INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

Université d'Ottawa · University of Ottawa

For my wife Xinyu and son Yuheng

ABSTRACT

The importance of cell surface carbohydrates in biological processes has led to remarkable progress in carbohydrate synthesis and glycobiology. However, efficient protocols for building oligosaccharides from monosaccharide components are still the major challenges in the field of carbohydrate synthesis. On the other hand, individual carbohydrate-protein interactions are characterized by low affinities. This drawback can be overcome by using the "multivalent or cluster effect". Therefore, the chemical synthesis of oligosaccharides and multivalent glycoconjugates is presented herein.

A very simple and efficient synthetic route towards *N*-acetyllactosamine derivatives was developed. This strategy has used "lightly protected" acceptors, 6-*O*-*tert*-butyldiphenylsilyl protected *N*-acetylglucosamine derivatives, to reduce tedious multiple protection and deprotection steps. Most importantly, these 6-*O*-*tert*-butyldiphenylsilyl protected *N*-acetylglucosamine acceptors allow the regiospecific introduction of a galactosyl moiety. In addition, this method constitutes a highly practical synthesis of Lewis X trisaccharide.

The "active-latent" glycosylation strategy made it possible to manipulate the reactivity of both glycosyl donors and acceptors by means of changing the electron density of the aryl substituents at the anomeric center. This strategy provides a facile approach toward the synthesis of complex oligosaccharides such as Lewis X pentasaccharide in a highly convergent manner.

The facile regio- and α-stereoselective glycosidation of Neu5Ac with several glycosyl acceptors was achieved using a thiophilic promoter (NIS/TfOH) in acetonitrile under kinetically controlled conditions. Under such conditions, the desired sialyl-α-(2→3)- and sialyl-α-(2→6)-sugar derivatives were obtained in good yields. The syntheses of sialyl α-(2→6) thioglycosides were also achieved in situ using diethylamine in chemoselective de-*S*-acetylation of thioacetate sialic acid derivative. These sialoside derivatives with their azido or *p*-nitrothiophenyl aglycone can be simply transformed into various neoglycoconjugates using well-established strategy.

Glycoclusters with various valencies were synthesized using transition metal-catalyzed reactions. Grubbs' ruthenium benzylidene catalyst had been successfully applied in olefin metathesis reactions from alkenyl sialic acid derivatives. The reactions afforded divalent sialoside derivatives in reasonable yields under mild reaction conditions. Using copper (I)-catalyzed Glaser reaction and palladium-catalyzed Sonogashira reaction, divalent 'rod-like' glycoclusters were prepared from terminal alkynes such as 2-propynyl thioglycosides in excellent yields. Such strategies also allowed 2-propynyl *O*-and *S*-sialic acid derivatives to form dimers, trimers and tetramers, respectively.

Divalent sialosides scaffolded on *p-tert*-butylcalix[4]arene were prepared using an isothiocyanate conjugation strategy. Following the same strategy, a GM_3 isothiocyanate derivative was coupled with multivalent amines based on γ-resorcylic acid- or gallic acid-cores to give multivalent GM_3 ligands in good yields. This strategy was extended to synthesize starburst® PAMAM-based glycodendrimers. PAMAM cores with generation G0 to G1 were conjugated with the GM_3 isothiocyanate derivative to give hypervalent thiourea derivatives containing four and eight GM_3 trisaccharide residues.

Lactosamine- and GM_3-containing glycopolymers were successfully prepared by incorporating the corresponding amine-terminated glycosides into pre-formed active poly(*N*-oxysuccinimidyl acrylate). The grafting polymerization reactions are carried out under mild conditions and afford fair to good yields.

ACKOWLEDGMENTS

I would like to thank Professor René Roy for allowing me to conduct my graduate research in his laboratory and for the financial support he provided over the last five years. Beyond this, Professor René Roy is a true mentor. His enthusiasm for chemistry, extensive knowledge of the field, and tremendous new ideas for the research made all the work possible generating good results. His intellectual and moral guidance, as well as his devotion to his graduate students will be forever remembered.

I would like to thank Dr. Suoding Cao and Mr. Qingquan Wu for their help when I first came to Ottawa. I am also grateful to my colleagues Dr. Serge J. Meunier, Dr. William K. C. Park, Dr. Diana Zanini, Dr. Myung-Gi Baek, Dr. Jin Mi Kim, Dr. Sanjoy K. Das, Dr. Muriel Llinares, Bradley Schmor, Denis Carrière, Daniel Pagé, Heba Abourahma for their helpful discussions and kindness.

Thanks also go to Dr. Glenn Facey, Raj Capoor, and Dr. Clem Kazakoff for their excellent technical assistance for NMR and mass spectra.

Lastly, I would like to thank my wife, Xinyu Wang for her love, encouragement, and understanding over the years. It is impossible for me to finish this thesis without her constant support. I also thank my little son, Yuheng for giving me a lot of pleasure even through he is still too young to speak. I should thank my parents Peining Gan and Liwen Du, my parents-in-law Jide Wang and Xiaofeng Liu, and my sister Zhongping Gan for their tremendous support and love.

TABLE OF CONTENTS

LIST OF SCHEMES

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Å	Angstrom
Ac	acetyl
AcCl	acetyl chloride
AcOH	acetic acid
ADEPT	Auto DEPT
Bn	benzyl
bp	boiling point
br	broad
bs	broad singlet
t-Bu	*tert*-Butyl
Bz	benzoyl
Cbz	carbobenzyloxy
CI	chemical ionization
COSY	correlation spectroscopy
d	doublet
DAST	diethylaminosulfur trifluoride
dba	dibenzylideneacetone
DBU	1,8-diazabicyclo[5,4,0]undec-7-ene
DCC	dicyclohexylcarbodiimide
dd	doublet of doublets
ddd	doublet of doublet of doublets
DEPT	distortionless enhanced polarization transfer
DIPEA	diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DMTST	dimethyl(methylthio)sulfonium triflate
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
equiv	equivalent

FAB-MS	fast atom bombardment ionization mass spectrometry
Fuc	fucose
Gal	galactose
GalNAc	*N*-acetylgalactosamine
Glc	glucose
GlcNAc	*N*-acetylglucosamine
h	hour(s)
HMQC	heteronuclear multiple quantum coherence
HOBt	1-hydroxybenzotriazole
HRMS	high resolution mass spectrometry
Hz	hertz
IDCP	iodonium dicollidine perchlorate
Le^x	Lewis X
Lit.	literature
m	multiple
M^+	parent molecular ion
Man	mannose
Me	methyl
min	minute(s)
μL	microliter
mL	milliliter
μmmol	micromolar
mmol	millimolar
MS	molecular sieves
mp	melting point
m/z	mass-to-charge ratio
Neu(5)Ac	*N*-acetylneuraminic acid
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
PAMAM	polyamidoamine

Ph	phenyl
Phth	phthaloyl
ppm	parts per million
PTC	phase transfer catalysis
py	pyridine
rt	room temperature
s	singlet
t	triplet
TBAHS	tetrabutylammonium hydrogen sulfate
TBDMSCl	*tert*-butyldimethylsilyl chloride
TBDPSCl	*tert*-butyldiphenylsilyl chloride
TEMEDA	*N,N,N',N'*-tetramethylethylenediamine
TfOH	trifluoromethanesulfonic acid
TLC	thin layer chromatography
Ts	*p*-toluenesulfonyl
TsOH	*p*-toluenesulfonic acid
Xyl	xylose

Chapter 1 Introduction

1.1 Biological significance of cell surface carbohydrates

Carbohydrates comprise the most abundant group of compounds found in natural sources. They are present both in plants and animals. They are the main source of energy supply in living organism and determine the structure of plants. Apart from these structural and energy storage roles, carbohydrates are now implemented in a wide range of biological processes such as cell-cell recognition, fertilization, embryogenesis, neuronal development, hormone activities, the proliferation of cells and their organization into specific tissues, viral and bacterial infection and tumor cell metastasis.[1]

Carbohydrates, nucleic acids and proteins are well-known biological information carriers. Among these, carbohydrates are capable of carrying higher biological information because of their greatest potential for structural variations. Carbohydrates are highly functionalized molecules. Each monosaccharide contains a number of hydroxyl groups to which another monosaccharide can be attached. This and the existence of other functional groups, substituents, two stereoisomers in the glycosidic linkage (α and β) and two ring forms (furanose and pyranose) render enormous possibilities of variation when monosaccharides assemble to form oligo- and polysaccharides. This means that a lot of biological information can be expressed in a rather short oligosaccharide sequence and the biological activity of carbohydrates is generally contained in structures smaller than a hexasaccharide.

Cell surface carbohydrates are mainly composed of glycolipids, glycoproteins, proteoglycans and capsular polysaccharides.[2] Oligosaccharides of glycoproteins and glycolipids predominantly on the cell surface serve as cell surface ligands for blood group

---

[1] Varki, A. *Glycobiology* **1993**, *3*, 97.
[2] Sharron, N. *Complex Carbohydrates, Their Chemistry, Biosynthesis and Function*, Addison-Wesly, Reading, MA, USA, **1975**.

and tumor associated antibodies, toxins, the cell attachment proteins of some bacteria,[3] mycoplasma,[4] various animal viruses,[5] and a variety of plant and animal lectins (Figure **1.1**). It was found that only a select group of monosaccharide residues were present in glycoproteins and glycolipids.[6] These include: *N*-acetyl-D-glucosamine (GlcNAc), *N*-acetyl-D-galactosamine (GalNAc), D-galactose (Gal), D-glucose (Glc), D-mannose (Man), L-fucose (Fuc), D-xylose (Xyl), and the sialic acids (Neu).

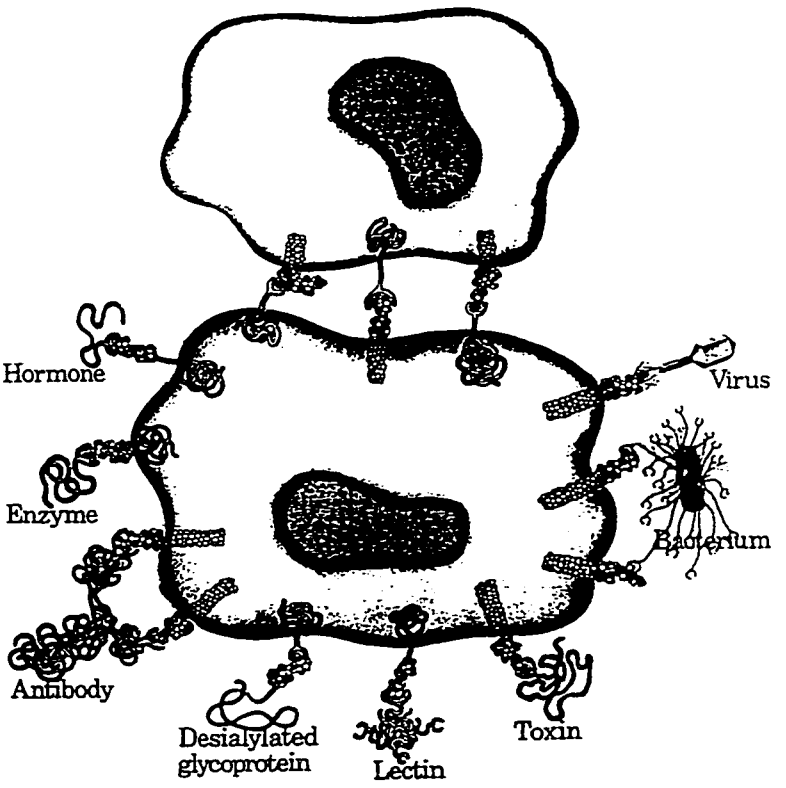


Figure 1.1 Cell surface carbohydrate interactions[7]

Sialic acids, which are exclusively α-linked, occupy the penultimate nonreducing end of the oligosaccharide chains in the glycoproteins and glycolipids. Among about 40 derivatives of neuraminic acid, *N*-acetylneuraminic acid (Neu5Ac) is the most widespread

[3] Sharon, N.; Lis, H. *The protein*, vol. 5 (Neurath, H.; Hill, R. L.; Eds.), Academic Press, **1982**, 1.
[4] Feizi, T. *Nature*, **1985**, *314*, 53.
[5] Paulson, J. C. *The receptors*, vol. 2 (Conn, P. M. Ed.) Academic Press, Orlando, **1985**, 131.
[6] Montreuil, J. *Adv. Carbohydr. Chem. Biochem.* **1980**, *37*, 158.

form of sialic acid (Figure **1.2**). The structural diversity of sialic acid is reflected in the variety of its biological functions.[8] Due to their negative charge, sialic acids are involved in binding and transport of positively charged molecules (e.g. Ca^{2+}) as well as in attraction and repulsion phenomena between cells and molecules.[9,10] As terminal substituents, they are ideally suited to participate in a variety of recognition processes between cells and molecules. Thus, the immune system can distinguish between self and nonself structures according their sialic acid pattern. Indeed, cell surface sialosides are known to serve as ligands for microbial toxins,[11] bacterial and viral adhesion,[12] and for mammalian lectins responsible for cell-cell adhesions (Table **1.1**).[13,14] In direct contrast to their recognition function, another important feature of sialic acids is the masking of cells and molecules such as erythrocytes.[15] However, masking of endogenous structures can also have a detrimental effect, as can be seen in the case of some tumors that are sialylated to a much higher degree than the corresponding normal tissues.[16,17]

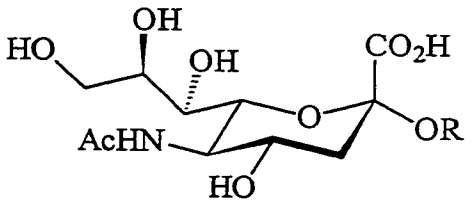


Figure 1.2 α-Sialosides as they appear on most natural cell surface gangliosides and sialyled glycoproteins

---

[7] Figure taken from BioCarb, **1990**.
[8] Schauer R.; Kelm, S. Reuter, G.; Roggentin, P.; Shaw, L. in *Biology of Sialic Acids*; Rosenberg, A. (Ed.); Plenum Press; New York; **1995**, 7.
[9] Kemp, R. B. *J. Cell Sci.* **1970**, *6*, 751.
[10] Brown, D. M.; Michael, A. F. *Proc. Soc. Exp. Biol. Med.* **1969**, *131*, 568.
[11] Karlsson, K.-A. *Curr. Opin. Struct. Bio.* **1995**, *5*, 622.
[12] Sharon, N.; Lis. H. *Science* **1989**, *246*, 227.
[13] Springer, T. A.; Larsky, L. A. *Nature* **1991**, *349*, 196.
[14] Paulson, J. C. in *Adhesion: Its Role in Inflammatory Diseases*; Harlan, J. M.; Liu, D. Y. (Eds.); New York: WH Freeman; **1992**, 19.
[15] Schauer, R. *Trends Biochem. Sci.* **1985**, *19*, 357.
[16] Furukawa, K.; Yamagachi, H.; Oettgen, H. F.; Old, L. J.; Lloyd, K. O. *Cancer Res.* **1989**, *49*, 191.
[17] Wargalla, U.; Sanders, D. J.; Reisfeld, R. A.; Mujos, K.; Kipps, T. J.; Yang, H. M.; Cheresh, D. A. *Cancer Res.* **1989**, *49*, 2857.

Table 1.1 Cell surface sialosides recognized as ligands by carbohydrate binding proteins

Carbohydrate binding protein	Cell surface sialoside ligand[a]
Influenza virus hemagglutinin (human)	NeuAcα2,6Galβ1,4GlcNAc-R
Influenza virus hemagglutinin (avian, equine)	NeuAcα2,3Galβ1,4GlcNAc-R
Sendai virus hemagglutinin	NeuAcα2,8NeuAcα2,3Gal-R
E. coli adhesin	NeuAcα2,3Galβ1,4Glcβ-ceramide (GM_3)
Cholera toxin	Galβ1,3GalNAcβ1,4(NeuAcα2,3)Galβ1,4-Glcβ-ceramide(GM_1)
E-selectin and P-selectin	NeuNAcα2,3Galβ1,4(Fucα1,3)GlcNAc-R(SLe^x) NeuNAcα2,3Galβ1,3(Fucα1,4)GlcNAc-R(SLe^a)
Bone marrow macrophage lectin	NeuAcα2,3Galβ1,3GalNAc-R

[a]R indicates the remaining core carbohydrate structure to which the sialoside sequence is attached

The influenza infection (common flu) is a well-known example of a cell-virus interaction mediated by sialic acid. Both of adhesion to the cell prior to infected cells as well as the release of newly formed viruses from the infected cells are accomplished by structures containing sialic acid. In this case, sialic acid plays two critical roles. Thus, it constitutes the key host receptor for the viral hemagglutinins and acts as a substrate for neuraminidase which is believed to be essential for the release of viron particles from the infected cells.

The roles of sialic acid involved in influenza virus infections have attracted interest by the pharmaceutical industry. Because bacterial adhesion is so critical to infection, the use of carbohydrates for prevention and treatment are being seriously considered. Blocking bacterial attachment is one strategy for combating infections. Thus, carbohydrates could act as molecular decoys, intercepting pathogenic bacteria before they reach their tissue targets (Figure **1.3**).

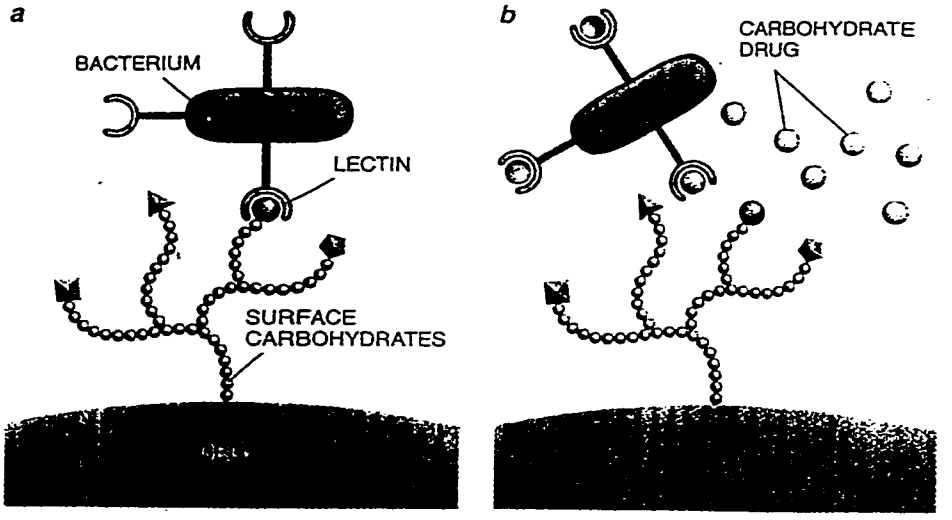


Figure 1.3[18] Blocking bacterial attachment using carbohydrate drugs. (a) As a prelude to infection, bacterial surface proteins (lectins) attach to surface carbohydrates on susceptible host cells. (b) Drug containing similar carbohydrates could prevent the attachment by binding to the lectins.

1.2 Recent advances in glycosidation methods and strategies

The importance of cell surface carbohydrates in biological events such as cell-cell recognition, growth, differentiation, pathogenic infection, and cancer has stimulated much synthetic activity in glycoside synthesis in the past years. Unlike peptides and oligonucleotides which can be readily produced in an automated manner, oligosaccharides are much more difficult to synthesize because of their multifunctionality and stereochemical variations. However, the use of various new leaving groups and activating agents in the

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

recent oligosaccharide synthesis[19] has dramatically improved the efficiency of glycosylation reactions (chemical yield, regioselectivity, and stereoselectivity).

Apart from liable glycosyl halides, glycosyl trichloroacetimidates,[20] thioglycosides,[21] 4-pentenyl glycosides,[22] glycal,[23] glycosyl phosphite,[24] and sulfoxides[25] as glycosyl donors are being drawn more and more attention in oligosaccharide synthesis. Among these glycosyl donors, glycosyl halides, trichloroacetimidates, and thioglycosides are most frequently used in the synthesis of *O*-glycosides.

Glycosyl halides used as effective glycosyl donors were first introduced by Koenigs and Knorr in 1901,[26] and are still frequently in use today because of their easy accessibility and high reactivity. However, the broad applications of these donors to the synthesis of complex oligosaccharides are often hampered due to the instability of the glycosyl bromides and chlorides, and the conditions involved in their preparation.

Glycosidation with anomeric trichloroacetimidate donors has proved to be a highly attractive and powerful alternative to the classical Koenigs-Knorr method. Its main advantages are that the glycosyl trichloroacetimidate donors are easily prepared from the corresponding hemiacetal sugar and readily activated by Lewis acids under mild conditions.

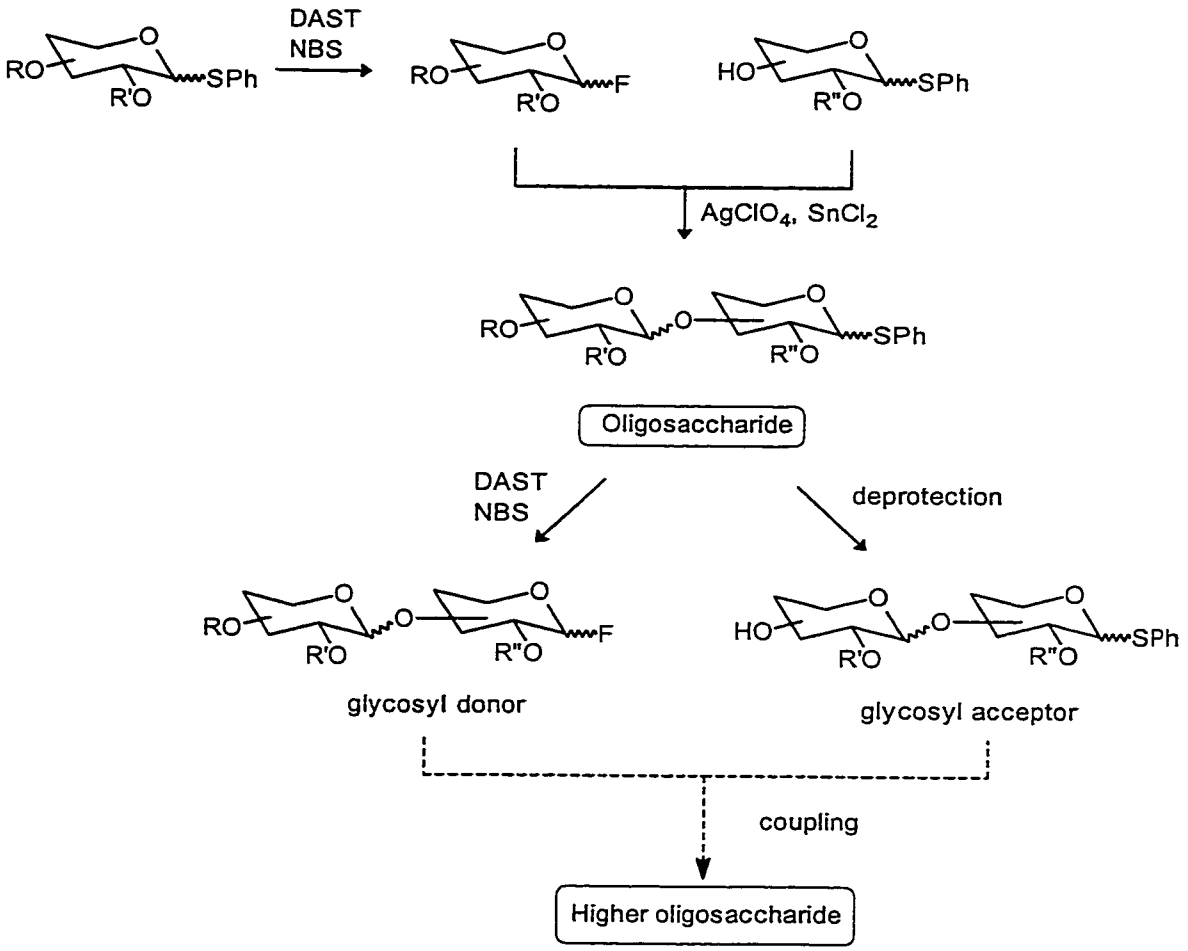
Thioglycosides are also used as glycosyl donors. In contrast to the donors discussed above, the thioglycosides are stable during most protection group manipulation as well as under many glycosidation conditions using other types of donors. Therefore, anomeric thio leaving group can be introduced early at the monosaccharide level. Another advantage with thioglycosides is that they are readily transformed into other types of glycosyl donors.

Although much effort has been devoted to the development of highly stereocontrolled methods for glycosylation, the strategies for the rapid and efficient assembly of oligosaccharide have also gained interest. The synthesis of large oligosaccharides is possible

---

[19] a) Paulson, H. *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 155; b) Schmidt, R. R. *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 155; c) Sinaÿ, P. *Pure Appl. Chem.* **1991**, *63*, 519; d) Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, 1503; e) Boons, G.-J. *Contemp. Org. Synth.* **1996**, *3*, 173.
[20] Schmidt, R. R.; Michel, J. *Angew. Chem. Int. Ed. Engl.* **1980**, 19, 731.
[21] Fügedi, P.; Garegg, P. J.; Lönn, H ; Norberg, T. *Glycoconjugate J.* **1987**, *4*, 97.
[22] Fraser-Reid, B.; Konradsson, P.; Mootoo, D. R.; Udodong, U. *J. Chem. Soc. Chem. Commun.* **1988**, 823.
[23] Halcomb, R. L.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 6661.
[24] a) Martin, T. J.; Schmidt, R. R. *Tetrahedron Lett.* **1992**, *33*, 6123; b) Kondo, H.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 8748.
[25] Kahne, D.; Walker, S.; Chang, Y.; Van Engen, D. *J. Am. Soc. Chem.* **1989**, *111*, 6881.
[26] Koenigs, W.; Knorr, E. *Ber.* **1901**, *34*, 957.

when a convergent strategy is used. In such a strategy, most efforts are directed towards the preparation of the monomeric glycosyl donors and acceptors. Earlier, the formation of stable oligosaccharide donors was a problem in convergent synthesis. However, with the introduction of the new types of donors (thioglycosides, pentenyl glycosides, trichloroacetimidates), the convergent strategy is applicable to almost any type of structures.

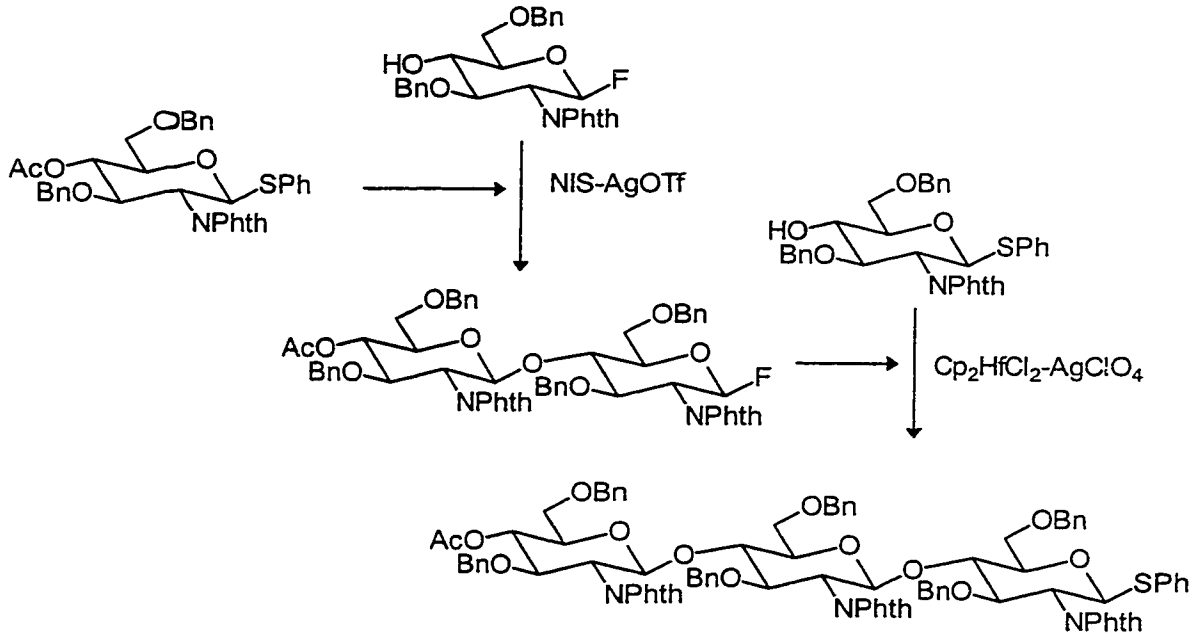


Scheme 1.1 The two-stage activation procedure for glycoside bond formation

The strategy of combining the chemistry of thioglycosides with that of glycosyl fluorides for the synthesis of oligosaccharides, known as the two-stage activation procedure,

was first suggested by Nicolaou *et al.*[27] Scheme **1.1** depicts this strategy. Thus, a stable phenylthioglycoside is first converted into a glycosyl fluoride donor. This donor is then coupled with a phenylthioglycoside acceptor. The procedure can be repeated by conversion of the anomeric phenylthio group into an anomeric fluoride which can be used in a further coupling reaction.

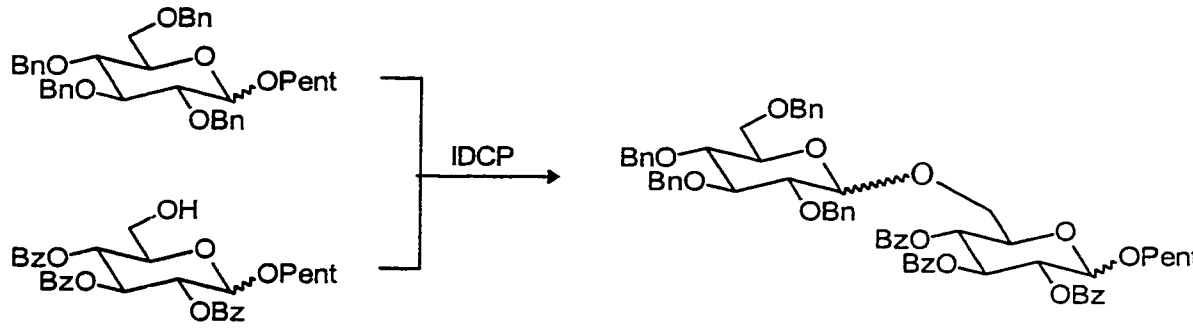
Recently, Ogawa *et al.*[28] reported an "orthogonal glycosidation strategy". In this approach, two sets of anomeric groups and activation conditions were used. Both of them act as anomeric protecting groups as well as leaving groups. The criteria for this strategy are that one anomeric group can be activated without affecting the other anomeric group and *vice versa*. The feasibility of this methodology was demonstrated in Scheme **1.2**. The thioglycosyl donor was activated in the presence of NIS-AgOTf as promoter and then coupled with a glycosyl fluoride acceptor to give disaccharide in 85% yield. Subsequent Cp_2HfCl_2-$AgClO_4$ mediated coupling of this disaccharide with thioglycosyl acceptor afforded trisaccharide in 72% yield.



Scheme 1.2 Orthogonal glycosylation strategy in oligosaccharide synthesis

[27] Nicolaou, K. C.; Dolle, R. E.; Papahatjis, D. P.; Randall, J. L. *J. Am. Soc. Chem.* **1984**, *106*, 4189.
[28] Kanie, O.; Ito, Y.; Ogawa, T. *J. Am. Soc. Chem.* **1994**, *116*, 12073.

During their extensive studies of 4-pentenyl glycosides, Fraiser-Reid *et al.*[29] introduced a new concept in glycosylation reactions, which is so called "armed-disarmed glycosylation strategy" (Scheme **1.3**). In this strategy, a C-2 ether protected pentenyl glycoside can be coupled chemoselectively with an acyl protected pentenyl glycoside under the mild promoter iodonium di-collidine perchlorate (IDCP). Such glycosidation depends on the fact that C-2 ether activates (arms) and C-2 ester deactivates (disarms) the anomeric center. Unlike orthogonal glycosylation strategy, this strategy requires only one type of anomeric group. This concept was also successfully applied to thioglycosides by Van Boom *et al.*[30] and to glycals by Danishefsky *et al.*[31]

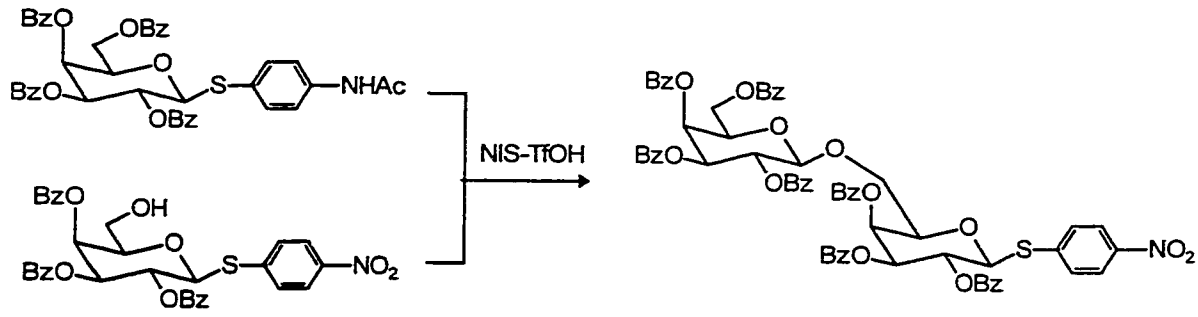


Scheme 1.3 "Armed-disarmed" glycosylation reaction

Almost one decade ago, our group[32] first proposed a "latent-active" concept for the oligosaccharide synthesis, in which para-substituted phenyl thioglycosides can be used as glycosyl donors or acceptors depending on the electron density of the aryl substituents. In this strategy, a stable anomeric group can be converted into a good leaving group by a simple chemical interconversion. An elegant example[33] is shown in Scheme **1.4**.

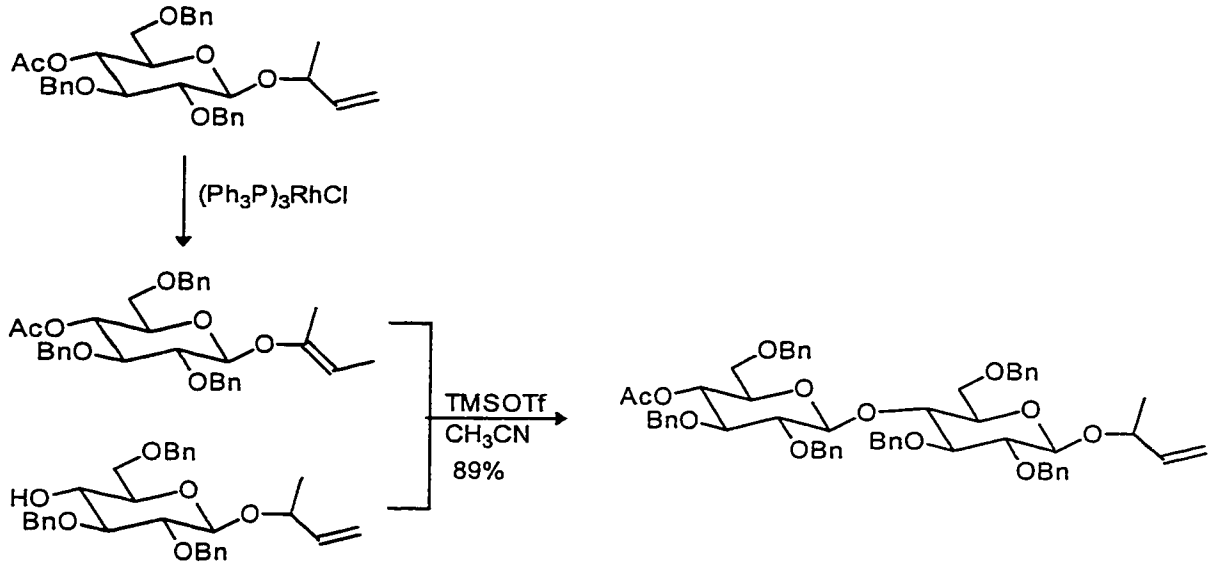
---

[29] Mootoo, D. R.; Konradsson, P.; Udodong, U.; Fraser-Reid, B. *J. Am. Soc. Chem.* **1988**, *110*, 5583.
[30] a) Veeneman, G. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 275; b) Veeneman, G. H.; van leeumen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331.
[31] Friesen, R. W.; Danishefsky, S. J. *J. Am. Soc. Chem.* **1989**, *111*, 6656.
[32] Roy, R.; Andersson, F. O.; Letellier, M *Tetrahedron Lett.* **1992**, *33*, 6053.
[33] Cao, S.; Hernández-Matéo, F.; Roy, R. *J. Carbohydr. Chem.* **1998**, *17*, 609.



Scheme 1.4 *p*-Nitrophenyl thioglycosides in "latent-active" glycosylation strategy

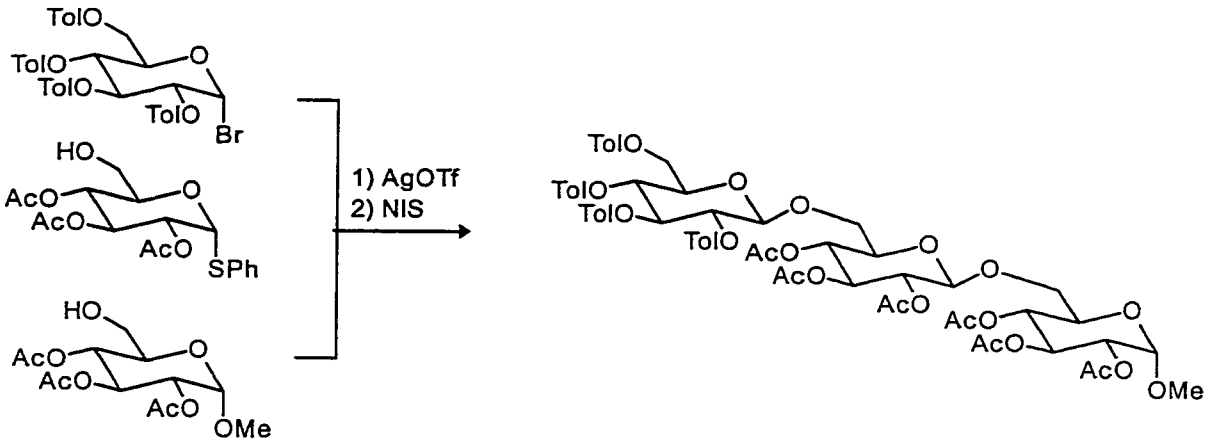
Boons *et al.*[34] have further extended the "latent-active" glycosylation strategy to vinyl glycosides (Scheme **1.5**). This strategy is based on isomerization of a substituted allyl glycoside to a 2-isobutenyl glycoside that may undergo a Lewis acid-promoted glycosylation reaction.



Scheme1.5 Vinyl glycosides in "latent-active" glycosylation strategy

[34] Boons, G. J.; Isles, S. *Tetrahedron Lett.* **1994**, *35*, 3593.

A one-pot multistep glycosylation strategy was reported by Takahashi *et al.*[35] The idea of this approach is that different glycosides could be selectively activated by using different promoters, thus the construction of several glycosidic linkages could be finished in the one-pot procedure. One example is shown in Scheme **1.6**. The glycosyl bromide could be coupled with thioglycoside in the presence of AgOTf to afford thiophenyl disaccharide. After addition of a second promoter (NIS) and glycosyl acceptor, the thiophenyl disaccharide was activated and further glycosylated with the acceptor to give a trisaccharide.

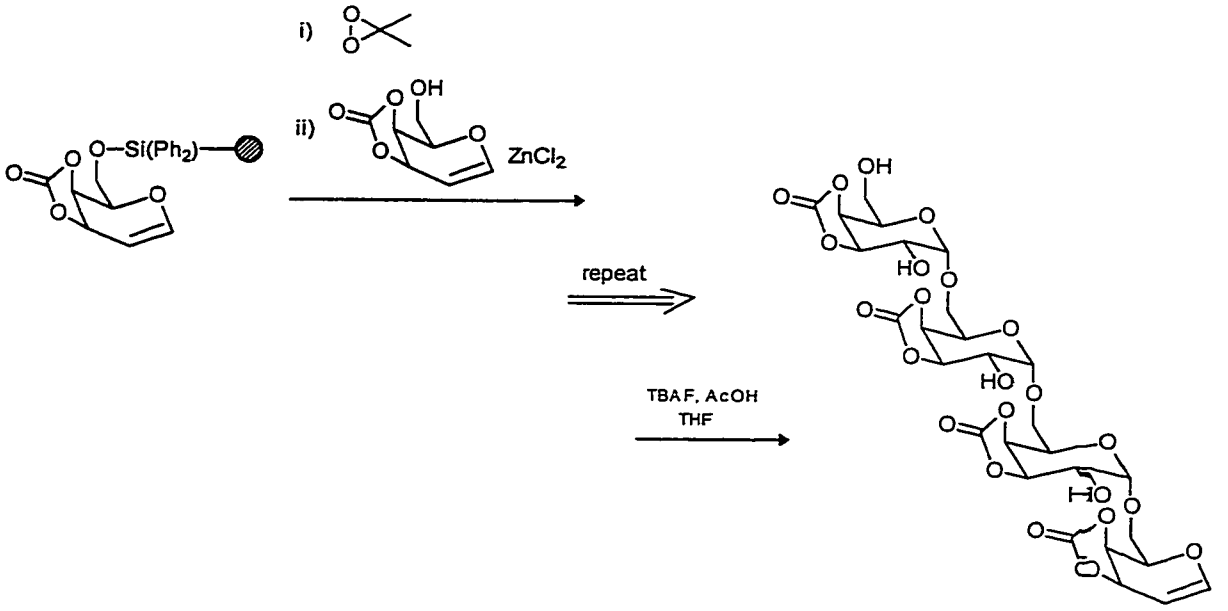


Scheme 1.6 Synthesis of trisaccharide using one-pot multistep strategy

As solid-phase synthesis has been demonstrated to be extremely valuable for routine preparation of peptides and oligonucleotides, much effort had been devoted to solid-phase *O*-glycosylation in the 1970's.[36] However, the success was limited due to lack of powerful glycosylation reactions compatible with solid-phase reaction conditions. This situation has drastically changed in the 1990's because a variety of modern glycosylation technologies have been developed suitable to solid-phase synthesis. For instance, Danishefsky *et al.*[37] successfully applied glycal glycosidation method to solid-phase oligosaccharide synthesis (Scheme **1.7**). In this case, chain elongation was performed from the nonreducing to the reducing end. The reaction commenced with resin-bound glycal and each chain elongation

[35] Yamada, H.; Harada, T.; Miyazaki, H.; Takahashi, T. *Tetrahedron Lett.* **1994**, *35*, 3979.
[36] a) Fréchet, J. M.; Schuerch, C. *Carbohydr. Res.* **1972**, *22*, 399; b) Chiu, S. H. L.; Anderson, L. *Carbohydr. Res.* **1976**, 50, 277.

cycle consists of two steps: oxidation of glycal to yield a 1,2-anhydro glycosyl donor, and Lewis acid promoted glycosylation. The major advantage of solid-phase oligosaccharide synthesis[38] is that time-consuming work-up procedures and purification steps are eliminated.



Scheme 1.7 Application of glycal glycosylation method in solid-phase synthesis

Soluble polymer supported solution synthesis[39] has also been explored in oligosaccharide synthesis.[40] The advantage of soluble polymers over solid phase supports is that the synthetic steps can be performed under homogeneous conditions, thus avoiding the pseudo high dilution problem of solid-phase reactions, and the soluble polymer-bound product can be readily precipitated during the work-up procedure. Scheme **1.8** illustrates the use of PEG as a soluble polymer support in oligomannoside synthesis.[41]

---

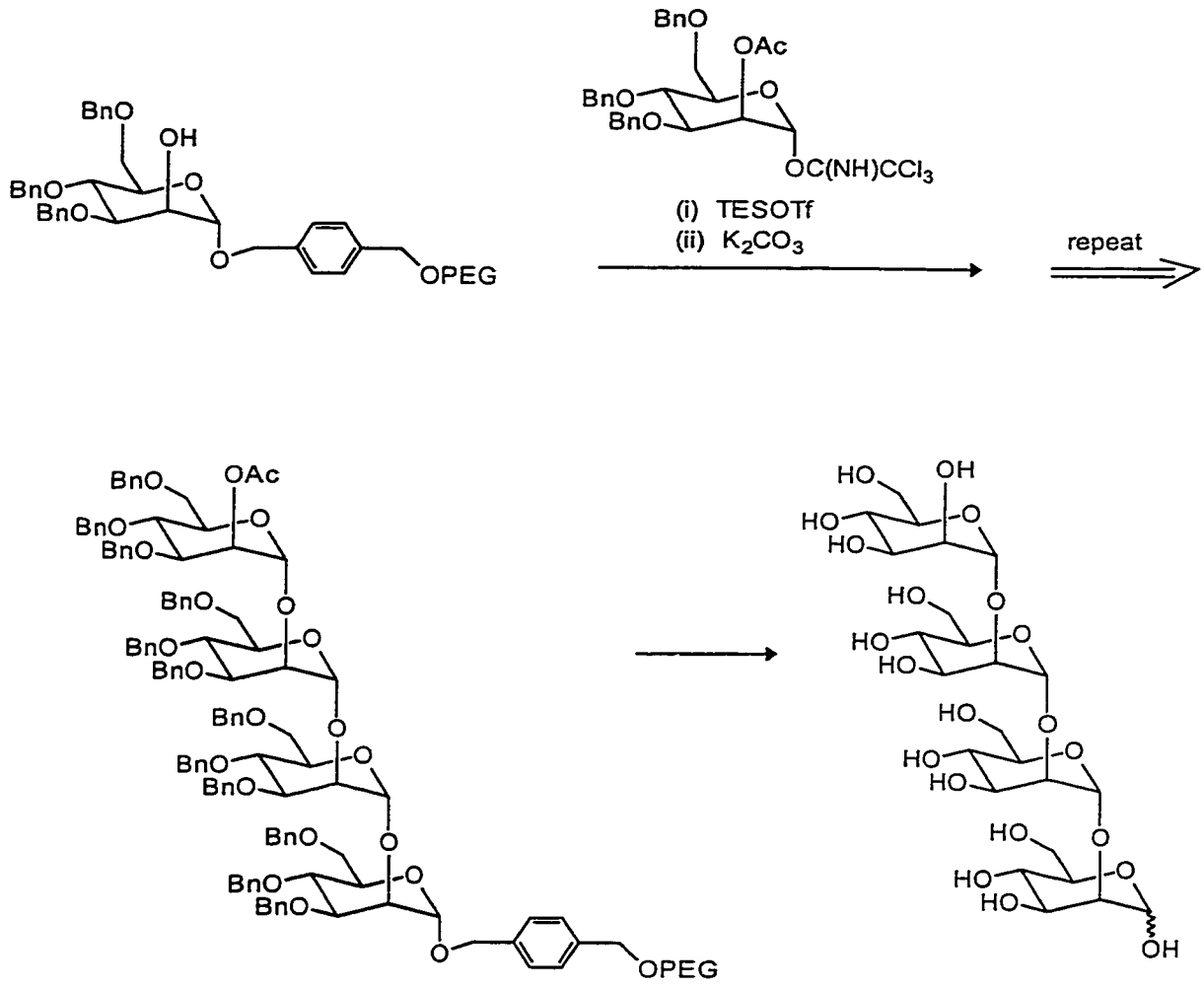
[37] a) Danishefsky, S. J.; McClure, K. F.; Randolph, J. T. Ruggeri, R. B. *Science* **1993**, *260*, 1307; b) Randolph, J. T.; McClure, K. F.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1995**, *117*, 5712.

[38] For a review on solid-phase oligosaccharide synthesis, see: a) Osborn, H. M. I.; Khan, T. H.; *Tetrahedron* **1999**, *55*, 1807; b) Ito, Y.; Manabe, S. *Curr. Opin. Chem. Bio.* **1998**, *2*, 701.

[39] Gravert, D. J.; Janda, K. D. *Chem. Rev.* **1997**, *97*, 489.

[40] Krepinsky, J. J. in *Modern Methods in Carbohydrate Synthesis*; Khan, S. H.; O'Neill R. A. (Eds.); Harwood Academic, Amsterdam, **1996**, 194.

[41] Douglas, S. P.; Whitfield, D. M.; Krepinsky, J. J. *J. Am. Soc. Chem.* **1995**, *117*, 2116.



Scheme 1.8 Oligosaccharide synthesis using PEG as a soluble polymer support

1.3 Glycoside cluster effect and glycoconjugates

1.3.1 Glycoside cluster effect

The recognition of cell-surface carbohydrates by protein (lectins) represents the basis of many biologically important events. However, individual carbohydrate-protein interactions are generally weak. Such binding events proceed with millimolar to micromolar dissociation

constants.[42] Since most lectins possess multiple carbohydrate-recognition domains (CRDS) and typically exist as oligomeric structures,[43] nature may deal with this problem through multivalency, where multiple simultaneous binding events overcome weak individual interaction. Therefore, suitably designed multivalent carbohydrate ligands should show high binding affinities.

From this basis, a number of multivalent carbohydrate ligands have been designed. These ligands span from synthetic low-valency glycoclusters to high valency glycopolymers. In general, these ligands show enhancements of $10\text{-}10^3$ over the appropriate monovalent ligand. These results have led to general acceptance of the "glycoside cluster effect" which is defined as "an affinity enhancement over and beyond what would be expected from the concentration increase of the determinant sugar in a multivalent ligand".[44]

The remainder of this chapter will focus on the chemical syntheses of glycoclusters, glycodendrimers, and glycopolymers.

1.3.2 Glycoclusters

Glycoclusters play important roles in the early studies of the cluster effect in carbohydrate-protein interactions. They are considered chemically well defined while lacking high valency. Their synthetic design, however, requires spatial and conformational optimization for all specific carbohydrate-protein interactions of interest.

The pioneering work by Lee *et al.*[45] on mammalian hepatic Gal/GalNAc receptors has resulted in developing a very potent trivalent *N*-acetylgalactosaminide inhibitor [YEE(GalNAcAH)$_3$], which is the best known synthetic ligand for human hepatic ASGP-Rs. Kichler and Schuber[46] have also synthesized trivalent β-D-galactopyranoside which is almost as potent as YEE(GalNAcAH)$_3$ towards Gal/GalNAc receptors of human hepatoma cells (HepG2). Glick and Knowles[47] have synthesized a number of divalent-sialoside clusters

---

[42] Toone, E. J. *Curr. Opin. Struc. Bio.* **1994**, *4*, 719.
[43] Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *96*, 637.
[44] a) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321; b) Ozaki, K.; Lee, R. T.; Lee, Y. C. Kawasaki, T. *Glycoconjugate J.* **1995**, *122*, 268.
[45] Lee, Y. C. in *Carbohydrate Recognition in Cellular Function, Ciba Foundation Symposium 145*; Bock, G.; Harnette, S. (Eds.); Wiley, Chichester, **1989**, 80.
[46] Kichler, A.; Schuber, F. *Glycoconjugate J.* **1995**, *12*, 275.
[47] Glick, G. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1991**, *113*, 4701.

grafted on aromatic derivatives with different distances between two sialic acids. They found the sialoclusters in which two sialic acids were 5.7 nm apart showed 100-fold more potency than methyl α-sialoside towards influenza virus hemagglutinin (Figure **1.4**).

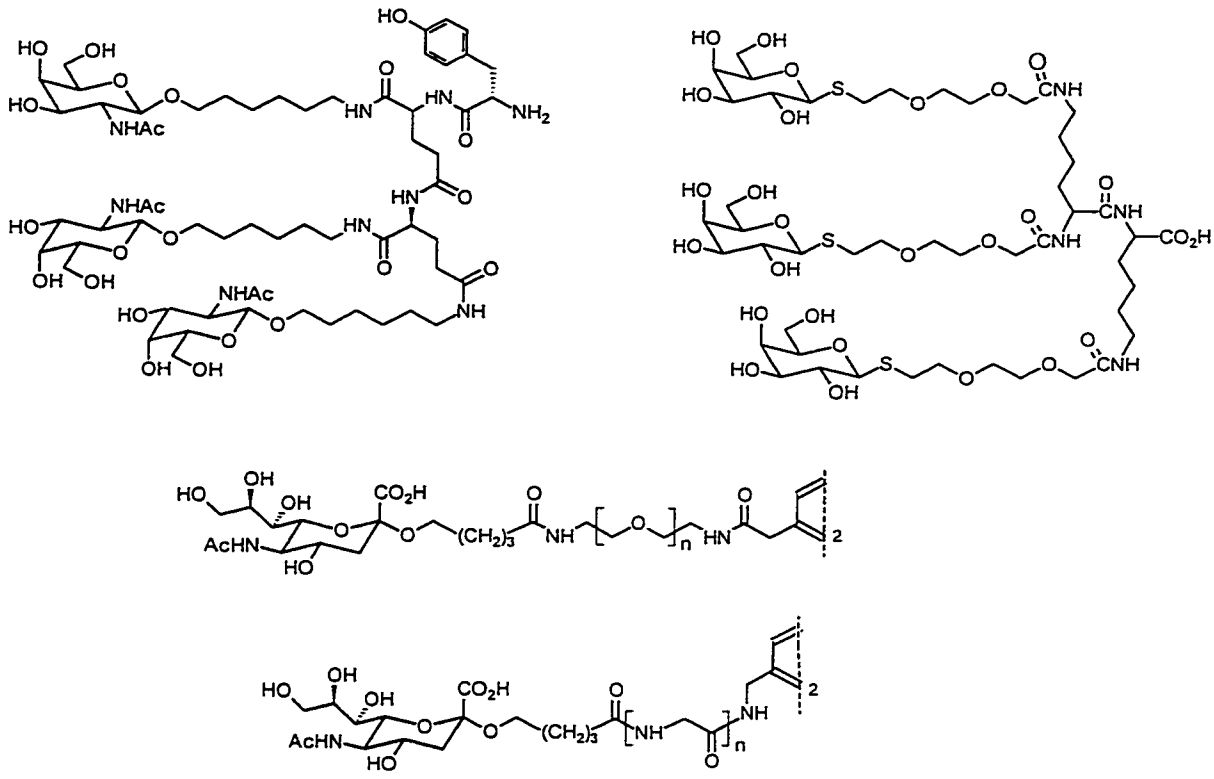
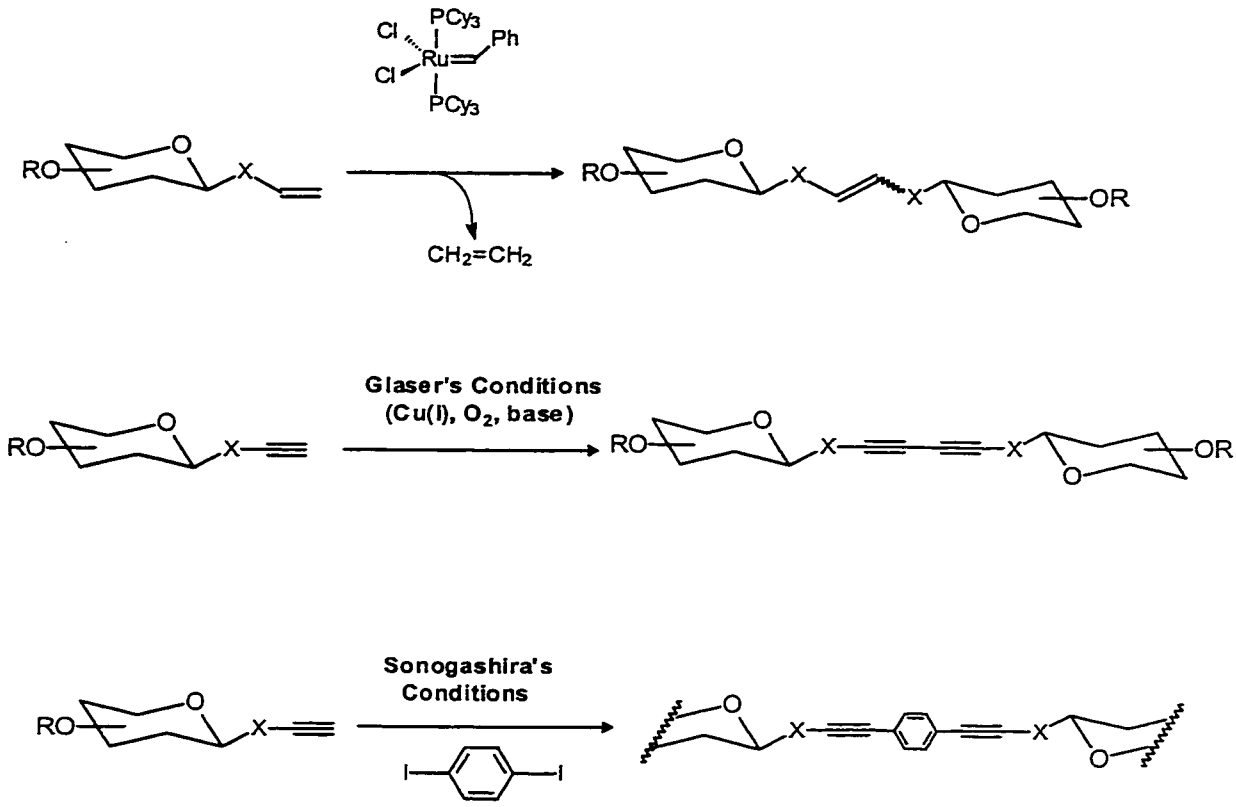


Figure 1.4 Examples of glycoclusters

Recently, we have applied transition metal-catalyzed reactions to generate various carbohydrate-containing clusters.[48] Thus, Grubbs' ruthenium benzylidene catalyst has been used successfully in olefin self-metathesis reactions from both *O*- and *C*-alkenyl glycosides. Oxidative dimerization of terminal acetylentic glycosides was accomplished with palladium and copper-catalyzed homo-coupling reactions. Sonogashira cross-coupling reaction was also used to incorporate aryl halide and propynyl glycosides. The reaction conditions are normally mild and the yields are relatively high. In general, transition metal-catalyzed reactions afford

[48] Roy, R.; Das, S. K.; Dominique, R.; Trono, M. C.; Hernández-Mateo, F.; Santoyo-González, F. *Pure Appl. Chem.* **1999**, *71*, 565.

rapid and facile ways to build glycoclusters. These glucoclusters will be discussed in detail in Chapter 4 of this dissertation. The general strategies are shown in Scheme **1.9**.



Scheme 1.9 Transition metal-catalyzed glycocluster syntheses

1.3.3 Glycodendrimers

Dendrimers are tree-like and three-dimensional polymers that are considered monodisperse macromolecules. They can be prepared by divergent[49] or convergent[50] approaches. Because they are built from AB_n monomers (where $n \geq 2$), dendrimers are highly branched and have three distinct structural features: a core, branching units, and multiple

[49] a) Buhleier, E.; Wehner, W.; Vögtle, F. *Synthesis* **1978**, 155; b) Tomalia, D. A.; Baker, H.; Dewald, J. R.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P. *Polym. J.* **1985**, *17*, 117; c) Newkome, G. R.; Yao, Z.-Q.; Baker, G. R.; Gupta, V. K. *J. Org. Chem.* **1985**, *50*, 2003.

[50] a) Hawker, C. J.; Fréchet, J. M. J. *J. Chem. Soc. Chem. Commun.* **1990**, 1010; b) Sayaraman, M.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1998**, *120*, 12996.

peripheral (end) groups. The high local concentration of end groups and chemically well-defined structures enable dendrimers to have a number of potential applications such as diagnostic agents, gene therapeutic agents, new chiral materials, chiral catalysts, coatings, and photocopier toners.[51]

Glycodendrimers[52] are low molecular weight multiantennary carbohydrate-containing biopolymers which are constructed by coating carbohydrates on dendritic molecules' exterior surfaces. They sit structurally between clusters and polymers. The first glycodendrimer based on L-lysine was synthesized by our group in 1993.[53] The hyperbranched poly-L-lysine dendritic cores have been efficiently achieved by using Fmoc protecting group strategy and carbodiimide-hydroxybenzotriazole (HOBt) coupling chemistry on Wang resin. The surface of poly-L-lysine dendritic cores was modified by the *N*-chloroacetyl group. Conjugation of a sialic acid thiol derivative to L-lysine cores afforded glycodendrimers having up to sixteen surface groups. Interestingly, the dendritic α-thiosialosides with 16 valencies was found to be as potent as homologous glycopolymers in inhibition of hemagglutination of human erythrocytes by influenza viruses. Other glycodendrimers based on poly-L-lysine core bearing *N*-acetylglucosaminides,[55] α-D-mannosides,[54] β-D-lactosides,[55] *N*-acetyllactosaminides,[55,56] and T-antigens[57] were also prepared in a similar manner in our laboratory (Figure **1.5**). It's worthy to mention that *N*-acetyllactosamine containing dendrimers[56] were the first chemo-enzymatically prepared glycodendrimers.

---

[51] Fischer, M.; Vögtle, F. *Angew. Chem. Int. Ed.* **1999**, *38*, 884.
[52] a) Roy, R. *Polymer News* **1996**, *21*, 226; b) Jayaraman, N.; Nepogodiev, S. A.; Stoddart, J. F. *Chem. Eur. J.* **1997**, *3*, 1193.
[53] a) Roy, R.; Zanini, D.; Meunier, S. J.; Romanowska, A. *J. Chem. Soc., Chem. Commun.* 1993, 1869; b) Roy, R.; Zanini, D.; Meunier, S. J.; Romanowska, A. *ACS Symp. Ser.* **1994**, *560*, 104.
[54] Pagé, D.; Zanini, D.; Roy, R. *Bioorg. Med. Chem.* **1996**, *11*, 1949.
[55] Zanini, D.; Park, W. K. C.; Roy, R.; *Tetrahedron Lett.* **1995**, *36*, 7383.
[56] Zanini, D.; Roy, R. *Bioconjugate Chem.* **1997**, *8*, 187.
[57] Baek, M. G. Ph.D. Dissertation, University of Ottawa, 1997.

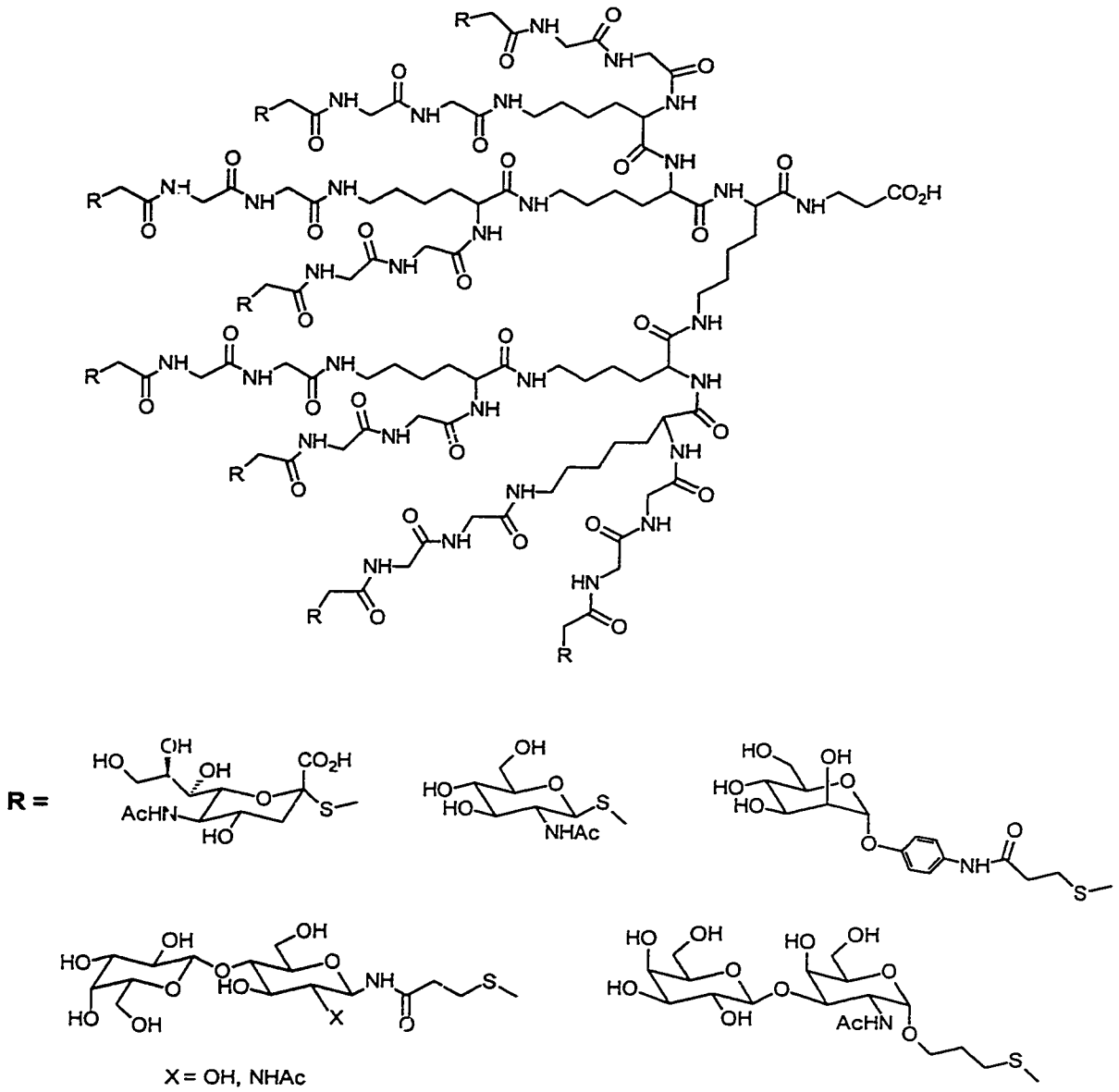


Figure 1.5 Glycodendrimers based on L-lysine dendritic core

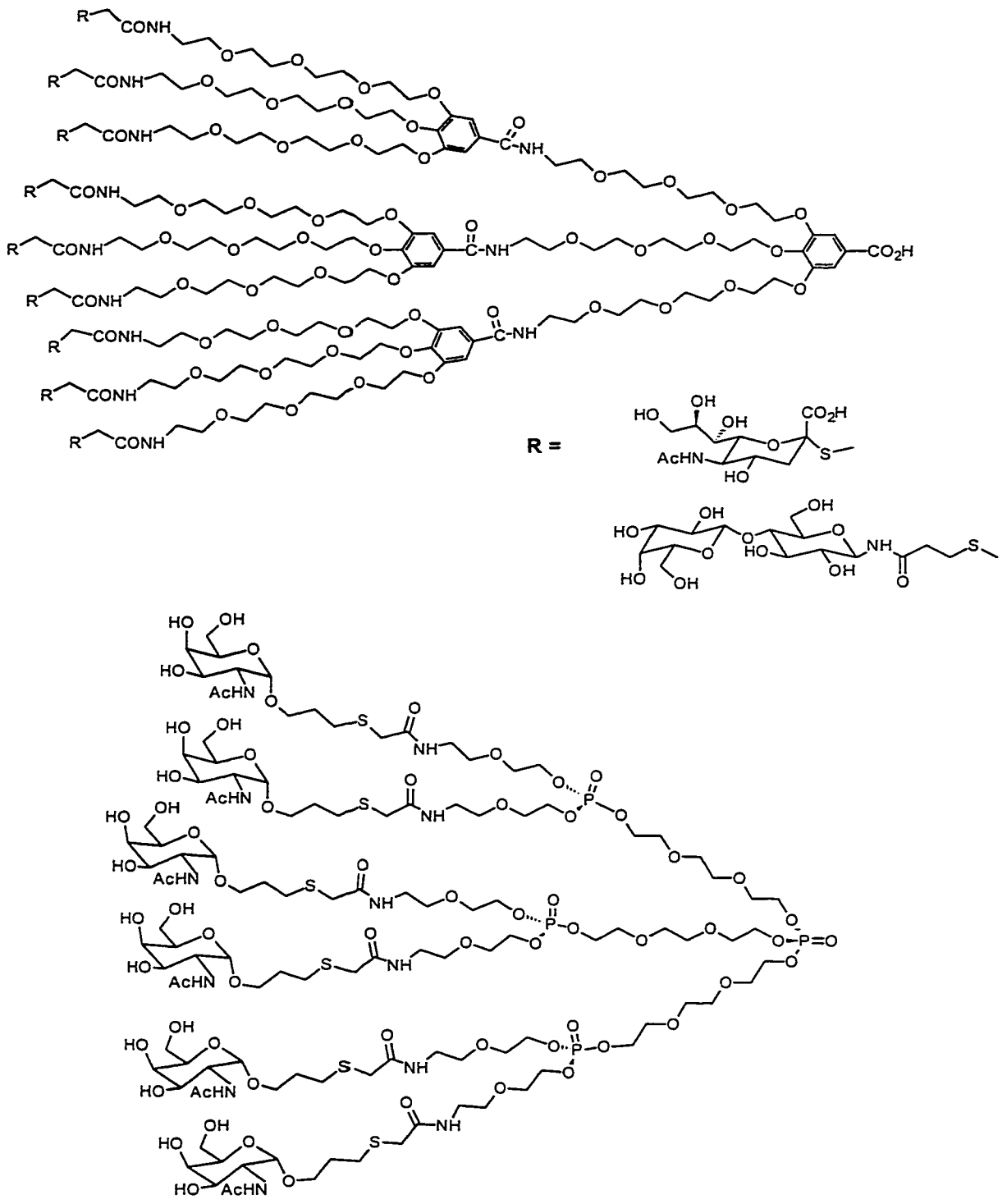


Figure 1.6 Glycodendrimers based on gallic acid core and phosphotriester backbone

Other types of glycodendrimers based on the different dendritic cores such as gallic acid core,[58,59] phosphotriester backbone,[60] and 3,3'-iminobis(propylamine) core[61,62,63] were also synthesized by us. The representative examples of these glycodendrimers are illustrated in Figure **1.6** and **1.7**.

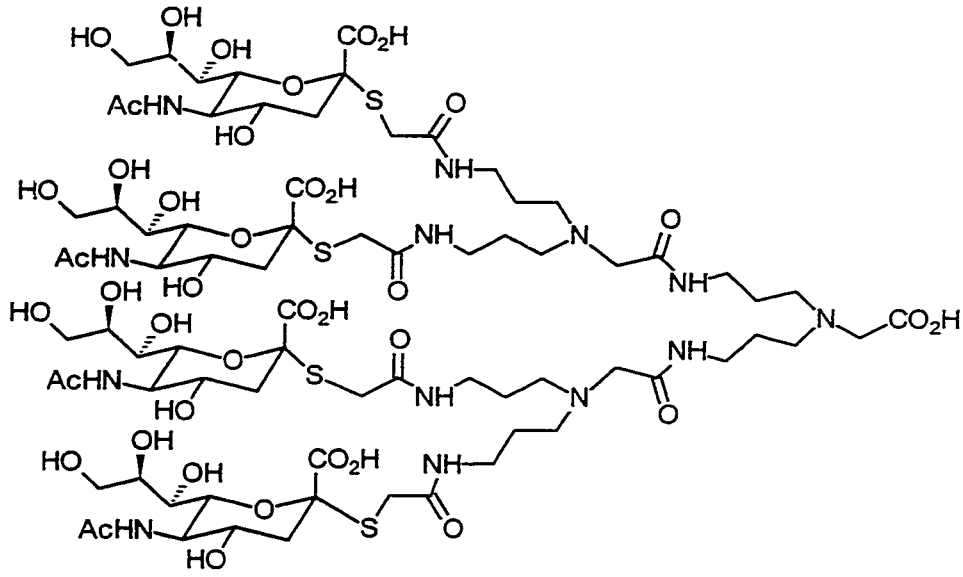


Figure 1.7 Example of glycodendrimers based on 3,3'-iminobis(propylamine) core

Spherical Starburst® (PAMAM) dendrimers[64] have been used to attach various carbohydrate derivatives by several research groups. Okada *et al.*[65] reported the functionalization of the terminal amine groups of the PAMAM dendrimers with disaccharide lactones of lactose and maltose by the formation of an amide bond. Singhal *et al.*[66] have

---

[58] Roy, R.; Park, W. K. C.; Wu, Q. Q.; Wang, S.-N. *Tetrahedron Lett.* **1995**, *36*, 4377.
[59] Meunier, S. L.; Wu, Q. Q.; Wang, S.-N.; Roy, R. *Can. J. Chem.* **1997**, *75*, 1472.
[60] Park, W. K. C.; Kratzer, B.; Zanini, D.; Wu, Q.-Q.; Meunier, S. J.; Roy, R. *Glycoconjugate J.* **1995**, *12*, 456.
[61] Zanini, D.; Roy, R. *J. Org. Chem.* **1996**, *61*, 7348.
[62] Zanini, D.; Roy, R. *J. Am. Chem. Soc.* **1997**, *119*, 2088.
[63] Llinares, M.; Roy, R. *Chem. Commun.* **1997**, 2119.
[64] a) Tomalia, D. A.; Naylor, A. N.; Goddard, W. A. *Angew. Chem. Int. Ed. Eng.* **1990**, *29*, 617; b)Tomalia, D. A.; Dvornic, P. R. *Nature* **1994**, 617.
[65] Aoi, K.; Itoh, K.; Okada, M. *Macromolecules* **1995**, *28*, 5391.
[66] Toyokumi, T.; Singal, K. *Chem. Soc. Rev.* **1995**, 231.

attached T_N antigen (α-D-GalNAc-*O*-Ser) peptide to PAMAM dendrimers to give non-immunogenic glycodendrimers (Figure **1.8**).

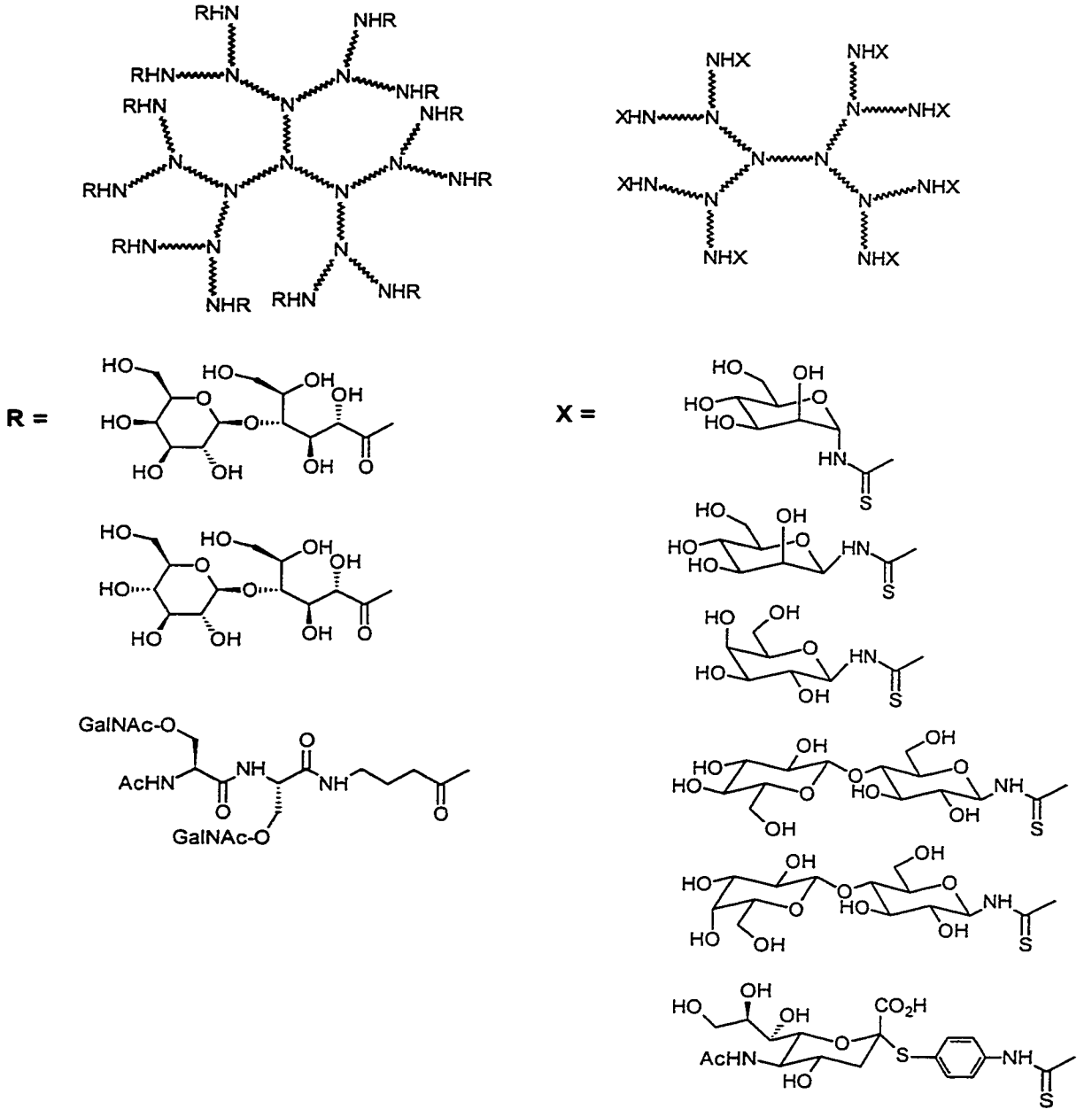
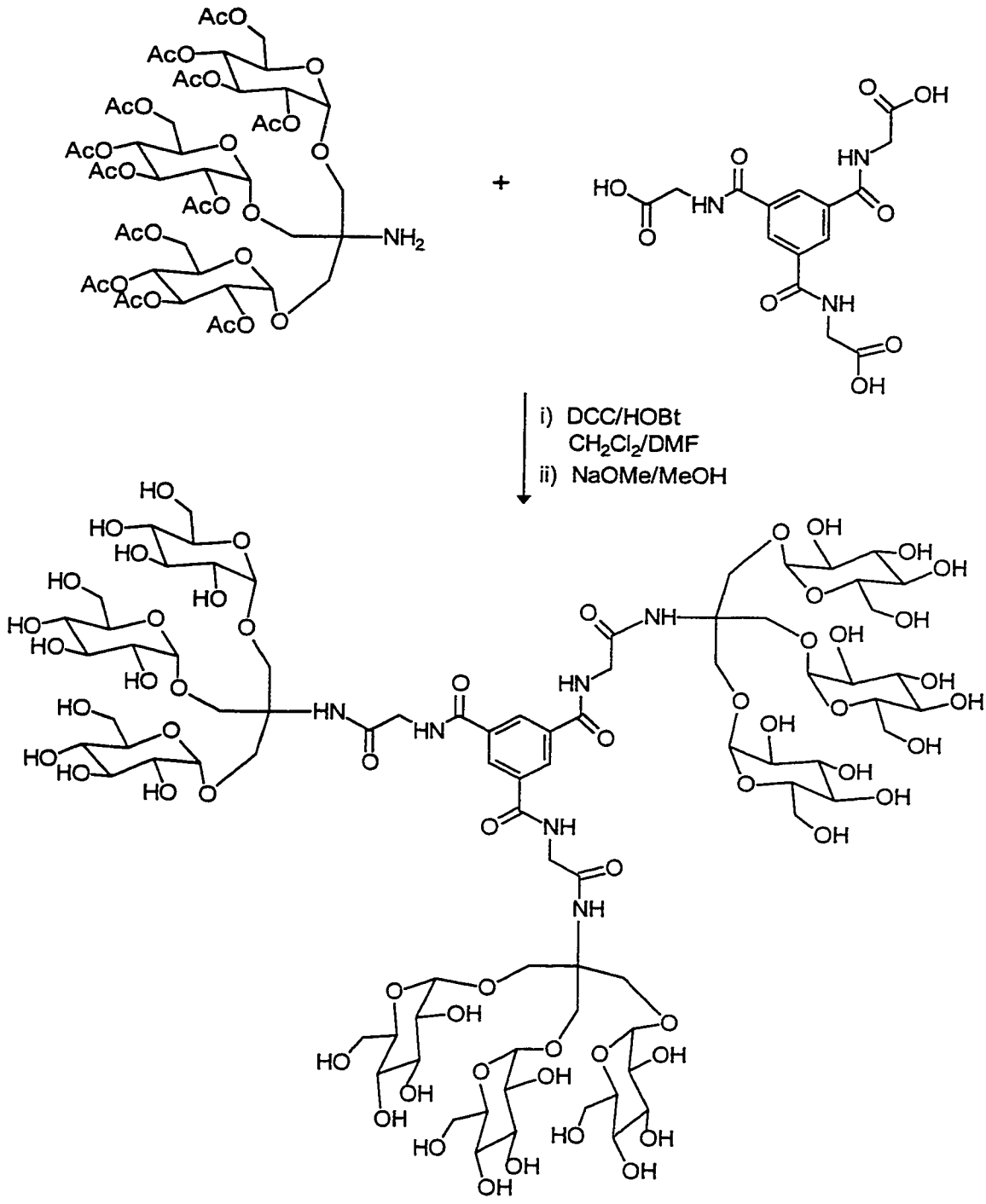


Figure1.8 Examples of PAMAM glycodendrimer



Scheme 1.10 Convergent synthesis of glycosylated dendrimer

An isothiocyanate coupling strategy has been employed to connect a number of glycosides to dendrimers. Lindhorst *et al.*[67] used peracetylated glycosyl isothiocyanates of mannose, glucose, galactose, cellobiose, and lactose to conjugate with PAMAM to form different PAMAM glycodendrimers. This strategy was also applied to prepare PAMAM dendrimers containing *p*-thiophenyl urea of α-D-mannoside[68] and α-sialoside[69] by our group (Figure **1.8**).

The above glycodendrimers were prepared in a divergent manner, in which saccharide residues were grafted on to the pre-existing dendritic molecules. Ashton *et al.*[70] employed a convergent strategy for the synthesis of glycodendrimers bearing β-D-glucopyranosyl residues (Scheme **1.10**). The first step of this strategy involved the attachment of two or more sugar residues to a polyfunctional branching unit. Convergent attachment of the branch units to a tricarboxylic acid core by an amide bond gave spherical glycodendrimers. The convergent synthetic method allows the creation of dendrimers with perfect chemical constitutions.

1.3.4 Glycopolymers

Of the various types of neoglycoconjugates developed for studying carbohydrate-receptor binding interactions, glycopolymers[71] have attacted much interest in recent years. Although neoglycoproteins have surely been the first class of synthetic neoglycoconjugates and long served as tools for the investigation of carbohydrate-protein interactions, they still have some drawbacks, such as difficult structural characterization, difficult preparation, and immunogenicity. In contrast to neoglycoproteins, glycopolymers offer many advantageous physical and immunochemical properties. They are cheap and easy to prepare in large quantities. Their purification and charaterization are easier than neoglocoproteins. They can

---

[67] Lindhorst, T. K.; Kieburg, C. *Angew. Chem. Int. Ed. Eng.* **1996**, *35*, 1953.
[68] Pagé, D.; Roy, R. *Bioconjugate Chem.* **1997**, *8*, 714.
[69] Zanini, D.; Roy, R. *J. Org. Chem.* **1998**, *63*, 3486.
[70] Ashton, P. R.; Boyd, S. E.; Brown, C. L.; Jayaraman, N.; Nepogodiev, S. A.; Stoddart, J. F. *Chem. Eur. J.* **1996**, *2*, 1115.
[71] a) Bovin, N. V.; Gabius, H.-J. *Chem. Soc. Rev.* **1995**, 413; b) Roy, R. *Trends Glycosci. Glycotechnol.* **1996**, *8*, 79; c) Kiessling, L. L.; Pohl, N. L. *Chem. Biol.* **1996**, *3*, 71; d) Miyata, T. Nakamae, K. *Trends Polym. Sci.* **1997**, *5*, 198.

be constructed with a wide range of molecular weights, any desired carbohydrate density, and functionality. They are also more stable to wide pH range and microbial degradation. Most importantly, they have been shown to be non-toxic, and poorly or non-immunogenic.

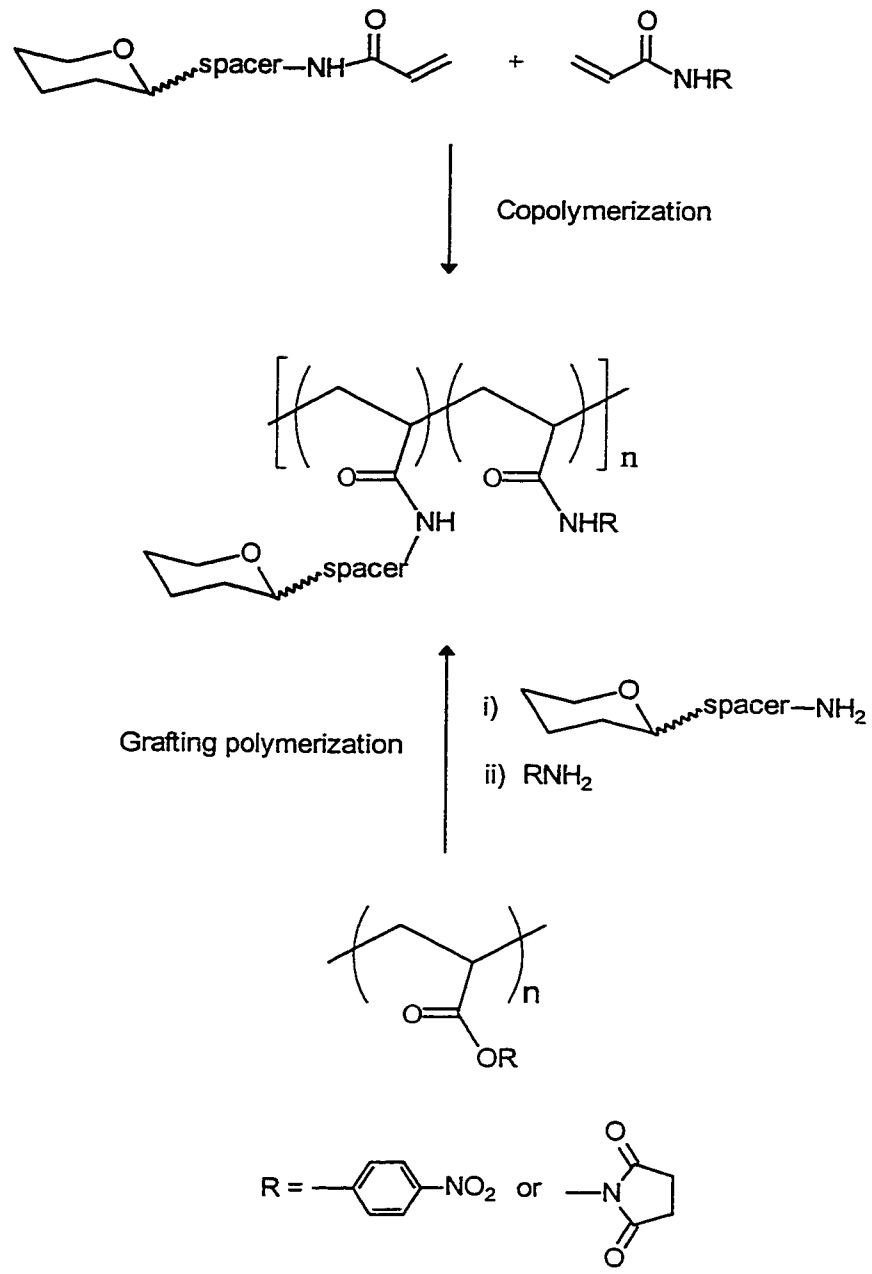
Although there are many strategies for the preparation of glycopolymers, most glycopolymers are generally prepared in two ways: copolymerization and grafting polymerization.

Early glycopolymers were made from allyl glycosides through copolymerization with acrylamide using persulfate as a radical inititator.[72] However, the ratios of applied and incorporated quantities of the monomers can vary significantly because the reactivity ratios between carbohydrate-containing monomers and acrylamide are quite different in the polymerization process. An improved strategy was achieved by using carbohydrate derivatives having *N*-acryloylated groups in the aglycon portions.[73] Both monomers have similar reactivities in the chain reaction process. Consequently, the incorporated monomers ratio within the glycomer is almost identical to the ratio of monomers added to the reaction.

Grafting polymerization is the other elegant approach toward glycopolymers with predictable composition. Thus, the desired carbohydrate haptens can be directly incorporated into a pre-formed polymers bearing reactive functionalities. The most common ones by this approach involve reacting amine-containing carbohydrates with active esters of polyacrylates, such as poly(4-nitrophenyl acrylate)[74] and poly(*N*-oxysuccinimidyl acrylate).[75] One of the major advantages of this approach is that pre-formed active polymers can be synthesized with the desired molecular weight. Therefore, the same basic polymer backbones used in grafting polymerization can avoid molecular weight variations in the previous strategy. Furthermore, the desired carbohydrate content in glycopolymers can be easily achieved by controlling the initial ratio of carbohydrate haptens and pre-formed active polymers.

---

[72] Horejsi, V.; Kocourek, J. *Biochem. Biophys. Acta* **1973**, *297*, 346.
[73] a) Roy, R.; Laferrière, C. A. *Carbohydr. Res.* **1988**, *177*, C1; b) Roy, R. Tropper, F. *Glycoconjugate J.* **1988**, *5*, 203.
[74] a) Bovin, N. V.; Korchagina, E. Y.; Zemlyanukhina, T. V.; Byramova, N. E.; Galanina, O. E.; Zemlyakov, A. E.; Ivanov, A. E.; Zubov, V. P.; Mochalova, L. V. *Glycoconjugate J.* **1993**, *10*, 142; b) Zemlyanukhina, T. V.; Nifant'ev, N. E.; Shashkov, A. S.; Tsvetkov, Y. E.; Bovin, N. V. *Carbohydr. Lett.* **1995**, *1*, 277.
[75] Sigal, G. B.; Mammen, M.; Dahmann, G.; Whitesides, G. M. *J. Am. Chem. Soc.* **1996**, *118*, 3789.



Scheme 1.11 Synthetic strategies for glycopolymers

Chapter 2 Regiospecific synthesis of *N*-acetyllactosamine derivatives

2.1 Introduction

N-Acetyllactosamine [Galβ(1→4)GlcNAc, LacNAc] is well known as a biologically important disaccharide core structure which has been found in glycoproteins and glycolipids, and is widely distributed in many different human and animal tissues.[76,77] Furthermore, it is also a key intermediate of sialyl Lewis X (sLe^x)[78] and sulfo-Le^x,[79] identified as a ligand for the endothelial leukocyte adhesion molecule-1 (ELAM-1) which mediates the early stage of adhesion of leukocytes to activated endothelial cells. Several chemical syntheses of *N*-acetyllactosamine derivatives have been developed by the glycosylations between galactose and *N*-acetylglucosamine derivatives.[80,81,82,83,84,85] The secondary hydroxyl group at C-4 of a hexopyranose with a $^4C_1$ conformation was particularly unreactive when the remaining hydroxyl groups were acylated.[86] This problem has been solved by using a non $^4C_1$ conformation precursor, such as 2-acetamido-1,6-anhydro-2-deoxy-β-D-glucopyranose with a $^1C_4$ conformation,[87] or an acyclic *N*-acetylglucosamine derivative.[88] Moreover, Sinaÿ *et al.*[80]

---

[76] Fukuda, M. *Biochim. Biophys. Acta* **1985**, *780*, 119.
[77] Feizi, T.; Childs, R. A. *J. Biochem.* **1987**, *245*, 1.
[78] a) Fukushima, K.; Hirota, H.; Terasaki, P. I.; Waranabe, A.; Togashi, H.; Chia, D.; Sayama, N.; Fukushi, Y.; Nudelman, S.; Hakomori, S. *Cancer Res.* **1984**, *44*, 5279; b) Phillips, M. L.; Nudelman, E.; Gaeta, F. C. A.; Perez, M.; Singhal, A. K.; Hakomori, S.; Paulson, J. C. *Science* **1990**, *250*, 1130; c) Mulligan, M. S.; Paulson, J. C.; Defrees, S.; Zheng, Z.-L.; Lowe, J. B.; Ward, P. A. *Nature* **1993**, *364*, 149.
[79] Yuen, C.-T.; Lawson, A. M.; Chai, W.; Larkin, M.; Stoll, M. S.; Stuart, A. C.; Sullivan, F. X.; Ahern, T. J.; Feizi, T. *Biochem.*, **1992**, *31*, 9126.
[80] Jacquinet, J.-C.; Sinaÿ, P. *Carbohydr. Res.* **1976**, *46*, 138.
[81] Nashed, M. A.; Anderson, L. *Carbohydr. Res.* **1983**, *114*, 43.
[82] Dahmén, J.; Gnosspelius, G.; Larsson, A.-C.; Lave, T.; Noori, G.; Pålsson, K.; Frejd, T.; Magnusson, G. *Carbohydr. Res.* **1985**, *138*, 17.
[83] Nicolaou, K. C.; Hummel, C. W.; Bockovich, N. J.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1991**, 870.
[84] Nicolaou, K. C.; Bockovich, N. J.; Carcanague, D. R. *J. Am. Soc. Chem.* **1993**, *115*, 8843.
[85] Von dem Bruch, K.; Kunz, H. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 101.
[86] Willams, J. M.; Richardson, A. C. *Tetrahedron* **1967**, *23*, 1369.
[87] Schmitt, F.; Sinaÿ, P. *Carbohydr. Res.* **1973**, *29*, 99.

demonstrated that OH-4 of the 2-acetamido-2-deoxy-D-glucose residue where the remaining hydroxyl groups were protected by benzyl groups was more reactive than the corresponding acyl counterpart under appropriate glycosylation conditions. However, the procedure often involved tedious multisteps of protections and deprotections. To overcome this drawback, a new strategy has been the use of "lightly protected" acceptors which have several hydroxyl groups unprotected, especially near the position where the glycosidic bond is formed. This "open" glycosylation in which one hydroxyl group is preferentially glycosylated in the presence of the other free hydroxyl group(s) may offer shorter and easier routes to oligosaccharide synthesis. The "lightly protected" acceptors, such as 6-*O*-benzyl or 6-*O*-pivaloyl-2-deoxy-2-phthalimido-β-D-glucopyranoside derivatives, were used as key intermediates in various glycosylations by Ogawa's group,[89] Sinaÿ's group,[90] Schmidt's group,[91] and Matta's group.[92] The bulky phthalimido group which was chosen to protect *N*-2 was claimed to reduce the reactivity of OH-3 by steric hindrance. However, the regioselectivity was unsatisfactory in these cases. The yields of undesired β-(1→3) linked disaccharides were from 8% to 25%. More recently, Verez-Bencomo *et al.* [93] have reported a complete regiospecific galactosylation of similar diol acceptor bearing a phthalimido-protecting group by using a 4,6-benzylidene galactosyl donor. No 1→3 isomer was detected. The presence of both the phthalimido group of the acceptor and the rigidified bicyclic system of the galactosyl donor were crucial for the regiospecific galactosylation (Table **2.1**).

