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Design and Syntheses of GM_3- and Lactose-containing Multivalent Glycoconjugates

William K. C. Park

Thesis submitted to

the School of Graduate Studies and Research

in partial fulfillment for the degree of

Doctor of Philosophy in Chemistry

University of Ottawa

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To my wife Jiyoung and my son Joshua

Design and Syntheses of GM_3- and Lactose-containing Multivalent Glycoconjugates

Abstract

Cell-surface carbohydrates are known to be responsible for various cellular activities. These carbohydrates are involved in many recognition and adhesion processes acting as receptors for viruses, bacteria, toxins, hormones and other cells. The carbohydrates are present on the cell surface in branched forms and are conjugated with proteins and lipids which are embedded in the plasma membrane. Isolation and purification of glycoconjugates from natural sources are difficult to achieve. Thus, syntheses of chemically well-characterized, carbohydrate hapten-containing compounds are called for. In order to achieve this goal, the recently found concept of multivalency is used as a part of synthetic strategy.

A ganglioside, GM_3 has been chosen as a carbohydrate hapten in syntheses of various glycoforms, and lactose has been used as a model system for the GM_3. Both carbohydrate haptens have been modified to glycosyl azide forms so that a versatile N-acrylamido function could be provided by facile reduction and acryloylation. The GM_3 carbohydrate moiety has been constructed by coupling a lactosyl azide derivative as glycosyl acceptor and a thiophenyl sialoside as glycosyl donor in the presence of NIS/TfOH as promotor.

Lactose- and GM_3-containing glycopolymers with different spacer arms and varied lengths have been synthesized. Double immunodiffusions of the GM_3-containing glycopolymers have been performed for evaluation of their binding interactions with WGA.

A single-step reaction to provide a series of lactose-containing telomers has been examined as a facile route to glycoconjugate syntheses. Monomeric N-acrylamidolactose has been telomerized with *t*-butylmercaptan as telogen in the presence of AIBN as initiator. Similarly, the lactose-containing monomer with hexanoate spacer arm has been telomerized. A dimer was prepared by tethering two lactose-containing monomers with 1,3-propanedithiol. Both the telomers and the tethered dimer have been compared for their inhibitory potencies using a serological assay, enzyme-linked lectin assay (ELLA). All telomers exihibited similar inhibitory potency to that of the free lactose, while the tethered dimer showed the highest inhibition against lactose-containing polymer among the compounds tested.

In order to mimic the dendritic nature of cell-surface carbohydrates, synthetic dendrimers using gallic acid as a seeding molecule have been prepared. The dendrimers have been constituted of a 3^n increase in valency where n represents the n^{th} generation. The second generation dendrimer has been assembled using carbodiimide chemistry by coupling the parent amino-ester with the azido-acid daughter molecules. Using chemoselective de-S-acetylation by hydrazinium acetate, thioacetates of lactose derivatives were transformed into the corresponding thiols. The thiols have been conjugated to the electrophilic peripheries of the first and second generation dendrimers. Similarly, the thiols of lactose and lactosamine derivatives have been anchored to already available L-lysine-core dendrimers. These lactose-containing dendrimers have been evaluated for their bindings with the plant lectin *Arachis Hypogaea* (peanut lectin). The second generation gallic acid-based dendrimer showed strong precipitin band formation with the lectin whereas the first generation dendrimer did not form precipitin band.

Acknowledgements

I wish to express the deepest appreciation to my supervisor, professor R. Roy for his guidance, encouragement and support not only as a supervisor but also as my mentor during my stay at University of Ottawa. His knowledge, enthusiasm and openness towards research was the major driving force of our achievement.

The appreciation is extended to professor W. Szarek at Queen's University and professor T. Durst of this department for their insightful advice and encouragement.

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Abbreviations.

AgOTf	Silver trifluoromethanesulfonate
ABTS	2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
Ac_2O	acetic anhydride
AIBN	azobisisobutyronitrile
ASGP-r	asialoglycoprotein receptor
°C	degree Celsius
COSY	shift correlation spectroscopy
CRD	carbohydrate-recognition domain
d	doublet
Da	dalton
DEPT	distortionless enhancement by polarization transfer
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DMSO-d_6	hexadeuterated dimethylsulfoxide
DMTST	dimethyl (methylthio) sulfonium trifluoromethane sulfonate
EDC	1-(3-dimethylaminopropyl)-3-ethyl carbodiimide
ELLA	enzyme-linked lectin assay
equiv.	equivalent(s)
EtOAc	ethyl acetate
EtOH	ethanol
Et_2O	diethyl ether
Et_3N	triethylamine
FAB-MS	fast atom bombardment ionization mass spectrometry
GM_3	monosialylated ganglioside 3
h	hour(s)
HETCOR	heteronuclear shift correaltion spectroscopy
HOBt	1-Hydroxybenzotriazole
KDO	3-deoxy-D-manno-octulosonic acid
min	minute(s)
m.p.	melting point

MeCN	acetonitrile
MSB	methyl sulfenylbromide
MW	molecular weight
Neu5NAc	N-acetylneuraminic acid
NIS	N-iodosuccinimide
NMR	nuclear magnetic resonance
p-TsOH	*p* -toluenesulfonic acid
q	quartet
RP	receptor protein
r.t.	room temperature
s	singlet
t-BuSH	*tert*-butanethiol
TLC	thin layer chromatography
TfOH	trifluoromethanesulfonic acid
WGA	wheat germ agglutinin

Table of Contents.

CHAPTER I. Introduction

I.1. Significance of Cell Surface Carbohydrates.

Carbohydrates are ubiquitous constituents of the cell surface and extracelluar matrix serving as important source of energy and as major structural components of cells and tissues. Beyond these classical roles, carbohydrates are now known to be participating in essential cellular activities.[1] These include various recognition and adhesion processes acting as receptors for enzymes, hormones, proteins, bacteria and viruses as shown in **Figure 1.1.**[2-5]

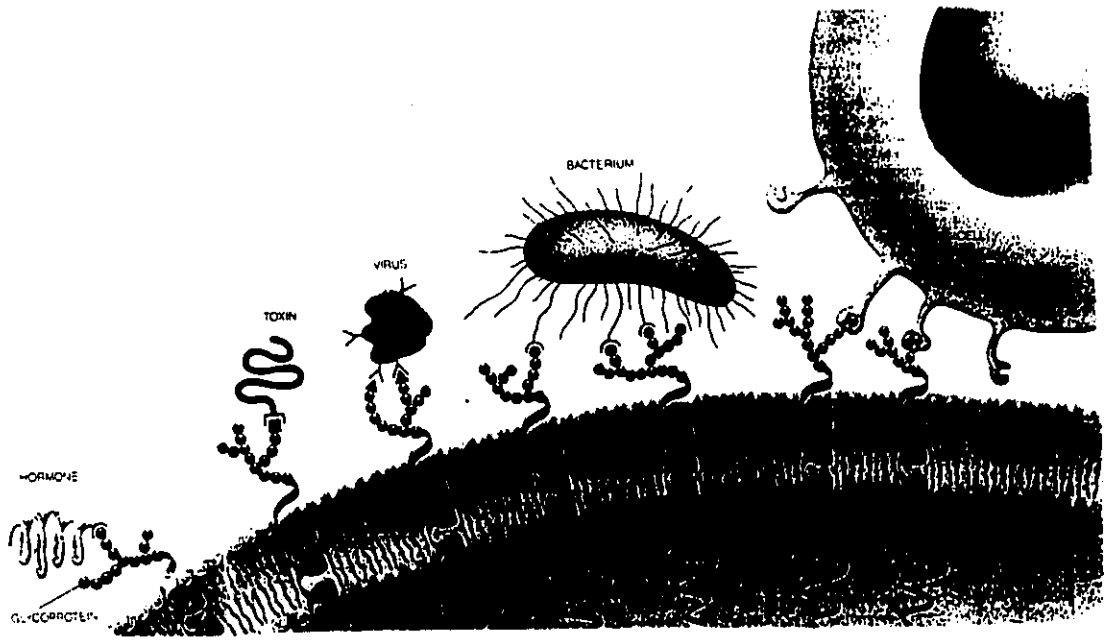

Figure 1.1. Carbohydrates on a cell surface and their biological roles.[6]

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1. H. Paulsen, *Angew. Chem., Int. Ed. in Engl.*, **1982**, 21, 155.
2. S. Hakomori, A. Kobata: *Blood group antigens in M. Sela:The Antigens 2,* Academic Press, New York, **1974**, 79.
3. R.C. Hughes: *Membrane Glycoproteins*, Butterworths, London, **1976**.
4. J. Montreuil, *Adv. Carbohydr. Chem. Biochem.*, **1980**, 37, 157.
5. J.C. Paulson, *The receptors*, 2, M.Conn (ed), Academic Press, Orlando, Florida, **1985**, 131.

The terminal carbohydrates are expressed multivalently on a layer of branched oligosaccharides which are in turn conjugated to proteins or lipid molecules. In the cellular environment, these glycoconjugates are externally expressed so that the hydrophilic nature of the carbohydrates embraces the aqueous environment while the hydrophobic aglycone part is embedded in the lipid bilayer of the plasma membranes. This orientational preference allows carbohydrates to be frontline mediators in a wide range of the above intercellular events.

The origins of the biological events taking place on the cell surface come from the complex structural nature of the carbohydrates. Such complexity can be easily envisaged if various combinations of structural differentiations such as the anomeric configuration, branching and functional substituents are considered. Thus carbohydrates can offer the highest information capacity.[6] For example, four monosaccharides can produce more than 35,000 possible tetrasaccharide structures whereas only 24 tetrapeptide arrangements are possible with four amino acids.[7] With vastly advancing current techniques, accurate sequences of naturally occuring oligosaccharide units have reaveled a remarkable complexity and diversity of molecules.[8-11] Such complex nature of oligosaccharides has been well described in the following statement: '··· while the functions of DNA and proteins are generally known, it is much less clear what carbohydrates do'.[11]

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6. N. Sharon, H. Lis, *Scientific American*, **1993**, Jan., 82.
7. B.N.N. Rao, M. Moreland and B.K. Brandly. *Med. Chem. Res. 1*,1, **1991.**
8. Bock, G. and Harret, S. *Carbohydrate Recognition in cellular Function*, Ciba Foundation Symposium 145, Wiley, New York, **1989**, 3.
9. M. Horowitz and W. Pigman, *The glycoconjugates*, Academic Press, New York, **1982.**
10. R. Schauer, *Sialic Acids, Chemistry, Metabolism and Function*, Springer-Verlag, New York, Vol. 10, **1982**, 5.
11. Ginsburg, V. and Robbins, P., *Biology of Carbohydrates*, J. Wiley, New York, **1991**, 3.

As previously mentioned, the roles of carbohydrates on the cell surface include structural support and participation in a wide range of biologically essential processes: more specifically, physical maintenance of tissue structure, integrity and porosity, protection of polypeptides from proteases or antibodies, receptors for viruses, bacteria and parasites, a hormone switching role, a tuning effect of heparin to antithrombin, a depot or sink function, intercellular binding with enzymes and cell-cell, cell-matrix recognitions.[12] Varki, in his recent review, has described a series of carbohydrates which are biologically significant. The **Table 1.1** shown below summarized the structures of some oligosaccharides and their biological functions.[12]

Table 1.1. Structures of some oligosaccharides and their biological roles.

Oligosaccharides	Biological effect	reference
Chondroitin sulfate and keratan sulfate	These constitute mulitiple components of cartilage and provide organization and tensile strength.	13-15
Gastric mucus (a glycoprotein with many O-linked sialylated and sulfated chains)	HCl in the lumen (pH 2) can't diffuse back to the epithelium because the high viscosity of the gastric mucus gel prevents the stomach from digesting itself.	16

12. A. Varki, *Glycobiology, 3* , **1993**, 97.
13. Hook, M, Kjellen, L. and Johansson, S., *Cell-Surface glycosaminoglycan*, **1984**, 53, 847.
14. T.E. Hardingham and A.J. Fosang, *Proteoglycans: FASEB J.*, **1992**, 6, 861.
15. V. Hascall, Proteoglycans: *Structure and Function.* In Ginsburg, V. and Robbins, P. (ed), *Biology of Carbohydrates*, J. Wiley, New York, 2, 523.
16. K. R. Baskar, P. Garik, B.S. Turner, J.D. Bradeley, R. Bansil, H.E. Stanley and J.T. LaMont, *Nature*, **1992**, 360, 458.

Table 1.1 (continued).

Oligosaccharides	Biological effects	reference
Heparan sulfate chains of proteoglycans	Interacting with fibronectin, laminin, collagen, organization of basement membrane and extracellular matrix, cell adhesion to matrix.	13-15
Terminal sialic acids	Receptors for a variety of virus hemagglutinins, first step in infectious process of viruses.	16-18
Polylactosamine on erythrocytes	Antigens for cold agglutinins and other autoimmune antibodies. Responsible for autoimmune destruction of cells.	19, 20
GM_1 ganglioside	Receptors for several bacterial toxins, e.g. *Vibrio cholerae* B subunits	21, 22
Sialylated N-linked Oligosaccharides	Expressed in increased amounts on transformed cells believed to enhances tumorigenecity and metastatic capability of tumor cells	23-25
Terminal 9-O-acetylated sialic acids	influenza C viruses	16,17 26,27
Terminal sialic acids on glycoproteins and cell surfaces	Masking recognitions of β–GalNAc residues, asialoglycoprotein receptor, macrophage Gal/GalNAc lectin.	28, 29
Terminal sialic acids on O-linked oligosaccharides	Masking recognition of core O-linked oligosaccharides by natural antibodies to T and Tn antigens	30, 31

14. E. Ruoslahti, *Annu. Rev. Cell Biology*, **1988**, 4, 229.
15. L. Kjellen and V. Lindahl, *Annu. Rev. Biochem.*, **1991**, 60, 443.
16. R. Schauer, *Trends Biochem. Sci.*, **1985**, 10, 357.
17. A. Varki, *Glycobiology*, **1992**, 2, 5.
18. M.A. Markwell and J.C. Paulson, *Proc. Natl. Acad. Sci.*, USA, **1980**, 77, 5693.
19. T. Feizi, *Nature*, **1985**, 314, 53.

Table 1.1 (continued.)

Oligosaccharides	Biological effects	reference
Plasma membrane gangliosides	Binds tightly with Ca^{2+}, local increase in Ca^{2+} concentration believed to affect receptor recognition processes	32
Sialyl $Lewis^x$	Present on the white blood cell surface during inflammation process. Binds with the lectin called E-Selectin. (Similar bindings are found in P- and L-Selectins)	33-35
α-(2-6) linked sialic acids on lactosamine	Immunogenecity related; Early stage in interactions of B-cells with activated T-cells or activated B-cells.	36, 37
	α-Linked Gal. Residue on O-Linked Oligosaccharides of egg protein Sperm-egg recognition	
GM_3 ganglioside	Interaction with Gg_3 or Lac-Cer glycolipids (carbohydrate-carbohydrate interaction). Proposed that they mediate interactions between different cell types. Abnormally high concentration expressed on tumor cells.	38-40

20. R. Kannagi, D. Roelcke, K.A. Peterson, Y. Okada, S.B.Lavery, S. Hakomori, *Carbohydr. Res.*, **1983**, 16, 143.
21. I. Ofeek and N. Sharon, *Curr. Top: Microbiol. Immunol.*, **1990**, 151, 91.
22. K.-A. Karlsson, *Annu. Rev. Biochem.*, **1989**, 58, 309.
23. J.W. Dennis and S. Laferte, *Cancer Metastatic Rev.*, **1987**, 5, 185.
24. K. Yamashita, Y. Tachibana, T. Ohkura and A. Kobata, *J. Biol. Chem.*, **1985**, 260, 3963.
25. J.W.Dennis, S. Laferte, C. Waghorne, M.L. Breitman and R.S. Kerbel, *Science*, **1987**, 236, 582.
26. R. Schauer, *Glycobiology*, **1991**, 1 , 449 J. B1
27. G.N. Rogers, G. Herrler, J.C. Paulson and H.D. Klenk, *J. Biol. Chem.*, **1986**, 261,5947.
28. G. Ashwell and J. Harford, *Annu. Rev. Biochem.*, **1982**, 51, 531.
29. A.L. Schwartz, *Annu. Rev. Immunol.*, **1990**, 8, 195.
30. M. Inoue, S. Ton, H. Ogawa and O. Tanizawa, *Am. J. Clin.Pathol*, **1991**, 96, 711.

From the examples enlisted above it is clear that the biological roles of oligosaccharides appear to span the spectrum from those that are trivial to those that are crucial for the development, growth, function or survival of an organism. It also implies that synthetic glycoconjugates which can imitate or change their cell-cell and cell-matrix interactions have enormous therapeutic potential as well as values as tools for studying fundamental concepts underlying protein-carbohydrate and/or carbohydrate-carbohydrate interactions.

It is of our interest, therefore, to synthesize carbohydrate-containing molecules which mimic the naturally occurring glycoconjugates. To achieve this goal it is necessary to comprehend the concept of the multivalency effect.[41] This effect suggests that both the number of carbohydrate residues and their respective proximity confer tc the glycosylated clusters their improved overall binding affinity (avidity). An individual glycoside usually exhibits a very weak binding affinity with its receptor. However, the clustered molecules with the same glycosides have very tight binding. For example, monomeric hemagglutinin (HA), the receptor for α-sialosides requires high concentration of the α-sia losides for binding ($K_D \sim 2 \times 10^{-3}$ M) while much less α-sialosides

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31. S. Itzkowitz, T. Kjeldsen, A. Friera, S. Hakomori, U.-S. Yang and Y.K. Kim, *Gastroenterology*, **1991**, 100, 1691.
32. B. Maggio, J.M. Sturterant and R.K. Yu, *Biochem. Biophys. Acta.* **1987**, 901, 173.
33. G. Waltz, A. Aruffo, W. Kolanus, M. Bevilacqua and B. Seed, *Science*, **1990**,250, 1132.
34. E.L. Berg, M.K. Robinson, O. Mansson, E.C. Butcher and J.L. Magnani, *J. Biol. Chem.*, **1991**, 266, 14869.
35. M. Larkin, T.J. Ahern, M.S. Stoll, M. Schaffer, D. Sako, J. O'Brien, C.-T. Yuen, A.M. Lawson, R.A. Childs, K.M. Barone, P.R. Lauqer-Safer, A. Hasegawa, M. Kiso, G.R. Larsen and T. Feizi, *J. Biol. Chem.*, **1992**, 267, 13661.
36. I. Stamenkovic, D. Sgroi, A. Aruffo, M. S. Sy and T. Anderson, *Cell*, **1991**, 66, 1133.
37. I. Stamenkovic, D. Sgroi, A. Aruffo, *Cell*, **1992**, 68, 1003.
38. N. Kojima, S. Hakomori, *J. Biol. Chem.*, **1991**, 266, 17552.
39. N. Kojima, M. Shiota, Y. Sadahira, K. Handa and S. Hakomori, *J. Biol. Chem.*, **1992**, 267, 17264.
40. R. A. Kinloch, S. Mortillo, C. I. Stewart and P.M. Wassarman, *J. Cell Biology*, **1991**, 115, 655.
41. Y.C. Lee, R.T. Lee, K. Rice, Y. Ichikawa, T.-C. Wong, *Pure Appl. Chem.* **1993**, 63, 499.

are needed to obtain the same binding effect (μM range) when clustered.[42,43] In another instance, the mammalian hepatic asialoglycoprotein Gal/GalNAc receptors (ASGP-r) exhibit dramatically increased binding affinity when two or three ligands are clustered. In general, the simple glycosides have binding affinity of 10^{-4} M whereas the binding affinity for clustered asialoglycoproteins is about 10^{-9} M.[44] Along with the multivalent effect, the flexibility of their attachment can be another parameter to control the binding affinities.[45,46] This aspect has been examined by synthesizing lactose-containing cluster molecules such as those shown in **Figure 1.2.**[7]

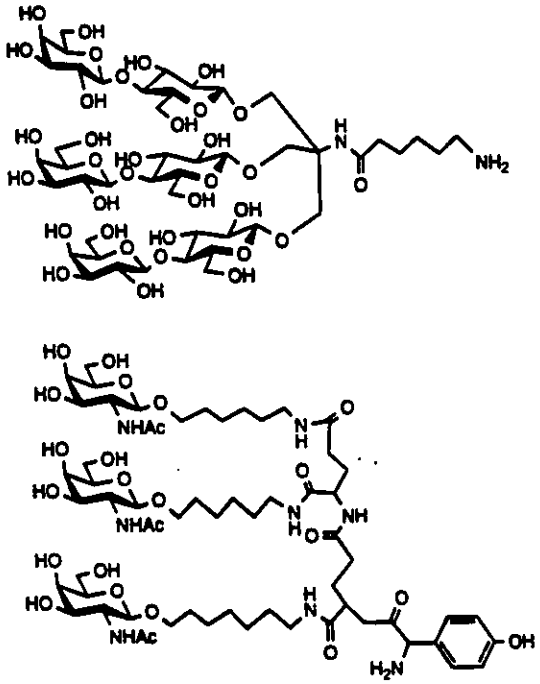

Figure 1.2. Clustered lactose and N-Acetylgalactosamine ligands with different spacer lengths.[7]

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42. R. Roy, F. O. Andersson, G. Harms, S. Helm, R. Schauer, *Angew. Chem., Int. Ed. Engl.*, **1992**, 31, 1478.
43. D.C. Wiley, J.J. Skeehel, *Annu. Rev. Biochem.*, **1987**, 56, 365.
44. R.T. Lee, Y.C. Lee, *Glycoconjugate J.* **1987**, 4, 317.
45. G.D. Glick, R. Knowles, *J.Am. Chem. Soc.*, **1991**, 113, 4703.
46. S.A. DeFrees, W. Kosch, W. Way, J.C. Paulson, S. Sabesan, R.L. Halcomb, D.-H. Huang, Y. Ichikawa, C.-H. Wong, *J. Am. Chem. Soc.*, **1995**, 117, 66.

In the present study, N-linked sialyl-α(2-3)-lactoside (the carbohydrate moiety of the GM_3 molecule) and lactoside were chosen as the carbohydrates of interest. The GM_3 and its structural analogues are known to be involved in cell proliferation, differentiation, oncogenesis, modulation of transmembrane signaling and immunosupression processes.[47,48] The GM_3 molecule is also found to be expressed in abnormally high concentration in tumor cells.[49,50] As seen in **Figure 1.3**, the naturally occurring GM_3 consists of two main features, a carbohydrate and lipidic ceramide part. The sialylated trisaccharide is β-O-linked to the ceramide moiety, a long hydrocarbon chain which is embedded in the plasma membrane. The ceramide portion of the GM_3 is believed to play a role mainly as structural support for the carbohydrate part. Thus, it was envisaged that the trisaccharide unit of GM_3 linked to an N-acrylamide function can be prepared as a monomer. This carbohydrate monomer can then be polymerized to provide GM_3-containing macromolecules which will be expected to possess the multivalent property. In order to achieve this goal lactose,

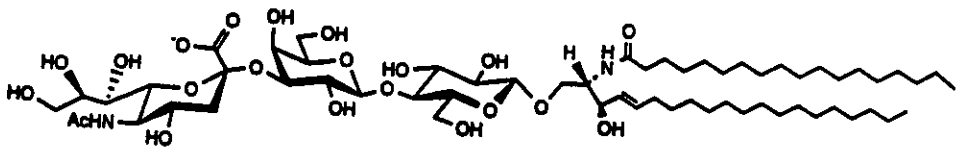

Figure 1.3. The structure of the GM_3.

47. S. Hakomori, J. Biol. Chem., **1990**, 265, 18713.
48. W. Song, M.F. Vacca, R. Welti, D.A. Rintoul, J. Biol. Chem., **1991**, 266, 10174.
49. J.M. Carubia, R.K. Yu, L.J. Macala, J.M. Kirkwood, J.M. Varga, Biochem. Biophys. Res. Commun. **1984**, 120, 500.
50. J. Portoukalian, G. Zwingelstein, J.-F. Dore, J.-J. *Biochemie*, **1976**, 58, 1285.

the asialoform of GM_3 was chosen as model system. Lactosides are known to be involved in the recognition process by macrophages[51] and have also been demonstrated to contain inhibitory properties in the metastasis of tumor cells.[52]

It is envisaged therefore that synthetically prepared GM_3- and lactose-containing clusters should not only be excellent tools for studying the multivalency effect but also possess therapeutic values. The running theme of this dissertation, at least in part, includes the synthesis of multivalent GM_3- and lactose-containing molecules.

I.2. The Design of Glycoconjugates.

Carbohydrates are expressed on the cell surface in conjugated forms with varieties of lipids (glycolipids) and proteins (glycoproteins). With respect to their structures, glycolipids are generally well understood. However, glycoproteins are complex and made of many different N- and O-linked glycan structures which are at various stages of their metabolic processes. Isolation of the homogeneous glycoproteins of interest from natural sources would be exceedingly difficult to achieve. Thus, direct use of the isolated glycoproteins from the natural sources as tools to study binding interactions or as possible therapeutic agents is impractical.

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51. R. Schauer, A.K. Shukla, C. Schröder, E. Müller, *Pure & Appl. Chem.*, **1984**, 56, 907.
52. B. Dean, H. Oguchi, S. Cai, E. Otsuji, K. Tashro, S. Hakomori, T. Toyokuni, *Carbohydr. Res.*, **1993**, 245, 175

Synthetic glycoconjugates constitute families of carbohydrate derivatives which include neoglycoproteins, neoglycolipids, their clusters and glycopolymers.[53,54] Most attention in the syntheses of such glycoconjugates has been paid to carbohydrate-protein interaction studies,[55,56] synthetic vaccines,[57] inhibitions of cell adhesions by viruses, bacteria, mycoplasma and toxins,[58] ligands in affinity chromatography,[59,60] diagnostics, probes and targeted drug-delivery systems.[61]

Setting aside the above structural problems of natural glycoconjugates and their use, the most fundamental difficulty lies in the fact that their concentrations in the cellular environment are not clearly defined. Therefore, understanding of the avidity or the multivalency effect is required. The multivalency effect refers to the enhancement of the overall binding interaction of carbohydrates towards their receptors. Since this effect was first noted by Lee et al.,[52] many different synthetic clusters have been introduced.[43,44]

Among many synthetic glycoconjugates, the most well established are the neoglycoproteins. The strategy employed in the syntheses of such molecules is relatively simple: suitably derivatized carbohydrate haptens are attached to the protein

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53. Y.C. Lee and R. T. Lee (eds), *Neoglycoconjugates: Preparation and Applications, Academic Press*, San Diego, CA **1994**.
54. R. Roy, *Design and Synthesis of Glycoconjugates*, in Modern Methods in Carbohydrate Synthesis, S.H. Khan, R. O'Neill Eds., Harwood Academic Publisher, **1995**.
55. I. J. Goldstein and R.O. Loretz, In the Lectins, *Properties, Functions and Applications in Biology and Medicine*, I. E. Liener, N. Sharon and I. J. Goldstein, eds, Academic Press, Orlando, FL, **1986**, 35-247.
56. R. U. Lemieux, *Chem. Soc. Rev.*, **1989**, 18, 347.
57. W.E. Dick and M. Beurret, *in Microbiology and Immunology, vol.10, Conjugate Vaccines* (J. M. Cruse, R. E. Lewis, Jr., eds.), Karger, Basel, **1989**, 48-114
58. J. Haensler and F. Schuber, *Glycoconjugate J.*, **1991**, 8, 116.
59. R.L. Schnar, *Anal. Biochem.*, **1984**, 143, 1.
60. J.H. Pazur, *Adv. carbohydr. Chem. Biochem.*, **1981**, 39, 405.
61. J. Kopecek, *J. Controlled Release*, **1990**, 11, 279.

carrier with activated side-arms as shown in **Figure 1.4.**

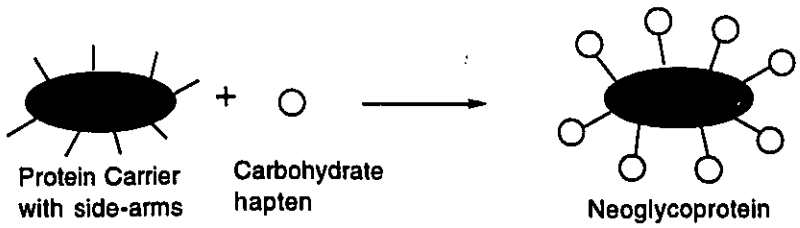

Figure1.4. Synthetic strategy of neoglycoproteins.

Reductive amination of free amine residues of proteins (ε-amine of lysine and N-terminus) has been used to link to carbohydrate haptens containing terminal aldehyde using sodium cyanoborohydride ($NaCNBH_3$).[62,63] This method can be performed under mild conditions without further modifications of the carbohydrates or alteration of the overall charge of the protein carrier. However, it suffers from some drawbacks. For example, the process is slow and the highly toxic $NaCNBH_3$ has to be used in excess.

p-Aminophenyl glycosides[64] and alditol[65,66] derivatives have been used for a long time in spite of their apparent disadvantages associated with the fact that diazotization of an aromatic amine is not chemoselective and a large excess of diazophenyl glycosides is necessary for the conjugation with the protein carrier. The isothiocyanate method[66] also requires the use of a large excess of toxic thiophosgene to make the thiocarbamoyl linkage with amine functions of protein carriers.

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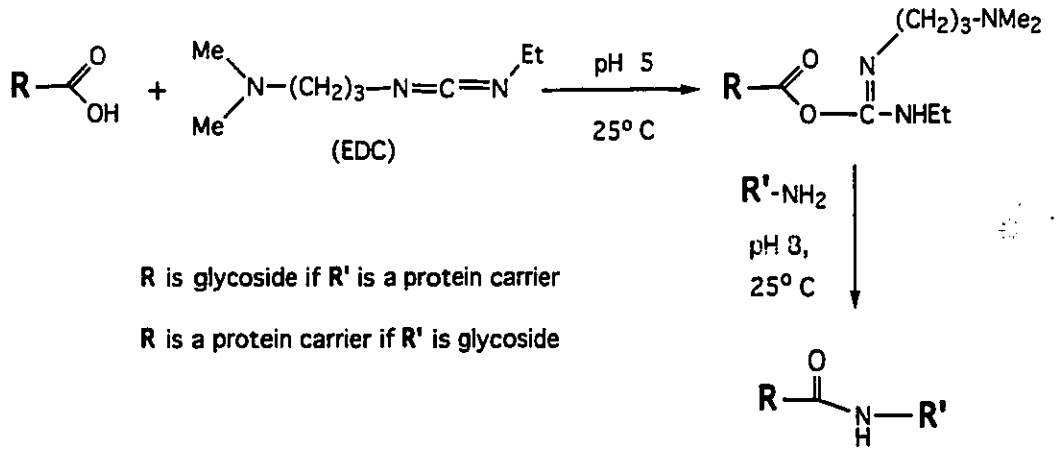
62. B.J. Kamicker, B.A. Schwartz, R.M. Olson, D.C. Drinkwitz, G.R. Gray, *Arch. Biochem. Biophys.* **1977**, 193, 393.
63. R. Roy, C.A. Laferrière, *Can. J. Chem.* **1990**, 68, 2045.
64. O. Westphal, H. Feir, *Chem. Ber.* **1956**, 89, 582.
65. S.B. Svenson, A.A. Lindberg, *J. Immunol. Methods*, **1979**, 25, 323.
66. D.A. Zopf, D.F. Smith, Z. Drzeniek, C.M. Tsai, V. Ginsburg, *Methods in Enzymol.* **1978**, 171, 50.

The syntheses of neoglycoproteins are also possible by using an amidation method (**Scheme 1.1**). A water soluble carbodiimide (1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC)) was used to link carbohydrate and protein carrier (each part can be either in an acid or an amine form). This method seems to proceed well but with several drawbacks associated with the nature of the protein used. For instance, great care and control of pH during the reaction

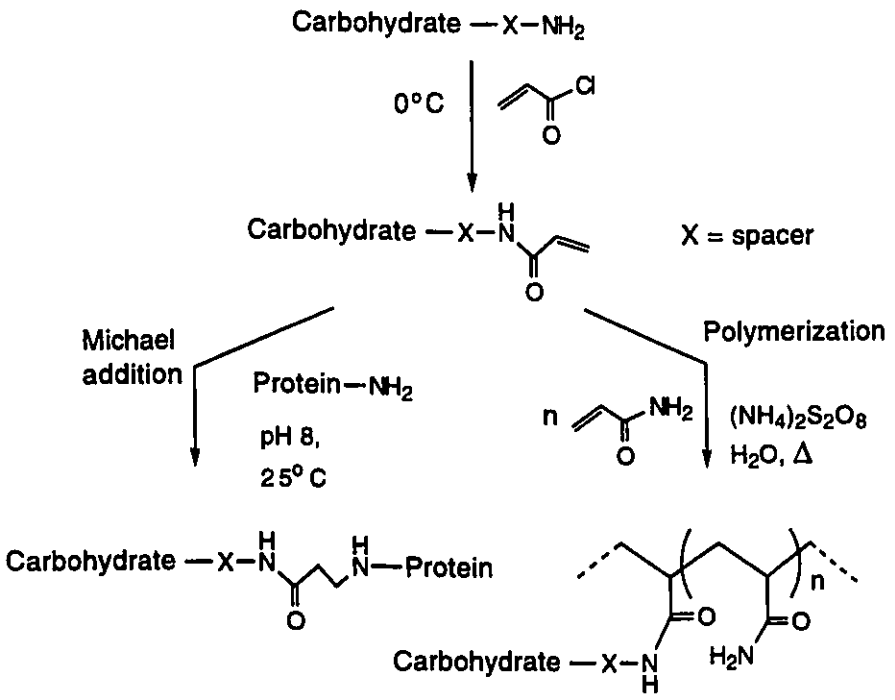


Scheme 1.1. EDC mediated neoglycoprotein synthesis.

is necessary to prevent the protein carrier molecules from crosslinking.[67] Furthermore, the overall charge (pI) of the protein molecules can be altered during the conjugation which may result in the denaturation of the protein carrier.

An effective conjugation of carbohydrate haptens to a protein carrier molecule has been reported by Roy et al.[68,69] In this method the nucleophilic termini of proteins are added onto an acrylamido function of the carbohydrate. The nucleophilic terminus of protein carrier can be either a thiol (a side chain of cysteine) or an amine (lysine ε-terminus and protein N-terminus). Furthermore, the N-linked acrylamide function can also be used as monomer for the copolymerization[70] shown in **Scheme I.2.**

By using this method the conjugated products satisfy the following requirements which are necessary for the syntheses of glycoproteins. First, the reagents used for the syntheses must be mild and selective as well as nontoxic. This is because the molecules to be prepared are multifunctional in nature and are potentially involved in medicinal applications. Second, the final products should be soluble in water. Third, the chemical procedures should have high yield since most of the carbohydrate haptens and proteins are expensive. Fourth, the procedures should also be applicable to different carbohydrate haptens.



Scheme 1.2. Neoglycoprotein and Neoglycopolymer syntheses from a versatile acrylamide precursor.

67. M.-T. B. Davis, J.F. Preston, *J. Anal. Biochem.* **1981**, 116, 402.
68. R. Roy, F.D. Tropper, T. Morrison, J. Boratynski, *J. Chem. Soc., Chem. Commun.* **1991**, 7, 536.
69. R. Roy, F.D. Tropper, A. Romanowska, R. K. Jain, C.F. Piskorz, K.L. Matta, *Bioorg. & Med. Chem. Lett.*, **1992**, 2, 9, 911.
70. F.D. Tropper, Ph.D. Thesis, University of Ottawa, **1991**, 25.

Despite the fact that the glycoproteins prepared by the various methods meet the above requirements, they suffer from certain drawbacks. First of all the characterizations of the carrier molecules are difficult due to the various arrangement of 20 different amino acids. Second, neoglycoproteins are biodegradable and also sensitive to nonphysiological environment such as acidic or basic media which is often inevitable during conjugation. Therefore, the resulting neoglycoproteins should be handled with great care and often have very short life time even under refrigerated conditions. Furthermore, the exact degree of carbohydrate incorporation to protein carrier molecule can not be controlled. Last, the carrier molecule itself can be a cause of non-specific binding and can disturb the true state of the binding interactions during various serological studies. These disadvantages severely limit their initially premised abilities to be used in related studies and thus syntheses of other types of glycoconjugates have been investigated. Especially, the above mentioned N-acrylamido-carbohydrates have been rigorously looked into as versatile precursors for the syntheses of glycoconjugates. This aspect is described in the following section.

I.3. Glycopolymers.

Since the first synthetic glycopolymers were introduced by Horejsi and Kocourek involving copolymers of allyl glycosides and acrylamide,[71] many glycopolymers have been synthesized and used as tools to study binding interactions.[72-74] One of the most important reasons why these glycopolymers have triggered much attention, comes from the pioneering observation of the multivalency effect by Lee.[75,76] This effect have demonstrated the significance of carbohydrate density on glycoproteins when some cells or specific ligand molecules interact with carbohydrates. For example, it has been clearly shown that, in Gal*p*β(1-3)Gal*p*NAc (T-antigen epitope)-hepatocyte binding systems, a 'cluster' or 'multipoint' carbohydrate-ligand interaction was found to be involved in the successful binding process.[77,78] This result led to the syntheses of various

carbohydrate-containing clusters such as glycopolymers in the premise that the polymer backbones could replace the protein carriers which have several disadvantages as discussed above. Polyacrylamide copolymers are one of them and possess the following advantages: they are easy to prepare, water soluble and have high molecular weight. In addition, the degree of carbohydrate incorporation can be chosen simply by altering the proportion of monomers in the reaction. Furthermore, they have simple and well-characterizable structures that minimize the risk for undesired immunological responses such as non-specific bindings as is often the case in neoglycoproteins.

Allyl glycosides have been first used as monomers for polymerization. These have distinct advantages for the following reasons: the allyl group is an effective protecting group in carbohydrate chemistry[79] and a precursor to reductive amination strategies to proteins.[80] However, glycopolymers prepared from this method suffer from a short distance between the polymer backbone and the carbohydrate hapten. It has been pointed out that this may not provide the critical distance necessary for an antibody-antigen binding interaction.[81] These difficulties come from steric constraints imposed by the proximity of the polymeric backbone and the antigenic determinant. In order to provide the distance necessary for binding, elongated spacer arms were introduced such as n-

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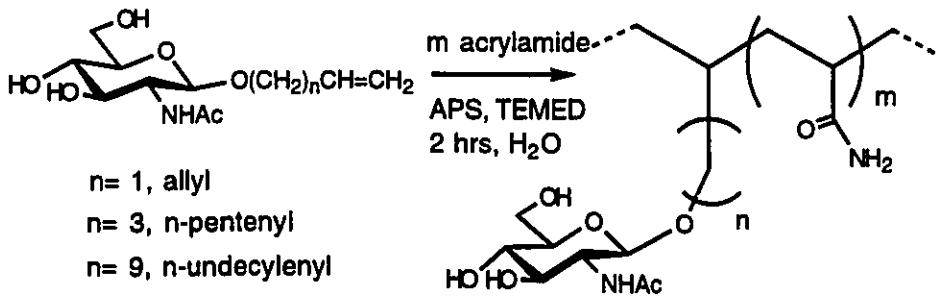
71. V. Horejsi and J. Kocourek, *Biochem. Biophys. Acta.*, **1973**, 297, 346.
72. A.Y. Chernyak, A.B. Levinski, B.A. Dmitriev and N.K. Kochetkov, *Carbohydr. Res.*, **1984**, 128, 269.
73. P.H. Wiegel, E. Schmell, Y.C. Lee and S. Roseman, *J.Biol.Chem.*, **1976**,253,330.
74. A. Spaltenstein and G.M. Whitesides, *J. Am. Chem. Soc.*, **1991**, 113, 686.
75. Y.C. Lee, *FASEB J.*, **1992**, 6, 3193.
76. Y.C. Lee, *Carbohydr. Res.*, **1978**, 67, 509.
77. R.T.Lee and Y.C. Lee, *Methods in Enzymol.* 138, 424.
78. R.T. Lee, P. Lin and Y.C. Lee, *Biochemistry* **1984**, 23, 4255.
79. R.W. Binkley (ed), *Modern Carbohydrate Chemistry*, Marcel Decker, New Yory, **1988**, 121-122.
80. M.A. Bernstein and L.D. Hall, *Carbohydr. Res.*, **1980**, 78, C1.
81. R. Roy and F.D. Tropper, *J. Chem. Soc., Chem. Commun.*, **1988**, 1058.

pentenyl or n-undecenyl glycosides (**Scheme 1.3**).[82,83] However, introduction of too long lipophilic chains for the sake of provision of longer distance between the haptens and the backbone structure can cause solubility problems.[84] Also, the reaction rate difference

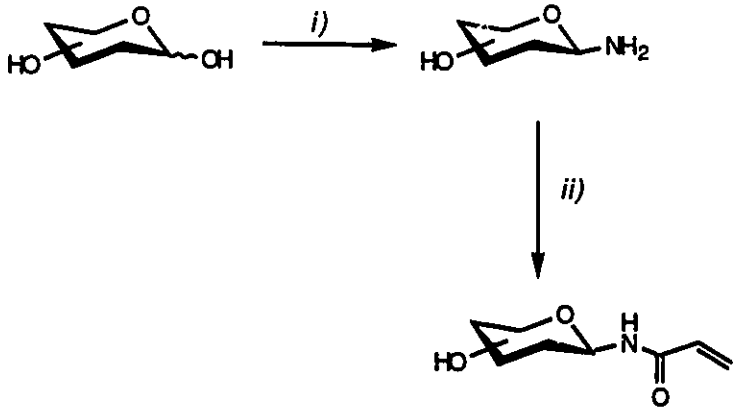


Scheme 1.3. Copolymerization of allyl glycosides and acrylamide.
APS, Ammonium peroxodisulfite; TEMED, N,N,N',N'-tetramethylethylenediamine.

during copolymerization with acrylamide is likely to result in undesirable block copolymers. This can make carbohydrate density in the polymer difficult to predict and control. To overcome this problem, N-acryloylated carbohydrate monomer has been suggested. The strategy, which employs acrylamide as comonomer, is believed to minimize the above mentioned block polymerization since the moieties of both monomers which participate in polymerization share the same N-acrylamido functionality. These monomers have

82. S.-I. Nishimura, T. Furuike, K. Matsuoka , *Methods in Enzymol.*, **1994**, 242 , 235.
83. N.K. Kochetkov, *Pure Appl. Chem.*, **1984**, 56, 923.
84. S.-I. Nishimura, K. Matsuoka and K. Kurita, *Macromolecules*, **1990**, 23, 4182.

been prepared by reductive amination followed by N-acryloylation.[85] The monomers are copolymerized with acrylamide (**Scheme 1.4**). The reductive amination step involves the treatment of free carbohydrates (reducing sugars) with saturated aqueous ammonium bicarbonate for a period of 6 days at 30°C. However, it is known that the reaction depends on temperature and pH[86] and often suffer from low yields. Once obtained, the N-acrylamide carbohydrate derivatives are copolymerized with acrylamide.



Scheme 1.4. Preparation of N-acryloylglycosylamine by amination.
i) NH_4HCO_3 ; *ii)* acryloyl chloride/ Na_2CO_3.

Easier methods have been developed to prepare N-acrylamido carbohydrate monomers by acryloylation of glycoside derivatives as shown in **Scheme 1.5**.[87,88] The N-acrylamido function was provided by reacting p-aminophenyl-O-glycoside derivatives with acryloyl chloride at 0°C. Distinct advantages of this method over the reductive amination include that the N-acryloylation proceeds well for both free hydroxylated or acylated carbohydrate derivatives and provides the desired monomer with excellent yields, and requires much shorter reaction time.

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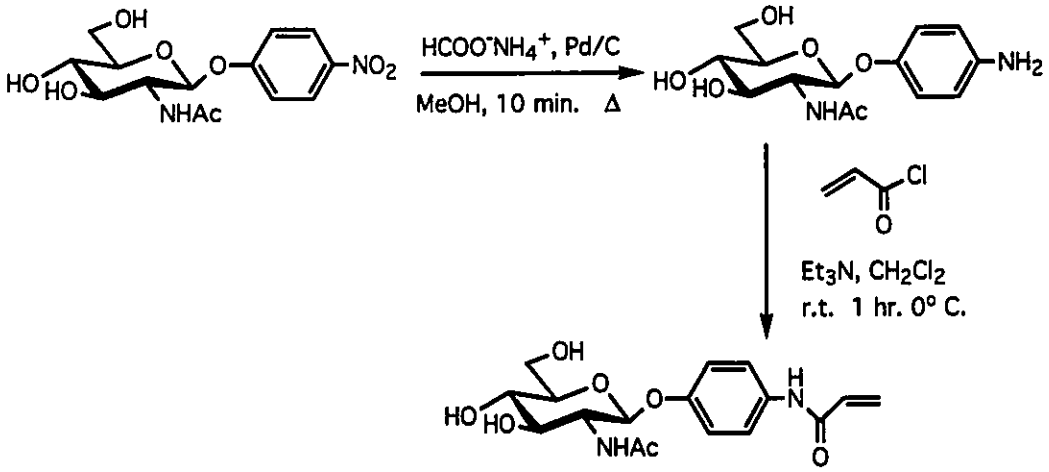
85. a. R. Roy, C.A. Laferrière, *Carbohydr. Res.*, **1988**, 177, C1; b. R. Roy, F.D. Tropper, *J. Chem. Soc., Chem. Commun.*, **1988**, 1058; c. E. Kallin, H. Lönn, T. Norberg and M. Elofsson, *J. Carbohydr. Chem.*, 1989, 8, 597.
86. R. Roy and C. Laferrière, *J. Chem. Soc., Chem. Commun.*, **1990**, 1709.



Scheme 1.5. Preparation of a p-N-acryloylphenyl glycoside by acryloylation.

Perhaps the most distinguishable virtue of the N-acrylamido function in carbohydrate derivatives can be found in its versatility. Various glycopolymers and glycoproteins have been prepared by this method as described earlier. In addition, this functionality on carbohydrate haptens can be utilized as precursors to telomerization and dendrimerization processes (*vide infra*).

In this dissertation, the syntheses of several glycopolymers are described. Lactose- and GM_3-containing monomers with different lengths of spacer arms are copolymerized with acrylamide to give the corresponding glycopolymers and their binding abilities towards plant lectins are discussed.

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87. R. Roy and F.D. Tropper, Glycoconjugate J., **1988**, 5, 203.
88. R. Roy and C. A. Laferrière, *Methods in Enzymol.*, **1994**, 242, 271.

I.4. Telomerization.

Carbohydrate-containing polymers (glycopolymers) have been specifically designed to access the multivalent properties of clusters.[89] However, such glycopolymers constitute poorly defined chemical entities and thus represent mediocre therapeutic agents. To overcome this drawback, we describe herein a single-step synthesis of a family of clusters derived from quenching polymer growth by chain transfer agents (telogen).[90] The classical example of this type involves the reaction of ethylene as monomer with CCl_4 as telogen as shown in Equation 1.1.

$$CCl_4 + n\ CH_2{=}CH_2 \longrightarrow Cl_3C(CH_2CH_2)_nCl \qquad n = 1, 2, 3..... \qquad \text{Eq. 1.1.}$$

The stepwise sequence of this process is shown below.

Initiation

$$\text{Initiator} \xrightarrow{CCl_4} \text{radicals} \longrightarrow {}^{\bullet}CCl_3 \qquad \text{Eq. 1.2.}$$

Chain growth

$$^{\bullet}CCl_3 + nH_2C{=}CH_2 \longrightarrow CCl_3(CH_2CH_2)_n^{\bullet} \qquad \text{Eq. 1.3.}$$

Chain transfer

$$CCl_3(CH_2CH_2)_n^{\bullet} + CCl_4 \longrightarrow CCl_3(CH_2CH_2)_nCl + {}^{\bullet}CCl_3 \qquad \text{Eq. 1.4.}$$

Termination

$$2\ R^{\bullet} \longrightarrow \text{non-radical products} \qquad \text{Eq. 1.5.}$$

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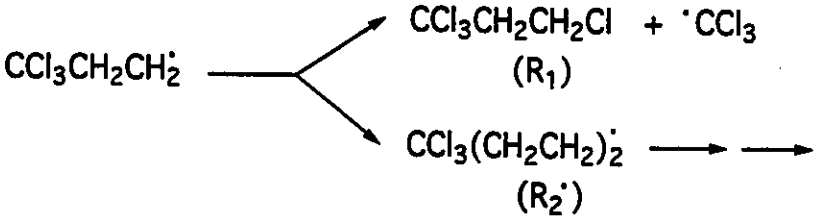
89. a. R. Roy, F.D. Tropper, A. Romanowska, *J. Chem. Soc., Chem. Commun.*, **1992**, 1611; b. R. Roy, F.D. Tropper, A. Romanowska, *Bioconjugate Chem.* **1992**, 3, 256; c. A. Kobayashi, T. Akaiki, K. Kobayashi, H. Sumimoto, *Makromol. Chem., Rapid Commun.* **1986**, 7, 645.
90. C.M. Starks, '*Free Radical Telomerizations*', Academic Press, **1974**, New York, Chapter 1.

In order for telomerization to occur, it is essential that the chain growth (Eq.1.3.) and the chain transfer (Eq.1.4.) steps have similar rates to each other. Apparently polymer can form if the rate of the chain growth is much greater than that of the chain transfer and an 1:1 adduct of monomer and telogen will form under the opposite condition. This relation is defined as C, the chain transfer coefficient.

$$C = \frac{\text{rate constant for chain transfer}}{\text{rate constant for chain growth}}$$

Assuming that the telomer itself cannot function as a chain transfer agent, the product distribution is determined by the rate of chain transfer relative to that of the chain growth. The first telomer radical $R_1^{\bullet}$, may either form the first telomer or elongate to the next telomer radicals as shown in **Scheme 1.6.**



Scheme1.6. The fate of the first radical monomer.

In addition, the temperature used during the telomerization affects the chain transfer coefficient C. Although some exceptions exist, the general trend seems to be that the values of C decrease as the temperature increases.[91]

The initiators suitable for polymerization are also generally suitable for telomerization. Although many initiators are available, azoisobutyronitrile (AIBN), benzoylperoxide and di-*tert*-butyl

peroxide are frequently used because of their nominal cost, availability and long storage life.[90] Furthermore, these three initiators have different but overlapping temperature ranges for operations: AIBN (40-100 °C), benzoyl peroxide (70-120 °C) and di-t-Butyl peroxide (120-170 °C).

Sulfur-centered radicals such as RS• or RSO2• readily participate in the chain transfer step. Most olefins react with the radicals such as hydrogen sulfide, thiols, sulfinic acids, sodium bisulfite and sulfonyl halides.[92] They frequently yield an 1:1 adduct even with high olefin/sulfur radical ratios. Thiols as telogens react reasonably well with monomers such as acrylates, methacrylate, styrene, vinyl chloride and acrylonitrile.[93] For example, alkane thiols such as ethane thiol (CH_3CH_2SH) and isopropane thiol (iso-C_3H_7SH) can react with methyl propenoate ($H_2C{=}CHCO_2CH_3$) and styrene to give low molecular weight telomers as shown below.

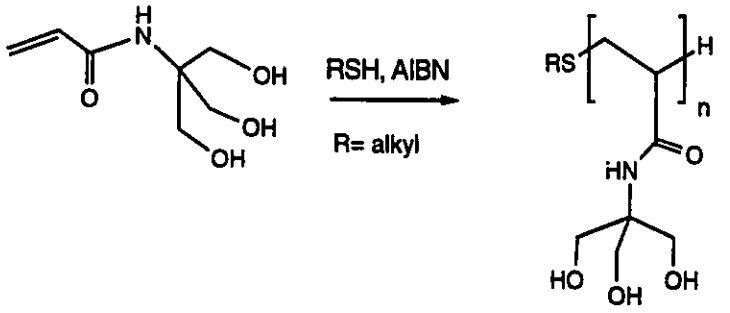
Table 1.2. Chain transfer coefficients of alkanethiols.

Telogen	Taxogen	C_1	C_2	C_3	C_∞	Ref.
CH_3CH_2SH	$H_2C{=}CHCO_2CH_3$	0.78	1.7	-	1.6	94
i-C_3H_7SH	$H_2C{=}CHCO_2CH_3$	0.54	0.64	0.57	0.6	95
CH_3CH_2SH	styrene	7	20	-	17	96

91. B.A. Englin and R. Kh. Freidlina, *Pokl. Akad. Nauk SSSR*, **1964**, 158, 922.
92. R.M. Kellog, *"Methods in Free Radical Chemistry"*, **1969**, 2, 1-117, Dekker, New York.
93. C.M. Starks, *'Free Radical Telomerizations'*, Academic Press, New York, **1974**, Chapter 8.
94. G.P. Scott, C. C. Soong, W.S. Huang and J.L. Reynolds, *J. Org. Chem.*, **1964**, 29, 83.
95. G.P. Scott, A.M. R. Elghoul, *J. Polym. Sci.*, Part A-1,**1920**, 8, 2255.
96. G.E. Ham, *J. Polym. Sci.*, Part B 3, **1965**, 459; G.P. Scott, J.C. Wang, *J. Org. Chem.*, **1963**, 28, 1314.

Chain transfer coefficients C for the thiols listed above range from 0.78 to 20. This satisfies the criterion which is required to form the telomers.[93]

Pavia et al.[97] have synthesized a new class of molecules which have amphiphilic properties using telomerization method as shown in **Scheme 1.7.** These compounds were obtained by reacting a tris(hydroxymethyl)-acrylamidomethane monomeric unit, in the presence of an alkane thiol as chain transfer agent.



Scheme 1.7. Synthetic scheme of telomer formation of Tris(hydroxymethyl)-acrylamidomethane. The alkane thiol used in the synthesis was n-decane thiol.

Another example of telomerization can be found in recent Kobayashi's work.[98] In this study, the telomeric monomer was designed to be reutilized for the synthesis of grafted glycopolymer as seen in **Scheme 1.8.**

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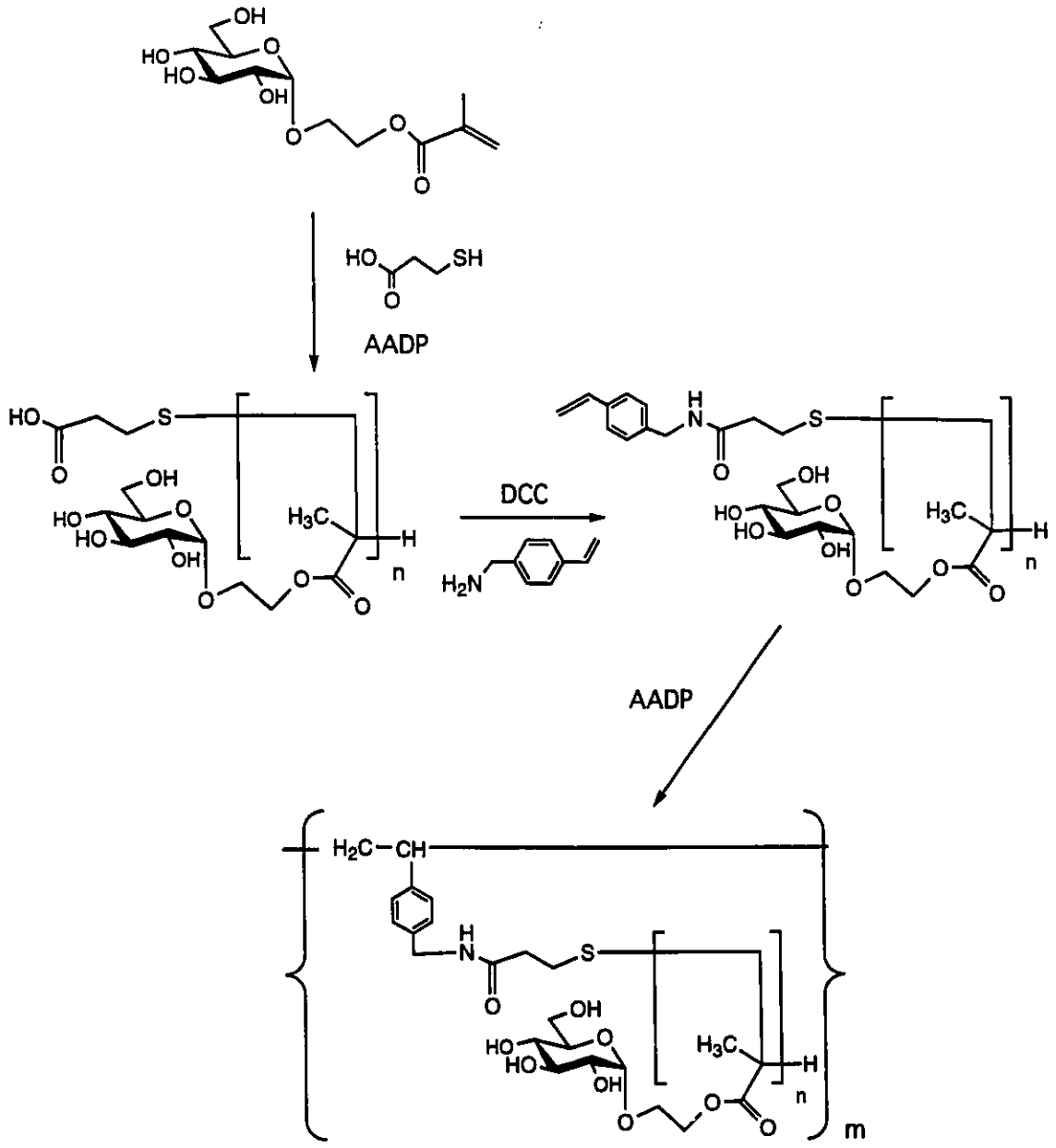
97. a. B,Pucci, A. Pavia, *Tet. Lett.*, **1991**, 32, 1437-1440; b. A. Pavia, B. Pucci, J.C. Reiss, L. Zarif, *Bioorg. Med. Chem. Lett.* **1991**, 1, 103.
98. K. Kobayashi, N. Kakishita, M. Okada, *Makromol. Chem., Rapid Commun.*, **1993**, 14, 293.



Scheme 1.8. The use of monomeric telomer in glycopolymer synthesis.

