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"Synthetic Studies
on the Lactosaminoglycan Models"
Functionalization of Lactose
as an Unused Disaccharide Resource.

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**"Synthetic Studies
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Functionalization of Lactose
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Abstract

Oligosaccharide resources can be utilized as fine materials to construct further complicated and biologically important glycoconjugates. In addition to the known oligosacchride resources such as cellobiose, maltooligosacchrides, and *N,N'*-diacetylchitobiose, lactose which is obtainable from whey in large amount should be also highly functionalized and applied for glycoconjugate synthesis. Since this disaccharide contains a precursor of *N*-acetylactosamine structure known as an important unit of complex carbohydrates, chemical conversion of lactose to further functional synthons for oligosacchride synthesis seems to be powerful and versatile strategy for glycosciences.

The thesis is composed of six chapters. The first chapter describes general introduction to the significance of molecular recognition in nature based on the interaction of carbohydrates and proteins. In addition, an importance of cluster effects in sugar-protein interaction is described. In order to investigate the significance and mechanism of the cluster effects, we have to prepare some appropriately designed sugar-ligands. For this purpose, use of lactose as a key starting material might facilitate the synthetic procedure of new clustered model-ligands.

In order to achieve more effective methodology to design "high density cluster ligands", it might be necessary to synthesize novel type of polymerizable

sugar derivatives. Thus, in the second chapter of this thesis, a simple and efficient method for the syntheses of clustering-sugar homopolymers from ω -acryloylaminoalkyl glycosides of *N*-acetyl- β -D-glucosamine (Glc_pNAc) as a simple model of sugar moiety was discussed in order to produce highly clustered glycoprotein models.¹⁻⁵ Apparent association constants of wheat germ agglutinin (WGA) with a variety of clustered glycopolymers were determined and evaluated by measuring the change in fluorescence intensity produced by various concentrations of polymeric ligand. It was suggested that addition of the clustered glycopolymer to WGA induced much greater enhancement of the fluorescence intensity and a significant blue shift of the fluorescence emission maximum of WGA than did addition of the low-density glycopolymer derived from *n*-pentenyl type.

In the third chapter, the syntheses of cluster-type homopolymers from ω -acryloylaminoalkyl glycosides of *N*-acetylactosamine [β -D-Galp-(1 $\rightarrow$ 4)- β -D-Glc_pNAc] derived from lactose were discussed in relation to the easy determination study of sugar-binding specificity of fluorescein isothiocyanated *Erythrina Corallodendron* lectin (FITC-ECorL).⁶ It was clearly demonstrated by using these polymers that fluorometric titration of FITC-ECorL with a variety of LacNAc polymers revealed an importance of the suitable spacing of the inter-sugar residues on the glycoprotein models for the efficient interaction with the binding sites of lectins

Since multivalency of glycoprotein-sugar moiety has not been synthesized on the basis of macromolecular models, attention is next directed towards design of triantennary polymeric sugar ligands using acrylamide type aglycon. Thus, in the chapter 4, an efficient synthesis of a new glycoprotein model having clustered triantennary *N*-acetyllactosamine (LacNAc) using 4-nitro-4-[1-(3-hydroxypropyl)]-1,7-heptandiol as a key starting material was examined.⁷ Using the Newkome's derivative, several model glycopolymers having different degrees of density of triantennary LacNAc residues were successfully prepared.

Finally, in the chapter 5, for the easy and versatile chemical synthesis of oligosaccharide moieties of branching lactosaminoglycan model, new approaches of chemical modification of lactose were described. Use of 1,6-anhydro- β -lactose as a starting material was proved to establish synthetic strategy that permits the syntheses of a series of lactosaminoglycan models, since all hydroxyl groups of 1,6-anhydro- β -lactose can be distinguished by chemical modifications.⁸

Concluding remarks will be described in Chapter 6.

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Abbreviations

Ac	Acetyl
All	Allyl
APS	Ammonium persulfate
Bn	Benzyl
CHCl ₃	Chloroform
CH ₂ Cl ₂	Dichloromethane
CDCl ₃	Chloroform- <i>d</i>
CSA	10-Camphorsulfonic acid
DMAP	4-Dimethylaminopyridine
DMF	<i>N,N'</i> -Dimethylformamide
DMSO	Dimethylsulfoxide
DMSO- <i>d</i> ₆	Dimethylsulfoxide- <i>d</i> ₆
D ₂ O	Deuterium oxide
DSS	3-(trimethylsilyl)-propanesulfonic acid sodium salt
ECorL	<i>Erythrina Corallodendron</i> lectin
Et	Ethyl
FITC	fluorescein isothiocyanate
Gal	D-Galactose
GalNAc	<i>N</i> -Acetyl-D-galactosamine
Glc	D-Glucose
GlcNAc	<i>N</i> -Acetyl-D-Glucose
GlcNPhth	<i>N</i> -Phthaloyl-D-Glucose
GPC	Gel permeation chromatography
Lac	Lactose: Galβ(1→4)Glc
LacNAc	<i>N</i> -Acetylactosamine: Galβ(1→4)GlcNAc
LacNPhth	<i>N</i> -Phthaloyllactosamine: Galβ(1→4)GlcNPhth
Le ^x	Lewis X: Galβ(1→4)[Fucα(1→3)]GlcNAc
Man	D-Mannose
Me	Methyl
MS	Molecular sieves
Neu5Ac	<i>N</i> -Acetylneuraminic acid
NMR	Nuclear magnetic resonance
PBS	Phosphate buffered saline
Pent	<i>n</i> -Pentenyl
Phth	Phthaloyl
TEMED	<i>N,N,N',N'</i> -Tetramethylethylenediamine
THF	Tetrahydrofuran

TIPBS	Triisopropylbenzenesulfonyl
TLC	Thin layer chromatography
TMS	Tetramethylsilane
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
Tr	Triphenylmethyl
TSP	3-(Trimethylsilyl)propanesulfonic acid sodium salt
WGA	Wheat germ agglutinine

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Dedication

I would like to dedicate this dissertation to my parents, Suekiyo and Setsuko Furuike.

Chapter 1

1.1. General Introduction

A water-soluble component except for lipid, casein and lipid-soluble vitamin from milk is referred to whey or milk serum. A main-component of the whey is lactose, comprising 70% of the all component. However, although it is also obtained a few hundred million ton per year as by-product for production of cheese and casein in the world, the purpose other than food-materials or fodder is little known. Therefore, fine use of this abundant material has long been expected to open new area of bio-resources.

On the other hand, with a remarkable development of cell biology and molecular biology, the study for a multiplicity of functions of glycoconjugates containing carbohydrate chains existing on the cell surfaces as shown in Figure 1 is rapidly in progress. It is considered that these sugar chains mainly play an important role as carriers of the specific information with regard to cell-cell interaction *in vivo*.^{1,2} For example, a specific sugar-sugar interaction in addition to sugar-protein interaction has been recently reported by Hakomori et al..³⁻⁵ This basic concept will be greatly applicable for elucidation of the various phenomena related to glycoconjugates such as fertilization, differentiation, aging, malignant alterations, and so on.

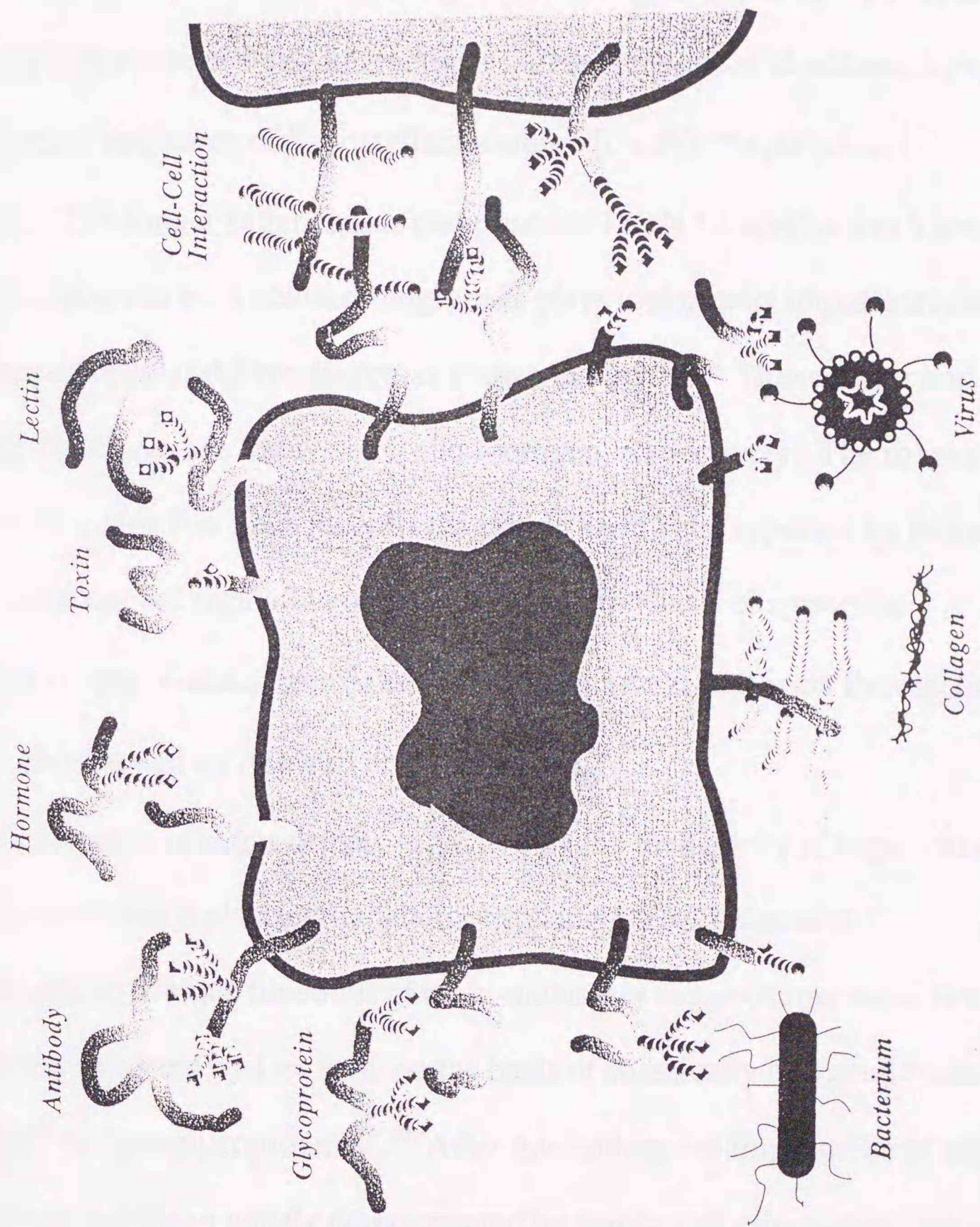


Figure 1. Cell Surface Carbohydrate Interactions

The asparagine-linked type glycoproteins in these glycoconjugates existing on the cell surfaces are known as multi-antennary chains such as high mannose-, complex-, and hybrid-type structures as indicated in Figure 2. It is suggested that the branching structure greatly participates with the appearance in a variety of specific cell-cell interactions. All these types of structures have a common sugar sequence of *N*-acetyllactosamine [$\text{Gal}\beta(1\rightarrow4)\text{GlcNAc}$] (LacNAc). The longer sugar chains composed of LacNAc residue are known as lactosaminoglycans. Lactosaminoglycans plays biologically important role in the process of cellular differentiation as shown in Figure 3.⁶ These linear and branching structures are known as i- and I-antigen, respectively. The roles as carriers of receptor functions for cell recognition have been reported by Fukuda et al.⁷ In addition, the regulation of endocytosis on the basis of a specific recognition of this *N*-acetyllactosamine structure, as a receptor on liver lectin, was also investigated by Ashwell et al. (Figure 4).⁸

A significance of sugar density regulated by the multiplicity of sugar chains on the glycoproteins is also spotlighted for explaining bio-functions of glycoconjugates. Unique functions of multi-antennary sugar chains were first proposed and reported by Lee et al. on the basis of chemically designed "cluster glycosides"⁹ or "neoglycoproteins".¹⁰ After this finding, the importance of sugar cluster effects has been widely demonstrated by using synthetic multivalent ligands and neoglycoconjugates.¹¹ For example, a typical cluster effect was demonstrated as shown in Figure 5 by means of isolated some multivalent

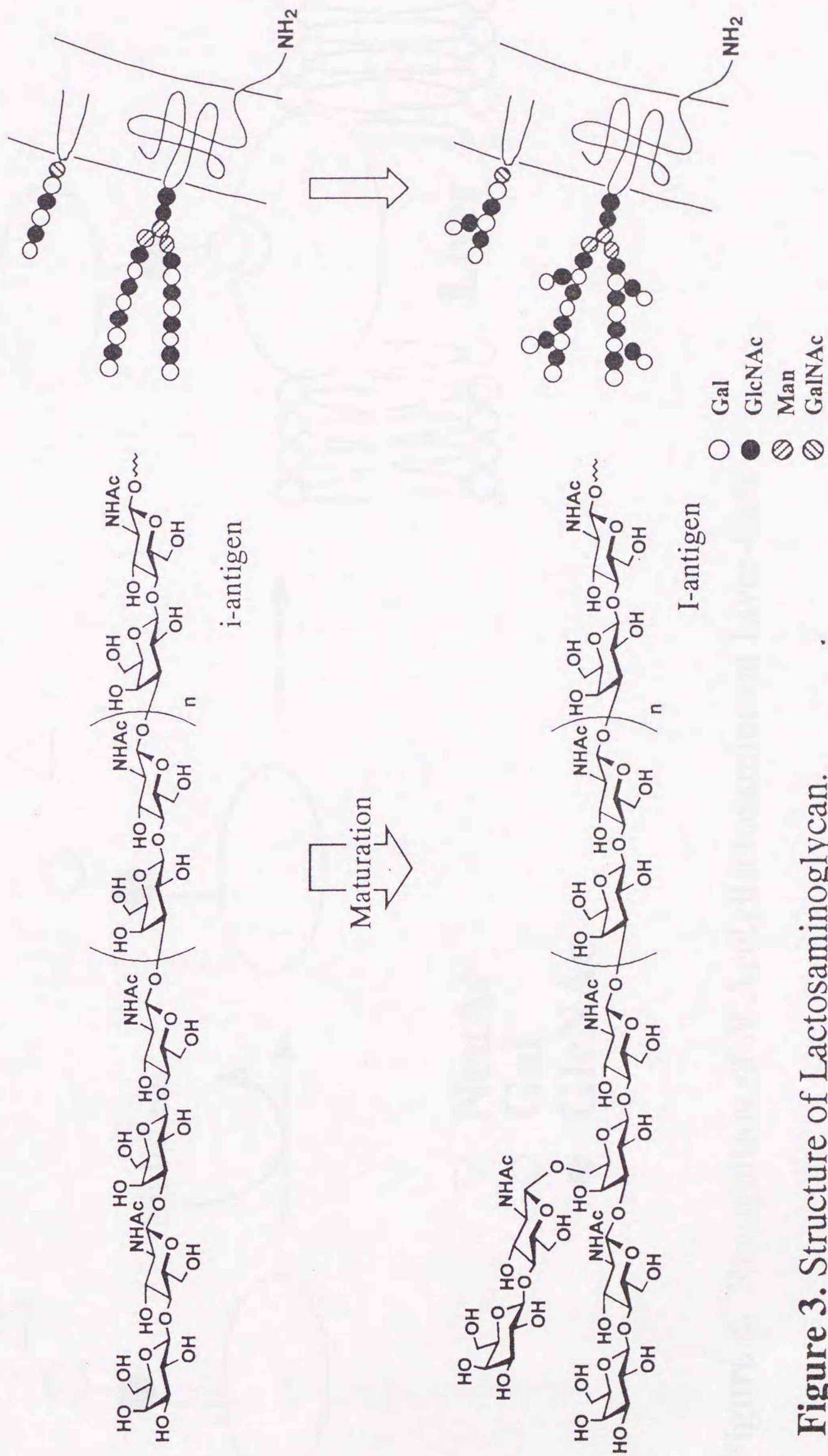


Figure 3. Structure of Lactosaminoglycan.

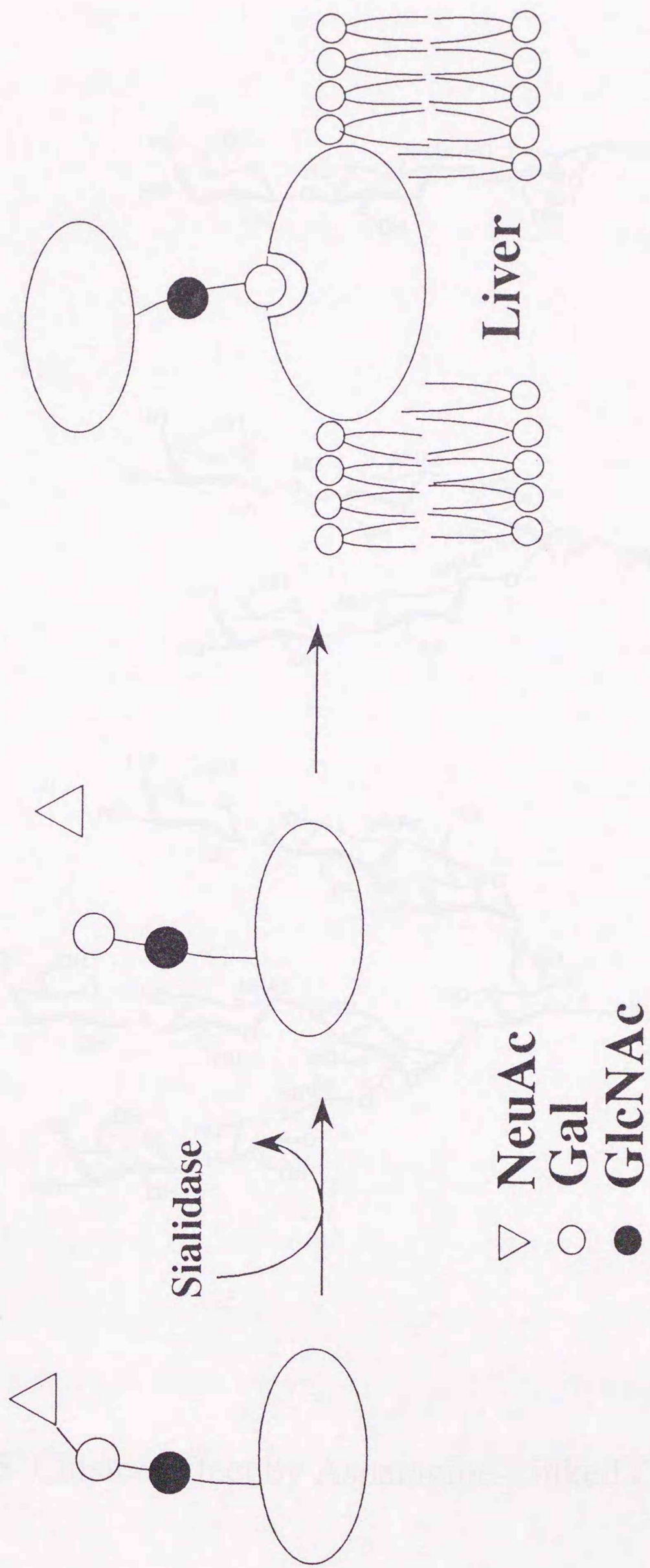


Figure 4. Recognition of N-Acetylglucosamine on Liver-Cell.

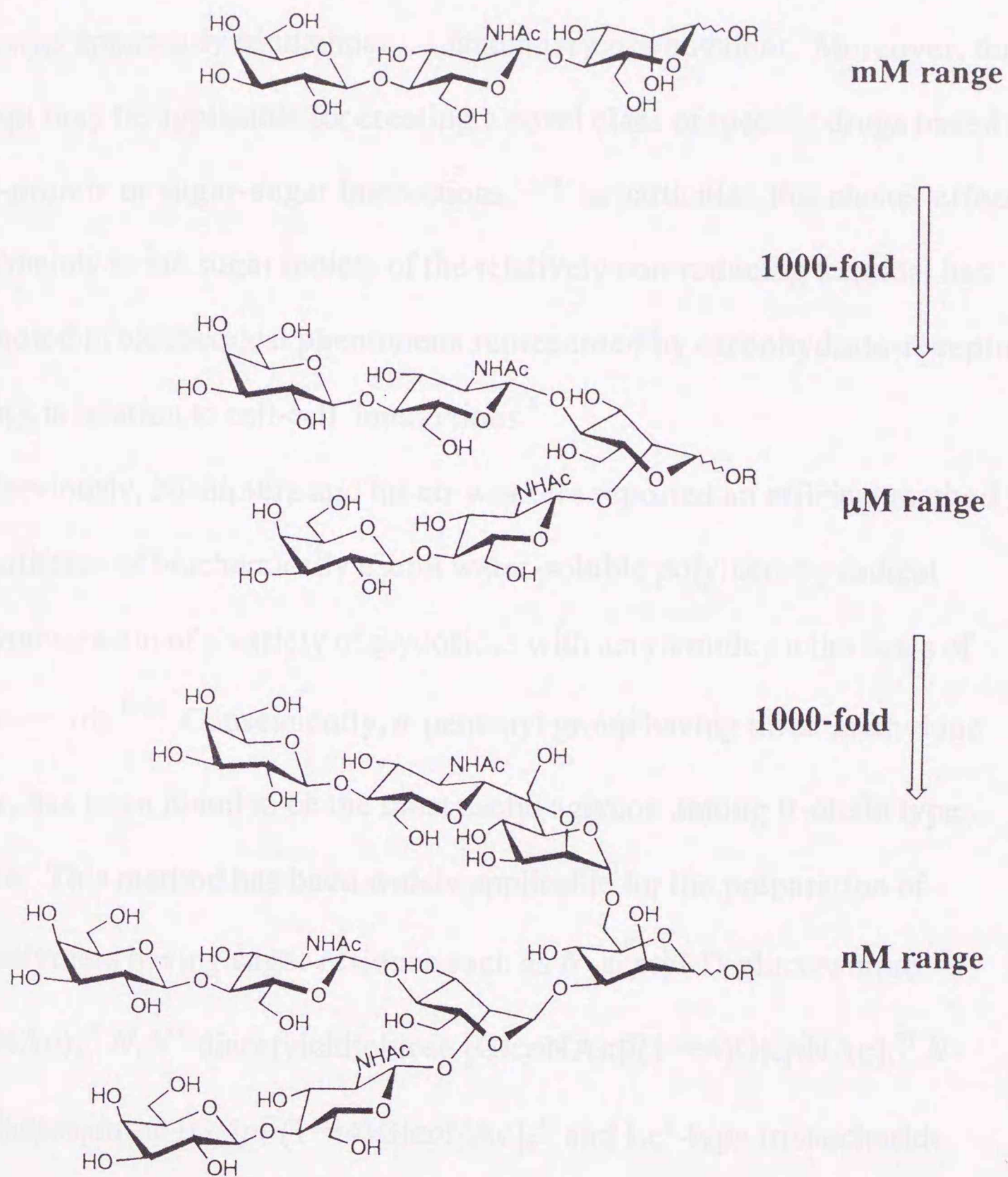


Figure 5. Cluster Effect by Asparagine-Linked Oligosaccharides.

ligands which have mono-, bi-, and triantennary *N*-acetylactosamine, respectively.¹² The order of the binding constant of these oligosaccharides with lectin was apparently triantennary>biantennary>monovalent. Moreover, this concept may be applicable for creating a novel class of specific drugs based on sugar-protein or sugar-sugar interactions.^{13, 14} In particular, this cluster effect found mainly in the sugar moiety of the relatively non-reducing terminal has been noted in biochemical phenomena represented by carbohydrate-receptor bindings in relation to cell-cell interactions.⁹

Previously, Nishimura and his co-workers reported an efficient method for the syntheses of biochemically useful water-soluble polymers by radical copolymerization of a variety of glycosides with acrylamide on the basis of spacer-length.¹⁵⁻¹⁸ Consequently, *n*-pentenyl group having three methylene anchor, has been found to be the most useful aglycon among α -olefin type spacers. This method has been widely applicable for the preparation of glycopolymers having sugar residues such as *N*-acetyl-D-glucosamine (Glc_pNAc),¹⁶ *N,N'*-diacetylchitobiose [Glc_pNAc β (1 $\rightarrow$ 4)Glc_pNAc],¹⁷ *N*-acetylactosamine [Gal_p β (1 $\rightarrow$ 4)Glc_pNAc],¹⁷ and Le^x-type trisaccharide {Gal_p β (1 $\rightarrow$ 4) [Fuc_p α (1 $\rightarrow$ 3)]Glc_pNAc} known as a sequence of tumor-associated carbohydrate antigen.¹⁸ It was also demonstrated that chemically synthesized glycopolymers are efficiently modified with enzymes to afford sialic acid-containing glycoconjugates.¹⁹ Using a variety of *N*-acetylactosamine

related polymers from *n*-pentenyl glycosides, it was clearly suggested that *Erythrina Corallodendron* lectin specifically recognizes $\beta(1\rightarrow4)$ linkage between galactose and *N*-acetyl-D-glucosamine and structural isomers such as $\beta(1\rightarrow3)$ - and $\beta(1\rightarrow6)$ -type models showed much lower affinity to the lectin than native lactosamine structure.¹⁵

Although *n*-pentenyl glycosides were useful for the preparation of "simple and low sugar-density ligands", higher sugar-density ligands could not be prepared owing to the inert reactivity of α -olefin structure. In order to achieve more effective procedure to design "high density cluster ligands", it might be necessary to synthesize novel type of polymerizable sugar derivatives. Thus, in the second chapter of this thesis, a simple and efficient method for the syntheses of clustering-sugar homopolymers from ω -acryloylaminoalkyl glycosides of *N*-acetyl- β -D-glucosamine (Glc_pNAc) as a simple model of sugar moiety will be discussed in order to produce highly clustered glycoprotein models.¹¹ Moreover, as an example of the availability of these compounds, the apparent association constants of wheat germ agglutinin (WGA) with a variety of clustered polymers are determined and evaluated by measuring the change in fluorescence intensity produced by various concentrations of polymeric ligand.

In the next chapter, as an application to lactosaminoglycan related oligosaccharides of ω -acryloylaminoalkyl derivatives obtained in the second chapter, a simple and facile method for the syntheses of cluster-type

homopolymers from ω -acryloylaminoalkyl glycosides of *N*-acetyllactosamine [β -D-Galp-(1 $\rightarrow$ 4)- β -D-GlcpNAc] derived from lactose will be discussed in relation to the easy determination study of sugar-binding specificity of *Erythrina Corallodendron* lectin (ECorL)

Since multivalent ligandxc of glycoprotein-sugar moiety has not been synthesized on the basis of macromolecular models, attention is next directed towards design of triantennary polymeric sugar ligands using acrylamide type aglycon. Although useful methods for the synthesis of low molecular weight of "triantennary glycosides" having galactose residues were reported using tris(hydroxymethyl)aminomethane by Lee et al.¹¹ and Polidori et al.,²⁰ there is no attempt to prepare polymers bearing clustered "triantennary glycoside" which is expecting to show specific affinity with liver lectins. Thus, in chapter 4, an efficient synthesis of a new glycoprotein model having clustered triantennary *N*-acetyllactosamine (LacNAc) using 4-nitro-4-[1-(3-hydroxypropyl)]-1,7-heptandiol²¹⁻²³ as a key starting material will be demonstrated.

In the case of chemical synthesis of oligosaccharide moieties of branching lactosaminoglycan model, a class of important target molecules from lactose, an efficient and selective chemical modification of lactose must be considered. Use of 1,6-anhydro- β -lactose as a starting material was previously reported by Kuzuhara et al.²⁴ and Tejima et al..²⁵⁻²⁶ Moreover, Nishimura et al. demonstrated using this compound as a key starting material that an important LewisX

trisaccharide can be synthesized through an easy manipulations of this conformationally regulated intermediate.^{18, 27} So, synthetic stragedy using the 1,6-anhydro- β -lactose will permit the syntheses of a series of lactosaminoglycan models, because all hydroxyl groups of 1,6-anhydro- β -lactose are supposed to be distinguished by chemical modifications. Thus, in chapter 5, simple and much more useful methods for synthesis of lactosaminoglycan models using 1,6-anhydrolactose as a starting material will be described.

In the series of this study, new methods for creating highly functional polymeric sugar-ligands and key intermediates of lactosaminoglycans are established. Especially, it should be noted that an unused disaccharide resource, lactose, would be utilized as an important bio-material to produce valuable bio-probes and modulators for investigating biological roles of glycoconjugates.

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Chapter 2

Lactosamine-Based Glycopolymers(1)

---Evaluation of the Functional Aglycons for the Preparation of Glycopolymer-Ligands

The simple and efficient methods for the syntheses of *n*-pentenyl and ω -acryloylamimoalkyl glycosides of *N*-acetyl- β -D-glucosamine (Glc_pNAc) has been demonstrated. Radical polymerization of the new glycosides proceeded smoothly in aqueous solution in the presence of ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine as initiator and gave water-soluble glycopolymers. Homopolymerization of the ω -acryloylamimoalkyl glycosides also gave homopolymers having high-density sugar branches as a novel class of cluster glycosides. The apparent association constant of wheat germ agglutinin (WGA) with poly[3-(*N*-acryloylamino)propyl 2-acetamido-2-deoxy- β -D-glucopyranoside] was determined by measuring the change in fluorescence intensity produced by various concentrations of polymeric ligand, and found to be approximately 10^8 M^{-1} . Addition of the cluster type Glc_pNAc-polymer to WGA induced much greater enhancement of the fluorescence intensity and a significant blue shift of the fluorescence emission maximum of WGA than did

addition of the low density GlcNAc-polymer derived from *n*-pentenyl glycoside.

2.1. Introduction

The significance of sugar density on glycoproteins in the specific interaction between oligosaccharide chains and receptors was first proposed and reported by Lee et al. on the basis of chemically designed "cluster glycosides"¹ or "neoglycoproteins".² They have elegantly demonstrated for the galactose/*N*-acetylgalactosamine-hepatocyte binding systems that a "cluster" or "multipoint" sugar-ligand interaction may be involved in the successful binding process.³ Actually, this cluster theory has been recently shown to be applicable to a number of carbohydrate-receptor bindings in relation to cell-cell interactions. For example, it was reported that some of inhibitors containing multivalent-type *N*-acetylneuramic acid showed markedly amplified inhibitory effects on hemagglutination by influenza virus.⁴ Furthermore, it was also found that bivalent-type sialyl LewisX (SLe^x) exhibited much higher affinity to the specific receptor proteins, selectin families.⁵

In this chapter, an efficient method for the syntheses of biochemically useful glycoprotein models having pendant oligosaccharides based on the radical copolymerization of *n*-pentenyl glycosides with acrylamide will be first found by using *N*-acetylglucosamine as sugar moiety.^{6,7} On the other hand, molecular design of water-soluble polymers containing sugar side chains is of gaining interest as one of the most effective method to increase the density of

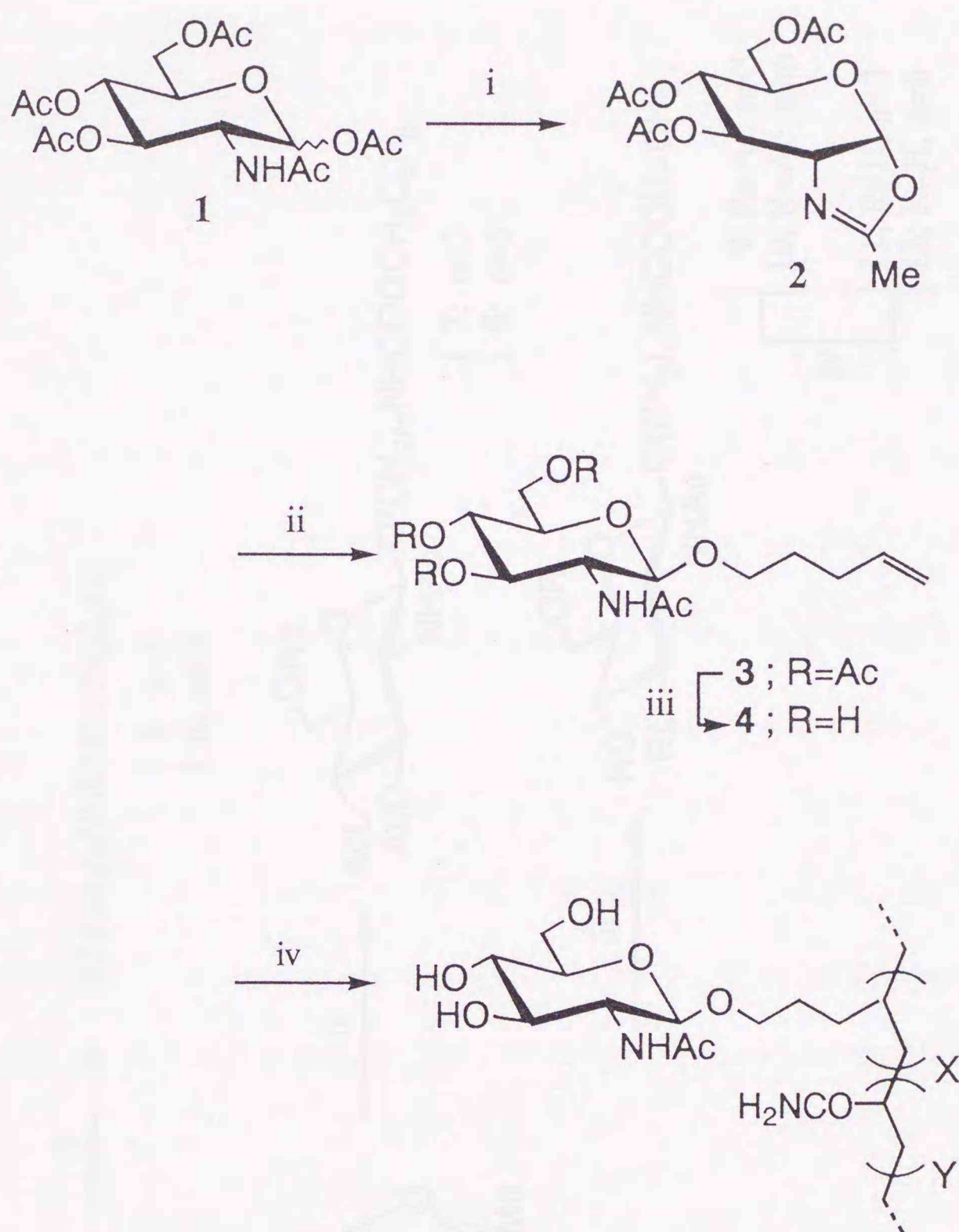
carbohydrate moiety. High density sugar-ligands based on the polymerizable glycosides seem to be one of the most effective and potential reagents not only for biomimetic models of glycoconjugates but also for therapeutic or diagnostic purposes in biomedical fields. Thus, the preparation of homopolymers from excellent polymerizable glycomonomers might serve high density and clustering carbohydrate-ligands. Although the *n*-pentenyl glycosides have been proved to be versatile polymerizable glycosides for design of a variety of glycoconjugate models and the sugar contents in the copolymers could be adjusted by the feed ratio of polymerizable glycosides and acrylamide as needed, homopolymers from these ω -alkenyl type glycosides could not be obtained under this reaction condition owing to the innate low chemical reactivity of simple olefin-type aglycons.⁷ So, a facile and efficient method for the preparation of a new type of sugar-homopolymers, "cluster glycopolymers", from highly reactive glycosides having ω -acryloylaminoalkyl-type aglycons is demonstrated in the present chapter. Moreover, as preliminary studies for an biochemically elucidation of sugar cluster effect, the specific interaction of this cluster glycopolymer with wheat germ agglutinin (WGA), a plant lectin which agglutinates erythrocytes and other types of cells through binding with oligosaccharides containing *N*-acetyl- β -D-glucosamine (Glc p NAc),⁸ will be also preliminarily discussed on the basis of a spectrofluorometric investigation.

2.2. Results and Discussion

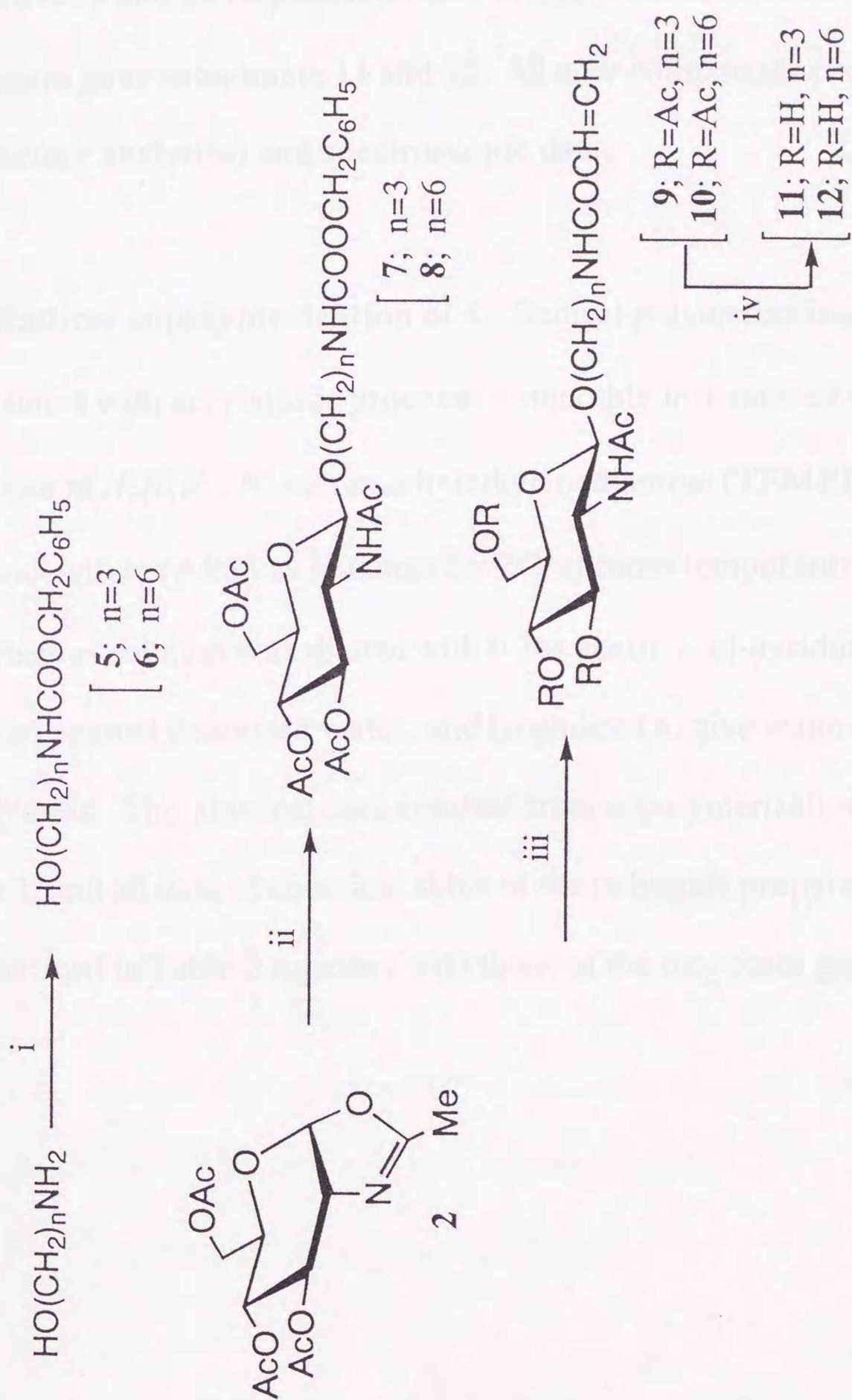
Synthesis of *n*-pentenyl glycosides and their polymers. An oxazoline derivative **2**, prepared from GlcpNAc peracetate **1** by using trimethylsilyl trifluoromethanesulfonate (TMSOTf)⁹ was allowed to react with 4-penten-1-ol as polymerizable alcohol in the presence of 10-camphorsulfonic acid (CSA) as the promoter and gave *n*-pentenyl glycoside **3** in high yields (Scheme 1). *O*-deacetylation of **3** by the Zemplen method afforded polymerizable glycoside **4** in quantitative yield. All new compounds prepared here gave satisfactory analytical and spectroscopic data.

