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FACULTÉ DE ÉTUDES SUPÉRIEURES ET POSTDOCTORALES

FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

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GRADE - DEGREE

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TITRE DE LA THÈSE - TITLE OF THE THESIS

Design and Transition Metal-Mediated Synthesis of Multivalent Neoglycoconjugates Bearing Terminal Galactosides with Various Valency and Structural Features

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Design and Transition Metal-Mediated Synthesis of Multivalent Neoglycoconjugates Bearing Terminal Galactosides with Various Valency and Structural Features

Bingcan Liu

Thesis submitted to the School of Graduate Studies and Research in partial fulfillment for the degree of Doctor of Philosophy in Chemistry

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Your file Votre référence
ISBN: 0-612-90003-7
Our file Notre référence
ISBN: 0-612-90003-7

ABSTRACT

In the past ten years, with the emergency of multivalent neoglycoconjugates such as glycopolymers, glycodendrimers, and glycoclusters, carbohydrates have gained a lot of attention in the hope of developing potent carbohydrate-based therapeutics, as well as the opportunities to investigate glycobiology.

So far many strategies have been explored to effectively assemble multivalent neoglycoconjugates. This work contains a discussion of the design and transition metal-mediated synthesis of terminal galactoside-carrying glycoclusters and glycodendrimers in detail.

Propargyl α-galactoside antigen monomer was synthesized using conventional glycosidation methods. Then analogues of α-galactoside antigen clusters were prepared utilizing the Sonogashira reaction as the key step. Various conditions for the Sonogashira have been investigated and an interesting finding is that this reaction does occur with Cu (I). These synthetic α-galactoside antigens have shown enhanced binding affinity toward human anti-α-galactoside antigen antibodies in biochemical assays.

Likewise, galactoclusters were prepared as potential galectin inhibitors. However, these fully deprotected compounds were barely soluble in aqueous conditions. To solve this problem, analogues of lactoclusters, having an extra glucosyl residue, with various valencies and structural features were synthesized. Kinetic precipitation tests demonstrated that most lactoclusters cross-linked with galectin-3 formed formed insoluble complexes quickly and this phenomenon was observed for the first time, thus suggesting the clustering of the receptors upon contact.

A sequence of olefin cross-metathesis, Sonogashira reaction, and cyclotrimerization was performed to assemble a hexameric C-linked glycopeptidomemetic.

Olefin cross-metathesis mediated by the second generation Grubbs catalyst was successfully effected to prepare extended glycoallyl halides and unnatural amino acids. These glycoallyl halides were proven to be useful in the synthesis of high order glycoclusters.

ACKNOWLEDGMENTS

Finally, this gruelling effort is coming to an end. In the course of my study, many people have helped me, and now I would like to exploit this opportunity to express my gratefulness to these people.

First I would like to thank my supervising professor R. Roy for giving me this wonderful opportunity to study in his group. Because of his offer I could come to Canada to unify my wife and daughter. His broad knowledge and masterful problem-solving skills deeply impress me. His enthusiasm and insightful thoughts about science inspired me a lot. I thank him for guiding me throughout my Ph. D. program.

I also want to thank Dr. Sanjoy K. Das. His contribution to this group is tremendous. I am grateful to his various help and feel lucky to meet such a fantastic chemist and good friend. My thanks also go to current and past members of Roy's group: Dr. Romyr Dominique, for a lot of helpful discussion and good times; Brad Schmor and Mary King, for all the humours, jokes and pleasurable times; and Mr. Qingquan Wu, Dr. Zhonghong Gan, Dr. Yunjin Hu, Cindy Smith, Alex Sene for all their help. I also cannot forget the help from my friends in the Department of Chemistry.

I would like to thank professors T. Durst, A. Fallis, L. Barriault for many helpful discussions and instructions.

The collaborations with Professors F. Brewer and U. Galili from the United States of America, H.-J. Gabius from Germany are well appreciated and are also essential to my work for the biological tests of my compounds.

Finally, I want to thank my family. They have been so patient and supportive to accompany me through this long journey. Without their support, this work could not be completed.

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LIST OF ABBREVIATIONS

Ac	acetyl
AcCl	acetyl chloride
Ac_2O	acetic anhydride
AcOH	acetic acid
b	broad
Boc	*t*-butoxycarbonyl
bs	broad singlet
BSA	bovine serum albumin
t-Bu	*t*-butyl
CBz	carbobenzyloxy
CH_2Cl_2	dichloromethaane
CI	chemical ionization
CSA	camphorsulphonic acid
COSY	correlation spectroscopy
d	doublet
d	day
Da	Dalton
DCC	dicyclohexylcarbodiimide
dd	doublet of doublets
ddd	doublet of doublet of doublets
DEPT	distortionless enhanced polarization transfer

DIPEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
ELISA	enzyme-linked immunosorption assay
eq.	equivalent(s)
ES	electrospray
EtOAc	ethyl acetate
EtOH	ethanol
Et_3N	triethylamine
FAB-MS	fast atom bombardment ionization mass spectrometry
Gal	galactose
GalNAc	*N*-acetylgalactosamine
Glc	glucose
GlcNac	*N*-acetylglucosamine
h	hour(s)
HATU	O-(7-Azabenzotriazaol-1-yl)-N,N,N',N'-tetramethyluranium hexafluorophosphate
HMQC	heteronuclear multiple quantum coherence
HRMS	high resolution mass spectrum
Hz	Hertz
IC_{50}	concentration required for 50% inhibition
kDa	kiloDalton
Lac	lactose

LacNAc	*N*-acetyllactosamine
Lit.	literature
Lys	lysine
m	multiplet
M+	parent molecular ion
MALDI-TOF	matrix assisted laser desorption ion time of flight
Me	methyl
MeOH	methanol
MIC	minimal inhibitory concentration
Min	minute(s)
mmol	millimolar
mol	molar
m.p.	melting point
MS	mass spectrometry
MW	molecular weight
m/z	mass to charge ratio
NHS	*N*-hydroxy succinimide
NMR	nuclear magnetic resonance
Nu	nucleophile
O.D.	optical density
PAMAM	polyamidoamine
Ph	phenyl
ppm	parts per million

PTC	phase transfer catalysis
*p*TSA	*para*-toluene sulphonic acid
RT	room temperature
s	singlet
t	triplet
TBASH	tetrabutylammonium hydrogen sulfate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
VVA	*Vicia villosa*

CHAPTER 1 INTRODUCTION

1.1. Introduction to glycobiology

Carbohydrate epitopes widely exist on the surface of all cells and within extracellular domains in the form of glycolipids, glycoproteins, and proteoglycans[1] (Fig. 1-1). The interaction between carbohydrates and proteins contributes to many important biological events such as cell adhesion, pathogenic infections, growth regulation, internal process, tumour cell apoptosis, and xenotransplantation.[2] As ligands, glycoforms can bind with many different types of receptors such as lectins, viruses, bacteria, antibodies, and toxins, and can play essential roles in molecular and cellular recognition (Fig. 2-2).[2e,3]

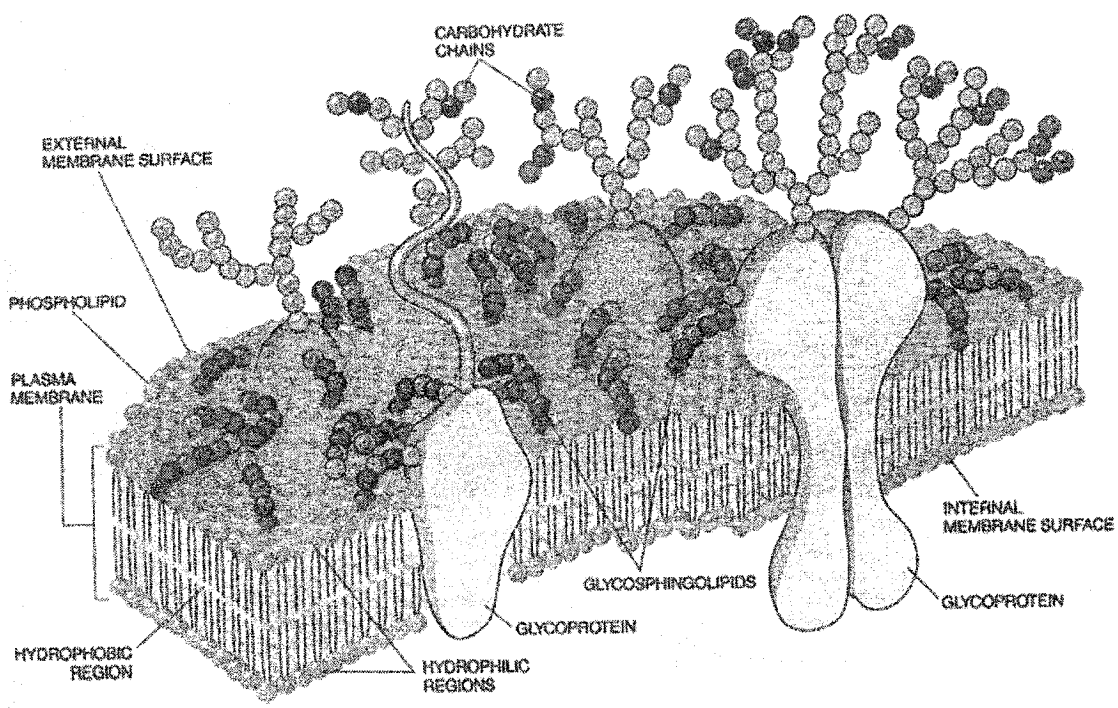


Fig. 1-1. Presentation of glycoforms on cell surface.