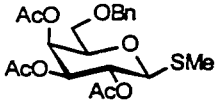
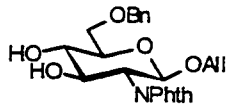
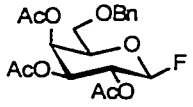
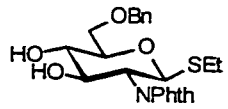
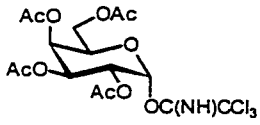
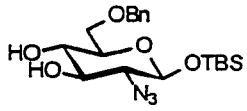
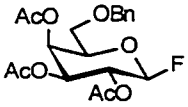
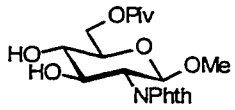
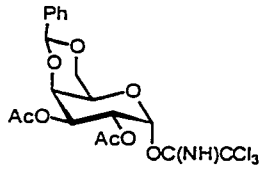
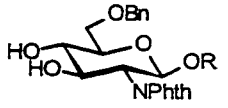
Recently, we found that an *O*-6 *tert*-butyldiphenylsilyl protecting group could also contribute to regiospecific glycosylation. We used *p*-nitrophenyl 2-phthalimido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-1-thio-β-D-glucopyranoside as glycosyl acceptor and phenyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside as donor to obtain only the corresponding β (1→4) linked disaccharide in 74% yield.[94] The regiospecificity was attributed to the bulky 6-*O*-*tert*-butyldiphenylsilyl and phthalimido groups. In order to explore the possibility that the enhanced regioselectivity was not a combination of these two

---

[88] Heyns, K.; Propp, K.; Harrison, R.; Paulsen, H. *Ber.* **1967**, *100*, 2655.
[89] Numomura, S.; Iida, M.; Numata, M.; Sugimoto, M.; Ogawa, T. *Carbohydr. Res.* **1994**, *263*, C1.
[90] Zhang, Y.-M.; Brodzky, A.; Sinaÿ, P.; Saint-Marcoux, G.; Perly, B. *Tetrahedron Asymmetry* **1995**, *6*, 1195.
[91] Toepfer, A.; Kinzy, W.; Schmidt, R. R. *Liebigs Ann. Chem.* **1994**, 449.
[92] Jain, R. K.; Vig, R.;Rampal, R.; Chandrasekaran, E. V.; Matta, K. L. *J. Am. Chem. Soc.* **1994**, *116*, 12123.
[93] Figueroa-Pérez S.; Verez-Bencomo, V. *Tetrahedron Lett.*, **1998**, *39*, 9143.
[94] Cao, S. *Ph. D. Dissertation*, U. of Ottawa, **1996**.

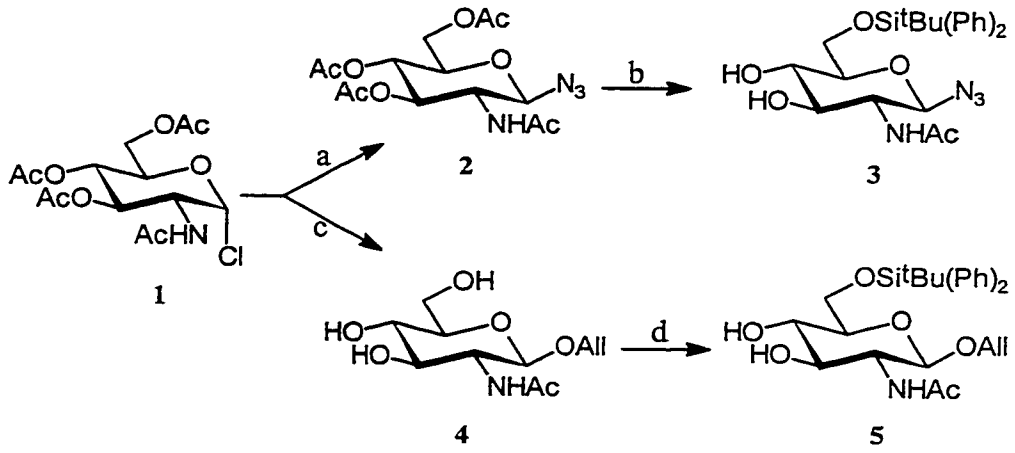
protecting groups, we chose the analogous 2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranoside derivative as glycosyl acceptor to synthesize *N*-acetyllactosamine derivatives. Furthermore, we also used this strategy toward the synthesis of a Lewis X trisaccharide derivative.

Table 2.1 Regioselectivities in the glycosylation of "lightly" protected glucosamine derivatives

Glycosyl donor	Glycosyl Acceptor	Reaction Conditions	1→4 linked yield (%)	1→3 linked yield (%)[Ref]
		MeOTf, Toluene, -15 °C	61	17[89]
		$AgOTf\text{-}SnCl_2$ Toluene-CH_2Cl_2 R. T.	45	25[90]
		$BF_3{\cdot}OEt_2$ Hexane-CH_2Cl_2 -25 °C	21	65[91]
		$AgOTf\text{-}SnCl_2$ Toluene-CH_2Cl_2 -15-20 °C	47	22[92]
		TMSOTf CH_2Cl_2 -25 °C	81	0[93]

2.2 Results and discussion

Compound **3** and **5** were selected as the key acceptors for the syntheses of *N*-acetyllactosamine disaccharides. These two compounds were obtained from acetochloro-*N*-acetylglucosamine **1** in three steps as shown in Scheme **2.1**.



Scheme 2.1 Preparation of "lightly" protected *N*-acetylglucosamine acceptors

Reagents and conditions: (a) NaN_3, TBAHS, 1M Na_2CO_3, 94%; (b) *i*: NaOMe/MeOH; *ii*: TBDPSCl, pyridine, 92%; (c) $NaOCH_2CH{=}CH_2/HOCH_2CH{=}CH_2$, 80%; (d) TBDPSCl, pyridine, 88%.

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl chloride **1**[95] was treated with sodium azide under improved liquid two-phase PTC conditions[96] (1 equiv of tetrabutylammonium hydrogen sulfate (TBATS), CH_2Cl_2, 1 M Na_2CO_3) to give glycosyl azide **2** in 94% yield. Compound **2** had a β configuration as confirmed by ^{1}H NMR ($J_{1,2}$ = 9.3 Hz). No trace of orthoester-like product was observed. Subsequent Zemplén[97] deacetylation (NaOMe, MeOH) and selective silylation of OH-6 with *tert*-butylchlorodiphenylsilane in

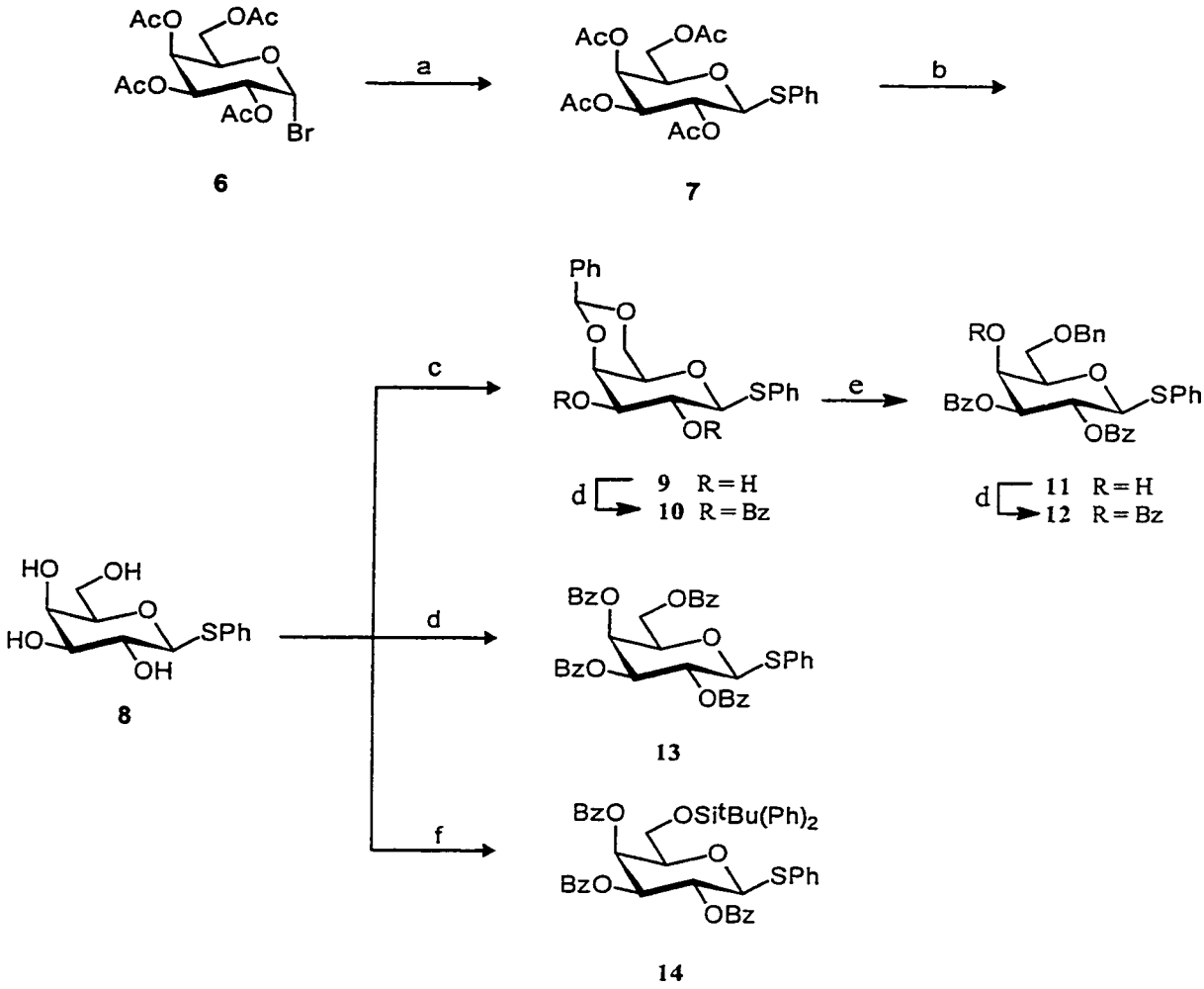
[95] Horton, D. *Methods Carbohydr. Chem.* **1972**, *6*, 282.
[96] a) Roy, R. In *Handbook of Phase Transfer Catalysis*; Sasson, Y.; Neumann, R., Eds.; Chapman & Hall: Hampshire, U. K., **1997**; 244; b) Roy, R.; Tropper, F. D.; Cao, S.; Kim, J. M. *ACS Symposium Series* **1997**, *659*, 163.

pyridine gave azide acceptor **3** in 88% overall yield. Compound **1** was also treated with 1M solution of sodium allyloxide-allyl alcohol to afford allyl β-glycoside **4** ($J_{1,2}$ = 8.4 Hz, 80% yield) and simultaneous de-*O*-acetylation. No oxazoline by-product formed under these conditions. Compared with other methods to prepare allyl β-glycosides, for example, via an oxazoline intermediate[98] or acetochloro-*N*-acetylglucosamine coupled with allyl alcohol using mercury salts as promoters,[99] this method was simpler and afforded a higher yield. Selective silylation of OH-6 with TBDPSCl yielded allyl β-glycoside acceptor **5** (88%) (Scheme **2.1**).

Acetobromogalactose **6**[100] was treated with thiophenol under PTC conditions to afford phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside **7** in 84% yield. The reaction occurred with complete stereocontrol to give the *trans* glycoside **7** as judged by ^{1}H NMR ($J_{1,2}$ = 9.9 Hz). Zemplén deacetylation of **7** afforded phenyl 1-thio-β-D-galactopyranoside **8** which was the precursor for the preparation of the galactosyl donors **12**, **13**, and **14**. Benzylidenation of **8** could be accomplished with benzaldehyde dimethyl acetal under acid catalyst (*p*-TsOH) to give acetal **9** in 90% yield. Benzoylation of **9** with benzoyl chloride in pyridine afforded compound **10** in quantitative yield. The reductive ring opening[101] of benzylidene acetal **10** with sodium cyanoborohydride provided galactosyl derivative **11** (84%). The reductive benzylidene ring opening is worthy of additional comments. It was found that the benzylidene ring opening was very slow and incomplete if the reaction system was under basic or neutral conditions. Enough hydrogen chloride should be added to keep the pH of the solution at about 5. The ring opening reaction was very fast and highly regioselective. Finally, benzoylation of **11** again finished the preparation of glycosyl donor **12**. Similarly, benzoylation of **8** gave another galactosyl donor **12** in quantitative yield. Galactosyl donor **13** was easily available by a simple one pot, two-step reaction from phenyl thiogalactoside **8**. Thus successive treatment of **8** with *tert*-butyldiphenylsilyl chloride and benzoyl chloride in dry pyridine gave galactosyl donor **13** in 92% overall yield (Scheme **2.2**).

---

[97] Zemplén, G. *Ber. Dtsch. Chem. Ges.* **1927**, *60*, 1555.
[98] Nishimura, S.-I.; Matsuoka, K.; Furuike, T.; Ishii, S.; Kurita, K.; Nishimura, K. M. *Macromolecules* **1991**, *24*, 4236.
[99] Lee, R. T.; Lee, Y. C. *Carbohydr. Res.* **1974**, *37*, 193.
[100] Jeanloz, R. W,; Stoffyn, P. J. *Methods in Carbohydr. Chem.* **1962**, *1*, 221.
[101] Garegg, P. J.; Hultberg, H. *Carbohydr. Res.* **1981**, *93*, C10.



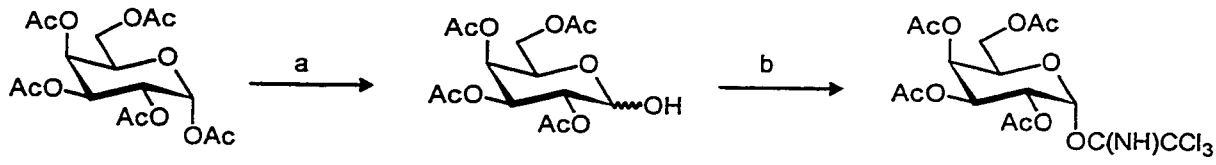
Scheme 2.2 Syntheses of thiogalactoside derivatives

Reagents and conditions: (a) PhSH, TBAHS, CH_2Cl_2, 1 M Na_2CO_3, 84 %; (b) NaOMe/MeOH, 99%; (c) $C_6H_5CH(OCH_3)_2$, *p*-TsOH, 90%; (d) BzCl, pyridine; (e) $NaBH_3CN$, HCl, 84%; (f) *i*: TBDPSCl, pyridine; *ii*: BzCl, 92%.

The other type of glycosyl donors such as *O*-galactosyl trichloroacetimidate[102] was also synthesized. Regioselective anomeric de-*O*-acetylation[103] of β-D-galactose pentaacetate **15** by using hydrazinium acetate in DMF afforded hemiacetal **16** (85%). Treatment of **16**

[102] Schmidt, R., R.; Kinzy, W. *Advances in Carbohydr. Chem. & BioChem.* **1994**, *50*, 21.
[103] Excoffier, G.; Gagnaire, D.; Utille, J.-P. *Carbohydr. Res.* **1975**, *39*, 368.

with trichloroacetonitrile under base catalyst (1,8-diazabicyclo[5,4,0]undec-7-ene) generated α- galactosyl trichloroacetimidate **17** in 88% yield (Scheme **2.3**).

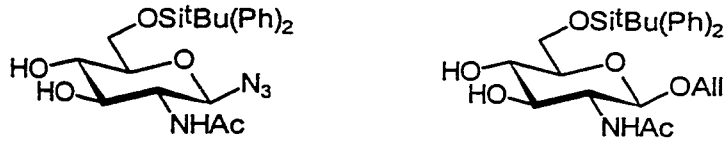


15 16 17

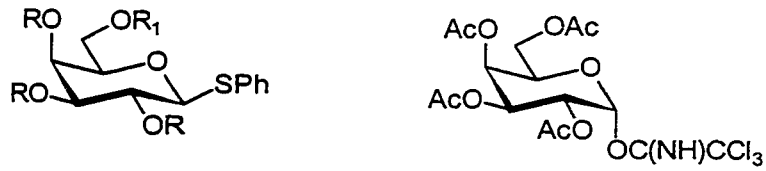
Scheme 2.3 Synthesis of galactosyl trichloroacetimidate

Reagents and conditions: (a) $NH_2NH_2{\cdot}HOAc$, DMF, 50 °C, 30 min, 85%; (b) CCl_3CN, DBU, 1 h, 88%.

In order to study the regioselectivity/specificity of the *tert*-butyldiphenylsilyl protecting group in glycosylation, the two glycosyl acceptors **3**, **5** and the five functionalized glycosyl donors **7**, **12-14**, and **17** (Figure **2.1**) were used as building blocks for the synthesis of *N*-acetyllactosamine derivatives.



3 5

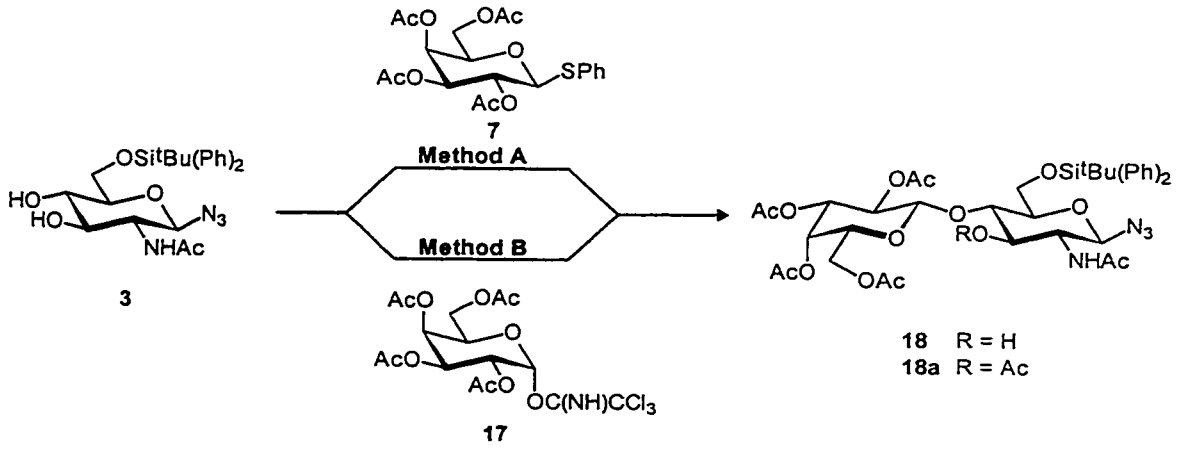


7 R=Ac, R_1=Ac
12 R=Bz, R_1=Bn
13 R=Bz, R_1=Bz
14 R=Bz, R_1=$SitBu(Ph)_2$

17

Figure 2.1 Glycosyl acceptors **3**, **5** and donors **7**, **12-14**, **17**

Condensation of phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside **7** with 6-*O*-*tert*-butyldiphenylsilyl acceptor **3** was performed using *N*-iodosuccinimide and trifluoromethanesulfonic acid[104] as promoter in dichloromethane-acetonitrile (1:1) at -45 °C. The reaction afforded the desired β-(1→4)-linked disaccharide **18** in 73% yield (Scheme **2.4**, Method **A**). Very impressively, no β-(1→3)-linked regioisomer was detected during this coupling reaction. The β-configuration of the disaccharide **18** was deduced from the ^{1}H-NMR spectrum which showed H-1' as a doublet at δ 4.69 ppm ($J_{1',2'}$ = 8.1 Hz). The regiochemistry of the newly introduced glycosidic linkage of **18** was proved from the ^{13}C NMR spectrum, which showed a downfield shift signal for C-4 at δ 79.3 ppm (Δδ= + 8.1 ppm) for disaccharide **18** compared to δ 71.2 ppm for C-4 of the *N*-acetylglucosamine acceptor **3**, and further confirmed by converting **18** to its corresponding acetate **18a** which showed H-3 at δ 4.88 ppm (Δδ = +1.0 ppm) as a downfield-shift signal in its ^{1}H-NMR spectrum. All the ^{1}H and ^{13}C signal assignments were unambiguously determined using 2-D COSY together with HMQC experiments (Figures **2.2** and **2.3**).



Scheme 2.4 Synthesis of disaccharide **18**

Reagents and conditions: Method A, NIS/TfOH, CH_2Cl_2/CH_3CN, -45 °C, 73%; Method B, $BF_3{\cdot}Et_2O$, CH_2Cl_2, -45 °C, 70%.

[104] a) Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331; b) Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313.

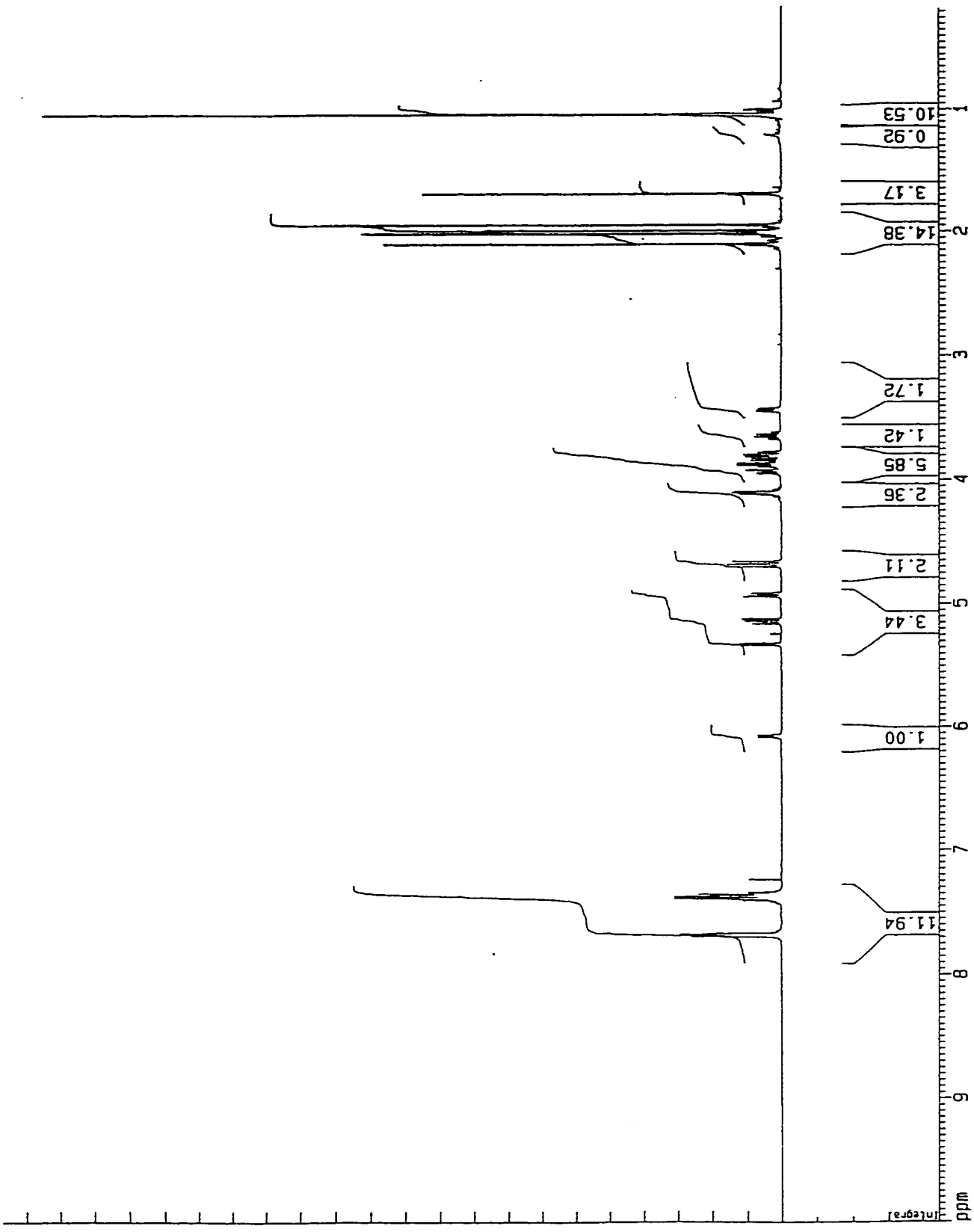


Figure 2.2 ^{1}H NMR (500MHz, $CDCl_3$) spectrum of disaccharide **18**

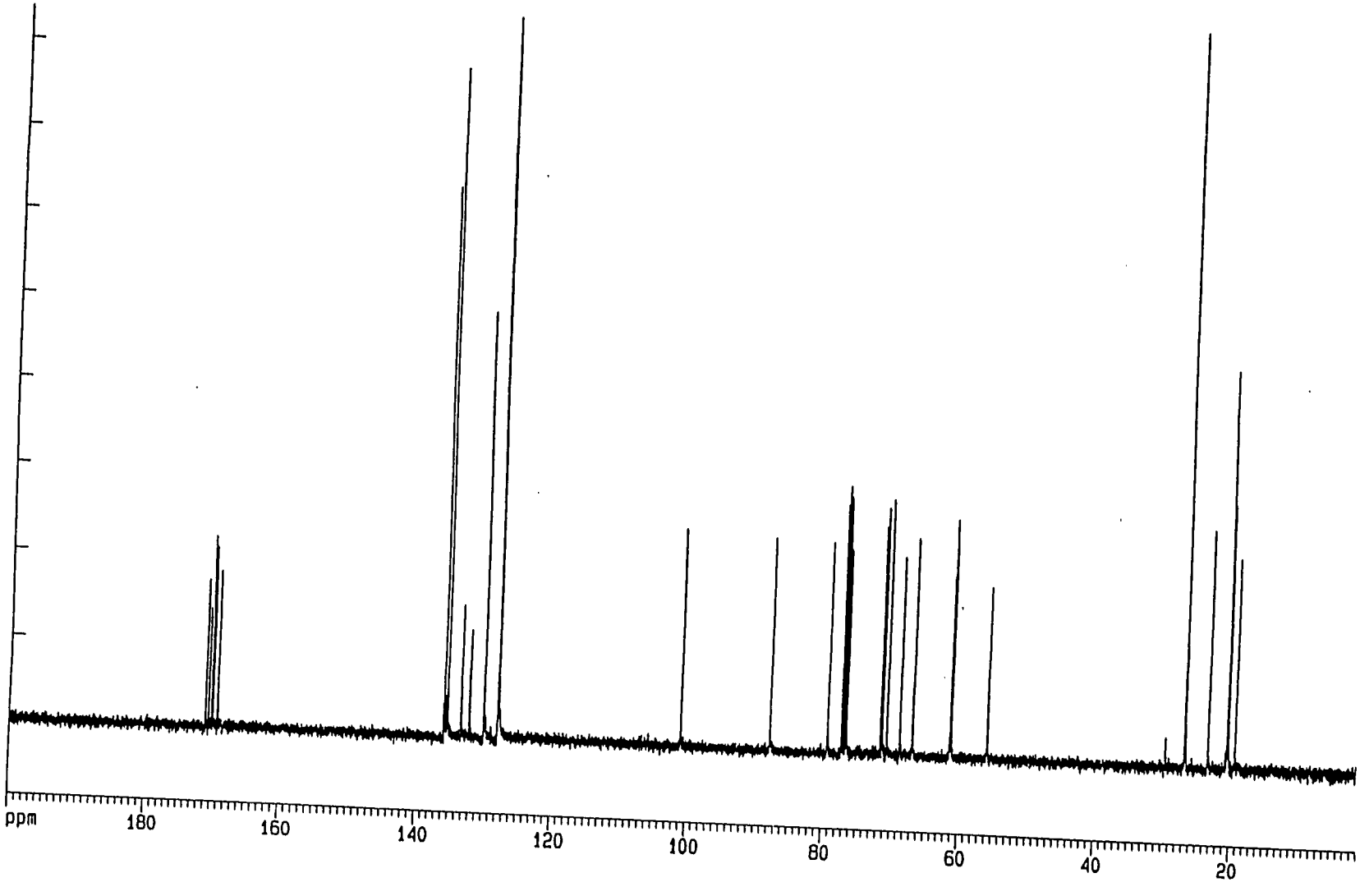
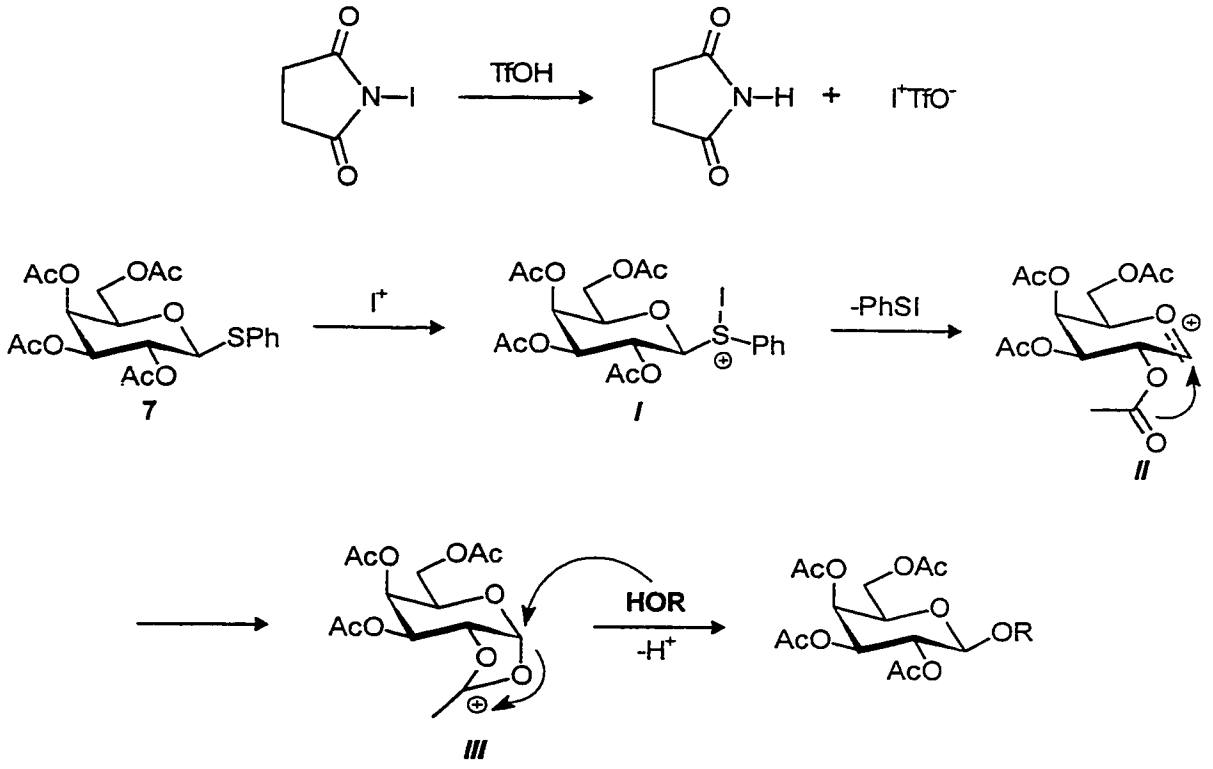


Figure 2.3 ^{13}C NMR (500MHz, $CDCl_3$) spectrum of disaccharide **18**

Disaccharide **18** was also synthesized by using boron trifluoride etherate as a promoter. Glycosylation of "lightly" protected acceptor **3** with *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-trichloroacetimidate (**17**) was achieved in dichloromethane using boron trifluoride etherate as the activator to provide disaccharide **18** in 70 % yield (Scheme **2.4**, Method **B**).

The formation of exclusive 1,2-*trans* linked *O*-glycoside **18** can be explained by neighboring group participation.[105] Thus, the iodonium ion, which was generated from NIS and triflic acid, reacted with thiogalactoside **7** to give iodosulfenium ion ***I***. After liberation of phenylsulfenyl iodide, the oxycarbonium ion ***II*** reverted to cyclic acyloxonium ion ***III***. Nucleophilic ring opening at C-1 with alcohol could only occur as a *trans* cleavage yielding the 1,2-*trans* oriented *O*-glycosidic linkage (Scheme **2.5**).



Scheme 2.5 Mechanism for the formation of 1,2 -*trans* linked *O*-glycosides

[105] Frush, H. L.; Isbell, H. S. **1949**, *43*, 161.

It appears that the bulky 6-*O*-*tert*-butyldiphenylsilyl protecting group in acceptor **3** played a key role in controlling the regiospecificity. Under such reaction conditions, the very bulky protecting group could cover the top side (3,5-*cis*) of the OH-3 of the acceptor **3** and block the glycosyl donor to approach the OH-3.

In order to test this hypothesis, other phenylthiogalactoside donors **12-14** (Figure **2.1**) were used to glycosylate acceptor **3.** The β (1→4) linked disaccharides **19-21** (Figure **2.4**) were obtained in 71-82% yield and no β (1→3) coupling products were obtained as products of this reaction. The sites of glycosylation in **19-21** were identified from the ^{13}C NMR spectra, which showed downfield-shift signals for C-4 at δ 78.6-77.9 ppm (Δδ= + 7.4-6.7 ppm) for disaccharides **19-21** compared to δ 71.2 ppm for C-4 of the *N*-acetylglucosamine acceptor **3** as well as by observation of downfield-shift signals of their H-3 protons in the ^{1}H NMR spectra of their acetylated derivatives **19a**, **20a**, and **21a**.

The utility of allyl glycosides is substantiated. The allyl group can be transformed into aldehyde or carboxylic acid by ozonolysis[106] or extended in length of spacer through a thiol addition.[107] The modified spacer carbohydrate haptens with different functional groups are very useful in the preparation of various glycoconjugates.[108]

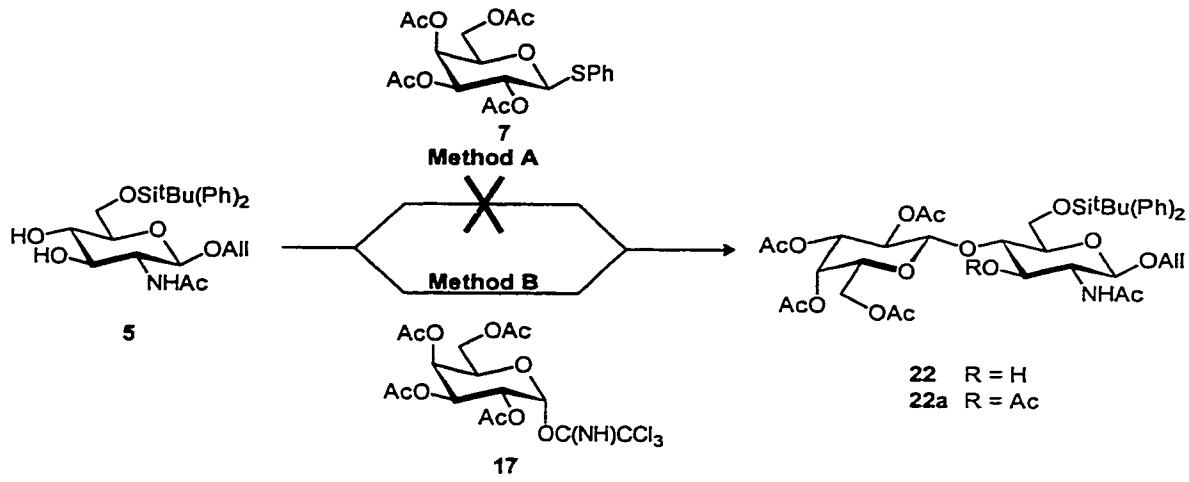
Synthesis of allyl lactosamine derivative **22** was first tested by using thiophenyl galactoside **7** as a donor and NIS/TfOH as promoters. Unfortunately, the glycosidation failed because the allyl group of acceptor **5** was labile to the iodonium ion generated from NIS and triflic acid. To avoid the side reaction, imidate derivative **17** as a donor and boron trifluoride etherate as a promoter were chosen for the glycosylation with allyl glucosaminide acceptor **5** in dichloromethane at -45 °C to afford the disaccharide **22** in 70% yield (Scheme **2.6**).

---

[106] a) Criegee, R. *Angew. Chem. Int. Ed. Engl.* **1975**, *14*, 745; b) Kuczkowski, R. L. *Acc. Chem. Res.* **1983**, *16*, 42.

[107] Chipowsky, S.; Lee, Y. C. *Carbohydr. Res.* **1973**, *31*, 339.

[108] a) Roy, R.; Laferrière, *Carbohydr. Res.* **1988**, *177*, C1; b) Roy, R.; Tropper, F. D.; Romanowska, A. *J. Chem. Soc., Chem. Commun.* **1992**, 1611.

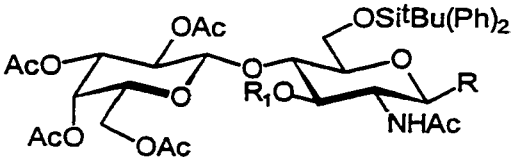


Scheme 2.6 Synthesis of disaccharide **22**

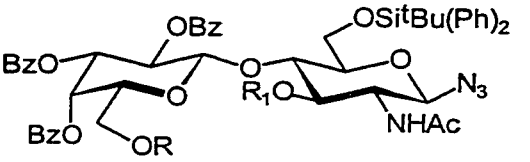
Reagents and conditions: Method A, NIS/TfOH, CH_2Cl_2/CH_3CN, -45 °C; Method B, $BF_3{\cdot}Et_2O$, CH_2Cl_2, -45 °C, 68%.

Similarly, the β-configuration of the disaccharide **22** was deduced from the ^{1}H-NMR spectrum which showed H-1' as a doublet at δ 4.68 ppm ($J_{1',2'}$ = 8.1 Hz). The site of glycosylation in compound **22** was identified by observation of significant deshielding for the signal of its C-4 carbon as well as by the H-3 proton in the ^{1}H NMR spectrum for its acetylated derivative **22a**.

The structures of *N*-acetyllactosamine derivatives **18-22** are shown in Figure **2.4**. The results of the glycosidation reactions between donors **7**, **12-14**, and **17** and acceptors **3** and **5** are summarized in Table **2.2**.



18 R=N_3, R_1=H
18a R=N_3, R_1=Ac
22 R=OAll, R_1=H
22a R=OAll, R_1=Ac



19 R=Bn, R_1=H
19a R=Bn, R_1=OAc
20 R=Bz, R_1=H
20a R=Bz, R_1=OAc
21 R=TBDPS, R_1=H
21a R=TBDPS, R_1=OAc

Figure 2.4 *N*-Acetyllactosamine derivatives **18-22**

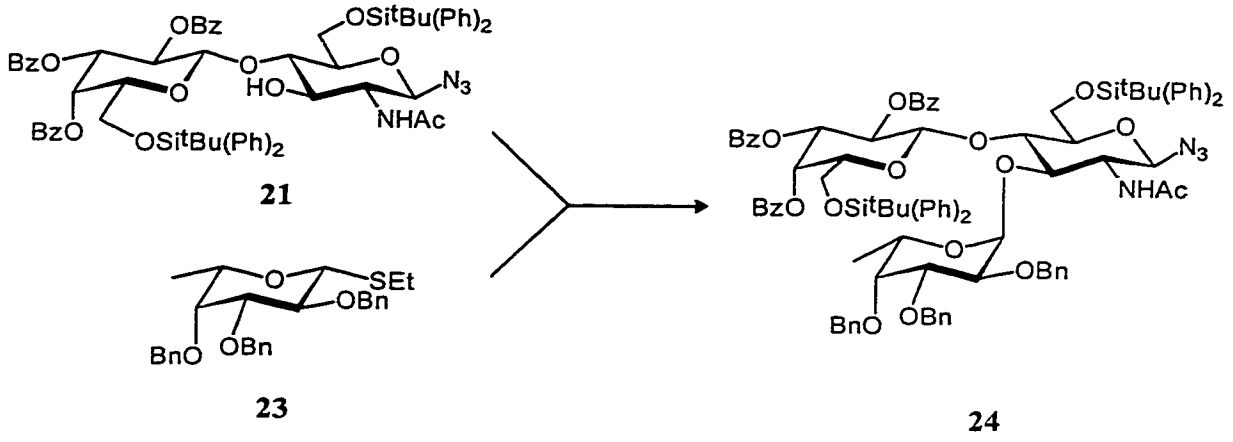
Further fucosylation of the *N*-acetyllactosamine derivative **21** with ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside **23** by using dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST)[109] as promoter at 0 °C afforded Le^x derivative **24** in good yield (78%) (Scheme **2.7**). The α configuration of the newly introduced anomeric center in compound **24** was assigned by ^{1}H NMR ($J_{1'',2''}$ = 3.5 Hz). The ^{1}H and ^{13}C NMR spectra of **24** are shown in Figures **2.5** and **2.6**.

Table 2.2 Glycosidation reactions of donors **7**, **12-14**, and **17** with acceptors **3** and **5**[a]

Entry	Donor	Acceptor	Promoter	Solvent	Product (yield%)[b]
1	**7** (1.5 equiv)	**3**	NIS/TfOH	CH_2Cl_2/CH_3CN	**18** (73%)
2	**17** (1.5 equiv)	**3**	$BF_3{\cdot}Et_2O$	CH_2Cl_2	**18** (70%)
3	**12** (1.3 equiv)	**3**	NIS/TfOH	CH_2Cl_2/CH_3CN	**19** (78%)
4	**13** (1.3 equiv)	**3**	NIS/TfOH	CH_2Cl_2/CH_3CN	**20** (71%)
5	**14** (1.3 equiv)	**3**	NIS/TfOH	CH_2Cl_2/CH_3CN	**21** (82%)
6	**17** (1.5 equiv)	**5**	$BF_3{\cdot}Et_2O$	CH_2Cl_2	**22** (68%)

[a]Reactions were performed at -45 °C in the presence of 4Å molecular sieves.
[b]After purification by column chromatography.

[109] a) Fügedi, P.; Garegg, P. J. *Carbohydr. Res.* **1986**, *149*, C-9; b) Andersson, F.; Fügedi, P.; Garegg, P. J.; Nashed, M. *Tetrahedron Lett.* **1986**, *27*, 3919.



Scheme 2.7 Synthesis of Le^x trisaccharide **24**

Reagents and conditions: DMTST, benzene:CH_2Cl_2 5:1, 0 °C, 78%.

2.3 Conclusion

A very simple and efficient synthetic route towards *N*-acetyllactosamine derivatives was developed. This procedure takes advantage of a 6-*O*-*tert*-butyldiphenylsilyl protected *N*-acetylglucosamine derivative, allowing the regiospecific introduction of a galactosyl moiety. In addition, this method constitutes a highly practical synthesis of Lewis X trisaccharide derivative.

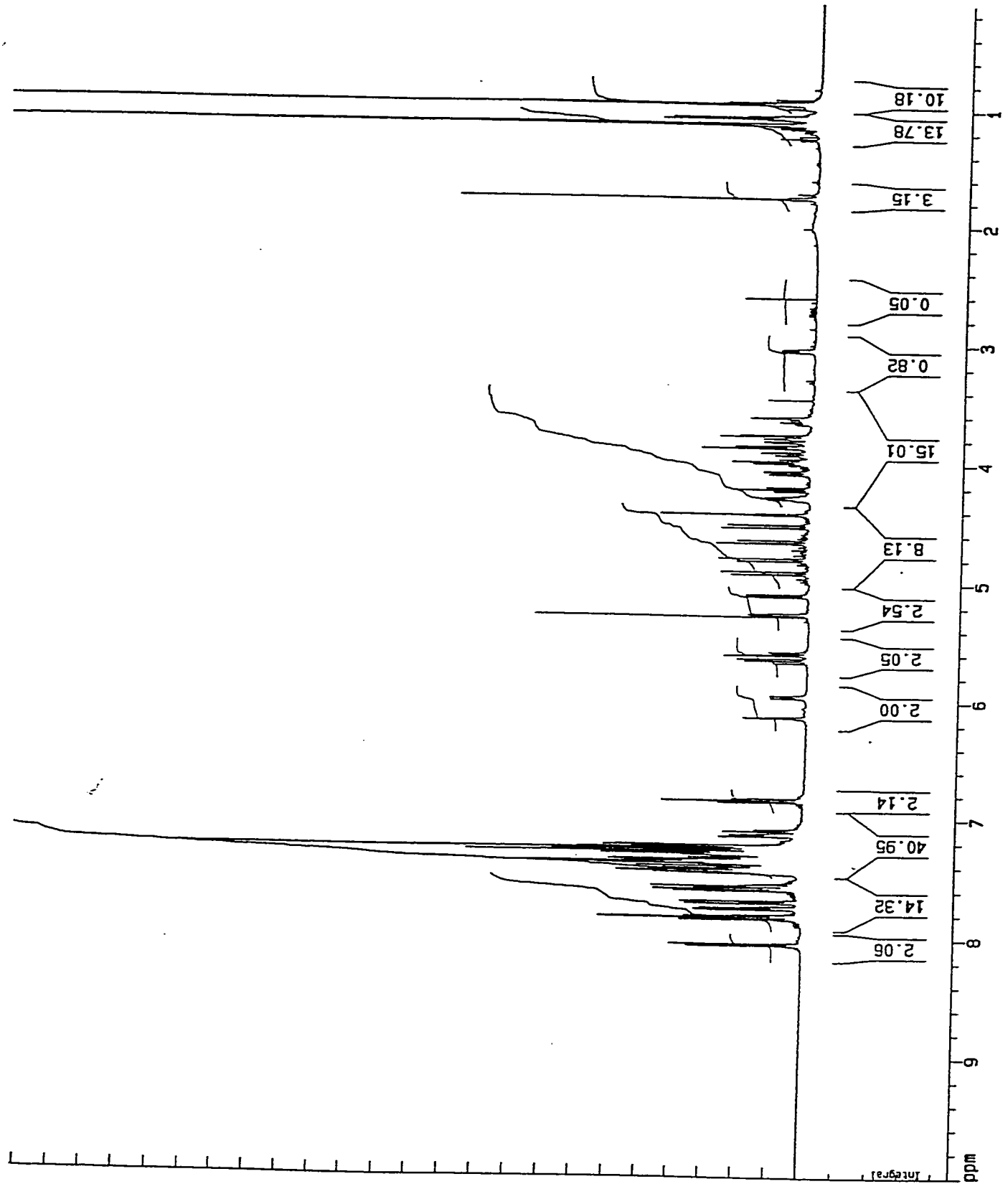


Figure 2.5 ^{1}H NMR (500MHz, $CDCl_3$) spectrum of Le^x trisaccharide **24**

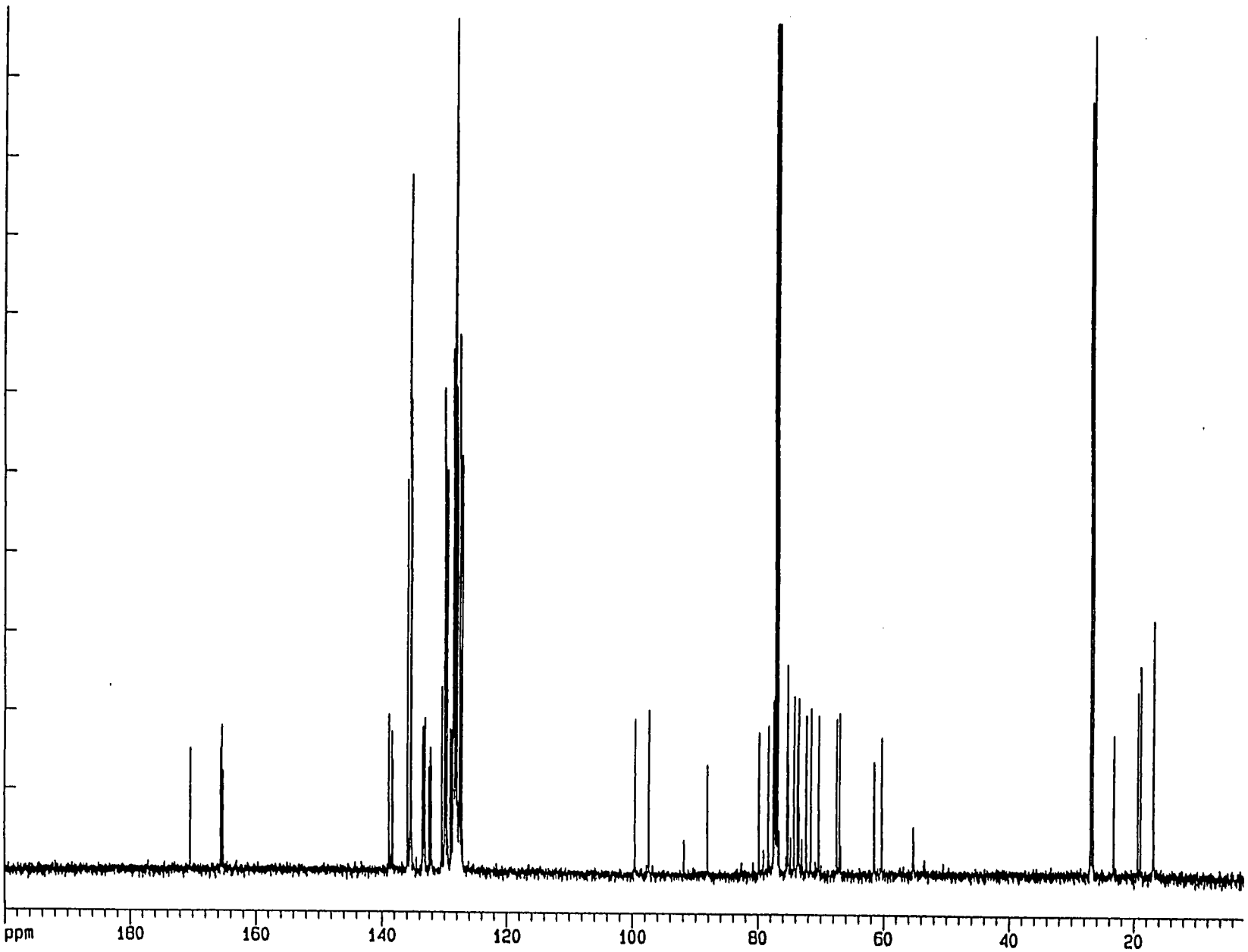


Figure 2.6 ^{13}C NMR (500MHz, $CDCl_3$) spectrum of Le^x trisaccharide **24**

2.4 Experimental

General methods

^{1}H- and ^{13}C-NMR spectra were recorded on a Brüker AMX 500 instrument or a Varian Gemini 200 spectrometer at 500 and 200 MHz for protons and 125.7 and 50.3 MHz for carbons, respectively. Proton chemical shifts (δ) are given relative to internal $CHCl_3$ (7.24 ppm) for $CDCl_3$ solutions, DMSO (2.49 ppm) for DMSO-d_6 solutions, and to HOD (4.76 ppm) for D_2O solutions unless indicated otherwise. Repeated exchange of protons for deuterium with D_2O and lyophilization for unprotected carbohydrates to simplify proton spectra was performed. Carbon chemical shifts were given relative to $CDCl_3$ (77.0 ppm) and DMSO-d_6 (39.5 ppm). Spectral analyses were performed as first-order approximations and were based on shift correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and 1- and 2-dimensional distortionless enhancement by polarization transfer (DEPT) experiments.

Melting points were determined on Gallenkamp apparatus and are uncorrected.

Optical rotations were measured on a Perkin-Elmer 241 polarimeter and were run at room temperature.

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

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

Elemental analyses were performed by the Chemistry or Geography Department, University of Ottawa.

Crude pH measurements were routinely performed with Hydron test paper.

Thin layer chromatography (TLC) was performed on silica gel 60 F-254 glass plates. Reagents used for developing plates include ceric sulfate (1% w/v) and ammonium molybdate (2.5% w/v) in aqueous sulfuric acid (10% v/v), isatin (0.2% w/v) in ethanol with sulfuric acid

(5% v/v), iodine, dilute aqueous potassium permanganate, ninhydrin (2% w/v) in ethanol, or UV light. TLC plates were heated to about 150 °C when necessary.

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

Purifications were also performed *via* preparative size exclusion chromatography. Columns were connected to a Pharmacia Peristaltic Pump P3 and eluted with distilled H_2O. A Waters Differential Refractomer R401 or R403 apparatus was used for detection, and recording on a Linear 1200 or 200 chart recorder. Fractions were collected using LKB 2112 Redirac or Pharmacia Model 5051 fraction collectors.

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

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

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

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

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (2)

To a solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride **1** (10 g, 27.3 mmol), TBAHS (13.9 g, 41 mmol) and sodium azide (3.55 g, 54.6 mmol) in dichloromethane (100 mL) was added 1M Na_2CO_3 solution (100 mL). The two phase reaction mixture was vigorously stirred at room temperature for 2 h. The organic phase was separated, washed with water, then dried over anhydrous sodium sulfate, and concentrated to give **2** (9.6 g, 94%). Compound **2** was crystallized from ethanol: mp 159-161 °C; $[\alpha]_D$ -45.7° (*c* 0.9, chloroform); Lit.[110] mp 160-161 °C; $[\alpha]_D$ -43°; ^{1}H NMR ($CDCl_3$) δ (ppm) 5.77 (d, 1 H, $J_{2,NH}$ 8.9 Hz, NH), 5.23 (dd, 1 H, $J_{3,4}$ 9.8 Hz, H-3), 5.07 (dd, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 4.74 (d, 1 H, $J_{1,2}$ 9.3 Hz, H-1), 4.24 (dd, 1 H, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.13 (dd, 1 H, $J_{5,6b}$ 2.4 Hz, H-6b), 3.92 (dd, 1 H,

[110] Thiem, J.; Wiemann, T. *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 80.

$J_{2,3}$ 10.1 Hz, H-2), 3.72 (ddd, 1 H, $J_{5,6b}$ 4.7 Hz, H-5), 2.07, 2.02, 2.01, 1.96 (4s, 3 x OAc, NHAc).

2-Acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide (3)

To a suspension of **2** (3.0 g, 8.04 mmol) in methanol (50 mL) was added 1M NaOMe/MeOH solution until the pH was 9-10. The mixture was stirred for 1 h at room temperature. TLC (Ethanol/CH_2Cl_2, 1:4) showed that deacetylation was complete. The solution was neutralized with H^+ resin (Dowex 50W-X8), filtered, and concentrated. The residue (1.7 g, 6.9 mmol) in dry pyridine (20 mL) was added *tert*-butylchlorodiphenylsilane (2.15 mL, 8.28 mmol). The solution was stirred at room temperature for 6 h. The reaction mixture was poured into ice-water and extracted with dichloromethane (3 x 20 mL). The combined extracts were washed successively with 5% hydrochloric acid, saturated $NaHCO_3$ solution, and water. The organic extract was dried (Na_2SO_4), filtered, and concentrated. The crude product was subjected to column chromatography with CH_2Cl_2-CH_3OH (15:1, v/v) as eluent to give **3** (3.08 g, 92%): $[\alpha]_D$ -53.6° (*c* 1.0, chloroform); ^1H NMR ($CDCl_3$) δ 7.67-7.34 (m, 10 H, aromatic), 6.14 (d, 1H, $J_{2,NH}$ 7.1 Hz, NH), 4.61 (d, 1 H, $J_{1,2}$ 9.1 Hz, H-1), 3.88 (m, 2 H, H-6, H-6'), 3.66 (dd, 1 H, $J_{3,4}$ 9.4 Hz, H-3), 3.60 (dd, 1 H, $J_{4,5}$ 9.1 Hz, H-4), 3.53 (dd, 1 H, $J_{2,3}$ 9.1 Hz, H-2), 3.44 (ddd, 1 H, $J_{5,6a}$ 4.3 Hz, $J_{5,6b}$ 4.5 Hz, H-5), 2.02 (s, 3 H, NHAc), 1.02 (s, 9 H, $SiCMe_3$); ^{13}C NMR ($CDCl_3$) δ (ppm) 172.6 (C=O), 137.6-127.7 (12 C, aromatic), 88.2 (C-1), 77.8 (C-5), 74.4 (C-3), 71.2 (C-4), 63.5 (C-6), 55.6 (C-2), 26.7 ($SiC(\underline{C}H_3)_3$), 23.3 (NHAc), 19.2 ($Si\underline{C}Me_3$).

Anal. Calcd for $C_{24}H_{32}N_4O_5Si$: C, 59.48; H, 6.66; N, 11.56. Found: C, 59.51; H, 6.55; N, 11.91.

Allyl 2-acetamido-2-deoxy-β-D-glucopyranoside (4)

To a solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride **1** (10 g, 27.3 mmol) dissolved in CH_2Cl_2 (25 mL) was added a solution of sodium allyloxide in allyl alcohol (1M, 54.6 mL). The mixture was stirred at room temperature for 30 min. The suspension was neutralized with Amberlite IR-120 (H^+ form) resin as judged by pH test paper, filtered, and the resin was washed with methanol (3 x 40 mL). The filtrate and washings were combined and concentrated to give the allyl β-glycoside **4** (6.79 g, 95%) as a solid.

Crystallization from ethanol gave white crystalline **4** (5.71 g, 80%): mp 168-169 °C (Lit.[99] 171-172 °C); $[\alpha]_D$ -23° (*c* 1.0, methanol); ^{1}H NMR (D_2O) δ 5.86 (m, 1 H, -C<u>H</u>=CH_2), 4.51 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 1.98 (s, 3 H, NHAc).

Allyl 2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranoside (5)

To a solution of compound **4** (1 g, 3.83 mmol) in dry pyridine (15 mL) was added *tert*-butylchlorodiphenylsilane (1.30 mL, 4.98 mmol). The solution was stirred at room temperature for 6 h. The reaction mixture was poured into ice-water and extracted with CH_2Cl_2 (3 x 20 mL). The combined extracts were washed successively with 5% hydrochloric acid, saturated $NaHCO_3$ solution, and water. The organic extract was dried (Na_2SO_4), filtered, and concentrated. The crude product was subjected to column chromatography with CH_2Cl_2-CH_3OH (15:1, v/v) as eluent to give **5** (1.68 g, 88%): $[\alpha]_D$ -42.6° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.70-7.24 (m, 10 H, aromatic), 6.83 (br, 1 H, NH), 5.91-5.83 (m, 1 H, -C<u>H</u>=CH_2), 4.48 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 3.94 (dd, 1 H, $J_{5,6a}$ 2.8 Hz, $J_{6a,6b}$ 11.0 Hz , H-6a), 3.84 (dd, 1 H, $J_{5,6b}$ 5.5 Hz, H-6b), 3.68-3.61 (m, 2 H, H-2, H-3), 3.47 (dd, 1 H, $J_{3,4}$ 9.4 Hz, $J_{4,5}$ 8.2 Hz, H-4), 3.39 (ddd, 1 H, H-5), 1.99 (s, 3 H, NHAc), 1.01 (s, 9 H, $SiCMe_3$); ^{13}C NMR ($CDCl_3$) δ (ppm) 172.6 (C=O), 135.5-127.6 (12 C, aromatic), 134.1 (-<u>C</u>H=CH_2), 117.4 (-CH=<u>C</u>H_2), (99.7 (C-1), 76.5 (C-5), 74.7 (C-3), 71.3 (C-4), 69.5 (O-<u>C</u>H_2CH=), 63.9 (C-6), 56.2 (C-2), 26.7 ($SiC(\underline{C}H_3)_3$), 23.2 (NHAc), 19.1 ($Si\underline{C}Me_3$).

Anal. Calcd for $C_{27}H_{37}NO_6Si$: C, 64.90; H, 7.46; N, 2.80. Found: C, 64.88; H, 7.44; N, 3.06.

Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (7)[111]

To a solution of acetobromogalactose **6** (10 g, 24.3 mmol), TBTAS (8.25 g, 24.3 mmol) and thiophenol (3.74 mL, 36.45 mmol) in dichloromethane (100 mL) was added 1M Na_2CO_3 solution (100 mL). The two-phase reaction mixture was vigorously stirred at room temperature for 1 h. The organic phase was separated, washed with water, then dried over anhydrous sodium sulfate, and concentrated under vacuum. The crude product was subjected to column chromatography with hexane-ethyl acetate (3:2, v/v) as eluent to give **7** (9.0 g, 84%): Compound **7** was crystallized from ethanol: mp 69-71 °C; $[\alpha]_D$ +7.5° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ (ppm) 7.50-7.23 (m, 5 H, aromatic H), 5.39 (dd, 1 H, $J_{4,5}$ 0.9

Hz, H-4), 5.21 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2), 5.01 (dd, 1 H, $J_{3,4}$ 3.3 Hz, H-3), 4.69 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 4.16 (dd, 1 H, $J_{5,6a}$ 7.1 Hz, $J_{6a,6b}$ 11.2 Hz, H-6a), 4.07 (dd, 1 H, $J_{5,6b}$ 6.1 Hz, H-6b), 3.90 (ddd, 1 H, H-5), 2.09, 2.06, 2.01, 1.94 (s, OAc's); ^{13}C NMR ($CDCl_3$) δ (ppm) 170.1, 170.0, 169.7, 169.2 (C=O's), 132.4, 132.2, 128.8 (aromatic C), 86.0 (C-1), 74.2 (C-5), 71.8 (C-3), 67.2 (C-4), 67.1 (C-2), 61.6 (C-6), 20.7, 20.5, 20.4 (OAc's); M.S. (C.I. ether) (M/Z): 441.4 ($[M+1]^+$, 2.9%).

Phenyl 1-thio-β-D-galactopyranoside (8)

Compound **7** (5 g, 11.33 mmol) was deacetylated using the standard Zemplén deacetylation procedure to afford compound **8** in quantitative yield. The pure compound **8** was recrystallized from ethanol: m.p. 120-122 °C; $[\alpha]_D$ -51.0° (*c* 1.15, CH_3OH); ^{1}H NMR ($CDCl_3$) δ (ppm) 7.63-7.37 (m, 5 H, aromatic H), 4.82 (d, 1 H, $J_{1,2}$ 9.3 Hz, H-1), 4.74-3.60 (m, 6 H, H-2, 3, 4, 5, 6a, 6b); M.S. (C.I. ether) (M/Z): 273.3 ($[M+1]^+$, 29.4%).

Phenyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (9)

Phenyl 1-thio-β-D-galactopyranoside **8** (1 g, 3.67 mmol) in acetonitrile (10 mL) was heated at 60 °C until the starting material was completely dissolved. The solution was then cooled to room temperature, benzaldehyde dimethyl acetal (1.65 mL, 11 mmol) and *p*-toluenesulfonic acid monohydrate (catalytic amount) were then added. The mixture was stirred for 4 h at room temperature, neutralized with triethylamine, and concentrated under reduced pressure. The residue was then purified by column chromatography using MeOH-CH_2Cl_2 (1:10, v/v) as eluent to give **9** (1.17 g, 90%): $[\alpha]_D$ -24.0° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.70-7.10 (m, 10 H, aromatic), 5.41 (s, 1 H, PhCH), 4.39 (d, 1 H, $J_{1,2}$ 9.2 Hz, H-1), 4.29 (dd, 1 H, $J_{5,6a}$ 1.4 Hz, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.11 (d, 1 H, $J_{3,4}$ < 1.0 Hz, H-4), 3.94 (dd, 1 H, $J_{5,6b}$ 1.7 Hz, H-6b), 3.58 (m, 2 H, H-2, H-3), 3.46 (m, 1 H, H-5).

Anal. Calcd for $C_{19}H_{20}O_5S$: C, 63.32; H, 5.59. Found: C, 63.51; H, 5.31.

Phenyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (10)

To a solution of **9** (1 g, 2.77 mmol) in dry pyridine (15 mL) at 0 °C was added benzoyl chloride (0.81 mL, 7 mmol) slowly. The solution was then stirred at room temperature for 2 h.

[111] Bogusiak, J.; Szeja, W. *Polish J. Chem.* **1981**, *55*, 1503.

TLC showed the reaction was complete. Methanol (1 mL) was added and stirred for additional 15 min. The mixture was concentrated under reduced pressure. The crude product was purified by column chromatography using ethyl acetate-hexane (1:4, v/v) as eluent to give **10** (1.54 g, 97%): $[\alpha]_D$ +54.9° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.95-7.10 (m, 20 H, aromatic), 5.66 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 5.41 (s, 1 H, PhCH), 5.22 (dd, 1 H, $J_{3,4}$ 3.2 Hz, H-3), 4.84 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1), 4.50 (d, 1 H, $J_{4,5}$ < 1.0 Hz, H-4), 3.87-3.67 (m, 3 H, H-6a, H-6b, H-5).
Anal. Calcd for $C_{33}H_{28}O_7S$: C, 69.70; H, 4.96. Found: C, 69.82; H, 4.90.

Phenyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-galactopyranoside (11)

To a solution of **10** (1 g, 1.76 mmol) in dry THF (10 mL) was added powdered molecule sieves 4 Å (1 g). The mixture was stirred for 2 h at room temperature, and sodium cyanoborohydride (1.5 g, 23.9 mmol) was gradually added. After the reagent was dissolved, saturated solution of hydrogen chloride in ether was added dropwise at room temperature until the evolution of gas ceased. TLC (EtOAc/Hexane 2:3) indicated the starting material **10** (Rf = 0.46) was clearly converted to product **11** (Rf = 0.54) after 10 min. The mixture was diluted with dichloromethane, and filtered. The organic phase was washed with saturated $NaHCO_3$ solution and water. The solution was dried (Na_2SO_4), filtered, and concentrated. Column chromatography on silica gel using ethyl acetate-hexane (1:3, v/v) as eluent gave **11** (0.85 g, 84%): $[\alpha]_D$ +77.0° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.99-7.20 (m, 20 H, aromatic), 5.75 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2), 5.29 (dd, 1 H, $J_{3,4}$ 3.2 Hz, H-3), 4.91 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 4.56 (AB pattern, 2 H, CH_2Ph), 4.41 (ddd, 1 H, $J_{4,OH}$ 1.4, $J_{4,5}$ < 1.0 Hz, H-4), 3.91-3.83 (m, 3 H, H-5, H-6a, H-6b), 2.67 (d, 1 H, OH).
Anal. Calcd for $C_{33}H_{30}O_7S$: C, 69.46; H, 5.30. Found: C, 69.32; H, 5.23.

Phenyl 2,3,4-tri-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-galactopyranoside (12)

Compound **11** was treated as described as the synthesis of **10**. Column chromatograph of the residue on silica gel using methanol-dichloromethane (1%, v/v) gave **12** in 97% yield as an amorphous mass: $[\alpha]_D$ +113.5° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.96-7.10 (m, 25 H, aromatic), 5.94 (dd, 1 H, $J_{4,5}$ < 1.0 Hz, H-4), 5.68 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2), 5.53 (dd, 1 H, $J_{3,4}$ 3.0 Hz, H-3), 4.98 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 4.52 (AB pattern, 1 H, *J* 11.8 Hz, H-A of CH_2Ph), 4.45 (AB pattern, 1 H, H-B of CH_2Ph), 4.19 (m, 1 H, H-5), 3.73 (dd, 1 H, $J_{5,6a}$ 6.3 Hz, $J_{6a,6b}$ 9.8

Hz, H-6a), 3.62 (dd, 1 H, $J_{5,6b}$ 6.3 Hz, H-6b); ^{13}C NMR ($CDCl_3$) δ (ppm) 165.5, 165.3, 165.2 (C=O's), 85.7 (C-1), 76.7 (C-5), 73.7 (CH_2), 73.2 (C-3), 68.6 (C-4), 68.2 (C-6), 68.0 (C-2). Anal. Calcd for $C_{40}H_{34}O_8S$: C, 71.20; H, 5.08. Found: C, 71.28; H, 4.96.

Phenyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside (13)

Compound **8** was treated as described as the synthesis of **10**. Column chromatograph of the residue on silica gel using hexane-ethyl acetate (3:1, v/v) gave **13** in 96% yield as an amorphous mass: $[\alpha]_D$ +92.2° (*c* 1.0, chloroform); ^1H NMR ($CDCl_3$) δ (ppm) 8.05-7.10 (m, 25 H, aromatic), 6.00 (dd, 1 H, $J_{4,5}$ < 1 Hz, H-4), 5.77 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2), 5.60 (dd, 1 H, $J_{3,4}$ 3.2 Hz, H-3), 5.04 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1), 4.64 (m, 1 H, H-6a), 4.48-4.41 (m, 2 H, H-5, H-6b); ^{13}C NMR ($CDCl_3$) δ (ppm) 166.0, 165.4, 165.3, 165.1 (C=O's), 138.9-123.2 (aromatic C), 85.8 (C-1), 75.1 (C-5), 72.9 (C-3), 67.8 (C-4), 67.3 (C-2), 62.5 (C-6); M.S. (C.I. ether) (M/Z): 688.8 ($[M+1]^+$, 1.9%).

Phenyl 2,3,4-tri-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-1-thio-β-D-galactopyranoside (14)

To a solution of compound **8** (3.3 g, 12.1 mmol) in dry pyridine (15 mL) was added TBDPSCl (3.5 mL, 13.3 mmol). The reaction mixture was stirred for 4 h at room temperature. TLC showed complete conversion of **8** to the silylated intermediate. The mixture was cooled to 0 °C, and benzoyl chloride (5.0 mL, 44 mmol) was added. The mixture was stirred at room temperature for 1 h and then poured into ice. The solution was extracted with chloroform. The extracts were washed successively with 5% hydrochloric acid, saturated $NaHCO_3$ solution, and water. The organic extract was dried (Na_2SO_4), filtered, and concentrated. The crude product was subjected to column chromatography with ethyl acetate/hexane (1:4, v/v) as eluent to give **14** (9.2 g, 92%): $[\alpha]_D$ +110° (*c* 1.0, chloroform); ^1H NMR ($CDCl_3$) δ 7.98-7.14 (m, 30 H, aromatic), 6.06 (dd, 1 H, $J_{4,5}$ < 1 Hz, H-4), 5.66 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2), 5.60 (dd, 1 H, $J_{3,4}$ 3.1 Hz, H-3), 4.98 (d, 1 H, $J_{1,2}$ 9.8 Hz, H-1), 4.14 (m, 1 H, H-5), 3.90 (dd, 1 H, $J_{5,6a}$ 6.1 Hz, $J_{6a,6b}$ 10.3 Hz, H-6a), 3.79 (dd, 1 H, $J_{5,6b}$ 7.6 Hz, H-6b), 1.02 (s, 9 H, $SiCMe_3$); M.S. (C.I. ether) (M/Z): 823.3 ($[M+1]^+$, 3.6%).

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (17)

To a solution of β-D-galactose pentaacetate **15** (5.75 g, 14.7 mmol) in DMF (20 mL) was added hydrazine acetate (1.77 g, 19.2 mmol). The mixture was stirred at 50 °C for 30 min. The mixture was poured into ice-NaCl solution (100 mL). The solution was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were successively washed with 5 % HCl, sat. $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography using hexane-ethyl acetate (1:1, v/v) as eluent to give compound **16** (4.36 g, 85 %).

To a solution of compound **16** (2.50 g, 7.18 mmol)) in dry dichloromethane (20 mL) were added trichloroacetonitrile (1.08 mL, 10.77 mmol) and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU, 5 drops). The solution was stirred at room temperature for 1 h. TLC showed the reaction was complete. The solution was concentrated under vacuum. The residue was purified by silica gel column chromatography using hexane-ethyl acetate (3:2, v/v) as eluent to give compound **17** (3.11 g, 88 %): m.p. 118-120 °C (dichloromethane-hexane), $[\alpha]_D$ +112° (*c* 1.0, chloroform); Lit.[112] m.p. 122-123 °C (benzene-hexane), $[\alpha]_D$ +115.5°; ^{1}H NMR ($CDCl_3$) δ 8.64 (s, 1 H, NH), 6.57 (d, 1 H, $J_{1,2}$ 3.1 Hz, H-1), 2.14, 2.00, 1.99.1.98 (s, 12 H, OAc's).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide (18)

Method A: To a solution of compound **3** (200 mg, 0.41 mmol) and phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside **7** (273 mg, 0.62 mmol) in dry acetonitrile and dichloromethane (10 mL, 1:1, v/v) under nitrogen was added powdered molecular sieves 4 Å (0.5 g). The mixture was stirred for 2 h at room temperature, then cooled to -45 °C. *N*-Iodosuccinimide (186 mg, 0.83 mmol) and trifluoromethanesulfonic acid (36 μL, 0.41 mmol) were added. After 1 h, the mixture was diluted with dichloromethane (10 mL), filtered through celite, and the residue was washed with dichloromethane. The combined filtrate and washings were successively washed with 10% $Na_2S_2O_3$ and sat. $NaHCO_3$ solution. The dried (Na_2SO_4) solution was concentrated under pressure. The syrupy residue was purified by silica gel

[112] Amvam-Zollo, P. H.; Sinaÿ, P. *Carbohydr. Res.* **1987**, *167*, 197.

chromatography using methanol-dichloromethane as eluent (1:30, v/v) to afford disaccharide **18** (246 mg, 73%).

Method B: To a solution of compound **3** (200 mg, 0.41 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate **17** (305 mg, 0.62 mmol) in dry dichloromethane (10 mL) under nitrogen was added powdered molecular sieves 4 Å (0.5 g). The mixture was stirred for 2 h at room temperature, then cooled to -45 °C. $BF_3{\cdot}Et_2O$ (50 μL, 0.41 mmol) was added dropwise. After 1 h, the mixture was neutralized with triethylamine, diluted with dichloromethane (10 mL), filtered through celite, and the residue was washed with dichloromethane. The combined filtrate and washings were dried with Na_2SO_4, filtered, and concentrated. The syrupy residue was purified by silica gel chromatography using methanol-dichloromethane (1:30, v/v) as eluent to give **18** (235 mg, 70%); mp 160-161 °C; $[\alpha]_D$ -11.6° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.72-7.33 (m, 10 H, aromatic), 6.08 (d, 1 H, $J_{2,NH}$ 8.4 Hz, NH), 5.33 (d, 1 H, $J_{3',4'}$ 3.4 Hz, H-4'), 5.15 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2'), 4.93 (dd, 1 H, H-3'), 4.69 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.67 (d, 1 H, $J_{1,2}$ 9.3 Hz, H-1), 4.17-4.06 (m, 2 H, H-6a', H-6b'), 3.94 (dd, 1 H, $J_{5,6a}$ 1.2, $J_{6a,6b}$ 10.9 Hz, H-6a), 3.89-3.77 (m, 4 H, H-3, H-4, H-6b, H-5'), 3.64 (ddd, 1 H, $J_{2,3}$ 9.1 Hz, H-2), 3.43 (m, 1 H, H-5), 2.10, 2.02, 2.00, 1.95, 1.70 (5s, 15 H, 4 x OAc, NHAc), 1.05 (s, 9 H, $SiCMe_3$); ^{13}C NMR ($CDCl_3$) δ 170.8, 170.4, 170.0, 169.8, 169.1 (5 x C=O), 135.8-127.6 (12 C, aromatic), 100.9 (C-1'), 87.8 (C-1), 79.3 (C-4), 76.6 (C-5), 71.6 (C-3), 71.4 (C-5'), 70.7 (C-3'), 68.7 (C-2'), 66.9 (C-4'), 61.3 (C-6), 61.1 (C-6'), 55.9 (C-2), 26.8 ($SiC(\underline{C}H_3)_3$), 23.5 (NHAc), 20.6, 20.5, 20.4, 20.3 (4 x OAc), 19.3 ($Si\underline{C}Me_3$); FAB-MS (glycerol) gave m/z (ion, relative intensity): 815.3 ($[M+1]^+$, 6.1%), 772.3 ($[M-N_3]^+$, 25.5%).

Anal. Calcd for $C_{38}H_{50}N_4O_{14}Si$: C, 56.01; H, 6.18; N, 6.88. Found: C, 56.29; H, 6.16; N, 6.81.

2,3,4-Tri-*O*-benzoyl-6-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide (19)

Compound **3** (100 mg, 0.21 mmol) was glycosylated with **12** (181 mg, 0.27 mmol) as described as the method A to give **19** (168 mg, 78%) as an amorphous mass: $[\alpha]_D$ +56.5° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 8.01-7.12 (m, 30 H, aromatic), 5.91 (d, 1 H, H-4'), 5.73 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2'), 5.57 (d, 1 H, $J_{2,NH}$ 8.1 Hz, NH), 5.51 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3'), 5.02 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.74 (d, 1 H, $J_{1,2}$ 9.3 Hz, H-1), 4.52 (AB pattern, 1 H, *J*

11.8 Hz, H-A of CH_2Ph), 4.40 (AB pattern, 1 H, H-B of CH_2Ph), 4.15 (dd, 1 H, $J_{5',6a'}$ 6.3, $J_{5',6b'}$ 6.5 Hz, H-5'), 4.03-4.00 (m, 2 H, H-3, H-4), 3.79 (dd, 1 H, $J_{5,6a}$ 1.4, $J_{6a,6b}$ 11.8 Hz, H-6a), 3.72-3.67 (m, 2 H, H-6b, H-6a'), 3.61 (dd, 1 H, $J_{6a',6b'}$ 9.5 Hz, H-6b'), 3.52 (ddd, 1 H, $J_{2,3}$ 8.9 Hz, H-2), 3.36 (m, 1 H, H-5), 2.01 (s, 3 H, NHAc), 1.06 (s, 9 H, $SiCMe_3$); ^{13}C NMR ($CDCl_3$) δ 170.6, 165.4, 165.4 and 165.1 (4 x C=O), 137.2-127.8 (24 C, Aromatic), 100.9 (C-1'), 87.6 (C-1), 78.6 (C-4), 76.6 (C-5), 73.7 ($\underline{C}H_2Ph$), 73.0 (C-5'), 71.6 (C-3'), 71.4 (C-3), 68.8 (C-2'), 68.1 (C-4'), 67.3 (C-6'), 61.5 (C-6), 56.5 (C-2), 26.9 ($SiC(\underline{C}H_3)_3$), 23.6 (NHAc), 19.5 ($Si\underline{C}Me_3$); FAB-MS (glycerol) gave m/z (ion, relative intensity): 1049.4 ($[M+1]^+$, 4.8%).
Anal. Calcd for $C_{58}H_{60}N_4O_{13}Si$: C, 66.40; H, 5.76; N, 5.34. Found: C, 66.11; H, 5.68; N, 5.32.

2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide (20)

Compound **3** (100 mg, 0.21 mmol) was glycosylated with **13** (185 mg, 0.27 mmol) as described as the method A to give **20** (156 mg, 71%) as an amorphous mass: $[\alpha]_D$ +67° (*c* 1.0, chloroform); ^1H NMR ($CDCl_3$) δ 8.09-7.14 (m, 30 H, aromatic), 5.97 (d, 1 H, H-4'), 5.85 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2'), 5.85-5.75 (b, 1 H, NH), 5.58 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, H-3'), 5.12 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.65 (d, 1 H, $J_{1,2}$ 9.1 Hz, H-1), 4.64 (dd, 1 H, $J_{5',6a'}$ 5.0 Hz, H-6a'), 4.49 (dd, 1 H, $J_{5',6b'}$ 8.0 Hz, $J_{6a',6b'}$ 11.6 Hz, H-6b'), 4.15 (dd, 1H, H-5'), 4.10 (dd, 1 H, $J_{3,4}$ 8.9 Hz, H-4), 3.97 (dd, 1 H, $J_{2,3}$ 9.3 Hz, H-3), 3.80 (dd, 1 H, $J_{5,6a}$ 1.3 Hz, H-6a), 3.75 (dd, 1 H, $J_{5,6b}$ 2.2, $J_{6a,6b}$ 11.8 Hz, H-6b), 3.64 (ddd, 1 H, $J_{2,NH}$ 8.4 Hz, H-2), 3.35 (ddd, 1 H, $J_{4,5}$ 9.6 Hz, H-5), 2.02 (s, 3 H, NHAc), 0.98 (s, 9 H, $SiCMe_3$); ^{13}C NMR ($CDCl_3$) δ 170.7, 166.0, 165.4, 165.3, 165.0 (5 x C=O), 135.8-127.8 (36 C, Aromatic), 100.8 (C-1'), 87.8 (C-1), 78.2 (C-4), 76.5 (C-5), 72.4 (C-5'), 71.4 (2C, C-3, C-3'), 69.6 (C-2'), 68.1 (C-4'), 62.1 (C-6'), 61.1 (C-6), 56.2 (C-2), 26.7 ($SiC(\underline{C}H_3)_3$), 23.4 (NHAc), 19.3 ($Si\underline{C}Me_3$); FAB-MS (glycerol) gave m/z (ion, relative intensity): 1063.3 ($[M+1]^+$, 0.2 %), 1021.3 ($[M-N_3]^+$, 0.2%).
Anal. Calcd for $C_{58}H_{58}N_4O_{14}Si$: C, 65.52; H, 5.50; N, 5.27. Found: C, 65.22; H, 5.47; N, 5.10.

2,3,4-Tri-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-galactopyranosyl-(1→4)-2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide (21)

Compound **3** (100 mg, 0.21 mmol) was glycosylated with **14** (220 mg, 0.27 mmol) as described by method A to give **21** (203 mg, 82%): mp 108-110 °C; $[\alpha]_D$ +45.2° (*c* 1.0,

chloroform); ^{1}H NMR ($CDCl_3$) δ 7.99-7.10 (m, 35 H, aromatic), 6.03 (d, 1 H, H-4'), 5.73 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2'), 5.61 (d, 1 H, $J_{3,NH}$ 8.3 Hz, NH), 5.59 (dd, 1 H, $J_{3',4'}$ 3.3 Hz, H-3'), 5.06 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.70 (d, 1 H, $J_{1,2}$ 9.2 Hz, H-1), 4.07 (m, 1 H, H-5'), 4.04 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.94 (dd, 1 H, $J_{3,4}$ 8.4 Hz, H-3), 3.88 (dd, 1 H, $J_{5',6a'}$ 6.3, $J_{6a',6b'}$ 10.1 Hz, H-6a'), 3.82-3.76 (m, 2 H, H-6b', H-6a), 3.74 (dd, 1 H, $J_{5,6b}$ 2.3, $J_{6a,6b}$ 11.6 Hz, H-6b), 3.54 (ddd, 1 H, $J_{2,3}$ 9.4 Hz, H-2), 3.32 (m, 1 H, H-5), 1.98 (s, 3 H, NHAc), 1.11, 1.01 (2s, 18 H, 2 x $SiCMe_3$); ^{13}C NMR ($CDCl_3$) δ 170.6, 165.4, 165.3, 165.2 (4 x C=O), 135.9-127.7 (42 C, Aromatic), 100.8 (C-1'), 87.7 (C-1), 77.9 (C-4), 76.7 (C-5), 74.3 (C-5'), 71.6 (C-3'), 71.3 (C-3), 69.9 (C-2'), 67.6 (C-4'), 61.3 (C-6), 60.9 (C-6'), 56.4 (C-2), 27.0 ($SiC(\underline{C}H_3)_3$), 26.7 ($SiC(\underline{C}H_3)_3$), 23.5 (NHAc), 19.5 ($Si\underline{C}Me_3$), 19.0 ($Si\underline{C}Me_3$); FAB-MS (glycerol) gave m/z (ion, relative intensity): 1154.5 ($[M-HN_3]^+$, 3.2 %).

Anal. Calcd for $C_{67}H_{72}N_4O_{13}Si_2$: C, 67.20; H, 6.06; N, 4.68. Found: C, 66.46; H, 6.01; N, 4.71.

Allyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranoside (22)

Compound **5** (200 mg, 0.40 mmol) was glycosylated with **17** (296 mg, 0.60 mmol) as described in method B to give **22** (226 mg, 68%): mp 179-180 °C; $[\alpha]_D$ -3.1° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.70-7.30 (m, 10 H, aromatic), 6.06 (d, 1 H, $J_{2,NH}$ 8.2 Hz, NH), 5.90-5.83 (m, 1 H, $CH_2C\underline{H}=CH_2$), 5.32 (d, 1 H, $J_{3',4'}$ 3.4 Hz, H-4'), 5.21 (dd, 1 H, J_{gem} 1.5 Hz, J_{trans} 17.1 Hz, $CH_2CH=C\underline{H}_2$), 5.15-5.11 (m, 2 H, H-2', $CH_2CH=C\underline{H}_2$), 4.93 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, H-3'), 4.74 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.68 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.29 (dd, 1 H, *J* 5.1, *J* 12.9 Hz, $OC\underline{H}_2CH=$), 4.12 (d, 2 H, $J_{5',6'}$ 6.6 Hz, H-6a', H-6b'), 4.03 (dd, 1 H, $OC\underline{H}_2CH=$), 3.98 (dd, 1 H, $J_{3,4}$ 8.6 Hz, H-3), 3.92-3.82 (m, 2 H, H-5', H-6a), 3.80-3.78 (m, 2 H, H-4, H-6b), 3.53 (ddd, 1 H, $J_{2,3}$ 9.3 Hz, H-2), 3.40 (m, 1 H, H-5), 2.13, 2.03, 2.01, 1.96, 1.69 (5s, 15 H, 4 x OAc, NHAc), 1.05 (s, 9 H, $SiCMe_3$); ^{13}C NMR ($CDCl_3$) δ 170.6, 170.3, 170.0, 169.8, 169.0 (5 x C=O), 135.9-127.6 (12 C, aromatic), 133.9 ($-\underline{C}H=CH_2$), 117.2 ($-CH=\underline{C}H_2$), 100.9 (C-1'), 99.0 (C-1), 79.8 (C-4), 74.5 (C-5), 71.4 (C-3), 71.2 (C-5'), 70.7 (C-3'),69.1 ($-O\underline{C}H_2CH=$), 68.8 (C-2'), 66.9 (C-4'), 61.7 (C-6'), 61.1 (C-6), 56.4 (C-2), 26.8 ($SiC(\underline{C}H_3)_3$), 23.5 (NHAc), 20.6, 20.5, 20.4, 20.2 (4 x OAc), 19.3 ($Si\underline{C}Me_3$); FAB-MS (glycerol) gave m/z (ion, relative intensity): 830.4 ($[M+1]^+$, 0.7%).

Anal. Calcd for $C_{41}H_{55}NO_{15}Si$: C, 59.33; H, 6.88; N, 1.69. Found: C, 59.10; H, 6.64; N, 1.81.

General procedure for the acetylation of disaccharides 18-22.

Disaccharide (100 mg) was dissolved in pyridine (1 mL) and acetic anhydride (0.75 mL) was added. The reaction solution was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure. The crude product was purified by column chromatography using methanol-dichloromethane (3%, v/v) as eluent to give the 3-*O*-acetyl disaccharide quantitatively.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide (18a)

$[\alpha]_D$ -31° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.71-7.34 (m, 10 H, aromatic), 5.97 (d, 1 H, $J_{2,NH}$ 9.4 Hz, NH), 5.29 (d, 1 H, $J_{3',4'}$ 3.5 Hz, H-4'), 5.02-4.97 (m, 2 H, H-2', H-3), 4.88 (dd, 1 H, $J_{2',3'}$ 10.3 Hz, H-3'), 4.76 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.44 (d, 1 H, $J_{1,2}$ 9.2 Hz, H-1), 4.17 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-4), 4.13-4.05 (m, 3 H, H-2, H-6a', H-6b'), 3.95 (dd, 1 H, $J_{5,6a}$ 1.2 Hz, $J_{6a,6b}$ 11.6 Hz, H-6a), 3.87 (dd, 1 H, $J_{5,6b}$ 2.3 Hz, H-6b), 3.73 (dd, 1 H, $J_{5',6a'}$, $J_{5',6b'}$ 6.6 Hz, H-5'), 3.37 (ddd, 1 H, $J_{4,5}$ 9.5 Hz, H-5), 2.11, 2.05, 2.04, 1.97, 1.95, 1.74 (6 s, 18 H, 5 x OAc, NHAc), 1.06 (s, 9 H, $SiCMe_3$).

2,3,4-Tri-*O*-benzoyl-6-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acteyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide (19a)

$[\alpha]_D$ +20.2° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 8.10-6.63 (m, 30 H, aromatic), 6.86 (d, 1 H, $J_{2,NH}$ 9.7 Hz, NH), 5.92 (d, 1 H, $J_{3',4'}$ 3.3 Hz, H-4'), 5.62 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, H-2'), 5.32 (dd, 1 H, H-3'), 5.13 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-3), 5.07 (d, 1H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.72 (d, 1 H, $J_{1,2}$ 9.3 Hz, H-1), 4.53 (AB pattern, 1 H, *J* 11.8 Hz, H-A of CH_2Ph), 4.47 (AB pattern, 1 H, H-B of CH_2Ph), 4.36 (dd, 1 H, $J_{4,5}$ 9.4 Hz, H-4), 4.24 (ddd, 1 H, H-2), 4.01 (dd, 1 H, $J_{5',6a'}$, $J_{5',6b'}$ 6.6 Hz, H-5'), 3.70-3.56 (m, 4 H, H-6a, H-6b, H-6a', H-6b'), 2.95 (ddd, 1 H, $J_{5,6a}$ < 1, $J_{5,6b}$ < 1 Hz, $J_{4,5}$ 9.7 Hz, H-5), 2.03, 2.00 (2s, 6 H, OAc, NHAc), 1.11 (s, 9 H, $SiCMe_3$).

2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acteyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide (20a)

$[\alpha]_D$ +24.4° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 8.12-6.64 (m, 30 H, aromatic), 6.78 (d, 1 H, $J_{2,NH}$ 9.5 Hz, NH), 5.91 (d, 1 H, $J_{3',4'}$ 3.4 Hz, H-4'), 5.71 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, H-2'),), 5.38 (dd, 1 H, H-3'), 5.20 (dd, 1 H, $J_{2,3}$ 10.5 Hz, H-3), 5.12 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.72 (d, 1 H, $J_{1,2}$ 9.4 Hz, H-1), 4.64 (dd, 1 H, $J_{5',6a'}$ 5.1, $J_{6a',6b'}$ 11.6 Hz, H-6a'), 4.39-4.34 (m, 2 H, H-4, H-6b'), 4.23 (ddd, 1 H, H-2), 4.16 (dd, 1 H, $J_{5',6b'}$ 8.1 Hz, H-5'), 3.63 (dd, 1 H, $J_{5,6a} < 1$, $J_{6a,6b}$ 11.3 Hz, H-6a), 3.53 (dd, 1 H, $J_{5,6b}$ 1.6 Hz, H-6b), 2.99 (ddd, 1 H, $J_{4,5}$ 9.7 Hz, H-5), 2.12, 2.03 (2s, 6 H, OAc, NHAc), 0.97 (s, 9 H, $SiCMe_3$).

2,3,4-Tri-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acteyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide (21a)

$[\alpha]_D$ +26.2° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.98-6.88 (m, 35 H, aromatic), 6.07 (d, 1 H, $J_{2,NH}$ 8.1 Hz, NH), 6.06 (d, 1 H, H-4'), 5.57 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, H-2'), 5.46 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3'), 5.03 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.98 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3), 4.46 (d, 1 H, $J_{1,2}$ 9.2 Hz, H-1), 4.25 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 4.06 (ddd, 1 H, $J_{2,3}$ 10.1 Hz, H-2), 3.96 (dd, 1 H, $J_{5',6a'}$ 5.5, $J_{5',6b'}$ 8.8 Hz, H-5'), 3.78 (dd, 1 H, $J_{6a',6b'}$ 9.7 Hz, H-6a'), 3.70 (dd, 1 H, $J_{5,6a}$ 1.4 Hz, $J_{6a,6b}$ 12.0 Hz, H-6a), 3.64-3.60 (m, 2 H, H-6b', H-6b), 3.06 (m, 1 H, H-5), 1.96, 1,76 (2s, 6 H, OAc, NHAc), 1.11, 0.99 (2s, 18 H, 2 x $SiCMe_3$).

Allyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranoside (22a)

$[\alpha]_D$ -16.2° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.74-7.34 (m, 10 H, aromatic), 5.87-5.80 (m, 1 H, $C\underline{H}=CH_2$), 5.59 (d, 1 H, $J_{2,NH}$ 9.4 Hz, NH), 5.30 (d, 1 H, $J_{3',4'}$ 3.2 Hz, H-4'), 5.23 (dd, 1 H, J_{gem} 1.5 Hz, J_{trans} 17.3 Hz, -CH=C$\underline{H}_2$), 5.15 (dd, 1 H, J_{cis} 11.4 Hz, -CH=C$\underline{H}_2$), 5.04 (dd, 1 H, $J_{2',3'}$ 10.3 Hz, H-2'), 4.99 (dd, 1 H, $J_{2,3}$ 9.7 Hz, $J_{3,4}$ 8.8 Hz, H-3), 4.92 (dd, 1 H, H-3'), 4.74 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.41 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.41-4.05 (m, 4 H, H-2, H-4, H-6a', H-6b'), 3.94 (dd, 1 H, $J_{5,6a}$ 2.3, $J_{6a,6b}$ 11.8 Hz, H-6a), 3.88 (dd, 1 H, $J_{5,6b}$ 0.8 Hz, H-6b), 3.75 (dd, 1 H, $J_{5',6a'}$, $J_{5',6b'}$ 6.7 Hz, H-5'), 3.31 (ddd, 1 H, $J_{4,5}$ 8.7 Hz, H-5), 2.12, 2.04, 2.03, 1.96, 1.96, 1.78 (6s, 18 H, 5 x OAc, NHAc), 1.06 (s, 9 H, $SiCMe_3$).

2,3,4-Tri-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)]-2-acetamido-6-*O*-*tert*-butyl-diphenylsilyl-2-deoxy-β-D-glucopyranosyl azide (24)

To a solution of **21** (100 mg, 0.084mmol) and ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-fucopyranoside **23**[113] (60 mg, 0.125 mmol) in 5 mL of 5:1 benzene/dichloromethane was added powdered molecular sieves 4Å (100 mg). The mixture was stirred for 2 h at room temperature under nitrogen. DMTST (70 mg, 0.342 mmol) was added to the stirred mixture at 0 °C. After 1 h, TLC (2% MeOH in CH_2Cl_2) showed complete conversion of the donor. Then triethylamine (100 μL) and methanol (200 μL) were added to the reaction mixture which was stirred for an additional 25 min. The reaction mixture was filtered through celite, and the residue was washed with dichloromethane. The combined filtrate and washings were successively washed with saturated sodium bicarbonate solution, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by silica gel chromatography using methanol-dichloromethane as eluent (1:30, v/v) to afford trisaccharide **24** (105 mg, 78%): $[\alpha]_D$ -15.1° (*c* 1.0, chloroform); ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.04-6.82 (m, 50 H, aromatic H), 6.13 (dd, 1 H, $J_{4',5'}$ 0.9 Hz, H-4'), 5.95 (d, 1 H, $J_{2,NH}$ 7.9 Hz, NH), 5.65 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2'), 5.59 (dd, 1 H, $J_{3',4'}$ 2.4 Hz, H-3'), 5.25 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1''), 5.09 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.91-4.50 (m, 4 H, 2 x CH_2Ph), 4.42 (s, 2 H, CH_2Ph), 4.28 (m, 2 H, $J_{1,2}$ 9.0 Hz, H-1, H-5''), 4.21 (dd, 1 H, $J_{4,5}$ 8.0 Hz, H-4), 4.08 (dd, 1 H, $J_{2'',3''}$ 10.2 Hz, H-2''), 4.02-3.95 (m, 2 H, H-5', H-3), 3.90 (dd, 1 H, $J_{5,6a}$ 2.4 Hz, $J_{6a,6b}$ 11.7 Hz, H-6a), 3.86-3.84 (m, 2 H, H-6a', H-6b'), 3.85-3.75 (m, 2 H, H-6b, H-3''), 3.64 (dd, 1 H, $J_{2,3}$ 9.0 Hz, H-2), 3.61 (d, 1 H, $J_{4'',5''}$ < 1.0 Hz, H-4''), 3.04 (m, 1 H, H-5), 1.76 (s, 3 H, NHAc), 1.12 (s, 9 H, $SiCMe_3$), 1.07 (d, 3 H, $J_{5'',6''}$ 6.6 Hz, H-6''), 0.95 (s, 9 H, $SiCMe_3$); ^{13}CNMR ($CDCl_3$) δ (ppm): 170.3, 165.5, 165.3, 165.2 (4 x C=O), 138.9-127.2 (60 C, aromatic), 99.6 (C-1'), 97.4 (C-1''), 88.1 (C-1), 79.8 (C-3''), 78.3 (C-4''), 77.5 (2C, C-5, C-2''), 75.3 (CH_2Ph), 75.1 (C-5'), 74.2 (CH_2Ph), 73.7 (C-4), 73.5 (C-3), 72.2 (CH_2Ph), 71.6 (C-3'), 70.3 (C-2'), 67.5 (C-4), 67.0 (C-5''), 61.5 (C-6), 60.3 (C-6'), 55.2 (C-2), 26.9 ($SiC(\underline{C}H_3)_3$), 26.6 ($SiC(\underline{C}H_3)_3$), 23.2 (NHAc), 19.3 ($Si\underline{C}Me_3$), 18.9 ($Si\underline{C}Me_3$), 16.9 (C-6''); FAB-MS (glycerol) gave m/z (ion, relative intensity): 1571.7 ($[M+1-N_3]^+$, 4.8 %).