Synthesis of ω -acryloylaminoalkyl glycosides and their polymers.

The Scheme 2 indicates the synthetic route of GlcpNAc derivatives **11** and **12**¹⁰ as tentative candidates of the reactive model monomers with appropriate spacer-arm structure for the polymerization of a variety of oligosaccharides containing GlcpNAc residue at the reducing end. Initially, precursors of polymerizable aglycons, *N*-protected simple aminoalcohols, 3-(*N*-benzyloxycarbonylamino)propan-1-ol **5** and 6-(*N*-benzyloxycarbonylamino)hexan-1-ol **6**,¹⁰ were prepared. Coupling reactions of oxazoline derivative **2** with *N*-protected aminoalcohols **5** and **6** proceeded smoothly in the presence of CSA



Scheme 1. Reagents and conditions: i) TMSOTf, Et₃N / ClCH₂CH₂Cl, 50°C, 15 h; ii) HO(CH₂)₃CH=CH₂, CSA / ClCH₂CH₂Cl, 90°C, 2 h; iii) NaOMe / MeOH, r.t., 3 h; iv) CH₂=CHCONH₂, TEMED, APS / H₂O, 25°C, 4 h.

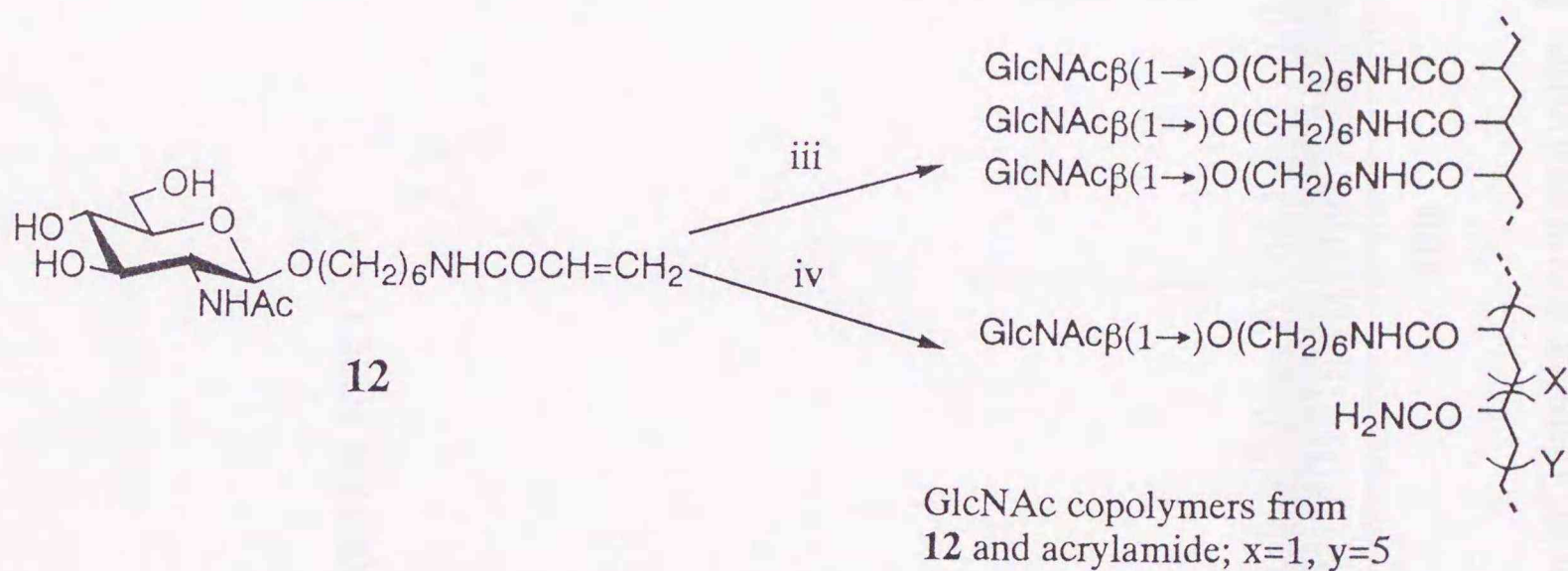
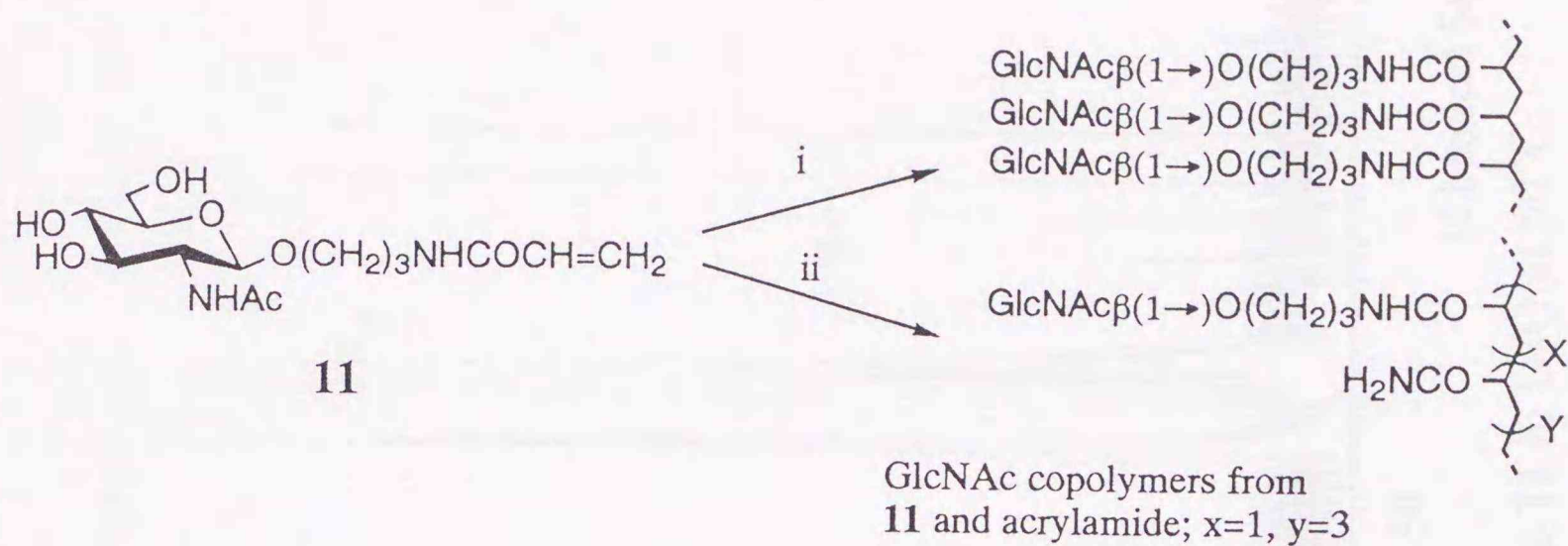


Scheme 2. Reagents and conditions : i) $\text{C}_6\text{H}_5\text{CH}_2\text{OCOCl}$, NaHCO_3 aq., r.t., 3 h; ii) **5** or **6**, CSA / $\text{ClCH}_2\text{CH}_2\text{Cl}$, 90°C , 2 h; iii) H_2 , Pd-C / MeOH , r.t., 2 h; then, $\text{CH}_2=\text{CHCOCl}$, Et_3N / THF , $0 \rightarrow 25^\circ\text{C}$, 24 h; iv) NaOMe / MeOH , r.t., 3 h.

as the promoter and gave the corresponding intermediates **7** and **8**. General *N*-deprotection by hydrogenation and the following *N*-acryloylation afforded derivatives **9** and **10** as peracetates. Finally, *O*-deacetylation by usual Zemplen procedure gave monomers **11** and **12**. All new compounds prepared here gave satisfactory analytical and spectroscopic data.

Radical copolymerization of 4. Radical polymerization of *n*-pentenyl glycoside **4** with acrylamide proceeded smoothly in deionized water in the presence of *N,N,N',N'*-tetramethylethylenediamine (TEMED) and ammonium peroxodisulfate (APS) as initiators for 24h at room temperature (Scheme 1). The viscous solution was diluted with 0.1M acetic acid-pyridine buffer (pH 5.1), dialyzed against deionized water, and lyophilized to give water-soluble copolymers. The physical data resulted from copolymerization are shown in Table 1, and all data of chemical shifts of the polymers prepared here are summarized in Table 2 together with those of the monomer glycosides.

Homo- and copolymerizations of 11 and 12. Radical polymerization of ω -acryloylaminoalkyl-type monomers **11** and **12** were carried out in deionized water in the presence of TEMED and APS and the products were purified according to the method described above (Scheme 3).^{6,7} As anticipated, these new compounds showed an excellent polymerizability comparing with those of the glycosides having simple ω -alkenyl type aglycons.⁷ As seen in Table 1, the polymerization reaction proceeded efficiently at room temperature even in the absence of acrylamide and afforded desired homopolymers from derivatives **11** and **12** in 100 and 35% yield, respectively. Moreover, polymerization of glycosides with acrylamide yielded the corresponding copolymers containing desirable amounts of GlcpNAc residues in high yields. Since polymers from **11** exhibited much higher molecular weight than those of polymers from **12**, the 3-acryloylaminoethyl group seems to be a practically available "polymerizable aglycon" having high reactivity, a moderate spacer-arm structure (8.822 Å), and a good solubility in water. Figure 6 indicates the ¹³C-NMR spectrum of homopolymer from **11**. Moreover, Fully assigned ¹³C-NMR spectra of derivative **12** and polymers from **12** are shown in Figure 7 and all data of chemical shifts of the polymers prepared here are summarized in Table 2 together with those of the monomer glycosides.



Scheme 3. Reagents and conditions : i) TEMED, APS / H₂O, 25°C, 4 h; ii) CH₂=CHCONH₂, TEMED, APS / H₂O, 25°C, 4 h; iii) TEMED, APS / H₂O-EtOH, 50°C, 4 h; iv) CH₂=CHCONH₂, TEMED, APS / H₂O-EtOH, 50°C, 4 h.

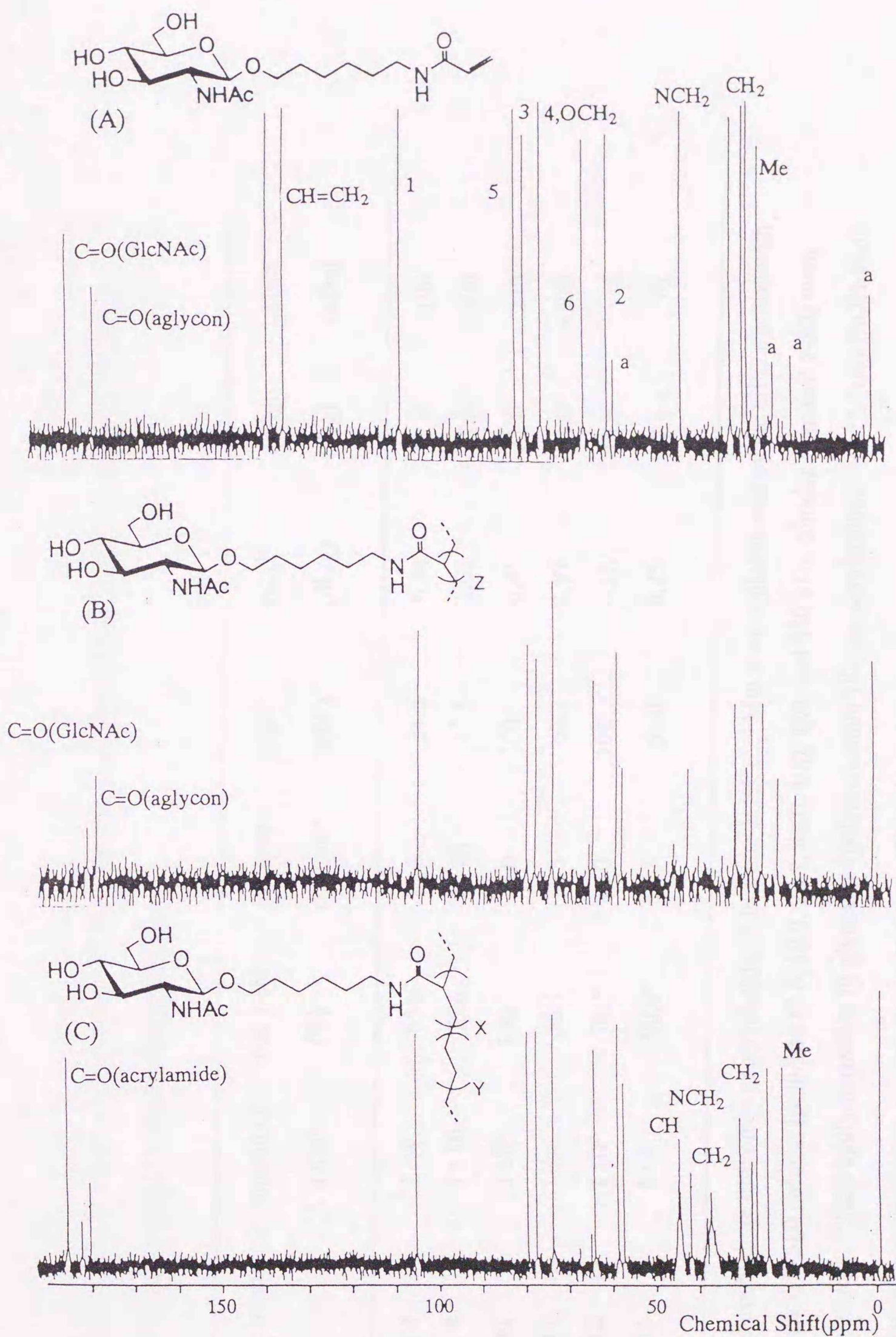


Figure 7. ^{13}C -NMR spectra of (A) 6-(acryloylamino)hexyl glycoside 12, (B) homopolymer from glycoside 12 and (C) copolymer from glycoside 12 with acrylamide, in D_2O at 50°C .

Table 1. Polymerizations of Polymerizable Glycosides Derived from *N*-Acetyl-D-Glucosamine with Acrylamide.

sugar monomer	monomer ratio ^a	total yield (%)	polymer compos ^a	sugar (wt%)	η_{inh}^b (dL/g)	$[\alpha]_D$ (deg)	M_w^c (kDa)
4	1 : 4	65.8	1 : 8	34.6	0.34	-11.4	190
4	1 : 10	75.6	1 : 28	12.5	1.03	-7.6	>300
11	1 : 0 ^d	100	1 : 0	100	0.41	-31.0	220
11	1 : 4	86.4	1 : 3	61.1	1.97	-21.9	>300
12	1 : 0 ^d	78.3 ^e	1 : 0	100	0.12	-18.6	9
12	1 : 4	90.0 ^e	1 : 5	51.0	0.35	-7.1	70

^a Ratio of carbohydrate monomer to acrylamide. ^b In water at 25°C. ^c M_w 's were determined by the GPC method with an Asahipack GS-510 column [pullulans (5.8, 12.2, 23.7, 48.0, 100, 186, and 380 kDa, Shodex Standard P-82) were used as standards]. ^d Homopolymerization of glycoside (polymerization without acrylamide). ^e 1 : 1 (v/v) EtOH-H₂O was used for the polymerization solvent owing to a poor solubility in water.

Table 2. ^{13}C -Chemical Shifts of Polymerizable Glycosides Derived from *N*-Acetyl-D-Glucosamine and the Other Polymers.
(in ppm from TSP ^a)

compds	carbohydr. monomers			homopolymers		copolymers		
	4	11	12	11	12	4	11	12
C-1	103.8	104.0	103.7	103.8	103.7	104.3	103.9	103.7
C-2	58.3	58.4	58.3	58.3	58.3	58.2	58.3	58.3
C-3	76.5	76.6	76.5	76.6	76.6	76.4	76.5	76.5
C-4	72.6	72.8	72.9	72.8	72.8	72.8	72.8	72.9
C-5	78.5	78.7	78.5	78.6	78.5	78.5	78.5	78.5
C-6	63.4	63.0	63.0	62.9	62.9	63.5	63.0	63.0
CH ₂ =CH	141.4	130.0	129.3					
CH ₂ =CH	117.5	132.5	132.9					
CH ₂	31.9	31.4	31.1	31.5	31.3	37.5	31.2	31.2
	30.6		30.8		31.2	37.5		30.9
			28.3		28.7			28.5
			27.3		27.5			27.3
OCH ₂	72.3	70.9	72.7	70.4	72.7	73.2	70.4	72.7
NCH ₂		39.4	41.9	39.1	42.1		39.1	42.0
CH ^b				45.0	45.0	44.5	44.5	44.5
CH ₂ ^b				38.0	38.0	37.5	38.3	38.0
C=O ^c	177.1	177.4	176.9	176.8	176.6	176.9	177.1	176.9
C=O ^d		171.3	171.0	178.7	178.7		179.0	178.8
CONH ₂						182.1	182.3	182.0
CH ₃	24.9	25.0	24.8	25.1	25.0	25.0	24.9	24.9

^a 3-(Trimethylsilyl)propanesulfonic acid sodium salt. ^b Methylene carbons due to main chain.

^c Signals due to the carbonyl group of GlcpNAc residue. ^d Signals due to the carbonyl groups of aglycons.

Interaction of cluster glycopolymer with WGA. It has long been suggested that the value of the binding constants of WGA with chitin oligomers (Glc_pNAc)_n increases with the length of these oligosaccharides. Results of equilibrium dialysis showed that association constants for the binding to WGA of Glc_pNAc, (Glc_pNAc)², and (Glc_pNAc)³ were found to be $1.3 \times 10^3 \text{ M}^{-1}$, $2.0 \times 10^4 \text{ M}^{-1}$, and $8.3 \times 10^4 \text{ M}^{-1}$, respectively.¹² Fluorescence methods have been also used extensively to study the specific interaction of WGA with sugars and to evaluate the binding site structure of WGA.¹³⁻¹⁵ For example, Privat et al.¹⁴ reported that changes of tryptophan fluorescence by addition of (Glc_pN_nAc)_n depend on the ligand size and all ligands but monomeric Glc_pNAc produce a 10 nm shift towards shorter wavelength and 46% enhancement of fluorescence intensity. The binding constants of chitin oligomers to WGA were determined to be $6.9 \times 10^2 \text{ M}^{-1}$ (Glc_pNAc), $4.5 \times 10^3 \text{ M}^{-1}$ (Glc_pNAc)₂, $2.0 \times 10^4 \text{ M}^{-1}$ (Glc_pNAc)₃, and $2.3 \times 10^4 \text{ M}^{-1}$ (Glc_pNAc)₄.¹² Sharon et al. clearly demonstrated by further systematic investigation on the interaction of WGA and 4-methylumbelliferyl chitooligosaccharides that each binding site of WGA consists of three adjacent subsites.¹⁴

If clustering Glc_pNAc residues on the glycopolymer possesses the appropriate flexibility to bind all subsites in the binding site, or bind at least two of them, the binding of cluster Glc_pNAc-polymer (cluster-Glc_pNAc) to WGA might induce significant enhancement of the fluorescence intensity and a shift of

the fluorescence emission maximum to shorter wavelength observed in the cases for the bindings of chitooligosaccharides with WGA. Figure 8 shows the emission spectra of WGA and of its complexes with cluster-GlcpNAc (Figure 8-A) and with the low density GlcpNAc-polymer (LD-GlcpNAc; the ratio of GlcpNAc residue / acrylamide residue of the polymer = 1 : 8) derived from the known *n*-pentenyl glycoside⁷ (Figure 8-B) at pH 7.8. When protein was saturated with cluster type polymer, the maximum fluorescence intensity was enhanced by 43% and the emission maximum was shifted from 349 to 343 nm (Figure 8-A). In contrast, LD-GlcpNAc induced only 14% enhancement of fluorescence intensity and a 3-nm shift towards shorter wavelengths (Figure 8-B).

Thus, although the results suggested that the environment of tryptophan residues located at or near the binding sites of WGA is altered from hydrophilic to relatively more hydrophobic upon interaction with both glycopolymers, the combining of cluster GlcpNAc to the subsites seems to be more effective than that of LD-GlcpNAc and very similar profile with those of chitooligosaccharides which tightly bind to the subsites. In a plot of $\Delta F/F_0$ versus $[S]$, apparent association constants (K_{app}) of these polymers with WGA based on the concentration required for 50% enhancement of fluorescence intensity are preliminarily estimated to be approximately $9 \times 10^7 \text{ M}^{-1}$ and $7 \times 10^7 \text{ M}^{-1}$, respectively. These results clearly indicated that the lectin showed much higher

affinity for polymeric GlcpNAc-ligands than chitooligosaccharides.

Interestingly, the combining curve of the cluster GlcpNAc has somewhat sigmoid shape characteristic of positive cooperativity in the binding of the two macromolecules. The plausible models we propose regarding the interaction between glycopolymers and WGA are shown in Figure 9 ; it illustrates the binding profile of each glycopolymer-ligand with the subsites of WGA.

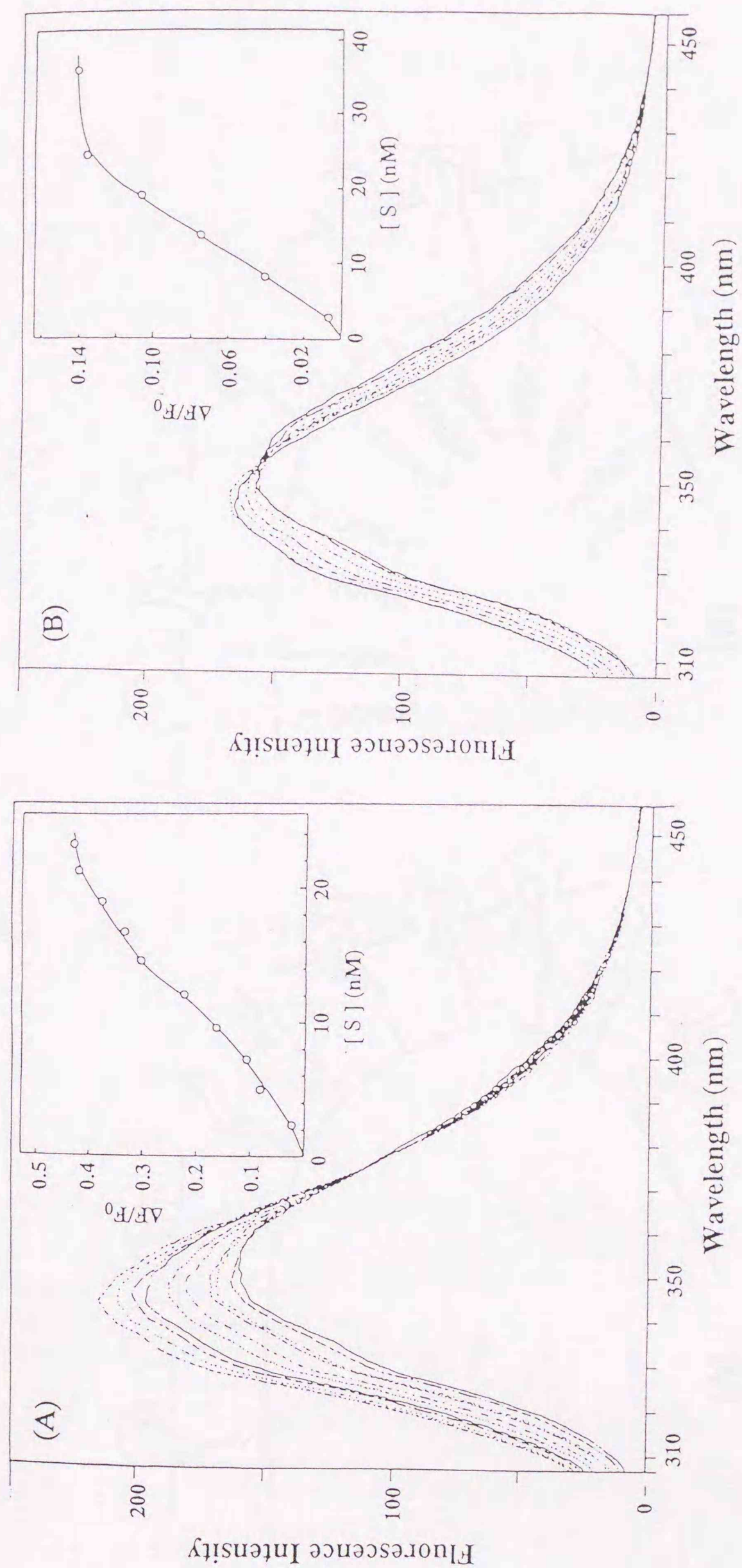


Figure 8. Changes in fluorescence emission spectra of WGA (0.65 μM , 3.0 mL 25 mM Tris-HCl buffer containing 1.25 M NaCl and 25 mM CaCl_2 , pH 7.8, 5.0°C) upon addition of (A) 10 μL aliquots of cluster GlcpNAc-polymer (0.76 μM) and (B) 20 μL aliquots of Low density GlcpNAc-polymer (0.88 μM). ΔF is a change of the fluorescence intensity at the fluorescence maximum wavelength of a solution containing the protein with a total ligand concentration $[S]$, and F_0 is the fluorescence intensity of protein alone.

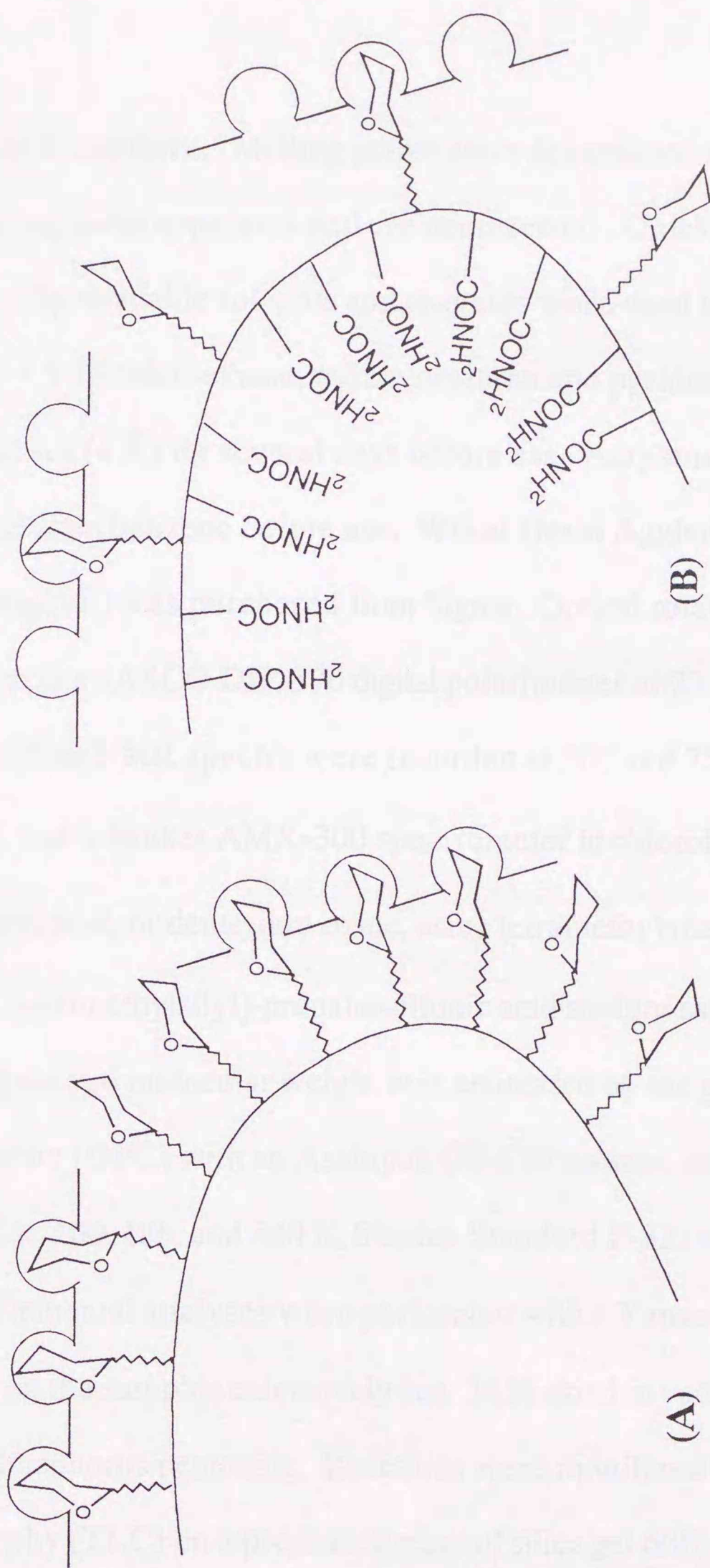


Figure 9. Models of multivalent binding of polymeric GlcpNAc ligands to the subsites of WGA.
 (A) Cluster GlcpNAc polymer; (B) Low-density GlcpNAc polymer.

2.3. Experimental Section

General Procedure. Melting points were determined with a Laboratory Devices melting point apparatus and are uncorrected. Unless otherwise stated, all commercially available solvents and reagents were used without further purification. 1,2-Dichloroethane, tetrahydrofuran and pyridine were stored over molecular sieves (4 Å) for several days before use. Acrylamide was recrystallized from benzene before use. Wheat Germ Agglutinin (WGA, activity <20mg/mL) was purchased from Sigma. Optical rotations were determined with a JASCO DIP-370 digital polarimeter at 23 °C. ^1H and proton decoupled carbon NMR spectra were recorded at 300 and 75.47 MHz, respectively, and a Bruker AMX-300 spectrometer in chloroform-*d*, dimethylsulfoxide-*d*₆ or deuterium oxide, using tetramethylsilane (TMS), methanol or 3-(trimethylsilyl)-propanesulfonic acid sodium salt (TSP) as internal standards. Average molecular weight was estimated by the gel permeation chromatography (GPC) with an Asahipak GS-510 column, and pullulans (5.8, 12.2, 23.7, 48.0, 100, 186, and 380 K, Shodex Standard P-82) were used as standards. Elemental analyses were performed with a Yanaco MT-3 CHNcorder on the samples extensively (ca. 24 h) dried in vacuo (50 °C, 0.1 Torr) over phosphorus pentoxide. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel 60F₂₅₄ (layer thickness,

0.25 mm; E. Merck, Darmstadt, Germany). For detection of the components, TLC sheets were sprayed with (a) a solution of 85:10:5 (v/v/v) methanol-conc. sulfuric acid-*p*-anisaldehyde and heated for a few minutes (for carbohydrates), (b) a solution of 5wt% ninhydrin in ethanol and heated for a few minutes (for amino groups), or (c) an aqueous solution of 5wt% potassium permanganate and heated similarly (for C-C double bonds). Column chromatography was performed on silica gel (Wakogel C-200; 100-200 mesh, Wako Pure Chemical Industries Co. Ltd., Japan). Solvent extracts were dried with anhydrous magnesium sulfate and concentrated below 40 °C under diminished pressure.

2-Methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy- α -D-glucopyrano)-[2,1-d]-2-oxazoline(2). A solution of *N*-acetyl-D-glucosamine per-*O*-acetate **1** (1.7 g, 2.51 mmol) in dry 1,2-dichloroethane (10 mL) was treated with trimethylsilyl trifluoromethanesulfonate (TMSOTf) (0.5 mL, 2.63 mmol) under a nitrogen atmosphere, and the mixture was stirred for 15 h at 50°C. After the solution was cooled to room temperature, triethylamine (1.8 mL) was added and applied directly to a column of silica gel and eluted with 300 : 100 : 1 (v/v/v) toluene-ethyl acetate-trimethylamine, to give oxazoline derivative **2** (1.55 g, q.y) as syrup: ¹H-NMR δ (CDCl₃): 2.09, 2.12, 2.16 (all s, 9H, 3COCH₃), 2.10 (s, 3H, CH₃-oxazoline), 3.61 (dt, 1H, $J_{5,6}$ 4.5Hz, H-5), 4.14 (m, 1H, H-2), 4.18 (d, 2H, H-6a

and 6b), 4.93 (dt, 1H, $J_{4,5}$ 9.3Hz, H-4), 5.27 (t, 1H, $J_{3,4}$ 2.4Hz, H-3), and 5.98 (d, 1H, $J_{1,2}$ 7.4Hz, H-1).

***n*-Pentenyl 2-acetamido-3,4,6-di-*O*-acetyl-2-deoxy- β -D-glucopyranoside(3).** To a solution of freshly prepared **2** (1.0 g, 1.61 mmol) in dry 1,2-dichloroethane were added 4-penten-1-ol (0.94 mL, 4.84 mmol) and 10-camphorsulfonic acid (CSA) (30 mg), and the solution was stirred for 2 h at 90°C. The mixture was cooled at room temperature, diluted with chloroform, and poured into ice-water. The extract with chloroform was washed successively with saturated sodium hydrogen carbonate and brine, dried, filtered and evaporated. The residue was chromatographed on silica gel with 50 : 1 (v/v) chloroform-methanol as the eluant to afford compound **3** (980 mg, 86%): mp 124-125°C; $[\alpha]_D^{25}$ -14.1° (c 0.350, CHCl₃); ¹H-NMR δ (CDCl₃): 1.67 (m, 2H, OCH₂CH₂), 1.94- 2.10 (all s, 12H, 4COCH₃), 2.10 (m, 2H, CH₂CH=CH₂), 3.69 (m, 2H OCH₂CH₂), 3.71 (m, 1H, H-5), 3.86 (ddd, 1H, $J_{1,2}$ 8.3Hz, H-2), 4.12 (dd, 1H, $J_{5,6a}$ 2.3 and $J_{6a,6b}$ 12.2Hz, H-6a), 4.26 (dd, 1H, $J_{5,6b}$ 4.5 and $J_{6a,6b}$ 12.2Hz, H-6b), 4.68 (d, 1H, $J_{1,2}$ 8.3Hz, H-1), 4.96 (m, 2H, CH=CH₂), 5.06 (t, 1H, $J_{3,4}$ 9.8Hz H-4), 5.30 (t, 1H, $J_{3,4}$ 9.9Hz H-3), 5.72 (d, 1H, $J_{2,NH}$ 8.8Hz, NH), and 5.78 (m, 1H, CH=CH₂); Anal. Calcd for C₁₉H₂₉NO₉: C, 54.93; H, 7.04; N, 3.37. Found: C, 54.85; H, 7.32; N, 3.38.

***n*-Pentenyl 2-acetamido-2-deoxy- β -D-glucopyranoside(4).** A solution of **3** (3.0 g, 7.22 mmol) and sodium methoxide(120 mg) in methanol(30 mL) were continuously stirred for 3h at room temperature. The mixture was neutralized with Dowex 50W-X8 (H^+) resin, filtrated and evaporated *in vacuo*. The residue was crystallized from ethanol to give **4** (2.1 g, q.y.): mp 184°C; $[\alpha]_D^{25}$ -27.3° (c 0.130, water); 1H -NMR δ (D_2O): 1.61 (m, 2H, OCH_2CH_2), 2.00 (s, 3H, $NHCOCH_3$), 2.05 (m, 2H, $CH_2CH=CH_2$), 3.86 (m, 2H, OCH_2CH_2), 4.46 (d, 1H, $J_{1,2}$ 8.3Hz, H-1), 5.01 (m, 2H, $CH=CH_2$), and 5.85 (m, 1H, $CH=CH_2$); Anal. Calcd for $C_{13}H_{23}NO_6 \cdot H_2O$: C, 50.51; H, 8.22; N, 4.53. Found: C, 50.48; H, 8.04; N, 4.40.

3-(*N*-Benzyloxycarbonyl)aminopropan-1-ol(5). To a solution of 3-amino-1-propanol (7.6 mL, 0.1 mol) and sodium hydrogen carbonate (21.8 g, 0.26 mol) in water were added dropwise to a mixture of benzyloxycarbonyl chloride (20.8 mL, 0.13 mol) and ether (20 mL), and the mixture was stirred for 3 h at room temperature. The mixture was extracted with ether (200 mL), washed with water, dried, and evaporated. The residual syrup was chromatographed on silica gel eluted first with 20 : 1 and then 2 : 1 (v/v) toluene-ethyl acetate as an eluant to give the *N*-Cbz-propanol **5** (15.7 g) : mp 43°C; Anal. Calcd for $C_{11}H_{15}NO_3$: C, 63.07; H, 7.16; N, 6.69. Found: C, 63.27; H, 7.25; N, 6.80.

6-(*N*-Benzyloxycarbonyl)aminohexan-1-ol(6). To a solution of 6-amino-1-hexanol (11.7 g, 0.1 mol) and sodium hydrogen carbonate (22.7 g, 0.39 mol) in water were added dropwise to a mixture of benzyloxycarbonyl chloride (20.8 mL, 0.13 mol) and ether (40 mL) and the mixture was stirred for 3 h at room temperature. The mixture was filtered with celite, extracted with ether, washed with water, dried, and evaporated. Recrystallization from ethanol gave the *N*-Cbz-hexanol **6** (15.0 g): mp 80°C; ¹H-NMR δ (CDCl₃): 1.20 and 1.42 (m, 8H, CH₂), 3.11 (m, 2H, NCH₂), 3.32-3.41 (m, 2H, OCH₂), 4.31 (t, 1H, OH), 4.98 (br s, 1H, NHCOO), 5.12 (dd, 2H, PhCH₂), and 7.30-7.37 (m, 5H, aromatic).

3-(*N*-Benzyloxycarbonyl)amino-propyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (7). A solution of oxazoline derivative **2** (3.9 g, 11.8 mmol) and *N*-Cbz-propanol **5** (5.0 g, 23.7 mmol) in 1, 2-dichloroethane (15 mL) were stirred under a nitrogen atmosphere for 2 h at 90°C in the presence of CSA (50 mg). The solution was cooled to room temperature, diluted with chloroform, and poured into ice-water. The extract with chloroform was washed successively with aqueous sodium hydrogen carbonate and water, dried, filtered, and evaporated. The residue was purified by chromatography on silica gel with 20 : 1 (v/v) toluene-ethyl acetate to afford compound **7** (5.7 g, 89%) : mp 145-146°C; [α]_D²⁵ -7.4° (c 0.229, CHCl₃); ¹H-

NMR δ (CDCl_3): 1.77 and 1.84 (m, 2H, CH_2), 1.94, 2.03, and 2.07 (all s, 12H, 4 COCH_3), 3.55 and 3.94 (m, 2H OCH_2), 3.56 (m, 1H, H-5), 3.96 (ddd, 1H, $J_{1,2}$ 8.9Hz, H-2), 4.12 (dd, 1H, $J_{5,6a}$ 2.3 and $J_{6a,6b}$ 12.2Hz, H-6a), 4.23 (dd, 1H, $J_{5,6b}$ 4.5 and $J_{6a,6b}$ 12.2Hz, H-6b), 4.31 (d, 1H, $J_{1,2}$ 8.3Hz, H-1), 4.95 (m, 1H, NHCOO), 5.0-5.16 (m, 4H, H-3, H-4, and PhCH_2), 6.38 (d, 1H, $J_{2,\text{NH}}$ 8.6 Hz, NH), and 7.31-7.40 (m, 5 H, aromatic); Anal. Calcd for $\text{C}_{25}\text{H}_{34}\text{N}_2\text{O}_{11}$: C, 55.73; H, 6.31; N, 5.20. Found: C, 55.51; H, 6.32; N, 5.26.

