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for my wonderful wife Isabelle,
my daughter Sandrine and son Jérémy
and my parents Mireille and Jean Meunier

ABSTRACT

In order to further understand fundamental carbohydrate-protein interactions as well as to supply carbohydrate clusters of high affinity, glycoconjugates with differing carbohydrate densities, conformations, and interglycosidic spacings were prepared. A series of multivalent α-sialosides were scaffolded onto gallic acid-based dendrimers and calix[4]arenes.

Carbohydrate residues involved in the syntheses of glycoconjugates were all prepared stereoselectively in high yields from phase transfer catalysis (PTC) reaction. These carbohydrate ligands were coupled to the dendritic and calixarene cores through nucleophilic displacement of chlorides by thioglycosides. The conjugation strategy employed herein lead to the study of different chemoselective deprotection of thioacetate glycosides. The methods studied involved, *i*) hydrazinium acetate in DMF (one- & two-pot reactions) and *ii*) sodium methoxide in MeOH at low temperature (Zemplén conditions).

Solid phase synthesis on Wang resin was used to construct the first dendritic α-thiosialosides. The design of these hyperbranched clusters was based on L-lysine core structures using established Fmoc protecting group and benzotriazolyl activated ester coupling procedures. Chain extension of the lysyl amino groups with chloroacetylglycylglycine active ester allowed introduction of the required functionality necessary for the coupling to an α-thiosialoside derivative.

The synthesis of hyperbranched glycodendrimers containing sialic acid residues having 3^n in valency, is also described. Gallic acid as trivalent core and oligoethylene glycol derivatives as hydrophilic spacers were used to scaffold the dendritic backbones. α-Thiosialoside derivative was conjugated onto N-chloroacetylated dendritic precursors by nucleophilic substitution to afford tri- and nona-valent sialodendrimers.

The synthesis of glycocalix[4]arenes was then effected since these glycoconjugates have an extra advantage over chemically well-defined glycodendrimers. They possess the ability to form drug inclusion complexes.

p-Tert-butylcalix[4]arene was transformed into its tetraethyl ester. This opened the way to the formation of the acid chloride which was treated with excess mono-Boc-1,4-butanediamine or mono-Boc-1,6-hexanediamine used as spacer arms. α-Thiosialoside derivative was covalently attached to the calix[4]arenes by nucleophilic substitution on the cone-shaped tetra-*N*-chloroacetylated calix[4]arenes. The glyco-calix[4]arenes were liberated from their protecting groups to afford biologically active clusters where sialic acid is the ligand.

A different tetravalent sialocalixarene was synthesized following a convergent approach. Tetraacyl chloride calix[4]arene derivative was reacted by peptide coupling with an aminosialoside having a long spacer arm. Finally, octavalent dendritic sialocalix[4]arene was then synthesized from a tetraamino calix[4]arene and *N*-bromoacetylated sialoside derivatives. Both reagents have built-in spacer arms which allowed for an efficient double N-alkylation reaction.

Binding studies *via* turbidimetric analysis confirmed the ability of the glycodendrimers and glycocalix[4]arenes to cross-link and precipitate appropriate lectins (WGA and LFA).

ACKNOWLEDGEMENTS

This thesis has been possible from the help, patience and support of the many people that I wish to thank with these following few words.

I would first like to thank my supervisor, Professor René Roy, for his enthusiasm, devotion and leadership of this research work. He brought so many new ideas and allowed "endless" discussions which lead to better and more exciting projects. His tremendous knowledge of the field, along with being always available for you, makes one jealous of having a supervisor like him. I am very grateful.

I also want to thank my lab colleagues who were my collaborators from which we had several helpful discussions and without forgetting some starting materials they provided me. I am thankful to Dr. Diana Zanini, Qing Quan Wu, Denis Carrière, Dr. Jin Mi Kim, Zhonghong Gan, Dr. Muriel Llinares, Daniel Pagé, Dr. W.K.C. Park, Dr. S. Cao, Dr. A. Romanowska, Dr. B. Kratzer, M.G. Baek, Dr. A. Sivasubramanian, Dr. Malo Nianga N., C. Chamoun and Jocelyn Renaud.

I am also grateful to my friends who were always there for me. There attention and support are greatly appreciated. Thanks especially to Isabelle Pilote, Denis Carrière, Diana Zanini, Alain B. Paquin, Mélina Dallaire, Sylvie Corneau, Neil Faibish and Scott Millan.

I am thankful for the efficient technical support at the University of Ottawa. I must express my sincere thanks to Dr. Glenn Facey and Raj Capoor for providing such high quality NMR spectra and Dr. Clem Kazakoff from the mass spectrometry laboratory.

I acknowledge and am grateful for the financial support, in the form of scholarships, from NSERC (PGS A, PGS B), FCAR (PGS 5) and the University of Ottawa.

Lastly, I would especially like to thank my wife Isabelle who I love very much. She always supported me and is the most important contributor to the success of this thesis. My parents Mireille and Jean Meunier always believed in me and were always there for me in every stage of my life, good and bad moments. I am grateful to my brother Yves for being such a good friend to me. Their support and understanding is deeply appreciated. Finally, I cannot forget my son Jérémy born in October 1996 and daughter Sandrine born in April 1998 for bringing so much joy in my life. Jérémy was very helpful while I was writing my thesis. He kept saying: "Moi aussi je veux écrire une thèse, Papa".

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LIST OF ABBREVIATIONS

Ac	acetyl
AcCl	acetyl chloride
AcOH	acetic acid
AcSH	thiol acetic acid
Ala	alanine
b	broad
BOC	*tert*-butoxycarbonyl
bs	broad singlet
t-Bu	*tert*-butyl
Cbz	carbobenzyloxy
CI	chemical ionization
COSY	correlation spectroscopy
d	doublet
D_2O	deuterated water
DCC	dicyclohexylcarbodiimide
dd	doublet of doublets
ddd	doublet of doublet of doublets
DEPT	distortionless enhanced polarization transfer
DIC	diisopropylcarbodiimide
DIPEA	diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DMSO-d_6	hexadeuterated dimethylsulfoxide
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
ELLA	enzyme linked lectin assay
equiv	equivalent(s)

Et	ethyl
EtOAc	ethyl acetate
EtOH	ethanol
Et_3N	triethylamine
FAB-MS	fast atom bombardment ionization mass spectrometry
Fmoc	9-fluorenylmethoxycarbonyl
Gal	galactose
Glc	glucose
GlcNAc	*N*-acetylglucosamine
h	hour(s)
HMQC	heteronuclear multiple quantum coherence
HOBt	1-hydroxybenzotriazole
Hz	hertz
LFA	*Limax flavus* lectin
LiBr	lithium bromide
Lit.	literature
Lys	lysine
m	multiplet
M^+	parent molecular ion
Me	methyl
min	minute(s)
mp	melting point
MS	mass spectrometry
MW	molecular weight
m/z	mass to charge ration
NaBr	sodium bromide
Neu5Ac	*N*-acetylneuraminic acid
NMR	nuclear magnetic resonance

Nu	nucleophile
O.D.	optical density
Ph	phenyl
pos.	positive
ppm	parts per million
PTC	phase transfer catalysis
R_f	retention factor
r.t.	room temperature
s	singlet
SPPS	solid phase peptide synthesis
t	triplet
TBAHS	tetrabutylammonium hydrogen sulfate
TFA	trifluoroacetic acid
TLC	thin layer chromatography
WGA	wheat germ agglutinin

Chapter 1

Introduction

1.1 Biological Importance of Cell-Surface Carbohydrates

Carbohydrates are very important in various aspects of life, they constitute the major components of plants and animals. It is well understood that an important role they serve is being a source of energy. However, carbohydrates play other important functions in biological systems, which include cell-growth, -regulation, and -differentiation, inflammation, hormonal actions, cellular trafficking, metastasis, and bacterial and viral infections.

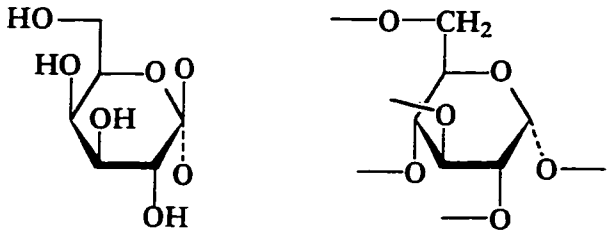
The fact that carbohydrates, especially in the forms of glycoconjugates, play many important roles in the field of biology (glycobiology) has made their study gain remarkable momentum in recent years.[1,2] These roles are not as simple as those of nucleic acids or amino acids, which can be attributed to the complexity of carbohydrates. The basic monomeric entity (monosaccharide) is by far more complex than that of other major groups of biological compounds and this complexity is greatly increased when the basic monomeric units are assembled together. This latter complexity arises since monosaccharides, unlike amino acids or nucleotides, have many ways of linking to each other. Although each monosaccharide has a single reducing group, when it cyclizes, it can assume either a 5-membered ring (furanose form) or a 6-membered ring (pyranose form), both of which can be found in nature. In addition to the ring forms, there is the stereochemistry of the glycosidic linkage to contend with, which can be α- or ß-, depending on the configuration of the anomeric carbon. Thus, the connection of two monomeric units, A and B, can have the sequence of A-B or B-A and in addition can take

---

1 Rademacher, T. W.; Parekh, R. B.; Dwek, R. A. *Ann. Rev. Biochem.* **1988**, *57*, 785.

2 Lasky, L. A. *Science* **1992**, *258*, 964.

one of four of α- or ß-furanose or α- or ß-pyranose forms. Furthermore, because of the polyhydroxy nature of monosaccharide, several positional isomers of glycosyl linkage are possible (Figure 1.1.1). When the number of sugar residues increases to more than three, the situation becomes more complicated because the presence of many hydroxyl groups on a single sugar allows formation of branched structures. This unique feature differentiates carbohydrates from peptides and nucleotides. When the branching is taking into consideration, the possible structures that can be generated by a finite number of monosaccharides increases exponentially.[3]



A **B**

Figure 1.1.1. Possibilities for the formation of polysaccharides.

All possible structural forms are fortunately not fully utilized in nature and the number of monosaccharides used for the construction of most natural oligosaccharides are quite limited. Among, the most widely found sugars in glycoconjugates, one can list D-glucose (Glc), D-galactose (Gal), *N*-acetyl-D-glucosamine (GlcNAc), *N*-acetyl-D-galactosamine (GalNAc), D-mannose (Man), sialic acid (NeuAc or SA), L-fucose (Fuc) and D-xylose (Xyl). Furthermore, certain sugars tend to be associated more with one anomeric form than the other. Sialic acid in glycoconjugates, usually has the α-pyranosyl configuration whereas GlcNAc is mostly found in the ß-pyranosyl form. Examples of

---

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

sugars that can either be found in the α- or ß-form are D-mannose and D-galactose. The latter not only can be α- or ß-linked but can also adopt the pyranose or furanose form.[4]

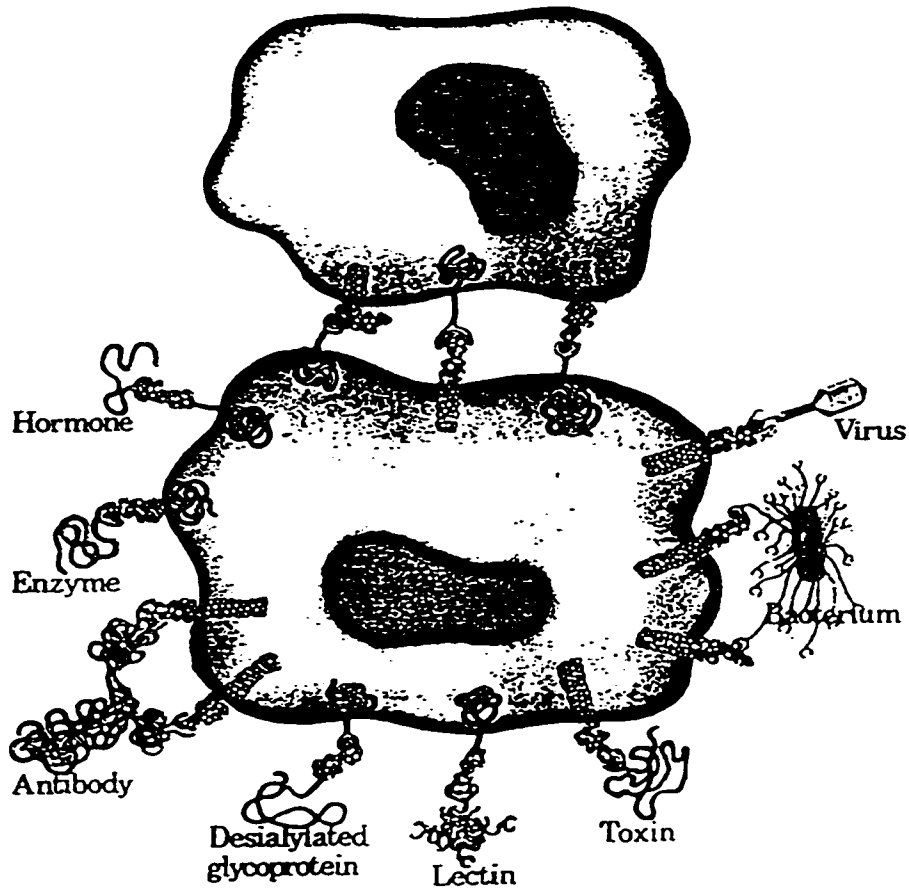


Figure 1.1.2.[5] Schematic of interactions involving cell surface carbohydrates.

Carbohydrates discussed so far, in addition to being present by themselves in nature, are mostly found as glycoconjugates wherein the carbohydrate residue is covalently linked to other molecules such as proteins and lipids. These glycoproteins and glycolipids along with proteoglycans and capsular polysaccharides form the main

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[4] Rick, P. D.; Drewes, L.R.; Gander, J.E. *J. Biol. Chem.* **1974**, *249*, 2073.

[5] Figure taken from BioCarb, **1990**.

components of cell surfaces.[6] These cell surface carbohydrates can act as intracellular events mediators and/or receptors for toxins, hormones, enzymes, lectins, antibodies, viruses and bacteria[7,8,9,10,11,12] (Figure 1.1.2). Table 1.1.1 illustrates some of the most frequently identified carbohydrate residues involved as ligands in carbohydrate-protein interactions.

Table 1.1.1. Sugar Specificities of Cell Surface Lectins

Saccharide	Bacterial/Viral Receptors
L-Fucose	*Vibrio cholerae*
Lactose	*E. coli*
	Lung tissues (Cancer metastasis)
D-Mannose	HIV-virus
	Pseudomonas aeruginosa
D-GalNAc	*E. coli*
D-Gal/D-GalNAc	Hepatocytes
D-GlcNAc	HIV-virus
	E. coli
Sialic Acid (NeuAc)	Mycoplasma
	Influenza virus (flu)
Sialyloligosaccharides	E, P, L Selectins (Inflammation)
	H. pilori (Gastric ulcer)

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

7 Reutter, W.; Köttgen, E.; Bauer, C.; and Gerok, W. in *Sialic Acids. Chemistry, Metabolism and Function.* Cell Biology monograph series. Vol. 10, R. Schauer (Ed.), Springer-Verlag, Vienna, **1982**.

8 Ankel, H.; Krishnamurti, C.; Besancon, F.; Stefanos, S. and Falcoff, E. *Proc. Natl. Acad. Sci. USA* **1980**, *77*, 2528.

9 Goldstein, I. J. and Poretz, R. D. in *The Lectins. Properties, Functions, and Applications in Biology and Medecine.* I. E. Liener, N. Sharon, I. J. Goldstein (Eds.), Academic Press, Orlando, FL, **1986**, 103.

10 Paulson, J. C. in *The Receptors*, vol. 2, P. M. Conn (Ed.), Academic Press, Orlando, Florida, **1985**, p. 131-219.

11 Wright, A.; McConnel, M.; and Kanegasaki, S. in *Virus Receptors*, L. L. Randall and L. Philipson (Eds.), *Receptors and Recognition*, series B, vol. 7, Chapman and Hall, London, **1980**.

12 Arbuthnott, J. P. and Smyth, C. J. in *Adhesion of Microorganisms to Surfaces*, D. C. Elwood, J. Melling and P. Rutter (Eds.), Academic Press, London, **1975**.

Cell-surface sialic acid (Figure 1.1.3) is of particular interest since it is involved in inflammation processes and in influenza virus infections. In the former case, many different sialyloligosaccharides have been identified as ligands for the cell adhesion molecules implicated in the cascade of events leading to inflammation. The flu virus infection case will be discussed here in greater details since sialic acid "alone" is involved in the process leading to infection (Figure 1.1.4)[13] and since most of the work done in this dissertation targeted the synthesis of sialosides (glycoconjugates of sialic acid).

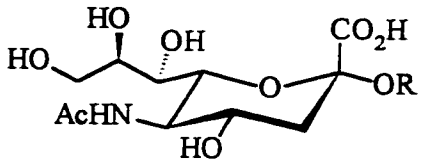

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Figure 1.1.3. α-Sialosides as they appear on most natural cell surface gangliosides and sialylated glycoproteins.

The first step leading to infection of a cell by the influenza A virus is the attachment mediated through binding of the virus to cell surface sialic acids. The attachment occurs through the interaction of hemagglutinin (HA), a sialic acid (SA) receptor on the virus, with SA groups linked to glycoproteins and glycolipids on the surface of the cell.[10,14] The name hemagglutinin comes from its ability to mediate viral agglutination of erythrocytes.[10] A second protein present at the surface of the virus, named sialidase (neuraminidase) is postulated to facilitate the elution of newly formed virus particles from the host cell, to help the movement of the virus in the mucus, and perhaps also to prevent irreversible adsorption to mucus glycoproteins. Both proteins have been the target of drugs.

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

[14] Wiley, D. C.; Skehel, J. J. *Annu. Rev. Biochem.* **1987**, *56*, 365.

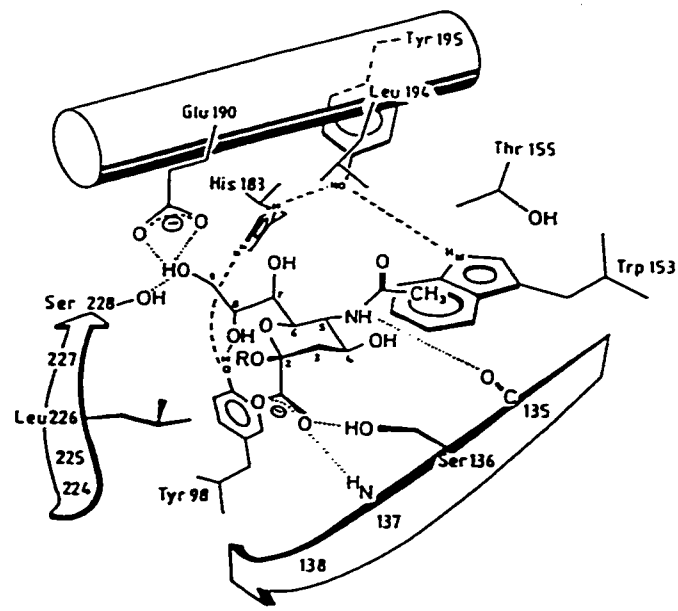

Figure 1.1.4.[13] Model for the position of α-sialoside in the binding pocket of influenza A (X-3, H3N2) virus hemagglutinin.

An approach which seems viable in the prevention of infection by the influenza virus is the design of sialic acid analogs that bind tightly to HA and therefore prevent attachment of the virus to the cells. However, it is difficult to find tight-binding inhibitors of HA because the binding pocket of the virus is small and shallow.[15-16] The interaction of a single HA binding site with a single sialic acid is weak (K_d ~ 3 mM for α-methylsialoside **1**, R = CH_3);[13] the binding of a particle of virus to the surface of a cell is strong.[10,17] It is believed that this strong binding reflects the interaction of multiple copies of HA on the viral surface simultaneously with multiple SA groups on the surface of the cell.[18] Based on the idea that a polyvalent interaction is required for tight binding of the cell to the virus, many research groups have developed inhibitors that present multiple

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15 Sauter, N. K.; Hanson, J. E.; Glick, G. D.; Brown, J. H.; Crowther, R. L.; Park, S. J.; Skehel, J. J.; Wiley, D. C. *Biochemistry* **1992**, *31*, 9609.

16 Weis, W.; Brown, J. H.; Cusack, S.; Paulson, J. C.; Skehel, J. J.; Wiley, D. C. *Biochemistry* **1992**, *31*, 9609.

17 Wharton, S. A.; Weis, W.; J. C.; Skehel, J. J.; Wiley, D. C. In *The Influenza Viruses*, R. M. Krug (Ed.) Plenum, New York, **1989**, Chapter 3.

18 a) Mastrosovich, M. N. *FEBS Lett.* **1989**, *252*, 1. b) Ellens, H.; Bentz, J.; Mason, D.; Zhang, F.; White, J. M. *Biochemistry* **1990**, *29*, 2697.

copies of SA to the virus and were effective at preventing the attachment of the virus to red blood cells.[19,20,21,22,23] In view of the prior art, this dissertation is an attempt to continue the study of understanding how these molecular interactions really work.

1.2 Glycoconjugates and Cluster Effect

1.2.1 Cluster or Multivalent Effect

The low binding affinity mentioned above is not only seen in the influenza virus case. It is known that individual carbohydrate-protein interactions are generally of low affinity.[24] In order to overcome these weak interactions, multiple copies of oligosaccharides must be present on branched structures in order to obtain optimal binding. The branched structures can offer multiple target sugars in a certain spatial arrangement which some lectins can bind simultaneously for enhanced binding affinity. The phenomenon where an affinity enhancement achieved by multivalent ligands over monovalent ones that is greater than would be expected from a simple effect of a local concentration increase is termed the "glycoside cluster effect" (or multivalent effect).[25] These amplifications can be by factors as high as thousands. One of the earliest example of this effect was the attachment of sugars on the hydroxyl groups of amino-(tris-hydroxymethyl)methane.[26] An example showing how important the change in binding

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19 Spevak, W.; Nagy, J. O.; Charych, D. H.; Schaefer, M. E.; Gilbert, J. H.; Bednarski, M. D. *J. Am. Chem. Soc.* **1993**, *115*, 1146.

20 Spaltenstein, A.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 686.

21 Roy, R.; Laferrière, C. A. *Carbohydr. Res.* **1988**, *177*, C1.

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

23 Sparks, M. A.; Williams, K. W.; Whitesides, G. M. *J. Med. Chem.* **1993**, *36*, 778.

24 Toone, E. J. *Curr. Opin. Struct. Biol.* **1994**, *4*, 719.

25 Lee, Y. C.; Townsend, R. R.; Hardy, M. R.; Lönngren, J.; Arnarp, J.; Haraldsson, M.; Lönn, H. *J. Biol. Chem.* **1983**, *258*, 199.

26 Lee, Y. C. *Carbohydr. Res.* **1978**, *67*, 509.

affinity can be, is the huge increase in affinity for the tri- and bi-antennary structures over the monovalent structures by the mammalian hepatic lectin.[27] Studies on the hepatocyte asialoglycoprotein receptor (ASGP-R) have shown that the most successful ligands (in terms of binding affinity) are those which possess several monosaccharide moieties separated by an average distance of 1.5 nm. In addition, Lee and Lee described the optimal ligand for the ASGP-R as having three galactose terminal residues with a triangular configuration, having inter-site distances of 1.5, 2.2 and 2.5 nm.[28,29] It is believed that a balance of three factors contributes to the effectiveness of polyvalent glycosides in preventing viral and bacterial attachment. First, there is an increase in the overall avidities that are much higher than the single site affinities. Second, multivalent glycosides can form high molecular weight entities when binding with a virus or bacterium, therefore preventing the pathogen from coming close enough to a cell for the receptors and cell-surface ligands to interact. The final factor is unfavorable; a ligand in a low molecular weight form binds more tightly to the receptor than a single ligand attached to a macromolecule.[30] All the evidence points in the direction that multivalency is required for tight binding interactions. Possible ways where multivalency is manifested are depicted in Figure 1.2.1.1.[31,32,33]

In order to demonstrate that multivalency does indeed amplify carbohydrate-protein interactions, the research community synthesized many different neoglycoconjugates, which are illustrated in Figure 1.2.1.2. The earliest and best known cases of neoglycoconjugates belong to the neoglycoproteins[34,35,36] and neoglycolipids.[37]

---

27 Lee, Y. C.; Lee, R. T.; Rice, K.; Ichikawa, Y.; Wong, T.-C. *Pure & Appl. Chem.* **1991**, *63*, 499.

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

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

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

31 Drickamer, K.; Taylor, M. E. *Ann. Rev. Cell. Biol.* **1993**, *9*, 237.

32 . Barondes, S. H.; Cooper, D. N.; Gitt, M. A.; Leffler, H. *J. Biol. Chem.* **1994**, *269*, 20807.

33 Kiessling, L. L.; Pohl, N. L. *Chem. Biol.* **1996**, *3*, 71.

34 Stowell, C. P.; Lee, Y. C. *Adv. Carbohydr. Chem. Biochem.* **1980**, *37*, 225.

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

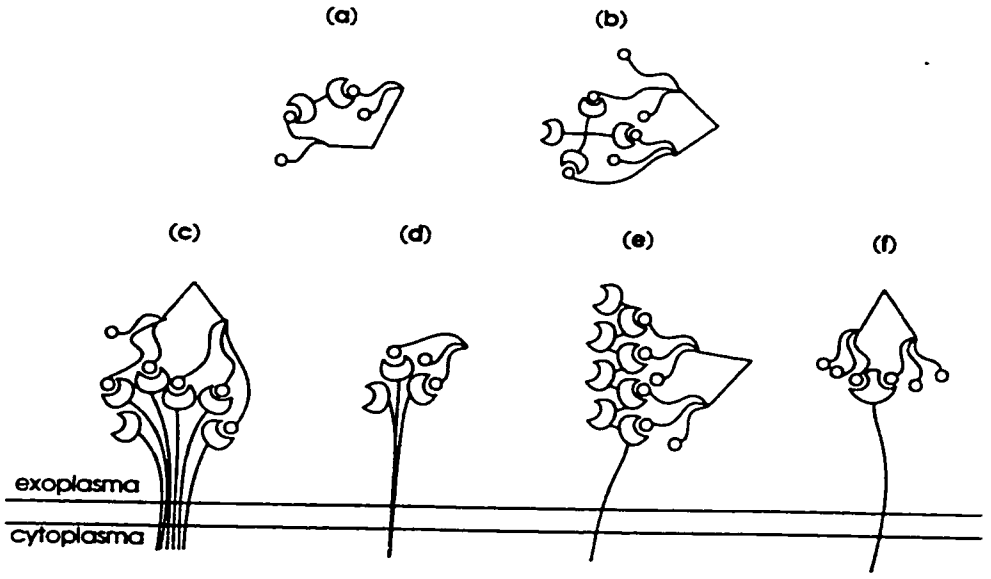

Figure 1.2.1.1.[38] Amplification of specific carbohydrate-protein recognition processes via multivalent interactions: (a) and (b) di- and tetra-valent receptors, (c) clustered monovalent receptors at the cell surface, (d) and (e) oligomeric receptors and (f) receptors that bind more than one carbohydrate simultaneously.

The neoglycoproteins were used *in vitro* for such things as screening and studying antibodies, lectins, enzymes as well as microorganisms, and *in vivo* as immunogens. Other more synthetic members of the neoglycoconjugates family, that have received much more attention in recent years, are shown in Figure 1.2.1.2 and consist of glycopolymers,[39,40,41,42,43] glycoclusters[35] having as little as two conjugated carbohydrate

---

36 Lee, Y. C.; Lee, R. T. *Methods Enzymol.* **1994**, *242* and *247*.

37 Feizi, T.; Childs, R. A. *Methods Enzymol.* **1994**, *242*, 205.

38 Zanini, D.; Roy, R. in *Carbohydrate mimics: concepts and methods* Y. Chapleur (Ed.) Wiley-VCH, Weinheim, **1998**, 385.

39 Roy, R. *Trends Glycosci. Glycotechnol.* **1996**, *8*, 79.

40 Magnusson, G.; Chernyak, A. Y.; Kihlberg, J.; Kononov, L. O. in *Neoglycoconjugates: Preparation and Applications* Y. C. Lee and R. T. Lee (Eds.) Academic Press. San Diego, **1994**, 53.

41 Chernyak, A. Y. *ACS Symposium Series* **1994**, *560*, 133.

42 Bovin, N. V.; Korchagina, E. Y.; Zemlyanukhina, T. V.; Byramova, N. E.; Ivanov, A. E.; Zubov, V. P.; Mochalova, L. V. *Glycoconjugate J.* **1993**, *10*, 142.

haptens, glycotelomers,[44-45] glycopeptides,[46] glycopeptoids,[47,48,49,50] glycodendrimers[51] and glycocalixarenes.[52,53,54] The remainder of the chapter will focus on describing these novel glycoconjugates with an emphasis on glycodendrimers and glycocalixarenes.

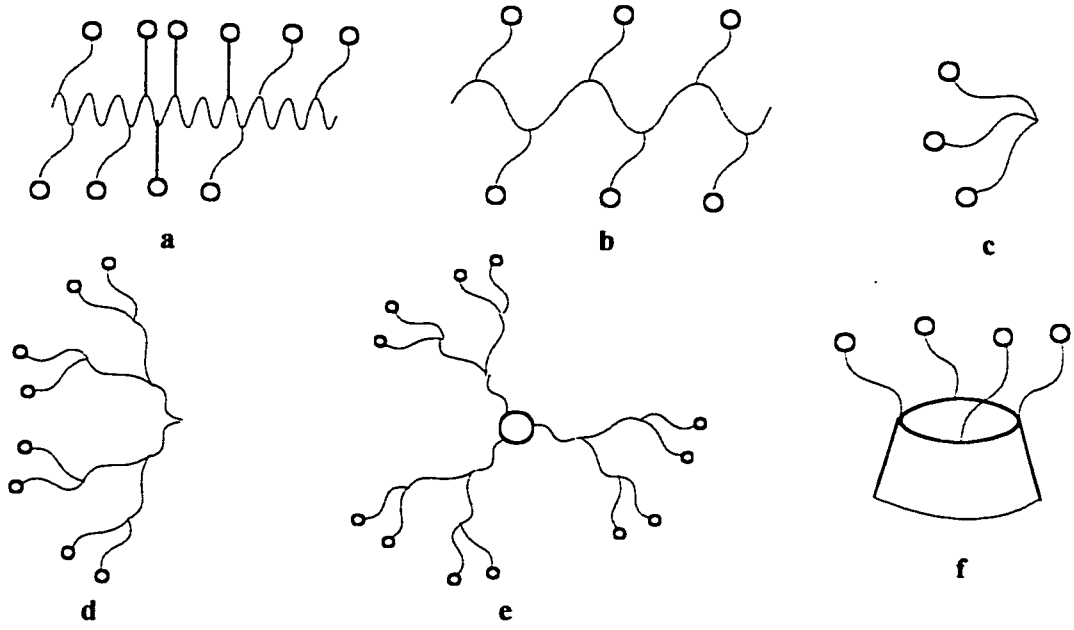

Figure 1.2.1.2. Multivalent carbohydrate ligands. (a) glycopolymers, (b) glycotelomers, glycopeptides and glycopeptoids, (c) glycoclusters, (d) glycodendrimers, (e) spherical glycodendrimers, and (f) glycocalixarenes.

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43 Roy, R. in *Carbohydrate Chemistry* G. J. Boons (Ed.) Blackie Academic & Professional, New York, **1998**, 243.

44 Park, W. K. C.; Aravind, S.; Romanowska, A.; Renaud, J.; Roy, R. *Methods Enzymol.* **1994**, *242*, 294.

45 Aravind, S.; Park, W. K. C.; Brochu, S.; Roy, R. *Tetrahedron Lett.* **1994**, *35*, 7739.

46 Wadhwa, M. S.; Knoell, D. L.; Young, A. P.; Rice, K. G. *Bioconjugate Chem.* **1995**, *6*, 283.

47 Saha, U. K.; Roy, R. *Tetrahedron Lett.* **1995**, *36*, 3635.

48 Saha, U. K.; Roy, R. *J. Chem. Soc., Chem. Commun.* **1995**, 2571.

49 Saha, U. K.; Roy, R. *Chem. Commun.* **1996**, 210.

50 Kim, J. M.; Roy, R. *Carbohydr. Lett.* **1996**, *1*, 465.

51 Roy, R. *Polymer News* **1996**, *21*, 226.

52 Meunier, S. J.; Roy, R. *Tetrahedron Lett.* **1996**, *37*, 5469.

53 Marra, A.; Schermann, M.-C.; Dondoni, A.; Casnati, A.; Minari, P.; Ungaro, R. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2479.

54 Marra, A.; Dondoni, A.; Sansone, F. *J. Org. Chem.* **1996**, *61*, 5155.

1.2.2 Neoglycoproteins

Neoglycoproteins were first introduced as animal vaccines to prepare anti-carbohydrate antibodies.[55] These glycoconjugates were required because carbohydrates by themselves are poorly immunogenic since they are T cell-independent antigens. As a result, they usually activate immunoglobulins of IgM class having low affinities. However, when conjugated to highly immunogenic protein carriers, carbohydrates become T cell-dependent antigens capable of stimulating antibodies of IgG classes having high affinities and specificities.

A wide variety of chemical methods for syntheses of neoglycoproteins are known and have been reviewed by Stowell and Lee.[56] Among these methods, the use of amino terminated glycosides find many different applications such as conjugation of the glycosides to proteins by diazotization or through conjugation using the isocyanate method. Coupling by amidation with a water soluble carbodiimide[57] is not only possible with amino sugars but also when a carboxyl group is present on the carbohydrate. Protein amidation can also be realized with glycosides bearing an acylating group such as mixed anhydrides,[58] nitrophenyl and *N*-hydroxysuccinimide esters.[59] Reductive amination of free amine residues on proteins (ε-amine of lysine and *N*-terminus) using sodium cyanoborohydride ($NaCNBH_3$) with both reducing sugars[22,60,61] and aglycons[62,63] containing a terminal aldehyde group offers the desired chemoselectivity and has proven to

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55 Dick, W. E.; Beurret, M. in *Contribution to Microbiology and Immunology*, vol. 10, *Conjugate Vaccines* J. M. Cruse and R. E. Lewis Jr. (Eds.), Karger, Basel, 1989, pp. 48-114.

56 Stowell, C. P.; Lee, Y. C. *Adv. Carbohydr. Chem. Biochem.* **1980**, *37*, 225.

57 Davies, M.-T.; Preston, J. F. *J. Anal. Biochem.* **1981**, *116*, 402.

58 King, R. R.; Cooper, F. P.; Bishop, C. T. *Carbohydr. Res.* **1977**, *55*, 83.

59 Klausner, Y. S.; Bodansky, M. *Synthesis* **1972**, 453.

60 Roy, R.; Katzenellenbogen, E.; Jennings, H. J. *Can. J. Chem. Cell Biol.* **1984**, *62*, 270.

61 Roy, R.; Laferrière, C. A.; Dettman, H. *Carbohydr. Res.* **1989**, *186*, C_1.

62 Roy, R.; Laferrière, C. A. *Can. J. Chem.* **1990**, *68*, 2045.

63 Kamicker, B. J.; Schwartz, B. A.; Olson, R. M.; Drinkwitz, D. C.; Gray, G. R. *Arch. Biochem. Biophys.* **1977**, *193*, 393.

be a very effective method for preparing neoglycoprotein conjugates. Another approach to neoglycoprotein synthesis is based on the Michael addition reaction. In this method, carbohydrates having terminally *N*-linked acrylamide aglycons are reacted with nucleophilic protein sites such as cysteines (thiol groups) and lysines (ϵ-terminal amines). In early work on the reductive amination method, allyl α-sialoside **5** was ozonolyzed and the resulting aldehyde conjugated to both bovine serum albumin (BSA) and tetanus toxoid (TT).[64] *p*-Formyl phenyl sialoside **3** was similarly conjugated to BSA (Scheme 1.2.2.1).[65] It was concluded that threshold carbohydrate densities are required for efficient binding to model lectins since conjugates with few carbohydrate residues on the proteins were poorly antigenic. The above sialoconjugates were only moderate inhibitors of hemagglutinations of influenza virus[22] and the ones with high NeuAc contents were highly immunogenic in rabbits.[66] An alternative to sialylated neoglycoproteins had to be considered since the immunogenicity of the neoglycoproteins caused a serious problem to their use as therapeutic antagonists in viral infections. This problem was first solved by the synthesis of non-immunogenic glycopolymers.

1.2.3 Glycopolymers

Glycopolymer antigens have numerous advantages over their neoglycoprotein counterparts. These advantages consist of more uniform and stable structures having a wide range of molecular weights, carbohydrate densities and functionalities. They also can be synthesized in large scale quite inexpensively, with easier purification and characterization steps.[39] Several different types of glycopolymers have been used as biomedical materials such as cell-specific culture substrata,[67,68,69] artificial antigens,[70,71]

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

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

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

67 Weigel, P. H.; Schnaar, R. L.; Kuhlenschmidt, M. S.; Schmell, E.; Lee, R. T.; Lee, Y. C.; Roseman, S. *J. Biol. Chem.* **1979**, *254*, 10830.

targeted drug delivery agents,[72] and as was mentioned before, they are also useful tools for investigating biological recognition phenomena.[73,74,75,76,77]

The first glycopolymer synthesis was performed by Horejsi and Kocourek[78] where simple allyl glycosides were copolymerized with acrylamide under aqueous radical conditions (Scheme 1.2.3.1). Numerous approaches exist to glycopolymer syntheses.[40,41,79,80] Copolymerization of monomers such as acrylamide with carbohydrate monomers having alkenyl[70,81] or styryl[69] glycosides or *N*-[82] or *O*-acryloyl[83] residues have thus been performed (Scheme 1.2.3.1). Another useful method is graft polymerization where the desired carbohydrate haptens can be directly incorporated into preformed polymers having reactive functionalities such as amines and active esters.[84] Consequently, the carbohydrate precursors are synthesized with either amino or reactive carboxyl functions on the aglycon moieties (Scheme 1.2.3.2).

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68 Kobayashi, A.; Akaibe, T.; Kobayashi, K.; Sumitomo, H. *Makromol. Chem., Rapid Commun.* **1986**, *7*, 645.

69 Kobayashi, K.; Kobayashi, A.; Tobe, S.; Akaibe, T. in *Neoglycoconjugates: Preparation and Applications* Y. C. Lee and R. T. Lee (Eds.) San Diego, Academic Press, **1994**, 261.

70 Kochetkov, N. K. *Pure Appl. Chem.* **1984**, *56*, 923.

71 Rozalski, A.; Brade, L.; Kuhn, H.-M.; Brade, J.; Kosma, P.; Appelmek, B. J.; Kusumoto, S.; Paulsen, H. *Carbohydr. Res.* **1989**, *193*, 257.

72 Duncan, R.; Kopeckova-Rojmanova, P.; Strohalm, J.; Hume, I.; Cable, H. C.; Pohl, J.; Lloyd, J. B.; Kopecek, J. *Br. J. Cancer* **1987**, *55*, 165.

73 Roy, R.; Tropper, F. D. *J. Chem. Soc., Chem. Commun.* **1988**, 1058.

74 Roy, R.; Tropper, F. D.; Romanowska, A. *J. Chem. Soc., Chem. Commun.* **1992**, 1611.

75 Roy, R.; Tropper, F. D.; Morrison, T.; Boratynski, J. *J. Chem. Soc., Chem. Commun.* **1991**, 536.

76 Nishimura, S.; Matsuoka, K.; Kurita, K. *Macromolecules* **1990**, *23*, 4182.

77 Hatanaka, K.; Ito, Y.; Muruyama, A.; Watanabe, Y.; Akaibe, T. *Macromolecules* **1993**, *26*, 1483.

78 Horejsi, V.; Kocourek, J. *Biochim. Biophys. Acta* **1973**, *297*, 346.

79 Roy, R. in *Modern Methods in Carbohydrate Synthesis* S.-H. Khan and R. O'Neil (Eds.) Harwood Academic, Amsterdam, **1996**, 378.

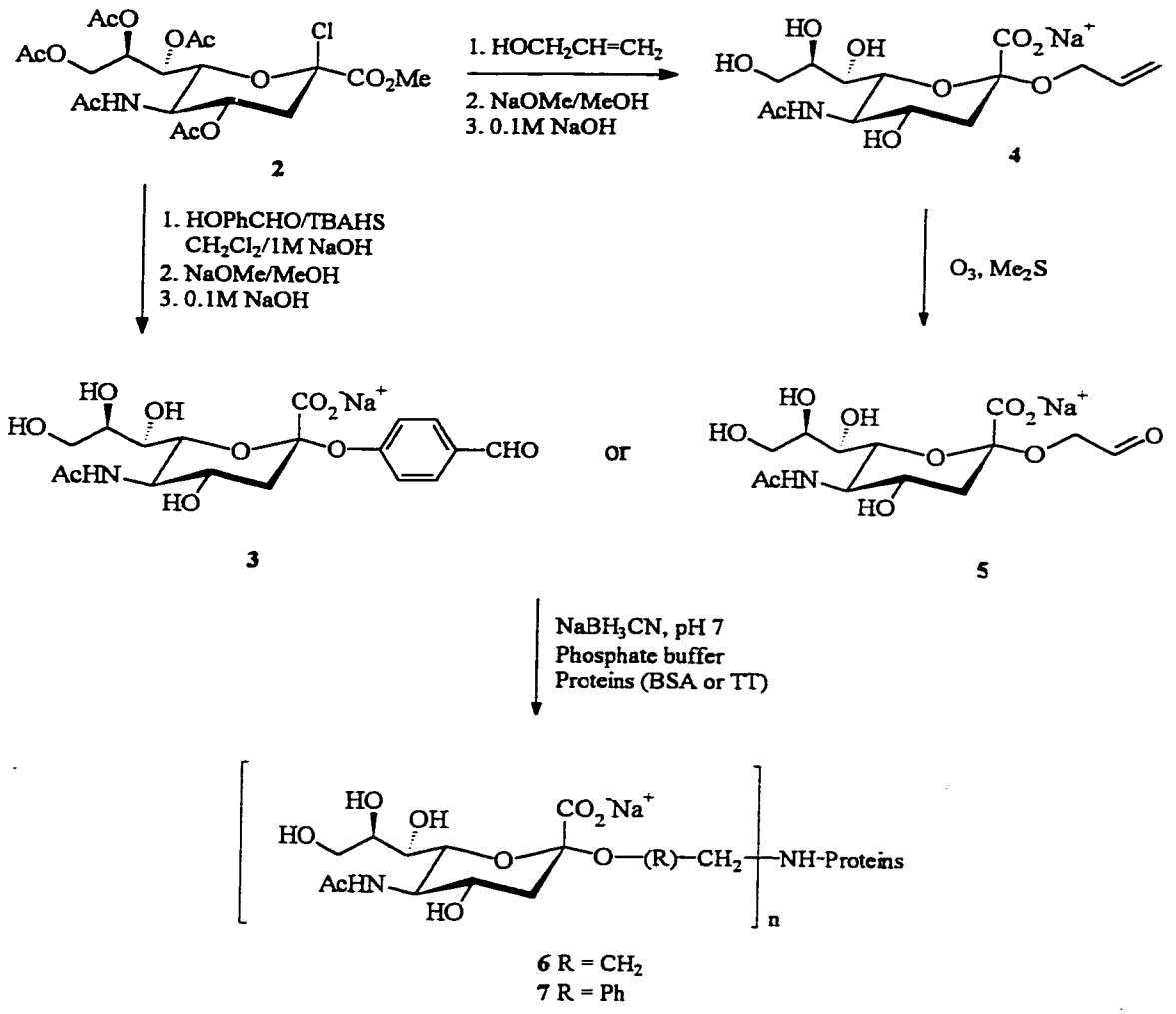
80 Bovin, N. V.; Gabius, H. J. *Chem. Soc. Rev.* **1995**, *24*, 413.

81 Horejsi, V.; Smolek, O.; Kocourek, J. *Biochim. Biophys. Acta* **1978**, *538*, 293.

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

83 Chytri, V.; Driguez, H. *Makromol. Chem., Rapid Commun.* **1992**, *13*, 499.

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



Scheme 1.2.2.1. Synthesis of neoglycoproteins.

One of the most striking influenza virus binding improvements (>10^3 - 10^6-fold) was achieved with polymeric α-sialosides.[85] This shows that glycopolymers are indeed useful as multivalent antigens. Figure 1.2.3.1 depicts some representative examples of glycopolymers. Sialopolymer **8** has been used for the inhibition of hemagglutination of human erythrocytes by influenza A virus (IC_{50} 2.3 μm).[22] However, polymer **10** appeared to be inactive against the A strain while its homologous 9-*O*-acetylated derivative **11**

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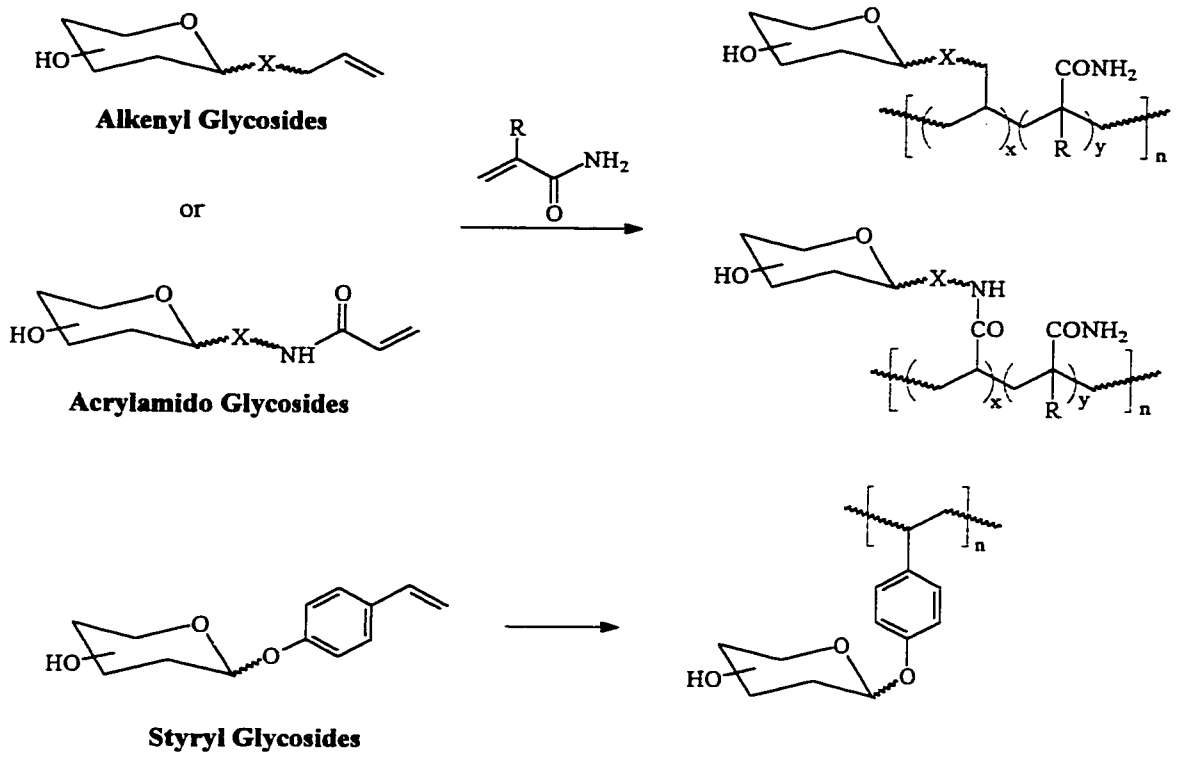
[85] Roy, R. in *Carbohydrates: Targets for Drug Design*. Z. J. Witczak (Ed.) Marcel Dekker, New York, **1996**, 84.

showed good inhibition of hemagglutinin of influenza C virus (IC_{50} 3.3 μm).[86] Polysialoside **12** is an example of a glycopolymer synthesized by the grafting method where the carbohydrate residue is added to a poly[*N*-(acryloyloxy)succinimide].[87] A more complex example of glycopolymer is represented by terpolymer **13** where poly-L-lysine is conjugated with mannopyranoside residues, gluconamide solubilizing moieties, and the AZT-drug.[88] This copolymer was used to target HIV-infected macrophages.



Scheme 1.2.3.1. Synthesis of glycopolymers by homo- or co-polymerization strategies.

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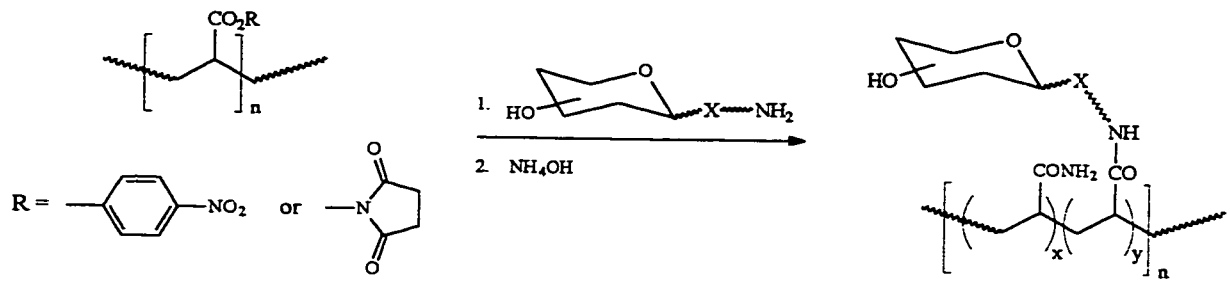
[86] Roy, R.; Andersson, F. O.; Harms, G.; Kelm, S.; Schauer, R. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1478.

[87] Sigal, G. B.; Mammen, M.; Dahmann, G.; Whitesides, G. M. *J. Am. Chem. Soc.* **1996**, *118*, 3789.

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



Scheme 1.2.3.2. Synthesis of glycopolymers involving the grafting method.

1.2.4 Glycoclusters

Glycoclusters were very important in the early studies of the multivalent effect seen in carbohydrate-protein interactions. They allowed some understanding of the requirements for conformations and geometries that cause improved binding. Glycoclusters, while lacking high valency, are considered chemically well defined, and offer effective tools at investigating supramolecular recognition phenomena. Figure 1.2.4.1 depicts many representative glycoclusters. Lee[89] examined a number of multivalent carbohydrate ligands and found the best known synthetic ligand for human hepatic ASGP-Rs, which is trivalent *N*-acetylgalactosaminide **14** [YEE(GalNAcAH)$_3$].

Glick and Knowles[90] synthesized bidentate α-sialoside ligands **15** and **16**, and studied the inhibition of hemagglutination of human erythrocytes by varying the distances between the two sialic acid residues. They found the optimal distance between the two sialosyl residues to be 5.7 nm and furthermore, ligand **16** (oligoglycine spacer) was more potent than the oligoethylene glycol series **15** due to the rigidity of **16**.

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[89] Lee, Y. C. in *Carbohydrate Recognition in Cellular Function. Ciba Foundation Symposium 145.* G. Bock and S. Harnette (Eds.) Wiley, Chichester, **1989**, 80.

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

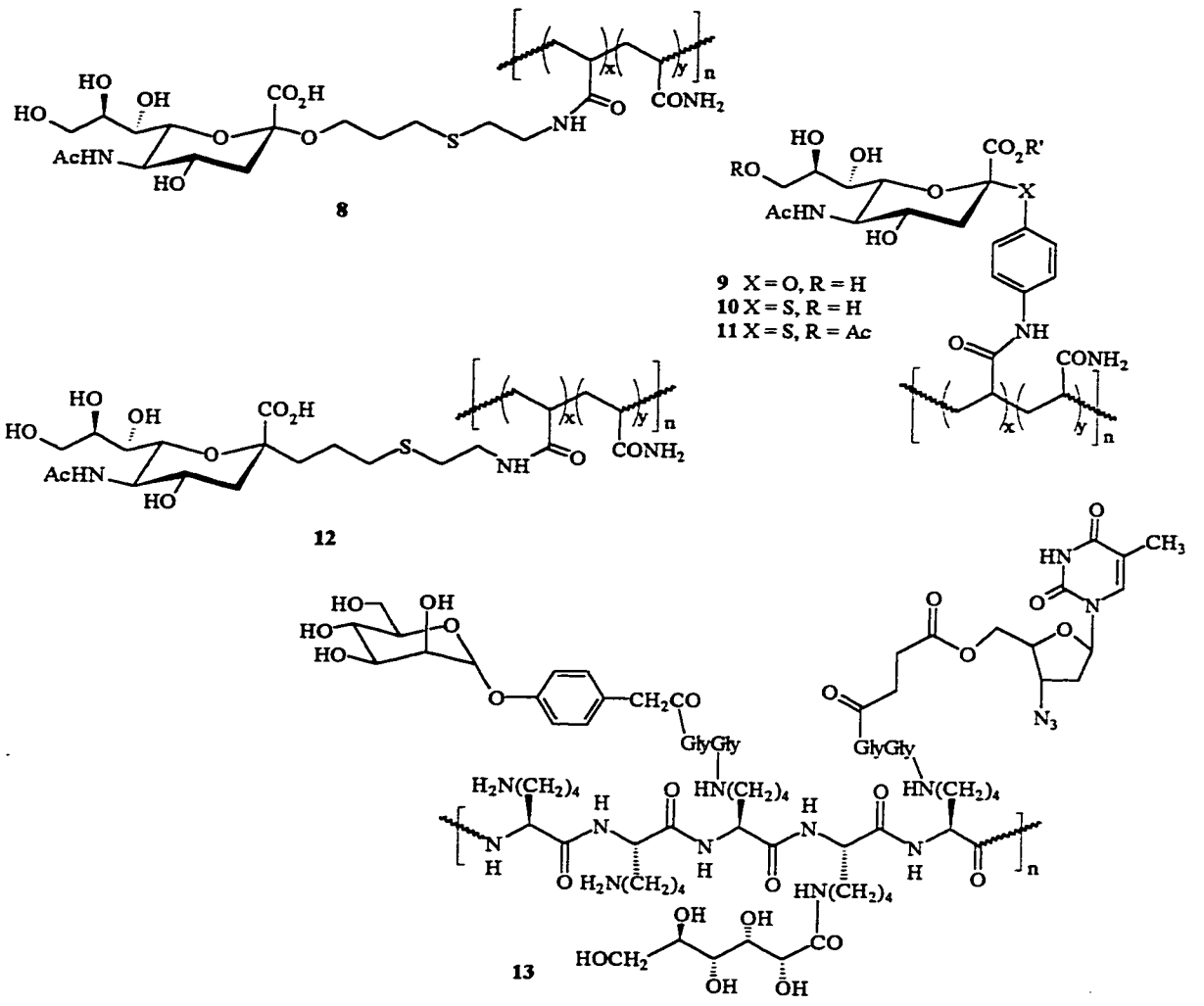

Figure 1.2.3.1. Representative examples of glycopolymers.

Other synthetic divalent α-sialosides were grafted onto peptides (glycopeptides) and gave dimeric clusters **17** and **18**, having glycine-rich (**17**) and proline-rich (**18**) peptides as linear backbone.[91] These clusters were eight- and four-fold more potent, respectively, compared to the corresponding parent monosialoside.

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

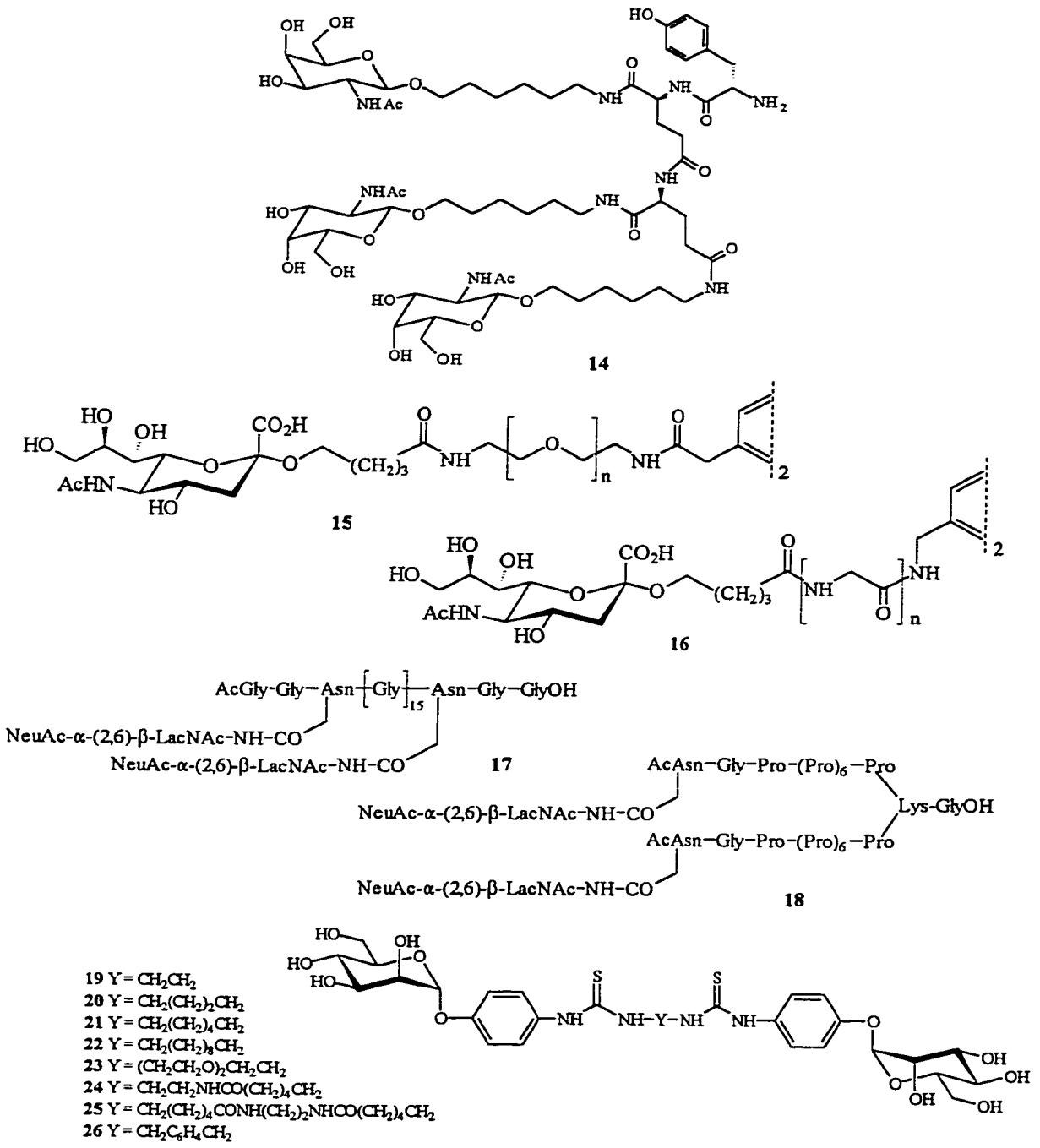

Figure 1.2.4.1. Representative examples of glycoclusters.

In our laboratory, a series of divalent α-D-mannopyranoside ligands with varying inter-mannoside distances were synthesized.[92] The study resulted in dimers **19-26** being ~10 to 90 fold more potent than methyl α-D-mannopyranoside. Cluster **21** was found to be the most potent with a spacer length of six methylene units whereas dimers having shorter and longer spacers were found to be less efficient.[92]

1.2.5 Glycotelomers

Telomers are low molecular weight, usually of less than ~10-12 monomer units, products of a free radical polymerization performed in the presence of a chain transfer agent (telogen).[93] Glycotelomers are considered chemically well defined having a higher valency than their glycocluster counterparts. However, glycotelomers lack the geometrical freedom possessed by the glycoclusters, due to their linear backbones.

Lactoside-containing telomers **29-34**[44-45] were synthesized by homopolymerization (using AIBN) of *N*-acryloylated lactosyl monomers **27** and **28** in the presence of adjusted concentrations of *tert*-butylmercaptan, 3-mercaptopropionic acid or its methyl ester as the telogen (Scheme 1.2.5.1). Telomers **29-34** were obtained in a single step as a family of low molecular weight lactosylated clusters. These telomers were only slightly better ligands than lactose in inhibition assays using peanut lectin.[44]

1.2.6 Glycopeptoids

Peptoids are *N*-substituted oligoglycines (NGs) whose carbonyl and side chain residues are superimposable to those of natural peptides (Figure 1.2.6.1). Glycopeptoids offer similar advantages over glycopeptides and glycotelomers with the exception that they exist as slowly equilibrating amide rotamers (E/Z), which may probe an ensemble of

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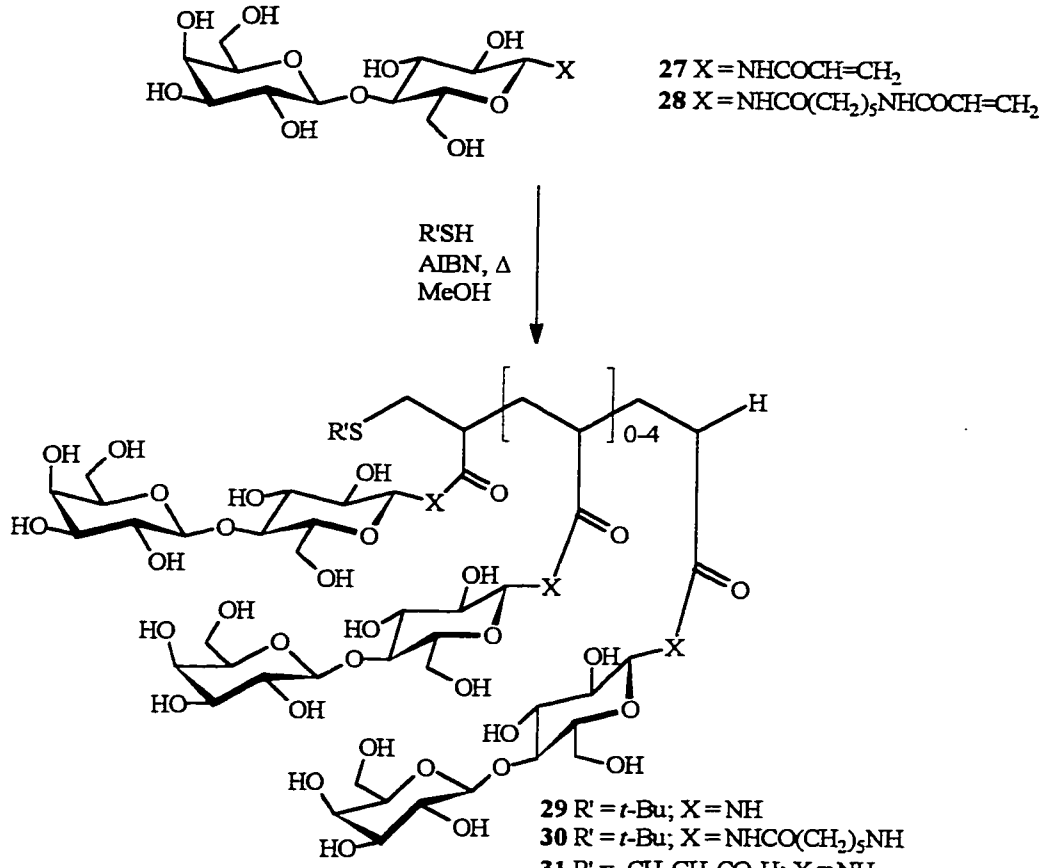
92 Pagé, D.; Roy, R. *Glycoconjugate J.* **1997**, *14*, 345.

93 Starks, C. M. in *Free Radical Telomerizations*, Academic Press, New York, **1974**, 4.

receptor *loci*. This equilibration occurs because of the tertiary amide linkage present at every branching point. Also, peptidomimetics are recognized to be metabolically stable, as compared to peptides. These glycopeptoids, some of which are represented in Figure 1.2.6.2, are readily obtained using principles taken from glycopeptide syntheses.[49] Oligomeric peptoids **35**[94] and **36**[49] were synthesized by a convergent blockwise approach using orthogonally protected derivatives (not shown). The multivalent glycopeptoids **35** and **36** were suitable for biological testing.



Scheme 1.2.5.1. Synthesis of lactoside-containing telomers.

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[94] Roy, R. in *Glycoscience: synthesis of substrate analogs and mimetics*, Topics in current chemistry, H. Driguez; J. Thiem and J. M. Beau (Eds.) Springer-Verlag, Berlin, **1997**, *187*, 241.

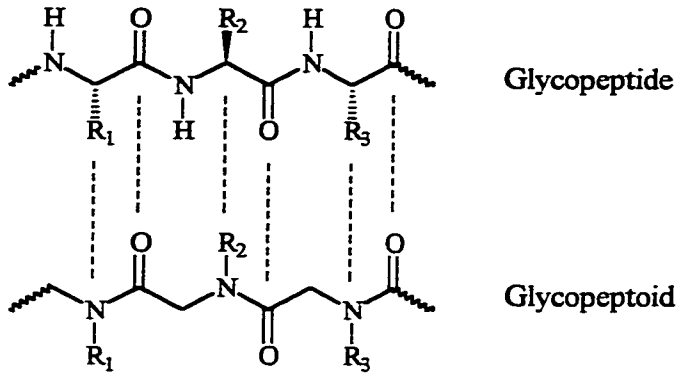

Figure 1.2.6.1. Structural similarities between glycopeptoids in relation to their analogous glycopeptides.

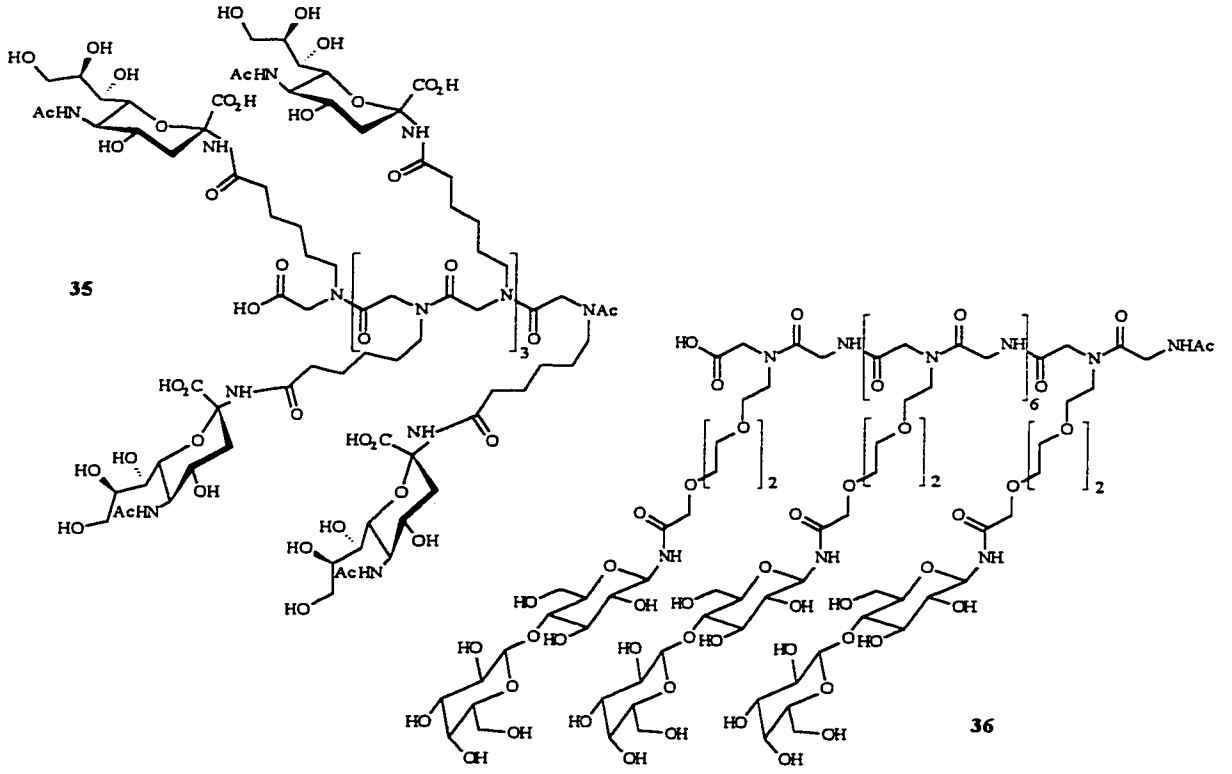

Figure 1.2.6.2. Representative examples of glycopeptoids.

1.3 Dendrimers and Glycodendrimers

1.3.1 Dendrimers

Dendrimers are macromolecules composed of a series of branched chains, each of which is termed a "dendron" (from Greek means tree or branch). Such dendra depart from a central, polyvalent organic core and propagate in a cascade fashion towards the periphery in concentric levels of successive growth (generations) by means of suitable repetitive units. This novel family of macromolecules is characterized by its high degree of branching which originates from a central point, a branch point at each monomer unit, and a large number of chain ends or surface functional groups, resulting in a unique, controlled macromolecular architecture. Their three-dimensional structure, which becomes progressively globular as the molecular weight increases, makes these materials a new form of matter, the closest analogues being globular proteins.