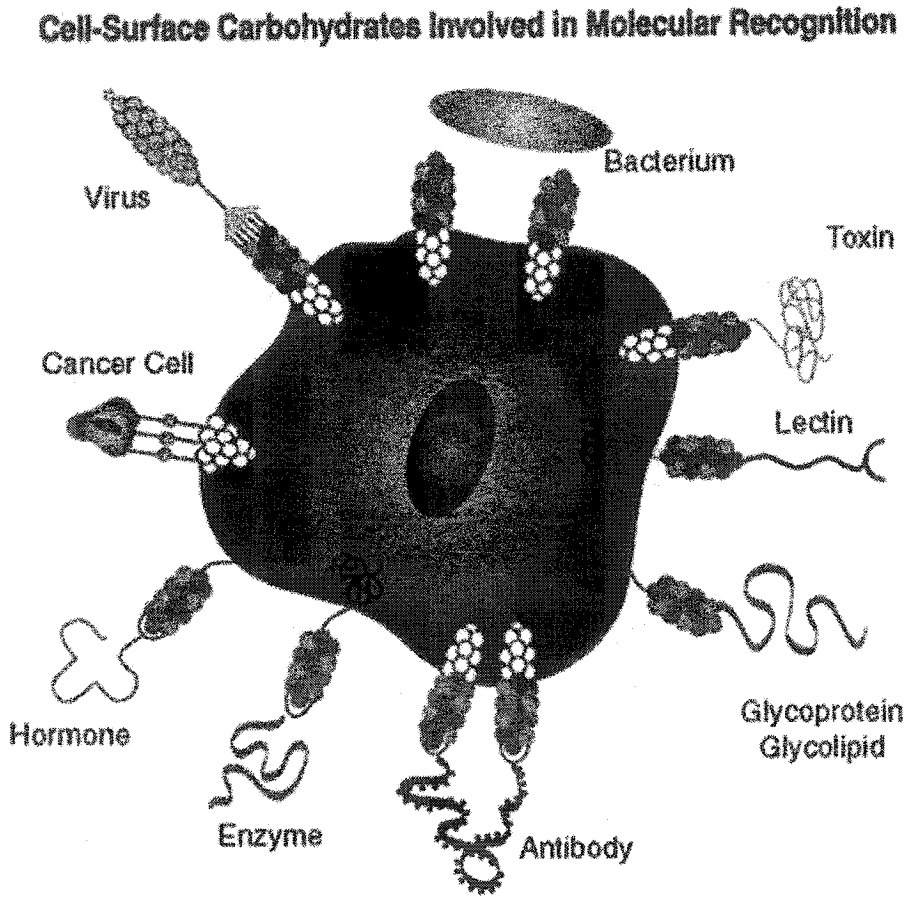


Fig. 1-2. Typical receptors of cell-surface carbohydrates.[3]

It has been proven that different sugar moieties in terms of sugar type and glycosidic linkage exert specific biological activities. For example, Galili antigen or α-Gal epitope (in the form of Galα1-3Galβ1-4Glcβ-R, Galα1-3Galβ1-4GlNAcβ-R' and Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ-R'') is present abundantly on the organ surfaces of many living organisms except that of humans, apes, and old world monkeys (Fig. 1-3).[4] It can be recognised and targeted by human anti α-Gal antibodies, which are responsible for

hyperacute xenograft rejection. Additionally, galabiose (Galα1-4Gal) in glycolipids mediates the adhesion and infection of pathogenic *E. coli* and *S. suis*.[5] Moreover, β-galactosides can bind with galectins to regulate tumour cell apoptosis and metastasis.[6]

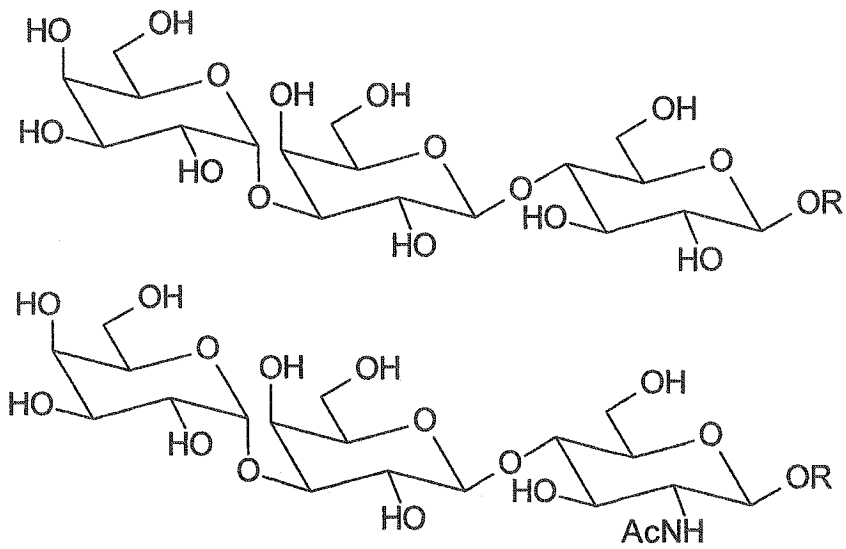


Fig. 1-3. Typical structures of Galili antigen epitopes.

Based on the interaction between carbohydrate ligands and their receptors it is logical to believe that one can use synthetic carbohydrate motifs to modulate or block a certain biological function by mimicking natural biological processes. Therefore, it might be possible to develop potential therapeutics such as enzyme inhibitors. On the other hand, the interaction mechanism at the molecular level is still illusive. As biological probes, synthetic compounds are useful in addressing this issue and deepening our insight into the basics of glycobiology which is helpful for the development of potent enzymatic inhibitors or receptor antagonists.[7]

Unfortunately, it is well known that the affinity between monovalent carbohydrate ligands and receptors is intrinsically low with Kds in the range of milimolar to milimolar.[8] To tackle this problem, nature has evolved a mechanism called "multivalent effect" or "cluster effect" to compensate the low binding affinity (Fig. 1-4)[9,10]. For instance, some receptors have multiple binding sites so they can interact with multivalent ligands. On the other hand, those monovalent receptors can cluster together to bind with multivalent ligands cooperatively to increase the binding affinity dramatically. Based on this principle, a plethora of neoglycoconjugates have been synthesised and have shown remarkable enhancement in binding efficiency.[11]

So far, two major families of neoglycoconjugates have been devised (Fig. 1-5).[11] These two families are the glycodendrimers,[11b] which include glycoclusters, and the glycopolymers,[11d-11h] which include random coil polymers, dendronized polymers, and dendrigrafts.