6-(*N*-Benzyloxycarbonyl)aminohexyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- β -D-glucopyranoside (8). A solution of oxazoline derivative **2** (8.0 g, 24.3 mmol) and *N*-Cbz-hexanol **6** (12.2 g, 48.6 mmol) in 1, 2-dichloroethane (50 mL) were stirred under a nitrogen atmosphere for 2 h at 90°C in the presence of CSA (50 mg). The solution was cooled to room temperature, diluted with chloroform, and poured into ice-water. The extract with chloroform was washed successively with saturated sodium hydrogen carbonate and water, dried, filtered, and evaporated. The residue was purified by chromatography on silica gel with 1 : 1 (v/v) toluene-ethyl acetate to afford compound **8** (8.8 g, 62%): mp $111\text{-}113^\circ\text{C}$ (^{lit.}¹⁰ $112\text{-}114^\circ\text{C}$, ^{lit.}¹¹ $96\text{-}98^\circ\text{C}$); $^1\text{H-NMR}$ δ (CDCl_3): 1.35 and 1.50 (m, 8H, CH_2), 1.93, 2.02, 2.03, and 2.08 (all s, 12H, COCH_3), 3.19 (m, 2H, NCH_2), 3.46 and 3.80 (m, 2H, OCH_2), 3.63 (br d, 1H, H-5), 3.85 (ddd, 1H, H-2), 4.09 (dd, 1H, $J_{5,6a}$ 2.3 and $J_{6a,6b}$ 12.2 Hz, H-6a), 4.25 (dd, 1H, $J_{5,6b}$ 4.5 and $J_{6a,6b}$

12.2Hz, H-6b), 4.63 (d, 1H, $J_{1,2}$ 8.2Hz, H-1), 4.87 (br s, 1H, NHCOO), 5.06 (t, 1H, $J_{3,4}$ 9.6Hz, H-4), 5.12 (dd, 2H, PhCH_2), 5.29 (t, 1H, $J_{3,4}$ 9.6Hz, H-3), 5.93 (d, 1H, $J_{2,\text{NH}}$ 8.6Hz, NH), and 7.30-7.37 (m, 5H, aromatic) ; Anal. Calcd for $\text{C}_{28}\text{H}_{40}\text{N}_2\text{O}_{11}$: C, 57.90; H, 6.89; N, 4.82. Found: C, 58.08; H, 6.96; N, 4.91.

3-(*N*-Acryloylamino)propyl 2-acetamido-3,4,6-tri-*O*-acetyl- 2-deoxy- β -D-glucopyranoside (9). Compound 7 (4.7 g, 8.72 mmol) in methanol (100 mL) was hydrogenated in the presence of 10% palladium on carbon (0.4 g) as catalyst for 2 h at room temperature. The reaction was monitored by tlc in 65 : 25 : 4 (v/v/v) chloroform-ethyl acetate-methanol. The mixture was filtered and evaporated to give the crude 3-aminopropyl glycoside.

To a solution of the crude 3-aminopropyl glycoside in tetrahydrofuran (THF) (40 mL) were added triethylamine (1.57 mL) and acryloyl chloride (0.90 mL) at 0°C and stirred for 24 h at room temperature. The mixture was poured into ice-water and extracted with chloroform. The organic layer was washed with brine, dried, and evaporated. The residue was subjected to silica gel chromatography with 1 : 2 (v/v) toluene-ethyl acetate as eluant to yield **9** (3.2 g, 80%): mp 149-150 °C; $[\alpha]_{\text{D}}^{25}$ -51.4 ° (c 0.230, CHCl_3); $^1\text{H-NMR}$ δ (CDCl_3): 1.37 and 1.53 (m, 8H, CH_2), 1.95, 2.03, 2.04, and 2.08 (all s, 12H, COCH_3), 3.19 and 3.46 (m, 2H, NCH_2), 3.75 and 4.05 (m, 2H, OCH_2), 3.67 (m, 1H, H-5), 4.08 (dd, 1H, H-2), 4.12 (dd, 1H, $J_{5,6a}$ 2.2 and $J_{6a,6b}$ 12.0Hz, H-6a), 4.27 (dd, 1H, $J_{5,6a}$ 4.5 and

$J_{6a,6b}$ 12.0 Hz, H-6b), 4.47 (d, 1H, J 8.5 Hz, H-1), 5.12 (t, 1H, J 9.3 Hz, H-4), 5.14 (t, 1H, J 9.7 Hz, H-3), 5.65 [dd, 1H, J 3.7 and 8.3 Hz, $\text{CH}=\text{CH}_2$ (*cis*)], 6.27-6.30 (m, 2H, $\text{CH}=\text{CH}_2$ (*trans*)], 6.31 (m, 1H, CH_2NHCO), and 6.53 (d, 1H, $J_{2,\text{NH}}$ 9.0 Hz, NH); Anal. Calcd. for $\text{C}_{20}\text{H}_{30}\text{N}_2\text{O}_{10}$: C, 51.79; H, 6.65; N, 6.04. Found: C, 51.79; H, 6.76; N, 6.14.

6-(*N*-Acryloylamino)hexyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- β -D-glucopyranoside (10).¹¹ Compound **8** (2.2 g, 3.79 mmol) was hydrogenated and subsequently *N*-acryloylated according to the method described for the preparation of **8** to afford compound **10** (1.0 g, 53%): mp 159°C; $[\alpha]_{\text{D}}^{25}$ -15.6° (c 0.205, CHCl_3); $^1\text{H-NMR}$ δ (CDCl_3): 1.37 and 1.54 (m, 8H, CH_2), 1.95, 2.03, 2.04, and 2.08 (all s, 12H, COCH_3), 3.35 (m, 2H, NCH_2), 3.48 and 3.88 (m, 2H, OCH_2), 3.70 (br d, 1H, H-5), 3.86 (ddd, 1H, $J_{1,2}$ 8.3 Hz, H-2), 4.15 (dd, 1H, $J_{6a,6b}$ 12.0 Hz, H-6a), 4.25 (dd, 1H, $J_{5,6b}$ 4.5 and $J_{6a,6b}$ 12.0 Hz, H-6b), 4.67 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.07 (t, 1H, $J_{3,4}$ 10.0 Hz, H-4), 5.32 (t, 1H, $J_{3,4}$ 9.6 Hz, H-3), 5.65 [dd, 1H, J 10.0 Hz, $\text{CH}=\text{CH}_2$ (*cis*)], 6.03 (br s, 1H, CH_2NH), 6.15-6.35 (m, 2H, $\text{CH}=\text{CH}_2$ (*trans*)], and 6.29 (d, 1H, NH).

3-(*N*-Acryloylamino)propyl 2-acetamido-2-deoxy- β -D-glucopyranoside (11). To a solution of compound **9** (0.8 g, 1.75 mmol) in dry methanol (30 mL) was added sodium methoxide (28 mg), and the mixture was

stirred for 1 h at room temperature. It was made neutral with Dowex 50W-X8 (H^+) resin, filtered, and evaporated *in vacuo* to give a quantitative yield of **11** (0.5 g): mp 168-169 °C; $[\alpha]_D^{25}$ -29.3 ° (c 0.232, water); 1H -NMR δ (D_2O) 1.80 (m, 2H, CH_2), 2.03 (s, 3H, $NHCOCH_3$), 3.30 (m, 2H, NCH_2), 3.44 (m, 2H, H-4 and H-5), 3.53 (t, 1H, $J_{3,4}$ 8.8Hz, H-3), 3.62 and 3.94 (each dd, 2H, OCH_2), 3.70 (ddd, 1H, $J_{2,3}$ 10.2Hz, H-2), 3.74 (dd, 1H, $J_{6a,6b}$ 12.5Hz, H-6a), 3.93 (dd, 1H, $J_{5,6b}$ 4.5 and $J_{6a,6b}$ 12.1 Hz, H-6b), 4.49 (d, 1H, $J_{1,2}$ 8.4Hz, H-1), 5.74 [dd, 1H, J 1.9 and 10.0 Hz, $CH=CH_2$ (*cis*)], and 6.13-6.30 [m, 2H, $CH=CH_2$ (*trans*)]; Anal. Calcd. for $C_{14}H_{24}N_2O_7$: C, 49.79; H, 7.34; N, 8.29. Found: C, 49.69; H, 7.31; N, 8.32.

6-(N-Acryloylamino)hexyl 2-acetamido-2-deoxy- β -D-glucopyranoside (12).¹¹ Treatment of acetate **10** (0.9 g, 1.8 mmol) with sodium methoxide (29 mg) in dry methanol, as described for the preparation of **11**, gave a quantitative yield of **12** (0.7 g): mp 171 °C; Anal. Calcd. for $C_{17}H_{30}N_2O_7$: C, 53.44; H, 8.14; N, 7.33. Found: C, 53.69; H, 8.44; N, 7.33.

Copolymerization of Carbohydrate Monomer 4 with Acrylamide. A solution of carbohydrate monomer (100 mg) and 4 or 10 equivalent molar of acrylamide in 1.0 mL of deionized water were deaerated for a while using water pump, to which were added TEMED (0.1 equivalent molar of the carbohydrate monomer) and APS (0.04 equivalent molar of the carbohydrate monomer). The

reaction mixture was continuously stirred for 4h at room temprature, diluted with 0.1M acetic acid-pyridine buffer(pH 5.1), dialyzed against deionized water, and lyophilized to give water-soluble copolymers as white powder.

Copolymerization of Carbohydrate Monomer 11 with Acrylamide. A solution of a carbohydrate monomer (100 mg) and 4 equivalent molar of acrylamide in 1.0 mL of deionized water were deaerated for a while using water pump, to which were added TEMED (0.1 equivalent molar of the carbohydrate monomer) and APS (0.04 equivalent molar of the carbohydrate monomer). The reaction mixture was treated by same procedure as described above and gave water-soluble copolymer as white powder.

Homopolymerization of Carbohydrate Monomer 11. A solution of only carbohydrate monomer (100 mg) in 1.0 mL of deionized water was deaerated for a while using water pump, to which were added TEMED (0.1 equivalent molar of the carbohydrate monomer) and APS (0.04 equivalent molar of the carbohydrate monomer). The reaction mixture was treated by same procedure as described above and gave water-soluble homopolymers as white powder.

Copolymerization of Carbohydrate Monomer 12 with Acrylamine and Homopolymerization of 12. A solution of a carbohydrate monomer (100mg) and 4 equivalent molar of acrylamide or only itself in deionized water (1.0 mL)

and ethanol (1.0 mL) were deaerated for a while using water pump, to which were added TEMED (0.1 equivalent molar of the carbohydrate monomer) and APS (0.04 equivalent molar of the carbohydrate monomer). The reaction mixture was treated according to same procedure as described above and gave water-soluble polymers as white powder.

The physical data and ^{13}C -NMR chemical shifts of all polymers prepared above are summarized in Table 1 and Table 2, respectively.

Fluorescence Measurements. Emission spectra of WGA induced by excitation at 295 nm were uncorrected and were recorded with Hitachi 650-60 fluorescence spectrophotometer or Perkin-Elmer LS-50B Luminescence Spectrometer. The solutions were contained in 1 cm quartz cuvettes, mounted in thermostated holders, and the measurements were carried out at 5°C in order to remove the effect of non-specific binding on the spectra. The concentration of WGA was estimated to be 0.65 mM by using the absorption coefficient at 280 nm ($1.27\text{ mg}^{-1}\text{ cm}^2$ in 0.1 M $\text{CH}_3\text{COONa}/\text{CH}_3\text{COOH}$, 0.5 M NaCl, pH 4.7).¹⁸

2.4. Conclusion

The simple and efficient methods for the syntheses of *n*-pentenyl and ω -acryloylamimoalkyl glycosides of *N*-acetyl- β -D-glucosamine (Glc_pNAc) has been established. Radical polymerization of the new glycosides with acrylamide proceeded smoothly in aqueous solution in the presence of ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine as initiator and gave water-soluble glycopolymers. It was suggested that this class of glycopolymers are specific and effective sugar-ligands for lectins. Moreover, Homopolymerization of the ω -acryloylamimoalkyl glycosides gave homopolymers having high-density sugar branches as a novel class of cluster glycosides. The apparent association constants of wheat germ agglutinin (WGA) with these polymers were determined by measuring the change in fluorescence intensity produced by various concentrations of polymeric ligand, and found to be approximately 10^8 M^{-1} . Addition of the cluster type Glc_pNAc-polymer to WGA induced much greater enhancement of the fluorescence intensity and a significant blue shift of the fluorescence emission maximum of WGA than did addition of the low density Glc_pNAc-polymer derived from *n*-pentenyl glycoside. This method will enhance considerably our ability to design and synthesize a variety of glycoconjugate mimetics for investigating the nature of cell surface receptors which specifically interact with sugar molecules. This

method will be applied for synthesizing of cluster glycoconjugates having more complex oligosaccharides.

2.5. References

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Chapter 3.

Lactosamine-Based Glycopolymers(2)

---Synthesis and Biochemical Evaluation of LacNAc-Containing Polymers

A simple and facile method for the syntheses of cluster-type homopolymers from ω -acryloylaminoalkyl glycosides of *N*-acetylactosamine [Galp β (1 $\rightarrow$ 4)Glc p NAc] is established on the basis of radical polymerization of the novel glycosides in the presence of ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine as initiators. These synthetic cluster glycopolymers exhibited good solubility in water and had much higher sugar density than the glycopolymers derived from known *n*-pentenyl glycosides. The association constants of *Erythrina Corallodendron* lectin (ECorL) with these cluster glycopolymers were evaluated by measuring the changes in fluorescence intensity of fluorescent-labeled ECorL induced by the addition of the polymeric ligands. Addition of the cluster-type glycopolymers having different degree of *N*-acetylactosamine density to ECorL resulted in a significant decrease of the fluorescence intensity at 520 nm, and this phenomenon was used for the determination of binding constants between the glycopolymers and ECorL.

3.1. Introduction

Recently, an efficient method for the syntheses of biochemically useful water-soluble polymers containing a variety of oligosaccharides by radical copolymerization of *n*-pentenyl glycosides with acrylamide is widely applied for the preparation of glycopolymers having sugar residues such as *N*-acetyl-D-glucosamine (Glc p NAc),¹ *N,N'*-diacetylchitobiose [Glc p NAc β (1 $\rightarrow$ 4)Glc p NAc],² and Le^x-type trisaccharide {Gal p β (1 $\rightarrow$ 4)[Fuc p α (1 $\rightarrow$ 3)]Glc p NAc} known as a sequence of tumor-associated carbohydrate antigen.³ It was also demonstrated that chemically synthesized glycopolymers are enzymatically modified to afford sialic acid-containing glycoconjugates.⁴ Using a variety of *N*-acetylglucosamine related polymers from *n*-pentenyl glycosides, it was clearly suggested that *Erythrina Corallodendron* lectin (ECorL) specifically recognizes β (1 $\rightarrow$ 4)-linkage between galactose and *N*-acetyl-D-glucosamine and structural isomers such as β (1 $\rightarrow$ 3)- and β (1 $\rightarrow$ 6)-type models showed much lower affinity to the lectin than native lactosamine structure.⁵ Thus, these *n*-pentenyl glycosides may be useful for the preparation of "simple and low sugar-density ligands", however, higher sugar-density ligands could not be prepared owing to the inert reactivity of α -

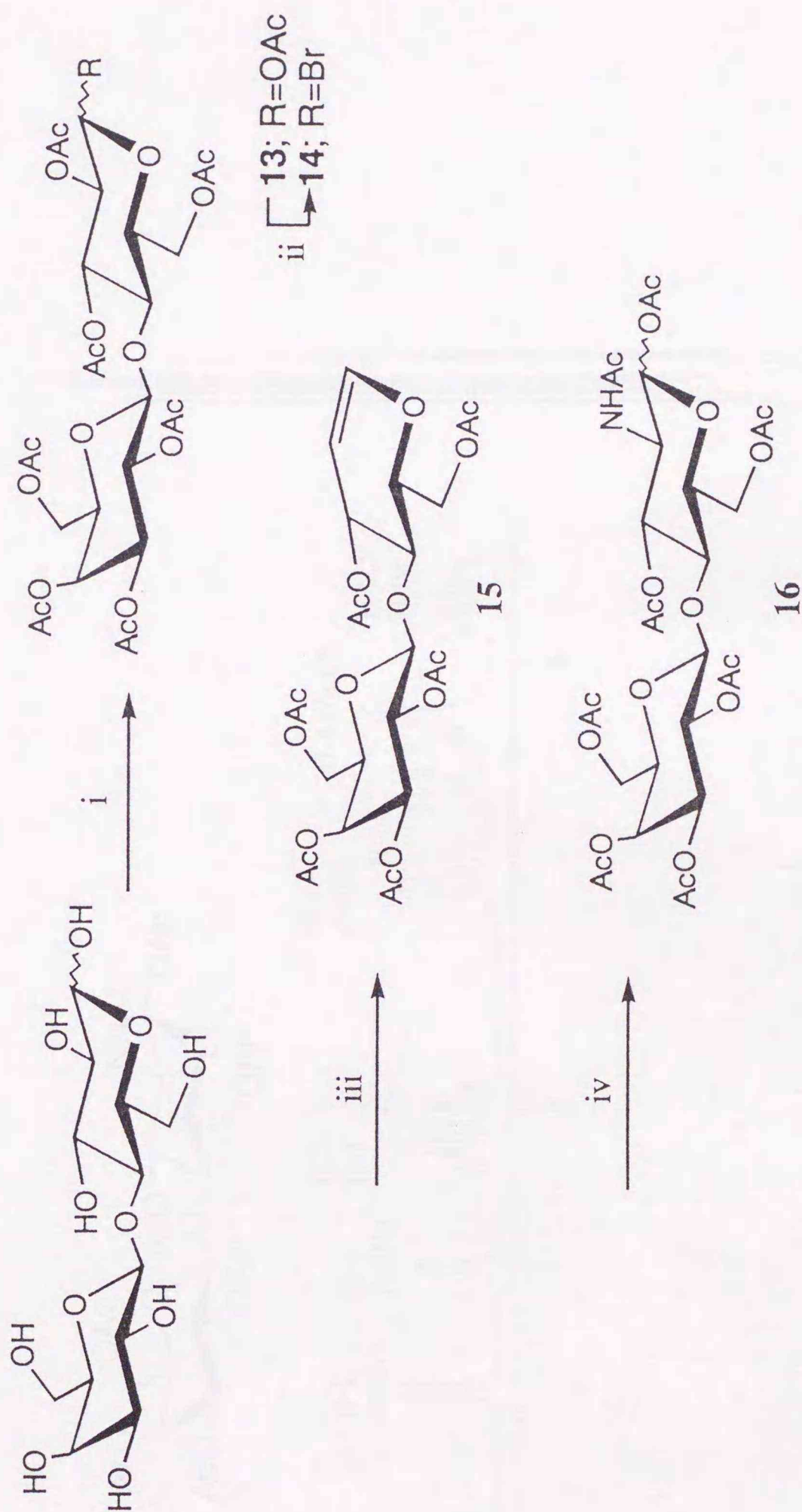
olefin structure. So, the preparation of clustered glycopolymer having much highly sugar density will be rapidly required as the next step.

In the previous chapter, a feasibility of ω -acryloylaminoalkyl glycosides for creating cluster-type high density ligands having *N*-acetyl-D-glucosamine has been discussed.⁶ These polymeric cluster ligands were used for the characterization of the specific interaction of wheat germ agglutinin (WGA) with synthetic glycopolymers. Thus, in this chapter, the versatility of the ω -acryloylaminoalkyl groups for the synthesis of novel cluster glycopolymers having *N*-acetylglucosamine with different degrees of sugar density is discussed. A biochemical availability of these novel glycoligands will be also evaluated on the basis of fluorometric analyses of the binding between FITC-labeled *Erythrina Corallodendron* lectin (ECorL)⁷ and glycopolymers in relation to the significance of the inter-lactosamine distance on the polymer main chains for the successful binding.

3.2. Results and Discussion

Synthesis of *N*-acetylactosamine per-*O*-acetate from lactose. The preparation of *N*-acetylactosamine derivative by using lactose as a starting material was attempted according to the procedure reported by Lemieux et al.⁸ and Lonngren et al..⁹ as shown in Scheme 4. Firstly, Lactal **15** was efficiently derived from lactose, via acetate and bromide. The lactal **15** was converted to azide derivative by azidonitration, followed by transesterification hydrogenation, and acetylation. Consequently, *N*-acetylactosamine peracetate **16** was obtained in 62% overall yield from lactose octaacetate **13**. ¹H-NMR spectrum of *N*-acetylactosamine acetate **16** (β -form) is shown in Figure 10.

Synthesis of *n*-pentenyl glycoside of *N*-acetylactosamine. An oxazoline derivative **17** was prepared from the *N*-acetylactosamine octaacetate **16** by using trimethylsilyl trifluoromethanesulfonate (TMSOTf) (Scheme 5).¹⁰ The oxazoline derivative **17** was allowed to react with 4-penten-1-ol as polymerizable alcohol in the presence of 10-camphorsulfonic acid (CSA) as the promoter and gave *n*-pentenyl glycoside **18** in 86% yield. De-*O*-acetylation of **18** by the Zemplen method afforded polymerizable glycoside **19** in high yields.



Scheme 4. Reagents and conditions : i) NaOAc / Ac₂O, 120°C, 3 h; ii) 25% HBr-AcOH / Ac₂O-CHCl₃, 0°C→r.t., 10 h; iii) Zn, NaOAc·3H₂O, CuSO₄·5H₂O / 60% AcOH, 0°C, 3 h; iv) NaN₃, Ce(IV)(NH₄)₂(NO₃)₆ / CH₃CN, -15°C, 20 h; NaOAc / AcOH, 110°C, 3 h; H₂, 10% Pd-C / EtOAc, r.t., 24 h; then Ac₂O / Pyridine, 0°C→r.t., 16 h.

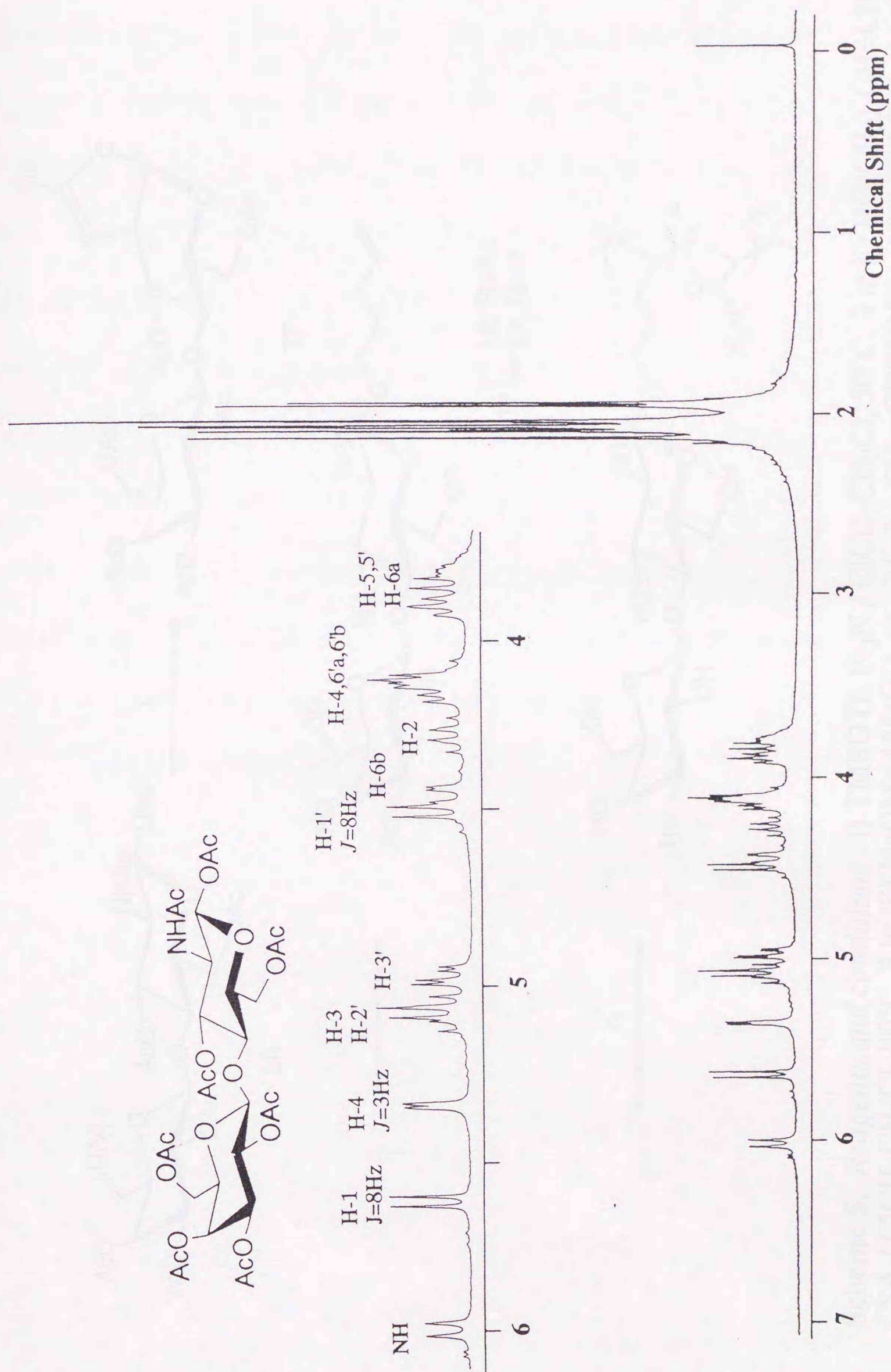
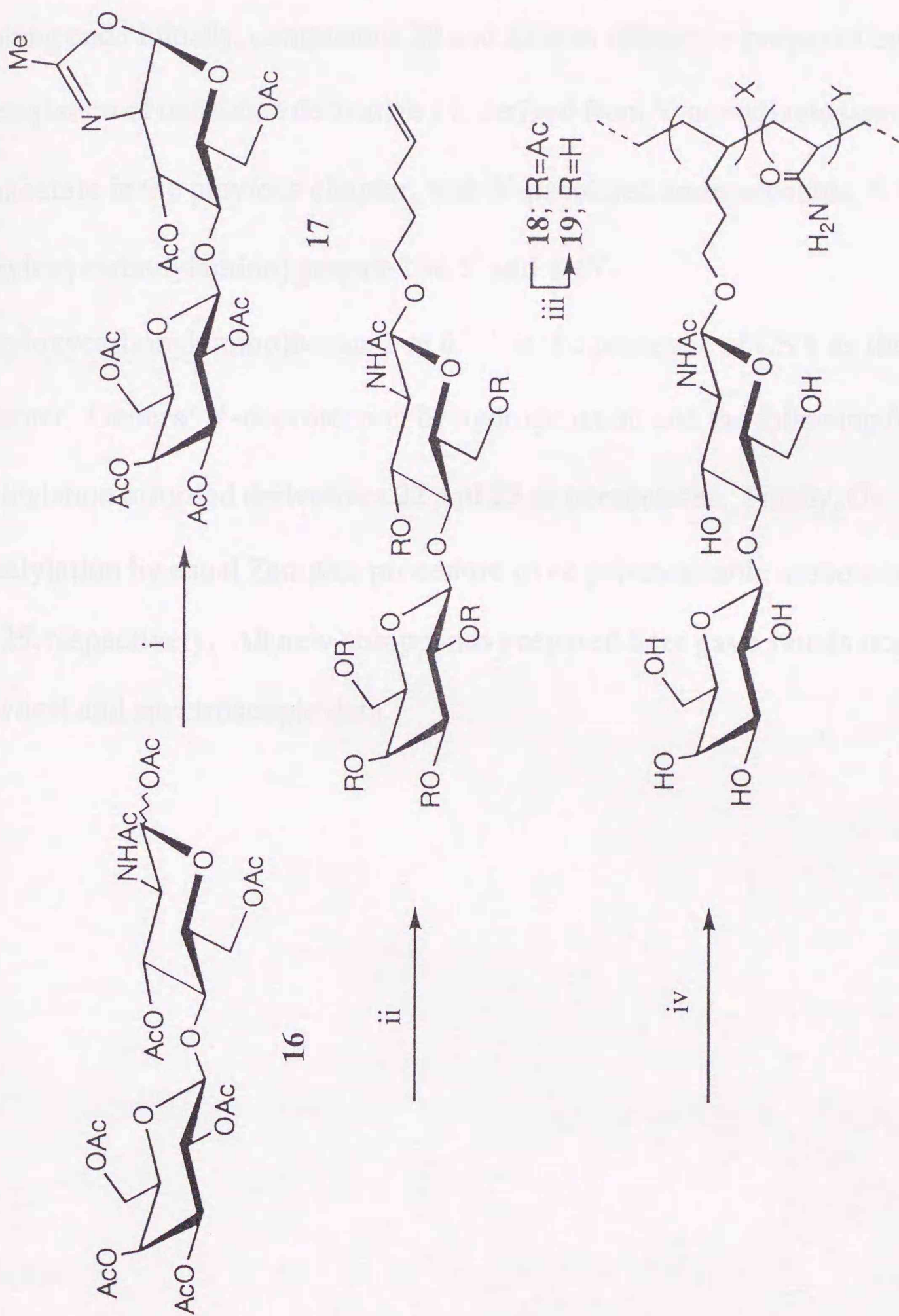


Figure 10. $^1\text{H-NMR}$ Spectrum of *N*-Acetylglucosamine Peracetate 16.

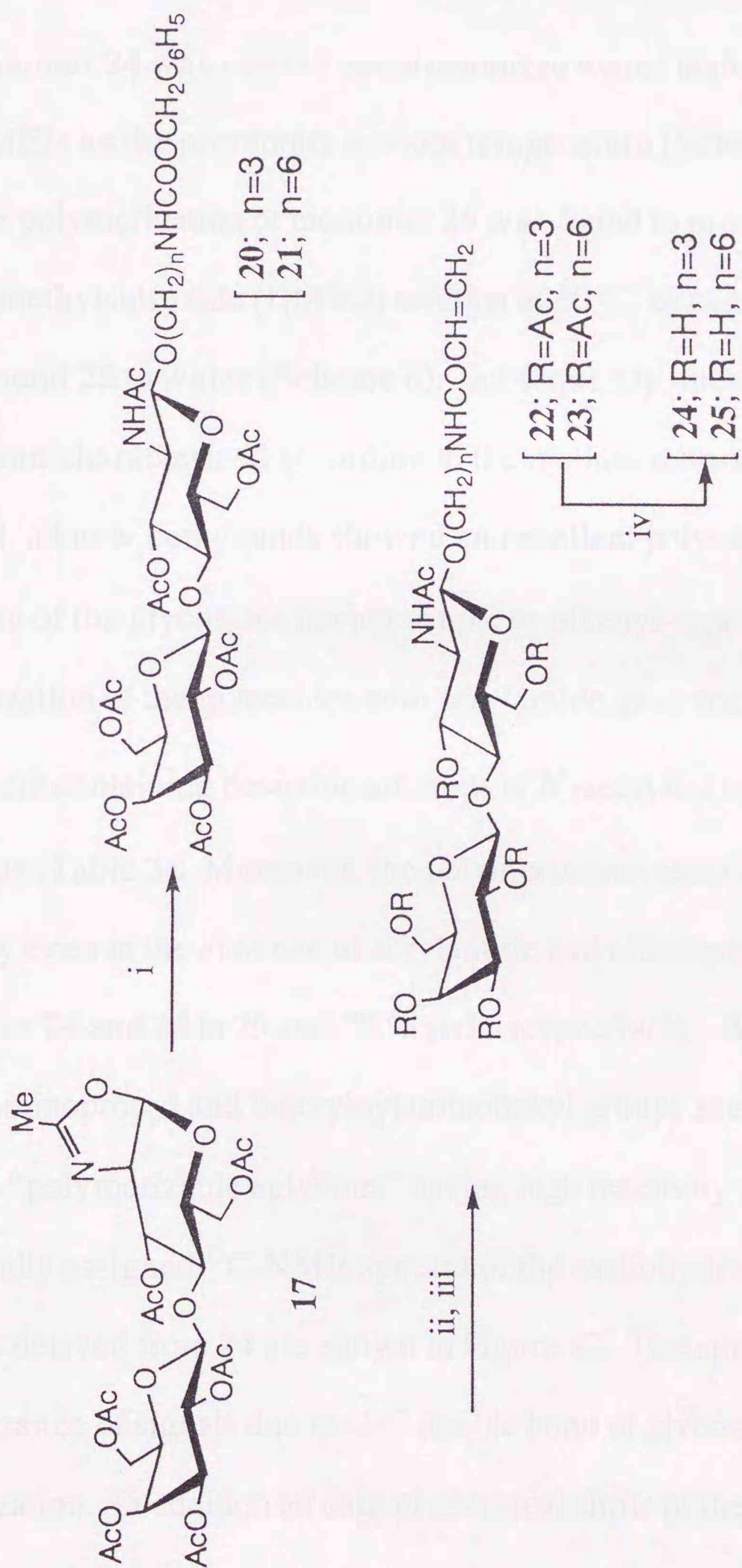


Scheme 5. Reagents and conditions : i) TMSOTf, Et₃N / ClCH₂CH₂Cl, 50°C, 3 h; ii) HO(CH₂)₃CH=CH₂, CSA / ClCH₂CH₂Cl, 90°C, 3 h; iii) NaOMe / MeOH, r.t., 3 h; iv) CH₂=CHCONH₂, APS, TEMED / H₂O, r.t., 4h.

Synthesis of ω -acryloylaminoalkyl glycosides of *N*-acetyllactosamine. Scheme 6 indicates the synthetic route of *N*-acetyllactosamine derivatives **24** and **25** having polymerizable aglycon at the reducing end. Initially, compounds **20** and **21** was efficiently prepared by glycosylation of oxazoline derivative **17**, derived from *N*-acetyllactosamine octaacetate in the previous chapter, with *N*-protected aminoalcohols, 3-(*N*-benzyloxycarbonylamino) propan-1-ol **5**⁶ and 6-(*N*-benzyloxycarbonylamino)hexan-1-ol **6**,^{6, 11} in the presence of CSA as the promoter. General *N*-deprotection by hydrogenation and the following *N*-acryloylation afforded derivatives **22** and **23** as peracetates. Finally, *O*-deacetylation by usual Zemplen procedure gave polymerizable monomers **24** and **25**, respectively. All new compounds prepared here gave satisfactory analytical and spectroscopic data.

Copolymerization of *n*-pentenyl glycoside **19** with acrylamide.

Radical polymerization of *n*-pentenyl glycoside **19** with acrylamide proceeded smoothly in deionized water in the presence of *N,N,N',N'*-tetramethylethylenediamine (TEMED) and ammonium persulfate (APS) as initiators for 24h at room temperature (Scheme 5). The viscous solution was diluted with 0.1M acetic acid-pyridine buffer (pH 5.1), dialyzed against deionized water, and lyophilized to give water-soluble copolymers. Fully assigned ^{13}C -NMR spectra of derivative **19** and the copolymer are shown in Figure 11. The physical data resulted from copolymerization are shown in Table 3, and all data of chemical shifts of the polymers prepared here are summarized in Table 4 together with those of the monomer glycosides.



Scheme 6. Reagents and conditions: i) **5** or **6**, CSA / ClCH₂CH₂Cl, 70°C, 3 h; ii) H₂, Pd-C / MeOH, r.t., 15 h; iii) CH₂=CHCOCl, Et₃N / DMF, 0→25°C, 3 h; iv) NaOMe / MeOH, r.t., 3 h.

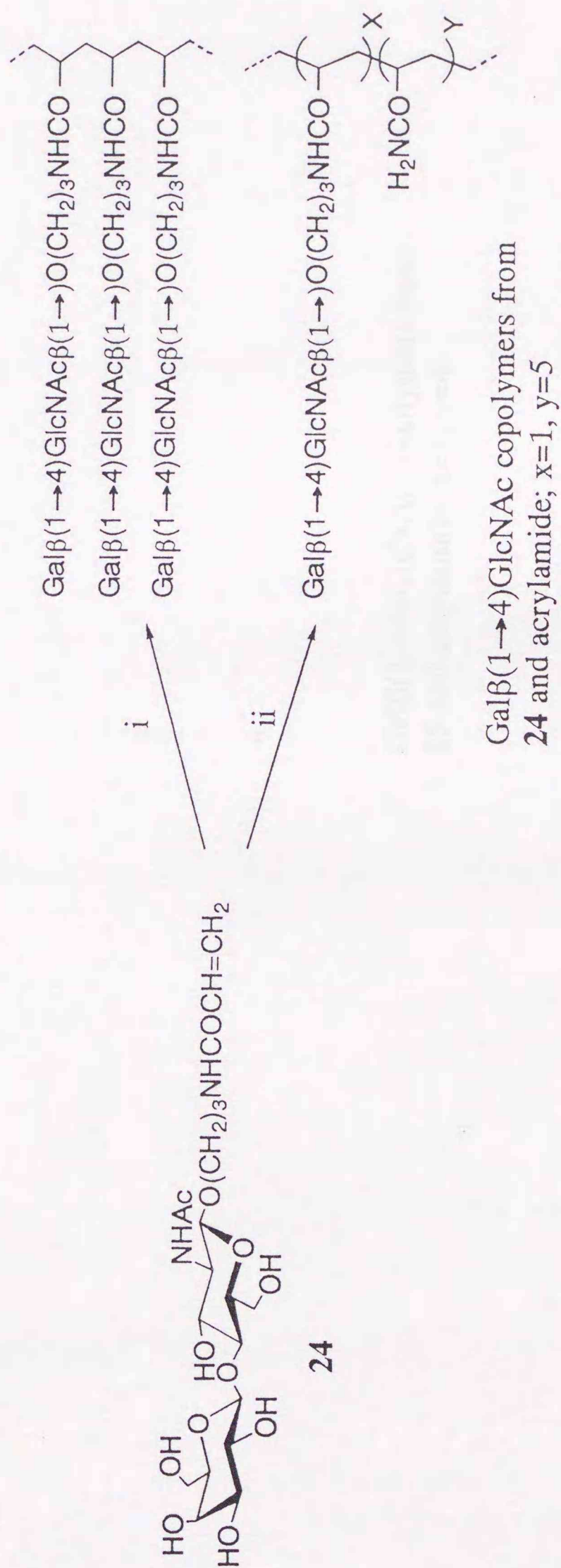
Preparation of water-soluble polymers containing *N*-

acetyllactosamine residue. Radical polymerization of the ω -acryloylamino-type monomer **24** was carried out in deionized water in the presence of APS and TEMED as the promoters at room temperature (Scheme 7). On the other hand, the polymerization of monomer **25** was found to proceed in deionized water-dimethylsulfoxide (DMSO) solution at 50 °C because of lower solubility of compound **25** to water (Scheme 8). Subsequently, these polymers were purified and characterized according to the method reported previously.^{1-3,5} As expected, all new compounds showed an excellent polymerizability comparing with those of the glycosides having simple ω -alkenyl-type aglycons.

Polymerization of the glycosides with acrylamide gave the corresponding copolymers containing desirable amounts of *N*-acetyllactosamine residues in high yields (Table 3). Moreover, the polymerization reaction proceeded efficiently even in the absence of acrylamide and afforded homopolymers from derivatives **24** and **25** in 79 and 78 % yield, respectively. Both 3-acryloylaminopropyl and 6-acryloylaminohexyl groups seem to be practically available "polymerizable aglycons" having high reactivity and good solubility in water. Fully assigned ¹³C-NMR spectra of the carbohydrate monomer **24** and polymers derived from **24** are shown in Figure 12. The spectra show a disappearance of signals due to C-C double bond of glycosides after polymerization. In addition all data of chemical shifts of these polymers and

each monomer glycosides prepared here are assigned and summarized in Table

4.



Scheme 7. Reagents and conditions: i) TEMED, APS / H₂O, 25°C, 4 h; ii) CH₂=CHCONH₂, TEMED, APS / H₂O, 25°C, 4 h.

