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ABSTRACT

A series of scaffolding glycoconjugates were synthesized with various carbohydrate densities, conformations, and interglycosidic spacers. These compounds include glycopeptoids, glycocalix[4]arenes, self-assembled glycoclusters and glycodendrimers, glyco PAMAM dendrimers, and glycopolymers.

The synthesis of Tn-antigen glycopeptidomimetic clusters, peptoids (N-substituted oligoglycines), was accomplished to generate metabolically stable glycopeptide analogs in multivalent fashion. By reiteration of the deprotection-coupling process using monomer and dimer as building blocks, trimer and tetramer were synthesized. Hexamer and octamer were obtained in the same manner.

Whereas glycopeptoids are arranged in linear fashion, structurally more defined, tree-like glycodendrimers were synthesized utilizing GalNAc derivatives as carbohydrate entities and calix[4]arene as a core to afford tetra-, octa-, and hexadecamers. Two tetramers, each having four equidistant GalNAc residues, with different lengths of spacer arms were synthesized by coupling GalNAc ligands to acyl chloride on a *p-tert*-butylcalix[4]arene core. Glycocalix[4]arenes with higher valencies, octa- and hexadecamers were obtained using a double *N*-alkylation strategy. This strategy was extended to synthesize glyco PAMAM dendrimers. The second generation (G2), Starburst® PAMAM core was dialkylated with bromoacetylated GalNAc derivatives to afford 32-mers.

A more convenient alternative to long and iterative procedures to construct hyperbranched glycodendrimers evaluated nucleated simple building blocks (dendrons) around metal ions. The synthesis of small building blocks and their non-covalent assembly around the metal ions, Fe(II) and Cu(II) were described.

Preparations of enzymetically stable *C*-glycosides of GalNAc were also presented. *O*-Acetyl protected galactal was azido-phenylselenylated and Keck allylation at the anomeric center afforded α-linked *C*-glycosides. Elongated *C*-glycosyl derivatives were used to synthesize small and rigid glycoclusters to investigate the cross-linking properties of *Vicia villosa* B_4 (VVA) lectin.

Turbidimetric assays confirmed the ability of the glycodendrimers to cross-link and precipitate the lectin. Solid phase enzyme-linked lectin assay (ELLA) of all the synthesized glycoconjugates showed improved inhibitory efficacy compared to allyl α-D-GalNAc which was used as a reference.

ACKNOWLEDGMENTS

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I am grateful to my colleagues specially in Marion Laboratory, Serge J. Meunier, Zhonghong Gan, Mr. Qing Quan Wu, and Heba Abourahma for their helpful discussions and the pleasant times we had in the lab. I can not forget other colleagues, Dr. Bernd Kratzer, Dr. Neil Faibish, Dr. Saha Uttam, Dr. Muriel Llinares, Daniel Pagé, Denis Carrière, and Cindy Smith, who also made those days happy and memorable.

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LIST OF ABBREVIATIONS

ABTS	2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)
Ac	acetyl
AcCl	acetyl chloride
AcOH	acetic acid
Ala	alanine
b	broad
Boc	*tert*-butoxycarbonyl
BOP	benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphoniumhexafluorophosphate
bs	broad singlet
BSA	bovine serum albumin
t-Bu	*tert*-butyl
Cbz	carbobenzyloxy
CI	chemical ionization
COSY	correlation spectroscopy
d	doublet
Da	dalton
DCC	dicyclohexylcarbodiimide
dd	doublet of doublets
ddd	doublet of doublet of doublets
DEPT	distortionless enhanced polarization transfer
2,5-DHB	2,5-dihydroxybenzoic acid
DICI	1,3-diisopropylcarbodiimide
DIPEA	diisopropylethylamine
DMF	*N,N*-dimethylformamide
ELLA	enzyme linked lectin assay
eq.	equivalent(s)
EtOAc	ethyl acetate
EtOH	ethanol

Et_3N	triethylamine
FAB-MS	fast atom bombardment ionization mass spectrometry
Fmoc	9-fluorenylmethoxycarbonyl
Gal	galactose
GalNAc	*N*-acetyl-D-galactosamine
Glc	glucose
GlcNAc	*N*-acetyl-D-glucosamine
h	hour(s)
HMQC	heteronuclear multiple quantum coherence
HRP	horse radish peroxidase
Hz	Hertz
IC_{50}	concentration required for 50% inhibition
kDa	kiloDalton
Lac	lactose
LacNAc	N-acetyl lactosamine
LFA	*Limax flavus* lectin
Lit.	literature
Lys	lysine
m	multiplet
M+	parent molecular ion
MAb	monoclonal antibodies
MALDI-TOF	matrix assisted laser desorption ion time of flight
MAP	multiple antigen peptide
Me	methyl
min	minute(s)
mmol	millimolar
mol	molar
m.p.	melting point
MS	molecular sieves
MW	molecular weight
m/z	mass to charge ratio

Neu(5)Ac	*N*-acetylneuramic acid
NMM	4-methylmorpholine
NMR	nuclear magnetic resonance
Nu	nucleophile
O.D.	optical density
PAMAM	polyamidoamine
PBS	phosphate bovine saline
PBST	phosphate bovine saline with Tween 20
Ph	phenyl
pm	picometer
pos.	positive
ppm	parts per million
PTC	phase transfer catalysis
PyBOP	benzotrizole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
R_f	retention factor
RT	room temperature
s	singlet
sLe^X	sialyl Lewis^X
t	triplet
TBAC	tetrabutylammonium chloride
TBAHS	tetrabutylammonium hydrogen sulfate
TBTU	2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TFA	trifluoroacetic acid
THAP	2,4,6-trihydroxyacetophenone
TLC	thin layer chromatography
VVA	*Vicia villosa*
VVA/HRP	peroxidase-labeled *Vicia villosa*
WGA	wheat germ agglutinin

Chapter 1. Introduction

1.1. Carbohydrates in cellular recognition

The information networks encoded by nucleic acids and proteins are essential to the normal maintenance of living organisms, so genetic defects in the primary structures of these molecules often lead to the death of the organism. However, many diseases are caused by abnormalities in control mechanisms which are not immediately essential for life but which maintain the normal social behavior of differentiated cells in multicellular organisms. The complex sugar chains of glycoproteins and glycolipids play important roles in the control of cellular functions and in recognition interactions between cells and its cellular and fluid environment. In contrast to nucleic acids and proteins, complex sugar chains are not formed by direct transfer of information from templates but rather by the concerted actions of glycotransferases (each enzyme being specific for a particular monosaccharide unit and linkage type) and glycosidases (attached carbohydrate chains being modified or trimmed by glycosidases). Thus, investigations into abnormalities of complex sugar chain assembly are yielding important new understanding onto the pathogenesis of human diseases.

Abnormalities in the profiles of cell surface carbohydrates have been found in all human cancers. The carbohydrate epitopes, resulting either from incomplete synthesis or neosynthesis which is controlled by a number of glycosyltransferases, accumulate in high density at the tumor cell surface. These carbohydrate changes then play specific and crucial roles in cell-cell communications, regulation of cell growth, and differenciation.[1] A variety of monoclonal antibodies (MAb) have been developed that recognize tumor-associated carbohydrate antigens and their aberrant organization at the cell surface.

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[1] Fenderson, B. A.; Eddy, E. M.; Hakomori, S. *BioEssays* **1990**, *12*, 173.

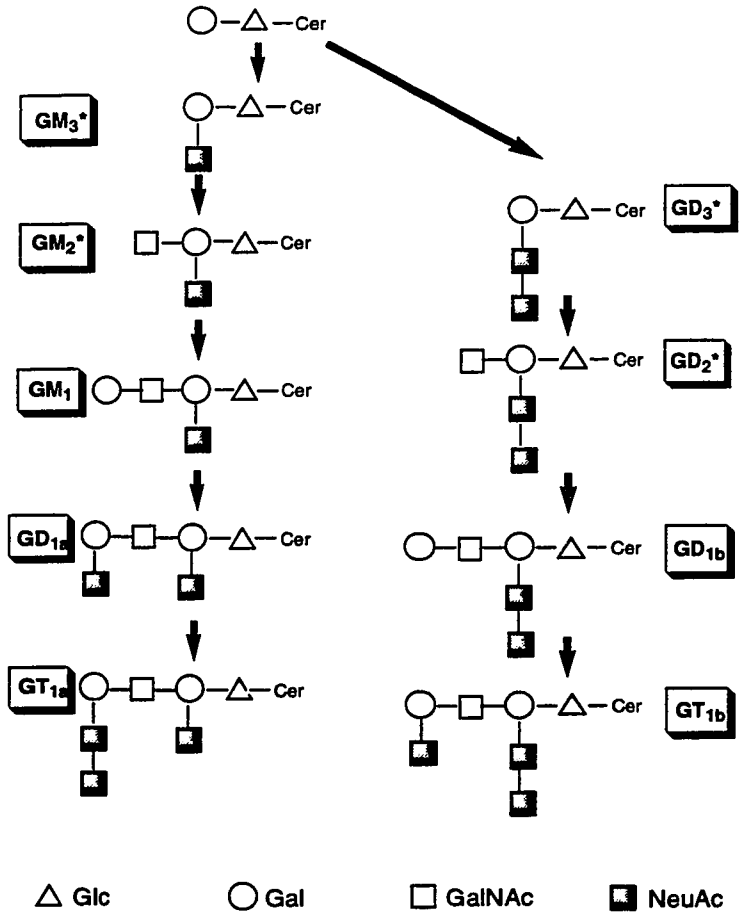

Figure 1.1.1 Increased accumulation of various gangliosides (*) in melanoma caused by increased precursor synthesis and blocked chain elongation. Cer:= Ceramide (long-chain amino alcohol, sphingosine plus a long-chain fatty acid).

Tumor-associated carbohydrate structures defined by these MAbs are: (1) Relatively novel structures expressed on the tumor cell surface, however may be present in normal tissues (e.g., GD_3 (Figure 1.1.1) in melanoma,[2,3,4] polymeric Le^x and sialyl Le^x [5,6,7](Figure 1.1.2) in gastrointestinal cancer). (2) Highly restricted structures,

[2] Nudelman, E.; Hakomori, S.; Kannagi, R.; Levery, S. B.; Yeh, M.-Y.; Hellstrom, K.-E.; Hellstrom, I. *J. Biol. Chem.* **1982**, *257*, 12752.
[3] Tai, T.; Paulson, J. C.; Cahan, L. D.; Irie, R. F. *Proc. Natl. Acad. Sci. USA* **1983**, *80*, 5392.
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immunologically detectable only in tumor cells (e.g., T, Tn, and sialyl-Tn (Figure 1.1.3) in various cancers).[8,9,10]

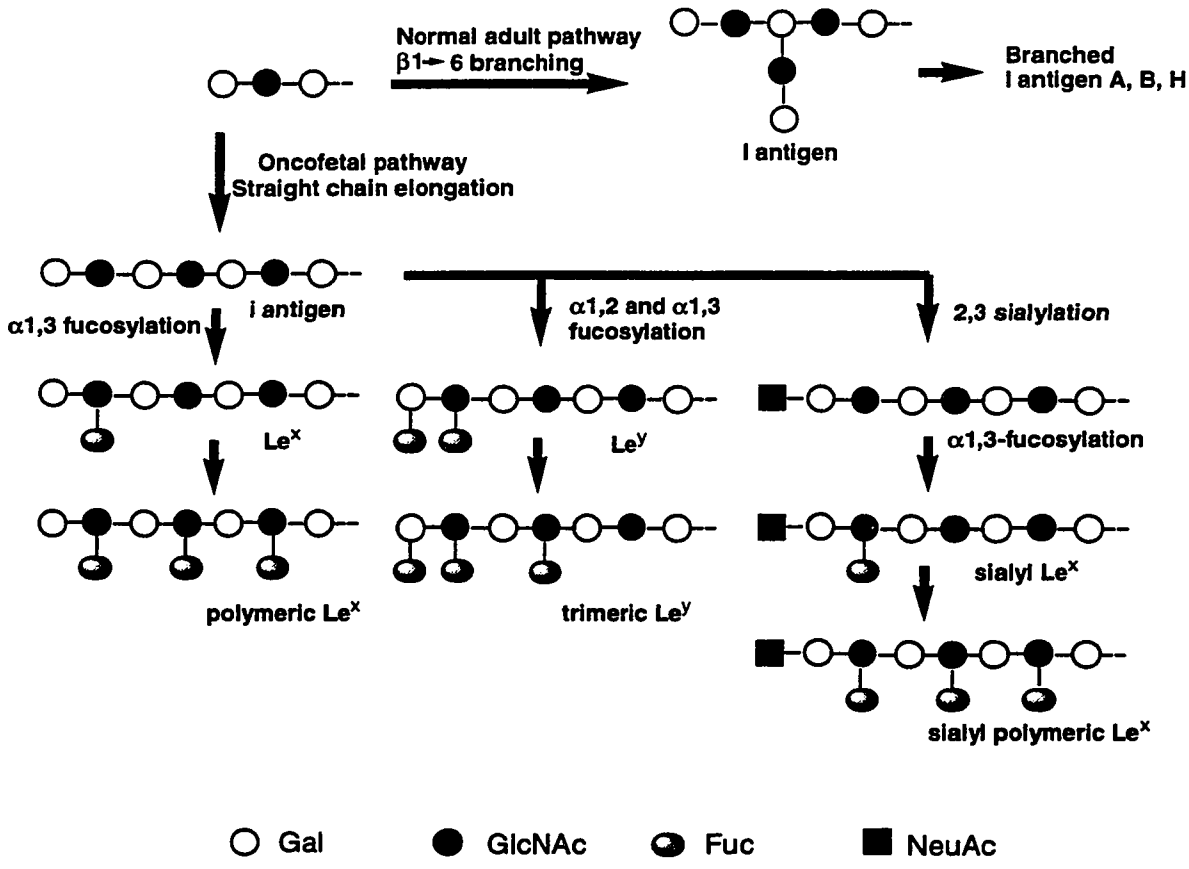

Figure 1.1.2. Normal and oncofetal biosynthetic pathways of lacto-series type 2 chain carbohydrates.

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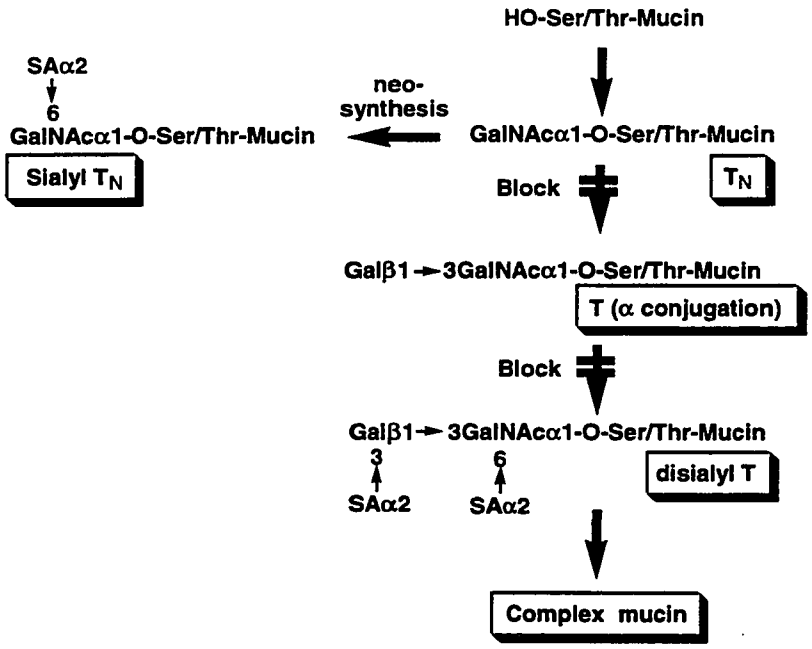

Figure 1.1.3. Accumulation of Tn- and T-epitopes as a result of blocked chain elongation, and of silayl Tn as a result of neosynthesis (sialylation of Tn-epitope).

These carbohydrate epitopes and the antibodies specific to these structures are therefore being exploited to develop novel diagnostic tools and therapeutic strategies for cancer.

There is another class of carbohydrate binding proteins, the lectins. Lectins bind mono- and oligosaccharides reversibly and with some degree of specificity. Each lectin molecule contains two or more carbohydrate-combining sites (di- or poly-valent); therefore lectins can cause cross-linking of the cells and their subsequent precipitation (referred to cell agglutination). Lectins also form cross-linked lattices between polysaccharides or glycoproteins in solution and induce their precipitation. Both the agglutination and precipitation of lectins are inhibited by the carbohydrate ligands for which the lectins are specific.

Lectins, therefore, are invaluable tools for the structural and functional investigation of complex carbohydrates, glycoproteins, and for the examination of

changes that occur on cell surfaces during physiological and pathological processes, from cell differentiation to cancer.[11,12]

1.2. Cluster (or multivalent) effect

It has been suggested that antigenicity of melanoma cells might depend on the density of ganglioside GM_3 expressed on those cells.[13] Recent binding studies[14,15] of GalNAc-modified peptides with Tn-specific antibodies also showed that the repeating units of at least two GalNAcα-O-Ser/Thr residues were necessary for the specific antigen-antibody interactions. Lee *et al*[16] demonstrated another example of "cluster effect". The synthesized trivalent glycoside (Figure 1.2.1) showed an increased affinity (500-fold) toward rabbit hepatic lectin over GalNAc.

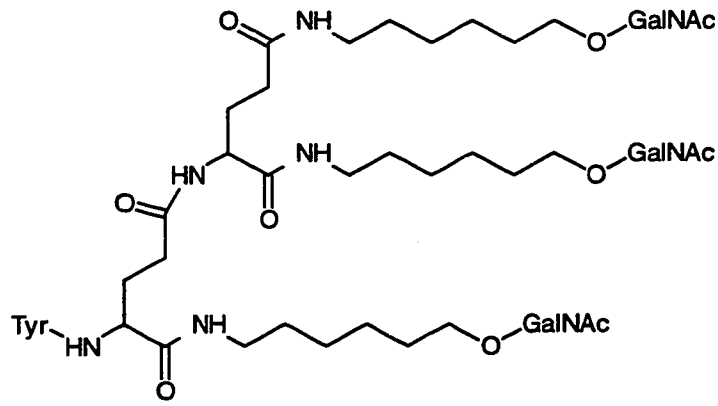

Figure 1.2.1. A trivalent glycoside reported by Lee *et al.*[16]

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[11] Goldstein, I. J.; Winter, H. C.; Poretz, R. D. In *Glycoproteins II*; Vliegenthart, J. F. G.; Montreuil, J.; Schachter, H., Eds.: Elsevier Science B. V.: Amsterdam, **1997**, p403.
[12] Lis, H.; Sharon, N. *The Lectins: Properties, Functions and Applications in Biology and Medicine*; Liener, I. E.; Sharon, N.; Goldstein, I. J., Eds.: Academic Press, Inc.: Orlando, **1986**, p293.
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[16] Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321.

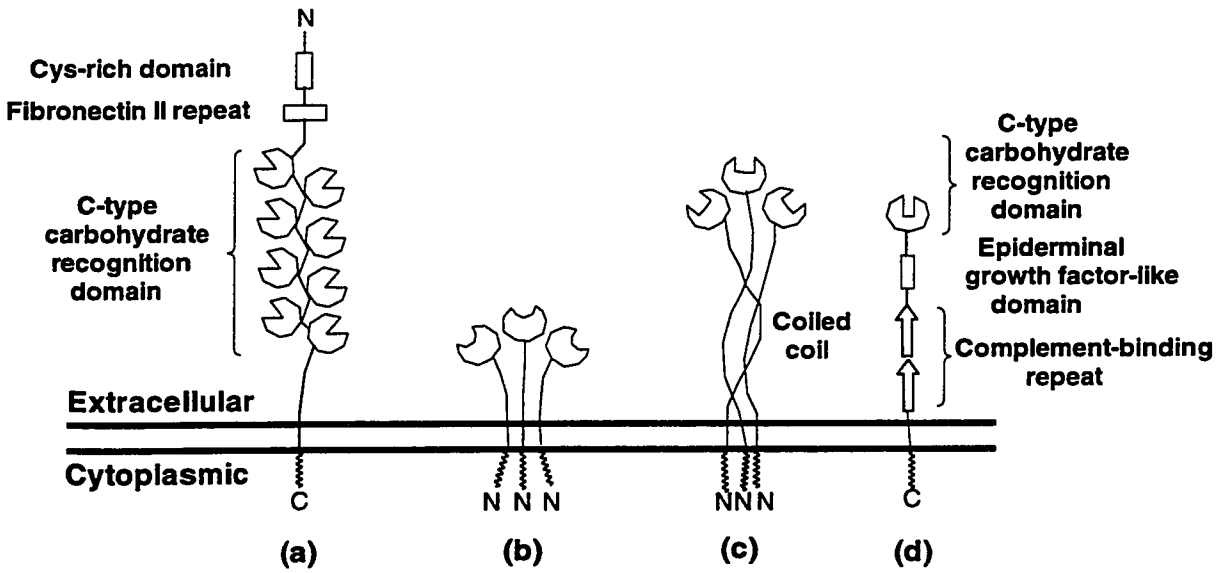

Figure 1.2.2. Organization of membrane-bound C-type animal lectins: (a) the mannose macrophage receptor; (b), (c) endocyclic receptors (the chicken hepatic lectin and the Kupffer cell receptor); (d) L-lectin.

This cluster effect can be explained by multiple binding sites present in many glycoproteins. There are numerous receptors known to have clustered carbohydrate domains. For example, the mannose-specific macrophage receptor (MW 175 kDa),[17,18,19,20] endocytic receptors[21,22,23] (the chicken hepatic lectin and Kupffer cell receptor[24]) and the highly assymetric membrane-bound proteins, E-(MW 115 kDa), P-(140 kDa), and L-selectin (90-110 kDa),[17,25,26,27,28,29] are thought to have clustered arrangements (Figure 1.2.2).

---

[17] Drickamer, K.; Taylor, M. E. *Annu. Rev. Cell Biol.* **1993**, *9*, 237.
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[27] Springer, T. A. *Annu. Rev. Physiol.* **1995**, *57*, 827.

Investigation of this "cluster effect" concept *via* the chemical syntheses of glycoconjugates with synthetically varied shapes and carbohydrate densities would generate useful tools to develop a better understanding of these important recognition processes. Subsequent studies of the interaction between these chemically well defined glycoconjugates and their receptors would offer better insights into the binding requirements of the receptors.

The remaining sections of this chapter will discuss the chemical syntheses of mulivalent glycoconjugates with various shapes and carbohydrate densities. The novel glycoconjugates prepared in this dissertation are depicted in Figure 1.2.3.

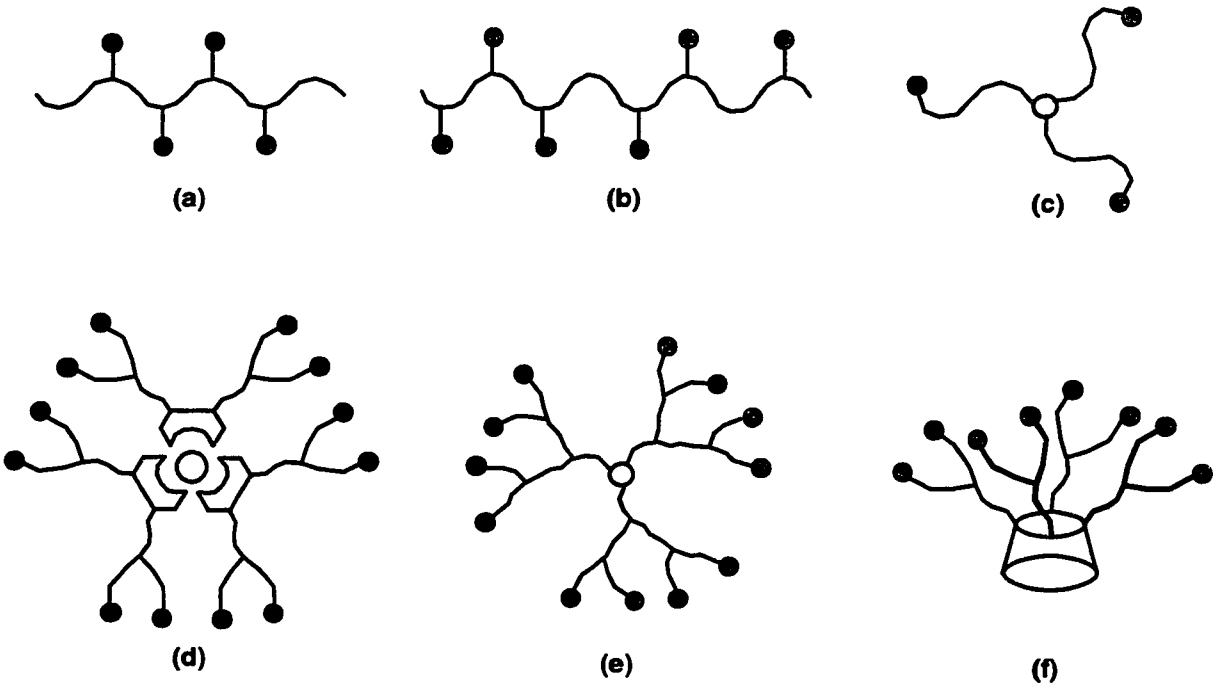

Figure 1.2.3. Multivalent glycoconjugates: (a) glycopeptoids and glycotelomers; (b) glycopolymers; (c) glycoclusters; (d) self-assembled glycodendrimers; (e) spherical PAMAM glycodendrimers; (f) glycocalix[4]arenes.

---

[28] McEver, R. P.; Moore, K. L.; Cummings, R. D. *J. Biol. Chem.* **1995**, *270*, 11025.
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1.3. Peptoids

As described earlier, molecular recognition of a target plays an important role not only in biological regulation and communication but also influences rational drug design. Finding inhibitors for target systems, which are macromolecular receptors, may prevent the progression of the disease or even provide a complete cure. An efficient screening protocol would therefore be required for testing the effect of a variety of spatial arrangements of different functional groups on the binding and activity against the target molecule. Up until the present, peptides have often been used in this type of drug design because of their large variety of functional groups, and because their chemical synthesis can be automated.[30,31] Peptide inhibitors, however, are unsuitable as drugs due to their many pharmacological problems:[32] (1) short serum half-life values and high susceptibility to hydrolysis by degradative enzymes present in the blood stream, gut and cells, (2) poor absorption and oral bioavailability, and (3) rapid liver clearance and biliary excretion.

Recently, there have been increasing efforts to design a new scaffold with a number of criteria: simple synthesis of monomers, increased resistance to hydrolytic enzymes, the ability to display a wide range of functionality, high-yielding coupling steps amenable to automation, and the use of achiral monomers.[33]

Oligo(*N*-substituted)glycines, or "peptoids" were proposed to meet these requirements (Scheme 1.3.1). The side chains of peptoids are displayed from the amide nitrogen of an oligoglycine backbone instead of the α-carbon atom, providing a protease-resistant[34] and achiral tertiary amide linkage. The standard solid-phase peptoid synthesis methods are shown in Scheme 1.3.2.

---

[30] Computer-Aided Drug Design, (Eds.: Perun, T. J.; Propst, C. L.), Marcel Dekker, New York, **1989.**
[31] Peptide Pharmaceuticals. Approaches to the Design of Novel Drugs, (Eds.: Ward, D.), Open University Press, Milton Keynes, **1991.**
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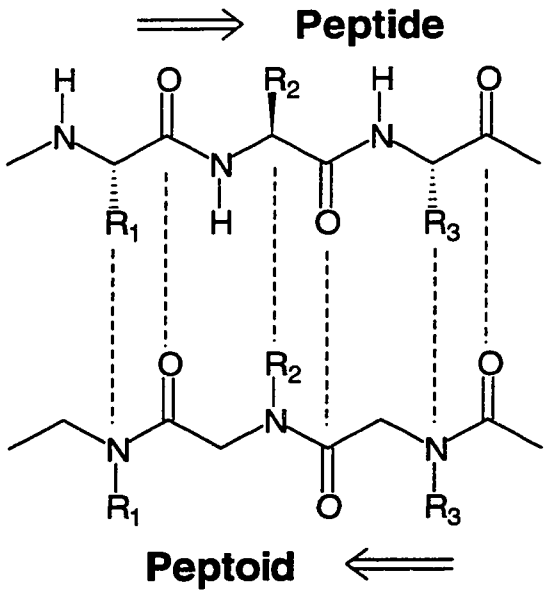
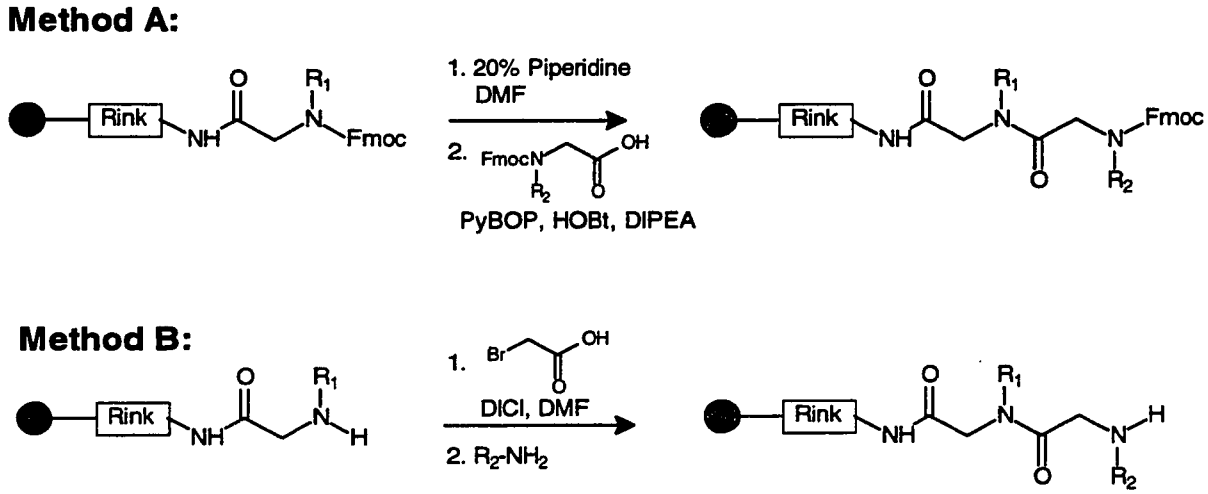
[34] (a) Miller, S. M.; Simon, R. J.; Ng, S.; Zuckermann, R. N.; Kerr, J. M.; Moos, W. H. *Drug Dev. Res.* **1995**, *35*, 20. (b) Miller, S. M.; Simon, R. J.; Ng, S.; Zuckermann, R. N.; Kerr, J. M.; Moos, W. H. *BioMed. Chem. Lett.* **1994**, *4*, 2657.



Scheme 1.3.1. Comparison of a portion of a peptide chain and a peptoid chain. The direction of the peptide bonds are reversed in the peptoid ("retro-sequence") to maintain the relative orientation of the carbonyl groups to the R groups.



Scheme 1.3.2. Solid-phase peptoid synthesis methods.

Whereas Simon *et al*[33] employed Fmoc-protected *N*-alkylglycines as the monomer components (Method A, Scheme 1.3.2), Zuckermann *et al*[35] proposed the method using submonomer (Method B, Scheme 1.3.2) to synthesize the same peptoid chain. In the latter method, each cycle of synthesis involves a two-step procedure: (1) amide bond formation with α-bromoacetic acid employing 1,3-diisopropylcarbodiimide (DICI) as an activating agent and (2) bromide displacement with a suitable primary amine to provide the secondary *N*-alkylglycine ready for the next coupling step. This approach allows for the direct incorporation of commercially available amines as building blocks, thereby eliminating costly, time-consuming monomer synthesis and preventing the need for α-amine protection.

Zuckermann *et al*[36] also reported the syntheses of peptoid library and screening of these high-affinity ligands for 7-transmembrane G-protein-coupled receptors (7TM/GPCR).[37] The 7TM/GPCRs are targets for various pharmaceuticals and among these are non-peptide adrenergic receptor ligands for treating asthma, congestion, glaucoma, heart failure, hypertension,[38] and benign prostatic hyperplasia.[39] The choice of side chains was biased to resemble known ligands to 7TM/GPCRs (Figure 1.3.1).

Low molecular weight tripeptoids were discovered that were ligands for both the α1-adrenergic receptor and μ-opiate receptor (Figure 1.3.2). The α1-adrenergic ligand CHIR 2279 (MW 565 g/mol, Ki 5 nM) competes with prazosin (Ki 0.2 nM).[40] These Ki values were obtained using a cloned human receptor. The endogenous ligands for this receptor are the non-peptidic neurotransmitters epinephrine (Ki 2-14 μM) and norepinephrine (Ki 0.5-4 μM).[40] The opiate ligand CHIR 4531 (MW 623 g/mol, Ki 6 μM) competes with DAMGO (Ki 4 nM),[41] a pentapeptide. Endogenous ligands for the opiate

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receptor can be either morphine (Ki 2 nM) or enkephalin (Ki 20 nM).[42] This work[40] demonstrated that low molecular weight, achiral tripeptoids can be potent competitors for chiral endogenous ligands.

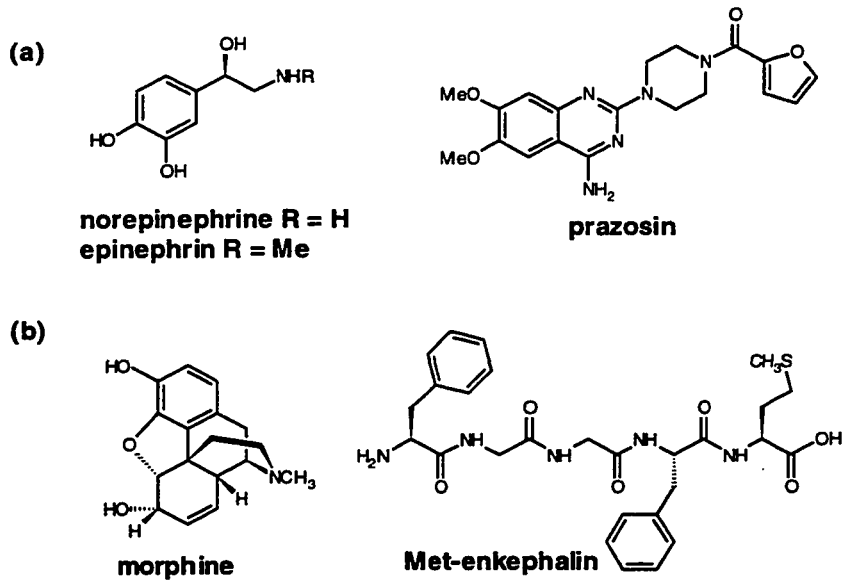

Figure 1.3.1. Known ligands for the (a) α1-adrenergic and (b) opiate receptor.

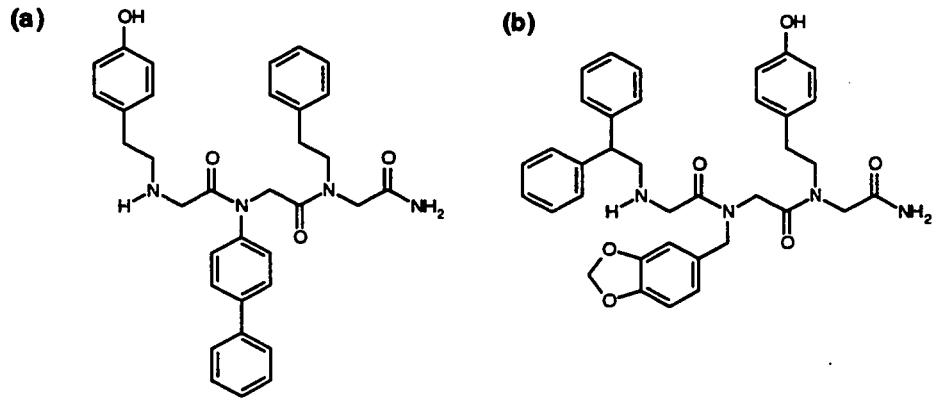

Figure 1.3.2. High-affinity ligand for (a) α1-adrenergic and (b) μ-specific opiate receptors discovered from combinatorial peptoid libraries.

[42] Rees, D. C.; Hunter, J. C. In *Comprehensive Medicinal Chemistry*, (Eds.: Hansch, C.; Sammes, P. G.; Taylor, J. B.), Pergamon Press: Oxford, **1990**, Vol 3, p805.

1.4. Dendrimers

Dendrimers,[43,44,45,46,47] also known as arborols, or cascade or starburst polymers can be characterized in two critical ways. First, they are constructed from AB_n (n usually 2 or 3) monomers resulting in hyper-branched structures, whereas linear polymers contain AB monomers. Secondly, the main synthetic approaches to dendrimers are iterative. Each repetition cycle leads to the addition of one more layer of branches ("generation") to the dendrimer framework. The hallmark of dendrimer synthesis is therefore the ability to synthesize in a controlled manner very high molecular weight polymers with narrower molecular weight distributions.

Whereas dendrimers of lower generation number tend to exist in relatively open forms, dendrimers of higher generations, often at the fifth generation, adopt spherical three-dimensional structures just like globular proteins. In addition to their structural similarity, dendrimers and proteins might function in the same ways. Dendrimers can act as hosts, having selective binding for guest molecule(s), just as proteins do.

Complexation in dendrimers can take place either in the interior or on the periphery. The types of interactions involved in the interior are hydrophobic interaction, hydrogen bonding, or physical encapsulation.

Dendrimers built for the complexation of guest molecules *via* hydrophobic interactions include Tomalia's poly(aminoamine) (PAMAM) dendrimers[48] and Newkome's "unimolecular micelle"[49,50] (Figure 1.4.1). Techniques used to study its micelle-like behavior include dynamic light scattering, hydrophobic probes (e.g., diphenylhexatriene and phenol blue), fluorimetry, fluorescence microscopy, and UV-visible spectroscopy.

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[43] Tomalia, D. A.; Naylor, A. M.; Goddart, W. A. *Angew. Chem.* **1990**, *102*, 119.
[44] Tomalia, D. A.; Durst, H. D. *Top. Curr. Chem.* **1993**, *165*, 193.
[45] Newkome, G. R.; Moorefield, C. N.; Vögtle, F. *Dendritic Macromolecules: Concepts, Syntheses, Perspectives*; VCH: Weinheim, Germany, **1996**.
[46] Fréchet, J. M. J. *Science*, **1994**, *263*, 1710.
[47] Ardoin, N.; Astruc, D. *Bull. Soc. Chim. Fr.* **1995**, B2, 875.
[48] Naylor, A. M.; Goddart, W. A. I.; Kiefer, G. E.; Tomalia, D. A. *J. Am. Chem. Soc.* **1989**, *111*, 2339.
[49] Newkome, G. R.; Gupta, V. K.; Baker, G. R.; Yao, Z.-Q. *J. Org. Chem.* **1985**, *50*, 2003.
[50] Newkome, G. R.; Moorefield, C. N.; Baker, G. R.; Saunders, M. J.; Grossman, S. H. *Angew. Chem. Int. Ed. Engl.* **1991**, *31*, 1178.

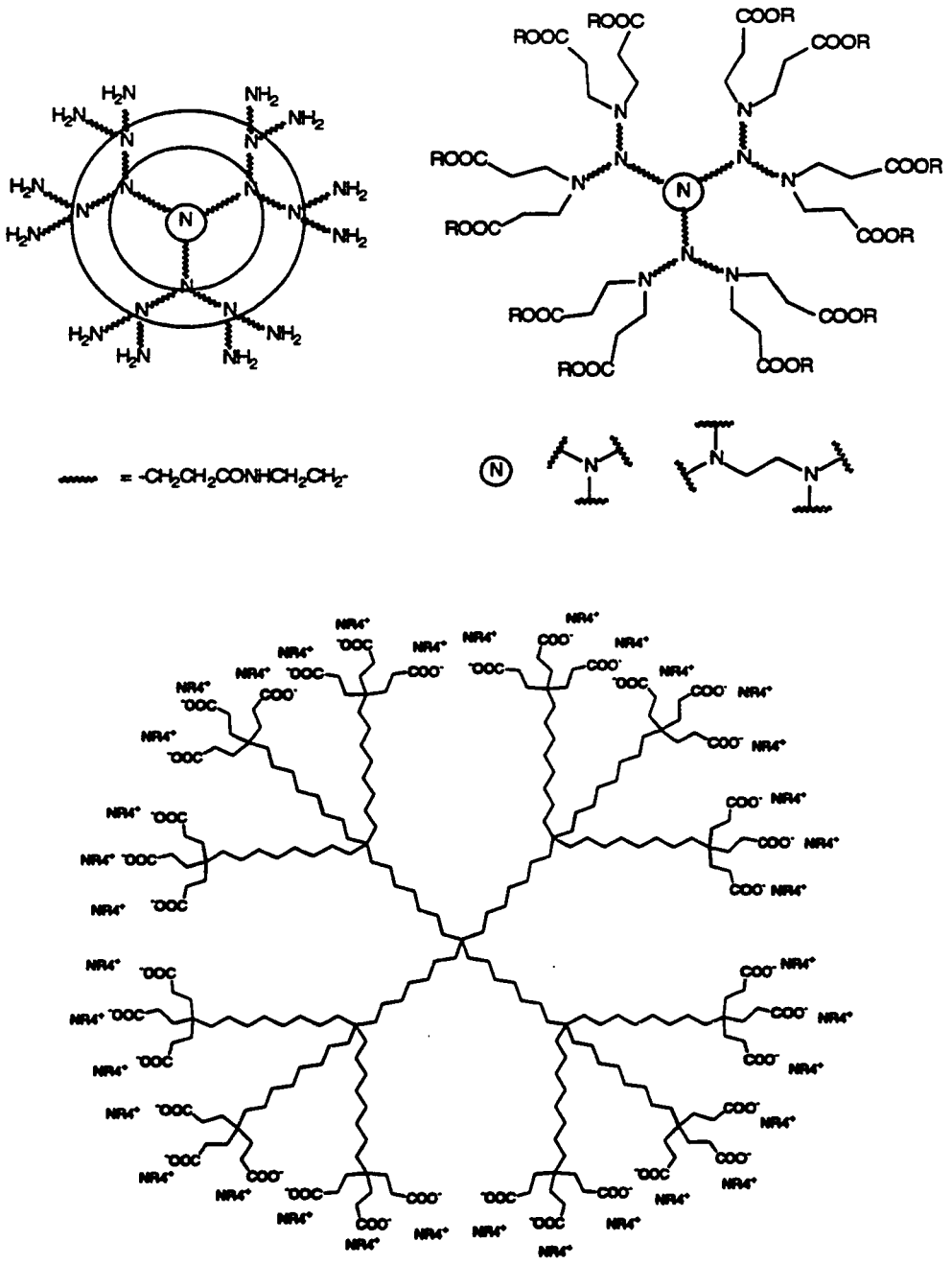

Figure 1.4.1. Tomalia's PAMAM dendrimers and Newkome's "unimolecular micelle".

Another types of dendrimer complexation takes place *via* physical encapsulation. Meijer and coworkers[51,52] reported the synthesis of the dendrimer host-guest complex by capping the peripheral functional groups. The entrapped small molecules can be released by removing the external functional groups in chemical approach.[53] Selective removal of the capping devices using photochemical or enzymetic methods would however be of great value in the development of a selective drug delivery system (Figure 1.4.2).

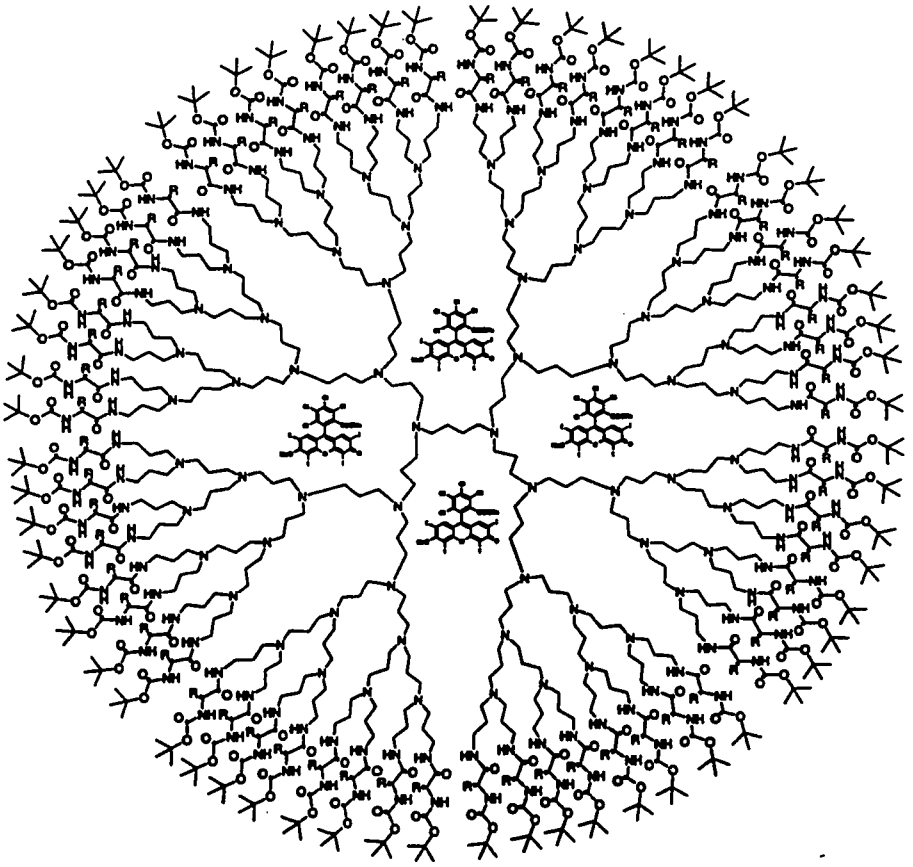

Figure 1.4.2. Meijer's dendritic box containing trapped Bengal Rose molecules.

[51] Jansen, J. F. G. A.; de Brabander-van den Berg, E. M. M.; Meijer, E. W. *Science*, **1994**, *266*, 1226.
[52] Jansen, J. F. G. A.; Jansen, R. A. J.; de Brabander-van den Berg, E. M. M.; Meijer, E. W. *Adv. Mater.* **1995**, *7*, 561.
[53] Jansen, J. F. G. A.; Meijer, E. W.; de Brabander-van den Berg, E. M. M. *J. Am. Chem. Soc.* **1995**, *117*, 4417.

As an example of complexation event on dendrimer periphery (selective binding of a guest molecule by a dendritic host), multiligand dendrimers on the surface were introduced. These multiligand dendrimers incorporate peptide dendrimers and glycodendrimers, which will be discussed in the following section (chapter 1.6). The presence of multiligands on a dendritic surface is particularly important in the area where the target receptors are multivalent and the diffusion of the particular ligand is a problem. Until recently, a variety of polymers was employed for this purpose. However, in contrast to dendrimers, polymers are heterogeneous and their structures are not defined. Furthermore, the flexibility of polymer chains make the three-dimensional structure variable and difficult to predict.

Tam *et al* [54,55] introduced dendrimers containing multiple peptides (MAP) for the production of antipeptide antibodies and synthetic vaccines. Compared to conventional peptide conjugates, where large protein carriers and small amounts of active peptides are present, more than 80% of the total weight of active peptides are conjugated to a relatively small dendritic lysine core (Figure 1.4.3).

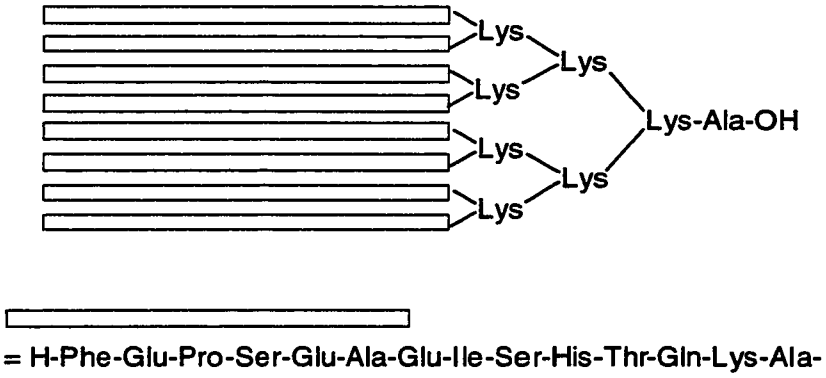

Figure 1.4.3. Multiple antigen peptide (MAP) system.

[54] Tam, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5409.
[55] Posnett, D. N.; Mcgrath, H.; Tam, J. P. *J. Biol. Chem.* **1988**, *263*, 1719.

Peptidic dendrimers were found to be more immunogenic than those conjugated to proteins and the antibodies induced by this peptidic dendrimer in rabbits and mice were specific to the peptidic dendrimers, as well as monovalent peptide without any reactivity toward the dendritic core.[54,55] Thus, peptidic dendrimers provide excellent scaffolds for high immunogenicity which eliminate the undesired immunological responses seen when carrier proteins are used.

1.5. Glycodendrimers

It is well established that multivalent ligands display the strong binding affinity to their carbohydrate specific lectins which present multiple binding sites. This result has been rationalized as "cluster (or multivalent) effect". As an extension of glycopolymers which exhibited the cluster effect very well in the biological assays, Roy and coworkers first reported many examples of glycodendrimers which showed the multivalent effect. The carbohydrates incorporated into dendritic cores include sialic acid,[56] D-mannose,[57] GlcNAc,[58] GalNAc,[59] β-lactose, *N*-acetyllactosaminide,[60] and T antigen.[61] A few examples are shown in Scheme 1.5.1.

The inhibitory enhancements of these synthetic glycodendrimers were measured using enzyme-linked lectin assay (ELLA). The results indicated that the carbohydrate valency required for maximum capacity is not necessarily correlated to the number of binding sites in the lectin. Also, higher generation glycodendrimers present weaker binding affinity due to the steric crowding on the surface or poor complementary molecular shapes.

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[56] (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 Symposium Series*, **1994**, *560*, 104.
[57] Pagé, D.; Zanini, D.; Roy, R. *Bioorg. Med. Chem.* **1996**, *4*, 1949.
[58] Zanini, D.; Roy, R. *Bioconjugate Chem.* **1997**, *8*, 187.
[59] Roy, R. *Topics Curr. Chem.* **1997**, *187*, 241.
[60] Zanini, D.; Park, W. K. C.; Roy, R. *Tetrahedron Lett.* **1995**, *36*, 7383.
[61] Baek, B. G. Ph.D. Dissertation, University of Ottawa, 1997.

Toyokuni[62] and Okada[63] employed amine-terminated PAMAM dendrimers as cores to construct PAMAM-based glycodendrimers. D-Glucose and D-mannose were attached to the dendrimer surface.[62a] Dimeric Tn antigen [63b] was, after conversion into the *N*-hydroxysuccinimide ester, incorporated to the PAMAM(G5) dendrimer (Scheme 1.5.2). These synthetic Tn antigens containing PAMAM-based glycodendrimers were however found to be nonimmunogenic in the antibody production experiment.

Since it was evident that the efficiency of multivalent carbohydrate-protein interactions was influenced by the ligand's shape, size, and valency number, the development of novel multivalent dendrimers was initiated.

Instead of building tethered or spherical glycodendrimers from the symmetrically polyfunctionalized core, structurally defined molecules, such as calix[n]arenes and cyclodextrins, were adopted as the focal points of dendrimers.

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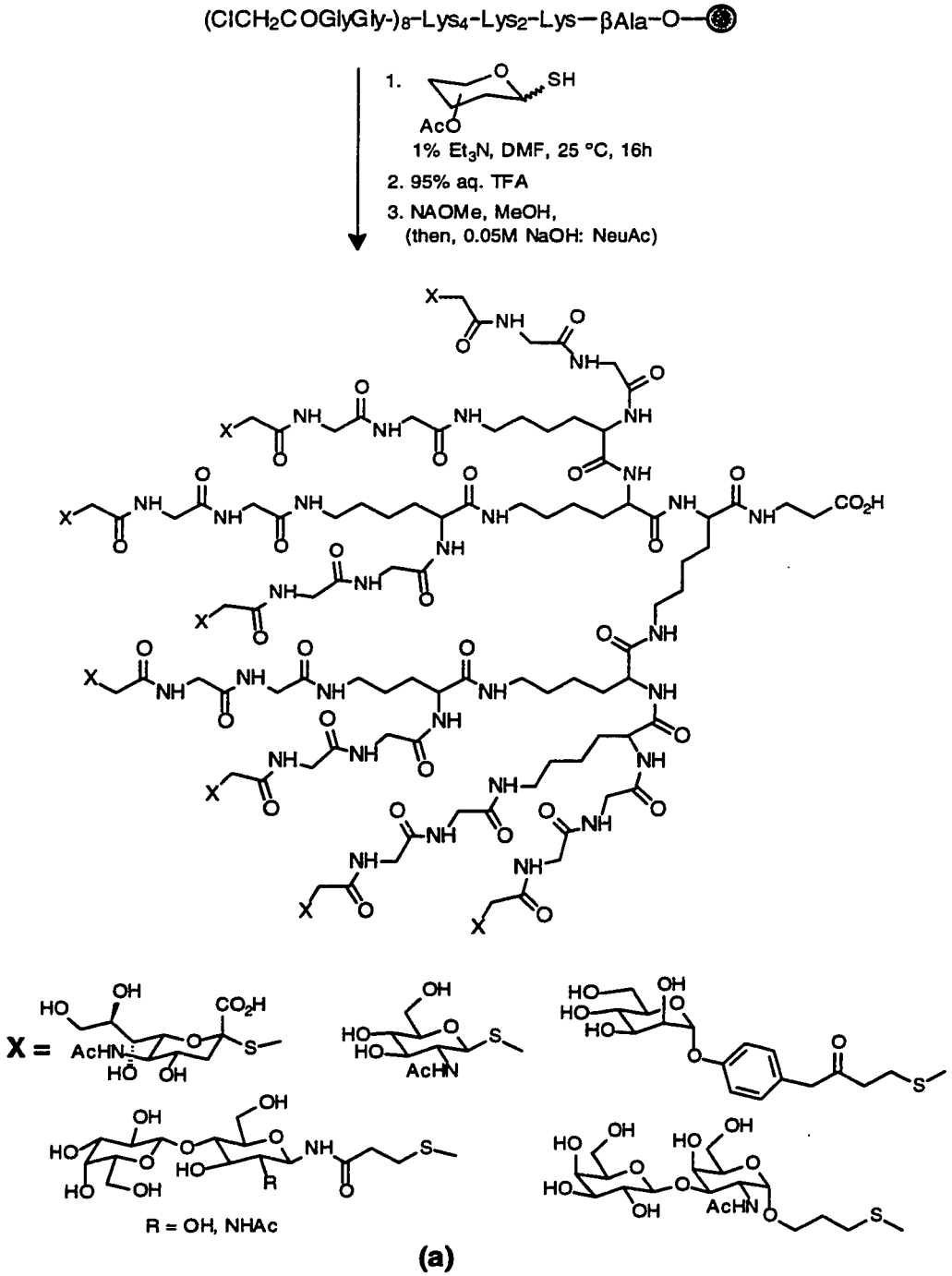
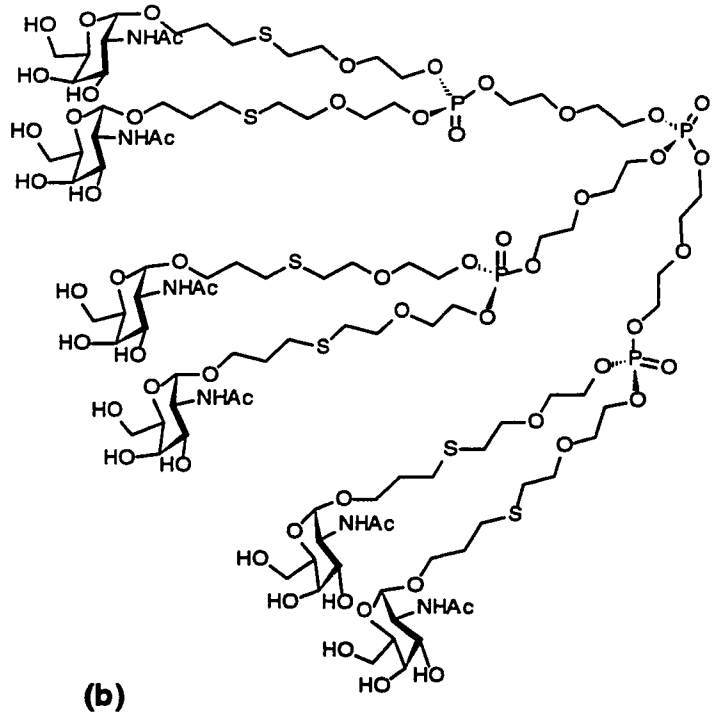
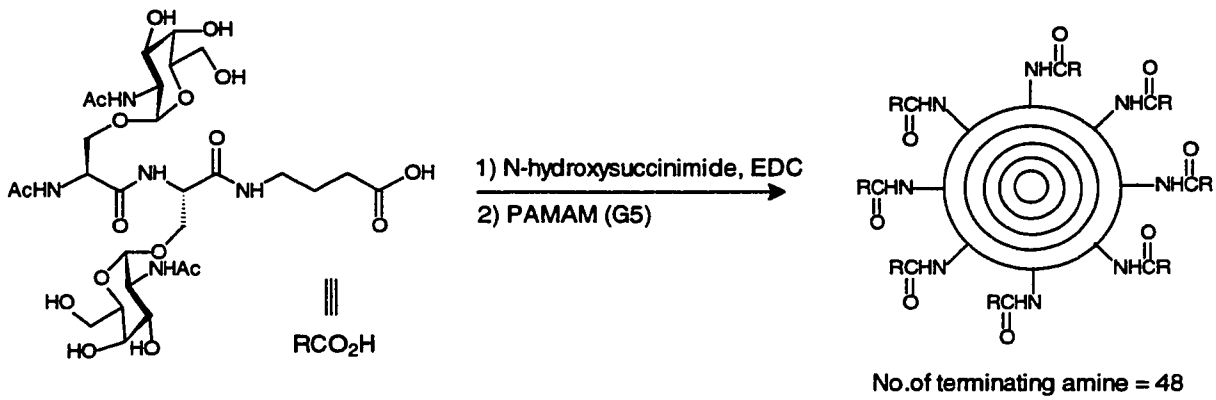
[62] (a) Toyokuni, T.; Hakomori, S.-I, Singhal, A. K. *Bioorg. Med. Chem.* **1994**, *2*, 1119. (b) Toyokuni, T.; Singhal, K. *Chem. Soc. Rev.* **1995**, 231.
[63] Aoi, K.; Itoh, K.; Okada, M. *Macromolecules* **1995**, *28*, 5391.


(ClCH2COGlyGly-)8-Lys4-Lys2-Lys—βAla-O—
1.
AcO
SH
1% Et3N, DMF, 25 °C, 16h
2. 95% aq. TFA
3. NAOMe, MeOH,
(then, 0.05M NaOH: NeuAc)
X =
R = OH, NHAc

(a)



Scheme 1.5.1. Glycodendrimers by Roy and coworkers[56-61]; (a) poly-L-lysine dendrimer, (b) phosphate dendrimer.



Scheme 1.5.2. PAMAM (G5) based glycodendrimers containing dimeric Tn antigen.

In many ways, calix[n]arenes are structurally and functionally related to cyclodextrins. These can be categorized as cyclic host molecules in the field of supramolecular chemistry. Cylodextrins (CDs) are cyclomaltooligosaccharides prepared from starch. The commonest forms are α-CD with six glucopyranose units and a cavity diameter of 500 pm, β-CD with seven units and a cavity diameter of 650 pm, and γ-CD which contains eight units and has a cavity 850 nm in diameter. The cavity of γ-CD is just large enough to encapsulate a C_{60} molecule if γ-CD forms a 2:1 complex with C_{60}[64](Figure 1.5.1). Similarly, the 1:1 complex of calix[8]arene and C_{60} can be employed for the purification of fullerenes[65,66] (Figure 1.5.2). These properties offer cheap and convenient methods for the rapid isolation of highly pure C_{60}. Also, the potential utility of inclusion phenomena can be employed to transport biologically active molecules[67] when they have biologically recognizable sites. Grafting bio-recognizable carbohydrate structures onto CDs[68,69,70,71,72,73] and calix[n]arenes[74,75,76] can therefore address this synthetic target (Scheme 1.5.3 and Scheme 1.5.4).

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[64] Anderson, T.: Nilsson, K.; Sundahl, M.; Westman, G.; Wennerstöm, O. *J. Chem. Soc., Chem. Commun.* **1992**, 604.
[65] Atwood, J. L.; Koutsantonis, G. A.; Raston, C. L. *Nature*, **1994**, *368*, 229.
[66] Suzuki, T.; Nakashima, K.; Shinkai, S. *Chem. Lett.* **1994**, 699.
[67] Szejtli, J. *Med. Res. Rev.* **1994**, *14*, 353.
[68] Derobertis, L.; Lancelonpin, C.; Driguez, H.; Attioui, F.; Bonaly, R.; Marsura, A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1127.
[69] Imata, H.; Kubota, K.; Hattori, K.; Aoyagi, M.; Jindoh, C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 109.
[70] Matsuda, K.; Inazu, T.; Haneda, K.; Mizuno, M.; Yamanoi, T.; Hattori, K.; Yamamoto, K.; Kumagai, H. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2353.
[71] Kassab, R.; Felix, C.; Parrot-Lópaz, H.; Bonaly, R. *Tetrahedron Lett.* **1997**, *38*, 7555.
[72] Hattori, K.; Imata, H.; Kubota, K.; Matsuda, K.; Aoyagi, M.; Yamamoto, K.; Jindoh, C.; Yamanoi, T.; Inazu, T. *J. Inclusion Phenom. Mol. Recgn.* **1996**, *25*, 69.
[73] Imata, H.; Kubota, K.; Hattori, K.; Aoyagi, M.; Jindoh, C. *Polym. J.* **1997**, *29*, 563.
[74] Marra, A.; Dondoni, A.; Sansone, F. *J. Org. Chem.* **1996**, *61*, 5155.
[75] Marra, A.; Scherrmann, M. -C.; Dondoni, A.; Casnati, A.; Minari, P.; Ungaro, R. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2479.
[76] Meunier, S. J.; Roy, R. *Tetrahedron Lett.* **1996**, *37*, 5496.

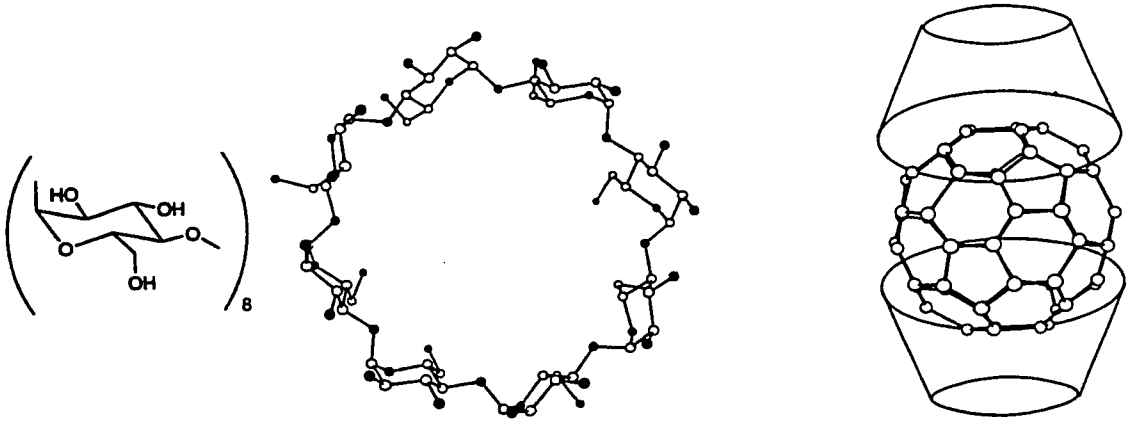


Figure 1.5.1. Two views of γ-CD and 2:1 complex of γ-CD and C_{60}.

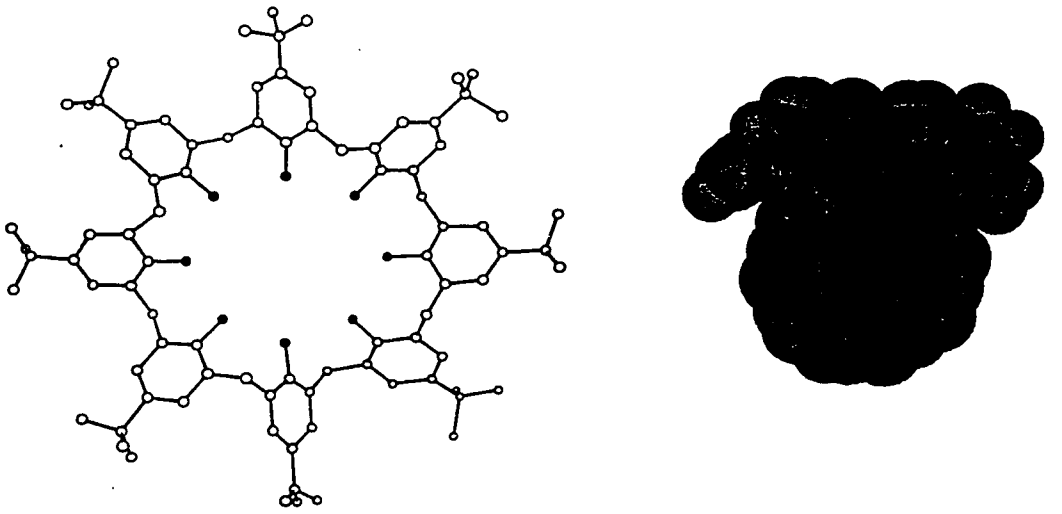
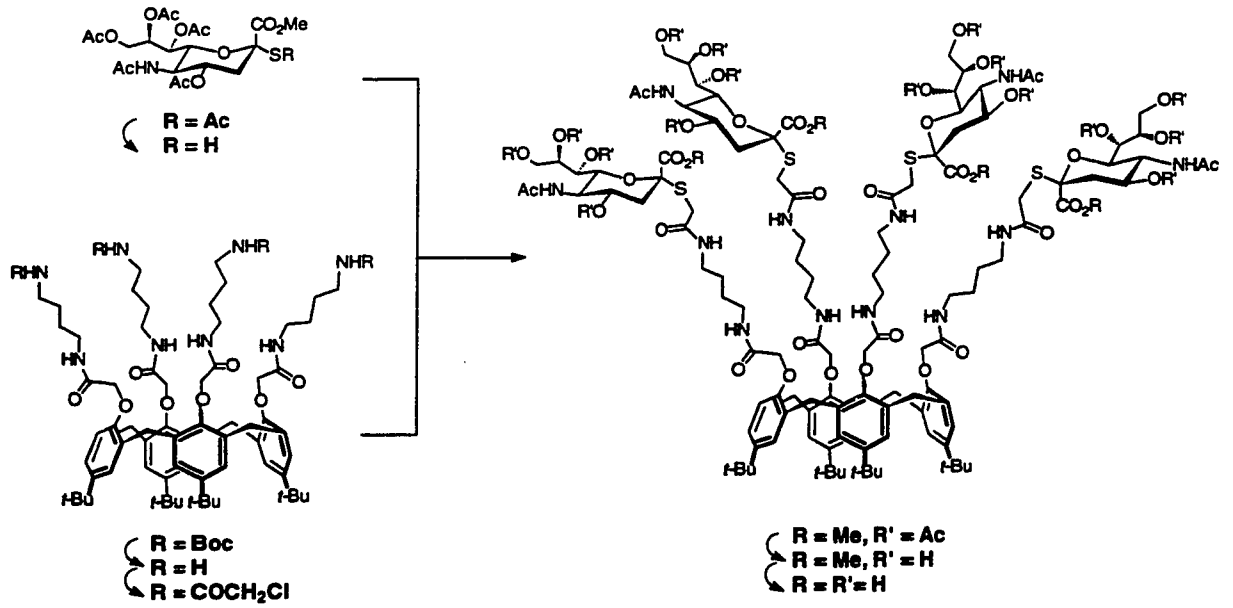
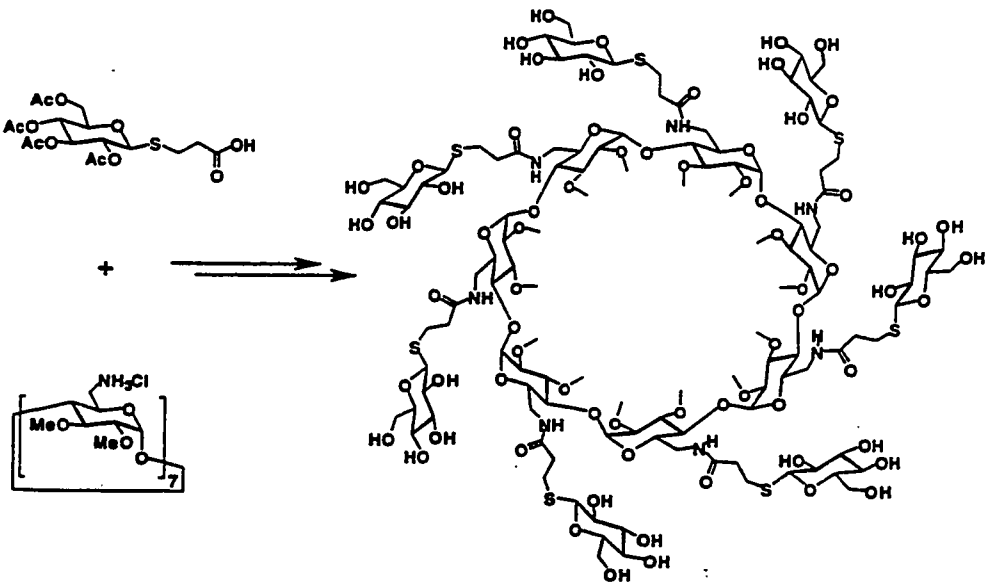


Figure 1.5.2. Structure of calix[8]arene and possible interaction with C_{60}.



Scheme 1.5.3.[76] Roy's glyco-calix[4]arene bearing sialic acid.



Scheme 1.5.4. Stoddart's β-cyclodextrin bearing sugar moiety.

1.6. Self-assembled dendrimers

Two main synthetic strategies for constructing dendrimers include the divergent approach[77] and the convergent approach.[78] In the divergent approach, succesive branches are added to a central core out to the periphery. In contrast, the convergent approach builds the dendrimers from the periphery toward the central core. However, these approaches require tedious and lengthy procedures to build high generation dendrimers. An attractive strategy induces the assembly of simpler hyper-branched building blocks (called dendrons) into dendritic structures.

Utilization of metal ion coordination to nucleate dendrons has received significant attention because organometallic dendrimers contain multi-electron transfer redox centers and thus have potential applications. These include novel catalysts,[79] molecular electronic and photochemical devices for information storage and switching,[80] and as energy transfer and conversion devices.[81,82]

Although many dendrimers that contain metal complexes at the surface[83] have been prepared, only a few examples describe dendrimers that have metal only at the central core.

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[77] (a) Denkewalter, R. G.; Kolc, J. F.; Lukasavage, W. J. U.S. Pat. 4,410,688, 1983, (b) Buhleier, E.; Wehner, W.; Vögtle, F. *Synthesis* **1978**, 155, (c) de Brabander-van den Berg, E. M. M.; Meijer, E. W. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1308, (d) Worner, C.; Mulhaupt, R. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1306, (e) Tomalia, D. A. *Aldrichimica Acta* **1993**, *26*, 91, (f) Newkome, G. R.; Gupta, V. K.; Baker, G. R.; Yao, Z.-Q. *J. Org. Chem.* **1985**, *50*, 2003.

[78] (a) Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1990**, *112*, 7638, (b) Miller, T. M.; Neenan, T. X.; *Chem. Mater.* **1990**, *2*, 346, (c) Xu, Z. F.; Moore, J. S. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1354, (d) Xu, Z. F.; Moore, J. S. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 246.

[79] (a) Wulff, G. *Angew. Chem.,Int.. Ed. Engl.* **1995**, *34*, 1812, (b) Bergbreiter, D. E. *Macromol. Symp.* **1996**, *105*, 9, (c) *Polymeric reagents and Catalysts* (Ed.: Ford, W. T.) ACS Symposium series 308, American Chemical Society: Washington, DC, **1986**.

[80] (a) Ward, M. D. *Chem. Soc. Rev.* **1995**, *24*, 121, (b) Fabbrizzi, L.; Poggi, A. *Chem. Soc. Rev.* **1995**, *24*, 197, (c) Diamond, D.; McKervey, M. A. *Chem. Soc. Rev.* **1996**, *25*, 1524, (d) Yu, L.; Chan, W. K.; Peng, Z.; Gharavi, A. *Acc. Chem. Res.* **1996**, *29*, 13.

[81] (a) Lehn, J. M. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1304, (b) Whitesides, J. M.; Mathias, J. P.; Seto, C. T. *Science*, **1991**, *254*, 1312.

[82] For new applications of metallic dendrimers: (a) Haggin, J. *Chem. Eng. News* **1995** (Feb. 6), 26, (b) Balzani, V. *New Sci.* **1994** (Nov), 31, (c) Knapen, J. W. J., van der Made, A. W.; de Wilde, J. C.; van Leeuwen, P. W. N. M.; Wijkens, P.; Grove, D. M.; van Koten, G. *Nature* **1994**, *372*, 659, (d) Constable, E. C.; Harverson, P. *J. Chem. Soc., Chem. Commun.* **1996**, 33, (e) Dagani, R. *Chem. Eng. News* **1996** (July 8), 26, (f) Balzani, V.; Juris, A.; Venturi, M.; Campagna, S.; Serroni, S. *Chem. Rev.* **1996**, *96*, 759.

[83] (a) Newkome, G. R.; Moorefield, C. N. *Macromol. Symp.* **1994**, *77*, 63, (b) Cloutet, E.; Fillaut, J. -L.; Gnanou, Y.; Astruc, D. *J. Chem. Soc., Chem. Commun.* **1994**, 2433, (c) Ottaviani, M. F.; Bossmann, S.;

These metal-coordinated dendrimers can be prepared by complexation[84,85,86] of pre-made dendrons around the metal ion or by synthetically linking dendrons to a preformed metal complex, e.g., a zinc porphyrin.[87] Dendrimers based on a metal porphyrin core have been reported to exhibit quite interesting electrochemical[87b] and photophysical properties.[87c]

This non-covalent approach to the assembly of dendritic structures has unique features: (1) the symmetry of resulting structure and geometrical arrangement are governed by the coordination number and geometry of the selected metal ion, as well as by the degree of branching in the dendron, (2) a stepwise synthesis is required only for the dendron, (3) morphologies that are not easily accessible from polyfunctional organic compounds can be assembled by utilizing metal ions with high coordination numbers, (4) unsymmetrical structures composed of two or three different dendrons (e.g., $[M(L)_3]^{n+}$, $[M(L)_2(L')]^{n+}$, $[M(L)(L')_2]^{n+}$, or $[M(L)(L')(L'')]^{n+}$) can be assembled by a controlled and stepwise complex formation.

Some examples of dendrimers induced by metal ion complexation are shown in Figure 1.6.1, Figure 1.6.2, and Figure 1.6.3.

Turro, N. J.; Tomalia, D. A. *J. Am. Chem. Soc.* **1994**, *116*, 661, (d) Liao, Y. -H.; Moss, J. R. *Organometallics* **1995**, *14*, 2130.

[84] Tzalis, D.; Tor, Y. *Tetrahedron Lett.* **1996**, *37*, 8293.

[85] Newkome, G. R.; Guther, R.; Moorefield, C. N.; Cardullo, F.; Echegoyen, L.; Pérez-Cordero, E.; Luftmann, H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2023.

[86] Chow, H. F.; Chan, I. Y. K.; Chan, D. T. W.; Kwok, R. W. M. *Chem. Eur. J.* **1996**, *2*, 1085.

[87] (a) Dandliker, P. J.; Diederich, F.; Gross, M.; Knobler, C. B.; Louati, A.; Sanford, E. M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1739, (b) Dandliker, P. J.; Diederich, F.; Gisselbrecht, J. -P.; Louati, A.; Gross, M. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2725, (c) Sadamoto, R.; Tomioka, N.; Aida, T. *J. Am. Chem. Soc.* **1996**, *118*, 3978, (d) Bhyrappa, P.; Young, J. K.; Moore, J. S.; Suslick, K. S. *ibid.* **1996**, *118*, 5708.

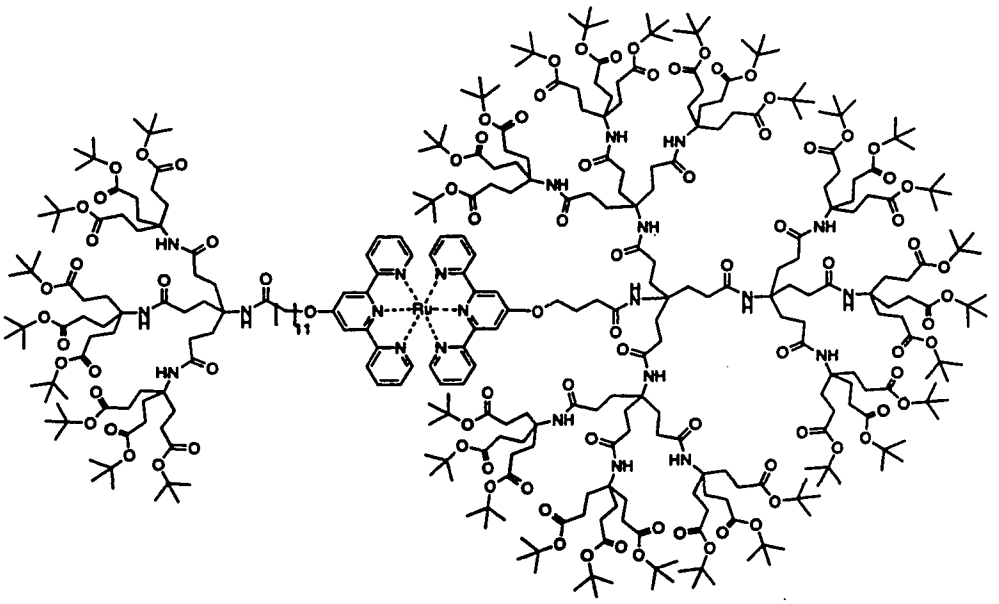


Figure 1.6.1. Newkome's dendritic ruthenium complex.

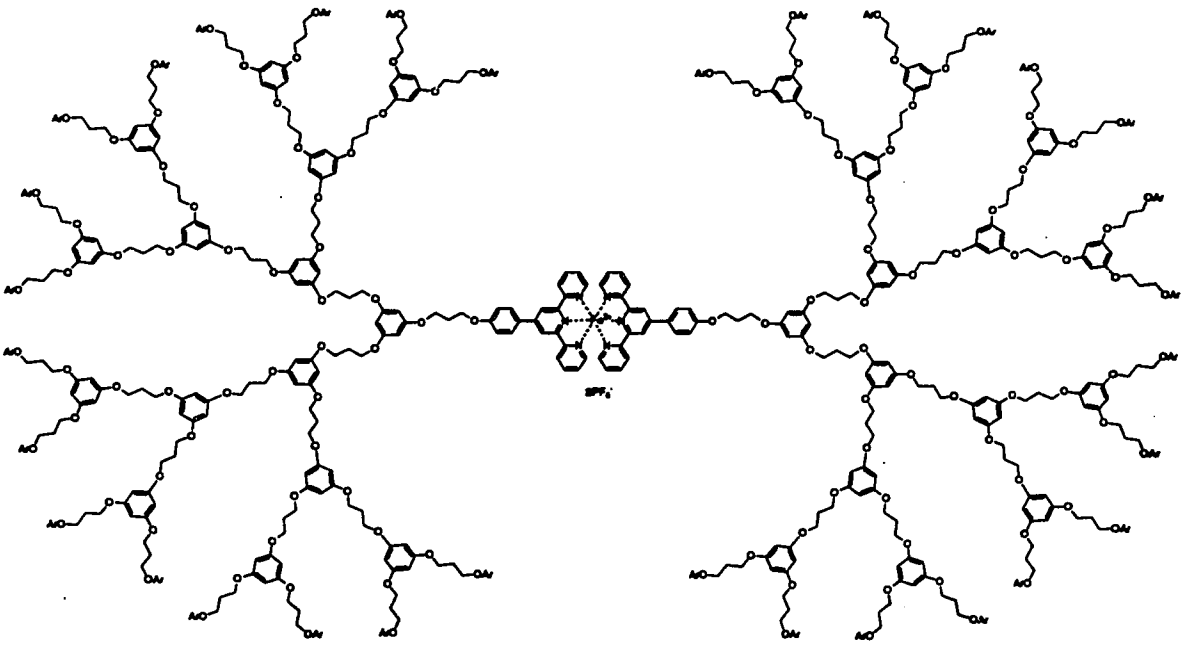
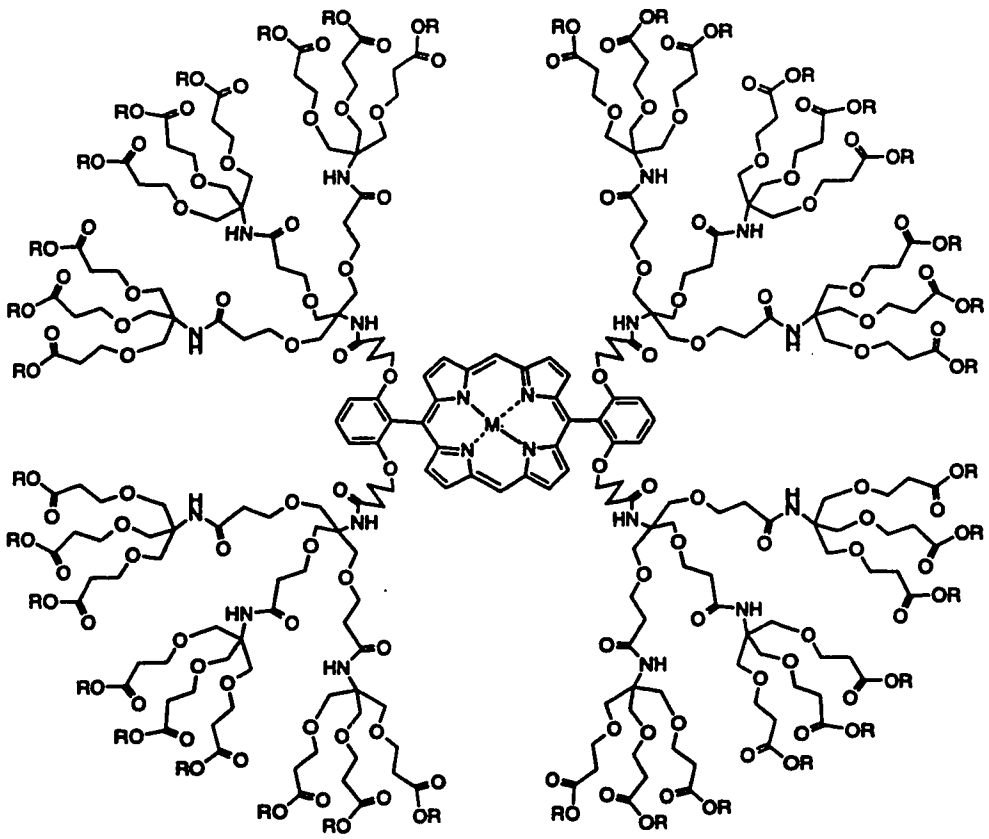


Figure 1.6.2. Chow's dendritic iron(II) complex.



M = Zn (II), R = CH_3 -
M = Fe (III) or Fe(II),R =$(CH_2CH_2O)_3$ CH_3 -

Figure 1.6.3. Dendritic metalloporphyrins.

1.7. Glycopolymers

Protein-protein, protein-nucleic acid and protein-hormone interactions are considered to guide recognition processes like information exchange and transfer. It is obvious that naturally occurring oligomers from sets of amino acids and nucleotides display coding ability. However, considering oligosaccharides of cellular glycoconjugates[88] such as glycoproteins and glycolipids, the ability to code and store information is not limited to peptides and nucleic acids.

The enhanced coding versatility of carbohydrates are explained by several factors: formation of anomers, variation in the positions of glycosidic linkage, branching, further modifications like site-specific sulfation, phosphorylation, O-acetylation or lactonisation.

As described previously (chapter 1.1), recognizable protein-carbohydrate interactions at the cell surfaces play important roles in understanding the pathogenesis of disease and designing rational therapeutics.[89] Therefore, analysis of expression of glycoconjugates and of glycoligand specific receptors such as endogenous lectins can contribute to various biomedical applications. However, the affinities of free carbohydrates in solution for carbohydrate receptors are often only in the millimolar range and their localization or quantity for binding is undetectable. Conjugation of the carbohydrate moieties to a macromolecular carrier can thus address these problems by increasing the avidity of a ligand for its receptor sites by means of clustering and spatially associating the ligand with a label (Figure 1.7.1).

These well defined polymers with pendent carbohydrate residues are of interest as cell-specific biomedical materials,[90,91,92] as pharmacological substances,[93,94] and

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[88] Springer, G. F. *Science* **1984**, *224*, 1198.

[89] (a) Gabius, H.-J. *Biochim. Biophys. Acta* **1991**, *1071*, 1, (b) Geisow, M. J. *Trends Biotechnol.* **1991**, *9*, 221, (c) Karlsson, K.-A, *Trends Pharmacol. Sci.* **1991**, *12*, 265, (d) Schnaar, R. L. *Adv. Pharmacol.* **1992**, *23*, 35, (e) *Lectins and Glycobiology* (Eds.: Gabius, H.-J., Gabius, S.; Springer, G. F.) Heidelberg, New York, **1993**.

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

[91] Kobayashi, K.; Sumitomo, H.; Ina, Y. *Polym. J.* **1985**, *17*, 567.

[92] Kugumiya, T.; Yagawa, A.; Maeda, A.; Nomoto, H.; Tobe, S.; Kobayashi, K.; Matsuda, T.; Onishi, T.; Akaike, T. *J. Bioactive Compatible Polym.* **1992**, *7*, 337.

also as tools for investigating biological recognition phenomena.[95,96,97] Clustered carbohydrates are known to be effective as recognition signals.[98]

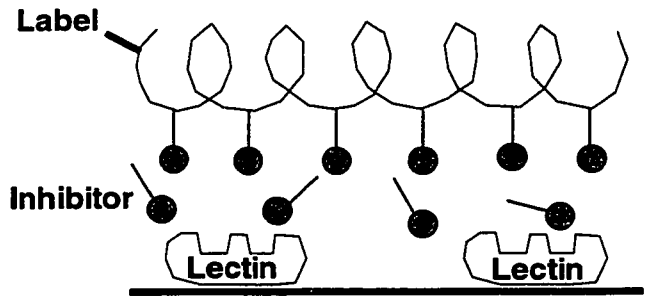

Figure 1.7.1. Schematic illustration of the application of carbohydrate ligand-bearing conjugates as lectin-seeking probes, whose access to the carbohydrate-binding sites can be blocked by the presence of free ligand inhibitors.

Matta *et al* [99] synthesized acrylamide copolymers containing either T-(Galβ1→3GalNAcα-) or Tn (-GalNAcα-) haptens and studied their interaction with the lectins of peanut (PNA), *Agaricus bisporus* (ABA), *Helix pomatia* (HPA) and *Vicia villosa* B_4 (VVA), using asialo Cowper's gland mucin (ACGM). This mucin contains both T and Tn epitopes and was used as the coating substrate in an enzyme-linked lectin assay (ELLA). Both T and Tn copolymers showed high affinity and specificity; the T-copolymer showed 50% inhibition of interaction of either PNA or ABA with ACGM at 0.05~0.07 μM concentration and the Tn-copolymer at 0.02-0.05 μM inhibited HPA or

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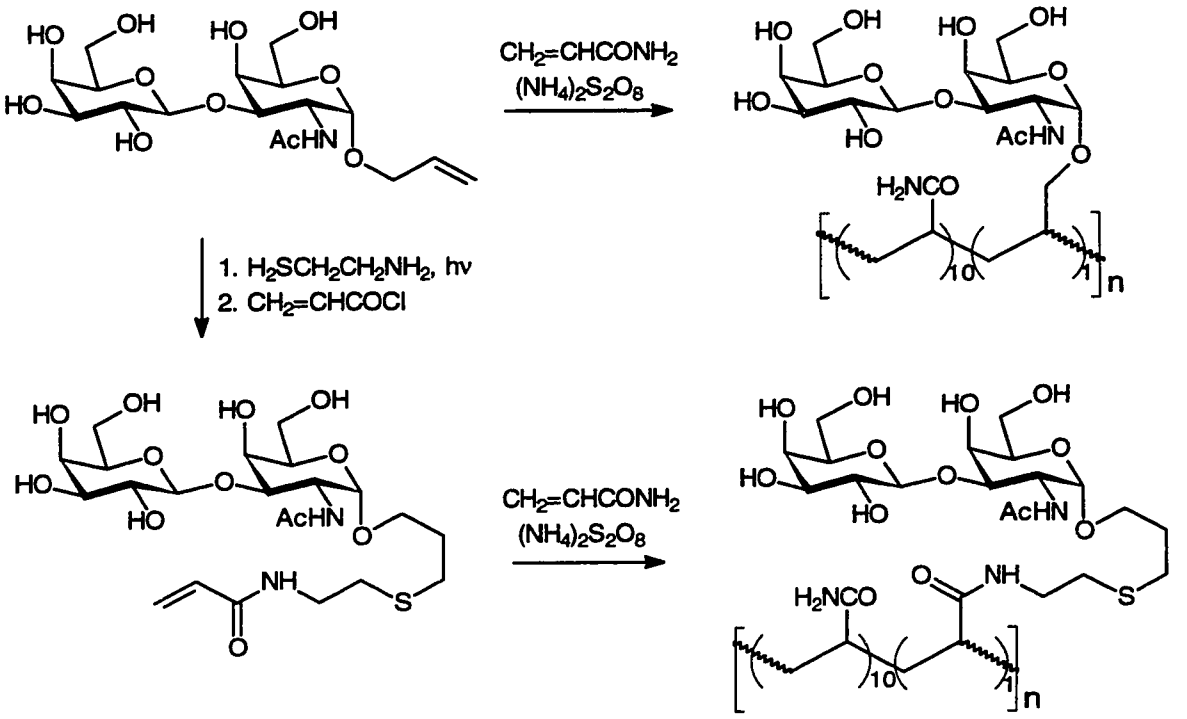
[93] Duncan, R.; Kopeckova-Rejmanova, P.; Strohalm, J.; Hume, I.; Cable, H. C.; Pohl, J.; Lloyd, J. B.; Kopecek, J. *Br. J. Cancer* **1987**, *55*, 165.
[94] Rozalski, A.; Brade, L.; Kuhn, H.-M.; Brade, J.; Kosma, P.; Appelmek, B. J.; Kusumoto, S.; Paulsen, H. *Carbohydr. Res.* **1989**, *193*, 257.
[95] Koyama, Y.; Yoshida, A.; Kurita, K. *Polym. J.* **1986**, *18*, 479.
[96] Roy, R.; Tropper, F. *J. Chem. Soc., Chem. Commun.* **1988**, 1058.
[97] Nishimura, S.; Matsuoka, K.; Furuike, T.; Ishii, S.; Kurita, K.; Nishimura, K.. *Macromolecules* **1991**, *24*, 4236.
[98] Lee, Y. C. *Carbohydrate Recognition in Cellular Function*, Wiley, Chichester **1989**, (Chiba Foundation Symp. 145), p80.
[99] Chen, Y.; Jain, R. K.; Chandrasekaran, E. V.; Matta, K. L. *Glycoconjugate J.* **1995**, *12*, 55.

VVA interaction with ACGM by 50%. These concentrations are much lower than those required for monomeric epitopes to inhibit the interactions. For instance, the typical T-structure (Galβ1→3GalNAcα-*O*-allyl) showed 50% inhibition of interactions of PNA and ABA at 0.23 mM and 1.25 mM, respectively, and the Tn-structure (GalNAcα-*O*-allyl) inhibited HPA and VVA interactions with ACGM by 30% at 4 mM and 50% at 1 mM, respectively.

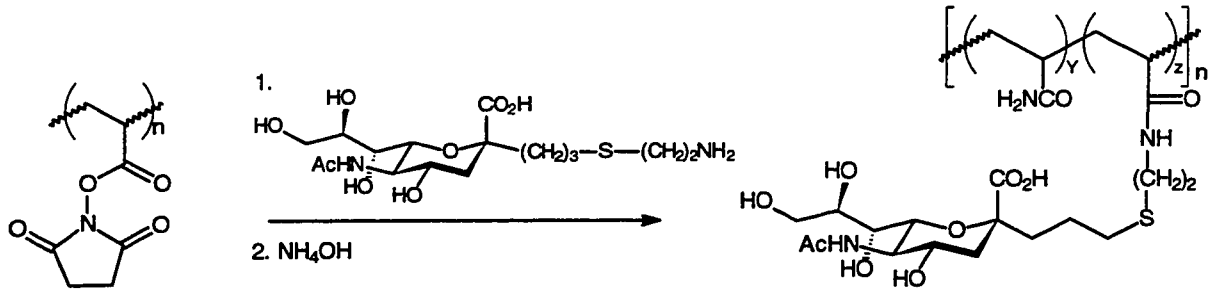
The acrylamide T- and Tn-copolymers were also used as coating substrates in enzyme-linked immunoassays to measure the serum level of anti-T and anti-Tn antibodies in breast cancer patients and normal females. The results indicated that a significant depression in the serum level of anti-T (two to three-fold decrease) and anti-Tn (two-fold decrease) antibodies in breast cancer compared with normal control subjects.



Scheme 1.7.1. Synthesis of copolymer containing T-antigen.

The copolyacrylamide polymer derived from allyl α-glycoside of the T-antigen disaccharide was prepared in our group[100] (Scheme 1.7.1).

Instead of preparing carbohydrate monomers, activated polymers of known molecular weight can also be synthesized.[101] This approach provides constant molecular weight for a given family of glycopolymers. As a pre-activated polymer, poly[*N*-(acryloxy)succinimide] has been prepared[100,102] and this was employed to synthesize copolyacrylamides containing a *C*-glycoside of sialic acid (Scheme 1.7.2).



Scheme 1.7.2. Synthesis of copolyacrylamide containing the *C*-glycoside of sialic acid.

1.8. C-Glycosides

Recently, there have been increasing attention to the chain elongation of saccharides at the anomeric position to give *C*-glycosides. This class of compounds are hydrolytically stable analogs of normal *O*-glycosides, thus attracts intense synthetic and biochemical interest.[103]

The synthesis of *C*-glycosides in ionic reactions involves the attack of an appropriate *C*-nucleophile onto the electrophilic anomeric center. An "umpolung"

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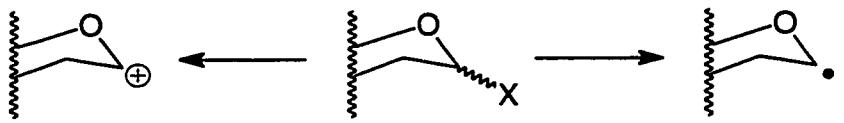
[100] Baek, B.G. Ph. D. Dissertation, University of Ottawa, 1997.
[101] Bovin, N. V.; Korchagina, E. Y.; Zemlyanukhina, T. V.; Byramova, N. E.; Ivanov, A. E.; Zubov, V. P.; Mochalova, L. V. *Glycoconjugate J.* **1993**, *10*, 142.
[102] Sigal, G. B.; Mammen, M.; Dahmann, G.; Whitesides, G. M. *J. Am. Chem. Soc.* **1996**, *118*, 3789.
[103] Recent reviews: (a) Hanessian, S.; Pernet, A. G. *Adv. Chem. Biochem.* **1976**, *33*, 111, (b) Postema, M. H. R. *Tetrahedron* **1992**, *48*, 8545, (c) Levy, D. E.; Tang, C. *The Chemistry of C-Glycosides* (Eds.: Baldwin, J. E.; Magnus, P. D.) Elsevier Science Ltd.: Oxford, **1995**.

method has been developed for this purpose. However, homolytic or radical reactions can also be applied to form C-C bonds (Scheme 1.8.1).

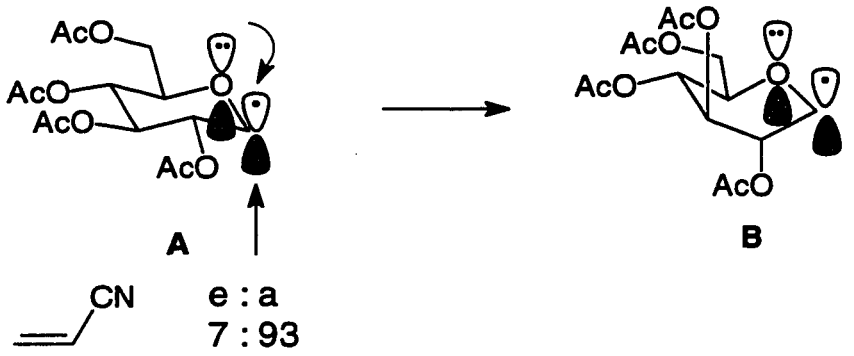


electrophile **nucleophile-like**

Scheme 1.8.1. Homolytic reaction for *C*-glycosidation formation.

Here, the high-lying SOMO of an alkoxyalkyl radical can interact with the LUMO of an electron-poor alkene. The presence of electron-withdrawing substituents in alkenes lowers the LUMO energy and increases the addition rate by reducing the SOMO-LUMO difference.[104] Therefore, the addition of a glycosyl radical to an electron-poor olefin is an extremely appealing approach for the construction of C-glycosides.

One important aspect of free radical chemistry at the anomeric center is the predictable stereochemistry of hexopyranosyl radicals.

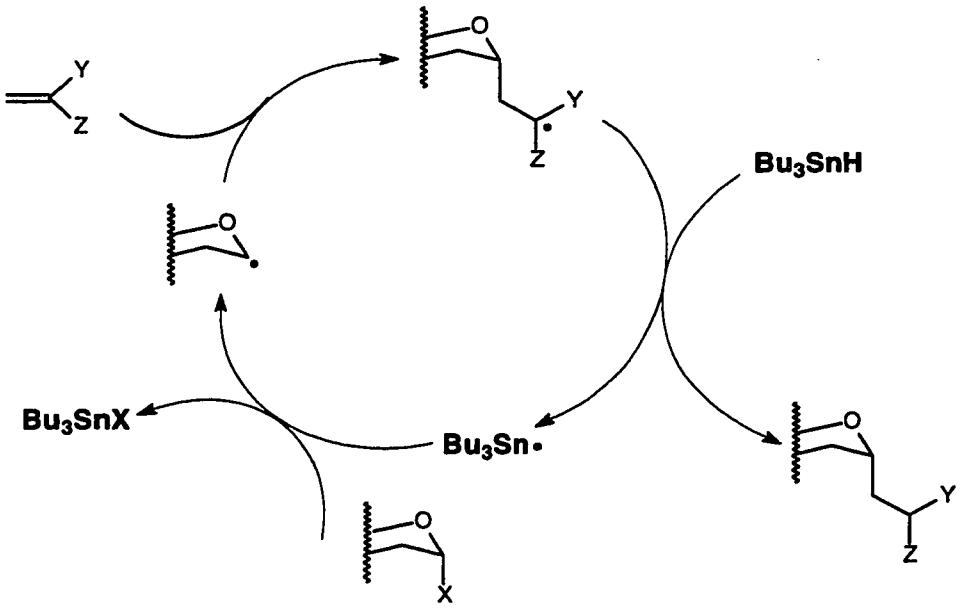


Scheme 1.8.2. Mechanism of *C*-glycoside formation by the radical pathway.

[104] Giese, B. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 753.

As shown in Scheme 1.8.2, the anomeric radical reacts with acrylonitrile to give an axial-substituted adduct preferentially. This phenomenon can be explained by a stereoelectronic effect.[105] The conformation adopted by a D-glucopyranosyl radical is not $^4C_1$, **A** but the distorted $B_{2,5}$ shape **B**.[106,107] The equatorial-like attack at the boat conformer leads to the predominant formation of the observed α-*C*-glycosides. In addition, during the attack the stabilizing conjugative interaction between the lone pair at the ring oxygen and SOMO is maintained.

One of the common methods to form a C-C bond by addition of a radical to a C-C multiple bond in intermolecular or intramolecular system is the tin hydride method.[108,109,110] The general reaction process is outlined in Scheme 1.8.3.



Scheme 1.8.3. *C*-glycosidation by tin hydride method.

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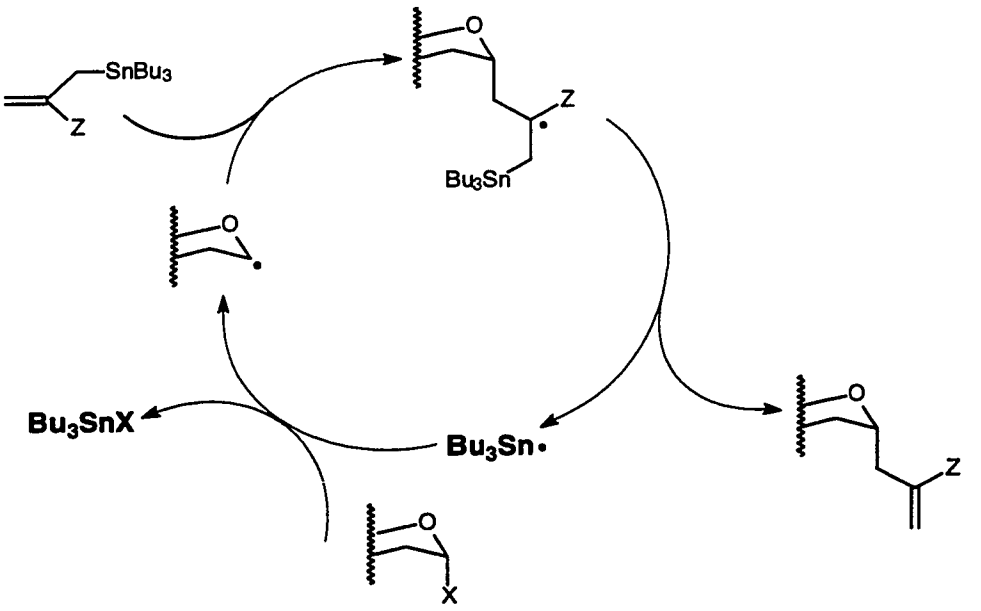
[105] Giese, B.; Dupuis, J.; Gröninger, K.;Hasskerl, T.; Nix, M.; Witzel, T. *Substituent Effects in Radical Chemistry* (Eds.: Viehe, H. G. et al), **1986**, p283.
[106] Dupuis, J.; Giese, B.; Rüegge, D.; Fischer, H.; Korth, H.-G.; Sustmann, R. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 896.
[107] Korth, H.-G.; Sustmann, R.; Dupuis, J.; Giese, B. *J. Chem. Soc. Perkin Trans.* **1986**, *2*, 1453.
[108] Giese, B. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 553.
[109] Giese, B.; Dupuis, J. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 622.
[110] Adlington, R. M.; Baldwin, J. E.; Basak, A.; Kozyrod, R. P. *J. Chem. Soc. Chem. Commun.* **1983**, 944.

The tin hydride method is useful only when the abstraction of X by the stannyl radical is rapid enough to compete with hydrostannylation of the alkene. Thus, this method is applicable to bromides, phenylselenides, thiocarbonyl esters, and to tertiary nitro glycosides.



Scheme 1.8.4. *C*-Glycosidation by addition of the allyl tin compounds.

Another useful method for the formation of *C*-glycosides is the fragmentation method.[111] The mechanism of this reaction is illustrated in Scheme 1.8.4.

Due to the absence of tin hydride, intermediate radicals are less susceptible to hydrogen atom abstraction. Thus, the alternative pathway is the fragmentation of the adduct radical to form allylated compound by β-bond cleavage. This method can be applied to relatively unreactive glycosyl precursors, such as glycosyl chlorides and phenylsulfides.

2,2'-Azobisisobutyronitrile is the most commonly employed initiator, with a half-life time for unimolecular scission of 1 hour at 80 °C.

1.9. Immunochemical Techniques

1.9.1. Lectins

As mentioned previously, the binding studies of carbohydrates and their specific protein receptors (lectins) are of great value in understanding diverse biological processes.[112,113,114]

Lectins have been used for the separation and characterization of glycopeptides and glycoproteins for typing blood cells, for separating erythrocytes from leucocytes and for studying cell-surface interactions.[115] The specificity of lectin is often very high, yet the affinity of the lectins for monosaccharides is usually weak with association constants in the millimolar range.[116,117] This is the point where the glycoside cluster effect becomes of great value.

The Tn epitope, *N*-acetyl-D-galactosamine-α-*O*-Ser/Thr (GalNAcα-*O*-Ser/Thr), represents one of the most specific carcinoma-associated antigens identified by monoclonal antibodies.[118] Immune recognition of the Tn antigen by antibodies is explained by an inherent genetic block of biosynthetic step to Galβ1→3GalNAcα-*O*-Ser/Thr and this blockage is associated with the inability of tumor cells to complete normal carbohydrate synthesis.[119,120] In addition to specific antibodies, some lectins have been reported to recognize the Tn epitope. Among them, the isolectin B_4 isolated[121] from *Vicia villosa* seeds displayed a specificity similar to that of anti-Tn

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[111] (a) Keck, G. E.; Enholm, E. J.; Yates, J. B.; Wiley, M. R. *Tetrahedron* **1985**, *41*, 4079, (b) Keck, G. E.; Yates, J. B. *J. Am. Chem. Soc.* **1982**, *104*, 5829.
[112] Sharon, N.; Lis, H. *Science* **1989**, *246*, 227.
[113] Sharon, N.; Lis, H. *Sci. Am.* **1993**, *268* (1) , 82.
[114] Kornfield, S.; Kornfield, R. In *The Glycoconjugates* (Eds.: Horowitz, M. I., Pigman, W.), Vol II, Academic Press Inc., New York, **1978**, p437.
[115] Sharon, N.; Lis, H. *Fed. Amer. Soc. Exp. Biol. J.* **1990**, *4*, 3198.
[116] Sharon, N.; Lis, H. *Lectins*; Chapman and Hall: London, **1989**, p127.
[117] Goldstein, I. J.; Poretz, R. D. In *The Lectins: Properties, Functions and Apllications in Biology and Medicine* (Eds.: Liener, I. E.; Sharon, N.; Goldstein, I. J.) Academic Press Inc., Orlando, **1986**, p35.
[118] Hirohashi, S.; Clausen, H.; Yamada, T.; Shimosato, Y.; Hakamori, S. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7039.
[119] Springer, G. *Science*, **1984**, *224*, 1198.
[120] Hakomori, S. *Adv. Cancer Res.* **1989**, *52*, 257.
[121] Tollefsen, S.; Kornfield, R. *J. Biol. Chem.* **1983**, *258*, 5165.

monoclonal antibodies.[122,123] Seeds of *Vicia villosa* contain different molecular forms of lectins assembled by combination of two subunits A (M.W. 33,600) and B (M.W. 35,900).[121] The pure isoforms A_4 and B_4 have distinct carbohydrate-binding specificity and the hybrid A_2B_2 shares the binding properties of both forms. Tollefsen and Kornfield[122] showed that the B_4 lectin agglutinated erythrocytes bearing the Tn antigen and that *N*-acetylgalactosamine was the best monosaccharide inhibitor of the binding of the B_4 lectin to Tn erythrocytes. The specificity of B_4 lectin for Tn antigen is presented in Table 1.9.1.

Table 1.9.1. Inhibition by various sugars of *Vicia villosa* B_4 lectin binding to erythrocytes bearing the Tn antigen[a].

Inhibitor	Relative inhibitory potency[b]
N-Acetylgalactosamine	1.0
p-Nitrophenyl *N*-acetyl-α-D-galactosaminide	0.85
p-Nitrophenyl *N*-acetyl-β-D-galactosaminide	1.05
Galactose	0.005
Methyl α-D-galactopyranoside	0.05
Methyl β-D-galactopyranoside	0.005
p-Nitrophenyl α-D-galactopyranoside	0.014
p-Nitrophenyl β-D-galactopyranoside	0.015
D-Galactosamine HCl	0.001
D-*N*-Acetylglucosamine	0.0002
D-Mannose	0.0002
Galβ1→3GalNAc	0.014

[a] From ref.122.
[b] *N*-Acetylgalactosamine is normalized to 1.0 (0.04 mM required for 50% inhibition).

[122] Tollefsen, S.; Kornfield, R. *J. Biol. Chem.* **1983**, *258*, 5172.
[123] Itzkowitz, S.; Yuan, M.; Montgomery, C.; Kjeldsen, T.; Takahashi, H.; Bigbee, W.; Kim, Y. *Cancer Res.* **1989**, *49*, 197

1.9.2. Turbidimetric assay

Turbidimetric analysis in one of the simple, qualitative forms of testing for agglutination. When an antibody or lectin combines with an antigen or glycoconjugate, it forms a precipitate of antibody (lectin)/antigen (glycoconjugate) complex.

The glycoconjugate being tested is placed in a microtiter well together with the lectin. Optical density (O. D.) is then measured as a function of time.

In the case of excess glycoconjugate all the lectin binding sites are occupied, resulting in the formation of small, soluble complex of lectin/glycoconjugate (Figure 1.9.2.1. a). If the lectin is in excess all of the glycoconjugate molecules may be bound and again small soluble complexes are formed (Figure 1.9.2.1.b). But if neither is in excess, then some of the reactions between lectin and glycoconjugate will be with adjacent molecules resulting in the formation of a lectin/glycoconjugate polymer or lattice (Figure 1.9.2.1.c). Precipitation occurs when the ratio of lectin to glycoconjugate favors multiple attachments between them ("equivalence") (Figure 1.9.2.2).

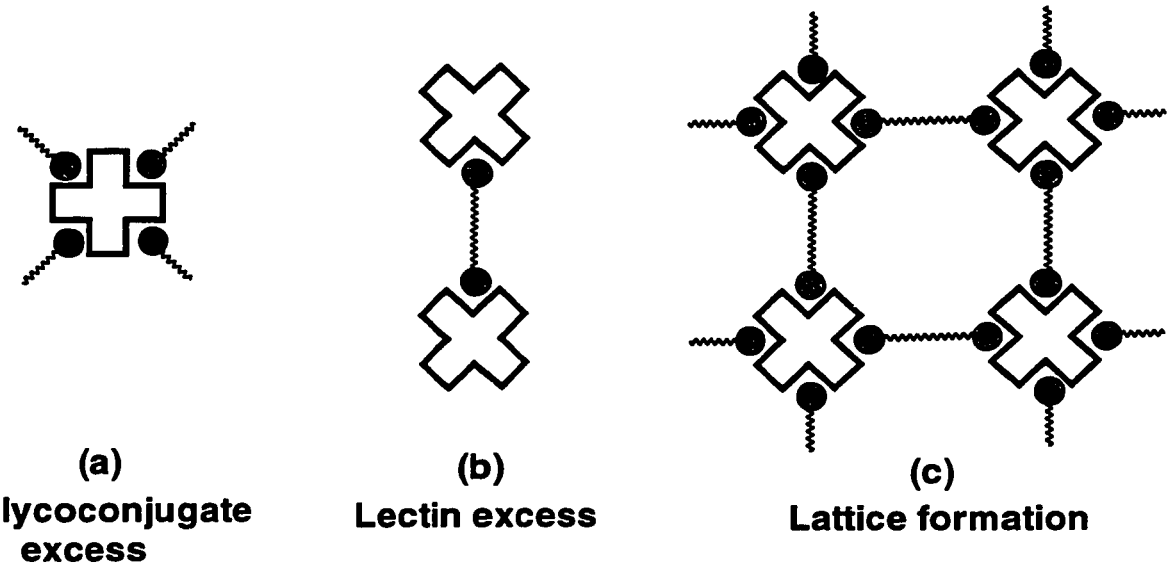

Figure 1.9.2.1. Schematic turbidimetric analysis.

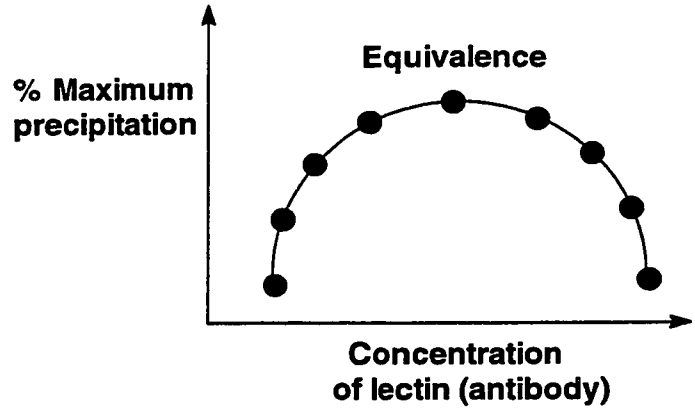

Figure 1.9.2.2. Plot of precipitation against lectin concentration. As lectin (antibody) concentration is increased so the amount of precipitate increases until equivalence is reached. In lectin (antibody) excess the amount of precipitate declines.

1.9.3. Enzyme Linked Lectin Assay (ELLA)

ELLA is a quantitative competitive assay and measures an estimate of an amount of a particular antigen (or glycoconjugate) necessary for 50% inhibition (IC_{50}).

Competitive assays measure competition in binding to labeled receptor, lectin, between a fixed amount of antigen and an unknown quantity of antigen, 'sample'. The microtiter plate is coated with the same antigen or antigen mixture and enzyme-labeled lectin specific for the test antigen added together with the sample. In a modification of this method, the enzyme-labeled lectin and sample are incubated together before being added to the antigen coated plate. If the corresponding antigen is present in the sample, the enzyme-labeled lectin will be prevented from binding (Figure 1.9.3.1).

One of the most common enzymes conjugated to lectins is Horseradish peroxidase (HRP) which is a plant glycohemeprotein. HRP readily combines with hydrogen peroxide (H_2O_2) and the resultant [HRP•H_2O_2] complex can oxidize a wide variety of chromogenic hydrogen donors (Table 1.9.3.1).

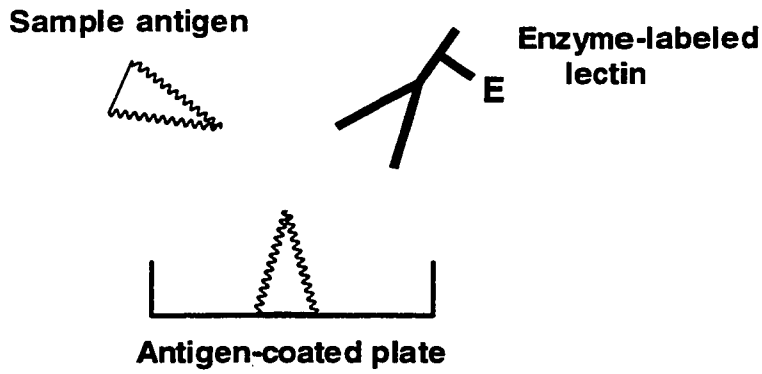

Figure 1.9.3.1. Competitive ELLA for detection of antigen using labeled lectin.

Table 1.9.3.1. HRP reactivity with chromogenic hydrogen donors in the presence of H_2O_2.

Enzyme label	Substrate system	Color reaction	End product	Application
	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)	Green	Soluble	ELISA
	o-Phenylenediamine (OPD)	Orange	Soluble	ELISA
	3,3',5,5'-Tetramethylbenzidine (TMB)	Blue	Soluble	ELISA
Peroxidase	o-Dianisidine	Yellow-orange	Soluble	ELISA
	5-Aminosalicylic acid (5AS)	Brown	Soluble	ELISA
	3,3'-Diaminobenzidine (DAB)	Brown	Soluble	Immuno-blotting
	3-Amino-9-ethylcarbazole (AEC)	Red	Insoluble	Immuno-blotting
	4-Chloro-1-naphthol (4C1N)	Blue	Insoluble	Immuno-blotting

The principle involved in the reaction between HRP and ABTS is shown in Figure 1.9.3.2.

$$H_2O_2 + 2\ ABTS \xrightarrow{Peroxidase} 2\ H_2O + 2\ Oxidized\ ABTS$$

ABTS:

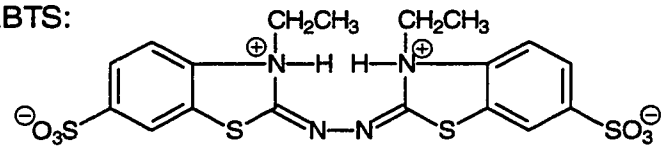

Figure 1.9.3.2. Principle of peroxidase enzyme assay.

Chapter 2. Glycopeptoids as small non-peptidic mimetics

2.1. Introduction

Glycopeptoids bearing xylopyranoside

In proteoglycans (e.g., heparin) the linkage region between protein and carbohydrate most often has the following structure: polysaccharide β-D-GlcA-(1→3)-β-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-Xyl-serine.[124,125] Unprotected mono- or oligosaccharide-serine[126,127,128,129] or oligosaccharide-peptide[130,131] fragments of this structure have been synthesized for biochemical or structural studies, as well as protected xylopyranosylserines and xylopyranosylpeptides.[132,133]

It has been recently argued that there is no amino acid recognition pattern necessary for the initial biosynthesis using xylosyltransferases, although some indications have suggested conformational requirements. Construction of glycopeptide libraries would be advantageous to solve this issue. Another approach which takes this strategy one step further would be to construct conformationally flexible "glycopeptoid" analogs[134,135,136,137] which could be used for primary structure and conformational

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[124] Kjellén, L.; Lindahl, U. *Ann. Rev. Biochem.* **1991**, *60*, 443.
[125] Garg, H. G.; Lyon, N. B. *Adv. Carbohydr. Chem. Biochem.* **1991**, *49*, 239.
[126] Erbing, B.; Lindberg, B.; Norberg, T. *Acta. Chem. Scand. Ser.* **1978**, *B 32*, 308.
[127] Garegg, P. J.; Lindberg, B.; Norberg, T. *Acta. Chem. Scand. Ser.* **1979**, *B 33*, 449.
[128] Goto, F.; Ogawa, T. *Tetrahedron Lett.* **1992**, *33*, 5099.
[129] Ekborg, G.; Curenton, T. Krishina, N. R.; Rodén, L. *J. Carbhydr. Chem.* **1990**, *9*, 15.
[130] Garg, H.; Hasenkamp, T.; Paulsen, H. *Carbohydr. Res.* **1986**, *151*, 225.
[131] Rio, S.; Beau, J.; Jacquinet, J. *Carbohydr. Res.* **1991**, *219*, 71.
[132] Friedrich-Bochnitschek, S.; Waldmann, H.; Kunz, H. *J. Org. Chem.* **1989**, *54*, 751.
[133] Kunz, H; Waldmann, H. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 71.
[134] Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646.
[135] Zuckermann, R. N.; Martin, E. J.; Spellmeyer, D. C.; Stauber, G. B.; Shoemaker, K. R.; Kerr, J. M.; Figliozzi, G. M.; Goff, D. A.; Siani, M. A.; Simon, R. J.; Banville, S. C.; Brown, E. G.; Wang, L.; Richter, L. S.; Moos, W. H. *J. Med. Chem.* **1994**, *37*, 2678.
[136] Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 543.
[137] Kruijtzer, J. A.; Liskamp, R. M. J. *Tetrahedron Lett.* **1995**, *36*, 6969.

screening at once. Moreover, glycopeptoids could be also used as potential therapeutic inhibitors.[138]

As an extension of ongoing activities of the syntheses of conformationally flexible *N*-linked glycopeptidomimetics, glycopeptoids (*N*-substituted oligoglycines) in our group,[139,140,141,142] we describe herein the first syntheses of xylose-containing peptoids corresponding to the NH_2-terminal amino acid sequence of human bone and cartilage proteoglycans-I (PG-I).[125] The structural sequences of the synthesized homoserine xylopeptoid analogs include Val-Phe-Ser-(β-D-Xyl)-Glu-Ala and Ala-Ser-(β-D-Xyl)-Gly-Ala.

Glycopeptoids bearing GalNAc

Malignant cells express abnormally substituted mucin glycoproteins as a result of incomplete or aberrant glycosylation. In adenocarcinomas, these mucins expose tumor-associated carbohydrate antigens that are cryptic in healthy tissues.[143,144,145] Of particular interest are the blood group antigens Tn (GalNAcα-O-Ser), T (Galβ1,3GalNAcα-O-Ser), and sialosyl-Tn (Neu5Acα2,6GalNAcα-O-Ser) of epithelial cancers.[146] These antigens appear as clusters of glycopeptide repeating units and a synthetic vaccine (neoglycoprotein) is now in clinical trials.[147] Moreover, a dimeric Tn antigen glycopeptidolipid has been shown to be highly immunogenic.[148] These vaccines were not derived from the natural glycopeptide and since there is still no concluding evidence as to the antigenic participation of the peptide backbone, it

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[138] Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Ng, S.; Wang, L. Rosenberg, S.; Marlowe, C. K.; Spellmeyer, D. C.; Tan, R.; Frankel, A. D.; Santi, D. V.; Cohen, F. E.; Bartlett, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367.

[139] Presented in part at the 8th *European Carbohydrate Symposium*, Seville, Spain, July 2-7, **1995**. Abstract C IL-5.

[140] Saha, U. K.; Roy, R. *Tetrahedron Lett.* **1995**, *36*, 3635.

[141] Saha, U. K.; Roy, R. *J. Chem. Soc., Chem. Commun.* **1995**, 2571.

[142] Roy, R.; Saha, U. K. *J. Chem. Soc., Chem. Commun.* **1996**, 201.

[143] Hakomori, S. *Adv. Cancer Res.* **1989**, *52*, 2214.

[144] Hakomori, S. *Curr. Opin. Immunol.* **1991**, *3*, 646.

[145] Singhal, A. K.; Hakomori, S. *BioAssays* **1990**, *12*, 223.

[146] Toyokuni, T.; Singhal, A. K. *Chem. Soc. Rev.* **1995**, 231.

[147] Longenecker, B. M.; Reddish, M.; Miles, D.; MacLean, G. D. *Vaccine Res.* **1993**, *2*, 151.

became of interest to generate glycomimetics. To evaluate the role of multivalency in antigen presentation and to generate metabolically stable glycopeptide analogs, the syntheses of Tn antigen glycopeptidomimetic clusters is performed herein.