[113] Lönn, H. *Carbohydr. Res.* **1985**, *139*, 105

Anal. Calcd for $C_{94}H_{100}N_4O_{17}Si_2$: C, 69.95; H, 6.24; N, 3.47. Found: C, 69.84; H, 6.27; N, 3.71.

Chapter 3 Active-latent glycosylation strategy toward Lewis X pentasaccharide synthesis

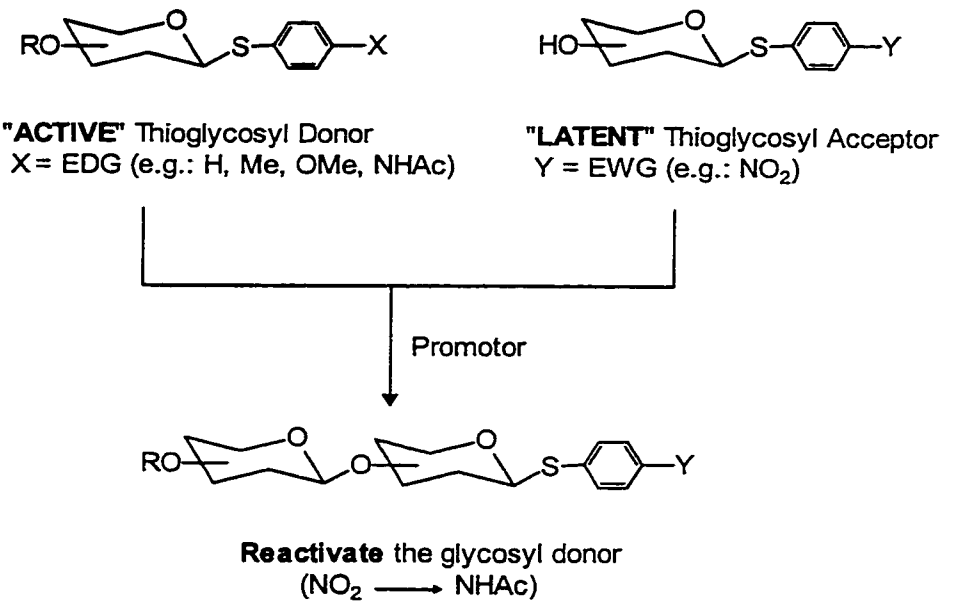
3.1 Introduction

The importance of cell surface carbohydrates in biological processes such as antibody-antigen interaction, cell-cell recognition, cellular-immune response, and cell oncogenic transformation and inflammation has led to a great deal of activities in glycobiology. As glycobiology moves forward, the need for efficient methods to prepare oligosaccharides available for further investigations becomes more urgent.

Earlier studies from our laboratory demonstrated that "active-latent" *para*-substituted phenyl 1-thioglycosides could be used efficiently as glycosyl donors or acceptors depending on the electron density of the aryl substituents.[32,114,115,33] Thus, *p*-nitrophenyl thioglycosides are inert (latent) towards thiophilic promoters, but the electron withdrawing *p*-nitro-substituent can be "turned on" into an electron donating *N*-acetyl group which is active with suitable thiophilic promoters. The glycosylation of this "active" thioglycoside with a "latent" *p*-nitrophenyl thioglycoside should be possible under suitable conditions (Scheme **3.1**). The "active-latent" glycosylation strategy was also exploited with aryl sulfoxides,[116] allyl *vs.* vinyl glycosides,[34,117] and bulky alkyl thioglycosides[118,119] by other groups. In order to further study the usefulness of "active-latent" glycosylation strategy, we choose the synthesis of Lewis X pentasaccharide[120] which is widely distributed in many different human and animal tissues, and also in human milk oligosaccharides.

---

[114] Cao, S.; Meunier, S. J.; Andersson, F. O.; Letellier, M.; Roy, R. *Tetrahedron: Asymmetry* **1994**, *5*, 2303;
[115] Cao S.; Roy, R. *Tetrahedron Lett.* **1996**, *37*, 3421.
[116] Raghavan, S.; Kahne, D. *J. Am. Chem. Soc.* **1993**, *115*, 1580.
[117] Boons, G.-J.; Isles, S. *J. Org. Chem.* **1996**, *61*, 4262.
[118] Boons, G.-J.; Geurtsen, R.; Holmes, D. *Terahedron Lett.* **1995**, *36*, 6325.
[119] Geurtsen, R.; Holmes, D.; Boons, G.-J. *J. Org. Chem.* **1997**, *62*, 8145.
[120] For previous syntheses of the Le^x epitopes, see the following: a) Sato, S.; Ito, Y.; Nukada, T.; Ogawa, T. *Carbohydr. Res.* **1987**, *167*, 197; b) Nicolaou, K. C.; Caulfield, T. J.; Kataoka, H.; Stylianides, N. A. *J. Am.*



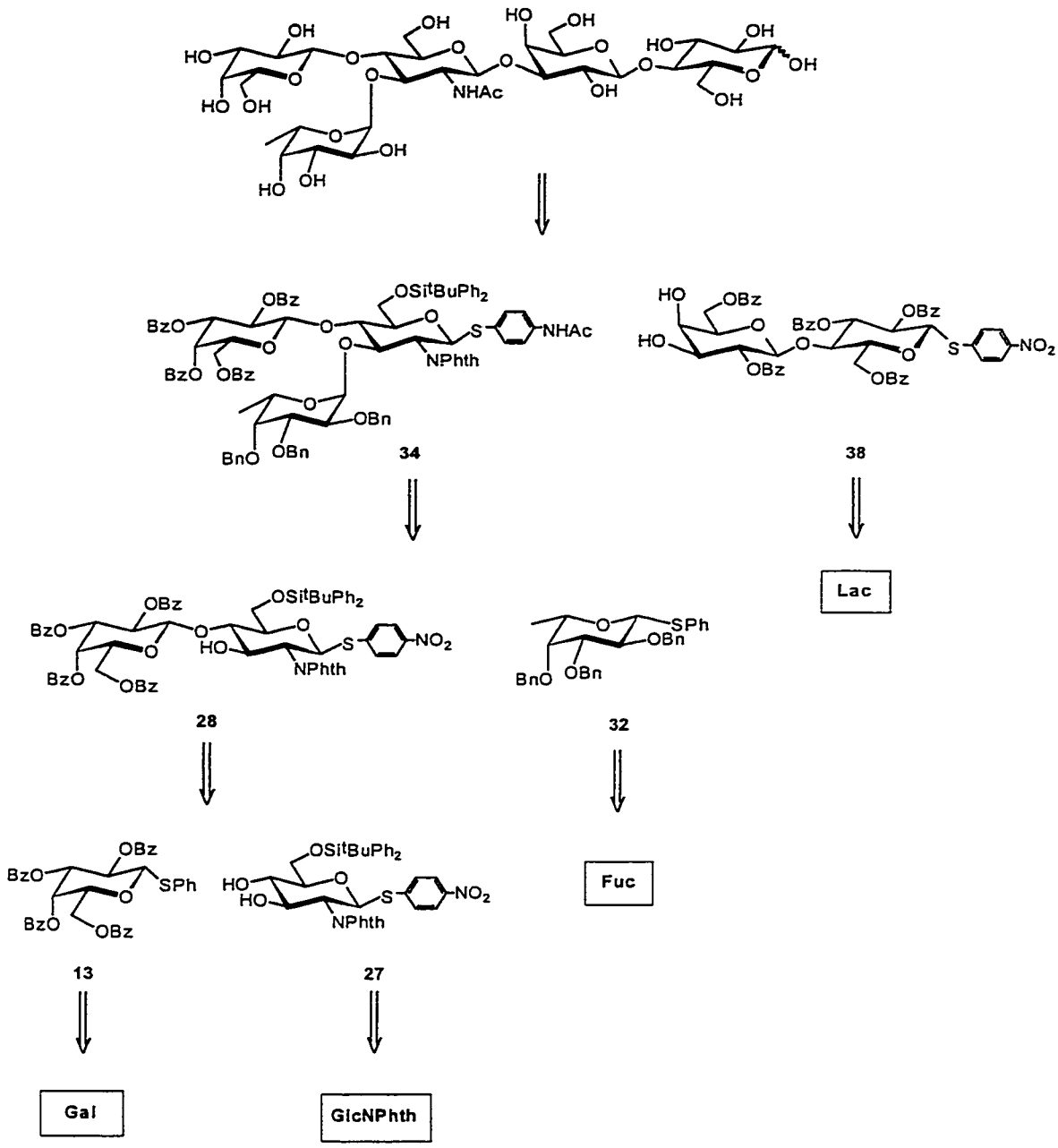
Scheme 3.1 "Active-latent" glycosylation strategy

3.2 Results and discussion

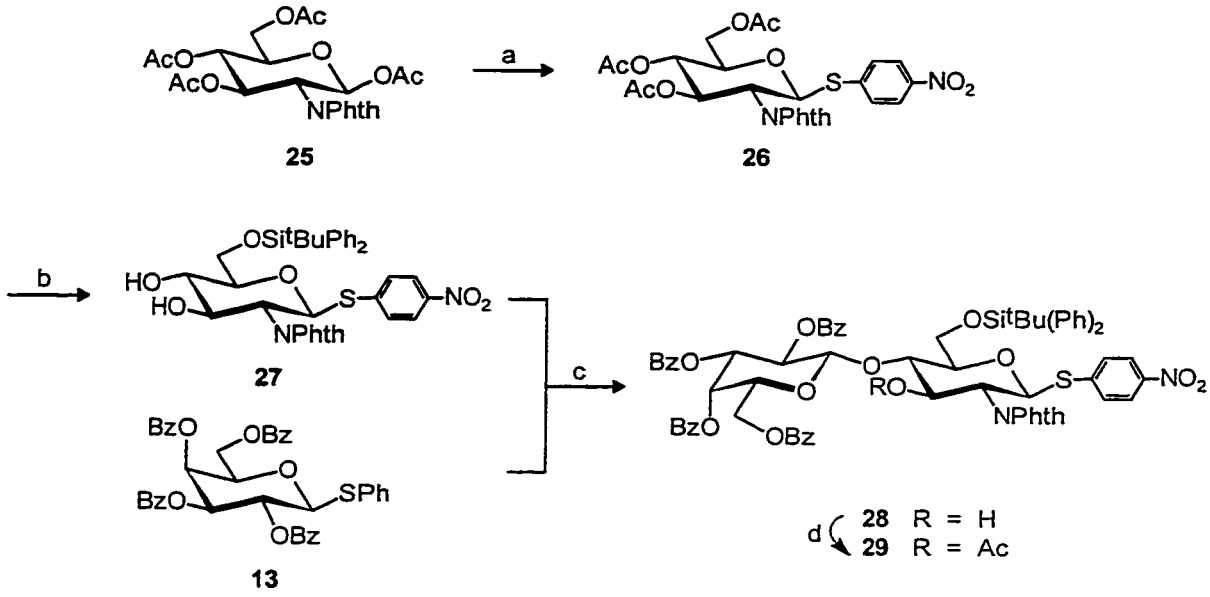
Retrosynthetic analysis of a suitable process to Le^x pentasaccharide led us to design the putative Le^x trisaccharide donor **34** that could be coupled with lactosyl acceptor **38**. The donor **34** and acceptor **38** were expected to be constructed from synthons derived from D-galactose, 2-amino-2-deoxy-D-glucose, L-fucose, and lactose, respectively (Scheme **3.2**). The efficiency of the *tert*-butyldiphenylsilyl auxiliary group at *O*-6 of **27** was established in previous studies.[121] Utilization of this silyl group can regiospecifically control the formation of 1→4 linked key intermediate disaccharide **28**.

Soc. Chem. 1990, 112, 3693; c) Lay, L.; Manzoni, L.; Schmidt, R. R. *Carbohydr. Res.* **1998**, *310*, 157; d) Manzoni, L.; Lay, L.; Schmidt, R. R. *J. Carbohydr. Chem.*, **1998**, *17*, 739 and ref. cited therein.

[121] See chapter 2.



Scheme 3.2 Retrosynthetic analysis of Lewis X pentasaccharide



Scheme 3.3 Synthesis of disaccharide **28**

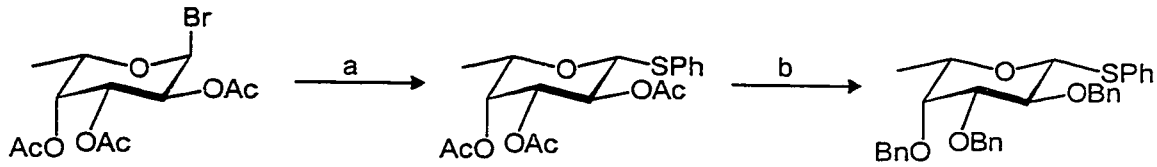
Reagents and conditions: (a) 4-nitrothiophenol, $SnCl_4$, 6 h, 91 %; (b) i: NaOMe/MeOH, ii: TBDPSCl, pyridine, 82%; (c) NIS/TfOH, CH_2Cl_2, -30 °C, 74%; (d) Ac_2O/pyridine

Tetra-*O*-acetyl-*N*-phthalimido protected glucosamine **25**[122] was treated with 4-nitrothiophenol in the presence of $SnCl_4$ to afford β-thioglycoside **26** in 91 % yield. Zemplén deacetylation of **26** and selective silylation of OH-6 with *tert*-butylchlorodiphenylsilane in pyridine gave 3,4-*O*-unprotected *p*-nitrophenyl acceptor **27** in 82% yield. Condensation of thiophenyl donor **13** with 6-*O*-*tert*-butyldiphenylsilyl acceptor **27** was performed using *N*-iodosuccinimide and trifluoromethanesulfonic acid as promoter in dichloromethane at -30 °C. The reaction afforded exclusively the desired β-(1→4)-linked disaccharide **28** in 74% yield (Scheme **3.3**). No (1→3)-linked regioisomer was detected during the coupling reaction. The β-configuration of the disaccharide **28** was deduced from the ^{1}H NMR spectrum which showed H-1' as a doublet at δ 5.09 ppm ($J_{1',2'}$ 8.1 Hz). The regiochemistry of the newly introduced glycosidic linkage of compound **28** was proved by its ^{13}C NMR spectrum which showed a downfield-shift signal for C-4 at δ 79.0 ppm (Δδ= + 7.1 ppm) for disaccharide **28** compared to δ 71.9 ppm for C-4 of the glucosamine acceptor **27**, and by converting

compound **28** into its corresponding 3-*O*-acetate **29** which showed H-3 at δ 5.87 ppm (Δδ = +1.2 ppm) as a downfield-shift signal in its ^{1}H-NMR spectrum.

It seemed that the bulky 6-*O*-*tert*-butyldiphenylsilyl protecting group in acceptor **27** played a key role for the regiospecificity. Under such reaction conditions, the very bulky protecting group could cover the top side (3,5-cis) of the OH-3 of the acceptor **27** and block the glycosyl donor to approach the OH-3. This strategy, coupled with the steric hindrance imparted by the neighboring 2-phthalimido group on OH-3, concurred to the total regiospecificity of the glycosylation.[121] Previous studies[89,90,91,92] have shown that the 2-phthalimido group alone could not be entirely responsible for the selectivity/specificity observed.

Fucosyl donor **32** was synthesized from acetobromofucopyranose **30**. Treatment of **30** with thiophenol under PTC conditions afforded β-thiophenyl fucoside **31** in excellent yield (92%). The β configuration of **31** was assigned from its ^{1}H NMR spectrum ($J_{1,2}$ 10.0 Hz). Removal of all *O*-acetyl groups could be performed under Zemplén conditions at room temperature; then reaction with sodium hydride followed by benzyl bromide gave donor **32**[123] in 87 % yield (Scheme **3.4**).



30 **31** **32**

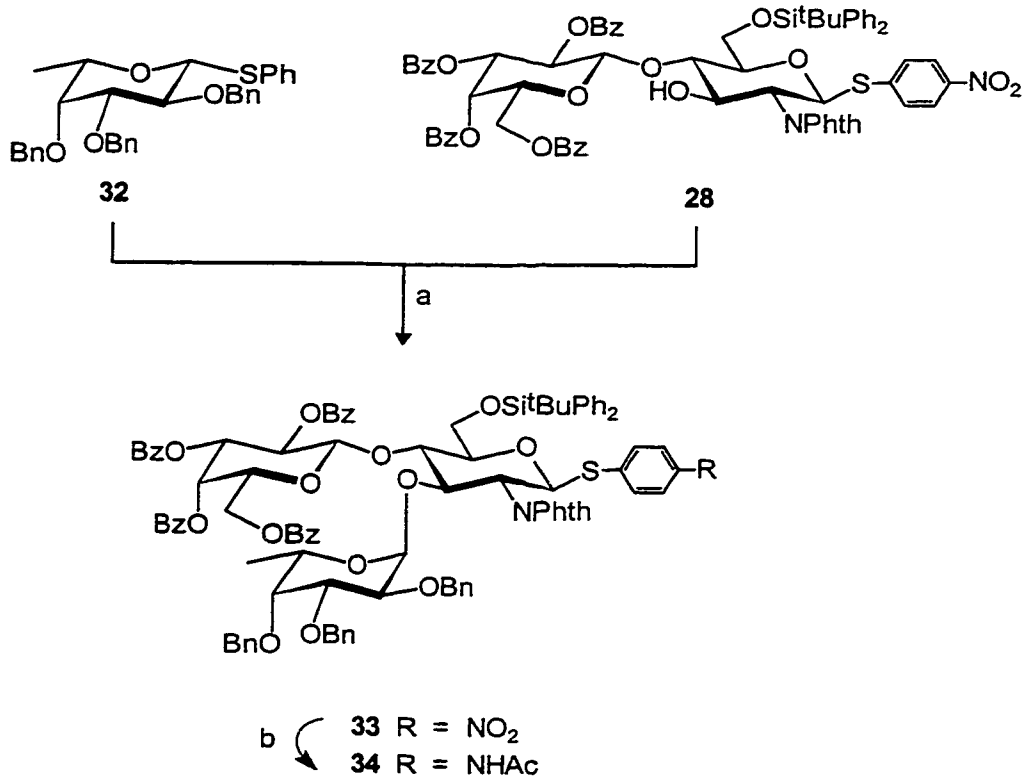
Scheme 3.4 Preparation of fucose donor **32**

Reagents and conditions: (a) thiophenol, TBAHS, EtOAc, 1 M Na_2CO_3, 1h, 92 %; (b) i: NaOMe/MeOH; ii: NaH, BnBr, 3h, 87 %.

Further 3-*O*-fucosylation of disaccharide **28** with fucosyl donor **32** using DMTST as promoter at 0 °C afforded Le^x trisaccharide **33** in good yield (72%). The α configuration of the newly introduced anomeric center in compound **33** was assigned from its ^{1}H NMR spectrum ($J_{1'',2''}$ 3.6 Hz). The *p*-nitrothiophenyl group of **33** was then reduced with zinc in acetic acid and

[122] Baker, B. R.; Joseph, J. P.; Schaub, R. E.; Williams, J. H. *J. Org. Chem.* **1954**, *19*, 1786.

the resulting amine was *N*-acetylated to give the active *p*-acetamidothiophenyl donor **34** in 84% yield (Scheme **3.5**).



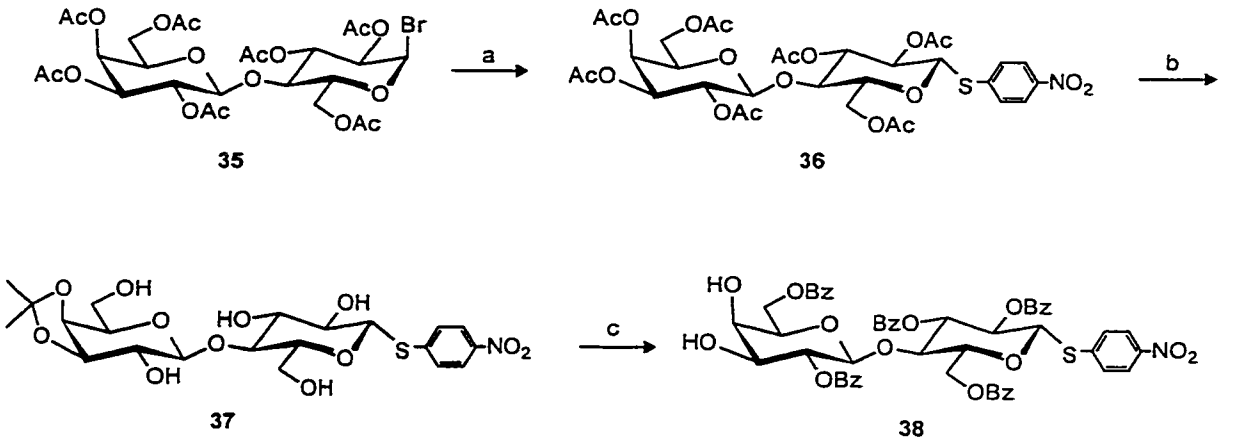
Scheme 3.5 Synthesis of Le^x trisaccharide donor **34**

Reagents and conditions: (a) DMTST, C_6H_6-CH_2Cl_2, 0 °C; 72%; (b) i: Zn/AcOH, ii: Ac_2O/pyridine, 84%.

4-Nitrophenyl hepta-*O*-acetyl-1-thio-β-lactoside **36** was conveniently prepared on a large scale using PTC methods. Catalytic deacetylation (NaOMe) of **36** and treatment with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid afforded 4-nitrophenyl 3',4'-*O*-isopropylidene-1-thio-β-D-lactopyranoside **37** in 80% yield. Sequential benzoylation and hydrolysis using cat. *p*-TsOH gave the latent diol lactoside acceptor **38** in good yield (92%) (Scheme **3.6**).

---

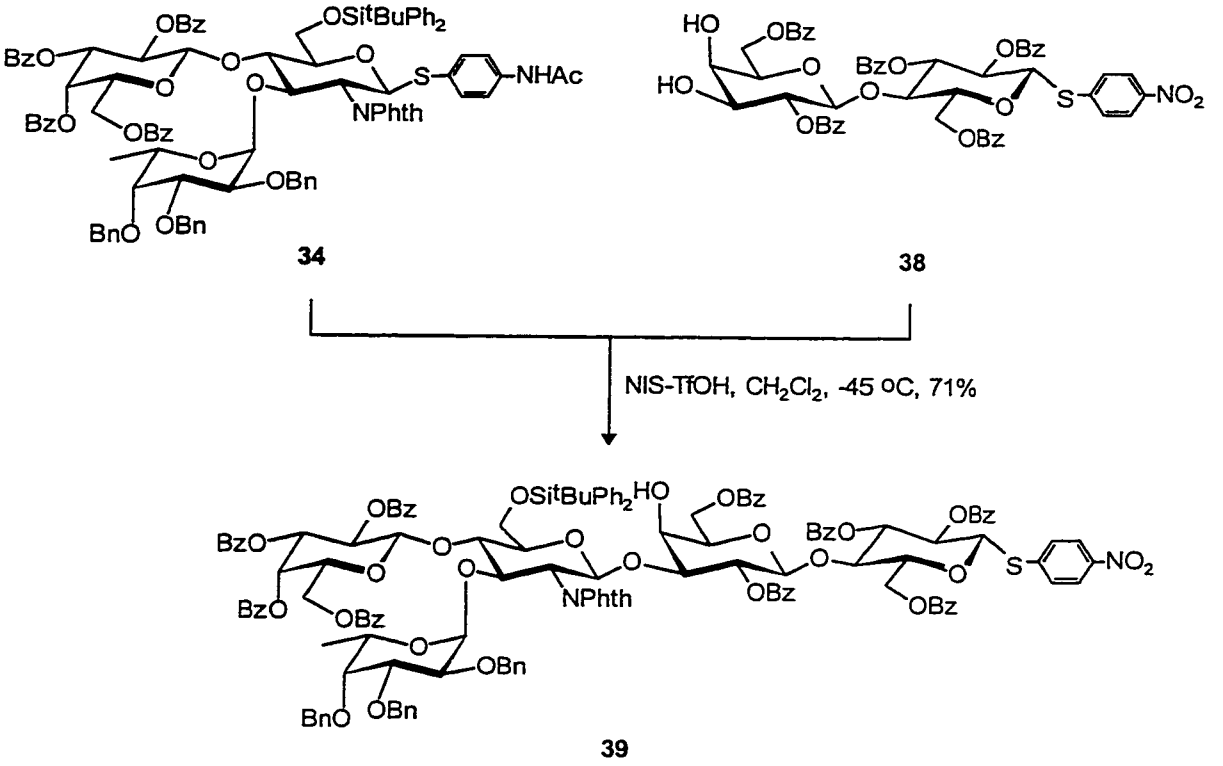
[123] Komba, S.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1996**, *285*, C1.



Scheme 3.6 Synthesis of lactose acceptor **38**

Reagents and conditions: (a) 4-nitrothiophenol, TBAHS, EtOAc, 1 M Na_2CO_3, 3.5 h, 90 %; (b) 2,2-dimethoxypropane, TsOH, 80 %; (c) i: BzCl/pyridine, ii: *p*-TsOH, 40 °C, 92%.

Condensation of *p*-acetamidothiophenyl donor **34** with the *p*-nitrothiophenyl acceptor **38** was performed using NIS and TfOH as promoters at -45 °C. The reaction afforded β-(1→3)-linked pentasaccharide **39** in 71% yield (Scheme **3.7**). The stereochemistry of the newly introduced β-anomeric center in compound **39** was assigned from the ^{1}H NMR spectrum which showed a doublet for H-1" at δ 5.15 ppm ($J_{1",2"}$ 8.5 Hz)(Figure **3.1**). The ^{1}H-NMR spectrum of **39** was less informative to confirm the regiochemistry of the newly introduced glycosidic linkage. However, the regioselectivity could be confirmed from the ^{13}C NMR spectrum which showed a downfield-shift signal for C-3' at δ 80.1 ppm (Δδ = +7.5 ppm) for pentasaccharide **39** compared to δ 72.6 ppm for C-3' of the lactose acceptor **38**.



Scheme 3.7 Synthesis of Le^x pentasaccharide **39**

3.3 Conclusion

In conclusion, the "active-latent" glycosylation strategy made it possible to manipulate the reactivity of both glycosyl donors and acceptors by means of changing the electron density of the aryl substituents at the anomeric center. This strategy provides a facile approach toward the synthesis of complex oligosaccharides in a highly convergent manner.

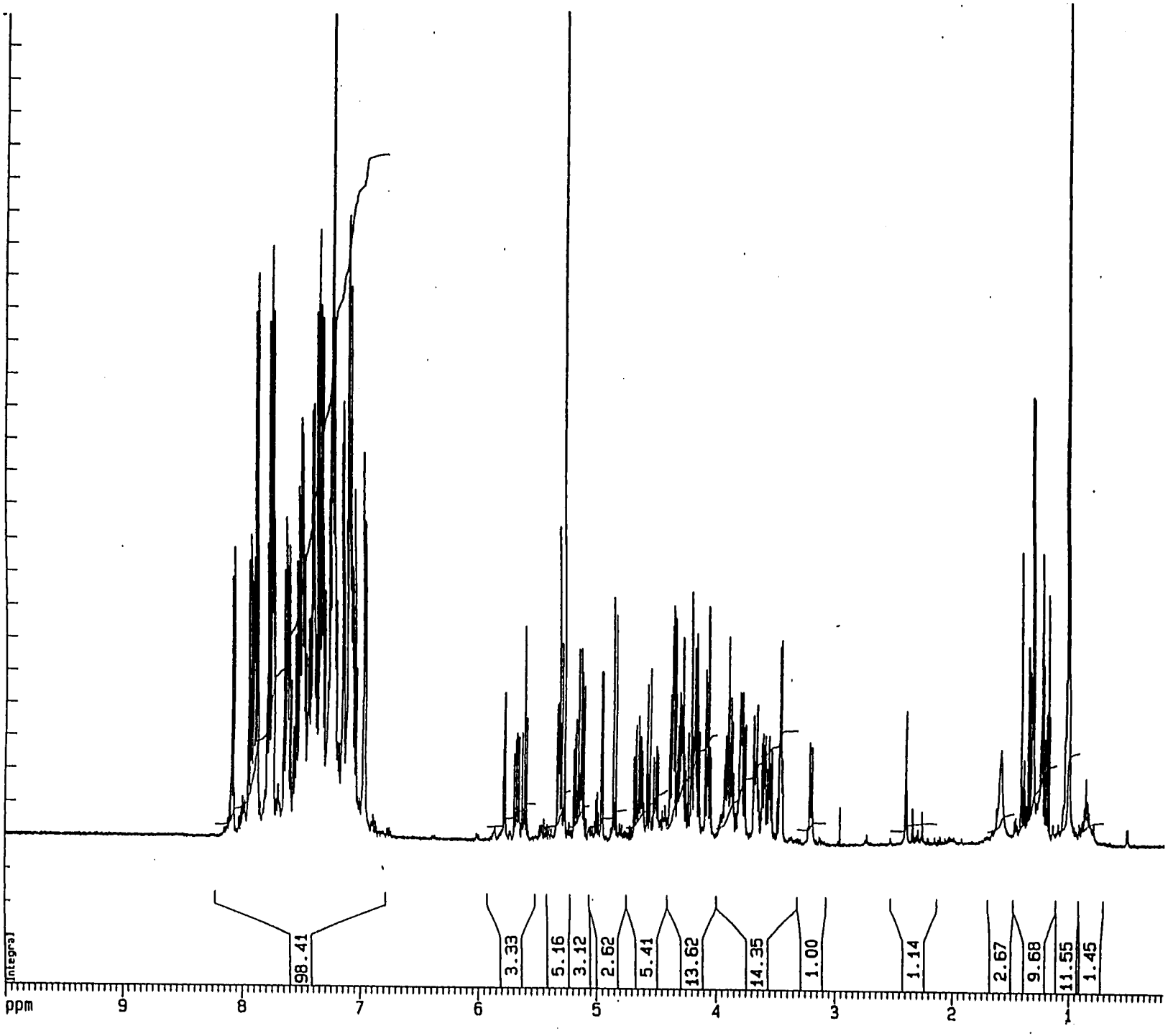


Figure 3.1 ^{1}H NMR (500MHz, $CDCl_3$) spectrum of Le^x pentasaccharide **39**

3.4 Experimental

4-Nitrophenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (26)

Tin (IV) chloride (1 mL) was added to a stirred mixture of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose **25** (1 g, 2.09 mmol), 4-nitrophenylthiol (0.65 g, 4.19 mmol), and ground molecular sieves (4 Å, 2 g) in dichloromethane (30 mL) at 0 °C. The mixture was stirred under nitrogen at room temperature for 6 h. The mixture was filtered through celite, and the filtrate was washed with ice-cold 1 M sulfuric acid, aqueous sodium bicarbonate, and water. The organic phase was dried over Na_2SO_4, evaporated under reduced pressure, and coevaporated with toluene. The residue was crystallized from dichloromethane-ethyl ether. Compound **26** (1.09 g) was obtained in 91 % yield: m.p. 217.2-217.5 °C; $[\alpha]_D$ +65.7° (*c* 0.68, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.11-7.48 (m, 8 H, aromatic H), 5.85 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1), 5.81 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3), 5.13 (dd, 1 H, $J_{4,5}$ 10.2 Hz, H-4), 4.39 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-2), 4.29 (dd, 1 H, $J_{5,6a}$ 5.2, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.20 (dd, 1 H, $J_{5,6b}$ 2.3 Hz, H-6b), 3.98 (ddd, 1 H, H-5), 2.10, 2.02, 1.82 (s, 9 H, OAc's); ^{13}C NMR ($CDCl_3$): δ 170.4, 170.0, 169.4, 168.4, 167.7 (C=O's), 147.0-123.8 (aromatic C), 81.9 (C-1), 76.2 (C-5), 71.3 (C-3), 68.4 (C-4), 62.1 (C-6), 53.3 (C-2), 20.9, 20.6,20.4 (OAc's); CI-MS (ether) gave m/z (ion, relative intensity): 420 ($[M+1-SPhNO_2]^+$, 2.3%).

4-Nitrophenyl 6-*O*-*tert*-butyldiphenylsilyl-2-phthalimido-2-deoxy-1-thio-β-D-glucopyranoside (27)

To a suspension of compound **26** (4.0 g, 6.99 mmol) in methanol (100 mL), sodium methoxide in methanol (0.3 mL, 1.0 M) was added. After stirring for 2 h at room temperature, the reaction was quenched with H^+ resin (Dowex 50w-X8) and then filtered. The filtrate was evaporated under reduced pressure. Toluene was added and distilled from the residue. The crude product was then dissolved in dry pyridine (20 mL), *tert*-butylchlorodiphenylsilane (2.1 mL, 8.20 mmol) was added in one portion. The mixture was stirred for 3 h at room temperature and poured into ice water, extracted with dichloromethane, and the extract was washed with saturated aq solution of $NaHCO_3$. The

organic extract was dried (Na_2SO_4) and concentrated. The crude product was subjected to column chromatography (methanol-methylene chloride, 1:10) to give pure **27** as a foamy solid (3.93 g, 82%): $[\alpha]_D$ +37.8° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.87-7.18 (m, 18 H, aromatic H), 5.75 (d, 1 H, $J_{1,2}$ 10.3 Hz, H-1), 4.40 (dd, 1 H, $J_{3,4}$ 8.3 Hz, H-3), 4.23 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 4.05 (dd, 1 H, $J_{5,6a}$ 2.7, $J_{6a,6b}$ 10.3 Hz, H-6a), 3.94 (dd, 1 H, $J_{5,6a}$ 3.0 Hz, H-6b), 3.80-3.68 (m, 2 H, H-4, H-5), 3.45-3.25 (b, 2 H, OH) 1.06 (s, 9 H, SiC$\underline{Me}_3$); ^{13}C NMR ($CDCl_3$): δ 168.2, 167.7 (2 x C=O), 148.8-123.3 (24 C, aromatic), 81.9 (C-1), 80.2 (C-5), 72.5 (C-3), 71.9 (C-4), 63.9 (C-6), 55.1 (C-2), 26.7 (SiC$\underline{Me}_3$), 19.2 (Si$\underline{C}Me_3$); FAB-MS (glycerol) gave m/z (ion, relative intensity): 530.3 ($[M-SPhNO_2]^+$, 0.9%).

Anal. Calcd for $C_{36}H_{36}N_2O_8SSi$: C, 63.14; H, 5.30; N, 4.09. found: C, 63.09; H, 5.32; N, 4.13.

4-Nitrophenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-6-*O*-tert-butyldiphenylsilyl-2-phthalimido-2-deoxy-1-thio-β-D-glucopyranoside (28)

To compound **27** (500 mg, 0.73 mmol) and phenyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside **13** (720 mg, 1.05 mmol) dissolved in dry dichloromethane (10 mL) under nitrogen, powdered molecular sieves 4 Å (1.2 g) was added. The mixture was stirred at room temperature for 2 h, and then cooled to -30 °C. *N*-Iodosuccinimide (330 mg, 1.46 mmol) and trifluoromethanesulfonic acid (40 μL, 0.44 mmol) were added. After stirring at -30 °C for 55 min, the mixture was diluted with dichloromethane (20 mL) and filtered through celite. The filtrate was washed with 10% aqueous sodium thiosulfate (30 mL), saturated aq solution of $NaHCO_3$ (2x30 mL), and brine (30 mL). The solution was dried (Na_2SO_4) and concentrated. Column chromatography (2% methanol in methylene chloride) of the residue on silica gel afforded disaccharide **28** (681 mg, 74%): $[\alpha]_D$+114.5° (*c* 1.0, $CHCl_3$); ^1H NMR (500 Hz, $CDCl_3$): δ 8.09-7.13 (m, 38 H, aromatic H), 5.95 (d, 1 H, H-4'), 5.84 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2'), 5.71 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1), 5.57 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3'), 5.09 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.69 (dd, 1 H, $J_{5',6a'}$ 4.2, $J_{6a',6b'}$ 12.5 Hz, H-6a'), 4.67 (dd, 1 H, $J_{3,4}$ 8.9 Hz, H-3), 4.36 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 4.34 (dd, 1 H, $J_{5',6b'}$ 4.7 Hz, H-6b'), 4.30 (dd, 1 H, H-5'), 4.15 (dd ,1 H, $J_{4,5}$ 9.2 Hz, H-4), 3.85 (dd, 1 H, $J_{5,6a}$ 1.4, $J_{6a',6b'}$ 11.8 Hz, H-6a), 3.79 (dd, 1 H, $J_{5,6b}$ 2.5 Hz, H-6b), 3.57 (ddd, 1 H, H-5), 0.95 (s, 9 H, SiC$\underline{Me}_3$); ^{13}C NMR ($CDCl_3$): δ 168.2, 167.2, 166.1, 165.4, 165.3, 165.0 (6 x C=O), 146.5-123.4 (48 C, aromatic), 101.0 (C-1'), 81.9

(C-1), 79.0 (C-4), 78.7 (C-5), 72.5 (C-5'), 71.4 (C-3'), 70.2 (C-3), 69.6 (C-2'), 68.1 (C-4'), 62.6 (C-6'), 61.5 (C-6), 55.0 (C-2), 26.8 (SiC<u>Me</u>$_3$), 19.4 (Si<u>C</u>Me$_3$); FAB-MS (glycerol) gave m/z (ion, relative intensity): 1108.4 ([M-SPhNO$_2$]$^+$, 0.5 %).

Anal. Calcd for $C_{70}H_{62}N_2O_{17}SSi$: C, 66.55; H, 4.95; N, 2.22. Found: C, 66.61; H, 4.81; N, 2.19.

4-Nitrophenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-tert-butyldiphenylsilyl-2-phthalimido-2-deoxy-1-thio-β-D-glucopyranoside (29)

Acetic anhydride (150 μL) was added to a solution of compound **28** (20 mg, 0.016 mmol) in dry pyridine (1 mL). The mixture was stirred at room temperature for 2 h. The solution was directly evaporated under reduced pressure to give compound **29** (21 mg) in quantitative yield: $[\alpha]_D$ +90.8° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 Hz, $CDCl_3$): δ 8.11-7.14 (m, 38 H, aromatic H), 5.93 (d, 1 H, H-4'), 5.87 (dd, 1 H, $J_{3,4}$ 9.1 Hz, H-3), 5.82 (d, 1 H, $J_{1,2}$ 10.5 Hz, H-1), 5.75 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, H-2'), 5.50 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3'), 5.20 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.60 (dd, 1 H, $J_{5',6a'}$ 5.3, $J_{6a',6b'}$ 11.5 Hz, H-6a'), 4.43(dd, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.40 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2), 4.39 (dd, 1 H, $J_{5',6b'}$ 7.8 Hz, H-6b'), 4.30 (m, 1 H, H-5'), 3.92 (s, 2 H, H-6a, H-6b), 3.53 (d, 1 H, H-5), 1.93 (s, 3 H, OAc), 1.03 (s, 9 H, SiC<u>Me</u>$_3$); ^{13}C NMR ($CDCl_3$): δ 170.1, 167.8, 167.0, 166.0, 165.4, 165.3, 164.6 (7 x C=O), 146.7-123.7 (48 C, aromatic), 101.2 (C-1'), 81.6 (C-1), 79.3 (C-5), 73.9 (C-4), 71.9 (C-3'), 71.5 (C-5'), 71.4 (C-3), 69.8 (C-2'), 68.1 (C-4'), 62.0 (C-6'), 60.9 (C-6), 53.6 (C-2), 26.8 (SiC<u>Me</u>$_3$), 20.6 (OAc), 19.4 (Si<u>C</u>Me$_3$).

Phenyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranoside (31)

To a solution of acetobromofucopyranose **30** (6 g, 17 mmol), TBAHS (8.5 g, 25.4 mmol), and thiophenyl (5.23 mL, 51 mmol) in ethyl acetate (70 mL) was added 1 M Na_2CO_3 (70 mL). The two-phase reaction mixture was vogorously stirred at room temperature for 1 h. EtOAc was added, and the organic phase was separated and successively washed with sat. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, evaporated under reduced pressure. The residue was purified by silica gel chromatography using EtOAc-hexane (1:2, v/v) as eluent to provide product **31** (5.97 g, 92 %): $[\alpha]_D$ -9.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR

($CDCl_3$): δ 7.49-7.27 (m, 5 H, aromatic), 5.24 (dd, 1 H, $J_{4,5}$ 1.0 Hz, H-4), 5.20 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 5.03 (dd, 1 H, $J_{3,4}$ 3.3 Hz, H-3), 4.68 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 3.80 (dq, 1 H, $J_{5,6}$ 6.4 Hz, H-5), 2.12, 2.06, 1.95 (s, OAc's), 1.21 (d, 3 H, H-6); ^{13}C NMR ($CDCl_3$): δ170.6, 170.1, 169.5 (OAc's), 132.4-127.9 (aromatic C), 86.5 (C-1), 73.2 (C-5), 72.4 (C-3), 70.3 (C-4), 67.4 (C-2), 20.8, 20.6, 20.6 (s, OAc's), 16.5 (C-6).

Phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (32)

To a solution of compound **31** (4.5 g, 11.8 mmol) in methanol (30 mL) was added 1 M sodium methoxide solution until pH of the reaction mixture was about 9. After the de-O-acetylation was complete, H^+ resin was added to neutralize the solution. The mixture was filtered and concentrated under reduced pressure. To the residue (2.7 g, 10.5 mmol) dissolved in DMF (30 mL) was added sodium hydride (60 %, 2.3 g) at 0 °C. The mixture was stirred for 30 min at 0 °C. Benzyl bromide (5.64 mL, 47.4 mmol) was added. The resulting mixture was then stirred under nitrogen for 3 h. Methanol (2 mL) was added to the reaction mixture to destroy excess sodium hydride. The mixture was diluted with dichloromethane. The organic layer was washed with 5 % HCl and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography using EtOAc-hexane (1:6, v/v) as eluent to provide compound **32** (4.85 g, 87 %): $[\alpha]_D$ −12.5° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.60-7.20 (m, 15 H, aromatic), 5.03-4.67 (6 H, 3 AB pattern, 3 x CH_2), 4.61 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1), 3.93 (dd, 1 H, $J_{2,3}$ 9.2 Hz, H-2), 3.64 (dd, 1 H, $J_{4,5}$ < 1.0 Hz, H-4), 3.60 (dd, 1 H, $J_{3,4}$ 2.8 Hz, H-3), 3.53 (dq, 1 H, $J_{5,6}$ 6.4 Hz, H-5), 1.27 (d, 3 H, H-6); M.S. (C.I. ether, m/z): 527.0 (M+1).

4-Nitrophenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2-phthalimido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-1-thio-β-D-glucopyranoside (33)

To a solution of **32** (200 mg, 0.38 mmol) and compound **28** (328 mg, 0.26 mmol) in 10 mL of 5:1 benzene/dichloromethane was added powdered molecular sieves 4Å (500 mg). The mixture was stirred for 2 h at room temperature under nitrogen. DMTST (156 mg, 0.76 mmol) was added to the stirred mixture at 0 °C. After 1 h, TLC (2% MeOH in CH_2Cl_2) showed complete conversion of the donor. Then triethylamine (200 μL) and methanol (200 μ

L) were added to the reaction mixture which was stirred for an additional 25 min. The mixture was then diluted with dichloromethane (15 mL) and filtered through celite. The filtrate was washed with 10% aqueous sodium thiosulfate (10 mL), saturated aq solution of $NaHCO_3$ (2x10 mL), and brine (10 mL). The solution was dried (Na_2SO_4) and concentrated. Column chromatography (2% methanol in dichloromethane) of the residue on silica gel afforded **33** (314 mg, 72%): $[\alpha]_D$ +24.5° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.16-7.00 (m, 53 H, aromatic H), 5.89 (d, 1 H, $J_{4',5'}$ 3.6 Hz, H-4'), 5.83 (dd, 1 H, $J_{2',3'}$ 10.2 Hz, H-2'), 5.61 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1), 5.54 (dd, 1 H, $J_{3',4'}$ 3.6 Hz, H-3'), 5.39 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1'), 5.02 (d, 1 H, $J_{1'',2''}$ 3.6 Hz, H-1''), 4.89 (dd 1 H, $J_{3,4}$ 9.3 Hz, H-3), 4.81 (m, 1 H, H-5''), 4.63 (dd, 1 H, $J_{5',6a'}$ 7.7, $J_{6a',6b'}$ 11.2 Hz, H-6a'), 4.62-4.11 (3 x AB pattern, 6 H, 3 x $PhCH_2$), 4.58 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2), 4.43 (dd, 1 H, $J_{4,5}$ 9.4 Hz, H-4), 4.29 (dd, 1 H, $J_{5',6b'}$ 3.6 Hz, H-6b'), 4.18 (m, 1 H, H-5'), 4.02(dd, 1 H, $J_{5,6a}$ 1.5, $J_{6a,6b}$ 12.0 Hz, H-6a), 3.97 (dd, 1 H, $J_{3'',4''}$ 2.7 Hz, H-3''), 3.88 (dd, 1 H, $J_{5,6b}$ 1.4 Hz, H-6b), 3.79 (dd, 1 H, $J_{2'',3''}$ 10.2 Hz, H-2''), 3.53 (d, 1 H, H-4''), 3.38 (m, 1 H, H-5), 1.43 (d, 3 H, $J_{5'',6''}$ 6.4 Hz, H-6''), 1.02 (s, 9 H, $SiC\underline{Me}_3$); ^{13}C NMR ($CDCl_3$): δ146.3-123.8 (66 C, aromatic), 100.2 (C-1'), 97.2 (C-1''), 82.2 (C-1), 79.6 (C-5), 79.4 (C-4''), 79.3 (C-3''), 75.3 (C-2''), 75.2 (CH_2), 74.9 (C-4), 73.0 (2 C, C-3, CH_2), 71.9 (C-3'), 71.8 (C-5'), 71.7 (CH_2), 69.7 (C-2'), 68.5 (C-4'), 66.9 (C-5''), 61.4 (C-6'), 61.0 (C-6), 55.0 (C-2), 26.8 ($SiC\underline{Me}_3$), 19.3 ($Si\underline{C}Me_3$), 16.8 (C-6''); FAB-MS (glycerol) gave m/z (ion, relative intensity): 1678.7 (M^+, 0.1%).

Anal Calcd for $C_{97}H_{90}N_2O_{21}SSi$: C, 69.35; H, 5.40; N, 1.67. Found: C, 69.29; H, 5.47; N, 1.66.

4-Acetamidophenyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2-phthalimido-6-*O*-tert-butyldiphenylsilyl-2-deoxy-1-thio-β-D-glucopyranoside (34)

To a solution of compound **33** (150 mg, 0.089 mmol) in dichloromethane (5 mL) was added acetic acid (1 mL) and zinc dust (75 mg). The mixture was stirred for 1 h at room temperature. TLC (1% ethanol in dichloromethane) indicated the transformation was completed. The reaction mixture was then added triethylamine (1.25 mL) and concentrated under reduced pressure. The resulting crude *p*-aminophenylthio glycoside was immediately treated overnight with pyridine (2 mL) and acetic anhydride (1 mL) at room temperature. The

solution was thoroughly evaporated under reduced pressure. The crude residue was purified by silica gel chromatography using 0.5% methanol in dichloromethane as eluent to give **34** (127 mg, 84%): $[\alpha]_D$ +28.6° (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 8.13-7.01 (m, 53 H, aromatic), 5.87 (d, 1 H, $J_{3',4'}$ 3.6 Hz, H-4'), 5.78 (dd, 1 H, $J_{2',3'}$ 10.3 Hz, H-2'), 5.49 (dd, 1 H, H-3'), 5.37 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1), 5.35 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1'), 5.04 (d, 1 H, $J_{1'',2''}$ 3.6 Hz, H-1''), 4.81 (dd, 1 H, $J_{3,4}$ 9.8 Hz, H-3), 4.80 (m, 1 H, H-5''), 4.62-4.11 (3 x AB pattern, 6 H, 3 x $PhCH_2$), 4.56 (dd, 1 H, $J_{5',6a'}$ 6.6, $J_{6a',6b'}$ 11.2 Hz, H-6a'), 4.48 (dd, 1 H, $J_{2,3}$ 10.7 Hz, H-2), 4.42 (dd, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 4.32 (dd, 1 H, $J_{5',6b'}$ 7.4 Hz, H-6b'), 4.10 (m, 1 H, H-5'), 3.98-3.95(m, 2 H, H-3'', H-6a), 3.88 (d, 1 H, $J_{6a,6b}$ 10.1 Hz, H-6b), 3.79 (dd, 1 H, $J_{2'',3''}$ 10.2 Hz, H-2''), 3.55 (d, 1 H, $J_{3'',4''}$ 1.7 Hz, H-4''), 3.24 (m, 1 H, H-5), 2.10 (s, 3 H, NHAc), 1.42 (d, $J_{5'',6''}$ 6.5 Hz, H-6''), 1.05 (s, 9 H, $SiCMe_3$); ^{13}C NMR ($CDCl_3$): δ 139.0-119.3 (66 C, aromatic), 100.1 (C-1'), 97.0 (C-1''), 84.6 (C-1), 79.6 (2 C, C-5, C-4''), 79.3 (C-3''), 75.2 (2 C, C-2'', CH_2), 75.0 (C-4), 73.2 (C-3), 72.9 (CH_2), 72.0 (C-3'), 71.7 (CH_2), 71.6 (C-5'), 69.7 (C-2'), 68.5 (C-4'), 66.7 (C-5''), 61.4 (2 C, C-6', C-6), 55.5 (C-2), 26.9 ($SiC\underline{Me}_3$), 24.6 (OAc), 19.4 ($Si\underline{C}Me_3$), 16.9 (C-6''); FAB-MS (glycerol) gave m/z (ion, relative intensity): 1691.8 (M^+, 0.4%).

Anal Calcd for $C_{99}H_{94}N_2O_{20}SSi$: C, 70.28; H, 5.60; N, 1.66. Found: C, 70.10; H, 5.63; N, 1.70.

4-Nitrophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (36)

To a solution of acetobromolactose **35** (10 g, 14.3 mmol) in ethyl acetate (50 mL) was added 1 M Na_2CO_3 (50 mL) containing TBAHS (4.78 g, 14.3 mmol) and 4-nitrothiophenol (4.44 g, 28.6 mmol). The two-phase reaction mixture was vigorously stirred at room temperature for 3.5 h. The separated organic phase was successively washed with sat. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by silica gel chromatography using EtOAc-hexane (3:5, v/v) as eluent to afford **36** (9.9 g, 90 %): $[\alpha]_D$ -28.2° (*c* 1.3, $CHCl_3$); m.p. 122-124 °C; ^1H NMR ($CDCl_3$): δ 8.12 (d, 2 H, *J* 9.0 Hz, aromatic), 7.53 (d, 2 H, aromatic), 4.80 (d, 1 H, $J_{1,2}$ 10.1 Hz, H-1), 4.44 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'); FAB-MS (glycerol) gave m/z (ion, relative intensity): 774.2 ($[M+1]^+$, 0.6%).

4-Nitrophenyl 3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→4)-1-thio-β-D-glucopyranoside (37)

To a solution of compound **36** (8 g, 10.3 mmol) in methanol (30 mL) was added 1 M sodium methoxide until pH of the reaction mixture was about 9. After the de-*O*-acetylation was complete, H^+ resin was added to neutralize the solution. The mixture was filtered and the filtrate was concentrated under reduced pressure to afford 4-nitrophenyl 1-thiolactoside. 4-Nitrophenyl 1-thiolactoside (2.0 g, 4.17 mmol) was suspended in 2,2-dimethoxypropane (50 mL). A catalytic amount of *p*-toluenesulfonic acid was added and the reaction mixture was then heated under reflux for 15 min. Triethylamine was added until the pH became lightly basic. The mixture was concentrated and the crude product was purified by silica gel chromatography using methanol/dichloromethane (1/20) as eluent to afford **37** (1.73 g, 80%): m.p. 175-176 °C; $[\alpha]_D$ -32° (*c* 1.0, DMSO); ^{1}H NMR (acetone-d6): δ 8.14 (d, 2 H, *J* 9.0 Hz, aromatic), 7.69 (d, 2 H, aromatic), 5.04 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 4.44 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1'), 1.57, 1.38 (s, 6 H, CH_3); ^{13}C NMR (acetone-d6): δ 146.7-124.5 (aromatic C), 110.1 (O-C-O), 104.3 (C-1'), 86.2 (C-1), 81.8, 80.7, 80.6, 80.0, 75.0, 74.5, 74.1, 74.0, 62.3 (C-6'), 61.8 (C-6), 28.4, 26.5 (2 x CH_3); FAB-MS (glycerol) gave m/z (ion, relative intensity): 519.5 ($[M+1]^+$, 1.8%).

4-Nitrophenyl 2,6-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (38)

4-Nitrophenyl 3', 4'-*O*-isopropylidene-β-lactoside derivative **37** (250 mg, 0.481 mmol) was dissolved in dry pyridine (10 mL), the solution was cooled to 0°C and benzoyl chloride (0.5 mL) was added. The mixture was then allowed to reach room temperature and was stirred overnight. The reaction mixture was poured onto ice water and extracted with CH_2Cl_2 (3x20 mL). The combined extracts were washed successively with 5% HCl solution, saturated aq solution of $NaHCO_3$, and water. The solution was dried (Na_2SO_4) and concentrated to a foam which was dissolved in 15 mL of a 1:1 mixture of CH_2Cl_2 and CH_3OH, and *p*-toluenesulfonic acid (100 mg) was added. The mixture was stirred at 40 °C for 2 h. The reaction mixture was neutralized with triethylamine and concentrated under reduced pressure. The residue was purified by silica gel chromatography using 1:2 hexane

and ethyl acetate as eluent to afford **38** (440 mg, 92%): m.p. 178-180 °C; $[\alpha]_D$ +11.8° (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 8.01-7.27 (m, 29 H, aromatic H), 5.72 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3), 5.41 (dd, 1 H, $J_{2,3}$ 9.7 Hz, H-2), 5.31 (dd, 1 H, $J_{2',3'}$ 7.7 Hz, H-2'), 4.97 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 4.65 (dd, 1 H, $J_{5,6a}$ 1.8, $J_{6a,6b}$ 12.1 Hz, H-6a), 4.62 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.51 (dd, 1 H, $J_{5,6b}$ 5.9 Hz, H-6b), 4.05 (dd, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 4.02 (dd, 1 H, $J_{6a',6b'}$ 11.3 Hz, H-6a'), 3.96 (ddd, 1 H, H-5), 3.84 (d, 1 H, H-4'), 3.73 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3'), 3.64 (dd, 1 H, $J_{5',6b'}$ 6.5 Hz, H-6b'), 3.65 (dd, 1 H, $J_{5',6a'}$ 6.4 Hz, H-5'); ^{13}C NMR ($CDCl_3$): δ 166.4, 166.1, 165.7, 165.7, 165.2, (5 x C=O), 146.8-123.7 (36 C, aromatic), 100.9 (C-1'), 84.1 (C-1), 77.4 (C-5), 76.1 (C-4), 73.8 (C-3), 73.7 (C-2'), 72.7 (C-5'), 72.6 (C-3'), 70.1 (C-2), 68.5 (C-4), 62.7 (C-6), 61.9 (C-6'); FAB-MS (glycerol) gave m/z (ion, relative intensity): 1000.3 ($[M+1]^+$, 3%).

Anal. Calcd for $C_{53}H_{45}NO_{17}S$: C, 63.66; H, 4.54; N, 1.40. Found: C, 63.11; H, 4.68; N, 1.30.

4-Nitrophenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)]--(6-*O*-*tert*-butyldiphenylsilyl-2-phthalimido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (39)

To compound **34** (81 mg, 0.048 mmol) and compound **38** (48 mg, 0.048 mmol) in dry dichloromethane (3 mL) was added powdered molecular sieves 4 Å (120 mg) under nitrogen. The mixture was stirred at room temperature for 3 h and then cooled to -30 °C. N-Iodosuccinimide (22 mg, 0.096 mmol) and trifluoromethanesulfonic acid (4.2 μL, 0.048 mmol) were added. After stirring at -45 °C for 45 min, the mixture was diluted with dichloromethane (12 mL) and filtered through celite. The filtrate was washed with 10% aq sodium thiosulfate (15 mL), saturated aq solution of $NaHCO_3$ (2 x 15 mL), and brine (15 mL). The solution was dried (Na_2SO_4) and concentrated to a foam that was chromatographed on silica gel (1% ethanol in methylene chloride) to afford pentasaccharide **39** (86.2 mg, 71%): $[\alpha]_D$ +12.8° (*c* 1.0, $CHCl_3$); ^1H NMR (500 Hz, $CDCl_3$): δ 8.08-6.97 (m, 78 H, aromatic H), 5.78 (d, 1 H, H-4d), 5.67 (dd, 1H, $J_{2,3}$ 10.4 Hz, H-2d), 5.61 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-3a), 5.32 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2a), 5.31 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-3d), 5.18 (dd, 1 H, $J_{2,3}$ 9.6 Hz, H-2b), 5.15 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1c), 5.12 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1d), 4.96 (d, 1 H, $J_{1,2}$ 3.5

Hz, H-1e), 4.85 (d, 1 H, $J_{1,2}$ 9.8 Hz, H-1a), 3.46 (m, 1 H, H-5c), 1.32 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6e), 1.02 (s, 9 H, $SiCMe_3$); ^{13}C NMR ($CDCl_3$): δ 166.0, 165.9, 165.8, 165.6, 165.2, 165.1, 164.6, 164.1 (9 x C=O) 146.7-123.2 (96 C, aromatic), 100.5 (C-1b), 99.6 (C-1d), 99.0 (C-1c), 96.6 (C-1e), 84.0 (C-1a), 80.1 (C-3b), 79.3 (C-4e), 79.1 (C-3e), 77.3 (C-2e), 75.5 (2 C, C-5a, C-5c), 75.2 (CH_2), 75.0 (2 C, C-4C, C-5d), 74.7 (C-3a), 73.2 (CH_2), 72.8 (C-5b), 72.2 (C-3c), 71.9 (CH_2), 71.8 (C-3d), 71.7(C-4a), 71.4 (C-2b), 70.8 (C-2a), 70.0 (C-2d), 69.7 (C-4d), 68.2 (C-4b), 67.8 (C-5e), 66.7 (C-6b), 62.8 (C-6a), 61.5 (C-6c), 61.2 (C-6d), 55.9 (C-2c), 26.9 ($SiCMe_3$), 19.5 ($SiCMe_3$), 16.8 (C-6e); FAB-MS (glycerol) gave m/z (ion, relative intensity): 2524.9 ($[M]^+$, 0.1%).

Anal. Calcd for $C_{144}H_{130}N_2O_{36}SSi$: C, 68.51; H, 5.19; N, 1.11. Found: C, 68.39; H, 5.23; N, 1.19.

Chapter 4 Syntheses of sialoside haptens

4.1 Introduction

Sialic acid-containing glycoconjugates, such as gangliosides and sialoglycoproteins, are cell-type specific markers that are widely distributed throughout the organism. They have been recognized to play a vital role in a large variety of biological processes.[124] These include embryogenesis, organ development, immune defense, migration and homing of leukocytes, metastasis of neoplastic cells, as well as inflammation and the infection by a variety of pathogens. One well-known example of cell/virus interaction mediated by sialic acid is the influenza infection (common flu).[125] Up to now more than 40 different sialic acids have been discovered, of which *N*-acetylneuraminic acid (Neu5Ac) is the most abundant one. Neu5Ac is usually attached to *O*-3 or *O*-6 of galactose, or *O*-6 of *N*-acetylgalactosamine with an α(2→3)- or α(2→6)-linkage, or to *O*-8 of another Neu5Ac with an α(2→8)-linkage, giving diverse structures and functions. To understand the structure-function relation of sialyl-oligosaccharides at the molecular level, efficient methods for regio- and α-stereoselective glycoside synthesis of sialic acids are desirable.

Glycosylation using sialic acid-derived donors has been recognized as one of the most challenging tasks in synthetic carbohydrate chemistry[126] because of the special structure of sialic acid. First, the carboxylic acid function at the anomeric position (C-2) not only electronically disfavors oxonium ion formation which is necessary for almost all known

---

[124] a) Traving, C.; Schauer, R. *Cell. Mole. Life Sci.* **1998**, *54*, 1330; b) Reutter, W.; Stäsche, R.; Stehling, P.; Baum, O. in *Glycoscience*, Gabius, H. J.; Gabius, S. Eds.; Chapman & Hall, Weinheim, **1997**, 245; c) R. Schauer, R. Kamering, J. P. in *Glycoproteins II*, Montreuil, J.; Vliegenthart, J. F. G.; Schachter H. Eds.; Elsevier, Amsterdam, **1997**, 241; d) Rosenberg, A. *Biology of Sialic Acids*, Plenum, New York, **1995**; e) Schauer, R. *Sialic Acids-Chemistry, Metabolism & Functions. Cell Biology Monographs*. Vol. 10, Wien, Springer Verlag, **1992**.

[125] Mammen, M.; Choi, S.-K.; Whitesides, G. *Angew. Chem. Int. Ed.* **1998**, *37*, 2754.

glycosidation reactions, but also sterically restricts glycoside formation. Second, the lack of a substituent at C-3 precludes the suitable neighboring participation leading to α-glycoside. Third, the thermodynamically favored product is the β-glycoside. These combined factors disfavor the desired α-glycoside formation, especially with secondary sugar hydroxyls, and promote an elimination pathway to the deoxy center at C-3, giving the 2,3-dehydro derivative as the major byproduct.

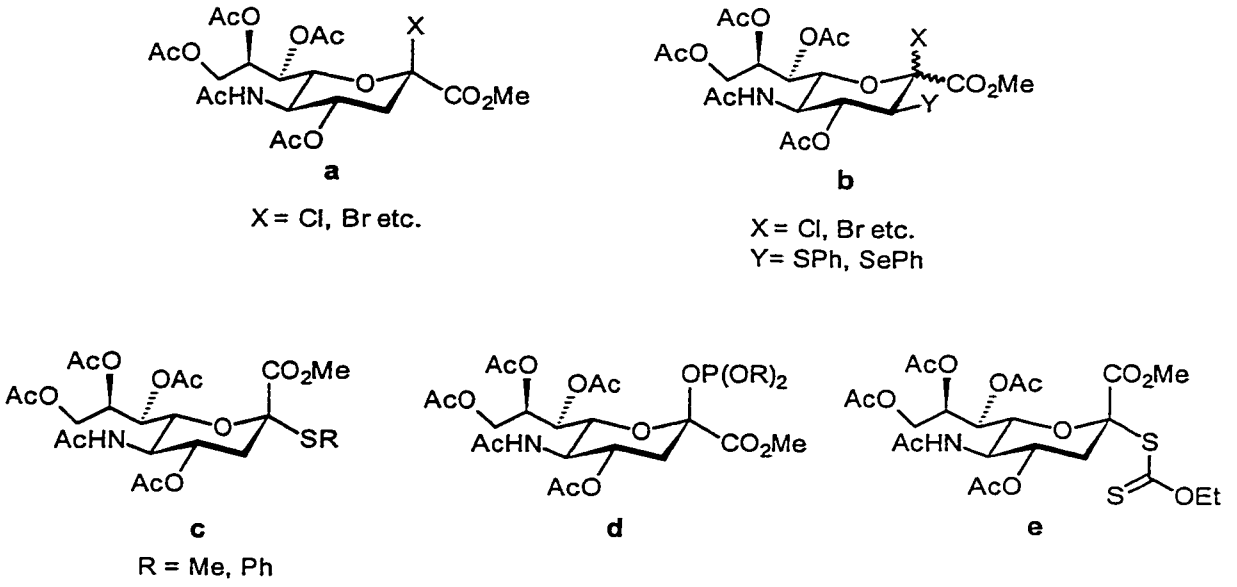


Figure 4.1 The structures of sialyl donors

Early attempts utilizing *N*-acetylneuraminyl halides (**a**) (Figure **4.1**) as glycosyl donors by the classic Koenigs-Knorr method were satisfactory only for the reactive alcohols and, once applied to relatively hindered glycosyl acceptor substrates, this method resulted in poor yields and little stereochemical preference for the desired α-isomer.[127] As a solution, the introduction of a temporary equatorial substituent at C-3 such as 3-β-thio- or selenyl-phenyl

[126] a) Ito, Y.; Gaudino, J. J.; Paulson, J. C. *Pure. & Appl. Chem.* **1993**, *65*, 753; b) DeNinno, M. P. *Synthesis* **1991**, 583; c) Okamoto, K.; Goto, T. *Tetrahedron* **1990**, *13*, 267.
[127] a) van der Vleugel, D. M. J.; Wassenberg, F. R.; Zwikker, J. W.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1982**, *104*, 221; b) Ogawa, T.; Sugimoto, M. *Carbohydr. Res.* **1985**, *135*, c5; c) Paulsen, H.; von Deessen, U. *Carbohydr. Res.* **1986**, *146*, 147.

group (**b**)[128] has been proposed. It can function either by participation, or steric blocking of the β-face. However, additional multiple steps for introduction and removal of the C-3 auxiliary group are required, even though the yield and stereoselectivity of the sialylation are greatly improved.

Recently, more dramatic improvement has come from examination of various leaving groups and activating agents. Thioglycosides of neuraminic acid (**c**) have been used as sialyl donors[129] and showed to be superior to conventional 2-halo derivatives in both yield and stereoselectivity. These compounds are stable under many different chemical conditions and can be activated by suitable thiophilic promoters (NIS-triflic acid, dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST), phenyl selenyl triflate (PhSeOTf)). Systematic ganglioside syntheses have been achieved based on this method. Given these preliminary findings, several new attempts were made by employing Neu5Ac glycosyl phosphites[130] (**d**) or xanthates[131] (**e**) as alternative glycosyl donors.

Phase transfer catalysis (PTC) has emerged as an effective general method for the preparation of different types of anomeric glycosyl derivatives including C-, N-, O-, S-, and Se-glycosides. Under a PTC approach, thiosialyl donors with inverted α-anomeric configuration can be easily synthesized in a large scale, although there is no substituent in β-acetochloroneuraminic acid to participate in any anchimeric fashion which may help to provide nucleophilic displacement with inversion of configuration. On the other hand, we chose glycosyl azide derivatives which can also be prepared by PTC method as glycosyl acceptors. The azido group of glycosyl acceptors can be further transformed into an isothiocyanate group or aglycon spacers which are important precursors to construct various glycoconjugates. Moreover, the *N*-linkage between the sugar moiety and aglycon instead of the usual *O*-linked form increases the stability toward the hydrolysis by glycohydrolases.

---

[128] a) Ito, Y.; Ogawa, T. *Tetrahedron lett.* **1988**, *29*, 3987; b) Kondo, T.; Abe, H.; Goto, T. *Chem. Lett.* **1988**, 1657; c) Nicolaou, K. C.; Hummel, C. W.; Bockovich, N. J.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1991**, 870.

[129] a) Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1988**, *29*, 1061; b) Ito, Y.; Ogawa, T.; Numata, M.; Sugimoto, M. *Carbohydr. Res.* **1990**, *201*, 165; c) Hasagawa, A.; Ohki, T.; Nagahama, H.; Ishida, M. Kiso, M. *Carbohydr. Res.* **1991**, 212, 277; d) Hasagawa, A.; Nagahama, H.; Ohki, T.; Hotta, T.; Ishida, M. Kiso, M. *J. Carbohydr. Chem.* **1991**, 10, 493.

[130] a) Martin, T. J.; Schmidt, R. R. *Tetrahedron Lett.* **1992**, *33*, 6123; b) Sim, M. M.; Kondo, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 2260.

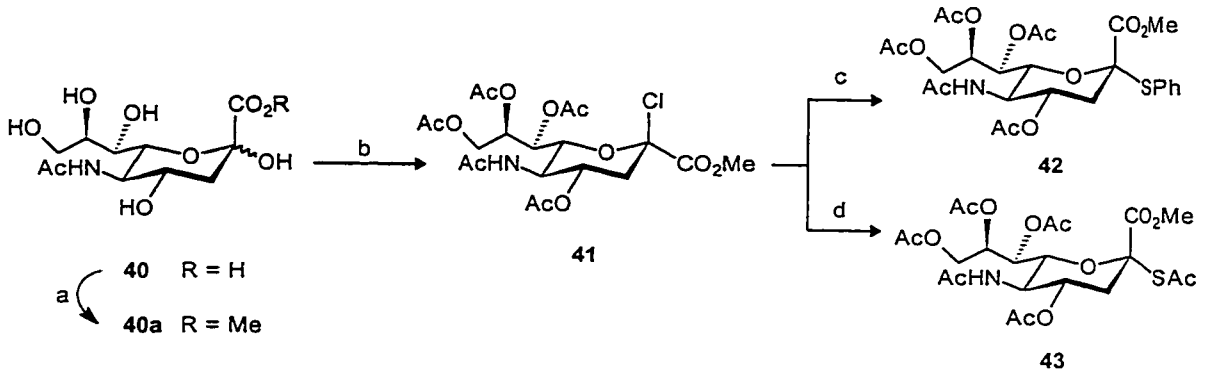
[131] a) Marra, A.; Sinäy, P. Carbohydr. Res. 1990, 195, 303; b) Lönn, H.; Stenvall, K. *Tetrahedron Lett.* **1992**, *33*, 115.

4.2 Results and discussion

4.2.1 Synthesis of Neu5Ac-α-(2→6)-β-D-galactoside

The Neu5Ac-α-(2→6)-β-D-galactoside unit is found in numerous glycoproteins and glycolipids, and is a receptor ligand for virus hemagglutinin and the neuraminidase.

For the synthesis of the required α-thiosialosyl donor **42**, a standard procedure which was first used by Kuhn *et al.*[132] was slightly modified to prepare acetochloroneuraminic acid **41** from sialic acid **40** (Scheme **4.1**). Under the previously mentioned PTC conditions, treatment of freshly prepared β-acetochloroneuraminic acid **41** with thiophenol afforded phenyl α-thiosialoside **42** in 87% yield. The α-configuration of **42** was determined according to well established empirical rules[133] (^{1}H NMR δ 2.77 for H_{3eq}). This method was also successfully applied to prepare 2-*S*-acetyl sialoside **43** in 72% yield.



Scheme 4.1 Synthesis of thiosialoside derivative by PTC reactions

Reagents and conditions: (a) MeOH, H^+ resin, 24 h, 94%; (b) AcCl, MeOH, 48 h, 98%; (c) PhSH, TBAHS, 1M Na_2CO_3, 1h, 87%; (d) AcSH, TBAHS, 1M Na_2CO_3, 1h, 72%.

Acetobromogalactose **44**[134] was treated with sodium azide under PTC conditions to afford 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl azide **45** in 95% yield. The reaction occurred with complete stereocontrol to give the 1,2-*trans* glycoside **45** as judged by ^{1}H

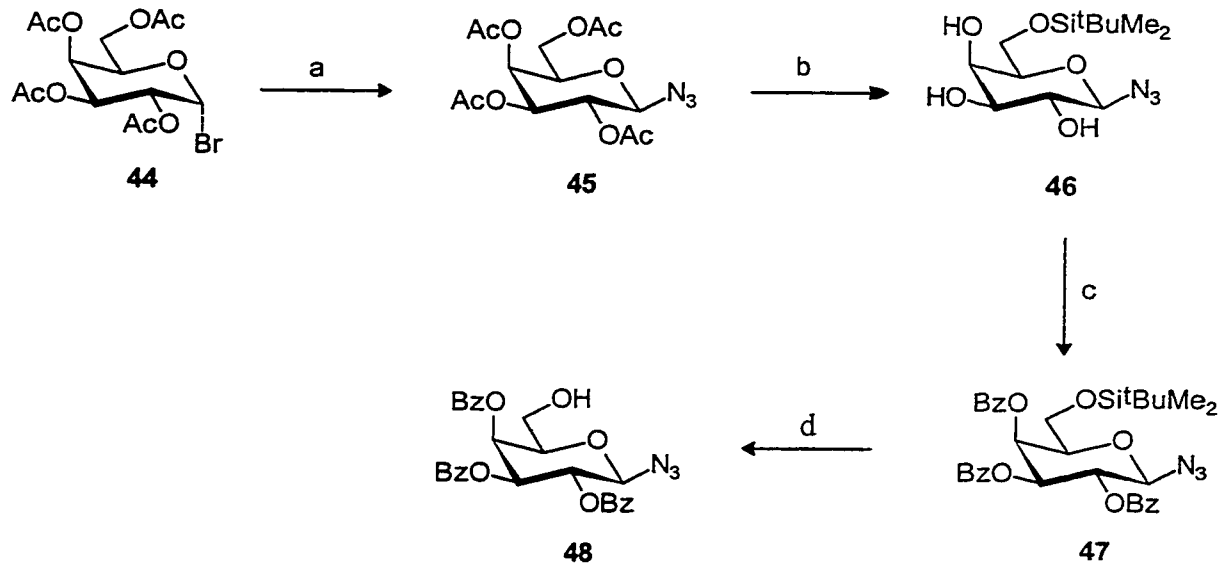
---

[132] Kuhn, R.; Luts, P.; MacDonald, D. L. *Chem. Ber.* **1966**, *99*, 611.

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

[134] Jeanloz, R. W,; Stoffyn, P. J. *Methods in Carbohydr. Chem.* **1962**, *1*, 221.

NMR ($J_{1,2}$ 9.9 Hz). Zemplén deacetylation of compound **45** afforded β-D-galactopyranosyl azide **45a** which was used in the next step directly without further purification. Regioselective silylation of the tetraol with *tert*-butyldimethylsilyl chloride using pyridine as solvent gave the 6-*O*-silyl derivative **46** in 86% yield. Benzoylation of compound **46** with benzoyl chloride in pyridine gave compound **47** (88%). To avoid extensive *O*-acyl migration, the most effective method[135] for de-*O*-silylation of galactoside **47** was based on the use of 3% hydrogen chloride in methanol/ether (1:1, v/v), freshly prepared from acetyl chloride and methanol. Under these conditions, the cleavage of the silyl group occurred smoothly at room temperature and the 6-*O*-deprotected galactoside acceptor **48** was obtained in 92% yield (Scheme **4.2**).



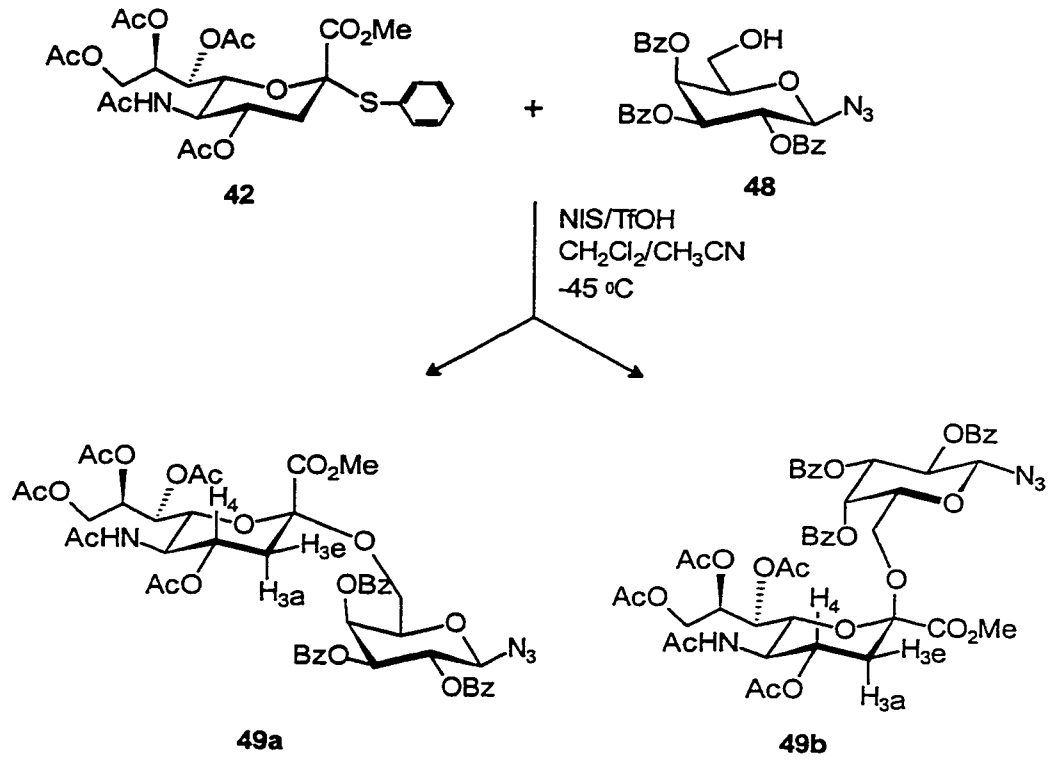
Scheme 4.2 Synthesis of galactosyl acceptor **48**

Reagents and conditions: (a)NaN_3, TBAHS, 1 M Na_2CO_3, CH_2Cl_2, 95%; (b) i: NaOMe/MeOH; ii: $^tBuMe_2SiCl$, pyridine; 86%; c) BzCl, pyridine, 88%; (d) AcCl, MeOH, 30 min, 92%.

Glycosylation of phenyl α-thiosialosyl donor **42** with the 6-OH galactopyranosyl azide acceptor **48** in acetonitrile using *N*-iodosuccinimide (NIS) and triflic acid (TfOH) as promoter proceeded well at -45 °C. After routine work up, the reaction afforded two

[135] Hanessian, S.; Lavallée, P. *Can. J. Chem.* **1975**, *53*, 2975.

compounds **49a** and **49b** in a ratio of about 7:1. The α,β mixtures were successfully resolved by silica gel column chromatography. The desired α disaccharide **49a** was obtained in 66% yield as a major product compared with the β anomer **49b** in 9% yield (Scheme **4.3**).

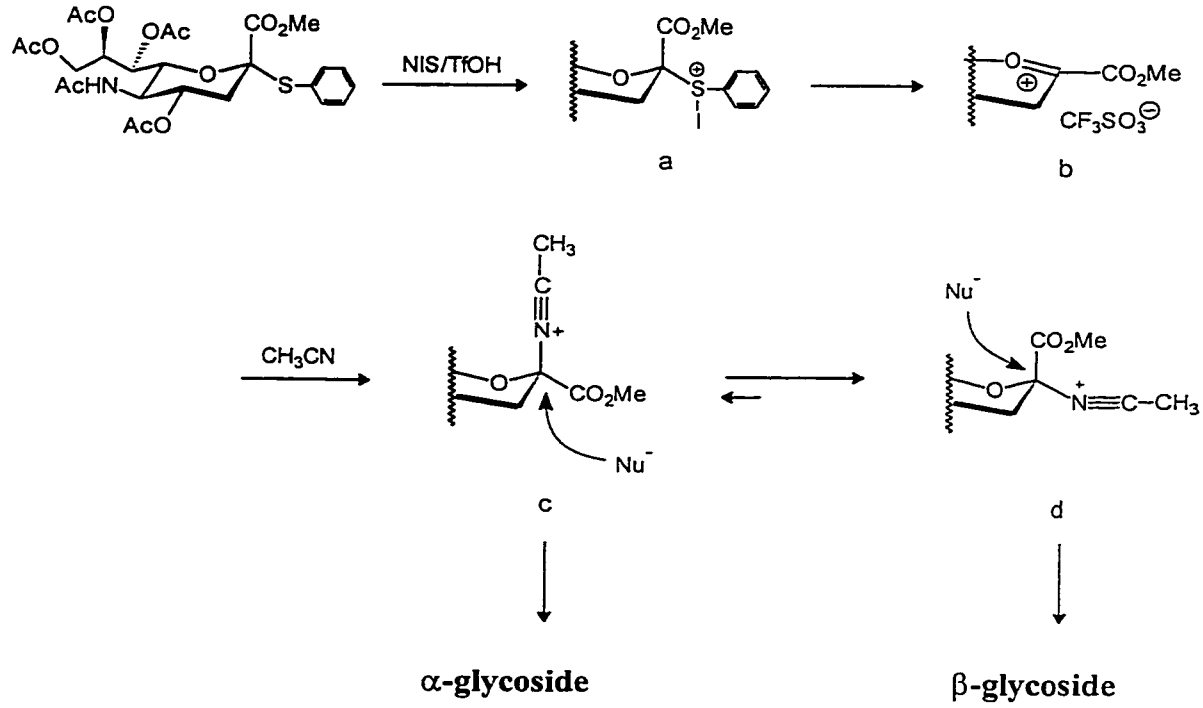


Scheme 4.3 Synthesis of Neu5Ac-α,β-(2→6)-β-D-Gal disaccharide **49a** and **49b**

Although sialosides lack an anomeric proton, the anomeric configuration can be inferred on the basis of empirical rules. These rules include: (i) α δ $H3_{eq}$ > β δ $H3_{eq}$;[136] (ii) α δ H4 < β δ H4;[137] (iii) α $J_{7,8}$ > β $J_{7,8}$.[138] Another NMR method for the determination of the anomeric configuration was based on coupling constants between C-1 and H-3a. In the $^2C_5$ chair conformation, the $^3J_{C1,\ H3a}$ of the α-anomer is larger than that of the β-anomer.[139] Accordingly, the α-confirguration of C-2' of disaccharide **49a** was confirmed by ^{1}H NMR

[136] Dabrowski, U.; Friebolin, H.; Brossmer, R.; Supp, M. *Tetrahedron Lett.* **1979**, *48*, 4637.
[137] Paulson, H.; Tietz, H. *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 927.
[138] van der Vleugel, D. M. J.; van Boom, J.H.; Heeswijk, W.A.R.; Vliegenthart, J.F.G. *Carbohydr. Res.* **1982**, *102*, 121.

spectrum which showed H-3e at δ 2.44 ppm and H-4' at δ 4.77 ppm by comparison with the β-anomer **49b**'s ^{1}H NMR spectrum which showed H-3e at δ 2.28 ppm and H-4' at δ 5.01 ppm. For comparison, the ^{1}H NMR and ^{13}C NMR spectra of **49a** and **49b** are illustrated in Figures **4.2-4.5**.



Scheme 4.4 The possible mechanism for α-predominant sialylation in acetonitrile

The high stereoselectivity of this glycosylation reaction can be explained by the effect of acetonitrile as participating solvent. The general principle of solvent participation is shown in Scheme **4.4**. When the glycosyl donor is specifically activated by the thiophilic promoter (NIS-TfOH), the cyclic oxocarbenium ion is formed by the initially produced intermediate. The oxocarbenium ion reacts with acetonitrile to generate the β-acetonitrilium ion at low temperature. The β-acetonitrilium ion then undergoes S_N2-like displacement at the anomeric center by sugar nucleophiles to give predominantly α-glycosides.[140]

---

[139] a) Hori, H.; Nakajima, T.; Nishida, Y.; Ohrui, H.; Meguro, H. *Tetrahedron Lett.* **1988**, *29*, 6317; b) Czarmiecki, M.F.; Thornton, E.R. *J. Am. Chem. Soc.* **1977**, *99*, 8173.
[140] Kirchner, E.; Thiem, F.; Demick, R. *J. Carbohydr. Chem.* **1988**, *7*, 453.

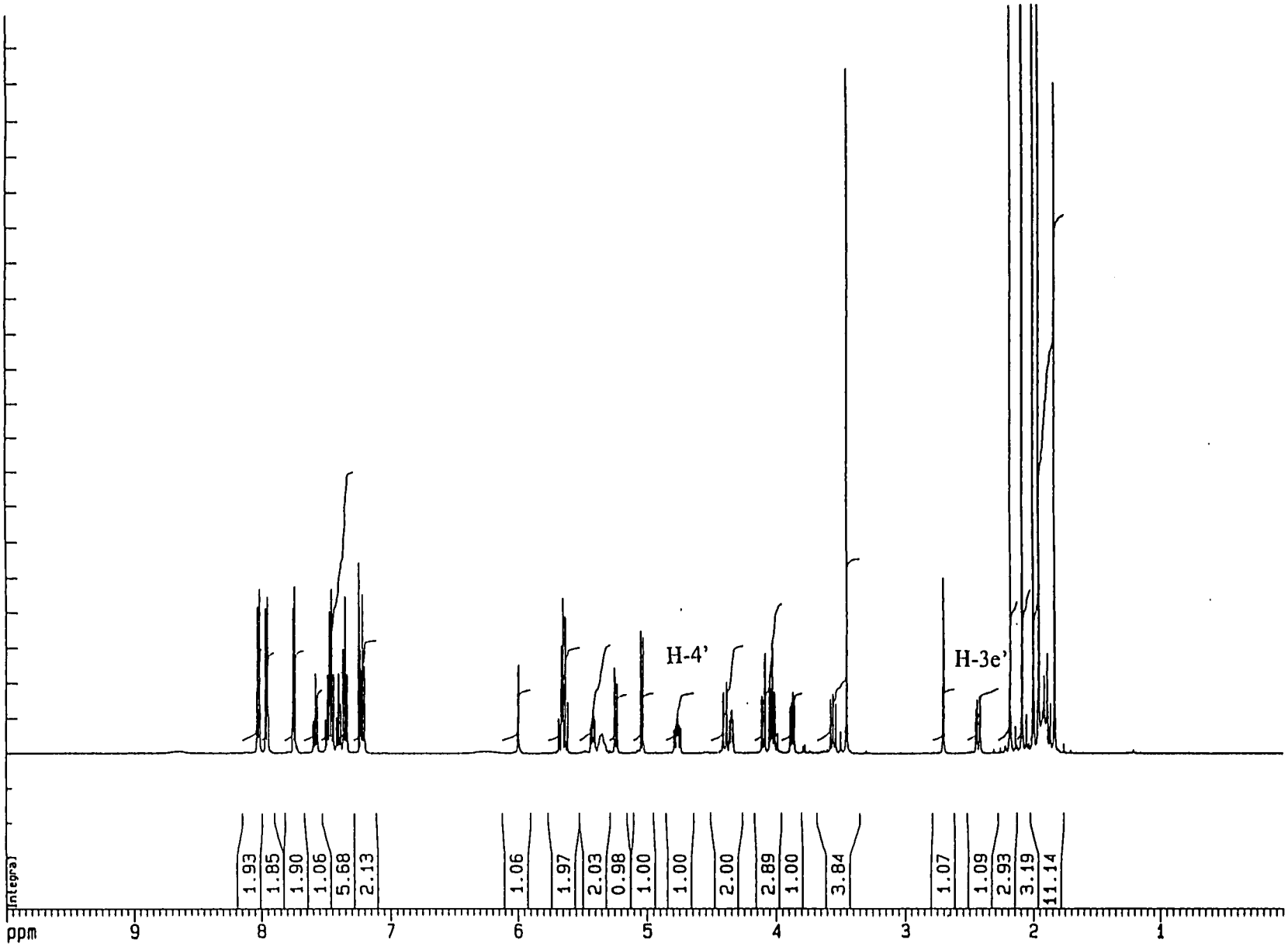


Figure 4.2 ^{1}H NMR (500MHz, $CDCl_3$) spectrum of disaccharide **49a**

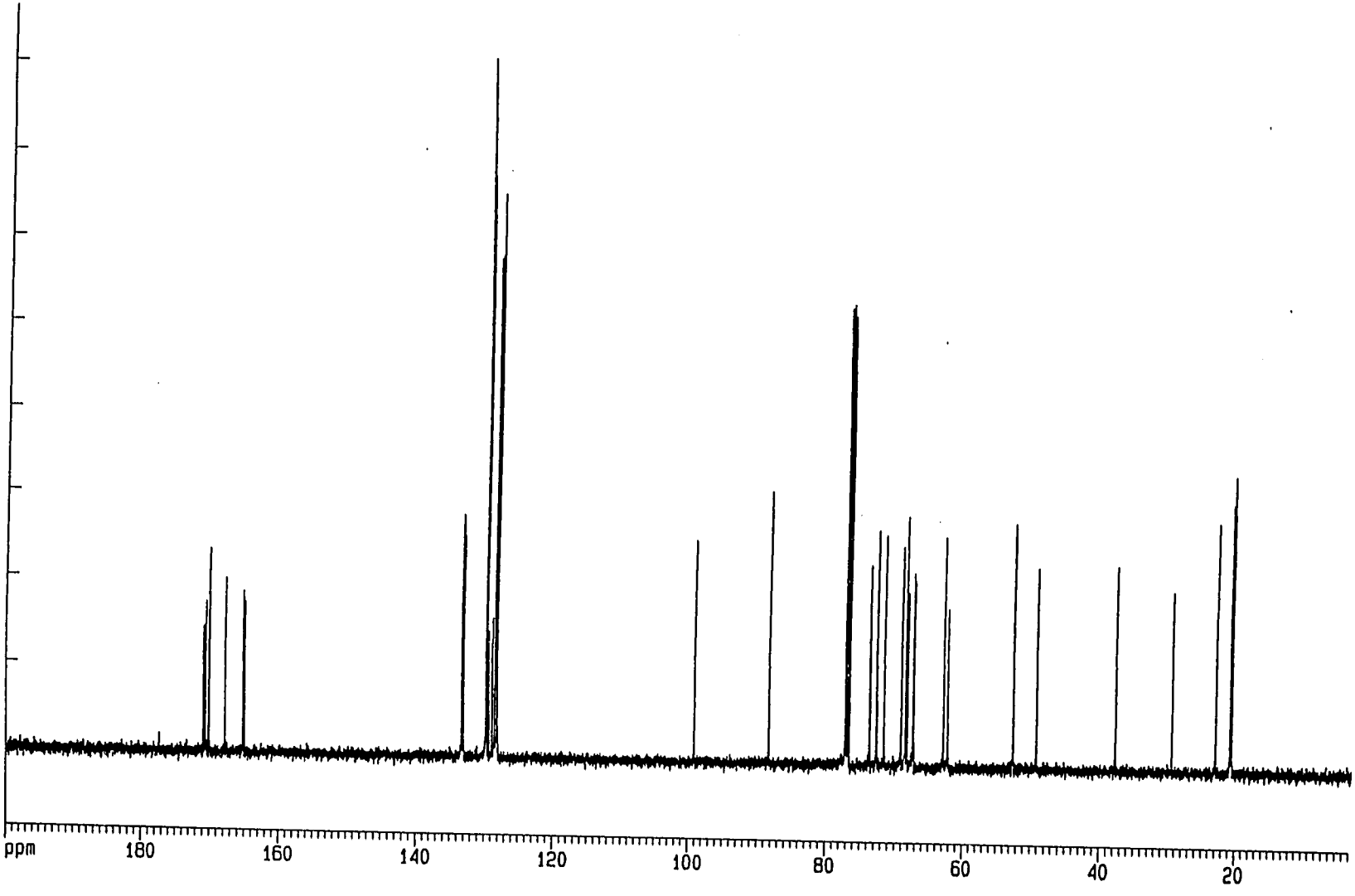


Figure 4.3 ^{13}C NMR (500MHz, $CDCl_3$) spectrum of disaccharide **49a**

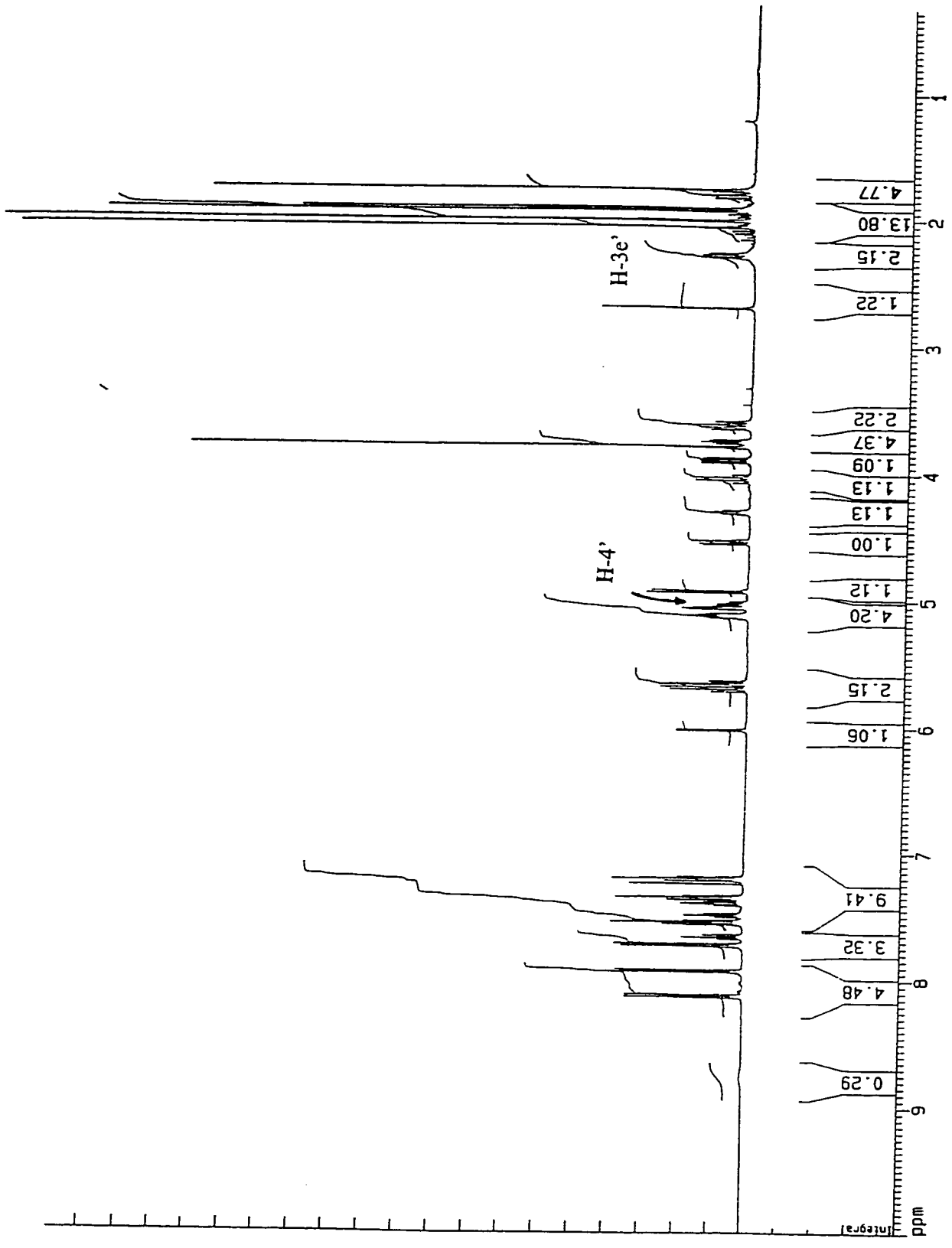


Figure 4.4 ^{1}H NMR (500MHz, $CDCl_3$) spectrum of disaccharide **49b**

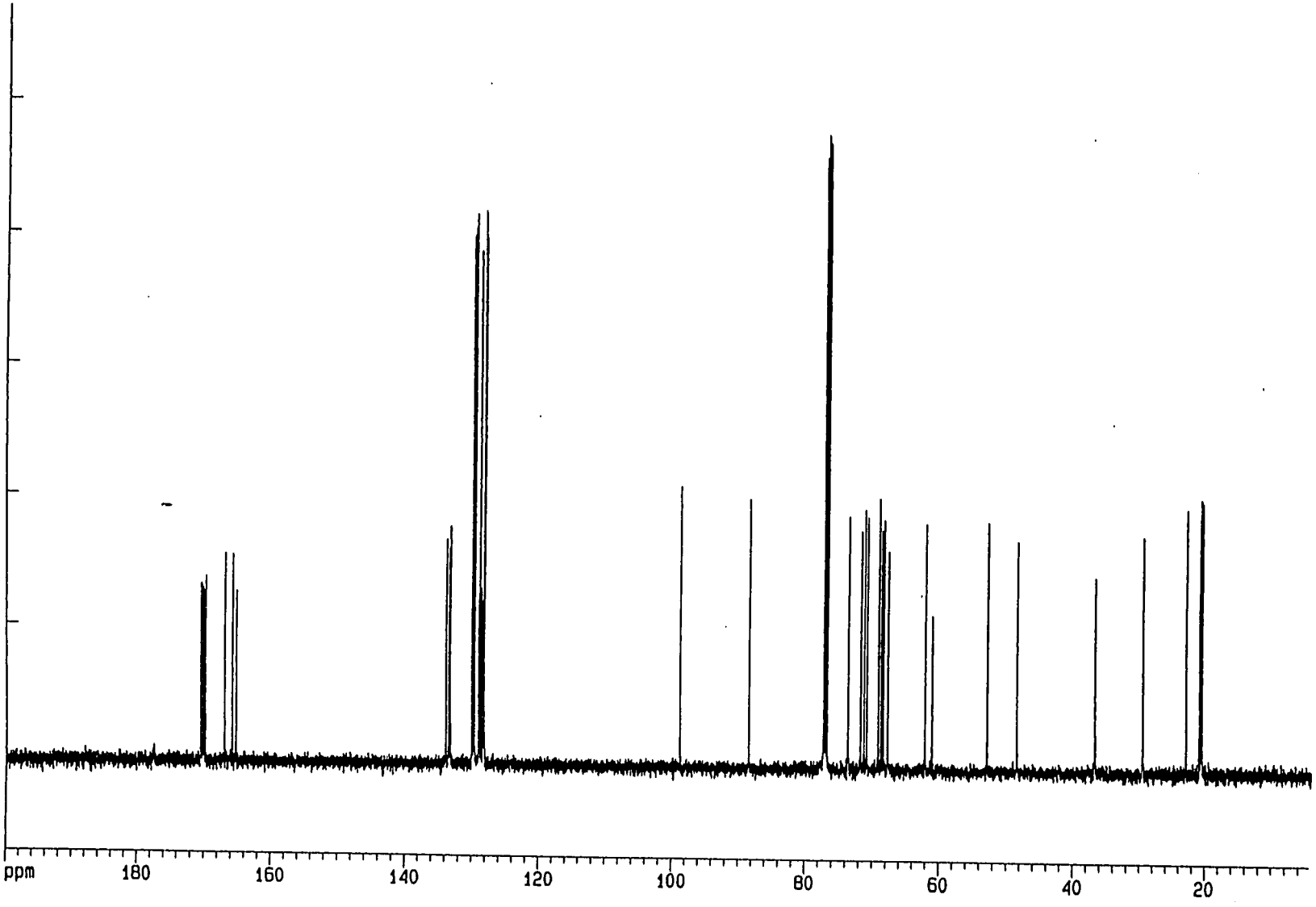


Figure 4.5 ^{13}C NMR (500MHz, $CDCl_3$) spectrum of disaccharide **49b**

4.2.2 Synthesis of thio-linked analogue of Neu5Ac-α-(2→6)-β-D-galactoside

The *O*-glycosidic linkage of the Neu5Ac-α-(2→6)-β-D-galactoside unit is the breakdown target for glycohydrolases present *in vivo*. To prevent enzymatic hydrolysis, one of the strategies was used by Sabesan to connect sugars via a peptide linkage.[141] Peptidosialoside Neu5Ac-α-(N-2→6-CO)-β-D-Gal was synthesized in reasonable yield by the coupling of sialylamine with activated galactopyranosyluronic acid (Figure **4.6**).[142] However, the possible anomerisation and steric hindrance around C-2 of sialylamine were unfavorable to the formation of peptide bond.