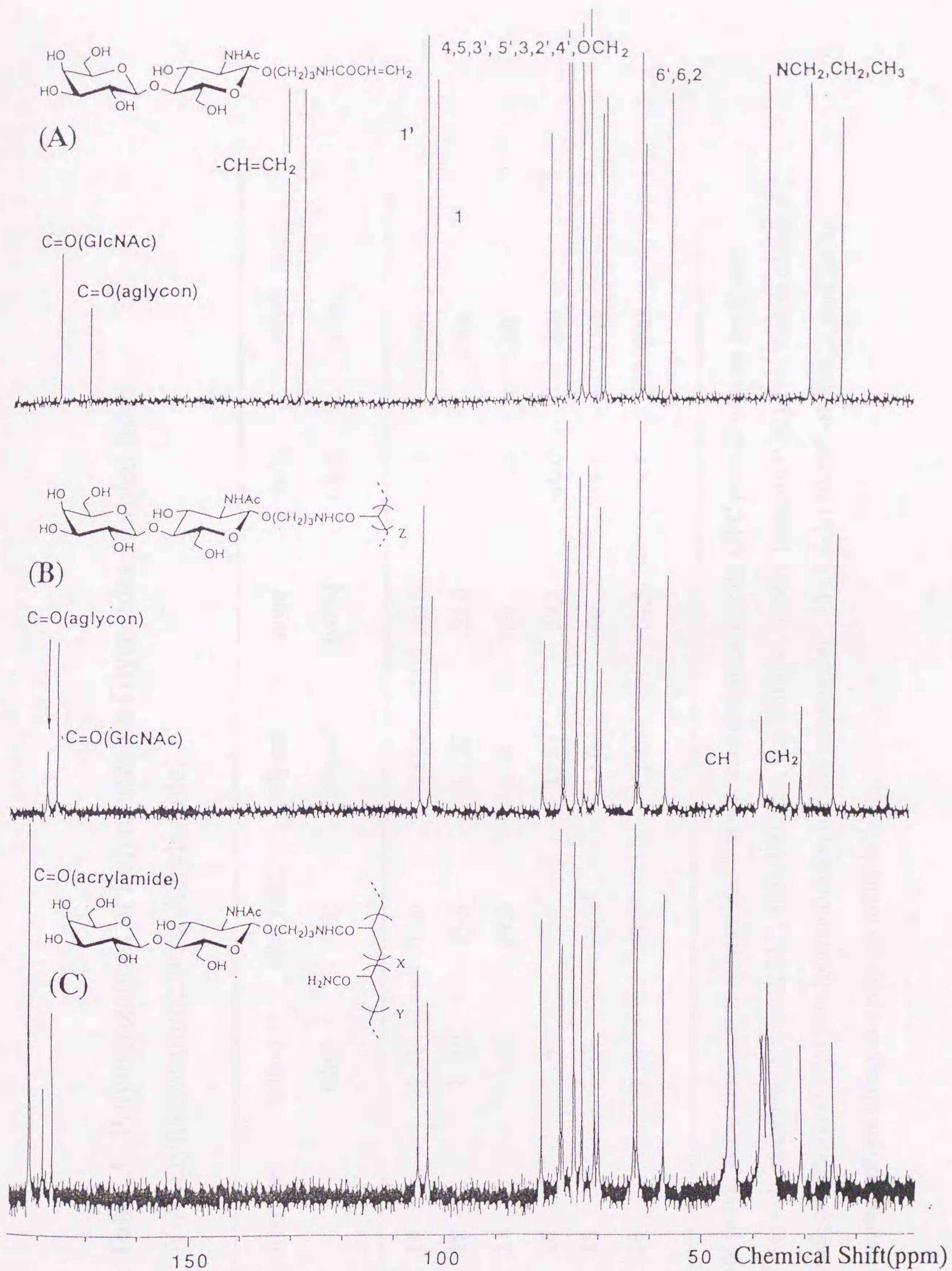


Figure 12. ¹³C-NMR spectra of (A) 3-(acryloylamino)propyl glycoside **24**, (B) homopolymer from glycoside **24** and (C) copolymer from glycoside **24** with acrylamide, in D₂O at 50°C.

Table 3. Polymerizations of Polymerizable Glycosides Derived from *N*-Acetyllactosamine with Acrylamide.

sugar monomer	monomer ratio ^a	total yield (%)	polymer compos ^a	sugar (wt%)	$[\alpha]_D$ (deg)	Mw ^b (kDa)
19	1 : 4	55.9	1 : 14	30.6	-6.6	180
19	1 : 10	76.5	1 : 23	23.0	-5.6	>300
24	1 : 0 ^c	79.3	1 : 0	100	-15.5	280
24	1 : 4	86.4	1 : 5	59.2	-10.0	200
25	1 : 0 ^c	78.3 ^d	1 : 0	100	-15.1	60
25	1 : 4	89.3 ^d	1 : 4	64.5	-8.5	230

^a Ratio of carbohydrate monomer to acrylamide. ^b Mw's were determined by the GPC method with an Asahipack GS-510 column [pullulans (5.8, 12.2, 23.7, 48.0, 100, 186, and 380 kDa, Shodex Standard P-82) were used as standards].

^c Homopolymerization of glycoside (polymerization without acrylamide). ^d 1 : 1 (v/v) DMSO-H₂O was used for the polymerization solvent owing to a poor solubility in water.

Table 4. ^{13}C -Chemical Shifts of Polymerizable Glycosides Derived from *N*-Acetyllactosamine and the Other Polymers.(in ppm from TSP ^a)

comps	carbohydr. monomers			homopolymers		copolymers		
	19	24	25	24	25	19	24	25
C-1	103.7	103.8	103.8	103.8	103.7	103.7	103.9	103.9
C-2	58.3	57.9	57.9	58.0	57.9	57.8	57.9	58.1
C-3	75.1	75.2	75.2	75.2	75.2	74.9	75.2	75.4
C-4	81.2	81.6	81.3	81.8	81.5	81.5	81.3	81.8
C-5	78.0	78.1	78.0	78.1	78.1	78.0	78.2	78.3
C-6	62.8	63.0	62.9	63.1	62.9	62.9	62.9	63.2
C-1'	105.6	105.7	105.7	105.8	105.7	105.6	105.7	105.8
C-2'	73.6	73.8	73.7	73.8	73.7	73.6	73.8	73.9
C-3'	77.4	77.6	77.4	77.6	77.5	77.4	77.6	77.7
C-4'	71.2	71.4	71.3	71.4	71.4	71.2	71.4	71.5
C-5'	75.2	75.4	75.2	75.4	75.3	75.0	75.3	75.6
C-6'	63.7	63.7	63.7	63.7	63.7	63.6	63.8	63.9
CH ₂ =CH	141.4	129.7	129.6					
CH ₂ =CH	117.5	132.9	132.9					
CH ₂	32.0	31.1	31.2	31.6	31.5	37.5	31.2	31.3
	30.5		31.0		31.4	37.5		31.5
			28.4		28.9			28.8
			27.4		27.7			27.6
OCH ₂	72.4	70.7	73.1	70.5	73.0	73.2	70.6	73.3
NCH ₂		39.2	42.0	39.3	42.3		39.1	42.3
CH ^b				45.9	45.4	44.5	44.6	44.9
CH ₂ ^b				38.0	38.2	37.5	37.7	37.8
C=O ^c	177.1	177.2	177.0	176.8	176.6	176.8	177.3	177.1
C=O ^d		171.3	171.1	178.9	178.6		179.3	179.1
CONH ₂						182.0	182.3	182.2
CH ₃	24.9	24.9	25.0	25.3	25.2	25.0	25.0	25.3

^a 3-(Trimethylsilyl)propanesulfonic acid sodium salt. ^b Methylene carbons due to main chain.

^c Signals due to the carbonyl group of GlcPNAc residue. ^d Signals due to the carbonyl groups of aglycons.

Interaction of cluster glycopolymers with ECorL. In the second chapter, a tight interaction of the homopolymer bearing clustered GlcNAc residues with subsites in the binding sites of WGA has been already demonstrated.⁶ Since WGA showed a much higher affinity for this clustered GlcNAc polymer due to polymeric sugar cluster effects than the known chitooligosaccharides such as (GlcNAc)₂, (GlcNAc)₃, and (GlcNAc)₄,^{7, 13} cluster-type glycopolymers seemed to be high performance ligands in order to investigate sugar-lectin interactions by amplified binding specificity which could not be detected in cases of simple sugar ligands with lower molecular weights.

Erythrina Corallodendron lectin (ECorL) is one of the well characterized plant lectins which mainly recognized lactose and *N*-acetyllactosamine (LacNAc).^{11, 14-16} Although the importance of inter-glycosidic bond between galactose and *N*-acetyl-D-glucosamine residues in the effective binding was clearly demonstrated by measuring the inhibitory effects of glycopolymers containing LacNAc or related regioisomers on the hemagglutination of blood cells induced by ECorL,⁵ there is no attempt to possess the information of the LacNAc density or its distribution on the globular glycoproteins on the binding process. Therefore, our interest was focused on the versatility of novel LacNAc glycopolymers prepared here for the characterization of the binding site of lectins, since the binding constants and the combining curves of synthetic glycopolymers and lectins were significantly affected by the multiplicity of interaction, the subsite structure, and the cooperativity of inter-subunit

structure.¹⁷ In the present study, we selected a fluorescein isothiocyanate-labeled ECorL (FITC-ECorL) as a modified lectin for the binding study, because ECorL has no tryptophan residue which is usually available for fluorescence analyses.¹⁸

Figure 13 shows the emission spectra of FITC-ECorL and its complexes with cluster LacNAc polymer (homopoly-LacNAc) at pH 7.3. Emission spectrum of FITC-ECorL was gradually reduced by addition of 10 μ L aliquots of cluster LacNAc polymer. When lectin was saturated with the polymers, the maximum fluorescence intensities were decreased by approximately 9~22% but no significant shift of the wavelength at the maximum fluorescence spectrum was observed. Therefore, it was suggested that the environment of the fluorescent probes of the lectin altered from hydrophobic to relatively more hydrophilic upon interaction with the glycopolymer. In the course of the combining of LacNAc polymer to the binding sites of lectin, there may be some conformational changes in the protein structure. Moreover these fluorescent probes seemed to locate apart from the binding sites, since it is commonly reported that the emission maximum is shifted to shorter wavelength and the intensity is enhanced if a fluorescent probe locates at or near the binding sites of ECorL.

The maximum intensity of the fluorescence spectra measured here were replotted against the glycopolymer concentration [S] (Figure 14) and association constant was calculated using a Steck-Wallack equation.

$$\frac{[S]}{\Delta F} = \frac{1}{\Delta F_{\max}} [S] + \frac{1}{\Delta F_{\max} \cdot K_a}$$

We have performed a preliminary survey of the affinity of FITC-ECorL for the several LacNAc polymers having different sugar density in order to determine the binding specificity of this lectin. Compounds used all exhibited a similar decrease in fluorescence as observed in the case of LacNAc homopolymer upon interaction with FITC-ECorL (Figure 14). From the Steck-Wallack analyses, we obtained the association constants (K_a) and the K_a values of low-density LacNAc polymers were proved to be slightly higher than that of LacNAc homopolymer (Table 5). Figure 15 shows a relationship between K_a value and average inter-LacNAc distance (r). The affinity of the low LacNAc-density polymer derived from *n*-pentenyl glycoside ($r=67.7\text{\AA}$) was approximately 2-fold higher than that of LacNAc homopolymer. Moreover, K_a value seems to be linearly enhanced by increasing inter-sugar spatial distance. Since no significant difference was observed in the inhibitory effects of these glycopolymers on the hemagglutination by ECorL (data was not shown), it should be noted that the fluorometric titration examined here is regarded as one of the most sophisticated methods for investigating the binding specificity of lectins with glycoconjugates. The results in these combining experiments can be summarized as follows : (i) All polymeric LacNAc ligands prepared here showed strong cluster effects of up to 1000-fold compared with

the corresponding LacNAc monomer or oligosaccharide ligands.¹⁹ (ii) Since the K_a values were enhanced by increasing the ratio of the acrylamide as a partner monomer, the maximal and special distance between the two LacNAc residues on the macromolecular ligand may be existing for the successful binding with each binding site of the two subunits of ECorL. (iii) At least, much shorter spacing of inter-LacNAc residues as in the case of LacNAc homopolymer reduced the binding affinity of glycopolymer, suggesting that the binding pocket of each subunit locates independently with an appropriate spacing.¹⁵ In contrast, it was demonstrated recently using GlcNAc polymers that wheat germ agglutinin (WGA) exhibits a specific and cooperative binding affinity with a clustered GlcNAc homopolymer.⁶ Such strong affinity enhancements probably occurred because of multipoint type binding between "subsite structure" of WGA and high-density GlcNAc residues. It appears, therefore, that the binding characteristics of polymeric sugar-cluster ligands with lectins are mainly dependent upon the number, multivalency (subunit), and subsite structures of sugar binding proteins.

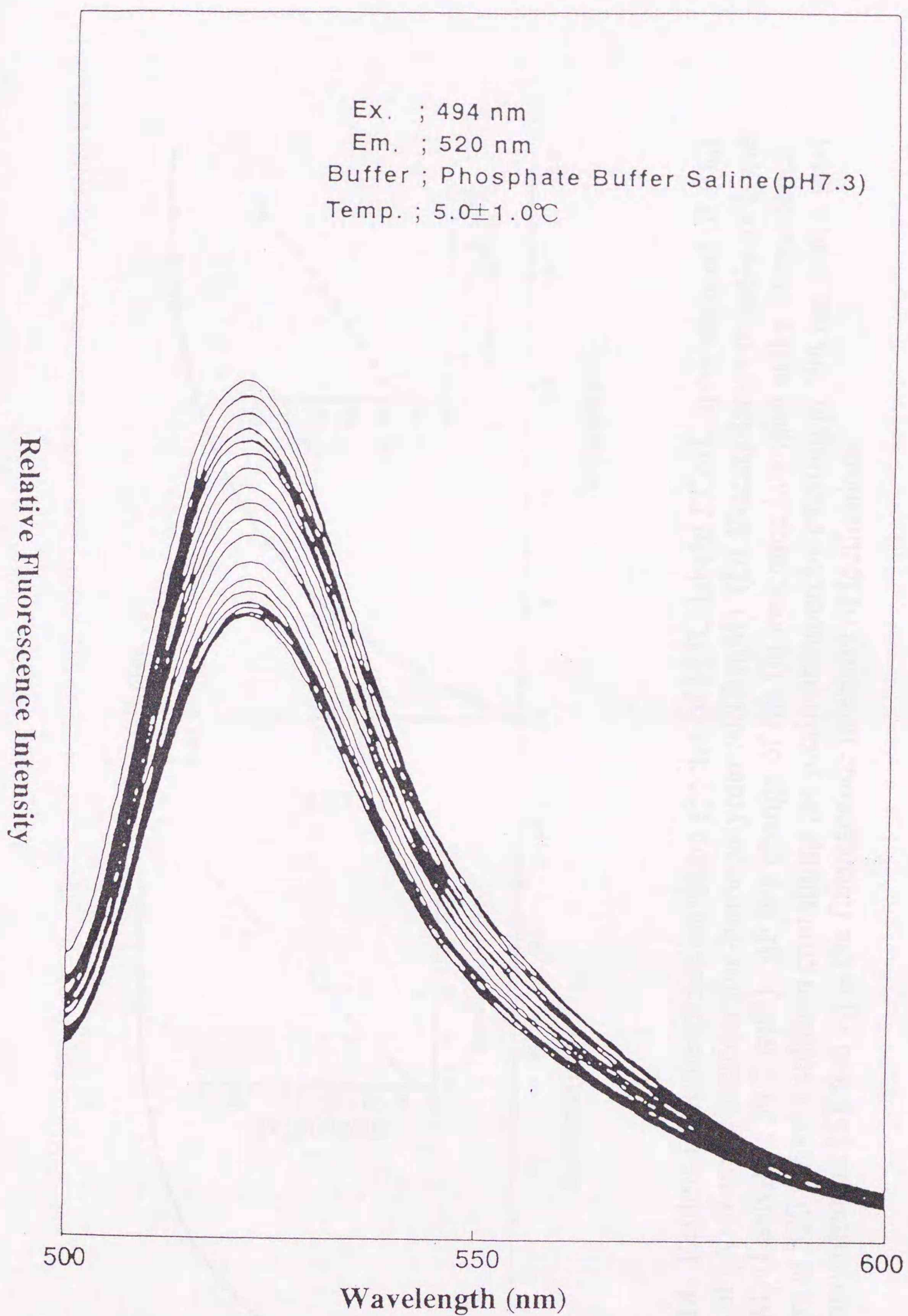


Figure 13. Changes in fluorescence spectrum of FITC-labeled ECorL (*Erythrina Corallodendron* lectin) ($0.86 \mu\text{M}$, 1.0 mL of 0.15 M phosphate buffered saline containing $50 \text{ mM Na}_2\text{HPO}_4$ and $50 \text{ mM KH}_2\text{PO}_4$, pH 7.3, 5.0°C) upon addition of $10 \mu\text{L}$ aliquots of the cluster *N*-acetyllactosamine-polymer (0.10 mM).

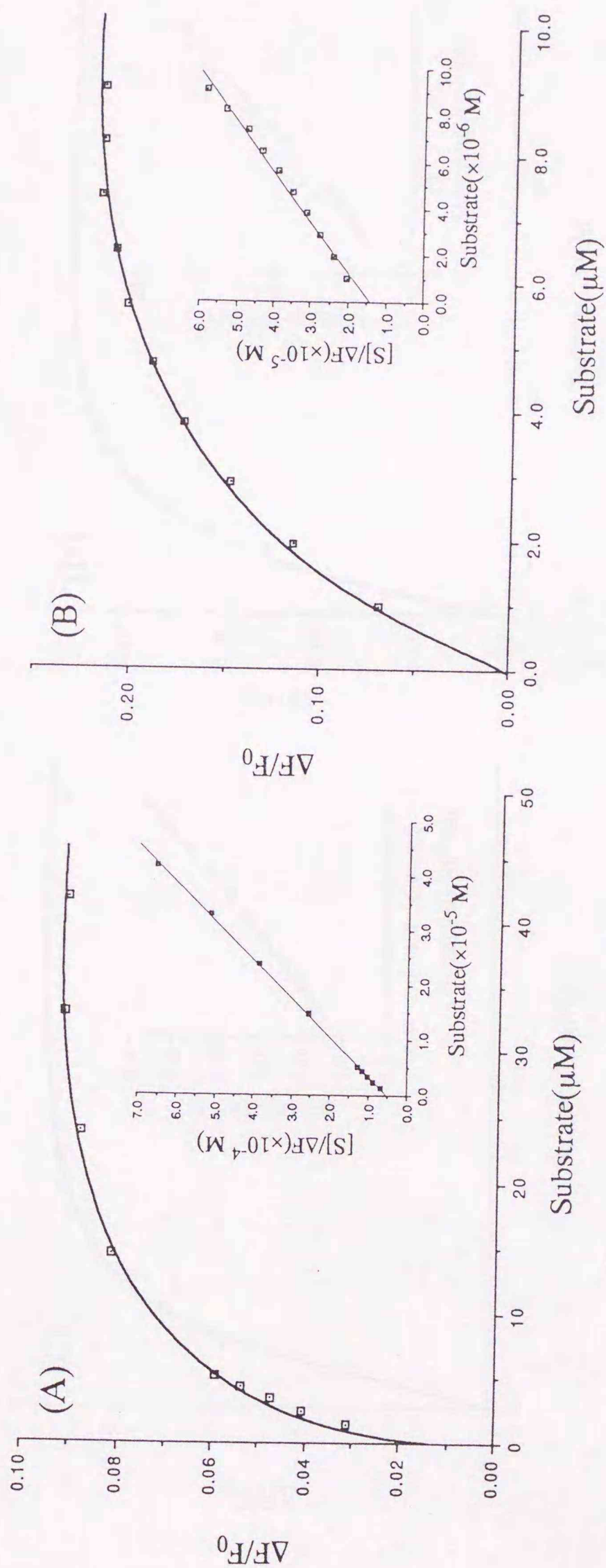


Figure 14. Replots in fluorescence emission spectra of FITC-labeled ECorL upon addition of 10 μl aliquots of (A) *N*-acetyllactosamine-homopolymer (0.10 mM), (B) *N*-acetyllactosamine-copolymer (0.10 mM) (LacNAc : 59.2 wt%). ΔF is a change of the fluorescence intensity at the fluorescence maximum at 520 nm of a solution containing the lectin obtained by exciting at 494 nm with a total ligand concentration $[S]$, and F_0 is the fluorescence intensity of lectin alone.

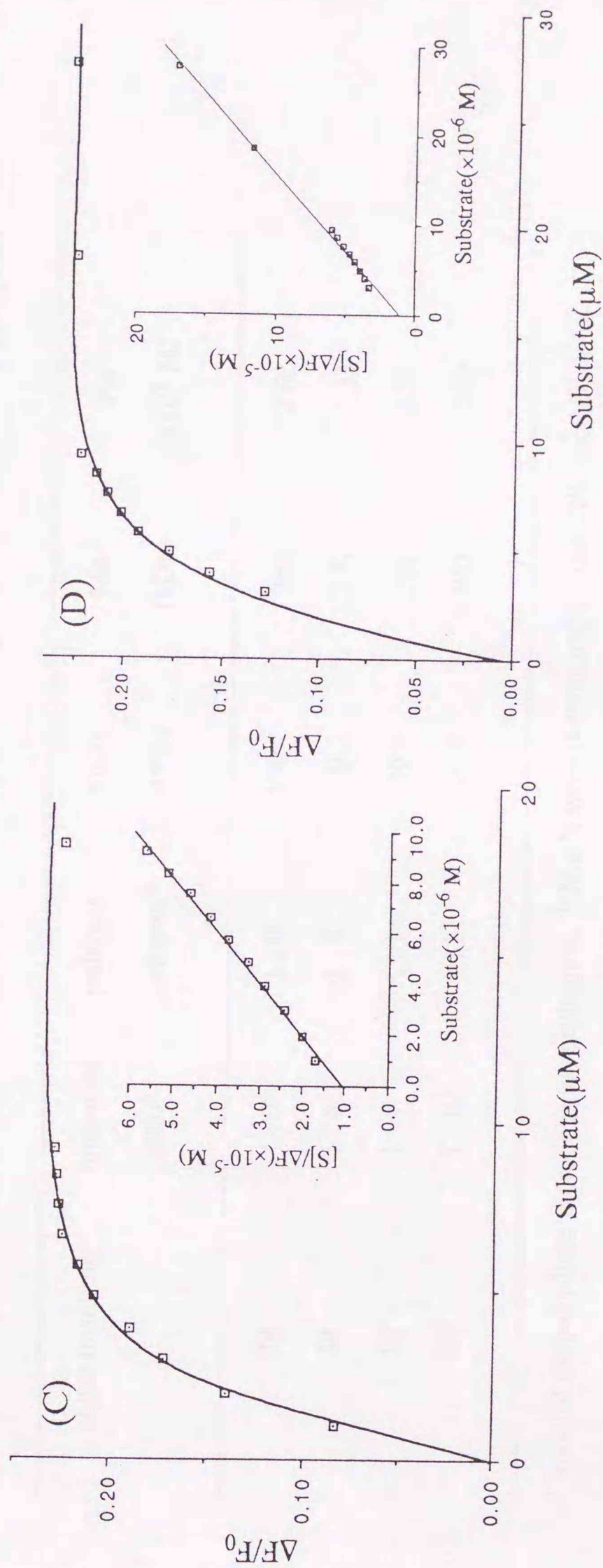


Figure 14. Replots in fluorescence emission spectra of FITC-labeled ECorL upon addition of 10 μl aliquots of (C) *N*-acetyllactosamine-copolymer (0.10 mM) (LacNAc : 30.6 wt%) and (D) *N*-acetyllactosamine-polymer (0.10 mM) (LacNAc : 23.0 wt%). ΔF is a change of the fluorescence intensity at the fluorescence maximum at 520 nm of a solution containing the lectin obtained by exciting at 494 nm with a total ligand concentration $[S]$, and F_0 is the fluorescence intensity of lectin alone.

Table 5. K_a of the Glycopolymers Containing *N*-Acetyllactosamine with ECorL.

sugar monomer	monomer ratio ^a	polymer compos. ^a	sugar (wt%)	M_w^b (kDa)	K_a^c ($\times 10^5 \text{ M}^{-1}$)
24	1:0 ^d	1:0	100	280	2.82
24	1:4	1:5	59.2	200	3.33
19 ^e	1:4	1:14	30.6	180	4.73
19 ^e	1:10	1:21	23.0	>300	5.67

^a Ratio of carbohydrate monomer to acrylamide. ^b M_w 's were determined by the GPC method with an Asahipack GS-510 column [pullulans (5.8, 12.2, 23.7, 48.0, 100, 186, and 380 kDa, Shodex Standard P-82) were used as standards]. ^c K_a is calculated from a Steck-Wallack plot using average residual molecular weights calculated from polymer composition. ^d Homopolymerization of glycoside (polymerization without acrylamide). ^e Polymer containing the *n*-pentenyl glycosides of *N*-acetyllactosamine.

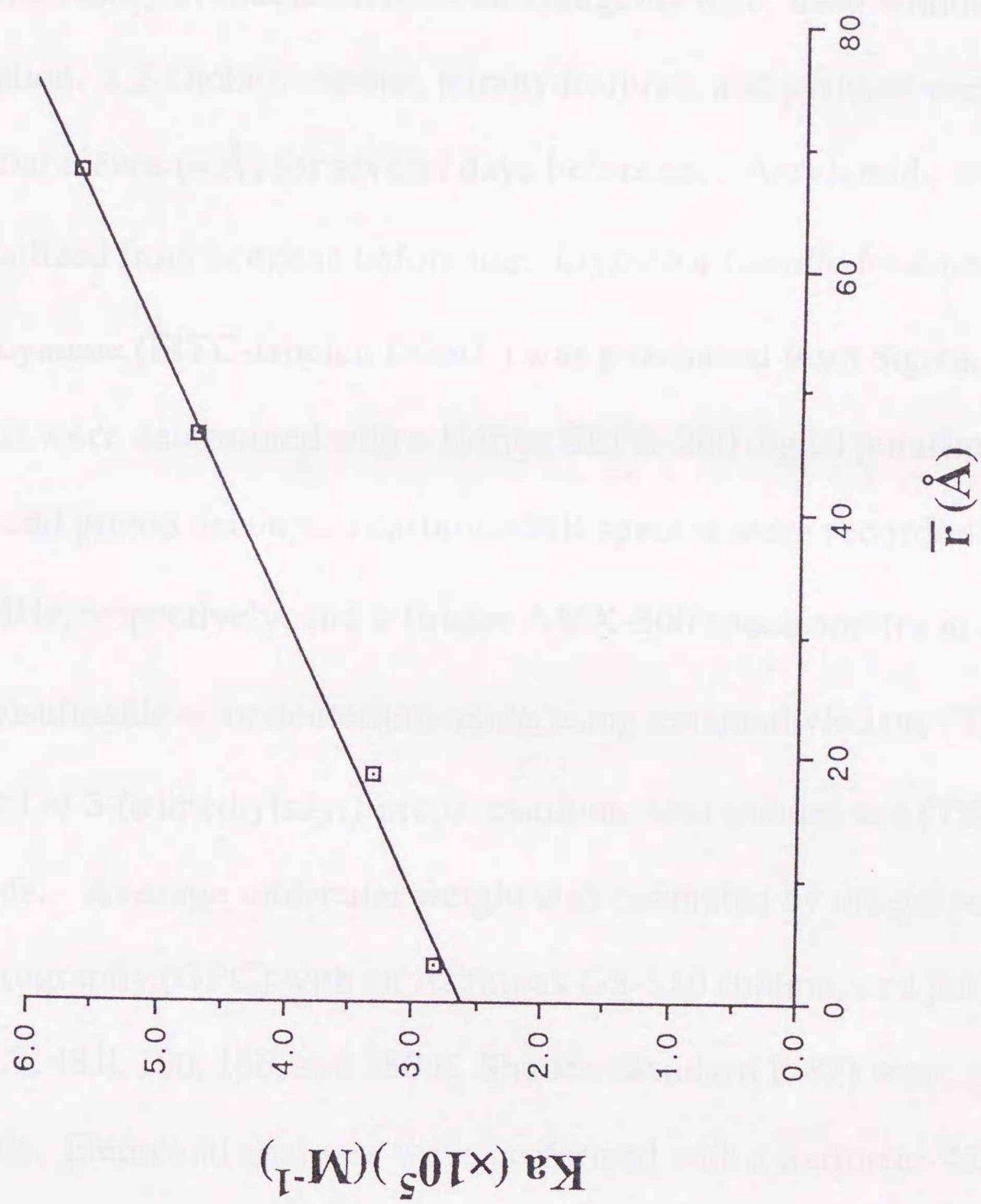


Figure 15. Relationship between K_a value and sugar density. $\bar{r}$ is average inter-LacNAc distance in the polymers.

3.3. Experimental Section

General Procedure. Melting points were determined with a Laboratory Devices melting point apparatus and are uncorrected. Unless otherwise stated, all commercially available solvents and reagents were used without further purification. 1,2-Dichloroethane, tetrahydrofuran, and pyridine were stored over molecular sieves (4 Å) for several days before use. Acrylamide was recrystallized from benzene before use. *Erythrina Corallodendron*-fluorescein isothiocyanate (FITC-labeled ECorL) was purchased from Sigma. Optical rotations were determined with a Horiba SEPA-200 digital polarimeter at 25 °C. ¹H and proton decoupled carbon NMR spectra were recorded at 300 and 75.47 MHz, respectively, and a Bruker AMX-300 spectrometer in chloroform-*d*, dimethylsulfoxide-*d*₆ or deuterium oxide, using tetramethylsilane (TMS), methanol or 3-(trimethylsilyl)-propanesulfonic acid sodium salt (TSP) as internal standards. Average molecular weight was estimated by the gel permeation chromatography (GPC) with an Asahipak GS-510 column, and pullulans (5.8, 12.2, 23.7, 48.0, 100, 186, and 380 K, Shodex Standard P-82) were used as standards. Elemental analyses were performed with a Sartorius 4503 Micro, 7079 printer on the samples extensively (ca. 24 h) dried in vacuo (50 °C, 0.1 Torr) over phosphorus pentoxide. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel 60F₂₅₄ (layer thickness,

0.25 mm; E. Merck, Darmstadt, Germany). For detection of the components, TLC sheets were sprayed with (a) a solution of 85:10:5 (v/v/v) methanol-conc. sulfuric acid-*p*-anisaldehyde and heated for a few minutes (for carbohydrates), (b) a solution of 5wt% ninhydrin in ethanol and heated for a few minutes (for amino groups), or (c) an aqueous solution of 5wt% potassium permanganate and heated similarly (for C-C double bonds). Column chromatography was performed on silica gel (Wakogel C-200; 100-200 mesh, Wako Pure Chemical Industries Co. Ltd., Japan). Solvent extracts were dried with anhydrous magnesium sulfate and concentrated below 40 °C under diminished pressure.

O-(2,3,4,6-Tetra-*O*-acetyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1,2,3,6-tetra-*O*-acetyl- β -D-glucopyranose (**13**). To a suspension of sodium acetate (85.9 g, 1.04 mol) in acetic anhydride (910 mL, 9.66 mol) was gradually added lactose monohydrate (300 g, 0.833 mol) and stirred at 120 °C for 3 h. The mixture was poured into ice-water and extracted with chloroform. The organic layer was successively washed with 1N sulfuric acid, saturated sodium hydrogen carbonate, and brine, dried, filtrated and evaporated. The residue was recrystallized from 1 : 1 (v/v) chloroform-ethanol and gave **13** (495.0 g, 88.0%).

O-(2,3,4,6-Tetra-*O*-acetyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-3,6-di-*O*-acetyl-D-glucal (**15**). To a solution of **13** (30 g, 44.2 mmol) in chloroform (300

mL) and acetic anhydride (15 mL) was dropwise added 25% hydrogen bromide in acetic acid (40.9 mL, 132.6 mmol) at 0°C and stirred at room temperature for 10 h under light exclusion. The mixture was poured into ice-water and extracted with chloroform. The organic layer was washed successively with water, saturated sodium hydrogen carbonate, and brine, dried, filtrated and evaporated. The residue **14** was used for next step without further purification. The solution of bromide **14** in acetic acid (100 mL) was gradually added to zinc powder (28.0 g, 442 mmol), sodium acetate trihydrate (6.05 g, 354mmol) and cupric sulfate pentahydrate (2.68 g, 10.7 mmol) in 50% aqueous acetic acid (400 mL) at 0°C and stirred for 3 h at room temperature. After carefully filtered, the filtrate was extracted with chloroform. The organic layer was successively washed with water, saturated sodium hydrogen carbonate and brine, dried, filterd and concentrated. The resulting residue was chromatographed on silica gel with 500 : 100 :1 (v/v/v) toluene-ethyl acetate-triethylamine as eluant to give syrup **15** (21.0 g, 85% from **13**) : ¹H-NMR δ (CDCl₃): 1.90-2.15 (all s, 18H, 6COCH₃), 3.94 (m, 1H, H-5), 4.01-4.27 (m, 5H, H-4, H-6a, H-6b, H-5', and H-6'a), 4.48 (m, 1H, H-6'b), 4.70 (d, 1H, *J*_{1,2} 8.0Hz, H-1'), 4.88 (dd, 1H, *J*_{2,3} 3.6Hz, H-2), 5.04 (dd, 1H, *J*_{3,4} 3.6Hz, H-3'), 5.23 (dd, 1H, *J*_{2',3'} 10.5Hz, H-2'), 5.41 (m, 1H, H-3), 5.44 (t, 1H, H-4'), and 6.46 (dd, 1H, *J*_{1,2} 5.8Hz, H-1).

O-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy- β -D-glucopyranose(**16**). To a solution of lactal **15** (21.7 g, 38.7 mmol) in acetonitrile (170 mL) were added cerium(IV) ammonium nitrate (45.0 g, 82.1 mmol) and sodium azide (3.25 g, 54.2 mmol) at -15°C and the mixture was stirred at -15°C for 20 h. The resulting solution was poured into ice-water, extracted with ethyl acetate. The extract was washed with water(twice), dried and evaporated *in vacuo*.

To the solution of the residue dissolved in glacial acetic acid (200 mL) was added sodium acetate (9.54 g, 116.1 mmol) and stirred at 100°C for 3 h. After cooled to room temperature, the mixture was concentrated and the residue obtained was poured into ice water and the organic layer, which extracted with chloroform, washed successively with water, saturated sodium hydrogen carbonate, and brine, dried, filtered and evaporated.

The mixture of azide derivative was dissolved in ethyl acetate (200 mL) and hydrogenated over 10%Pd on carbon for 24 h. After filtered and concentrated, the reduction mixture was treated with acetic acid (100 mL) in pyridine (200 mL) for 16 h at room temperature. After evaporated and poured into ice-water, extracted with chloroform, the organic layer was washed with 1N sulfuric acid, saturated sodium hydrogen carbonate and brine, dried and concentrated. The resulting residue was chromatographed on silica gel with 200 : 1 and then 50 : 1 (v/v) chloroform : ethanol as eluant, to give **16** (β -form) (15.44 g, 58.9%), and **16** (α -form) (3.87 g, 14.7%): $^1\text{H-NMR}(\beta\text{-form}) \delta (\text{CDCl}_3)$:

1.90-2.15 (all s, 24H, 8COCH₃), 3.78 (m, 1H, H-5), 3.85-3.93 (m, 2H, H-5' and H-6a), 4.09-4.18 (m, 3H, H-4, H-6'a and H-6'b), 4.27 (dd, 1H, H-2), 4.47 (dd, 1H, H-6b), 4.51 (d, 1H, $J_{1,2}$ 7.8Hz, H-1'), 4.95-5.15 (m, 3H, H-2' and H-3, H-3'), 5.37 (t, 1H, $J_{3,4}$ H-4'), 5.64 (d, 1H, $J_{1,2}$ 8.2Hz, H-1), and 6.03 (d, 1H, $J_{2,NH}$ 10.2Hz, NH);
 Anal. Calcd for C₂₈H₃₉NO₁₈ • 0.5 H₂O: C, 48.98; H, 5.87; N, 2.04. Found: C, 49.10; H, 6.15; N, 2.11.

2-Methyl-[*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano]-[2,1-d]-2-oxazoline(17). A solution of peracetate **16** (1.7 g, 2.51 mmol) in dry 1,2-dichloroethane (10 mL) was treated with TMSOTf (0.5 mL, 2.63 mmol) under a nitrogen atmosphere and stirred for 15 h at 50°C. After cooled to room temperature, triethylamine (1.8 mL) was added and the solution was applied directly to a column of silica gel and eluted with 300 : 100 : 1 (v/v/v) toluene-ethyl acetate-trimethylamine, to give oxazoline derivative **17** (1.55 g, q.y) as syrup: ¹H-NMR δ (CDCl₃): 1.90-2.15 (all s, 18H, 6COCH₃), 2.10 (s, 3H, CH₃-oxazoline), 3.61 (dt, 1H, $J_{5,6}$ 4,5Hz, H-5), 3.79 (dt, 1H, $J_{4,5}$ 9.2Hz, H-4), 3.82-3.91 (m, 2H, H-6'a and H-6'b), 4.10-4.16 (m, 3H, H-6a, H-6b and H-5'), 4.16 (m, 1H, H-2), 4.54 (d, 1H, $J_{1,2}$ 7.8Hz, H-1'), 4.97 (m, 1H, H-2'), 5.00 (d, 1H, $J_{3,4}$ Hz, H-3'), 5.28 (t, 1H, $J_{3,4}$ 2.4Hz, H-3), 5.38 (t, 1H, H-4'), 5.80 (d, 1H, $J_{2,NH}$ 9.8Hz, NH), and 5.96 (d, 1H, $J_{1,2}$ 7.4Hz, H-1).

n-Pentenyl *O*-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy- β -D-glucopyranoside (**18**). To a solution of freshly prepared **17** (1.0 g, 1.61 mmol) in dry 1,2-dichloroethane were added 4-penten-1-ol (0.94 mL, 4.84 mmol) and CSA (30 mg), and the solution was stirred for 2h at 90°C. After cooled to room temperature, diluted with chloroform, and poured into ice-water. The extract with chloroform was washed successively with saturated sodium hydrogen carbonate and brine, dried, filtered and evaporated. The residue was chromatographed on silica gel with 50 : 1 (v/v) chloroform-methanol as the eluant to afford compound **18** (980 mg, 86%): $[\alpha]_D^{25}$ -19.6° (c 0.121, CHCl₃); ¹H-NMR δ (CDCl₃): 1.68 (m, 2H, OCH₂CH₂), 1.90-2.15 (all s, 21H, 7COCH₃), 2.10 (m, 2H, CH₂CH=CH₂), 3.44 (m, 2H, OCH₂), 3.63 (m, 1H, H-5), 3.78 (t, 1H, $J_{3,4}$ 8.0Hz, H-4), 3.85 (m, 2H, H-6'a and H-6'b), 4.05 (dd, 1H, $J_{2,3}$ 7.5Hz, H-2), 4.10-4.16 (m, 3H, H-5', H-6a and H-6b), 4.43 (d, 1H, $J_{1,2}$ 8.4Hz, H-1), 4.51 (d, 1H, $J_{1',2'}$ 7.8Hz, H-1'), 4.94 (d, 1H, H-2'), 5.00 (d, 1H, $J_{3',4'}$ 3.4Hz, H-3'), 5.05-5.15 (m, 3H, H-3 and CH=CH₂), 5.37 (t, 1H, H-4'), 5.77 (m, 1H, CH=CH₂), and 5.80 (d, 1H, $J_{2,NH}$ 9.8Hz, NH); ¹³C-NMR δ (CDCl₃): 24.8 (CH₃), 30.6, 31.9 (CH₂), 53.1 (C-1), 60.8 (C-6), 62.4 (C-6'), 66.7 (C-4'), 68.8 (C-4'), 68.8 (C-2'), 69.1 (C-5'), 70.8 (C-3 and C-3'), 72.4 and 72.6 (C-5 and OCH₂), 75.8 (C-4'), 100.9 (C-1), 101.0 (C-1'), 117.5 (CH=CH₂), 141.4 (CH=CH₂), 177.1 (C=O); Anal. Calcd for C₃₁H₄₅NO₁₇ • 0.1 H₂O: C, 52.78; H, 6.46; N, 1.99. Found: C, 52.54; H, 6.40; N, 2.22.

n-Pentenyl *O*-(β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- β -D-glucopyranoside(**19**). A solution of **18** (388 mg, 0.550 mmol) and sodium methoxide(10 mg) in methanol (20 mL) were continuously stirred for 3h at room temperature. The mixture was neutralized with ion-exchange resin(H⁺), filtrated and evaporated *in vacuo*. The residue was crystallized in ethanol to give **19** (218 mg, 87%): mp 234°C(from ethanol-ether); $[\alpha]_D^{25}$ -19.0° (c 0.255, water); ¹H-NMR δ (DMSO-*d*₆): 1.52 (m, 2H, OCH₂CH₂), 1.78 (s, 3H, NHCOCH₃), 2.04 (m, 2H, CH₂CH=CH₂), 4.21 (d, 1H, *J*_{1,2} 8.3Hz, H-1), 4.32 (d, 1H, *J*_{1',2'} 8.3Hz, H-1'), 5.00 (m, 2H, CH=CH₂), 5.80 (m, 1H, CH=CH₂), and 7.76 (d, 1H, *J*_{2,NH} 8.5Hz, NH); Anal. Calcd for C₃₁H₄₅NO₁₇ • 1.5 H₂O: C, 47.69; H, 7.58; N, 2.93. Found: C, 47.57; H, 7.19; N, 2.93.