Dendrimers have a promising future, in which they possibly can replace a wide variety of materials currently used. Possible applications include those in specialized drug-delivery systems, catalyst carriers, adhesives, coatings, photocopier toners.[95-96] Furthermore, the presence of intramolecular spaces within the molecular structure of dendrimers enables them to withhold bioactive molecules used in agrochemical, pharmaceutical and cosmetic applications.

Work in the field of dendritic molecules dates back to the early fifties with the publication of a theoretical paper,[97] but it is only in 1978 that Vögtle and coworkers[98] first reported synthetic dendrimer molecules. The pioneering cascade approach involved a branching reaction sequence through cyanoethylation of amines with acrylonitrile followed

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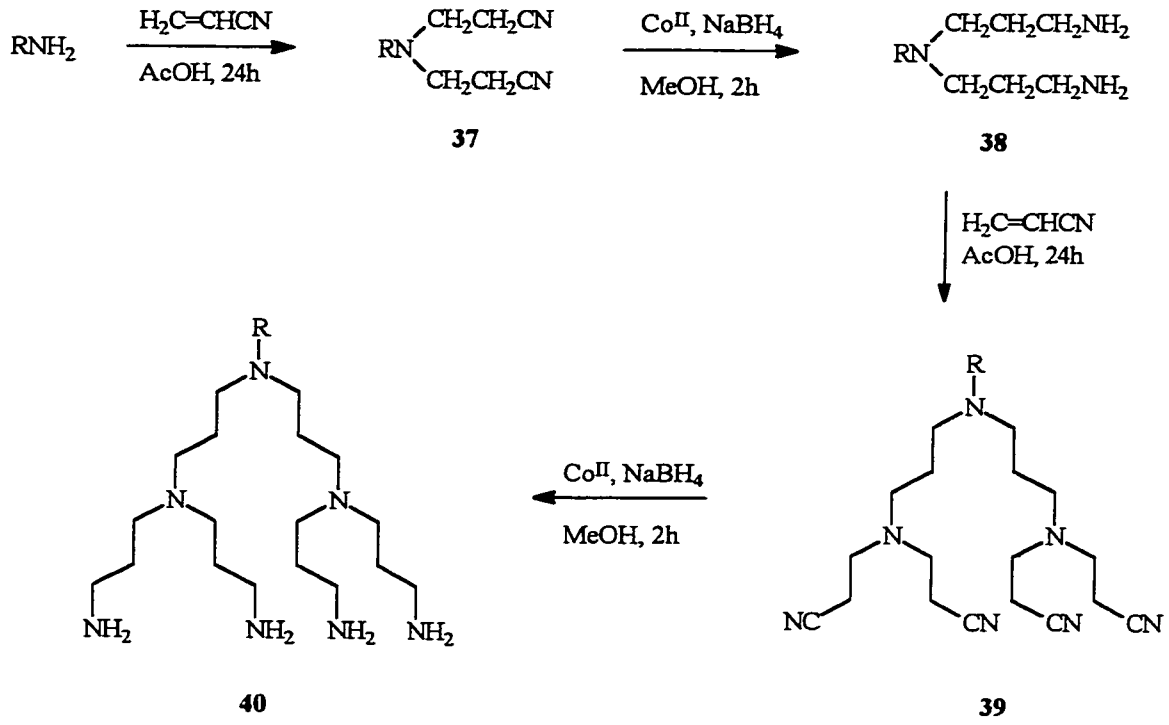
[95] a) Fréchet, J. M. J. *Science* **1994**, *263*, 1710. b) Dvornic, P. R.; Tomalia, D. A. *Curr. Opin. Colloid Interface Sci.* **1996**, *1*, 221. c) Issberner, J.; Moors, R.; Vögtle, F. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2413.

[96] Ardoin, N.; Astruc, D. *Bull. Soc. Chim. Fr.* **1995**, *132*, 875.

[97] Flory, P. J. *J. Am. Chem. Soc.* **1952**, *74*, 2719.

[98] Buhleier, E.; Wehner, W.; Vögtle, F. *Synthesis* **1978**, 155.

by reduction to double the amine end group functionality, thus forming dendritic poly(trimethylene imine) **40** (Scheme 1.3.1.1). This first attempt was not very useful because very low yields were obtained which restricted the synthesis to low molecular weight dendritic polyamines.



Scheme 1.3.1.1. Vögtle's pioneering cascade molecules.

Two distinct approaches to dendrimer synthesis[99,100,101] have evolved from the pioneering work of Vögtle.[98] Tomalia[102,103,104] and Newkome[105,106] developed the

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99 Tomalia, D. A.; Durst, H. D. *Top. Curr. Chem.* **1993**, *165*, 193.

100 Newkome, G. R.; Moorefield, C. N.; Vögtle, F. *Dendritic Molecules, Concepts, Syntheses, Perspectives*; VCH, Weinheim, 1996.

101 Hawker, C. J.; Fréchet, J. M. J. in *Comprehensive Polymer Science* S. L. Aggarwal and S. Russo (Eds.) Pergamon, Oxford, **1996**, 140.

divergent route based on step reactions starting from the centre of the dendrimer. Thus, reaction of a core with two or more moles of a reagent containing at least two protected branching sites, followed by removal of the protecting groups and subsequent reaction of the liberated reactive sites, leads to the first generation. This cycle is repeated until an ideal size for the macromolecule has been reached. On the other hand, Fréchet[107-108] and Miller[109] developed the convergent route (Convergent Growth Strategy) in which the synthesis is started at what will become the boundary of the dendrimer. Unique features of the convergent approach include the control over the nature and placement of the groups **S** (Scheme 1.3.1.2) that are placed at the periphery of the molecule. Also, the fact that each successive growth step consists of the reaction of these small groups **S** having only one reactive centre **41** (f_r) to a monomer **42** having two different functional groups (c and f_p) wherein c is the coupling site and fp is unreactive when the coupling reaction occurs but becomes reactive by simple deprotection step. Each generation occurs after the reaction of two of the newly formed growing molecules **44** (1st) or **46** (2nd generation) to the monomer **42** and so on. After reaching a satisfying size of dendritic wedges, the dendrimer is completed by coupling of these wedges to a suitable core containing typically 2, 3, or 4 reacting sites. The hypothetical core **47** (Scheme 1.3.1.2) contains 4 reacting centers (c) which will lead to a four-directional cascade polymer **48**.

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

[103] Tomalia, D. A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P. *Macromolecules* **1986**, *19*, 2466.

[104] Tomalia, D. A.; Naylor, A. M.; Goddard, W. A., III *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 138.

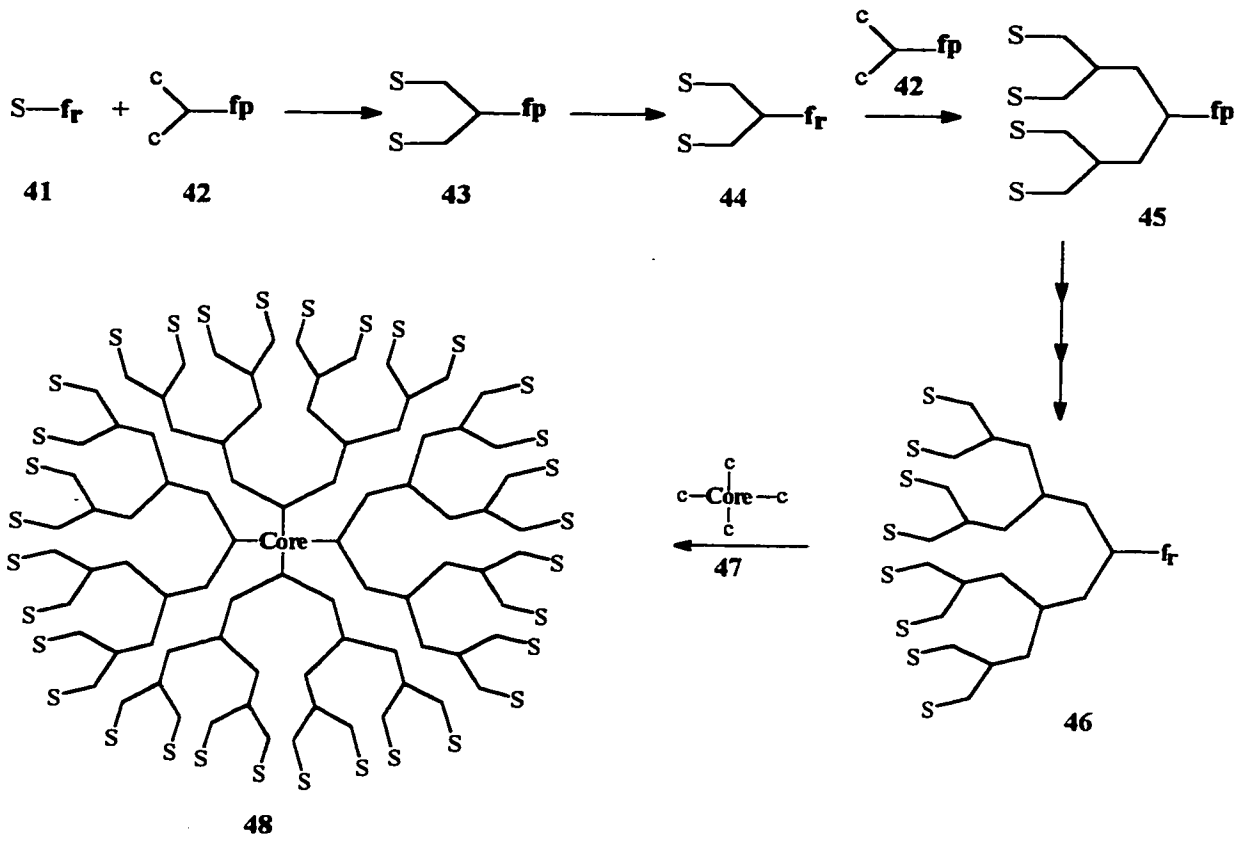
[105] Newkome, G. R.; Yao, Z.; Baker, G. R.; Gupta, V. K. *J. Org. Chem.* **1985**, *50*, 2003.

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

[107] Hawker, C. J.; Fréchet, J. M. J. *J. Chem. Soc., Chem. Commun.* **1990**, 1010.

[108] Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1990**, *112*, 7638.

[109] Miller, T. M.; Neenan, T. X. *Chem. Mat.* **1990**, *2*, 346.



Scheme 1.3.1.2. Basic concept of the Convergent Growth Strategy.

Newkome used a nucleophilic displacement reaction on a multifunctional core **49** to produce (Scheme 1.3.1.3), after two stages of reaction, a cascade molecule **51** coined "arborol" or polyamidoalcohol.[105-106] Newkome also synthesized many different dendrimers (Figure 1.3.1.1), ranging from the dendritic polyamidoacid **52**[110] obtained from DCC coupling of a polycarboxylic acid core and an aminotriester residue, to the ruthenium-based cascade polymer **53**.[111]

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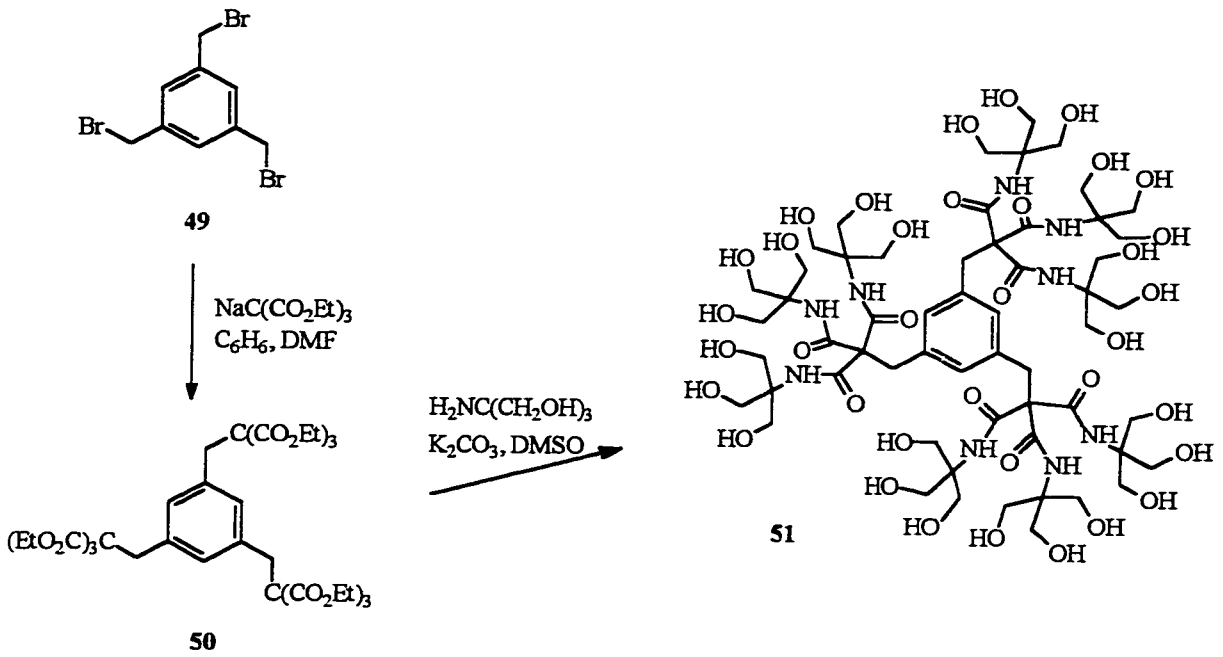
[110] Newkome, G. R.; Lin, X.; Weis, C. D. *Tetrahedron Asymmetry* **1991**, *2*, 957.

[111] Newkome, G. R.; Cardullo, F.; Constable, E. C.; Moorefield, C. N.; Cargill Thompson, A. M. W. *J. Chem. Soc., Chem. Commun.* **1993**, 925.



Scheme 1.3.1.3. Newkome's synthesis of dendritic molecules.

The most extensive published studies of dendritic molecules are directed to Starburst™ polymers, which were introduced by Tomalia.[102] These Starburst™ polymers are highly branched polyamidoamine (PAMAM) dendrimers built up to the tenth generation.[104,112] Scheme 1.3.1.4 illustrates the synthesis of two different PAMAM molecules. The synthesis of dendritic PAMAM **57** and **58** involves the reaction of a nucleophilic initiator core such as ammonia with a multifunctional electrophilic reagent (methyl acrylate) which carries functional groups of significantly different reactivities toward the initiator core (Scheme 1.3.1.4). Reaction of the resulting adduct **54** with a large molar excess of ethylene diamine (multifunctional nucleophile) produces a compound **55** of increased terminal multiplicity, with terminal nucleophilic sites that can serve to expand the system further upon repetition of the growth cycle. The dendrimers are terminated by a final amidation step performed in the presence of 2-aminoethanol or

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

ethylene diamine which gives dendrimers **57** and **58** having either hydroxyls or amines as end groups.

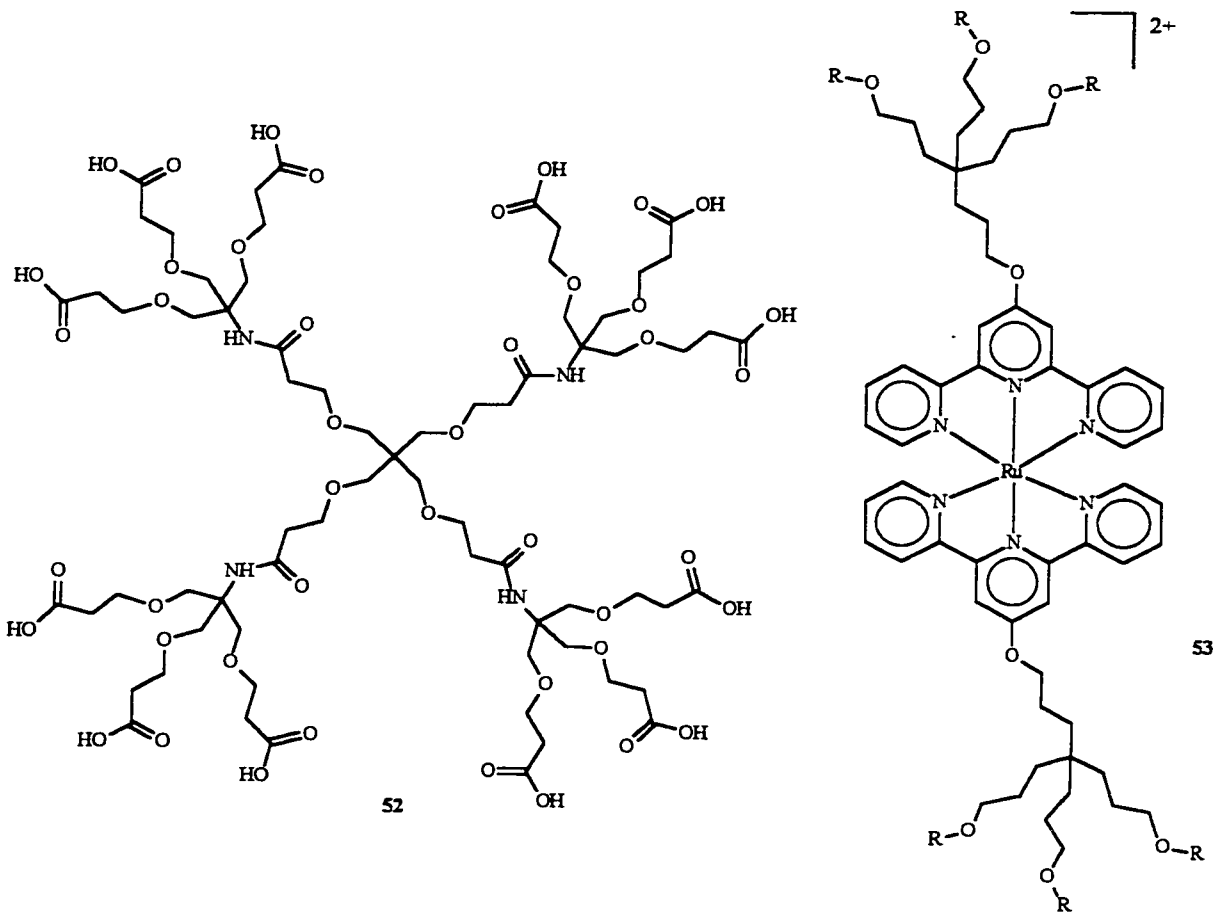
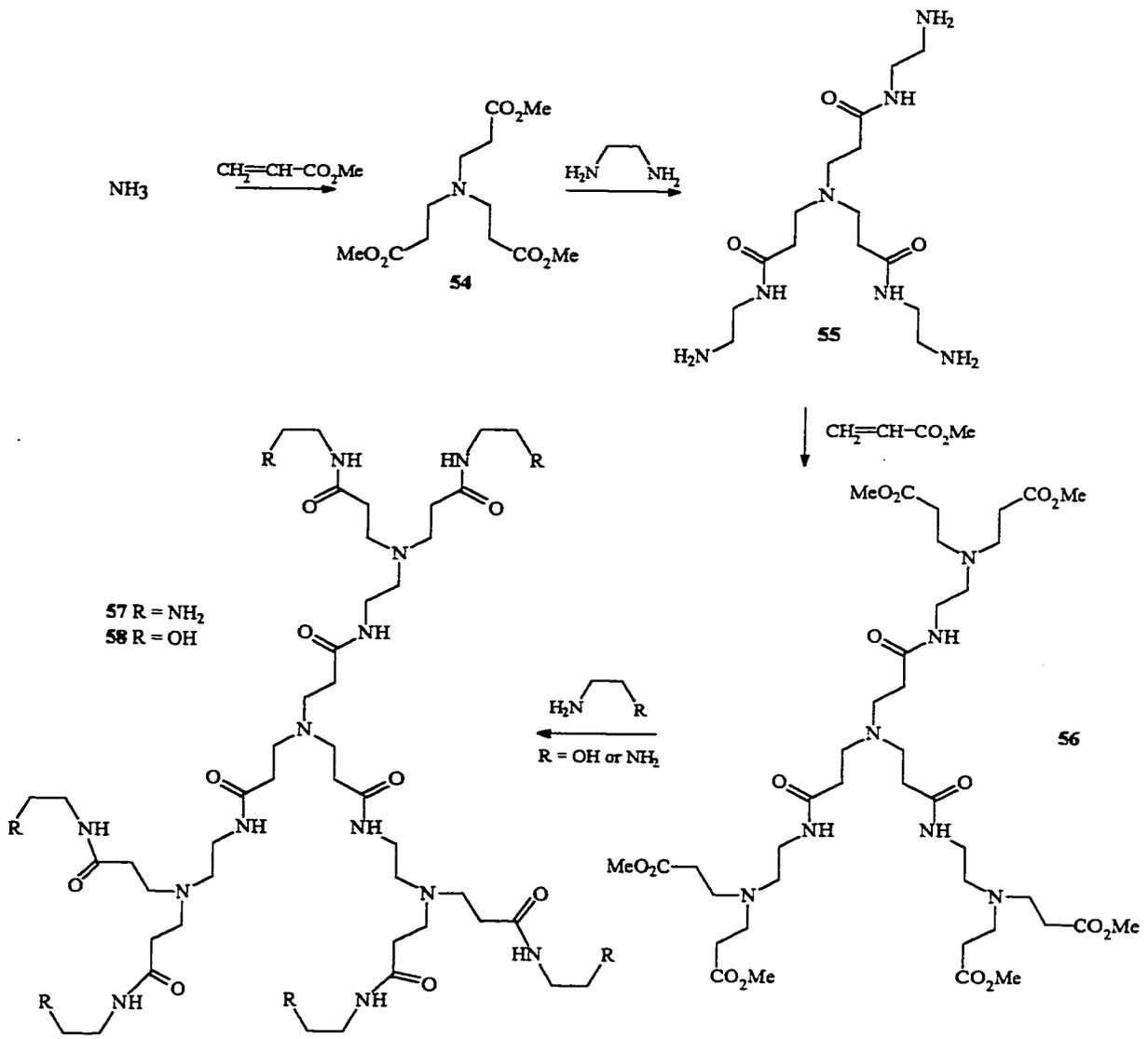


Figure 1.3.1.1. Representative examples of Newkome's dendrimers.

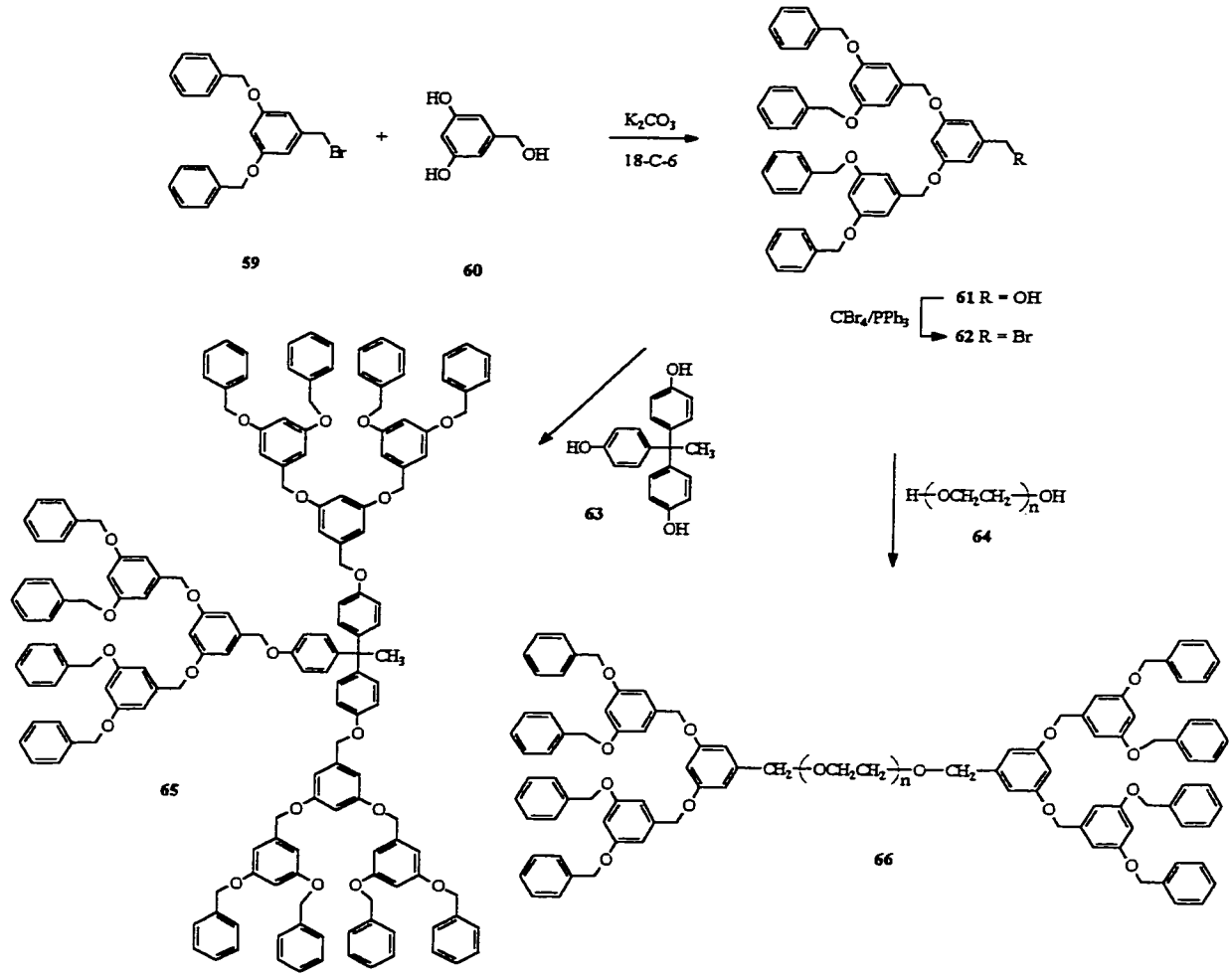
Fréchet's synthesis of dendrimers using the convergent approach is depicted in Scheme 1.3.1.5.[108,113] The polyether dendritic fragments are prepared from benzylic bromide residues condensed with the two phenolic groups of the monomer, 3,5-dihydroxybenzyl alcohol **60**. After transformation of the benzylic alcohol functionality of the growing molecule into the corresponding bromide **59**, the procedure is repeated with

[113] Gitsov, I.; Wooley, K. L.; Fréchet, J. M. J. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1200.

stepwise addition of monomer **60** followed again by activation of the benzylic site. After several generations of growth, the resulting dendritic wedges, in their benzylic bromide form **62**, can be coupled to a polyfunctional core such as 1,1,1-tris(4'-hydroxyphenyl)ethane **63** or polyethylene glycols **64** of different sizes to form the final hyperbranched macromolecules **65**[108] or **66**.[113]



Scheme 1.3.1.4. Tomalia's synthesis of dendritic PAMAM.



Scheme 1.3.1.5. Fréchet's synthesis of dendrimers.

1.3.2 Glycodendrimers

Following the development of the numerous elegant synthetic routes for the synthesis of different types of dendrimers,[96,99-101] using various kinds of building blocks, dendrimer chemistry now appears to offer the most highly controllable methodology for synthesizing large three-dimensional macromolecules with very precise chemical constitutions and increasingly well-defined molecular structures. It was then obvious to take advantage of dendrimers as carrier molecules to covalently attach carbohydrate

moieties. This gave birth to a novel class of glycoconjugate. The valency of glycodendrimers place them between small glycoclusters and glycopolymers which fills the gap present between these two types of glycoconjugates.

The first glycodendrimer synthesis was performed in our laboratory in 1993[114,115] and was based on sialic acid attached to a hyperbranched L-lysine dendritic core. These glycodendrimers will be discussed in some detail in Chapter 4 of this thesis dissertation, along with an in-depth discussion of sialic acid-based gallic acid dendrimers.[116-117] Many other dendrimers having different carbohydrate haptens attached to L-lysine and gallic acid dendritic cores originated directly from work done in this thesis dissertation, and are listed below without going in details. Poly L-lysine glycodendrimers containing ß-D-lactosides,[118] *N*-acetyllactosaminides,[118] *N*-acetylglucosaminides,[118] α-D-mannosides,[119] T-antigens,[38] and 3'-sulfo-Lewis^X glucoside analogs[120] were synthesized. Glycodendrimers based on gallic acid core bearing ß-D-lactosides[121] and 3'-sulfo-Lewis^X glucoside analogs[120] were also prepared in our laboratory.

Figure 1.3.2.1 illustrates representative examples of various glycodendrimers originating from Roy's research group. Bi-directional glycodendrimer **67** is based on a phosphotriester backbone containing *N*-acetylgalactosaminides.[51,122,123] Dendrimer **67** showed enhance binding affinity using ELLA inhibition experiments. Two other series of glycodendrimers **68** and **69**, one of which is spherical (**69**), are formed from a dendritic

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114 Roy, R.; Zanini, D.; Meunier, S. J.; Romanowska, A. *J. Chem. Soc., Chem. Commun.* **1993**, 1869.

115 Roy, R.; Zanini, D.; Meunier, S. J.; Romanowska, A. *ACS Symp. Ser.* **1994**, *560*, 104.

116 Wang, S.-N.; Meunier, S. J.; Wu, Q.-Q.; Roy, R. *Proceedings of the XVIIth Int. Carbohydr. Symp.* **1994**, Ottawa, Canada, July 17-22, 304.

117 Meunier, S. J.; Wu, Q.-Q.; Wang, S.-N.; Roy, R. *Can. J. Chem.* **1997**, *75*, 1472.

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

119 Pagé, D.; Zanini, D.; Roy, R. *Bioorg. Med. Chem.* **1996**, *4*, 1949.

120 Zanini, D.; Roy, R.; Park, W. K. C.; Foxall, C.; Srivastava, O. P. *Proceedings of the XVIIIth Int. Carbohydr. Symp.* **1996**, Milan, Italy, July 21-26, 543.

121 Roy, R.; Park, W. K. C.; Wu, Q.-Q.; Wang, S.-N. *Tetrahedron Lett.* **1995**, *36*, 4377.

122 Zanini, D.; Park, W. K. C.; Meunier, S. J.; Wu, Q.-Q.; Aravind, S.; Kratzer, B.; Roy, R. *Polym. Mater. Sci. Eng.* **1995**, *73*, 82.

123 Park, W. K. C.; Kratzer, B.; Zanini, D.; Wu, Q.-Q.; Meunier, S. J.; Roy, R. *Glycoconjugate J.* **1995**, *12*, 456.

backbone based on a 3,3'-iminobis(propylamine) core.[124,125] These glycodendrimers (**68** and **69**), having valencies up to sixteen, were all found to be better inhibitors of the binding of human α_1-acid glycoprotein (orosomucoid) to the lectin *Limax flavus* than the monomeric sialoside on a per sialoside basis.[124,125]

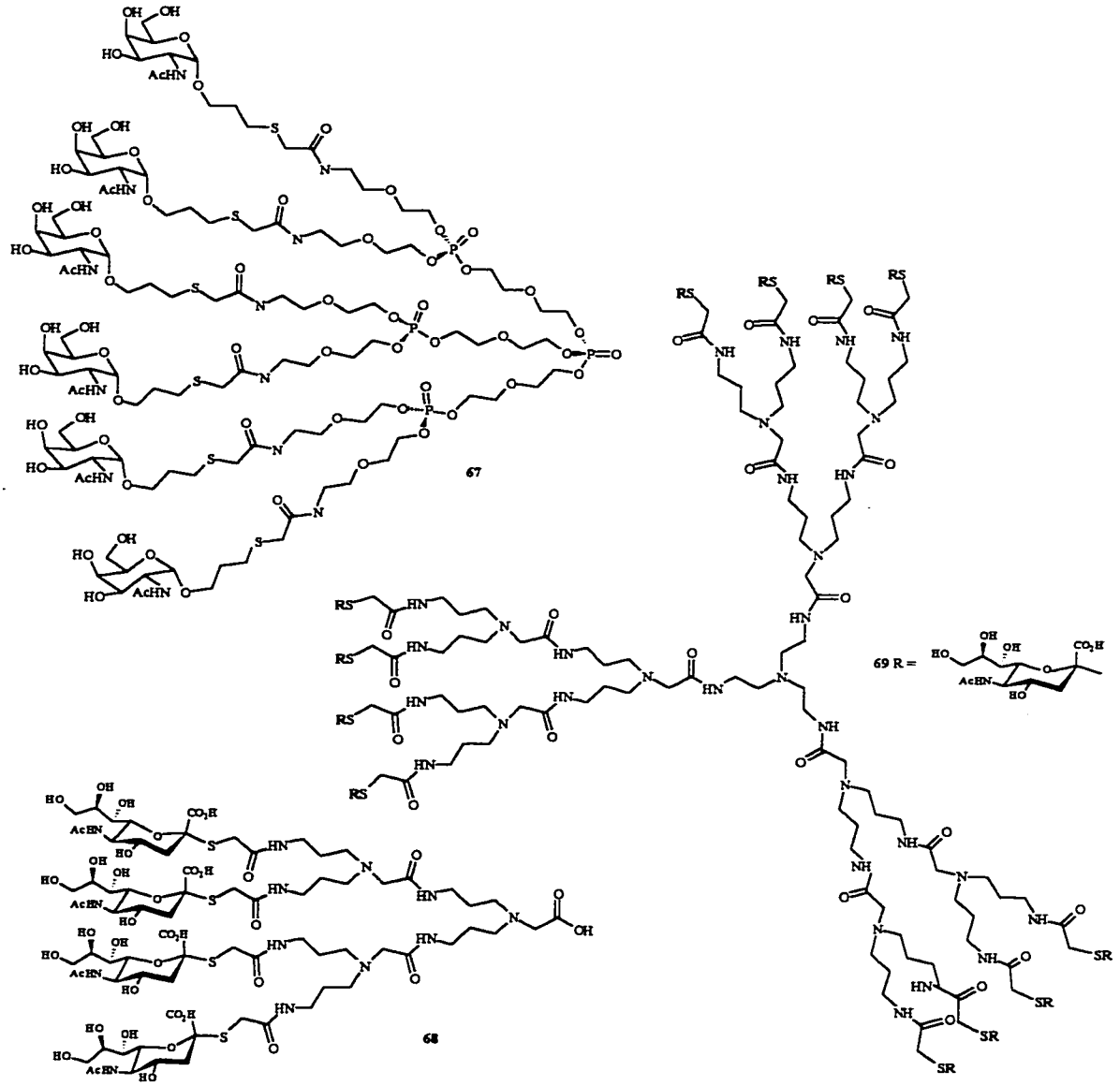

Figure 1.3.2.1. Representative examples of Roy's glycodendrimers.

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[124] Zanini, D.; Roy, R. *J. Org. Chem.* **1996**, *61*, 7348.

[125] Zanini, D.; Roy, R. *J. Am. Chem. Soc.* **1997**, *119*, 2088.

Spherical Starburst™ PAMAM dendrimers **70** and **71**[99,104] have been used by many different research groups to attach a number of carbohydrate derivatives. Disaccharide lactones related to lactose **72** (*O*-ß-D-galactopyranosyl-(1,4)-D-glucono-1,5-lactone) and maltose **73** (*O*-ß-D-glucopyranosyl-(1,4)-D-glucono-1,5-lactone) have been attached to PAMAM dendrimers **70** via amide bond formation (Scheme 1.3.2.1).

Dendrimers having 12 (**78** and **79**), 24, and 48 glycan residues were found to have strong binding towards Concanavalin A and peanut lectins.[126] T_N-antigen (α-D-GalNAc-*O*-Ser) peptide **74** was conjugated onto PAMAM dendrimers **70** to obtain non-immunogenic glycodendrimers **80** up to the fifth generation (Scheme 1.3.2.1).[127]

PAMAM dendrimers **70** and **71** have been linked to *p*-aminophenyl α-D-mannopyranoside through formation of thioureas (Scheme 1.3.2.1).[92,119,128] These compounds (**81**) were found to have enhanced binding affinity for Concanavalin A lectin.[128]

Lindhorst and Kieburg[129,130] prepared PAMAM dendrimers **82** and **83** up to eight in valency using an isothiocyanate coupling reaction to connect the carbohydrate residues to the dendritic core (Scheme 1.3.2.1). The coupling is very efficient when peracetylated glycosyl isothiocyanates of ß-D-glucose, α-D-mannose **76**, ß-D-galactose **77**, ß-cellobiose, and ß-lactose are used.

The isothiocyanate coupling reaction was also used for the reaction of *p*-isothiocyanato α-D-mannopyranoside **75** with dendritic core **84** (Scheme 1.3.2.2).[131] The spherical mannosylated dendrimer **85** obtained showed only slight improvements over a monomeric mannoside in solid phase inhibition assays of the binding of yeast mannan to both Concanavalin A and *Pisum Sativum* (pea) lectins.

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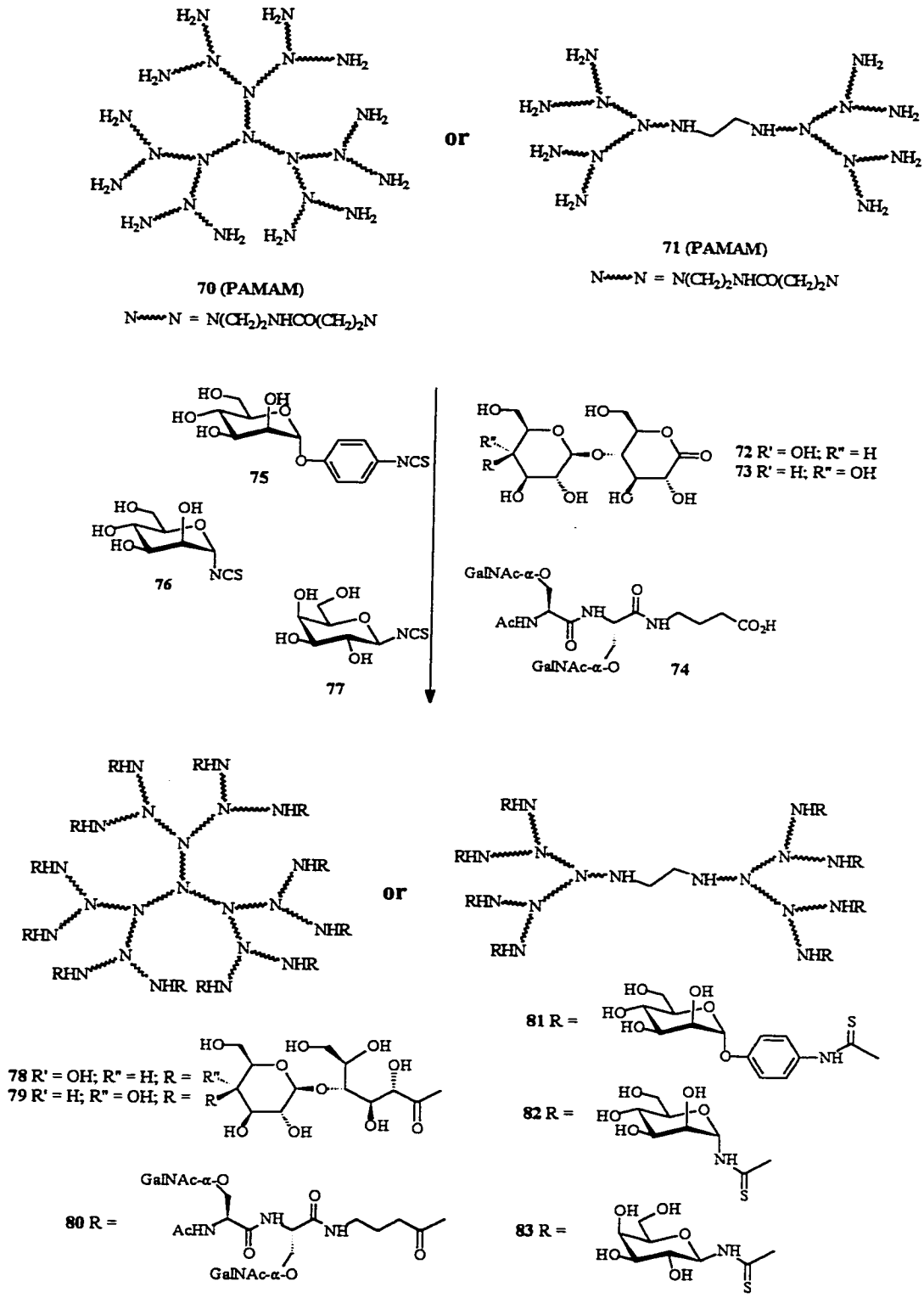
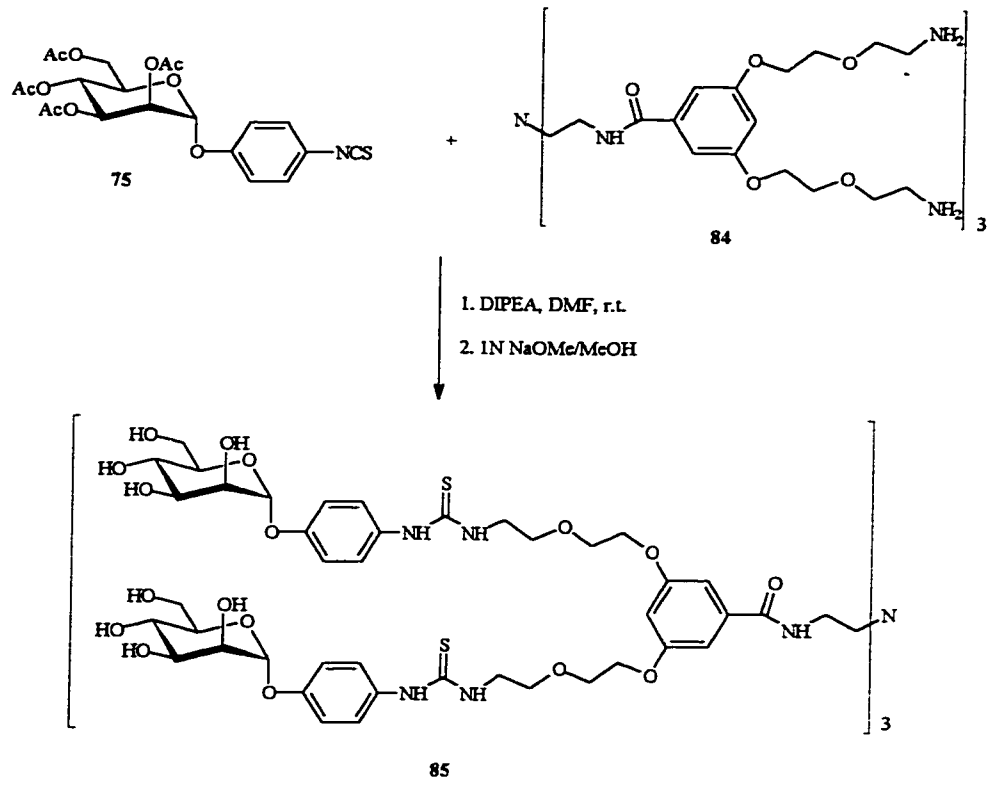
[126] Aoi, K.; Itoh, K.; Okada, M. *Macromolecules* **1995**, *28*, 5391.
[127] Toyokuni, T.; Singhal, K. *Chem. Soc. Rev.* **1995**, 231.
[128] Pagé, D.; Roy, R. *Bioconjug. Chem.* **1997**, *8*, 714.
[129] Lindhorst, T. K.; Kieburg, C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1953.
[130] Lindhorst, T. K.; Kieburg, C. *Synthesis* **1995**, 1228.
[131] Pagé, D.; Aravind, S.; Roy, R. *Chem. Commun.* **1996**, 1913.



Scheme 1.3.2.1. Synthesis of various glycodendrimers based on polyamidoamines.



Scheme 1.3.2.2. Synthesis of hexavalent α-D-mannopyranoside dendrimer.

Another example of spherical non-PAMAM dendrimers is given in Scheme 1.3.2.3. Ashton *et al.*[132,133] employed a repetitive synthetic sequence for forming amide bonds in the preparation of various dendritic wedges having 3 (**86**), 6, and 12 mannoside residues.[133] Spherical dendrimers **88** are obtained in the final step by convergent attachment of the dendritic wedges to a 1,3,5-trisubstituted benzenoid core **87**. ELLA experiments on these glycodendrimers showed some improvement in inhibiting the binding of Con A lectin to purified yeast mannan.[133]

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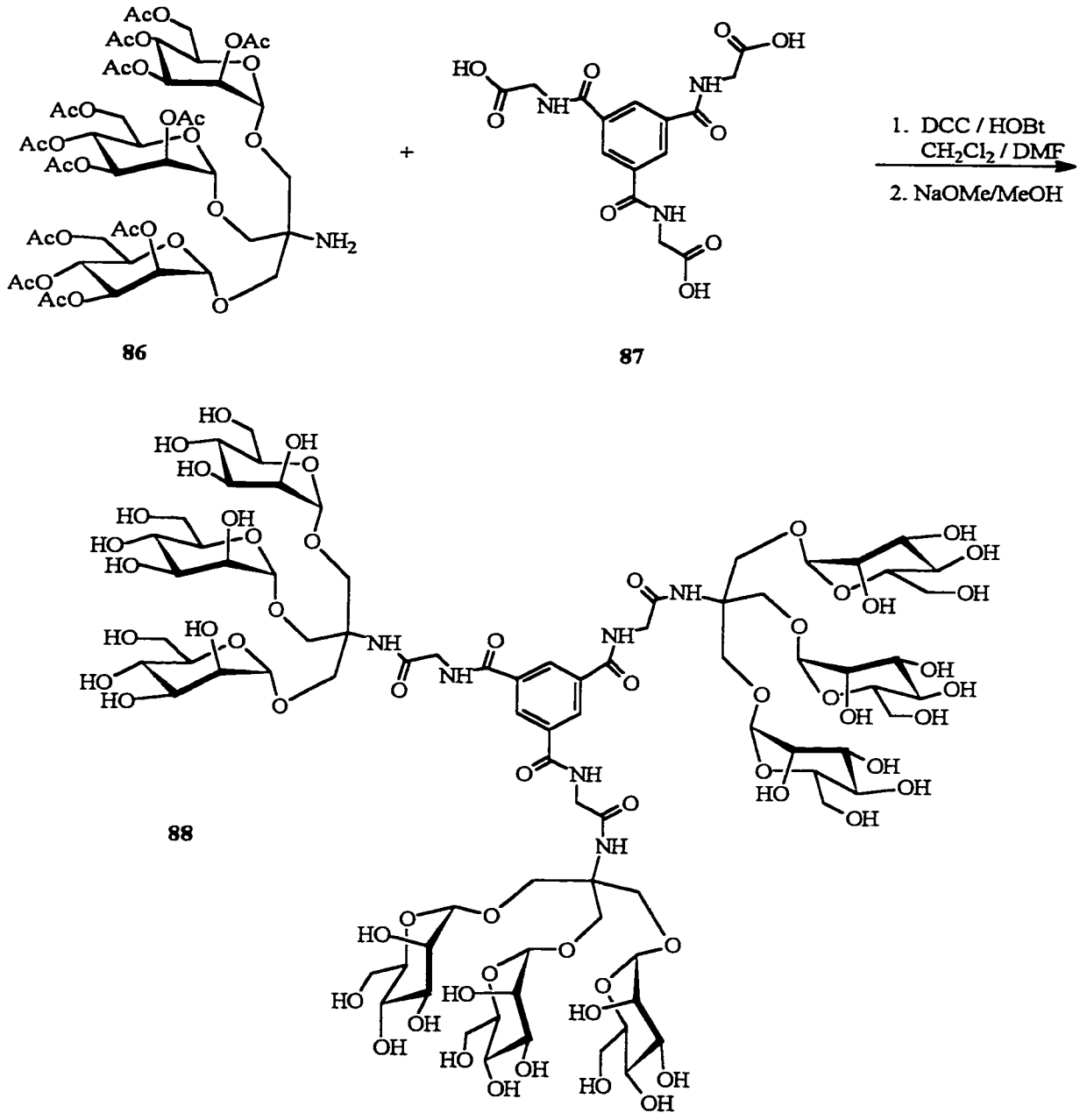
[132] Ashton, P. R.; Boyd, S. E.; Brown, C. L.; Jayaraman, N.; Nepogodiev, S. A.; Stoddart, J. F. *Chem. Eur. J.* **1996**, *2*, 1115.

[133] Ashton, P. R.; Hounsell, E. F.; Jayaraman, N.; Nilsen, T. M.; Spenser, N.; Stoddart, J. F.; Young, M. *J. Org. Chem.* **1998**, *63*, 3429.



86 87 88

Scheme 1.3.2.3. Synthesis of α-D-mannopyranoside dendrimers.

1.4 Calix[n]arenes and Glycocalix[4]arenes

Calix[n]arenes are [1_n]metacyclophanes made up of phenolic units meta-linked by methylene bridges and possessing basket-shaped cavities.[134] They were first discovered in the 1940's by Zinke[135] but it was not until the late 1970's that their structures were completely elucidated by Gutsche.[136,137] The name calixarene was given by Gutsche[136-137] who had recognized, in molecular models of the tetramer, a chalice- or cup-like shape similar to that of a Greek crater vase (in Greek : calix). The suffix arene indicates the presence of aryl rings in the molecular framework.[134]

Calixarenes have been used in many different applications. Applications of non-modified calixarenes include recovery of cesium,[138] lanthanide sequestration,[139] stabilizers for organic polymers (antioxidants),[140] separation of neutral organic molecules such as isomeric xylenes,[141] and pollution control,[142] whereas modified calixarenes are useful in applications such as recovery of uranium,[143] selective complexation of metal cations,[144] stabilizers for organic polymers,[145] phase transfer agents,[146] accelerators for instant

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134 a) Gutsche, C. D. *Acc. Chem. Res.* **1983**, *16*, 161. b) Gutsche, C. D. in *Calixarenes, monographs in supramolecular chemistry* J. F. Stoddart (Ed.) The Royal Society of Chemistry, Cambridge, **1989**.

135 a) Zinke, A.; Ziegler, E. *Chem. Ber.* **1944**, *77*, 264. b) Zinke, A.; Zigewerer, G.; Hössinger, K.; Hoffman, G. *Monatsh.* **1948**, *79*, 438.

136 Gutsche, C. D.; Muthukrishnan, R. *J. Org. Chem.* **1978**, *43*, 4905.

137 Gutsche, C. D.; Gutsche, A. E.; Karaulov, A. I. *J. Inclusion Phenom.* **1985**, *3*, 447.

138 Izatt, R. M.; Christensen, J. J.; Hawkins, R. T. *US Patent 4,477,377* **1984**.

139 Harrowfield, J. M.; Ogden, M. I.; White, A. H.; Wilner, F. R. *Austr. J. Chem.* **1989**, *42*, 949.

140 Seiffarth, K.; Schulz, M.; Goermar, G.; Bachmann, J. *Polymer Degradation and Stability* **1989**, *24*, 73.

141 Perrin, R.; Bourakhouadar, M.; Perrin, M.; Oehler, D.; Gharnati, F.; Lecocq, S.; Royer, J.; Decoret, C.; Bayard, F. *C. R. Acad. Sci.* Paris, **1991**, *312*, 1135.

142 Wainwright, K. P. *PCT Int. Appl.* WO 89-08092, **1989**.

143 Kondo, Y.; Yamamoto, T.; Manabe, O.; Shinkai, S. *Jpn. Kokai Tokkyo Koho*, JP 88-197544, **1988**.

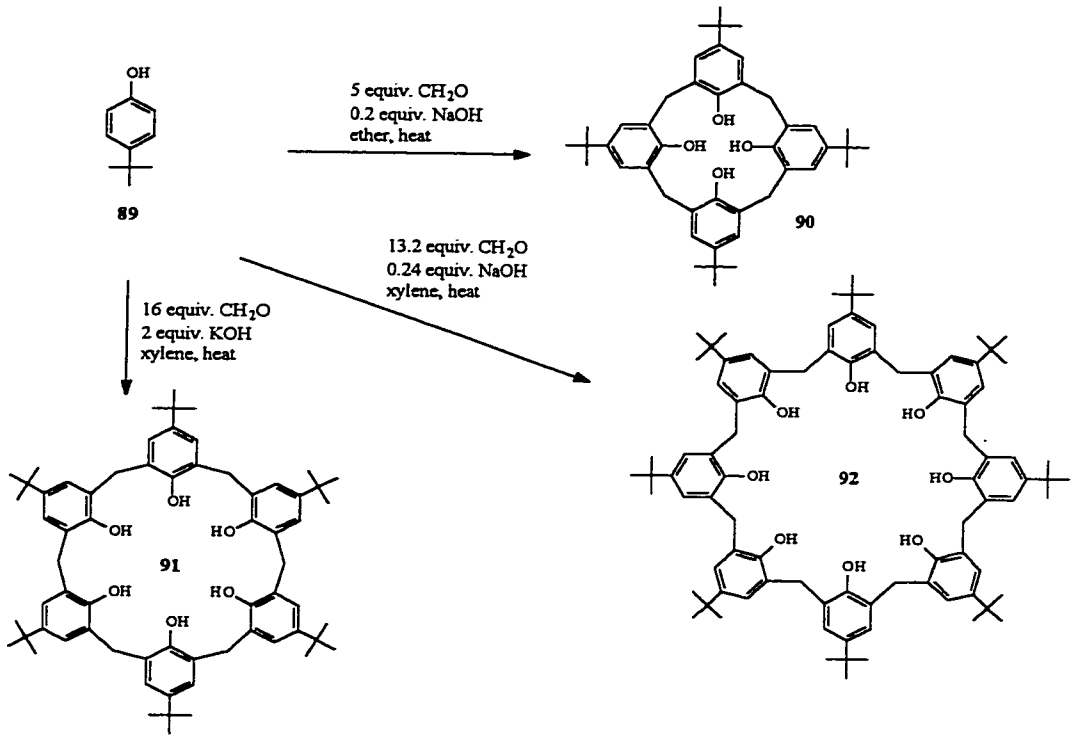
144 Harris, S. J.; Guthrie, J.; MacManus, M.; McArdle, C.; McKervey, M. A. *Eur. Patent Appl.*, EP 432 989, **1991**.

145 Seiffarth, K.; Schulz, M.; Goermar, G.; Bachmann, J. *Ger. East.* DD 273844, **1989**.

146 Taniguchi, H.; Nomura, E. *Chem. Lett.* **1988**, 1773.

adhesives,[147] Langmuir-Blodgett films and membranes,[148] catalytic properties,[149] and deodorant compositions.[150]

Calixarenes are usually prepared by the condensation of *p-tert*-butylphenol and formaldehyde (either as an aqueous solution or as paraformaldehyde) under alkaline conditions in apolar solvents.[151] Depending on the reaction conditions, such as amount of catalyst, reaction time and temperature, the cyclic tetra- **90**, hexa- **91**, or octamer **92** can be obtained in yields of 50%, 83-88% or 62-65%, respectively (Scheme 1.4.1).



Scheme 1.4.1. One-pot synthesis of calixarenes.

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147 a) Harris, S. J. *UK Patent Appl.*, GB 2 200 909, **1988**. b) Harris, S. J.; Guthrie, J.; MacManus, M. *Eur. Patent Appl.*, EP 262 910, **1988**.

148 a) Nakamoto, Y.; Kallinowski, G.; Böhmer, V.; Vogt, W. *Langmuir* **1988**, *4*, 276. b) Markowitz, M. A.; Bielski, R.; Regen, S. L. *J. Am. Chem. Soc.* **1988**, *110*, 7545. c) Naoko, M. *Eur. Patent Appl.*, EP 456 497, **1991**.

149 a) Arimura, T.; Nagasaki, T.; Shinkai, S.; Matsuda, T. *J. Org. Chem.* **1989**, *54*, 3766. b) Kneafsey, B.; Rooney, J. M.; Harris, S. J. *US Patent 4,912,183* **1990**.

150 Tanaka, M.; Miyabe, M. . *Jpn. Kokai Tokkyo Koho*, JP 90-022345, **1989**.

151 Gutsche, C. D.; Iqbal, M. in *Organic Syntheses* J. D. White (Ed.) vol. 68.

One of the most appealing features of calixarenes is their three-dimensional structure. Molecular models show that they cannot be planar. Each phenolic unit of the calixarene rotates around the Ar-CH_2-σ-bonds at relatively high rates around room temperature.[152] Owing to this freedom, a calixarene is present as several conformational isomers. For example, in calix[4]arene, four principal conformations can be distinguished such as cone, partial cone, 1,2-alternate and 1,3-alternate conformations (Figure 1.4.1). It is now well established that calix[4]arenes normally assume the cone conformation. Here, all hydroxyl groups are on the same side of the molecule, which permits stabilization by a cyclic array of intramolecular hydrogen bonds where each hydroxyl group simultaneously acts as donor and acceptor. This has been shown in the crystalline state by single crystal X-ray analysis for numerous examples.[153]

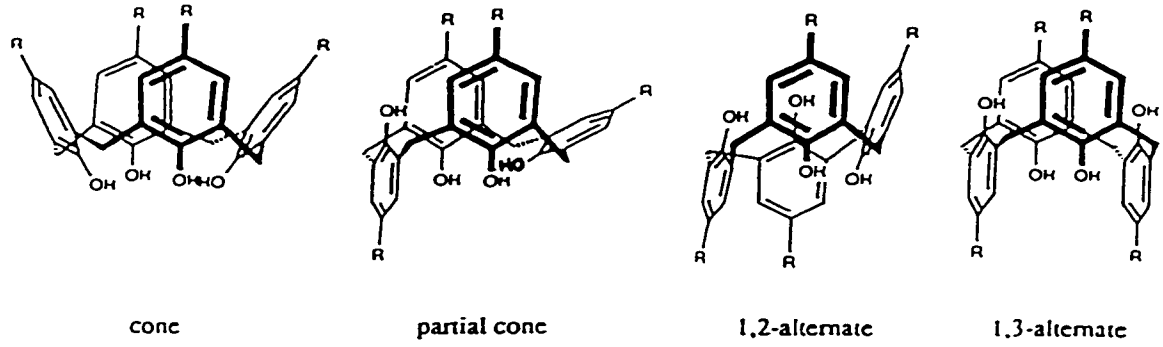

Figure 1.4.1.[154] Four basic conformations of calix[4]arenes.

In solution, the cone conformation follows unambiguously from the ^{1}H-NMR spectrum, which shows, for *tert*-butylcalix[4]arene, one singlet for OH, Ar-H and *tert*-butyl protons (not shown). At low temperature, the Ar-CH_2-Ar protons appear as a pair of doublets (geminal coupling), indicating that all four methylene groups are equivalent, but that the two protons of each methylene group are different (diastereotopic). With

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[152] Gutsche, C. D.; Levine, J. A. *J. Am. Chem. Soc.* **1982**, *104*, 2652.

[153] Vicens, J.; Böhmer, V. (Eds.) in *Calixarenes: A Versatile Class of Macrocyclic Compounds* Kluwer Academic Publishers, Dordrecht, **1991**.

[154] Figure taken from Grootenhuis, P. D. J.; Kollman, P. A.; Groenen, L. C.; Reinhoudt, D. N.; van Hummel, G. J.; Ugozzoli, F.; Andreeti, G. D. *J. Am. Chem. Soc.* **1990**, *112*, 4165.

increasing temperature, these signals broaden, then coalesce and finally become a sharp singlet, a behavior which is best understood as a rapid (on the NMR timescale) interconversion of opposite (but identical) cone conformations (Figure 1.4.2). During this interconversion, the hydroxyl groups pass through the annulus (the intramolecular hydrogen-bond system is broken or at least weakened), and the originally equatorial CH_2-proton finally assumes the axial position, and vice versa.[134b] It has been demonstrated that the rotation of the phenolic units of a calix[4]arene can be hindered by converting the calix[4]arene into a suitable derivative.[155] When the phenolic hydroxyl groups are functionalized with substituents larger than ethyl groups, interconversion between the different conformations is no longer possible,[156] and the conformation of the tetrasubstituted calix[4]arene is determined during the alkylation process.

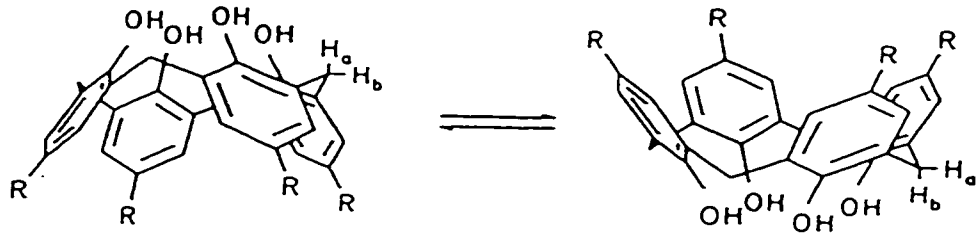

Figure 1.4.2.[157] Ring inversion of calix[4]arenes.

Calixarenes can be chemically modified at three reactive sites by using i) reactions of hydroxyl groups; ii) reactions of aromatic rings; and iii) reactions of methylene bridges. The most common modifications are: introduction of new functionality through the phenolic oxygen atoms and substitution at the *para* position after removal of the *tert*-butyl group.[134b] The first type of modification is easily performed by alkylation,[155,158] acylation[155] or silylation[158] as generally shown in Scheme 1.4.2.

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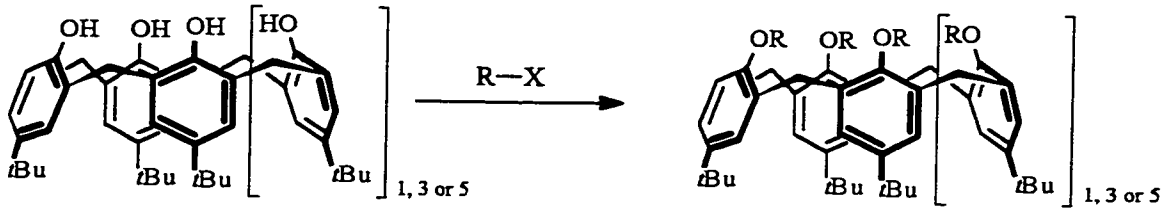
[155] Gutsche, C. D.; Dwanan, J. A.; Levine, J. A.; No, K. H.; Bauer, L. J. *Tetrahedron* **1983**, *39*, 409.

[156] a) Araki, K.; Iwamoto, K.; Shinkai, S.; Matsuda, T. *Chem. Lett.* **1989**, 1747. b) Iwamoto, K.; Araki, K.; Shinkai, S. *J. Org. Chem.* **1991**, *56*, 4955.

[157] Figure taken from Böhmer, V.; O'Sullivan, P. *TRIP* **1993**, *1*, 267.

[158] Gutsche, C. D.; Dwanan, J. A.; No, K. H.; Muthukrishnan, R. *J. Am. Chem. Soc.* **1985**, *103*, 409.



93 **94**

Scheme 1.4.2. Reaction of hydroxyl groups.

Reinhoudt *et al.*[159] have found experimental procedures that give tetrasubstituted calix[4]arenes with conformations that can be predicted based on the nature of the substituents and the different base and solvent combinations used in their preparations. Derivatives in the cone,[134b] partial cone,[160] 1,2-alternate[160] and 1,3-alternate[161] conformation have been obtained as pure diastereomers and most have been confirmed by X-ray analysis.

The second type of modification of calixarenes involves the elimination of the *tert*-butyl group by transalkylation with $AlCl_3$ in the presence of a suitable acceptor (e.g. toluene or phenol).[162] This reaction plays a key role in calixarene chemistry since nearly all types of electrophilic substitution are possible, leading to calixarenes with various substituents or functional groups in *para* positions. Figure 1.4.3 contains a collection of interesting and important examples that have been realized, demonstrating the variety of possible structures.

The increasing interest devoted to calixarenes brought the research community to synthesize a novel class of glycoconjugate comprising carbohydrates scaffolded on calix[4]arenes. These glycoconjugates possess an extra advantage over already known

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[159] van Dienst, E.; Iwema Bakker, W. I.; Engbersen, J. F. J.; Verboom, W.; Reinhoudt, D. N. *Pure & Appl. Chem.* **1993**, *65*, 387.

[160] Groenen, L. C.; van Loon, J.-D.; Verboom, W.; Harkema, S.; Casnati, A.; Ungaro, R.; Pochini, A.; Ugozzoli, F.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1991**, *113*, 2385.

[161] Verboom, W.; Datta, S.; Asfari, Z.; Harkema, S.; Reinhoudt, D. N. *J. Org. Chem.* **1992**, *57*, 5394.

[162] Gutsche, C. D.; Lin, L. G. *Tetrahedron* **1985**, *42*, 1633.

neoglycoproteins, glycopolymers and glycodendrimers[163] which lies in their ability to form drug inclusion complexes. The first sugar calixarenes were prepared in **1994** by Dondoni and Ungaro,[53] but were not primarily used for carbohydrate-protein interaction studies. These glycoconjugates did not have spacer arms suitable for efficient access to the carbohydrate residues. They were successful in synthesizing bis-glucosides in α,α- (**95**) and α,β-configurations (**96**) using standard Mitsunobu conditions.[164] These glycocalix[4]arenes were not soluble in water whereas the tetrasugar derivative **97** proved to be the only water-soluble glycoconjugate synthesized (Figure 1.4.4). Galactocalix[4]arene **97** was prepared under glycosylation conditions using ethyl 1-thio-2,3,4,6-tetrabenzoyl-β-D-galactopyranoside as the glycosyl donor.[53]

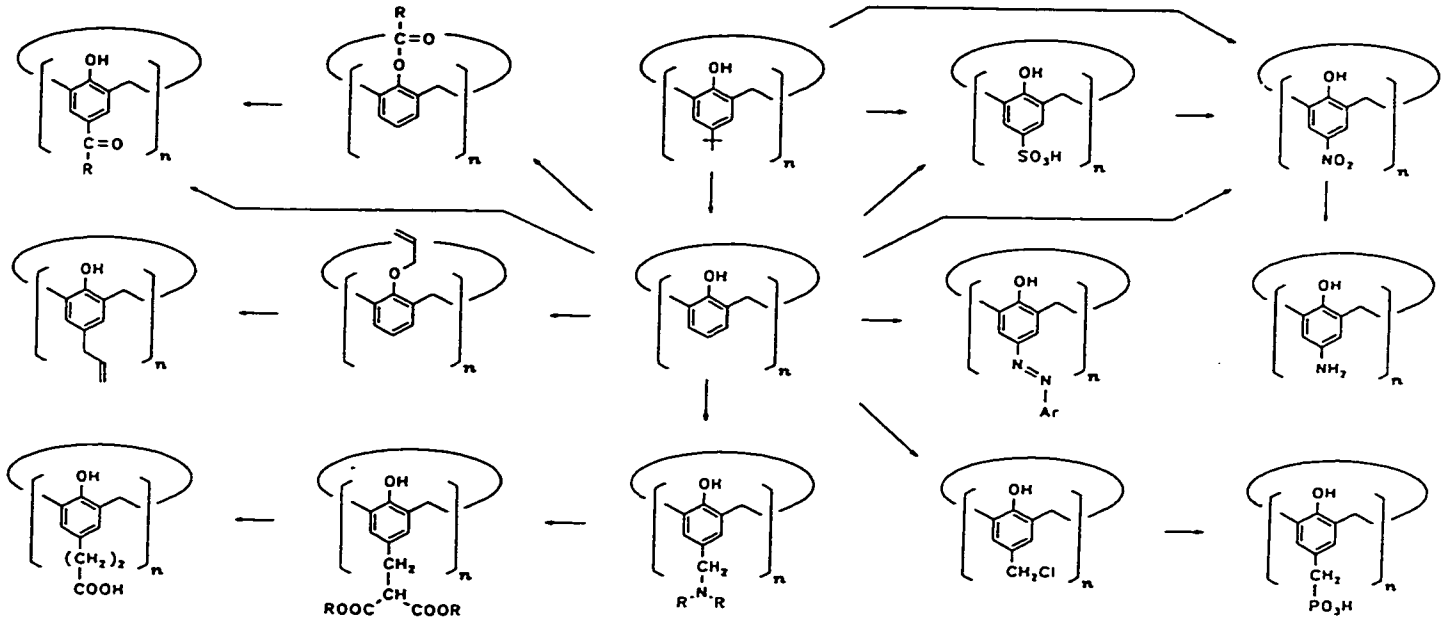

Figure 1.4.3.[165] Electrophilic substitution and Claisen rearrangement routes for synthesizing a variety of *para*-functionalized calixarene derivatives.

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163 Roy, R. *Curr. Opin. Struct. Biol.* **1996**, *6*, 692.