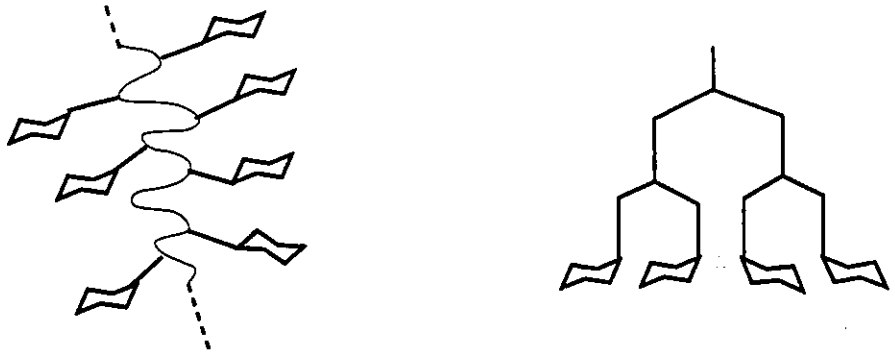
As previously stated, glycopolymers were originally envisaged as source of optimun multivalent cluster molecules. However, such molecules of relatively high molecular weight seem to obscure their chemical identity such as the exact numbers of carbohydrate haptens incorporated into the polymer. Thus, glycotelomers with controlled numbers of repeating units should afford structural information with respect to the incorporated extent of carbohydrate haptens in the telomers. Because the separation of each individual telomer unit is possible it is feasible to assess the minimum quantity of carbohydrate haptens for optimum binding with their receptors.

In Chapter IV, a one-step synthesis of lactosylated telomers using *tert*-butanethiol as telogen is described. The choice of the telogen was made primarily because of its convenience for characterization of isolated telomers using ^1H NMR spectroscopy. The spacer-arm effect on binding with the peanut lectin from *Arachis Hypogaea* are also discussed.

I.5. Dendrimers.

As importance of multivalency effect (avidity) has been realized, the syntheses of various macromolecules with multiple incorporations of antigenic epitopes have been vigorously sought. A number of glycopolymers has exhibited the multivalency effect by copolymerizing the suitably derivatized antigenic monomer and acrylamide. The polymers made in this manner have been found useful in various applications (*vide supra*). Without exception, however, these glycopolymers have a wide range of molecular weights and at best one can expect a normal distribution when synthesized. Thus, their molecular weights can be estimated only by an averaged value. For quantitative investigations of the multivalency effect, these polymeric forms do not serve as reliable tools.

To circumvent the problems of undefined molecular weight associated with glycopolymers, the use of 'cascade' (dendritic) polymers have been introduced. These dendritic polymers are based upon the application of branched progressions to organic synthesis, and thus possess a well-defined molecular topology.[99] As **Figure 1.5** shows, dendrimers have distinct advantages over polymers with respect to chemical definitions as well as their possible abilities to mimic multi-antennary glycoproteins present on the cell surface.



Glycopolymer Glycodendrimer

Figure 1.5. Schematic structural difference between glycopolymer and dendrimer.

Many dendrimers have been synthesized since Vögtle introduced the first 'cascade' molecule in 1978.[100] The common synthetic theme of dendrimers involves the preparation of molecular building blocks possessing polyfunctional terminal groups attached to a central core. This would provide a covalently linked macromolecule with well-defined structure.[101-103] For example, treatment of the tetra-bromide central core(**1**) with the alkyne(**2**) generated the dodecabenzyl ether as shown in **Scheme 1.9.**

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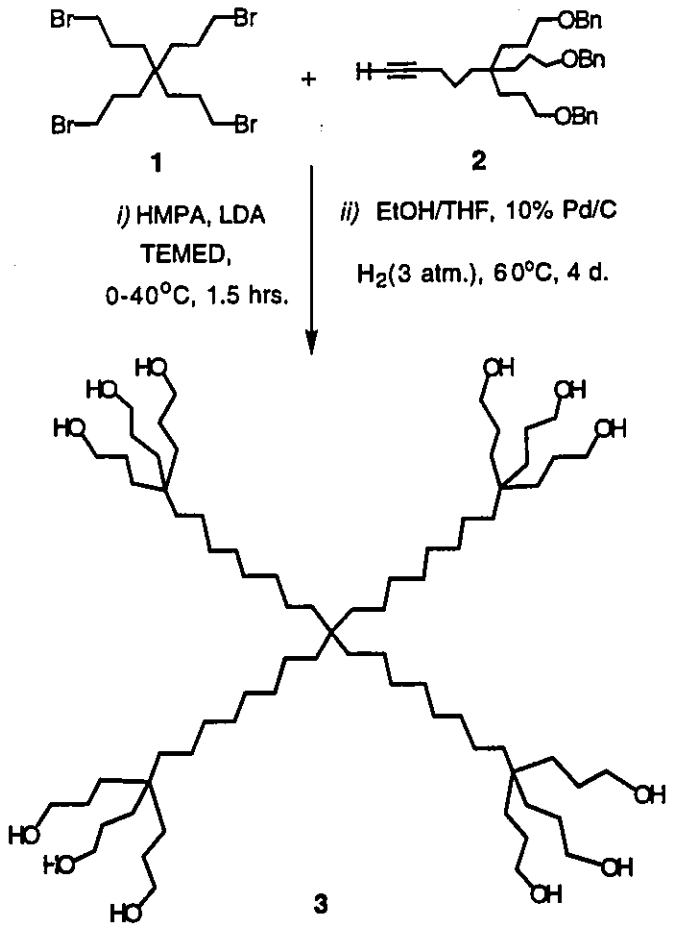
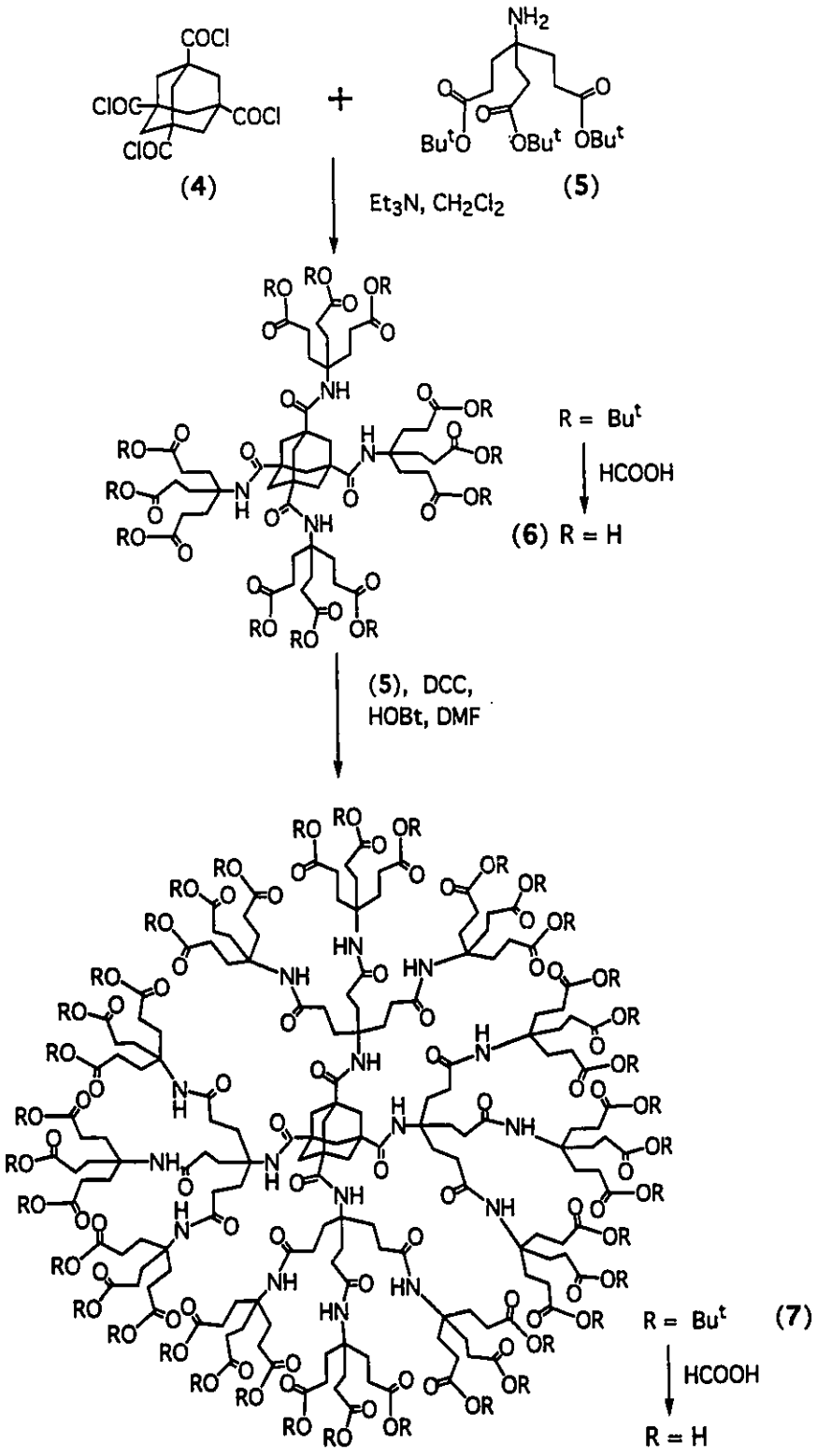
99. G.R. Newkome, C.N. Moorefield, G.R. Baker, *Aldrich Chem. Acta*, **1992**, 25, 31. 100. E.Buhleier, W.Wehner, F. Vögtle. *Synthesis*, **1978**,155.
101. G.R. Newkome, C.N. Moorefield, G.R. Baker, R.K. Behera, A.L. Johnson, *Angew. Chem. Int. Ed. Engl.*, **1991**, 30, 1176.
102. G.R. Newkome, C.N. Moorefield, G.R. baker, S.H. Grossman, *Angew. Chem., Int. Ed. Engl.*, **1991**, 30, 1178.
103. D.A. Tomalia, New Scientist, **1991**, 23, 30.



Scheme 1.9. Synthesis of dodecahydroxy dendrimer.

Simultaneous reduction of triple bonds followed by hydrogenolysis of the benzyl ethers yielded (**3**), 12-cascade: methane [4]: (nonylidyne):propanol. The next generation dendrimers can be made through the iterative processes.[99]

A similar example can also be found in dendrimer synthesis with adamantane derivative(**4**) and amine monomer(**5**) as the initiator core as illustrated in **Scheme 1.10**. The first generation, (**6**) (12-cascade: tricyclo [3.3.1.1.3,7] decane [4-1,3,5,7]:(3-oxo-2-aza propylidyne): *tert*-butyl propanoate) was then deesterified to give a terminal acid, ready to couple to (**5**), with DCC and HOBt to afford the second generation (**7**).



Scheme 1.10. Preparation of adamantane-core dendrimer.

Fréchet et al.[104] have used a convergent approach to minimize the problems associated with the divergent approach, i.e. any incomplete reaction of terminal groups would lead to imperfect or failed sequences in the next generation; chances of this occurring increases as the generation increases. A large excess of reagents is, therefore, required in the late stages of growth in the divergent approach. Using the convergent method dendrimers of up to the sixth generation are synthesized.[105]

Various types of dendrimers have been reported using either convergent or divergent method having unique characteristics and properties within. Engel et al.[106] described the syntheses of poly phosphonium and polyammonium dendrimers containing a large number of charges. Dendrimers containing nine diaza[18]crown-6 units have been synthesized by Shinkai et al.[107] Selective alkali-metal ions binding is expected from these dendrimers. Balzani et al.[108] reported the syntheses of polynuclear transition metal complexes of a dendritic nature. Ruthenium (II) ions were complexed with three 2,3-bis(2-pyridyl)pyrazine ligands. The metal centers of this dendritic structures were oxidized and showed some luminescence.

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104. J.M.J. Fréchet, C. J. Hawker, *J.Am. Chem.Soc.*, **1990**,112, 7638.
105. I. Gitsov, K.I. Wooley, J.M.J. Fréchet, *Angew. Chem. Int. Ed. Engl.*, **1992**, 31, 1200.
106. K. Rengan, R. Engel, *J. Chem.Soc. , Chem. Commun.*, **1992**, 757.
107. T. Nagasaki, M. Ukon, S. Arimori, S. Shinkai, *J. Chem. Soc. Chem. Commun*, **1992**, 608-610.
108. S. Serroni, G. Denti, S. Campagna, A. Juris, M. Ciano, V. Balzani, *Angew. Chem. Int. Ed. Engl.*, **1992**, 31, 1493.

Carbohydrate-containing dendrimers were first introduced by our group.[109] In this work, L-lysine was used as a branching monomer unit to construct a multiple antigen peptide (MAP)[110] dendritic core using solid-phase peptide synthesis.[111] Thus, the systematic 2^n increase of valency (where n represents nth generation) was obtained as the generation grows. To each end of MAP dendritic structure, a sialic acid (Neu5NAc) was attached and di-, tetra-, octa-, hexadecameric glycodendrimers were prepared. The serological evaluation of these dendrimers against influenza A virus showed that even the dimer, the first generation dendrimer was 5 times as potent as its monomer, and the hexadecamer was comparable to its polymer.

In Chapter V, the preparation of various glycodendrimers is described using N-linked sialyl lactoside, lactose and lactosamine as carbohydrate haptens. As complementary to the L-lysine core dendrimers having 2^n valency increase, dendrimers of gallic acid methyl ester as a seeding molecule are designed to have 3^n valency. An oligoethyleneglycol derivative was used as a hydrophilic and biocompatible spacer-arm which structurally linked the core unit to the carbohydrate haptens. The syntheses of the first and second generations of carbohydrate-containing dendrimers and the testing for their binding abilities with the plant lectin, *Arachis Hypogaea* from peanut lectin are described.

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109. R. Roy, D. Zanini, S.J. Meunier and A. Romanowska, *J. Chem. Soc. Chem. Commun.*, **1993**, 1869.
110. Y.A. Lu, P. Clavijo, M. Galantino, Z.-Y. Shen, W. Liu, J.P. Tam, *Molecular immunology*, **1991**, 28, 623.
111. R.B. Merrifield, Solid-phase synthesis, *Science*, **1986**, 232, 341.

I.6. Lectins.

By definition, lectins are non-enzymatic, carbohydrate-binding proteins of non-immunological origin that bind to specific carbohydrate structures.[112] Along with antibodies they represent one of the major subclasses which contain carbohydrate-recognition domains (CRD) as complementary structures to carbohydrates. Lectins are present in virtually every living cell: this ranges from plant to animal cells; from viruses to mammals including human. Their functions and significance as cell surface carbohydrate receptors are now well appreciated.[113]

Although incomplete, structural elucidations reveal that lectins are small proteins (usually ranging from 40 to 160kDa), made up of several subunits and some of them chelate with metals to complete the binding with carbohydrate haptens. Lectins are also involved in the selectin-mediated cell-cell binding, the binding of sperm lectins to sugar epitopes at the surface of the *zona pellucida* of the egg (fertilization), cell growth, cell differentiation and migration processes.[112]

Perhaps the most important aspect of lectins includes their specificities towards carbohydrates. That is, a lectin molecule contains a CRD, specific to the particular glycoside but not to others as **Table 1.3.** shows. This specificity has provided many useful applications of lectins. For example, lectins are used in the elucidation of blood typing, cell separation, and structural variations of complex carbohydrates during malignant cell transformation.[114]

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112. D.H. Vanden Eijnden and D.H. Joziasse, *Carbohydrates in Europe*, 1994, 11.
113. I. E. Liener, N. Sharon, I.J. Goldstein (eds.)*The lectins, Properties, Functions and Application in Biolcgy and Medicine*, Academic Press, Orlando, Florida, 1986.
114. N. Sharon, H. Lis, *FASEB. J.* 1990, 4, 3198.

Table 1. 3. A list of the lectin-carbohydrate specificities and their sources.

Lectin	Source	Carbohydrate specificity	Characteristics
Wheat Germ Agglutinin (WGA)	*Triticum Vulgaris*	GlcNAc NeuNAc	M.W. 43kDa plant lectin
Peanut lectin	*Arachis Hypogaea*	β-D-Gal	110 kDa plant lectin
RCA_{120}	*Ricinus Communis* (Castor Bean)	β-D-Gal α-L-Rha	120 kDa plant lectin
Maclura Pomifera	Osage Orange	β-D-Gal	42 kDa plant lectin
Sambucus Nigra	Elder stems and branches	Lactose	140 kDa plant lectin
Galectin	animal cells, extracellular & cytoplasm	β-D-Gal	No Ca^{2+} required, 36kDa, animal lectin
Mannose receptor	animal cell surface	mannose 6-phosphate	275 kDa folding stabilizition by dissulfide bridge
Selectins	E, Endothelial L, Leukocyte surface P, Platelet	Sialyl-$Lewis^x$ Sialyl dimeric $Lewis^x$ Sialyl-$Lewis^a$ 3'-sulfo-$Lewis^x$	

In general, the individual CRDs present in lectins show only a weak affinity for their carbohydrate ligands. However, lectins usually overcome this weak binding by clustering themselves. For example, hepatic lectins associate into hexamers while the calcium-independent mannose 6-phosphate receptor, (C1) MPR, contains multiple CRDs in a single polypeptide strand.[115] This tendency of lectins is complementary to our effort to synthesize clusters of various forms to compensate for weak binding interactions. During this research, glycoconjugates with varying degrees of hapten densities were synthesized in order to examine their binding effectiveness towards lectins.

I.7. Serological Assays.

Three main types of serological assays are available to give a qualitative and/or quantitative analyses of binding interactions between carbohydrates and their receptors: precipitation, double immunodiffusion and enzyme-linked assays. All assays are of precipitin (an antigen-lectin complex) formation in nature, only two types of assays were used in the present research, the double immunodiffusion technique and enzyme-linked lectin assay. More detailed description of experimental protocols are given in the appropriate chapters. Brief introductions of the two assays used in the present research are provided in this section.

The double immunodiffusion technique[116] is a simple way to test agglutination. In this method a drop of glycoconjugate solution (10-20 μL, 1 mg/mL stock solution) is put in a well cut in an agar-

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115. S. Kornfeld, *Annu. Rev. Biochem.*, 1992, 61, 307-330.
116. D.M. Kemeny, *A practical guide to ELISA*, 1st ed., Pergamon Press, 8, 1991.

coated glass plate and lectin in an adjacent well. The two diffuse outwards and a precipitin forms between the two wells if there is molecular recognition. This method is easy to perform and is ideal for monitoring the relative concentration of carbohydrates in the conjugate although the assessment will be qualitative. No specialized equipment is necessary and reagents can be used directly without further processing, such as extra purifications of purchased lectins. The precipitate line, called precipitin band can be visible without magnification and permanently stained or photographed.

The enzyme-linked assay is a very sensitive method for evaluating antigen-receptor affinity.[117,118] For example, the inhibitory behavior of newly prepared glycoconjugates can be evaluated using the enzyme-linked lectin assay (ELLA) technique. This is illustrated in **Scheme 1.11**. The enzyme-labeled lectin can be exposed to the polymer-supported carbohydrate matrix in the presence of the glycoconjugate which contains the same haptens. Thus, the same haptens from two different sources compete for the lectins. Washing and exposing to the substrate (e.g. p-nitrophenylenediamine for horse radish peroxidase) will give photochemically measurable amount of lectins which still bound to the glycopolymer used as support. Therefore, the washed amount of lectins should represent the inhibitiory capacity of the glycoconjugate. This procedure is known as an enzyme linked lectin assay (ELLA), used for the evaluation of the inhibitory capacity of lactose-containing telomers against the corresponding polymer.

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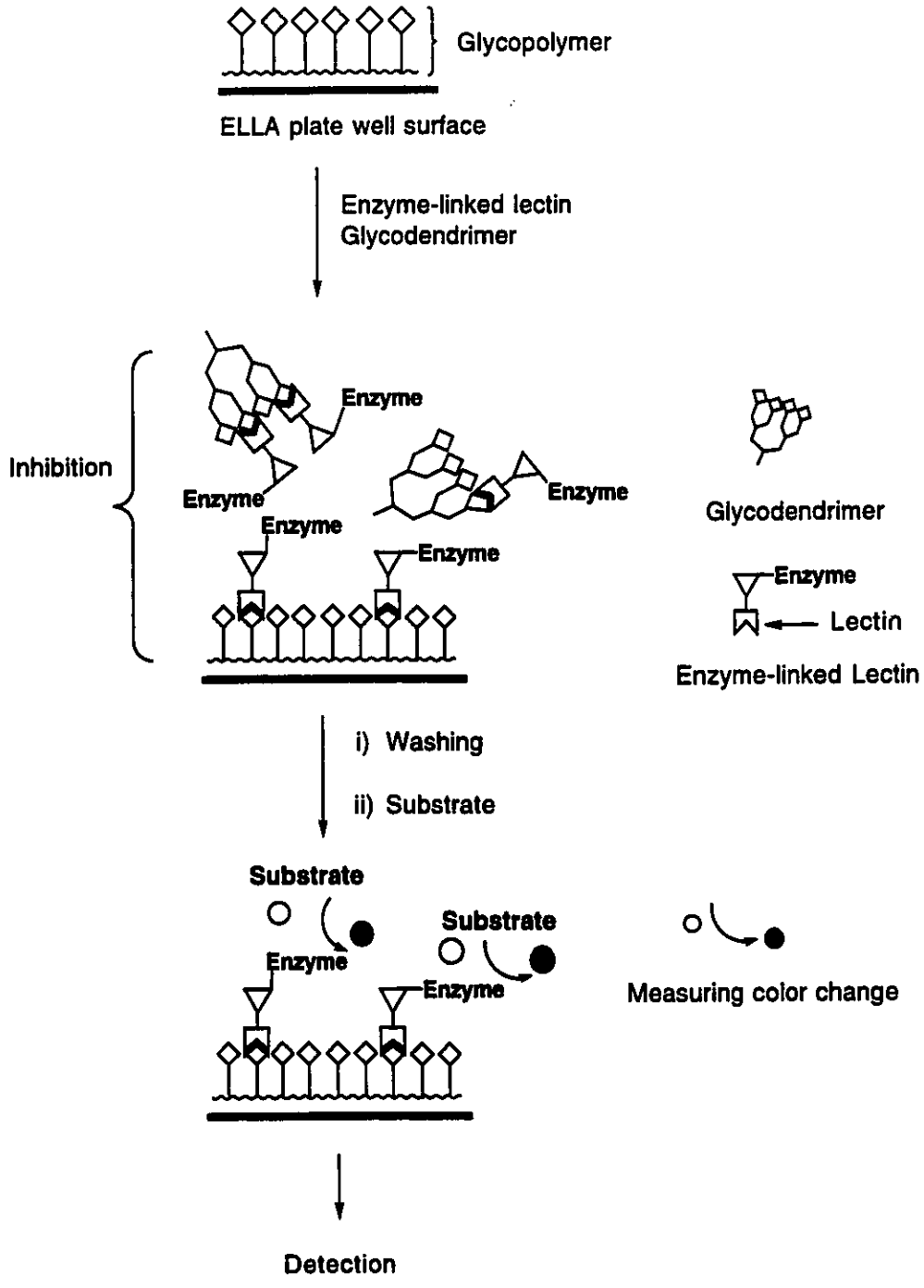
117. B.K. Van Weeman, A.H.W.M. Schuurs, *FEBS Lett.* **1971**, 15, 232.
118. E. Engvall, P. Perlman, *Immunochemistry*, **1971**, 8, 871.



Scheme 1.11. Schematic representation of Inhibition Enzyme-Linked Lectin Assay of Glycodendrimer.

Chapter II. Syntheses of the Carbohydrate Haptens.

II.1. Introduction.

A ganglioside GM_3 (**Figure 2.1**) was first isolated from *equine* erythrocytes by Yamakawa and Suzuki in 1952.[119] Since then, it has been known that this ganglioside plays a key role as precursor for many complex gangliosides in the biosynthetic pathways.[120] It also has been shown to modulate the epidermal growth factor (EGF), and platelet-derived growth factor (PDGF) receptors[121] and to be expressed in abnormally high concentration in tumor cells.[122] As with most glycolipids, isolation of homogeneous GM_3 from natural sources is very difficult[123] and thus effective syntheses have been desired.

The following two aspects have been the focus in chemical synthesis of GM_3[124]: regio- and stereo-specific glycosidation of the glycosyl donor, Neu5NAc to the lactoside acceptor; β-glycosidation of the ceramide moiety to a suitable lactosyl donor. It is known that the ceramide has its function as a support for the carbohydrate moiety to be exposed to extracellular environment while most part of itself is embedded in the lipidic layer of the plasma membrane as the structure of GM_3 suggests in **Figure 2.1**.

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119. T. Yamakawa, S. Suzuki, *J. Biochem.* (Tokyo), **1952**, 39, 383.
120. D. Bouhours, J.-F. Bouhours, *J. Biol. Chem.*, **1991**, 266, 12944.
121. a. E. Bremer, J. Schlessinger, S. Hakomori, *J. Biol. Chem.* **1986**, 261, 2434. b. N. Hanai, G.A. Nores, C. R. Torres-Mendez, S. Hakomori, *Biochem. Biophys. Res. Commun.* **1987**, 147, 127. c. G.A. Nores, N. Hanai, S.B. Levery, H.L. Eaton, M. E. Salyan, S. Hakomori, *Carbohydr. Res.*, **1988**, 179, 393.
122. a. J.M. Carubia, R.K. Yu, L. J. Macala, J.M. Kirkwood, J.M. Varga, *Biochem. Biophys. Res. Commun.*, **1984**, 120, 500. b. J. Portoukalian, G. Zwingelstein, J.-F. Dore, J.-J. Borgoin, *Biochemistry*, **1976**, 56, 1285
123. S. Ando, H. Waki, K. Kon, Y. Kishimoto, in *Gangliosides and Modulation of Neuronal Functions*; H. Rahamann Ed. Springer-Verlag: Berlin-Heidelberg, **1987**, 167.
124. a. K.-C. Kevin, S. Danishfsky, *J. Am. Chem. Soc.*, **1993**, 115, 4933; b. A. Hasegawa, K. Adachi, M. Yoshida, M. Kiso, *J. Carbohydr. Chem.*, **1992**, 11(1), 95; c. H. Lönn, K. Stenvall, *Tet. Lett.*, **1992**, 33(1), 115; d. A. K. Ray, U. Nilsson, G. Magnusson, J. Am. Chem. Soc., **1992**, 114, 2256.

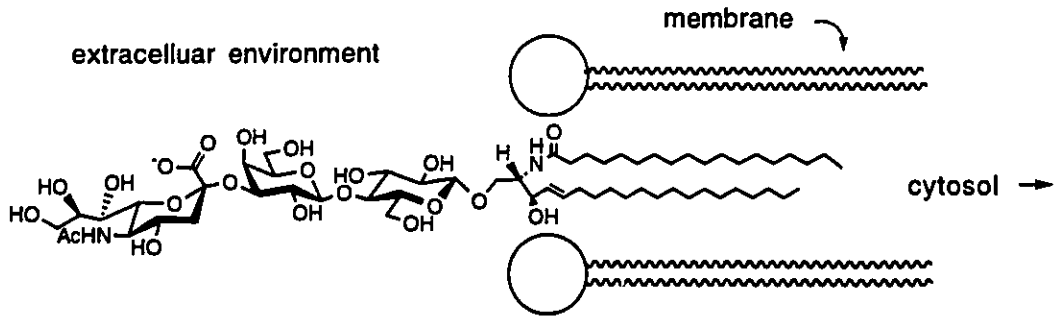

Figure 2.1. Schematic orientation GM_3 in the plasma membrane.

Although the ceramide residue has been suggested to be involved in monoclonal antibody-GM_3 interactions[125] the structure of the lipid component is not critically important for the purpose of coating microtitre plates to be used in the determination of the carbohydrate-binding specificities of lectins.[126] Our interest in synthesizing of GM_3 involved the constructions of not only the GM_3 oligosaccharide moieties themselves but also a molecule in which many GM_3 trisaccharides were incorporated so that multivalency effects can be examined.

The syntheses of the trisaccharide unit of GM_3 can be achieved by reacting the glycosyl donor with the glycosyl acceptor using NIS/TfOH as a promotor in a kinetically controlled manner. The glycosyl donor can be prepared from Neu5NAc, a sialic acid via formation of the sialyl chloride intermediate. The acceptor can be synthesized by kinetically controlled benzoylation of lactosyl azide. This synthetic strategy is illustrated in **Figure 2.2.**

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125. S. Itonori, K. Hidari, Y. Sanai, M. Tanigushi, Y. Nagai, *Glycoconjugate J.*, **1989**, 6, 551.
126. I. J. Glodstein, R. D. Poretz in " *The lectins: Properties, Functions and Application in Biology and Medicine*" (I.E. Liener, N. Sharon, and I.J. Goldstein, eds. Academic Press, Orlando, Florida, **1986**, b. R. Roy, A. Romanowska, F. O. Andersson, *Methods in Enzymol.*, **1994**, 242, 198.

Scheme 2.2.

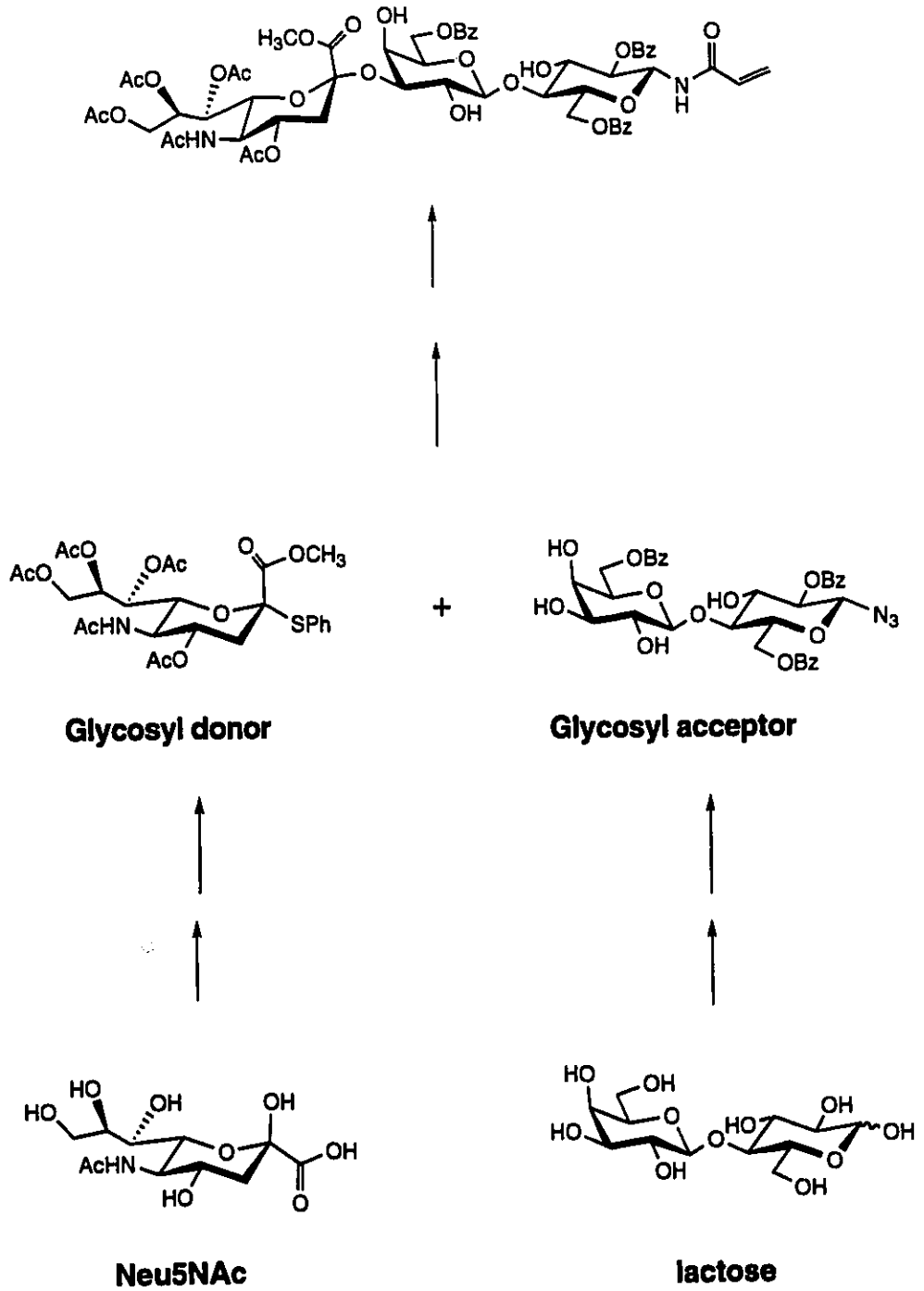


Figure 2.2. A retrosynthetic analysis of the construction of the β-N-linked GM_3.

The synthesis of a β-N-linked GM_3 trisaccharide moiety is considered instead of the usual β–O-linked form as seen in the naturally occurring case. This β–N-linkage can provide not only a versatile functionality for the construction of various glycoconjugates but also prevent the removal of a β–O-linked glycoside from an enzymatic attack by glucohydrolase.

The derivatization of the anomeric center can be achieved by the reaction of glycosyl halide with NaN_3 under the phase transfer catalysis(PTC) condition using Bu_4NHSO_4 as phase transfer catalyst as shown in **Figure 2.3.** This aspect was examined by using lactose as model system. The azido group can act as a temporary protecting group and can be readily reduced and N-acryloylated for further modifications towards the constructions of glycopolymers, glycotelomers and glycodendrimers.

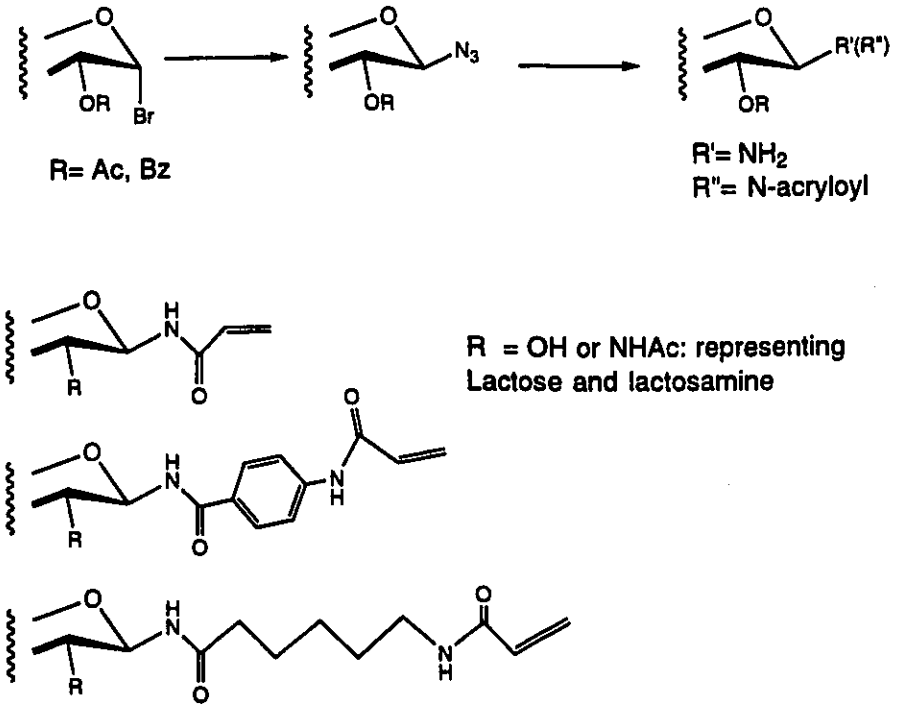
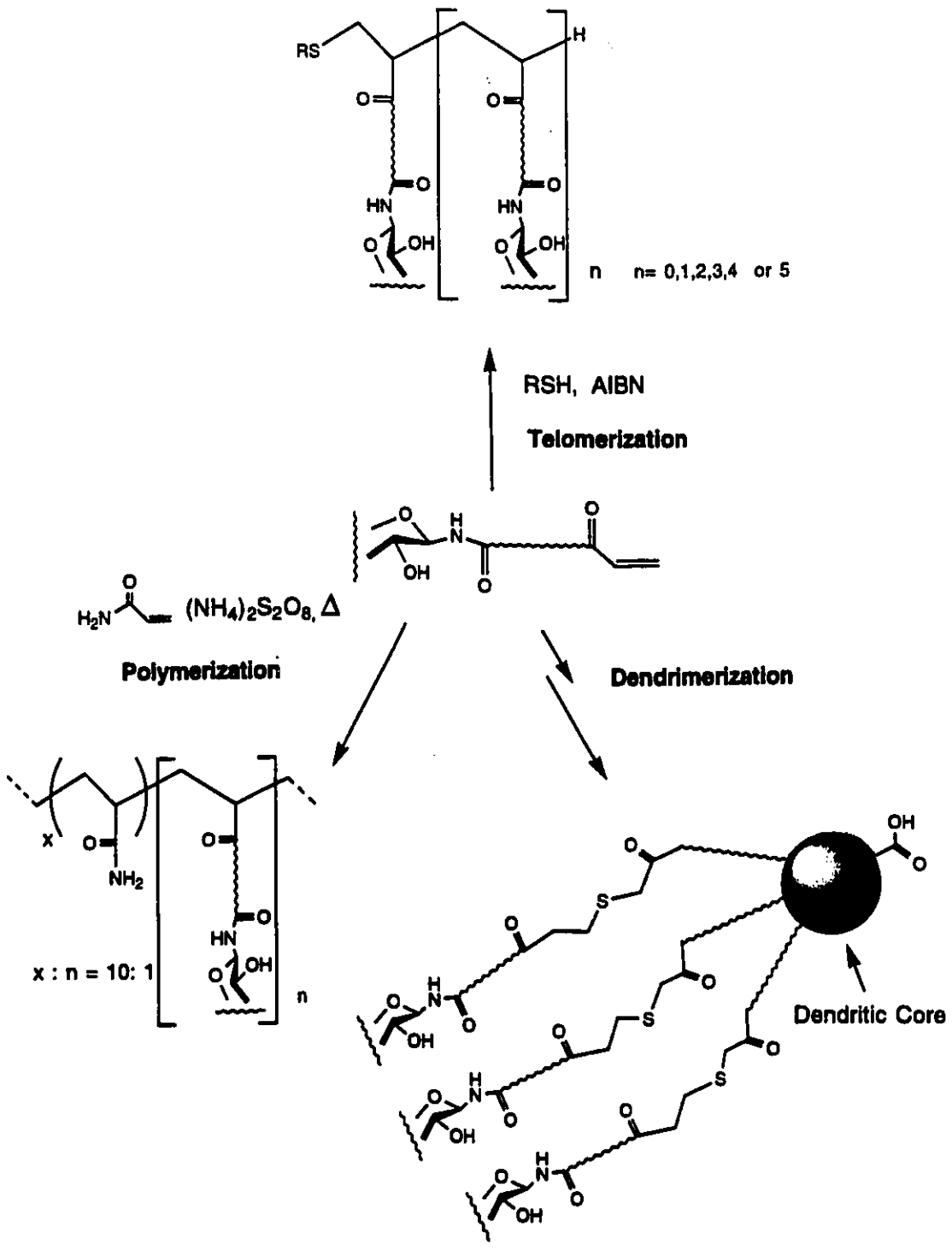
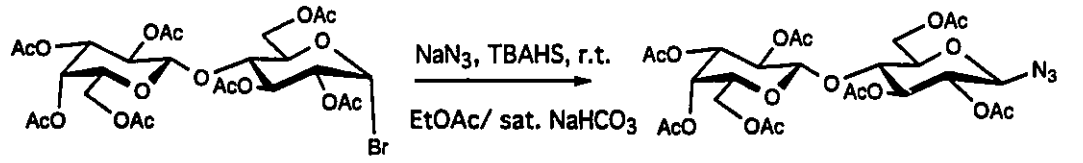


Figure 2.3. Glycosyl azide formation and its derivatization to N-acrylamido function.


RS
H
HN
OH
n
n= 0,1,2,3,4 or 5
RSH, AIBN
Telomerization
$(NH_4)_2S_2O_8, \Delta$
Polymerization
Dendrimerization
x : n = 10: 1
NH_2
Dendritic Core

II. 2. Results and Discussion

The N-acryloyl functionality of carbohydrate derivatives has been envisioned as a versatile monomeric unit towards the constructions of various glycoforms. Formation of β–glycosyl azide was considered as a precursor to the N-acryloylated monomer. This aspect was examined by using the model carbohydrate, lactose. Bromination of peracetylated lactose was performed by following the standard method.[127] This facile glycosyl halide formation was complete within 1 hour and gave exclusively the α-anomer 1 in quantitative yield.



1 2

Figure 2.4. Formation of peracetylated β–lactosyl azide.

As **Figure 2.4.** shows the β-lactosyl azide 2 was obtained from the reaction with 1 by employing a PTC condition : sat. $NaHCO_3$ (pH 9), NaN_3(5 equiv.) and Bu_4NHSO_4(1 equiv.).[128] This two-phase reaction was complete within 2.5 hours and gave exclusively the β-product in quantitative yield. The stereoselectivity at the anomeric center was confirmed by the ^1H NMR spectrum of the crude reaction mixture showing the characteristic 1,2-trans-β–D-anomeric configuration with $J_{1,2}$ = 8.6 Hz. The IR spectrum of 2 gave $\upsilon_{N\equiv N}$ = 2120 cm^{-1} in $CHCl_3$.

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127. R.W. Jeanloz, P.J. Stoffin, *Methods in Carbohydr. Chem.*, **1962**, 1, 221.
128. a. R. Roy, F. D. Tropper, F. O. Andersson, C. Grand-Maître, *Synthesis*, **1991**, 7, 734,
b. R. Roy, F. D. Tropper, C. Grand-Maître, *Can. J. Chem.*, **1991**, 69, 1462.

This facile, stereoselective and mild reaction is in contrast to the previously published one-phase system reactions. For example, a glycosyl azide was synthesized by treating glycosyl halides (either bromide or chloride) with silver or sodium azide in chloroform.[129] Lewis acid catalysed glycosyl azide synthesis from 1-O-acyl glycosides has also been reported.[130] These reactions were always resulted in mixtures of α- and β-anomers because the addition of azide anions takes place after oxonium formation catalyzed by Lewis acid such as Ag^+ as illustrated in **Figure 2.5.**

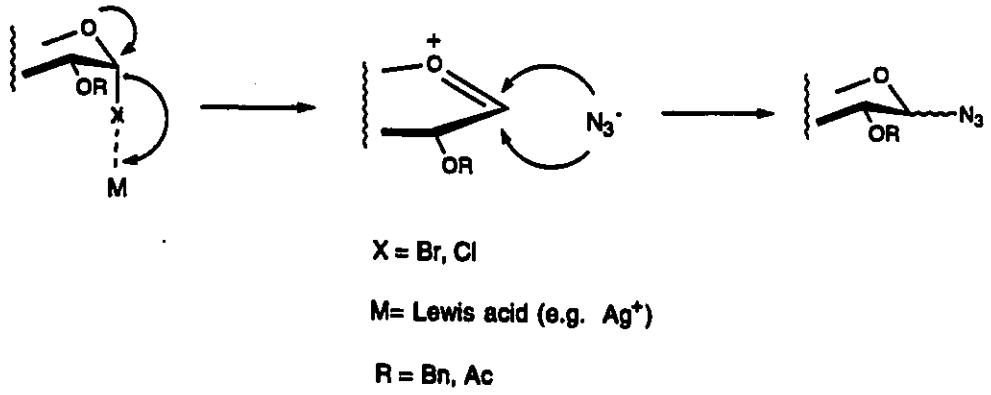

Figure 2.5. Lewis acid catalyzed glycosyl azide formation.

Glycal formations during the PTC reactions have been noted.[131] Under a typical PTC condition (tetrabutylammonium hydrogen sulfate, CH_2Cl_2/1M NaOH, 25° C, 1 hour) the glycosyl bromide should undergo nucleophilic substitution by the incoming nucleophile with the complete inversion of the configuration at the anomeric center.

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129. K. Okamoto, T. Goto, *Tetrahedron*, **1990**, 46, 5835.
130. H. Kunz, W. Sager, D. Schanzenbach, M. Decker, *Liebigs Ann. Chem.*, **1991**, 649.
131. F. D. Tropper, Ph.D. Thesis, University of Ottawa, **1992**, 190.

Glycal formation can be explained by the E_2 process in which the basic hydroxide anion attacks the axial proton at C-2. Under such condition, the nucleophilic substitution and the elimination process are thought to compete during the reaction as illustrated in **Figure 2.6.**

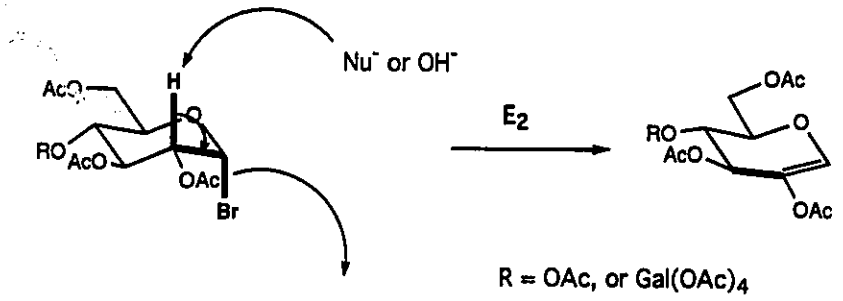


Figure 2.6. Glycal formation under a typical PTC condition.

If the rate of the elimination is greater than that of the substitution by hydroxide, glycal formation would be the dominant process. For the preparation of the glycosyl azide **2**, the basicity of the aqueous phase was controlled by using a buffer solution of 1M Na_2CO_3. Within this range no adverse effect of basicity was observed.

The lactosyl azide **2** was used to prepare the glycosyl acceptor. Under Zemplén condition (pH of the reaction solution 9.5, using a 1 M NaOMe/MeOH stock solution), peracetylated lactosyl azide **2** was de-O-acetylated to give unprotected lactosyl azide **3**.

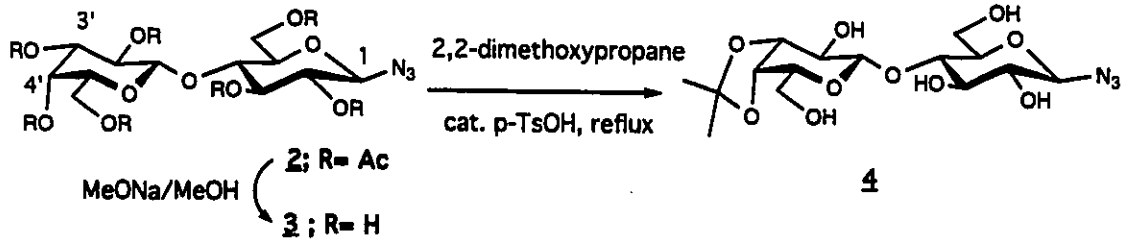
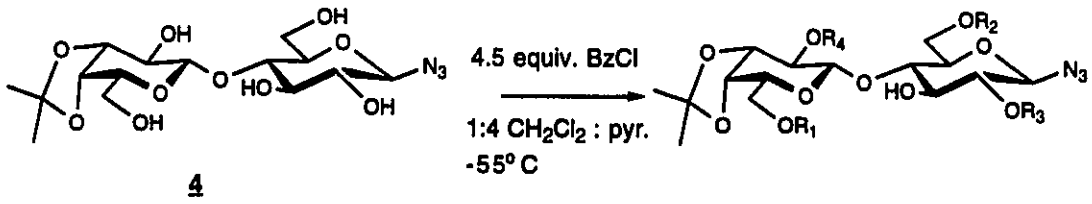


Figure 2.7. Preparation of 3',4'-isopropylidene protected lactosyl azide.

Regioselective protection of the 3' and 4' OH groups of **3** by isopropylidene formation was achieved by refluxing it with 2,2-dimethoxypropane containing catalytic amount of p-TsOH. Briefly boiling the solution prior to the addition of the catalyst (p-TsOH) resulted in a significant improvement in yield (86%) of the desired product **4** over the other possible regioisomeric acetals.(**Figure 2.7**)

Regiospecific protection of **4** with benzoyl chloride proceeded in a kinetically controlled manner. It was hoped that two primary and one secondary hydroxyl groups at 6, 6' and 2 positions were protected leaving 2' position unprotected. Because it is known that the benzoylated 2' OH group greatly diminishes the nucleophilicity of the 3' OH group during the glycosidation.[132] The protection of the OH group at 2 position was found to be difficult due to its steric hindrance. The partial benzoylations at 2, 6 and 6' were achieved using an 1: 4 mixture of CH_2Cl_2 and pyridine at -55° C as shown in **Figure 2.8.**



4

5; R_1 = Bz, R_2= R_3 = R_4 = H

6; R_1 =R_2 = Bz, R_3 = R_4 = H

7; R_1 =R_2 = R_3= Bz, R_4 = H

8; R_1 =R_2 = R_3=R_4 = Bz

Figure 2.8. The partial benzoylation of **4**.

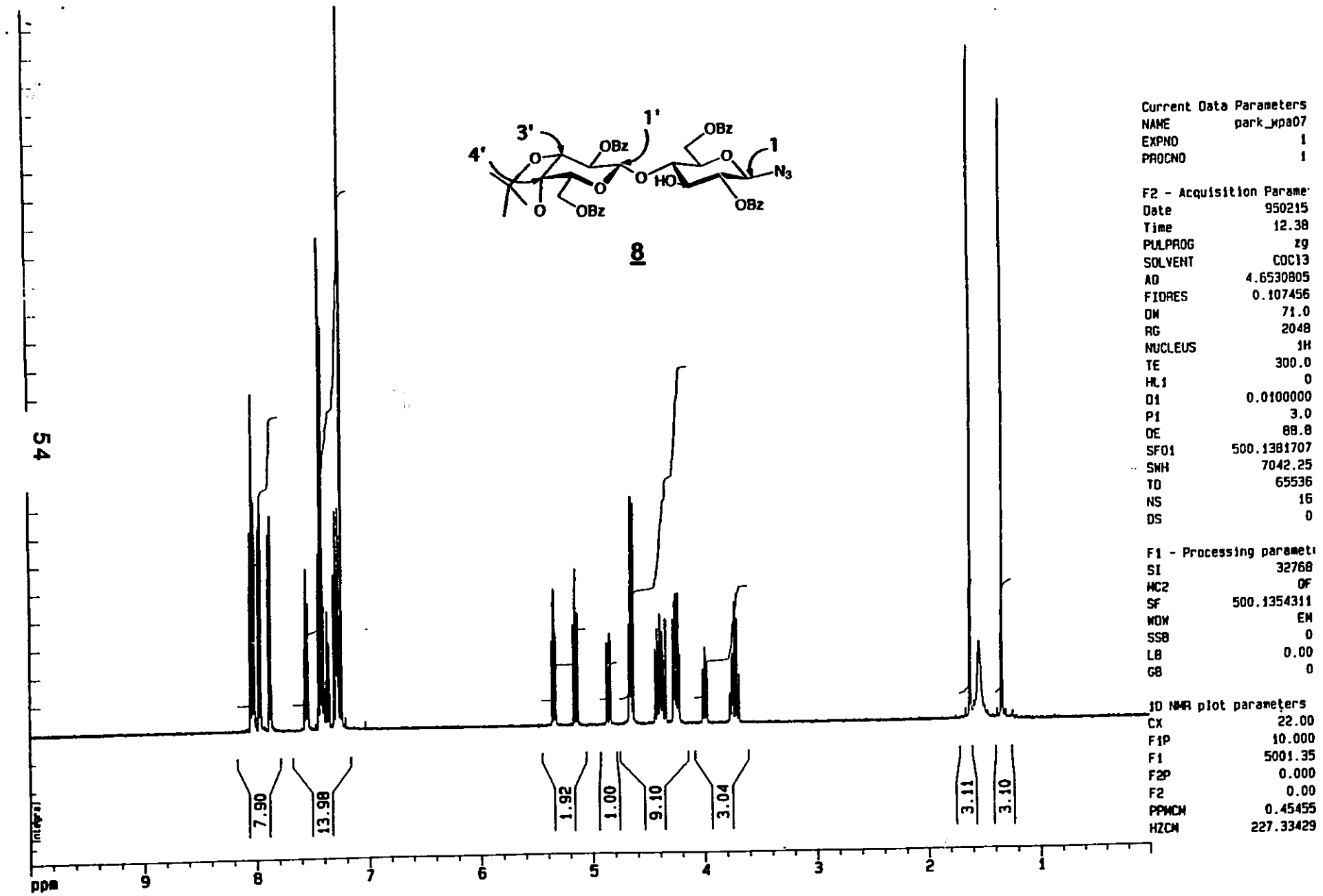
132. H. Lönn, K. Stenvall, *Tetrahedron Lett.*, **1992**, 33, 115.

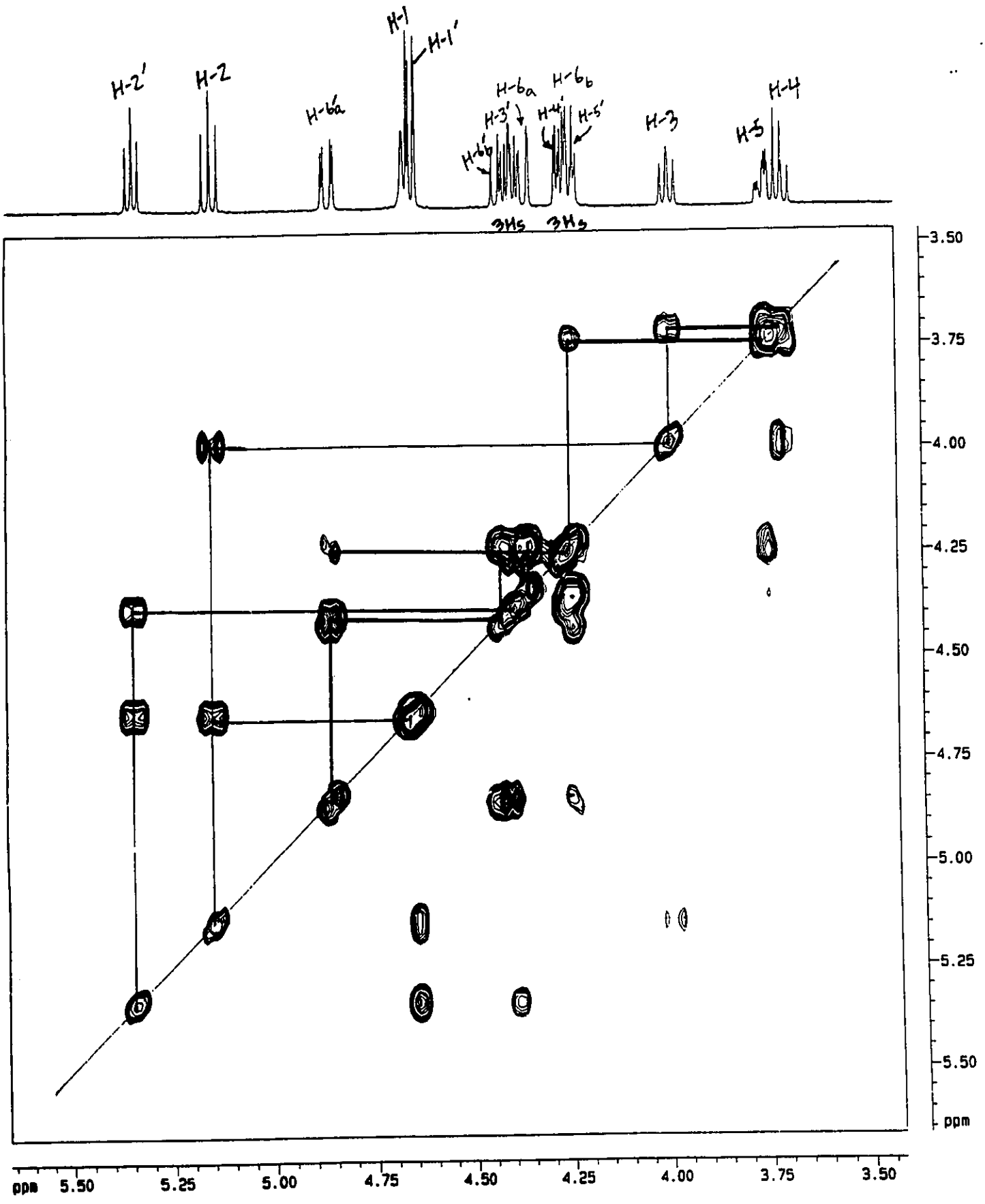
This kinetically controlled regiospecific benzoylation was optimized by changing the amount of benzoyl chloride to maximize the desired product **7**. The physical properties of these compounds **5-8** are summarized in **Table 2.1** and the 1H- and 13C-spectral data are tabulated in **Tables 2.2 and 2.3**. The chemical shifts and coupling constants of compounds were initially assigned by obtaining ^{1}H- and ^{13}C-NMR spectra. If not sufficient, the COSY, HETCOR and HMQC experiments were performed to assign the peaks. The NMR spectra of **8**, shown on the following pages, illustrate the typical steps involved in identifying and assigning the structure and individual chemical shifts.

Table 2.1. Physical properties of the partially benzoylated compounds **5-8**.