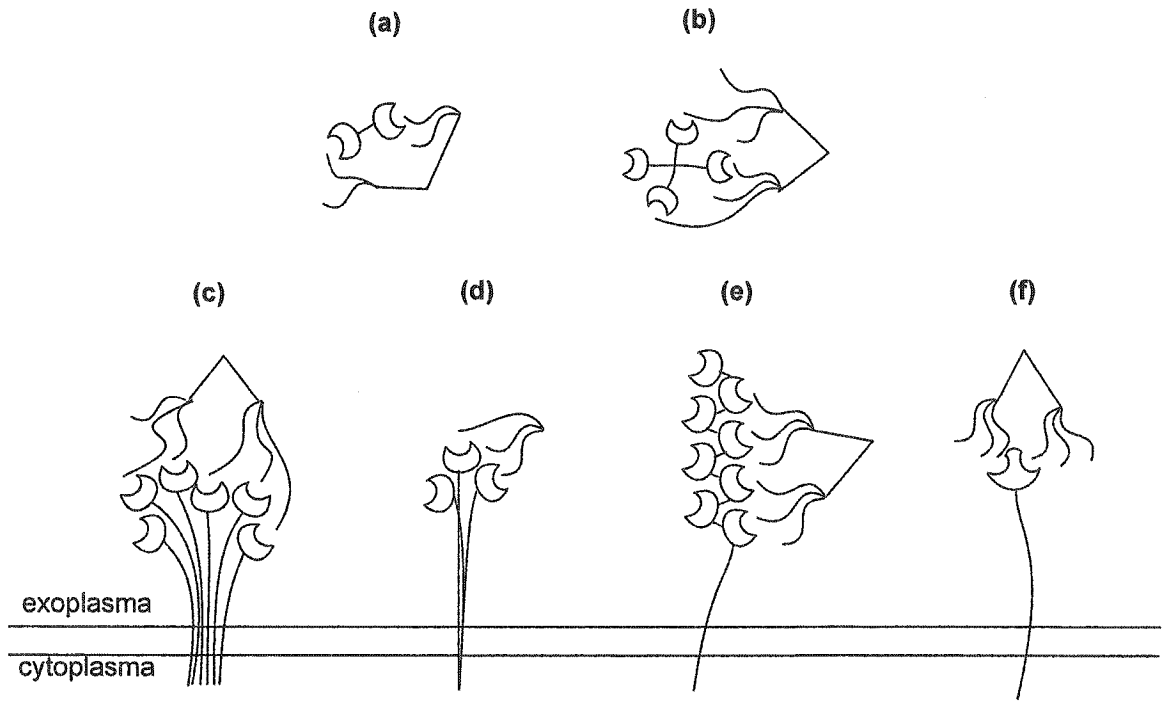


Fig. 1-4. Schematic diagram of specific carbohydrate-protein recognition processes via multivalent effect. (a) and (b) di- and tetra-valent receptors; (c) clustered monovalent receptors on the cell surface; (d) and (e) oligomeric receptors; (f) receptors that bind with more than one carbohydrates simultaneously.

Glycopolymers offer some advantages[9c] such as high valency, structural flexibility and simplicity for synthesis. These properties make them good candidates for vaccines and diagnostic reagents. However the major drawback is of structural heterogeneity including degree of polymerization, uneven distribution in copolymerization, and incomplete derivatization in dendigrafts. Glycoclusters and glycodendrimers,[12] with well-defined structures, fall into the second category. Since they own tailorable length and rigidity of the linkers, controllable geometry, predictable spatial arrangements of the sugar arrays, these

make them ideal biological probes for the investigation of glycobiology. They have helped us to better refine our understanding of multiple carbohydrate-protein interactions.

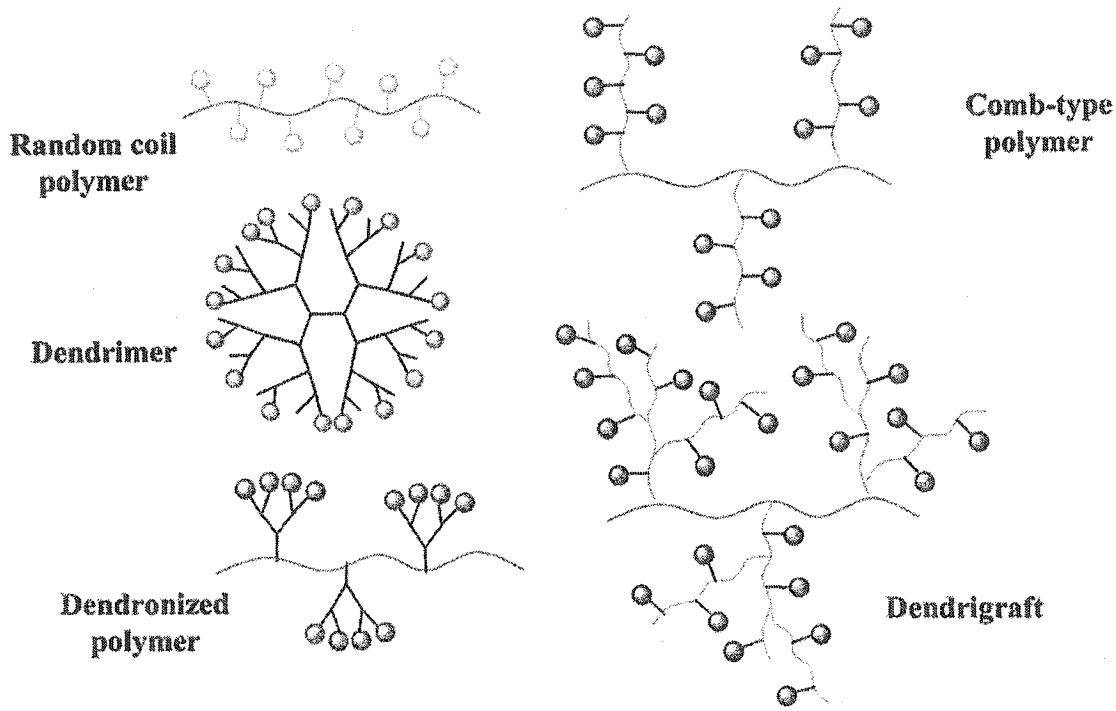


Fig. 1-5. Design of hyperbranched glycans.[12]

Galactose or galactose-terminated epitopes such as α-Gal, lactose, lactosamine, galabiose, melibiose, Gb3, *etc*, have significant biological activities, and are involved in a numerous biological processes (see Table 1-1). So far neoglycoconjugates with nonreducing galactoside ends have been studied extensively. Herein the synthesis, biological activities of glycoclusters, glycodendrimers, and glycopolymers with terminal galactosides will be summarized.

Table 1-1 Multivalent glycosides with terminal galactosides and their structures, receptors, and applications

Carbohydrate epitope	Structure	Receptor	Application	Ref.
β-Galactoside	Galβ	Asialoglycoprotein receptor, galectins	Anticancer, model study	13
α-GalNAc	GalNAcα	Asialoglycoprotein receptor, lectin (*Vicia villosa*)		14
Galabiose	Gal(α1-4)Galβ	*S. suis, E. coli*		15
Lactose	Gal(b1-4)Glcβ	Galectins	anticancer	16
Lactosamine	Gal(β1-4)GlcNAcβ	Lectin (*E. cristagalli*)		17
Melibiose	Gal(α1-6)Glc	Anti α-Gal antibodies	Xenotranspl-antation	18
T-antigen	Gal(β1-3)GalNAcα	IgG3, IgM	Cancer vaccine	19
B-disaccharide	Gal(α1-3)Galβ	Anti-B IgG		20
α-Gal	Gal(α1-3)Gal(β1-4)GalNAcβ	Anti α-Gal antibodies	Xenotranspl-antation	21
Gb_3 trisaccharide	Gal(α1-4)Gal(β1-4)Glcβ	Shiga toxins, cholera toxin		22
$isoGb_3$ trisaccharide	Gal(α1-3)Gal(β1-4)Glcβ	Toxin A (*Clostridium difficle*)		22b
GM1	Gal(β1-3)GalNAc(β1-4)[Neu5Ac(α2-3)]Lactose	Cholera toxin B; *E. coli* toxin		23