2.2 Synthesis of glycopeptoids

Xylose-containing pentapeptoid

The synthesis of the key glycan portion, 2-azidoethyl β-D-xylopyranoside homoserine mimic **6** was illustrated (Scheme 2.2.1). Due to difficulties encountered in the direct Koenigs-Knorr glycosylation of acetobromoxylose **3** with 2-azidoethanol, thioglycoside chemistry was chosen to synthesize 2-azidoethyl β-D-xylopyranose (**6**). β-D-Xylopyranosyl bromide (**3**) was prepared by treating xylopyranose pentaacetate (**2**) with 30% HBr in acetic acid in quantitative yield. Bromide **3** was then stereospecifically transformed into phenyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranoside (**4**) in 95% yield using phase transfer catalyzed glycosylation (PhSH, TBAHS, EtOAc, 1M Na_2CO_3, 23 °C, 30 min.). As per-acetylated phenyl-1-thio-xylopyranoside **4** was prone to orthoester formation, it was further transformed into its per-benzoylated derivative **5** in a two-step sequence involving Zemplén de-*O*-acetylation (1M NaOMe, MeOH) and *O*-benzoylation (BzCl, pyridine, quant.). Glycosylation of phenyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-xylopyranoside (**5**) with azidoethanol using dimethyl(methylthio)sulfonium triflate (DMTST) in dichloromethane occurred in 78% yield. 2-Azidoethanol was prepared by the S_N2 reaction of commercially available 2-chloroethanol with NaN_3 (10 eq) and NaI (1 eq) in acetonitrile. Hydrogenation of azide **6** afforded amine **7** (H_2-Pd/C, MeOH) in 95% yield.

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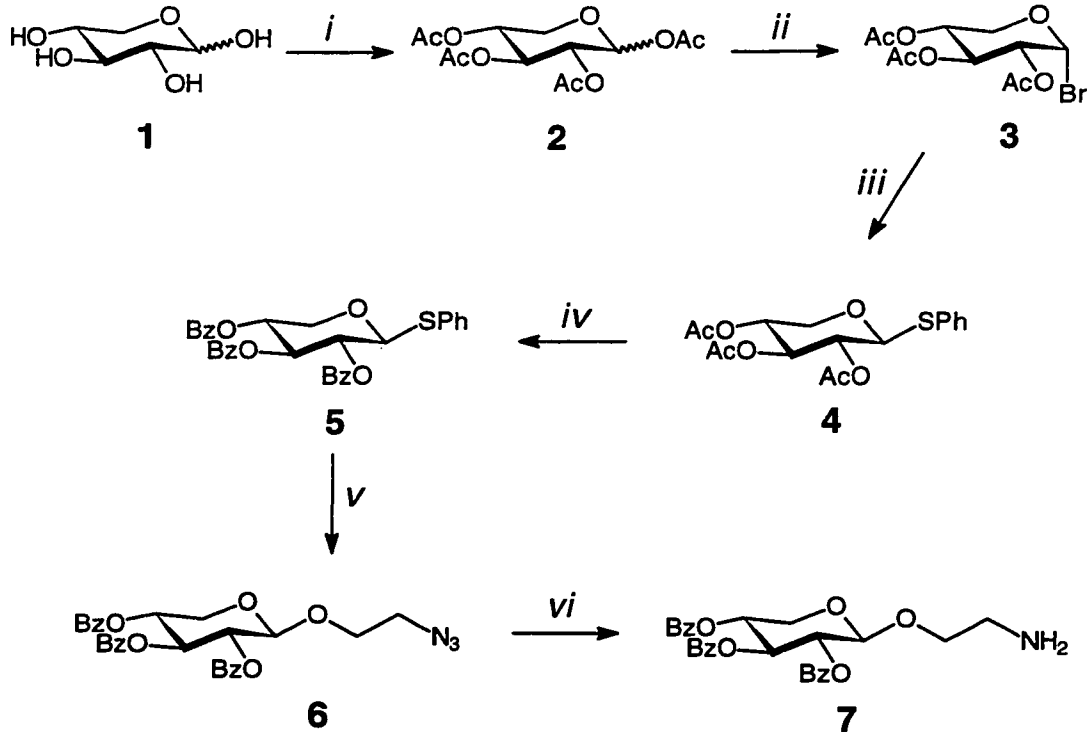
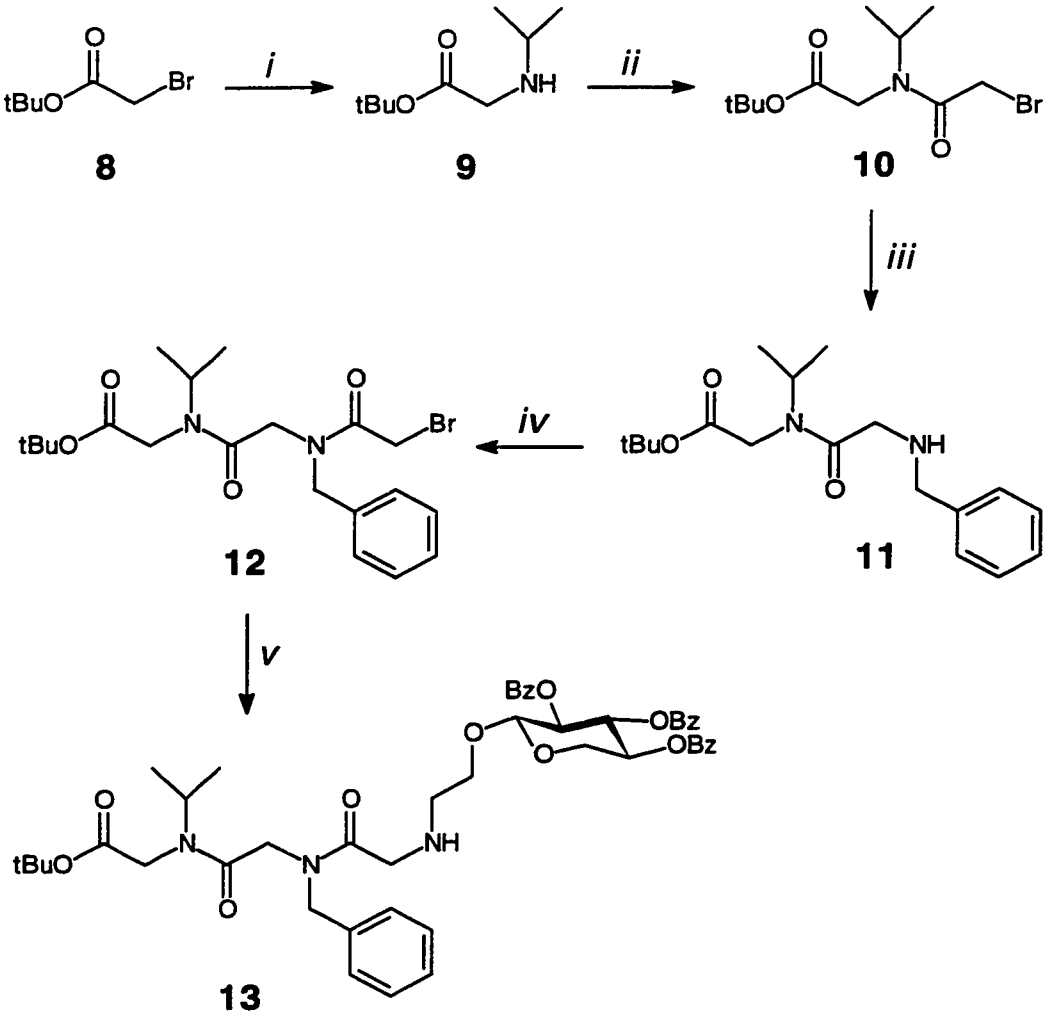
[148] Toyokuni, T.; Dean, B.; Cai, S.; Boivin, D.; Kakomori, S.; Singhal, A. K. *J. Am. Chem. Soc.* **1994**, *116*, 395.



Scheme 2.2.1. Synthesis of 2-aminoethyl β-D-xylopyranoside homoserine mimic **7**. *i*) Ac_2O, NaOAc; *ii*) 30% HBr, AcOH; *iii*) PhSH, TBAHS, EtOAc, 1M Na_2CO_3, 30 min., 23 °C, 95%; *iv*) (1) 1M NaOMe, MeOH, pH 9, quant. (2) BzCl, pyridine, 1.5 h, 23 °C, quant.; *v*) $HOCH_2CH_2N_3$ (3 eq), DMTST (4 eq), CH_2Cl_2, 24 h, 0-23 °C, 78%; *vi*) H_2-Pd/C, MeOH, 5 h, 95%.

The *N*-terminus of the *N*-substituted glycine portion corresponding to Val-Phe **12** was prepared from *t*-butyl bromacetate by a series of re-iterative *N*-alkyltion (Me_2CHNH_2, then $PhCH_2NH_2$) and bromoacetylation ($BrCH_2COCl$, DIPEA, CH_2Cl_2, 20-30 min., 0 °C) sequences as described in Scheme 2.2.2. The resulting secondary amine **11** was obtained in 59% yield after three steps. It was shown to exist as slowly equilibrating *E,Z*-conformers in a ratio of 1:1.6 as measured from the relative integration of the two *t*-butyl signals shown at 1.39 and at 1.43 ppm in its ^{1}H-NMR spectrum. For dipeptoids and higher homologues, 2^n slow equilibrating *E,Z*-conformers, where n represents the number of tertiary amide bonds, are possible. These early observation allowed us to speculate that peptoids resulting from such a strategy would offer distinct

advantages for probing large conformational spaces in potential receptors. N-Bromoacetylation of the secondary amine **11** provided bromoacetylated dipeptoid unit **12** in 78% yield, which was shown to exist as four *E,Z*-conformers in a ratio of 1.3:1.7:1.1:1.0. Coupling of this fragment to 2-aminoethyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-xylopyranoside (**7**) (DIPEA, CH_3CN) afforded peptoid block **13** in 47% yield.



Scheme 2.2.2. Synthesis of dipeptoid block **13**. *i*) Me_2CHNH_2 (3 eq), CH_3CN, 0 °C, 30 min.; *ii*) $ClCOCH_2Br$, DIPEA, CH_2Cl_2, 30 min., 0 °C, 75% (2 steps); *iii*) $PhCH_2NH_2$ (3 eq), CH_3CN, 20 min., 0 °C, 78%; *iv*) $ClCOCH_2Br$, DIPEA, CH_2Cl_2, 20 min., 0 °C, 78%; *v*) xylopyranoside **7**, DIPEA, CH_3CN, 0 °C, 1h, 47%.

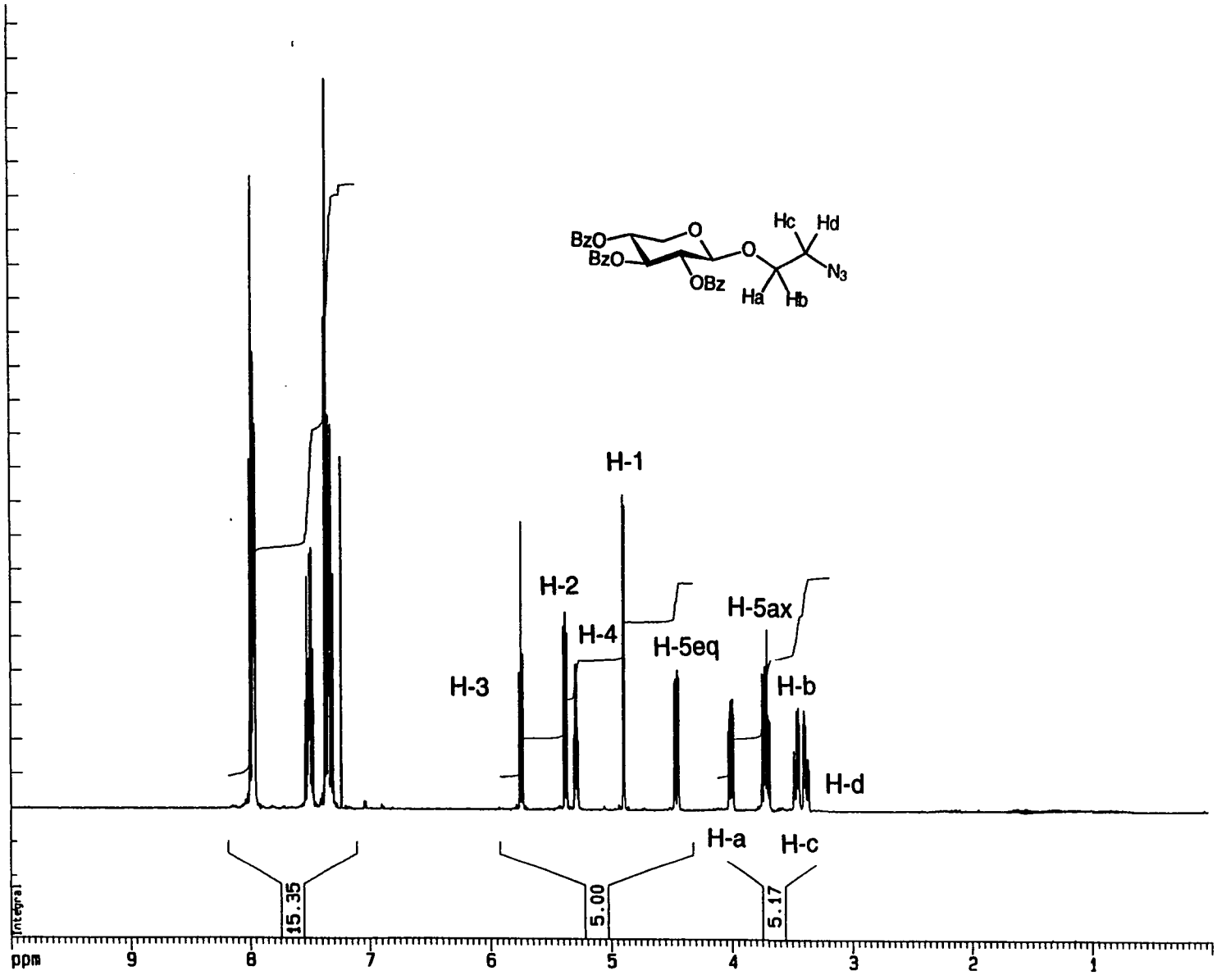


Figure 2.2.1. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of 2-azidoethyl β-D-xylopyranoside homoserine mimic **6**.

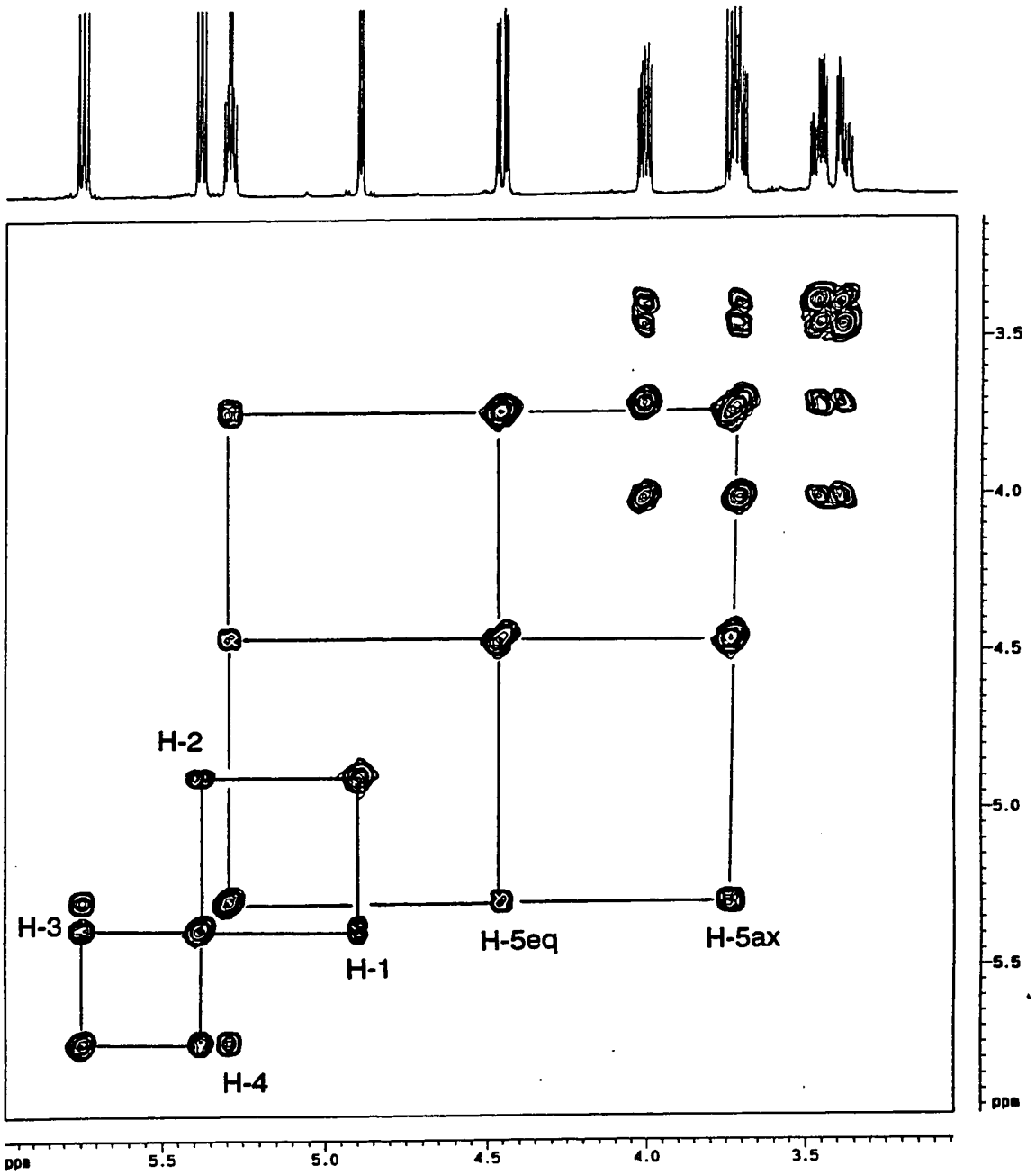


Figure 2.2.2. COSY ($CDCl_3$, 500 MHz) spectrum of 2-azidoethyl β-D-xylopyranoside homoserine mimic **6**.

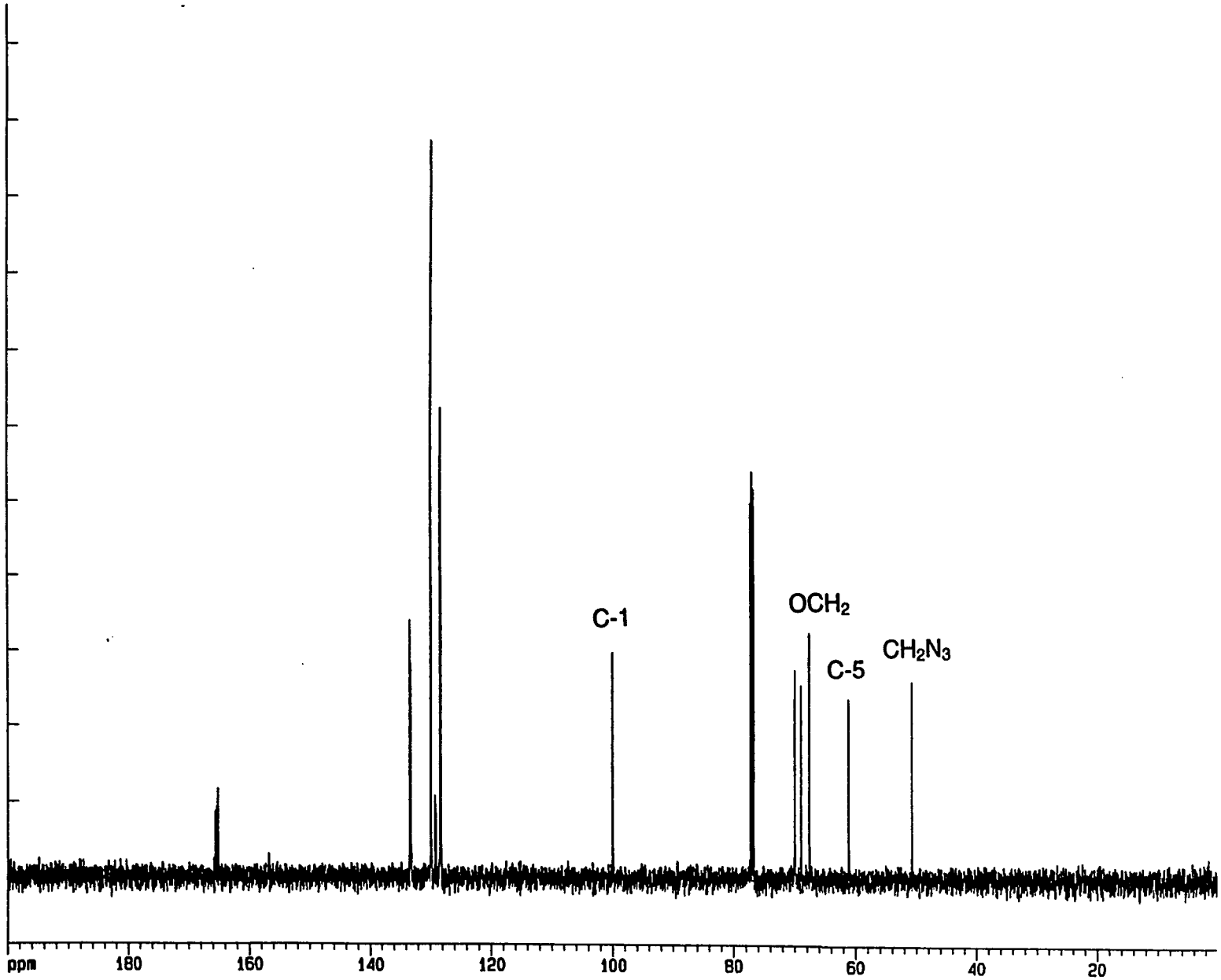
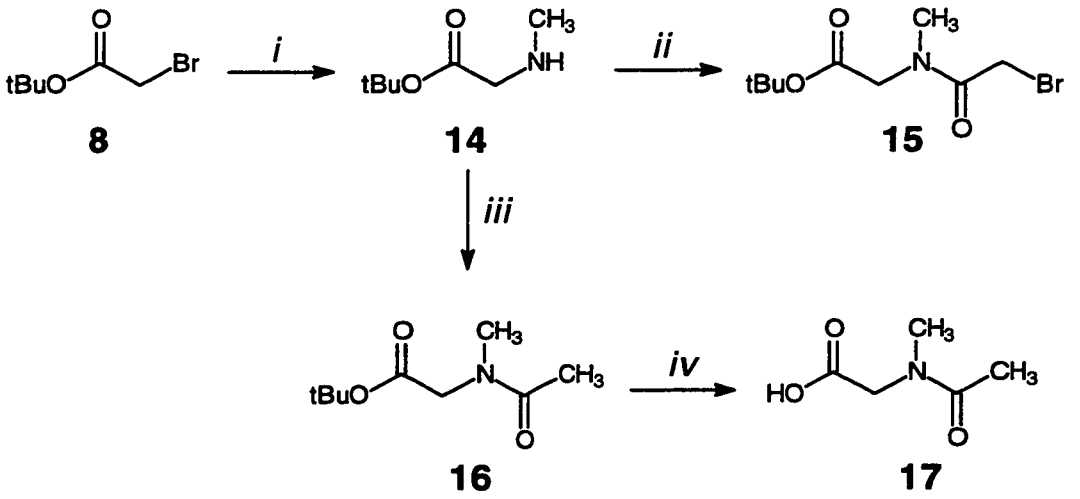


Figure 2.2.3. ^{13}C-NMR ($CDCl_3$, 125 MHz) spectrum of 2-azidoethyl β-D-xylopyranoside homoserine mimic **6**.

The synthesis of the right-hand side of the target molecule corresponding to the *C*-terminal Glu-Ala mimetic **21** is illustrated in Schemes 2.2.3 and 2.2.4. The *N*-acetyl-Ala mimic **16** was obtained by treating *t*-butyl N-methyl bromoacetate **8** with methylamine (30% aqueous) to give *t*-butyl *N*-methylglycinate **14** which was transformed (AcCl, pyridine, CH_2Cl_2) into *N*-acetyl-Ala peptoid unit **16** in 89% yield for two steps. The ^{1}H-NMR spectrum of *N*-acetyl-Ala unit **16** showed a mixture of *E,Z*-conformers in a 1:2.4 ratio, as judged from the relative integration of the two *t*-butyl signals at 1.34 and 1.36 ppm, respectively. Hydrolysis of the *t*-butyl ester using 20% trifluoroacetic acid (TFA) in CH_2Cl_2 provided free acid **17** in 92% yield (Scheme 2.2.3).

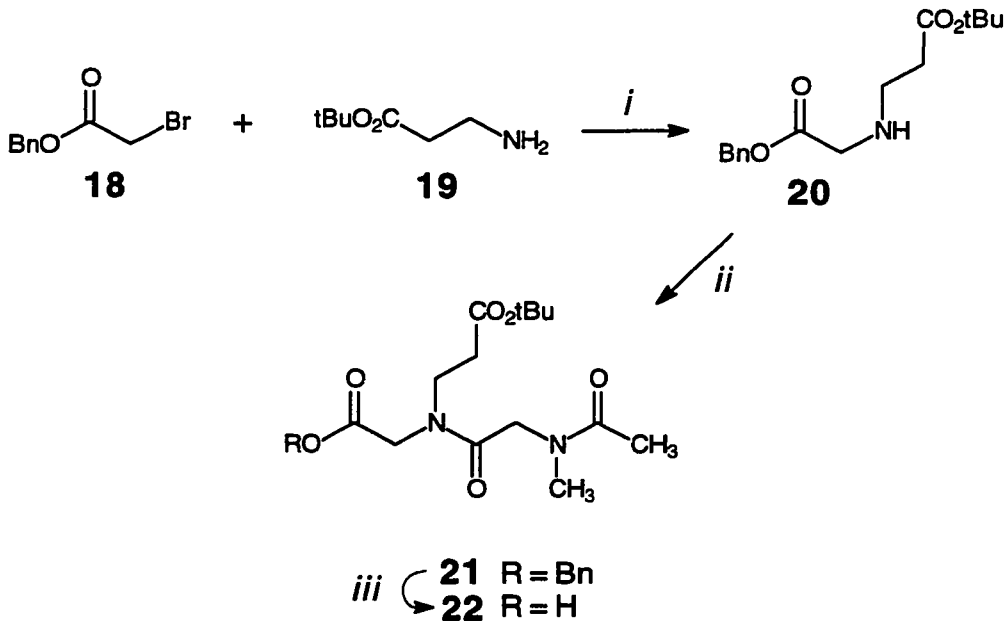


Scheme 2.2.3. Synthesis of compound **17**. *i*) 30% CH_3NH_2 in H_2O (3 eq), CH_3CN, 30 min., 0 °C; *ii*) $ClCOCH_2Br$, DIPEA, 30 min., 0 °C, 74% (2 steps); *iii*) AcCl, pyridine, CH_2Cl_2, 30 min., 0 °C, 89%; *iv*) 20% TFA, CH_2Cl_2, 2h, 23 °C, 92%.

Benzyl bromoacetate was used to *N*-alkylate *t*-butyl 2-aminopropionate to provide glutamic acid mimic **20** in 79% yield (Scheme 2.2.4). The resulting secondary amine **20** was coupled with acid **17** using DCC to afford the *C*-terminal Glu-Ala mimetic

21 in 98% yield. Hydrogenolysis (H_2-Pd/C, MeOH) of the benzyl ester furnished acid **22** in 98% yield.

Final coupling of amine **13** and acid **22** was also accomplished with DCC (CH_2Cl_2, 23 °C, 2 h) to provide protected xylopeptoid **23** in 92% yield (Scheme 2.2.5). Mild deprotection of the benzoate group of the xylose moiety under Zemplén condition (1M NaOMe, MeOH, pH 9) followed by treatment with 20% TFA in CH_2Cl_2 afforded final compound **25** in quantitative yield.



Scheme 2.2.4. Synthesis of compound **22**. *i*) DIPEA, 0 °C, 15 min., 79%; *ii*) $HO_2CCH_2N(CH_3)COCH_3$ (**17**), DCC, CH_2Cl_2, 23 °C, 5h, 98%; *iii*) H_2-Pd/C, MeOH, 3h, 98%.

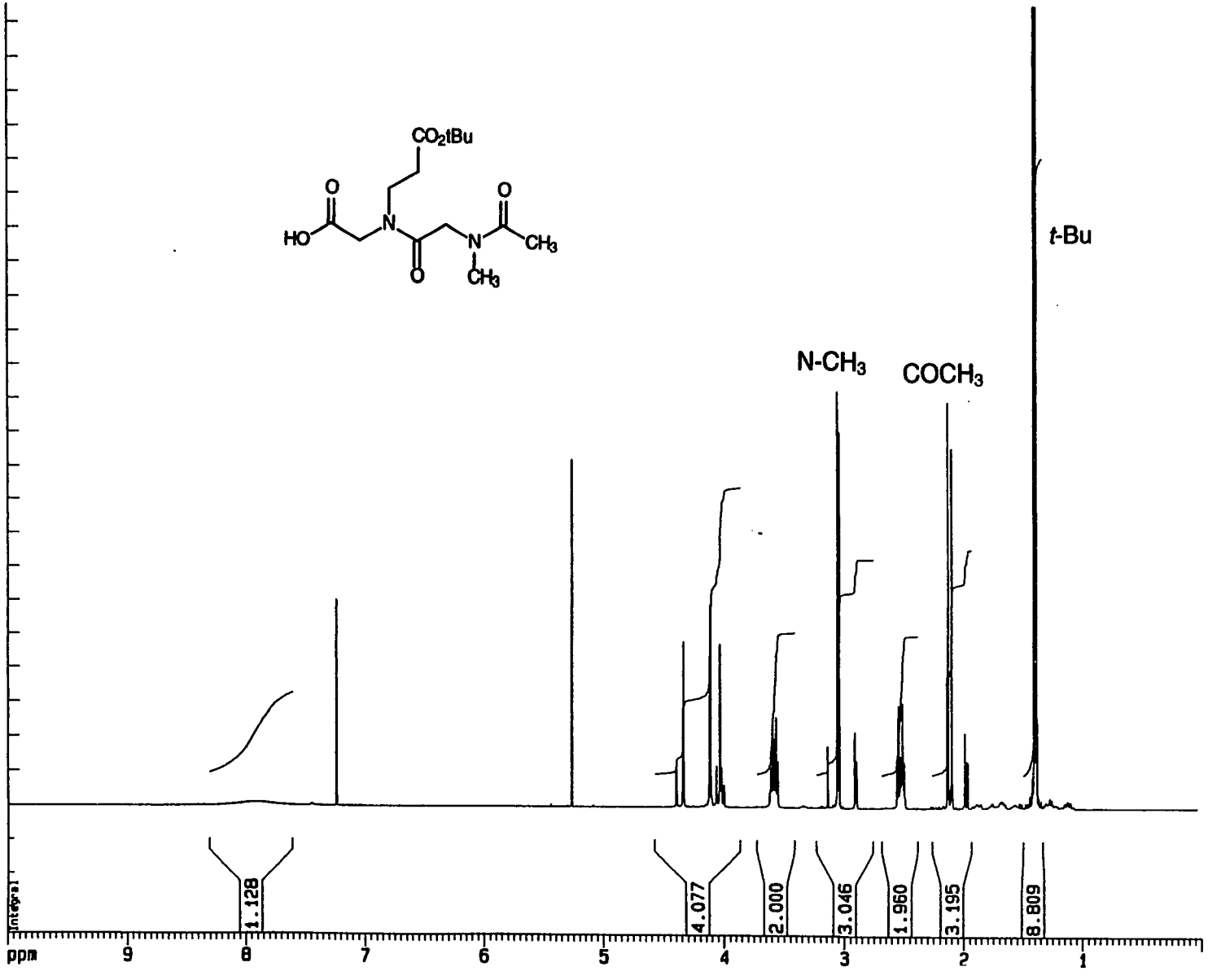
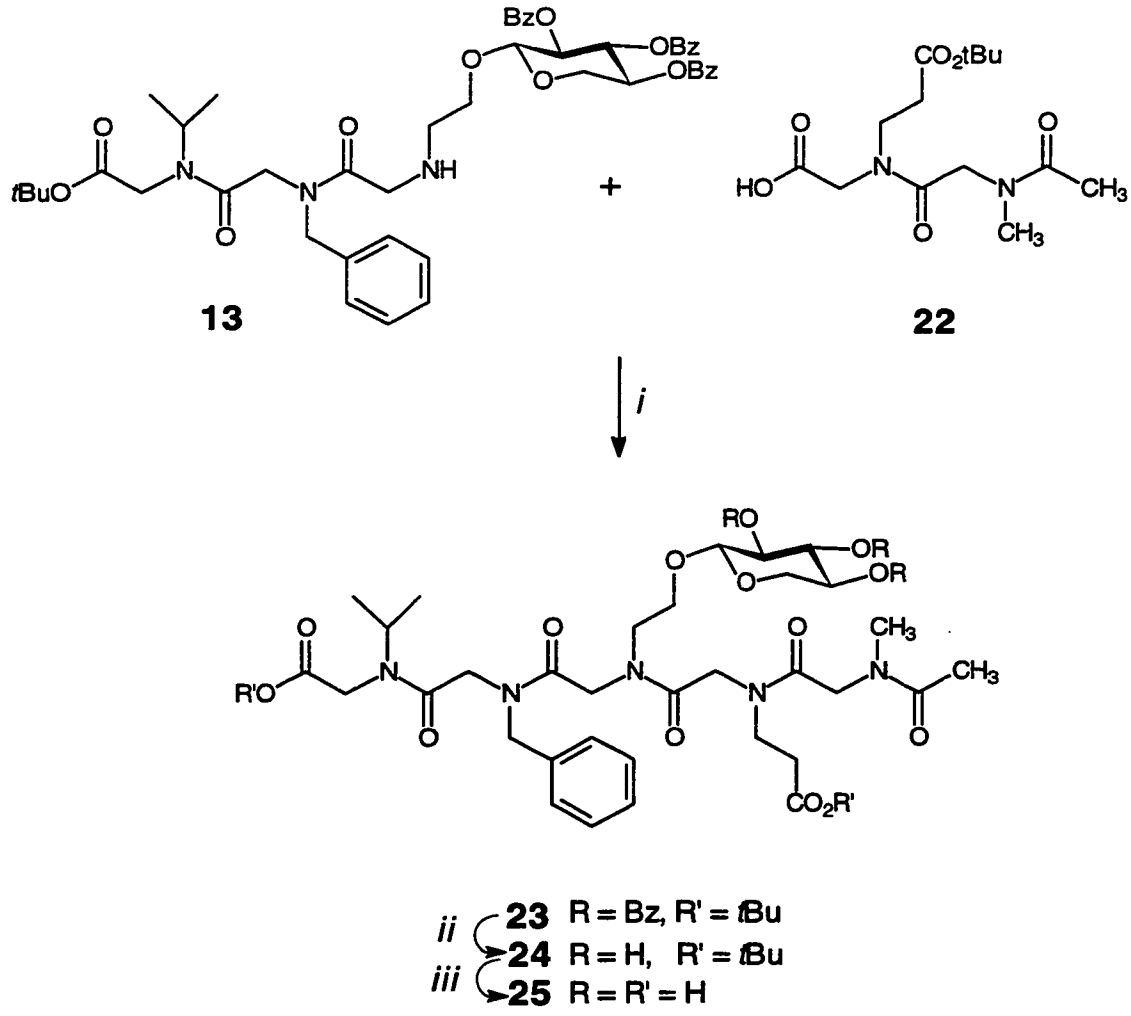


Figure 2.2.4. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of compound **22**.



Scheme 2.2.5. Synthesis of pentapeptoid **25**. *i*) DCC, CH_2Cl_2 23 °C, 2h, 92%; *ii*) 1M NaOMe, MeOH, 23 °C, 2h, quant.; *iii*) 20% TFA, CH_2Cl_2, 23 °C, 1h, quant.

Xylose-containing tetrapeptoid

Following the initial investigation on the synthesis of the xylose-containing pentapeptoid, another conformationally flexible glycopeptidomimetic was synthesized. The structural analogy between the sequence Ala-Ser (β-D-Xyl)-Gly-Ala and the homoserine xylopeptoid analog is depicted in Figure 2.2.5.

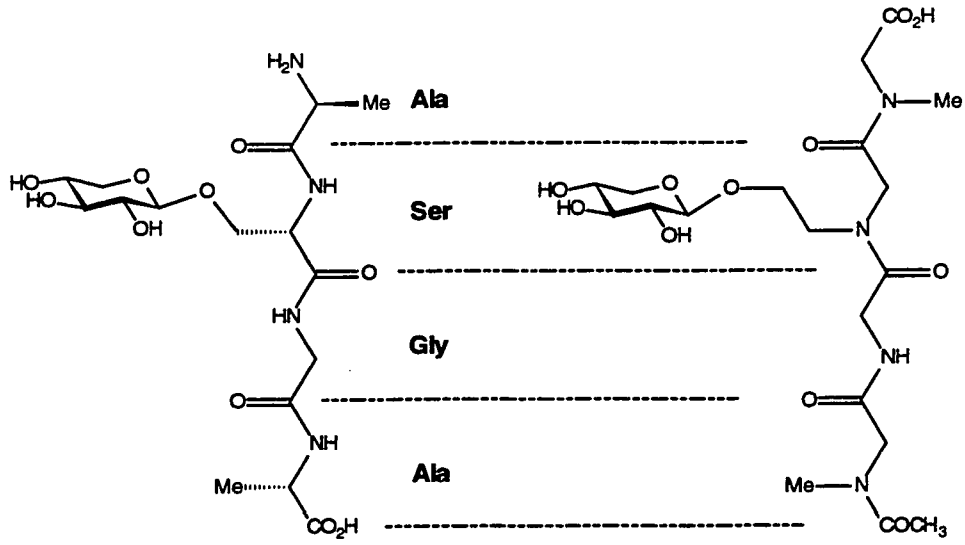
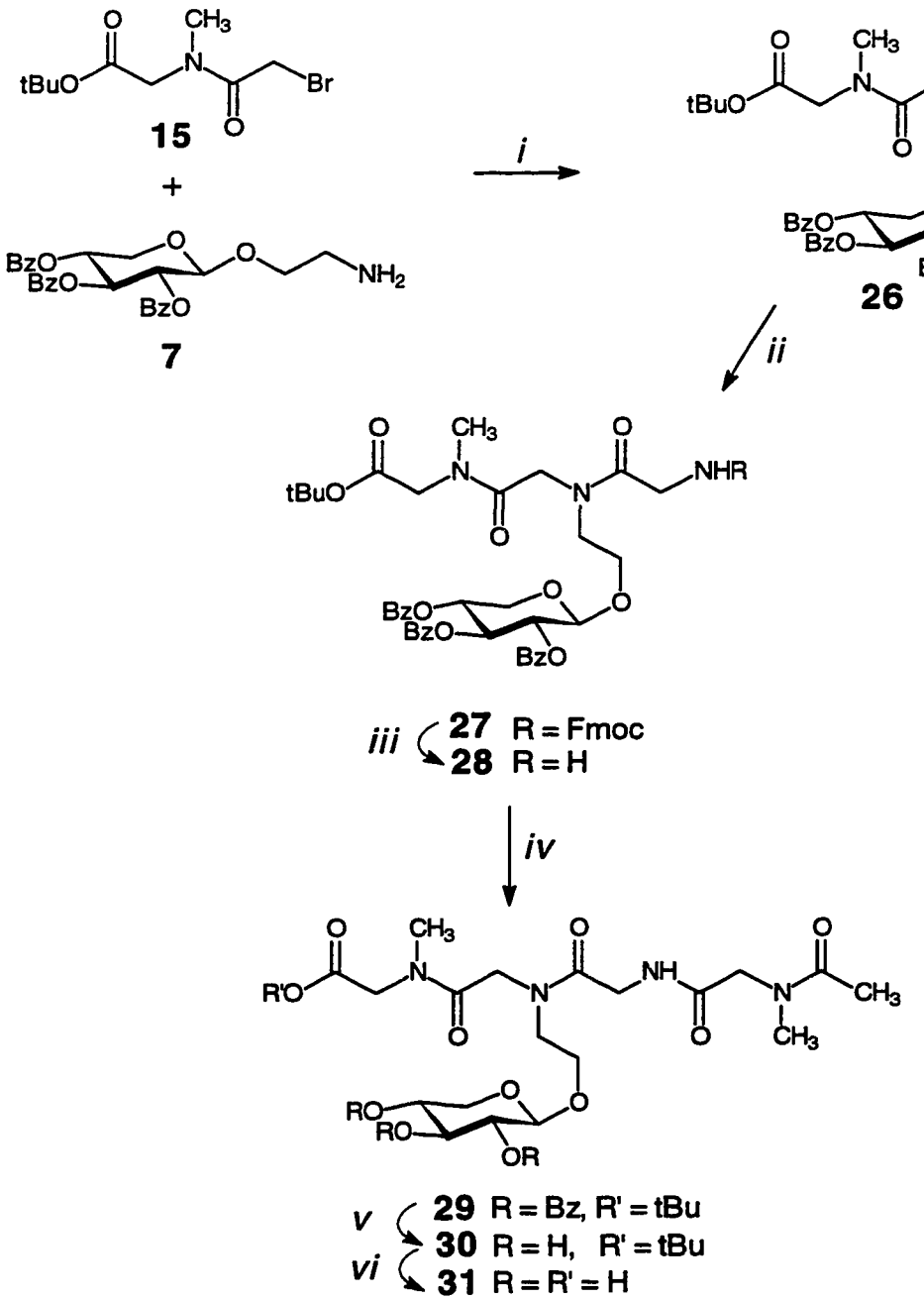


Figure 2.2.5. Structural relationships between *O*-linked xylopeptide and *N*-substituted oligoglycine with an homoserine mimetics.

The left-hand *N*-terminus corresponding to bromoacetylated Ala **15** was prepared by alkylating methylamine with *t*-butyl bromoacetate (**8**) in acetonitrile and promptly treating the resulting secondary amine with bromoacetyl chloride (74% yield for two steps) (Scheme 2.2.3). This secondary amide **15** was shown to exist as slowly equilibrating *E,Z*-conformers. From the integration of ^{1}H-NMR signals, the observed conformer ratio was 1:2.3. Xylose-containing dipeptoid precursor **26** was obtained in 37% yield after reacting bromoacetylated derivative **15** with 2-aminoethyl β-D-xylopyranoside **7** (DIPEA, CH_3CN) (Scheme 2.2.6), which was prepared previously (see Scheme 2.2.1). The resulting secondary amine **26** was transformed into dipeptoid unit **27** by coupling with Fmoc-glycine using DDC (76%). Removal of Fmoc-protecting group (20% piperidine) furnished glycine derivative **28** which was directly coupled to acid **17** (DCC, 62%, 2 steps) providing fully protected tetraacid **29**. Deprotection of the benzoate groups on the xylose residue under Zemplén condition (1M NaOMe, MeOH, pH 9) afforded **30** quantitatively. The *t*-Butyl ester protecting group in **30** was removed by treating with 20% TFA in CH_2Cl_2 to provide fully deprotected tetrapeptoid **31**.



Scheme 2.2.6. Synthesis of tetrapeptoid **31**. *i*) DIPEA, CH_3CN, 1h, 0 °C, 37%; *ii*) $FmocHNCH_2CO_2H$, DCC, CH_2Cl_2, 30 min., 23 °C, 76%; *iii*) 20% piperidine, DMF, 30 min., 23 °C; *iv*) $HO_2CCH_2N(CH_3)COCH_3$ (**17**), DCC, CH_2Cl_2, 30 min., 23 °C, 62% (2 steps); *v*) 1M NaOMe, MeOH, pH 9, 2h, 23 °C; *vi*) 20% TFA, CH_2Cl_2, 1h, 23 °C, quant.

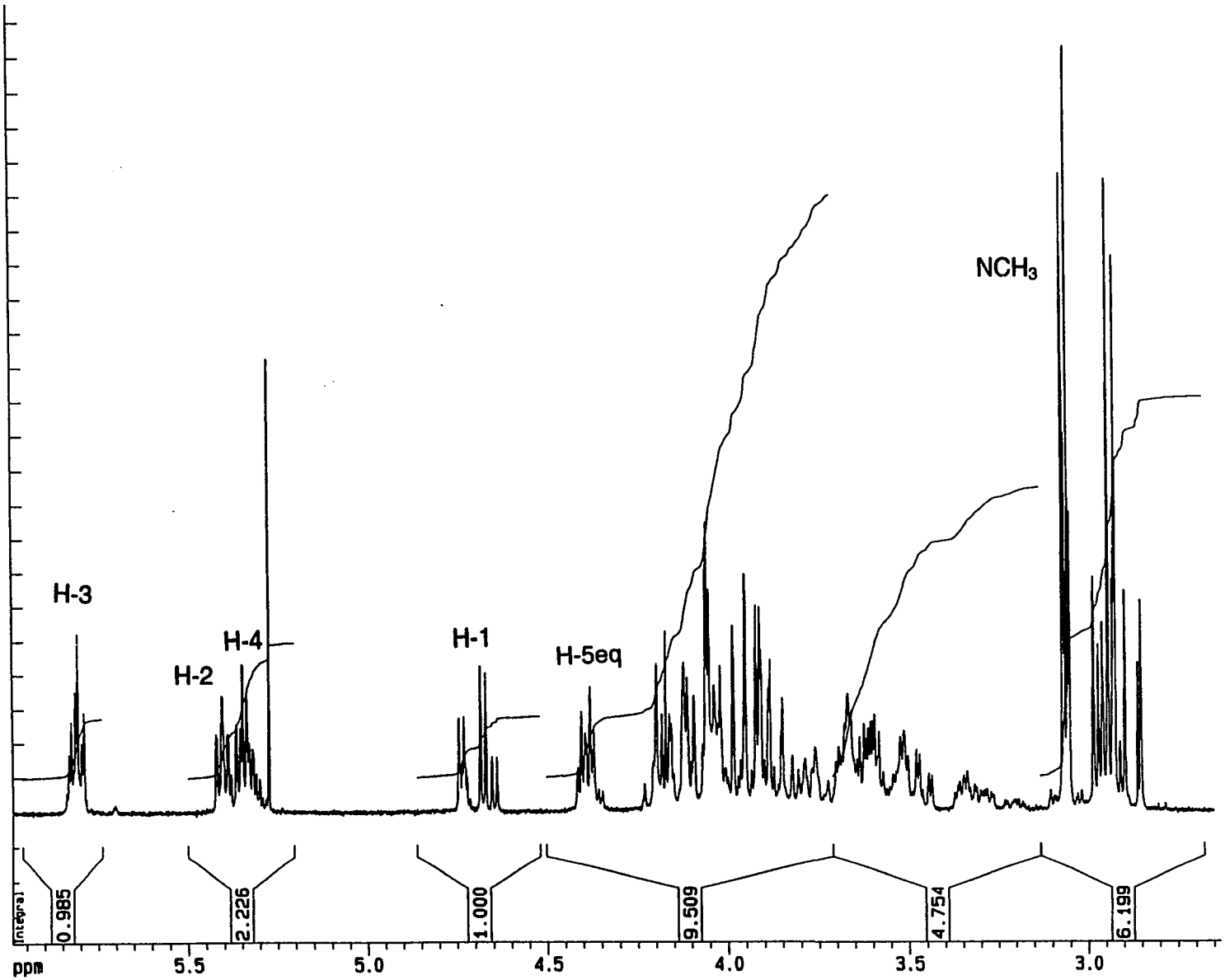


Figure 2.2.6. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of fully protected tetrapeptoid **29**.

Glycopeptoid containing GalNAc

N-substituted oligoglycines (peptoids) have been considered as scaffolding peptide surrogates. Figure 2.2.7 illustrates structural similarities between representative divalent analogs.

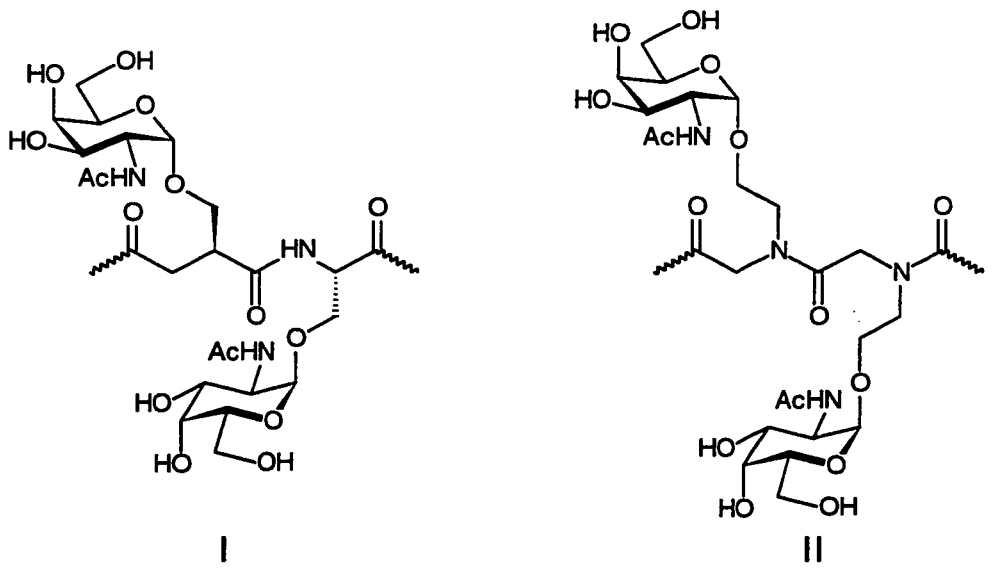
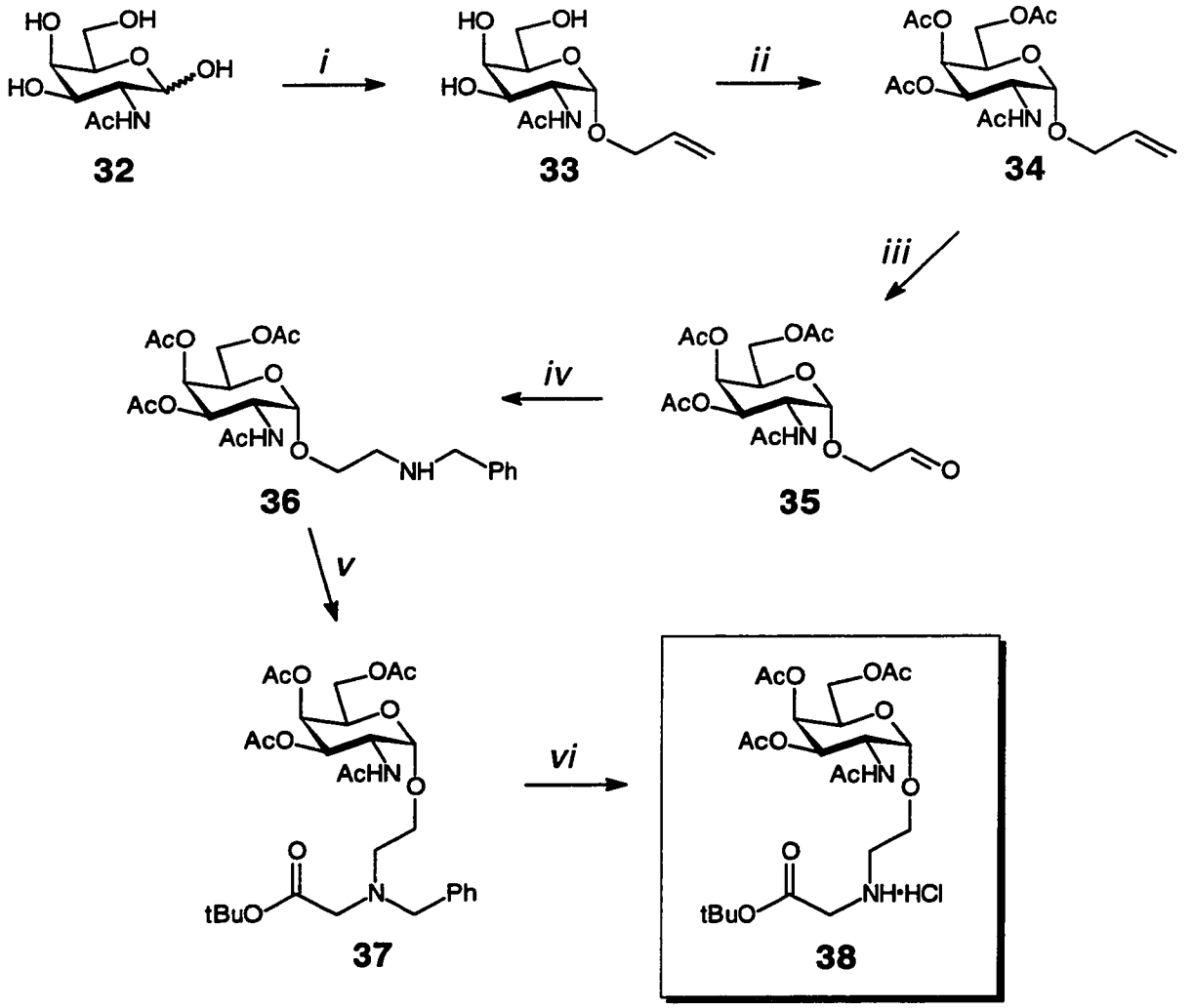


Figure 2.2.7. Structural similarities between dimeric α-D-GalNAc-*O*-Ser dipeptide (I) and α-D-GalNAc-*O*-(homo)Ser dipeptoid mimetic (II).

The strategy described herein was based on the reiterative scaffolding of a protected key building block **38** that was prepared from readily available allyl N-acetyl-α-D-galactopyranoside derived from commercial GalNAc by treatment with allyl alcohol and $BF_3 \bullet OEt_2$ (67%) (Scheme 2.2.7). Ozonolysis and reductive amination of the resulting peracetylated aldehyde **35** with benzylamine afforded secondary amine **36** in 74% yield. *N*-Alkylation of **36** with *t*-butyl bromoacetate provided tertiary amine **37** in 98% yield. After standard hydrogenolysis of the benzyl group (quant.), the resulting *C*-terminal amino ester unit **38** was transformed into either an internal *N*-Cbz protected acid **42** (Cbz-Cl, DIPEA, CH_2Cl_2, 77%; then 20% TFA in CH_2Cl_2, quant.) or into an *N*-

terminal *N*-Ac-protected acid **40** (AcCl, DIPEA, CH_2Cl_2, 98%; then 20% TFA in CH_2Cl_2, quant.) (Scheme 2.2.8).



Scheme 2.2.7. Synthesis of compound **38**. *i*) Allyl alcohol, $BF_3 \bullet OEt_2$, reflux, 2h, 23 °C, 16h, 82%; *ii*) Ac_2O, pyridine, 23 °C, 6h, 93%; *iii*) (1) O_3, CH_2Cl_2, -76°C, 10 min., (2) CH_3SCH_3, CH_2Cl_2, 23 °C, 16h; *iv*) $PhCH_2NH_2$ (5 eq), $NaCNBH_3$ (5 eq), conc. HCl (cat.), THF, 23 °C, 6h, 74%; (*v*) $BrCH_2CO_2tBu$ (1.5 eq), DIPEA (1.5 eq), CH_2Cl_2, 23 °C, 16h, 97%; *vi*) (1) H_2-Pd/C, AcOH, MeOH, 3h, (2) Amberlite IRA 400 (Cl), MeOH, 16h, 92%.

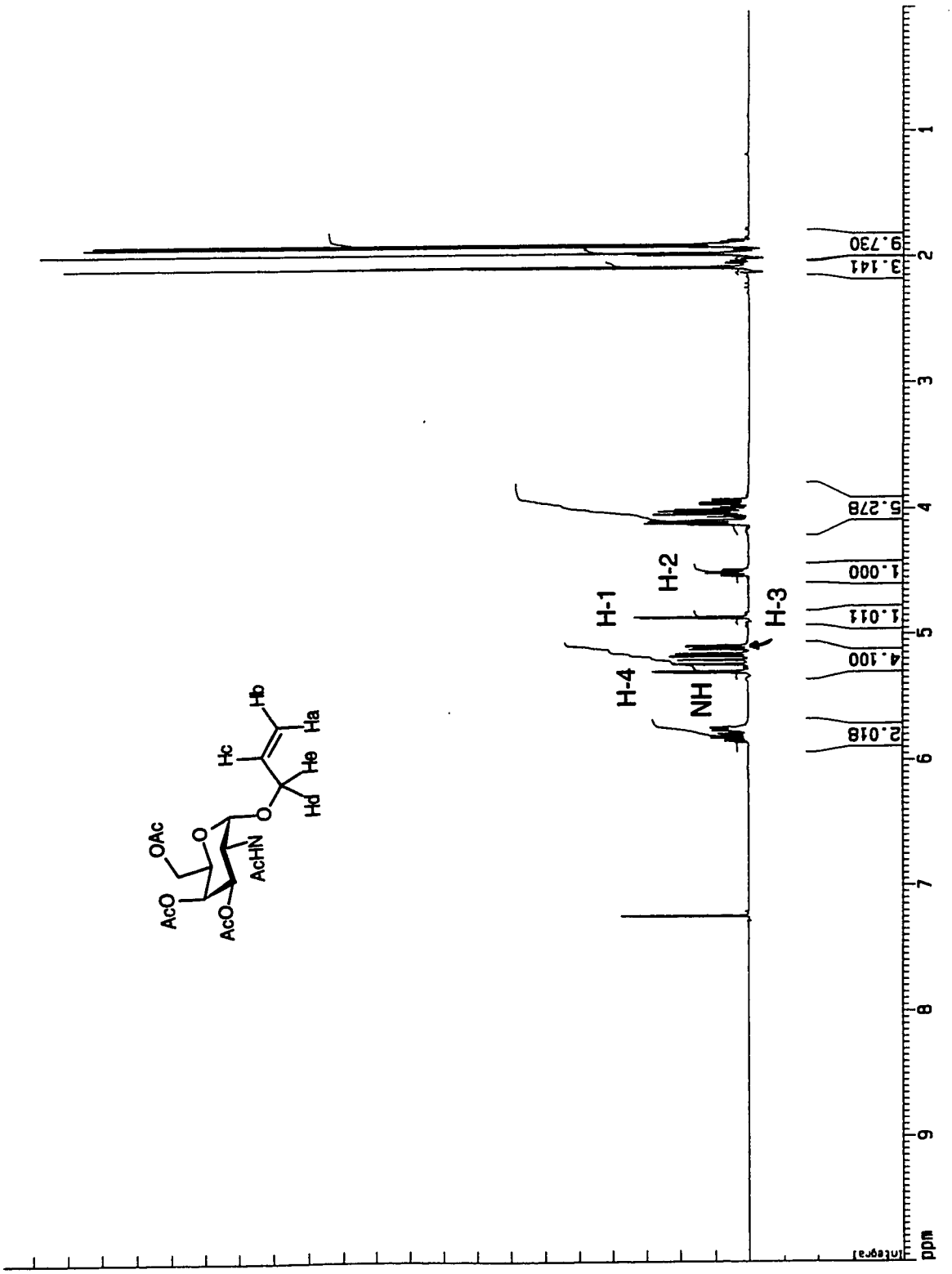


Figure 2.2.8. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of allyl α-D-GalNAc **34**

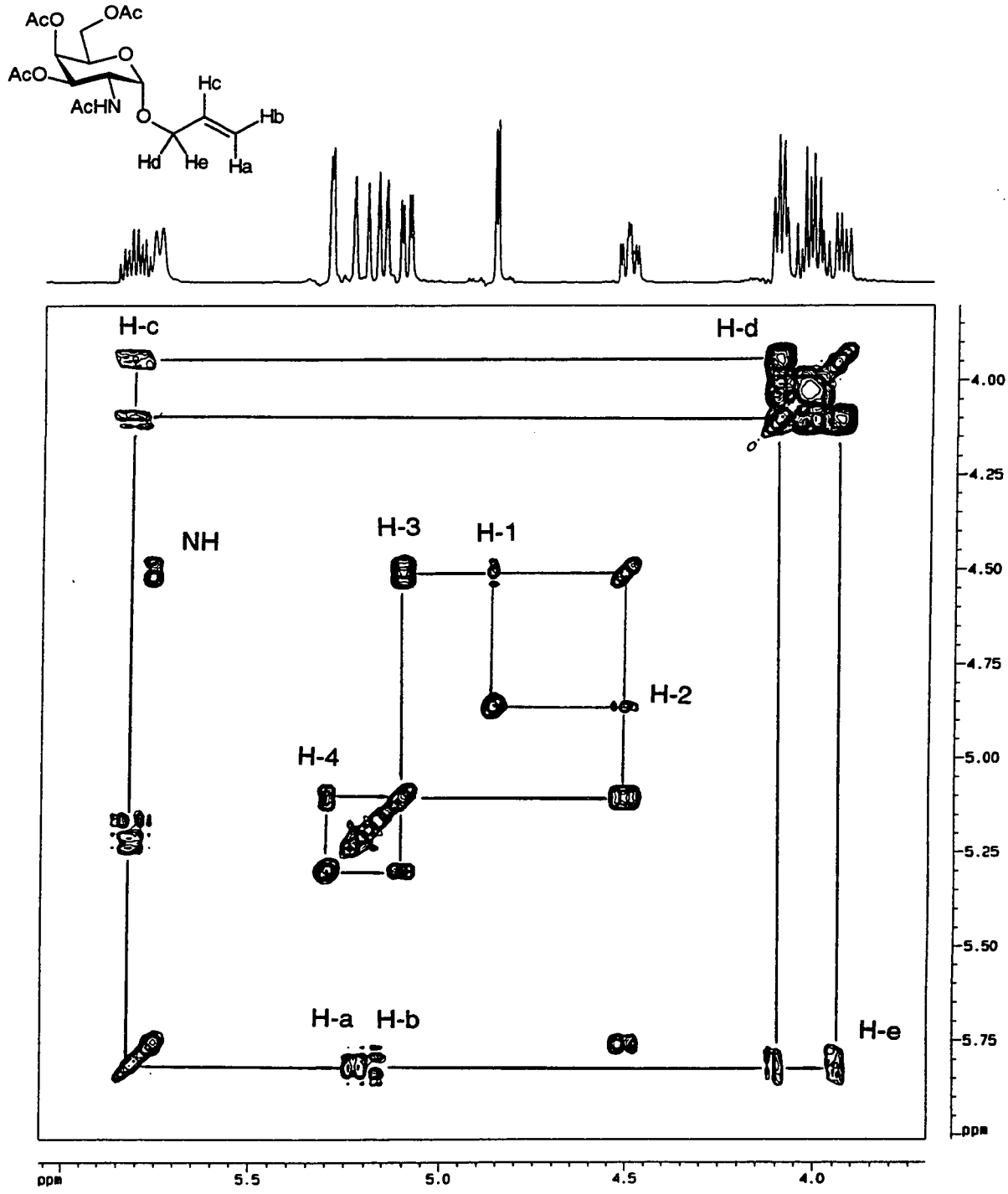


Figure 2.2.9. COSY ($CDCl_3$, 500 MHz) spectrum of allyl α-D-GalNAc **34**.

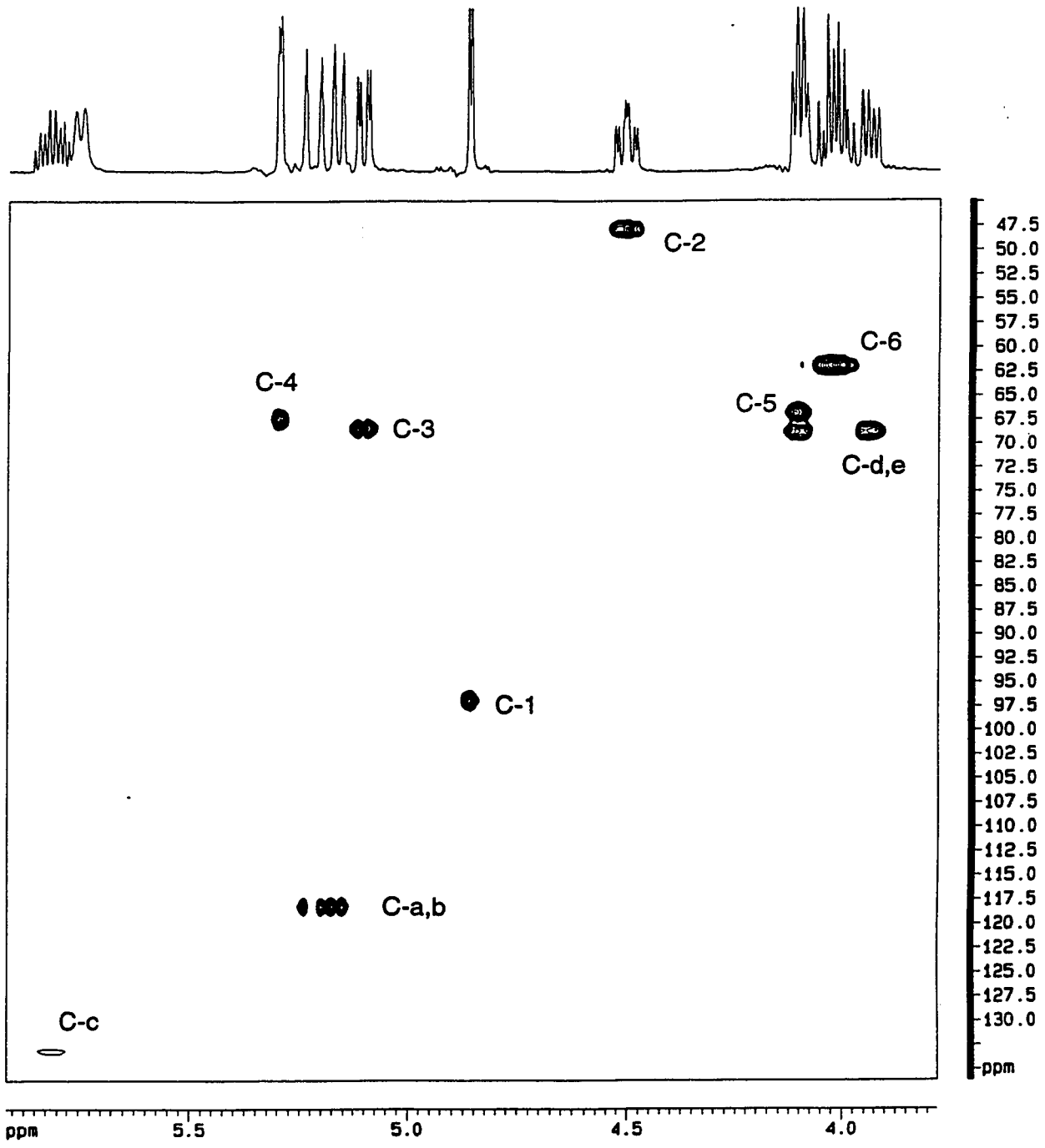


Figure 2.2.10. HMQC ($CDCl_3$, 500 MHz) spectrum of allyl α-D-GalNAc **34**.

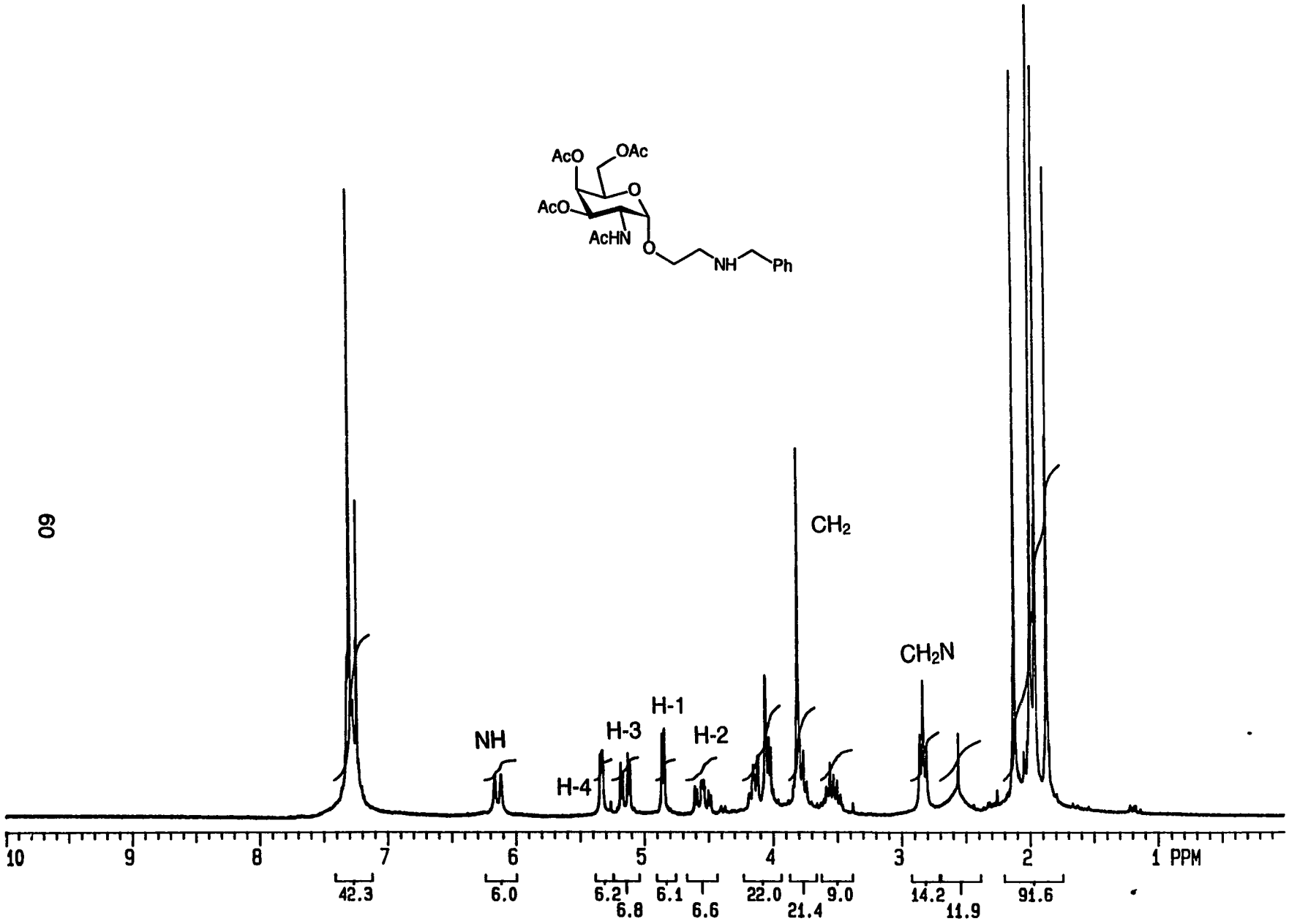


Figure 2.2.11. ^{1}H-NMR ($CDCl_3$, 200 MHz) spectrum of compound **36**.

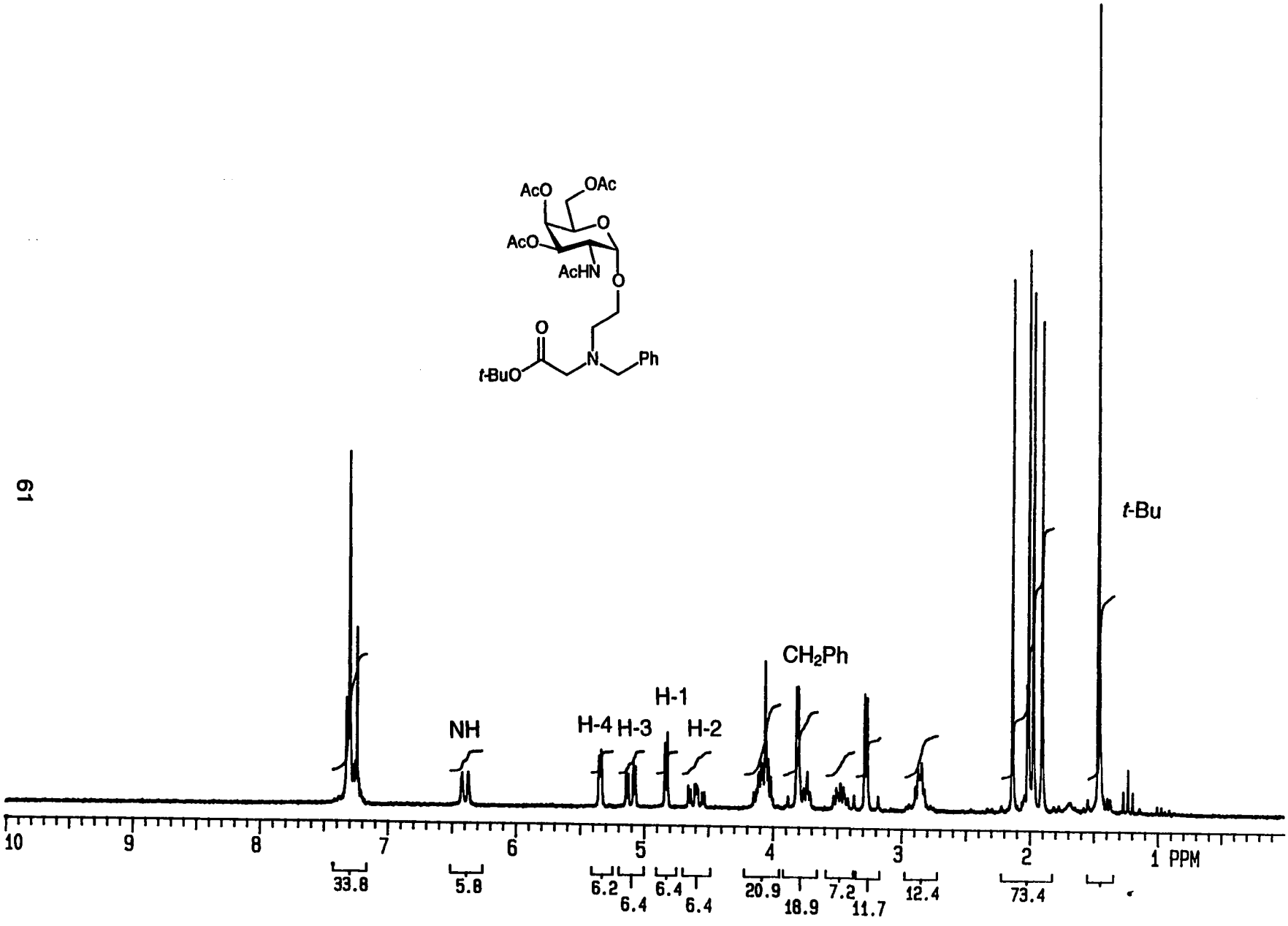
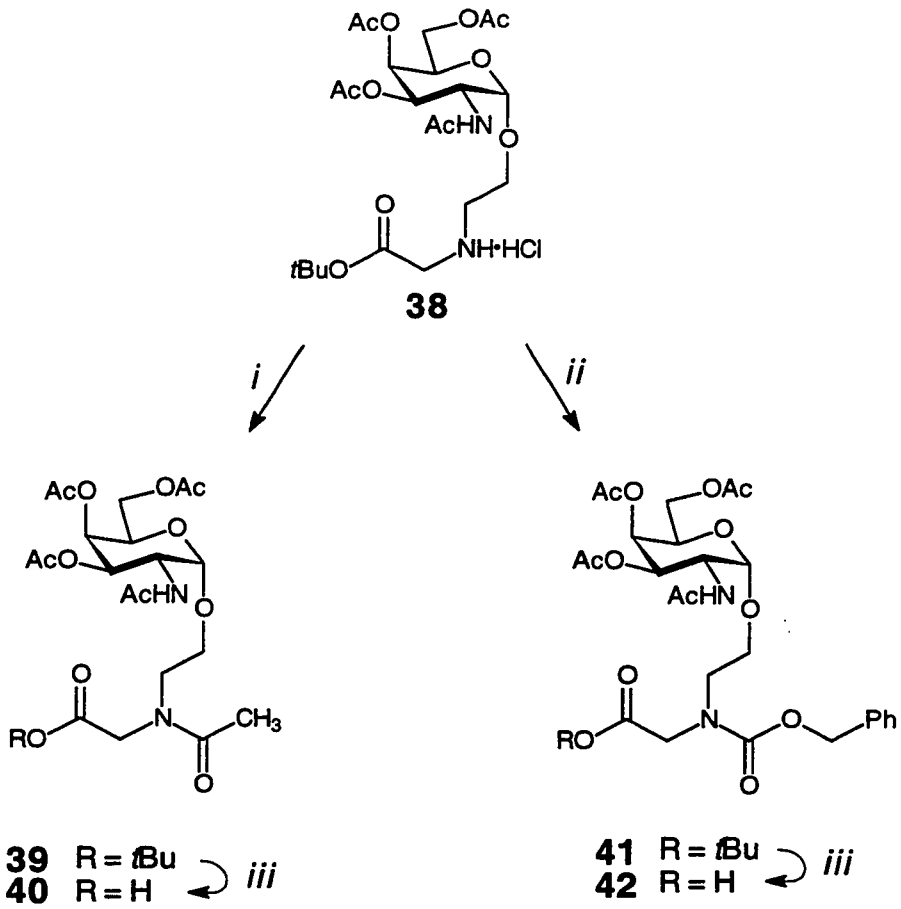
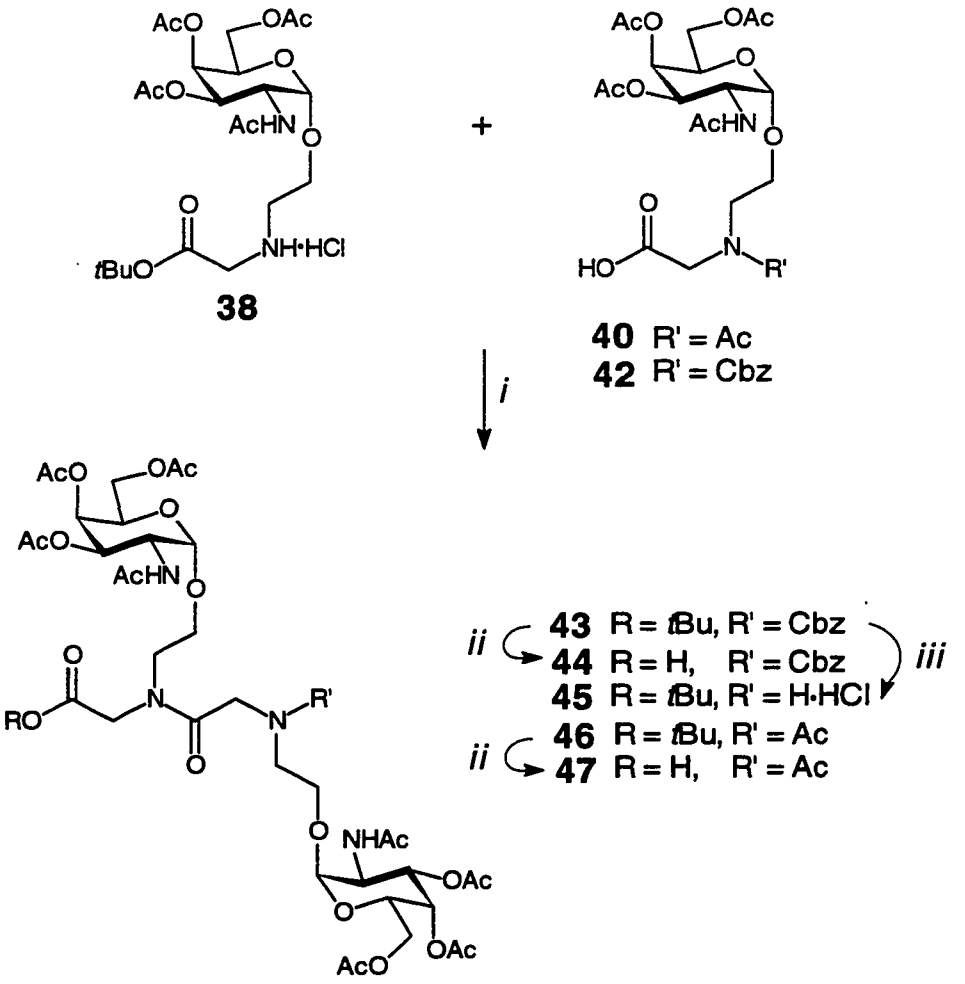


Figure 2.2.12. ^{1}H-NMR ($CDCl_3$, 200 MHz) spectrum of compound **37**.



Scheme 2.2.8. Syntheses of monovalent building blocks. *i*) AcCl, DIPEA, CH_2Cl_2, 0 °C, 94%; *ii*) Cbz-Cl, DIPEA, CH_2Cl_2, 0 °C, 1h, 77%; *iii*) 20% TFA in CH_2Cl_2, 23 °C, 2h, quant.

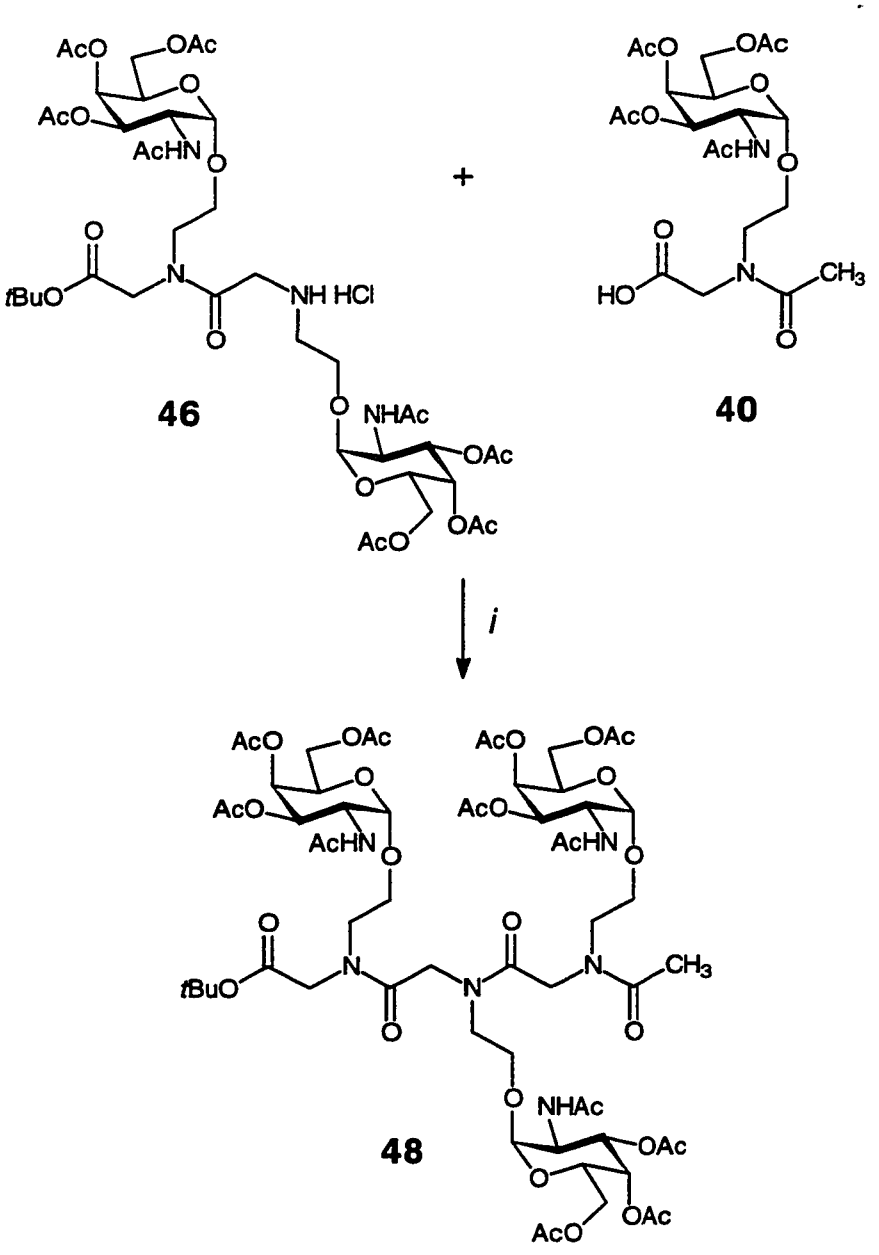
Coupling of amine **38** with either acid **40** or **42** (TBTU, DIPEA, CH_2Cl_2/CH_3CN 2:1) afforded intermediate dimer **46** or **43**, respectively (81% yield for both). Divalent *N*-Cbz-protected ester **43** was further transformed into acid **44** or amine **45** following the procedure described above, while dimeric *t*-butyl ester **46** was converted into acid **47** in a quantitative yield (20% TFA in CH_2Cl_2) (Scheme 2.2.9). Then, dimeric amine **46** was coupled to monomeric acid **40** to provide protected trimer **48** in 60% yield (BOP, NMM, CH_2Cl_2) (Scheme 2.2.10).



Scheme 2.2.9. Syntheses of divalent building blocks. *i*) TBTU, DIPEA, CH_2Cl_2/CH_3CN 2:1, 23 °C, 2h, 81% for both; *ii*) 20% TFA in CH_2Cl_2, 23 °C, 2h, quant.; *iii*) (1) H_2-Pd/C, AcOH, MeOH, 3h, (2) Amberlite IRA-400 (Cl), MeOH 16h, 23 °C.

Tetravalent *N*-Cbz-protected ester **49** and N-Ac-protected ester **51** were prepared by coupling dimeric amine **46** with N-Cbz-protected acid **44** and *N*-Ac-protected acid **47**, respectively (TBTU, DIPEA, CH_2Cl_2/CH_3CN 2:1, 68%: *N*-Cbz, 79%: *N*-Ac) (Scheme 2.2.11). By reiteration of the deprotection of *t*-butyl group from **51** and the coupling (TBTU, DIPEA) process, dimer **46** and tetramer **52** afforded hexamer **53** (62%) (Scheme 2.2.12). Alternatively, peptide coupling between tetrameric amine **50**

and its analogous tetrameric acid **52** furnished octamer **54** in 59% yield (Scheme 2.2.13).



Scheme 2.2.10. Synthesis of trimer **48**. *i*) BOP, NMM, CH_2Cl_2, 23 °C, 2h, 60%.

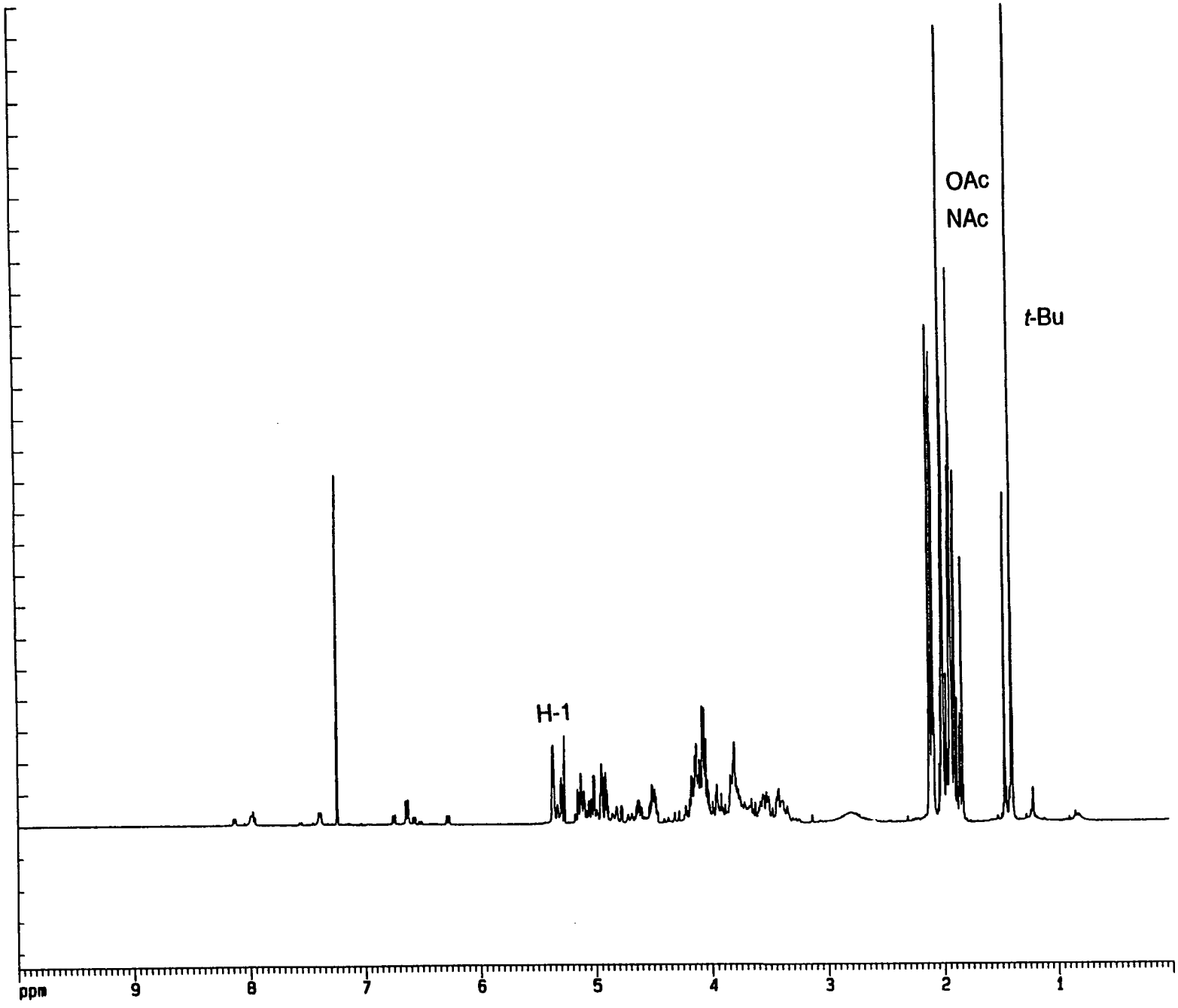
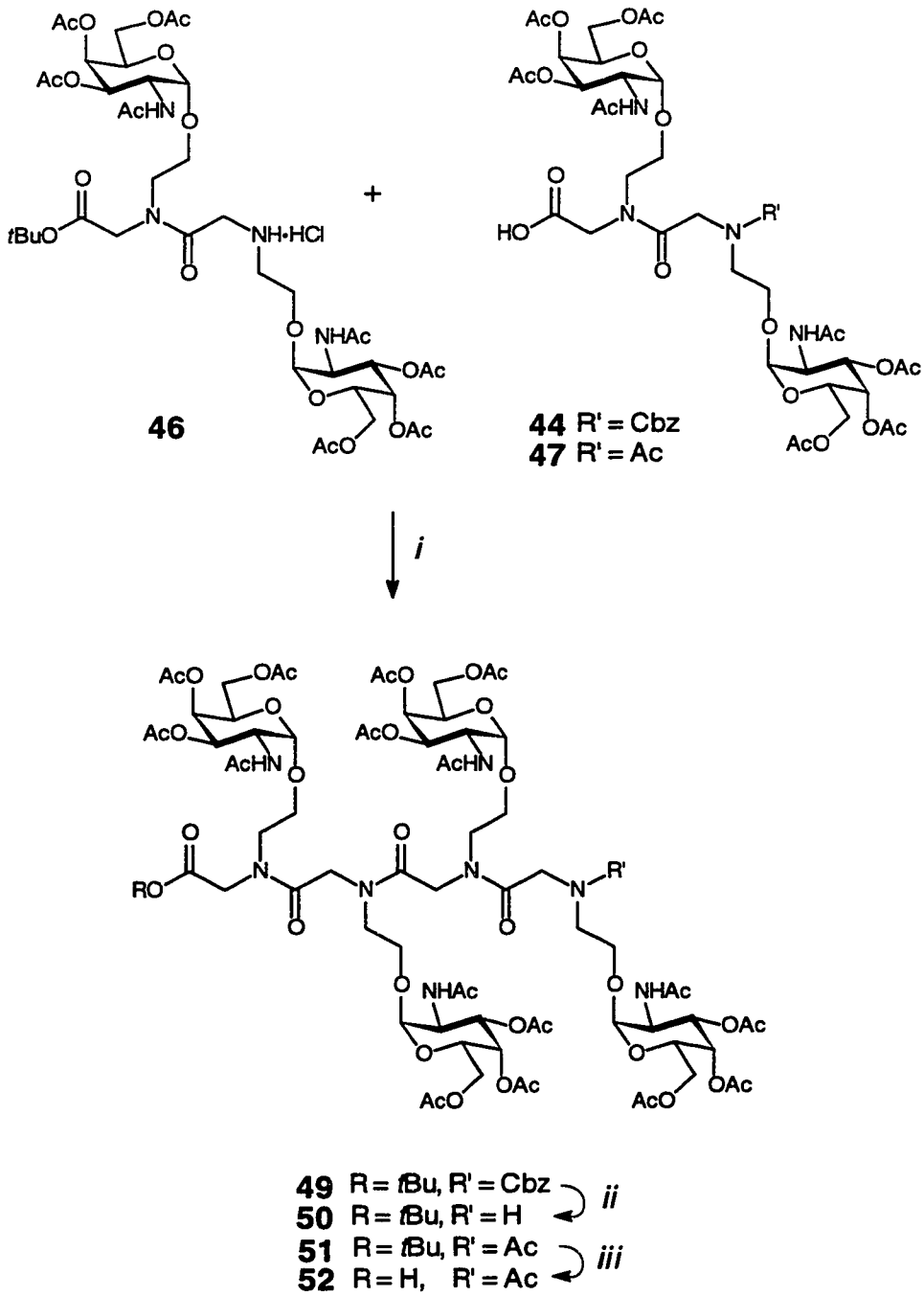
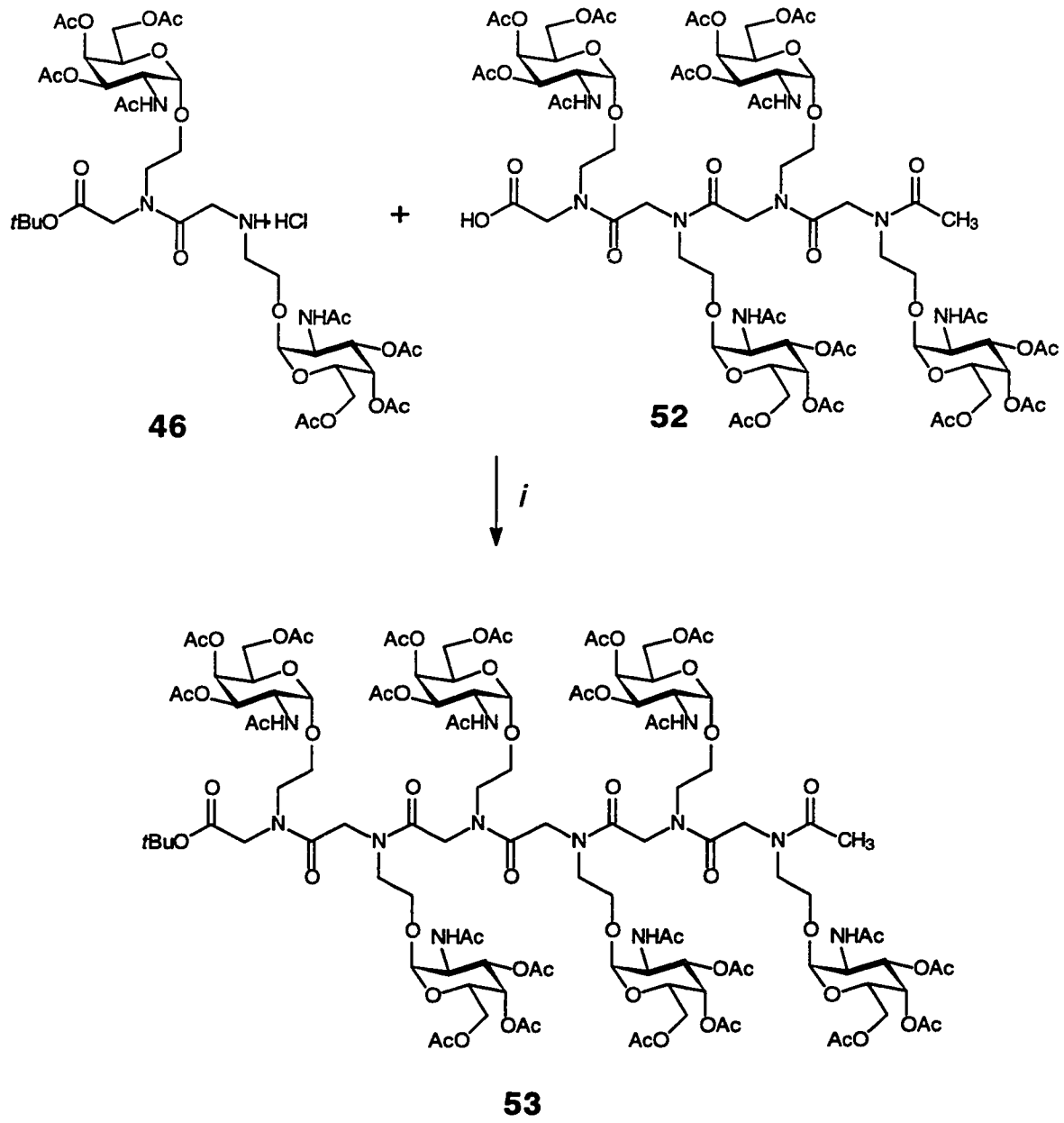


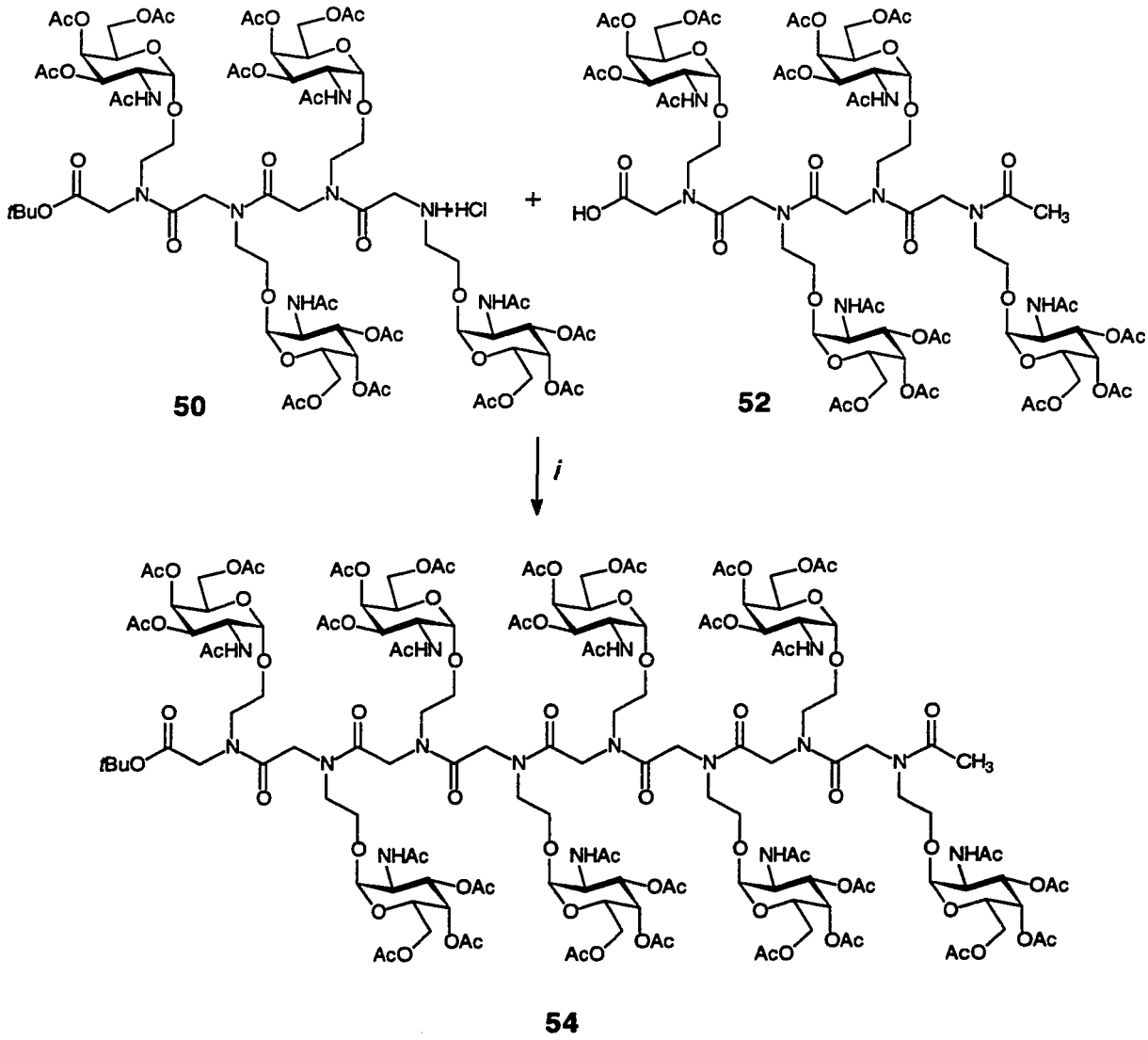
Figure 2.2.13. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of tripeptoid **48**.



Scheme 2.2.11. Syntheses of tetravalent building blocks. *i*) TBTU, DIPEA, CH_2Cl_2/CH_3CN 2:1, 23 °C, 2h, 68%: N-Cbz, 79%: N-Ac; *ii*) H_2-Pd/C, AcOH, MeOH, 3h; *iii*) 20% TFA in CH_2Cl_2, 23 °C, 2h, quant.



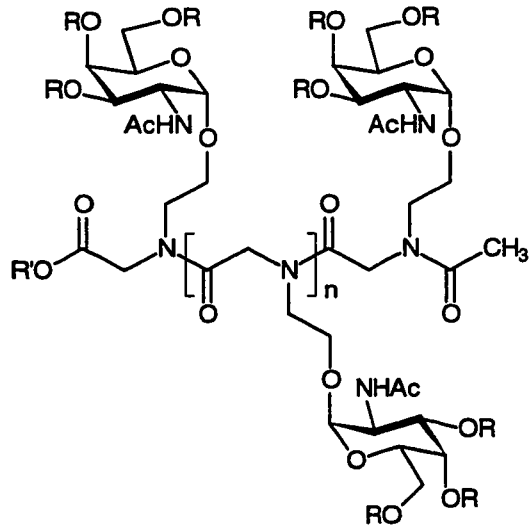
Scheme 2.2.12. Synthesis of hexamer **53**. *i*) TBTU, DIPEA, CH_2Cl_2/CH_3CN 2:1, 23 °C, 2h, 62%.



Scheme 2.2.13. Synthesis of octamer **54**. *i*) TBTU, DIPEA, CH_2Cl_2/CH_3CN 2:1, 23 °C, 16h, 59%.

All the above well-structured oligomers **46** (dimer), **48** (trimer), **51** (tetramer), **53** (hexamer), **54** (octamer) were fully deprotected in essentially quantitative yields by treatment with a catalytic amount of 1M NaOMe in MeOH (Zemplén condition, pH 9) followed by trifluoroacetolysis (20% TFA in CH_2Cl_2) (Scheme 2.2.14). All compounds

provided spectroscopic data in agreement with their structures. Per-*O*-acetylated intermediates were readily purified by silica gel chromatography, while final deprotected oligopeptoids were purified by size exclusion column chromatography (Sephadex LH20, CH_3OH). It is worth mentioning that by virtue of their tertiary amide contents, these oligomers gave mixtures of *E,Z*-conformers which could be detected on the NMR time scale. Integration of key signals, together with mass spectra were used to confirm the structures. These slowly interconverting conformers are capable of induced fit, a property which can be exploited to scan an ensemble of receptors. Moreover, these glycopeptodomimetics showed MS-fragmentation patterns similar to peptides, thus enabling easy structural determination.



i → **46** n = 0, R = Ac, R' = *t*Bu
→ **55** n = 0, R = R' = H
i → **48** n = 1, R = Ac, R' = *t*Bu
→ **56** n = 1, R = R' = H
i → **51** n = 2, R = Ac, R' = *t*Bu
→ **57** n = 2, R = R' = H
i → **53** n = 4, R = Ac, R' = *t*Bu
→ **58** n = 4, R = R' = H
i → **54** n = 6, R = Ac, R' = *t*Bu
→ **59** n = 6, R = R' = H

Scheme 2.2.14. Syntheses of fully deprotected glycopeptoids. *i*) (1) 1M NaOMe, MeOH, pH 9, 23 °C, 2-6h; (2) 20% TFA in CH_2Cl_2, 23 °C, 2h.

2.3. Binding properties of GalNAc-containing glycopeptoids

The binding properties of all the glycopeptoids bearing GalNAc were performed by solid phase competitive inhibition assay.

When the peroxidase-labeled lectin is used for the detection purpose in a competitive fashion, it should be used at a limiting concentration because at high lectin concentration a small portion will bind non-specifically to the coating antigen or directly to the plate. The limiting concentration can be determined by adding sufficient detector (labeled lectin), in the absence of sample, to give an optical density as close as possible to the maximum that can be read ('titration'). This will normally be in the steep part of the binding curve and will give the widest detectable range. If there is no plateau, then a dilution close to the highest recorded optical density reading can be used. If the curve is sigmoid, then the top of the steep part of the curve should be used.

Titration of VVA/HRP on asialoglycophorin

Prior to performing the competitive inhibition assays for the synthetic glycopeptoids bearing GalNAc, titration of lectin on antigen coated on the microtiter plate was accomplished. GalNAc specific lectin *Vicia villosa* B_4 (VVA) and asialoglycophorin, a glycoprotein found in human erythrocyte membrane and containing several GalNAc repeating units, were employed as carbohydrate binding protein and competing antigen against sample inhibitor, respectively.

The amount of coating antigen was also determined by using different amounts of asialoglycophorin (e.g. 1 μg/1 mL PBS, 5 μg/1 mL PBS, 10 μg/ 1 mL PBS). The wells were coated with 100 μL/well of asialoglycophorin solution for 2 hours at 37 °C and 100 μL/well of 10-fold serial dilutions of horseradish peroxidase-labeled VVA (VVA/HRP) from 10^{-1} to 10^{-5} mg/mL in PBS was added after blocking non-coated area of the wells with 150 μL/well of 1% BSA in PBS solution for 1 hour at 37 °C. Allowing binding of *VVA*/HRP to asialoglycophorin for 2 hours at 37 °C, the bound lectin was

detected by treating with substrate, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), diammonium salt (ABTS, 1 mg/4 mL, 50 µL/well) in citrate-phosphate buffer (0.2 M, pH 4.0 with 0.015% H_2O_2). The generation of color was detected at 410 nm relative to 570 nm after stopping the reaction with 1M H_2SO_4 (50 µL/well, 20 min.). The curves were plotted in a logarithmic scale for the lectin dilution (Figure 2.3.2). Since the resultant curve is sigmoid, the top of the steep part of the curve was chosen as a limiting concentration. As shown in Figure 2.3.1, the most fitting concentration of coating antigen, asialoglycophorin would be 5 µg/mL and the lectin VVA/HRP would be used at a 500-fold diluted concentration.

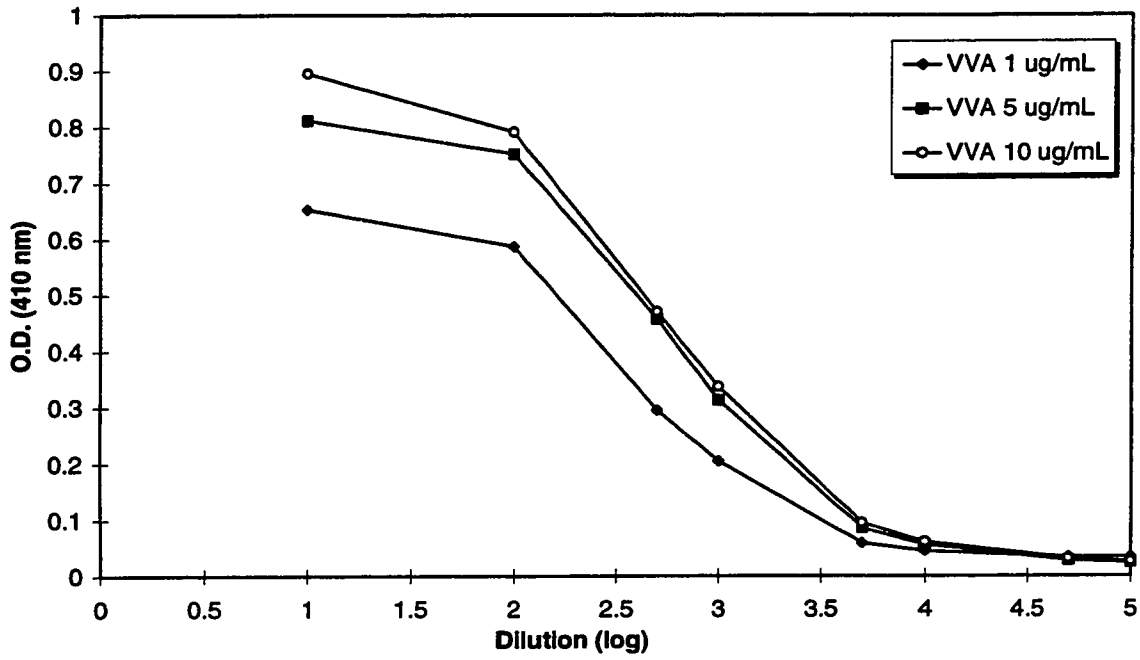


Figure 2.3.1. Titration of VVA/HRP on asialoglycophorin.

Enzyme Linked Lectin Assay (ELLA)

Once the limiting concentrations were fixed, the competitive inhibition assays including the synthetic GalNAc-containing glycopeptoids were performed.

The microtiter wells were coated with asialoglycophorin (0.5 μg/well) for 2 hours at 37 °C and then blocked with 150 μL/well of 1% BSA in PBS solution for 1 hour at 37 °C. After washing the wells with 300 μL/well of PBST three times, a mixture of glycopeptoid ligands and VVA/HRP (100 μL/well) in PBS was added and allowed to compete for the binding to VVA/HRP. The results from ELLA tests were represented in Figure 2.3.2, Figure 2.3.3, and Table 2.3.1.

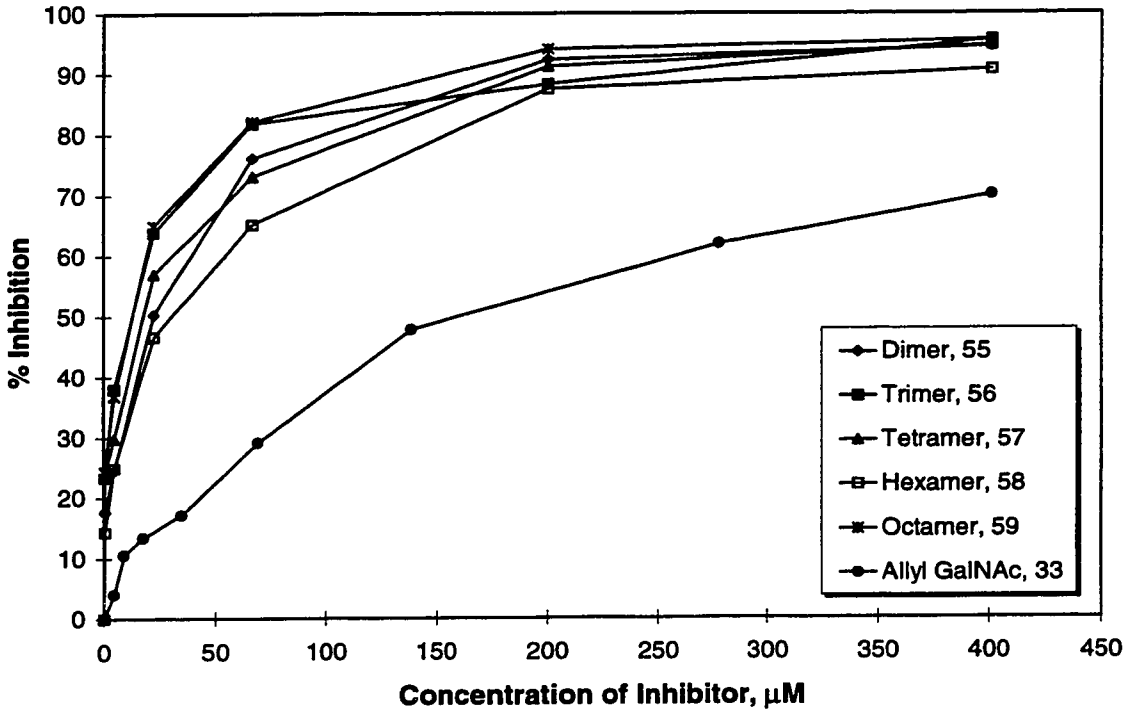


Figure 2.3.2. ELLA inhibition of binding of asialoglycophorin to VVA/HRP by glycopeptoids **55-59**.

Table 2.3.1. IC_{50}'s of GalNAc-containing glycopeptoids, **33**, **55-59**.

Glycopeptoids	IC_{50}'s (μM)	Relative potency[a]
Allyl α-D-GalNAc **33**	158.3	1
Dimer **55**	21.6	7.3 (3.7)
Trimer **56**	12.3	12.9 (4.3)
Tetramer **57**	17.3	9.2 (2.3)
Hexamer **58**	29.8	5.3 (0.9)
Octamer **59**	12.5	12.7 (1.6)

[a] Values in parentheses are based on a per-hapten in a molecule.

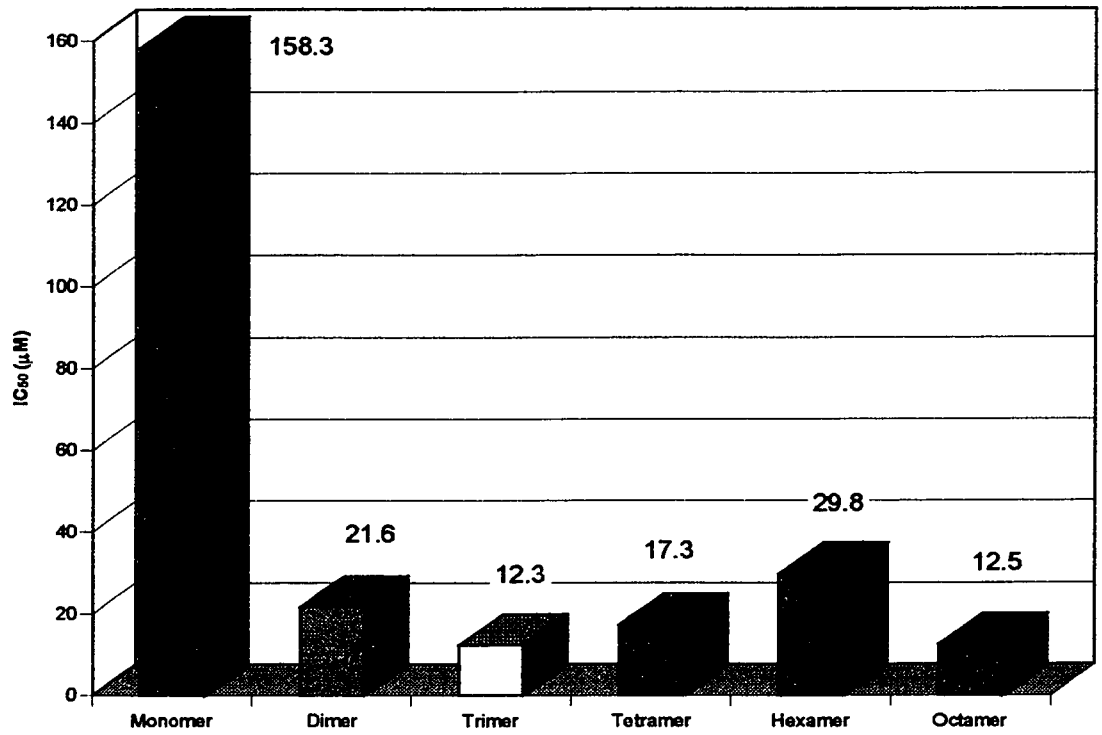


Figure 2.3.3. IC_{50}'s of GalNAc-containing glycopeptoids **33**, **55-59**.

The inhibitory properties of the glycopeptoids clearly demonstrated the cluster effect as shown in Table 2.3.1. Trimer **56** and octamer **59** had IC_{50} values as low as 12.3 μM and 12.5 μM, respectively. Taking into consideration GalNAc residues in a molecule, trimer **56** exhibited the most potent inhibitory power with 4.3-fold increase over that of allyl α-D-GalNAc **33**. The second most potent inhibitor was found to be dimer **55** (IC_{50} 21.6 μM) with 3.7-fold increase followed by tetramer **57** (IC_{50} 17.3 μM, 2.3-fold increase) and octamer **59** (IC_{50} 12.5 mM, 1.6-fold increase). These results showed that high density of carbohydrate residues in a linearly arranged scaffold was not optimally accessible for binding to lectin, probably due to the steric crowding in the binding sites and short aglycon spacer.

2.4. Conclusions

Conformationally flexible glycopeptidomimetics, glycopeptoids, were synthesized using the *N*-substituted oligoglycine strategy. These metabolically stable glycopeptoid analogs were used to investigate the role of valency in antigen presentation in a linear cluster and their conformational flexibilities. As preliminary examples of glycopeptoids, xylose-containing pentapeptoid Val-Phe-Ser-(β-D-Xyl)-Glu-Ala and tetrapeptoid Ala-Ser-(β-D-Xyl)-Gly-Ala were prepared. The tumor-associated carbohydrate antigen, Tn-antigen (GalNAcα-*O*-Ser) was also incorporated in glycopeptoid clusters. The reiterative deprotection and coupling processes afforded dimeric, trimeric, tetrameric, hexameric, and octameric glycopeptoids in good yields.

The inhibitory properties were tested by ELLA. These synthetic glycopeptoids were allowed to compete with natural glycoprotein, asialoglycophorin, for binding to the GalNAc specific lectin, VVA/HRP. These glycopeptoids showed increased inhibitory potentials demonstrating a modest cluster effect.

2.5. Experimental Methods

General Methods

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

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

Melting points were determined on Gallenkamp apparatus and are uncorrected.

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

Infrared spectra were recorded on Bomem Michelson series FT-IR apparatus. For solution, NaCl sealed cells were used.

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

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

Elemental analyses were performed by the analytical services of the Department of Chemistry of the University of Ottawa.

The pH values of aqueous solutions for the ELLA and turbidimetric assays were measured using a Fischer Scientific Model 805NP instrument fitted with a Fischer

Scientific E-N5 pencil electrode. The pH measurements for routine reactions were performed with Hydron test paper.

Thin layer chromatography (TLC) was performed using silica gel 60-F254 glass plates. Reagents used for developing plates include ceric sulfate (1% w/v) and ammonium molybdate (2.5% w/v) in 10% (v/v) aqueous sulfuric acid, iodine, ninhydrin (0.4% w/v) in aqueous pyridine (4% v/v), or UV light. TLC plates were heated to ≈150 °C when necessary.

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

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

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

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

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

Amberlite IRA-400(Cl) ion-exchange resin and Amberlite IR-120 (H) ion-exchange resin were used for synthetic purposes unless stated otherwise.

1,2,3,4-Tetra-*O*-acetyl-D-xylopyranose (2).

A suspension of anhydrous sodium acetate (20 g, 0.244 mol) in acetic anhydride (200 mL) in a round-bottomed flask was heated until the solution was homogeneous. D-Xylose (25 g, 0.167 mol) was added to the solution in small portions and stirred for 1 hour. The reaction mixture was cooled and poured into ice-water (500 mL) with stirring. The solution was extracted with CH_2Cl_2 (3 × 200 mL) and the organic layer was washed with saturated Na_2CO_3 (5 × 200 mL), water (3 × 200 mL), and then brine. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was recrystallized from ethanol to give 45.5 g of white crystals (86% yield); ^{1}H-NMR ($CDCl_3$) δ 2.01, 2.02, 2.07 (3s, 12H, 4OAc), 3.48 (dd, 1H, $J_{5a,5e}$ 12.0 Hz, $J_{5a,4}$ 8.4 Hz, H-5a), 4.11 (dd, 1H, $J_{5e,4}$ 4.9 Hz, H-5e), 4.90-5.03 (m, 2H, H-2, H-4), 5.17 (t, 1H, $J_{2,3}$ 8.1 Hz, H-3), 5.68 (d, 1H, $J_{1,2}$ 6.9 Hz, H-1). The product was predominantly the β-conformer.

2,3,4-Tri-*O*-acetyl-α-D-xylopyranosyl bromide (3).

Peracetylated xylopyranose **2** (5.0 g, 15.7 mmol) was placed in a round-bottomed flask and 45% HBr in acetic acid (10 mL) was added. The reaction mixture was stirred for 20 min. at room temperature and the progress of the reaction was monitored by TLC (R_f for peracetate: 0.21, R_f for bromide: 0.69, 3:2 Hexanes/EtOAc). When the reaction was complete, the reaction mixture was diluted with CH_2Cl_2 (100 mL) and poured into an ice-saturated $NaHCO_3$ solution. The organic phase was separated from the aqueous solution and washed with water (2 × 200 mL), then brine (1 × 200 mL). The dried organic solution (Na_2SO_4) was concentrated and the residue was recrystallized from anhydrous ether (4.90 g, 92%); mp, 101.8-102.0 °C; ^{1}H-NMR ($CDCl_3$) δ 1.99, 2.03 (2s, 9H, 3OAc), 3.80 (dd, 1H, $J_{5a,5e}$ 11.1 Hz, $J_{5a,4}$ 11.0 Hz, H-5a), 3.98 (dd, 1H, $J_{5e,4}$ 6.1 Hz, H-5e), 4.71 (dd, 1H, $J_{2,3}$ 10.0 hZ, H-2), 4.97 (ddd, 1H, $J_{3,4}$ 9.7 Hz, H-4), 5.48 (t, 1H, H-3), 6.51 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1).

Phenyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranoside (4).