164 Mitsunobu, O. *Synthesis* **1981**, 1.

165 Modified Figure taken from reference 134b.

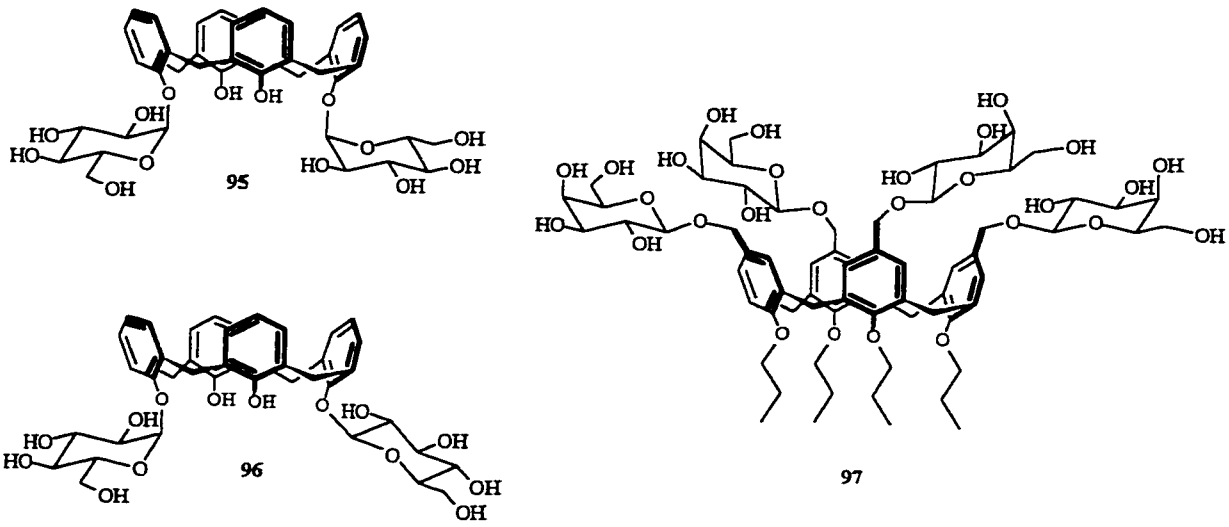

Figure 1.4.4. Representative examples of sugar calix[4]arenes.

Dondoni *et al.*[54] also synthesized upper rim *O*-galactopyranosyl calix[4]arenes **101** and **102** by glycosylation of calix[4]arenes **99** and **100** with thiazolyl galactopyranoside **98** as a glycosyl donor (Scheme 1.4.3). The choice of the thiazole ring as aglycon was made due to the facile transformation of the ring into readily manipulable functionality such as a formyl group.[166] The tetrasubstituted galactocalix[4]arene **102** was obtained in poor yield which was explained by the restricted cone conformation of the macrocycle and the increasing congestion of the system by the sequential insertion of the bulky ketosyl fragments.

Chapter 5 of this thesis dissertation discusses the synthesis of sialocalix[4]arenes which were the first calixarene derivatives to have a spacer arm between the calixarene core and the carbohydrate moiety.

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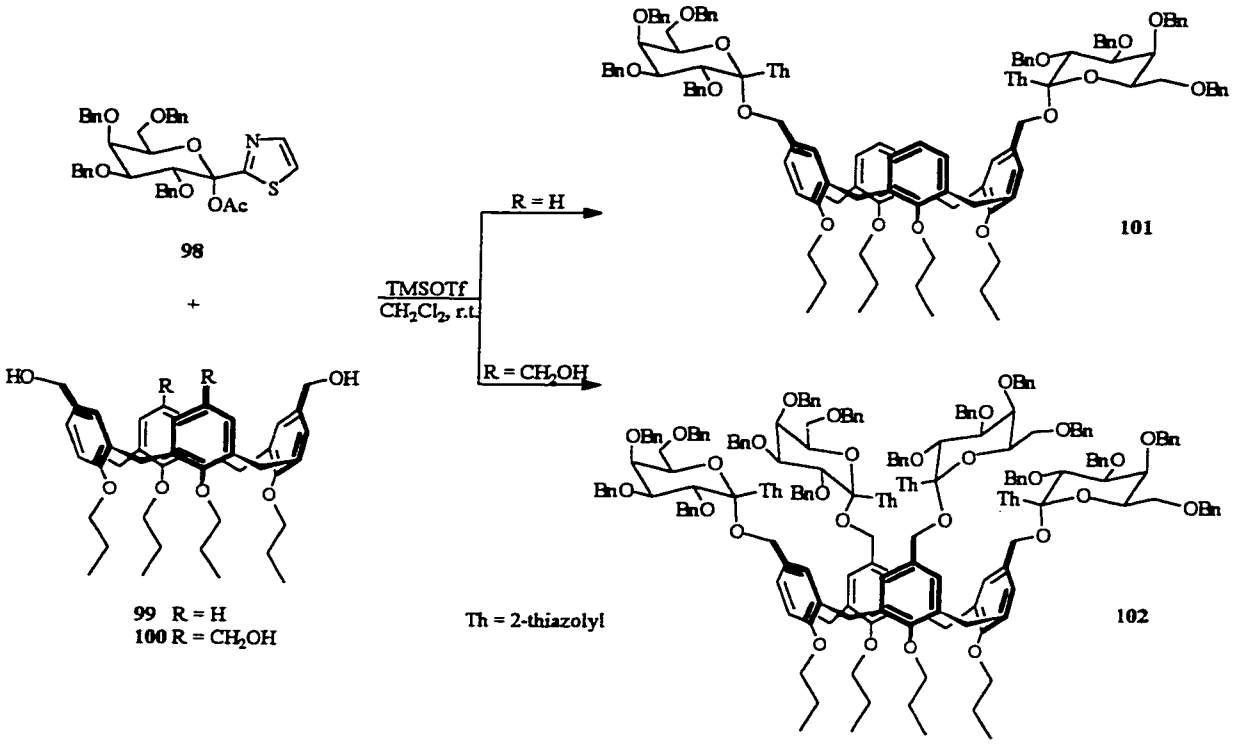
[166] Dondoni, A. in *Modern Synthetic Methods* R. Scheffold (Ed.) Verlag Helvetica Chimica Acta, Basel, **1992**, 377.



Scheme 1.4.3. Synthesis of upper rim *O*-galactopyranosyl calix[4]arenes.

1.5 Lectins and Immunochemical Techniques

1.5.1 Sialic Acid Specific Lectins

Carbohydrate-binding proteins called lectins were first discovered in plants more than 100 years, and they have been tremendously useful as tools for chemistry and biology that involve carbohydrate recognition. Lectins are very useful in investigation of carbohydrate-protein interactions, which gives one an idea of how the binding affinity differs from different synthetic multivalent glycoconjugates. Lectins can be isolated from plants, animals and microorganisms and are usually relatively small proteins (order of 40 to 160 kDa) made up of subunits. Lectins, using their carbohydrate recognition domains

(CRD), can discriminate among the myriad of complex carbohydrate structures.[167] Thus, particular lectin only binds with specific glycosides. In order to assess the binding ability of the sialoglycoconjugates present in this dissertation, two lectins were chosen, wheat germ agglutinin (WGA) and *Limax flavus* lectin (LFA).

Wheat Germ Agglutinin

Wheat germ agglutinin is considered a well characterized cereal lectin[168] which has a binding specificity for *N*-acetylglucosamine (GlcNAc) and *N*-acetylneuraminic acid (sialic acid) (Table 1.5.1.1). WGA is a dimeric carbohydrate-free protein composed of two identical 17.5-24 kDa subunits,[9] each consisting of four homologous domains of 43 amino acids. These domains are similarly folded, with each having four identically positioned disulfide bridges which makes WGA a highly stable molecule and appears to be essential for the activity of the lectin. The lectin occurs in hexaploid wheat in three isoforms, (differing isoelectric points) termed isolectins: WGA1, WGA2 and WGA3. The carbohydrate-binding specificity of WGA has been studied by a variety of techniques,[167] such as hapten inhibition of hemagglutination and of specific precipitation of glycoconjugates, changes in fluorescence of the lectin (intrinsic) or of chromatogenic ligands (extrinsic), equilibrium dialysis, NMR, and x-ray diffraction.[167]

Table 1.5.1.1. Carbohydrate-binding specificity of wheat germ agglutinin.[169]

Inhibitor	Relative inhibitory potency[a]
N-Acetylglucosamine	1.0
Sialic Acid	0.25
N-Acetylgalactosamine	0.19
Glucosamine	<0.1

[a] *N*-Acetylglucosamine is normalized to 1.0 (5.9 μm *N*-acetylglucosamine required for 50% inhibition.

[167] Liener, I. E.; Sharon, N.; Goldstein, I. J. (Eds.) *The Lectins. Properties, Functions, and Applications in Biology and Medicine*, Academic Press, Orlando, **1986**.

[168] Wright, C. S. *J. Mol. Biol.* **1989**, *209*, 475.

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

Equilibrium-dialysis measurements showed that each WGA monomeric subunit contains two identical and independent binding sites for GlcNAc. However, in the case of NeuAc, only one sialic acid ligand per subunit of WGA was present.[170] A schematic representation of the binding site of WGA towards NeuAc (α anomer preferred)[170] is depicted in Figure 1.5.1.1.[171] WGA exhibits little or no anomeric preference for either the α- or β-glycoside of GlcNAc. Hapten-inhibition analysis showed that *N*-acetylneuraminic acid (α-methyl ketosides) all bind to WGA with an affinity fourfold less than *N*-acetylglucosamine. The specific binding of NeuAc to WGA is based on the similarity in configuration of this sugar to *N*-acetylglucosamine at positions C-2 (*N*-acetamido group) and C-3 (hydroxyl group) of the pyranose ring (Figure 1.5.1.2); these are the positions critical to productive contact with the WGA combining site. Though *N*-GalNAc also possesses the above configurational determinants (Figure 1.5.1.2), it binds to WGA only weakly because it also contains an axial C-4 hydroxyl group.

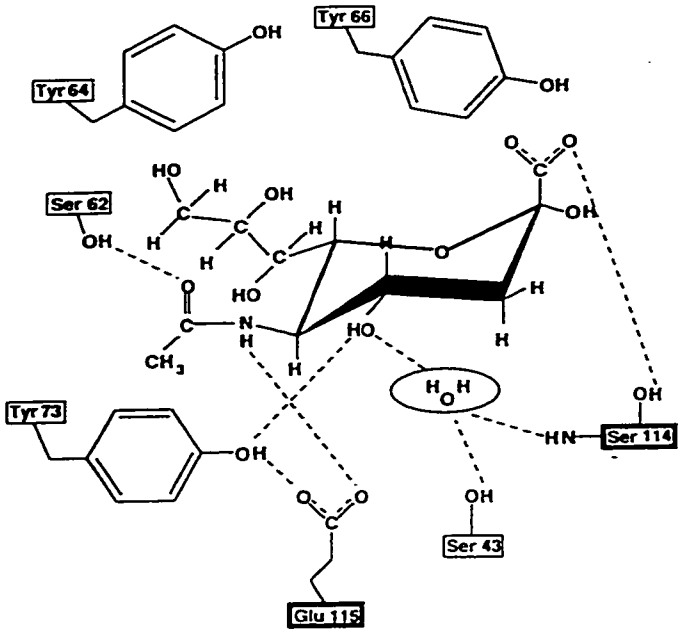


Figure 1.5.1.1.[171] Schematic representation of the amino acids forming the hydrogen bond network and the hydrophobic side chains that bind *N*-acetylneuraminic acid to wheat germ agglutinin. The amino acid residues in bold belong to different subunits of the lectin.

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[170] Wright, C. S. *J. Mol. Biol.* **1990**, *215*, 635.

[171] Figure taken from Sharon, N. *Trends Biochem. Sci.* **1993**, *18*, 221.

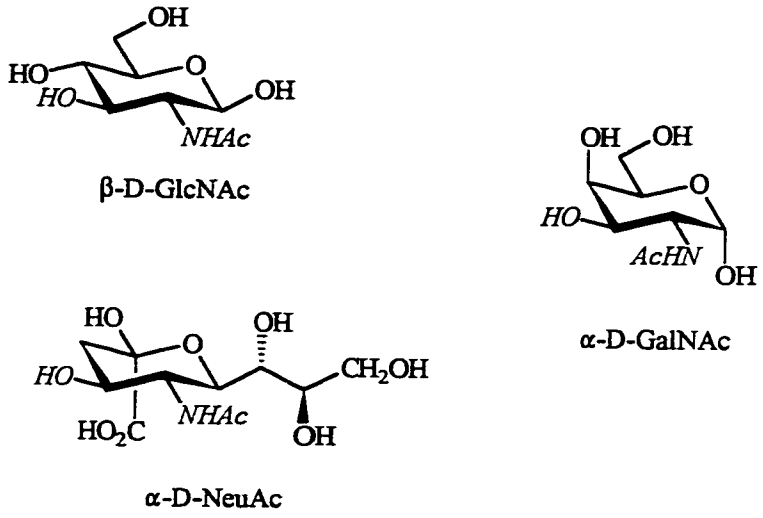

Figure 1.5.1.2. Comparison of common structural features shared by GlcNAc, NeuAc and GalNAc. Substituents critical for the interaction with WGA are italicized.

Limax flavus

LFA is the lectin isolated from the slug *Limax flavus* which has a high degree of specificity and selectivity for binding sialic acid of sialoproteins and sialopeptides. The lectin has a high degree of hemagglutinative activity which is selectively inhibited by sialic acid or sialoproteins. The slug lectin is a relatively small protein consisting of two non-covalently associated subunits of ~22 kDa each.[9] It is highly specific for *N*-acetylneuraminic acid residues and to a lesser extent for *N*-glycolylneuraminic acid, as determined by hemagglutination inhibition experiments.[172]

1.5.2 Turbidimetric analysis

The lectin binding experiments were performed by a simple qualitative test known as turbidimetric analysis. It consists of producing a solution of the lectin and of a synthetic

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[172] Miller, R. L. *US Patent 4,457,865* **1984**.

glycoconjugate which is then placed in a microtiter well where the optical density (O.D.) is then measured as a function of time. Multidimensional cross-linked lattices can be formed from the reaction of the lectin and the antigen with the condition that neither the lectin nor the antigen are in excess. Hence, the complex formed precipitates out of the solution due to its insolubility. This phenomenon is illustrated in Figure 1.5.2.1.

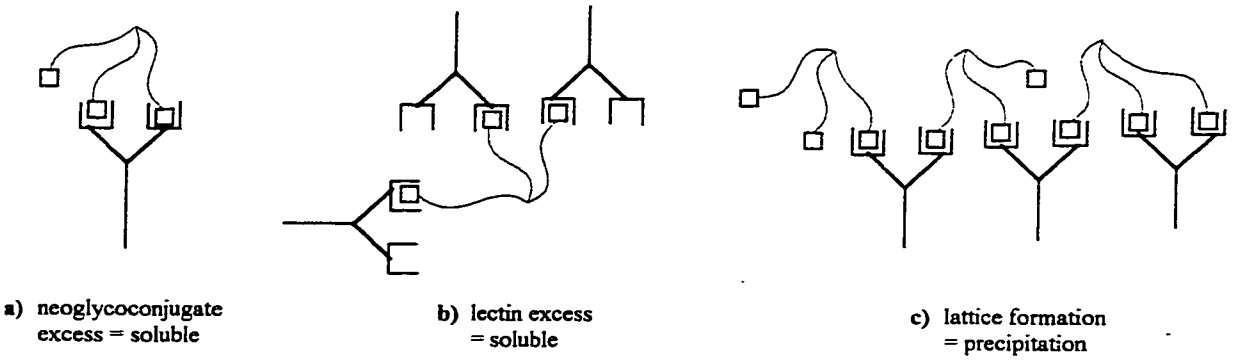

Figure 1.5.2.1. Schematic of turbidimetric analysis.

Chapter 2

Carbohydrate Synthons

2.1 Introduction

The starting point of glycoconjugates syntheses involves the preparation of carbohydrate synthons. In the design of stable and viable inhibitors, one must consider the action of glycohydrolase enzymes. Thioglycosides are very stable compounds even in acidic aqueous solutions. They are also stable towards enzymatic hydrolysis,[173] and many thioglycosides are known as glycosidase inhibitors.[174] They are also fairly easy to prepare in high yields. Thus, thioglycoside derivatives represent the ideal carbohydrate synthons in the preparation of glycodendrimers (Chapter 4) and glycocalixarenes (Chapter 5).

As previously stated in Chapter 1, sialic acid was chosen to be the carbohydrate synthon used in the syntheses of glycoconjugate inhibitors. Sialic acid was efficiently functionalized using the phase transfer catalysis (PTC) method.

Over the years, PTC has proven to be a very useful and convenient method for various synthetic transformations. This methodology allows synthetic modifications under mild conditions that were heretofore reserved to very drastic reagents and reaction media. PTC was first devised to permit simple reactions where various reagents had incompatible solubilities. It has now developed into a far reaching field of synthetic transformations with complex substrates, as well as differing reaction media. Many natural products are synthesized with PTC conditions included in their procedures. Among these natural products, carbohydrates have proved to be challenging molecules because of the variety of functional groups, ring sizes, and stereocenters they include. Classical carbohydrate transformations are quite tedious as a result of the various protection and deprotection

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[173] Horton, D.; Hutson, D. H. *Adv. Carbohydr. Chem.* **1963**, *18*, 123.

[174] Cuatrecasas, P. *Adv. Enzymol.* **1972**, *36*, 58.

steps needed. These often require reactive promoters and reagents that can be quite toxic, costly, and difficult to manipulate. Also, the use of very polar solvents (e.g. DMF) can prove to be tedious because of the need for drying, purifying and their removal after completion of the reaction. PTC is well suited for carbohydrate chemistry as the conditions can be quite mild and applicable to large scale synthesis. The use of non-anhydrous, as well as technical grade solvents, can alleviate much of the preparative work. Another important fact is that glycosides are susceptible to hydrolysis under acidic conditions, whereas PTC conditions are generally carried out under neutral or basic conditions.

Even though this methodology as proven itself quite useful, its practice is not common.[175] Known examples of its use include complete or regioselective protection of hydroxyl groups,[176] oxidation,[177] and reduction.[178] Although these transformations are quite useful, the major stereoselective modifications of interest to carbohydrate chemists involve the anomeric center. The ability to control the stereochemistry at this position can be quite challenging and is perhaps the most important to the field of carbohydrate chemistry. So far, PTC conditions have been successfully applied to mono- and di-saccharides as well as sialic acid,[175, 179] providing a variety of *O*-, *N*-, *S*-, *Se*- and *C*-glycosides[175] with complete anomeric inversion (i.e. stereospecifically). PTC reactions are based on phase transfer catalyzed reactions between glycosyl halides and nucleophiles (e.g. thiols, azide anions, and selenols). The general PTC reaction is outlined in Scheme 2.1.1.

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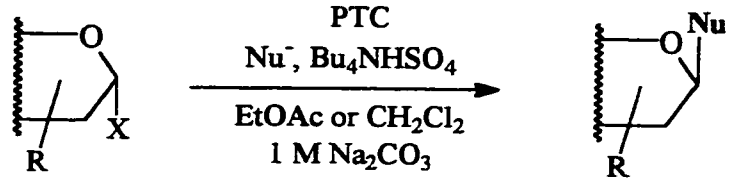
[175] Roy, R. *Phase Transfer Catalysis in Carbohydrate Chemistry.* In: Handbook of Phase Transfer Catalysis; Y. Sasson and R. Neumann (Eds.) Chapman & Hall, Glascow, **1997**, Chap. 7, 244.

[176] a) Kim, K. S.; Szarek, W. *Synthesis* **1978**, 48; b) Garegg, P. J.; Kvarnström, I.; Niklasson, G.; Svensson, S. C. T. *J. Carbohydr. Chem.* **1993**, *12*, 933; c) Szeja, W.; Kokt, I.; Grynkiewicz, G. *Recl. Trav. Chim. Pays-Bas* **1989**, *108*, 224.

[177] Morris, P. E.; Kiely, D. E.; Vigee, G. S. *J. Carbohydr. Chem.* **1990**, *9*, 661.

[178] Bessodes, M.; Antonakis, K. *Tetrahedron Lett.* **1985**, *26*, 1305.

[179] Roy, R.; Tropper, F. D.; Cao, S.; Kim, J. M. *ACS Symp. Ser.* **1997**, *659*, 163.



Scheme 2.1.1. General PTC reaction.

2.2 Glycosyl Derivatives

PTC reactions were used in the preparation of glycosyl derivatives **113** and **118** needed for the synthesis of glycoconjugates. The liquid-liquid two-phase system was also applied to other nucleophiles and glycosyl halides in order to show the versatility of the PTC method and to obtain new compounds useful in other situations, one of which is the study of an efficient chemoselective thioacetate deprotection method (Chapter 3).

Glycosyl halides **103**, **106**,[180] **109**[181] and **2**[182] were synthesized according to previously published procedures.[180-182] Isolated halides **103**, **106** and **109** or freshly prepared acetochloroneuraminic acid **2** were treated at room temperature with tetrabutylammonium hydrogen sulfate (Bu_4NHSO_4 or TBAHS, 1 equiv) as the lipophilic phase transfer catalyst with a small excess of specific nucleophiles (1.5-3 equiv) in equal volumes of ethyl acetate (organic phase) and 1-2 M sodium carbonate (mildly basic aqueous phase) to give the corresponding *S*-, *N*-, and *Se*-glycosides. As for the organic solvent, the original conditions for PTC have included dichloromethane but this was a poor choice in the case of thiols and selenols. It was previously demonstrated that large proportions of the thiols reacted with dichloromethane to give products such as bis(4-

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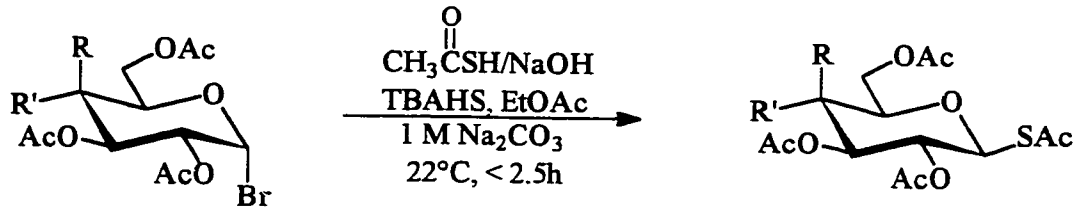
[180] Jeanloz, R. W.; Stoffyn, P. J. *Methods Carbohydr. Chem.* **1962**, *1*, 221.
[181] Horton, D. *Methods Carbohydr. Chem.* **1972**, *6*, 282.
[182] Kuhn, R.; Luts, P.; MacDonald, D. L. *Chem. Ber.* **1966**, *99*, 611.

nitrophenylthio)methane.[183] It has since been shown that ethyl acetate was a much better choice of solvent, which allowed fast and stereospecific routes toward thio- and seleno-glycosides.

PTC procedures afforded, in high yields, the desired β-anomers in the case of galactose, glucose and *N*-acetylglucosamine, and the α-anomer in the case of sialic acid. The complete inversion of configuration at the anomeric carbon is indicative of an S_N2 type reaction mechanism. The only side-products seen following the present PTC reactions were a result of elimination (base catalyzed dehydrohalogenation of starting sugar halides) and hydrolysis which proceeded generally to only a limited extent.

The glycosyl halides **103**, **106**, and **2** (1 equiv) were each reacted with TBAHS (1 equiv) and the thioacetic acid mixture in the two-phase system EtOAc/1 M Na_2CO_3. However, the thioacetic acid was first neutralized by an equivalent amount of sodium hydroxide and then dissolved in the 1 M aqueous Na_2CO_3 solution. Thioacetates **104** and **107** (Scheme 2.2.1) and **113** (Scheme 2.2.3) were obtained as white solids in 89%, 78%, and 66% yields, respectively. Thioacetyl sialoside **113**[115,184] is the key precursor (carbohydrate moiety) in the syntheses of glycodendrimers and glycocalixarenes.



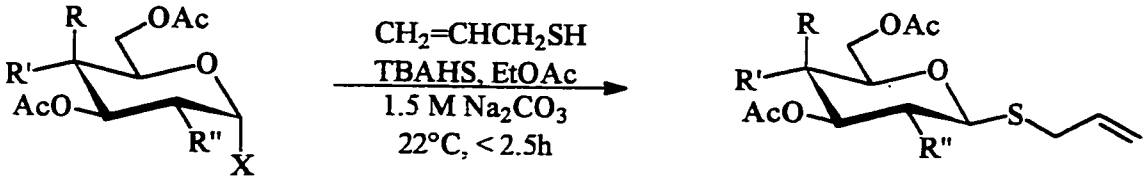
103 R = OAc; R' = H
106 R = H; R' = OAc

104 R = OAc; R' = H
107 R = H; R' = OAc

Scheme 2.2.1 PTC synthesis of thioacetate glycoside derivatives.

[183] Cao, S.; Roy, R. *Carbohydr. Lett.* **1996**, *2*, 27.
[184] Rothermel, J.; Faillard, H. *Biol. Chem. Hoppe-Seyler* **1989**, *370*, 1077.

Allyl mercaptan was efficiently conjugated onto glycosyl halides **103**, **106**, **109**, and **2** under PTC conditions which were described above. The thioallyl glycosides **105**, **108**, and **110** (Scheme 2.2.2)[185] and **114**[186] (Scheme 2.2.3) were obtained in 80% to 86% yields. These latter compounds were made to demonstrate the usefulness of PTC reactions but can be very useful in situation where the double bond is functionalized by introduction of new functional groups.



103 R=R"=OAc; R'=H; X=Br
106 R=H; R'=R"=OAc; X=Br
109 R=H; R'=OAc; R"=NHAc; X=Cl

105 R=R"=OAc; R'=H
108 R=H; R'=R"=OAc
110 R=H; R'=OAc; R"=NHAc

Scheme 2.2.2 PTC synthesis of thioglycoside derivatives.

Other thiol nucleophiles such as benzenethiol, *p*-nitrothiophenol, and *p*-methoxy benzenethiol and nucleophiles such as sodium azide and benzeneselenol reacted with acetochloroneuraminic acid **2** efficiently under similar PTC conditions (see above). Thioglycosides **115-117**[184,186] were produced in 65-74% crystalline yields, azido derivative **118**[187,188] was synthesized as a white solid in 83% yield and finally seleno-glycoside **119** was produced as an off-white solid in 72% yield (Scheme 2.2.3).[185,189]

The fully deprotected version of thiophenyl sialoside **115** was synthesized to serve as an inhibitor in turbidimetric assays performed with glycocalixarenes of Chapter 5.

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185 Carrière, D.; Meunier, S. J., Tropper, F.D., Cao, S.; Roy, R. *J. Mol. Cat. A: Chemical*, **1999**, in press.

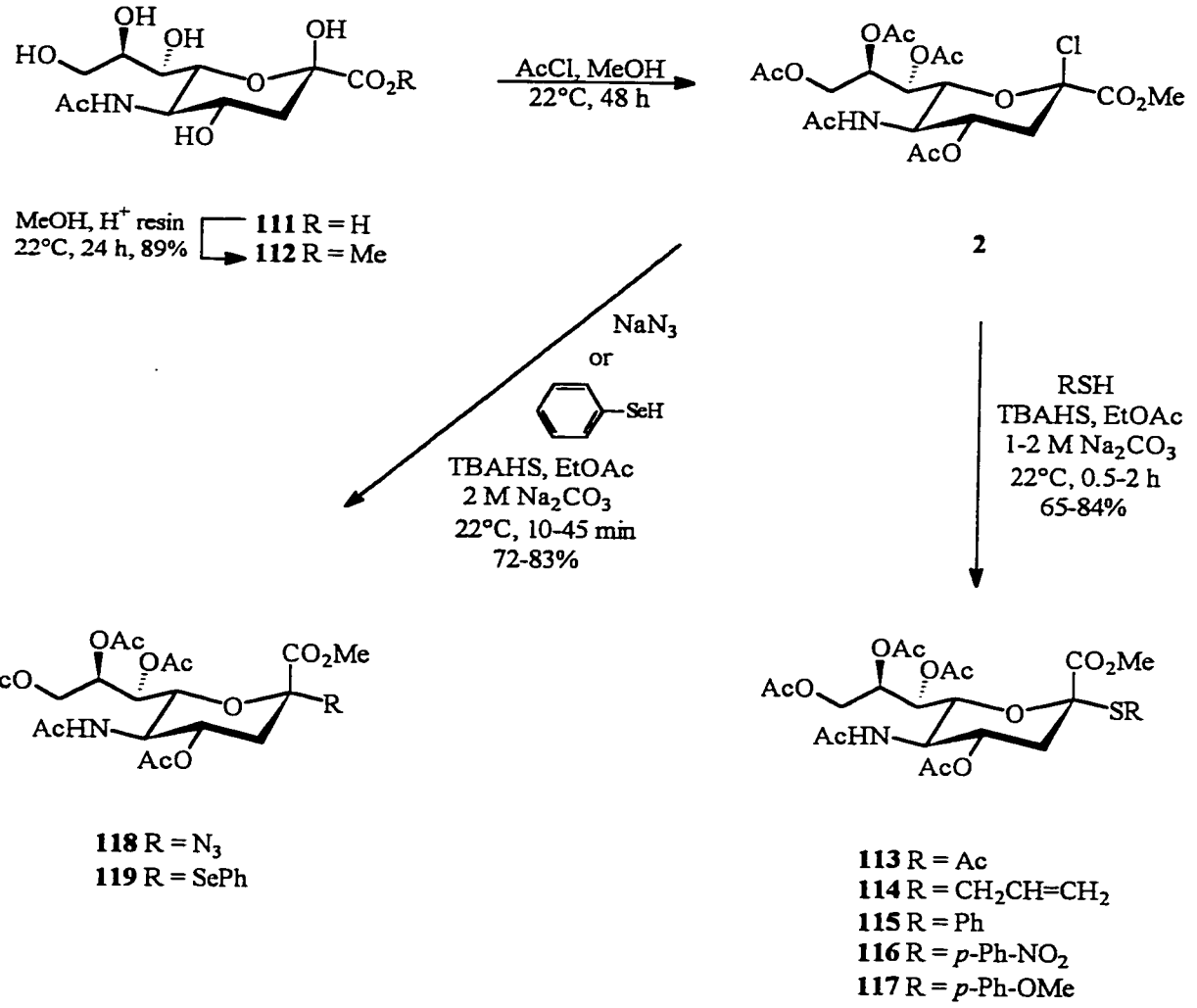
186 Cao, S.; Meunier, S. J.; Andersson, F. O.; Letellier, M.; Roy, R. *Tetrahedron Asymmetry* **1994**, *5*, 2303.

187 Tropper, F. D.; Andersson, F. O.; Braun, S.; Roy, R. *Synthesis* **1992**, 618.

188 Rothermel, J.; Weber, B.; Faillard, H. *Liebigs Ann. Chem.* **1992**, 799.

189 Rothermel, J.; Faillard, H. *Carbohydr. Res.* **1990**, *208*, 251.

Azido sialoside **118** was made as a precursor in the synthesis of a different glycocalixarene. Catalytic hydrogenation of the azido group afforded the corresponding amine which in turn could easily be functionalized into a compatible aglycone for its attachment onto the calixarene backbone (Chapter 5). The other sialosides (**116-117**, and **119**) were synthesized to expand the library of glycosides that can be prepared using PTC and their use as glycosyl donors and acceptors is beyond the scope of this thesis dissertation.



Scheme 2.2.3 PTC synthesis of sialoside derivatives.

The stereochemistry of galactosides **104** and **105**, glucosides **107** and **108**, and glucosaminide **110** was easily determined from the appearance of the signals of the anomeric protons ($J_{1,2}$ = 10.0-10.3 Hz) in the ^{1}H NMR spectra. This large coupling constant between the H-1 (anomeric proton) and H-2 is indicative of a *trans* coupling, which necessarily puts the aglycone in the β position (Figure 2.2.1).

Sialic acid derivatives were fully characterized and the physical properties of known compounds were in good agreement with reported values. The fully assigned ^{1}H- and ^{13}C-NMR spectroscopic data of **113-119** confirmed their anomeric configurations (Experimental Methods). Although these sialic acid derivatives lack anomeric protons, the anomeric configurations were inferred on the basis of the characteristic downfield shifts of their H-3e signals relative to those of the β-anomers.[190] The H-3e signals in α-sialoside anomers are usually observed ~0.5 ppm downfield to those of the β-anomers.[191] This assignment was also confirmed by the chemical shifts of H-4 which are shifted upfield in the α-anomers.[192] Moreover, the coupling constants of $J_{7,8}$ (6.2-8.2 Hz) in α-sialosides are usually larger than those in β-sialosides (~2.4 ± 0.3 Hz) (Figure 2.2.2). One last test to determine the anomeric configuration of thiosialosides is the high positive optical rotation values of the α-anomers relative to the β-anomers.[184,193]

2.3 Conclusion

A mild, completely stereoselective and high yielding entry into useful thioglycosides, glycosyl azides, and seleno-glycosides has been achieved under PTC conditions. The procedure is compatible with acid- and base-labile protecting groups.

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[190] Hasegawa, A.; Ohki, H.; Nagahama, T.; Ishida, H.; Kiso, M. *Carbohydr. Res.* **1991**, *212*, 277.

[191] Paulsen, H.; von Deessen, H. *Carbohydr. Res.* **1986**, *146*, 147.

[192] Vliegenthart, J. F. G.; Dorland, L.; van Halbeek, H.; Haverkamp, J. in *Sialic Acids (Cell Biology Monographs)*, vol.10, R. Schauer (Ed.), Springer Verlag, New York, **1982**.

[193] Eschenfelder, V.; Brossner, R. *Glycoconjugate J.* **1987**, *4*, 171.

Readily available reagents were used without the requirements of heavy metals, high boiling solvents or Lewis acids. The reaction occurred with complete inversion of configuration at the anomeric centers and was also effective with the tertiary center of sialic acid.

2.4 Experimental Methods

2.4.1 General methods

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

Optical rotations ($[\alpha]_D$) were measured at room temperature (23 ± 1°C) in a 1 dm cell on a Perkin-Elmer 241 polarimeter.

The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Gemini-200, XL-300 and/or Brüker AMX-500 spectrometers at 200, 300, and 500 MHz for protons and 50.3, 75.4, and 125.7 MHz for carbons, respectively. Proton chemical shifts (δ) are given relative to internal chloroform (δ 7.24 ppm) for $CDCl_3$ solutions, DMSO (2.49 ppm) for DMSO-d_6 solutions, HOD (4.76 ppm) for H_2O solutions, and to methanol (3.30 ppm) for CD_3OD solutions. Spectra taken in D_2O were performed after repeated exchange of protons for deuterium with D_2O followed by freeze drying. Carbon chemical shifts are given relative to deuteriochloroform (77.0 ppm), DMSO-d_6 (39.5 ppm), and to CD_3OD (49.0 ppm). Spectral analyses were performed as first order approximations and were based on shift correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and 1- and 2-dimensional distortionless enhancement by polarization transfer (DEPT) experiments.

Mass spectra were recorded on a VG 7070-E spectrometer (EI and CI, ether) and/or Kratos Concept IIH spectrometer (FAB-MS, thioglycerol).

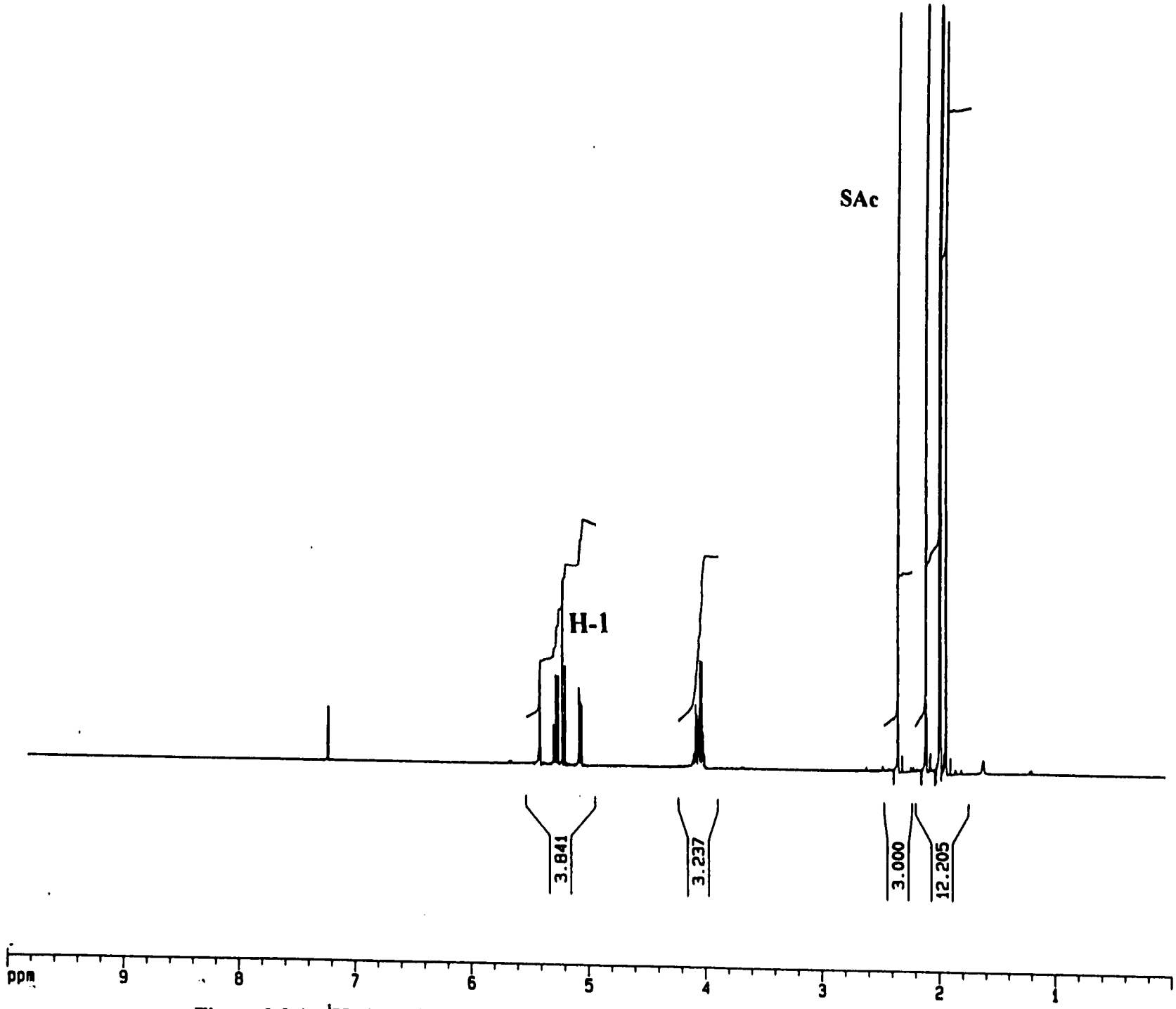

Figure 2.2.1. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of thioacetyl galactoside **104**.

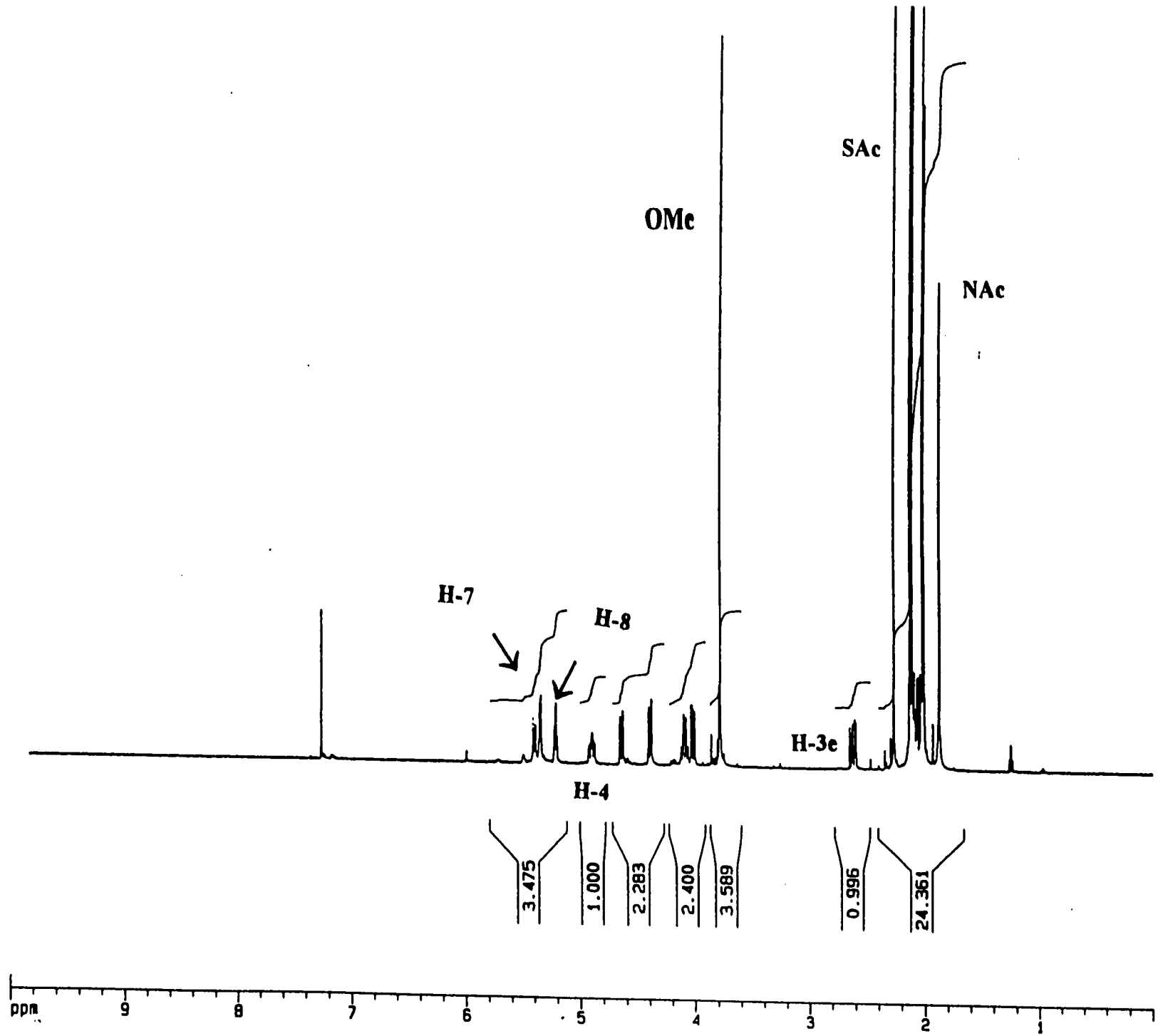

Figure 2.2.2. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of thioacetyl sialoside **113**.

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

Optical densities (O.D.) for turbidimetric analyses were measured on a Dynatech MR600 Microplate Reader.

The lectins, *Limax flavus* (LFA) and *Triticum vulgaris* (WGA), were obtained from E-Y Laboratories (cat. No. L-5101-1) and from Sigma (cat. No. L-9640), respectively.

Elementary analyses were performed by Guelph Chemical Laboratories Ltd. (Ontario), by M-H-Z Laboratories (Phoenix, AZ), or by the University of Ottawa Chemistry Department.

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

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

Thin-layer chromatography (TLC) was performed using silica gel 60 F-254 glass and aluminum plates. Reagents used for the development of plates include (a) ceric sulfate (1% w/v) and ammonium molybdate (2.5% w/v) in 10% (v/v) aqueous sulfuric acid, (b) isatin (0.2% w/v) in ethanol with sulfuric acid (5% v/v), (c) iodine, (d) dilute aqueous potassium permanganate, (e) UV light, and (f) ninhydrin (2% w/v) in aqueous pyridine (4% v/v). TLC plates were heated a few seconds on a hot plate (low setting) when necessary.

The ninhydrin (Kaiser)[194] color test was used to detect primary amines for solution samples. The reagent includes: (a) ninhydrin (10% w/v) in ethanol, (b) phenol (80% w/v) in ethanol, and (c) potassium cyanide (1×10^{-4} w/v) in pyridine. Equal volumes of

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

reagents and test solutions (~200 μL) were combined and heated for 2-3 minutes at ~120°C.

Purifications were performed by gravity or flash column chromatography on silica gel 60 (230-400 mesh, E. Merck No. 9385), by preparative thin layer chromatography on silica gel 60 (1 mm thick), or by chromatotron. Other purifications were performed via preparative scale size exclusion chromatography (LH-20).

All solvents and reagents used in experiments were reagent grade. When necessary, further purifications were performed following published procedures.[195]

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

2.4.2 Synthesis

2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl bromide (103)

Galactose pentaacetate[176] (2.9 g, 7.43 mmol) was dissolved in 30% HBr in acetic acid in an amount sufficient to dissolve the sugar (~7 mL). The reaction mixture was stirred at room temperature for 10 min, after which time the TLC indicated complete disappearance of the starting material. Methylene chloride (35 mL) was added to the reaction mixture and the organic phase was poured in a Na_2CO_3/ice solution. The organic layer was separated and washed with saturated $NaHCO_3$ solution until the aqueous layer became non-acidic (~4 x 50 mL). The organic layer was then washed with water (2 x 40 mL) followed by saturated NaCl (1 x 30 mL). The organic phase was dried (Na_2SO_4) and the residue obtained after concentration under vacuum was recrystallized from ether to afford 2.61 g of α-acetobromogalactose **103** (85 %). Compound **103** has mp 80-81.8°C; (lit.[180] mp 79-81°C); R_f 0.64 (EtOAc/Hexanes, 1/1); ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.67 (d,

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[195] Perrin, D. D.; Armarego, W. C.; Perrin, D. R. *Purification of Laboratory Compounds, 2nd Edition*, Pergammon Press, London, **1980**.

1H, $J_{1,2}$ = 3.9 Hz, H-1), 5.49 (dd, 1H, $J_{3,4}$ = 3.3, $J_{4,5}$ = 1.3, H-4), 5.38 (dd, 1H, $J_{2,3}$ = 10.6, H-3), 5.02 (dd, 1H, H-2), 4.46 (dd, 1H, H-6'), 4.06-4.21 (m, 2H, H-5 & H-6), 2.13, 2.09, 2.04, 1.99 (4s, 12H, 4OAc).

2,3,4,6-Tetra-O-acetyl-1-S-acetyl-1-thio-β-D-galactopyranose (104)

To a solution of galactosyl bromide **103** (3.06 g, 7.43 mmol) in ethyl acetate (40 mL) was added a solution of sodium thiolacetate (10.3 mmol) and tetrabutylammonium hydrogen sulfate (2.55 g, 7.43 mmol) in 1 M sodium carbonate (40 mL). The mixture was stirred at room temperature for 2 h, the reaction being monitored by TLC (R_f 0.48, EtOAc:hexanes, 1:1). The reaction mixture was then diluted with 75 mL each of ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was separated and washed with saturated aqueous $NaHCO_3$ (2 x 100 mL), H_2O (2 x 100 mL) followed by saturated sodium chloride (60 mL). The dried organic layer (Na_2SO_4) was concentrated near dryness under reduced pressure, and purified by silica gel column chromatography using a gradient from 1/2 to 1/1 EtOAc:hexanes as eluent. Title compound **104** was isolated as a white foam in 89% yield (2.69 g, 6.62 mmol). Recrystallization of the foam from ethanol afforded pure compound **104** as white needles in 83% yield (2.50 g, 6.15 mmol); mp 114.9-116.4°C; $[\alpha]_D$ +30.0° (*c* 1.03, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.42 (m, 1H, H-4), 5.29 (dd, 1H, $J_{2,3}$ = 9.7 Hz, H-2), 5.22 (d, 1H, $J_{1,2}$ = 10.3, H-1), 5.08 (dd, 1H, $J_{3,4}$ = 3.4, H-3), 4.03-4.10 (m, 3H, H-5, H-6 & H-6'), 2.36 (s, 3H, SAc), 2.12, 2.01, 2.00, 1.95 (4s, 12H, 4OAc); ^{13}C-NMR ($CDCl_3$) δ (ppm): 191.7 (SAc), 170.0, 169.8, 169.5, 169.1 (4C=O), 80.2 (C-1), 74.6 (C-5), 71.5 (C-3), 66.8 (C-4), 66.0 (C-2), 60.8 (C-6), 30.4 (SAc), 20.2 (x2), 20.1 (x2) (4OAc); M.S. (CI ether, rel. intensity) m/z: 406.9 ($[M+H]^+$, 0.9%), 331.9 ($[M+H\text{-aglycone}]^+$, 16.1%), 330.9 ($[M\text{-aglycone}]^+$, 100.0%).
Anal. Calcd for $C_{16}H_{22}O_{10}S$: C, 47.29; H, 5.46. Found: C, 47.36; H, 5.47.

Allyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside (105).

Acetobromogalactose **103** (200 mg, 0.486 mmol) and Bu_4NHSO_4 (165 mg, 0.486 mmol) were dissolved in EtOAc (2 mL). A solution of allyl mercaptan (276 μL, 2.43 mmol) in 1.5M Na_2CO_3 (2 mL) was added to the reaction mixture and stirred vigorously at room temperature. After 2.5 hours, TLC showed complete consumption of **103** to give crude **105** as the major product. The reaction was worked up by addition of EtOAc (20 mL) and washed with $NaHCO_3$ (2 x 20 mL), distilled water (2 x 20 mL) and sat. NaCl (15 mL). The organic phase was dried (Na_2SO_4) and the residue obtained after concentration of the organic phase was chromatographed on silica gel column using 1/3 ethyl acetate/hexanes with 0.5% isopropanol as eluant. Pure product **105** was obtained in 80% yield (158.0 mg). Compound **105** has ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.69-5.89 (m, 1H, CH=), 5.40 (dd, 1H, $J_{3,4}$ = 3.3 Hz, $J_{4,5}$ = 0.9, H-4), 5.09-5.30 (m, 3H, H_2, $=CH_2$), 5.02 (dd, 1H, $J_{2,3}$ = 10.0, H-3), 4.45 (d, 1H, $J_{1,2}$ = 10.0, H-1), 4.03-4.16 (m, 2H, H-6, H-6'), 3.86 (ddd, 1H, $J_{5,6}$ = 7.1, H-5), 3.39 (dd, 1H, J_{vic} = 8.3, J_{gem} 13.5, SCH_x), 3.21 (dd, 1H, J_{vic} = 6.2, SCH_y), 2.13, 2.05, 2.03, 1.96 (4s, 12H, 4OAc). ^{13}C-NMR ($CDCl_3$) δ (ppm): 170.3, 170.2, 170.0, 169.5 (4C=O), 133.3 (CH=), 117.8 ($=CH_2$), 82.3 (C-1), 74.3 (C-5), 71.8 (C-3), 67.3 (C-4), 67.1 (C-2), 61.6 (C-6), 32.7 (SCH_2), 20.7, 20.6, 20.6, 20.5 (4OAc). MS (CI ether, rel. intensity) m/z: 405 ($[M+H]^+$, 1.8%), 331 ($[M\text{-aglycone}]^+$, 100%).

Anal. Calcd for $C_{17}H_{24}O_9S$: C, 50.49; H, 5.98. Found: C, 50.60; H, 5 91.

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide (106)

Glucose pentaacetate[192] (50.0 g, 0.128 mol) was dissolved in 30% HBr in acetic acid in an amount sufficient to dissolve the sugar (~30 mL). The reaction mixture was stirred at room temperature for 20 min, after which time the TLC indicated complete disappearance of the starting material. Methylene chloride (500 mL) was added to the reaction mixture and the organic phase was poured in a Na_2CO_3/ice solution. The organic

layer was separated and washed with saturated $NaHCO_3$ solution until the aqueous layer became non-acidic (~4 x 500 mL). The organic layer was then washed with water (2 x 500 mL) followed by saturated NaCl (1 x 400 mL). The organic phase was dried (Na_2SO_4) and the residue obtained after concentration under vacuum was recrystallized from ether to afford 45.3 g of α-acetobromoglucose **106** (86%). Title compound **106** has mp 85.2-86.9°C; (lit.[196] R_f 0.80 (EtOAc/Hexanes, 2/1); ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.58 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1), 5.54 (dd, 1H, $J_{3,4}$ = 9.5, H-3), 5.15 (dd, 1H, $J_{4,5}$ = 10.1, H-4), 4.81 (dd, 1H, $J_{2,3}$ = 10.0, H-2), 4.07-4.33 (m, 3H, H-5, H-6 & H-6'), 2.08, 2.07, 2.03, 2.01 (4s, 12H, 4OAc).

2,3,4,6-Tetra-O-acetyl-1-S-acetyl-1-thio-β-D-glucopyranose (107)

To a solution of glucosyl bromide **106** (3.0 g, 7.3 mmol) in ethyl acetate (20 mL) was added a solution of sodium thiolacetate (10.9 mmol) and tetrabutylammonium hydrogen sulfate (2.48 g, 7.3 mmol) in 1 M sodium carbonate (20 mL). The mixture was stirred at room temperature for <2.5 h, the reaction being monitored by TLC (R_f 0.45, EtOAc:hexanes, 1:1). The reaction mixture was then diluted with 75 mL each of ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was separated and washed with saturated aqueous $NaHCO_3$ (3 x 75 mL), H_2O (2 x 75 mL) followed by saturated sodium chloride (75 mL). The dried organic layer (Na_2SO_4) was concentrated near dryness under reduced pressure, and purified by silica gel column chromatography using 2/5 EtOAc:hexanes as eluent. Compound **107** was isolated as a white foam in 78% yield (2.31 g, 5.68 mmol). Recrystallization of the foam from ethanol afforded pure compound **107** as white urchins in 68% yield (2.0 g, 4.9 mmol); mp 115-116.1°C; $[\alpha]_D$ +6.08° (*c* 1.02, $CHCl_3$); (lit.[197] mp 121°C; $[\alpha]_D$ +9° (*c* 2.1, 1,1,2,2-tetrachloroethane)); ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.21-5.30 (m, 2H, H-1 & H-3), 5.04-5.15 (m, 2H, H-2 & H-

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[196] Lemieux, R. U. *Methods Carbohydr. Chem.* **1963**, *2*, 221.

[197] Horton, D. *Methods Carbohydr. Chem.* **1963**, *2*, 433.

4), 4.25 (dd, 1H, $J_{5,6'}$ = 4.4 Hz, $J_{6,6'}$ = 12.5, H-6'), 4.07 (dd, 1H, $J_{5,6}$ = 2.1, H-6), 3.83 (ddd, 1H, $J_{4,5}$ = 9.9, H-5), 2.37 (s, 3H, SAc), 2.06, 2.01, 2.00, 1.99 (4s, 12H, 4OAc); ^{13}C-NMR ($CDCl_3$) δ (ppm): 191.8 (SAc), 170.2, 169.9, 169.5, 169.2 (4C=O), 80.5 (C-1), 76.7 (C-5), 74.1 (C-3), 69.8 (C-2), 68.9 (C-4), 62.3 (C-6), 30.9 (SAc), 20.6, 20.5, 20.4 (x2) (4OAc); M.S. (CI ether, rel. intensity) m/z: 406.9 ($[M+H]^+$, 0.4%), 346.9 ($[M-OAc]^+$, 6.6%), 331.9 ($[M+H\text{-aglycone}]^+$, 16.5%), 330.8 ($[M\text{-aglycone}]^+$, 100.0%).
Anal. Calcd for $C_{16}H_{22}O_{10}S$: C, 47.29; H, 5.46. Found: C, 47.57; H, 5.52.

Allyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (108)

To a solution of α-acetobromoglucose **106** (100 mg, 0.243 mmol) and tetrabutylammonium hydrogen sulfate (82.5 mg, 0.243 mmol) in ethyl acetate (1 mL) was added a solution of allyl mercaptan (138 μL, 1.22 mmol) in 1.5M sodium carbonate (1 mL). The reaction mixture was stirred at room temperature until TLC indicated complete transformation of the starting material (<2.5h). Ethyl acetate (10 mL) was added to the reaction mixture and the organic layer was separated and washed three times with saturated sodium hydrogen carbonate (10 mL each), twice with water (10 mL) and once with saturated sodium chloride (10 mL). The organic phase was then dried using sodium sulfate and evaporated near dryness. The oily residue was purified by silica gel column chromatography using 1/3 ethyl acetate/hexanes containing 0.5% isopropanol as eluant, to obtain pure thioallyl glucoside **108** (82.5 mg) in 84% yield. Title compound **108** has ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.72-5.78 (m, 1H, CH=), 5.20 (dd, 1H, $J_{3,4} \sim J_{2,3} \sim 9.3$ Hz, H-3), 5.00-5.15 (m, 4H, H-2, H-4, $=CH_2$), 4.45 (d, 1H, $J_{1,2}$ = 10.0, H-1), 4.20 (dd, 1H, $J_{5,6}$ = 5.1, $J_{6,6'}$ = 12.3, H-6), 4.10 (dd, 1H, $J_{5,6'}$ = 2.4, H-6'), 3.63 (m, 1H, H-5), 3.38 (dd, 1H, J_{vic} = 8.5, J_{gem} = 13.5, SCH_x), 3.20 (dd, 1H, J_{vic} = 6.3, SCH_y), 2.06, 2.03, 2.00, 1.98 (4s, 12H, 4OAc). ^{13}C-NMR ($CDCl_3$) δ (ppm): 169.4 (x2), 169.3, 169.2 (4C=O), 133.3 (CH=), 118.0 ($=CH_2$), 81.9 (C-1), 75.6 (C-5), 73.9 (C-3), 69.9 (C-4), 68.4 (C-2), 62.2

(C-6), 32.9 (SCH_2), 20.8, 20.7 (x2), 20.5 (4OAc). MS (CI ether, rel. intensity) m/z: 405 ($[M+H]^+$, 2.3%), 331 ($[M\text{-aglycone}]^+$, 100%).

Anal. Calcd for $C_{17}H_{24}O_9S$: C, 50.49; H, 5.98. Found: C, 50.67; H, 5.75.

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride (109)

N-Acetylglucosamine (3.0 g, 13.56 mmol) was dissolved in 200 μL MeOH and acetyl chloride (60 mL). The mixture was carefully sealed with a fritted glass stopper and stirred at room temperature for 48h. The reaction was monitored by TLC (R_f (EtOAc) 0.56). The resultant clear solution was evaporated under reduced pressure and co-evaporated with toluene. Glycosyl chloride **109** was dried under vacuum and purified by silica gel column chromatography using a gradient of hexanes to EtOAc as eluent. Title compound **109** was isolated as a beige solid in 67% yield (2.22 g, 6.07 mmol); mp 125.0-128.1°C; $[\alpha]_D$ +105.0° (*c* 1.0, $CHCl_3$). Lit.[181] mp 127.0-128.0°C; $[\alpha]_D$ +110.0° (*c* 1.0, $CHCl_3$).

Allyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-1-thio-β-D-glucopyranoside (110).

N-acetylglucosaminyl chloride **109** (100 mg, 0.273 mmol) and Bu_4NHSO_4 (82.8 mg, 0.273 mmol) were dissolved in EtOAc (1 mL). A solution of allyl mercaptan (140μL, 1.37 mmol) in 1.5M Na_2CO_3 (1 mL) was added to the reaction mixture and stirred vigorously at room temperature. After 2.5 hours, TLC showed complete consumption of **109** to give crude **110** as the major product. The reaction was worked up by addition of EtOAc (10 mL) and washed with $NaHCO_3$ (2 x 10 mL), distilled water (2 x 10 mL) and sat. NaCl (8 mL). The organic phase was dried (Na_2SO_4) and the residue obtained after concentration of the organic phase was chromatographed on silica gel column using 3/1 ethyl acetate/hexanes with 0.5% isopropanol as eluant. Pure product **110** was obtained in 86% yield (84.0 mg). Compound **110** has ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.70-5.84 (m, 1H,

CH=), 5.41 (d, 1H, $J_{2,NH}$ = 9.2 Hz, NH), 5.06-5.15 (m, 4H, H_3, H_4, $=CH_2$), 4.48 (d, 1H, $J_{1,2}$ = 10.3 Hz, H-1), 4.20 (dd, 1H, $J_{5,6}$ = 5.2, $J_{6,6'}$ = 12.3, H-6), 4.13 (m, 1H, H-2), 4.11 (dd, 1H, $J_{5,6'}$ = 2.4, H-6'), 3.60 (m, 1H, H-5), 3.38 (dd, 1H, J_{vic} = 8.8, J_{gem} = 13.4, SCH_x), 3.20 (dd, 1H, J_{vic} = 5.8, SCH_y), 2.07, 2.01, 2.00 (3s, 9H, 3OAc), 1.94 (s, 3H, NAc). ^{13}C-NMR ($CDCl_3$) δ (ppm): 171.5, 171.0, 170.3, 169.6 (4C=O), 133.7 (CH=), 118.1 ($=CH_2$), 83.0 (C-1), 76.1 (C-5), 74.2 (C-3), 68.7 (C-4), 62.7 (C-6), 53.3 (C-2), 33.0 (SCH_2), 23.6 (NAc), 21.0, 20.9 (x2) (3OAc). MS for $C_{17}H_{25}NO_8S$ (CI ether, rel. intensity) m/z: 404 ($[M+H]^+$, 62.9%), 330 ([M-aglycone]$^+$, 7.5%).

Methyl 5-acetamido-3,5-dideoxy-D-glycero-D-galacto-2-nonulopyranosonate (112)

A solution of *N*-acetylneuraminic acid **111** (2.00 g, 6.47 mmol) and Amberlite IR-120 H^+ resin (4 g) in methanol (50 mL) was stirred for 24 h at room temperature. The reaction was monitored by TLC (R_f 0.76, isopropanol:H_2O, 7:3). The clear solution was filtered and evaporated to a volume of ~ 5 mL whereupon, ether was added to turbidity. Methyl ester **112** was isolated as white crystals in 89% yield (1.86 g, 5.76 mmol); mp 186.7-187.8°C; (lit.[198] mp 180.0-182.0°C); $[\alpha]_D$ -29.5° (*c* 1.0, H_2O); MS for $C_{12}H_{21}NO_9$ (CI ether, rel. intensity) m/z: 324 ($[M+H]^+$, 15.8%), 306 ([M-OH]$^+$, 100.0%).

Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosonyl chloride)onate (2)

Methyl ester **112** (150.0 mg, 0.464 mmol) was combined with 100 μL MeOH in acetyl chloride (3.5 mL). The mixture was sealed and left stirring for 48 h at room temperature. Reaction progress was monitored by TLC (R_f 0.41, EtOAc). The resultant clear solution was evaporated under reduced pressure and co-evaporated with toluene to remove residual HCl. Title compound **2** was dried under vacuum and used for subsequent

[198] Ogura, H.; Furuhata, K. *Carbohydr. Res.* **1986**, *158*, 37.

reactions without further purification since ^{1}H NMR showed the product to be >95% pure. It was isolated in 98% yield (232 mg, 0.455 mmol). This product could be recrystallized from CH_2Cl_2 ether to give pure **2** as white crystals in 80% yield (189.1 mg, 0.372 mmol); mp 104.1-107.7°C; $[\alpha]_D$ -63.2° (*c* 1.1, $CHCl_3$); (lit.[199] mp 105.0°C (dec.); $[\alpha]_D$ -68.0° (*c* 1.0, $CHCl_3$)); ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.85 (d, 1H, $J_{5,NH}$ = 10.0 Hz, NH), 5.42 (dd, 1H, $J_{7,8}$ = 6.6, $J_{6,7}$ = 2.1, H-7), 5.32 (ddd, 1H, $J_{4,5}$ = 10.4, H-4), 5.11 (ddd, 1H, $J_{8,9'}$ = 2.6, H-8), 4.36 (dd, 1H, $J_{9,9'}$ = 12.5, H-9'), 4.18 (m, 1H, H-5), 4.01 (dd, 1H, $J_{8,9}$ = 6.0, H-9), 3.74-3.85 (m, 1H, H-6), 3.82 (s, 3H, OMe), 2.72 (dd, 1H, $J_{3e,4}$ = 4.7, $J_{3e,3a}$ = 13.9, H-3e), 2.20 (dd, 1H, $J_{3a,4}$ = 11.1, H-3a), 2.06, 2.02, 2.00, 1.99 (4s, 12H, 4OAc), 1.85 (s, 3H, NAc).

Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-S-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosonate (113)

To a solution of freshly prepared β-acetochloroneuraminic acid **2** (3.39 g, 7.73 mmol) in ethyl acetate (20 mL) was added a solution of sodium thiolacetate (12.8 mmol) and tetrabutylammonium hydrogen sulfate (2.89 g, 7.73 mmol) in 1 M sodium carbonate (20 mL). The mixture was stirred at room temperature for 30 min, the reaction being monitored by TLC (ethyl acetate). The reaction mixture was then diluted with 75 mL each of ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was separated and washed with saturated aqueous $NaHCO_3$ (2 x 75 mL) followed by saturated sodium chloride (50 mL). The dried organic phase (Na_2SO_4) was concentrated near dryness under vacuum, and purified by crystallization from ethyl acetate/hexanes to afford 66% of pure compound **113** as white needles (2.8 g); mp 89-92°C, $[\alpha]_D$ +52.0° (*c* 1.0, $CHCl_3$), (lit.[184] mp 75-80°C, $[\alpha]_D$ +46.7°); R_f(EtOAc) = 0.38; ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.40 (d, 1H, $J_{5,NH}$ = 10.1 Hz, NH), 5.35 (dd, 1H, $J_{7,8}$ = 6.2, $J_{6,7}$ = 2.3, H-7), 5.22 (ddd, 1H, $J_{8,9}$ = 6.2, H-8), 4.90 (ddd, 1H, $J_{3a,4}$ = 11.7, $J_{3e,4}$ = 4.6, $J_{4,5}$ = 10.4, H-4), 4.65 (dd, 1H, $J_{5,6}$

[199] Sharma, M.N.; Edy, R. *Carbohydr. Res.* **1984**, *127*, 201.

= 10.8, H-6), 4.40 (dd, 1H, $J_{9,9'}$ = 12.4, $J_{8,9'}$ = 2.5, H-9'), 4.09 (m, 1H, H-5), 4.02 (dd, 1H, H-9), 3.79 (s, 3H, OMe), 2.62 (dd, 1H, $J_{3e,4}$ = 4.6, $J_{3e,3a}$ = 13.0, H-3e), 2.27 (s, 3H, SAc), 2.13, 2.12, 2.03, 2.01 (4s, 12H, 4OAc), 2.02 (dd, 1H, $J_{3a,4}$ = 11.7, H-3a), 1.88 (s, 3H, NAc). ^{13}C-NMR ($CDCl_3$) δ (ppm): 191.5 (SAc), 170.8, 170.7, 170.4, 170.3, 170.1 (5C=O), 169.4 (C-1), 84.5 (C-2), 75.2 (C-6), 70.6 (C-8), 68.9 (C-4), 67.8 (C-7), 62.4 (C-9), 53.4 (OMe), 49.0 (C-5), 37.5 (C-3), 30.1 (SAc), 23.1 (NAc), 20.9, 20.8 (x2), 20.7 (4OAc). MS (CI ether, rel. intensity) m/z: 550 ($[M+H]^+$, 55.7%), 474 ($[M\text{-aglycone}]^+$, 31.6%).

Anal. Calcd for $C_{22}H_{31}NO_{13}S$: C, 48.08; H, 5.68; N, 2.55; S, 5.83%. Found: C, 48.04; H, 5.79; N, 2.49; S, 6.02.

Methyl (allyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosid) onate (114)

To a solution of β-acetochloroneuraminic acid **2** (118 mg, 0.232 mmol) and Bu_4NHSO_4 (78.8 mg, 0.232 mmol) in ethyl acetate (1.2 mL) was added a solution of allyl mercaptan (75 μL, 0.928 mmol) in 1 M Na_2CO_3 (1.2 mL). The reaction mixture was stirred at room temperature until TLC indicated complete transformation of the starting material (1h). Ethyl acetate (18 mL) was added to the reaction mixture and the organic phase was separated and washed three times with sat. $NaHCO_3$ (20 mL each), twice with water (20 mL) and once with sat. NaCl (10 mL). The organic layer was then dried using Na_2SO_4 and evaporated near dryness. The oily residue was purified by silica gel chromatography 5/2 EtOAc/hexanes with 0.5% i-PrOH as eluant. Pure product **114** was obtained in 84% yield (106.4 mg) as white crystals from ether/hexanes. Compound **114** has mp 109-111°C (ether/hexanes); $[\alpha]_D$ +38.9° (*c* 1.01, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.73 (dddd, 1H, J_{cis} = 10.0 Hz, J_{trans} = 17.0 Hz, CH=), 5.37 (ddd, 1H, $J_{8,9'}$ = 2.7, $J_{8,9}$ = 5.4, H-8), 5.29 (dd, 1H, $J_{7,8}$ = 8.2, H-7), 5.19 (d, 1H, $J_{5,NH}$ = 9.9, NH), 5.06-5.18 (m, 2H, $=CH_2$), 4.83 (ddd, 1H, $J_{4,5}$ = 10.4, H-4), 4.29 (dd, 1H, $J_{9,9'}$ = 12.5, H-9'), 4.09

(dd, 1H, H-9), 4.02 (ddd, 1H, $J_{5,6}$ = 10.7, H-5), 3.84 (dd, 1H, $J_{6,7}$ = 2.2, H-6), 3.76 (s, 3H, OMe), 3.35 (dd, 1H, J_{gem} = 13.8, J_{vic} = 7.5, SCH_x), 3.26 (dd, 1H, J_{vic} = 6.3, SCH_y), 2.69 (dd, 1H, $J_{3e,4}$ = 4.6, $J_{3e,3a}$ = 12.7, H-3e), 2.15, 2.12, 2.01, 2.00 (4s, 12H, 4OAc), 1.96 (dd, 1H, $J_{3a,4}$ = 11.7, H-3a), 1.84 (s, 3H, NAc). ^{13}C-NMR ($CDCl_3$) δ (ppm): 170.9, 170.6, 170.1 (x2), 170.1 (5C=O), 168.3 (C-1), 132.9 (CH=), 118.0 (=CH_2), 82.9 (C-2), 74.1 (C-6), 69.5 (C-4), 68.6 (C-8), 67.3 (C-7), 62.2 (C-9), 52.8 (OMe), 49.3 (C-5), 37.8 (C-3), 31.6 (SCH_2), 23.1 (NAc), 21.1, 20.8, 20.7, 20.6 (4OAc). MS (CI ether, rel. intensity) m/z: 548 ($[M+H]^+$, 68.4%), 488 ($[M-CO_2Me]^+$ and/or $[M-OAc]^+$, 55.8%), 474 ([M-aglycone]$^+$, 22.1%).