Thioglycosides have proved to be much more stable to glycosidases action than glycosides, thus being potential inhibitors of glycosidases.[143] α-(2→6)-Thioglycosides of Neu5Ac have been first prepared by Hasegawa *et al.*[144] 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 **43** was selectively *S*-deacetylated with sodium methoxide. The sodium salt of sialic acid was then coupled with a glycosyl acceptor to afford the desired α-(2→6)-thioglycoside. However, the use of sodium methoxide to selective *S*-deacetylation resulted in partial *O*-deacetylation, which necessitated a reacetylation step.

A more efficient method for the synthesis of α-(2→6)-thioglycoside has been reported,[145] in which thiodeacetylation was achieved in situ using diethylamine. Thus treatment of *S*-acetyl sialic acid and 6-bromo glucosyl acceptor with diethylamine afforded the desired disaccharide in 82% yield. Encouraged by these results, we applied this method to synthesize α-(2→6)-thiogalactosides of Neu5Ac.

---

[141] Sabesan, S. *Tetrahedron Lett.* **1997**, *38*, 3127.

[142] a) Nicolaou, K. C.; Florke, H.; Egan, M. G.; Bath, T.; Estevez, V. A. *Tetrahedron Lett.* **1995**, *36*, 1775; b) Suhara, Y.; Hildreth, J. E. K.; Ichikawa, Y. *Tetrahedron Lett.* **1996**, *37*, 1575; c) Muller, C.; Kitas, E.; Wessel, H. P. *J. Chem. Soc., Chem. Commoun.* **1995**, 2425.

[143] a) Witczak, Z. J.; Chhabra, R.; Chen. H.; Xie, X.-Q. *Carbohydr. Res.* **1997**, *301*, 167; b) Bär, T.; Schmidt, R. R. *Liebigs Ann. Chem.* **1991**, 185; c) Shafizadeh, F.; Furneaux, R. H.; Stevenson, T. T. *Carbohydr. Res.* **1979**, *71*, 169-191.

[144] a) Hasegawa, A.; Terada, T.; Ogawa, H.; Kiso, M. *J. Carbohydr. Chem.* **1992**, *11*, 319; b) Hasegawa, A.; Morita, M.; Ito, Y.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1990**, *9*, 369.

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

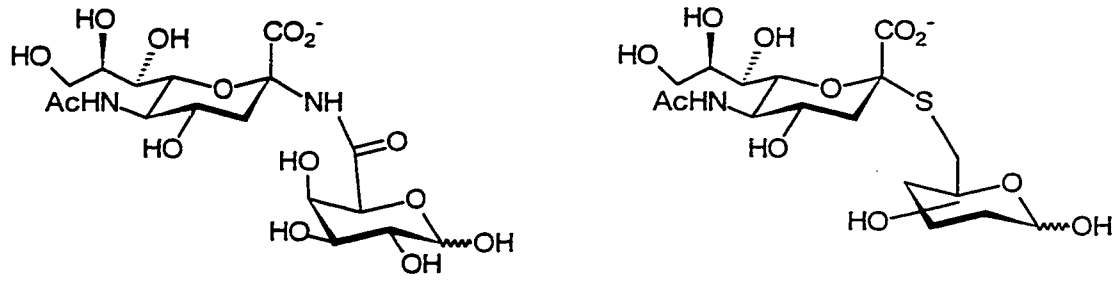
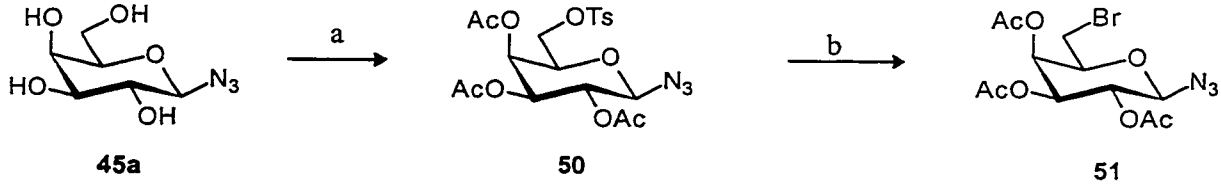


Figure 4.6 Mimics of α-(2→6)-glycosides of Neu5Ac

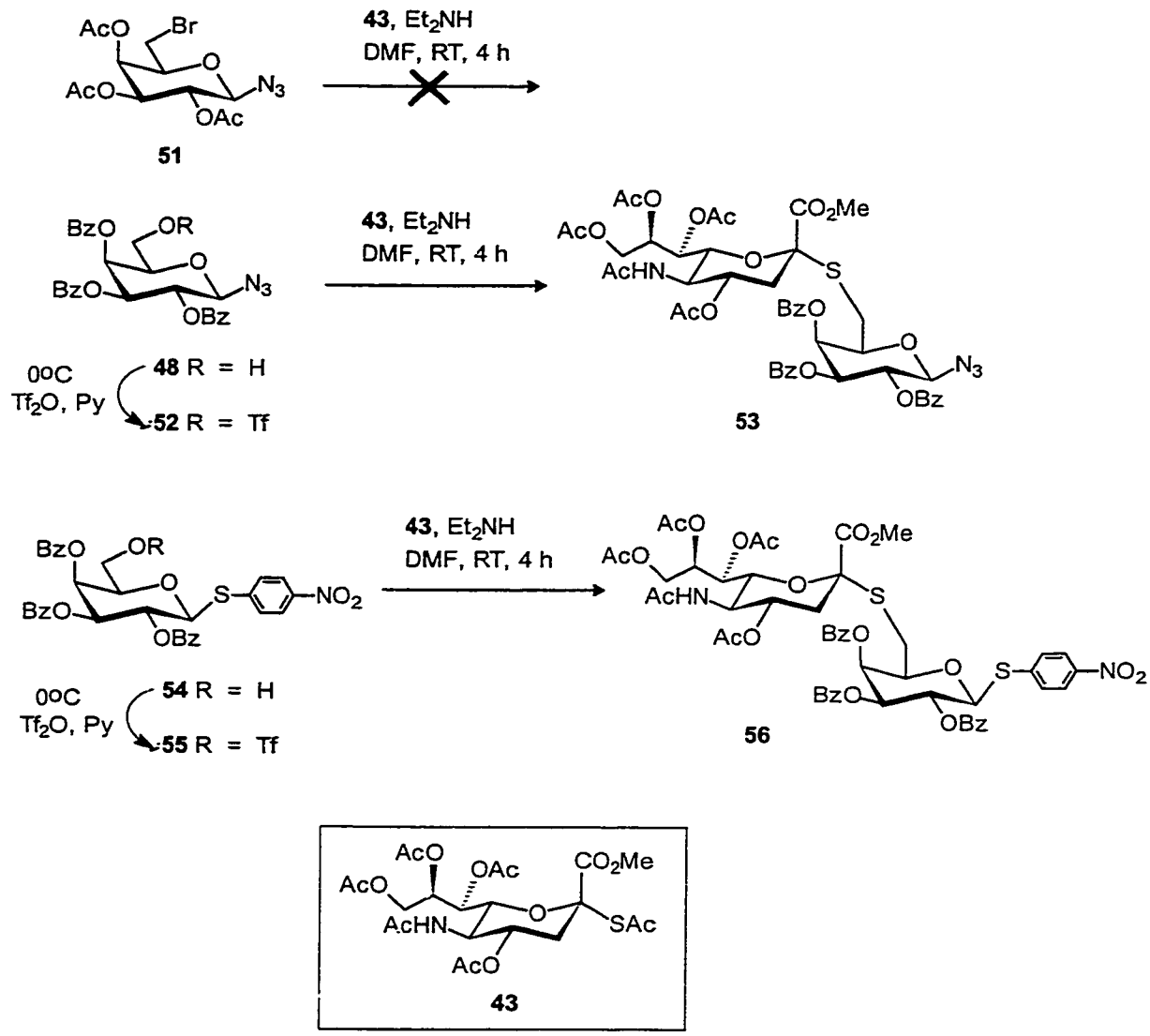
The 6-tosylate derivative **50** was prepared from β-D-galactopyranosyl azide **45a** by treatment with tosyl chloride in pyridine, followed by in situ acetylation (acetic anhydride-pyridine). The tosylate derivative **50** was reacted with lithium bromide in butanone under reflux to afford 6-bromo derivative **51** in 72% yield (Scheme **4.5**).



45a **50** **51**

Scheme 4.5 Synthesis of 6-bromogalactosyl azide **51**

Reagents and conditions: (a) i) TsCl, pyridine; ii) Ac_2O, pyridine; 85% (b) LiBr, 2-butanone, reflux, 24 h, 72%.



Scheme 4.6 Syntheses of α-(2→6)-thioglycoside of Neu5Ac

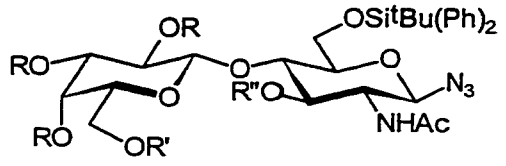
6-Bromo galactose derivative **51** and methyl 5-acetamide-4,7,8,9-tetra-*O*-acetyl-2-*S*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate (**43**) were treated with diethylamine in DMF. Unlike 6-bromo glucose or GlcNAc derivatives,[145] no desired α-(2→6)-thioglycoside was observed even after 1 day. The steric hindrance of the *O*-4 group reduced the reactivity of Br-6, thus leading to this unsuccessful coupling reaction. However, a better leaving group such as triflate group can solve this problem. 2,3,4-Tri-*O*-benzoyl-β-D-galactopyranosyl azide (**48**) was treated with triflic anhydride in pyridine to afford the 6-

triflate derivative **52**. Exposure of sialic acid **43** and the 6-triflate derivative **52** to diethylamine furnished the α-(2→6)-thioglycoside **53** in 73% yield after only 4 h at room temperature. In this reaction, selective deprotection of the anomeric thioacetate **43** by diethylamine successfully generated the sialic acid thiol intermediate to couple with the 6-triflate derivative **52**. Encouraged by the result, we also tried the coupling reaction of thioacetate sialic acid **43** with the other galactosyl acceptor **54**. This reaction was performed under the same conditions described above for the synthesis of disaccharide **53**. Again, α-(2 →6)-thioglycoside derivative **56** was obtained in 66% yield.

4.2.3 Synthesis of Neu5Acα(2→6)Galβ(1→4)GlcNAc trisaccharide

Neu5Acα(2→6)Galβ(1→4)GlcNAc trisaccharide is also a receptor ligand for virus hemagglutinin and the neuraminidase.

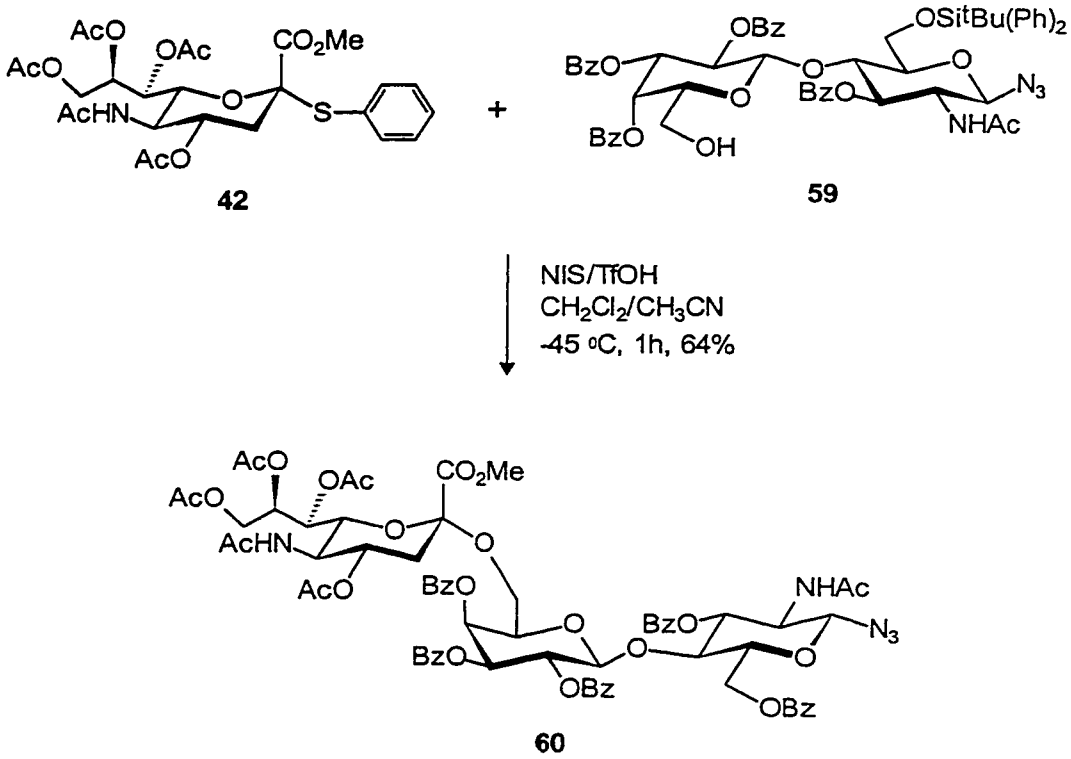
The starting material, 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide (**18**), was conveniently prepared by using the regiospecific synthetic method well described in Chapter 2. Catalytic deacetylation (NaOMe/MeOH) of compund **18** afforded β-D-galactopyranosyl-(1 →4)-2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide (**57**) quantitatively. Successive treatment of with *tert*-butyldimethylsilyl chloride and benzoyl chloride in dry pyridine gave lactosamine derivative **58** in 91% overall yield. *Tert*-butyldiphenylsilyl (TBDPS) was shown to be about 250 times more stable than *tert*-butyldimethylsilyl (TBDMS) toward acid-catalysed hydrolysis.[146] Thus selective desilylation of TBDMS and TBDPS sugars can be performed well under acidic conditions like HCl in MeOH. Treatment of disilyl ethers of the lactosamine derivative with 3% hydrogen chloride in methanol/ether (1:1, v/v), which was freshly prepared from acetyl chloride and methanol, afforded the 6'-O-deprotected lactosamine acceptor **59** in 88% yield (Scheme **4.7**).



		R	R'	R"
a	**18**	Ac	Ac	H
b	**57**	H	H	H
	58	Bz	$^tBuMe_2Si$	Bz
c	**59**	Bz	H	Bz

Scheme 4.7 Preparation of lactosamine acceptor **59**

Reagents and conditions: (a) NaOMe/MeOH; (b) i: $^tBuMe_2SiCl$, pyridine; ii: BzCl, pyridine, 91%; (c) AcCl, MeOH, 30 min, 88%.



Scheme 4.8 Synthesis of NeuAcα2,6Galβ1,4GlcNAc trisaccharide **60**

---

[146] Kocienski, P. J. *Protecting Groups*, Georg Thieme, Stuttgart, **1994**.

The glycosylation of lactosamine acceptor **59** with phenyl thiosialoside donor **42** in acetonitrile and dichloromethane for 1 h at -45 °C in the presence of NIS/TfOH gave the expected α-sialoside in 64% yield. No β-glycoside of Neu5Ac was isolated (Scheme **4.8**). The observed chemical shift for H-3"e in the Neu5Ac moiety is characteristic of the α-sialoside linkage.

4.2.4 Synthesis of Neu5Acα(2→3)Galβ(1→4)Glc (GM_3) trisaccharide

Ganglioside GM_3 (Figure **4.7**) was first isolated from equine erythrocytes by Yamakawa and Suzuki in 1952.[147] It has been known to serve as the precursor of many complex gangliosides in the biosynthetic pathway[148] and is overexpressed in tumor cells.[149] It also has been shown to modulate the epidermal growth factor (EGF) and the platelet-derived growth factor (PDGF) receptors[150]. Because of difficult isolation of homogeneous GM_3 gangliosides from natural sources, effective syntheses are required.

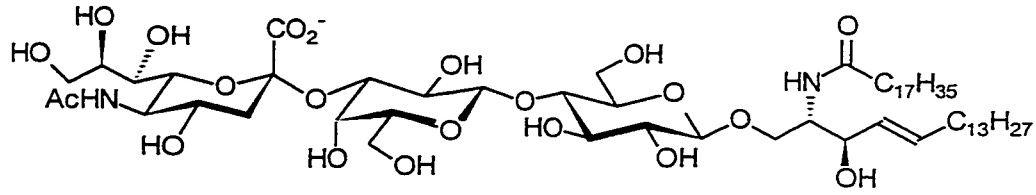


Figure 4.7 Structure of GM_3

The benefits of using lightly protected acceptors, which result mostly from the lack of additional steric impediments, was already recognized by several groups.[151] The synthesis of

---

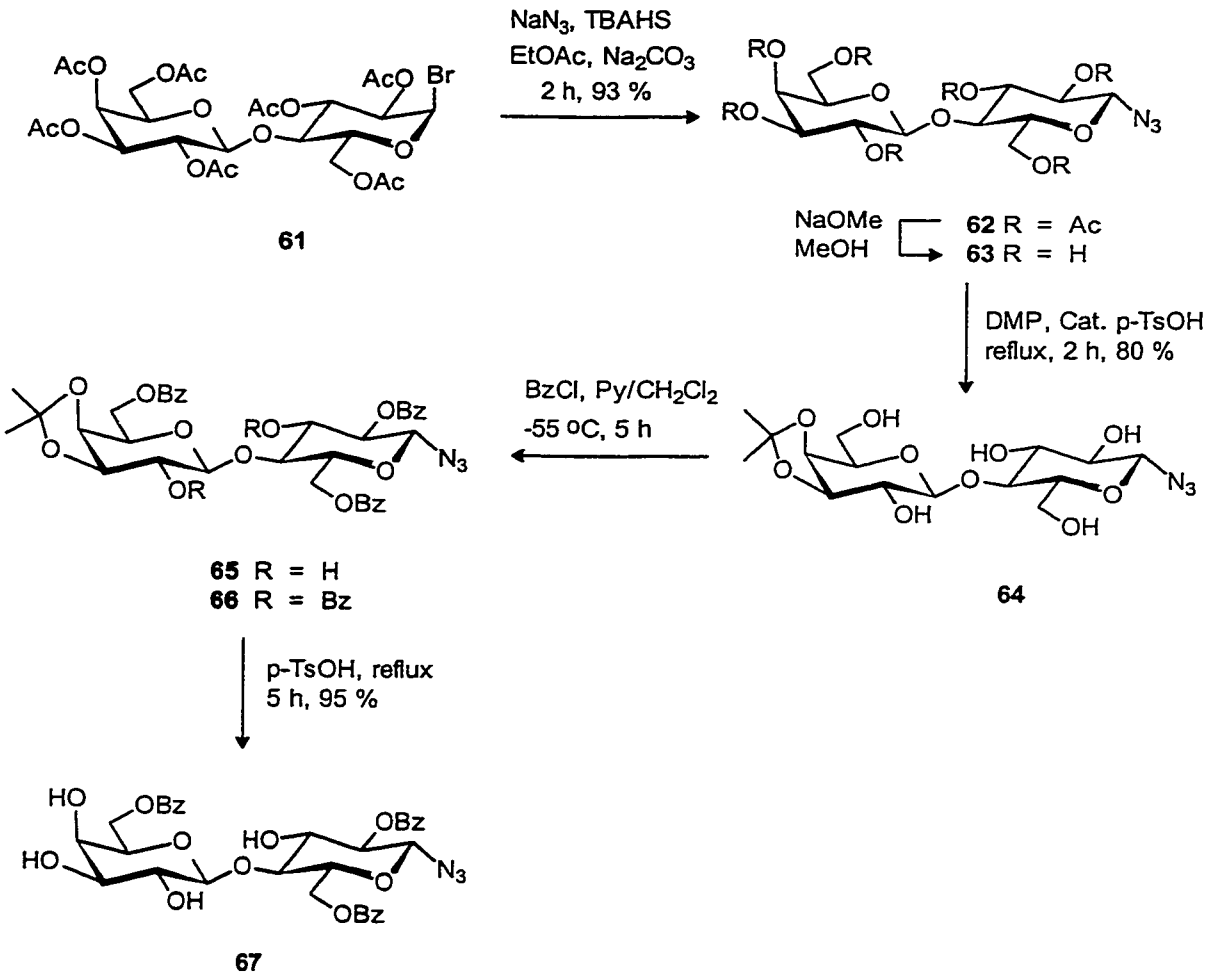
[147] Yamakawa, T.; Suzuki, S. *J. Biochem.* (Tokyo), **1952**, *39*, 383.
[148] Bouhours, D.; Bouhours, J.-F. *J. Bio. Chem.* **1991**, *266*, 12944.
[149] Portoukalian, J.; Zwingelstein, G.; Dore, J.-F.; Borgion, J.-J. *Biochem.* **1976**, *56*, 1285.
[150] a) Bremer, E.; Schlessinger, J.; Hakomori, S. *J. Bio. Chem.* **1986**, *261*, 2434; b) Hanai, N.; Nores, G. A.; Torres-Mendez, C. R.; Hakomori, S. *Biochem. Biophys. Res. Commun.* **1987**, *147*, 127.
[151] a) Kanie, O.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1988**, *7*, 501; b) Schmidt, R. R. *Pure Appl. Chem.* **1989**, *61*, 1257; c) Ito, Y.; Ogawa, T. *Tetrahedron* **1990**, *46*, 89.

the GM_3 trisaccharide analog first required the preparation of lightly protected lactoside building blocks.

Hepta-*O*-acetyl-D-lactosyl azide **62** was prepared in 93% yield under PTC conditions by treatment of lactosyl bromide **61** with sodium azide. The β configuration of the lactosyl azide was confirmed by ^{1}H NMR spectroscopy ($J_{1,2}$ 8.6 Hz). Subsequent Zemplén deacetylation of compound **62** afforded β-D-lactosyl azide **63** quantitatively. Regioselective isopropylidene protection of the 3' and 4' OH groups of compound **63** was achieved by refluxing it with 2,2-dimethoxypropane in the presence of a catalytic amount of *p*-toluenesulfonic acid. Briefly boiling the solution prior to the addition of the *p*-TsOH resulted in a significant improvement in yield (80%) of the desired product **64**.

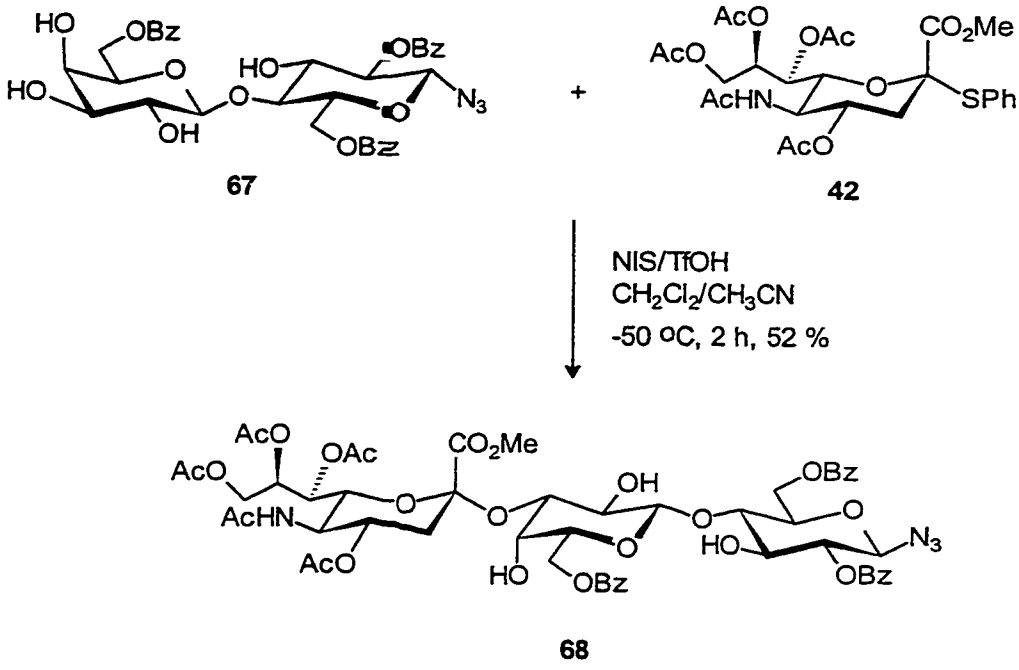
Regioselective benzoylation of compound **64** was achieved by using pyridine-dichloromethane as solvent and low temperature (-55 °C). The desired 2,6,6'-tri-*O*-benzoylated derivative **65** was obtained in 64% yield as major product, together with 2,6,2',6'-tetra-*O*-benzoylated derivative **66** (10%) and other partially benzoylated products. The benzoyl groups' positions of compound **65** can be unambiguously assigned from the H-2, H-6 and H-6' signals in the ^{1}H NMR spectrum which showed a downfield-shifted H-2 signal at δ 5.17 ppm, H-6a signal at δ 4.92 ppm, and H-6a' signal at δ 4.79 ppm.

The isopropylidene group in tribenzoylated lactoside **65** could be removed by refluxing in methanol in the presence of *p*-TsOH as catalyst. This procedure gave lightly protected lactosyl acceptor **67** in 95% yield (Scheme **4.9**). The 2',3',4'-OH free lactosyl acceptor allows selective 3'-*O*-α-sialylation with thiophenyl sialyl donor **42** to produce GM_3 trisaccharide analog **68**.



Scheme 4.9 Synthesis of lactosyl acceptor **67**

Again, the glycosylation of lactosyl acceptor **67** with phenyl thiosialyl donor **42** in acetonitrile and dichloromethane for 2 h at -50 °C in the presence of NIS/TfOH gave the expected α-sialoside **68** in 52% yield (Scheme **4.10**). No β-glycoside of Neu5Ac was isolated. The α-configuration at C-2 in GM_3 derivative was readily assigned from the ^1H NMR spectrum which contained a downfield-shifted doublet for H-3e at δ 2.68 ppm. The regioselectivity for the introduced interglycosidic linkage in **68** was determined by its ^1H and ^{13}C NMR spectra. The H-3' signal in **68** was shifted downfield from 3.64 to 4.14 ppm upon sialylation and the anomeric carbon (C-2") of sialic acid was shifted downfield from 87.5 to 97.5 ppm by substituting the anomeric sulfur atom by oxygen (Figures **4.8** and **4.9**).



Scheme 4.10 Synthesis of GM_3 trisaccharide **68**

2,2',6,6'-Tetra-*O*-benzoyl-β-D–lactosyl azide **66**, obtained from the selective benzoylation as a byproduct, was considered as a precursor for synthesis of Lewis A trisaccharide. Fucosylation of the lactosyl acceptor **66** with phenyl 2,3,4-tri-*O*-acetyl-1-thio-β -L-fucopyranoside (**32**) by using DMTST as promoter in benzene-dichloromethane at 0°C afforded trisaccharide **69** in good yield (89%). The α-configuration of the newly introduced anomeric center of **69** was determined from the ^{1}H NMR spectrum which showed a signal for H-1 (fucosyl moiety) at δ 5.40 ppm ($J_{1'',2''}$ 3.7 Hz). Deprotection of the isopropylidene group in **69** was first tested by using refluxing methanol in the presence of *p*-TsOH as catalyst. Unfortunately, the glycosidic bond between lactosyl moiety and fucosyl moiety was unstable. Thus a weaker acid such as acetic acid was used to deprotect the isopropylidene group. Treatment of trisaccharide **69** with 80% acetic acid at 60 °C afforded product **70** in 94% yield (Scheme **4.11**).

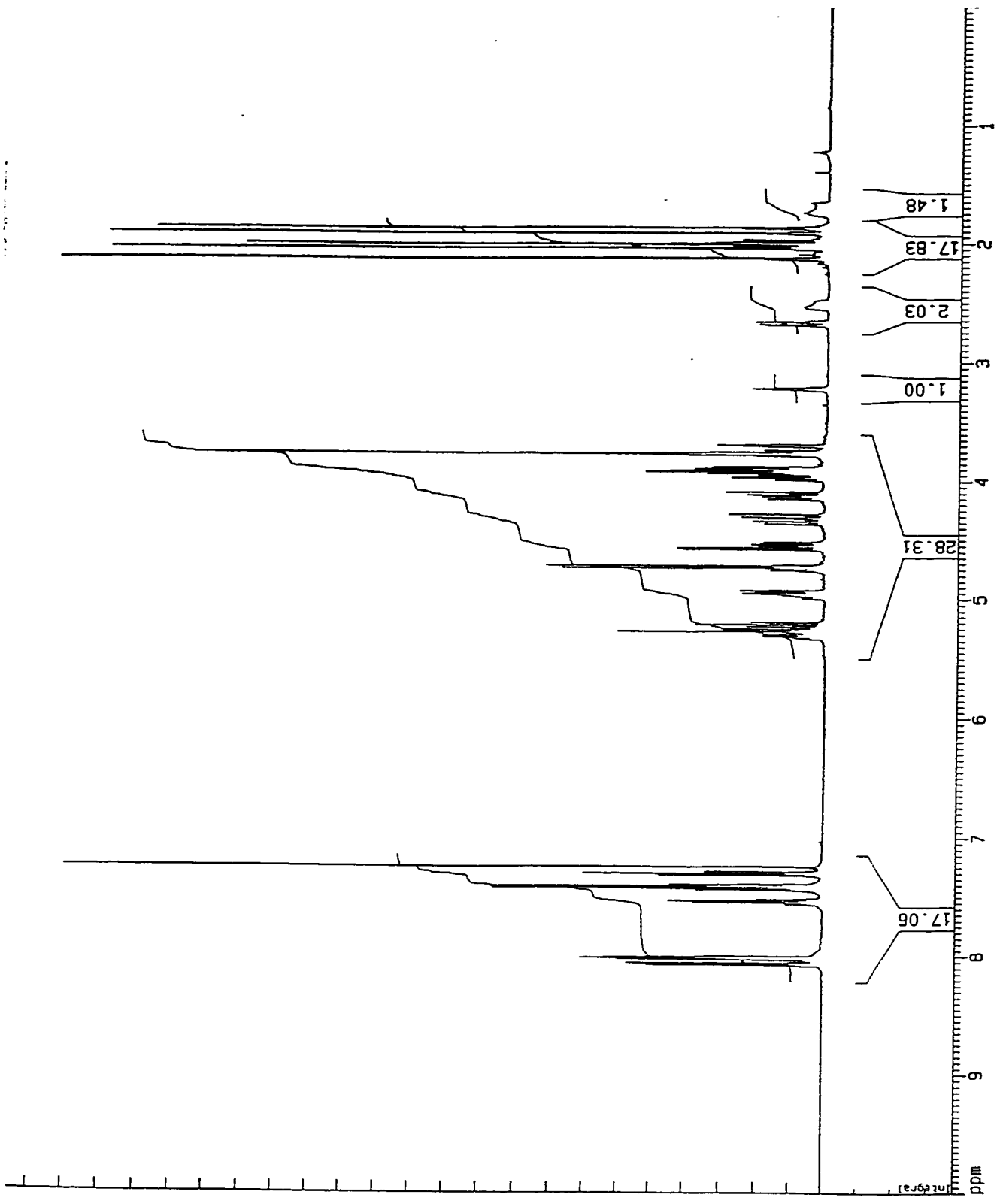


Figure 4.8 ^{1}H NMR (500MHz, $CDCl_3$) spectrum of GM_3 trisaccharide **68**

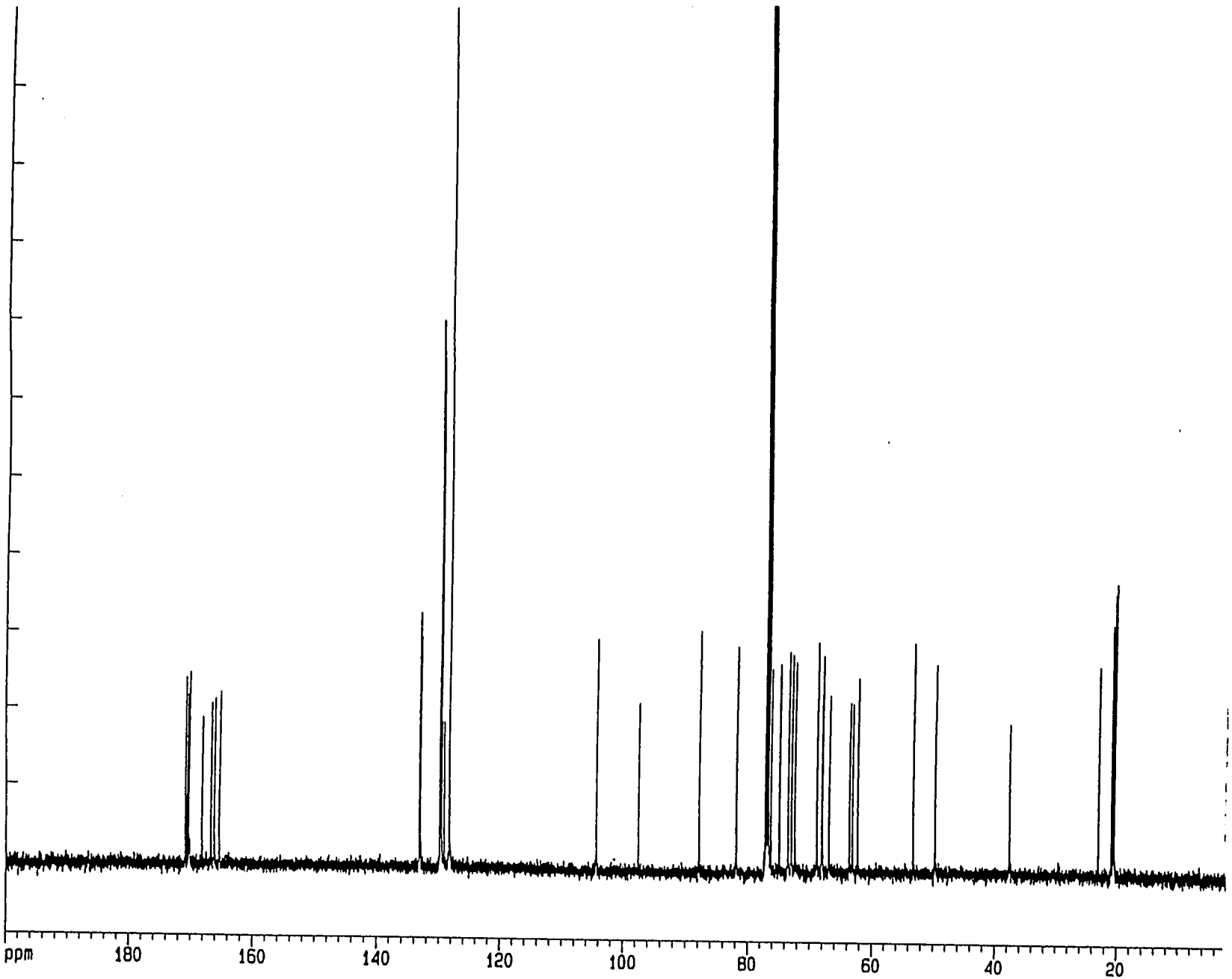
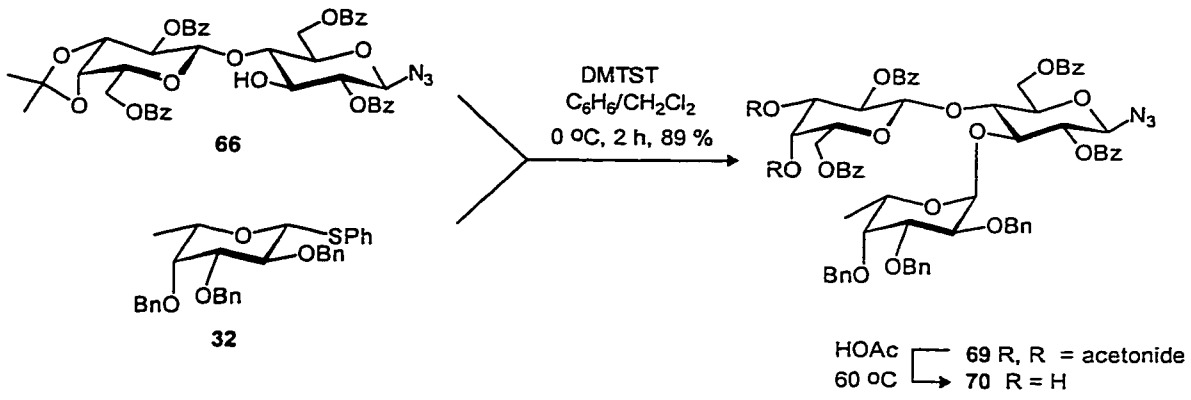


Figure 4.9 ^{13}C NMR (500MHz, $CDCl_3$) spectrum of GM_3 trisaccharide **68**



Scheme 4.11 Fucosylation of lactosyl acceptor **66**

4.3 Conclusion

In conclusion, the facile regio- and α-stereoselective glycosidation of Neu5Ac with several glycosyl acceptors was achieved using a thiophilic promoter (NIS/TfOH) in acetonitrile under kinetically controlled conditions. The syntheses of α(2→6) thioglycosides of Neu5Ac were also achieved in situ using diethylamine. These sialoside derivatives with their azido or *p*-nitrothiophenyl aglycone can be simply transformed into various neoglycoconjugates using well-established strategy.[152]

4.4 Experimental

Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate (41)

N-Acetylneuraminic acid **40** (5.00 g, 16.2 mmol) and Amberlite IR-120 H^+ resin (10 g) in methanol (250 mL) were stirred for 24 h at room temperature until completion of the

reaction (TLC isopropyl alcohol-H_2O, 7:3). The clear solution was filtered and evaporated to dryness. The residue was recrystallized from methanol/ether. Methyl ester **40a** (4.91 g) was isolated as white crystals in 94% yield: m.p. 182-185 °C; $[\alpha]_D$ -27.6° (*c* 1.1, H_2O). Lit.[153] m.p. 180-182 °C; $[\alpha]_D$ -28° (*c* 1.0, H_2O).

Methyl ester **40a** (4.00 g, 12.37 mmol) was combined with 200 μL MeOH in acetyl chloride (100 mL). The mixture was sealed tightly and kept stirring for 48 h at room temperature. The progress of the reaction was monitored by TLC (ethyl acetate). The resulting clear solution was evaporated under reduced pressure and co-evaporated with toluene to remove residual HCl. The chloride product was dried under vacuum and used for subsequent reactions without further purification. It was isolated in 98% yield. This product can be recrystallized from CH_2Cl_2/ether to give pure **41** as white crystals: m.p. 105-108 °C; $[\alpha]_D$ -62.4° (*c* 1.0, $CHCl_3$). Lit.[154] m.p. 105.0 °C (dec.); $[\alpha]_D$ -68.0° (*c* 1.0, $CHCl_3$).

Phenyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosid) onate (42)

To a solution of freshly prepared acetochloroneuraminic acid **41** (6.0 g, 11.77 mmol) in ethyl acetate (100 mL) was added 1 M sodium carbonate (100 mL) containing TBAHS (4.0 g, 11.77 mmol). To the reaction mixture was added thiophenol (1.81 mL, 17.66 mmol). The mixture was stirred vigorously for 1 h at room temperature. The reaction mixture was next diluted with EtOAc (100 mL). The separated organic phase was washed with sat. aq. sodium bicarbonate and brine. The organic phase was then dried (Na_2SO_4), filtered and evaporated under reduced pressure. The resulting syrup was purified by silica gel chromatography using ethyl acetate as eluent to give **42** (5.87 g, 87%): m.p. 140-141 °C; $[\alpha]_D$ +24.8° (*c* 1.0, $CHCl_3$). Lit.[155] m.p. 139-140 °C; $[\alpha]_D$ +23° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ (ppm) 7.49-7.27 (m, 5 H, aromatic H), 5.27 (bs, 1 H, NH), 5.26 (dd, 1 H, $J_{7,8}$ 7.2 Hz, H-7), 5.23 (m, 1 H, H-8), 4.80 (ddd, 1 H, $J_{4,5}$ 10.1 Hz, H-4), 4.34 (d, 1 H, $J_{8,9a}$ 2.5, $J_{9a,9b}$ 12.4 Hz, H-9a), 4.18 (dd, 1 H, $J_{8,9b}$ 4.9 Hz, H-9b), 3.95 (dd, 1 H, $J_{5,6}$ 9.7 Hz, H-5), 3.85 (dd, 1 H, $J_{6,7}$ 1.6 Hz, H-6), 3.53 (s, 3 H, OCH_3), 2.77 (dd, 1 H, $J_{3a,3e}$ 12.8 Hz, $J_{3e,4}$ 4.6 Hz, H-3e), 2.05 (dd, 1 H,

---

[152] Roy, R. in *Carbohydrate Chemistry*; Boons, G.-J. (Ed.); Chapman & Hall, UK; **1998**, 243.
[153] Ogura, H.; Furuhata, K. *Carbohydr. Res.* **1986**, *158*, 37.
[154] Sharma, M. N.; Edy, R. *Carbohydr. Res.* **1984**, *127*, 201.
[155] Rothermel, J.; Faillard, H. *Biol. Chem. Hoppe-Seyler* **1989**, *370*, 1077.

$J_{3a,4}$ 11.6 Hz, H-3a), 2.10, 2.02, 2.00, 1.98, 1.82 (s, 15 H, OAc's, NHAc); ^{13}C NMR ($CDCl_3$) δ 171.0-170.0 (O=C's), 168.0 (C-1), 87.5 (C-2), 74.7, 70.1, 69.7, 67.8 (C-4, C-6, C-7, C-8), 62.0 (C-9), 52.6 (OCH_3), 49.2 (C-5), 38.0 (C-3), 23.0, 21.0, 20.8, 20.6 (NHAc, OAc's); M.S. (C. I. ether) M/Z: 584.0 ($[M+1]^+$, 36.2 %).

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 (43)

To a solution of freshly prepared acetochloroneuraminic acid **41** (2.30 g, 4.51 mmol) in EtOAc (25 mL) was added a solution of thioacetic acid (0.70 mL, 9 mmol) and TBAHS (1.53 g, 4.51 mmol) in 1 M Na_2CO_3 (25 mL). The mixture was stirred vigorously for 1 h at room temperature and next diluted with EtOAc (50 mL). The organic phase was separated and washed with brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatograph using EtOAc to give compound **43** (1.85 g, 72%): m.p. 80-85 °C; $[\alpha]_D$ +49.0 ° (*c* 1.1, $CHCl_3$); Lit.[155] m.p. 75-80 °C; $[\alpha]_D$ +46.7 ° (*c* 1.0, MeOH).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl azide (45)

To a solution of freshly prepared acetobromogalactose **44** (10 g, 24.3mmol) in ethyl acetate (100 mL) was added 1 M sodium carbonate (100 mL) containing TBAHS (8.26 g, 24.3 mmol). To the reaction mixture was added sodium azide (2.37 g, 36.5 mmol). The mixture was stirred vigorously for 2 h at room temperature. The reaction mixture was next diluted with EtOAc (100 mL). The separated organic phase was washed with sat. aq. sodium bicarbonate and brine. The organic phase was then dried (Na_2SO_4), filtered and evaporated under reduced pressure. The resulting syrup was purified by silica gel chromatography using ethyl acetate as eluent to give **45** (8.61 g, 95%): m.p.102-104 °C; $[\alpha]_D$ -14.5° (*c* 1, $CHCl_3$); Lit.[156] m.p. 103-104 °C; $[\alpha]_D$ -14.0° (*c* 1, $CHCl_3$).

6-*O*-*Tert*-butyldimethylsilyl-β-D-galactopyranosyl azide (46)

To a suspension of peracetyl-β-D-galactopyranosyl azide **45** (7.20 g, 19.3 mmol) in methanol (100 mL), sodium methoxide in methanol (0.3 mL, 1.0 M) was added. After

stirring for 2 h at room temperature, the reaction was quenched with H^+ resin (Dowex 50W-X8) and then filtered. The filtrate was evaporated under reduced pressure. Toluene was added and distilled from residue. The crude product **45a** was then dissolved in dry pyridine (20 mL). *Tert*-butyldimethylsilyl chloride was added at 0 °C. The reaction mixture was then allowed to attain room temperature and stirred for 3 h. The reaction mixture was poured into ice-water and extracted with dichloromethane (3 x 20 mL). The combined extracts were washed successively with 5% hydrochloric acid, saturated $NaHCO_3$ solution, and water. The organic extract was dried (Na_2SO_4), filtered, and concentrated. The crude product was subjected to column chromatography with dichloromethane and methanol (10:1, v/v) as eluent to give **46** (5.29 g, 86%): $[\alpha]_D$ -31.5° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.50 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 0.87 (s, 9 H, t-Butyl), 0.06 (s, 6 H, $SiMe_2$); ^{13}C NMR ($CDCl_3$) δ 90.7 (C-1), 76.6 (C-5), 73.7 (C-3), 70.6 (C-2), 69.1 (C-4), 62.6 (C-6), 25.8 ($SiC\underline{Me}_3$), 18.2 ($Si\underline{C}Me_3$), -4.1, -4.2 ($Si\underline{Me}_2$); HRMS calcd for $C_{12}H_{25}N_3O_5Si_1$ (M^+) *m/z* 319.1563, found 319.1585.

2,3,4-Tri-*O*-benzoyl-6-*O*-*tert*-butyldimethylsilyl-β-D-galactopyranosyl azide (47)

To a solution of **46** (1.85 g, 5.80 mmol) in dry pyridine (10 mL) at 0°C was added benzoyl chloride (3.0 mL, 26.1 mmol) slowly. The solution was then stirred at room temperature for 2 h. TLC showed the reaction was complete. Methanol (1 mL) was added and stirred for additional 15 min. The mixture was concentrated under reduced pressure. The crude product was purified by column chromatography using ethyl acetate-hexane (1:4, v/v) as eluent to give **47** (3.22 g, 88%): $[\alpha]_D$ +76.3° (*c* 1.4, chloroform); ^1H NMR ($CDCl_3$) δ 8.10-7.16 (m, 15 H, aromatic H), 6.05 (d, 1 H, $J_{3,4}$ 1.9 Hz, H-4), 5.78-5.73 (m, 2 H, H-2, H-3), 5.00 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.12-3.88 (m, 3 H, H-5, H-6a, H-6b), 0.86 (s, 9 H, t-Butyl), 2.61, -7.28 (2s, 6 H, $SiMe_2$);^{13}C NMR ($CDCl_3$) δ 165.4, 165.2 (C=O's), 133.4-128.2 (18 C, aromatic C), 88.5 (C-1), 76.1 (C-5), 71.7 (C-3), 69.2 (C-2), 68.6 (C-4), 60.7 (C-6), 25.7 ($SiC\underline{Me}_3$), 18.1 ($Si\underline{C}Me_3$), -4.2, -4.3 ($Si\underline{Me}_2$).

2,3,4-Tri-*O*-benzoyl-β-D-galactopyranosyl azide (48)

Acetyl chloride (1 mL) was added dropwise to methanol (20 mL) and the hydrogen chloride solution obtained was cooled to 20 °C. A solution of the silyl derivative **47** (3.00 g,

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

4.75 mmol) in ethyl ether (20 mL) was added. The reaction mixture was stirred at room temperature for 30 min (TLC solvent). The reaction mixture was poured into aqueous sat. $NaHCO_3$. The solution was extracted with ethyl ether. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by silica gel chromatography using hexane and ethyl acetate (1.5:1, v/v) as eluent to afford **48** (2.26 g, 92%); $[\alpha]_D$ +127° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.13-7.16 (m, 15 H, aromatic H), 5.85 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 5.76 (dd, 1 H, H-2), 5.61 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-3), 4.94 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.12 (t, 1 H, $J_{5,6a,5,6b}$ 6.6 Hz, H-5), 3.85 (dd, 1 H, $J_{6a,6b}$ 10.2 Hz, H-6a), (3.65 (dd, 1 H, H-6b); ^{13}C NMR ($CDCl_3$) δ 166.7, 165.4 (C=O's), 133.9-128.4 (18 C, aromatic C), 88.6 (C-1), 76.1 (C-5), 71.6 (C-3), 69.1 (C-2), 68.6 (C-4), 60.4 (C-6); HRMS calcd for $C_{27}H_{24}N_3O_8$ (MH^+) *m/z* 518.1563, found 518.1602.

(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α or (β)-D-*galacto*-2-nonulopyranosylonate)-(2→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl azide (49a or 49b)

To a solution of galactose acceptor **48** (200 mg, 0.39 mmol) and sialic acid donor (410 mg, 0.70 mmol) in dry CH_2Cl_2/CH_3CN (1:1, 5 mL) was added molecular sieves 4 Å (650 mg) and the mixture was stirred for 1 h at room temperature. The mixture was then cooled to -45 °C and *N*-iodosuccinimide (176 mg, 0.78 mmol)) and trifluoromethanesulfonic acid (35 μL, 0.39 mmol) were added. The reaction mixture was stirred at -45 °C under nitrogen. The progress of the reaction was monitored by TLC until the donor was completely consumed. The reaction mixture was diluted with CH_2Cl_2 and filtered through a layer of celite. The filtrate was washed with 10 % $Na_2S_2O_3$, sat. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography using ethanol-dichloromethane as eluent (1:20, v/v) to afford disaccharides α form **49a** (252 mg, 66 %) and β form **49b** (35 mg, 9%).

49a (α): m.p. 154-155 °C; $[\alpha]_D$ +31.5° (*c* 1.0, chloroform); ^1H NMR ($CDCl_3$) δ 8.03-7.20 (m, 15 H, aromatic H), 6.00 (dd, 1 H, $J_{4,5}$ 1.0, $J_{3,4}$ 3.0 Hz, H-4), 5.66 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-3), 5.64 (dd, 1 H, H-2), 5.42 (m, 1 H, H-8'), 5.37-5.33 (m, 1 H, NH), 5.24 (dd, 1 H, $J_{6',7'}$ 2.0, $J_{7',8'}$ 10.7 Hz, H-7'), 5.04 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.77 (ddd, 1 H, H-4'), 4.40 (dd, 1 H, $J_{9a',9b'}$ 12.3, $J_{8',9a'}$ 2.7, H-9a'), 4.35 (ddd, 1 H, H-5), 4.10 (dd, 1 H, $J_{5',6'}$ 10.6 Hz, H-6'), 4.06-

3.99 (m, 2 H, H-9b', H-5'), 3.88 (dd, 1 H, $J_{5,6a}$ 5.8, $J_{6a,6b}$ 10.0 Hz, H-6a), 3.56 (dd, 1 H, $J_{5,6b}$ 8.7 Hz, H-6b), 3.46 (s, 3 H, OCH_3), 2.44 (dd, 1 H, $J_{3e',4'}$ 4.7, $J_{3a',3e'}$ 13.0 Hz, H-3e'), 2.19, 2.10, 2.01, 1.97, 1.85 (OAc's, NHAc), 1.90 (dd, 1 H, $J_{3a',4'}$ 12.7 Hz, H-3a'); ^{13}C NMR ($CDCl_3$) δ 170.9-165.0 (C=O's), 133.4-128.2 (18 C, aromatic C), 99.2 (C-2'), 88.5 (C-1), 73.8 (C-5), 72.6 (C-6'), 71.7 (C-3), 69.2 (C-2), 68.6 (C-4'), 68.4 (C-8'), 67.6 (C-4), 67.4 (C-7'), 63.0 (C-9'), 62.4 (C-6), 52.6 (OCH_3), 49.3 (C-5'), 37.8 (C-3'), 23.1 (NHAc), 20.9-20.7 (OAc's); HRMS calcd for $C_{47}H_{51}N_4O_{20}$ (MH^+) *m/z* 991.3097, found 991.3042.

49b (β): m.p. 102-104 °C; $[\alpha]_D$ +25.6° (*c* 1.0, chloroform); ^1H NMR ($CDCl_3$) δ 8.12-7.18 (m, 15 H, aromatic H), 6.02 (d, 1 H, $J_{3,4}$ 3.1 Hz, H-4), 5.70 (dd, 1 H, H-2), 5.65 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-3), 5.13 5.10 (m, 2 H, H-8', NH), 5.07-5.01 (m, 2 H, H-7', H-4'), 5.92 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.53 (dd, 1 H, $J_{9a',9b'}$ 12.4, $J_{8',9a'}$ 2.6, H-9a'), 4.30 (dd, 1 H, $J_{5,6b}$ 6.7 Hz, H-5), 4.03 (m, 1 H, H-5'), 3.89 (dd, 1 H, $J_{8',9b'}$ 6.8 Hz, H-9b'), 3.78 (s, 3 H, OCH3), 3.74 (dd, 1 H, $J_{5,6a}$ 6.6, $J_{6a,6b}$ 9.8 Hz, H-6a), 3.64-3.58 (m, 2 H, H-6b, H-6'), 2.28 (dd, 1 H, $J_{3e',4'}$ 5.0, $J_{3a',3e'}$ 12.9 Hz, H-3e'), 2.05, 2.00, 1.93, 1.91, 1.76 (OAc's, NHAc), 1.80 (dd, 1 H, $J_{3a',4'}$ 12.8 Hz, H-3a'); ^{13}C NMR ($CDCl_3$) δ 170.6-165.2 (C=O's), 133.9-128.3 (18 C, aromatic C), 98.9 (C-2'), 88.4 (C-1), 73.7 (C-5), 71.8 (C-6'), 71.3 (C-3), 70.9 (C-8'), 69.2 (C-2), 68.8 (C-4'), 68.5 (C-4), 67.8 (C-7'), 62.2 (C-9'), 61.1 (C-6), 53.0 (OCH_3), 48.5 (C-5'), 36.8 (C-3'), 23.0 (NHAc), 21.0, 20.8, 20.7, 20.6 (OAc's); HRMS calcd for $C_{47}H_{51}N_4O_{20}$ (MH^+) *m/z* 991.3097, found 991.3081.

2,3,4-Tri-*O*-acetyl-6-*O*-*p*-toluenesulfonyl-β-D-galactopyranosyl azide (50)

To a solution of β-D-galactopyranosyl azide **45a** (2.80 g, 13.7 mmol) in dry pyridine (30 mL) was added *p*-toluenesulfonyl chloride (3.89 g, 20.5 mmol). After the reaction mixture was stirred at room temperature for 3 h, TLC (6 % CH_3OH in CH_2Cl_2) showed complete conversion of the starting material to the 6-tosylated derivative. To this reaction mixture was added acetic anhydride (10 mL), and stirring was continued for an additional 4 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel chromatography using hexane and ethyl acetate (2:1, v/v) as eluent to afford product **50** (5.64 g, 85 %): $[\alpha]_D$ -14.8° (*c* 1.0, chloroform); ^1H NMR ($CDCl_3$) δ 7.74 (d, 2 H, *J* 8.4 Hz, aromatic H), 7.33 (d, 2 H, aromatic H), 5.37 (dd, 1 H, $J_{3,4}$ 3.1 Hz, $J_{4,5}$ 0.7 Hz, H-4), 5.07-4.94 (m, 2 H, H-2, H-3), 4.54 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.10-3.95 (m, 3 H, H-5, H-6a, H-6b), 2.42 (s, 3 H,

Me), 2.04, 2.03, 1.94 (s, 9 H, OAc's); ^{13}C NMR ($CDCl_3$) δ 169.8, 169.8, 169.3 (C=O's), 145.4, 132.0, 130.0, 128.0 (aromatic C), 88.1 (C-1), 72.6 (C-5), 70.4 (C-3), 67.7 (C-2), 66.6 (C-4), 66.0 (C-6), 21.6 (Me), 20.6, 20.4 (OAc's).

2,3,4-Tri-*O*-acetyl-6-bromo-6-deoxy-β-D-galactopyranosyl azide (51)

To a solution of the 6-tosylated derivative **50** (2 g, 4.12 mmol) in 2-butanone (10 mL) was added lithium bromide (1.07 g, 12.3 mmol). The mixture was refluxed for 24 h. The mixture was then poured into water (50 mL). The solution was extracted with dichloromethane (20 mL x 2). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography using hexane-ethyl acetate (3:1, v/v) as eluent to afford compound **51** (1.17 g, 72 %) as white crystals: m.p. 110-111 °C; $[\alpha]_D$ -9.1° (*c* 1.0, chloroform); ^1H NMR ($CDCl_3$) δ 5.55 (dd, 1 H, $J_{3,4}$ 3.0 Hz, $J_{4,5}$ 1.2 Hz, H-4), 5.15-4.98 (m, 2 H, H-2, H-3), 4.60 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 3.96 (dt, 1 H, $J_{5,6a} = J_{5,6b} = 6.4$ Hz, H-5), 3.45-3.26 (m, 2 H, H-6a, H-6b), 2.15, 2.05, 1.95 (s, 9 H, OAc's); ^{13}C NMR ($CDCl_3$) δ 169.8, 169.3 (C=O's), 88.2 (C-1), 75.1 (C-5), 70.6 (C-3), 67.7 (C-2), 67.2 (C-4), 27.2 (C-6), 20.6, 20.5, 20.4 (OAc's).

Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→6)-2,3,4-tri-*O*-benzoyl-6-thio-β-D-galactopyranosyl azide (53)

To compound **48** (353 mg, 0.68mmol) in dry CH_2Cl_2 (5 mL) at -15 °C were added triflate anhydrate (289 μL, 1.02 mmol) and pyridine (166 μL, 2.05 mmol). The solution was stirred at 0 °C for 30 min. The organic solution was washed with ice-cold 1% HCl, brine, and sat. $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product **52** was directly used in next step. To compound **52** and thioacetate sialic acid derivative **43** (250 mg, 0.45 mmol) in dry DMF (10 mL) was added diethylamine (0.94 mL, 9 mmol) under nitrogen. The solution was stirred at room temperature for 5 h. The solution mixture was concentrated under reduced pressure. The residue was purified by silica gel chromatography using dichloromethane and methanol (30:1, v/v) as eluent to afford disaccharide **53** (336 mg, 73%): m.p. 111-114 °C; $[\alpha]_D$ +34.3° (*c* 1.0, chloroform); ^1H NMR ($CDCl_3$) δ 8.01-7.18 (m, 15 H, aromatic H), 6.12 (d, 1 H, $J_{3,4}$ 3.2

Hz, H-4), 5.75 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-3), 5.63 (dd, 1 H, H-2), 5.48-5.38 (m, 2 H, NH, H-8'), 5.28 (m, 1 H, H-7'), 5.28 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 4.90 (ddd, 1 H, H-4'), 4.41 (dd, 1 H, $J_{9a',9b'}$ 12.4, $J_{8',9a'}$ 2.9 Hz, H-9a'), 4.35 (dd, 1 H, H-5), 4.15 (dd, 1 H, $J_{8',9b'}$ 5.9 Hz, H-9b'), 3.99-3.91 (m, 2 H, H-5', H-6'), 3.78 (s, 3 H, OCH_3), 2.90 (dd, 1 H, $J_{5,6a}$ 6.1, $J_{6a,6b}$ 14.9 Hz, H-6a), 2.75 (dd, 1 H, $J_{5,6b}$ 8.9 Hz, H-6b), 2.69 (dd, 1 H, $J_{3e',4'}$ 4.7, $J_{3a',3e'}$ 12.8 Hz, H-3e'), 2.24, 2.18, 1.99, 1.98, 1.87 (s, 15 H, OAc's, NHAc), 1.98 (dd, 1 H, $J_{3a',4'}$ 12.3 Hz, H-3a'); ^{13}C NMR ($CDCl_3$) δ 170.7-165.1 (C=O's), 133.4-128.1 (18 C, aromatic C), 87.9 (C-1), 84.6 (C-2'), 74.7 (C-5), 73.9 (C-6'), 71.8 (C-3), 69.1 (C-2), 69.0 (C-4'), 68.5 (C-4), 67.8 (C-8'), 67.0 (C-7'), 62.7 (C-9'), 53.1 (OCH_3), 49.4 (C-5'), 38.1 (C-3'), 29.8 (C-6), 23.1 (NHAc), 21.0-20.6 (OAc's); HRMS calcd for $C_{47}H_{51}N_4O_{19}S$ (MH^+) *m/z* 1007.2868, found 1007.2834.

4-Nitrophenyl methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→6)-2,3,4-tri-*O*-benzoyl-1,6-dithio-β-D-galactopyranoside (56)

To 4-nitrophenyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (**54**) (450 mg, 0.715 mmol) in dry CH_2Cl_2 (5 mL) at -15 °C were added triflate anhydrate (252 mg, 0.894 mmol) and pyridine (144 μL, 1.79 mmol). The solution was stirred at 0 °C for 30 min. The organic solution was washed with ice-cold 1% HCl, brine, and sat. $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product **55** was directly used in next step. To compound **55** and the thioacetyl sialic acid derivative **43** (200 mg, 0.364 mmol) in dry DMF (10 mL) was added diethylamine (0.81 mL, 7.28 mmol) under nitrogen. The solution was stirred at room temperature for 5 h. The solution mixture was concentrated under reduced pressure. The residue was purified by silica gel chromatography using dichloromethane and methanol (30:1, v/v) as eluent to afford disaccharide **56** (261 mg, 64%): m.p. 122-125 °C; $[\alpha]_D$ +34.3° (*c* 1.0, chloroform); ^1H NMR ($CDCl_3$) δ 8.14-7.15 (m, 15 H, aromatic H), 6.18 (d, 1 H, $J_{3,4}$ 2.8 Hz, H-4), 5.84 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-3), 5.76 (dd, 1 H, H-2), 5.67 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 5.59 (d, 1 H, $J_{5',NH}$ 4.1 Hz, NH,), 5.48 (m, 1 H, H-8'), 5.32 (d, 1 H, $J_{7',8'}$ 9.9 Hz, H-7'), 4.92 (m, 1 H, H-4'), 4.50-4.44 (m, 2 H, H-9a', H-5), 4.18 (dd, 1 H, $J_{9a',9b'}$ 12.4, $J_{8',9b'}$ 5.9 Hz, H-9b'), 4.05-3.95 (m, 2 H, H-5', H-6'), 3.81 (s, 3 H, OCH_3), 2.93 (dd, 1 H, $J_{5,6a}$ 6.7, $J_{6a,6b}$ 14.8 Hz, H-6a), 2.81 (dd, 1 H, $J_{5,6b}$ 7.6 Hz, H-6b), 2.73 (dd, 1 H, $J_{3e',4'}$ 4.3, $J_{3a',3e'}$ 12.9 Hz, H-3e'), 2.25, 2.20, 1.98, 1.92, 1.89 (s, 15 H,

OAc's, NHAc), 1.98 (dd, 1 H, $J_{3a',4'}$ 12.6 Hz, H-3a'); ^{13}C NMR ($CDCl_3$) δ 170.6-165.0 (C=O's), 146.3-123.5 (24 C, aromatic C), 84.3 (C-2'), 83.1 (C-1), 76.6 (C-5), 73.8 (C-6'), 72.7 (C-3), 69.0 (C-4'), 68.7 (C-4), 67.8 (C-8'), 67,5 (C-2), 66.8 (C-7'), 62.6 (C-9'), 53.1 (OCH_3), 49.3 (C-5'), 38.3 (C-3'), 30.3 (C-6), 23.0 (NHAc), 21.1-20.5 (OAc's); HRMS calcd for $C_{53}H_{54}N_2O_{21}S_2$ (MH^+) *m/z* 1119.2739, found 1119.2794.

β-D-Galactopyranosyl-(1→4)-2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide (57)

To compound **18** (600 mg, 0.74 mmol) in methanol (10 mL) was added 1 M sodium methoxide solution until pH was about 9. The solution was stirred at room temperature for 2 h. Then the solution was neutralized with H^+ resin. The mixture was filtered and concentrated under reduce pressure to afford product **57** in quantitative yield: m.p. 170 °C (dec); $[\alpha]_D$ -14.8° (*c* 0.4, MeOH); ^1H NMR (CD_3OD) δ 7.40-7.00 (m, 10 H, aromatic), 4.24 (d, 2 H, $J_{1,2}$, $J_{1',2'}$ 8.1 Hz, H-1, H-1'), 1.65 (s, 3 H, NHAc), 0.67 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (CD_3OD) δ 174.0 (C=O), 136.9-128.7 (aromatic C), 104.6 (C-1'), 89.7 (C-1), 87.8 (C-1), 78.7 (C-4), 78.5, 76.9, 74.8, 74.1, 72.4, 70.5, 63.0 (C-6'), 62.6 (C-6), 56.1 (C-2), 27.4 ($SiC(\underline{C}H_3)_3$), 22.9 (NHAc), 20.3 ($Si\underline{C}Me_3$); FAB-MS (glycerol) gave m/z (ion, relative intensity): 647.3 (M+1, 1.2%).

2,3,4-Tri-*O*-benzoyl-6-*O*-*tert*-butyldimethylsilyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide (58)

To compound **57** (400 mg, 0.62 mmol) in dry pridine (10 mL) was added *tert*-butyldimethylsilyl chloride (140 mg, 0.93 mmol). The solution was stirred at room temperature for 4 h. TLC showed the starting material dispeared. Then benzoyl chloride (0.43 mL, 3.72 mmol) was slowly added. The solution was stirred at room temperature for 2 h. TLC showed the reaction was complete. Methanol (1 mL) was added and stirred for additional 15 min. The mixture was concentrated under reduced pressure. The crude product was purified by column chromatography using dichloromethane-methanol (30/1, v/v) as eluent to give compound **58** (662 mg, 91%): m.p. 128-132 °C; $[\alpha]_D$ -33.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.99-6.50 (m, 31 H, aromatic H, NH), 5.76 (d, 1 H, H-4'), 5.67 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, H-2'),), 5.53 (dd, 1 H, $J_{2,3}$ 10.6 Hz, $J_{3,4}$ 9.5 Hz, H-3), 5.26 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3'), 5.10 (d, 1 H,

$J_{1,2}$ 9.4 Hz, H-1), 5.05 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.56 (ddd, $J_{2,NH}$ 9.5 Hz, 1 H, , H-2), 4.45 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.63 (dd, 1 H, $J_{5',6a'}$ 5.4, $J_{5',6b'}$ 9.5 Hz, H-5'), 3.57 (d, 1 H, $J_{6a,6b}$ 10.6 Hz, H-6a), 3.44 (d, 1 H, H-6b), 3.14 (dd, 1 H, $J_{6a',6b'}$ 9.7 Hz, H-6a'), 3.01 (d, 1 H, H-5), 2.45 (dd, 1 H, H-6b'), 1.91 (s, 3 H, NHAc), 1.15, 0.79 (2s, 18 H, 2 x $SiCMe_3$), -0.16, -0.20 (2s, 6 H, $SiMe_2$); ^{13}C NMR ($CDCl_3$) δ 170.5, 167.5, 165.1, 165.1, 164.6 (5 x C=O), 135.9-127.9 (Aromatic), 99.5 (C-1'), 88.6 (C-1), 77.0 (C-5), 74.3 (C-3), 73.4 (C-4), 73.4 (C-5'), 71.9 (C-3'), 69.9 (C-2'), 67.3 (C-4'), 60.3 (C-6), 58.9 (C-6'), 52.5 (C-2), 26.9, 25.7 (2x$SiC(\underline{C}H_3)_3$), 23.4 (NHAc), 19.4 ($Si\underline{C}Me_3$), 18.0 ($Si\underline{C}Me_3$), -5.7, -5.8 ($SiMe_2$); FAB-MS (glycerol) gave m/z (ion, relative intensity): 1177.5 (M+1, 0.2%).

Anal. Calcd for $C_{64}H_{72}N_4O_{14}Si_2$: C, 65.29; H, 6.16; N, 4.76. Found: C, 65.43; H, 6.18; N, 4.77.

2,3,4-Tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide (59)

Acetyl chloride (0.5 mL) was added dropwise to methanol (10 mL) and the hydrogen chloride solution obtained was cooled to 20 °C. A solution of the silyl derivative **58** (325 mg, 0.28 mmol) in ethyl ether (10 mL) was added. After stirred at room temperature for 30 min, the reaction mixture was poured into aqueous sat. $NaHCO_3$. The solution was extracted with ethyl ether. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by silica gel chromatography using methanol-dichloromethane as eluent (1:30, v/v) to afford disaccharide **59** (293 mg, 88%): m.p. 143-146 °C; $[\alpha]_D$ -14.7° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.12-6.49 (m, 31 H, aromatic H, NH), 5.75 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2'), 5.54 (dd, 1 H, $J_{2,3}$ 10.2 Hz, $J_{3,4}$ 9.5 Hz, H-3), 5.51 (d, 1 H, H-4'), 5.20 (dd, 1 H, $J_{3',4'}$ 3.2 Hz, H-3'), 5.12 (d, 1 H, $J_{1,2}$ 9.4 Hz, H-1), 5.05 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.56 (ddd, $J_{2,NH}$ 9.5 Hz, 1 H, H-2), 4.44 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.66 (dd, 1 H, $J_{5',6a'}$, $J_{5',6b'}$ 7.0 Hz, H-5'), 3.59 (d, 1 H, $J_{6a,6b}$ 10.7 Hz, H-6a), 3.42 (d, 1 H, H-6b), 3.01 (d, 1 H, H-5), 2.79 (m, 1 H, H-6a'), 2.41 (m, 1 H, H-6b'), 2.23 (t, 1 H, $J_{6a',OH}$, $J_{6b',OH}$ 7.5 Hz, OH), 1.80 (s, 3 H, NHAc), 1.14 (s, 9 H, $SiCMe_3$); ^{13}C NMR ($CDCl_3$) δ 170.5, 167.4, 166.4, 165.1, 164.7 (5 x C=O), 135.9-127.8 (Aromatic), 99.2 (C-1'), 88.7 (C-1), 77.0 (C-5), 74.2 (C-3), 73.6 (C-4), 73.5 (C-5'), 71.7 (C-3'), 69.8 (C-2'), 68.8 (C-4'), 60.2 (C-6), 59.2 (C-6'), 52.4 (C-2), 26.9 ($SiC(\underline{C}H_3)_3$), 23.4 (NHAc), 19.4 ($Si\underline{C}Me_3$); FAB-MS (glycerol) gave m/z (ion, relative intensity): 1063.4 (M+1, 0.5%).

Anal. Calcd for $C_{58}H_{58}N_4O_{14}Si$: C, 65.52; H, 5.50; N, 5.27. Found: C, 65.13; H, 5.61; N, 5.24.

Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→6)-(2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide (60)

To a solution of lactosamine acceptor **59** (50 mg, 0.047 mmol) and thiophenyl sialosyl donor **42** (49.5 mg, 0.085 mmol) in dry CH_2Cl_2/CH_3CN (1:1, 5 mL) was added molecular sieves 4 Å (100 mg) and the mixture was stirred for 4 h at room temperature. The mixture was then cooled to -45 °C and *N*-iodosuccinimide (21 mg, 0.094 mmol) and trifluoromethanesulfonic acid (4.2 μL, 0.047 mmol) were added. The reaction mixture was stirred at -45 °C under nitrogen. The progress of the reaction was monitored by TLC until the donor was completely consumed. The reaction mixture was diluted with CH_2Cl_2 and filtered through a layer of celite. The filtrate was washed with 10% $Na_2S_2O_3$, sat. $NaHCO_3$, and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography using methanol-dichloromethane as eluent (1:20, v/v) to afford trisaccharide **60** (46 mg, 64%): ^{1}H NMR ($CDCl_3$) δ 8.12-6.49 (m, 31 H, aromatic H, NH), 5.75 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2'), 5.54 (dd, 1 H, $J_{2,3}$ 10.2 Hz, $J_{3,4}$ 9.5 Hz, H-3), 5.51 (d, 1 H, H-4'), 5.20 (dd, 1 H, $J_{3',4'}$ 3.2 Hz, H-3'), 5.12 (d, 1 H, $J_{1,2}$ 9.4 Hz, H-1), 5.05 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.56 (ddd, $J_{2,NH}$ 9.5 Hz, 1 H, H-2), 4.44 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.66 (dd, 1 H, $J_{5',6a'}$, $J_{5',6b'}$ 7.0 Hz, H-5'), 3.59 (d, 1 H, $J_{6a,6b}$ 10.7 Hz, H-6a), 3.42 (d, 1 H, H-6b), 3.01 (d, 1 H, H-5), 3.46 (s, 3 H, OCH_3), 2.44 (dd, 1 H, $J_{3e'',4''}$ 4.7, $J_{3a'',3e''}$ 13.0 Hz, H-3e"), 2.19, 2.10, 2.01, 1.97, 1.85, 1.80 (s, 18 H, OAc's, NHAc), 1.90 (dd, 1 H, $J_{3a'',4''}$ 12.7 Hz, H-3a"), 1.14 (s, 9 H, $SiCMe_3$); ^{13}C NMR ($CDCl_3$) δ 170.5, 167.4, 166.4, 165.1, 164.7 (5 x C=O), 135.9-127.8 (Aromatic), 99.2 (C-1'), 88.7 (C-1), 77.0 (C-5), 74.2 (C-3), 73.6 (C-4), 73.5 (C-5'), 71.7 (C-3'), 69.8 (C-2'), 68.8 (C-4'), 60.2 (C-6), 59.2 (C-6'), 52.4 (C-2), 26.9 ($SiC(\underline{C}H_3)_3$), 23.4 (NHAc), 19.4 ($Si\underline{C}Me_3$).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-β-D-glucopyranosyl azide (62)

To a solution of freshly prepared acetolactosyl bromide **61** (1 g, 1.43 mmol) in ethyl acetate (5 mL) was added a solution of sodium azide (280 mg, 4.3 mmol) and tetrabutylammonium hydrogen sulfate (486 mg, 1.43 mmol) in 1M sodium carbonate (5 mL). The reaction mixture was vigorously stirred at room temperature for 2 h. The reaction was diluted with ethyl acetate (10 mL). The organic layer was separated and washed with saturated sodium chloride solution. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using hexane-ethyl acetate (1:1) as eluent to obtain pure product **62** (0.88 g, 93%): m.p. 67-70 °C; $[\alpha]_D$ -20.7° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.33 (dd, 1 H, $J_{4',5'}$ 1.1 Hz, H-4'), 5.22 (dd, 1 H, $J_{3,4}$ 9.4 Hz, H-3), 5.09 (dd, 2 H, $J_{2,3}$ 9.5 Hz, $J_{2',3'}$ 9.5 Hz, H-2, H-2'), 4.92 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3'), 4.64 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.45 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.48 (dd, 1 H, H-6a), 4.13-4.02 (m, 3 H, H-6b, H-6'), 3.85 (m, 1 H, H-5'), 3.68 (1 H, H-5); ^{13}C NMR ($CDCl_3$) δ 170.2, 170.1, 169.9, 169.8, 169.4, 169.3, 168.9, 101.0, 87.7, 75.7, 74.8, 72.5, 71.0, 70.9, 70.7, 69.0, 66.6, 61.7, 60.8, 20.9, 20.8, 20.7, 20.5.

Anal. Calcd for $C_{26}H_{35}N_3O_{17}$: C, 47.20; H, 5.33; N, 6.35. Found: C, 46.93; H, 5.31; N, 6.07.

β-D-Galactopyranosyl-(1→4)-β-D-glucopyranosyl azide (63)

To a solution of compound **62** (10.1 g, 15.3 mmol) in methanol (30 mL) was added 1 M NaOMe/MeOH solution until the pH of the solution reached 9. The reaction was complete after 1 h and the solution was neutralized to pH 7 by adding acetic acid. The solution was evaporated under reduced pressure to afford compound **63** (5.44 g, 99%) as white crystals: m.p. 113-114.5 °C; $[\alpha]_D$ -2.8° (*c* 1.0, H_2O); ^{1}H NMR (D_2O) δ 4.81 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 4.49 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'); ^{13}C NMR (D_2O) δ 102.4 (C-1'), 89.4 (C-1), 77.3 (C-4), 76.2 (C-5), 74.9 (C-5'), 73.8 (C-3), 72.1 (C-2, C-3'), 70.5 (C-2'), 68.1 (C-4'), 60.6 (C-6), 59.4 (C-6'); FAB-MS for $C_{12}H_{21}N_3O_{10}$ 368.3 (M+1).

Anal. Calcd for $C_{12}H_{21}N_3O_{10}$: C, 44.23; H, 6.19; N, 10.32. Found: C, 44.00; H, 6.19; N, 10.38.

3,4-*O*-Isopropylidene-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl azide (64)

To lactosyl azide **63** (3.00 g, 8.17 mmol) was added 2,2-dimethoxypropane (15 mL). The solution was refluxed for 1 h, and then catalytic amount of *p*-TsOH was added. The solution continued to be refluxed until white precipitates appeared. The reaction mixture was cooled to room temperature, and more precipitate was formed. The white solid was filtered and washed with cold EtOAc. The resulting solid was recrystallized with MeOH to give white needle crystals **64** (2.66 g, 80%): m.p. 180-181 °C; $[\alpha]_D$ +12.0° (*c* 1.0, CH_3OH); ^1H NMR (D_2O) δ 4.77 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 4.49 (d, 1 H, $J_{1',2'}$ 8.4 Hz, H-1'), 4.37 (dd, 1 H, $J_{4',5'}$ 2.0 Hz, H-4'), 4.22 (dd, 1 H, $J_{3',4'}$ 5.2, $J_{2',3'}$ 8.0 Hz, H-3'), 1.55, 1.39 (2 s, 6 H, Me); ^{13}C NMR (D_2O) δ 111.9, 103.0, 90.8, 79.4, 78.9, 77.5, 75.1, 74.6, 74.3, 73.6, 73.4, 61.6, 60.6, 28.0, 26.2.

Anal. Calcd for $C_{15}H_{25}N_3O_{10}$: C, 51.78; H, 5.49; N, 8.23. Found: C, 51.78; H, 5.74; N, 8.03.

6-*O*-Benzoyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl-(1→4)-2,6-di-*O*-benzyol-β-D-glucopyranosyl azide (65) and 2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-2,6-di-*O*-benzoyl-β-D-glucopyranosyl azide (66)

The 3',4'-*O*-isopropylidene β-D-lactoside derivative **64** (3 g, 7.37 mmol) was dissolved in a 2:1 mixture solution (45 mL) of pyridine and dichloromethane. The solution was cooled to -55 °C. A solution of diluted benzoyl chloride (4.3 mL, 37 mmol) with dichloromethane (30 mL) was added dropwise to this cooled solution. The reaction was proceeded under nitrogen at -55 °C and carefully monitored by TLC. After 5 h, methanol (2 mL) was added and the mixture was stirred for 15 min. The mixture was concentrated under reduced pressure. Column chromatograph (dichloromethane/methanol, 100:1) of the residue on silica gel gave tribenzoyl lactoside **65** (3.88 g, 64%) and tetrabenzoyl lactoside **66** (0.68 g, 10%).

Compound **65**: m.p. 90-92 °C; $[\alpha]_D$ +15.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.17-7.23 (m, 15 H, aromatic), 5.17 (dd, 1 H, $J_{2,3}$ 9.0 Hz, H-2), 4.92 (dd, 1 H, $J_{5,6a}$ 2.1, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.79 (dd, 1 H, $J_{5',6a'}$ 2.4, $J_{6a',6b'}$ 12.3 Hz, H-6a'), 4.69 (d, 1 H, $J_{1,2}$ 9.0 Hz, H-1), 4.46 (dd, 1 H, $J_{5,6b}$ 5.1 Hz, H-6b), 4.37 (dd, 1 H, $J_{5',6b'}$ 9.0 Hz, H-6b'), 4.32 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1'), 4.19-4.13 (m, 3 H, H-3', H-4',H-5'), 3.94 (dd, 1 H, $J_{3,4}$ 8.3 Hz, H-3), 3.84 (ddd, 1 H, H-5), 3.70 (dd, 1 H, $J_{2',3'}$ 7.0 Hz, H-2'), 3.55 (dd, 1 H, $J_{4,5}$ 10.0 Hz, H-4), 1.54, 1.45 (2s, 6 H,

Me); ^{13}C NMR ($CDCl_3$) δ 166.9, 166.5, 165.2 (C=O's), 133.6-128.3 (aromatic C), 110.8 (O-C-O), 103.8 (C-1'), 87.8 (C-1), 82.3 (C-4), 78.9 (C-3'), 75.9 (C-5), 73.5 (C-3), 73.2 (C-4'), 72.2 (C-5', C-2'), 72.1 (C-2), 63.8 (C-6, C-6'), 28.0, 26.2 (2xMe); FAB-Ms for $C_{36}H_{36}N_3O_{13}$: 720.3 (M+1).

Anal. Calcd for $C_{36}H_{36}N_3O_{13}$: C, 60.08; H, 5.18; N, 5.84. Found: C, 59.98; H, 5.17; N, 5.72.

Compound **66**: m.p. 203-204 °C (dec.); $[\alpha]_D$ +4.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.13-7.26 (m, 20 H, aromatic), 5.35 (dd, 1 H, $J_{2',3'}$ 7.8 Hz, H-2'), 5.17 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2), 4.85 (dd, 1 H, $J_{5',6a'}$ 2.4, $J_{6a',6b'}$ 12.1 Hz, H-6a'), 4.64 (d, 1 H, $J_{1,2}$ 9.0 Hz, H-1), 4.63 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.45-4.36 (m, 2 H, H-6b', H-3'), 4.35 (dd, 1 H, $J_{5,6a}$ 2.1, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.30-4.20 (m, 3 H, H-4', H-6b, H-5'), 3.99 (dd, 1 H, $J_{3,4}$ 8.3 Hz, H-3), 3.74 (m, 1 H, H-5), 3.70 (dd, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 1.54, 1.45 (2s, 6 H, Me); ^{13}C NMR ($CDCl_3$) δ 166.5, 165.6, 165.3, 165.2 (C=O's), 133.5-128.4 (aromatic C), 111.3 (O-C-O), 101.6 (C-1'), 87.9 (C-1), 81.9 (C-4), 77.0 (C-3'), 74.2 (C-5), 73.4 (C-3, C-4'), 73.1 (C-2'), 72.2 (C-5', C-2), 63.6 (C-6'), 62.2 (C-6), 27.6, 26.3 (2xMe); FAB-Ms for $C_{43}H_{40}N_3O_{14}$: 824.3 (M+1).

Anal. Calcd for $C_{43}H_{40}N_3O_{14}$: C, 62.70; H, 5.02; N, 5.10. Found: C, 62.41: H, 4.99; N, 5.04.

6-*O*-Benzoyl-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-benzyol-β-D-glucopyranosyl azide (67)

To a solution of compound **65** (0.8 g, 1.1 mmol) in methanol (15 mL) was added a catalytic amount of *p*-TsOH. The solution was refluxed for 5 hr. TLC (CH_2Cl_2/MeOH, 20/1) showed the reaction was complete. The reaction mixture was cooled and neutralized with triethylamine and evaporated under reduced pressure. The residue was pufiried by silica gel chromatography using to CH_2Cl_2/MeOH (20/1) as eluent to give crystalline compound **67** (0.72g, 95%): m.p. 163°C; $[\alpha]_D$ +0.8° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.96-7.22 (m, 15 H, aromatic), 5.10 (dd, 1 H, $J_{2,3}$ 9.3 Hz, H-2), 4.79 (dd, 1 H, $J_{5,6a}$ 2.1, $J_{6a,6b}$ 11.3 Hz, H-6a), 4.67 (d, 1 H, $J_{1,2}$ 8.9 Hz, H-1), 4.64 (m, 1 H, H-6a'), 4.44 (dd, 1 H, $J_{5,6b}$ 5.7 Hz, H-6b), 4.33 (m, 2 H, H-6b', H-1'). 3.98-3.84 (m, 5 H, H-3, H-5, H-2', H-4',H-5'), 3.64 (bs, 1 H, H-3'), 3.56 (dd, 1 H, $J_{4,5}$ 9.1 Hz, H-4); ^{13}C NMR ($CDCl_3$) δ 167.1, 166.8, 165.3 (C=O's), 133.6-128.4 (aromatic C), 104.2 (C-1'), 87.8 (C-1), 81.5 (C-4), 78.9 (C-3'), 75.9 (C-5), 73.5 (C-3), 73.2

(C-4'), 72.2 (C-5', C-2'), 72.1 (C-2), 64.0 (C-6), 63.7(C-6); FAB-MS for $C_{33}H_{33}N_3O_{13}$: 680.2 (M+1).

Anal. Calcd for $C_{33}H_{33}N_3O_{13}$: C, 58.32; H, 4.89; N, 6.18. Found: C, 58.20; H, 4.86; N, 6.19.

Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-(6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,6-di-*O*-benzyol-β-D-glucopyranosyl azide (68)

To a solution of lactosyl acceptor **67** (500 mg, 0.74 mmol) and thiophenyl sialic acid donor **42** (800 mg, 1.37 mmol) in dry CH_2Cl_2/CH_3CN (1:2, 15 mL) was added molecular sieves 4 Å (2 g) and the mixture was stirred for 2 h at room temperature. The mixture was then cooled to -50 °C and *N*-iodosuccinimide (500 mg, 2.22 mmol) and trifluoromethanesulfonic acid (75 μL, 0.85 mmol) were added. The reaction mixture was stirred at -50 °C under nitrogen. The progress of the reaction was monitored by TLC until the donor was completely consumed. After 2h, the reaction mixture was diluted with CH_2Cl_2 and filtered through a layer of celite. The filtrate was washed with 10% $Na_2S_2O_3$, sat. $NaHCO_3$, and brine. The organic was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography using methanol-dichloromethane as eluent (1:20, v/v) to afford GM_3 trisaccharide **68** (450 mg, 53%): m.p. 115-116°C; $[\alpha]_D$ +3.1° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.06-7.26 (m, 15 H, aromatic), 5.33-5.25 (m, 3 H, NH, H-8", H-7"), 5.21 (dd, 1 H, $J_{2,3}$ 9.3 Hz, H-2), 4.97 (m, 1 H, H-4"), 4.94 (dd, 1 H, $J_{5,6a}$ 1.3 Hz, $J_{6a,6b}$ 12.1 Hz, H-6a), 4.74 (dd, 1 H, $J_{5',6a'}$ 2.7 Hz, $J_{6a',6b'}$ 12.0 Hz, H-6a'), 4.73 (d, 1 H, $J_{1,2}$ 9.3 Hz, H-1), 4.57 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.53 (dd, 1 H, $J_{5,6b}$ 5.7Hz, H-6b), 4.34 (dd, 1 H, $J_{5',6b'}$ 8.0 Hz, H-6b'), 4.28 (dd, 1 H, $J_{8",9a"}$ 2.5 Hz, $J_{9a",9b"}$ 12.5 Hz, H-9a"), 4.14 (dd, 1 H, $J_{2',3'}$ 9.6 Hz, $J_{3',4'}$ 3.1 Hz, H-3'), 4.10 (d, 1 H, $J_{5",6"}$ 10.7 Hz, H-6"), 3.97 (dd, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 3.94-3.88 (m, 4 H, H-5, H-5', H-5", H-9"), 3.79-3.73 (m, 5 H, OMe, H-4, H-2'), 3.70 (bs, 1 H, H-4'), 2.68 (dd, 1 H, $J_{3e",4"}$ 4.7, $J_{3a",3e"}$ 13.0 Hz, H-3e"), 2.13, 2.04, 2.01, 1.92, 1.88 (5s, 15 H, OAc's, NHAc), 2.00 (dd, 1 H, $J_{3a",4"}$ 12.1 Hz, H-3a"); ^{13}C NMR ($CDCl_3$) δ 170.8-165.3 (C=O's), 133.2-128.4 (aromatic C), 104.4 (C-1'), 97.5 (C-2"), 87.9 (C-1), 82.0 (C-4), 76.4 (C-3'), 75.0 (C-5'), 73.5 (C-3), 73.1 (C-6"), 72.9 (C-5), 72.5 (C-2), 69.0 (C-2'), 68.9 (C-8"), 68.2 (C-4"), 68.1 (C-4'), 67.0 (C-7"), 63.7 (C-6'), 63.2 (C-6), 62.4 (C-9"), 53.3 (OMe), 49.8

(C-5"), 37.7 (C-3"), 23.2 (NHAc), 21.1, 20.8, 20.7, 20.6 (OAc's); FAB-MS for $C_{53}H_{60}N_4O_{25}$: 1110.2 (M-N_3, 1.9%).

Anal Calcd for $C_{53}H_{60}N_4O_{25}$: C, 55.21; H, 5.24; N, 4.86. Found: C, 55.08; H, 5.22; N, 4.90.

2,6-Di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→4)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2,6-di-*O*-benzoyl-β-D-glucopyranosyl azide (69)

To a solution of compound **66** (0.24 g, 0.29 mmol) and phenyl 2,3,4-tri-O-acetyl-1-thio-β-L-fucopyranoside (**32**) (0.46 g, 0.88 mmol) in 5 mL of 10:1 benzene-dichloromethane was added powdered molecular sieves 4Å (0.4 g). The mixture was stirred for 3 h at room temperature under nitrogen at 0 °C. DMTST (0.262 g, 1.02 mmol) was added to the stirred mixture at 0 °C. After 2 h, TLC (Hexane/ethyl acetate, 3/1) showed complete conversion of the donor. Then triethylamine (0.5 mL) and methanol (1 mL) were added to the reaction mixture which was stirred for an additional 25 min. The mixture was then diluted with dichloromethane (15 mL) and filtered through celite. The filtrate was washed with saturated aq solution of $NaHCO_3$ (2x10 mL), and brine (10 mL). The solution was dried (Na_2SO_4) and concentrated. Column chromatography (hexanes/ethyl acetate, 3/1) of the residue on silica gel afforded trisaccharide **69** (0.321 g, 89%): $[\alpha]_D$ -35.5° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.19-6.83 (m, 35 H, aromatic H), 5.40 (d, 1 H, $J_{1",2"}$ 3.7 Hz, H-1"), 5.36 (dd, 1 H, $J_{2,3}$ 9.0 Hz, H-2), 5.23 (dd, 1 H, $J_{2',3'}$ 8.1 Hz, H-2'), 4.94 (AB pattern, 1 H, *J* 11.7 Hz, $PhCH_a$), 4.84 (m, 1 H, H-5"), 4.79-4.72 (m, 3 H, $PhCH_2$, H-6a'), 4.64 (AB pattern, 1 H, $PhCH_b$), 4.53 (m, 2 H, H-6), 4.49 (d, 1 H, $J_{1',2'}$ 8.7 Hz, H-1'), 4.46 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1), 4.33-4.19 (m, 6 H, H-6b', H-3, H-3', $PhCH_2$, H-3"), 4.16 (dd, $J_{3',4'}$ 5.2 Hz, $J_{4',5'}$ 1.9 Hz, H-4'), 4.08 (dd, $J_{3,4}$ 9.5 Hz, $J_{4,5}$ 9.5 Hz, H-4), 3.92 (dd, 1 H, $J_{2",3"}$ 10.2 Hz, H-2"), 3.83 (m, 1 H, H-5'), 3.77 (s, 1 H, H-4"), 3.56 (m, 1 H, H-5), 1.49 (s, 3 H, CMe), 1.31 (d, 3 H, $J_{5",6"}$ 6.8 Hz, H-6"), 1.30 (s, 3 H, CMe); ^{13}C NMR ($CDCl_3$): δ 166.2, 166.0, 164.7, 164.6 (C=O's), 139.1-126.8 (aromatic C), 110.9 (-O-C-O), 100.3 (C-1'), 97.7 (C-1"), 88.1 (C-1), 79.2 (C-4"), 78.2 (C-3"), 77.4 (C-3'), 75.5 (C-2"), 75.4 (C-5), 74.9 (CH_2), 74.4 (C-4), 74.1 (2 C, C-2, C-3), 73.3, 73.2 (2 C, C-2', C-4'), 72.7, 72.6 (2 x CH_2), 71.5 (C-5'), 66.6 (C-5"), 62.6 (C-6'), 62.1 (C-6), 27.8, 26.2 (2 x Me), 16.9 (C-6"); FAB-MS (glycerol) gave m/z (ion, relative intensity): 1239.5 (M^+, 0.1%).

Anal Calcd for $C_{70}H_{69}N_3O_{18}$: C, 67.79; H, 5.61; N, 3.39. Found: C, 67.52; H, 5.72; N, 3.36.