Cpd.	yield(%)	m.p.(° C)	$[\alpha]_D$*	FAB-MS	Elemental Analyses
5	< 1	143-146	-	-	-
6	2	173	+19.1	Calculated for $C_{29}H_{32}N_3O_{12}$ 615.2, found 616.2 (M+1)	-
7	75	90.1 -91.8	+15.0	Calculated for $C_{36}H_{36}N_3O_{13}$ 719.4, found 720.3 (M+1)	Calculated : C 60.08, H 5.18, N 5.84, found: C 59.98, H 5.17, N 5.72
8	18	203.0-204(dec)	+4.00	Calculated for $C_{43}H_{40}N_3O_{14}$ 823.0, found 824.3 (M+1)	Calculated : C 62.70, H 5.02, N 5.10, found : C 62.41, H 4.99, N 5.04

* c= 1.0 in $CHCl_3$


3'
1'
4'
OBz
OBz
OBz
OBz
HO
1
N_3
O
8
54
7.90
13.98
1.92
1.00
9.10
3.04
3.11
3.10
ppm
9
8
7
6
5
4
3
2
1
Current Data Parameters
NAME park_xpa07
EXPNO 1
PROCNO 1
F2 - Acquisition Parame
Date 950215
Time 12.38
PULPROG zg
SOLVENT CDCl3
AD 4.6530805
FIDRES 0.107456
DW 71.0
RG 2048
NUCLEUS 1H
TE 300.0
HL1 0
O1 0.0100000
P1 3.0
DE 88.8
SFO1 500.1381707
SWH 7042.25
TD 65536
NS 16
DS 0
F1 - Processing paramet
SI 32768
MC2 DF
SF 500.1354311
WDW EM
SSB 0
LB 0.00
GB 0
1D NMR plot parameters
CX 22.00
F1P 10.000
F1 5001.35
F2P 0.000
F2 0.00
PPMCM 0.45455
HZCM 227.33429


H-2'
H-2
H-6a
H-1
H-1'
H-6a
H-6b
H-3'
H-4'
H-5'
H-6b'
H-3
H-5
H-4
3Hs
3Hs
3.50
3.75
4.00
4.25
4.50
4.75
5.00
5.25
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4.75
4.50
4.25
4.00
3.75
3.50

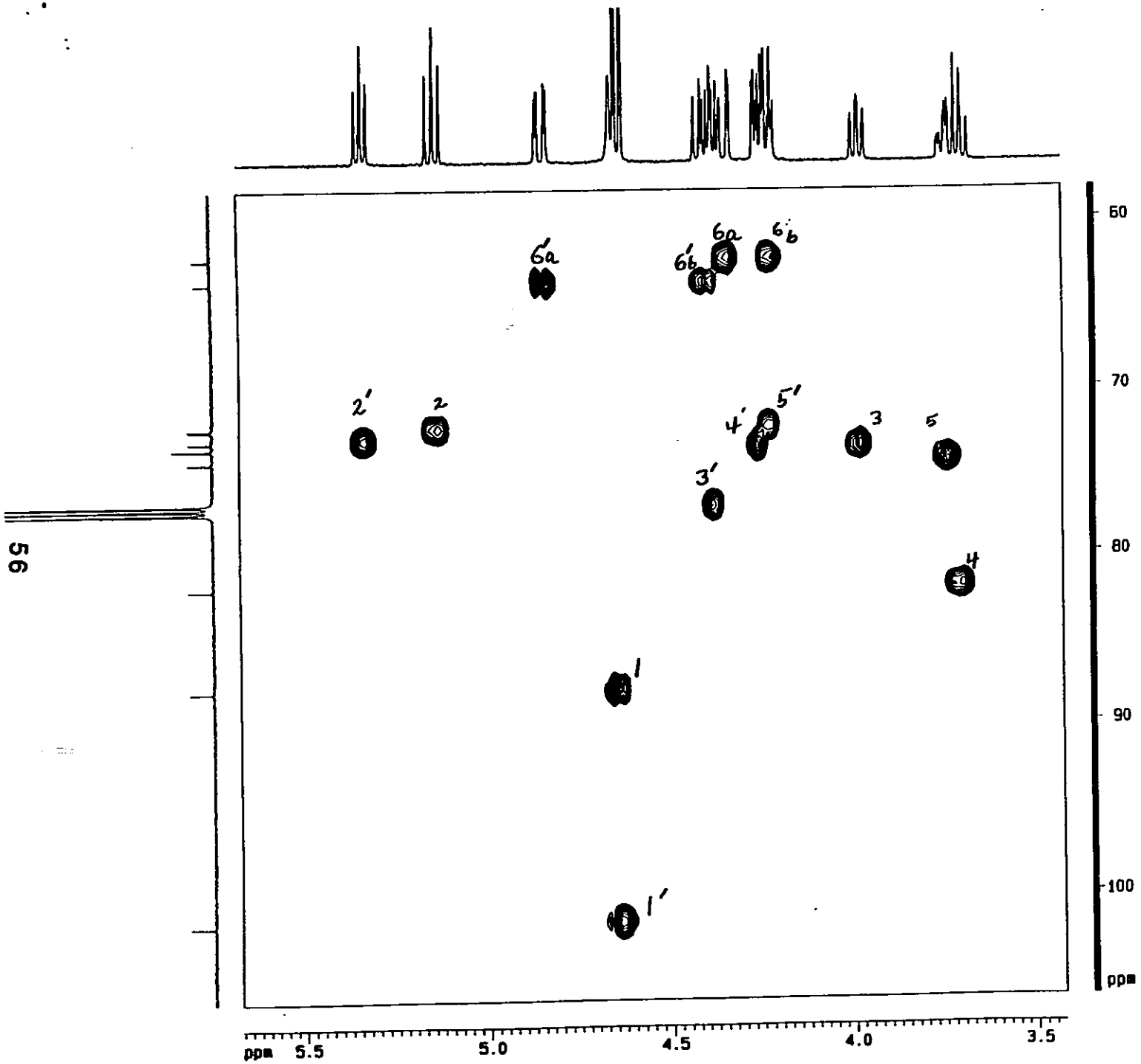

6a'
6b'
6a
6b
2'
2
5'
4'
3
5
3'
4
1
1'
96
60
70
80
90
100
ppm
ppm 5.5
5.0
4.5
4.0
3.5

Table 2.2. ^{1}H NMR spectral data of the partially benzoylated derivatives **5-8**($CDCl_3$, 500 MHz)[a]

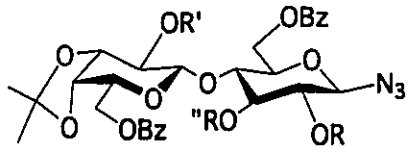
Com-pounds[b,c]		H-1, d ($J_{1,2}$) Hz	H-2, dd ($J_{2,3}$) Hz	H-3, dd ($J_{3,4}$) Hz	H-4, dd ($J_{4,5}$) Hz	H-5, ddd ($J_{5,6a}$) Hz	H-6a, dd ($J_{6a,6b}$) Hz	H-6b, dd ($J_{5,6b}$) Hz
5	Glc	4.57 (8.75)	3.47-3.85 (m)	3.47-3.85 (m)	3.27 (7.3)	3.47-3.85 (m)	3.86 (m)	3.86 (m)
	Gal	4.32 (8.43)	3.47-3.85 (m)	4.14 (2.0)	4.74 (2.4)	3.47-3.85 (m)	4.19-4.23 (m)	4.19-4.23 (m)
6	Glc	4.55 (8.7)	3.32 (8.7)	3.61 (9.2)	3.36 (8.5)	3.73 (1.7)	4.76 (12.3)	4.39 (2.5)
	Gal	4.28 (8.1)	3.67 (7.2)	4.12 (5.4)	4.85 (1.2)	4.47 (12.1)	4.03-4.15 (m)	4.03-4.15 (m)
7	Glc	4.69 (9.0)	5.17 (9.0)	3.94 (8.3)	3.55 (10.0)	3.84 (2.1)	4.92 (12.3)	4.46 (m)
	Gal	4.32 (8.2)	3.70 (7.0)	4.13-4.19 (m)	4.79 (1.2)	4.37 (9.0)	4.13-4.19 (m)	4.13-4.19 (m)
8	Glc	4.64 (9.0)	5.16 (9.5)	3.99 (8.3)	3.70 (9.8)	3.74 (2.5)	4.30 (12.3)	4.21-4.26 (m)
	Gal	4.63 (8.1)	5.35 (7.8)	4.20 (4.4)	4.25 (2.2)	4.25 (1.7)	4.85 (12.1)	4.43-4.46 (m)

a: Assignments are based on HMQC and COSY experiments. Chemical shifts are referenced to internal $CHCl_3$ at 7.24 ppm and are given in ppm.
b: OBz: aromatic protons 7.23-8.13 ppm; **5** (5 H= 1Bz), **6** (10 H= 2Bz), **7** (15 H= 3Bz) and **8** (20 H= 4Bz).
c: isopropylidene CH_3 groups appear at 1.45 and 1.54 ppm.

Table 2.3. ^{13}C-NMR data of the partially benzoylated derivatives **4-8** ($CDCl_3$, 125.8 MHz).

Compound		C-1	C-2	C-3	C-4	C-5	C-6	Benzoyl group			
								C_{ipso}	C_m	C_o	C_p
5	Glc	79.4									
	Gal	90.8									
6	Glc	87.7	71.4	73.3	75.4	73.6	70.1	127.7	126.7	128.1	131.7
	Gal	101.4	71.2	74.7	71.1	72.2	62.1		126.8	128.2	
7	Glc	87.8	72.1	73.5	82.3	75.9	63.8				
	Gal	103.8	72.2	78.9	73.2	72.2	63.8				
8	Glc	87.9	72.2	73.4	81.9	74.2	63.6	128.8	128.4	129.6	133.1
	Gal	101.6	72.3	76.9	73.0	72.2	62.2	129.1	128.5	129.8	133.2
								129.5	-	129.9	133.3
								129.6		130.0	133.5

The incorporation of the position of the third benzoyl group in **7** was confirmed over the other possible regioisomers **7a** and **7b** on the basis of the following observations: a comparative down-field shift of H-2 (5.17 ppm) of **7** from that of the 6 and 6' benzoylated derivative **6** (3.32 ppm) was noted and this excludes the possibility of **7b**. Likewise, a comparative down-field shift of H-2' (5.35 ppm) of tetrabenzoylated product **8** from that of **7** (3.70 ppm) eliminates the possible formation of **7a**. This observations provides benzoylation of **4** at -55° C with the preferred order of 6'≈6 > 2 > 2'.



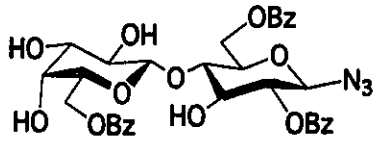
7; R= Bz, R',R"= H

7_a; R'= Bz, R= R"= H

7_b; R"= Bz, R= R'= H

Figure 2.9. The possible tribenzoylated regioisomers.

Deprotection of the isopropylidene groups in 7 was accomplished by refluxing in MeOH in the presence of p-TsOH used as catalyst. This gave the glycosyl acceptor 9 in excellent yield (95%). **(Figure 2.10.)**



9

Figure 2.10. Preparation of the Glycosyl acceptor.

For the synthesis of the sialosyl donor 13, a standard procedure, first used by Kuhn et al[133] was slightly modified to prepare acetochloroneuraminic acid 11 from sialic acid 10 as illustrated in **Figure 2.11.** The β-chloro configuration in 11 reflects the fact that the anomeric effect is operative.

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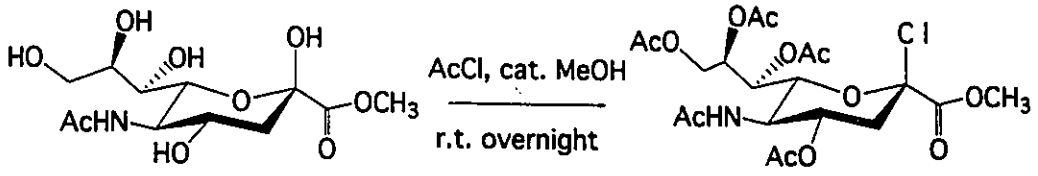
133. R. Kuhn, P. Lutz, D.L. MacDonald, *Chem. Ber.* **1966**, 99, 611.



10 11

Figure 2.11. Preparation of acetochloro nuraminic acid methyl ester 11 from sialic acid 10.

Using the previously mentioned PTC condition, the phenylseleno-(12) and the phenylthio-(13) sialosides were prepared from 11 in 70% and 82% yield, respectively. **(Figure 2.12)**

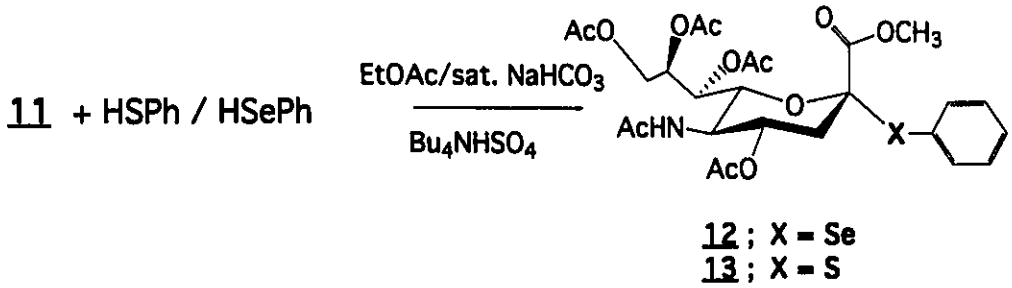
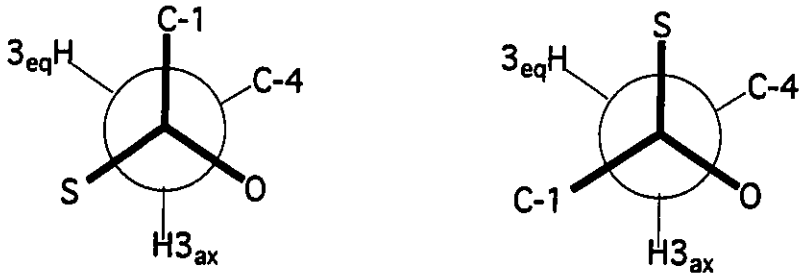


Figure 2.12. Preparation of phenylthio/seleno sialosides, 12 and 13, respectively.

Since there is no anomeric proton in sialic acid derivatives, the configuration at C-2(anomeric center) of 13 was examined by an ^{1}H-coupled ^{13}C-NMR experiment in which can be calculated. From these values the coupling constants between C-1 and H-3_e or H-3_a were determined that the configuration at C-2 was α with $J_{C1,H3a}$= 8.1 Hz and $J_{C1,\ H3e}$= 3.9 Hz. This is consistent with the previously observed results: for 2-methyl Neu5NAc having the α anomer $J_{C1,H3a}$= 5.1 Hz, $J_{C1,H3e}$= 2.0 Hz[134] ; $J_{C1,H3a}$= 6.1 Hz for another 2-substituted Neu5NAc.[135] In contrast, $J_{C1,H3a}$ values for β anomer do not exceed 1.5 Hz. These coupling constant values seem to obey the Karplus relationship.

Similar observations have been made with 3-deoxy-D-manno-octulosonic acid (KDO) (C-2, 176.3 ppm, $J_{C2,H3a}$= 5.2 Hz).[136] In the Newman projection of C-2 through C-3 of **13** is made the CO_2Me group is *gauche* to H_{3eq} and simultaneouly *anti* to H_{3ax} for the α anomer as depicted in **Figure 2.13.** If **13** were the β anomer C-1 would bisect H_{3ax} and H_{3eq} resulting *gauche* relationship to each proton.



α-configuration β-configuration

Figure 2.13. The Newman projections of the two possible anomers of phenylthio sialoside.

The α–glycosidation of Neu5NAc with various alcohols has been reported.[137] The Koenigs-Knorr type of reaction, direct coupling of sialosyl halides with reactive alcohols, phenols and primary sugar hydroxyl groups using a Lewis acid such as AgOTf as promotor has been carried out with moderate success: these usually resulted in α- and β-anomeric mixtures. Similar sialylations with the less reactive acceptors such as secondary or hindered hydroxyl groups of sugars gave the 2,3-dehydro-Neu5Ac derivative as the major product (**Figure 2.14**) formed from an elimination process.

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134. M. F. Czarniecki, E. R. Thornton, *J. Am. Chem. Soc.*, **1977**, 99, 8273.
135. H. Hori, T. Nakajima, Y. Nishida, H. Ohrui, H. Meguro, *Tet. Lett.*, **1988**,29, 6317.
136. W. E. Kohlbrenner, S. W. Fesik, *J. Biol. Chem.*, **1985**, 260(27), 14695.
137. K. Okamoto, T. Goto, *Tetrahedron*, **1990**, 46, 5835 and references cited therein.

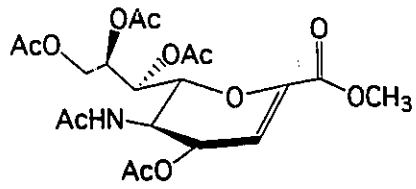

Figure 2.14. The structure of 2,3-dehydro-Neu5Ac.

Recently, it have been reported that sialylation is possible by using the sulfur-containing glycosyl donor such as **13** and effective promotors such as NIS/TfOH, methyl sulfenylbromide (MSB) or dimethyl (methylthio) sulfonium trifluoromethanesulfonate (DMTST). Yields of the reactions range from 38 to 55% containing α- and β-anomeric mixtures.[138-139] H. Lönn et al. have reported that, in synthesis of a GM_3 glycoside, a Neu5Ac glycosyl xanthate with MSB/AgOTf as promotor in $MeCN/CH_2Cl_2$ as solvent at -50° C gave exclusively an α product in exceptionally high yield (63-82%).[140] The observed α stereoselectivity is believed to originate from acetonitrile participation during the reaction as **Figure 2.15** illustrates. This implies that a delicate balance of charges and steric effect is present during the nucleophilic substitution.

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138. F. Dasgupta, P. Garegg, *Carbohydr. Res.*, **1988**, 177, C13; T. Murase, H. Ishida, M. Kiso, A. Hasegawa, *Carbohydr. Res.*, **1989**, 188, 71.
139. A. Hasegawa, K. Adachi, M. Yoshida, M. Kiso, *J. Carbohydr. Chem.*, **1992**, 11(1), 95.
140. H. Lönn, K. Stenvall, *Tet. Lett.*, **1992**, 33, 115.

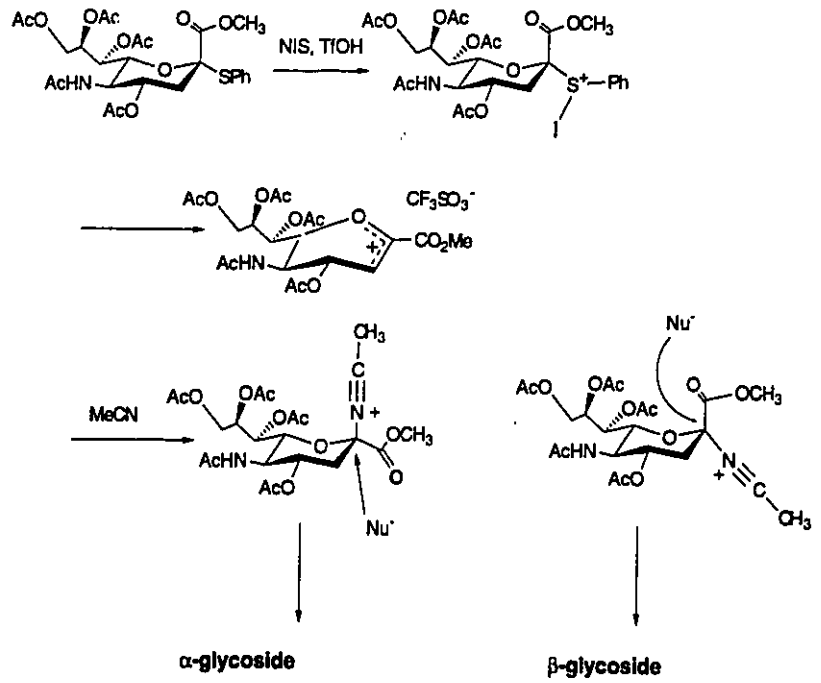
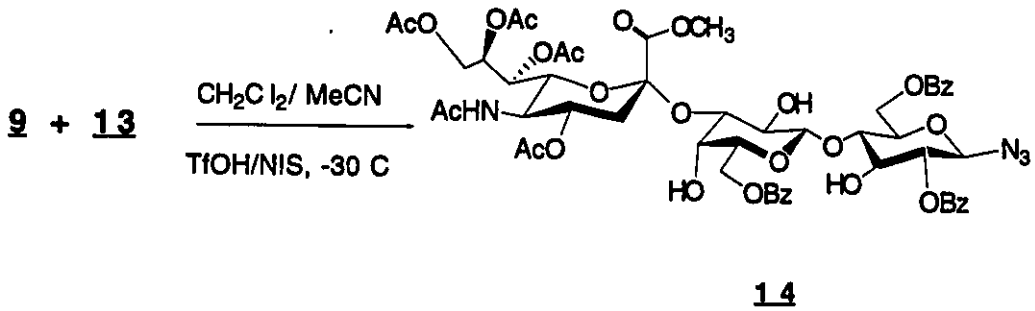


Figure 2.15. The possible mechanism of the iodonium ion mediated sialylation.

Glycosylation of **9** with thiosialoside **13** proceeded well in a mixture of CH_2Cl_2 and MeCN (14 : 86(v/v)) at -30 °C using TfOH/NIS as promotor. This iodonium ion mediated glycosylation gave the α-sialylated trisaccharide **14** as the major product in respectable yield of 81 %. **(Figure 2.16)**



14

Figure 2.16. Sialylation of **13** with the glycosyl acceptor **9**.

The regiochemical outcome of the reaction was confirmed by ^{1}H-NMR spectroscopy. The H-3' in **14** shifted downfield from 3.64 to 4.14 ppm upon sialylation and the methyl ester (CH_3) appeared at 3.78 ppm. In addition, the anomeric carbon (C"-2) of sialic acid was shifted downfield from 87.5 to 97.6 ppm as a result of substituting the vicinal sulfur atom by oxygen, respectively.

Further derivatization of **14** was considered in order to transform the azido group into the N-acrylamide function. As the model compound, peracetylated lactosyl azide **2** was reduced to the corresponding amine **24** by using Raney Ni. The N-acryloylation to the amine afforded **25** which was purified by silica gel column chromatography to give 95% yield. Similarly, the azido group of the GM_3 derivative **14** was reduced by hydrogenation in the presence of 10 % Pd/C catalyst in methanol and was complete within 15 minutes. The reduced product **15** was then reacted with acryloyl chloride at 0° C to give the N-acryloylated GM_3 **16** in 90% yield. The following pages show the ^{1}H-NMR and its COSY experiment spectra of **16** obtained from the acryloylation.

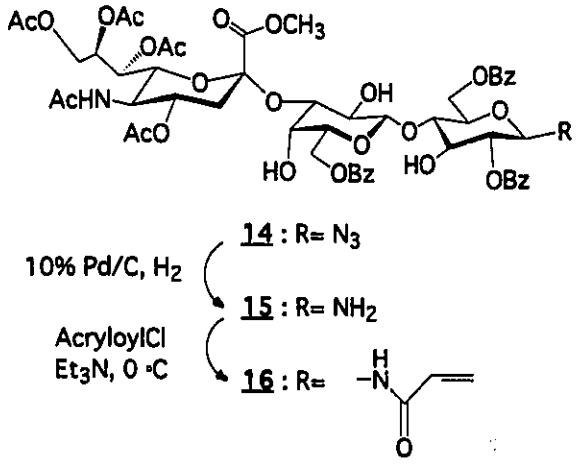
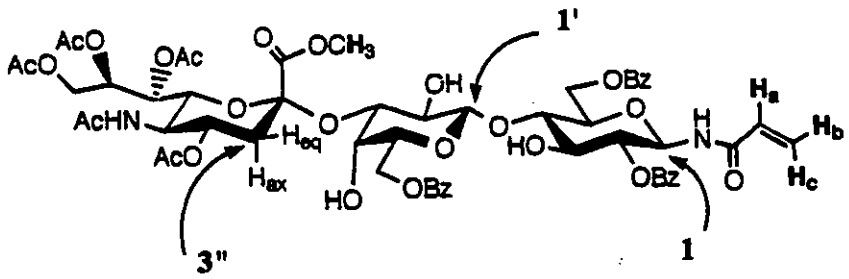


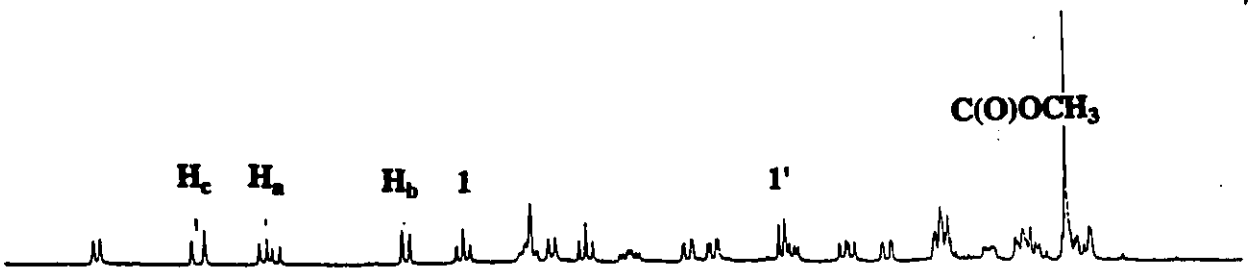
Figure 2.17. N-acryloylation of the key intermediate molecule **14** via reduction of azido function.

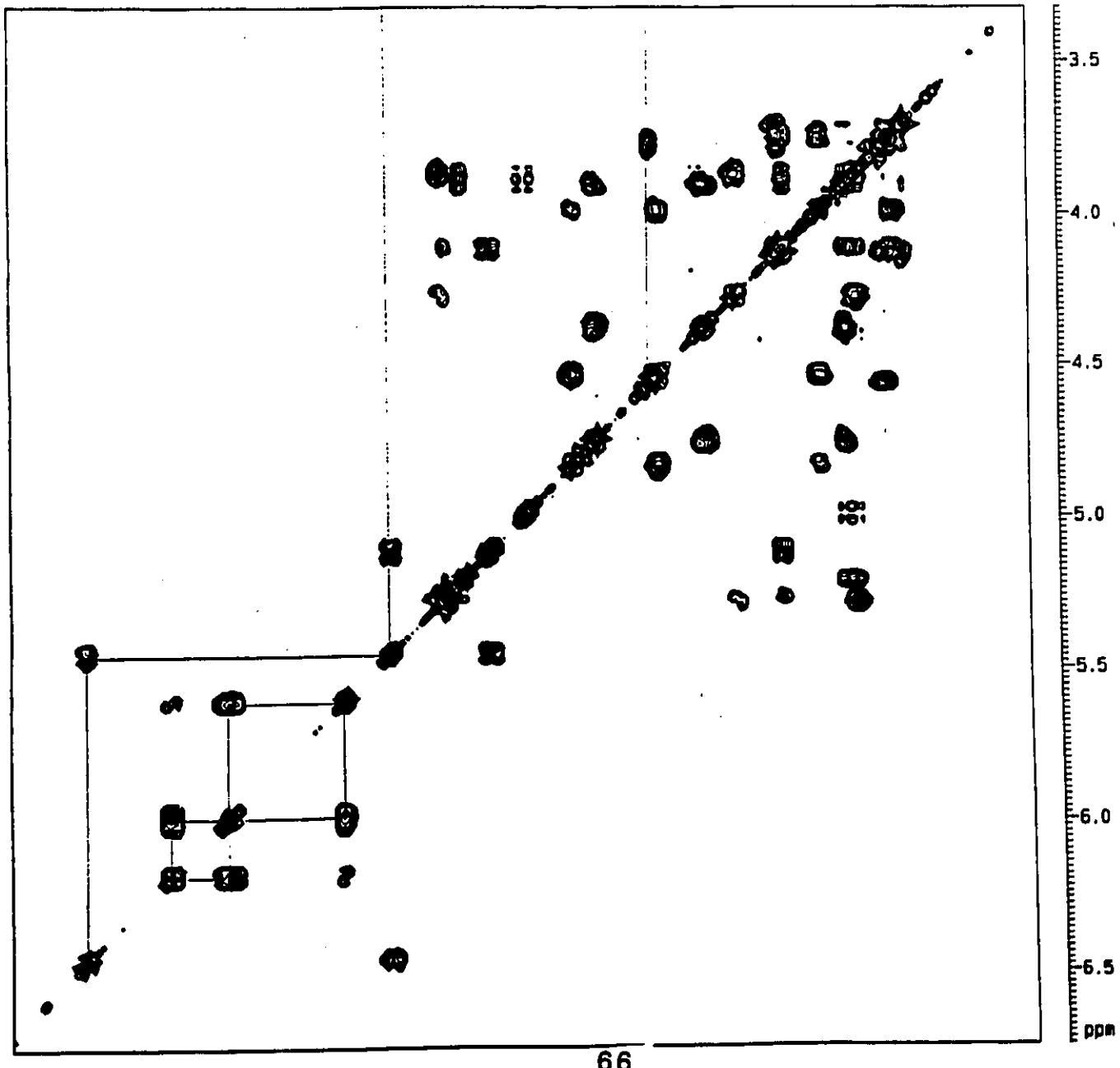
Integral
6.882
2.222
6.400
7.706
1.000
0.971
1.013
0.864
0.996
3.216
1.068
1.109
2.539
2.215
1.366
0.978
3.638
11.257
1.030
29.091
ppm
C(O)OCH3
3"eq
3"ax
1'
1
3"
OBz
OAc
AcO
AcHN
OCH3
OH
HO
Ha
Hb
Hc
Hax
Heq



Park—wPa 11

COSY.





II.3. Experimental Methods

General Methods.

All chemicals and solvents used in the experiments were of reagent grade. Further purifications were performed, when necessary, following published procedures.[141]

Thin-layer chromatography (TLC) was performed using Silica Gel 60-F254 glass plates. The developed TLC plates were dipped in a solution of ceric sulfate (1% (w/v)) and ammonium molybdate (2.5 %(w/v)) in 10% (v/v) aqueous sulfuric acid and heated at *ca* 150-200° C. Other reagents used for developing TLC plates include 0.2% (w/v) isatin in ethanol with 5% (v/v) sulfuric acid; iodine; dilute aqueous potassium permanganate; 2% (w/v) ninhydrin in 4 % (v/v) aqueous pyridine for primary amines; and ultraviolet light for compounds containing chromophores. Column chromatography was conducted on Silica Gel 60 (230-400 Mesh, E. Merck No. 9385).

Solvents were evaporated under reduced pressure using a Büchi rotary evaporator connected to a water aspirator. Melting points were measured with a Gallenkamp apparatus and are uncorrected. Optical rotations($[\alpha]_D$) were recorded with a Perkin Elmer 241 polarimeter and were run at ambient temperature.

^{1}H- and ^{13}C-NMR spectra were recorded on a Brüker AMX 500 or Gemini 200 spectrometer at 500 and 200 MHz for protons and 125.8

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141. D.D. Perrin, W.L.F. Armarego, D. R. Perrin, " *Purification of Laboratorty Chemicals.*", 2nd Ed. 1980, Pergamon Press.

and 50.3 MHz for carbon, respectively. Proton chemical shifts (δ) for protons are given relative to internal chloroform (7.24 ppm) for $CDCl_3$ solutions, to DMSO (2.50 ppm) for DMSO-d_6 solutions and acetone (2.216 ppm) for D_2O solutions unless indicated otherwise. Repeated exchange of protons with deuterium using D_2O and lyophilization for unprotected carbohydrates were necessary to simplify the proton spectra. Carbon chemical shifts are given relative to $CDCl_3$ (77.00 ppm), to DMSO-d_6 (39.50 ppm) and to acetone (31.07 ppm). Spectral analyses were performed as first order approximations and were based on ^1H-^1H COSY, ^1H-^{13}C HETCOR, 1- and 2-dimensional distortionless enhancement by polarization transfer (DEPT), HMQC and HMBC experiments. Mass spectra were obtained using a VG 7070-E spectrometer (EI and CI) or Kratos II H instrument (FAB-glycerol, CI-ether). Xenon was used as the neutral carrier atom in FAB-MS experiments. Infrared spectra were recorded on a Bomen Michelson series FT-IR instrument. Anhydrous KBr chip was prepared as support for solid compound.

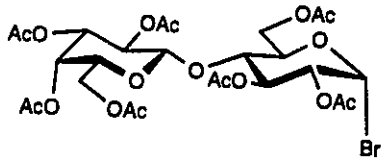
The pH of aqueous solutions were measured with a Fischer Scientific model 805 NP instrument fitted with a Fischer Scientific E-N5 pencil electrode at ambient temperature. Crude pH measurements were routinely performed with Hydrion test paper.

For telomers preparative scale size exclusion chromatography was used. The column was connected to a Pharmacia Peristaltic Pump P3 and was eluted with distilled water. Detection of eluted compounds were made with a Waters Differential Refractometer R401 or R403 and recorded on a Linear 1200 or 2000 chart recorder. Collection of fractions were made using LKB 2112 Redirac or Pharmacia model 5051 fraction collectors. Glycopolymers were dialysed repeatedly against water using cellulose tubing (Sigma, molecular weight cut-off 12 kDa). Glycodendrimers were also dialysed against water using the cellulose tubing with smaller molecular weight cut-off (Sigma, 2000 Da). Once dialysed, the tubing was open and lyophilized using a VIRTIS-24 freeze drier.

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

Experimental

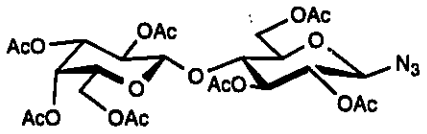
(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1-4)-O-2,3,6-tri-O-acetyl-α-D-glucopyranosyl bromide, (1).



1

To a solution of peracetylated lactose (20.0 g, 29.5 mmol) dissolved in 20 mL of ethylacetate (EtOAc), was slowly added HBr solution in acetic acid (12 g (≈ 1.5 equiv.), 30 wt % HBr in acetic acid, Aldrich) while stirring. Progress of the reaction was monitored by TLC (a 4 :1 mixture of EtOAc : Hexane as eluent). The reaction was complete within 1 hour. The reaction mixture was slowly poured onto an ice- cold saturated sodium bicarbonate solution (0.5 L). The organic phase was separated , washed with water (2 x 25 mL) and followed by saturated NaCl solution (2 x 25 mL). The organic phase was separated, dried over Na_2SO_4 and filtered. The organic solution, then, was concentrated under reduced pressure to give 1 as a white foam (20.1 g, 98%). The product was directly used for the next step without further purification. ^{1}H-NMR spectral data[128] ($CDCl_3$, δ ppm); 4.45 (d, 1H, $J_{1',2'}$ =7.9 Hz, H-1'), 5.09 (dd, 1H, $J_{2',3'}$=10.8 Hz, H-2'), 4.92 (dd, 1H, $J_{3',4'}$ =3.4 Hz, H-3'), 5.33 (dd, 1H, $J_{4',5'}$=1 Hz, H-4'), 3.85 (ddd, 1H, H-5'), 5.64 (d, 1H, $J_{1,2}$ =8.2 Hz, H-1), 5.09 (dd, 1H, $J_{2,3}$=9.4 Hz, H-2), 5.22 (dd, 1H, $J_{3,4}$ =9.1 Hz, H-3), 3.82 (dd, 1H, $J_{4,5}$=8.7 Hz, H-4), 3.68 (m, 1H, H-5), 4.02-4.13 (m, 4H, H-6, H-6'); ^{13}C-NMR spectral data: 20.4, 20.5, 20.6, 20.7, 20.8, 60.8, 61.7, 66.5, 68.9, 70.4, 70.6, 70.9, 72.5, 73.4, 75.6, 91.4, 100.8, 168.8, 169.0, 169.5, 169.6, 170.0, 170.1, 170.2, 170.3.

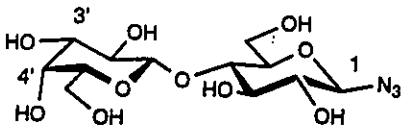
(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1-4)-O-2, 3,6-tri-O-acetyl-β-D-glucopyranosyl azide, (2).



2

A saturated $NaHCO_3$ solution (30 mL) containing NaN_3 (9.3 g, 143 mmol, 5 equiv.) and Bu_4NHSO_4 (10.7 g, 31.5 mmol, 1.1 equiv.) was placed in a round bottom flask while an EtOAc solution (20 mL) containing 1 (20 g, 28.6 mmol) was added. The two-phase solution mixture was vigorously stirred for 2.5 hours at ambient temperature. The progress of the reaction was monitored by TLC using EtOAc as eluent. After the reaction was complete, the organic layer was separated, washed with water (2 x 10 mL) and saturated NaCl solution (2 x 10 mL). The organic phase was washed with Na_2SO_4 and was filtered. The filtrate was evaporated under reduced pressure using a rotatory evaporator to give a white amorphous foam (18.7 g, 99 %); m.p.[129] 67-70 °C (amorphous); $[\alpha]_D$ -20.7° (c=1.0, $CHCl_3$); ^{1}H-NMR spectral data ($CDCl_3$, δ ppm): 4.45 (d, 1H, $J_{1',2'}$ =7.8 Hz, H-1'), 5.09 (dd, 1H, $J_{2',3'}$=9.5 Hz, H-2'), 4.92 (dd, 1H, $J_{3',4'}$= 3.4 Hz, H-3'), 5.33 (dd, 1H, $J_{4',5'}$ =1.1 Hz, H-4'), 3.85 (ddd, 1H, H-5'), 5.64 (d, 1H, $J_{1,2}$ =8.6 Hz, H-1), 5.09 (dd, 1H, $J_{2,3}$=9.5 Hz, H-2), 5.22 (dd, 1H, $J_{3,4}$=9.4Hz, H-3), 3.82 (dd, 1H, $J_{4,5}$=8.9 Hz, H-4), 3.68 (m, 1H, H-5), 4.02-4.13 (m, 3H, H-6, H-6') 4.48 (dd, 1H, H-6); ^{13}C-NMR spectral data: 20.5, 20.7, 20.8, 20.9, 60.8, 61.7, 66.6, 69.0, 70.7, 70.9, 71.0, 72.5, 74.8, 75.7, 87.7, 101.0, 168.9, 169.3, 169.4, 169.8, 169.9, 170.1, 170.2. Anal. Calcd for $C_{26}H_{35}N_3O_{17}$: C 47.20, H 5.33, N 6.35. Found: C 46.93, H 5.31, N, 6.07.

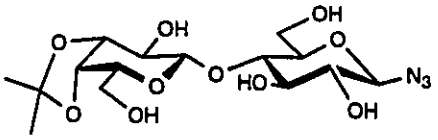
(β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl azide, (3).



3

To a MeOH solution (15 mL) containing 2 (10.0 g, 27.2 mmol) was added a freshly prepared I M $NaOCH_3$/ MeOH solution until the pH of the solution reached *ca* 9.5. The reaction mixture was stirred until a single spot was observed in TLC using a mixture of $CHCl_3$: MeOH : H_2O (10 : 10 : 0.1, respectively) as eluent. The reaction was complete within 1/2 hour and the pH of the reaction mixture was brought to *ca* 7 using H^+ resin. The methanolic solution was filtered and the filtrate was evaporated under reduced pressure to yield white crystalline compound 3 (9.48 g, 95 %). This was recrystallized using ethanol; m.p. 113.0-114.5° C; $[\alpha]_D$ -2.80° (c=1.0, H_2O); ^{1}H-NMR spectral data (D_2O, δ ppm); 4.81(d, 1H, $J_{1,2}$=8.8 Hz, H-1), 4.49(d, 1H, $J_{1',2'}$=7.8 Hz, H-1'), 4.03(dd, 1H, $J_{6a,b}$= 11.9 Hz, H-6a), 3.97 (dd, 1H, $J_{4',5'}$ <1.0 Hz, H-4'), 3.88(dd, 1H, $J_{6b,5}$= 3.8 Hz, H-6b), 3.79-3.86(m, 2H, H-6'a,6'b), 3.70 (dd, 1H, $J_{3',4'}$=3.4 Hz, H-3'), 3.59 (dd, 1H, $J_{2',3'}$=10.0 Hz, H-2'), 3.34-3.38(m, 1H, H-2); ^{13}C-NMR spectral data: 102.4(C-1'), 89.4(C-1), 77.3(C-4), 76.2(C-5), 74.9(C-5'), 73.8(C-3), 72.1(C-2, 3'), 70.5(C-2'), 68.1(C-4'), 60.6(C-6), 59.4(C-6') ; FAB-MS calcd for $C_{12}H_{21}N_3O_{10}$ 367.1. Found 368.3 (M+1); Anal. Calcd for $C_{12}H_{21}N_3O_{10}$: C 44.23, H 6.19, N 10.32. Found C 44.0, H 6.19, N, 10.38.

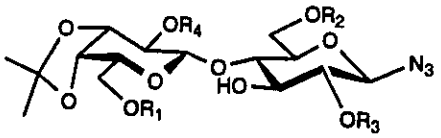
(3,4-O-isopropylidene-β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl azide, (4).



4

Lactosyl azide 3 (5.00 g, 12.3 mmol) was placed in 2,2-dimethoxypropane (20 mL) and refluxed for 15 minutes. Catalytic amount of p-TsOH was added to the refluxing solution in which no solid was observed. Approximately 1 hour after the addition of p-TsOH, a white precipitate appeared. As reflux was stopped and the reaction mixture was cooled to room temperature, more precipitate was formed. The white solid was filtered and washed with cold EtOAc. The resulting solid was recrystallized with MeOH to give transparent needle shaped crystals (4.30 g, 86 %) ; m.p. 180.4-181.3 °C; $[\alpha]_D$ +12.0 (c= 1.0, MeOH); ^{1}H-NMR spectral data (D_2O, δ ppm); 4.49 (d, 1H, $J_{1',2'}$=8.4 Hz, H-1'), 3.52 (dd, 1H, $J_{2',3'}$ =8.0 Hz, H-2'), 4.22 (dd, 1H, $J_{3',4'}$=5.2 Hz, H-3'), 4.37 (dd, 1H, $J_{4',5'}$=2.0 Hz, H-4'), 4.01-4.12 (m, 1H, H-5'), 4.77 (d, 1H, $J_{1,2}$=8.8 Hz, H-1), 3.28-3.34 (m, 1H, H-2), 3.67-3.69 (m, 3H, H-3,4,5), 3.79-3.97 (m, 4H, H-6 and H-6') 1.39 (s, 3H, Me), 1.55 (s, 3H, Me); ^{13}C-NMR spectral data: 26.2, 28.0, 60.6, 61.6, 73.4, 73.6, 74.3, 74.6, 75.1, 77.5, 78.9, 79.4, 90.8, 103.0, 111.9; Anal. Calcd. for $C_{15}H_{25}N_3O_{10}$: C 51.78, H 5.49, N 8.23. Found C 51.78, H 5.74, N, 8.03.

(3,4-O-isopropylidene 6-O-benzoyl-β-D-galactopyranosyl) -(1-4)-O-β-D-glucopyranosyl azide, (5);
(3',4'-O-isopropylidene 6-O-benzoyl-β-D-galactopyranosyl) -(1-4)-O-6-O-benzoyl-β-D-glucopyranosyl azide, (6);
(3',4'-O-isopropylidene 6-O-benzoyl-β-D-galactopyranosyl) -(1-4)-O-2,6-O-dibenzoyl-β-D-glucopyranosyl azide, (7);
(3',4'-O-isopropylidene 2,6-O-benzoyl-β-D-galactopyranos yl)-(1-4)-O-2,6-O-dibenzoyl-β-D-glucopyranosyl azide, (8).



5; R_1 = Bz, R_2= R_3 = R_4 = H

6; R_1 =R_2 = Bz, R_3 = R_4 = H

7; R_1 =R_2 = R_3= Bz, R_4 = H

8; R_1 =R_2 = R_3=R_4 = Bz

Compound 4 (1.00 g, 2.46 mmol) was dissolved in a 4:1 mixture (15 mL) of pyridine and CH_2Cl_2. The solution was cooled to -55° C before a solution of diluted BzCl (1.29 mL, 4.5 equiv.) with CH_2Cl_2 (7 mL) was added dropwise through a pressure equalized dropping funnel. The reaction was proceeded under dry condition (N_2) and carefully monitored by TLC using an 1:1 mixture of EtOAc and hexanes as eluent. The benzoylated products were separated by column chromatography using the same sovent mixture (1:2). The relative yields for **5, 6, 7 and 8** were < 1 %, 2 %, 75 % and 18 %, respectively. The ^{13}C-NMR spectral data of 7 are representatively assigned.

Compound **5** has : m.p. 143-146 °C; ^{1}H-NMR spectral data ($CDCl_3$, δ ppm); 7.34-8.11 (m, 5H, aromatic), 4.74 (dd, 1H, $J_{4',5'}$ =2.4 Hz, H-4'), 4.57 (d, 1H, $J_{1,2}$=8.75 Hz, H-1), 4.32 (d, 1H, $J_{1',2'}$=8.43 Hz, H-1'), 4.19-4.23 (m, 2H, H-6'), 4.14 (dd, 1H, $J_{3',4'}$=2.0 Hz, H-3'), 3.86 (m, 2H, H-6), 3.47-3.85 (m, 4H, H-2,3,5 and 2'), 3.27 (dd, 1H, $J_{4,5}$ 7.3 Hz, H-4), 1.35 (s, 3H, Me), 1.52 (s, 3H, Me); ^{13}C-NMR spectral data: 26.2, 28.0, 60.6, 61.6, 73.4, 73.6, 74.3, 74.6, 75.1, 77.5, 78.9, 79.4, 90.8, 103.0, 111.9.

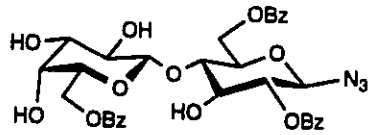
Compound **6** : m.p. 173° C; $[\alpha]_D$ +19.1° (c= 1.0, $CHCl_3$); FAB-MS calcd for $C_{29}H_{32}N_3O_{12}$ 615.2, found 616.2 (M +1); ^{1}H-NMR spectral data ($CDCl_3$, δ ppm); 7.39-8.11 (m, 10 Hs, aromatic), 4.85 (dd, 1H, $J_{4',5'}$=12.2 Hz, H-4'), 4.76 (dd, 1H, $J_{6a,5}$=12.3 Hz, H-6a), 4.55 (d, 1H, $J_{1,2}$=8.7 Hz, H-1), 4.47 (dd, 1H, $J_{5',6'}$=12.1 Hz, H-5'), 4.39 (dd, 1H, $J_{6b,5}$=12.5 Hz, H-6b), 4.28 (d, 1H, $J_{1',2'}$=8.1 Hz, H-1'), 4.12 (dd, 1H, $J_{3',4'}$ =5.4 Hz H-3'), 3.73 (ddd, 1H, $J_{5,6ab}$ =5.5, 1.7 Hz, H-5), 3.67 (dd, 1H, $J_{2',3'}$ =7.2 Hz, H-2'), 3.61 (dd, 1H, $J_{3,4}$ =9.2 Hz, H-3), 3.36 (dd, 1H, $J_{4,5}$ =8.5 Hz, H-4), 3.32 (dd, 1H, $J_{2,3}$ =8.7 Hz, H-2), 1.36 (s, 3H, Me), 1.55 (s, 3H, Me); ^{13}C-NMR spectral data: 24.5, 26.3, 62.1, 70.1, 71.1, 71.2, 71.4, 73.3,73.6, 74.7, 75.4, 87.7, 101.4, 108.9, 126.7, 126.8, 127.7, 128.1, 128.2, 131.7, 164.7, 165.0.

Compound **7** : m.p. 90.1-91.8° C; $[\alpha]_D$ +15.00° 1c $CHCl_3$; FAB-MS calcd. for $C_{36}H_{36}N_3O_{13}$ 719.4, found 720.3 (M +1), 677.25 (M-N_3, 16.3 %); ^{1}H-NMR spectral data ($CDCl_3$, δ ppm): 7.23-8.17 (m, 15 Hs, aromatic), 5.17 (dd, 1H, $J_{2,3}$ =9.0 Hz, H-2), 4.92 (dd, 1H, $J_{6a,5}$ =12.3 Hz, H-6a), 4.79 (dd, 1H, $J_{4',5'}$ =12.3 Hz, H-4'), 4.69 (d, 1H, $J_{1,2}$ =9.0 Hz, H-1), 4.46 (dd, 1H, H-6b), 4.37 (dd, 1H, $J_{5',6'}$=9.0 Hz, H-5'), 4.32 (d, 1H, $J_{1',2'}$ =8.2 Hz H-1'), 4.19-4.13 (m, 3H, H-6', H-3'), 3.94 (dd, 1H, $J_{3,4}$ =8.3 Hz, H-3), 3.84 (dd, 1H, $J_{5,6}$ =5.1Hz, H-5), 3.70 (dd, 1H, $J_{2',3'}$ =7.0 Hz, H-2'), 3.55 (dd, 1H, $J_{4,5}$ =10.0 Hz, H-4), 1.45 (s, 3H, Me), 1.54 (s, 3H, Me); ^{13}C-NMR spectral data : 26.2 , 28.0 (methyl groups of isopropyledine), 63.8(C-4',5' and 6), 72.1(C-2), 72.2(C-2'), 73.2(C-4), 73.3(C-6'), 73.5(C-3), 75.9(C-5), 78.9(C-3'), 82.3(C-4), 87.8(C-1), 103.8(C-1'), 110.8(quarternary C), 133.6-128.3 (aryl), 165.2, 166.5, 166.9(C=O); Anal. Calcd. for $C_{36}H_{36}N_3O_{13}$: C 60.08, H 5.18, N 5.84 ; Found C 59.98, H 5.17, N, 5.72.

Compound **8** : m.p. 203.0-204° C(dec.) ; $[\alpha]_D$ +4.00° (c= 1.0, $CHCl_3$); FAB-MS calcd. for $C_{43}H_{40}N_3O_{14}$ 823.0, found 824.3 (M +1), 781.24 (M-N_3, 13.9 %); ^{1}H-NMR spectral data ($CDCl_3$, δ ppm): 7.26-8.13 (m, 20 Hs, aromatic), 5.35 (dd, 1H, $J_{2',3'}$ =7.8 Hz, H-2'), 5.16 (dd, 1H, $J_{2,3}$ =9.5 Hz, H-2), 4.64 (d, 1H, $J_{1,2}$ =9.0 Hz, H-1), 4.63 (d, 1H, $J_{1',2'}$ =8.1

Hz, H-1'), 4.20 (m, 6H, H-6,6',3' and 5'), 3.99 (dd, 1H, $J_{3,4}$ =7.7 Hz, H-3), 3.74 (m, 2H, H-4,5), 1.45 (s, 3H, Me), 1.54 (s, 3H, Me); ^{13}C-NMR spectral data : 26.3 , 27.6 (methyl groups of isopropyledine), 62.2(C-6'), 63.6(C-6), 72.2(C-2), 72.3(C-2'), 73.0(C-4'), 73.4(C-3), 74.2(C-5), 77.0(C-3'), 81.9(C-4), 87.9(C-1), 101.5(C-1'), 110.8(quarternary C), 128.3-133.4 (aryl), 165.2, 166.5, 166.5(C=O); Anal. Calcd. for $C_{43}H_{40}N_3O_{14}$: C 62.70, H 5.02, N 5.10. Found C 62.41, H 4.99, N, 5.04.

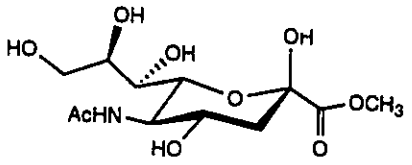
(6'-O-benzoyl-β-D-galactopyranosyl)-(1-4)-O-2,6-di-O-benzoyl-β-D-glucopyranosyl azide, (9).



9

To a solution of 7 (500 mg, 0.70 mmol) dissolved in MeOH (15 mL) was added a catalytic amount of p-TsOH. The solution was refluxed overnight. The progress of the reaction was examined by TLC using an 1 : 1 mixture of EtOAc and hexanes. The reaction mixture was cooled, neutralized with triethylamine and evaporated under reduced pressure. Small quantities of contaminants were removed by column chromatography using a 9:1 mixture of $CHCl_3$ and MeOH, respectively to give crylstalline compound **9** (452 mg, 95 %); m.p. 163° C; $[\alpha]_D$ +0.80° (c=1.0, $CHCl_3$), ^{1}H-NMR spectral data ($CDCl_3$, δ ppm): 7.22-7.96 (m, 15 Hs, aromatic), 5.10 (dd, 1H, $J_{2,3}$ =9.3 Hz, H-2), 4.79 (d, 1H, $J_{6a,b}$ =11.3 Hz, H-6a), 4.67(d, 1H, $J_{1,2}$ =8.9 Hz, H-1), 4.64 (m, 1H, H-6'), 4.44 (dd, 1H, $J_{6b,5}$ =5.7 Hz, H-6b), 4.33 (m, 2H, H-1', 6'), 3.84-3.98 (m, 5H, H-3, 5', 2', 4', 5), 3.64 (broad b, 1H, H-3'), 3.56 (d, 1H, $J_{4,5}$ 9.1 Hz, H-4); ^{13}C-NMR spectral data: 63.7(C-6), 64.0(C-6'), 68.9(C-4'), 70.9(C-5), 72.5(C-2), 73.1(C-3), 73.5(C-5'), 74.8(C-2'), 81.5(C-4), 87.8(C-1), 104.2(C-1'), 128.4-133.6 (aromatic), 165.3, 166.8, 167.1(C=O); FAB-MS calcd for $C_{33}H_{33}N_3O_{13}$; 679.2. Found 680.2 (M+1, 8.9 %) and 637.2 (M-N_3, 13.9 %); Anal. Calcd for $C_{33}H_{33}N_3O_{13}$: C 58.32, H 4.89, N 6.18. Found, C 58.20, H 4.86, N 6.19.

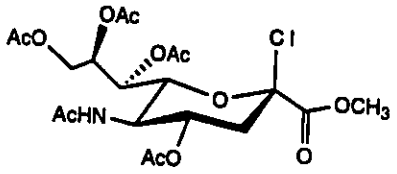
Methyl 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate, (10).



10

N-acetylneuraminic acid (5.00 g, 16.2 mmol) and Amberlite IR-20 H^+ resin(5.0 g) in MeOH (100 mL) were stirred overnight at ambient temperature. The initially cloudy and turbid solution became clear. When the reaction was over, the resin was filtered off and the filtrate was evaporated under reduced pressure until the final volume was ≈ 7-8 mL. The esterified product was crystallized upon addition of ether (5.11 g, 98 %). M.p.= 194.3-196.8° C; $[\alpha]_D$ = -28° (c=3.5, H_2O).

Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosid)onate, (11).

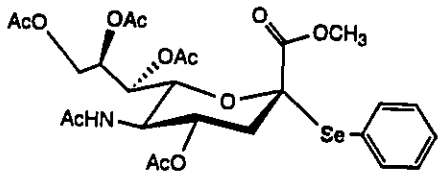


11

To an AcCl solution (23 mL) was added a small quantity of MeOH (0.1 mL) at 0° C. The mixture was kept closed and stirred for 1 hour at the same temperature. The reaction was then initiated by adding 11 (1.00 g, 3.1 mmol) to the mixture and sealed for 24 hours. The progress of the reaction was monitored by TLC using EtOAc as eluent. The reaction mixture was concentrated under reduced pressure to give a syrupy material. A small amount of toluene was used to coevaporate the residual AcCl and this resulted in a white

foam. The product was vacuum dried for two days and used for the next step without further purification (1.60 g, 99 %)

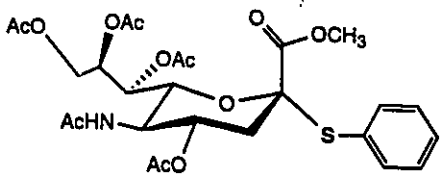
[2-(phenylselenyl) methyl 5-acetamido-4,7,8,9,-tetra-O-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosid]onate, (12).



12

To a solution of **11** (560 mg, 1.07 mmol) dissolved in EtOAc (1 mL) was added a saturated Na_2CO_3 solution (2 mL) containing Bu_4NHSO_4 (370 mg, 1.1 mmol). Benzeneselenol (150 μL, 1.1 mmol) was added while the two-phase mixture was vigorously stirred. Immediately after commencing the reaction, fleeting green colour was observed. The reaction was complete within 5 minutes. The reaction mixture was diluted by adding ≈ 20 mL of EtOAc and the organic layer was separated from the aqueous phase. The EtOAc layer was washed with saturated $NaHCO_3$ solution (2 x 20 mL), water (2 x 20 mL) followed by saturated NaCl solution (2 x 20 mL). The organic phase was then dried over $MgSO_4$, filtered and evaporated under reduced pressure. The desired product was recrystallized from a mixture of hexane and benzene at 4° C (457 mg, 70 %); ^{1}H-NMR spectral data: 7.27-7.48(m, 5H, aromatic), 5.25(m, 2H, H-7,8), 5.19(d, 1H, $J_{NH,5}$ 10.0 Hz, NH), 4.78(ddd, 1H, $J_{4,3e}$ =4.7, $J_{4,3a}$ =11.5 Hz, $J_{4,5}$ =20.9 Hz, H-4), 4.38(dd, 1H, $J_{9a,8}$ =2.5 Hz, H-9a), 4.16(dd, 1H, $J_{9b,a}$ =12.4, $J_{9b,8}$ =5.6 Hz, H-9b), 3.94(dd, 1H, $J_{5,6}$ =9.2 Hz, H-5), 3.85(dd, 1H, $J_{6,7}$ =1.7Hz, H-6), 3.54(s, 3H, OCH_3), 2.83(dd, 1H, $J_{3a,e}$ =12.9, $J_{3e,4}$ =4.6 Hz, H-3eq), 2.00(m, 1H, H-3ax), 2.09, 2.05, 2.03, 1.97(OAc), 1.82(NHAc); Anal. Calcd. for: $C_{26}H_{33}NO_{11}Se$: C,50.84, H, 5.38, N, 2.28. Found, C, 50.61, H, 5.49, N, 2.23.

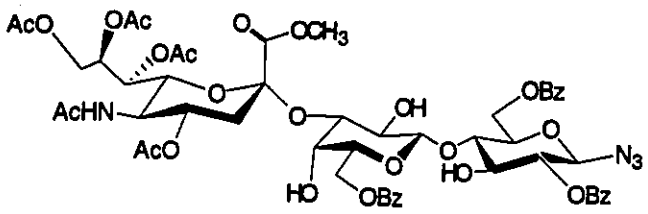
[2-(thiophenyl)methyl 5-acetamido-4,7,8,9,-tetra-O-acetyl 3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosid]onate, (13).