Anal. Calcd for $C_{23}H_{33}NO_{12}S$: C, 50.45; H, 6.07; N, 2.56%. Found: C, 50.65; H, 5.97; N, 2.38.

Methyl (phenyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosid) onate (115)

To a solution of freshly prepared β-acetochloroneuraminic acid **2** (495 mg, 0.97 mmol) and tetrabutylammonium hydrogen sulfate (330 mg, 0.97 mmol) in ethyl acetate (5 mL) was added a solution of benzenethiol (150 μL, 1.45 mmol) in 2 M sodium carbonate (5 mL). The mixture was stirred at room temperature for 2 h, the reaction being monitored by TLC (R_f 0.45, ethyl acetate). The reaction mixture was then diluted with 45 mL each of ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was separated and washed with saturated aqueous $NaHCO_3$ (2 x 40 mL), H_2O (2 x 30 mL) and by saturated sodium chloride (20 mL). The dried organic phase (Na_2SO_4) was concentrated near dryness under vacuum, and purified by crystallization from benzene/hexanes to afford 417.4 mg of title compound **115** (74 % yield). Compound **115** has mp 140.2-141.8°C; $[\alpha]_D$ +19.8° (*c* 1.09, $CHCl_3$); (lit.[200] mp 139-140°C; $[\alpha]_D$ +21° (*c* 1.0, $CHCl_3$)); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.49 (d, 2H, H-ortho), 7.37 (t, 1H, H-para), 7.32

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

(dd, 2H, H-meta), 5.23-5.30 (m, 2H, H-7 & H-8), 5.09 (d, 1H, $J_{5,NH}$ = 9.8 Hz, NH), 4.82 (ddd, 1H, $J_{3a,4}$ = 11.7, $J_{3e,4}$ = 4.7, $J_{4,5}$ = 10.1, H-4), 4.36 (dd, 1H, $J_{8,9'}$ = 2.4, $J_{9,9'}$ = 12.5, H-9'), 4.18 (dd, 1H, $J_{8,9}$ = 4.9, H-9), 3.96 (ddd, 1H, $J_{5,6}$ = 10.7, H-5), 3.84 (dd, 1H, $J_{6,7}$ = 1.7, H-6), 3.55 (s, 3H, OMe), 2.81 (dd, 1H, $J_{3e,3a}$ = 12.9, H-3e), 2.00 (dd, 1H, H-3a), 2.14, 2.04, 2.03, 2.00 (s, 12H, 4AOc), 1.86 (s, 3H, NAc); ^{13}C-NMR ($CDCl_3$) δ (ppm): 171.0, 170.7, 170.4, 170.2, 170.2 (5C=O), 167.9 (C-1), 138.5 (C_m), 129.9 (C_p), 128.9 (C_{ipso}), 128.3 (C_o), 87.5 (C-2), 74.7 (C-6), 69.8 (C-4), 69.6 (C-8), 67.6 (C-7), 62.0 (C-9), 52.6 (OMe), 49.2 (C-5), 38.0 (C-3), 23.1 (NAc), 20.8, 20.6, 20.6, 20.6 (4OAc); MS (CI ether, rel. intensity) m/z: 584 ($[M+H]^+$, 31.2%), 524 ($[M-CO_2Me]^+$ and/or $[M-OAc]^+$, 24.1%), 474 ($[M-aglycone]^+$, 13.6%).

Anal. Calcd for $C_{26}H_{33}NO_{12}S$: C, 53.51; H, 5.70; N, 2.40; S, 5.49%. Found: C, 53.75; H, 5.84; N, 2.31; S, 5.39.

Methyl (4-nitrophenyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosid) onate (116)

To a solution of freshly prepared acetochloroneuraminic acid **2** (3.0 g, 5.89 mmol) in EtOAc (60 mL) was added a solution of *p*-nitrothiophenol (1.7 g, 8.83 mmol) and Bu_4NHSO_4 (2.0 g, 5.89 mmol) in 2 M Na_2CO_3 (60 mL). The mixture was stirred vigorously for 1 h at room temperature while the reaction was monitored by TLC (R_f 0.5, EtOAc). The reaction mixture was then diluted with 100 mL each of EtOAc and saturated $NaHCO_3$. The organic phase was separated and successively washed with saturated $NaHCO_3$ (2 x 100 mL), water (3 x 90 mL) and finally by saturated NaCl (50 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a gradient of 2/1 to 4/1 of EtOAc/hexanes to give compound **116** as an off-white foam in 73% yield (2.72 g, 4.32 mmol). The foam was then recrystallized from $CHCl_3$. Pure glycoside **116** has mp 169.6-171.5°C; $[\alpha]_D$ +20.1° (*c* 1.06, $CHCl_3$); (lit.[184] mp 168.0-172.0°C; $[\alpha]_D$ 27.6°

(*c* 1.0, MeOH)); ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.17 (d, 2H, $J_{o,m}$ = 9.0 Hz, H-meta), 7.63 (d, 2H, H-ortho), 5.30 (dd, 1H, $J_{6,7}$ = 2.2, H-7), 5.27 (ddd, 1H, $J_{8,9}$ = 1.2, $J_{8,9'}$ = 2.1, H-8), 5.14 (d, 1H, $J_{5,NH}$ = 9.8, NH), 4.86 (ddd, 1H, $J_{3a,4}$ = 11.8, $J_{3e,4}$ = 4.7, $J_{4,5}$ = 9.9, H-4) 4.30 (dd, 1H, $J_{9,9'}$ = 12.5, H-9'), 4.10 (dd, 1H, $J_{5,6}$ = 11.3, H-6), 4.06 (dd, 1H, H-9), 3.99 (ddd, 1H, H-5), 3.59 (s, 3H, OCH3), 2.85 (dd, 1H, $J_{3e,3a}$ = 12.8, H-3e), 2.14, 2.04, 2.04, 2.02 (4s, 12H, 4OAc), 2.08 (dd, 1H, H-3a), 1.87 (s, 3H, NAc); ^{13}C-NMR ($CDCl_3$) δ (ppm): 170.8, 170.7, 170.2, 170.1, 169.7 (5C=O), 167.3 (C-1), 148.2 (C_{ipso}), 137.7 (C_p), 135.0 (C_m), 123.8 (C_o), 86.9 (C-2), 74.5 (C-6), 69.1 (C-8), 68.6 (C-4), 67.3 (C-7), 62.1 (C-9), 53.0 (OMe), 49.2 (C-5), 38.3 (C-3), 23.1 (NAc), 20.9, 20.7, 20.7,20.7 (4OAc); MS (CI ether, rel. intensity) m/z: 629 ($[M+H]^+$, 66.2%), 569 ($[M-CO_2Me+H]^+$ and/or $[M-OAc+H]^+$, 41.3%), 474 ($[M-aglycone]^+$, 34.8%).

Anal. Calcd for $C_{26}H_{32}N_2O_{14}S$: C, 49.68; H, 5.13; N, 4.46; S, 5.10; Found: C, 49.83; H, 5.36; N, 4.29; S, 4.92.

Methyl (4-Methoxyphenyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-a-D – glycero-D-galacto-2-nonulopyranosid)onate (117)

To a solution of freshly prepared acetochloroneuraminic acid **2** (400 mg, 0.785 mmol) in EtOAc (4 mL) was added a solution of *p*-methoxybenzenethiol (145 μL, 1.18 mmol) and Bu_4NHSO_4 (266.6 mg, 0.785 mmol) in 2 M Na_2CO_3 (4 mL). The mixture was stirred vigorously for 1 h at room temperature and the reaction monitored by TLC (R_f 0.45, EtOAc). The reaction mixture was then diluted with 50 mL each of EtOAc and saturated $NaHCO_3$. The organic phase was separated and successively washed with saturated $NaHCO_3$ (2 x 45 mL), water (2 x 45 mL) followed by saturated NaCl (20 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude thioglycoside **117** was crystallized from benzene/hexanes to afford pure compound **117** in 65% yield (311.9 mg); mp 132.2-133.5°C; $[\alpha]_D$ +17.3° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.40 (d, 2H $J_{o,m}$ = 8.8 Hz, H-meta), 6.83 (d, 2H, H-ortho), 5.28 (dd,

1H, $J_{6,7}$ = 1.8, $J_{7,8}$ = 6.8, H-7), 5.22 (ddd, 1H, $J_{8,9}$ = 2.6, $J_{8,9'}$ = 5.5, H-8), 5.15 (d, 1H, $J_{NH,5}$ = 9.8, NH), 4.83 (ddd, 1H, $J_{3a,4}$ = 11.7, $J_{3e,4}$ = 4.6, $J_{4,5}$ = 10.2, H-4), 4.37 (dd, 1H, $J_{9,9'}$ = 12.2, H-9'), 4.18 (dd, 1H, H-9), 3.96 (ddd, 1H, $J_{5,6}$ = 10.8, H-5), 3.83 (dd, 1H, H-6), 3.80 (s, 3H, PhOCH3), 3.60 (s, 3H, OCH3), 2.78 (dd, 1H, $J_{3e,3a}$ = 12.9, H-3e), 2.11, 2.05, 2.02, 1.99 (4s, 12H, 4OAc), 2.01 (dd, 1H, H-3a), 1.84 (s, 3H, NAc); ^{13}C-NMR ($CDCl_3$) δ (ppm): 171.0, 170.8, 170.2, 170.3, 170.2 (5C=O), 168.0 (C-1), 161.2 (C_{ipso}), 138.5 (C_m), 119.1 (C_p), 114.4 (C_o), 87.4 (C-2), 74.7 (C-6), 70.1 (C-8), 69.6 (C-4), 67.5 (C-7), 61.9 (C-9), 55.4 (PhOMe), 52.8 (OMe), 49.1 (C-5), 38.0 (C-3), 23.0 (NAc), 20.8, 20.6, 20.6, 20.6, (4OAc); MS (CI ether, rel. intensity) m/z: 614 ($[M+H]^+$, 20%), 554 ($[M-CO_2Me]^+$ and/or $[M-OAc]^+$, 40%), 474 ($[M-aglycone]^+$, 23%).

Anal. Calcd for $C_{27}H_{35}NO_{13}S$: C, 52.85; H, 5.75; N, 2.28; S, 5.22; Found: C, 52.91; H, 5.89; N, 2.13; S, 4.88.

Methyl (azido 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosid) onate (118)

To a solution of β-acetochloroneuraminic acid **2** (241 mg, 0.473 mmol) and Bu_4NHSO_4 (161 mg, 0.473 mmol) in ethyl acetate (2.5 mL) was added a solution of sodium azide (125 mg, 1.89 mmol) in 2 M Na_2CO_3 (2.5 mL). The reaction mixture was stirred at room temperature until TLC indicated complete transformation of the starting material (45 min). Ethyl acetate (50 mL) was added to the reaction mixture and the organic layer was separated and washed three times with sat. $NaHCO_3$ (50 mL each), twice with water (50 mL) and once with saturated NaCl (45 mL). The organic layer was then dried using Na_2SO_4 and evaporated near dryness. The oily residue was chromatographed on a silica gel column with a gradient from 3/1 EtOAc/hexanes to pure EtOAc as eluant. Pure product **118** was obtained in 83% yield (202.8 mg, 0.392 mmol) as white foam, which could then be recrystallized from chloroform/hexanes in 74% yield as white crystals (180.8 mg, 0.350 mmol). Title compound **118** has mp 83.1-84.2°C; $[\alpha]_D$ -

29.2° (*c* 1.19, $CHCl_3$); lit.[188] mp 93-98°C; and $[\alpha]_D$ -23.16° (*c* 1.675, MeOH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.31-5.33 (m, 2H, H-7, H-8), 5.16 (d, 1H, $J_{5,NH}$ = 9.8 Hz, NH), 5.03 (ddd, 1H, $J_{3e,4}$ = 4.8, $J_{3a,4}$ = 11.8, $J_{4,5}$ = 10.2, H-4), 4.34 (dd, 1H, $J_{8,9'}$ = 2.2, H-9'), 4.11 (dd, 1H, $J_{9,9'}$ = 12.5, $J_{8,9}$ = 5.6, H-9), 4.02 (ddd, 1H, H-5), 3.87 (dd, 1H, $J_{5,6}$ = 10.7, $J_{6,7}$ = 1.8, H-6), 3.87 (s, 3H, OMe), 2.55 (dd, 1H, $J_{3e,3a}$ = 13.0, H-3e), 2.13, 2.11, 2.02, 2.01 (4s, 12H, 4OAc), 1.82 (dd, 1H, H-3a), 1.87 (s, 3H, NAc). ^{13}C-NMR ($CDCl_3$) δ (ppm): 170.8, 170.6, 170.2 (x2), 170.0 (5C=O), 167.1 (C-1), 88.9 (C-2), 73.9 (C-6), 69.4 (C-8), 68.7 (C-4), 67.4 (C-7), 62.1 (C-9), 53.4 (OMe), 49.2 (C-5), 36.5 (C-3), 23.1 (NAc), 21.0, 20.8, 20.7, 20.7 (4OAc); MS (CI ether, rel. intensity) m/z: 517 ($[M+H]^+$, 100%), 489 ($[M-N_2+H]^+$, 18.4%), 474 ($[M\text{-aglycone}]^+$, 15.4%).

Anal. Calcd for $C_{20}H_{28}N_4O_{12}$: C, 46.51; H, 5.46; N, 10.85%. Found: C, 46.22; H, 5.29; N, 10.52.

Methyl [2-(phenylselenyl) 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosid) onate (119)

To a solution of β-acetochloroneuraminic acid **2** (235.4 mg, 0.462 mmol) and Bu_4NHSO_4 (156.9 mg, 0.462 mmol) dissolved in EtOAc (2 mL) was added 2 M Na_2CO_3 (2 mL). Benzeneselenol (110 μL, 0.924 mmol) was added to the mixture while vigorously stirred at room temperature. Within 5 min., a fleeting green color was observed and the reaction was completed in 10 min. The mixture was diluted by adding EtOAc (30 mL) and the organic layer was separated from the aqueous phase. The organic phase was washed with cold 1M NaOH (2 x 50 mL), water (2 x 50 mL) followed by saturated NaCl solution (40 mL). The organic extract was then dried (Na_2SO_4), filtered and evaporated under reduced pressure. The desired product was recrystallized from EtOAc/petroleum ether or hexanes/benzene to afford 72% yield of **119** (209.1 mg, 0.332 mmol). Title compound **119** has mp 102.8-105.6°C (EtOAc/pet. ether); $[\alpha]_D$ +18.0° (*c* 1, MeOH); lit.[189] mp 106-109°C; and $[\alpha]_D$ +24.3° (*c* 1.26, MeOH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.27-

7.48 (m, 5H, H_{ar}), 5.25 (m, 2H, H-7, H-8), 5.19 (d, 1H, $J_{5,NH}$ = 10.0 Hz, NH), 4.78 (ddd, 1H, $J_{3e,4}$ = 4.7, $J_{3a,4}$ = 11.5, H-4), 4.38 (dd, 1H, $J_{8,9'}$ = 5.6, H-9'), 4.16 (dd, 1H, $J_{9,9'}$ = 12.4, $J_{8,9}$ = 2.5, H-9), 3.94 (ddd, 1H, $J_{5,6}$ = 9.2, H-5), 3.85 (dd, 1H, $J_{6,7}$ = 1.7, H-6), 3.54 (s, 3H, OMe), 2.83 (dd, 1H, $J_{3e,3a}$ = 12.9, H-3e), 2.09, 2.05, 2.03, 1.97 (4s, 12H, 4OAc), 2.00 (dd, 1H, H-3a), 1.82 (s, 3H, NAc). ^{13}C-NMR ($CDCl_3$) δ (ppm): 170.5, 170.3 (x2), 170.0, 169.8 (5C=O), 169.0 (C-1), 137.7 (C_m), 129.6 (C_p), 128.9 (C_o), 125.8 (C_{ipso}), 81.7 (C-2), 74.6 (C-6), 70.0 (C-4), 69.7 (C-8), 67.6 (C-7), 62.0 (C-9), 52.6 (OMe), 49.2 (C-5), 38.9 (C-3), 23.1 (NAc), 21.1, 20.8, 20.7, 20.6 (4OAc). MS (CI ether, rel. intensity) m/z: 631 ($[M+H]^+$, 76%), 475 ($[M\text{-aglycone}+H]^+$, 20%).

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.

Chapter 3

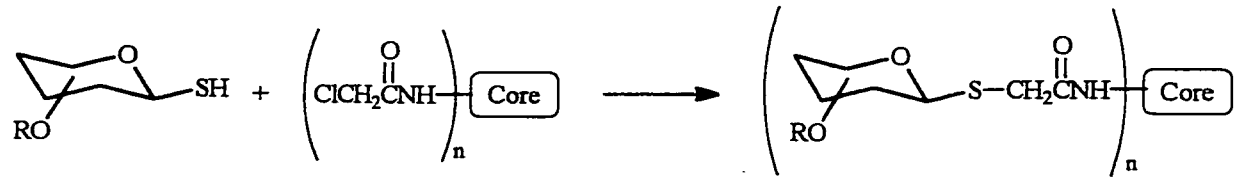
Chemoselective Deprotection of Thioacetates

3.1 Introduction

In an ongoing program directed at the syntheses of various glycoforms including glycodendrimers[79,115,117] and glycocalixarenes,[51] an efficient conjugation strategy was required to allow for the incorporation of carbohydrate residues onto preformed cores (dendrimers and calixarenes) at a late stage in the syntheses.[39,51] This permits the preparation of a variety of glycoconjugates containing different sugar moieties.

The approach chosen for this conjugation was based on the convergent syntheses of multi-branched structures having terminal *N*-chloroacetyl groups to which thiolated carbohydrate derivatives could be added (Scheme 3.1.1). This reaction, involving nucleophilic chlorine displacement (S_N2) by thiolated glycosides, was shown to proceed in very high yields.[51,115,117] Furthermore, a definite advantage of the *N*-chloroacetyl strategy is that it enables the determination of the extent of glycoside incorporation by ^{1}H NMR spectroscopy. The ^{1}H NMR chloromethylene (*N*-chloroacetyl) signals appear as well resolved singlets in the achiral starting materials at ~4.01 ppm ($CDCl_3$) and can be precisely integrated relative to other well resolved signals. Following carbohydrate attachment, the chloromethylene signal is shielded due to chlorine displacement by sulfur and appears as two separated doublets due to their diastereotopic relationship between the methylene protons when the methylene group is directly attached to an anomeric thiolate (chiral anomeric center). This behavior is illustrated in Figures 3.1.1 and 3.1.2, and Schemes 3.3.3 and 3.3.4. Thioglycoside **134** having an ethoxycarbonylmethyl group as aglycone features (^{1}H NMR) two upfield protons as two doublets ($J_{a,b}$ = 16.8 Hz) at 3.57

($S\text{-}CH_a$) and 3.40 ppm ($S\text{-}CH_b$) (Figure 3.1.1). The behavior arises due to the aglycone being directly bonded to the anomeric sulfur. However, this behavior (for SCH_2CO protons) is not observed when the same ethoxycarbonylmethyl group is bonded to the sulfur atom in azido thioether **125** (Figure 3.1.2 and Scheme 3.3.1). The two protons appear as a singlet at 3.26 ppm which is still upfield from the chloroacetyl singlet signal at 4.01 ppm.



Scheme 3.1.1 *N*-chloroacetylated conjugation strategy in the synthesis of multi-branched glycoforms.

Since the necessary thiol functionality could be readily incorporated onto the carbohydrate moieties in the form of thioacetates, selective deprotection of the thioacetates was therefore essential for subsequent nucleophilic displacement. 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 were also present. Other mild conditions for selective deprotection of anomeric *S*-acetates were reported to occur under low temperature (-40°C) Zemplén conditions[200] and more recently with diethylamine[201] or with cysteamine in hexamethylphosphoramide (HMPA) containing 1,4-dithioerythritol (DTE).[202] As these conditions were not widely applicable to achieve the desired transformations, different combinations of reagents were sought.

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201 Bennett, S.; von Itzstein, M.; Kiefel, M. J. *Carbohydr. Res.* **1994**, *259*, 293.

202 a) Schou, C.; Rasmussen, G.; Schulein, M.; Henrissat, B.; Driguez, H. *J. Carbohydr. Chem.* **1993**, *12*, 743; b) Blanc-Muesser, M.; Driguez, H. *J. Chem. Soc. Perkin Trans. I* **1988**, 3345.

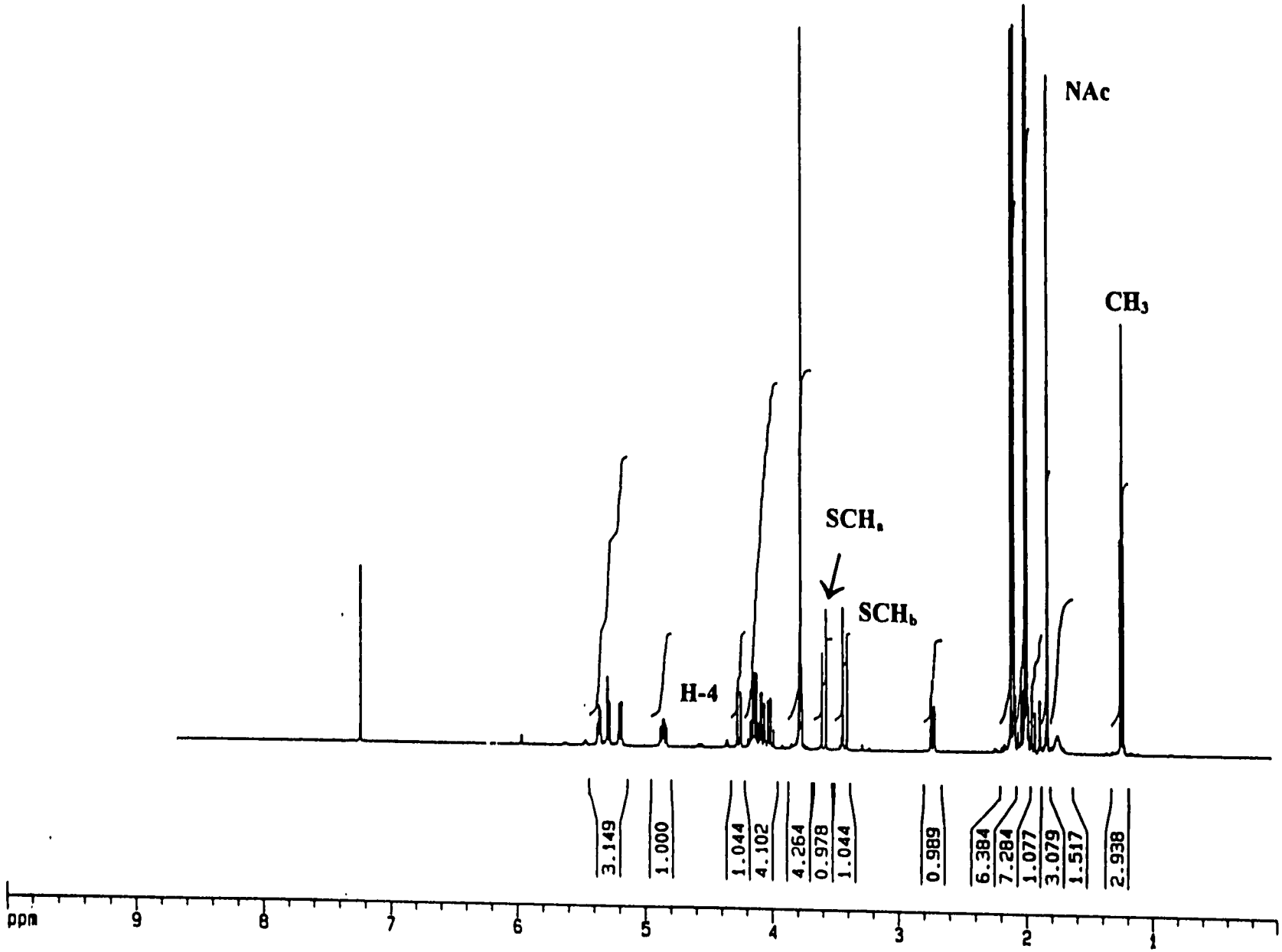

Figure 3.1.1. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of thiosialoside **134** having an ethoxycarbonylmethyl group as aglycone.

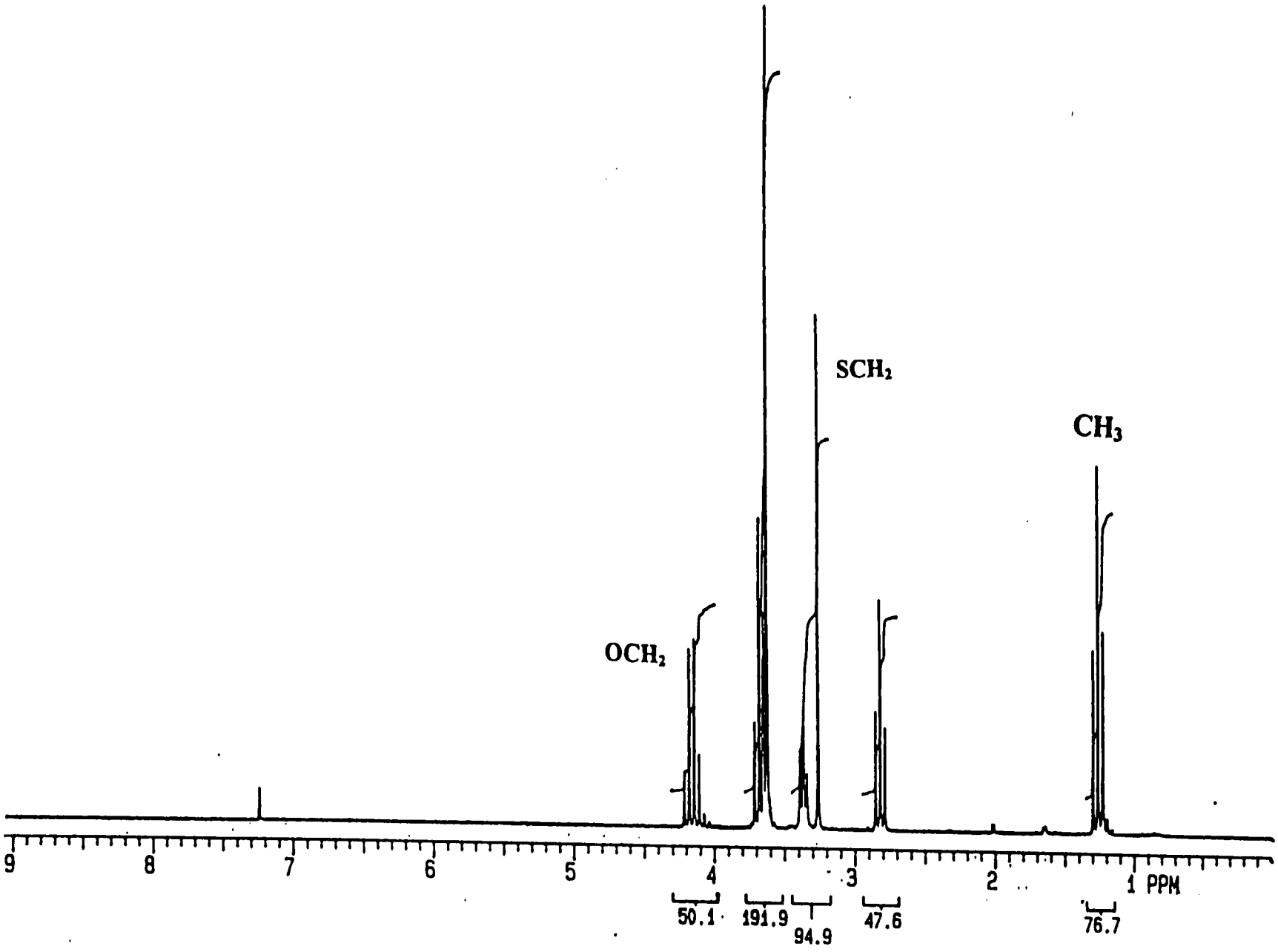

Figure 3.1.2. ^{1}H-NMR ($CDCl_3$, 200 MHz) spectrum of non-carbohydrate thioether **125**.

3.2 Chemoselective Deprotection of Thioacetates

Chemoselective low temperature Zemplén de-*S*-acetylation (0.95 equiv NaOMe, MeOH, -40°C, 15 min) was successful only with the more electrophilic anomeric *S*-acetates **104**, **107** and **113** or when there was no chemoselectivity involved such as in **123**.[203] However, these conditions were inadequate when the thioacetate group on the glycoside was not at the anomeric position. Furthermore, under such conditions extensive oxidation to disulfides was inevitable.

Although hydrazinium acetate (H_2NNH_2-HOAc) has been widely used to accomplish anomeric de-*O*-acetylations,[204] this reagent has not been fully exploited for de-*S*-acetylations. It was found that the treatment of thioacetates with hydrazinium acetate in DMF under nitrogen exhibited complete chemoselectivity not only at the anomeric centers but also at primary thioesters. Furthermore, even after prolonged reaction time at ambient temperature, complete chemoselectivity was still observed (i.e. no detectable de-*O*-acetylation occurred).

Chemoselective de-*S*-acetylation was accomplished using three different procedures or methods (namely Methods A, B, and C). The ultimate purpose for these deprotections is to easily produce thiolated glycosides in high yields for the conjugation onto any multi-branched cores such as dendrimers, polymers and calixarenes. However, since the present chapter deals with the study of de-*S*-acetylation, freshly deprotected thiol derivatives produced from any of the 3 methods were subsequently treated with a wide variety of electrophiles to provide thioethers in good to excellent overall yields (Table 3.2.1). The results from the thioethers formation allowed us to determine the most suitable method that would give the highest yields when applied to multivalent structures.

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

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

Table 3.2.1. Deprotection of thioacetates and thioethers formation.

Entry	Thioacetate	Electrophiles	Thioether	Method A[a]	Method B[b]	Method C[c]
1	**123**	$BrCH_2CO_2Et$	**125**	85%	—	81%
2	**104**	$BrCH_2Ph$	**127**	77%	71%	—
3	**104**	CH_2=$CHCO_2Me$	**128**	78%	—	75%
4	**104**	$Br(CH_2)_5CO_2Me$	**130**	82%	—	84%
5	**107**	CH_2=$CHCO_2Me$	**132**	—	52%	67%
6	**113**	$BrCH_2CO_2Et$	**134**	83%	—	80%
7	**113**	$BrCH_2Ph$-*p*-NO_2	**135**	74%	—	—
8	**113**	$ClCH_2CO$-GlyGlyOH	**136**	91%	—	—

[a] One-pot hydrazinium acetate (H_2NNH_2•AcOH)
[b] Two-pot hydrazinium acetate
[c] Sodium methoxide

Three procedures are presented herein for the deprotection-thioether formation. These procedures are depicted in Scheme 3.2.1. The first two procedures involve hydrazinium acetate, one of which describes a single-pot two step reaction, whereas the third method involves thioacetate deprotection using modified low temperature Zemplén conditions.

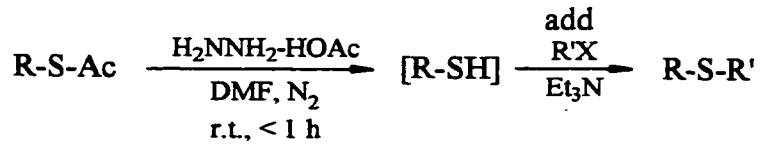
Method A refers to a single-pot two step hydrazinium acetate method and involves the treatment of any thioacetate derivatives with hydrazinium acetate (1 equiv) in DMF under nitrogen (22°C, quantitative). After a period of ~1 h, an electrophile (1.5 equiv) and triethylamine (1 equiv) are added into the initial mixture and the reaction is stirred (N_2, 22°C) until the transformation (<3 h) into a thioether is complete.

Method B, on the other hand, refers to a two-pot two step hydrazinium acetate method. The difference with method A lies in the fact that three times the amount of hydrazinium acetate (3 equiv) is added, resulting in a shorter reaction time (<30 min).

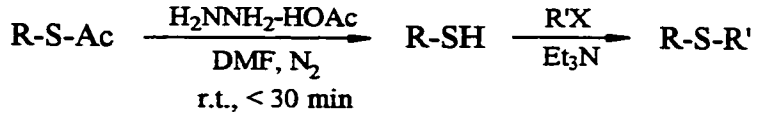
Also in method B, the intermediate thiol derivative is isolated (aqueous work-up) and reacted with an electrophile (1.5 equiv) and a base (Et_3N, 1 equiv) in EtOAc (N_2, 22°C).

Method C corresponds to mild trans-esterification low temperature Zemplén conditions (0.95 equiv NaOMe, MeOH, -40°C, 15 min) which was slightly modified[115] from the published procedure.[200] The modification involves a low temperature treatment of the reaction mixture with H^+ resin (-40°C, 15 min). The resin is then filtered off and the resulting filtrate evaporated near dryness at room temperature under reduced pressure. The crude thiol residue is then dissolved in CH_2Cl_2 or MeCN, and quenched with an electrophile (1.5 equiv) and Et_3N (1 equiv, 22°C, N_2).

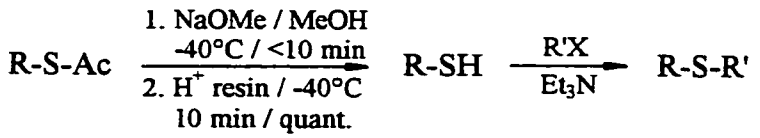
Method A: Single-pot two-step hydrazinium acetate method



Method B: Two-pot two-step hydrazinium acetate method



Method C: Chemoselective low temperature Zemplén de- *S*-acetylation method



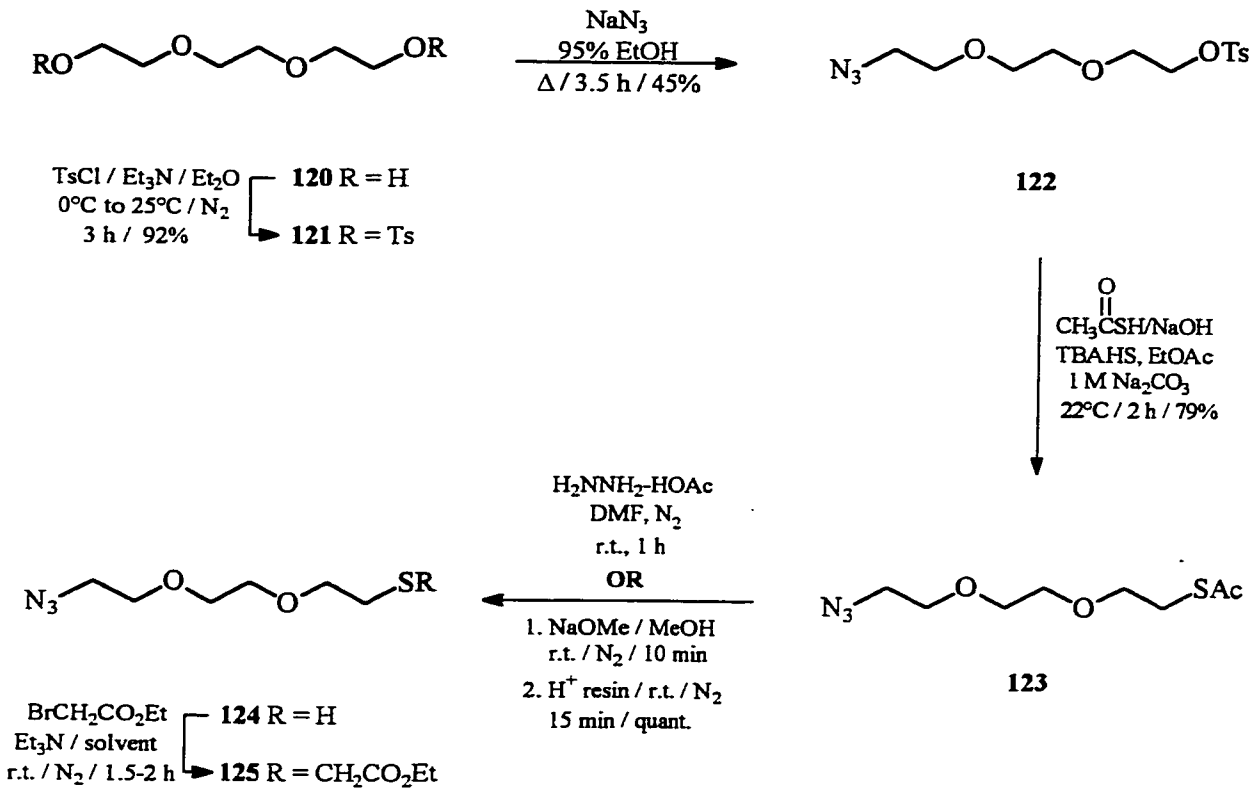
Scheme 3.2.1. Different methods for chemoselective deprotection of thioacetates.

The results from the two-step deprotection-thioether formations are summarized in Table 3.2.1. It can be seen that Methods A and C give good and similar results whereas Method B is less efficient. The lower yields obtained, may be the result of the greater number of manipulation steps involved in this method (two work-ups instead of one). As mentioned previously, Method C (NaOMe) is not always adequate. This situation arises when the thioacetate on the glycoside is non-anomeric. Moreover, if this thiolated derivative is needed for multi-branched structure conjugation, then Method B would be useful since it requires an excess of that glycosyl derivative.

3.3 Thioacetate Deprotection-Thioether Formation

De-*S*-acetylation of thioacetate was performed on non-carbohydrate thioacetate derivatives **123**, galactosyl **104**, glucosyl **107** and sialosyl thioacetate **113**. The non-carbohydrate thioacetate derivative **123** was synthesized from commercially available triethylene glycol **120**. Diol **120** was ditosylated in 92% yield using *p*-toluenesulfonyl chloride (TsCl, triethylamine, Et_2O, reflux). Bistosylated derivative **121** was then treated with one equivalent of sodium azide (95% EtOH, reflux, 4 h) to afford azidotosylate **122** in moderate yield (45 %) (Scheme 3.3.1). Azidotosylate **122** was reacted at room temperature in a two phase system using pre-neutralized (NaOH) thioacetic acid, TBAHS as catalyst in EtOAc and 1 M Na_2CO_3. The reaction afforded thioacetate **123** in 79% yield. Hydrolysis of the thioacetate group in **123** was achieved following either the one-pot hydrazinium acetate method (Method A) or the sodium methoxide method (Method C). The NaOMe conditions applied to non-carbohydrate thioacetate **123** were slightly different than the general procedure since no *O*-acetates were present on the molecule. The reaction was not performed at low temperature. Furthermore, reaction under nitrogen was essential due to the primary thioacetate, that make the oxidation to dissulfide an easy process. Intermediate thiol **124** was treated with ethyl bromoacetate as

electrophile to afford thioether **125** in 85% (A)[203] and 81% (C) (Scheme 3.3.1). A small amount of disulfides could be detected from the TLC.



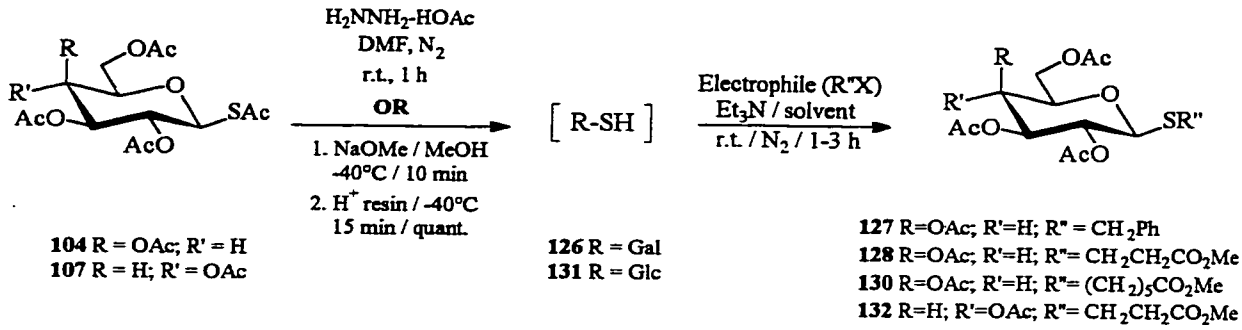
Scheme 3.3.1. Synthesis of non-carbohydrate thioethers.

Galactosyl thioacetate **104** was chemoselectively de-*S*-acetylated using the three different methods. The two hydrazinium acetate methods (A and B) were first used on thioacetate **104** to furnish thiol **126** that was then reacted with benzyl bromide (electrophile) to afford thioether **127** in 77% from Method A and 71% yield following Method B (Scheme 3.3.2).

Thiogalactoside **126** was obtained from hydrolysis of the thioacetate group in **104** following the one-pot hydrazinium acetate method (A) and/or the NaOMe low temperature method (C). Thiogalactoside **126** was quenched with specific electrophiles;

methyl acrylate as quenching electrophile afforded thioether **128** in 78% or 75% yields following Method A or C, respectively; when the quenching electrophile was methyl bromohexanoate **129**, thioether **130** could be obtained in 82% (A) or 84% yields (C).

Methyl acrylate was used as the electrophile in the synthesis of thioether **132**, that was obtained in 52% yield when Method B (two-pot hydrazinium acetate) was employed to de-*S*-acetylate glucosyl thioacetate **107**. A yield of 67% for the preparation of thioether **132** was achieved following the NaOMe method (C) in the production of thiol **131** (Scheme 3.3.2). A side product was isolated in 13% yield which corresponded to the disulfide.



Scheme 3.3.2. Synthesis of thioether gluco- and galactosides.

The thiogluco- and thiogalacto- sides synthesized were fully characterized and the β anomeric configuration of each was conserved during the deprotection-thioether formation reaction steps. The configuration was easily proven from the ^{1}H NMR spectrum of thioglycosides. The large coupling constant between H-1 (anomeric proton) and H-2 ($J_{1,2}$ = 10.0 Hz) corresponded to the β anomer (Figure 3.3.1). Figures 3.3.2 and 3.3.3 illustrate, respectively, a COSY and a HMQC spectrum of thiogalactoside **127**. The two spectra are shown to exemplify the tools used to correctly characterize each proton and carbon peak on newly synthesized molecules.

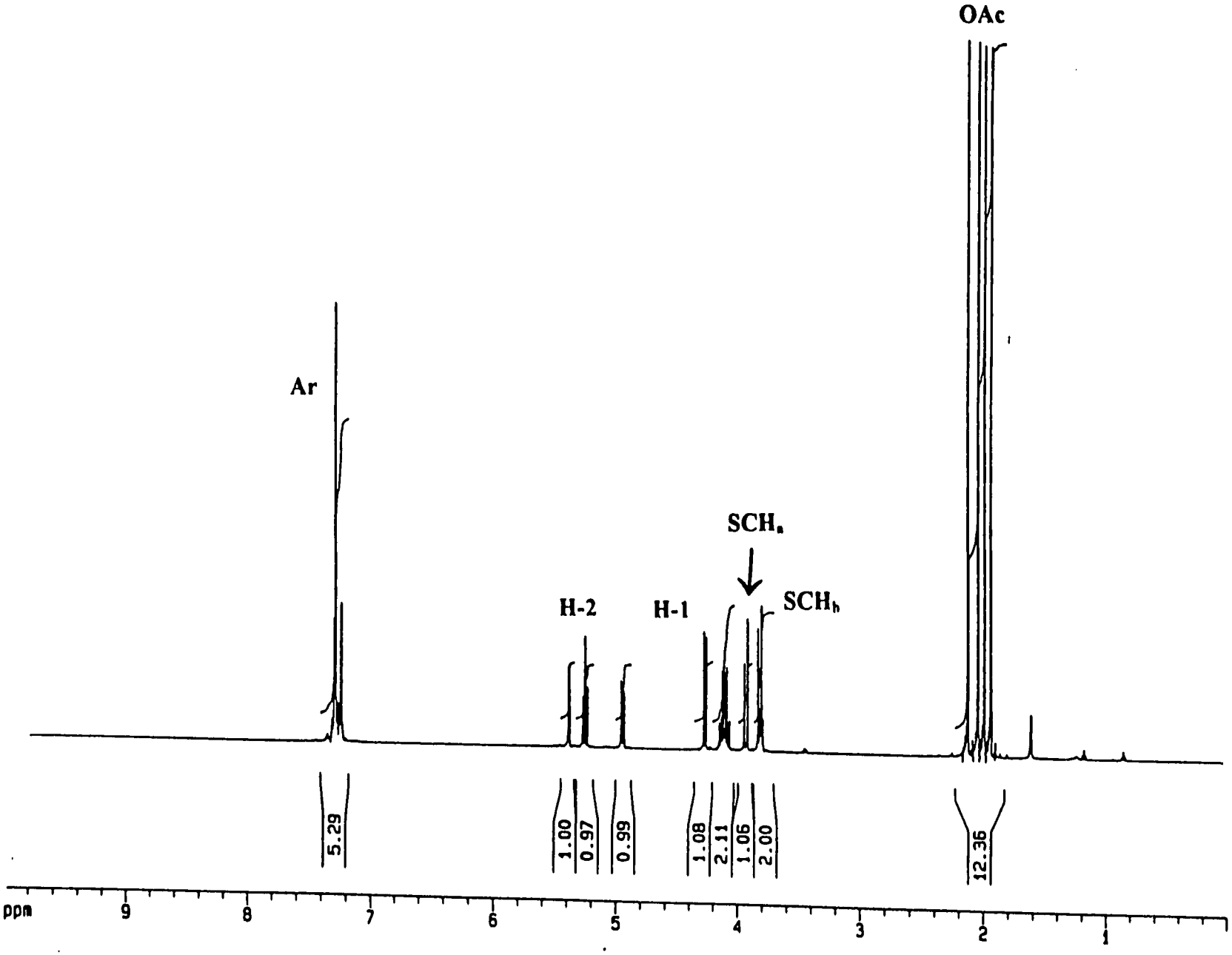


Figure 3.3.1. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of thiobenzyl galactoside **127**.

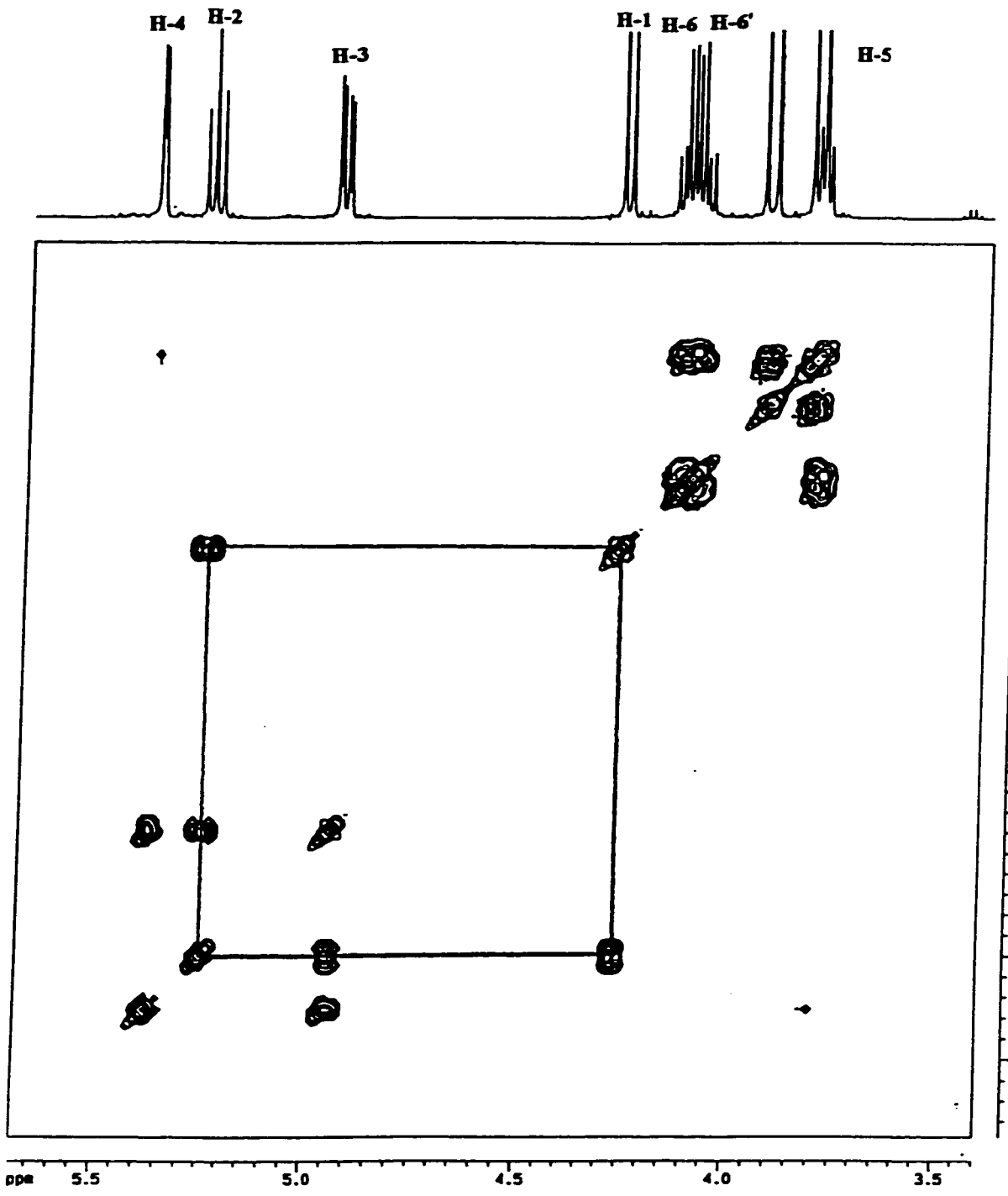


Figure 3.3.2: ^{1}H-NMR (CDCl$_3$, 500 MHz) COSY spectrum of thiobenzyl galactoside **127**.

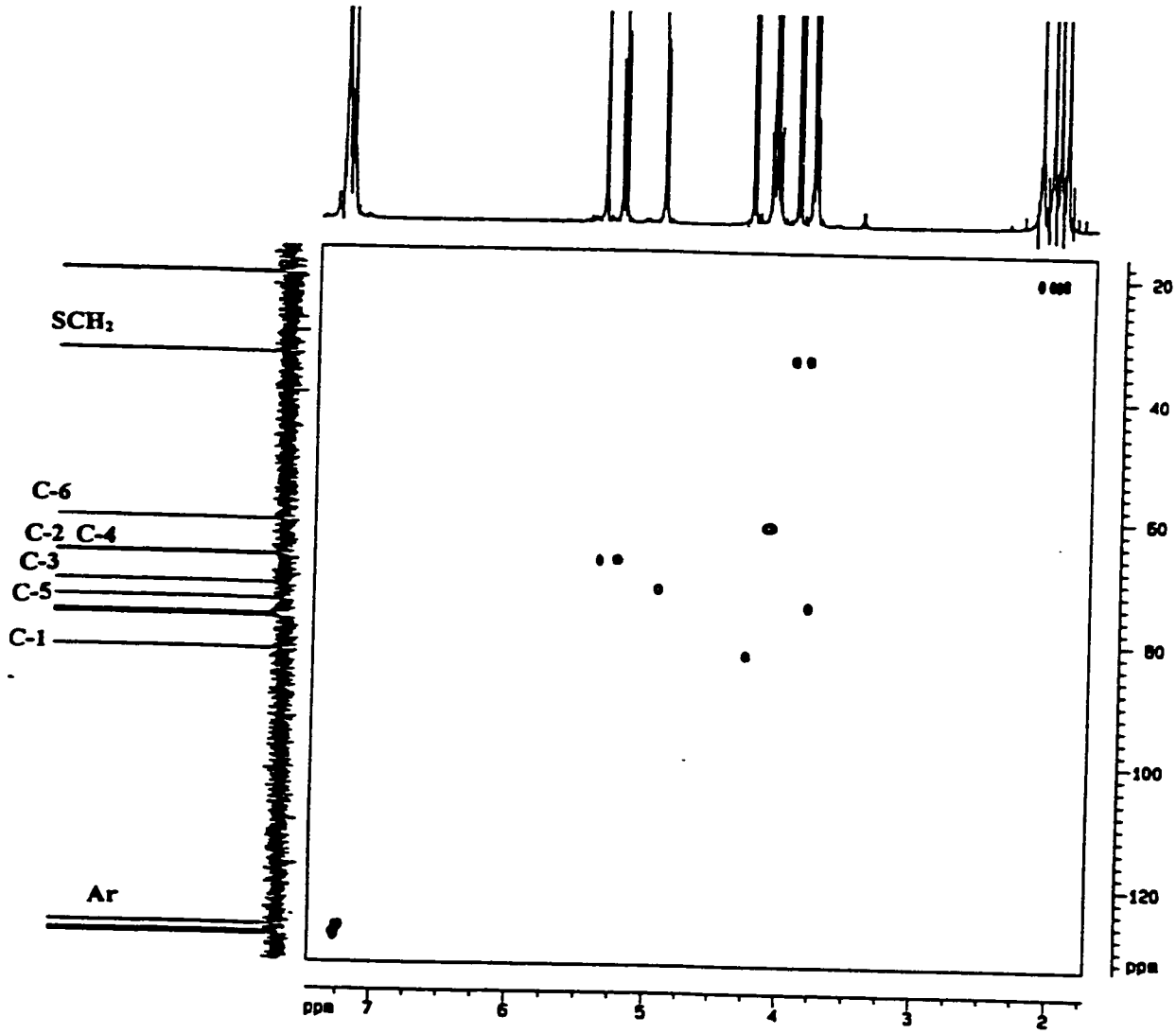
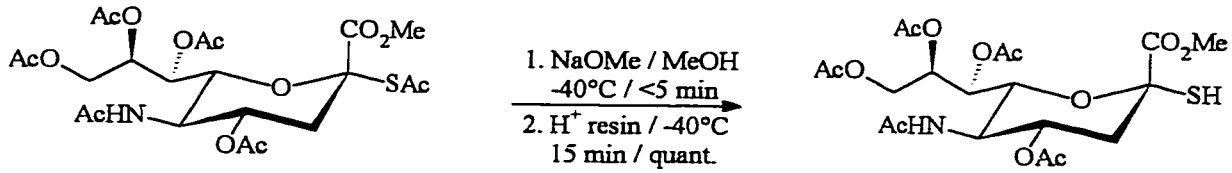


Figure 3.3.3. ^{1}H- and ^{13}C-NMR ($CDCl_3$, 500 MHz) HMQC spectrum of thiobenzyl galactoside **127**.

Thioacetyl sialoside **113** reacted in the same way as the other thioacetates under these conditions. The thioacetate group at the anomeric position of sialic acid could also be hydrolyzed chemoselectively using either method A or C. Under the sodium methoxide low temperature conditions (0.95 equiv NaOMe, MeOH, -40°C) followed by H^+ resin treatment (-40°C), thioacetate **113** furnished thiol **133** in quantitative yield (Scheme 3.3.3). Thiosialoside **133** was characterized by optical rotation, ^1H- and ^{13}C-NMR spectroscopy ($CDCl_3$), and by mass spectrometry.



113 **133**

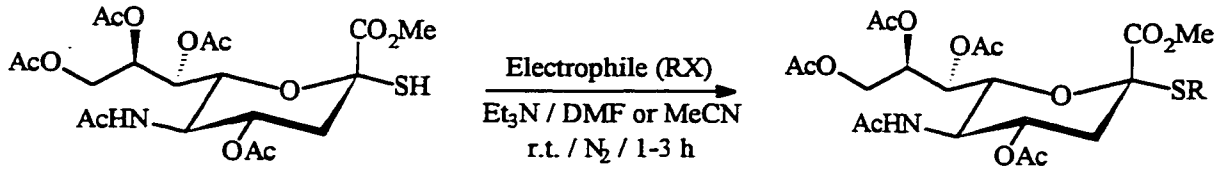
Scheme 3.3.3. Synthesis of thiolated sialoside **133**.

The NaOMe low temperature conditions were found to be best suited for the preparation of thiol **133** when added onto multi-branched structures such as dendrimers and calixarenes (see following chapters).

Thiosialoside **133** obtained from Method A and/or Method C was quenched with either ethyl bromoacetate or 4-nitrobenzyl bromide as electrophile to afford thioether **134** in 83% (A) or 80% yields (C) or thioether **135** in 74% yield subsequent to deprotection Method A (Scheme 3.3.4). The conditions applied for the hydrolysis of thioacetate groups and the quenching with electrophiles were the same as described above for procedures A and C.

Thioether **136** was prepared from thioacetyl sialoside **113** subsequent to de-*S*-acetylation using the one-pot hydrazinium acetate method (A) and quenching with *N*-chloroacetylated glycylglycine. The latter reaction was executed differently than the previous deprotection-thioether formations (see above) in the fact that the electrophile

was added as the limiting reagent (thiol in excess). It allowed for an easier purification procedure due to the high polarity of thioether **136**. The reaction mixture was rendered basic and treated with AcO^- resin. The resin having the thioether bonded to it, was filtered to remove excess sugars and reagents. Treatment of the resin with aqueous acetic acid solution (0.5 M) released the thioether in the aqueous solution which was then lyophilized to provide pure thioether **136** as a white foam.



133 Obtained from Method A and/or C

134 R = CH_2CO_2Et
135 R = CH_2Ph-*p*-NO_2
136 R = CH_2CO-GlyGlyOH

Scheme 3.3.4. Synthesis of thioether sialosides.

The anomeric configuration of thiosialosides **134-136** was determined by ^1H NMR spectroscopy (see Chapter 2.2). It was demonstrated that the α configuration was also conserved during the reaction steps described above. The H-3e signal was at 2.64-2.72 ppm, the H-4 signal was upfield (4.70-4.84 ppm), and the large constant coupling between H-7 and H-8 ($J_{7,8}$ = 8.3-9.3 Hz) all suggested that the anomeric configuration was α ($J_{7,8}$ of β-sialosides ~2.4 ± 0.3 Hz) (Figure 3.1.1).[190-192] The high positive optical rotation values (+29.7°, +36.5°, +53.6°) of the α-thiosialosides relative to the β-anomers provided additional proof of the α configuration.[184,193]

3.4 Conclusion

Suitable methods for the chemoselective deprotection of thioacetates were demonstrated. The de-*S*-acetylation proceeded in high yields on anomeric thioacetate glycosides as well as on non-carbohydrate thioacetate derivatives as illustrated by the high yields of the two steps de-*S*-acetylation-thioether formation.

The one-pot hydrazinium acetate method (A) and the low temperature Zemplén (NaOMe) method (C) provided highly efficient methods in the preparation of thiol glycosides. These thiols are an important part of the conjugation strategy proposed in the syntheses of glycoconjugates. The strategy involves the S_N2 displacement of the chlorine atom in preformed *N*-chloroacetylated multi-branched structures by thiol glycosides.

3.5 Experimental Methods

The three (3) methods used for the chemoselective deprotection of the thioacetates are:

Method A: One-pot hydrazinium acetate (H_2NNH_2•AcOH) method.

Method B: Two-pot hydrazinium acetate (H_2NNH_2•AcOH) method.

Method C: Sodium methoxide method.

1,8-Bis-[(p-toluenesulfonyl)oxy]-3,6-dioxaoctane (121)

A solution of triethylene glycol **120** (5.0 g, 33 mmol) in anhydrous diethyl ether (30 mL) was cooled to 0°C under a nitrogen atmosphere. Triethylamine (5 mL) was added followed by *p*-toluenesulfonyl chloride (6.88 g, 36 mmol) over a 1 h period, and the solution was warmed to room temperature. After stirring for 3 h, the reaction mixture was evaporated near dryness under vacuum. The resulting residue was dissolved in CH_2Cl_2 and washed successively with saturated sodium bicarbonate solution (10 mL) and twice with water (10 mL). The organic layer was dried using anhydrous sodium sulfate

and concentrated under vacuum to afford 12.6 g (83%) of **121** as a white solid. After filtration, the remaining crude product was purified by silica gel column chromatography using ethyl acetate/hexanes (1:1, v/v) as eluent to afford 1.2 g (8%) of **121** (total yield 92%): mp 82.2-84°C (EtOH); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.75 (d, 4H, J 8.3 Hz, H_{ar}), 7.31 (d, 4H, J 8.3 Hz, H_{ar}), 4.11 (t, 4H, J 4.8 Hz, TsO*CH*$_2$), 3.62 (t, 4H, J 4.8 Hz, TsOCH$_2$*CH*$_2$), 3.49 (s, 4H, OCH_2), 2.41 (s, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 144.8, 132.8, 129.8, 127.9 (C_{ar}), 70.6 (TsO*CH*$_2$), 69.2 (CH_2O), 68.6 (CH_2O), 21.6 (CH_3); MS (CI ether, rel. intensity) m/z: 459 ([M+H]$^+$, 36%), 287 (74).

1-Azido-8-[(p-toluenesulfonyl)oxy]-3,6-dioxaoctane (122)

To a solution of 250 mg (0.545 mmol) of bistosylate **121** in 95% ethanol (5 mL) was added sodium azide (17.7 mg, 0.272 mmol). The reaction was stirred under reflux for 3.5 h and monitored by TLC. After cooling the reaction mixture to room temperature and removing the precipitated solid, the filtrate was evaporated under reduced pressure. The oily residue was dissolved in diethyl ether and washed twice with water and brine. The dried organic phase (Na_2SO_4) was concentrated and the crude product purified by silica gel chromatography using a gradient of 1:5 to 1:1 (v/v) ethyl acetate/hexanes as eluent to afford 80.9 mg (45%) of **122** as a colorless oil: IR ν_{max} (cm^{-1}): 3015, 2904, 2108, 1738, 1598; ^{1}H NMR ($CDCl_3$) δ (ppm): 7.75 (d, 2H, J 8.3 Hz, H_{ar}), 7.32 (d, 2H, J 8.3 Hz, H_{ar}), 4.12 (t, 2H, J 4.7 Hz, TsO*CH*$_2$), 3.68-3.56 (m, 8H, OCH_2), 3.33 (t, 2H, CH_2N_3), 2.41 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 144.6, 132.6, 129.6, 127.6 (6C_{ar}), 70.4, 70.2, 69.7, 69.1, 68.4 (CH_2O), 50.3 (CH_2N_3), 21.3 (CH_3); MS (CI ether, rel. intensity) m/z: 330 ([M+H]$^+$, 3%), 302 ([M-N_2+H]$^+$, 61).

Anal. Calcd. for $C_{13}H_{19}N_3O_5S$: C 47.40, H 5.81, N 12.76; found: C 47.23, H 6.00, N 12.96.

1-S-Acetyl-1-thio-8-azido-3,6-dioxaoctane (123)

To a solution of mono-tosylated **122** (500 mg, 1.52 mmol) and tetrabutylammonium hydrogen sulfate (515 mg, 1.52 mmol) in ethyl acetate (4 mL) was added thioacetic acid (217 μL, 3.04 mmol, 2 equiv) pre-neutralized with 1M NaOH. 1M Na_2CO_3 (4 mL) was then added and the mixture was vigorously stirred at room temperature for 2 h. The reaction was monitored by TLC (R_f 0.57, EtOAc:hexanes, 1:2) which showed complete transformation of the starting material. The reaction mixture was then diluted with 30 mL each of ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was separated and washed with saturated aqueous $NaHCO_3$ (2 x 35 mL), H_2O (3 x 30 mL) followed by saturated sodium chloride (20 mL). The dried organic layer (Na_2SO_4) was concentrated near dryness under reduced pressure, and purified by silica gel column chromatography using a gradient from 1/6 to 1/3 EtOAc:hexanes as eluent. Title compound **123** was isolated as a pure oil in 79% yield (280 mg, 1.2 mmol): IR ν_{max} (cm^{-1}): 2109, 1688; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.56-3.68 (m, 8H, OCH_2), 3.37 (t, 2H, J_{vic} = 5.0 Hz, CH_2N_3), 3.07 (t, 2H, J_{vic} = 4.7, SCH_2), 2.31 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 195.2 (C=O), 70.3, 70.1, 69.8, 69.5 (CH_2O), 50.4 (CH_2N_3), 30.3 (CH_3), 28.5 (SCH_2); MS (CI ether, rel. intensity) m/z: 234 ($[M+H]^+$, 100%), 206 ($[M-N_2+H]^+$, 62.5), 190 ($[M-Ac+H]^+$, 2.1).

Anal. Calcd. for $C_8H_{15}N_3O_3S$: C 41.19, H 6.48, N 18.01; found: C 41.36, H 6.39, N 17.83.

1-S-(Ethyl)acetyl-1-thio-8-azido-3,6-dioxaoctane (125)

Method A: To a solution of thoroughly degassed (N_2) thioacetate **123** (90 mg, 0.386 mmol) in dry DMF (250 μL) was added 2.5 M (DMF) hydrazinium acetate (154 μL, 0.386 mmol). The stock solution of hydrazinium acetate was most efficiently degassed by repeated freeze-thaw cycles. The reaction was monitored by TLC (R_f 0.30,

EtOAc/hexanes + 0.5% *i*-prOH), 1/3) and after stirring for 1 h at room temperature and under a N_2 atmosphere, ethyl bromoacetate (85.6 μL, 0.772 mmol) and triethylamine (53.7 μL, 0.386 mmol) were added. The reaction mixture was stirred at room temperature until TLC showed complete transformation (1.5 h) after which time EtOAc (8 mL) and water (7 mL) were added. The resulting organic phase was washed once with water (7 mL) followed by sat. NaCl (6 mL) then dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by silica gel column chromatography using EtOAc/hexanes (1:4 v/v) as eluent. Compound **125** was obtained as a colorless oil in 85% yield (90.9 mg, 0.328 mmol).

Method C: To a solution of thioacetate **123** (68 mg, 0.294 mmol) in dry methanol (1.5 mL) at room temperature under a N_2 atmosphere, was added 1 M sodium methoxide in methanol (294 μL, 0.294 mmol). The reaction, monitored by TLC, took less than 10 min after which time the reaction mixture was treated with H^+ resin (Amberlite IR-120) for 15 min. The solution was filtered and evaporated under reduced pressure to give compound **124** as an oil in quantitative yield. Thio-derivative **124** was dissolved, without further purification, in CH_2Cl_2 (3 mL) under nitrogen and ethyl bromoacetate (65.2 μL, 0.588 mmol) and triethylamine (61.4 μL, 0.441 mmol) were added to the reaction mixture. The reaction mixture was stirred at room temperature until TLC showed complete transformation (<2 h) after which time CH_2Cl_2 (7 mL) and water (7 mL) were added. The resulting organic phase was washed twice with water (7 mL) followed by sat. NaCl (5 mL) then dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by silica gel column chromatography using EtOAc/hexanes (1:4 v/v) as eluent. Compound **125** was obtained as a colorless oil in 81% yield (66 mg, 0.238 mmol).

Compound **125** has ^{1}H-NMR ($CDCl_3$) δ (ppm): 4.16 (q, J_{vic} = 7.1 Hz, 2H, O*CH*$_2$CH$_3$), 3.61-3.71 (m, 8H, H-2, H-4, H-5, & H-7), 3.36 (t, 2H, CH_2N_3 or H-8), 3.26 (s, 2H, CH_2CO), 2.82 (t, $J_{1,2}$ = 6.5, 2H, H-1), 1.26 (t, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ (ppm): 170.4 (C=O), 70.6, 70.6, 70.3, 70.0 (C-2, C-4, C-5, & C-7), 61.3 (O*CH*$_2$CH$_3$),

50.6 (C-8), 34.0 (CH_2CO), 31.9 (C-1), 14.1 (CH_3); M.S. for $C_{10}H_{19}N_3O_4S$ (CI ether, rel. intensity) m/z: 277.9 ($[M+H]^+$, 1.1%), 249.8 ($[M+H-N_2]^+$, 6.3%), 190.9 ($[M+H-CO_2Et]^+$, 2.2%).

Anal. Calcd for $C_{10}H_{19}N_3O_4S$: C, 43.31; H, 6.91; N, 15.15; S, 11.56. Found: C, 43.47; H, 6.70; N, 14.94; S, 11.57.