3-(Benzyloxycarbonyl)aminopropyl *O*-(2,3,4,6-Tetra-*O*-acetyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2-acetamido-3,6-di-*O*- acetyl-2-deoxy- β -D-glucopyranoside (**20**). A solution of oxazoline derivative **17**² (1.0 g, 1.62 mmol) and **5**¹¹ (1.02 g, 4.86 mmol) in 1,2-dichloroethane (15 mL) was stirred at 70°C under a nitrogen atmosphere for 3 h in the presence of CSA (200 mg). The solution was cooled to room temperature, diluted with chloroform, and poured into ice-water. The organic layer was successively washed with saturated sodium hydrogen carbonate and brine, dried sulfate, filtered, and evaporated. The residue was purified by chromatography on silica gel with 1 : 0 ~ 50 : 1 (v/v)

chloroform-methanol to afford compound **20** (950 mg, 71%): mp 86 °C; $[\alpha]_D^{25}$ -7.5 ° (c 0.327, CHCl₃); ¹H-NMR δ (CDCl₃): 1.60 and 1.76 (m, 2H, CH₂), 1.94-2.15 (all s, 21H, 7 COCH₃), 3.07 (m, 2H, NCH₂), 3.33 (ddd, 1H, H-5), 3.49 (m, 1H, OCH₂), 3.77 (t, 1H, $J_{3,4}$ 8.9Hz, H-4), 3.84-3.93 (m, 2H, H-6'a and H-6'b), 4.05 (m, 2H, H-6a and H-6b), 4.11-4.17 (m, 3H, H-2, H-5' and OCH₂), 4.46 (d, 1H, $J_{1,2}$ 9.5Hz, H-1), 4.48 (d, 1H, $J_{1',2'}$ 7.9Hz, H-1'), 4.96 (dd, 1H, $J_{2',3'}$ 10.5Hz, H-3'), 5.01 (br s, 1H, NHCOO), 5.03-5.16 (m, 3H, H-3, and PhCH₂), 5.15 (d, 1H, $J_{1',2'}$ 7.5Hz, H-2'), 5.35 (d, 1H, $J_{3',4'}$ 2.6Hz, H-4'), 6.42 (d, 1H, $J_{2,NH}$ 8.9Hz, NH), and 7.29-7.39 (m, 5H, aromatic); Anal. Calcd for C₃₇H₅₀N₂O₁₉ · 0.5 H₂O: C, 53.17; H, 6.15; N, 3.35. Found: C, 53.02; H, 6.10; N, 3.46.

6-(Benzyloxycarbonyl)aminohexyl O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranoside (21). A solution of oxazoline derivative **17** (1.5 g, 2.43 mmol) and **6**¹¹ (1.83 g, 7.29 mmol) in 1,2-dichloroethane (40 mL) was stirred under a nitrogen atmosphere for 3 h at 70 °C in the presence of CSA (200 mg). The solution was cooled to room temperature, diluted with chloroform, and poured into ice-water. The extract with chloroform was washed successively with saturated sodium hydrogen carbonate and water, dried, filtered, and evaporated. The residue was purified by chromatography on silica gel with 1 : 0 ~ 80 : 1 (v/v) chloroform-methanol to afford compound **21** (1.5 g, 71%): mp 70-72 °C; $[\alpha]_D^{25}$

-2.7 ° (c 0.326, CHCl₃); ¹H-NMR δ (CDCl₃): 1.32 and 1.51 (m, 8H, CH₂), 1.94-2.15 (all s, 21H, 7COCH₃), 3.18 (m, 2H, NCH₂), 3.43 (m, 1H, H-5), 3.56 and 3.81-3.90 (m, 4H, H-6'a and H-6'b and OCH₂), 3.77 (t, 1H, *J*_{3,4} 8.4Hz, H-4), 4.02 (dd, 1H, *J*_{1,2} 7.4Hz, H-2), 4.07-4.13 (m, 3H, H-5', H-6a and H-6b), 4.40 (d, 1H, *J*_{1,2} 7.5Hz, H-1), 4.49 (d, 1H, *J*_{1',2'} 7.5Hz, H-1'), 4.93 (br s, 1H, NHCOO), 4.97 (dd, 1H, *J*_{3',4'} 3.3 and *J*_{2,3'} 10.5Hz, H-3'), 5.07 (t, 1H, *J*_{3,4} 9.5Hz, H-3), 5.11 (m, 2H, PhCH₂), 5.14 (d, 1H, H-2'), 5.35 (d, 1H, *J*_{3',4'} 2.6Hz, H-4'), 5.98 (d, 1H, *J*_{2,NH} 9.5Hz, NH), and 7.30-7.36 (m, 5H, aromatic); Anal. Calcd for C₄₀H₅₆N₂O₁₉ • CH₃OH: C, 54.65; H, 6.71; N, 3.11. Found: C, 54.66; H, 6.88; N, 3.28.

3-(*N*-Acryloylamino)propyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (22). Compound **20** (190 mg, 0.230 mmol) was hydrogenated in the presence of 5% palladium on carbon (100 mg) in methanol (10 mL) for 15 h at room temperature. The reaction was monitored by tlc in 5 : 4 : 1 (v/v/v) chloroform-ethyl acetate-methanol. The mixture was filtered and evaporated to give the crude 3-aminopropyl glycoside.

To a solution of the crude 3-aminopropyl glycoside in DMF (5.0 mL) were added triethylamine (38.5 mL) and acryloylchloride (22.4 mL) at 0 °C and stirred for 3 h at room temperature. The mixture was evaporated under reduced pressure, and the residue was poured into ice-water and extracted with

chloroform. The organic layer was washed with brine, dried, filtrated and evaporated. The residue was subjected to silica gel chromatography with 1 : 0 ~ 20 : 1 (v/v) chloroform-methanol as eluant and was crystallised from chloroform-ether-*n*-hexane to give **22** (120 mg, 70%) as amorphous powder : mp 94-96 °C; $[\alpha]_D^{25}$ -27.4 ° (c 0.316, CHCl₃); ¹H-NMR δ (CDCl₃): 1.70 and 1.84 (m, 2H, CH₂), 1.97-2.17 (all s, 21H, 7COCH₃), 3.19 and 3.43 (m, 2H, NCH₂), 3.59 (m, 1H, H-5), 3.67 and 3.96 (m, 2H, OCH₂), 3.81 (t, 1H, H-4), 3.88 (dd, 2H, H-6'a and H-6'b), 4.03-4.17 (m, 4H, H-2, H-5, H-6a and H-6b), 4.33 (d, 1H, $J_{1,2}$ 8.2Hz, H-1), 4.51 (d, 1H, $J_{1,2}$ 7.5Hz, H-1'), 4.97 (dd, 1H, $J_{3,4'}$ 3.3 and $J_{2,3'}$ 10.2Hz, H-3'), 5.07 (d, 1H, $J_{3,4}$ 7.9 Hz, H-3), 5.14 (d, 1H, H-2'), 5.35 (d, 1H, $J_{3,4'}$ 2.6 Hz, H-4'), 5.63 [dd, 1H, J 3.4 and 7.4 Hz, CH=CH₂ (*cis*)], and 6.27-6.31 [m, 4H, CH=CH₂ (*trans*), CH₂NHCO and NH]; Anal. Calcd. for C₃₂H₄₆N₂O₁₈ • H₂O: C, 50.25; H, 6.33; N, 3.66. Found: C, 50.28; H, 6.13; N, 3.57.

6-(*N*-Acryloylamino)hexyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (23). Compound **21** (1.7 g, 1.96 mmol) was hydrogenated in the presence of 5% palladium on carbon (300 mg) in methanol (30 mL) for 12 h at room temperature. The reaction was monitored by tlc in 5 : 4 : 1 (v/v/v) chloroform-ethyl acetate-methanol. The mixture was filtered and evaporated to give the crude 6-aminohexyl glycoside.

To the solution of the crude 6-aminohexyl glycoside in DMF (20 mL) were added triethylamine (328 mL) and acryloylchloride (191 mL) at 0 °C and stirred for 3 h at room temperature. The mixture was evaporated *in vacuo*, and the residue was poured into ice-water and extracted with chloroform. The organic layer was washed with brine, dried, filtrated and concentrated. The residue obtained was subjected to silica gel chromatography with 1 : 0~50 : 1(v/v) chloroform-methanol as eluant and crystallised from chloroform-ether-*n*-hexane to give **23** (1.28 g, 83%) as amorphous powder : mp 79-81 °C; $[\alpha]_D^{25}$ -14.1° (c 0.326, CHCl₃); ¹H-NMR δ (CDCl₃): 1.35 and 1.57 (m, 8H, CH₂), 1.96-2.13 (all s, 21H, 7COCH₃), 3.27 and 3.40 (m, 2H, NCH₂), 3.44 (m, 2H, OCH₂), 3.61 (m, 1H, H-5), 3.78 (t, 1H, H-4), 3.88 (m, 2H, H-6'a and H-6'b), 4.00 (m, 1H, H-2), 4.12 (m, 2H, H-6a and H-6b), 4.44 (d, 1H, $J_{1,2}$ 7.5Hz, H-1), 4.50 (d, 1H, $J_{1',2'}$ 7.9Hz, H-1'), 4.97 (dd, 1H, $J_{3',4'}$ 3.3 and $J_{2',3'}$ 10.5Hz, H-3'), 5.09 (m, 1H, H-3), 5.13 (d, 1H, $J_{1',2'}$ 7.9Hz, H-2'), 5.35 (d, 1H, $J_{3',4'}$ 2.6Hz, H-4'), 5.64 [dd, 1H, J 1.6 and 9.9Hz, CH=CH₂ (*cis*)], 6.07-6.23 (m, 2H, CH=CH₂ (*trans*)], 6.17 (d, 1H, $J_{2,NH}$ 9.8Hz, NH), and 6.32 (m, 1H, CH₂NHCO); Anal. Calcd. for C₃₅H₅₂N₂O₁₈: C, 53.29; H, 6.64; N, 3.55. Found: C, 53.13; H, 6.62; N, 3.73.

3-(*N*-Acryloylamino)propyl *O*-(β-D-Galactopyranosyl)-(1→4) -2-acetamido-2-deoxy-β-D-glucopyranoside (24). To a solution of compound **22** (300 mg, 0.402 mmol) in dry methanol (10 mL) was added sodium methoxide

(20 mg), and the mixture was stirred for 3 h at room temperature. It was neutralized by Dowex 50W-X8 (H⁺) resin, filtered, and evaporated *in vacuo* to give **24** (170 mg, 86%) : mp 188 °C; $[\alpha]_D^{25}$ -18.2 ° (c 0.250, water) ; ¹H-NMR δ (D₂O): 1.82 (m, 2H, CH₂), 2.04 (s, 3H, COCH₃), 3.28 and 3.35 (m, 2H, NCH₂), 4.48 (d, 1H, $J_{1,2}$ 7.8Hz, H-1), 4.53 (d, 1H, $J_{1',2'}$ 7.9Hz, H-1'), 5.76 [dd, 1H, J 1.8 and 9.7 Hz, CH=CH₂ (*cis*)], 6.14-6.32 [m, 2H, CH=CH₂ (*trans*)], 6.25 (d, 1H, $J_{2,NH}$ 9.7Hz, NH), and 6.30 (m, 1H, CH₂NHCO); Anal. Calcd. for C₂₀H₃₄N₂O₁₂ • H₂O: C, 48.87; H, 7.08; N, 5.47. Found: C, 46.70; H, 6.80; N, 5.31.

6-(N-Acryloylamino)hexyl O-(β -D-Galactopyranosyl)-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- β -D-glucopyranoside (25).⁶ To a solution of compound **23** (290 mg, 0.368 mmol) in dry methanol (10 mL) was added sodium methoxide (10mg), and the mixture was stirred for 2 h at room temperature. It was neutralized by Dowex 50W-X8 (H⁺) resin, filtered, and evaporated *in vacuo* to give **25** (187 mg, 95%) : m.p. 176 °C.; ¹H-NMR δ (CDCl₃): 1.26 and 1.47 (m, 8H, CH₂), 1.96 (s, 3H, COCH₃), 3.20 and 3.40 (m, 2H, NCH₂), 3.37 (m, 2H, OCH₂), 4.41 (d, 1H, $J_{1,2}$ 7.7Hz, H-1), 4.45 (d, 1H, $J_{1',2'}$ 7.3Hz, H-1'), 5.68 [dd, 1H, J 1.8 and 9.9Hz, CH=CH₂ (*cis*)], 6.06-6.24 [m, 2H, CH=CH₂ (*trans*)], 6.17 (d, 1H, $J_{2,NH}$ 9.8 Hz, NH), and 6.22 (m, 1H, CH₂NHCO); Anal. Calcd. for C₂₃H₄₀N₂O₁₂ • 0.5H₂O: C, 50.63; H, 7.57; N, 5.13. Found: C, 50.80; H, 7.37; N, 5.06.

Copolymerization of 19 with Acrylamide. A solution of monomer **19** (100 mg) and 4 or 10 equivalent molar of acrylamide in deionized water (1.0 mL) were deaerated by using a water aspirator for 20 min, followed by addition of TEMED (0.1 equivalent molar of the carbohydrate monomer) and APS (0.04 equivalent molar of the carbohydrate monomer). The mixture was stirred for 4 h at room temperature, diluted with 1.0 mL of 0.1 M pyridine-acetic acid buffer (pH 5.1), dialyzed against deionized water, and freeze-dried to give water-soluble copolymers as white powdery.

Homopolymerization of 24. A solution of monomer **24** (140 mg, 0.283 mmol) in deionized water (2.0 mL) was deaerated by using a water aspirator for 20 min, followed by addition of TEMED (4.24 mL) and APS (2.58 mg). The mixture was stirred for 4 h at room temperature, diluted with 1.5 mL of 0.1 M pyridine-acetic acid buffer (pH 5.1), dialyzed against deionized water, and freeze-dried to give a white powdery polymer (111 mg, 79%).

Copolymerization of 24 with Acrylamide. A solution of monomer **24** (150 mg, 0.303 mmol) and acrylamide (86.2 mg, 1.21 mmol) in deionized water (5.0 mL) were deaerated by using a water aspirator for 20 min, followed by addition of TEMED (4.55 mL) and APS (2.77 mg). The mixture was stirred for 4 h at room temperature, diluted with 2.0 mL of 0.1 M pyridine-acetic acid buffer

(pH 5.1), dialyzed against deionized water, and freeze-dried to give a white powdery polymer (204 mg, 86%).

Homo- and copolymerization of 25. A solution of monomer **25** (140 mg, 0.224 mmol) in deionized water (2.0 mL) and dimethylsulfoxide (2.0 mL) was deaerated by using a water aspirator for 20 min, followed by addition of TEMED (5.04 mL) and APS (4.09 mg). The mixture was stirred for 4 h at room temperature, diluted with 2.0 mL of 0.1 M pyridine-acetic acid buffer (pH 5.1), dialyzed against deionized water, and freeze-dried to give as a white powdery homopolymer (94 mg, 78%).

The monomer **25** (150 mg, 0.280 mmol) and acrylamide (79.6 mg, 1.12 mmol) were dissolved in deionized water (3.0 mL) and dimethylsulfoxide (3.0 mL), and the mixture was treated with procedure as described above to give a white powdery copolymer (205 mg, 89 %).

The physical data and ^{13}C -NMR chemical shifts of all polymers prepared above are summarized in Table 3 and Table 4, respectively.

Fluorescence Measurements. Emission spectra of a FITC in the ECorL induced by excitation at 494 nm were recorded with Hitachi 650-60 fluorescence spectrophotometer. The solutions were contained in 1 cm quartz cuvettes, and the measurements were carried out at 5 °C in order to remove the

effect of non-specific binding on the spectra. The concentration of FITC-labeled ECorL was estimated to be $0.87 \mu\text{M}$ by using an absorption coefficient at 280 nm ($1.53 \text{ mg}^{-1}\text{cm}^2$ in $50 \text{ mM Na}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$, 0.15 M NaCl , pH 7.3).²⁰

3.4. Conclusion

A simple and versatile method for the syntheses of cluster-type homopolymers from ω -acryloylaminoalkyl glycosides of *N*-acetylactosamine [Galp β (1 $\rightarrow$ 4)Glc α NAc] was established on the basis of radical polymerization of the novel glycosides in the presence of ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine as initiators. These synthetic cluster glycopolymers exhibited good solubility in water and had much higher sugar density than the glycopolymers derived from known *n*-pentenyl glycosides. The association constants of *Erythrina Corallodendron* lectin (ECorL) with these cluster glycopolymers were evaluated by measuring the changes in fluorescence intensity of fluorescent-labeled ECorL induced by the addition of the polymeric ligands. Addition of the cluster-type glycopolymers having different degree of *N*-acetylactosamine density to ECorL resulted in a significant decrease of the fluorescence intensity at 520 nm. It was clearly suggested that fluorometric titration of FITC-ECorL with a variety of LacNAc polymers revealed an importance of the suitable spacing of the inter-sugar residues on the glycoprotein models for the efficient interaction with the binding sites of lectins.

3.5. References

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Chapter 4.

Synthesis of Novel Clustered Glycopolymers Containing Triantennary Glycosides of *N*-Acetylactosamine

An efficient method for the first synthesis of novel glycopolymers containing triantennary glycosides of *N*-acetylactosamine [Galp β (1 $\rightarrow$ 4) GlcpNAc] is described. Radical copolymerization of the triantennary glycosides with acrylamide proceeded smoothly in an aqueous solution and gave water-soluble clustered glycopolymers.

4.1. Introduction

A biological significance of oligosaccharides of asparagine-linked type glycoproteins existing on the cell surfaces has been rapidly investigated. Unique functions of multi-antennary sugar chains were referred to "cluster effect" by Lee et al.¹ In particular, this cluster effect, in the sugar moiety of the relatively non-reducing terminal, has been recently noted in biochemical phenomena represented by carbohydrate-receptor bindings in relation to cell-cell interactions.²

In the preceding chapters, an efficient and facile method for the syntheses of biochemically useful glycoprotein models based on the radical copolymerization of a variety of *n*-pentenyl glycosides with acrylamide have been demonstrated.³ In addition, aglycones of ω -acryloylamino-type structure have been proved to permit homopolymerization of polymerizable sugar derivatives in order to produce highly clustered glycoprotein models.^{4,5} Although useful methods for the synthesis of "triantennary glycosides" having galactose residues were reported using tris(hydroxymethyl)aminomethane by Lee et al.⁶ and Polidori et al.,⁷ there is no attempt to prepare polymers bearing clustered "triantennary glycoside" which is expecting to show specific affinity with liver lectins and other carbohydrate-binding proteins.⁸

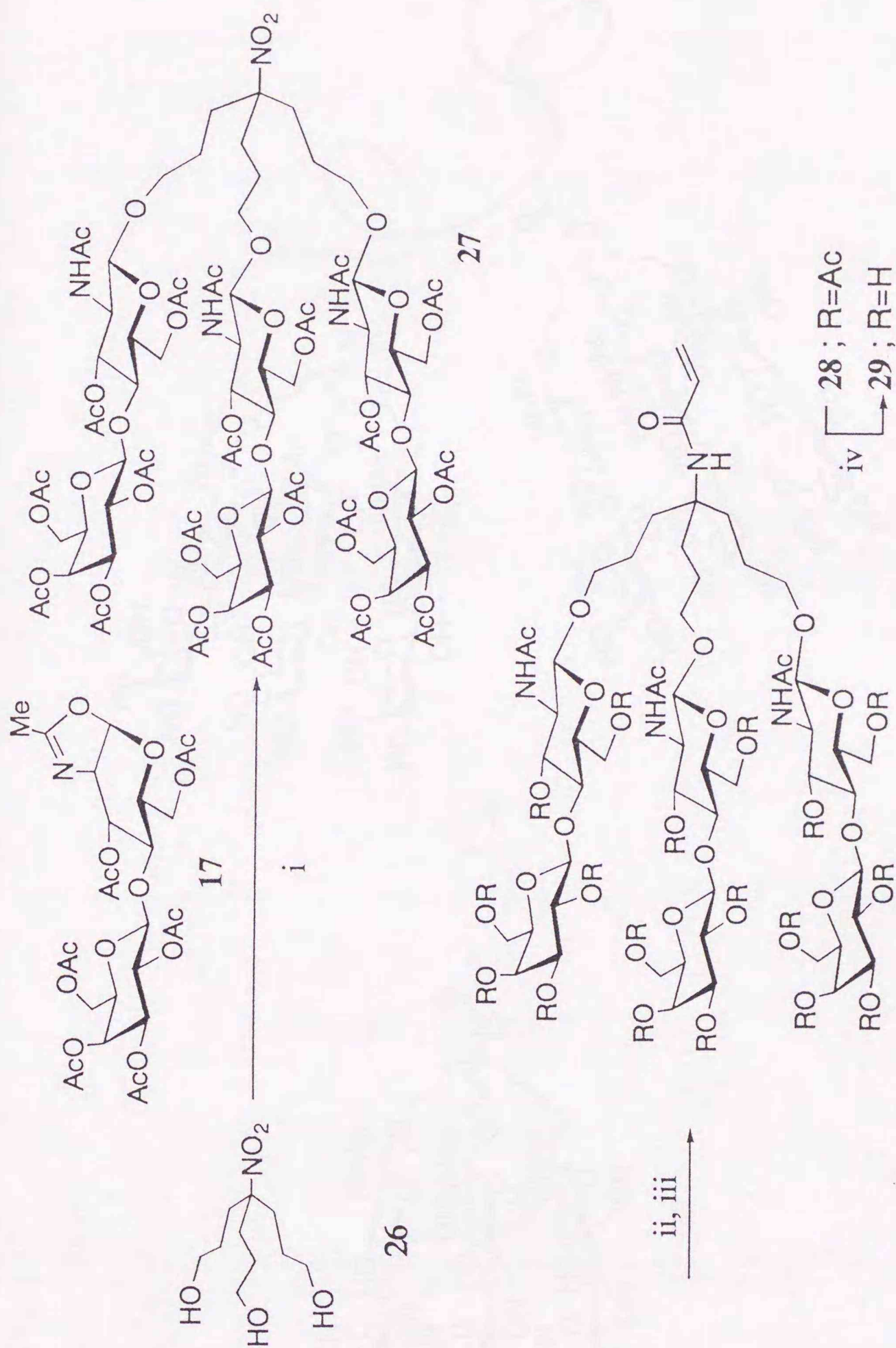
In this chapter, a simple and versatile synthesis of a new glycoprotein model having clustered triantennary *N*-acetylglucosamine (LacNAc) will be described by using 4-nitro-4-[1-(3-hydroxypropyl)]-1,7-heptandiol⁹⁻¹¹ as a key starting material.

4.2. Results and Discussion

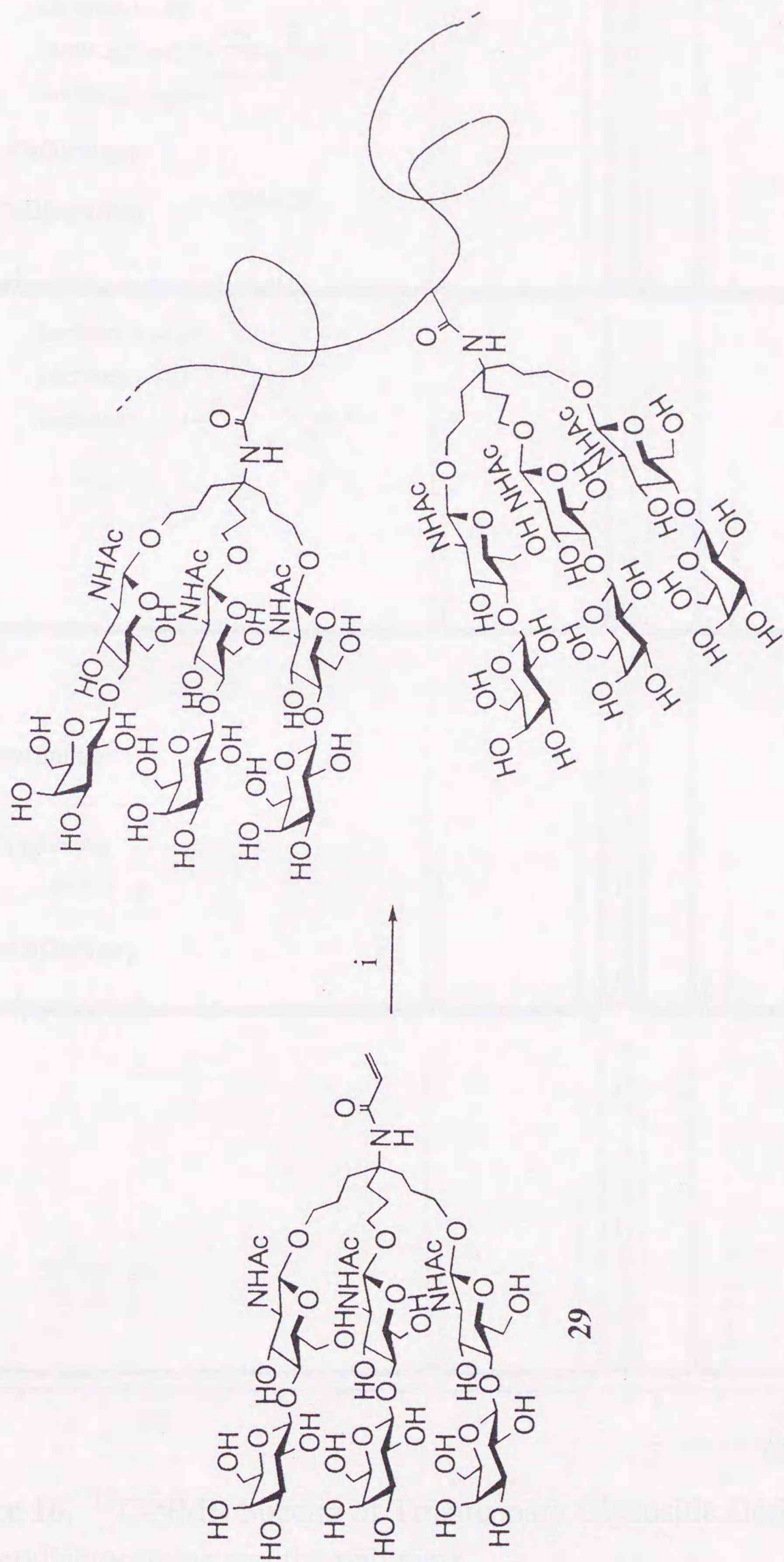
Scheme 9 indicates a synthetic route of a target glycomonomer having triantennary LacNAc residues. Firstly, oxazoline derivative **17**,⁴ readily obtained from *N*-acetylactosamine octaacetate described in the second chapter, was coupled with 4-nitro-4-[1-(3-hydroxypropyl)]-1,7-heptandiol **26**⁹⁻¹¹ as a key starting material in the presence of 10-camphorsulfonic acid (CSA) as the promoter in 1,2-dichloroethane to give nitromethanetrakis[propyl *O*-(2',3',4',6'-tetra-*O*-acetyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy- β -D-glucopyranoside] **3** in 75% yield. The tris-glycoside **3** was hydrogenated in the presence of Raney Ni (T-1)¹² as catalyst, followed by *N*-acryloylation to afford *O*-protected tris-LacNAc monomer **4** in 65% overall yield from **3**. After de-*O*-acetylation by Zemplen method, a desired key glycomonomer **5** was obtained in 95% yield. Next, copolymerization of this triantennary glycomonomer **5** with acrylamide in deionized water was carried out at room temperature in the presence of *N,N,N',N'*-tetramethylethylenediamine (TEMED) and ammonium peroxodisulfate (APS) as initiators (Scheme 10). After 6 h, the viscous solution was diluted with 0.1M acetic acid-pyridine buffer (pH 5.1), subjected to Sephadex G-50 column chromatography with 10 mM ammonium acetate solution as elutant, and the

crude polymer fractions were dialyzed against deionized water. Finally, the solution was lyophilized to afford water-soluble copolymers in 52-91% yield. The sugar content of these copolymers was determined according to the previous chapters.³⁻⁵

The results of copolymerization are summarized in Table 6 together with physical data. Fully assigned ¹³C-NMR spectra of triantennary glycoside **29** and the copolymers in D₂O is shown in Figure 16. The spectrum shows a disappearance of signals due to C-C double bond of glycosides after polymerization and characteristic signals attributed to the ring carbons of sugar residues besides the carbons of polyacrylamide. The method described in this section using Newkome's building block of cascade molecules⁹⁻¹¹ as a polymerizable aglycon seems to be a simpler and more convenient method in terms of the sugar content (41.2-68.2%) than those previously described.³⁻⁵ It was clearly suggested from these trials that the Newkome's building block employed here had excellent properties as a key aglycone to convert a polymerizable triantennary sugar-derivatives, because this structure contains both tris-hydroxyl groups with an appropriate spacer-arm and nitro group as precursor for converting reactive amino group.



Scheme 9. Reagents and conditions : i) CSA / ClCH₂CH₂Cl, 70°C, 2 h; ii) H₂, Raney Ni(T-1) / EtOAc-EtOH, r.t., 48 h; iii) ClCOCH=CH₂, Et₃N / THF, 0°C→r.t., 3 h; iv) NaOMe / MeOH, r.t., 2 h.



Scheme 10. Reagents and conditions : i) $\text{CH}_2=\text{CHCONH}_2$, APS, TEMED / H_2O , r.t., 4 h.

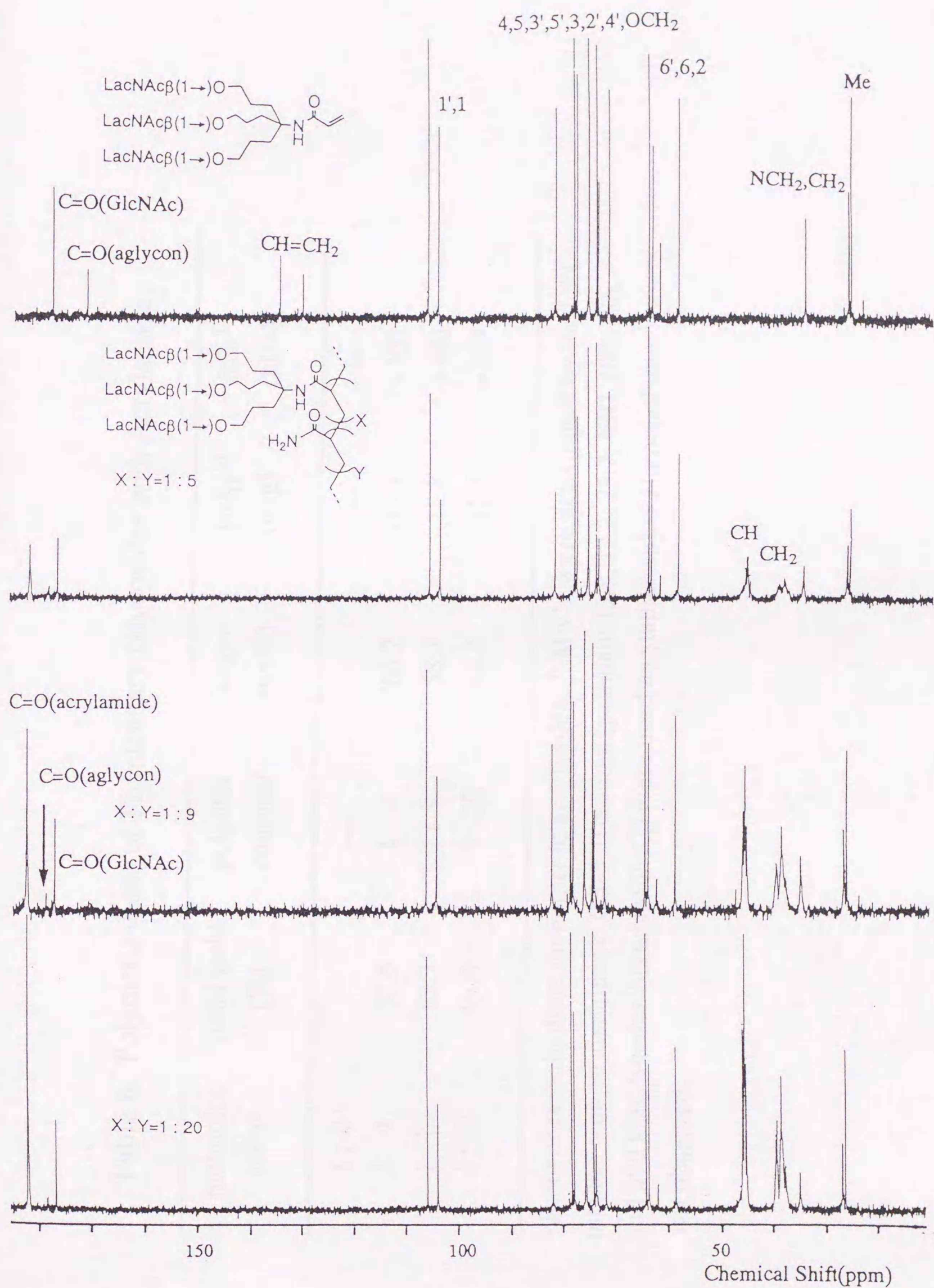


Figure 16. ^{13}C -NMR Spectra of Triantennary Glycoside Derived from *N*-Acetylglucosamine and the polymers.

Table 6. Polymerizations of Triantennary Glycosides with Acrylamide.

monomer ratio ^a	total yield (%)	polymer compos ^a	sugar (wt%)	$[\alpha]_D^{25}$ (deg)	M_w^b (kDa)
1 : 0 ^c	—	—	—	—	—
1 : 4	52.8	1 : 5	68.2	-18.1	>300
1 : 10	83.7	1 : 9	58.7	-15.8	>300
1 : 20	91.4	1 : 20	41.2	-11.3	>300

^a Ratio of carbohydrate monomer to acrylamide. ^b M_w 's were determined by the GPC method with an Asahipack GS-510 column [pullulans (5.8, 12.2, 23.7, 48.0, 100, 186, and 380 kDa, Shodex Standard P-82) were used as standards]. ^c Homopolymer was not observed.

Table 7. ^{13}C -Chemical Shifts of Triantennary Glycosides and the Polymers.(in ppm from TSP ^a)

	Carbohydr. monomers	Copolymers
C-1	103.8	103.6
C-2	58.0	57.8
C-3	75.3	75.1
C-4	81.6	81.6
C-5	78.1	77.9
C-6	63.0	63.0
C-1'	105.7	105.6
C-2'	73.8	73.6
C-3'	77.6	77.4
C-4'	71.4	71.2
C-5'	75.4	75.3
C-6'	63.8	63.6
CH ₂ =CH	129.5	
CH ₂ =CH	133.8	
CH ₂	26.5	25.7
	33.8	34.0
OCH ₂	73.4	73.1
<i>tert</i> -C	61.6	61.3
CH ^b		44.6
CH ₂ ^b		37.7
C=O ^c	176.9	176.5
C=O ^d	170.4	178.1
CONH ₂		181.9
CH ₃	25.1	25.1

^a 3-(Trimethylsilyl)propanesulfonic acid sodium salt. ^b Methylene carbons due to main chain. ^c Signals due to the carbonyl group of GlcpNAc residue. ^d Signals due to the carbonyl groups of aglycons.

4.3. Experimental Section

General Procedure. Melting points were determined with a Laboratory Devices melting point apparatus and are uncorrected. Unless otherwise stated, all commercially available solvents and reagents were used without further purification. 1,2-Dichloroethane, and ethyl acetate were stored over molecular sieves (4 Å) for several days before use. Acrylamide was recrystallized from benzene before use. Nitromethanetrispropanol was purchased from Aldrich Co. Ltd., U.S.A.. Optical rotations were determined with a Horiba SEPA-200 digital polarimeter at 25 °C. ^1H and proton decoupled carbon NMR spectra were recorded at 300 and 75.47 MHz, respectively, on a Bruker AMX-300 spectrometer in chloroform-*d* or deuterium oxide, using tetramethylsilane (TMS), methanol or 3-(trimethylsilyl)-propanesulfonic acid sodium salt (TSP) as internal standards. Average molecular weight was estimated by the gel permeation chromatography (GPC) with an Asahipak GS-510 column, and pullulans (5.8, 12.2, 23.7, 48.0, 100, 186, and 380 K, Shodex Standard P-82) were used as standards. Elemental analyses were performed with a Sartorius 4503 Micro, 7079 printer on the samples extensively (ca. 24 h) dried in vacuo (50 °C, 0.1 Torr) over phosphorus pentoxide. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel 60F₂₅₄ (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany). For detection of the components,

TLC sheets were sprayed with (a) a solution of 85:10:5 (v/v/v) methanol-conc. sulfuric acid-*p*-anisaldehyde and heated for a few minutes (for carbohydrates), (b) a solution of 5wt% ninhydrin in ethanol and heated for a few minutes (for amino groups), or (c) an aqueous solution of 5wt% potassium permanganate and heated similarly (for C-C double bonds). Column chromatography was performed on silica gel (Wakogel C-200; 100-200 mesh, Wako Pure Chemical Industries Co. Ltd., Japan). Solvent extracts were dried with anhydrous magnesium and concentrated below 40 °C under diminished pressure.

Nitromethanetrakis[propyl *O*-(2,3,4,6-Tetra-*O*-acetyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy- β -D-glucopyranoside](27). A solution of oxazoline derivative **17** (940 mg ; 1.53 mmol) and nitromethanetrispropanol (120 mg, 0.510 mmol) in 1,2-dichloroethane (10 mL) were stirred at 70°C in the presence of CSA (150 mg) under a nitrogen atmosphere. After 1 h, a solution of oxazoline derivative **17** (940 mg, 1.53 mmol) in 1,2-dichloroethane (10 mL) was further added to reaction mixture and stirred at 70°C for 1 h. The solution was cooled to room temperature, diluted with chloroform, and poured into ice-water. The organic layer was washed successively with saturated sodium hydrogen carbonate and brine, dried, filtered, and evaporated. The residue was purified by chromatography on silica gel first with 50 : 1 and then with 20 : 1 (v/v) chloroform-methanol to afford compound **27** (800 mg, 75%): mp 132°C; $[\alpha]_D^{25}$

-19.8° (c 0.238, chloroform); $^1\text{H-NMR}$ δ (CDCl_3): 1.45 (m, 6H, $3\times \text{OCH}_2\text{CH}_2\text{CH}_2$), 1.90 (m, 6H, $3\times \text{OCH}_2\text{CH}_2$), 1.96-2.14 (all s, 63H, $21\times \text{OCOCH}_3$), 3.42 (m, 6H, $3\times \text{OCH}_2$), 3.62 (m, 3H, $3\times \text{H-5}$), 3.77 (t, 3H, $J_{3,4}$ 8.5Hz, $3\times \text{H-4}$), 3.80 (m, 3H, $3\times \text{OCH}_2$), 3.88 (m, 3H, $3\times \text{H-5'}$), 3.92 (d, 3H, $J_{1,2}$ 7.6Hz, $3\times \text{H-2}$), 4.05-4.30 (m, 9H, $3\times \text{H-6a}$, $3\times \text{H-6'a}$ and $3\times \text{H-6'b}$), 4.47 (m, 3H, $3\times \text{H-6b}$), 4.49 (d, 3H, $J_{1,2}$ 7.8Hz, $3\times \text{H-1}$), 4.55 (d, 3H, $J_{1',2'}$ 7.9Hz, $3\times \text{H-1'}$), 4.99 (dd, 3H, $J_{3',4'}$ 3.4Hz and $J_{2',3'}$ 10.4Hz, $3\times \text{H-3'}$), 5.09 (m, 3H, $3\times \text{H-2'}$), 5.35 (d, 3H, $3\times \text{H-4'}$), and 6.36 (d, 3H, $J_{2,\text{NH}}$ 9.3Hz, $3\times \text{NH}$); Anal. Calcd for $\text{C}_{88}\text{H}_{126}\text{N}_4\text{O}_{53} \cdot \text{H}_2\text{O}$: C, 50.19; H, 6.12; N, 2.66. Found: C, 50.02; H, 6.12; N, 2.94.