2,6-Di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2,6-di-*O*-benzoyl-β-D-glucopyranosyl azide (70)

To compound **69** (200 mg, mmol) was added 80% acetic acid solution (5 mL). The solution was stirred at 60 °C overnight. The solution was poured into water and extracted with dichloromethane (10 mL x 3). The combined organic layers were washed with saturated aq solution of $NaHCO_3$ (2x10 mL), and brine (10 mL). The solution was dried (Na_2SO_4) and concentrated. Column chromatography (hexanes/ethyl acetate, 3/2) of the residue on silica gel afforded trisaccharide **70** (182 mg, 94%): $[\alpha]_D$ -58.7° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.26-6.93 (m, 35 H, aromatic H), 5.40 (dd, 1 H, $J_{2,3}$ 9.0 Hz, H-2), 5.36 (d, 1 H, $J_{1'',2''}$ 3.8 Hz, H-1''), 5.23 (dd, 1 H, $J_{1',2'}$ 8.1 Hz, $J_{2',3'}$ 9.6 Hz, H-2'), 4.94 (AB pattern, 1 H, *J* 11.3 Hz, $PhCH_a$), 4.85 (m, 1 H, H-5''), 4.87, 4.84 (AB pattern, 2 H, *J* 12 Hz, $PhCH_2$), 4.66 (dd, 1 H, H-6a'), 4.63-4.61 (m, 5 H, $PhCH_b$, H-6, H-1'), 4.45 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1), 4.29-4.16 (m, 4 H, H-6b', H-3, $PhCH_2$), 4.17 (dd, $J_{3,4}$ 9.3 Hz, $J_{4,5}$ 9.3 Hz, H-4), 4.10 (dd, 1 H, H-3''), 3.90 (dd, 1 H, $J_{2'',3''}$ 10.2 Hz, H-2''), 3.85 (d, 1 H, $J_{3'',4''}$ 1.3 Hz, H-4''), 3.81 (d, 1 H, $J_{3',4'}$ 3.0 Hz, H-4'), 3.69 (dd, 1 H, H-3'), 3.57 (m, 1 H, H-5), 3.52 (t, 1 H, $J_{5',6a'}$, $J_{5',6b'}$ 6.9 Hz, H-5'), 3.08 (bs, 1 H, OH), 2.99 (bs, 1 H, OH), 1.30 (d, 3 H, $J_{5'',6''}$ 6.5 Hz, H-6''); ^{13}C NMR ($CDCl_3$): δ 166.6, 166.4, 166.1, 164.6 (C=O's), 139.1-126.9 (aromatic C), 100.0 (C-1'), 98.0 (C-1''), 88.2 (C-1), 79.1 (C-3''), 78.4 (C-4''), 75.5 (C-5), 75.4 (C-2''), 75.2 (CH_2), 74.7 (C-3), 74.0 (C-2), 73.7 (2 C, C-2', C-4), 72.9, 72.4 (2 x CH_2), 72.3 (C-3'), 71.9 (C-5'), 67.8 (C-4'), 66.7 (C-5''), 62.4 (C-6), 61.7 (C-6'), 16.6 (C-6''); FAB-MS (glycerol) gave m/z (ion, relative intensity): 1199.4 (M^+, 0.2%).

Anal Calcd for $C_{67}H_{65}N_3O_{18}$: C, 67.05; H, 5.46; N, 3.50. Found: C, 67.38; H, 5.51; N, 3.52.

Chapter 5 Transition metal-catalyzed carbohydrate clusters syntheses

5.1 Introduction

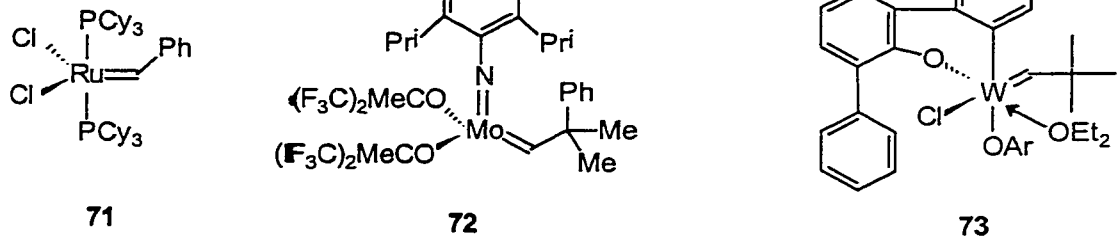
A number of essential biological processes, such as the inflammatory response, lymphocyte homing, and fertilization, were mediated by protein-carbohydrate recognition.[157] Some of these protein-carbohydrate interactions also facilitate a variety of pathogenic events, including bacterial and viral infection and tumor metastases. Therefore, the development of small-molecule inhibitors of pathogenic carbohydrate-mediated interactions has been the subject of intense activity in recent years. Unfortunately, such efforts are hampered by the low-affinity binding of protein-carbohydrate interaction. The binding events proceed uniformly with millimolar to micromolar dissociation constant.[42] Nature has however dealt with the binding problem through multivalency, where both proteins and carbohydrates with multivalent binding sites offer multiple simultaneous binding events to overcome weak individual interactions. Multivalent neoglycoconjugates from low-valency clusters to high-valency glycopolymers have demonstrated enhanced potency through increased avidty.[158] Such neoglycoconjugates offer great promises as antiadhesins toward pathogenic infections.[159] The family of low-valency carbohydrate clusters are attractive synthetic targets because of their enhanced binding properties. To further expand methodologies to generate carbohydrate clusters, transition metal-catalyzed syntheses will be described.

---

[157] Varki, A. *Glycobiology* **1993**, *3*, 97.
[158] a) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321; b) Roy, R. *Topics Curr. Chem.* **1997**, *187*, 241.

5.2 Syntheses of divalent sialoside derivatives by olefin metathesis reaction

Olefin metathesis catalyzed by transition metals has recently emerged as a powerful tool for the formation of C-C bonds in organic syntheses.[160] Grubbs' ruthenium carbene complex **71**[161] and Schrock's molybdenum complex **72**[162] (Figure **5.1**) have been recently used in carbohydrate-related ring-closing metathesis,[163] cross-metathesis,[164] and ring-opening metathesis[165] reactions. Olefin metathesis reaction has been rarely applied towards the synthesis of carbohydrate homodimers. The only example of carbohydrate homodimerization was reported by Descotes *et al.*[166] in sugar bolaform syntheses using tungsten aryoxoalkylidene complex **73**. However, these conditions were unsuccessful with *O*-allyl glycosides as well as benzyl-protected sugar derivatives.



71 **72** **73**

Figure 5.1 The structures of transition metal catalysts

---

[159] Karlsson, K.-A. *Curr. Opin. Struct. Bio.* **1995**, *5*, 622.

[160] For reviews, see: (a) Grubbs, R. H.; Chang, S. *Tetrahedron* **1998**, *54*, 4413. (b) Schuster, M.; Blechert, S. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2036. (c) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446.

[161] Schwab, P.; France, M. B.; Ziller, J. W.; Grubbs, R. H. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2039.

[162] (a) Schrock, R. R.; Murdzek, S. J.; Bagan, G. C.; Dimare, M.; O'Regan, M. *J. Am. Chem. Soc.* **1990**, *112*, 3875; (b) Bagan, G. C.; Oskam, J. H.; Cho, H.-N.; Park, L. Y.; Schrock, R. R. *J. Am. Chem. Soc.* **1991**, *113*, 6899.

[163] (a) Overkleeft, H. S.; Pandit, U. K. *Tetrahedron lett.* **1996**, *37*, 547; (b) Armstrong, S. K. *J. Chem. Soc., Perkin Trans. 1* **1998**, 371.

[164] (a) Schuster, M. F.; Lucas, N.; Blechert, S. *Chem. Commun.* **1997**, 823; (b) Feng, J.; Schuster, M.; Blechert, S. *Synlett.* **1997**, 129.

[165] (a) Fraser, C.; Grubbs, R. H. *Macromolecules* **1995**, *28*, 7248; (b) Nomura, K.; Schrock, R. R. *Macromolecules* **1996**, *29*, 540; (c) Manning, D. D.; Strong, L. E.; Hu, X.; Beck, P. J.; Kiessling, L. L. *Tetrahedron.* **1997**, 53, 11937.

[166] (a) Descotes, G.; Ramza, J.; Basset, J.-M.; Pagano, S. *Terahedron Lett.* **1994**, *35*, 7379; (b) Ramza, J.; Descotes, G.; Basset, J.-M.; Mutch, A. *J. Carbohydr. Chem.* **1996**, *15*, 125.

Ruthenium carbenoid **71** is especially useful in this area because of its high reactivity, stability to air, and tolerance to many functional groups. Recent developments in our laboratory have showed that O-allyl, O-pentenyl, C-vinyl, and C-allyl glycopyranosides can undergo efficient homodimerization by using Grubbs' ruthenium carbene complex **71**.[167]

N-Acetylneuraminic acid (NeuAc) frequently terminates oligosaccharide chains of glycoproteins and glycolipids of cell membranes and plays vital roles in many biological processes. Unfortunately, individual carbohydrate-protein interactions, as compared to protein-protein interactions, are of low affinity (K_D's). To compensate for their low affinity, divalent sialoclusters built on aromatic,[168] galactoside,[169] or peptide scaffolds[170] have been synthesized.

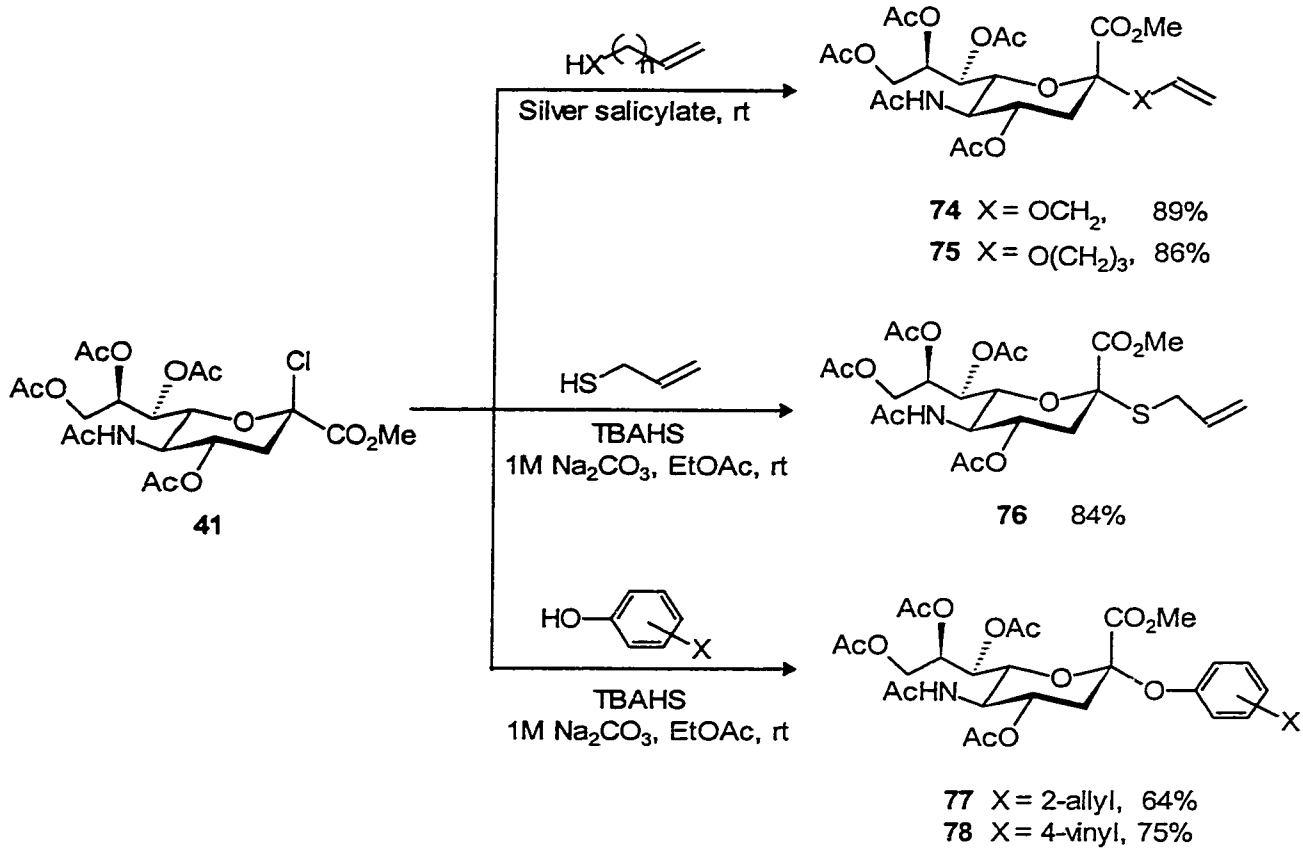
Since ruthenium catalyzed homodimerization offers a simple and efficient way to generate divalent carbohydrate clusters and hasn't been employed to sialic acid, it's worthy to apply this method for the syntheses of divalent sialoside derivatives.

The requisite allyl **74**[171] and *n*-pentenyl *O*-α-sialoside **75**[172] were readily accessible from the corresponding alcohols using β-acetochloroneuraminic acid **41** in the presence of silver salicylate.[173] The Koenigs-Knorr glycosylation ocurred with complete stereocontrol giving rise to the α form **74** and **75** in 89% and 86% yields respectively. Allyl thiosialoside **76** was also prepared from **41** using phase transfer catalysis (PTC) as previously reported.[174] Following improved phase transfer catalyzed conditions developed in our laboratory,[175] the synthesis of aryl α-sialosyl derivatives **77** and **78** was achieved at room temperature in a two-phase system using ethyl acetate, 1 M sodium carbonate and tetra-n-butylammonium hydrogen sulfate as phase transfer catalyst. The reactions were entirely stereoselective and provided crystalline **77** and **78** in 64% and 75% yield, respectively (Scheme **5.1**).

---

[167] (a) Dominique, R.; Das, S. K.; Roy, R. *Chem. Commun.* **1998**, 2437. (b) Das, S. K.; Dominique, R.; Smith, C.; Nahra, J.; Roy, R. *Carbohydr. Lett.* **1999**, *3*, 361. (c) Roy, R.; Dominique, R.; Das, S. K. *J. Org. Chem.* **1999**, *64*, 5408. (d) Roy, R.; Das, S. K.; Dominique, R.; Trono, M. C.; Hernández-Mateo, F.; Santoyo-González, F. *Pure Appl. Chem.* **1999**, *71*, 565; (e) Roy, R.; Das, S. K. *Chem. Commun.* **2000**, 513.
[168] Glick, G. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1991**, *113*, 4701.
[169] (a) Sabesan, S.; Duus, J. Ø.; Domaille, P.; Kelm, S.; Paulson, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 5865. (b) Sabesan, S.; Duus, J. Ø.; Neira, S.; Domaille, P.; Kelm, S.; Paulson, J. C.; Bock, K. *J. Am. Chem. Soc.* **1992**, *114*, 8363.
[170] Unverzagt, C.; Kelm, S.; Paulson, J. C. *Carbohydr. Res.* **1994**, *251*, 285.
[171] Roy, R.; Laferrière, C. A. *Can. J. Chem.* **1990**, *68*, 2045.
[172] Allanson, N. M.; Davidson, A. H.; Floyd, C. D.; Martin, F. M. *Tetrahedron: Asymmetry* **1994**, *5*, 2061.
[173] van der Vleugel, D. J. M.; van Heeswijk, W. A. R.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1982**, *102*, 121.
[174] Cao, S.; Meunier, S. J.; Andersson, F. O.; Letellier, M.; Roy, R. *Tetrahedron: Asymmetry* **1994**, *5*, 2303.

Treatment of allyl *O*-α-sialoside **74** with 5 mol% of Grubbs' catalyst **71** in refluxing CH_2Cl_2 under nitrogen afforded homodimer **79** in 82% yield as a mixture of 7:1 *E* and *Z* isomers (Scheme **5.2**). Some starting material together with a trace amount of cross-metathesis product from the initially released styrene was also obtained.

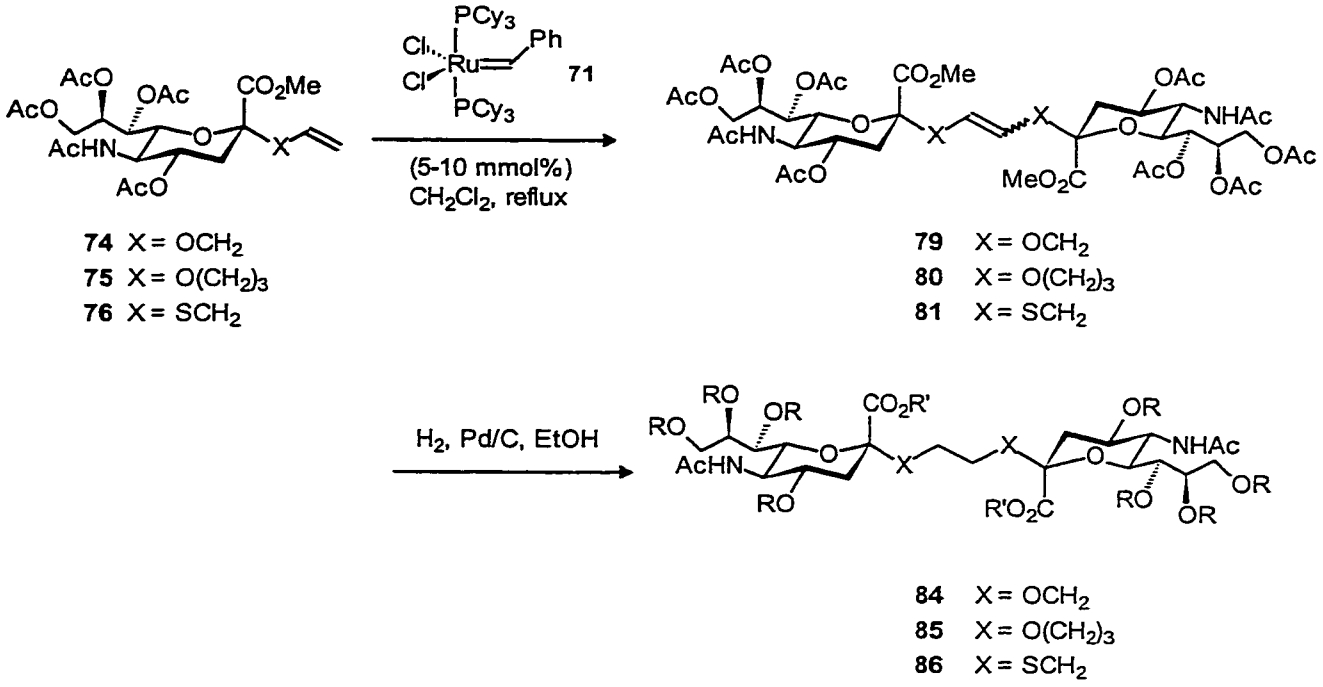


Scheme 5.1 Preparation of alkenyl α-sialoside derivatives

The ratio of the inseparable *E* and *Z* isomers couldn't be determined from the ^1H NMR spectrum of the dimer **79** because of overlapping signals (Figure **5.2**). However, it could be assigned after compound **79** was deacetylated under Zemplén conditions (NaOMe, MeOH). The ^{13}C NMR spectrum was more informative to confirm the identity of the *E* and *Z* isomers. It is generally accepted that the carbon α to the double bond in the *Z* isomer is more

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

shielded than that in the *E* isomer due to the γ effect.[176] The empirical relationship $\delta_{\alpha(Z)} < \delta_{\alpha(E)}$ allowed us to assign the relative configuration of the *E* and *Z* isomers. For instance, the ^{13}C NMR spectrum of **79** showed a downfield shift for C-2' in the *E* isomer (δ 64.6 ppm, Δδ 3.7 ppm) compared to δ 60.9 ppm for that of the *Z* isomer (Figure **5.3**).



Scheme 5.2 Preparation of divalent sialoside derivatives **79-81**

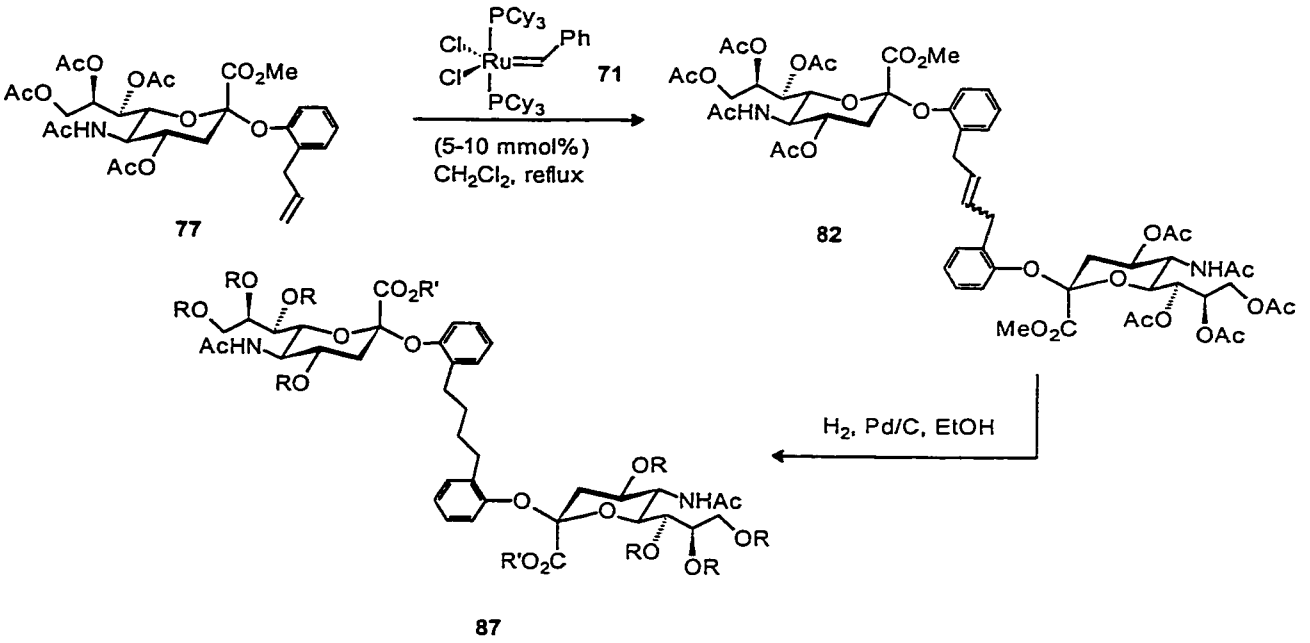
Olefin metathesis of the longer spacer *n*-pentenyl *O*-α-sialoside **75** under similar reaction conditions afforded homodimer **80** in 88% yield as a 3:1 mixture of *E* and *Z* isomers. Monomer **75** was more reactive than **74** because of less steric hindrance around the quaternary anomeric center; as a consequence, the *E* and *Z* stereoselectivity decreased from 7:1 to 3:1.

Sulfur-containing compounds have long been known to act as poisons for transition metal catalysts because of their strong coordinating properties, which cause them to block the reactive sites of the metals.[177] As expected, allyl thiosialoside **76** showed less reactivity

[176] Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy. High Resolutions Methods and Applications in Organic Chemistry and Biochemistry*, VCH, New York, 1987, 192.
[177] Hegedus, L. L.; McCabe, R. W. *Catalyst Poisoning*; Marcel Deker: New York, 1984.

toward Grubbs' catalyst **71** when compared to the corresponding *O*-glycoside analogs (**74, 75**). However, the expected homodimer **81** could be obtained in 26% yield together with recovered starting material **76** by using more catalyst and prolonged heating.

To further explore the scope of this method, the more rigid 2-allylphenyl sialoside **77** and 4-vinylphenyl sialoside **78** were treated with Grubbs' catalyst under similar reaction conditions to give compounds **82** and **83** in 78 and 37% yields, respectively (Scheme **5.3, 5.4**). Interestingly, compound **83** was obtained as a single *E* isomer. The results and conditions are summarized in Table **5.1**.



Scheme 5.3 Preparation of divalent sialoside derivative **82**

Each of the α-sialodimers **79-83** was transformed into a single compound by hydrogenation at atmospheric pressure (10% Pd/C, 1-12 h) to give their corresponding saturated α-sialodimers **84-88**, respectively, in quantitative yields.

In summary, the olefin metathesis reaction provides a facile approach toward the synthesis of divalent sialoside derivatives. The reactions afforded reasonable yields under mild reaction conditions. Vinyl substituted aryl sialosides provided only the *trans* isomer as previously expected on the basis of analogous results.[167]

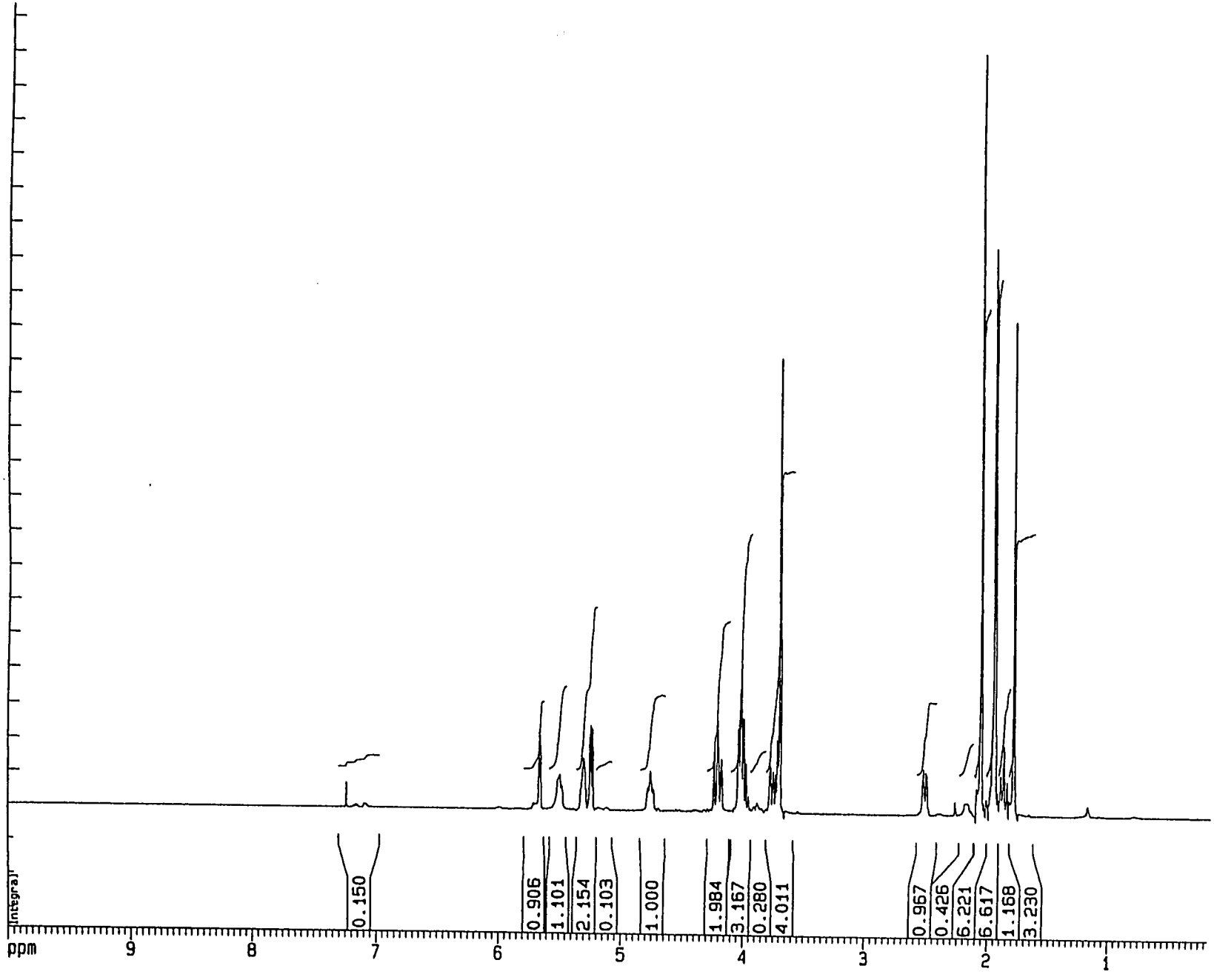


Figure 5.2 ^{1}H NMR (500MHz, $CDCl_3$) spectrum of divalent sialosides **79**

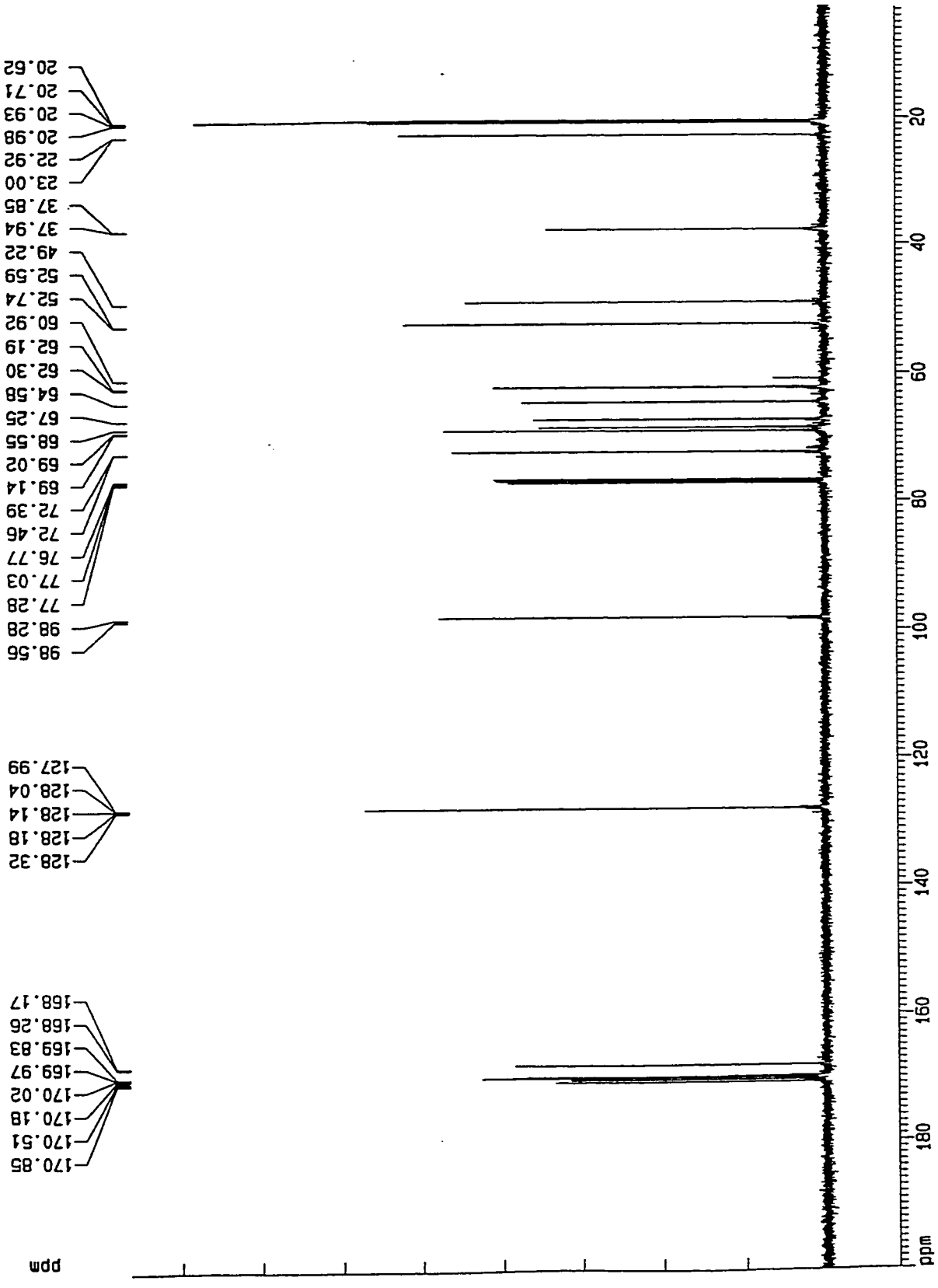
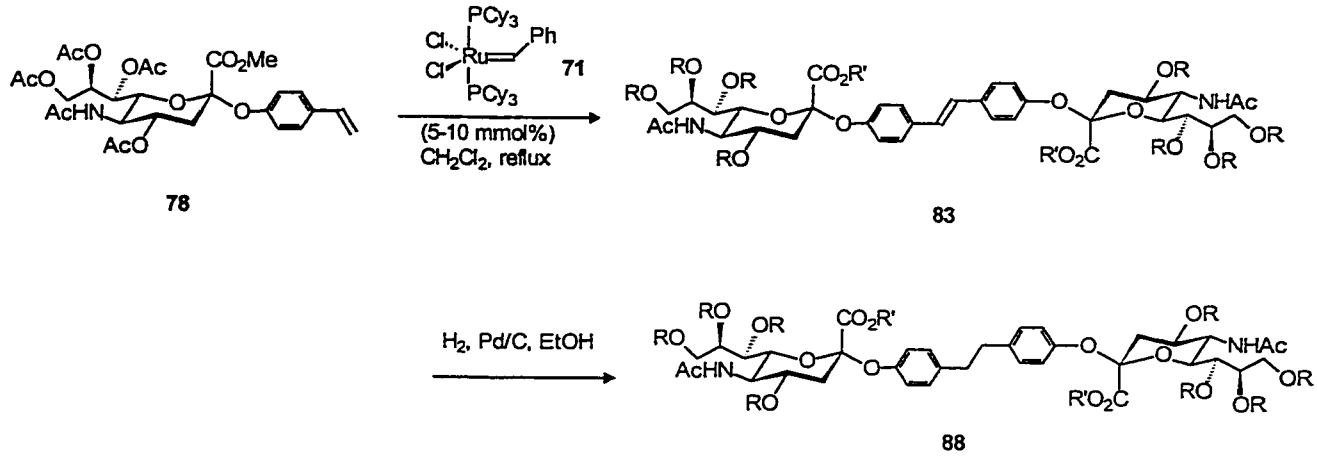


Figure 5.3 ^{13}C NMR (500MHz, $CDCl_3$) spectrum of divalent sialosides **79**



Scheme 5.4 Preparation of divalent sialoside derivative **83**

Table 5.1 Olefin self-metathesis of alkenyl sialic acids

Entry	Substrate	Product	Catalyst amount (mol %)	time (h)	Yield (%)[a]	(E/Z) Ratio[b]
1	**74**	**79**	5	6	82	7/1[c]
2	**75**	**80**	5	2	88	3/1[c]
3	**76**	**81**	10	24	26	2.5/1
4	**77**	**82**	5	2	78	1.7/1
5	**78**	**83**	10	24	37	1/0

[a]After purification by column chromatography.
[b]Determined by ^{1}H NMR.
[c]The ratio was determined from its deacetylated derivatives.

5.3 Transition metal-catalyzed syntheses of "rod-like" thioglycoside dimers

Conjugated acetylenic compounds are very valuable materials for antitumor antibiotics and nanometer-sized molecular objects.[178] In this respect, homo-coupling between sp and sp carbon atoms and cross-coupling between sp^2 and sp carbon atoms (such as

[178] (a) Stang, P. J.; Diederich, F. *Modern Acetylene Chemistry*; Wiley-VCH: Weinheim, Germany, 1995; (b) Siemsen, P.; Livingston, R. C.; Diederich, F. *Angew. Chem. Int. Ed. Eng.* **2000**, *39*, 2632.

Glaser[179] and Sonogashira reactions[180]) as methods to build such compounds have gained great interest. However, much less attention has been paid to reactions with organic sulfur compounds[181] which have long been known to act as poisons for transition metal catalysts because of their strong coordinating properties. Additionally, these useful reactions have rarely been used in carbohydrate chemistry. Some examples of these reactions have been described by Vasella *et al*[182] in the synthesis of higher oligomers of acetylenosaccharides. Moreover, no such reactions has been applied in thioglycosides which are of special interest as enzyme inhibitors because they offer the advantage of being resistant to enzymatic hydrolysis, thus being potential inhibitors of glycosidases.[183]

Recently, molecular rods[184] which are considered as spacers and construction elements in giant molecules and supramolecular assemblies have drawn widespread attention. This encouraged us to look for a facile way to build so called "rod-like" carbohydrate dimers. Glaser and Sonogashira reactions make it possible to prepare such a kind of sugar rods, where the acetylenic bonds of two carbohydrate moieties are connected to each other or onto an aromatic ring. Such dimers should have more rigid structures that can diminish entropic loss that is usually associated with flexible carbohydrate ligands.

The requisite starting material 2-propynyl thioglycosides were synthesized by the alkylation of the corresponding 1-thioglycoses with 2-propynyl bromide under base conditions. Although a number of useful procedures to prepare 1-thioglycoses,[185] phase transfer catalysis or similar S_N2 processes appeared to be most reliable in terms of

---

[179] For reviews, see: (a) Sonogashira, K. in *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I.; Pergamon Press: New York, 1991; Vol. 3, 551. (b) Brandsma, L.; Vasilevsky, S. F.; VerKruijsse, H. D. *Application of Transition Metal Catalysts in Organic Synthesis*; Springer: Berlin, 1998; 179.

[180] For reviews, see: (a) Sonogashira, K. in *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I.; Pergamon Press: New York, 1991; Vol. 3, 521. (b) Brandsma, L.; Vasilevsky, S. F.; VerKruijsse, H. D. *Application of Transition Metal Catalysts in Organic Synthesis*; Springer: Berlin, 1998; 67. (c) Sonogashira, K. In *Metal-Catalyzed Cross-Coupling Reactions*; Diederich, F.; Stang, P. J., Eds.; Wiley-VCH: Weinheim, Germany, 1998; 203.

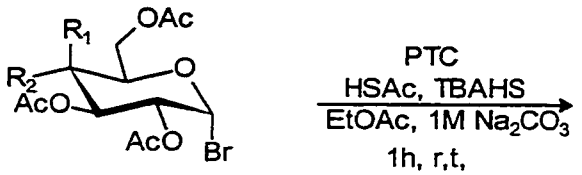
[181] For examples of Glaser reaction and Sonogahira reaction using organosulfur compounds, see: (a) Müller, T. J. J. *Tetrahedron Lett.* **1999**, *40*, 6563. (b) Fossatelli, M.; Kerk, A. C. T. H. M. van der; vasilevsky, S. F.; Brandsma, L. *Tetrahedron Lett.* **1992**, *33*, 4229. (c) Neen, T. X.; Whitesides, G. M. *J. Org. Chem.* **1988**, *53*, 2489. (d) Fritzsche, V.; Hunig, S. *Tetrahedron Lett.* **1972**, 4831.

[182] Vasella, A. *Pure Appl. Chem.* **1998**, *70*, 425.

[183] (a) Steers, Jr., E.; Cuatrecasas, P.; Pollard, H. B. *J. Biol., Chem.* **1971**, *246*, 196; (b) Shafizadeh, F.; Furneaux, R. H.; Stevenson, T. T. *Carbohydr. Res.* **1979**, *71*, 169; (c) Witczak, Z. J.; Chhabra, R.; Chen. H.; Xie, X.-Q. *Carbohydr. Res.* **1997**, *301*, 167.

[184] Schwab, P. F. H.; Levin, M. D.; Michl, J. *Chem. Rev.* **1999**, *99*, 1863.

stereoselectivity.[186] Thus, glycosyl bromides were stereoselectively transformed into anomeric thioacetates in a two-phase system with thioacetic acid and TBAHS used as phase transfer catalyst (EtOAc, 1 M Na_2CO_3, r.t.). Glycosyl thioacetates **91**, **92**, and **93** were obtained under these PTC conditions (Scheme **5.5**).



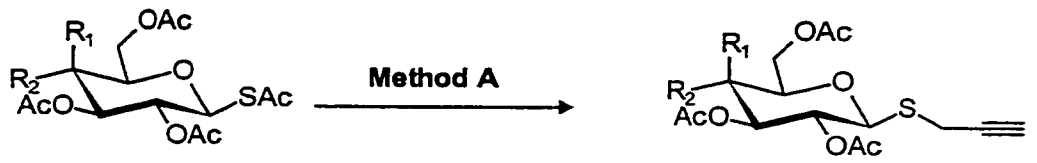
	R_1	R_2
90	H	OAc
6	OAc	H
35	H	Gal

	R_1	R_2	yield
91	H	OAc	72%
92	OAc	H	86%
93	H	Gal	75%

Scheme 5.5 PTC synthesis of thioacetateglycoside derivatives

Selective deprotection of the anomeric thioacetates was essential for subsequent nucleophilic alkylation. This process can perform well under low temperature (-40 °C) Zemplén conditions. This method sometimes caused partial de-*O*-acetylation when *O*-acetates were also present.[187] However, this side reaction could be minimized by controlling the reaction and work-up temperature (-40 °C) carefully. The freshly deprotected thiol derivatives were subsequently treated with 2-propynyl bromide and triethylamine to provide *S*-propynyl glycosides **94**, **95**, and **96** in good yields (Scheme **5.6**).

[185] (a) Horton, D.; Hudson. D. *Adv. Cabohydr. Chem.* **1963**, *18*, 123; (b) Horton, D. *Methods Carbohydr. Chem.* **1963**, *2*, 433.
[186] Tropper, F. D.; Andersson,F. D.; Grand-Maître, C; Roy, R. *Carbohydr. Res.* **1992**, *229*, 149.
[187] Hasegawa, A.; Nakamura, J.; Kiso, M. *J. Carbohydr. Chem.* **1986**, *5*, 11.



	R_1	R_2
91	H	OAc
92	OAc	H
93	H	Gal

	R_1	R_2	yield
94	H	OAc	83%
95	OAc	H	86%
96	H	Gal	81%

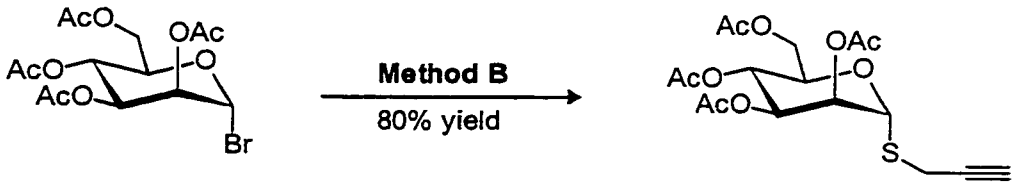
Scheme 5.6 Preparation of *S*-propynyl glycosides from thioacetates

Reagents and conditions: **Method A**: i) NaOMe/MeOH, -40 °C, 30 min; ii) H^+ resin, -40 °C, 15 min; iii) propargyl bromide, Et_3N, 1 h.

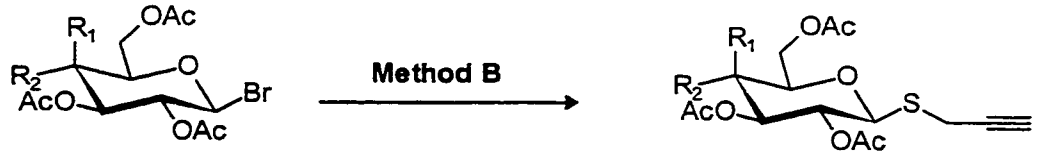
The main disadvantage of the method was that column chromatography had been done twice during the process. To overcome this drawback, a simple, efficient, one-pot procedure was chosen. Thus, glycosyl bromides were treated with thiourea to give the corresponding glycosyl 2-thiopseudourea hydrobromide intermediates. The pseudothioureas were directly used into the next step without purification. Nucleophilic displacement of bromide from propargyl bromide by the potassium thiolates generated in situ from the pseudothioureas using K_2CO_3 provided *S*-propynyl glycosides **94**, **95**, **96**, and **97** in 84%, 78%, 75%, and 80% yields, respectively (Scheme **5.7**).

Copper(I) halide-catalyzed oxidative coupling of acetylenes represents a convenient way to prepare diynes.[188] Pyridine or tetramethylethylenediamine (TMEDA) are normally used to solubilize the copper salt and also to facilitate proton abstraction from the terminal alkyne. However, when pyridine was used in homo-coupling of acetylenic thiosugar **97**, the yield was rather low (30 %) when compared to the corresponding *O*-mannopyranoside.[167] Addition of more CuCl, or diazabicycloundecene (DBU), had no effect on the yield. The yield could be dramatically increased when TMEDA was used instead of pyridine. In a typical experimental procedure, in a mixture of thiosugar **97** (1 mmol), CuCl (0.3 mmol), and TMEDA (0.6 mmol) in *N*,*N*-dimethylformamide (10 mL) was bubbled O_2 at 40 °C for 2 h. After usual work-up and purification by silica-gel column chromatography, dimer **98** was

obtained in 84% yield. The structure of the product was fully confirmed by ^{1}H NMR which showed no signal at δ 2.24 ppm for the acetylenic proton of the monomer. It was also determined by ^{13}C NMR which showed the acetylene's signals shifted upfield from δ 78.4 and 72.0 ppm of **97** to δ 73.6 and 67.8 ppm, respectively. The mass spectrum of compound **98** gave a M^++1 peak at 803.2. This procedure was successfully applied to other thiopropynyl glycosides (**94**, **95**, and **96**) to afford the corresponding dimers in good yields. The results are summarized in Table **5.2**.



89 **97**



	R_1	R_2
90	H	OAc
6	OAc	H
35	H	Gal

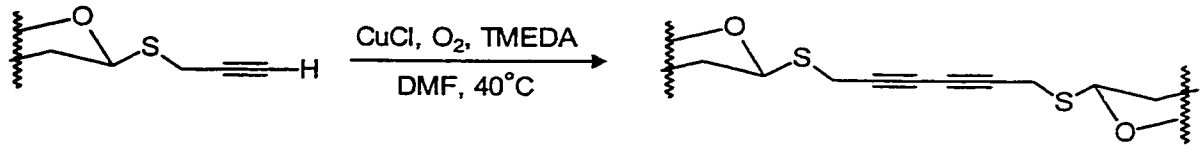
	R_1	R_2	yield
94	H	OAc	84%
95	OAc	H	78%
96	H	Gal	75%

Scheme 5.7 One-pot preparation of *S*-propynyl glycosides from glycosyl bromides

Reagents and conditions: **Method B**: i) $(H_2N)_2CS$, acetone, reflux; ii) K_2CO_3, H_2O, propargyl bromide, 1h.

[188] Hay, A. S. *J. Org. Chem.*, **1962**, *27*, 3320.

Table 5.2 Cu(I) catalyzed homo-coupling reactions of 2-propynyl glycosides



98-101

Entry	Sugar substitute	Reaction time (h)	Product	Yield (%)
1	**Man (97)**	2	**98**	84
2	**Glc (94)**	2	**99**	80
3	**Gal (95)**	2	**100**	85
4	**Lac(96)**	3	**101**	82

In order to further increase the interglycosidic distance, while the rigidity within the linker is maintained, palladium-catalyzed Sonogashira cross-coupling between alkynes and aryl iodides was next considered.

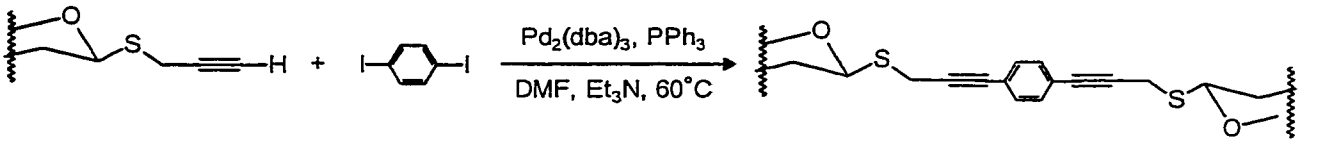
The Sonogashira coupling reaction between 2-propynyl thiomannopyranoside **97** and 1,4-diiodobenzene was investigated by using tris(dibenzylideneacetone)dipalladium [$Pd_2(dba)_3$], CuI, and PPh_3 in DMF and Et_3N solution (1:1, v/v) under nitrogen at room temperature for 1 h. The sulfur-containing compound **97** showed not to poison the palladium catalyst. Interestingly, the cross-coupling dimer **102** was formed in 82% yield. However, the oxidative homo-coupling of acetylenic mannopyranoside **97** to symmetrical diyne **98** (10% yield) was also catalyzed under such conditions. The separation of the diyne from the desired product was very difficult because of their close polarity. To reduce the side reaction, some methods such as strict exclusion of oxygen[189] or slow addition of alkyne[190] could be used. Copper(I) iodide was a particularly effective cocatalyst to facilitate the coupling and to allow the reaction to occur at room temperature. A likely assumption was that a copper alkynylide was formed. We thought this intermediate could cause the side reaction. Thus the modified

[189] Austin, W. B.; Bilow, N.; Kelleghan, W. J.; Lau, K. S. Y. *J. Org. Chem.* **1981**, *46*, 2280-2286.
[190] Thorand, S.; Krause, N. *J. Org. Chem.* **1998**, *63*, 8551-8553.

Sonogashira reaction without addition of copper salt was carried out. In a typical reaction, treatment of 2-propynyl thiomannopyranoside **97** (1 mmol) and 1,4-diiodobenzene (0.45 mmol) in a degassed solution of DMF and Et_3N (1:1, v/v) in the presence of $Pd_2(dba)_3$ (0.05 mmol) and PPh_3 (0.1 mmol) at 60 °C under a nitrogen atmosphere for 2 h afforded the desired dimer **102** in 95% yield. No diyne was found under these conditions. The difference is very clear between the homodimer and crossdimer in ^1H and ^{13}C NMR spectra. The methylene peaks of the crossdimer were at δ 3.59 and at 3.42 ppm compared with those of the homodimer at δ 3.42 and at 3.27 ppm from ^1H NMR spectra. Moreover, the acetylene peaks of the crossdimer were shifted downfield at δ 87.5 and at 83.3 ppm compared with those of the homodimer at δ 73.6 and at 67.8 ppm.

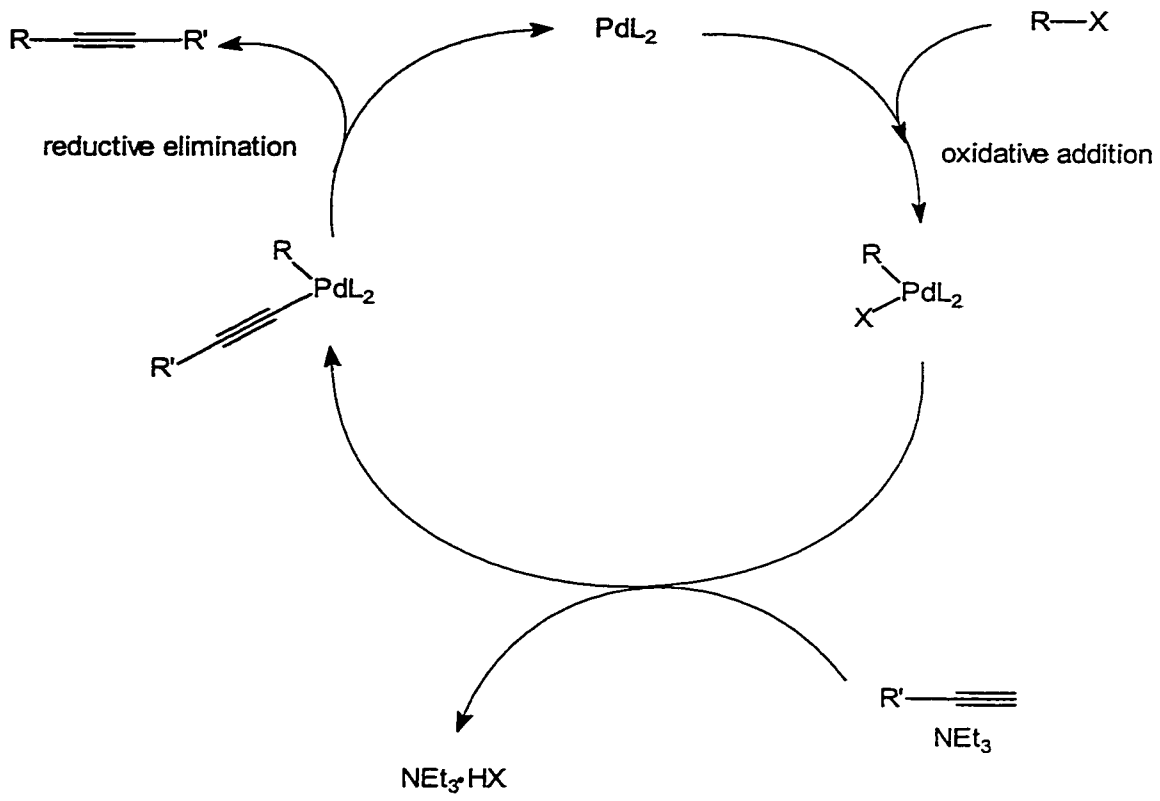
To demonstrate the scope of this new application, we also examined Sonogashira coupling of diiodobenzene with other carbohydrates (**94, 95**, and **96**). All of them provided good yields of dimer **103-105** in yield ranging from 89% to 93%. The results are summarized in Table **5.3**.

Table 5.3 Palladium catalyzed Sonogashira coupling of 2-propynyl glycosides



102-105

Entry	Sugar substitute	Reaction Time (h)	Product	Yield (%)
1	**Man (97)**	2	**102**	95
2	**Glc (94)**	2	**103**	90
3	**Gal (95)**	2	**104**	93
4	**Lac (96)**	4	**105**	89



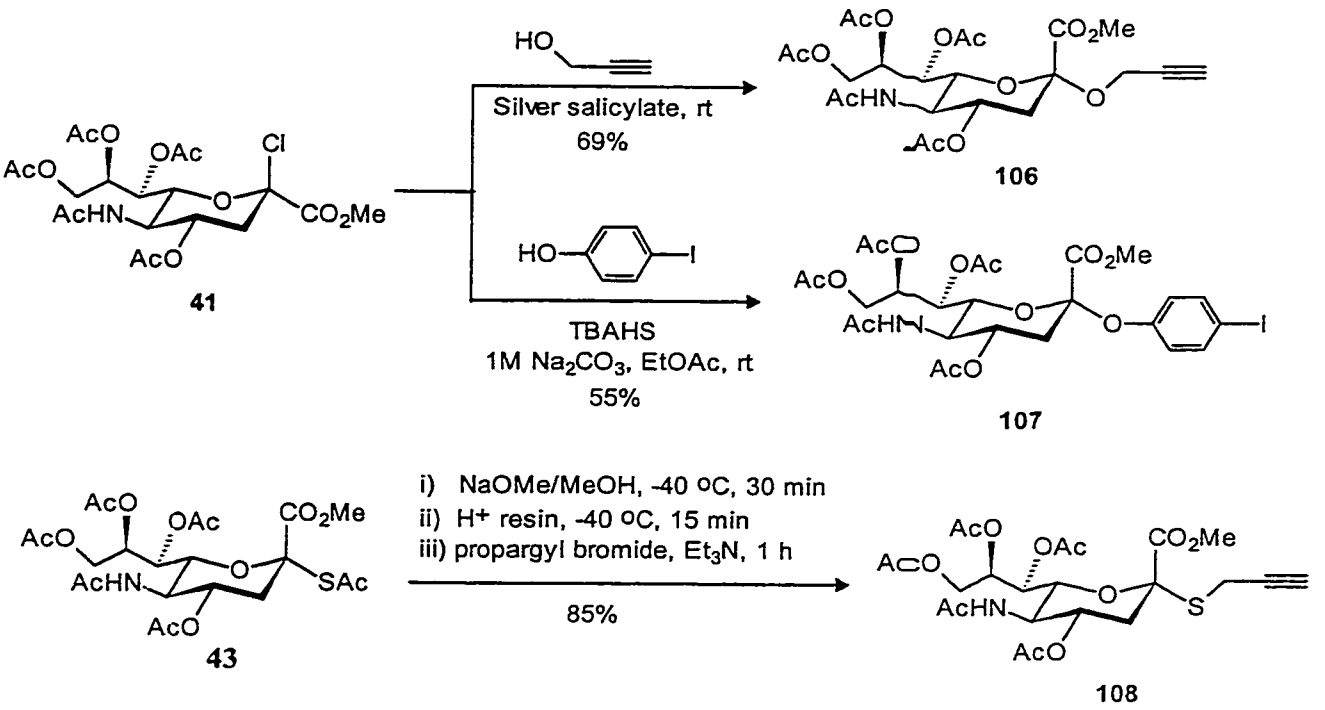
Scheme 5.8 Mechanism for cross-coupling of terminal alkynes with aryl halides

The formation of cross-coupling product can be explained by Scheme **5.8**. The coordinatively unsaturated Pd(0) species and the aryl halide form the intermediate by oxidative addition. This intermediate undergoes nucleophilic attack by the terminal alkyne. Reductive elimination subsequently affords the disubstituted acetylene and Pd(0) species. Interestingly, only a catalytic amount of Pd(0) was necessary since Pd(0) is generated in the catalytic cycle after the release of disubstituted acetylene.

In conclusion, the copper(I)-catalyzed Glaser reaction and palladium-catalyzed Sonogashira reaction have been applied toward the synthesis of divalent "rod-like" thioglycoside clusters. The reactions are general, high yielding, and compatible with the usual acetate protecting groups.

5.4 Synthesis of sialoside clusters by palladium catalysts

All of the above strategies were met with success using several monosaccharides and disaccharides. Encouraged by these results, we also applied these methods to *N*-acetylneuraminic acid which is the key epitope recognized as being essential for a number of virus infections.

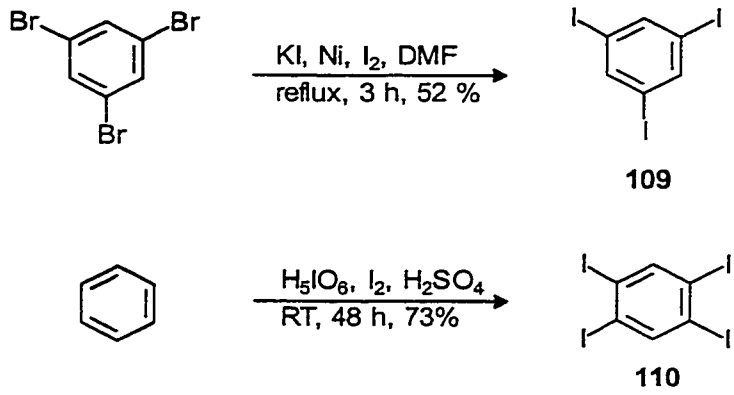


Scheme 5.9 Preparation of alkynyl α-sialoside derivatives

The requisite 2-propynyl *O*-α-sialoside **106** was readily accessible from β-acetochloroneuraminic acid **41** using propargyl alcohol in the presence of silver salicylate. The Koenigs-Knorr glycosylation occurred with complete stereocontrol giving rise to the α form. The pure product can be obtained by recrystallizing the crude sample in ethanol. The final product showed poor solubility in chloroform. 4-Iodophenyl *O*-α-sialoside **107** was prepared from **41** using improved phase transfer catalysis (PTC) conditions developed in our laboratory.[96] The synthesis of aryl α-sialosyl derivatives was achieved at room temperature in

a two-phase system using ethyl acetate, 1 M sodium carbonate and tetra-n-butylammonium hydrogen sulfate as phase transfer catalyst. The reaction was entirely stereoselective and provided product **107** in 55% yield. 2-Propynyl *S*-α-sialoside **108** was synthesized from the corresponding thioacetate derivative **43**. Chemoselective de-*S*-acetylation of **43** under low temperature Zemplén conditions and low temperature quenching by H^+ resin generated a sialic acid thiol intermediate. The thiol intermediate was then coupled to propargyl bromide to give **108** in 85% yield (Scheme **5.9**).

1,3,5-Triiodobenzene (**109**) was prepared from 1,3,5-tribromobenzene following the known procedure[191]. Treatment of 1,3,5-tribromobenzene with KI, I_2, and Ni powder in refluxing DMF afforded 1,3,5-triiodobenzene in 52% yield. Direct polyiodination[192] of benzene with periodic acid and iodine in sulfuric acid at room temperature offered 1,2,4,5-tetraiodobenzene (**110**) in good yield (73%) (Scheme **5.10**).

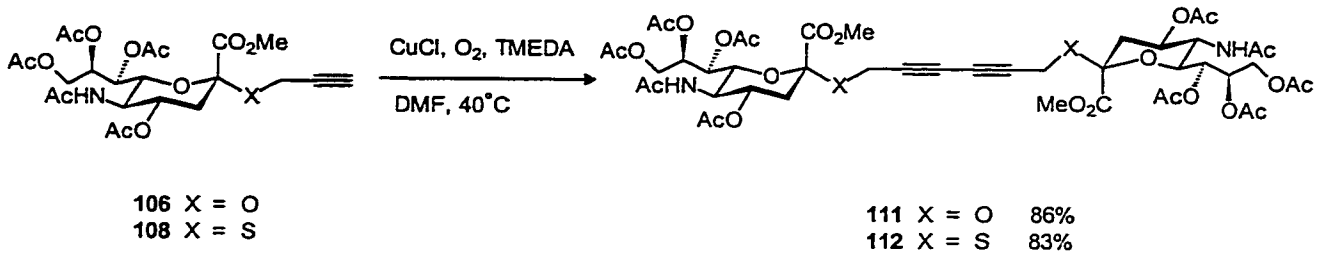


Scheme 5.10 Preparation of polyiodinated benzene derivatives

The syntheses of homo-coupling symmetrical diynes can be accomplished by using the Glaser reaction. Thus, to a solution of 2-propynyl *O*-α-sialoside **106,** CuCl, and TMEDA in DMF was bubbled O_2 at 40 °C for 2 h. The desired dimer **111** was obtained in 86% yield. The structure of **111** was fully confirmed by ^{1}H NMR which showed no signal at δ 2.24 ppm for the acetylenic proton of the monomer (Figures **5.4** and **5.5**). The mass spectrum of compound **111** gave a M^++1 peak at 1057.2. This procedure was also successfully applied to

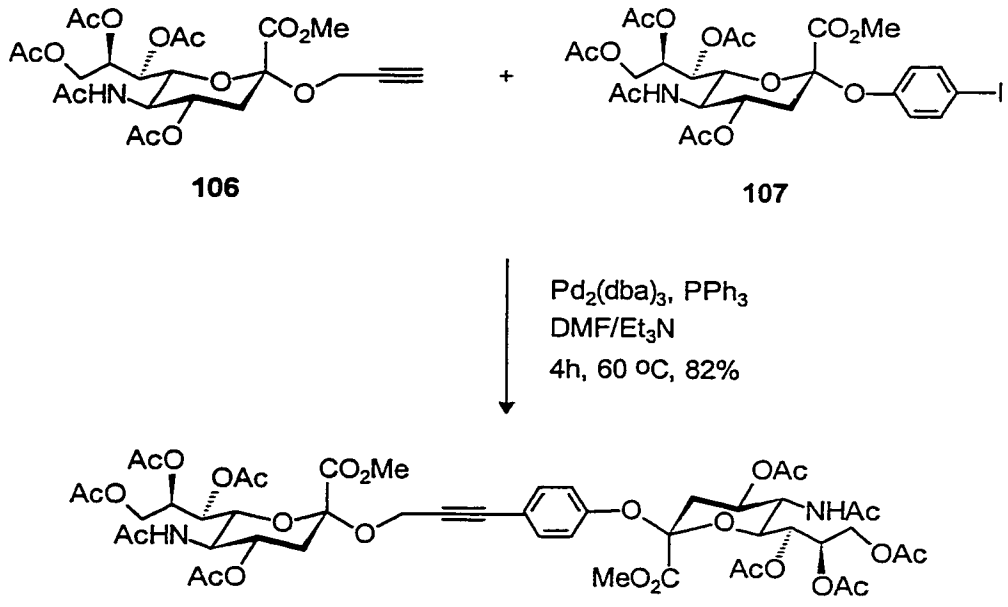
[191] Schöberl, U.; Magnera, T. F.; Harrison, R. M.; Fleischer, F.; Pflug, J. L.; Schwab, P. F. H.; Meng, X.; Lipiak, D.; Noll, B. C.; Allured, V. S.; Rudalevige, T.; Lee, S.; Michl, J. *J. Am. Chem. Soc.* **1997**, *119*, 3907.

2-propynyl *S*-α-sialoside **108** to afford the corresponding dimer **112** in 83% yield (Scheme **5.11**).



Scheme 5.11 Preparation of homodimers by Glaser reaction

According to our previous study, the modified Sonogashira cross-coupling conditions without CuI cocatalyst can eliminate the formation of symmetrical diyne. Treatment of 2-propynyl *O*-α-sialoside **106** (1.1 equiv) and 4-iodophenyl *O*-α-sialoside **107** (1 equiv) with $Pd_2(dba)_3$ at 60 °C afforded cross-coupling dimer **113** in 82% yield. No symmetrical diyne **111** was found (Scheme **5.12**).



Scheme 5.12 Synthesis of asymmetrical sialoside dimer **113** by Sonogashira reaction

[192] Mattern, D. L. *J. Org. Chem.* **1984**, *49*, 3051.

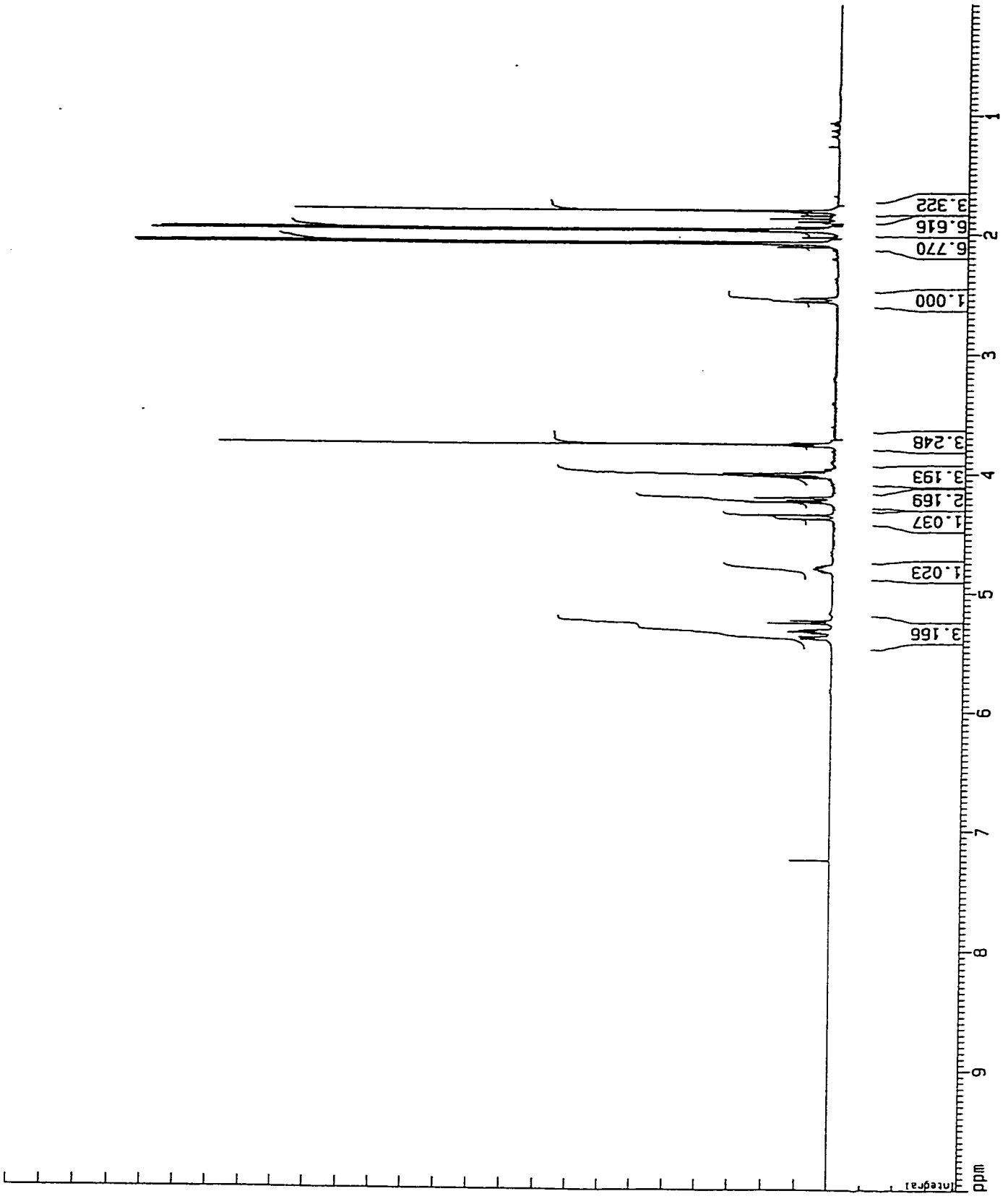


Figure 5.4 ^{1}H NMR (500MHz, $CDCl_3$) spectrum of homodimer **111**

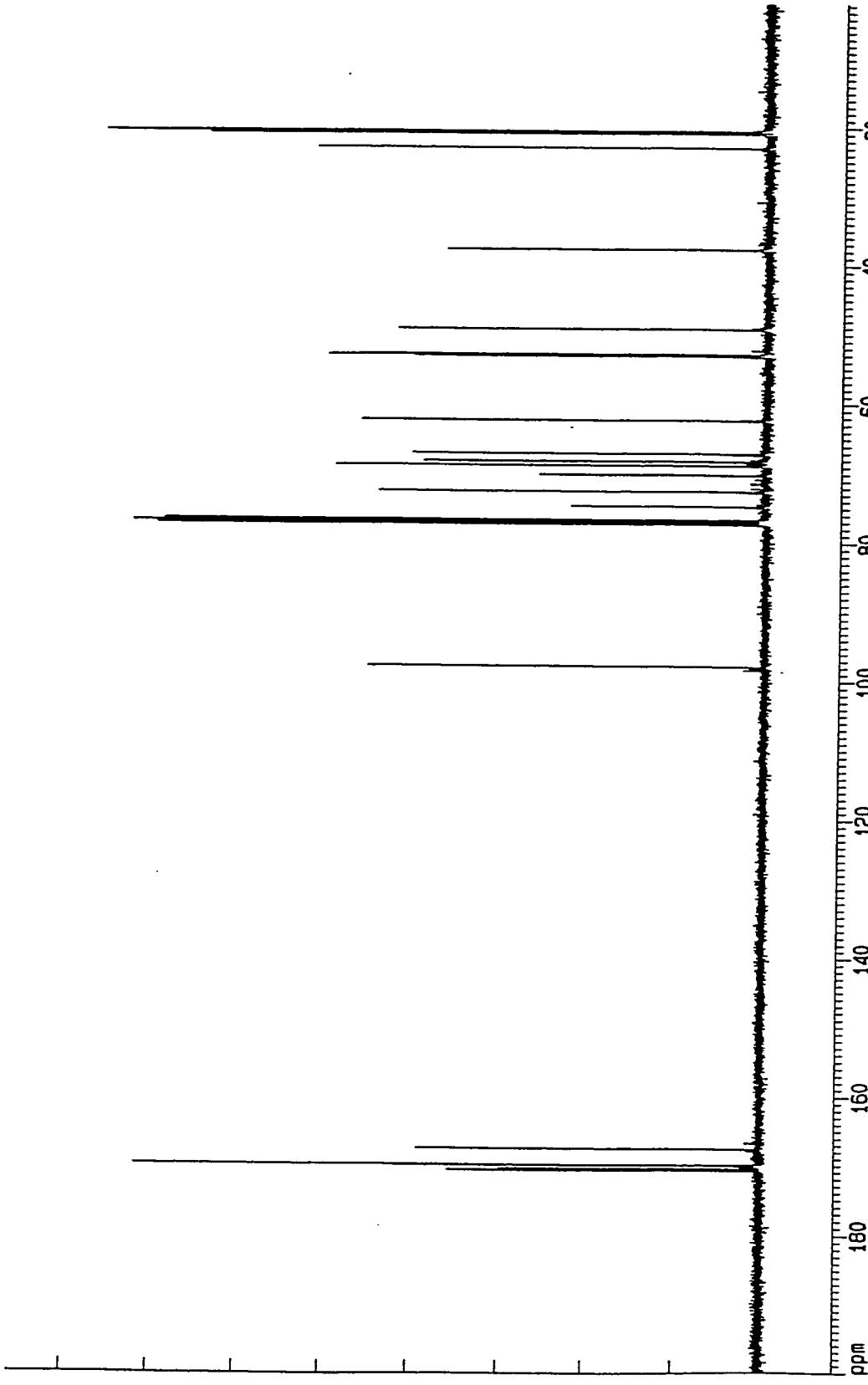


Figure 5.5 ^{13}C NMR (500MHz, $CDCl_3$) spectrum of homodimer **111**

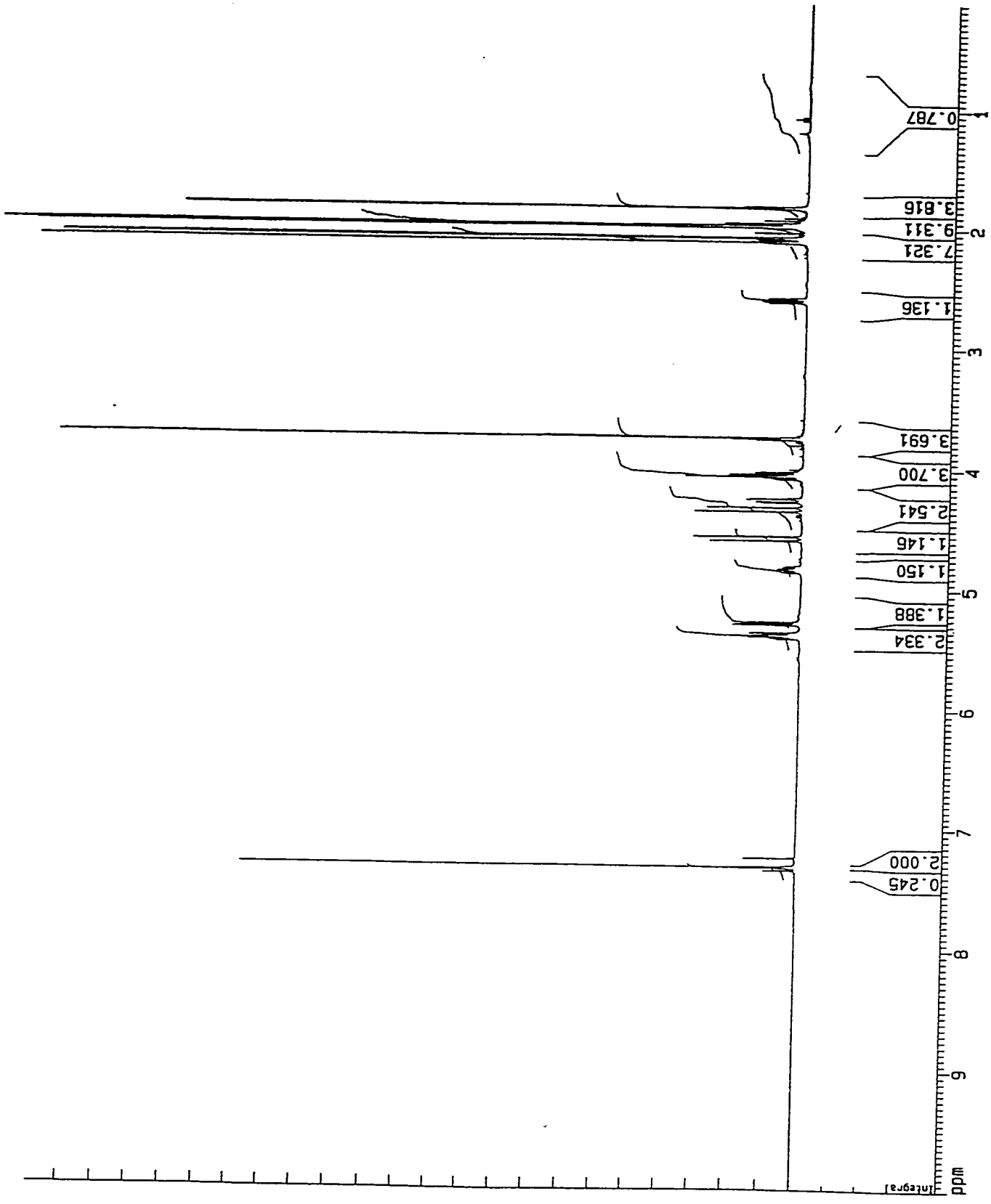


Figure 5.6 ^{1}H NMR (500MHz, $CDCl_3$) spectrum of dimer **114**

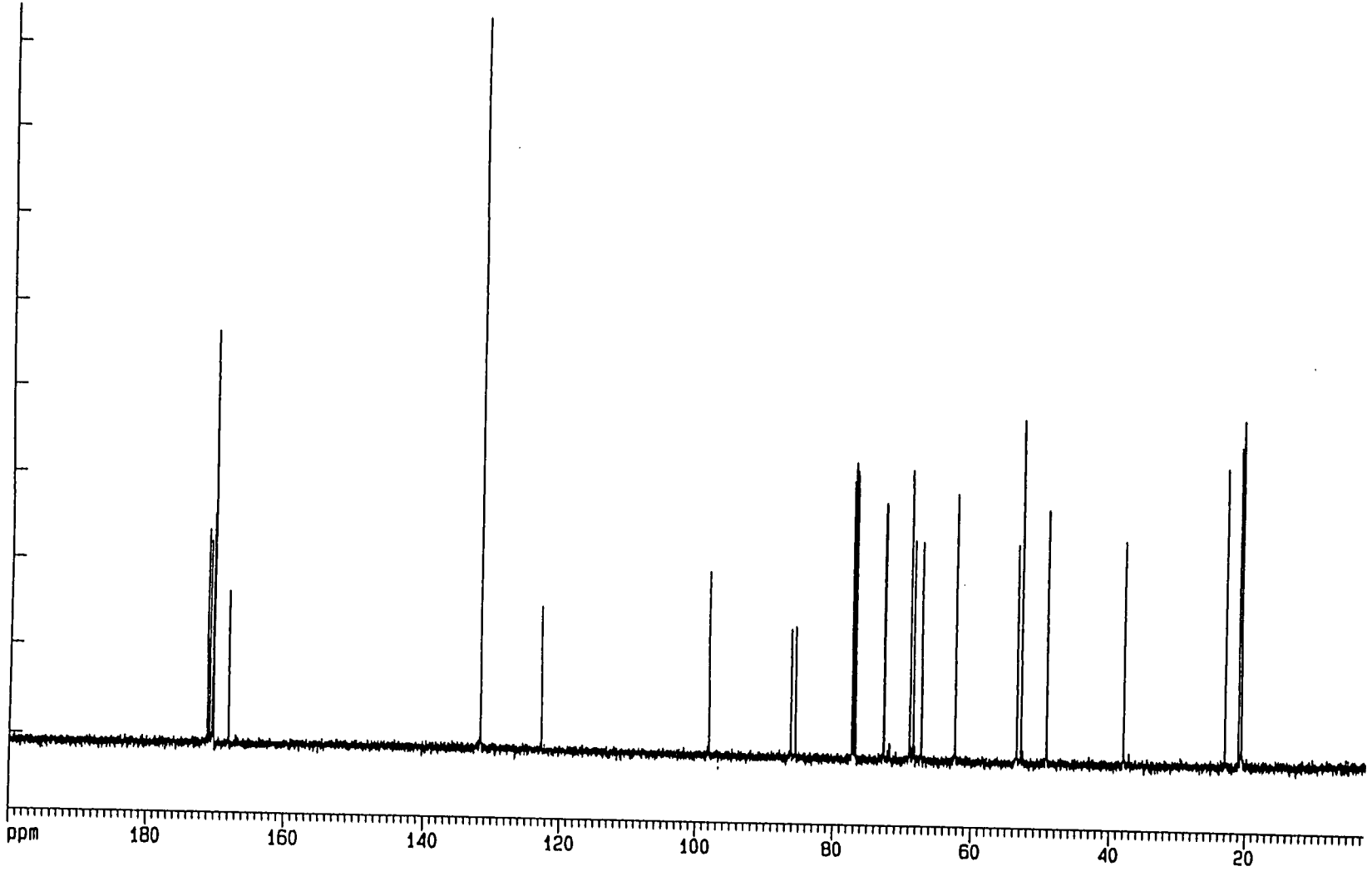
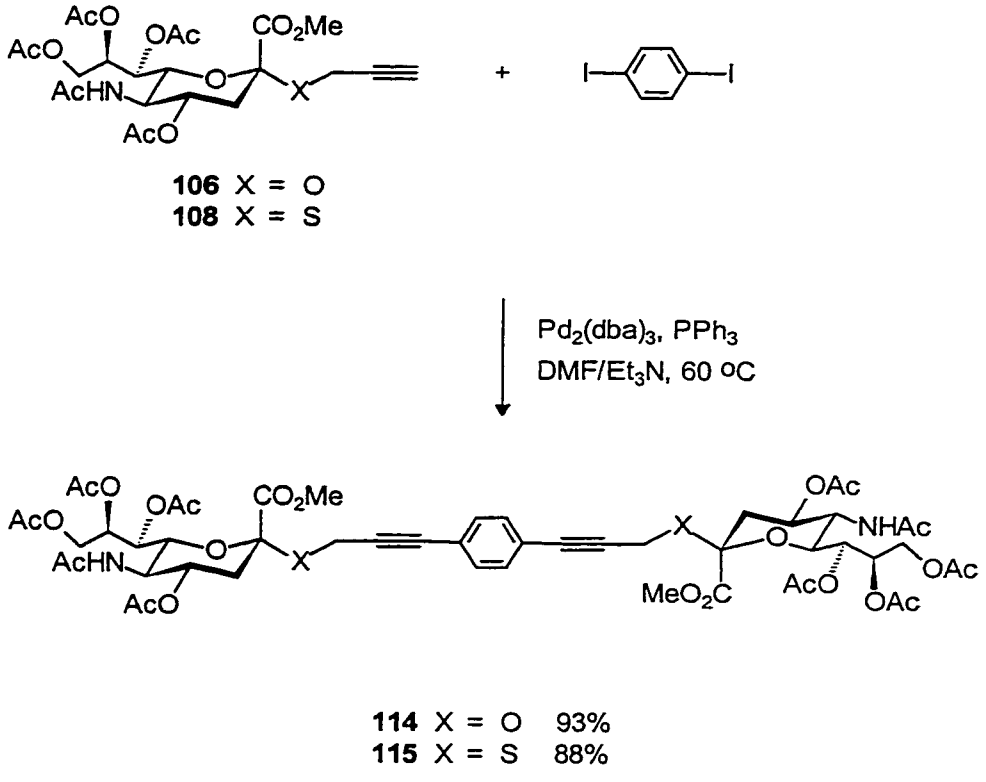


Figure 5.7 ^{13}C NMR (500MHz, $CDCl_3$) spectrum of dimer **114**

Treatment of 2-propynyl *O*-α-sialosides **106** with 1,4-diiodobenzene (1 equiv) under the similar conditions generated dimer **114** in 93% yield. No symmetrical diyne **111** was found. The distinction between the homodimer **111** and the crossdimer **114** was very clear from their ^{13}C NMR spectra. The acetylene peaks of the crossdimer **114** were shifted downfield at δ 86.2 and at 85.6 ppm compared with those of the homodimer **111** at δ 74.7 and at 70.1 ppm (Figures **5.6** and **5.7**). The previous results showed that thiopropynyl glycosides successfully undergo cross-coupling with 1,4-diiodobenzene under Sonogashira conditions. Thus, 2-propynyl *S*-α-sialosides **108** reacted with 1,4-diiodobenzene in the presence of $Pd_2(dba)_3$ at 60 °C to afford dimer **115** in 88% yield (Scheme **5.13**).

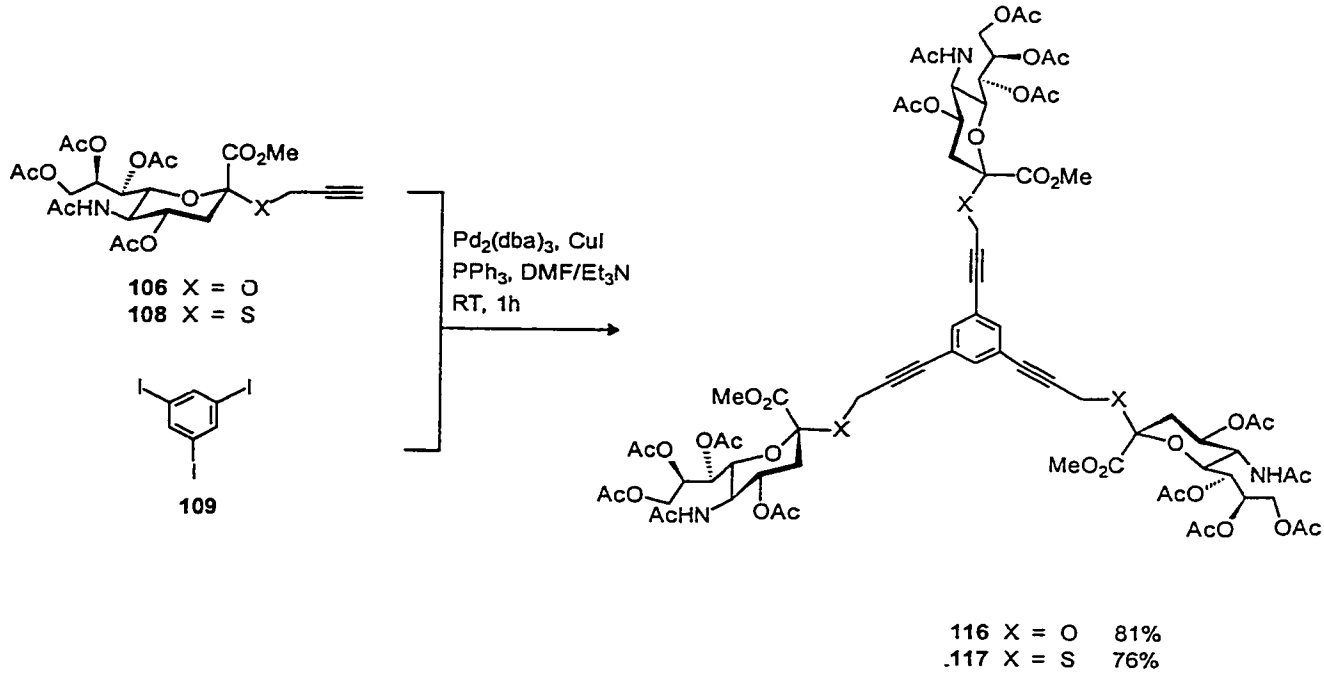


Scheme 5.13 Preparation of divalent sialoside derivatives by Sonogashira reaction

Encouraged by the successful synthesis of divalent sialoside derivatives by Sonogashira reaction, we further expanded this reaction toward the synthesis of higher valency sialoside derivatives. To avoid the formation of symmetrical diynes as minor

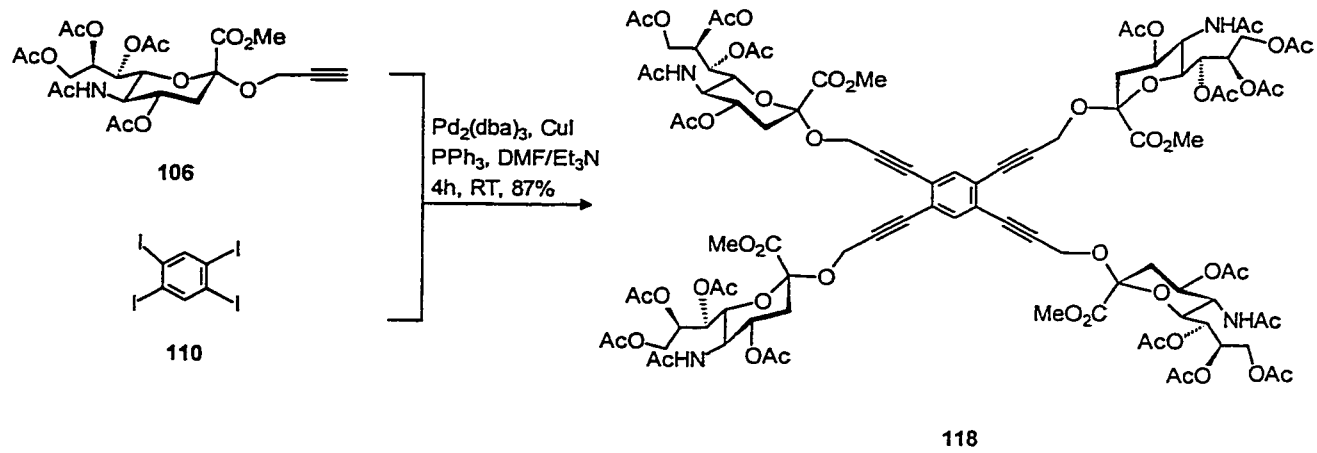
byproducts which cause purification problems, CuI is not used in the synthesis of crossdimers. However, CuI is known as a particularly effictive cocatalyst which allows the reaction to occur at room temperature. Without CuI, the reaction has to be accomplished at higher temperature and longer reaction time. Since sialic acid trimers and tetramers are easily separated from symmetrical diynes, the addition of CuI would help reactions occur smoothly with more hindered tri- or tetra-iodobenzenes.

2-Propynyl *O*-α-sialosides **106** was treated with 1,3,5-triiodobenzene (**109**) under $Pd_2(dba)_3$, CuI, and PPh_3 in DMF/Et_3N at room temperature for 1 h. The desired trimer **116** was obtained in 81% yield. The trimer **116** was easily separated from a small amount of byproduct, symmetrical diyne **111**, by chromatography column. Trimer **117** was also prepared from 2-propynyl *S*-α-sialoside **108** under the same conditions in 76% yield (Scheme **5.14**).



Scheme 5.14 Preparation of trivalent sialoside derivatives by Sonogashira reaction

Treatment of 2-propynyl *O*-α-sialosides **106** with 1,2,4,6-tetraiodobenzene (**110**) under $Pd_2(dba)_3$, CuI, and PPh_3 in DMF/Et_3N generated tetramer **118** in 87% yield. Because of the steric hindrance of tetraiodobenzene, the reaction needed much more time to complete (Scheme **5.15**). The structure of **118** was confirmed by its ^1H NMR and ^{13}C NMR spectra (Figures **5.8** and **5.9**).



Scheme 5.15 Synthesis of tetravalent sialoside derivative **118**

Grubbs' catalyst **71** can also be used to catalyze intermolecular cyclotrimerization of terminal alkynes.[193] 2-Propynyl *O*-α-sialoside **106** was treated with Grubbs' catalyst **71** in refluxing 1,2-dichloroethane for 24 h. The desired trisubstituted benzene derivatives **119a, b** were isolated as a mixture of 1,2,4-(**119a**) and 1,3,5-(**119b**) regioisomers in 68% yield (Scheme 5.). The reaction was found to be highly regioselective in favor of the 1,2,4-regioisomer **119a** over that of **119b** (4.5:1). The regioisomeric structures and the ratio were determined from the ^1H NMR spectrum by comparing the data of analogous products reported in the literature.[194] The aromatic protons for the 1,2,4-isomer **119a** appeared at δ 7.27 ppm (d, *J* 7.8 Hz), 7.23 (s), and 7.19 (d, *J* 7.8 Hz) and for the symmetrical 1,3,5-isomer **119b** as a singlet at δ 7.14 ppm. The ^{13}C NMR showed aromatic carbon signals at 136.4, 135.4, 135.0 ppm (quaternary carbons) and 128.7, 127.9, 127.1 ppm (tertiary carbons) for the 1,2,4-isomers and those for 1,3,5-isomer appeared at 137.2 ppm (quaternary carbon) and at 126.4 ppm (tertiary carbon).

[193] Das, S. K.; Roy, R. *Tetrahedron Lett.* **1999**, *40*, 4015.
[194] Kaufman, R. J.; Sidhu, R. S. *J. Org. Chem.* **1982**, *47*, 4941.