To a solution of 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (**3**) (0.50 g, 1.47 mmol) and tetrabutylammonium hydrogen sulfate (TBAHS) (0.50 g, 1.47 mmol) in ethyl

acetate (5 mL) was added thiophenol (0.49 g, 4.42 mmol) in 1M sodium carbonate (5 mL). The reaction mixture was vigorously stirred at room temperature for 40 min. Ethyl acetate (15 mL) was added and the organic phase was separated from the aqueous phase. The organic solution was washed with 1M NaOH (20 mL), water (20 mL × 2), and brine (15 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. The crude compound was purified by silica gel column chromatography eluting with hexanes/ethyl acetate (7:3) to give the title compound **7** (0.53 g, 1.43 mmol) in 95 % yield; mp 77.6-77.9 °C; $[\alpha]_D$ -54.9 (*c* 1.0 $CHCl_3$); Lit.[149] mp 79-80 °C, $[\alpha]_D$ -55 (*c* 1.0 $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 2.02 (s, 6H, 2 × OAc), 2.07 (s, 3H, OAc), 3.40 (dd, 1H, $J_{5a,4}$ 8.8 Hz, H-5a), 4.26 (dd, 1H, $J_{5e,4}$ 4.9 Hz, $J_{5e,5a}$ 11.8 Hz, H-5e), 4.78 (d, 1H, $J_{1,2}$ 8.3 Hz, H-1), 4.90 (m, 1H, H-4), 4.92 (t, 1H, $J_{2,3}$ 8.2 Hz, H-2), 5.16 (t, 1H, $J_{3,4}$ 8.2 Hz, H-3), 7.28-7.30 (m, 3H, *o,p*-Ar), 7.44-7.45 (m, 2H, *m*-Ar); ^{13}C NMR ($CDCl_3$) δ 20.7 (OAc), 65.2 (C-5), 68.4 (C-4), 69.8 (C-2), 72.0 (C-3), 86.3 (C-1), 128.2 (*o*-C_{Ar}), 129.0 (*p*-C_{Ar}), 132.2 (*ipso*-C_{Ar}), 132.7 (*m*-C_{Ar}); Anal. Calcd for $C_{17}H_{20}O_7S$: C, 55.42; H, 5.48. Found: C, 55.55; H, 5.43.

Phenyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-xylopyranoside (5).

Phenyl 1-thio-β-D-xylopyranoside was obtained from phenyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranoside **(4)** under the usual Zemplén conditions (NaOMe, MeOH, r.t., 4 h.). To a solution of phenyl 1-thio-β-D-xylopyranoside (1.97 g, 8.15 mmol) in pyridine (10 mL) at 0 °C was added dropwise benzoyl chloride (5.16 g, 36.7 mmol). The solution was then stirred for 1.5 h at room temperature. The reaction mixture was then treated with ice-water (15 mL) for 1 h. to destroy the excess benzoyl chloride. The organic phase was separated and washed with saturated $NaHCO_3$ (20 mL × 2), water (20 mL × 2), and brine (20 mL). The organic phase was dried over anhydrous Na_2SO_4, concentrated under vacuum and the residue was purified by silica gel column chromatography using hexane/ethyl acetate (4:1) as eluant. Compound **5** (3.96 g, 7.16 mmol) was obtained as a white solid in 88 % yield; mp 60.5-61.6 °C; $[\alpha]_D$ -29.3 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 3.81 (dd, 1H, $J_{5a,5e}$ 12.2 Hz, $J_{5a,4}$ 6.6 Hz, H-5a), 4.70 (dd,

[149] Ferrier, R. J.; Furneaux, R. H. *Carbohydr. Res.* **1976**, *52*, 63.

1H, $J_{5e,4}$ 3.9 Hz, H-5e), 5.27 (d, 1H, $J_{1,2}$ 6.2 Hz, H-1), 5.26-5.30 (m, 1H, H-4), 5.45 (t, 1H, $J_{2,3}$ 6.3 Hz, H-2), 5.77 (t, 1H, $J_{3,4}$ 6.6 Hz, H-3), 7.18-7.57 (m, 6H, *o*-Ar), 7.95-8.09 (m, 14H, Ar); ^{13}C-NMR ($CDCl_3$) δ 64.2 (C-5), 69.3 (C-4), 70.6 (C-2), 71.0(C-3), 87.0 (C-1), 128.7, 129.0, 129.5, 129.7, 129.8, 130.5, 130.6, 130.8, 133.2, 133.7, 134.0, 134.1(Ar), 165.7, 165.8, 166.1(C=O's); Anal. Calcd for $C_{32}H_{26}O_7S$: C, 69.30; H, 4.73. Found: C, 69.45; H, 4.77.

2-Azidoethyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranoside (6).

To the solution of phenyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-xylopyranoside **(5)** (1.0 g, 1.80 mmol) and 2-azidoethanol (0.47 g, 5.40 mmol) in CH_2Cl_2 containing 4 Å molecular sieves (3.0 g) was added DMTST (1.86 g, 7.22 mmol) at 0 °C under nitrogen. The reaction mixture was stirred for 7 h at 0 °C and for another 18 h at room temperature until the reaction was complete as judged by TLC. The reaction mixture was then filtered through a celite pad and the filtrate was concentrated under vacuum. The crude residue was purified by silica gel column chromatography using ethyl acetate/hexane (1:4) as eluant to give **6** (0.74 g, 1.40 mmol) as a white solid in 78% yield; mp 115.0-115.5 °C; $[\alpha]_D$ -38.8 (*c* 0.34, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 3.36-3.41, 3.44-3.49 (m, 2H, CH_2N_3), 3.69-3.73, 3.99-4.03 (m, 2H, OCH_2), 3.73 (dd, 1H, $J_{5a,5e}$ 12.2 Hz, $J_{5a,4}$ 6.7 Hz, H-5a), 4.46 (dd, 1H, $J_{5e,4}$ 4.2 Hz, H-5e), 4.89 (d, 1H, $J_{1,2}$ 5.2 Hz, H-1), 5.28-5.31 (m, 1H, H-4), 5.38 (dd, 1H, $J_{2,3}$ 7.1 Hz, H-2), 5.75 (t, 1H, $J_{3,4}$ 7.0 Hz, H-3), 7.30-7.38 (m, 6H, *m*-Ar), 7.47-7.54 (m, 3H, *p*-Ar), 7.94-8.00 (m, 6H, *o*-Ar); ^{13}C NMR ($CDCl_3$) δ 50.7 (CH_2N_3), 61.2 (C-5), 67.6 (OCH_2), 69.0 (C-4), 70.0 (C-2, C-3), 100.0 (C-1), 128.33, 128.40, 128.43 (3 × *m*-C_{Ar}), 129.12, 129.24, 129.29 (3 × *ipso*-C_{Ar}), 129.89, 129.91(3 × *o*-C_{Ar}), 133.27, 133.37, 133.40 (3 × *p*-C_{Ar}), 165.17, 165.36, 165.56 (C=O's); CI-MS (*m/z*) calcd. for $C_{28}H_{25}N_3O_8$: 531.16; found: 531.9 (M^+ + 1, 0.9%), 504.0 (M^+ + 1-N_2, 7.7%), 444.9 (M^+ + 1-$OCH_2CH_2N_3$, 55.3%); FTIR ($CHCl_3$) ν 2105 cm^{-1} (N_2).

2-Aminoethyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranoside (7).

A solution of 2-azidoethyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranoside **(6)** (0.53 g, 1.00 mmol) in MeOH (5 mL) was added to a suspension of 10% Pd/C (0.10 g) in

methanol (15 mL). Nitrogen was bubbled into the solution for 5 min and then hydrogen was bubbled overnight under atmospheric pressure. The suspension was filtered through a celite pad and the filtrate was concentrated under vacuum to provide **7** (0.48 g, 0.95 mmol) in 95% yield; ^{1}H-NMR ($CDCl_3$) δ 1.56 (bs, 2H, NH_2), 2.81-2.90 (m, 2H, CH_2N), 3.54-3.58 (m, 1H, OCH_2), 3.69 (dd, 1H, $J_{5a,5e}$ 12.1 Hz, $J_{5a,4}$ 7.4 Hz, H-5a), 3.87-3.91 (m, 1H, OCH_2), 4.42 (dd, 1H, $J_{5e,4}$ 4.4 Hz, H-5e), 4.83 (d, 1H, $J_{1,2}$ 5.7 Hz, H-1), 5.28-5.32 (m, 1H, H-4), 5.38 (dd, 1H, $J_{2,3}$ 7.6 Hz, H-2), 5.76 (t, 1H, $J_{3,4}$ 7.5 Hz, H-3), 7.32-7.40 (m, 6H, *m*-Ar), 7.45-7.53 (m, 3H, *p*-Ar), 7.94-8.03 (m, 6H, *o*-Ar); ^{13}C-NMR ($CDCl_3$) δ 41.8 (CH_2N), 61.5 (C-5), 69.3 (C-4), 70.5 (C-2, C-3), 71.8 (OCH_2), 100.5 (C-1), 128.4 (*m*-C_{Ar}), 129.1 (*ipso*-C_{Ar}), 129.8 (*o*-C_{Ar}), 133.4 (*p*-C_{Ar}), 165.2, 165.4, 165.6 (C=O's); FAB-MS (*m/z*) calcd. for $C_{28}H_{27}NO_8$: 505.17; found: 506.30 (M^+ + 1, 1.1%), 446.23 (M^+ + 1-$OCH_2CH_2NH_2$, 1.1%); Anal. Calcd for $C_{27}H_{27}NO_8$: C,66.51; H 5.39; N 2.77; found: C, 66.77; H, 5.42; N 2.80.

t-Butyl *N*-isopropylglycinate (9).

t-Butyl bromoacetate (1.0 g, 5.13 mmol) in CH_3CN (10 mL) was added dropwise to a solution of diisopropylethylamine (DIPEA) (0.91 g, 15.4 mmol) in CH_3CN (10 mL) at 0 °C. The solution was stirred at that temperature for 30 min. The solvent was removed under vacuum and the residue was dissolved in CH_2Cl_2. The solution was washed with water (30 mL × 2) and the organic phase was dried over anhydrous Na_2SO_4 and concentrated under vacuum to give crude compound **2** which was used for the next step without further purification; ^{1}H-NMR ($CDCl_3$) δ 0.90 (d, 6H, J 6.2 Hz, CMe_2), 1.31 (s, 9H, CMe_3), 1.45 (bs, 1H, NH), 2.62 (m, 1H, C*H*Me_2), 3.14 (s, 2H, $COCH_2$).

t-Butyl *N*-bromoacetyl-*N*-isopropylglycinate (10).

To a solution of **2** and diisopropylethylamine (5.13 mmol) in CH_2Cl_2 (20 mL) was added dropwise bromoacetyl chloride (0.81 g, 5.13 mmol). The solution was stirred at 0 °C for 30 min after which time it was washed with water (15 mL), 5% aqueous HCl and 5% aqueous $NaHCO_3$. The organic phase was dried over anhydrous Na_2SO_4, concentrated, and purified by silica gel column chromatography using hexane/ethyl

acetate (4:1, v/v, R_f 0.28) as eluant to give pure compound **10** as a colorless oil (1.13g, 3.85 mmol) in 75% yield for the two steps; ^{1}H-NMR ($CDCl_3$) δ 1.05, 1.21 (2d, 6H, J 6.9 Hz, CHC*Me*$_2$), 1.43, 1.45 (2s, 9H, C*Me*$_3$), 3.76, 3.78, 3.88, 3.89 (4s, 4H, 2CH_2), 4.12, 4.77 (2m, 1H, C*H*Me$_3$); ^{13}C-NMR ($CDCl_3$) δ 19.5, 20.8 (C*Me*$_2$), 25.8, 27.2 (CH_2Br), 28.0 (C*Me*$_3$), 43.4, 45.7 ($COCH_2N$), 49.2, 49.7 (*C*HMe$_2$), 81.6, 82.8 (*C*Me$_3$), 166.2, 168.0, 168.8 (C=O's) ; ratio of rotamers=1:1.6; CI-MS (*m/z*) calcd. for $C_{11}H_{20}NO_3Br$: 293.06; found: 294.0 (M^+ + 1, 73.3%);.

t-Butyl (*N*-phenylglycyl)-*N*-isopropylglycinate (11).

Benzylamine (368 mg, 3.43 mmol) was added to a solution of **3** (336 mg, 1.14 mmol) in CH_3CN (15 mL) at 0 °C and the solution was stirred for 20 min. The solvent was evaporated and the residue was dissolved in CH_2Cl_2 which was then washed with water (15 mL), saturated $NaHCO_3$ (15 mL) and dried over anhydrous Na_2SO_4. Silica gel column chromatography of the residue using a mixture of methylene chloride and methanol (96:4, v/v, R_f 0.44) afforded **11** (658 mg, 2.06 mmol) as a colorless oil in 78% yield; ^{1}H-NMR ($CDCl_3$) δ 1.05, 1.11 (2d, 6H, J 6.9 Hz, CH*Me*$_2$), 1.39, 1.43 (2s, 9H, C*Me*$_3$), 2.32 (bs, 1H, NH), 3.26-3.82 (m, 6H, 3 × CH_2), 3.94, 4.86 (2m, 1H, C*H*Me$_2$), 7.16-7.41 (m, 5H, Ph).

t-Butyl (*N*-bromoacetyl-*N*-phenylglycyl)-*N*-isopropylglycinate (12).

Diisopropylethylamine (121 mg, 936 μmol) was added to a solution of **4** (250 mg, 780 μmol) in CH_2Cl_2 (10 mL). Then, bromoacetyl chloride (147 mg, 936 μmol) was added dropwise at 0 °C and the resulting solution was stirred at that temperature for 20 min. The reaction mixture was washed with 5% HCl (10 mL), saturated $NaHCO_3$ (10 mL), and water (10 mL). The dried organic solution (anhydrous Na_2SO_4) was concentrated under vacuum and the residue was purified by silica gel column chromatography using 2:3 ethyl acetate/hexane (R_f 0.36) as eluant to give **12** (267 mg, 605 μmol) as a colorless oil in 78% yield; ^{1}H-NMR ($CDCl_3$) δ 1.02, 1.03, 1.11(3d, 6H, J 6.9 Hz, CH*Me*$_2$), 1.31, 1.42, 1.43 (3s, 9H, C*Me*$_3$), 3.63-4.90 (m, 9H, 4 × CH_2, C*H*Me$_2$),

7.20-7.37 (m, 5H, Ph); CI-MS (*m/z*) for $C_{20}H_{29}N_2O_4Br$: 440.13; found 440.9/442.9 (M^++1/M^++3, 8.6%/10.0%), 384.9/386.9 (M^++1/M^++3-*t*Bu, 37.4%/33.9%), 267.9/269.9 (M^++1/M^++3-*t*$BuO_2CCH_2NCHMe_2$, 100%/100%).

_N_-Alkylation of 7 with 12 to give 13.

To a solution of dipeptoid unit **12** (154 mg, 350 μmol) and diisopropylethylamine (54 mg, 420 μmol) in CH_3CN (15 mL) was added 2-aminoethyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranoside **(7)** (212 mg, 420 μmol). The solution was stirred for 1 h at 0 °C. The reaction mixture was then concentrated and the residue was purified by silica gel column chromatography using a mixture of $CHCl_3$/MeOH/MeCN (18:1:1, R_f 0.40) as eluant to give **13** (152 mg, 175 μmol) as a white foam in 47% yield; ^{1}H NMR ($CDCl_3$) δ 1.04, 1.12, 1.13 (3d, 6H, J 6.9 Hz, CH*Me*$_2$), 1.34, 1.39, 1.44 (3s, 9H, CMe_3), 2.08 (bs, 1H, NH), 2.83, 2.91 (2dd, 2H, J 5.5 Hz, 2.0 Hz, NCH_2CH_2OXyl), 3.38-4.21 (m, 10H), 4.41 (dd, 1H, $J_{5a,5e}$ 12.1 Hz, $J_{5e,4}$ 4.2 Hz, H-5e), 4.58-4.61 (m, 2H), 4.85 (d, 1H, $J_{1,2}$ 5.3 Hz, H-1), 5.26 (m, 1H, H-4), 5.32 (t, 1H, $J_{2,3}$ 7.1 Hz, H-2), 5.72 (t, 1H, $J_{3,4}$ 7.3 Hz, H-3), 7.17-7.40 (m, 11H, *m*-Ar, Ph), 7.44-7.53 (m, 3H, *p*-Ar), 7.93-8.00 (m, 6H, *o*-Ar); ^{13}C-NMR ($CDCl_3$) δ 19.7, 20.9, 27.8, 27.9, 28.0, 30.9, 45.3, 46.1, 47.6, 48.9, 50.3, 51.0, 61.3, 69.2, 70.4, 81.3, 82.4, 100.2, 100.4, 126.9, 128.3, 128.4, 128.6, 128.7, 128.9, 129.2, 129.3, 129.8, 129.9, 133.2, 165.4, 165.5, 167.4, 168.6; FAB-MS (pos. m/z) calcd. for $C_{48}H_{55}N_3O_{12}$: 865.38; found: 866.10 (M^+ + 1, 19.7%), 444.96 (M^+ + 1-aglycon, 2.0%).

***t*-Butyl *N*-methylglycinate (14).**

To a solution of methylamine (30% w/w in H_2O, 4.0 g) in CH_3CN (5 mL) was added dropwise *t*-butyl bromoacetate (1.0 g, 5.13 mmol) in CH_3CN (2 mL) under nitrogen atmosphere at 0 °C. The reaction was monitored by TLC (R_f for reactant: 0.78, R_f for product: 0.31, 3:2 EtOAc/Hexanes), staining plate with ninhydrin solution. After 30 min., the reaction solution was concentrated to a volume of 3~4 mL. The solution was then extracted with CH_2Cl_2 (3 × 20 mL) and the organic phase was washed with water (20 mL). The organic solution was dried over anhydrous Na_2SO_4 and

concentrated to a volume of about 5 mL. This solution was directly used for the next step.

t-Butyl *N*-bromoacetyl-*N*-methylglycinate (15).

Compound **14** in CH_2Cl_2 was treated with DIPEA (0.80 g, 0.16 mmol) at 0 °C and bromoacetyl chloride (0.81 g, 5.13 mmol) in CH_2Cl_2 (5 mL) was added to the reaction mixture under a nitrogen atmosphere. The reaction solution was stirred for 30 min. at 0 °C and then washed with saturated $NaHCO_3$ (2 × 5 mL), 5% HCl (1 × 5 mL), and water (1 × 5 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Silica gel column chromatography of the crude product eluting with 3:2 hexanes/EtOAc yielded 1.01 g of colorless oil (74% yield for two steps); ^{1}H-NMR ($CDCl_3$) δ 1.39, 1.41 (2s, 9H, C*Me*$_3$), 2.91, 3.06 (2s, 3H, NCH_3), 3.73, 3.84, 3.93, 4.06 (4s, 4H, $2CH_2$); ^{13}C-NMR ($CDCl_3$) δ 24.8, 27.2 (C*Me*$_2$), 34.4, 36.0 (CH_2Br), 26.8, 40.2 (C*Me*$_3$), 49.6, 52.0 ($COCH_2$), 82.4, 83.2 (*C*Me$_3$), 167.8, 168.2 (C=O's); CI-MS(m/z) calcd. for $C_9H_{16}NO_3Br$: 265.03; found: 266.0/268.6 ($M^+ + 1$/ $M^+ + 3$, 44.1/42.9%); ratio of two rotamers=1:2.3.

t-Butyl *N*-acetyl-*N*-methylglycinate (16).

t-Butyl bromoacetate **(8)** (1.0 g, 5.13 mmol) in CH_3CN (10 mL) was added dropwise to a solution of methylamine (30 % w/w in water, 2.4 g, 15.39 mmol) in CH_3CN. The reaction was allowed to proceed at 0 °C for 30 min. The solution was concentrated and the residue was diluted with CH_2Cl_2. The organic solution was washed with water, dried over anhydrous Na_2SO_4 and then concentrated under vacuum to give crude intermediate **14** which was used for the next step without further purification. To a solution of **14** and diisopropylethylamine in CH_2Cl_2 (10 mL) was added acetyl chloride (0.40 g, 5.13 mmol) in CH_2Cl_2 (5 mL). The solution was then stirred for 30 min. at 0 °C. The reaction mixture was washed with water, 5% aqueous HCl, and saturated $NaHCO_3$. The organic solution was dried over Na_2SO_4 and concentrated. Purification of the residue by silica gel column chromatography gave **16** as a white solid in 89% yield;^{1}H-NMR ($CDCl_3$) δ 1.34, 1.36 (2s, 9H, CMe_3), 1.91, 2.01 (2s, 3H, $COCH_3$), 2.83, 2.95 (2s, 3H, NCH_3), 3.81, 3.89 (2s, 2H, CH_2); FAB-MS (pos.

m/z) calcd. for $C_9H_{17}NO_3$: 187.12; found: 188.03 (M^++1, 54.3%); ratio of two rotamers 1:2.4.

_N_-Acetyl-_N_-methylglycine (17).

Compound **16** (1.69 g, 9.04 mmol) was treated with a 20 % solution of TFA in CH_2Cl_2 (120 mL) for 2 h at room temperature. The reaction mixture was then concentrated and coevaporated a few times with toluene to give **17** (1.09 g, 8.34 mmol) in 92% yield); ^{1}H-NMR ($CDCl_3$) δ 2.15, 2.23 (2s, 3H, $COCH_3$), 3.03, 3.14 (2s, 3H, NCH_3), 4.13, 4.20 (2s, 2H, CH_2), 11.73 (bs, 1H, CO_2H); ratio of two rotamers 1:4.4.

Benzyl _N_-[2-(_t_-butyloxycarbonyl)ethyl]glycinate (20).

To a solution of β-alanine _t_-butyl ester hydrochloride **(19)** (825 mg, 4.54 mmol) in CH_3CN was added diisopropylethylamine (1.47 g, 11.35 mmol). The resulting solution was stirred for 10 min and then benzyl bromoacetate **(18)** (800 mg, 3.49 mmol) was added. The reaction mixture was then stirred for 15 min at 0 °C. The concentrated solution was purified by chromatography as above using a mixture of hexane and ethyl acetate (3:2, R_f 0.28) as eluant. Compound **20** (813 mg, 2.77 mmol) was obtained as a colorless oil in 79% yield; ^{1}H-NMR ($CDCl_3$) δ 1.43 (s, 9H, CMe_3), 1.84 (bs, 1H, NH), 2.40 (t, 2H, J 6.5 Hz, CH_2CO), 2.83 (t, 2H, J 6.5 Hz, NCH_2), 3.44 (s, 2H, $COCH_2N$), 5.15 (s, 2H, $PhCH_2O$), 7.33-7.35 (m, 5H, Ph).

Coupling of 20 to 17 to give 21.

To a mixture of **20** (500 mg, 1.71 mmol) and **17** (224 mg, 1.71 mmol) in CH_2Cl_2 (20 mL) was added 1,3-dicyclohexylcarbodiimide (DCC, 528 mg, 2.56 mmol). The solution was stirred for 5 h at room temperature. The white solid dicyclohexylurea formed during the reaction was filtered through a cotton wool and the filtrate was concentrated. Column chromatography with CH_2Cl_2/MeOH (98:2, R_f 0.30) as eluant afforded **21** (679 mg, 1.67 mmol) as a colorless oil in 98% yield); ^{1}H-NMR ($CDCl_3$) δ 1.39, 1.41 (2s, 9H, CMe_3), 1.88, 1.97, 2.04, 2.11 (4s, 3H, $COCH_3$), 2.52, 2.55 (2t, 2H, J 6.8 Hz, CH_2CO_2), 2.85, 2.91, 2.99, 3.05 (4s, 3H, CH_3N), 3.56, 3.61 (2t, 2H, J 6.8 Hz,

NCH_2), 4.03-4.30 (m, 4H, 2 × CH_2), 5.12-5.15 (m, 2H, $PhCH_2O$), 7.32-7.34 (m, 5H, Ph); rotamers ratio 5:3:1.5:1.

Compound 22.

Nitrogen was passed for 5 min in a solution of **21** (55.6 mg, 137 μmol) in methanol (10 mL). Pd/C (10 % w/w, 6.0 mg) was added to the solution and then hydrogen was passed through the solution for 3 h. The reaction mixture was filtered through a celite pad and the filtrate was concentrated to afford **22** (42.5 mg, 134 μmol) as a colorless oil in 98 % yield; ^{1}H-NMR ($CDCl_3$) δ 1.39, 1.40, 1.41 (4s, 9H, CMe_3), 1.97, 1.99, 2.10, 2.13 (4s, 3H, $COCH_3$), 2.51, 2.54 (2t, 2H, J 6.8 Hz, CH_2CO_2), 2.90, 2.91, 3.05, 3.06 (4s, 3H, NCH_3), 3.57, 3.60 (2t, 2H, J 6.8 Hz, NCH_2), 4.00-4.40 (m, 4H, 2 × CH_2), 7.90 (bs, 1H, CO_2H); ^{13}C-NMR ($CDCl_3$) δ 21.2 (OAc), 28.0 (CMe_3), 33.9, 34.1, 34.4 (CH_2CO_2tBu), 37.5, 37.6 (NCH_3), 44.1, 44.7 ($CONCH_2$), 48.1, 49.1, 49.2, 50.4 (CH_2's), 80.9, 81.5 (CMe_3), 168.7, 169.0, 170.5, 171.6, 172.2, 172.4 (C=O's); rotamers ratio 6.8:6.5:1.5:1; CI-MS (m/z) calcd. for $C_{14}H_{24}N_2O_6$: 316.16; found: 317.0 (M^+ + 1, 2.6%); Anal. Calcd for $C_{14}H_{24}N_2O_6$: C 53.14; H, 7.65; N, 8.86: found: C, 53.33; H, 7.71; N 8.60.

Compound 23.

To a mixture of **13** (119 mg, 137 μmol) and **22** (43.4 mg, 137 μmol) in CH_2Cl_2 (10 mL) was added 1,3-dicyclohexylcarbodiimide (28.4 mg, 137 μmol). The reaction mixture was stirred for 2 h at room temperature. The white precipitate formed was filtered and the solution was concentrated, the residue was then purified by column chromatography using a mixture of $CHCl_3$/MeOH/MeCN (18:1:1, R_f 0.31) as eluant to give **23** (147 mg, 126 μmol) as a white solid in 92 % yield; mp 85.0-86.5 °C; $[\alpha]_D$ -9.22 (*c* 1.8, $CHCl_3$); FAB-MS (pos. m/z) calcd. for $C_{62}H_{77}N_5O_{17}$: 1163.53 ; found: 1164.33 (M^+ + 1, 0.5%); Anal. Calcd. for $C_{62}H_{77}N_5O_{17}$: C, 63.94; H, 6.67; N, 6.02; found: C, 63.97; H, 6.64; N, 5.93.

Compound 25.

A solution of **23** (40 mg, 34.4 μmol) in methanol (5 mL) was treated with a 1M $NaOCH_3$ solution in MeOH until pH 9. The solution was then stirred for 2 h at room temperature. The reaction mixture was treated with H^+ resin for 30 min, filtered, and then concentrated to give **24** as a white solid in quantitative yield. Compound **24** was used for the next step without further purification. It was dissolved in CH_2Cl_2 (2 mL) containing 20 % TFA and the resulting solution was stirred for 1 h at room temperature to give the fully deprotected pentapeptoid **22** in essentially quantitative yield; FAB-MS (pos. *m/z*) calcd. for $C_{33}H_{49}N_5O_{14}$: 739.33; found: 740.20 (M^+ + 1, 1.1%).

***t*-Butyl [*N*-(2-aminoethyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl)glycyl]-*N*-methylglycinate (26).**

To a solution of 7 (0.42 g, 0.83 mmol) and DIPEA (0.16 g, 1.25 mmol) in CH_3CN (15 mL) was added dropwise compound **15** (0.22 g, 0.83 mmol) in CH_3CN (3 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred at 0 °C for 1 hour and the reaction solution was directly chromatographed eluting with 18:1:1 $CHCl_3$/MeCN/MeOH to afford 0.22 g of a colorless oil (37%); ^{1}H-NMR ($CDCl_3$), δ 1.42, 1.43 (2s, 9H, C*Me*$_3$), 2.18 (bs, 1H, NH), 2.83-3.00 (m, 5H), 3.31-3.45 (m, 2H), 3.64-3.75 (m, 2H), 3.86-4.11 (m, 3H), 4.43 (dd, 1H, $J_{5a,5e}$ 12.0 Hz, $J_{5e,4}$ 4.5 Hz, H-5e), 4.85 (d, 1H, $J_{1,2}$ 5.5 Hz, H-1), 5.24-5.33 (m, 1H, H-4), 5.37 (dd, 1H, $H_{2,3}$ 7.5Hz, H-2), 5.74 (t, 1H, $J_{3,4}$ 7.3 Hz, H-3); ^{13}C-NMR ($CDCl_3$) δ 28.0, 28.1 (CH_3), 35.2, 35.9, 48.9, 49.3, 50.2, ,56.2, 61.9, 69.3, 70.7, 70.8, 100.7, 128.4, 129.2, 129.8, 129.9, 133.3, 133.4, 165.2, 165.4, 165.5; FAB-MS (pos. m/z) calcd. for $C_{37}H_{42}N_2O_{11}$: 690.28; found: 691.42 (M^+ + 1, 11.6%), 445.30 (glycon, 1.7%).

Coupling of 26 to Fmoc-Gly to give 27 (27).

A reaction mixture of compound **26** (83 mg, 0.12 mmol) and Fmoc-Gly (36 mg, 0.12 mmol) in CH_2Cl_2 (5 mL) was treated with 1,3-dicyclohexylcarbodiimide (30 mg, 0.14 mmol) for 30 min at room temperature. When the reaction was complete, the reaction solution was washed with saturated $NaHCO_3$ (2 × 5 mL), water (1 × 5 mL), and dried over anhydrous Na_2SO_4. The concentrated residue was purified by silica gel

column chromatography eluting with 38:1:1 $CHCl_3$/MeCN/MeOH to give 89 mg of a white solid (76%); ^{1}H-NMR ($CDCl_3$) δ 1.44, 1.45, 1.46, 1.49 (4s, 9H, C*Me*$_3$), 2.88, 2.92, 2.96 (3s, 3H, NCH_3), 3.30-4.45 (m, 15H, 6CH_2, H-5's), 4.70, 4.75 (2d, 1H, $J_{1,2}$ 7.0 Hz), 5.30-5.45 (m, 2H, H-2, H-4), 5.65-5.76 (m, 1H, NH), 5.83 (t, 1H, $J_{3,4}$ 8.2 Hz, H-3), 7.28-7.65, 7.72-7.78, 7.89-8.02 (m, 23H, Ar); FAB-MS(pos. m/z) calcd. for $C_{54}H_{55}N_3O_{14}$: 969.37; found: 970.43 (M^+ + 1, 1.7%).

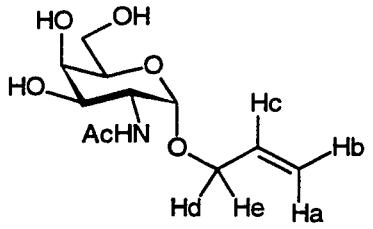
Coupling of 27 to 17 to give 29 (29).

A solution of compound **27** (89 mg, 0.092 mmol) was treated with 20% piperidine in DMF (1 mL) for 30 min. at room temperature and to this reaction solution was added the solution of acid **17** (12 mg, 0.092 mmol) in CH_2Cl_2 (5 mL) and 1,3-dicyclohexylcarbodiimide (23 mg, 0.11 mmol). The reaction mixture was stirred for 30 min. at room temperature. When the reaction was complete (R_f for amine: 0.1, R_f for product: 0.3, 18:1:1 $CHCl_3$/MeCN/MeOH), the reaction solution was concentrated. Then, the residue was dissolved in CH_2Cl_2 (10 mL), and the solution was washed with saturated $NaHCO_3$ (2 × 5 mL), and water (1 × 5 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by silica gel column chromatography eluting with 18:1:1 $CHCl_3$/MeCN/MeOH to yield 49 mg of a white solid (62%); mp 90.2-91.0 °C; $[\alpha]_D$ –6.54 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.43, 1.47, 1.54, 1.65 (4s, 9H, CMe3), 2.05, 2.08, 2.11, 2.12, 2.15 (5s, 3H, Ac), 2.92-3.07 (m, 6H, NCH3), 3.45-4.21 (m, 13H, CH2's, H-5a), 4.31-4.48 (m, 1H, H-5e), 4.64, 4.68, 4.74 (3s, 1H, J1,2 7.0 Hz, H-1), 5.30-5.45 (m, 2H, H-2, H-4), 5.78-5.89 (m, 1H, H-3), 6.70-6.89 (m, 1H, NH), 7.25-7.60, 7.82-8.00 (m, 15H, Ar); ^{13}C-NMR ($CDCl_3$) δ 21.4, 21.5 (CH_3), 28.1 (C*Me*$_3$), 37.3, 40.7, 41.0, 47.2, 47.9, 49.9, 50.3, 51.4, 62.5, 69.8, 71.1, 71.4, 101.0, 101.3 (C1), 128.4, 128.5, 128.8, 129.0, 129.6, 129.7, 129.8, 133.4, 133.9, 165.5, 167.7, 168.5; FAB-MS (pos. m/z) calcd. for $C_{44}H_{52}N_4O_{14}$: 860.35; found: 861.28 (M^+ + 1, 1.2%).

Compound 31.

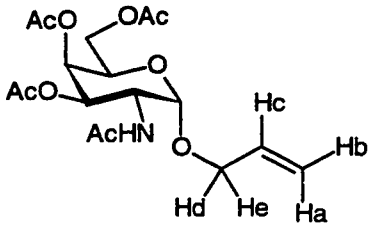
This compound was prepared as described previously for the pentapeptoid **25**; FAB-MS (pos. m/z) calcd. for $C_{19}H_{32}N_4O_{11}$: 492.21; found: 493.27 (M^+ + 1, 2.1%).

Allyl 2-acetamido-2-deoxy-α-D-galactopyranoside (33).



N-Acetyl-D-galactosamine (0.52 g, 2.33 mmol) was treated with allyl alcohol (10 mL) and $BF_3 \bullet OEt_2$ (14 mL, 0.12 mmol) was added. The reaction mixture was refluxed for 2 h and stirred at room temperature for 16 h. The solidified product from the reaction was then filtered and the concentrated filtrate was recrystallized from EtOH. The combined product yielded 0.50 g (82%) of a white solid; mp 190.0-191.5 °C; $[\alpha]_D$ +203.6 (*c* 0.5, DMSO); ^{1}H-NMR (D_2O) δ 2.10 (s, 3H, NAc), 3.83-3.86 (m 2H, H-6's), 3.99 (dd, 1H, $J_{2,3}$ 11.1 Hz, H-3), 4.04-4.08 (m, 2H, H-4, H-5), 4.10 (dd, 1H, $J_{c,d}$ 6.2 Hz, $J_{d,e}$ 13.1 Hz, H-d), 4.24 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 11.1 Hz, H-2), 4.28 (dd, 1H, $J_{c,e}$ 5.3 Hz, $J_{d,e}$ 13.0 Hz, H-e), 5.02 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.32 (dd, 1H, $J_{a,b}$ 1.5 Hz, $J_{b,c}$ 10.3 Hz, H-b), 5.30 (dd, 1H, $J_{a,c}$ 17.3 Hz, H-a), 6.00-6.08 (m, 1H, H-c); ^{13}C-NMR (D_2O) δ 21.5 (CH_3), 49.4 (C2), 60.8 (C6), 67.2 (C3), 68.0 (Cd), 68.1 (C4), 70.5 (C5), 95.8 (C1), 117.4 (Cab), 133.2 (Cc), 174.2 (C=O); FAB-MS (pos. m/z) calcd. for $C_{11}H_{19}NO_6$: 261.12; found: 262.34 (M^+ + 1, 100%); Anal. Calcd for $C_{11}H_{19}NO_6$: C, 50.57; H, 7.33; N, 5.36. Found: C, 50.22; H, 7.23; N, 5.24.

Allyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranoside (34).



Allyl 2-acetamido-2-deoxy-α-D-galctopyranoside (**33**) (0.72 g, 2.74 mmol) was dissolved in pyridine (27 mL) and acetic anhydride (31 mL) was added to the reaction mixture. The solution was stirred at room temperature for 6 h and concentrated under

the reduced pressure. The residue was diluted with ethyl acetate (50 mL) and washed with 5% aqueous HCl (3 × 30 mL), saturated $NaHCO_3$ (2 × 30 mL) and then water (1 × 30 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Purification of the crude product by silica gel chromatography with 4:1 EtOAc/hexanes afforded 0.98 g (93%) of a white foam; $[\alpha]_D$ +98.3 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.90 (s, 3H, NAc), 1.91, 1.97, 2.08 (3s, 9H, OAc), 3.94 (dd, 1H, $J_{He,Hd}$ 12.8 Hz, $J_{He,Hc}$ 6.3 Hz, H-e), 3.99-4.08 (m, 2H, H-6), 4.09-4.12 (m, 2H, H-d, H-5), 4.51 (ddd, 1H, H-2), 4.86 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.10 (dd, 1H, $J_{2,3}$ 11.4 Hz, $J_{3,4}$ 3.2 Hz, H-3), 5.17 (dd, 1H, $J_{b,c}$ 10.4 Hz, $J_{b,a}$ 1.3 Hz, H-b), 5.22 (dd, 1H, $J_{a,c}$ 17.2 Hz, H-a), 5.30 (d, 1H, H-4), 5.75 (d, 1H, $J_{2,NH}$ 9.6 Hz, NH), 5.82 (m, 1H, H-c); ^{13}C-NMR ($CDCl_3$) δ 20.5 (OAc), 23.0 (NAc), 47.5 (C-2), 61.8 (C-6), 66.6 (C-3), 67.2 (C-4), 68.2 (C-5), 68.6 (CH_2), 96.7 (C-1), 118.0 (CH=*C*H_2), 133.1 (*C*H=CH_2), 170.0, 170.1, 170.2, 170.6 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{17}H_{25}NO_9$: 387.15; found: 388.17 (M^++1, 83.6%), 346.15 (25.5%), 330.12 (91.4%); Anal calcd for $C_{17}H_{25}NO_9$: C, 52.69; H, 6.51; N, 3.62. Found: C, 52.71; H, 6.53; N, 3.64.

2-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)ethanal (35).

This compound was prepared following the general method for ozonolysis; ^{1}H-NMR ($CDCl_3$) δ 1.95, 1.97, 2.00, 2.11 (4s, 12H, Ac), 4.01-4.28 (m, 3H, H-5, H-6's), 4.27 (s, 2H, CH_2), 4.57 (ddd, 1H, $J_{2,3}$ 11.2 Hz, $J_{2,NH}$ 9.4 Hz, H-2), 4.86 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.17 (dd, 1H, $J_{3,4}$ 3.3 Hz, H-3), 5.35 (d, 1H, H-4), 6.18 (d, 1H, NH), 9.63 (s, 1H, HCO); ^{13}C-NMR ($CDCl_3$) δ 21.3 (OAc), 23.9 (NAc), 41.4, 48.2, 62.4, 67.8, 68.1, 68.6, 74.0, 99.5 (C1), 170.8, 171.1, 171.5, 198.1; FAB-MS (pos. m/z) calcd. for $C_{16}H_{23}NO_{10}$: 389.13; found: 390.15 (M^++1, 26.3%).

General procedure for ozonolysis.

The allyl glycoside was dissolved in CH_2Cl_2 and the solution was purged with O_2 for 10 min at -76 °C. The reaction solution was then treated with O_3 at -76 °C until the color of the solution turned blue. Oxygen was bubbled through the bluish solution for another 10 min at -76 °C to remove excess O_3 and excess CH_3SCH_3 was added to the colorless solution. The reaction mixture was slowly allowed to reach room temperature

and stirred overnight. The solution was concentrated and the residue was co-evaporated with toluene to remove DMSO which was produced during the reaction.

2-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-1-*N*-benzylaminoethane (36).

2-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-1-ethanal (**35**) (0.10 mg, 0.26 mmol) was dissolved in THF (15 mL). Benzylamine (0.14 mL, 1.29 mmol) and a catalytic amount of conc. HCl were added to the reaction solution which was then stirred at room temperature for 20 min under N_2. $NaCNBH_3$ (81 mg, 1.29 mmol) was added to the solution and the reaction mixture was stirred for another 6 hours. When the reaction was complete, the excess $NaCNBH_3$ was destroyed by adding conc. HCl until pH 3 and stirring for another 1 hour. Saturated $NaHCO_3$ (20 mL) was added to the reaction mixture to make the solution basic (pH 8) and extracted with EtOAc (3 × 20 mL). The organic phase was washed with brine, dried over Na_2SO_4 and then concentrated. Silica gel chromatography of the crude product eluting with 17:2:1 $CHCl_3/CH_3CN$/MeOH yielded 92 mg (74%) of a white foam; $[\alpha]_D$ +93.0 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.85, 1.95, 1.99, 2.21 (4s, 12H, OAc, NAc), 2.56 (bs, 1H, NH), 2.83 (t, 2H, J 5.3 Hz, CH_2N), 3.50-3.58 (m, 1H, OC*H*H), 3.74-3.83 (m, 3H, OCH*H*, CH_2Ph), 4.02-4.18 (m, 3H, H-5, H-6's), 4.54 (ddd, 1H, $J_{2,3}$ 11.3 Hz, $J_{2,NH}$ 9.4 Hz, H-2), 4.85 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.15 (dd, 1H, $J_{3,4}$ 3.3 Hz, H-3), 5.33 (d, 1H, H-4), 6.11 (d, 1H, NH), 7.22-7.36 (m, 5H, Ar); ^{13}C-NMR ($CDCl_3$) δ 20.7 (OAc), 23.1 (NAc), 47.7 (C-2), 48.0 (CH_2), 53.5 (CH_2), 61.9 (C-6), 66.8 (CH_2), 67.3 (C-3), 67.5 (C-4), 68.4 (C-5), 98.0 (C-1), 127.3 (Ar_{para}), 128.1 (Ar_{meta}), 128.6 (Ar_{ortho}), 139.3 (Ar_{ipso}), 170.2, 170.3, 170.4, 170.9 (C=O's); FAB-MS calcd. for $C_{23}H_{32}N_2O_9$: 480.21; found: 481.84 (M^++1, 100%), 437.26 (23.1%); Anal. calcd. for C, 57.47; H, 6.72; N, 5.83; found: 57.50; H, 6.79; N, 5.90.

1-(*N*-*t*-Butyloxycarbonylmethyl-*N'*-benzyl)-2-(2-acetamido-2-deoxy-3,4,6-tri-*O*-tri-acetyl-α-D-galactopyranosyl)ethane (37).

To a solution of 2-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-1-*N*-benzylaminoethane (**36**) (0.32 g, 0.661 mmol) and DIPEA (0.71 mL, 0.991 mmol) in

CH_2Cl_2 (10 mL) was added *t*-butyl bromoacetate (0.16 mL, 0.991 mmol) in one portion. The mixture was stirred at room temperature for 16 hours under a nitrogen atmosphere. When the reaction was complete, the reaction was washed with 5% aqueous HCl (1 × 5 mL), saturated $NaHCO_3$ (1 × 5 mL), water (1 × 5 mL), and then dried (Na_2SO_4). Silica gel column chromatography of the concentrated residue eluting with 38:1:1 CH_2Cl_2/ MeCN/MeOH yielded 0.38 g (98%) of a white foam; $[\alpha]_D$ +41.8 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.46 (s, 9H, C*Me*$_3$), 1.90, 1.97, 2.14 (4s, 12H, OAc, NAc), 2.81-2.89 (m, 2H, CH_2N), 3.27 (d, 2H, J 3.8 Hz, $COCH_2N$), 3.41-3.52 (m, 1H, C*H*HO), 3.71-3.76 (m, 1H, CH*H*O), 3.81 (d, 1H, J 2.6 Hz, CH_2Ph), 4.02-4.15 (m, 3H, H-5, H-6's), 4.60 (ddd, 1H, $J_{2,3}$ 11.3 Hz, $J_{2,NH}$ 9.7 Hz, H-2), 4.83 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.11 (dd, 1H, $J_{3,4}$ 3.2 Hz, H-3), 5.34 (d, 1H, H-4), 6.40 (d, 1H, NH), 7.24-7.32 (m, 5H, Ar); ^{13}C-NMR ($CDCl_3$) δ 21.4 (OAc), 23.7 (NAc), 28.8 (C*Me*$_3$), 48.0, 53.5, 56.5, 59.7, 62.7, 67.3, 67.5, 68.0, 69.3, 80.2 (*C*Me$_3$), 98.8 (C1), 127.9, 129.0, 129.3, 139.2, 171.0, 171.6, 171.8 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{29}H_{42}N_2O_{11}$: 594.28; found: 596.08 (M^++1, 100%), 539.15 (24.8%), 490.95 (13.2%).

1-*N*-*t*-Butyloxycarbonylmethylamino-2-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)ethane, hydrochloride (38).

1-(*N*-*t*-Butyloxycarbonylmethyl-*N'*-benzyl)-2-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)ethane (**37**) (0.40 g, 0.671 mmol) was dissolved in MeOH (20 mL) and to the solution were added Pd/C (80 mg) and AcOH (38 µL, 0.671 mmol). The mixture was purged with N_2 for 10 min. and H_2 was bubbled through it for 24 h. The reaction mixture was filtered through a celite pad and the filtrate was concentrated. The resulting residue was dissolved in MeOH (20 mL) and Amberlite IRA-400 (Cl) resin was added to the solution. The mixture was gently stirred for 24 h and the resin was filtered off. The reaction was monitored by ^{1}H-NMR spectroscopy (acetate peak at ≈2.0 ppm disappeared after complete exchange with chloride). The filtrate was concentrated to afford 0.36 g (98%) of a white foam; $[\alpha]_D$ +66.6 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.43 (s, 9H, CMe_3), 1.92, 1.97, 2.00, 2.11 (s, 12H, 3OAc, NAc), 3.03 (t, 2H, J 4.8 Hz, NCH_2), 3.49 (s, 2H, O_2CCH_2), 3.58 (td, 1H, J 11.3 Hz, J 4.9 Hz, OCH), 3.88 (td, 1H, J

11.3 Hz, J 4.9 Hz, OCH), 4.06-4.03 (m, 2H, H-6), 4.16 (ddd, 1H, $J_{4,5}$ 1.0 Hz, $J_{5,6}$ 6.0 Hz, H-5), 4.60 (ddd, 1H, $J_{2,3}$ 11.4 Hz, $J_{2,NH}$ 9.9 Hz, $J_{1,2}$ 3.5 Hz, H-2), 4.86 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.17 (dd, 1H, $J_{2,3}$ 11.4 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.35 (dd, 1H, $J_{3,4}$ 3.3 Hz, $J_{4,5}$ 1.0 Hz, H-4), 6.98 (d, 1H, J 9.9 Hz, NH), 7.35 (bs, 2H, NH_2); ^{13}C-NMR ($CDCl_3$) δ 170.9, 170.6, 170.4, 170.3, 168.8 (C=O's), 98.1 (C-1), 82.9, 68.3, 67.3, 66.9, 64.9, 62.0, 49.1, 47.2, 28.0, 23.0, 20.7; FAB-MS (pos. m/z) calcd. for $C_{22}H_{36}N_2O_{11}$: 504.23; found: 506.05 (M^+ + 1, 100%), 448.38 (26.7%), 330.07 (33.5%).

_t_-Butyl-monomer-Ac (39).

To a solution of 1-*N*-*t*-butyloxycarbonylmethylamino-2-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)ethane, hydrochloride (**38**) (0.10 g, 0.185 mmol) and DIPEA (0.097 mL, 0.555 mmol) in CH_2Cl_2 (5 mL) was added dropwise acetyl chloride (0.020 mL, 0.278 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The reaction solution was stirred at 0 °C for 30 min, and washed with 5% aqueous HCl (1 × 5 mL), saturated $NaHCO_3$ (1 × 5 mL), and water (1 × 5 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Silica gel column chromatography of the crude compound eluting with 19:1 EtOAc/MeOH yielded 94.3 mg (94%) of a white foam; $[\alpha]_D$ +53.5 (*c* 1.0, $CHCl_3$);^{1}H-NMR ($CDCl_3$) δ 1.41, 1.44 (2s, 9H, C*Me*$_3$), 1.93, 1.94, 1.97, 2.00, 2.01, 2.11, 2.25, 2.18 (8s, 12H, OAc, NAc), 3.40-3.85 (m, 4H, OCH_2CH_2N), 3.90-4.16 (m, 5H, H-5, H-6's, $COCH_2N$), 4.50-4.64 (m, 1H, H-2), 4.80, 4.84 (2s, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.00, 5.06 (2dd, 1H, $J_{2,3}$ 11.2 Hz, $J_{3,4}$ 3.2 Hz, H-3), 5.31-5.35 (m, 1H, H-4), 6.01, 6.38 (2d, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ 21.3 (OAc), 22.0, 22.2, 23.7, 23.8, 28.6 (C*Me*$_3$), 47.7, 48.0, 48.1, 49.8, 50.2, 52.9, 62.6, 66.6, 67.4, 67.5, 67.7, 67.9, 68.9, 69.3, 82.6, 83.6 (*C*Me$_3$), 99.0 (C1), 169.0, 169.2, 170.9, 171.0, 171.1, 171.3, 171.4, 171.8, 172.3 (C=O's); ratio of two rotamers = 1.6:1; FAB-MS (pos. m/z) calcd. for $C_{24}H_{38}N_2O_{12}$: 546.24; found: 547.29 (M^++1, 49.9%); Anal. Calcd for $C_{24}H_{38}N_2O_{12}$: C, 52.72; H, 7.01; N, 5.13. Found: C, 52.79; H, 7.06; N, 5.20.

General procedure for deprotecting _t_-Butyl ester.

t-Butyl protecting groups were removed by treating the compound with 20% TFA in CH_2Cl_2 at room temperature for 2 h. When the reaction was complete, the solution was concentrated and the residual TFA was removed by co-evaporating with toluene.

Carboxylic acid-monomer-Ac (40).

^{1}H-NMR ($CDCl_3$) δ 1.97, 1.98, 1.99, 2.04, 2.14, 2.15, 2.18, 2.26 (8s, 12H, Ac), 3.74-3.89, 3.54-3.69, 3.42-3.48 (m, 4H, NCH_2CH_2O), 4.00-4.26 (m, 5H, O_2CCH_2N, H-5, H-6), 4.50-4.54 (m, 1H, H-2), 4.81, 4.93 (2d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.09, 5.10 (2dd, 1H, $J_{2,3}$ 11.2 Hz, $H_{3,4}$ 3.2 Hz, H-3), 5.34, 5.37 (2d, 1H, J 2.2 Hz, H-4), 6.70, 6.92 (2d, 1H, J 9.6 Hz, NH); ^{13}C-NMR ($CDCl_3$) δ 20.6 (OAc), 20.9, 21.1, 22.3 (NAc), 47.8, 48.0, 48.8,, 49.3, 50.3, 52.0, 61.8, 61.9, 65.9, 66.1, 66.8, 67.1, 68.0, 68.5, 97.8, 97.9 (C1), 170.3, 170.4, 170.6, 170.7, 171.0, 171.4, 173.1, 173.4, 173.5; FAB-MS (pos. m/z) calcd. for $C_{20}H_{30}N_2O_{12}$: 490.18; found: 491.20 (M^+ + 1, 83.2%), 449.2 (27.8%); ratio of two rotamers = 1:1.3.

***t*-Butyl- monomer-Cbz (41).**

1-*N*-*t*-Butyloxycarbonylmethylamino-2-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)ethane hydrochloride (**38**) (0.20 g, 0.370 mmol) was dissolved in CH_2Cl_2 (5 mL) and DIPEA (0.19 mL, 1.11 mmol) was added. The solution was cooled to 0 °C and benzyl chloroformate (79 mL, 0.555 mmol) in CH_2Cl_2 (2 mL) was added dropwise to the reaction mixture. After stirring at 0 °C for 1 h, the reaction solution was washed with 5% aqueous HCl (1 × 5 mL), saturated $NaHCO_3$ (1× 5 mL), water (1 × 5 mL), and then dried (Na_2SO_4). The solution was concentrated and the residue was purified by silica gel column chromatography eluting with EtOAc to yield 0.21 g (77%) of a white foam; $[\alpha]_D$ +44.8 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.44, 1.36 (9H, 2s, *t*-Bu), 2.13, 2.01, 2.00, 1.96, 1.95, 1.93 (12H, 5s, Ac), 3.32-3.37, 3.45-3.52, 3.54-3.65, 3.72-3.84, (4H, m, OCH_2CH_2N), 3.87, 3.93 (2H, 2s, O_2CCH_2N), 4.01-4.16 (3H, m, H-5, H-6), 4.57-4.61 (1H, m, H-2), 4.79, 4.85 (1H, 2s, $J_{1,2}$ 3.5 Hz, H-1), 5.02, 5.06 (1H, 2dd, $J_{2,3}$ 11.2 Hz, $J_{3,4}$ 3.2 Hz, H-3), 5.10, 5.16 (2H, 2s, CH_2Ph), 5.30, 5.34 (1H, 2d, J=2.2 Hz, H-4), 6.15, 6.43 (1H, 2d, J 9.7 Hz, NH), 7.27-7.35 (5H, m, Ph), ;^{13}C-NMR ($CDCl_3$) δ 20.7 (OAc), 23.1 (NAc), 27.9 (CMe3), 28.0, 47.2, 49.1, 50.8, 51.1, 62.0, 66.1, 66.8, 67.3,

67.6, 67.7, 68.5, 68.7, 82.2, 97.9, 98.3, 127.8, 128.0, 128.2, 128.4, 128.6, 156.3, 170.4, ,170.7; FAB-MS (pos. m/z) calcd. for $C_{30}H_{42}N_2O_{13}$: 638.27; found: 639.32 (M^+ + 1, 12.7%), 583.26 (4.0%), 505.27 (3.6%); Anal. calcd for $C_{30}H_{42}N_2O_{13}$: C, 56.40; H, 6.63; N, 4.39. Found: C, 56.47; H, 6.65; N, 4.36; ratio of two rotamers = 2:1.

Carboxylic acd-monomer-Cbz (42).

^{1}H-NMR ($CDCl_3$) δ 1.96, 1.97, 2.00, 2.02, 2.14 (s, 12H, OAc, NAc), 3.30-3.85 (m, 4H, NCH_2CH_2O), 3.95-4.20 (m, 5H, CH_2, H-5, H-6's), 4.46-4.60 (m, 1H, H-2), 4.79, 4.83 (2d, 1H, $J_{1,2}$ 3.3 Hz, H-1), 5.02-5.20 (m, 3H, H-3, CH_2), 5.26-5.35 (m, 1H, H-4), 6.73, 6.91 (2d, 1H, $J_{2,NH}$ 9.2 Hz, NH), 7.24-7.33 (m, 5H, Ar), 8.80 (bs, 1H, CO_2H); ^{13}C-NMR ($CDCl_3$) δ 20.6 (OAc), 22.3 (NAc), 47.9, 48.9, 49.5, 50.0, 61.8, 61.9, 66.6, 66.7, 66.9, 67.2, 67.8, 68.1, 68.4, 68.6, 97.6, 97.9 (C1), 127.7, 128.1, 128.3, 128.4, 128.5, 128.6, 135.9, 156.3, 156.5, 158.6, 170.4, 170.5, 170.6, 170.7, 171.3, 172.1, 173.2 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{26}H_{34}N_2O_{13}$: 582.21; found: 583.24 (M^++1, 36.1%).

***t*-Butyl-dimer-Cbz (43).**

To a mixture of amine salt **38** (0.093 g, 0.171 mmol) and carboxylic acid **42** (0.091 g, 0.156 mmol) in 2:1 CH_2Cl_2/CH_3CN were added TBTU (0.17 g, 0.513 mmol) and DIPEA (0.18 mL, 1.03 mmol) at room temperature. The reaction was stirred for 2 hours. When the reaction was complete (acid: R_f 0.20, amine: R_f 0.28, product: R_f 0.48, 9:1 EtOAc/MeOH), the solution was concentrated. The residue was dissolved in EtOAc (5 mL) and washed with 5% aqueous HCl (1 × 3 mL), saturated $NaHCO_3$ (1 × 3 mL), and water (1 × 3 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Purification of the crude compound by silica gel column chromatography eluting with 19:1 EtOAc/MeOH yielded 0.14 g (81%) of a colorless syrupy residue; $[\alpha]_D$ +56.7 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.40, 1.43 (2s, 9H, *t*-Bu), 1.77, 1.91, 1.93, 1.94, 2.00, 2.01, 2.10, 2.11(8s, 24H, Ac), 3.32-3.64, 3.68-3.93, 3.98-4.22 (m, 17H), 4.50-4.57 (m, 2H, H-2), 4.83, 4.96 (2d, 2H, $J_{1,2}$ 3.5 Hz, H-1), 4.88, 4.91, 4.98, 5.09, 5.11 (5s, 3H), 5.01, 5.19 (2dd, 2H, $J_{2,3}$ 11.9 Hz, $J_{3,4}$ 3.1 Hz, H-3), 5.27, 5.39 (2d, 2H, J 2.0 Hz, H-4), 7.26-7.34 (m, 5H, Ph), 6.57, 7.55 (2d, 2H, J 9.4 Hz, J 8.4 Hz, NH); ^{13}C-NMR ($CDCl_3$) δ 20.5, 20.6 (OAc), 22.6, 23.0 (NAc), 27.9 (CMe_3), 38.5, 47.2, 47.3, 47.5,, 47.7,

47.8, 48.1, 49.9, 62.0, 62.1, 65.4, 66.5, 66.8, 67.1, 67.5, 67.6, 68.1, 82.0, 98.1, 98.6 (C1), 127.6, 128.0, ,128.2, 128.5, 135.9, 156.6, 167.9, 168.7, 170.3, 170.5, 171.3; FAB-MS (pos. m/z) calcd. for $C_{48}H_{68}N_4O_{23}$: 1068.43; found: 1069.30 (M^+ + 1, 3.4%), 935.30 (1.5%), 565.23 (1.9%); ratio of two rotamers = 1:1.

Carboxylic acid-dimer-Cbz (44).

^{1}H-NMR ($CDCl_3$) δ 1.77, 1.91, 1.93, 1.94, 2.00, 2.02, 2.11, 2.12 (8s, 24H, OAC, NAc), 3.35-4.25 (m, 16H), 4.45-4.62 (m, 2H, H-2), 4.80-5.45 (m, 8H), 6.61, 7.56 (2s, 2H, $J_{2,NH}$ 9.3 Hz, NH), 7.26-7.34 (m, 5H, Ar); FAB-MS (pos. m/z) calcd. for $C_{44}H_{60}N_4O_{23}$: 1012.36; found: 1013.38 (M^+ + 1, 7.5%), 971.37 (2.0%), 879.36 (1.7%).

General procedure for deprotecting Cbz group.

Cbz-derivatives (1 eq) was dissolved in MeOH and to the solution were added Pd/C (20%, w/w) and AcOH (1 eq). The mixture was purged with N_2 for 10-15 min. and H_2 was bubbled through the solution for 2-16 h. The reaction was monitored by TLC. When the reaction was complete, the solution was filtered through a celite pad and the filtrate was concentrated. The resulting residue was dissolved in fresh MeOH and Amberlite IRA-400 (Cl) resin was added to the solution. The solution was stirred gently for 16-24 hours and then the resin was filtered off. The filtrate was concentrated and the residue was co-evaporated with $CHCl_3$.

***t*-Butyl-dimer-NH_2 (45).**

^{1}H-NMR ($CDCl_3$) δ 1.40, 1.42 (2s, 9H, C*Me*$_3$), 1.87, 1.91, 1.93, 1.97, 1.99, 2.08, 2.10 (7s, 24H, OAc, NAc), 3.10-4.21 (m, 17H), 4.32-4.72 (m, 3H), 4.89, 5.05 (2s, 2H, $J_{1,2}$ 3.4 Hz, H-1), 4.88, 5.22 (2dd, 2H, H-3), 5.31-5.38 (m, 2H, H-4), 7.38, 8.02 (2d, 2H, NH); FAB-MS (pos. m/z) calcd. for $C_{40}H_{62}N_4O_{21}$: 934.39; found: 935.86 (M^++1, 76.5 %).

***t*-Butyl-dimer-Ac (46).**

The amine **38** (0.011 g, 21.4 μmol) and the acid **40** (0.010 g, 20.4 μmol) were dissolved in 2:1 CH_2Cl_2/CH_3CN (3 mL) and to the reaction mixture were added TBTU (0.020 g, 61.2 μmol) and DIPEA (0.021 mL), 122 μmol) at room temperature. The

mixture was stirred for 2 h and the reaction was monitored by TLC (amine: R_f 0.42, acid: R_f 0.14, product: R_f 0.42, 19:1 EtOAc/MeOH). When the reaction was complete, the solution was concentrated. The residue was diluted with EtOAc (5 mL) and washed with 5% aqueous HCl (1 × 3 mL), saturated $NaHCO_3$ (1 × 3 mL), water (1 × 3 mL), and then dried (Na_2SO_4). The organic phase was concentrated and purified by silica gel column chromatography eluting with 9:1 EtOAc/MeOH to afford 0.016 g (81%) of a colorless syrupy residue; $[\alpha]_D$ +54.6 (*c* 0.88, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.37, 1.42 (2s, 9H, C*Me*$_3$), 1.82, 1.87, 1.90, 1.91, 1.93, 1.97, 1.99, 2.06, 2.08, 2.09 (10s, 27H, OAc, NAc, Ac), 3.22-3.85 (m, 10H), 4.00-4.20 (m, 8H), 4.40-4.65 (m, 2H, H-2), 4.86, 4.92 (2s, 2H, $J_{1,2}$ 3.4 Hz, H-1), 4.96-5.13 (m, 2H, H-3), 5.28-5.36 (m, 2H, H-4), 6.80, 7.95 (2s, 2H, $J_{2,NH}$ 9.2 Hz, NH); ^{13}C-NMR ($CDCl_3$) δ 21.2, 21.3 OAc), 21.6, 21.9, 22.0, 23.4, 23.6, 28.6 (C*Me*$_3$), 47.9, 48.0, 48.1, 48.3, 48.6, 50.1, 61.0, 62.6, 62.8, 65.9, 66.8, 67.3, 67.8, 68.1, 68.4, 82.8, 98.8, 99.2 (C1), 167.7, 168.7, 169.0, 170.9, 171.0, 171.1, 171.3, 171.4, 171.8 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{42}H_{64}N_4O_{22}$: 976.40; found: 977.42 (M^+ + 1, 4.3%), 935.40 (1.7%), 630.30 (11.4%), 574.25 (26.6%), 473.20 (26.2%); Anal. Calcd for $C_{42}H_{64}N_4O_{22}$: C, 51.62; H, 6.61; N, 5.74. Found: C, 51.70; H, 6.67; N, 5.80.

Carboxylic acid-dimer-Ac (47).

^{1}H-NMR ($CDCl_3$) δ 1.94, 1.96, 2.01, 2.03, 2.12, 2.13, 2.14 (7s, 27H, Nac, OAc), 3.25-4.60 (m, 20H), 4.85-5.18 (m, 4H, H-1, H-3), 5.28-5.40 (m, 2H, H-4), 7.25, 8.82 (2bs, 2H, NH); FAB-MS (pos. m/z) calcd. for $C_{38}H_{56}N_4O_{22}$: 920.34; found: 921.18 (M^++1, 6.1%), 574.52 (26.6%), 473.22 (19.3%).

***t*-Butyl-trimer-Ac (48).**

A solution mixture of amine **46** (50.0 mg, 51.5 μmol) and acid **40** (25.2 mg, 51.5 μmol) in CH_2Cl_2 (2 mL) was treated with BOP (68.4 mg, 155 μmol) under nitrogen atmosphere. To this solution was added 4-methylmorpholine (NMM, 34 μL, 309 μmol) and the reaction mixture was stirred for 2 h at room temperature. When the reaction was complete, the solution was diluted with CH_2Cl_2 (5 mL) and washed with saturated $NaHCO_3$ (2 × 5 mL) and brine. The organic phase was dried over anhydrous Na_2SO_4

and concentrated to give an oil. Silica gel column chromatography of the crude product eluting with 4:1 EtOAc/ hexanes afforded 43.0 mg (60%) of an colorless syrupy residue; $[\alpha]_D$ +126.5 (*c* 1.1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.40, 1.47 (2s, 9H, C*Me*$_3$), 1.92, 1.93, 1.94, 2.00, 2.01, 2.08, 2.09, 2.10, 2.12 (s, 39H, OAc, NAc, Ac), 3.25-4.24 (m, 27H), 4.45-4.84 (m, 3H), 4.90-5.17 (m, 6H), 5.27-5.40 (m, 3H), 6.25, 6.64, 6.75, 7.38, 7.92, 8.12 (d, 3H, NH); ^{13}C-NMR ($CDCl_3$) δ 20.7, 21.2, 22.9, 28.0, 46.2, 47.3, 47.4, 47.5, 47.6, 47.8, 61.9, 62.0, 62.1, 66.5, 67.0, 67.1, 67.2, 67.4, 67.5, 67.8, 82.3, 97.8, 98.2 (C1), 168.5, 169.1, 170.3, 170.4, 170.6, 170.7, 170.8, 171.0 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{60}H_{90}N_6O_{32}$: 1406.56; found: 1407.90 (M^+ + 1, 4.8%), 935.31 (10.1%), 473.17 (19.8%).

t-Butyl-tetramer-Cbz (49).

The title compound was prepared using the same method described previously for the synthesis of **43**; Yield 68%; $[\alpha]_D$ +134.6 (*c* 1.1, $CHCl_3$);^{1}H-NMR ($CDCl_3$) δ 1.41, 1.46 (2s, 9H, C*Me*$_3$), 1.82-2.14 (m, 48H, OAc, NAc), 3.21-4.25 (m, 34H), 4.35-5.38 (m, 20H), 6.38, 6.41, 6.55, 7.16, 7.40, 7.68 (m, 4H, NH), 7.26-7.34 (Ar); FAB-MS (pos. m/z) calcd. for $C_{84}H_{120}N_8O_{43}$: 1928.74; found: 1929.69 (M^+ + 1, 0.6%), 1795.67 (0.2%), 1582.61 (1.6%), 1425.50 (0.5%), 995.35 (2.8%), 935.38 (3.0%).

t-Butyl-tetramer-NH_2 (50).

FAB-MS (pos. m/z) calcd. for $C_{76}H_{114}N_8O_{41}$: 1794.71; found: 1796.40 (M^++1, 18.2%), 1465.45 (1.3%), 860.24 (0.9%).

t-Butyl-tetramer-Ac (51).

The title compound was prepared using the same method described previously for the synthesis of **46**; Yield 79%; $[\alpha]_D$ +79.0 (*c* 1.1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.41, 1.45 (2s, 9H, C*Me*$_3$), 1.83-2.13 (m, 51H, OAc, NAc, Ac), 3.30-4.20 (m, 34H), 4.38-5.15 (m, 14H), 5.27-5.38 (m, 4H), 6.42, 6.61, 6.70, 7.16, 7.45, 7.61, 8.15 (d, 4H, NH); FAB-MS (pos. m/z) calcd. for $C_{78}H_{116}N_8O_{42}$: 1836.72; found: 1838.76 (M^+ + 1, 1.4%), 1366.22 (21.0%), 935.43 (2.4%), 903.37 (5.1%).

Carboxylic acid-tetramer-Ac (52).

FAB-MS (pos. m/z) calcd. for $C_{74}H_{108}N_8O_{42}$: 1780.66; found: 1782.20 (M^+ + 1, 0.7%), 1309.35 (0.4%).

***t*-Butyl-hexamer-Ac (53).**

The amine **46** and the acid **52** were coupled following the same method used for the synthesis of 46; Yield 62%; $[\alpha]_D$ +58.9 (*c* 1.6, $CHCl_3$);^{1}H-NMR ($CDCl_3$) δ 1.42-1.47 (m, 9H, C*Me*$_3$), 1.84-2.15 (m, 75H), 3.30-4.25 (m, 48H), 4.40-5.20 (m, 24H), 5.25-5.40 (m, 6H); FAB-MS (pos. m/z) calcd. for $C_{114}H_{168}N_{12}O_{62}$: 2697.04; found: 2698.67 (M^+ + 1, 0.1%), 2227.03 (1.2%), 1366.37 (31.3%).

***t*-Butyl-octamer-Ac (54).**

The amine **50** and the acid **52** were coupled following the same method used for the synthesis of **46**; Yield 59%; $[\alpha]_D$ +92.6 (*c* 1.4, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.40-1.48 (m, 9H, CMe3), 1.82-2.14 (m, 99H, OAc, NAc, Ac), 3.30-4.25 (m, 64H), 4.40-5.20 (m, 32H), 5.27-5.40 (m, 8H).

Fully deprotected glycopeptoids.

O-Acetyl groups on carbohydrate moieties were removed under Zemplén condition. Compounds were dissolved in MeOH and 1M NaOMe was added to the solution until pH ≈ 9. The methanolic solution was stirred at room temperature for 2-6 hours and treated with Amberlite IRA-120 (plus) resin for 15 min. The resin was filtered off and the filtrate was concentrated under reduced pressure. The resulting residue was then treated with 20% TFA in CH_2Cl_2 at room temperature for 2 h to remove the *t*-butyl group. The solution was concentrated and the residual TFA was removed by co-evaporating the residue with toluene. The resulting crude compounds were purified using size exclusion column chromatography (LH20) eluting with MeOH.

Chapter 3. Phase transfer catalysis

3.1. Introduction

Liquid-liquid phase transfer catalysis (PTC)[150] has been used for the synthesis of diverse anomeric glycosyl derivatives including acryloxides,[151] alkyl- and arylthiolates,[152] thioacetates,[153] xanthates,[154] arylselenoxides,[155] azides,[156] phosphates,[157] and oxysuccinimides[158] (Scheme 1.3.1).

Whereas the traditional synthetic methods can be often tedious, costly, and difficult to handle as a result of reactive promotors and sensitive reaction conditions, the PTC methodology is well suited to carbohydrate synthesis as reaction conditions can be quite mild and large scale preparations can be easily accommodated.

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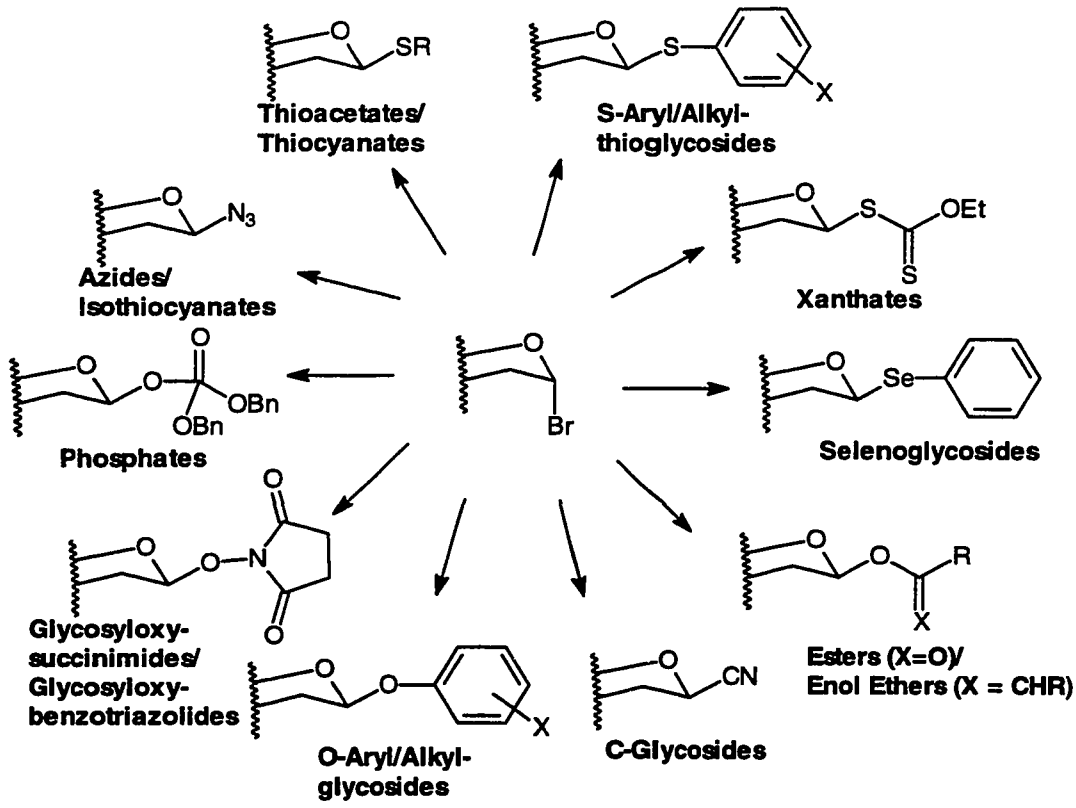
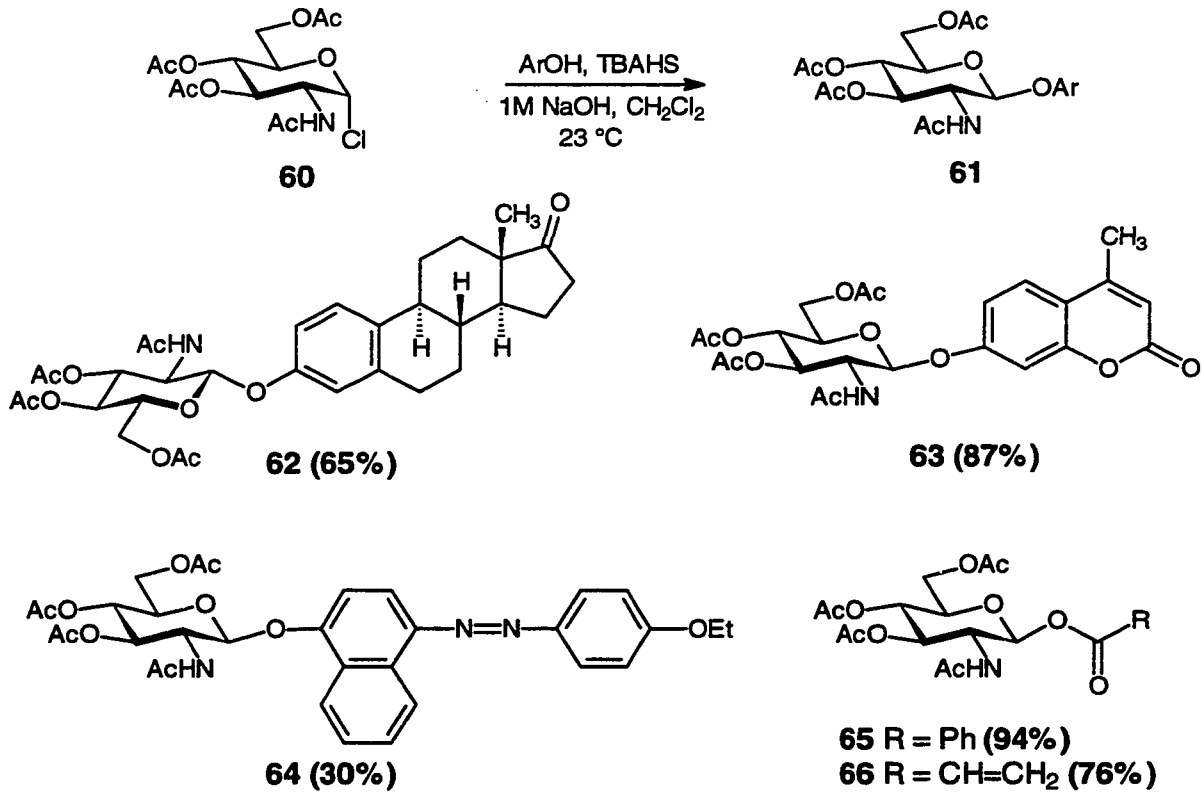


Scheme 3.1.1. General transformation describing the usefulness of PTC in anomeric nucleophilic substitution.

Using a PTC approach, a wide range of important glycosides were prepared in our laboratory. These glycosides include prodrugs, glycoprobes such as lectin and enzyme substrates, precursors for neoglycoconjugates, glycopeptide and glycopeptoid precursors, as well as various glycosyl donors for oligosaccharide syntheses. As illustrated in Scheme 3.1.2, anomeric nucleophilic substitutions under PTC conditions transformed peracetylated 2-acetamido-2-deoxy-α-D-glucopyranosyl chloride into O-aryl glycosides[151a,b] and other useful derivatives. The prepared numerous aryl glycosides **61** having electron donating and electron withdrawing substituents were exercised to probe potential electronic contributions originating from their binding to a plant lectin, wheat germ agglutinin (WGA). The results showed that WGA recognized to bind with better

affinity to aryl glycosides than the alkyl glycosides.[155a] The PTC conditions were also successfully applied for the syntheses of estrone prodrug **62**, glycohydrolase enzyme substrates 7-hydroxy-4-methylcoumarin **63**, the new chromogenic substrate Fat Brown B® **64**, and the anomeric esters **65**, **66**.[155a]

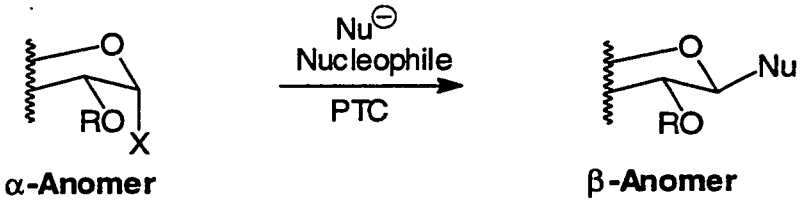


Scheme 3.1.2. Transformations of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride **60** into *O*-aryl glycosides and other derivatives under PTC.

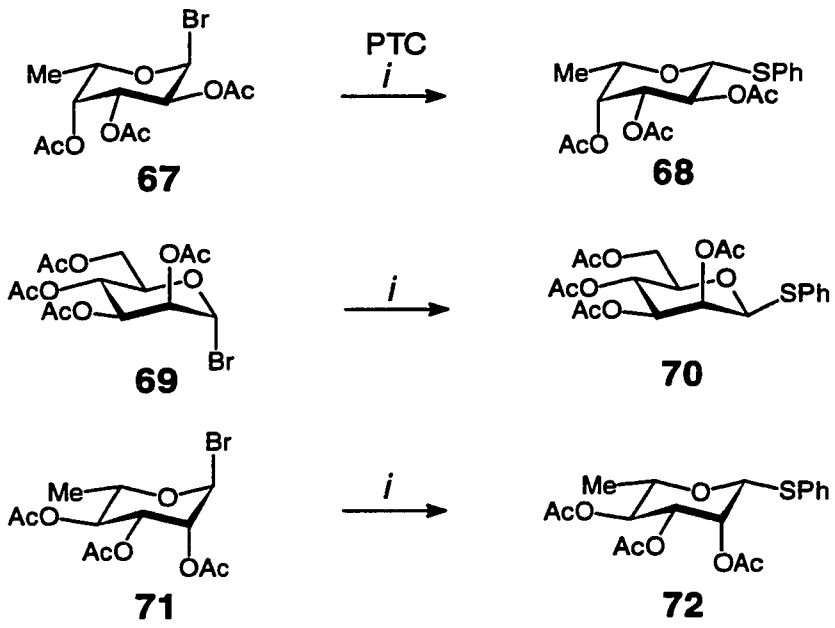
In most cases studied, 1,2-*cis* peracetylated glycosyl halides were used as starting materials. The resulting glycosyl derivatives usually had 1,2-*trans*-diequatorial arrangements (Scheme 3.1.3). In a more recent study,[159] peracetylated glycopyranosyl bromides of L-fucose (1,2-*cis*, $^1C_4$), D-mannose (1,2-*trans*-diaxial, $^4C_1$), and L-

[159] Cao, S.; Roy, R. *Carbohydr. Lett.* **1996**, *2*, 27.

rhamnose (1,2-*trans*-diaxial, $^1C_4$) were all shown to proceed with complete anomeric inversion (S_N2) using thiophenol and sodium azide as nucleophiles (Scheme 3.1.4).



Scheme 3.1.3. Anomeric nucleophile substitutions under PTC conditions.



Scheme 3.1.4. Stereochemical outcome for the PTC transformation of 1,2-*cis* and 1,2-*trans* glycosyl bromide into phenyl 1-thio-glycopyranosides: *i*) EtOAc, 1M Na_2CO_3, TBAHS, 23 °C, **68** (91%); **70** (84%); **72** (92%).

Nucleophilic substitution of acetochloroneuramic acid having an axial chloride and no participating group, was also shown to occur with net anomeric inversion under PTC conditions using a wide range of nucleophiles.[152-155] Therefore, in all cases, anomeric inversions occurred. It has been generally postulated that the anomeric inversions were due to anchimeric group participation from the neighboring 2-acyloxy group.[151f,g,160] When non-participating benzyloxy substituents were used, the reactions gave mixtures of anomers.[161] However, in this last case, it is likely that the observed lack of stereoselectivity was due to the fact that anomeric mixtures of glycosyl halides were used as starting materials. As anticipated from these reactions, hydrolysis and elimination accounted for some of the by-products obtained.[162]

The remainder of this chapter will discuss a case study of phase transfer catalyzed anomeric nucleophilic substitutions of peracetylated α-D-xylopyranosyl bromide **3** and α- and β-chlorides **83** and **82** having both 1,2-*cis*- or 1,2-*trans*-stereochemistry with a series of nucleophiles to further demonstrate that these anomeric substitutions occur with complete anomeric inversion. Although the reactions are likely to proceed by a direct inversion mechanism, anchimeric group participation cannot be totally ruled out. If the general trends hold, α- and β-glycosyl halides would provide β- and α-glycosyl derivatives respectively. Acyloxonium ions, if formed as intermediates, would result in orthoester-like products, while free oxonium ions would inevitably result in anomeric mixtures. As orthoesters are stable under basic conditions, it is unlikely that they would constitute transient intermediates. Under the basic PTC conditions used herein, it was hypothesized that the peracetylated β-D-xylopyranosyl chloride would provide α-xylopyranosyl derivatives exclusively.

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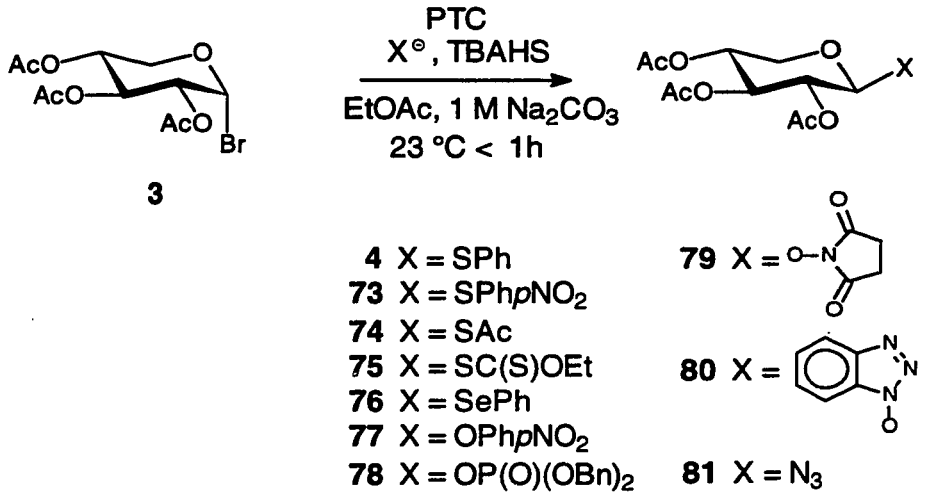
[161] (a) Szeja, W. *Synthesis* **1988**, 223, (b) Szeja, W.; Bogusiak, J. *Synthesis* **1988**, 224.

[162] (a) Roy, R.; Tropper, F. D.; Romanowska, A.; Letellier, M.; Cousineau, L.; Meunier, S. J.; Boratynski, J. *Glycoconjugate J.* **1991**, *8*, 75, (b) Shah, R. H.; Bahl, O. P. *Carbohydr. Res.* **1979**, *74*, 105.

3.2. Synthesis of glycosyl donor-xylose analogs

Treatment of 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (**3**)[163] with the respective nucleophiles, under improved liquid two phase PTC conditions[152a-c] (1 equiv of tetrabutylammonium hydrogen sulfate (TBAHS), EtOAc, 1 M Na_2CO_3, 23 °C, < 1h) afforded exclusively the inverted β-D-xylopyranosyl anomers **4, 73-81** in good to excellent yields (65-95%) as judged from the ^{1}H-NMR spectra of the crude reaction mixtures (Scheme 3.2.1, Table 3.2.1). No trace of orthoester-like products was observed. The only side reaction observed was the formation of a small amount of hydrolysis product. The amount of hydrolyzed halide was dependent on the relative nucleophilicities of the incoming nucleophiles which appeared to be higher for *para*-nitrophenoxide (**77**), dibenzylphosphate (**78**), and oxysuccinimide (**79**). All anomeric configurations were clearly established from the $^3J_{1,2}$ coupling constants (4.1-8.3 Hz, Table 3.2.2) which indicated 1,2-*trans*-relationships between the nucleophiles and the 2-acyloxy groups. Interestingly, the smallest value ($^3J_{1,2}$ of 4.1 Hz) was observed for compound **79**; the size of this coupling constant indicated that **79** largely existed in the $^1C_4$ chair conformation rather than the $^4C_1$ chair conformation.[164]



Scheme 3.2.1. Syntheses of glycosyl donor-xylose analogs

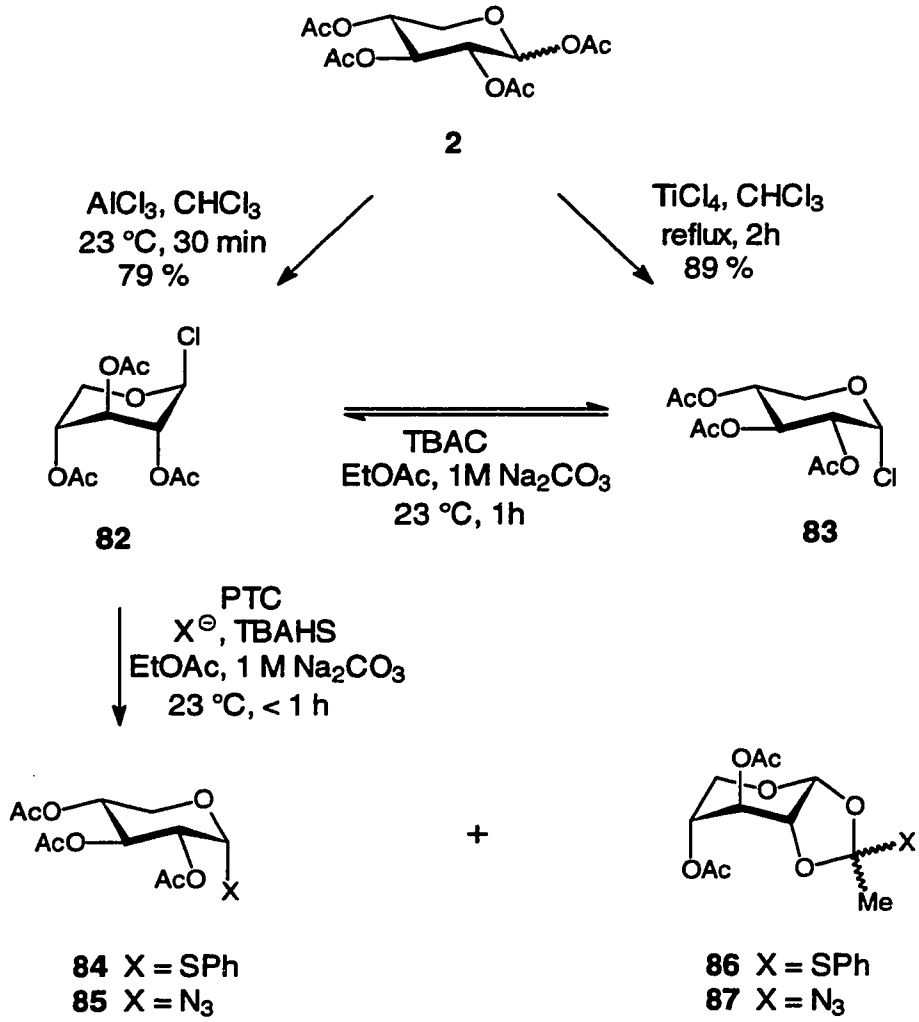
[163] Durette, P. L.; Horton, D. *Carbohydr. Res.* **1971**, *18*, 57.
[164] Mattok, G. L.; Phillips, G. O. *J. Chem. Soc. C.* **1958**, 130.

To unambiguously prove the stereochemical outcome of these PTC reactions, the opposite anomer, the β-halide was required. Owing to the high instability of the β-bromide, the more readily available 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl chloride (**82**) was prepared instead. Slight modifications of published procedures (Scheme 3.2.2)[163-165] were used to prepare compound **82** and its corresponding α-anomer **83** from an anomeric mixture of 1,2,3,4-tetra-*O*-acetyl-D-xylopyranose (**2**) (α/β 1:6). However, β-anomer **82** is known to exist in its $^1C_4$ conformation in spite of severe 1,3-diaxial interactions between the four axially oriented substituents (Scheme 3.2.3),[163-165] as confirmed by the sizes of $J_{1,2}$ (3.0 Hz), $J_{4eq,5ax}$ (3.7 Hz) and $J_{4eq,5eq}$ (3.0 Hz). The structures of **82** and **83** were clearly demonstrated by the differences in their ^{1}H-NMR spectra which showed the anomeric proton of **82** as a doublet of doublets at 5.77 ppm ($J_{1,2}$ 3.0, $J_{1,3}$ 0.4 Hz, long range coupling) while that of the α-anomer **83** appeared as a well resolved doublet at 6.21 ppm ($J_{1,2}$ 4.0 Hz). More convincing perhaps were the coupling constants between the H-4 and H-5 protons. For **82**, the two coupling constants between H-4eq and both H-5eq/H-5ax were small ($J_{4eq,5eq}$ 3.0, $J_{4eq,5ax}$ 3.7 Hz) indicating gauche relationships in both cases. For **83**, a large $J_{4ax,5ax}$ *trans*-diaxial coupling constant (11.2 Hz) was observed, while that of $J_{4ax,5eq}$ was 6.1 Hz. Taken together, this information confirmed[163-165] the anomeric as well as the conformational identities of both β-anomer **82** ($^1C_4$) and α-anomer **83** ($^4C_1$).

Treatment of the $^1C_4$ β-xylopyranosyl chloride **82**, having a 1,2-*trans*-diaxial stereochemistry, with either thiophenoxide or azide anions under exactly the same PTC conditions as described above for the α-bromo anomer **3** provided the corresponding α-D-xylopyranosyl derivatives **84** and **85** in 82% and 67% yields, respectively (Scheme 3.2.2). Again, in the ^{1}H-NMR spectra of the crude reaction mixtures, no signals were observed in the regions of the spectra where the β-glycosyl derivatives (**4**, **81**) absorb, suggesting complete anomeric inversions. We did however obtain *ca.* <5% of side-products to which orthoester-like structures **86** and **87** were assigned (Scheme 3.2.2). Compounds **86** and **87** were purified by concentration of mixed fractions obtained during silica gel column chromatography of the crude products. The structural assignments for these minor by-products were based on the observed typical chemical shifts of their *endo*-methyl signals (1.80 ppm). These by-products were derived from

[165] Holland, C. V.; Horton, D.; Jewell, J. S. *J. Org. Chem.* **1967**, *32*, 1818.

the nucleophilic attack of the nucleophiles (PhS^- and N_3^-) on the acyloxonium intermediates. Therefore, the 1,2-*trans*-xylopyranosyl chloride **82** appears to be slightly more prone to anchimeric group participation by its 2-acyloxy group than is its 1,2-*cis*-α-bromo analog **3**. However, this event constituted a very minor side reaction and was not unexpected owing to a favorable 1,2-*trans*-diaxial orientation of the anomeric chloride and the 2-acetoxy group in the preferred $^1C_4$ conformation of **82**. When 1,2-*cis*-α-chloride **83** was used under the above PTC conditions, the reaction rates were dramatically reduced (not shown).



Scheme 3.2.2. Preparation of α-D-xylopyranoside **84** and **85** using β-xylopyranosyl chloride under PTC condition.

Table 3.2.1. Selected Physical Properties of Compounds **4, 73-81, 84,** and **85.**

Cpd[a,ref]	Yield (%)	mp (°C)	$[\alpha]_D$	Formula	Combustion analyses Calculated (%) C/H/N	Found (%) C/H/N
4[149]	95	77.6-77.9	-54.9°	$C_{17}H_{20}O_7S$	55.42/5.48	55.55/5.43
73[166]	82	145.8-146.8	-60.1°	$C_{17}H_{19}NO_9S$	49.39/4.64/3.39	49.10/4.22/4.51
74	74	95.8-96.0	-7.5°	$C_{13}H_{18}O_8S$	46.70/5.43	46.64/5.21
75	84	103.4-103.7	+4.1°	$C_{14}H_{20}O_8S_2$	44.20/5.30	44.16/5.21
76	79	75.0-75.4	-90.3°	$C_{17}H_{20}O_7Se$	49.03/4.84	49.52/4.70
77[167]	67	136.6-136.7	-67.6°	$C_{17}H_{19}NO_{10}$	51.37/4.82/3.53	51.59/4.72/4.01
78	65	-	+40.8°	$C_{25}H_{29}O_{11}P$		
79	66	132.4-132.5	-148.6°	$C_{15}H_{19}NO_{10}$	48.24/5.13/3.75	48.34/5.00/3.80
80	78	-	-74.8°	$C_{17}H_{19}N_3O_8$	51.89/4.87/10.69	51.89/4.53/10.59
81	88	83.8-84.0	-80.5°	$C_{11}H_{15}N_3O_7$	43.84/5.02/13.95	43.58/4.67/13.93
84	82	-	+203.5°	$C_{17}H_{20}O_7S$		
85[168]	67	-	+170.5°	$C_{11}H_{15}N_3O_7$	43.84/5.02/13.95	44.06/4.95/13.58

[a] Physical data of known compounds agreed with lit. values.

[166] Marshall, P. J.; Sinnott, M. L.; Smith, P. J.; Widdows, D. *J. Chem. Soc. Perkin Trans.* **1981**, *1*, 366.
[167] Lootiens, F. G.; De Bruyne, C. K. *Naturwissenschaften*, **1964**, *51*, 359.
[168] Györgydeák, Z.; Szilágyi, L. *Liebigs Ann. Chem.* **1986**, 1393.

Table 3.2.2. ^{1}H-NMR (500 MHz) Chemical Shifts δ (ppm) and Coupling Constants J (Hz) for Compounds **4**, **73-81**, **84**, **85**.

Cpd	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5e}$)	H-5e ($J_{5e,5a}$)	H-5a ($J_{4,5a}$)	OAc	Aglycon H's	
4	4.78 (8.3)	4.92 (8.2)	5.16 (8.2)	4.90 (4.9)	4.26 (11.8)	3.40 (8.8)	2.07(3H) 2.02(6H)	7.45 (m, 2H)	7.29 (o, p, 3H)
73	5.05 (7.4)	4.97 (7.3)	5.18 (7.4)	4.92 (4.5)	4.32 (12.0)	3.53 (7.7)	2.01(3H) 2.06(6H)	8.14 (m, 2H)	7.54 (o, 2H)
74	5.34 (8.3)	4.99 (7.9)	5.17 (7.9)	4.90 (4.8)	4.12 (11.9)	3.51 (8.5)	2.03(9H)	2.35 (CH_3)	
75	5.65 (7.7)	5.03 (7.4)	5.19 (7.4)	4.90 (4.6)	4.20 (12.1)	3.55 (7.7)	2.05(9H)	4.65 (CH_2)	1.41 (CH_3)
76	5.16 (6.6)	5.01 (6.7)	5.10 (6.8)	4.85 (4.2)	4.33 (12.2)	3.51 (6.9)	2.06(6H) 2.04(3H)	7.56 (m, 2H)	7.28 (o, p, 3H)
77	5.31 (5.3)	5.16 (7.3)	5.22 (7.2)	4.98 (4.3)	4.20 (12.3)	3.60 (6.7)	2.08(9H)	8.19 (m,2H)	7.06 (o, 2H)
78	4.98 (7.8) ($^3J_{1,P}$ 5.9)	5.11 (7.6)	5.35 (6.6)	4.91 (4.6)	4.15 (12.4)	3.48 (7.8)	2.04(3H) 1.99(3H) 1.93(3H)	7.32 (m, 10H)	
79	5.22 (4.1)	5.12-5.13 (m)		4.93 (3.6)	4.58 (13.2)	3.59 (3.7)	2.05(3H) 2.10(6H)	2.70 (CH_2, 4H)	
80	5.52 (5.5)	5.25 (7.4)	5.32 (5.5)	5.03 (4.3)	4.44 (12.5)	3.57 (6.4)	2.18(3H) 2.10(3H) 2.00(3H)	7.99, 7.58 (o) 7.50, 7.37 (m)	
81	4.61 (8.1)	4.85 (8.9)	5.16 (8.9)	4.96 (5.3)	4.19 (11.7)	3.41 (9.6)	2.05(3H) 2.02(6H)	-	
84	5.75 (5.3)	5.01 (9.6)	5.37 (9.2)	4.94 (5.5)	3.84 (11.5)	4.11 (11.4)	2.08(3H) 2.03(6H)	7.41 (m, 2H)	7.27 (o, p, 3H)
85	5.46 (4.1)	4.83 (9.8)	5.30 (9.5)	4.88 (5.8)	3.87 (11.3)	3.71 (11.0)	2.03(3H) 1.97(6H)	-	

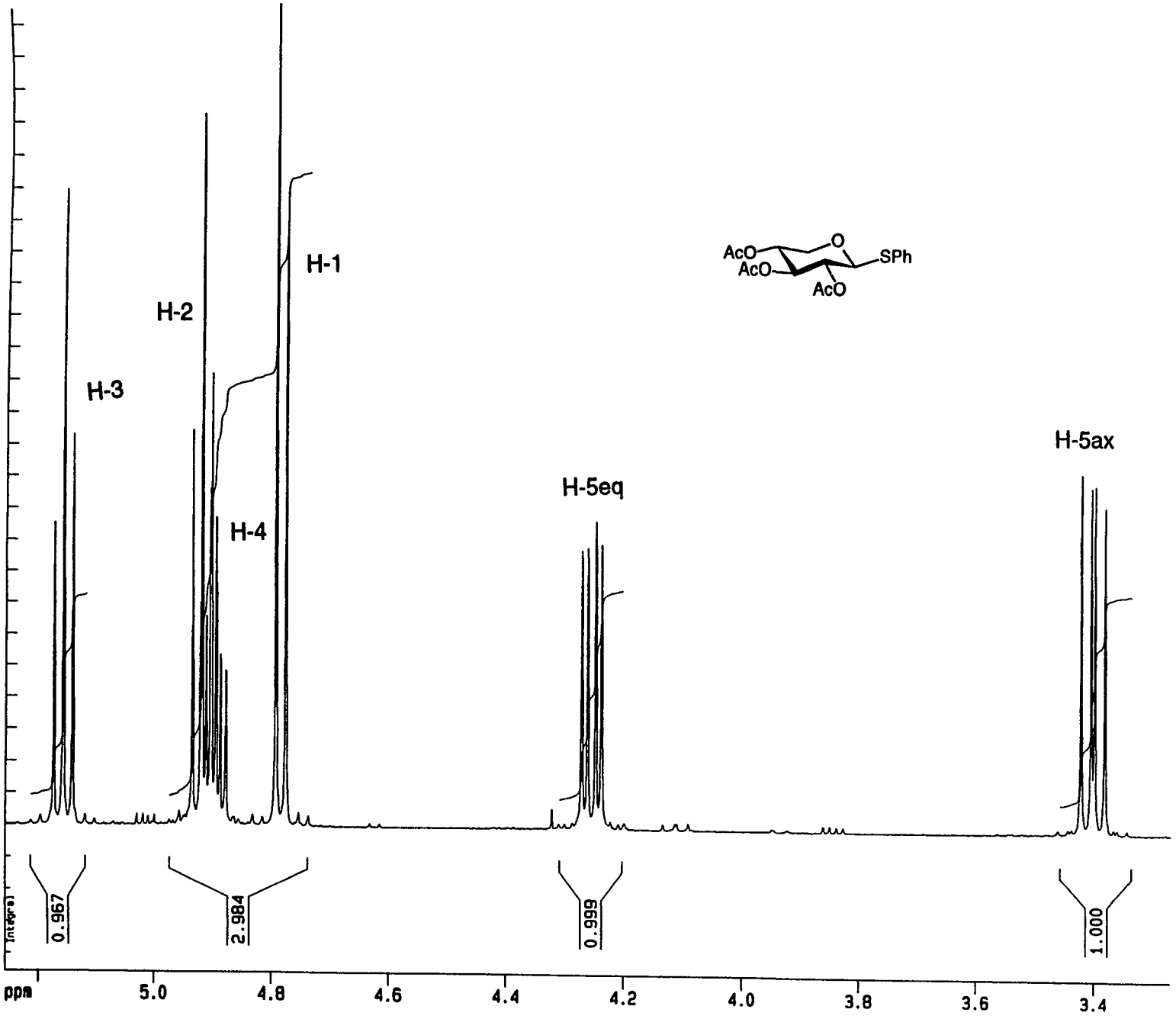


Figure 3.2.1. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of thiophenyl β-D-xylopyranoside **4**.

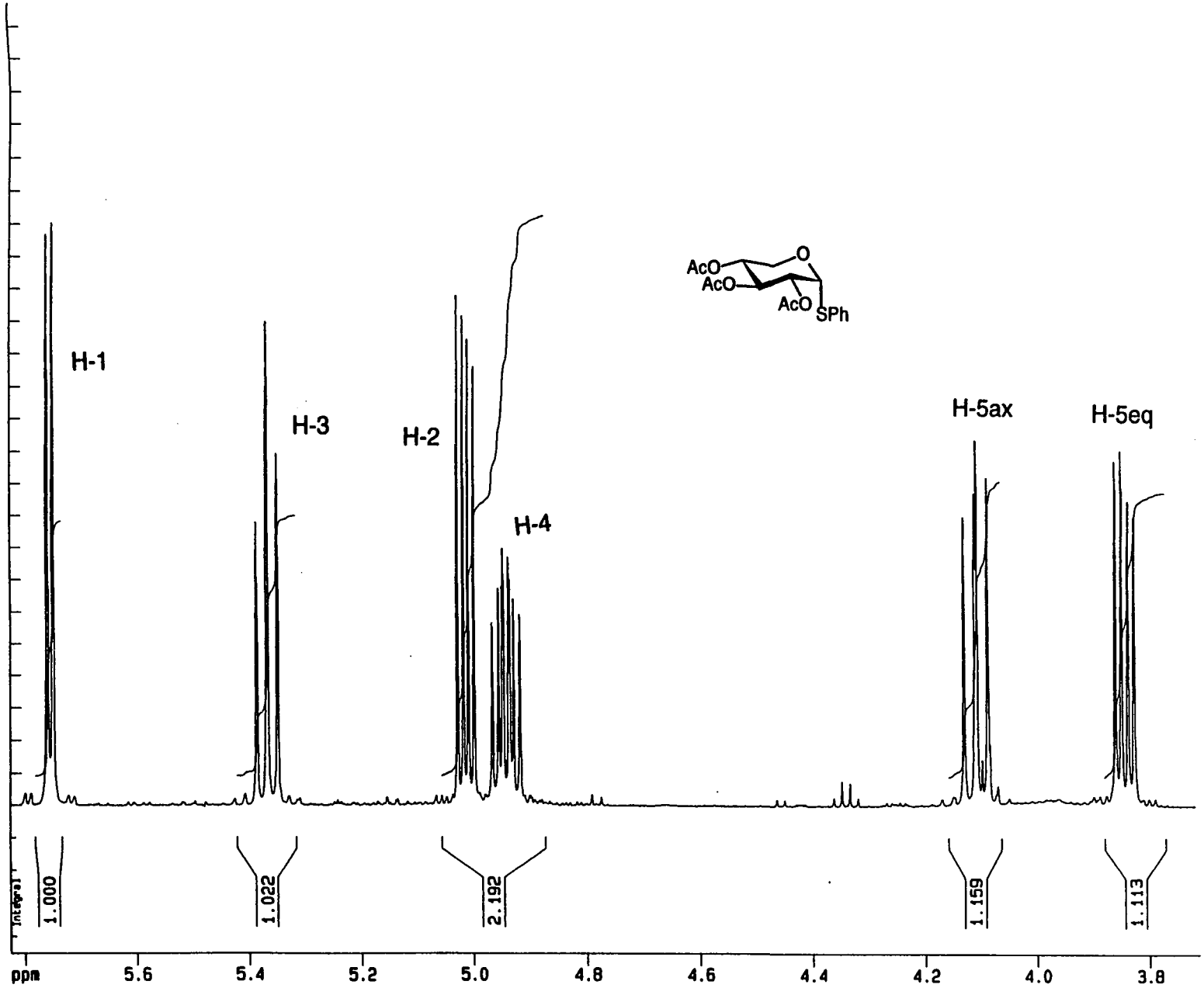
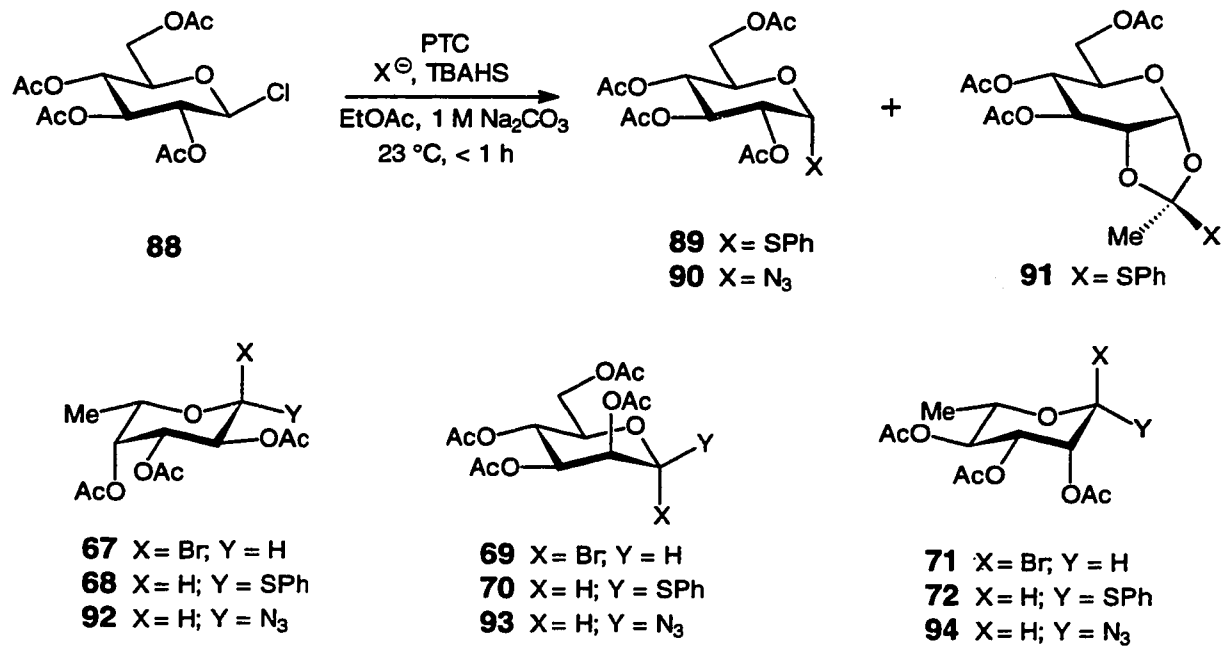


Figure 3.2.2. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of thiophenyl α-D-xylopyranoside **84**.

Since β-D-xylopyranosyl chloride adopts a $^1C_4$ conformation, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl chloride (**88**) having a fixed $^4C_1$ chair conformation was also investigated. β-D-Glucopyranosyl chloride **88** was similarly treated with thiophenol and sodium azide (Scheme 3.2.3). With the soft thiophenol nucleophile, 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranoside (**89**) and its corresponding 1,2-*O*-thiophenoxyethylidene **91** were obtained as a 10.5:1 mixture (69% yield). When the reaction was conducted with the more hydrophilic azide anion, the only substituted product 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl azide (**90**) was obtained (31% yield) without any detectable orthoester-like product. The poorer reactivity of the glycosyl chloride can account for the fact that more hydrolysis occurred in the case of azide anions.



Scheme 3.2.3. Anomeric substitution reactions of glucosyl **88**, fucosyl **67**, mannosyl **69**, and rhamnosyl **71** halides.

Table 3.2.3. Chemical Shifts (ppm) in ^{13}C NMR spectra[a] for compounds **4**, **73-81**, **84**, and **85**

Compound[b]	C_1	C_2	C_3	C_4	C_5	Aglycon C's			
						Ci	Co	Cm	Cp
4	86.3	69.8	72.0	68.4	65.2	132.2	128.2	132.7	129.0
73	84.7	69.3	70.8	67.9	64.6	130.3	124.0	124.4	126.4
74	80.3	69.0	71.5	68.2	65.6	30.8(CH_3)		192.1(C=O)	
75	85.7	68.5	70.8	68.0	65.1	20.6 (CH_2),		13.7 (CH_3),	210.1(C=S)
76	82.3	70.2	70.3	67.9	64.8	128.4	128.2	134.4	129.1
77	97.5	69.4	69.8	68.0	61.8	161.0	116.5	125.8	143.1
78[c]	96.4	69.9	70.0	69.7	62.2	169.6	127.9	128.6	128.6
79	102.7	68.3	68.3	66.9	62.0	25.4(CH_2)		170.5(C=O)	
80	105.6	68.0	69.6	67.8	62.3	143.5, 128.3, 128.5, 124.8, 120.2, 109.1			
81	88.3	68.4	71.5	70.4	64.3	-			
84	85.4	69.6	70.8	69.0	60.0	133.0	127.7	131.7	129.1
85	86.4	68.5	70.1	68.9	60.5	-			

[a] Recorded at 125 MHz.
[b] OAc: 20.6-20.7 ppm.
[c] $^2J_{C1,P}$ 5.0 Hz.

In none of the above PTC reactions and the previously described situations[159] with acetobromo-L-fucose (**67**), acetobromo-D-mannose (**69**) and acetobromo-L-rhamnose (**71**) leading to **68**, **92**, **70**, **93**, **72**, **94** were both anomers formed. We can therefore conclude that these PTC catalyzed anomeric nucleophilic substitutions were highly stereoselective since each anomeric glycosyl halide gave its respective inverted glycosyl derivatives. It is however also possible to argue that double anomeric inversions might have occurred during the process. To examine this possibility further,

we treated β-xylosyl chloride **82** with one equivalent of tetrabutylammonium chloride (TBAC) in the absence of any additional nucleophile under the same PTC conditions described above (Scheme 3.2.2). The anomerization process was rather slow as the reaction showed the formation of an anomeric mixture in a ratio of 1.2:1 in favor of the β-anomer **82** after one hour. These results suggest that in the presence of a large amount of phase transfer catalyst containing a halide anion which can compete with the added nucleophile, double inversions can occur. In the above set of experiments, we deliberately used TBAHS as catalyst to avoid this complication. This result also suggests that a nucleophile having a low partition coefficient between the organic and the aqueous phases would face competitive hydrolysis and nucleophilic displacement by the halide anion released from the glycosyl halide during reaction.

3.3. Conclusions

Phase transfer catalyzed reactions were used to provide a variety of xylosyl donors in mild and high yielding manner. These xylosyl donors could be further transformed into useful glycoside derivatives. It was also proposed that phase transfer catalyzed reactions occurred in a highly stereoselective fashion. Under PTC condition, anomeric nucleophilic substitutions of peracetylated α-D-xylopyranosyl bromide, and α- and β-chlorides provided direct inverted products.

3.4. Experimental Methods

Typical PTC reactions for the syntheses of compounds 4, 73-81, 84, and 85.

To a solution of 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (**3**)[163] (1 equiv) and tetrabutylammonium hydrogen sulfate (1 equiv) in ethyl acetate (1.0 mL/100 mg of sugar) were added the nucleophiles (1.2-3 equiv) and 1M sodium carbonate (1.0 mL/100 mg of sugar). The reaction mixture was stirred vigorously at room temperature for 1 h until the starting material was completely consumed as judged by TLC

monitoring using a mixture of ethyl acetate and hexane (4:6 v/v) as eluent. Then, the solution was diluted with ethyl acetate and the organic phase was separated from the aqueous phase. The organic solution was washed with saturated sodium bicarbonate (2 × 20 mL), water (1 × 20 mL), and brine (20 mL). It was then dried over anhydrous sodium sulfate and concentrated. The crude compounds were purified by silica gel column chromatography using a mixture of ethyl acetate and hexane (3/7 v/v) as eluent. The solid residues obtained after column chromatography were recrystallized from ethanol (Table 3.2.1).

PTC equilibration between 82 and 83.

2,3,4-Tri-*O*-acetyl-β-D-xylopyranosyl chloride (**82**)[169] (50 mg, 0.17 mmol) was dissolved in ethyl acetate (0.5 mL). To this solution, tetrabutylammonium chloride (47 mg, 0.17 mmol) in 1M aqueous Na_2CO_3 (0.5 mL) was added. The reaction mixture was stirred for 1 hour at room temperature. TLC indicated a mixture of α- and β-anomers ($R_{F,\alpha}$ 0.46, $R_{F,\beta}$ 0.37, hexane/ethyl acetate 7:3 v/v). The reaction mixture was diluted with ethyl acetate (5 mL) and the separated organic phase was washed with saturated $NaHCO_3$ (5 mL), water (5 mL), and then brine. The organic solution was dried over anhydrous Na_2SO_4 and concentrated. The ^{1}H-NMR spectrum of the crude product showed the anomeric ratio of α- (**83**) and β- (**82**) anomers to be: α:β = 1:1.2, as judged from the relative integration of the signals of their respective anomeric protons at 6.21 and 5.77 ppm, respectively.

3,4-Di-*O*-acetyl-1,2-*O*-thiophenoxyethylidene-α-D-xylopyranose (86).

Compound **86** was partially purified from the crude reaction mixture resulting from the treatment of **82** and thiophenol under the general PTC conditions described above. The following ^{1}H NMR data were extracted from an enriched ≈1:1 mixture of **84** and **86**: 7.51-7.56 (m, 2H, Ar), 7.31-7.34 (m, 3H, Ar), 5.62 (d, 1H, $J_{1,2}$ = 4.8 Hz, H-1), 5.25 (dd, 1H, $J_{2,3}$ = 2.7 Hz, H-3), 4.86-4.89 (m, 1H, H-4), 4.43 (ddd, 1H, long range J 1.1 Hz, H-2), 3.92 (dd, 1H, $J_{4,5e}$ = 6.3 Hz, H-5e), 3.59 (dd, 1H, $J_{4,5a}$ = 8.5, $J_{5a,5e}$ = 11.9

Hz, H-5a), 2.12 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.80 (s, 3H, *endo*-Me). These spectroscopic data are similar to those previously observed for an analogous *p*-methylthiophenoxy thioorthoester.[170]

Thiophenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (89).

This compound was prepared by PTC using penta-*O*-acetyl-β-D-glucopyranosyl chloride (**88**).[169] However, the time taken to consume the starting material was longer than in the case of β-D-xylopyranosyl chloride (6 h); yield 63%; mp 84.7-85.3 °C; $[\alpha]_D$ +193.0 (*c* 1.0, $CHCl_3$); ^{1}H-NMR δ 7.42-7.40 (m, 2H, Ar), 7.29-7.24 (m, 3H, Ar), 5.89 (d, 1H, $J_{1,2}$ 5.7 Hz, H-1), 5.41 (t, 1H, $J_{3,4}$ 10.0 Hz, H-3), 5.08 (dd, 1H, $J_{2,3}$ 10.3 Hz, H-2), 5.05 (dd, 1H, $J_{4,5}$ 10.2 Hz, H-4), 4.54 (ddd, 1H, $J_{5,6a}$ 5.2 Hz, $J_{5,6b}$ 2.2 Hz, H-5), 4.25 (dd, 1H, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.01 (dd, 1H, H-6b), 2.07, 2.03, 2.01, 1.99 (4s, 12H, OAc); ^{13}C-NMR δ 171.1, 170.5, 170.4, 170.2 (C=O), 132.4 (Ar_{meta}), 129.7 (Ar_{ortho}), 129.5 (Ar_{ipso}), 128.4 (Ar_{para}), 85.5 (C-1), 71.3 (C-3), 71.0 (C-2), 69.1 (C-4), 68.7 (C-5), 62.5 (C-6), 21.3 (OAc), 21.2 (NAc).

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl azide (90).

For this reaction the reaction rate was very slow and it took 19 h to consume the starting material; yield 31%; mp 102.7-103.2 °C; $[\alpha]_D$ = +155.6 (*c* 1.0, $CHCl_3$); ^{1}H-NMR δ 5.58 (d, 1H, $J_{1,2}$ 4.4 Hz, H-1), 5.36 (t, 1H, $J_{3,4}$ 9.7 Hz, H-3), 5.02 (t, 1H, $J_{4,5}$ 9.9 Hz, H-4), 4.92 (dd, 1H, $J_{2,3}$ 10.1 Hz, H-2), 4.07-4.26 (m, 3H, H-5, H-6's), 2.07×2, 2.00, 1.98 (3s, 12H, OAc); ^{13}C-NMR δ 171.2, 170.5, 170.1 (C=O), 86.8 (C-1), 70.7 (C-3), 70.2 (C-4), 70.1 (C-2), 68.4 (C-5), 62.1 (C-6), 21.3, 21.2, 21.2 (Me).

3,4,6-Tri-*O*-acetyl-1,2-thiophenoxyethylidene-α-D-glucopyranose (91).

This compound was a by-product from the PTC reaction using β-D-glucopyranosyl chloride and thiophenol as a nucleophile. This compound was purified from the column and its NMR was obtained: yield 6 %; ^{1}H-NMR δ 7.50-7.53 (m, 2H, Ar),

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[169] Lemieux, R. U. In *Methids in Carbohydrate Chemistry* Vol II, Eds.: Whistler, R. L.; Wolfrom, M. L..; Academic press: New York, 1963, p. 224.
[170] (a) Magnusson, G. *J. Org. Chem.* **1976**, *41*, 4110; (b) Magnusson, G. *J. Org. Chem.* **1977**, *42*, 913.

7.30-7.36 (m, 3H, Ar), 5.75 (d, 1H, $J_{1,2}$ 5.3 Hz, H-1), 5.22 (t, 1H, $J_{3,4}$ 2.5 Hz, H-3), 4.89 (ddd, 1H, $J_{4,5}$ 9.7 Hz, long range J 1.0 Hz, H-4), 4.58 (ddd, 1H, $J_{2,3}$ 2.7 Hz, long range J 1.0 Hz, H-2), 4.17, 4.16, 4.16 (3s, 2H, H-6's), 3.93 (ddd, 1H, $J_{5,6a}$ 4.14 Hz, $J_{5,6b}$ 3.9 Hz, H-5), 2.12, 2.06, 2.05 (3s, 9H, OAc), 1.80 (s, 3H, *endo*-Me).

Chapter 4. Self-assembling glycodendrimers

4.1. Introduction

Recently, there have been numerous studies in constructing various neoglycoconjugates[171] in a multivalent fashion to demonstrate the "cluster effect", which is considered as a form of carbohydrate-protein interaction on cell surfaces.[172] This cluster effect is expected when the multivalent glycosides interact with more than one lectin binding site simultaneously and cooperatively, resulting in better cellular recognition. Therefore, the preparation of carbohydrate ligands in the cluster domains which bind to the carbohydrate recognition sites on the cell protein would contribute to the development of better therapeutic inhibitors.

The conventional methods for the preparation of carbohydrate clusters and carbohydrate dendrimers includes convergent or divergent approaches (see Section 1.4). However, these procedures require lengthy and reiterative stepwise synthesis. This issue can be overcome by employing newly developed synthetic method, metal associated self-assembly,[173,174] where hyper-branched dendrimers are prepared by nucleating readily accessible building blocks (dendrons) around the metal ions such as ruthenium(II),[175,176,177] iron(II),[174,178] or copper(II).[179] In this self-assembling method, pre-made dendrons are non-covalently assembled around a coordinated metal and the resulting dendritic structure is governed by the coordination of the selected metal and the degree of branching in the dendron.

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[171] (a) Roy, R. In *Carbohydrate Chemistry*, Ed: Boons, G.-J., Thomson Science, London, **1998**, p. 241. (b) Jayaraman, N.; Nepogodiev, S. A.; Stoddart, J. F. *Chem. Eur. J.* **1997**, *3*, 1193.
[172] (a) Varki, A. *Glycobiology*, **1993**, *3*, 97, (b) Dwek, R. *Chem. Rev.* **1996**, *96*, 683.
[173] Zimmerman, S. C.; Zeng, F. W.; Reichert, D. E. C.; Kolotuchin, S. V. *Science* **1996**, *271*, 1095
[174] Tzalis, D.; Tor, Y. *Tetrahedron Lett.* **1996**, *37*, 8293.
[175] Beer, P. D.; Szemes, F. *J. Chem. Soc., Chem. Commun.* **1995**, 2245.
[176] Lamba, J. J. S.; Fraser, C. L. *J. Am. Chem. Soc.* **1997**, *119*, 1801.
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[178] Chow, H.-F.; Chan, I. Y. -K.; Chan, D. T. W.; Kwok, R. W. M. *Chem. Eur. J.* **1996**, *2*, 1085.

It has been previously demonstrated that Fe^{II}-induced trimeric GalNAc ligand,[180,181] where GalNAc was directly coupled to 5-(bromomethyl)-5'-methylbipyridine, exhibited increased binding affinity toward *Vicia villosa* B_4 (VVA) lectin. Notwithstanding the enhancement of binding affinity observed in carbohydrate dendrimers, an extension of this self-assembly concept to the synthesis of glycodendrimers is unprecedented.

Herein, the syntheses and the relative binding properties of Fe^{II} and Cu^{II} assisted self-assembled glycoclusters and glycodendrimers are presented. The carbohydrate moiety in these glycoclusters and glycodendrimers includes tumor-associated Tn-antigen (GalNAcα-O-Ser).[182,183]

Enzyme Linked Lectin Assay (ELLA)[184] was used to evaluate the inhibitory capacities of the synthetic GalNAc-containing ligands which inhibited binding of lectin VVA to asialoglycophorin.