13

To an EtOAc solution (10 mL) containing 11 (3.15 g, 6.14 mmol) was added a saturated Na_2CO_3 solution (15 mL) containing Bu_4NHSO_4 (2.61 g, 7.68 mmol). While the two-phase solution mixture was vigorously stirred, PhSH(1.26 g, 11.52 mmol) was added at once. The reaction was complete within 1/2 hour. The separated organic phase was washed with saturated Na_2CO_3 solution (2 x 20 mL), water (2 x 20 mL) followed by saturated NaCl solution (2 x 20 mL). The organic phase was then dried over Na_2SO_4, filtered and evaporated under reduced pressure. The resultant syrup was recrystallized with a solution of benzene and hexane mixture at 4° C to give a white crystalline compound (2.94 g, 82 %); m.p.= 116-117° C; ^{1}H-NMR spectral data : 7.27-7.48(m, 5H, aromatic), 5.31(d, 1H, $J_{NH,5}$ 10.0 Hz, NH), 5.25(dd, 1H, $J_{7,8}$ 7.0 Hz, H-7), 5.22(m, 1H, H-8), 4.80(ddd, 1H, $J_{4,3e}$ 4.7, $J_{4,3a}$ 11.5 Hz, $J_{4,5}$ 20.9 Hz, H-4), 4.35(dd, 1H, $J_{9a,8}$ 2.5 Hz, H-9a), 4.16(dd, 1H, $J_{9b,a}$ 12.4, $J_{9b,8}$ 5.6 Hz, H-9b), 3.94(dd, 1H, $J_{5,6}$ 9.2 Hz, H-5), 3.85(dd, 1H, $J_{6,7}$ 1.7Hz, H-6), 3.53(s, 3H, OCH_3), 2.77(dd, 1H, $J_{3a,e}$ 12.9, $J_{3e,4}$ 4.6 Hz, H-3e), 2.10, 2.01, 2.00(m, 1H, H-3a), 1.95, 1.82(acetyl H); ^{13}C-NMR spectral data : 20.7, 20.8(2), 20.9, 23.1(-OC(O)CH3), 38.1(C-3), 49.2(C-5), 52.7(OCH_3), 62.0(C-9), 67.8(C-7), 69.7(C-4), 70.1(C-8), 74.8(C-6), 87.5(C-2), 128.8(C_m), 129.9(C_p), 136.5(C_o), 167.9, 170.0, 170.1, 170.2, 170.6, 170.9(-C(O)-); FAB-MS calcd. for $C_{26}H_{33}NO_{12}$ 583.18. Found 584.3 (M +1, 23.4%).

(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosidonate)-(2-3)-O-(6-O-benzoyl-β-D-galactopyranosyl)-(1-4)-O-2,6-di-O-benzoyl-β-D-glucopyranosyl azide, (14).

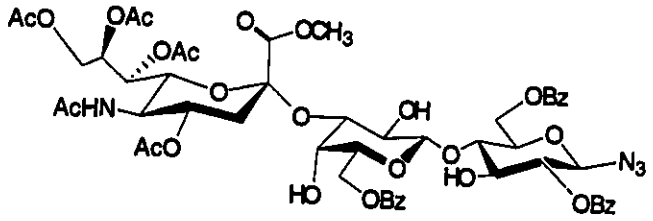


14

Molecular sieve-dried MeCN (14 mL) was added to a mixture of glycosyl donor **13** (1.50 g, 2.65 mmol) and acceptor **9** (1.00 g, 1.47 mmol). The solution was occasionally warmed until no solid was observed. The solution was then cooled to -30° C under nitrogen flushing through the reaction vessel. Already cooled NIS solution (0.74 g, 5.88 mmol) in a mixture of MeCN and CH_2Cl_2 (0.5 mL and 1mL respectively) was added to the reaction mixture and immediately after, followed by TfOH (neat). The reaction was over within 20 minutes leaving less than 10 % of the acceptor unreacted judging from intensity of TLC spots(Rf values: 0.21, 0.43 and 0.33 for **9**, **13** and **14**, respectively). The temperature was elevated to ambient temperature. To the reaction mixture was added $Na_2S_2O_3$ to remove the remaining iodo compound. Under reduced pressure, a yellow syrupy material was obtained. This was redissolved in EtOAc and water to separate the desired compound from salts. The EtOAc layer was then washed with saturated $NaHCO_3$ (30 mL), water (2 x 20 mL), followed by saurated NaCl solution (2 x 20 mL). The solution was then dried over Na_2SO_4, filtered and then evaporated under reduced pressure. The resulting mixture was then separated using silica gel column chromatography (EtOAc: hexanes, 7 : 3). The isolated product was then concentrated *in vacuo* to give a white foam(1.37 g, 81.0 %). M.p. 114.9-116.2° C; $[\alpha]_D$ +3.12° 1c $CHCl_3$; FAB-MS calcd for $C_{53}H_{60}N_4O_{25}$: 1152.4, found 1153.3 (M+1) and 1111.3 (M-N_3); ^{1}H-NMR spectral data ($CDCl_3$, δ ppm): 7.28-8.07(m, 15 H,

aromatic), 5.25-5.31 (m, 2H, H-7", 8"), 5.21(dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 4.97(ddd, 1H, H-4"), 4.94(dd, 1H, $J_{6a,b}$ 12.1, $J_{6a,5}$ 1.5 Hz, H-6a), 4.73(d, 1H, $J_{1,2}$ 8.9 Hz, H-1), 4.74(dd, 1H, $J_{6'a,b}$ 11.8 Hz, H-6'a), 4.57(d, 1H, $J_{1',2'}$ 7.9 Hz, H-1'), 4.53(dd, 1H, $J_{6b,5}$ 5.6 Hz, H-6b), 4.29-4.37(m, 2H, H-6'b, 9a"), 4.14(dd, 1H, $J_{3',4'}$ 3.3 Hz, H-3'), 4.10(dd, 1H, $J_{4',5'}$ 1.7 Hz, H-4'), 3.97(dd, 1H, $J_{3,4}$ 8.6 Hz, H-3), 3.88-3.94(m, 4H, H-5, 5', 6", 9"), 3.78 (s, 3H, methyl ester), 3.76-3.77 (m, 3H, H-4, 2', 5"), 2.13, 2.05, 2.01, 1.91, 1.88 (s, 3H each, acetyl); ^{13}C-NMR spectral data : 20.6, 20.7, 20.8, 21.1, 23.1 (-C(O)CH$_3$), 37.7(C-3"), 49.8(C-5"), 53.3(methyl ester), 62.4(C-9"), 63.2(C-6), 63.7(C-6'), 67.0(C-7"), 68.1(C-2'), 68.2(C-4"), 68.9(C-8"), 69.0(C-4), 72.5(C-2), 72.9(C-5), 73.1(C-4'), 73.5(C-3), 75.0(C-6"), 76.4(C-3'), 81.9(C-4), 87.9(C-1), 97.6(C-2"), 104.4(C-1'), 128.3, 128.4, 129.3, 129.6, 129.7, 129.8, 129.9, 130.0, 133.0, 133.2 (aromatic), 165.3, 166.6, 166.6, 168.1, 170.2, 170.2, 170.3, 170.5, 170.8 (C=O).

Amino N-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosidonate)-(2-3)-O-(6-O-benzoyl-β-D-galactopyranosyl)-(1-4)-2,6-di-O-benzoyl-β-D-glucopyranose, (15).

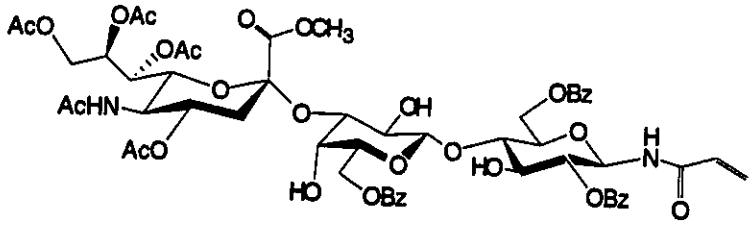


15

To methanol(0.5 mL) was added **14** (200 mg, 0.17 mmol) and 10 % Pd/C (40 mg). Hydrogen gas was bubbled through the solution while stirring. The reaction was complete within 15 minutes. Ninhydrin test was performed to ensure reduction of the azide :A small quantity of sample in question as well as equal volumes of each monitoring reagent; monitor 1: 76 % w/w phenol/EtOH, monitor 2: 0.0002 M KCN/pyr. and monitor 3: 0.28 M ninhydrin/EtOH, were placed in a small test tube and gently heated. Positive reaction turned violet. The palladium catalyst was filtered and the filtrate

was evaporated under reduced pressure to give a white foam (184 mg, 94 %). The resultant compound was directly used for the next step without further purification.

N-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosidonate)-(2-3)-O-(6-O-benzoyl-β-D-galactopyranosyl)-(1-4)-O-(2,6-di-O-benzoyl-β-D-glucopyranosyl) acrylamide, (16).



16

A solution containing 15 (90 mg, 0.08 mmol) in EtOAc (2 mL) was brought to 0° C and kept under nitrogen gas. A diluted acryloyl chloride (8.1 μL, 0.10 mmol in 100 μL EtOAc) solution was added. The reaction mixture was stirred for 5 minutes, after which triethylamine was added until the pH reached *ca* 9. A white precipitate appeared. After the reaction was comlete, the ionic by-products were separated by washing with water. The organic phase obtained from routine washing and drying processes was evaporated under reduced pressure. The resulting syrupy material was purified by column chromatography using EtOAc as eluent to give a white foam (84.9 mg, 90 %). ^{1}H -NMR spectral data ($CDCl_3$, δ ppm): 7.17-8.03(m, 15 H, aromatic), 6.45(d, 1H, $J_{NH,1}$ =9.2 Hz, anomeric NH), 6.17(d, 1H, $J_{c,a}$ =17.1 Hz, Hc), 5.98(dd, 1H, $J_{b,a}$ =10.4, $J_{a,c}$ =17.1 Hz, Ha), 5.61(d, 1H, $J_{b,a}$ =10.4 Hz, Hb), 5.45(dd, 1H, $J_{1,2}$ =9.4 Hz, H-1), 5.25-5.30 (m, 2H, H-7", 8"), 5.20(d, 1H, $J_{N"H,5"}$ =9.4 Hz, N"H), 5.11(dd, 1H, $J_{2,3}$ =9.6 Hz, H-2), 4.99(ddd, 1H, H-4"), 4.82(dd, 1H, $J_{6a,b}$ =10.6 Hz, H-6a), 4.75(dd, 1H, $J_{6'a,b}$ =12.0 Hz, H-6'a), 4.56(d, 1H, $J_{1',2'}$ =7.8 Hz, H-1'), 4.53(dd, 1H, $J_{6b,5}$ =5.6 Hz, H-6b), 4.27(dd, 1H, 9a"), 4.11(m, 3H, H-3, 4', 3'), 3.99(m, 1H, H-5), 3.79-3.92(m, 2H, H-9"a, 5"), 3.78 (s, 3H, methyl ester), 3.70-3.77 (m, 4H, H-4, 2', 5'), 2.67(dd, 1H,

$J_{3"e,a}$ =13.0, $J_{3"e,4}$ =4.6 Hz, H-3"e), 2.13, 2.03, 2.02, 2.00(dd, 1H, $J_{3"a,e}$ =13.0, $J_{3"a,4"}$ =12.0 Hz, H-3"a), 1.92, 1.88 (s, 3H each, acetyl); ^{13}C-NMR spectral data : 20.6, 20.7, 20.8, 21.1, 23.2(-C(O)$\underline{C}H_3$), 37.7(C-3"), 49.8(C-5"), 53.3(methyl ester), 62.4(C-9"), 63.2(C-6), 63.7(C-6'), 67.0(C-7"), 68.1(C-2'), 68.2(C-4"), 68.9(C-8"), 69.0(C-4), 72.5(C-2), 72.9(C-5), 73.1(C-4'), 73.5(C-3), 75.0(C-6"), 76.4(C-3'), 78.2(C-1), 82.3(C-4), 97.5(C-2"), 104.4(C-1'), 127.9(C-b), 130.3(C-a), 128.3, 128.4, 129.3, 129.6, 129.7, 129.8, 129.9, 130.0, 133.0, 133.2 (aromatic), 165.3, 166.6, 166.6, 168.1, 170.2, 170.2, 170.3, 170.5, 170.8 (C=O).

Chapter III. Glycopolymers.

III.1. Introduction.

The multivalency effect, commonly referred as "the cluster effect", greatly enhances the overall avidity of carbohydrate ligands towards their receptors.[53] Encouraged by this recent observation, chemists have prepared many synthetic clustered macromolecules containing biologically active carbohydrates. Among these clusters, neoglycoproteins were first introduced as animal vaccines to prepare anti-carbohydrate antibodies.[141] Since the neoglycoproteins are immunogenic,[142] syntheses of potentially nonimmunogenic glycopolymers are considered as an alternative. The glycopolymers have several advantages with respect to the following aspects. They can minimize the undesired high background and cross-reacting antibodies against the carrier molecule during serological evaluations. The glycopolymers are thermally more stable than the corresponding glycoproteins and can possess uniform structures with a wide range of molecular weights, carbohydrate densities and functionalities. Their preparations, purifications and characterizations are also easier than the protein counterparts. In addition, their nonimmunogenic properties can be advantageously applied to the glycopolymers as multivalent inhibitors or as cell targeting agents.

As already mentioned, the O-allyl glycosides have been frequently used as monomers.[143] However, glycopolymers prepared from this method suffer from a short distance between the polymer

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141. W.E. Dick and M. Beurret, In *Contribution to Microbiology and Immunology*, Vol. 10, *Conjugate Vaccines (J.M. Cruse, R.E. Lewis, Jr., Eds.)*, Karger, Basel, 1989, 48.
142. R. Roy, C.A. Laferrière, R.A. Pon and A. Gamian, *Methods in Enzymol.*, 247, 1994.

backbone and the carbohydrate hapten. In order to circumvent this problem carbohydrate monomers having alkenyl spacers with varied lengths have been prepared and copolymerized with acrylamide.[144] However, it was found that the longer the hydrocarbon spacers in the carbohydrate monomer, the less water soluble the glycopolymers were. These solubility problems can be overcome by providing hydrophilic spacers to carbohydrate monomers. The monomers with an N-acrylamido function have been prepared and copolymerized with acrylamide to produce various glycopolymers in our laboratory.[145] As an extended effort several lactose- and GM_3-containing glycopolymers will be synthesized. The corresponding monomers can be obtained by reducing the common precursor lactosyl azide followed by acryloylation.

The syntheses of lactose- and GM_3-containing glycopolymers are described herein dealing with the following aspects. First, a number of the carbohydrate monomers with spacer arms of varied lengths are synthesized. Second, the copolymerization was designed in such a way that the carbohydrate monomer and co-monomer (acrylamide) share common terminal functionality. From this,

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143. a. R. Roy, C.A. Laferrière, A. Gamian, H.J. Jennings, *J. Carbohydr. Chem.*, **1987**, 6, 161.
b. V. Horejsi, J. Kokourek, *Methods in Enzymology*, **1973**, 34, 361.
c. K. Sutoh, L. Rosenfeld, Y. C. Lee, *Anal. Biochem.*, **1977**, 79, 329.
d. V. Horejsi, P. Smoleck, J. Kokourek, *Biochim. Biophys. Acta* **1978**, 538, 293.
e. A. Y. Chernyak, A.B. Levinsky, B.A. Dmitriev, N.K. Kochetkov, *Carbohydr. Res.* **1982**, 110, c16.
f. A. Y. Chernyak, A.B. Levinsky, B.A. Dmitriev, N.K. Kochetkov, *Carbohydr. Res.* **1984**, 128, 269.
g. N. K. Kochetkov, *Pure and Appl. Chem.* **1984**, 56, 923.
h. A.Y. Chernyak, K. Antonov, N. K. Kochetkov, L. N. Padyukov, N. V. Tsetkova, *Carbohydr. Res*, **1985**, 141, 199.
i. P. Kosma, J. Gass, G. Schultz, R. Christian, F. M. Unger, *Carbohydr. Res.* **1987**, 167, 39.

144. S.-I. Nishimura, T. Furuike, K. Matsuoka, *Methods in Enzymlo.* Vol. 242 [22], **1994**, 235.

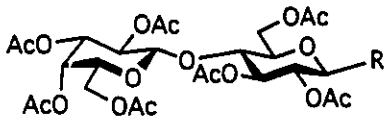
145. a. R. Roy, F. D. Tropper, *Glycoconjugate J.* **1988**, 5, 203.
b. R. Roy, F. D. Tropper, *J. Chem. Soc., Chem. Commun.*, **1988**, 1058.
c. R. Roy, F. D. Tropper, T. Morrison, J. Boratynski, *J. Chem. Soc., Chem. Commun.*, **1991**, 536.

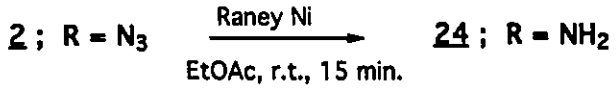
it is expected to maximize homogeniety of the resultant polymers and thus minimize the discrete block polymer formation. Third, the carbohydrate incorporation ratio to acrylamide polymer backbone was set at 1 : 10 (carbohydrate : acrylamide, respectively), which is chosen to balance the solubility of polymer with the multivalency effect, throughout all the polymerizations. This consistency of the carbohydrate-incorporation ratio in the polymers gives a basis of comparison with other polymers during the serological assays.

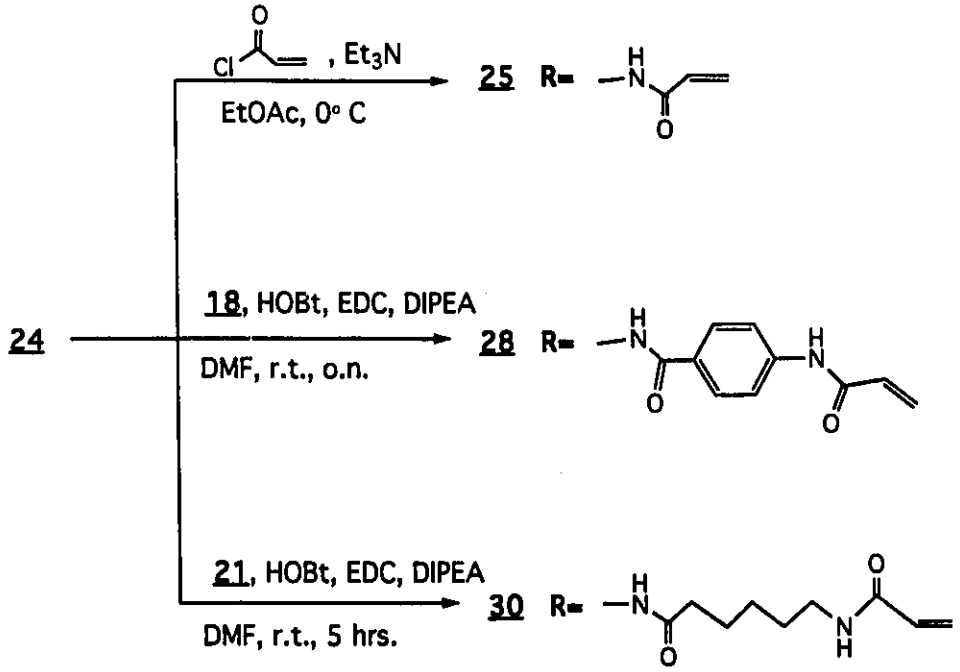
As N-linked acryloyl functions of carbohydrate haptens have emerged as useful tools for the synthesis of various glycopolymers, copolymerizations of N-acryloylated lactose- and GM_3-containing monomers and acrylamide have been sought out. As described earlier, the carbohydrate-containing monomers having N-acrylamido groups can be prepared by reduction of the azide group followed by N-acryloylation. In addition spacer arms with varied lengths are introduced to the monomers by employing carbodiimide chemistry. Thus, the effect of the distance between the carbohydrate haptens and the polymer backbone structures can be examined once they are polymerized.

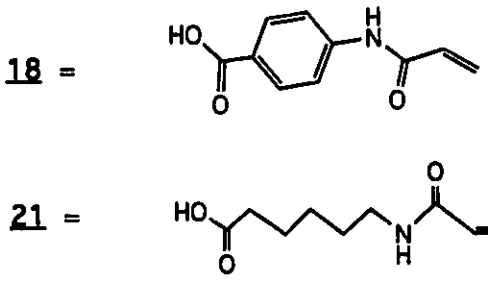
III.2. Results and Discussion.

As a model compound, peracetylated lactosyl azide **2** was reduced by using Raney Ni to give the corresponding amine **24** in 86% yield. The N-acryloylation of the amine afforded **25** in 95% yield. The crude product showed a small quantity of α–anomer (<1%) as judged from the TLC which was removed by silica gel column chromatography. As shown in **Scheme 3.1**, spacer arms with varied lengths were introduced by using carbodiimide chemistry. The condensation of **24** with p-acrylamidobenzoic acid **18** and 6-N-









Scheme 3.1. Syntheses of lactose-containing monomers with various spacer arms.

acrylamidohexanoic acid **21**, respectively in the presence of HOBt and EDC in DMF resulted in the corresponding peracetylated lactose derivatives **28** (79%) and **30** (83%) with different spacer arms. Compound **25** represents the monomer without a spacer arm. The precursor **28** with p-acrylamidobenzamido function is believed to provide not only a longer spacer arm than that of **25**, but also a more rigid carbohydrate entity with respect to the polymer backbone. Therefore, the accessibility of the carbohydrate towards an oncoming lectin molecule, for instance, is improved. The third monomer **30** with N-acrylamidohexanoyl spacer should provide more flexibility than that of **28** and a longer spacer arm than that of **25**.

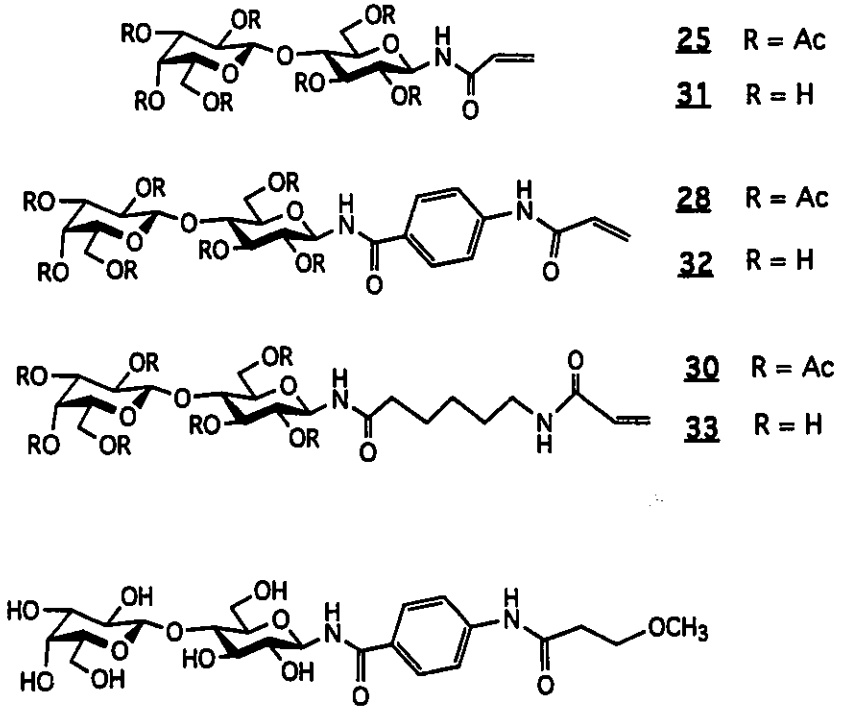
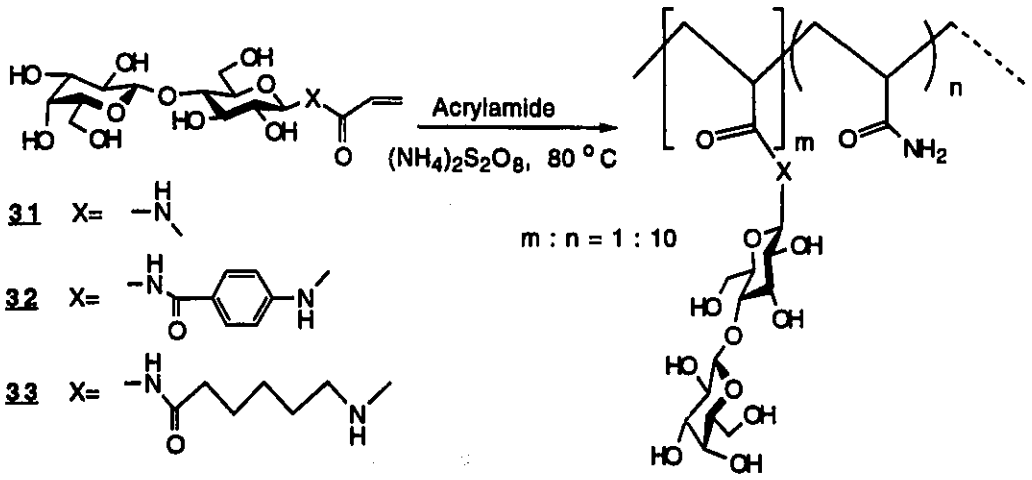


Figure 3.1. Deacetylation of the lactose-containing monomers under Zemplen condition and Michael addition product of **28**.

Under Zemplén condition (MeONa/MeOH) the three peracetylated monomers gave their de-O-acetylated derivatives, **31**, **32**, **33**, respectively in quantitative yields except for **32** as shown in **Figure 3.1**. Under pH 9.5 the p-acrylamidobenzamido function of **28** suffered from a side reaction. Michael addition of MeOH to the N-acrylamido group was observed in 15 % yield judged from ^1H NMR spectrum showing the characteristic $CH_2OC\underline{H}_3$ singlet at 3.40 ppm in D_2O. On the contrary, the other monomers **25** and **30** showed no Michael addition products. It is believed that the electronic effect of carbonyl group through phenyl ring of **28** seems to be operative.

Each lactose-containing monomer was copolymerized with acrylamide in a molar ratio of 1 : 10 in the presence of ammonium persulfate ($(NH_4)_2S_2O_8$) used as initiator in deoxygenated H_2O as illustrated in **Scheme 3.2**. This incorporation ratio was chosen on the basis of a delicate balance between solubility of reagents



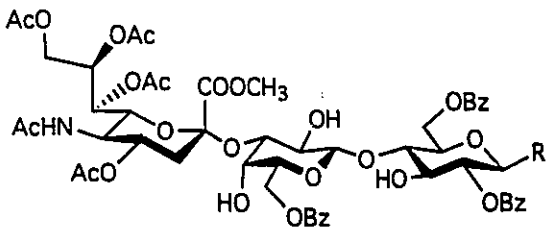
Scheme 3.2. Copolymerizations of the lactose-containing monomers.

(both monomers and polymers) and the cluster effect. If the ratio is too high (too much for the carbohydrate monomer), the polymer should have poor solubility in aqueous medium and thus the little value to study the multivalency effect. This is valid if the carbohydrate monomer contains a long lipophilic spacer. In contrast, if the ratio is too low the polymer would be soluble in water but the multivalency effect would be lessen owing to the sparsity of the carbohydrate antigen. The incorporation ratio (1:10) of lactose-containing monomers (**31**-**33**) to acrylamide gave the corresponding polymers **37**-**39** in 72%, 59% and 77% yields.

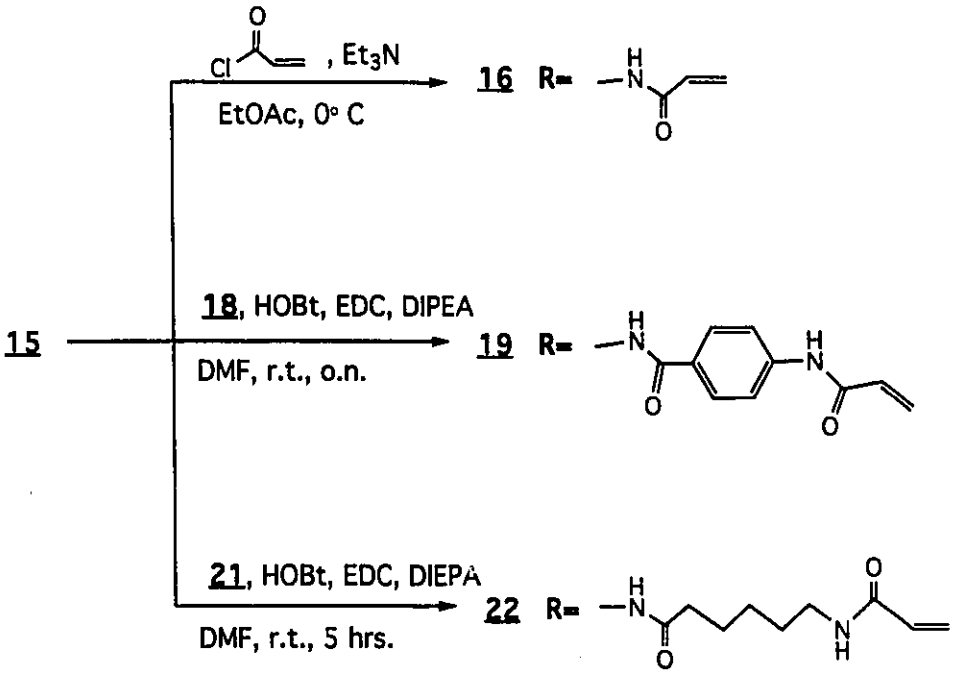
Molecular weights of the polymers were indirectly estimated by comparing the relative distance of the polymer and the peanut lectin (120kDa) in a double radial immunodiffusion plate. Since all polymers **37**-**39** traveled shorter distance than the lectin, the molecular weights of the polymers were thought to be greater than the weight of the lectin (120kDa).

During the polymerization, it was observed that the consumption rate of the carbohydrate monomer was not the same as that of acrylamide. For example, the monomer **32** disappeared in TLC before all the acrylamide was consumed during the copolymerization to give **38**. In another case, **31** was still observed when acrylamide was no longer seen in the reaction mixture. The possible difference in the reaction rates suggest the generation of a series of discrete block-polymers as opposed to the originally expected random incorporations of two monomers and acrylamide. The origin of such observation seems to be attributed to the fact that the solvation environment of the carbohydrate monomer was different than that of acrylamide since the carbohydrate monomer requires the larger space than the acrylamide. In addition the electronic effect seems also operative if the acryloyl function is adjacent to the electron withdrawing group as seen in the formation of Michael addition product of **28** under Zemplén condition.

Similar to the preparations of the lactose-containing monomers, reduction of the GM_3 azide **14** using 10% Pd/C with H_2



14 ; R = N_3 —Raney Ni, EtOAc, r.t., 15 min.→ **15** ; R = NH_2



Scheme 3.3. Syntheses of the GM_3-containing monomers with various spacer arms.

gas followed by N-acryloylation of the amine **15** afforded **16** in 90% yield. As shown in **Scheme 3.3**, carbodiimide chemistry was employed to prepare the N-p-acrylamidobenzoyl GM_3 precursor **19** (75% in yield) and N-acrylamidocaproic GM_3 precursor **22** (88% yield) by reacting **15** with the corresponding acids **18** and **21**, respectively in the presence of HOBt and EDC as coupling agents in DMF. None of these reactions contained detectable amount of the α-anomers which are possible to form during the reduction step.

As illustrated in **Figure 3.2**, the GM_3-containing monomers **16**, **19** and **22** were deacylated under Zemplén condition. The N-p-acrylamidobenzamido compound **35** gave 18% of Michael addition product while deacylation of the other monomers proceeded smoothly to provide **34** and **36**.

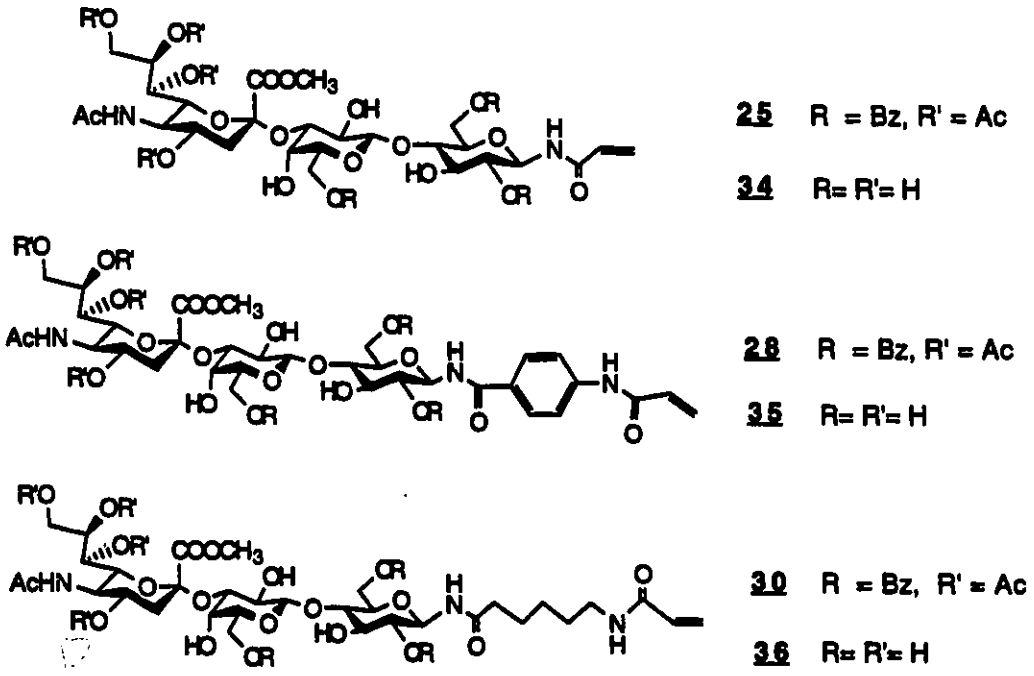
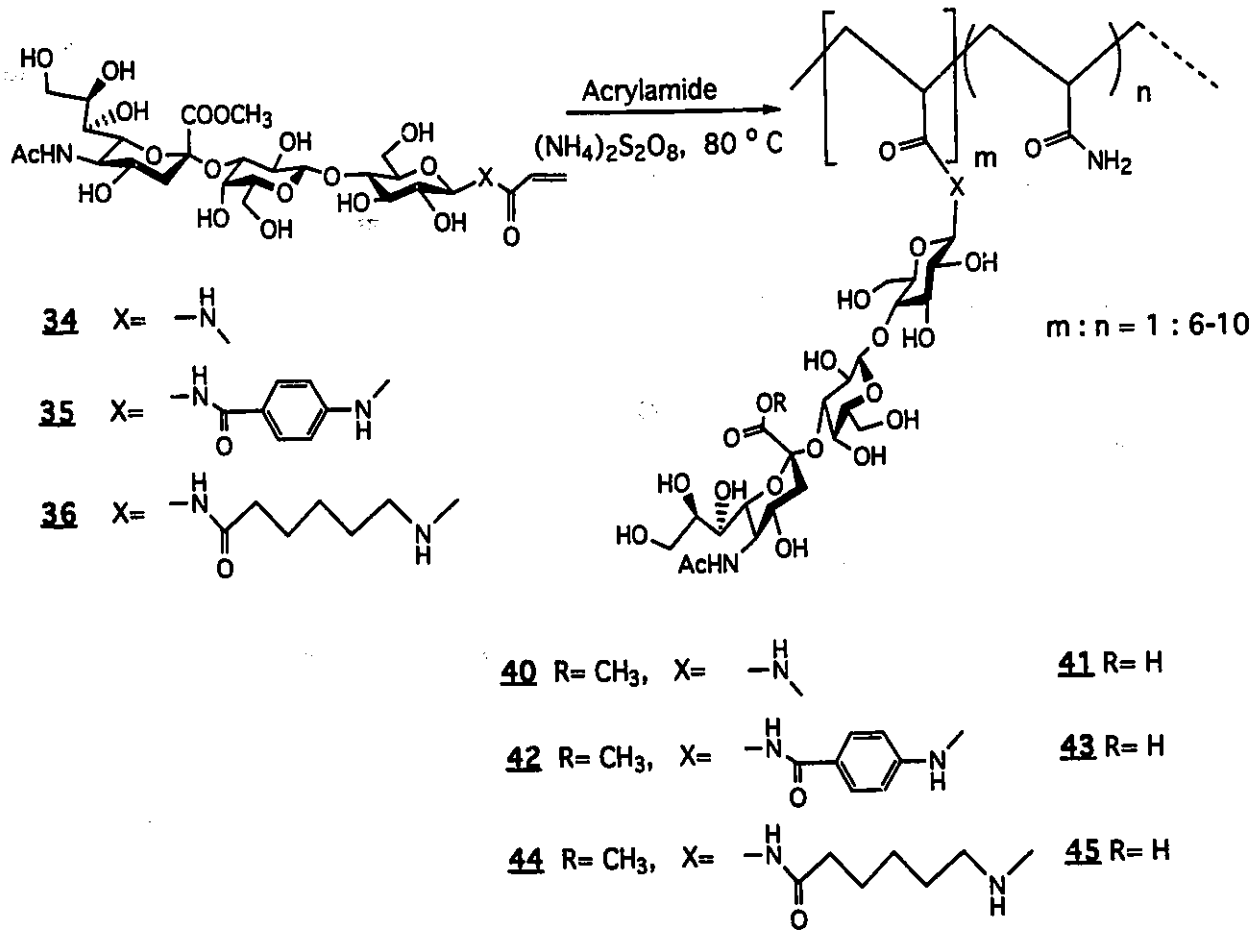


Figure 3.2. Deacylation of the GM_3-containing monomers.

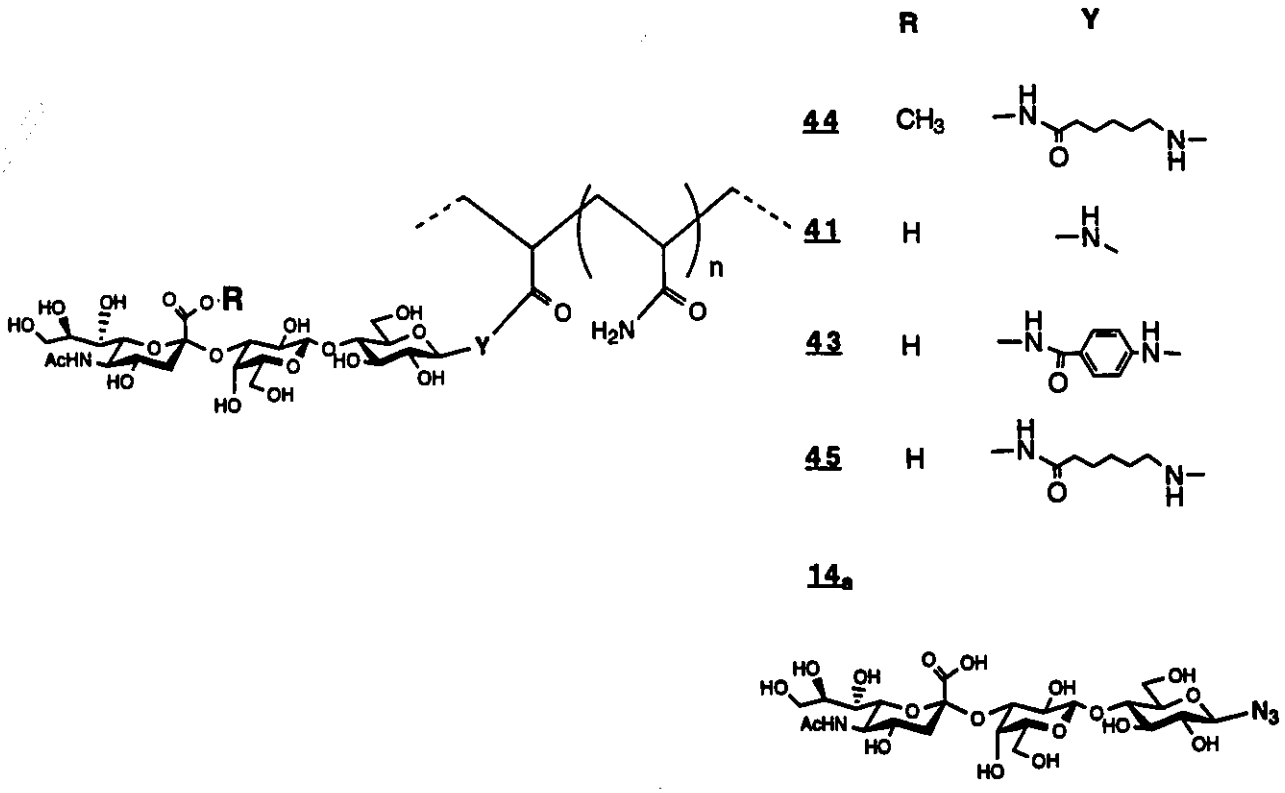
The GM_3-containing monomers **34**-**36** were then copolymerized with acrylamide in the presence of ammonium persulfate as radical initiator. Each polymer was then repeatedly dialysed against distilled water using cellulose tubing with the molecular weight cut-off 12kDa. The yields of the corresponding polymers **40**, **42** and **44** were 69%, 52% and 76%, respectively with the consistent carbohydrate to acrylamide incorporation ratio (1: 6-10, respectively) after lyophylization.

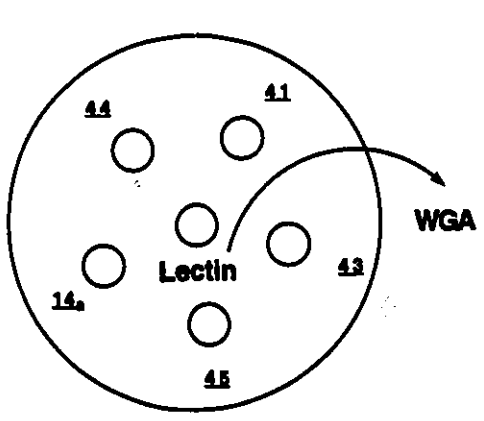


Scheme 3.4. Copolymerization of the GM_3-containing monomers and acrylamide and deesterifications.

Each polymer was then treated with ethanolic NaOH solution (0.05 M, 10% EtOH/H_2O) to saponify the sialic acid methyl ester and gave the acid polymers **41**, **43** and **45**, respectively in quantitative yields for all three polymers.

The GM_3-containing polymers **41**, **43** and **45** were qualitatively tested by using double immunodiffusion technique for their binding abilities with a plant lectin, wheat germ agglutinin (WGA). This carbohydrate-specific lectin is known to bind with Neu5NAc as well as GlcNAc. As shown in **Figure 3.3**, all three polymers formed bands with the lectin whereas the deacylated azide and the polymer with methyl ester **42** as a negative control showed no band formations. Since the interaction between the monomer **14_a** and the lectin depends on the individual affinity, the binding force should be very weak as previously pointed out. The polymers exerted strong binding interactions with WGA, suggesting that the avidity (multivalency effect) is in effect. The polymer with methyl ester showing the negative result implies the structural specificity of the GM_3 hapten required for binding with the lectin. It is interesting to point out, although it is qualitative, that there is no apparent spacer arm effect. As noted earlier the spatial requirement for binding between carbohydrate and lectin is shallow. The results of the three polymers with the different spacer arms seem to confirm this aspect.





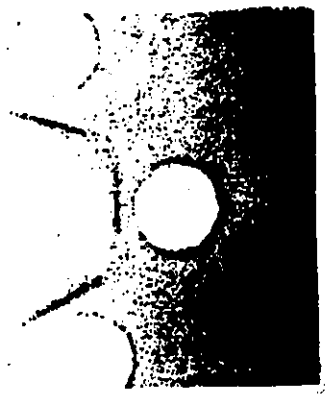


Figure 3.3. Double immunodiffusion of GM_3 polymers with WGA.

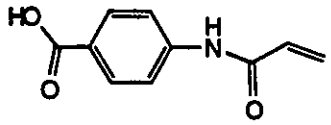
III.3. Experimental Methods.

III.3.1. *General Methods.*

General methods are as described in section II.3.

III.3.2. Preparations of N-acrylamidoglycoside monomers.

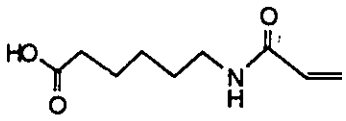
p-Acrylamido benzoic acid, (18).



18

To a basic solution (1M, Na_2CO_3, 20 mL) containing p-aminobenzoic acid (500 mg, 3.64 mmol) cooled to 0° C, was added slowly acryloyl chloride (330 µL, 4 mmol) diluted with dioxane (1 mL). The reaction mixture was stirred for 1 h at 0° C and acidified with conc. HCl. A white precipitate was formed. The precipitate was then filtered and washed repeatedly with distilled water. The product was crystalline and recrystallized from a mixture of EtOH and water(1:1). The recrystallized product was vacuum-dried under P_2O_5 and gave 601 mg, 86.0 % in yield. M.p.= 86° C(dec.); ^{1}H-NMR spectral data (δ, DMSO-d_6 ppm): 10.1(s, 1H, C(O)OH), 7.50 (dd, 4H, J= 8.6 Hz, H_o, H_m), 5.88-6.12(m, 3H, NH, $H_{c,a}$), 5.41(dd, 1H, $J_{b,a}$=9.6 Hz, $J_{b,c}$=1.8 Hz, H_b) ; ^{13}C-NMR spectral data: 167.0, 163.6(C(O)), 143.1,131.6, 130.5,127.7, 125.4, 118.7.

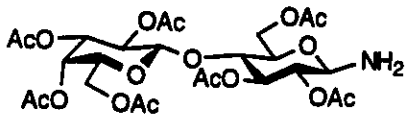
6-N-Acrylamidocaproic Acid, (21).



21

6-Aminocaproic acid (1.00 g, 7.6 mmol) was dissolved in 1N NaOH solution (10 mL). To this solution, cooled to 0 °C, was added an aqueous solution mixture (total volume, 4 mL) of acryloyl chloride (0.76 mL, 8.4 mmol) and 2,6-di-*tert*-butyl-4-methylphenol (TBMP, 10 mg, 0.045 mmol). The reaction mixture was stirred for 1 h at 0 °C and continued until it reached ambient temperature. The reaction was complete in 4 h. TBMP was removed from the reaction mixture by EtOAc extraction. The aqueous layer was then cooled to 0 °C before acidified with 6 N HCl. Upon acidification, a white precipitation was observed. The precipitate was then filtered and repeatedly washed with cold water. The product was then dried under vacuum over P_2O_5 (0.860 g, 61 %). M.p.= 83.5-85.5 ° C; ^{1}H-NMR spectral data ($CDCl_3$, δ ppm), 6.15 (m, 3H), 5.58 (dd, 1H), 3.29 (q, 2H), 2.32 (t, 2H), 1.53 (m, 6H): ^{13}C-NMR spectral data (δ ppm); 179.0 (C(O)), 166.6, 166.5 (acryl carbons), 39.9, 35.4, 29.6, 26.8, 24.8 (CH_2).

(2,3,4,6,-tetra-O-acetyl-β-D-galactopyranosyl)-(1-4)-O-2,3,6-tri-O-acetyl-β-D-glucopyranosyl amine, (24).

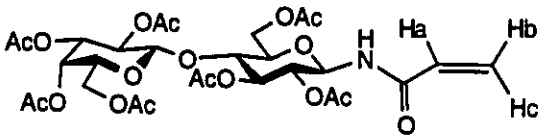


24

To a solution of **2** (4.00 g, 6.06 mmol) in EtOAc (4 mL) was added Raney Ni (*ca* 1.0 g), freshly washed with EtOH (2x5 mL) and followed by EtOAc (2x5 mL). Progress of the reaction was monitored

by TLC using EtOAc as eluent. The reaction was complete within 20 minutes. No detectable α anomer was observed in TLC. The reaction mixture was filtered. The filtrate was then concentrated under reduced pressure to give a white foam (3.31 g, 86 %). The product was then used for the next step without further purification. M.p. 81-84 °C; $[\alpha]_D$= +7.40° (1c $CHCl_3$); Anal. Calcd: C 49.29, H 5.57, N 2.21. Found C 49.21, H 5.77, N 1.98.

N-Acryloyl(2,3,4,6,-tetra-O-acetyl-β-D-galactopyranosyl)-(1-4)-O-(2,3,6-tri-O-acetyl-β-D-glucopyranosyl) amine, (25).

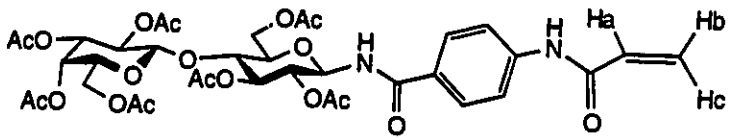


25

Freshly prepared **24** (4.00 g, 6.3 mmol) was dissolved in EtOAc (7.0 mL). To this was added a solution containing acryloylchloride (0.600 mL, 7.2 mmol) dissolved in 10 mL of EtOAc. The solution was cooled to 0 °C. To the reaction mixture, triethylamine (1.3 mL, 9.5 mmol) was added until the pH became *ca* 9. The reaction was complete within 1.5 hours when a white precipitate was observed. The excess acryloyl chloride was destroyed by adding water. The organic phase was separated and processed with routine washing and drying. The resultant organic-phase filtrate was then concentrated under reduced pressure to give a white foam. The desired β anomer was isolated and purified by column chromatography using a 8:2 mixture of EtOAc and hexane (4.12 g, 94.8 %). M.p. 97-97.9 °C ; $[\alpha]_D$= +10.4° (c=1.0 $CHCl_3$) ; ^{1}H-NMR spectral data ($CDCl_3$, δ, ppm): 6.36(d, 1H, $J_{NH,1}$= 9.3 Hz, NH), 6.24(dd, 1H, $J_{c,b}$= 1.1, $J_{c,a}$= 17.0 Hz, H_c), 6.00(dd, 1H, $J_{a,b}$= 10.4 Hz, H_a), 5.67(dd, 1H, $J_{b,c}$= 1.1Hz, H_b), 5.30(dd, 1H, $J_{4',5'}$= 0.9Hz, H-4'), 5.25(dd, 1H, $J_{1,2}$= 9.3Hz, H-1), 5.25(dd, 1H, $J_{3,4}$= 9.5Hz, H-3), 5.06(dd, 1H, $J_{2',3'}$=10.5Hz, H-3'), 4.90(dd, 1H, $J_{3',4'}$= 3.4 Hz, H-3'), 4.82(dd, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 4.42(d, 1H, $J_{1',2'}$= 7.9 Hz, H-1'), 4.38(dd, 1H, $J_{6a,b}$= 12.4 Hz, H-6a), 3.99-4.14(m, 4H, H-6b,

5, 6'a and 6'b), 3.84(ddd, 1H, H-5'), 3.74(dd, 1H, H-4), 2.11, 2.07, 2.02, 2.01, 2.00, 1.99, 1.92 (acetyl); ^{13}C NMR spectral data: 20.6, 2 x 20.7, 2 x 20.7, 20.8, 20.9 (acetyl $\underline{C}H_3$), 60.8(C-6), 62.0(C-6'), 66.6(C-4'), 69.0(C-2'), 70.7(C-2), 70.8(C-3'), 71.0(C-3), 72.3(C-5'), 74.5(C-5), 75.9(C-4), 78.2(C-1), 100.8(C-1'), 128.3(C-b,c), 129.9(C-a), 165.2, 168.8, 169.1, 169.9, 170.0, 170.2, 171.2 (C(O)).

<u>N</u>-(p-acrylamidobenzoyl)-(2,3,4,6,-tetra-<u>O</u>-acetyl-β-D-galactopyranosyl)-(1-4)-<u>O</u>-2,3,6-tri-<u>O</u>-acetyl-β-D-gluco pyranosyl amine, (<u>28</u>).

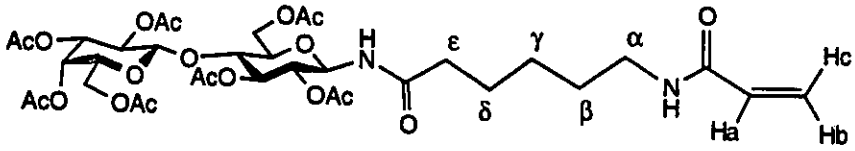


<u>28</u>

To a mixture of <u>24</u> (200 mg, 0.315 mmol) and <u>18</u> (150 mg, 0.788 mmol) in DMF (0.5 mL) was added HOBt (216 mg, 1.60 mmol) and diisopropylethylamine (DIPEA, 44.8 mg, 0.3465mmol). The solution was stirred for 5 minutes to dissolve all reagents. To this reaction mixture was added EDC (306 mg, 1.60 mmol). The solution was kept under N_2 at ambient temperature for 12 h to allow the reaction to complete. To the reaction mixture was added EtOAc and saturated $NaHCO_3$ solution. The separated organic layer was then washed twice with water (5 mL), followed by saturated NaCl solution. The EtOAc phase was then dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The resultant white solid was purified by column chromatography using a 1:1 mixture of acetone and hexane (198 mg, 78.7 %). ^1H-NMR spectral data ($CDCl_3$, δ ppm), 7.68(dd, 4H, $J_{a,b}$ 8.7 Hz, aromatic), 6.89(d, 1H, anomeric NH), 6.45(dd,1H, $J_{c,b}$ 0.9, $J_{c,a}$ 16.9 Hz, Hc), 6.23(dd, 1H, $J_{b,a}$ 10.3, $J_{a,c}$ 16.8 Hz, Ha), 5.80(dd, 1H, $J_{b,c}$ 0.9, $J_{b,a}$ 10.3 Hz, Hb), 5.33-5.38(m, 3H, H-1,3,4'), 5.10(dd, 1H, $J_{2',3'}$ 10.4 Hz, H-2'), 4.94(dd, 1H, $J_{3',4'}$< 1Hz, H-3'), 4.94(dd, 1H, $J_{2,3}$ 9.7Hz, H-2), 4.45(d, 1H, $J_{1',2'}$ 7.9

Hz, H-1'), 4.43(m, 1H, $J_{5,6}$ 13.1 Hz, H-6a), 4.11-4.17(m, 2H, H-6'b, H-6'), 4.06(dd, 1H, $J_{5',6'a}$ 7.3 Hz, H6'b), 3.86(dd, 1H, $J_{5,6}$ 13.9 Hz, H-5), 3.80(m, 2H, H-4, 5), 2.14, 2.10, 2.06, 2.06, 2.04, 2.01, 1.95(-C(O)$\underline{C}H_3$); ^{13}C NMR: 20.5, 20.6, 20.6, 20.7, 20.8, 20.9, 22.7(-C(O)$\underline{C}H_3$), 60.9(C-6'), 62.0(C-6), 66.7(C-4'), 69.0(C-2'), 70.8, 71.0, 71.2(C-2,3',5), 72.2(C-3), 74.5, 76.0(C-4, 5), 78.9(C-1), 100.9(C-1'), 119.4, 128.5, 128.8(aromatic), 130.7(C-b), 141.4(C-a), 163.4, 166.2, 169.3, 170.0, 170.1, 170.3, 170.3, 171.8(-$\underline{C}$(O)-).

$\underline{N}$-(6-N-acrylamido)-(2,3,4,6,-tetra-$\underline{O}$-acetyl-β-D-galacto pyranosyl)-(1-4)-$\underline{O}$-2,3,6-tri-$\underline{O}$-acetyl-β-D-glucopyranosyl hex anamide, ($\underline{30}$).

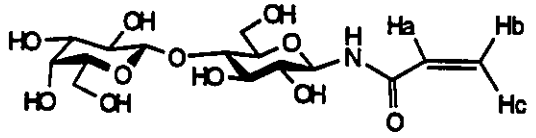


$\underline{30}$

To a mixture of $\underline{24}$ (200 mg, 0.315 mmol) and $\underline{21}$ (146 mg, 0.788 mmol) in DMF (0.5 mL) was added HOBt (216 mg, 1.60 mmol) and diisoprpylethylamine (DIPEA, 44.8 mg, 0.3465mmol). The solution was stirred for 5 min to dissolve all reagents. To this reaction mixture was added EDC (306 mg, 1.60 mmol). The reaction was kept under N_2 at ambient temperature for 7 h to be complete. Work-up and isolation of the desired product were performed in the similar manner as described in **25**. Yield; 210 mg, 83.0 %. $[\alpha]_D$ +5.7° (c 1.03 H_2O); ^1H NMR ($CDCl_3$, δ ppm), 6.24(dd,1H, $J_{c,b}$ 1.3, $J_{c,a}$ 17.0 Hz, Hc), 6.16(d, 1H, $J_{NH,1}$ 9.3 Hz, anomeric N$\underline{H}$), 6.07(dd, 1H, $J_{b,a}$ 10.3, $J_{a,c}$ 16.8 Hz, Ha), 5.83(m,1H, acryloyl N$\underline{H}$), 5.60(dd, 1H, $J_{b,c}$ 1.3, $J_{b,a}$ 10.2 Hz, Hb), 5.32(dd, 1H, $J_{4',5'}$< 1.0 Hz, H-4'), 5.26(dd, 1H, $J_{3,4}$ 8.9 Hz, H-3), 5.17(dd, 1H, $J_{1,2}$ 9.4 Hz, H-1), 5.08(dd, 1H, $J_{2',3'}$ 10.5 Hz, H-2'), 4.93(dd, 1H, $J_{3',4'}$ 3.4 Hz, H-3'), 4.79(dd, 1H, $J_{2,3}$ 9.6Hz, H-2), 4.43(d, 1H, $J_{1',2'}$ 7.9 Hz, H-1'), 4.41(dd, 1H, $J_{5,6a}$ 13.0 Hz, H-6a), 4.10-4.14(m,

2H, H-6'b, H-6'a), 4.04(dd, 1H, $J_{5',6'b}$ 7.3 Hz, H6'b), 3.83(dd, 1H, $J_{5',6'b}$ 7.3 Hz, H-5'), 3.72(dd, 1H, $J_{4,5}$ 9.9 Hz, H-4), 3.69(m, 1H, H-5), 3.30(m, 2H, H-α), 2.14(m, 2H, H-ε), 1.59(m, 2H, H-δ), 1.52(m, 2H, H-β), 1.31(m, 2H, H-γ), 2.13, 2.09, 2.04, 2.03, 2.02, 2.01,1.94(-C(O)C$\underline{H}_3$) ; ^{13}C NMR: 20.5, 20.6(3C), 20.7(2C), 20.9(-C(O)$\underline{C}H_3$), 24.4(C-δ), 26.1(C-γ), 29.0(C-β), 36.2(C-ε), 39.2(C-α), 60.9(C-6'), 62.0(C-6), 66.7(C-4'), 69.0(C-2'), 70.8, 71.0, 71.2(C-2,3',5), 72.2(C-3), 74.5, 76.0(C-4, 5), 78.9(C-1), 100.9(C-1'), 119.4, 128.5, 128.8(aromatic), 130.7(C-b), 141.4(C-a), 163.4, 166.2, 169.3, 170.0, 170.1, 170.3, 170.3, 171.8(-$\underline{C}$(O)-); FAB-MS (pos): Calcd for $C_{35}H_{50}N_2O_{12}$, 802.3. Found, 803.3 (M+1, 16.%).