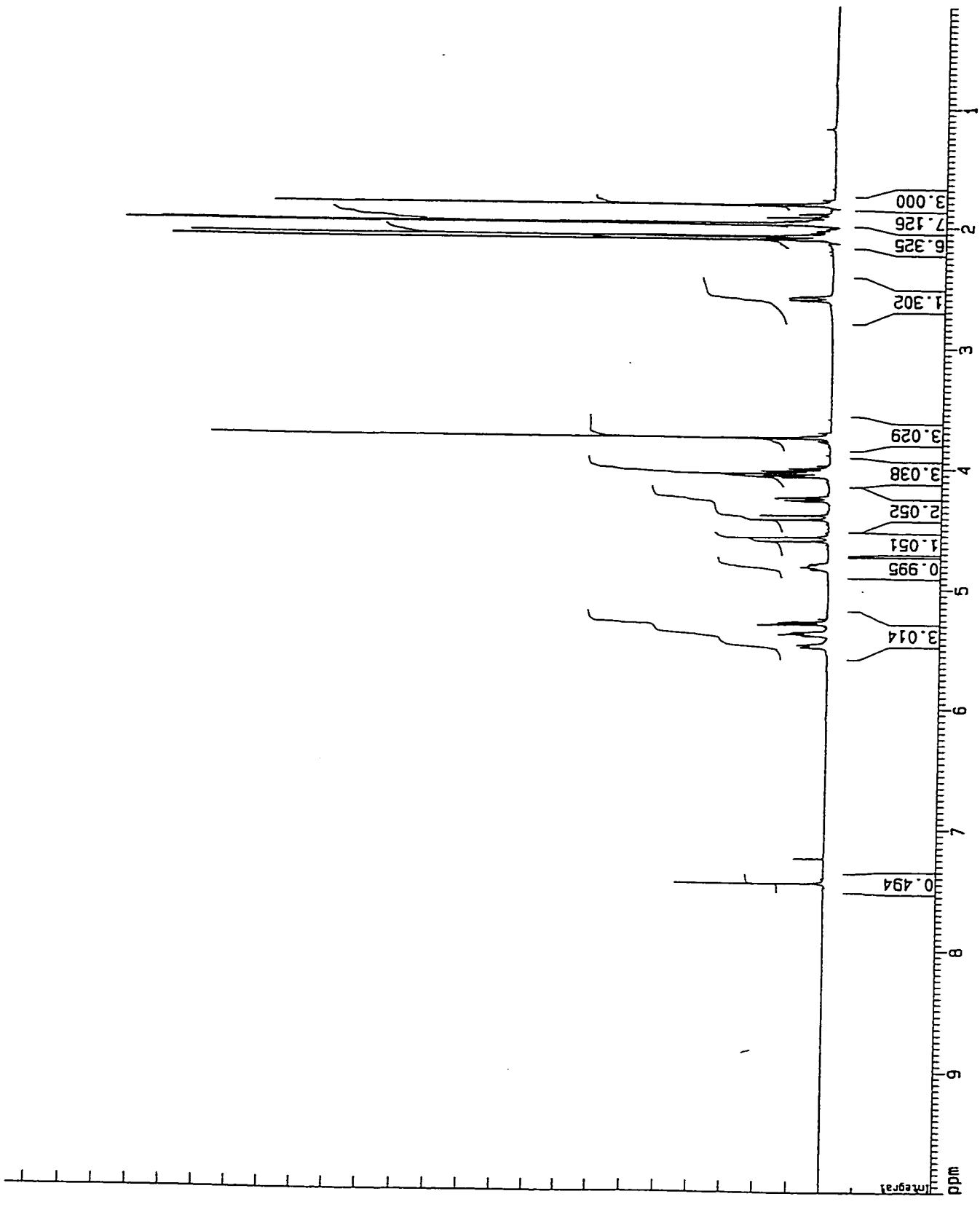


Figure 5.8 ^{1}H NMR (500MHz, $CDCl_3$) spectrum of tetramer **118**

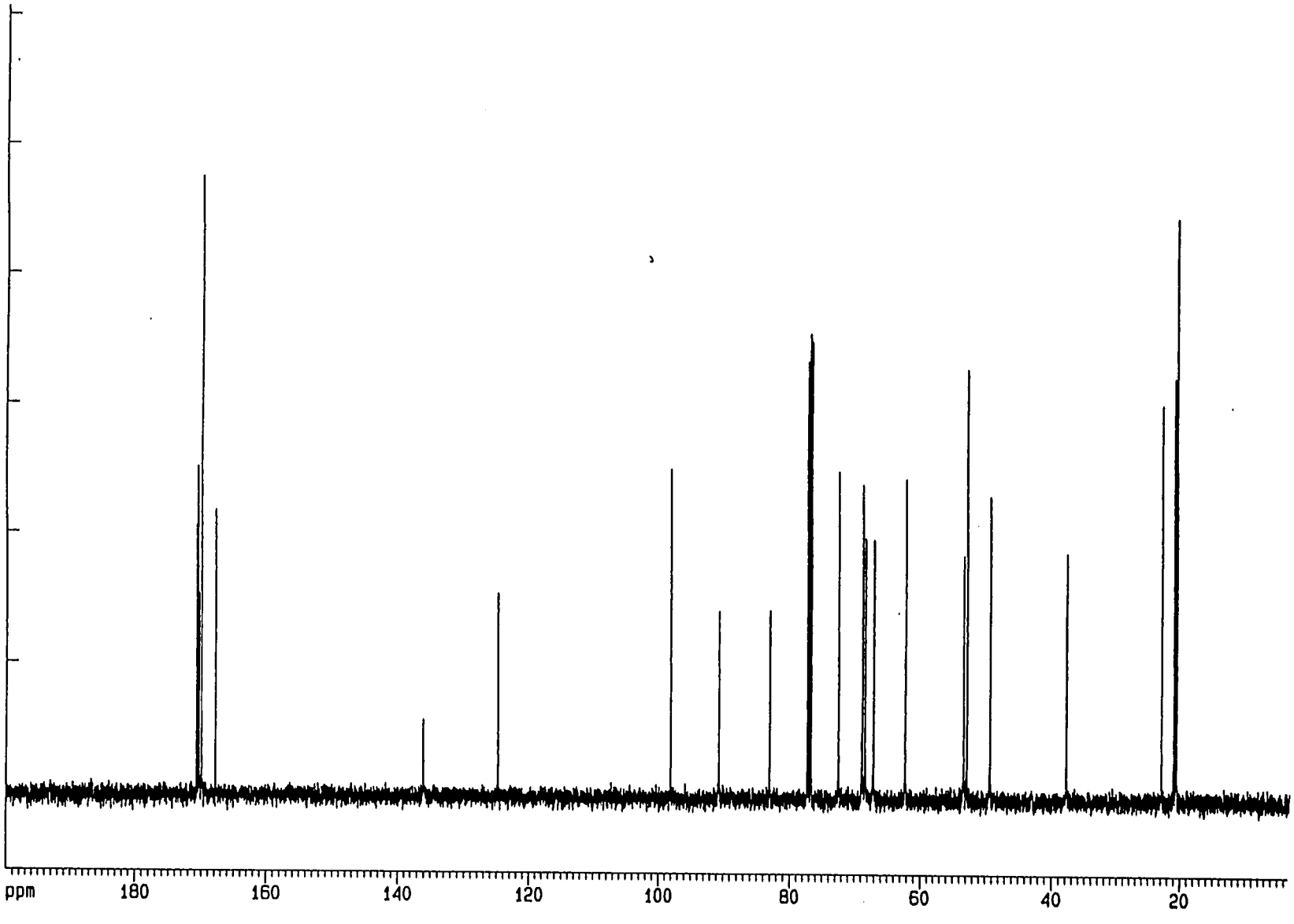
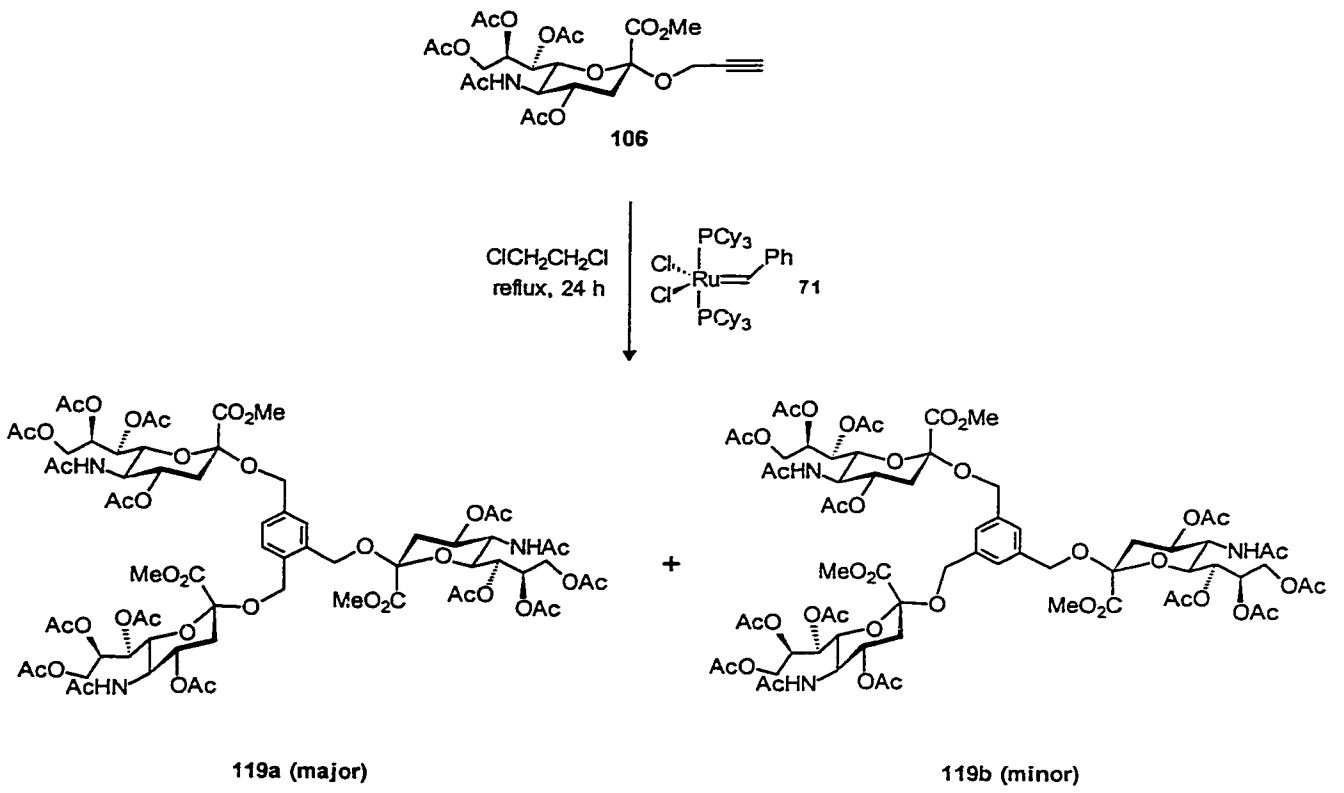


Figure 5.9 ^{13}C NMR (500MHz, $CDCl_3$) spectrum of tetramer **118**



Scheme 5.16 Cyclotrimerization of 2-propynyl sugar derivative **106**

In summary, the palladium-catalyzed Sonogashira reaction has been successfully applied for the synthesis of rigid sialic acid clusters. The procedure is general, high yielding, compatible with the easily removable acetate group and the sialic acid clusters were isolated in excellent yields.

5.5 Experimental

Methyl (allyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate (74)

To a solution of freshly prepared β-acetochloroneuraminic acid **41** (0.63 g, 1.2 mmol) in dry allyl alcohol (10 mL) containing 4Å molecular sieves (0.5 g) was added freshly prepared silver salicylate (0.45 g). The reaction was stirred for 2 h under nitrogen at room

temperature in the dark. The slurry was filtered over Celite, washed with dichloromethane, and evaporated to dryness. The residue was dissolved in dichloromethane (30 mL). The organic solution was successively washed with sat. $NaHCO_3$ solution, 5% $Na_2S_2O_3$ solution, and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane-methanol (30:1) as eluent to obtain pure product **74** (586 mg, 89 %): m.p. 154-158 °C (methanol-ether); $[\alpha]_D$ -13.8° (*c* 1.0, $CHCl_3$); Lit.[195] 154-156 °C; $[\alpha]_D$ -13° (methanol).

Methyl (4-pentenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate (75)

n-Pentenyl *O*-α-sialoside **75** was prepared as the same procedure as allyl *O*-α-sialoside **74**. Purification by silica gel column chromatography using ether-ethanol (50:1) as eluent afforded product **75** in 86% yield: ^{1}H NMR ($CDCl_3$) δ 5.73 (m, 1 H, -C<u>H</u>=CH_2), 5.46 (bs, 1 H, NH), 5.31 (m, 1 H, H-8), 5.26 (dd, 1 H, $J_{6,7}$ 1.6 Hz. $J_{7,8}$ 7.9 Hz, H-7), 4.97-4.88 (m, 2 H, CH=C<u>H</u>$_2$), 4.77 (m, 1 H, H-4), 4.27 (dd, 1 H, $J_{8,9a}$ 2.6 Hz, $J_{9a,9b}$ 12.4 Hz, H-9a), 4.06-3.98 (m, 3 H, H-9b, H-6, H-5), 3.72 (s, 3 H, OCH_3), 3.70 (m, 1 H, -OC<u>H</u>$_2$-), 3.17 (m, 1 H, -OC<u>H</u>$_2$-), 2.52 (dd, 1 H, $J_{3e,4}$ 4.4 Hz, $J_{3a,3e}$ 12.8 Hz, H-3e), 1.99, 1.89, 1.74 (5s, 15 H, NHAc, OAc's), 1.95 (m, 2 H, C<u>H</u>$_2$CH=), 1.80 (dd, 1 H, $J_{3a,4}$ 12.3 Hz, H-3a), 1.49 (m, 2 H, -OCH_2C<u>H</u>$_2$-); ^{13}C-NMR ($CDCl_3$) δ 170.6-168.3 (C=O's), 137.7 (CH_2<u>C</u>H=), 114.6 (CH=<u>C</u>H_2), 98.5 (C-2), 72.3 (C-6), 69.1 (C-4), 68.9 (C-8), 67.3 (C-7), 63.9 (O<u>C</u>$H_2(CH_2)_2$), 62.2 (C-9), 52.3 (OCH_3), 49.0 (C-5), 37.8 (C-3), 29.7 ($O(CH_2)_2$<u>C</u>H_2), 28.5 (OCH_2<u>C</u>H_2CH_2), 22.7 (NHAc), 20.8, 20.5, 20.4 (OAc's).

Methyl (allyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate (76)

To a solution of freshly prepared β-acetochloroneuraminic acid **41** (0.4 g, 0.78 mmol) in ethyl acetate (10 mL) was added a solution of allyl mercaptan (2 eq.) and tetrabutylammonium hydrogen sulfate (265 mg, 0.78 mmol) in 1M sodium carbonate (10 mL). The reaction mixture was vigorously stirred at room temperature until TLC indicated

[195] Eschenfelder, V.; Brossmer, R. *Carbohydr. Res.* **1980**, *78*, 190.

complete transformation of the starting material (1h). The reaction was diluted with ethyl acetate (20 mL). The organic layer was separated and washed with saturated sodium chloride solution (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 dichloromethane-methanol (30:1) as eluent to obtain pure product **76** as a white solid (84%); mp 108.5-110 °C (Ether/hexanes); $[\alpha]_D$ +38.9° (*c* 1.01, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.73 (m, 1 H, CH=), 3.35 (dd, 1 H, J_{gem} 13.8, J_{vic} 6.3 Hz, SCHa), 3.26 (dd, 1 H, J_{vic} 7.5 Hz, SCHb), 2.69 (dd, 1 H, $J_{3e,4}$ 4.6 Hz, $J_{3a,3e}$ 12.7 Hz, H-3e), 1.96 (dd, 1 H, $J_{3a,4}$ 11.7 Hz, H-3a); ^{13}C NMR ($CDCl_3$) δ 132.9 (CH=), 118.0 (=CH_2), 82.9 (C-2), 74.1 (C-6), 69.5 (C-8), 68.6 (C-4), 67.3 (C-7), 62.2 (C-9), 52.8 (CH_3O), 49.3 (C-5), 37.8 (C-3), 31.6 (SCH_2); M.S. for $C_{23}H_{33}NO_{12}S$: 547.9 (M+1).

General procedure for the preparation of aryl sialosides by PTC reaction

To a solution of freshly prepared β-acetochloroneuraminic acid **41** (0.4 g, 0.78 mmol) in ethyl acetate (10 mL) was added a solution of phenol derivatives (2-allyl phenol, or 4-vinyl phenol) (2 eq.) and tetrabutylammonium hydrogen sulfate (265 mg, 0.78 mmol) in 1M sodium carbonate (10 mL). The reaction mixture was vigorously stirred at room temperature until TLC indicated complete transformation of the starting material (1h). The reaction was diluted with ethyl acetate (20 mL). The organic layer was separated and washed with saturated sodium chloride solution (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 dichloromethane-methanol (30:1) as eluent to obtain pure product.

Methyl (2-allylphenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate (77)

White solid (64%); mp 86-88 °C; $[\alpha]_D$ +0.2 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.09-6.40 (m, 4 H, aromatic H), 5.88-5.81 (m, 1 H, -C<u>H</u>=), 5.79 (d, 1 H, $J_{5,NH}$ 10.1 Hz, NH), 5.31-5.30 (m, 2 H, H-7, H-8), 4.99-4.94 (m, 2 H, =C<u>H</u>$_2$), 4.87 (m, 1 H, H-4), 4.38 (d, 1 H, $J_{5,6}$ 10.9 Hz, H-6), 4.23 (dd, 1 H, $J_{8,9a}$ 2.0 Hz, $J_{9a,9b}$ 12.4 Hz, H-9a), 4.12-4.09 (m, 2 H, H-9b, H-5), 3.53 (s, 3 H, OCH_3), 3.03-3.28 (m, 2 H, -C<u>H</u>$_2$CH=), 2.63 (dd, 1 H, $J_{3e,4}$ 4.7 Hz, $J_{3a,3e}$ 12.9 Hz, H-3e), 2.16 (dd, 1 H, $J_{3a,4}$ 12.4 Hz, H-3a), 2.06, 2.05, 1.96, 1.95, 1.82 (5s, 15 H, NAc, OAc's).

^{13}C NMR ($CDCl_3$) δ 170.7-169.9 (C=O's), 168.1 (C-1), 150.2, 131.1, 129.9, 127.2, 123.8 (aromatic C), 136.5 (C-2'), 115.5 (C-1'), 100.1 (C-2), 73.4 (C-6), 69.5 (C-8), 68.9 (C-4), 68.0 (C-7), 62.0 (C-9), 52.7 (CH_3O), 49.1 (C-5), 37.9 (C-3), 34.4 (C-3'), 23.0, 20.9, 20.7, 20.6 (NAc, OAc's); FAB-MS: 608.3 ($[M+1]^+$, 41.1%).

Anal. Calcd. for $C_{29}H_{37}NO_{13}$: C, 57.33; H, 6.14; N: 2.31. Found: C, 57.03; H, 5.99; N, 2.37.

Methyl (4-vinylphenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate (78)

White solid (75%); mp 81-82 °C; $[\alpha]_D$ +14.8 (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.23 (d, 2 H, *J* 8.8 Hz, aromatic H), 6.93 (d, 2 H, aromatic H), 6.57 (dd, 1 H, -C<u>H</u>=), 5.77 (d, 1 H, $J_{5,NH}$ 11.1 Hz, NH), 5.58 (d, 1 H, J_{trans} 17.6 Hz, C<u>H</u>$_2$=), 5.30 (m, 2 H, H-7, H-8), 5.10 (d, 1 H, J_{cis} 11.0 Hz, C<u>H</u>$_2$=), 4.87 (ddd, 1 H, H-4), 4.36 (dd, 1 H, $J_{5,6}$ 10.7 Hz, $J_{6,7}$ < 1 Hz, H-6), 4.25 (dd, 1 H, $J_{8,9a}$ 1.5 Hz, $J_{9a,9b}$ 12.7 Hz, H-9a), 4.10 (dd, 1 H, $J_{8,9b}$ 2.6 Hz, H-9b), 4.04 (ddd, 1H, $J_{4,5}$ 10.4 Hz, H-5), 3.57 (s, 3 H, OCH_3), 2.62 (dd, 1 H, $J_{3e,4}$ 4.7 Hz, $J_{3a,3e}$ 13 Hz, H-3e), 2.14 (dd, 1 H, $J_{3a,4}$ 12.7 Hz, H-3a), 2.07, 2.05, 1.96, 1.95, 1.82 (5s, 15 H, NAc, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.8-169.9 (C=O's), 168.1 (C-1), 153.3, 133.4, 127.1, 119.8 (aromatic C), 135.9 (C-2'), 113.0 (C-1'), 99.9 (C-2), 76.8 (C-6), 69.5 (C-8), 68.8 (C-4), 67.4 (C-7), 62.0 (C-9), 52.8 (CH_3O), 49.1 (C-5), 38.0 (C-3), 23.0, 20.9, 20.7, 20.6 (NAc, OAc's); FAB-MS: 594.3 ($[M+1]^+$, 28.7%)

Anal. Calcd. for $C_{28}H_{35}NO_{13}$: C, 56.66; H, 5.94; N: 2.36. Found: C, 56.42; H, 5.86; N, 2.48.

Typical procedure for the preparation of divalent sialoside derivatives 79-83 by olefin metathesis reaction

To a solution of alkenyl glycoside (100 mg) in dry dichloromethane (2 mL) was added ruthenium catalyst (5-10 mol %). The reaction mixture was refluxed under nitrogen for 2-24 h. The reaction was monitored by TLC. The reaction mixture was concentrated and purified by silica gel column chromatography using dichloromethane-methanol (20:1) as eluent to obtain pure product.

(*E*, *Z*) 1,4-Di-*O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-but-2-ene (79)

^{1}H NMR ($CDCl_3$) δ 5.66 (m, 2 H, H-1' trans, H-1' cis), 5.51-5.23 (m, 6 H, NH, H-8, H-7), 4.76 (m, 2 H, H-4), 4.23-4.18 (m, 4 H, H-9a, -C$\underline{H}_2$C=), 4.04-3.96 (m, 6 H, H-9b, H-6, H-5), 3.77-3.71 (m, 2 H, -C$\underline{H}_2$C=), 3.70 (s, 6 H, OCH_3), 2.52 (dd, 2 H, $J_{3e,4}$ 4.5 Hz, $J_{3a,3e}$ 12.8 Hz, H-3e), 1.87 (dd, 2 H, $J_{3a,4}$ 12.6 Hz, H-3a), 2.06, 1.96, 1.95, 1.79 (5s, 30 H, NHAc, OAc's); ^{13}C-NMR ($CDCl_3$) δ 170.85-169.97 (C=O's), 168.26 (C-1 cis), 168.18 (C-1 trans), 128.18 (C-1' cis), 128.14 (C-1' trans), 98.56 (C-2 cis), 98.28 (C-2 trans), 72.46 (C-6 cis), 72.39 (C-6 trans), 69.14 (C-4 cis), 69.02 (C-4 trans), 68.55 (C-8), 67.25 (C-7), 64.58 (C-2' trans), 62.30 (C-9 trans), 62.19 (C-9 cis), 60.92 (C-2' cis), 52.59 (OCH_3), 49.22 (C-5), 37.94 (C-3 trans), 37.85 (C-3 cis); FAB-MS: 1035.4 ([M+1]$^+$, 5.3%).

Anal. Calcd. for $C_{44}H_{62}N_2O_{26}$: C, 51.06; H, 6.04; N: 2.71. Found: C, 50.67; H, 5.97; N, 2.72.

(*E*, *Z*) 1,8-Di-*O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-oct-4-ene (80)

^{1}H NMR ($CDCl_3$) δ 5.53 (d, 2 H, $J_{5,NH}$ 9.5 Hz, NH), 5.36-5.24 (m, 6 H, H-1' trans, H-1' cis, H-8, H-7), 4.77 (m, 2 H, H-4), 4.25 (dd, 2 H, $J_{8,9a}$ 2.6 Hz, $J_{9a,9b}$ 12.4 Hz, H-9a), 4.08-3.86 (m, 6 H, H-9b, H-6, H-5), 3.71 (s, 6 H, OCH_3), 3.69 (m, 2 H, -OC$\underline{H}_2$-), 3.15 (m, 2 H, -OC$\underline{H}_2$-), 2.51 (dd, 2 H, $J_{3e,4}$ 4.6 Hz, $J_{3a,3e}$ 12.8 Hz, H-3e), 1.87 (dd, 2 H, $J_{3a,4}$ 12.5 Hz, H-3a), 2.07, 2.06, 1.97, 1.96, 1.81 (5s, 30 H, NHAc, OAc's), 1.98 (m, 4 H, -C$\underline{H}_2$CH=), 1.52 (m, 4 H, -OCH$_2$C$\underline{H}_2$-); ^{13}C-NMR ($CDCl_3$) δ 170.88-169.97 (C=O's), 168.45 (C-1), 129.78 (C-1' trans), 129.32 (C-1' cis), 98.68 (C-2), 72.41 (C-6), 69.15 (C-4), 68.86 (C-8), 67.38 (C-7), 64.35 (C-4' trans), 64.29 (C-4' cis), 62.28 (C-9), 52.51 (OCH_3), 37.98 (C-3), 29.51 (C-3' cis), 29.43 (C-3' trans), 28.70 (C-2' trans), 23.32 (C-2' cis); FAB-MS: 1091.5 ([M+1]$^+$, 4.3%).

Anal. Calcd. for $C_{48}H_{70}N_2O_{26}$: C, 52.84; H, 6.47; N: 2.57. Found: C, 52.49; H, 6.46; N, 2.59.

(*E*, *Z*) 1,4-Di-*S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-but-2-ene (81)

^{1}H NMR ($CDCl_3$) δ 5.54 (t, 1.14 H, *J* 3.9 Hz, H-1' trans), 5.48 (t, 0.86 H, *J* 5.2 Hz, H-1' cis), 5.36-5 18 (m, 6 H, H-8, H-7, NH), 4.83 (m, 2 H, H-4), 4.28-4.25 (m,2 H, H-9a cis, H-9a trans), 4.08-4.06 (m, 2 H, H-9b cis, H-9b trans), 4.00 (ddd, 2 H, *J* 10.4 Hz, H-5), 3.71-3.76

(m, 2 H, H-6), 3.78 (s, 1.71 H, OCH_3 cis), 3.76 (s, 4.29 H, OCH_3 trans), 3.34-3.18 (m, 2 H, H-2' cis, H-2' trans), 2.69-2.65 (m, 2 H, H-3e cis, H-3e trans), 1.93 (m, 2 H, H-3a), 2.12, 2.10, 1.99, 1.98, 1.83 (5s, 30 H, NHAc, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.84-169.97 (C=O's), 168.46 (C-1 cis), 168.22 (C-1 trans), 128.39 (C-1' trans), 127.89 (C-1' cis), 83.25 (C-2 cis), 82.87 (C-2 trans), 74.20 (C-6 cis), 74.02 (C-6 trans), 69.53 (C-4), 68.70 (C-8 cis), 68.44 (C-8 trans), 67.34 (C-7 cis), 67.25 (C-7 trans), 62.16 (C-9), 53.01 (OCH_3 trans), 52.99 (OCH_3 cis), 49.36 (C-5), 37.82 (C-3), 30.41 (C-2' trans), 25.40 (C-2' cis); FAB-MS: 1067.4 ($[M+1]^+$, 3.9%).

Anal. Calcd. for $C_{44}H_{62}N_2O_{24}S_2$: C, 49.52; H, 5.86; N: 2.63. Found: C, 49.41; H, 5.97; N, 2.63.

Compound (82)

^1H NMR ($CDCl_3$) δ 7.24-6.93 (m, 8 H, aromatic H), 5.60 (t, 0.74 H, *J* 5.2 Hz, H-1' cis), 5.56 (t, 1.26 H, *J* 3.7 Hz, H-1' trans), 5.50 (d, 2 H, $J_{5,NH}$ 10.1 Hz, NH), 5.35-5.31 (m, 4 H, H-7, H-8), 4.92-4.87 (m, 2 H, H-4), 4.48-4.37 (m, 2 H, H-6 cis, H-6 trans), 4.26 (dd, 2 H, $J_{8,9a}$ 2.3 Hz, $J_{9a,9b}$ 12.8 Hz, H-9a), 4.12 (dd, 2 H, $J_{8,9b}$ 4.7 Hz, H-9b), 4.09-4.05 (m, 2 H, H-5 cis, H-5 trans), 3.55 (s, 2.22 H, OCH_3 cis), 3.53 (s, 3.78 H, OCH_3 trans), 3.41 (t, 1.48 H, H-2' cis), 3.29 (t, 2.52 H, H-2' trans), 2.65 (dd, 0.74 H, H-3e cis), 2.68 (dd, 1.26 H, $J_{3e,4}$ 4.7 Hz, $J_{3a,3e}$ 13.0 Hz, H-3e trans), 2.20-2.11 (m, 2 H, H-3a cis, H-3a trans), 2.06, 2.05, 1.96, 1.95, 1.82 (5s, 30 H, NHAc, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.73-169.87 (C=O's), 168.06 (C-1 cis), 168.02 (C-1 trans), 129.74 (C-1' trans), 128.34 (C-1' cis), 100.23 (C-2), 73.34 (C-6 cis), 73.31 (C-6 trans), 69.40 (C-8 trans), 69.36 (C-8 cis), 68.80 (C-4 trans), 68.76 (C-4 cis), 67.42 (C-7), 61.96 (C-9), 52.80 (CH_3O cis), 52.75 (CH_3O trans), 49.25 (C-5), 37.94 (C-3 cis), 37.80 (C-3 trans), 32.99 (C-2' trans), 27.67 (C-2' cis); FAB-MS: 1187.5 ($[M+1]^+$, 11.1%).

Anal. Calcd. for $C_{56}H_{70}N_2O_{26}$: C, 55.64; H, 5.95; N: 2.36. Found: C, 55.38; H, 5.98; N, 2.30.

Compound (83)

White solid (CH_2Cl_2/Hexane); mp 110-111 °C; $[\alpha]_D$ +201°(*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.36 (d, 4 H, *J* 8.8 Hz, aromatic H), 7.01 (d, 4 H, aromatic H), 6.93 (s, 2 H, -C<u>H</u>=), 5.37-5.33 (m, 6 H, NH, H-7, H-8), 4.94 (ddd, 2 H, H-4), 4.40 (dd, 2 H, $J_{5,6}$ 10.8 Hz, $J_{6,7}$ 1.7 Hz, H-6), 4.30 (dd, 2 H, $J_{8,9a}$ 2.4 Hz, $J_{9a,9b}$ 12.5 Hz, H-9a), 4.15 (dd, 2 H, $J_{8,9b}$ 4.7 Hz, H-9b),

4.07 (ddd, 2 H, $J_{4,5}$ 10.4 Hz, $J_{5,NH}$ 11.1 Hz, H-5), 3.63 (s, 6 H, OCH_3), 2.68 (dd, 2 H, $J_{3e,4}$ 4.6 Hz, $J_{3a,3e}$ 12.9 Hz, H-3e), 2.20 (dd, 2 H, $J_{3a,4}$ 12.5 Hz, H-3a), 2.13, 2.11, 2.02, 2.01, 1.89 (5s, 30 H, NAc, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.9-170.0 (C=O's), 168.1 (C-1), 153.2, 133.2, 127.3, 120.0 (aromatic C), 127.1 (-H<u>C</u>=), 100.0 (C-2), 73.3 (C-6), 69.2 (C-8), 68.7 (C-4), 67.3 (C-7), 62.0 (C-9), 52.9 (CH_3O), 49.4 (C-5), 38.1 (C-3); FAB-MS: 1159.3 ($[M+1]^+$, 0.4%).

Anal. Calcd. for $C_{54}H_{66}N_2O_{26}$: C, 56.86; H, 5.74; N: 2.42. Found: C, 56.04; H, 5.66; N, 2.42.

Typical procedure for preparation of divalent sialoside derivatives 84-88 by reduction reaction

To α-sialodimer **79** (20 mg,) in ethanol (2 mL) was added 10% Pd/C (2 mg). The mixture was stirred at room temperature for 1 h under hydrogen atmosphere. The reaction mixture was filtered and concentrated to yield compound **84** as a white solid (20 mg, 100%).

α-Sialodimers **85**, **87**, and **88** were obtained in the same manner from glycodimers **80**, **82**, and **83** in quantitative yields. However, α-thiosialodimer **81** was treated with the same mass amount of palladium catalyst for 12 h to afford compound **86** in 100% yield.

1,4-Di-*O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-butane (84)

mp 104-105 °C (CH_2Cl_2/Hexane); $[\alpha]_D$ -18.2° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.34 (m, 2 H, H-8), 5.29 (dd, 2 H, $J_{6,7}$ 2.1 Hz. $J_{7,8}$ 8.2 Hz, H-7), 5.21 (d, 2 H, $J_{NH,5}$ 9.7 Hz, NH), 4.80 (m, 2 H, H-4), 4.27 (dd, 2 H, $J_{8,9a}$ 2.7 Hz, $J_{9a,9b}$ 12.4 Hz, H-9a), 4.09-3.98 (m, 6 H, H-9b, H-6, H-5), 3.75 (s, 6 H, OCH_3), 3.74 (m, 2 H, -OC<u>H</u>$_2$-), 3.16 (m, 2 H, -OC<u>H</u>$_2$-), 2.53 (dd, 2 H, $J_{3e,4}$ 4.6 Hz, $J_{3a,3e}$ 12.8 Hz, H-3e), 1.90 (dd, 2 H, $J_{3a,4}$ 12.6 Hz, H-3a), 2.11, 2.10, 2.00, 1.99, 1.84 (5s, 30 H, NHAc, OAc's), 1.55 (m, 4 H, -OCH$_2$C<u>H</u>$_2$-); ^{13}C-NMR ($CDCl_3$) δ 170.0-168.3 (C=O's), 98.7 (C-2), 72.4 (C-6), 69.1 (C-4), 68.6 (C-8), 67.2 (C-7), 64.7 (-O<u>C</u>H$_2$CH$_2$-), 62.2 (C-9), 52.7 (OCH_3), 49.4 (C-5), 38.0 (C-3), 26.1 (-OCH$_2$<u>C</u>H$_2$-), 23.2 (NHAc), 21.0, 20.8, 20.7 (OAc's); HRMS calcd for $C_{44}H_{65}N_2O_{26}$ (MH^+) *m/z* 1037.3826, found 1037.3836.

1,8-Di-*O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-octane (85)

mp 82-84 °C (CH_2Cl_2/Hexane); $[\alpha]_D$ -15.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.35 (m, 2 H, H-8), 5.29 (dd, 2 H, $J_{6,7}$ 2.0 Hz. $J_{7,8}$ 8.1 Hz, H-7), 5.24 (d, 2 H, $J_{NH,5}$ 9.4 Hz, NH), 4.80 (m, 2 H, H-4), 4.27 (dd, 2 H, $J_{8,9a}$ 2.7 Hz, $J_{9a,9b}$ 12.4 Hz, H-9a), 4.09-3.99 (m, 6 H, H-9b, H-6, H-5), 3.75 (s, 6 H, OCH_3), 3.70 (m, 2 H, -OC$\underline{H}_2$-), 3.15 (m, 2 H, -OC$\underline{H}_2$-), 2.53 (dd, 2 H, $J_{3e,4}$ 4.7 Hz, $J_{3a,3e}$ 12.8 Hz, H-3e), 1.90 (dd, 2 H, $J_{3a,4}$ 12.5 Hz, H-3a), 2.10, 2.09, 2.00, 1.98, 1.84 (5s, 30 H, NHAc, OAc's), 1.48 (m, 4 H, -$OCH_2C\underline{H}_2$-), 1.29-1.21 (m, 8 H, -$O(CH_2)_2(C\underline{H}_2)_2$-); ^{13}C-NMR ($CDCl_3$) δ 171.0-168.5 (C=O's), 98.7 (C-2), 72.4 (C-6), 69.1 (C-4), 68.7 (C-8), 67.3 (C-7), 65.0 ($O\underline{C}H_2(CH_2)_2$), 62.3 (C-9), 52.6 ($OCH_3$), 49.4 (C-5), 37.7 (C-3), 29.3 ($OCH_2\underline{C}H_2CH_2$), 25.8 ($O(CH_2)_2\underline{C}H_2$), 23.1 (NHAc), 21.1, 20.8, 20.7 (OAc's); HRMS calcd for $C_{48}H_{73}N_2O_{26}$ (MH^+) *m/z* 1093.4452, found 1093.4441.

1,4-Di-*S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-butane (86)

mp 88-90 °C (CH_2Cl_2/Hexane); $[\alpha]_D$ +27.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.34-5.28 (m, 4 H, H-8, H-7), 5.20 (d, 2 H, $J_{NH,5}$ 10.1 Hz, NH), 4.84 (m, 2 H, H-4), 4.27 (dd, 2 H, $J_{8,9a}$ 2.4 Hz, $J_{9a,9b}$ 12.4 Hz, H-9a), 4.08 (dd, 2 H, $J_{8,9b}$ 4.7 Hz, H-9b), 4.00 (ddd, 2 H, *J* 10.4 Hz, H-5), 3.82-3.80 (m, 2 H, H-6), 3.78 (s, 6 H, OCH_3), 2.70 (m, 2 H, -SC$\underline{H}_2$-), 2.68 (dd, 2 H, $J_{3e,4}$ 4.7 Hz, $J_{3a,3e}$ 12.7 Hz, H-3e), 2.54 (m, 2 H, -SC$\underline{H}_2$-), 1.94 (dd, 2 H, $J_{3a,4}$ 12.4 Hz, H-3a), 2.13, 2.11, 2.01, 2.00, 1.85 (5s, 30 H, NHAc, OAc's), 1.57 (m, 4 H, -$SCH_2C\underline{H}_2$-); ^{13}C NMR ($CDCl_3$) δ 171.0-170.0 (C=O's), 83.0 (C-2), 74.0 (C-6), 69.6 (C-4), 68.5 (C-8), 67.2 (C-7), 62.1 (C-9), 53.0 (OCH_3), 49.4 (C-5), 38.0 (C-3), 28.4 (-S$\underline{C}H_2$-), 28.3 (-$SCH_2\underline{C}H_2$-), 23.2 (NHAc), 21.2, 20.9, 20.8 (OAc's); HRMS calcd for $C_{54}H_{65}N_2O_{24}S_2$ (MH^+) *m/z* 1069.3369, found 1069.3352.

Compound (87)

mp 93-94 °C (CH_2Cl_2/Hexane); $[\alpha]_D$ -3.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.14-6.93 (m, 8 H, aromatic H), 5.39-5.30 (m, 4 H, H-7, H-8), 5.27 (d, 2 H, $J_{5,NH}$ 10.1 Hz, NH), 4.91 (m, 2 H, H-4),), 4.41 (dd, 2 H, $J_{6,7}$ 1.8 Hz, $J_{5,6}$ 10.8 Hz, H-6), 4.27 (dd, 2 H, $J_{8,9a}$ 2.6 Hz, $J_{9a,9b}$ 12.5 Hz, H-9a), 4.14 (dd, 2 H, $J_{8,9b}$ 4.9 Hz, H-9b), 4.07 (ddd, 2 H, $J_{4,5}$ 10.4 Hz, H-5), 3.55

(s, 6 H, OCH_3), 2.62 (dd, 2 H, $J_{3e,4}$ 4.7 Hz, $J_{3a,3e}$ 12.9 Hz, H-3e), 2.57 (m, 4 H, -Ph-C$\underline{H}_2$-), 2.18 (dd, 2 H, $J_{3a,4}$ 12.6 Hz, H-3a), 2.12, 2.10, 2.01, 2.00, 1.88 (5s, 30 H, NHAc, OAc's), 1.57 (m, 4 H, -Ph-$CH_2C\underline{H}_2$-);^{13}C NMR ($CDCl_3$) δ 170.9-168.1 (C=O's), 151.9, 133.4, 126.8, 124.7, 118.9 (6 C, aromatic), 100.2 (C-2), 73.3 (C-6), 69.3 (C-8), 68.8 (C-4), 67.4 (C-7), 62.0 (C-9), 52.80 (CH_3O), 49.3 (C-5), 37.8 (C-3), 30.2, 29.6 (-CH_2CH_2-), 23.1 (NHAc), 21.0, 20.8, 20.7 (OAc's); HRMS calcd for $C_{54}H_{73}N_2O_{26}$ (MH^+) *m/z* 1189.4452, found 1189.4432.

Compound (88)

mp 102-103 °C (CH_2Cl_2/Hexane); $[\alpha]_D$ +3.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.98 (d, 4 H, *J* 8.6 Hz, aromatic H), 6.92 (d, 4 H, aromatic H), 5.37 (m, 2 H, H-8), 5.34 (dd, 2 H, $J_{6,7}$ 1.8 Hz. $J_{7,8}$ 8.2 Hz, H-7), 5.31 (d, 2 H, $J_{5,NH}$ 10.1 Hz, NH), 4.90 (m, 2 H, H-4),), 4.34 (dd, 2 H, $J_{6,7}$ 1.8 Hz, $J_{5,6}$ 10.8 Hz, H-6), 4.30 (dd, 2 H, $J_{8,9a}$ 2.5 Hz, $J_{9a,9b}$ 12.5 Hz, H-9a), 4.16 (dd, 2 H, $J_{8,9b}$ 4.7 Hz, H-9b), 4.05 (ddd, 2 H, $J_{4,5}$ 10.4 Hz, H-5), 3.58 (s, 6 H, OCH_3), 2.80 (m, 4 H, -Ph-C$\underline{H}_2$-), 2.66 (dd, 2 H, $J_{3e,4}$ 4.7 Hz, $J_{3a,3e}$ 12.9 Hz, H-3e), 2.16 (dd, 2 H, $J_{3a,4}$ 12.6 Hz, H-3a), 2.12, 2.10, 2.03, 2.00, 1.88 (5s, 30 H, NHAc, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.9-168.0 (C=O's), 151.8, 137.3, 129.3, 120.0 (aromatic C), 100.2 (C-2), 73.2 (C-6), 69.2 (C-8), 68.9 (C-4), 67.3 (C-7), 62.0 (C-9), 52.9 (CH_3O), 49.3 (C-5), 38.0 (C-3), 37.0 ($\underline{C}H_2$), 23.2 (NHAc), 21.0, 20.8, 21.7 (OAc's); HRMS calcd for $C_{54}H_{69}N_2O_{26}$ (MH^+) *m/z* 1161.4139, found 1161.4138.

Glycosyl halides

2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl bromide **89**, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **90**, 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **6**, and 2,2',3,3',4',6,6'-hepta-*O*-acetyl-α-D-lactosyl bromide **35** were prepared by the usual HBr/HOAc (35% w/w) treatment of the corresponding peracetates. All peracetylated glycosyl bromides were directly used in the next reaction without further purification.

Typical procedure for PTC thioacetates formation

To a solution of freshly prepared acetoglycosyl bromide (10 mmol) and TBAHS (1 eq) in EtOAc (50 mL) were added thioacetic acid (2 eq) and 1 M Na_2CO_3 (50 mL). The reaction mixture was vigorously stirred at room temperature for 1 h, The reaction mixture

was diluted with EtOAc (50 mL). The organic phase was successively washed with sat. $NaHCO_3$, and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography.

2,3,4,6-Tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-glucopyranose (91)

Chromatographed with hexanes/EtOAc (2:1, v/v); white solid (72%): m.p. 115-116 °C; $[\alpha]_D$ +7.2° (*c* 1.0, $CHCl_3$); Lit.[185] m.p. 121 °C (methanol); $[\alpha]_D$ +9° (*c* 2.1, 1,1,2,2-tetrachloroethane).

2,3,4,6-Tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-galactopyranose (92)

Chromatographed with hexanes/EtOAc (2:1, v/v); white solid (86%); m.p. 113-115 °C (ethanol); $[\alpha]_D$ +31.4° (*c* 1.0, $CHCl_3$); Lit.[196] m.p. 112-114 °C (ether); $[\alpha]_D$ +32° (*c* 1.25, $CHCl_3$).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-glucopyranose (93)

Chromatographed with hexanes/EtOAc (2:1, v/v); yield (75%); $[\alpha]_D$ -2.10° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.06 (d, 1 H, $J_{1,2}$ 10.5 Hz, H-1), 4.35 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'), 2.20 (s, 3 H, SAc); ^{13}C NMR ($CDCl_3$) δ 191.7 (SC=O), 100.6 (C-1'), 79.9 (C-1), 30.6 (SAc); FAB-MS for $C_{28}H_{38}O_{18}S$: 495.3 (M+1, 0.9 %).

General procedure for the syntheses of *S*-propynyl glycosides 94, 95, 96, and 97

Method A:

To a solution of thioacetate (1 mmol) in dry methanol and CH_2Cl_2 (1/1, 10 mL) at -40 °C was added 1M sodium methoxide solution (0.95 eq). The mixture was stirred at -40 °C for 30 min under nitrogen. The solution was then neutralized with Amberlite IR-120 H^+ resin at -40 °C for 15 min. The solution was filtered. Propargyl bromide (1.5 eq) and triethyl amine (3 eq) were added to the organic solution. The solution was stirred at room temperature for 1 h.

[196] Defaye, J.; Driguez, H.; Ohleyer, E.; Orgeret, C.; Viet, C. *Carbohydr. Res.* **1992**, *229*, 149.

The solution was concentrated under reduced pressure and purified by silica gel column chromatography.

Method B:

To a solution of glycosyl bromide (5 mmol) in dry acetone (20 mL) was added thiourea (1.5 eq). The mixture was refluxed for 6 h. TLC showed the starting material was completely converted into 2-thiopseudourea hydrobromide salt. The thiopseudourea hydrobromide salt was directly used in the next step without purification. Potassium carbonate solution (20 mL, 0.1 g/mL) and propargyl bromide (1.5 eq) were added to the mixture. The mixture was stirred at room temperature for 1 h. The mixture was diluted with water (100 mL). The solution was extracted with CH_2Cl_2 (3 x 30 mL). The combined organic layers were successively washed with 5% HCl, sat. $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography.

2-Propynyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (94)

Method A and B: Chromatographed with hexanes/EtOAc (2:1, v/v); $[\alpha]_D$ -82.1° (*c* 1.0, $CHCl_3$); m.p. 57-58 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 5.16 (dd, 1 H, $J_{3,4}$ 9.6 Hz, H-3), 4.99 (dd, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 4.95 (dd, 1 H, $J_{2,3}$ 10.1 Hz, H-2), 4.67 (d, 1 H, $J_{1,2}$ 10.2 Hz, H-1), 4.15 (dd, 1 H, $J_{5,6a}$ 4.9 Hz, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.03 (dd, 1 H, $J_{5,6b}$ 2.1 Hz, H-6b), 3.65 (ddd, 1 H, H-5), 3.45 (dd, 1 H, *J* 16.5, 2.5 Hz, -SC<u>H</u>$_2$C≡), 3.20 (dd, 1 H, *J* 2.6 Hz, -SC<u>H</u>$_2$C≡), 2.21 (t, 1 H, ≡CH), 1.97, 1.95, 1.92, 1.90, 1.94 (s, 12 H, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.4, 170.0, 169.3, 169.2 (C=O's), 81.9 (C-1), 78.6 (-<u>C</u>≡CH), 75.8 (C-5), 73.7 (C-3), 71.9 (-C≡ <u>C</u>H), 69.7 (C-2), 68.2 (C-4), 61.8 (C-6), 20.6, 20.5, 20.4, 20.4 (OAc's), 17.3 (-S<u>C</u>H$_2$C≡); FAB-MS for $C_{17}H_{22}O_9S$: 403.1 (M+1, 1.7 %).

2-Propynyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (95)

Method A and B: Chromatographed with hexanes/EtOAc (2:1, v/v); $[\alpha]_D$ -46.9° (*c* 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.35 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4), 5.17 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2), 5.01 (dd, 1 H, H-3), 4.67 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1), 4.10-4.04 (m, 2 H, H-6a, H-6b), 3.89 (m, 1 H, H-5), 3.49 (dd, 1 H, *J* 16.5, 2.6 Hz, -SC<u>H</u>$_2$C≡), 3.22 (dd, 1 H, *J* 2.7 Hz, -SC<u>H</u>$_2$C≡),

2.22 (t, 1 H, ≡CH), 2.07, 1.99, 1.96, 1.90 (s, 12 H, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.2, 170.0, 169.9, 169.5 (C=O's), 82.3 (C-1), 78.6 (-C≡CH), 74.3 (C-5), 71.7 (-C≡CH), 71.6 (C-3), 67.1 (C-4), 66.9 (C-2), 61.2 (C-6), 20.6, 20.5, 20.4 (OAc's), 17.3 (-SCH$_2$C≡); FAB-MS for $C_{17}H_{22}O_9S$: 402.1 (M, 51.6 %).

2-Propynyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (96)

Method A and B: Chromatographed with hexanes/EtOAc (2:1, v/v); $[\alpha]_D$ -45.2° (*c* 1.0, $CHCl_3$); m.p. 72-74 °C (CH_2Cl_2-hexanes); ^{1}H NMR ($CDCl_3$) δ 5.25 (d, 1 H, $J_{3',4'}$ 2.6 Hz, H-4'), 5.17 (dd, 1 H, $J_{3,4}$ 9.0 Hz, H-3), 5.01 (dd, 1 H, $J_{1',2'}$ 7.7 Hz, $J_{2',3'}$ 10.4 Hz, H-2'), 4.87 (dd, 1 H, $J_{2,3}$ 9.2 Hz, H-2), 4.84 (dd, 1 H, H-3'), 4.66 (d, 1 H, $J_{1,2}$ 10.1 Hz, H-1), 4.42-4.38 (m, 2 H, H-1', H-6a), 4.06-3.97 (m, 3 H, H-6b, H-6'a, H-6'b), 3.84-3.57 (m, 3 H, H-5', H-4, H-5), 3.44 (dd, 1 H, *J* 16.6, 2.6 Hz, -SCH$_2$C≡), 3.17 (dd, 1 H, *J* 2.6 Hz, -SCH$_2$C≡), 2.21 (t, 1 H, ≡CH), 2.06, 2.03, 1.98,1.96, 1.96 1.87 (s, 21 H, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.1, 169.9, 169.8, 169.5, 168.9 (C=O's), 100.8 (C-1'), 81.5 (C-1), 78.5 (-C≡C), 76.6 (C-5), 75.9 (C-4), 73.4 (C-3), 71.8 (-C≡C), 70.8 (C-5'), 70.6 (C-3'), 69.9 (C-2), 68.9 (C-2'), 66.5 (C-4'), 61.9 (C-6), 60.7 (C-6'), 20.7, 20.6, 20.4, 20.3 (OAc's), 17.3 (-CH$_2$C≡); FAB-MS for $C_{29}H_{38}O_{17}S$: 691.2 (M+1, 0.1 %).

2-Propynyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (97)

Method A: Chromatographed with hexanes/EtOAc (2:1, v/v); $[\alpha]_D$ +135.7° (*c* 1.0, $CHCl_3$); m.p. 104-105 °C (CH_2Cl_2-hexanes); ^{1}H NMR ($CDCl_3$) δ 5.45 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 5.33 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-2), 5.29 (dd, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 5.18 (dd, 1 H, $J_{3,4}$ 10.0 Hz, H-3), 4.32-4.27 (m, 2 H, H-6a, H-5), 4.08-4.04 (m, 1 H, H-6b), 3.36 (dd, 1 H, *J* 16.8, 2.6 Hz, -SCH$_2$C≡), 3.20 (dd, 1 H, *J* 2.6 Hz, -SCH$_2$C≡), 2.24 (t, 1 H, ≡CH), 2.13, 2.06, 2.00, 1.94 (s, 12 H, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.5, 169.7, 169.7, 169.6 (C=O's), 81.4 (C-1), 78.4 (-C≡CH), 72.0 (-C≡CH), 70.4 (C-2), 69.5 (C-3), 69.3 (C-5), 66.2 (C-4), 62.2 (C-6), 20.8, 20.6, 20.6, 20.5 (OAc's), 18.1 (-SCH$_2$); FAB-MS for $C_{17}H_{22}O_9S$: 403.1 (M+1, 14.3 %).

General procedure for the homo-coupling of acetylenic thiosugar by Glaser reaction

In a mixture of thiosugar (1 mmol), CuCl (0.3 mmol), and TMEDA (0.6 mmol) in *N,N*-dimethylformamide (10 mL) was bubbled O_2 at 40 °C for 2 h. Then the brownish green solution was poured into water (100 mL). The mixture was extracted with CH_2Cl_2 (20 mL x 3). The combined organic phases were washed with brine, dried over dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography.

1,6-Di-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosylthio)-hex-2,4-di-yne (98)

Chromatographed with ether; $[\alpha]_D$ +243° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.37 (d, 2 H, $J_{1,2}$ 1.4 Hz, H-1), 5.29 (dd, 2 H, $J_{2,3}$ 3.4 Hz, H-2), 5.25 (dd, 2 H, $J_{4,5}$ 9.7 Hz, H-4), 5.13 (dd, 2 H, $J_{3,4}$ 10.0 Hz, H-3), 4.27-4.22 (m, 4 H, H-6a, H-5), 4.02 (m, 2 H, H-6b), 3.42 (ABq, 2 H, *J* 16.7 Hz, -SC$\underline{H}_2$C≡), 3.27 (ABq, 2 H, -SC$\underline{H}_2$C≡), 2.10, 2.02, 1.98, 1.91 (s, 24 H, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.4, 169.6, 169.5, 169.4 (C=O's), 81.5 (C-1), 73.6 (-$\underline{C}$≡C), 70.1 (C-2), 69.5 (C-3), 69.3 (C-5), 67.8 (-C≡$\underline{C}$), 66.0 (C-4), 62.1 (C-6), 20.7, 20.7, 20.6, 20.5 (OAc's), 18.9 (-CH_2C≡); FAB-MS for $C_{34}H_{42}O_{18}S_2$: 803.2 (M+1).

1,6-Di-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylthio)-hex-2,4-di-yne (99)

Chromatographed with ether; $[\alpha]_D$ -143.4° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.20 (dd, 2 H, $J_{3,4}$ 9.4 Hz, H-3), 5.03 (dd, 2 H, $J_{4,5}$ 9.5 Hz, H-4), 4.97 (dd, 2 H, $J_{2,3}$ 9.3 Hz, H-2), 4.66 (d, 2 H, $J_{1,2}$ 10.1 Hz, H-1), 4.21-4.04(m, 2 H, H-6a, H-6b), 3.70 (m, 2 H, H-5), 3.58 (ABq, 2 H, *J* 17.2 Hz, -SC$\underline{H}_2$C≡), 3.33 (ABq, 2 H, -SC$\underline{H}_2$C≡), 2.02, 2.00, 1.95, 1.94 (s, 24 H, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.4, 169.9, 169.3, 169.2 (C=O's), 82.0 (C-1), 75.8 (C-5), 73.6 (C-3, -$\underline{C}$≡C), 69.7 (C-2), 68.1 (C-4), 67.7 (-C≡$\underline{C}$), 61.8 (C-6), 20.6, 20.5, 20.4, 20.4 (OAc's), 18.3 (-S$\underline{C}H_2$C≡); FAB-MS for $C_{34}H_{42}O_{18}S_2$: 820 ($M+NH_4$).

1,6-Di-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosylthio)-hex-2,4-di-yne (100)

Chromatographed with ether; $[\alpha]_D$ -125° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.38 (dd, 2 H, $J_{3,4}$ 3.4 Hz, H-4), 5.17 (dd, 2 H, $J_{2,3}$ 10.0 Hz, H-2), 5.05 (dd, 2 H, H-3), 4.65 (d, 2 H, $J_{1,2}$ 10.0 Hz, H-1), 4.11-4.04 (m, 4 H, H-6a, H-6b), 3.93 (ddd, 2 H, $J_{4,5}$ 1.0 Hz, $J_{5,6a}$, $J_{5,6b}$ 6.6 Hz, H-5), 3.61 (ABq, 2 H, *J* 16.2 Hz, -SC$\underline{H}_2$C≡), 3.34 (ABq, 2 H, -SC$\underline{H}_2$C≡), ≡), 2.09, 2.01, 1.98,

1.91 (s, 24 H, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.2, 170.1, 169.8, 169.5 (C=O'S), 82.5 (C-1), 74.5 (C-5), 73.7 (-C≡C), 71.6 (C-3), 67.7 (-C≡C), 67.2 (C-4), 67.1 (C-2), 61.3 (C-6), 20.6, 20.5, 20.4 (OAc's), 18.2 (-SCH$_2$C≡); FAB-MS for $C_{34}H_{42}O_{18}S_2$: 820 (M+NH$_4$).

1,6-Di-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosylthio]-hex-2,4-di-yne (101)

Chromatographed with ether; $[\alpha]_D$ -81.7° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.25 (d, 2 H, $J_{3',4'}$ 2.6 Hz, H-4'), 5.17 (dd, 2 H, $J_{3,4}$ 9.0 Hz, H-3), 5.01 (dd, 2 H, $J_{1',2'}$ 7.7 Hz, J2',3' 10.4 Hz, H-2'), 4.87 (dd, 2 H, $J_{2,3}$ 9.2 Hz, H-2), 4.86 (dd, 2 H, H-3'), 4.62 (d, 2 H, $J_{1,2}$ 10.1 Hz, H-1), 4.43-4.40 (m, 4 H, H-1', H-6a), 4.06-3.98 (m, 6 H, H-6b, H-6'a, H-6'b), 3.98-3.61 (m, 6 H, H-5', H-4, H-5), 3.54 (ABq, 2 H, *J* 16.6 Hz, -SCH$_2$C≡), 3.28 (ABq, 2 H, -SCH$_2$C≡), 2.06, 2.04, 1.98, 1.87 (s, 42 H, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.1, 169.9, 169.8, 169.5, 169.4, 168.8 (C=O's), 100.8 (C-1'), 81.7 (C-1), 76.6 (C-5), 75.9 (C-4), 73.6 (-C≡C), 73.4 (C-3), 70.8 (C-5'), 70.6 (C-3'), 70.0 (C-2), 68.9 (C-2'), 67.6 (-C≡C), 66.5 (C-4'), 61.9 (C-6), 60.7 (C-6'), 20.6, 20.6, 20.5, 20.4, 20.3 (OAc's), 18.1 (-CH$_2$C≡); FAB-MS for $C_{58}H_{74}O_{34}S_2$: 1396 (M+NH$_4$).

General procedure for the cross-coupling of acetylenic thiosugar by Sonogashira reaction

To acetylenic thiosugar (1 mmol) and 1,4-diiodobenzene (0.45 mmol) in a degassed solution of DMF and Et_3N (5 mL, 1:1, v/v) were added $Pd_2(dba)_3$ (0.05 mmol) and PPh_3 (0.1 mmol). The solution was stirred at 60 °C under nitrogen atmosphere for 2 h. The solution was diluted with CH_2Cl_2 (20 mL), and then washed successively with 5 % HCl and brine. The organic phase was dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography.

1,4-Bis-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosylthio-prop-2-ynyl)benzene (102)

Chromatographed with ether; $[\alpha]_D$ +227.4° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.28 (s, 4 H, aromatic H), 5.48 (d, 2 H, $J_{1,2}$ 1.4 Hz, H-1), 5.33 (dd, 2 H, $J_{2,3}$ 3.4 Hz, H-2), 5.28 (dd, 2 H, $J_{4,5}$ 9.8 Hz, H-4), 5.18 (dd, 2 H, $J_{3,4}$ 10.0 Hz, H-3), 4.33-4.25 (m, 4 H, H-6a, H-5), 4.03

(dd, 2 H, $J_{5,6b}$ 2.2 Hz, $J_{6a,6b}$ 12.1 Hz, H-6b), 3.59 (ABq, 2 H, J 16.8 Hz, -SC$\underline{H}_2$C≡), 3.42 (ABq, 2 H, -SC$\underline{H}_2$C≡), 2.10, 2.02, 1.98, 1.92 (s, 24 H, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.4, 169.7, 169.6, 169.5 (C=O's), 131.6, 122.5 (aromatic C), 85.7 (-$\underline{C}$≡C), 83.3 (-C≡$\underline{C}$), 81.5 (C-1), 70.4 (C-2), 69.5 (C-3), 69.4 (C-5), 66.1 (C-4), 62.2 (C-6), 20.8, 20.6, 20.6, 20.5 (OAc's), 19.1 (-S$\underline{C}$H$_2$C≡); FAB-MS for $C_{40}H_{46}O_{18}S_2$: 896 (M+NH_4).

1,4-Bis-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylthio-prop-2-ynyl)benzene (103)

Chromatographed with ether; $[\alpha]_D$ -144.9° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.32 (s, 4 H, aromatic H), 5.21 (dd, 2 H, $J_{3,4}$ 9.4 Hz, H-3), 5.06 (dd, 2 H, $J_{4,5}$ 9.9 Hz, H-4), 5.04 (dd, 2 H, $J_{2,3}$ 9.4 Hz, H-2), 4.73 (d, 2 H, $J_{1,2}$ 10.1 Hz, H-1), 4.21 (dd, 2 H, $J_{6a,6b}$ 12.4, $J_{5,6a}$ 4.9 Hz, H-6a), 4.09 (dd, 2 H, $J_{5,6b}$ 2.3 Hz H-6b), 3.74 (ABq, 2 H, J 16.5 Hz, -SCH_AC≡), 3.69 (ddd, 2 H, H-5), 3.51 (ABq, 2 H, -SCH_BC≡), 2.01, 2.00, 1.97, 1.95 (s, 24 H, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.5, 170.0, 169.3, 169.3 (C=O's), 131.6, 122.7 (aromatic C), 86.0 (C≡C), 83.2 (C≡C), 82.2 (C-1), 76.0 (C-5), 73.8 (C-3), 69.8 (C-2), 69.2 (C-4), 61.9 (C-6), 20.6, 20.5, 20.5 (OAc's), 18.5 (S$\underline{C}$H$_2$C≡); FAB-MS for $C_{40}H_{46}O_{18}S_2$: 896 (M+NH_4).

1,4-Bis-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosylthio-prop-2-ynyl)benzene (104)

Chromatographed with ether; $[\alpha]_D$ -125° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.32 (s, 4 H, aromatic H), 5.39 (dd, 2 H, $J_{3,4}$ 3.4 Hz, H-4), 5.23 (dd, 2 H, $J_{2,3}$ 10.0 Hz, H-2), 5.03 (dd, 2 H, H-3), 4.70 (d, 2 H, $J_{1,2}$ 10.0 Hz, H-1), 4.12 (dd, 2 H, $J_{6a,6b}$ 11.3, $J_{5,6a}$ 6.7 Hz, H-6a), 4.06 (dd, 2 H, $J_{5,6b}$ 6.5 Hz H-6b), 3.92 (ddd, 2 H, $J_{4,5}$ 1.0 Hz, H-5), 3.74 (ABq, 2 H, J 16.5 Hz, SCH_AC≡), 3.53 (ABq, 2 H, SCH_BC≡), 2.08, 2.01, 1.97, 1.93 (s, 24 H, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.2, 170.1, 169.9, 169.5 (C=O's), 131.6, 122.7 (aromatic C), 86.1 (C≡C), 83.1 (C≡C), 82.7 (C-1), 74.5 (C-5), 71.8 (C-3), 67.2 (C-4), 67.1 (C-2), 61.3 (C-6), 20.7, 20.5, 20.5 (OAc's), 18.5 (S$\underline{C}$H$_2$C≡); FAB-MS for $C_{40}H_{46}O_{18}S_2$: 896 (M+NH_4).

1,4-Bis-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosylthio-prop-2-ynyl]benzene (105)

Chromatographed with ether; $[\alpha]_D$ -76.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.31 (s, 4 H, aromatic H), 5.27 (d, 2 H, $J_{3',4'}$ 2.9 Hz, H-4'), 5.19 (dd, 2 H, $J_{2,3}$, $J_{3,4}$ 9.0 Hz, H-3), 5.03

(dd, 2 H, $J_{1,'2'}$ 7.9 Hz, $J_{2',3'}$ 10.4 Hz, H-2'), 4.95-4.85 (m, 4 H, H-2, H-3'), 4.70 (d, 2 H, $J_{1,2}$ 10.0 Hz, H-1), 4.48-4.37 (m, 4 H, H-1', H-6a), 4.14-3.95 (m, 6 H, H-6b, H-6'a, H-6'b), 3.88-3.54 (m, 8 H, H-5', H-4, H-5, $SCH_AC\equiv$), 3.46 (ABq, 2 H, J 16.6 Hz, $-SCH_BC\equiv$), 2.08, 2.03, 1.99, 1.97, 1.89 (s, 42 H, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.2, 170.0, 169.9, 169.5, 168.9 (C=O's), 131.5, 122.5 (aromatic C), 100.8 (C-1'), 85.9 (C≡C), 83.0 (C≡C), 81.8 (C-1), 76.7 (C-5), 76.0 (C-4), 73.6 (C-3), 70.8 (C-5'), 70.5 (C-3'), 70.1 (C-2), 68.9 (C-2'), 66.5 (C-4'), 61.9 (C-6), 60.7 (C-6'), 20.6, 20.5, 20.4, 20.3 (OAc's), 18.4 ($S\underline{C}H_2C\equiv$); FAB-MS for $C_{64}H_{78}O_{34}S_2$: 1472 ($M+NH_4$).

Methyl (2-propypnyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate (106)

To a solution of freshly prepared β-acetochloroneuraminic acid **41** (1.18 g, 2.31 mmol) in dry propargy alcohol (20 mL) containing 4Å molecular sieves (2 g) was added freshly prepared silver salicylate (0.85 g, 3.47 mmol). The reaction was stirred for 2 h under nitrogen at room temperature in the dark. The slurry was filtered over Celite, washed with dichloromethane, and evaporated to dryness. The residue was dissolved in dichloromethane (30 mL). The organic solution was successively washed with sat. $NaHCO_3$ solution, 5% $Na_2S_2O_3$ solution, and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude syrup was crystallized from ethanol to afford pure **106** (840 mg, 69%) as white solids: m.p. 162-164 °C; $[\alpha]_D$ +69.4° (*c* 0.5, CH_3CN); ^1H NMR ($CDCl_3$) δ 5.44 (d, 1 H, $J_{5,NH}$ 9.0 Hz, NH), 5.34 (m, 1 H, H-8), 5.25 (dd, 1 H, $J_{6,7}$ 1.3 Hz, $J_{7,8}$ 8.3 Hz, H-7), 4.80 (m, 1 H, H-4), 4.35 (dd, 1 H, J 15.7 Hz, $-OC\underline{H}_2C\equiv$), 4.22 (dd, 1 H, $J_{9a,9b}$ 12.4 Hz, $J_{8,9a}$ 2.7 Hz, H-9a), 4.10 (dd, 1 H, $-OC\underline{H}_2C\equiv$), 4.04-3.97 (m, 3 H, H-5, H-6, H-9b), 3.75 (s, 3 H, OCH_3), 2.57 (dd, 1 H, $J_{3a,3e}$ 12.8 Hz, $J_{3e,4}$ 4.6 Hz, H-3e), 2.39 (dd, 1 H, J 2.4 Hz, $-C\equiv C\underline{H}$), 2.09, 2.07, 1.98, 1.96, 1.81 (s, 15 H, OAc's, NHAc), 1.91 (dd, 1 H, $J_{3a,4}$ 12.5 Hz, H-3a); ^{13}C NMR ($CDCl_3$) δ 170.8-170.0 (O=C's), 167.8 (C-1), 98.2 (C-2), 78.9 ($-\underline{C}\equiv CH$), 74.4 ($-C\equiv\underline{C}H$), 72.6 (C-6), 68.8 (C-4), 68.4 (C-8), 67.2 (C-7), 62.3 (C-9), 52.8 (OCH_3), 52.7 ($-O\underline{C}H_2C\equiv$), 49.2 (C-5), 37.8 (C-3), 23.0, 21.0, 20.9, 20.7, 20.6 (NHAc, OAc's); HRMS calcd for $C_{23}H_{32}N_1O_{13}(MH^+)$ *m/z* 530.1874, found 530.1895.

Methyl (4-iodophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate (107)

To a solution of freshly prepared acetochloroneuraminic acid **41** (680 mg, 1.33 mmol) in EtOAc (20 mL) was added a solution of *p*-iodophenol (587 mg, 2.67 mmol) and TBAHS (453 mg, 1.33 mmol) in 1 M Na_2CO_3 (20 mL). The mixture was stirred vigorously for 1 h at room temperature and next diluted with EtOAc (20 mL). The organic phase was separated and washed with brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatograph using CH_2Cl_2/EtOH (30/1, v/v) as eluent to give compund **107** (509 mg, 55%) as a white solid: m.p. 88-90 °C; $[\alpha]_D$ +10.8° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.49 (d, 2 H, *J* 8.8 Hz, aromatic H), 6.76 (d, 2 H, aromatic H), 5.58 (bs, 1 H, NH), 5.29 (m, 2 H, H-7, H-8), 4.87 (m, 1 H, H-4), 4.38 (d, 1 H, $J_{5,6}$ 10.8 Hz, H-6), 4.21 (d, 1 H, $J_{9a,9b}$ 12.6 Hz, H-9a), 4.09-4.01 (m, 2 H, H-9b, H-5), 3.59 (s, 3 H, OCH_3), 2.62 (dd, 1 H, $J_{3a,3e}$ 12.9 Hz, $J_{3e,4}$ 4.6 Hz, H-3e), 2.14 (dd, 1 H, $J_{3a,4}$ 12.5 Hz, H-3a), 2.08, 2.06, 1.99, 1.97, 1.84 (s, 15 H, OAc's, NHAc); ^{13}C NMR ($CDCl_3$) δ 170.8-170.0 (O=C's), 168.0 (C-1), 153.7, 138.2, 121.8, 87.1 (aromatic C), 99.8 (C-2), 73.4 (C-6), 69.1 (C-8), 68.6 (C-4), 67.3 (C-7), 62.0 (C-9), 52.9 (OCH_3), 49.2 (C-5), 38.1 (C-3), 23.0, 20.9, 20.7, 20.6 (NHAc, OAc's); FAB-MS for $C_{26}H_{32}I_1O_{13}N_1$: 694.1 ($[M+1]^+$, 33.9%).

Methyl (2-propypnyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate (108)

To a solution of thioacetate sialoside **43** (220 mg, 0.40 mmol) in dry methanol (10 mL), cooled to -40 °C, was added a 1 M solution of sodium methoxide (380 μL, 0.38 mmol). The mixture was stirred at -40 °C under nitrogen for 30 min. The solution was then neutralized with Amberlite IR-120 H^+ resin at -40 °C for 15 min. The solution was filtered. To the filtrate were added prapargyl bromide (80% in toluene, 67 μL, 0.60 mmol) and triethyl amine (112 μL, 0.8 mmol). The solution was stirred at room temperature for 1 h. The solution was poured then into water and exacted by dichloromethane (10 mL x 3). The combined organic layers were washed with 5 % HCl and brine. The organic phase was dired over Na_2SO_4 and concentrated under reduced pressure. The compound **108** (185 mg, 85%) was purified by recrystallization from CH_2Cl_2/hexanes as white solids: m.p. 186-187 °C; $[\alpha]_D$ +51° (*c* 0.2, CH_3CN); ^{1}H NMR ($CDCl_3$) 5.37 (m, 1 H, H-8), 5.31-5.27 (m, 2 H, NH, H-7),

4.84 (m, 1 H, H-4), 4.25 (dd, 1 H, $J_{9a,9b}$ 12.5 Hz, $J_{8,9a}$ 2.7 Hz, H-9a), 4.08-3.99 (m, 2 H, H-9b, H-5), 3.84 (dd, 1 H, $J_{5,6}$ 10.8 Hz, $J_{6,7}$ 2.2 Hz, H-6), 3.79 (s, 3 H, OCH_3), 3.41, 3.40 (d, 2 H, J 2.6 Hz, -$SCH_2C{\equiv}$), 2.69 (dd, 1 H, $J_{3a,3e}$ 12.7 Hz, $J_{3e,4}$ 4.6 Hz, H-3e), 2.14 (t, 1 H, -C≡C<u>H</u>), 2.13, 2.09, 2.00, 1.99, 1.83 (s, 15 H, OAc's), 1.95 (dd, 1 H, $J_{3a,4}$ 12.3 Hz, H-3a); ^{13}C NMR ($CDCl_3$) δ 170.8-170.0 (O=C's), 168.0 (C-1), 82.7 (C-2), 79.5 (-<u>C</u>≡CH), 74.2 (C-6), 70.6 (-C ≡<u>C</u>H), 69.4 (C-4), 68.4 (C-8), 67.2 (C-7), 62.2 (C-9), 53.0 (OCH_3), 49.2 (C-5), 37.4 (C-3), 23.1, 21.1, 20.7, 20.7 (NHAc, OAc's), 16.8 (-S<u>C</u>H_2C≡); HRMS calcd for $C_{23}H_{32}N_1O_{12}S_1(MH^+)$ *m/z* 546.1645, found 546.1653.

1,3,5-Triiodobenzene (109)

1,3,5-Tribromobenzene (1.10 g, 3.5 mmol), KI (3.50 g, 21 mmol), Ni powder (2.00 g), I_2 (5.10 g), and DMF (12.5 mL) were added into 50 mL of round-bottomed flask. The flask was evacuated on the vacuum line at 0°C for 15 min. The mixture was refluxed under N_2 for 3 h. After cooling, the solution was poured into a separation funnel. The flask was washed with 3 % aqueous HCl (50 mL) and CH_2Cl_2 (50 mL) until all material, except for Ni powder, was transferred into the separation funnel. The CH_2Cl_2 layer was separated, and the aqueous layer was extraxted with CH_2Cl_2. The combined organic phase was washed with distilled water and dried over Na_2SO_4. The solvent was evaporated, leaving a light-brown crude product. This was further purified by sublimation at 60 °C overnight. The residue was sublimed at 120-140 °C to afford compound **109** (0.83 g, 52%): ^{13}C NMR ($CDCl_3$) δ 144.4, 95.2.

1,2,4,5-Tetraiodobenzene (110)

H_5IO_6 (1.59 g, 6.97 mmol) was dissolved with stirring in concentrated H_2SO_4 (25 mL), Iodine (5.20 g, 20.5 mmol) was crushed and added to the clear solution. After 30 min of stirring, the dark mixture was placed in an ice bath. Benzene (0.95 g, 12.20 mmol) was then added slowly. The reaction was allowed to stir at room temperature for 2 days. The mixture was poured onto crushed ice. The resulting solid was collected by suction filtration and washed well with methanol to remove iodine. The crude lavender powder was crystallized

from 2-methoxyethanol, giving white needles **110** (5.16 g, 73%): m.p. 253-254 °C; Lit.[197] m.p. 253 °C; ^{1}H NMR ($CDCl_3$) δ 7.98 (s, 2 H, aromatic H).

General procedure for the homo-coupling of acetylenic sialic acid derivatives

In a mixture of propargyl glycoside (0.2 mmol), CuCl (0.06 mmol), and TMEDA (0.12 mmol) in DMF (5 mL) was bubbled O_2 at 40 °C. Then the brownish green solution was poured into water (50 mL). The mixture was extracted with CH_2Cl_2 (20 mL x 3). The combined organic phases were washed with 5% HCl and brine, dried over dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography CH_2Cl_2/MeOH (20:1, v/v) as eluent.

1,6-Di-*O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-hex-2,4-di-yne (111)

Reaction time: 2 h; yield: 86%; m.p. 118-120 °C; $[\alpha]_D$ +23.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.39-5.31 (m, 4 H, NH, H-8), 5.24 (dd, 2 H, $J_{6,7}$ 1.7 Hz, $J_{7,8}$ 8.6 Hz, H-7), 4.80 (m, 2 H, H-4), 4.36 (ABq, 2 H, *J* 16.2 Hz, -OC$\underline{H}_2$C≡), 4.22 (ABq, 2 H, -OC$\underline{H}_2$C≡), 4.21 (dd, 2 H, $J_{9a,9b}$ 12.4 Hz, $J_{8,9a}$ 2.7 Hz, H-9a), 4.03-3.98 (m, 6 H, H-5, H-6, H-9b), 3.76 (s, 6 H, OCH_3), 2.55 (dd, 2 H, $J_{3a,3e}$ 12.8 Hz, $J_{3e,4}$ 4.6 Hz, H-3e), 2.09, 2.08, 1.98, 1.97, 1.81 (s, 30 H, OAc's, NHAc), 1.88 (dd, 2 H, $J_{3a,4}$ 12.6 Hz, H-3a); ^{13}C NMR ($CDCl_3$) δ 170.8-169.9 (O=C's), 167.7 (C-1), 97.9 (C-2), 74.7 (-OCH$_2\underline{C}$≡C-), 72.6 (C-6), 70.1 (-OCH$_2$C≡$\underline{C}$-), 68.8 (C-4), 68.2 (C-8), 67.1 (C-7), 62.3 (C-9), 53.1 (-O$\underline{C}$H$_2$C≡), 52.9 (OCH_3), 49.2 (C-5), 37.7 (C-3), 23.0, 21.0, 20.7, 20.7, 20.6 (NHAc, OAc's); FAB-MS: 1057.2 ([M+1]$^+$, 2.3%).
Anal. Calcd. for $C_{46}H_{60}N_2O_{26}$: C, 52.26; H, 5.72; N: 2.65. Found: C, 51.92; H, 5.75; N, 2.65.

1,6-Di-*S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-hex-2,4-di-yne (112)

Reaction time: 3 h; yield: 83%; m.p. 131-132 °C; $[\alpha]_D$ +131.7° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.40 (d, 2 H, $J_{5,NH}$ 10.1 Hz, NH), 5.33 (m, 2 H, H-8), 5.25 (dd, 2 H, $J_{6,7}$ 2.2 Hz, $J_{7,8}$ 8.6 Hz, H-7), 4.80 (m, 2 H, H-4), 4.20 (dd, 2 H, $J_{9a,9b}$ 12.5 Hz, $J_{8,9a}$ 2.7 Hz, H-9a), 4.02

[197] Suzuki, H.; Goto, R. *Bull. Chem. Soc. Jpn.* **1963**, *36*, 389.

(dd, 2 H, $J_{8,9b}$ 5.5 Hz, H-9b), 3.99 (ddd, 2 H, $J_{4,5}$ 10.6 Hz, H-5), 3.83 (dd, 2 H, $J_{5,6}$ 10.8 Hz, H-6), 3.78 (s, 6 H, OCH_3), 3.51 (ABq, 2 H, J 16.9 Hz, -SC$\underline{H}_2$C≡), 3.38 (ABq, 2 H, -SC$\underline{H}_2$C≡), 2.65 (dd, 2 H, $J_{3a,3e}$ 12.6 Hz, $J_{3e,4}$ 4.6 Hz, H-3e), 2.11, 2.07, 1.98, 1.96, 1.81 (s, 30 H, OAc's, NHAc), 1.89 (dd, 2 H, $J_{3a,4}$ 12.2 Hz, H-3a); ^{13}C NMR ($CDCl_3$) δ 170.7-170.0 (O=C's), 167.9 (C-1), 82.5 (C-2), 74.2 (-SC$H_2\underline{C}$≡C-), 74.1 (C-6), 69.3 (C-4), 68.2 (C-8), 67.1 (C-7, -SCH_2C≡ $\underline{C}$-), 62.2 (C-9), 53.3 (OCH_3), 49.1 (C-5), 37.4 (C-3), 23.0, 21.1, 20.7, 20.6, 20.6 (NHAc, OAc's), 17.6 (-S$\underline{C}H_2$C≡); FAB-MS: 1089.2 ([M+1]$^+$, 12.2%).

Anal. Calcd. for $C_{46}H_{60}N_2O_{24}S_2$: C, 50.73; H, 5.55; N: 2.57. Found: C, 50.83; H, 5.69; N, 2.56.

Methyl [4-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyloxyonate prop-2-ynyl) phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (113)

To a degassed solution of the propargyl sialic acid **106** (58.7 mg, 0.11 mmol) and iodophenyl sialic acid **107** (70 mg, 0.1 mmol) in DMF/Et_3N (4 mL, 1/1) were added $Pd_2(dba)_3$ (5.0 mg, 5.5 μmol) and PPh_3 (5.8 mg, 22 μmol). The mixture was stirred under N_2 at 60 °C for 4 h. The solution was poured into water (50 mL). The mixture was extracted with CH_2Cl_2 (10 mL x 3). The combined organic phases were washed with 5% HCl and brine, dried over dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (20:1, v/v) as eluent to afford dimer (91 mg, 82%): m.p. 120-121 °C; $[\alpha]_D$ +20° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.29 (d, 2 H, J 8.6 Hz, aromatic H), 6.93 (d, 2 H, aromatic H), 5.47-5.25 (m, 6 H, NH, NH', H-7, H-7', H-8, H-8'), 4.89 (m, 1 H, H-4'), 4.82 (m, 1 H, H-4), 4.55 (ABq, 2 H, J 15.6 Hz, -OC$\underline{H}_2$C≡),4.41 (dd, 1 H, $J_{5',6'}$ 10.3 Hz, $J_{6',7'}$ 1.1 Hz, H-6'), 4.29 (ABq, 2 H, -OC$\underline{H}_2$C≡), 4.25 (dd, 1 H, $J_{9a,9b}$ 12.4 Hz, $J_{8,9a}$ 2.8 Hz, H-9a), 4.23 (d, 1 H, $J_{9a',9b'}$ 12.6 Hz, H-9a'), 4.10-4.02 (m, 5 H, H-5, H-5', H-6, H-9b, H-9b'), 3.75 (s, 3 H, OCH_3), 3.57 (s, 3 H, OCH_3'), 2.64 (dd, 1 H, $J_{3a',3e'}$ 12.9 Hz, $J_{3e',4'}$ 4.6 Hz, H-3e'), 2.60 (dd, 1 H, $J_{3a,3e}$ 12.8 Hz, $J_{3e,4}$ 4.6 Hz, H-3e), 2.17 (dd, 1 H, $J_{3a',4}$ 12.6 Hz, H-3a'), 2.12, 2.09, 2.08, 2.07, 2.00, 1.99, 1.99, 1.98, 1.85, 1.83 (s, 30 H, OAc's, NHAc's), 1.96 (dd, 1 H, $J_{3a,4}$ 12.5 Hz, H-3a); ^{13}C NMR ($CDCl_3$) δ 170.9-169.9 (O=C's), 168.0 (C-1'), 167.9 (C-1), 154.0, 133.0, 119.4, 117.8 (aromatic C), 99.9 (C-2'), 98.0 (C-2), 85.6 (-OC$H_2\underline{C}$≡C-), 83.8 (-OCH_2C≡$\underline{C}$-), 73.4 (C-6'), 72.6 (C-6), 69.1 (C-8'), 68.9 (C-4), 68.6

(C-4'), 68.4 (C-8), 67.3 (C-7'), 67.2 (C-7), 62.3 (C-9), 62.0 (C-9'), 53.6 (-O<u>C</u>H$_2$C≡), 52.9 (OCH_3, OCH_3'), 49.2 (C-5, C-5'), 38.2 (C-3'), 37.9 (C-3), 23.1, 21.0, 20.9, 20.7, 20.6 (NHAc's, OAc's).

Anal. Calcd. for $C_{49}H_{62}N_2O_{26}$: C, 53.75; H, 5.71; N: 2.56. Found: C, 53.63; H, 5.76; N, 2.59.

General procedure for the cross-coupling of acetylenic sialic acid derivatives with 1,4-diiodobenzene

To a degassed solution of the propargyl sialic acid (0.2 mmol) and 1,4-diiodobenzene (30 mg, 91 μmol) in DMF/Et_3N (4 mL, 1/1) were added $Pd_2(dba)_3$ (9.2 mg, 10 μmol) and PPh_3 (10.5 mg, 40 μmol). The mixture was stirred under N_2 at 60 °C. The solution was poured into water (50 mL). The mixture was extracted with CH_2Cl_2 (10 mL x 3). The combined organic phases were washed with 5% HCl and brine, dried over dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (20:1, v/v) as eluent.

1,4-Bis-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyloxyonate prop-2-ynyl)benzene (114)

Reaction time: 2 h; yield (93%); m.p. 119-121 °C; $[\alpha]_D$ +18.8° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.31 (s, 4 H, aromatic H), 5.56-5.36 (m, 4 H, NH, H-8), 5.27 (dd, 2 H, $J_{6,7}$ 2.0 Hz, $J_{7,8}$ 8.5 Hz, H-7), 4.83 (m, 2 H, H-4), 4.36 (ABq, 2 H, *J* 15.8 Hz, -OC<u>H</u>$_2$C≡), 4.33 (ABq, 2 H, -OC<u>H</u>$_2$C≡), 4.25 (dd, 2 H, $J_{9a,9b}$ 12.4 Hz, $J_{8,9a}$ 2.8 Hz, H-9a), 4.12-4.00 (m, 6 H, H-5, H-6, H-9b), 3.74 (s, 6 H, OCH_3), 2.60 (dd, 2 H, $J_{3a,3e}$ 12.8 Hz, $J_{3e,4}$ 4.6 Hz, H-3e), 2.11, 2.08, 1.99, 1.98, 1.83 (s, 30 H, OAc's, NHAc), 1.96 (dd, 2 H, $J_{3a,4}$ 12.8 Hz, H-3a); ^{13}C NMR ($CDCl_3$) δ 170.8-170.0 (O=C's), 167.9 (C-1), 131.6, 122.6 (aromatic C), 98.1 (C-2), 86.2 (-OCH$_2$<u>C</u>≡C-), 85.6 (-OCH$_2$C≡<u>C</u>-), 72.6 (C-6), 68.8 (C-4), 68.4 (C-8), 67.2 (C-7), 62.3 (C-9), 53.5 (-O<u>C</u>H$_2$C≡), 52.9 (OCH_3), 49.3 (C-5), 37.9 (C-3), 23.1, 21.1, 20.8, 20.7, 20.6 (NHAc, OAc's); FAB-MS: 1133.3 ($[M+1]^+$, 3.6%).

Anal. Calcd. for $C_{52}H_{64}N_2O_{26}$: C, 55.12; H, 5.69; N: 2.47. Found: C, 54.82; H, 5.75; N, 2.49.

1,4-Bis-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylthioonate prop-2-ynyl)benzene (115)

Reaction time 3 h; yield 88 %; m.p. 134-136 °C; $[\alpha]_D$ +95° (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.23 (s, 4 H, aromatic H), 5.50 (d, 2 H, $J_{5,NH}$ 9.9 Hz, NH), 5.36 (m, 2 H, H-8), 5.26 (dd, 2 H, $J_{6,7}$ 1.6 Hz, $J_{7,8}$ 8.3 Hz, H-7), 4.81 (m, 2 H, H-4), 4.23 (dd, 2 H, $J_{9a,9b}$ 12.4 Hz, $J_{8,9a}$ 2.4 Hz, H-9a), 4.04-3.98 (m, 4 H, H-9b, H-5), 3.83 (dd, 2 H, $J_{5,6}$ 10.7 Hz, H-6), 3.71 (s, 6 H, OCH_3), 3.63 (ABq, 2 H, *J* 16.9 Hz, -SC$\underline{H}_2$C≡), 3.58 (ABq, 2 H, -SC$\underline{H}_2$C≡), 2.67 (dd, 2 H, $J_{3a,3e}$ 12.6 Hz, $J_{3e,4}$ 4.5 Hz, H-3e), 2.12, 2.05, 1.96, 1.95, 1.81 (s, 30 H, OAc's, NHAc), 1.93 (dd, 2 H, $J_{3a,4}$ 12.2 Hz, H-3a); ^{13}C NMR ($CDCl_3$) δ 170.7-170.0 (O=C's), 168.0 (C-1), 131.4, 122.8 (aromatic C), 86.6 (C-2), 82.3 (-$SCH_2\underline{C}$≡C-), 81.8 (-SCH_2C≡$\underline{C}$-), 74.2 (C-6), 69.4 (C-4), 68.5 (C-8), 67.2 (C-7), 62.2 (C-9), 53.1 (OCH_3), 49.1 (C-5), 37.5 (C-3), 23.0, 21.1, 20.7, 20.7, 20.6 (NHAc, OAc's), 18.0 (-S$\underline{C}H_2$C≡); FAB-MS: 1165.2 ($[M+1]^+$, 3.5%).

Anal. Calcd. for $C_{46}H_{60}N_2O_{24}S_2$: C, 50.73; H, 5.55; N: 2.57. Found: C, 50.83; H, 5.69; N, 2.56.

General procedure for the cross-coupling of acetylenic sialic acid derivatives with 1,3,5-triiodobenzene

To a degassed solution of the propargyl sialic acid (0.2 mmol) and 1,3,5-triiodobenzene (**109**) (27.8 mg, 61 μmol) in DMF/Et_3N (4 mL, 1/1) were added $Pd_2(dba)_3$ (9.2 mg, 10 μmol), CuI (3.8 mg, 20 μmol), and PPh_3 (10.5 mg, 40 μmol). The mixture was stirred under N_2 at room temperature for 1 h. The solution was poured into water (50 mL). The mixture was extracted with CH_2Cl_2 (10 mL x 3). The combined organic phases were washed with 5% HCl and brine, dried over dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (15:1, v/v) as eluent.

1,3,5-Tris-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyloxyonate prop-2-ynyl)benzene (116)

Yield (81%); m.p. 129-132 °C; $[\alpha]_D$ +16.2° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.37 (s, 3 H, aromatic H), 5.40-5.37 (m, 6 H, NH, H-8), 5.28 (dd, 3 H, $J_{6,7}$ 1.9 Hz, $J_{7,8}$ 8.5 Hz, H-7), 4.82 (m, 3 H, H-4), 4.53 (ABq, 3 H, *J* 15.9 Hz, -OC$\underline{H}_2$C≡), 4.28 (ABq, 3 H, -OC$\underline{H}_2$C≡),

4.24 (dd, 3 H, $J_{9a,9b}$ 12.6 Hz, $J_{8,9a}$ 2.7 Hz, H-9a), 4.08-4.00 (m, 9 H, H-5, H-6, H-9b), 3.74 (s, 9 H, OCH_3), 2.60 (dd, 3 H, $J_{3a,3e}$ 12.8 Hz, $J_{3e,4}$ 4.6 Hz, H-3e), 2.12, 2.09, 1.99, 1.98, 1.83 (s, 45 H, OAc's, NHAc), 1.95 (dd, 3 H, $J_{3a,4}$ 12.8 Hz, H-3a); ^{13}C NMR ($CDCl_3$) δ 171.1-170.0 (O=C's), 167.8 (C-1), 134.6, 123.2 (aromatic C), 98.1 (C-2), 85.8 (-$OCH_2\underline{C}\equiv C$-), 84.2 (-$OCH_2C\equiv\underline{C}$-), 72.6 (C-6), 68.8 (C-4), 68.3 (C-8), 67.2 (C-7), 62.4 (C-9), 53.4 (-$O\underline{C}H_2C\equiv$), 52.9 (OCH_3), 49.3 (C-5), 37.9 (C-3), 23.1, 21.1, 20.8, 20.7, 20.6 (NHAc, OAc's); FAB-MS: 1660.5 ($[M+1]^+$, 1.5%).

Anal. Calcd. for $C_{75}H_{93}N_3O_{39}$: C, 54.25; H, 5.64; N: 2.53. Found: C, 54.55; H, 5.63; N, 2.50.

1,3,5-Tris-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylthioonate prop-2-ynyl)benzene (117)

Yield (76%); $[\alpha]_D$ +104.7° (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.26 (s, 3 H, aromatic H), 5.53 (d, 3 H, $J_{5,NH}$ 9.9 Hz, NH), 5.36 (m, 3 H, H-8), 5.27 (dd, 3 H, $J_{6,7}$ 2.2 Hz, $J_{7,8}$ 8.3 Hz, H-7), 4.83 (m, 3 H, H-4), 4.24 (dd, 3 H, $J_{9a,9b}$ 12.5 Hz, $J_{8,9a}$ 2.6 Hz, H-9a), 4.05-3.99 (m, 6 H, H-9b, H-5), 3.85 (dd, 3 H, $J_{5,6}$ 10.8 Hz, H-6), 3.72 (s, 9 H, OCH_3), 3.59 (s, 6 H, -$SC\underline{H}_2C\equiv$), 2.68 (dd, 3 H, $J_{3a,3e}$ 12.6 Hz, $J_{3e,4}$ 4.6 Hz, H-3e), 2.13, 2.07, 1.98, 1.97, 1.82 (s, 45 H, OAc's, NHAc), 1.93 (dd, 3 H, $J_{3a,4}$ 12.0 Hz, H-3a); ^{13}C NMR ($CDCl_3$) δ 170.8-170.0 (O=C's), 168.0 (C-1), 134.1, 123.5 (aromatic C), 86.3 (C-2), 82.7 (-$SCH_2\underline{C}\equiv C$-), 81.0 (-$SCH_2C\equiv\underline{C}$-), 74.2 (C-6), 69.4 (C-4), 68.6 (C-8), 67.2 (C-7), 62.2 (C-9), 53.1 (OCH_3), 49.1 (C-5), 37.5 (C-3), 23.0, 21.1, 20.7, 20.7, (NHAc, OAc's), 17.8 (-$S\underline{C}H_2C\equiv$); FAB-MS: 1708.9 ($[M+1]^+$, 2.5%).

Anal. Calcd. for $C_{75}H_{93}N_3O_{36}S_3$: C, 52.72; H, 5.49; N: 2.46. Found: C, 52.75; H, 5.67; N, 2.45.

1,2,4,6-Tetrakis-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyloxyonate prop-2-ynyl)benzene (118)

To a degassed solution of the *O*-propargyl sialic acid **106** (100 mg, 0.19 mmol) and 1,2,4,5-tetraiodobenzene (**110**) (25 mg, 43 μmol) in DMF/Et_3N (4 mL, 1/1) were added $Pd_2(dba)_3$ (8.6 mg, 9.4 μmol), CuI (3.6 mg, 18.9 μmol), and PPh_3 (9.9 mg, 37.7 μmol). The mixture was stirred under N_2 at room temperature for 4 h. The solution was poured into water (50 mL). The mixture was extracted with CH_2Cl_2 (10 mL x 3). The combined organic phases were washed with 5% HCl and brine, dried over dried over Na_2SO_4, and concentrated

under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (10:1, v/v) as eluent to afford tetramer (82 mg, 87%): $[\alpha]_D$ +25.6° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.44 (s, 2 H, aromatic H), 5.47 (d, 4 H, $J_{5,NH}$ 8.4 Hz, NH), 5.37 (m, 4 H, H-8), 5.28 (dd, 4 H, $J_{6,7}$ 1.8 Hz, $J_{7,8}$ 8.4 Hz, H-7), 4.82 (m, 4 H, H-4), 4.58 (ABq, 4 H, *J* 16.1 Hz, -OC$\underline{H}_2$C≡), 4.40 (ABq, 4 H, -OC$\underline{H}_2$C≡), 4.25 (dd, 4 H, $J_{9a,9b}$ 12.5 Hz, $J_{8,9a}$ 2.6 Hz, H-9a), 4.07-3.98 (m, 12 H, H-5, H-6, H-9b), 3.75 (s, 12 H, OCH_3), 2.60 (dd, 4 H, $J_{3a,3e}$ 12.8 Hz, $J_{3e,4}$ 4.6 Hz, H-3e), 2.12, 2.08, 1.98, 1.97, 1.82 (s, 60 H, OAc's, NHAc), 1.93 (dd, 4 H, $J_{3a,4}$ 12.5 Hz, H-3a); ^{13}C NMR ($CDCl_3$) δ 170.8-170.0 (O=C's), 167.8 (C-1), 136.1, 124.8 (aromatic C), 98.1 (C-2), 90.7 (-OCH$_2\underline{C}$≡C-), 83.1 (-OCH$_2$C≡$\underline{C}$-), 72.5 (C-6), 68.8 (C-4), 68.4 (C-8), 67.1 (C-7), 62.3 (C-9), 53.4 (-O$\underline{C}$H$_2$C≡), 52.9 (OCH_3), 49.3 (C-5), 37.8 (C-3), 23.0, 21.0, 20.7, 20.7, 20.6 (NHAc, OAc's); FAB-MS: 1696.6.2 (M^+-$C_{20}H_{28}N_1O_{13}$, 0.7%).
Anal. Calcd. for $C_{98}H_{122}N_4O_{52}$: C, 53.75; H, 5.71; N: 2.56. Found: C, 53.46; H, 5.67; N, 2.50.

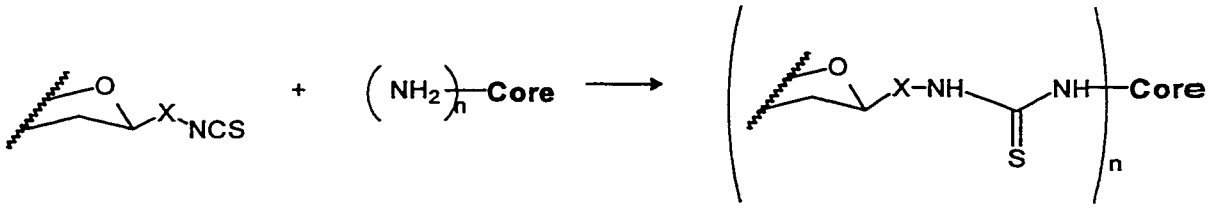
1,3,4-and 1,3,5-Tris-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyloxyonate methylene)benzene (119)

To a solution of the *O*-propargyl sialic acid **106** (100 mg, 0.19 mmol) in dry 1,2-dichloroethane (4 mL) was added ruthenium catalyst **71** (8 mg, 9.7 μmol). The mixture was refluxed under N_2 for 24 h. The solution was evaporated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (10:1, v/v) as eluent to afford two isomers **119** (68 mg, 68%) (1,2,4 and 1,3,5 regioisomers; 9:2): ^{1}H NMR ($CDCl_3$) δ 7.27 (d, 1 H, *J* 7.8 Hz, H-Ar), 7.23 (s, 1 H, H-Ar), 7.19 (d, 1 H, H-Ar), 7.14 (s, 1 H, H-Ar for the sym. isomer), 3.69, 355, 3.54 (s, OMe), 3.68 (s, OMe for the syn. isomer), 2.64-2.55 (m, 3 H, H-3e);^{13}C NMR ($CDCl_3$) δ 170.9-168.1 (C=O's), 137.2, 126.4 (Ar for sym. Isomer), 136.4, 135.4, 135.0, 128.7, 127.9, 127.1 (Ar), 98.6, 98.3, 98.3 (C-2), 72.5, 72.4, 72.3 (C-6), 69.4 (C-4), 68.5, 68.4 (C-8), 67.3, 67.2 (C-7), 66.5, 64.3, 64.1 (CH_2), 62.3, 62.2, 62.1 (C-9'), 52.6, 52.5, 52.5 (OCH_3), 49.4 (C-5), 38.0, 38.0, 37.9 (C-3), 23.1 (NHAc), 21.1, 20.8, 20.7, 20.7 (OAc's).

Chapter 6 Syntheses of multivalent sialosides using isothiocyanate conjugation strategy

6.1 Introduction

The recognition and binding of carbohydrate ligands by biological receptors is often enhanced when the sugars are presented in a multivalent, clustered format. Synthetic clusters of biologically important carbohydrates have therefore attracted great interest.[198] One of the most straightforward ways to synthesize glycoclusters is through the grafting of carbohydrate residues onto pre-existing multivalent cores. This approach can be done by isothiocyanate conjugation strategy by which glycosyl isothiocyanate derivatives could be conjugated to amine-terminated cores (Scheme **6.1**). The transformation of isothiocyanates and amines to thiourea derivatives has been applied to generate multivalent glycoconjugates.[199]



Scheme 6.1 Isothiocyanate conjugation strategy in the synthesis of glycoclusters

---

[198] a) Lee, R. T.; Lee, Y. C. in *Neoglycoconjugates: Preparation and Applications*; Lee, Y. C., Lee, R. T., Eds.; Academic Press, San Diego; **1994**, 23; b) Kichler, A.; Schuber, F.; *Glycoconjugate J.* 1995, 12, 275; c) Lehman, J.; Weitzel, U. P., *Carbohydr. Res.* **1996**, *294*, 65; d) Sprengard, U.; Schudok, M.; Schmidt, W.; Kretzschmer, Kunz, H. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 321.

6.2 Synthese of divalent sialosides scaffolded on *tert*-butylcalix[4]arene

Calixarenes are cyclic molecules containing a cavity useful in host-guest chemistry.[200] Their particular structural features, such as variation of conformational organization, substitution of the upper and lower rims, shape, and size, make them adequate candidates to function as scaffolds of carbohydrate moieties. These multivalent carbohydrate molecules can be potentially used for cell targeting and drug delivery. Until now, only limited efforts have been made to construct these kinds of molecules.[201] Moreover, the tedious synthetic procedures also limited their applications. Here, we describe a simple way to build such molecules.