1.2. Structures of various multivalent glycosides with terminal galacoside residues and their biological activities

1.2.1. α-Galactosides targeting anti α-galactoside antibodies

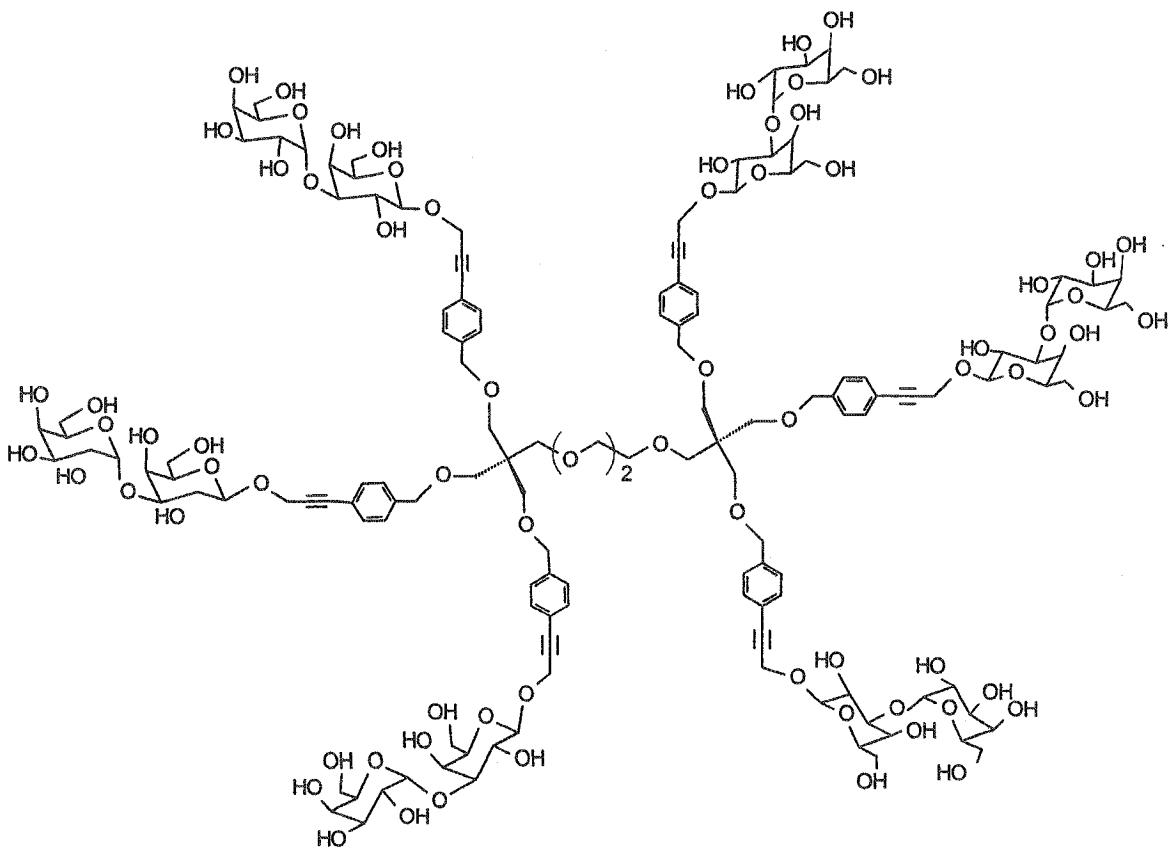
As mentioned before, xenotransplantation has been emerging as a promising strategy to tackle the problem of the severe shortage of human organ donation. Unfortunately, hyperacute xenograft rejection has dampened efforts thus far. It is believed that the immunoreaction between α-Gal (abundantly expressed on the surface of many animals' organs) and human anti α-Gal antibodies (IgG, IgM and IgA) is the major cause of xenograft rejection. A possible means to fix this problem is to "neutralize" the activity of those anti α-Gal antibodies by infusing a certain amount of synthetic α-Gal-containing epitopes prior to the operation.[4] Other groups and this group have synthesised a variety of glycoclusters, glycodendrimers and polymers bearing α-Gal motifs,[21] and some of them have shown interesting activities.

In my work, I started with the propynyl α-Gal epitope.[21g,21h] After a palladium-catalysed oxidative homocoupling reaction, or Sonogashira reactions with a series of aryl iodo scaffolds, dimer, trimer, tetramer and hexamer (Scheme 1-1) have been synthesised. Syntheses using PAMAM G0, G1 and G2 have also been performed by using the amide-coupling strategy. Enzyme-Linked Immunosorbent Assays (ELISA) against the human antibody IgG have shown certain cluster effect (at micromolar level) compared to monovalent α-Gal (at milimolar level).

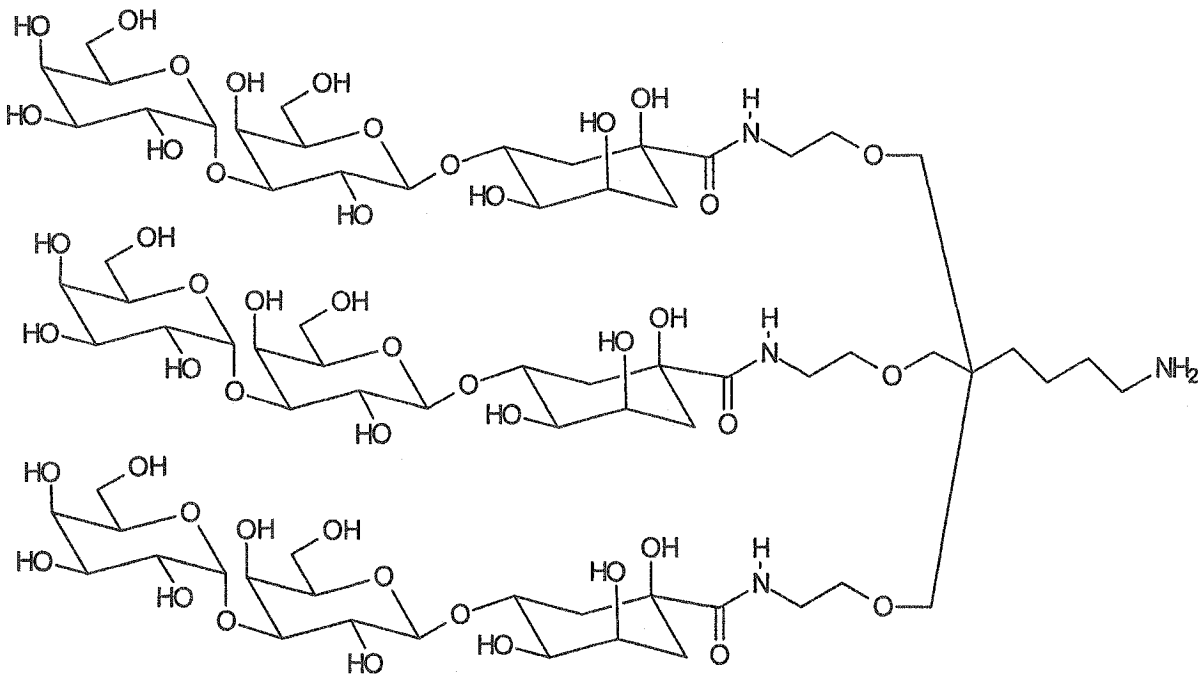
Hanessian *et al.*[21b,21c] have also reported the syntheses of trimeric clusters containing a pseudo-disaccharide and a pseudo-trisaccharide epitope respectively (Scheme 1-2). Their biological activites have not been disclosed so far.

Wang *et al.*[21d] have reported an analogue of α-Gal copolymers with acrylamide. Bioassays against mouse laminin glycoproteins and mammalian PK15 cells showed that the effectiveness of those polymers depended on the antibody isotypes and the density of the α-Gal epitopes conjugated to the polymers (Scheme 1-3). These results have demonstrated a pronounced enhancement in binding affinity toward human antibodies, especially toward the IgM and IgA with up to 4.4 X 10^4-fold increase.

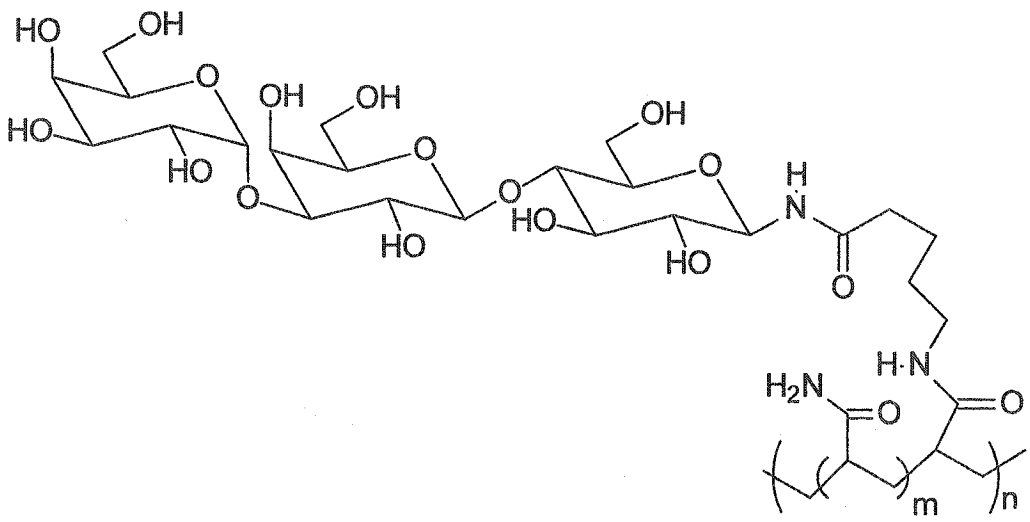
Rieben *et al.*[21a] also disclosed the synthesis and biological assays of a series of α-Gal oligomeric glycoconjugates and polymers. In their cases, those compounds demonstrated IC_{50} against human anti-α Gal IgG at micromolar level, as compared to milimolar level for the monomer.