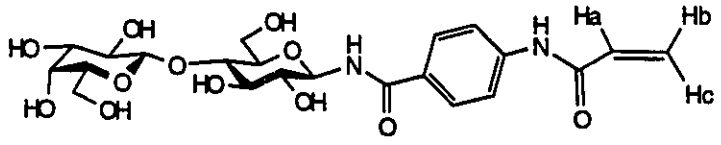
$\underline{N}$-acryloyl-(β-D-galactopyranosyl)-(1-4)-$\underline{O}$-β-D-glucopyranosyl amine, ($\underline{31}$).



To a methanolic solution (10 mL) containing peracetylated N-acryloylated lactose **$\underline{25}$** (1.42 g, 2.06 mmol) was added a NaOMe solution in MeOH until the pH of the reaction mixture became *ca.* 9. The de-$\underline{O}$-acetylated product was precipitated after 15 minutes. The crystalline compound was filtered. The resultant solid was then dried under reduced pressure (0.75 g, 92%). M.p. 241-246° C; $[\alpha]_D$ = + 15.1° (c=1.0 DMSO); ^{1}H-NMR spectral data (D_2O, δ ppm): 6.32(dd, 1H, $J_{a,b}$=10.2, $J_{a,c}$= 17.2 Hz, H_a), 6.24(dd, 1H, $J_{b,c}$ = 1.4 Hz, H_c), 5.82(dd, $J_{b,a}$ = 10.2 Hz, H_b), 5.06(d, 1H, $J_{1,2}$ = 9.0 Hz, H-1), 4.54(d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1'), 4.01-4.03(m, 2H, H-4',3'), 3.70-3.94(m, 8H, H-3, 4, 5, 5', 6, 6'), 3.64(dd, 1H, $J_{2,3}$= 10.0 Hz, H-2), 3.52(dd, 1H, $J_{2',3'}$ = 8.7 Hz, H-2'); ^{13}C NMR: 169.9(C=O), 130.0(C-b), 129.9(C-a), 103.5(C-1'), 79.9(C-1), 78.5(C-4), 77.2(C-5), 76.1(C-5'), 75.8(C-3), 73.2(C-3'),

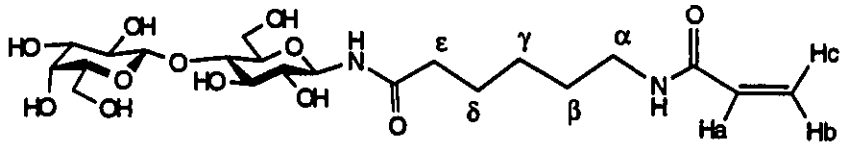
72.2(C-2), 71.1(C-2'), 69.3(C-4'), 61.8(C-6'), 60.6(C-6); FAB-MS Calcd for $C_{15}H_{25}O_{11}N$: 395.2. Found 396.3 (M+1).

N-p-acrylamidobenzoyl-(β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl amine, (32).



A small quantity of **28** (50 mg, 0.062 mmol) was dissolved in MeOH (0.3 mL). To this, a NaOMe/ MeOH (1 M) solution was added to make pH of the solution *ca* 9. The reaction was complete within 2 h. The solution was then neutralized by adding H^+ resin and filtered. The filtrate was evaporated to give white power (25.8 mg, 81 %). The crude product was purified by column chromatography using a 8: 2 mixture of MeCN: water. ^{1}H-NMR (D_2O, δ ppm): 7.86(d, 2H, $J_{o,m}$= 8.7 Hz, H_o), 7.65(d, 2H, J_m = 8.5 Hz, H_m), 6.43(dd, 1H, $J_{a,c}$= 17.0 Hz, H_a), 6.38(dd, 1H, $J_{b,c}$ = 1.9 Hz, H_c), 5.90(dd, $J_{b,a}$ = 9.5 Hz, H_b), 5.21(d, 1H, $J_{1,2}$ = 8.8 Hz, H-1), 4.47(d, 1H, $J_{1',2'}$ = 7.6 Hz, H-1'), 3.93-3.95(m, 2H, H-4',3'), 3.55-3.91(m, 8H, H-2, 2', 3, 4, 5, 5', 6, 6').

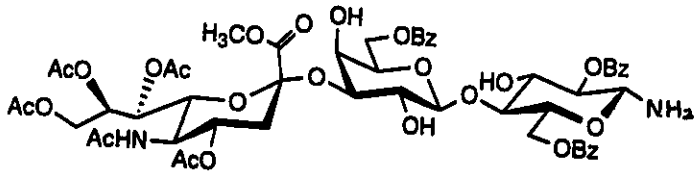
N-6-N-acrylamidohexano-(β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl amine, (33).



To a solution of **30** (55 mg, 0.0687 mmol) dissolved in MeOH (0.3 mL) was added a small quantity of NaOMe/MeOH solution (1M) to make pH *ca* 9. The reaction was monitored by TLC using a 8: 2

mixture of MeCN and water, respectively. The reaction was complete within 2 hours. The basic reaction medium was neutralized with Amberlite IR-120 cation-exchange resin (H^+) and then filtered. The filtrate was evaporated under reduced pressure to yield white solid (33 mg, 94.5 %). ^1H NMR (D_2O, δ ppm): 6.32(dd, 1H, $J_{a,b}$=10.2, $J_{a,c}$= 17.2 Hz, H_a), 6.23(dd, 1H, $J_{b,c}$ = 1.4 Hz, H_c), 5.82(dd, $J_{b,a}$ = 10.2 Hz, H_b), 5.06(d, 1H, $J_{1,2}$ = 9.0 Hz, H-1), 4.54(d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1'), 4.01-4.03(m, 2H, H-4',3'), 3.70-3.94(m, 8H, H-3, 4, 5, 5', 6, 6'), 3.64(dd, 1H, $J_{2,3}$= 10.0 Hz, H-2), 3.52(dd, 1H, $J_{2',3'}$ = 8.7 Hz, H-2'), 3.25(m, 2H, Hα), 2.42(m, 2H, Hε), 1.72-2.07(m, 4H, H-β,δ), 1.60(m, 2H, Hγ); ^{13}C-NMR spectral data: 179.9 (C(O)), 169.9(C(O)). 131.5 (CH=), 128.5 (=CH_2), 104.7 (C-1'), 80.6 (C-1). $[\alpha]_D$ +3.30° (c 1.10 water); FAB-MS (pos) Calcd for $C_{21}H_{36}N_2O_{12}$, 508.2. Found, 509.2 (M+1, 0.1 %).

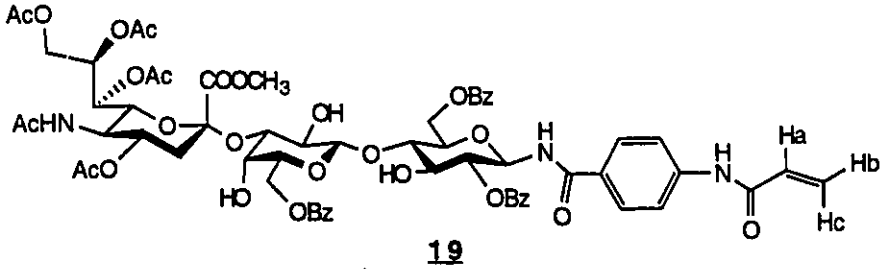
Amino N-(methyl 5-acetamido-4,7,8,9,-tetra-O-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosidonate)-(2-3)-O-(6-O-benzoyl-β-D-galactopyranosyl)-(1-4)-O-2,6-di-O-benzoyl-β-D-glucopyranose, (15).



To a methanolic solution (0.5 mL) was added **14** (200 mg, 0.17 mmol) and 10 % Pd/C (40 mg, 20 wt % of **14**). Hydrogen gas was bubbled through the solution while stirring. The reaction was complete within 15 minutes. A ninhydrin test was performed to ensure reduction of the azide. A small quantity of sample in question as well as equal volumes of each monitoring reagent; monitor 1: 76 % w/w phenol/EtOH, monitor 2: 0.0002 M KCN/pyr. and monitor 3: 0.28 M ninhydrin/EtOH, were placed in a small test tube and gently heated. Positive reactions turned violet. The palladium catalyst was filtered and the filtrate was evaporated under reduced pressure (195

mg, quantitative). The resultant compound was directly used for the next step without further purification.

N-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosidonate)-(2-3)-O-(6-O-benzoyl-β-D-galactopyranosyl)-(1-4)-O-2,6-di-O-benzoyl-β-D-glucopyranosyl p-acrylamidobenzamide, (19).

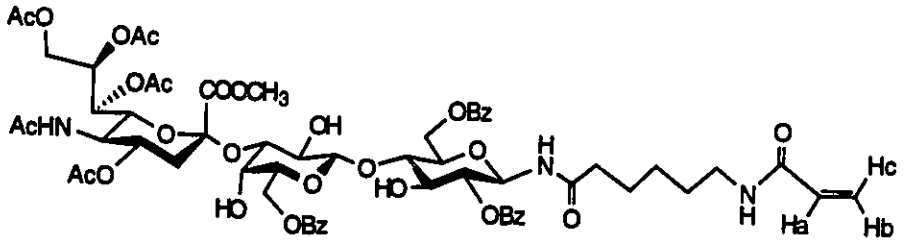


19

Freshly prepared 15 (195 mg, 0.17 mmol) was put in a round bottom flask with HOBt (114 mg, 0.85 mmol), 18 (80 mg, 0.425 mmol) and dissolved in DMF (0.4 mL). The reaction mixture was stirred under N_2. To this, was added 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDC, 163 mg, 0.85 mmol) dissolved in DMF (0.25 mL) and diisopropylethylamine (107 mg, 0.85 mmol). The reaction mixture was kept at ambient temperature overnight under N_2. It was washed with two phase solvent system, EtOAc and H_2O. The organic phase was separated and subsequently washed with 1M HCl (2 mL), saturated $NaHCO_3$ (2 x 2 mL), H_2O (2 x 2 mL) and sat. NaCl solution (2 x 2 mL). The separated organic phase was then dried over Na_2SO_4 and filtered. The filtrate was evaporated under reduced pressure and purified by column chromatography using a 3:7 mixture of acetone and benzene, respectively to give a white foam (182 mg, 75%). ^{1}H-NMR spectral data ($CDCl_3$, δ ppm): 7.03-8.06(m, 15 H, aromatic), 6.43(dd, 1H, $J_{c,b}$ 1.1, $J_{c,a}$ 16.9 Hz, Hc), 6.21(dd, 1H, $J_{b,a}$ 10.3, $J_{a,c}$ 16.8 Hz, Ha), 5.76(dd, 1H, $J_{b,a}$ 10.3 Hz, Hb), 5.53(dd, 1H, $J_{1,2}$ 9.2 Hz, H-1), 5.26-5.38 (m, 2H, H-7", 8"), 5.19(dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 4.97(ddd, 1H, H-4"), 4.83(m, 1H, H-6a), 4.75(m, 1H, H-6'a), 4.57(d, 1H, $J_{1',2'}$ 7.7 Hz, H-1'), 4.57(m, 1H, H-6b), 4.38(m, 1H, H-6'b), 4.27(dd, 1H, $J_{9",8"}$ 2.0Hz, 9a"), 4.18(dd, 1H, J3,4 8.7 Hz, H-3), 4.10-4.14(m, 2H, H-3',4'), 4.04(m, 1H, H-5), 3.85-3.97(m, 3H,

H-9"b, 5", 5'), 3.78 (s, 3H, methyl ester), 3.36-3.81 (m, 3H, H-4, 2', 6"), 2.67(dd, 1H, $J_{3"e,a}$ 13.0, $J_{3"e,4}$ 2.6 Hz, H-3"e), 1.99(dd, 1H, $J_{3"a,e}$ 13.0, $J_{3"a,4"}$ 12.0 Hz, H-3"a), 2.12, 2.02, 2.01, 1.91, 1.87 (s, 3H each, acetyl); ^{13}C-NMR spectral data : 20.6, 20.7, 20.8, 21.1, 23.2(-C(O)CH3), 37.7(C-3"), 49.8(C-5"), 53.3(methyl ester), 62.3(C-9"), 63.7(C-6, 6'), 67.0(C-7"), 68.1(C-2'), 68.2(C-4"), 68.9(C-8"), 69.0(C-6"), 72.8(C-5'), 72.9(C-4'), 73.2(C-2), 73.3(C-3), 74.4(C-5), 76.4(C-3'), 82.2(C-1), 97.6(C-4), 104.4(C-1'), 129.1(C-b), 132.9(C-a), 128.3, 128.4, 129.3, 129.6, 129.7, 129.8, 129.9, 130.0, 133.0, 133.2 (aromatic), 141.2, 165.3, 166.2, 166.6, 168.1, 170.2, 170.2, 170.3, 170.5, 170.8 (C=O).

N-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosidonate)-(2-3)-O-(6-O-benzoyl-β-D-galactopyranosyl)-(1-4)-O-2,6-di-O-benzoyl-β-D-glucopyranosyl 6-N-acrylamidohexanoicamide, (22).

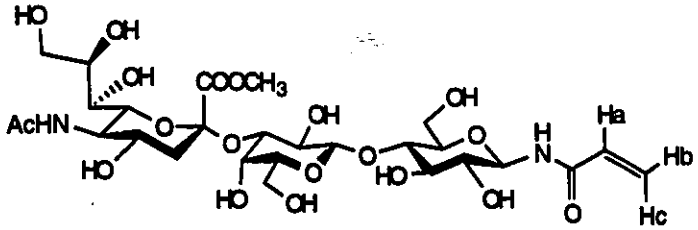


22

To a DMF solution (0.5 mL) containing freshly prepared **15** (200 mg, 0.17 mmol), **21** (78 mg, 0.425 mmol), diisopropylethylamine (DIPEA, 24.2 mg, 0.187 mmol) and HOBt (115 mg, 0.85 mmol). was added EDC (163 mg, 0.85 mmol). The reaction mixture was stirred under N_2 for 5 h. Work-up was proceeded in a similar manner as described in **16**. The isolated **22** was 238 mg, 88% in yield; ^{1}H-NMR spectral data ($CDCl_3$, δ ppm): 7.18-8.04(m, 15 H, aromatic), 6.29(d, 1H, $J_{NH,1}$ 9.4 Hz, anomeric NH), 6.24(dd, 1H, $J_{c,a}$ 17.0, $J_{c,b}$ 1.5 Hz, Hc), 6.02(dd, 1H, $J_{b,a}$ 10.3, $J_{a,c}$ 17.0 Hz, Ha), 5.61(m, 1H, acryloyl NH), 5.58(d, 1H, $J_{b,a}$ 10.3 Hz, Hb), 5.39(dd, 1H, $J_{1,2}$ 9.5 Hz, H-1), 5.25-5.30 (m, 2H, H-7", 8"), 5.08(dd, 1H, $J_{2,3}$ 9.6 Hz, H-2), 4.98(ddd, 1H, H-4"), 4.83(dd, 1H, $J_{6a,b}$ 12.1 Hz, H-6a), 4.74(dd, 1H, $J_{6'a,b}$ 12.0 Hz, H-6'a), 4.56(d, 1H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.52(dd, 1H, $J_{6b,5}$ 5.0, $J_{6b,a}$ 12.0 Hz, H-

6b), 4.37(dd, 1H, $J_{6'b,5'}$ 7.8, $J_{6'b,a}$ 12.0 Hz, H-6'b), 4.26(dd, 1H, $J_{9"a,b}$ 2.1, $J_{9"a,8"}$ 12.7 Hz, 9a"), 4.09-4.13(m, 4H, H-3, 3', 4', 5"), 3.95(m, 1H, H-5), 3.85-3.92(m, 2H, H-9"b, 5), 3.77 (s, 3H, methyl ester), 3.70-3.78 (m, 2H, H-4, 2'), 3.08(q, 2H, H-α), 2.67(dd, 1H, $J_{3"e,a}$ 13.1, $J_{3"e,4}$ 4.7 Hz, H-3"e), 2.05(m, 1H, H-3"a), 2.14(m, 2H, H-ε), 1.44(m, 2H, H-δ), 1.30(m, 2H, H-β), 1.10(m, 2H, H-γ), 2.12, 2.03, 2.01, 1.92, 1.88 (s, 3H each, acetyl); ^{13}C NMR : 20.6, 20.7, 20.8, 21.1, 24.5(NHAc), 26.0(C-γ), 28.8(C-β), 29.7(C-δ), 36.3(C-ε), 37.7(C-3"), 39.0(C-α), 49.8(C-5"), 53.3(methyl ester), 62.3(C-9"), 63.3(C-6), 63.6(C-6'), 67.0(C-7"), 68.1(C-2'), 68.2(C-4"), 68.8(C-8"), 69.0(C-4), 72.8(C-2), 72.9(C-5), 73.0(C-4'), 73.4(C-3), 74.5(C-6"), 76.4(C-3'), 77.9(C-1), 82.2(C-4), 97.5(C-2"), 104.4(C-1'), 126.1(C-b), 130.9(C-a), 128.2, 128.4, 128.5, 129.2, 129.3, 129.7, 129.8, 129.9, 130.0, (aromatic), 133.0, 133.1, 133.6 , 166.2, 166.5, 168.1, 170.2, 170.3, 170.5, 170.8 173.2 (C=O).

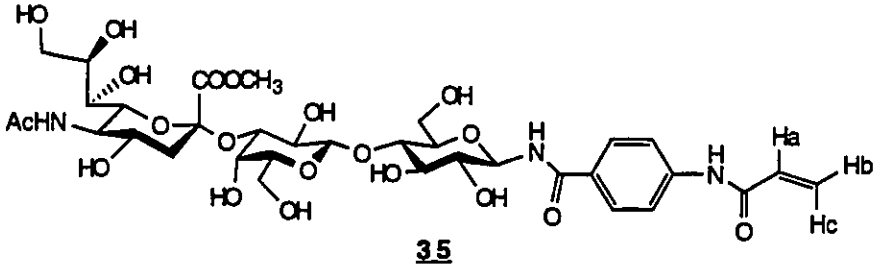
N-(methyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosidonate)-(2-3)-O-(β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl acrylamide, (34).



34

To a solution of 16 (30 mg, 0.0254 mmol) in MeOH (0.5 mL) was added 1 M NaOMe solution in MeOH until the pH of solution mixture became *ca* 8.5. The reaction was stirred overnight to be complete. The basicity of the solution was adjusted back to neutral by stirring the reaction mixture with H^+ resin for 15 minutes. The resin was then filtered. The filtrate was then evaporated under reduced pressure to result white powder 29 mg, 95 % in yield.

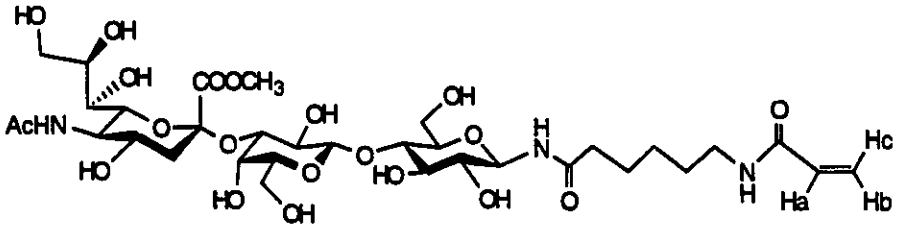
N-(methyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosidonate)-(2-3)-O-(β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl p-acrylamidobenzoylamide, (35).



35

To a solution of **19** (30 mg, 0.0230 mmol) in MeOH (0.5 mL) was added 1 M NaOMe solution in MeOH until the pH of solution mixture became *ca* 9. The reaction was stirred for 6 h. The pH of the solution was adjusted back to neutral by stirring the reaction mixture with H^+ resin for 30 min. The resin was then filtered and the filtrate was evaporated under reduced pressure to result white powder (27 mg, 80 %). ^{1}H-NMR spectral data (D_2O, δ ppm): 7.75(dd, 4H, aromatic), 6.42(m, 2H, H_a, H_c), 5.82(dd, $J_{b,a}$ = 10.2 Hz, H_b), 5.22(d, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 4.63(d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1'), 3.52-4.03(m, ring Hs), 3.77(s, 3H, -OCH_3), 2.83(dd, 1H, $J_{3"e,a}$= 12.5, $J_{3"e,4"}$= 4.7 Hz, H-3"e), 2.03(s, 3H, NHAc), 1.80(dd, 1H, $J_{3"a,4}$= 2.0 Hz, H-3"a).

N-(methyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosidonate)-(2-3)-O-(β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl 6-N-acryloylamidohexanamide, (36).



36

To a solution of **22** (30 mg, 0.0266 mmol) in MeOH (0.5 mL) was added 1 M NaOMe solution in MeOH until the pH of solution mixture became *ca* 9. The reaction was stirred for 4 hours to be complete. The pH was adjusted to neutral by stirring the reaction mixture with H^+ resin for 30 minutes. The resin was then filtered and the filtrate was evaporated under reduced pressure to result white solid (20 mg, 92 %). ^{1}H-NMR spectral data (D_2O, δ ppm), 6.32(dd, 1H, $J_{a,b}$=10.2, $J_{a,c}$= 17.2 Hz, H_a), 6.23(dd, 1H, $J_{b,c}$ = 1.4 Hz, H_c), 5.82(dd, $J_{b,a}$ = 10.2 Hz, H_b), 5.05(d, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 4.61(d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1'), 4.01-4.03(m, 2H, H-4',3'), 3.70-3.94(m, 15H, H-3, 4, 4", 5, 5', 5", 6, 6', 6", 7", 8", 9"), 3.64(dd, 1H, $J_{2,3}$= 10.0 Hz, H-2), 3.52(dd, 1H, $J_{2',3'}$ = 8.7 Hz, H-2'), 3.34(dd, 2H, Hα), 2.83(dd, 1H, $J_{3"e,a}$= 12.5, $J_{3"e,4"}$= 4.7 Hz, H-3"e), 2.42(m, 2H, Hε), 2.10(s, 3H, NHAc), 1.87(dd, 1H, $J_{3"a,4}$= 2.0 Hz, H-3"a), 1.71(m, 2H, Hδ), 1.63(m, 2H, Hβ), 1.43(m, 2H, Hγ); ^{13}C-NMR spectral data : 129.6(C_a), 126.4($C_{b,c}$), 102.1(C-1'), 78.6(C-1), 77.2(C-2"), 75.9(C-4), 75.0(C-3'), 74.7(C-6"), 74.6(C-3), 72.3(C-4'), 71.3(C-5), 71.0(C-2), 70.2(C-5'), 68.9(C-8"), 67.9(C-4"), 67.6(C-2'), 66.9(C-7"), 62.1(C-6'), 60.6(C-6), 59.4(C-9"), 51.2(C-5"), 39.2(Cα), 38.7(C-3"), 35.1(Cε), 27.3(Cδ), 25.0(Cβ), 24.1(Cγ), 21.5(C-NHAc).

Copoly(acrylamide/ N-acrylamidoglycosides).

Polymerization was initiated with $(NH_4)_2S_2O_8$ and carried out with acrylamide (10 equiv.) at 95° C for 10 minutes. After completion the reaction mixture was diluted with water, dialyzed (Dialysis Tubing, Benzoylated SIGMA®, D-2272) and lyophilized. Double-distilled water was used as solvent and deoxygenated before use. Deoxygenation was accomplished by repeating freeze-thaw cycles.

Copoly(acrylamide/N-(β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl acrylamide), (37).

To a small quantity of aqueous solution (200 μL) containing **31** (20 mg, 50 μmol) was added acrylamide (17.5 mg, 246 μmol) and

$(NH_4)_2S_2O_8$ (0.24 mg, 1.05 μmol, injection of 10 μL of a stock solution 4.8 mg/ 200 μL). The reaction mixture was then heated to 95 °C for 10 min. Progress of the reaction was monitored qualitatively by TLC using a 10:10: 0.1 mixture of $CHCl_3$, MeOH and H_2O, respectively. The polymer then was purified by dilution, dialysis followed by lyophilization to give 27 mg, 72 % in yield. ^1H NMR (D_2O, δ ppm): 5.06(m, 1H, H-1), 4.54(d, 1H, $J_{1',2'}$ = 7.3 Hz, H-1'), 4.01-4.03(m, 2H, H-4', 6_a), 3.70-3.94(m, 8H, H-3, 3', 4, 5, 5', 6_b, 6'), 3.64(dd, 1H, $J_{2,3}$= 10.0 Hz, H-2), 3.52(dd, 1H, $J_{2',3'}$ = 8.7 Hz, H-2'), 2.34(m, 1H, carbohydrate methine), 2.23(m, 8.8 H, acrylamide methine), 1.75(m, 2H, carbohydrate methylene), 1.69(m, 17.6H, acrylamide methylene).
Lactose incorporation ratio = 1: 8.8; estimated molecular weight: >120 KDa.

Copoly (acrylamide/N-(β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl p-acrylamidobenzamide), (38).

To a solution containing **32** (3.6 mg, 0.007 mmol) acrylamide (5.0 mg, 0.07 mmol) was added $(NH_4)_2S_2O_8$ (0.12 mg, 0.53 μmol, injection of 5μL of a stock solution 4.8 mg/ 200 μL). The solution was heated to 80° C for 15 minutes. The purification of the polymer product was followed as described above. Yield: 5 mg, 59 %; ^1H NMR (D_2O, δ ppm): 7.81(m, 4H, aromatic), 7.45(m,1H, NH), 5.29(m, 1H, H-1), 4.57(m, 1H, H-1'), 4.01-4.03(m, 2H, H-4', 6_a), 3.70-3.94(m, 8H, H-3, 3', 4, 5, 5', 6_b, 6'), 3.64(dd, 1H, $J_{2,3}$= 10.0 Hz, H-2), 3.52(dd, 1H, $J_{2',3'}$ = 8.7 Hz, H-2'), 2.34 (m, 1H, carbohydrate methine), 2.23(m, 10.4 H, acrylamide methine), 1.75(m, 2H, carbohydrate methylene), 1.69(m, 17.6H, acrylamide methylene). Lactose incorporation ratio = 1: 10.4; estimated molecular weight: >120 KDa.

Copoly(acrylamide/N-(β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl 6-N-acrylamidohexanoamide), (39).

To an aqueous solution (250 μL) containing **33** (5.0 mg, 0.0098 mmol) acrylamide (7.0 mg, 0.098 mmol) was injected $(NH_4)_2S_2O_8$ (0.12 mg, 0.53 μmol, injection of 5μL of a stock solution 4.8 mg/ 200 μL). DMSO was added (50 μL) to aid solubilization of **33** in aqueous medium. The solution was heated to 80° C for 15 minutes. The purification of the polymer product was followed as described in **1**. Yield : 9 mg, 77 % ; ^{1}H-NMR spectral data (D_2O, δ ppm): 5.06(d, 1H, $J_{1,2}$ = 9.0 Hz, H-1), 4.54(d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1'), 4.01-4.03(m, 2H, H-4',6_a), 3.70-3.94(m, 8H, H-3, 3', 4, 5, 5', 6_b, 6'), 3.64(dd, 1H, $J_{2,3}$= 10.0 Hz, H-2), 3.52(dd, 1H, $J_{2',3'}$ = 8.7 Hz, H-2'), 3.25(m, 2H, Hα), 2.42(m, 2H, Hε), 2.14-2.42(m, 12Hs, methine Hs), 1.72-2.07(m, 25H polymer backbone methylene, also 4H, H-β,δ), 1.60(m, 2H, Hγ); ^{13}C-NMR spectral data: 179.0(C=O), 102.4(C-1'), 78.6(C-1), 75.9(C-4), 74.9(C-5, 5'), 74.7(C-3), 72.1(C-3'), 71.0(C-2), 70.5(C-2'), 68.1(C-4'), 60.6(C-6'), 59.5(C-6), 42.2(methine Cs), 39.8(Cα), 35.1(Cε), 34.4(methylene Cs), 27.0(Cβ), 24.5(Cδ), 24.0(Cγ); lactose incorporation ratio = 1: 11; estimated molecular weight: >120 KDa.

Copoly (acrylamide/N-(methyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto* -2-nonulopyranosidonate)-(2-3)-O-(β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl acrylamide, (40).

Compound **34** (5 mg, 0.0061 mmol) was dissolved in H_2O (250 μL) solution in which also contained acrylamide (4.33 mg, 0.061 mmol) and a catalytic amount of $(NH_4)_2S_2O_8$ (0.073 mg, 0.32 μmol, injection of 3μL of a stock solution 4.8 mg/ 200 μL). The reaction mixture was then heated up to 95° C for 10 minutes. The polymer product was purfied by the similar method as described above. The obtained product was 6 mg, 69% in yield. ^{1}H-NMR spectral data (D_2O, δ ppm), 5.10(m, 1H, H-1), 4.57(m, 1H, H-1'), 3.50-4.25(m, 25H, carbohydrate Hs), 2.78(m, 1H, H-3"e), 2.25-2.41(m, 6H, polymer backbone methine Hs), 2.10(s, 3H, NHAc), 1.51-1.90(m, 12H, polymer backbone methylene Hs).

Copoly (acrylamide/N-(methyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto* -2-nonulopyranosidonate)-(2-3)-O-(β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl p-acryla midobenzamide, (42).

Compound **35** (5 mg, 0.0061 mmol) was dissolved in H_2O (250 μL) solution in which also contained acrylamide (4.33 mg, 0.061 mmol) and a catalytic amount of $(NH_4)_2S_2O_8$ (0.073 mg, 0.32 μmol, injection of 3μL of a stock solution 4.8 mg/ 200 μL). The reaction mixture was then heated up to 95° C for 10 minutes. The polymer product was purfied by the similar method as described above. The yield was 5 mg, 52%. ^{1}H-NMR spectral data (D_2O, δ ppm), 7.89(m, 4H, aromatic), 7.40(m, 2H, NH), 5.82(m, 1H, NH), 5.23(m, 1H, H-1), 4.53(m, 1H, H-1'), 3.50-4.25(m, 25H, carbohydrate Hs), 2.78(m, 1H, H-3"e), 2.25-2.41(m, 6H, polymer backbone methine Hs), 2.10(s, 3H, NHAc), 1.51-1.90(m, 12H, polymer backbone methylene Hs).

Copoly (acrylamide/N-(methyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto* -2-nonulopyranosidonate)-(2-3)-O-(β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl N-acryla midohexanamide, (44).

To an aqueous solution (250 μL) containing **36** (5 mg, 0.0061 mmol) and acrylamide (4.33 mg, 0.061 mmol) was added a catalytic amount of $(NH_4)_2S_2O_8$ (0.073 mg, 0.32 μmol, injection of 3μL of a stock solution 4.8 mg/ 200 μL). The reaction mixture was brought to 95° C for 10 minutes. The polymer product was purified by the similar method as described in **III.3.3.** The yield was 7 mg, 76%. ^{1}H-NMR spectral data (D_2O, δ ppm), 5.06(d, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 4.53(d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1'), 4.01-4.03(m, 2H, H-4',3'), 3.54-4.20(m, 16H, carbohydrate Hs), 3.34(dd, 2H, Hα), 2.83(m, 1H, H-3"e), 2.42(m, 2H, Hε), 2.25-2.41(m, 11H, polymer backbone methine Hs), 2.10(s, 3H, NHAc), 1.87(dd, 1H, $J_{3"a,4}$= 2.0 Hz, H-3"a), 1.51-1.90(m, 22H, polymer backbone methylene Hs), 1.71(m, 2H, Hδ), 1.63(m, 2H, Hβ), 1.43(m, 2H, Hγ); The GM_3 incorporation ratio 1: 11.2.

Copoly(acrylamide/N-(5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosidonate)-(2-3)-O-(β-D-galacto pyranosyl)-(1-4)-O-β-D-glucopyranosyl acrylamide, (41).

Polymer **40** (3 mg) was dissolved in 0.05M NaOH solution (0.5 mL) and stirred at room temperature. The reaction was complete in 3 hours. The product **41** was precipitated out upon adding cold Et_2O (10 mL), filtered and dried under reduced pressure to give 3 mg, quantitative yield. ^1H NMR (D_2O, δ ppm), 5.10(m, 1H, H-1), 4.57(m, 1H, H-1'), 3.50-4.25(m, 25H, carbohydrate Hs), 2.78(m, 1H, H-3"e), 2.25-2.41(m, 6H, polymer backbone methine Hs), 2.10(s, 3H, NHAc), 1.51-1.90(m, 12H, polymer backbone methylene Hs); The GM_3 incorporation ratio 1: 6.6; estimated molecular weight: >36 KDa.

Copoly(acrylamide/acrylamide/N-(5-acetamido-3,5dideoxy -D-*glycero*-β-D-*galacto*-2-nonulopyranosidonate)-(2-3)-O-(β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl p-acry lamidobenzamide, (43).

Polymer **42** (3 mg) was dissolved in 0.05M NaOH solution (0.5 mL) and stirred at room temperature. The reaction was complete in 3 hours. The product **43** was precipitated out upon adding cold Et_2O (10 mL), filtered and dried under reduced pressure to give 3 mg, quantitative yield. ^1H-NMR spectral data (D_2O, δ ppm): 7.89(m, 4H, aromatic), 7.40(m, 2H, NH), 5.82(m, 1H, NH), 5.23(m, 1H, H-1), 4.53(m, 1H, H-1'), 3.50-4.25(m, 25H, carbohydrate Hs), 2.78(m, 1H, H-3"e), 2.25-2.41(m, 6H, polymer backbone methine Hs), 2.10(s, 3H, NHAc), 1.51-1.90(m, 12H, polymer backbone methylene Hs); The GM_3 incorporation ratio 1: 6.2; estimated molecular weight: >36 KDa.

Copoly(acrylamide/acrylamide/N-(5-acetamido-3,5dideoxy -D-*glycero*-β-D-*galacto*-2-nonulopyranosidonate)-(2-3)-O-(β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl 6-acry lamidohexamide, (45).

Polymer **44** (3 mg) was dissolved in 0.05M NaOH solution (0.5 mL) and stirred at room temperature. The reaction was complete in 3 hours. The product **45** was precipitated out upon adding cold Et_2O (10 mL), filtered and dried under reduced pressure to give 3 mg, quantitative yield. ^{1}H-NMR spectral data (D_2O, δ ppm), 5.06(d, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 4.53(d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1'), 4.01-4.03(m, 2H, H-4',3'), 3.54-4.20(m, 16H, carbohydrate Hs), 3.34(dd, 2H, Hα), 2.83(m, 1H, H-3"e), 2.42(m, 2H, Hε), 2.25-2.41(m, 11H, polymer backbone methine Hs), 2.10(s, 3H, NHAc), 1.87(dd, 1H, $J_{3"a,4}$= 2.0 Hz, H-3"a), 1.51-1.90(m, 22H, polymer backbone methylene Hs), 1.71(m, 2H, Hδ), 1.63(m, 2H, Hβ), 1.43(m, 2H, Hγ); The GM_3 incorporation ratio 1: 11.2; estimated molecular weight: >36 KDa.

IV. Glycotelomers.

IV.1. Introduction.

Carbohydrate-containing polymers have been specifically designed to access the previouly described multivalency effect of clustered molecules.[146-148] However, these glycopolymers have poor chemical definitions and their molecular weights are difficult to control and thus represent mediocre therapeutic values. In order to circumvent the problems associated with the structural definition of the glycopolymers, severe restriction in the propagation step, thus resulting in small-size, low molecular-weight polymers was sought out. This process, called telomerization, constitutes a single step synthesis of a family of clustered molecules derived from quenching polymeric growth by chain transfer reagents (telogens).[149] It has given access to small size polymers (telomers) depending on the concentration and nature of the chain transfer reagent used.[97b]

The most attractive aspect of telomerization includes the followings. First, the size of the telomers can be controlled by varing the concentrations of the chain transfer reagent. Second, it is a single step process and thus saves many synthetic steps if one attempts to synthesize for example, a particular lactosylated cluster. Third, since the radicals are quenched by a telogen during the process, low-molecular weight telomers can be easily prepared . The isolation of each telomer is feasible and thus provides clusters of defined chemical structure. Diastereomeric mixtures can result from telomerization as commonly seen in polymerization processes. However, there is little evidence that this mixed tacticity of the molecules affects the binding nature of the carbohydrates with their receptors.

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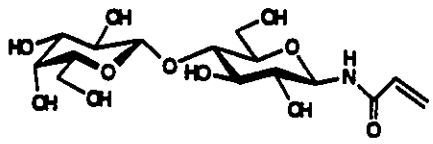
146. R. Roy, F.D. Tropper, A. Romanowska, *J. Chem. Soc., Chem. Commun.*, **1992**, 1161.
147. R. Roy, F. D. Tropper, A. Romanowska, *Bioconjugate Chem.* **1992**, 3, 256.
148. A. Kobayashi, T. Akaiki, K. Kobayashi, H. Sumimoto, *Makromol. Chem., Rapid Commun.* **1986**, 7, 645.
149. C. M. Starks, "*Free Radical Telomerizations*", Acaademic Press, New York, **1974** Chapter 8, 193.

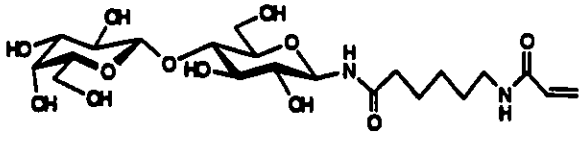
t-Butylmercaptan was first used as a telogen. As described earlier, alkanethiols have excellent radical quenching abilities and the choice of a tertiary alkanethiol was based on the fact that this would provide a good reporter group. For example, in ^{1}H-NMR spectrum, the *t*-butyl group should appear as a singlet, isolated from the carbohydrate protons. In addition, since each glycotelomer contains one *t*-butyl group, the integration of the *t*-butyl group and the corresponding carbohydrate protons will provide a good parameter for determination of the structure of the telomers.

In this chapter, two types of carbohydrate-containing monomers were used in the telomerizations, the lactose derivative **31** without a spacer arm and the lactose derivative **52** equipped



31

with an N-acrylamido hexanoyl spacer arm. The longer distance from the telomeric backbone given to the carbohydrate haptens should provide better accessibility in binding with receptors. After telomerization, each telomer was isolated by using size-exclusion chromatography and lyophylized. The isolated telomers were then evaluated for their inhibitory capacities in terms of the spacer arm using Enzyme-linked Lectin Assay (ELLA).



52

IV.2. Results and Discussion.

The preparation of a family of low molecular weight carbohydrate clusters was accomplished in a single-step reaction. The choice of *t*-butylmercaptan (*t*-BuSH) was made not only because it is an excellent reporter group in ^{1}H-NMR spectrum, but also because its rate of chain transfer is comparable with that of the chain growth by the carbohydrate monomer **31**. The monomer **31** was telomerized using various thiols such as ethanethiol, p-methoxybenzenethiol, thioacetic acid and *t*-butylmercaptan as telogens. Under the given condition (1 equiv. of thiol with respect to **31**, AIBN, in refluxing MeOH for 15 min.), the telomerizations with ethanethiol, p-methoxybenzenethiol and thioacetic acid gave mainly the Michael addition products, **31a**, **31b** and **31c**, respectively as shown in **Figure 4.1**.

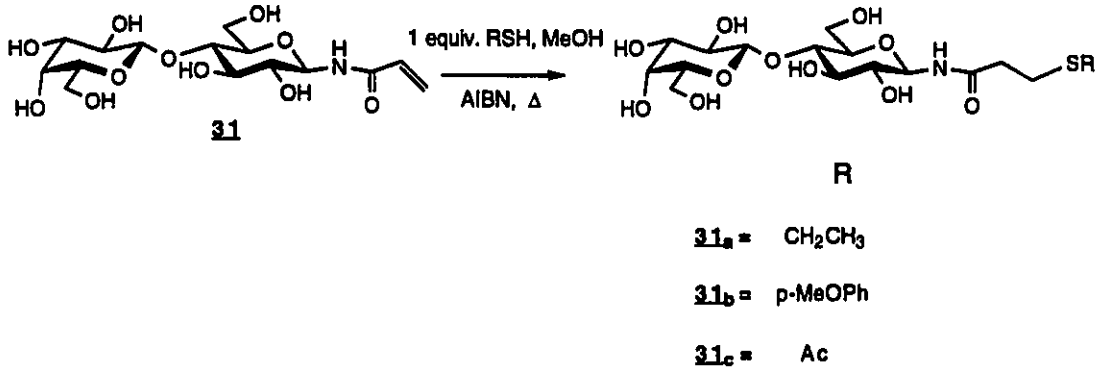


Figure 4.1. The Michael addition of various thiols to **31** via radical process.

In contrast, the monomer **31** without the spacer arm was telomerized using *t*-BuSH as telogen in refluxing MeOH in the presence of 2,2'-azobisisobutyronitrile (AIBN) as initiator to give the following telomers. (**Figure 4.2**)

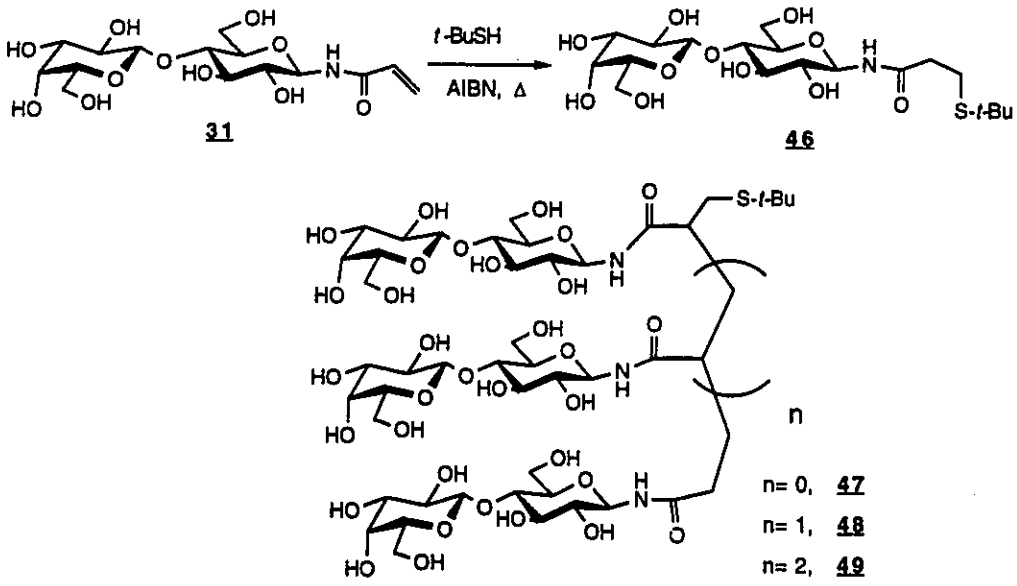


Figure 4.2. Telomerization of 31 using *t*-BuSH as telogen.

By varying the concentration of t-BuSH and keeping the other variables constant i.e. the concentration of the monomer and the initiator, optimization of the size of the telomers was exercised and the results are shown in **Table 4.1.**

Table 4.1. Effect of Telogen Content on Average Telomer Size.

Telogen[a] (equivalents)	Average number of lactose residues in the telomer mixtures[b]
0.1	190
0.2	85
1.0	43
5	6
10	2[c]

a The telogen was t-BuSH. Based on telomerization of 50 mg(0.127 mmol) of 31.

b. Based on ^{1}H NMR spectral data.

c. The distribution of telomers is as follows: 29.4% monomer 46, 15.8% dimer 47, 8.4% trimer 48, 5.8% tetramer 49 and 41% higher telomers.

By the same method described above, the monomer **52** equiped with an N-acrylamidohexanoyl spacer arm was telomerized as illustrated in **Figure 4.3.**

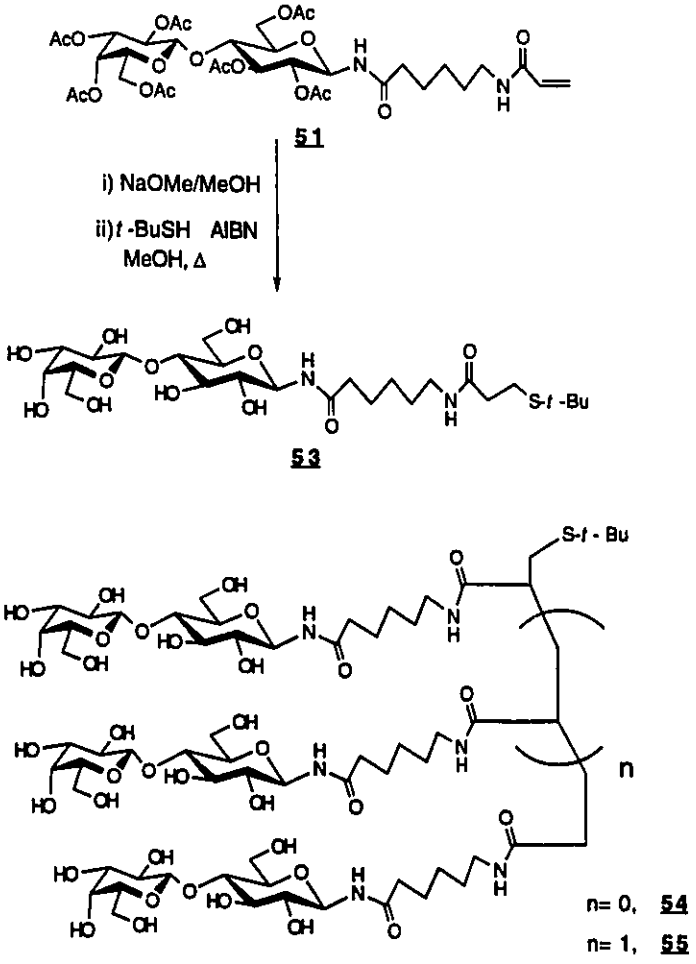
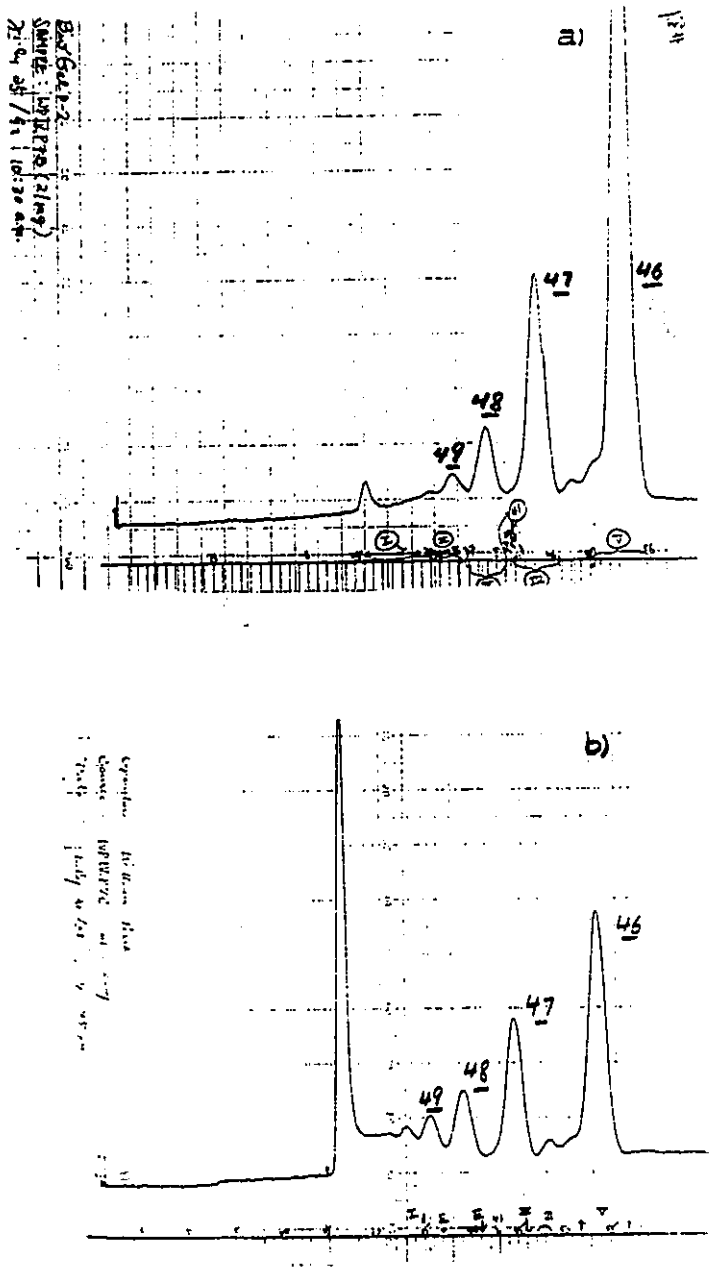


Figure 4.3. Telomerization of **52** using *t*-BuSH as telogen.

The separation of each telomer was accomplished by size exclusion chromatography on a Bio-Gel P-2 column using water as eluent. The telomers were then lyophilized. **Figure 4.4.** illustrates the effect of t-BuSH concentration on the distribution of telomers.



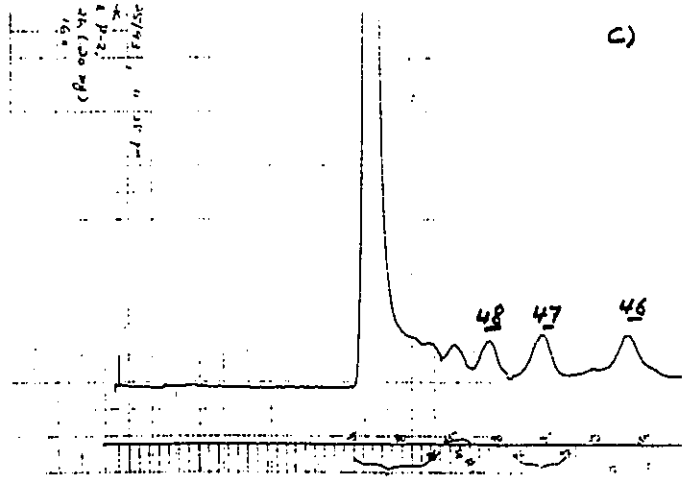
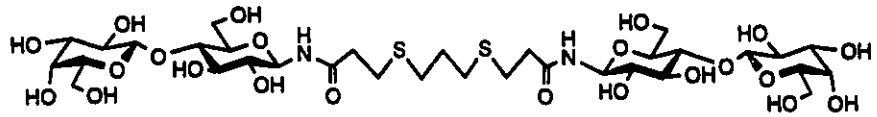


Figure IV.4. The reaction of 31 (taxogen) with *t*-BuSH (telogen) :a) the relative ratio of taxogen : telogen = 1: 20, b) 1:10, c) 1:1, respectively.

For comparison, compound **31** was dimerized by tethering the terminal N-acryloyl function with 1,3-propanedithiol under ionic Michael conditions to provide dimer **50** in 82% yield.



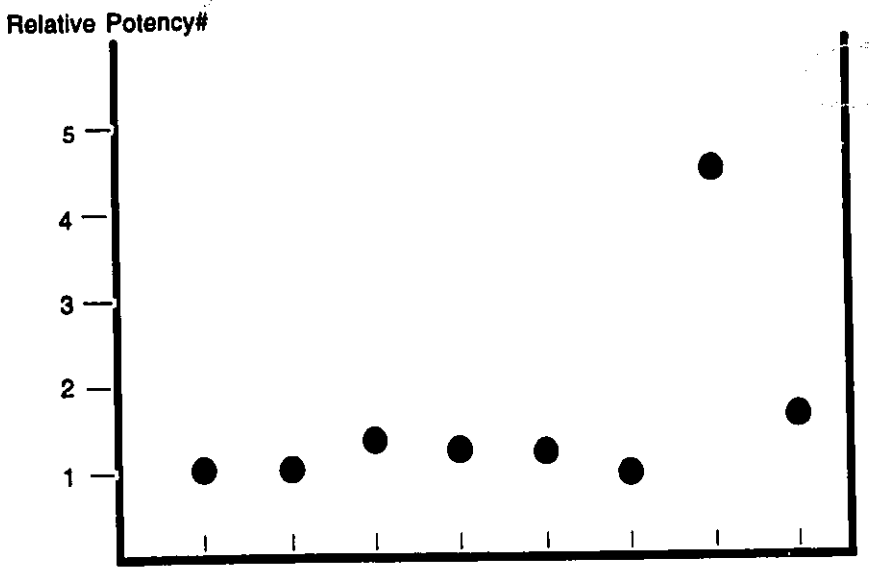
50

Each individual telomer **46**-**49** and the tethered dimer **50** were evaluated with respect to their relative abilities to inhibit the binding of peanut lectin against a model lactosylated polymer synthesized previously poly(acrylamide-co-p-N-acrylamidophenyl β-D-lactoside).[150] The results are shown in **Figure 4.4** and tabulated in **Table 4.2.** The telomers without spacer arm exhibited no improvement in inhibition in comparison to free lactose. On the contrary, the tethered dimer showed 4-fold increased inhibitory capacity over that of the free lactose.

150. R. Roy, F.D. Tropper, A. Romanowska, *Bioconjugate Chem.* **1992**, 3, 256.

compound	concentration (nM) required for 50 % inhibition	Relative potency
lactose	248	1.00
31	255	0.97
46	201	1.23
47	211	1.18
48	220	1.13
49	250	0.99
50	56	4.43
55	150	1.65

Table 4.2. : Relative potencies of lactosylated telomers as inhibitors of peanut lectin binding to lactosylated polymer.



Relative potency (●) is based on the normalized value 1 as free lactose.

Figure 4.5. Relative potencies of lactosylated telomers as inhibitors of peanut lectin binding to lactosylated polymer 39.

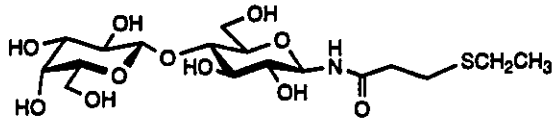
This clearly suggests that the telomers equiped with longer spacer arms can offer much better binding ability with their protein receptors than those without. The trivalent telomer **55** equipped with a spacer arm was compared to free lactose and was shown to be twice as potent. This relatively low inhibitory action by the telomer **55** implies a possible intramolecular hydrogen bonding network formed by the amide function of the spacer arms. Then, the degrees of freedom of the lactosyl units could be relatively restricted and thus less exposed to the receptors for binding.

As the size of the telomer increases, the number of structural isomers increases. For example, the dimer **47** has two possible diastereomers while the trimer **48** could form four different diastereomers. The number of structural isomers increases in the order of 2^n where n is the number of chiral centers in the telomers keeping the lactosyl units as constant. These isomer fomations were observed in the ^{1}H-NMR spectra of the isolated telomers. The signal for the *t*-butyl group of the dimer **47** appeared at 1.41 and at 1.40 ppm. For the trimer **48** the same group appeared at 1.41, 1.40, 1.39 and 1.38 ppm. This complicates the structual definitions of the telomers to some extent. However, it is speculative that the different telomer tacticities would affect the overall binding processes between the carbohydrate haptens and their protein receptors.

Since a series of telomer products can be obtained with a single-step reaction, one can prepare a library of useful telomers with systematic molecular weight increase from a single scale-up reaction.

IV.3. Experimental Methods.

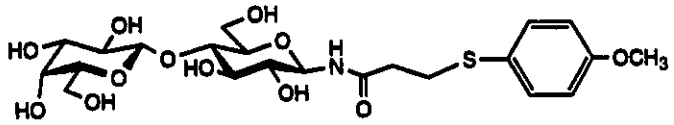
N-2-ethylthiopropyl (β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl amine, (31a).



31a

To MeOH (1 mL) solution containing **31** (20 mg, 0.05 mmol) was added ethanethiol (3 μL, 1 equiv.) and AIBN (10 μL of 1.2 M stock solution, 0.01 mmol). The reaction mixture was then refluxed for 20 min. The reaction was monitored by TLC using a mixture of MeCN : H_2O (8:2), respectively as eluent. The solution was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography to give **31a** 22 mg, 92% in yield. M.p. 129° C; ^{1}H-NMR spectral data (D_2O, δ ppm): 5.01(d, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 4.47 (d, 1H, $J_{1',2'}$ = 7.7 Hz, H-1'), 3.42-3.98(m, 12H, lactose H), 2.85(dd, 2H, -C<u>H</u>$_2$SEt), 2.67(dd, 2H, C(O)C<u>H</u>$_2$), 2.65(q, 2H, SC<u>H</u>$_2$CH$_3$), 1.24(t, 3H, -SCH$_2$C<u>H</u>$_3$); $[\alpha]_D$ = -11.0° (c 1.0, H_2O); FAB-MS (pos.): calcd for $C_{17}H_{38}NO_{11}S$, 457.4. Found, 458.2 (M+1, 22.0%).

N-2-p-methoxybenzenethiopropyl (β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl amine, (31b).

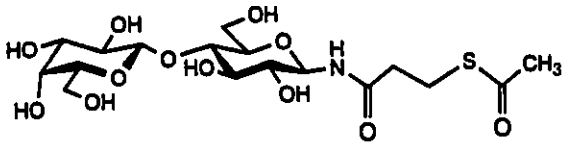


31b

To MeOH (1 mL) solution containing **31** (20 mg, 0.05 mmol) was added p-methoxybenzenethiol (7 μL, 1 equiv.) and AIBN (10 μL of 1.2

M stock solution, 0.01 mmol). The reaction mixture was then refluxed for 15 minutes. The reaction was monitored by TLC using a mixture of MeCN : H_2O (8:2), respectively as eluent. The solution was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography to give **31b** (27 mg, quantitative). M.p. 87-81° C; ^{1}H-NMR spectral data (DMSO-d_6, δ ppm): 7.21(dd, 4H, aromatic), 5.01(d, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 4.47 (d, 1H, $J_{1',2'}$ = 7.7 Hz, H-1'), 3.78(s, 3H, OMe), 3.10-3.71(m, 12H, lactose H), 3.00(dd, 2H, -CH_2SPh-), 2.38(m, 2H, C(O)CH_2); FAB-MS (pos.): calcd. for $C_{21}H_{44}NO_{12}S$, 535.3. Found, 536.2 (M+1, 10.5%).

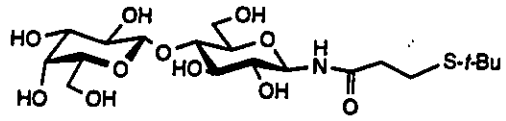
3-Acetylthiopropylamido-N-(β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranose, (31c).