2,3,4,6-Tetra-O-acetyl-1-S-benzyl-1-thio-β-D-galactopyranose (127)

Method A: To a solution of thoroughly degassed (N_2) thioacetate **104** (111.5 mg, 0.274 mmol) in dry DMF (250 μL) was added 2.5 M (DMF) hydrazinium acetate (230 μL, 0.576 mmol). The reaction was monitored by TLC (R_f 0.60, EtOAc/hexanes, 1/1) and after stirring for 1 h at room temperature and under a N_2 atmosphere, benzyl bromide (65.3 μL, 0.549 mmol) and triethylamine (40 μL, 0.287 mmol) were added. The reaction mixture was stirred at room temperature until TLC showed complete transformation (2 h) after which time EtOAc (10 mL) and water (10 mL) were added. The resulting organic phase was washed once with water (10 mL) followed by sat. NaCl (7 mL) then dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by silica gel column chromatography using EtOAc/hexanes (1:1 v/v) as eluent. Compound **127** was obtained as a white solid in 77% yield (96 mg, 0.211 mmol).

Method B: To a solution of thioacetate **104** (100 mg, 0.246 mmol) dissolved in degassed DMF (1 mL) was added 2.5 M hydrazinium acetate in DMF (295 μL, 0.738 mmol). The progress of the reaction was monitored by TLC. The reaction was complete after 30 min. The reaction mixture was diluted with EtOAc (15 mL) and successively washed with 1M HCl (5 mL), sat. $NaHCO_3$ (5 mL), water and sat. NaCl (5 mL). The dried organic phase (Na_2SO_4) was concentrated under reduced pressure. The residue was dissolved in EtOAc (0.5 mL) to which was added the electrophile, benzyl bromide (35 μL, 0.295 mmol) and Et_3N (34 μL, 0.246 mmol). The reaction was completed within 2.5 h.

After evaporation of the reaction mixture and usual washings, the residue was purified by silica gel column chromatography using EtOAc/hexanes 1:1, v/v as eluent. Compound **127** was obtained as a white solid in 71% overall yield (79.4 mg, 0.174 mmol).

Title compound **127** has mp (Ether/Hexanes) 95.5-96.5°C; $[\alpha]_D$ -80.4° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.25-7.31 (m, 5H, H_{ar}), 5.38 (m, 1H, H-4), 5.25 (dd, 1H, H-2), 4.95 (dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.4, 1H, H-3), 4.26 (d, $J_{1,2}$ = 10.0, 1H, H-1), 4.15-4.06 (m, 2H, H-6 & H-6'), 3.93 (d, J_{vic} = 13.0, 1H, SCH-a), 3.81 (d, J_{vic} = 13.0, 1H, SCH-b), 3.81 (m, 1H, H-5), 2.13, 2.04, 1.99, 1.94 (4s, 12H, 4OAc); ^{13}C-NMR ($CDCl_3$) δ (ppm): 170.3, 170.2, 170.0, 169.6 (4C=O), 136.9 (C_i), 129.1, 128.6 (C_o and C_m), 127.4 (C_p), 82.5 (C-1), 74.4 (C-5), 71.8 (C-3), 67.3 (C-4), 67.1 (C-2), 61.6 (C-6), 33.8 (SCH_2), 20.7, 20.7, 20.6, 20.5 (4OAc); M.S. for $C_{21}H_{26}O_9S$ (CI ether, rel. intensity) m/z: 454.9 ($[M+H]^+$, 0.6%), 394.9 ($[M-OAc]^+$, 16.1%), 330.8 ([M-aglycone]$^+$, 100%).
Anal. Calcd for $C_{21}H_{26}O_9S$: C, 55.50; H, 5.77. Found: C, 55.61; H,5.72.

2,3,4,6-Tetra-O-acetyl-1-S-methylpropionyl-1-thio-β-D-galactopyranose (128)

Method A: To a solution of thoroughly degassed (N_2) thioacetate **104** (100 mg, 0.246 mmol) in dry DMF (250 μL) was added 2.5 M (DMF) hydrazinium acetate (98.4 μL, 0.246 mmol). The reaction was monitored by TLC and after stirring for 1.5 h at room temperature and under a N_2 atmosphere, methyl acrylate (45 μL, 0.492 mmol) and triethylamine (17 μL, 0.123 mmol) were added. The reaction mixture was stirred at room temperature until TLC (R_f 0.39, EtOAc/hexanes, 1/1) showed complete transformation (2 h) after which time EtOAc (8 mL) and water (8 mL) were added. The usual washings were performed and the resulting organic layer was dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by silica gel column chromatography using a gradient EtOAc/hexanes from (1:1.8 to 2:3, v/v) as eluent. Compound **128** was obtained in 78% yield (86.5 mg, 0.192 mmol).

Method C: To a solution of thioacetate **104** (100 mg, 0.246 mmol) in dry methanol (2.5 mL), cooled to –40°C, was added 1 M sodium methoxide in methanol (234 μL, 0.234 mmol). The reaction, monitored by TLC, took less than 10 min at -40°C after which time the reaction mixture was treated with H^+ resin (Amberlite IR-120) still at -40°C for 15 min. The solution was filtered and evaporated at room temperature under reduced pressure to give compound **126** as an oil in quantitative yield. Thio-derivative **126** was dissolved, without further purification, in acetonitrile (5 mL) under nitrogen and methyl acrylate (45 μL, 0.492 mmol) and triethylamine (34 μL, 0.246 mmol) were added to the reaction mixture. The reaction mixture was stirred at room temperature until TLC showed complete transformation (<2 h) of the starting material. The usual washings were performed and the resulting organic layer was dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by silica gel column chromatography using a gradient EtOAc/hexanes from (1:1.8 to 2:3, v/v) as eluent. Compound **128** was obtained in 75% yield (82.9 mg, 0.184 mmol).

Title compound **128** has $[\alpha]_D$ -8.7° (*c* 1.63, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.40 (dd, 1H, H-4), 5.20 (t, $J_{2,3}$ = 9.9 Hz, 1H, H-2), 5.01 (dd, $J_{3,4}$ = 3.1, 1H, H-3), 4.50 (d, $J_{1,2}$ = 9.9, 1H, H-1), 4.06-4.11 (m, 2H, H-6 & H-6'), 3.89 (m, 1H, H-5), 3.67 (s, 3H, OMe), 2.79-3.05 (m, 2H, SCH_2), 2.65 (t, J_{vic} = 7.6, 2H, CH_2CO), 2.13, 2.04, 2.02, 1.96 (4s, 12H, 4OAc); ^{13}C-NMR ($CDCl_3$) δ (ppm): 172.0 (CO_2Me), 170.3, 170.1, 170.0, 169.5 (4C=O), 84.5 (C-1), 74.4 (C-5), 71.8 (C-3), 67.2 (C-4), 67.0 (C-2), 61.4 (C-6), 51.8 (OMe), 35.2 (CH_2CO), 25.5 (SCH_2), 20.7, 20.6, 20.6, 20.5 (4OAc); M.S. for $C_{18}H_{26}O_{11}S$ (CI ether, rel. intensity) m/z: 390.7 ([M-CO_2Me]$^+$, 4.8%), 331.9 ([M+H-aglycone]$^+$, 23.6%), 330.8 ([M-aglycone]$^+$, 100%).

Anal. Calcd for $C_{18}H_{26}O_{11}S$: C, 48.00; H, 5.82; S, 7.12. Found: C, 47.78; H, 5.98; S, 6.99.

Methyl 6-bromohexanoate (129)

To a solution of thionyl chloride (1 mL, 13.8 mmol) and methanol (20 mL) at -10°C was added 2 g of 6-bromohexanoic acid (10.2 mmol). The reaction mixture was then stirred at room temperature for 2 h where it was monitored by TLC (R_f 0.95, EtOAc/hexanes, 1/1). The reaction solvent was evaporated near dryness and co-evaporated a few times using methanol and then put on a vacuum pump for 36 h. Pure methyl ester was obtained in 97% yield (2.07 g). Title compound **129** has ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.64 (s, 3H, OMe), 3.38 (t, 2H, J_{vic} = 6.7 Hz, CH_2Br), 2.30 (t, 2H, J_{vic} = 7.2, CH_2CO), 1.39-1.92 (3m, 6H, internal CH_2's); ^{13}C-NMR ($CDCl_3$) δ (ppm): 173.8 (C=O), 51.4 (OMe), 33.7, 33.3, 32.3, 27.5, 23.9 ($5CH_2$'s); MS (CI ether, rel. intensity) m/z: 211 ($[M(^{79}Br)+H]^+$, 100%), 209 ($[M(^{81}Br)+H]^+$, 100%), 179 ($[M(^{79}Br)-OMe+H]^+$, 8.6%), 177 ($[M(^{81}Br)-OMe+H]^+$, 9.3%), 129 ($[M-Br+H]^+$, 46.5).

2,3,4,6-Tetra-O-acetyl-1-S-methylhexanoyl-1-thio-β-D-galactopyranose (130)

Method A: To a solution of thoroughly degassed (N_2) thioacetate **104** (100 mg, 0.246 mmol) in dry DMF (250 μL) was added 2.5 M (DMF) hydrazinium acetate (98.4 μL, 0.246 mmol). The reaction was monitored by TLC (R_f 0.48, EtOAc/hexanes, 1/1) and after stirring for 45 min at room temperature and under a N_2 atmosphere, methyl 6-bromohexanoate (62 μL, 0.369 mmol) and triethylamine (34.2 μL, 0.246 mmol) were added. The reaction mixture was stirred at room temperature until TLC showed complete transformation (<2h) after which time EtOAc (8 mL) and water were added. The resulting organic phase was washed once with water followed by sat. NaCl (6 mL) then dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by silica gel column chromatography using EtOAc/hexanes (2:3 v/v) as eluent. Title compound **130** was obtained as a colorless oil in 82% yield (99.5 mg, 0.201 mmol).

Method C: To a solution of thioacetate **104** (100 mg, 0.246 mmol) in dry methanol (2.5 mL), cooled to –40°C, was added 1 M sodium methoxide in methanol (234 μL, 0.234 mmol). The reaction, monitored by TLC, was stirred at -40°C for 10 min after which time the reaction mixture was treated as described above for method C. Thio-derivative **126** was dissolved in acetonitrile (5 mL) under nitrogen and methyl acrylate (45 μL, 0.492 mmol) and triethylamine (34 μL, 0.246 mmol) were added to the reaction mixture. The reaction mixture was stirred at room temperature until TLC showed complete transformation (<2 h) of the starting material. The usual washings were performed and the resulting organic layer was dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by silica gel column chromatography using EtOAc/hexanes (2:3, v/v) as eluent. Compound **130** was obtained as a syrup in 84% yield (101.6 mg, 0.206 mmol).

Compound **130** has $[\alpha]_D$ -12.4° (*c* 1.035, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.40 (dd, $J_{4,5}$ = 1.0 Hz, 1H, H-4), 5.20 (t, $J_{2,3}$ = 9.9, 1H, H-2), 5.01 (dd, $J_{3,4}$ = 3.3, 1H, H-3), 4.44 (d, $J_{1,2}$ = 9.9, 1H, H-1), 4.02-4.15 (m, 2H, H-6 & H-6'), 3.89 (m, 1H, H-5), 3.63 (s, 3H, OMe), 2.66 (dt, J = 3.5, J = 7.1, 2H, SCH_2), 2.28 (t, J_{vic} = 7.3, 2H, CH_2CO), 2.12, 2.04, 2.01, 1.95 (4s, 12H, 4OAc), 1.53-1.68 (m, 4H, 2 CH_2's), 1.33-1.45 (m, 2H, middle CH_2); ^{13}C-NMR ($CDCl_3$) δ (ppm): 173.8 (CO_2Me), 170.2, 170.0, 169.9, 169.4 (4C=O), 84.0 (C-1), 74.2 (C-5), 71.7 (C-3), 67.2 (C-4), 67.1 (C-2), 61.3 (C-6), 51.4 (OMe), 33.7, 29.7, 29.2, 28.0, 24.3 (aglycone CH_2's), 20.7, 20.5, 20.4 (x2) (4OAc); M.S. for $C_{21}H_{32}O_{11}S$ (CI ether, rel. intensity) m/z: 432.9 ($[M-CO_2Me]^+$; 10.3%), 331.8 ([M+H-aglycone]$^+$, 32.1%), 330.7 ([M-aglycone]$^+$, 100%).

Anal. Calcd for $C_{21}H_{32}O_{11}S$: C, 51.21; H, 6.55; S, 6.51. Found: C, 51.54; H, 6.81; S, 6.40.

2,3,4,6-Tetra-O-acetyl-1-S-methylpropionyl-1-thio-β-D-glucopyranose (132)

Method B: To a solution of thioacetate **107** (150 mg, 0.369 mmol) dissolved in degassed DMF (1 mL) was added 1.3 M hydrazinium acetate in DMF (568 μL, 0.738 mmol). The progress of the reaction was monitored by TLC. The reaction was complete after 45 min. The reaction mixture was diluted with EtOAc (8 mL) and successively washed with 1M HCl (5 mL), sat. $NaHCO_3$ (2 x 5 mL), water (2 x 5 mL)and finally sat. NaCl (5 mL). The dried organic phase (Na_2SO_4) was concentrated under reduced pressure. The residue was dissolved in DMF (1 mL) to which was added the electrophile, methyl acrylate (66 μL, 0.738 mmol) and Et_3N (77 μL, 0.554 mmol). The reaction was completed within 3 h. After evaporation of the reaction mixture and usual washings, the residue was purified by silica gel column chromatography using a gradient EtOAc/hexanes from 1:2 to 2:3, v/v as eluent. Title compound **132** was obtained in 52% yield (86.4 mg, 0.192 mmol).

Method C: To a solution of thioacetate **107** (500 mg, 1.23 mmol) in dry methanol (15 mL), cooled to –40°C, was added 1 M sodium methoxide in methanol (1.17 mL, 1.17 mmol). The reaction, monitored by TLC, took less than 10 min at -40°C after which time the reaction mixture was treated with H^+ resin (Amberlite IR-120) for 15 min at -40°C. The solution was filtered and evaporated at room temperature under reduced pressure to give compound **131** as an oil in quantitative yield. Thio-derivative **131** was dissolved, without further purification, in acetonitrile (15 mL) and DMF (1 mL) under nitrogen and methyl acrylate (144 μL, 1.60 mmol) and triethylamine (171 μL, 1.23 mmol) were added to the reaction mixture. The reaction mixture was stirred at room temperature until TLC showed complete transformation (4 h) of the starting material. The usual washings were performed and the resulting organic layer was dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by silica gel column chromatography using a gradient EtOAc/hexanes from (1:3 to 2:3, v/v) as eluent. Compound **132** was obtained in 67% yield (369 mg, 0.820 mmol). A side product of the reaction was isolated in 13% yield

(118 mg, 0.162 mmol) which corresponded to two molecules of thioglucose linked through a disulfide bond.

Title compound **132** has $[\alpha]_D$ -25.2° (*c* 1.1, $CHCl_3$); R_f 0.44, EtOAc/hexanes, 1/1; ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.19 (t, 1H, $J_{2,3} \sim J_{3,4}$ = 9.2 Hz, H-3), 4.95-5.09 (m, 2H, H-2 & H-4), 4.51 (d, 1H, $J_{1,2}$ = 9.9, H-1), 4.08-4.28 (m, 2H, $J_{5,6'}$ = 4.9 Hz, $J_{6,6'}$ = 12.1, $J_{5,6}$ = 2.4, H-6 & H-6'), 3.83 (ddd, 1H, H-5), 3.68 (s, 3H, OMe), 2.79-3.01 (m, 2H, SCH_2), 2.66 (t, J_{vic} = 7.7, 2H, CH_2CO), 2.07, 2.04, 2.01, 1.99 (4s, 12H, 4OAc); ^{13}C-NMR ($CDCl_3$) δ (ppm): 172.7 (CO_2Me), 171.2, 170.7, 170.0, 169.9 (4C=O), 84.5 (C-1), 76.5 (C-5), 74.3 (C-3), 70.3 (C-2), 68.8 (C-4), 62.7 (C-6), 52.4 (OMe), 35.8 (CH_2CO), 25.9 (SCH_2), 21.3 (x2), 21.2 (x2) (4OAc); M.S. for $C_{18}H_{26}O_{11}S$ (CI ether, rel. intensity) m/z: 451 ($[M+H]^+$, 2.6%), 391 ($[M-CO_2Me$ or $OAc]^+$, 15.2%), 332 ($[M+H$-aglycone$]^+$, 61.1%), 330.8 ($[M$-aglycone$]^+$, 100%).

Anal. Calcd for $C_{18}H_{26}O_{11}S$: C, 48.00; H, 5.82; S, 7.12. Found: C, 47.85; H, 6.01; S, 6.91.

Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulo-pyranosidonate (133)

Method C: To a solution of thioacetylated sialic acid **113**[115] (63.1 mg, 0.115 mmol) in dry methanol (1.5 mL) cooled to -40°C, was added 1 M sodium methoxide in methanol (109 μL, 0.109 mmol). The reaction, monitored by TLC, took less than 5 min after which time the reaction mixture was treated with H^+ resin (Amberlite IR-120) at -40°C for 15 min. The solution was filtered and evaporated at room temperature under reduced pressure to give title compound **133** as a yellow oil in quantitative yield (58.3 mg, 0.115 mmol). Thio-derivative **133** was used in subsequent reaction without further purification. Compound **133** has $[\alpha]_D$ +15.6° (*c* 0.99, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ (ppm): 5.33-5.28 (m, 2H, H-7 and H-8), 5.17 (d, 1H, $J_{NH,5}$ = 10.5 Hz, NH), 4.91 (ddd, 1H, $J_{4,5}$ = 10.3, $J_{3a,4}$ = 11.7, $J_{3e,4}$ = 4.8, H-4), 4.49 (dd, 1H, $J_{8,9'}$ = 2.1, $J_{9,9'}$ = 12.4, H-9'), 4.10 (dd,

1H, $J_{8,9}$ = 5.5, H-9), 4.06 (m, 1H, H-5), 3.83 (s, 3H, OCH_3), 3.73 (dd, 1H, $J_{6,7}$ = 1.9, $J_{5,6}$ = 10.8, H-6), 3.17 (s, 1H, SH), 2.80 (dd, 1H, $J_{3e,3a}$ = 12.8, H-3e), 2.14, 2.13, 2.05, 2.03 (4s, 12H, 4 OAc), 2.04 (dd, 1H, H-3a), 1.88 (s, 3H, NAc); ^{13}C NMR ($CDCl_3$) δ (ppm): 170.9, 170.6, 170.4, 170.4, 170.1, 170.0 (5C=O), 81.6 (C-2), 75.1 (C-6), 70.1 (C-8), 69.4 (C-4), 67.7 (C-7), 62.2 (C-9), 53.4 (OCH_3), 49.1 (C-5), 38.9 (C-3), 23.1 (NAc), 21.1, 20.8, 20.8, 20.7 (4 OAc); MS (CI ether, rel. intensity) m/z: 508 ($[M+H]^+$, 100%), 476 ($[M-Ac]^+$, 85).

Methyl (ethylacetyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosid) onate (134)

Method A: To a solution of thoroughly degassed (N_2) thioacetate **113** (75 mg, 0.136 mmol) in dry DMF (200 μL) was added 2.5 M (DMF) hydrazinium acetate (54.6 μL, 0.136 mmol). The reaction was monitored by TLC and after stirring for 1.5 h at room temperature and under a N_2 atmosphere, ethyl bromoacetate (30 μL, 0.273 mmol) and triethylamine (19 μL, 0.136 mmol) were added. The reaction mixture was stirred at room temperature until TLC (R_f 0.50, EtOAc) showed complete transformation (2 h) after which time EtOAc (10 mL) and water (10 mL) were added. The usual washings were performed and the resulting organic layer was dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by silica gel column chromatography using a gradient of 2% to 3% MeOH in CH_2Cl_2 as eluent. Compound **134** was obtained in 83% yield (66.8 mg, 0.113 mmol) and could be crystallized from ether/hexanes to form a white solid.

Method C: To a solution of thioacetate **113** (100 mg, 0.182 mmol) in dry methanol (2.5 mL) at room temperature under a N_2 atmosphere, was added 1 M sodium methoxide in methanol (173 μL, 0.173 mmol). The reaction, monitored by TLC, took less than 10 min after which time the reaction mixture was treated with H^+ resin (Amberlite IR-120) for 15 min. The solution was filtered and evaporated under reduced pressure to give

compound **133** as an oil in quantitative yield. Derivative **133** was dissolved, without further purification, in acetonitrile (6 mL) under nitrogen and ethyl bromoacetate (40 μL, 0.364 mmol) and triethylamine (26 μL, 0.182 mmol) were added to the reaction mixture. The reaction mixture was stirred at room temperature until TLC showed complete transformation (<2 h) of the starting material. The usual washings were performed and the resulting organic layer was dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by silica gel column chromatography using a gradient of 2% to 3% MeOH in CH_2Cl_2 as eluent. Compound **134** was obtained in 80% yield (86.3 mg, 0.146 mmol).

Title compound **134** has mp (Ether/Hexanes) 132.8-135.0°C; $[\alpha]_D$ +53.6° (*c* 1.09, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.45-5.24 (m, 3H, H-7, H-8, and NH), 4.84 (ddd, $J_{3e,4}$ = 4.7 Hz, $J_{4,5}$ = 10.3, $J_{3a,4}$ = 11.7, 1H, H-4), 3.94-4.35 (m, 5H, OCH_2, H-5, H-9, & H-9'), 3.76 (dd, $J_{5,6}$ = 10.4, $J_{6,7}$ = 2.2, 1H, H-6), 3.75 (s, 3H, OMe), 3.57 (d, $J_{a,b}$ = 16.8, 1H, SCH-a), 3.40 (d, $J_{a,b}$ = 16.8, 1H, SCH-b), 2.72 (dd, $J_{3e,4}$ = 4.7, $J_{3e,3a}$ = 12.6, 1H, H-3e), 2.10, 2.08, 2.00, 1.98 (4s, 12H, 4OAc), 1.98 (dd, 1H, H-3a), 1.82 (s, 3H, NAc), 1.23 (t, $J_{CH2,CH3}$ = 7.1, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ (ppm): 170.9, 170.6, 170.2, 170.1, 170.0, 169.3, 168.1 (7C=O), 82.3 (C-2), 74.2 (C-6), 69.4 (C-8), 68.4 (C-4), 67.2 (C-7), 62.2 (C-9), 61.6 (CH_2CH_3), 53.2 (MeO), 49.2 (C-5), 37.4 (C-3), 31.0 (SCH_2), 23.1 (NAc), 21.1, 20.8, 20.7, 20.6 (4OAc) 14.1 (CH_2CH_3); M.S. for $C_{24}H_{35}N_1O_{14}S$ (CI ether, rel. intensity) m/z: 594.0 ($[M+H]^+$, 45.5%), 534.0 ($[M-CO_2Me]^+$, and/or $[M-OAc]^+$, 80.5%), 474.0 ([M-aglycone]$^+$, 61.7%).

Anal. Calcd for $C_{24}H_{35}N_1O_{14}S$: C, 48.56; H, 5.94; N, 2.36. Found: C, 48.75; H, 6.01; N, 2.56.

Methyl (4-nitrobenzyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosid) onate (135)

Method A: To a solution of thoroughly degassed (N_2) thioacetate **113** (66.4 mg, 0.121 mmol) in dry DMF (200 μL) was added 2.5 M (DMF) hydrazinium acetate (48.3 μL, 0.121 mmol). The reaction was monitored by TLC and after stirring for 1 h at room temperature and under a N_2 atmosphere, *p*-nitrobenzyl bromide (52.2 mg, 0.242 mmol) and triethylamine (17 μL, 0.121 mmol) were added. The reaction mixture was stirred at room temperature until TLC (R_f 0.51, EtOAc) showed complete transformation (1.5 h) after which time EtOAc (10 mL) and water (10 mL) were added. The usual washings were performed and the resulting organic layer was dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by silica gel column chromatography using a gradient of 2% to 3% MeOH in CH_2Cl_2 as eluent. Compound **135** was obtained in 74% yield (57.4 mg, 0.089 mmol): $[\alpha]_D$ +36.5° (*c* 1.09, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.13 (d, $J_{o,m}$ = 8.7 Hz, 2H, H-m), 7.52 (d, $J_{o,m}$ = 8.7, 2H, H-o), 5.48-5.40 (m, 1H, H-8), 5.30 (dd, $J_{6,7}$ = 2.2, $J_{7,8}$ = 9.3, 1H, H-7), 5.07 (d, $J_{NH,5}$ = 10.1, 1H, NH), 4.83 (ddd, $J_{4,5}$ = 10.4, 1H, H-4), 4.28 (dd, $J_{8,9'}$ = 2.6, $J_{9,9'}$ = 12.3, 1H, H-9'), 4.09-3.98 (m, 2H, H-5, & H-9), 3.98 (s, 2H, SCH_2), 3.83 (dd, $J_{5,6}$ = 10.8, 1H, H-6), 3.48 (s, 3H, OMe), 2.70 (dd, $J_{3e,4}$ = 4.5, 1H, H-3e), 2.18, 2.15, 2.02, 2.01 (4s, 12H, 4OAc), 1.98 (dd, $J_{3e,3a}$ = 12.7, $J_{3a,4}$ = 12.1, 1H, H-3a), 1.87 (s, 3H, NAc); ^{13}C-NMR ($CDCl_3$) δ (ppm): 170.8, 170.6, 170.3, 170.2, 170.1, 168.0 (6 C=O), 146.9 (C-p), 144.9 (C-i), 130.0 (C-o), 123.4 (C-m), 82.6 (C-2), 73.9 (C-6), 69.3 (C-8), 67.9 (C-4), 67.0 (C-7), 62.3 (C-9), 52.8 (MeO), 49.3 (C-5), 37.7 (C-3), 32.2 (SCH_2), 23.2 (NAc), 21.3, 20.8, 20.8, 20.7 (4OAc); M.S. for $C_{27}H_{34}N_2O_{14}S$ (CI ether, rel. intensity) m/z: 642.8 ($[M+H]^+$, 4.3%), 582.9 ($[M+H\text{-}OAc]^+$, 3.2%), 476.0 ($[M+H\text{-aglycone}]^+$, 16.0%).

Anal. Calcd for $C_{27}H_{34}N_2O_{14}S$: C, 50.46; H, 5.33; N, 4.36; S, 4.99. Found: C, 50.17; H, 5.06; N, 4.52; S, 5.06.

Methyl ((glycylglycyl N-acetyl) 4-nitrophenyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosid) onate (136)

Method A: To a solution of thoroughly degassed (N_2) thioacetate **113** (97.1 mg, 0.177 mmol) in dry DMF (220 μL) was added 2.5 M (DMF) hydrazinium acetate (70.2 μL, 0.177 mmol). The reaction was monitored by TLC and after stirring for 1.25 h at room temperature and under a N_2 atmosphere, *N*-chloroacetyl glycylglycine (28 mg, 0.134 mmol) and triethylamine (25 μL, 0.177 mmol) were added. The reaction mixture was stirred at room temperature until TLC (R_f 0.29, CH_3CN/H_2O, 9/1) showed complete transformation (2.5 h) after which time the reaction mixture was diluted with methanol (20 mL) and 5g of AcO^- resin was added. The mixture was stirred very slowly for 30 min after which the resin was filtered off and washed with methanol. The resin was then treated three times with 0.5M acetic acid (20 mL) for 20 min each time and once for 1 hour. The acidic solution was freeze dried to yield 43.1 mg of a white foam.

Since the former methanol solution still contained a considerable amount of product, the solution was brought to a basic pH and retreated with AcO^- resin as described above. The resin was filtered off and the same treatment was effected to give after freeze drying, 40.1 mg of the white foam. The total yield gave 83.2 mg (91%, 0.122 mmol) of title compound **136**. The second resin treatment was completely successful since no more final product could be detected from the methanol solution. Compound **136** has mp 106-107.5° (sintered); 139.8-141.2° (melted); $[\alpha]_D$ +29.7° (*c* 0.99, MeOH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.07 (m, 2H, NH), 7.66 (d, $J_{NH,5}$ = 9.5 Hz, 1H, NHAc), 5.26 (ddd, $J_{8,9}$ = 5.8, 1H, H-8), 5.13 (dd, $J_{6,7}$ = 2.0, $J_{7,8}$ = 8.3, 1H, H-7), 4.70 (ddd, $J_{3e,4}$ = 4.5, $J_{3a,4}$ = 11.2, $J_{4,5}$ = 10.4, 1H, H-4), 4.18 (dd, $J_{8,9'}$ = 2.9, $J_{9,9'}$ = 12.2, 1H, H-9'), 4.01 (dd, $J_{8,9}$ = 5.8, $J_{9,9'}$ =12.2, 1H, H-9), 3.87 (m, 1H, H-5), 3.79-3.70 (m, 8H, H-6, MeO, 2-NH*CH*$_2$), 3.45 (d, $J_{a,b}$ = 14.6, 1H, SCH-a), 3.37 (d, $J_{a,b}$ = 14.6, 1H, SCH-b), 2.64 (dd, $J_{3e,4}$ = 4.5, $J_{3e,3a}$ = 12.4, 1H, H-3e), 2.08, 2.00, 1.97, 1.92 (4s, 12H, 4OAc), 1.78 (dd, $J_{3e,3a}$ = 12.4, 1H, H-3a), 1.66 (s, 3H, NAc); ^{13}C-NMR ($CDCl_3$) δ (ppm): 170.1, 169.6, 169.2, 169.0 (x2), 168.6, 168.1, 167.4 (x2) (9C=O), 82.3 (C-2), 73.7 (C-6), 69.5 (C-4), 67.8 (C-8), 67.0 (C-7), 61.8 (C-9), 53.1

(MeO), 47.7 (C-5), 42.2 (x2) ($2NHCH_2$), 37.4 (C-3), 32.0 (SCH_2), 22.6 (NAc), 20.9, 20.6, 20.6, 20.5 (4OAc); M.S. for $C_{26}H_{37}N_3O_{16}S$ (FAB^+ thioglycerol, rel. intensity) m/z: 680.2 ($[M+H]^+$, 40.0%), 620.2 ($[M-OAc]^+$, 3.1%), 474.2 ($[M-aglycone]^+$, 5.7%).

Anal. Calcd for $C_{26}H_{37}N_3O_{16}S \cdot 2.5\ H_2O$: C, 43.09; H, 5.84; N, 5.80. Found: C, 43.15; H, 5.66; N, 5.69.

Chapter 4

Synthesis of Gallic Acid-based Glycodendrimers

4.1 Introduction

As already stated, it is now well established that cell surface carbohydrates and sialosides, in particular, are involved as anchoring motifs onto which various pathogenic agents such as viruses (influenza, sendai, and rota-viruses), bacteria (*Helicobacter pilori*, *Escherichia coli*, *Bordetella pertussis*, *Vibrio cholerae*, *Pseudomonas aeruginosa*), and parasites (*Plasmodium falciparum*) adhere and colonize host tissues.[205] It has also been demonstrated that bacterial infections[206] and cancer metastasis[207] can be prevented by blocking pathogenic carbohydrate receptors (toxins, lectins, etc.) with high serum carbohydrate concentrations. Additionally, Roy *et al.*[38,79,85,94,163] and others[28,29,90,91,208,209,210] have established that multivalent neoglycoconjugates constitute powerful antiadhesins in a number of generally low affinity carbohydrate-protein binding interactions.

Potent multivalent neoglycoconjugate antiadhesins have been scaffolded onto a wide range of carriers including polymers,[39,43] polyamino acids,[88,211] cyclodextrins,[212] calix[4]arenes,[52-54] carbohydrates,[213] and dendrimers.[51] These last classes of scaffolds

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205 Karlsson, K. A. *Curr. Opin. Struct. Biol.* **1995**, *5*, 622.

206 Sharon, N. *FEBS Lett.* **1987**, *217*, 145.

207 Beuth, J.; Ko, H. L.; Pulverer, G.; Uhlenbruck, G. and Pichlmaier, H. *Glycoconjugate J.* **1995**, *12*, 1.

208 Lee, Y. C. and Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321, and references cited therein.

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

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

211 Duncan, R. and Kopecek, J. *Adv. Polymer Sci.* **1984**, *57*, 51.

212 (a) de Robertis, L.; Lancelon-Pin, C.; Driguez, H.; Attioui, F.; Bonaly, R.; Marsura, A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1127.

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

proved particularly appealing since the effects of size, shapes, and valency can be controlled at will.[114,115,118,124,125]

As the first example of glycoconjugate included in this thesis dissertation, we present herein the synthesis of tri- and nona-meric dendrimers bearing α-thiosialosides using gallic acid **162** as the seeding trivalent core molecule.

4.2 L-lysine-based Sialodendrimers

The first glycodendrimers were synthesized in our laboratory and I was part of the whole synthetic process.[114,115] However, this thesis dissertation will not discuss all the experimental data in details since this was extensively done in Dr. Diana Zanini's thesis dissertation.[214] She deserved most of the credit since she was involved in more than 50% of the work done on the L-lysine sialodendrimers. This thesis dissertation will, however, briefly discuss the synthetic sequences that were actually performed in the synthesis of those first sialodendrimers since it is also part of the work accomplished through my doctoral studies.

Schemes 4.2.1 and 4.2.2 depict the synthesis of the L-lysine sialodendrimers. In synthetic Scheme 4.2.1, dendritic L-lysine cores[215] were constructed on a β-alanyl spacer anchored to poly[styrene-*co*-4-(hydroxymethyl)phenoxymethyl] resin (Wang resin, 0.58 mmol/g) using fluoren-9-ylmethoxycarbonyl (Fmoc) chemistry (Scheme 4.2.1). The hyperbranched poly-L-lysine scaffolding was prepared using preformed N^{α},N^{ε}-di-Fmoc-L-lysine benzotriazolyl ester (2 equiv) [1-hydroxybenzotriazole, diisopropylcarbodiimide, *N*,*N*-dimethylformamide, 0°C, then 25°C, 1 h]. Removal of the Fmoc protecting group was effected by treatment with 20% piperidine in DMF (3 x 10 min). The products

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[214] Zanini, D. *Ph.D. Thesis*, **1997**, Univ. of Ottawa.

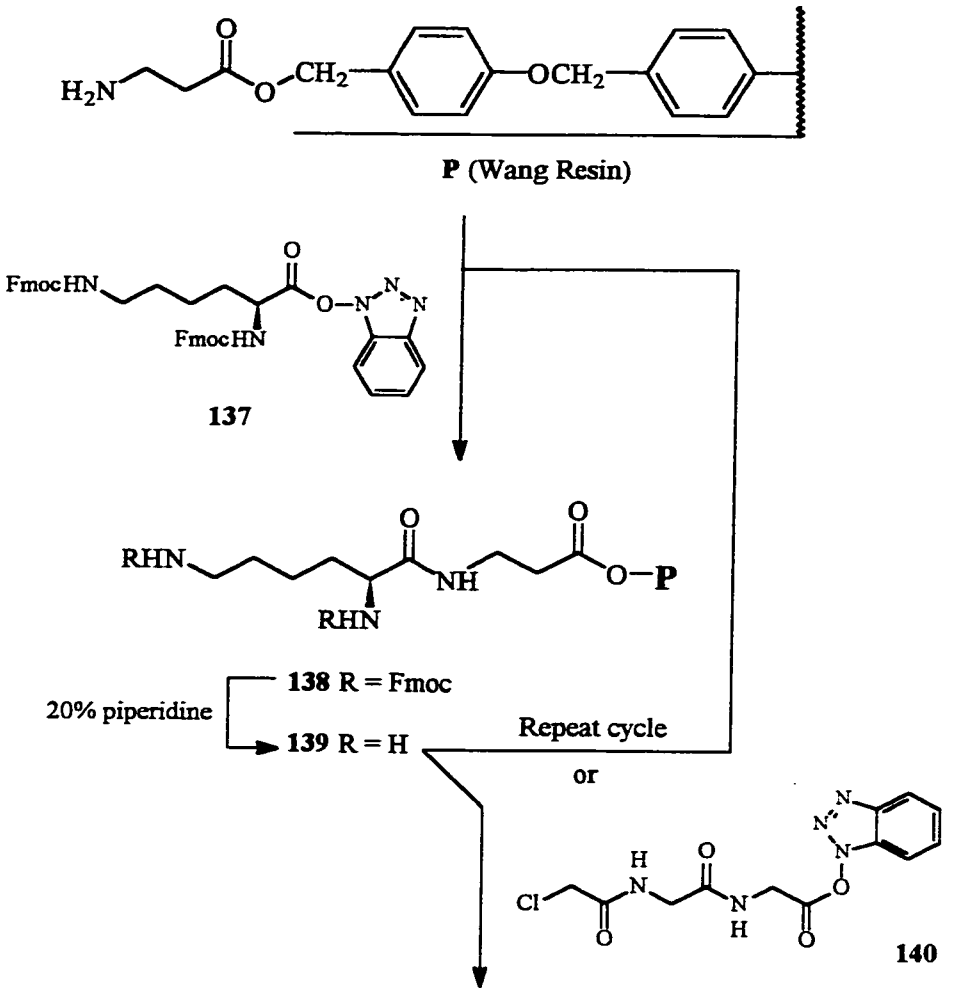
[215] Denkewalter, R. G.; Kolc, J.; Luskasavage, W. J. *US Patent 4,289,872* **1981**; Tam, J. P. *Proc. Natl. Acad. Sci., USA* **1988**, *85*, 5409.

resulting from each sequential generation were then treated with the preformed benzotriazolyl ester of chloroacetylglycylglycine as described above. In this manner, di- **141**, tetra- **142**, octa- **143** and hexadeca- valent **144** chloroacetylated dendrimers were obtained in the first, second, third and fourth generations, respectively.

While still attached to the resin, each dendrimer generation was treated with an excess of 2-thiosialic acid derivative **133** (1% Et_3N-DMF, 16 h, 25°C) (Scheme 4.2.2). Before the bulk of the dendrimers were released from the polymeric support, aliquots were withdrawn and hydrolysed with 95% aqueous trifluoroacetic acid (TFA, 1.5 h). The completeness of the couplings was estimated from the ^{1}H-NMR spectrum of the sialylated dendrimers which showed characteristic signals for any residual chloroacetylmethylene groups δ 4.2 (DMSO-d_6). Where required, the couplings were repeated.

The peracetylated sialyl dendrimers **149**, **152**, **154**, and **157** were released from the polymer support as above (TFA, 1.5 h) and obtained in 66-99% yields after removal of the solvent under reduced pressure. The ^{1}H and ^{13}C NMR spectra (DMSO-d_6) of the dendrimers revealed the integrity of the α-sialoside linkages as well as the ratio of the β-alanyl residues relative to those of both L-lysyl and sialyl signals. Each of the protected dendrimers (**149**, **152**, **154**, and **157**) were de-esterified with NaOMe-MeOH (25°C, 1 h) (**150** and **155**) followed by 0.05 M NaOH (25°C, 2 h, then H^+ resin) to afford dendrimers **151**, **153**, **156**, and **158** in essentially quantitative yields. The larger octa- **156** (Figure 4.2.1) and hexadeca-meric **158** dendrimers can be dialysed using benzoylated dialysis tubing (M.W. cutoff 2000).

The octa- **156** and hexadeca-meric **158** dendrimers were almost as efficient coating antigens as the poly(acrylamide-*co*-NeuAc-SPh-NH-acryloyl) copolymer (not shown) in ELLA experiment. The octameric dendrimer **156** was ~30 times better as an inhibitor of binding than the monovalent sialic acid in model studies using the plant lectin WGA. Preliminary experiments with influenza A virus (strain X-31) showed that the dendrimers were potent inhibitors of hemagglutination of human erythrocytes.[115]



$(ClCH_2CO\text{-}Gly\text{-}Gly\text{-})_2\text{-}Lys\text{-}\beta\text{-}Ala\text{-}O\text{-}R$

95% TFA → **141** R = **P**; **145** R = H

$(ClCH_2CO\text{-}Gly\text{-}Gly\text{-})_4\text{-}Lys_2\text{-}Lys\text{-}\beta\text{-}Ala\text{-}O\text{-}R$

95% TFA → **142** R = **P**; **146** R = H

$(ClCH_2CO\text{-}Gly\text{-}Gly\text{-})_8\text{-}Lys_4\text{-}Lys_2\text{-}Lys\text{-}\beta\text{-}Ala\text{-}O\text{-}R$

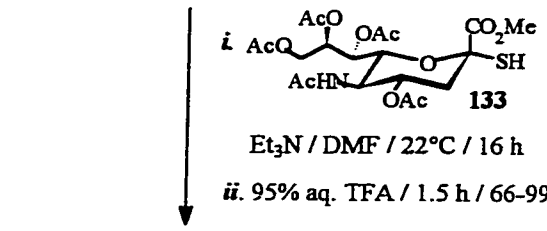
95% TFA → **143** R = **P**; **147** R = H

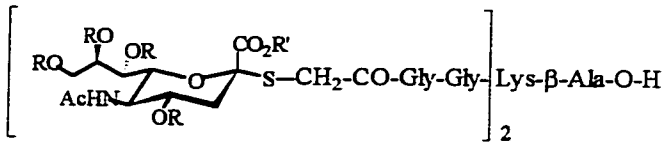
$(ClCH_2CO\text{-}Gly\text{-}Gly\text{-})_{16}\text{-}Lys_8\text{-}Lys_4\text{-}Lys_2\text{-}Lys\text{-}\beta\text{-}Ala\text{-}O\text{-}R$

95% TFA → **144** R = **P**; **148** R = H

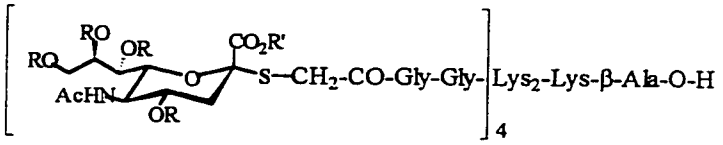
Scheme 4.2.1 Synthetic sequence for *N*-chloroacetylated L-lysine-based dendrimers.

141, 142, 143, or 144

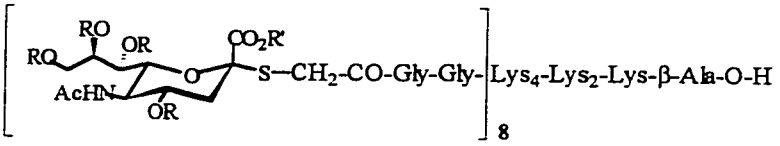




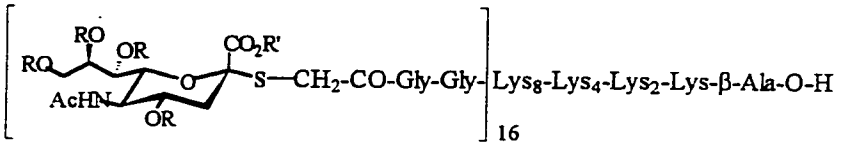
i → **149** R = Ac; R' = Me
ii → **150** R = H; R' = Me
→ **151** R = R' = H



i-ii → **152** R = Ac; R' = Me
→ **153** R = R' = H



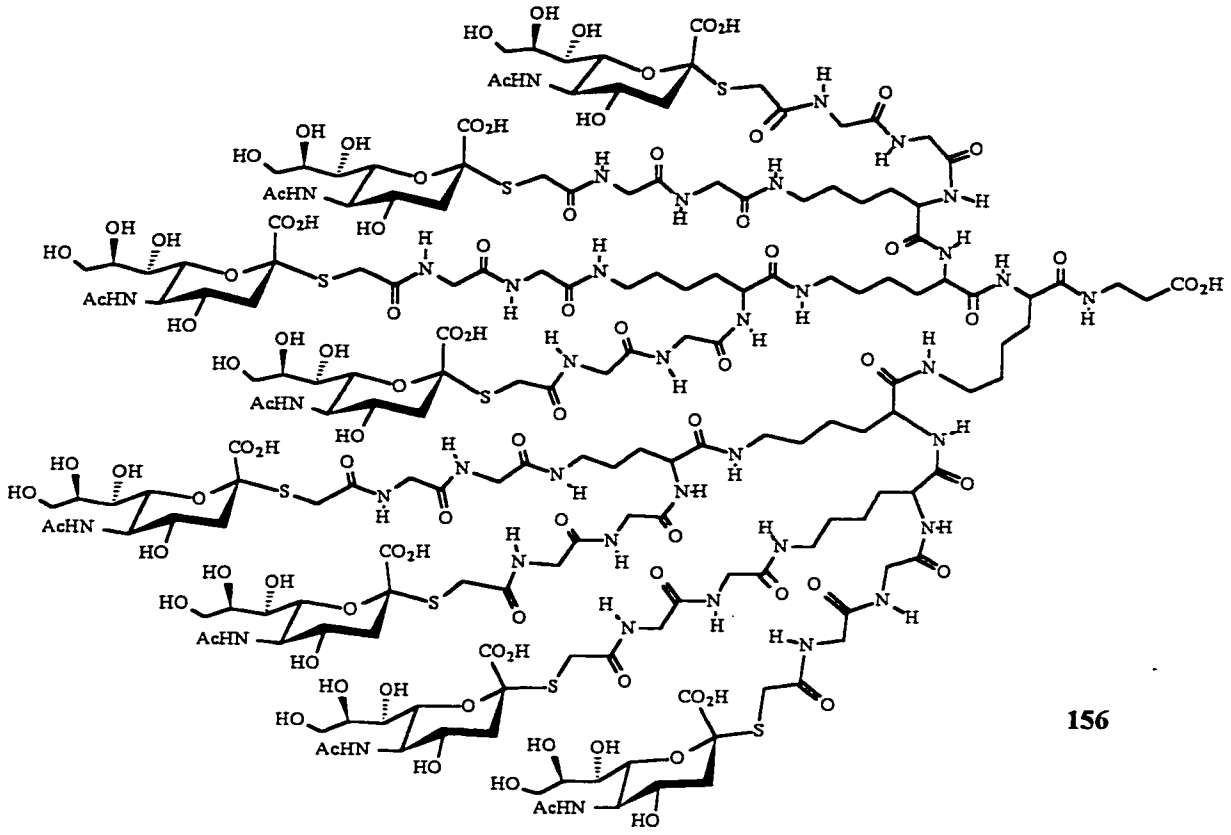
i → **154** R = Ac; R' = Me
ii → **155** R = H; R' = Me
→ **156** R = R' = H



i-ii → **157** R = Ac; R' = Me
→ **158** R = R' = H

i. NaOMe, MeOH, 1 h, 22°C, then H^+ resin treatment
ii. 0.05 M NaOH, 2 h, 22°C, then H^+ resin

Scheme 4.2.2 Synthesis of L-lysine-based sialodendrimers.



156

Figure 4.2.1 Octavalent L-lysine-based sialodendrimer.

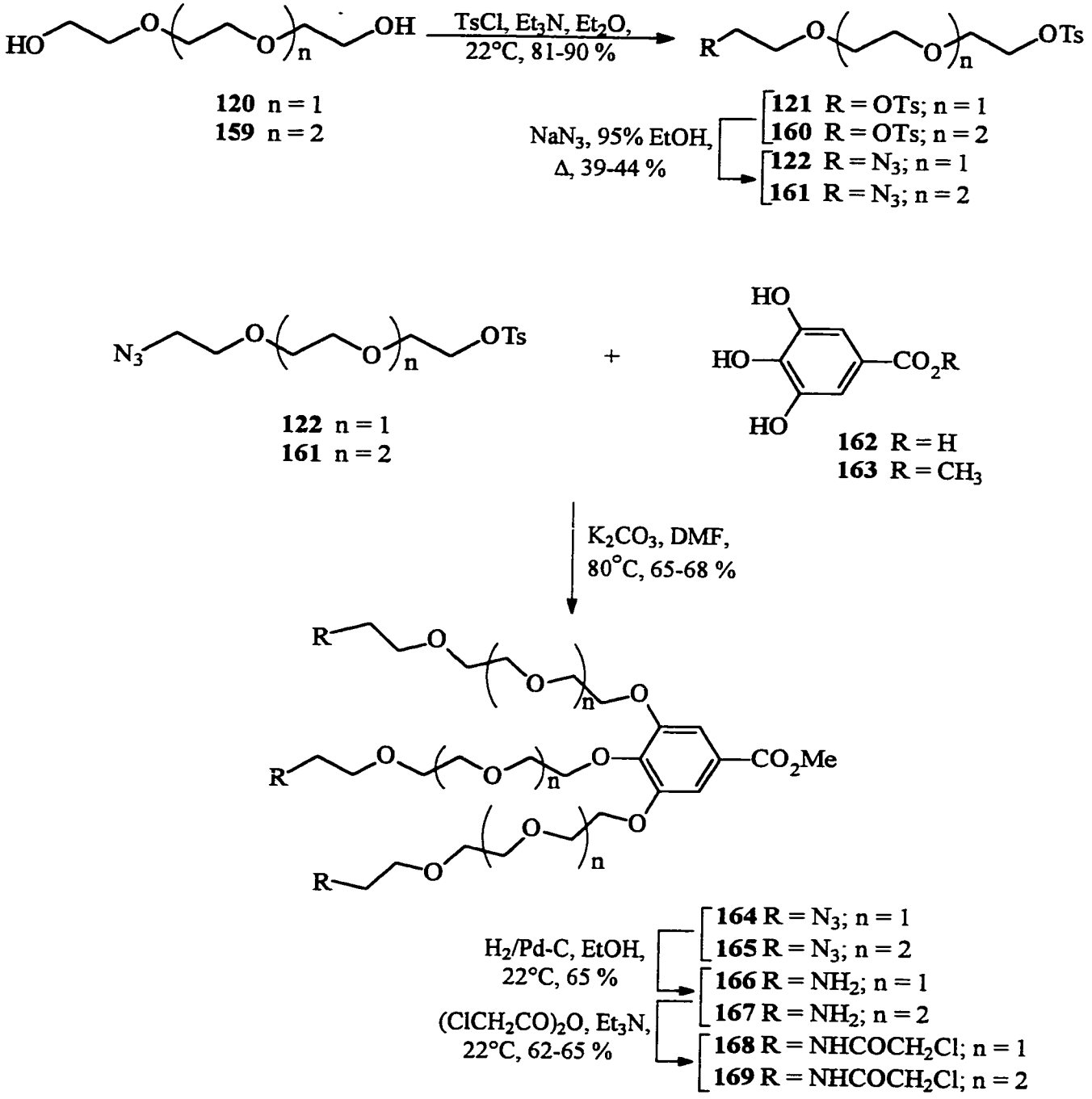
4.3 Gallic Acid-based Sialodendrimers

3,4,5-Trihydroxybenzoic acid (gallic acid, **162**) based sialodendrimers were synthesized using commercially available tri- and tetra-ethylene glycol **120** and **159** as spacer arms. The latter were chosen to ensure water solubility of the final product and to counteract the hydrophobic effect of the aromatic backbone. They were also chosen since they are commercially available in different lengths, and they provide sufficient spacing to allow the carbohydrate moieties to be readily accessible by receptor sites. Diols **120** and **159** were ditosylated in 81-90% yields using *p*-toluenesulfonyl chloride (TsCl,

triethylamine, Et_2O, reflux). Bistosylated spacers **121** and **160** were then treated with one equivalent of sodium azide (95% EtOH, reflux, 4 h) to afford azidotosylates **122** and **161** in moderate yields (39-44 %) (Scheme 4.3.1). Under these conditions, substantial amount of starting material could be recovered (40 to 50%), together with traces of bisazides and EtOH substitution products, which were minimized when 95% instead of 99% EtOH was used.

The choice of gallic acid as core unit allows dendrimer scaffolding to reach 3^n surface groups at the n^{th} generation. Furthermore, it can be used to generate dendrimers with different valencies than those previously reported (2^n) for sialylated dendrimers[51] in which the seeding molecules L-lysine[114,115,118] and 3,3'-iminobis(propylamine)[124-125] were used. To provide an iterative approach, gallic acid was transformed into an orthogonally protected amino acid derivative.[121] Methyl ester **163** was prepared from gallic acid **162** using standard procedure ($SOCl_2$, 22°C, 2 h, then MeOH, 98%). The white solid ester **163** (mp 199.5-201°C) was then carefully *O*-alkylated with a slight excess azidotosylate spacers **122** or **161** using K_2CO_3 as base (DMF at 80°C) to afford key dendrimer precursors **164** and **165** in 65 and 68% yields, respectively (Scheme 4.3.1). Trisamino esters **166** and **167** were obtained by reduction of azides **164** and **165** (H_2, 10 % Pd-C, 93 % crude yield for **167**) and used directly in the next step without further purification. An analytical sample of **167** was, however, purified by silica gel chromatography (65% yield). The extent of the reduction was monitored by FTIR spectroscopy, which showed total disappearance of the azido frequency (2106 cm^{-1}). Intermediate amines **166** and **167** were then sequentially transformed into either first generation precursors **168** and **169** or into orthogonally protected amino acid derivatives for further generation build up. Thus, treatment of **166** or **167** with chloroacetic anhydride [$(ClCH_2CO)_2O$, Et_3N, EtOH] provided tris-*N*-chloroacetylated esters **168** or **169** in 62-65% yields. Completion of the reaction could be readily monitored by a negative ninhydrin reaction and by the appearance of a sharp *N*-chloroacetyl methylene singlet at δ 4.01 ppm ($CDCl_3$) integrating

for the expected six proton signals relative to those of the two aromatic protons (δ 7.25 ppm, s) and the three carbomethoxy protons signal at δ 3.85 ppm (Figure 4.3.1).



Scheme 4.3.1 Synthesis of first generation gallic acid dendritic backbone.

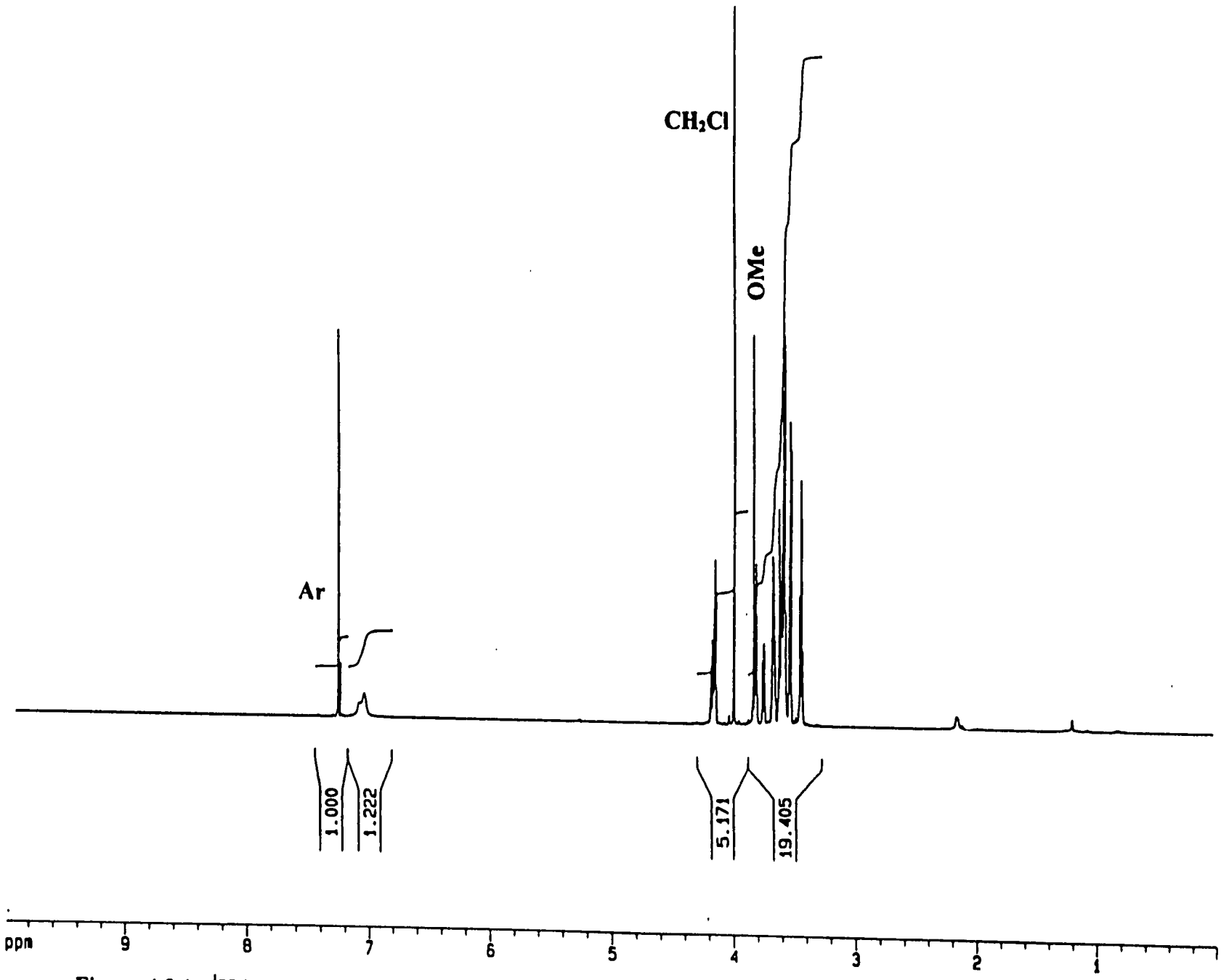
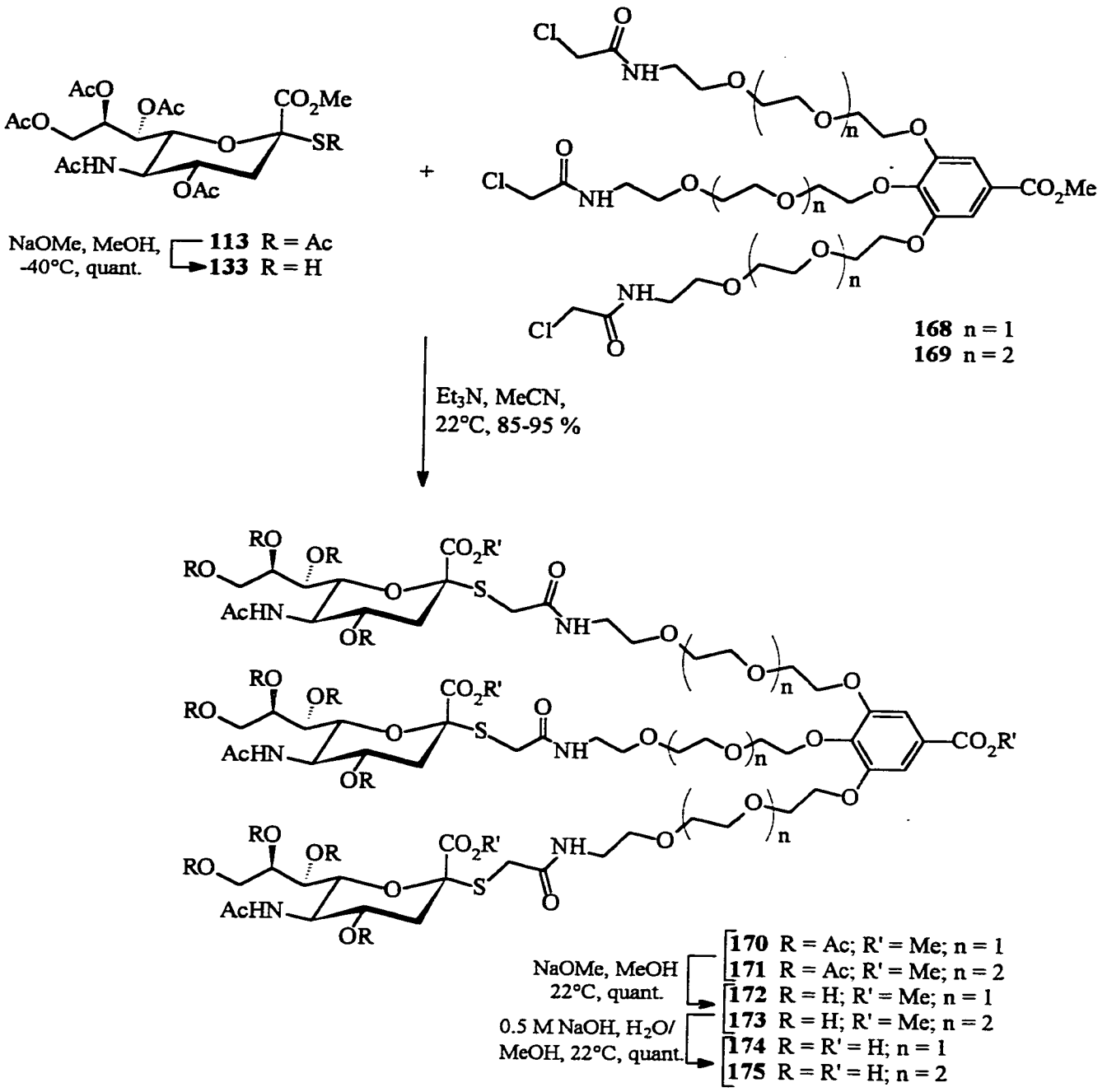


Figure 4.3.1. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of *N*-chloroacetylated trivalent gallic acid-based dendrimer **168**.



Scheme 4.3.2 Synthesis of trivalent gallic acid-based sialodendrimers.

As was previously demonstrated in other glycodendrimer syntheses,[51,119,126,127,129,131] it was found advantageous to attach the carbohydrate residues after the build up of the dendritic cores. Not only does this strategy permit incorporation of any other carbohydrate haptens at a late stage but it also allows convergent synthesis as well as

tethering glycodendrimer building blocks.[125] In this last case, tethered glycodendrimers are formed with spherical shapes in all three directions rather than being unidirectional.

Thus, known α-sialosyl thioacetate **113**[115] was chosen as the nucleophilic carbohydrate moiety to be initially used on the trimeric *N*-chloroacetylated precursors **168** and **169**. Chemoselective hydrolysis of the thioacetate group in **113** was first achieved by mild transesterification conditions (NaOMe, MeOH, -40°C, 15 min), followed by acidic resin treatment at -40°C[115,203] (Scheme 4.3.2). This mild treatment protected the anomeric thiol from both oxidation and anomerization. Thiol derivative **133** was always freshly prepared prior to coupling onto dendrimers. First-generation trivalent α-thiosialosides **170** and **171** were obtained from the coupling of thiol **133** to trimeric *N*-chloroacetylated esters **168** and **169** under a nitrogen atmosphere (CH_3CN, Et_3N, 22°C, 85-95% yields). The extent of thiosialoside incorporation in glycodendrimers **170** and **171** was readily determined by ^{1}H-NMR spectroscopy. The disappearance of the signal due to all three equivalent *N*-chloroacetyl residues at δ 4.01 ppm (6H), along with the integration of well-separated sialic acid associated signals at δ 4.84 ppm (H-4, 3H) and at δ 2.72 ppm (H-3e, 3H) relative to that of the aromatic protons at δ 7.27 ppm (2H), confirmed complete substitution (Figure 4.3.2). Peracetylated glycodendrimers **170** and **171** were then treated with NaOMe in MeOH (pH~9, 22°C) to provide partially deprotected intermediates **172** and **173** in quantitative yields. Their ^{1}H NMR spectra showed the presence of sialic acid methyl esters and *N*-acetyl residues as well as the disappearance of all *O*-acetyl signals. Methyl ester hydrolysis from both sialosides and gallic acid residues of **172** and **173** was performed simultaneously by treatment with 0.5 M aqueous NaOH in MeOH (1:1, v/v, 22°C , 16 h). This reaction afforded unprotected water-soluble glycodendrimers **174** and **175** in nearly quantitative yields after freeze-drying. Completion of hydrolysis was best monitored using ^{13}C NMR spectroscopy where the methoxy signals at δ ~52-54 ppm were well separated from the remaining carbon signals (Figure 4.3.3).

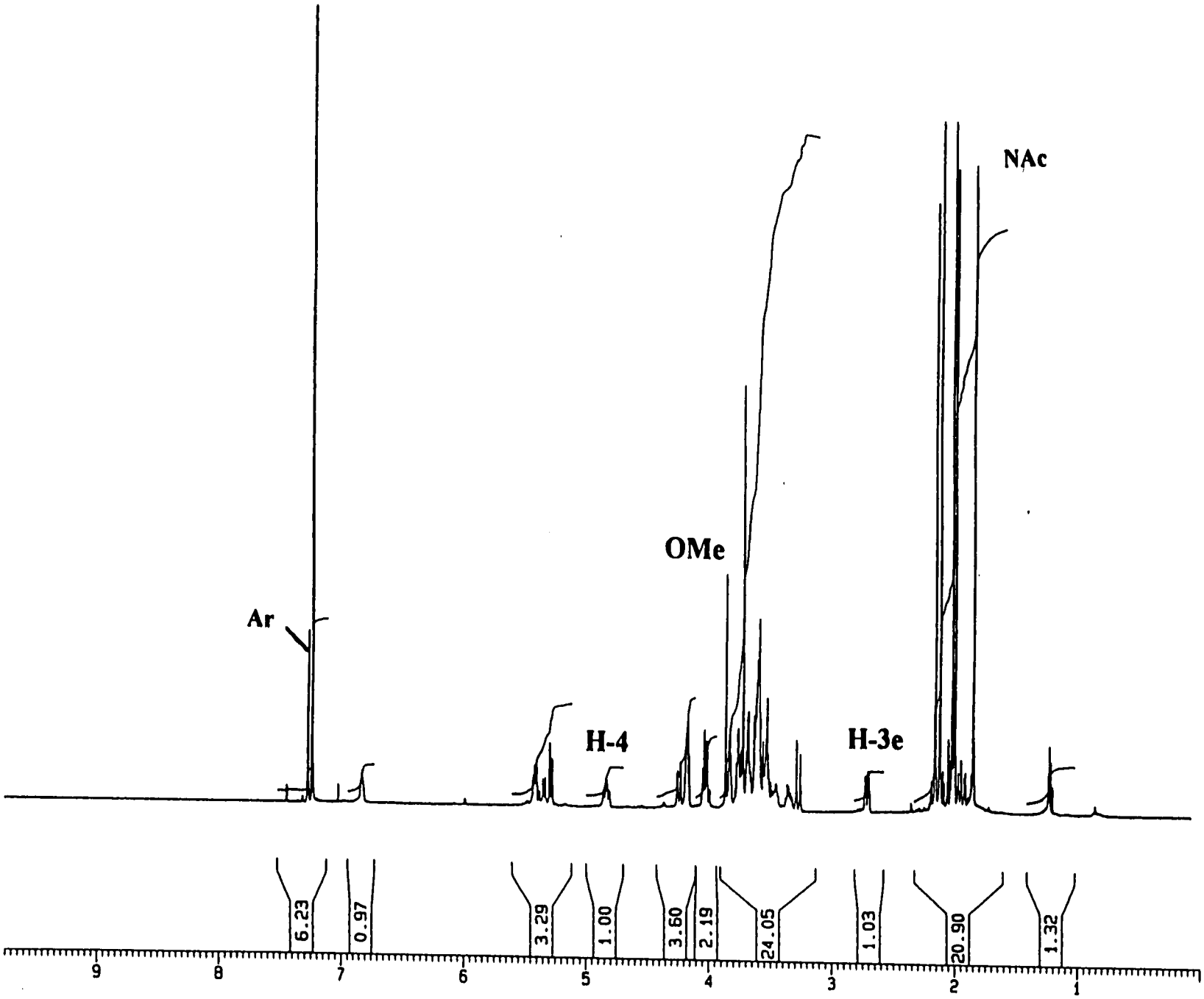


Figure 4.3.2. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of trivalent gallic acid-based sialodendrimer **170**.