4.2. Synthesis of self-assembling glycodendrimers

Synthesis of GalNAc derivatives from GlcNAc

Since *N*-acetyl-D-galactosamine (GalNAc) (**32**) is costly starting material, a method to prepare GalNAc glycosides in an inexpensive manner was accomplished by using a modification of Lee's method.[185]

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[179] Morgan, G.; Burstall, F. H. *J.* **1937**, 1649, (b) Hogg, R.; Wilkins, R. G. *J.* **1962**, 341.
[180] Sakai, S.; Sasaki, T. *J. Am. Chem. Soc.* **1994**, *116*, 1587.
[181] Sakai, S.; Shigemasa, Y.; Sasaki, T. *Tetrahedron Lett.* **1997**, *38*, 845.
[182] (a) Springer, G. F. *Science* **1984**, *224*, 1198, (b) Toyokuni, T.; Singhal, A. K. *Chem. Soc. Rev.* **1995**, 231.
[183] (a) Desai, P. R.; Tegmeyer, H.; Chandrasekaran, E. V.; Springer, G. F. *J. Tumor Maker Oncol.* **1987**, *2*, 233, (b) Springer, G. F.; Taylor, C. R.; Howard, D. R.; Tegmeyer, E. V.; Desai, P. R.; Murthy, S. M.; Felder, B.; Scalon, E. F. *Cancer*, **1985**, *55*, 561.
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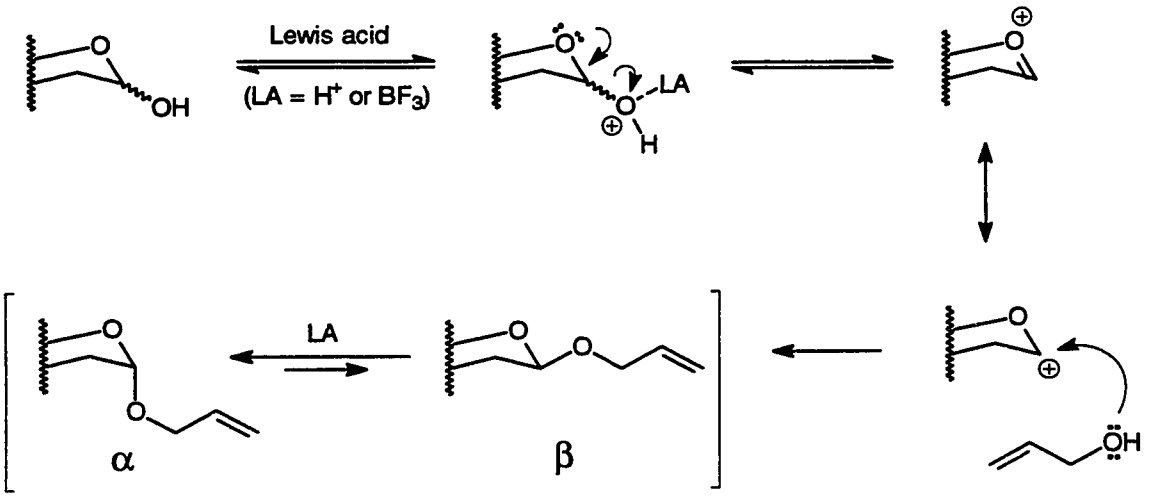
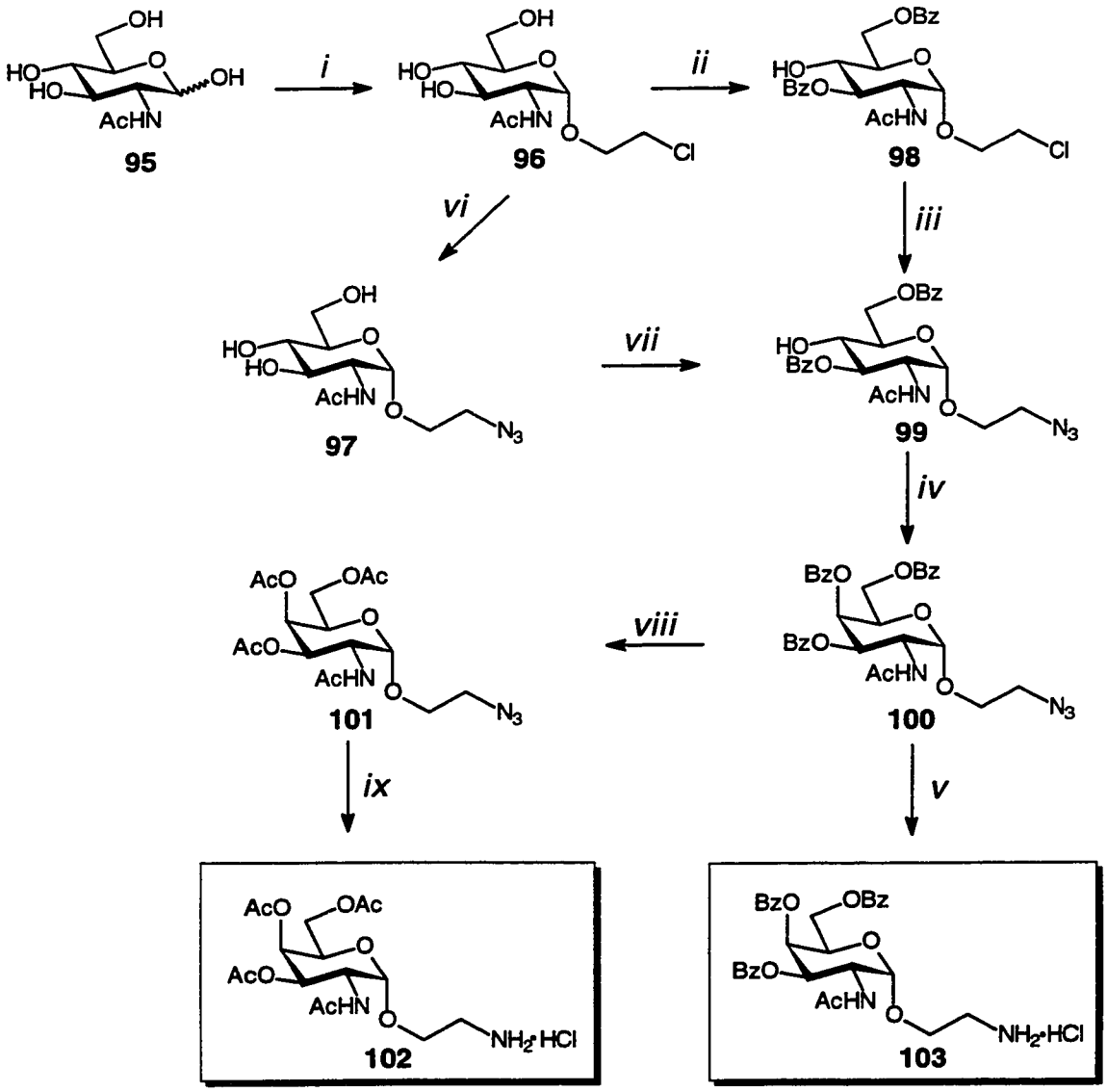
The essential core for further synthesis of the carbohydrate ligand was either 2-aminoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside (α-GalNAc homoserine) (**102**) or per-*O*-benzoylated derivative **103**. This amine salt can be synthesized mainly by two pathways; first, reduction of azide to amine and secondly, hydrogenation of 2°-benzylamine into 1°-amine (Scheme 4.2.1 and Scheme 4.2.2, respectively). Both methods provided *O*-benzoylated GalNAc residue in five consecutive steps. The corresponding *O*-acetylated GalNAc residue was also prepared by deprotecting the *O*-benzoyl groups under Zemplén conditions and then protecting the resulting free hydroxyl groups with acetate functionalities.

Relatively inexpensive *N*-acetyl-D-glucosamine (GlcNAc) (**95**) was used as a starting material for the synthesis of the key compound, the α-GalNAc homoserine derivative. First, *N*-acetyl-D-glucosamine (**95**) was glycosidated with 2-chloroethanol using Fischer conditions (Scheme 4.2.1).



Scheme 4.2.1. Fischer glycosidation.

A solution of GlcNAc (**95**) in chloroethanol was heated at ≈70 °C for 2 to 5 hours in the presence of acetic acid and stirred overnight at room temperature until one major compound was observed on the TLC plate. The acid used in the glycosidation process was either a Lewis acid such as $BF_3 \bullet OEt_2$ as a catalyst or an equimolar amount of acetic acid. When the reaction was complete, the solution was concentrated and the resulting syrupy residue was purified by silica gel column chromatography. Substitution of chloride by azide can be done at this stage with free hydroxyl group on GlcNAc residue. However, monitoring of the reaction was not facile because the reactant **96** and the product **97** had the same R_f values. Even though ^{1}H-NMR spectroscopy indicated different patterns for 2-chloroethyl GlcNAc **96** and 2-azidoethyl GlcNAc **97**, the quantitative evaluation of the transformation was difficult based only on the appearance of the ethyl signals in the ^{1}H-NMR spectra. 2-Chloroethyl GlcNAc **96** with free hydroxyl group was first di-*O*-benzoylated selectively at C-3 and C-6 positions (BzCl, pyridine, -60 °C, CH_2Cl_2) following Lee's method[185] and then the chloride was converted into the azide by a simple S_N2 reaction (Scheme 4.2.2). Due to the relatively slow reaction rate, a large excess of NaN_3 (10 eq) and the auxiliary NaI (1 eq) were used. The reaction was monitored by ^{1}H-NMR spectroscopy and the well resolved aglycon-protons of the product clearly indicated complete transformation. Epimerization of 2-azidoethyl GlcNAc **99** at C-4 was also accomplished by S_N2 reaction again following Lee's method.[185] The hydroxyl group on C-4 was transformed into a triflate group which served as a good leaving group. Nucleophilic substitution of the triflate by a benzoate salt (NaOBz) resulted in the amine precursor, 2-azidoethyl GalNAc **100**. Azide functionality was then transformed into an amine by hydrogenation. Hydrogenation of *O*-acetyl or *O*-benzoyl protected 2-azidoethyl GalNAc (**101** and **100**, respectively) was done in the presence of an equimolar amount of acetic acid. The resulting acetate was then converted into hydrochloride because acetamide was obtained as a by-product when the acetic acid salt was used in any peptide coupling process.



Scheme 4.2.2. Syntheses of GalNAc homoserine **102** and **103**; *i*) 2-chloroethanol, acetic acid (1 eq), 70 °C for 4h, then 23 °C for 4h, 75%; *ii*) benzoyl chloride (2.3 eq), pyridine, CH_2Cl_2, -60 °C, 3h, 77%; *iii*) NaN_3 (10 eq), NaI (1 eq), CH_3CN, 60 °C, 48h, 96%; *iv*) (1) triflic anhydride (1.5 eq), pyridine, CH_2Cl_2, -15 °C, 2h, (2) NaOBz (5 eq), DMF, 23 °C, 20h, 64%; *v*) (1) H_2, Pd/C, AcOH, MeOH, 16h, (2) Amberlite IRA-400 (Cl), 24h, MeOH, 98%; *vi*) NaN_3 (10 eq), NaI (1 eq), CH_3CN, 60 °C, 7h, 95%; *vii*) benzoyl chloride (2.3 eq), pyridine, CH_2Cl_2, -60 °C, 4h, 74%; *viii*) (1) 1M NaOMe, MeOH, pH 9, 3h, (2) Ac_2O, pyridine, 16h, 23 °C, 85%; *ix*) (1) H_2, Pd/C, AcOH, MeOH, 24h, (2) Amberlite IRA-400 (Cl), 24h, MeOH, 95%.

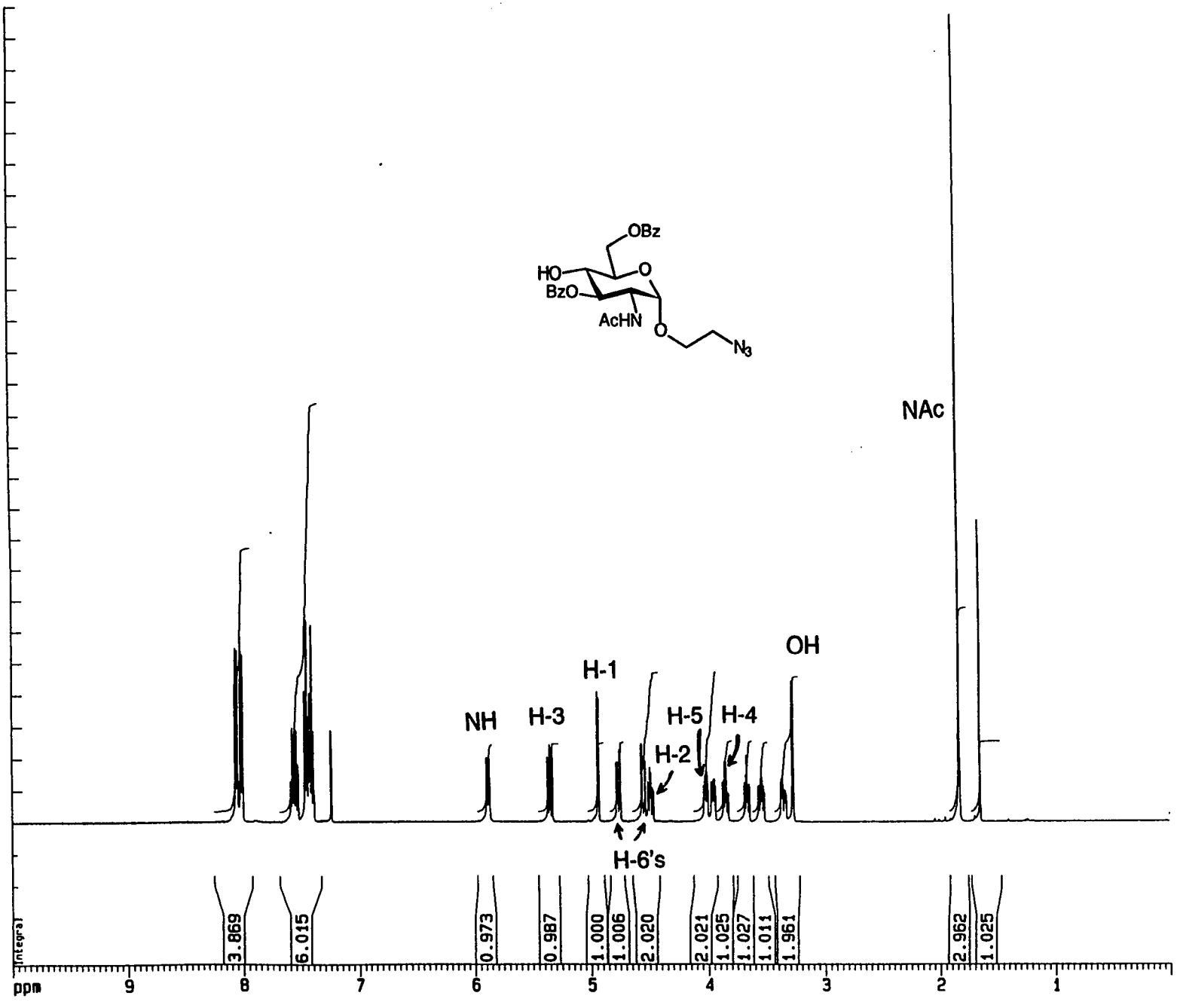


Figure 4.2.1. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of 2-azidoethyl 2-acetamido-3,6-di-O-benzoyl-2-deoxy-α-D-glucopyranoside (**99**).

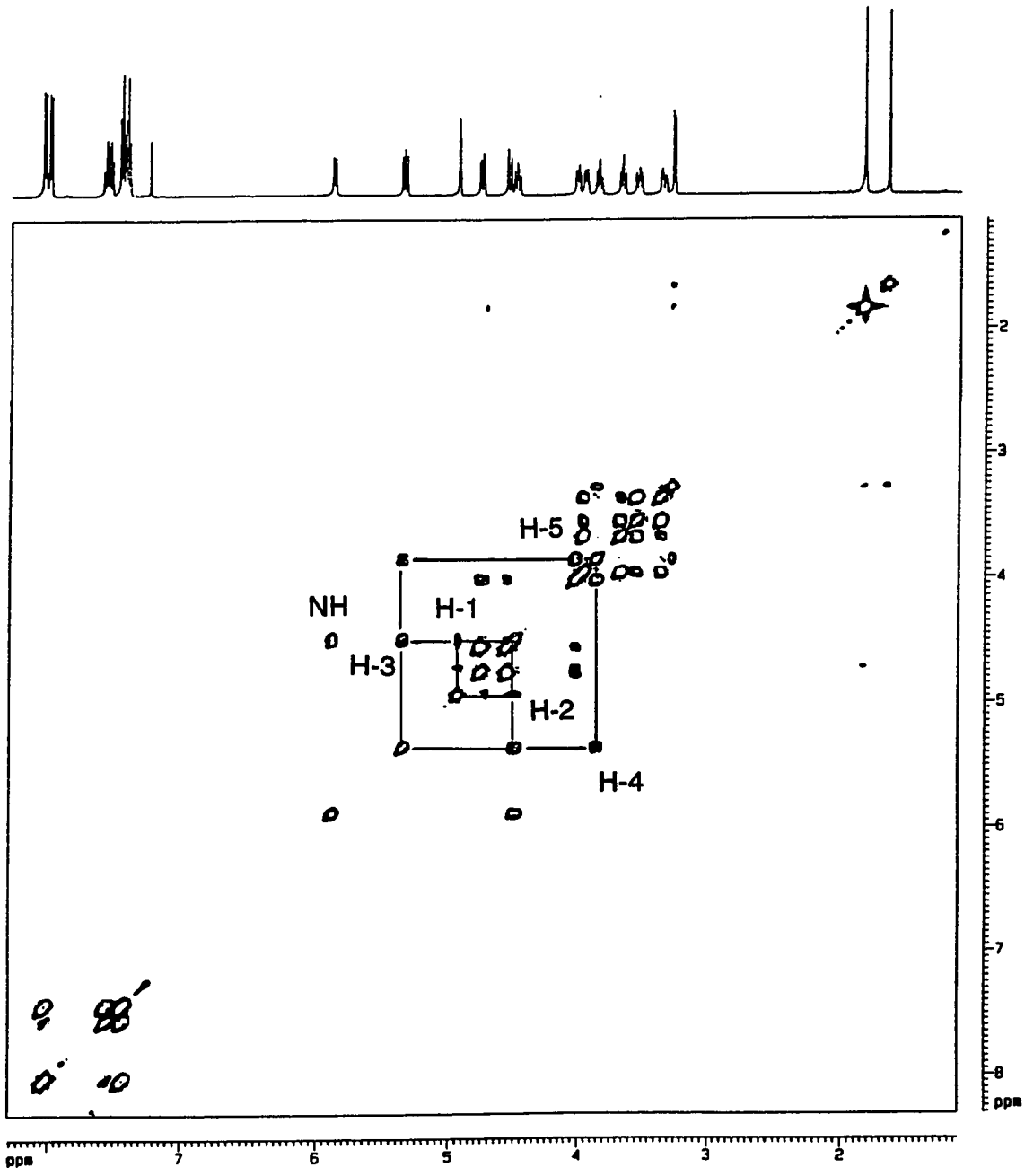


Figure 4.2.2. COSY ($CDCl_3$, 500 MHz) spectrum of 2-azidoethyl 2-acetamido-3,6-di-O-benzoyl-2-deoxy-α-D-glucopyranoside (**99**).

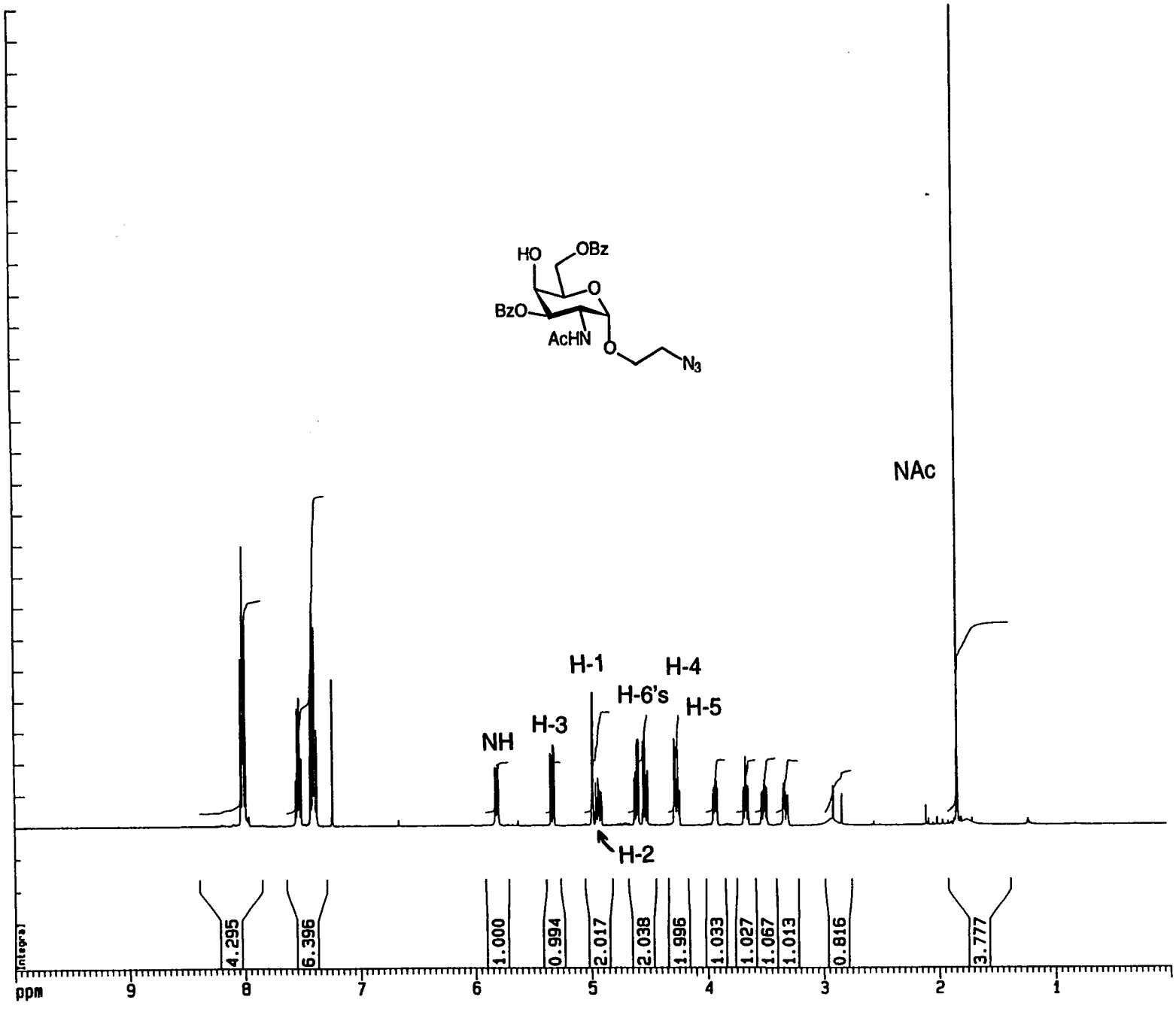


Figure 4.2.3. ^{1}H-NMR ($CDCl_3$, 500 MHz) specrum of 2-azidoethyl 2-acetamido-3,6-di-O-benzoyl-2-deoxy-α-D-galactopyranoside.

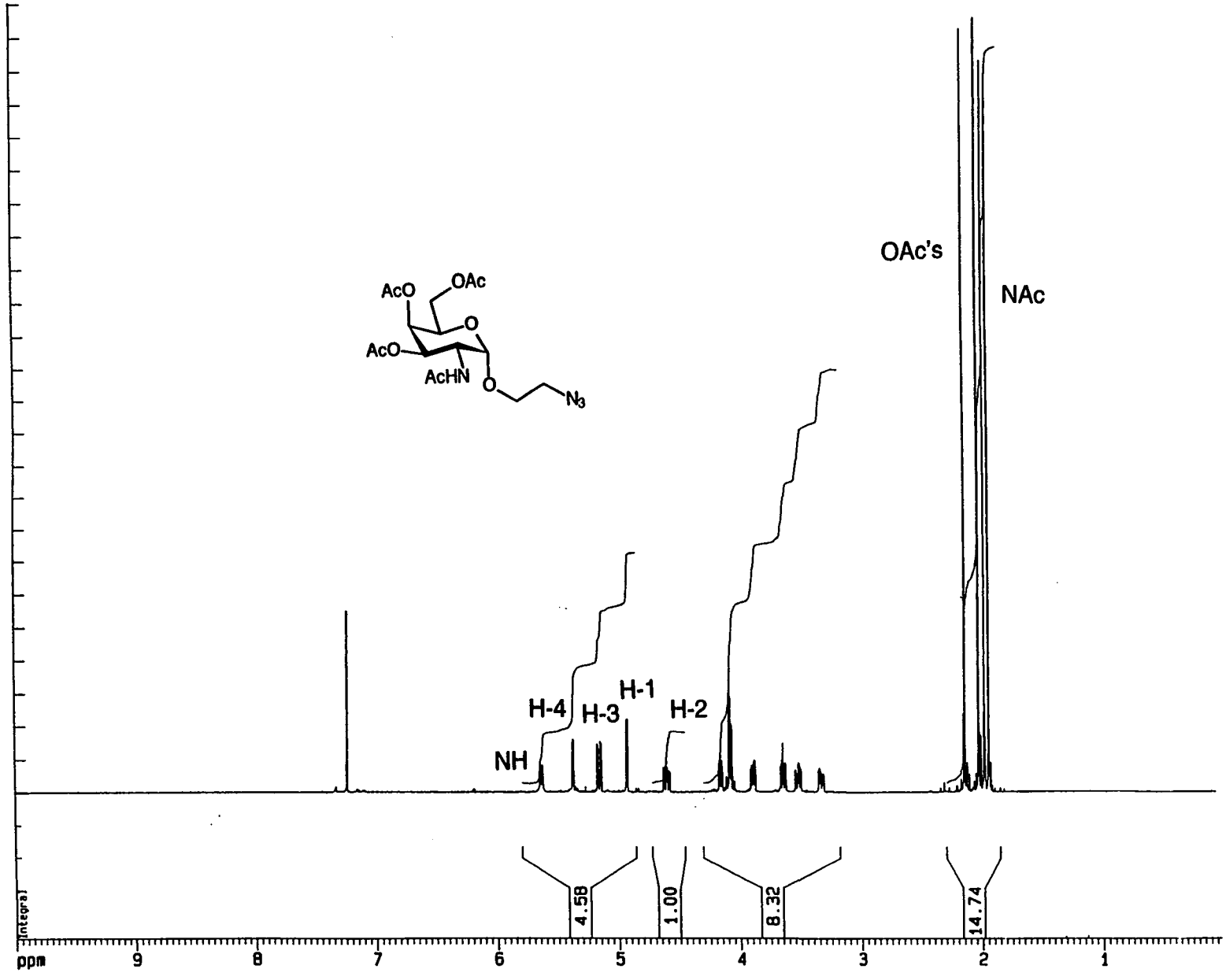
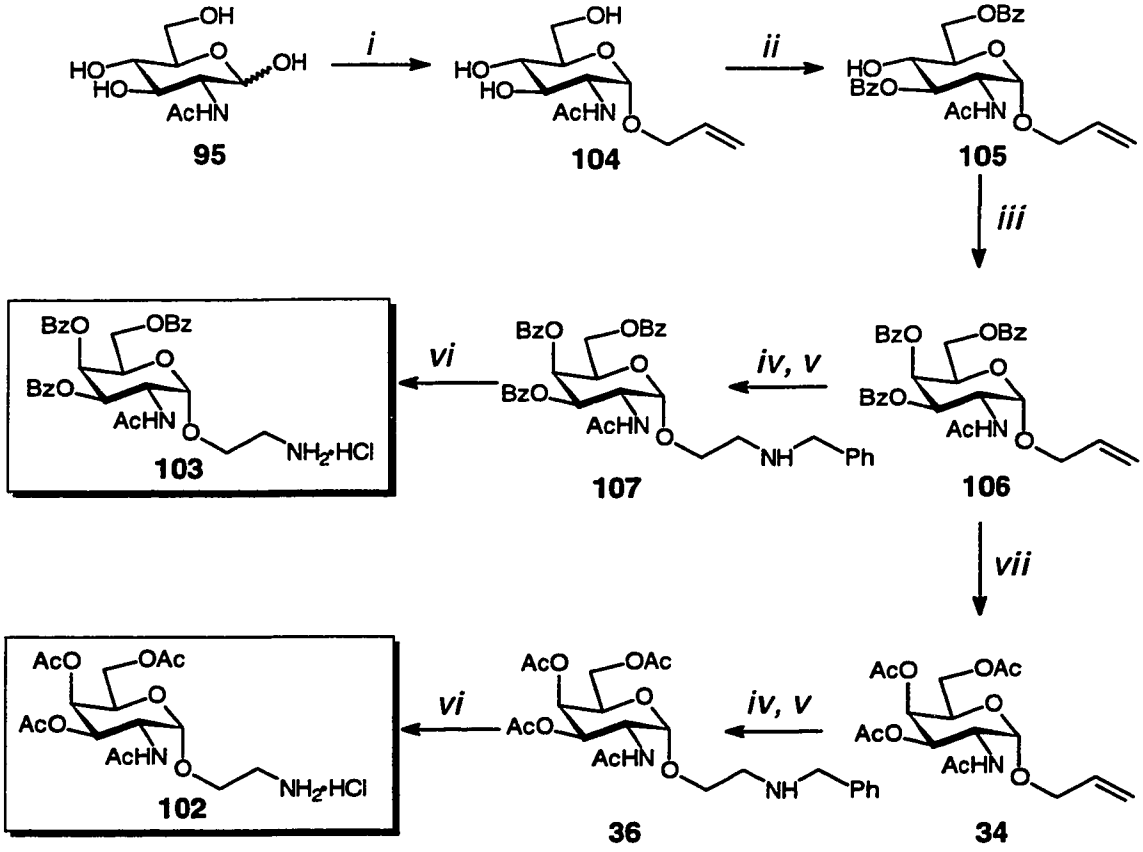


Figure 4.2.4. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of 2-azidoethyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranoside (**101**).

Amberlite® IRA-400 (Cl) resin was therefore used for the exchange of ammonium counteranion. Treating the acetate salt with Cl^- resin in methanol for 16 to 24 h converted acetate into chloride.



Scheme 4.2.3. Syntheses of GalNAc homoserine **102** and **103**; *i*) allyl alcohol, $BF_3{\bullet}OEt_2$, reflux, 5h, 23 °C, 16h, 67%; *ii*) benzoyl chloride (2.3 eq), pyridine, CH_2Cl_2, -60 °C, 3h, 64%; *iii*) (1) triflic anhydride (1.5 eq), pyridine, CH_2Cl_2, -15 °C, 2h, (2) NaOBz (5 eq), DMF, 23 °C, 20 h, 61%; *iv*) (1) O_3, CH_2Cl_2, -76°C, 15 min, (2) CH_3SCH_3, CH_2Cl_2, 16 h; *v*) $PHCH_2NH_2$ (5 eq), conc. HCl (cat), $NaCNBH_3$ (5 eq), THF, 23 °C, 6 h, 82% (OBz); 74% (OAc), *vi*) (1) H_2, Pd/C, AcOH, MeOH, 23 °C, 48 h, (2) Amberlite IRA-400 (Cl), MeOH, 94% (OBz), 96% (OAc); *vii*) 1M NaOMe, MeOH, pH 9, 23 °C, 2h, (2) Ac_2O, pyridine, 23 °C, 16 h, 91%.

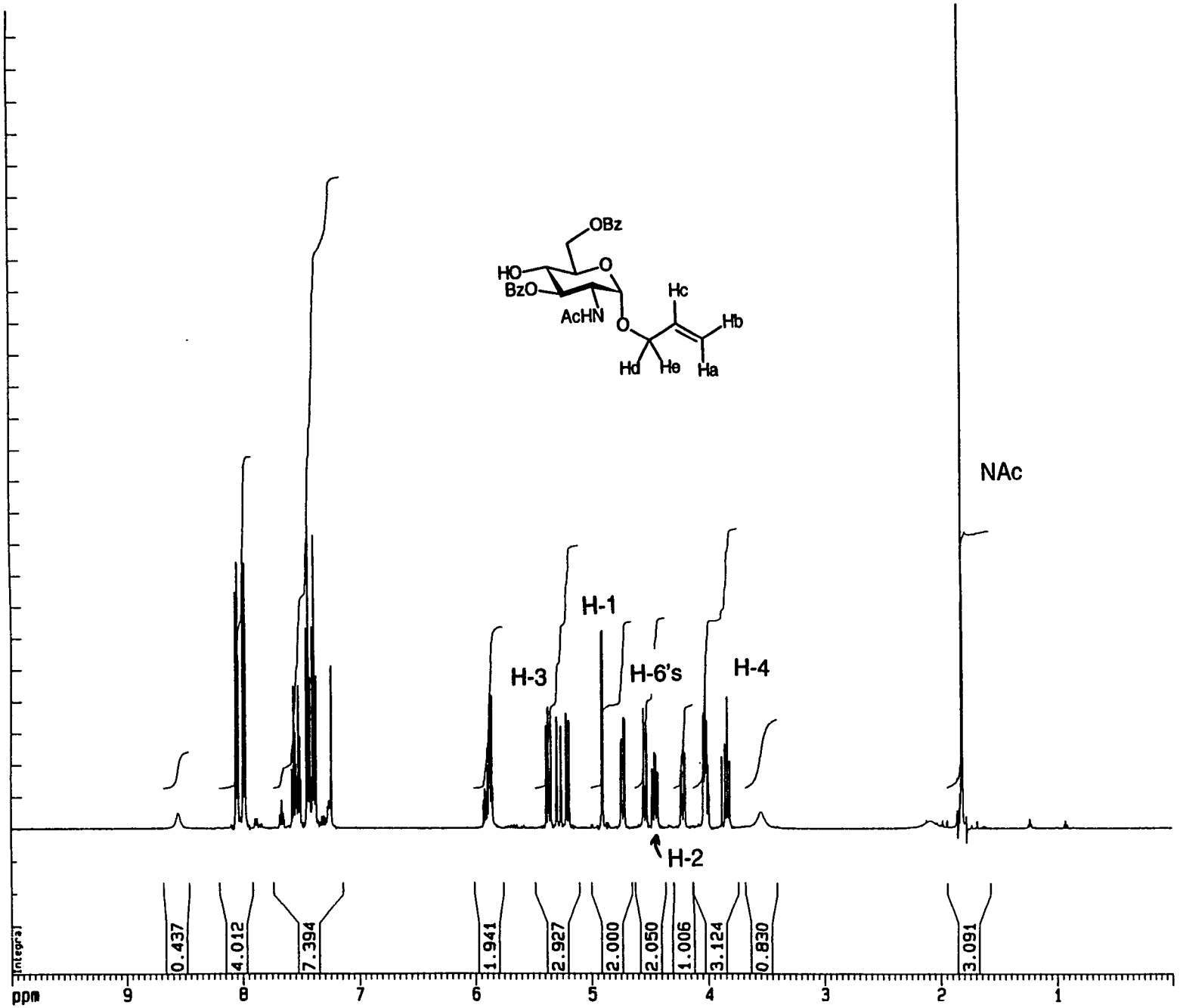


Figure 4.2.5. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of allyl 2-acetamido-3,6-di-O-benzoyl-2-deoxy-α-D-glucopyranoside (**105**).

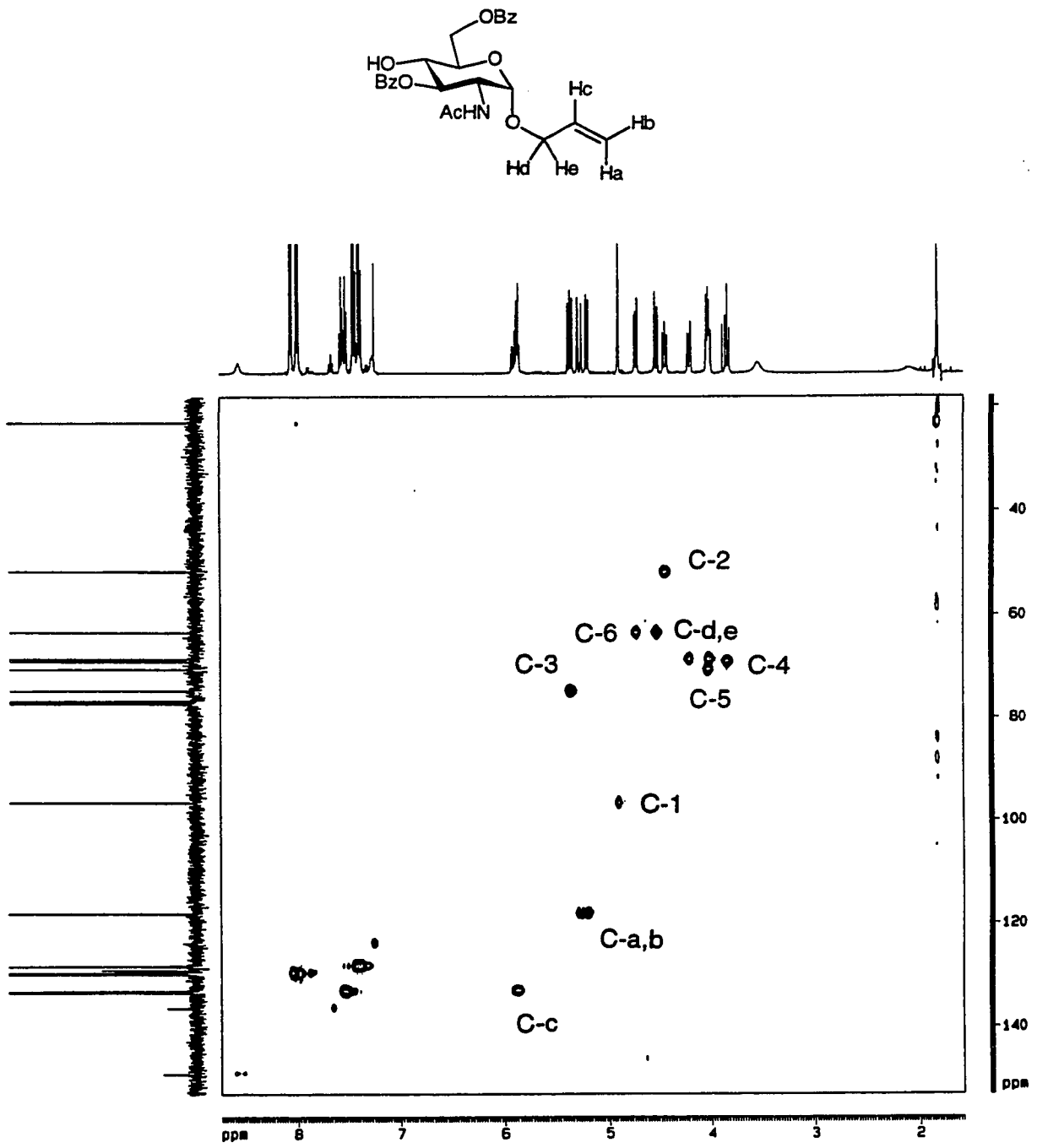


Figure 4.2.6. HMQC ($CDCl_3$, 500 MHz) spectrum allyl 2-acetamido-3,6-di-O-benzoyl-2-deoxy-α-D-glucopyranoside (**105**).

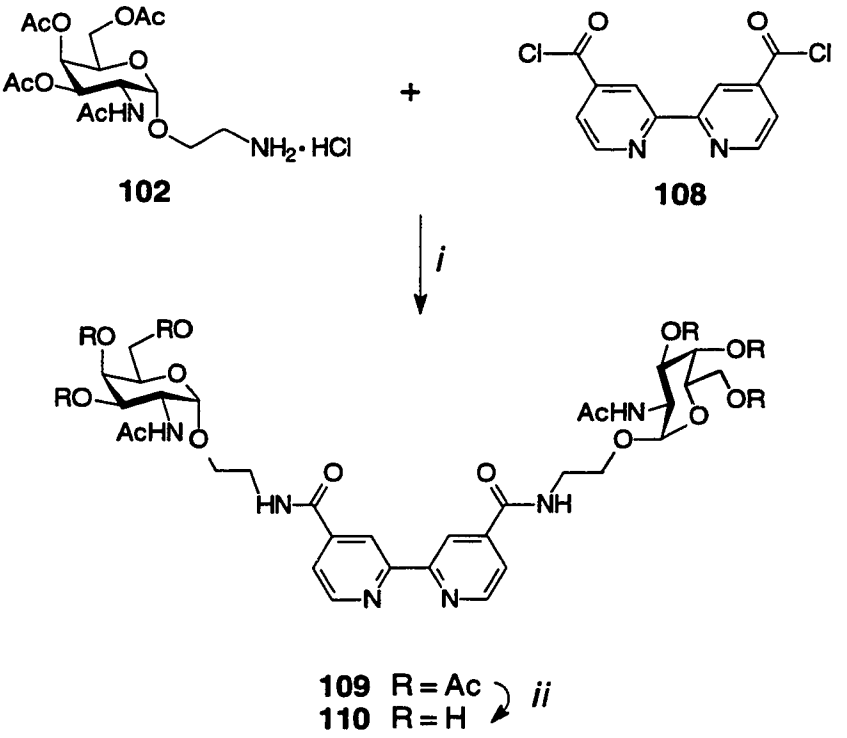
Another route to prepare 2-aminoethyl GalNAc was also successful (Scheme 4.2.3). Using the same Fischer glycosylation method, allyl GlcNAc **104** was obtained. Selective benzoylation of allyl GlcNAc **104** followed by epimerization of hydroxyl group on C-4 afforded allyl GalNAc **106**. *O*-Benzoyl protected GalNAc residue **106** can be transformed into *O*-acetyl protected glycosides **34** at this stage. Desired amine functionality was obtained by converting alkene **106** into aldehyde followed by reductive amination using $NaCNBH_3$. Direct conversion of aldehyde to 1°-amine was attempted using ammonium acetate as an aminating agent. However, monitoring the reaction was very complex because the spot for the product was located underneath $NaCNBH_3$ on the TLC plate. When benzylamine was used instead of ammonium acetate, the reaction was well analyzed on the TLC, where R_f for the product was much greater than the one for the reagent, $NaCNBH_3$. The excess $NaCNBH_3$ was destroyed by adding conc. HCl upto pH ≈3 and stirring vigorously for 30 min until no gas was evolved. The resulting 2°-benzylamines **36** and **107** were then transformed into 1°-amines **102** and **103** by hydrogenation in the presence of an equimolar amount of acetic acid followed by anion exchange.

Both pathways afforded the desired products, *O*-acetylated/*O*-benzoylated 2-aminoethyl GalNAc **102** and **103**, respectively, in 5 to 7 consecutive steps each of which occurred in good yield.

Synthesis of building blocks

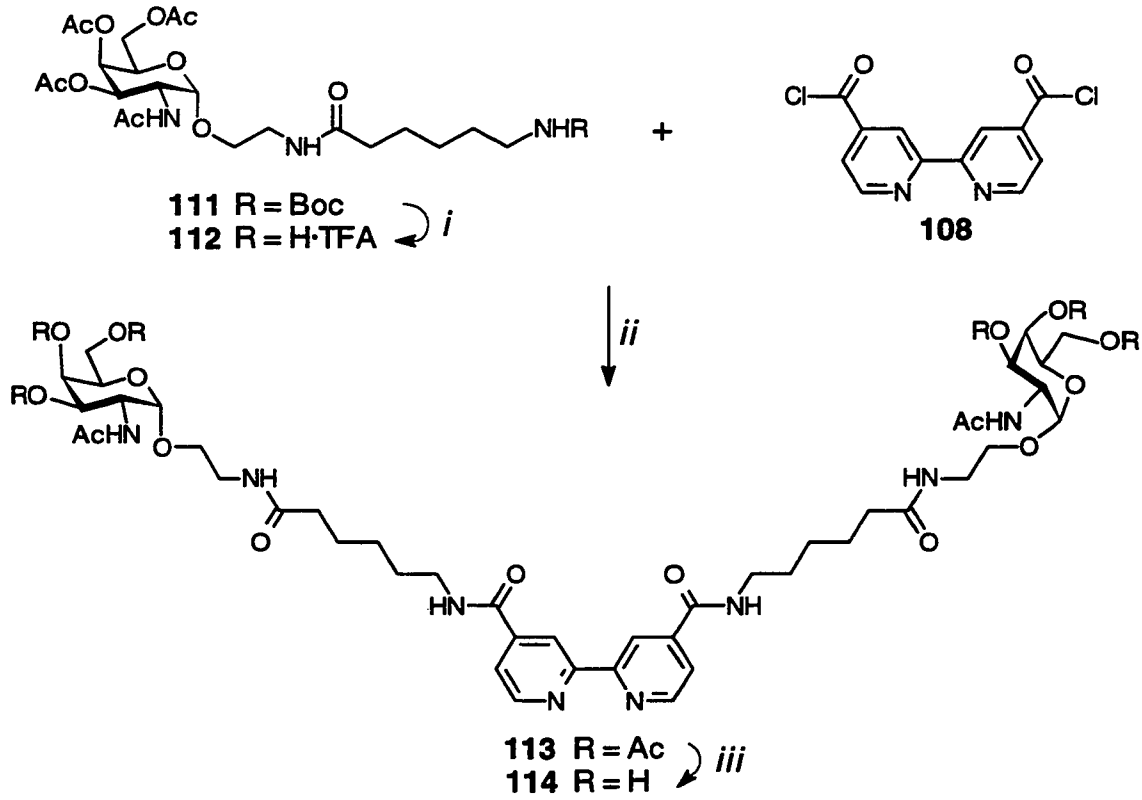
The building blocks (dendrons) were synthesized by coupling the corresponding glycosylated amine to bipyridyl residue. Commercially available 2,2'-bipyridine-4,4'-dicarboxylic acid was activated into the acid chloride **108** by refluxing it with thionyl chloride ($SOCl_2$) for 2 hours. In general, coupling was accomplished by adding the 2,2'-bipyridine-4,4'-diacid chloride (**108**) in CH_2Cl_2 to

a solution containing GalNAc-amine **102** and Et_3N at 0 °C. The progress of the reaction was monitored by TLC. As the reaction proceeded, the color of the solution turned into pinkish brown. However, this solution was decolorized by treating it with 1M NaOMe in MeOH for de-*O*-acetylation. When divalent bipyridine ligand **109** containing the *O*-acetylated GalNAc residue with a short spacer arm was treated with 1M NaOMe in MeOH, the deprotected compound **110** precipitated out from the solution (Scheme 4.2.4). This precipitate was filtered through a fritted glass filter. During the filtration, contact of the bipyridine compounds with any metal (e.g., spatula) was avoided because of latent contamination from any metal source.



Scheme 4.2.4. Synthesis of short-spacer-armed bipyridyl dimer **110** ; *i*) Et_3N (5 eq), CH_2Cl_2, 0 °C, 3 h; *ii*) 1M NaOMe, MeOH, pH 9, 23 °C, 3 h, 81%.

Divalent bipyridine ligands with long spacer arms were synthesized using a long spacer armed amine residue. 2-Aminoethyl GalNAc **102** was first elongated with *N*-Boc-caproic acid[186] (TBTU, DIPEA, CH_2Cl_2, 1h, 76%) to afford **111** and then coupled with bipyridine core (Et_3N, 3h) after removing the *N*-Boc protecting group (20% TFA, CH_2Cl_2, 2h). The coupling was confirmed by TLC analysis and the *O*-acetyl protecting groups were removed under Zemplén conditions. The resulting precipitate was filtered to afford unprotected divalent bipyridine ligand **114** (76%) (Scheme 4.2.5).



Scheme 4.2.5. Synthesis of the long-spacer-armed bipyridyl dimer **114**; *i*) 20% TFA, CH_2Cl_2, 23 °C, 2h; *ii*) Et_3N (5 eq), CH_2Cl_2, 0 °C, 3h; *iii*) 1M NaOMe, MeOH, pH 9, 23 °C, 3h, 76%.

[186] For the preparation for Boc-6-aminocaproic acid, see chapter 6.2 for compound **154**.

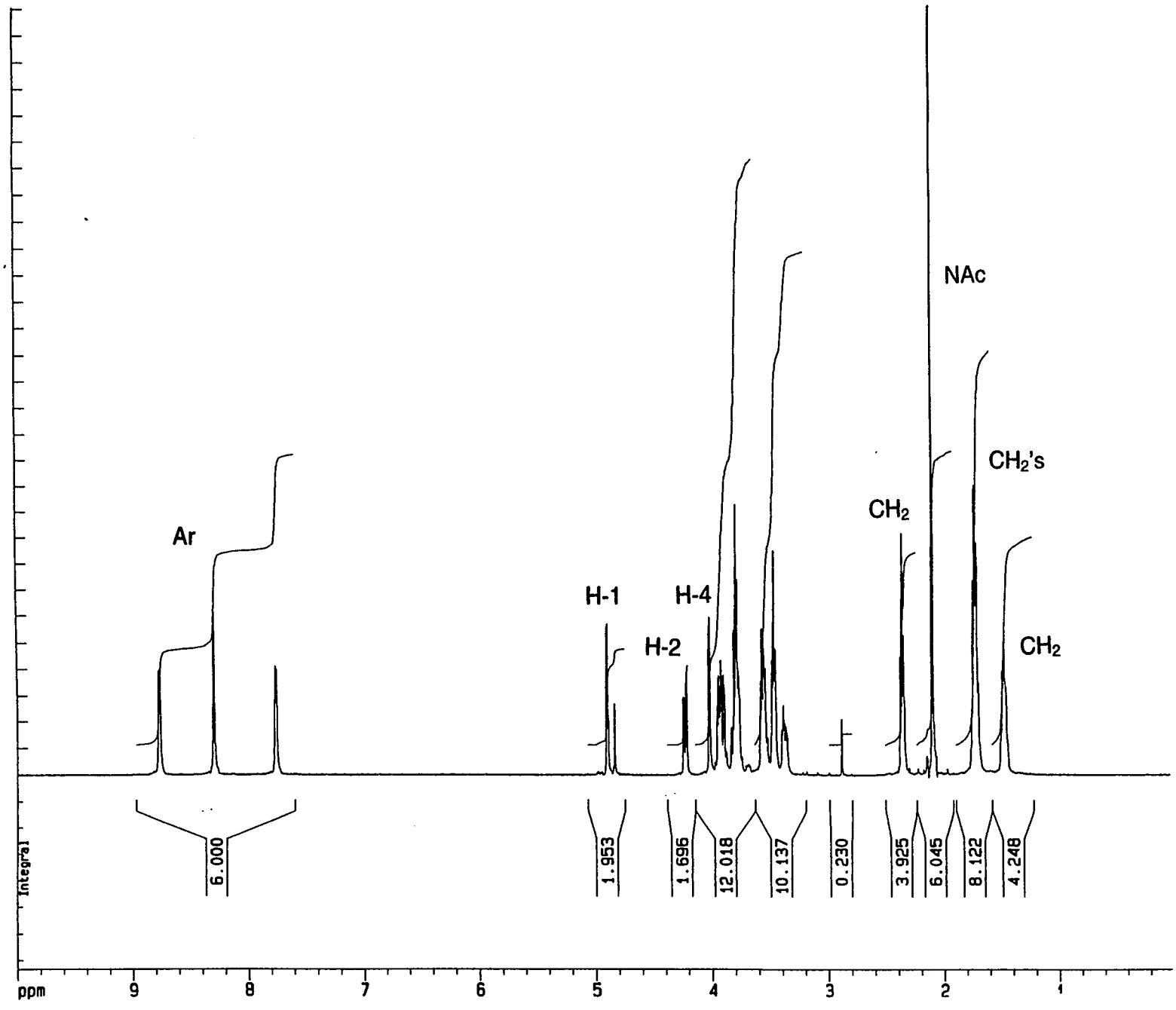
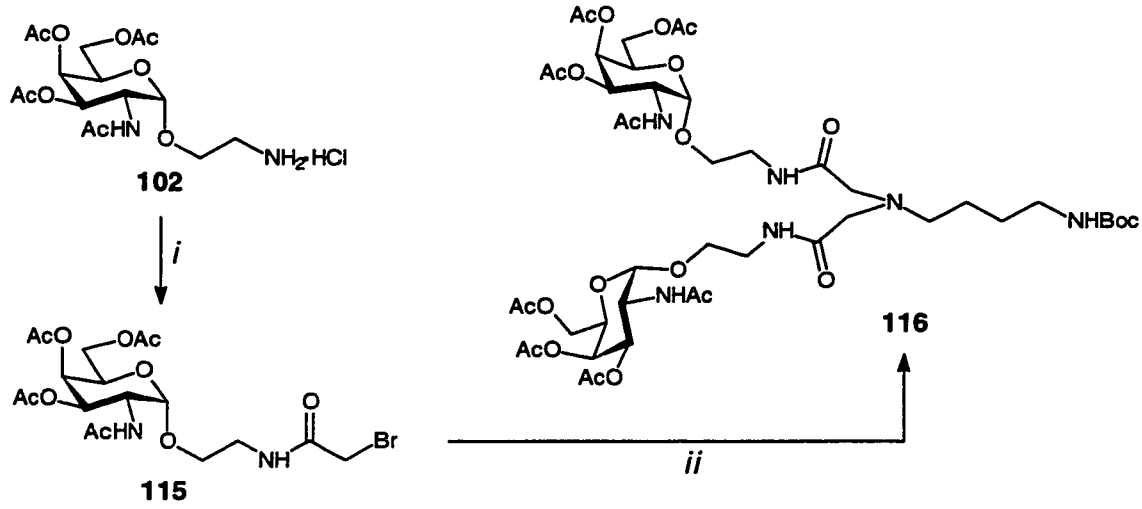


Figure 4.2.7. ^{1}H-NMR (D_2O, 500 MHz) spectrum of long spacer armed bipyridyl GalNAc ligand **114**.

Bipyridine dendrons with higher valency were prepared employing a *N,N*-dialkylation strategy (Schemes 4.2.6 and 4.2.7). Both short- and long-spacer-armed bromides (**115** and **117**, respectively) were derived from the corresponding GalNAc glycosylated amines (**102** and **112**, respectively) by treatment with bromoacetyl chloride (1.2 eq) in the presence of DIPEA (2.5 eq) in 85-92% yield. A minimum of two equimolar amount of GalNAc bromoacetylated compounds **115** and **117** were mixed with *N*-Boc-1,4-diaminobutane (1 eq) and DIPEA (6 eq), and the mixture was heated at 60 °C in CH_3CN for 48 hours. The progress of the reaction was monitored by TLC. The mono-substituted product was formed first and then the di-substituted one started to appear on the TLC plate with a higher R_f value than that of the mono-substituted product. These dialkylated products were purified by silica gel column chromatography (72-73%) and the *N*-Boc group was removed with 20% TFA in CH_2Cl_2.



Scheme 4.2.6. Synthesis of the short-spacer-armed dimer **116** by a dialkylation strategy; *i*) $ClCOCH_2Br$ (1.2 eq), DIPEA (2.5 eq), CH_2Cl_2, 0 °C, 20 min, 92%; *ii*) $BocHN(CH_2)_4NH_2$ (0.5 eq), DIPEA (1.5 eq), CH_3CN, 60 °C, 48 h, 72%.

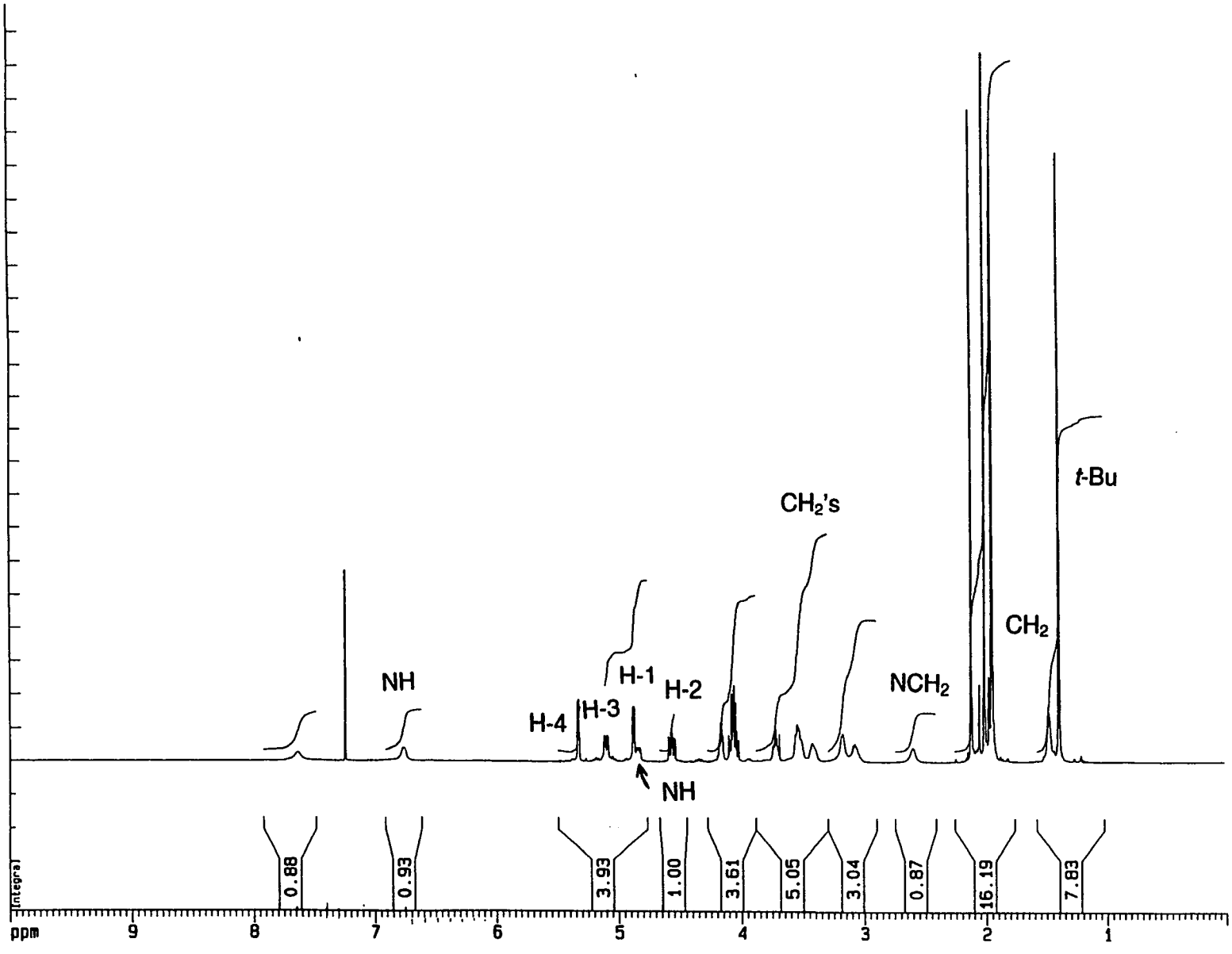


Figure 4.2.8. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of short spacer armed branched dimer **116**.

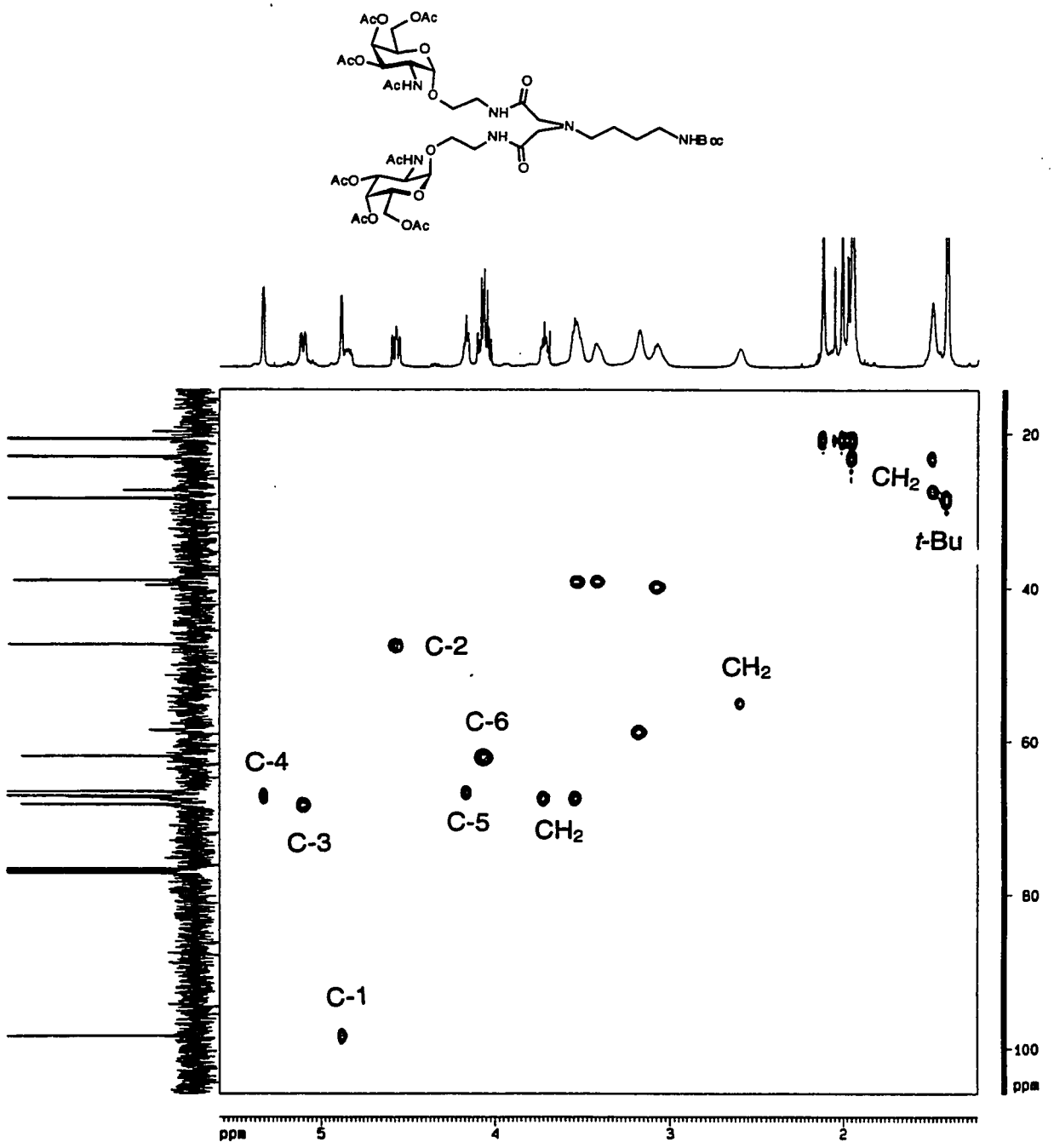
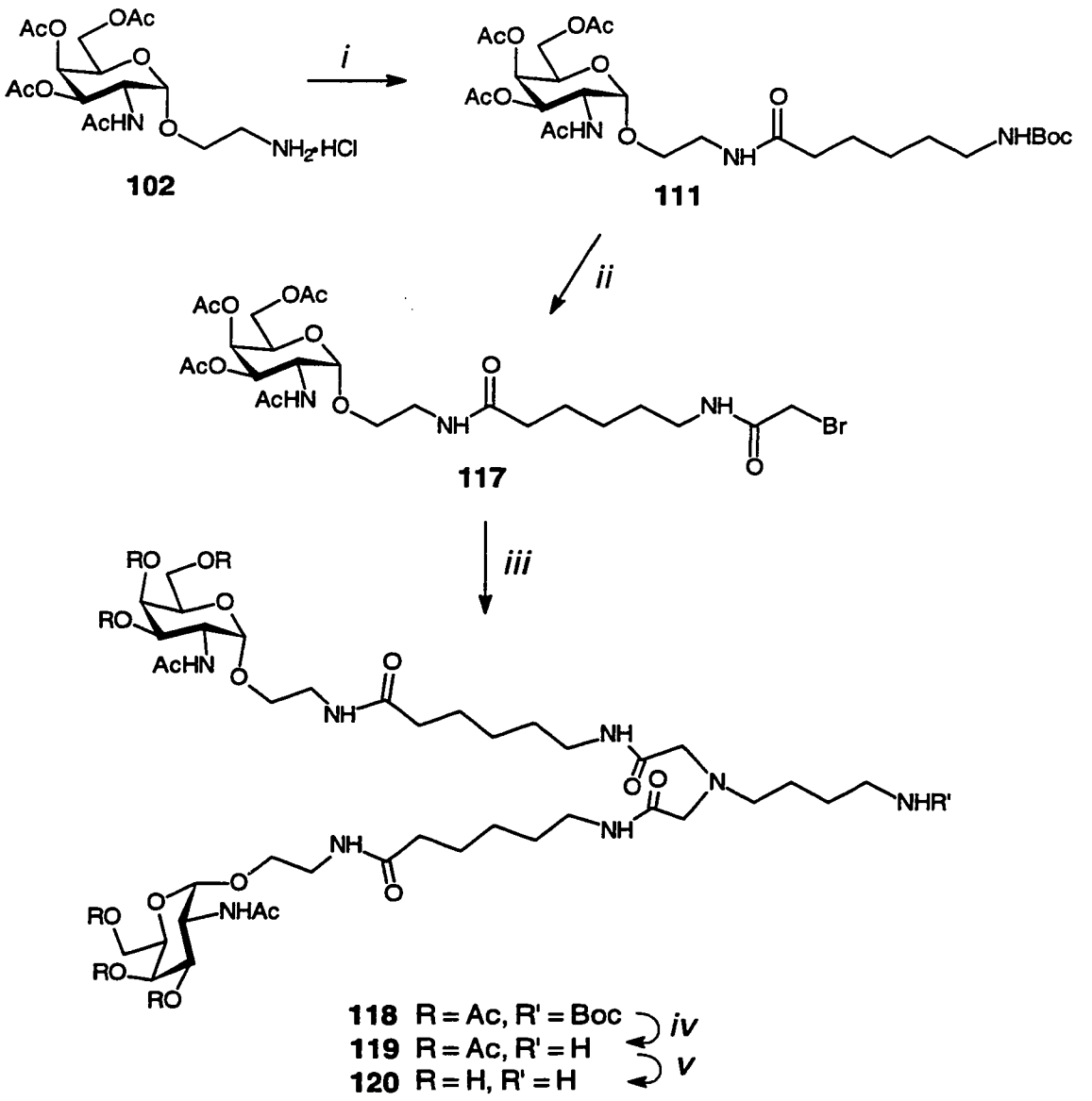


Figure 4.2.9. HMQC ($CDCl_3$, 500 MHz) spectrum of short spacer armed branched dimer **116**



Scheme 4.2.7. Synthesis of long-spacer-armed dimer **120** by the dialkylation strategy; *i*) $HO_2C(CH_2)_5NHBoc$ (1.5 eq), TBTU (1.5 eq), DIPEA (2.8 eq), CH_2Cl_2, 1 h, 76%; *ii*) (1) 20% TFA, CH_2Cl_2, 23 °C, 2 h, (2) $ClCOCH_2Br$ (1.2 eq), DIPEA (2.5 eq), CH_2Cl_2, 0 °C, 20 min, 85%; *iii*) $H_2N(CH_2)_4NHBoc$ (0.49 eq), DIPEA (3 eq), CH_3CN, 60 °C, 48 h, 73%; *iv*) 20% TFA, CH_2Cl_2, 23 °C, 2 h; *v*) 1M NaOMe, MeOH, pH 9, 23 °C, 3 h, quant.

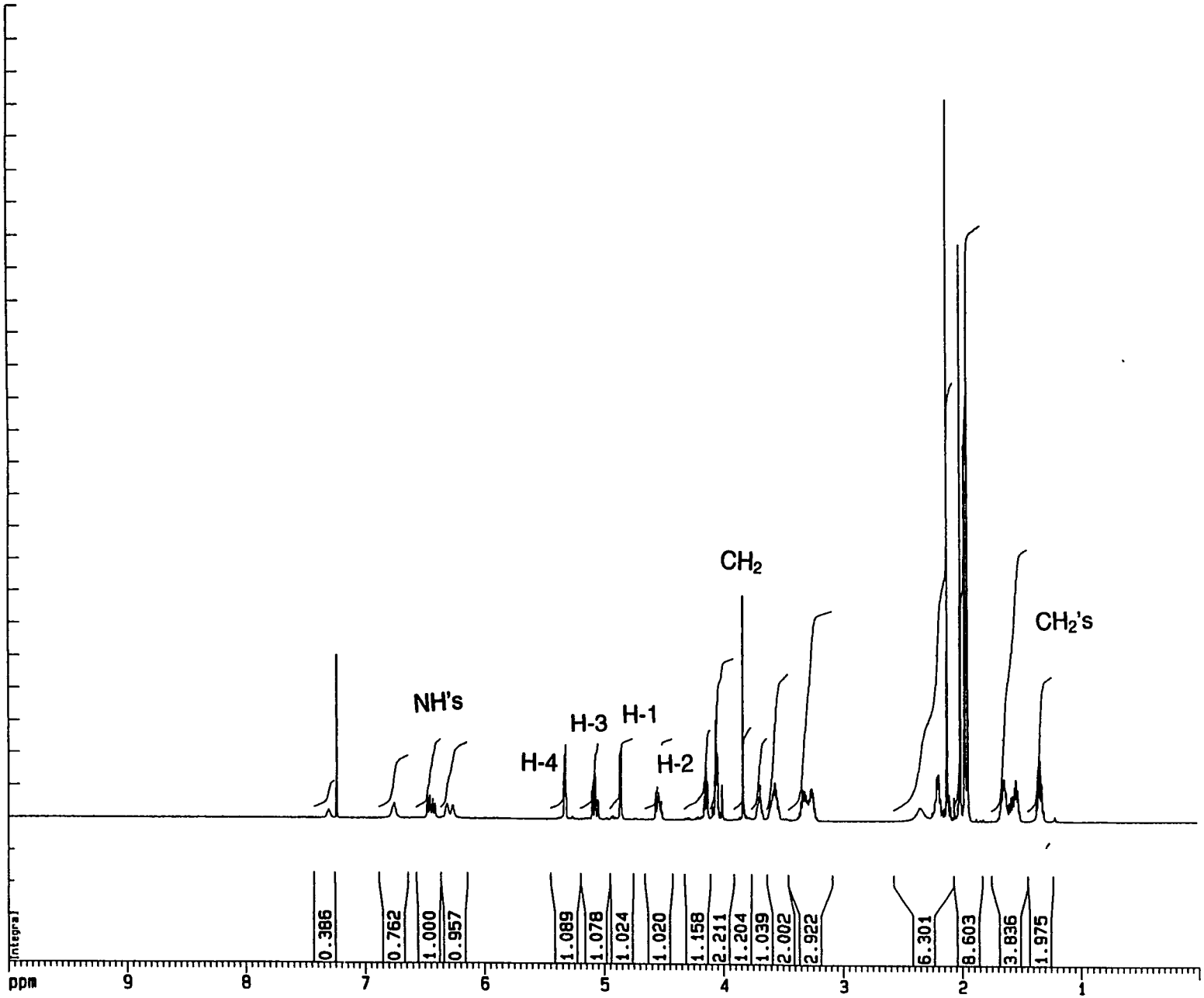


Figure 4.2.10. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of compound **117**

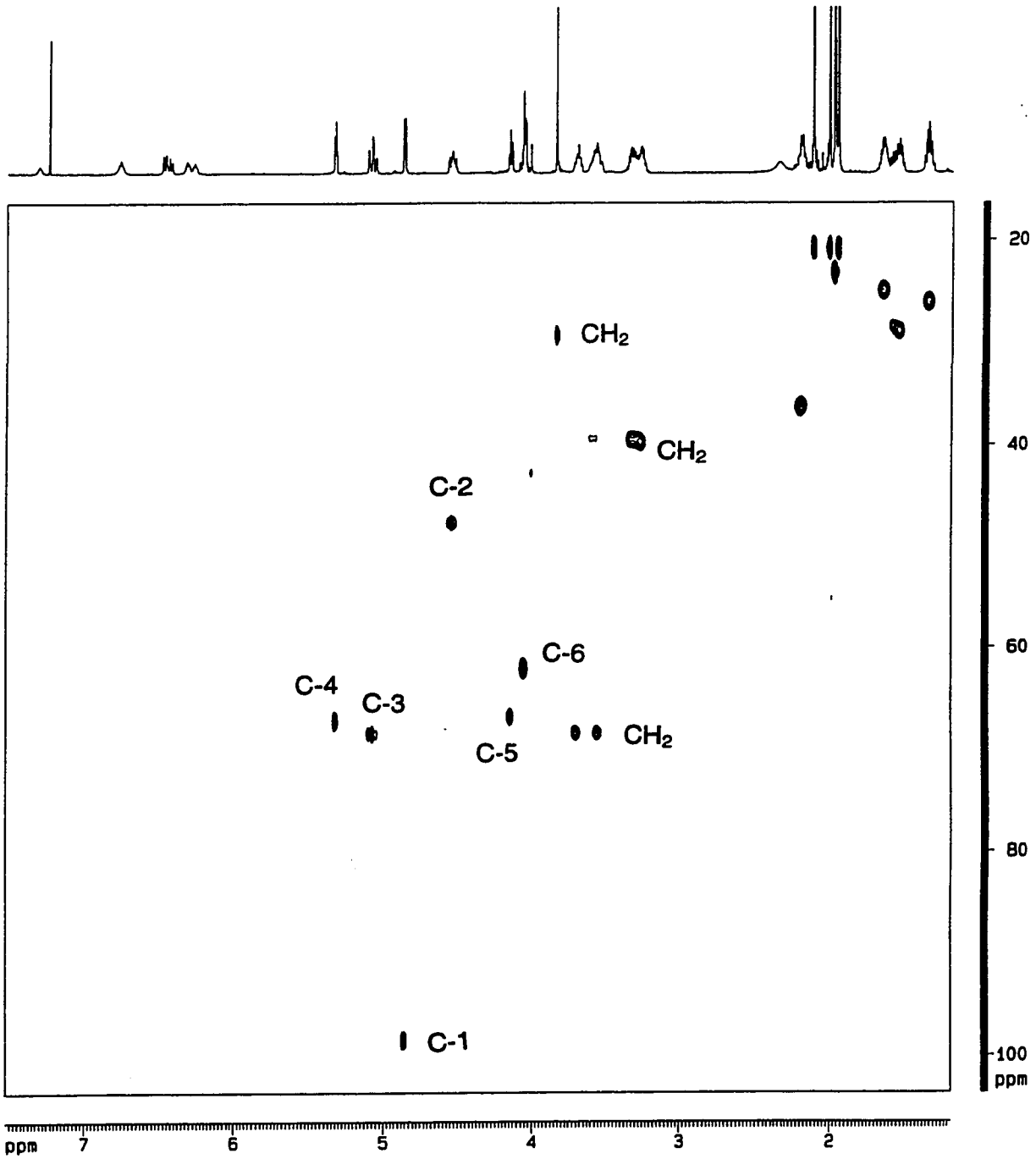


Figure 4.2.11. HMQC ($CDCl_3$, 500 MHz) spectrum of compound **117**

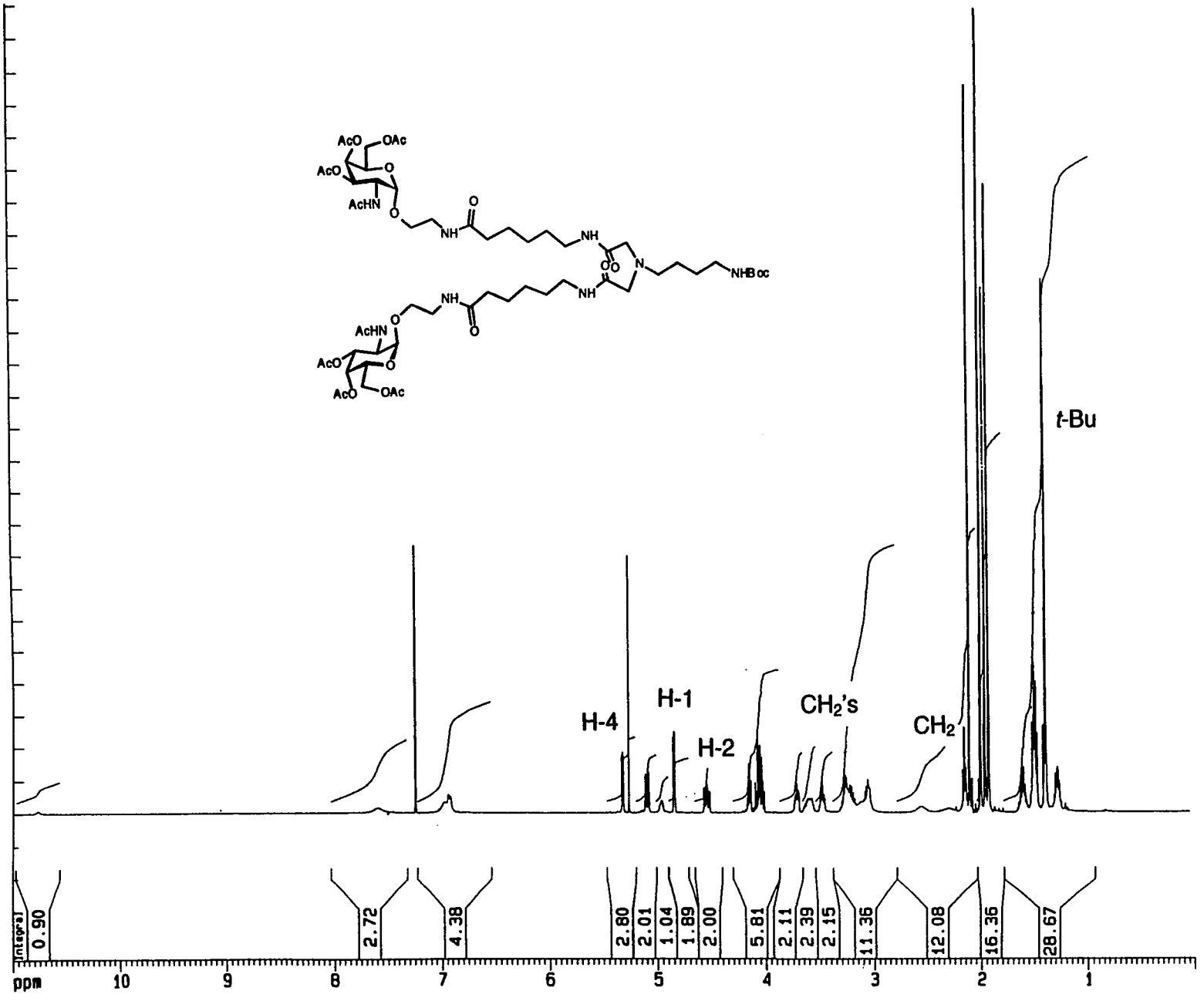


Figure 4.2.12. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of long spacer armed branched dimer **118**.

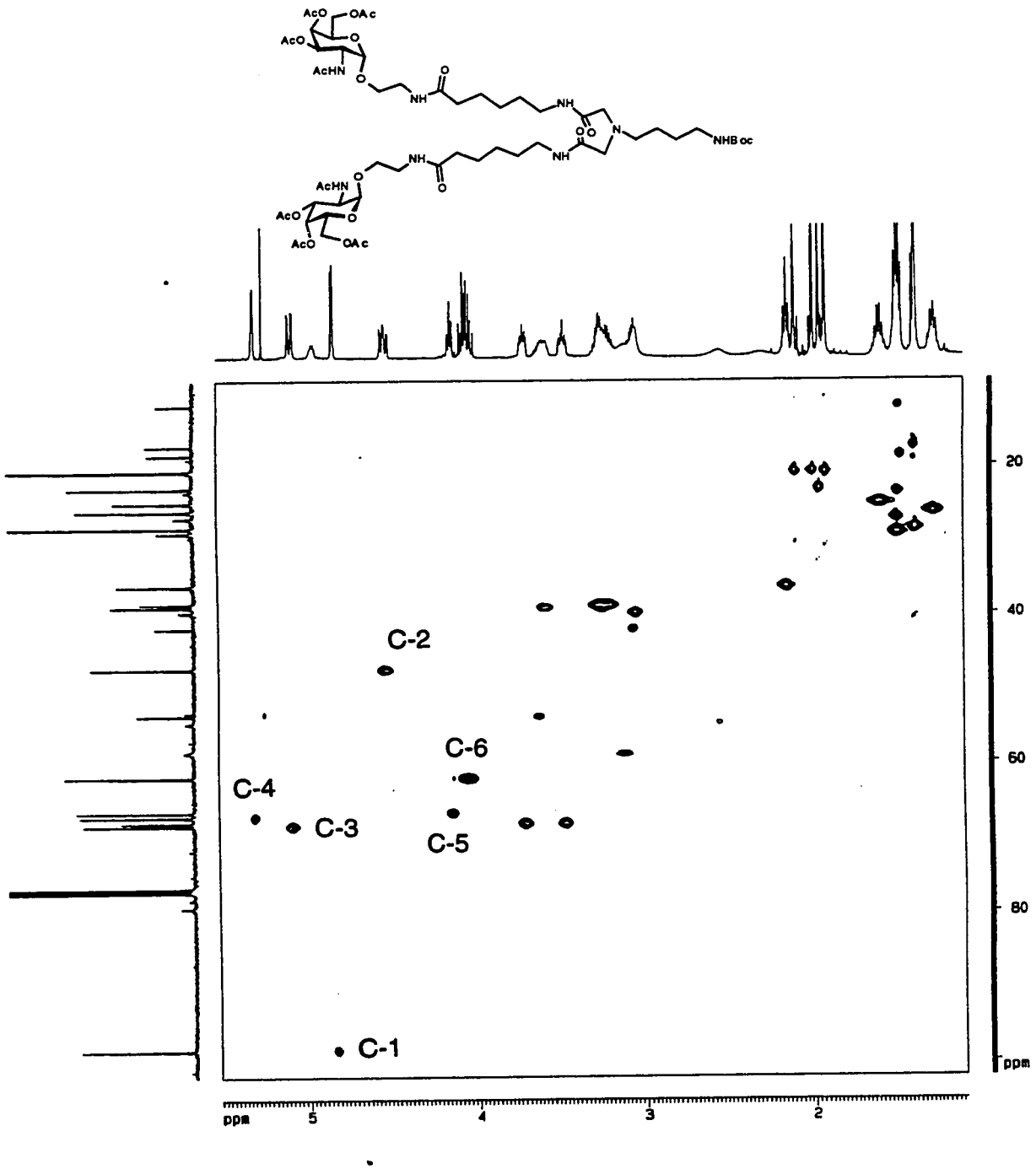
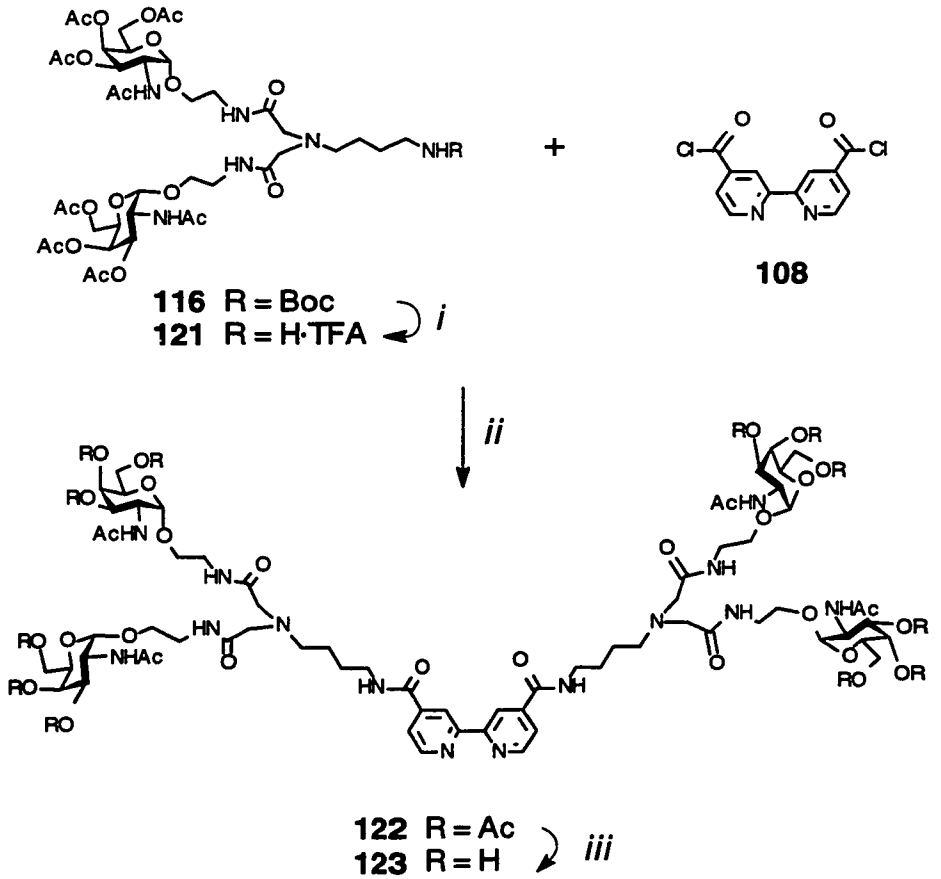


Figure 4.2.13. HMQC ($CDCl_3$, 500 MHz) spectrum of long spacer armed branched dimer **118**

Coupling the resulting dimers **121** and **119** to the bipyridine moiety afforded tetravalent bipyridine ligands **122** and **124**, respectively, which incorporated short and long spacer arms between the bipyridine core and the pendent GalNAc residues (Scheme 4.2.8 and Scheme 4.2.9, respectively). In these cases, de-*O*-acetylated GalNAc ligands were soluble in MeOH. Therefore, the crude products were purified by size exclusion column chromatography (LH20, MeOH, 89-94%).



Scheme 4.2.8. Synthesis of short-spacer-armed bipyridyl tetramer **123**; *i*) 20% TFA, CH_2Cl_2, 23 °C, 3 h; *ii*) Et_3N (5 eq), CH_2Cl_2, 0 °C, 3 h; *iii*) 1M NaOMe, MeOH, pH 9, 16 h, 94%.

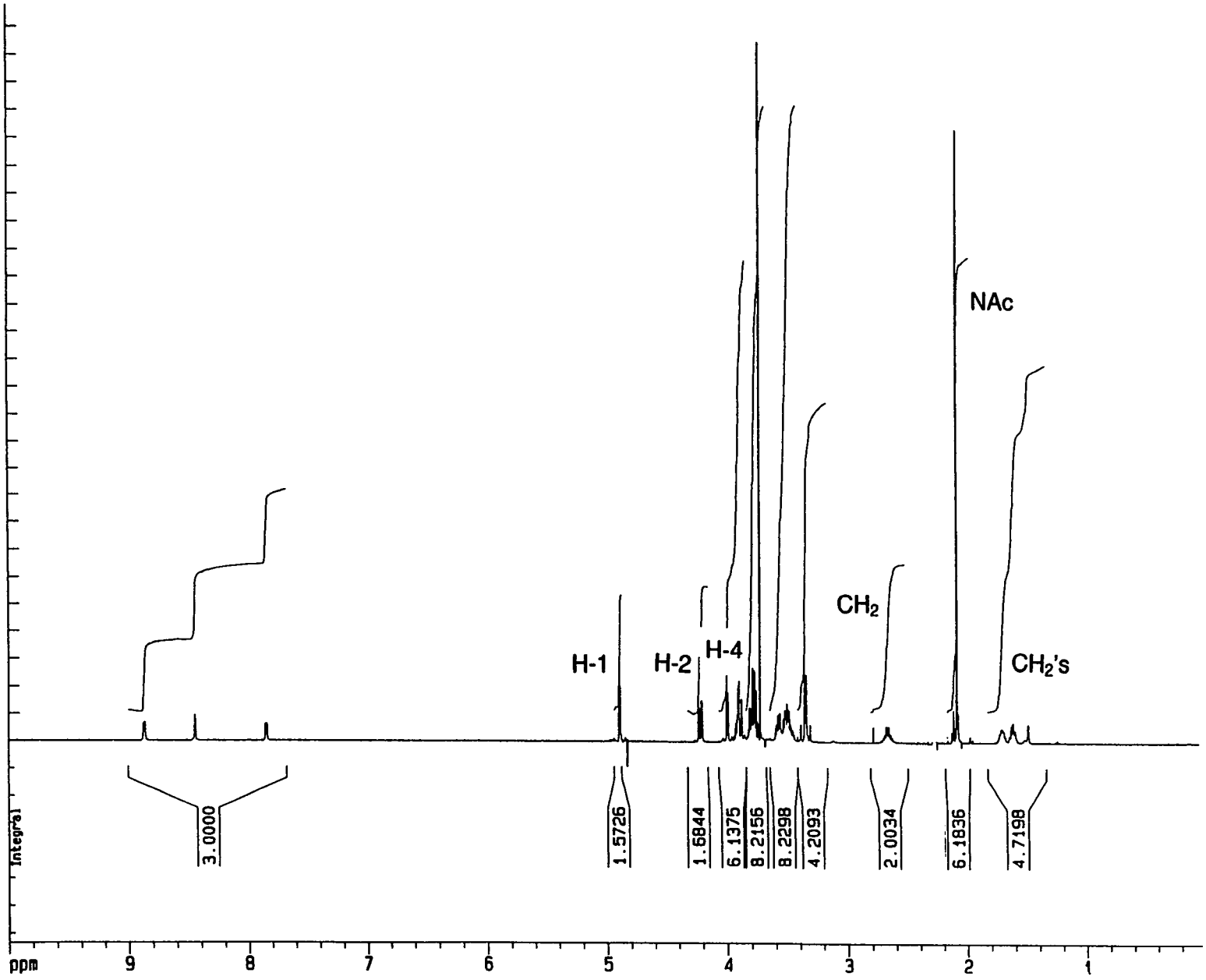


Figure 4.2.14. ^{1}H-NMR (D_2O, 500 MHz) spectrum of short spacer armed bipyridyl tetramer **123**

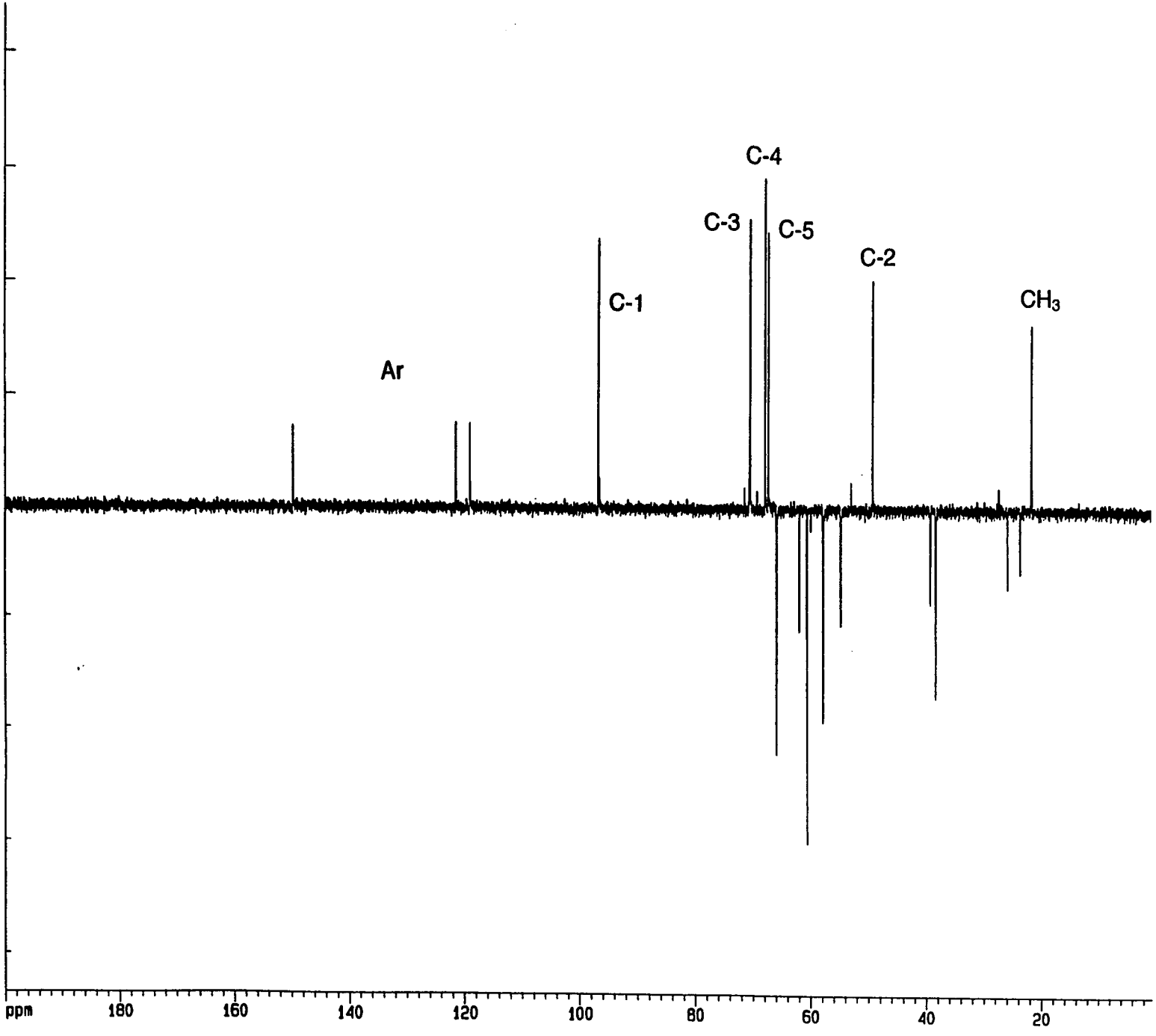
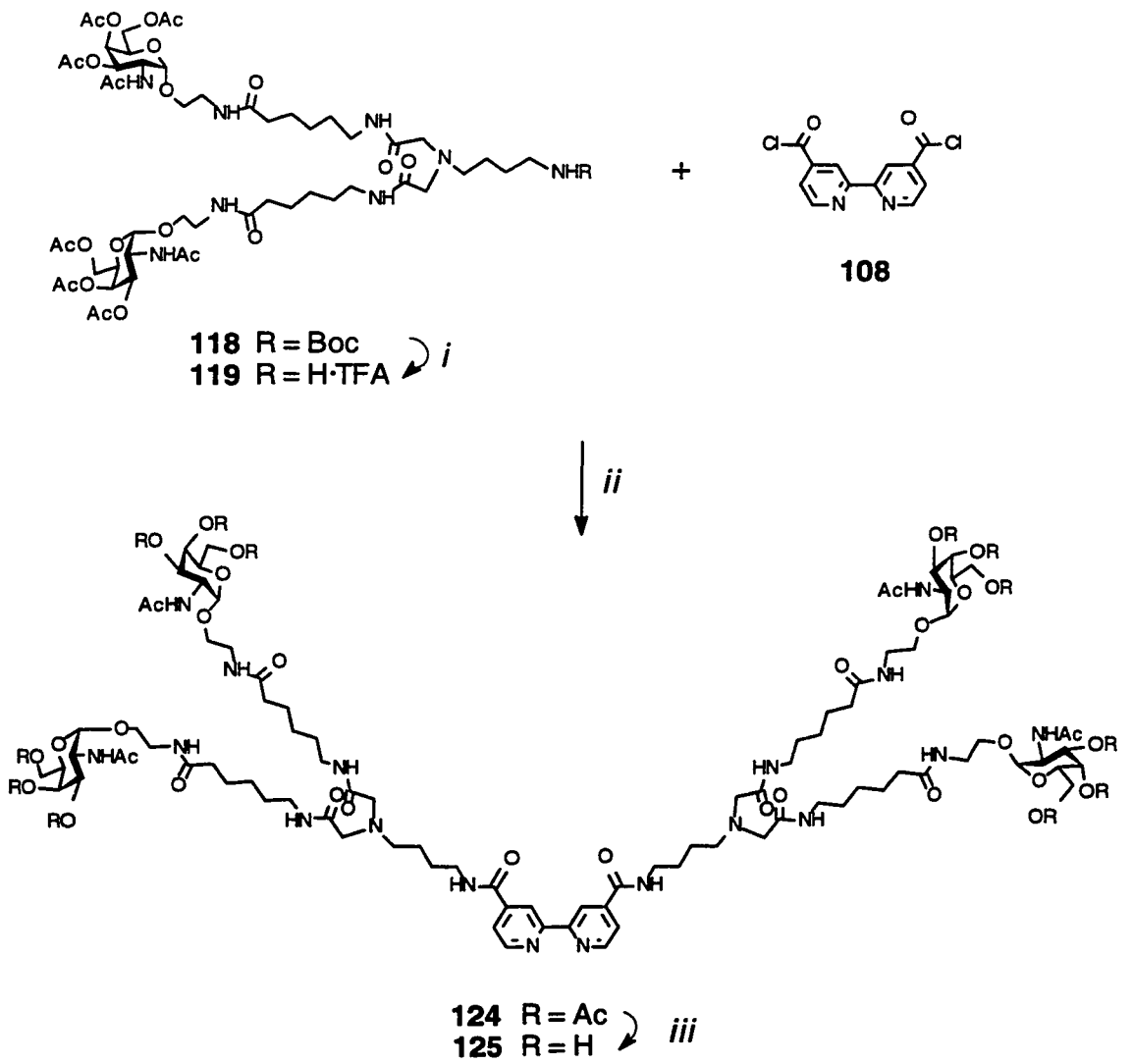


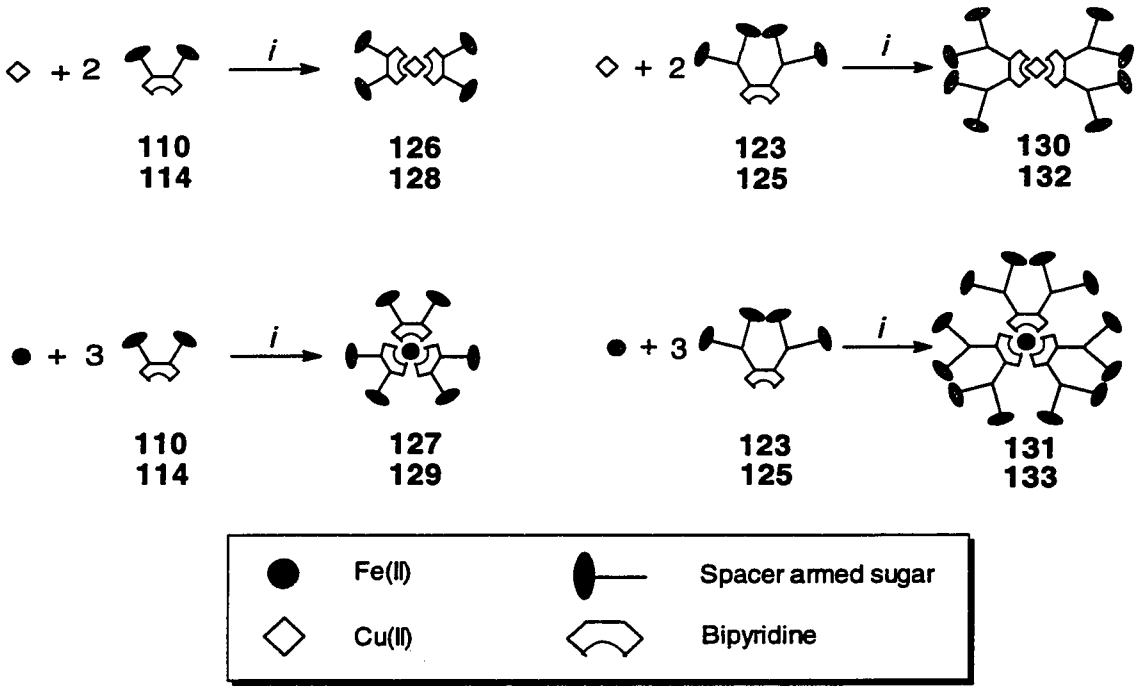
Figure 4.2.15. DEPT (D_2O, 125 MHz) spectrum of short spacer armed bipyridyl tetramer **123**



Scheme 4.2.9. Synthesis of long-spacer-armed bipyridyl tetramer **125**; *i*) 20% TFA, CH_2Cl_2, 23 °C, 2 h; *ii*) Et_3N (5 eq), CH_2Cl_2, 0 °C, 3 h; *iii*) 1M NaOMe, MeOH, pH 9, 23 °C, 16 h, 89%.

Assembling of dendrons around metal ions

The prepared dendrons, short-spacer-armed divalent bipyridine GalNAc ligands **110**, long-spacer-armed divalent bipyridine GalNAc ligands **114**, short-spacer-armed tetravalent bipyridine GalNAc ligands **123**, and long-spacer-armed tetravalent bipyridine GalNAc ligands **125**, were nucleated around iron(II) and copper(II) ions (Scheme 4.2.10).



Scheme 4.2.10. i) Deionized H_2O, 23 °C, 48h.

Three equivalents of bipyridine ligands were mixed with one equivalent of $FeSO_4 \bullet 7H_2O$ and the mixture was stirred at room temperature for 48 hours. As soon as iron(II) was added to the bipyridine solution in deionized water, the reaction color changed to dark pinkish red. This change indicated the formation of

an iron complex. After 48 hours, the red solution was lyophilized to afford a pinkish red powder.

Self-assembling of dendrons around copper(II) was accomplished by mixing two equivalents of bipyridine ligands and one equivalent of $CuSO_4 \bullet 5H_2O$. The assembled complex in aqueous solution was light bluish purple. After been stirred for 48 hours at room temperature, the solution was lyophilized to yield a light bluish purple colored powder. The prepared self-assembled GalNAc ligands are shown in Scheme 4.2.11 to Scheme 4.2.17.

Table 4.2.1. FAB mass spectrometry of bipyridyl GalNAc building blocks.

Ligands	Formular	M.W. (calculated)[a]	M.W.+1 (found)[a]
110	$C_{32}H_{44}N_6O_{14}$	736.30(737.2994)	737.38 (737.2830)
114	$C_{44}H_{66}N_8O_{16}$	962.47 (963.4675)	963.47 (963.4680)
123	$C_{68}H_{108}N_{14}O_{30}$	1600.74 (1601.7434)	1601.78 (1601.6491)
125	$C_{92}H_{152}N_{18}O_{34}$	2053.07	2054.67

[a] The values in parenthesis are from high resolution mass spectrometry.

Table 4.2.2. MALDI-TOF[a] mass spectrometry of self-assembled GalNAc ligands.

Ligands	Formular	M.W. (calculated)	M.W. (found)
126	$C_{64}H_{88}N_{12}O_{28}Cu$	1535.51	1536.06
127	$C_{96}H_{132}N_{18}O_{42}Fe$	2264.81	2265.10
128	$C_{88}H_{132}N_{16}O_{32}Cu$	1987.85	1989.39
129	$C_{132}H_{198}N_{24}O_{48}Fe$	2943.31	2943.44
130	$C_{136}H_{216}N_{28}O_{60}Cu$	3264.40	3267.76
131	$C_{204}H_{324}N_{42}O_{90}Fe$	4858.14	4859.72
132	$C_{184}H_{304}N_{36}O_{68}Cu$	4169.07	4170.19
133	$C_{276}H_{456}N_{54}O_{102}Fe$	6215.15	6217.87

[a] The matrixes used for these experiments were THAP (2,4,6-trihydroxyacetophenone) and 2,5-DHB (2,5-dihydroxybenzoic acid).

All bipyridyl dimers and tetramers were characterized by FAB-MS (Table 4.2.1). Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) was employed to characterize the tri-dentate and di-dentate metal coordinated complexes (Fe^{II} and Cu^{II}, respectively), using 2,5-dihydroxybenzoic acid (2,5-DHB) or 2,4,6-trihydroxyacetophenone (THAP) as the matrixes (Table 4.2.2).

Table 4.2.3. UV-vis (water) data of bipyridyl GalNAc building blocks and their self-assembled ligands.

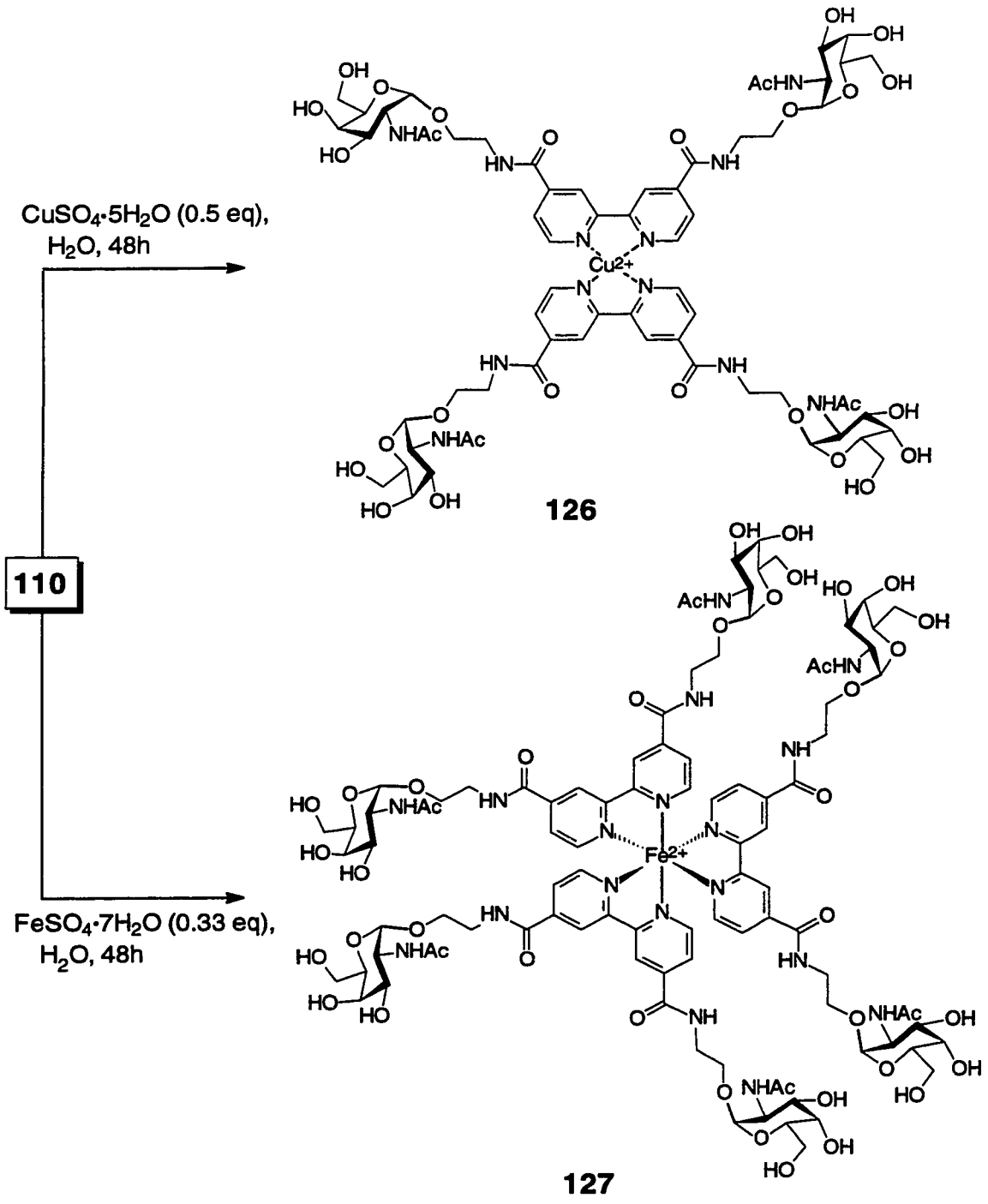
Ligands	M.W.	C (10^{-6} M)	λ (nm) (ε, M^{-1} cm^{-1})
short 2-mer **110**	736.32	27.2	236 (25331), 294 (15206)
(short 2-mer)$_2$Cu•SO_4 **126**	1632.19	12.3	312 (13306)
(short 2-mer)$_3$ Fe•SO_4 **127**	2360.87	8.47	254 (34943), 316 (34589), 384 (6422), 544 (8110)
long 2-mer **114**	962.49	20.8	240 (12849), 293 (5775)
(long 2-mer)$_2$Cu•SO_4 **128**	2085.70	9.59	256 (10950), 314 (3650)
(long 2-mer)$_3$Fe•SO_4 **129**	3039.37	6.58	254 (30699), 314 (29787), 384 (5775), 544 (7143)
short 4-mer **123**	1600.77	12.5	240 (9768), 293 (5604)
(short 4-mer)$_2$Cu•SO_4 **130**	3360.46	5.95	314 (12433)
(short 4-mer)$_3$Fe•SO_4 **131**	4954.20	4.04	314 (33688), 364 (6688), 542 (7184)
long 4-mer **125**	2053.10	9.74	238 (15399), 296 (8213)
(long 4-mer)$_2$Cu•SO_4 **132**	4265.13	4.69	314 (14075)
(long 4-mer)$_3$Fe•SO_4 **133**	6311.21	3.17	314 (35342), 362 (7258), 546 (8520), 662 (947)

The absorption spectra of the iron(II) complexes, **127**, **129**, **131**, and **133** showed similar patterns (λ_{max} (ε, $M^{-1}cm^{-1}$)=254 (sh), 314-316 (3.0-3.5 × 10^4), 362-384 (5.8-7.3 × 10^3), 542-546 (7.2-8.5 × 10^3)). The UV-vis spectra of the bipyridyl

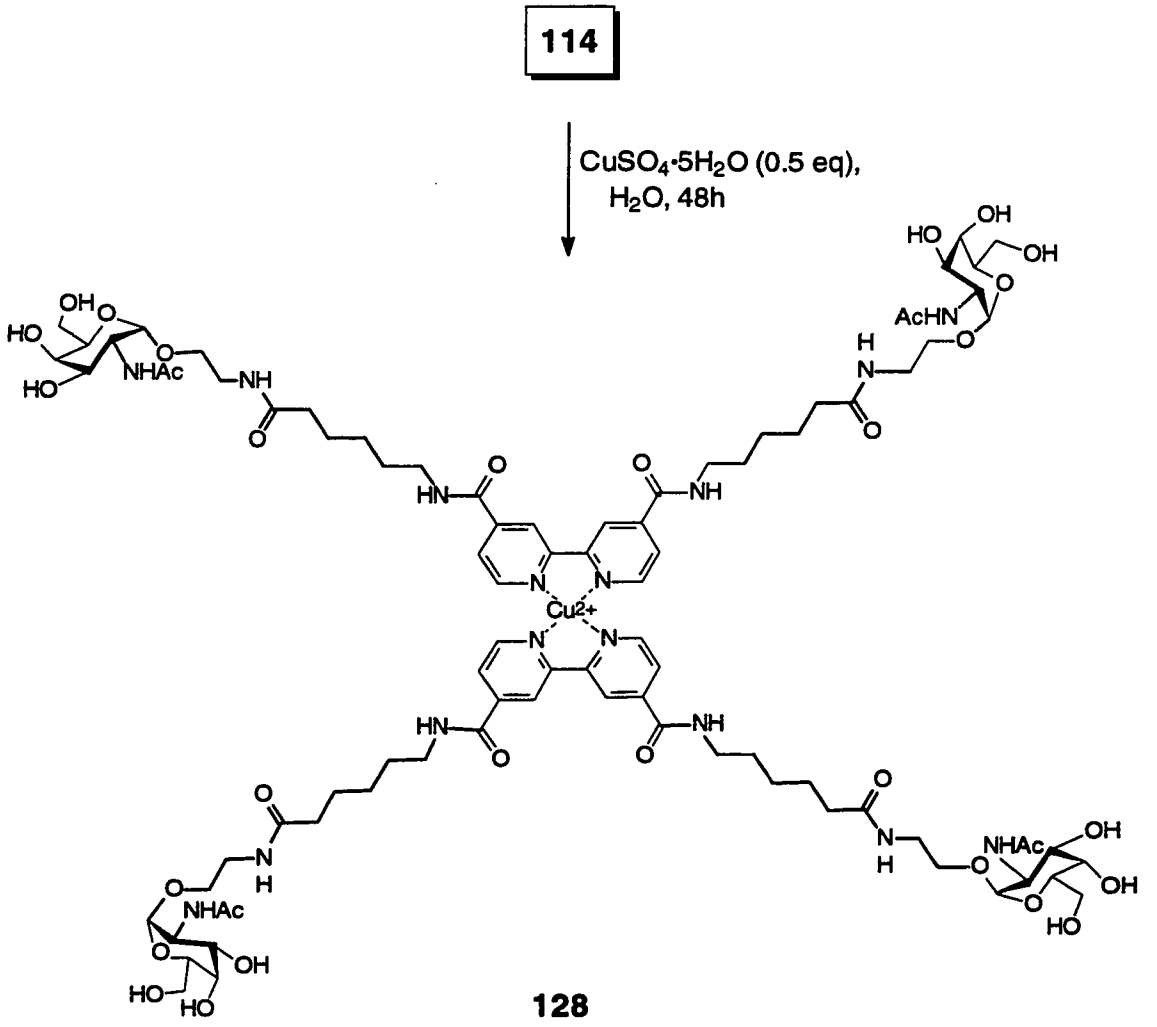
ligands without the metal ion **110**, **114**, **123**, and **125** only showed UV absorption due to the aromatic bipyridyl moiety (λ_{max} (ε, $M^{-1}cm^{-1}$) = 236-240 (1.3-2.5 $\times$ 10^4), 293-296 (5.6-15.0 $\times$ 10^3)). The red shift effect was also observed with copper(II) complexes, **126**, **128**, **130**, and **132** (λ_{max} (ε, $M^{-1}cm^{-1}$) = 312-314 (1.2-1.4 $\times$ 10^4)).

Fe^{II} and Cu^{II} complexes with GalNAc bipyridyl ligands appeared to adopt symmetric conformations with octahedral and square planar geometries, respectively. Their stereochemistries were proved based on the peaks shown in the size exclusion chromatography on a LH20 column in water. In addition, the 500 MHz ^{1}H-NMR spectra in D_2O displayed only three resonances in the pyridine region, confirming the symmetries in their structural arrangements. Since these Fe^{II} complexes are symmetrical, only two diastereomers could be formed. However, these isomers were not observable in the size exclusion column chromatography.

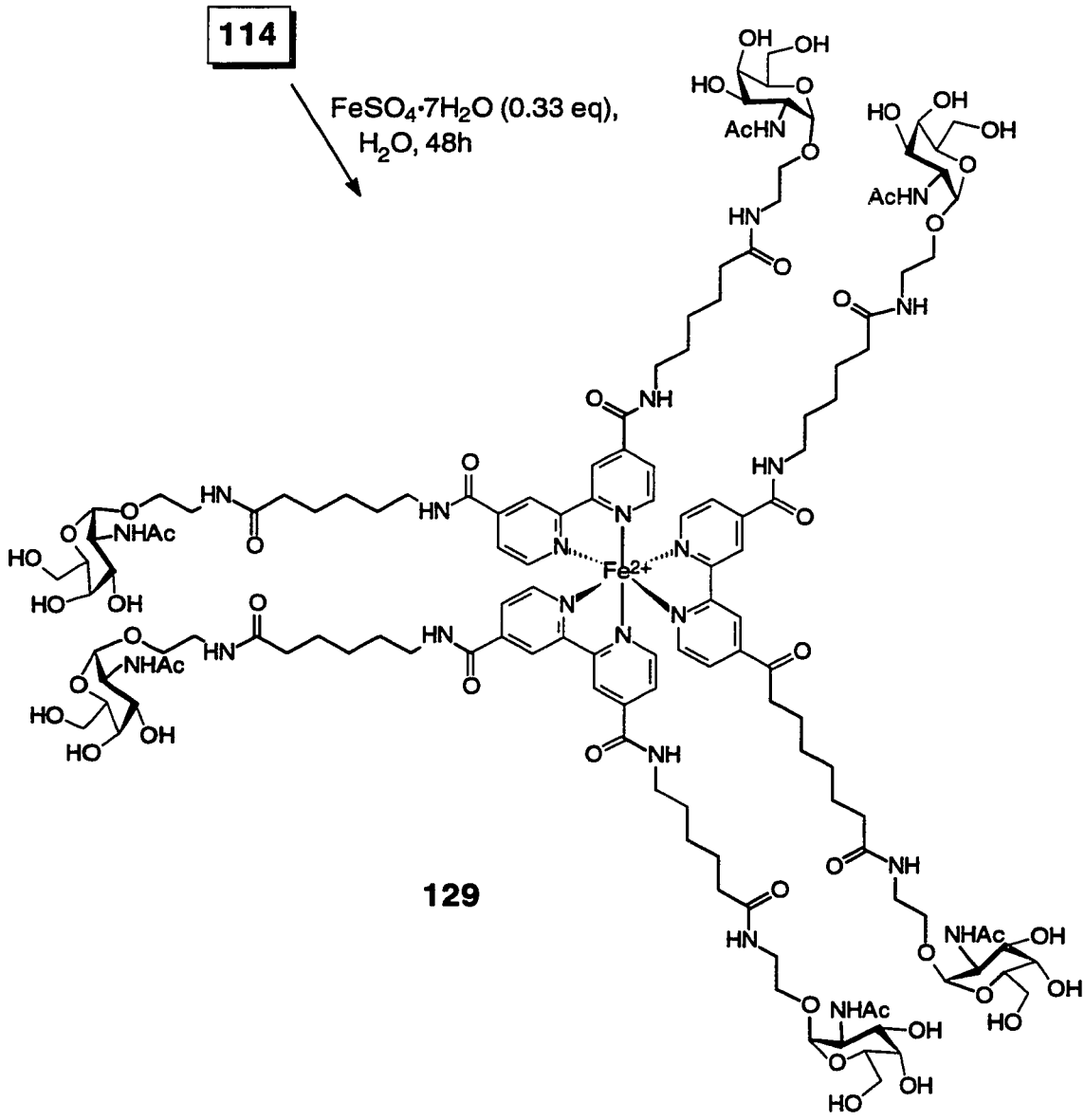
The stability of the iron(II) complexes was demonstrated with the formation of lectin-ligand complex. When the iron(II) complexes were mixed with *Vicia villosa* B_4, insoluble pink precipitates were observed with colorless supernatent. This experiment established that the carbohydrate-lectin interaction occurred as an aggregated tridentate Fe_{II}(bipy-GalNAc)$_3$ complex instead of dissociated bipyridine ligand.



Scheme 4.2.11. Syntheses of short-spacer-armed tetramer **126** and hexamer **127**.



Scheme 4.2.12. Synthesis of long-spacer-armed tetramer **128**.



Scheme 4.2.13. Synthesis of long-spacer-armed hexamer, **129**.