Scheme 1-1. Roy's hexameric α-Gal cluster.[21h]



Scheme 1-2. Hanessian's α-Gal cluster.[21b]



Scheme 1-3. Wang's α-Gal polymer.[21d]

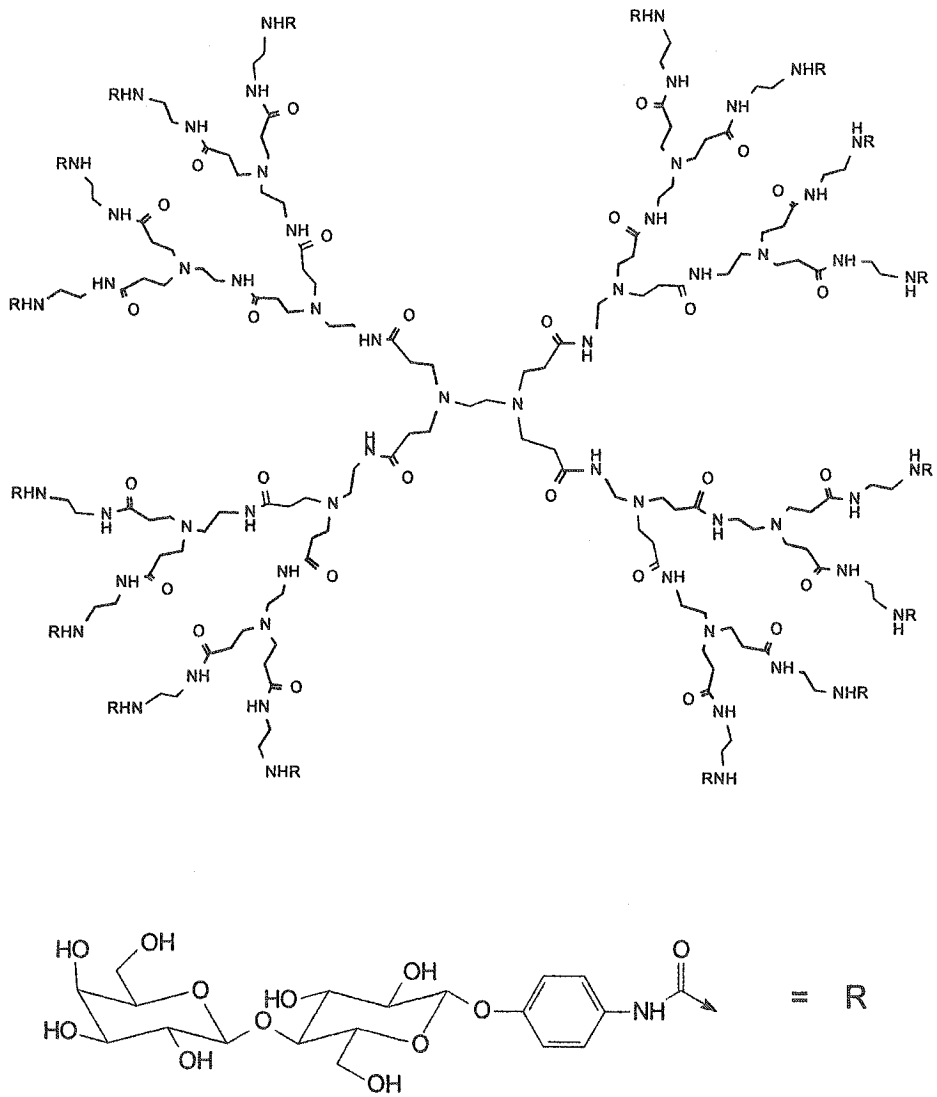
1.2.2. Galactosides and lactosides interacting with galectins and other lectins

Lectins are carbohydrate-binding proteins of non-immune origins. Galectins are a family of calcium-independent lectins comprising 14 members,[6] based on the difference in their structures and binding properties, among which, galectin-1 and galectin-3 have been widely investigated. Galectins possess a variety of biological activities such as modulating cell adhesion, regulating cell growth, and promoting cancer cell apoptosis and metastasis as well.[2e] Galectins exist on cell surfaces, within extracellular matrices, in cytoplasm, and in cell nuclei. It has been found that all galectins target β-galactose-containing glycoforms specifically. So far, many galactose and lactose-derived (lactose is usually considered as a source of terminal galactose) neoglycoconjugates have been reported.[13,16]

Polyamidoamine (PAMAM) is a family of synthetic polyamines with primary amino groups on the surface of the molecules. They are widely used as scaffolds in dendrimer synthesis. André *et al.*[16d] have reported (Scheme 1-4) analogues of lactosylated PAMAM dendrimers with remarkable cluster effect.[5] They were synthesised using the conventional thiourea strategy. The IC_{50} values for binding with galectin-1 and galectin-3 under various conditions range from 10 times to 100 times better than that of lactose on a per sugar moiety base.

Since both geometry and rigidity of the molecules, together with the topography of the sugar array play important roles in binding events, analogues of β-D-galactoside-clusters (Scheme 1-5)[13a] were designed using Sonogashira coupling reactions in order to investigate the relationship between these factors and binding properties. However, after full deprotection, these molecules were barely soluble in water due to their high hydrophobicity.

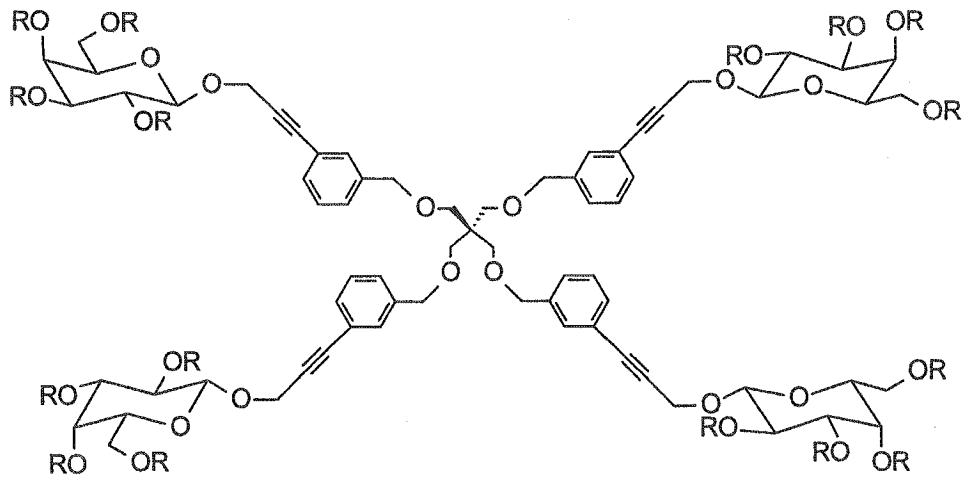
To overcome the solubility problem, lactosides were used to synthesize analogues of glycoclusters.



Scheme 1-4. Structure of Roy's lactose-bearing PAMAM G2 dendrimer.[16d]

André *et al.*[16b] have reported a set of wedge-like clusters with terminal lactosides (Scheme 1-6). They demonstrated a remarkable cluster effect toward galectin-1 and galectin-3 with a potency of thousands times better than that of lactose monomer at the

micromolar level. They have also synthesized another set of lactosylated clusters. From ELISA tests, however, those compounds did not show cluster effect against galectins. It may mean that the topographic features of the carbohydrate ligands and the topographic distributions of the binding sites on the receptors all play an important role in binding process.