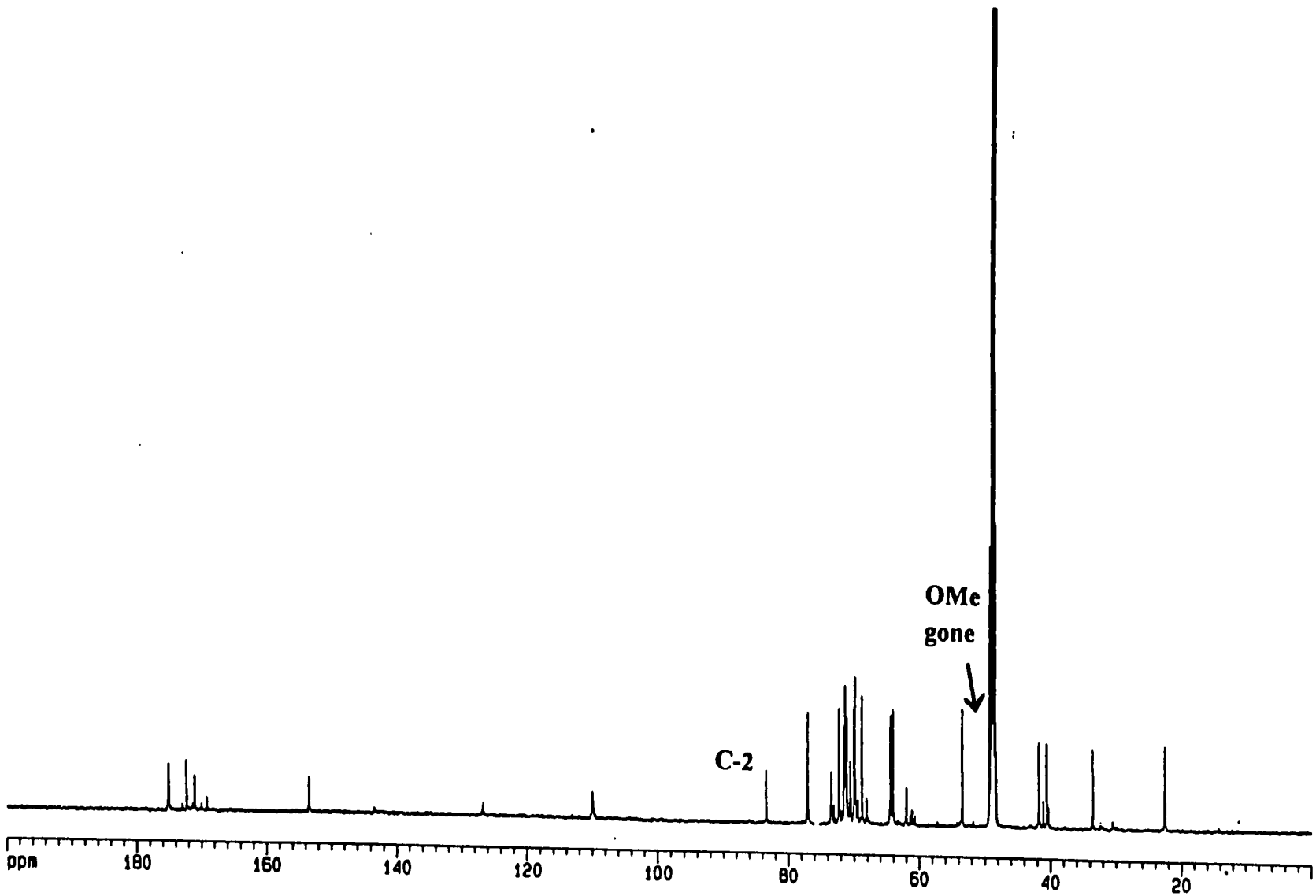
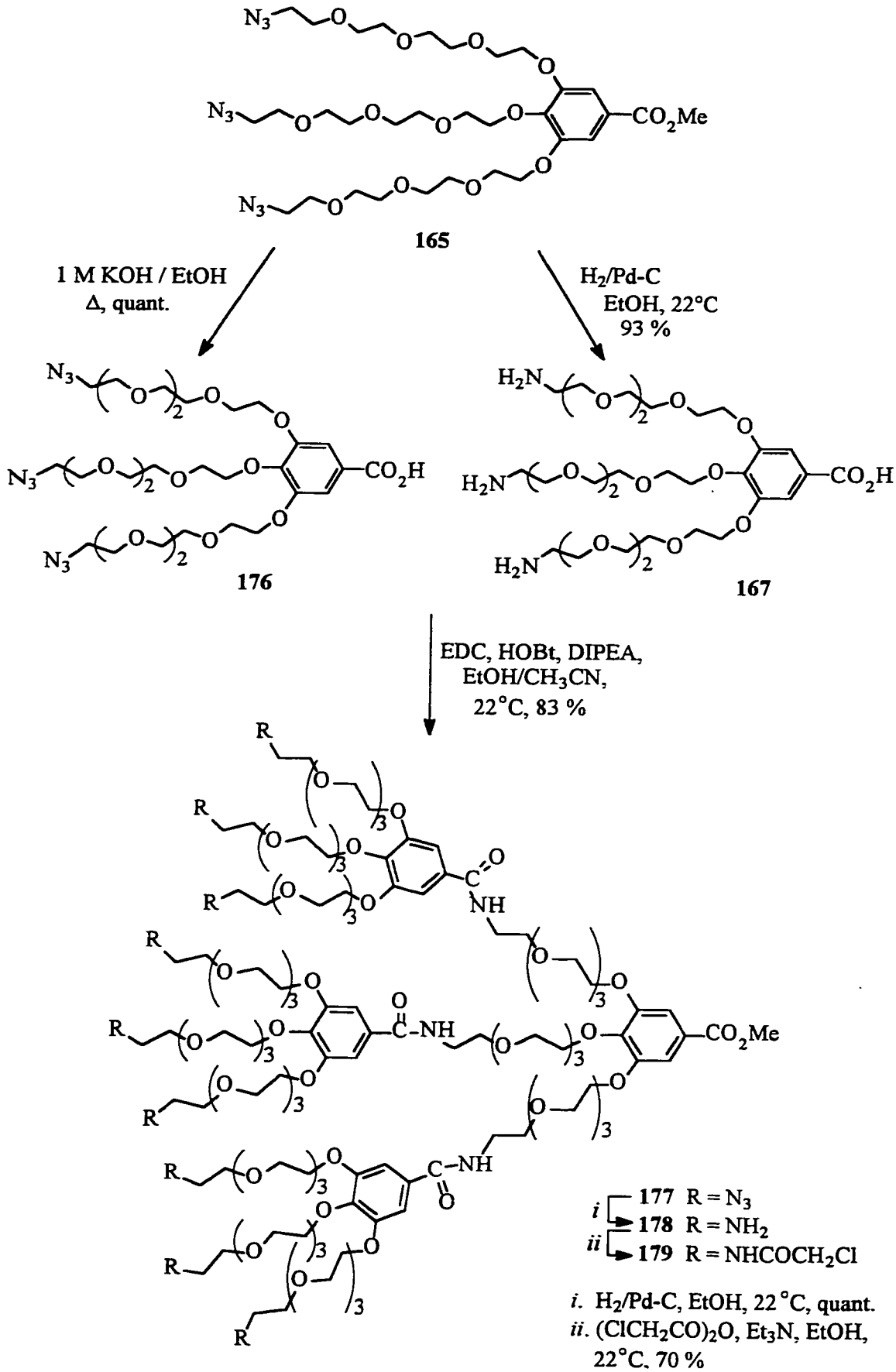


Figure 4.3.3. ^{13}C-NMR (DMSO-d_6, 500 MHz) spectrum of fully deprotected trivalent gallic acid-based sialodendrimer **174**.

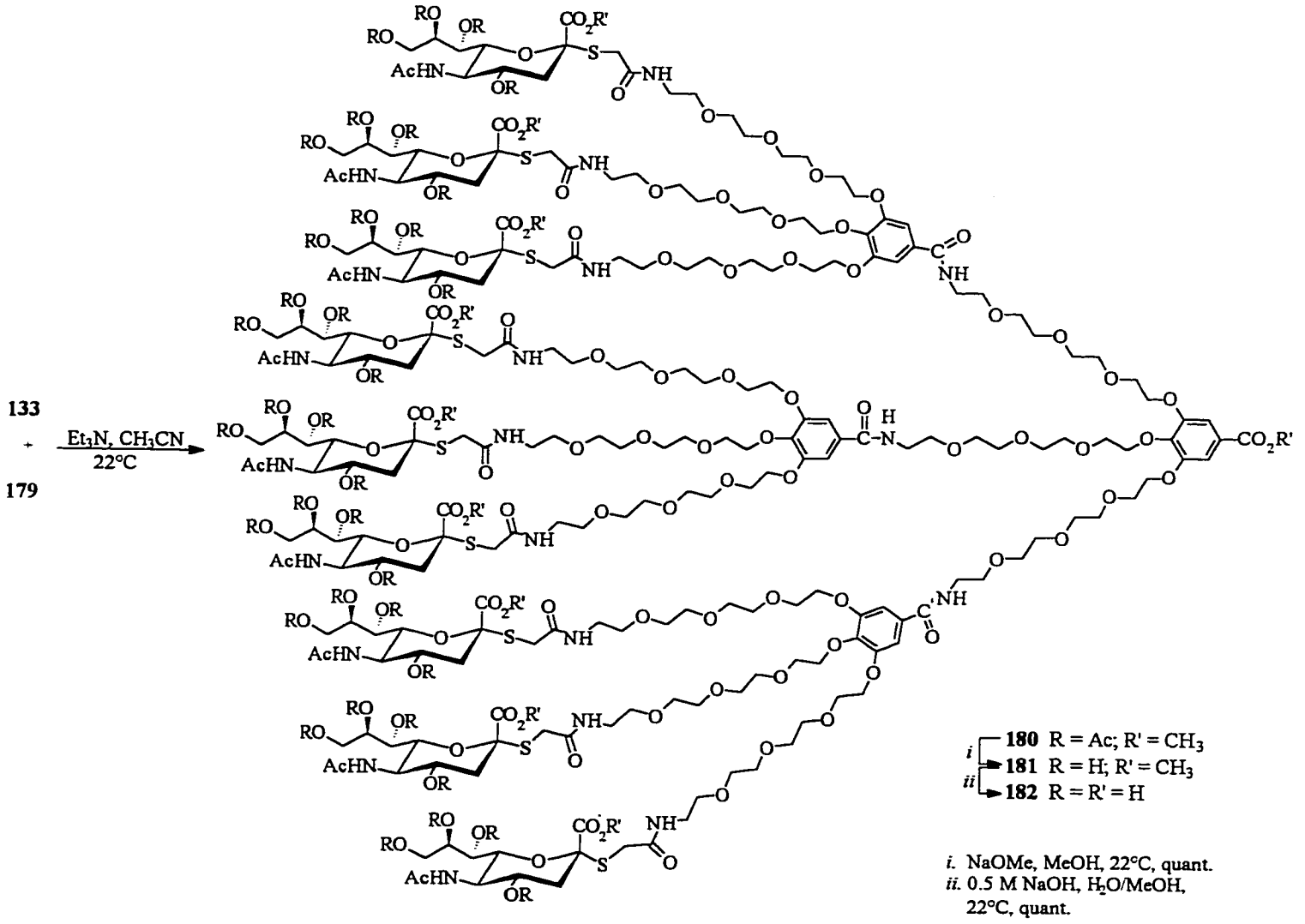


Scheme 4.3.3 Synthesis of second generation gallic acid dendritic backbone.

For the preparation of the second generation, readily available amide bond linkages between the subunits was chosen. To this end, orthogonally protected amino acid derivatives were required. Trisamino ester **167**, already obtained from reduction of trisazido ester **165** (see above), was used as an amine partner, while acid precursor **176** was obtained from the same trisazido ester **165** by hydrolysis (Scheme 4.3.3). Methyl ester **165** was hydrolyzed under basic conditions (KOH, EtOH, reflux, 2h) to afford **176** quantitatively. Coupling a slight excess of trisazido acid **176** to trisamino ester **167** using carbodiimide chemistry and hydroxybenzotriazole (EDC, HOBt, EtOH-CH_3CN, 1:1 v/v, DIPEA, 22°C, 3h) afforded nona-azido ester **177** in 83% yield. After usual work-up and purification by silica gel column chromatography, the ^{1}H-NMR spectrum of **177** showed the expected 1:3 ratio of inner (δ 7.24 ppm, ester) and outer (δ 7.02 ppm, amide) aromatic proton signals (Figure 4.3.4). Catalytic hydrogenation (H_2, 10% Pd-C) of azido ester **177** gave nona-amino ester **178**, which was not further purified since it was directly *N*-chloroacetylated as previously [$(ClCH_2CO)_2O$, Et_3N], to give **179** in 78% yield. The extent of reduction was ascertained by IR spectroscopy wherein the sensitive azide frequency (2106 cm^{-1}) could be monitored.

Electrophilic nona-*N*-chloroacetylated dendrimer **179** was suitable for nucleophilic substitution with sialic acid thiol **133** (11 equiv) as described earlier for compounds **170** and **171**. The coupling was done at room temperature overnight (CH_3CN, Et_3N) under nitrogen to provide fully protected nona-sialoside dendrimer **180** in 86% yield as a colorless oil after purification by silica gel column chromatography (Scheme 4.3.4). The incorporation level of α-thiosialosides was evaluated as before by using ^{1}H NMR spectroscopy. The aromatic protons of the inner residue (δ 7.25 ppm) integrated for two protons when compared to either H-4 (δ 4.83 ppm) or H-3e (δ 2.71 ppm) of the sialic acid residues, which both integrated for nine protons (Figure 4.3.5). De-*O*-acetylation of glycodendrimer **180** was achieved under standard Zemplén conditions (NaOMe, MeOH, pH~9, 22°C, 45 min) to give compound **181** in quantitative yield. Methyl esters of **181**

were hydrolyzed following the procedure described above (aq. NaOH, MeOH), and unprotected nona-α-thiosialylated dendrimer **182** was obtained in quantitative yield.



Scheme 4.3.4 Synthesis of nonavalent gallic acid-based sialodendrimer.

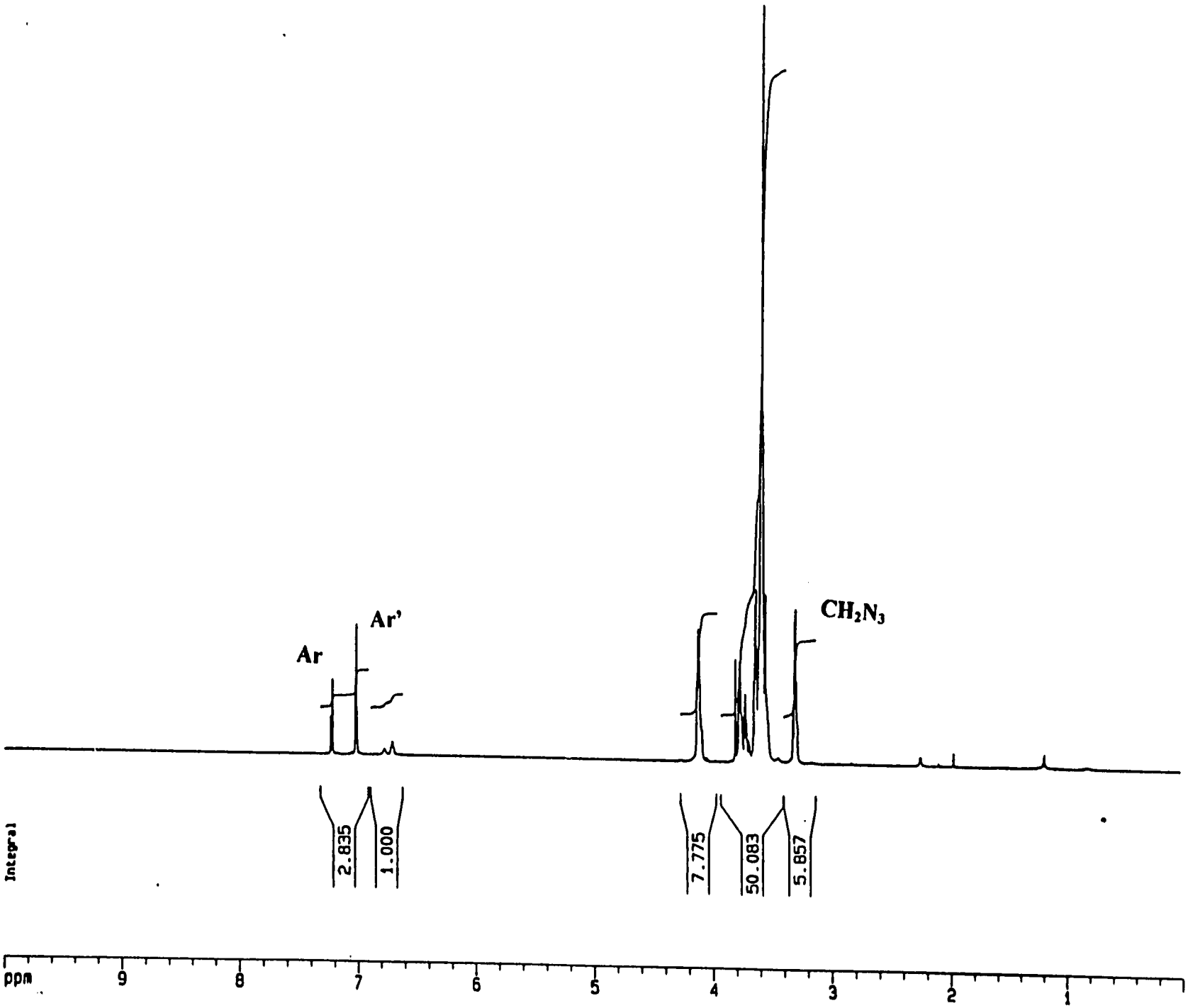


Figure 4.3.4. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of azido nonavalent gallic acid-based dendrimer **177**.

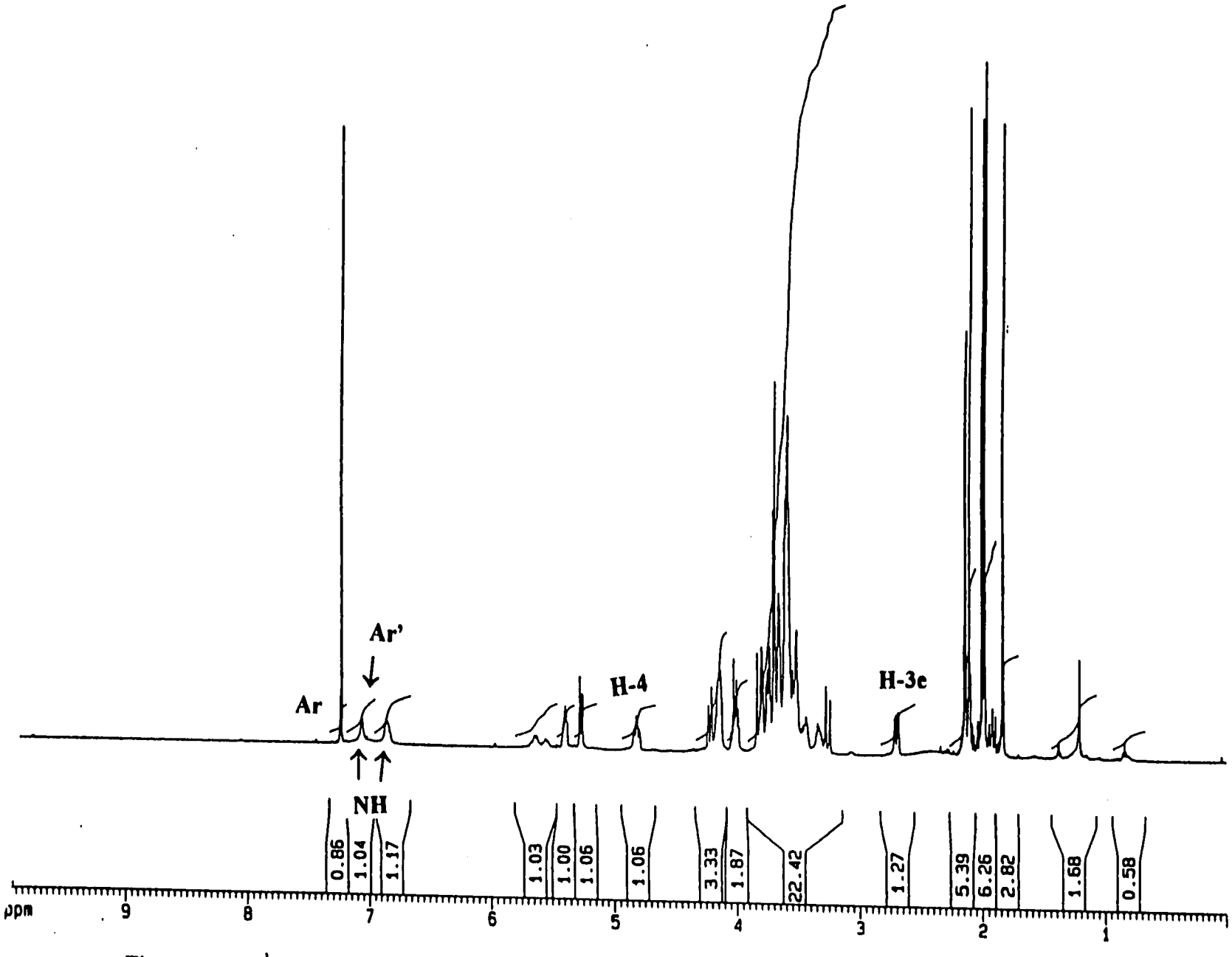


Figure 4.3.5. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of nonavalent gallic acid-based sialodendrimer **180**.

4.4 Lectin Binding

Preliminary lectin-binding experiments were achieved using microtiter plate turbidimetric analysis[216] wherein solutions of lectin-containing trimeric **174** or **175** and nonameric **182** sialodendrimers were allowed to form insoluble cross-linked complexes that could be monitored at 490 nm (nephelometry). To this end, two different lectins known to bind α-sialosides were used. Wheat germ agglutinin (WGA)[217] and the lectin from the slug *Limax flavus* (LFA)[218] were used as models. Both lectins are divalent and can therefore form linear one-dimensional (sometimes soluble) or multidimensional cross-linked lattices.[219] The results from the time course of turbidimetric analysis are shown in Figure 4.4.1. Interestingly, 9-mer α-thiosialoside **182** showed a clear and rapid cross-linked insoluble complex with both WGA and LFA lectins. This last result with LFA is similar to that previously observed with analogous multivalent α-thiosialosides scaffolded on a 3,3'-iminobis(propylamine) core.[124,125] It differs, however, from both previous studies[124,125] and the present WGA binding in that the complex seems less stable since there is a steady decrease of cross-linking after the initial 10 min of reaction. Additionally, trimeric α-thiosialosides **174** and **175**, only differing by the length of their ethylene glycol spacers, showed no stable insoluble complexes with either lectin. These results can be rationalized by the low valency of the trimers which fail to form multidimensional cross-linked complexes with the dimeric lectins. This implies that the third arm of the dendrimers does not allow further cross-linking with another lectin molecule. Further work to determine the intrinsic binding abilities of each sialodendrimer by inhibition assays would be needed. This work would supply the scientific community with a better understanding of carbohydrate-protein interactions of glycodendrimers varying in shape,

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[216] Singer, J. M.; Hengevels, J.; Edberg, S. C.; Lichtenbelt, J. W. T.; Wiersema P. H. *Immunol. Commun.* **1977**, *6*, 517.

[217] Peters, B. P.; Ebisu, S.; Goldstein, I. J.; Flashner, M. *Biochemistry* **1979**, *24*, 5505.

[218] Knibbs, R. N.; Osborne, S. E.; Glick, G. D.; Goldstein, I. J. *J. Biol. Chem.* **1993**, *268*, 18524.

[219] Brewer, C. F. *Chemtracts-Biochem. Molec. Biol.* **1996**, *6*, 165.

size, conformation (rigidity), and valency. As antigen presentation is becoming crucial in the design of potent carbohydrate ligands, which can be used as antiadhesins and as vectors for drug delivery, this further work becomes very important.

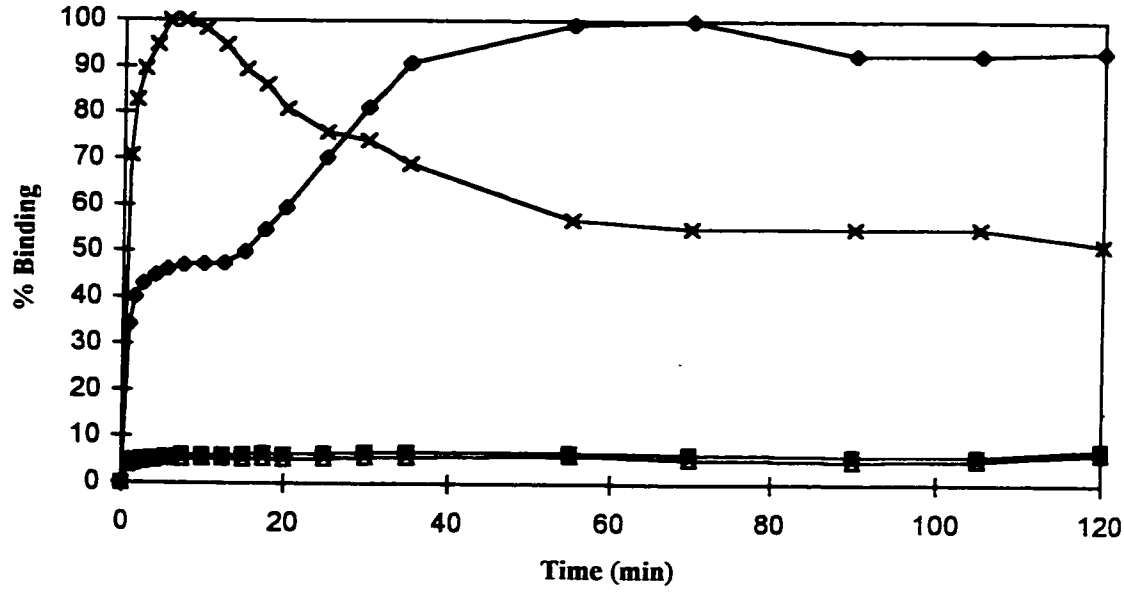

Figure 4.4.1 Time course of turbidimetric analysis of Wheat germ agglutinin (WGA) with 9-mer α-thiosialoside **182** (◆), trimers **174** (Δ) and **175** (■), and *Limax flavus* lectin (LFA) with **182** (X).

4.5 Conclusion

Hyperbranched glycodendrimers containing sialic acid residues were synthesized in good yields. Gallic acid **162** as the trivalent core and oligoethylene glycol derivatives as hydrophilic spacers proved to be very useful scaffolds for dendrimers. The fully deprotected glycodendrimers were shown to be water-soluble. The ability of these dendrimers to bind and cross-link sialic acid selective plant (phytohemagglutinins) and

animal lectins from wheat germ agglutinin and from the slug *Limax flavus* was also demonstrated. The preliminary immunoassay results indicated that the nonavalent α-sialodendrimer **182** has a greater affinity towards the above mentioned lectins than the corresponding trivalent glycodendrimers **174** and **175**.

4.6 Experimental Methods

4.6.1 Synthesis

1,8-Bis-[(p-toluenesulfonyl)oxy]-3,6-dioxaoctane (121)

A solution of triethylene glycol (50.0 g, 0.33 mol) in anhydrous diethyl ether (300 mL) was cooled to 0°C under a nitrogen atmosphere. Triethylamine (50 mL) was added followed by *p*-toluenesulfonyl chloride (68.84 g, 0.36 mol) over a 1 h period, and the solution was warmed to room temperature. After stirring for 3 h, the reaction mixture was evaporated in vacuo. The resulting residue was dissolved in CH_2Cl_2 and washed successively with saturated sodium bicarbonate solution (50 mL) and twice with water (50 mL). The organic layer was dried using anhydrous sodium sulfate and concentrated under vacuum to afford 120 g (79%) of **121** as a white solid. After filtration, the remaining crude product was purified by silica gel column chromatography using ethyl acetate/hexanes (1:1, v/v) as eluent to afford 16 g (11%) of **121** (total yield 90%): mp 82-84°C (EtOH); (lit.[220] mp 81-82°C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.75 (d, 4H, J 8.3 Hz, H_{ar}), 7.31 (d, 4H, J 8.3 Hz, H_{ar}), 4.11 (t, 4H, J 4.8 Hz, TsOCH_2), 3.62 (t, 4H, J 4.8 Hz, TsOCH$_2CH_2$), 3.49 (s, 4H, OCH_2), 2.41 (s, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 144.8, 132.8, 129.8, 127.9 (C_{ar}), 70.6 (TsOCH_2), 69.2 (CH_2O), 68.6 (CH_2O), 21.6 (CH_3); MS (CI ether, rel. intensity) m/z: 459 ($[M+H]^+$, 36%), 287 (74).

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[220] *Beilstein Handbuch, 10*, 483.

1,11-Bis-[(p-toluenesulfonyl)oxy]-3,6,9-trioxaundecane (160)

An identical procedure was used to synthesize compound **160** in 81% yield, the difference being that the final product was a colorless oil that was purified by silica gel column chromatography: ^{1}H NMR ($CDCl_3$) δ (ppm): 7.76 (d, 4H, J 8.2 Hz, H_{ar}), 7.31 (d, 4H, J 8.2 Hz, H_{ar}), 4.12 (t, 4H, J 4.8 Hz, TsO*CH*$_2$), 3.64 (t, 4H, J 4.8 Hz, TsOCH$_2$*CH*$_2$), 3.53 (s, 8H, OCH_2), 2.41 (s, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 144.6, 132.6, 129.6, 127.7 (C_{ar}), 70.4, 70.2, 69.1, 68.4 (CH_2O), 21.4 (CH_3); MS (CI ether, rel. intensity) m/z: 503 ($[M+H]^+$, 26%), 331 (46).

1-Azido-8-[(p-toluenesulfonyl)oxy]-3,6-dioxaoctane (122)

To a solution of 500 mg (1.09 mmol) of bistosylate **121** in 95% ethanol (10 mL) was added sodium azide (35.4 mg, 0.545 mmol). The reaction was stirred under reflux for 4 h and monitored by TLC. After cooling the reaction mixture to room temperature and removing the precipitated solid, the filtrate was evaporated under reduced pressure. The oily residue was dissolved in diethyl ether and washed twice with water and brine. The dried organic phase (Na_2SO_4) was concentrated and the crude product purified by silica gel chromatography using a gradient of 1:5 to 1:1 (v/v) ethyl acetate/hexanes as eluent to afford 157.6 mg (44%) of **122** as a colorless oil: IR ν_{max} (cm^{-1}): 3015, 2904, 2108, 1738, 1598; ^{1}H NMR ($CDCl_3$) δ (ppm): 7.75 (d, 2H, J 8.3 Hz, H_{ar}), 7.32 (d, 2H, J 8.3 Hz, H_{ar}), 4.12 (t, 2H, J 4.7 Hz, TsO*CH*$_2$), 3.68-3.56 (m, 8H, OCH_2), 3.33 (t, 2H, CH_2N_3), 2.41 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 144.6, 132.6, 129.6, 127.6 (C_{ar}), 70.4, 70.2, 69.7, 69.1, 68.4 (CH_2O), 50.3 (CH_2N_3), 21.3 (CH_3); MS (CI ether, rel. intensity) m/z: 330 ($[M+H]^+$, 3%), 302 (61).

Anal. Calcd. for $C_{13}H_{19}N_3O_5S$: C 47.40, H 5.81, N 12.76; found: C 47.23, H 6.00, N 12.96.

1-Azido-11-[(p-toluenesulfonyl)oxy]-3,6,9-trioxaundecane (161)

Compound **161**, prepared under the same conditions described above for **122**, was obtained as an oily residue in 39% yield (15.2 g): IR ν_{max} (cm^{-1}): 3015, 2930, 2110, 1727, 1599; ^1H NMR ($CDCl_3$) δ (ppm): 7.72 (d, 2H, J 8.3 Hz, H_{ar}), 7.27 (d, 2H, J 8.3 Hz, H_{ar}), 4.12 (t, 2H, J 4.8 Hz, TsO*CH*$_2$), 3.63-3.52 (m, 12H, OCH_2), 3.31 (t, 2H, CH_2N_3), 2.39 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 144.7, 132.7, 129.7, 127.8 (C_{ar}), 70.6, 70.5, 70.4, 69.9, 69.2, 68.5 (CH_2O), 50.5 (CH_2N_3), 21.5 (CH_3); MS (CI ether, rel. intensity) m/z: 374 ($[M+H]^+$, 3%), 346 (67).

Methyl 3,4,5-trihydroxybenzoate (163)

Gallic acid **162** (3.4g, 20 mmol) was treated with thionyl chloride (10 mL) at room temperature for 2 h after which time the solvent was removed under vacuum. The oily residue was cooled down using an ice bath followed by addition of methanol (10 mL). The mixture was allowed to react for 30 min and concentrated in vacuo. The crude residue was recrystallized from ethanol to afford **163** (3.6 g, 98%) as a white solid. Compound **163** has mp 199.5-201°C (EtOH); (lit.[221] mp 199-201°C); ^1H NMR (D_2O) δ (ppm): 7.06 (s, 2H, H_{ar}), 3.86 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ (ppm): 164.9 (C=O), 144.2, 137.0, 117.1, 107.1 (C_{ar}), 50.2 (OCH_3); HREIMS Calcd. for $C_8H_8O_5$: 184.0367; found: 184.0372, 15.4%.

Methyl 3,4,5-tri-(8-azido-3,6-dioxaoctyloxy)benzoate (164)

To a solution of methyl gallate **163** (184 mg, 1 mmol) in DMF (25 mL) was added potassium carbonate (1.4 g, 10 mmol) and tosylate **122** (1.3 g, 4.0 mmol) dissolved in DMF (5 mL). The mixture was stirred at 80°C for 24 h and allowed to cool to room

[221] Haas, G. J.; Zentner, M. R. *J. Am. Pharm. Assoc.* **1954**, *43*, 635.

temperature. The solid was filtered off and the filtrate was evaporated under vacuum, co-evaporated with *tert*-butanol, and the remaining residue was partitioned between CH_2Cl_2 and water. The organic layer was separated and washed further with water, saturated NaCl solution, and dried over sodium sulfate. After removing the solvent, the crude material was purified by silica gel chromatography using a gradient of 1:3 to 2:1 (v/v) ethyl acetate/hexanes to give 426 mg (65%) of **164** as a pure colorless oil: IR ν_{max} (cm^{-1}): 3018, 2901, 2107, 1717, 1587; ^1H NMR ($CDCl_3$) δ (ppm): 7.27 (s, 2H, H_{ar}), 4.23-4.15 (m, 6H, PhO*CH*$_2$), 3.86 (s, 3H, OCH_3), 3.88-3.61 (m, 24H, OCH_2), 3.35 (t, 6H, J 5.0 Hz, CH_2N_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 166.5 (C=O), 152.2, 142.4, 124.9, 108.9 (C_{ar}), 72.4, 72.3, 70.8, 70.7, 70.5, 70.0, 69.9, 69.6, 68.8 (CH_2O), 52.1 (OCH_3), 50.6 (CH_2N_3); MS (FAB thioglycerol, rel. intensity) m/z: 656 ($[M+H]^+$, 11%), 628 (14).

Anal. Calcd. for $C_{26}H_{41}N_9O_{11}$: C 47.62, H 6.30, N 19.23; found: C 47.69, H 6.22, N 18.97.

Methyl 3,4,5-tri-(11-azido-3,6,9-trioxaundecyloxy)benzoate (165)

Compound **165** was obtained, the same way as previously used for **164**, as a colorless oil in 68% yield (498 mg): IR ν_{max} (cm^{-1}): 3009, 2948, 2106, 1717, 1586; ^1H NMR ($CDCl_3$) δ (ppm): 7.26 (s, 2H, H_{ar}), 4.22-4.14 (m, 6H, PhO*CH*$_2$), 3.85 (s, 3H, OCH_3), 3.87-3.61 (m, 36H, OCH_2), 3.35 (t, 6H, CH_2N_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 166.2 (C=O), 152.0, 142.2, 124.6, 108.6 (C_{ar}), 72.1, 70.4, 70.3, 70.3, 69.7, 69.3, 68.5 (CH_2O), 51.8 (OCH_3), 50.3 (CH_2N_3); MS (FAB thioglycerol, rel. intensity) m/z: 788 ($[M+H]^+$, 2%), 760 (4).

Anal. Calcd. for $C_{32}H_{53}N_9O_{14}$: C 48.79, H 6.78, N 16.00; found: C 49.00, H 7.00, N 16.06.

Methyl 3,4,5-tri-(8-amino-3,6-dioxaoctyloxy)benzoate (166)

To a solution of azide **164** (132 mg, 0.206 mmol) in ethanol (10 mL) was added 50 mg of 10% palladium on carbon. Hydrogen was constantly bubbled through the

solution at room temperature and the reaction was monitored by IR (complete disappearance of azide peak). After 3 h, the catalyst was removed by filtration and the filtrate was concentrated under vacuum. The crude product obtained was used in the next step without further purification: IR ν_{max} (cm^{-1}): 3450, 3020, 2921, 1716, 1587; MS (FAB thioglycerol, rel. intensity) m/z: 579 ($[M+H]^+$, 32%).

Methyl 3,4,5-tri-(11-amino-3,6,9-trioxaundecyloxy)benzoate (167)

Following the procedure described previously, trisamino **167** was obtained as a yellowish syrup in 93% crude yield (128 mg). The product was purified by silica gel chromatography using 10% MeOH in CH_2Cl_2 as eluent to give pure **167** in 65% yield (89.5 mg): IR ν_{max} (cm^{-1}): 3369, 3050, 2897, 1715, 1586; ^1H NMR ($CDCl_3$) δ (ppm): 7.25 (s, 2H, H_{ar}), 4.16 (m, 6H, PhO*CH*$_2$), 3.85 (s, 3H, OCH_3), 3.84-3.54 (m, 30H, OCH_2), 3.45 (t, 6H, J 5.4 Hz, OCH_2), 2.82 (t, 6H, J 5.4 Hz, *CH*$_2NH_2$), 1.29 (m, 6H, NH_2); ^{13}C NMR ($CDCl_3$) δ (ppm): 166.4 (C=O), 152.1, 142.3, 124.8, 108.8 (C_{ar}), 73.3, 72.2, 70.7, 70.5, 70.4, 70.3, 70.1, 69.4, 68.6 (CH_2O), 52.0 (OCH_3), 41.6 (CH_2NH_2); MS (FAB thioglycerol, rel. intensity) m/z: 710 ($[M+H]^+$, 45%).

Methyl 3,4,5-tri-[8-(2-chloroacetamido)-3,6-dioxaoctyloxy]benzoate (168)

To a cooled solution (0°C) of trisamine **166** (119 mg, 0.206 mmol) in ethanol (10 mL) was added triethylamine (0.5 mL) and chloroacetic anhydride (132 mg, 0.772 mmol) in small portions over a 30 min period. The mixture was then stirred at room temperature for 2 h after which time the solvent was evaporated under vacuum. The reaction was monitored by a ninhydrin test. The oily residue was dissolved in H_2O and extracted with CH_2Cl_2. The organic extracts were dried (Na_2SO_4), concentrated under vacuum to give crude **168**, which was purified by silica gel chromatography using 5% methanol in CH_2Cl_2 as eluent to afford 108 mg (65%) of **168** as a colorless oil: IR ν_{max} (cm^{-1}): 3316, 3015,

2904, 1715, 1668, 1589; ^{1}H NMR ($CDCl_3$) δ (ppm): 7.25 (s, 2H, H_{ar}), 7.18 (m, 3H, NH), 4.22-4.15 (m, 6H, PhOCH_2), 4.01 (s, 6H, CH_2Cl), 3.85 (s, 3H, OCH_3), 3.85-3.40 (m, 30H, OCH_2 and CH_2NH); ^{13}C NMR ($CDCl_3$) δ (ppm): 166.3, 165.9 (C=O), 152.0, 142.0, 124.9, 108.6 (C_{ar}), 72.2, 70.5, 70.4, 70.3, 70.1, 69.4, 69.2, 68.6 (CH_2O), 52.0 (OCH_3), 42.4 (CH_2NH), 39.4 (CH_2Cl); MS (FAB thioglycerol, rel. intensity) m/z: 808 ($[M+H]^+$, 14%), 810 (5).

Anal. Calcd. for $C_{32}H_{50}N_3O_{14}Cl_3$; C 47.62, H 6.24, N 5.21; found: C 47.80, H 6.40, N 5.05.

Methyl 3,4,5-tri-[11-(2-chloroacetamido)-3,6,9-trioxaundecyloxy]benzoate (169)

Compound **169** was obtained from the amino **167** by the same procedure described above. The pure product was obtained in 62% yield as a colorless syrup. IR ν_{max} (cm^{-1}): 3425, 3035, 2904, 1714, 1671, 1588; ^{1}H NMR ($CDCl_3$) δ (ppm): 7.26 (s, 2H, H_{ar}), 7.08-7.04 (m, 3H, NH), 4.18 (t, 2H, J 4.9 Hz, PhOCH_2), 4.16 (t, 4H, J 5.0 Hz, PhOCH_2), 4.00 (s, 6H, CH_2Cl), 3.84 (s, 3H, OCH_3), 3.82 (t, 4H, OCH_2), 3.76 (t, 2H, OCH_2), 3.70-3.66 (m, 6H, OCH_2), 3.64-3.57 (m, 18H, OCH_2), 3.54 (t, 6H, OCH_2), 3.47-3.44 (m, 6H, CH_2NH); ^{13}C NMR ($CDCl_3$) δ (ppm): 166.4, 166.0 (C=O), 152.2, 142.2, 125.0, 108.8 (C_{ar}), 72.4, 70.8, 70.6, 70.5, 70.3, 69.6, 69.3, 68.7, (CH_2O), 52.2 (OCH_3), 42.6 (CH_2NH), 39.5 (CH_2Cl); HR-FABMS Calcd. for $C_{38}H_{63}N_3O_{17}Cl_3$: 938.330; found: 938.3223, 99 %).

Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulo-pyranosidonate (133)

To a solution of thioacetylated sialic acid **113** (63.1 mg, 0.115 mmol) in dry methanol (1.5 mL) cooled to -40°C, was added 1 M sodium methoxide in methanol (109 μL, 0.109 mmol). The reaction, monitored by TLC, took less than 15 min after which time the reaction mixture was treated with H^+ resin (Amberlite IR-120) at -40°C for 15

min. The solution was filtered and evaporated at room temperature under reduced pressure to afford **133**, which was used without further purification. $[\alpha]_D$ +15.6° (*c* 0.99, $CHCl_3$); ^1H NMR ($CDCl_3$) δ (ppm): 5.33-5.28 (m, 2H, H-7 and H-8), 5.17 (d, 1H, $J_{NH,5}$ 10.5 Hz, NH), 4.91 (ddd, 1H, $J_{4,5}$ 10.3 Hz, $J_{3a,4}$ 11.7, $J_{3e,4}$ 4.8, H-4), 4.49 (dd, 1H, $J_{8,9'}$ 2.1 Hz, $J_{9,9'}$ 12.4, H-9'), 4.10 (dd, 1H, $J_{8,9}$ 5.5 Hz, H-9), 4.06 (m, 1H, H-5), 3.83 (s, 3H, OCH_3), 3.73 (dd, 1H, $J_{6,7}$ 1.9 Hz, $J_{5,6}$ 10.8, H-6), 3.17 (s, 1H, SH), 2.80 (dd, 1H, $J_{3e,3a}$ 12.8 Hz, H-3e), 2.14, 2.13, 2.05, 2.03 (4s, 12H, 4 OAc), 2.04 (dd, 1H, H-3a), 1.88 (s, 3H, NAc); ^{13}C NMR ($CDCl_3$) δ (ppm): 170.9, 170.6, 170.4, 170.4, 170.1, 170.0 (6C=O), 81.6 (C-2), 75.1 (C-6), 70.1 (C-8), 69.4 (C-4), 67.7 (C-7), 62.2 (C-9), 53.4 (OCH_3), 49.1 (C-5), 38.9 (C-3), 23.1 (NAc), 21.1, 20.8, 20.8 (4 OAc); MS (CI ether, rel. intensity) m/z: 508 ($[M+H]^+$, 100%), 476 (85).

Methyl 3,4,5-tri-{8-[2-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulo-pyranosid)-acetamido]-3,6-dioxaoctyloxy}benzoate (170)

To a solution of α-thiosialoside **133** (75.4 mg, 0.149 mmol) in dry acetonitrile (4.5 mL) was added Et_3N (50 μL, 0.358 mmol) and trischloroacetamide precursor **168** (30 mg, 0.037 mmol). The mixture was stirred at room temperature overnight under a nitrogen atmosphere. The solution was concentrated under vacuum, and the crude residue was purified by preparative TLC plate using, first, ethyl acetate, followed by 7% methanol in CH_2Cl_2 as eluent. Pure **170** was extracted using 5% methanol in CH_2Cl_2 and, after evaporation, the residue was dissolved in ethyl acetate and filtered to remove soluble impurities from silica gel. The colorless oil was obtained in 85% (70.7 mg) yield: $[\alpha]_D$ +24.2° (*c* 1.02, $CHCl_3$); ^1H NMR ($CDCl_3$) δ (ppm): 7.27 (s, 2H, H_{ar}), 6.89 (m, 3H, NH), 5.44 (m, 6H, H-8 and NH), 5.30 (dd, 3H, $J_{6,7}$ 2.0 Hz, $J_{7,8}$ 9.0, H-7), 4.84 (ddd, 3H, $J_{3a,4}$ 11.6 Hz, $J_{3e,4}$ 4.5, H-4), 4.25 (dd, 3H, $J_{8,9'}$ 2.8 Hz, H-9'), 4.21-4.17 (m, 6H, PhOCH_2), 4.06-4.01 (m, 6H, H-5 and H-9), 3.87 (s, 3H, OCH_3), 3.84 (t, 6H, J 4.8 Hz, OCH_2), 3.78-3.34 (m, 30H, OCH_2, CH_2N, CH_2S and H-6), 3.73 (s, 9H, OCH_3), 3.29 (d, 3H, J 16.2 Hz,

CH_bS), 2.72 (dd, 3H, $J_{3e,3a}$ 12.7 Hz, $J_{3e,4}$ 4.5, H-3e), 2.17, 2.13, 2.03, 2.01 (4s, 36H, 4 OAc), 1.95 (dd, 3H, H-3a), 1.86 (s, 9H, NAc); ^{13}C NMR ($CDCl_3$) δ (ppm): 171.1, 170.8, 170.8, 170.7, 170.4, 170.4, 170.1, 168.6, 168.5, 168.4 (C=O), 152.2, 142.2, 125.0 (C_{ar}), 108.9 (C_{ar}-H), 82.1 (C-2), 73.9 (C-6), 72.3, 70.6, 70.5, 70.4, 70.2, 69.6, 69.6, 69.5, 68.7 (OCH_2), 69.4 (C-4), 68.0 (C-8), 67.0 (C-7), 62.3 (C-9), 53.1 (OCH_3), 52.2 (OCH_3), 49.1 (C-5), 39.7 (CH_2N), 37.3 (C-3), 32.4 (CH_2S), 23.1 (NAc), 21.3, 20.8, 20.8, 20.7 (4 OAc); MS (FAB thioglycerol, rel. intensity) m/z: 2223 ($[M+H]^+$, 20%).

Anal. Calcd. for $C_{92}H_{134}N_6O_{50}S_3$; C 49.77, H 6.08, N 3.79; found: C 49.80, H 6.43, N 3.63.

Methyl 3,4,5-tri-{11-[2-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulo-pyranosid)-acetamido]-3,6,9-trioxaundecyloxy}benzoate (171)

The sialylated glycodendrimer **171** was obtained in 95% (71.4 mg) yield as a colorless oil in the same way as described for glycodendrimer **170**: $[\alpha]_D$ +22.4° (*c* 1.01, $CHCl_3$); ^1H NMR ($CDCl_3$) δ (ppm): 7.27 (s, 2H, H_{ar}), 6.83 (m, 3H, NH), 5.41 (ddd, 3H, $J_{8,9'}$ 2.7 Hz, H-8), 5.34 (d, 3H, $J_{NH,5}$ 10.0 Hz, NH), 5.29 (dd, 3H, $J_{6,7}$ 2.1 Hz, $J_{7,8}$ 9.2, H-7), 4.84 (ddd, 3H, $J_{4,5}$ 10.4 Hz, $J_{3a,4}$ 11.6, $J_{3e,4}$ 4.6, H-4), 4.24 (dd, 3H, $J_{8,9'}$ 2.7 Hz, $J_{9,9'}$ 12.4, H-9'), 4.20-4.16 (m, 6H, PhOCH_2), 4.06-4.02 (m, 3H, H-5), 4.03 (dd, 3H, $J_{8,9}$ 5.8 Hz, H-9), 3.87 (s, 3H, OCH_3), 3.83 (t, 4H, J 4.9 Hz, OCH_2), 3.79-3.75 (m, 2H, OCH_2), 3.76 (dd, 3H, $J_{5,6}$ 10.7 Hz, H-6), 3.73 (s, 9H, OCH_3), 3.70-3.66 (m, 6H, OCH_2), 3.64-3.60 (m, 18H, OCH_2), 3.54 (d, 3H, J 16.0 Hz, CH_aS), 3.58-3.32 (m, 12H, CH_2CH_2N and CH_2CH_2N), 3.28 (d, 3H, J 16.0 Hz, CH_bS), 2.72 (dd, 3H, $J_{3e,3a}$ 12.7 Hz, $J_{3e,4}$ 4.6, H-3e), 2.16, 2.12, 2.02, 2.00 (4s, 36H, 4 OAc), 1.95 (dd, 3H, H-3a), 1.85 (s, 9H, NAc); ^{13}C NMR ($CDCl_3$) δ (ppm): 171.0, 170.7, 170.6, 170.6, 170.2, 170.0, 168.5, 168.2 (C=O), 152.2, 142.3, 124.9 (C_{ar}), 109.0 (C_{ar}-H), 82.1 (C-2), 73.9 (C-6), 72.3, 72.3, 70.7, 70.5, 70.5, 70.4, 70.2, 69.6, 69.5, 69.4, 69.4, 68.7 (C-4 and OCH_2), 67.9 (C-8), 67.0 (C-7),

62.3 (C-9), 53.1 (OCH_3), 52.2 (OCH_3), 49.1 (C-5), 39.6 (CH_2N), 37.3 (C-3), 32.4 (CH_2S), 23.1 (NAc), 21.3, 20.8, 20.8, 20.7 (4OAc); MS (FAB thioglycerol, rel. intensity) m/z: 2353 ($[M+H]^+$, 26%), 1879 (6), 1405 (2).

Anal. Calcd. for $C_{98}H_{146}N_6O_{53}S_3$; C 50.04, H 6.26, N 3.57; found: C 49.66, H 6.54, N 3.46.

Methyl 3,4,5-tri-{8-[2-(Methyl 5-acetamido-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulo-pyranosid)-acetamido]-3,6-dioxaoctyloxy}benzoate (172)

The dendritic α-thiosialoside **170** (45 mg, 0.020 mmol) was dissolved in dry methanol (3 mL) and a 1 M solution NaOMe/MeOH (pH~9) was added. The solution was stirred at room temperature for 45 min and then treated with H^+ resin (Amberlite IR-120). The filtrate was evaporated near dryness under reduced pressure and freeze dried to afford a white powder in quantitative yield. $[\alpha]_D$ +16.3° (*c* 0.49, CH_3OH); ^1H NMR (CD_3OD) δ (ppm): 7.30 (s, 2H, H_{ar}), 4.24-4.14 (m, 6H, PhO*CH*$_2$), 3.94-3.34 (m, 69H, OCH_3, OCH_2, OCH_3, H-4, H-5, H-6, H-7, H-8, H-9, H-9', CH_2S and CH_2N), 2.70 (dd, 3H, H-3e), 1.94 (s, 9H, NAc), 1.74 (dd, 3H, H-3a); ^{13}C NMR (CD_3OD) δ (ppm): 175.0, 171.5, 171.2, 168.1 (C=O), 153.7, 143.7, 126.3 (C_{ar}), 109.9 (C_{ar}-H), 83.6 (C-2), 77.2 (C-6), 73.6, 71.8, 71.4, 70.8, 70.4, 70.1 (OCH_2), 72.1 (C-8), 70.1 (C-4), 68.8 (C-7), 64.8 (C-9), 53.8 (C-5), 53.6 (OCH_3), 52.8 (OCH_3), 41.8 (C-3), 40.8 (CH_2N), 33.5 (CH_2S), 22.7 (NAc).

Methyl 3,4,5-tri-{11-[2-(Methyl 5-acetamido-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulo-pyranosid)-acetamido]-3,6,9-trioxaundecyloxy}benzoate (173)

Zemplén conditions as described above were applied to compound **171** to afford de-*O*-acetylated glycodendrimer **173** as a white foam (freeze-dried) in quantitative yield. $[\alpha]_D$ +14.4° (*c* 0.52, CH_3OH); ^1H NMR (CD_3OD) δ (ppm): 7.34 (s, 2H, H_{ar}), 4.25-4.11

(m, 6H, PhOCH_2), 3.88-3.85 (m, 9H, OCH_3 and PhOCH_2CH_2), 3.81-3.46 (m, 57H, OCH_2, H-4, H-5, H-7, H-8, H-9, H-9' and OCH_3), 3.50-3.45 (m, 9H, H-6 and CH_2S), 3.40-3.35 (m, 6H, CH_2N), 2.76 (dd, 3H, $J_{3e,3a}$ 12.8 Hz, $J_{3e,4}$ 4.5, H-3e), 1.98 (s, 9H, NAc), 1.80 (dd, 3H, H-3a); ^{13}C NMR (CD_3OD) δ (ppm): 174.9, 171.4, 171.0, 168.0 (C=O), 153.6, 143.5, 126.2 (C_{ar}), 109.9 (C_{ar}-H), 83.5 (C-2), 77.1 (C-6), 73.5, 71.6, 71.5, 71.5, 71.2, 70.7, 70.2, 70.0 (OCH_2), 72.0 (C-8), 70.1 (C-4), 68.7 (C-7), 64.7 (C-9), 53.8 (C-5), 53.6 (OCH_3), 52.7 (OCH_3), 41.7 (C-3), 40.7 (CH_2N), 33.4 (CH_2S), 22.6 (NAc).

3,4,5-Tri-{8-[2-(5-acetamido-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulo-pyranosid)-acetamido]-3,6-dioxaoctyloxy}benzoic acid (174)

A solution of α-thiosialoside methyl ester **172** (33 mg, 0.019 mmol), dissolved in a mixture of methanol (2 mL) and 0.5 M NaOH (2 mL), was stirred at room temperature overnight. After treatment with H^+ resin, the filtrate was first evaporated to remove most of the methanol and then freeze-dried. The pure product was obtained in nearly quantitative yield as a yellowish oil. $[\alpha]_D$ +3.4° (*c* 0.5, CH_3OH); ^1H NMR (CD_3OD) δ (ppm): 7.36 (s, 2H, H_{ar}), 4.24-4.20 (m, 6H, PhOCH_2), 3.88-3.43 (m, 51H, OCH_2, H-4, H-5, H-6, H-7, H-8, H-9, H-9' and CH_2S), 3.42-3.35 (m, 6H, CH_2N), 2.78 (dd, 3H, $J_{3e,3a}$ 12.2 Hz, $J_{3e,4}$ 4.3, H-3e), 1.99 (s, 9H, NAc), 1.81 (dd, 3H, H-3a); ^{13}C NMR (CD_3OD) δ (ppm): 175.2, 172.5, 171.2, 169.3 (C=O), 153.6, 143.7, 127.0 (C_{ar}), 110.1 (C_{ar}-H), 83.5 (C-2), 77.2 (C-6), 73.6, 71.8, 71.5, 71.3, 70.8, 70.1 (OCH_2), 72.5 (C-8), 70.3 (C-4), 69.0 (C-7), 64.5 (C-9), 53.6 (C-5), 42.0 (C-3), 40.8 (CH_2N), 33.7 (CH_2S), 22.7 (NAc).

3,4,5-Tri-{11-[2-(5-acetamido-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulo-pyranosid)-acetamido]-3,6,9-trioxaundecyloxy}benzoic acid (175)

The methyl esters hydrolysis of **173** was performed in the same manner as above to give a colorless oil after freeze-drying in quantitative yield. $[\alpha]_D$ + 5.5° (*c* 0.56, CH_3OH);

^{1}H NMR (CD_3OD) δ (ppm): 7.35 (s, 2H, H_{ar}), 4.25-4.20 (m, 6H, PhOCH_2), 3.90-3.40 (m, 63H, OCH_2, H-4, H-5, H-6, H-7, H-8, H-9, H-9' and CH_2S), 3.40-3.35 (m, 6H, CH_2N), 2.78 (dd, 3H, $J_{3e,3a}$ 12.6 Hz, $J_{3e,4}$ 4.3, H-3e), 1.99 (s, 9H, NAc), 1.82 (dd, 3H, H-3a); ^{13}C NMR (CD_3OD) δ (ppm): 175.1, 172.4, 171.1, 169.2 (C=O), 153.5, 143.5, 126.8 (C_{ar}), 110.1 (C_{ar}-H), 83.5 (C-2), 77.1 (C-6), 73.5, 71.6, 71.5, 71.4, 71.1, 70.7, 70.2, 69.9 (OCH_2), 72.4 (C-8), 70.0 (C-4), 68.9 (C-7), 64.5 (C-9), 53.5 (C-5), 41.8 (C-3), 40.6 (CH_2N), 33.6 (CH_2S), 22.6 (NAc).

3,4,5-Tri-(11-azido-3,6,9-trioxaundecyloxy)benzoic acid (176)

To a solution of methyl ester precursor **165** (170 mg, 0.215 mmol) in 95% EtOH (6 mL) was added 1 M KOH (400 μL). The reaction mixture was stirred and refluxed for 2 h after which time TLC showed complete hydrolysis of the ester. The solution was cooled to room temperature and neutralized with H^+ resin. The filtrate was concentrated under vacuum to afford 167 mg (quantitative yield) of pure acid **176** as a colorless residue. IR ν_{max} (cm^{-1}): 3009, 2903, 2109, 1690, 1587; ^{1}H NMR ($CDCl_3$) δ (ppm): 7.26 (s, 2H, H_{ar}), 4.15-4.10 (m, 6H, PhOCH_2), 3.83-3.78 (m, 6H, OCH_2), 3.66-3.57 (m, 30H, OCH_2), 3.31 (t, 6H, CH_2N_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 169.7 (C=O), 152.0, 142.6, 124.4, 109.1 (C_{ar}), 72.2, 70.6, 70.4, 70.4, 69.8, 69.4, 68.6 (CH_2O), 50.4 (CH_2N_3); MS (FAB thioglycerol, rel. intensity) m/z: 774 ($[M+H]^+$, 5 %), 746 (17).

Methyl 3,4,5-tri-{11-[3,4,5-tri-(11-azido-3,6,9-trioxaundecyloxy)-benzamido]-3,6,9-trioxaundecyloxy}benzoate (177)

To a solution of acid **176** (197 mg, 0.254 mmol) in a mixture of EtOH/CH_3CN (20 mL, 1:1 v/v) were added 1-hydroxybenzotriazole hydrate (HOBt, 52 mg, 0.381 mmol), trisamino precursor **167** (50 mg, 0.070 mmol), and diisopropylethylamine (DIPEA, Hünig's base, 89 μL). The mixture was stirred for 5 min under a nitrogen atmosphere

after which 73 mg (0.381 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) was added. The suspension was stirred for 3 h at room temperature, the solvent evaporated under vacuum, and the residue dissolved in $CHCl_3$. The organic layer was washed with saturated NH_4Cl, water, brine, and dried over Na_2SO_4. The crude product was concentrated and purified by silica gel chromatography using 5% MeOH in CH_2Cl_2 as eluent to afford 174 mg (83%) of **177** as a colorless oil: IR ν_{max} (cm^{-1}): 3430, 3025, 2899, 2108, 1713, 1653, 1585; ^1H NMR ($CDCl_3$) δ (ppm): 7.24 (s, 2H, H_{ar}), 7.02 (s, 6H, $H_{ar'}$), 6.78-6.71 (m, 3H, NH), 4.15-4.10 (m, 24H, Ph'OCH_2 and PhOCH_2), 3.82 (s, 3H, OCH_3), 3.80-3.56 (m, 150H, OCH_2), 3.31 (m, 18H, CH_2N_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 166.9, 166.5 (C=O), 152.5, 152.2, 142.5, 141.5, 129.7, 125.0 (C_{ar} and C'_{ar}), 109.1, 107.2 (C_{ar}-H and C'_{ar}-H), 72.4, 70.8, 70.7, 70.7, 70.6, 70.6, 70.5, 70.5, 70.2, 70.0, 69.8, 69.7, 68.6, 69.1, 68.8 (CH_2O and CH_2O'), 52.1 (OCH_3), 50.7 (CH_2N_3'), 39.9 (CH_2N).

Methyl 3,4,5-tri-{11-[3,4,5-tri-(11-amino-3,6,9-trioxaundecyloxy)-benzamido]-3,6,9-trioxaundecyloxy}benzoate (178)

Reduction of the nona-azide **177** was performed by the same method used to synthesize amino precursors **166** and **167**. Compound **178** was obtained by evaporation of the solvent and used without further purification for the next step. IR ν_{max} (cm^{-1}): 3358, 3243, 3042, 2896, 1716, 1647, 1582.

Methyl 3,4,5-tri-{11-[3,4,5-tri-(11-(2-chloroacetamido)-3,6,9-trioxaundecyloxy)-benzamido]-3,6,9-trioxaundecyloxy}benzoate (179)

The chloroacetylation was performed in the same manner as for compounds **168** and **169**. The oily residue was purified by preparative TLC plate to afford 70% yield of pure **179**: IR ν_{max} (cm^{-1}): 3344, 3035, 2903, 1717, 1670, 1558; ^1H NMR ($CDCl_3$) δ

(ppm): 7.24 (s, 2H, H_{ar}), 7.08-6.95 (m, 18H, $H_{ar'}$, NH and NH'), 4.30-4.13 (m, 24H, Ph'OCH_2 and PhOCH_2), 4.01, 4.01 (2s, 18H, CH_2Cl), 3.84 (s, 3H, OCH_3), 3.82-3.53 (m, 150H, OCH_2, OCH_2' and CH_2N), 3.47-3.43 (m, 18H, CH_2N'); ^{13}C NMR ($CDCl_3$) δ (ppm): 167.0, 166.5, 166.2 (C=O), 152.4, 152.2, 142.4, 141.2, 129.8, 125.1 (C_{ar} and C'_{ar}), 109.0, 107.2 (C_{ar}-H and C'_{ar}-H), 72.4, 70.7, 70.7, 70.6, 70.6, 70.5, 70.5, 70.5, 70.3, 70.2, 69.9, 69.7, 69.6, 69.4, 69.0, 68.8 (CH_2O and CH_2O'), 52.2 (OCH_3), 42.6 (CH_2N'), 39.9 (CH_2N), 39.6 (CH_2Cl).

Methyl 3,4,5-tri-{11-[3,4,5-tri-O-[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulo-pyranosid)-acetamido]-benzamido}-3,6,9-trioxaundecyl oxy}benzoate (180)

Conjugation between α-thiosialoside **133** and chloroacetylated monodendron **179** was performed under the same conditions described above for compounds **170** and **171**. The colorless oil obtained after silica gel chromatography was shown to be pure product **180** in 86% yield. $[\alpha]_D$ +19.0° (*c* 1.02, $CHCl_3$); ^1H NMR ($CDCl_3$) δ (ppm): 7.25 (s, 2H, H_{ar}), 7.07-7.03 (m, 9H, H'_{ar} and NH), 6.89-6.83 (m, 9H, N'H), 5.66-5.55 (m, 9H, NH), 5.41 (m, 9H, H-8), 5.28 (dd, 9H, $J_{7,8}$ 9.0 Hz, H-7), 4.83 (ddd, 9H, $J_{3a,4}$ 11.4 Hz, $J_{3e,4}$ 4.6, H-4), 4.23 (dd, 9H, $J_{8,9'}$ 2.5 Hz, $J_{9,9'}$ 12.4, H-9'), 4.20-4.15 (m, 24H, PhOCH_2' and PhOCH_2), 4.06-4.00 (m, 18H, H-5 and H-9), 3.85 (s, 3H, OCH_3), 3.84-3.79 (m, 18H, OCH_2), 3.77-3.31 (m, 159H, OCH_2, OCH_2', CH_2N, CH_2N' and H-6), 3.71 (s, 27H, OCH_3), 3.54 (d, 9H, J 16.1 Hz, CH_aS), 3.27 (d, 9H, J 16.1 Hz, CH_bS), 2.71 (dd, 9H, $J_{3e,3a}$ 12.6 Hz, $J_{3e,4}$ 4.4, H-3e), 2.15, 2.11, 2.01, 1.99 (4s, 108H, 4 OAc), 1.93 (dd, 9H, H-3a), 1.85 (s, 27H, NAc); ^{13}C NMR ($CDCl_3$) δ (ppm): 171.1, 170.7, 170.3, 170.1, 168.6, 168.4, 167.0, 166.5, 166.2 (C=O), 152.4, 152.2, 142.4,, 141.4, 129.7, 125.0 (C_{ar} and C'_{ar}), 109.1, 107.4 (C_{ar}-H and C'_{ar}), 82.2 (C-2), 73.9 (C-6), 72.4, 70.7, 70.6, 70.5, 70.3, 69.7, 69.1 (CH_2O and CH_2O'), 69.5 (C-4), 68.0 (C-8), 67.0 (C-7), 62.4 (C-9), 53.2 (OCH_3),

52.3 (OCH_3), 49.2 (C-5), 39.7 (CH_2N'), 39.7 (CH_2N), 37.4 (C-3), 32.5 (CH_2S), 23.1 (NAc), 21.3, 20.8, 20.8, 20.8 (4OAc).

Methyl 3,4,5-tri-{11-[3,4,5-tri-O-[(Methyl 5-acetamido-3,5-dideoxy-4,7,8,9-tetra-O-hydroxyl-2-thio-D-glycero-α-D-galacto-2-nonulo-pyranosid)-acetamido]-benzamido]-3,6,9-trioxaundecyl oxy}benzoate (181)

De-*O*-acetylation of **180** was done by using the Zemplén conditions described earlier for compounds **172** and **173**. After neutralization with H^+ resin, the solution was concentrated, dissolved in H_2O and freeze dried to give a yellowish half powder, half syrup compound in 98% yield. $[\alpha]_D$ +12.9° (*c* 1.05, CH_3OH); ^1H NMR (CD_3OD) δ (ppm): 7.28 (s, 2H, H_{ar}), 7.18 (s, 6H, H'_{ar}), 4.25-4.15 (m, 24H, PhOCH_2' and PhOCH_2), 3.87 (s, 3H, OCH_3), 3.86-3.32 (m, 276H, OCH_2, OCH_2', CH_2N, CH_2N', CH_2S, OCH_3, H-4, H-5, H-6, H-7, H-8, H-9' and H-9), 2.76 (dd, 9H, H-3e), 2.01 (s, 27H, NAc), 1.80 (dd, 9H, H-3a); ^{13}C NMR (CD_3OD) δ (ppm): 175.3, 171.5, 171.2, 169.4 (C=O), 153.8, 153.7, 143.1, 142.2, 130.6, 126.3 (C_{ar} and C'_{ar}), 109.9, 107.9 (C_{ar}-H and C'_{ar}), 83.7 (C-2), 77.1 (C-6), 72.1 (C-8), 73.7, 71.8, 71.7, 71.6, 71.3, 71.2, 70.8, 70.6, 70.3, 70.1 (CH_2O and CH_2O'), 70.2 (C-4), 68.8 (C-7), 64.8 (C-9), 53.9 (C-5), 53.8 (OCH_3), 53.0 (OCH_3), 41.8 (C-3), 41.2 (CH_2N), 40.9 (CH_2N'), 33.6 (CH_2S), 22.7 (NAc).

3,4,5-tri-{11-[3,4,5-tri-O-[(5-Acetamido-3,5-dideoxy-4,7,8,9-tetra-O-hydroxyl-2-thio-D-glycero-α-D-galacto-2-nonulo-pyranosid)-acetamido]-benzamido]-3,6,9-trioxaundecyloxy}benzoate (182)

The hydrolysis of the methyl esters on compound **181** was accomplished by treatment with NaOH as described before for the sialosylated glycodendrimers **174** and **175**. The yellowish residue was obtained after freeze-drying in nearly quantitative yield. $[\alpha]_D$ +5.2° (*c* 0.5, CH_3OH); ^1H NMR (CD_3OD) δ (ppm): 7.30 (s, 2H, H_{ar}), 7.18 (s, 6H,

H'$_{ar}$), 4.25-4.12 (m, 24H, PhOCH_2' and PhOCH_2), 3.94-3.33 (m, 249H, OCH_2, OCH_2', CH_2N, CH_2N', CH_2S, H-4, H-5, H-6, H-7, H-8, H-9' and H-9), 2.78 (dd, 9H, $J_{3e,4}$ 4.4 Hz, H-3e), 2.01 (s, 27H, NAc), 1.82 (dd, 9H, H-3a); ^{13}C NMR (CD_3OD) δ (ppm): 175.3, 172.4, 171.2, 169.5 (C=O), 153.7, 153.6, 143.7, 142.2, 130.6, 126.9 (C_{ar} and C'$_{ar}$), 110.3, 107.9 (C_{ar}-H and C'$_{ar}$), 83.6 (C-2), 77.2 (C-6), 72.5 (C-8), 73.7, 71.8, 71.7, 71.6, 71.5, 71.3, 70.8, 70.6, 70.3, 70.0 (CH_2O and CH_2O'), 70.1 (C-4), 69.0 (C-7), 64.6 (C-9), 53.7 (C-5), 42.0 (C-3), 41.2 (CH_2N), 40.8 (CH_2N'), 33.8 (CH_2S), 22.7 (NAc).

4.6.2 Turbidimetric Analysis

Turbidimetric experiments were performed in Linbro (Titertek) microtitration plates where 50 μL of stock lectin solutions prepared from WGA (4 mg/mL PBS) were mixed with 12.5, 11.2 and 10.6 μL (molar basis) of glycodendrimer solutions **182**, **175** and **174**, respectively (0.5 mg/mL PBS). Also, 25 μL of *L. flavus* lectin solution (2 mg/mL PBS) was mixed with 6.25 μL of 9-mer **182** (0.5 mg/mL PBS). The turbidity of the solutions was monitored by reading the optical density at 490 nm for 2 h. Each test was performed in triplicate.