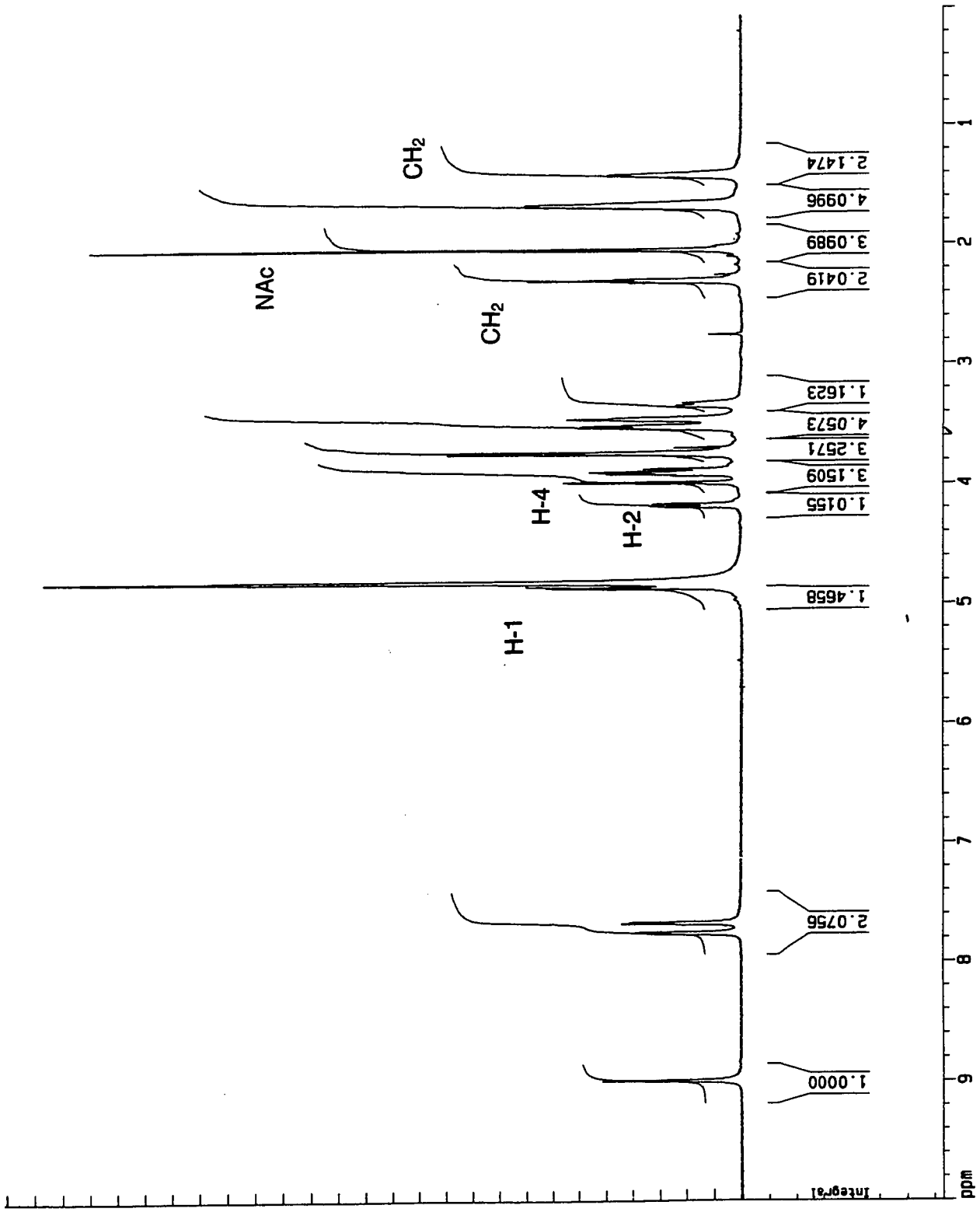
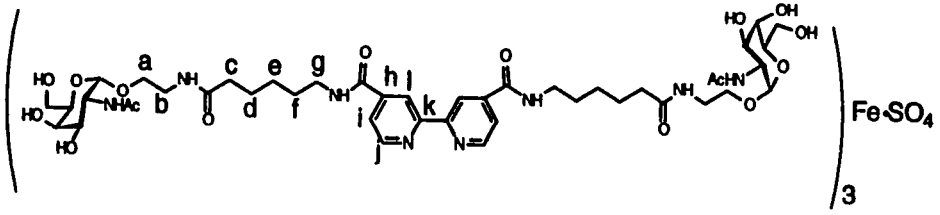


Figure 4.2.16. ^{1}H-NMR (D_2O, 500 MHz) spectrum of Fe^{II} (long dimer)$_3$•SO_4 **129.**



INDEX	FREQUENCY (PPM)	HEIGHT
1	174.397	66.0
2	171.843	29.1
3	163.420	55.0
4	156.822	58.7
5	152.441	30.3
6	141.397	48.7
7	122.172	35.4
8	119.354	31.4
9	119.274	15.8
10	94.663	82.2
11	68.477	96.2
12	65.900	85.6
13	65.300	94.1
14	63.857	66.0
15	58.609	75.8
16	47.205	95.0
17	37.580	58.7
18	36.362	66.6
19	33.141	83.0
20	25.412	14.7
21	25.335	80.2
22	23.095	93.3
23	22.568	94.3
24	19.457	74.8

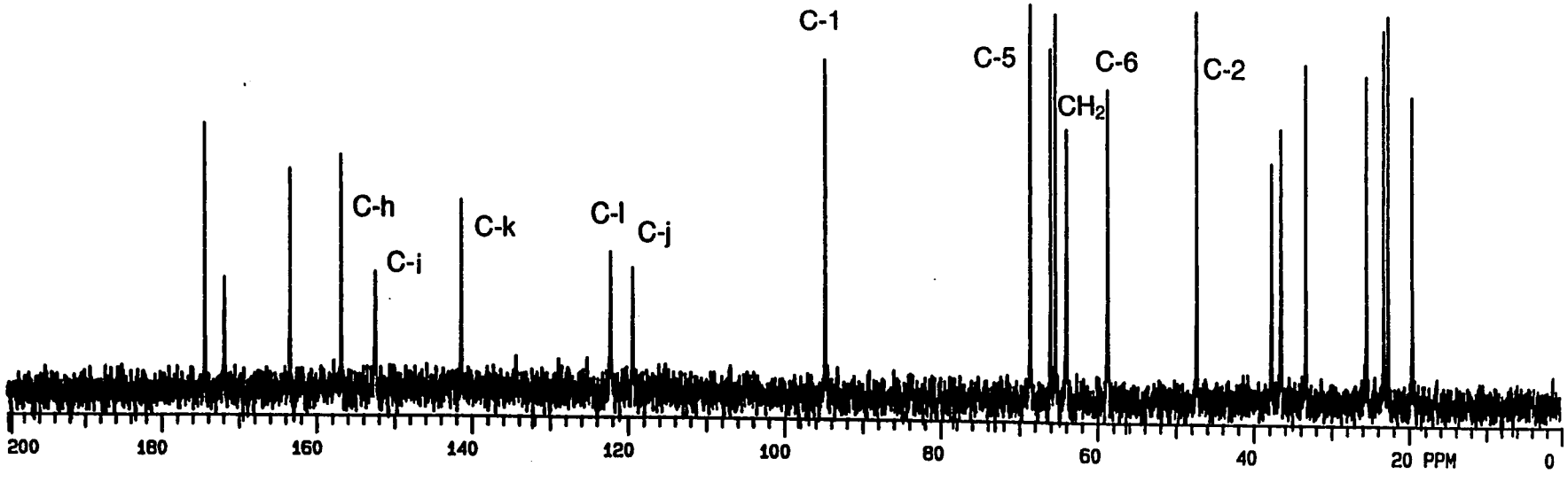
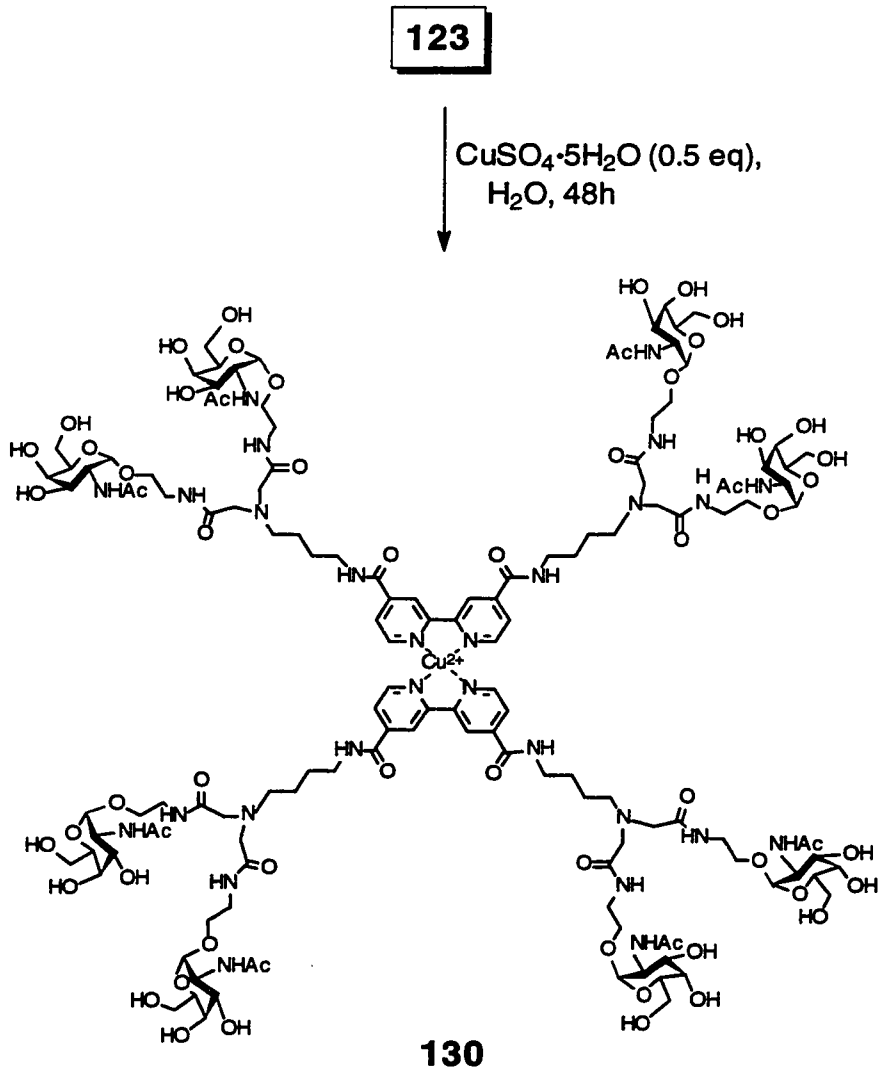


Figure 4.2.17. ^{13}C-NMR (D_2O, 75 MHz) spectrum of Fe^{II}(long dimer)$_3$•SO_4 **129.**



Scheme 4.2.14. Synthesis of short-spacer-armed octamer, **130**.

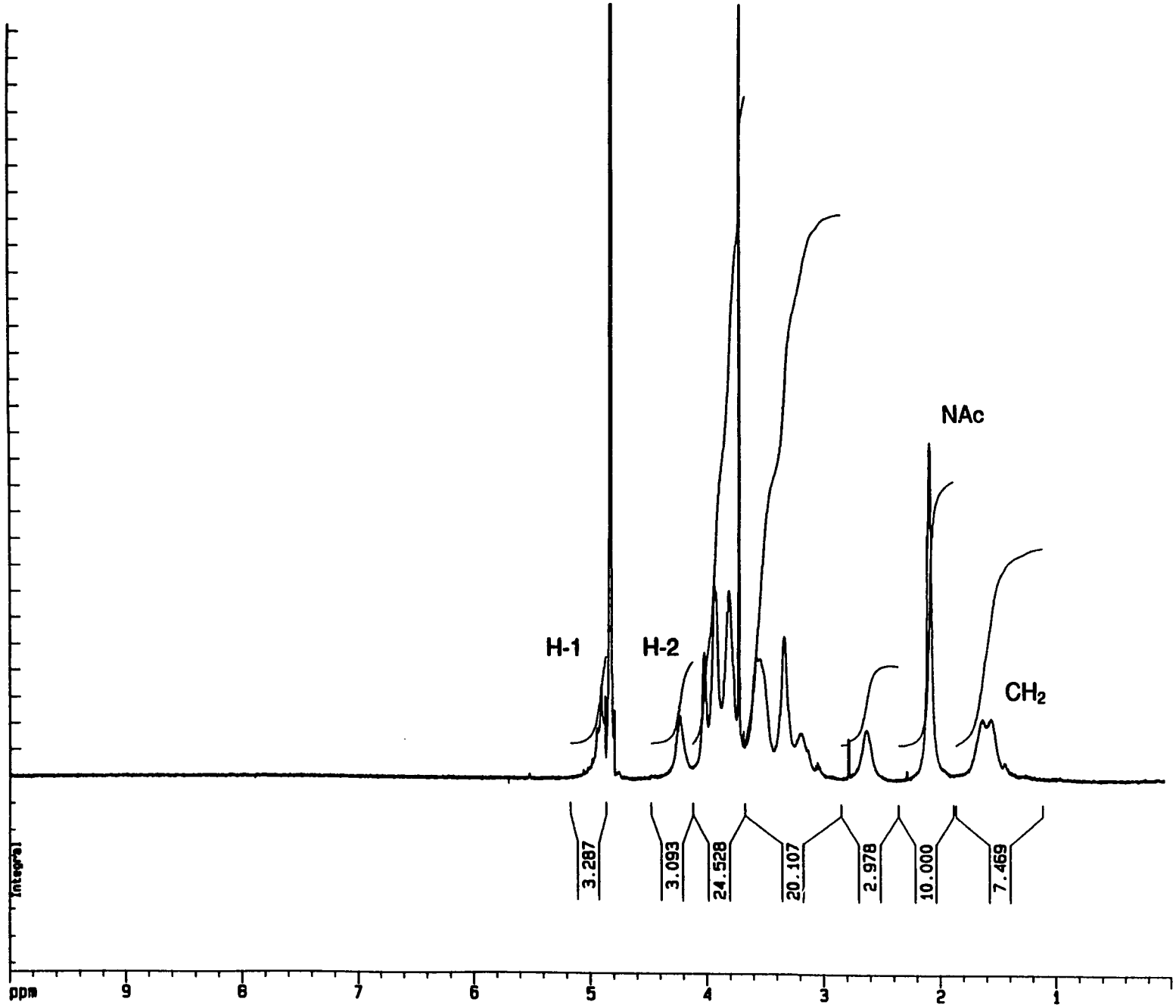


Figure 4.2.18. ^{1}H-NMR (D_2O, 500 MHz) spectrum of Cu^{II}(short tetramer)$_2$•SO_4 **130.**

INDEX	FREQUENCY (PPM)	HEIGHT
1	171.827	35.4
2	171.234	27.5
3	171.179	18.4
4	94.850	72.1
5	68.621	69.9
6	65.967	76.7
7	65.392	74.3
8	65.309	17.4
9	63.990	55.5
10	60.001	97.3
11	58.713	63.9
12	55.843	27.5
13	52.773	14.9
14	52.695	19.7
15	52.641	15.4
16	47.258	66.4
17	47.166	14.8
18	37.405	15.5
19	37.362	21.1
20	36.389	46.0
21	36.270	15.2
22	22.835	17.3
23	22.802	16.3
24	21.525	14.3
25	19.563	59.0

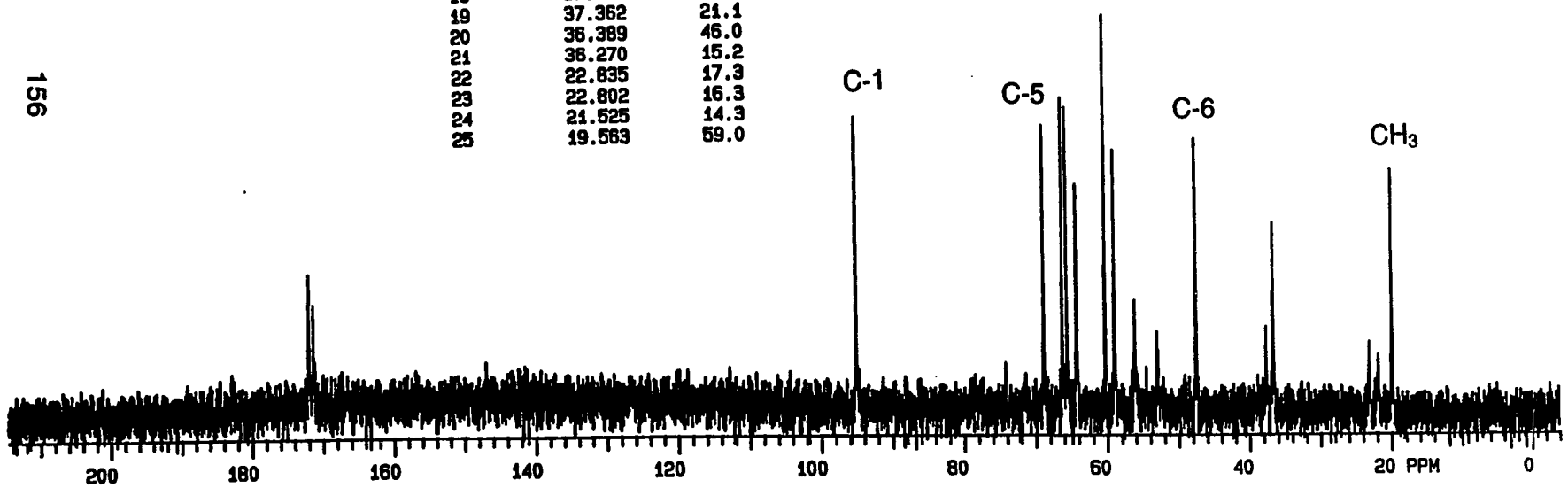
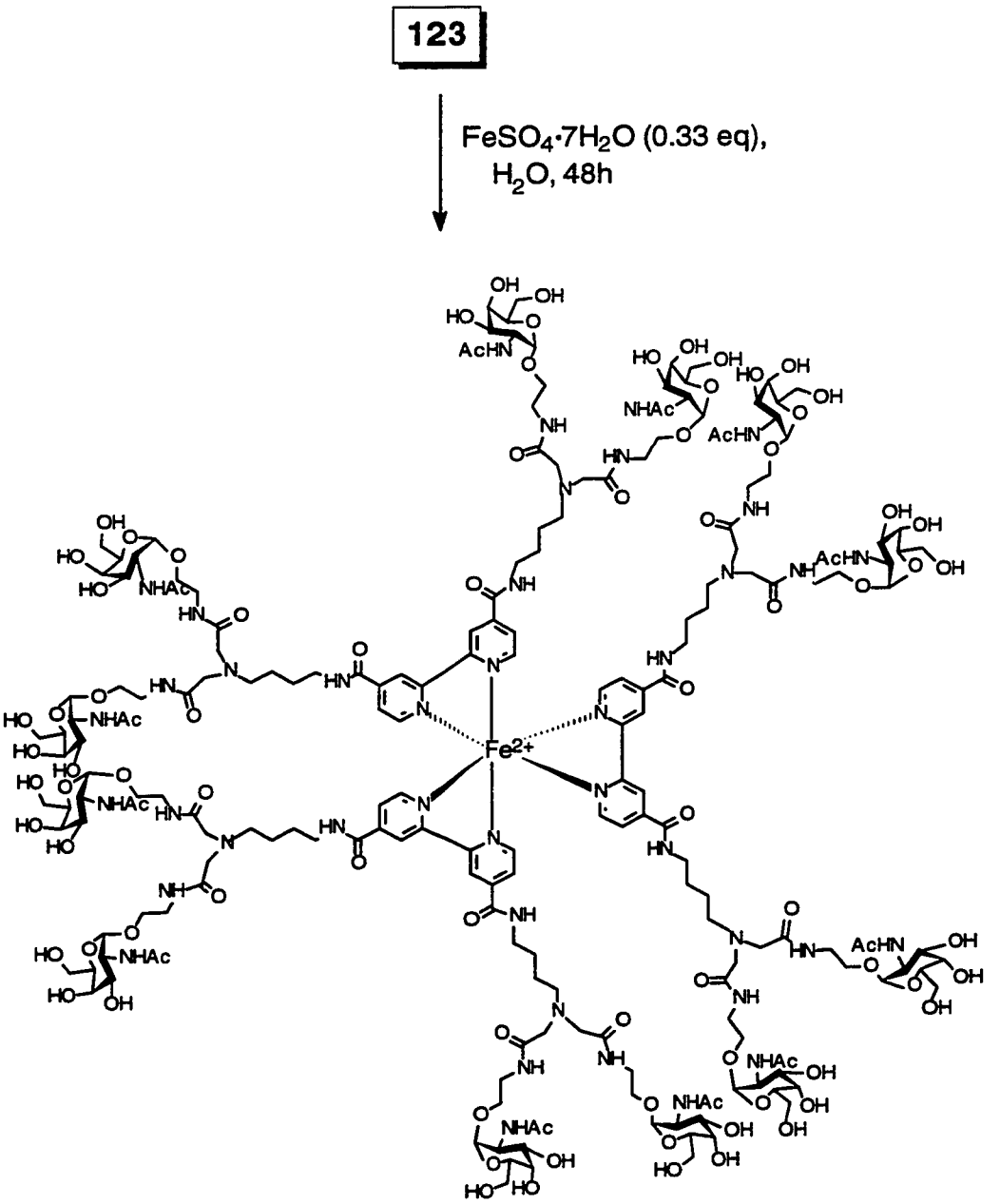
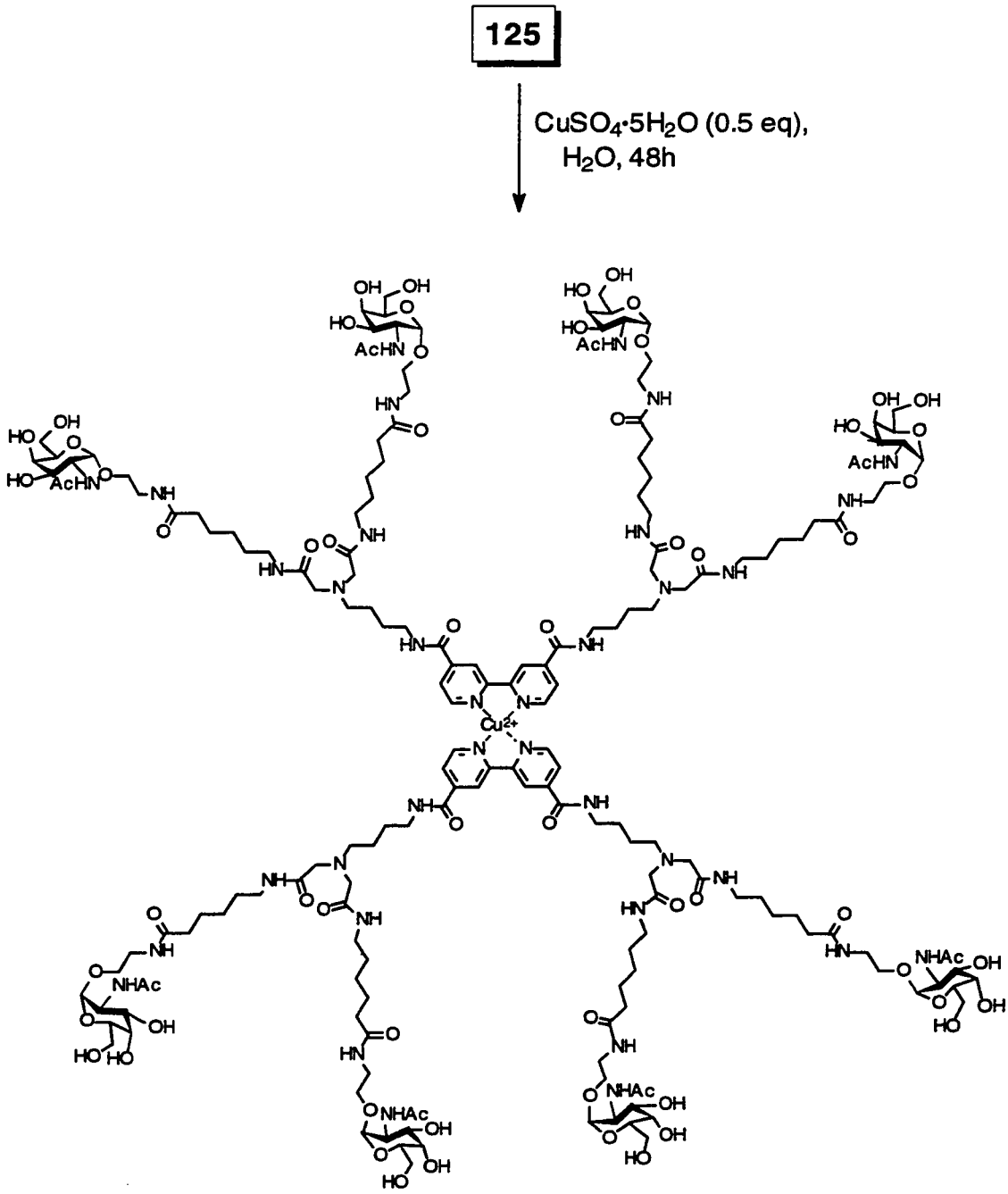


Figure 4.2.19. ^{13}C-NMR (D_2O, 75 MHz) spectrum of Cu^{II}(short tetramer)$_2$•SO_4 **130**.



131

Scheme 4.2.15. Synthesis of short-spacer-armed dodecamer, **131**.



Scheme 4.2.16. Synthesis of long-spacer-armed octamer, **132**.

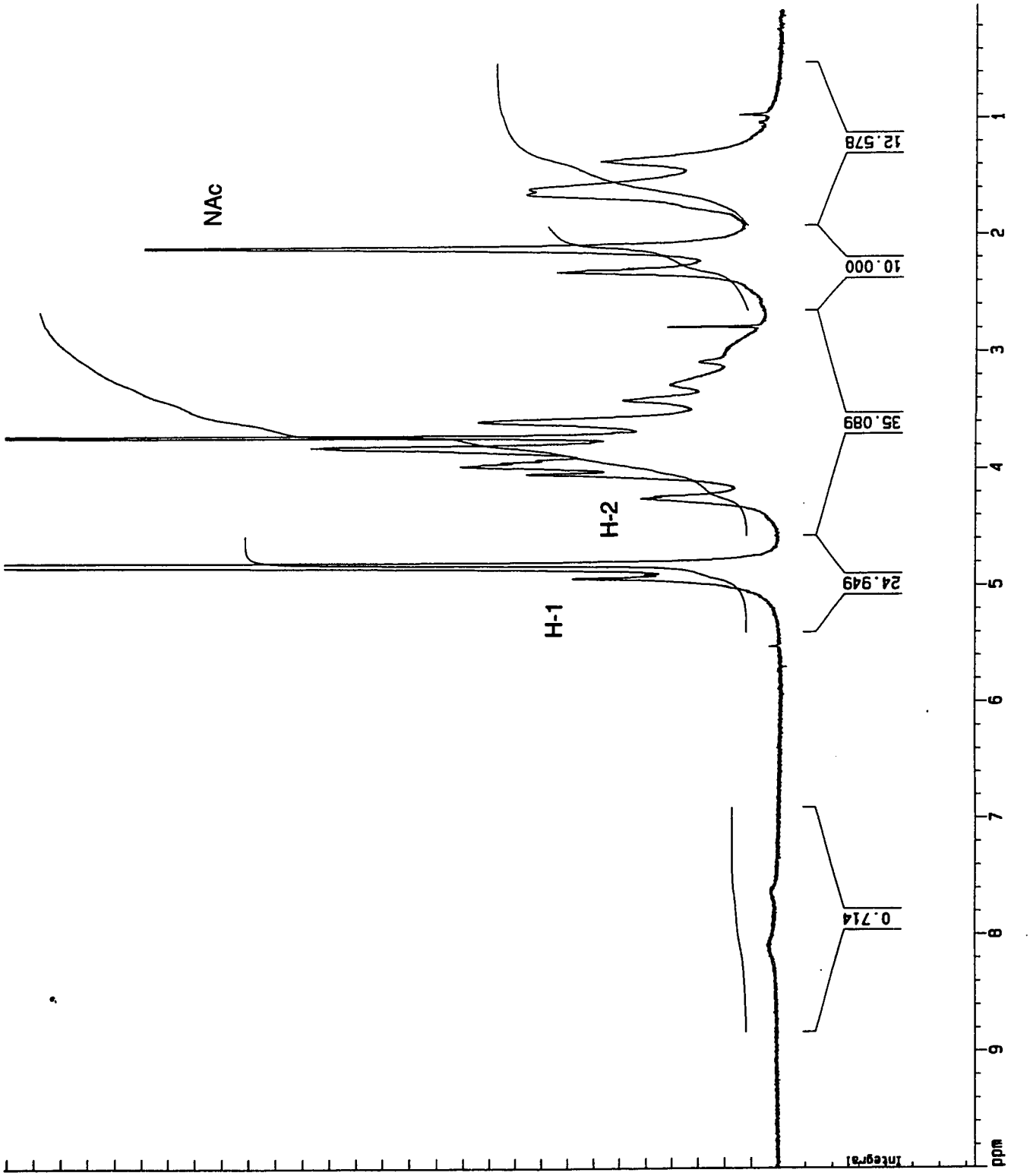
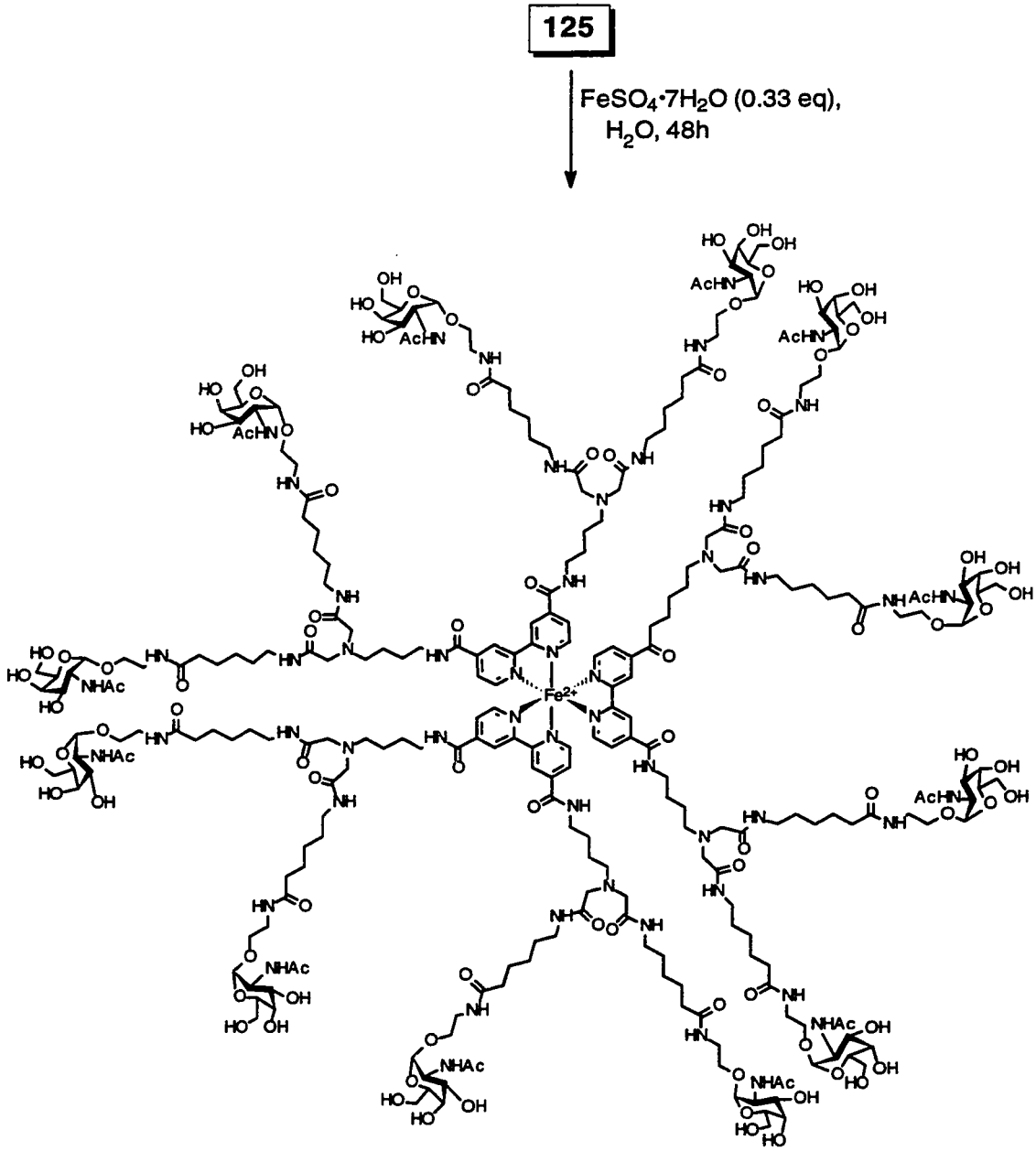


Figure 4.2.20. ^{1}H-NMR (D_2O, 500 MHz) spectrum of Cu^{II}(long tetramer)$_2$•SO_4 132.



133

Scheme 4.2.17. Synthesis of long-spacer-armed dodecamer, **133.**

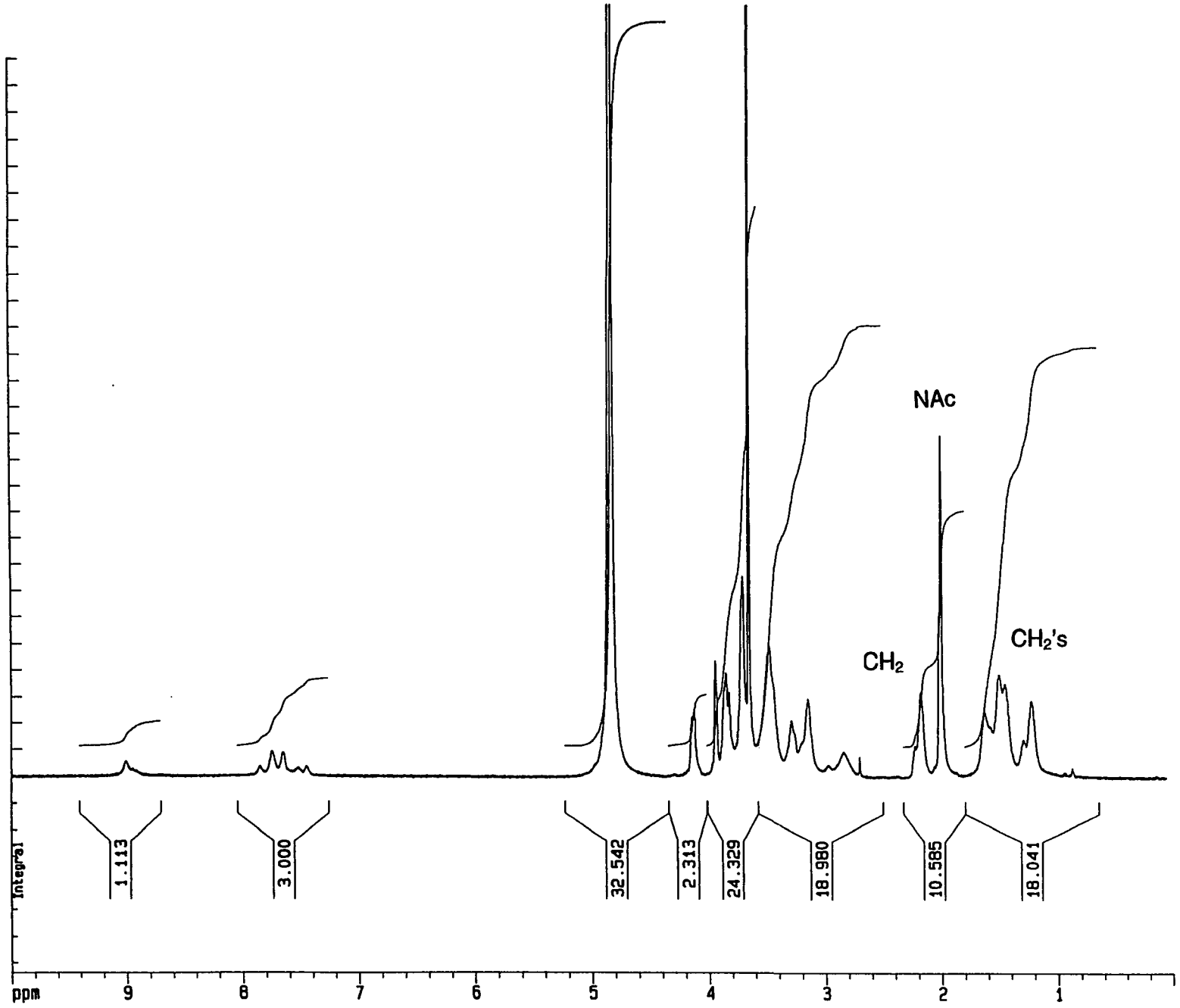


Figure 4.2.21. ^{1}H-NMR (D_2O, 500 MHz) spectrum of Fe^{II}(long tetramer)$_3$•SO_4 **133**.

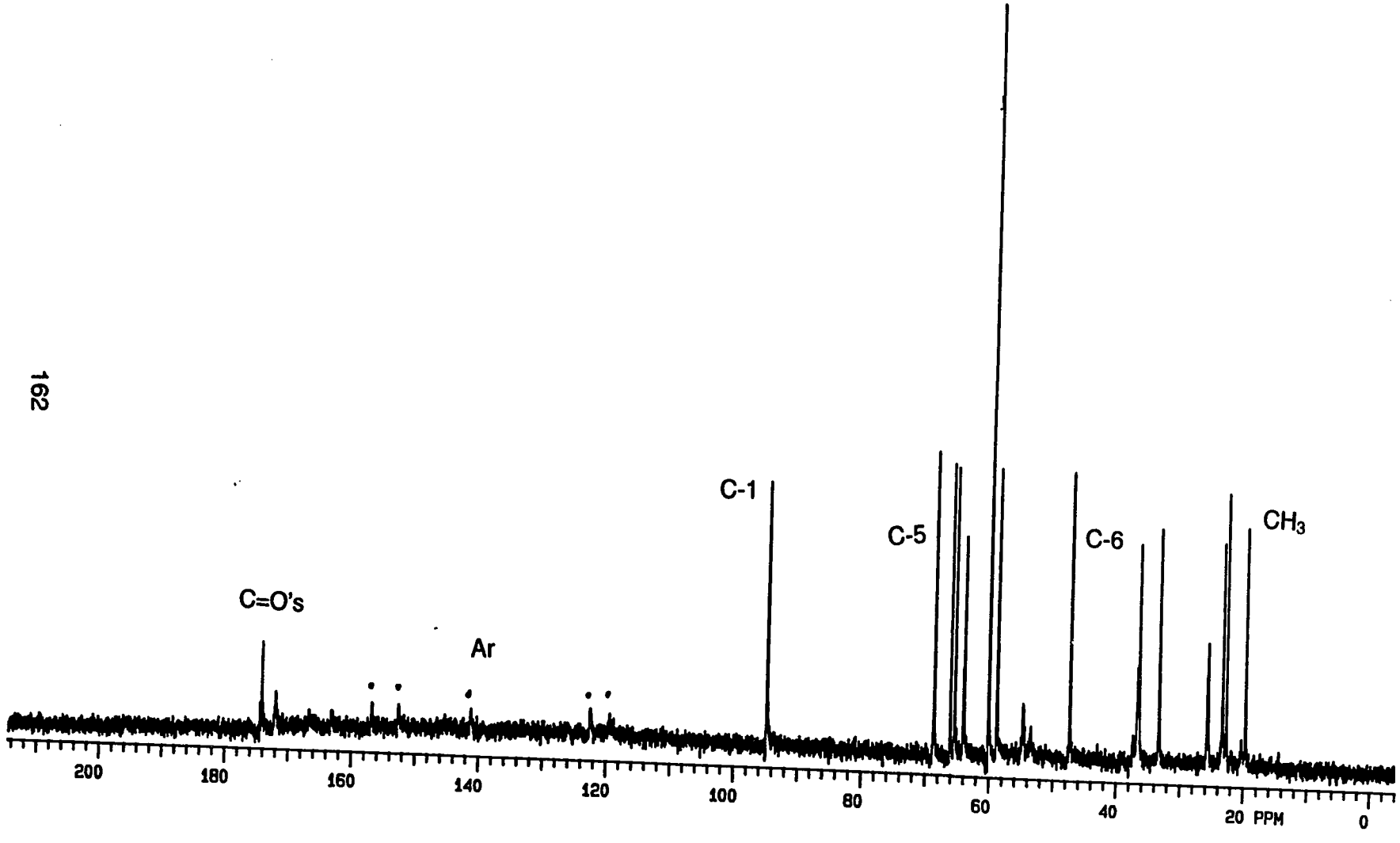


Figure 4.2.22. ^{13}C-NMR (D_2O, 75 MHz) spectrum of Fe^{II}(long tetramer)$_3$•SO_4 **132**

4.3. Binding properties of the self-assembled GalNAc clusters

The efficiency of each bipyridine building block and self-assembled GalNAc clusters to inhibit the binding of asialoglycophorin to *Vicia villosa* B_4 (VVA) was measured by ELLA. Asialoglycophorin was coated on the ELLA plate and a mixture of GalNAc clusters and carbohydrate binding protein, VVA/HRP was added to the coated microtiter plates for the competitive inhibition assay. The competition for binding to lectin VVA occurred between GalNAc residues on asialoglycophorin and the synthetic ligands. After washings, the adsorbed *VVA*/HRP on the plate was then detected quantitatively on the basis of color change using ABTS/H_2O_2 as enzyme substrates. The results for the inhibition of binding of VVA to asialoglycophorin are shown in Figure 4.3.1.

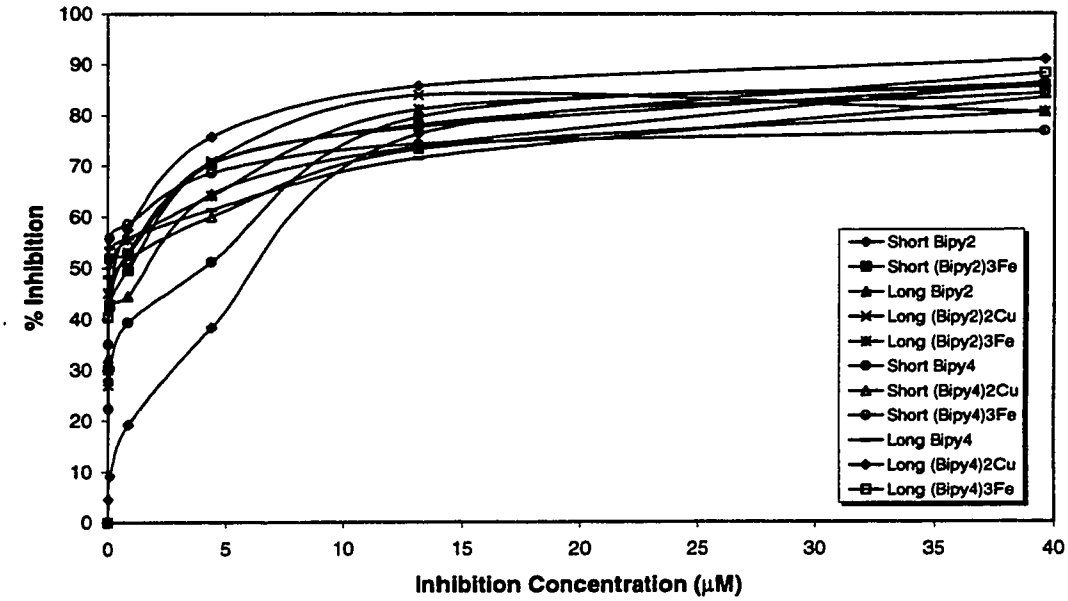


Figure 4.3.1. ELLA inhibition of binding of VVA to asialoglycophorin by self-assembled GalNAc ligands.

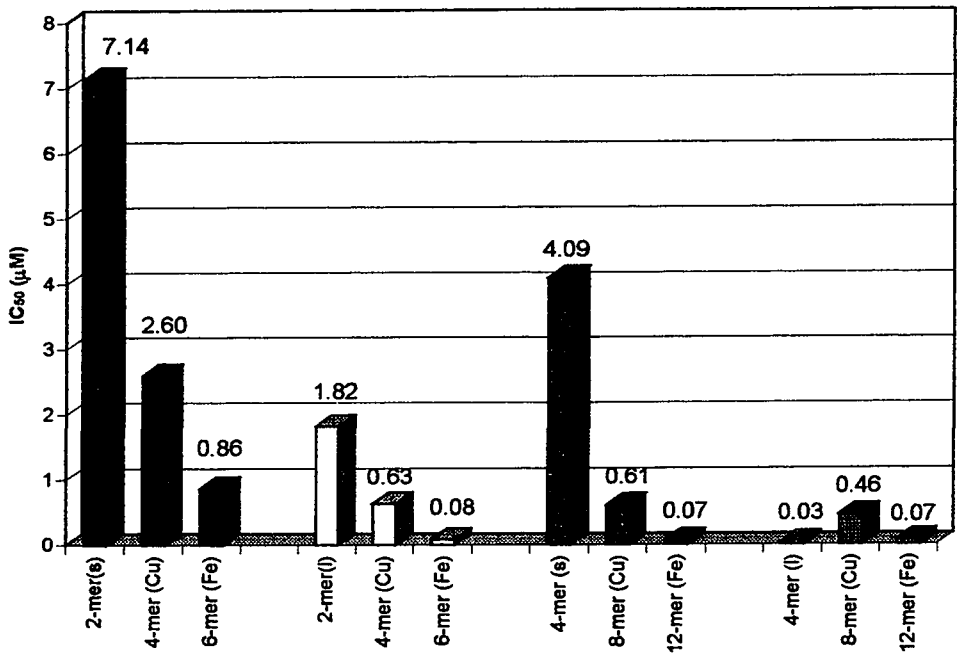


Figure 4.3.2. IC_{50}'s of self-assembled GalNAc ligands

As shown from the IC_{50} values (Figure 4.3.2), all the synthetic GalNAc ligands inhibited the binding of asialoglycophorin to peroxidase labeled *VVA* with greater efficacy than allyl α-D-GalNAc monomer **33** which was used as a standard inhibitor. Iron (II) and copper (II) complexes also demonstrated the multivalency effect. The bipyridine GalNAc dimers **110** and **114** were more efficient inhibitors than the allyl GalNAc monomer **33** (IC_{50} 158.3 μM) by 22 and 87 times, respectively. The Cu^{II}(bipy-GalNAc)$_2$ tetramers **126** and **128** (61 and 251 times, respectively) and bipy-GalNAc tetramers **123** and **125** (39 and 5277 times, respectively) also displayed much stronger inhibitory power than the monomer **33**. The Fe^{II}(bipy-GalNAc)$_3$ dodecamers **131** and **133**, and Cu^{II}(bipy-GalNAc)$_2$ octamers **130** and **132** appeared to be very strong inhibitors with IC_{50} values as low as 0.07-0.61 μM, which were 260-2261 times more efficient than monomer **33**. The structural arrangement and flexibility of the molecule played important roles in

the binding activity. The longer spacer armed ligands showed better inhibition than the shorter ones.

Table 4.3.1. IC_{50}'s of the self-assembled GalNAc ligands, **33**, **110**, **114**, **123**, **125**, and **126-133**.

GalNAc ligands	IC_{50}'s (μM)	Relative potency [a]
allyl α-D-GalNAc **33**	158.3	1
short bipy 2-mer **110**	7.14	22.2 (11.1)
(short bipy 2-mer)$_2$Cu•SO_4 **126**	2.60	60.9 (15.2)
(short bipy 2-mer)$_3$Fe•SO_4 **127**	0.86	184.1 (30.7)
long bipy 2-mer **114**	1.82	87.0 (43.5)
(long bipy 2-mer)$_2$Cu•SO_4 **128**	0.63	251.3 (62.8)
(long bipy 2-mer)$_3$Fe•SO_4 **129**	0.08	1978.8 (329.8)
short bipy 4-mer **123**	4.09	38.7 (9.7)
(short bipy 4-mer)$_2$Cu•SO_4 **130**	0.61	259.5 (32.4)
(short bipy 4-mer)$_3$Fe•SO_4 **131**	0.07	2261.4 (188.5)
long bipy 4-mer **125**	0.03	5276.7 (1319.2)
(long bipy 4-mer)$_2$Cu•SO_4 **132**	0.46	344.1 (43.0)
(long bipy 4-mer)$_3$Fe•SO_4 **133**	0.07	2261.4 (188.5)

[a] Values in parentheses are based on a per-hapten in a molecule.

4.4. Conclusions

The conventional method for the synthesis of glycodendrimers required lengthy and reiterative procedures. This issue was surmounted by a novel self-assembling methodology. In this synthetic strategy, the pre-made building blocks (dendrons) were assembled around the metal ions, Cu^{II} and Fe^{II} to generate

copper coordinated tetramers and octamers, and iron coordinated hexamers and dodecamers. These metal associated GalNAc-bearing glycodendrimers were characterized using spectrometric analyses, ^{1}H- and ^{13}C-NMR spectroscopy, MALDI-TOF mass spectrometry and UV-vis spectrometry.

The potential of these self-assembled GalNAc-bearing dendrimers to cross-link and precipitate lectin VVA was confirmed by the formation of pink precipitates between VVA and iron(II) coordinated glycodendrimers. When tested in ELLA using asialoglycophorin as coating antigen and horseradish peroxidase-labeled VVA for detection, these metal associated glycodendrimers exhibited markedly increased inhibitory potential. The best candidates for efficient binding were found to be longer spacer armed iron(II) coordinated hexamer **129** and bipyridyl tetramer **125** which had 330-fold and 1320-fold increases, respectively, on a GalNAc residue basis. These findings confirm that aglycon spacer and higher valency of sugar residues in neoglycoconjugates are responsible for an increase in binding of carbohydrate-protein interactions.

4.5. Experimental Methods

2-Chloroethyl 2-acetamido-2-deoxy-α-D-glucopyranoside (96).

Acetyl chloride (4.26 g, 54.2 mmol) was added dropwise to a solution of N-acetyl-D-glucosamine (**95**) (10.0 g, 45.2 mmol) in 2-chloroethane (150 mL) at 0 °C. The reaction mixture was heated at 70 °C for 4 h and stirred at room temperature for another 4 h. The solution was concentrated and the brownish oily residue was dissolved in ethanol. The solution was decolorized using charcoal and filtered through a celite pad. The concentrated residue was purified by silica gel chromatography to yield 9.64 g (75%) of an syrupy residue; $[\alpha]_D$ +147.9 (*c* 1.0, MeOH); ^{1}H-NMR (D_2O) δ 1.98 (s, 3H, NAc), 3.40-3.50 (m, 1H), 3.65-3.95 (m, 9H), 4.89 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1); ^{13}C-NMR (D_2O) δ 23.3 (CH_3), 45.0 (CH_2), 55.1 (C-2),

62.0 (C-6), 69.7 (CH_2), 71.4 (C-4), 72.3 (C-3), 73.5 (C-5), 98.6 (C-1), 176.0 C=O); CI-MS (m/z) calcd. for $C_{10}H_{18}NO_6Cl$: 283.1; found: 283.9 (M^+ + 1, 72.8%), 285.9 (32.6%).

2-Azidoethyl 2-acetamido-2-deoxy-α-D-glucopyranoside (97).

2-Chloroethyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**96**) (0.10 g, 0.353 mmol) was dissolved in CH_3CN (3 mL) by heating the solution. NaN_3 (0.23 g, 3.53 mmol) and NaI (0.053 g, 0.353 mmol) were added to this solution. The reaction mixture was then heated at 60 °C for 7 h. The reaction mixture was concentrated and the purification of the residue using short silica gel column eluting with 95:5 $CHCl_3$/MeOH yielded 0.097 g (95%) of an syrupy oil; $[\alpha]_D$ +103.9 (*c* 1.0, MeOH); ^{1}H-NMR (D_2O) δ 1.00 (s, 3H, NAc), 3.35-3.50 (m, 3H), 3.55-3.95 (m, 7H), 4.89 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1); CI-MS (m/z) calcd. for $C_{10}H_{18}N_4O_6$: 290.1; found: 291.0 (M^+ + 1, 12.6%).

2-Chloroethyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (98).

2-Chloroethyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**96**) (6.89 g, 24.3 mmol) was dissolved in a mixture of pyridine (66 mL) and CH_2Cl_2 (33 mL) at -60 °C and benzoyl chloride (7.86 g, 55.9 mmol) in CH_2Cl_2 (10 mL) was added through a dropping funnel over 1 h period at -60 °C. The reaction was monitored by thin layer chromatography (TLC) and the reaction was quenched by adding MeOH when the tri-*O*-benzoylated product started to appear on TLC. The reaction mixture was diluted with $CHCl_3$ (50 mL) and washed with 5% aqueous HCl (2 × 100 mL), saturated $NaHCO_3$ (2 × 100 mL) and then water (1 × 100 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated to afford an oily residue. Purification of the crude product by silica gel chromatography eluting with 98:2 CH_2Cl_2/MeOH yielded 9.20 g (77%) of a white foam: ^{1}H-NMR ($CDCl_3$) δ 1.82 (s, 3H, NAc), 3.62-4.18 (m, 7H, H-5, H-4, $2CH_2$, OH), 4.45 (ddd, 1H, $J_{2,3}$ 10.3

Hz, $J_{2,NH}$ 9.3 Hz, H-2), 4.56 (dd, 1H, $J_{6,6'}$ 12.6 Hz, $J_{5,6'}$ 2.6 Hz, H-6'), 4.73 (dd, 1H, $J_{5,6}$ 3.9 Hz, H-6), 4.90 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.39 (dd, 1H, $J_{3,4}$ 10.3 Hz, H-3), 6.00 (d, 1H, NH), 7.30-7.68 (m, 6H, Ar_{meta}, Ar_{para}), 7.98, 8.03 (2dd, J 8.6 Hz, J 1.2 Hz, Ar_{ortho}); CI-MS (m/z) calcd. for $C_{24}H_{26}NO_8Cl$: 491.1; found: 492.1 (M^+ + 1, 79.1%), 493.9 (45.8%), 494.9 (12.2%).

2-Azidoethyl 2-Acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (99).

2-Chloroethyl 2-acetamido-3,4-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (**98**) (6.86 g, 14.0 mmol) was dissolved in CH_3CN (100 mL) and NaN_3 (9.10 g, 0.14 mmol) and NaI (2.10g, 14.0 mmol) were added to the solution. The reaction mixture was heated at 60 °C for 48 h and then concentrated. The residue was treated with CH_2Cl_2 (100 mL) and the solution was washed with water (2 × 30 mL), saturated $NaHCO_3$ (2 × 30 mL), then brine (1 × 20 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Silica gel chromatography eluting with 9:1 EtOAc/Hexanes provided 6.68 g (96%) of a white foam: $[\alpha]_D$ +77.7 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.84 (s, 3H, NAc), 3.27 (d, 1H, $J_{4,OH}$ 4.6 Hz, OH), 3.35 (ddd, 1H, $J_{a,b}$ 13.4 Hz, $J_{a,d}$ 5.5 Hz, $J_{a,c}$ 2.9 Hz, $CH_aH_bN_3$), 3.54 (ddd, 1H, $J_{b,a}$ 13.4, $J_{b,c}$ 7.9 Hz, $J_{b,d}$ 3.0 Hz, CH_aH_bN3), 3.67 (ddd, 1H, $J_{c,d}$ 10.7 Hz, $J_{c,b}$ 7.9 Hz, $J_{c,a}$ 2.9 Hz, OCH_cH_d), 3.95 (ddd, 1H, $J_{d,c}$ 10.7 Hz, $J_{d,a}$ 5.5 Hz, $J_{d,b}$ 3.0 Hz, OCH_cH_d), 3.85 (ddd, 1H, $J_{4,5}$ 9.5 Hz, H-4), 4.02 (ddd, 1H, H-5), 4.49 (ddd, 1H, $J_{2,3}$ 10.2 Hz, $J_{2,NH}$ 9.9 Hz, H-2), 4.55 (dd, 1H, $J_{5,6}$ 2.2 Hz, $J_{6,6'}$ 12.2 Hz, H-6), 4.76 (dd, 1H, $J_{5,6'}$ 4.2 Hz, H-6'), 4.93 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.35 (dd, 1H, $J_{3,4}$ 9.3 Hz, H-3), 5.88 (d, 1H, NH), 7.41 7.45 (2dd, 4H, $J_{m,o}$ 7.3 Hz, $J_{m,p}$ 7.4 Hz, Ar_{meta}), 7.54, 7.58 (2dd, 2H, Ar_{para}), 8.01, 8.05 (2d, 4H, Ar_{ortho}); ^{13}C-NMR ($CDCl_3$) δ 23.0 (CH_3), 50.4 (CH_2N_3), 51.4 (C-2), 63.3 (C-6), 67.3 (OCH_2), 68.9 (C-4), 70.7 (C-3), 74.4 (C-5), 97.8 (C-1), 128.5 (Ar_{meta}), 129.1, 129.5 (Ar_{ipso}'s), 129.7, 129.9 (Ar_{ortho}'s), 133.3, 133.5 (Ar_{para}'s), 166.9, 167.8, 170.3 (C=O's); CI-MS (m/z) calcd. for $C_{24}H_{26}N_4O_8$:

498.2; found: 499.0 (M^+ + 1, 64.8%), 411.9 (glycon, 100%); Anal. Calcd for $C_{24}H_{26}N_4O_8$: C, 57.83; H, 5.26; N, 11.24. Found: C, 57.73; H, 5.42; N, 10.71.

2-Azidoethyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactopyranoside (100).

A solution of pyridine (1.95 mL, 24.1 mmol) in CH_2Cl_2 (10 mL) was added dropwise through a dropping funnel to a solution of trifluoromethanesulfonic anhydride (2.0 mL, 12.1 mmol) in CH_2Cl_2 (30 mL) at -15 °C. A solid appeared during the initial phase of the reaction, but dissolved after the complete addition of pyridine. A solution of 2-azidoethyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (**99**) (4.0 g, 8.03 mmol) in CH_2Cl_2 (10 mL) was added to the reaction mixture and the solution was stirred at -15 °C for 1 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with cold 5% aqueous HCl (1 × 30 mL), saturated $NaHCO_3$ (2 × 30 mL) and then water (1 × 30 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated to afford the triflate as a white foam: ^{1}H-NMR ($CDCl_3$) δ 1.83 (s, 3H, NAc), 3.35-3.46 (m, 1H, CH_aN_3), 3.52-3.77 (m, 2H, $OCH_cCH_bN_3$), 3.90-4.02 (m, 1H, OCH_d), 4.30-4.47 (m, 2H, H-6', H-5), 4.58 (ddd, 1H, $J_{2,3}$ 10.8 Hz, $J_{2,NH}$ 9.6 Hz, H-2), 4.81 (dd, 1H, $J_{6,5'}$ 11.9 Hz, $J_{5,6}$ 1.4 Hz, H-6), 4.96 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.33 (dd, 1H, $J_{3,4}$ 9.7 Hz, H-3), 5.75 (dd, 1H, $J_{4,5}$ 10.7 Hz, H-4), 5.92 (d, 1H, NH), 7.40-7.66 (m, 6H, Ar_{meta}, Ar_{para}), 8.04, 8.09 (2dd, $J_{o,m}$ 6.6 Hz, $J_{o,p}$ 1.2 Hz, Ar_{ortho}); FAB-MS (pos. m/z) calcd. for $C_{25}H_{25}N_4O_{10}SF_3$: 630.12; found: 631.19 (M^+ + 1, 25.2%). This product was used for the next reaction without further purification.

The triflate obtained was dissolved in DMF (20 mL) and sodium benzoate (5.78 g, 0.040 mmol) was added. The reaction mixture remained heterogeneous and was stirred at room temperature for 20 h. The reaction mixture was diluted with $CHCl_3$ (30 mL) and washed thoroughly and successively with brine (2 × 30 mL) and water (3 × 30 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Purification of the crude product by silica gel chromatography

eluting with 7:3 EtOAc/Hexanes yielded 3.07 g (64%) of a white foam: $[\alpha]_D$ +103.3 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.84 (s, 3H, NAc), 3.33 (ddd, 1H, $J_{a,b}$ 13.4 Hz, $J_{a,d}$ 5.5 Hz, $J_{a,c}$ 2.9 Hz, $CH_aH_bN_3$), 3.50 (ddd, 1H, $J_{b,a}$ 13.4 Hz, $J_{b,c}$ 7.9 Hz, $J_{b,d}$ 3.0 Hz, $CH_aH_bN_3$), 3.69 (ddd, 1H, $J_{c,d}$ 10.7 Hz, $J_{c,b}$ 7.9 Hz, $J_{c,a}$ 2.9 Hz, OCH_cH_d), 3.95 (ddd, 1H, $J_{d,c}$ 10.7 Hz, $J_{d,a}$ 5.5 Hz, $J_{d,b}$ 3.0 Hz, OCH_cH_d), 4.37 (dd, 1H, $J_{6,6'}$ 9.8 Hz, $J_{5,6'}$ 4.3 Hz, H-6'), 4.51-4.57 (m, 2H, H-5, H-6), 4.94 (ddd, 1H, $J_{2,3}$ 11.3 Hz, $J_{2,NH}$ 9.5 Hz, H-2), 5.12 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.57 (dd, 1H, $J_{3,4}$ 3.3 Hz, H-3), 5.92 (d, 1H, H-4), 6.01 (d, 1H, NH), 7.25, 7.36 (2t, 4H, Ar_{meta}), 7.43 (t, 3H, Ar_{para}, Ar_{meta}), 7.49, 7.56 (2t, 2H, $J_{m,p}$ 7.4 Hz, Ar_{para}), 7.80, 7.96, 8.06 (3d, 6H, $J_{o,m}$ 7.3 Hz, Ar_{ortho}); ^{13}C-NMR ($CDCl_3$) δ 23.15 (CH_3), 48.21 (C-2), 50. 43 (CH_2), 62.53 (C-6), 67.50 (CH_2), 67.60 (C-3), 68.25 (C-4), 69.03 (C-5), 98.20 (C-1), 128.37, 128.42, 128.62 (Ar_{meta}'s), 128.84, 129.07, 129.37 (Ar_{ipso}'s), 129.63, 129.83, 129.94 (Ar_{ortho}'s), 133.24, 133.38, 133.55 (Ar_{para}'s), 165.71, 166.00, 166.39, 170.43 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{31}H_{30}N_4O_9$: 602.20; found: 603.19 (M^+ + 1, 2.0%); Anal. Calcd for $C_{31}H_{30}N_4O_9$: C, 61.79; H, 5.02; N, 9.30. Found: C, 61.77; H, 4.95; N, 8.75.

2-Azidoethyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-galactopyranoside.

The procedure described above was modified to prepare the title compound. To a solution of the triflate in DMF was added sodium nitrite (10 eq) and the reaction was mixture was stirred at room temperature for 20 hours. The reaction mixture was then diluted with $CHCl_3$ (20 mL) and washed with brine (2 × 20 mL) and water (3 × 20 mL). The dried (Na_2SO_4) organic phase was concentrated. Silica gel chromatography of the crude product eluting with 38:1:1 $CHCl_3$/MeCN/MeOH afforded 0.31 g (76%) of a white foam; $[\alpha]_D$ +73.0 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.84 (s, 3H, NAc), 2.90 (bs 1H, OH), 3.35 (ddd, 1H, $J_{a,b}$ 13.4 Hz, $J_{a,d}$ 5.5 Hz, $J_{a,c}$ 2.9 Hz, $CH_aH_bN_3$), 3.54 (ddd, 1H, $J_{b,a}$ 13.4 Hz, $J_{b,c}$ 7.9 Hz, $J_{b,d}$ 3.0 Hz, $CH_aH_bN_3$), 3.67 (ddd, 1H, $J_{c,d}$ 10.7 Hz, $J_{c,b}$ 7.9 Hz, $J_{c,a}$ 2.9 Hz, OCH_cH_d), 3.95 (ddd, 1H, $J_{d,c}$ 10.7 Hz, $J_{d,a}$ 5.5 Hz, $J_{d,b}$ 3.0 Hz, OCH_cH_d), 4.25 (dd,

1H, $J_{5,6}$ 6.3 Hz, H-5), 4.28 (d, 1H, H-4), 4.53 (dd, 1H, $J_{6,6'}$ 11.5 Hz, $J_{5,6}$ 6.8 Hz, H-6), 4.61 (dd, 1H, $J_{5,6'}$ 5.7 Hz, H-6'), 4.93 (ddd, 1H, $J_{2,3}$ 11.1 Hz, $J_{2,NH}$ 9.8 Hz, H-2), 4.99 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.33 (dd, 1H, $J_{3,4}$ 3.0 Hz, H-3), 5.81 (d, 1H, NH), 7.41, 7.45 (2dd, 4H, $J_{m,o}$ 7.3 Hz, $J_{m,p}$ 7.4 Hz, Ar_{meta}'s), 7.54, 7.58 (2t, 2H, Ar_{para}'s), 8.01, 8.05 (2dd, 4H, Ar_{ortho}'s); CI-MS (m/z) calcd. for $C_{24}H_{26}N_4O_8$: 498.2; found: 499.0 (M^+ + 1, 12.7%), 411.9 (glycon, 69.9%).

2-Azidoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside (101).

2-Azidoethyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactopyranoside (**100**) (8.5 g, 17.1 mmol) was dissolved in MeOH (100 mL) and 1M sodium methoxide was added dropwise until the pH of the solution reached ~9.0. The solution was stirred at room temperature for 3 hours. When the reaction was complete, the reaction solution was treated with Amberlite IR (H) resin for 15 min. to neutralize. The resin was filtered off and the filtrate was concentrated to dryness to provide de-O-acetylated compound.

The dried residue was dissolved in pyridine (20 mL) and acetic anhydride (15 mL) was added. The solution was stirred at room temperature for 16 hours and then concentrated under reduced pressure. The residue was dissolved in $CHCl_3$ (30 mL) and washed with 5% aqueous HCl (2 × 20 mL), saturated $NaHCO_3$ (2 × 20 mL), water (1 × 20 mL), then dried over anhydrous Na_2SO_4. Purification of the crude product by silica gel chromatography eluting with 4:1 EtOAc/Hexanes yielded 6.05 g (85%) of a white foam: $[\alpha]_D$ +50.2 (*c* 1.1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.94, 1.98, 2.03, 2.14 (4s, 12H, OAc, NAc), 3.33 (ddd, 1H, $J_{d,c}$ 13.5 Hz, $J_{d,b}$ 2.9 Hz, $J_{d,a}$ 5.5 Hz, H-d), 3.52 (ddd, 1H, $J_{c,d}$ 13.5 Hz, $J_{c,a}$ 3.0 Hz, $J_{c,b}$ 8.0 Hz, H-c), 3.65 (ddd, 1H, $J_{b,a}$ 10.8 Hz, $J_{b,c}$ 8.0 Hz, $J_{b,d}$ 2.9 Hz, H-b), 3.89 (ddd, 1H, $J_{a,b}$ 10.8 Hz, $J_{a,c}$ 3.0 Hz, $J_{a,d}$ 5.5 Hz, H-a), 4.05-4.12 (m, 2H, H-6's), 4.16 (dt, 1H, $J_{5,6}$ 6.9 Hz, $J_{4,5}$ 1.0 Hz, H-5), 4.60 (ddd, 1H, $J_{2,3}$ 11.4 Hz, $J_{2,NH}$ 9.6 Hz, H-2), 4.93 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.16 (dd, 1H, $J_{2,3}$ 11.4 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.38 (dd, 1H, $J_{3,4}$ 3.3 Hz,

$H_{4,5}$ 1.1 Hz, H-4), 5.64 (d, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ 20.6 (OAc), 23.1 (NAc), 47.4 (C-2), 50.3 (CH_2), 61.8 (C-6), 66.9 (CH_2), 67.2 (C-3), 674 (C-4), 68.0 (C-5), 97.9 (C-1), 170.1, 170.2, 170.3, 170.8 (C=O's); CI-MS (m/z) calcd. for $C_{16}H_{24}N_4O_9$: 416.15; found: 417.0 (M^+ + 1, 91.0%); Anal Calcd for $C_{16}H_{24}N_4O_9$: C, 46.14; H, 5.81; N, 13.46. Found: 46.21; H, 5.85; N, 13.40.

2-Aminoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactpyranoside hydrochloride (102).

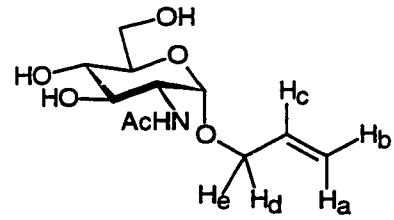
To a solution of 2-azidoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside (**101**) (1.0 g, 2.40 mmol) in MeOH (100 mL) were added Pd/C (0.20 g) and acetic acid (0.14 g, 2.40 mmol). H_2 was bubbles through the heterogeneous reaction mixture for 24 h. The reaction mixture was filtered through a celite pad and the filtrate was gently stirred with Amberlite IR (Cl) resin (2.0 g) for 24 h. The resin was filtered off and the filtrate was concentrated to provide 0.97 g (95%) of a white foam: ^{1}H-NMR ($CDCl_3$) δ 1.94, 1.99, 2.01, 2.11 (4s, 12H, OAc, NAc), 3.27 (bs, 2H, CH_2N), 3.64-3.73 (m, 3H, OC*H*H, NH_2), 3.90-4.08 (m, 3H, OCH*H*, H-6's), 4.28 (dd, 1H, $J_{5,6}$ 6.0 Hz, H-5), 4.53 (ddd, 1H, $J_{2,NH}$ 9.4 Hz, $J_{2,3}$ 11.3 Hz, H-2), 4.91 (d, 1H, $J_{1,2}$ 3.3 Hz, H-1), 5.27 (dd, 1H, $J_{3,4}$ 3.1 HZ, H-3), 5.35 (d, 1H, H-4), 7.67 (d, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ 20.6 (OAc), 22.8 (NAc), 39.3 (CH_2), 47.0 (C-2), 62.0 (C-6), 63.5 (CH_2), 66.9 (C-3), 67.1 (C-4), 68.2 (C-5), 98.1 (C-1), 170.4, 170.4, 171.0, 171.2 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{16}H_{26}N_2O_9$: 390.16; found: 391.23 (M^+ + 1, 98.4%).

2-Aminoethyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactpyranoside hydrochloride (103).

The same procedure described previously for the preparation of 2-aminoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactpyranoside hydrochloride (**102**) was used: $[\alpha]_D$ +133.3 (*c* 0.7, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.80 (s, 3H, NAc), 3.20 (b, 2H, CH_2N), 3.60-3.75 (m, 3H, OC*H*H, NH_2), 3.95-4.06 (m,

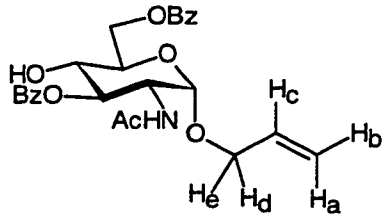
1H, OCH*H*), 4.31-4.40 (m, 1H, H-6), 4.50-4.68 (m, 2H, H-5, H-6'), 5.00 (ddd, 1H, H-2), 5.10 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1), 5.61 (dd, 1H, H-3), 5.91 (d, 1H, $J_{3,4}$ 3.6 Hz, H-4), 7.24-7.58 (m, 10H, Ar_{meta}'s, Ar_{para}'s, NH), 7.76, 7.98, 8.03 (3d, 6H, Ar_{ortho}'s); ^{13}C-NMR ($CDCl_3$) δ 23.5 (NAc), 41.4 (C-2), 48.3 (CH_2), 63.5 (C-6), 65.9 (CH_2), 68.07 (C-3), 68.94 (C-4), 70.20 (C-5), 99.04 (C-1), 129.1, 129.2 (Ar_{meta}'s), 129.4, 129.7, 130.1 (Ar_{ipso}'s), 130.1, 130.4, 130.6 (Ar_{ortho}'s), 133.8, 134.1 (Ar_{para}'s), 166.4, 166.7, 167.3, 171.5 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{31}H_{32}N_2O_9$: 576.21; found: 577.29 (M^+ + 1, 62.1%).

Allyl 2-acetamido-2-deoxy-α-D-glucopyranoside (104).



A solution of *N*-acetyl-D-glucosamine (20 mg, 90.4 mmol) in allyl alcohol (400 mL) was refluxed for 5 h and stirred at room temperature overnight. The reaction solution was concentrated and the brownish residue was recrystallized from the EtOH to yield 15.8 g (67%) of a white solid: mp 165-166 °C; $[\alpha]_D$ +58.4 (*c* 1.0, DMSO); ^{1}H-NMR (D_2O) δ 2.01 (s, 3H, NAc), 3.44-3.50 (m, 1H, H-5), 3.71-3.93 (m, 5H, H-2, H-3, H-4, H-d, H-e), 4.01 (dd, 1H, $J_{5,6'}$ 6.1 Hz, $J_{6,6'}$ 13.2 Hz, H-6'), 4.25 (dd, 1H, $J_{5,6}$ 5.3 Hz, H-6), 4.93 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.20-5.31 (m, 2H, H-b, H-a), 5.90-6.05 (m, 1H, H-c), 8.00 (d, 1H, $J_{2,NH}$ 8.0 Hz, NH); FAB-MS (pos. m/z) Calcd. for $C_{11}H_{19}NO_6$: 261.12; found: 262.26 (M^+ + 1, 100%); Anal. Calcd for $C_{11}H_{19}NO_6$: C, 50.57; H, 7.33; N, 5.36. Found: C, 50.01; H, 7.01; N, 5.15.

Allyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (105).



Allyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**104**) (10.0 g, 38.3 mmol) was treated with a solution of pyridine (100 mL) in CH_2Cl_2 (50 mL) at -60 °C. A solution of benzoyl chloride (10.2 mL, 88.1 mmol) in CH_2Cl_2 (10 mL) was then added to the solution dropwise through a dropping funnel and stirred at -60 °C for 3 hours. When the tri-*O*-benzoylated product started to appear on the TLC plate, the reaction was quenched by adding MeOH and the reaction solution was diluted with $CHCl_3$ (50 mL). The solution was washed with 5% aqueous HCl (3 × 50 mL), saturated $NaHCO_3$ (2 × 20 mL) and then water (1 × 50 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Silica gel chromatography of the crude product eluting with 99:1 CH_2Cl_2/MeOH afforded 11.5 g (64%) of a white foam: $[\alpha]_D$ +100.2 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.82 (s, 3H, NAc), 3.60 (bs, 1H, OH), 3.84 (dd, 1H, $J_{3,4}$ H-4), 4.00-4.05 (m, 2H, H-5, H-d), 4.21 (dd, 1H, $J_{He,Hc}$ 12.8 Hz, $J_{Hd,He}$ 5.4 Hz, H-e), 4.45 (ddd, 1H, $J_{2,3}$ 10.6 Hz, $J_{2,NH}$ 9.8 Hz, H-2), 4.54 (dd, 1H, $J_{6,6'}$ 12.0 Hz, $J_{5,6}$ 2.2 Hz, H-6), 4.73 (dd, 1H, $J_{5,6'}$ 4.4 Hz, H-6'), 4.91 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.20 (dd, 1H, $J_{Hb,Hc}$ 10.4 Hz, $J_{Hb,Ha}$ 1.4 Hz, H-b), 5.28 (dd, 1H, $J_{Ha,Hc}$ 17.2 Hz, H-a), 5.37 (dd, 1H, H-3), 5.87 (d, 1H, NH), 5.88 (m, 1H, H-c), 7.39, 7.43 (2t, $J_{m,o}$ 7.8 Hz, Ar_{meta}'s), 7.52, 7.56 (2tt, $J_{m,p}$ 7.4 Hz, $J_{o,p}$ 1.2 Hz, Ar_{para}'s), 7.98, 8.04 (2dd, $J_{o,m}$ 7.8 Hz, Ar_{ortho}'s); ^{13}C-NMR ($CDCl_3$) δ 23.1 (NAc), 51.6 (C-2), 63.4 (C-6), 68.5 (CH_2), 69.0 (C-4), 70.5 (C-3), 74.7 (C-5), 96.7 (C-1), 118.2 (CH=*C*H_2), 128.3, 128.4 (Ar_{meta}'s), 129.5, 129.6 (Ar_{ipso}'s), 129.7, 129.9 (Ar_{ortho}'s), 133.2 (*C*H=CH_2), 133.3, 133.4 (Ar_{para}'s), 166.9, 167.8, 170.0 (C=O's); FAB-MS (pos. m/z) Calcd. for $C_{25}H_{26}NO_8$: 468.17; Found: 469.45 (M^++1, 15.9%);

Anal. Calcd for $C_{25}H_{26}NO_8$: C, 64.10; H, 5.59; N, 2.99. Found: C, 63.72; H, 5.69; N, 2.88.

Allyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactopyranoside (106).

The same procedure was used as described previously for the preparation of 2-azidoethyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactopyranoside in 61% yield.

Allyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside (34).

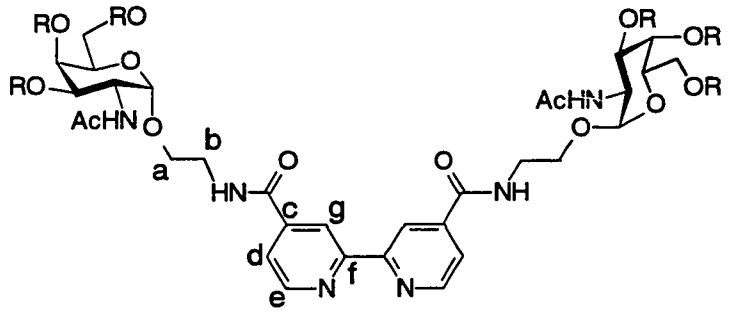
Allyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactopyranoside was de-*O*-benzoylated under Zemplén condition and *O*-acetylated using acetic anhydride and pyridine as described previously for the preparation of compound **101**.

2-(2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactosyl)-1-*N*-benzylaminoethane (107).

Title compound was prepared using the same procedure described previously for the preparation of 2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-1-*N*-benzylaminoethane: Yield: 82%; ^{1}H-NMR ($CDCl_3$) δ 1.79 (s, 3H, NAc), 2.87 (t, 2H, J 5.3 Hz, CH_2N), 3.57-3.68 (m, 1H, OC*H*H), 3.81 (s, 2H, CH_2Ph), 3.81-3.92 (m, 1H, OCH*H*), 4.33-4.47 (m, 1H, H-5), 4.50-4.57 (m, 2H, H-6), 4.91 (ddd, 1H, $J_{2,3}$ 11.2 Hz, $J_{2,NH}$ 9.3 Hz, H-2), 5.07 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.56 (dd, 1H, $J_{3,4}$ 3.3 Hz, H-3), 5.89 (d, 1H, H-4), 6.10 (d, 1H, NH), 7.20-7.62 (m, 14H, Ar_{meta}, Ar_{para}, Ph), 7.83, 7.98, 8.08 (3dd, 6H, $J_{o,m}$ 7.8 Hz, $J_{o,p}$ 1.3 Hz, Ar_{ortho}); ^{13}C-NMR ($CDCl_3$) δ 23.1 (NAc), 48.2 (C-2), 48.4 (CH_2), 53.7 (CH_2), 62.6 (C-6), 67.3 (CH_2), 67.9 (C-3), 68.3 (C-4), 69.3 (C-5), 98.1 (C-1), 127.1 (Ph_{para}), 128.1 (Ph_{meta}), 128.3 (Ph_{ortho}), 128.4, 128.5, 128.6 (Ar_{meta}'s), 128.9, 129.1, 129.4 (Ar_{ipso}'s), 129.6, 129.8, 129.9 (Ar_{ortho}'s), 133.2, 133.3, 133.5 (Ar_{para}'s), 139.7

(Ph_{ipso}); FAB-MS (pos. m/z) calcd. for $C_{38}H_{38}N_2O_9$: 666.26; found: 667.34 (M^++1, 88.9%).

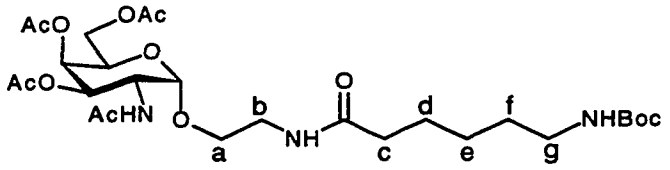
Coupling of 102 with bipyridine (110).



A solution of 2,2'-bipyridine-4,4'-dicarboxylic acid (54.2 mg, 0.222 mmol) in $SOCl_2$ (3 mL) was refluxed for 2 h and the solution was concentrated to provide a yellowish solid. To a solution of 2-aminoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galctopyranoside hydrochloride (**102**) (0.19 g, 0.444 mmol) in CH_2Cl_2 (10 mL) were added Et_3N (0.15 mL, 1.11 mmol) at 0 °C and previously prepared solution of 4,4'-bis(chlorocarbonyl)-2,2'-bipyridine (**108**) in CH_2Cl_2 through a dropping funnel. The solution was stirred at 0 °C for 3 h. During the reaction, the solution turned pink in color. After the reaction was complete, the solution was concentrated and the residue was dissolved in MeOH (10 mL). The methanolic solution was treated with 1M NaOMe until pH 9 and stirred at room temperature for 3 hours. The de-*O*-acetylated product was precipitated out from the solution and the filtration of the product through a fritted glass funnel provided 0.13 g (81%) of a white solid: **109**: ^{1}H-NMR ($CDCl_3$) δ 1.93, 2.10 (2s, 24H, OAc, NAc), 3.55-3.72 (m, 4H, H-b, H-b'), 3.75-3.86 (m, 4H, H-a, H-a'), 4.02-4.10 (m, 4H, H-5, H-6'), 4.21 (t, 2H, $J_{5,6}$ 6.3 Hz, H-6), 4.54 (ddd, 2H, $J_{2,NH}$ 9.5 Hz, $J_{2,3}$ 11.2 Hz, H-2), 4.89 (d, 2H, $J_{1,2}$ 3.5 Hz, H-1), 5.14 (dd, 2H, $J_{3,4}$ 2.0 Hz, H-3), 5.33 (d, 2H, H-4), 6.45 (d, 2H, NHAc), 7.62 (b, 2H, NHCO), 7.76 (d, 2H, H-d), 8.66 (s, 2H, H-g), 8.71 (d, 2H, H-e); ^{13}C-NMR ($CDCl_3$) δ 21.3 (OAc), 23.5 (NAc), 40.6 (C-b), 48.1 (C-2),

62.8 (C-6), 67.5 (C-3), 67.9 (C-a), 68.7 (C-4), 68.9 (C-5), 99.8 (C-1), 118.6 (C-g), 123.1 (C-d), 143.3 (C-f), 150.6 (C-e), 156.3 (C-c), 166.5, 170.9, 171.1, 171.2, 171.4 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{44}H_{56}N_6O_{20}$: 988.35; found: 989.37 (M^+ + 1, 54.8%); **110**: $[\alpha]_D$ +38.8 (*c* 0.5, DMSO); FAB-HRMS (pos. m/z) calcd. for $C_{32}H_{45}N_6O_{14}$: 737.2994; found: 737.2830 (M^+ + 1, 38.4%); Anal Calcd for $C_{32}H_{44}N_6O_{14}$: C, 66.64; H, 7.69; N, 14.57. Found: C, 66.84; H, 7.78; N, 14.45.

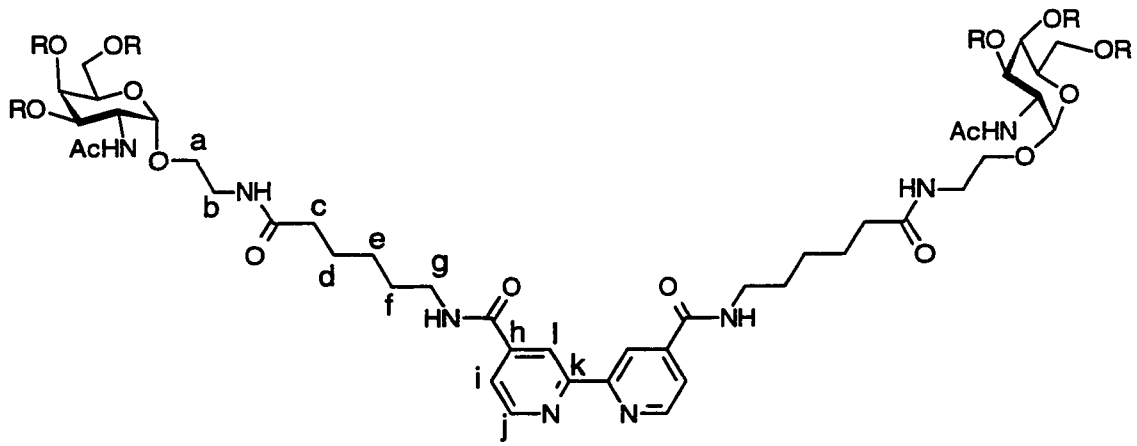
Boc-6-aminocaproic acid coupled GalNAc(OAc)$_3$ (111).



2-Aminoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside hydrochloride (**102**) (1.06 g, 2.48 mmol) and Boc-6-aminocaproic acid (0.86 g, 3.72 mmol) were dissolved in CH_2Cl_2 (20 mL) and the solution was cooled to 0 °C. TBTU (1.19 g, 3.72 mmol) and DIPEA (1.20 mL, 6.89 mmol) were added to the solution and the reaction mixture was stirred at 0 °C for 1 h. The reaction solution was washed with 5% aqueous HCl (2 × 10 mL), saturated $NaHCO_3$ (2 × 10 mL) and water (1 × 10 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Purification of the crude product by silica gel chromatography eluting with 18:1:1 $CHCl_3$/CH_3CN/MeOH afforded 1.14 g (76%) of a white foam: $[\alpha]_D$ +68.0 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.25-1.69 (m, 6H, H-d, H-e, H-f), 1.38 (s, 9H, t-Bu), 1.94, 1.97, 2.00, 2.11 (4s, 12H, OAc, NAc), 2.14-2.27 (m, 2H, H-c), 3.05 (t, 2H, J 6.6 Hz, H-g), 3.25-3.40 (m, 1H, H-b), 3.50-3.69 (m, 3H, H-a, H-b), 4.02-4.05 (m, 2H, H-6's), 4.14-4.20 (m, 1H, H-5), 4.53 (ddd, 1H, $J_{2,3}$ 11.4 Hz, $J_{2,NH}$ 9.5 Hz, H-2), 4.71-4.75 (m, 1H, N*H*Boc), 4.83 (d, 1H, $J_{1,2}$ 3.0 Hz, H-1), 5.06 (dd, 1H, $J_{2,3}$ 11.4 Hz, $J_{3,4}$ 3.1 Hz, H-3), 5.31 (d, 1H, H-4), 6.40-6.55 (b, 2H, NHAc, NHCO); ^{13}C-NMR ($CDCl_3$) δ 20.7 (OAc), 23.0 (NAc), 25.2 (C-d), 26.2 (C-e), 28.3 (t-Bu), 29.6 (C-f), 36.3 (C-c), 39.3 (C-g), 41.3 (C-b),

47.4 (C-2), 62.0 (C-6), 66.7 (C-3), 67.2 (C-4), 68.4 (C-5), 68.4 (C-a), 79.2 (CMe_3), 98.6 (C-1), 156.1, 170.3, 170.5, 170.7, 170.8, 173.7 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{27}H_{45}N_3O_{12}$: 603.30; found: 604.34; Anal. Calcd for $C_{27}H_{45}N_3O_{12}$: C, 53.72; H, 7.51; N, 6.96. Found: C, 53.70; H, 7.52; N 6.96.

Coupling of 112 with bipyridine (114).



4,4'-Bis(chlorocarbonyl)-2,2'-bipyridine (**108**) was prepared by refluxing 2,2'-bipyridine-4,4'-dicarboxylic acid (51.8 mg, 0.212 mmol) in $SOCl_2$ (3 mL) for 2 h. Compound **111** (0.256 g, 0.424 mmol) was treated with 20% TFA in CH_2Cl_2 (5 mL) for 2 h at room temperature. When the reaction was complete, the solvent was evaporated and the residual TFA was removed by co-evaporating the residue with toluene. Then, the de-protected amine salt was dissolved in CH_2Cl_2 (10 mL) and Et_3N (0.12 mL, 0.848 mmol) was added at 0 °C. A solution of 4,4'-bis(chlorocarbonyl)-2,2'-bipyridine (**108**) in CH_2Cl_2 (5 mL) was added to the reaction mixture through a dropping funnel and the solution was stirred at 0 °C for 1 hour and at room temperature for another 2 h. The reaction turned into a pink in color. This solution was concentrated and dissolved in MeOH (10 mL). 1M NaOMe solution was added to the pinkish solution until pH 9 and it was stirred at room temperature for 3 h. As the reaction proceeded, white precipitates came out from the solution and were filtered through a fritted glass funnel to afford 0.15 g

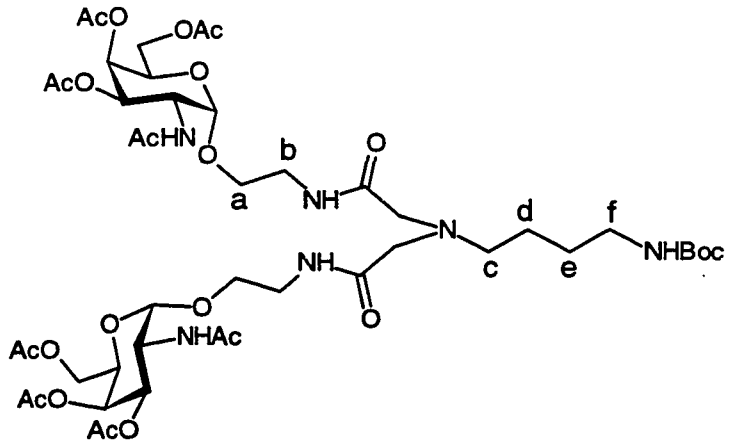
(76%) of a white solid: **113**: FAB-MS (pos. m/z) calcd. for $C_{56}H_{78}N_8O_{22}$: 1214.52; found: 1215.86 (M^+ + 1, 6.2%); **114**: $[\alpha]_D$ +54.5 (*c* 0.2, DMSO); ^{1}H-NMR (D_2O) δ 1.47 (quintet, 4H, J 7.6 Hz, H-e), 1.72 (quintet, 8H, J 7.4 Hz, H-d, H-f), 2.09 (s, 6H, NAc), 2.36 (t, 4H, J 7.3 Hz, H-c), 3.33-3.40 (m, 2H, H-b'), 3.46 (t, 4H, J 6.8 Hz, H-g), 3.52-3.58 (m, 4H, H-a', H-b), 3.74-3.83 (m, 6H, H-6's, H-a), 3.88-3.96 (m, 4H, H-3, H-5), 4.01 (d, 2H, $J_{3,4}$ 2.8 Hz, H-4), 4.22 (dd, 2H, $J_{2,3}$ 11.0 Hz, H-2), 4.89 (d, 2H, $J_{1,2}$ 3.6 Hz, H-1), 7.75 (d, 2H, J 4.6 Hz, H-i), 8.29 (s, 2H, H-l), 8.76 (d, 2H, J 4.6 Hz, H-j); ^{13}C-NMR (D_2O) δ 21.5 (C-e), 24.6 (NAc), 25.2 (C-d), 27.5 (C-f), 35.2 (C-c), 38.4 (C-b), 39.4 (C-g), 49.3 (C-2), 60.7 (C-6), 65.9 (C-3), 67.4 (C-a), 68.0 (C-4), 70.6 (C-5), 96.7 (C-1), 118.9 (C-l), 121.5 (C-i), 142.6 (C-k), 149.7 (C-j), 154.7 (C-h), 167.1, 173.9, 176.4 (C=O's); FAB-HRMS (pos. m/z) calcd. for $C_{44}H_{67}N_8O_{16}$: 963.4675; found: 963.4680 (M^+ + 1, 5.6%); Anal. Calcd for $C_{44}H_{66}N_8O_{16}$ C, 54.88; H, 6.91; N, 11.64. Ffound: C, 54.98; H, 6.75; N, 11.25.

2-Bromoacetamidoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside (115).

A solution of 2-aminoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside hydrochloride (**102**) (0.40 g, 0.89 mmol) in CH_2Cl_2 (10 mL) was treated with DIPEA (0.39 mL, 2.22 mmol) at 0 °C. Bromoacetyl chloride (88 μL, 1.07 mmol) in CH_2Cl_2 (5 mL) was then added dropwise through a dropping funnel at 0 °C. After 20 min, the reaction solution was washed with 5% aqueous HCl (1 × 10 mL), saturated $NaHCO_3$ (1 × 10 mL) and water (1 × 10 mL). The dried (Na_2SO_4) organic phase was concentrated and silica gel chromatography of the crude product eluting with 19:1 $CHCl_3$/MeOH yielded 0.42 g (92%) of a white foam: $[\alpha]_D$ +75.1 (*c* 1.0, $CHCl_3$), ^{1}H-NMR ($CDCl_3$) δ 1.96, 2.02, 2.13 (3s, 12H, OAc, NAc), 3.45-3.64 (m, 3H, OC*H*H, CH_2N), 3.70-3.78 (m, 1H, OCH*H*), 3.89 (s, 2H, CH_2Br), 4.05-4.18 (m, 3H, H-5, H-6's), 4.56 (ddd, 1H, $J_{2,NH}$ 9.6 Hz, $J_{2,3}$ 11.3 Hz, H-2), 4.87 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.12 (dd, 1H, $J_{3,4}$ 3.3 Hz, H-3), 5.33 (d, 1H, H-4), 6.00 (d, 1H, NHAc), 6.93 (b, 1H, NHCO); ^{13}C-NMR ($CDCl_3$) δ 20.7 (OAc), 23.3

(NAc), 29.2 (CH_2), 39.8 (CH_2), 47.6 (C-2), 62.0 (C-6), 66.9 (C-3), 67.2 (C-4), 67.3 (CH_2), 68.3 (C-3), 98.2 (C-1), 166.0, 170.3, 170.6, 171.0 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{18}H_{27}N_2O_{10}Br$: 510.08; found: 511.06 (M^+ + 1, 14.6%), 513.05 (M^+ + 3, 13.6%).

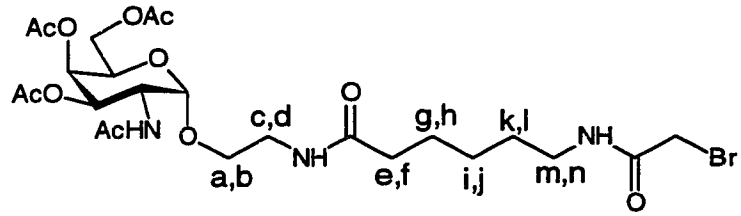
Short spacer armed dimers by *N,N'*-dialkylation (116).



A solution containing 2-bromoacetamidoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside (**115**) (0.42 g, 0.819 mmol), *N*-Boc-1,4-diaminobutane (77 mg, 0.410 mmol) and DIPEA (0.2 mL, 1.23 mmol) in CH_3CN (5 mL) was heated at 60 °C for 48 h. The reaction solution was concentrated and dissolved in CH_2Cl_2 (10 mL). The organic solution was washed with saturated $NaHCO_3$ (1 × 5 mL) and water (1 × 5 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Purification by silica gel chromatography of the crude product eluting with 18:1:1 $CHCl_3$/MeCN/MeOH afforded 0.31 g (72%) of a white foam: $[\alpha]_D$ +91.4 (*c* 0.22, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.40 (s, 9H, *t*-Bu), 1.48 (bs, 4H, H-d, H-e), 1.95, 1.96, 2.01, 2.12 (4s, 24H, OAc, NAc), 2.67 (b, 2H, H-c), 3.10-3.15 (m, 2H, H-f), 3.18 (bs, 4H, $COCH_2N$), 3.37-3.45 (m, 2H, H-b'), 3.52-3.61 (m, 4H, H-a', H-b), 3.70-3.76 (m, 2H, H-a), 4.02-4.11 (m, 4H, H-6's), 4.17 (dd, 2H, $J_{5,6}$ 6.3 Hz, H-5), 4.57 (ddd, 2H, $J_{2,3}$ 11.4 Hz, $J_{2,NH}$ 9.5 Hz, H-2), 4.82-4.86 (m, 1H, NHBoc), 4.87 (d, 2H, $J_{1,2}$ 3.4 Hz, H-1), 5.10 (dd, 2H, $J_{3,4}$ 2.9 Hz,

H-3), 5.33 (d, 2H, H-4), 6.76 (b, 2H, NHAc), 7.63 (b, 2H, NHCO); ^{13}C-NMR ($CDCl_3$) δ 20.7 (OAc), 22.9 (NAc), 23.0 (C-d), 27.3 (C-e), 28.4 (t-Bu), 39.0 (C-b), 39.6 (C-f), 47.4 (C-2), 55.0 (C-c), 58.6 ($COCH_2N$), 62.0 (C-6), 66.7 (C-3), 67.2 (C-4), 67.4 (C-a), 68.4 (C-5), 79.4 (CMe_3), 98.4 (C-1), 156.4, 170.4, 170.6, 170.8, 171.4 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{45}H_{72}N_6O_{22}$: 1048.47; found: 1049.50 (M^+ + 1, 18.1%); Anal. Calcd for $C_{45}H_{72}N_6O_{22}$: C, 51.50; H, 6.92; N, 8.01. Found: C, 51.18; H, 6.86; N, 7.88.

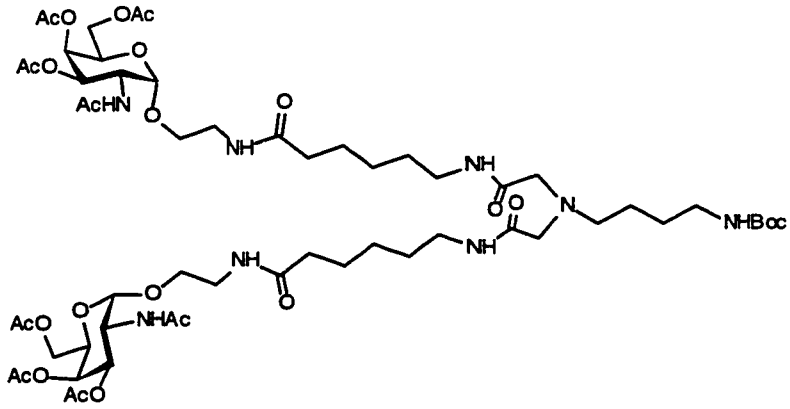
GalNAc(OAc)$_3$ coupled with bromoacetylated caproic acid (117).



Compound **111** (0.43 g, 0.72 mmol) was treated with 20% TFA in CH_2Cl_2 (5 mL) at room temperature for 2 h and the solvent was evaporated. The residue was then co-evaporated with toluene to remove residual TFA. The de-protected amine salt was dissolved in CH_2Cl_2 (20 mL) and DIPEA (0.31 mL, 1.8 mmol) was added at 0 °C. A solution of bromoacetyl chloride (70 µL, 0.87 mmol) in CH_2Cl_2 (5 mL) was added dropwise and the reaction solution was stirred at 0 °C for 20 min. The solution was washed with 5% aqueous HCl (1 × 10 mL), saturated $NaHCO_3$ (1 × 10 mL) and water (1 × 10 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Silica gel chromatography of the crude product eluting with 18:1:1 $CHCl_3$/CH_3CN/MeOH yielded 0.38 g (85%) of an off-white foam: $[\alpha]_D$ +74.1 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.34 (quintet, 2H, J 7.5 Hz, H-i, H-j), 1.51-1.62 (m, 2H, H-k, H-l), 1.63-1.70 (m, 2H, H-g, H-h), 1.95, 1.98, 2.01, 2.13 (4s, 12H, OAc, NAc), 2.15-2.28 (m, 2H, H-e, H-f), 3.22-3.38 (m, 3H, H-d, H-m, H-n), 3.53-3.62 (m, 2H, H-b, H-c), 3.68-3.72 (m, 1H, H-a), 3.84 (s, 2H, CH_2Br), 4.04-4.09 (m, 2H, H-6's), 4.15 (dd, 1H, $J_{5,6}$ 6.5 Hz, H-5), 4.54 (ddd, 1H, $J_{2,3}$ 11.4 Hz,

$J_{2,NH}$ 9.5 Hz, H-2), 4.86 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.07 (dd, 1H, $J_{2,3}$ 11.4 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.33 (d, 1H, H-4), 6.29 (m, 1H, NHCO), 6.46 (d, 1H, AcNH), 6.75 (m, 1H, NHCO); ^{13}C-NMR ($CDCl_3$) δ 20 6 (OAc), 23.0 (NAc), 24.8 (C-g,h), 25.9 (C-i,j), 28.8 (C-k,l), 29.0 (CH_2Br), 36.1 (C-e,f), 39.1 (C-m,n), 39.3 (C-c,d), 47.4 (C-2), 61.9 (C-6), 66.6 (C-3), 67.1 (C-4), 68.3 (C-5), 68.3 (C-a,b), 98.4 (C-1), 166.3, 170.3, 170.5, 170.8, 173.3 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{24}H_{38}N_3O_{11}Br$: 623.17; found: 624.26 (M^+ + 1, 9.9%), 626.26 (M^+ + 3, 10.1%) Anal. Calcd for $C_{24}H_{38}N_3O_{11}Br$: C, 46.22; H, 6.15; N, 6.74. Found: 46.43; H, 6.03; N, 6.65.

Long spacer armed dimer by *N,N'*-dialkylation (118).



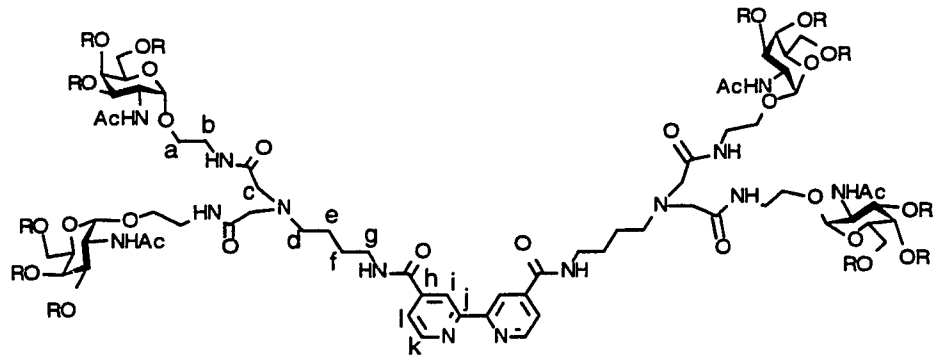
A solution mixture of bromide **117** (0.44 g, 0.71 mmol), *N*-Boc-1,4-diaminobutane (66 mg, 0.35 mmol) and DIPEA (0.18 mL, 1.06 mmol) in CH_3CN (3 mL) was heated at 60 °C for 48 h. The reaction solution was concentrated and the residue was dissolved in $CHCl_3$ (10 mL). The solution was washed with saturated $NaHCO_3$ (1 × 5 mL), water (1 × 5 mL) and dried over anhydrous Na_2SO_4. Silica gel chromatography of the concentrated residue eluting with 18:1:1 $CHCl_3$/CH_3CN/MeOH yielded 0.33 g (73%) of a white foam: $[\alpha]_D$ +59.1 (*c* 1.0, $CHCl_3$), ^{1}H-NMR ($CDCl_3$) δ 1.24-1.32 (quintet, 4H, J 7.4 Hz, $2CH_2$), 1.39 (s, 9H, *t*-Bu), 1.47-1.53 (m, 8H, $4CH_2$), 1.93, 1.96, 2.00, 2.1 (4s, 24H, 6OAc, 2NAc), 2.13-2.17 (t, 4H, J 7.4 Hz, $2CH_2CONH$), 3.02-3.33 (m, 14H, $5CH_2$, 4C*H*H), 3.45-3.52 (m, 2H, 2CH*H*), 3.55-3.66 (m, 2H, CH_2), 3.68-3.75 (m, 2H, 2C*H*H), 4.02-4.10

(m, 4H, 2H-6's), 4.12-4.16 (m, 2H, 2H-5), 4.54 (ddd, 2H, $J_{2,3}$ 11.4 Hz, $J_{2,NH}$ 9.5 Hz, 2H-2), 4.84 (d, 2H, $J_{1,2}$ 3.6 Hz, 2H-1), 4.93-4.99 (m, 1H, NHBoc), 5.10 (dd, 2H, $J_{3,4}$ 3.3 Hz, 2H-3), 5.32 (d, 2H, 2H-4), 6.90-7.05 (b, 4H, 4NH), 7.50-7.65 (b, 2H, 2NH); ^{13}C-NMR ($CDCl_3$) δ 20.7 (OAc), 23.0 (NAc), 25.0 (CH_2), 26.1 (CH_2), 27.0 (CH_2), 28.4 (t-Bu), 29.0 (CH_2), 36.2 (CH_2), 38.6 (CH_2), 39.0 (CH_2), 39.7 (CH_2), 42.0 (CH_2), 47.4 (C-2), 58.8 (CH_2), 62.0 (C-6), 66.7 (C-3), 67.3 (C-4), 68.1 (CH_2), 68.5 (C-5), 79.4 (*C*Me_3), 98.4 (C-1), 156.6, 170.4, 170.5, 170.6, 170.7, 173.5 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{57}H_{94}N_8O_{24}$: 1274.64; found: 1275.58 (M^+ + 1, 10.2%).

Fully deprotected divalent long spacer armed GalNAc ligand (120).

To a solution of **118** (0.10 g, 78.5 μmol) in MeOH (5 mL) was added 1M NaOMe and stirred at room temperature for 2 h. The solution was treated with Amberlite IR H^+ resin for 15 min and filtered. The filtrate was concentrated and the residue was treated with 20% TFA in CH_2Cl_2 (5 mL) for 1 hour. The solution was concentrated and co-evaporated with toluene to remove residual TFA. The residue was then dissolved in water (1 mL) and neutralized with 1M NaOMe. The residue was purified by size exclusion column chromatography (LH20) eluting with MeOH to afford 60 mg (83%) of an off-white foam: ^{1}H-NMR (D_2O) δ 1.14-1.32 (m, 4H, H-e), 1.38-1.68 (m, 12H, H-d, H-f, H-j, H-k), 1.98 (s, 6H, NAc), 1.99 (t, 4H, H-c), 2.52-2.68 (m, 2H, H-l), 2.85-2.99 (m, 2H, H-i), 3.10-3.38 (m, 10H, H-h, H-g, H-b'), 3.39-3.54 (m, 4H, H-a', H-b), 3.60-3.78 (m, 6H, H-6's, H-a), 3.75-3.86 (m, 4H, H-3, H-5), 3.91 (bs, 2H, H-4), 4.12 (dd, 2H, H-2), 4.89 (bs, 2H, H-1); FAB-MS (pos. m/z) calcd. for $C_{40}H_{74}N_8O_{16}$: 922.52; found: 923.59 (M^+ + 1, 3.2%).

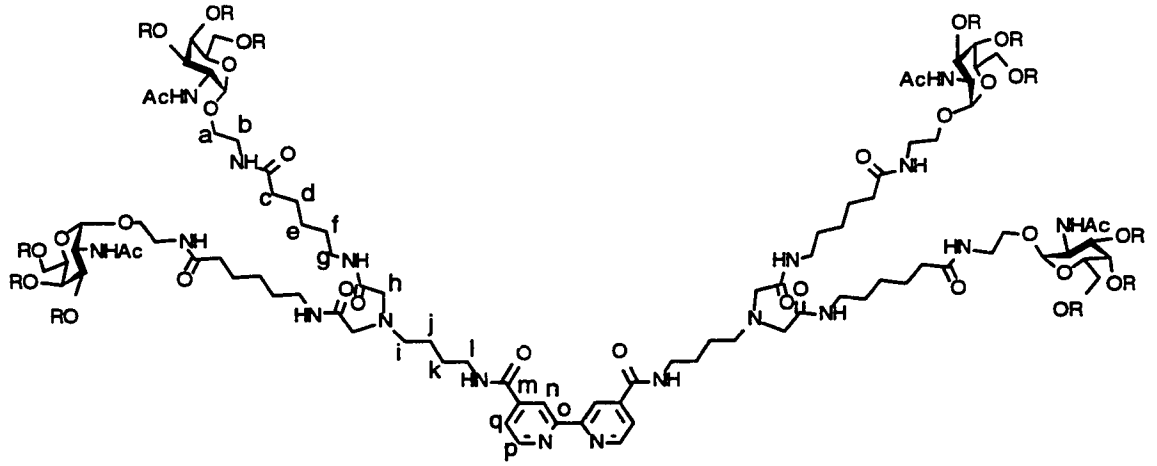
Coupling of short spacer armed dimer 121 with bipyridine (123).



4,4'-Bis(chlorocarbonyl)-2,2'-bipyridine (**108**) was prepared as described previously by refluxing 2,2'-bipyridine-4,4'-bicarboxylic acid (10 mg, 0.041 mmol) with $SOCl_2$ (3 mL) for 2 h. Compound **116** (86 mg, 0.082 mmol) was treated with 20% TFA in CH_2Cl_2 (5 mL) for 3 h. The solvent and the residual TFA were co-evaporated with toluene. To a solution of amine salt and Et_3N (28 µL, 0.205 mmol) in CH_2Cl_2 (2 mL) was added a solution of carbonyl chloride in CH_2Cl_2 (2 mL) at 0 °C and stirred for 3 h. The pinkish solution was concentrated and the residue was purified by size exclusion column chromatography (LH 20) eluting with MeOH. The purified product was then treated with 1M NaOMe in pH 9 for 16 h. The solution was concentrated and size exclusion column chromatography (LH 20) of the crude product eluting MeOH yielded 61.5 mg (94%) of a white foam: **122**: ^{1}H-NMR ($CDCl_3$) δ 1.49-1.68 (m, 8H, H-e, H-f), 1.92, 199, 2.10 (3s, 48H, OAc, NAc), 2.55-2.68 (m, 4H, H-d), 3.10-3.25 (m, 8H, H-c), 3.31-3.55 (m, 12H, H-a', H-b, H-b'), 3.60-3.70 (m, 8H, H-a, H-g), 3.98-4.15 (m, 12H, H-5, H-6's), 4.52 (ddd, 4H, $J_{2,3}$ 11.4 Hz, $J_{2,NH}$ 9.4 Hz, H-2), 4.84 (d, 4H, $J_{1,2}$ 3.6 Hz, H-1), 5.07 (dd, 4H, $J_{3,4}$ 3.1 Hz, H-3), 5.29 (d, 4H, H-4), 6.92 (d, 4H, NHAc), 7.65 (m, 2H, NHCOAr), 7.73 (d, 2H, J 4.6 Hz, H-k), 7.80-7.88 (m, 2H, NH), 8.67 (s, 2H, H-i), 8.75 (d, 2H, J 4.6 Hz, H-l); FAB-MS (pos. m/z) calcd, for $C_{92}H_{132}N_{14}O_{42}$: 2104.86; found: 2106.56 (M^+ + 1, 8.0%); **123**: $[\alpha]_D$ +81.8 (*c* 0.22, DMSO); ^{1}H-NMR (D_2O) 1.62 (quintet, 4H, J 7.4 Hz, H-f), 1.67-1.74 (m, 4H, H-e), 2.09 (s, 12H, NAc), 2.62-2.72 (m, 4H, H-d), 3.90 (d, 8H, J 3.1 Hz, H-c), 3.44-3.62 (m, 16H, H-a, H-b, H-b',

H-g), 3.76-3.83 (m, 12H, H-6's, H-a), 3.87-3.93 (m, 8H, H-3, H-5), 4.00 (d, 4H, $J_{3,4}$ 3.2 Hz, H-4), 4.22 (dd, 4H, $J_{2,3}$ 11.0 Hz, H-2), 4.89 (d, 4H, $J_{1,2}$ 3.7 Hz, H-1), 7.85 (d, 2H, J 5.1 Hz, H-l), 8.45 (s, 2H, H-i), 8.87 (d, 2H, J 5.1 Hz, H-k); ^{13}C-NMR (D_2O) δ 21. 6 (NAc), 23.6 (C-f), 25.8 (C-e), 38.4 (C-b), 39.3 (C-g), 49.3 (C-2), 54.8 (C-d), 57.9 (C-c), 60.7 (C-6), 66.0 (C-a), 67.4 (C-3), 68.0 (C-4), 70.6 (C-5), 96.9 (C-1), 119.1 (C-i), 121.6 (C-l), 142.9 (C-k), 149.8 (C-j), 155.0 (C-h), 167.4 (C=OAr), 173.31, 173.86 (C=O's); FAB-HRMS (pos. m/z) calcd. for $C_{68}H_{109}N_{14}O_{30}$: 1601.7434; found: 1601.6491 (M^+ + 1, 3.6%); Anal. Calcd for $C_{68}H_{108}N_{14}O_{30}$: C, 50.99; H, 6.80; N, 12.24. Found: C, 50.82; H, 6.82; N, 12.66.

Coupling of long spacer armed dimer 119 with bipyridine (125).



2,2'-Bipyridine-4,4'-bicarboxylic acid (22.2 mg, 0.091 mmol) was treated with $SOCl_2$ (3 mL) and the solution was refluxed for 2 h. The *N*-Boc protecting group was removed by treating **118** (0.232 g, 0.182 mmol) with 20% TFA in CH_2Cl_2 (5 mL) for 2 h and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (5 mL) and Et_3N (62 µL, 0.456 mmol) was added at 0 °C. A solution of carbonyl chloride **108** in CH_2Cl_2 (3 mL) was added through a dropping funnel to the reaction mixture and the solution was stirred for 3 hours. The concentrated pinkish residue was purified by size exclusion column chromatography (LH 20, MeOH). The purified product was then dissolved in MeOH (10 mL) and 1M

NaOMe was added until pH 9. The basic solution was stirred at room temperature for 16 h. After which time, the reaction solution had become colorless. The solvent was evaporated and purification of the crude product by size exclusion column chromatography eluting with MeOH afforded 0.16 g (89%) of a white foam: **124**: FAB-HRMS (pos. m/z) calcd. for $C_{116}H_{177}N_{18}O_{46}$: 2559.2098; found: 2559.2719 (M^+ + 1, 0.5%); **125**: ^{1}H-NMR (D_2O) 1.23-1.32 (m, 8H, H-e), 1.43-1.62 (m, 16H, H-f, H-j, H-k), 1.73-1.84 (m, 8H, H-d), 2.08 (s, 12H, NAc), 2.23 (t, 8H, J 7.4 Hz, H-c), 3.17-3.25 (m, 12H, H-g, H-i), 3.31-3.37 (m, 4H, H-b'), 3.48-3.60 (m, 12H, H-l, H-h), 3.75-3.83 (m, 12H, H-a', H-b, H-b'), 3.87-3.97 (m, 16H, H-3, H-5, H-6, H-a), 3.99-4.03 (bs, 4H, H-4), 4.21 (dd, 4H, $J_{2,3}$ 10.9 Hz, H-2), 4.91 (d, 4H, $J_{1,2}$ 3.4 Hz, H-1), 7.83 (bs, 2H, H-q), 8.66 (bs, 2H, H-n), 8.82 (bs, 2H, H-p); ^{13}C-NMR (D_2O) δ 19.5 (NAc), 22.5 (C-f), 23.1 (C-e, C-k, C-j), 25.4 (C-d), 33.1 (C-c), 36.4 (C-b, C-g), 36.7 (C-l), 47.2 (C-2), 53.9 (C-i), 58.7 (C-h), 60.0 (C-6), 63.9 (C-a), 65.3 (C-3), 66.0 (C-o), 68.5 (C-5), 94.7 (C-1), 116.9 (C-n), 119.6 (C-q), 140.5 (C-p), 147.7 (C-o), 152.9 (C-m), 164.3, 164.9, 171.8, 174.2 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{92}H_{152}N_{18}O_{34}$: 2053.07; found: 2054.67 (M^+ + 1, 0.1%).

General method for the preparation of self-assembling clusters.

(A) Fe(II) coordinated clusters

Three equivalents of de-*O*-acetylated divalent bipyridine ligand **110** or **114**, or tetravalent ligand **123** or **125** was dissolved in deionized water (3 mL) and one equivalent of $FeSO_4 \bullet 7H_2O$ was added to the aqueous solution. As soon as the iron (II) was added, the color of the solution turned dark pinkish red. The reaction mixture was stirred at room temperature for 48 h. The reddish solution was then freeze-dried to afford a red lyophilized powder, **127**, **129**, **131**, and **133**.

(B) Cu(II) coordinated clusters

To a solution containing two equivalents of de-*O*-acetylated divalent bipyridine ligand **110** or **114**, or tetravalent ligand **123** or **125** in deionized water (3

mL) was added one equivalent of $CuSO_4 \bullet 5H_2O$. The reaction solution was then stirred at room temperature for 48 h. The color of the reaction solution was light bluish purple. The solution was then lyophilized to yield Cu(II) coordinated cluster, **126**, **128**, **130**, and **132**.

(Short dimer)$_3$ Fe • SO_4 (127).

^{1}H-NMR (D_2O) δ 1.82 (s, 18H, NAc), 3.64-3.82 (m, 30H, CH_2N, OC*H*H, H-6's), 3.85-3.98 (m, 24H, H-3, H-4, H-5, OCH*H*), 4.17-5.02 (m 6H, H-2), 4.80-4.92 (m, 6H, H-1), 7.75-7.78 (m, 6H, H-d), 7.80-7.84 (m, 6H, H-g), 9.08-9.10 (m, 6H, H-e); ^{13}C-NMR (D_2O) δ 21.8 (NAc), 39.4 (C-b), 49.3 (C-2), 60.6 (C-6), 65.7 (C-a), 67.2 (C-3), 68.0 (C-4), 70.5 (C-5), 96.9 (C-1), 122.3 (C-e), 124.6 (C-g); MALDI-TOF calcd. for $C_{96}H_{132}N_{18}O_{42}Fe$: 2264.81; found: 2265.10.

(Long dimer)$_2$ Cu • SO_4 (128).

^{1}H-NMR (D_2O) δ 1.38 (s, 8H, H-e), 1.65 (s, 16H, H-d, H-f), 2.08 (s, 12H, NAc), 2.31 (s, 8H, H-c), 3.09 (s, 8H, H-g), 3.37 (s, 4H, H-b'), 3.55 (s, 8H, H-a', H-b), 3.79 (s, 12H, H-6's, H-a), 3.92, 3.95 (2s, 8H, H-3, H-5), 4.03 (s, 4H, H-4), 4.22 (s, 4H, H-2), 4.87 (s, 4H, H-1), 7.70, 7.80, 9.03 (bs, H-i, H-j, H-l); ^{13}C-NMR (D_2O) δ 19.5 (NAc), 22.5 (C-f), 23.1 (C-e), 24.3 (C-d), 33.1 (C-c), 36.4 (C-b), 37.4 (C-g), 47.3 (C-2), 58.6 (C-6), 63.9 (C-a), 65.3 (C-3), 65.9 (C-4), 68.5 (C-5), 94.7 (C-1), 172.0, 174.4 (C=O's), arometic peaks are not detectable; FAB-MS (pos. m/z) calcd. for $C_{88}H_{132}N_{16}O_{32}Cu$: 1987.85; found: 1988.14 (M^+ + 1, 0.8%), 1025.45 (monomer + Cu, 8.1%); MALDI-TOF calcd for $C_{88}H_{132}N_{16}O_{32}Cu$: 1987.85; found: 1989.39.

(Long dimer)$_3$ Fe • SO_4 (129).

^{1}H-NMR (D_2O) δ 1.37-1.46 (m, 12H, H-e), 1.62-1.72 (m, 24H, H-d, H-f), 2.04 (s, 18H, NAc), 2.30 (t, 12H, J 6.8Hz, H-c), 3.30-3.38 (m, 6H, H-b'), 3.42-3.50 (m, 12H, H-g), 3.50-3.57 (m, 12H, H-a', H-b), 3.72-3.78 (m, 18H, H-a, H-6's), 3.87-

3.93 (m, 12H, H-3, H-5), 3.91 (d, 6H, H-4), 4.18 (dd, 6H, H-2), 4.86 (d, 6H, H-1); ^{13}C-NMR (D_2O) δ 19.5 (NAc), 22.6 (C-d), 23.1 (C-e), 25.3 (C-f), 33.1 (C-c), 36.4 (C-b), 37.6 (C-g), 47.2 (C-2), 58.6 (C-6), 63.9 (C-a), 65.3 (C-3), 65.9 (C-4), 68.5 (C-5), 94.7 (C-1), 119.4 (C-j), 122.2 (C-l), 141.3 (C-k), 152.4 (C-i), 156.8 (C-h), 163.4, 171.8, 174. 4 (C=O's); MALDI-TOF calcd. for $C_{132}H_{198}N_{24}O_{48}Fe$: 2943.31; found: 2943.44.

(Short tetramer)$_2$ Cu • SO_4 (130).

^{1}H-NMR (D_2O) δ 1.56 (s, 8H, H-f), 1.64 (s, 8H, H-e), 2.10 (s, 24H, NAc), 2.63 (s, 8H, H-d), 3.33 (s, 16H, H-c), 3.47-3.63 (m, 32H, H-a', H-b, H-b', H-g), 3.81 (s, 24H, H-a, H-6's), 3.93 (s, 16H, H-3, H-5), 4.02 (s, 8H, H-4), 4.23 (s, 8H, H-2), 4.94 (s, 8H, H-1); ^{13}C-NMR (D_2O) δ 19.6 (NAc), 22.8 (C-f), 22.8 (C-e), 36.4 (C-b), 37.4 (C-g), 47.3 (C-2), 52.7 (C-d), 55.8 (C-c), 58.7 (C-6), 64.0 (C-a), 65.4 (C-3), 66.0 (C-4), 68.6 (C-5), 94.9 (C-1), 171.2, 171.8 (C=O's); MALDI-TOF calcd. for $C_{136}H_{216}N_{28}O_{60}Cu$: 3264.41; found: 3267.76.

(Short tetramer)$_3$ Fe • SO_4 (131).

^{1}H-NMR (D_2O) δ 1.60 (s, 24H, H-f, H-e), 2.09 (s, 36H, NAc), 2.64 (s, 12H, H-d), 3.35 (s, 24H, H-c), 3.53 (s, 48H, H-a, H-b, H-b', H-g), 3.75-4.12 (m, 72H, H-a', H-6's, H-3, H-5, H-4), 4.21 (s, 12H, H-2), 4.93 (s, 12H, H-1), 7.75 (s, 6H, H-l), 7.83 (s, 6H, H-i), 9.03 (s, 6H, H-k); ^{13}C-NMR (D_2O) δ 21.6 (NAc), 23.6 (C-f), 25.8 (C-e), 38.4 (C-b), 39.6 (C-g), 49.3 (C-2), 54.7 (C-d), 57.9 (C-c), 60.8 (C-6), 66.1 (C-a), 67.5 (C-3), 68.0 (C-4), 70.7 (C-5), 96.9 (C-1), 121.5 (C-i), 124.3 (C-l), 143.4 (C-k), 154.6 (C-j), 158.9 (C-h), 165.4 (C=OAr), 173.3, 173.8 (C=O's); MALDI-TOF calcd. for $C_{204}H_{324}N_{42}O_{90}Fe$: 4858.14; found: 4859.72.

(Long tetramer)$_2$ Cu • SO_4 (132).

^{1}H-NMR (D_2O) δ 1.37 (s, 16H, H-e), 1.49-1.81 (m, 48H, H-f, H-d, H-j, H-k), 2.11 (s, 24H, NAc), 2.32 (s, 16H, H-c), 2.82-3.49 (m, 32H, H-i, H-g, H-b, H-b'),

3.60 (s, 32H, H-l, H-h, H-a'), 3.82 (s, 24H, H-a, H-6's), 3.90-4.02 (m, 16H, H-3, H-5), 4.04 (s, 8H, H-4), 4.21-4.32 (m, 8H, H-2), 4.94 (s, 8H, H-1); ^{13}C-NMR (D_2O) δ 19.5 (NAc), 22.6 (C-f), 23.1 (C-e, C-k, C-j), 25.5 (C-d), 33.2 (C-c), 36.3 (C-b), 36.4 (C-g), 36.6 (C-l), 47.3 (C-2), 58.7 (C-h), 60.0 (C-6), 63.9 (C-a), 65.3 (C-5), 66.0 (C-4), 68.6 (C-3), 94.7 (C-1), 171.9, 171.9, 174.4 (C=O's); MALDI-TOF calcd. for $C_{184}H_{304}N_{36}O_{68}Cu$: 4169.07; found: 4170.19.

(Long tetramer)$_3$ Fe • SO_4 (133).

^{1}H-NMR (D_2O) δ 1.22 (s, 24H, H-e), 1.44-1.60 (m, 48H, H-f, H-j, H-k), 1.61-1.70 (m, 24H, H-d), 2.00 (s, 36H, NAc), 2.22 (s, 24H, H-c), 2.84 (s, 12H, H-i), 3.15-3.26 (m, 24H, H-b, H-g), 3.28-3.40 (m, 12H, H-b'), 3.45-3.60 (m, 48H, H-a', H-h, H-l), 3.70 (s, 36H, H-a, H-6's), 3.76-3.86 (m, 24H, H-3, H-5), 3.93 (s, 12H, H-4), 4.13 (d, 12H, H-2), 4.88 (s, 12H, H-1), 7.65 (s, 6H, H-q), 7.74 (s, 6H, H-n), 9.00 (s, 6H, H-p); ^{13}C-NMR (D_2O) δ 19.5 (NAc), 22.6 (C-f), 23.1 (C-e, C-k, C-j), 25.5 (C-d), 33.1 (C-c), 36.4 (C-b), 36.5 (C-q), 36.6 (C-l), 47.2 (C-2), 54.6 (C-i), 58.6 (C-h), 60.0 (C-6), 63.8 (C-a), 65.3 (C-3), 65.9 (C-4), 68.5 (C-5), 94.7 (C-1), 119.3 (C-n), 122.5 (C-q), 141.1 (C-p), 152.5 (C-o), 156.7 (C-m), 163.0, 171.9, 174.0, 174.1 (C=O's) MALDI-TOF calcd. for $C_{276}H_{456}N_{54}O_{102}Fe$: 6215.15; found: 6217.87.

Enzyme-Linked Lectin Assay (ELLA).