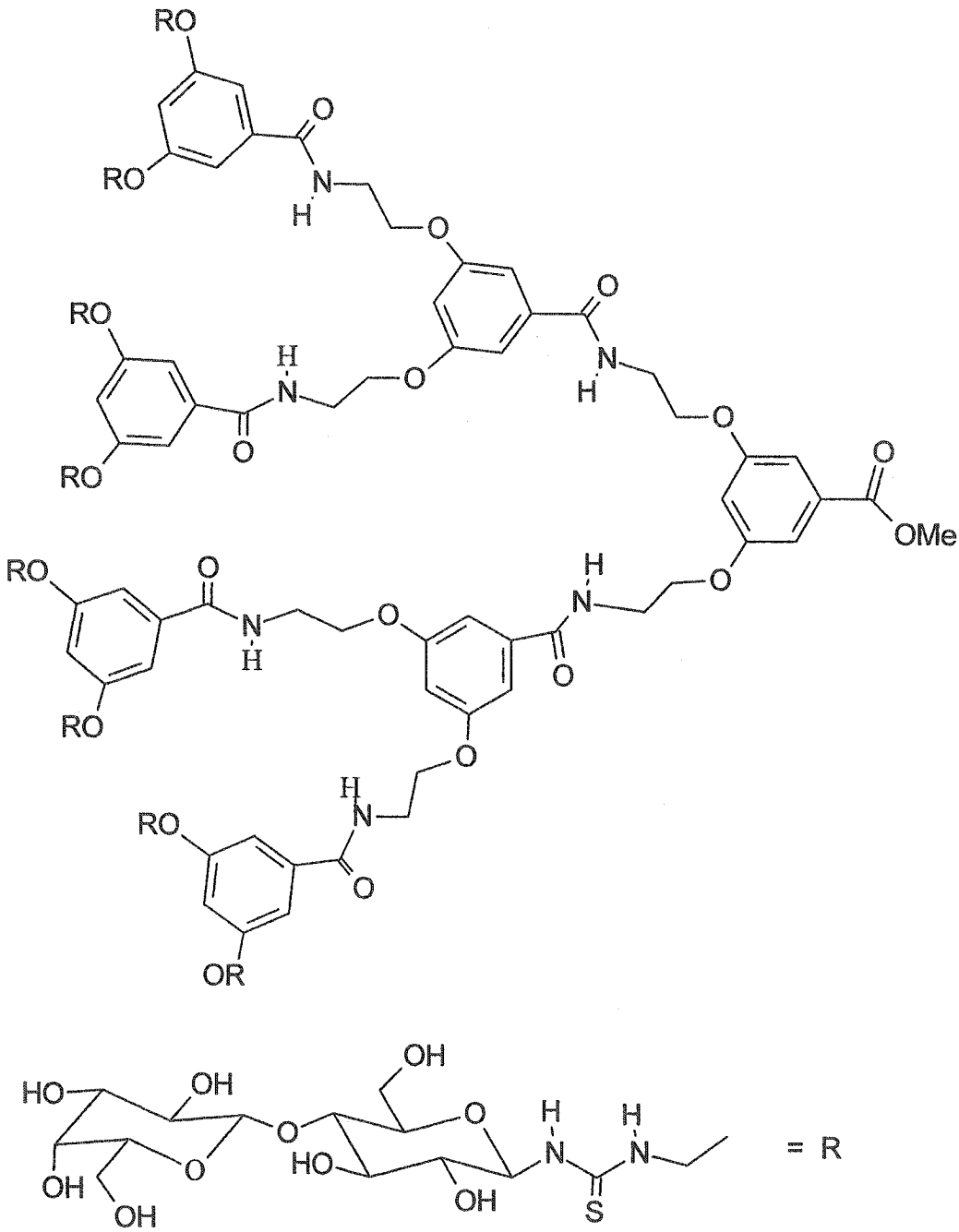


Scheme 1-5. Tetrameric cluster containing galactosides.[13a]

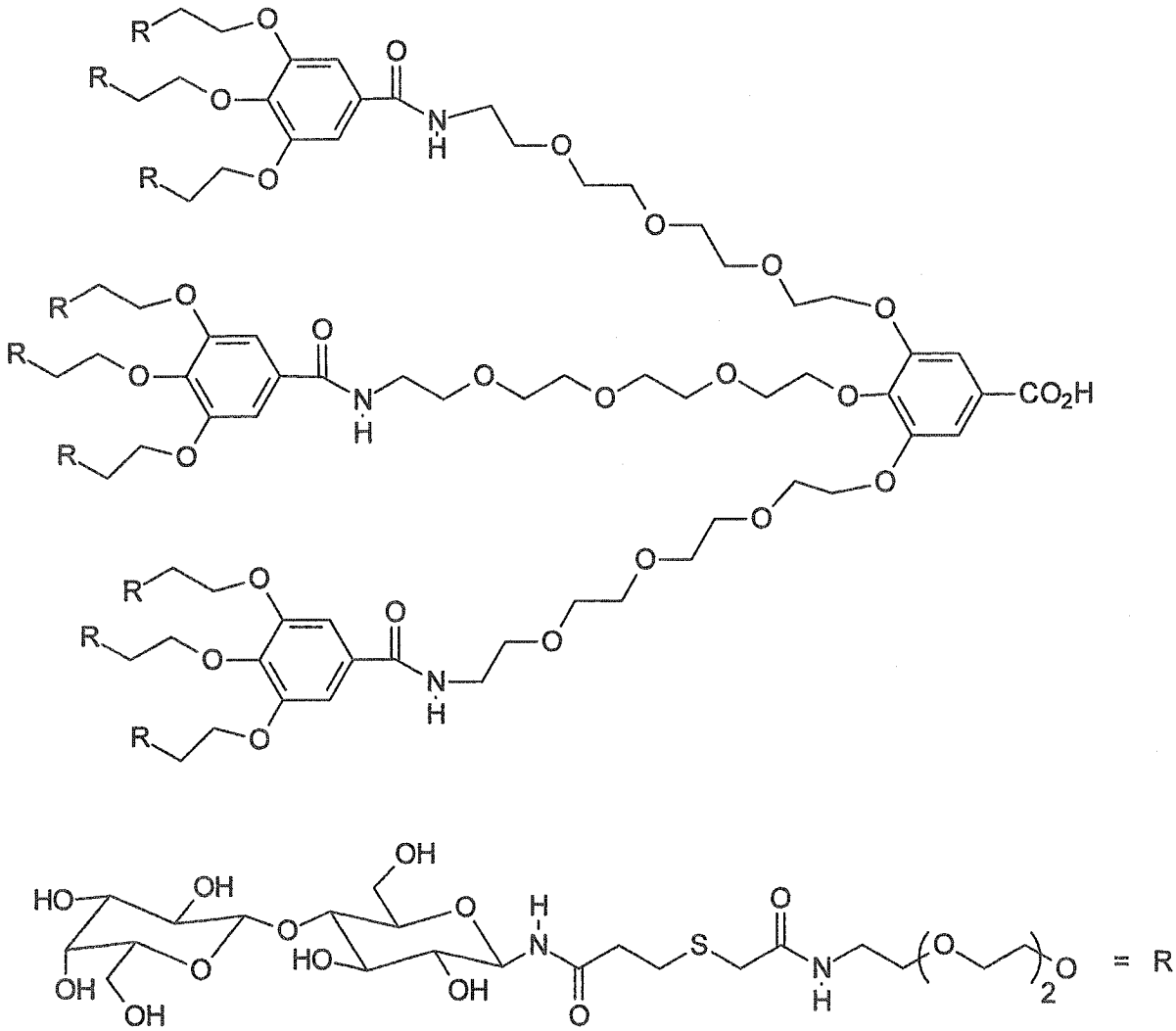
The binding specificity of galactosides and lactosides are not high and they can interact with other types of lectins. For instance, Roy and Park[16f] have reported the synthesis of gallic acid (Scheme 1-7) and polylysine-based dendrimers with terminal lactose. As indicated by double-immunodiffusion assays against peanut lectin from *Arachis Hypogaea*, the higher order dendrimers showed stronger binding toward the lectin used as a model.

Stoddart *et al.*[13d] have reported the synthesis of galacto- and lactodendrimers using polypropylene imine (DAB-*dendr*-$(NH_2)_x$) as scaffolds. However, their biological activities were not disclosed (Scheme 1-8). Li *et al.*[16a] also demonstrated a heptameric lactosyl cluster

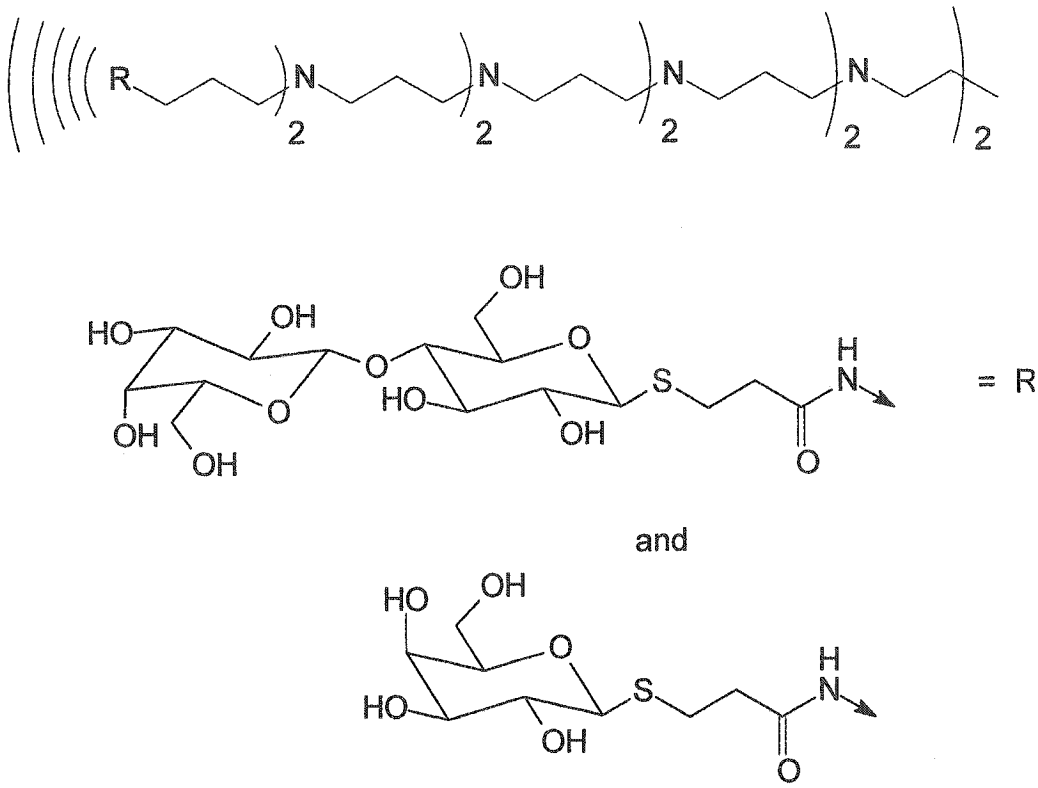
using lactose as the scaffold and terminated with lactose (Scheme 1-9). The related bioassay results have not been reported.