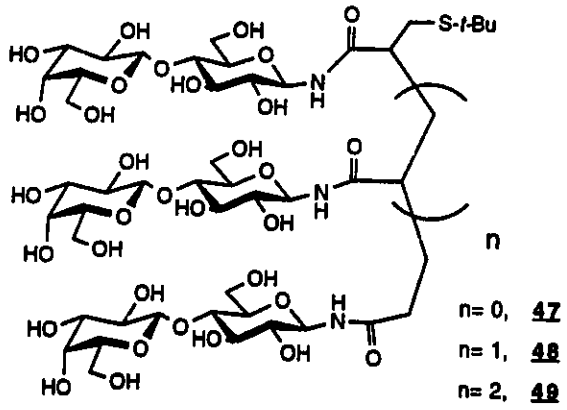
31c

To a MeOH (1 mL) solution containing **31** (20 mg, 0.05 mmol) was added ethanethiol (4.5 μL, 1 equiv.) and AIBN (10 μL of 1.2 M stock solution, 0.01 mmol). The reaction mixture was then refluxed for 20 min. The reaction was monitored by TLC using a mixture of MeCN : H_2O (8:2), respectively as eluent. The solution was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography to give **31c** (21 mg, 88%). ^{1}H-NMR spectral data (D_2O, δ ppm): 5.00(d, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 4.47 (d, 1H, $J_{1',2'}$ = 9.8 Hz, H-1'), 3.42-3.98(m, 12H, lactose H), 3.16(dd, 2H, -CH_2S), 2.66(dd, 2H, C(O)CH_2-), 2.38(s, 3H, C(O)CH_3); ^{13}C-NMR spectral data: 200.5, 170.0 (C=O), 102.5 (C-1'), 78.7 (C-1), 77.4, 76.0, 75.0, 74.7, 72.1, 71.0, 70.6, 68.2, 60.7(C-6), 59.5(C-6'), 34.7(C(O)CH_2), 29.5(CH_3), 23.9(CH_2S); $[\alpha]_D$ = -15.0° (c 1.0, H_2O); FAB-MS (pos.): calcd for $C_{17}H_{29}NO_{12}S$, 471.3. Found, 472.2 (M+1, 22.0%).

Telomerizations of N-Acryloylated Lactosylamine : Telomers without spacer-arm



46



n= 0, 47
n= 1, 48
n= 2, 49

Mixtures of the N-acryloylated lactosylamine **31** (50 mg, 0.127 mmol), the telogen *tert*-butylmercaptan in various molar ratios (see **Table 4.1**), and a catalytic amount of AIBN (0.2 mg) used as a radical initiator were refluxed in methanol (0.57 mL) for 4 h under a nitrogen atmosphere. The progress of the reaction was monitored by TLC using a 8: 2 mixture of MeCN and water, respectively as eluent.. When all reactant was consumed the reaction was stopped simply by cooling the reaction mixture. The solvent was evaporated under reduced pressure, and the resultant crude mixture of telomers were dissolved in water and separated by the size exclusion chromatography (2.0 x 90 cm, Bio-Rad) using BioGel P-2 and water as eluent. Using 10 equivalent of the telogen (*t*-butanethiol) as shown in **Table 4.1**, the telomers **46**-**49** were obtained with the following distribution: 29.4 % (14.5 mg), 15.8 % (7.9 mg), 8.4 % (4.2 mg), and 5.8 % (3 mg), respectively (total of 59 %): the remainder of the material was isolated as higher size telomers which were not processed further.

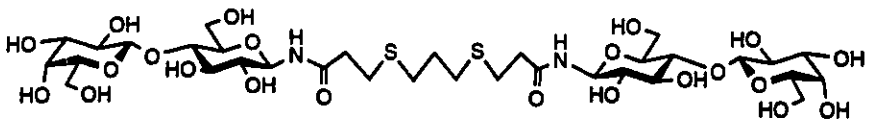
Monomer **46**: ^{1}H-NMR spectral data (D_2O, δ ppm): 5.10 (d, 1H, $J_{1,2}$ = 9.3 Hz, H-1), 4.55 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1'), 2.98 (dd, 2H, -SCH_2), 2.71[m, 2H, -CH_2-C(O)-], 1.42 (s, 9H, ^{t}Bu) ; ^{13}C-NMR spectral data: 99.7 (C-1'), 75.8 (C-1), 32.4 [-$\underline{C}H_2$-C(O)-], 26.6 (^{t}Bu), 19.7 (S-CH_2); $[\alpha]_D$ +3.62° (c 1.0, water).

Dimer **47**: ^{1}H-NMR spectral data: 5.10 (d, 1H, $J_{1,2}$ = 9.3 Hz, H-1), 4.55 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1'), 2.89 (m, 2H, -S$\underline{CH_2}$), 2.67 [m, 1H, CH-C(O)], 2.48 [m, 2H, $\underline{CH_2}$-C(O)], 2.03 [m, 2H, $\underline{CH_2}$-C(O)], 1.40,1.41 (two singlets, 9 H, diastereomric ^{t}Bu); ^{13}C-NMR spectral data: 99.7 (C-1'), 75.8 (C-1), 42.9 [$\underline{C}$H-C(O)], 29.6, 26.2, 24.3 (CH_2), 26.6 (^{t}Bu); $[\alpha]_D$= + 9.85° (c 1.0, water); FAB-MS (pos): calcd for $C_{34}H_{60}N_2O_{22}S$: 881.0. Found, 882.3 (M+1).

Trimer **48**: ^{1}H-NMR spectral data: 5.10 (broad d, 1H, H-1), 4.55 (broad d, 1H, H-1), 3.75-2.27 [m, 4H, two CH, CH_2-C(O)], 2.91-2.81 (m, 2H, S-$\underline{CH_2}$), 2.07-1.90 (m, 4H, backbone -CH_2), 1.41, 1.40, 1.39, 1.38 (four singlets, 9H, diastereomeric ^{t}Bu); ^{13}C-NMR spectral data: 99.7 (C-1'), 75.8 (C-1), 42.9 [$\underline{C}$H-C(O)], 31.5, 29.6, 26.2, 24.3 ($\underline{C}H_2$), 26.6 (^{t}Bu); $[\alpha]_D$= +11.6° (c 1.0, water); FAB-MS (pos): calculated for $C_{49}H_{85}N_3O_{33}S$, 1276.3; found, 1277.8 (M+1).

Tetramer **49**: ^{1}H-NMR spectral data: 5.10 (broad d, 1H, H-1), 4.55 (broad d, 1H, H-1'), 2.95-2.78, 2.66-2.44, 2.44-2.38, 2.16-1.80 (4m, 10CH_2, 3CH, backbone), 1.41, 1.40, 1.39 (s, 9H, diasteromeric ^{t}Bu); ^{13}C-NMR spectral data: 99.7 (C-1'), 75.8 (C-1), 42.9 (CH), 31.5, 29.6, 26.2, 24.3 (CH_2), 26.6 (^{t}Bu); $[\alpha]_D$= +5.43° (c 1.0, water); FAB-MS (pos): calculated for $C_{64}H_{110}N_4O_{44}S$, 1671.6; found, 1672.4 (M+1, 6.5 %).

Tethered Dimer, (50)



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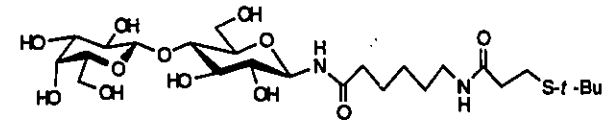
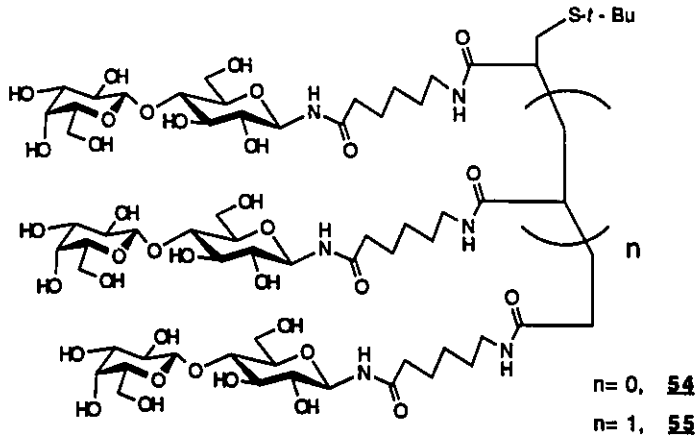
To a 10 mL round-bottom flask containing compound **31** (53.6 mg, 0.136 mmol) in MeOH (2 mL) was added propanedithiol (7.36 mg, 68 μmol, 0.5 equiv.) and triethylamine (13.8 mg, 0.136 mmol, 1.0 equiv.). The reaction mixture was refluxed for 5 h. The progress of the reaction was monitored by TLC using a 97: 3 (v/v) mixture of methanol and water as eluent, respectively. The new product has R_f 0.57. The solvent and volatile reagents were evaporated under reduced pressure, and the residue was applied to a BioGel P-2 column using water as eluent. Pure compound **50** was obtained as a white and amorphous solid in 82% yield. Relevant NMR data are provided as follows. ^{1}H-NMR spectral data (D_2O, δ ppm): 4.83 (d, 2H, $J_{1,2}$ = 9.2 Hz, H-1), 4.28 (d, 2H, $J_{1',2'}$ = 7.5 Hz, H-1'), 2.67 [t, 4H, J=7.1 Hz, -C(O)-CH_2-], 2.51 (t, 4H, J= 7.2 Hz, -CH_2S-), 2.47 (t, 4H, J = 7.3 Hz, SCH_2-), 1.70 (q, 2H, J = 7.1 Hz, mid CH_2); ^{13}C-NMR spectral data (δ ppm): 175.5, 175.4 (C=O), 102.5 (C-1'), 78.7 (C-1), 36.2, 35.4 [-C(O)-CH_2], 29.5, 29.3 (-CH_2S), 27.9, 27.5 (-SCH_2), 26.1 (mid CH_2).

Telomerization of 6-N-Acrylamidohexanoyl lactosylamine.

A mixture of monomer **52** (200 mg, 0.393 mmol), *tert*-butylmercaptan(195 mg, 245 mL, 2.16 mmol, 5.5 equiv.), and a catalytic amount of AIBN (10 mg) in MeOH-water (3.2 mL and 0.5 mL, respectively) was refluxed for 5 h under nitrogen atmosphere. After complete consumption of the monomer **52**, the reaction mixture was evaporated to dryness under a reduced pressure. The resultant mixture of the telomers in the minimum volume of water(200 μL) was separated by the size-exclusion chromatography (BioGel P-2



53



n= 0, 54

n= 1, 55

(2.0 x 90 cm)) using water as eluent. The column allowed the separation of the monomer **53**, the dimer **54** and the trimer **55** with the percent weight ratio of 26.6: 13.9: 1.22, respectively. ^{1}H- and ^{13}C-NMR spectral data (D_2O, δ ppm) for the monomer **53** : 5.13(d, 1H, $J_{1,2}$= 9.2 Hz, H-1), 4.60(d, 1H, $J_{1',2'}$= 7.8 Hz, H-1'), 1.81-1.47 (m, s, 15 H, ^{t}Bu, CH_2); 176.0, 175.1 (two C(O)), 99.6 (C-1'), 75.8 (C-1), 26.7 (^{t}Bu); $[\alpha]_D$= +6.9° (c 0.56, water); FAB-MS (pos): calcd for $C_{25}H_{46}N_2O_{12}S$, 598.3. Found, 599.2 (M+1, 19.5 %). The dimer **54** : $[\alpha]_D$ + 8.1° (c 0.48, water); FAB-MS: calcd for $C_{46}H_{82}N_4O_{24}S$, 1106.5; Found, 1107.5 (M+1, 3.5 %). The trimer **55** : $[\alpha]_D$ +14.9° (c 0.25, water); FAB-MS: calcd for $C_{67}H_{118}N_6O_{36}S$, 1614.7. Found, 1615.9 (M+1, 1.1 %).

Enzyme-Linked Lectin Assay

Inhibition of binding of Peanut Lectin to Lactose-Containing Polymer by Lactosylated Telomers.

Linbro microtitration plates (Flow Laboratories, Mississauga, ON, Canada) were coated overnight with poly(acrylamide-*co*-*p*-*N*-acrylamidophenyl-β-D-lactoside) (2.5 μg/ 100μL/well). The stock solution of polymer was prepared at 1mg/mL in phosphate-buffered saline (PBS). After washing the excess coating capture antigen, the wells were blocked with 1% (w/v) bovine serum albumin (BSA) in PBS at 200 μL/well for 1 hour at room temperature. After washing the plates five times with PBS-Tween, the inhibitors were added in serial dilutions starting from stock solutions at 2 mg/mL PBS. Thus 50 μL/well of between 50 and 500 nmol inhibitors were used in each well. A solution of horseradish peroxidase-labelled peanut lectin from *Arachis hypogaea* (Sigma Chemical Co., St, Louis, MO, L-7759, 20 U/mg protein) in PBS (0.25 μg/ 100μL/well) was added. The inhibitors and lectin in the wells were allowed to equilibrate for 3 hours at room temperature. The plates were then washed 5 times with PBS-Tween and 2 times with PBS alone. The peroxidase substrate, 2,2'-azinobis(3-ethylbenzo thiazoline-6-sulfonic acid) (ABTS, Aldrich, 1mg/4mL) (50 μL/well) in 0.2 M citrate-phosphate buffer, pH 4.0, containing 0.015 % (v/v) H_2O_2 was then added. After 15 minutes the absorbance were measured at 410 nm. The concentration of inhibitors required for 50 % inhibition are tabulated in **Table 4.2.**

Chapter V. Glycodendrimers.

V.1. Introduction.

The cell surface carbohydrates found in the forms of glycoproteins, glycolipids and proteoglycans are now known to be forefront mediators responsible for the various cell to cell and cell to matrix phenomena: these include recognitions of virus, bacteria, hormones and other cells, cell growth, metastasis of normal and tumor cells as mentioned previously. Further understanding of these phenomena at the molecular level require suitable synthetic models that imitate the architectural aspect of the natural molecules. The multivalency is the prime example of this kind, and its mimickry has taken the forms of glycopolymers and glycotelomers as described in the two previous chapters. However, the glycopolymers have failed to deliver well defined chemical identities in spite of their excellent mutivalent effect. Similarly, the corresponding telomers showed much improved chemical homogeneity and yet mediocre multivalent effect, at least in the case of the lactose-containing telomers. Thus, a method has been sought to synthesize carbohydrate-containing molecules which are chemically well-defined and simultaneously exhibit the multivalent effect.

Molecules with tree-like shapes, named dendrimers have been prepared by employing various strategies[151-152] and have been described earlier(Chapter I). The common factor of the strategies employed for the construction of such molecules lies in the choice of a seeding molecule. This molecule should contain two functional groups which can be differentiated from each other, and one of the two functionalities must exist in a multiple form. Thus, a tree-like generation growth can be made by attaching the two different functional groups of the seeding molecules.

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151. G. R. Newkome, C. N. Moorefield, G. R. Baker, *Aldrichmica Acta*, **1992**, 25 (2), 31.
152. C. J. Hawker, J.M.J. Fréchet, *J. Am. Chem. Soc.* **1990**, 112, 7683.

Tam et al. has used this fundamental idea and introduced multiple antigen peptide (MAP) system as an efficient and chemically well-defined approach to produce peptide immunogen without the use of a protein carrier.[153] In this study, the two amino groups of L-lysine anchored to solid support with β-alanine were condensed with the next set of L-lysine and thus the second layer (generation) contained four lysine structures. The structure of the first MAP is shown in **Figure 5.1** and clearly exhibits multivalent functions at its periphery.

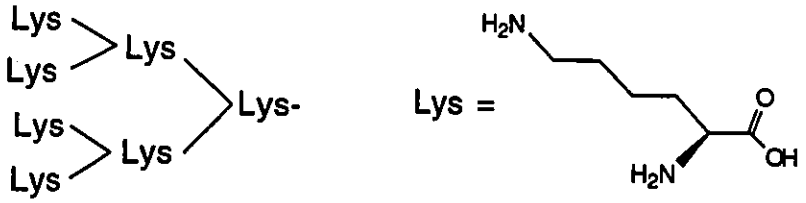

Figure 5.1. The structures of L-Lysine and MAP.

Using this MAP as a dendritic support, the first glycodendrimer was synthesized. A sialic acid (Neu5NAc)-containing MAP was designed and prepared by employing the strategies of the efficient solid-phase peptide synthesis and Fmoc chemistry from our laboratory. (see **Scheme 5.1.**)[154] This strategy has provided a powerful tool for the quantitative investigation of the multivalent effect. The sialic acid-containing dendritic structures (di-, tetra-, octa- and hexadeca-mers) were evaluated for their inhibitory properties against influenza virus A. The result shows that even the dimer was five times more potent than a monosialoside and the hexadecamer was as potent as the sialic acid-containing polymer.[154]

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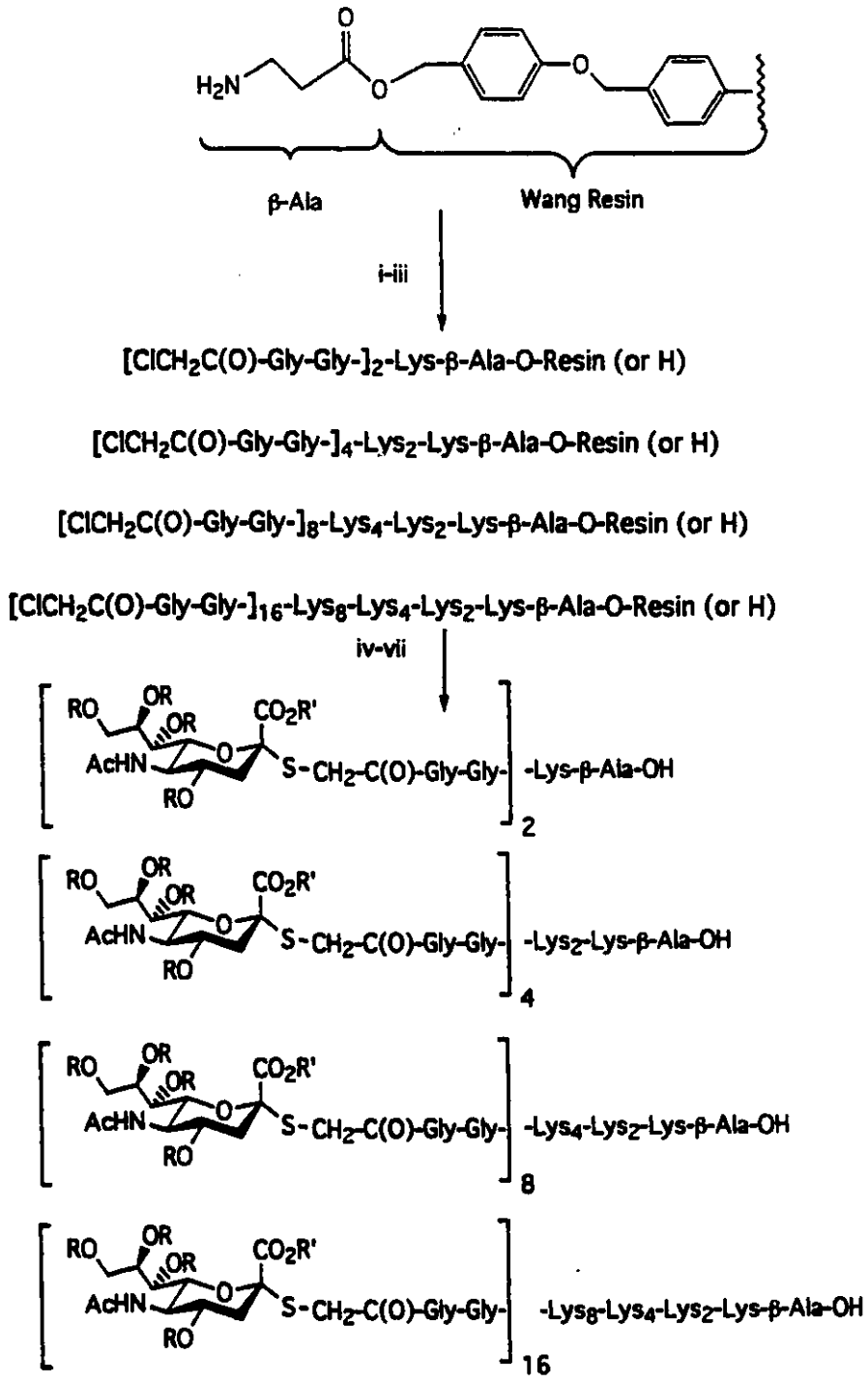
153. Y.A. Lu, P. Clavijo, M. Galantino, Z.-Y. Shen, W. Liu, J. P. Tam, *Molecular Immunology*, **1991**, 28, 623.
154. R. Roy, D. Zanini, S. Meunier, A. Romanowska, *J. Chem. Soc., Chem. Commun.*, **1993**, 1869.



Scheme 5.1. Reagents and conditions: i, N^{α}-N^{ε}-di-Fmoc-L-Lys-OBt, DMF; ii, 20% piperidine (3 x 10 min); iii, repeat cycle or $ClCH_2C(O)$-Gly-Gly-OBt, DMF; iv, thiosialoside (R=Ac, R'= CH_3), 1 % Et_3N, DMF, 16 h, 25° C; v, 95% aq. TFA, 1.5 h, 66-99 %; vi, NaOMe, MeOH, 1h; vii, 0.05 M NaOH, 2h, 25° C, then H^+ resin treatment, quantitative, the sialic acid moiety (R=R'=H).

The synthetic strategy for the conjugation of carbohydrates to the dendritic core can be approached in two ways. The first one involves a carbohydrate derivative containing an electrophilic center while the periphery of the dendrimer is equipped with a nucleophilic center such as thiol. The second option would be the opposite approach as **Figure 5.2.** illustrates.

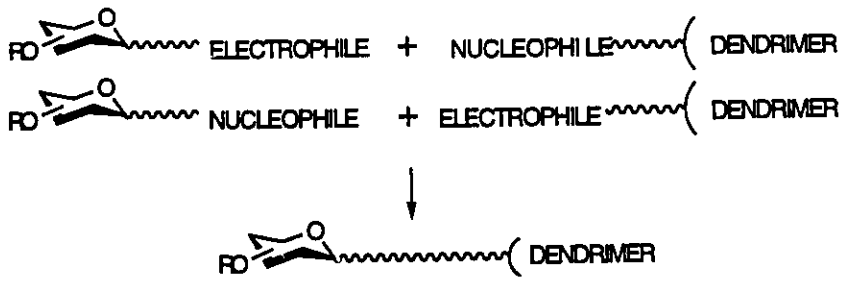


Figure 5.2. Two possible synthetic approaches for conjugations.

When thiols are utilized as the nucleophilic periphery of the dendritic core molecule, the former approach is difficult to accomplish because the intra- as well as the inter-molecular disulfide bridge formations are possible between the dendritic core molecules. This will interfere with the originally designed nucleophilic substitution reaction and consequently bear the partially glycosylated and partially disulfide bridged dendrimers. This makes the characterization process (e.g. separation, structural analyses) even more difficult and complex than what already is. The latter approach, on the contrary, should eliminate the difficulties associated with disulfide formation. Since the nucleophilic thiols are part of the carbohydrate, the disulfide formations should occur between the carbohydrate thiols. If the nucleophilic substitutions with electrophilic dendritic core were incomplete, the partially glycosylated dendrimers can be re-exposed to the carbohydrate thiols until the reaction is complete.

These thiol functionalities can be readily incorporated onto the carbohydrate moieties in the form of thioacetates. Thus, chemoselective deprotection of the thioacetyl group can easily generate the thiol which is essential for the subsequent nucleophilic displacement reaction. This process has been known to proceed well under Zemplén conditions. However, partial or complete de-O-acetylation has been frequently observed when O-acetates are present in the same carbohydrate molecule. Other mild conditions for selective deprotection of anomeric S-acetates were reported to occur at low temperature (-40° C) Zemplén conditions[155a] and more recently with diethylamine[155b] or with cysteamine in hexamethylphosphoramide (HMPA) containing 1,4-dithioerythritol (DTE).[155c] However, some of these conditions are not applicable to achieve the non-anomeric de-S-acetylation in the presence of the other O-acetyl groups, and thus different combinations of reagents were sought. The chemoselective de-S-acetylation of various carbohydrate derivatives can be achieved using hydrazinium acetate.

As the lysine core dendrimer increases its valency by 2^n (where n= integer) for the growth of each generation, another dendritic structure with different valency increment was designed. The construction of the core unit on which the dendrimers are scaffolded was initiated with gallic acid methyl ester (**Figure 5.3**).

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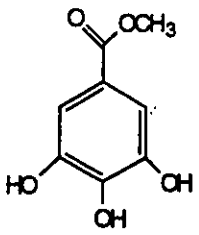
155. a. A. Hasegawa, J. Nakamura, M. Kiso, *J. Carbohydr. Chem.* **1986**, 5, 11.
b. S. Bennett, M. von Itzstein, M. J. Kiefel, *Carbohydr. Res.* **1994**, 259, 293.
c. C. Schou, G. Rasmussen, M. Schlein, B. Henrissac, H. Driguez, *J. Carbohydr. Chem.* **1993**, 12, 743.



77b

Figure 5.3. Structure of the gallic acid methyl ester(77b).

This choice allowed the scaffolding of the dendrimer valency to be 3^n (where n= integer). Tetraethylene glycol (TEG) (**Figure 5.4**) was chosen as a hydrophilic spacer of sufficient length to allow the carbohydrate ligands to be readily accessible to their receptors. The TEG as a small fragment of the polyethylene glycol (PEG) family is known to play an important part in cell fusions and in transport processes through cellular membranes.[156] In addition, PEG has been used in protein chemistry for stabilization and shielding of proteins.[157]

Figure 5.4. Structure of the tetraethylene glycol (TEG).

156. D. Hoekstra, L. Rupert, J. Engberts, S. Nir, H. Hoff, K. Klappe, S. Novick, *Stud. Biophys.* **1988**, 127, 105.
157. M. S. Hershfeld, S. Chaffe, L. Koro-Johnson, A. Mary, A.A. Smith, S.A. Short, *Proc. Natl. Acad. Sci.* USA **11991**, 88, 9185.

Oligoethylene glycol like TEG has also been used as linkers for membrane and glass bound oligonucleotides for hybridization experiments.[158,159] These studies provide confidence with respect to the biocompatibility of TEG and thus its use as spacer.

Functionalization of the periphery of the gallic acid methyl ester-core dendrimer is provided by anchoring N-chloroacetyl function at the end of the spacer arm as in the case of the L-lysine-core dendrimer. The generation growth was envisaged by scaffolding the methyl ester with amine at the periphery of the gallic acid derivative by condensation using DCC and HOBt (**Scheme 5.2**).

Using hydrazinium acetate, the thiols of the carbohydrate derivatives can be produced and conjugated at the periphery of the dendrimer *via* nucleophilic substitution. The carbohydrates used in the present chapter to produce the corresponding glycodendrimers include lactose and GM_3 derivatives. In particular, the model carbohydrate lactose and lactosamine derivatives were also conjugated to the dendrimers having a lysine-core using the same thiol activation method.

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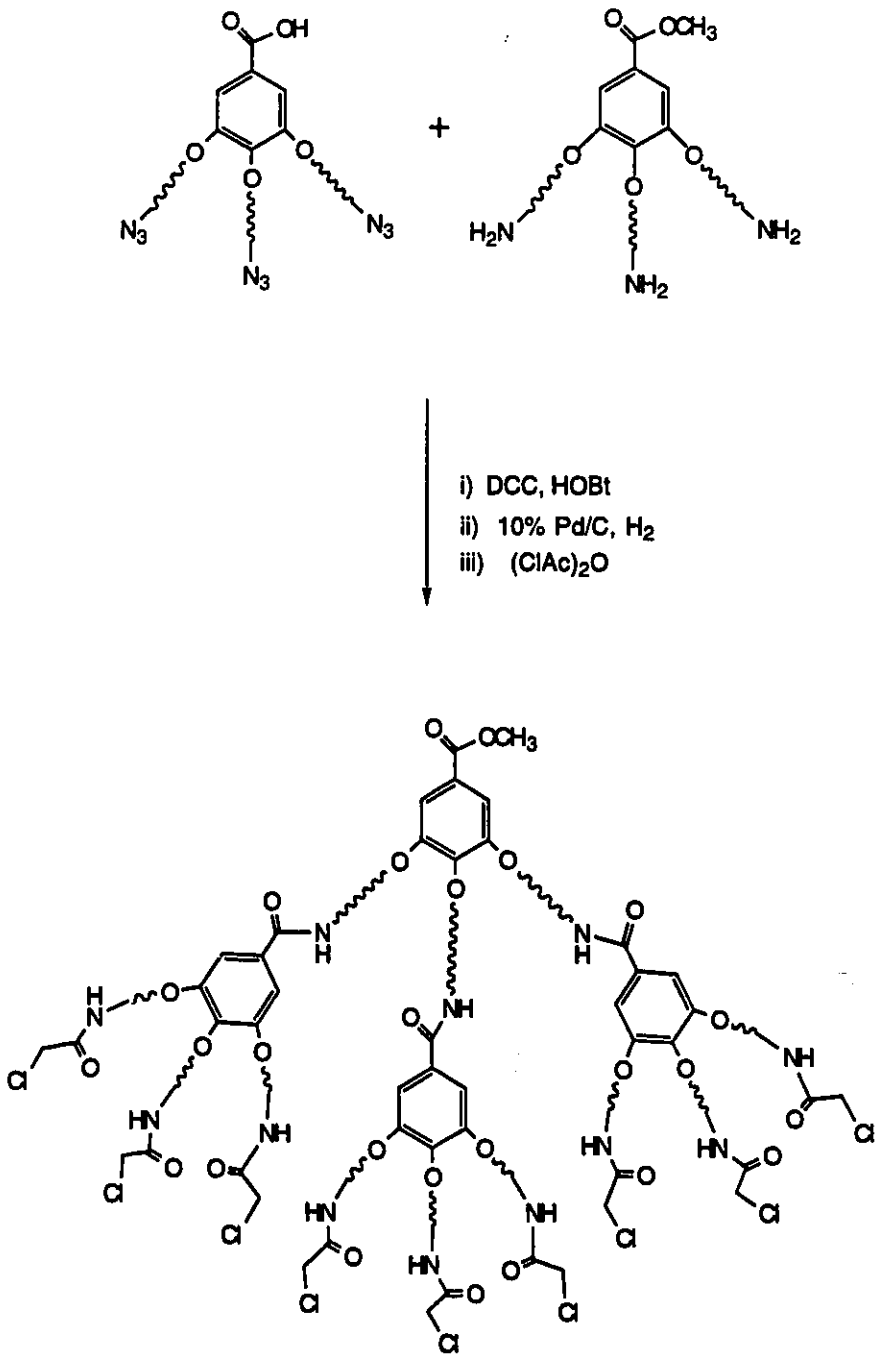
158. Y. Zhang, M.Y. Coyne, S.G. Will, C.H. Levensen, E.S. Kawasaki, *Nucl. Acids Res.* **1990**, 19, 3929.
159. U. Maskos, E.M. Southern, *Nucl. Acids Res.* **1992**, 20, 1679.

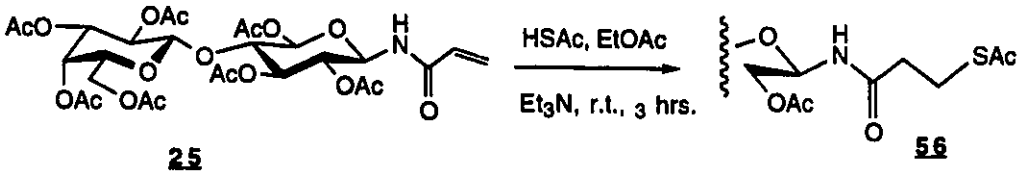


Scheme 5.2. The scaffolding strategy of the gallic acid-core dendrimers.

V.2. Results and Discussion.

V.2.1. Chemoselective De-S-acetylation with Hydrazinium acetate.[160]

As a part of model studies, a primary thioacetate of the lactosyl derivative **56** was synthesized via Michael addition of thioacetic acid onto the N-acryloylated derivative **25**. The resulting thioacetate was de-S-acetylated by using hydrazinium acetate. The thiol was, in turn, reacted with various electrophiles. The primary thioacetate functionality was introduced by Michael addition of thiolacetic acid (Et_3N, EtOAc, r.t., 3 hours) onto N-acryloylated lactosyl amine **25** to afford the corresponding thioacetate **56** in 95% yield. **(Scheme 5.3)**



25 **56**

Scheme 5.3. Preparation of the thioacetylated model compound.

Chemoselective de-S-acetylation under low temperature Zemplén condition (0.95 equiv. NaOMe, MeOH, -40° C, 15 min.) has been successfully carried out for anomeric S-acetates.[155a] However, the conditions were not adequate for primary thioacetates, which often resulted in partial de-O-acetylation.[160] Furthermore, under such condition extensive oxidation of the thiol intermediates to form more stable disulfide products was inevitable **(Figure 5.5)**.

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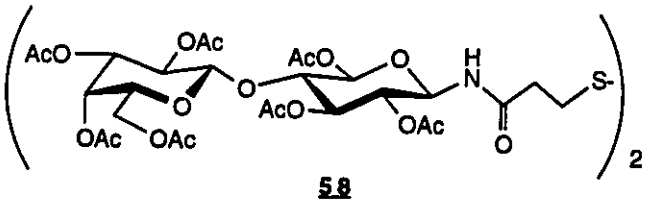
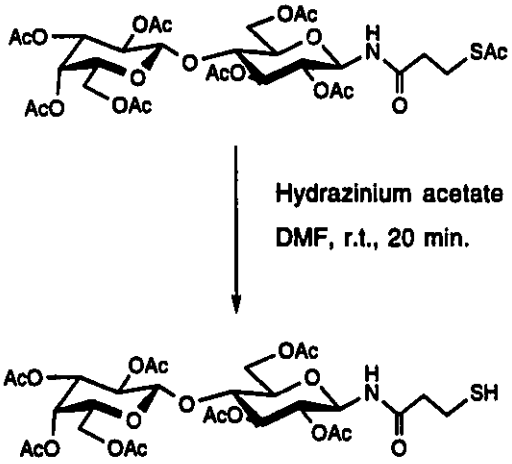
160. W.K.C. Park, S.J. Meunier, D. Zanini, R. Roy, *Carbohydr. Lett.*, **1994**, 1, 179.



58

Figure V.5. A disulfide biproduct (58).

Although hydrazinium acetate ($H_2NNH_2 \cdot HOAc$) has been widely used to accomplish anomeric de-O-acetylations,[161] this reagent has not been fully exploited for de-S-acetylations. Treatment of the thioacetate **56** with hydrazinium acetate in DMF under nitrogen exhibited complete chemoselectivity at the primary thioesters (**Scheme 5.4**). In addition, even after prolonged reaction time at ambient temperature, complete chemoselectivity was still observed (i.e. no detectable de-O-acetylation occurred).



Scheme 5.4. The chemoselective de-S-acetylation of **56** by hydrazinium acetate.

161. G. Excoffier, D. Gagnaire, J.-P. Utille, *Carbohydr. Res.* **1975**, 39, 368.

Freshly prepared thiol derivative was subsequently treated with various electrophiles to provide the corresponding thioethers in the equation shown below from good to excellent overall yields. (**Table 5.1**) It is also worth mentioning that compounds **61** is the prototype product which constitutes the integral subunit of the glycodendrimers as will be described later.

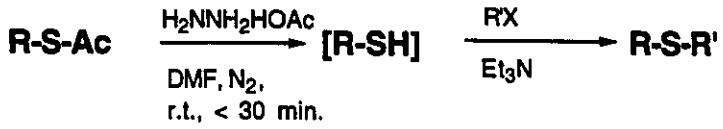


Table 5.1. De-S-acetylation of **58** and thioether formations.

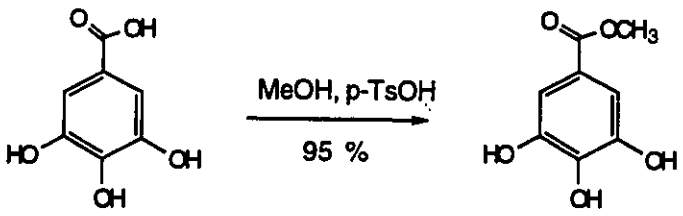
Thioacetate	Electrophile	Thioether	Yield (%)*
56	$BrCH_2CO_2Et$	**59**	88
56	$Br(CH_2)_5CO_2CH_3$	**60**	82
56	$ClCH_2CO$-GlyGlyOH	**61**	76

* The values represent the overall yields of the two-step reactions: the de-S-acetylation and followed by the reactions with the corresponding electrophiles.

V.2.2. Syntheses of gallic acid-core glycodendrimers.

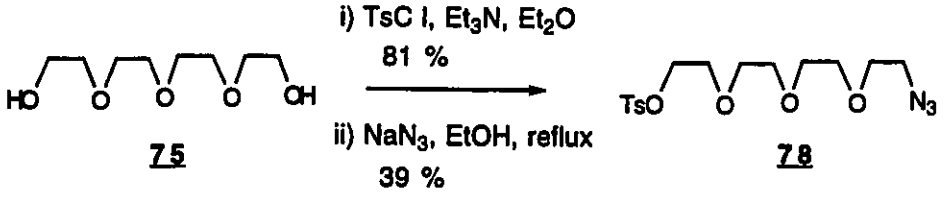
V.2.2.1. Preparation of the core unit.

The construction of the core unit on which the dendrimers were scaffolded was initiated with gallic acid (3,4,5-trihydroxybenzoic acid **77a**) and its subsequent esterification with methanol, gave the corresponding methyl ester **77b** (MeOH, p-TsOH, 95 %) as shown below.



77a 77b

Scheme 5.5. Synthesis of the gallic acid methyl ester.

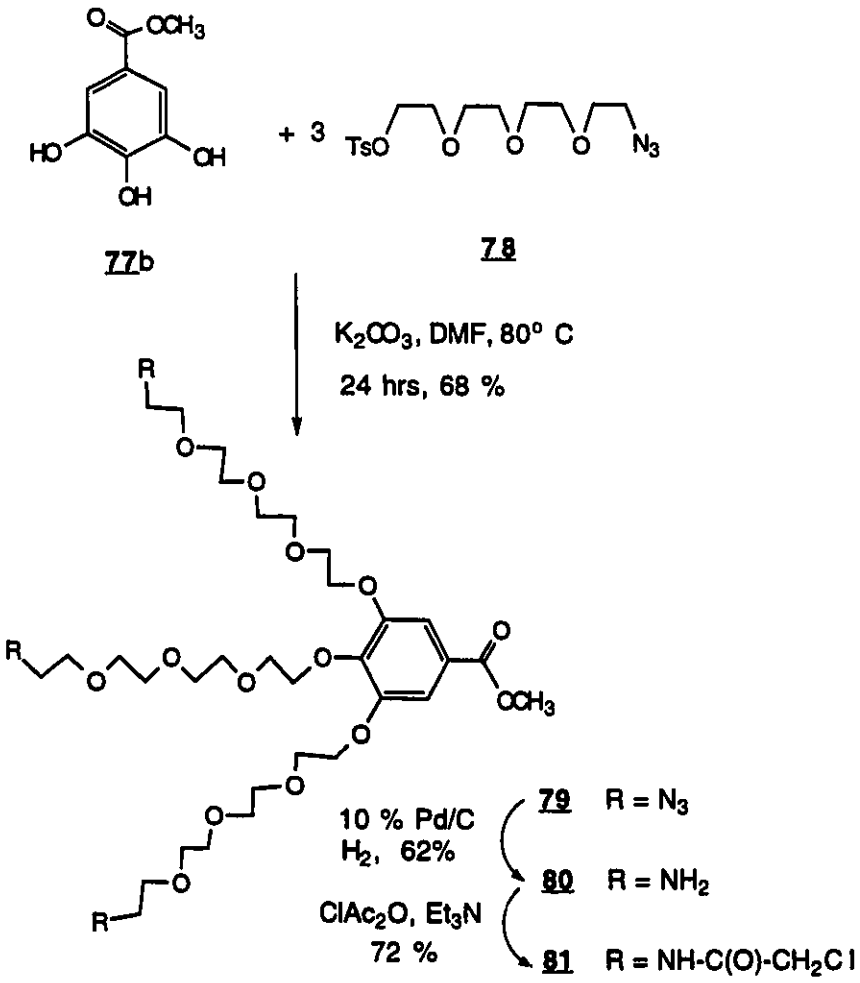


75 78

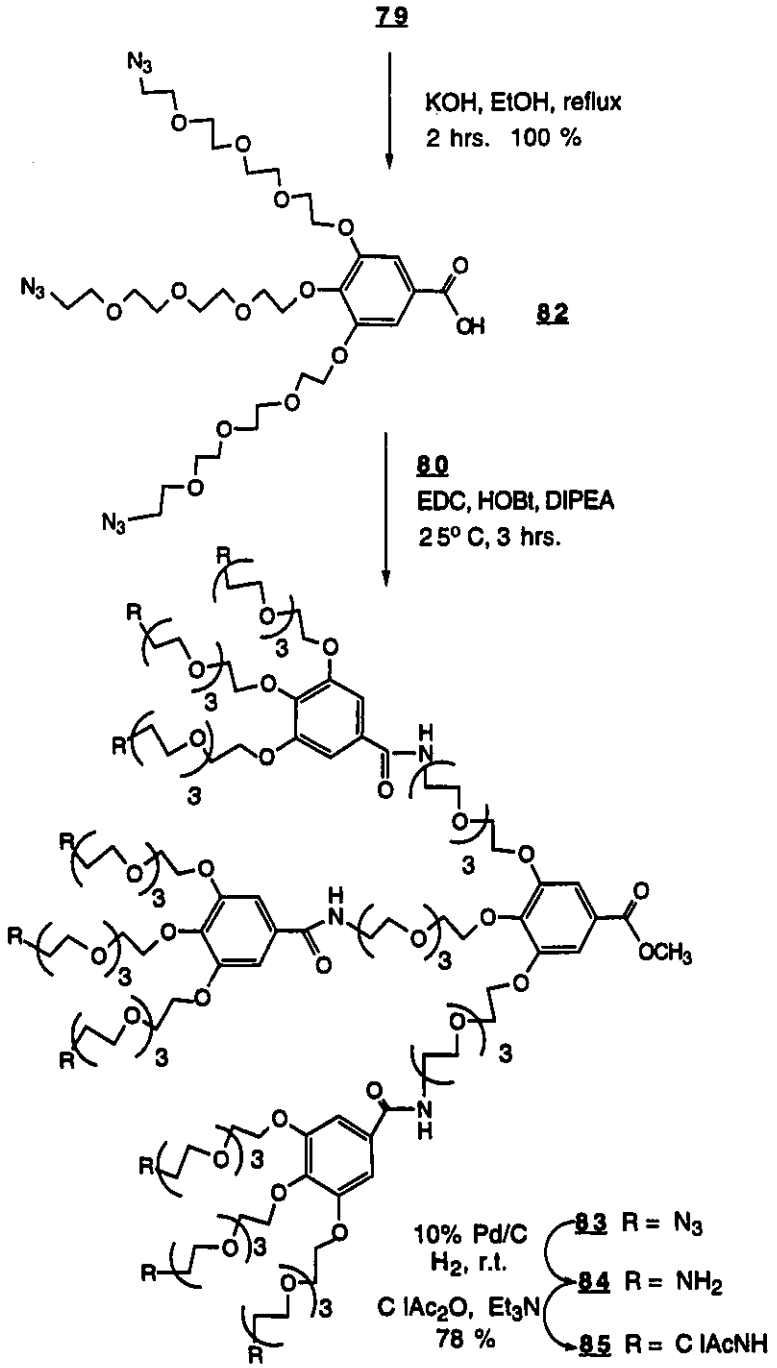
Scheme 5.6. Derivatization of tetraethylene glycol (TEG).

Tetraethylene glycol (TEG) **75** was chosen as a hydrophilic spacer with a sufficient length. The diol **75** was tosylated (TsCl, Et_3N, Et_2O) to provide the bis-tosylated product **76**. Simple nucleophilic displacement of a tosyl group of **76** by azide under refluxing condition in EtOH afforded the azidotosylate **78**. The structure of **78** was confirmed by comparing the integration of the methyl group (2.41 ppm, s, 3H) with that of TEG methylene groups (3.30-3.40(2H), 3.54-3.68(12H), 4.09-4.16(2H)) and by appearance of an IR absiorption band of N_3 at 2106 cm^{-1}. This reaction was also accompanied by other side products such as bis-azide (25 %), unreacted bis-tosylated **76** (20 %) and a small quantity of ethyl ether products (7%). Gallic acid methyl ester **77b** was then alkylated with a slight excess of azido tosylate **78** (3.6 equiv., K_2CO_3, DMF, 80° C, 24 hours) to afford the key dendritic unit precursor **79** in 68 % yield after purification by column chromatography. Compound **79** was then reduced to the corresponding amine **80** by using 10% Pd/C and H_2 gas and subsequent N-chloroacetylation gave the first

generation of dendrimer with electrophilic periphery 81 in 72% yield. (**Scheme 5.7**)



Scheme 5.7. Preparation of the first generation of the gallic acid-core dendritic structure.

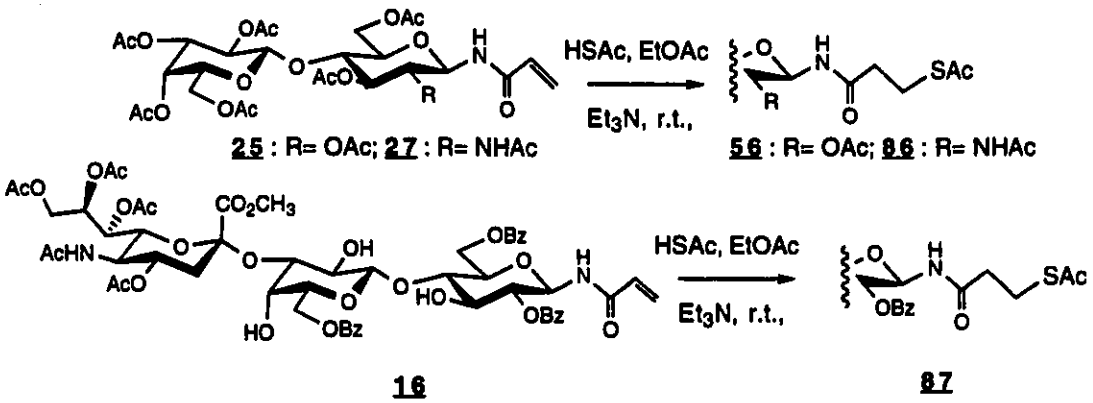


Scheme 5.8. Preparation of the second generation of the gallic acid-core dendritic structure.

Construction of the second generation was accomplished by coupling a slight excess of azido acid **82** obtained from hydrolysis of **81** (KOH, EtOH, reflux, 2 hours, 100%) to amino ester **80** using carbodiimide chemistry and hydroxybenzotriazole(HOBt) activator (EDC, HOBt, DIPEA, 25° C, 3 hours) to provide nona-azido ester **83** in 83 % yield. As in the first generation, the peripheral azido groups of **83** were reduced to amine **84** by catalytic hydrogenation (H_2, 10 % Pd/C). The integrity of the product was verified by the complete absence of the N=N streching band in its infrared spectrum (2106 cm^{-1}). The corresponding amine was directly N-chloroacetylated as above to give **85** in 78 % yield after silica gel column chromatography (**Scheme 5.8**).

V.2.2.2. Conjugations of Gallic acid-core glycodendrimers.

As a synthetic strategy, it was sought out that a nucleophilic carbohydrate thiol attacks the electrophilic periphery of the dendritic core. In order to achieve this goal, three carbohydrate thioacetates **56**, **86** and **87** were prepared. Among these, only the model thioacetate **56** was used in conjugation with the gallic acid-core dendrimers. Preparations of **86** and **87** were followed by the same method used for **56** by simple addition of HSAc to their corresponding N-acryloylated precursors **27** and **16** in 88 % and 82 % yields, repectively (**Scheme 5.9**).

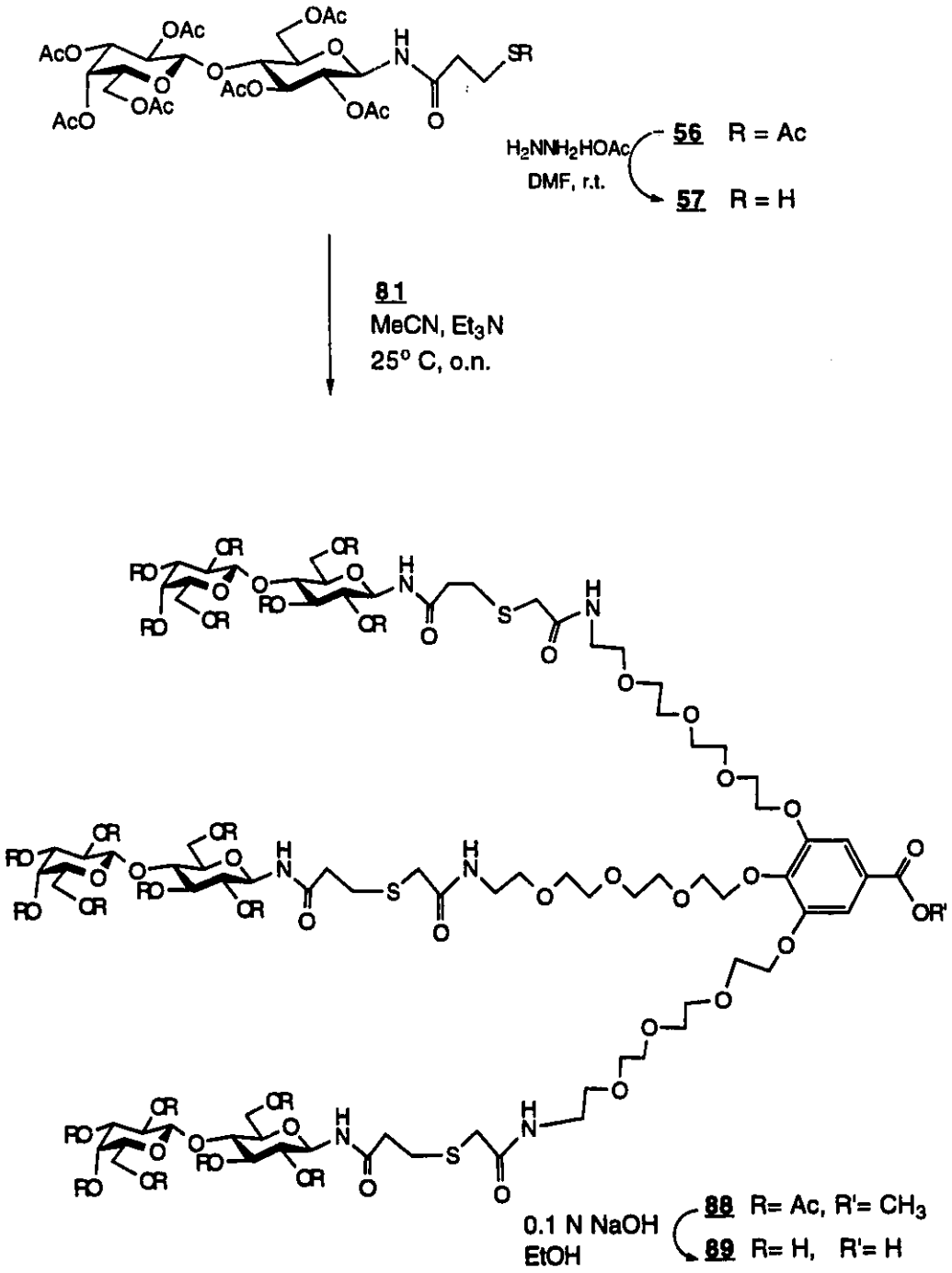


Scheme 5.9. Preparation of the thioacetate of **56**, **86** and **87**.

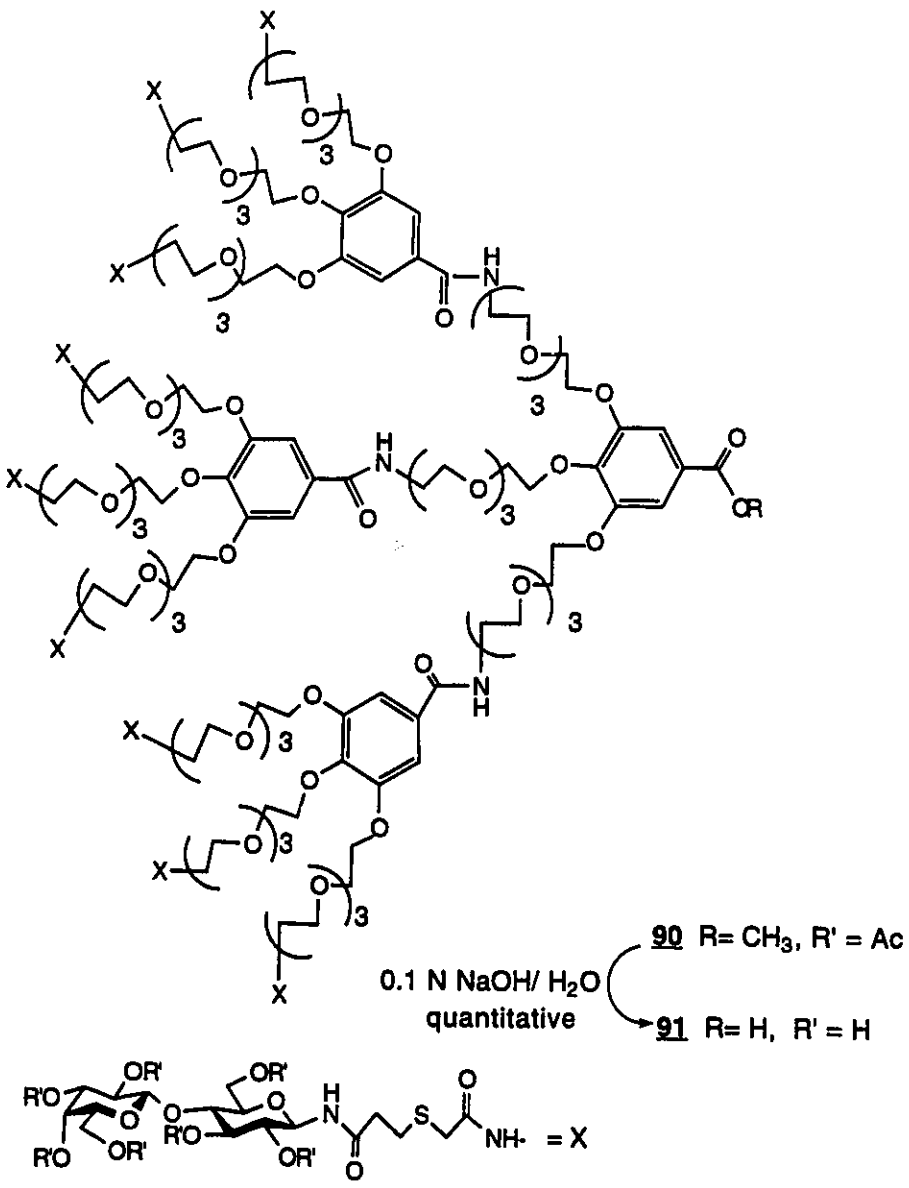
The thiol was generated by treating **56** with hydrazinium acetate in DMF. No de-O-acetylation occurred even when **56** was treated with excess hydrazinium acetate. Only acceleration of the de-S-acetylation was observed. Excess hydrazinium acetate was removed simply by washing with H_2O. The thiol derivative was then subsequently reacted with the first generation of the gallic acid -core dendrimer **81** (MeCN, Et_3N, 25° C, overnight) to produce **88** in 98 % yield.

The integrity of the lactoside incorporation was readily established by ^{1}H-NMR spectroscopy which revealed the absence of the well separated N-chloroacetyl signal at 4.12 ppm in DMSO-d_6 together with a new -SCH_2CO- signal at 3.20 ppm. This signal was integrated relative to those of the anomeric glucosyl signals at 5.23 ppm (H-1, dd) and the aromatic protons at 7.37 ppm. Treatment of peracetylated **88** with 0.1 M NaOH in aqueous ethanol (20% EtOH in H_2O) provided de-O-acetylated lactose-containing dendrimer **89** in quantitative yield. (**Scheme 5.10**).

The lactosyl thiol derivative was coupled to the second generation of electrophilic dendrimer **85** under the condition (11 equiv. MeCN, DMSO (1:5, v/v), Et_3N, 25° C, overnight). The reaction afforded the nona-lactoside **90** in 88 % yield. The reaction was monitored by TLC and the level of lactoside incorporation was confirmed by disappearance of the peak representing ClCH_2C(O) protons at 4.12 ppm in the ^{1}H-NMR spectroscopy. The well separated aromatic protons of the inner and outer-shell ortho protons were observed at 7.22 and 7.16 ppm, respectively with a ratio of 1 : 3. The de-O-acetylated acid **91** was quantitatively obtained after base treatment of **90** with 0.1 N NaOH solution. (**Scheme 5.11**).



Scheme 5.10. Syntheses of the lactose-containing gallic acid-core dendrimer, first generation.



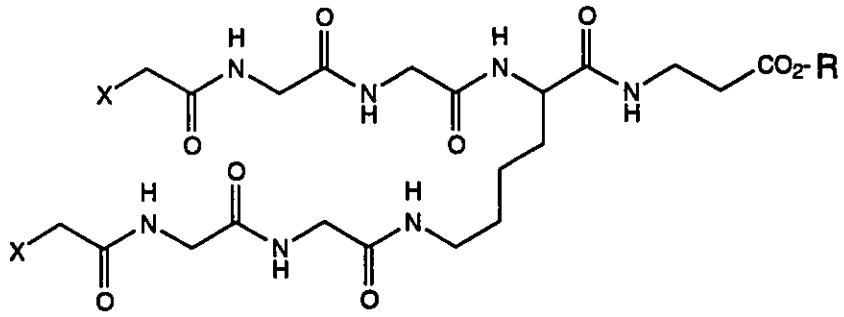
Scheme 5.11. Syntheses of the lactose-containing gallic acid-core dendrimer, second generation.

It is believed that the chemical environment of 4-tetraethylene glycol (TEG) unit is different from that of 3,5-TEG units. In the ^{1}H-NMR spectrum of **81** in D_2O, for example, the farthest methylene group, adjacent to the amido function appeared at 3.49 and 3.46 ppm for 4-TEG (2H) and 3,5-TEG (4H), respectively with the same coupling constant (J= 5.40 Hz) for both methylene groups. This difference comes from the fact that the relative chemical environments are different from one another, i.e. the 4-TEG (para-alkylated) is surrounded by two TEG units (3 and 5 alkylated TEG) whereas 3 and 5-TEGs are equally neighboured by the same 4-TEG. This implies that once cojugated the para-positioned carbohydrate moiety would have different accessibility towards their receptors from the meta-positioned ones. In principle, it can be extended to which the difference in their chemical environment and thus their accessibility to their receptors should exist in the second generation dendritic structures.