Nunc microtitration plates were coated with asialoglycophorin at 100 μL/well of a stock solution of 5 μg/mL in 0.01M phosphate buffer (PBS, pH 7.3) for 2 h at 37 °C. The wells were then washed three times with 300 μL/well of 0.01 phosphate buffer (ph 7.3) containing 0.05% (v/v) Tween 20 (PBST). This washing procedure was repeated after each incubation period. Wells were then blocked with 150 μL/well of 1% BSA/PBS for 1 h. Inhibitors used include allyl α-D-GalNAc as a reference monovalent compound and synthesized multivalent GalNAc-containing ligands which were used as stock solution of 0.317 μM in PBS. Each inhibitor was added in serial 2- to 10-fold dilutions (60 μL/well) in PBS with

appropriate lectin-enzyme conjugate concentration (60 µL/well of 500-fold dilution of a 1 mg/mL stock solution of *vicia villosa* in PBS) on Linbro (Titertek) microtiter plates. These inhibitor solutions (100 µL) was then transferred to an antigen-coated plates and incubated for another hour at 37 °C. The plates were washed and 50 µL/well of 2,2'-azinobis(3-ethylbenzothiazolin-6-sulonic acid), diammonium salt (ABTS, 1 mg/4 mL) in citrate-phosphate buffer (0.2M, pH 4.0 with 0.015% H_2O_2) was added. The reaction was stopped after 20 min. by adding 50 µL/well of 1M H_2SO_4 and optical density measured at 410 nm relative to 570 nm. The percent inhibition was calculated as follows:

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

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

Chapter 5. Glycodendrimers based on the *t*-butyl calix[4]arene

5.1. Introduction

During the last decade, calixarenes have been attracting great interest not only in pure science, but also in various industrial applications[187] utilizing host-guest chemistry.[188] In spite of their novel properties including pre-organization, diversity on either the upper or lower rim and dimensionality, no effort was made to evaluate the biological behaviors of calixarenes which contain carbohydrate moieties.[189]

In this chapter, the synthesis of water soluble carbohydrate-containing *t*-butylcalix[4]arenes and their lectin binding properties on the basis of their well-defined structure at the molecular level are described. Carbohydrate-containing calix[4]arenes can serve as another model to demonstrate the multivalent effect which is shown by many other glycodendrimers.[190] Hydrophobic substituent on the lower rim of calix[4]arene core, such as *t*-butyl groups, can also be anticipated to provide the driving force for stable surface adhesion or assembly, while the hydrophilic carbohydrates mimic the cell's saccharide-rich surface. This type of molecule can, for example, be employed as a coating hapten in competitive solid-phase immunoassays (Figure 5.1.1).

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[187] For example, C_{60} has been isolated using *p-t*-butylcalix[8]arene in large quantity and with high purity: Raston, C. L.; Atwood, J. L.; Nichols, P. J.; Sudria, I. B. N. *J. Chem. Soc., Chem. Commun.* **1996**, 2615.

[188] For recent reviews, see: (a) Akida, A.; Shinkai, S. *Chem Rev.* **1997**, *97*, 1713, (b) Takeshita, M.; Shinkai, S. *Bull. Chem. Soc., Jpn.* **1995**, *68*, 1008, (c) Gutsche, C. D. *Aldrichimica Acta* **1995**, *28*, 3, (d) Lhotak, P.; Shinkai, S. *J. Synth. Org. Chem., Jpn.* **1995**, *53*, 963.

[189] For recent calixsugars, see: (a) Marra, A.; Scherrmann, M.-C.; Dondoni, A.; Casnati, A.; Minari, P.; Ungaro, R. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2479, (b) Marra, A.; Dondoni, A.; Sansone, F. *J. Org. Chem.* **1996**, *61*, 5155, (c) Meunier, S. J.; Roy, R. *Tetrahedron Lett.* **1996**, *37*, 5496.

[190] (a) Zanini, D.; Roy, R. *J. Am. Chem. Soc.* **1997**, *119*, 2089, (b) Pagé, D.; Aravind, S.; Roy, R. *Chem. Commun.* **1996**, *1913*, (c) Zanini, D.; Roy, R. *J. Org. Chem.* **1996**, *61*, 7348, (d) Roy, R. *Curr. Opin. Struct. Biol.* **1996**, *6*, 692, and references therein.

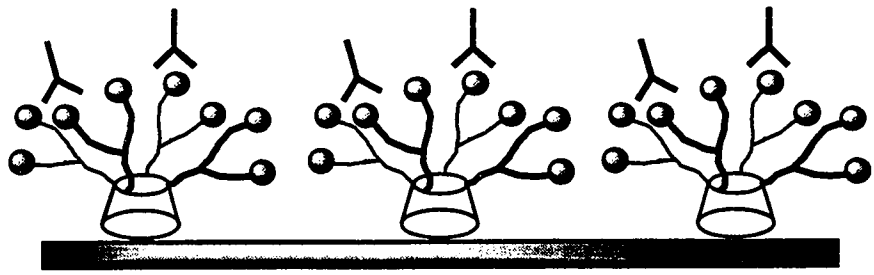


Figure 5.1.1. Glycocalix[4]arene as hybrid molecule used as coating antigen on polystyrene surface; ● sugar, ⊱ carbohydrate-binding protein.

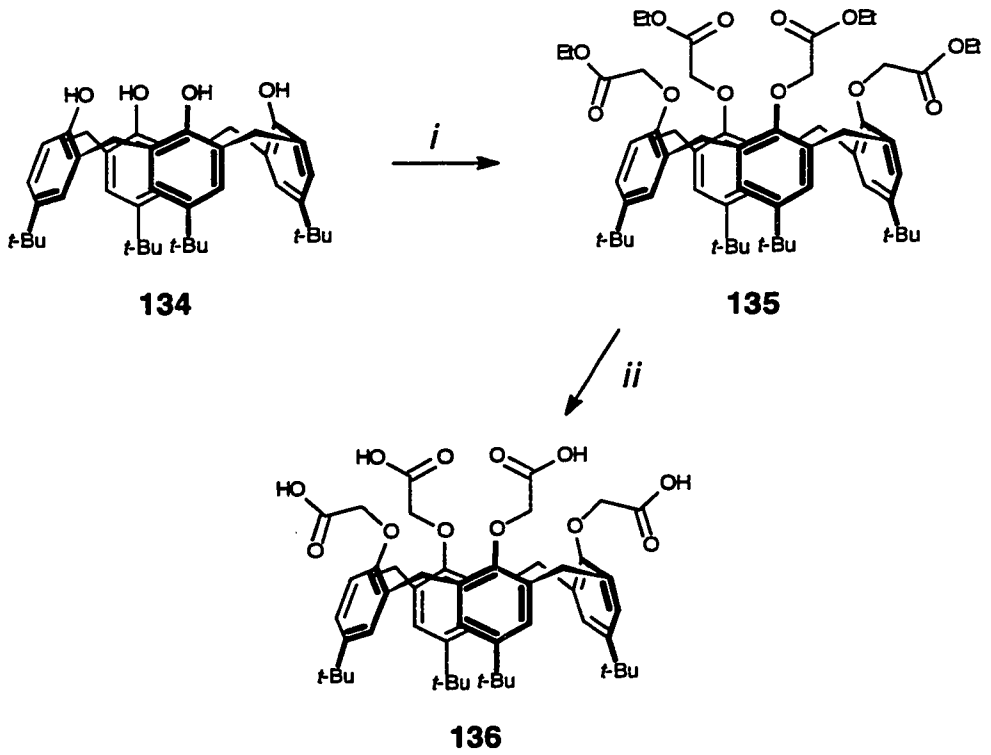
The model carbohydrate involved herein consists of Tn-antigen (GalNAcα1-*O*-Ser/Thr) corresponding to one of the immunodominant epitopes found in human adenocarcinomas' mucins.[191] This family of carbohydrate-associated tumor markers are normally cryptic in normal cells. Roy and coworkers have also recently shown that the *O*-linked Ser/Thr residues in the analogous T-antigen (Galβ1→3GalNAcα1-*O*-Ser/Thr) were not essential to generate mouse monoclonal antibodies that recognize cancer tissues.[192] Consequently, the α-linked GalNAc moieties described herein was deprived of the *O*-Ser/Thr aglycone.

[191] (a) Toyokuni, T.; Singhal, A. *Chem. Soc. Rev.* **1995**, *24*, 231, (b) Makomori, S. *Curr. Opin. Immunol.* **1991**, *3*, 646.
[192] Rittenhouse-Diakun, K.; Xia, Z.; Pickhardt, D.; Baek, M.-G.; Roy, R. *Hybridoma* **1998**, *17*, 165.

5.2. Synthesis of glycocalix[4]arenes

The main synthetic strategy was to attach a spacer-armed carbohydrate residue to the calix[4]arene core in a convergent or a divergent manner.

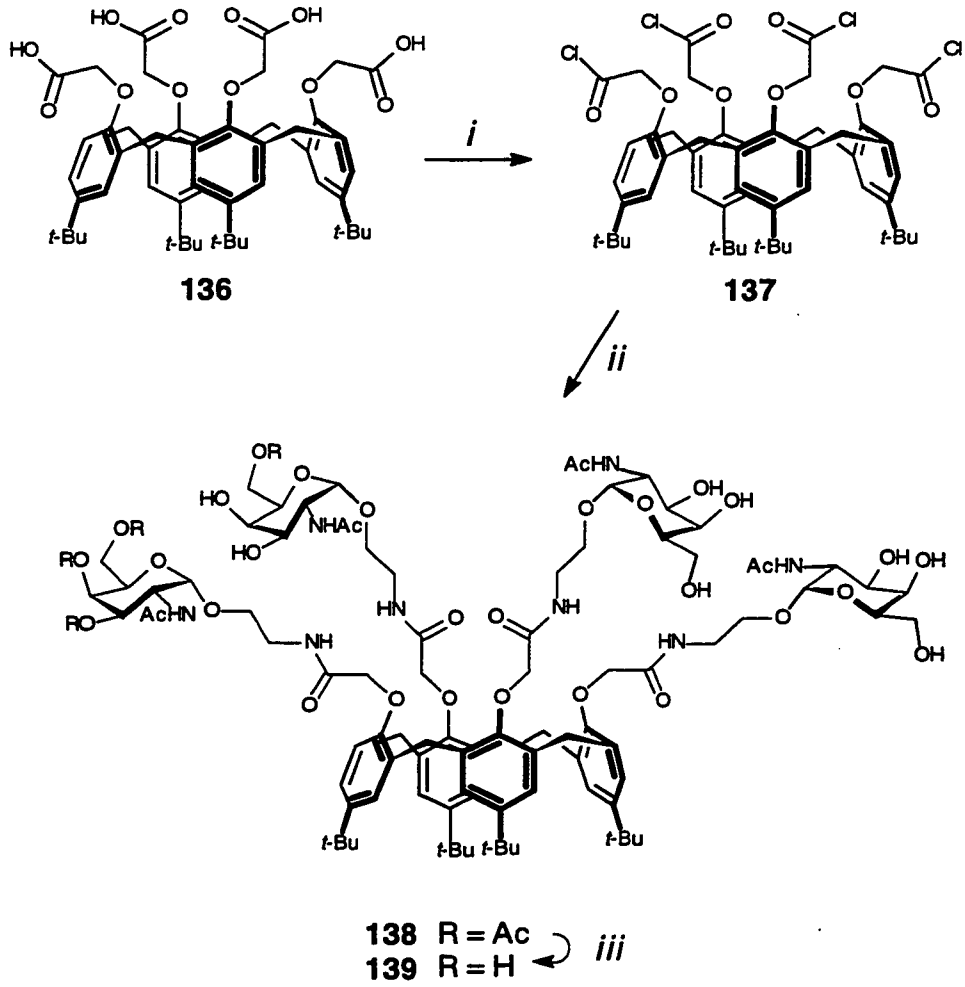
The calix[4]arene core was prepared by transformation of commercial *p-t*-butylcalix[4]arene (**134**) into tetraethyl ester **135** followed by hydrolysis to form tetraacid **136** (Scheme 5.2.1). The calix[4]arene core was stored as tetraacid **136** form and converted into the acid chloride **137** by treating with thionyl chloride ($SOCl_2$, reflux, 2h) as needed.



134 **135** **136**

Scheme 5.2.1. Synthesis of tetraacid **136**. *i*) $BrCH_2CO_2Et$ (20 eq), K_2CO_3 (20 eq), acetone, MS-4Å, reflux, 24 h, 84%; *ii*) 1M KOH, EtOH, (1:1.1, v/v), reflux, 9 h, 90%.

The tetravalent glycocalix[4]arene **138** was prepared by coupling α-GalNAc homoserine **102** to the calix[4]arene core in acid chloride form **137** in the presence of Et_3N (Scheme 5.2.2).



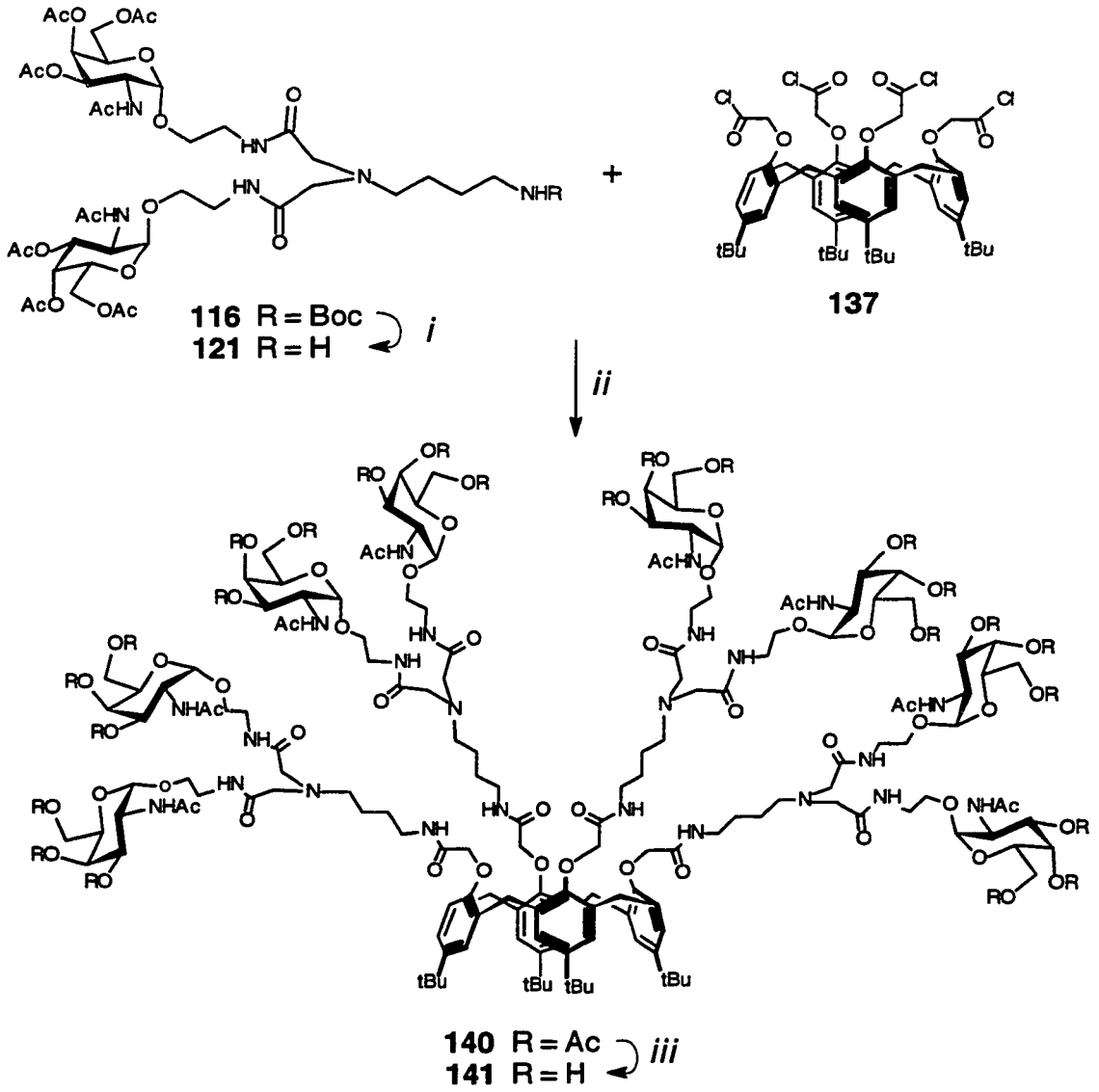
Scheme 5.2.2. Synthesis of tetramer **139**. *i*) $SOCl_2$, reflux, 2 h, quant.; *ii*) α-GalNAc homoserine **102** (6 eq), Et_3N (12 eq), CH_2Cl_2, 0 °C, 2h, 74%; *iii*) 1M NaOMe, MeOH, pH 9, 23 °C, 2 h, 94%.

For the purpose of complete conversion from amine to amide in each reaction site, 1.5 equivalents of amine **102** were used for each acid chloride (CH_2Cl_2, 2 h, 74%). The reaction was monitored by TLC and the crude product was purified by silica gel chromatography. De-*O*-acetylation of the resulting tetramer **138** was done under Zemplén condition (1M NaOMe, MeOH, pH 9). However, insolubility of the deprotected tetramer in MeOH caused precipitation as the reaction proceeded. The precipitates were collected on a fritted glass funnel to afford a white solid, tetramer **139** (94% yield).

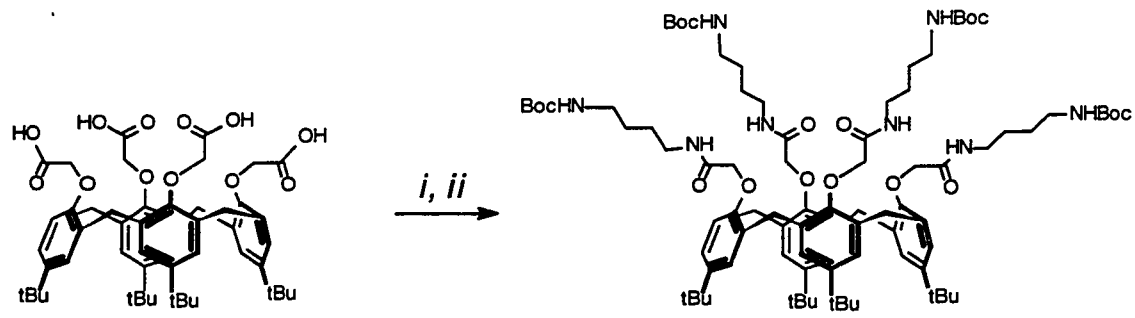
The octavalent glycocalix[4]arenes **140** and **144** were synthesized by convergent and divergent manners, respectively. For the synthesis of short-spacer-armed octavalent glycocalix[4]arene **140**, the pre-synthesized divalent GalNAc ligand **116** (see chapter 4.2) was attached to the calix[4]arene core after removing N-Boc protecting group (20% TFA, Ch_2Cl_2). The coupling process was evaluated on the TLC plate and the reaction was complete after 3 hours at 0 °C. After simple washing with water, the crude product was de-*O*-acetylated in a sodium methoxide solution. The solution remained clear as the reaction proceeded, therefore the product was purified by size exclusion column chromatography (LH20, MeOH, 73%). The resulting compound was then lyophilized to afford an off-white powdered **141** (Scheme 5.2.3).

This convergent method was initially applied to try to synthesize octavalent glycocalix[4]arene **144** which bears a long spacer arm. However, the reaction did not provide the desired product. It was presumed that the congestion of the area by the approaching flexible long-spacer-armed GalNAc ligand inhibited completion of the reaction. Therefore, the divergent method was employed. First *N*-Boc-1,4-diaminobutane was attached to the calix[4]arene carbonyl chloride core **137** to give **142** (DIPEA, CH_2Cl_2, 3h, 63%) (Scheme 5.2.4) and then each *N*-Boc terminating reaction site on **142** was *N,N'*-dialkylated with long-spacer-armed bromoacetylated GalNAc ligand **117** after *N*-Boc deprotection. Each amine on the

calix[4]arene **143** was treated with 2.5 equivalents of bromoacetylated GalNAc ligand **117**.



Scheme 5.2.3. Synthesis of short-spacer-armed octamer **141**. *i*) 20% TFA, CH_2Cl_2, 23 °C, 2 h, quant.; *ii*) DIPEA, CH_2Cl_2, 0 °C, 3 h; *iii*) 1M NaOMe, MeOH, pH 9, 23 °C, 16 h, 73%.



136 142

Scheme 5.2.4. Synthesis of tetravalent N-Boc protected amine **142**. *i*) $SOCl_2$, reflux, 2 h, quant.; *ii*) $BocHN(CH_2)_4NH_2$ (6 eq), DIPEA (12 eq), CH_2Cl_2, 0 °C, 3 h, 63%.

After 48 hours, the reaction mixture was washed with water to remove residual salt produced from the reaction and treated with sodium methoxide solution for de-*O*-acetylation of the carbohydrate residue. The resulting de-*O*-acetylated octavalent glycocalix[4]arene **145** was purified by size exclusion column chromatography (LH20, MeOH) and then lyophilized to afford an off-white powder (64%) (Scheme 5.2.5).

Since the *N,N'*-dialkylation strategy performed successfully the synthesis of octavalent glycocalix[4]arene **145**, it was also applied to the synthesis of doubly branched hexadecamer **150**. Tetraaminocalix[4]arene, after *N*-Boc deprotection, was dialkylated with bromoacetylated *N*-Boc-1,4-diaminobutane **147** (Scheme 5.2.6) to produce branched *N*-Boc-octaamino-calix[4]arene **148** in 51% yield (Scheme 5.2.7). Hexadecavalent glycocalix[4]arene **149** was then prepared utilizing the *N*-Boc-octaamino-calix[4]arene **148** and the long-spacer-armed bromoacetylated GalNAc ligand **117** (Scheme 5.2.8). The reaction mixture was heated at 60 °C for 48 hours in acetonitrile with DIPEA and then the *O*-acetyl groups on the carbohydrate residues were removed under Zemplén conditions. Purification (LH20, MeOH) of the crude product followed by lyophilization yielded an off-white powdered hexadecamer **150** in 69% yield.

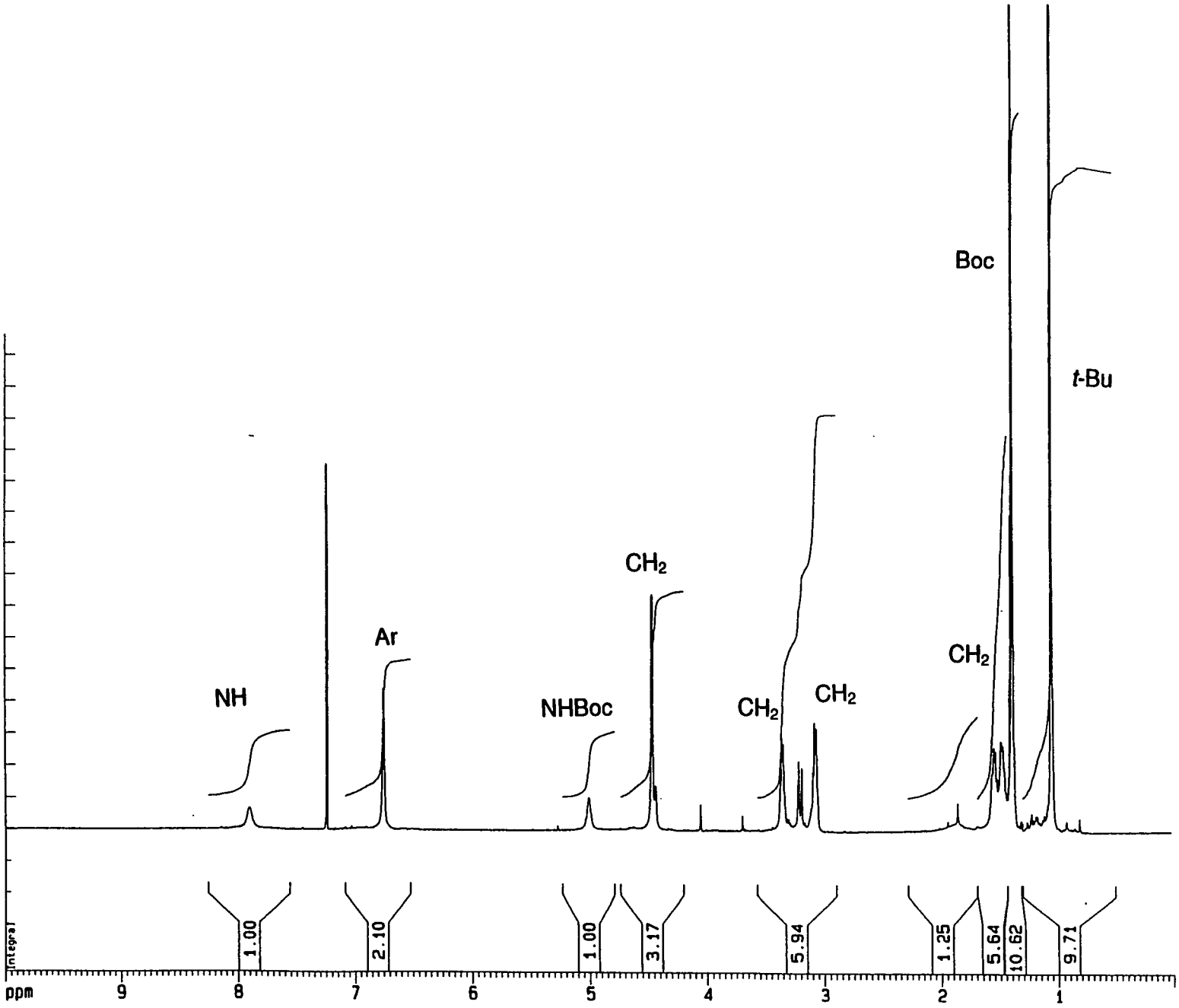
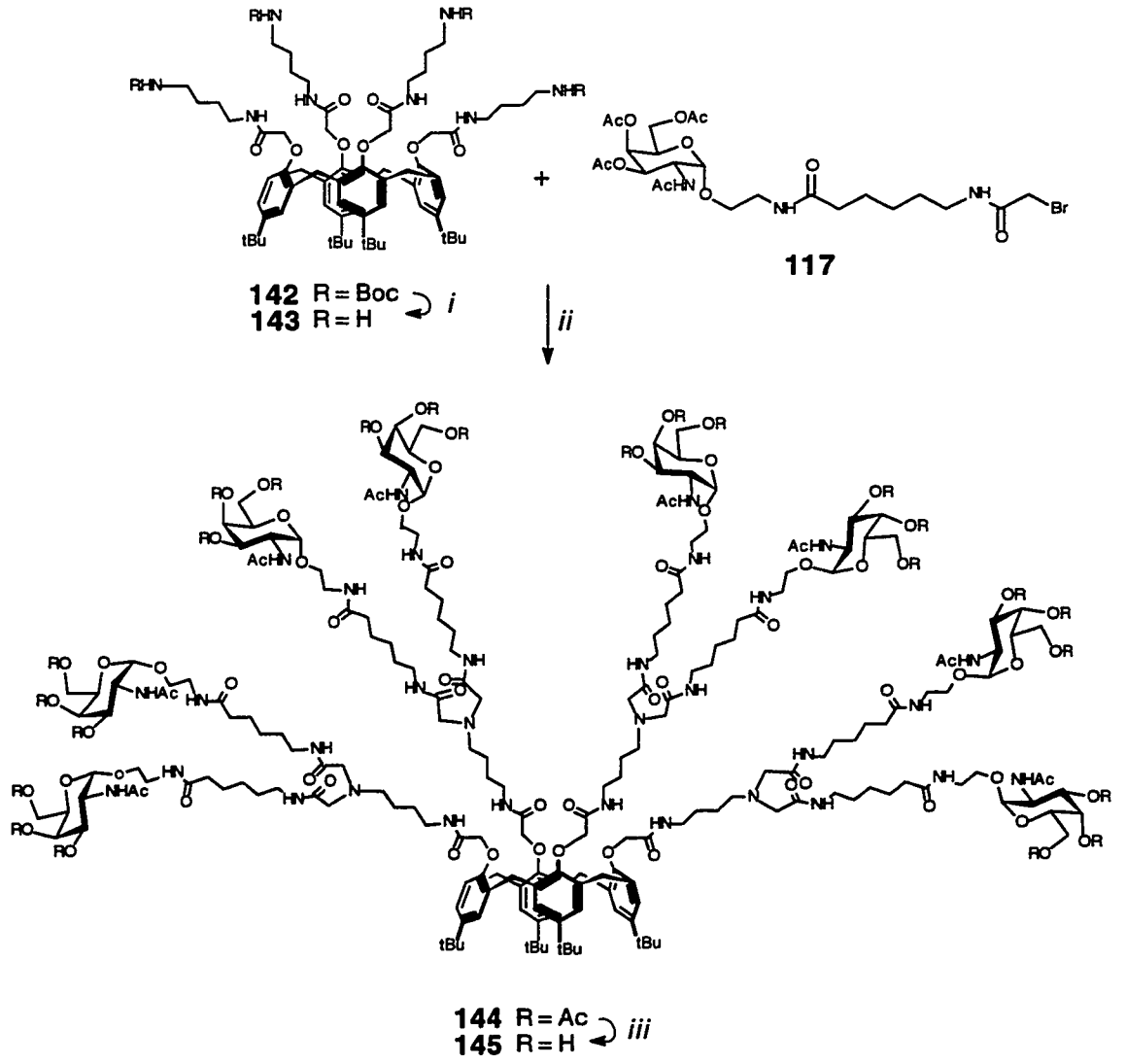
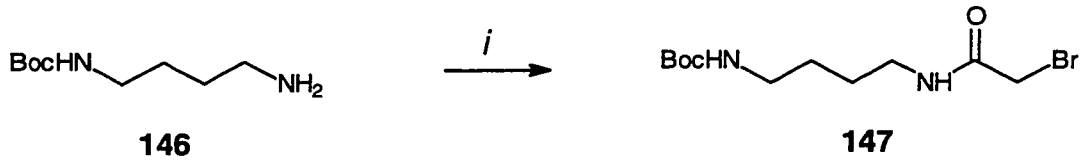


Figure 5.2.2. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of tetravalent N-Boc protected amine **142**.

^{1}H-NMR spectroscopy (in D_2O) suggested that these glycocalix[4]arenes are in the *cone* conformation based on the singlets for the aromatic (6.8 ppm) and the *t*-butyl protons (1.1 ppm).

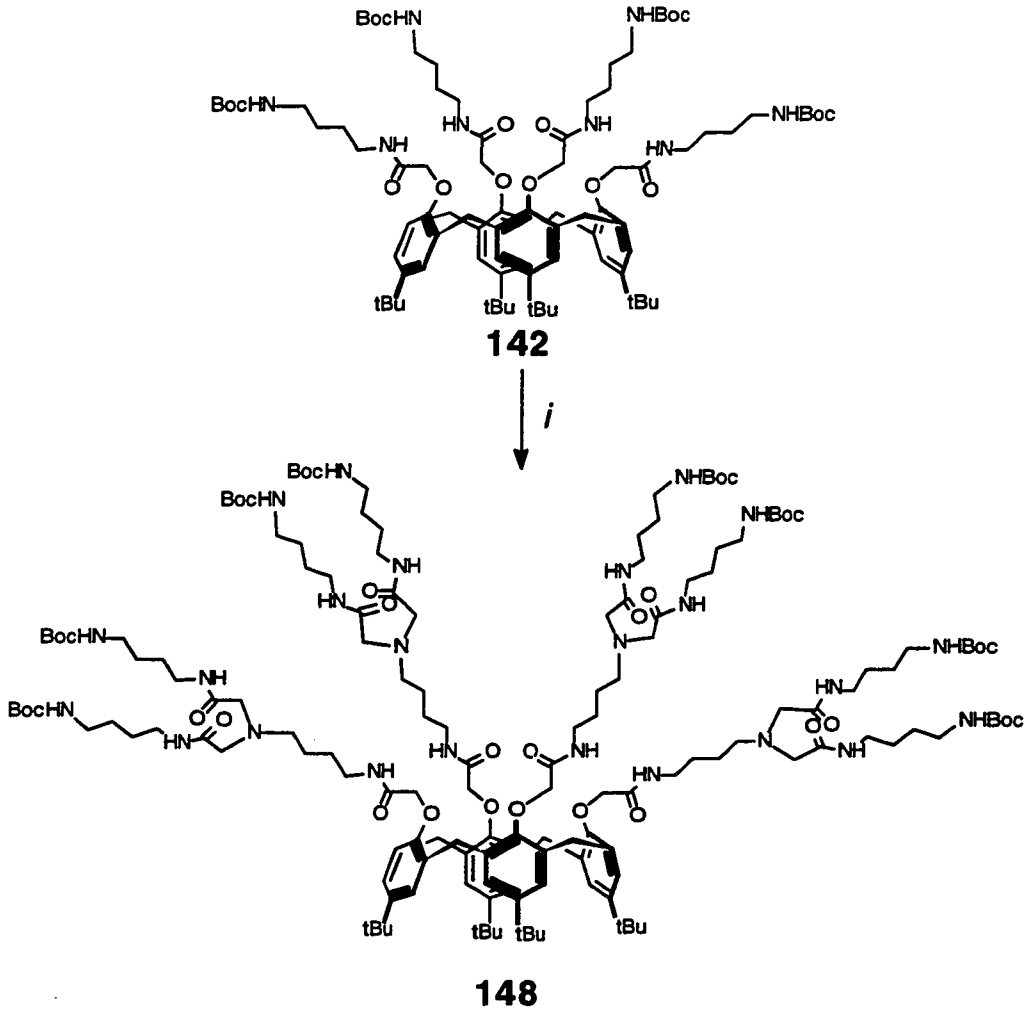


Scheme 5.2.5. Synthesis of long-spacer-armed octamer **145**. *i*) 20% TFA, CH_2Cl_2, 23 °C, 2 h; *ii*) DIPEA, CH_3CN, 60 °C, 48 h; *iii*) 1M NaOMe, MeOH, pH 9, 23 °C, 16 h, 64%.



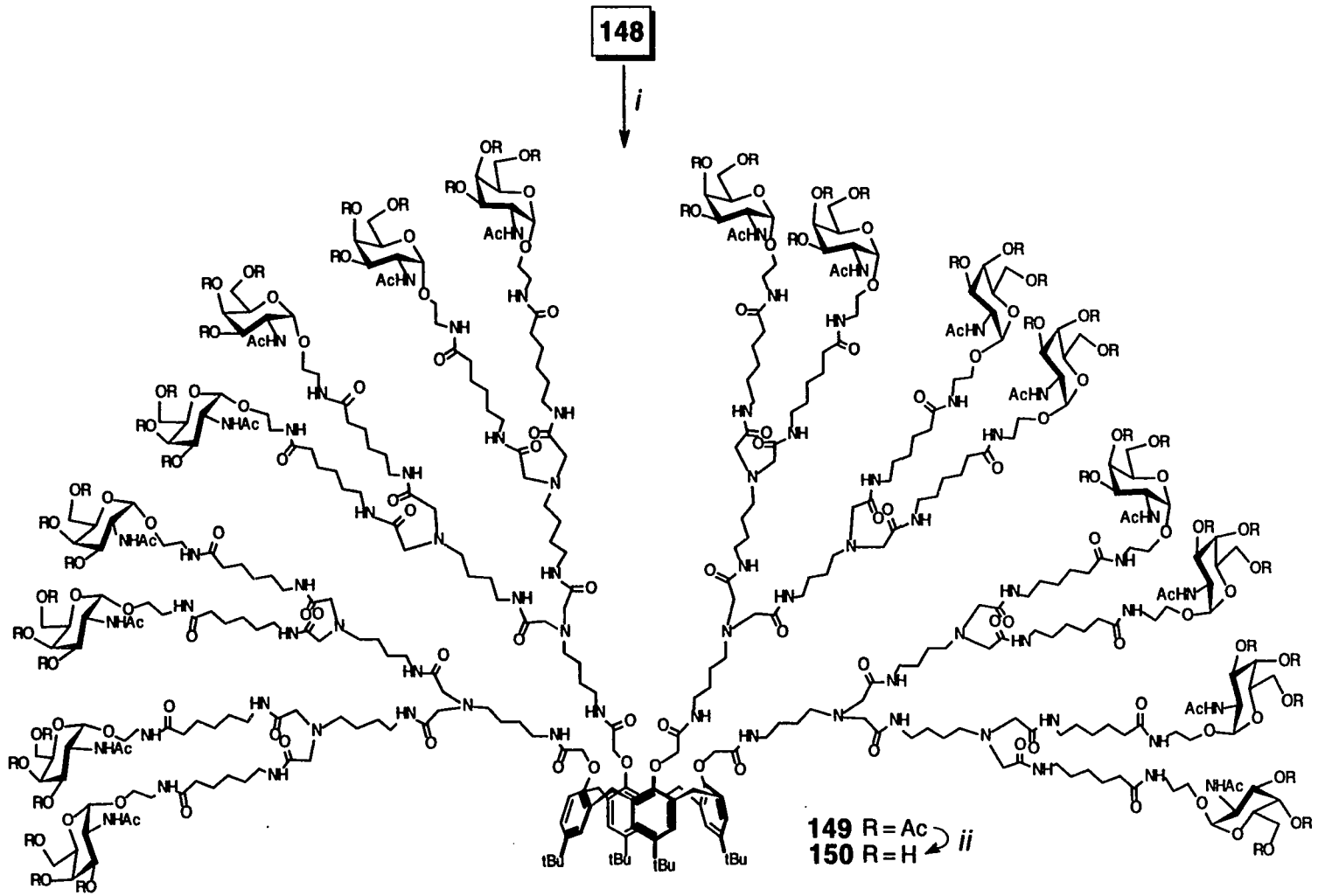
146 147

Scheme 5.2.6. Synthesis of bromoacetylated *N*-Boc-1,4-diaminobutane **147**. *i*) $ClCOCH_2Br$ (1.2 eq), DIPEA (1.2 eq), CH_2Cl_2, 0 °C, 30 min., 99%.



142 148

Scheme 5.2.7. Synthesis of octavalent *N*-Boc calix[4]arene **148**. *i*) (1) 20% TFA, CH_2Cl_2, 23 °C, 2 h; (2) $BocHN(CH_2)_4NHCOCH_2Br$ (**147**) (10 eq), DIPEA (14 eq), CH_3CN, 60 °C, 48 h, 51%.



Scheme 5.2.8. Synthesis of hexadecamer **150**. *i*) (1) 20% TFA, CH_2Cl_2, 23 °C, 2 h, (2) **117** (20 eq), DIPEA (30 eq), CH_3CN, 60 °C, 48 h, 71%; *ii*) 1M NaOMe, MeOH, pH 9, 23 °C, 16 h, 97%.

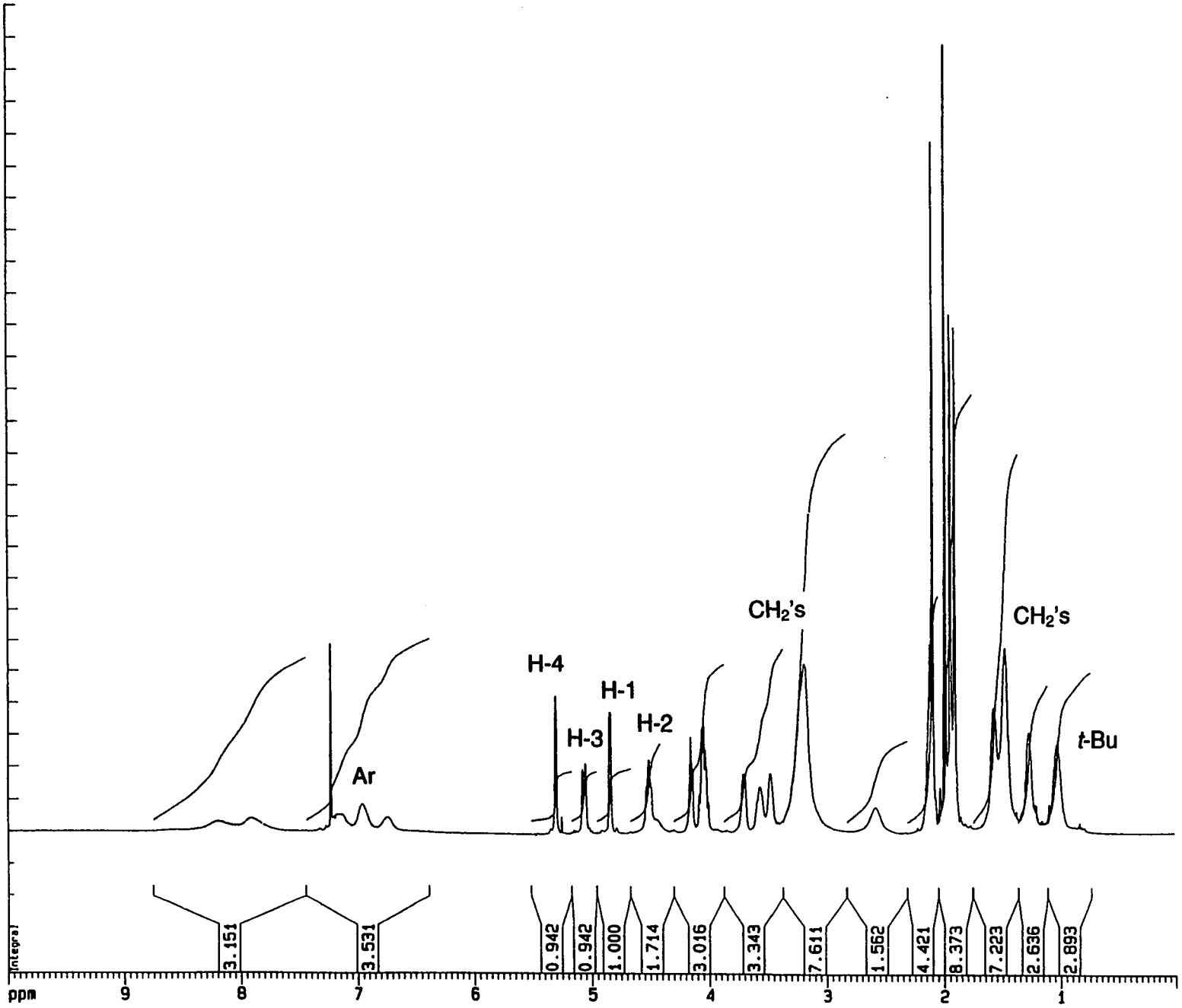


Figure 5.2.3. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of fully protected hexadecamer **149**.

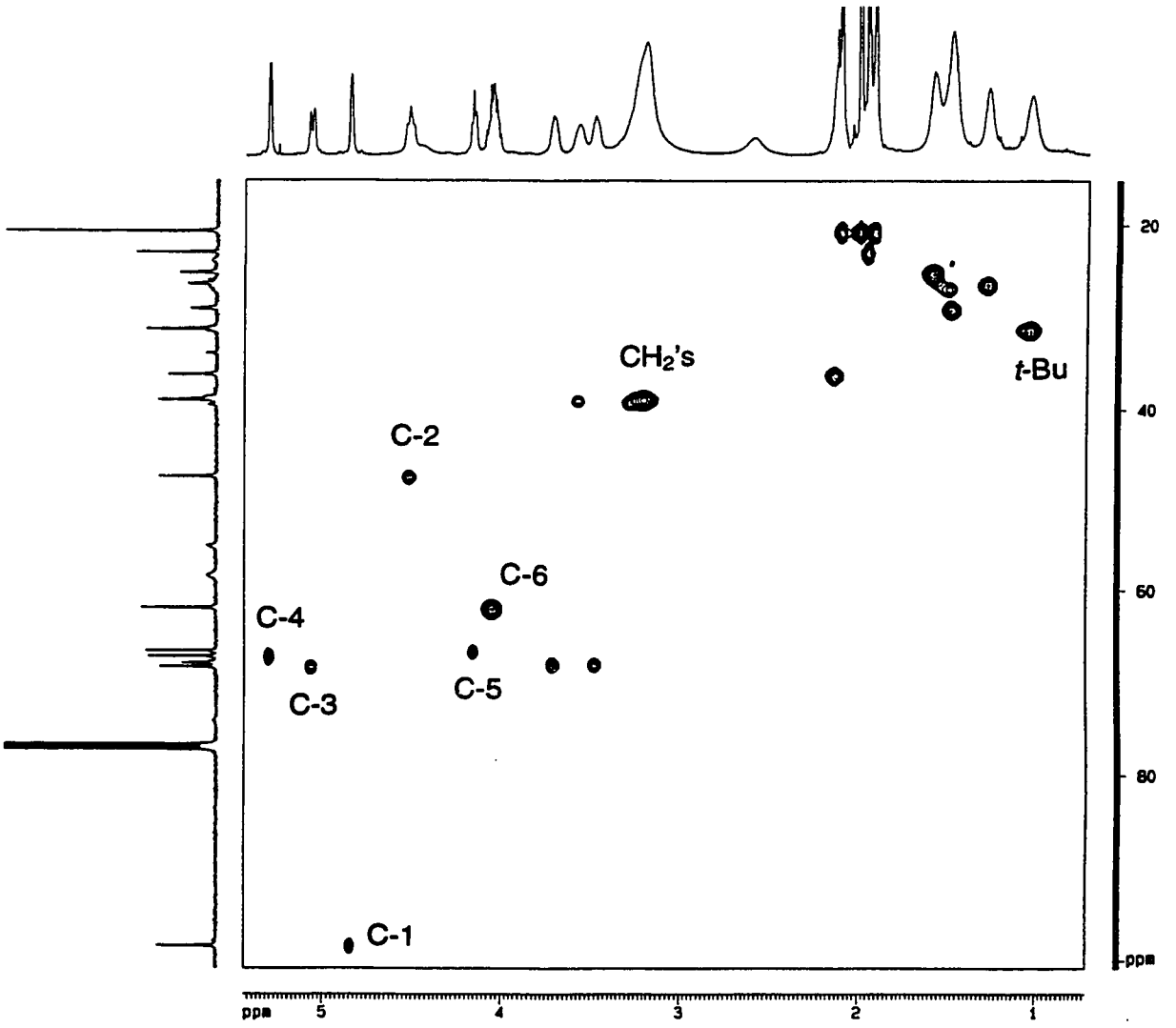


Figure 5.2.4. HMQC ($CDCl_3$, 500 MHz) spectrum of fully protected hexadecamer **149**.

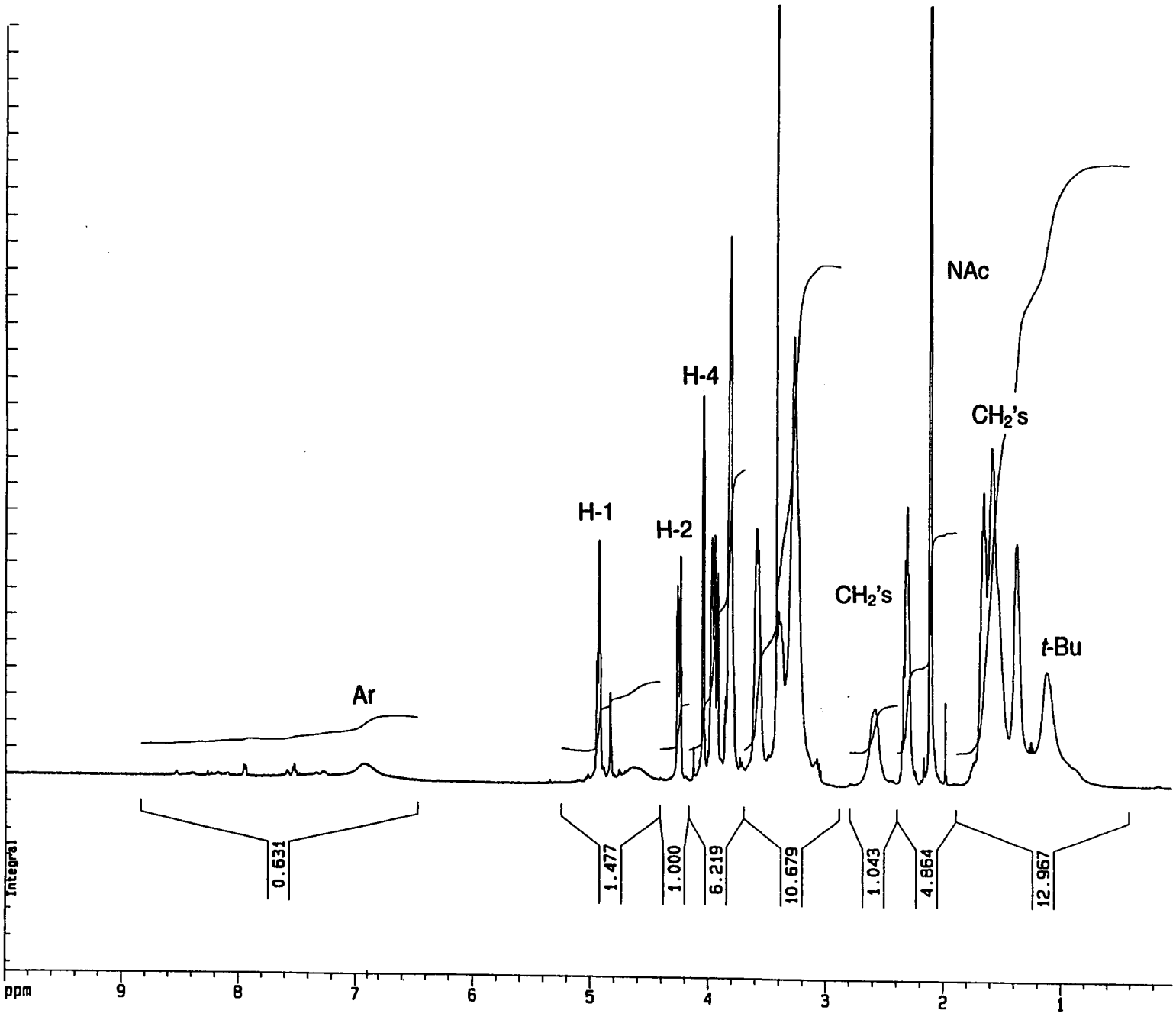


Figure 5.2.5. ^{1}H-NMR (D_2O, 500 MHz) spectrum of deprotected hexadecamer **150**.

In the case of long-spacer-armed octamer **144** and hexadecamer **149**, the progress of the reaction was monitored by ^{1}H-NMR spectroscopy because newly formed spots stayed on the bottom of the TLC plates due to the high molecular weights. The ratio of anomeric protons (4.9 ppm) on GalNAc residue and *t*-butyl protons (1.1 ppm) on the phenyl ring therefore was used to infer completion of the dialkylation process instead of the existence of mixtures of mono- and di-alkylated products.

5.3. Binding properties of glycocalix[4]arenes

Direct binding of glycocalix[4]arenes to lectins

The binding ability of synthetic glycocalix[4]arenes **139**, **141**, **145**, and **150** to appropriate lectins was first performed by turbidimetric analysis. The lectins used in direct binding studies were VVA and *Maclura pomifra* which were isolated from hairy vetch and osage orange tree, respectively. Both lectins have an affinity for *N*-acetyl-D-galactosamine.

As shown in Figure 5.3.1 and Figure 5.3.2, all glycocalix[4]arenes demonstrated direct binding to VVA and *Maclura pomifera* with formation of insoluble precipitates over the course of time. In both experiments, long-spacer-armed octamer **145** showed the best cross-linking property to the lectins followed by short-spacer-armed octamer **141** and then tetramer **139**. The dimer **120** with long spacer arm did not exhibit good cross-linking, and neither did 16-mer **150**. This observation may be due to the fact that too long a spacer might be too flexible to allow the formation of a stable lattice, reducing the cross-linking efficiency of the ligand.

As an qualitative inhibitory assay, allyl 2-acetamido-2-deoxy-α-D-galactopyranoside (allyl α-D-GalNAc) (**33**) was used as an inhibitor for the cross-linking of octavalent glycocalix[4]arene **145** with VVA (Figure 5.3.1). The binding interaction between the glycocalix[4]arene **145** and VVA was so strong that almost 250 equivalents of allyl α-D-GalNAc monomer **33** was required to disrupt the cross-linking.

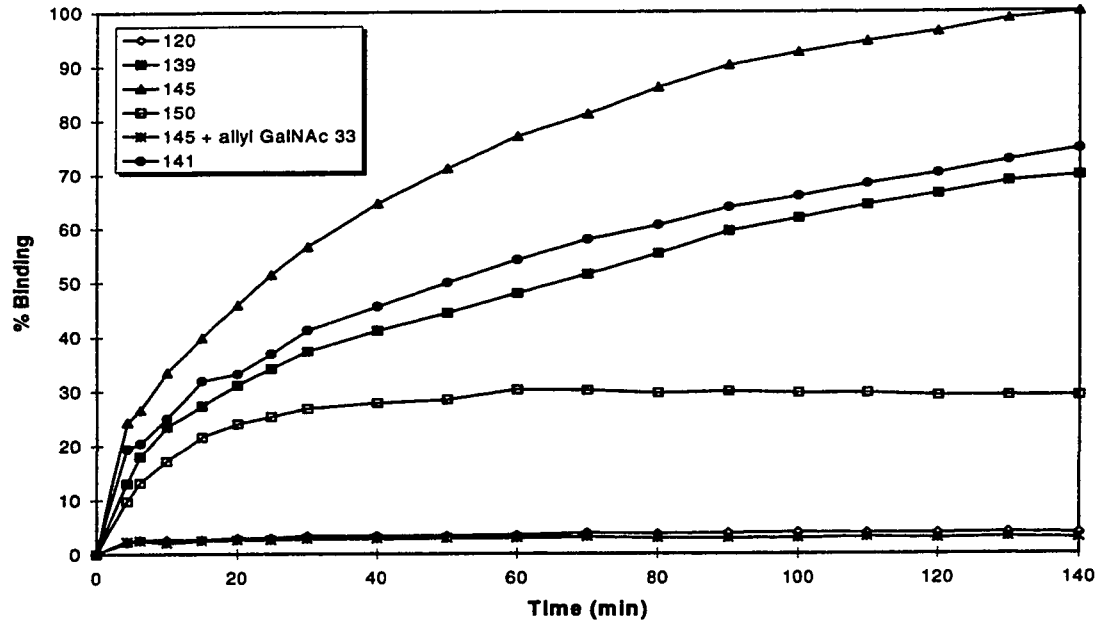


Figure 5.3.1. Time course turbidimetric assay of glycocalix[4]arenes **139**, **141**, **145**, and **150** with VVA.

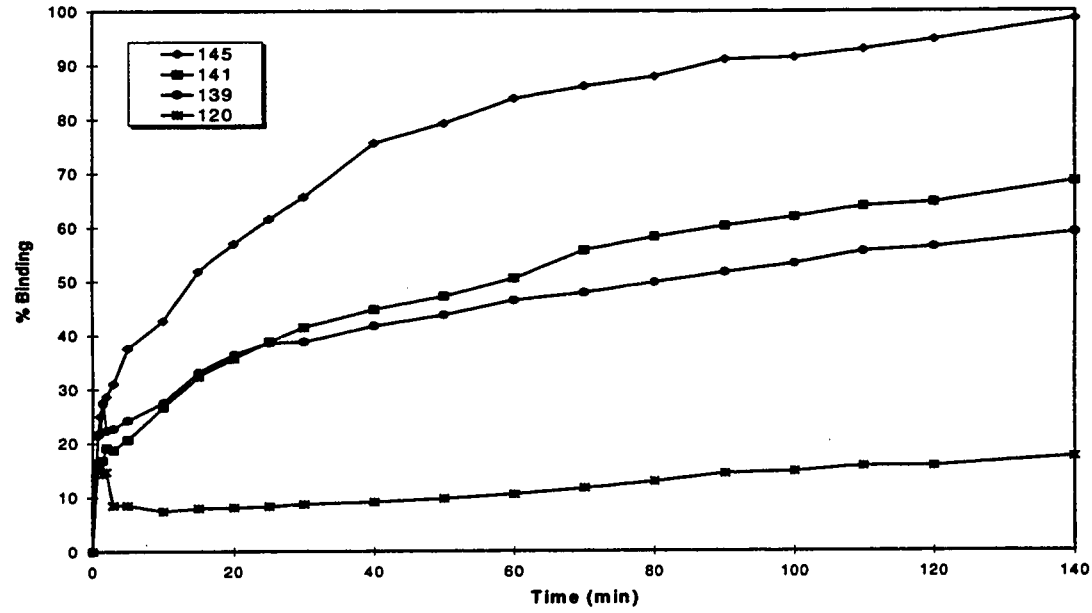


Figure 5.3.2. Time course turbidimetric assay of glycocalix[4]arenes with *Maclura pomifera*.

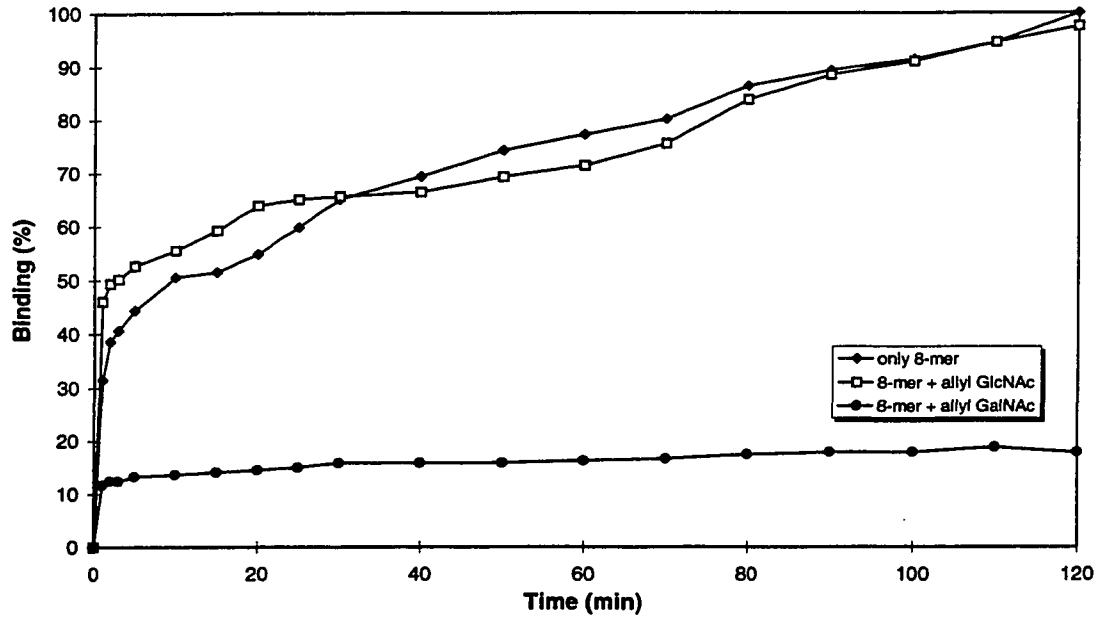


Figure 5.3.3. Time course turbidimetric assay of octamer **145** with *Maclura pomifera* in the presence of allyl α-D-GalNAc **33** and allyl α-D-GlcNAc **104** as inhibitors.

Another qualitative inhibitory assay was performed using allyl α-D-GalNAc **33** and allyl α-D-GlcNAc **104** as inhibitors. The long-spacer-armed octamer **145** was mixed with 225-fold more concentrated allyl α-D-GalNAc **33** and allyl α-D-GlcNAc **104** separately. Along with octamer **104** alone, two solutions with each inhibitor were incubated with *Maclura pomifera* lectin for 2 hours. As illustrated in Figure 5.3.3, allyl α-D-GalNAc inhibited the binding of octamer **145** to *Maclura pomifera*, resulting in no precipitate. Allyl α-D-GlcNAc however did not have any inhibitory capacity of binding of octamer **145** to the lectin. These findings clearly indicated the lectin specificity of *Maclura pomifera* to GalNAc residue and the effective interaction between the carbohydrate and the protein's binding site.

Competitive inhibition assay using asialoglycophorin as coating antigen and VVA

The competitive inhibition between naturally occurring GalNAc glycoprotein and synthetic GalNAc clusters to bind to their receptor was tested by ELLA experiment. Human blood group glycoprotein, asialoglycophorin (0.5 μg/well), was coated onto microtiter plates and allowed to compete with synthetic GalNAc-containing glycocalix[4]arenes **139**, **141**, **145**, and **150** at various concentrations for the binding to horseradish peroxidase-labeled VVA (VVA/HRP). After 2 hours of incubation and washings, the presence of adsorbed VVA/HRP was detected using 2,2'-azinobis(3-ethylbenzothiazolin-6-sulfonic acid) (ABTS) and hydrogen peroxide as enzyme substrates. The results for the inhibition assays are shown in Table 5.3.1, Figure 5.3.4 and Figure 5.3.5.

Table 5.3.1. IC_{50}'s of GalNAc-containing glycocalix[4]arenes **139**, **141**, **145**, and **150** using asialoglycophorin and VVA.

GalNAc ligands	M.W.	IC_{50}'s (μM)	Relative potency[a]
allyl α-D-GalNAc **33**	261.12	158.3	1
2-mer **120**	922.52	39.6	4.0 (2.0)
4-mer **139**	1887.92	15.0	10.6 (2.6)
short 8-mer **141**	3616.80	33.1	4.8 (0.6)
long 8-mer **145**	4473.48	18.5	8.6 (1.1)
16-mer **150**	8883.93	13.4	11.8 (0.7)

[a] Values in parenthesis are given on a per-hapten basis in a molecule.

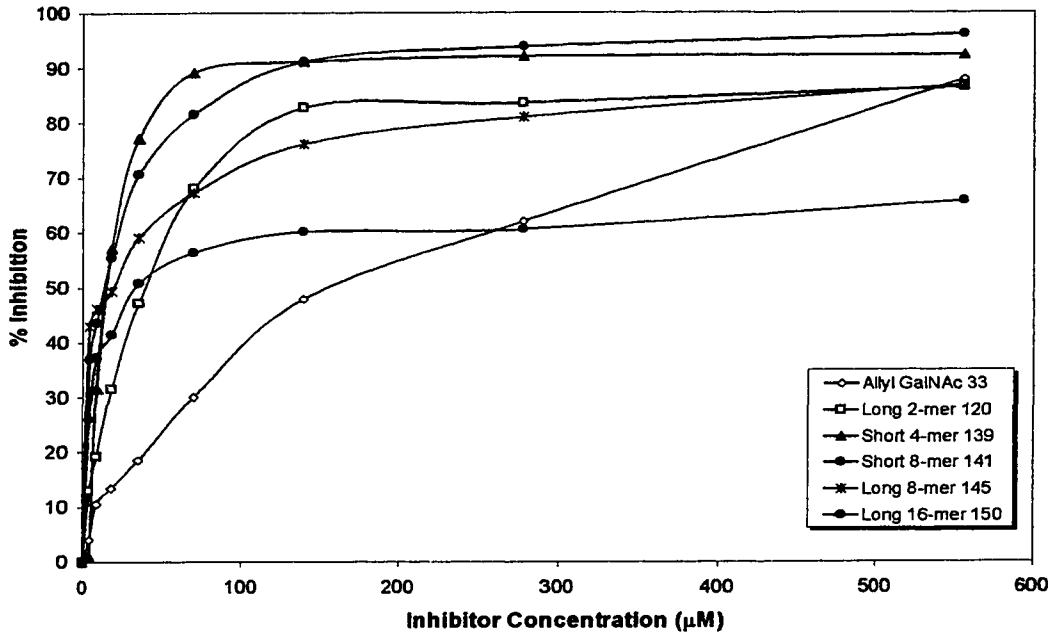


Figure 5.3.4. ELLA inhibition of binding of *VVA* B_4 to asialoglycophorin by glycocalix[4]arenes, **139**, **141**, **145**, and **150**.

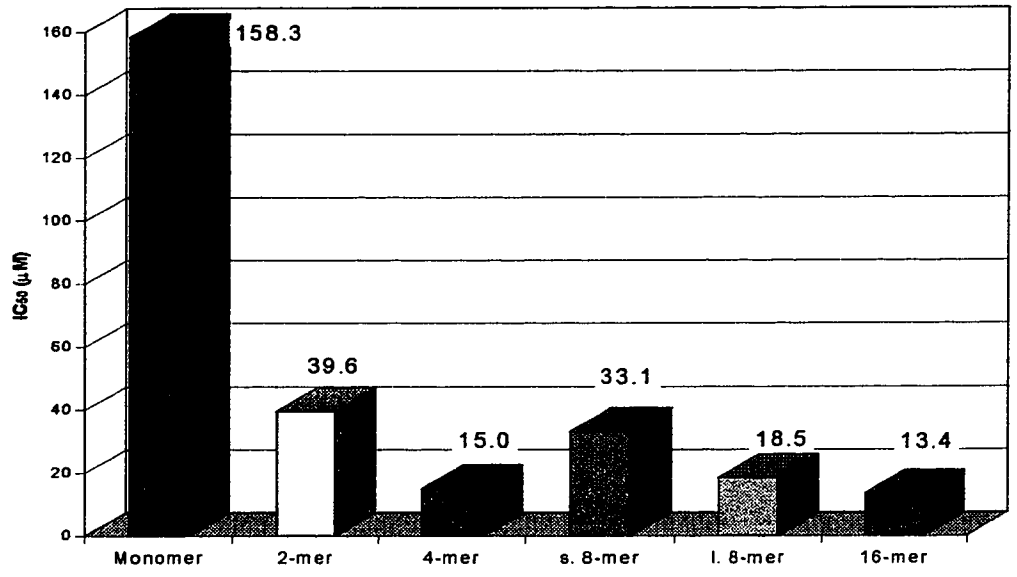


Figure 5.3.5. IC_{50}'s of GalNAc-containing glycocalix[4]arenes **139**, **141**, **145**, and **150** using asialoglycophorin and *VVA* B_4.

Hexadecavalent glycocalix[4]arene **150** indicated the lowest IC_{50} value (13.4 μM) which represents a 12-fold increase in potency over that of allyl α-D-GalNAc **33** (IC_{50} 158.3 μM). Tetravalent calix[4]arene **139** also showed a 11-fold increase in inhibitory potency with an IC_{50} of 15.0 μM. However, considering the number of GalNAc residues in each molecule, hexadecamer **150** showed only a 0.7-fold relative potency while tetramer **139** had a 2.6-fold increase per GalNAc residue compared to allyl α-D-GalNAc monomer **33**. When two octamers **141** and **145** were compared with their IC_{50} values, **141** with the short spacer arm had almost twice as high an IC_{50} as the long-spacer-armed octamer **145**. This illustrated again that intra-GalNAc distance in the molecule plays an important role for efficient binding.

Competitive inhibition assay using glycopolymer as coating antigen and VVA as binding protein

Whereas asialoglycophorin found in human erythrocyte membrane contains several repeating units of GalNAc modified Ser or Thr residues, synthetic glycopolymers are known to have irregular carbohydrate density expressed in the backbone. The synthesis of GalNAc-containing glycopolymer will be described in the following chapter (chapter 6.2). The synthetic GalNAc-containing glycopolymer **164** included in average 1:7 ratio of GalNAc residue and propylamide after aminolysis of succinimide with propylamine. This GalNAc-containing glycopolymer was used as a coating antigen for the competitive inhibition assay for the synthetic glycocalix[4]arenes. While 0.5 μg/well of asilaoglycophorin was used in the previous experiment, 1.0 μg/well of glycopolymer was coated on the microtiter plate. The same procedure was applied as described previously. The results from this experiment are shown in Table 5.3.2, Figure 5.3.6 and Figure 5.3.7.

Table 5.3.2. IC_{50}'s of GalNAc-containing glycocalix[4]arenes **139**, **141**, **145**, and **150** using glycopolymer and VVA.

GalNAc ligands	IC_{50}'s (μM)	Relative potency[a]
allyl α-D-GalNAc **33**	500.0	1
2-mer **120**	13.0	38.5 (19.2)
4-mer **139**	28.1	17.8 (4.4)
short 8-mer **141**	256.9	1.9 (0.2)
long 8-mer **145**	236.5	2.1 (0.3)
16-mer **150**	37.3	13.4 (0.8)

[a] Values in parentheses are given on a per-hapten basis in a molecule.

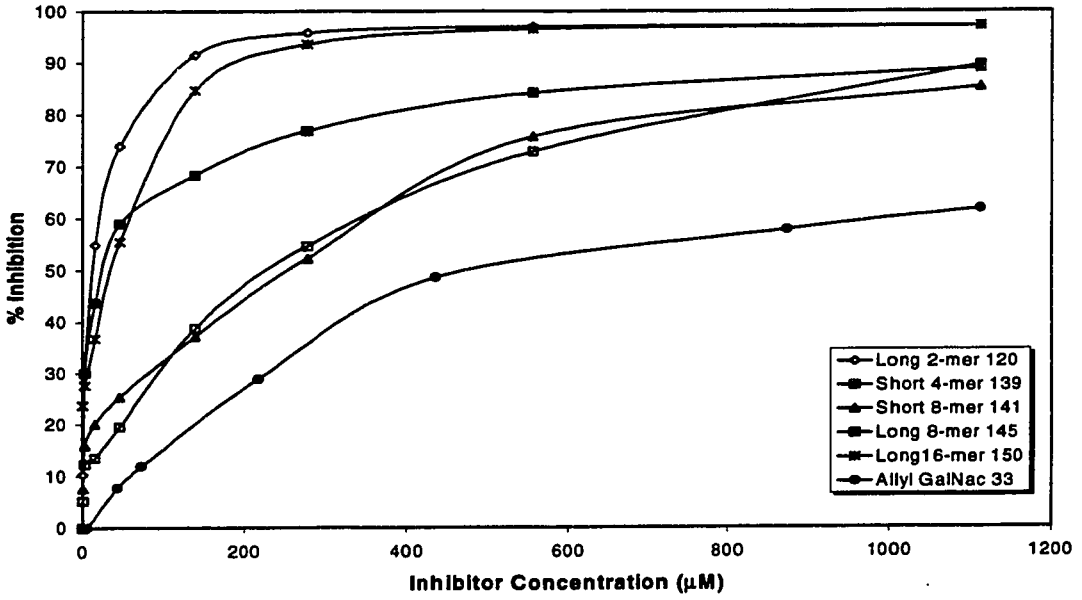


Figure 5.3.6. ELLA inhibition of binding of VVA to glycopolymer by GalNAc-containing glycocalix[4]arenes, **139**, **141**, **145**, and **150**.

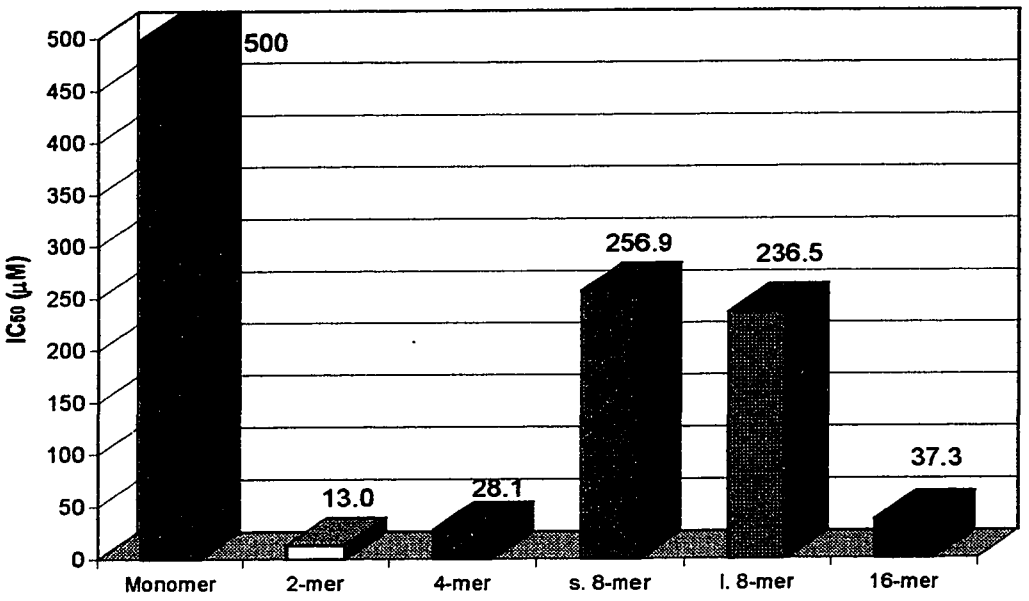


Figure 5.3.7. IC_{50}'s of GalNAc-containing glycocalix[4]arenes **139** (tetramer), **141** (short octamer), **145** (long octamer), and **150** (hexadecamer) using glycopolymer and *VVA*.

As demonstrated in the results, the IC_{50} values for the binding of glycopolymer to *VVA* B_4 by the GalNAc-containing glycocalix[4]arenes **139**, **141**, **145**, and **150** were generally greater than those obtained with the natural asialoglycophorin. The best inhibitory efficiency was however shown in dimer **120** with IC_{50} of 13. 0 μM. Taking into an account the number of GalNAc residue per molecule, dimer **120** had a 19-fold increase over allyl α-D-GAlNAc monomer **33** and tetramer **139** increased the inhibitory potency by 4-fold. This outcome clearly demonstrates the cluster effect in the tetramer **139** while the octamers **141** and **145** and hexadecamer **150** showed small increase in their relative inhibitory potential and even had less potential than the monomer **33** on per GalNAc residue basis. It is presumed that the structure of the molecule in a *cone* shape could not be arranged for the best binding to the lectin with the maximum cluster effect.

Competitive inhibition assay using glycopolymer as coating antigen and Maclura pomifera as binding protein

Competitive ELLA experiments were also performed using *Maclura pomifera* as the binding protein for the GalNAc residues and asialoglycophorin that was coated on the microtiter plate. However, none of the GalNAc-containing calix[4]arenes could inhibit the binding of *Maclura pomifera*/HRP to asialoglycophorin. This phenomenon could be explained by the less selective affinity of *Maclura pomifera* which was reported to bind more to specially T antigen (Gal β1→3αGalNAc) and also has an affinity for terminal α-D-galactosyl and *N*-acetyl-D-galactosaminyl residues. Since asialoglycophorin is a natural glycoprotein, it contains other carbohydrate residues other than GalNAc even though it occupies more than 20% of total glycosides. Therefore, the coating antigen, asialoglycophorin was replaced with a GalNAc-containing glycopolymer and ELLA experiment was performed, following the same procedure described previously. The results were presented in Table 5.3.3, Figure 5.3.8 and Figure 5.3.9.

The data obtained from this experiment exhibited less specific binding of GalNAc ligands to lectin *Maclura pomifera* according to the lower IC_{50} values in general (Figure 5.3.9). The monomeric allyl α-D-GalNAc **33** and dimer **120** did not reach 50% inhibition at 556 μM concentration. Thus, IC_{50} values of these compounds were extrapolated from the graph (Figure 5.3.8).

Table 5.3.3. IC_{50}'s of GalNAc-containing glycocalix[4]arenes **139**, **145**, and **150** using glycopolymer and *Maclura pomifera*.

GalNAc ligands	IC_{50}'s (μM)	Relative potency[b]
allyl α-D-GalNAc **33**	1180.0[a]	1
2-mer **120**	700.0[a]	1.7 (0.8)
4-mer **139**	155.8	7.6 (1.9)
long 8-mer **145**	272.7	4.3 (0.5)
16-mer **150**	19.5	60.5 (3.8)

[a] Values were extrapolated from the graph.

[b] Values in parenthesis are given on a per-hapten basis in a molecule.

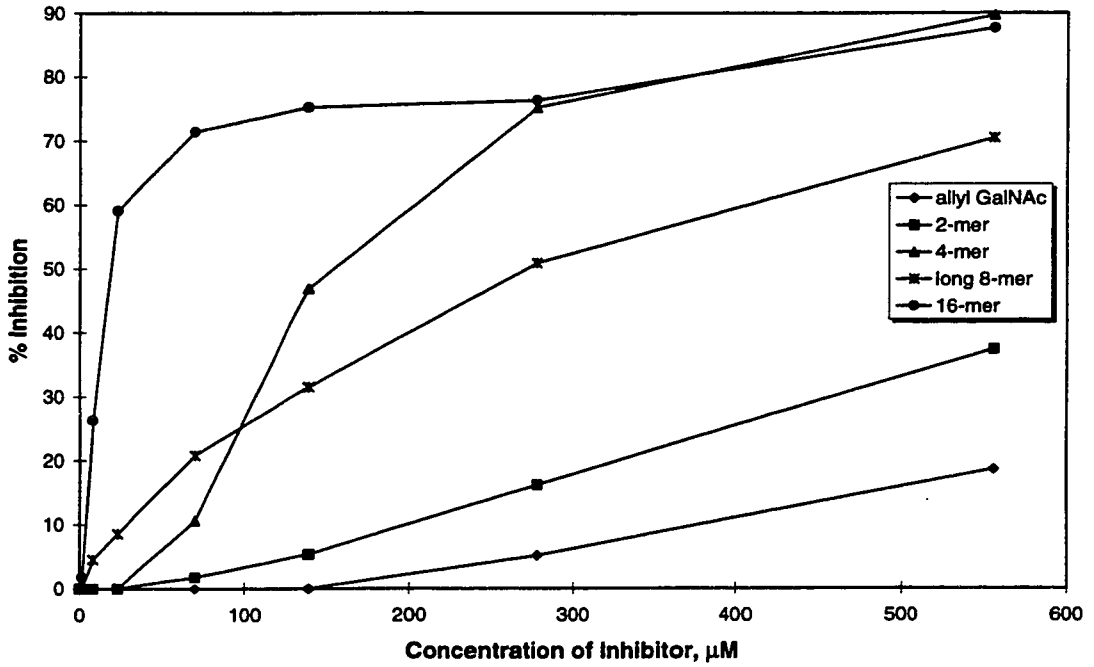


Figure 5.3.8. ELLA inhibition of binding of *Maclura pomifera* to glycopolymer by GalNAc-containing glycocalix[4]arenes **139** (tetramer), **145** (long octamer), and **150** (hexadecamer).

Not like the previous findings, hexadecamer **150** showed the most potent inhibitory capacity with IC_{50} 19.5 μM. Even further, the highest valency effect was obtained at a value of 16 (glycocalix[4]arene **150**) where each GalNAc residue was 3.8 times more potent than the monomeric allyl α-D-GalNAc. These data suggested that an amplication in carbohydrate-protein interactions is also related to the binding sites in a protein (lectin) as well as the valency of sugar residues in neoglycoconjugates.

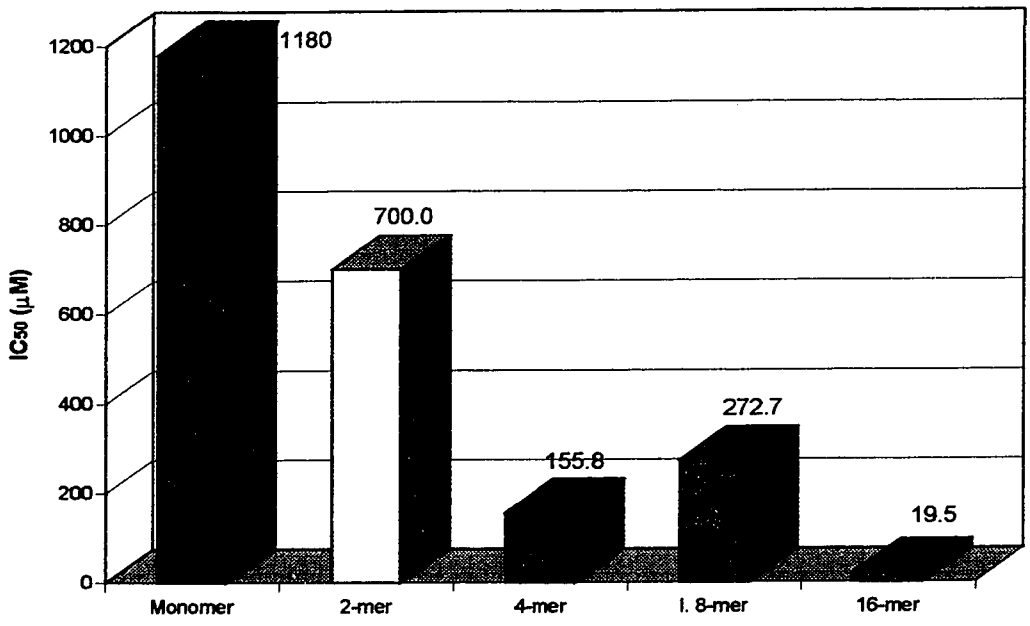


Figure 5.3.9. IC_{50}'s of GalNAc-containing glycocalix[4]arenes using glycopolymer **164** and *Maclura pomifera*.

5.4. Glycocalix[4]arenes as coating antigen

The unique structural features of *t*-butylcalix[4]arene can be employed in designing novel biological properties of glycocalix[4]arenes. For example, the hydrophobic *tert*-butyl group on the lower rim of calix[4]arene core can provide the driving force for the stable surface adhesion while the hydrophilic carbohydrates mimic the cell's saccharide-rich surface. This unique property of GalNAc-containing calix[4]arene was exercised as coating antigen in competitive ELLA experiments. For comparison purpose, GalNAc-containing glycopolymer **164** (chapter 6.3), glyco PAMAM dendrimer **158** (32-mer, chapter 6.2) and glycopeptoid **59** (8-mer, chapter 2.2) were tested along with glycocalix[4]arenes **139** (tetramer) and **150** (hexadecamer).

These compounds were coated onto microtiter plates at various concentrations. After 2 hours of incubation at 37 °C, blocking with 1% bovine serum albumin (BSA) and then washing, the plates were incubated with VVA/HRP. The adsorbed GalNAc residues were confirmed using ABTS and hydrogen peroxide as enzyme substrates. The details of these ELLA tests are illustrated in Figure 5.4.1.

Although hexadecameric glycocalix[4]arene **150** was not as efficient as coating antigen compared to glycopolymer **164**, it was still as good a coating antigen as 32-valent glyco PAMAM dendrimer **158**. As expected, the octameric glycopeptoid **59** and the tetrameric glycocalix[4]arene **139** showed poor coating properties. These results were attributed to their lack of lipophilic components. Even though the tetrameric glycocalix[4]arene **139** included hydrophobic *t*-butyl component, the intramolecular distance between the GalNAc residues was too short for **139** to exhibit efficient binding to VVA/HRP.

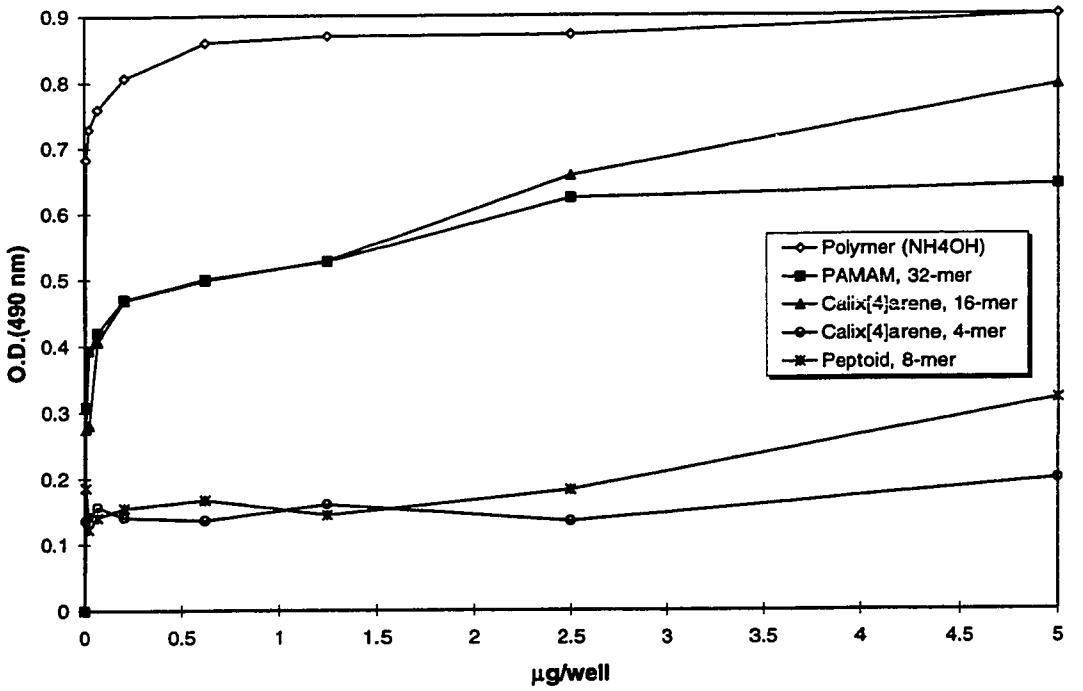


Figure 5.4.1. ELLA using glycocalix[4]arenes **139** and **150**, glyco PAMAM dendrimers **158**, glycopolymer **164**, and glycopeptoid **59** as coating antigens on the microtiter plates using VVA.

5.5. Conclusions

Glycocalix[4]arenes bearing GalNAc glycosides were synthesized using an *N,N'*-dialkylation strategy. Tetravalent and short-spacer-armed octavalent glycocalix[4]arenes were prepared in a convergent manner whereas long-spacer-armed octavalent and hexadecavalent glycocalix[4]arenes were prepared in a divergent manner.

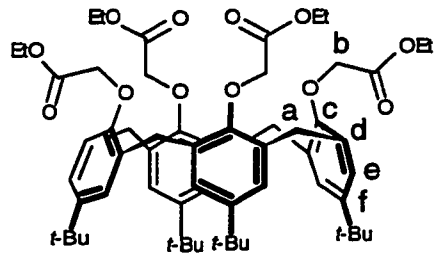
The cross-linking properties of these glycocalix[4]arenes were confirmed by turbidimetric analyses. ELLA inhibition of binding of *VVA*/HRP lectin to asialoglycophorin and GalNAc-containing glycopolymer indicated that, indeed, the cluster effect was observed with lower valency molecules, dimer and tetramer, when the number of sugar residues in each ligand was taken into account.

However, when tested in ELLA using GalNAc-containing glycopolymer and horseradish peroxidase-labeled *Maclura pomifera* for detection, the best inhibitory potential was observed with the higher valency molecule, hexadecamer (IC_{50} 19.5 μM, 3.8-fold increase in relative potency per sugar in a molecule).

The multivalency effect is valuable for each individual interaction and to what extent multivalency plays a role in an enhanced binding interactions.

5.6. Experimental methods

4-*t*-Butylcalix[4]arene-*O,O',O'',O'''*-tetraacetic acid tetraethyl ester (135).



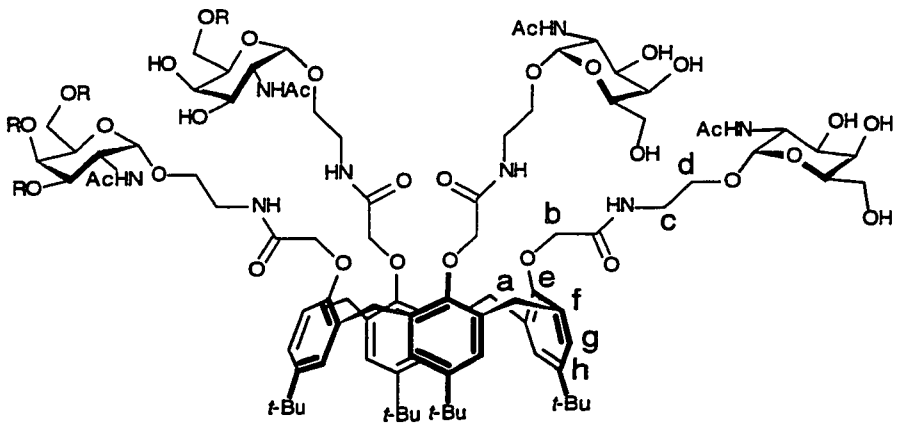
4-*t*-butylcalix[4]arene (**134**) (5.0 g, 7.70 mmol) was suspended in dry acetone (100 mL) containing anhydrous potassium carbonate (21.3 g, 0.154 mol) and ethyl bromoacetate (17. 1 mL, 0.154 mol), and the mixture was refluxed with molecular sieves (4 Å) for 24 h (until TLC analysis showed the disappearance of calix[4]arene). The cooled mixture was filtered and the solid residue was washed several times with CH_2Cl_2. The combined organic solutions were concentrated to an oil that contained residual ethyl bromoacetate. The latter was removed by distillation under high vacuum, leaving a residue to which sufficient ethanol was added to effect dissolution. After standing in the fridge overnight, the solution deposited a crystalline mass in almost quantitative yield. Recrystallization from

ethanol-dichloromethane gave 6.50 g (84%) of a pure colorless compound: mp 154.2-154.8 °C; ^{1}H-NMR ($CDCl_3$) δ 1.06 (s, 36H, 4 C*Me*$_3$), 1.27 (t, 12H, 4 CH_3), 3.17 (d, 4H, J 13.0 Hz, 4 H-a), 4.19 (q, 8H, J 7.1 Hz, 4 CH_2), 4.79 (s, 8H, 4 H-b's), 4.84 (d, 4H, J 13.0 Hz, 4 H-a'), 6.76 (s, 8H, 4 H-e); ^{13}C-NMR ($CDCl_3$) δ 14.2 (CH_3), 31.4 (C*Me*$_3$), 31.9 (*C*Me$_3$), 33.8 (CH_2), 60.3 (CH_2), 71.3 (CH_2), 125.3 (C-e), 133.4 (C-d), 145.1 (C-f), 152.9 (C-c), 170.5 (C=O); Anal. Calcd. for $C_{60}H_{80}O_{12}Na$: C, 70.91; H, 7.93. Found: C, 71.27; H, 7.93.

4-*t*-Butylcalix[4]arene-*O,O',O'',O'''*-tetraacetic acid (136).

4-*t*-Butylcalix[4]arene-*O,O',O'',O'''*-tetraacetic acid tetraethyl ester (**135**) (2.75 g, 2.77 mmol) was placed in a flask and was added a mixture of ethanol (33 mL) and 1M aq. KOH (30 mL). The mixture was refluxed for 9 hours resulting in a clear homogeneous solution. The reaction solution was then cooled down and treated with Amberlite IR(H) resin for 15 min. During this acidifying process, a white precipitate deposited from the solution. The solution was decanted carefully from the resin as much as possible and the remainder was filtered through a coarse fritted filter. The resin was washed several times with methanol and the combined filtrates were concentrated under reduced pressure to afford a white solid (2.20 g, 90%): mp, 272-273 °C dec.; FAB-MS calcd. for $C_{52}H_{64}O_{12}Na$: 903.43; found: 903.76 (2.0%).

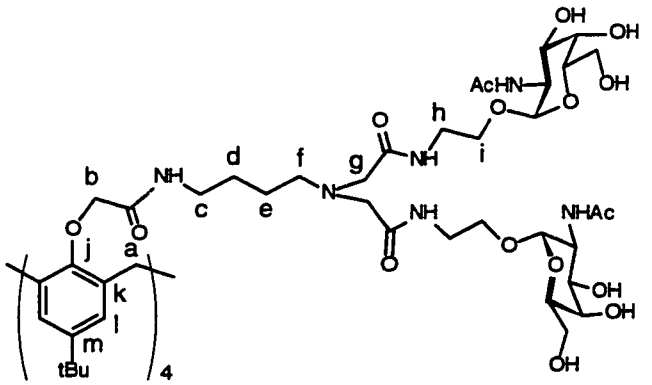
Calix[4]arene tetramer (139).



4-*t*-Butylcalix[4]arene-*O,O',O'',O'''*-tetraacetic acid (**136**) (0.10 g, 0.114 mmol) was treated with $SOCl_2$ (5 mL) and the solution was refluxed for 2 h. The reaction solution was then concentrated to dryness. 2-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactosyl)-1-aminoethane, hydrochloride salt **102** (0.29 g, 0.681 mmol) was dissolved in CH_2Cl_2 (10 mL) and Et_3N (0.19 mL, 1.35 mmol) was added at 0 °C. A solution of calix[4]arene tetraacetyl chloride in CH_2Cl_2 (3 mL) was then added dropwise to the solution and stirred for 2 h. The reaction was monitored by TLC. When the reaction was complete, the solution was diluted with CH_2Cl_2 (10 mL) and washed with 5% aqueous HCl (1 × 10 mL), saturated $NaHCO_3$ (1 × 10 mL) and then water (1 × 10 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Purification was done by silica gel chromatography eluting with 19:1 $CHCl_3$/MeOH to afford 0.20 g (74%) of a white foam. The product was then de-*O*-acetylated under Zemplén condition. As the reaction proceeded, the solution was getting turbid. The solution was stirred 16 hours and stored in the fridge for 24 h. The precipitates were filtered and dried under the reduced pressure to yield 0.15 g (94%) of a white solid: **138**: $[\alpha]_D$ +74.9 (*c* 0.78, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.05 (s, 9H, t-Bu), 1.92, 1.93. 2.12 (3s, 12H, OAc, NAc), 3.27 (d, 1H, J 12.6 Hz, H-a), 3.50-3.65 (m, 3H, H-c, H-c', H-d'), 3.87

(bs, 1H, H-d), 4.03-4.07 (m, 2H, H-6's), 4.10-4.20 (m, 1H, H-5), 4.50 (d, 1H, H-a), 4.54-4.63 (m, 3H, H-2, H-b), 4.87 (bs, 1H, H-1), 5.12 (bd, 1H, H-3), 5.33 (bs, 1H, H-4), 6.79 (s, 2H, H-g), 8.10 (bs, 1H, CONH); ^{13}C-NMR ($CDCl_3$) δ 20.6 (OAc), 22.9 (NAc), 31.2 (t-Bu), 31.5 (C-a), 33.9 (CMe_3), 39.3 (C-c), 47.4 (C-2), 62.0 (C-6), 66.6 (C-3), 67.4 (C-4), 67.9 (C-d), 68.3 (C-5), 74.3 (C-b), 98.3 (C-1), 126.1 (C-g), 132.3 (C-f), 146.2 (C-h), 152.9 (C-e), 170.3, 170.6, 170.7 (C=O's); Anal. Calcd for $C_{116}H_{160}N_8O_{44}$: C, 58.77; H, 6.80; N, 4.73; found C, 58.64; H, 6.56; N, 4.40; **139**: FAB-MS (pos. m/z) calcd. for $C_{92}H_{136}N_8O_{32}$: 1864.93; found: 1865.84 (M^+ + 1, 2.4%).

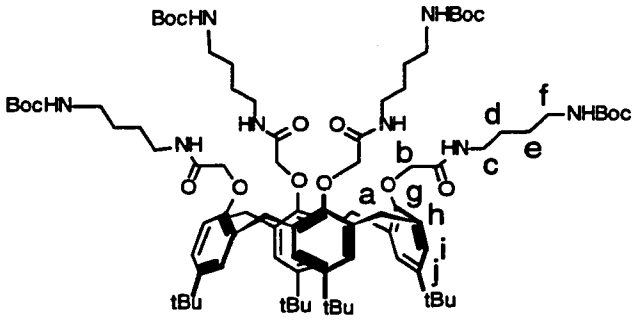
Calix[4]arene octamer with short-spacer-armed GalNAc (141).



4-*t*-Butylcalix[4]arene-*O,O',O'',O'''*-tetraacetyl chloride **137** was prepared by refluxing tetraacid **136** (20 mg, 22.7 μmol) in $SOCl_2$ for 2 h. Divalent GalNAc ligand **116** with a short spacer arm (0.14 g, 0.136 mmol) was treated with 20% TFA in CH_2Cl_2 (5 mL) for 1 h. The solution was concentrated and the residue was co-evaporated with toluene to remove excess TFA. The amine **121** in a salt foam was then dissolved in CH_2Cl_2 (10 mL) and Et_3N (40 mL, 0.273 mmol) was added at 0 °C. Calix[4]arene tetraacetyl chloride **137** in CH_2Cl_2 (3 mL) was added to the reaction mixture dropwise at 0 °C and the solution was stirred for 3 h. The solution was diluted with CH_2Cl_2 (10 mL) and washed with 5% aqueous HCl (1 ×

10 mL) and then water (1 × 10 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. The residue was dissolved in MeOH (15 mL) and 1M NaOMe was added dropwise to the solution until pH 9. The reaction solution was stirred at room temperature for 16 h and concentrated. Size exclusion column chromatography (LH 20) of the crude product eluting with MeOH followed by lyophilization yielded 60 mg (73%) of an off-white powder: $[\alpha]_D$ +47.4 (*c* 0.46, DMSO); ^{1}H-NMR presented broad peaks in general. ^{1}H-NMR (D_2O) δ 1.07 (s, 9H, t-Bu), 1.55 (s, 4H, H-e, H-d), 2.09 (s, 6H, NAc), 2.68 (s, 2H, H-f), 3.02-3.63 (m, 13H, H-i', H-h, H-h', H-c, H-g, H-a'), 3.81 (s, 6H, H-6's, H-i), 3.95 (s, 4H, H-3, H-5), 4.04 (s, 2H, H-4), 4.25 (d, 2H, $J_{2,3}$ 10.0 Hz, H-2), 4.90 (s, 2H, H-1), 6.88 (s, 2H, H-l); ^{13}C-NMR (D_2O) 21.7 (NAc), 24.0 (C-d), 26.3 (C-e), 30.9 (t-Bu), 33.2 (C-a), 38.5 (C-h), 38.6 (C-c), 49.4 (C-2), 55.0 (C-f), 57.6 (C-g), 60.8 (C-6), 66.1 (C-i), 67.5 (C-3), 68.1 (C-4), 70.7 (C-5), 73.6 (C-b), 97.0 (C-1), 125.1 (C-l), 132.7 (C-k), 140.1 (C-m), 152.4 (C-j), 170.2, 172.2, 173.6 (C=O's).

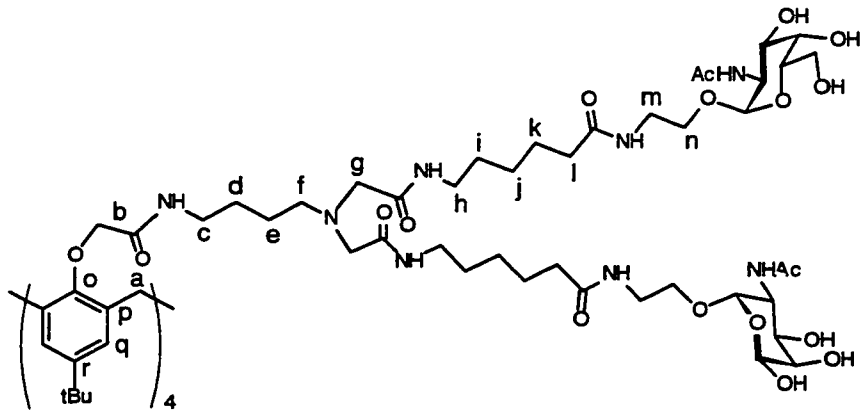
_N_-Boc protected calix[4]arene tetramer (142).



Title compound was prepared using the same procedure as for the synthesis of calix[4]arene tetramer, **138**. Purification was done by silica gel chromatography eluting with 18:1:1 $CHCl_3$/MeCN/MeOH to afford a pale yellowish product **142** (63%): ^{1}H-NMR ($CDCl_3$) δ 1.05 (s,9H, *t*-Bu-Ar), 1.40 (s, 9H, Boc),

1.44-1.52 (m, 2H, H-e), 1.53-1.55 (m, 2H, H-d), 3.05-3.10 (m, 2H, H-f), 3.20 (d, 1H, J 13.1 Hz, H-a), 3.30-3.44 (m, 2H, H-c), 4.45 (d, 1H, H-a'), 4.47 (s, 2H, H-b), 5.01 (bs, 1H, NHBoc), 6.75 (s, 2H, H-j), 7.90 (bs, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ 26.9 (C-d), 27.4 (C-e), 28.4 (Boc), 31.3 (t-Bu-Ar, C-a), 33.9 (CMe_3, Ar), 38.9 (C-c), 40.2 (C-f), 74.4 (C-b), 79.0 (CMe_3, Boc), 125.8 (C-i), 132.6 (C-h), 145.8 (C-j), 152.6 (C-g), 156.2, 169.6 (C=O's); FAB-HRMS (pos. m/z) calcd. for $C_{88}H_{137}N_8O_{16}$: 1562.0153; $C_{88}H_{136}N_8O_{16}Na$: 1583.9972; found: 1562.9765 (M^+ + 1, 6.5%), 1583.9516 (M^+ + Na, 24.2%); Anal. Calcd for $C_{88}H_{136}N_8O_{16}$: C, 66.68; H, 8.65; N, 7.07. Found C, 66.55, H, 8.57; N, 7.37.

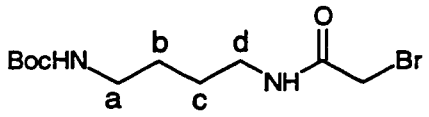
Calix[4]arene octamer with long-spacer-armed GalNAc (145).



N-Boc protected calix[4]arene tetramer **142** (50.0 mg, 32.1 μmol) was treated with 20% TFA in CH_2Cl_2 (5 mL) and stirred for 2 h. The solution was concentrated and co-evaporated with toluene twice. The amine **143** in a salt form was dissolved in CH_3CN (10 mL) and Et_3N (60 mL, 449 μmol) and bromide **117** (0.20 g, 321 μmol) were added. The reaction solution was heated at 60 °C for 48 h. The solution was evaporated and the residue was dissolved in CH_2Cl_2 (20 mL). The solution was washed with water (2 × 10 mL) and the organic phase was dried

over anhydrous Na_2SO_4. The concentrated residue was dissolved in MeOH (10 mL) and 1M NaOMe was added until pH 9. The methanolic solution was stirred at room temperature for 16 h and treated with Amberlite IR (H) resin for 15 min. The resin was filtered off and the filtrate was concentrated. Size exclusion column chromatography (LH 20) of the crude product eluting with MeOH followed by lyophilization afforded 91 mg (64%) of an off-white powder: $[\alpha]_D$ +53.6 (*c* 0.5, DMSO); ^{1}H-NMR (D_2O) 1.09 (s, 9H, *t*-Bu), 1.28-1.42 (m, 6H, H-j, H-d), 1.48-1.83 (m, 10H, H-i, H-k, H-e), 2.10 (s, 6H, NAc), 2.24-2.35 (m, 4H, H-l), 3.05-3.13 (b, 2H, H-f), 3.20-3.30 (m, 7H, H-h, H-b, H-a'), 3.34-3.43 (m, 6H, H-g, H-m'), 3.52-3.62 (m, 6H, H-m, H-n', H-c), 3.78-3.87 (m, 7H, H-6's, H-n, H-a), 3.91-4.00 (m, 4H, H-3, H-5), 4.04 (bs, 2H, H-4), 4.25 (dd, 2H, J1,2 3.3 Hz, J2,3 10.9 Hz, H-2), 4.91 (bs, 2H, H-1), 6.89 (b, 2H, H-g); ^{13}C-NMR (D_2O) δ 19.6 (NAc), 22.7 (C-k), 23.2 (C-d), 23.4 (C-j), 25.6 (C-e), 25.9 (C-i), 28.8 (t-Bu), 33.2 (C-l), 36.4 (C-m), 36.8 (C-c, C-h), 47.3 (C-2), 55.2 (C-f), 58.7 (C-6), 64.0 (C-n), 65.4 (C-3), 66.0 (C-4), 68.6 (C-5), 94.8 (C-1), 123.1 (C-g), 130.5 (C-p), 142.8 (C-r), 150.5 (C-o), 170.2, 171.6, 171.7, 173.7 (C=O's).

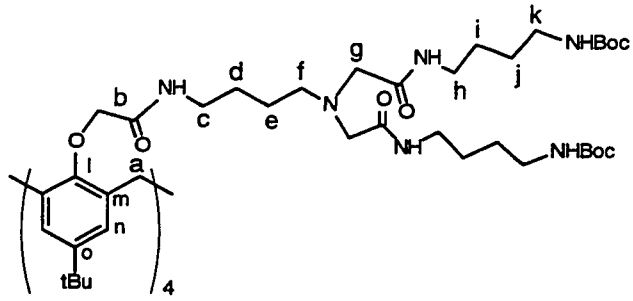
4-*N*-Boc-*N'*-(bromoacetamidyl)diaminobutane (147).



To a solution of *N*-Boc-1,4-diaminobutane **146** (0.30 mg, 1.60 mmol) and DIPEA (0.33 mL, 1.91 mmol) in CH_2Cl_2 (30 mL) was added dropwise bromoacetyl chloride (0.16 mL, 1.91 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The reaction solution was stirred at 0 °C for 30 min and washed with 5% aqueous HCl (1 × 10 mL), saturated $NaHCO_3$ (1 × 10 mL) and then water (1 × 10 mL). The dried (Na_2SO_4) organic phase was concentrated and the residue was purified by silica gel

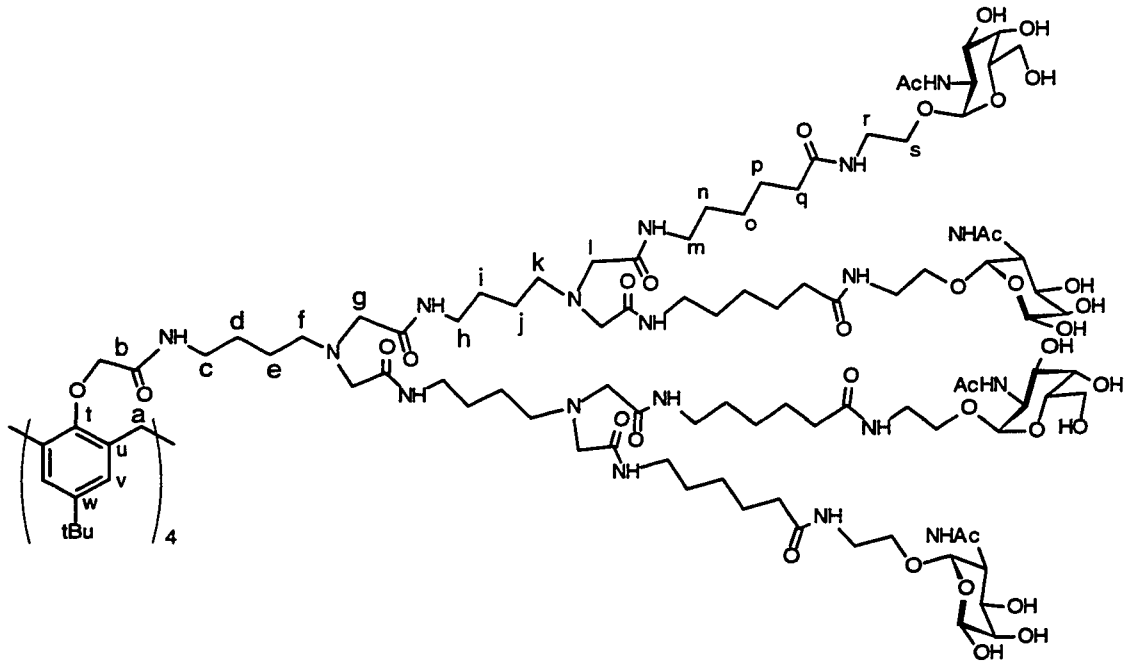
chromatography eluting with 18:1:1 $CHCl_3$/MeCN/MeOH to yield 0.49 g (99%) of a brownish oil: ^{1}H-NMR ($CDCl_3$) δ 1.41 (s, 9H, *t*-Bu), 1.46-1.69 (m, 4H, H-b, H-c), 3.08-3.14 (m, 2H, H-a), 3.26-3.31 (m, 2H, H-b), 3.84 (s, 2H, CH_2Br), 4.59 (b, 1H, NHBoc), 6.63 (b, 1H, NHCO); ^{13}C-NMR ($CDCl_3$) δ 26.7 (C-c), 27.3 (C-b), 28.5 (t-Bu), 29.2 (CH_2Br), 39.7 (C-d), 40.0 (C-a), 79.1 (CMe_3), 156.0, 165.6 (C=O's).

***N,N'*-Dialkylated Boc-protected calix[4]arene octamer (148).**



The title compound was prepared using the same procedure as for the preparation for **144**: yield: 51%; ^{1}H-NMR ($CDCl_3$) δ 1.11 (s, 9H, t-Bu-Ar), 1.37 (s, 18H, Boc), 1.45-1.70 (m, 12H, H-e, H-d, H-i, H-j), 2.56 (bs, 2H, H-f), 3.05 (bs, 4H, H-k), 3.21 (bs, 10H, H-c, H-h, H-g), 3.32 (bs, 2H, H-b), 3.39 (bd, 1H, J 10.8 Hz, H-a), 4.00 (bd, 1H, H-a'), 5.09 (bs, 2H, NHBoc), 7.10 (bs, 2H, H-n), 8.30 (b, 2H, NH), 9.20 (b, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ 26.5 (C-e, C-i), 27.3 (C-d, C-j), 28.4 (Boc), 30.1 (C-a), 31.3 (t-Bu-Ar), 34.0 (CMe_3, Ar), 39.0 (C-g), 41.2 (C-k), 50.5 (C-c), 55.1 (C-f), 58.3 (C-h), 79.0 (CMe_3, Boc), 126.2 (C-n), 134.3 (C-m), 148.9 (C-o), 149.6 (C-l), 156.3, 171.4 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{156}H_{264}N_{24}O_{32}$: 2985.98; found: 2987.76 (0.2%).

Calix[4]arene hexadecamer (150).



N-Boc protected calix[4]arene octamer **148** (0.10 g, 33.5 μmol) was treated with 20% TFA in CH_2Cl_2 (5 mL) for 2 h and the solution was concentrated to dryness. The residue was then dissolved in 4:1 CH_3CN/DMF, and DIPEA (0.18 mL, 1.01 mmol) and bromide **117** (0.42 g, 0.67 mmol) were added. The reaction solution was heated at 60 °C for 48 hours and then concentrated. The residue was purified using size exclusion column chromatography (LH20) eluting with MeOH to afford 0.26 g (71%) of yellowish foam of **149**: ^{1}H-NMR ($CDCl_3$) 1.03 (s, 9H, *t*-Bu), 1.27 (s, 8H, H-o), 1.45-1.63 (m, 28H, H-p, H-n, H-i, H-j, H-d, H-e), 1.91, 1.95, 2.00, 2.10 (4s, 48H, OAc, NAc), 2.12-2.17 (s, 8H, H-q), 3.19 (s, 37H, H-l, H-g, H-f, H-c, H-m, H-n, H-k, H-r, H-a'), 3.48 (s, 4H, H-r'), 3.57 (s, 4H, H-s'), 3.71 (s, 4H, H-s), 4.01-4.09 (m, 8H, H-6's), 4.13-4.18 (m, 4H, H-5), 4.40-4.57 (m, 7H, H-2, H-b, H-a), 4.85 (s, 4H, H-4), 5.07 (d, 4H, J 11.4 Hz, H-2), 5.31 (s, 4H, H-1), 6.75 (s, 2H, H-v), 6.96 (s, 4H, NH), 7.20 (s, 4H, NH), 7.90 (b, 3H, NH), 8.20 (b, 4H, NH); ^{13}C-NMR ($CDCl_3$) δ 20.7 (OAc), 23.0 (NAc), 23.8 (C-i, C-d), 25.2 (C-n), 25.2 (C-o), 26.0 (C-e), 26.4 (C-j), 31.3 (t-Bu), 36.2 (C-q), 38.7, 39.0, 39.5 (C-r, C-a, C-

m, C-l, C-g), 47.5 (C-2), 58.5 (C-f, C-k, C-c, C-h), 62.0 (C-6), 66.7 (C-3), 67.3 (C-4), 68.0 (C-s), 68.4 (C-5), 98.4 (C-1), 125.9 (C-v), 132.5 (C-u), 146.0 (C-w), 153.0 (C-t), 170.4, 170.5, 170.7, 173.5 (C=O's).

The product was de-*O*-acetylated under Zemplén condition. The methanolic solution was treated with Amberlite IR (H) resin for 15 min. and the resin was filtered off. Size exclusion column chromatography of the crude product eluting with MeOH followed by lyophilization yielded 0.20 g (97%) of an off-white powdered product **150**. ^{1}H-NMR presented broad peaks in general: $[\alpha]_D$ +66.4 (*c* 1.0, MeOH); ^{1}H-NMR (D_2O) δ 1.11 (s, 9H, t-Bu), 1.32-1.43 (m, 8H, H-o), 1.46-1.70 (m, 28H, H-p, H-n, H-i, H-j, H-d, H-e), 2.11 (s, 12H, NAc), 2.25-2.36 (m, 8H, H-q), 2.50-2.66 (m, 6H, H-k, H-f), 3.14-3.44 (m, 31H, H-s', H-m, H-h, H-l, H-c, H-g, H-a), 3.53-3.62 (m, 8H, H-r', H-s), 3.76-3.87 (m, 13H, H-6's, H-r, H-a'), 3.93-4.00 (m, 8H, H-2, H-5), 4.04 (s, 4H, H-4), 4.25 (dd, 4H, $J_{2,3}$ 11.0 Hz, H-2), 4.82 (s, 4H, H-1), 6.92 (s, 2H, H-v); ^{13}C-NMR (D_2O) δ 21.7 (NAc), 23.4 (C-i, C-j), 24.7 (C-p), 25.4 (C-o), 27.9 (C-n), 30.8 (t-Bu), 35.3 (C-q), 38.5 (C-c, C-s, C-m, C-h), 49.3 (C-2), 54.8 (C-k, C-f), 57.9 (C-l, C-g), 60.1 (C-4), 70.6 (C-5), 96.8 (C-1), C-t, C-u, C-v, C-w not observable on NMR. 172.4, 173.7, 174.0, 175.9, 176.3 (C=O's).

Turbidimetric assay with *Maclura pomifera.*

Turbidimetry experiments were performed in Linbro (Titertek) microtitration plates where 50 µL/well of stock solution prepared from *Maclura pomifera* (1 mg/mL) PBS) was mixed with 50 µL of stock solutions of inhibitors containing GalNAc (3.54 µmol of glycoside residue/mL PBS) to obtain a final volume of 100 µL per well. The solutions were then incubated at room temperature for 2 to 3 h. The turbidity of the solution was monitored by reading the optical density (O.D.) at 490 nm at regular time intervals until no noticeable change could be observed. Each test was done in duplicate.

Turbidimetric assay with *Maclura pomifera* with mono-valent inhibitors.

50 μL/well of stock solution prepared from *Maclura pomifera* (1 mg/mL PBS) was mixed with 50 μL of stock solution of inhibitor **145** (3.54 μmol of glycoside residue/mL PBS) and 10 μL of stock solution of inhibitors (0.5 mM PBS), allyl α-D-GalNAc **33** or allyl α-D-GlcNAc **104** to obtain a final volume of 110 μL per well. The solutions were incubated at room temperature for 2 to 3 h. The turbidity of the solution was monitored by reading the optical density (O.D.) at 490 nm at regular time intervals until no noticeable change could be observed. Each test was done in duplicate.

Enzyme Linked Lectin Assay (ELLA) using glycopolymer (allyl α-D-GalNAc with $PrNH_2$ on PNAS backbone polymer) and asialoglycophorin as coating antigen.

Nunc microtitration plates were coated with glycopolymer **164** (allyl α-D-GalNAc with $PrNH_2$ on PNAS polymer backbone) and asialoglycophorin at 100 μL/well of a stock solution of 10 μg/mL (glycopolymer) and 5 μg/mL (asialoglycophorin) in 0.01M phosphate buffer (PBS, pH 7.3) for 2 h at 37 °C. The wells were then washed three times with 300 μL/well of 0.01 phosphate buffer (pH 7.3) containing 0.05% (v/v) Tween 20 (PBST). This washing procedure was repeated after each incubation period. Wells were then blocked with 150 μL/well of 1% BSA/PBS for 1 h. Inhibitors used include allyl α–D-GalNAc **33** as a reference monovalent compound and synthesized multivalent GalNAc-containing ligands which were used as stock solution of 1.11 mM in PBS. Each inhibitor was added in serial 2- to 10-fold dilutions (60 μL/well) in PBS with appropriate lectin-enzyme conjugate concentration (60 μL/well of 500-fold dilution of a 1 mg/mL stock solution of *Vicia villosa* in PBS) on Linbro (Titertek) microtiter plates. These inhibitor solutions (100 μL) were then transferred to an antigen-coated plates and incubated for another hour at 37 °C. The plates were washed and 50 μL/well of 2,2'-azinobis(3-ethylbenzothiazolin-6-sulfonic acid), diammonium salt (ABTS, 1

mg/4 mL) in citrate-phosphate buffer (0.2M, pH 4.0 with 0.015% H_2O_2) was added. The reaction was stopped after 20 min. by adding 50 µL/well of 1M H_2SO_4 and optical density measured at 410 nm relative to 570 nm. The percent inhibition was calculated as follows:

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

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

Glycocalix[4]arenes as coating antigens.

Nunc microtitration plates were coated with glycocalix[4]arenes **139** and **150**, glyco PAMAM dendrimer **158**, glycopolymer **164**, and glycopeptoid **59** (100 µL/well) diluted from each stock solution of 50 µg/mL in 0.01M phosphate buffer saline (PBS, pH 7.3) at 37 °C for 2 h. The wells were then washed three times with 300 µL/well of washing buffer (PBS containing 0.05% (v/v) Tween 20) (PBST). This washing procedure was repeated after each incubation throughout the assay. The wells were then blocked with 150 µL/well of 1% BSA/PBS for 1 h at 37 °C. After washing, the wells were filled with 50 µL/well of horseradish peroxidase-labeled *Vicia villosa* B_4 and incubated 37 °C for 1 h. The plates were washed with as above and the ABTS substrate in citrate-phosphate buffer (0.2M, pH 4.0 with 0.015% H_2O_2) was added. The color development was stopped after 20 minutes by adding 1M H_2SO_4. The O.D. was then measured at 410 nm relative to 570 nm. Each test was done in duplicate.

Chapter 6. PAMAM-based glycodendrimers and glycopolymers

6.1. Introduction

PAMAM-based glycodendrimers

Tomalia et al[193,194] first reported the synthesis of Starburst® PAMAM dendrimers, where each generation was added to the initial core of ammonia or ethylenediamine stepwise in a divergent manner. The synthesis of PAMAM dendrimers is mainly composed of two iterative steps: (1) the addition of excess ethylenediamine to methyl acrylate, and (2) amidation by an excess ethylenediamine (Scheme 6.1.1).

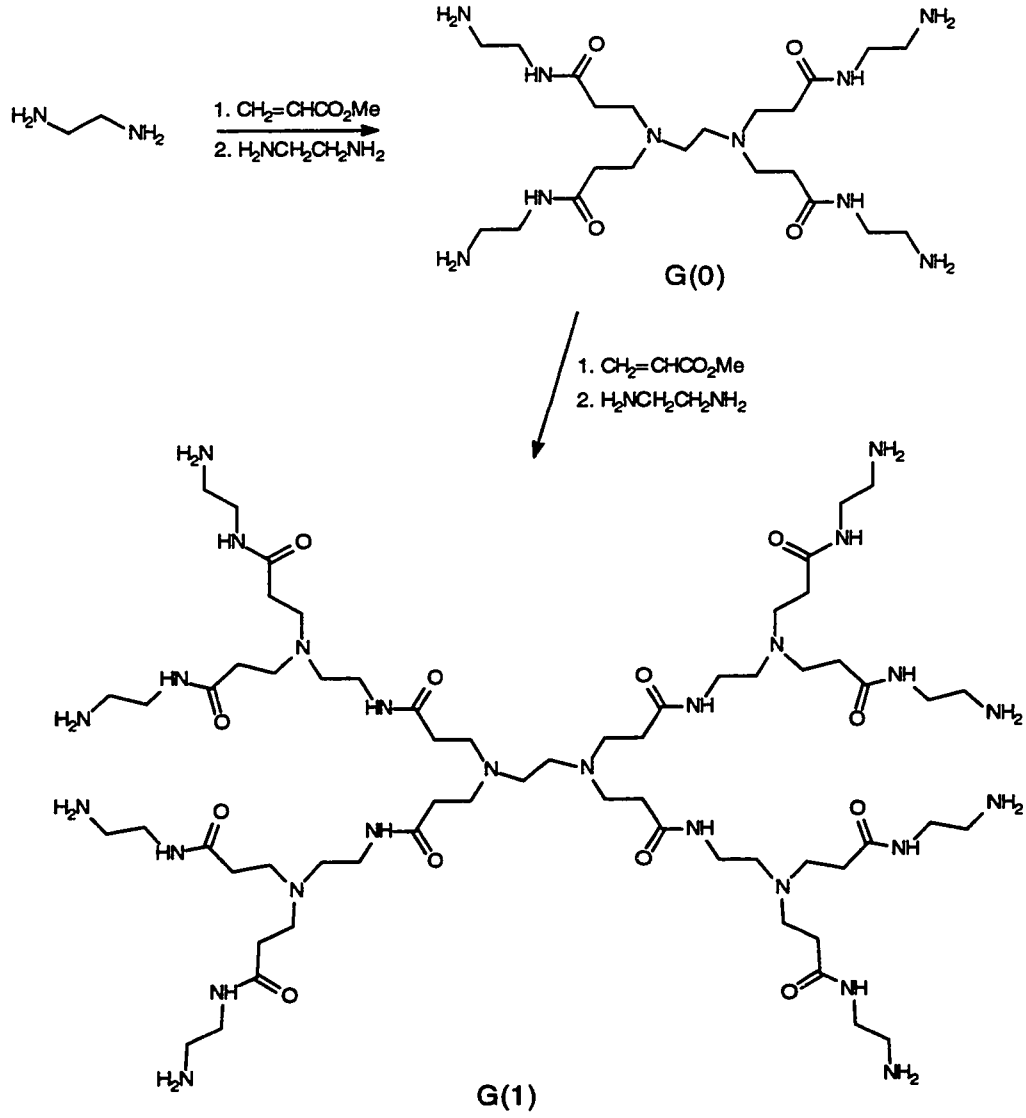
The physical characterization of PAMAM dendrimers are summarized in Table 6.1.1.

Table 6.1.1. Characterization of Starburst® PAMAM dendrimers.