Scheme 1-6. Wedge-like dendrimer with terminal lactosides.[16b]

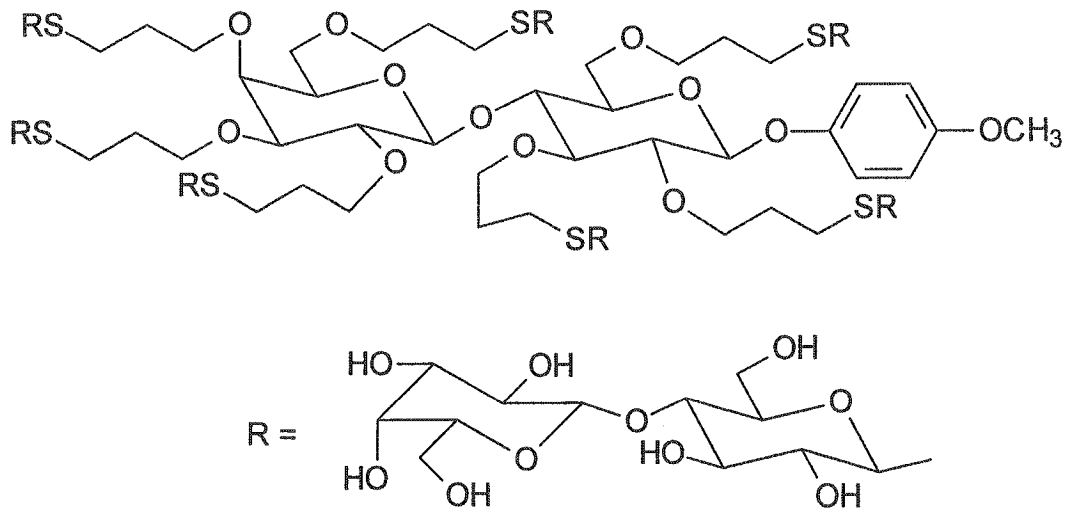


Scheme 1-7. Galactodendrimer with a gallic acid scaffold from Roy's group.[16f]



Scheme 1-8. Galacto- and lactodendrimers from Stoddart's group.[13d]

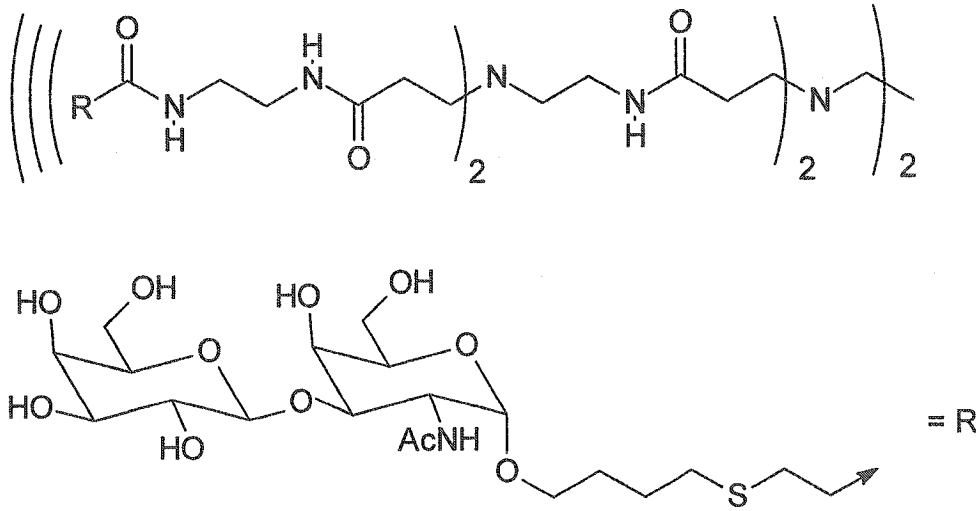
Vargas-Berenguel *et al.*[13e] reported the syntheses of galacto- and lactoclusters using a β-cyclodextrin core to investigate the potential of those compounds as molecular carriers. They concluded that a clustered lactoside might be used as a molecular carrier to transport a guest molecule towards a specific lactoside receptor such as peanut lectin (PNA).



Scheme 1-9. Lactodendrimer using lactose as a core.[16a]

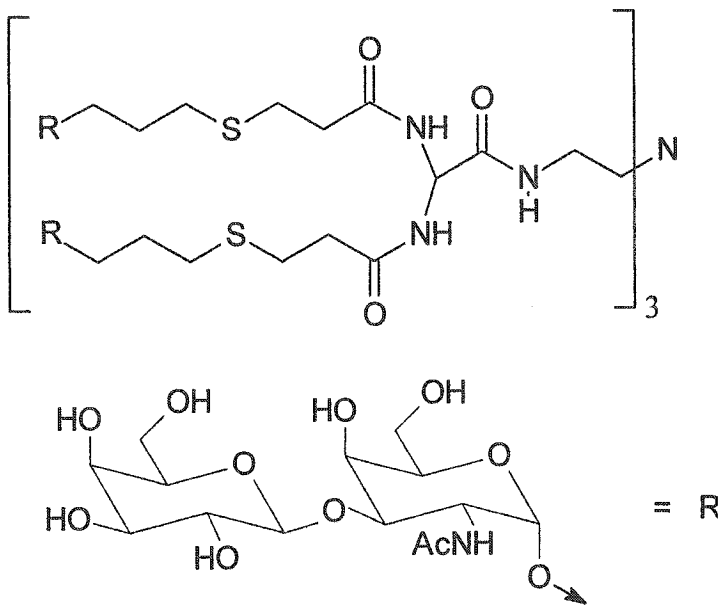
1.2.3. T-antigen/Anti T-antibody

T-antigen (Thomsen-Friedenrich antigen, Galβ1-4GalNAcα-OR) is a disaccharide moiety expressed on the surface of erythrocytes and epithelial cells, and has been used for the detection and immunotherapy of tumours, especially involved with breast cancer. Roy *et al.*[19e] have synthesized 4 PAMAM-scaffolded T-antigen dendrimers with valencies ranging from 4 to 32 (Scheme 1-10). Results from ELISA tests targeting mouse antibody IgG_3 showed that all four dendrimers were 115-fold more potent than that of the monomer on a per saccharide basis (at the nanomolar level). Interestingly, although those compounds demonstrated a remarkable cluster effect, they did not show any trend in terms of binding affinity.



Scheme 1-10. Octomeric PAMAM-based T-antigen dendrimer (G2).[19e]

Roy *et al.*[19b] also reported another analogue of T-antigen clusters based on N,N'-bis(acrylamido)acetic acid (Scheme 1-11). From ELISA tests, these compounds demonstrated more than 10-fold increase in binding affinity toward mouse antibody IgG_3 compared to the monomeric T-antigen, with IC_{50} values at the submicromolar level. Among the four compounds, the two tetramers showed much higher potency than the dimer and hexamer. Interestingly, biotinylated lysine-based T-antigen dendrimers did not bind to breast cancer tissue, thus demonstrating that there is no receptor for this ligand on the surface.