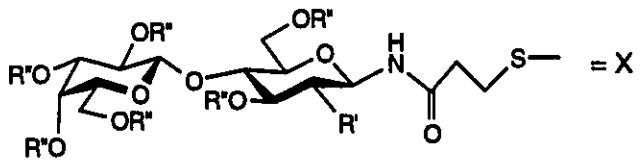
V.2.2.3. Syntheses of L-lysine-core glycodendrimers.

Another set of glycodendrimer was synthesized (**Schemes 5. 12-14**) using lactose **56** and lactosamine **86** derivatives as carbohydrate pendants. The preparation of the di-, tetra- and octa-lysine core dendrimers **94a**, **94b** and **94c** has been published and described earlier.[154] Conjugations of the corresponding thiols of **56** and **86** unto the resin-bound dimeric structure **94a** were accomplished by direct solid-phase chemistry (1% Et_3N/ DMF, agitation with bubbling N_2 gas, overnight). The resin-bound glycosylated divalent products **95** and **96** were hydrolysed with 95% trifluoroacetic acid (TFA) to give the fully protected lysine core dimer glycosides, **97** (75 %) and **98** (37 %), respectively.

For tetra- and octamers solution-chemistry was used to conjugate the carbohydrates, **56** and **86**. The pre-cleaved tetra- and octameric chloroacetyl dendrimers, **94b**, and **94c** were reacted with the thiol forms of the above carbohydrate derivatives (1.6 equiv. to each ClAc- group) in degassed 1 % Et_3N/DMSO to afford the following

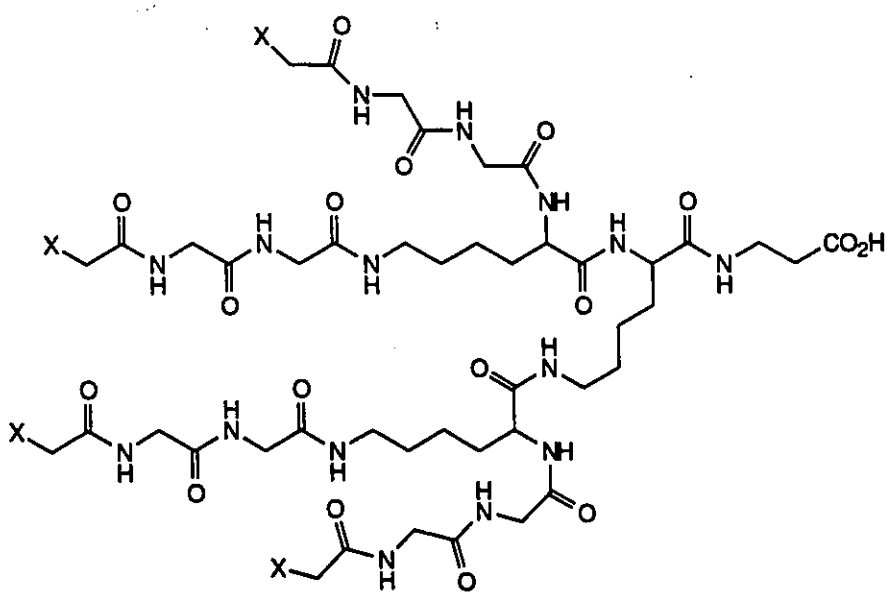


94a R= Resin, X= C l

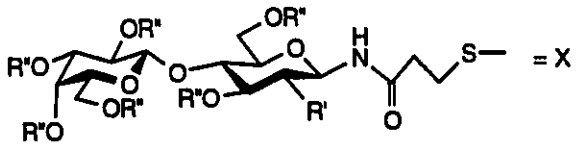


The lactose-containing dendrimer	The lactosamine containing dendrimer
95 R =Resin, R' = OAc, R"= Ac	**96** R = Resin, R' = NHAc, R"= Ac
97 R =H, R' = OAc, R"= Ac	**98** R =H, R' = NHAc, R"= Ac
99 R=H, R'= OH, R"= H	**100** R= H, R'= NHAc, R"= H

Scheme 5.12. Lactose- and lactosamine-containing L-lysine-core dendrimer, first generation.



94a R= Resin, X= C l



The Lactose-containing dendrimer

101 R =H, R' = OAc, R"= Ac

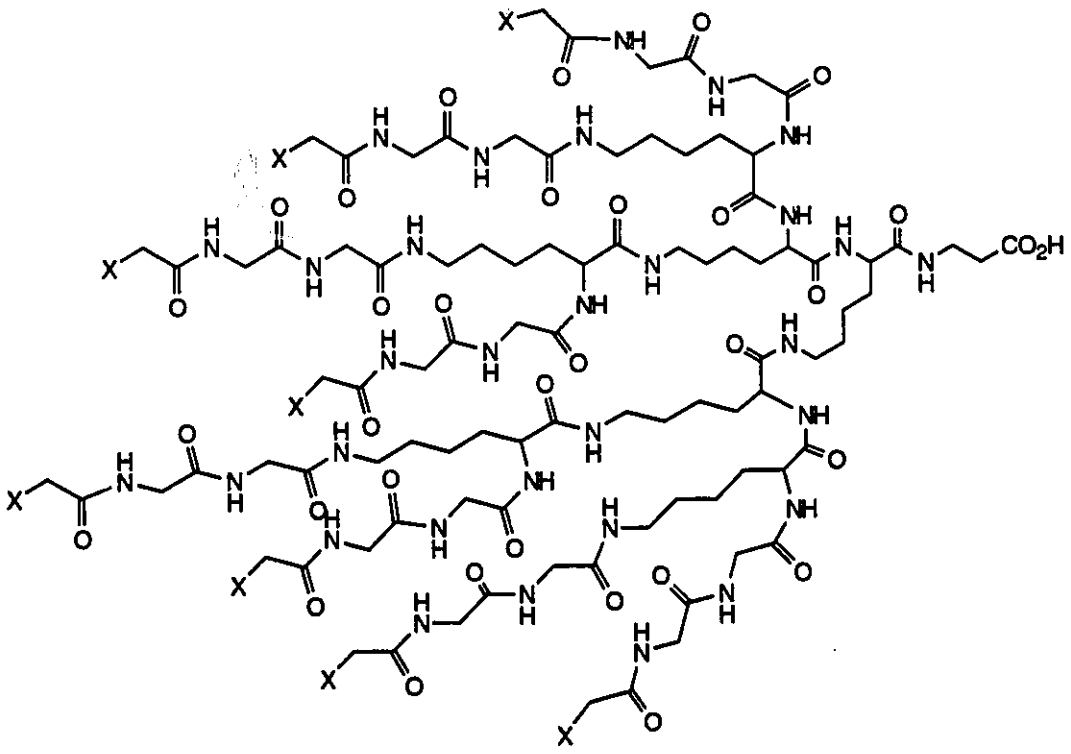
103 R=H, R'= OH, R"= H

The Lactosamine-containing dendrimer

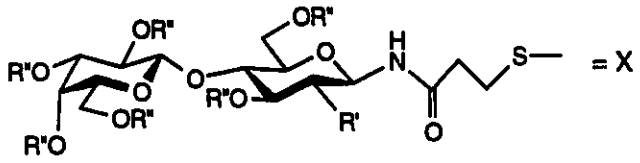
102 R =H, R' = NHAc, R"= Ac

104 R= H, R'= NHAc, R"= H

Scheme 5.13. Lactose- and lactosamine-containing L-lysine-core dendrimer, second generation



94c X= C l



The lactose-containing dendrimer

105 R =H, R' = OAc, R"= Ac

107 R=H, R'= OH, R"= H

The lactosamine-containing dendrimer

106 R =H, R' = NHAc, R"= Ac

108 R= H, R'= NHAc, R"= H

Scheme 5.14. Lactose- and lactosamine-containing L-lysine-core dendrimer, third generation.

fully protected glycodendrimers: **101** and **105** for lacose-containing tetra- and octamer, **102** and **106** for lactosamine-containing tetra- and octamer, respectively. These results are tabulated in **Table 5.2.**

Table 5.2. Yields of the lysine-core glycodendrimers.

Compound	Yield (%)	Compound	Yield (%)
97	75	**99**	92
101	88	**103**	57
105	93	**107**	65
98*	37	**100**	72
102*	44	**104**	71
106*	36	**108**	79

* Particularly low yields of the lactosamine-containing glyco- dendrimers **98**, **102** and **106** are believed to originate from the dialyzation step which lactose-containing compounds have not been taken.

De-O-acetylation of the fully protected compounds were performed in 0.05M NaOH solution for 2 hours. For lactosamine-containing dendrimers **98**, **102** and **106** which were partially insoluble in aqueous media, DMSO was added to the solution to help solubilization. However, these compounds turned out suffering from β-elimination (the corresponding elimination products, acryloyl protons were observed at 6.33 and 5.93 ppm for $H_{c,a}$ ($J_{a,c}$= 10.3, $J_{c,b}$ <1 Hz) and H_b($J_{b,a}$= 8.6 Hz), respectively in the ^{1}H-NMR spectra of **100**, **104** and **108** with 10-15 % of the integration.) The ^{1}H NMR spectra of lactose-containing dendrimers **99**, **103** and **107** did not show these β-elimination products which were obtained under the same condition without DMSO. This observation can be explained by understanding the property of DMSO as solvent. DMSO possesses a dual properties as solvent, i.e. DMSO molecules solvate cation extremely well but anion very poorly.[161]

161. C. Reichardt, *"Solvents and Solvent Effects in Organic Chemistry"*, 2nd ed., VCH Verlagsgeselschaft GmbH, Weinheim, 1988.

V.2.3. Double-Immunodiffusion of the Glycodendrimers.

In order to evaluate the binding properties of the prepared glycodendrimers, double-immunodiffusion experiments were performed. The glycodendrimers used in this experiment include the first and second generation gallic acid-core dendritic lactosides **89** and **91**, the di-, tetra- and octamers of the lysine-core dendritic lactosides **99**, **103** and **107**. These lactose-containing dendrimers were let diffuse against peanut lectin from *Arachis Hypogaea*. Along with these dendrimers, lactose-containing copolymer **39** was used as a positive control and glucosamine-containing copolymer **109** was used as a negative control (**Scheme 5.15**). The results obtained from the above experiments are listed in **Table 5.3.**

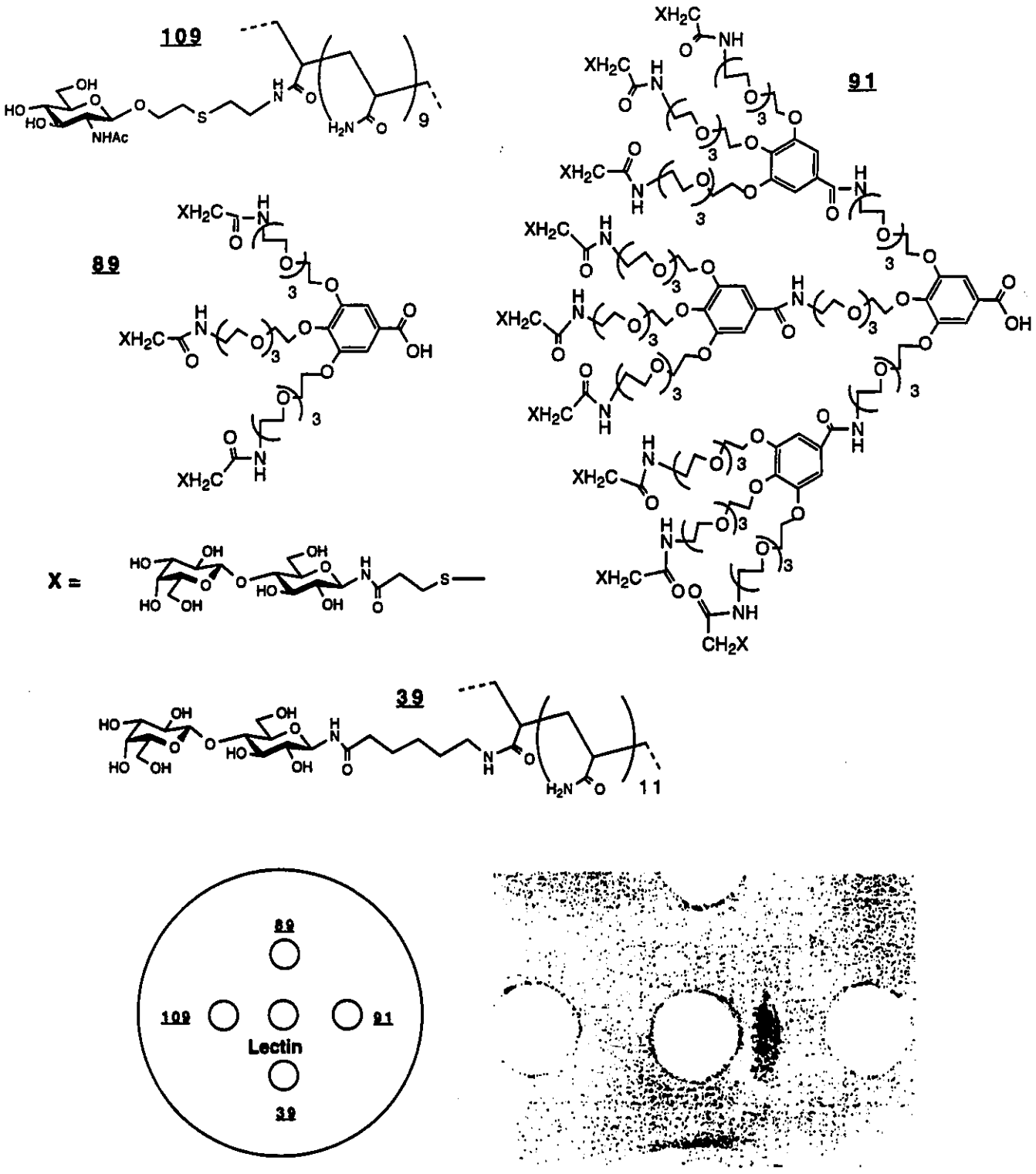
Table 5.3. Double-immunodiffusion results of the glycodendrimers vs Peanut Lectin from *Arachis Hypogaea*.

Compounds[a]	Results
89	-
91	+
99	-
103	+
107	+
39	+
109	-

a. The stock solutions were prepared by dissolving *ca* . 2 mg of the sample in 1 mL of H_2O.

The representative results of the double-immunodiffusion experiment of **89** (well **B**) and **91** (well **C**) are shown in **Scheme 5.15.** The most striking result includes that the nona-valent dendrimer **91** showed an intense precipitin band which is indicative

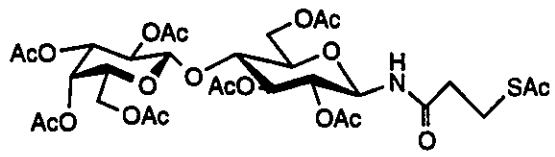
of a strong binding interaction between the dendrimer and the lectin. However, the tri-valent dendrimer **89** showed no pricipitin band. The concentrations of the dendrimer were 0.23 μM for **91** and 0.56 μM for **89**. This indicates the tri-valent dendrimer was nearly three times more concentrated and thus nearly equal numbers of the lactose units must be available for binding. Even when the same experiment was performed with a doubled concentration (1.12 μM) of **89** keeping the others the same, still no binding was observed for **89**. This implies that the tri-valent dendrimer may exert weaker binding than the nona-valent dendrimer towards the lectin. Another possibility involves that the precipitin complex of trivalent dendrimer is more soluble than that of nonavalent dendrimer. The above observation is consistent with the concept of the multivalency effect, and persistent to the L-lysine-core dendrimers. While tetra- and octameric lactosides **103** and **107**, respectively were showing the precipitin bands with the lectin, the dimer **99** showed no band formation. These results, although qualitative, clearly show the existence of the multivalency effect and one aspect of usefulness of the glycodendrimers.



Scheme 5.15. Double immunodiffusion of lactose dendrimers with *peanut lectin* from Arachis Hypogaea.

V.3. Experimental Methods.

N-3-thioacetylpropyl (2,3,4,6-tetra-O-acetyl-β-D-galacto pyranosyl)-(1-4)-O-2,3,6-tri-O-acetyl-β-D-glucopyranosyl amine, (56).

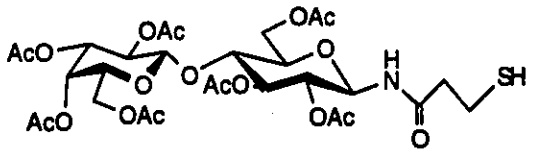


56

To an EtOAc (10 mL) solution containing **25** (2.84 g, 4.1 mmol) was added thiolacetic acid (0.32 mL, 4.5 mmol) which was diluted with EtOAc (2.0 mL). The reaction was performed at ambient temperature and monitored by TLC using an 8: 2 mixture of EtOAc and hexane, respectively. The reaction rate was found to be accelerated by adding a catalytic amount of triethylamine. The reaction was complete within 5 h. The reaction mixture was treated with saturated $NaHCO_3$ solution (2 x 5 mL), washed with water (2 x 10 mL) and a saturated NaCl solution (2 x 10 mL). The organic phase was dried over $MgSO_4$ and filtered. The filtrate was then evaporated under reduced pressure to give **56** as a white amorphous solid (2.98 g, 95 %). M.p. 77-79° C; $[\alpha]_D$ +1.12 (c=1.0, $CHCl_3$); ^{1}H-NMR spectral data :($CDCl_3$, δ ppm): 6.24 (d, 1H, $J_{NH,1}$ = 9.3 Hz, N-H), 5.31 (dd, 1H, $J_{4',5'}$= 1, $J_{3',4'}$ = 3.4 Hz, H-4'), 5.24 (dd, 1H, $J_{3,4}$ = 9.5, $J_{3,2}$ = 9.5 Hz, H-3), 5.17 (dd, 1H, $J_{1,2}$ = 9.4, $J_{NH,1}$= 9.4 Hz, H-1), 5.06 (dd, 1H, $J_{2',3'}$ =10.4 Hz, $J_{1',2'}$ = 7.9Hz H-2') , 4.90 (dd, 1H, $J_{3',4'}$=3.4, $J_{2',3'}$ = 10.4 Hz, H-3'), 4.78 (dd, 1H, $J_{1,2}$ = 9.6Hz, $J_{2,3}$= 9.6 Hz, H-2) , 4.42 (d, 1H, $J_{1',2'}$=7.9 Hz, H-1'), 4.37-4.40 (m, 1H, H-6_b), 4.08- 4.12 (m, 1H, H-6_a), 4.01-4.17 (m, 2H, H-6'), 3.82-3.85 (m, 1H, H-5'), 3.72 (dd, 1H, $J_{4,3}$ = 9.9, $J_{4,5}$ = 8.7 Hz, H-4) 3.67-3.70 (m, 1H, H-5), 3.03-3.08 (m, 2H, C(O)-CH_2-CH_2-SC(O)-), 2.35-2.51 (m, 2H, CH_2-SC(O)-), 2.28 (s, 3H -SC(O)CH_3), 1.92, 2 x 2.00, 2.01, 2.02, 2.08, 2.11, (7 s, 3H each 7 x C(O)CH_3) ; ^{13}C-NMR spectral data: 20.5, 20.6,20.8,20.9 (7 x CO<u>C</u>H_3), 24.2 (1C,-<u>C</u>H_2-CH_2SC(O)-), 30.6 (1C, -SCO<u>C</u>H_3), 36.2 (1C, -CH_2-<u>C</u>H_2-SAc), 60.8 (C-6'), 61.9 (C-6), 66.6

(C-4'), 69.0 (C-2'), 70.7 (C-5'), 70.9 (C-2), 71.0 (C-3'), 72.5 (C-3), 74.6 (C-5), 75.9 (C-4), 78.0 (C-1), 100.9 (C-1'); FAB-MS: calcd for $C_{31}H_{43}NO_{19}S$, 765.22; found, 766.2 (M+1, 21.4 %); Anal. Calcd. for $C_{31}H_{43}NO_{19}S$, C 48.66, H 5.66, N 1.83. Found C 48.40, H 5.51, N 1.77.

N-3-thiopropyl (2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1-4)-O-2,3,6-tri-O-acetyl-β-D-glucopyranosyl amine, (57).



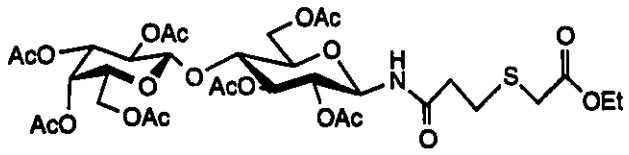
57

To a previously degassed DMF solution (1 mL) containing **56** (0.46 g, 0.60 mmol) was added a small quantity of hydrazinium acetate (460 µL of a 2.5 M stock solution in DMF) under nitrogen atmosphere at ambient temperature. The reaction was monitored by TLC using EtOAc as an eluent and was complete within 20 minutes. Using degassed EtOAc and water as two phase solvent system, the thiol **57** was separated from the aqueous layer. The organic phase was washed with water several times to minimize DMF being mixed in the organic phase and followed by saturated NaCl solution (2 x 10 mL). The organic phase was then dried over Na_2CO_3, filtered and evaporated under reduced pressure. The resulting amorphous white solid was then used immediately for the next step without further purification.

Upon exposure of some **57** to air for several days the disulfide **58** was formed: m.p. 130-134° C; $[\alpha]_D$ = -23.3° (c=1.0, $CHCl_3$); 'H-NMR spectral data ($CDCl_3$, δ ppm) are as follows: 1,94, 2.02, 2 x 2.03, 2.04, 2.09, 2.13 (7 s, 3H each, 14x $COCH_3$), 2.50-2.60 (m, 4H, S-S-CH_2-), 2.83-2.92 (m, 4H, -S-S-CH_2-CH_2-C(O)), 3.72-3.74 (m, 2H, H-5), 3.76 (dd, 2H, $J_{3,4}$ = 9.8, $J_{4,5}$ = 8.8Hz, H-4), 3.84 - 3.86 (m, 2H, H-5'), 4.01-4.15 (m, 6H, H-6, 2 X H-6'), 4.40 (dd, 2H, $J_{6,6}$=12.6, $J_{6,5}$ = 2.2 Hz, H-6), 4.45 (d, 2H, $J_{1',2'}$ = 7.9 Hz, H-1'), 4.82 (dd, 2H,

$J_{2,1}$= 9.5, $J_{2,3}$ = 9.6Hz, H-2), 4.93 (dd, 2H, $J_{3',2'}$ = 10.4, $J_{3',4'}$ = 3.4 Hz, H-3'), 5.08 (dd, 2H, $J_{2',3'}$=10.5, $J_{2',1'}$ = 8.0 Hz, H-2'), 5.22 (dd, 2H, $J_{NH,1}$= 9.3, $J_{1,2}$ = 9.4 Hz, H-1), 5.27 (dd, 2H, $J_{3,4}$ = 9.2Hz, $J_{3,2}$ = 9.3 Hz, H-3), 5.33 (dd, 2H, $J_{4',3'}$ = 3.3 Hz, H-4'), 6.52 (d, 2H, $J_{NH,1}$ =9.2Hz, N-H) ;^{13}C-NMR spectral data: 20.5, 20.6, 20.8,20.9 (14 x $C(O)CH_3$), 33.1 (2C, S-S-CH_2-), 35.6 (2C, S-S-CH2-$\underline{C}H_2$-C(O)-), 60.8 (2C, C-6'), 61.9 (2C, C-6), 66.6 (2C, C-4'), 69.0 (2C, C-2'), 70.7 (2C, C-5'), 70.9 (2C, C-2), 71.0 (2C, C-3'), 72.5 (2C, C-3), 74.6 (2C, C-5), 75.9 (2C, C-4), 78.0 (2C, C-1), 100.9(2C, C-1'). 168.9, 170.0, 170.1, 170.3, 171.1, 171.3 (16C, C(O)); FAB-MS Calcd. for: $C_{58}H_{80}N_2O_{36}S_2$, 1445.42. Found, 1446.3 (M+1, 10.3 %)

N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1-4)-O-2,3,6-tri-O-acetyl-β-D-glucopyranosyl 3-thiopropanamido ethylacetate (59)

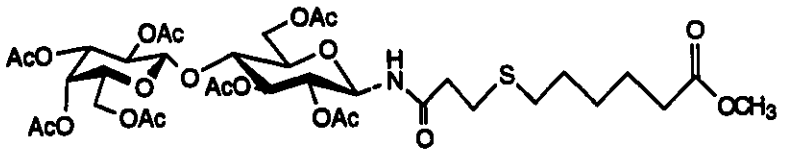


59

Freshly prepared 57 (50 mg, 0.065 mmol) was dissolved in degassed EtOAc (0.5 mL). To this solution under N_2 atmosphere was added ethylbromoacetate (13 mg, 0.078 mmol), followed by triethylamine (6.6 mg, 9μL, 0.065 mmol) while stirring at ambient temperature. The reaction was allowed to proceed overnight and no disulfide 58 was observed. The reaction mixture was washed with H_2O (2 x 5 mL) and saturated NaCl solution (2 x 5 mL). The organic layer was then dried with Na_2CO_3 and filtered. The filtrate was concentrated under reduced pressure to give a light-yellow foam of mixtures. This mixture was then separated by column chromatography using a gradient of solvent mixtures (1: 1 to 4: 1 EtOAc: Hexane, respectively) as eluent to give an amorphous solid (46 mg, 87 %). The physical data of the desired product are given as follows: m.p. 71-74° C; $[\alpha]_D$ = -2.73° (c=1.0, $CHCl_3$); ^{1}H-NMR spectral data ($CDCl_3$, δ ppm): 1.26 (t, 3H, -CH_2-$C\underline{H}_3$), 1.94, 2.02, 2x2.03, 2.04, 2.09, 2.13 (7 s, 3 H each, 7x $C(O)CH_3$), 2.41-2.54 (m, 2H,

-C(O)CH_2-CH_2-S-), 2.83-2.91 (m, 2H, -C(O)CH_2-CH_2-S-), 3.20(s, 2H, -S-CH_2-C(O)-), 3.67-3.72(m, 1H, H-5), 3.75(dd, 1H, $J_{4,3}$= 10.0, $J_{4,5}$= 8.7 Hz, H-4), 3.84(m, 1H, H-5'), 4.02-4.14 (m, 2H, H-6'), 4.06-4.14 (m, 1H, H-6), 4.16(q, 2H, -CH_2-CH_3), 4.40 (m, 1H, H-6), 4.43 (d, 1H, $J_{1',2'}$= 7.9 Hz, H-1'), 4.82(dd, 1H, $J_{1,2}$= 9.5, $J_{2,3}$= 9.6 Hz, H-2), 4.91(dd, 1H, $J_{2',3'}$= 10.4, $J_{3',4'}$= 3.4 Hz, H-3'), 5.08 (dd, 1H, $J_{1',2'}$= 10.4, $J_{1',2'}$= 3.4 Hz, H-2'), 5.20 (dd, 1H, $J_{NH,1}$= 9.3, $J_{1,2}$= 9.3 Hz, H-1), 5.26 (dd, 1H, $J_{2,3}$= 9.5, $J_{3,4}$= 8.9 Hz, H-3), 5.32 (dd, 1H, $J_{3',4'}$=3.4, $J_{4',5'}$= 0.8 Hz, H-4'), 6.41(d, 1H, J= 9.2 Hz, N-H); ^{13}C-NMR spectral data (based on HMQC results): 20.5, 20.6, 20.9 (14x C(O)CH_3), 28.2 (C(O)-CH_2-CH_2-S-), 34.0(-S-CH_2-C(O)-OEt), 36.6(C(O)-CH_2-CH_2-S-), 60.8(C-5'), 61.9(C-6), 62.0 (C(O)-O-CH_2CH_3), 66.6 (C-4'), 69.0 (C-2'), 70.7 (C-5'), 70.9 (C-2), 71.0 (C-3'), 72.5 C-5), 75.9 (C-4), 78.0 (C-1), 100.9 (C-1'); FAB-MS Calcd. for: $C_{33}H_{47}NO_{20}S$, 809.24. Found, 810.2 (M+1, 22.4 %).

N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1-4)-O-2,3,6-tri-O-acetyl-β-D-glucopyranosyl 3-thiopropanamido methylhexanoate, (60).

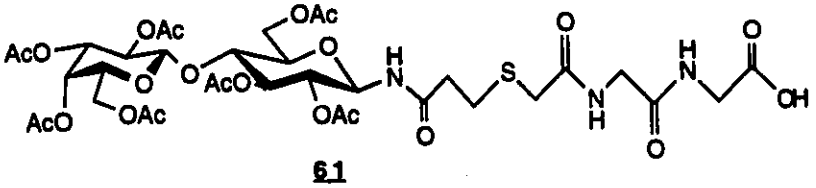


60

To a EtOAc solution (0.5 mL) containing freshly prepared **57** (250 mg, 0.326 mmol) was added methyl 6-bromohexanoate (68.2 mg, 0.326 mmol) and 1 equiv. of triethylamine. The reaction mixture was kept under N_2 atmosphere at ambient temperature for 2 h. The reaction was monitored by TLC using EtOAc as eluent. There was no detectable amount of **57** left after 1 h. The separation of the desired product and purification were performed in a similar manner as described previously to give a light yellow liquid (227 mg, 82%). The physical data obtained are as follows: m.p. liquid; $[\alpha]_D$ = +6.98° (c=1.0, $CHCl_3$); ^{1}H-NMR spectral data ($CDCl_3$, δ ppm): 1.38(m, 2H, H_c),

1.61(m, 2H, H_d), 1.62(m, 2H, H_b), 2x 2.02, 2.04, 2.09, 2.13, 2.14 (7 s, 3H each, 7x C(O)CH_3), 2.29(t, 2H, $J_{a,b}$=7.5 Hz, H_a), 2.34(m, 1H, H_g), 2.48(m, 1H, $H_{g'}$), 2.74(m, 2H, H_f), 3.64(s, 3H, -OCH_3), 3.68-3.72(m, 1H, H-5), 3.73(dd, 1H, $J_{4,3}$= 9.9, $J_{4,5}$= 8.7 Hz, H-4), 3.83-3.86 (m, 1H, H-5'), 4.03-4.14 (m, 2H, H-6'), 4.09-4.12(m, 1H, H-6), 4.38-4.41(m, 1H, H-6), 4.43 (d,1H, $J_{1',2'}$= 7.9 Hz, H-1'), 4.81 (dd, 1H, $J_{1,2}$= 9.6, $J_{2,3}$=9.6 Hz, H-2), 4.92 (dd,1H, $J_{3',4'}$= 3.4, $J_{2',3'}$= 10.4 Hz, H-3'), 5.07(dd, 1H, $J_{2',3'}$=10.4, $J_{1',2'}$= 7.9 Hz, H-2'), 5.19 (dd, 1H, $J_{NH,1}$= 9.3, $J_{1,2}$= 9.3 Hz, H-1), 5.27 (dd, 1H, $J_{3,4}$= 9.6, $J_{2,3}$= 9.5 Hz, H-3), 5.32 (dd, 1H, $J_{4',5'}$=1, $J_{3',4'}$= 3.4 Hz, H-4'), 6.31(d 1H, $J_{NH,1}$= 9.2 Hz, N-H); ^{13}C-NMR spectral data: 2x 20.7,4x 20.8, 20.9 (7x C(O)CH3), 24.5(C_b), 27.1(C_f), 28.2(C_c), 29.1(C_b), 32.1(C_e), 33.9(C_a), 36.8(C_g), 51.5(O-CH_3), 60.9(C-6'), 62.0(C-6), 66.6(C-4'), 69.0(C-2), 71.0(C-3'), 72.3(C-3), 74.5(C-5), 76.0(C-4), 78.0(C-1), 100.9(C-1'), 168.9, 169.3, 170.0, 170.1, 2x 170.3, 2x 171.3(7C, C(O)-CH_3), 170.4(-NHC(O)-), 174.0(-C(O)-OCH_3); FAB-MS calcd. for: $C_{36}H_{53}NO_{20}S$, 851.3; found, 852.3 (M+1, 33.5 %).

N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1-4)-O-2,3,6-tri-O-acetyl-β-D-glucopyranosyl 3-thiopropanamido acetylglycylglycine, (61).



61

To a freshly degassed DMF (0.5 mL) solution containing 57 (50 mg, 0.065 mmol) were added chloroacetylglysylglysine (9.5 mg, 0.046 mmol) and triethylamine (1 equivalent). The reaction was stirred under N_2 atmosphere and at ambient temperature for overnight. After 15 h no detectable amount of 57 was observed. The separation of the desired product was performed by a column chromatography using a mixture of MeCN and water (8:2) as eluent. The purified product was then concentrated under reduced pressure to give a white solid (44 mg, 76%). The physical data obtained are

as follows: m.p. 113-119° C; $[\alpha]_D$ = +2.93° (c=1.0, DMSO); ^{1}H-NMR spectral data ($CDCl_3$, δ ppm): 1.88, 1.91, 1.95, 1.98, 2.00, 2.05, 2.09 (7 s, 3H each, 7x $C(O)CH_3$), 2.39(m, 2H, $C(O)C\underline{H}_2$-CH_2-S-), 2.73(m, 2H, C(O)-CH_2-$C\underline{H}_2$-S-), 3.15(d, 2H, J=1.1 Hz, -S-CH_2-C(O)-), 3.38(broad, 2H, -CH_2-C(O)OH), 3.69(d, 2H, $J_{NH,CH}$=5.7 Hz, -NH-CH_2-C(O)NH-), 3.74(dd, 1H, $J_{3,4}$= 9.4, $J_{4,5}$= 9.5 Hz, H-4), 3.86(m, 1H, H-5), 3.97(m, 1H, H-6), 3.99-4.02(m, 2H, H-6'), 4.22(m, 1H, H-5'), 4.25(m, 1H, H-6), 4.74(m, 2H, H-1', H-2), 4.83(dd, 1H, $J_{1',2'}$= 8.0, $J_{2',3'}$= 10.2 Hz, H-2'), 5.12(dd, 1H, $J_{3',2'}$= 10.3, $J_{3',4'}$= 3.6 Hz, H-3'), 5.18 (dd, 1H, $J_{2,3}$= 9.3, $J_{3,4}$= 9.4 Hz, H-3), 5.21 (dd, $J_{3',4'}$= 4.3, $J_{4',5'}$< 1 Hz, H-4'), 5.27(dd, 1H, $J_{1,2}$= 9.4, $J_{NH,1}$= 9.4 Hz, H-1), 7.45(broad s, 1H, NH(anomeric)), 8.30 (1H, $J_{NH,CH}$= 5.7 Hz, -C(O)-N$\underline{H}$$CH_2$C(O)NH-), 8.79 (broad, 1H, -N$\underline{H}$-CH_2C(O)OH); ^{13}C-NMR spectral data (based on HMQC spectra): 26.8-27.8 (7x $C(O)CH_3$), 34.1 ($C(O)CH_2\underline{C}H_2$-S-), 40.3 (-S-$\underline{C}H_2$C(O)-), 41.4 (-C(O)$\underline{C}H_2CH_2$-S-), 48.4 (NH$\underline{C}H_2$C(O)NH-), 49.5 (NH$\underline{C}H_2$C(O)OH), 67.2 (C-6'), 69.0 (C-6), 73.2 (C-4'), 75.0 (C-2'), 75.8(C-5'), 76.6(C-3'), 76.9(C-2), 79.0(C-3), 79.2(C-5), 82.1(C-4), 83.0(C-1), 104.7(C-1'); FAB-MS Calcd. for: $C_{35}H_{49}N_3O_{22}S$, 895.3. Found, 896.3 (M+1, 40.3 %).

1,11-di-*p*-tosyl tetraethylene glycol, (<u>76</u>).

<u>76</u>

A solution of tetraethyleneglycol (29.1 g, 0.15 mol) in anhydrous diethylether (170 mL) was cooled to 0 °C under nitrogen atmosphere. To this, was added triethylamine (50 mL) and p-toluenesulfonyl chloride (68.6g, 0.36 mol, 2.4 equiv.). The reaction mixture was stirred for 1 h at 0 °C and was allowed to reach r.t. The reaction was complete within 3 h. The ethereal solution was evaporated under reduced pressure and the residue was then redissolved in CH_2Cl_2. The solution was neutralized with saturated $NaHCO_3$ and washed with water (2 x 25 mL). The CH_2Cl_2 layer was

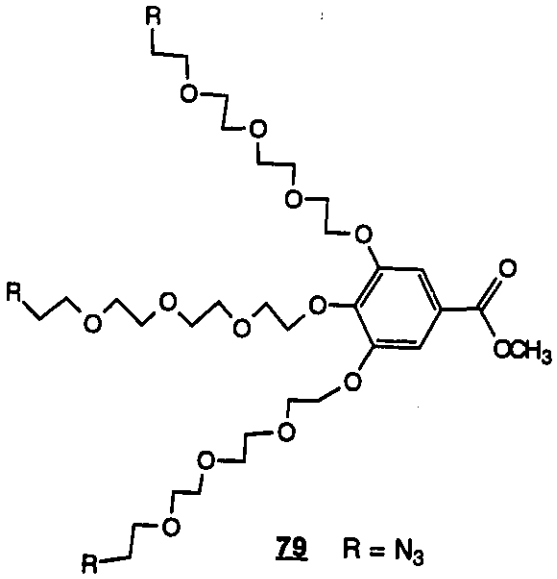
dried over Na_2SO_4 and concentrated under vacuum. Purification of the crude product was performed by column chromatography using a 1: 1 mixture of EtOAc and hexane, respectively as eluent. Removal of the solution yielded **76** as a colorless liquid (61.0 g, 81 %).^{1}H-NMR spectral data ($CDCl_3$, δ ppm): 2.35-2.40 (6H, CH_3), 3.46-3.52 (8H, $-CH_2O-C\underline{H_2}C\underline{H_2}-$), 3.57-3.66 (4H, $-SO_3-CH_2C\underline{H_2}-O-$), 4.05-4.14 (4H, $-SO_3-C\underline{H_2}CH_2-O-$) and 7.42-7.78 (8H, aromatic); ^{13}C-NMR spectral data: 22.2, 69.2, 69.9, 71.1, 71.3, 128.5, 130.4, 133.5, 145.5.

1-Azido, 11-*p*-tosyl tetraethylene glycol, (78).

78

To an ethanolic solution (95%, 300 mL) of **76** (53.3 g, 0.106 mol), was added sodium azide (6.9 g, 0.106 mol). The mixture was refluxed for 4 h and cooled to r.t.. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The concentrated residue was then redissolved in CH_2Cl_2 and washed with water (2 x 70 mL). The organic phase was separated and dried over Na_2SO_4 and filtered. The filtrate was concentrated under vacuum. The desired product was obtained by column chromatography with a gradient mixture of EtOAc and hexane (1: 5 to 1: 1, respectively) as eluent to give **78** as a transparent oil, (16.1 g, 39% : other products, bisazide (7.31 g, 25%) and **76** (10.66 g, 20%). ^{1}H-NMR spectral data ($CDCl_3$, δ ppm): 2.41(s, 3H, CH_3), 3.30-3.40 (2H), 3.54-3.68 (12 H), 4.09-4.16 (2H), and 7.35-7.80 (4H, aromatic); ^{13}C-NMR spectral data: 22.16, 51.18, 69.24, 69.86, 70.61, 71.17, 71.23, 71.32, 128.46, 130.42, 133.46, 145.43.

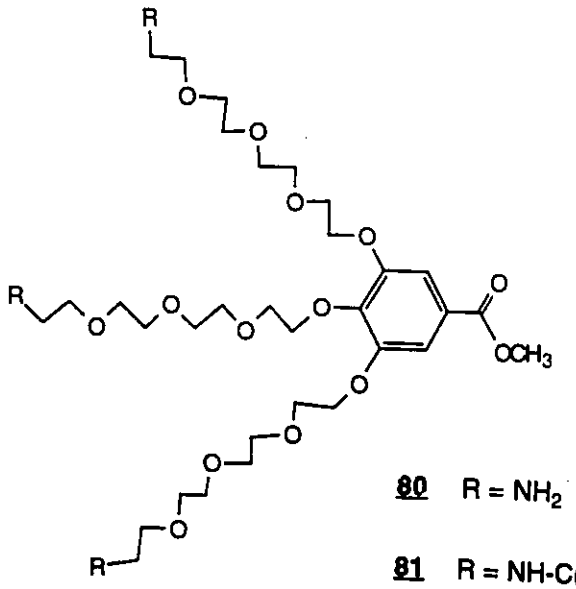
Methyl tri(11-azido tetraethylene glycol) 3,4,5-tri-O-benz oate, (79).



79 R = N_3

To a solution of **78** (4.03 g 10.8 mmol) dissolved in DMF (60 mL) was added methyl 3,4,5-trihydroxybenzoate **77b** (0.564g, 3 mmol) and potassium carbonate (2 g). The mixture was stirred at 80° C for 24 hours to complete the reaction. The mixture was concentrated to *ca.* 10 mL under vacuum. The residue was extracted with CH_2Cl_2 (30 mL) and then washed with water. The organic phase was repeatedly washed with water (2 x 10 mL), dried with Na_2SO_4 and filtered. The filtrate was then evaporated under reduced pressure under vacuum to afford **79** as crude product. This was in turn purified by column chromatography using EtOAc as eluent to give **79** (1.5 g, 68 %) as a colorless syrup; IR (neat): 2897, 2106 (N_3),1717, 1586, 1433, 1334, 1231, 1119, 937, 765 cm^{-1}; ^{1}H-NMR spectral data ($CDCl_3$, δ ppm) 3.31-3.42 (6H), 3.60-3.92 (m, 39H), 4.13-4.25 (6H), 7.27(s 2H, aromatic); ^{13}C-NMR spectral data: 51.17, 52.70, 69.37, 70.13, 70.54, 71.06, 71.18, 71.25, 71.34, 72.94, 109.43, 125.45, 143.02, 152.81, 167.06; FAB-MS Calcd for $C_{32}H_{53}N_9O_{14}$ 787.4. Found 788.4 (M+1).

Methyl tri(11-chloroacetylamido tetraethylene glycol) 3,4,5-tri-O-benzoate, (81).



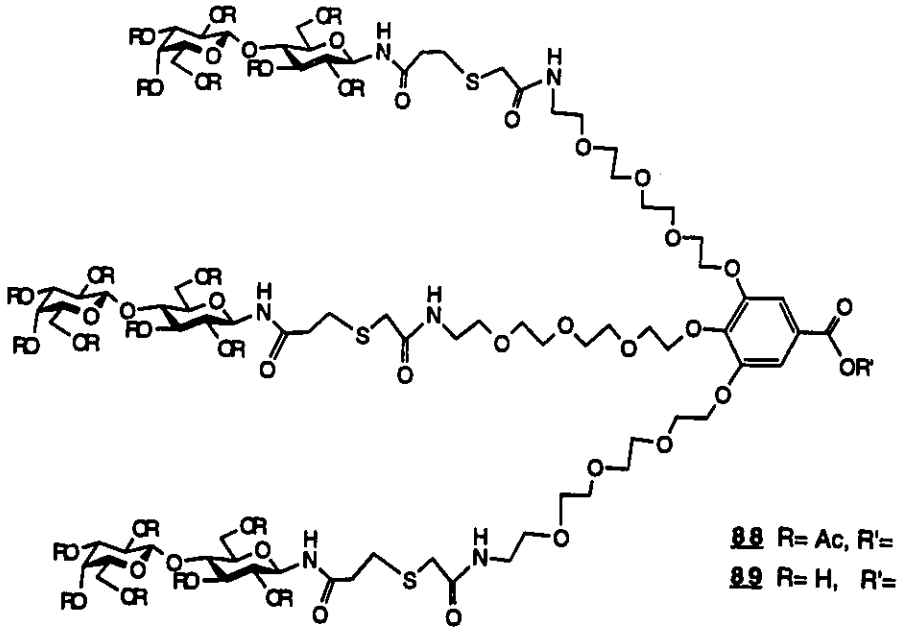
80 R = NH_2

81 R = NH-C(O)-CH_2Cl

To a solution of **79** (102 mg, 0.13 mmol) in EtOH (2 mL) was added Pd catalyst (10% Pd/C, 50 mg, Aldrich). The suspension was stirred for 3 hours under H_2 environment and filtered. The filtrate was directly added to a mixture of triethylamine (0.5 mL, 3.6 mmol) and chloroacetic anhydride (276 mg, 1.4 mmol). The mixture was stirred for 2 hours at ambient temperature. The solution was evaporated under reduced pressure. The resulting residue was diluted with water and extracted with methylene chloride. The organic layer was dried over Na_2SO_4 and filtered. The filtrate was then concentrated under vacuum to yield the crude product which was purified by column chromatography. MeOH in CH_2Cl_2 (5 v %) was used as eluent to give **81** as a transparent syrup(76 mg, 62 %). IR (neat): 3425, 2903, 2247, 1714, 1671, 1588, 1534, 1434, 1337, 1242, 1116, 925 cm^{-1}; ^{1}H-NMR spectral data ($CDCl_3$, δ ppm): 3.40-3.89 (m, 45H), 4.01 (s, 6H, Cl-CH_2-), 4.12-4.24 (m, 4H), 7.10 (b, 3H), 7.28(s, 2H); ^{13}C-NMR spectral data: 40.17, 43.25, 52.83, 69.39, 69.97, 70.22, 70.93, 71.15, 71.25, 71.41, 73.02, 109.50, 125.64,

152.80, 166.66; FAB-MS Calcd. for $C_{38}H_{62}N_3O_{17}Cl_3$ 939.3. Found 940.3.

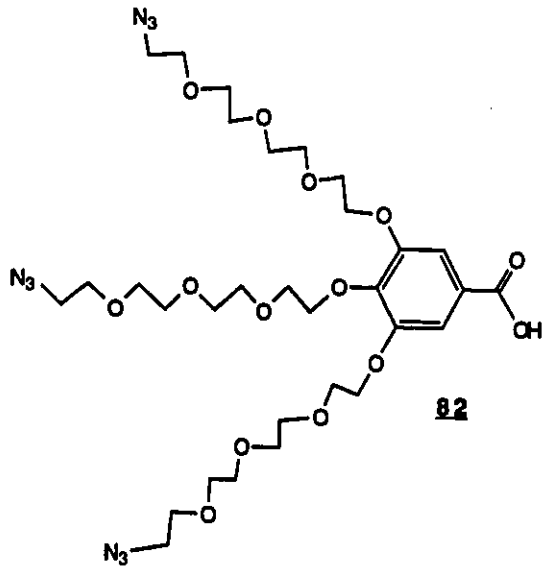
Methyl tri(N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1-4)-O-2,3,6-O-acetyl-β-D-glucopyranosyl 3-thio-propanamido 11-acetamido-tetraethylene glycol)-3,4,5-tri-O-benzoate, (88).



To a MeCN (0.5 mL) solution containing **81** (13 mg, 0.014 mmol) was added freshly prepared **57** (43 mg, 0.055 mmol) and triethylamine (added until pH 9) while nitrogen was bubbled through the solution. The reaction mixture was stirred overnight at room temperature. The reaction was monitored by TLC using acetone as eluent. The reaction was complete within 12 h. The reaction mixture was evaporated under reduced pressure and the resultant residue was purified by column chromatography using acetone as eluent to give **88** as a concentrated syrup (41 mg, 98 %). ^{1}H-NMR spectral data ($CDCl_3$, δ, ppm): peracetylated lactose unit(all integrations were 3 x of magnitude): 5.32(dd, 1H, $J_{4',5'}$= 3.4 Hz, H-4'), 5.21-5.26(m, 2H, H-1,3), 5.07(dd, 1H, $J_{2',3'}$= 10.4Hz, H-2'), 4.92(dd,

1H, $J_{3',4'}$= 3.4 Hz, H-3'), 4.83(dd, 1H, $J_{2,3}$= 9.5 Hz, H-2), 4.45(d, 1H, $J_{1',2'}$= 7.9 Hz, H-1'), 4.40(b.d. 1H, H-6a), 4.09-4.14(m, 2H, H-6_b, 6'a), 4.02-4.06(m, 1H, H-6'$_b$), 3.82-3.86(m, 1H, H-5'), 3.46-3.71(m, 1H, H-5), 2.60-2.82(m, 2H, NHC(O)-C$\underline{H_2}$-CH_2-S-), 2.43-2.56(m, 2H, NHC(O)-CH_2-C$\underline{H_2}$-S-), 2.15, 2.13, 2.07, 2.03, 2.02, 2.01, 1.94 (O$\underline{Ac}$); dendrimer core unit: 7.27(s, 2H, aromatic), 4.17(m, 6H, Ph-O-C$\underline{H_2}$-CH_2-O), 3.86(s, 3H, OCH_3), 3.82-3.86(m, 6H, TEG-CH_2), 3.46-3.71(m, 42H, TEG-CH_2-), 3.20(m, 6H, -SCH_2-C(O)); ^{13}C-NMR spectral data: 166.4-171.5(C=O), 152.1, 124.1, 188.8(C_o) (aromatic), 100.8(C-1'), 77.7(C-1), 75.9(C-4), 74.5(C-5), 72.7(C-3), 68.7-28.4 (14 TEG carbons were found), 66.5(C-4'), 61.9(C-6), 60.6(C-6'), 52.2(OCH3), 42.5(TEG-O-$CH_2\underline{C}H_2$-NHC(O)-), 39.6(-S$CH_2\underline{C}H_2$C(O)-), 39.5(S-$\underline{C}H_2$C(O)-), 36.2(-S$\underline{C}H_2CH_2$C(O)-), 20.4, 20.5x2, 20.6x2, 20.7, 20.8 (-C(O)$\underline{C}H_3$).

Tri(11-azido tetraethylene glycol) 3,4,5-tri-$\underline{O}$-benzoic acid, ($\underline{82}$).

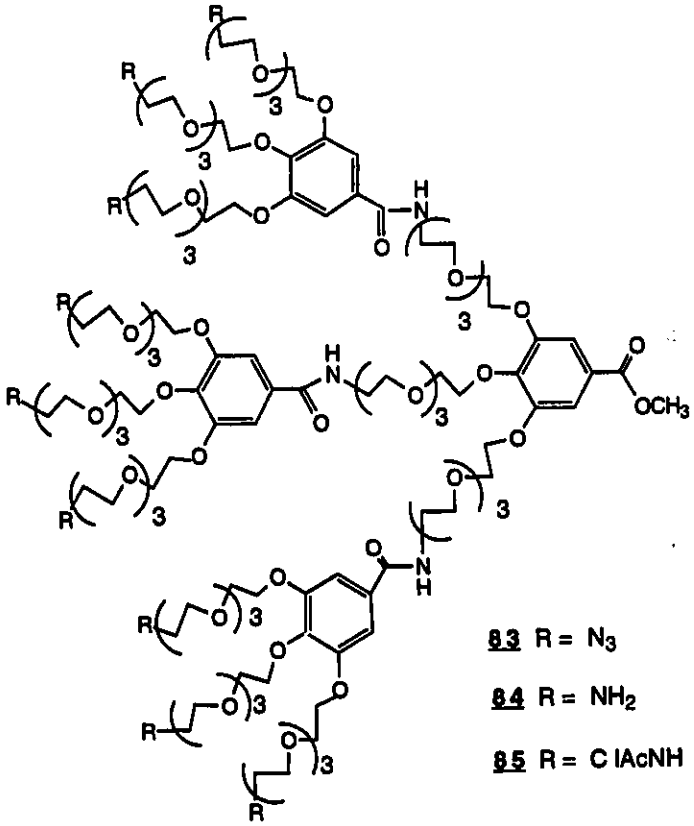


82

To an ethanolic solution (6 mL) of **$\underline{81}$** (170 mg, 0.215 mmol) was added 1 M KOH and refluxed for 2 hours. The solution was then cooled and neutralized with Amberlite Cation Exchange resin (H^+120) and filtered. The filtrate was evaporated under reduced pressure. The residue was then extracted with CH_2Cl_2, washed, dried

and concentrated to yield the **82** (167 mg, quantitative). The product was immediately used for the next step without further purification.

Methyl tri [tri(11-azido tetraethylene glycol) 3,4,5-tri-O-benzamido tetraethylene glycol] 3,4,5-tri-O-benzoate, (83).



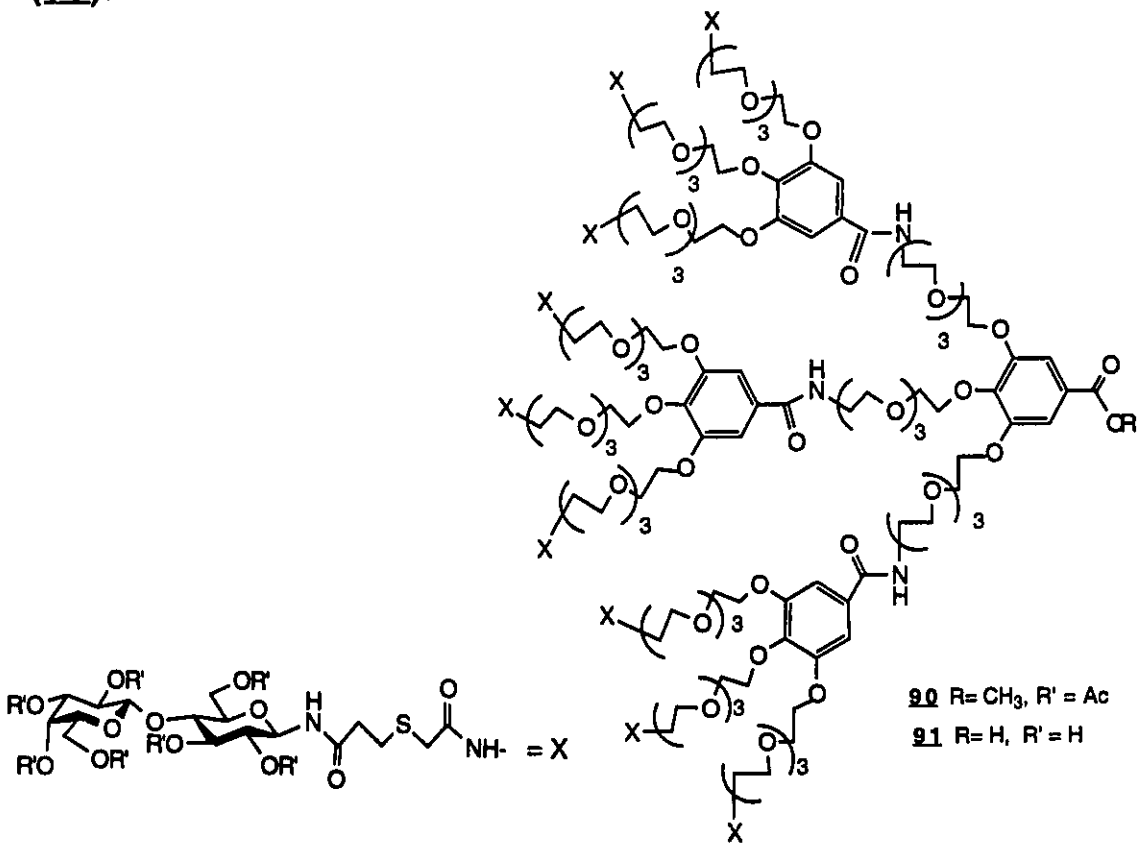
A solution of **82** (197 mg, 0.254 mmol) in a 1: 1 mixture of EtOH and MeCN (2.0 mL), respectively was added to a mixture of 1-hydroxybenzotriazole hydrate (HOBt, 50 mg, 0.0704 mmol) and the

reduction product of **80** (50 mg, 0.07 mmol). The mixture was stirred for 5 minutes and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide· hydrochloride (EDC, 73 mg, 0.381 mmol). The mixture was then further reacted for 3 h at ambient temperature. After removing the solvent, the residue was washed with water and dried over Na_2SO_4. The crude product was then evaporated and purified by column chromatography using 5 % (v/v) MeOH in CH_2Cl_2 (165 mg, 83%). IR (neat); 3686, 2898, 2246, 2108, 1713, 1653, 1584, 1525, 1330, 1117 cm^{-1}; ^{1}H-NMR spectral data ($CDCl_3$, δ ppm) 3.30-3.33 (m, 18 H), 3.56-3.82 (m, 153H), 4.10-4.15 (m, 24H), 6.70-7.26 (m, 11H); ^{13}C-NMR spectral data: 39.90, 39.93, 50.66, 52.14, 68.81, 69.12, 69.58, 69.69, 69.82, 69.98, 70.24, 70.51, 70.55, 70.60, 70.65, 70.69, 70.72, 70.77, 72.38, 107.21, 109.09, 124.99, 129.70, 129.75, 141.46, 142.53, 152.22, 152.46, 166.47, 166.95.

Methyl tri [tri (11-N-acetylchloroamido tetraethylene glycol) 3,4,5-tri-O-benzamido tetraethylene glycol] 3,4,5 -tri-O-benzoate, (85).

The same procedure for the preparation of **81** was used. Yield: 78 %, Purification by column chromatography using 5 v% MeOH in CH_2Cl_2 gave pure product **85**. IR (neat): 3344, 2903, 1664, 1558, 1497, 1433, 1335, 1241, 1108 cm^{-1}; ^{1}H-NMR spectral data ($CDCl_3$, δ ppm): 3.42-3.85 (m, 171H), 4.01(d, 18H), 4.13-4.18 (m, 24H), 7.00-7.24 (m, 20H); ^{13}C-NMR spectral data: 39.61, 39.94, 42.65, 52.20, 68.77, 69.02, 69.38, 69.53, 69.57, 69.70, 69.91, 70.27, 70.33, 70.47, 70.51, 70.54, 70.62, 70.65, 70.71, 70.73, 72.40, 107.23, 109.04, 125.09, 129.76, 129.82, 141.24, 142.37, 152.19, 152.36, 166.17, 166.47, 167.03.

Methyl tri[tri(N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1-4)-O-2,3,6-O-acetyl-β-D-glucopyranosyl 3-thiopropanamido 11-acetamido tetraethylene glycol) 3,4,5-tri-O-benzamido tetraethylene glycol] 3,4,5-tri-O-benzoate, (90).