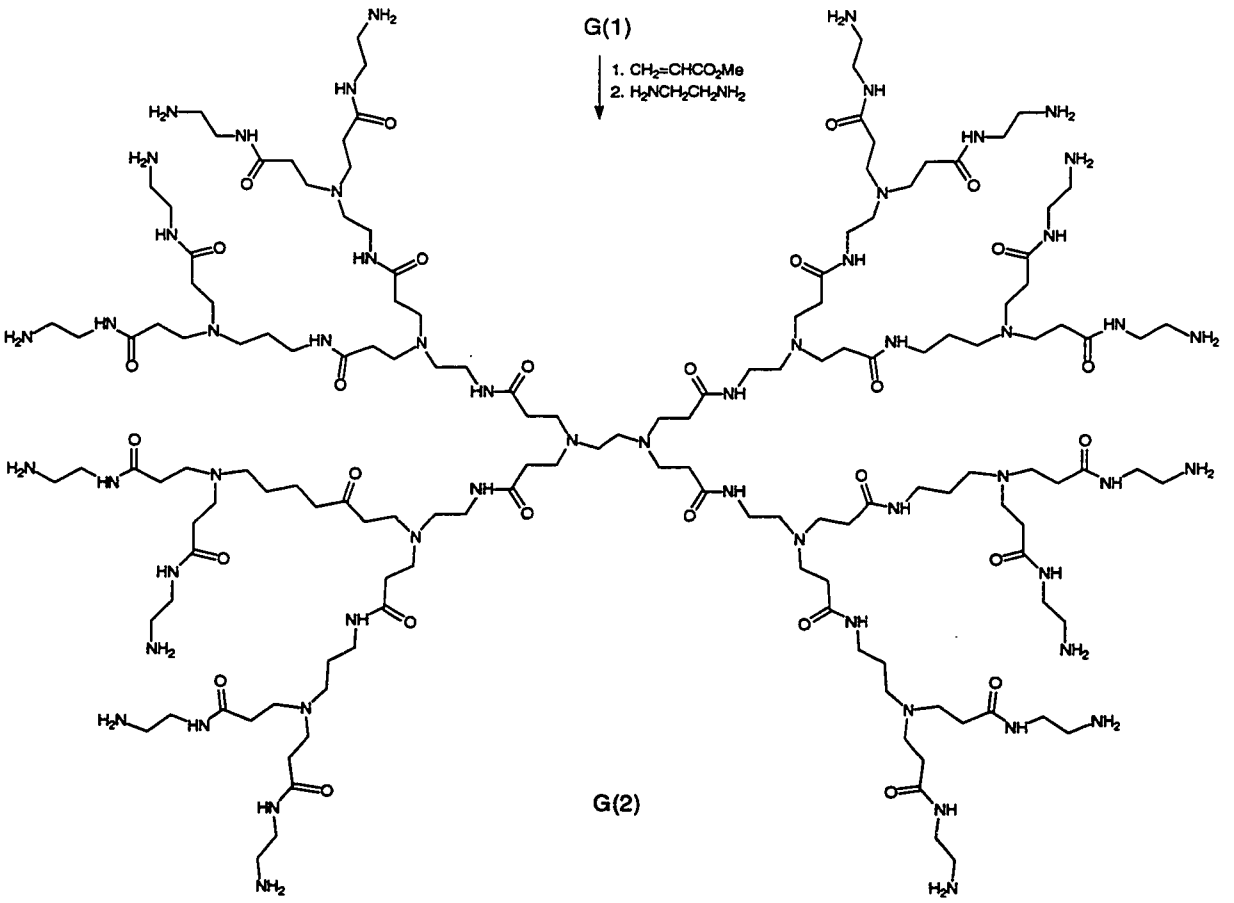
Generations	M.W.	Diameter (Å)	Surface groups
0	517	15	4
1	1,430	22	8
2	3,256	29	16
3	6,909	36	32
4	14,215	45	64
5	28,826	54	128
6	58,048	67	256
7	116,493	81	512
8	233,383	97	1024
9	467,162	114	2048
10	934,720	135	4096

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

[194] (a) Tomalia, D. A.; Durst, H. D. *Topics Curr. Chem.* **1994**, *165*, 193, (b) Tomalia, D. A. *Aldrichimica Acta* **1993**, *26*, 91, (c) Tomalia., D. A.; Naylor, A. M.; Goddart III, W. A. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 138.



Scheme 6.1.1. Synthesis of Starburst® PAMAM G(0) and G(1)dendrimers.



Scheme 6.1.2. Synthesis of Starburst® PAMAM (G2) dendrimer.

PAMAM dendrimers have been widely used to synthesize glycodendrimers in a systematically designed manner. Their biological properties have been studied with lectins specific for the incorporated carbohydrates.

To date, disaccharide lactones of lactose and maltose,[195] and Tn antigen peptide[195] have been conjugated to PAMAM dendrimers. Moreover, isothiocyanate derivatives of α-[196] and β-D-mannose, β-D-glucose, β-cellobiose, and β-lactose have

[195] Aoi, K.; Itoh, O.; Okada, M. *Macromolecules* **1995**, *28*, 5391.
[196] Pagé, D.; Roy, R. *Bioconjugate Chem.* 1998, in press.

been employed to generate PAMAM based glycodendrimers.[197] Sialic acid residues[198] have been also incorporated into these PAMAM dendrimers and their lectin (*Limax flavus*, LFA) binding assays confirmed an amplication in carbohydrate-protein interactions with an increase in the valency of sugar residues in neoglycoconjugates.

It is shown in this chapter that an extension of *N,N'*-dialkylation strategy which was employed as a branching methodology (see Chapter 4.2 and Chapter 5.2), can be used to synthesize PAMAM based glycodendrimers containing the modified Tn antigen structure (GalNAcα-*O*-homoserine). Starburst® PAMAM dendrimer (G2) was used to construct spherical glycodendrimers carrying 32 terminal GalNAc residues. The length between the final branching point and the terminal sugar residue was differentiated to probe the effect of spacer arm upon the binding interactions with their binding protein, lectin *Vicia villosa* B_4.

Glycopolymers

There have been examples of using acrylamide T- and Tn-copolymers as coating substrates in enzyme-linked immunoassays.[199] Roy et al[200] also prepared chemically well-defined water-soluble carbohydrate copolymers bearing *N*-acetylglucosamine,[201] sialic acid,[202] rhamnose,[203] lactose[204] and mannose residues[205,206] for various biological assays. Furthermore, a pre-activated polymer,

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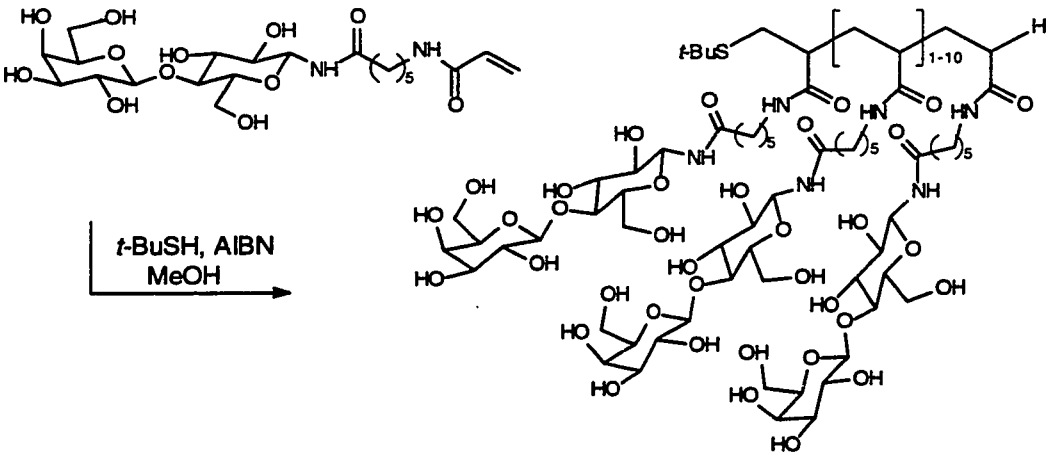
[197] Lindhorst, T. K.; Kieburg, C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1953.
[198] Zanini, D. *Ph.D. Dissertation* **1997**, Department of Chemistry, University of Ottawa.
[199] Chen, Y.; Jain, R. K.; Chandrasekaran, E. V.; Matta, K. L. *Glycoconjugate J.* **1995**, *12*, 55.
[200] Roy, R. *Trends Glycosci. Glycotechnol.* **1996**, *8*, 79.
[201] Roy, R.; Tropper, F. D. *Glycoconjugate J.* **1988**, *5*, 203.
[202] Roy, R.; Lafferière, C. A. *Carbohydr. Res.* **1988**, *88*, C1-C4.
[203] Roy, R.; Tropper, F. D. *J. Chem. Soc., Chem. Commun.* **1988**, 1058.
[204] Roy, R.; Tropper, F. D.; Romanowska, A. *Bioconjugate Chem.* **1992**, *3*, 256.
[205] Pagé, D. M.Sc. Dissertation, **1997**, Department of Chemistry, University of Ottawa.
[206] (a) Fan, J.-Q.; Quesenberry, M. S.; Takegawa, K.; Iwahara, S.; Kando, A.; Kato, I.; Lee, Y. C. *J. Biol. Chem.* **1995**, *270*, 17730, (b) Horejsi, V.; Kocourek, J. *Methods Enzymol.* **1974**, *34*, 361, (c) Mortell, K. H.; Weatherman, R. V.; Kiessling, L. L. *J. Am. Chem. Soc.* **1996**, *118*, 2297.

poly[*N*-(acryloxy)succinimide] has been used to prepare glycopolymers with known molecular weight.[207]

Telomers, short homopolymers having less than 10 residues, constitute a family of "clusters". These are readily available by quenching homopolymerization reaction with telogens which are essentially radical scavengers. Thiols are effective telogens and have been used in the syntheses of glycotelomers bearing lactose[208] in our laboratory (Scheme 6.1.3). These telomers can be also employed in their further attachment to other carriers such as L-lysine[209] and polymers[210] thus providing graft polymers in the latter case.



Scheme 6.1.3. Glycotelomer bearing lactose residue.

As well-defined polymers with pendant carbohydrate residues are of interest as cell-specific biomedical materials[211,212,213,214] and clustered carbohydrates are effective

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[207] Baek, B. G. *Ph.D. Dissertation*, **1997**, Department of Chemistry, University of Ottawa.
[208] Aravind, S.; Park, W. K. C.; Brochu, S.; Roy, R. *Tetraheron Lett.* **1994**, *35*, 7739.
[209] Park, W. K.C.; Aravind, S.; Romanowska, A.; Renaud, J.; Roy, R. *Methods Enzymol.* **1994**, *242*, 294.
[210] Kobayashi, K.; Kakishita, N.; Okada, M.; Akaike, T.; Kitazawa, S. *Makromol. Chem., Rapid Commun.* **1993**, *14*, 293.
[211] Weigel, P. H.; Scnaar, R. L.; Kuhlenschmidt, M. S.; Schmell, E.; Lee, R. T.; Lee, Y. C.; Roseman, S. *J. Biol. Chem.* **1979**, *254*, 10830.
[212] Kobayashi, K.; Sumitomo, H.; Ina, Y. *Polym. J.* **1985**, *17*, 567.

as recognition signals,[215] it would be useful to synthesize polymers, telomers and their graft copolymers bearing tumor-associate Tn antigen.

6.2. Synthesis of glyco-PAMAM dendrimers

As shown in the previous chapters (chapter 4.2, and chapter 5.2), the *N,N'*-dialkylation strategy successfully fulfilled our synthetic goal of building a dendritic structure that was branched. This method was therefore applied to construct hyper-branched glyco-PAMAM dendrimers bearing GalNAc moieties. The amine ending structure of PAMAM required two equivalents of bromoacetylated GalNAc ligand **151** per amine group. Compound **151** was readily obtained in high yield (98%) as described in Scheme 6.2.1.

To maximize the efficiency of substitution reaction in the *N*-alkylation process, two and half equivalents of bromoacetylated GalNAc ligand **151** and three equivalents of DIPEA were used for each amine functionality of PAMAM (G2). Due to the high molecular weight, the reaction could not be monitored by TLC. After 48 hours of its reaction, an aliquot was taken and analyzed by ^{1}H-NMR spectroscopy. The integration of key signals were used to assign the molecular structure. The benzoate protecting groups on the GalNAc moieties were removed under Zemplén condition (1M NaOMe, MeOH, pH 9, 24h). The residue was then dialyzed against water for 48 hours. Lyophilization of the solution afforded fully de-*O*-benzoylated glyco-PAMAM 32-mer **153** (Figure 6.2.1).

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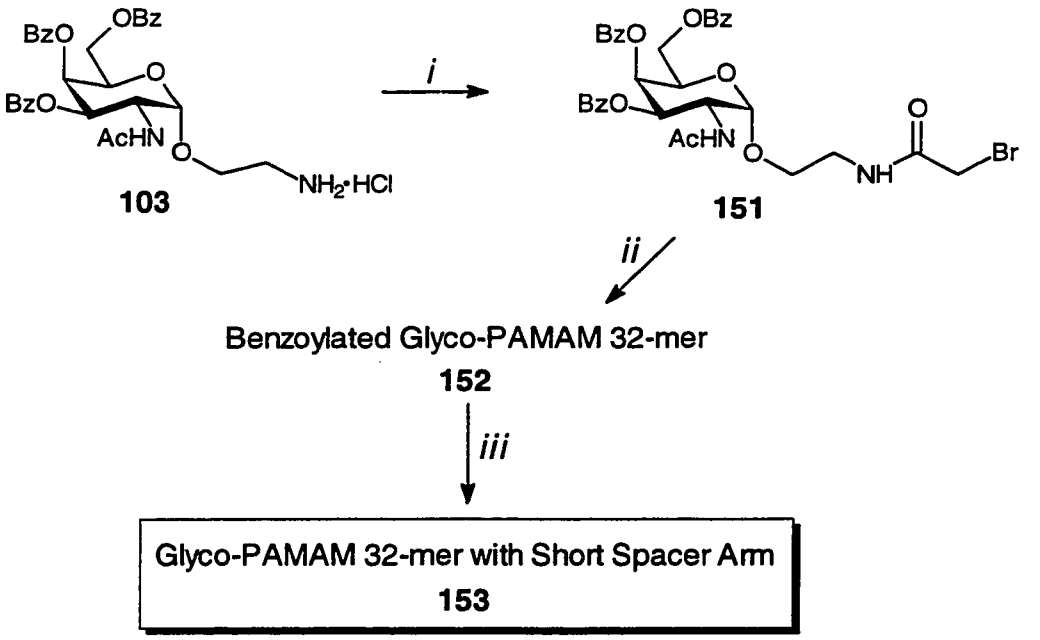
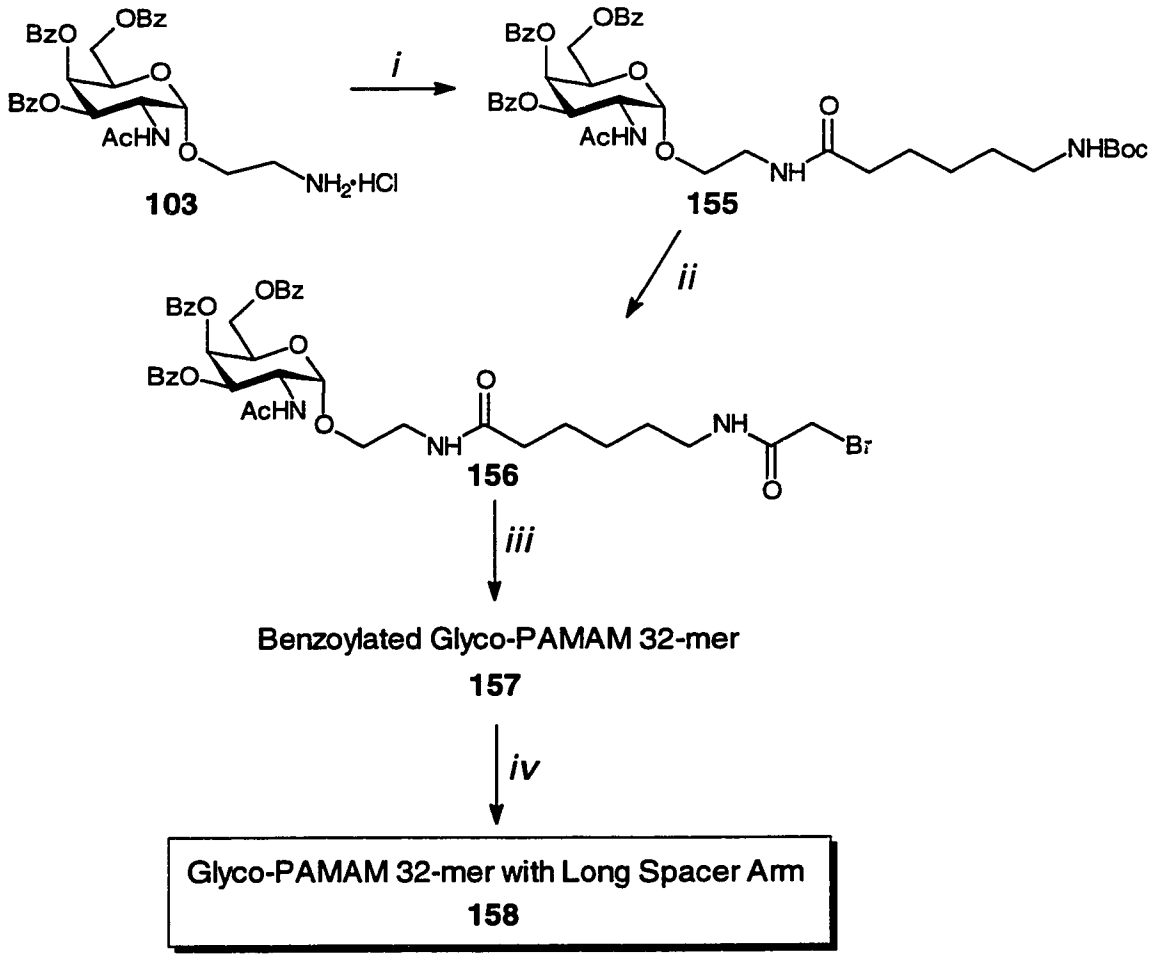
[213] Kobayashi, A.; Akaike, T.; Kobayashi, A.; Sumitomo, H. *Makromol. Chem., Rapid Commun.* **1986**, *7*, 645.
[214] Kugumiya, T.; Yagawa, A.; Maeda, A.; Nomoto, H.; Tobe, S.; Kobayashi, K.; Matsuda, T.; Onishi, T.; Akaike, T. *J. Bioactive Compatible Polym.* **1992**, *7*, 337.
[215] Lee, Y. C. *Carbohydrate Recognition in Cellular Function*, Wiley, Chichester, **1989** (Chiba Foundation Symp. 145) p 80.



Scheme 6.2.1. Synthesis of glyco-PAMAM 32-mer with a short spacer arm, **153**. *i*) $ClCOCH_2Br$ (1.2 eq.), DIPEA (2.5 eq.), CH_2Cl_2, 0 °C, 30 min., 98%; *ii*) PAMAM(G2), DIPEA, DMF, 60 °C, 48 h; *iii*) 1M NaOMe, MeOH, pH 9, 23 °C, 24 h, 62%.

In order to study the effect of flexibility of a molecule upon its binding capacity to the carbohydrate binding protein, another glyco-PAMAM 32-mer **158** was synthesized, which had a longer distance between the carbohydrate moiety and the branching point (tertiary amine at the end of the second generation of PAMAM) (Scheme 6.2.2).

2-Aminoethyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-benzoyl-α-D-galactopyranoside, **103**, was coupled to the *N*-hydroxysuccinimide active ester of *N*-Boc-caproic acid **154** (DIPEA, 85% yield) which was prepared treating *t*-butyl *N*-carbamylhexanoic acid with *N*-hydroxysuccinimide by the conventional amide coupling method (TBTU, DIPEA, 90%). This elongated GalNAc ligand **155** was bromoacetylated (89%) after deprotection of Boc group (20% TFA in CH_2Cl_2). This bromoacetylated GalNAc ligand **156** with an extended spacer arm was employed to generate *N,N'*-dialkylated glyco-PAMAM 32-mer **157** (Scheme 6.2.2). After deprotection of benzoate group of sugar residues in conventional manner (1M NaOMe, MeOH, pH 9), the glyco-PAMAM 32-mer **158** was purified by dialysis against water.



Scheme 6.2.2. Synthesis of glyco-PAMAM 32-mer with long spacer arm, **158**. *i*) $BocHN(CH_2)_5CO_2N(COCH_2)_2$ (**154**) (1.2 eq.), DIPEA (1.2 eq.), CH_2Cl_2, 23 °C, 30 min, 85%; *ii*) (1) 20% TFA, CH_2Cl_2, 23 °C, 2 h, (2) $ClCOCH_2Br$ (1.2 eq.), DIPEA (2.5 eq.), CH_2Cl_2, 0 °C, 30 min, 89%; *iii*) PAMAM(G2), DIPEA, DMF, 60 °C, 48 h; *iv*) 1M NaOMe, MeOH, pH 9, 23 °C, 24 h, 75%.

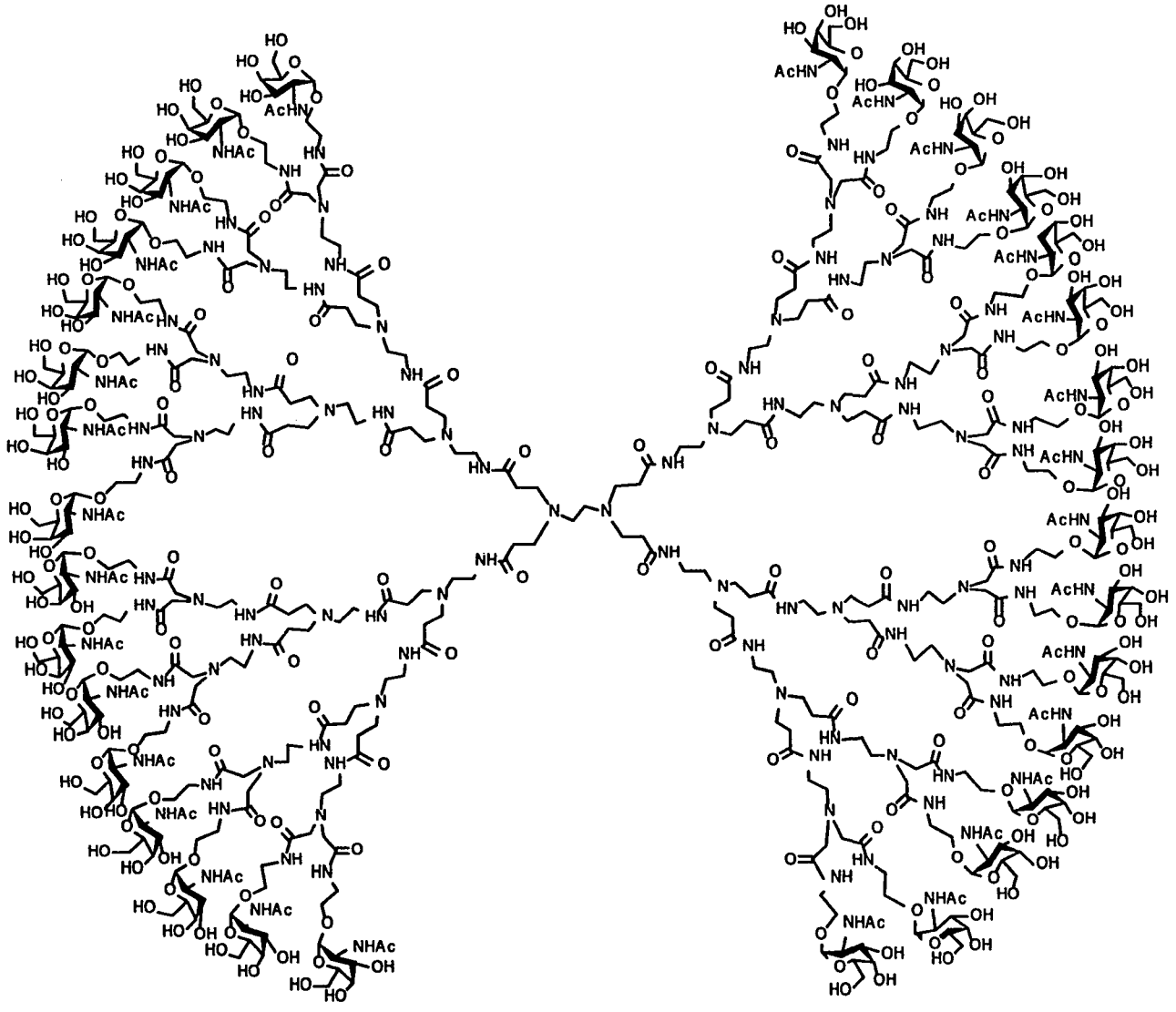


Figure 6.2.1. Short-spacer-armed glyco-PAMAM 32-mer **153**.

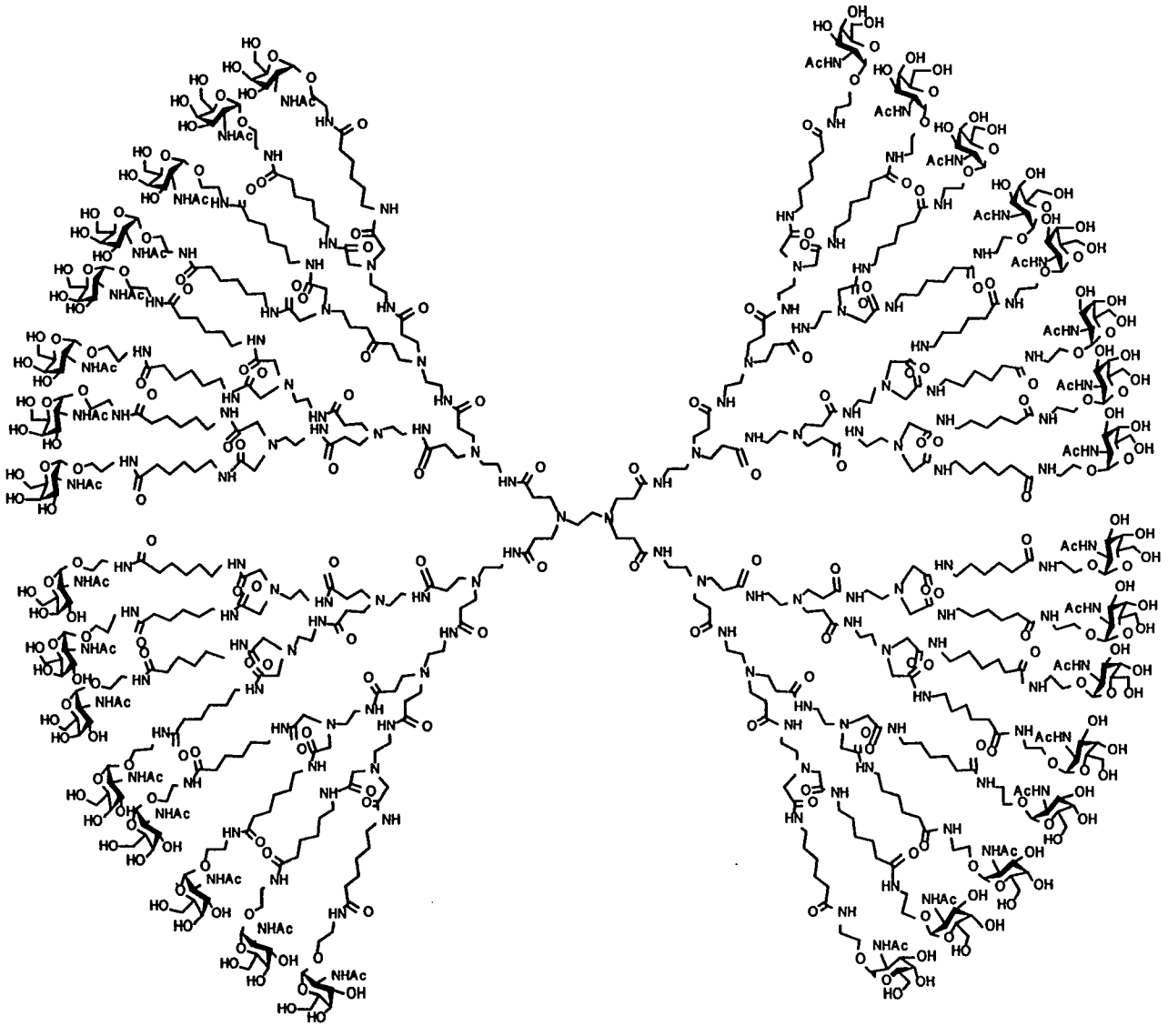


Figure 6.2.2. Long-spacer-armed glyco-PAMAM 32-mer **158**

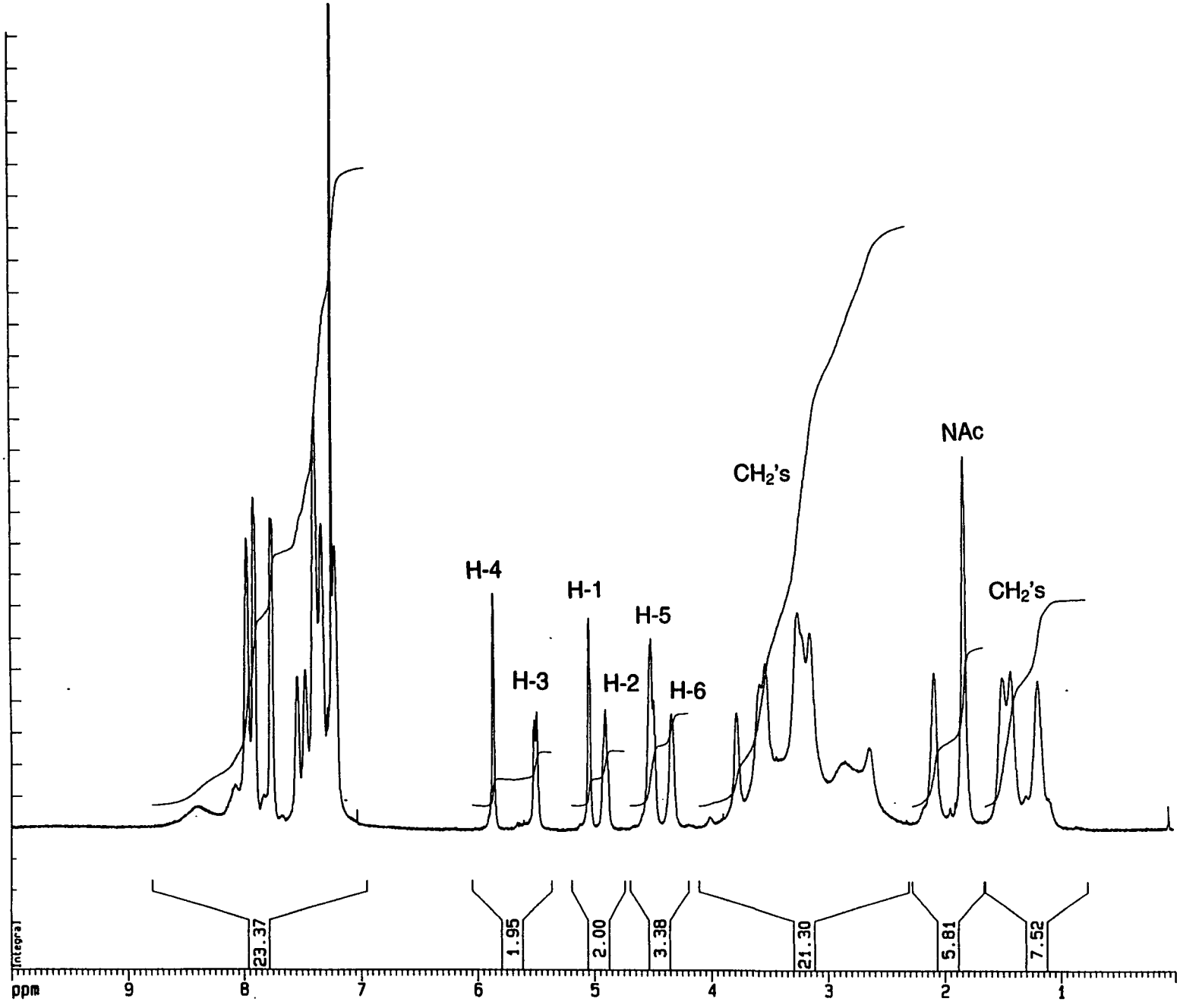


Figure 6.2.3. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of long spacer armed fully protected glyco PAMAM 32-mer **157**.

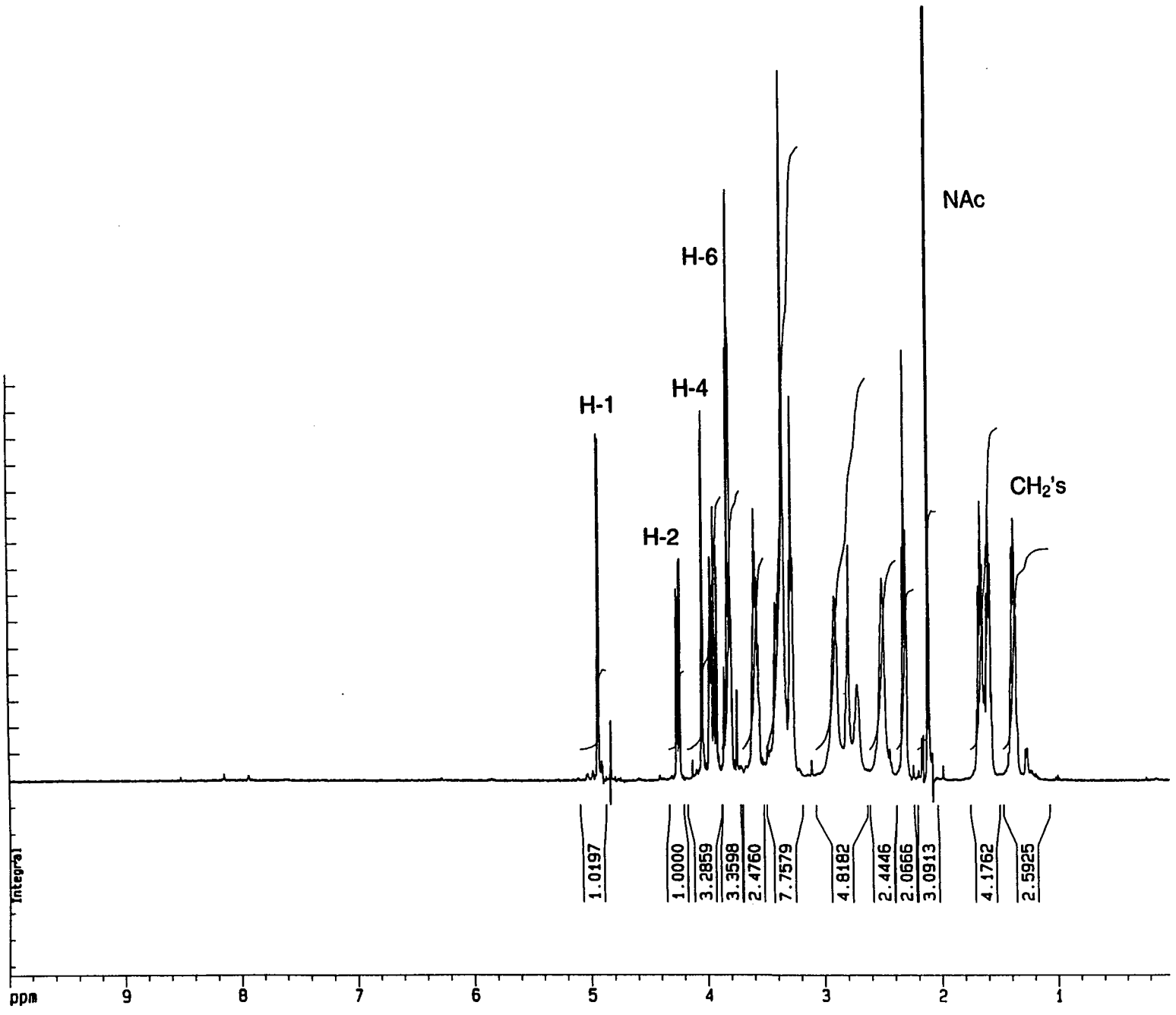


Figure 6.2.4. ^{1}H-NMR (D_2O, 500 MHz) spectrum of long spacer armed fully deprotected glyco PAMAM 32-mer **158**.

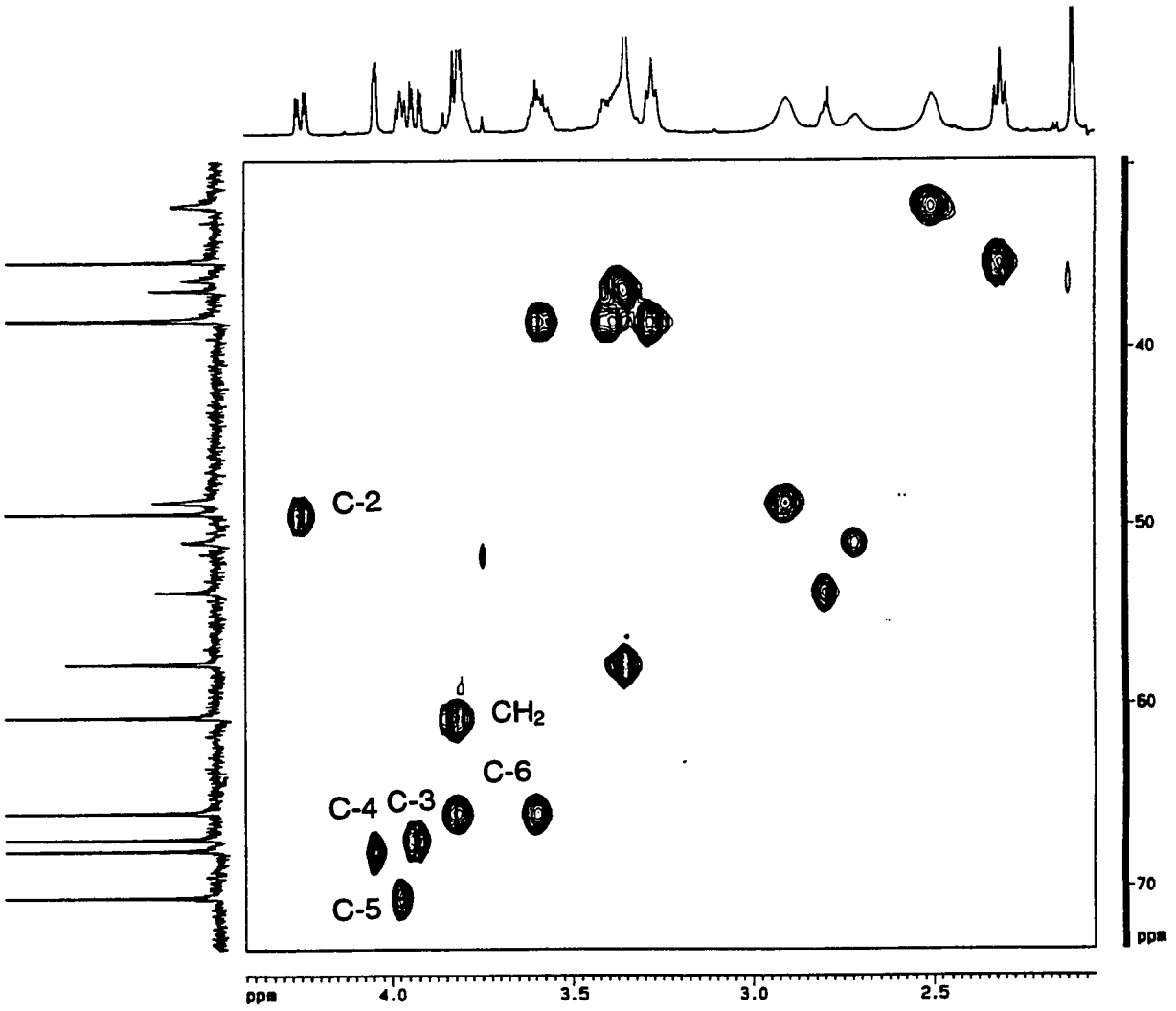
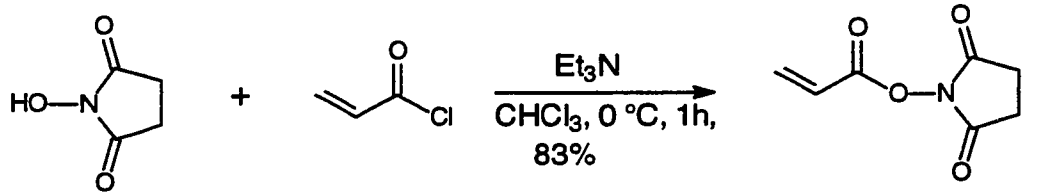


Figure 6.2.5. HMQC (D_2O, 500 MHz) spectrum of long spacer armed fully deprotected glyco PAMAM 32-mer **158**.

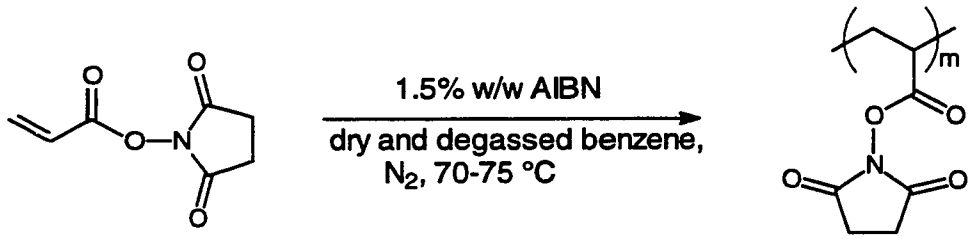
6.3. Syntheses of glycopolymers and glycotelomers

Preparation of glycopolymers from poly(N-acryloxysuccnimide) (159) using 2-aminoethyl GalNAc 160 and divalent GalNAc ligand 120 as monomers

Poly(*N*-acryloxysuccinimide) (**159**), a preactivated polymer with an *N*-hydroxysuccinimide active ester, was employed to prepare glycopolymers incorporating the GalNAc moiety. This poly(*N*-acryloxysuccinimide) (**159**) was prepared in our group and its preparation is shown in Scheme 6.3.1. The molecular weight of poly(N-acryloxysuccinimide) (**159**) was determined to be 42.1 kDa by measuring its viscosity.



N-Acryloxysuccinimide

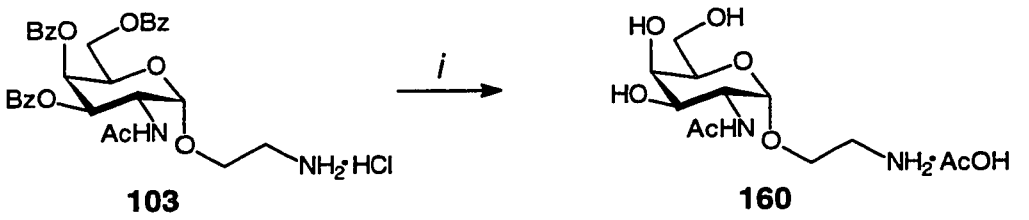


159
Poly(N-acryloxysuccinimide)

Scheme 6.3.1. Preparation of poly(*N*-acryloxysuccinimide) (**159**) from its monomer.

The synthesis of glycopolymer was accomplished by conjugating 2-aminoethyl 2-acetamido-2-deoxy-α-D-galactopyranoside (**160**) into the preactivated poly(*N*-

acryloxysuccinimide) (**159**). Fully deprotected 2-aminoethyl GalNAc **160** was obtained in conventional manner (1M NaOMe, MeOH, pH 9) from its benzoylated precursor **103** (Scheme 6.3.2).



103 **160**

Scheme 6.3.2. Preparation of fully deproteced 2-aminoethyl α-D-GAlNAc **160**. *i*) (1) 1M NaOMe, MeOH, pH 9, 23 °C, 2 h, (2) AcOH (1.2 eq.), LH20 column (MeOH), 89%.

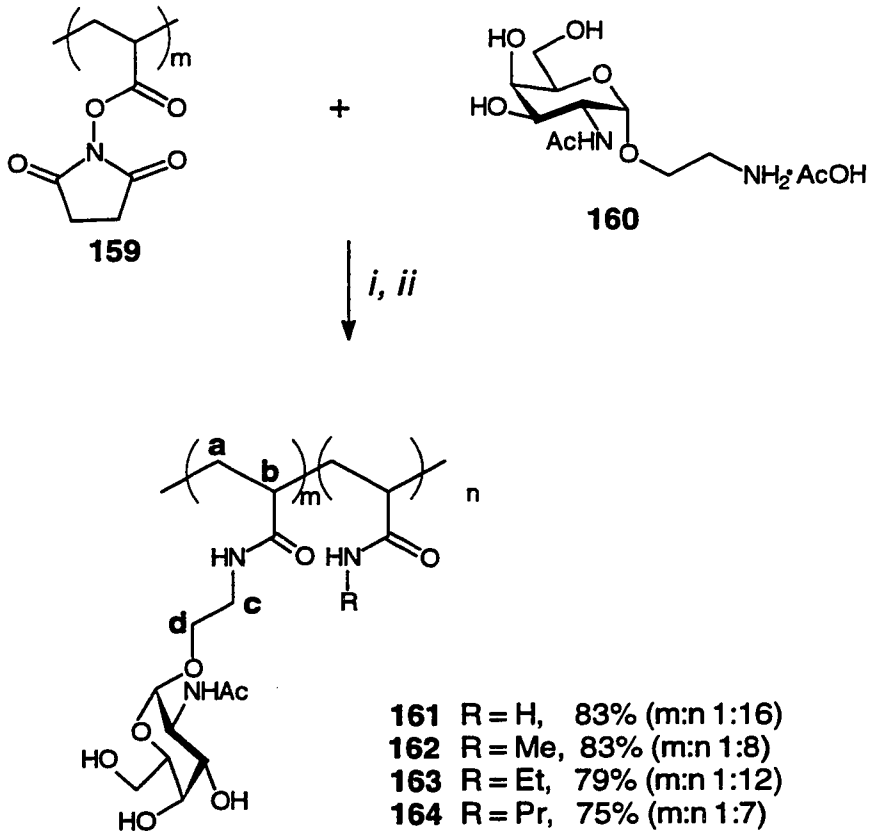
Poly(*N*-acryloxysuccinimide) (**159**) was stirred in DMF at room temperature for one hour to obtain a homogeneous solution and to this solution was added 2-aminoethyl GalNAc (**103**) in DMF. The reaction mixture was stirred for 24 hours at room temperature in the presence of Et_3N. Then, excess of active ester in the polymer backbone was quenched with ammonium hydroxide (NH_4OH), methylamine ($MeNH_2$), ethylamine ($EtNH_2$), or propylamine ($PrNH_2$) to afford acrylamide copolymers with different side chains (Scheme 6.3.3).

Table 6.3.1. Results from copolymerization of 2-aminoethyl GalNAc (**160**) with poly(*N*-acryloxysuccinimide) (**159**).

Copolymer	Monomer ratio[a]	Yield %[b]	Copolymer composition[c]
161 R = H	1:10	83	1:16
162 R = Me	1:10	83	1:8
163 R = Et	1:10	79	1:12
164 R = Pr	1:10	75	1:7

[a] Molar ratio of carbohydrate monomer to poly(acryloxysuccinimide).
[b] Based on the weight.
[c] Based on ^{1}H-NMR.

The compositions of the prepared copolymers (Table 6.3.1) were determined based on the relative size of the integrations of the ^{1}H-NMR (D_2O) signals of the backbone methylene (δ ≈1.4-1.8 ppm) and methine protons (δ ≈2.0-2.4 ppm) to the anomeric proton at δ ≈5.0 ppm. In the cases of ethylamine and propylamine conjugation, methyl protons (δ ≈ 1.0 ppm) or ethylene (δ ≈ 3.2 ppm) protons were also taken as the reference signals.



161 R = H, 83% (m:n 1:16)
162 R = Me, 83% (m:n 1:8)
163 R = Et, 79% (m:n 1:12)
164 R = Pr, 75% (m:n 1:7)

Scheme 6.3.3. Preparation of glycopolymers **161-164**; *i*) Et_3N, DMF, 23 °C, 24 h; *ii*) NH_4OH / $MeNH_2$ / $EtNH_2$ /$PrNH_2$, 23 °C, 24 h.

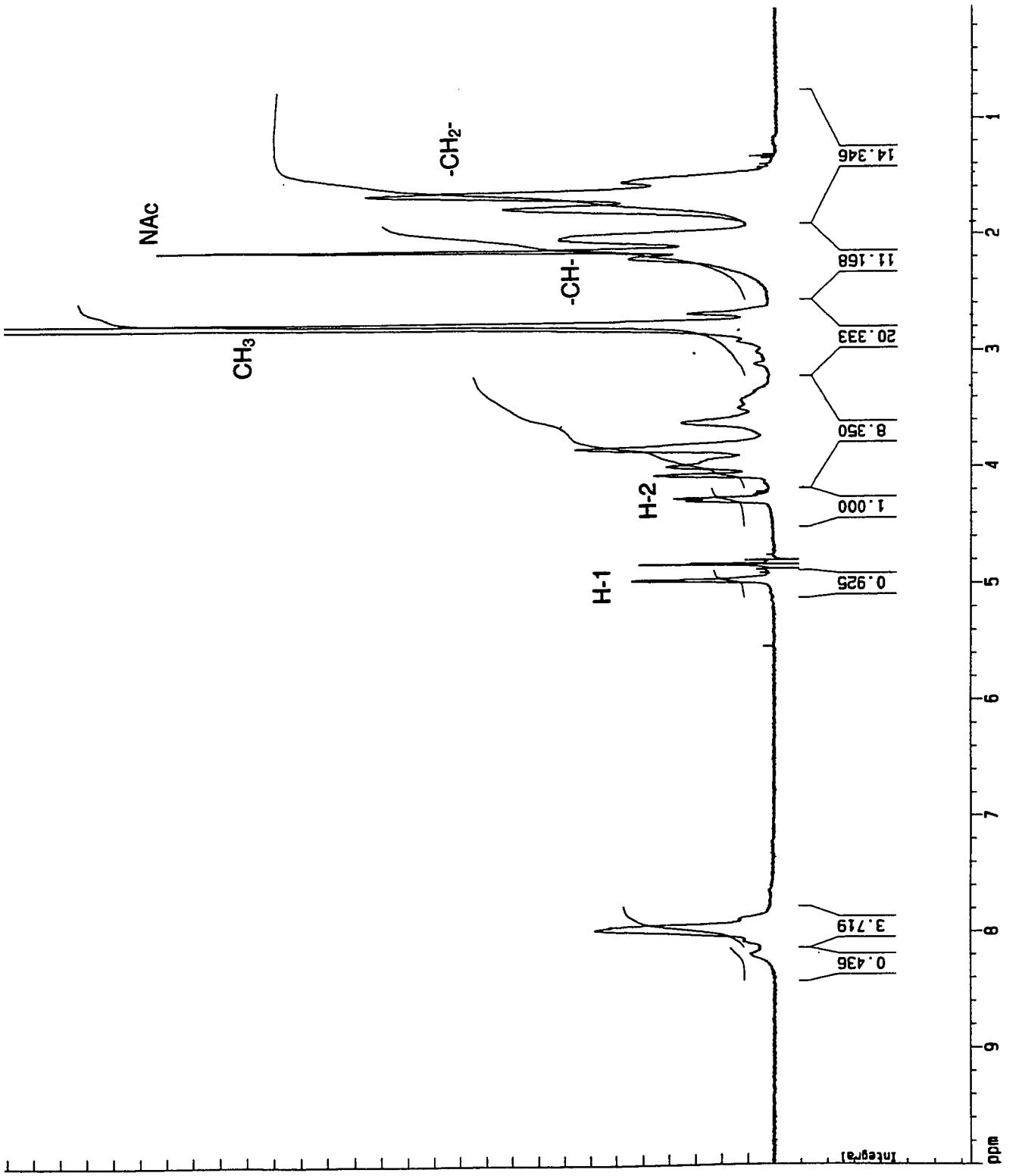
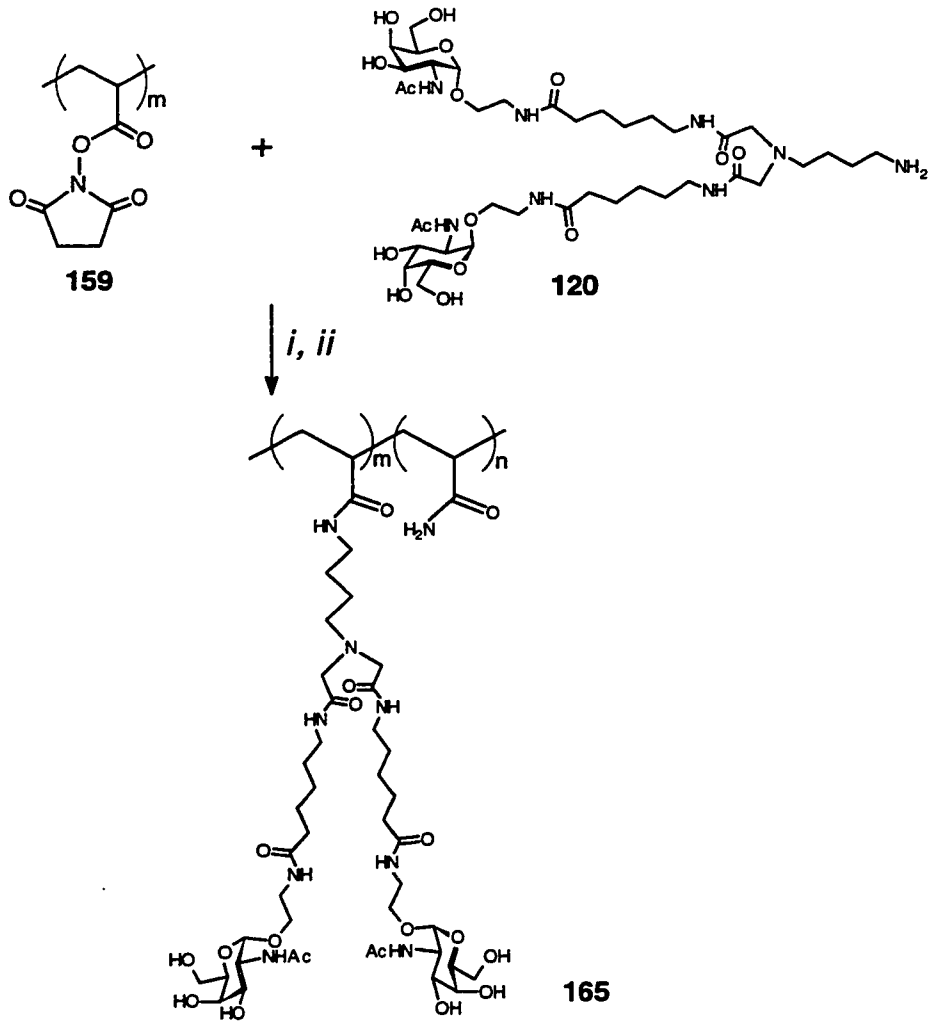


Figure 6.3.1. ^{1}H-NMR (D_2O, 500 MHz) spectrum of copolymer **162**.

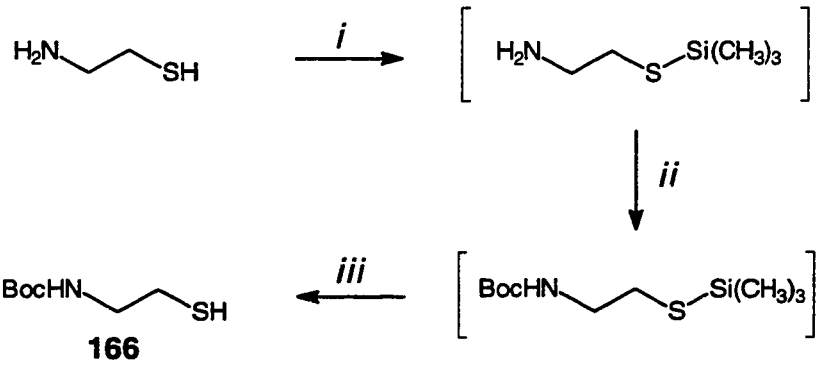
The synthesis of copolymer **165** of the branched divalent GalNAc ligand **120** was also fulfilled (Scheme 6.3.4). The divalent GalNAc ligand **120** was conjugated to the poly(N-acryloxysuccinimide) (**159**) using the same procedure described previously in order to provide the branched copolymer bearing the GalNAc moiety **165** (82% yield, w/w).



Scheme 6.3.4. Preparation of copolymer **165** using divalent GalNAc ligand **120**.

Preparation of telomers from mono- and divalent GalNAc ligands

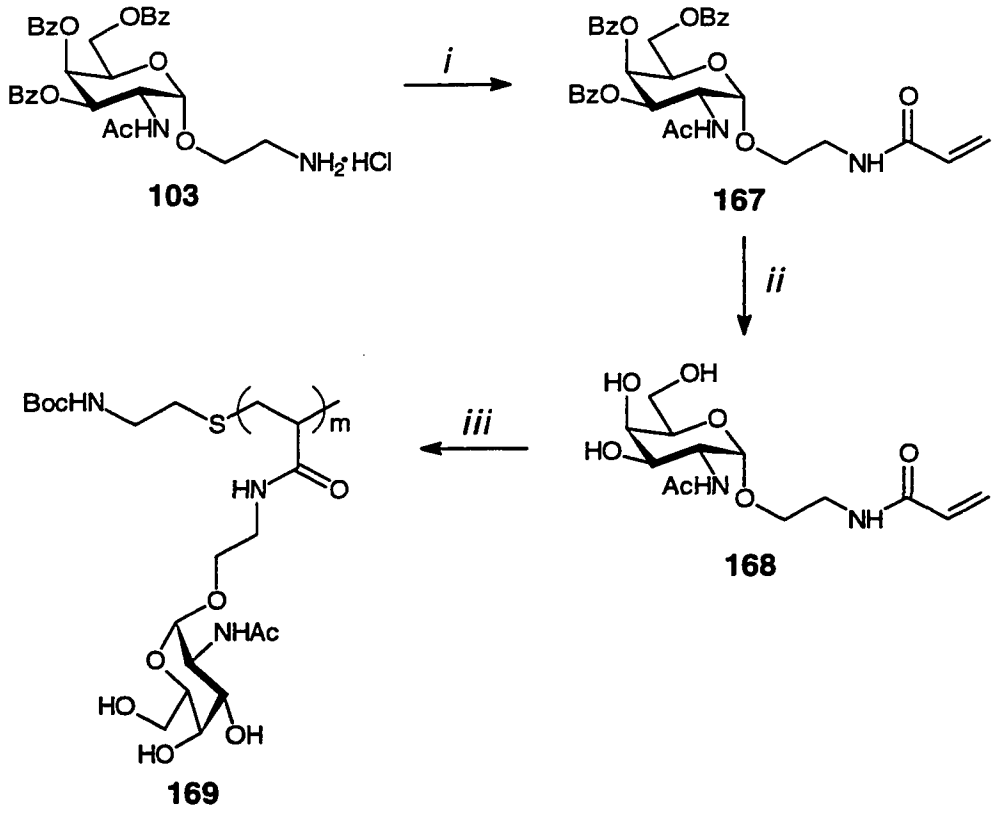
The preparation of low molecular weight clusters was accomplished in a single-step reaction using *N*-Boc-cysteamine ($BocNHCH_2CH_2SH$) (**166**). *N*-Boc-cysteamine (**166**) was chosen as a telogen because it contains an excellent reporter group, the *t*-butyl group, which is observed at ≈1.5 ppm in ^1H-NMR spectrum (D_2O). *N*-Boc-cysteamine (**166**) was obtained from cysteamine using chlorotrimethylsilane followed by di-*t*-butyl dicarbonate. The thiol functionality was protected first with a trimethylsilyl group. The S-Si linkage was readily hydrolyzed during the aqueous work-up after *N*-Boc protection (Scheme 6.3.5).



166

Scheme 6.3.5. Preparation of *N*-Boc-cysteamine **166**; *i*) $(CH_3)_3SiCl$ (1.3 eq.), DIPEA (2.3 eq.), CH_3CN, 0 °C, 10 min; *ii*) (*t*-$BuO_2C)O$ (1 eq.), DIPEA (1 eq.), CH_3CN, 0 °C, 30 min, 23 °C, 1 h; *iii*) H_2O, 91%.

The carbohydrate monomer **167** equipped with acrylamidoethyl spacer arm was telomerized using *N*-Boc-cysteamine **166** and 2,2'-azobisisobutylronitrile (AIBN) as telogen and initiator, respectively. The reaction solution in $MeOH/H_2O$ (1:4, v/v) was refluxed for 24 hours to afford telomers (86% yield) (Scheme 6.3.6).

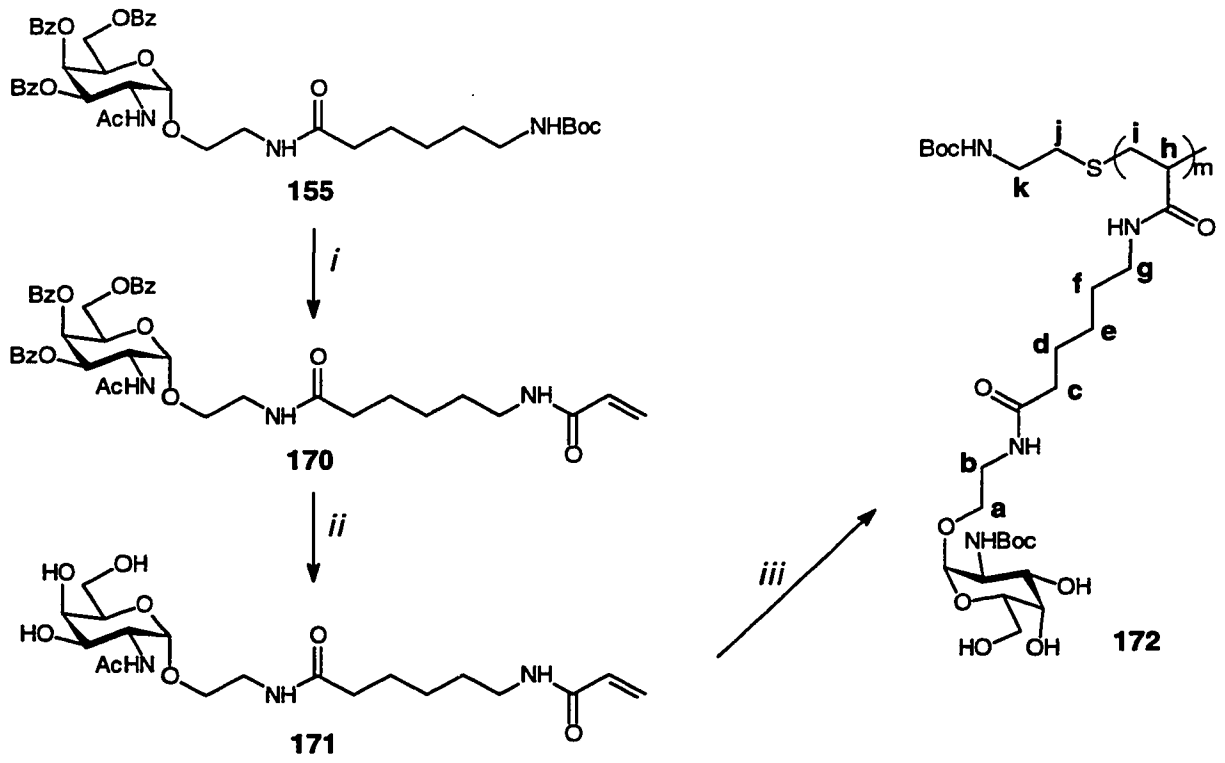


Scheme 6.3.6. Preparation of telomer **169** (m=7) with short aglycon spacer; *i*) $ClCOCH{=}CH_2$ (1.2 eq.), DIPEA (2.5 eq.), 0 °C, 30 min; *ii*) 1M NaOMe, MeOH, pH 9, 23 °C, 2 h, 90%; *iii*) $BocHNCH_2CH_2SH$ (**166**), AIBN, H_2O/MeOH (4:1), reflux, 24 h, 86% (w/w).

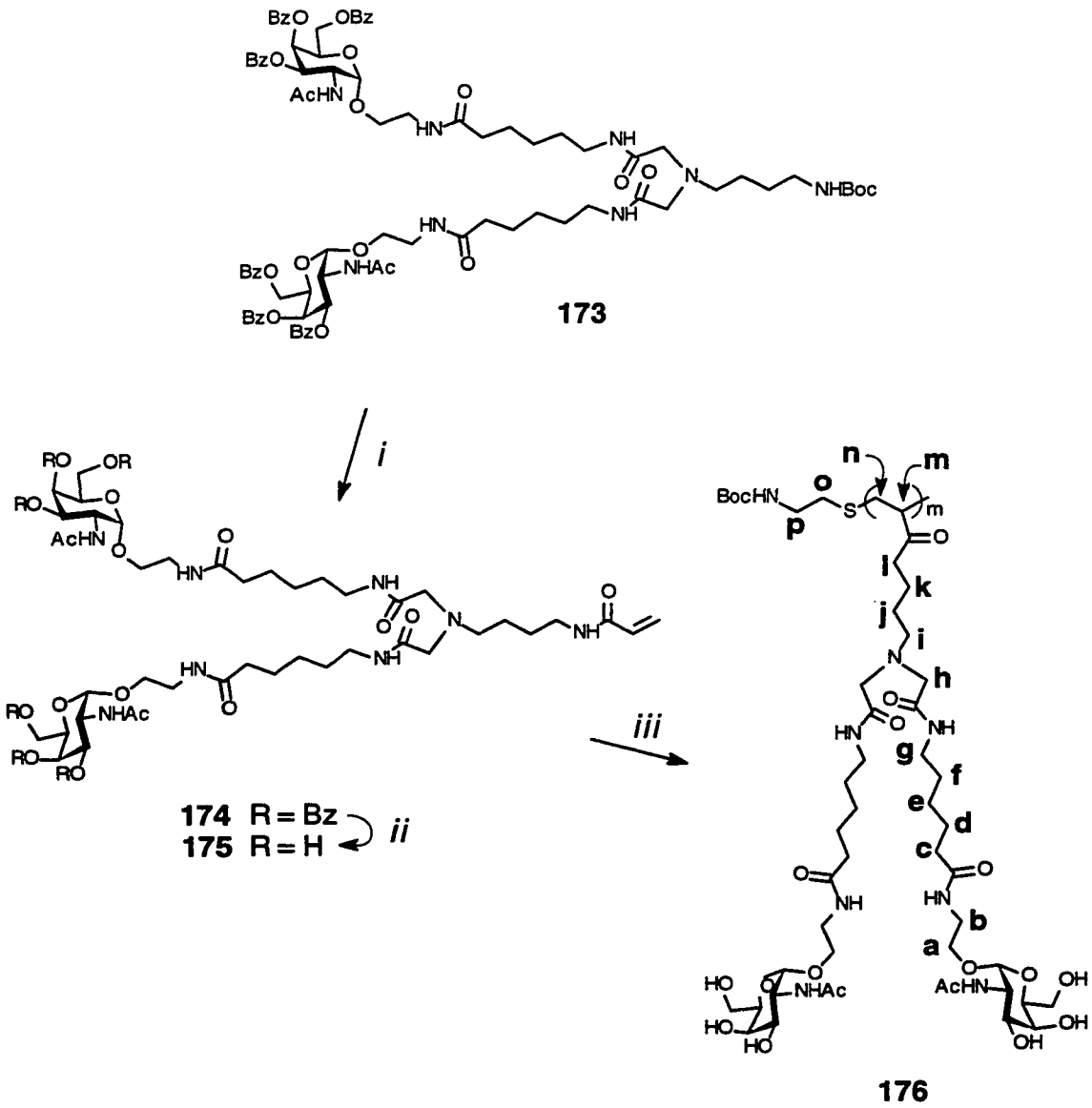
Using the same method, the longer-spacer-armed monomer **171** was telomerized in 70% yield as illustrated in Scheme 6.3.7. *N*-Boc-caproic acid-coupled GalNAc ligand **155** was acrylated ($ClCOCH{=}CH_2$, DIPEA, 87%) after deprotecting Boc group (20% TFA, CH_2Cl_2). The benzoate protecting group on the carbohydrate moiety of the acrylated monomer **170** was removed under Zemplén condition (1M NaOMe, MeOH, 23 °C, 1h, 80%) and telomerization of this monomer **170** was established in the presence of *N*-Boc-cysteamine **166** and AIBN (70% yield)).

Construction of telomer using a divalent monomer was also performed. Acrylation of the divalent GalNAc ligand **173** followed by deprotection of the Boc group

provided a precursor **174** in 98% yield (Scheme 6.3.8). Telomerization of this divalent acrylate, however, did not result in high yield (30%). This is probably due to the molecular hindrance of the divalent GalNAc ligand.



Scheme 6.3.7. Preparation of telomer **172** (m=6) with long aglycon spacer; *i*) (1) 20% TFA, CH_2Cl_2, 23 °C, 1 h, (2) $ClCOCH{=}CH_2$ (1.2 eq.), DIPEA (3.5 eq.), CH_2Cl_2, 0 °C, 1 h, 87%; *ii*) 1M NaOMe, MeOH, pH 9, 23 °C, 1h, 80%; *iii*) $BocHNCH_2CH_2SH$ (**166**), AIBN, H_2O/MeOH (3:1), 24 h, reflux, 70%.



Scheme 6.3.8. Preparation of telomer **176** (m=3) with divalent GalNAc ligand; *i*) (1) 20% TFA, CH_2Cl_2, 23 °C, 1 h, (2) $ClCOCH{=}CH_2$ (1.2 eq.), DIPEA (3.5 eq.), CH_2Cl_2, 0 °C, 1 h, 98%; *ii*) 1M NaOMe, MeOH, pH 9, 23 °C, 1 h, 95%; *iii*) $BocHNCH_2CH_2SH$ (**166**), AIBN, H_2O/MeOH (1:1), 24 h, reflux, 30%.

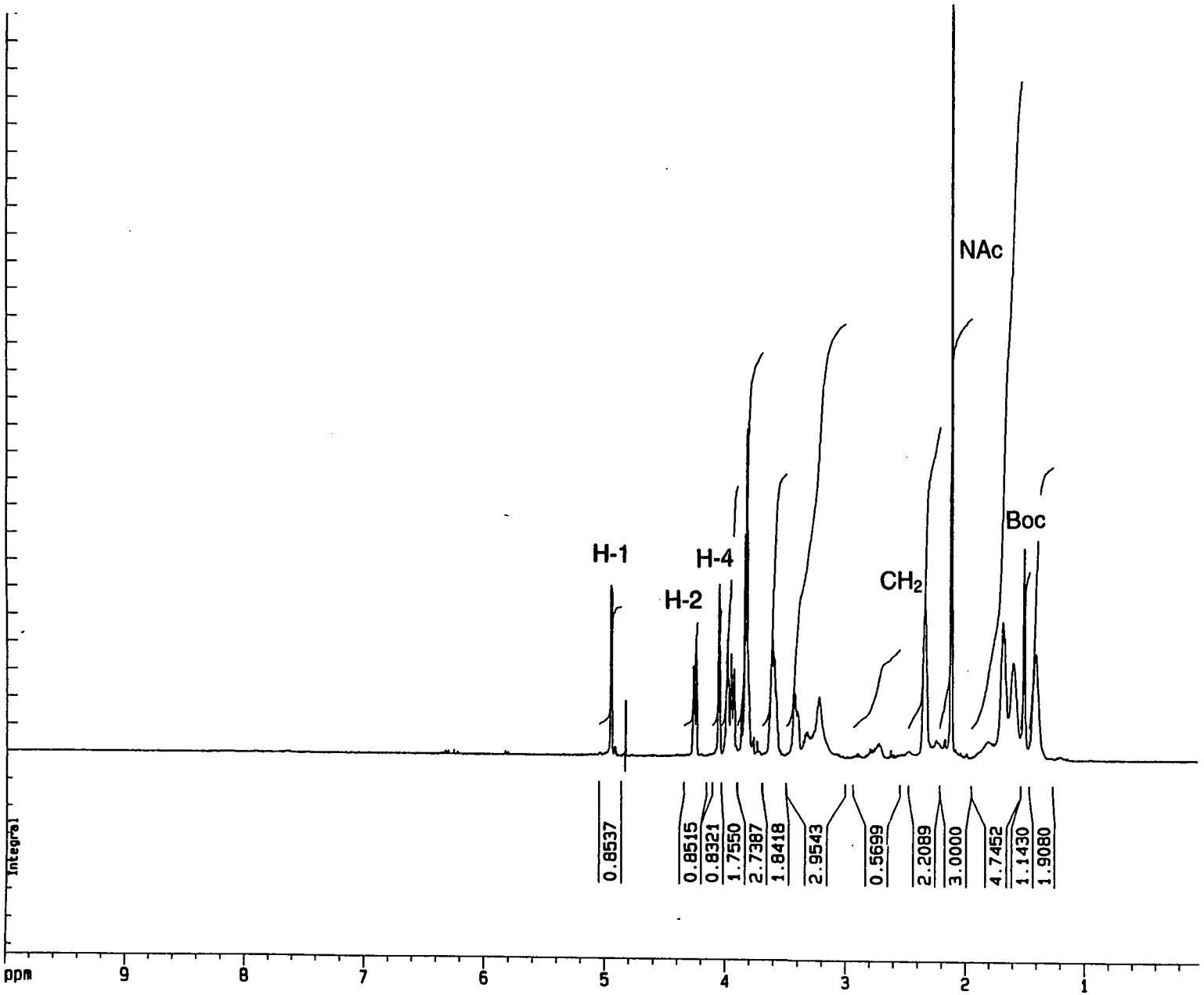


Figure 6.3.2. ^{1}H-NMR (D_2O, 500 MHz) spectrum of telomer **172** (m=3) with long aglycon spacer.

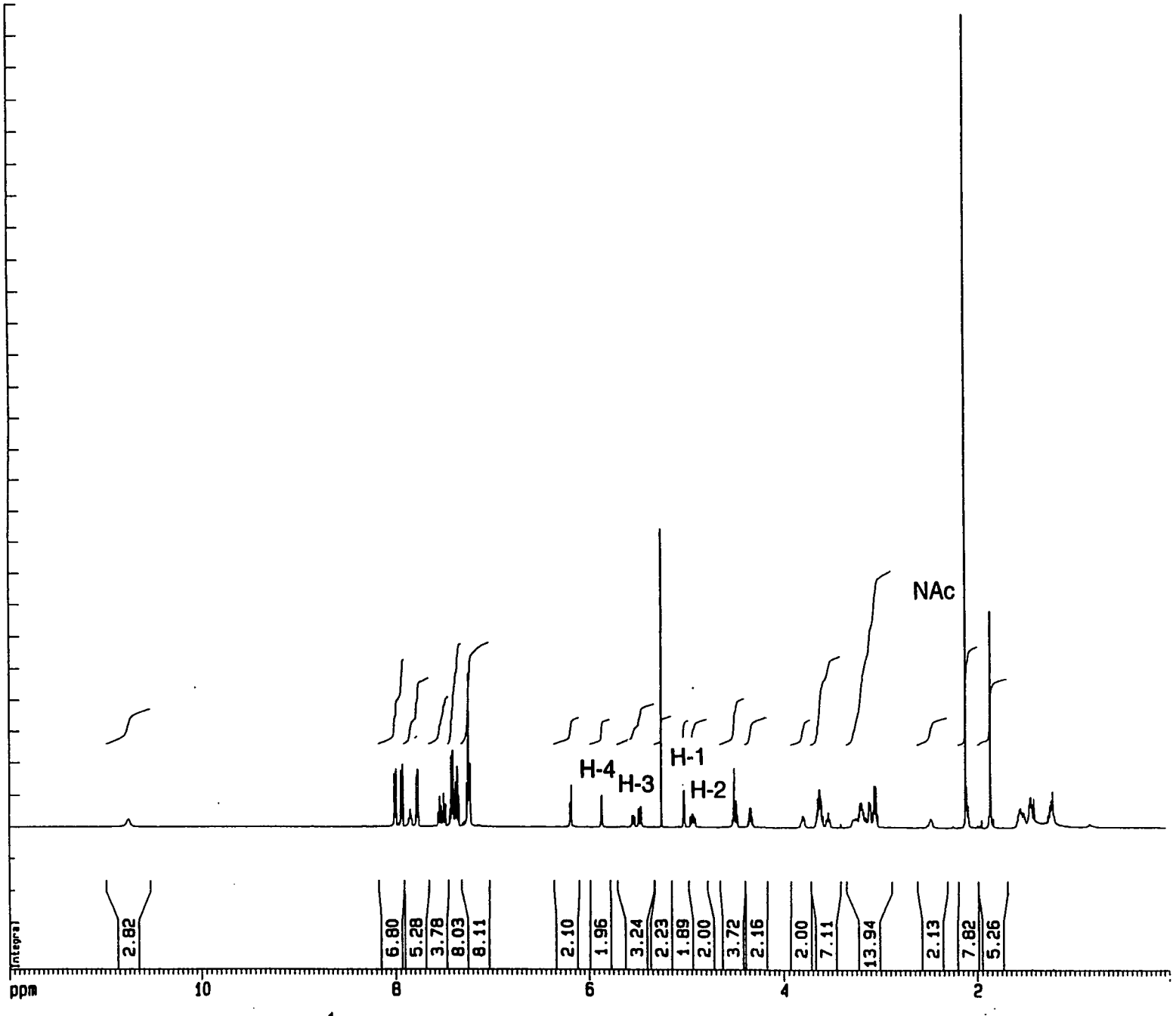


Figure 6.3.3. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of acrylamide of divalent GalNAc ligand **174**.

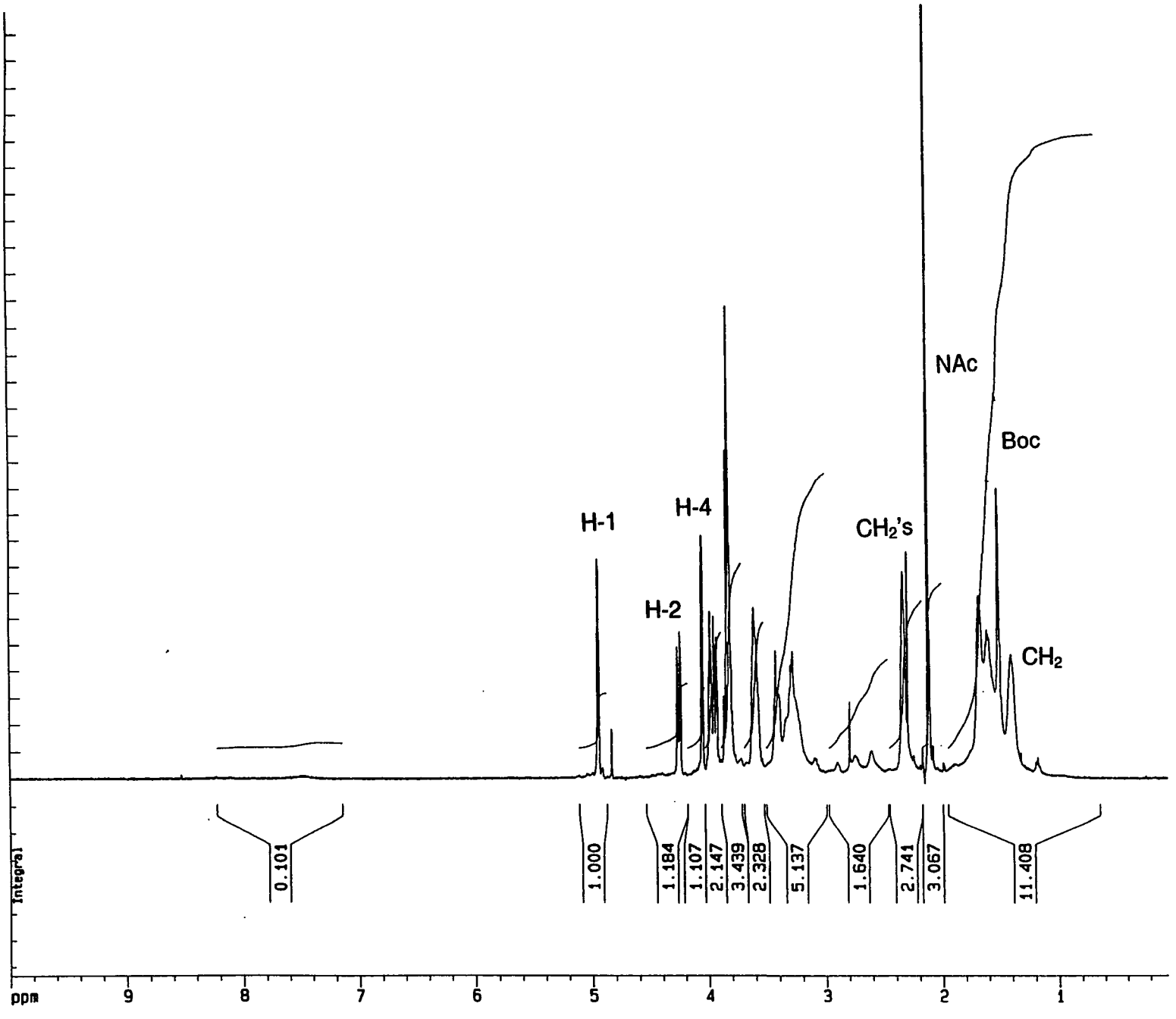


Figure 6.3.4. ^{1}H-NMR (D_2O, 500 MHz) spectrum of telomer **176** (m=3) with divalent GalNAc ligand.

6.4. Binding assays

Direct binding of glyco-PAMAM 32-mers 153 and 158 to VVA

Turbidimetric analyses were performed to determine the direct binding of these glyco-PAMAM 32-mers **153** and **158** to the GalNAc specific lectin VVA. The time course of formation of precipitate is shown in Figure 6.4.1.

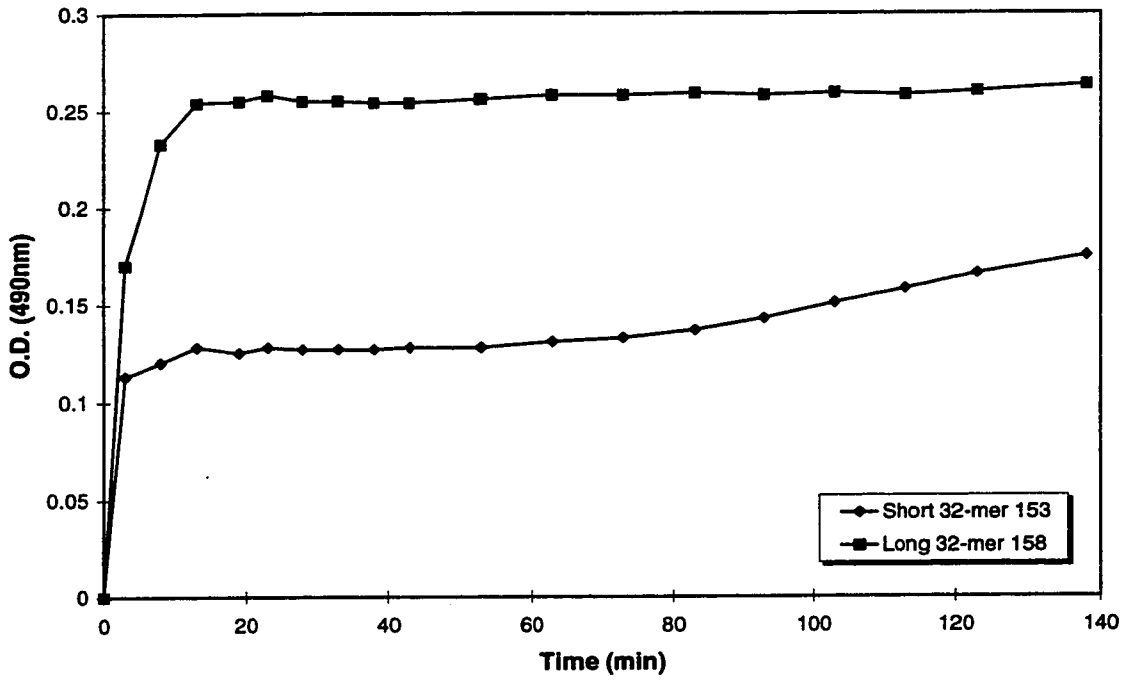


Figure 6.4.1. Turbidimetric analyses of glyco-PAMAM 32-mers bearing GalNAc **153** and **158** using VVA.

As illustrated in Figure 6.4.1, the distances between the GalNAc and the branching points of PAMAM based glycodendrimers played an important role in their binding ability. Notwithstanding the fact that both glycodendrimers **153** and **158** had the

same valencies (32-mer), the optical density measurements of their turbidities, which resulted from the cross-linking between the carbohydrate moiety and its binding protein, clearly demonstrated that the longer spacer arm allowed more efficient binding to its binding protein than the short one. The results of the turbidimetric experiments also indicated that the maximum turbidity was reached within 20 minutes in both cases.

ELLA inhibition of glyco-PAMAM 32-mers 153 and 158

The inhibitory capacities of these PAMAM based 32-meric glycodendrimers **153** and **158** were tested using asialoglycophorin as coating antigen and VVA/HRP as carbohydrate binding protein. Their results were illustrated in Figure 6.4.2. and Figure 6.4.3.

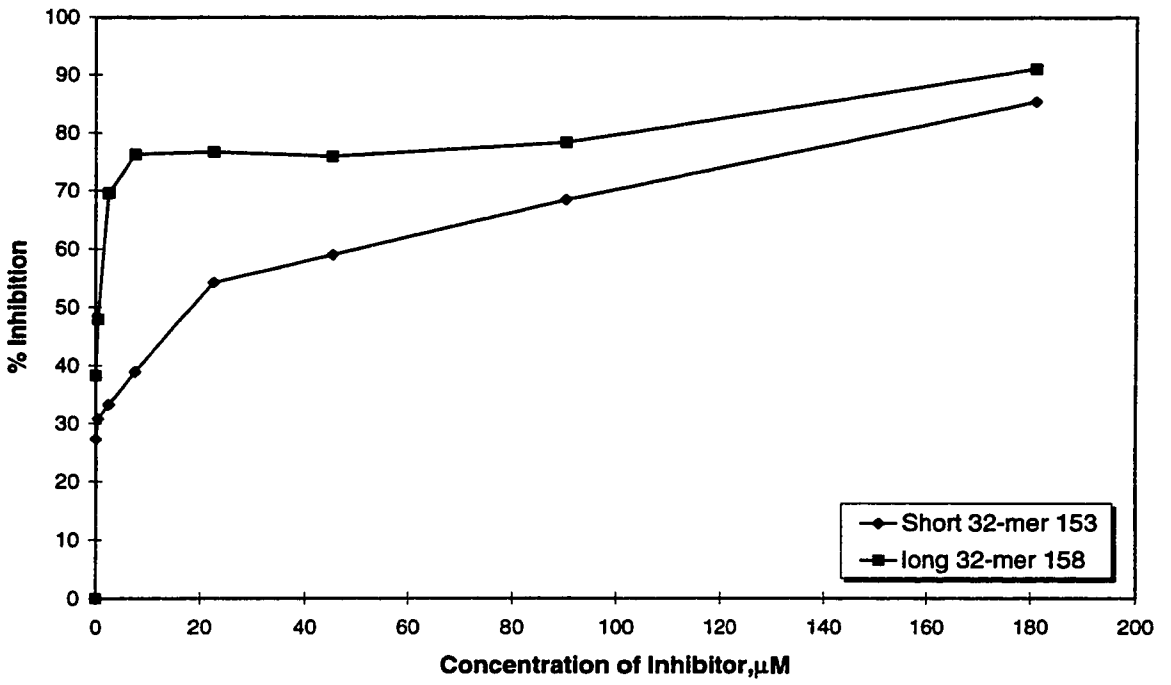


Figure 6.4.2. ELLA inhibition of binding to VVA/HRP to asialoglycophorin by glyco-PAMAM 32-mers **153** and **158**.

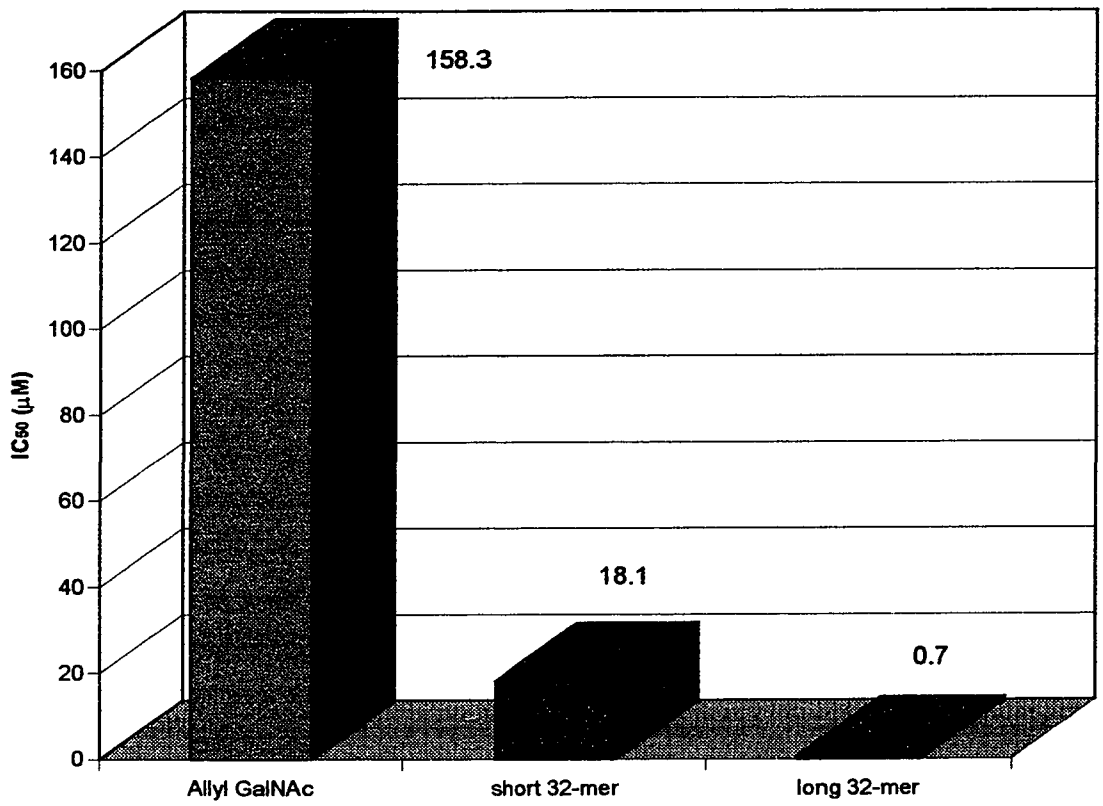


Figure 6.4.3. IC_{50}'s of glyco-PAMAM 32-mers **153** and **158**.

Table 6.4.1. IC_{50}'s of glyco-PAMAM 32-mers **153** and **158**

GalNAc ligands	IC_{50}'s, μM	Relative potency[a]
allyl α-D-GalNAc **33**	158.3	1
short PAMAM 32-mer **153**	18.1	8.7 (0.3)
long PAMAM 32-mer **158**	0.7	226 (7.1)

[a] Values in parentheses are based on a per-hapten in a molecule.

As shown in Table 6.4.1, the longer aglycon spacer is intensely accountable for efficient binding of PAMAM dendrimers to lectin *VVA* B_4. Whereas short spacer armed

dendrimer **153** did not show a cluster effect, ligand **158** with a long spacer had a 7.1-fold increase with respect to the monomer on per sugar basis.

6.5. Conclusions

PAMAM based dendrimers (32-mer) were prepared using the *N,N'*-dialkylation strategy which was employed for the syntheses of branched dimers in chapters 4 and 5. The bromoacetylated GalNAc ligands were successfully conjugated to amine terminating PAMAM (G2) by *N,N'*-dialkylation in good yields. Both 32-valent PAMAM based dendrimers exhibited direct binding properties to GalNAc-specific lectin (VVA) as demonstrated by turbidimetric analyses. ELLA inhibition of binding of VVA/HRP lectin to asialoglycophorin indicated that the structure of the aglycon spacer influences the inhibitory potential greatly as demonstrated in two glyco PAMAM 32-mers having different lengths of aglycon spacers.

Glycopolymers bearing GalNAc residues were prepared by conjugating GalNAc homoserine derivatives to the preactivated poly(*N*-acryloxysuccinimide) in good yields. Telomers having small repeating units (n=3 to 7) were also synthesized *via* radical telomerization using mono- and divalent acrylamide.

These glycopolymers demonstrated their utilities as excellent coating antigens in solid-phase enzyme-linked lectin assays. This property of glycopolymers could be employed in diagnostic procedures.

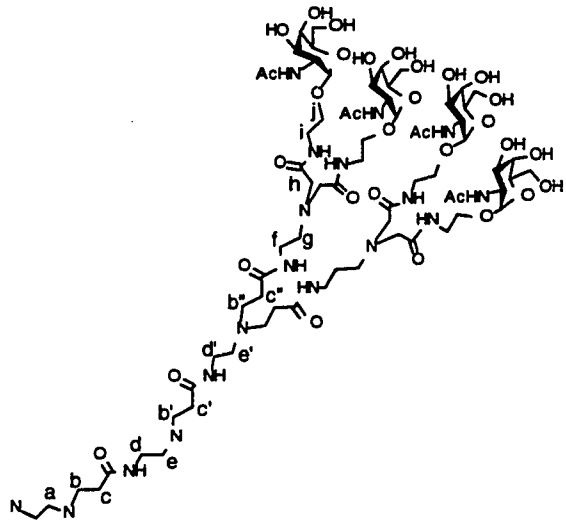
6.6. Experimental Methods

2-Bromoacetamidoethyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactopyranoside (151).

To a solution of 2-aminoethyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactopyranoside hydrochloride (**103**) (0.73 g, 1.19 mmol) in CH_2Cl_2 (40 mL) was

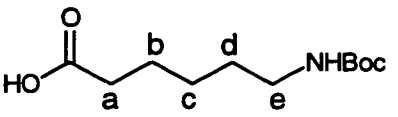
added DIPEA (0.52 mL, 2.98 mmol) and dropwise bromoacetyl chloride (0.12 mL, 1.43 mmol) in CH_2Cl_2 (10 mL) at 0 °C. After 20 min, the reaction solution was washed with 5% aqueous HCl (1 × 30 mL), saturated $NaHCO_3$ (1 × 30 mL), and water (1 × 20 mL). Dried (Na_2SO_4) organic phase was concentrated and silica gel column chromatography of the crude compound eluting with 38:1:1 CH_2Cl_2/CH_3CN/MeOH yielded 0.82 g (98%) of an off-white foam: $[\alpha]_D$ +98.4 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.89 (s, 3H, NAc), 3.45-3.83 (m, 4H, H-a's, H-b's), 4.43-4.40 (m, 1H, H-5), 4.49-4.59 (m, 4H, H-6's, H-c's), 4.93 (ddd, 1H, $J_{2,3}$ 11.2 Hz, $J_{2,NH}$ 9.5 Hz, H-2), 5.04 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.49 (dd, 1H, $J_{3,4}$ 3.1 Hz, H-3), 5.88 (d, 1H, H-4), 6.34 (d, 1H, NHAc), 6.70 (t, 1H, $J_{Hb,NH}$ 5.9 Hz, NH), 7.21-7.62 (m, 9H, Ar_{meta}'s, Ar_{para}'s), 7.80, 7.96, 8.06 (dd, $J_{o,m}$ 7.8 Hz, $J_{o,p}$ 1.5 Hz, Ar_{ortho}'s); ^{13}C-NMR ($CDCl_3$) δ 23.25 (NAc), 29.27 (CH_2), 39.84 (CH_2), 48.20 (C-2), 62.60 (C-6), 67.41 (C-3), 67.46 (CH_2), 68.21 (C-4), 69.24 (C-5), 98.42 (C-1), 128.40, 128.50, 128.65 (Ar_{meta}'s), 128.88, 129.09, 129.32 (Ar_{ipso}'s), 129.66, 129.85, 129.97 (Ar_{ortho}'s), 133.36, 133.41, 133.59 (Ar_{para}'s), 165.73, 165.94, 166.18, 166.44, 170.31 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{33}H_{33}N_2O_{10}Br$: 696.13; found: 697.10 (M^+ + 1, 58.0%), 699.08 (M^+ + 3, 57.1%); Anal. Calcd for $C_{33}H_{33}N_2O_{10}Br$: C, 56.82; H, 4.77; N, 4.02. Found C, 57.05; H, 5.03; N, 4.00.

PAMAM 32-mer with short spacer armed GalNAc bromoacetylamide 153.



PAMAM (G2) (9.14 μmol, 0.12 g of 24.81% w/w methanolic solution) was obtained as a powder by evaporating MeOH followed by co-evaporating with $CHCl_3$. Resulting PAMAM (G2) compound was dissolved in DMF (2 mL) and DIPEA (80 μL, 0.457 mmol) was added to the solution. 2-Bromoacetamidoethyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactopyranoside (**151**) (0.255 g, 0.365 mmol) in DMF (2 mL) was added to the mixture and the reaction mixture was allowed to stir at 60 °C for 48 h. When the reaction was complete, the reaction solution was concentrated and the resulting residue was dissolved in MeOH. The methanolic solution was then treated with 1M NaOMe until pH 9 and stirred at room temperature for 24 h. After evaporating the solvent, the residue was dissolved in water and dialyzed against water for 48 h. The aqueous solution was lyophilized to afford 74 mg (62%) of an off-white powder: $[\alpha]_D$ +60.3 (*c* 0.5, DMSO); ^{1}H-NMR (D_2O) δ 2.11 (s, 96H, NAc), 2.52 (bs, 56H, H-b), 2.70-2.84 (m, 56H, H-e, H-g), 2.94 (m, 56H, H-c), 3.28-3.45 (m, 124H, H-d, H-f, H-h, H-a), 3.53 (bs, 32H, H-i'), 3.57 (bs, 32H, H-i), 3.64 (bs, 32H, H-j'), 3.76-3.88 (m, 96H, H-6's, H-j), 3.90-3.97 (m, 64H, H-3, H-5), 4.03 (bs, 32H, H-4), 4.24 (dd, 32H, $J_{2,3}$ 11.0 Hz, $J_{1,2}$ 3.4 Hz, H-2), 4.94 (bs, 32H, H-1); ^{13}C-NMR (D_2O) δ 21.65 (NAc), 31.88 (C-b), 36.06 (C-a), 36.84 (C-d, C-f), 38.49 (C-i), 48.63 (C-c), 49.32 (C-2), 50.95 (C-e), 53.50 (C-g), 57.70 (C-h), 60.77 (C-6), 66.03 (C-j), 67.44 (C-3), 68.06 (C-4), 70.69 (C-5), 96.88 (C-1).

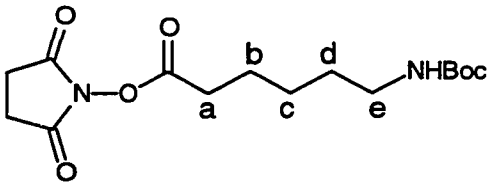
Boc-6-Aminocaproic acid.



ε-Amino-n-caproic acid (1.2 g, 9.15 mmol) and NaOH (0.73 g, 18.3 mmol) were dissolved in water (5 mL). A solution of di-*t*-butyl dicarbonate (2.0 g, 9.15 mmol) in CH_2Cl_2 (15 mL) was added to the aqueous solution at 0 °C and stirred at room temperature for 48 h. The progress of the reaction was monitored by ninhydrin test. When the reaction was complete, the solution was acidified by adding conc. HCl dropwise. The organic layer was separated from the aqueous layer. The aqueous phase was extracted with $CHCl_3$ (2 × 20 mL) and the combined organic phase was dried over anhydrous Na_2SO_4 and then concentrated. Purification of the crude product

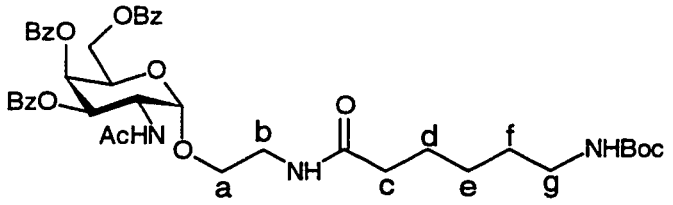
by silica gel chromatography eluting with 18:1:1 $CHCl_3/CH_3CN$/MeOH yielded 1.94 g (92%) of a colorless oil: ^{1}H-NMR ($CDCl_3$) δ 1.32 (quintet, 2H, J 6.7 Hz, H-c), 1.39 (s, 9H, t-Bu), 1.45 (quintet, 2H, J 7.4 Hz, H-d), 1.59 (quintet, 2H, J 7.6 Hz, H-b), 2.29 (t, 2H, J 7.4 Hz, H-a), 3.05 (t, 2H, J 6.8 Hz, H-e), 4.59 (bs, 1H, NH), 10.3 (bs, 1H, CO_2H); ^{13}C-NMR ($CDCl_3$) δ 24.3 (H-b), 26.1 (H-c), 28.3 (t-Bu), 29.6 (H-d), 33.9 (H-a), 40.2 (H-e), 79.1 (CMe_3), 156.0, 178.9 (C=O's); CI-MS (m/z) calcd. for $C_{11}H_{21}NO_4$: 231.1; found: 232.0 ($M^+ + 1$, 2.3%).

Boc-6-Aminocaproic acid succinimide (154).



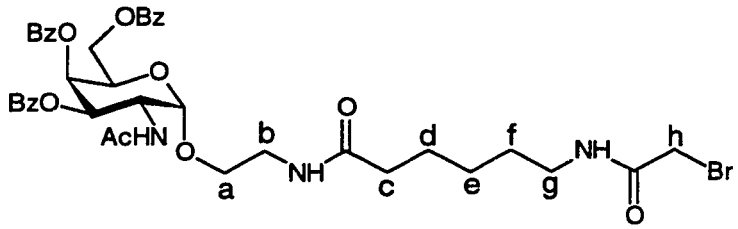
To a solution of Boc-6-aminocaproic acid (see above) (1.0 g, 4.33 mmol) and *N*-hydroxysuccinimide (0.75 g, 6.49 mmol) in CH_2Cl_2 (15 mL) was added TBTU (2.08 g, 6.49 mmol) at 0°C. DIPEA (1.13 mL, 6.49 mmol) was added and the reaction solution was stirred at 0 °C for 1.5 h. Ninhydrin test on the TLC after spraying the plate with TFA indicated the completion of the reaction. The reaction solution was washed with saturated $NaHCO_3$ (2 × 10 mL) and water (1 × 10 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Silica gel chromatography of the crude product eluting with 49:1 CH_2Cl_2/MeOH yielded 1.28 g (90%) of a white solid: ^{1}H-NMR ($CDCl_3$) δ 1.40 (s, 9H, t-Bu), 1.41-1.56 (m, 4H, H-c, H-d), 1.73 (quintet, 2H, J 7.3 Hz, H-b), 2.57 (t, 2H, J 7.2 Hz, H-a), 2.80 (s, 4H, $2CH_2$), 3.09 (q, 2H, J 6.1 Hz, H-e), 4.60 (bs, 1H, NH).

Long spacer armed GalNAc(OBz)$_3$ ligand (155).



A solution of 2-aminoethyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactopyranoside hydrochloride (**103**) (1.50 g, 2.45 mmol) and *t*-butyl *N*-(*N*-hydroxysuccinimidylhexanoyl)carbamate (**154**) (0.96 g, 2.94 mmol) in CH_2Cl_2 (10 mL) was treated with DIPEA (0.51 mL, 2.94 mmol) at room temperature for 30 min. When the amine was consumed completely on the TLC, the solution was washed with 5% aqueous HCl(2 × 5 mL), saturated $NaHCO_3$ (2 × 5 mL), water (1 × 5 mL) and then dried over anhydrous Na_2SO_4. The concentrated residue was purified by silica gel column chromatography eluting with 18:1:1 CH_2Cl_2/MeCN/MeOH to yield 1.65 g (85%) of a white foam: $[\alpha]_D$ +73.0 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.20-1.68 (m, 6H, H-e, H-d, H-f), 1.39 (s, 9H, C*Me*$_3$), 1.89 (s, 3H, NAc), 2.12-2.20 (m, 2H, H-c), 3.00-3.11 (m, 2H, H-g), 3.25-3.41 (m, 1H, H-b'), 3.50-3.82 (m, 3H, H-a, H-b), 4.31-4.54 (m, 3H, H-5, H-6's), 4.61-4.72 (m, 1H, NH), 4.92 (ddd, 1H, H-2), 5.04 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.48 (dd, $J_{2,3}$ 11.2 Hz, $J_{3,4}$ 3.2 Hz, H-3), 5.89 (d, 1H, H-4), 6.22-6.23, 6.45-6.58 (m, 2H, NH), 7.18-7.60 (m, 9H, Ar_{meta}, Ar_{para}), 7.81, 7.93, 8.05 (3d, 6H, Ar_{ortho}); FAB-MS (pos. m/z) calcd. for $C_{42}H_{51}N_3O_{12}$: 789.35; found: 790.45 (M^+ + 1, 1.6%), 690.38 (M^+ - Boc, 30.2%).

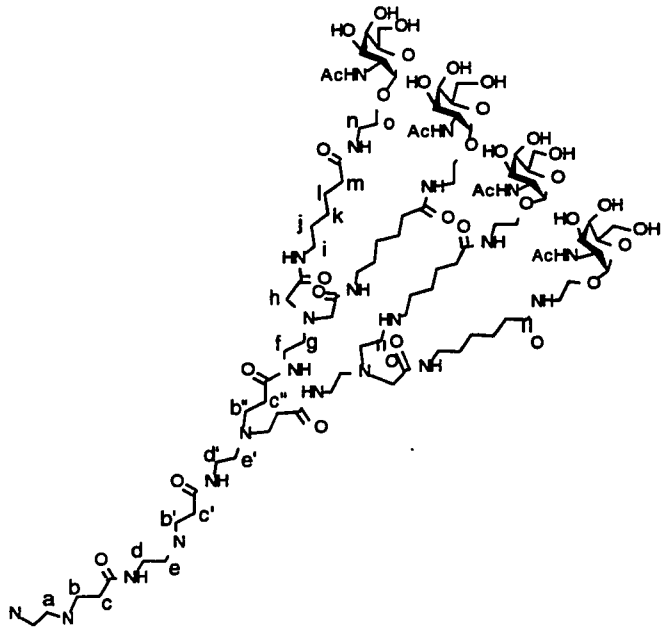
Bromoacetylated compound 156.



Long spacer armed GalNAc(OBz)$_3$ ligand (**155**) (1.34 g, 1.70 mmol) was treated with 20% TFA in CH_2Cl_2 (10 mL) at room temperature for 2 h and the solvent was evaporated. The resulting residue was dissolved in CH_2Cl_2 (100 mL) and DIPEA (0.74

mL, 4.25 mmol) was added. Bromoacetyl chloride (0.17 mL, 2.04 mmol) in CH_2Cl_2 (10 mL) was then added dropwise to the reaction mixture at 0 °C and the solution was allowed to stir for 30 min. The reaction solution was washed with 5% aqueous HCl (2 × 20 mL), saturated $NaHCO_3$ (2 × 20 mL), water, and then dried (Na_2SO_4). Purification by silica gel column chromatography eluting with 18:1:1 CH_2Cl_2/MeCN/MeOH afforded 1.23 g (89%) of an off-white foam: ^{1}H-NMR ($CDCl_3$) δ 1.24-1.40 (m, 2H, H-e), 1.42-1.70 (m, 4H, H-d, H-f), 2.12-2.20 (m, 2H, H-c), 3.18-3.32 (m, 3H, H-b', H-g), 3.45-3.71 (m, 3H, H-a, H-b), 3.98 (s, 2H, H-h), 4.00-4.16 (m, 3H, H-5, H-6's), 4.51 (ddd, 1H, $J_{NH,2}$ 9.4 Hz, H-2), 4.82 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1), 5.04 (dd, 1H, $J_{2,3}$ 11.3 Hz, $J_{3,4}$ 3.1 Hz, H-3), 5.29 (d, 1H, H-4), 6.41-6.50, 6.52-6.61, 6.81-6.90 (m, 3H, NH's), 7.18-7.60 (m, 9H, Ar_{meta}, Ar_{para}), 7.81, 7.97, 8.04 (3d, 6H, J 7.3 Hz, Ar_{ortho}); FAB-MS (pos. m/z) calcd. for $C_{39}H_{44}N_3O_{11}Br$: 809.22; found: 810.26 (M^+ + 1, 0.2%), 812.23 (M^+ + 3, 0.2%).

PAMAM 32-mer with long-spacer-armed GAlNAc bromoacetylamide 158.



The title compound was prepared using the same method foe the preparation of PAMAM 32-mer with short spacer armed GalNAc with 75% yield: **157**: $[\alpha]_D$ +65.1 (*c* 1.0, $CHCl_3$) ^{1}H-NMR ($CDCl_3$) δ 1.08-1.30 (m, 64H, H-i), 1.35-1.60 (m, 128H, H-h, H-j), 1.83 (s, 96H, NAc), 2.04-2.20 (m, 64H, H-k), 2.55-3.34 (m, 324H, H-b, H-a, H-c, H-d, H-e, H-

g, H-l'), 3.42-3.67 (m, 128H, H-f, H-l, H-m'), 3.75-3.85 (m, 32H, H-m), 4.33 (bs, 32H, H-6'), 4.51 (bs, 64H, H-5, H-6), 4.90 (bs, 32H, H-2), 5.04 (bs, 32H, H-1), 5.49 (d, 32H, $J_{2,3}$ 11.0Hz, H-3), 5.86 (bs, 32H, H-4), 7.15-7.60 (m, 384H, Ar_{meta}, Ar_{para}, 48NH's), 7.75, 7.91, 7.97 (3s, 192H, Ar_{ortho}), 8.20-8.55 (b, 14H, NH's); ^{13}C-NMR ($CDCl_3$) δ 22.88 (NAc), 25.22 (C-j), 26.41 (C-i), 29.08 (C-h), 36.15 (C-k), 37.40 (C-d, C-e), 38.99 (C-f, C-l), 47.90 (C-2), 54.82 (C-b, C-c, C-a), 59.00 (C-g), 62.72 (C-6), 67.05 (C-5), 67.92 (C-m), 68.28 (C-4), 69.34 (C-3), 98.53 (C-1), 128.37, 128.45, 128.58 (Ar_{meta}'s), 129.02, 129.08, 129.32 (Ar_{ipso}'s), 129.58, 129.69, 129.81 (Ar_{ortho}'s), 133.31, 133.51 (Ar_{para}'s), 165.77, 166.07, 166.17, 170.77, 171.18, 173.68 (C=O's); **158**: $[\alpha]_D$ +57.5 (*c* 0.24, DMSO); ^{1}H-NMR (D_2O) δ 1.37 (quintet, 64H, J 7.7 Hz, H-k), 1.59 (quintet, 64H, J 7.4 Hz, H-j), 1.66 (quintet, 64H, J 7.4 Hz, H-l), 2.11 (s, 96H, NAc), 2.32 (t, 64H, J 7.4 Hz, H-m), 2.51 (bs, 56H, H-b), 2.72 (bs, 24H, H-e), 2.80 (bs, 32H, H-g), 2.91 (bs, 56H, H-c), 3.28 (t, 64H, J 6.6 Hz, H-i), 3.32-3.44 (m, 156H, H-h, H-n', H-d, H-f, H-a), 3.54-3.63 (m, 64H, H-n, H-o'), 3.78-3.86 (m, 96H, H-o, H-6's), 3.93 (dd, 32H, $J_{2,3}$ 11.0 Hz, $J_{3,4}$ 3.2 Hz, H-3), 3.97 (t, 32H, $J_{5,6}$ 6.2 Hz, H-5), 4.04 (d, 32H, H-4), 4.25 (dd, 32H, $J_{1,2}$ 3.7 Hz, H-2), 4.93 (d, 32H, H-1); ^{13}C-NMR (D_2O) δ 21.60 (NAc), 24.70 (C-l), 25.28 (C-k), 27.77 (C-j), 32.20 (C-b), 35.29 (C-m), 36.26 (C-a), 36.84 (C-d), 38.47 (C-f, C-i), 38.50 (C-n), 48.68 (C-c), 49.33 (C-2), 50.94 (C-e), 53.76 (C-g), 57.79 (C-h), 60.73 (C-6), 65.99 (C-o), 67.43 (C-3), 68.06 (C-4), 70.61 (C-5), 96.78 (C-1), 172.43, 173.84, 176.16 (C=O's).

General procedure for polymerization.

PNAS **159** (MW 42K, a polymer of N-acryloyloxysuccinimide) was stirred in DMF at room temperature for 2 h and a solution of glycosyl amine in DMF was added to the PNAS solution. The reaction mixture was stirred at room temperature for 24 h. Completion of the reaction was indicated by Kaiser test. The reaction solution was then treated with aqueous concentrated ammonium hydroxide, $MeNH_2$, $EtNH_2$, or $PrNH_2$ for another 24 h to aminolyze the remaining active ester. The reaction mixture was concentrated under reduced pressure and the resulting residue was dialyzed against water for 48 h. The aqueous solution was then lyophilized to afford a white powder: See Scheme 6.3.3 for the structural assignment; NH_4OH aminolysis to give **161** (m:n 1:16) ^{1}H-NMR (D_2O) δ 1.55-1.95 (m, 34H, H-a), 2.14 (s, 3H, NAc), 2.23-2.53 (m, 17H,

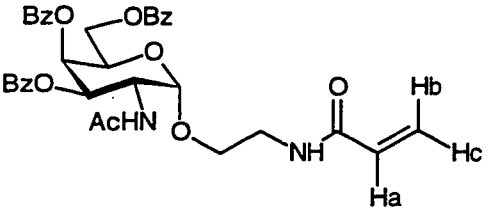
H-b), 3.32-3.70 (m, 3H, H-d', H-c's), 3.76-3.90 (m, 3H, H-d, H-6's), 3.92-4.04 (m, 2H, H-3, H-5), 4.07 (s, 1H, H-4), 4.27 (d, 1H, J 11.0 Hz, H-2), 4.83 (s, 1H, H-1); $MeNH_2$ aminolysis to give **162** (m:n 1:8) ^{1}H-NMR (D_2O) δ 1.49-1.92 (m, 18H, H-a), 1.90-2.40 (m, 9H, H-b), 2.14 (s, 3H, NAc), 2.71-2.96 (s, 24H, CH_3), 3.30-3.72 (m, 3H, H-d', H-c's), 3.76-3.90 (m, 3H, H-d, H-6's), 3.92-4.03 (m, 2H, H-3, H-5), 4.09 (s, 1H, H-4), 4.27 (d, 1H, J 10.0 Hz, H-2), 4.97 (s, 1H, H-1); ^{13}C-NMR (D_2O) δ 21.65 (NAc), 25.39 (CH_3), 34.42 (multiple around this peak, C-a), 42.36 (multiple around this peak, C-b), 49.30 (C-2), 60.82 (C-6), 65.85 (C-d), 67.43 (C-5), 70.68 (C-3), 68.08 (C-4), 97.00 (C-1), 176.41 (C=O); $EtNH_2$ aminolysis to give **163** (m:n 1:12) ^{1}H-NMR (D_2O) δ 1.19 (bs, 36H, CH_3), 1.44-1.88 (m, 26H, H-a), 2.00-2.33 (m, 13H, H-b), 2.13 (s, 3H, NAc), 3.26 (bs, 24H, CH_2), 3.52-3.70 (m, 3H, H-d', H-c's), 3.75-3.89 (m, 3H, H-d, H-6's), 3.90-4.03 (m, 2H, H-3, H-5), 4.04-4.09 (m, 1H, H-4), 4.28 (d, 1H, J 10.6 Hz, H-2), 4.96 (s, 1H, H-1); $PrNH_2$ aminolysis to give **164** (m:n 1:7) ^{1}H-NMR (D_2O) δ 0.98 (bs, 21H, CH_3), 1.48-1.92 (m, 30H, CH_2, H-a), 2.04-2.34 (m, 11H, NAc, H-b), 3.05-3.38 (m, 14H, CH_2), 3.52-3.68 (m, 3H, H-c's, H-d'), 3.73-3.90 (m, 3H, H-d, H-6's), 3.92-4.02 (m, 2H, H-3, H-5), 4.03-4.09 (m, 1H, H-4), 4.23-4.32 (m, 1H, H-2), 4.96 (bs, 1H, H-1).

***N*-Boc-Cysteamine (166).**

2-Aminoethanethiol hydrochloride (2.0 g, 17.6 mmol) was dissolved in CH_3CN (15 mL) by adding DIPEA (7.1 mL, 40.5 mmol) at 0 °C under a nitrogen atmosphere. After 5 min, trimethylsilyl chloride (2.9 mL, 22.9 mmol) was added with a syringe in one portion. The solution was allowed to stir at 0 °C for 10 min. To the mixture was then added slowly a solution of di-*t*-butyl carbonate (3.84 g, 17.6 mmol) in CH_3CN (5 mL) and DIPEA (3.1 mL, 17.6 mmol) at 0 °C. The resulting mixture was stirred for 30 min. at 0 °C and then for additional hour at room temperature. The mixture was poured into ice/water (50 mL) and extracted with CH_2Cl_2 (2 × 25 mL). The extract was washed with water (1 × 10 mL), 5% aqueous HCl (1 × 10 mL), saturated $NaHCO_3$ (1 × 10 mL) and then brine (1 × 10 mL). After drying over anhydrous Na_2SO_4, the organic solution was concentrated to afford 2.85 g (91%) of a colorless oil: ^{1}H-NMR ($CDCl_3$) δ 1.32 (t, 1H, J 8.2 Hz, SH), 1.38 (s, 9H, t-Bu), 2.57 (q, 2H, J 6.5 Hz, CH_2S), 3.27 (q, 2H, J 6.4 Hz,

CH_2N), 4.92 (b, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ 24.89 (CH_2S), 28.25 (t-Bu), 43.54 (CH_2N), 79.37 (CMe_3), 155.66 (C=O).

2-Acrylamidoethyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-benzoyl-α-D-galactopyranoside (167).



^{1}H-NMR ($CDCl_3$) δ 1.88 (s, 3H, NAc), 3.45-3.82 (m, 4H, CH_2's), 4.31-4.58 (m, 3H, H-5, H-6's), 4.90 (ddd, 1H, $J_{2,3}$ 11.2 Hz, H-2), 5.06 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.48 (dd, 1H, $J_{3,4}$ 3.3 Hz, H-3), 5.60 (dd, 1H, J_{gem} 1.8 Hz, J_{cis} 9.6 Hz, H-c), 5.87 (d, 1H, H-4), 6.11 (dd, 1H, J_{trans} 17.0 Hz, H-a), 6,26 (dd, 1H, H-b), 6.45-6.62 (m, 2H, NH), 7.25-7.61 (m, 9H, Ar_{para}, Ar_{meta}), 7.79, 7.98, 8.06 (3d, 6H, Ar_{ortho}); ^{13}C-NMR ($CDCl_3$) δ 23.7 (NAc), 40.1 (C-2), 48.8 (CH_2), 63.3 (C-6), 67.9 (CH_2), 68.8 (C-5), 68.9 (C-4), 70.0 (C-3), 99.2 (C-1), 127.9 (Cb,c), 129.0, 129.1, 129.3 (Ar_{meta}), 129.5, 129.7, 129.9 (Ar_{ipso}), 130.3, 130.4, 130.6 (Ar_{ortho}), 131.0 (C-a), 134.0, 134.2 (Ar_{para}), 166.8, 167.0, 171.4 (C=O's); FAB-MS (pos. m/z) calcd. for $C_{34}H_{34}N_2O_{10}$ 630.22; found 631.45 (M^+ + 1, 25.7%).

2-Acrylamidoethyl 2-acetamido-2-deoxy-α-D-galactopyranoside (168).

^{1}H-NMR (D_2O) δ 1.97 (NAc), 3.30-3.90 (m, 9H, H-3, H-4, H-5, H-6's, $2CH_2$), 4.05-4.16 (m, 1H, H-2), 4.90 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.73 (dd, 1H, J_{gem} 2.5 Hz, J_{cis} 9.0 Hz, H-c), 6.21 (dd, 1H, J_{trans} 17.2 Hz, H-a), 6.24 (dd, 1H, H-b); ^{13}C-NMR (D_2O) δ 23.5 (NAc), 40.5 (C-2), 51.2 (CH_2), 62.6 (C-6), 67.6 (CH_2), 69.2 (C-5), 69.9 (C-4), 72.5 (C-3), 98.5 (C-1), 129.0 (C-b,c), 131.3 (C-a), 176.0 (C=O); CI-MS (pos. m/z) calcd. for $C_{13}H_{22}N_2O_7$ 318.1; found 318.9.

Acylamide of long spacer armed monovalent GalNAc ligand (171).

The title compound was prepared using the same method for the synthesis of divalent acrylamide in 87% yield. De-*O*-benzoylation was done under Zemplén condition and the crude product was purified by size exclusion column chromatography (80% yield).

***O*-Benzoyl protected divalent long-spacer-armed GalNAc ligand 173.**

N,N-Dialkylation of bromoacetylated long spacer armed GalNAc ligand was accomplished using the same method as for the preparation of *O*-acetylated divalent long-spacer-armed GalNAc **118** (chapter 4.5) in 92% yield: $[\alpha]_D$ +78.8 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.25 (quintet, 4H, J 7.3 Hz, H-e), 1.39 (s, 9H, t-Bu), 1.43-1.52 (m, 8H, H-f, H-j, H-k), 1.57 (quintet, 4H, J 7.1 Hz, H-d), 1.87 (s, 6H, NAc), 2.13 (t, 4H, J 7.3 Hz, H-c), 2.98-3.35 (m, 12H, H-g, H-i, H-l, H-b, H-h), 3.53-3.59 (m, 2H, H-a'), 3.62-3.69 (m, 2H, H-b'), 3.76-3.83 (m, 2H, H-a), 4.33-4.38 (m, 2H, H-6), 4.48-4.56 (m, 4H, H-5, H-6'), 4.93 (ddd, 2H, $J_{2,3}$ 11.3 Hz, $J_{2,NH}$ 9.5 Hz, H-2), 4.86-4.93 (m, 1H, NHBoc), 5.05 (d, 2H, $J_{1,2}$ 3.6 Hz, H-1), 4.50 (dd, 2H, $J_{3,4}$ 3.2 Hz, H-3), 5.87 (d, 2H, H-4), 6.95 (b, 2H, NHCO), 7.07-7.15 (b, 2H, NHAc), 7.26, 7.38, 7.42 (3t, 12H, J 7.9 Hz, Ar_{meta}'s), 7.44, 7.52, 7.56 (3t, 6H, J 7.4 Hz, Ar_{para}'s), 7.79, 7.95, 8.01 (3dd, 12H, J 8.2 Hz, J 0.9 Hz, Ar_{ortho}'s); ^{13}C-NMR ($CDCl_3$) δ 22.94 (NAc), 23.29 (C-f), 24.85 (C-d), 25.97 (C-e), 26.86 (C-j), 28.32 (t-Bu), 28.93 (C-k), 36.21 (C-c), 38.57 (C-i, C-l), 39.02 (C-b, C-h), 39.61 (C-g), 47.87 (C-2), 62.75 (C-6), 67.17 (C-3), 68.11 (C-a), 68.25 (C-4), 69.45 (C-5), 79.43 (CMe_3), 98.59 (C-1), 128.35, 128.45, 128.56 (Ar_{meta}'s), 129.04, 129.12, 129.36 (Ar_{ipso}'s), 129.60, 129.72, 129.88 (Ar_{ortho}'s), 133.29, 133.47 (Ar_{para}'s), 156.55, 165.77, 166.18, 170.63, 173.46 (C=O'); FAB-HRMS (pos. m/z) calcd. for $C_{87}H_{107}N_8O_{24}$: 1647.7398; found: 1647.7373 (M^+ + 1, 15.2%); Anal. Calcd for $C_{87}H_{106}N_8O_{24}$: C, 63.41; H, 6.48; N, 6.80. Found C, 63.78; H, 6.63; N, 6.26.