Chapter 5

Polysialosides Scaffolded on *p-Tert*-Butylcalix[4]arene

5.1 Introduction

Chapter 4 demonstrated that sialylated dendrimers scaffolded onto multi-branched gallic acid afforded conjugates having the ability to strongly bind and cross link sialic acid selective plant and animal lectins.[117] Furthermore, many other glycoconjugates such as divalent cluster,[90,91,222] neoglycoproteins,[66,223] polymers,[21,22,64,84,87] and dendrimers[51,115,117] containing α-sialosides have been prepared[85] in order to study factors influencing carbohydrate-protein interactions and to provide high affinity ligands for potential anti-adhesion therapy. Although very promising, each of these conjugates has its own drawback spanning from low affinity, immunogenicity,[66] heterogeneity and synthetic complexity. As an extension of these finding, an interest was acquired for chemically well defined carbohydrate clusters of high affinity with the potential for cell targeting and drug delivery. To this end, it is described herein a novel prototype structure which has the intrinsic abilities for both carbohydrate scaffolding and drug inclusion complex formation.

Calix[n]arenes have been previously used as scaffolding elements for carbohydrate attachment.[53] However, previous syntheses afforded structures deprived of suitable spacer arm and of low water solubility. Since calix[n]arenes can be prepared with different ring sizes and conformations,[224] they represent an interesting family of precursors for the synthesis of biologically active carbohydrate clusters. Their built-in architecture allows desired carbohydrate orientations to be obtained easily.

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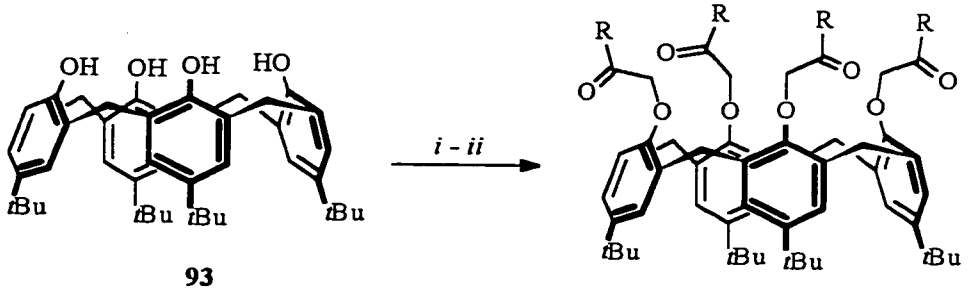
5.2 Divergent Synthesis of Tetravalent Sialocalix[4]arenes

Commercially available *p-tert*-butylcalix[4]arene **93** was transformed into the known tetraethyl ester **183** following slight modifications of the published procedure ($BrCH_2CO_2Et$, K_2CO_3, acetone, reflux, 85%).[225] Chromatographic purification followed by exhaustive aqueous washings were necessary to remove excess ethyl bromoacetate and cations encapsulated in the cavity formed by the four ionophoric OCH_2CO_2Et groups.[226] Compound **183**, having a fixed cone-shape conformation allowing carbohydrate orientation in only one direction, was hydrolyzed (1 M KOH, H_2O, EtOH, reflux, 95%) to form tetraacid **184**[227] in 95% yield (Scheme 5.2.1). The acid **184** was transformed into acid chloride **185** ($SOCl_2$, reflux, 2 h, 100%) which, after treatment with excess (5 equiv) mono-*N*-Boc-1,4-butanediamine or mono-*N*-Boc-1,6-hexanediamine (Et_3N, CH_2Cl_2, 0°C→22°C, 16 h), afforded key precursors **186** and **187** in 72 and 91% yield, respectively (Scheme 5.2.2).



93

183 R = OCH_2CH_3
184 R = OH

Scheme 5.2.1. Synthesis of *O*-alkylated calix[4]arene **185**. i) $BrCH_2CO_2Et$, K_2CO_3, acetone, N_2, reflux, 20 h, 85%; ii) 1M KOH, EtOH (1:1.1, v/v), reflux, 3.5 h, 95%.

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[225] Iwamoto, K.; Shinkai, S. *J. Org. Chem.*, **1992**, *57*, 7066.

[226] Arimura, T.; Kubota, M.; Matsuda, T.; Manabe, O.; Shinkai, S. *Bull. Chem. Soc. Jpn.*, **1989**, *62*, 1674.

[227] Arnaud-Neu, F.; Barrett, G.; Cremin, S.; Deasy, M.; Ferguson, G.; Harris, S.J.; Lough, A.J.; Lourdes, G.; McKervey, M.A.; Schwing-Weill, M.J.; Schwinte, P. *J. Chem. Soc., Perkin Trans. 2*, **1992**, 1119.

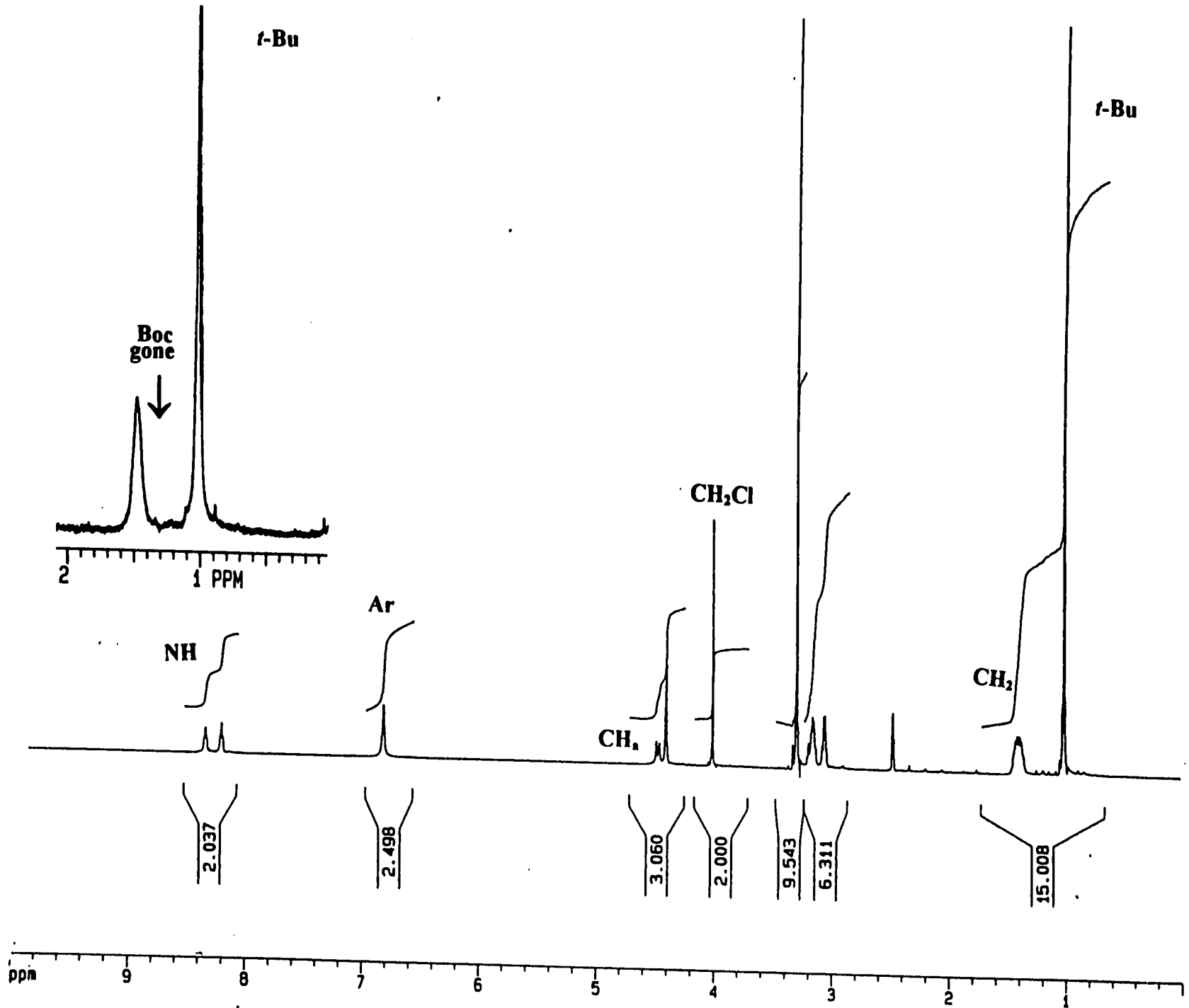
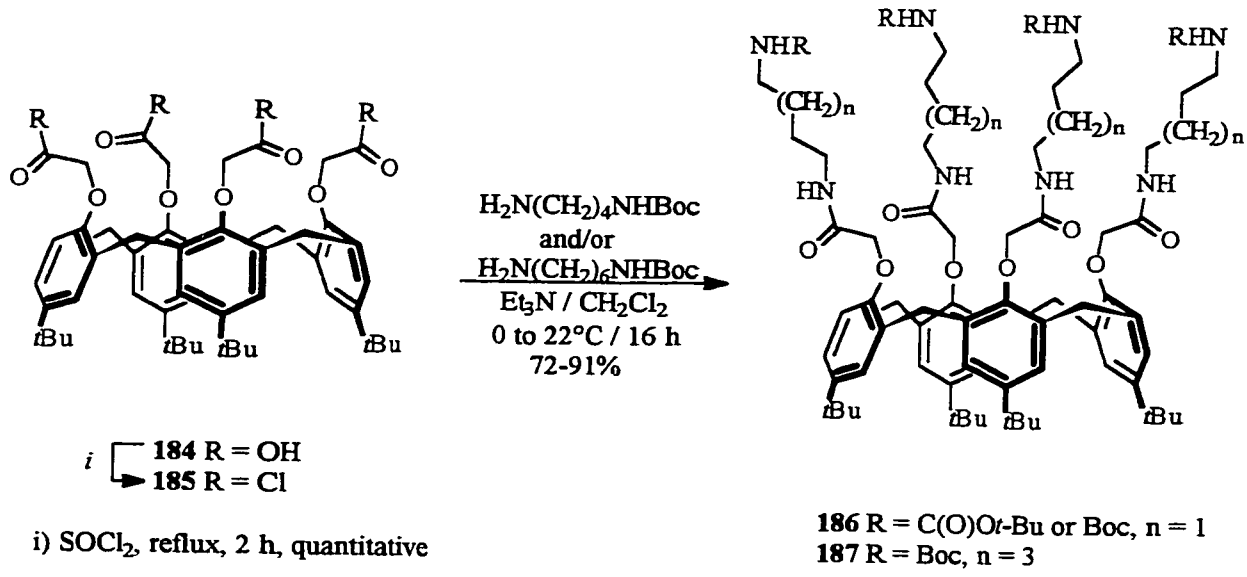


Figure 5.2.1. ^{1}H-NMR (DMSO-d_6, 500 MHz) spectrum of tetravalent *N*-chloroacetylcalix[4]arene **190** and 0-2 ppm region (inset, DMSO-d_6, 200 MHz) of tetravalent aminocalix[4]arene **188**.

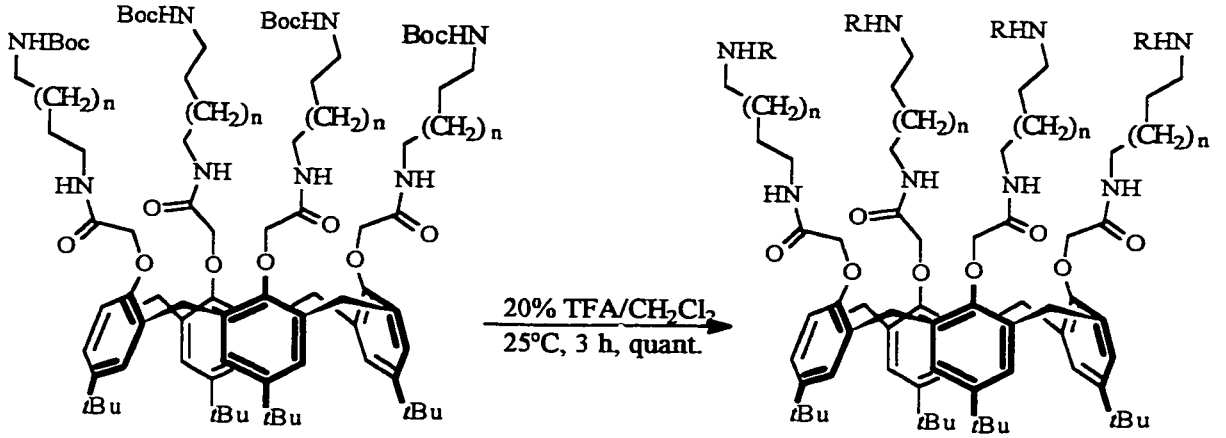
Boc-protecting groups of intermediates **186** and **187** were removed (20% TFA in CH_2Cl_2, 22°C, 3 h) to provide tetraamines **188** and **189** quantitatively (Scheme 5.2.3). Complete amine deprotection was monitored by ^{1}H-NMR spectroscopy (200 MHz, DMSO-d_6) which revealed the absence of any remaining *tert*-butyl signal (Boc) at 1.40 ppm (inset of Figure 5.2.1). Tetraamine calix[4]arenes **188** and **189** were then treated with chloroacetic anhydride (Et_3N, CH_2Cl_2, 22°C, 5 h) to give key electrophilic tetra-*N*-chloroacetylated calix[4]arenes **190** and **191** (70-80%) (Figure 5.2.1). Previous studies from this laboratory have established that *N*-chloroacetyl groups constitute efficient precursors for multivalent attachment of thiolated sugars.[51,115,117]



Scheme 5.2.2. Synthesis of tetravalent *N*-Boc protected amines **186** and **187**.

For the first glycocalix[4]arene prototype, α-thiolated sialoside was chosen as carbohydrate precursor since it had proven useful in dendrimer syntheses.[51,115,117] Thus, chemoselective de-*S*-acetylation (NaOMe, MeOH, 15 min, -40°C, 95%) and low temperature quenching (H^+ resin) of the known 1-thio-α-sialosyl acetate **113**[117] afforded

thiol intermediate **133** which was then coupled to tetra-*N*-chloroacetylated calix[4]arenes **190** and **191** (Et_3N, CH_3CN, 16 h, 22°C) to give sialylated calix[4]arenes **192** and **193** in 65 and 72% yield, respectively (Scheme 5.2.4). The extent of α-thio-sialoside incorporation was readily determined by ^{1}H-NMR spectroscopy. The total disappearance of the *N*-chloroacetyl signal at 4.01 ppm ($CDCl_3$) along with the integration of the well separated H-4 (4.86 ppm) and H-3e (2.72 ppm) sialosyl signals relative to the *tert*-butyl signal at 1.11 ppm showed complete incorporation of the four residues (Figure 5.2.2). Removal of the *O*-acetates from peracetylated ester **192** (cat. NaOMe, MeOH) gave **194** quantitatively which upon treatment with 1 M aqueous NaOH in EtOH (1:5, v/v, 16 h, 22°C) afforded fully deprotected α-sialylated calix[4]arene **196** in quantitative yield. Complete de-esterification of α-sialylated calix[4]arene **193** having the longer spacer arm was effected in one step by a treatment at room temperature with 0.5 M aqueous NaOH in EtOH (1:5, v/v, 16 h, 22°C). Fully deprotected α-sialylated calix[4]arene **197** was thus obtained as a lyophilized powder in quantitative yield (Scheme 5.2.4).



Scheme 5.2.3. Synthesis of *N*-chloroacetylated calix[4]arene derivatives **190** and **191**.

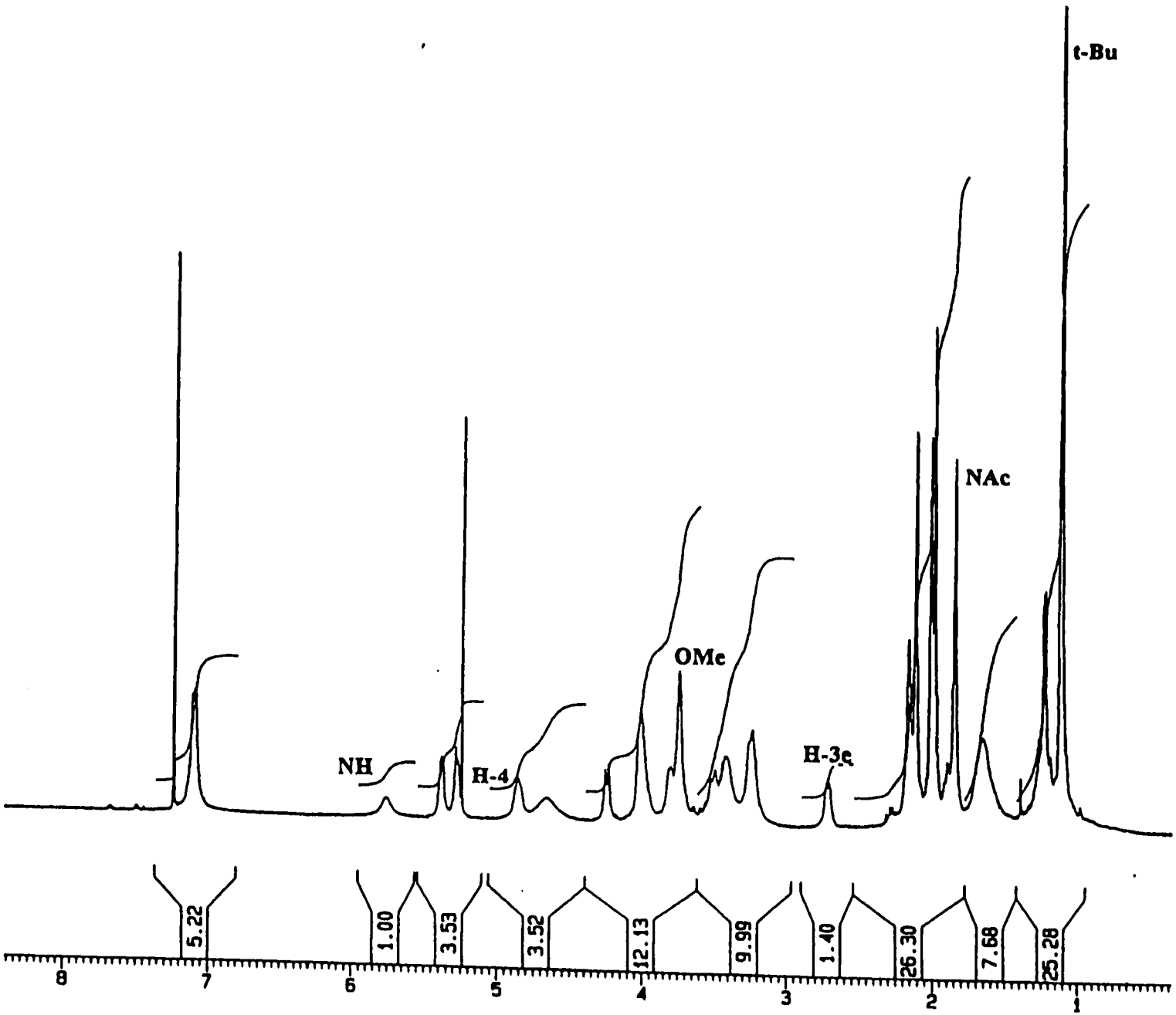
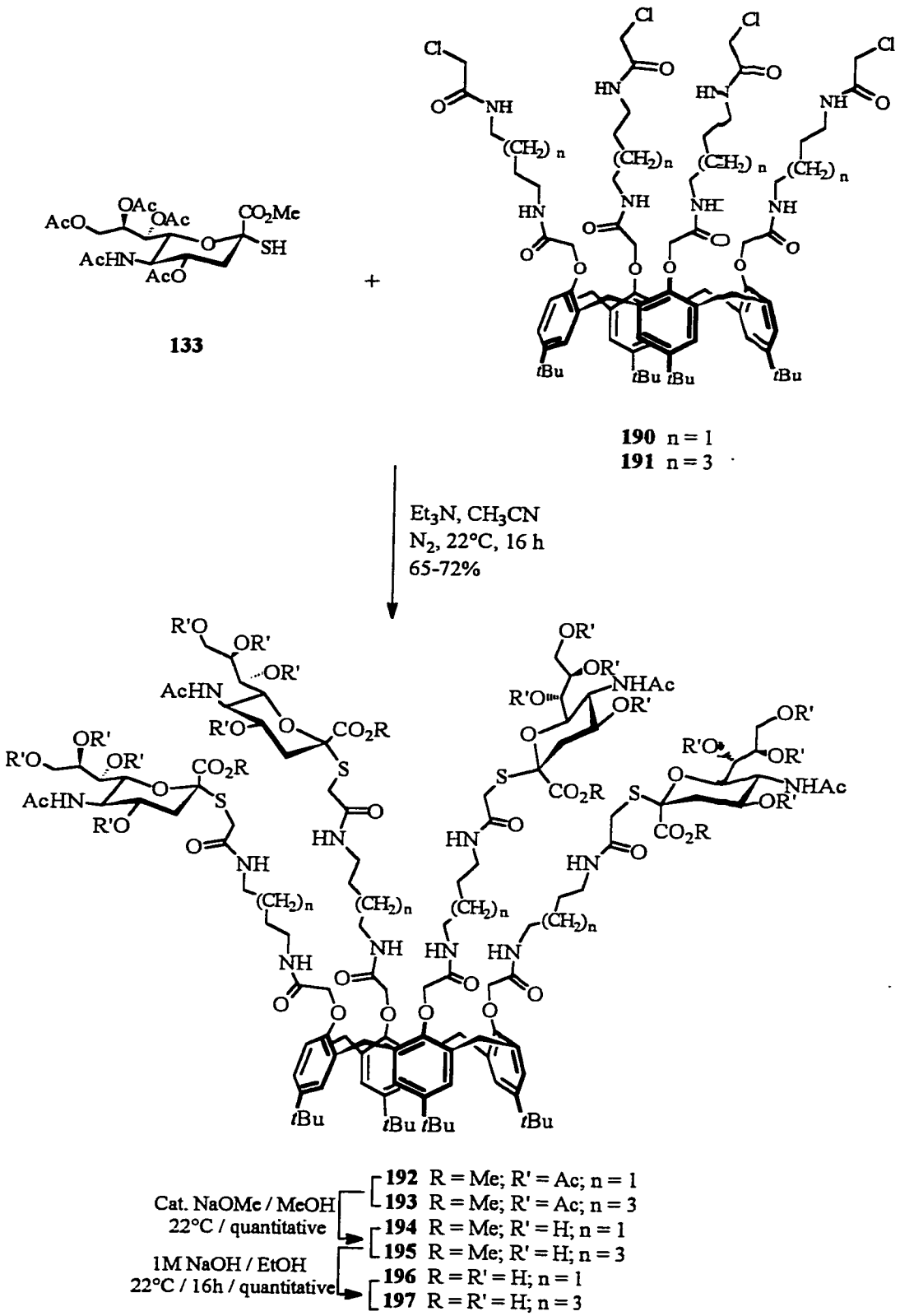


Figure 5.2.2. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of tetravalent sialocalix[4]arene **193**.



Scheme 5.2.4. Conjugation of thiosialoside **133** onto *N*-chloroacetylated calix[4]arene derivatives **190** and **191**.

In spite of its amphiphatic structure, acid **196** was fairly water soluble (~1.1 mM, 3 mg/mL). Moreover, after titration (pH 7.0) with 0.5 M NaOH (sodium form) the solubility increased to 4.8 mM (13 mg/mL). The solubility of both compounds was thus adequate for protein binding studies.

5.3 Convergent Synthesis of Tetravalent Sialocalix[4]arenes

Tetravalent sialocalix[4]arene **203** was prepared in order to demonstrate that the synthesis of glycocalixarenes was feasible using a convergent approach. The convergent approach is based on the functionalization of the outer components (i.e. sugar moieties) which are then, at a final stage, conjugated to a central core (i.e. calixarene). Amino sialoside **202** (sugar moiety) (Scheme 5.3.1) was synthesized from sialosyl azide **118** and commercially available aminocaproic acid derivative **199**. Reduction of azide **118** using H_2 and palladium on carbon (22°C, 30 min) in MeOH afforded anomeric amine **198** which was then coupled, without further purification, onto freshly prepared acid chloride **200** ($SOCl_2$, reflux, 3 h, 100%). Removal of the *N*-Cbz protecting group on amine derivative **201** was effected using conditions similar to the reduction of the azide group (H_2/Pd-C, MeOH, 22°C, 4 h). The deprotection afforded amine derivative **202** in quantitative yield (Scheme 5.3.1). Peptide coupling of amine **202** with tetraacid chloride calix[4]arene (Section 5.2) provided tetravalent sialocalix[4]arene **203** (Scheme 5.3.2) in moderate yield (Et_3N, CH_2Cl_2, 0°C $\rightarrow$ 22°C, 17 h, N_2, 50%). The extent of α-thio-sialoside incorporation was readily determined by ^{1}H-NMR spectroscopy. The integration of the well separated H-4 (4.92 ppm) and H-3e (2.62 ppm) sialosyl signals relative to the *tert*-butyl signal at 1.05 ppm showed complete incorporation of the four residues. The ^{13}C-NMR spectrum (Figure 5.3.1) revealed the integrity of the α-sialoside linkages from the C-2 signal at 82.2 ppm.

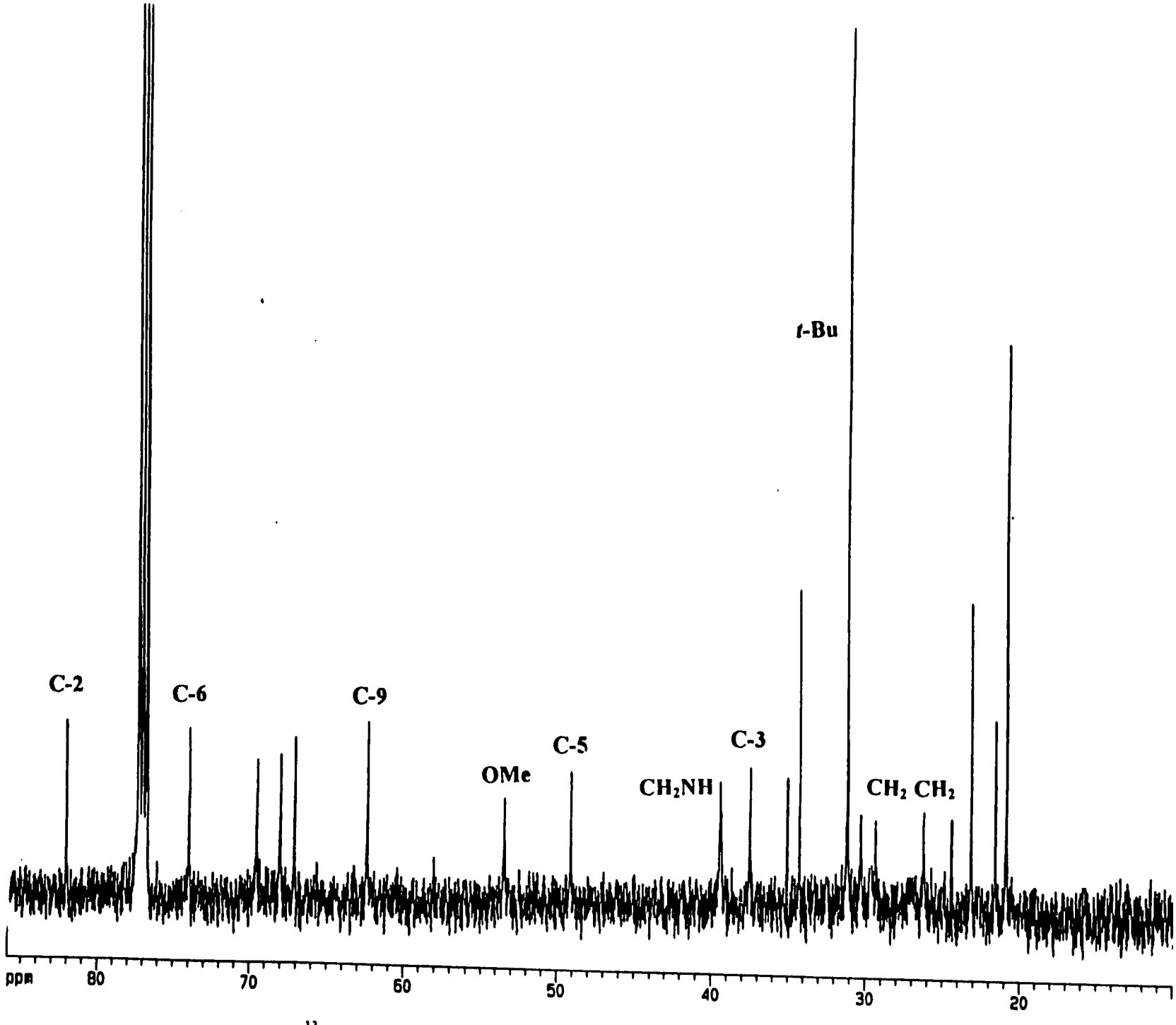
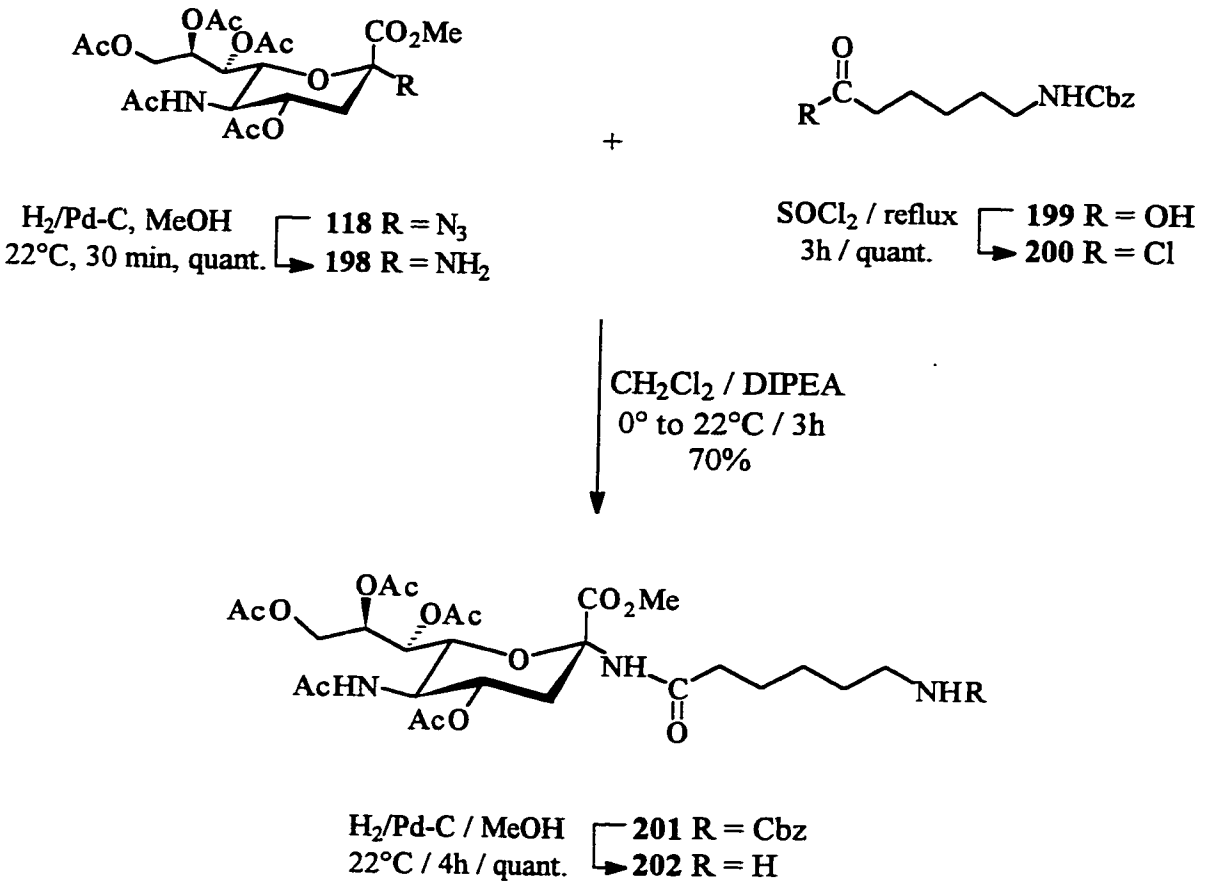


Figure 5.3.1. ^{13}C-NMR ($CDCl_3$, 500 MHz) spectrum of tetravalent sialocalix[4]arene **203** (aromatic and carbonyl regions not shown).

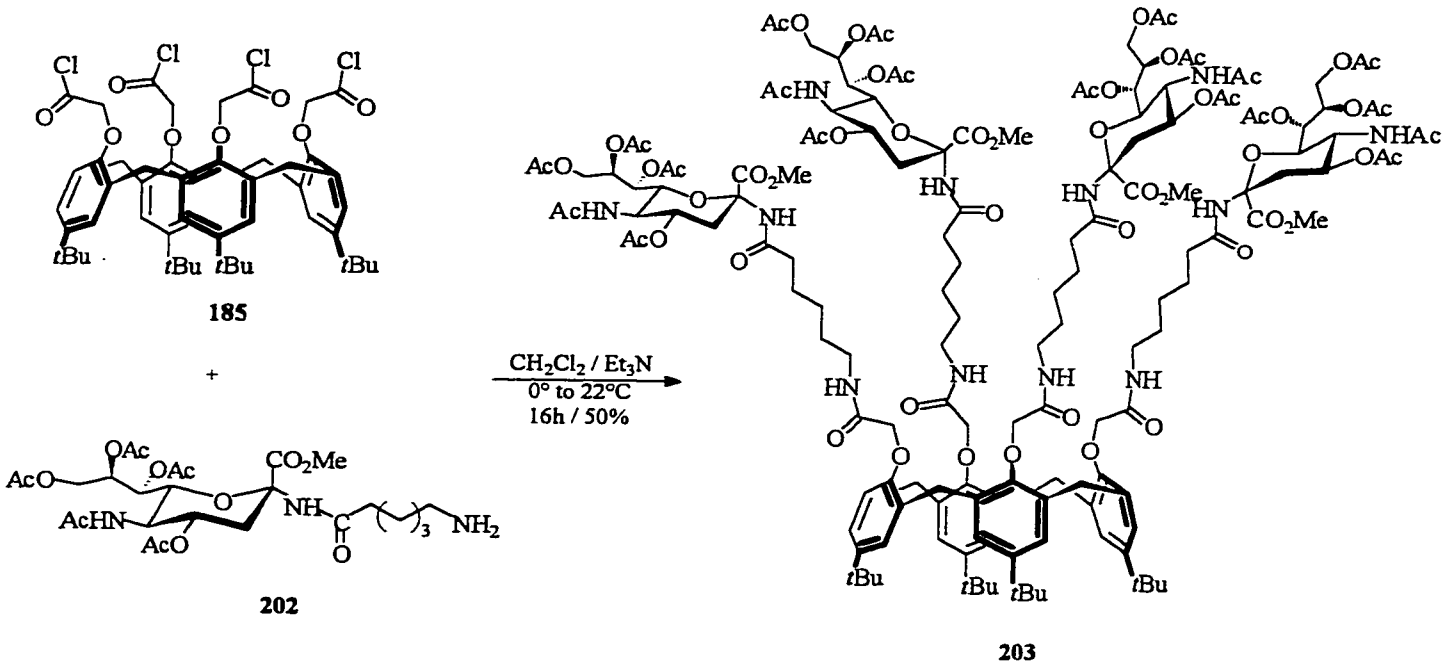


Scheme 5.3.1. Synthesis of 6-aminohexanamidosialoside precursor **202**.

5.4 Synthesis of Octavalent Sialocalix[4]arenes through *N*,*N*-Dialkylation

Octavalent dendritic glycocalix[4]arene **214** was synthesized using a double *N*-alkylation strategy. The synthesis involved a divergent-convergent combination approach since both moieties, namely calixarene core and carbohydrate ligand, were extensively modified prior to their coupling.

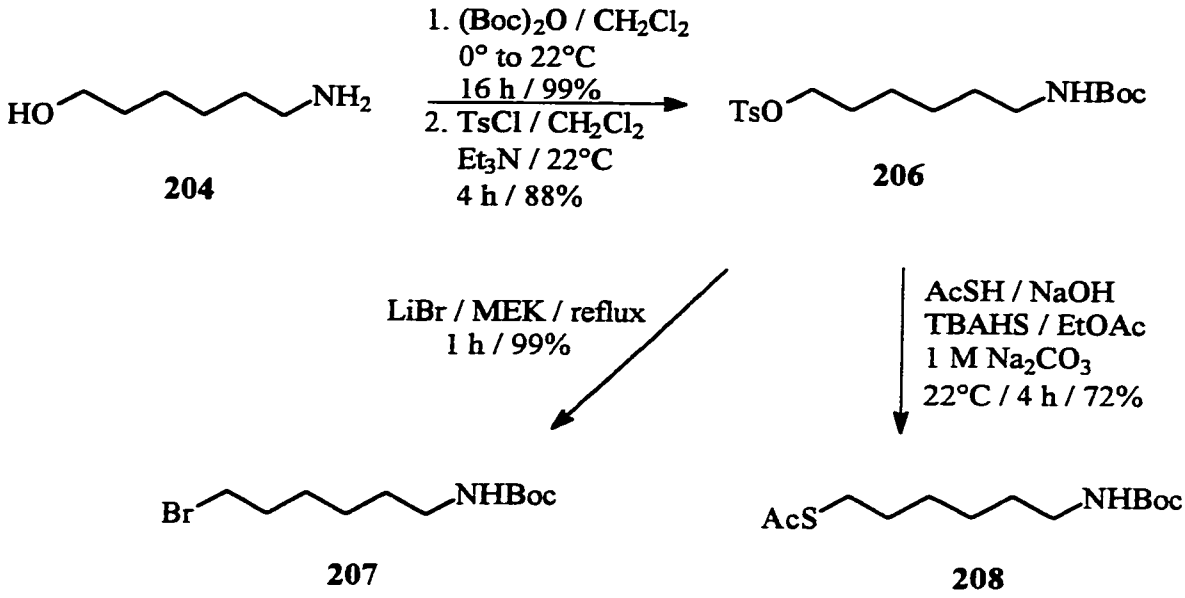
The spacer arm attached on the sialic acid residue was derived from 6-amino-1-hexanol **204**. The amine was first protected with a *N-tert*-butyloxycarbonyl group (Boc_2O, CH_2Cl_2, 0°C → 22°C, 16 h, 99%) to allow functionalization of the alcohol (Scheme 5.4.1) into electrophilic tosylate **206** (TsCl, CH_2Cl_2, Et_3N, 22°C, 88%). Tosylate **206** was transformed into electrophilic bromide **207** in 99% yield (LiBr, 2-butanone, reflux, 1 h). Tosylate **206** was also submitted to a phase transfer catalysis reaction (Chapter 2) using sodium thioacetate as nucleophile (Bu_4NHSO_4, EtOAc, 1 M Na_2CO_3, 22°C) which produced thioacetate derivative **208** in 72% yield.



Scheme 5.3.2. Convergent synthesis of sialocalix[4]arene **203**.

N-Boc protected α-thiosialoside **210** was prepared via two different synthetic routes (Scheme 5.4.2). The first method involved PTC reaction between acetochloroneuraminic acid **2** and de-*S*-acetylated thioacetate spacer arm derivative **208**. The thioacetate group was hydrolyzed using modified Zemplén conditions[115] (NaOMe,

MeOH, 22°C, N_2, then H^+ resin treatment) to afford nucleophilic thiol **209**, which was directly reacted with chloride **2** under PTC conditions (Bu_4NHSO_4, EtOAc, 1 M Na_2CO_3).



Scheme 5.4.1. Synthesis of spacer arm derivatives **207** and **208**.

The latter reaction yielded desired *N*-Boc protected α-thiosialoside **210** in non-satisfactory quantity (10% yield). A second method was necessary in order to obtain *N*-Boc protected α-thiosialoside **210** in a more efficient manner (Scheme 5.4.2). The first attempt of nucleophilic displacement by thiosialoside **133** involved tosylate displacement from derivative **206**. The reaction was unsuccessful due to the hard-soft acid-base incompatibility between the soft thiolate nucleophile and the hard tosyl leaving group. Bromide **207** was found to be the electrophile of choice for nucleophilic displacement by thiol **133**, since it afforded *N*-Boc protected α-thiosialoside **210** (Figure 5.4.1) in 77% yield (CH_2Cl_2, Et_3N, 22°C, N_2). The Boc protecting group of intermediate **210** was removed (20% TFA in CH_2Cl_2, 22°C, 1 h) to provide amine **211** quantitatively, which was used without further purification for the peptide coupling with the calixarene core. Complete amine deprotection was monitored by ^{1}H-NMR spectroscopy (200 and 500 MHz, $CDCl_3$) which revealed the absence of any remaining *tert*-butyl signal at 1.41 ppm (inset of Figure 5.4.1).

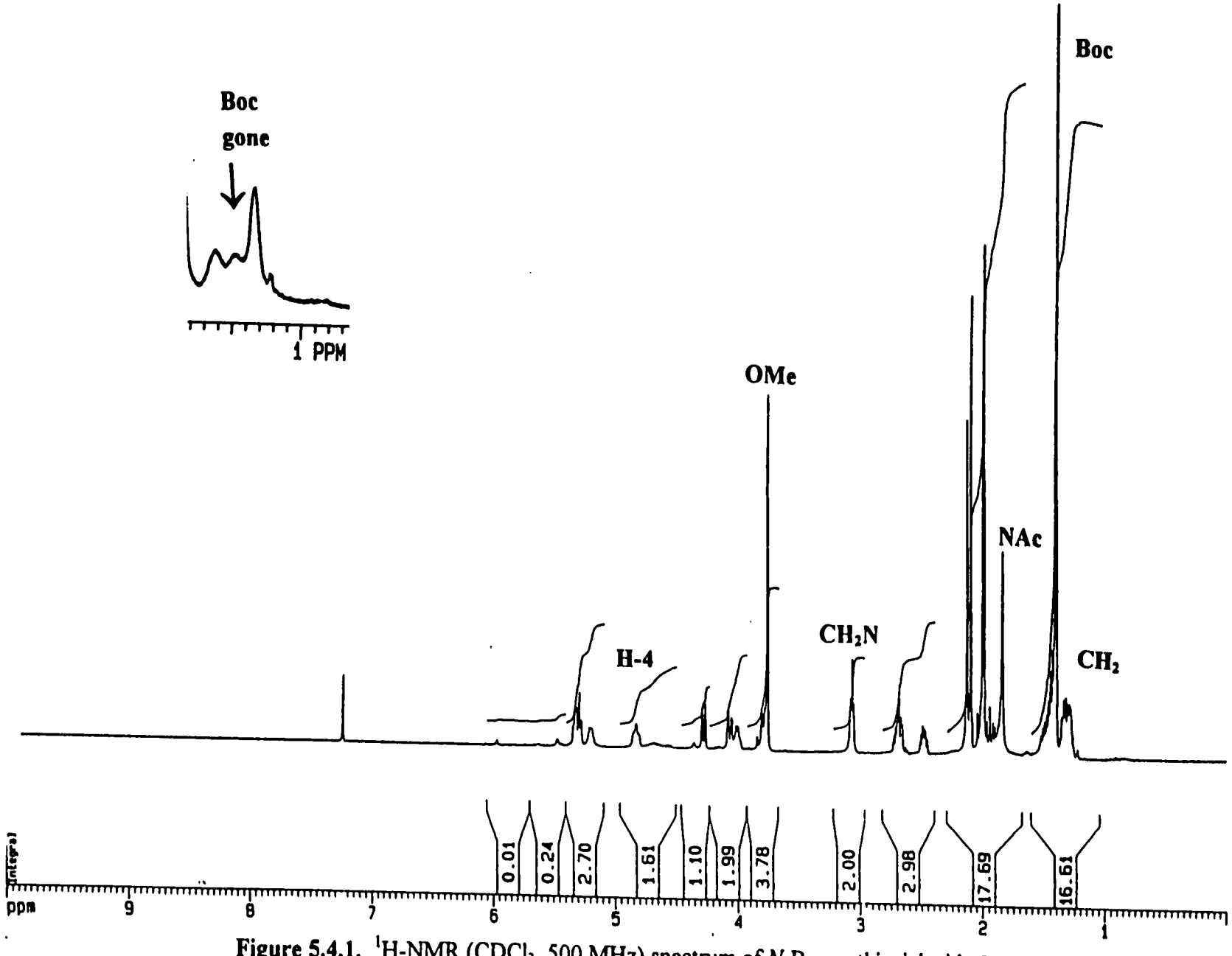
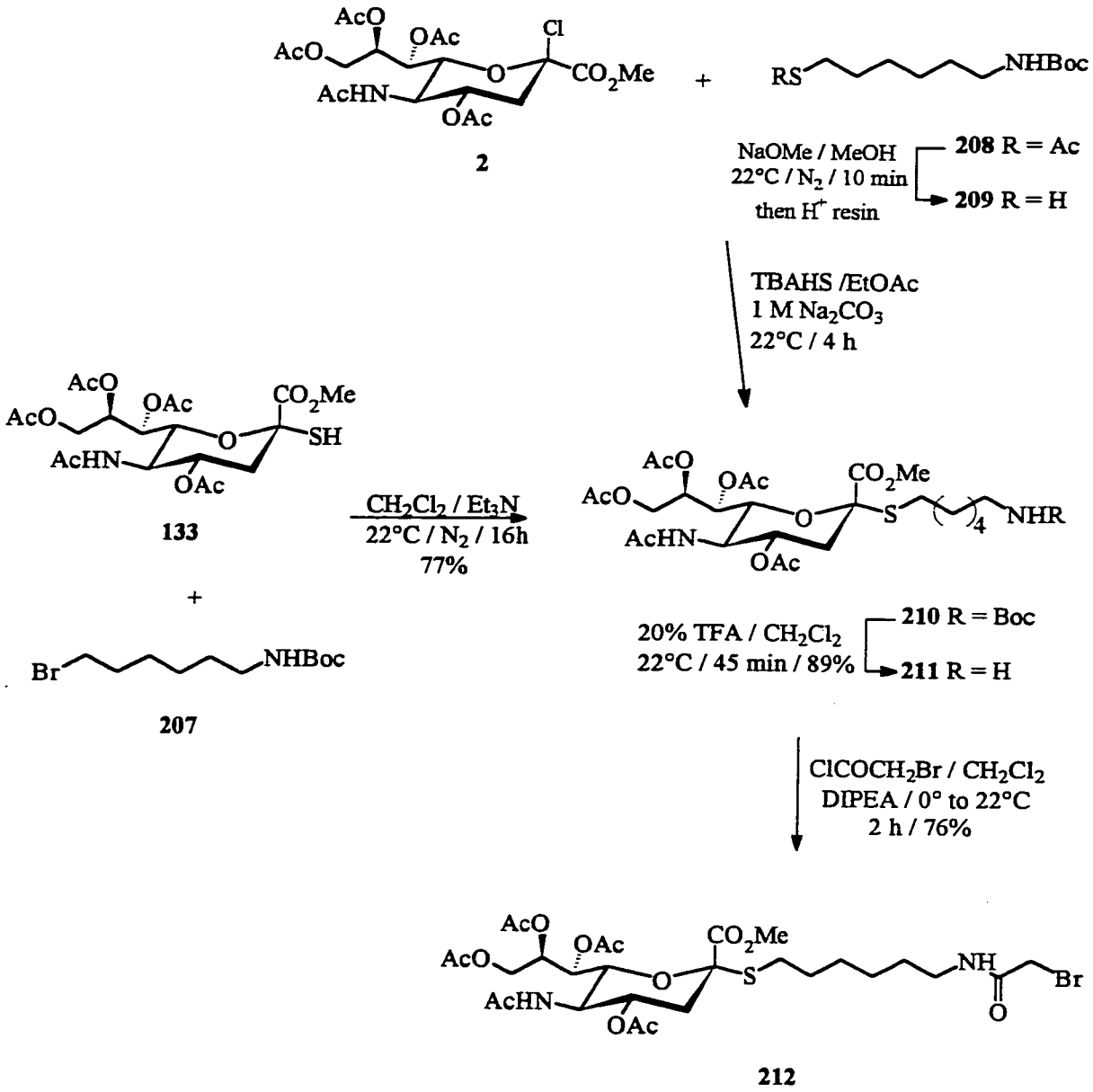


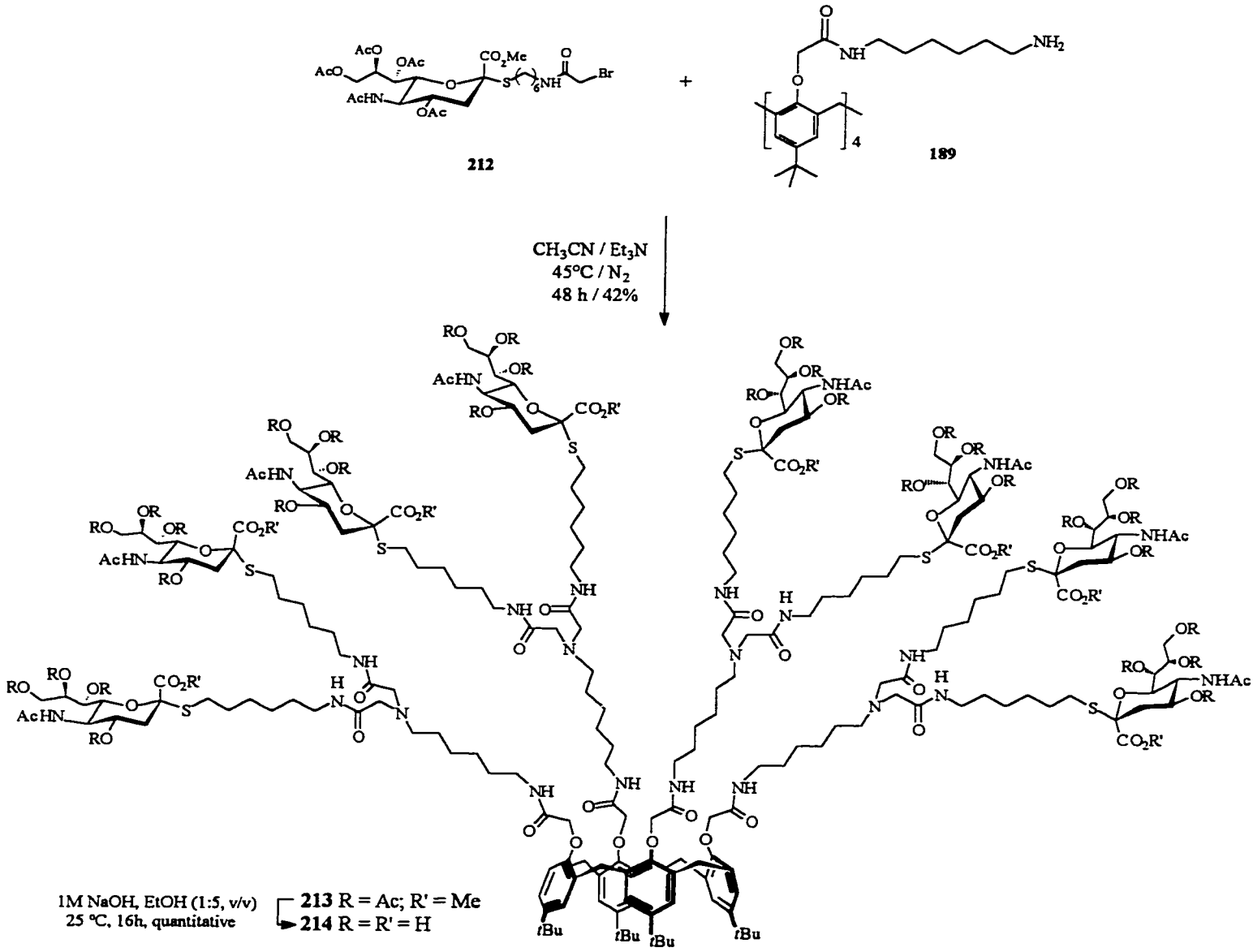
Figure 5.4.1. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of *N*-Boc α-thiosialoside **210** and 1-1.5 ppm region (inset) of Boc deprotected thiosialoside **211**.



Scheme 5.4.2. Synthesis of *N*-bromoacetylated thiosialoside precursor **212**.

Tetraamine calix[4]arene **189** (Section 5.2), after *N*-Boc deprotection (20% TFA in CH_2Cl_2) was *N,N*-dialkylated (Scheme 5.4.3) with *N*-bromoacetylated sialic acid ligand **212** in CH_3CN and Et_3N at 45°C for 48 h. The double *N*-alkylation produced octavalent sialocalix[4]arene **213** in 42% yield (Figure 5.4.2). Complete removal of ester groups

(acetates and methyl esters) was effected by basic hydrolysis (0.05 M NaOH, 22°C, 16 h) which afforded fully deprotected octavalent glycocalix[4]arene **214**. The ^{13}C-NMR signal for C-2 (83.6 ppm), the absence of methyl ester carbon peak and the integration of NAc (1.95 ppm) or H-3a (1.87 ppm) sialosyl signals relative to the *tert*-butyl signal at 1.08 ppm showed the presence of water-soluble octavalent sialocalix[4]arene **214**.



Scheme 5.4.3. Synthesis of octavalent dendritic sialocalix[4]arene **214** via double *N*-alkylation.

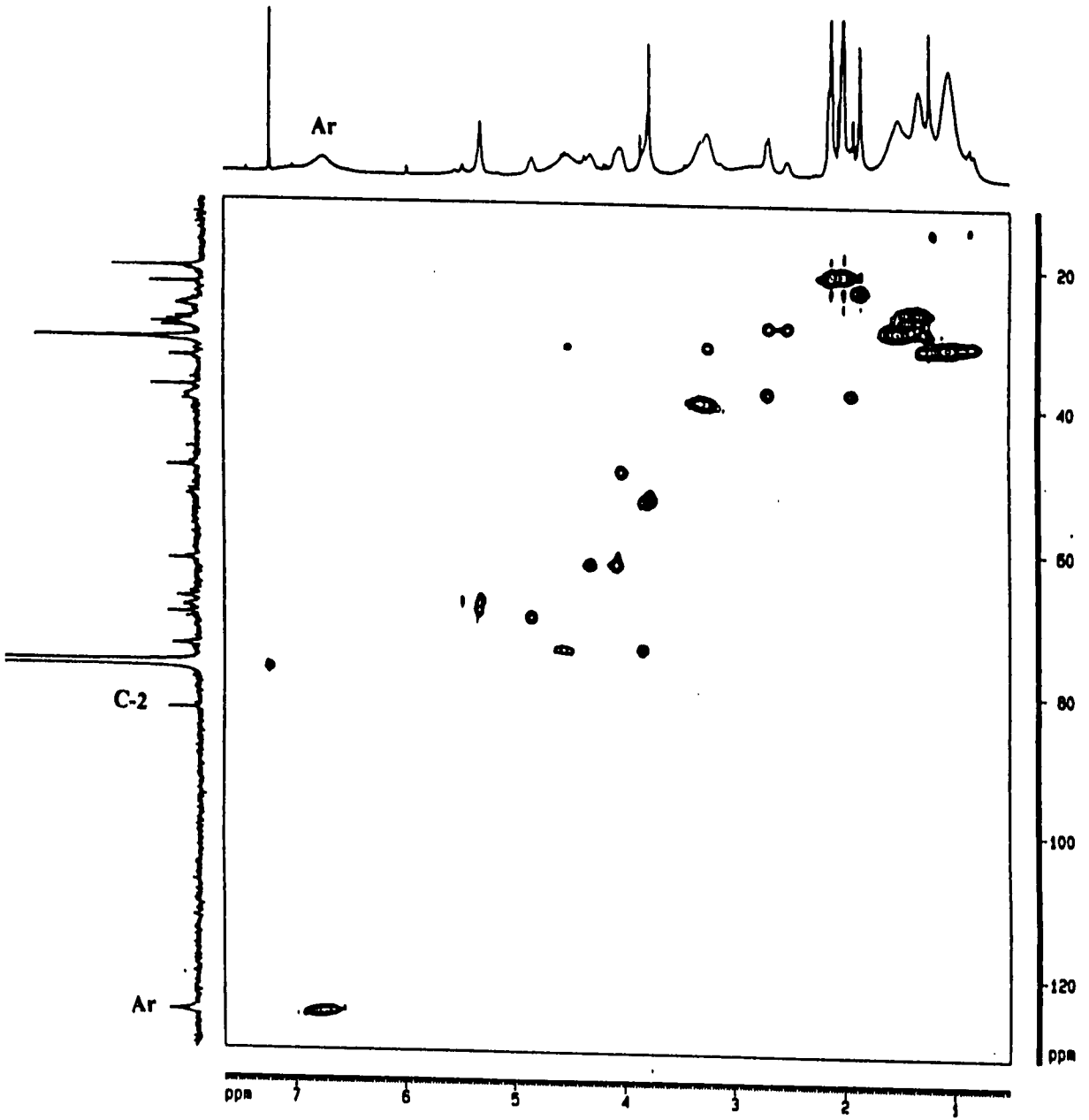


Figure 5.4.2. ^{1}H- and ^{13}C-NMR ($CDCl_3$, 500 MHz, 320°K) HMQC spectrum of octavalent sialocalix[4]arene **213**.

5.5 Lectin Binding

Preliminary lectin-binding experiments were achieved using microtiter plate turbidimetric analysis[214] wherein solutions of lectin-containing tetrameric **196** or **197** and octameric **214** sialocalix[4]arenes were allowed to form insoluble cross-linked complexes with wheat germ agglutinin (WGA) that could be monitored at 490 nm. The lectin is divalent and can therefore form linear one-dimensional (sometimes soluble) or multidimensional cross-linked lattices.[217] The results from the time course of turbidimetric analysis are shown in Figure 5.5.1. Interestingly, all three glycocalixarenes showed clear and rapid cross-linked insoluble complexes with WGA lectin. Additionally, octavalent sialocalix[4]arene **214** was only slightly better at the formation of insoluble precipitin complexes than the tetravalent counterparts (**196** or **197**). This is probably attributed to the fact that not all of the eight sugar moieties are involved in the direct binding with the lectin due to either the flexibility of the spacer arms or the steric hindrance between the lectin and the macromolecular glycocalix[4]arene **214**. The two tetrameric α-thiosialosides (**196** or **197**), only differing by the length of their spacers, showed similar insoluble complexes formation with WGA. These results can be rationalized by the length of the spacers having no effect on the binding since these differ only by two methylene groups (2 carbon atoms).

Figure 5.5.2 depicts the results of a different turbidimetric assay (490 nm) involving the sodium form of tetrameric sialocalix[4]arene **196** (0.5 mg/mL, PBS, 50 μL, pH 7.4) which exhibited strong crosslinking ability with wheat germ agglutinin (WGA, 2 mg/mL, PBS, 50 μL) (see also above). Formation of the crosslinked lattice (80 min) could be inhibited by monomeric phenylthio α-sialoside (20 mg/mL PBS, 10μL). Methyl ester **194**, also water soluble, formed a stable complex with WGA at the same concentration, demonstrating the absence of simple electrostatic binding interactions (not shown).

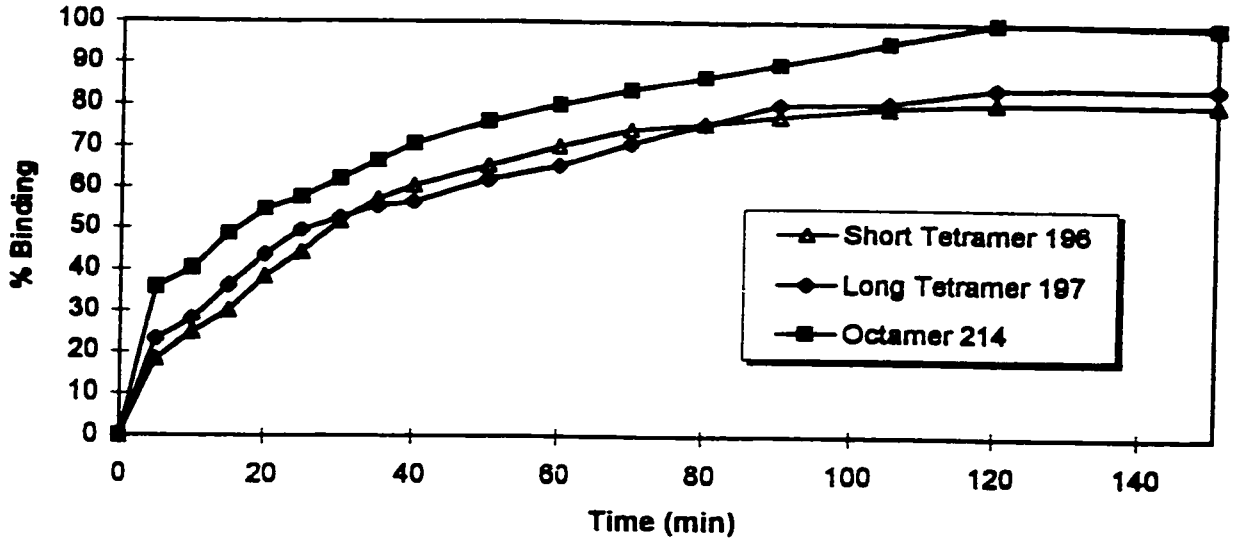


Figure 5.5.1 Time course of turbidimetric analysis of Wheat germ agglutinin (WGA) with octamer α-thiosialoside **214** (■), tetramers **196** (Δ) and **197** (◆).

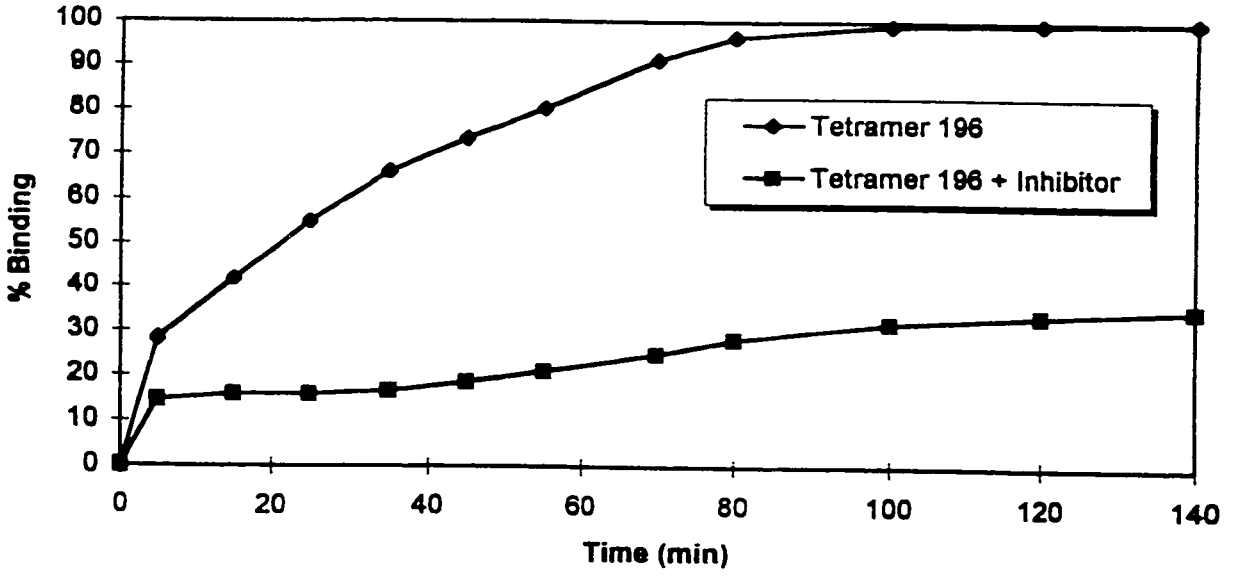


Figure 5.5.2 Time course of turbidimetric analysis of WGA and tetramer α-thio-sialoside **197** with (■) and without (◆) α-thiophenyl Neu5Ac as inhibitor.

Tetrameric sialocalix[4]arene **197** and nonameric sialodendrimer **182** were further tested for their binding properties to LFA *via* turbidimetric analysis. The time course formation of insoluble precipitin complexes between LFA and glycoconjugates **197** and **182** is illustrated in Figure 5.5.3. Maximum turbidity was reached after only 6 min in the case of sialodendrimer **182** whereas the maximum was attained after 90 min with sialocalix[4]arene **197**. The 9-mer sialodendrimer **182** is faster to form the insoluble complex and also seems to have a less stable complex than the tetrameric sialocalix[4]arene **197**. The decrease in optical density can also be explained by the formation of a well-organized precipitin complex which allow the beam of light to be deflected through the crystals, hence a decrease in optical density.

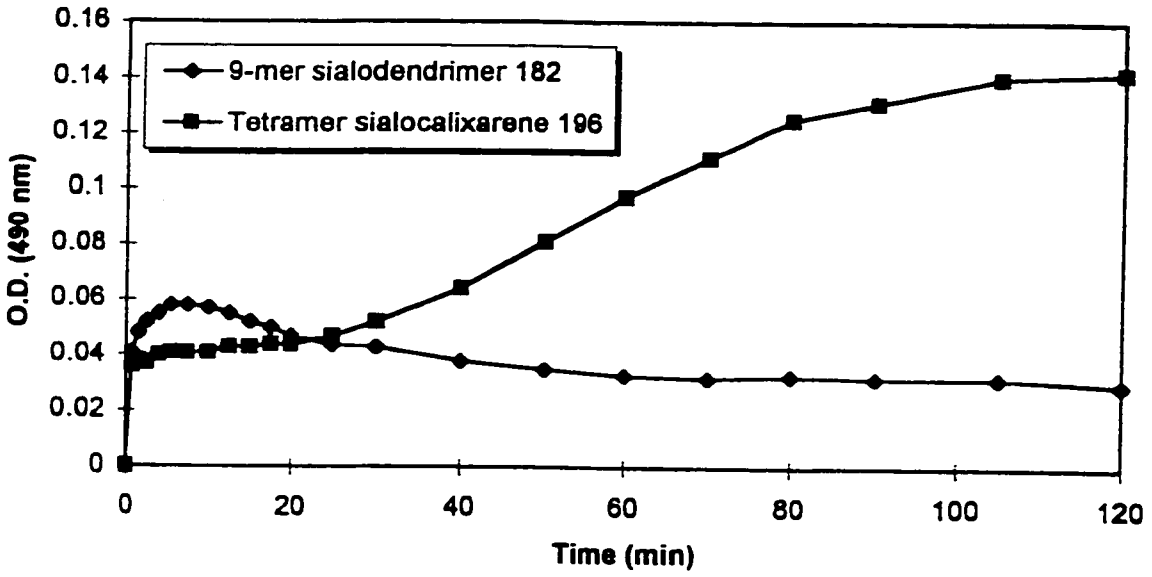


Figure 5.5.3 Time course of turbidimetric analysis of *Limax flavus* lectin (LFA) with tetrameric sialocalixarene **197** (■) and 9-mer sialodendrimer **182** (◆).

These micro-quantitative precipitation experiments confirmed the direct binding and cross-linking properties of α-thiosialosyl calix[4]arenes with both WGA and LFA lectins.

5.6 Conclusion

Water-soluble tetravalent α-sialosides were efficiently scaffolded on the upper rim of *p-tert*-butylcalix[4]arene using a convergent approach and nucleophilic displacement of a chloride by a thiol. A convergent approach was shown to be successful in the synthesis of a tetravalent glycocalix[4]arene, wherein the carbohydrate moiety was functionalized and coupled onto an electrophilic calixarene core. Water-soluble octavalent dendritic sialocalix[4]arene was efficiently synthesized using a double *N*-alkylation strategy. Preliminary binding experiments on tetra- **196** & **197** and octa- **214** valent sialosides with the lectins wheat germ agglutinin (WGA) and *Limax flavus* (LFA) showed promising results. All glycocalix[4]arenes exhibited strong binding (turbidimetric analysis) with the above lectins.