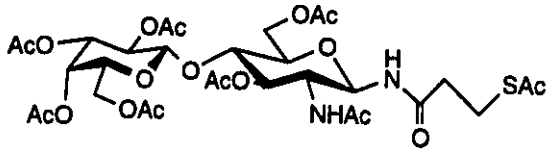
To a solution of **85** (16 mg, 4.7 μmol) dissolved in a mixture (0.50 mL) of 5% MeCN in DMSO, was added freshly prepared **57** (42 mg, 0.054 mmol) and triethylamine (10 μL). The reaction was stirred under N_2 atmosphere overnight. The progress of the reaction was monitored by TLC using a 8: 92 mixture of MeOH and CH_2Cl_2, respectively as eluent. After the reaction was completed the solution was evaporated under reduced pressure followed by lyophilization to remove remaining DMSO. The residue was then further purified by preparative TCL plate (41 mg, 88 %). ^1H NMR spectral data (DMSO-d_6, δ ppm): peracetylated (All integration were x 9 in magnitude): 5.27(dd, 1H, $J_{1,2}$= 9.4Hz, H-1), 5.21(dd, 1H, $J_{4',5'}$<1

Hz, H-4), 5.18(dd, $J_{3,4}$= 9.4Hz, H-3), 5.12(dd, 1H, $J_{3',4'}$= 3.6Hz, H-3'), 4.83(dd, 1H, $J_{2',3'}$= 8.1 Hz, H-2'), 4.73(dd, 1H, $J_{2,3}$= 8.0 Hz, H-2), 4.71(d, 1H, $J_{1',2'}$= 9.4Hz, H-1'), 4.19-4.26(m, 2H, $H6_a$, H-5'), 3.89-4.04(m, 4H, H-$6_{a',b}$, 6_b, 4), 3.76-3.87(m, 1H, H-5), 2.08, 2.04, 1.99, 1.98, 1.95, 1.91, 1.88(OAc); dendron 2nd generation core unit: 7.22(s, 2H, aromatic H_o, 1st generation), 7.16(s, 6H, aromatic H_o, 2nd generation), 4.05-4.12(m, 36H, TEG-CH2-), 3.17-3.74(m, TEG-CH2-), 3.15(s, 18H, -SCH_2-C(O)-), 2.66-2.72(m, 18H, -SCH_2-CH_2-C(O)-), 2.35-2.45(m, 18H, -SCH_2-CH_2-C(O)-). Both FAB-MS and Ion spray-MS have failed to yield the molecular ion peak.

Tri[tri(N-(β-D-galactopyranosyl)-(1-4)-O-β-D-glucopyranosyl 3-thio-propanamido 11-acetamido tetraethylene glycol) 3,4,5-tri-O-benzamido tetraethylene glycol] 3,4,5-tri-O-benzoic acid, (91).

To an ethanolic solution (200 μL) containing **90** (25 mg, 2.5 μmol) was added a NaOH solution (100 μL of 1.0 M stock solution (95% EtOH)). The reaction was stirred for 6 hours at room temperature to complete. The resultant solution was neutraized with H^+ resin and filtered. The filtrate was then diluted with Et_2O at 4° C to result in a light yellow solid (18 mg, quatitative). ^{1}H-NMR spectral data (DMSO-d_6, δ ppm): the spectrum was complicated to analyze. However, key signals were found with intergral consistency: 7.22(s, 2H, aromatic H_o, 1st generation), 7.16(s, 6H, aromatic H_o, 2nd generation), 4.74(dd, 1H, $J_{1,2}$= 9.0Hz, H-1), 4.20(d, 1H, $J_{1',2'}$= 7.0 Hz, H-1'), 3.10(s, 18H, -SCH_2C(O)-), 2.71-2.76 (m, 18H, -SCH_2CH_2C(O)-), 2.37-2.42 (m, 18H, -SCH_2CH_2C(O)-).

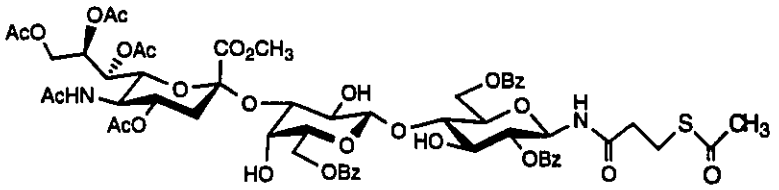
N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1-4)-O-2-N-acetamido-3,6-di-O-acetyl-β-D-glucopyranosyl 3-thio acetylpropanamide, (86).



86

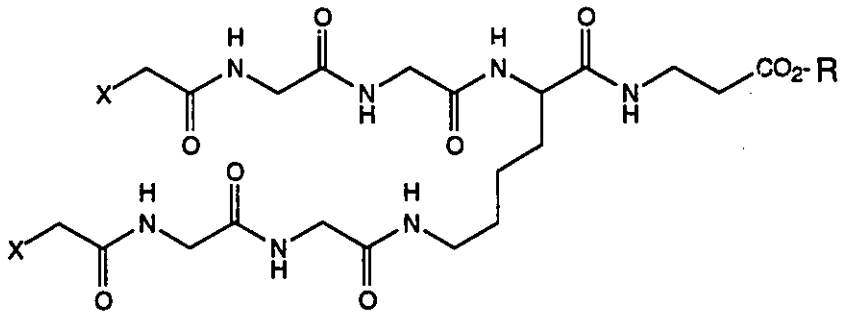
To an EtOAc solution (1 mL) containing 27 (183 mg, 0.266 mmol) was added AcSH(20 μL, 1.1 equiv.) followed by a catalytic amount of Et_3N (2 μL). The reaction mixture was stirred at room temperature under N_2 atmosphere for 3 hours. The progress of the reaction was monitored by TLC using EtOAc as eluent. The solution was simply evaporated under reduced pressure without purification. The resulting amorphous solid was further dried in *vacuo* to give 86, (204 mg, quantitatively). The followings are the physical data of the product: m.p. 123° C, $[\alpha]_D$ = +18° (c=1.0, $CHCl_3$); ^{1}H-NMR spectral data: ($CDCl_3$, δ, ppm) 6.98(d, 1H, $J_{NH,1}$= 8.4 Hz, anomeric NH), 6.25(d, 1H, $J_{NH,2}$= 8.3Hz, NHAc), 5.32(dd, 1H, $J_{4',5'}$= 2.7 Hz, H-4'), 5.06(dd, 1H, $J_{2',3'}$= 10.4 Hz, H-2'), 4.98-5.02(m, 2H, H-1, 3), 4.92 (dd, 1H, $J_{3',4'}$= 3.4 Hz, H-3'), 4.45(d, 1H, $J_{1',2'}$= 7.9 Hz, H-1'), 4.37(dd, 1H, $J_{6a,b}$= 10.2 Hz, H-6a), 4.08-4.12(m, 2H, H6b, 6'a), 4.03(dd, 1H, J6'a,b= 11.2, J6'b,5'= 7.4 Hz, H-6'b), 3.98(dd, 1H, J2,3= 10.1 Hz, H-2), 3.85(ddd, 1H, $J_{5',6'b}$= 7.1 Hz, H-5'), 3.73(dd, 1H, $J_{4,5}$= 9.6 Hz, H-4), 3.64(ddd, 1H, H-5), 3.06(dd, 2H, CH_2-SAc), 2.46(m, 2H, CH_2-CH_2SAc), 2.27(s, 3H, SAc), 2.11, 2.08, 2.07, 2.02, 2.01, 1.92 (s, 3H each, OAc), 1.93 (s, 3H, NHAc); ^{13}C-NMR spectral data: 100.9(C-1'), 80.0(C-1), 76.5(C-4), 75.0(C-5), 73.5(C-3), 70.1(C-3',5'), 68.8(C-2'), 66.6(C-4'), 62.1(C-6), 61.0(C-6'), 53.3(C-2), 36.9(-CH_2-CH_2-S-), 30.4(SAc), 24.0(-CH_2SAc), 23.0(NAc), 19.0-21.5(OAc); FAB-MS calculated for $C_{31}H_{44}N_2O_{18}S$, calculated 764.3, found 765.2 (M+1, 34.6%); Anal. Calcd for $C_{31}H_{44}N_2O_{18}S$: C 48.71, H 5.80, N 3.67. Found: C 48.52, H 5.95, N 3.78.

N-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyrasidonate)-(2-3)-O-(6-O-benzoyl-β-D-galactopyranosyl)-(1-4)-O-2,6-di-O-benzoyl-β-D-glucopyranosyl 3-thioacetylpropanamide, (87).

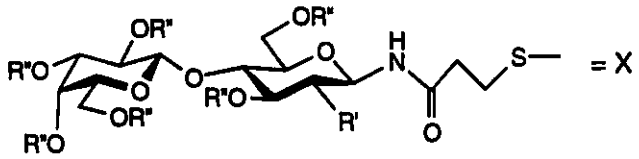


87

To an EtOAc solution (0.5 mL) containing 16 (50 mg, 0.042 mmol) was added AcSH (4.8 mg, 4.5 μL, 0.063 mmol, 1.5 equiv.) without triethylamine. The reaction mixture was stirred under N_2 atmosphere for 8 hours at ambient temperature to be complete. The progress of the reaction was monitored by TLC using a 2 : 3 mixture of acetone and benzene as eluent. The reaction solution was simply evaporated under reduced pressure overnight *in vacuo* to give light-yellow amorphous solid (43 mg, 82 %). M.p.= 94-97° C; $[\alpha]_D$ = +7.8° (c=1.0, $CHCl_3$); ^1H NMR spectral data ($CDCl_3$, δ ppm): 7.17-8.03 (m, 15 H, aromatic), 6.45(d, 1H, $J_{NH,1}$ 9.2 Hz, anomeric NH), 5.45(dd, 1H, $J_{1,2}$ 9.4 Hz, H-1), 5.25-5.30 (m, 2H, H-7", 8"), 5.20(d, 1H, $J_{N"H,5"}$ 9.4 Hz, N"H), 5.11(dd, 1H, $J_{2,3}$ 9.6 Hz, H-2), 4.99(ddd, 1H, H-4"), 4.82(dd, 1H, $J_{6a,b}$ 10.6 Hz, H-6a), 4.75(dd, 1H, $J_{6'a,b}$ 12.0 Hz, H-6'a), 4.56(d, 1H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.53(dd, 1H, $J_{6b,5}$ 5.6 Hz, H-6b), 4.27(dd, 1H, 9a"), 4.11(m, 3H, H-3, 4', 3'), 3.99(m, 1H, H-5), 3.79-3.92(m, 2H, H-9"a, 5"), 3.78 (s, 3H, methyl ester), 3.70-3.77 (m, 4H, H-4, 2', 5'), 3.06(dd, 2H, CH_2-SAc), 2.67(dd, 1H, $J_{3"e,a}$ 13.0, $J_{3"e,4}$ 4.6 Hz, H-3"e) 2.46(m, 2H, CH_2-CH_2SAc), 2.17(s, 3H, SAc), , 2.13, 2.03, 2.02, 2.00(dd, 1H, $J_{3"a,e}$ 13.0, $J_{3"a,4"}$ 12.0 Hz, H-3"a), 1.92, 1.88 (s, 3H each, acetyl); ^{13}C-NMR spectral data (based on HMQC experiment): 20.6-23.2(acetyl), 24.0(-CH_2SAc), 30.4(SAc), 37.0(-CH_2-CH_2-S-), 37.7(C-3"), 49.8(C-5"), 53.3(methyl ester), 62.4(C-9"), 63.2(C-6), 63.7(C-6'), 67.0(C-7"), 68.1(C-2'), 68.2(C-4"), 68.9(C-8"), 69.0(C-4), 72.5(C-2), 72.9(C-5), 73.1(C-4'), 73.5(C-3), 75.0(C-6"), 76.4(C-3'), 78.2(C-1), 82.3(C-4), 97.5(C-2"), 104.4(C-1'), 128.3-133.2 (aromatic).



94a R= Resin, X= C l



The lactose-containing dendrimer

95 R =Resin, R' = OAc, R"= Ac

97 R =H, R' = OAc, R"= Ac

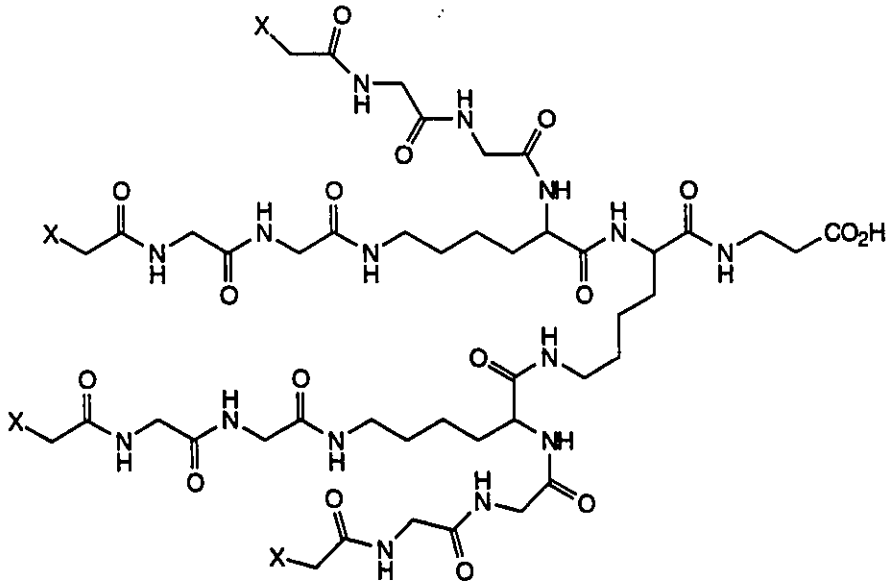
99 R=H, R'= OH, R"= H

The lactosamine containing dendrimer

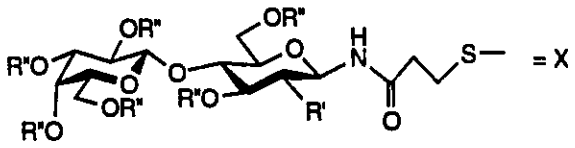
96 R = Resin, R' = NHAc, R"= Ac

98 R =H, R' = NHAc, R"= Ac

100 R= H, R'= NHAc, R"= H



24b X= C l



The Lactose-containing dendrimer

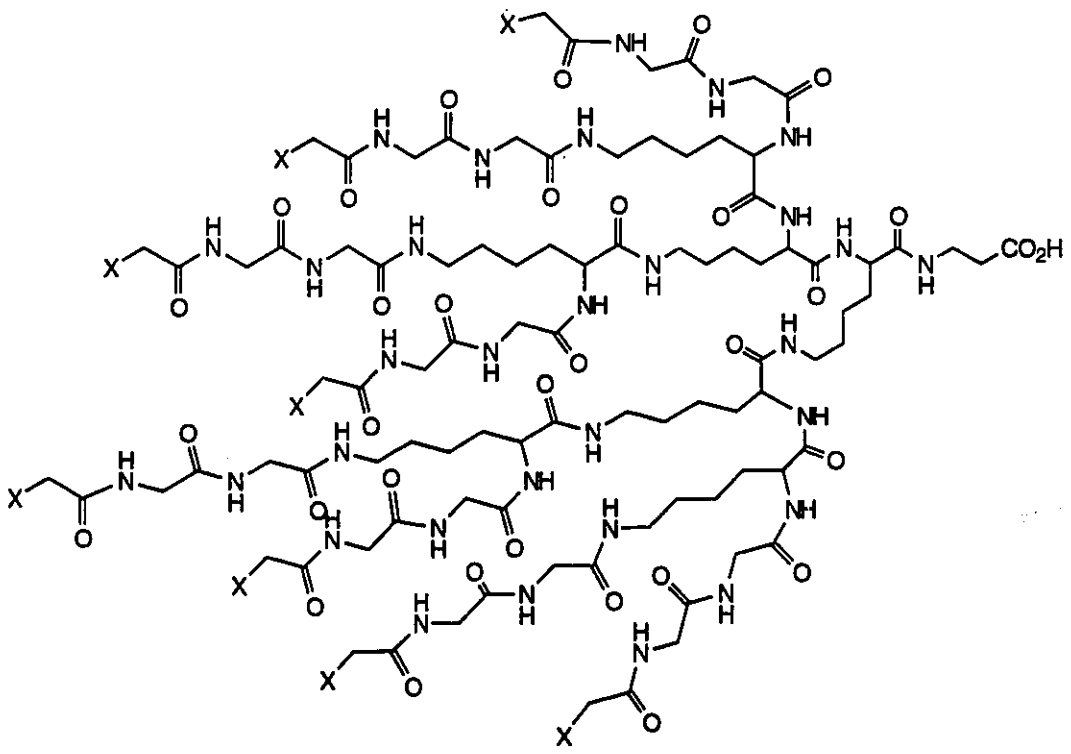
101 R =H, R' = OAc, R"= Ac

103 R=H, R'= OH, R"= H

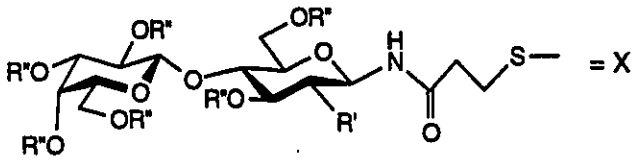
The Lactosamine-containing dendrimer

102 R =H, R' = NHAc, R"= Ac

104 R= H, R'= NHAc, R"= H



94c X= C l



The lactose-containing dendrimer

105 R =H, R' = OAc, R"= Ac

107 R=H, R'= OH, R"= H

The lactosamine-containing dendrimer

106 R =H, R' = NHAc, R"= Ac

108 R= H, R'= NHAc, R"= H

Divalent peracetylated lactose dendrimer, (97).

The resin-bound divalent, N-chloroacetylated dendrimer **94a**(37 mg with resin, estimated 6.1 x 10^{-5} mol) was washed with DMF (5 x 1 mL). A solution of thiol **57** (50 mg, 1.6 equiv. per each ClAc- group) dissolved in 1 % Et_3N/DMF was added to the resin and agitated by N_2 bubbling overnight. The liquid was drained and washed with DMF (5 x 1 mL). The resin-bound glycodendrimer **95** was treated with 95% trifluoroacetic acid(TFA, *ca* . 1mL/ 10 mg) and the mixture was stirred at ambient temperature for 1.5 hours. The filtrate was collected and the resin beads were rinsed three times with neat TFA. The different collections of the filtrate were combined and evaporated under reduced pressure. Remaining traces of TFA were co-evaporated with diethyl ether. From this a white solid was afforded (9 mg, 75 %): the yield calculation is based on the difference in weight of the dry resin when purchased and that after the reaction. ^{1}H-NMR spectral data (DMSO-d_6, δ ppm): peptide backbone: 10.80 (bs, 1H, CO_2H), 8.45(m, 2H, glycyl NH), 8.18(m, 2H, glycyl NH) , 7.92(m, 2H, β-alanyl NH and lysyl α-NH), 7.72(t, 1H, J= 5.6 Hz, lysyl ε-NH)2.99(m, 2H, lysyl ε-CH_2-), 4.13(m, 1H, lysyl α-CH), 3.20(m, 2H, β-alanyl β-CH_2-), 3.65 and 3.74(2d, 4H, J= 5.8 Hz, glycyl CH_2s), 3.15(s, 4H, -SCH_2C(O)), 2.88(s, 2H, SCH_2) , 2.36(t, 2H, J= 7 Hz, β-alanyl α-CH_2-),1.46 and 1.60 (2m, 2H, lysyl β-CH_2-, unequiv.), 1.33(m, 2H, lysyl δ-CH_2), 1.16(m, 2H, lysyl γ-CH_2-) ; lactose unit: 5.27(dd, 2H, $J_{1,2}$= 9.3 Hz, H-1), 5.21(dd, 2H, $J_{4',5'}$< 1Hz, H-4'), 5.19(dd, 2H, $J_{3,4}$= 9.3 Hz, H-3), 4.84(dd, 2H, $J_{2',3'}$= 10.2 Hz, H-2'), 4.66-4.80(m, 4H, H-1', 2), 4.12-4.26(m, 2H, H-6'a), 3.97-4.03(m, 6H, H6b, 6'a, 6'b), 3.75-3.88(m, 2H, H-5), 3.64-3.74(m, 4H, H-4, 5'), 2.70-2.76(m, 4H, -SCH_2CH_2), 2.34-2.40(m, 4H, -SCH_2CH_2), 2.08, 2.05, 2.00, 1.98, 1.95, 1.92, 1.89(OAc): ^{13}C-NMR spectral data (based on HMQC experiment): peptide backbone: 52.5(lysyl α-C), 41.7(4 x glycyl CH_2s), 38.7(lysyl ε-C), 34.3(β alanyl β-C), 33.8(β alanyl α-C), 31.7(lysyl β-C), 28.7(lysyl δ-C), 22.7(lysyl γ-C); lactose unit: 168.6-172.8(7 x C(O)CH_3), 99.8(C-1'), 77.5(C-4), 76.6(C-1), 73.3(C-3), 73.1(C-5), 70.9(C-3'), 70.4(C-5), 69.7(C-2), 68.9(C-2'), 67.1(C-4'), 62.0(C-6),

60.8(C-6'), 42.1($SCH_2\underline{C}H_2$-), 34.2($S\underline{C}H_2CH_2$-), 20.3, 20.4, 20.5, 20.7(7 x $C(O)\underline{C}H_3$).

Divalent de-O-acetylated lactose dendrimer, (99).

The dendritic peracetylated thioglycoside **97** (9 mg) was dissolved in 0.05 M NaOH solution and stirred at room temperature for 2 hours after completetly dissolved. The solution was then treated with H+ resin and the filtrate was lyophilized. The product was obtained as white powder (5.8 mg, 92 %). ^{1}H NMR spectral data (D_2O, δ ppm): peptide backbone: 4.10(m, 1H, lysyl α-CH_2) 3.34(m, 2H, lysyl ε-CH_2), 2.70(m, 2H, β-alanyl α-CH_2), 1.78 and 1.84 (2m, 2H, lysyl β-CH_2, unequiv.), 1.64(m, 2H, lysyl δ-CH_2), 1.42(m, 2H, lysyl γ-CH_2-), lactose unit (2 x): 5.07(d, 1H, J1,2= 9.3 Hz, H-1), 4.53(d, 1H, J1',2'=7.8 Hz, H-1'), 3.47(s, 2H, -$SC\underline{H}_2C(O)$), 2.96(m, 2H, $C\underline{H}_2SCH_2$), 2.74(m, 2H, CH_2CH_2S).

Divalent peracetylated lactosamine dendrimer, (98).

The resin-bound divalent, N-chloroacetylated dendrimer **94a**(8 mg with resin, estimated 1.11 x 10^{-5} mol) was washed with DMF (5 x 1 mL). A solution of thiol **86** (35 mg) dissolved in 1 % Et_3N/DMF was added to the resin and agitated by N_2 bubbling overnight. The liquid was drained and washed with DMF (5 x 1 mL). The resin-bound glycodendrimer **95** was treated with 95% trifluoroacetic acid(TFA, *ca* . 1mL/ 10 mg) and the mixture was stirred at ambient temperature for 1.5 hours. The filtrate was collected and the resin beads were rinsed three times with neat TFA. The different collections of the filtrate were combined and evaporated under reduced pressure. Remaining traces of TFA were co-evaporated with diethyl ether. From this a white solid was afforded (8.0 mg, 37 %): the yield calculation is based on the difference in weight of the dry resin when purchased and that after the reaction. ^{1}H-NMR spectral data (DMSO-d_6, δ ppm): peptide backbone: 10.80 (bs, 1H, CO_2H), 8.45(m, 2H, glycyl NH), 8.18(m, 2H, glycyl NH) , 7.92(m, 2H, β-alanyl

NH and lysyl α-NH), 7.72(t, 1H, J= 5.6 Hz, lysyl ε-NH), 4.13(m, 1H, lysyl α-CH), 3.20(m, 2H, β-alanyl β-CH_2-), 3.65 and 3.74(2d, 4H, J= 5.8 Hz, glycyl CH_2s), 3.15(s, 4H, -SC<u>H_2</u>C(O)), 2.99(m, 2H, lysyl ε-CH_2-), 2.88(s, 2H, SCH_2), 2.36(t, 2H, J= 7 Hz, β-alanyl α-CH_2-),1.46 and 1.60 (2m, 2H, lysyl β-CH_2-, unequiv.), 1.33(m, 2H, lysyl δ-CH_2), 1.16(m, 2H, lysyl γ-CH_2-), lactosamine unit: 5.21(dd, 1H, $J_{4',5'}$= 2.7 Hz, H-4'), 5.13(dd, 1H, $J_{2',3'}$= 10.4 Hz, H-2'), 5.05-5.10(m, 2H, H-1, 3), 4.90 (dd, 1H, $J_{3',4'}$= 3.4 Hz, H-3'), 4.67(d, 1H, $J_{1',2'}$= 7.9 Hz, H-1'), 3.28(s, 2H, SC<u>H_2</u>C(O)), 3.16(dd, 2H, C<u>H_2</u>-SCH_2C(O)), 2.46(m, 2H, C<u>H_2</u>-CH_2S), 2.09, 2.05, 2.00, 1.99, 1.93, 1.88 (s, 3H each, O<u>Ac</u>), 1.71 (s, 3H, NH<u>Ac</u>): ^{13}C-NMR spectral data (based on HMQC experiment): peptide backbone: 52.5(lysyl α-C), 41.7(4 x glycyl CH_2s), 38.7(lysyl ε-C), 34.3(β alanyl β-C), 33.8(β alanyl α-C), 31.7(lysyl β-C), 28.7(lysyl δ-C), 22.7(lysyl γ-C); lactosamine unit: 99.9(C-1'), 77.8(C-1), 76.1(C-4), 73.7(C-3), 73.5(C-5), 70.3(C-3'), 69.6(C-5'), 68.9(C-2'), 67.1(C-4'), 62.5(C-6), 60.9(C-6'), 52.3(C-2), 42.1(-SCH_2C(O)), 35.0(S<u>C</u>H_2CH_2-), 33.4 (SCH_2<u>C</u>H_2), 22.6(NAc), 20.3-20.7(<u>O</u>-acetyl).

Divalent de-<u>O</u>-acetylated lactosamine dendrimer (<u>100</u>).

The dendritic peracetylated thioglycoside **<u>98</u>** (8 mg) was placed in 1 mL of 0.05 M NaOH solution. Since it was not soluble in aqueous NaOH solution in 30 minutes, a small quantity of DMSO(200 μL) was added to aid solubilization. After completely dissolved the reaction mixture was stirred at ambient temperature for 2 h. The solution was then treated with H^+ resin and filtered. The filtrate was lyophilized. The product was obtained as white powder (4.2 mg, 72 %). ^{1}H NMR spectral data (D_2O, δ ppm): peptide backbone: 4.10(m, 1H, lysyl α-CH_2) 3.34(m, 2H, lysyl ε-CH_2), 2.70(m, 2H, β-alanyl α-CH_2), 1.78 and 1.84 (2m, 2H, lysyl β-CH_2, unequiv.), 1.64(m, 2H, lysyl δ-CH_2), 1.42(m, 2H, lysyl γ-CH_2-), lactosamine unit (2 x): 5.07(d, 1H, $J_{1,2}$= 9.7 Hz, H-1), 4.55(d, 1H, $J_{1',2'}$=7.8 Hz, H-1'), 3.62(m, 2H, C<u>H_2</u>SCH_2), 2.80(s, 2H, -SC<u>H_2</u>C(O)), 2.74(m, 2H, C<u>H_2</u>CH_2S), 2.05(s, 3H, NH<u>Ac</u>).

Tetravalent peracetylated lactose dendrimer (101).

The pre-cleaved, tetrameric chloroacetylated dendrimer **94b** (10 mg with the resin) was dissolved in degassed 1 % Et_3N/DMF(v/v) solution. The freshly prepared thiol **57** (1.6 equiv. to each N-chloroacetyl group) dissolved in 1 % Et_3N/DMF(v/v) solution was allowed to react overnight at room temerature. The solution was dialyzed against 50 % DMSO/H_2O mixture (v/v) using the dialysis tubing with molecular cut-off 2000 and lyophilized after. The glycodendrimer **101** was isolated as white solid (28.2 mg, 88 %). ^{1}H- and ^{13}C-NMR spectral data are consistent with compound **97** including the corresponding integration signals. ^{1}H-NMR spectral data (DMSO-d_6, δ ppm): peptide backbone: 10.80 (bs, 1H, CO_2H), 8.45(m, 2H, glycyl NH), 8.18(m, 2H, glycyl NH) , 7.92(m, 2H, β-alanyl NH and lysyl α-NH), 7.72(t, 1H, J= 5.6 Hz, lysyl ε-NH)2.99(m, 2H, lysyl ε-CH_2-), 4.13(m, 1H, lysyl α-CH), 3.20(m, 2H, β-alanyl β-CH_2-), 3.65 and 3.74(2d, 4H, J= 5.8 Hz, glycyl CH_2s), 3.15(s, 4H, -SCH_2C(O)), 2.88(s, 2H, SCH_2) , 2.36(t, 2H, J= 7 Hz, β-alanyl α-CH_2-),1.46 and 1.60 (2m, 2H, lysyl β-CH_2-, unequiv.), 1.33(m, 2H, lysyl δ-CH_2), 1.16(m, 2H, lysyl γ-CH_2-) ; lactose unit: 5.27(dd, 2H, $J_{1,2}$= 9.3 Hz, H-1), 5.21(dd, 2H, $J_{4',5'}$< 1Hz, H-4'), 5.19(dd, 2H, $J_{3,4}$= 9.3 Hz, H-3), 4.84(dd, 2H, $J_{2',3'}$= 10.2 Hz, H-2'), 4.66-4.80(m, 4H, H-1', 2), 4.12-4.26(m, 2H, H-6'a), 3.97-4.03(m, 6H, H6b, 6'a, 6'b), 3.75-3.88(m, 2H, H-5), 3.64-3.74(m, 4H, H-4, 5'), 2.70-2.76(m, 4H, -SC$H_2$$CH_2$), 2.34-2.40(m, 4H, -$SCH_2CH_2$), 2.08, 2.05, 2.00, 1.98, 1.95, 1.92, 1.89(OAc): ^{13}C-NMR spectral data (based on HMQC experiment): peptide backbone: 52.5(lysyl α-C), 41.7(4 x glycyl CH_2s), 38.7(lysyl ε-C), 34.3(β alanyl β-C), 33.8(β alanyl α-C), 31.7(lysyl β-C), 28.7(lysyl δ-C), 22.7(lysyl γ-C); lactose unit: 168.6-172.8(7 x C(O)CH_3), 99.8(C-1'), 77.5(C-4), 76.6(C-1), 73.3(C-3), 73.1(C-5), 70.9(C-3'), 70.4(C-5), 69.7(C-2), 68.9(C-2'), 67.1(C-4'), 62.0(C-6), 60.8(C-6'), 42.1(SCH_2CH_2-), 34.2(SC$H_2$$CH_2$-), 20.3, 20.4, 20.5, 20.7(7 x C(O)CH_3).

Tetravalent de-O-acetylated lactose dendrimer (103).

The dendritic peracetylated thioglycoside (10 mg) was dissolved in 0.05 M NaOH solution and stirred at room temperature for 2 h. The solution was then treated with H^+ resin and the filtrate was lyophilized. The product **103** was obtained as white powder (4 mg, 57 %). ^{1}H- and ^{13}C-NMR spectral data are consistent with compound **99** including the corresponding integration signals. ^{1}H-NMR spectral data (D_2O, δ ppm): peptide backbone: 4.10(m, 1H, lysyl α-CH_2) 3.34(m, 2H, lysyl ε-CH_2), 2.70(m, 2H, β-alanyl α-CH_2), 1.78 and 1.84 (2m, 2H, lysyl β-CH_2, unequiv.), 1.64(m, 2H, lysyl δ-CH_2), 1.42(m, 2H, lysyl γ-CH_2-), lactose unit (2 x): 5.07(d, 1H, J1,2= 9.3 Hz, H-1), 4.53(d, 1H, J1',2'=7.8 Hz, H-1'), 3.47(s, 2H, -SCH_2C(O)), 2.96(m, 2H, CH_2SCH_2), 2.74(m, 2H, CH_2CH_2S).

Tetravalent peracetylated lactosamine dendrimer (102).

Essentially the same procedure was used as in the preparation of **101**: The pre-cleaved, tetrameric chloroacetylated dendrimer **94b** (9.4 mg with the resin) was dissolved in degassed 1 % Et_3N/DMF(v/v) solution. The freshly prepared thiol **87** (50 mg,1.6 equiv. to each N-chloroacetyl group) dissolved in 1 % Et_3N/DMF(v/v) solution was allowed to react overnight at r.t.. The solution was dialyzed against 50 % DMSO/H_2O mixture (v/v) using the dialysis tubing with molecular cut-off 2000 and lyophilized after. The glycodendrimer **102** was isolated as white solid (8.0 mg, 44 %). ^{1}H- and ^{13}C NMR spectral data are consistent with compound **98** including the corresponding integration signals. ^{1}H-NMR spectral data (DMSO-d_6, δ ppm): peptide backbone: 10.80 (bs, 1H, CO_2H), 8.45(m, 2H, glycyl NH), 8.18(m, 2H, glycyl NH) , 7.92(m, 2H, β-alanyl NH and lysyl α-NH), 7.72(t, 1H, J= 5.6 Hz, lysyl ε-NH), 4.13(m, 1H, lysyl α-CH), 3.20(m, 2H, β-alanyl β-CH_2-), 3.65 and 3.74(2d, 4H, J= 5.8 Hz, glycyl CH_2s), 3.15(s, 4H, -SCH_2C(O)), 2.99(m, 2H, lysyl ε-CH_2-), 2.88(s, 2H, SCH_2), 2.36(t, 2H, J= 7 Hz, β-alanyl α-CH_2-),1.46 and 1.60 (2m, 2H, lysyl β-CH_2-, unequiv.), 1.33(m, 2H, lysyl δ-CH_2), 1.16(m, 2H, lysyl γ-CH_2-),

lactosamine unit: 5.21(dd, 1H, $J_{4',5'}$= 2.7 Hz, H-4'), 5.13(dd, 1H, $J_{2',3'}$= 10.4 Hz, H-2'), 5.05-5.10(m, 2H, H-1, 3), 4.90 (dd, 1H, $J_{3',4'}$= 3.4 Hz, H-3'), 4.67(d, 1H, $J_{1',2'}$= 7.9 Hz, H-1'), 3.28(s, 2H, $SCH_2C(O)$), 3.16(dd, 2H, CH_2-$SCH_2C(O)$), 2.46(m, 2H, CH_2-CH_2S), 2.09, 2.05, 2.00, 1.99, 1.93, 1.88 (s, 3H each, OAc), 1.71 (s, 3H, NHAc): ^{13}C-NMR spectral data (based on HMQC experiment): peptide backbone: 52.5(lysyl α-C), 41.7(4 x glycyl CH_2s), 38.7(lysyl ε-C), 34.3(β alanyl β-C), 33.8(β alanyl α-C), 31.7(lysyl β-C), 28.7(lysyl δ-C), 22.7(lysyl γ-C); lactosamine unit: 99.9(C-1'), 77.8(C-1), 76.1(C-4), 73.7(C-3), 73.5(C-5), 70.3(C-3'), 69.6(C-5'), 68.9(C-2'), 67.1(C-4'), 62.5(C-6), 60.9(C-6'), 52.3(C-2), 42.1(-$SCH_2C(O)$), 35.0(SCH_2CH_2-), 33.4 (SCH_2CH_2), 22.6(NAc), 20.3-20.7(O-acetyl).

Tetravalent de-O-acetylated lactosamine dendrimer (104).

Essentially the same procedure was used as in the preparation of **100**. The dendritic peracetylated thioglycoside **102** (8 mg) was placed in 1 mL of 0.05 M NaOH solution. Since it was not soluble in aqueous NaOH solution in 30 min, a small quantity of DMSO(200 μL) was added to aid solubilization. After completely dissolved the reaction mixture was stirred at ambient temperature for 2 h. The solution was then treated with H^+ resin and filtered. The filtrate was lyophilized. The product **104** was obtained as white powder (7.2 mg, 71%). ^{1}H- and ^{13}C-NMR spectral data are consistent with compound **100** including the corresponding integration signals. ^{1}H-NMR spectral data (D_2O, δ ppm): peptide backbone: 4.10(m, 1H, lysyl α-CH_2) 3.34(m, 2H, lysyl ε-CH_2), 2.70(m, 2H, β-alanyl α-CH_2), 1.78 and 1.84 (2m, 2H, lysyl β-CH_2, unequiv.), 1.64(m, 2H, lysyl δ-CH_2), 1.42(m, 2H, lysyl γ-CH_2-), lactosamine unit (4 x): 4.57(d, 1H, $J_{1,2}$= 9.7 Hz, H-1), 4.55(d, 1H, $J_{1',2'}$=7.8 Hz, H-1'), 3.62(m, 2H, CH_2SCH_2), 2.80(s, 2H, -$SCH_2C(O)$), 2.74(m, 2H, CH_2CH_2S), 2.05(s, 3H, NHAc).

Octavalent peracetylated lactose dendrimer, (105).

The pre-cleaved, tetrameric chloroacetylated dendrimer **94c** (10 mg with the resin) was dissolved in degassed 1 % Et_3N/DMF(v/v) solution. The freshly prepared thiol **57** (1.6 equiv. to each N-chloroacetyl group) dissolved in 1 % Et_3N/DMF(v/v) solution was allowed to react overnight at room temerature. The solution was dialyzed against 50 % DMSO/H_2O mixture (v/v) using the dialysis tubing with molecular cut-off 2000 and lyophilized after. The glycodendrimer **105** was obtained as white solid (30 mg, 93 %). ^{1}H- and ^{13}C-NMR spectral data are consistent with compound **97** including the corresponding integration signals except for the β-alanyl residues become part of the background noise level. ^{1}H-NMR spectral data (DMSO-d_6, δ ppm): peptide backbone: 10.80 (bs, 1H, CO_2H), 8.45(m, 2H, glycyl NH), 8.18(m, 2H, glycyl NH) , 7.92(m, 2H, β-alanyl NH and lysyl α-NH), 7.72(t, 1H, J= 5.6 Hz, lysyl ε-NH)2.99(m, 2H, lysyl ε-CH_2-), 4.13(m, 1H, lysyl α-CH), 3.65 and 3.74(2d, 4H, J= 5.8 Hz, glycyl CH_2s), 3.15(s, 4H, -SC$\underline{H}_2$C(O)), 2.88(s, 2H, SCH_2) , 2.36(t, 2H, J= 7 Hz, β-alanyl α-CH_2-),1.46 and 1.60 (2m, 2H, lysyl β-CH_2-, unequiv.), 1.33(m, 2H, lysyl δ-CH_2), 1.16(m, 2H, lysyl γ-CH_2-) ; lactose unit: 5.27(dd, 2H, $J_{1,2}$= 9.3 Hz, H-1), 5.21(dd, 2H, $J_{4',5'}$< 1Hz, H-4'), 5.19(dd, 2H, $J_{3,4}$= 9.3 Hz, H-3), 4.84(dd, 2H, $J_{2',3'}$= 10.2 Hz, H-2'), 4.66-4.80(m, 4H, H-1', 2), 4.12-4.26(m, 2H, H-6'$_a$), 3.97-4.03(m, 6H, H6$_b$, 6'$_a$, 6'$_b$), 3.75-3.88(m, 2H, H-5), 3.64-3.74(m, 4H, H-4, 5'), 2.70-2.76(m, 4H, -SC$\underline{H}_2$$CH_2$), 2.34-2.40(m, 4H, -$SCH_2C\underline{H}_2$), 2.08, 2.05, 2.00, 1.98, 1.95, 1.92, 1.89(O$\underline{Ac}$): ^{13}C-NMR spectral data (based on HMQC experiment): peptide backbone: 52.5(lysyl α-C), 41.7(4 x glycyl CH_2s), 38.7(lysyl ε-C), 34.3(β alanyl β-C), 33.8(β alanyl α-C), 31.7(lysyl β-C), 28.7(lysyl δ-C), 22.7(lysyl γ-C); lactose unit: 168.6-172.8(7 x $\underline{C}$(O)CH_3), 99.8(C-1'), 77.5(C-4), 76.6(C-1), 73.3(C-3), 73.1(C-5), 70.9(C-3'), 70.4(C-5), 69.7(C-2), 68.9(C-2'), 67.1(C-4'), 62.0(C-6), 60.8(C-6'), 42.1($SCH_2\underline{C}H_2$-), 34.2(S$\underline{C}H_2CH_2$-), 20.3, 20.4, 20.5, 20.7(7 x C(O)$\underline{C}H_3$).

Octavalent de-O-acetylated lactose dendrimer (107).

The dendritic peracetylated thioglycoside **105** (8 mg) was dissolved in 0.05 M NaOH solution and stirred at r.t. for 2 h. The solution was then treated with H^+ resin and the filtrate was lyophilized. The product was obtained as white powder (4.2 mg, 72 %). ^{1}H- and ^{13}C-NMR spectral data are consistent with compound **99** including the corresponding integration signals. ^{1}H-NMR spectral data (D_2O, δ ppm): peptide backbone: 4.10(m, 1H, lysyl α-CH_2) 3.34(m, 2H, lysyl ε-CH_2), 2.70(m, 2H, β-alanyl α-CH_2), 1.78 and 1.84 (2m, 2H, lysyl β-CH_2, unequiv.), 1.64(m, 2H, lysyl δ-CH_2), 1.42(m, 2H, lysyl γ-CH_2-), lactose unit (2 x): 5.07(d, 1H, J1,2= 9.3 Hz, H-1), 4.53(d, 1H, J1',2'=7.8 Hz, H-1'), 3.47(s, 2H, -SCH_2C(O)), 2.96(m, 2H, CH_2SCH_2), 2.74(m, 2H, CH_2CH_2S).

Octavalent peracetylated lactosamine dendrimer (106).

The same procedure was used as in the preparation of **101**. The pre-cleaved, tetrameric chloroacetylated dendrimer **94$_c$** (10 mg with the resin) was dissolved in degassed 1 % Et_3N/DMF(v/v) solution. The freshly prepared thiol **87** (50 mg) dissolved in 1 % Et_3N/DMF(v/v) solution was allowed to react overnight at room temerature. The solution was dialyzed against 50 % DMSO/H_2O mixture (v/v) using the dialysis tubing with molecular cut-off 2000 and lyophilized after. The glycodendrimer **106** was obtained as white solid (8 mg, 36 %). ^{1}H- and ^{13}C-NMR spectral data are consistent with compound **98** including the corresponding integration signals. ^{1}H-NMR spectral data (DMSO-d_6, δ ppm): peptide backbone: 10.80 (bs, 1H, CO_2H), 8.45(m, 2H, glycyl NH), 8.18(m, 2H, glycyl NH) , 7.92(m, 2H, β-alanyl NH and lysyl α-NH), 7.72(t, 1H, J= 5.6 Hz, lysyl ε-NH), 4.13(m, 1H, lysyl α-CH), 3.20(m, 2H, β-alanyl β-CH_2-), 3.65 and 3.74(2d, 4H, J= 5.8 Hz, glycyl CH_2s), 3.15(s, 4H, -SCH_2C(O)), 2.99(m, 2H, lysyl ε-CH_2-), 2.88(s, 2H, SCH_2), 1.46 and 1.60 (2m, 2H, lysyl β-CH_2-, unequiv.), 1.33(m, 2H, lysyl δ-CH_2),

1.16(m, 2H, lysyl γ-CH_2-), lactosamine unit: 5.21(dd, 1H, $J_{4',5'}$= 2.7 Hz, H-4'); 5.13(dd, 1H, $J_{2',3'}$= 10.4 Hz, H-2'), 5.05-5.10(m, 2H, H-1, 3), 4.90 (dd, 1H, $J_{3',4'}$= 3.4 Hz, H-3'), 4.67(d, 1H, $J_{1',2'}$= 7.9 Hz, H-1'), 3.28(s, 2H, SC$\underline{H_2}$C(O)), 3.16(dd, 2H, C$\underline{H_2}$-SCH_2C(O)), 2.46(m, 2H, C$\underline{H_2}$-CH_2S), 2.09, 2.05, 2.00, 1.99, 1.93, 1.88 (s, 3H each, O$\underline{Ac}$), 1.71 (s, 3H, NH$\underline{Ac}$): ^{13}C-NMR spectral data (based on HMQC experiment): peptide backbone: 52.5(lysyl α-C), 41.7(4 x glycyl CH_2s), 38.7(lysyl ε-C), 34.3(β alanyl β-C), 33.8(β alanyl α-C), 31.7(lysyl β-C), 28.7(lysyl δ-C), 22.7(lysyl γ-C); lactosamine unit: 99.9(C-1'), 77.8(C-1), 76.1(C-4), 73.7(C-3), 73.5(C-5), 70.3(C-3'), 69.6(C-5'), 68.9(C-2'), 67.1(C-4'), 62.5(C-6), 60.9(C-6'), 52.3(C-2), 42.1(-SCH_2C(O)), 35.0(S$\underline{C}H_2CH_2$-), 33.4 ($SCH_2\underline{C}H_2$), 22.6(NAc), 20.3-20.7($\underline{C}$-acetyl).

Octavalent de-$\underline{O}$-acetylated lactosamine dendrimer, (<u>108</u>).

The dendritic peracetylated thioglycoside **<u>106</u>** (8 mg) was placed in 1 mL of 0.05 M NaOH solution. Since it was not soluble in aqueous NaOH solution in 30 min, a small quantity of DMSO(200 μL) was added to aid solubilization. After completely dissolved the reaction mixture was stirred at ambient temperature for 2 h. The solution was then treated with H^+ resin and filtered. The filtrate was lyophilized. The product **<u>108</u>** was obtained as white powder (6.6 mg, 79%). ^{1}H- and ^{13}C-NMR spectral data are consistent with compound **<u>100</u>** including the corresponding integration signals. ^{1}H-NMR spectral data (D_2O, δ ppm): peptide backbone: 4.10(m, 1H, lysyl α-CH_2) 3.34(m, 2H, lysyl ε-CH_2), 2.70(m, 2H, β-alanyl α-CH_2), 1.78 and 1.84 (2m, 2H, lysyl β-CH_2, unequiv.), 1.64(m, 2H, lysyl δ-CH_2), 1.42(m, 2H, lysyl γ-CH_2-), lactosamine unit (8 x): 5.07(d, 1H, $J_{1,2}$= 9.7 Hz, H-1), 4.55(d, 1H, $J_{1',2'}$=7.8 Hz, H-1'), 3.62(m, 2H, C$\underline{H_2}$SCH_2), 2.80(s, 2H, -SC$\underline{H_2}$C(O)), 2.74(m, 2H, C$\underline{H_2}CH_2$S), 2.05(s, 3H, NH$\underline{Ac}$).

Chapter.VI. Conclusions

In the present research various GM_3- and lactose-containing multivalent glycoconjugates were designed and synthesized. Introduction of N-acrylamide function was found to be useful to prepare the monomers of the carbohydrate haptens. The monomers were then used either directly or with further modification to synthesize the various glycoforms such as glycopolymers, glycotelomers and glycodendrimers. These synthetic glycoconjugates were evaluated for their binding capacities with various plant lectins.

The GM_3 hapten was constructed by coupling a lactosyl azide derivative as glycosyl acceptor and a thiophenyl sialoside as glycosyl donor using NIS/TfOH as the promotor at low themperature. The trisaccharide GM_3 azide as well as the lactosyl azide as model system were reduced and then N-acryloylated to produce the GM_3 and lactose monomers.

Introduction of the spacer arms with varied lengths to the GM_3 and lactose derivatives were accomplished using carbodiimide chemistry by coupling amine forms of the carbohydrates and the acid forms of the spacer arms. The spacer arms were designed to provide the critical distance required for binding between the carbohydrate epitope and its receptor.

The GM_3- and lactose-containing monomers with different spacer arms were copolymerized with acrylamide to give the corresponding glycopolymers. Double immunodiffusions of the GM_3-containing glycopolymers were performed for evaluation of their binding interactions with a sialic acid-specific lectin, WGA. All of the glycopolymers were active in terms of the formation of pricipitin whereas a monomeric GM_3 hapten appeared negative. These results were regarded as existence of the multivalent effect.

A series of lactose-containing clusters was prepared in a single step by reacting the monomeric N-acrylamido lactose with *t*-butylmercaptan as telogen in the presence of catalytic amount of AIBN as initiator. Similarly, the lactose monomer with hexanoate spacer arm was telomerized. The telomerized lactose-containing clusters were separated by using size-exclusion chromatography. This facile, single-step reaction could be applied to syntheses of other glycoconjugates. These telomers were compared to a dimeric lactose-containing cluster for their inhibitory potencies by enzyme-linked lectin assay (ELLA) where the lectin was *Arachis Hypogaea* from peanut lectin. Surprisingly, the dimeric cluster was five times more potent than the telomers and more than twice as potent as the spacer-armed trivalent telomer. The observed low affinity of the telomers was possibly attributed to the restricted availabilities of the lactose haptens caused by inter- and intramolecular hydrogen bonding formations of the amide functions.

The most chemically well-defined glycoforms were designed and prepared by mimicking the dendritic presentation of natural cell-surface carbohydrates. The dendrimers of gallic acid as a seeding molecule with 3^n valency increase were synthesized. The synthesis of second generation dendrimer was accomplished by assembling amino-ester parent molecule and azido-acid daughter molecules using carbodiimide chemistry. Using chemoselective de-S-acetylation by hydrazinium acetate, thiol forms of the lactose derivative were conjugated to the electrophilic peripheries of the dendrimers of both generations. Similarly, thiols of the lactose and lactosamine derivatives were anchored to the premade L-lysine-core dendrimers with 2^n valency increase. The lactose-containing dendrimers were evaluated for their bindings with *Arachis Hypogaea* from peanut lectin using the double immunodiffusion technique. Octa- and nonavalent lactose-containing dendrimers exerted very strong bindings with the lectin whereas di- and trivalent dendrimers appeared to have very weak binding interactions with the lectin if

there was at all. This suggested that the trivalent dendrimer was not enough to generate the multivalent effect with the lectin. Thus, more than three valencies are required to initiate the effect.

As described above, the various glycoforms containing GM_3 and lactose can be used as tools to study the fundamental concept underlying the carbohydrates and the receptors such as multivalency effect. In addition, these compounds can be used as potential therapeutics such as inhibitory drugs or as affinity columns to separate the desired receptors.

Claims to Original Research

1. The N-lactosyl and the N-GM_3 acrylamides have been prepared by reduction of the glycosyl azide and N-acryloylations.

2. Three different N-linked lactose-containing glycopolymers have been prepared: copoly[acrylamide/N-acrylamido(β-D-galactopyranosyl)-(1-4)-β-D-glucopyranose], copoly[acrylamide/N-p-acrylamidobenzamido (β-D-galacto pyranosyl)-(1-4)-β-D-glucopyranose] and copoly[acrylamide/6-N-acrylamido hexanamido-N-(β-D-galactopyranosyl)-(1-4)-β-D-glucopyranose].

3. Three different N-linked GM_3-containing glycopolymers have been synthesized: copoly[acrylamide/N-acrylamido (5-acetamido-3,5-dideoxy-D-*glycero*-β-D-galacto-2-nonulopyranosidonate)-(2-3)-O-(β-D-galactopyranosyl)-(1-4)-(β-D-glucopyranose], copoly[acrylamide/N-p-acrylamidobenzamido(5-acetamido-3,5-dideoxy-D-*glycero*-β-D-galacto-2-nonulopyranosidonate)-(2-3)-O-(β-D-galactopyranosyl)-(1-4)-(β-D-galacto pyranosyl)-(1-4)-β-D-glucopyranose] and copoly[acrylamide/6-N-acrylamidohexanamido-N-(5-acetamido-3,5-dideoxy-D-*glycero*-β-D-galacto-2-nonulopyranosidonate)-(2-3)-O-(β-D-galactopyranosyl)-(1-4)-(β-D-galactopyranosyl)-(1-4)-β-D-glucopyranose].

4. A facile, single-step reaction to prepare a series of lactose-containing clusters has been developed using N-acrylamido(β-D-galactopyranosyl)-(1-4)-β-D-glucopyranose as monomer. This new method called telomerization has provided the lactose-containing mono-, di-, tri- and tetramers. Similarly, the monomer with spacer arm, 6-N-acrylamidohexanamido N-(β-D-galactopyranosyl)-(1-4)-β-D-glucopyranose was telomerized to yield the corresponding telomers.

5. The chemically well-defined dendritic structures with 3^n valency increase have been designed and synthesized using gallic acid as seeding molecule. The second generation of the dendrimer has been synthesized by using carbodiimide chemistry.

6. Thioacetates of lactose, lactosamine and GM_3 derivatives have been designed and synthesized as precursors to the corresponding nucleophilic thiols. The thiols generated by hydrazinium acetate via chemoselective de-S-acetylation have been conjugated to the electrophilic peripheries of various dendrimers.

7. The glycodendrimers generated by the above method include lactose-containing gallic acid-core dendrimers of both first and second generations. In addition, the thiols of lactose and lactosamine derivatives have been conjugated to the premade L-lysine core dendrimers up to the third generation.

Personal Curriculum Vitae

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Publications:

1. R. Roy, William K.C. Park, Q.-Q. Wu, S.-N. Wang, 'Synthesis of Hyper-branched Lactosides', *Tetrahedron Lett.*, **1995**, 36, 4377.

2. Willam K. C. Park, Diana Zanini, Serge J. Meunier and Rene Roy, 'Chemoselective Removal of Thiolacetates with Hydrazinium acetate' , *Carbohydr. Lett.*, **1995**, 1, 179.

3. William K.C. Park, S. Aravind, Anna Rommanowska, Jocelyn Renaud and Rene Roy, 'Syntheses of Clustered Lactosides by Telomerization', *Methods in Enzymol.*, **1994**, 242, 292.

4. E. Buncel, William K.C. Park, R. Tarkka, P. Liu and S. Hoz, 'Solvent Independent Transition Structures (III), Sulfonyl Transfer Reactions', *J. C. S. Perkin Trans. II*, **1994**, 2439.

5. S. Aravind, William K. C. Park, S. Brochu and R. Roy, 'Single Step Syntheses of Lactosylated Clusters by Telomerizations', *Tetrahedron Lett.* , **1994**, 35, 7739.

6. Robert D. Gordon and William K.C. Park, 'The 353 nm ^1n-π^* transition of 4H-pyran-4-one and a deuterated derivative', *Can. J. Chem.*, **1993**, 71, 1672, .

7. Diana Zanini, William K.C. Park and René Roy, 'Synthesis of Novel Dendritic Glycosides', *Tetrahedron Lett.*, in press.

Conference Contributions:

8. William K.C. Park, B. Kratzer, D. Zanini, Q. Wu, S.J. Meunier, and R.Roy, 'Glycodendrimers: New Powerful Inhibitors of Carbohydrate-Protein Interactions', *XIIIth International Symposium on Glycoconjugates*, Seattle, WA. Aug. 20-26, **1995**.

9. D. Zanini, William K.C. Park, S.J. Meunier, Q. Wu, S. Aravind, B. Kratzer, and R. Roy, 'Syntheses and Biological Properties of Glycodendrimers.' *ACS Symposium Series*, Chicago, **1995**.

10. William K.C. Park, S.J. Meunier, D. Zanini and R. Roy 'Selective Hydrosis of Thiolacetates with Hydrazinium Acetate', Abstracts of *the XVIIth International Carbohydrate Symposium*, Ottawa, Canada, B1.146, **1994**.

11. William K.C. Park, Diana Zanini, S.-N. Wang and R. Roy 'Solid Phase Synthesis of Dendritic Glycopeptides' , Abstracts of *the XVIIth International Carbohydrate Symposium*, Ottawa, Canada, B1.147, **1994**.

12. William K.C. Park, S. Aravind, S. Brochu and R. Roy, ' One Step Synthesis of Families of Lactosylated Clusters by Telomerization', Abstracts of *the XVIIth International Carbohydrate Symposium*, Ottawa, Canada, B1.143, **1994**.

13. S. Cao, William K.C. Park, F. Hernandez-Mateo, S. Aravind and R. Roy, 'Synthesis of Glycopolymers contaning Sialyl α-(2-3)-Lactosides', Abstracts of *the XVIIth International Carbohydrate Symposium*, Ottawa, Canada, B2.028, **1994**.

14. R. Roy, D. Zanini, A. Romanowaska, S.J. Meunier, William K.C. Park, M.A.Gidney, B. Harrison, D.R. Bundle and R.W. Williams,' Synthesis of Sialylated Multiple Antigen Peptides Having Tetanus Toxin T-cell Epitopes ' , Abstracts of *the XVIIth Internatonal Carbohydrate Symposium*, Ottawa, Canada, **1994**.

15. R. Roy, D. Zanini, William K.C. Park, S. J. Meunier, S.-N. Wang, S. Kelm and R. Schauer, ' Syntheses of Carbohydrate based Dendrimers ' at *2nd Annual Conference on Glycotechnology*, La Jolla, CA. USA. May 16-18, **1994**.

Conference Presentations:

16. William K.C. Park, 'Syntheses of Carbohydrate-Containing Dendrimers and Biological Aspects', at *5th Ontario-Quebec Minisymposium in Synthetic and Bioorganic Chemistry*, Oct. 14-16, **1994**, University of Toronto, Ontario, Canada.

17. R. Roy, D. Zanini, William K.C. Park, S. J. Meunier, S.-N. Wang, S. Kelm and R. Schauer, 'Syntheses of Carbohydrate based Dendrimers ' at *2nd Annual Conference on Glycotechnology*, La Jolla, CA. USA. May 16-18, **1994**.

18. William K.C. Park, S. J. Meunier, D. Zanini, 'Selective Hydrolysis of Thioacetates with Hydrazinium acetate ' at *4th Ottawa-Carleton Chemistry Institute Spring Symposium*, May 2-3, **1994** University of Ottawa, Ontario, Canada.

19. S.-N. Wang, William K.C. Park, Serge J. Meunier, ' Syntheses of Gallic Glycosides of Biological Interest', at *4th Ottawa-Carleton Chemistry Institute Spring Symposium*, May 2-3, **1994**, University of Ottawa, Ontario, Canada.

20. William K.C. Park, 'N-Acryloylated Lactosyl Derivatives as Novel Precursors to Glycoconjugates' at *3rd Ontario-Quebec Minisymposium in Synthetic and Bioorganic Chemistry*, October 24-25, **1992**, University of Guelph, Ontario, Canada.

Appendix

1. List of Figures

2. List of Schemes

3. List of Tables