Acryloylaminomthanetris[propyl *O*-(2,3,4,6-Tetra-*O*-acetyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy- β -D-glucopyranoside](28). Compound **27** (470 mg, 0.225 mmol) was hydrogenated at room temperature in the presence of Raney Ni (T-1) (1.0 g) in ethanol (10 mL) and ethyl acetate (5 mL) for 48 h. The reaction was monitored by tlc in 5 : 4 : 1 (v/v/v) chloroform-ethyl acetate-methanol. The mixture was filtered and evaporated to give the crude triantennary glycosyl amine.

To a solution of the crude triantennary glycosyl amine in THF (5.0 mL) were added triethylamine (47.1 μL) and acryloylchloride (27.4 μL) at 0 °C and stirred for 3 h at room temperature. The mixture was evaporated *in vacuo*, and the residue was poured into ice-water and extracted with chloroform. The

organic layer was washed with brine, dried, filtrated and concentrated. The residue was subjected to silica gel chromatography first with 50 : 1 and then with 20 : 1 (v/v) chloroform-methanol as eluant and the pure material was obtained by crystallization from chloroform-ether-*n*-hexane to give **29** (310 mg, 65%) as amorphous powder : mp 142-144°C; $[\alpha]_D^{25}$ -21.2° (c 0.287, chloroform); $^1\text{H-NMR}$ δ (CDCl_3): 1.43 (m, 6H, $3\times\text{OCH}_2\text{CH}_2\text{CH}_2$), 1.90 (m, 6H, $3\times\text{OCH}_2\text{CH}_2$), 1.97-2.15 (all s, 63H, $21\times\text{OCOCH}_3$), 3.57 (m, 3H, $3\times\text{OCH}_2$), 3.69 (m, 3H, $3\times\text{H-5}$), 3.78 (t, 3H, $J_{3,4}$ 8.6Hz, $3\times\text{H-4}$), 3.89 (m, 3H, $3\times\text{H-5'}$), 3.96 (d, 3H, $3\times\text{H-2}$), 4.03 (d, 3H, $J_{6'a,6'b}$ 13.5Hz, $3\times\text{H-6'a}$), 4.09-4.17 (m, 6H, $3\times\text{H-6a}$ and $3\times\text{H-6'b}$), 4.41 (d, 3H, $J_{1,2}$ 7.8Hz, $3\times\text{H-1}$), 4.51 (m, 3H, $3\times\text{H-6b}$), 4.54 (d, 3H, $J_{1',2'}$ 7.7Hz, $3\times\text{H-1'}$), 4.99 (dd, 3H, $J_{3',4'}$ 3.3Hz and $J_{2',3'}$ 10.7Hz, $3\times\text{H-3'}$), 5.05 (d, 3H, $J_{3,4}$ 8.7Hz, $3\times\text{H-3}$), 5.11 (dd, 3H, $J_{1',2'}$ 7.7Hz and $J_{2',3'}$ 10.4Hz, $3\times\text{H-2'}$), 5.36 (d, 3H, $3\times\text{H-4'}$), 5.69 [dd, 1H, $\text{CH}=\text{CH}_2(\text{cis})$], 5.86 [br s, 1H, $\text{NH}(\text{aglycon})$], 6.22 [dd, 1H, $-\text{CH}=\text{CH}_2(\text{trans})$], 6.43 (dd, 1H, $\text{CH}=\text{CH}_2$), 6.56 (d, 3H, $3\times\text{NH}$); Anal. Calcd. for $\text{C}_{91}\text{H}_{130}\text{N}_4\text{O}_{52}$: C, 51.75; H, 6.20; N, 2.65. Found: C, 51.65; H, 6.34; N, 2.72.

Acryloylaminomethanetrakis[propyl *O*-(β -D-Galactopyranosyl)-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- β -D-glucopyranoside](29**).** To a solution of compound **28** (250 mg, 0.118 mmol) in dry methanol (10 mL) was added sodium methoxide (40mg), and the mixture was stirred for 2 h at room temperature. After water

was added, it was neutralized with Dowex 50W-X8 (H^+) resin, filtered, and evaporated *in vacuo*. The residue was crystallised from 95% ethanol to give **29** (187 mg, 95%): m.p. 222°C(dec.); $[\alpha]_D^{25}$ -28.3° (c 0.231, water); 1H -NMR δ ($CDCl_3$): 1.49 (m, 6H, $3 \times OCH_2CH_2CH_2$), 1.74 (m, 6H, $3 \times OCH_2CH_2$), 2.13 (s, 9H, $3 \times NHCOCH_3$), 4.48 (d, 3H, $J_{1,2}$ 7.8Hz, $3 \times H-1$), 4.52 (d, 3H, $J_{1',2'}$ 7.5Hz, $3 \times H-1'$), 5.71 [d, 1H, $-CH=CH_2(cis)$], 6.15 [d, 1H, $-CH=CH_2(trans)$], 6.29 (dd, 1H, $-CH=CH_2$); Anal. Calcd. for $C_{54}H_{94}N_4O_{34}$: C, 48.28; H, 7.05; N, 4.17. Found: C, 48.05; H, 7.06; N, 4.01.

Copolymerization of 29 with Acrylamide. A solution of triantennary glycoside **29** (100 mg ; 0.202 mmol) and 4,10 or 20 equivalent molar of acrylamide in 1.0 mL of deionized water was deaerated for a while using water pump, to which were added TEMED (0.1 equivalent molar of the carbohydrate monomer) and APS (0.04 equivalent molar of the carbohydrate monomer). The reaction mixture was continuously stirred for 4 h at room temperature, diluted with 0.1M acetic acid -pyridine buffer(pH 5.1), dialyzed against deionized water, and lyophilized to give water-soluble copolymers as white powder.

The physical data and ^{13}C -NMR chemical shifts of all polymers prepared above are summarized in Table 6 and Table 7, respectively.

4.4. Conclusion

An efficient synthetic procedure of triantennary glycopolymers having pendant *N*-acetylglucosamine residues was established. It was demonstrated from the polymerization experiment that 4-nitro-4-[1-(3-hydroxypropyl)]-1,7-heptandiol seemed to be an excellent and convenient compound to prepare clustered glycopolymers bearing triantennary glycoside. These glycopolymers will be much useful for investigating binding-interaction with liver lectins, a variety of sugar-binding proteins that specifically recognize clustered *N*-acetylglucosamine residues.

4.5. References

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Chapter 5.

A Facile Synthetic Method of Lactosaminoglycan Models Using 1,6-Anhydrolactose Derivatives as Key Materials

The simple and useful methods for synthesis of lactosaminoglycan models using 1,6-anhydrolactose as a starting material have been established by regioselective chemical modifications of 1,6-anhydro- β -lactose as a key starting material. Each synthetic routes through two useful intermediates such as 3'-*O*-allyl-6'-*O*-triphenylmethyl or 3',6'-di-*O*-allyl derivatives was used for the selective deprotections at C-3' and C-6' position and gave novel standerdized intemediates. Moreover, glycosidations of the 3',6'-dihydroxyl derivative with *N*-phthalimido amino-sugars afforded corresponding tri- and tetrasaccharides, respectively.

5.1. Introduction

The lactosaminoglycans are high molecular weight carbohydrate bound to proteins or lipids and unique in having *N*-acetylglucosaminyl repeats $[\text{Gal}\beta(1\rightarrow4)\text{GlcNAc}\beta(1\rightarrow3)]_n$. These essentially antigenic determinants have been known as Ii-antigenic carbohydrates. The branching lactosaminoglycan, especially named as I-antigen, was greatly interesting and important structures not only the immunochemical roles but also as unique carbohydrate sequences.¹

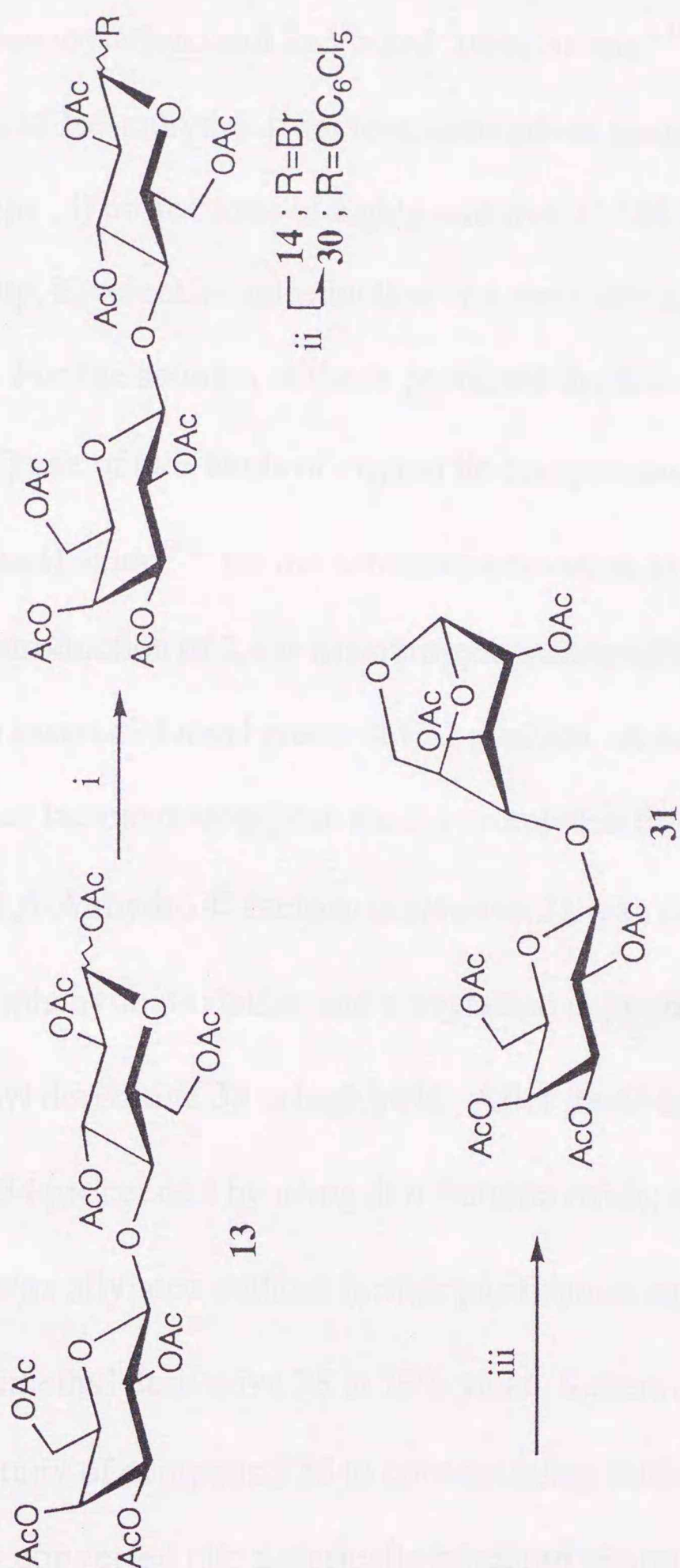
In the case of chemical synthesis of oligosaccharide moieties of branching lactosaminoglycan models, the sequential synthesis of a protected monosaccharide have been mainly reported.²⁻⁴ However, the method included a few difficult problems, for example, requirement of complicated synthetic strategies involving protection and deprotection, purification of anomeric mixture in glycosylation reaction and so on. Therefore, more easier and versatile synthetic procedures should be designed by using oligosaccharides existing in nature.

The chemistry of 1,6-anhydro- β -lactose was partly reported by Kuzuhara et al.⁵ and Tejima et al..⁶⁻¹⁰ Moreover, Nishimura et al. demonstrated using this compound as a key starting material that an important LewisX(Le^x) trisaccharide can be synthesized through an easy manipulations of this conformationally regulated intermediate.^{11,12} Therefore, synthetic strategy

using the 1,6-anhydro- β -lactose will permit the efficient syntheses of a series of lactosaminoglycan models, because all hydroxyl groups of 1,6-anhydro- β -lactose can be distinguished by selective chemical modifications. Thus, in this chapter, simple and much more useful methods for synthesis of lactosaminoglycan models using 1,6-anhydrolactose as a starting material will be described.

5.2. Results and Discussion

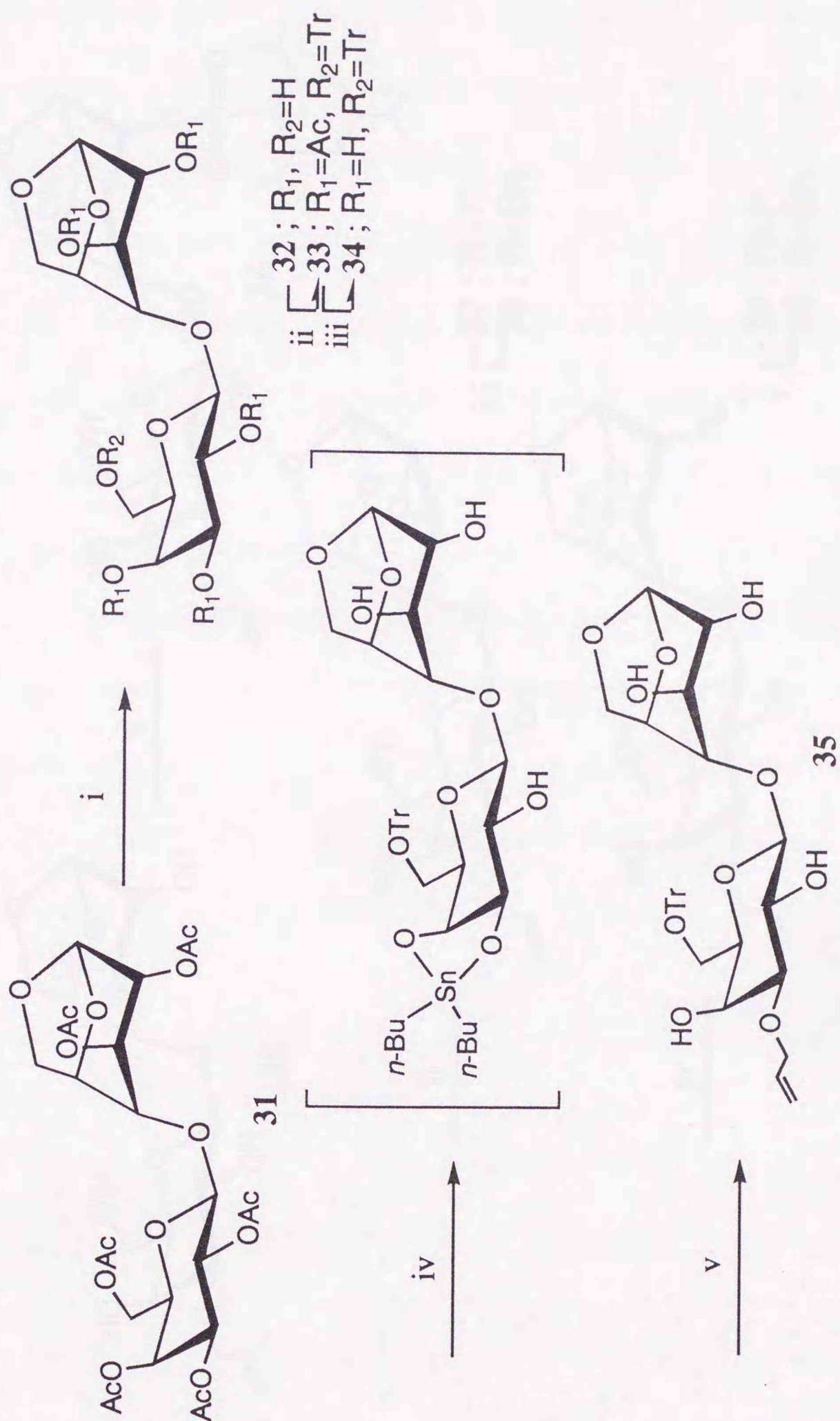
Preparation of 1,6-anhydro- β -lactose as a key starting material. In versatile syntheses of lactosaminoglycan models, a readily available 1,6-anhydro- β -lactose derivatived from lactose monohydrate was selected as a key starting material for the efficient synthesis of a key glycosyl acceptor having an unprotected hydroxyl group only at C-3' or C-6' position (Scheme 11). According to the procedure reported by Kuzuhara et al.,⁵ The lactose pentachlorophenoxide was prepared *via* bromide **14** from per-*O*-acetylated lactose **13**. The syrupy **30** was heated with aqueous potassium hydroxide and then acetylated, the desired 1,6-anhydrolactose per-*O*-acetate **31** was obtained as crystals in 47% yield from octaacetyl lactose **13**.



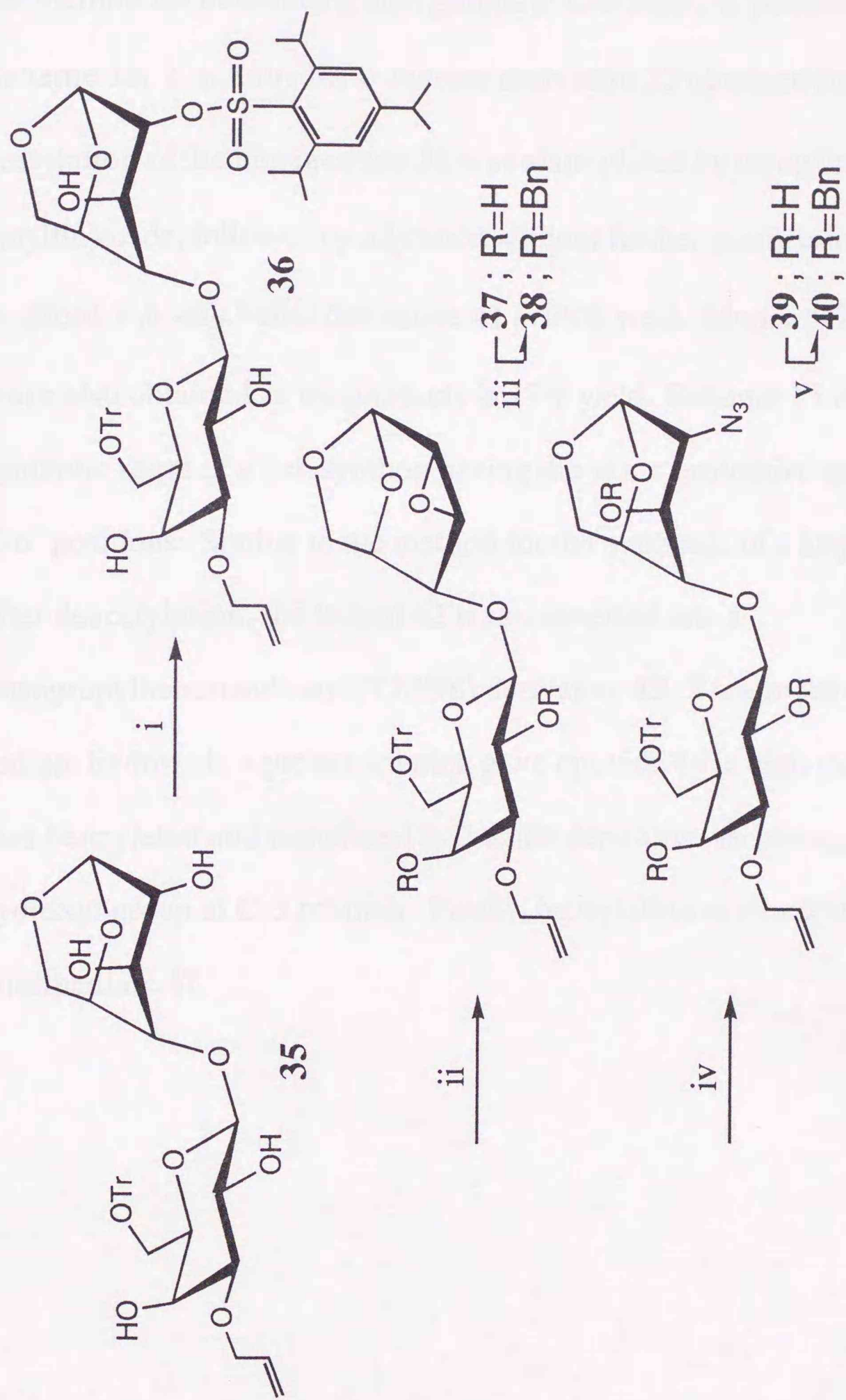
Scheme 11. Reagents and conditions : i) 25% HBr-AcOH, Ac₂O / CHCl₃, 0°C→r.t., 10 h;
 ii) NaOC₆Cl₅ / acetone, 60°C, 19 h; 5N KOH aq., 100°C, 24 h; then Ac₂O, DMAP /
 Pyridine, 0°C→r.t., 14 h.

Synthesis of 3'-O-allyl-6'-O-triphenylmethyl derivative 41. Tejima et al. reported that the order of reactivities of the secondary hydroxyl groups were $3' > 2 > 3 > 2'$ (benzoylation) and $2 > 3' > 3 > 2'$ (tosylation).⁶⁻¹⁰ The regioselective modifications of 1,6-anhydro- β -lactose derivatives seem to require two important steps ; i) protections of highly reactive 3'-OH group prior to other hydroxyl group, ii) selective introduction of a sterically hindered leaving group at C-2 position. For the solution of these problems the following procedures were considered ; i) use of two kinds of organo tin compounds, di-*n*-butyltin oxide¹³ or bis(tri-*n*-butyltin) oxide¹⁴⁻¹⁶ for the selective activation at C-3' or C-6' position ; ii) selective introduction of 2,4,6-triisopropylbenzenesulfonyl group as a bulky leaving group instead of tosyl group at C-2 position. A selective protection for the synthesis of lactosaminoglycan model selected in this study is shown in Scheme 12. 1,6-Anhydro- β -lactose derivative **31** was de-*O*-acetylated, followed by triphenylmethylation and acetylation in pyridine to give 6'-*O*-triphenylmethyl derivative **33** in high yield. After de-*O*-acetylation, stannylation of compound **34** proceeded by using di-*n*-butyltin oxide, and the instable intermediate was allylated without further purification and afforded 3'-*O*-allyl-6'-*O*-triphenylmethyl derivative **35** in 73% yield. Scheme 13 indicates further modification study of compound **35** to convert a key intermediate **40**. The tetraol **35** was converted into a sterically hindered triisopropylbenzenesulfonyl (TIPBS) derivative **36** having a leaving group at C-2 position. Intramolecular

nucleophilic substitution reaction of compound **36** with 1N sodium hydroxide aqueous solution efficiently gave an epoxide **37**. 2'4'-di-*O*-benzyl derivative **38** was subsequently prepared by usual benzylation of the epoxide **37**. Furthermore, nucleophilic attack with azide ion in the presence of cesium salt as accelerator of the perfectly protected epoxide **38** was carried out stereospecifically to yield 2-azido derivative **39**. Finally, benzylation of an unprotected hydroxyl group at C-3 position gave a fully functionalized disacchride acceptor **40** in high yield.

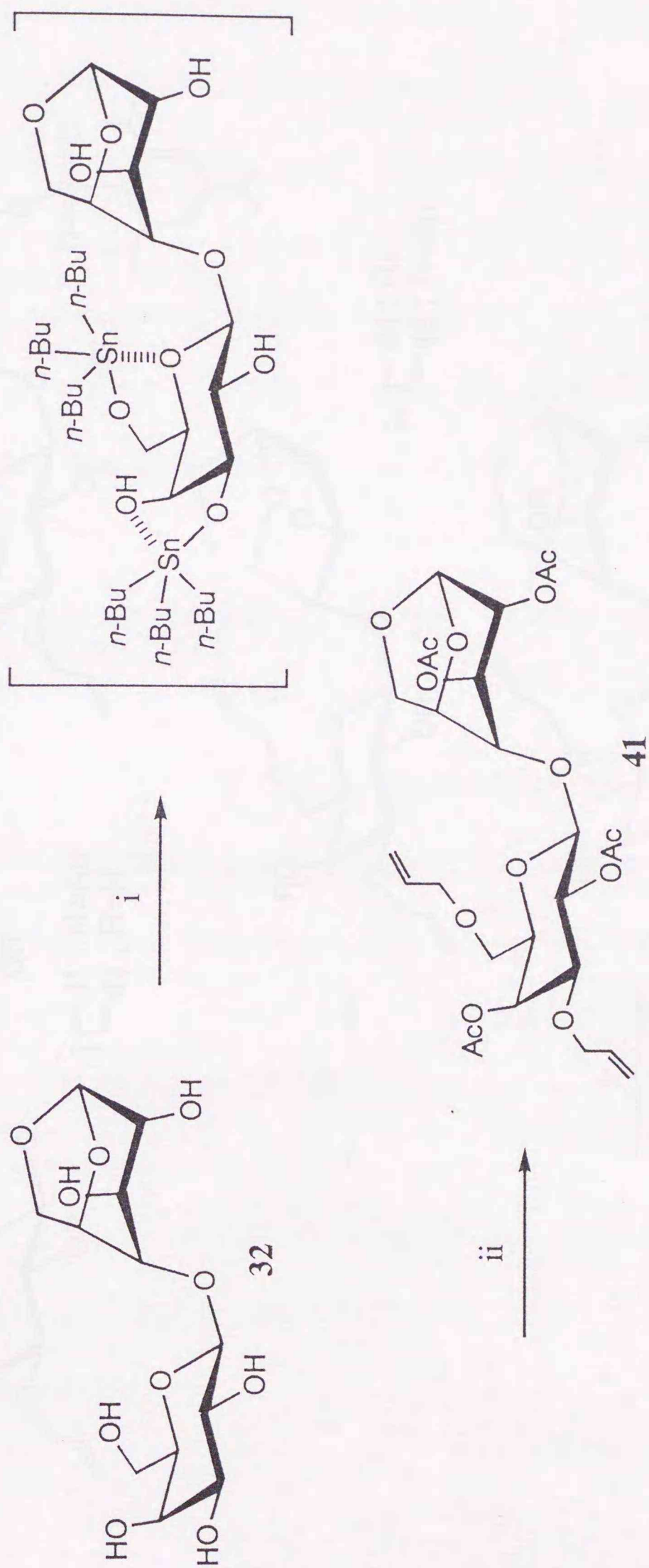


Scheme 12. Reagents and conditions : i) NaOMe / MeOH-THF, r.t., 6 h; ii) TrCl, DMAP / Pyridine, 90°C, 24 h; then added Ac₂O, 0°C→r.t., 12 h; iii) NaOMe / MeOH-THF, r.t., 3 h; iv) *n*-Bu₂SnO / toluene, azeotrope, 4 h; v) allyl bromide, reflux, 5 h.

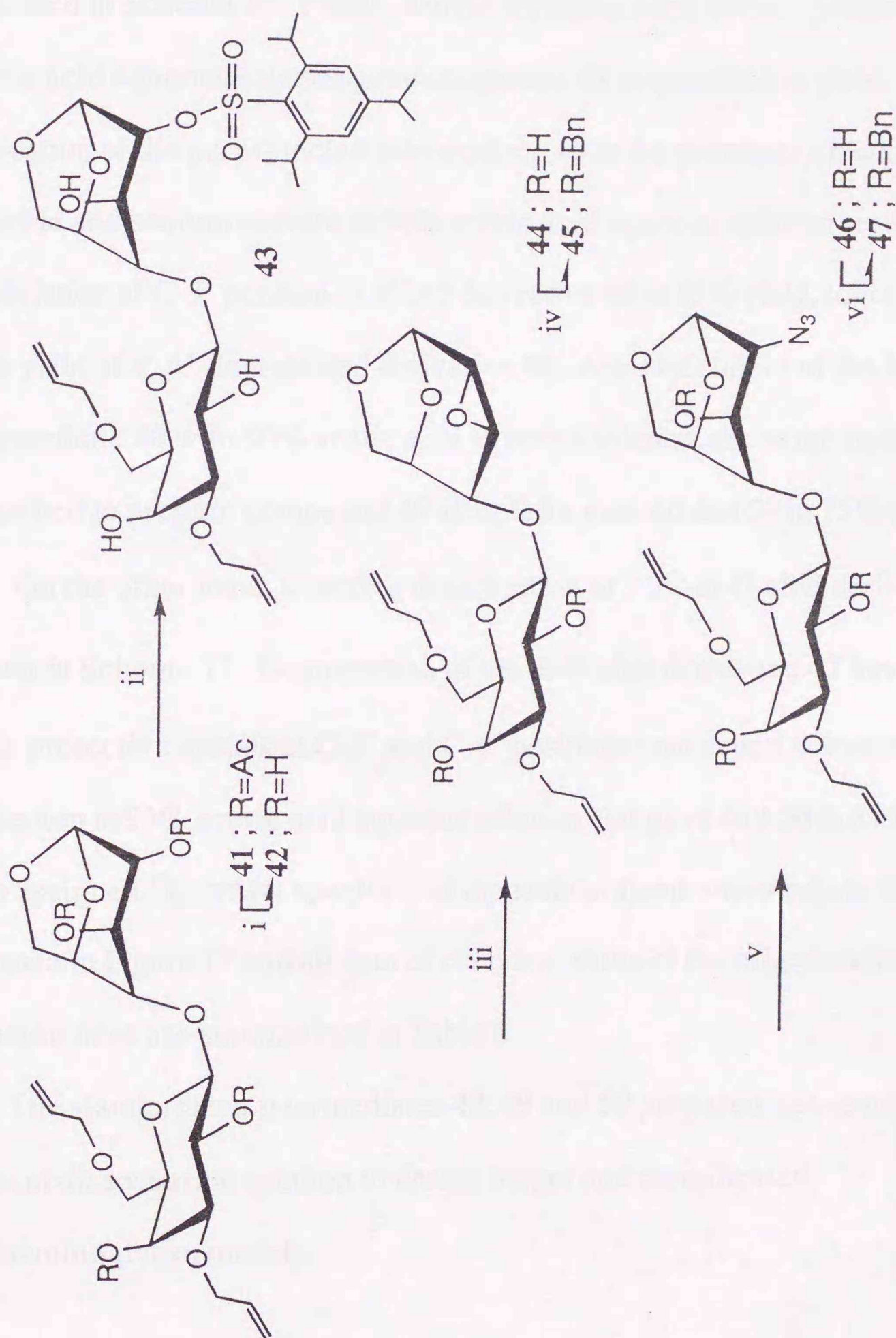


Scheme 13. Reagents and Conditions : i) TIPBS-Cl, DMAP / Pyridine, $0^{\circ}\text{C} \rightarrow \text{r.t.}$, 16 h; ii) 1N NaOHaq. / MeOH-THF, $0^{\circ}\text{C} \rightarrow \text{r.t.}$, 24 h; iii) BnBr, NaH / DMF, $0^{\circ}\text{C} \rightarrow \text{r.t.}$, 42 h; iv) NaN₃, CsF / DMF, 110°C , 15 h; v) BnBr, NaH / DMF, $0^{\circ}\text{C} \rightarrow \text{r.t.}$, 12 h.

Synthesis of 3',6'-di-*O*-allyl derivative 47. As an alternative strategy, the method for introducing allyl groups at C-3' and C-6' positions is shown in Scheme 14. 1,6-Anhydro- β -lactose derivative **32** obtained from the de-*O*-acetylation of the hexaacetate **31** was stannylated by using bis (tri-*n*-butyltin)oxide, followed by allylation without further purification and acetylation to afford 3',6'-di-*O*-allyl derivative **41** in 68% yield. Monoallylated products were also obtained as by-products in 17% yield. Scheme 15 indicates the synthetic route of a key synthon having the same protective groups at C-3' and C-6' positions. Similar to the method for the synthesis of a key intermediate **40**, after deacetylation, the tetraol **42** was converted into a triisopropylbenzensulfonyl (TIPBS) derivative **43**. Treatment of triol **43** with 1N sodium hydroxide aqueous solution gave epoxide **44** in high yield, the epoxide was benzylated and transferred to 2-azido derivative **46** having an unprotected hydroxyl group at C-3 position. Finally, benzylation of compound **46** gave a key intermediate **47**.



Scheme 14. Reagents and Conditions : i) $(n\text{-Bu}_3\text{Sn})_2\text{O}$ / toluene, azeotrope, 4 h; ii) Allyl bromide, 80°C , 12 h; then Ac_2O / Pyridine, $0^\circ\text{C} \rightarrow \text{r.t.}$, 15 h.

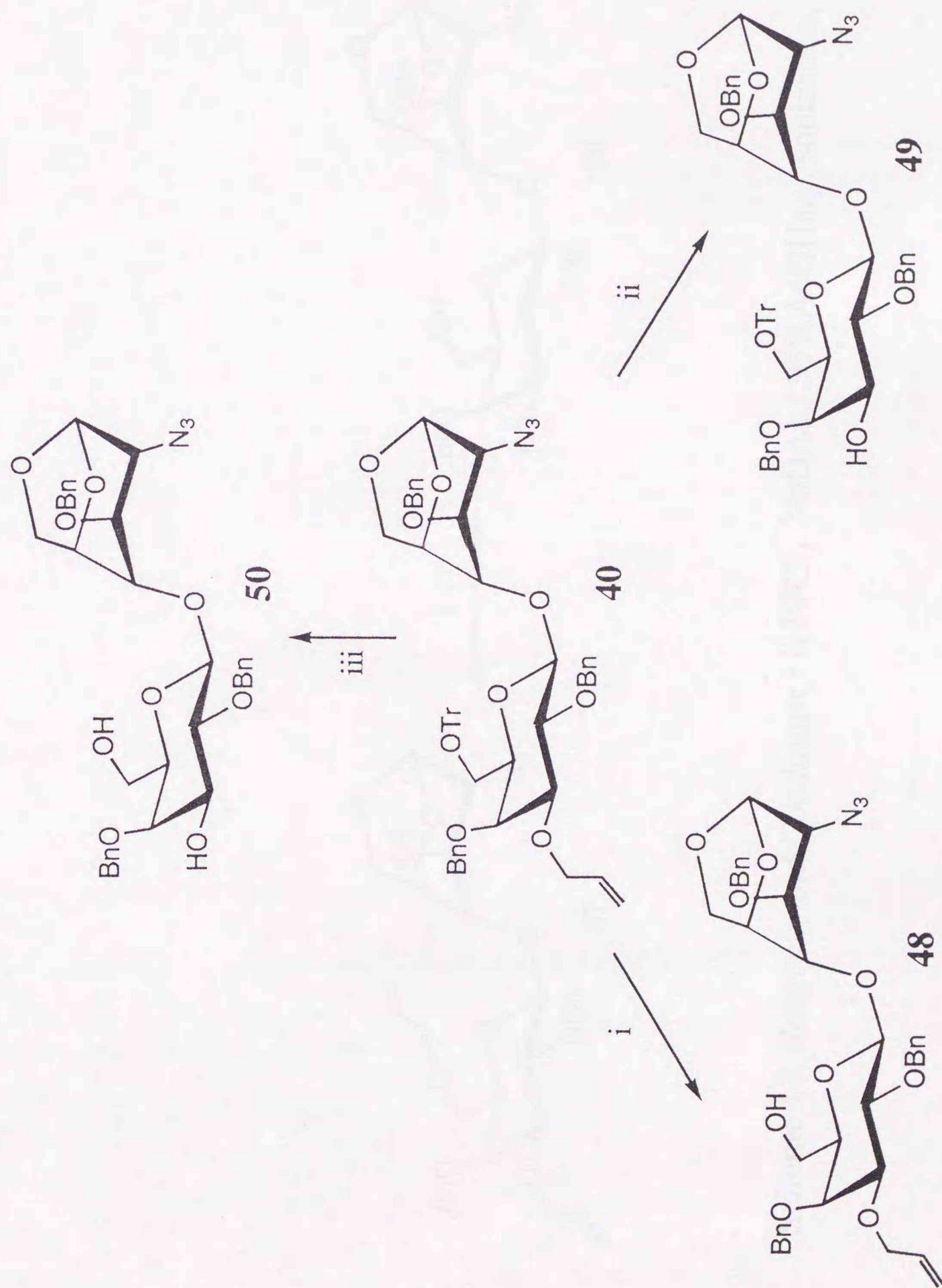


Scheme 15. Reagents and Conditions : i) NaOMe / MeOH-THF, r.t., 3 h; ii) TIPBS-Cl, DMAP / Pyridine, 0°C → r.t., 72 h; iii) 1N NaOHaq. / MeOH-THF, 0°C → r.t., 24 h; iv) BnBr, NaH / DMF, 0°C → r.t., 24 h; v) NaN₃, CsF / DMF, 110°C, 24 h; vi) BnBr, NaH / DMF, 0°C → r.t., 14 h.

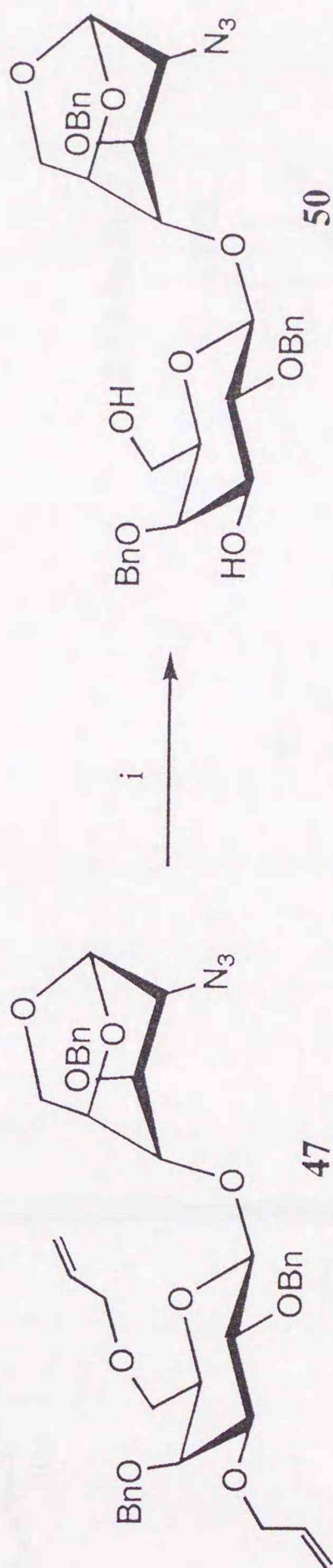
Deprotections of key intermediates 40 and 47. Selective deprotection at C-3' or C-6' position of 3'-*O*-allyl-6'-*O*-triphenylmethyl derivative **40** was indicated in Scheme 16. Firstly, detriphenylmethylation at C-6' position with 90% acetic acid aqueous solution gave compound **48** in quantitative yield. Next, sonication of the perprotected intermediate **40** in the presence of palladium (II) chloride and sodium acetate in 90% acetic acid aqueous solution resulted in the deallylation at C-3' position to afford derivative **49** in 65% yield, together with 18% yield of 3',6'-deprotected derivative **50**. After treatment of the key intermediate **40** with 90% acetic acid aqueous solution, the same condition as described to prepare compound **49** afforded a desired diol **50** in 75% yield.