5.7 Experimental Methods

5.7.1 Synthesis

5,11,17,23-Tetra-tert-butyl-25,26,27,28-tetrakis-((ethoxycarbonyl)methoxy) calix[4]arene (183)

Calix[4]arene **93** (1.0 g, 1.54 mmol) was suspended in dry acetone (40 mL) containing anhydrous potassium carbonate (3.75 g, 27 mmol) and ethyl bromoacetate (4.8 mL, 43.2 mmol). The reaction mixture was refluxed under a nitrogen atmosphere with 4Å molecular sieves for 18 h. The cooled solution was filtered and the solid washed with CH_2Cl_2 (30 mL). The organic phase was concentrated under reduced pressure to an oil that contained excess ethyl bromoacetate. The $BrCH_2CO_2Et$ was removed by high vacuum distillation leaving crude compound **183** which was purified by silica gel column chromatography using a gradient of 0 to 7% MeOH in CH_2Cl_2 as eluent. The residue

obtained was dissolved in CH_2Cl_2 (50 mL) and submitted to exhaustive washings (6 x 75 mL H_2O). The organic phase was evaporated under reduced pressure and the residue recrystallized from ethanol to afford 1.30 g (85% yield, 1.31 mmol) of title compound **183** as a white solid. Tetra-ethylester calix[4]arene **183** has mp 131.0-132.8°C; R_f 0.30 (10% MeOH in CH_2Cl_2); ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.75 (s, 8H, H_{ar}), 4.84 (d, 4H, $J_{a,b}$ = 13.0 Hz, CH-a), 4.78 (s, 8H, OCH_2), 4.18 (q, 8H, $J_{CH2,CH3}$ = 7.1, CH_2CH_3), 3.17 (d, 4H, $J_{a,b}$ = 13.0, CH-b), 1.26 (t, 12H, $J_{CH2,CH3}$ = 7.1, CH_2CH_3), 1.05 (s, 36H, *t*-Bu); ^{1}H-NMR ($CDCl_3$) data of cation encapsulated calix[4]arene tetraethyl ester **183** δ (ppm): 7.09 (s, 8H, H_{ar}), 4.21 (d, 4H, $J_{a,b}$ = 13.0 Hz, CH-a), 4.44 (s, 8H, OCH_2), 4.35 (q, 8H, $J_{CH2,CH3}$ = 7.1, CH_2CH_3), 3.37) (d, 4H, $J_{a,b}$ = 13.0, CH-b), 1.39 (t, 12H, $J_{CH2,CH3}$ = 7.1, CH_2CH_3), 1.12 (s, 36H, *t*-Bu); ^{13}C-NMR ($CDCl_3$) δ (ppm): 170.5 (C=O), 153.0, 145.2, 133.5 (C_{ar}), 125.4 (C_{ar}-H), 71.4 (OCH_2), 60.3 (CH_2CH_3), 33.8 (CMe_3), 32.0 ($ArCH_2$), 31.4 (*t*-Bu), 14.2 (CH_2CH_3); MS for $C_{60}H_{80}O_{12}$ (FAB$^+$ thioglycerol, rel. intensity) m/z: 1016.6 ($[M+H+Na]^+$, 14.1%), 1015.6 ($[M+Na]^+$, 22.2%).

5,11,17,23-Tetra-tert-butyl-25,26,27,28-tetracarboxymethoxycalix[4]arene (184)

A suspension of 4-*tert*-butylcalix[4]arene-*O*,*O*',*O*'',*O*'''-tetraacetic acid tetraethyl ester **183** (2 g, 2.01 mmol) in EtOH (22 mL) containing 1 M aqueous KOH (20 mL) was heated under reflux for 3.5 h. The cooled solution was acidified with H^+ resin (Amberlite IR-120) for 30 min, during which time a white solid appeared. The resin was filtered off and rinsed with MeOH (60 mL) in order to dissolve the entire crude product. The filtrate was concentrated under reduced pressure and a white solid corresponding to tetraacid **184** was obtained in 95% yield (1.68 g, 1.90 mmol). Title compound **184** has mp 272-274°C (dec.); ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.92 (s, 8H, H_{ar}), 4.76 (d, 4H, $J_{a,b}$ = 12.6 Hz, CH-a), 4.58 (s, 8H, OCH_2), 3.21 (d, 4H, $J_{a,b}$ = 12.6, CH-b), 1.05 (s, 36H, *t*-Bu); ^{13}C-NMR (DMSO-d_6) δ (ppm): 170.7 (C=O), 152.2, 145.0, 133.2 (C_{ar}), 125.2 (C_{ar}-H), 71.6 (OCH_2), 33.5 (CMe_3), 30.9 (*t*-Bu), 30.6 ($ArCH_2$); MS for $C_{52}H_{64}O_{12}$ (FAB$^+$ thioglycerol,

rel. intensity) m/z: 904.5 ($[M+H+Na]^+$, 4.7%), 903.5 ($[M+Na]^+$, 8.2%), 881.5 ($[M+H]^+$, 4.9%).

5,11,17,23-Tetra-tert-butyl-25,26,27,28-tetrakis-((chloroformyl)methoxy)calix[4]arene (185)

Tetraacid **184** (120 mg, 0.136 mmol) was dissolved in thionyl chloride (2 mL, 27.2 mmol) and the solution was heated under reflux for 2 h. The cooled solution was evaporated to dryness to afford acid chloride **185** (128.5 mg, 0.134 mmol) which was used without purification. Title compound **185** has IR ν_{max} ($CHCl_3$)/cm^{-1}: 1807; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.78 (s, 8H, H_{ar}), 5.09 (s, 8H, OCH_2), 4.59 (d, 4H, $J_{a,b}$ = 13.1 Hz, CH-a), 3.25 (d, 4H, $J_{a,b}$ = 13.1, CH-b), 1.05 (s, 36H, *t*-Bu).

5,11,17,23-Tetra-tert-butyl-25,26,27,28-tetrakis-[4-(N-tert-butylcarbamoylbutyl) aminocarbonylmethoxy] calix[4]arene (186)

Tetraacyl chloride **185** (488 mg, 0.511 mmol) in dry CH_2Cl_2 (10 mL) was added, dropwise with stirring under nitrogen, to a cooled (0°C) solution of mono *N*-Boc-1,4-butanediamine (782 μL, 4.09 mmol) and Et_3N (569 μL, 4.09 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was allowed to warm up to room temperature where the reaction was stirred for 16 h. The solution was diluted with CH_2Cl_2 (20 mL) and washed with 5% aqueous HCl (15 mL), saturated $NaHCO_3$ (15 mL), H_2O (15 mL) and brine (10 mL). The dried (Na_2SO_4) organic phase was concentrated under reduced pressure and purified by silica gel column chromatography using a gradient of 5/1 to 1/4 CH_2Cl_2/THF as eluent. The crude product was crystallized from CH_2Cl_2/hexanes to afford compound **186** as a white solid in 72% yield (577.7 mg, 0.370 mmol). Title compound **186** has mp 198.5-200°C; R_f 0.43 (10% MeOH in CH_2Cl_2); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.90-7.98 (m, 4H, NH), 6.76 (s, 8H, H_{ar}), 4.97-5.07 (m, 4H, NH), 4.47 (s, 8H, OCH_2), 4.38-4.47 (d, 4H,

CH-a), 3.34-3.38 (m, 8H, NCH_2), 3.21 (d, 4H, $J_{a,b}$ = 13.1 Hz, CH-b), 3.06-3.10 (m, 8H, CH_2NHBoc), 1.44-1.59 (m, 16H, 2 internal CH_2), 1.40 (s, 36H, O-*t*-Bu), 1.06 (s, 36H, *t*-Bu); ^{13}C-NMR ($CDCl_3$) δ (ppm): 169.6, 156.2 (C=O), 152.8, 145.9, 132.7 (C_{ar}), 125.9 (C_{ar}-H), 79.1 ($OCMe_3$), 74.5 (OCH_2), 40.4, 39.0 (2 NCH_2), 33.9 (CMe_3), 31.4 (ArCH_2), 31.3 (*t*-Bu), 28.5 (O-*t*-Bu), 27.6, 27.0 (2 internal CH_2); MS for $C_{88}H_{136}N_8O_{16}$ (FAB$^+$ thioglycerol, rel. intensity) m/z: 1562.8 ($[M+H]^+$, 6.7%).

Anal. Calcd. for $C_{88}H_{136}N_8O_{16}Na$: C, 66.68; H, 8.65; N, 7.07. Found: C, 66.55; H, 8.57; N, 7.37.

5,11,17,23-Tetra-tert-butyl-25,26,27,28-tetrakis-[6-(N-tert-butylcarbamoylhexyl) aminocarbonylmethoxy] calix[4]arene (187)

Tetraacyl chloride **185** (270 mg, 0.283 mmol) in dry CH_2Cl_2 (7 mL) was added, dropwise with stirring under nitrogen, to a cooled (0°C) solution of mono *N*-Boc-1,6-hexanediamine hydrochloride salt (572 mg, 2.26 mmol) pre-treated with OH^- resin (20 min) and Et_3N (315 μL, 2.26 mmol) in CH_2Cl_2 (4 mL). The reaction mixture was allowed to warm up to room temperature where the reaction was stirred for 16 h. The solution was diluted with $CHCl_3$ (20 mL) and washed with 0.2 M HCl (2 x 15 mL), saturated $NaHCO_3$ (2 x 15 mL), H_2O (2 x 15 mL) and brine (10 mL). The dried (Na_2SO_4) organic phase was evaporated under reduced pressure and purified by silica gel column chromatography using a gradient of 5/1 to 1/4 CH_2Cl_2/THF as eluent. Title compound **187** was obtained in 91% amorphous yield (431.6 mg, 0.258 mmol). Compound **187** has mp 204.2-206.1°C (CH_2Cl_2/hexanes); R_f 0.49 (10% MeOH in CH_2Cl_2); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.72-7.79 (m, 4H, NH), 6.75 (s, 8H, H_{ar}), 4.73-4.82 (m, 4H, NH), 4.48 (s, 8H, OCH_2), 4.43-4.48 (d, 4H, CH-a), 3.18-3.36 (m, 12H, CH-b & NCH_2), 3.02-3.11 (m, 8H, CH_2NHBoc), 1.49-1.56 (m, 16H, 2 CH_2), 1.28-1.40 (m, 16H, 2 internal CH_2), 1.40 (s, 36H, O-*t*-Bu), 1.05 (s, 36H, *t*-Bu); ^{13}C-NMR ($CDCl_3$) δ (ppm): 169.5, 156.3 (C=O), 152.6, 145.8, 132.6 (C_{ar}), 125.8 (C_{ar}-H), 79.0 ($OCMe_3$), 74.4 (OCH_2), 40.3, 38.9 (2

NCH_2), 33.9 (CMe_3), 31.4 (ArCH_2), 31.3 (*t*-Bu), 28.9, 28.4, 28.2 (O-*t*-Bu, & 2 CH_2), 27.5, 26.8 (2 internal CH_2); MS for $C_{96}H_{152}N_8O_{16}$ (FAB$^+$ thioglycerol, rel. intensity) m/z: 1675.3 ([M+H]$^+$, 1.8%), 1674.3 ([M]$^+$, 2.5%).

Anal. Calcd. for $C_{96}H_{152}N_8O_{16}Na$: C, 67.94; H, 9.03; N, 6.60. Found: C, 68.06; H, 8.89; N, 6.73.

5,11,17,23-Tetra-tert-butyl-25,26,27,28-tetrakis-[4-(N-chloroacetylaminobutyl) amino-carbonylmethoxy] calix[4]arene (190)

N-Boc protected calix[4]arene tetramer **186** (100.5 mg, 0.0643 mmol) was treated with 20% TFA in CH_2Cl_2 (2 mL) and stirred at room temperature for 3 h. The solution was evaporated under reduced pressure and co-evaporated twice with toluene. Tetraamine **188** as a trifluoroacetate salt (0.0643 mmol) was dissolved in CH_2Cl_2 (2 mL) and Et_3N (72 μL, 0.515 mmol) and the mixture cooled with an ice bath (~0°C). Chloroacetic anhydride (63.6 mg, 0.335 mmol) dissolved in CH_2Cl_2 (3 mL) was added dropwise to the cooled reaction mixture over 10 min. The reaction was then stirred at room temperature for 5 h. The reaction was monitored by a ninhydrin test. The solution was diluted with CH_2Cl_2 (30 mL) and washed with 5% aqueous HCl (2 x 20 mL), saturated $NaHCO_3$ (2 x 20 mL), H_2O (2 x 20 mL) and brine (10 mL). The dried (Na_2SO_4) organic phase was evaporated near dryness and purified by silica gel chromatography (1 mm chromatotron plate) using 5% MeOH in CH_2Cl_2 as eluent. The crude product was obtained as a white foam in 70% yield (66.1 mg, 0.0450 mmol), which could be recrystallized from CH_2Cl_2/hexanes to afford a white solid as pure title compound **190**. Compound **190** has mp 243-245°C; R_f 0.46 (10% MeOH in CH_2Cl_2); ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.34 (t, 4H, $J_{NH,CH2}$ = 5.2 Hz, NH), 8.21 (t, 4H, $J_{NH,CH2}$ = 5.4, NH), 6.82 (s, 8H, H_{ar}), 4.47 (d, 4H, $J_{a,b}$ = 12.7, CH-a), 4.41 (s, 8H, OCH_2), 4.02 (s, 8H, CH_2Cl), 3.15-3.21 (m, 12H, CH-b & NCH_2), 3.05-3.09 (m, 8H, CH_2N), 1.39-1.45 (m, 16H, 2 internal CH_2), 1.04 (s, 36H, *t*-Bu); ^{13}C-NMR (DMSO-d_6) δ (ppm): 168.9, 165.8 (C=O), 152.8,

144.6, 132.9 (C_{ar}), 125.3 (C_{ar}-H), 74.0 (OCH_2), 42.8 (CH_2Cl), 38.2, 38.7 (2 NCH_2), 33.6 (CMe_3), 31.4 (ArCH_2), 31.1 (*t*-Bu), 26.5, 26.3 (2 internal CH_2); MS for $C_{76}H_{108}N_8O_{12}Cl_4$ (FAB$^+$ thioglycerol, rel. intensity) m/z: 1491.5 ([M+Na+H]$^+$, 12.1%), 1469.2 ([M+H]$^+$, 15.5%).

5,11,17,23-Tetra-tert-butyl-25,26,27,28-tetrakis-[6-(N-chloroacetylaminohexyl) amino-carbonylmethoxy] calix[4]arene (191)

Tetrachloroacetylated calix[4]arene **191** was obtained following an identical procedure as described above (for **190**) in 80% yield (94 mg, 0.0595 mmol). Title compound **191** has mp 253-254.5°C (CH_2Cl_2/hexanes); R_f 0.49 (10% MeOH in CH_2Cl_2); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.68-7.77 (m, 4H, NH), 6.80-6.88 (m, 4H, NH), 6.76 (s, 8H, H_{ar}), 4.50 (s, 8H, OCH_2), 4.46 (d, 4H, $J_{a,b}$ = 12.9, CH-a), 4.01 (s, 8H, CH_2Cl), 3.15-3.38 (m, 20H, CH-b & 2 NCH_2), 1.49-1.62 (m, 16H, 2 CH_2), 1.28-1.41 (m, 16H, 2 internal CH_2), 1.06 (s, 36H, *t*-Bu); ^{13}C-NMR ($CDCl_3$) δ (ppm): 168.7, 165.6 (C=O), 152.9, 144.8, 133.1 (C_{ar}), 125.1 (C_{ar}-H), 74.2 (OCH_2), 42.7 (CH_2Cl), 38.6, 38.9 (2 NCH_2), 33.5 (CMe_3), 31.4 (ArCH_2), 31.1 (*t*-Bu), 27.9, 27.3, 26.3, 26.0 (4 internal CH_2); MS for $C_{84}H_{124}N_8O_{12}Cl_4$ (FAB$^+$ thioglycerol, rel. intensity) m/z: 1603.8 ([M+Na+H]$^+$, 8.1%), 1580.79 ([M+H]$^+$, 9.6%).

Calix[4]arene tetramer with short spacer armed Neu5Ac (192)

To a solution of α-thiosialoside **133** (61 mg, 0.120 mmol) in dry acetonitrile (5 mL) was added Et_3N (25 μL, 0.175 mmol) and tetrachloroacetamide precursor **190** (32.2 mg, 0.0219 mmol). The mixture was stirred at room temperature overnight under a nitrogen atmosphere. The solution was concentrated under vacuum, and the crude residue was dissolved in CH_2Cl_2 (15 mL) and the organic mixture washed with sat. $NaHCO_3$ (2 x 10 mL), water (2 x 10 mL) and sat. NaCl solution (8 mL). The dried (Na_2SO_4) organic

layer was evaporated under vacuum and the crude residue purified by silica gel column chromatography using first, EtOAc to get rid of unreacted starting material (sugar) and second a gradient from 5 to 10% MeOH in CH_2Cl_2 as eluent. Title compound **192** was obtained as a white foam in 65% yield (47.5 mg, 0.0142 mmol). Compound **192** has $[\alpha]_D$ +10.3° (*c* 1.0, $CHCl_3$); R_f 0.40 (10% MeOH in CH_2Cl_2); R_f 0.0 (EtOAc); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.09 (s, 8H, H_{ar}), 7.03-7.14 (m, 4H, NH), 5.71-5.80 (m, 4H, NH), 5.35-5.46 (m, 4H, H-8), 5.28 (dd, 4H, $J_{7,8}$=8.8, H-7), 4.86 (ddd, 4H, H-4), 4.64-4.75 (m, 4H, NH), 4.25 (dd, 4H, $J_{9,9'}$=11.6, H-9), 3.99-4.10 (m, 12H, CH-a, H-5 and H-9'), 3.75-3.88 (m, 8H, H-6 and OCH-a), 3.75 (s, 12H, OMe), 3.35-3.53 (m, 20H, CH_2N, SCH-a, CH-b and OCH-b), 3.20-3.31 (m, 12H, CH_2N and SCH-b), 2.72 (dd, 4H, H-3e), 2.17, 2.12, 2.02, 1.99 (4s, 48H, 4OAc), 1.90 (dd, 4H, H-3a), 1.85 (s, 12H, NAc), 1.55-1.75 (m, 16H, 2 internal CH_2), 1.11 (s, 36H, *t*-Bu); ^{13}C-NMR ($CDCl_3$) δ (ppm): 171.4, 170.8, 170.6, 170.4, 170.0, 169.3, 168.7 (C=O) 149.6, 149.0, 134.3 (C_{ar}), 126.2 (C_{ar}-H), 82.0 (C-2), 77.0 (OCH_2), 74.0 (C-6), 69.5 (C-4), 68.0 (C-8), 67.1 (C-7), 62.4 (C-9), 53.4 (OMe), 49.1 (C-5), 39.4 (2 NH*CH*$_2$), 37.5 (C-3), 34.2 (*C*Me_3), 32.4 (SCH_2), 31.2 (*t*-Bu), 30.3 (Ar*CH*$_2$), 27.0, 26.2 (2 internal CH_2), 23.1 (NAc), 21.6, 20.8 (OAc).

Anal. Calcd. for $C_{156}H_{220}N_{12}O_{60}S_4$: C, 55.90; H, 6.62; N, 5.01%. Found: C, 55.58; H, 6.89; N, 4.86%.

Calix[4]arene tetramer with long spacer armed Neu5Ac (193)

Conjugation of α-thiosialoside **133** and chloroacetylated calix[4]arene **191** was performed under the same conditions described above for tetrameric sialocalix[4]arene **192**. The off-white foam obtained after silica gel chromatography (1 mm chromatotron plate) was found to be pure product **193** in 72% yield (44.7 mg, 0.0129 mmol). Title compound **193** has $[\alpha]_D$ +10.0° (*c* 0.5, $CHCl_3$); R_f 0.43 (10% MeOH in CH_2Cl_2); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.06 (s, 8H, H_{ar}), 7.05-7.15 (m, 4H, NH), 5.69-5.77 (m, 4H, NH), 5.38 (m, 4H, H-8), 5.27 (dd, 4H, $J_{7,8}$=8.8, H-7), 4.84 (ddd, 4H, H-4), 4.66-4.75 (m,

4H, NH), 4.25 (dd, 4H, $J_{9,9'}$=11.5, H-9), 3.98-4.12 (m, 12H, CH-a, H-5 and H-9'), 3.76-3.90 (m, 8H, H-6 and OCH-a), 3.76 (s, 12H, OMe), 3.37-3.58 (m, 20H, CH_2N, SCH-a, CH-b and OCH-b), 3.21-3.34 (m, 12H, CH_2N and SCH-b), 2.73 (dd, 4H, H-3e), 2.15, 2.11, 2.03, 2.00 (4s, 48H, 4OAc), 1.91 (dd, 4H, H-3a), 1.86 (s, 12H, NAc), 1.65-1.77 (m, 16H, 2 CH_2), 1.48-1.63 (m, 16H, 2 internal CH_2), 1.10 (s, 36H, *t*-Bu); ^{13}C-NMR ($CDCl_3$) δ (ppm): 171.5, 170.7, 170.6, 170.3, 170.0, 169.5, 168.6 (C=O) 149.8, 148.9, 134.4 (C_{ar}), 126.1 (C_{ar}-H), 82.1 (C-2), 77.0 (OCH_2), 74.0 (C-6), 69.4 (C-4), 68.0 (C-8), 67.0 (C-7), 62.4 (C-9), 53.3 (OMe), 49.1 (C-5), 39.4 (2 NH*CH*$_2$), 37.5 (C-3), 34.1 (*C*Me_3), 32.4 (SCH_2), 31.1 (*t*-Bu), 30.3 (Ar*CH*$_2$), 27.8, 27.2, 26.5, 26.0 (4 internal CH_2), 23.1 (NAc), 21.6, 20.9, 20.8 (OAc).

Anal. Calcd. for $C_{164}H_{236}N_{12}O_{60}S_4$: C, 56.86; H, 6.87; N, 4.85%. Found: C, 56.59; H, 6.98; N, 4.60%.

Fully deprotected calix[4]arene tetramer with short spacer armed Neu5Ac (196)

Tetrameric sialocalix[4]arene **192** (40 mg, 0.0119 mmol) was dissolved in dry methanol (3 mL) and a 1 M solution NaOMe/MeOH (pH~9) was added. The solution was stirred at room temperature for 45 min and then treated with H^+ resin (Amberlite IR-120). The filtrate was evaporated near dryness under reduced pressure and freeze dried to afford a white powder in quantitative yield (compound **194**). A solution of α-thiosialoside methyl ester **194** (31 mg, 0.0116 mmol), dissolved in a mixture of methanol (2.5 mL) and 1 M aqueous NaOH (0.5 mL), was stirred at room temperature overnight (16 h). After treatment with H^+ resin, the filtrate was first evaporated to remove most of the methanol and then freeze-dried. Pure product **196** was obtained in nearly quantitative yield (~30 mg) as a yellowish oil. Compound **194** has ^{1}H-NMR (D_2O) δ (ppm): 7.05 (s, 8H, H_{ar}), 3.06-4.02 (m, 80H, H-4, H-5, H-6, H-7, H-8, H-9, H-9', OMe, CH-a, CH-b, OCH-a, OCH-b, 2 CH_2N, SCH-a and SCH-b), 2.73 (dd, 4H, H-3e), 1.95 (s, 12H, NAc), 1.86 (m, 4H, H-3a), 1.44-1.65 (m, 16H, 2 internal CH_2), 1.09 (s, 36H, *t*-Bu); ^{13}C-NMR (D_2O) δ

(ppm): 173.9, 173.1, 171.5, 168.4 (C=O) 152.6, 149.1, 134.7 (C_{ar}), 126.0 (C_{ar}-H), 83.5 (C-2), 77.4 (OCH_2), 76.2 (C-6), 71.4 (C-8), 70.0 (C-4), 68.3 (C-7), 64.6 (C-9), 53.3 (OMe), 53.3 (C-5), 40.2 (2 NH*CH_2*), 41.6 (C-3), 36.5 (*C*Me_3), 33.5 (SCH_2), 32.1 (*t*-Bu), 30.8 (Ar*CH_2*), 28.0, 27.5 (2 internal CH_2), 22.7 (NAc). Compound **196** has $[\alpha]_D$ +4.3° (*c* 0.45, MeOH); ^{1}H-NMR (D_2O) δ (ppm): 7.10 (s, 8H, H_{ar}), 3.10-4.00 (m, 68H, H-4, H-5, H-6, H-7, H-8, H-9, H-9', CH-a, CH-b, OCH-a, OCH-b, 2 CH_2N, SCH-a and SCH-b), 2.76 (dd, 4H, H-3e), 1.99 (s, 12H, NAc), 1.90 (m, 4H, H-3a), 1.45-1.65 (m, 16H, 2 internal CH_2), 1.10 (s, 36H, *t*-Bu); ^{13}C-NMR (D_2O) δ (ppm): 173.9, 172.5, 171.6, 169.3 (C=O) 152.7, 149.1, 135.1 (C_{ar}), 126.2 (C_{ar}-H), 83.4 (C-2), 77.5 (OCH_2), 76.2 (C-6), 71.5 (C-8), 70.0 (C-4), 68.4 (C-7), 64.3 (C-9), 53.0 (C-5), 40.3 (2 NH*CH_2*), 41.5 (C-3), 36.6 (*C*Me_3), 33.6 (SCH_2), 32.2 (*t*-Bu), 30.9 (Ar*CH_2*), 28.1, 27.5 (2 internal CH_2), 22.8 (NAc).

Fully deprotected calix[4]arene tetramer with long spacer armed Neu5Ac (197)

A solution of the tetravalent α-thiosialocalix[4]arene **193** (35 mg, 0.0101 mmol) dissolved in 0.5 M aqueous NaOH in EtOH (3 mL, 1:5, v/v) was stirred at room temperature overnight (16 h). The solution was then treated with Amberlite IR-120 cation exchange resin and the filtrate freeze dried. Title compound **197** was obtained as a white, lyophilized powder in quantitative yield (27.5 mg, 0.0100 mmol). Compound **197** has $[\alpha]_D$ +3.8° (*c* 0.5, MeOH); ^{1}H-NMR (D_2O) δ (ppm): 7.09 (s, 8H, H_{ar}), 3.11-4.03 (m, 68H, H-4, H-5, H-6, H-7, H-8, H-9, H-9', CH-a, CH-b, OCH-a, OCH-b, 2 CH_2N, SCH-a and SCH-b), 2.76 (dd, 4H, H-3e), 1.97 (s, 12H, NAc), 1.90 (m, 4H, H-3a), 1.56-1.66 (m, 16H, 2 CH_2), 1.38-1.55 (m, 16H, 2 internal CH_2), 1.10 (s, 36H, *t*-Bu); ^{13}C-NMR (D_2O) δ (ppm): 173.9, 172.4, 171.6, 169.3 (C=O) 152.5, 149.0, 135.1 (C_{ar}), 126.1 (C_{ar}-H), 83.3 (C-2), 77.5 (OCH_2), 76.2 (C-6), 71.4 (C-8), 70.0 (C-4), 68.4 (C-7), 64.2 (C-9), 53.0 (C-5), 40.3 (2 NH*CH_2*), 41.5 (C-3), 36.5 (*C*Me_3), 33.6 (SCH_2), 32.1 (*t*-Bu), 30.7 (Ar*CH_2*), 28.5, 28.1, 27.5, 26.9 (4 internal CH_2), 22.6 (NAc).

Methyl (amino 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosid) onate (198)

Azidosialoside **118** (1.0 g, 1.93 mmol) was dissolved in anhydrous methanol (30 mL). Then 10% palladium on carbon (Pd-C, 150 mg) was added to the solution and the resulting slurry was stirred at room temperature under hydrogen (H_2 was bubbled through the solution). After 2 h, the catalyst was filtered off (celite bed) and washed with methanol (2 x 15 mL). The methanolic solution was then evaporated under reduced pressure. The resulting colorless aminoketoside **198** (0.93 g, 1.90 mmol) was used for subsequent reactions without further purification. Title compound **198** has $[\alpha]_D$ -50.2° (*c* 1.1, $CHCl_3$); MS (CI ether, rel. intensity) m/z: 491 ($[M+H]^+$, 1.8%), 431 ($[M-CO_2Me]^+$ and/or $[M-OAc]^+$, 47.1%).

Methyl {[6-(Carbobenzyloxyamino)hexanoylamido] 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosid} onate (201)

N-(Carbobenzyloxyamino)caproic acid **199** (560 mg, 2.11 mmol) was dissolved in thionyl chloride (5 mL, 68 mmol) and the solution was heated under reflux for 3 h. The cooled solution was evaporated to dryness and co-evaporated twice using toluene to afford acid chloride **200** (quantitative). Freshly prepared aminosialoside **198** (0.93 g, 1.90 mmol) was dissolved in dry methylene chloride (10 mL). The reaction mixture was cooled to 0°C and stirred under nitrogen wherein newly prepared *N*-(Carbobenzyloxy-amino)hexanoyl chloride **200** (600 mg, 2.11 mmol) was added. The reaction was stirred at room temperature for 3 h. The reaction mixture was then diluted with 50 mL each of CH_2Cl_2 and saturated $NaHCO_3$. The organic phase was separated and successively washed with saturated $NaHCO_3$ (40 mL), water (2 x 40 mL) followed by saturated NaCl (20 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a gradient

of 1% to 2% MeOH in $CHCl_3$ as eluent. Title compound **201** was obtained as an amorphous solid in 71% yield (1.0 g, 1.36 mmol). Compound **201** has $[\alpha]_D$ +13.1° (*c* 1.28, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.47 (s, 1H, NH), 7.18-7.30 (m, 5H, H_{ar}), 5.55 (d, 1H, NH), 5.36 (dd, 1H, $J_{6,7}$ = 2.3 Hz, $J_{7,8}$ = 7.7, H-7), 5.15 (ddd, 1H, $J_{8,9'}$ = 2.1, $J_{8,9}$ = 6.6, H-8), 5.03 (s, 2H, O*CH*$_2$Ph), 4.90 (ddd, 1H, $J_{4,5}$ = 10.6, $J_{3a,4}$ = 11.9, $J_{3e,4}$ = 4.5, H-4), 4.60 (dd, 1H, $J_{5,6}$ = 10.4, H-6), 4.37 (dd, 1H, $J_{9,9'}$ = 12.4, H-9'), 4.05 (ddd, 1H, $J_{5,NH}$ = 10.4, H-5), 3.95 (dd, 1H, $J_{8,9}$ = 6.6, H-9), 3.70 (s, 3H, OCH_3), 3.15 (dt, 2H, J = 6.4, $J_{CH2,NH}$ = 12.4, *CH*$_2$NHCbz), 2.60 (dd, 1H, $J_{3e,3a}$ = 13.0, H-3e), 2.16 (t, 2H, J = 6.3, CH_2CO), 2.00-2.11 (4s, 12H, 4 OAc), 1.95-2.04 (m, 1H, H-3a), 1.85 (s, 3H, NAc), 1.60 (m, 2H, CH_2), 1.48 (m, 2H, CH_2), 1.30 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ (ppm): 169.1-172.4 (C=O), 156.5, 136.7, 128.5, 128.0 (C_{ar}), 82.4 (C-2), 73.1 (C-6), 70.5 (C-8), 69.3 (C-4), 67.9 (C-7), 66.4 (O*CH*$_2$Ph), 64.5 (C-9), 52.8 (OCH_3), 49.8 (C-5), 40.8 (CH_2NH), 37.8 (C-3), 35.2 (*CH*$_2$CO), 29.4 (CH_2), 26.1 (CH_2), 24.4 (CH_2), 23.1 (NAc), 20.9, 20.9, 20.8, 20.7 (4 OAc); MS (FAB^+ thioglycerol, rel. intensity) m/z: 738 ($[M+H]^+$, 43%), 678 ($[M-CO_2Me]^+$ and/or $[M-OAc]^+$, 18%), 630 ($[M-OCH_2Ph]^+$, 36.8%).

Tetravalent sialocalix[4]arene (203)

Sialoside **201** (125.6 mg, 0.170 mmol) was dissolved in anhydrous methanol (10 mL). Then 10% Pd-C (15 mg) along with 10 drops of acetic acid were added to the solution and the resulting slurry was stirred at room temperature with hydrogen bubbling in the solution. After 2 h, the catalyst was filtered off (celite bed) and washed with methanol (2 x 5 mL). The methanolic solution was then evaporated near dryness. The resulting oily acetate salt of aminosialoside **202** was dissolved in dry CH_2Cl_2 (5 mL) and Et_3N (32 μL, 0.227 mmol). The solution was then added dropwise over a period of 15 min, into a cooled (0°C) freshly prepared 4-*tert*-butylcalix[4]arene-*O*,*O*',*O*'',*O*'''-tetraacetyl chloride **185** (0.0284 mmol) dissolved in CH_2Cl_2 (5 mL) and Et_3N (24 μL, 0.170 mmol) solution. The reaction mixture was stirred at room temperature under

nitrogen for 17 h. The reaction mixture was then diluted with 25 mL each of CH_2Cl_2 and saturated $NaHCO_3$. The organic phase was separated and successively washed with saturated $NaHCO_3$ (20 mL), water (2 x 20 mL) followed by saturated NaCl (15 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a gradient of 2% to 10% MeOH in CH_2Cl_2 as eluent. Title compound **203** was obtained as an amorphous solid in 50% yield (45.8 mg, 0.0142 mmol). Compound **203** has $[\alpha]_D$ +8.3° (*c* 0.45, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.90-7.29 (m, 16H, 2 NH & H_{ar}), 5.35 (dd, 4H, H-7), 5.17 (m, 8H, NH and H-8), 4.92 (ddd, 4H, H-4), 3.92-4.70 (m, 20H, H-5, H-6, H-9, H-9', & CH-a), 3.71 (s, 12H, OCH_3), 3.62-3.78 (m, 4H, OCH-a), 3.18-3.46 (m, 16H, CH_2N, CH-b and OCH-b), 2.62 (dd, 4H, H-3e), 2.16 (t, 8H, CH_2CO), 1.97-2.07 (4s, 48H, 4 OAc), 1.95-2.04 (m, 4H, H-3a), 1.87 (s, 12H, NAc), 1.60 (m, 8H, CH_2), 1.42 (m, 8H, CH_2), 1.30 (m, 8H, CH_2), 1.05 (s, 36H, *t*-Bu); ^{13}C NMR ($CDCl_3$) δ (ppm): 169.1-171.6 (C=O), 149.7, 149.1, 134.3 (C_{ar}), 126.4 (C_{ar}-H), 82.2 (C-2), 77.2 (OCH_2), 73.4 (C-6), 69.6 (C-4), 69.1 (C-8), 67.5 (C-7), 63.6 (C-9), 53.0 (OCH_3), 49.5 (C-5), 40.5 (CH_2NH), 37.5 (C-3), 35.1 (*CH_2*CO), 34.2 (*C*Me_3), 31.4 (*t*-Bu), 30.2 (Ar*CH_2*), 29.5 (CH_2), 26.3 (CH_2), 24.4 (CH_2), 23.1 (NAc), 21.3, 20.9, 20.8, 20.7 (4 OAc).

6-(N-tert-Butoxycarbonylamino)-1-hexanol (205)

6-Amino-1-hexanol **204** (2.5 g, 21.3 mmol) was dissolved in CH_2Cl_2 (13 mL) in a 100 mL round-bottom flask equipped with a pressure equalizing addition funnel and cooled to 4°C. Di-*tert*-butyl dicarbonate (5.03 g, 23.0 mmol) in CH_2Cl_2 (13 mL) is added dropwise over 30 min. After stirring for 12 h at room temperature, the solution is diluted with CH_2Cl_2 (25 mL) and washed with 0.1 M HCl (10 mL), sat. $NaHCO_3$ (2 x 15 mL), H_2O (15 mL), brine (10 mL) and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford upon standing in the refrigerator a white solid corresponding to title compound **205** in 99% yield (4.61 g, 21.2 mmol). Compound **205** has R_f 0.39 (7%

MeOH in CH_2Cl_2); mp 37.2-39°C; (lit.[228] ^{1}H-NMR ($CDCl_3$) δ (ppm): 4.35-4.75 (bs, 1H, NH), 3.60 (t, 2H, J = 6.4 Hz, CH_2O), 3.08 (q, 2H, J = 6.8, CH_2N), 1.41 (s, 9H, Boc), 1.31-1.60 (m, 8H, 4 CH_2); ^{13}C-NMR ($CDCl_3$) δ (ppm): 156.1 (C=O), 79.2 (*C*Me$_3$), 62.6 (OCH_2), 40.5 (CH_2N), 32.5, 30.0 (2 CH_2), 28.4 (C*Me$_3$*), 26.3, 25.2 (2 CH_2); MS (CI ether, rel. intensity) m/z: 218 ([M+H]$^+$, 59.8%), 162 ([M+H-*t*-Bu]$^+$, 100%), 118 ([M+H-Boc]$^+$, 100%).

1-(N-tert-Butoxycarbonylamino)-6-[(p-toluenesulfonyl)oxy]hexane (206)

A solution of *N*-Boc 6-aminohexanol **205** (5.55 g, 25.5 mmol) in anhydrous methylene chloride (25 mL) was cooled to 0°C. Triethylamine (5.35 mL, 38.4 mmol) was added followed by *p*-toluenesulfonyl chloride (7.32 g, 38.4 mmol) over a 15 min period, and the solution was warmed to room temperature. After stirring for 3 h, the dark orange reaction mixture was diluted with CH_2Cl_2 (35 mL) and washed successively with 5% HCl solution (30 mL), saturated sodium bicarbonate solution (30 mL), twice with water (30 mL) and finally with brine (20 mL). The organic layer was dried using anhydrous Na_2SO_4 and concentrated under vacuum to afford an amorphous white solid. The crude product was purified by silica gel column chromatography using a gradient of 1/5 to 1/3 EtOAc/hexanes as eluent. Title compound **206** was obtained as a white amorphous solid in 88% yield (8.40 g, 22.6 mmol). Compound **206** has R_f 0.41 (1/2, EtOAc/hexanes); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.75 (d, 2H, J = 8.4 Hz, H_{ar}), 7.32 (d, 2H, H_{ar}), 4.36-4.61 (bs, 1H, NH), 3.98 (t, 2H, J = 6.4, CH_2O), 3.03 (m, 2H, CH_2N), 2.42 (s, 3H, $ArCH_3$), 1.40 (s, 9H, *O-t*-Bu), 1.22-1.68 (m, 8H, 4 CH_2); ^{13}C-NMR ($CDCl_3$) δ (ppm): 155.9 (C=O), 144.7, 133.1, 129.8, 127.8 (C_{ar}), 79.1 (*C*Me$_3$), 70.4 (OCH_2), 40.4 (CH_2N), 29.8, 28.7 (2 CH_2), 28.4 (C*Me$_3$*), 26.1, 25.0 (2 CH_2), 21.6 (CH_3); MS (CI ether, rel. intensity) m/z: 371.9 ([M+H]$^+$, 25.1%), 316 ([M+H-*t*-Bu]$^+$, 100%), 271.9 ([M+H-Boc]$^+$, 100%).

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[228] a) Voss, H. F.; Plattner, J.; Herrin, T. R. *US Patent 4,273,866* **1981**; b) Dutton, J. K. *et al. J. Chem. Soc., Perkin I* **1985**, 2581.

Anal. Calcd for $C_{18}H_{29}NO_5S$: C, 58.20; H, 7.87; N, 3.77%. Found: C, 58.11; H, 7.92; N, 3.92.

6-Bromo-1-(N-tert-butoxycarbonylamino)hexane (207)

To a solution of tosylate **206** (2.5 g, 6.73 mmol) in freshly distilled 2-butanone (MEK or methylethyl ketone) (12 mL) was added lithium bromide (0.88 g, 10.1 mmol). The reaction was protected with a drying tube and stirred under reflux for 1 h. After cooling the reaction mixture to room temperature and removing the precipitated solid, the filtrate was evaporated under reduced pressure. The oily residue was purified by silica gel column chromatography using a gradient of 1:10 to 1:6 (v/v) ethyl acetate/hexanes as eluent to afford 1.86 g (99% yield, 6.64 mmol) of **207** as a colorless oil. Title compound **207** has R_f 0.34 (1:4, EtOAc/hexanes); ^{1}H-NMR ($CDCl_3$) δ (ppm):[229] 4.41-4.61 (bs, 1H, NH), 3.35 (t, 2H, J = 6.8 Hz, CH_2Br), 3.06 (m, 2H, CH_2N), 1.71-1.90 (m, 2H, CH_2), 1.39 (s, 9H, Boc), 1.31-1.50 (m, 6H, 3 CH_2); ^{13}C-NMR ($CDCl_3$) δ (ppm): 155.9 (C=O), 79.0 (CMe_3), 40.4 (CH_2N), 33.7 (CH_2Br), 32.6, 29.9 (2 CH_2), 28.3 (CMe_3), 27.7, 25.8 (2 CH_2); MS (FAB^+ thioglycerol, rel. intensity) m/z: 280 ($[M+H]^+$, 5.5%), 226 ($[M+4H-t\text{-}Bu]^+$, 24.3%), 224 ($[M+2H-t\text{-}Bu]^+$, 26.2%), 182 ($[M+4H-Boc]^+$, 3.5%), 180 ($[M+2H-Boc]^+$, 6.6%).

Anal. Calcd for $C_{11}H_{22}NO_2Br$: C, 47.15; H, 7.91; N, 5.00%. Found: C, 47.00; H, 7.82; N, 5.09.

1-S-Acetyl-6-(N-tert-butoxycarbonylamino)-1-thiohexane (208)

To a solution of tosylate **206** (600 mg, 1.62 mmol) and tetrabutylammonium hydrogen sulfate (548 mg, 1.62 mmol) in EtOAc (5 mL) was added thioacetic acid (289

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[229] Egbertson, M. S.; Chang, C. T.-C.; Duggan, M. E.; Gould, R. J.; Halczenko, W.; Hartman, G. D.; Laswell, W. L.; Lynch, J. J.; Lynch, J. R. *J. Med. Chem.* **1994**, *37*, 2537.

μL, 4.04 mmol, 2.5 equiv) pre-neutralized with 1M NaOH (4.0 mL). 1M Na_2CO_3 (5 mL) was then added and the mixture was vigorously stirred at room temperature for 3 h. The reaction was monitored by TLC (R_f 0.60, EtOAc:hexanes, 1:2) which showed complete transformation of the starting material. The reaction mixture was then diluted with 30 mL each of ethyl acetate and saturated aqueous $NaHCO_3$. The organic layer was separated and washed with saturated $NaHCO_3$ (2 x 20 mL), H_2O (3 x 20 mL) followed by saturated NaCl (15 mL). The dried organic layer (Na_2SO_4) was concentrated near dryness under reduced pressure, and purified by silica gel chromatography (2 mm chromatotron plate) using a gradient from 1/7 to 1/4 EtOAc/hexanes as eluent. Title compound **208** was isolated as a pure oil in 72% yield (321.2 mg, 1.17 mmol). Compound **208** has ^{1}H-NMR ($CDCl_3$) δ (ppm): 4.37-4.55 (bs, 1H, NH), 3.06 (m, 2H, J = 6.6 Hz, J = 7.0, CH_2N), 2.82 (t, 2H, J = 7.1 Hz, CH_2S), 2.29 (s, 3H, CH_3CO), 1.41 (s, 9H, *O-t*-Bu), 1.29-1.54 (m, 8H, 4 CH_2); ^{13}C-NMR ($CDCl_3$) δ (ppm): 195.9 (SAc), 155.9 (C=O), 78.9 (CMe_3), 40.4 (CH_2N), 30.5 (CH_3CO), 29.8, 29.3 (2 CH_2), 28.9 (SCH_2), 28.4 (CMe_3), 28.3, 26.2 (2 CH_2); MS (CI ether, rel. intensity) m/z: 276 ($[M+H]^+$, 57.5%), 232 ($[M-Ac]^+$, 3.6%), 220.9 ($[M+2H-t\text{-}Bu]^+$, 14.5%), 219.9 ($[M+H-t\text{-}Bu]^+$, 100%), 218 ($[M+H-OAc]^+$, 11.5%), 175.9 ($[M+H-Boc]^+$, 100%).

Anal. Calcd for $C_{13}H_{25}NO_3S$: C, 56.69; H, 9.15; N, 5.08%. Found: C, 56.90; H, 9.01; N, 5.27.

Methyl {[6-(N-tert-butoxycarbonylamino)hexyl] 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosid} onate (210)

PTC Method:

To a solution of thioacetate **208** (64 mg, 0.232 mmol) in dry methanol (1 mL) at room temperature under a N_2 atmosphere, was added 1 M sodium methoxide in methanol (232 μL, 0.232 mmol). The reaction became dark brown as soon as the NaOMe was added to the solution. The reaction was stirred at room temperature for 10 min after

which time the solution was treated with H^+ resin (Amberlite IR-120) for 5 min. The solution was filtered and evaporated under reduced pressure to give compound **209** as an oil in quantitative yield. Thiol **209** (54 mg, 0.232 mmol) was dissolved in degassed EtOAc (1 mL) and added to a solution of freshly prepared β-acetochloroneuraminic acid **2** (78.8 mg, 0.155 mmol) in degassed ethyl acetate (2 mL). Nitrogen was bubbled through a solution of Bu_4NHSO_4 (53 mg, 0.155 mmol) in EtOAc (1 mL) and 1 M Na_2CO_3 (3 mL) for 2 h, after which time it was transferred into the sugar-thiol reaction mixture. The two-phase solution was stirred under nitrogen at room temperature for 4 h, the reaction being monitored by TLC (ethyl acetate). The reaction mixture was then diluted with 10 mL each of ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was separated and washed with saturated $NaHCO_3$ (2 x 10 mL), water (2 x 10 mL) followed by brine (7 mL). The dried organic phase (Na_2SO_4) was concentrated under reduced pressure, and purified by silica gel chromatography (1 mm chromatotron plate) using a gradient of CH_2Cl_2 to 7% MeOH in CH_2Cl_2. Title compound **210** was obtained as a white foam in 10% yield (10.5 mg, 0.015 mmol).

Thioacetate Deprotection-Thioether Formation Method:

To a solution of freshly prepared α-thiosialoside **133** (46.1 mg, 0.091 mmol) in CH_2Cl_2 (5 mL) under nitrogen were added bromide **207** (38.2 mg, 0.136 mmol) and triethylamine (19 μL, 0.136 mmol). The reaction mixture was stirred at room temperature (N_2 atmosphere) for 16 h (overnight), since it was hard to determine the end of the reaction. The reaction mixture was then diluted with 10 mL of CH_2Cl_2 and the organic layer washed successively with saturated aqueous sodium bicarbonate (2 x 8 mL), H_2O (2 x 8 mL) and saturated NaCl (5 mL). The dried (Na_2SO_4) organic layer was evaporated to dryness under vacuum and the residue purified by silica gel preparative TLC plate. The plate was eluted twice using 5% and 6% MeOH in CH_2Cl_2. The compound was extracted from the preparative plate using EtOAc. Filtration of the silica gel and evaporation of the EtOAc solution afforded pure thiosialoside **210** 76% yield (48.7 mg, 0.0689 mmol).

Title compound **210** has R_f 0.39 (EtOAc); $[\alpha]_D$ +21.4° (*c* 0.5, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.34 (ddd, 1H, $J_{8,9'}$ = 2.7 Hz, $J_{8,9}$ = 5.2, $J_{7,8}$ = 8.2, H-8), 5.29 (dd, 1H, $J_{6,7}$ = 1.7, H-7), 5.11 (d, 1H, NH), 4.85 (ddd, 1H, $J_{3e,4}$ = 4.4, $J_{3a,4}$ = 11.4, $J_{4,5}$ = 10.6, H-4), 4.65 (bs, 1H, *NH*Boc), 4.29 (dd, 1H, $J_{9,9'}$ = 12.4, H-9'), 4.08 (dd, 1H, H-9), 4.00 (ddd, $J_{5,NH}$ = 9.9, 1H, H-5), 3.81 (dd, 1H, H-6), 3.78 (s, 3H, OMe), 3.08 (m, 2H, J = 6.6, CH_2N), 2.68-2.75 (m, 2H, SCH-a & H-3e), 2.48-2.53 (m, 1H, SCH-b), 2.14, 2.11, 2.01, 2.00 (4s, 12H, 4OAc), 1.95 (dd, 1H, H-3a), 1.85 (s, 3H, NAc), 1.41-1.50 (m, 4H, 2 CH_2), 1.41 (s, 9H, *O-t*-Bu), 1.28-1.38 (m, 4H, 2 CH_2); ^{13}C-NMR ($CDCl_3$) δ (ppm): 170.9, 170.6, 170.2, 170.1, 170.0 (C=O), 168.4 (C-1), 156.0 (Boc C=O), 83.1 (C-2), 78.9 (CMe_3), 74.0 (C-6), 69.6 (C-4), 68.6 (C-8), 67.3 (C-7), 62.1 (C-9), 52.8 (OMe), 49.4 (C-5), 40.6 (CH_2N), 38.0 (C-3), 29.8, 29.6, 29.0 (3 CH_2), 28.8 (SCH_2), 28.4 (CMe_3), 26.1 (CH_2), 23.1 (NAc), 21.1, 20.8, 20.8, 20.7 (4OAc); MS (FAB^+ thioglycerol, rel. intensity) m/z: 707.4 ($[M+H]^+$, 25.6%), 647.4 ($[M-CO_2Me]^+$ and/or $[M-OAc]^+$, 4.0%), 607.3 ($[M+H-Boc]^+$, 100%), 547.3 ($[M+H-Boc-CO_2Me]^+$ and/or $[M+H-Boc-OAc]^+$, 10.2%), 474.2 ([M-aglycone]$^+$, 12.6%).

Anal. Calcd for $C_{31}H_{50}N_2O_{14}S$: C, 52.68; H, 7.13; N, 3.96%. Found: C, 52.48; H, 7.19; N, 4.11.

Methyl [(6-aminohexyl) 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosid]onate (211)

N-Boc protected thiosialoside **210** (200 mg, 0.283 mmol) was treated with 20% TFA in CH_2Cl_2 (5 mL) and stirred at room temperature for 1 h. The solution was evaporated under reduced pressure, co-evaporated twice with toluene and put on a vacuum pump for 1 h. The amine **211** was purified by a short silica gel column chromatography using a gradient of 5% to 12% MeOH in CH_2Cl_2 as eluent. Title compound **211** in a TFA salt form was obtained in 89% yield (180.8 mg, 0.251 mmol). Compound **211** has R_f 0.21 (10% MeOH in CH_2Cl_2); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.61 (bs,

1H, NH_2), 5.69 (d, 1H, $J_{5,NH}$ = 10.0 Hz, NH), 5.20-5.31 (m, 2H, H-7 & H-8), 4.82 (ddd, 1H, $J_{3e,4}$ = 4.5, $J_{3a,4}$ = 11.3, H-4), 3.92-4.34 (m, 3H, H-5, H-9, & H-9'), 3.69-3.82 (m, 1H, H-6), 3.76 (s, 3H, OMe), 2.94 (m, 2H, CH_2N), 2.56-2.75 (m, 2H, SCH-a & H-3e), 2.40-2.55 (m, 1H, SCH-b), 2.12, 2.10, 2.00, 2.00 (4s, 12H, 4OAc), 1.92-2.00 (m, 1H, H-3a), 1.85 (s, 3H, NAc), 1.55-1.70 (m, 2H, CH_2), 1.42-1.55 (m, 2H, CH_2), 1.27-1.42 (m, 4H, 2 CH_2); MS (FAB^+ thioglycerol, rel. intensity) m/z: 607.6 ($[M+H]^+$, 47.2%), 606.6 ($[M]^+$, 72.4%), 547.8 ($[M-CO_2Me]^+$ and/or $[M-OAc]^+$, 4.0%), 474.0 ($[M-aglycone]^+$, 2.3%).

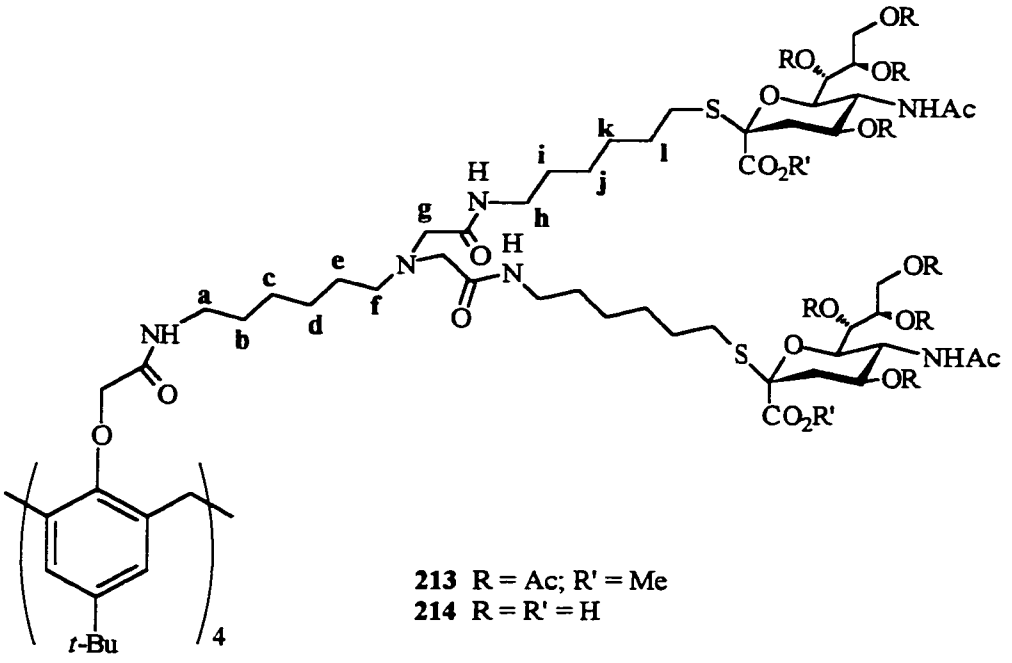
Methyl {[N-(bromoacetyl)-6-aminohexyl] 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosid} onate (212)

To a solution of amino thiosialoside **211** (250 mg, 0.347 mmol) and diisopropylethylamine (DIPEA, 151 μL, 0.868 mmol) in dry CH_2Cl_2 (10 mL) at 0°C was added dropwise bromoacetyl chloride (34.7 μL, 0.416 mmol) in CH_2Cl_2 (10 mL) over a 30 min period. The reaction solution was stirred without the ice-water bath for an extra 1.5 h. The reaction mixture diluted with CH_2Cl_2 (10 mL) and washed with 5% aqueous HCl (10 mL), saturated $NaHCO_3$ (10 mL), H_2O (10 mL) and brine (7 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel chromatography (1 mm chromatotron plate) using 4% MeOH in CH_2Cl_2 as eluent to afford *N*-bromoacetylated thiosialoside **212** in 76% yield (192 mg, 0.264 mmol). Title compound **212** has $[\alpha]_D$ +19.5° (*c* 0.9, $CHCl_3$); R_f 0.45 (10% MeOH in CH_2Cl_2); ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.65 (m, 1H, NH), 5.33 (ddd, 1H, $J_{8,9'}$ = 2.4 Hz, $J_{8,9}$ = 5.2, H-8), 5.29 (dd, 1H, $J_{6,7}$ = 2.0, $J_{7,8}$ = 8.4, H-7), 5.26 (d, 1H, $J_{5,NH}$ = 9.8, NH), 4.84 (ddd, 1H, $J_{3e,4}$ = 4.6, $J_{3a,4}$ = 11.6, $J_{4,5}$ = 10.4, H-4), 4.31 (dd, 1H, H-9'), 4.06 (dd, 1H, H-9), 4.01 (ddd, 1H, H-5), 3.85 (s, 2H, CH_2Br), 3.80 (dd, 1H, $J_{5,6}$ = 10.2, H-6), 3.77 (s, 3H, OMe), 3.27 (m, 2H, J = 6.5, CH_2N), 2.64-2.74 (m, 2H, SCH-a & H-3e), 2.49 (dt, 1H, $J_{a,b}$ = 12.6, SCH-b), 2.14, 2.11, 2.01, 2.00 (4s, 12H, 4OAc), 1.93 (dd, 1H, $J_{3e,3a}$ = 12.6, H-3a), 1.86 (s, 3H, NAc), 1.45-1.55 (m, 4H, 2 CH_2), 1.30-1.41 (m, 4H, 2 CH_2); ^{13}C-NMR

($CDCl_3$) δ (ppm): 171.0, 170.7, 170.5, 170.2, 170.1 (C=O), 168.5 (C-1), 166.0 (C=O), 83.2 (C-2), 74.0 (C-6), 69.7 (C-4), 68.7 (C-8), 67.4 (C-7), 62.2 (C-9), 53.0 (OMe), 49.5 (C-5), 40.1 (CH_2N), 38.1 (C-3), 29.1 (CH_2Br), 29.0, 29.1, 29.0 (3 CH_2), 28.7 (SCH_2), 26.2 (CH_2), 23.1 (NAc), 21.2, 20.8, 20.8, 20.7 (4OAc); MS (FAB^+ thioglycerol, rel. intensity) m/z: 730.3 ([M(^{81}Br)+H]$^+$, 9.7%), 729.3 ([M(^{81}Br)]$^+$, 29.4%), 728.3 ([M(^{79}Br)+H]$^+$, 10.0%), 727.3 ([M(^{79}Br)]$^+$, 27.7%), 685.3 ([M(^{81}Br)-Ac]$^+$, 22.7%), 683.3 ([M(^{79}Br)-Ac]$^+$, 47.1%), 669.2 ([M(^{81}Br)-CO_2Me]$^+$ and/or [M(^{81}Br)-OAc]$^+$, 10.9%), 667.2 ([M(^{79}Br)-CO_2Me]$^+$ and/or [M(^{79}Br)-OAc]$^+$, 10.3%), 475.2 ([M+H-aglycone]$^+$, 14.9%), 474.2 ([M-aglycone]$^+$, 29.0%).

Anal. Calcd for $C_{28}H_{43}N_2O_{13}BrS$: C, 46.22; H, 5.96; N, 3.85%. Found: C, 46.50; H, 5.71; N, 3.99.

Octavalent sialocalix[4]arene (214)



213 R = Ac; R' = Me
214 R = R' = H

N-Boc protected calix[4]arene tetramer **187** (25 mg, 0.0149 mmol) was treated with 20% TFA in CH_2Cl_2 (2 mL) and stirred at room temperature for 3 h. The solution was evaporated under reduced pressure and co-evaporated twice with toluene. To a solution of tetraamine **189** (0.0149 mmol) dissolved in CH_3CN (10 mL) and Et_3N (25 μL, 0.179 mmol) was added bromide **212** (108.6 mg, 0.149 mmol). The reaction mixture was stirred at 45°C under a nitrogen atmosphere for 48 h. The solvent was evaporated under reduced pressure and the residue purified by size exclusion column chromatography (LH20) using MeOH as eluent to afford off-white foam **213** in 42% yield (40.4 mg, 0.00627 mmol). A solution of fully protected sialocalix[4]arene **213** (30 mg, 0.00466 mmol) dissolved in 0.05 M NaOH (2.5 mL) was stirred at room temperature overnight (16 h). The solution was then treated with Amberlite IR-120 cation exchange resin and the filtrate freeze dried. Fully deprotected octavalent sialocalix[4]arene **214** was obtained as a yellowish, lyophilized powder in nearly quantitative yield (22.7 mg, 0.00456 mmol). Compound **213** has $[\alpha]_D$ +15.1° (*c* 0.47, $CHCl_3$); ^{1}H-NMR presented broad peaks; ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.24 (m, 4H, NH), 6.75 (bs, 16, NH & H_{ar}), 5.27-5.54 (m, 24H, NH, H-7, & H-8), 4.85 (ddd, 8H, H-4), 4.40-4.58 (m, 12H, OCH_2 & ArCH-a), 4.37 (dd, 8H, H-9'), 4.05 (dd, 8H, H-9), 3.99 (m, 8H, H-5), 3.78-3.89 (m, 8H, H-6), 3.78 (s, 24H, OMe), 3.12-3.45 (m, 44H, ArCH-b, H-a, H-g, & H-h), 2.68-2.78 (m, 16H, SCH-a & H-3e), 2.48-2.56 (m, 12H, SCH-b & H-f), 2.11, 2.10, 2.01, 1.99 (4s, 96H, 4OAc), 1.91 (dd, 8H, H-3a), 1.85 (s, 24H, NAc), 1.05 (s, 36H, *t*-Bu), 0.95-1.68 (m, 96H, H-b, H-c, H-d, H-e, H-i, H-j, H-k, & H-l); ^{13}C-NMR ($CDCl_3$) δ (ppm): 168.6-170.8 (C=O) 152.7, 145.8, 132.6 (C_{ar}), 125.7 (C_{ar}-H), 83.2 (C-2), 74.1 (C-6), 74.0 (OCH_2), 70.4 (C-4), 69.7 (C-8), 67.4 (C-7), 62.1 (C-9), 53.1 (OMe), 49.2 (C-5), 39-40.1 (C-a, C-g, & C-h), 38.0 (C-3), 33.8 (CMe_3), 31.4 (ArCH_2), 31.2 (*t*-Bu), 26-30 (SCH_2, C-b, C-c, C-d, C-e, C-f, C-i, C-j, C-k, & C-l), 23.1 (NAc), 20.6-21.2 (OAc). **214**: ^{1}H-NMR (D_2O) δ (ppm): 7.08 (s, 8H, H_{ar}), 3.09-4.05 (m, 112H, H-4, H-5, H-6, H-7, H-8, H-9, H-9', ArCH-a, ArCH-b, OCH-a, OCH-b, H-a, H-g, & H-h), 2.45-2.76 (m, 32H, SCH-a, SCH-b, H-f, & H-3e), 1.95 (s, 24H, NAc), 1.87 (m, 8H, H-3a), 1.01-1.70 (m, 96H, H-b, H-c, H-d, H-e, H-i, H-j, H-k, &

H-l), 1.08 (s, 36H, *t*-Bu); ^{13}C-NMR (D_2O) δ (ppm): 169.1-173.4 (C=O) 152.9, 148.2, 134.6 (C_{ar}), 126.0 (C_{ar}-H), 83.6 (C-2), 75.6 (OCH_2), 75.2 (C-6), 71.0 (C-8), 70.1 (C-4), 68.4 (C-7), 63.8 (C-9), 52.7 (C-5), 39.2-40.3 (C-a, C-g, & C-h), 41.8 (C-3), 36.4 (CMe_3), 32.2 (*t*-Bu), 31.9 (ArCH_2), 26.2-29.4 (SCH_2, C-b, C-c, C-d, C-e, C-f, C-i, C-j, C-k, & C-l), 22.5 (NAc).

5.7.2 Turbidimetric Analysis

Turbidimetric Assay with Wheat Germ Agglutinin

Turbidimetric experiments were performed in Linbro (Titertek) microtitration plates where 50 μL of stock lectin solutions prepared from WGA (2 mg/mL PBS) were mixed with 25.0, 26.1 and 24.0 μL of glycocalix[4]arene solutions **196**, **197** and, **214** respectively (0.5 mg/mL PBS). The turbidity of the solutions was monitored by reading the optical density at 490 nm for 2.5 h (Figure 5.5.1).

Turbidimetric Assay with Wheat Germ Agglutinin and Monovalent Inhibitor

Binding interactions were tested with 50 μL of 2 mg/mL wheat germ agglutinin (WGA) in phosphate buffer saline (PBS, pH 7.3) added to wells of a microtiter plate along with 50 μL of tetrameric sialocalix[4]arene **196** (0.5 mg/mL PBS). Half of the wells were then filled with 10 μL of α-thiophenyl sialoside (20 mg/mL PBS) which served as reference monovalent inhibitor. After mixing, the optical density (O.D. at 490 nm) was measured as a function of the time (Figure 5.5.2).

Turbidimetric Assay with *Limax flavus* of glycodendrimers and glycocalix[4]arenes

Binding experiments were performed using microtitration plates where 50 μL of 1 mg/mL LFA in phosphate buffer saline (PBS, pH 7.3) were mixed in the wells with 6.25

μL of nonameric sialodendrimer **182** (0.5 mg/mL PBS) and 6.13 μL of sialocalix[4]arene **197** (0.5 mg/mL PBS). The turbidity of the solutions was monitored by reading the optical density (490 nm) for 2 h (Figure 5.5.3).

All of the above turbidimetric tests were performed twice where consistency occurred, otherwise the tests were done in triplicate.

Conclusions

Syntheses of glycodendrimers and glycocalix[4]arenes have diversified the field of neoglycoconjugates. These hypervalent, chemically well-defined novel glycoconjugates were prepared in order to further explore multivalency (cluster effect) and their role in carbohydrate-protein interactions.

The carbohydrate moieties of the glycoconjugates were efficiently synthesized using mild, completely stereoselective phase transfer catalysis conditions. All the glycosides were obtained in high yields and occurred with complete inversion of configuration at the anomeric center, even with sialic acid's tertiary center.

An efficient conjugation strategy was employed in the syntheses of glycodendrimers and glycocalix[4]arenes. The strategy involved S_N2 displacement of the chlorine atom in pre-formed *N*-chloroacetylated multi-branched structures by thiol glycosides. These latter thiol glycosides were prepared from chemoselective deprotection of glycosides' thioacetates using two different methods. The de-*S*-acetylation proceeded in high yields on anomeric thioacetate glycosides as well as on non-carbohydrate thioacetate derivatives as illustrated by the high yields of the two steps de-*S*-acetylation-thioether formation. The two principal methods employed were, *i*) one-pot hydrazinium acetate and *ii*) a low temperature Zemplén reaction (NaOMe, MeOH).

Using solid phase synthesis on Wang resin, along with HOBt/DIC coupling chemistry, the first reported glycodendrimers were prepared based on hyperbranched L-lysine cores which were functionalized with *N*-chloroacetylglycylglycine spacers. Peracetylated thiosialosides were added to the solid phase for nucleophilic substitution of the *N*-chloroacetyl groups.

In addition, hyperbranched glycodendrimers containing sialic acid residues were synthesized from gallic acid as trivalent core and oligoethylene glycol derivatives as hydrophilic spacers. Fully deprotected gallic acid-based sialodendrimers exhibited good water solubility. These glycodendrimers were shown to bind and cross-link sialic acid

selective plant and animal lectins from wheat germ agglutinin and from the slug *Limax flavus*. Preliminary immunoassays from turbidimetric analyses indicated that nonavalent α-sialodendrimer **182** had a greater affinity towards the above mentioned lectins than the corresponding trivalent glycodendrimers **174** and **175**.

Sialic acid derivatives were finally conjugated onto calix[4]arenes which permitted the synthesis of a new class of glycoconjugates, namely glycocalix[4]arenes. Water-soluble tetravalent α-sialosides were efficiently scaffolded on the upper rim of *p-tert*-butylcalix[4]arene using a divergent approach and nucleophilic chloride displacement by thiosialosides. A different tetravalent sialocalix[4]arene was obtained using a more convergent approach in which the carbohydrate moiety was functionalized and coupled onto an electrophilic calixarene core.

Water-soluble octavalent dendritic sialocalix[4]arene was efficiently synthesized using double *N*-alkylation strategy from the reaction of a tetraamino calixarene derivative and *N*-bromoacetylated sialosides having a spacer arm therebetween.

Preliminary binding experiments on tetra- **196** & **197** and octa- **214** valent sialosides with the lectins WGA and LFA showed promising results, since they all exhibited strong binding (turbidimetric analysis) with the above lectins.

The glycodendrimers and glycocalix[4]arenes presented herein represent synthetically challenging and innovative neoglycoconjugates. Evaluation of the cluster effect *via* turbidimetric testing revealed that with a proper multivalent design, binding affinity between carbohydrates and proteins may be greatly enhanced depending on the biological systems under investigation. L-lysine-based α-thiosialodendrimers were shown to be as potent as their analogous polymers in the inhibition of the hemagglutination of *Influenza* virus.

Glycodendrimers and glycocalix[4]arenes represent potential therapeutic agents in cell surface carbohydrate interactions. Glycodendrimers, in particular, have already generated strong interest from the glycobiological community.

Claims to Original Research

1. Anomeric thio- and seleno- glycopyranoses were synthesized *via* phase transfer catalysis. These stable derivatives were synthesized in an easy and high yielding manner. They represent key monomeric precursors in the syntheses of glycodendrimers and glycocalix[4]arenes.

2. The *S*-acetate functionality on glycosides as well as on non-carbohydrate derivatives was chemoselectively deprotected in the presence of *O*-acetates by treatment with hydrazinium acetate in a one- or two-pot procedure. The reaction efficiency was demonstrated by the deprotection-thioether formation sequence.

3. *S*-Acetate functionality according to claim 2 can also be chemoselectively deprotected by treatment with sodium methoxide in methanol at low temperature (-40°C). This fast (less than 10 min) and easy method provided the source of thiol glycosides in the syntheses of glycodendrimers and glycocalix[4]arenes.

4. The first biologically active dendrimers were synthesized. The dendrimers are based on hyperbranched L-lysine cores and contain surface sialic acid residues. First through fourth generation sialodendrimers were prepared based on a solid phase, HOBt/DIC coupling strategy.

5. Pie-shaped divergent dendrimers with an increase in valency of 3^n where n represents the n'th generation, were prepared based on gallic acid core and oligoethylene glycol derivatives as spacers. Thiosialoside derivatives were conjugated to gallic acid-based dendrimers to provide divergent tri- and nona- valent sialodendrimers.

6. The first ever water soluble tetravalent glycocalix[4]arenes were synthesized from the scaffolding of sialoside derivatives onto functionalized calix[4]arene cores. These sialocalix[4]arenes were synthesized either by following a divergent or a convergent approach.

7. Calix[4]arene derivatives were also used to scaffold sialoside derivatives using a double *N*-alkylation reaction strategy which provided a water-soluble octavalent dendritic sialocalix[4]arene.

8. In biological evaluations, all glycodendrimers and glycocalix[4]arenes exhibited enhanced binding. This confirms the role of multivalency in carbohydrate-protein interactions.

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