Diethylene glycol was chosen as a hydrophilic spacer with a sufficient length. The diol was tosylated with tosyl chloride to provide bis-tosylated product **120** in 90% yield. Nucleophilic displacement of one tosyl group of compound **120** by sodium azide in ethanol under refluxing condition afforded the monoazido product **121** (46%) together with other side products such as bis-azide and unreacted bis-tosylate.

With the suitable spacer in hand, the distally disubstituted calixarene **122** was first prepared by alkylation of *tert*-butylcalix[4]arene with 1-azido-5-(*p*-toluenesulfonyloxy)-3-oxapentane (**121**) in the presence of K_2CO_3 in acetonitrile under refluxing condition. Further reaction of disubstituted calixarene **122** with the same alkylating reagent **121** and NaH at room temperature in DMF gave the tetraazidocalixarene derivative **123** in 75% yield. The diazido and tetraazido derivatives show very close polarity. In our opinion, this two-step procedure, rather than a one-step exhaustive alkylation, was preferable for the easy isolation of the desired diazido and tetraazido derivatives. The ^{1}H NMR splitting pattern of the $ArCH_2Ar$ methylene protons and the singlet peak for the aromatic protons suggest "cone"

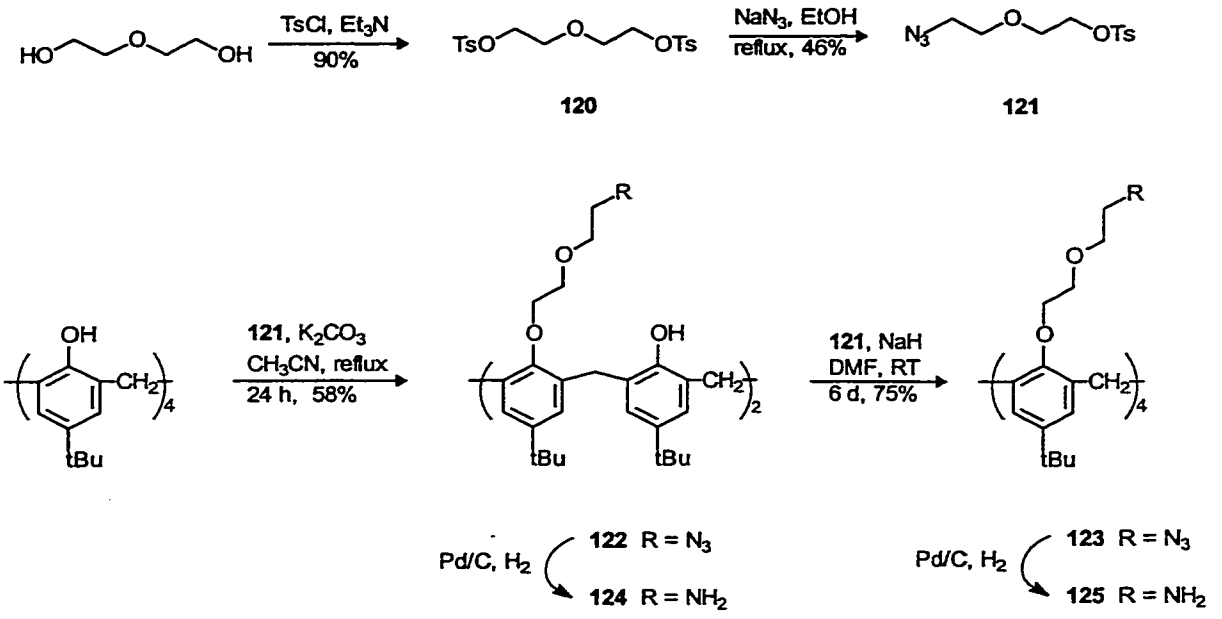
---

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

[200] a) Böhmer, V. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 713; b) Gutsche, C. D. *Aldrichimica Acta* **1995**, *28*, 3; c) Takeshita, M.; Shinkai, S. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 1088; d) Ikida, A.; Shinkai, S. *Chem. Rev.* **1997**, *97*, 1713.

[201] a) Meunier, S. J.; Roy, R. *Tetrahedron Lett.* **1996**, *37*, 5649; b) Marra, A.; Dondoni, A.; Sansone, F. *J. Org. Chem.* **1996**, *61*, 5155; c) Dondoni, A.; Kleban, M.; Marra, A. *Tetrahedron Lett.* **1997**, *38*, 7801; d) Dondoni, A.; Marra, A.; Scherrmann, M.-C.; Casnati, A.; Sansone, F.; Ungaro, R. *Chem. Eur. J.* **1997**, *3*, 1774; e) Fujimoto, T.; Shimizu, C.; Hayashida, O.; Aoyama, Y. *J. Am. Chem. Soc.* **1997**, *119*, 6676; f) Hayashida, O.; Shimizu, C.; Fujimoto, T.; Aoyama, Y. *Chem. Lett.* **1998**, 13; g) Roy, R.; Kim, J. M. *Angew. Chem. Int. Ed. Engl.* **1998**, *38*, 369.

conformations[202] for these two compounds. Hydrogenolysis (Pd/C, H_2) of compound **122** and **123** afforded di- and tetra-amines **124** and **125** in quantitative yields, respectively (Scheme **6.2**).

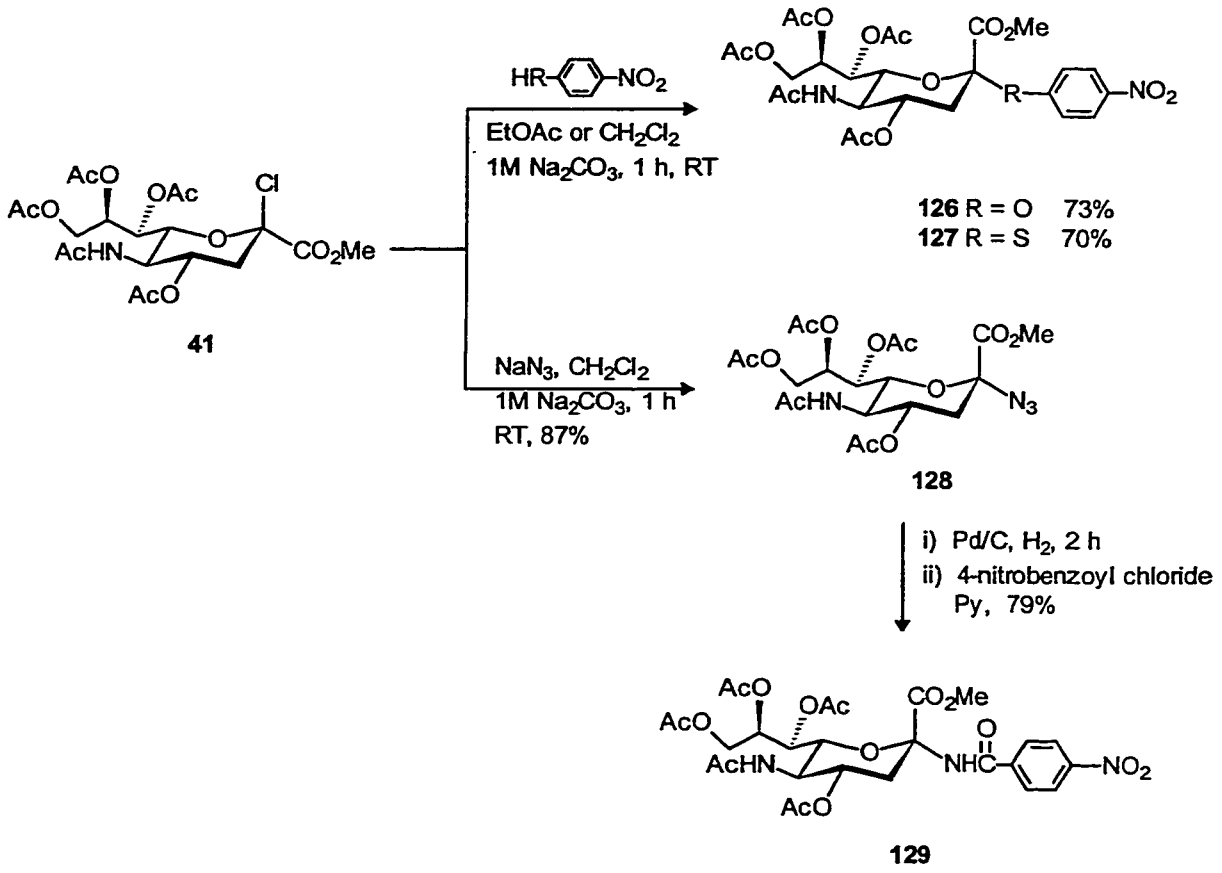


Scheme 6.2 Synthesis of amine-terminated calixarene cores

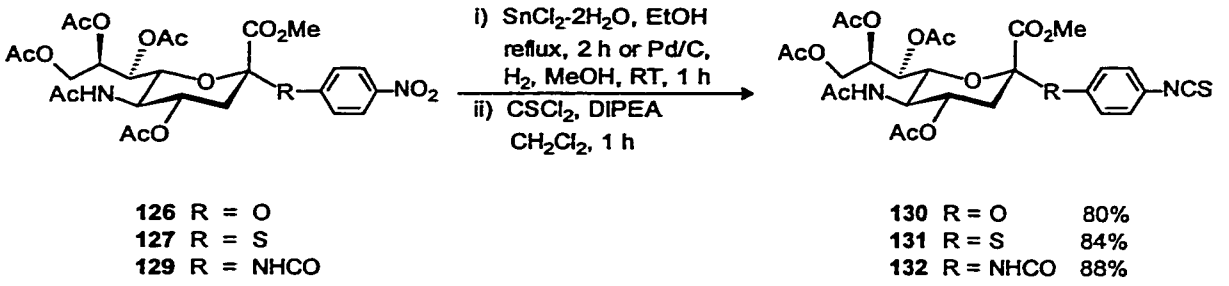
Bisamine-terminated calixarene core **124** was conjugated to three types of *p*-isothiocyanatophenyl α-sialosides to give dithiourea derivatives. In the last two types of α-sialosides, *S*- and amide linkages should offer a better stability towards glycosidases. The preparation of glycosyl isothiocyanates have been reported by several groups.[203] While these methods may be applied efficiently to most simple carbohydrates, sialic acid, with its unique anomeric configuration, is not suitable to these techniques. Instead, PTC was used to synthesize these key *p*-nitrophenyl α-sialosides. Acetochloroneuraminic acid **41** was treated with nucleophiles (*p*-nitrophenol, *p*-nitrothiophenol, or sodium azide) and TBAHS in equal volumes of ethyl acetate or dichloromethane and 1 M Na_2CO_3 to give *O*-, *S*-, and *N*-glycosides **126**, **127**, and **128** in 73%, 70%, and 87% yields, respectively. The α

[202] Shinkai, S.; Fujimoto, K.; Otsuka, T.; Ammon, H.L. *J. Org. Chem.* **1992**, *57*, 1516.
[203] a) Camarosa, M. J.; Fernandez-Resa, P.; Garcia-Lopez, M. T.; de las Heras, F. G. *Synthesis* **1984**, 504; b) Rothermal, J.; Weber, B.; Faillard, H. *Leibigs Ann. Chem.* **1992**, 799; c) Lindhorst, T. K.; Kieburg, C. *Synthesis* **1995**, 1228.

configurations of these compounds were judged by well-documented data. Hydrogenolysis (H_2, Pd/C) of the azide derivative **128** afforded the α-sialic acid amine derivative in quantitative yield. To minimize anomerisation, the next acylation step was performed within 1 h. Treatment of the amine derivative with *p*-nitrobenzoyl chloride in pyridine gave *p*-nitrobenzamido α-sialoside **129** in 79% yield (Scheme **6.3**). The nitro groups in sialic acid derivatives **126**, **127**, and **129** were efficiently reduced with tin(II) chloride in refluxing ethanol or hydrogenolysis (Pd/C, H_2) to give the corresponding amino α-sialoside intermediates. These intermediates were then treated with thiophosgene in dichloromethane (3 equiv. DIPEA) to provide *p*-isothiocyanato sialosides **130**, **131**, and **132** in 80%, 84%, and 88% yields, respectively (Scheme **6.4**).



Scheme 6.3 PTC synthesis of *p*-nitrophenyl sialic acid derivatives



126 R = O
127 R = S
129 R = NHCO

130 R = O 80%
131 R = S 84%
132 R = NHCO 88%

Scheme 6.4 Synthesis of sialoside isothiocyanate derivatives

Sialyl isothiocyanate derivatives **130-131** were conjugated to bisamine-teminated calixarene core **124** in the presence of DIPEA. The reactions were complete in one hour. After purification by silica gel chromatography, sialoside dimers **133-135** were obtained in 73%, 81%, and 83% yields, respectively (Scheme **6.5**). The strucutres of **133-135** were confirmed by their NMR and mass spectra. The ^{1}H NMR and ^{13}C NMR spectra of **133** are shown in Figures **6.1** and **6.2**.

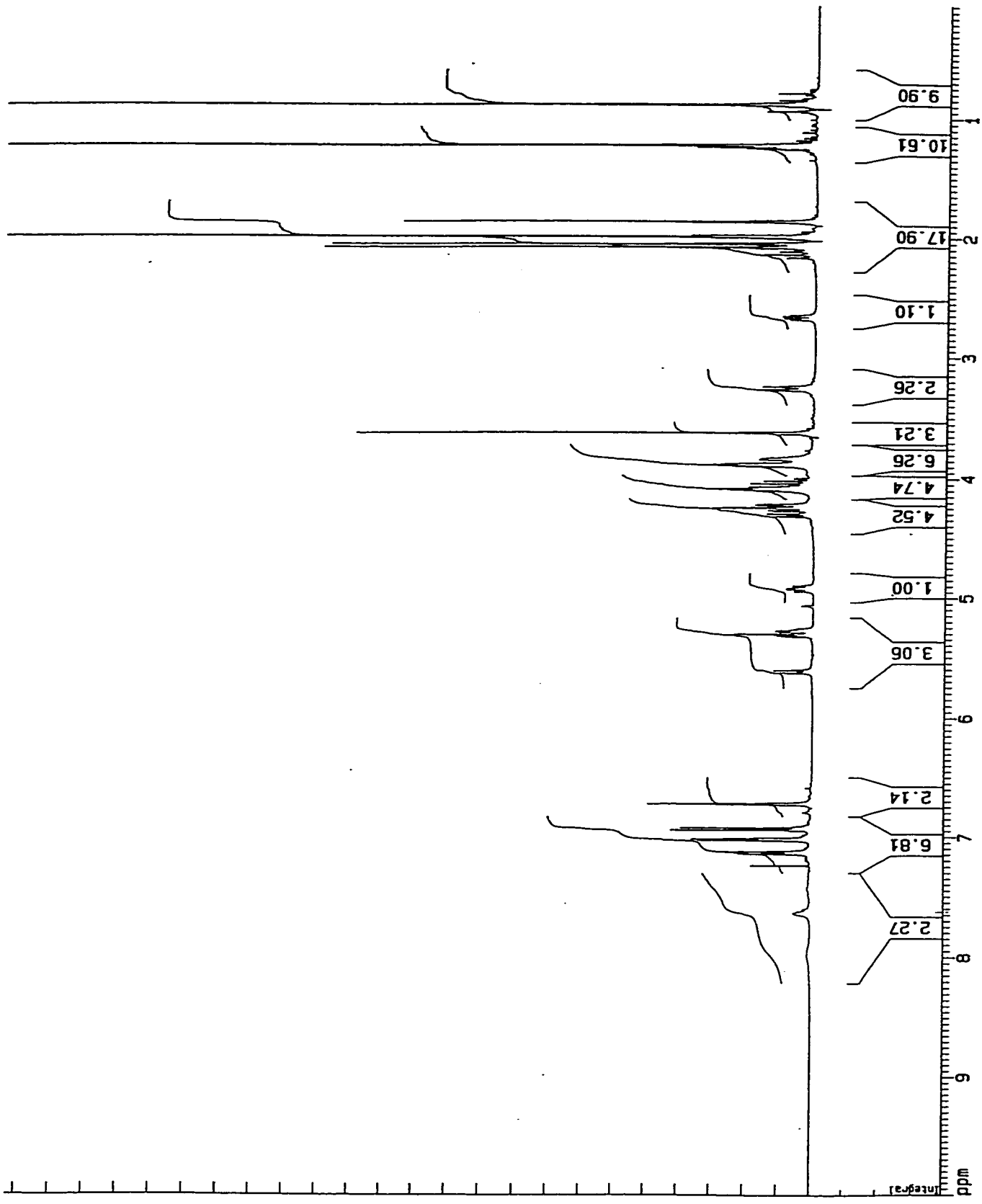


Figure 6.1 ^{1}H NMR (500MHz, $CDCl_3$) spectrum of **133**

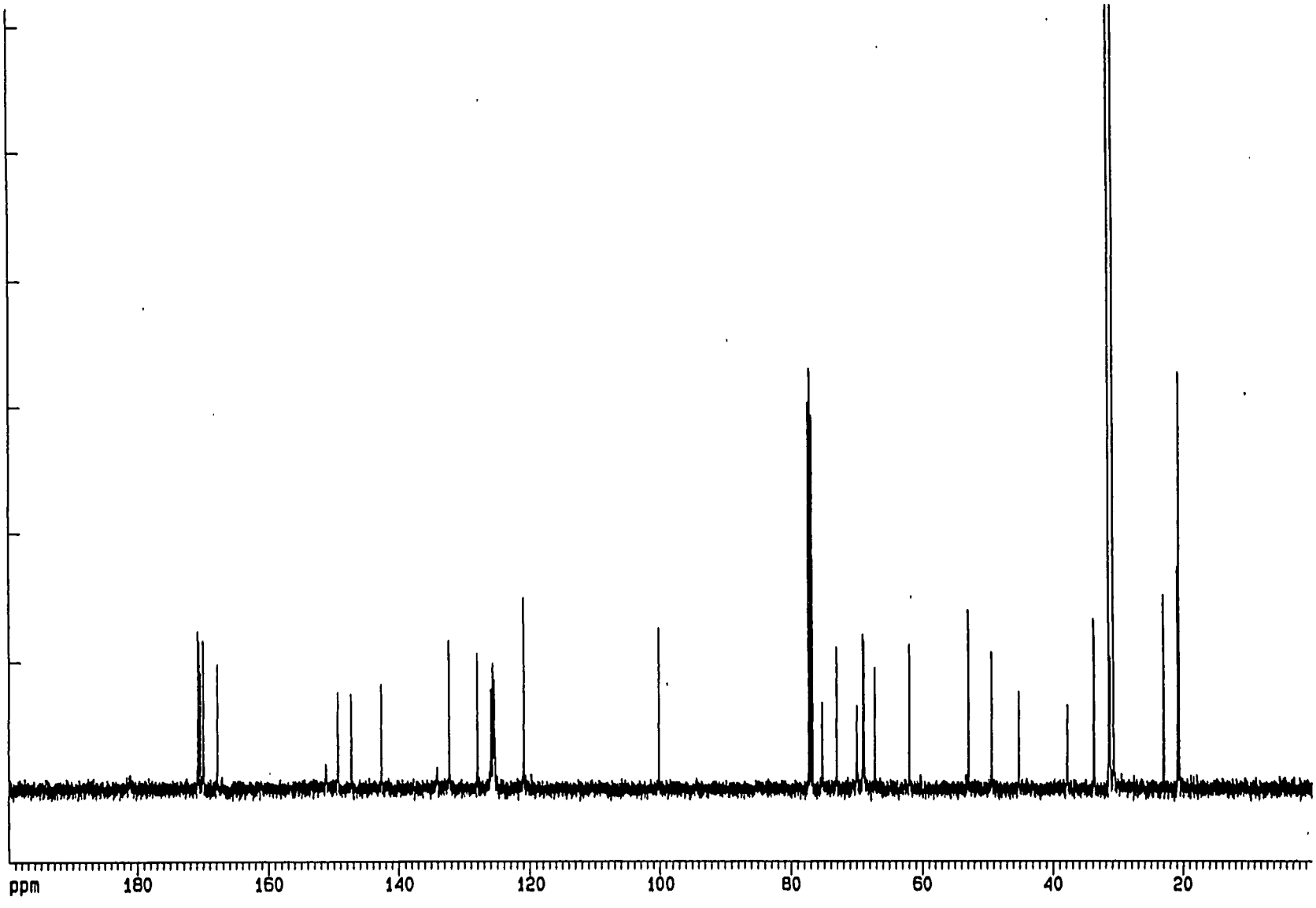
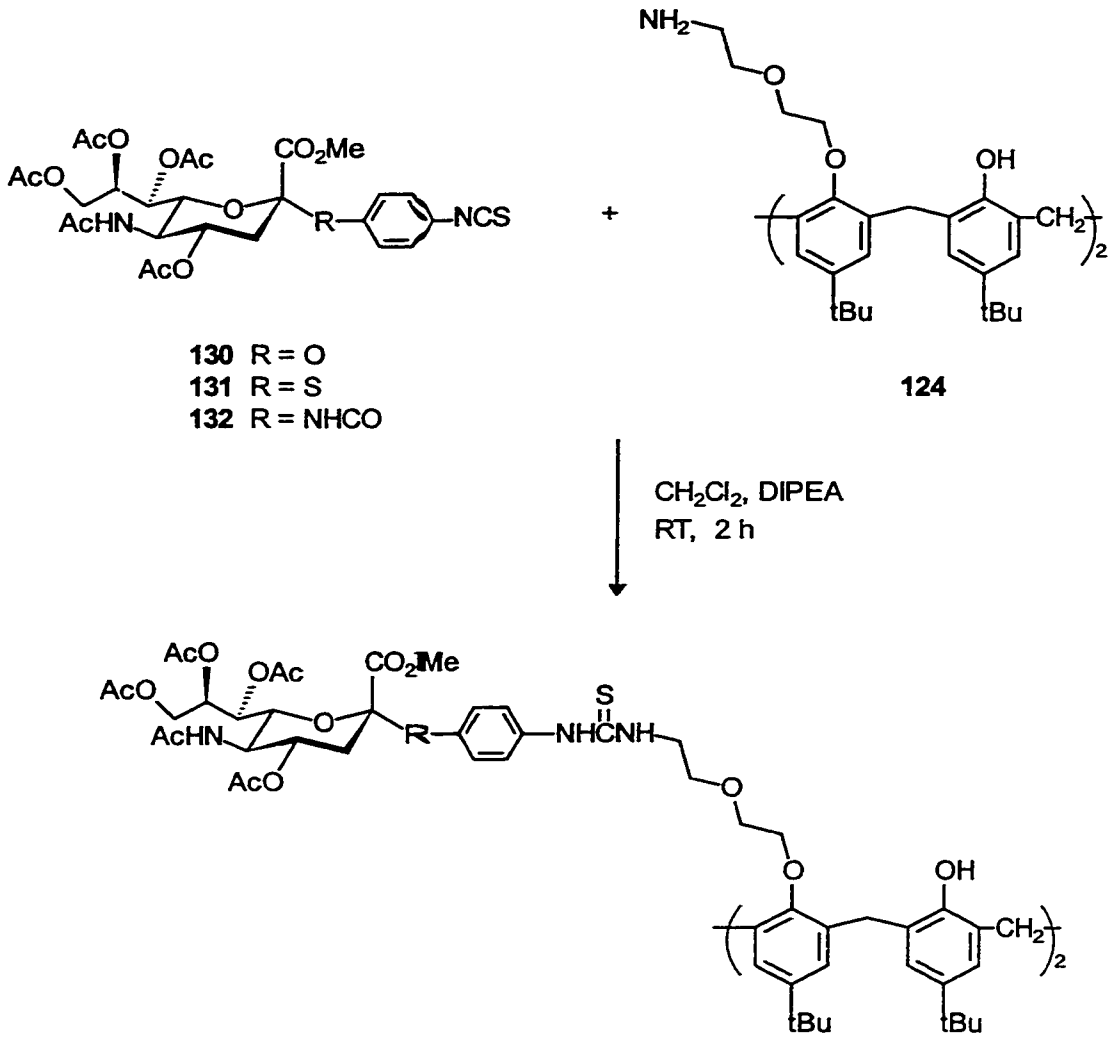


Figure 6.2 ^{13}C NMR (500MHz, $CDCl_3$) spectrum of **133**



Scheme 6.5 Synthesis of sialoside dimers scaffolded on calix[4]arene

6.3 Syntheses of multivalent GM_3 ligands based on resorcylic acid- and gallic acid-core

Since the first reported synthesis of L-lysine-based α-thiosialodendrimers,[53] much effort has gone into the preparation of other glycodendrimers. Divergent lactose-[58] and

sialodendrimers[59] based on a gallic acid core have been previously reported by our group. Recently, dendronized polyacrylates with glucose units based on a γ-resorcylic acid core have also been synthesized by Schlüter *et al.*[204]

As demonstrated in section **6.2**, the isothiocyanate coupling strategy has been successfully employed to build sialic acid calixarene dimers. This strategy combining with γ-resorcylic acid and gallic acid cores can afford an easy way to prepare GM_3-like clusters which could be potential antigens in therapeutic vaccines against melanoma.[205]

The synthesis started with esterification of γ-resorcylic acid (**136**) with methanol to yield the methyl ester **137** in 99% yield. The methyl ester **137** was then alkylated with a slight excess of azido tosylate **121** and potassium carbonate in refluxing acetonitrile to afford the key G-0 dendron **138** in 88% yield. The methyl ester of bisazide **138** was hydrolyzed in the presence of KOH solution under reflux to give azido acid **139**. Alternatively, hydrogenolysis of the azide groups of compound **138** afforded bisamino ester **140** (H_2, 10%Pd/C, 99% yield). Construction of tetra-azide ester **141** (G-1 dendron) was accomplished by coupling a slight excess of azido acid **139** to bisamino ester **140** using EDC and HOBt in 83% yield. Reduction of tetra-azide ester **141** by hydrogenation provided tetrakisamino ester **142** in quantitative yield (Scheme **6.6**).

A slight excess of bisamino acid **139** was coupled to 2,2'-(ethylenedioxy) bis(ethylamine) to give tethered tetrakisazido ester **143** (EDC, HOBt, DIPEA, 97% yield) which upon hydrogenolysis afforded tethered tetrakisamine **144** (Scheme **6.7**).

Similarly, gallic acid methyl ester **145** was alkylated with mono-tosylated azide **121** to afford trisazido ester **146** in 89% yield. Reduction of trisazido ester **146** gave trisamino ester **147** (H_2, 10%Pd/C, 99% yield) (Scheme **6.8**).

Sialyl α-(2→3)-lactosyl azide trisaccharide **68** (GM_3) was reduced (H_2, 10% Pd/C, 99% yield) to a sialyl lactosylamine intermediate which was immediately treated with thiophosgene to give sialyl α-(2→3)-lactosyl isothiocyanate **148** in 81% yield (Scheme **6.9**).

Dendritic bisamine **140** and trisamine **147** were each treated with an excess of GM_3 trisaccharide isothiocyanate derivative **148** (1.2 equivalents per amine) to provide GM_3 ligands **149** and **151** in good yields (82% and 76%), respectively. These compounds can be

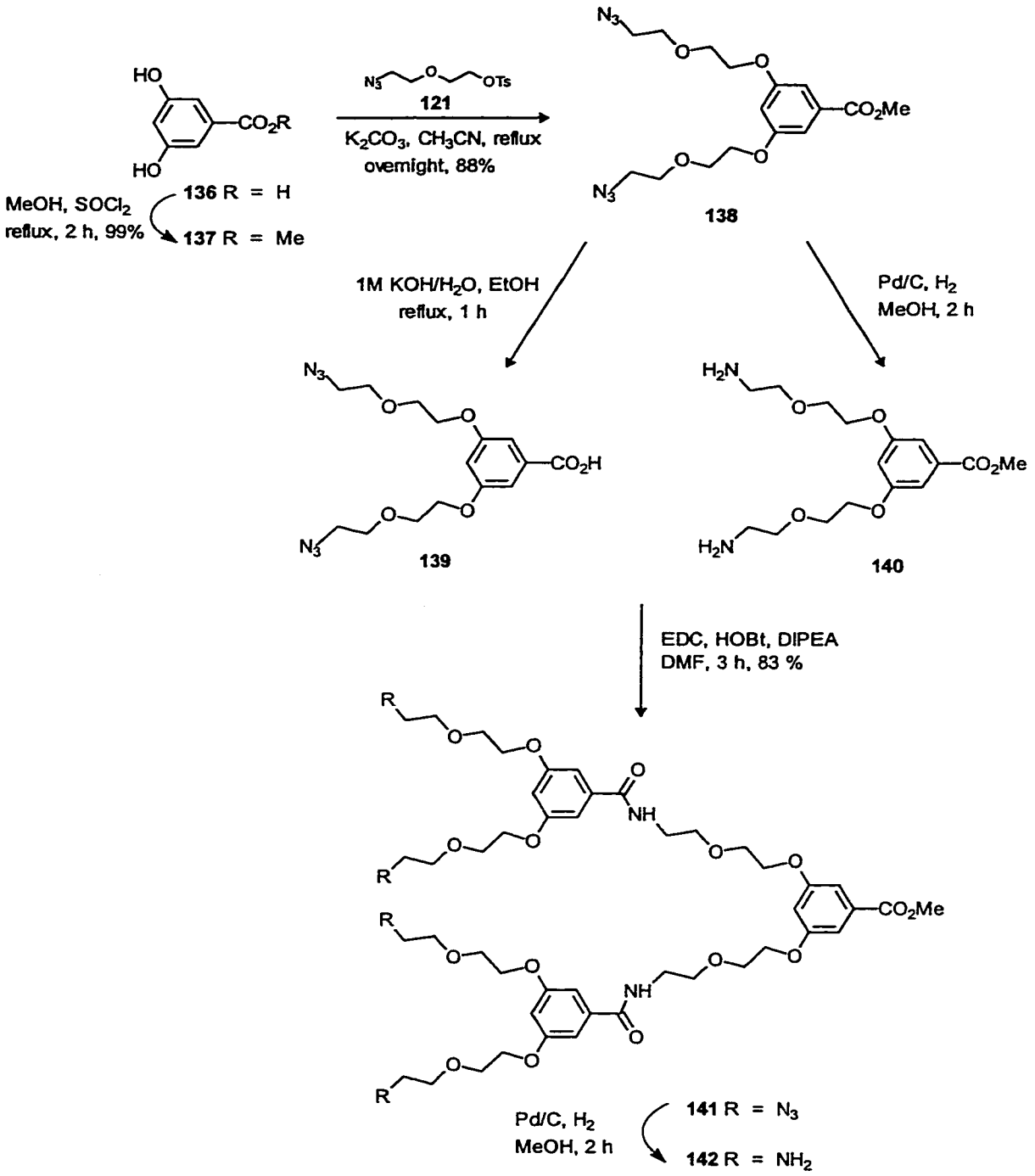
---

[204] Zistler, A.; Koch, S.; Schlüter, A. D. *J. Chem. Soc., Perkin, Trans. 1* **1999**, 1233.
[205] a) Nores, G. A.; Dohi, T.; Taniguchi, M.; Hakomori, S.-I. *J. Immunol.* **1987**, *139*, 3171.

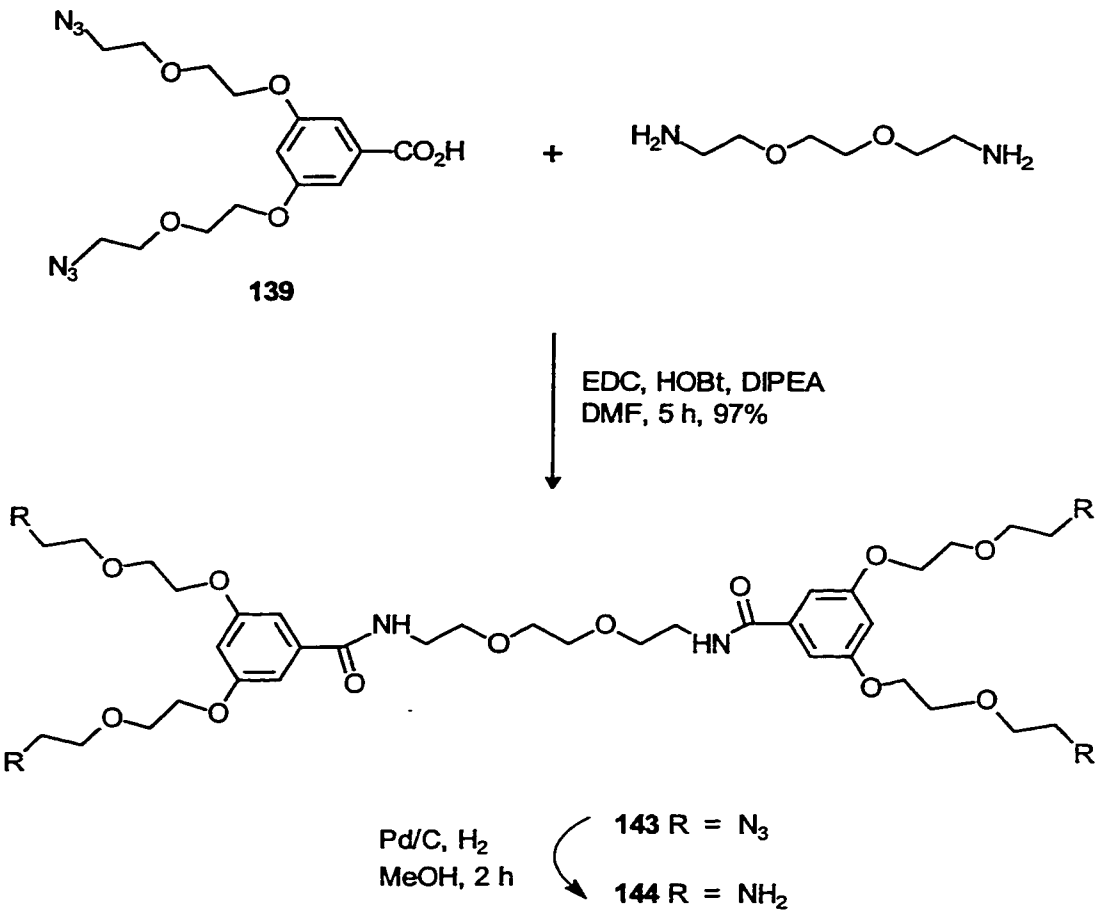
purified by silica gel chromatography. Deprotection of compound **149** and **151** by sequential ester hydrolysis ((i) NaOMe/MeOH, 8 h, 25 °C; (ii) H_2O, 12 h, 25 °C) followed by gel permeation chromatography (Biogel-P2, H_2O as eluent) afforded fully deprotected glycoclusters **150** and **152** with two and three GM_3 trisaccharide residues, respectively (87% and 90%) (Schemes **6.10** and **6.11**).

The tetravalent GM_3 ligands **153** and **154** were obtained by coupling isothiocyanate derivative **148** to the corresponding tetrakismines **142** and **144**, respectively. However, the polarity of GM_3 trisaccharide residues dismisses the possibility of purification by silica gel chromatography for glycoclusters of higher valency. The reaction mixtures were directly used in the deprotection step without purification by silica gel. Again, deprotection of clusters by sequential ester hydrolysis ((i) NaOMe/MeOH, 8 h, 25 °C; (ii) H_2O, 12 h, 25 °C) followed by gel permeation chromatography (Biogel-P4, H_2O as eluent) afforded fully deprotected glycoclusters **153** and **154** with four GM_3 trisaccharide residues in 64% and 58% yields, respectively (Schemes **6.12** and **6.13**).

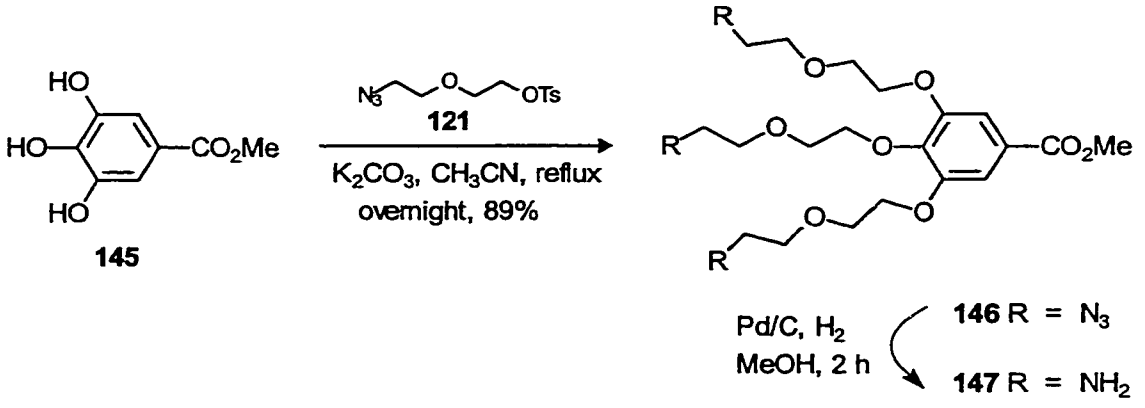
Complete coupling between the dendritic core and the GM_3 trisaccharide was confirmed by the ^1H NMR spectra of compound **150**, **152**, **153**, and **154**. The key signals included the aromatic protons of the dendritic core and the H-3a, NHAc and H-3e of the NeuAc moieties at δ 1.88, 2.10, and 2.83 ppm, respectively. The integration showed complete incorporation of GM_3 trisaccharide residues. The ^1H NMR spectrum of **154** is shown in Figure **6.3**.



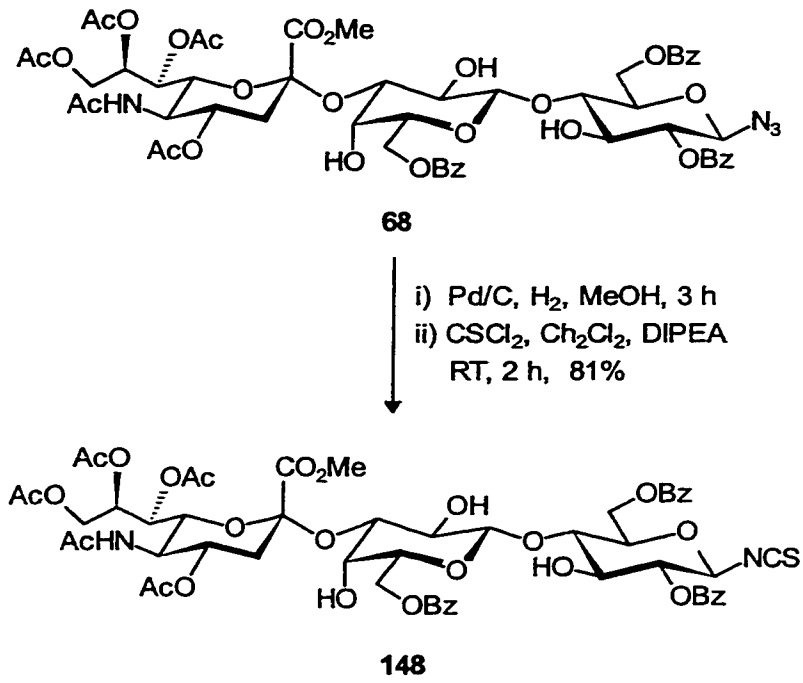
Scheme 6.6 Synthesis of divalent and tetravalent γ-resorcylic acid-based dendritic cores



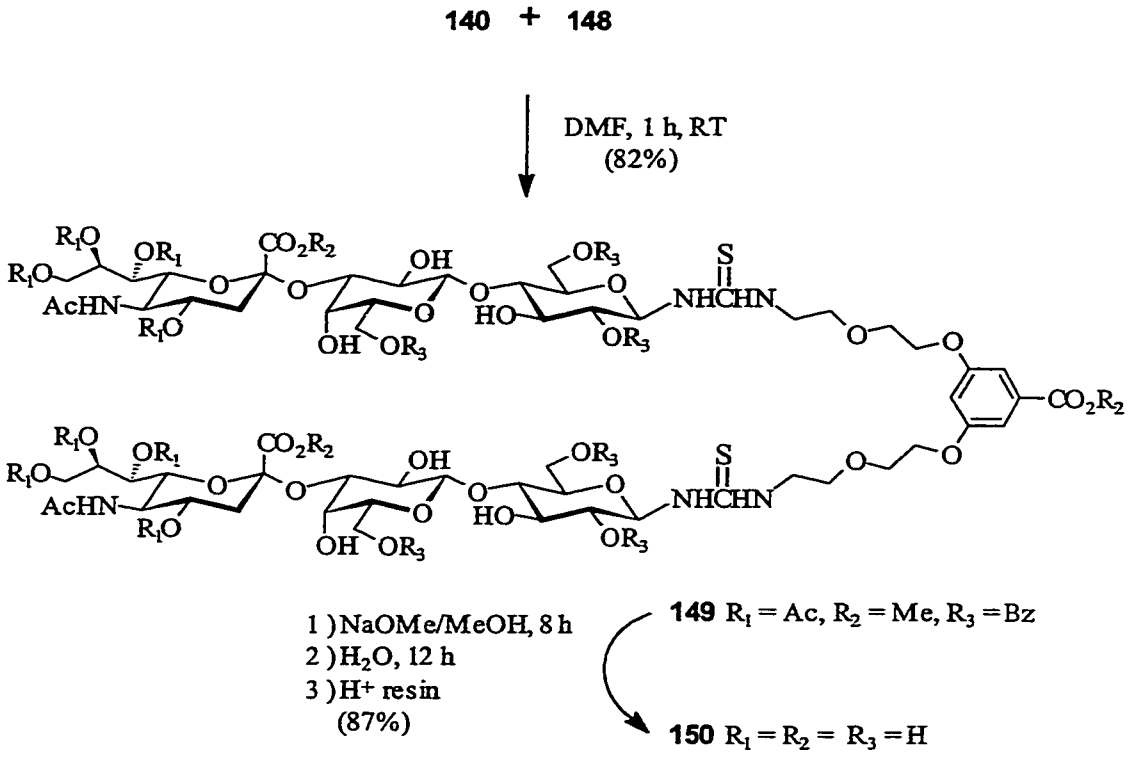
Scheme 6.7 Synthesis of tethered tetravalent γ-resorcylic acid-based dendritic core



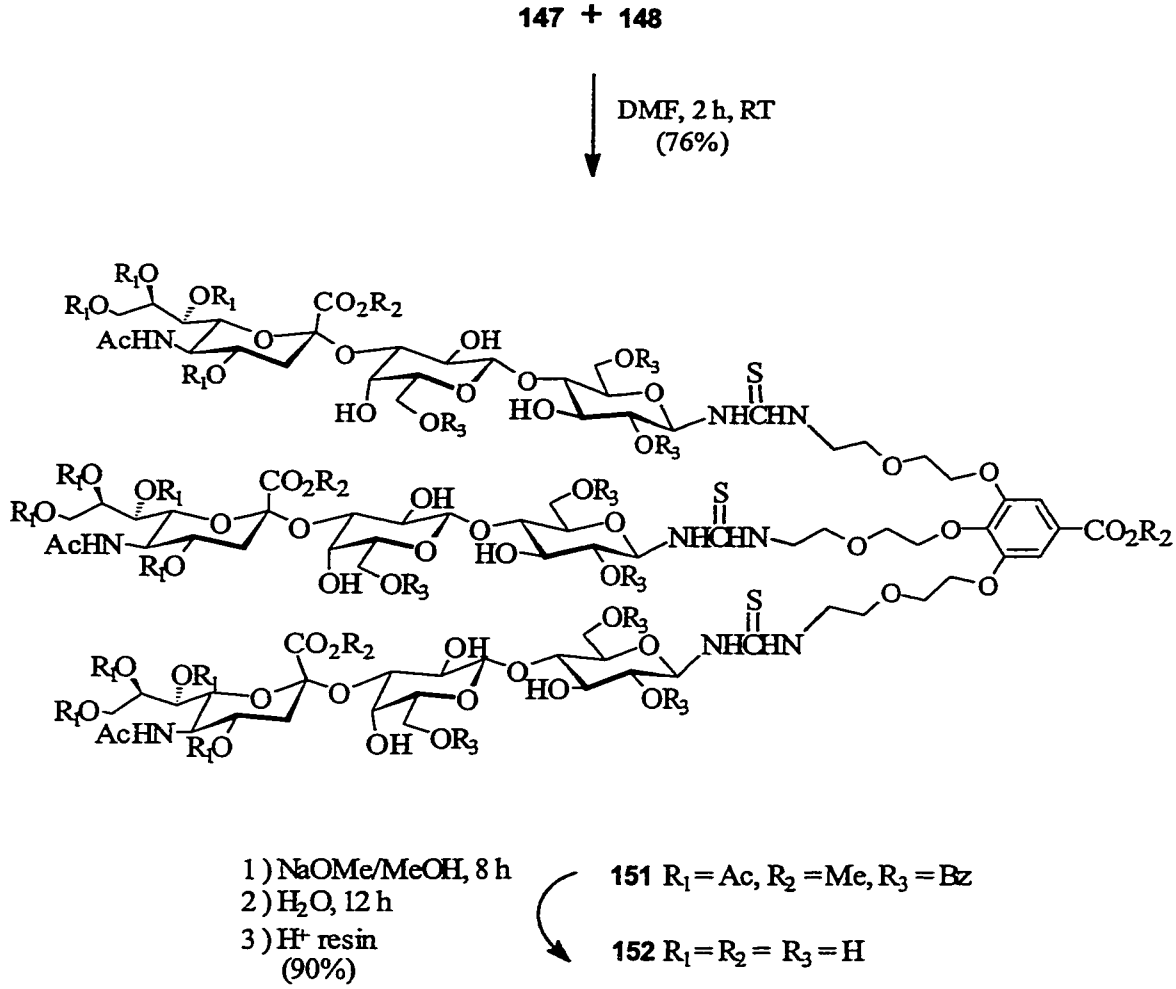
Scheme 6.8 Synthesis of trivalent gallic acid-based dendritic core



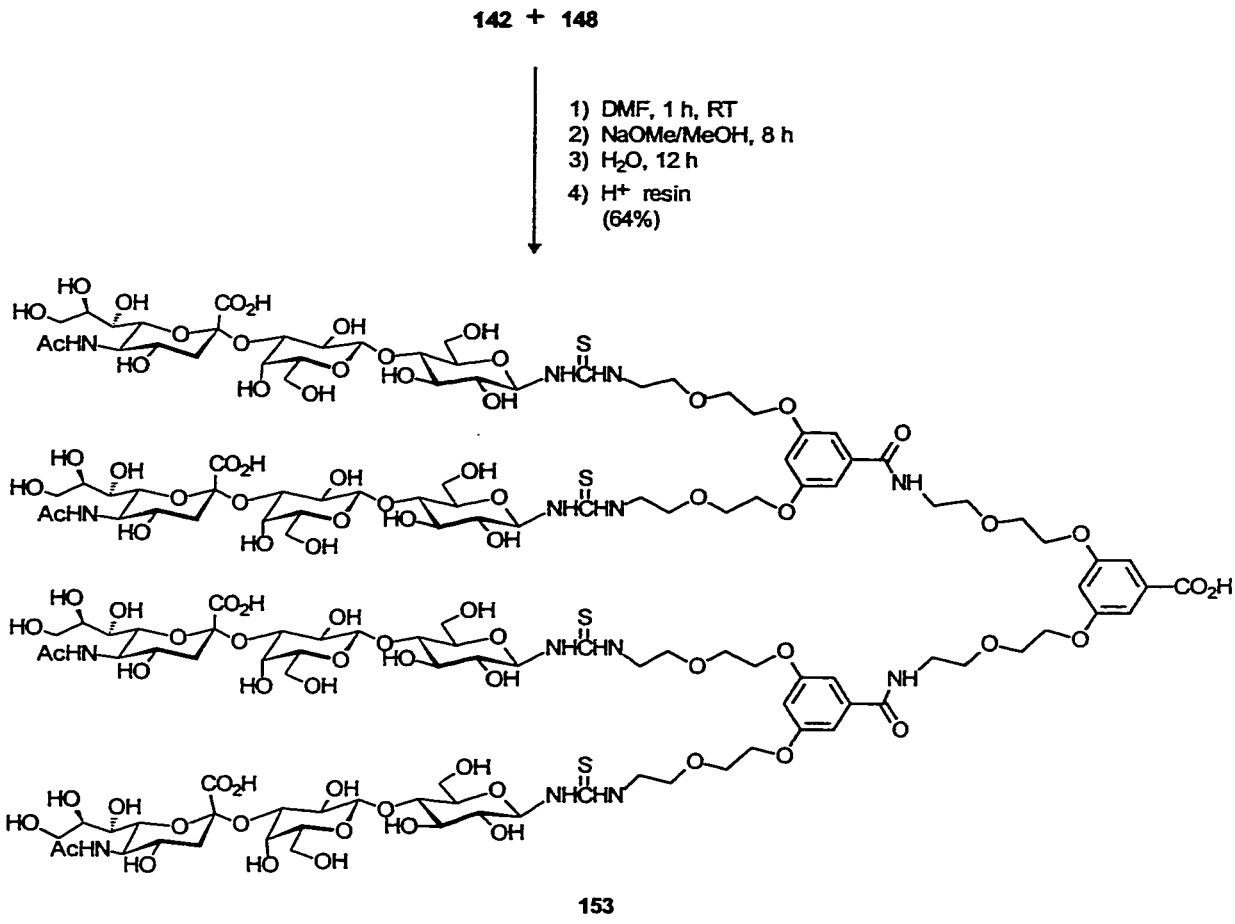
Scheme 6.9 Synthesis of sialyl α-(2→3)-lactosyl isothiocyanate derivative **148**



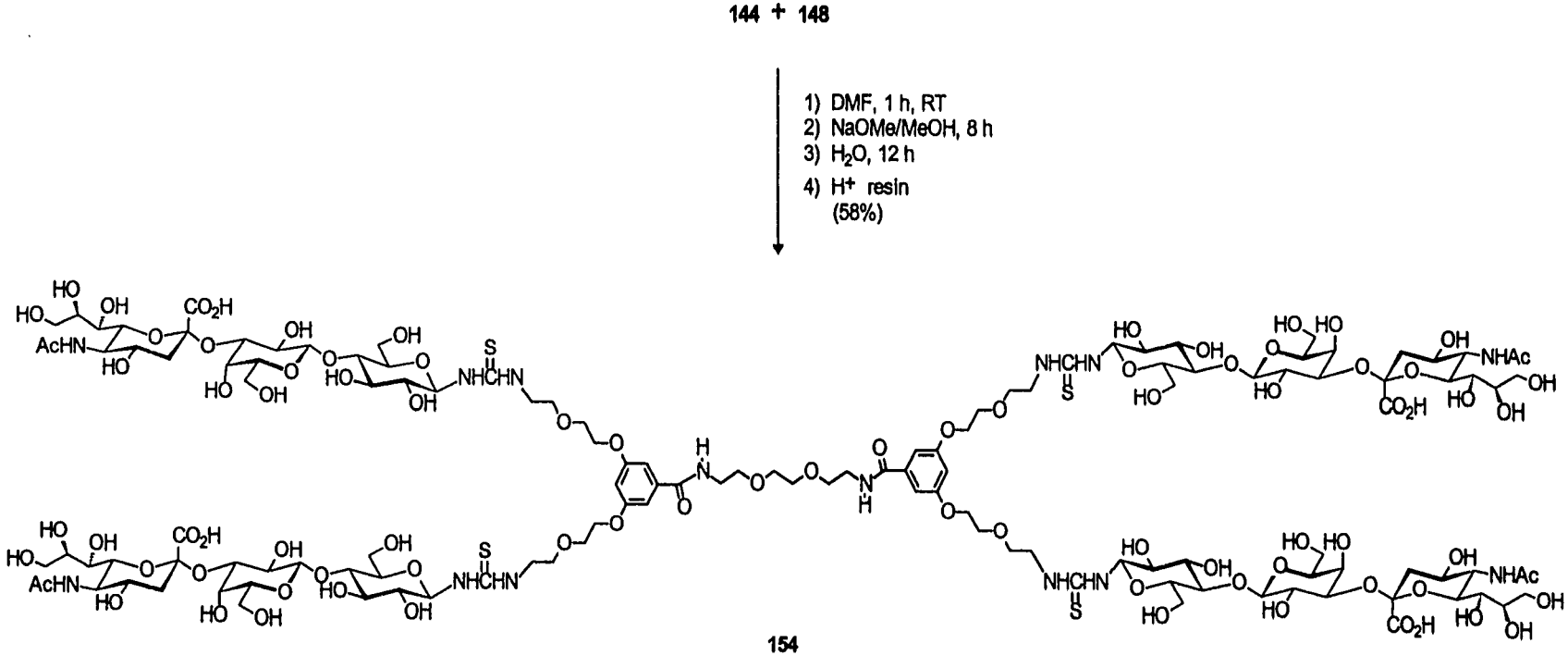
Scheme 6.10 Synthesis of deprotected divalent GM_3 ligand **150**



Scheme 6.11 Synthesis of deprotected trivalent GM_3 ligand **152**



Scheme 6.12 Synthesis of deprotected tetravalent GM_3 ligand **153**



Scheme 6.13 Synthesis of deprotected tethered tetravalent GM_3 ligand **154**

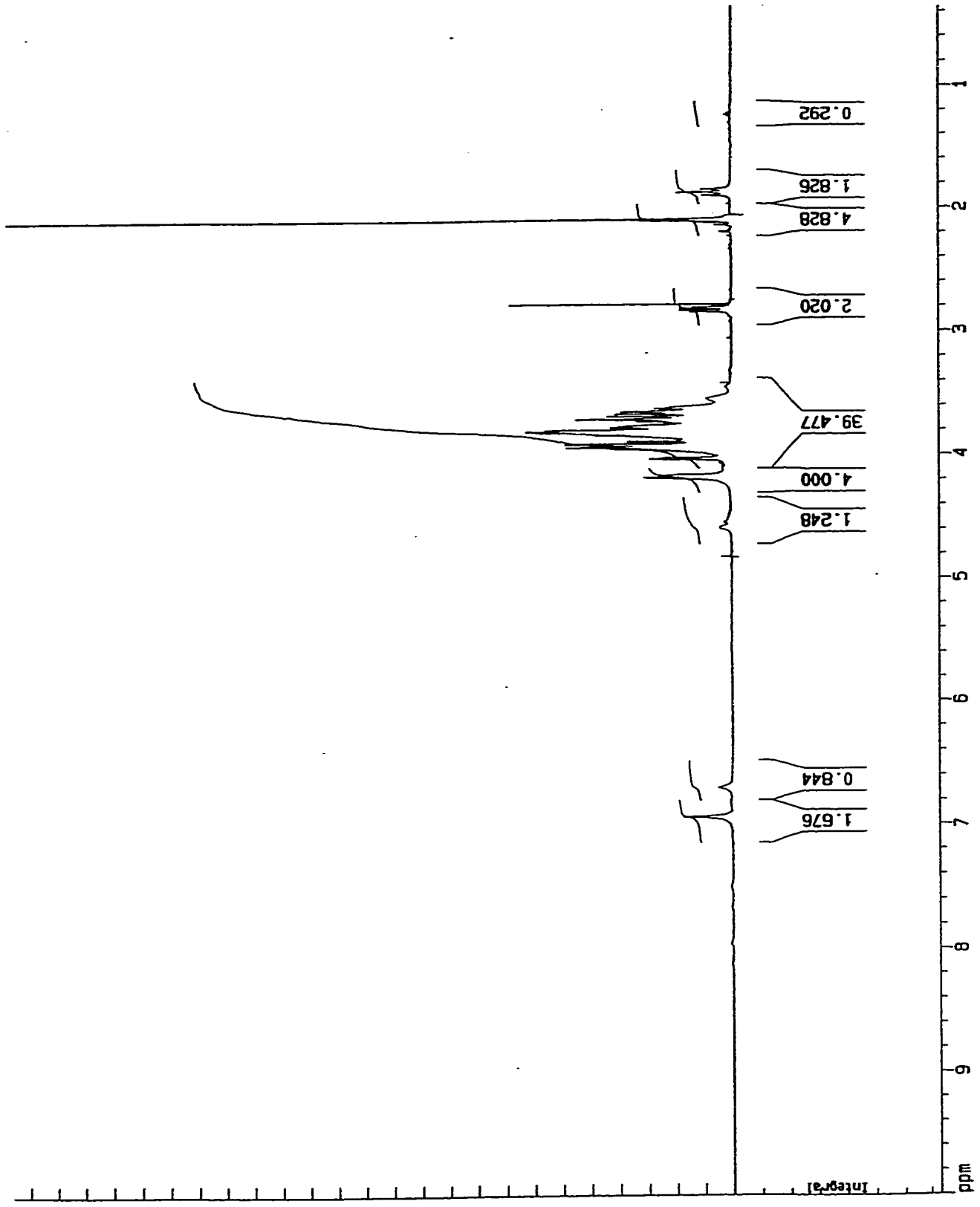


Figure 6.3 ^{1}H NMR (500MHz, D_2O) spectrum of tetravalent GM_3 ligand **154**

6.4 Syntheses of PAMAM-based GM_3 dendrimers

Starburst PAMAM dendrimers were first synthesized in a divergent manner by Tomalia *et al.*[64] They are made of polyamidoamine and are commercially available. The synthesis starts with the conjugate addition of ethylenediamine to methyl acrylate followed by amidation of excess ethylenediamine to give spherical, hyperbranched dendrimers with a defined number of amine surface groups (Scheme **6.14**).

Due to their hydrophilic backbones, PAMAM dendrimers could be used advantageously over other dendrimers for biological investigations. Much effort has been made by attaching various carbohydrate derivatives to Starburst PAMAM dendrimers. To date, disaccharide lactones of lactose and maltose,[65] and a T_N-antigen peptide[66] have been coupled to PAMAM dendrimers. Moreover, the isothiocyanate coupling strategy has been employed for the isothiocyanate derivatives of α-[206] and β-D-mannose, β-D-glucose, β-D-cellobiose, β-lactose,[67] and sialic acid[69] to conjugate with PAMAM dendrimers.

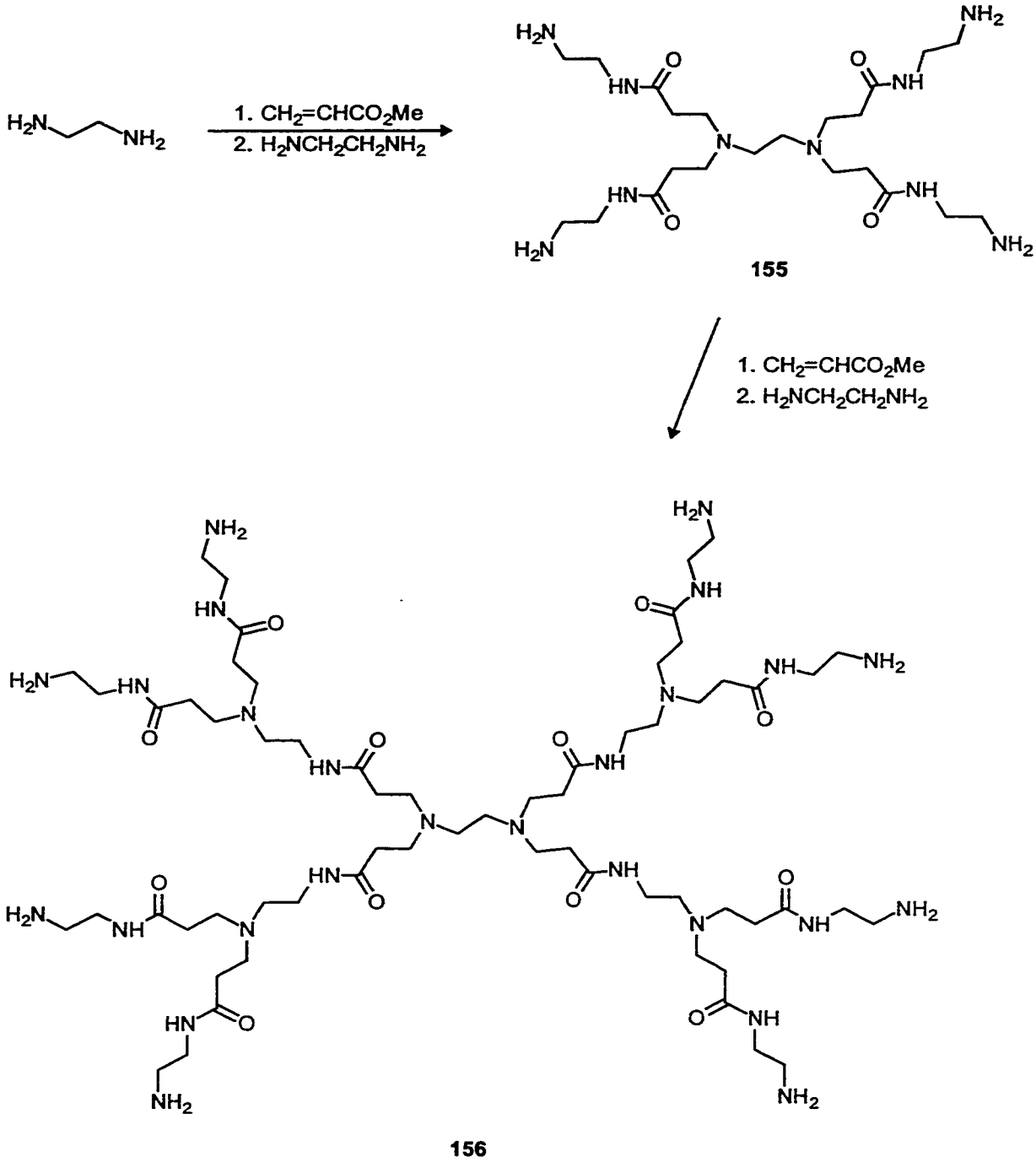
Commercially available methanolic solution of PAMAM dendrimers **155** and **156** were dried under vacuum. The resulting residues were dissolved in CH_2Cl_2 for **155** or DMF for **156**. GM_3 isothiocyanate derivative **148** (1.2 equiv. per amine moiety) and 1 drop of DIPEA were added to the solutions which were stirred at room temperature for 3 h. The reaction mixtures were concentrated under vacuum and used directly in the deprotection step.

Deprotection of these mixtures by sequential ester hydrolysis ((i) NaOMe/MeOH, 8 h, 25 °C; (ii) H_2O, 12 h, 25 °C) followed by gel permeation chromatography (Biogel-P4, H_2O as eluent) afforded fully deprotected PAMAM based GM_3 dendrimers, with four and eight GM_3 trisaccharide residues, **157** and **158**, respectively (78% and 57%) (Scheme **6.15** and **6.16**).

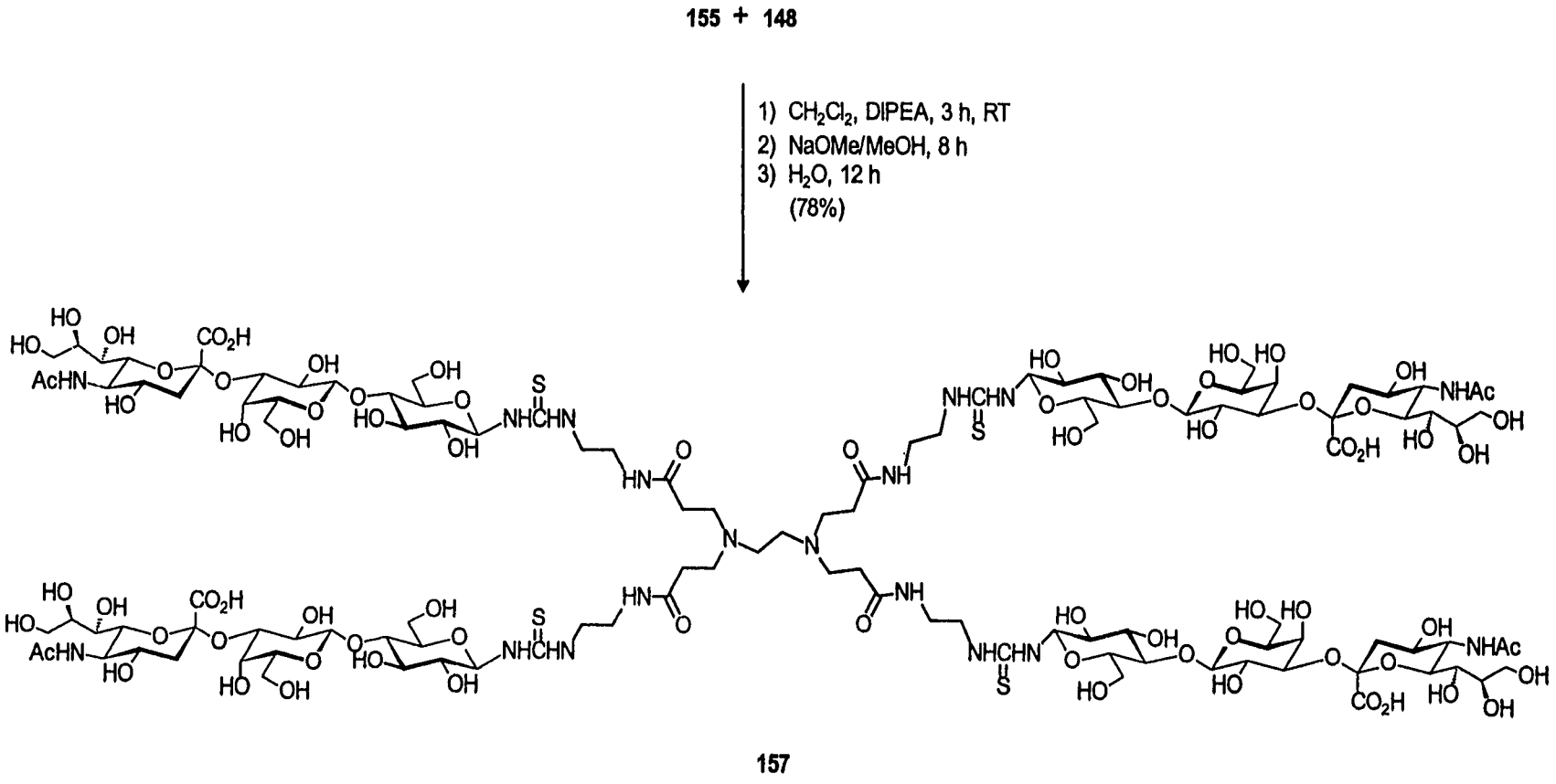
The ^{1}H NMR spectra of compounds **157** and **158** confirmed the total incorporation of the GM_3 trisaccharide residues. The key signals included the methylene units of the dendritic core at δ 2.80 ppm and the H-3a, NHAc and H-3e of the NeuAc moieties at δ 1.88, 2.10, and 2.83 ppm, respectively. The ^{1}H NMR spectrum of **157** is shown in Figure **6.4**.

---

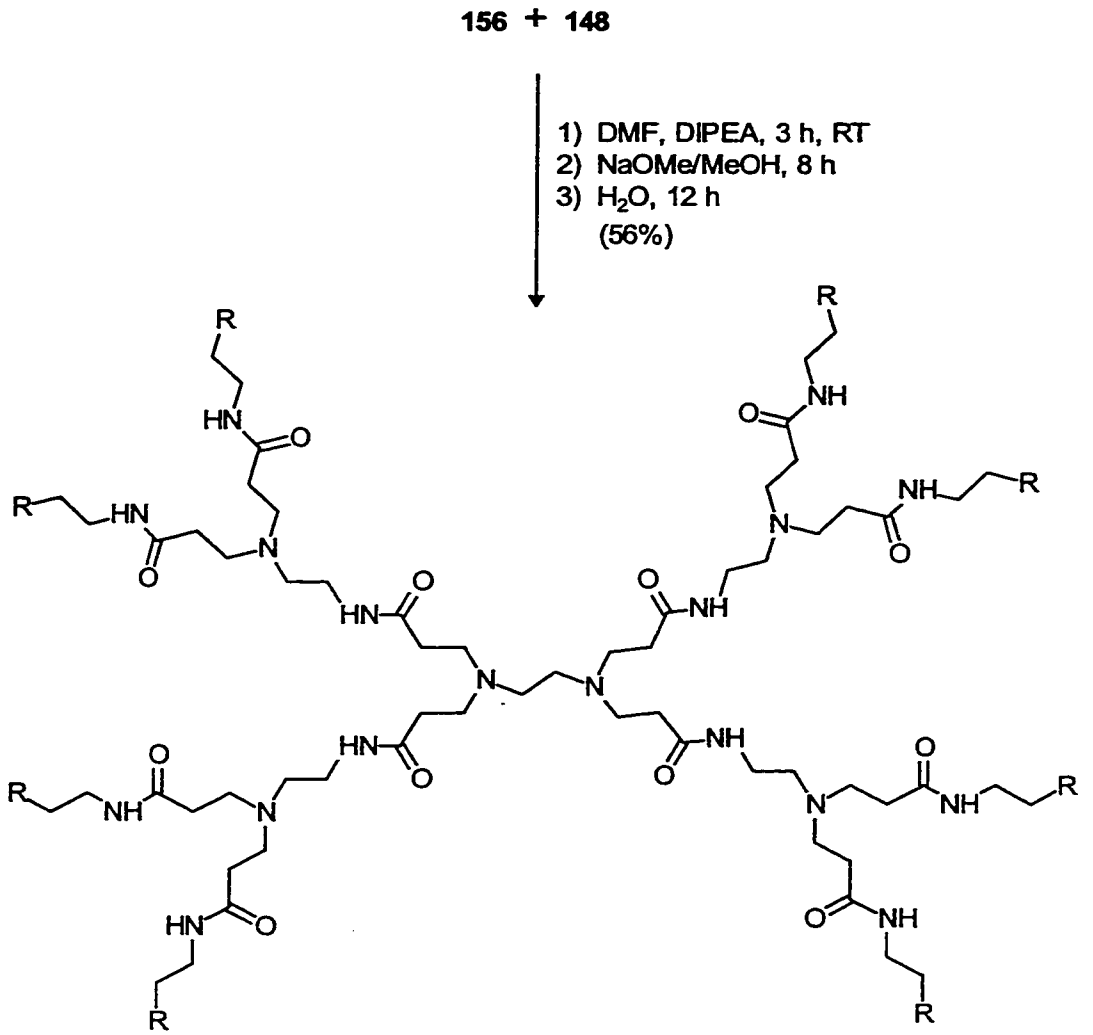
[206] a) Pagé, D.; Aravind, S.; Roy, R. *Chem. Commun.* **1996**, 1913; b) Pagé, D.; Roy, R. *Bioconjugate Chem.* **1997**, *8*, 714.



Scheme 6.14 Synthesis of spherical Starburst® PAMAM dendrimers.



Scheme 6.15 Synthesis of tetravalent PAMAM-based GM_3 dendrimer



Scheme 6.16 Synthesis of octavalent PAMAM-based GM_3 dendrimer

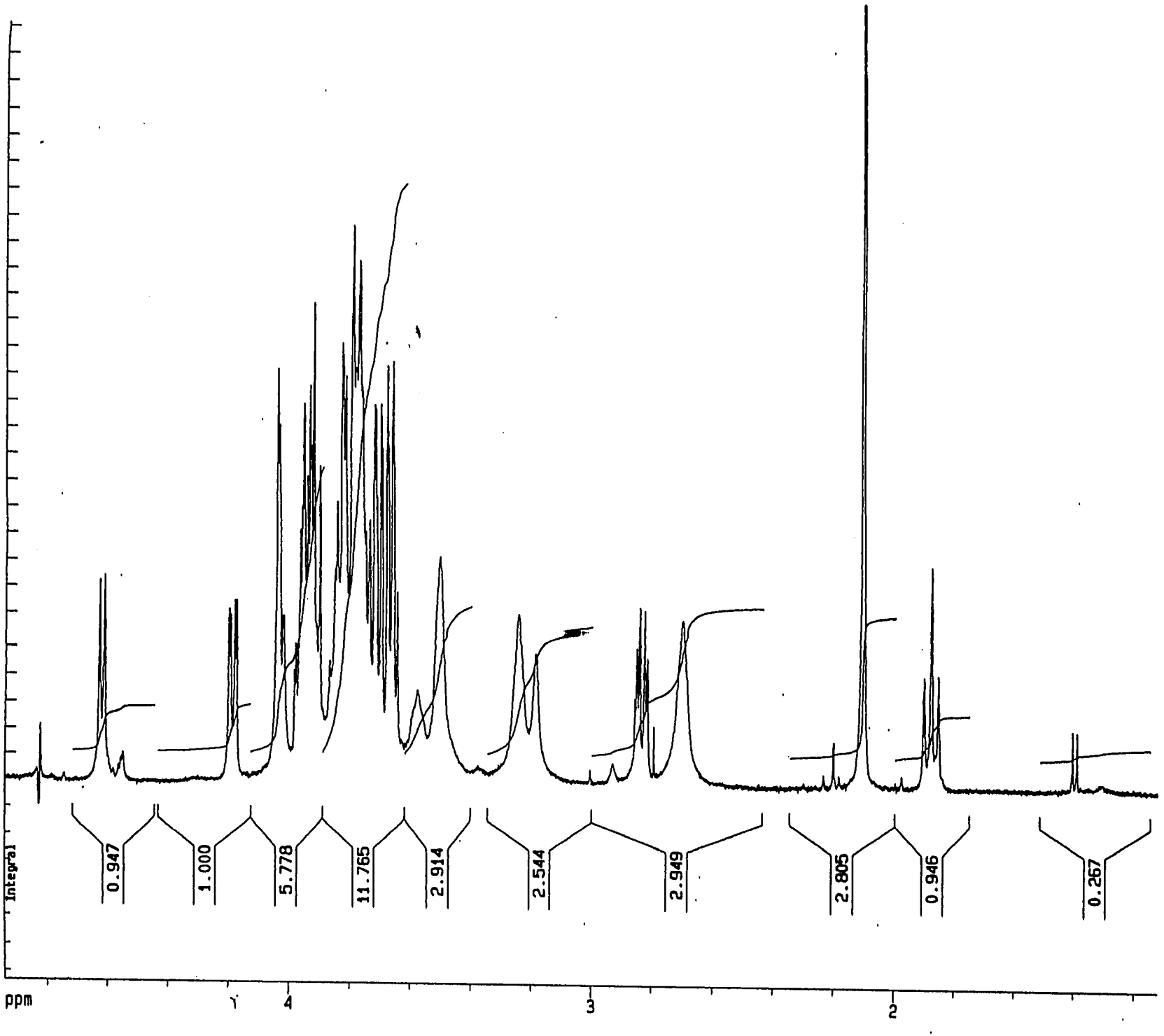


Figure 6.4 ^{1}H NMR (500MHz, D_2O) spectrum of PAMAM-based GM_3 dendrimer **157**

6.5 Conclusion

Divalent sialosides scaffolded on *tert*-butylcalix[4]arene were synthesized using the isothiocyanate conjugation strategy. This strategy was also successfully applied to synthesize multivalent γ-resorcylic acid- or gallic acid-based GM_3 ligands in good yields. Moreover, Starburst® PAMAM-based glycodendrimers containing four and eight GM_3 trisaccharide residues were prepared in the same manner.

6.6 Experimental

1,5-Bis-(*p*-toluenesulfonyloxy)-3-oxapentane (120)

To a solution of diethylene glycol (5 mL, 53 mmol) in ether (40 mL) were added tosyl chloride (25.1 g, 132 mmol) and Et_3N (18.33 mL, 132 mmol) at 0°C. The reaction was stirred at room temperature for 4 h. The solvent was evaporated under vacuum. The residue was diluted with dichloromethane (50 mL). The organic layer was washed successively with 5% HCl, sat. $NaHCO_3$, and brine. The organic phase was dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography using hexane/EtOAc (1:1, v/v) as eluent to give compound **120** (19.57 g, 90 %): m.p. 87-89 °C; ^{1}H NMR ($CDCl_3$) δ 7.76 (d, *J* 8.4 Hz, 4 H, aromatic H), 7.32 (d, 4 H, aromatic H), 4.06 (t, *J* 4.7 Hz, 4 H, CH_2), 3.58 (t, 4 H, CH_2), 2.43 (s, 6 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 144.9, 132.7, 129.8, 127.8 (aromatic C), 68.9, 68.6 (CH_2O), 21.6 (CH_3); EI *m/z*: 414.4 (M^+, 19.0%).

1-Azido-5-(*p*-toluenesulfonyloxy)-3-oxapentane (121)

To a solution of compound **120** (4.14 g, 10 mmol) in 95% ethanol (20 mL) was added sodium azide (0.65 g, 10 mmol). The mixture was refluxed for 2 h. The solution was concentrated under vacuum. The mixture was then diluted with dichloromethane (50 mL) and washed with brine. The organic phase was dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography using hexane/EtOAc

(3:1, v/v) as eluent to give compound **121** (1.30 g, 46%): ^{1}H NMR ($CDCl_3$) δ 7.64 (d, *J* 8.4 Hz, 2 H, aromatic H), 7.22 (d, 2 H, aromatic H), 4.02 (t, *J* 4.7 Hz, 2 H, CH_2), 3.54 (t, 2 H, CH_2), 3.44 (t, 2 H, CH_2), 3.15 (t, 2 H, CH_2), 2.29 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 144.5, 132.2, 129.4, 127.3 (aromatic C), 69.4, 68.9, 67.9, 50.0 (CH_2), 21.0 (CH_3); EI *m/z*: 286.0 (M^++1, 7.5%).

25,27-Bis-2-(2-azidoethoxy)ethoxy-26,28-dihydroxy-*p-tert*-butylcalix[4]arene (122)

p-Tert-butylcalix[4]arene (1.30 g, 2.0 mmol) was first treated with anhydrous potassium carbonate (1.10 g, 8.0 mmol) in acetonitrile (30 mL) for preliminary deprotonation for 2 h at room temperature. Then, the tosylate **121** (2.28 g, 8.0 mmol) was added and the reaction mixtures were refluxed for additional 24 h. The mixture was filtered. The clear filtrate was concentrated and diluted with CH_2Cl_2 (40 mL). The organic layer was washed successively with 5 % HCl and brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography using EtOAc-hexane (1:3,v/v) as eluent to give the desired dialkylated product **122** (1.02 g, 58%) as white crystals: ^{1}H NMR ($CDCl_3$) δ 7.52 (s, 2 H, OH), 7.14 (s, 4 H, aromatic H), 6.94 (s, 4 H, aromatic H), 4.45 (d, *J* 12.9 Hz, 4 H, Ha of CH_2), 4.24 (unresolved t, 4 H, CH_2), 4.06 (unresolved t, 4 H, CH_2), 3.95 (t, *J* 5.1 Hz, 4 H, CH_2), 3.55 (t, *J* 5.1 Hz, 4 H, CH_2), 3.40 (d, *J* 12.9 Hz, 4 H, Hb of CH_2), 1.37 (s, 18 H, t-Butyl), 1.09 (s, 18 H, t-Butyl); ^{13}C NMR ($CDCl_3$) δ 150.5, 149.5, 146.9, 141.3, 132.5, 127.5, 125.5 125.0 (aromatic C), 75.1, 70.1, 69.9, 50.8 (4xCH_2), 33.8, 33.7 ($\underline{C}Me_3$), 31.6 (CH_3), 31.5 ($ArCH_2Ar$), 31.0 (CH_3); FAB-MS (glycerol) *m/z*: 874.6 ([M-2]$^+$, 27.7%).

25,26,27,28-Tetrakis-2-(2-azidoethoxy)ethoxy-*p-tert*-butylcalix[4]arene (123)

To a solution of dialkylated compound **122** (400 mg, 0.46 mmol) in dry DMF (5 mL) was added sodium hydride (60% in oil, 109 mg, 2.73 mmol) at 0°C under N_2, and the mixture was stirred at 0°C for 20 min. Monotosylate azide **121** (520 mg, 1.82 mmol) was added to the reaction. The reaction was allowed to stir under N_2 at 0°C for 1h, and then at room temperature for 6 days. The reaction was quenched and diluted with water (20 mL). The product was extracted with dichloromethane (3x20 mL). The organic layer was dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography using EtOAc-hexane (1:3,v/v) as eluent to give the all-cone product **123**

(377 mg, 75%): ^{1}H NMR ($CDCl_3$) δ 6.78 (s, 8 H, aromatic H), 4.42 (d, *J* 12.6 Hz, 4 H, Ha of CH_2), 4.12 (t, *J* 5.5 Hz, 8 H, CH_2), 4.00 (t, *J* 5.5 Hz, 8 H, CH_2), 3.72 (t, *J* 5.0 Hz, 8 H, CH_2), 3.40 (t, *J* 5.0 Hz, 8 H, CH_2), 3.13 (d, *J* 12.6 Hz, 4 H, Hb of CH_2), 1.07 (s, 36 H, t-Butyl); ^{13}C NMR ($CDCl_3$) δ 153.0, 144.7, 133.7, 125.0 (aromatic C), 72.7, 70.2, 69.7, 50.8 (4xCH_2), 33.8 ($\underline{C}Me_3$), 31.3 (CH_3), 30.9 ($ArCH_2Ar$).

25,27-Bis-2-(2-aminoethoxy)ethoxy-26,28-dihydroxy-*p-tert*-butylcalix[4]arene (124)

To a solution of **122** (220 mg, 0.20 mmol) in methanol/EtOAc (10 mL, 1:1) was added 10% Pd/C (22 mg). H_2 was bubbled through the solution and the mixture was stirred for 1 h at room temperature. The mixture was filtered and the Pd/C was washed with MeOH. All of the filtrates were combined and concentrated under vacuum to give compound **124** (195 mg, 98 %). The compound **124** is readily used in the next step without further characterization.

25,26,27,28-Tetrakis-2-(2-aminoethoxy)ethoxy-*p-tert*-butylcalix[4]arene (125)

To a solution of **123** (170 mg, 0.15 mmol) in methanol/EtOAc (10 mL, 1:1) was added 10 % Pd/C (17 mg). H_2 was bubbled through the solution and the mixture was stirred for 1 h at room temperature. The mixture was filtered and the Pd/C was washed with methanol. All of the filtrates were combined and concentrated under vacuum to give compound **6** (152 mg, 99 %). The compound **125** is readily used in the next step without further characterization.

Methyl (4-Nitrophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (126)

To a solution of freshly prepared acetochloroneuraminic acid **41** (780 mg, 1.53 mmol) in CH_2Cl_2 (10 mL) was added a solution of *p*-nitrophenol (426 mg, 3.06 mmol) and TBAHS (520 mg, 1.53 mmol) in 1 M Na_2CO_3 solution (10 mL). The mixture was stirred vigorously for 1 h at room temperature. The organic phase was separated and washed with sat. $NaHCO_3$ solution followed by brine. The organic phase was dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2-CH_3OH (30:1, v/v) as eluent to give compound **126** (680 mg, 73%): m.p. 112-115 °C; $[\alpha]_D$

+21° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.09 (d, 2 H, *J* 8.8 Hz, aromatic H), 6.97 (d, 2 H, aromatic H), 5.55 (d, 1 H, $J_{5,NH}$ 9.9 Hz, NH), 5.30 (m, 2 H, H-7, H-8), 4.88 (m, 1 H, H-4), 4.40 (d, 1 H, $J_{5,6}$ 10.7 Hz, H-6), 4.21 (d, 1 H, $J_{9a,9b}$ 12.5 Hz, H-9a), 4.13-3.98 (m, 2 H, H-5, H-9b), 3.58 (s, 3 H, OMe), 2.65 (dd, 1 H, $J_{3e,4}$ 4.5 Hz, $J_{3a,3e}$ 12.9 Hz, H-3e), 2.16 (dd, 1 H, $J_{3a,4}$ 12.3 Hz, H-3a), 2.10, 2.07, 2.00, 1.99, 1.85 (5s, 15 H, NHAc, OAc's); FAB-MS (glycerol) *m/z*: 625.2 (M+1, 18.1%).

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 (127)

To a solution of freshly prepared acetochloroneuraminic acid **41** (5.0 g, 9.80 mmol) in EtOAc (100 mL) was added a solution of *p*-nitrothiophenol (1.83 g, 11.7 mmol) and TBAHS (3.33 g, 9.80 mmol) in 1 M Na_2CO_3 solution (100 mL). The mixture was stirred vigorously for 1 h at room temperature and next diluted with EtOAc (100 mL). The organic phase was separated and washed with sat. $NaHCO_3$ solution followed by brine. The organic phase was dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography using a gradient of hexane to EtOAc to give compound **127** (4.32 g, 70%): m.p. 166-172 °C; $[\alpha]_D$ +28.2° (*c* 1.0, $CHCl_3$). Lit.[155] m.p. 168.0-172.0 °C; $[\alpha]_D$ +27.6° (*c* 1.0, MeOH).

Methyl (Azido 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (128)

To a solution of freshly prepared acetochloroneuraminic acid **41** (1.50 g, 2.94 mmol) in CH_2Cl_2 (20 mL) was added a solution of sodium azide (0.38 g, 5.85 mmol) and TBAHS (1.00 g, 2.95 mmol) in 1 M Na_2CO_3 solution (20 mL). The mixture was stirred vigorously for 1 h at room temperature. The organic phase was separated and washed with sat. $NaHCO_3$ solution followed by brine. The organic phase was dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography using EtOAc as eluent to give compound **128** (1.31 g, 87%): m.p. 83-85 °C; $[\alpha]_D$ -27.2° (*c* 1.0, $CHCl_3$); Lit.[156] m.p. 84°C; $[\alpha]_D$ -26.5° (*c* 1.0, $CHCl_3$).

Methyl (4-Nitrobenzamido 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (129)

To a solution of compound **128** (530 mg, 1.03 mmol) in methanol (10 mL) was added 10% Pd/C (53 mg). H_2 was bubbled through the solution and the mixture was stirred for 2 h at room temperature. The mixture was filtered and the Pd/C was washed with methanol. All of the filtrates were combined and concentrated under vacuum to give the amino derivative quantitatively. To a solution of the amino derivative in dry pyridine (10 mL) was added 4-nitrobenzoyl chloride (380 mg, 2.05 mmol). The solution was stirred at room temperature for 1 h. Pyridine was evaporated under vacuum. The residue was diluted with dichloromethane (20 mL). The organic phase was washed successively with 5 % HCl, sat. $NaHCO_3$ solution, and brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2-CH_3OH (20:1,v/v) as eluent to give the desired product **129** (520 mg, 79%): m.p. 144-145 °C; $[\alpha]_D$ +45.2° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 9.17 (s, 1 H, NH), 8.22 (d, 2 H, *J* 8.9 Hz, aromatic H), 8.14 (d, 2 H, aromatic H), 6.35 (d, 1 H, $J_{5,NH}$ 10.3 Hz, NH), 5.46 (dd, 1 H, $J_{6,7}$ 2.0 Hz. $J_{7,8}$ 5.5 Hz, H-7), 5.18 (m, 1 H, H-8), 5.00 (m, 1 H, H-4), 4.97 (dd, 1 H, $J_{5,6}$ 10.2 Hz, H-6), 4.38 (dd, 1 H, $J_{8,9a}$ 2.1 Hz, $J_{9a,9b}$ 12.1 Hz, H-9a), 4.13 (ddd, 1 H, $J_{4,5}$ 10.5 Hz, H-5), 3.89 (dd, 1 H, $J_{8,9b}$ 7.2 Hz, H-9b), 3.78 (s, 3 H, OMe), 2.64 (dd, 1 H, $J_{3a,4}$ 12.5 Hz, H-3a), 2.57 (dd, 1 H, $J_{3e,4}$ 4.2 Hz, $J_{3a,3e}$ 12.8 Hz, H-3e), 2.08, 1.99, 1.96, 1.95 (5s, 15 H, NHAc, OAc's); ^{13}C-NMR ($CDCl_3$) δ 171.3-164.9 (C=O's), 149.9, 138.3, 129.3, 123.4 (aromatic C), 83.0 (C-2), 73.3 (C-6), 70.8 (C-8), 69.2 (C-4), 67.8 (C-7), 62.4 (C-9), 53.0 (OCH_3), 50.2 (C-5), 37.0 (C-3), 23.1 (NHAc), 20.9, 20.8, 20.7, 20.6 (OAc's).

Methyl (4-Isothiocyanatophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (130)

To a solution of compound **126** (250 mg, 0.41 mmol) in methanol (10 mL) was added 10 % Pd/C (25 mg). H_2 was bubbled through the solution and the mixture was stirred for 1 h at room temperature. The mixture was filtered and the Pd/C was washed with methanol. All of the filtrates were combined and concentrated under vacuum to give amino derivative quantitatively. The amino derivative was dissolved in CH_2Cl_2 (10 mL). Thiophosgene (48 μ L, 0.63 mmol) and DIPEA (213 μL, 1.2 mmol) were added and the solution was stirred at

room temperature for 1 h. The solvent was evaporated under vacuum. The residue was purified by silica gel column chromatography CH_2Cl_2-CH_3OH (30:1, v/v) as eluent to give compound **130** (204 mg, 80%): m.p. 128-131 °C; $[\alpha]_D$ +62.2° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.09 (d, 2 H, *J* 8.8 Hz, aromatic H), 6.97 (d, 2 H, aromatic H), 5.55 (d, 1 H, $J_{5,NH}$ 9.9 Hz, NH), 5.30 (m, 2 H, H-7, H-8), 4.88 (m, 1 H, H-4), 4.40 (d, 1 H, $J_{5,6}$ 10.7 Hz, H-6), 4.21 (d, 1 H, $J_{9a,9b}$ 12.5 Hz, H-9a), 4.13-3.98 (m, 2 H, H-5, H-9b), 3.58 (s, 3 H, OMe), 2.65 (dd, 1 H, $J_{3e,4}$ 4.5 Hz, $J_{3a,3e}$ 12.9 Hz, H-3e), 2.16 (dd, 1 H, $J_{3a,4}$ 12.3 Hz, H-3a), 2.10, 2.07, 2.00, 1.99, 1.85 (5s, 15 H, NHAc, OAc's); FAB-MS (glycerol) *m/z*: 625.2 (M+1, 18.1%).

Methyl (4-Isothiocyanatophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (131)

To *p*-nitrophenyl derivative **127** (250 mg, 0.40 mmol) in absolute ethanol (10 mL) was added tin (II) chloride dihydrate (450 mg). The reaction mixture was refluxed for 2 h and then cooled. The mixture was poured onto ice-water and the final pH adjusted to ~8 with sat. $NaHCO_3$ solution. The resulting mixture was filtered, and the clear filtrate was extracted with CH_2Cl_2. The organic layers were combined, washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The *p*-aminophenyl derivative was obtained as an off-white powder and used directly in subsequent reactions without further purification. *P*-Aminophenyl derivative was dissolved in CH_2Cl_2 (5 mL). Thiophosgene (46 μL, 0.60 mmol) and DIPEA (213 μL, 1.2 mmol) were added and the solution was stirred at room temperature for 1 h. The solvent was evaporated under vacuum. The residue was purified by silica gel column chromatography CH_2Cl_2-CH_3OH (30:1, v/v) as eluent to give compound **131** (215 mg, 84%): m.p. 125-130.1 °C; $[\alpha]_D$ +26.3° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.45 (d, 2 H, *J* 8.6 Hz, aromatic H), 7.16 (d, 2 H, aromatic H), 5.27-5.23 (m, 2 H, H-7, H-8), 5.10 (d, 1 H, $J_{5,NH}$ 9.9 Hz, NH), 4.83 (ddd, 1 H, $J_{4,5}$ 10.3 Hz, H-4), 4.31 (d, 1 H, H-9a), 4.11-3.96 (m, 1 H, H-9b), 3.90-3.78 (m, 2 H, H-5, H-6), 3.57 (s, 3 H, OMe), 2.80 (dd, 1 H, $J_{3e,4}$ 4.7 Hz, $J_{3a,3e}$ 12.8 Hz, H-3e), 2.02 (dd, 1 H, $J_{3a,4}$ 12.3 Hz, H-3a), 2.13, 2.04, 2.03, 1.99, 1.85 (5s, 15 H, NHAc, OAc's); ^{13}C NMR ($CDCl_3$) δ 170.8-167.5 (C=O's), 137.2, 137.1, 132.9, 126.0 (aromatic C), 127.9 (NCS), 87.3 (C-2), 74.5 (C-6), 69.4 (C-8), 69.2 (C-4), 67.5 (C-7), 62.7 (C-7), 60.3 (C-9), 52.8 (OCH_3), 49.3 (C-5), 38.1 (C-3), 23.1 (NHAc), 20.9, 20.7 (OAc's); FAB-MS (glycerol) *m/z*: 641.2 (M+1, 18.0%).

Methyl (4-Isothiocyanatobenzamido 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (132)

To a solution of compound **129** (150 mg, 0.23 mmol) in methanol (5 mL) was added 10% Pd/C (15 mg). H_2 was bubbled through the solution and the mixture was stirred for 1 h at room temperature. The mixture was filtered and the Pd/C was washed with methanol. All of the filtrates were combined and concentrated under vacuum to give the amino derivative quantitatively. The amino derivative was dissolved in CH_2Cl_2 (5 mL). Thiophosgene (27 μL, 0.34 mmol) and DIPEA (120 μL, 0.69 mmol) were added and the solution was stirred at room temperature for 1 h. The solvent was evaporated under vacuum and the residue was purified by silica gel column chromatography using EtOAc as eluent to give compound **132** (132 mg, 88%): m.p. 105-110 °C; $[\alpha]_D$ +32° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.76 (s, 1 H, NH), 7.96 (d, 2 H, *J* 8.5 Hz, aromatic H), 7.22 (d, 2 H, aromatic H), 6.03 (d, 1 H, $J_{5,NH}$ 10.4 Hz, NH), 5.43 (dd, 1 H, $J_{6,7}$ 2.0 Hz. $J_{7,8}$ 5.7 Hz, H-7), 5.16 (m, 1 H, H-8), 5.01 (m, 1 H, H-4), 4.89 (dd, 1 H, $J_{5,6}$ 10.7 Hz, H-6), 4.36 (dd, 1 H, $J_{8,9a}$ 2.3 Hz, $J_{9a,9b}$ 12.3 Hz, H-9a), 4.11 (ddd, 1 H, $J_{4,5}$ 10.5 Hz, H-5), 3.91 (dd, 1 H, $J_{8,9b}$ 6.9 Hz, H-9b), 3.77 (s, 3 H, OMe), 2.60-2.55 (m, 2 H, H-3a, H-3e), 2.09, 2.02, 2.00, 1.97, 1.96, 1.94 (5s, 15 H, NHAc, OAc's); FAB-MS (glycerol) *m/z*: 652.2 (M+1, 5.7%).

Calix[4]arene dimer with *O*-Neu5Ac (133)

To a solution of amine terminated divalent calixarene **124** (30 mg, 42.4 μmol) in CH_2Cl_2 (5 mL) were added isothiocyanate derivative **130** (57 mg, 91.3 μmol) and 1 drop of DIPEA. The solution was stirred at room temperature for 2 h and concentrated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2-CH_3OH (30:1, v/v) as eluent to give compound **133** (55 mg, 73%): m.p. 105-107 °C; $[\alpha]_D$ +52° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.62 (s, 2 H, OH), 7.13 (d, 4 H, *J* 8.7 Hz, aromatic H), 7.02 (d, 2 H, *J* 2.1 Hz, Ar-H of calix.), 7.01 (d, 2 H, *J* 2.1 Hz, Ar-H of calix.), 6.93 (d, 4 H, aromatic H), 6.72 (s, 4 H, aromatic H), 5.61 (d, 2 H, $J_{5,NH}$ 10.0 Hz, NH), 5.31 (dd, 2 H, $J_{6,7}$ 1.7 Hz. $J_{7,8}$ 8.3 Hz, H-7), 5.28 (m, 2 H, H-8), 4.92 (ddd, 2 H, $J_{4,5}$ 10.5 Hz, H-4), 4.30 (dd, 2 H, $J_{5,6}$ 11.0 Hz, H-6), 4.27-4.22 (m, 6 H, CHa, Hºa), 4.10-3.99 (m, 8 H, H-9b, H-5, CH_2), 3.88-3.82 (m, 12 H, CH_2), 3.61 (s, 6 H, OMe), 3.25 (d, *J* 12.9 hz, CHb), 2.66 (dd, 2 H, $J_{3e,4}$ 4.6, $J_{3a,3e}$ 12.8 Hz, H-

3e), 2.14 (dd, 2 H, $J_{3a,4}$ 12.5 Hz, H-3a), 2.07, 2.04, 1.98, 1.86 (5s, 30 H, NHAc, OAc's), 1.22 (s, 18 H, t-Butyl), 0.89 (s, 18 H, t-Butyl); ^{13}C-NMR ($CDCl_3$) δ 181.5 (C=S), 170.8-167.8 (C=O's), 151.2-120.9 (aromatic C), 100.1 (C-2), 75.2 (CH_2), 73.1(C-6), 69.9 (CH_2), 69.0 (CH_2, C-8), 68.8 (C-4), 67.2 (C-7), 61.9 (C-9), 52.9 (OCH_3), 49.3 (C-5), 45.1 (CH_2), 37.8 (C-3), 33.9, 33.8 ($\underline{C}Me_3$), 31.5 (CH_3), 31.4 ($ArCH_2Ar$), 30.9 (CH_3), 23.1 (NHAc), 20.9, 20.8, 20.7 (OAc's); FAB-MS (glycerol) *m/z*: 2073.9 (M+1, 1.6%).