Acrylamide of *O*-benzoylated dimer (175).

N-Boc protecting group was removed by treating the compound **173** (0.32 g, 0.194 mmol) with 20% TFA in CH_2Cl_2 (10 mL) at room temperature for 1 h. The solution was concentrated and the residue was dissolved in CH_2Cl_2 (5 mL) with DIPEA

(0.12 mL, 0.679 mmol). The reaction mixture was cooled to 0 °C and acryloyl chloride (19 mL, 0.233 mmol) in CH_2Cl_2 (2 mL) was added. The reaction was monitored by TLC (*N*-Boc: R_f 0.42, amine: R_f 0.0, acrylamide: 0.36, 8:1:1 $CHCl_3$/MeCN/MeOH) and after 1 hour the reaction solution was washed with 5% aqueous HCl (1 × 5 mL), saturated $NaHCO_3$ (1 × 5 mL), water (1 × 5 mL) and then dried (Na_2SO_4). The residue after evaporating solvent was purified by silica gel column chromatography eluting with 18:1:1 CH_2Cl_2/MeCN/MeOH to yield 0.30 g (98%) of **174** in a white foam; FAB-MS (pos. m/z) calcd. for $C_{85}H_{100}N_8O_{23}$: 1600.69; found: 1601.82 (M^+ + 1, 3.6%).

The resulting product **174** was then de-*O*-benzoylated under Zemplén condition. After 1 h the solution was treated with Amberlite IR 120 (H) resin at 0 °C for 10 min. The resin was filtered off and the filtrate was concentrated. Size exclusion column chromatography of the residue eluting with MeOH afforded 0.18 g (95%) of **175** in an off-white foam: FAB-MS (pos. m/z) calcd. for $C_{43}H_{76}N_8O_{17}$: 976.53; found: 977.51 (M^+ + 1, 10.0%).

General procedure for telomerization.

An acrylamide (5 eq) was dissolved in H_2O and the solution degassed by freeze-thaw method. *N*-Boc-cysteamine (**166**) (1 eq) in MeOH was degassed by bubbling N_2 through the solution. The two solutions were mixed and AIBN (1 eq) was added to the mixture. The solution was heated ≈70 °C for 24 h and concentrated. The reaction was monitored by TLC eluting with 10:6:1 $CHCl_3$/MeOH/H_2O. R_f value for telomers was almost zero. Purification of the crude compound was done by size-exclusion column chromatography (LH20) eluting with MeOH.

Telomer with monovalent short spacer armed GalNAc (m=7) (169).

See the Scheme 6.3.6 for the structural assignment: yield 86%; ^{1}H-NMR (D_2O) δ 1.51 (s, 9H, t-Bu), 2.10-2.14 (m, 21H, NAc), 2.64-2.82 (m, 14H, CH_2S), 3.12-3.36 (m, 14H, CH_2NBoc), 3.38-3.68 (m, 21H, OCH_2, C*H*HN), 3.77-3.90 (m 21H, CH*H*N, H-6's), 3.92-4.08 (m, 21H, H-3, H-4, H-5), 4.20-4.30 (m, 7H, H-2), 4.90-5.00 (m, 7H, H-1).

Telomer with monovalent long-spacer-armed GalNAc (m=6) (172).

See the Scheme 6.3.7 for the structural assignment: yield 70%; ^{1}H-NMR (D_2O) δ 1.36-1.48 (m, 12H, H-e), 1.50 (s, 9H, t-Bu), 1.56-1.91 (m, 36H, H-f, H-d, H-j), 2.34 (s, 18H, NAc), 2.31-2.39 (m, 18H, H-c, H-i), 2.69-2.79 (m, 2H, H-l), 3.14-3.30 (m, 12H, H-g), 3.31-3.38 (m, 2H, H-k), 3.39-3.48 (m, 6H, H-a'), 3.56-3.66 (m, 12H, H-b, H-b'), 3.80-3.88 (m, 18H, H-6's, H-a), 3.92-4.01 (m, 12H, H-3, H-5), 4.05 (s, 6H, H-4), 4.25 (dd, 6H, $J_{2,3}$ 10.9 Hz, $J_{1,2}$ 3.5 Hz, H-2), 4.94 (d, 6H, H-1), ^{13}C-NMR (D_2O) δ 21.57 (NAc), 24.67 (C-d), 25.42 (C-e), 27.24 (t-Bu), 27.68 (C-f), 35.30 (C-c), 38.47 (C-b), 38.86 (C-g, C-l), 49.32 (C-2), 60.72 (C-6), 65.99 (C-a), 67.43 (C-3), 68.05 (C-4), 70.60 (C-5), 96.76 (C-1), 173.95, 176.35 (C=O's).

Telomer with divalent long spacer armed GalNAc (m=3) (176).

See the Scheme 6.3.8 for the structural assignment: yield 30%; ^{1}H-NMR (D_2O) δ 1.33-1.46 (m, 12H, H-e), 1.50 (s, 9H, t-Bu), 1.51-1.80 (m, 42H, H-d, H-f, H-j, H-k, H-n), 2.12 (s, 18H, NAc), 2.28-2.38 (m, 15H, H-c, H-m), 2.57-2.81 (m, 6H, H-l), 2.87-2.94 (m, 2H, H-o), 3.05-3.13 (m, 2H, H-p), 3.15-3.46 (m, 36H, H-g, H-h, H-b', H-i), 3.55-3.65 (m, 12H, H-a', H-b), 3.77-3.87 (m, 18H, H-6's, H-a), 3.93 (dd, 6H, $J_{2,3}$ 11.0 Hz, $J_{3,4}$ 2.9 Hz, H-3), 3.98 (t, 6H, $J_{5,6}$ 6.0 Hz, H-5), 4.04 (d, 6H, H-4), 4.25 (dd, $J_{1,2}$ 3.5 Hz, H-2), 4.95 (d, 6H, H-1); ^{13}C-NMR (D_2O) δ 21.57 (NAc), 24.65 (C-d), 25.19 (C-e, C-f), 27.31 (t-Bu), 27.56 (C-j, C-k, C-n), 35.30 (C-c, C-m), 38.47 (C-b), 38.90 (C-g, C-h), 49.32 (C-2), 60.72 (C-6), 65.98 (C-a), 67.43 (C-3), 68.05 (C-4), 70.60 (C-5), 96.76 (C-1), 173.91 (C=O).

Chapter 7. Glycoclusters using *C*-glycosides

7.1. Introduction

It is well documented that 2-acetamido-2-deoxy-α-D-galactopyranose-*O*-Ser/Thr is present in a wide variety of glycoproteins[216] including glycoprotein A,[217] epiglycanin,[218] antifreeze glycoproteins,[219] and the human blood specific glycoproteins.[220] Thus, the significant roles of these compounds has caused numerous synthetic methods to be developed to give compounds for immunochemical and enzymological studies.

Due to the small size of the azido group which does not cause steric hindrance for glycosylation nor anchimeric group participation leading to 1,2-trans glycoside, 2-azido-2-deoxy glycopyranosyl donors have been generally employed as reactive intermediates.

In 1979, Lemieux and Ratcliffe introduced an efficient method for the preparation of 2-azido-2-deoxy-glycopyranoses by azidonitration of *O*-protected glycals.[221] The nitrate adduct is readily converted into various glycosyl donors including halide ions[221] and S-glycosides.[222] Trichloroacetimidates[223] and fluorides[224] have also been prepared *via* hydrolysis of anomeric nitrates (Scheme 7.1.1).

Since the glycosidic bond of GalNAc is enzymatically hydrolyzable in *vivo*, designing non-hydrolyzable analogs of GalNAc-α-O-Ser/Thr, is an attractive approach to control, at the molecular level, events of crucial importance to glycobiology and immunology.

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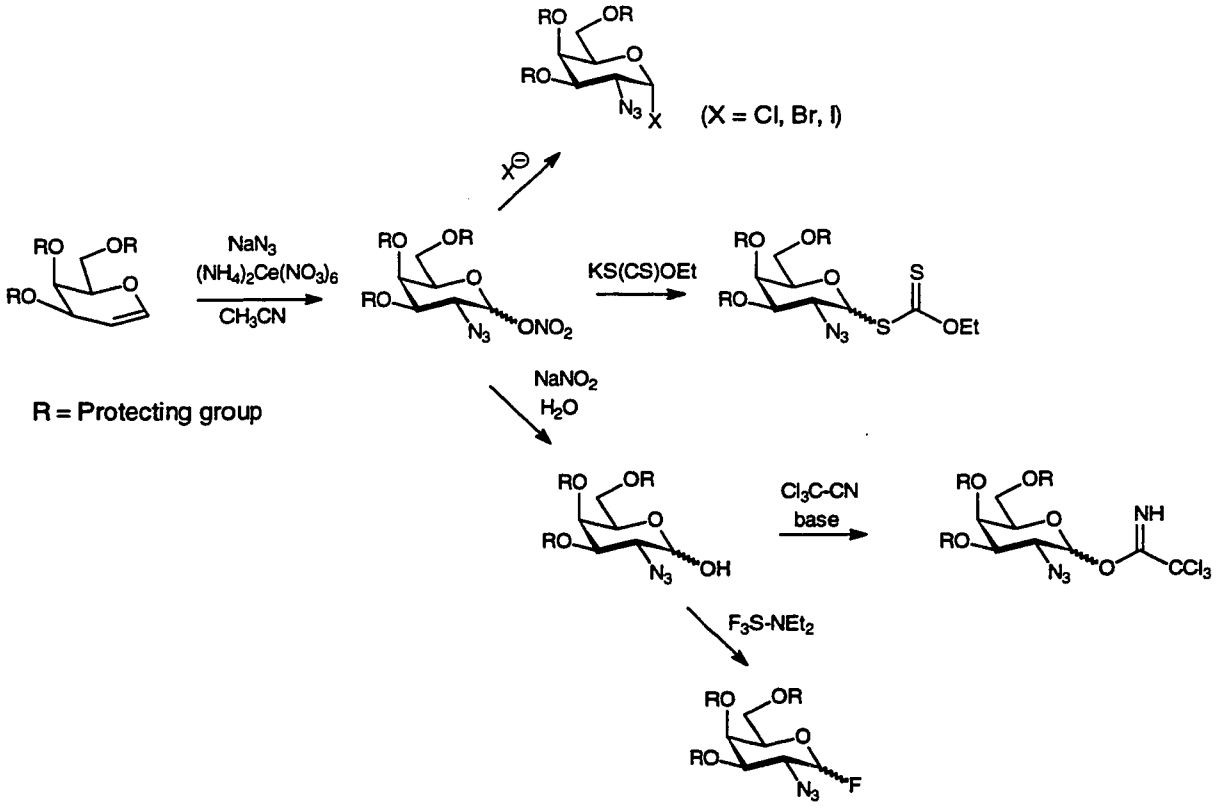
One of the most common methods to prepare *C*-glycosides is through free radical intermediates derived from glycosyl precursors which include chlorides,[225] bromides,[226,227,228] phenylselenides,[229,230,231] or thiocarbonyl esters.[232]



Scheme 7.1.1. Azido-nitration of D-galactal and some of the products derived therefrom.

As an extension of azido-nitration, azido-phenylselenylation methodology[233] would afford the glycosyl precursor directly from the protected glycal for the radical induced C-C bond formation.

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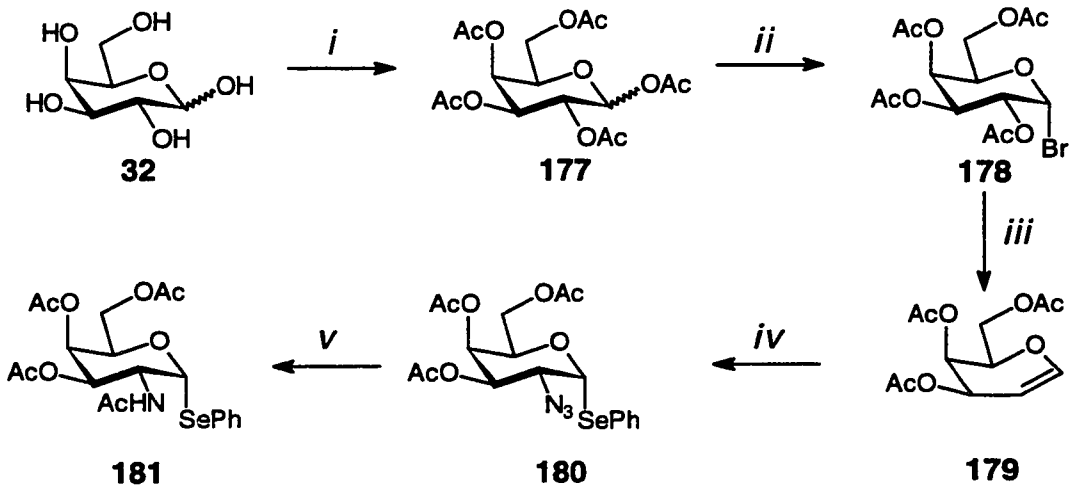
7.2. Syntheses of glycoclusters

Synthesis of radical-induced glycosyl donor

The synthesis of glycosyl donor, phenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-seleno-α-D-galactopyranoside (**181**), was accomplished as a precursor to radically induced *C*-glycosides. Peracetylated galactopyranoside **177** was converted into known bromogalactopyranoside **178** in good yield (98%) (Scheme 7.2.1). Then, this was treated with zinc dust and catalytic hydrogen hexachloroplatinate(IV) hydrate ($H_2PtCl_6 \bullet xH_2O$) in aqueous acetic acid (1:1 v/v) to provide galactal **179** in 68% yield. The galactal was obtained as a colorless syrup and this crude product was purified by flash silica gel column chromatography. It is worth mentioning that the major by-product was derived from hydrolysis at the C-1 position.



Scheme 7.2.1. Synthesis of *C*-glycoside precursor **181**. *i*) NaOAc, Ac_2O, 87%; *ii*) 30% HBr in AcOH, 10 min, 23 °C, 90%; *iii*) Zn dust, AcOH/H_2O 1:1 (v/v), H_2PtCl_6, xH_2O (cat.), 68%; *iv*) $PhI(OAc)_2$ (1.4 eq.), NaN_3 (2.4 eq.), $(PhSe)_2$ (0.6 eq.), CH_2Cl_2, 23 °C, 48 h, 88%; *v*) (1) $NiCl_2 \bullet 6H_2O$ (8.6 eq.), H_3BO_3 (16.2 eq.), $NaBH_4$ (2.8 eq.), EtOH, 23 °C, 2 h, (2) Ac_2O, pyridine, 23 °C, 2 h, 75%.

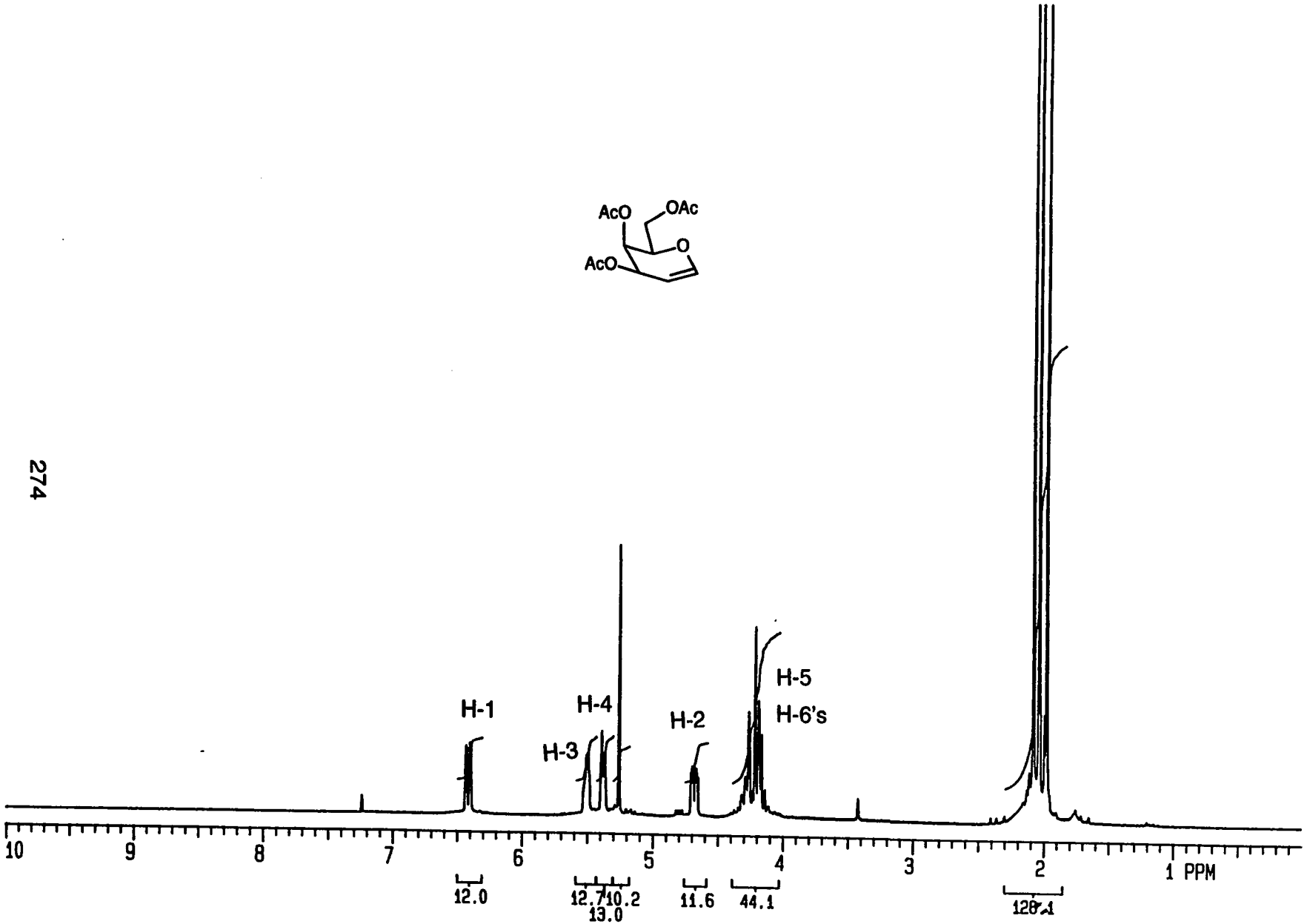


Figure 7.2.1. ^{1}H-NMR ($CDCl_3$, 200 MHz) spectrum of galactal **179**.

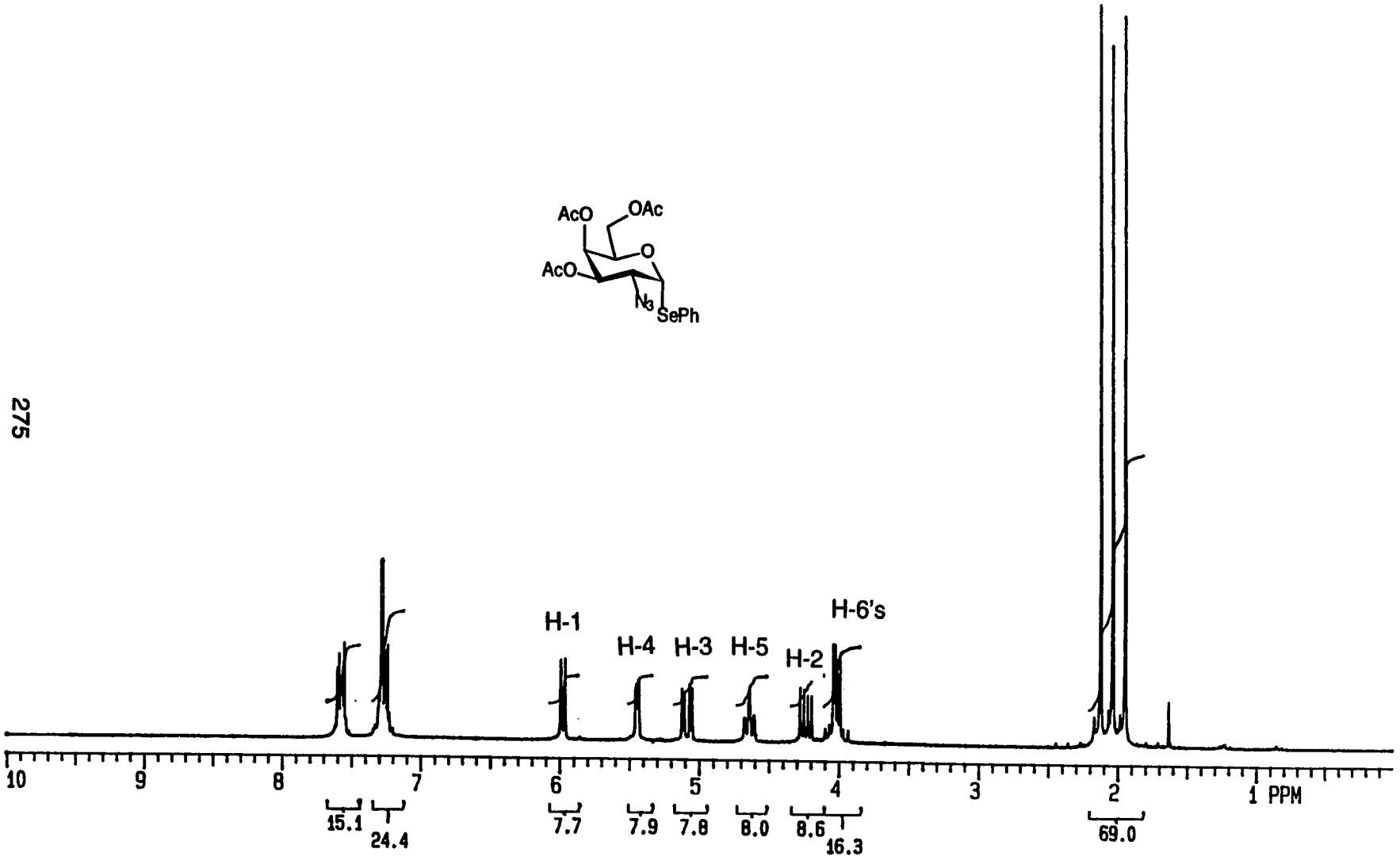


Figure 7.2.2. ^{1}H-NMR ($CDCl_3$, 200 MHz) spectrum of phenyl 2-azido-3,4,6-tri-O-acetyl-2-deoxy-1-seleno-α-D-galactopyranoside (**180**).

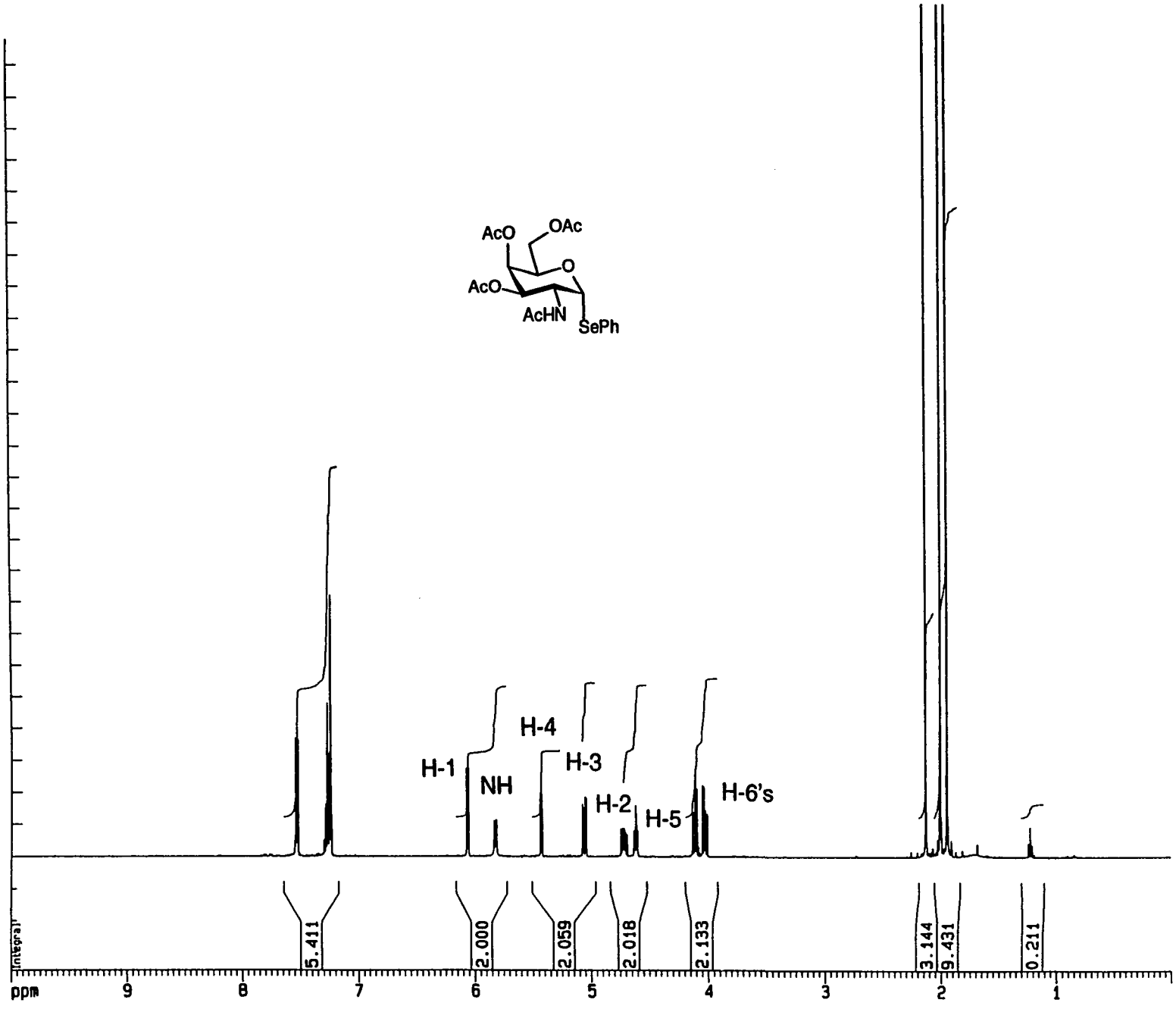


Figure 7.2.3. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of phenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-1-seleno-α-D-galactopyranoside (**181**).

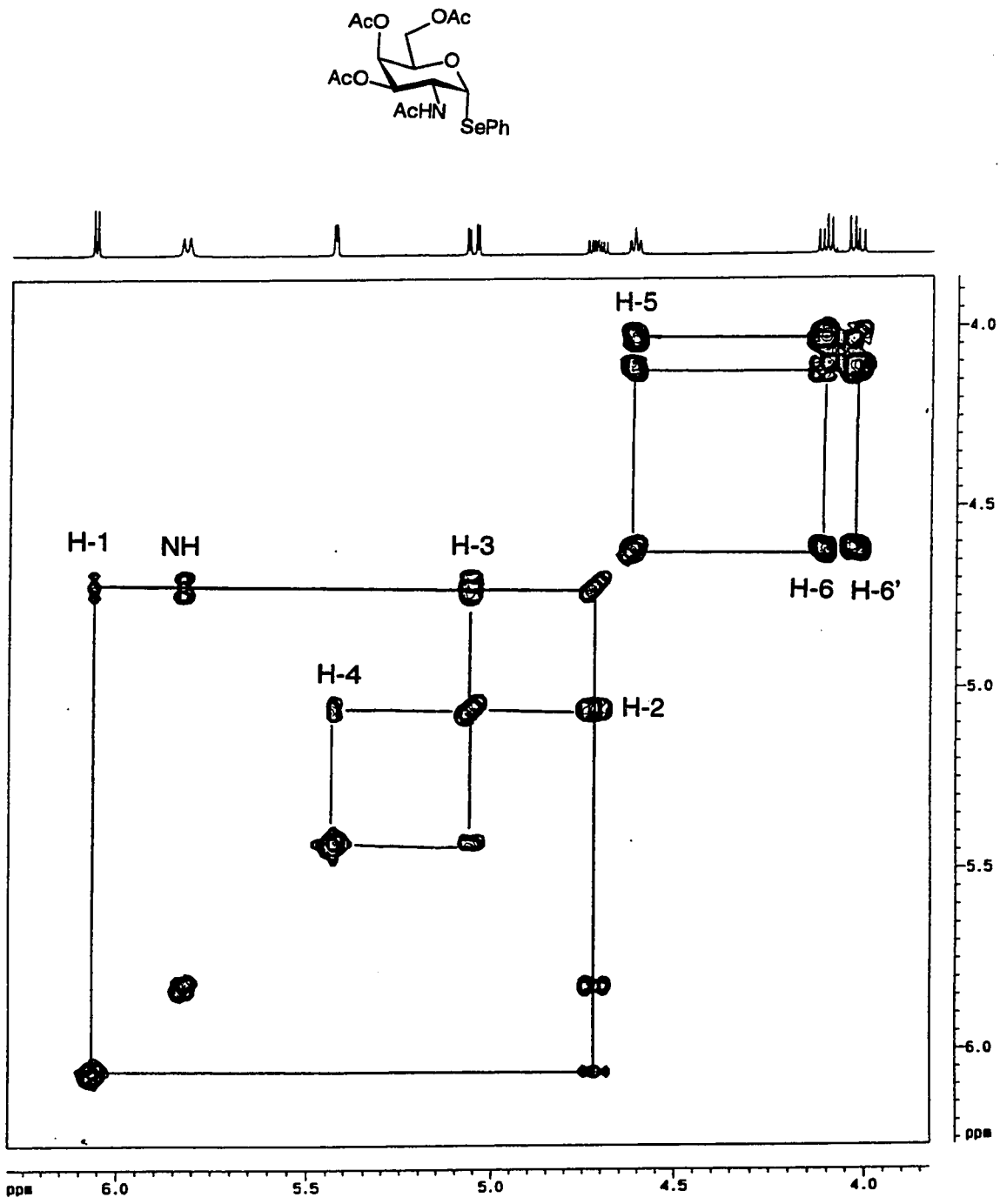


Figure 7.2.4. COSY ($CDCl_3$, 500 MHz) spectrum of phenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-1-seleno-α-D-galactopyranoside (**181**).

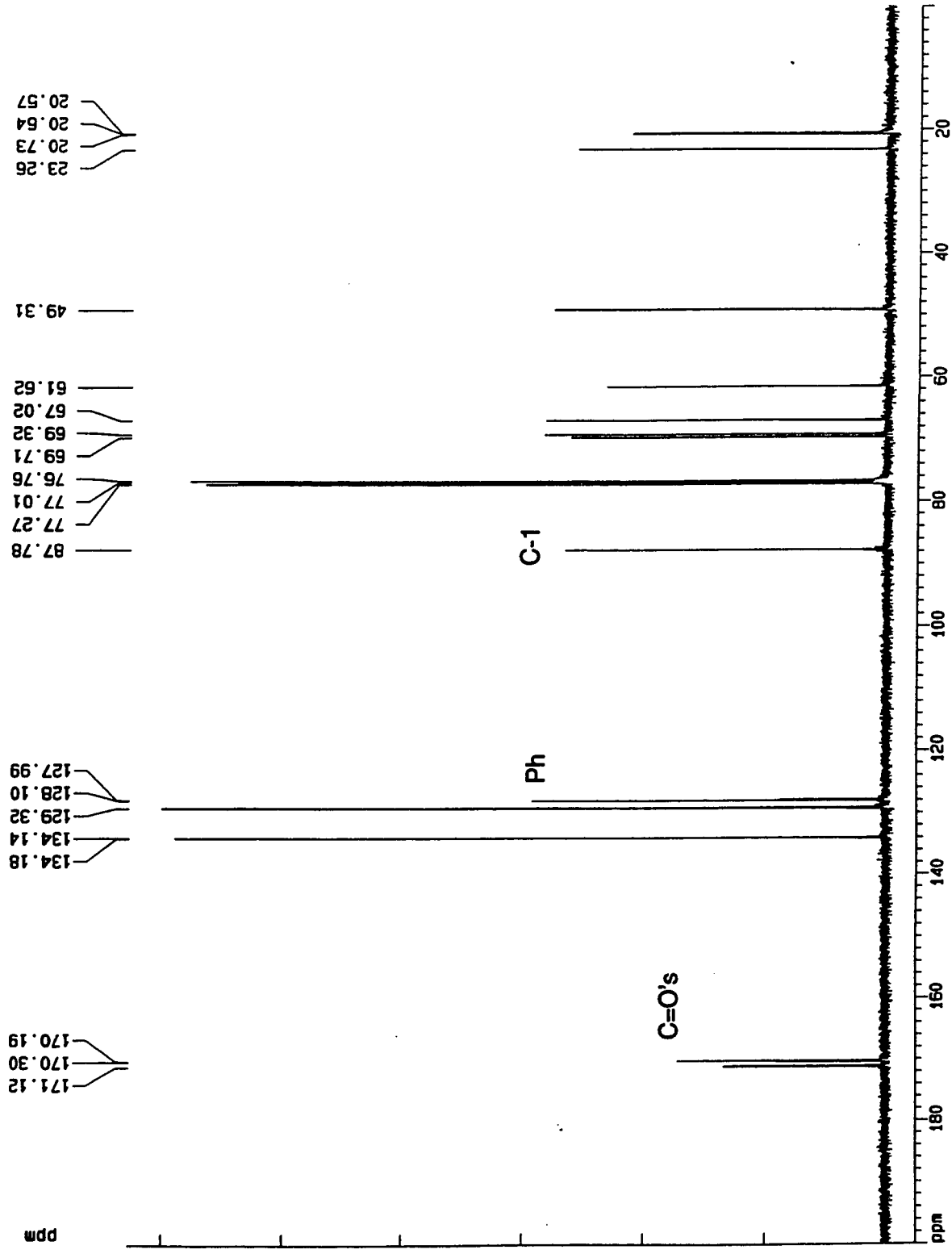
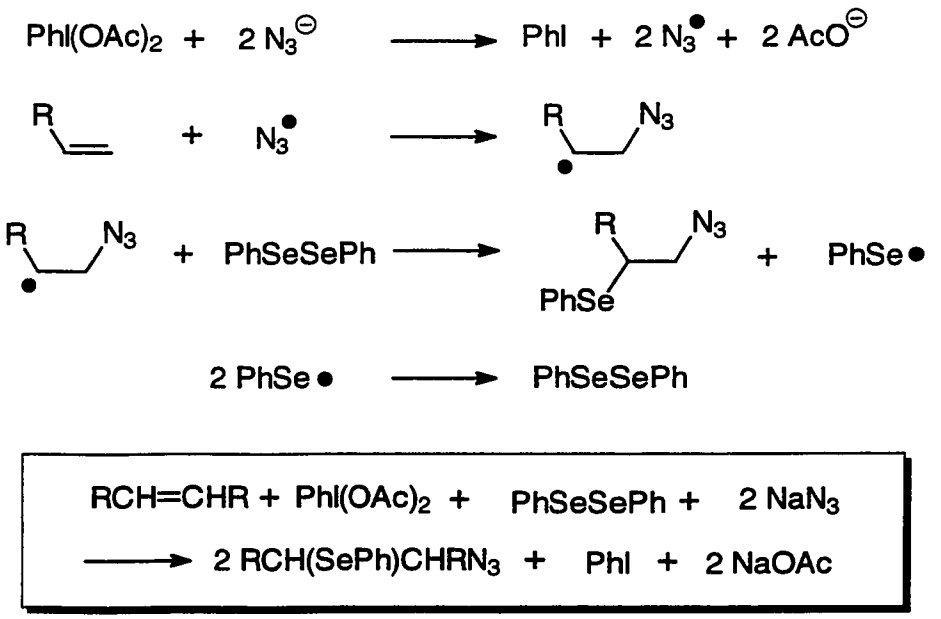


Figure 7.2.5. ^{13}C-NMR ($CDCl_3$, 125 MHz) spectrum of phenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-1-seleno-α-D-galactopyranoside (**181**).

Glycal **179** is a very versatile intermediate and has useful synthetic applications including azidonitration.[221] As a modification of this, azido-phenylselenylation was employed to provide a radical-induced glycosyl donor as a precursor for *C*-glycosides. The azido-phenylselenylation was simply carried out by stirring a mixture of the galactal **179** (1 eq) with (diacetoxyiodo)benzene (1.4 eq), diphenyl diselenide (0.6 eq), and sodium azide (2.4 eq) in CH_2Cl_2 at room temperature for 48 hours (Scheme 7.2.2).



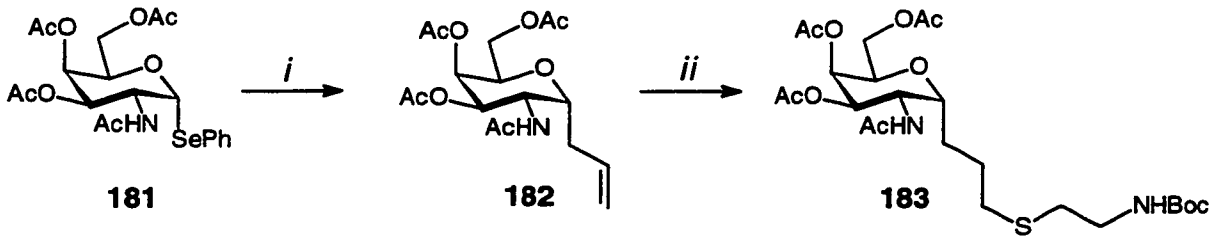
Scheme 7.2.2. Mechanism for the azido-phenylselenylation of alkenes.

The azido-phenylselenylation of galactal **179** (Scheme 7.2.1) was highly regioselective and stereoselective. The TLC analysis of the crude reaction mixture indicated that the other isomers were present only in a minute amount (less than 5%). Excess diphenyl diselenide was easily removed by silica gel chromatography eluting with hexane/ethyl acetate (7:3). During column chromatography, the pure product crystallized in the receiving containers for the collection of each fraction. The product obtained after column chromatography was therefore crystallized using hexane/ethyl

acetate (88% yield). The reduction[234] of azide to amine was accomplished using nickel chloride ($NiCl_2 \bullet 6H_2O$, 8.6 eq.), boric acid (H_3BO_3, 16.2 eq.), and sodium borohydride ($NaBH_4$, 2.8 eq.) in EtOH. The reaction was monitored by TLC, where the newly formed amine had an R_f close to zero. *N*-Acetylation of the resulting amine under conventional condition (Ac_2O, pyridine) afforded phenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-seleno-α-D-galactopyranoside **181** (75% yield).

Synthesis of clusters bearing C-α-GalNAc

The free radical allylation of phenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-1-seleno-galactopyranoside **181** was performed using allyltributyltin (10 eq) and AIBN (0.8 eq) in dry THF (Scheme 7.2.3).



181 **182** **183**

Scheme 7.2.3. Synthesis of elongated GalNAc *C*-glycoside **183**. *i*) Allyltributyltin (10 eq), AIBN (0.8 eq), THF, reflux, 1 h, 92%; *ii*) $BocHNCH_2CH_2SH$ (**166**) (2 eq), AIBN (0.8 eq), CH_3CN, reflux, 6 h, 87%.

When lesser amounts of allyltributyltin (e.g., 8 eq instead of 10 eq) were used, the reaction was slowed down and the formation of an unidentified by-product was observed. When the reaction was complete in one hour, the concentrated reaction mixture was partitioned between acetonitrile and pentane. Consecutive washing of acetonitrile solution with pentane removed the excess allyltributyltin. The crude mixture was crystallized from hexane/ethyl acetate by triturating the solution with a glass rod (92% yield).

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[234] Bencomo, V. V.; Jacquinet, J. -C.; Sinaÿ, P. *Carbohydr. Res.* **1982**, *110*, C9.

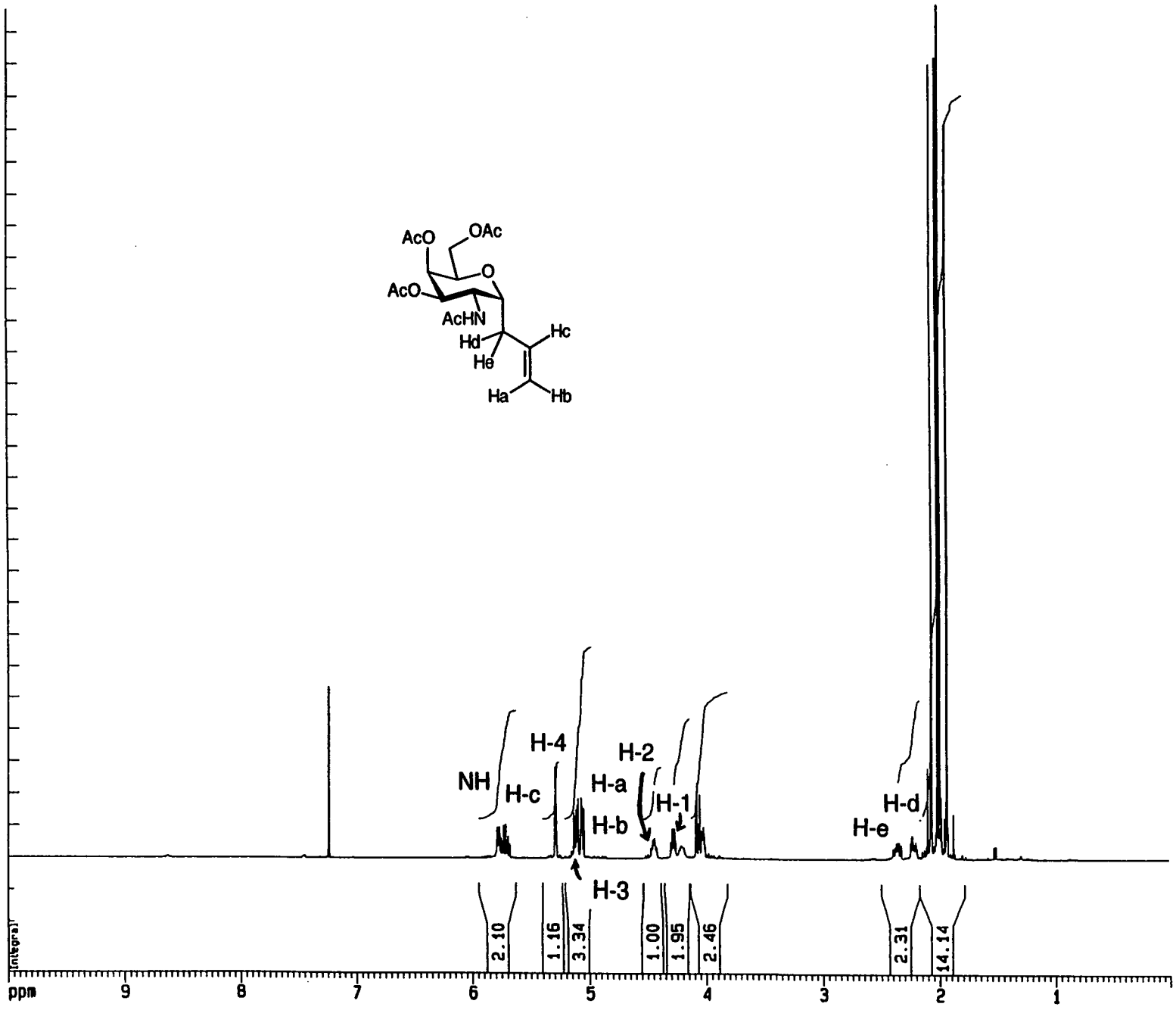


Figure 7.2.6. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of 3-(2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-galactopyranosyl)propene (**182**).

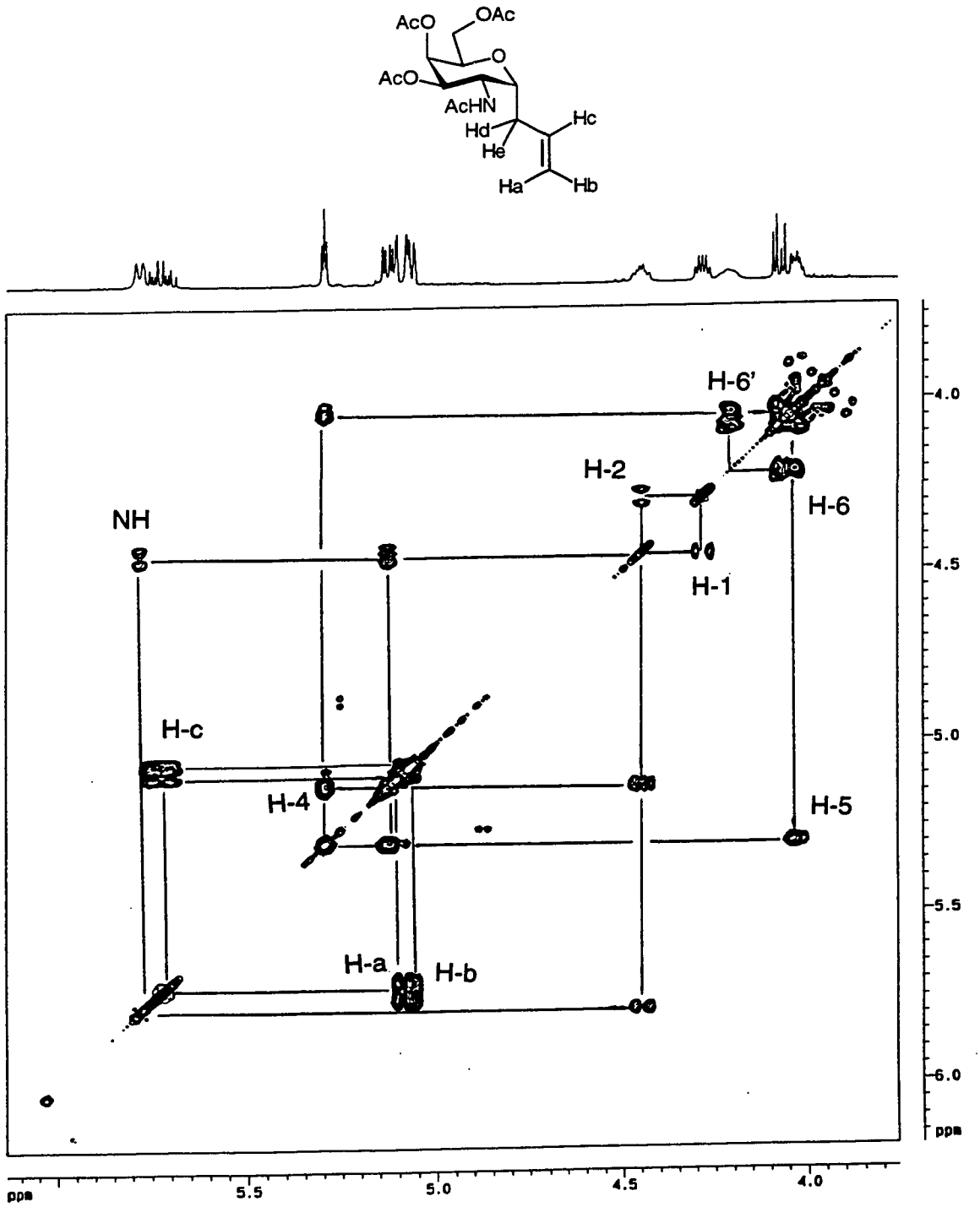


Figure 7.2.7. COSY ($CDCl_3$, 500 MHz) spectrum of 3-(2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-galactopyranosyl)propene (**182**).

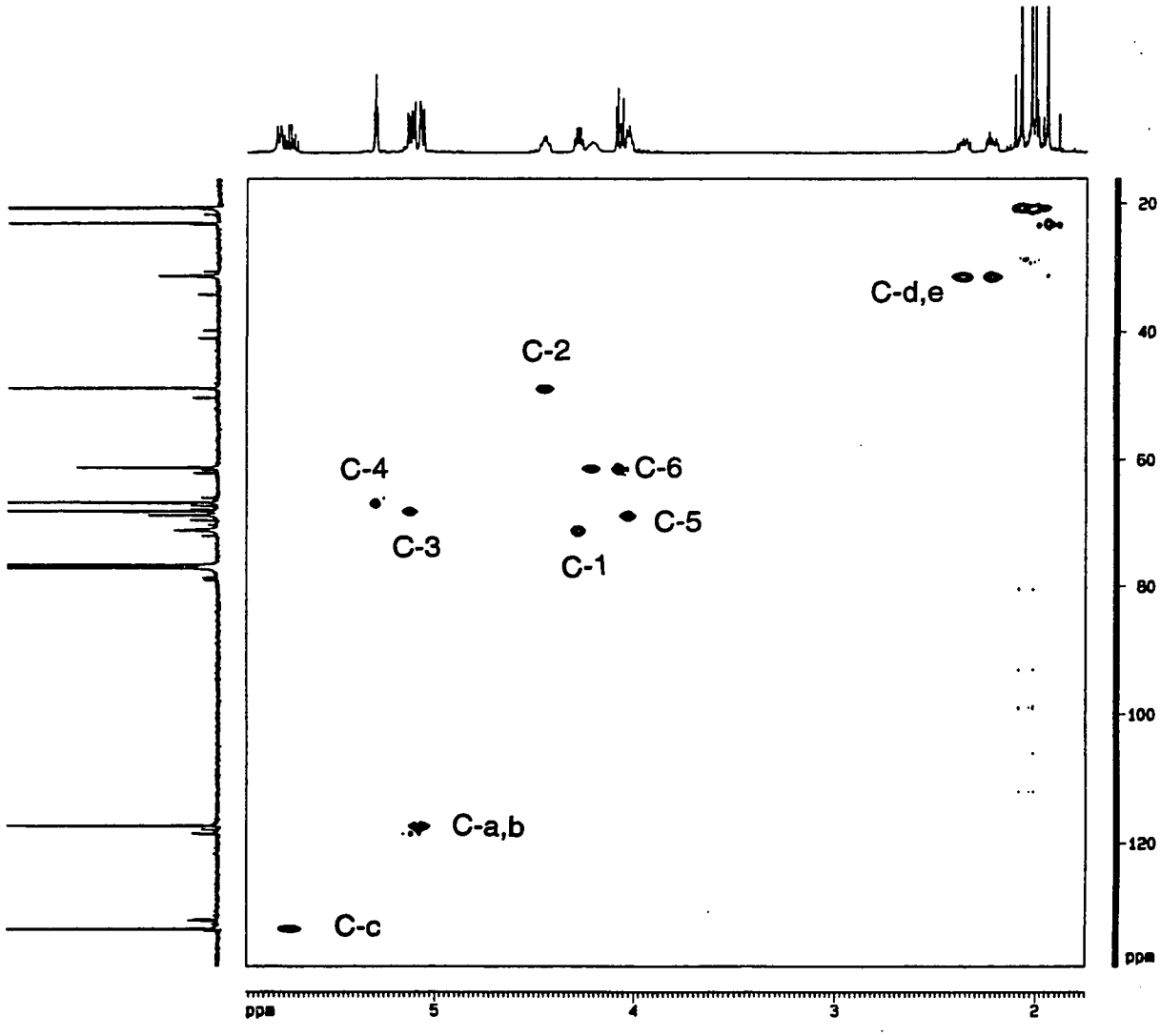
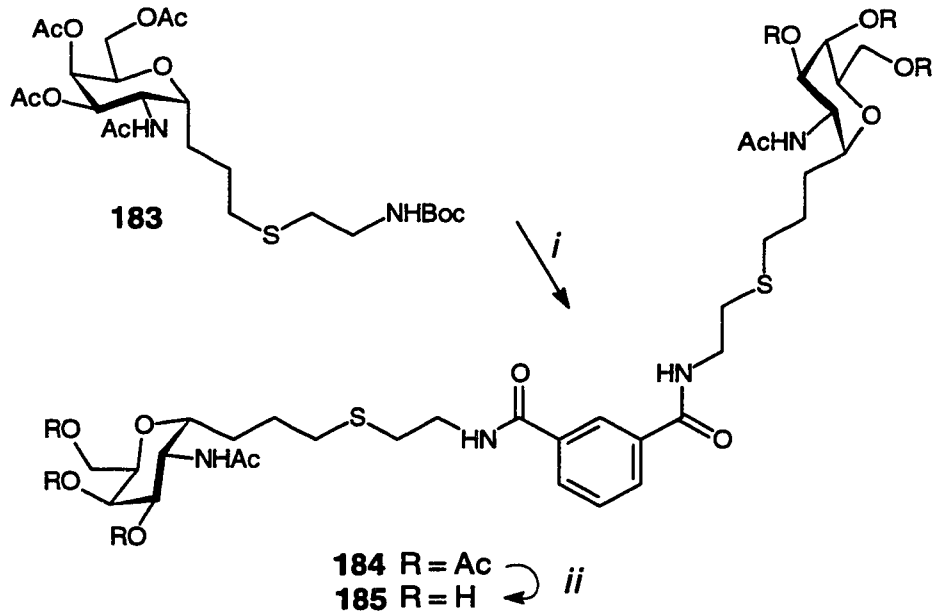


Figure 7.2.8. HMQC ($CDCl_3$, 500 MHz) spectrum of 3-(2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-galactopyranosyl)propene (**182**).

The ^{1}H-NMR spectrum of the allyl *C*-GalNAc indicated that the compound existed as epimeric mixture of α and β anomers (α:β, >10:1). This allyl *C*-α-GalNAc (major anomer) was further treated with *N*-Boc-cysteamine (**166**) and AIBN to furnish an adduct by a free radical process. The *C*-α-GalNAc derivative conjugated with *N*-Boc cysteamine, **183** was then coupled to both isophthaloyl chloride and 1,3,5-benzenetricarbonyl trichloride, after removing the Boc group (20% TFA in CH_2Cl_2), to generate dimeric cluster **184** (Scheme 7.2.4, 68% yield) and trimeric cluster **186** (Scheme 7.2.5, 83% yield). The acetate functionalities on the GalNAc moieties were removed under Zemplén conditions in a good yield (92-95%).



Scheme 7.2.4. Synthesis of dimer **185**; *i*) (1) 20% TFA, CH_2Cl_2, 23 °C, 2 h, (2) isophthaloyl chloride (0.45 eq), Et_3N (2.3 eq), CH_2Cl_2, 0 °C, 1 h, 68%; *ii*) 1M NaOMe, MeOH, pH 9, 23 °C, 16 h, 92%.

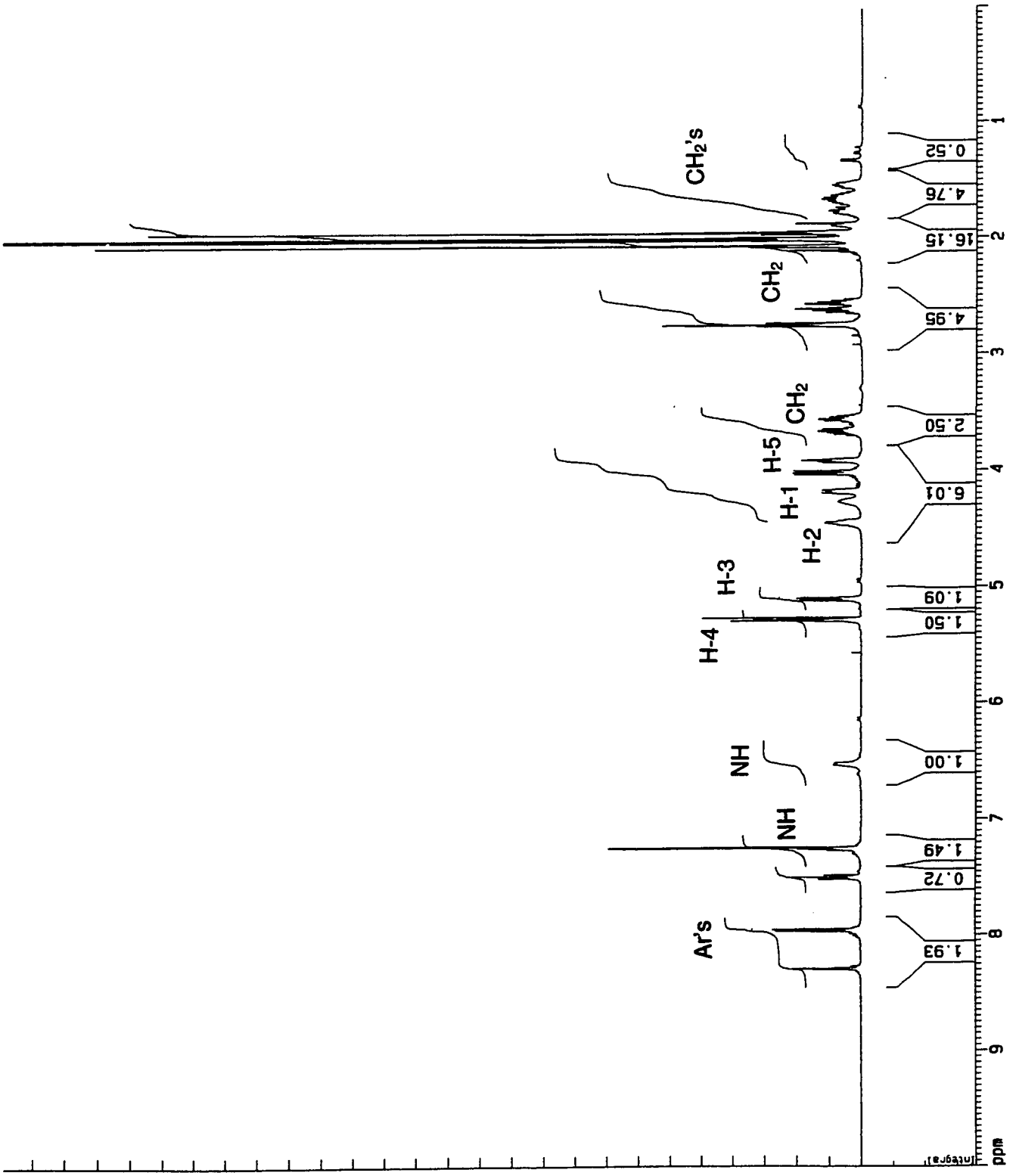


Figure 7.2.9. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of fully protected dimer **184**

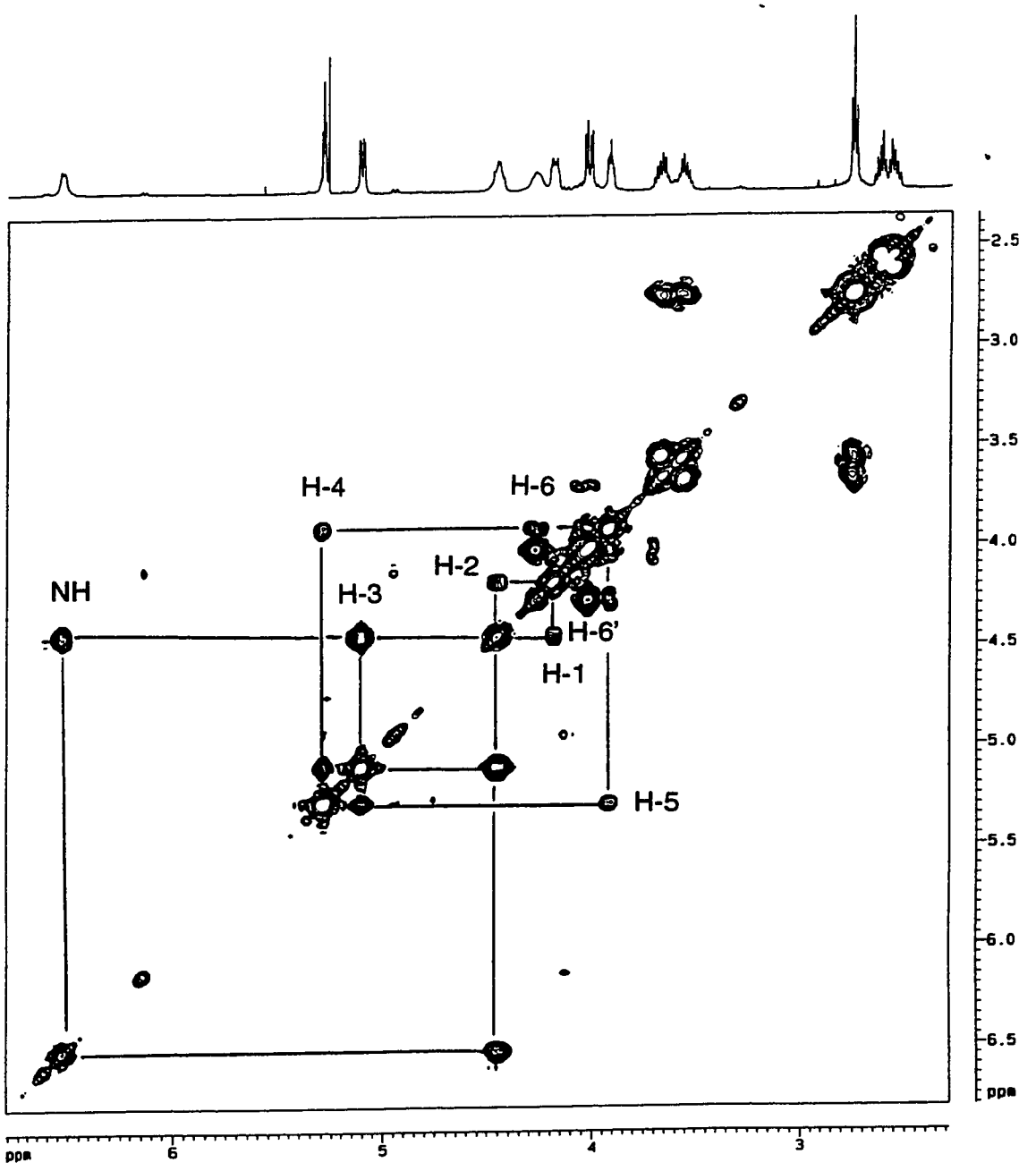


Figure 7.2.10. COSY ($CDCl_3$, 500 MHz) spectrum of fully protected dimer **184**

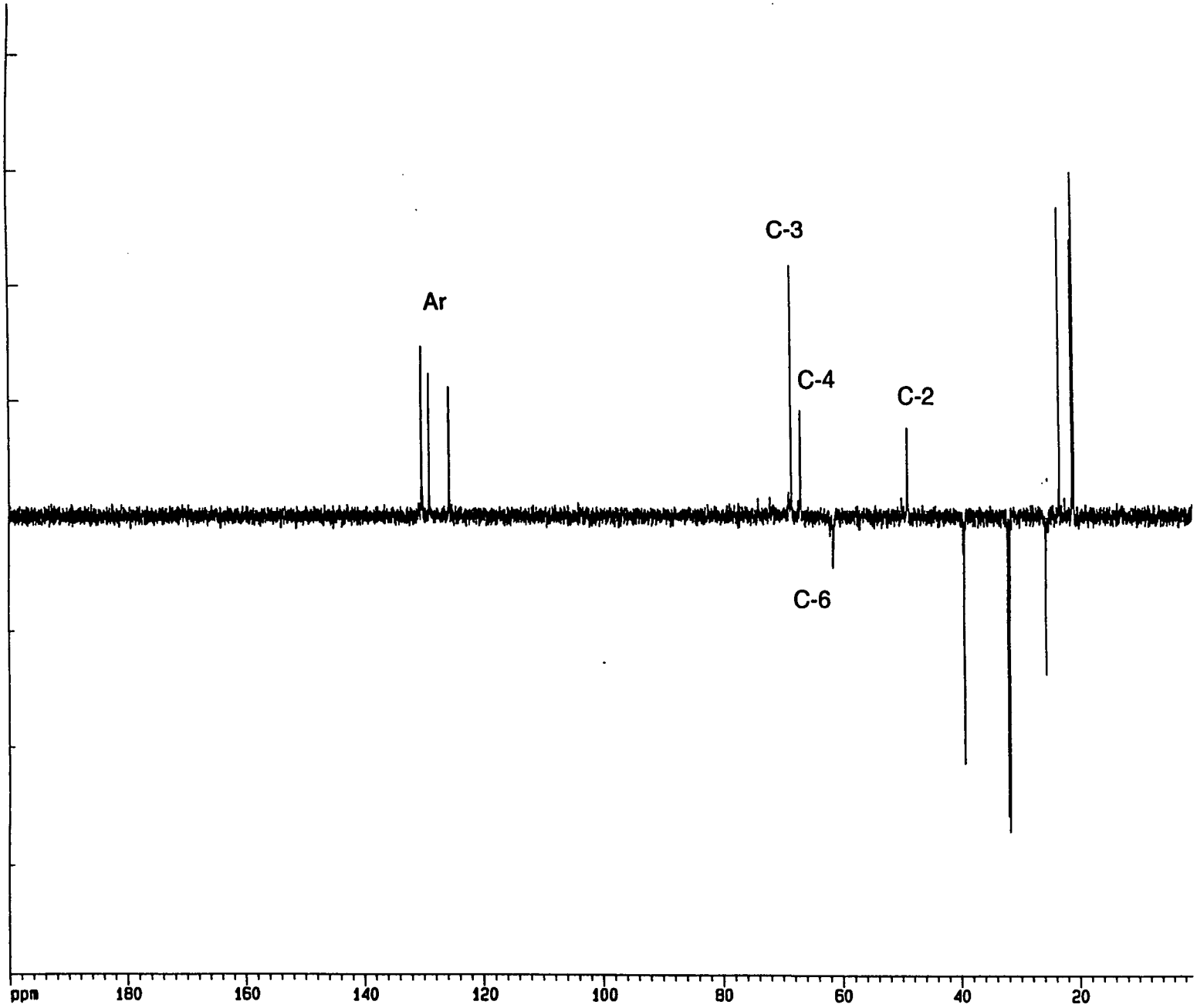


Figure 7.2.11. DEPT ($CDCl_3$, 125 MHz) spectrum of fully protected dimer **184**

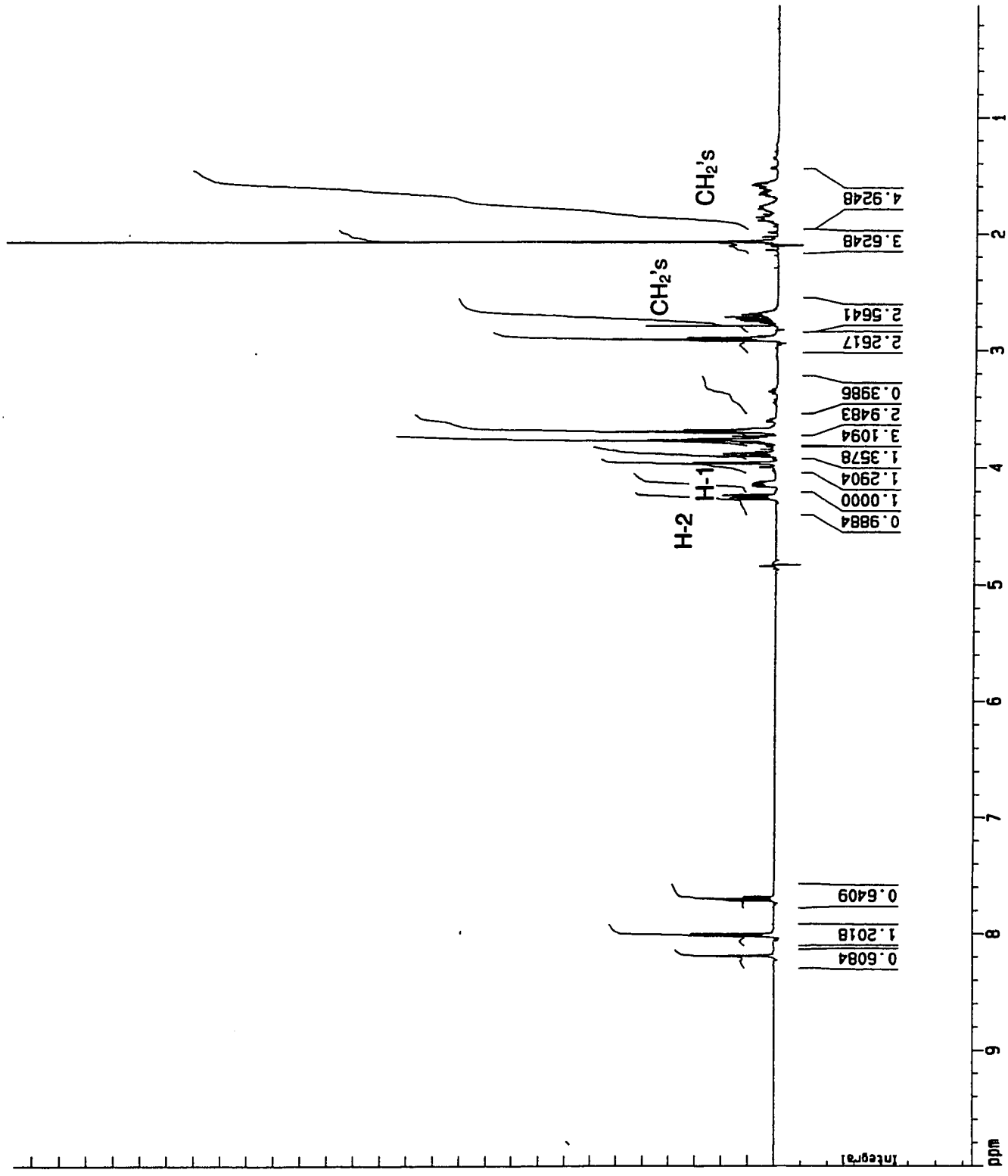


Figure 7.2.12. ^{1}H-NMR (D_2O, 500 MHz) spectrum of deprotected dimer **185**

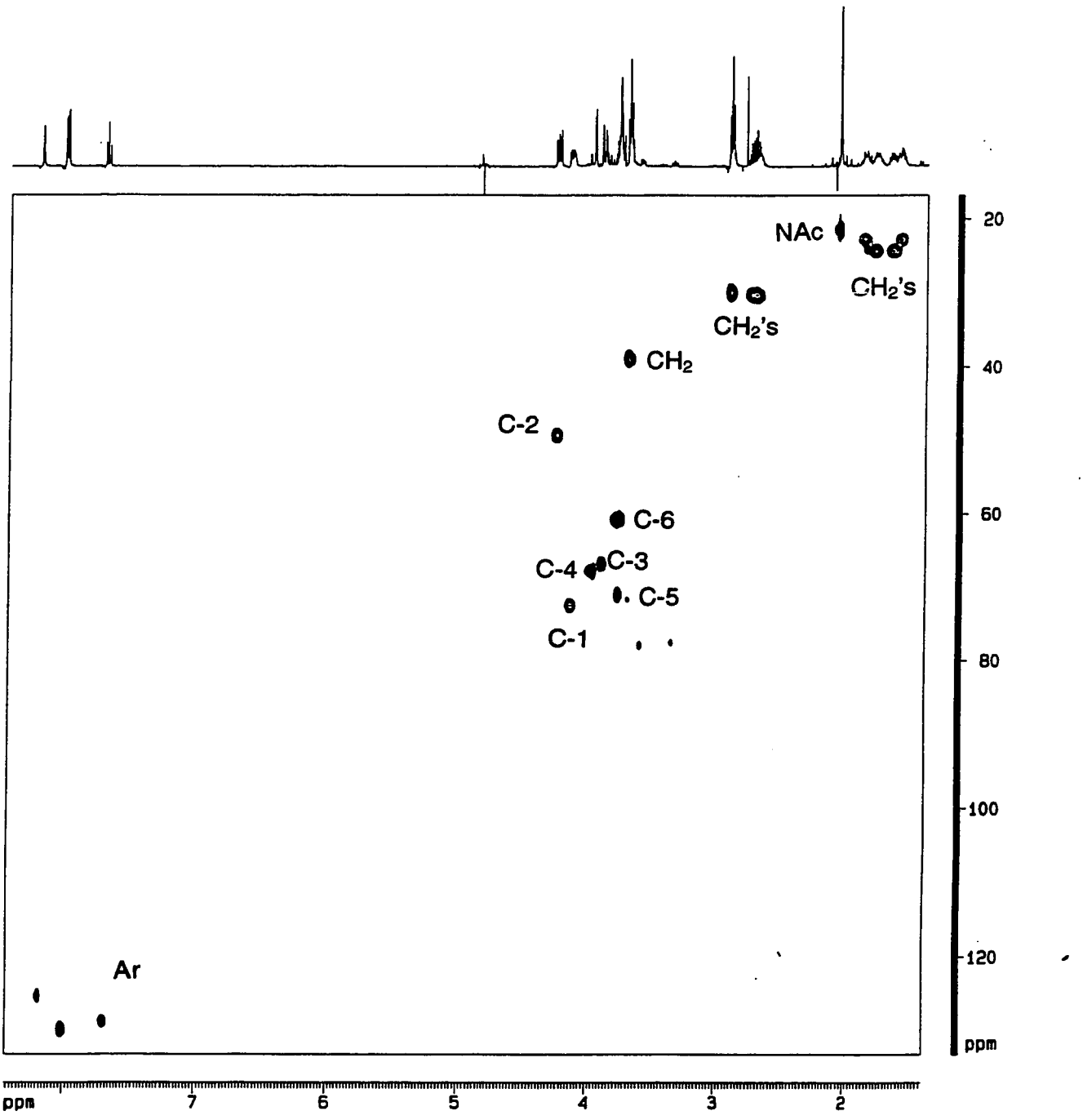
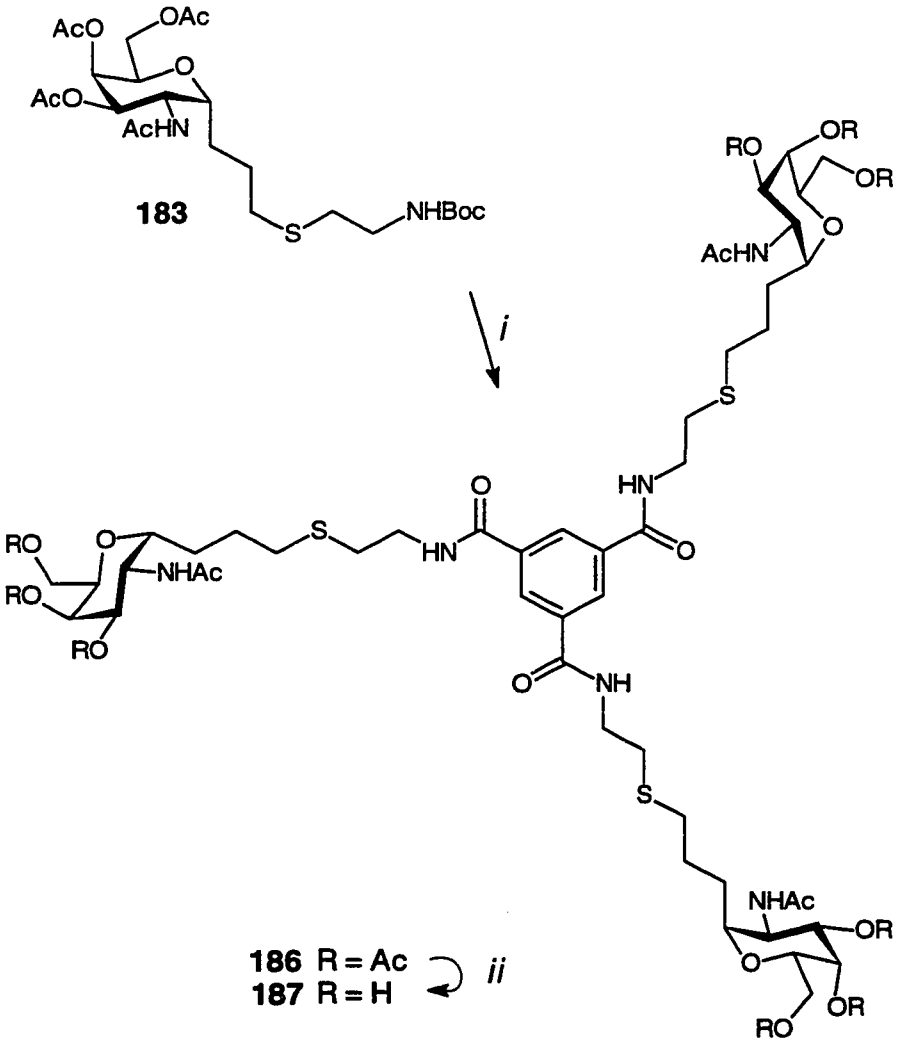


Figure 7.2.13. HMQC (D_2O, 500 MHz) spectrum of deprotected dimer **185**



Scheme 7.2.5. Synthesis of trimer **187.** *i*) (1) 20% TFA, CH_2Cl_2, 23 °C, 2 h, (2) 1,3,5-benzenetricarbonyl trichloride (0.3 eq), Et_3N (3.5 eq), CH_2Cl_2, 0 °C, 1 h, 83%; *ii*) 1M NaOMe, MeOH, pH 9, 23 °C, 16 h, 95%.

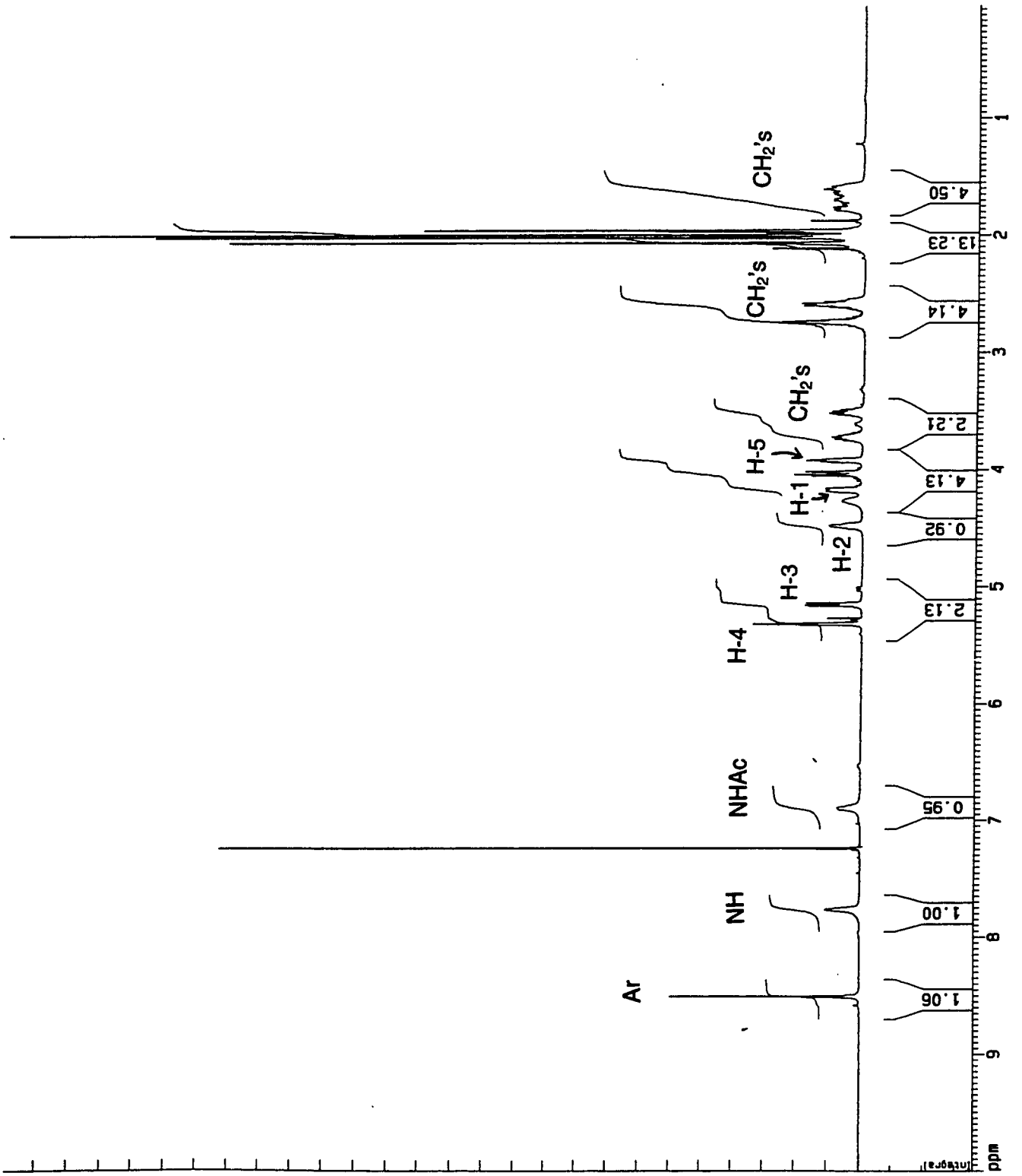


Figure 7.2.14. ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of fully protected trimer **187**

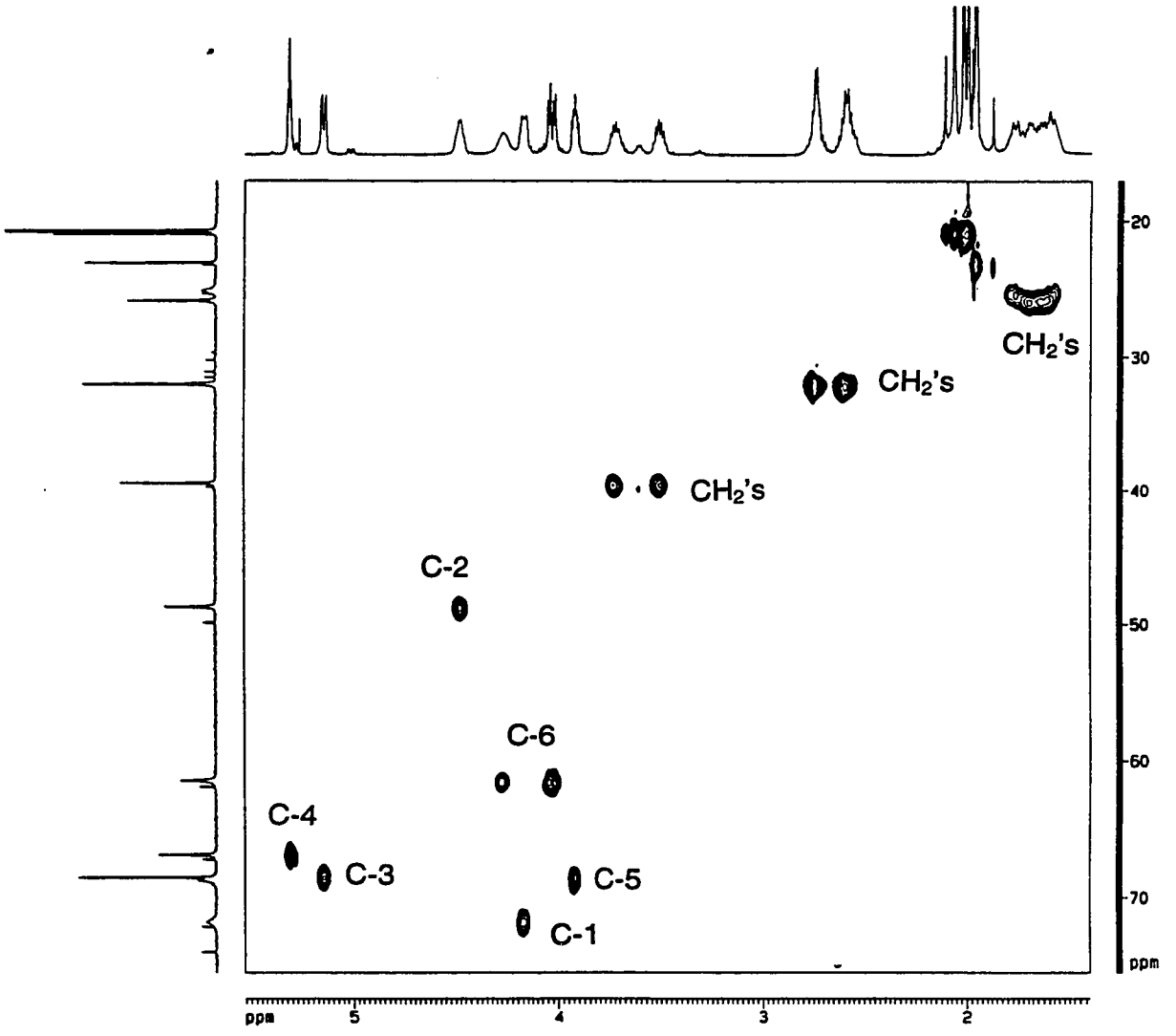


Figure 7.2.15. HMQC ($CDCl_3$, 500 MHz) spectrum of fully protected trimer **187**.

7.3. Conclusions

Hydrolytically stable carbohydrate mimetic of GalNAcα-*O*-Ser was synthesized *via* radical *C*-glycosylation process. α-linked *C*-glycoside, 3-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)propene was obtained from the reaction of anomeric radical with allyltributyltin in the presence of AIBN. The radical glycosyl donor, GalNAc phenylselenide was derived form the azido-phenylselenylation of galactal followed by reduction of azide to acetamide in good yield. The synthesized *C*-glycoside was conjugated to a rigid aromatic core to afford dimer and trimer in 68% and 83% yields.

7.3. Experimental Methods

D-Galactopyranose pentaacetate (177).

The title compound was prepared using the same method described previously for the preparation of 1,2,3,4-tetra-*O*-acetyl-D-xylopyranoside (**2**); yield: 87% (recrystallized from EtOH), ratio of α:β anomers (1:4).

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide (178).

Peracetylated D-galactopyranose **177** (10.0 g, 2.56 mol) was treated with 30% HBr in acetic acid (30 mL) at room temperature for 30 min. The solution was diluted with CH_2Cl_2 (30 mL) and poured into a beaker containing ice in saturated $NaHCO_3$ (30 mL). The solution mixture was stirred vigorously for 10 min and the organic phase was separated from the aqueous phase. The organic phase was washed with water (2 × 30 mL), dried over anhydrous Na_2SO_4 and then concentrated to yield 10.4 g (98%) of a white foam. The product was recrystallized from anhydrous ether to provide a white crystal (90%): mp, 82.0-83.5 °C; $[\alpha]_D$ +208.0 (*c* 1.0, $CHCl_3$): Lit. mp, 83-84 °C; $[\alpha]_D$ +215 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.96, 2.01, 2.06, 2.10 (4s, 12H, OAc), 4.01-4.18 (m,

2H, H-6's), 4.40-4.48 (m, 1H, H-5), 4.99 (dd, 1H, $J_{2,3}$ 10.6 Hz, H-2), 5.35 (dd, 1H, $J_{3,4}$ 3.3 Hz, H-3), 5.46 (dd, 1H, $J_{4,5}$ 1.2 Hz, H-4), 6.65 (d, 1H, $J_{1,2}$ 3.9 Hz, H-1).

3,4,6-Tri-*O*-acetyl-D-galactal (179)

A mixture of 50% acetic acid (240 mL), 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**178**) (15.0 g, 36.5 mmol), zinc dust (15.0 g), and 3% aqueous hydrogen hexachloroplatinate (IV) solution (0.14 mL) was placed in a round-bottomed flask and stirred vigorously to prevent caking. The mixture was further supplemented with a few drops of the platinic chloride solution and zinc dust (30.0 g) slowly added over a period of 2 h. Stirring was continued another 2 h. The mixture was then filtered and the residue was washed with water. The filtration residue must be kept wet to prevent rapid oxidation of the remaining zinc dust on suction of air through the filter or the exposure of air. The filtrate was evaporated under reduced pressure to about 50 mL and diluted with water (100 mL). The aqueous solution was extracted with $CHCl_3$ (4 × 100 mL) and the extract was washed with saturated $NaHCO_3$ (2 × 50 mL) and then water (1 × 50 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Purification by short silica gel column chromatography eluting with 7:3 Hexanes/EtOAc yielded 6.7 g (68%) of a colorless syrupy residue: $[\alpha]_D$ -17.0 (*c* 3.0, $CHCl_3$), Lit. $[\alpha]_D$ -16.5 (*c* 3.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 2.00, 2.06, 2.10 (3s, 9H, 3OAc), 4.15-4.24 (m, 3H, H-5, H-6's), 4.70 (ddd, 1H, $J_{1,2}$ 5.9 Hz, $J_{2,3}$ 3.8 Hz, $J_{2,4}$ 1.7 Hz, H-2), 5.36-5.42 (m, 1H, H-4), 5.50-5.56 (m, 1H, H-3), 6.43 (dd, 1H, $J_{1,2}$ 5.9 Hz, $J_{1,3}$ 1.7 Hz, H-1); ^{13}C-NMR ($CDCl_3$) δ 21.3 (OAc), 62.3 (C6), 64.3 (C3, C4), 73.0 (C5), 99.2 (C2), 145.7 (C1), 170.8 (C=O's).

Phenyl 2-azido-3,4,6-tri-*O*-acetyl-2-deoxy-1-seleno-α-D-galactopyranoside (180).

3,4,6-Tri-*O*-acetyl-D-galactal (**179**) (5.0 g, 18.4 mmol) was dissolved in CH_2Cl_2 (200 mL) and the solution was purged with N_2 for 1 h. Then, (diacetoxyiodo)benzene (8.3 g, 25.7 mmol), sodium azide (2.9 g, 44.1 mmol), and diphenyldiselenide (3.4 g, 11.0 mmol) were added to the solution containing galactal. The reaction solution was stirred at room temperature for 48 h. The reaction was monitored by TLC (galactal: R_f 0.40, product: R_f 0.47, 3:2 Hexanes/EtOAc). When the reaction was complete, the

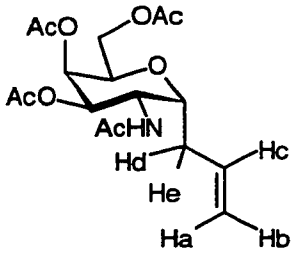
solution was diluted with CH_2Cl_2 (100 mL) and washed with saturated $NaHCO_3$ (2 × 200 mL), water (2 × 200 mL), then brine (1 × 100 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated to afford an orange oily residue. Purification by silica gel column chromatography eluting with 7:3 Hexanes/EtOAc yielded 7.6 g (88%) of a pale yellowish powder: $[\alpha]_D$ +256.3 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.95, 2.04, 2.12 (3s, 9H, OAc), 3.99-4.04 (m, 2H, H-6's), 4.24 (dd, 1H, $J_{2,3}$ 10.8 Hz, H-2), 4.64 (ddd, 1H, $J_{5,6}$ 6.6 Hz, $J_{4,5}$ 0.9 Hz, H-5), 5.08 (dd, 1H, $J_{3,4}$ 3.2 Hz, H-3), 5.44 (dd, 1H, H-4), 5.44 (dd, 1H, $J_{1,2}$ 5.4 Hz, H-1), 7.23-7.31 (m, 3H, Ar_{para}, Ar_{meta}), 7.55-7.61 (m, 2H, Ar_{ortho}); ^{13}C-NMR ($CDCl_3$) δ 20.60 (OAc), 58.67 (C-2), 61.49 (C-6), 67.12 (C-4), 68.91 (C-3), 71.13 (C-5), 84.05 (C-1), 127.48 (Ar_{ipso}), 128.18 (Ar_{para}), 129.18 (Ar_{meta}), 134.74 (Ar_{ortho}), 169.60, 169.92, 170.32 (C=O's); MS-FAB (pos. m/z) cald. For $C_{18}H_{21}N_3O_7Se$: 471.05; found: 472.18 (M^+ + 1, 38.6%); Anal. Calcd for $C_{18}H_{21}N_3O_7Se$: C, 45.97; H, 4.50; N, 8.93. Found C, 45.76; H, 4.43; N, 9.12.

Phenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-seleno-α-D-galactopyranoside (181).

To a solution of phenyl 2-azido-3,4,6-tri-*O*-acetyl-2-deoxy-1-seleno-α-D-galactopyranoside (**180**) (0.10 g, 0.21 mmol) in EtOH (10 mL) were added $NiCl_2 \bullet 6H_2O$ (0.43 g, 1.81 mmol) and boric acid (0.21 g, 3.41 mmol). Then, a suspension of $NaBH_4$ (22 mg, 0.58 mmol) in EtOH (2 mL) was added slowly to the reaction solution. As soon as $NaBH_4$ was added, the color of the solution was changed from green to dark gray. The reaction mixture was stirred at room temperature for 2 h until no starting material was observable on TLC (starting material: R_f 0.50, product: R_f 0.0, 7:3 Hexanes/EtOAc). The solvent was evaporated under reduced pressure and the dark residue was dissolved in pyridine (10 mL) and then acetic anhydride (2 mL) was added. The solution was stirred at room temperature for 2 h and the reaction was monitored by TLC (starting material: R_f 0.0, product: R_f 0.10, 7:3 Hexanes/EtOAc). The solution was concentrated under reduced pressure and the residue was dissolved in CH_2Cl_2 (20 mL). The organic solution was washed with aqueous 3% $KHSO_4$ (2 × 10 mL), water (1 × 10 mL), and dried over anhydrous Na_2SO_4. Silica gel column chromatography of the crude product eluting with 1:1 EtOAc/Hexanes yielded 77 mg (75%) of a white foam: $[\alpha]_D$

+228.6 (*c* 0.35, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.95, 2.01, 2.13 (3s, 12H, OAc, NAc), 4.03 (dd, 1H, $J_{6,6'}$ 11.4 Hz, $J_{5,6'}$ 7.0 Hz, H-6'), 4.11 (dd, 1H, $J_{5,6}$ 6.0 Hz, H-6), 4.62 (ddd, 1H, $J_{4,5}$ 1.3 Hz, H-5), 4.72 (ddd, 1H, $J_{2,3}$ 11.7 Hz, $J_{2,NH}$ 9.1 Hz, H-2), 5.06 (dd, 1H, $J_{3,4}$ 3.2 Hz, H-3), 5.43 (dd, 1H, H-4), 5.83 (d, 1H, NH), 6.06 (d, 1H, $J_{1,2}$ 5.0 Hz, H-1); ^{13}C-NMR ($CDCl_3$) δ 20.57, 20.64, 20.73 (OAc), 23.26 (NAc), 49.31 (C-2), 61.62 (C-6), 67.02 (C-4), 69.32 (C-3), 69.71 (C-5), 87.78 (C-1), 127.99 (Ar_{ipso}), 128.10 (Ar_{para}), 129.32 (Ar_{meta}), 134.14 (Ar_{ortho}), 170.19, 170.30, 171.12 (C=O's); FAB-HRMS (pos. m/z) calcd. for $C_{20}H_{26}NO_8Se$: 488.0823; found: 488.0865 (M^+ + 1, 42.6%); Anal Calcd for $C_{20}H_{25}NO_8Se$: C, 49.39; H, 5.18; N, 2.88. Found C, 49.23; H, 5.22; N, 2.81.

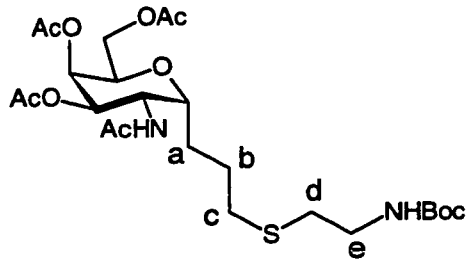
3-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)propene (182).



Phenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-seleno-α-D-galactopyranoside (**181**) (1.89 g, 3.89 mmol) was dissolved in THF (30 mL) which was freshly distilled over sodium and benzophenone, and allyltributyltin (12.0 mL, 38.9 mmol) was added to the solution. The reaction solution was degassed by bubbling N_2 through it for 30 min and then a half portion of AIBN (0.51 g, 3.11 mmol) was added. AIBN was recrystallized from EtOH. The reaction mixture was refluxed for 30 min under a nitrogen atmosphere and the other half portion of AIBN was added. After refluxing for another 30 min, the starting material was consumed completely (starting material: R_f 0.52, product: R_f 0.42, 18:1:1 $CHCl_3$/MeCN/MeOH). The solvent was evaporated and the residue was partitioned between CH_3CN (20 mL) and pentane (50 mL). The acetonitrile layer was washed with pentane (5 × 50 mL) and concentrated. The oily residue was treated with EtOAc and Hexanes. An off-white solid (0.96 g) was crystallized by triturating the solution with a glass rod. The crystal was filtered and the filtrate was concentrated. Silica gel column chromatography of the residue from the filtrate eluting with 18:1:1

$CHCl_3/CH_3CN$/MeOH yielded 0.36 g of an off-white solid (total yield 92%): $[\alpha]_D$ +48.0 (*c* 1.1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.94, 2.01, 2.03, 2.08 (4s, 12H, OAc, NAc), 2.20-2.27 (m, 1H, H-d), 2.32-2.44 (m, 1H, H-e), 4.01-4.05 (m, 1H, H-5), 4.07 (dd, 1H, $J_{6,6'}$ 11.5 Hz, $J_{5,6'}$ 4.9 Hz, H-6'), 4.17-4.25 (m, 1H, H-6), 4.28 (ddd, 1H, $J_{1,2}$ 5.0 Hz, $J_{1,e}$ 5.0 Hz, $J_{1,d}$ 10.1 Hz, H-1), 4.42-4.48 (m, 1H, H-2), 5.06 (dd, 1H, $J_{a,b}$ 1.6 Hz, $J_{b,c}$ 7.8 Hz, H-b), 5.09 (dd, 1H, $J_{a,c}$ 13.7 Hz, H-a), 5.13 (dd, 1H, $J_{2,3}$ 9.4 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.29 (t, 1H, $J_{4,5}$ 3.2 Hz, H-4), 5.73 (dddd, 1H, $J_{a,c}$ 13.7 Hz, $J_{b,c}$ 7.8 Hz, $J_{c,e}$ 7.8 Hz, $J_{c,d}$ 10.0 Hz, H-c), 5.78 (d, 1H, $J_{2,NH}$ 8.4 Hz, NH); ^{13}C-NMR ($CDCl_3$) δ 20.58, 20.64, 20.69 (OAc's), 23.16 (NAc), 31.49 (C-d, C-e), 48.88 (C-2), 61.35 (C-6), 66.91 (C-4), 68.27 (C-3), 68.88 (C-5), 71.31 (C-1), 117.51 (C-a, C-b), 133.51 (C-c), 169.99, 170.09, 170.42, 170.55 (C=O's); FAB-HRMS (pos. m/z) calcd. for $C_{17}H_{26}NO_8$: 372.1658; found: 372.1666 (M^+ + 1, 100.0%); Anal. Calcd for $C_{17}H_{25}NO_8$: C, 54.96; H, 6.79; N, 3.77. Found C, 54.97; H, 6.83; N, 3.83.

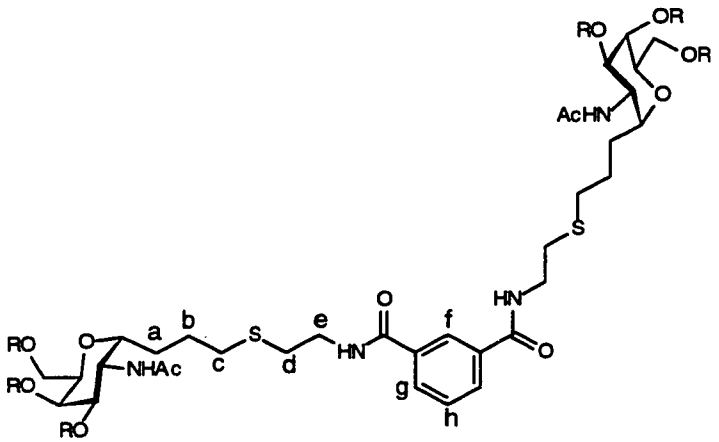
Conjugation of 3-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)propene with *N*-Boc-cysteamine (183).



A solution of 3-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)propene (**182**) (83 mg, 0.224 mmol) and *N*-Boc-cysteamine (**166**) (80 mg, 0.447 mmol) in CH_3CN (3 mL) was degassed by bubbling with N_2 through it for 30 min. A half portion of AIBN (29 mg, 0.179 mmol) was added to the reaction solution and the solution was refluxed for 6 h under a nitrogen atmosphere. Then, the solution was treated with the other half portion of AIBN and refluxed for another 18 h. The reaction was monitored by spraying TFA on the TLC to observe the Boc group (starting material: R_f 0.16, product: R_f 0.09, 7:3 EtOAc/Hexanes). When the reaction was complete, the solution was concentrated. Purification by silica gel column

chromatography eluting with 38:1:1 $CHCl_3/CH_3CN$/MeOH afforded 0.11 g (87%) of a white foam: $[\alpha]_D$ +54.0 (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.41 (s, 9H, t-Bu), 1.47-1.55 (m, 1H, H-a'), 1.51-1.63 (m, 1H, H-b'), 1.65-1.74 (m, 2H, H-b, H-a), 1.96, 2.03, 2.04, 2.09 (4s, 12H, OAc, NAc), 2.48-2.58 (m, 2H, H-c), 2.60 (t, 2H, J 7.7 Hz, H-d), 3.20-3.33 (m, 2H, H-e), 3.96-4.02 (m, 1H, H-5), 4.06 (dd, 1H, $J_{6,6'}$ 11.6 Hz, $J_{5,6}$ 5.1 Hz, H-6), 4.18-4.30 (m, 2H, H-1, H-6'), 4.42-4.50 (m, 1H, H-2), 4.96 (bs, 1H, NHBoc), 5.10 (dd, 1H, $J_{2,3}$ 9.7 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.30 (t, 1H, $J_{3,4}$ 3.1 Hz, H-4), 5.77-5.85 (m, 1H, NHAc); ^{13}C-NMR ($CDCl_3$) δ 20.62, 20.69, 20.79 (OAc), 23.17 (NAc), 24.80 (C-a), 25.37 (C-b), 28.32 (t-Bu), 31.41 (C-c), 32.15 (C-d), 39.83 (C-e), 48.84 (C-2), 61.46 (C-6), 66.90 (C-4), 68.23 (C-3), 68.48 (C-5), 71.89 (C-1), 79.40 (CMe_3), 170.07, 170.52, 170.79 (C=O's); FAB-HRMS (pos. m/z) calcd. for $C_{24}H_{41}N_2O_{10}S$: 549.2482; found: 549.2510 (M^+ + 1, 44.7%); Anal. Calcd for $C_{24}H_{40}N_2O_{10}S$: C, 52.53; H, 7.35; N, 5.1. Found C, 52.18; H, 7.41; N, 5.24.

Dimer with *C*-GalNAc glycoside (185).



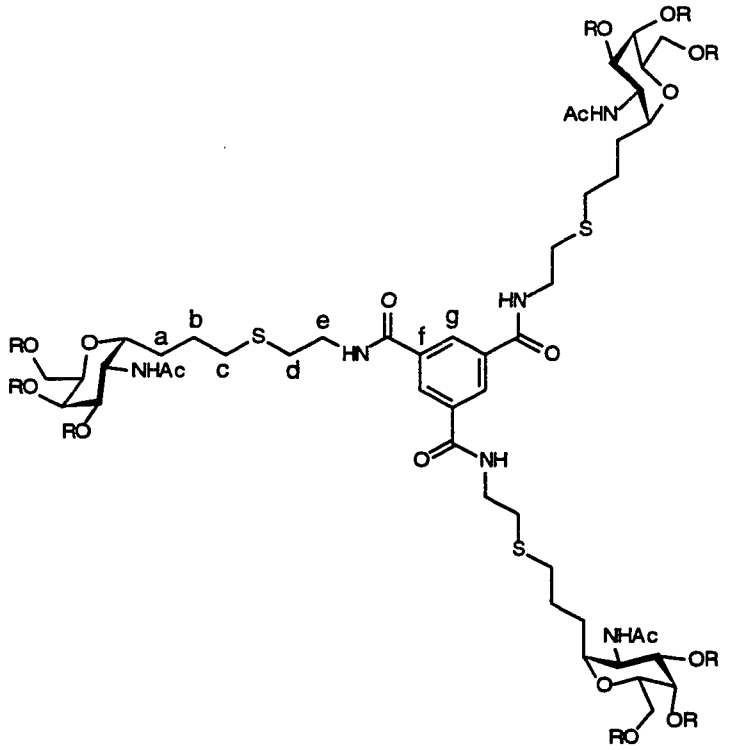
Compound **183** (0.11 g, 0.192 mmol) was treated with 20% TFA in CH_2Cl_2 (5 mL) at room temperature for 2 h and the solution was concentrated. The residual TFA was removed by co-evaporating with toluene. Commercially available isophthaloyl chloride (17.7 mg, 87.3 μmol) was refluxed with $SOCl_2$ (3 mL) for 2 h to increase the reactivity and the solution was concentrated to dryness. To a solution of amine salt in CH_2Cl_2 (10 mL) were added Et_3N (61 μL, 0.436 mmol) followed by isophthaloyl chloride

in CH_2Cl_2 (3 mL) at 0 °C. The reaction solution was allowed to stir at 0 °C for 1 h and monitored by TLC (*N*-Boc: R_f 0.68, NH_2: R_f 0.0, product: R_f 0.52, 8:1:1 $CHCl_3$/MeCN/MeOH). When the reaction was complete, the solution was washed with 5% aqueous HCl (1 × 5 mL), saturated $NaHCO_3$ (1 × 5 mL), and water (1 × 5 mL). Dried (Na_2SO_4) organic phase was concentrated under reduced pressure and silica gel column chromatography of the crude product eluting with 18:1:1 $CHCl_3$/MeCN/MeOH yielded 61 mg (68%) of an off-white foam: **184**: ^{1}H-NMR ($CDCl_3$) δ 1.50-1.58 (m, 2H, H-a'), 1.60-1.72 (m, 4H, H-b), 1.73-1.83 (m, 2H, H-a), 1.95, 2.00, 2.02, 2.07 (4s, 24H, OAc, NAc), 2.54 (quintet, 2H, J 6.5 Hz, H-c), 2.64 (quintet, 2H, J 6.7 Hz, H-c'), 2.75 (t, 4H, J 6.6 Hz, H-d), 3.56 (septaplet, 2H, J 6.7 Hz, H-e), 3.66 (setptaplet, 2H, J 6.7 Hz, H-e'), 3.88-3.94 (m, 2H, H-5), 4.02 (dd, 2H, $J_{6,6'}$ 11.7 Hz, $J_{5,6'}$ 4.9 Hz, H-6'), 4.15-4.22 (m, 2H, H-1), 4.23-4.33 (m, 2H, H-6), 4.40-4.50 (m, 2H, H-2), 5.11 (dd, 2H, $J_{2,3}$ 9.2 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.29 (t, 2H, H-4), 6.53 (d, 2H, $J_{2,NH}$ 7.8 Hz, NHAc), 7.23-7.28 (m, 2H, NH), 7.50 (t, 1H, J 7.8 Hz, H-h), 7.96 (dd, 2H, J 7.8 Hz, J 1.6 Hz, H-g), 8.29 (s, 1H, H-f); ^{13}C-NMR ($CDCl_3$) δ 20.67, 20.75, 20.88 (OAc), 23.75 (NAc), 24.98 (C-a), 25.53 (C-b), 31.67 (C-c), 31.93 (C-d), 39.22 (C-e), 48.88 (C-2), 61.39 (C-6), 66.85 (C-4), 68.42 (C-3), 68.82 (C-5), 71.50 (C-1), 125.59 (H-f), 128.95 (H-h), 130.22 (H-g), 134.55 (Ar_{ipso}), 166.65, 170.06, 170.40, 170.53, 170.66 (C=O's).

The product was then dissolved in MeOH (% mL) and 1M NaOMe was added dropwise to the solution until pH 9. As the reaction proceeded, the solution became turbid and after 6 hours de-*O*-acetylated compound precipitated out. The reaction solution was stored in the fridge overnight and the precipitate was filtered and lyophilized to afford 42 mg (92%) of an off-white powder: **185**: $[\alpha]_D$ +63.6 (*c* 0.45, DMSO); ^{1}H-NMR (D_2O) δ 1.53-1.61 (m, 2H, H-a'), 1.62-1.69 (m, 2H, H-b'), 1.72-1.81 (m, 2H, H-b), 1.82-1.91 (m, 2H, H-a), 2.06 (s, 6H, NAc), 2.66-2.78 (m, 4H, H-c), 2.90 (t, 4H, J 6.5 Hz, H-d), 3.66-3.72 (m, 6H, H-6', H-e), 3.74-3.79 (m, 4H, H-6, H-5), 3.89 (dd, 2H, $J_{2,3}$ 10.9 Hz, $J_{3,4}$ 3.3 Hz, H-3), 3.96 (d, 2H, H-4), 4.13 (ddd, 2H, $J_{1,a}$ 10.5 Hz, $J_{1,a'}$ 4.6 Hz, $J_{1,2}$ 5.8 Hz, H-1), 4.25 (dd, 2H, H-2), 7.70 (t, 1H, J 7.9 Hz, H-i), 8.01 (dd, 2H, J 7.8 Hz, J 1.8 Hz, H-h), 8.19 (t, 1H, H-g); ^{13}C-NMR (D_2O) δ 21.41 (NAc), 22.75 (C-a), 24.34 (C-b), 29.94 (C-d), 30.26 (C-c), 38.90 (C-e), 49.32 (C-2), 60.78 (C-6), 66.90 (C-3), 67.96 (C-4), 71.09 (C-5), 72.56 (C-1), 125.40 (H-g), 128.89 (H-i), 129.92 (H-h), 133.73

(H-f), 169.25, 173.96 (C=O's); FAB-HRMS (pos. m/z) calcd. for $C_{34}H_{55}N_4O_{12}S_2$: 775.3258; found: 775.2724 (M^+ + 1, 4.7%).

Trimer with *C*-GalNAc glycoside (187).



The title compound was prepared using the same method used as for the preparation of dimer **184** described previously (83% yield): **186**: ^{1}H-NMR ($CDCl_3$) δ 1.55-1.83 (m, 12H, H-a', H-b, H-a), 1.96, 2.00, 2.03, 2.07 (4s, 36H, OAc, NAc), 2.53-2.67 (m, 6H, H-c), 2.70-2.82 (m, 6H, H-d), 3.47-3.57 (m, 3H, H-e'), 3.67-3.78 (m, 3H, H-e), 3.90-3.96 (m, 3H, H-5), 4.04 (dd, 3H, $J_{6,6'}$ 11.7 Hz, $J_{5,6}$ 4.8 Hz, H-6), 4.15-4.22 (m, 3H, H-1), 4.23-4.33 (m, 3H, H-6'), 4.45-4.53 (m, 3H, H-2), 5.15 (dd, 3H, $J_{2,3}$ 9.3 Hz, $J_{3,4}$ 3.1 Hz, H-3), 5.32 (t, 3H, H-4), 6.89 (b, 3H, NHAc), 7.76 (b, 3H, NH), 8.50 (s, 3H, H-g); ^{13}C-NMR ($CDCl_3$) δ 20.69, 20.77, 20.94 (OAc), 23.07 (NAc), 25.10 (C-a), 25.84 (C-b), 32.02 (C-d, C-c), 39.50 (C-e), 48.77 (C-2), 61.48 (C-6), 66.88 (C-4), 68.53 (C-3), 68.70 (C-5), 71.84 (C-1), 128.75 (C-g), 134.88 (C-f), 165.88, 170.14, 170.68, 170.72, 170.78 (C=O's).

De-*O*-acetylation was done under Zemplén conditions and the precipitate was filtered and lyophilized to afford a white foam (90%): **187**: $[\alpha]_D$ +49.0 (*c* 0.2, DMSO); FAB-HRMS (pos. m/z) calcd. for $C_{48}H_{79}N_6O_{18}S_3$: 1123.4613; found 1123.4632 (M^+ + 1, 0.4%).

Chapter 8. Comparitive binding studies of di- and tetravalent GalNAc ligands having different scaffolding backbones

8.1. Introduction

The previous chapters have described the effective direct bindings and inhibitory properties of neoglycoconjugates in their individual biological assays. As illustrated in Figure 1.2.3, the various glycoconjugates have different shapes and geometries through variations in bond angles and aglycon spacers. Optimization of these variables could create potent ligands with high inhibitory properties. Moreover, the design of small molecules with optimized geometric factors that have better carbohydrate-protein interactions would provide ideal candidates for potential therapeutic uses. In this chapter, the biological properties of previously synthesized dimers and tetramers that have different shapes and aglycon spacers were compared.

8.2. Binding properties

Turbidimetric analyses of tetramers

The tetramers tested herein included glycopeptoid **57**, (bipy-GalNAc)$_2$Cu•SO_4 with short spacer arm **126**, and glycocalix[4]arene **139**. These tetrameric ligands were incubated with lectin VVA resulting in the formation of insoluble precipitates over the course of time (Figure 8.2.1). As expected, the copper-coordinated complex exhibited the best cross-linking property followed by calix[4]arene tetramer. The peptoid did not have the correct geometry for cross-linking with lectin probably due to the short aglycon spacer as well as steric crowding.

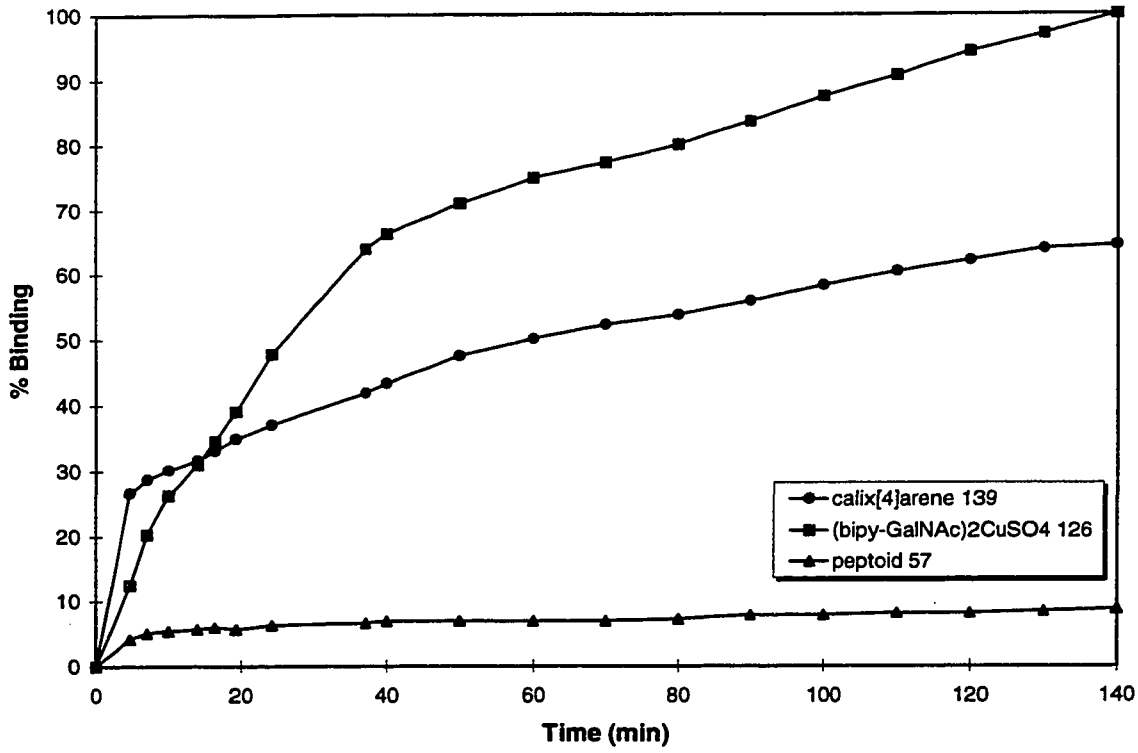


Figure 8.2.1. Turbidimetric analyses of tetramers **57** (peptoid), **129** (Cu^{II} complex), **139** (calix[4]arene).

ELLA inhibition of glycopolymer to lectin VVA B_4 by dimers and tetramers

The efficiency of each ligand to inhibit the binding of GalNAc-containing glycopolymer to *vicia villosa* B_4 was measured by ELLA. GalNAc-containing polymer **164** (1.0 μg/well) was coated on the microtiter plates and a mixture of tetramers and and horseradish peroxidase-labeled *VVA* was added for competition. The results of the assays were shown in Figure 8.2.2, Figure 8.3.3. Among a series of tetramers, the best result was obtained with copper(II) coordinated GalNAc tetramer. An IC_{50} of 2.6 μM was measured, representing a 192-fold-increase over that of monomeric allyl α-D-GalNAc. On a per-GalNAc basis, the inhibitory potential of this complex was increased by 48 times in comparison to that of the monomer.

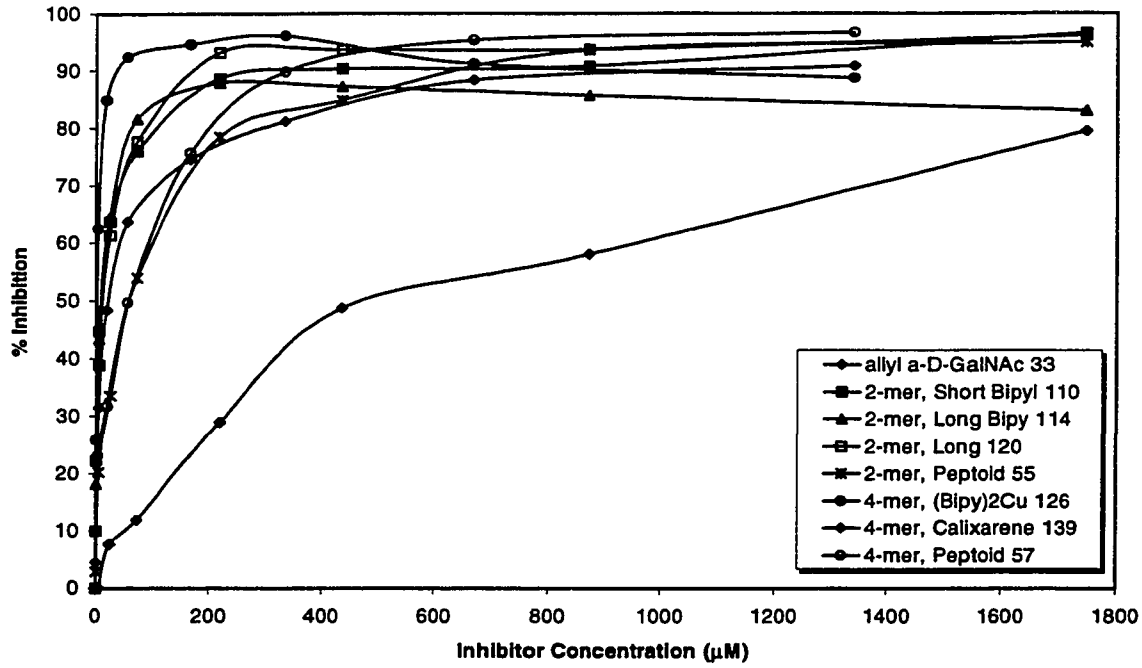


Figure 8.2.2. ELLA inhibition of binding of GalNAc-containing polymer to VVA/HRP by dimers (**55**, **110**, **114**, and **120**) and tetramers (**57**, **126**, and **139**).

Table 8.2.1. IC_{50}'s of dimers and tetramers.

GalNAc ligands	M.W.	IC_{50}'s (μM)	Relative potency[a]
Dimers			
Allyl α-D-GalNAc **33**	261.12	500.0	1
Peptoid **55**	668.28	63.2	7.9 (4.0)
Short bipyridyl **110**	736.32	10.2	49.0 (24.5)
Long bipyridyl **114**	962.49	11.0	45.5 (22.8)
Branched **120**	922.52	14.4	34.7 (17.4)
Tetramers			
Peptoid **57**	1276.53	56.6	8.8 (2.2)
$(Bipy)_2CuSO_4$ **126**	1632.19	2.6	192.3 (48.1)
Calix[4]arene **139**	1887.92	23.1	21.6 (5.4)

[a] Values in parentheses are given on a per-hapten basis in a molecule.

A series of dimers were also tested in ELLA experiments, which included peptoid **55**, short and long spacer armed bipyridyl dimers (**110** and **114**, respectively) and branched dimer **120**. These dimers exhibited IC_{50} values of 63.2, 10.2, 11.0, and 14.4 μM, respectively. Dimers with long spacer arms and greater bond angles were more accessible for binding to lectin VVA. These data confirm previous findings that the aglycon spacer and geometry determine the efficiency of binding in carbohydrate-protein interactions.

Claims to original research

1. Conformatinally flexible glycopeptidomimetics, "glycopeptoids", were synthesized using a strategy based on the reiterative scaffolding of a key structure. The synthesized glycopeptoid clusters included GalNAc-valencies of between two and eight in a linearly arranged manner.

2. New prototypical *O*-linked-glycopeptidomimetics based on peptoids were synthesized mimicking the β-D-Xyl-(1→3)-*O*-L-Ser linkage region of proteoglycans. These glycopeptoids contained Val-Phe-Ser-(β-D-Xyl)-Glu-Ala and Ala-Ser-(β-D-Xyl)-Gly-Ala mimics.

3. Phase transfer catalyzed anomeric nucleophilic substitutions of peracetylated α-D-xylopyranosyl bromide, and α- and β-chlorides were investigated using a series of nucleophiles to demonstrate that these anomeric substitutions occurred with complete anomeric inversion.

4. Symmetrical GalNAc-containing clusters and dendrimers were synthesized by employing a rapid facile self-assembling approach. Relatively simple divergent building blocks (dendrons) were prepared on the bipyridyl core in a convergent manner and then associated with metal ions such as Fe^{II} and Cu^{II} to furnish tetra-, hexa-, octa-, and dodecavalent GalNAc ligands.

5. A structurally defined calix[4]arene core was used to synthesize cone shaped glycodendrimers with valencies of between four and sixteen. *t*-Butyl groups on the lower rim of the calix[4]arene core served as a primary structure for binding on the hydrophobic surface. This property of glycocalix[4]arenes allowed this type of glycodendrimers to serve as a coating antigen in solid phase immunoassays.

6. Symmetrical, spherical GalNAc-containing glycodendrimers were synthesized based on Starburst® PAMAM dendritic core. The *N,N'*-Dialkylation strategy was adopted to prepare 32-valent glycodendrimers by conjugating bromoacetylated GalNAc ligands to the amine-terminated PAMAM (G2) core.

7. Glycopolymer bearing GalNAc residues were prepared by conjugating amine terminated GalNAc ligands to pre-activated poly(N-acryloxysuccinimide). These glycopolymers were employed in the solid phase immunoassay as excellent coating antigens.

8. An Enzymetically non-hydrolyzable *C*-glycoside mimetic of GalNAcα-*O*-Ser was synthesized *via* a radical *C*-glycosylation process. The resulting α-linked GalNAc *C*-glycoside was conjugated to a rigid aromatic core to afford di- and trivalent clusters.

9. In biological evaluations, glycoclusters and glycodendrimers exhibited enhanced inhibitory potentials to variable extents. Many were highly inhibitory, others were less inhibitory than the monomer. Thus, multivalency is very significant in carbohydrate-protein interactions but, the geometry and the density of the clusters are also significant. Considering various factors studied in this dissertation, the octahedral iron-coordinated dodecamer with a long spacer arm (13 carbons) (**133**) showed the most efficient inhibitory power.

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