On the other hand, selective deprotection of 3',6'-di-*O*-allyl derivative is shown in Scheme 17. Deprotection of the di-*O*-allyl derivative **47** having the same protective groups at C-3' and C-6' positions proceeded smoothly by sonication in 95% acetic acid aqueous solution and gave diol **50** in 81% yield. Fully assigned ¹³C-NMR spectrum of the standardized intermediate **50** in CDCl₃ is shown in Figure 17 and all data of chemical shifts of the oligosaccharides prepared here are summarized in Table 8.

The standardized intermediates **48**, **49** and **50** prepared above will be much efficient disaccharide synthon to design longer and complicated lactosaminoglycan models.



Scheme 16. *Reagents and Conditions* : i) 80% AcOHaq., 70°C, 1 h; ii) PdCl₂, NaOAc / 95% AcOHaq., sonication, 6 h; iii) 90% AcOHaq., 70°C, 1 h; then PdCl₂, NaOAc / 95% AcOHaq., sonication, 2 h.



Scheme 17. Reagents and Conditions : i) PdCl_2 , NaOAc / 95% AcOH_{aq} , sonication, 2 h.

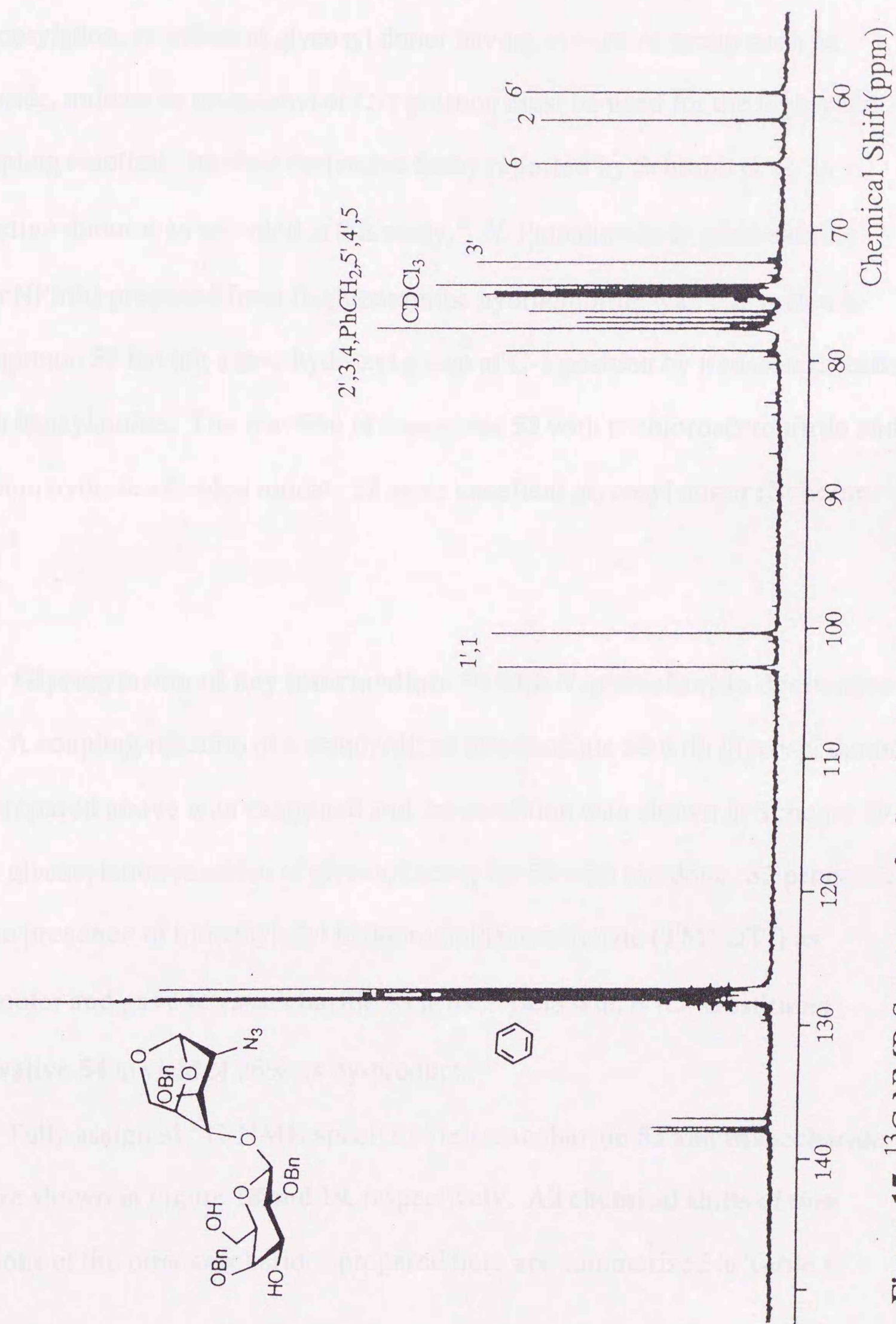
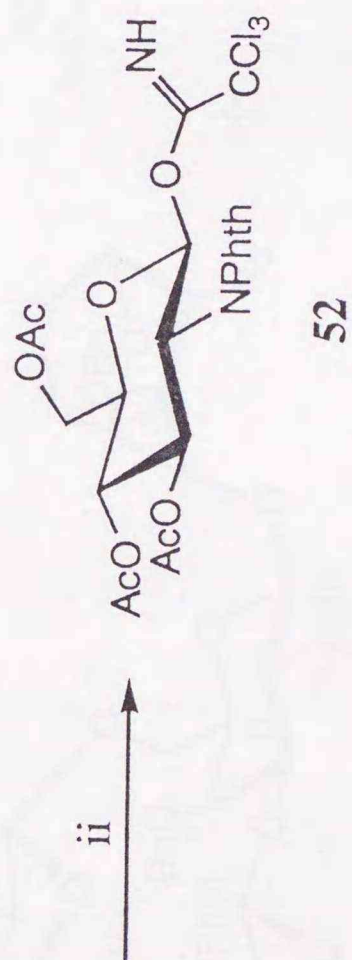
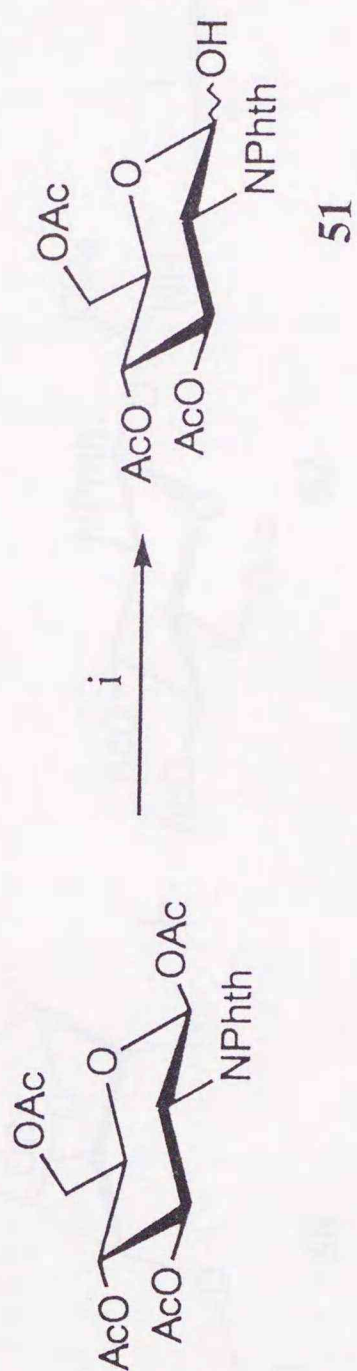


Figure 17. ¹³C-NMR Spectrum of a Standardized Intermediate 50.

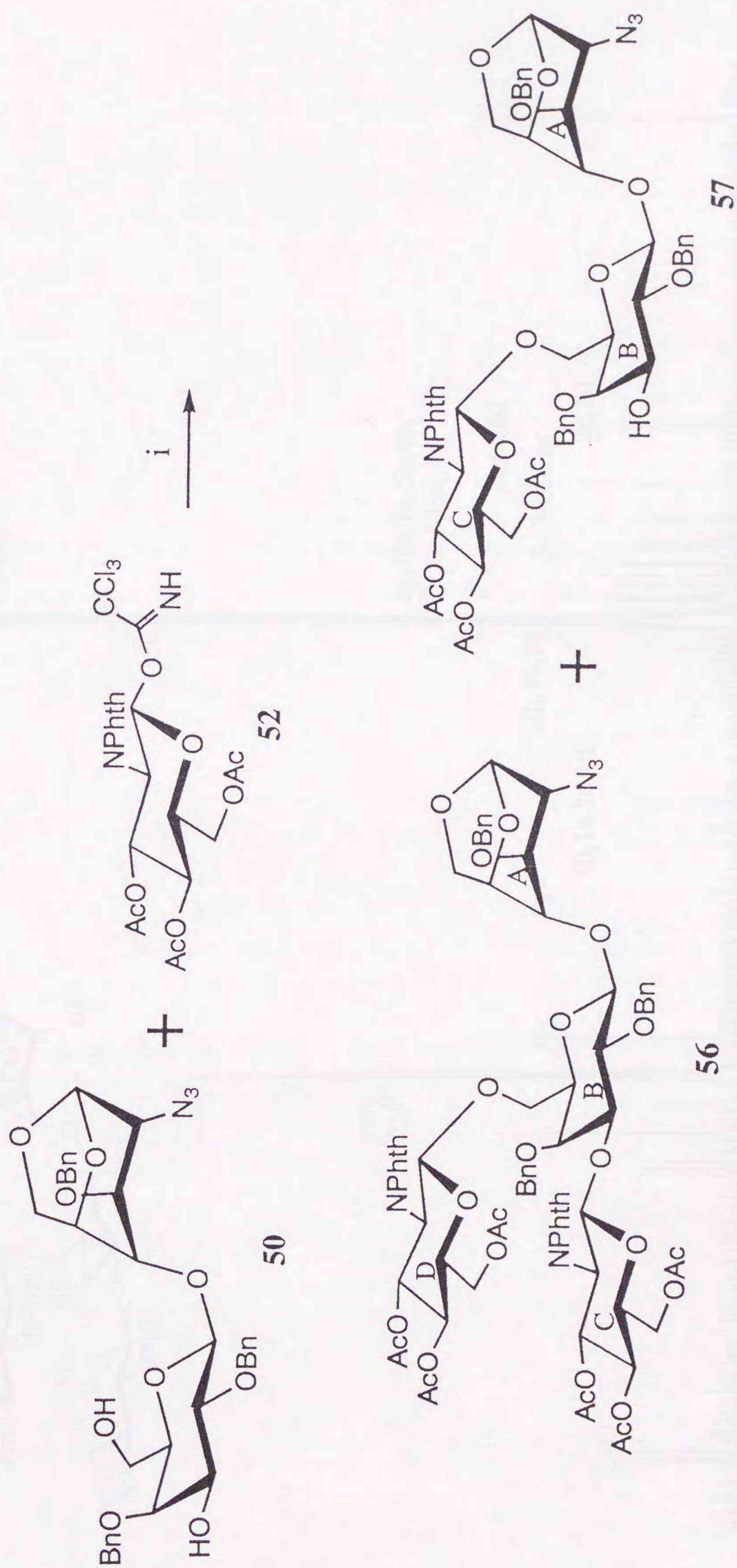
***N*-Phthalimide derivatives 52 as glycosyl donor.** In general glycosylation, an efficient glycosyl donor having a reactive group such as fluoride, imidate or thiophenyl at C-1 position must be used for the high-yield coupling reaction. Imidate derivative firstly reported by Schmidt et al. as reactive donor was selected in this study.¹⁷ *N*-Phthalimido-D-glucosamine (GlcNPhth) prepared from D-glucosamine hydrochloride was converted to compound **51** having a free hydroxyl group at C-1 position by transesterification with benzylamine. The reaction of compound **51** with trichloroacetonitrile and sodium hydride afforded imidate **52** as an excellent glycosyl donor (Scheme 18).¹⁸

Glycosylation of key intermediate 50 with *N*-phthalimido derivative 52. A coupling reaction of a standardized intermediate **50** with glycosyl donors **52** prepared above was examined and the condition was shown in Scheme 19. The glycosylation reaction of glycosyl acceptor **50** with the donor **52** proceeded in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as promoter and gave tetrasaccharide **53** in 69% yield with 6'-*O*-substituted derivative **54** in yield of 26% as by-product..

Fully assigned ¹³C-NMR spectra of tetrasaccharide **53** and trisaccharide **54** are shown in Figure 18 and 19, respectively. All chemical shifts of ring carbons of the oligosaccharides prepared here are summarized in Table 8.



Scheme 18. Reagents and Conditions : i) BnNH_2 / CH_2Cl_2 , r.t., 2 h; ii) CCl_3CN , NaH / CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{r.t.}$, 2 h.



Scheme 20. Reagents and Conditions : i) TMSOTf, MS4Å / CH₂Cl₂, -15°C, 1h.

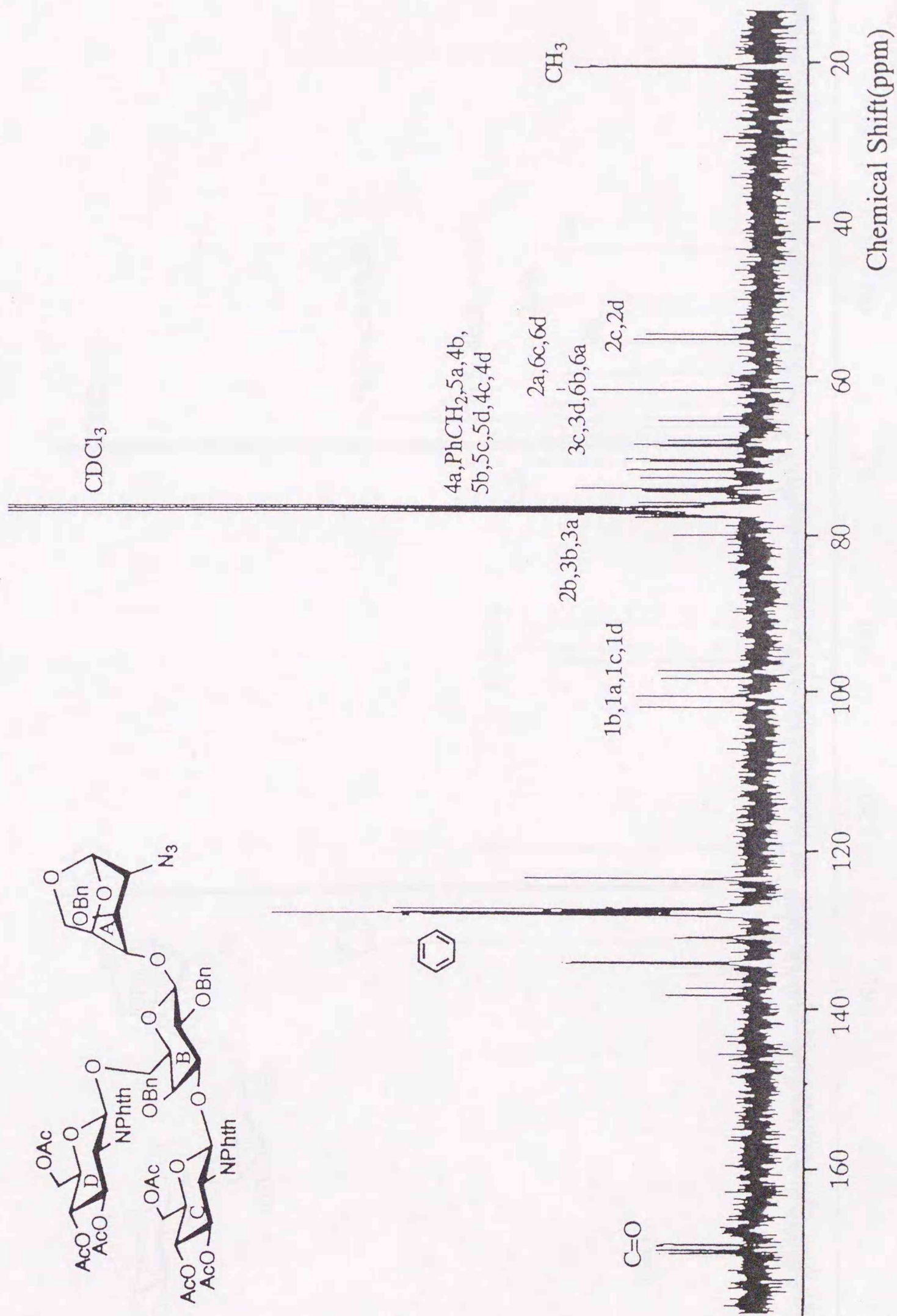


Figure 18. ^{13}C -NMR Spectrum of Tetrasaccharide **53** Derived from Standardized Intermediate **50** with Glycosyl Imidate **52**.

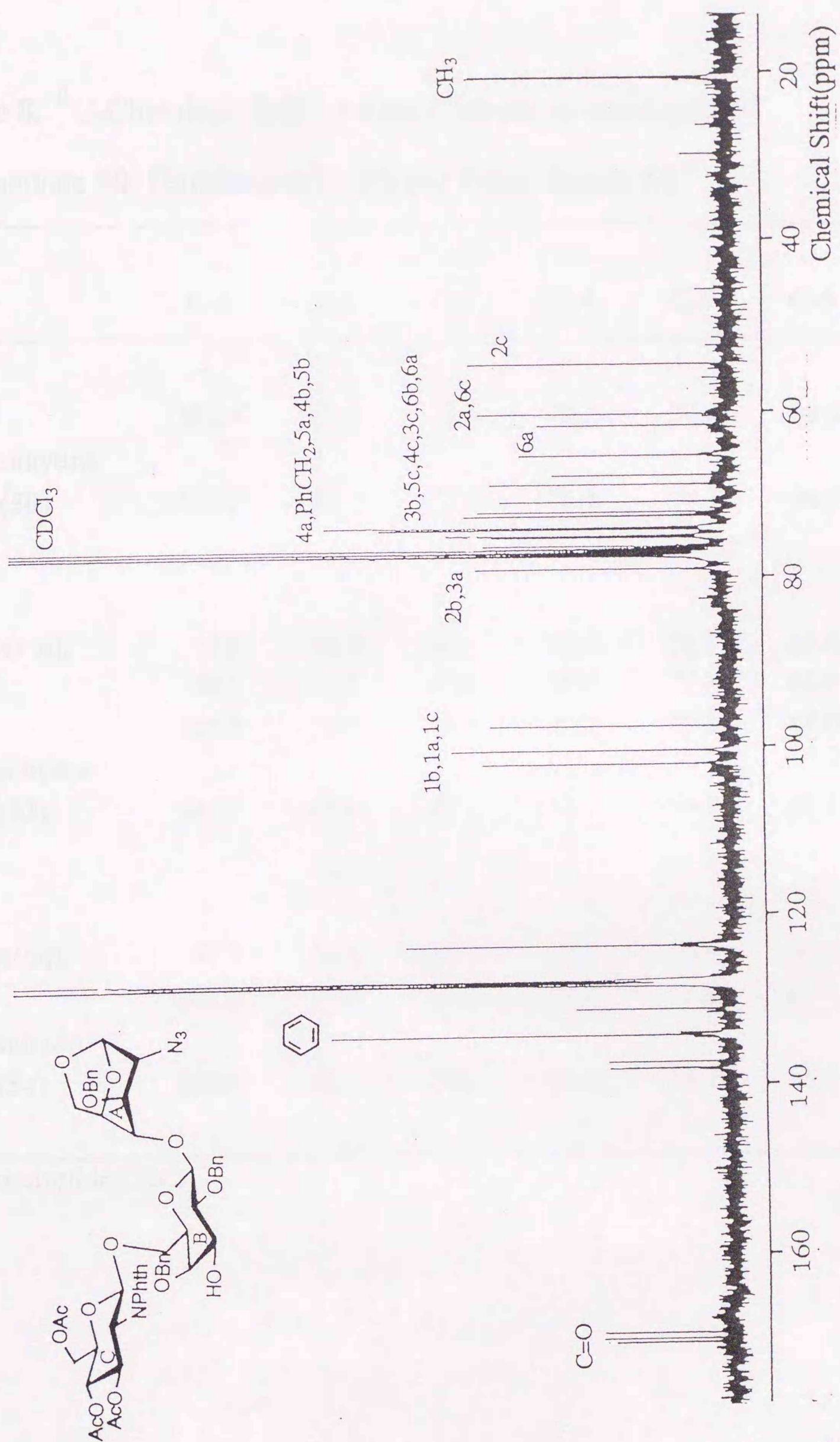


Figure 19. ^{13}C -NMR Spectrum of Trisaccharide **54** Derived from Standardized Intermediate **50** with Glycosyl Imidate **52**.

Table 8. ^{13}C -Chemical Shifts of Ring Carbons of Standardized Intermediate **50**, Tetrasaccharide **53** and Trisaccharide **54**.^a

	C-1	C-2	C-3	C-4	C-5	C-6
Gal	103.1	79.0	72.3	74.1	74.4	59.6
1,6-anhydro						
Glc (50)	100.5	61.7	77.4	76.1	74.1	64.9
GlcNPhth	97.5	54.4	68.5	70.4	71.7	61.6
	98.5	55.1	68.8	70.8	71.8	61.6
Gal	102.2	78.1	80.3	74.2	72.8	68.0
1,6-anhydro						
Glc (53)	100.7	61.6	77.7	76.1	74.2	65.5
GlcNPhth	97.7	54.5	68.7	70.7	71.9	60.4
Gal	102.2	79.0	72.4	73.8	73.8	67.6
1,6-anhydro						
Glc (54)	100.6	61.7	77.4	76.1	74.1	65.2

^a measured in CDCl_3 .

5.3. Experimental Section

General Procedure. Unless otherwise stated, all commercially available solvents and reagents were used without further purification. Chloroform (CHCl_3), methanol (MeOH) and toluene were stored over molecular sieves 4 Å (MS 4 Å) before used. Pyridine was dried over potassium hydroxide, and tetrahydrofuran (THF) and *N,N*-dimethylformamide (DMF) were distilled before use. ^1H -NMR spectra were recorded at 400 MHz with a JEOL EX-400 or JEOL ALPHA-400 spectrometer in chloroform-*d* at 27°C, using tetramethylsilane (TMS) as internal standard. Ring-proton assignments in NMR were made by first-order analysis of the spectra and supported by HH-COSY experiments. Reactions were monitored by thin-layer chromatography (t.l.c.) on a precoated plate of silica gel 60F₂₅₄ (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany). For detection of the sugar components, t.l.c. sheets were sprayed with (a) a solution of 85:10:5 (v/v/v) methanol-conc. sulfuric acid-*p*-anisaldehyde, and heated for a few minutes (for carbohydrate detection), or with (b) an aqueous solution of 5 wt% potassium permanganate and heated similarly (for detection of double bonds). Column chromatography was performed on silica gel (Wakogel C-200; 100-200 mesh, Wako Pure Chemical Industries Co. Ltd. Japan). Solvent extracts were dried with anhydrous magnesium and concentrated below 40 °C under diminished pressure.

O-(2,3,4,6-Tetra-*O*-acetyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,3-di-*O*-acetyl-1,6-anhydro- β -D-glucopyranose (**31**). Lactosyl bromide **14** prepared from lactose octaacetate **13** (150 g, 0.221 mol) according to same manner as described in the chapter 2 was dissolved in acetone (500 mL). To the solution was added sodium pentachlorophenoxide (85.0 g, 0.265 mol) and vigorously stirred for 19 h at 60°C. The resulting solution was filtered, the filtrate was evaporated and extracted with dichloromethane. The organic layer was washed with water, dried and concentrated to syrup **30**. An aqueous solution of approximately 5N potassium hydroxide (500 mL) was added to syrup **30** and the mixture was heated at 100°C for 24 h with stirring. After having been cooled, the resulting solution was adjusted to pH 5 with 4N sulfuric acid to give insoluble inorganic salt, which was filtered off. The filtrate was readjusted to pH 7 with solid sodium hydrogen carbonate and evaporated *in vacuo*. The residue was suspended in pyridine (300 mL) and to the mixture was added acetic anhydride (200 mL) at 0°C. The suspension was vigorously stirred at room temperature for 14 h. After evaporation, the residue was poured into ice-water and extracted with chloroform. The extract was washed with 1N sulfuric acid, saturated sodium hydrogen carbonate and brine, dried and concentrated. Recrystallization from 1 : 1(v/v) chloroform-ethanol gave **31** (60.1 g, 47% overall from lactose per-*O*-acetate) : mp 205-206°C ; $^1\text{H-NMR}$ $\delta(\text{CDCl}_3)$: 1.87-2.04 (all s, 18H, 6COCH₃), 3.57 (t, 1H, $J_{5',6'a}$ 8.6Hz, H-5'), 3.35 (dd, 1H, $J_{5,6'a}$ 8.8 and $J_{6'a,6'b}$ 5.6Hz, H-6'a), 3.56 (s, 1H, H-5'), 3.79 (dd, 1H, $J_{5,6a}$ 5.9 and $J_{6a,6b}$ 7.5Hz, H-6a), 4.00 (m,

2H, H-4 and H-6b), 4.05 (dd, 1H, $J_{5',6'a}$ 6.5 and $J_{6'a,6'b}$ 11.0Hz, H-6'a), 4.15 (dd, $J_{5',6'b}$ 6.3 and $J_{6'a,6'b}$ 11.0Hz, H-6'b), 4.55 (s, 1H, H-3), 4.60 (d, 1H, $J_{5,6a}$ 5.8Hz, H-5), 4.79 (d, 1H, $J_{1',2'}$ 7.8Hz, H-1'), 5.02 (dd, 1H, $J_{2',3'}$ 10.3 and $J_{3',4'}$ 3.4Hz H-3'), 5.16 (s, 1H, H-2), 5.27 (t, 1H, $J_{1',2'}$ 7.8 and $J_{2',3'}$ 10.2Hz, H-2'), 5.38 (d, 1H, $J_{3',4'}$ 3.2Hz, H-4'), and 5.43 (s, 1H, H-1).

***O*-(β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1,6-anhydro- β -D-glucopyranose (32).**

To a solution of **31** (44.5 g, 77.2 mmol) in 1 : 1 (v/v) methanol-tetrahydrofuran (THF) (300 mL) was added sodium methoxide (1.25 g) and the mixture was stirred for 6 h at room temperature. The solution was naturalized with Dowex 50W-X8(H⁺) resin, filtered and evaporated to give a quantitative yield of **32** (10.9 g) as amorphous powder: ¹H-NMR δ (D₂O): 3.39 (s, 1H, H-2), 3.41 (dd, 1H, H-2'), 3.45-3.56 (m, 2H, H-3' and H-5'), 3.58-3.62 (m, 3H, H-6a, H-6'a and H-6'b), 3.65 (s, 1H, H-4), 3.73 (s, 1H, H-3), 3.78 (br s, 1H, H-4'), 3.93 (dd, 1H, H-6b), 4.39 (dd, 1H, $J_{1',2'}$ 7.6Hz, H-1'), 4.62 (s, 1H, H-5), and 5.29 (s, 1H, H-1).

***O*-(2,3,4-Tri-*O*-acetyl-6-*O*-triphenylmethyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,3-di-*O*-acetyl-1,6-anhydro- β -D-glucopyranose (33).** To a solution 1,6-anhydro- β -lactose **32** (13.0 g, 22.9 mmol) in pyridine (268 mL) were added triphenylmethyl chloride (26.2 g, 92 mmol) and 4-(dimethylamino)pyridine (DMAP) (1.4 g, 11.5 mmol), and the mixture was stirred under a nitrogen atmosphere for 24 h at 90°C. After cooled to 0°C, to the solution added acetic

anhydride (67 mL). The mixture was stirred for 12 h at room temperature, then evaporated and extracted with chloroform. The organic layer was washed successively with 1N sulfuric acid, saturated sodium hydrogen carbonate and brine, dried, filtered off through celite bed and evaporated. The residue was subjected to silica gel chromatography with 20 : 1 and 10 : 1 (v/v) toluene-ethyl acetate as an eluant. Recrystallization from 1 : 1 (v/v) ethanol-chloroform gave **33** (15.2 g, 85%): mp 187-189°C ; $[\alpha]_D^{25}$ -63.4° (c 0.10, CHCl₃) ; ¹H-NMR δ (CDCl₃) : 1.89, 1.98, 2.00, 2.03, and 2.04 (all s, 15H, 5COCH₃), 3.05 (t, 1H, $J_{5',6'a}$ 8.6Hz, H-5'), 3.35 (dd, 1H, $J_{5,6'a}$ 8.8 and $J_{6'a,6'b}$ 5.6Hz, H-6'a), 3.59 (s, 1H, H-4), 3.77 (t, 1H, $J_{6a,6b}$ 7.5Hz, H-6a), 3.85 (br t, 1H, $J_{6'a,6'b}$ 5.6Hz, H-6'b), 3.96 (d, 1H, $J_{6a,6b}$ 7.6Hz, H-6b), 4.48 (s, 1H, H-2), 4.61 (d, 1H, $J_{5,6a}$ 5.1Hz, H-5), 4.74 (d, 1H, $J_{1',2'}$ 7.8Hz, H-1'), 4.98 (s, 1H, H-3), 5.07 (dd, 1H, $J_{2',3'}$ 10.3 and $J_{3',4'}$ 3.2Hz, H-4'), 5.20 (dd, 1H, $J_{1',2'}$ 7.8 and $J_{2',3'}$ 10.5Hz, H-2'), 5.41 (s, 1H, H-1), 5.57 (d, 1H, $J_{3',4'}$ 3.2Hz, H-4'), and 7.19-7.37 (m, 15H, aromatic); Anal. Calcd. for C₄₁H₄₄O₁₅: C, 63.44; H, 5.64. Found: C, 63.40; H, 5.71.

O-(6-O-triphenylmethyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1,6-anhydro- β -D-glucopyranose (34). A solution of compound **33** (15.2 g, 19.4mmol) and sodium methoxide (140 mg) in 1 : 1 (v/v) methanol-THF (400 mL) were stirred for 3 h at room temperature. The mixture was made natural with Dowex 50W-X8(H⁺) resin, and the suspension was filtered. The filtrate was evaporated to give a quantitative yield of **34** (10.9 g) as amorphous powder : $[\alpha]_D^{25}$ -48.1° (c

0.262, water) ; $^1\text{H-NMR}$ δ (CDCl_3): 3.39 (s, 1H, H-2), 3.37-4.42 (dd, 2H, H-2' and H-6'a), 3.45-3.56 (m, 2H, H-3' and H-5'), 3.58-3.62 (m, 1H, H-6a), 3.65 (s, 1H, H-4), 3.73 (s, 1H, H-3), 3.80-3.88 (m, 2H, H-4' and H-6'b), 3.93 (dd, 1H, H-6b), 4.41 (d, 1H, $J_{1,2}$ 7.6Hz, H-1'), 4.65 (s, 1H, H-5), and 5.34 (s, 1H, H-1) ; Anal. Calcd. for $\text{C}_{31}\text{H}_{34}\text{O}_{10} \cdot 0.5\text{H}_2\text{O}$: C, 64.69; H, 6.13. Found: C, 64.35; H, 6.01.

***O*-(3-*O*-Allyl-6-*O*-triphenylmethyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1,6-anhydro- β -D-glucopyranose (35).** To a solution of **34** (6.57 g, 11.6 mmol) in toluene (100 mL) was added di-*n*-butyltin oxide (3.17 g, 12.7 mmol) and the mixture was stirred for 4 h at 140°C with continuous azeotropic removal of water. After evaporation to the half volume, 3-bromo-1-propene (50 mL) was added and stirred for 5 h at 80°C. The mixture was evaporated and the residual syrup was chromatographed on silica gel first with 60 : 1 and then 20 : 1 (v/v) chloroform-methanol as the eluant to afford compound **35** (5.12 g, 73%): mp 132°C; $[\alpha]_{\text{D}}^{23}$ -48.1° (c 0.26, CHCl_3); $^1\text{H-NMR}$ δ (CDCl_3): 2.18, 2.96, 3.19, and 3.90 (each br, 4H, 2-OH, 3-OH, 2'-OH and 4'-OH), 3.30 (dd, 1H, $J_{3',4'}$ 3.0 and $J_{2',3'}$ 9.9Hz, H-3'), 3.35-3.48 (m, 4H, H-2, H-5', H-6'a, and H-6'b), 3.58-3.69 (m, 3H, H-4, H-6a, and H-6b), 3.80 (dd, 1H, $J_{1',2'}$ 7.8 and $J_{2',3'}$ 9.9Hz, H-2'), 3.95 (br s, 1H, H-3), 3.99 (d, 1H, $J_{3',4'}$ 3.0Hz, H-4'), 4.19 (m, 2H, $\text{CH}_2=\text{CH}-\text{CH}_2$), 4.36 (d, 1H, $J_{1',2'}$ 7.8Hz, H-1'), 4.65 (d, 1H, $J_{5,6a}$ 5.9Hz, H-5), 5.38 (br s, 1H, H-1), 5.20 [dd, 1H, J 10.5 and 1.5Hz, $\text{CH}_2=\text{CH}$ (*cis*)], 5.34 [dd, 1H, J 17.1 and 1.5Hz, $\text{CH}_2=\text{CH}$ (*trans*)], 5.94 (ddd, 1H, J 17.1, 10.5 and 5.7Hz, $\text{CH}_2=\text{CH}$), and 7.19-7.44 (m, 15H,

aromatic) ; Anal. Calcd. for $C_{34}H_{38}O_{10}$: C, 67.31; H, 6.31. Found: C, 67.21; H, 6.30.

O-(3-*O*-Allyl-6-*O*-triphenylmethyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1,6-anhydro-2-*O*-triisopropylbenzenesulfonyl- β -D-glucopyranose (**36**). To a solution of **35** (4.0 g, 6.58 mmol) in pyridine (60 mL) were added DMAP (2.01 g, 16.5 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (TIPBS-Cl) (4.99 g, 16.5 mmol), and the mixture was stirred under nitrogen atmosphere for 16 h at room temperature. The reaction solution was evaporated and extracted with chloroform. The organic layer was washed successively with 1N sulfuric acid, saturated sodium hydrogen carbonate and brine, dried, filtered and concentrated. The residue was chromatographed on silica gel with 4 : 1 and then 1 : 1 (v/v) toluene-ethyl acetate to give **36** (4.2 g, 73%) as a crystalline: mp 96-97°C; $[\alpha]_D^{23}$ -2.0° (c 0.25, $CHCl_3$); 1H -NMR δ ($CDCl_3$): 1.24 and 1.26 [each s, each 6H, $(CH_3)_2CH$], 2.44 (br, 1H, 3-OH), 2.87 (br, 2H, 2'-OH and 4'-OH), 3.30 (dd, 1H, $J_{3',4'}$ 3.2 and $J_{2',3'}$ 9.4 Hz, H-3'), 3.41-3.47 (m, 3H, H-5', H-6'a, and H-6'b), 3.61-3.65 (m, 2H, H-4, and H-6a), 3.82 (m, 1H, H-2'), 3.85 (d, 1H, $J_{6a,6b}$ 7.2 Hz, H-6b), 3.93 (m, 1H, H-3), 3.96 (d, 1H, $J_{3',4'}$ 3.2 Hz, H-4'), 4.13 [m, 3H, $(CH_3)_2-CH$], 4.19 (m, 2H, $CH_2=CH-CH_2$), 4.35 (m, 1H, H-2), 4.37 (d, 1H, $J_{1',2'}$ 7.4 Hz, H-1'), 4.63 (d, 1H, $J_{5,6a}$ 5.0 Hz, H-5), 5.22 [dd, 1H, J 10.3 and 1.5 Hz, $CH_2=CH$ (*cis*)], 5.31 [dd, 1H, J 17.1 and 1.5 Hz, $CH_2=CH$ (*trans*)], 5.43 (br s, 1H, H-1), 5.94 (ddd, 1H, J 17.2, 10.3 and 5.8 Hz, $CH_2=CH$), and 7.20-7.32 (m, 17H,

aromatic) ; Anal. Calcd. for $C_{49}H_{60}O_{12}S$: C, 67.41;H, 6.93. Found : C, 66.98 ;H, 6.81.

***O*-(3-*O*-Allyl-6-*O*-triphenylmethyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1,6 :**

2,3-dianhydro- β -D-mannopyranose (37). To a solution of **36** (1.30 g, 1.49 mmol) in 1:1 (v/v) methanol-THF (20 mL) was added aqueous solution of 1N sodium hydroxide (4.47 mL) and stirred for 24 h at room temperature. The mixture was concentrated and the residual oil was dissolved in chloroform, washed with brine, dried and evaporated *in vacuo*. The syrup obtained was purified by chromatography on silica gel with 6 : 1 and then 2 : 1 (v/v) toluene-ethyl acetate as an eluant to give **37** (810 mg, 92%) as a crystalline powder: mp 98° - 100° C; $[\alpha]_D^{23}$ -17.1° (c 0.24, $CHCl_3$); 1H -NMR δ ($CDCl_3$): 2.78 (br, 2H, 2'-OH and 4' -OH), 3.36 (dd, 1H, $J_{3',4'}$ 3.2 and $J_{2',3'}$ 9.4Hz, H-3'), 3.42-3.46 (m, 4H, H-2, H-4, H-6'a, and H-6'b), 3.54 (t, 1H, $J_{5',6'b}$ 7.8Hz, H-5'), 3.73 (d, 2H, H-6a, and H-6b), 3.81 (dd, 1H, $J_{1',2'}$ 7.7 and $J_{2',3'}$ 9.4Hz, H-2'), 4.00 (br s, 1H, H-3), 4.05 (br, 1H, H-4'), 4.21 (m, 2H, $CH_2=CH-CH_2$), 4.44 (d, 1H, $J_{1',2'}$ 7.7Hz, H-1'), 4.55 (t, 1H, $J_{5,6a}$ 4.3, and $J_{5,6b}$ 4.3Hz, H-5), 5.23 [dd, 1H, J 10.3 and 1.4 Hz, $CH_2=CH$ (*cis*)], 5.33 [dd, 1H, J 17.12 and 1.4Hz, $CH_2=CH$ (*trans*)], 5.70 (d, 1H, $J_{1,2}$ 2.8Hz, H-1), 5.96 (ddd, 1H, J 17.2, 10.4 and 4.4Hz, $CH_2=CH$), and 7.21-7.46 (m, 15H, aromatic) ; Anal. Calcd. for $C_{34}H_{36}O_9$: C, 69.37;H, 6.16. Found: C, 69.40 ;H, 6.15.