Calix[4]arene dimer with *S*-Neu5Ac (134)

To a solution of amine terminated divalent calixarene **124** (35 mg, 36.3 μmol) in CH_2Cl_2 (5 mL) were added isothiocyanate derivative **131** (68 mg, 0.106 mmol) and 1 drop of DIPEA. The solution was stirred at room temperature for 2 h and concentrated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2-CH_3OH (30:1, v/v) as eluent to give compound **134** (72 mg, 81%): m.p. 118-120 °C; $[\alpha]_D$ +36° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.49 (bs, 2 H, OH), 7.34 (d, 4 H, *J* 8.5 Hz, aromatic H), 7.29 (d, 4 H, aromatic H), 7.05 (d, 2 H, *J* 2.3 Hz, Ar-H of calix.), 7.03 (d, 2 H, *J* 2.3 Hz, Ar-H of calix.), 6.70, 6.70 (2s, 4 H, aromatic H), 5.54 (d, 2 H, $J_{5,NH}$ 9.9 Hz, NH), 5.25 (dd, 2 H, $J_{6,7}$ 1.8 Hz. $J_{7,8}$ 7.6 Hz, H-7), 5.16 (m, 2 H, H-8), 4.83 (ddd, 2 H, $J_{4,5}$ 10.6 Hz, H-4), 4.33 (dd, 2 H, $J_{8,9a}$ 2.8 Hz, $J_{9a,9b}$ 12.5 Hz, H-9a), 4.23 (d, 2 H, *J* 13.3 Hz, CHa), 4.22 (d, 2 H, *J* 13.3 Hz, CHa'), 4.12 (dd, 2 H, $J_{8,9b}$ 5.3 Hz, H-9a), 4.08 (m, 4 H, CH_2), 3.94 (ddd, 2 H, $J_{5,6}$ 10.3 Hz, H-5), 3.92-3.82 (m, 14 H, H-6, 3 x CH_2), 3.54 (s, 6 H, OMe), 3.28 (d, 2 H, CHb), 3.24 (d, 2 H, CHb'), 2.75 (dd, 2 H, $J_{3e,4}$ 4.7, $J_{3a,3e}$ 12.8 Hz, H-3e), 2.09, 2.00, 1.98, 1.82 (s, 30 H, NHAc, OAc's), 1.26 (s, 18 H, t-Butyl), 0.87 (s, 18 H, t-Butyl); ^{13}C NMR ($CDCl_3$) δ 181.0 (C=S), 170.8-167.8 (C=O's), 149.3-123.7 (aromatic C), 87.3 (C-2), 75.2 (CH_2), 74.4 (C-6), 69.9 (CH_2), 69.7(C-4), 69.6 (C-8), 68.7 (CH_2), 67.4 (C-7), 61.8 (C-9), 52.7 (OCH_3), 49.2 (C-5), 45.1 (CH_2), 37.9 (C-3), 33.9, 33.8 ($\underline{C}Me_3$), 31.6 (CH_3), 31.3 ($ArCH_2Ar$), 30.9 (CH_3), 23.0 (NHAc), 21.0, 20.8, 20.7 (OAc's); FAB-MS (glycerol) *m/z*: 2104.9 (M^+, 0.4%).

Calix[4]arene dimer with *NHCO*-Neu5Ac (135)

To a solution of amine terminated divalent calixarene **124** (30 mg, 42.4 μmol) in CH_2Cl_2 (5 mL) were added isothiocyanate derivative **132** (60 mg, 92.1 μmol) and 1 drop of DIPEA. The solution was stirred at room temperature for 2 h and concentrated under vacuum.

The residue was purified by silica gel column chromatography using CH_2Cl_2-CH_3OH (20:1, v/v) as eluent to give compound **135** (64 mg, 83%): m.p. 123-125 °C; $[\alpha]_D$ +45.2° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.54 (bs, 2 H, NH), 7.77 (d, 4 H, *J* 8.3 Hz, aromatic H), 7.59 (s, 2 H, OH), 7.46 (d, 4 H, aromatic H), 7.07 (s, 4 H, Ar-H of calix), 6.41 (s, 4 H, Ar-H of calix), 5.94 (bs, 2 H, NH), 5.40 (d, 2 H, $J_{7,8}$ 5.3 Hz, H-7), 5.19 (m, 2 H, H-8), 5.02 (m, 2 H, H-4), 4.67 (d, 2 H, $J_{5,6}$ 8.2 Hz, H-6), 4.38 (d, 2 H, $J_{9a,9b}$ 10.6 Hz, H-9a), 4.25 (d, 2 H, CHa), 4.23 (d, 2 H, CHa'), 4.17-3.94 (m, 8 H, H-5, CH_2, H-9b), 3.85-3.77 (m, 12 H, 3 x CH_2), 3.75 (s, 6 H, OMe), 3.30 (d, 2 H, *J* 12.3 Hz, CHb), 3.27 (d, 2 H, *J* 13.3 Hz, CHb'), 2.70 (bs, 2 H, H-3a), 2.37 (bs, 2 H, H-3e), 2.07, 2.06, 1.97, 1.88 (s, 30 H, NHAc, OAc's), 1.27 (s, 18 H, t-Butyl), 0.87 (s, 18 H, t-Butyl); ^{13}C-NMR ($CDCl_3$) δ 180.8 (C=S), 170.9-166.3 (C=O's), 149.1-122.5 (aromatic C), 83.4 (C-2), 75.2 (CH_2), 73.2(C-6), 70.5 (C-8), 70.0 (CH_2), 69.4 (C-4), 68.4 (CH_2), 67.9 (C-7), 62.4 (C-9), 52.9 (OCH_3), 49.8 (C-5), 45.1 (CH_2), 37.5 (C-3), 33.9, 33.9 ($\underline{C}Me_3$), 31.6 (CH_3), 31.2 ($ArCH_2Ar$), 30.8 (CH_3), 23.0 (NHAc), 20.9, 20.8, 20.8, 20.7 (OAc's).

Methyl 3,5-dihydroxybenzoate (137)

To a mixture of 3,5-dihydroxybenzoic acid (**136**) (10 g, 65 mmol) and methanol (125 mL) was slowly added thionyl chloride (7.11 mL, 98 mmol) at 0°C. The solution was allowed to reach to room temperature and then refluxed for 2 h. TLC showed the starting material to be completely transformed (CH_2Cl_2/MeOH, 15:1). The solution was evaporated under vacuum to afford compound **137** as white crystals (10.83g, 99%): m.p. 165-169 °C; Lit.[207] m.p. 167-170 °C.

Methyl 3,5-bis-[2-(2-azidoethoxy)ethoxy]benzoate (138)

A solution of diol ester **137** (2.00 g, 12 mmol), 2-(2-azidoethoxy)ethyl tosylate (**121**) (8.55 g, 30 mmol), and potassium carbonate (4.97 g, 36 mmol) in dry acetonitrile (50 mL) was refluxed overnight. The solution was filtered and the filtrate was evaporated under reduced pressure. The residue was purified by silica gel chromatography using hexane-ethyl acetate (3:1, v/v) as eluent to afford compound **138** (4.12 g, 88 %); ^{1}H NMR ($CDCl_3$) δ 7.17 (d, 2 H, *J* 2.4 Hz, aromatic H), 6.68 (t, 1 H, aromatic H), 4.03 (m, 4 H, CH_2), 3.86 (s, 3 H, OMe), 3.83 (t,

4 H, *J* 4.4 Hz, CH_2), 3.71 (t, 4 H, *J* 5.2 Hz, CH_2), 3.38 (t, *J* 5.2 Hz, 4 H, CH_2); ^{13}C NMR ($CDCl_3$) δ 166.5 (C=O), 159.5, 131.8, 107.9, 106.7 (aromatic C), 70.1, 69.4, 67.6 (CH_2), 52.1 (OMe), 50.5 (CH_2); CI *m/z*: 385.0 (M^++1, 12.6%).

3,5-Bis-[2-(2-azidoethoxy)ethoxy]benzonic acid (139)

To a solution of compound **138** (450 mg, 1.14 mmol) in ethanol (10 mL) was added 1 M KOH solution. The solution was refluxed for 2 h. TLC (CH_2Cl_2/CH_3OH, 20/1) showed the completion of the reaction. The solution was neutralized with H^+ resin. The mixture was filtered and washed with methanol. All of the filtrates were combined and concentrated under vacuum to afford compound **139** quantitatively: ^1H NMR ($CDCl_3$) δ 10.62 (bs, 1 H, COOH), 7.23 (d, 2 H, *J* 2.3 Hz, aromatic H), 6.72 (t, 1 H, aromatic H), 4.13 (m, 4 H, CH_2), 3.83 (m, 4 H, CH_2), 3.71 (t, 4 H, CH_2), 3.38 (t, *J* 5.2 Hz, 4 H, CH_2); ^{13}C NMR ($CDCl_3$) δ 171.0 (C=O), 159.4, 130.8, 108.2, 107.3 (aromatic C), 69.8, 69.1, 67.4, 50.2 (CH_2); CI *m/z*: 362.9 (M^+-OH, 56%).

Methyl 3,5-bis-[2-(2-aminoethoxy)ethoxy]benzoate (140)

To a solution of compound **138** (0.5 g, 1.27 mmol) in methanol (10 mL) was added 10 % Pd/C (50 mg). H_2 was bubbled through the solution and the mixture was stirred for 2h at room temperature. The completeness of the reaction was estimated from the IR spectrum of the compound which showed the absence of any residual azide peak at about 2100 cm^{-1}. The mixture was filtered and the Pd/C was washed with methanol. All of the filtrates were combined and concentrated under vacuum to give compound **140** quantitatively. The compound **140** is readily used in the next step without further characterization.

Methyl 3,5-bis-{5-[3,5-bis-(5-azido-3-oxapentyloxy)-benzamido]-3-oxapentyloxy}-benzoate (141)

A solution of bisazido acid **139** (830 mg, 2.18 mmol) and HOBT (300 mg, 2.22 mmol) in DMF (10 mL) was stirred for 10 min. Bisamino ester **140** (300 mg, 0.88 mmol), EDC (420 mg, 2.19 mmol), and DIPEA (370 μL, 2.18 mmol) were added and the solution was stirred at room temperature for 3 h. The solution was diluted with dichloromethane (50

[207] *Aldrich* catalog.

mL), and washed successively with 5 % HCl and brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2-CH_3OH (40:1,v/v) as eluent to give compound **141** (780 mg, 83 %): ^1H NMR ($CDCl_3$) δ 7.14 (d, 2 H, *J* 2.2 Hz, aromatic H), 6.89 (d, 4 H, *J* 2.2 Hz, aromatic H), 6.69 (bs, 2 H, NH), 6.61 (t, 1 H, aromatic H), 6.57 (t, 2 H, aromatic H), 4.09-3.58 (m, 40 H, CH_2), 3.84 (s, 3 H, OMe), 3.35 (t, *J* 4.9 Hz, 8 H, CH_2); ^{13}C NMR ($CDCl_3$) δ 166.9, 166.3 (C=O's), 159.4, 159.3, 136.2, 131.5, 107.7, 106.4 (aromatic C), 69.8, 69.5, 69.1, 69.0, 67.4, 67.3 (CH_2),51.9 (OMe), 50.2 (CH_2N_3), 39.5 ($NHCO\underline{C}H_2$); FAB-MS for $C_{46}H_{62}N_{14}O_{16}$: 1068.0 (M+1, 27.2 %).

Methyl 3,5-bis-{5-[3,5-bis-(5-amino-3-oxapentyloxy)-benzamido]-3-oxapentyloxy}-benzoate (142)

To a solution of compound **141** (60 mg, 0.056 mmol) in methanol (5 mL) was added 10% Pd/C (6 mg). H_2 was bubbled through the solution and the mixture was stirred for 2h at room temperature. The completeness of the reaction was estimated from the IR spectrum of the compound which showed the absence of any residual azide peak at about 2100 cm^{-1}. The mixture was filtered and the Pd/C was washed with methanol. All filtrated were combined and concentrated under vacuum to give compound **142** quantitatively. The compound **142** is readily used in the next step without further characterization.

1,9-Bis-[3,5-bis-(5-azido-3-oxapentyloxy)-benzamido]-3,6-dioxanonane (143)

A solution of bisazido acid **139** (100 mg, 0.26 mmol) and HOBT (35 mg, 0.26 mmol) in CH_3CN (2 mL) was stirred for 10 min. 2,2'-(Ethylenedioxy)bis(ethylamine) (14.6 μL, 0.10 mmol), EDC (50 mg, 0.26 mmol), and DIPEA (45 μL, 0.26 mmol) were added and the solution was stirred at room temperature for 5 h. The solution was concentrated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2-CH_3OH (30:1,v/v) as eluent to give compound **143** (84 mg, 97%): ^1H NMR ($CDCl_3$) δ 6.90 (d, 4 H, *J* 2.2 Hz, aromatic H), 6.75 (bs, 2 H, NH), 6.57 (t, 2 H, aromatic H), 4.09 (t, 8 H, *J* 4.5 Hz, CH_2), 3.80-3.62 (m, 28 H, CH_2), 3.36 (t, 8 H, *J* 5.0 Hz, CH_2); ^{13}C NMR ($CDCl_3$) δ 167.0 (C=O), 159.3, 136.5, 105.8, 104.5 (aromatic C), 70.2, 69.7, 69.5, 67.5, 50.5, 39.6 (CH_2); FAB-MS for $C_{36}H_{52}N_{14}O_{12}$: 873.3 (M+1, 2.8 %).

1,9-Bis-[3,5-bis-(5-amino-3-oxapentyloxy)-benzamido]-3,6-dioxanonane (144)

To a solution of compound **143** (30 mg, 0,034 mmol) in methanol (5 mL) was added 10% Pd/C (3 mg). H_2 was bubbled through the solution and the mixture was stirred for 2h at room temperature. The completeness of the reaction was estimated from the IR spectrum of the compound which showed the absence of any residual azide peak at about 2100 cm^{-1}. The mixture was filtered and the Pd/C was washed with methanol. All of the filtrates were combined and concentrated under vacuum to give compound **144** quantitatively. The compound **144** is readily used in the next step without further characterization.

Methyl 3,4,5-tris-[2-(2-azidoethoxy)ethoxy]benzoate (146)

A solution of triol ester **145** (300 mg, 1.63 mmol), 2-(2-azidoethoxy)ethyl tosylate **121** (1.95 g, 6.85 mmol), and potassium carbonate (0.95 g, 6.85 mmol) in dry acetonitrile (10 mL) was refluxed for 24 h. The solution was filtered and the filtrate was evaporated under reduced pressure. The residue was purified by silica gel chromatography using hexane-ethyl acetate (3:2, v/v) as eluent to afford compound **146** (0.76 g, 89%); ^1H NMR ($CDCl_3$) δ 7.21 (s, 2 H, aromatic H), 4.15 (m, 6 H, CH_2), 3.78 (s, 3 H, OMe), 3.76 (m, 6 H, CH_2), 3.64 (t, 6 H, *J* 5.4 Hz, CH_2), 3.39 (t, 6 H, *J* 5.0 Hz, CH_2); ^{13}C NMR ($CDCl_3$) δ 166.1 (C=O), 151.9, 142.0, 124.7, 108.5 (aromatic C), 72.0, 70.3, 69.8, 69.5, 69.3, 68.5 (CH_2), 51.9 (OMe), 50.4 (CH_2); CI-MS (m/z) for $C_{20}H_{29}N_9O_8$: 523.0 (M+, 3.0%).

Methyl 3,4,5-tris-[2-(2-aminoethoxy)ethoxy]benzoate (147)

To a solution of compound **146** (50 mg, 0.096 mmol) in methanol (2 mL) was added 10% Pd/C (5 mg). H_2 was bubbled through the solution and the mixture was stirred for 2h at room temperature. The completeness of the reaction was estimated from the IR spectrum of the compound which showed the absence of any residual azide peak at about 2100 cm^{-1}. The mixture was filtered and the Pd/C was washed with methanol. The mixture was filtered and the Pd/C was washed with methanol. All of the filtrates were combined and concentrated under vacuum to give compound **147** (41 mg, 96 %). The compound **147** is readily used in the next step without further characterization.

(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,6-di-*O*-benzyol-β-D-glucopyranosyl isothiocyanate (148)

To a solution of GM_3 trisaccharide **68** (150 mg, 0.13 mmol) in methanol (5 mL) was added 10% Pd/C (15 mg). H_2 was bubbled through the solution and the mixture was stirred for 3h at room temperature. The mixture was filtered and the Pd/C was washed with methanol. All of the filtrates were combined and concentrated under vacuum to give compound quantitatively. The GM_3 amine derivative was used directly into the next step without further purification. To the amine in dry dichloromethane (5 mL) were added thiophosgene (15 μL, 0.20 mmol) and DIPEA (66 μL, 0.40 mmol). The solution was stirred for 1 h at room temperature. TLC (CH_2Cl_2/MeOH, 20:1) showed the reaction was complete. The solution was concentrated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2-CH_3OH (20:1,v/v) as eluent to give compound **148** (125 mg, 82 %): m.p. 105-107°C; $[\alpha]_D$ +46.3° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.06-7.26 (m, 15 H, aromatic), 5.33-5.25 (m, 3 H, NH, H-8", H-7"), 5.21 (dd, 1 H, $J_{2,3}$ 9.3 Hz, H-2), 4.97 (m, 1 H, H-4"), 4.94 (dd, 1 H, $J_{5,6a}$ 1.3 Hz, $J_{6a,6b}$ 12.1 Hz, H-6a), 4.74 (dd, 1 H, $J_{5',6a'}$ 2.7 Hz, $J_{6a',6b'}$ 12.0 Hz, H-6a'), 4.73 (d, 1 H, $J_{1,2}$ 9.3 Hz, H-1), 4.57 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.53 (dd, 1 H, $J_{5,6b}$ 5.7Hz, H-6b), 4.34 (dd, 1 H, $J_{5',6b'}$ 8.0 Hz, H-6b'), 4.28 (dd, 1 H, $J_{8",9a"}$ 2.5 Hz, $J_{9a",9b"}$ 12.5 Hz, H-9a"), 4.14 (dd, 1 H, $J_{2',3'}$ 9.6 Hz, $J_{3',4'}$ 3.1 Hz, H-3'), 4.10 (d, 1 H, $J_{5",6"}$ 10.7 Hz, H-6"), 3.97 (dd, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 3.94-3.88 (m, 4 H, H-5, H-5', H-5", H-9"), 3.79-3.73 (m, 5 H, OMe, H-4, H-2'), 3.70 (bs, 1 H, H-4'), 2.68 (dd, 1 H, $J_{3e",4"}$ 4.7, $J_{3a",3e"}$ 13.0 Hz, H-3e"), 2.13, 2.04, 2.01, 1.92, 1.88 (5s, 15 H, OAc's, NHAc), 2.00 (dd, 1 H, $J_{3a",4"}$ 12.1 Hz, H-3a"); ^{13}C NMR ($CDCl_3$) δ 170.8-165.3 (C=O's), 133.2-128.4 (aromatic C), 126.2 (NCS), 104.4 (C-1'), 97.5 (C-2"), 87.9 (C-1), 82.0 (C-4), 76.4 (C-3'), 75.0 (C-5'), 73.5 (C-3), 73.1 (C-6"), 72.9 (C-5), 72.5 (C-2), 69.0 (C-2'), 68.9 (C-8"), 68.2 (C-4"), 68.1 (C-4'), 67.0 (C-7"), 63.7 (C-6'), 63.2 (C-6), 62.4 (C-9"), 53.3 (OMe), 49.8 (C-5"), 37.7 (C-3"), 23.2 (NHAc), 21.1, 20.8, 20.7, 20.6 (OAc's); FAB-MS for $C_{54}H_{60}N_2O_{25}S$: 1110.2 (M-NCS, 0.2%).

Synthesis of protected divalent GM_3 ligand (149)

To a solution of bisamine ester **140** (5 mg, 15 μmol) in DMF (1 mL) were added GM_3 isothiocyanate **148** (44 mg, 38 μmol) and 1 drop of DIPEA. The solution was stirred at room temperature for 1 h. The solution was diluted with dichloromethane (15 mL), and washed successively with 5 % HCl and brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2-CH_3OH (25:1,v/v) as eluent to give compound **149** (32 mg, 82 %): m.p. 112-115°C; $[\alpha]_D$ +28.3° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.01-6.60 (m, 33 H, aromatic), 5.38 (d, 2 H, $J_{5,NH}$ 10.0 Hz, NH), 5.33-5.25 (m, 4 H, H-8", H-7"), 5.15 (dd, 2 H, $J_{1,2}$ 9.3, $J_{2,3}$ 9.3 Hz, H-2), 4.97 (m, 2 H, H-4"), 4.94 (dd, 2 H, $J_{6a,6b}$ 11.3 Hz, H-6a), 4.71 (dd, 2 H, $J_{5',6a'}$ 3.2 Hz, $J_{6a',6b'}$ 11.7 Hz, H-6a'), 4.67 (bs, 2 H, H-1), 4.50 (bs, 2 H, H-1'), 4.37 (d, 2 H, H-6b), 4.35 (d, 2 H, H-6b'), 4.26 (dd, 2 H, $J_{9a",9b"}$ 10.8 Hz, H-9a"), 4.11-4.08 (m, 4 H, H-3', H-6"), 4.01 (bs, 4 H, CH_2), 3.84 (s, 3 H, OMe), 3.74 (s, 6 H, OMe), 3.97-3.50 (m, 30 H, H-3, H-4, H-5, H-2', H-4', H-5', H-4", H-5", H-9", CH_2), 2.67 (dd, 2 H, $J_{3e",4"}$ 4.5, $J_{3a",3e"}$ 12.8 Hz, H-3e"), 2.09, 2.02, 2.01, 1.91, 1.85 (s, 30 H, OAc's, NHAc); ^{13}C NMR ($CDCl_3$) δ 182.0 (C=S), 170.6-166.2 (C=O's), 159.8-107.3 (aromatic C), 104.4 (C-1'), 97.6 (C-2"), 82.2 (C-1), 76.4 (C-3'), 73.7, 72.9, 72.8, 69.5, 68.7, 68.4, 68.0, 67.7, 66.9 (C-7"), 63.7 (C-6'), 63.5 (C-6), 62.3 (C-9"), 53.2 (OMe), 52.4 (OMe), 49.7 (C-5"), 37.5 (C-3"), 23.1 (NHAc), 21.1, 20.7, 20.7, 20.6 (OAc's).

Synthesis of deprotected divalent GM_3 ligand (150)

To compound **149** (25 mg, 9.3 μmol) was added 1 M NaOMe in MeOH (5 mL) for 8 h. Then water (5 mL) was added and the solution was stirred at room temperature for another 12 h. The solution was neutralized with H^+ resin and filtered. The filtrate was concentrated under vacuum and purified by size exclusion chromatography (P-2) using distilled water as eluent. The resulting solution was lyophilized to give compound **150** (13.6 mg, 87%):^1H NMR (D_2O) δ 7.26 (s, 2 H, aromatic H), 6.88 (s, 1 H, aromatic H), 4.60 (bs, 2 H, H-1'), 4.30 (s, 4 H, CH_2), 4.18 (dd, 2 H, $J_{2',3'}$ 9.8, $J_{3',4'}$ 3.1 Hz, H-3'), 4.04-3.55 (m, 48 H, CH_2 and GM_3 residues excluding H-1, H-1', H-3', H-3e", and H-3a"), 2.83 (dd, 2 H, $J_{3e",4"}$ 4.5, $J_{3a",3e"}$ 12.3 Hz, H-3e"), 2.11 (s, 6 H, NHAc), 1.89 (dd, 2 H, $J_{3a",4"}$ 12.1 Hz, H-3a"); ^{13}C NMR (D_2O) δ 174.6, 173.4 (C=O's), 158.7, 131.3, 108.2, 106.8 (aromatic C), 102.3 (C-1'), 99.4 (C-2"),

82.7 (C-1), 77.4, 75.6, 75.1, 74.7, 74.6, 72.4, 71.3, 68.9, 68.5, 68.1, 67.9, 67.7, 67.1, 67.0, 62.2 (C-9"), 60.6, 59.5 (C-6, C-6'), 51.3 (C-5"), 43.8 (CH_2), 39.2 (C-3"), 21.6 (NHAc).

Synthesis of protected trivalent GM_3 ligand (151)

To a solution of trisamine ester **147** (8 mg, 18 μmol) in DMF (2 mL) were added GM_3 isothiocyanate **148** (82 mg, 70 μmol) and 1 drop of DIPEA. The solution was stirred at room temperature for 2 h. The solution was diluted with dichloromethane (30 mL), and washed successively with 5 % HCl and brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2-CH_3OH (20:1,v/v) as eluent to give compound **151** (54 mg, 76%): m.p. 122-124°C; $[\alpha]_D$ +63.1° (*c* 1.0, $CHCl_3$); ^1H NMR δ 8.01-6.60 (m, 33 H, aromatic), 5.38 (d, 2 H, $J_{5,NH}$ 10.0 Hz, NH), 5.33-5.25 (m, 4 H, H-8", H-7"), 5.15 (dd, 2 H, $J_{1,2}$ 9.3, $J_{2,3}$ 9.3 Hz, H-2), 4.97 (m, 2 H, H-4"), 4.94 (dd, 2 H, $J_{6a,6b}$ 11.3 Hz, H-6a), 4.71 (dd, 2 H, $J_{5',6a'}$ 3.2 Hz, $J_{6a',6b'}$ 11.7 Hz, H-6a'), 4.67 (bs, 2 H, H-1), 4.50 (bs, 2 H, H-1'), 4.37 (d, 2 H, H-6b), 4.35 (d, 2 H, H-6b'), 4.26 (dd, 2 H, $J_{9a",9b"}$ 10.8 Hz, H-9a"), 4.11-4.08 (m, 4 H, H-3', H-6"), 4.01 (bs, 4 H, CH_2), 3.84 (s, 3 H, OMe), 3.74 (s, 6 H, OMe), 3.97-3.50 (m, 30 H, H-3, H-4, H-5, H-2', H-4', H-5', H-4", H-5", H-9", CH_2), 2.67 (dd, 2 H, $J_{3e",4"}$ 4.5, $J_{3a",3e"}$ 12.8 Hz, H-3e"), 2.09, 2.02, 2.01, 1.91, 1.85 (s, 30 H, OAc's, NHAc); ^{13}C NMR ($CDCl_3$) δ 182.0 (C=S), 170.6-166.2 (C=O's), 159.8-107.3 (aromatic C), 104.4 (C-1'), 97.6 (C-2"), 82.2 (C-1), 76.4 (C-3'), 73.7, 72.9, 72.8, 69.5, 68.7, 68.4, 68.0, 67.7, 66.9 (C-7"), 63.7 (C-6'), 63.5 (C-6), 62.3 (C-9"), 53.2 (OMe), 52.4 (OMe), 49.7 (C-5"), 37.5 (C-3"), 23.1 (NHAc), 21.1, 20.7, 20.7, 20.6 (OAc's).

Synthesis of deprotected trivalent GM_3 ligand (152)

To compound **151** (40 mg, 10 μmol) was added 1 M NaOMe in MeOH (5 mL) for 8 h. Then water (5 mL) was added and the solution was stirred at room temperature for another 12 h. The solution was neutralized with H^+ resin and filtered. The filtrate was concentrated under vacuum and purified by size exclusion chromatography (P-4) using distilled water as eluent. The resulting solution was lyophilized to give compound **152** (22.4 mg, 90%): ^1H NMR (D_2O) δ 7.36 (s, 2 H, aromatic H), 4.59 (bs, 3 H, H-1'), 4.36 (s, 6 H, CH_2), 4.19 (dd, 3 H, $J_{2',3'}$ 9.6, $J_{3',4'}$ 2.8 Hz, H-3'), 4.04-3.55 (m, 78 H, CH_2 and GM_3 residues excluding H-1, H-1', H-3', H-3e", and H-3a"), 2.83 (dd, 3 H, $J_{3e",4"}$ 4.6, $J_{3a",3e"}$ 12.5 Hz, H-3e"), 2.11 (s, 9 H,

NHAc), 1.89 (dd, 4 H, $J_{3a'',4''}$ 12.1 Hz, H-3a"); ^{13}C NMR (D_2O) δ 174.6, 173.4 (C=O's), 151.0, 138.6, 107.8 (aromatic C), 102.2 (C-1'), 99.4 (C-2"), 82.6 (C-1), 77.4, 75.5, 75.1, 75.0, 74.7, 74.6, 72.4, 71.7, 71.3, 69.6, 68.9, 68.3, 67.9, 67.7, 67.1, 62.2 (C-9"), 60.6, 59.5 (C-6, C-6'), 51.3 (C-5"), 43.9 (CH_2), 39.2 (C-3"), 21.6 (NHAc).

Synthesis of deprotected tetravalent GM_3 ligand (153)

To a solution of tetravalent amine derivative **142** (18 mg, 18.7 μmol) in DMF (5 mL) were added GM_3 isothiocyanate **148** (104.8 mg, 89.7 μmol) and 1 drop of DIPEA. The solution was stirred at room temperature for 1 h. The reaction mixture was concentrated under vacuum. To the residue was added 1 M NaOMe in MeOH (5 mL) for 8 h. Then water (5 mL) was added and the solution was stirred at room temperature for another 12 h. The solution was neutralized with H^+ resin and filtered. The filtrate was concentrated under vacuum and purified by size exclusion chromatography (P-4) using distilled water as eluent. The resulting solution was lyophilized to give compound **153** (43.8 mg, 64%): ^{1}H NMR (D_2O) δ 6.85 (bs, 6 H, aromatic H), 6.62 (bs, 3 H, aromatic H), 4.90 (d, 4 H, $J_{1,2}$ 9.3 Hz, H-1), 4.58 (d, 4 H, $J_{1',2'}$ 6.4 Hz, H-1'), 4.18 (d, 4 H, $J_{2',3'}$ 9.6 Hz, H-3'), 4.17-3.42 (m, 120 H, CH_2 and GM_3 residues excluding H-1, H-1', H-3', H-3e", and H-3a"), 2.83 (dd, 4 H, $J_{3e'',4''}$ 4.3, $J_{3a'',3e''}$ 12.2 Hz, H-3e"), 2.10 (s, 12 H, NHAc), 1.88 (dd, 4 H, $J_{3a'',4''}$ 12.0 Hz, H-3a"); ^{13}C NMR (D_2O) δ 174.6, 173.4 (C=O's), 158.7, 158.5, 135.0, 107.5, 105.7 (aromatic C), 102.3 (C-1'), 99.4 (C-2"), 82.7 (C-1), 77.5, 75.5, 75.0, 74.7, 74.6, 72.4, 71.3, 69.1, 68.9, 68.3, 67.9, 67.7, 67.0, 62.2 (C-9"), 60.6, 59.5 (C-6, C-6'), 51.3 (C-5"), 43.7 (CH_2), 39.2 (C-3"), 21.6 (NHAc).

Synthesis of deprotected tethered tetravalent GM_3 ligand (154)

To a solution of tethered tetravalent amine **144** (15 mg, 19.5 μmol) in DMF (5 mL) were added GM_3 isothiocyanate **148** (109.5 mg, 93.8 μmol) and 1 drop of DIPEA. The solution was stirred at room temperature for 1 h. The reaction mixture was concentrated under vacuum. To the residue was added 1 M NaOMe in MeOH (5 mL) for 8 h. Then water (5 mL) was added and the solution was stirred at room temperature for another 12 h. The solution was neutralized with H^+ resin and filtered. The filtrate was concentrated under vacuum and purified by size exclusion chromatography (P-4) using distilled water as eluent. The resulting solution was lyophilized to give compound **154** (39 mg, 58%): ^{1}H NMR (D_2O)

δ 6.94 (s, 4 H, aromatic H), 6.70 (s, 2 H, aromatic H), 4.58 (bs, 4 H, H-1'), 4.19-4.17 (m, 12 H, H-3', CH_2), 4.04-3.55 (m, 108 H, CH_2 and GM_3 residues excluding H-1, H-1', H-3', H-3e", and H-3a"), 2.83 (dd, 4 H, $J_{3e",4"}$ 4.6, $J_{3a",3e"}$ 12.5 Hz, H-3e"), 2.10 (s, 12 H, NHAc), 1.88 (dd, 4 H, $J_{3a",4"}$ 12.0 Hz, H-3a"); ^{13}C NMR (D_2O) δ 174.6, 173.4 (C=O's), 158.8, 135.1, 105.9, 104.6 (aromatic C), 102.3 (C-1'), 99.4 (C-2"), 82.7 (C-1), 77.5, 75.5, 75.0, 74.7, 74.6, 72.4, 71.3, 69.1, 68.9, 68.3, 67.9, 67.7, 67.1, 62.2 (C-9"), 60.5, 59.5 (C-6, C-6'), 51.3 (C-5"), 43.8 (CH_2), 39.1 (C-3"), 21.6 (NHAc).

Synthesis of deprotected PAMAM-based tetravalent GM_3 dendrimer (157)

To a solution of amine terminated tetravalent Starburst® PAMAM dendritic core **155** (G-0) (3 mg, 5.8 μmol) in dichloromethane (5 mL) were added GM_3 isothiocyanate **148** (35 mg, 30 μmol) and 1 drop of DIPEA. The solution was stirred at room temperature for 3 h. The reaction mixture was concentrated under vacuum. To the residue was added 1 M NaOMe in MeOH (5 mL) for 8 h. Then water (5 mL) was added and the solution was stirred at room temperature for another 12 h. The solution was concentrated under vacuum and purified by size exclusion chromatography (P-4) using distilled water as eluent. The resulting solution was lyophilized to give tetravalent PAMAM-based GM_3 dendrimer **157** (14.5 mg, 78%): ^1H NMR (D_2O) δ 4.62 (d, 4 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.19 (dd, 4 H, $J_{2',3'}$ 9.9, $J_{3',4'}$ 3.0 Hz, H-3'), 4.04-3.51 (m, 86 H, CH_2 of PAMAMA and GM_3 residues excluding H-1, H-1', H-3', H-3e", and H-3a"), 3.38-3.18 (m, 10 H, CH_2 of PAMAM), 2.82 (dd, 4 H, $J_{3e",4"}$ 4.4, $J_{3a",3e"}$ 12.4 Hz, H-3e"), 2.70 (m, 8 H, CH_2 of PAMAM), 2.11 (s, 12 H, NHAc), 1.88 (dd, 4 H, $J_{3a",4"}$ 12.1 Hz, H-3a").

Synthesis of deprotected PAMAM-based octavalent GM_3 dendrimer (158)

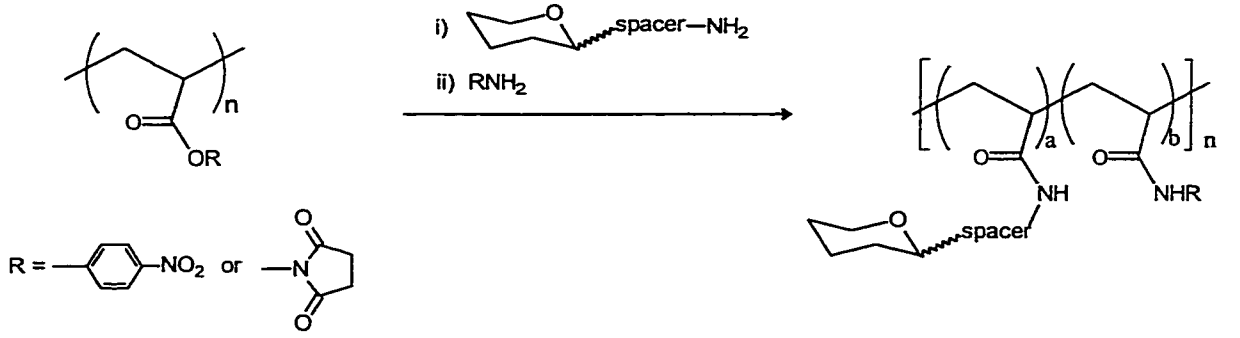
To a solution of amine terminated octavalent Starburst® PAMAM dendritic core **156** (G-1) (6 mg, 4.2 μmol) in DMF (5 mL) were added GM_3 isothiocyanate **148** (51 mg, 44 μ mol) and 1 drop of DIPEA. The solution was stirred at room temperature for 3 h. The reaction mixture was concentrated under vacuum. To the residue was added 1 M NaOMe in MeOH (5 mL) for 8 h. Then water (5 mL) was added and the solution was stirred at room temperature for another 12 h. The solution was concentrated under vacuum and purified by size exclusion chromatography (P-4) using distilled water as eluent. The resulting solution

was lyophilized to give octavalent PAMAM-based GM_3 dendrimer **158** (16 mg, 56%): ^1H NMR (D_2O) δ 4.62 (d, 8 H, $J_{1',2'}$ 7.6 Hz, H-1'), 4.19 (d, 8 H, $J_{2',3'}$ 9.9Hz, H-3'), 4.04-3.20 (m, 228 H, CH_2 of PAMAMA and GM_3 residues excluding H-1, H-1', H-3', H-3e", and H-3a"), 2.85-2.79 (m, 32 H, H-3e", CH_2 of PAMAM), 2.11 (s, 24 H, NHAc), 1.88 (dd, 8 H, $J_{3a",4"}$ 12.2, $J_{3a",3e"}$ 12.5 Hz, H-3a").

Chapter 7 Glycopolymers

7.1 Introduction

Glycopolymers are gaining increasing interest owing to their many advantageous physical and immunochemical properties over other forms of neoglycoconjugates. For instance, glycopolymers can be constructed with a wide range of molecular weights, any desired carbohydrate density, and functionality. They are inexpensive to produce in a large scale and their uniformity and quality are easy to control. They are also more stable to a wide pH range and microbial degradation. In addition, they have been shown to be non-toxic, and poorly or non-immunogenic. Moreover, the multivalency effect of glycopolymers by virtue of their numerously exposed carbohydrate moieties enhances the binding avidity toward their receptors.[208] Today, glycopolymers have been used not only in affinity chromatograph,[209,210] but also as potential candidates for drug carriers,[211] and as anti-inflammatory agents.[212]



Scheme 7.1 Synthesis of glycopolymers using grafting polymerization strategy

---

[208] Lee, Y. C.; Lee, R. T.; Rice, K.; Ichikawa, Y.; Wong, T.-C. *Pure Appl. Chem.* **1991**, *63*, 499.
[209] Pazur, J. H. *Adv. Carbohydr. Chem. Biochem.* **1981**, *39*, 405.
[210] Schnaar, R. L. *Anal. Biochem.* **1984**, *143*, 1.
[211] a) Seymour, L. W. *Adv. Drug. Deliv. Rev.* **1994**, *14*, 89; b) Molema, G.; Meijer, D. K. F. *Adv. Drug. Deliv. Rev.* **1994**, *14*, 25; c) Monsigny, M.; Roche, A.-C.; Midoux, P.; Mayer, R. *Adv. Drug. Deliv. Rev.* **1994**, *14*, 1.
[212] a) Spaltenstein, A.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 686; b) Sparks, M. A.; Williams, K. W.; Whitesides, G. M. *J. Med. Chem.* **1993**, *36*, 778.

One of the methods toward glycopolymers with predictable composition is grafting polymerization, in which the desired carbohydrate haptens can be directly incorporated into a pre-formed polymer bearing reactive functionalities. The most common ones by this approach involve reacting amine-containing carbohydrates with active esters of polyacrylates, such as poly(4-nitrophenyl acrylate) and poly(*N*-oxysuccinimidyl acrylate) (Scheme **7.1**). The major advantage of this approach is that pre-formed active polymers can be synthesized with the desired molecular weight. Therefore, the same basic polymer backbones used in grafting polymerization can avoid molecular weight variations. Furthermore, the desired carbohydrate content in glycopolymers can be easily achieved by controlling the initial ratio of carbohydrate haptens and pre-formed active polymers.

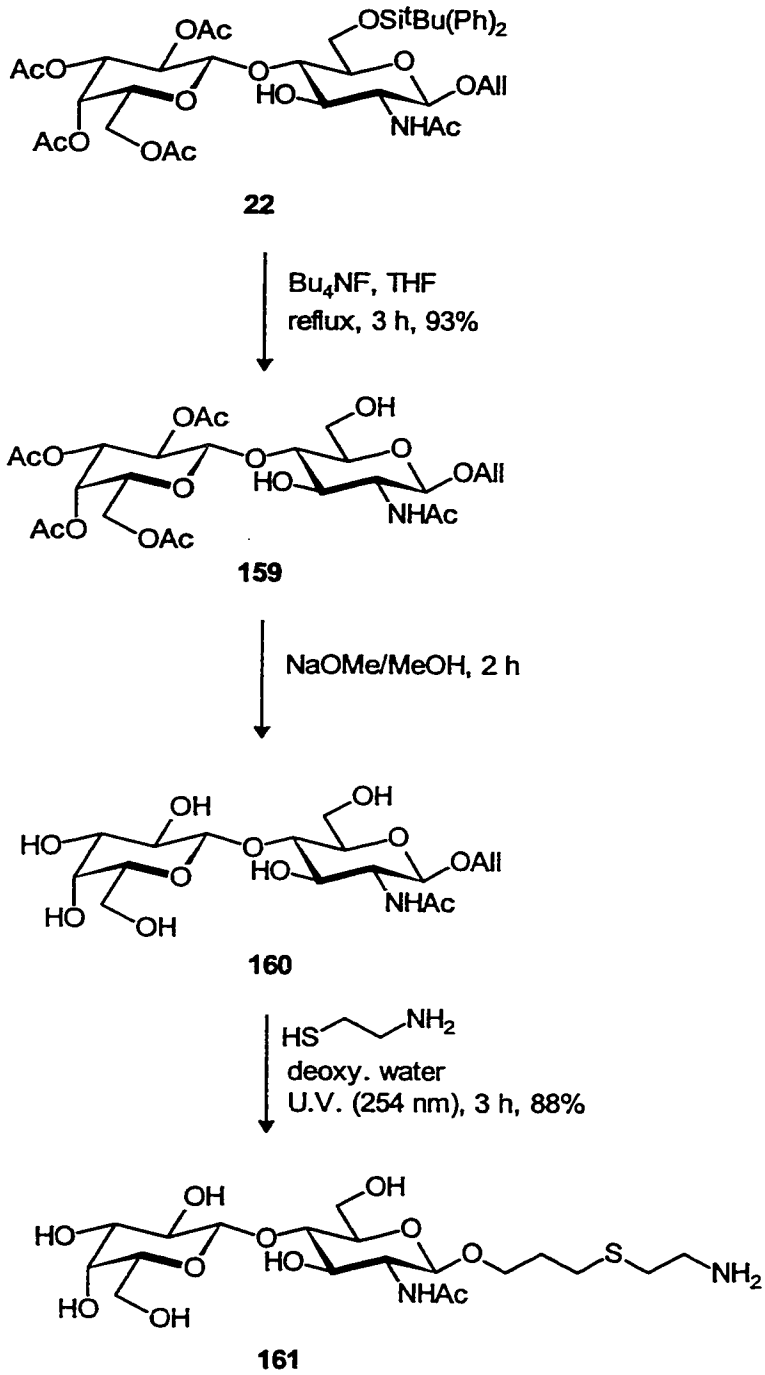
In the following sections, syntheses of lactosamine- and GM_3-containing glycopolymers using grafting polymerization strategy will be described.

7.2 Graft conjugation of *N*-acetyllactosamine to poly(*N*-oxysuccinimidyl acrylate)

N-Acetyllactosamine [Galβ(1→4)GlcNAc, LacNAc] is well known as a biologically important disaccharide core structure which has been found in glycoproteins and glycolipids, and is widely distributed in many different human and animal tissues.[76,77]

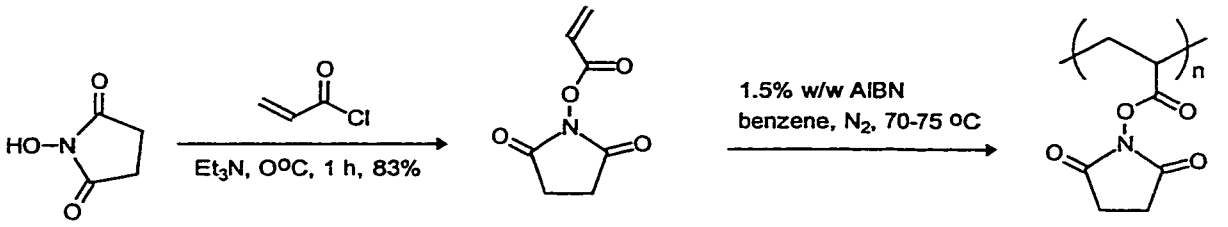
Allyl lactosamine derivative **22** was prepared as described in the Section **2.2**. Desilylation of compound **22** was performed using tetra-n-butylammonium fluoride in refluxing THF. However, the basic solution also caused partial deacetylation and made the purification more difficult. To avoid this problem, a small amount of acetic acid was carefully added into the solution to adjust the pH to about 7. If too much acetic acid was added, desilylation would be hampered due to lack of fluoride ion. Under such conditions, the cleavage of the silyl group occurred smoothly in 3 h and the 6-*O*-deprotected allyl lactosamine derivative **159** was obtained in 93% yield. Zemplén de-*O*-acetylation of compound **159** afforded compound **160** quantitatively. Allyl glycoside derivative **160** and cysteamine hydrochloride in deoxygenated water were irradiated with a U. V. developing

lamp operating at 254 nm for 3 h to generate the desired 3-(2-aminoethylthio)propyl lactosamine derivative **161** in 88% yield (Scheme **7.2**).



Scheme 7.2 Preparation of 3-(2-aminoethylthio)propyl lactosamine derivative **161**

With the suitable carbohydrate hapten in hand, poly(*N*-oxysuccinimidyl acrylate) was employed as a preactived polymer to prepare glycopolymers bearing the *N*-acetyllactosamine moiety. The polymer was prepared in our group, and its molecular weight was determined to be 42.1 kDa by measuring its intrinsic viscosity. The procedure for polymer synthesis is shown in Scheme **7.3**.



162

Scheme 7.3 Preparation of poly(*N*-oxysuccinimidyl acrylate) **162**

Poly(*N*-oxysuccinimidyl acrylate) **162** was stirred in DMF at room temperature until the solution became homogeneous. To this solution was added compound **161** in DMF. The resulting mixture was stirred overnight at room temperature. To increase the lipophilicity of the glycopolymers, propylamine was used as the quenching material. Thus an excess of propylamine was then directly added to quench the remaining active ester groups. The water-soluble lactosamine copolymer was purified by dialysis and freeze drying to give lactosamine copolymer **163** in 86% yield (Scheme **7.4**).

^{1}H NMR spectroscopy is a simple and effective tool to determine the carbohydrate content in glycopolymers. From the ^{1}H NMR spectrum of copolymer **163**, the ratio of the carbohydrate unit in the polymer was judged as 1:15 based on the relative integration of the anomeric proton at δ 5.3 ppm and methyl protons at δ 1.0 ppm (Figure **7.1**).

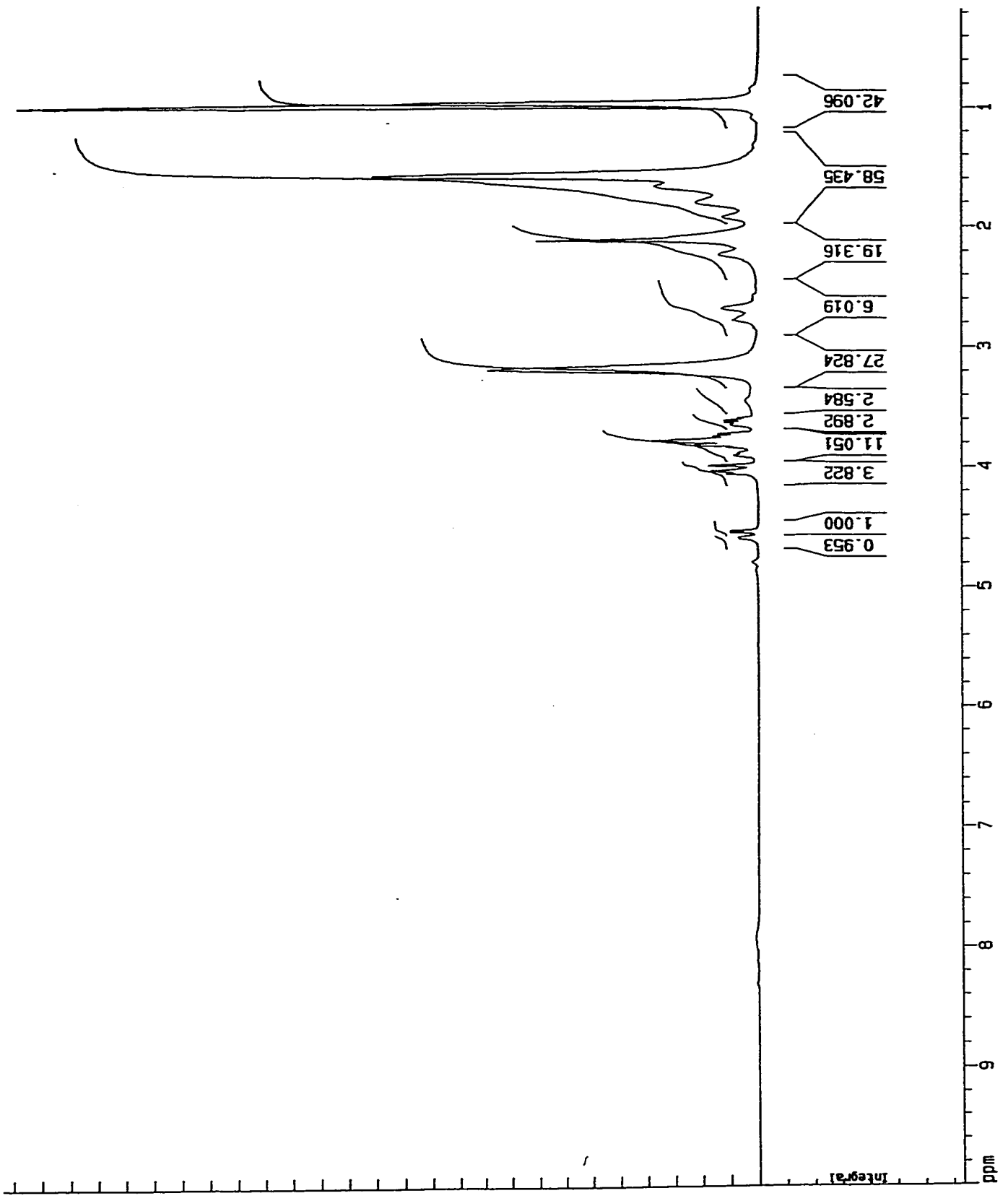
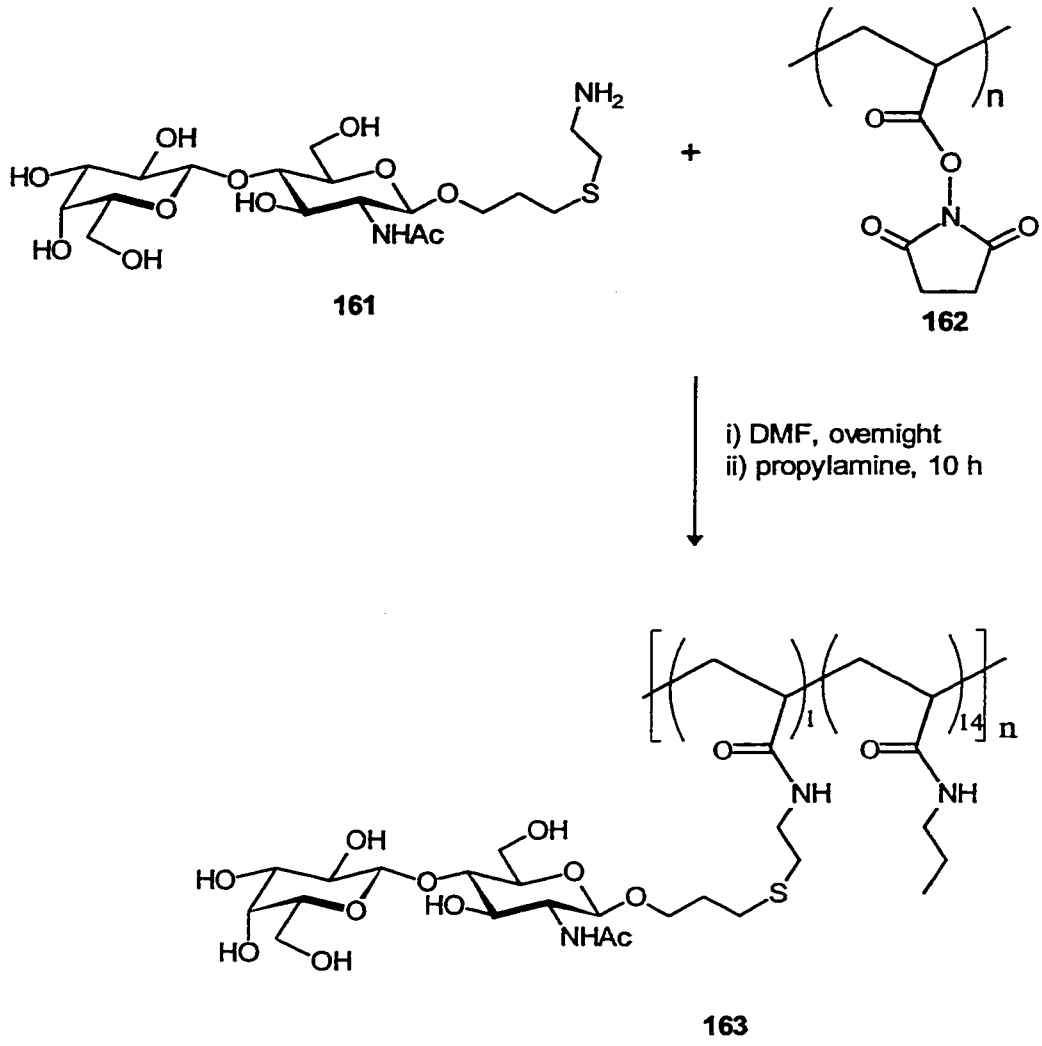


Figure 7.1 ^{1}H NMR (500MHz, D_2O) spectrum of lactosamine glycopolymer **163**



Scheme 7.4 Preparation of lipophilic lactosamine glycopolymer **163**

7.3 Graft conjugation of GM_3 trisaccharide to poly(*N*-oxysuccinimidyl acrylate)

Although ganlioside GM_3 and GM_3 trisaccharide were often chosen as targets for the evaluation of the present synthetic methodology, syntheses of water-soluble glycopolymers

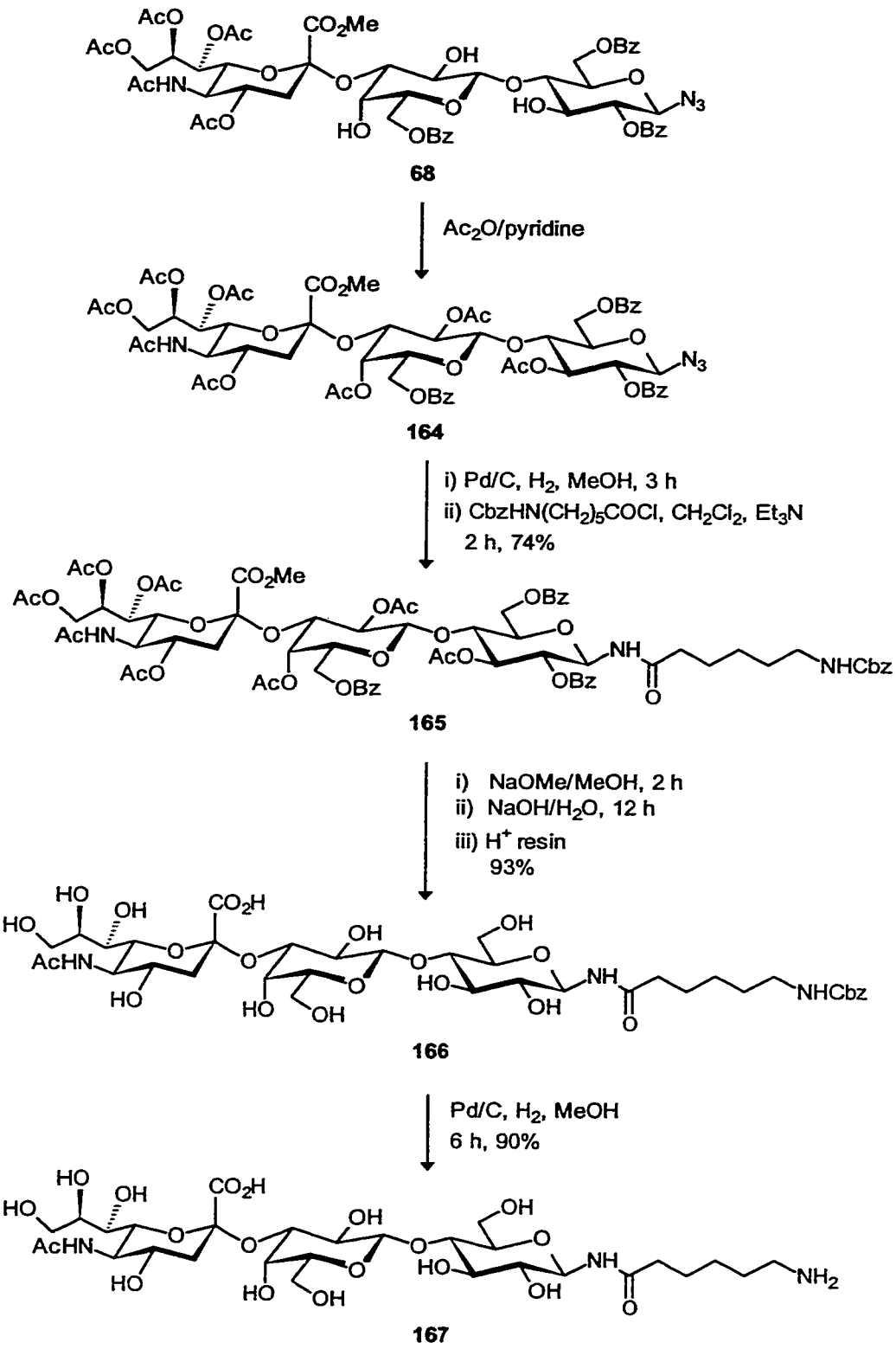
containing GM_3 trisaccharide units have rarely been reported.[213] Therefore, it's worth to apply grafting polymerization strategy to prepare GM_3 glycopolymer.

As shown in Scheme **7.5**, acetylation of GM_3 trisaccharide **68** in pyridine afforded fully protected trisaccharide **164** in quantitative yield. The azide derivative **164** was reduced by hydrogenation to give the amino derivative which was directly used in the next step. Treatment of the amino intermediate with freshly prepared *N*-(carbobenzyloxyamino)caproyl chloride in dichloromethane using triethylamine as base generated the key compound **165** in 74% yield. The preparation of unprotected GM_3 derivative **166** was completed by Zemplén de-*O*-acetylation (NaOMe, MeOH) and saponification (NaOH, H_2O). After purification by gel permeation chromatography (Biogel-P2, H_2O as eluent), compound **166** was obtained in 93%yield. Hydrogenolysis of the Cbz group of compound **166** afforded unprotected trisaccharide **167** with a primary amine spacer (10%Pd/C, H_2, MeOH, 6 h, 90%) (Scheme **7.5**).

Similar to the preparation of lactosamine glycopolymer **163**, the trisaccharide derivative **167** in DMF was added into a solution of poly(*N*-oxysuccinimidyl acrylate) **162** in DMF. After quenching with an excess of aqueous ammonium hydroxide and purifying by dialysis, water-soluble GM_3 glycopolymer **168** was obtained in 72% yield (Scheme **7.6**).

The incorporation ratio of GM_3 in this copolymer **168** is about 1:25, based on the relative integration of the ^1H NMR (D_2O) signals of the anomeric proton at δ 4.60 ppm to the backbone methylene and methine protons (δ 1.4-2.4 ppm) (Figure **7.2**).

[213] a) Cao, S.; Roy, R. *Tetrahedron Lett.* **1996**, *37*, 3421; b) Nishimura, S.-I.; Yamada, K. *J. Am. Chem. Soc.* **1997**, *119*, 10555; c) Kamitakahara, H.; Suzuki, T.; Nishigori, N.; Suzuki, Y.; Kanie, O.; Wong, C.-H. *Angew. Chem. Int. Ed.* **1998**, *37*, 1524.



Scheme 7.5 Preparation of GM_3 derivative **167**

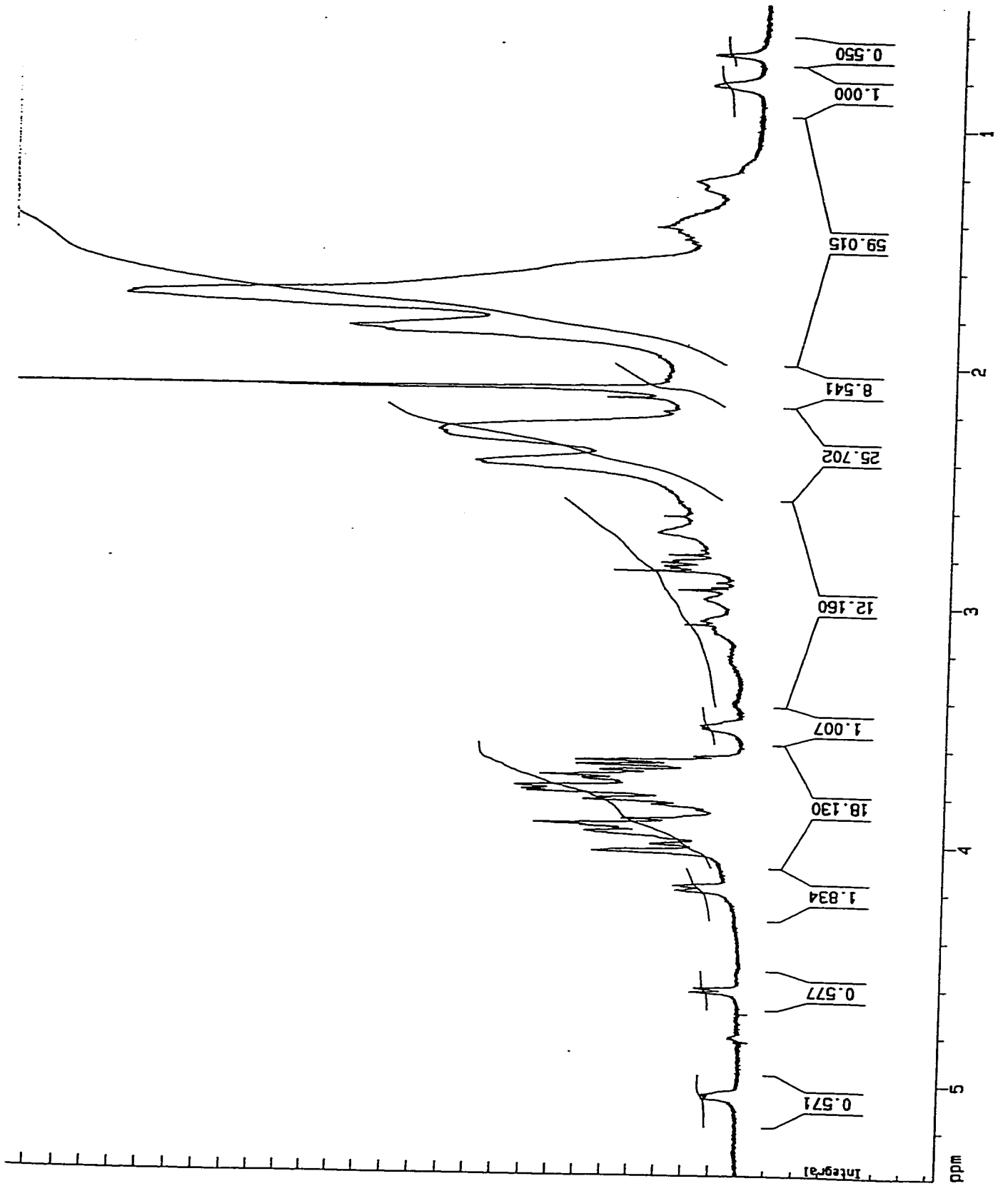
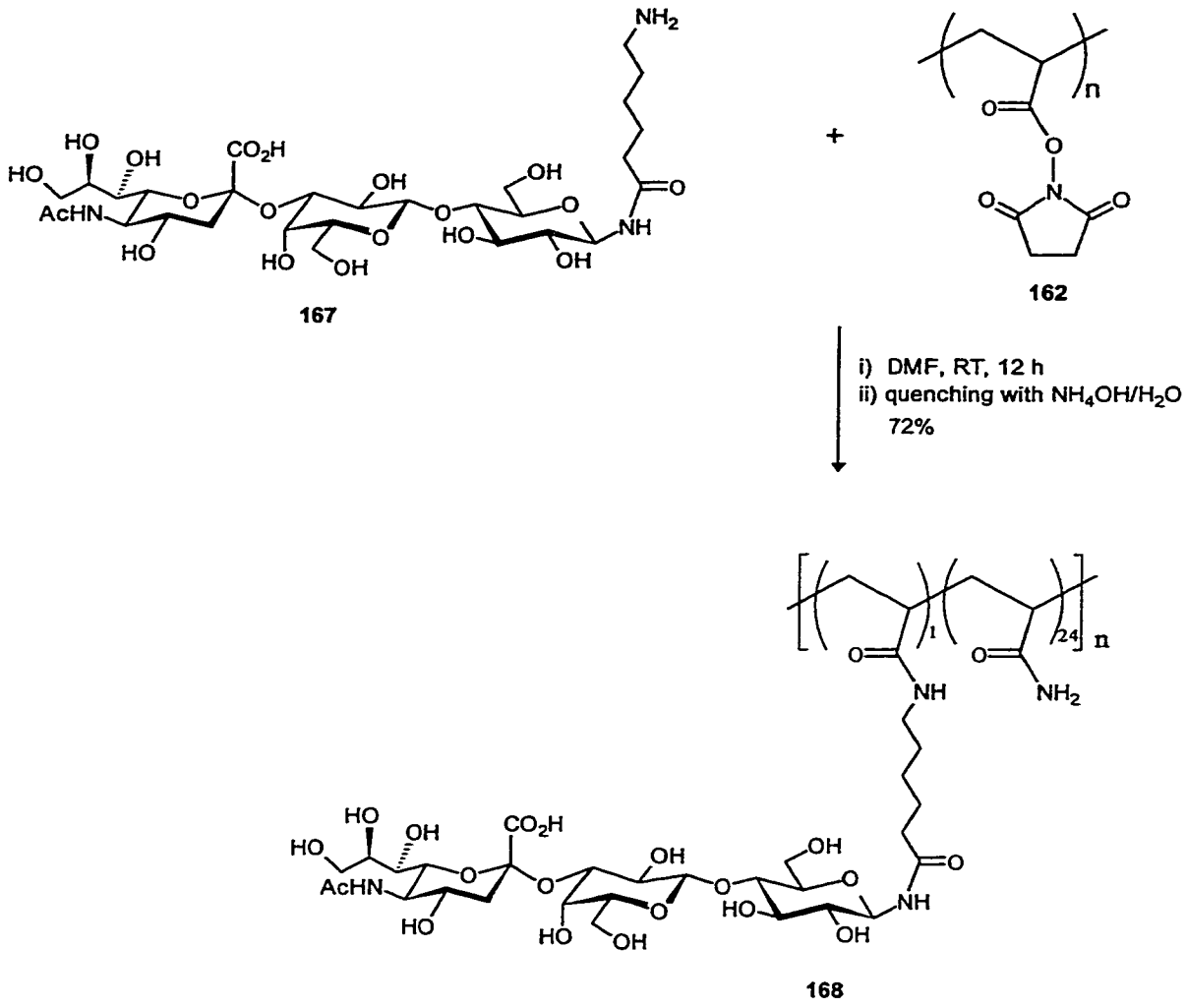


Figure 7.2 ^{1}H NMR (500MHz, D_2O) spectrum of GM_3 glycopolymer **168**



Scheme 7.6 Preparation of GM_3-containing glycopolymer **168**

7.4 Conclusion

Lactosamine- and GM_3-containing glycopolymers were successfully prepared by incorporating the corresponding amine-terminated glycosides into pre-formed active poly(*N*-oxysuccinimidyl acrylate). The grafting polymerization reactions are carried out under mild conditions and afford fair to good yields. One of the major advantages of this strategy is that the characteristics of the polymers such as chain length and polydispersity keep constant.

7.5 Experimental

Allyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (159)

To lactosamine derivative **22** (650 mg, 0.78 mmol) in THF (20 mL) was added 1 M tetra-n-butylammonium fluoride in THF solution (1.5 mL). Acetic acid was added to the solution until the pH of the solution was about 7. The solution was refluxed for 3 h. TLC (CH_3OH-CH_2Cl_2, 1:20) showed the reaction was complete. The solution was concentrated under reduced pressure. The syrupy residue was purified by silica gel chromatography using methanol-dichloromethane as eluent (1:20, v/v) to afford **159** (430 mg, 93%): $[\alpha]_D$ +0.5° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 6.44 (d, 1 H, $J_{2,NH}$ 8.2 Hz, NH), 5.90-5.70 (m, 1 H, $CH_2C\underline{H}=CH_2$), 5.31 (d, 1 H, $J_{3',4'}$ 3.1 Hz, H-4'), 5.24-5.09 (m, 3 H, $CH_2CH=C\underline{H}_2$, H-2'), 4.98 (dd, 1 H, $J_{2',3'}$ 10.6 Hz, H-3'), 4.64 (d, 2 H, $J_{1,2}$, $J_{1',2'}$ 7.7 Hz, H-1, H-1'), 4.24 (dd, 1 H, *J* 4.8, *J* 12.9 Hz, $OC\underline{H}_2CH=$), 4.05-3.30 (m, 10 H, H-6a', H-6b', $OC\underline{H}_2CH=$, H-3, H-5', H-4, H-6a, H-6b, H-2, H-5), 2.08, 2.03, 1.98, 1.91, 1.90 (5s, 15 H, 4 x OAc, NHAc); ^{13}C NMR ($CDCl_3$) δ 171.0, 170.4, 170.0, 169.9, 169.4 (5 x C=O), 133.9 ($-\underline{C}H=CH_2$), 117.3 ($-CH=\underline{C}H_2$), 101.6 (C-1'), 99.7 (C-1), 81.2 (C-4), 74.2 (C-5), 71.9 (C-3), 71.1 (C-5'), 70.8 (C-3'), 70.1 (C-2'), 69.0 ($-O\underline{C}H_2CH=$), 67.0 (C-4'), 61.5 (C-6'), 60.8 (C-6), 56.4 (C-2), 23.2 (NHAc), 20.6, 20.5, 20.4 (4 x OAc); HRMS calcd for $C_{25}H_{37}N_1O_{15}$ (M^+) *m/z* 591.2163, found 591.2082.

Allyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (160)

To compound **159** (600 mg, 1.01 mmol) in methanol (10 mL) was added 1 M sodium methoxide solution until pH was about 10. The solution was stirred at room temperature for 2 h. The solution was then neutralized by Amberlite® IRA-120 H^+ resin . The mixture was filtered and concentrated under pressure to afford product **160** in quantitative yield; m.p. 117-120°C; $[\alpha]_D$ +32.5° (*c* 1.0, H_2O); ^{1}H NMR (D_2O) δ 5.96-5.75 (m, 1 H, $CH_2C\underline{H}=CH_2$), 5.30-5.19 (m, 2 H, $CH_2CH=C\underline{H}_2$), 4.53 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.42 (d, 1 H, $J_{1',2'}$ 7.4 Hz, H-1'), 4.30 (dd, 1 H, *J* 3.5, *J* 13.2 Hz, $OC\underline{H}_2CH=$), 4.10 (dd, 1 H, *J* 6.1 Hz, $OC\underline{H}_2CH=$), 3.98-3.44 (m, 12 H, H-2, H-2', H-3, H-3', H-4, H-4', H-5, H-5', H-6a, H-6b, H-6a', H-6b'), 1.99 (s, 3 H, NHAc); ^{13}C NMR (D_2O) δ 176.1 (C=O), 134.8 ($-\underline{C}H=CH_2$), 119.7 ($-CH=\underline{C}H_2$), 104.3 (C-1'),

101.5 (C-1), 79.9 (C-4), 76.8, 76.2, 74.0, 72.4, 72.0, 70.0, 62.5 (C-6'), 61.5 (C-6), 56.5 (C-2), 23.6 (NHAc); FAB-MS (glycerol) gave m/z (ion, relative intensity): 446.3 ($[M+Na]^+$, 69%).

3-(2-amnioethylthio)propyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (161)

Compound **160** (440 mg, 1.04 mmol) and cysteamine hydrochloride (141 mg, mmol) were dissolved in deoxygenated distilled water (5 mL) and irradiated (λ = 254 nm) under nitrogen for 3 h. The solution became pale brown. TLC (3/2/1, EtOAc/HOAc/H_2O) showed the reaction was complete. The solution was concentrated under pressure. The crude product was purified by cation exchange chromatography (Dowex 50W, NH_4^+ form) using water to ammonium solution as eluent to afford **161** (460mg, 88%): $[\alpha]_D$ +36.7° (*c* 1.0, H_2O); ^1H NMR (D_2O) δ 4.58 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 4.53 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.10-4.02 (m, 2 H, H-6a, OCHa), 3.99 (d, 1 H, $J_{3',4'}$ 3.4 Hz, H-4'), 3.89 (dd, 1 H, $J_{5,6b}$ 5.2, $J_{6a,6b}$ 12.3 Hz, H-6b), 3.84-3.72 (m, 9 H, OCHb, H-2, H-3, H-3', H-4, H-4', H-5', H-6a', H-6b'), 3.65 (m, 1 H, H-5), 3.60 (dd, 1 H, $J_{2',3'}$ 9.9 Hz, H-2'), 2.96 (t, 2 H, *J* 6.6 Hz, -SC$\underline{H}_2$CH_2NH_2), 2.76 (dt, 2 H, *J* 2.3 Hz, -C$\underline{H}_2$NH_2), 2.66 (m, 2 H, -CH_2S-), 2.11 (s, 3 H, NHAc), 1.91 (m, 2 H, -OCH_2C$\underline{H}_2$-); ^{13}C NMR (D_2O) δ 174.0 (C=O), 102.4 (C-1'), 100.7 (C-1), 78.1 (C-4), 74.9 (C-5'), 74.3 (C-5), 72.1 (C-3), 71.9 (C-3'), 70.5 (C-2'), 68.2 (C_a), 68.1 (C-4'), 60.5 (C-6'), 59.6 (C-6), 54.7 (C-2), 39.0 (C_d), 31.9 (C_e), 28.2 (C_c), 26.7 (C_b), 21.8 (NHAc); HRMS calcd for $C_{19}H_{37}N_2O_{11}S_1$ (M^+) *m/z* 501.2118, found 501.2092.

Lactosamine-containing glycopolymer (163)

Poly(*N*-oxysuccinimidyl acrylate) (**162**) (1.18g) was added into DMF (50 mL). The mixture was stirred until the polymer dissolved completely. Then disaccharide **161** (350 mg, 0.70 mmol) in DMF (5 mL) solution was added to the polymer solution. The solution was stirred at room temperature overnight. After complete consumption of the sugar moitey, excess amount of propylamine (1.15 mL, 14 mmol) was added. The reaction was stirred for 10 h,. The solution was concentrated under reduced pressure. The syrup was dialyzed with 2k M.W. cut-off tube thoroughly against distilled water for 2 days and freeze-dried to afford lactosamine polymer **163** (1.01 g, 86%): ^1H NMR (D_2O) δ 4.58 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 4.53 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.10-3.57 (m, the rest protons of the disaccharide, OCH_2), 3.07 (br,

$NHC\underline{H}_2$ of backbone), 2.80-1.40 (m, CH_2 of the spacer, NHAc, methine and methylene of backbone, $NHCH_2C\underline{H}_2$ of backbone), 1.0 (br, $NH(CH_2)_2C\underline{H}_3$ of backbone); ^{13}C NMR (D_2O) δ 177.7 (C=O of backbone), 175.7 (NHAc's C=O), 104.4 (C-1'), 102.7 (C-1), 80.0 (C-4), 76.8, 76.3, 74.0, 73.9, 73.8, 72.5, 70.0, 62.5 (C-6'), 61.6 (C-6), 56.6 (C-2), 44.6-42.5 (br, methine of backbone), 42.8 ($NH\underline{C}H_2$), 37.6-30.1 (br, methylene of backbone), 23.8 (NHAc), 23.44 ($NHCH_2\underline{C}H_2$), 12.42 ($NH(CH_2)_2\underline{C}H_3$).

(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-3-*O*-acetyl-2,6-di-*O*-benzyol-β-D-glucopyranosyl azide (164)

To GM_3 trisaccharide **68** (300 mg, 0.26 mmol) were added acetic anhydride (1.5 mL) and pyridine (2 mL). The solution was stirred for 5 h at room temperature. The solution was poured into ice water and extracted with dichloromethane (2 x 10 mL). The organic phase was washed with 5% HCl, water, a saturated aq solution of $NaHCO_3$, and brine. The solution was dried over Na_2SO_4 and concentrated under reduced pressure to afford compound **164** (330 mg, 99%): m.p. 111-112°C; $[\alpha]_D$ +28° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.02-7.37 (m, 15 H, aromatic), 5.50 (m, 1 H, H-8"), 5.46 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-3), 5.32 (dd, 1 H, $J_{6",7"}$ 2.7 Hz, $J_{7",8"}$ 8.9 Hz, H-7"), 5.18 (d, 1 H, $J_{5",NH}$ 10.2 Hz, NH), 5.11 (dd, 1 H, $J_{2,3}$ 9.7 Hz, H-2), 4.99 (dd, 1 H, $J_{2',3'}$ 10.2 Hz, H-2'), 4.95 (d, 1 H, $J_{3',4'}$ 3.3 Hz, H-4'), 4.87 (dd, 1 H, $J_{5,6a}$ 2.0 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.85 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.83 (m, 1 H, H-4"), 4.75 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 4.56 (dd, 1 H, H-3'), 4.38-4.34 (m, 2 H, H-9a", H-6b), 4.31 (dd, 1 H, $J_{5',6a'}$ 6.7 Hz, $J_{6a',6b'}$ 11.1 Hz, H-6a'), 4.16 (dd, 1 H, $J_{5',6b'}$ 7.3 Hz, H-6b'), 4.08 (dd, 1 H, $J_{4,5}$ 9.4 Hz, H-4), 4.00-3.93 (m, 3 H, H-5', H-5", H-9b"), 3.91 (m, 1 H, H-5), 3.66 (s, 3 H, OMe), 3.56 (dd, 1 H, $J_{5",6"}$ 10.7 Hz, H-6"), 2.53 (dd, 1 H, $J_{3e",4"}$ 4.7, $J_{3a",3e"}$ 12.7 Hz, H-3e"), 2.18, 2.07, 1.98, 1.97, 1.97, 1.95, 1.93, 1.80 (8s, 24 H, OAc's, NHAc), 1.63 (dd, 1 H, $J_{3a",4"}$ 12.3 Hz, H-3a"); ^{13}C NMR ($CDCl_3$) δ 170.8-165.2 (C=O's), 133.6-128.4 (aromatic C), 101.1 (C-1'), 96.8 (C-2"), 87.9 (C-1), 76.0 (C-4), 75.3 (C-5), 72.7 (C-3), 72.1 (C-6"), 71.4 (2 C, C-2, C-3'), 70.5 (C-5'), 69.9 (C-2'), 69.3 (C-4"), 68.0 (C-8"), 67.3 (C-4'), 67.0 (C-7"), 62.4 (2 C, C-6, C-9"), 61.3 (C-6'), 53.0 (OMe), 49.1 (C-5"), 37.4 (C-3"), 23.1 (NHAc), 21.1, 20.9, 20.7, 20.7, 20.6, 20.6 (OAc's).

***N*-(6-*N*-Carbobenzyloxyamino) (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-3-*O*-acetyl-2,6-di-*O*-benzyol-β-D-glucopyranosyl hexanamide (165)**

To compound **164** (250 mg, 0.167 mmol) in methanol (15 mL) was added 10% Pd/C (30 mg). H_2 gas was bubbled through the mixture for 3 h at room temperature. TLC (CH_2Cl_2:EtOH, 30:1) showed the azide derivative was completely transformed into the amino product. The mixture was filtered and washed with methanol. The filtrate was concentrated under reduced pressure. The residue was dried under the oil pump and then dissolved in dry dichloromethane (5 mL). To the solution were added freshly *N*-(carbobenzyloxyamino)caproyl chloride (66 mg, 0.25 mmol) and DIPEA (58 μL). The solution was stirred under N_2 for 4 h at room temperature. The solution was concentrated and purified by silica gel chromatography using methanol-dichloromethane as eluent (1:30, v/v) to afford compound **165** (217 mg, 74%): m.p. 103-107°C; $[\alpha]_D$ +36.8° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.02-7.37 (m, 15 H, aromatic), 6.35 (d, 1 H, $J_{1,NH}$ 9.4 Hz, NH), 5.55 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-3), 5.45 (m, 1 H, H-8"), 5.37 (d, 1 H, $J_{1,2}$ 9.3 Hz, H-1), 5.32 (dd, 1 H, $J_{6",7"}$ 2.5 Hz, $J_{7",8"}$ 8.9 Hz, H-7"), 5.28 (bs, 1 H, NH), 5.05-4.95 (m, 5 H, $PhCH_2$, H-2, H-2', H-4'), 4.84 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.82 (m, 1 H, H-4"), 4.80 (dd, 1 H, $J_{5,6a}$ 2.0 Hz, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.70 (bs, 1 H, NHCbz), 4.55 (dd, 1 H, $J_{2',3'}$ 10.2 Hz, $J_{3',4'}$ 3.3 Hz, H-3'), 4.37-4.31 (m, 3 H, H-9a", H-6b, H-6a'), 4.14 (dd, 1 H, $J_{5',6b'}$ 7.4 Hz, $J_{6a',6b'}$ 11.1 Hz, H-6b'), 4.04 (dd, 1 H, $J_{4,5}$ 9.4 Hz, H-4), 3.98-3.91 (m, 4 H, H-5', H-5", H-9b", H-5), 3.65 (s, 3 H, OMe), 3.59 (dd, 1 H, $J_{5",6"}$ 8.6 Hz, H-6"), 2.92 (m, 2 H, (e)CH_2), 2.52 (dd, 1 H, $J_{3e",4"}$ 4.7, $J_{3a",3e"}$ 12.7 Hz, H-3e"), 2.15, 2.07, 1.98, 1.96, 1.94, 1.86, 1.78 (8s, 24 H, OAc's, NHAc), 2.10 (m, 2 H, (a)CH_2), 1.62 (dd, 1 H, $J_{3a",4"}$ 12.4 Hz, H-3a"), 1.42 (m, 2 H, (b)CH_2), 1.22 (m, 2 H, (d)CH_2), 1.07 (m, 2 H, (c)CH_2); ^{13}C NMR ($CDCl_3$) δ 173.0-165.7 (C=O's), 156.3-125.7 (aromatic C), 101.1 (C-1'), 96.8 (C-2"), 78.2 (C-1), 76.2 (C-4), 74.8 (C-5), 72.8 (C-3), 72.0 (C-6"), 71.7 (C-2), 71.4 (C-3'), 70.4 (C-5'), 70.0 (C-2'), 69.3 (C-4"), 68.0 (C-8"), 67.3 (C-4'), 67.0 (C-7"), 66.5 ($PhCH_2$), 62.5 (C-6), 62.3 (C-9"), 61.4 (C-6'), 52.9 (OMe), 49.1 (C-5"), 40.6 ((e)CH_2), 37.4 (C-3"), 36.3 ((a)CH_2), 29.4 ((d)CH_2), 25.9 ((c)CH_2), 24.6 ((b)CH_2), 23.1 (NHAc), 21.1, 20.9, 20.7 (OAc's); FAB-MS (glycerol) gave m/e: 1501.1 (M, 1.3%).

***N*-(6-*N*-Carbobenzyloxyamino) (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl hexanamide (166)**

To a solution of compound **165** (110 mg, 73 μmol) in MeOH (5 mL) was added 1M NaOMe solution (5 mL). The solution was stirred at room temperature for 2 h. Then water (5 mL) was added to the solution. The reaction mixture was stirred at room temperature for 12 h. The solution was neutralized by Amberlite® IRA-120 H^+ resin. After filtration, the filtrate was concentrated under reduced vacuum, and the residue was purified by size exclusion chromatography (P-2) using distilled water as eluent. The resulting solution was lyophilized to give compound **166** (60 mg, 93%): $[\alpha]_D$ +25.3° (*c* 1.0, H_2O); ^1H NMR (D_2O) δ 7.53-7.44 (m, 5 H, aromatic), 5.17 (s, 2 H, $PhCH_2$), 5.04 (d, 1 H, $J_{1,2}$ 9.2 Hz, H-1), 4.60 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.19 (dd, 1 H, $J_{2',3'}$ 9.9 Hz, $J_{3',4'}$ 3.1 Hz, H-3'), 4.03 (d, 1 H, H-4'), 3.50 (dd, 1 H, $J_{2,3}$ 9.2 Hz, H-2), 3.18 (t, 2 H, *J* 6.5 Hz, e-CH_2), 2.83 (dd, 1 H, $J_{3e'',4''}$ 4.7, $J_{3a'',3e''}$ 12.4 Hz, H-3e"), 2.37 (t, 2 H, *J* 7.1 Hz, (a)CH_2), 2.10 (s, 3 H, NHAc), 1.87 (dd, 1 H, $J_{3a'',4''}$ 12.1 Hz, H-3a"), 1.68 (m, 2 H, (b)CH_2), 1.55 (m, 2 H, (d)CH_2), 1.40 (m, 2 H, (c)CH_2); ^{13}C NMR (D_2O) δ 177.9, 174.6 (C=O's), 173.4 (C-1"), 128.3, 127.9, 127.1 (aromatic C), 102.2 (C-1'), 99.2 (C-2"), 78.9 (C-1), 77.4, 75.9, 75.1, 74.7 (C-3'), 74.6, 72.4, 71.3, 71.0 (C-2), 68.9 , 67.9, 67.8, 67.0 (C-4'), 66.3 (Ph$\underline{C}H_2$), 62.1 (C-9"), 60.6, 59.5 (C-6, C-6'), 51.3 (C-5"), 39.8 ((e)CH_2), 39.2 (C-3"), 35.2 ((a)CH_2), 28.0 ((d)CH_2), 24.8 ((c)CH_2), 24.1 ((b)CH_2), 21.6 (NHAc).

(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl 6-aminohexanamide (167)

To a solution of compound **166** (46 mg, 0.096 mmol) in methanol (3 mL) was added 10% Pd/C (10 mg). H_2 was bubbled through the solution and the mixture was stirred for 6 h at room temperature. The mixture was filtered and the Pd/C was washed with methanol. All of the filtrates were combined and concentrated under vacuum to give compound **167** (35 mg, 90%): $[\alpha]_D$ +27.6° (*c* 1.0, H_2O); ^1H NMR (D_2O) δ 5.04 (d, 1 H, $J_{1,2}$ 9.2 Hz, H-1), 4.60 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.19 (dd, 1 H, $J_{2',3'}$ 9.9 Hz, $J_{3',4'}$ 3.1 Hz, H-3'), 4.03 (d, 1 H, H-4'), 3.50 (dd, 1 H, $J_{2,3}$ 9.2 Hz, H-2), 2.95 (t, 2 H, *J* 6.5 Hz, e-CH_2), 2.83 (dd, 1 H, $J_{3e'',4''}$ 4.7, $J_{3a'',3e''}$ 12.4 Hz, H-3e"), 2.37 (t, 2 H, *J* 7.1 Hz, (a)CH_2), 2.10 (s, 3 H, NHAc), 1.87 (dd, 1 H, $J_{3a'',4''}$ 12.1 Hz, H-3a"), 1.62 (m, 4 H, (b)CH_2, (d)CH_2), 1.40 (m, 2 H, (c)CH_2); ^{13}C NMR (D_2O) δ 174.6

(C=O's), 173.4 (C-1"), 102.2 (C-1'), 99.2 (C-2"), 78.9 (C-1), 77.4, 75.9, 75.1, 74.7 (C-3'), 74.6, 72.4, 71.3, 71.0 (C-2), 68.9 , 67.9, 67.8, 67.0 (C-4'), 62.1 (C-9"), 60.6, 59.5 (C-6, C-6'), 51.3 (C-5"), 39.8 ((e)CH_2), 39.2 (C-3"), 35.2 ((a)CH_2), 28.0 ((d)CH_2), 24.8 ((c)CH_2), 24.1 ((b)CH_2), 21.6 (NHAc).

GM_3-containing glycopolymer (168)

Poly(*N*-oxysuccinimidyl acrylate) **162** (90.7 mg) was added into DMF (5 mL). The mixture was stirred until the polymer dissolved completely. Then trisaccharide **167** (20 mg, 26.8 μmol)) in DMF (1 mL) solution was added to the polymer solution. The solution was stirred at room temperature overnight. After complete consumption of the sugar moitey, excess amount of ammonium hydroxide solution (0.5 mL) was added. The reaction was stirred for 10 h,. The solution was concentrated under reduced pressure. The syrup was dialyzed with 2k M.W. cut-off tube thoroughly against distilled water for 2 days and freeze-dried to afford GM_3 polymer **168** (41.5 mg, 72%): ^{1}H NMR (D_2O) δ 5.05 (d, 1 H, $J_{1,2}$ 9.0 Hz, H-1), 4.60 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1'), 4.19 (d, 1 H, $J_{2',3'}$ 9.7 Hz, H-3'), 3.41 (1 H, H-2), 2.83 (dd, 1 H, $J_{3e'',4''}$ 4.7, $J_{3a'',3e''}$ 12.1 Hz, H-3e"), 2.60-2.14 (br, methine of backbone), 2.10 (s, 3 H, NHAc), 2.06-1.60 (br, methylene of backbone); ^{13}C NMR (D_2O) δ 179.1 (br, C=O of backbone), 174.6 (C-1"), 102.2 (C-1'), 95.7 (C-2"), 78.7 (C-1), 77.4, 75.9, 75.0, 74.7 (C-3'), 74.6, 72.4, 71.3, 71.0 (C-2), 68.9, 67.9, 67.7, 67.1 (C-4'), 62.2 (C-9"), 60.6, 59.5 (C-6, C-6'), 51.3 (C-5"), 42.2-40.9 (br, methine of backbone), 39.2 (C-3"), 35.8-32.6 (br, methylene of backbone), 21.6 (NHAc).

Claims to original research

1. An efficient synthetic route towards *N*-acetyllactosamine derivatives was developed. This method allows the synthesis of Lewis X trisaccharide on a large scale without difficulty.

2. Lewis X pentasaccharide was synthesized using "active-latent" glycosylation strategy.

3. Sialyl-α-(2→3)- and sialyl-α-(2→6)-sugar derivatives were obtained using a thiophilic promoter (NIS/TfOH) in acetonitrile under kinetically controlled conditions. The syntheses of sialyl α(2→6) thioglycosides were also achieved in situ using diethylamine in the chemoselective de-S-acetylation of a thioacetate sialic acid derivative.

4. An olefin metathesis reaction catalyzed by Grubbs' ruthenium benzylidene catalyst had been applied in alkenyl sialic acid derivatives to afford divalent sialoside derivatives.

5. Using a copper (I)-catalyzed Glaser reaction and a palladium-catalyzed Sonogashira reaction, divalent 'rod-like' glycoclusters were prepared from 2-propynyl thioglycosides.

6. Di-, tri-, and tetra-valent sialyl glycoclusters were synthesized from 2-propynyl *O*-and *S*-sialic acid derivatives using Glaser and Sonogashira reactions.

7. Divalent sialosides scaffolded on *p-tert*-butylcalix[4]arene were prepared by conjugating isothiocyanate sialyl derivatives to distally disubstituted amine terminated *tert*-butylcalix[4]arene.

8. A GM_3 isothiocyanate derivative was coupled to multivalent amines based on γ-resorcylic acid- or gallic acid-cores to give multivalent GM_3 ligands.

9. Symmetrical, spherical GM_3-containing glycodendrimers were synthesized based on the Starburst® PAMAM dendritic core. An isothiocyanate conjugation strategy was used to prepare 4- and 8-valent glycodendrimers by conjugating a GM_3 isothiocyanate derivative to

amine terminated PAMAM cores (G0 and G1).

10. Glycopolymers bearing lactosamine and GM_3 trisaccharide residues were synthesized by coupling amine terminated lactosamine and GM_3 trisaccharide derivatives to pre-activated poly(*N*-acryloxysuccinimide).

Publications

1. Reuter, J. D.; Myc, A.; Hayes, M. M.; Gan, Z.; Roy, R.; Qin, D.; Yin, R.; Piehler, L. T.; Esfand, R.; Tomalia, D. A.; Baker, Jr., J. R. "Inhibition of Viral Adhesion and Infection by Sialic-Acid-Conjugated Dendritic Polymers" *Bioconjugate Chem.* **1999**, 10, 271.
2. Gan, Z.; Cao, S.; Wu, Q.; Roy, R. "Regiospecific Syntheses of *N*-Acetyllactosamine Derivatives and Application toward a Highly Practical Synthesis of Lewis X Trisaccharide" *J. Carbohydr. Chem.* **1999**, **18**, 755.
3. Cao, S.; Gan. Z.; Roy, R. "Active-Latent Glycosylation Strategy toward Lewis X Pentasaccharide in a Form Suitable for Neoglycoconjugate Syntheses" *Carbohydr. Res.* **1999**, *318*, 75.
4. Gan, Z.; Roy, R. "Transition Metal-Catalyzed Syntheses of 'Rod-like' Thioglycoside dimers" *Tetrahedron Lett.* **2000**, *41*, 1155.
5. Gan, Z.; Roy, R. "Facile Preparation of Divalent Sialoside Derivatives by Olefin Metathesis Reaction" *Tetrahedron* **2000**, *56*, 1423.
6. Roy, R.; Das, S. K.; Hernández-Mateo, F.; Santoyo-González, F.; Gan, Z. "Palladium Mediated Oxidative Homocoupling of Terminal Alkynes: Application toward the Synthesis of Symmetical Conjugated Diynes" Org. Lett., submitted.

Conference proceedings

Gan, Z.; Roy, R. "Synthesis of Multivalent Sialyl α-(2→3)-Lactoside Ligands" XVIII International Carbohydrate Symposium, Milan, Italy, July **1996**.