O-(3-*O*-Allyl-2,4-di-*O*-benzyl-6-*O*-triphenylmethyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1,6:2,3-dianhydro- β -D-mannopyranose (**38**). To **37** (800 mg, 1.36 mmol) dissolved in DMF (30 mL) were added sodium hydride (60% oil suspension ;163 mg, 4.08 mmol) and benzyl bromide (0.83 mL, 6.80 mmol). The mixture was stirred at room temperature. After 18 h, the solution was monitored by t.l.c., but it showed that the reaction wasn't completed. So the same amount of sodium hydride (163 mg) and benzyl bromide (0.83 mL) were added and stirred for another 24 h. Excess NaH was destroyed by carefully using MeOH. After evaporation, the residue was dissolved in ethyl acetate, successively washed with water, brine and dried. The mixture was filtered and separated by silica gel column chromatography with 4 : 1 (v/v) *n*-hexane-ethyl acetate as an eluant, to give **38** (980 mg, 94 %) as amorphous powdery: mp 88°C; $[\alpha]_D^{23}$ -22.3° (c 0.31, CHCl₃); ¹H-NMR δ (CDCl₃): 3.22 (dd, 1H, $J_{5',6'a}$ 6.0, and $J_{6'a,6'b}$ 9.1Hz, H-6'a), 3.38-3.43 (m, 4H, H-2, H-4, H-3', and H-5'), 3.50 (dd, 1H, $J_{5',6'b}$ 6.4, and $J_{6'a,6'b}$ 9.1Hz, H-6'b), 3.67 (d, 2H, H-6a, and H-6b), 3.77 (dd, 1H, $J_{1',2'}$ 7.7 and $J_{2',3'}$ 9.7Hz, H-2'), 3.86 (d, 1H, $J_{3',4'}$ 3.0Hz, H-4'), 4.20 (m, 2H, CH₂=CH-CH₂), 4.46 (m, 1H, H-5), 4.46 (d, 1H, $J_{1',2'}$ 7.8Hz, H-1'), 4.48-4.91 (m, 4H, PhCH₂), 5.20 [dd, 1H, J 10.4 and 1.5Hz, CH₂=CH (*cis*)], 5.33 [dd, 1H, J 17.3 and 1.5Hz, CH₂=CH (*trans*)], 5.70 (d, 1H, $J_{1,2}$ 2.9Hz, H-1), 5.93 (ddd, 1H, J 17.3, 10.4 and 5.2Hz, CH₂=CH), and 7.16-7.41 (m, 25H, aromatic) ; Anal. Calcd. for C₄₈H₄₈O₉: C, 74.98;H, 6.29. Found: C, 74.58;H, 6.38.

O-(3-*O*-Allyl-2,4-di-*O*-benzyl-6-*O*-triphenylmethyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1,6-anhydro-2-azido-2-deoxy- β -D-glucopyranose (39). The mixture of 38 (350 mg, 0.455 mmol), cesium fluoride (690 mg, 4.55 mmol) and sodium azide (300 mg, 4.55 mmol) dissolved in DMF (20 mL) were stirred under nitrogen atmosphere for 15 h at 110°C. After cooled to room temperature, the mixture was diluted with water, extracted with ethyl acetate, washed with brine and dried. After filtered and concentrated, the residue was subjected to chromatograph on silica gel column to give 39 (285 mg, 77%) as an crystalline powder with 3 : 1 and then 2 : 1 (v/v) *n*-hexane-ethyl acetate as an eluant: mp 83°C; $[\alpha]_D^{23}$ -20.0° (c 0.22, CHCl₃); ¹H-NMR δ (CDCl₃): 1.56 (br s, 1H, 3-OH), 3.14 (dd, 1H, $J_{5',6'a}$ 5.6 and $J_{6'a,6'b}$ 9.9Hz, H-6'a), 3.22 (d, 1H, $J_{2,3}$ 6.6Hz, H-2), 3.26 (br t, 1H, H-5'), 3.32 (dd, 1H, $J_{3,4'}$ 3.0 and $J_{2',3'}$ 9.9Hz, H-3'), 3.41 (d, 1H, $J_{3,4}$ 7.6Hz, H-4), 3.52 (dd, 1H, $J_{5',6'b}$ 5.6 and $J_{6'a,6'b}$ 9.9Hz, H-6'b), 3.56 (m, 1H, H-6a), 3.68-3.69 (m, 2H, H-6b and H-4'), 3.76-3.79 (m, 2H, H-3 and H-2'), 4.18 (m, 2H, CH₂=CH-CH₂), 4.32 (d, 1H, $J_{1',2'}$ 7.6Hz, H-1'), 4.39 (m, 1H, H-5), 4.45-4.83 (m, 4H, PhCH₂), 5.21 [dd, 1H, J 10.6 and 1.7 Hz, CH₂=CH (*cis*)], 5.27 (br s, 1H, H-1), 5.33 [dd, 1H, J 17.2 and 1.7 Hz, CH₂=CH (*trans*)], 5.93 (ddd, 1H, J 17.2, 10.6 and 5.3Hz, CH₂=CH), and 7.11-7.64 (m, 25H, aromatic) ; Anal. Calcd. for C₄₈H₄₉N₃O₉: C, 71.07; H, 6.08; N, 5.18. Found: C, 71.00; H, 6.00; N, 5.12.

O-(3-*O*-Allyl-2,4-di-*O*-benzyl-6-*O*-triphenylmethyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy- β -D-glucopyranose (**40**). To a solution of **39** (270 mg, 0.33 mmol) in DMF (20 mL) were added sodium hydride (60% oil suspension ; 40 mg, 1.0 mmol) and benzyl bromide (0.21 mL, 1.7 mmol), and stirred at room temperature for 12 h. The same manner as described for **38** was carried out, and the residue obtained was subjected to a silica gel chromatography with 5 : 1 and then 3 : 1 (v/v) *n*-hexane-ethyl acetate as an eluant to give **40** (281 mg, 94%) as crystalline powder: mp 104°C; $[\alpha]_D^{23}$ -9.9° (c 0.24, CHCl₃); ¹H-NMR δ (CDCl₃): 3.20 (br s, 1H, H-2), 3.32 (m, 2H, H-5' and H-6'a), 3.37 (dd, 1H, $J_{3',4'}$ 3.0 and $J_{2',3'}$ 9.5Hz, H-3'), 3.47 (ddd, 1H, $J_{5',6'b}$ 8.8 and $J_{6'a,6'b}$ 11.7Hz, H-6'b), 3.72 (t, 1H, $J_{5,6a}$ 6.0 and $J_{6a,6b}$ 7.3Hz, H-6a), 3.78 (m, 2H, H-4 and H-2'), 3.82 (br s, 1H, H-3), 3.87 (d, 1H, $J_{3',4'}$ 2.9Hz, H-4'), 4.02 (d, 1H, $J_{6a,6b}$ 7.3Hz, H-6b), 4.20 (m, 2H, CH₂=CH-CH₂), 4.40 (d, 1H, $J_{1',2'}$ 8.1Hz, H-1'), 4.64 (d, 1H, $J_{5,6a}$ 6.0Hz, H-5), 4.49-4.97 (m, 6H, PhCH₂), 5.20 [dd, 1H, J 10.3 and 1.5 Hz, CH₂=CH (*cis*)], 5.34 [dd, 1H, J 16.9 and 1.5 Hz, CH₂=CH (*trans*)], 5.49 (br s, 1H, H-1), 5.95 [ddd, 1H, J 16.9, 10.7 and 5.1 Hz, CH₂=CH], and 7.12-7.34 (m, 30H, aromatic) ; Anal. Calcd. for C₅₅H₅₅N₃O₉: C, 73.23; H, 6.15; N, 4.66. Found: C, 73.19; H, 6.16; N, 4.61.

O-(2,4-Di-*O*-acetyl-3,6-di-*O*-allyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,3-di-*O*-acetyl-1,6-anhydro- β -D-glucopyranose (**41**). To a solution of **32** (2.5 g, 6.18 mmol) in toluene (300 mL) was added bis(tri-*n*-butyltin)oxide (4.27 mL,

8.03 mmol), and the mixture was stirred for 4 h at 140°C with continuous azeotropic removal of water. After evaporated to the half volume, 3-bromo-1-propene (20 mL) was added and stirred for 12 h at 80°C. After concentration, the residual syrup was dissolved in pyridine (50 mL) and to the mixture were added DMAP (0.76 g, 6.18 mmol) and acetic anhydride (50 mL), the mixture was stirred for 15 h at room temperature. After concentrated and extracted with chloroform. The extract was washed successively with 1N sulfuric acid, saturated sodium hydrogen carbonate and brine, dried, filtered and evaporated. The residue was chromatographed on silica gel with 6 : 1 and then 2 : 1 (v/v) toluene-ethyl acetate as the eluant to afford compound **41** (2.4 g, 68%): mp 116°C; $[\alpha]_D^{23}$ -37.4° (c 0.149, CHCl₃); ¹H-NMR δ(CDCl₃): 2.08-2.13 (all s, 12H, 4COCH₃), 3.45-3.55 (br, 2H, H-3' and H-5'), 3.59 (s, 1H, H-4), 3.86-4.19 (m, 6H, CH₂=CH-CH₂, H-6a, and H-6b), 4.10-4.17 (br, 2H, H-6'a and H-6'b), 4.53 (s, 1H, H-3), 4.61 (d, 1H, $J_{5,6a}$ 5.0Hz, H-5), 4.71 (d, 1H, $J_{1,2'}$ 7.9Hz, H-1'), 5.10 (s, 1H, H-2), 5.11-5.21 (m, 5H, CH₂=CH and H-2'), 5.44 (s, 1H, H-1), 5.47 (d, 1H, $J_{3',4'}$ 3.3Hz, H-4'), and 5.70-5.90 (m, 2H, CH₂=CH) ; Anal. Calcd. for C₂₆H₃₆O₁₄ · 0.5H₂O: C, 53.70; H, 6.41. Found: C, 53.77; H, 6.26.

O-(3,6-Di-*O*-allyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2-*O*-triisopropylbenzenesulfonyl-β-D-glucopyranose (**43**). A solution of compound **41** (3.26g, 5.69 mmol) and sodium methoxide (100 mg) in 1 : 1 (v/v) methanol-THF (50 mL) were stirred for 3 h at room temperature. The mixture

was made natural with the Dowex 50W-X8(H⁺) resin, and the suspension was filtered. The filtrate was evaporated to give a quantitative yield of **42** (2.3 g). To a solution of **42** in pyridine (30 mL) were added DMAP (2.09 g, 17.1 mmol) and TIPBS-Cl (5.16 g, 17.1 mmol), and the mixture was stirred under nitrogen atmosphere for 72 h at room temperature. After concentrated and extracted with chloroform, the organic layer was washed successively with 1N sulfuric acid, saturated sodium hydrogen carbonate and brine, dried, and evaporated. The residue was chromatographed on silica gel with 3 :1 (v/v) toluene-ethyl acetate to give **43** (2.4 g, 63%) as a crystalline mass: mp 80°C; $[\alpha]_D^{23} -7.6^\circ$ (c 0.240, CHCl₃); ¹H-NMR δ (CDCl₃): 1.25-1.27 [each s, each 6H, (CH₃)₂CH], 2.50 (br, 1H, 3-OH), 2.90 (br, 2H, 2'-OH and 4' -OH), 3.37 (dd, 1H, $J_{3',4'}$ 3.2 and $J_{2',3'}$ 10.4Hz, H-3'), 3.55 (d, 1H, H-5'), 3.63-3.70 (m, 5H, H-4, H-6a, H-6b, H-6'a, and H-6'b), 3.81 (t, 2H, H-2'), 3.93-4.02 (m, 2H, H-3, and H-4'), 4.14 [m, 3H, (CH₃)₂-CH], 4.20 (m, 2H, CH₂=CH-CH₂), 4.36 (d, 1H, $J_{1,2}$ 5.6Hz, H-2), 4.38 (d, 1H, $J_{1',2'}$ 7.8Hz, H-1'), 4.60 (d, 1H, $J_{5,6a}$ 6.8Hz, H-5), 5.15-5.35 (m, 4H, CH₂=CH), 5.43 (br s, 1H, H-1), 5.88 (m, 2H, CH₂=CH), and 7.17 (s, 2H, aromatic); Anal. Calcd. for C₃₃H₅₀O₁₂S: C, 59.08;H, 7.51; S, 4.78. Found : C, 59.05 ;H, 7.57 ; S, 5.07.

***O*-(3,6-Di-*O*-allyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1,6 : 2,3-dianhydro- β -D-mannopyranose (**44**).** To a solution of **43** (650 mg, 0.969 mmol) in 1:1 (v/v) methanol-THF (10 mL) was added aqueous solution of 1N sodium hydroxide

(2.90 mL) and the mixture was stirred for 24 h at room temperature. After concentration, the residual oil was dissolved in chloroform, washed with brine, dried and evaporated *in vacuo*. The syrup obtained was purified by chromatography on silica gel with 100 : 1 (v/v) chloroform-methanol as an eluant to give a syrup **44** (290 mg, 78%): $[\alpha]_D^{23} -16.4^\circ$ (c 0.251, CHCl_3); $^1\text{H-NMR}$ δ (CDCl_3): 2.77 (br, 2H, 2'-OH and 4'-OH), 3.38 (dd, 1H, $J_{3',4'}$ 3.3 and $J_{2',3'}$ 9.4Hz, H-3'), 3.42-3.47 (m, 4H, H-2, H-4, H-5' and H-6'a), 3.63 (dd, 1H, $J_{5',6'a}$ 5.7 and $J_{6'a,6'b}$ 11.6Hz, H-6'b), 3.68-3.78 (m, 1H, H-6a), 3.79-3.84 (m, 2H, H-2' and H-6b), 3.98 (br s, 1H, H-3), 4.04-4.06 (m, 3H, H-4' and $\text{CH}_2=\text{CH}-\text{CH}_2$), 4.21 (m, 2H, $\text{CH}_2=\text{CH}-\text{CH}_2$), 4.45 (d, 1H, $J_{1',2'}$ 7.7Hz, H-1'), 4.54 (t, 1H, H-5), 5.19-5.36 (m, 4H, $\text{CH}_2=\text{CH}$), 5.71 (d, 1H, $J_{1,2}$ 3.0Hz, H-1), and 5.84-6.00 (m, 2H, $\text{CH}_2=\text{CH}$); Anal. Calcd. for $\text{C}_{18}\text{H}_{26}\text{O}_9 \cdot 0.5\text{H}_2\text{O}$: C, 54.67; H, 6.88. Found: C, 54.50 ;H, 6.58.

O-(3,6-Di-*O*-allyl-2,4-di-*O*-benzyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1,6:2,3-dianhydro- β -D-mannopyranose (**45**). To **44** (200 mg, 0.518 mmol) dissolved in DMF (10 mL) were added sodium hydride (60% oil suspension; 62 mg, 1.55 mmol) and benzyl bromide (0.32 mL, 2.59 mmol). The mixture was stirred at room temperature. After 24 h, excess NaH was destroyed by carefully using MeOH. The resulting solution was dissolved in ethyl acetate, washed with water, brine and dried. After filtered and evaporated, the residue was separated by silica gel column chromatography with 5 : 1 (v/v) toluene-ethyl acetate as an eluant, and recrystallization from ethanol gave **45** (270 mg,

92 %): mp 138°C; $[\alpha]_D^{23}$ -23.3° (c 0.227, CHCl₃); ¹H-NMR δ (CDCl₃): 3.41-3.45 (m, 4H, H-2, H-4, H-3', and H-5'), 3.52 (m, 2H, H-6'a and H-6'b), 3.67 (d, 1H, H-6a), 3.82 (dd, 1H, $J_{1,2}$ 7.6 and $J_{2,3}$ 9.7Hz, H-2'), 3.86-3.91 (m, 4H, H-3, H-6b and CH₂=CH-CH₂), 3.89 (d, 1H, H-4'), 4.20-4.22 (m, 2H, CH₂=CH-CH₂), 4.46 (m, 1H, H-5), 4.50 (d, 1H, $J_{1,2}$ 7.7Hz, H-1'), 4.64-4.97 (m, 4H, PhCH₂), 5.15-5.37 (m, 4H, CH₂=CH), 5.72 (d, 1H, $J_{1,2}$ 2.8Hz, H-1), 5.78-5.90 (m, 2H, CH₂=CH), and 7.25-7.42 (m, 10H, aromatic); Anal. Calcd. for C₃₂H₃₈O₉: C, 67.83;H, 6.76.

Found: C, 67.71;H, 6.89.

***O*-(3,6-Di-*O*-allyl-2,4-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (46).** The mixture of **45** (100 mg, 0.176 mmol), cesium fluoride (270 mg, 1.76 mmol) and sodium azide (114 mg, 1.76 mmol) dissolved in DMF (2.0 mL) were stirred under nitrogen atmosphere for 24 h at 110°C. After cooled to room temperature, the mixture was diluted with water, extracted with ethyl acetate, washed with brine, dried and filtered. After evaporated *in vacuo*, the syrup obtained was subjected to chromatograph on silica gel column with 3 : 1 (v/v) *n*-hexane-ethyl acetate as an eluant to give **46** (70 mg, 65%) as a crystalline powder: mp 87°C; $[\alpha]_D^{23}$ -40.7° (c 0.229, CHCl₃); ¹H-NMR δ (CDCl₃): 1.64 (br s, 1H, 3-OH), 3.22 (d, 1H, $J_{2,3}$ 8.6Hz, H-2), 3.26 (d, 1H, $J_{5',6'a}$ 6.3Hz, H-5'), 3.36 (d, 1H, $J_{3,4}$ 6.3Hz, H-4), 3.43 (dd, 1H, $J_{3',4'}$ 2.9 and $J_{2',3'}$ 9.8Hz, H-3'), 3.53-3.64 (m, 4H, H-6a, H-6b, H-6'a and H-6'b), 3.75 (d, 1H, $J_{3',4'}$ 3.3Hz, H-4'), 3.79-3.88 (m, 1H, H-3), 3.84 (d, 1H, $J_{1',2'}$

7.7Hz, H-2'), 3.91 (m, 2H, CH₂=CH-CH₂), 4.23 (m, 2H, CH₂=CH-CH₂), 4.34 (br t, 1H, H-5), 4.38 (d, 1H, *J*_{1',2'} 7.8Hz, H-1'), 4.51-4.97 (m, 4H, PhCH₂), 5.20 (d, 1H, H-1), 5.16-5.38 (m, 4H, CH₂=CH), 5.80-6.00 (m, 2H, CH₂=CH), and 7.30-7.35 (m, 10H, aromatic); Anal. Calcd. for C₃₂H₃₉N₃O₉: C, 63.04; H, 6.45; N, 6.89. Found: C, 63.08; H, 6.45; N, 6.74.

***O*-(3,6-Di-*O*-allyl-2,4-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy-β-D-glucopyranose (47).** To a solution of **46** (180 mg, 0.295 mmol) in DMF (10 mL) were added sodium hydride (60% oil suspension ; 24 mg, 0.590 mmol) and benzyl bromide (0.11 mL ; 0.885 mmol), and stirred for 14 h at room temperature. The same manner as described for **38** was carried out, and the syrup obtained was isolated on the silica gel chromatography first with 5 : 1 and then 2 : 1(v/v) *n*-hexane-ethyl acetate as an eluant to give **47** (170 mg, 82%): [α]_D²³ -6.6° (c 0.290, CHCl₃); ¹H-NMR δ (CDCl₃): 3.13 (s, 1H, H-2), 3.32-3.43 (m, 4H, H-3', H-5' H-6'a and H-6'b), 3.63 (t, 1H, *J*_{5,6a} 6.1 and *J*_{6a,6b} 7.2Hz, H-6a), 3.70-3.82 (m, 5H, H-4, H-2', H-4' and CH₂=CH-CH₂), 3.85 (s, 1H, H-3), 3.94 (d, 1H, *J*_{6a,6b} 7.1Hz, H-6b), 4.14 (m, 2H, CH₂=CH-CH₂), 4.38 (d, 1H, *J*_{1',2'} 7.6Hz, H-1'), 4.55 (d, 1H, H-5), 4.50-4.95 (m, 6H, PhCH₂), 5.02-5.30 (m, 4H, CH₂=CH), 5.42 (s, 1H, H-1), 5.65-5.97 (m, 2H, CH₂=CH], and 7.18-7.38 (m, 15H, aromatic) ; Anal. Calcd. for C₃₉H₄₅N₃O₉ • CH₃OH: C, 65.65; H, 6.75; N, 5.74. Found: C, 65.80; H, 6.53; N, 5.70.

O-(3-*O*-Allyl-2,4-di-*O*-benzyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy- β -D-glucopyranose (**48**). The solution of **40** (500 mg, 0.554 mmol) in acetic acid (18.0 mL) and deionized water (2.0 mL) was stirred at 70°C for 1h. The solution was extracted with chloroform and the organic layer was washed successively with ice water, saturated sodium hydrogen carbonate and brine, dried and filtered. After evaporated, the residual syrup was applied on silica gel chromatography first with 8 : 1 and then 5 : 1 (v/v) toluene-ethyl acetate as an eluant, to give **48** (360 mg, 98%) as a syrup foam : $[\alpha]_D^{23}$ -15.4° (c 0.20, CHCl₃); ¹H-NMR δ (CDCl₃): 3.09 (br m, 1H, 6'-OH), 3.25 (brs, 1H, H-2), 3.34 (t, 1H, $J_{5',6'a}$ 5.9 and $J_{5',6'b}$ 6.6Hz, H-5'), 3.42 (dd, 1H, $J_{2',3'}$ 9.5 and $J_{3',4'}$ 3.0Hz, H-3'), 3.54 (m, 1H, H-6'b), 3.68 (m, 1H, H-6'a), 3.72 (t, 1H, $J_{5,6a}$ 5.9 and $J_{6a,6b}$ 7.3Hz, H-6a), 3.76 (br s, 1H, H-4), 3.79 (d, 1H, $J_{3',4'}$ 2.9Hz, H-4'), 3.85 (dd, 1H, $J_{1',2'}$ 8.1 and $J_{2',3'}$ 9.5Hz, H-2'), 3.90 (br s, 1H, H-3), 4.03 (d, 1H, $J_{6a,6b}$ 7.3Hz, H-6b), 4.25 (m, 2H, CH₂=CH-CH₂), 4.43 (d, 1H, $J_{1',2'}$ 8.1Hz, H-1'), 4.62 (d, 1H, $J_{5,6a}$ 5.9Hz, H-5), 4.65-5.01 (m, 6H, PhCH₂), 5.23 [dd, 1H, J 11.0 and 1.5 Hz, CH₂=CH (*cis*)], 5.36 [dd, 1H, J 16.9 and 1.5Hz, CH₂=CH (*trans*)], 5.51 (br s, 1H, H-1), 5.98 (ddd, 1H, J 16.9, 11.0 and 5.9Hz, CH₂=CH), and 7.45-7.30 (m, 15H, aromatic) ; Anal. Calcd. for C₃₆H₄₁N₃O₉: C, 65.54; H, 6.26; N, 6.37. Found: C, 65.41; H, 6.32; N, 6.12.

***O*-(2,4-Di-*O*-benzyl-6-*O*-triphenylmethyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy- β -D-glucopyranose (49).**

To a solution of **40** (480 mg, 0.532 mmol) in acetic acid (9.0 mL) and H₂O (1.0 mL) were added sodium acetate (218 mg, 2.60 mmol) and palladium (II) chloride (189 mg, 1.06 mmol) and the mixture was setted into a sonicator for 6 h (the water temperature was kept under 35°C). The reaction solution was filtered off through celite bed and extracted with chloroform. The organic layer was washed with saturated sodium hydrogen carbonate and brine. After dried and evaporated, the residue was chromatographed over a silica gel with 2 : 1 (v/v) *n*-hexane-ethyl acetate to give **49** (300 mg, 65%) as an amorphous powder, with 3',6'-*O*-diol **50** (60 mg, 18%) : $[\alpha]_D^{23} +13.4^\circ$ (c 0.208, CHCl₃): ¹H-NMR δ (CDCl₃): 2.23 (br s, 1H, 3'-OH), 3.21 (br s, 1H, H-2), 3.31 (br t, 1H, $J_{5',6'a}$ 5.8 and $J_{5,6'b}$ 6.3Hz, H-5'), 3.48 (m, 2H, H-3' and H-6'a), 3.57-3.60 (m, 2H, H-6a, and H-6'b), 3.76 (m, 2H, H-4 and H-2'), 3.84 (d, 1H, $J_{3',4'}$ 2.9Hz, H-4'), 3.97 (br s, 1H, H-3), 4.05 (d, 1H, $J_{6a,6b}$ 7.1Hz, H-6b), 4.44 (d, 1H, $J_{1',2'}$ 7.0Hz, H-1'), 4.49-5.04 (m, 6H, PhCH₂), 4.52 (m, 1H, H-5), 5.49 (br s, 1H, H-1), and 7.12-7.37 (m, 30H, aromatic) ; Anal. Calcd. for C₅₂H₅₁N₃O₉: C, 72.46; H, 5.96; N, 4.87. Found: C, 72.17; H, 6.01; N, 4.72.

***O*-(2,4-Di-*O*-benzyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy- β -D-glucopyranose (50). (*Synthesis of diol 50*)**

from **40**) The solution of **40** (300 mg, 0.333 mmol) in acetic acid (9.0 mL) and deionized water (1.0 mL) was stirred at 70°C for 1 h. After cooled to room temperature, to the solution were added sodium acetate (136 mg, 1.66 mmol) and palladium (II) chloride (118 mg, 0.665 mmol). The mixture was sonicated for 2 h (the water temperature was kept under 35°C). After filtered, the filtrate was extracted with chloroform and the organic layer washed with saturated sodium hydrogen carbonate and brine, dried, filtered, and concentrated. The residue was chromatographed over silica gel with 60 : 1 (v/v) toluene-ethyl acetate as the eluant to give **50** (154 mg, 75%) as a syrup foam:

(*Synthesis of diol 50 from 47*) To a solution of **47** (80 mg, 0.114 mmol) in acetic acid (1.9 mL) and H₂O (0.1 mL) were added sodium acetate (93 mg, 1.14 mmol) and palladium (II) chloride (81 mg, 0.456 mmol) and the mixture was setted into a sonicator for 2 h (the water temperature was kept under 35°C). After filtered off through celite bed and extracted with chloroform, the organic layer was washed with saturated sodium hydrogen carbonate and brine, dried and concentrated. The residue obtained was chromatographed over a silica gel with 60 : 1 (v/v) chloroform : methanol as an eluant, to afford **50** (57 mg, 81%) as a syrup foam: $[\alpha]_D^{23} +2.0^\circ$ (c 0.19, CHCl₃); ¹H-NMR δ (CDCl₃): 3.09 (br, 2H, 3'-OH and 6'-OH), 3.27 (br s, 1H, H-2), 3.41 (t, 1H, $J_{5',6'a}$ 5.6 and $J_{5',6'b}$ 6.5Hz, H-5'), 3.56 (dd, 1H, $J_{5',6'a}$ 5.6 and $J_{6'a,6'b}$ 10.1Hz, H-6'a), 3.68 (dd, 1H, $J_{3',4'}$ 3.2 and $J_{2',3'}$ 9.9Hz, H-3'), 3.68 (m, 1H, H-6'b), 3.72 (d, 1H, $J_{5,6a}$ 6.1 and $J_{6a,6b}$ 7.4Hz, H-6a),

3.75(br s, 1H, H-4), 3.78 (m, 2H, H-2' and H-4'), 3.91 (br s, 1H, H-3), 4.07 (d, 1H, $J_{6a,6b}$ 7.4Hz, H-6b), 4.44 (d, 1H, $J_{1',2'}$ 7.0Hz, H-1'), 4.61 (d, 1H, $J_{5,6a}$ 6.1Hz, H-5), 4.64-5.10 (m, 6H, PhCH₂), 5.51 (br s, 1H, H-1), and 7.30-7.40 (m, 15H, aromatic); ¹³C-NMR δ (CDCl₃): 127.5-128.67, 137.3, 138.0, 138.3 (Bn), and the data of the ring carbons are given in Table 8; Anal. Calcd. for C₃₃H₃₇N₃O₉: C, 63.96; H, 6.02; N, 6.78. Found: C, 63.78; H, 6.20; N, 6.80.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (51).

To a solution of *N*-phthalimido-D-glucosamine per-*O*-acetate (3.0 g, 6.28mmol) in tetrahydrofuran (20 mL) was added benzylamine (1.03 mL, 9.42 mmol) and stirred for 2 h at room temperature. The mixture was poured into chloroform and washed with 1N hydrochloric acid and water, dried, and filtered. After concentration, the residue was chromatographed on silica gel with 5 : 1 and then 3 : 1 (v/v) toluene : ethyl acetate as the eluant to afford **51** (2.6 g, 87%): ¹H-NMR δ (CDCl₃): 1.86, 2.03, and 2.11 (all s, 9H, 3COCH₃), 3.80 (br, 1H, 1-OH), 3.94 (ddd, 1H, H-5), 4.22 (dd, 1H, $J_{6a,6b}$ 11.6Hz, H-6b), 4.25-4.33 (m, 2H, H-2 and H-6a), 5.18 (t, 1H, $J_{4,5}$ 10.0Hz, H-4), 5.64 (d, 1H, $J_{1,2}$ 8.1Hz, H-1), 5.90 (dd, 1H, $J_{3,4}$ 9.2Hz, H-3), and 7.72-7.90 (2m, 4H, aromatic).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (52). Trichloroacetonitrile (3.52 mL, 34.5 mmol) and

sodium hydride (60% oil suspension ; 826 mg, 20.7 mmol) were added to a solution of **51** (1.5 g, 3.45 mmol) in dichloromethane (20 mL) and stirred for 2 h at room temperature. The suspension was carefully filtered on glass filter and the filtrate obtained was evaporated *in vacuo*. The resulting residue was subjected to chromatograph on silica gel with 400 : 100 : 1(v/v/v) toluene-ethyl acetate-triethylamine as eluant to give **52** (1.43 g, 72%): $^1\text{H-NMR}$ δ (CDCl_3): 1.90, 2.06, and 2.13 (all s, 9H, 3COCH_3), 4.07 (ddd, 1H, H-5), 4.20 (dd, 1H, $J_{5,6b}$ 1.7Hz, H-6b), 4.38 (dd, 1H, $J_{5,6a}$ 4.5 and $J_{6a,6b}$ 12.2Hz, H-6a), 4.63 (dd, 1H, $J_{2,3}$ 10.1Hz, H-2), 5.27 (dd, 1H, $J_{4,5}$ 9.0Hz, H-4), 5.90 (dd, 1H, $J_{3,4}$ 9.4Hz, H-3), 6.61 (d, 1H, $J_{1,2}$ 8.9Hz, H-1), 7.68-7.90 (2m, 4H, aromatic), and 8.63 (s, 1H, NH).

O-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-(1 $\rightarrow$ 3)-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)]-*O*-(2,4-di-*O*-benzyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy- β -D-glucopyranose (**53**). *O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-(1 $\rightarrow$ 6)-*O*-(2,4-di-*O*-benzyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy- β -D-glucopyranose (**54**). To a mixture of 1,6-anhydrolactose derivative **50** (160 mg, 0.259 mmol), glucosaminy l imidate **52** (360 mg, 0.622 mmol) and MS4Å (300 mg) in dry dichloromethane (4.0 mL) was added trimethylsilyl trifluoromethanesulfonate (TMSOTf) (40 μL , 0.207 mmol) in dry

dichloromethane (0.5 mL) at -15°C under nitrogen atmosphere. After stirred at room temperature for 1 h, the mixture was neutralized with triethylamine and concentrated. The resulting residue was purified by passing through a silica gel column first with 3 : 1 and then 1 : 1 (v/v) *n*-hexane-ethyl acetate to give tetrasaccharide **53** (260 mg, 69%) and trisaccharide **54** (70 mg, 26%).

Tetrasaccharide **53** : $[\alpha]_{\text{D}}^{25} +13.0^{\circ}$ (c 0.258, CHCl_3); $^1\text{H-NMR}$ δ (CDCl_3): 1.82-2.04 (all s, 18H, 6 COCH_3), 3.15 (d, 1H, H-2A), 3.36 (br t, 1H, $J_{5\text{B},6\text{Ba}}$ 5.7Hz, H-5B), 3.62 (s, 1H, H-3A), 3.59-3.68 (m, 4H, H-3B, H-6Ba, H-5C, and H-6Db), 3.73 (d, 1H, H-4A), 3.70-3.78 (m, 5H, H-4A, H-6Aa, H-4B, H-6Ca, and H-5D), 3.96 (d, 1H, $J_{1\text{B},2\text{B}}$ 7.4Hz, H-2B), 4.22 (d, 1H, $J_{1\text{D},2\text{D}}$ 8.6Hz, H-2D), 4.10-4.28 (m, 3H, H-5A, H-6Ab, and H-6Da), 4.35 (dd, 1H, $J_{1\text{C},2\text{C}}$ 8.4 and $J_{2\text{C},3\text{C}}$ 10.9Hz, H-2C), 4.36 (d, 1H, $J_{1\text{B},2\text{B}}$ 7.3Hz, H-1B), 4.31-4.38 (m, 2H, H-6Ca, and PhCH_2), 5.11 (t, 1H, $J_{3\text{D},4\text{D}}$ 9.4 and $J_{4\text{D},5\text{D}}$ 9.6Hz, H-4D), 5.18 (t, 1H, $J_{3\text{C},4\text{C}}$ 9.2 and $J_{4\text{C},5\text{C}}$ 9.5Hz, H-4C), 5.32 (s, 1H, H-1A), 5.48 (d, 1H, $J_{1\text{D},2\text{D}}$ 8.4Hz, H-1D), 5.62 (dd, 1H, $J_{2\text{D},3\text{D}}$ 10.6 and $J_{3\text{D},4\text{D}}$ 9.2Hz, H-3D), 5.70 (d, 1H, $J_{1\text{C},2\text{C}}$ 8.3Hz, H-1C), 5.70 (dd, 1H, $J_{2\text{C},3\text{C}}$ 10.6 and $J_{3\text{C},4\text{C}}$ 9.1Hz, H-3C), 7.20-7.31 [m, 15H, aromatic(Bn)], and 7.57-7.81 [4m, 8H, aromatic(Phth)]; $^{13}\text{C-NMR}$ δ (CDCl_3): 20.4-20.7 (COCH_3), 123.5, 123.6 (Phth 3/6), 127.3-128.4 (Bn), 131.1, 131.2 (Phth 1/2), 134.1, 134.4 (Phth 4/5), 137.5, 138.3, 138.4 (Bn), 169.5-170.6 (COCH_3), and the data of the ring carbons are given in Table 8 ; Anal. Calcd. for $\text{C}_{73}\text{H}_{75}\text{N}_5\text{O}_{27} \cdot \text{H}_2\text{O}$: C, 59.55; H, 5.27; N, 4.76. Found: C, 59.64; H, 5.07; N, 4.78.

Trisaccharide **54** : $[\alpha]_D^{25} +14.0^\circ$ (c 0.253, CHCl_3); $^1\text{H-NMR}$ δ (CDCl_3): 1.84, 1.99 and 2.00 (all s, 9H, 3COCH_3), 2.16 (br s, 1H, 3B-OH), 3.24 (s, 1H, H-2A), 3.39 (m, 1H, H-3B), 3.44 (dd, 1H, $J_{1B,2B}$ 7.5 and $J_{2B,3B}$ 9.7Hz, H-2B), 3.62-3.78 (m, 4H, H-6Ab, H-4B, H-6Ba, and H-6Bb), 3.79 (s, 1H, H-4A), 3.82 (s, 1H, H-3A), 3.86 (dd, 1H, $J_{4C,5C}$ 10.2Hz, H-5C), 3.97 (dd, 1H, $J_{6Ca,6Cb}$ 12.5Hz, H-6Cb), 4.08 (dd, 1H, $J_{5A,6Ab}$ 7.4Hz, H-6Ab), 4.22 (dd, 1H, $J_{5C,6Ca}$ 4.4 and $J_{6Ca,6Cb}$ 12.5Hz, H-6Ca), 4.27 (dd, 1H, $J_{1C,2C}$ 8.5 and $J_{2C,3C}$ 10.4Hz, H-2C), 4.39 (d, 1H, $J_{1B,2B}$ 7.4Hz, H-1B), 4.62 (m, 1H, H-5A), 4.57-5.03 (m, 6H, PhCH_2), 5.12 (t, 1H, $J_{3C,4C}$ 9.1 and $J_{4C,5C}$ 9.9Hz, H-4C), 5.46 (s, 1H, H-1A), 5.47 (d, 1H, $J_{1C,2C}$ 8.5Hz, H-1C), 5.70 (dd, 1H, $J_{2C,3C}$ 10.5 and $J_{3C,4C}$ 9.1Hz, H-3C), 7.32-7.36 [m, 15H, aromatic(Bn)], and 7.69-7.82 [2m, 4H, aromatic(Phth)]; $^{13}\text{C-NMR}$ δ (CDCl_3): 20.4-20.7 (COCH_3), 123.6 (Phth 3/6), 127.7-128.4 (Bn), 131.0 (Phth 1/2), 134.4, (Phth 4/5), 137.5, 138.2, 138.3 (Bn), 169.3, 170.0, 170.5 (COCH_3), and the data of the ring carbons are given in Table 8 ; Anal. Calcd. for $\text{C}_{53}\text{H}_{56}\text{N}_4\text{O}_{18} \cdot \text{H}_2\text{O}$: C, 60.34; H, 5.54; N, 5.31. Found: C, 60.34; H, 5.39; N, 5.34.

5.4. Conclusion

In the present study, new methods for creating highly functional key intermediates of lactosaminoglycans were established. An available 1,6-anhydro- β -lactose derived from lactose was proved to be a key intermediate for the efficient synthesis of a series of fully functionalized disacchride acceptors in high yields. Selective deprotections of the 1,6-anhydrolactose derivatives gave key glycosyl acceptors having an unprotected hydroxyl group only at C-3' or C-6' position. A glycosylation of the glycosyl acceptor with *N*-phthalimido-D-glucosamine derivative proceeded smoothly by using an imidate procedure and afforded corresponding tri- and tetrasaccharides, respectively

5.5. References

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Chapter 6.

Concluding Remarks

It has been described as major subjects through this study that new methods for creating highly functional polymeric sugar-ligands and key intermediates of lactosaminoglycans are established by employing an unused disaccharide resource, lactose. The contents of the findings and conclusions are summarized as follows:

- The simple and efficient methods for the syntheses of *n*-pentenyl and ω -acryloylamimoalkyl glycosides of *N*-acetyl- β -D-glucosamine (Glc_pNAc) have been demonstrated. Radical polymerization of the new glycosides proceeded smoothly in aqueous solution in the presence of ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine as initiator and gave water-soluble glycopolymers. Homopolymerization of the ω -acryloylamimoalkyl glycosides also gave homopolymers having high-density sugar branches as the novel class of cluster glycosides.
- The apparent association constant of wheat germ agglutinin (WGA) with the poly[3-(*N*-acryloylamino)propyl 2-acetamido-2-deoxy- β -D-glucopyranoside] was determined by measuring the change in fluorescence intensity produced by various concentrations of polymeric

ligand, and found to be approximately 10^{-8}M^{-1} . Addition of the cluster type GlcpNAc-polymer to WGA induced much greater enhancement of the fluorescence intensity and a significant blue shift of the fluorescence emission maximum of WGA than did addition of the low density GlcpNAc-polymer derived from *n*-pentenyl glycoside.

- A simple and facile method for the syntheses of cluster-type homopolymers from ω -acryloylaminoalkyl glycosides of *N*-acetyllactosamine [Galp β (1 $\rightarrow$ 4) GlcpNAc] was also established on the basis of radical polymerization of the novel glycosides in the presence of ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine. These synthetic cluster glycopolymers exhibited good solubility in water and had much higher sugar density than the glycopolymers derived from the known *n*-pentenyl glycosides.
- The association constants of *Erythrina Corallodendron* lectin (ECorL) with these cluster glycopolymers were evaluated by measuring the changes in fluorescence intensity of fluorescent-labeled ECorL induced by the addition of the polymeric ligands. Addition of the cluster-type glycopolymers having different degree of *N*-acetyllactosamine density to ECorL induced a significant decrease of the fluorescence intensity at 520

nm, and this phenomenon was used for the determination of binding constants between the glycopolymers and ECorL.

- An efficient method for the first synthesis of novel glycopolymers containing triantennary glycosides of *N*-acetylactosamine [Galp β (1 $\rightarrow$ 4) GlcpNAc] is described. Radical copolymerization of the triantennary glycosides with acrylamide proceeded smoothly in an aqueous solution and gave water-soluble clustered glycopolymers.
- The versatile and useful methods for synthesis of lactosaminoglycan models using 1,6-anhydrolactose as a starting material have been established by synthetic routes *via* two useful intermediates such as 3'-*O*-allyl-6'-*O*-triphenylmethyl or 3',6'-di-*O*-allyl derivative. The selective deprotection of these derivatives at C-3' and C-6' position gave novel standardized intermediates. Moreover, glycosidations of the 3',6'-dihydroxyl derivative with *N*-phthalimido-D-glucosaminyl imidate afforded corresponding tri- and tetrasaccharides, respectively.