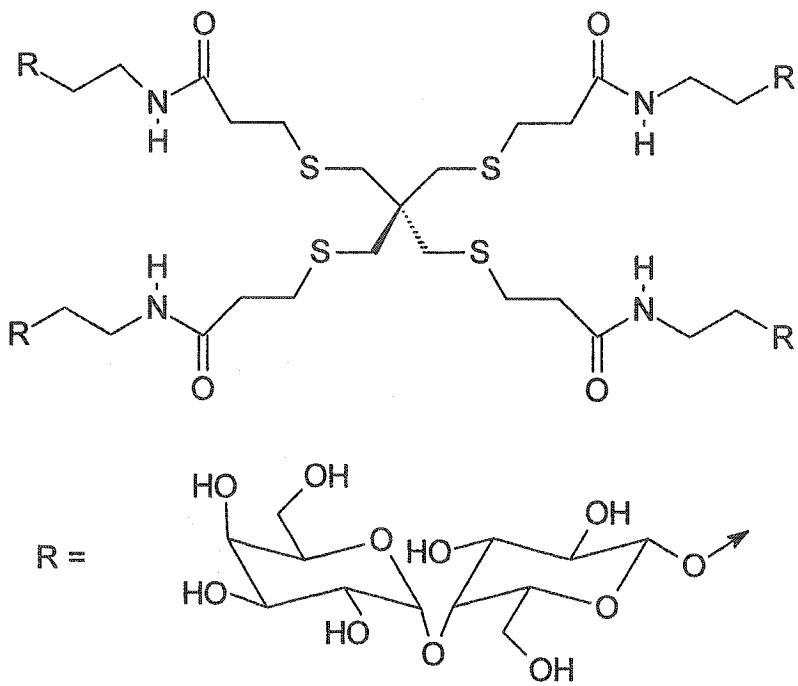
Scheme 1-11. Hexameric T-antigen dendrimer based on N, N'-bis(acrylamido)acetic core.[19b]

1.2.4. Galabiose interacting with *E. coli* and *S. Suis*

Galabiose (Galα1-4Gal) is one of the characteristic structural entities of glycolipids expressed on the surface of human erythrocytes and serves as an inhibitor of pathogenic *E. coli* and of *S. suis* hemagglutination. Magnusson *et al.*[15a] have reported the synthesis of cluster analogues containing several galabioside residues (Scheme 1-12). Inhibition of agglutination of human erythrocytes by *S. suis* demonstrated that all the dimers, trimers, and tetramers showed much enhanced binding affinities. It also showed that longer spacers tend to increase the binding affinity. While dimers and tetramers were better inhibitors than trimers, thus the geometry of these inhibitors may play some role as well. These results reminded us again that fine-tuning of the chosen structures might be helpful in deciphering a biological event.

1.2.5. Gb_3 and $isoGb_3$ binding to Shiga or Shiga-like toxins

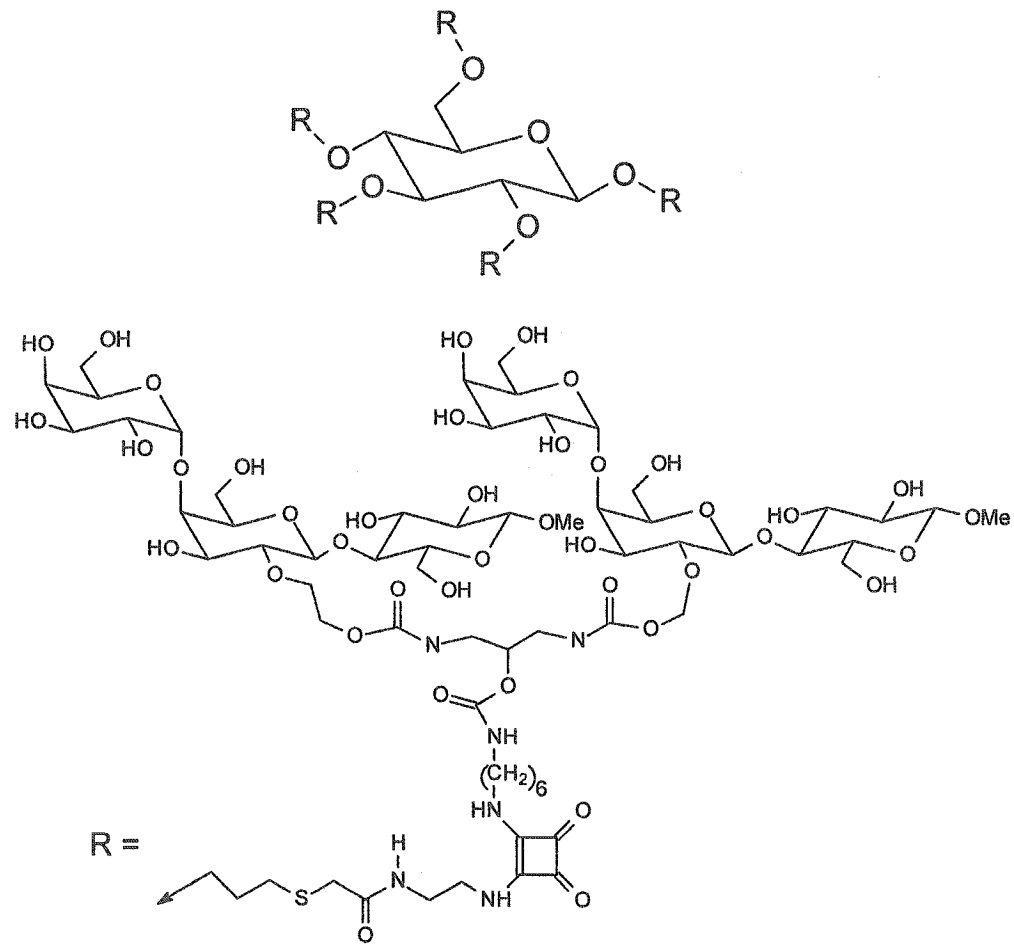
Gb_3 or globotrioside (Galα1-4Galβ1-4Glc) is actually the same as galabiose in source, specifically targeted by Shiga toxin, Shiga-like toxins, heat-labile enterotoxin (LT), and pertussis toxin, a family of AB_5 bacterial toxins. Based on a rational-design strategy, Bundle *et al.*[22a] reported the synthesis of a STARFISH molecule using glucose as the pentavalent core (Scheme 1-13). With the well-tailored length of spacers and the incorporation of rigid domains in the structure, the STARFISH molecule showed 1 million times more potent than its monomer against Shiga-like toxin-I in ELISA assays.



Scheme 1-12. Magnusson's tetravalent galabiocluster.[15a]

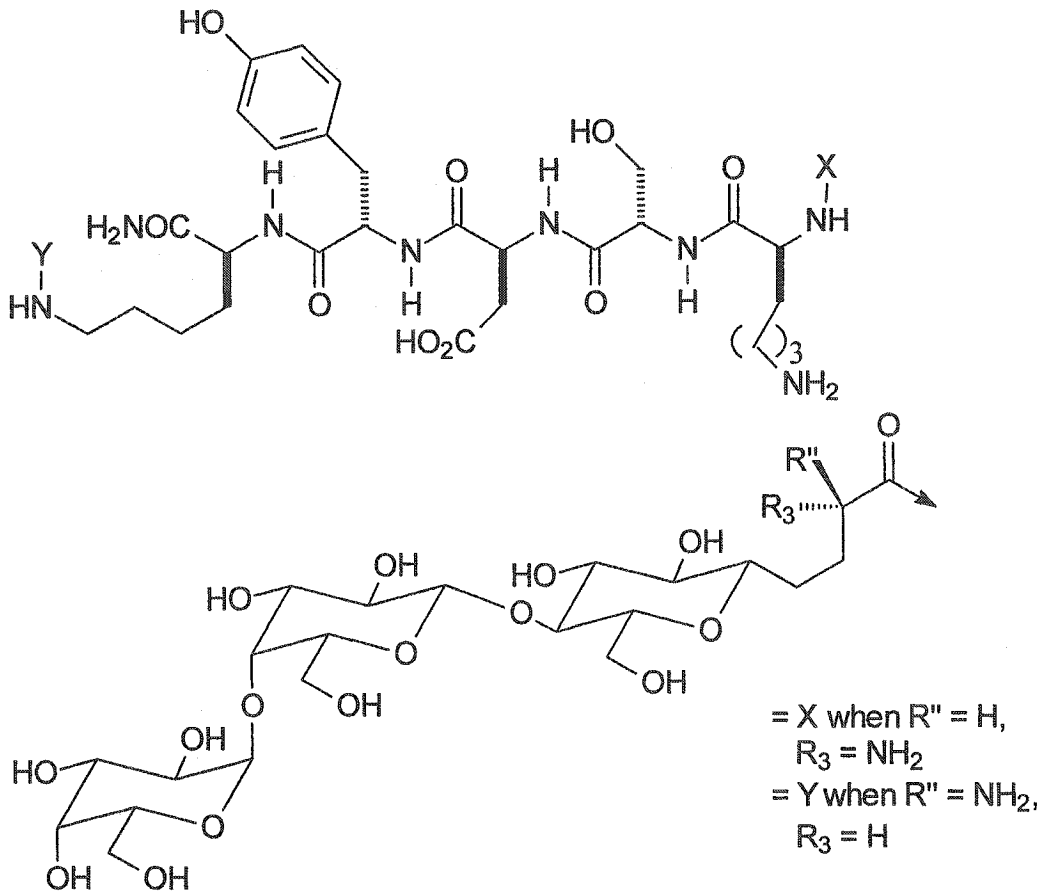
Shiga or Shiga-like toxin consists of 2 AB_5 units. This well-distinguished structure has prompted researchers to investigate the multivalency effect. For example, Toone *et al.*[22d]

have synthesised C-linked Gb3-based bivalent glycopeptides (Scheme 1-14) as a model to study the binding efficiency toward SLT-1. These bivalent glycopeptides have shown enhanced binding affinity compared to monovalent glycopeptides.



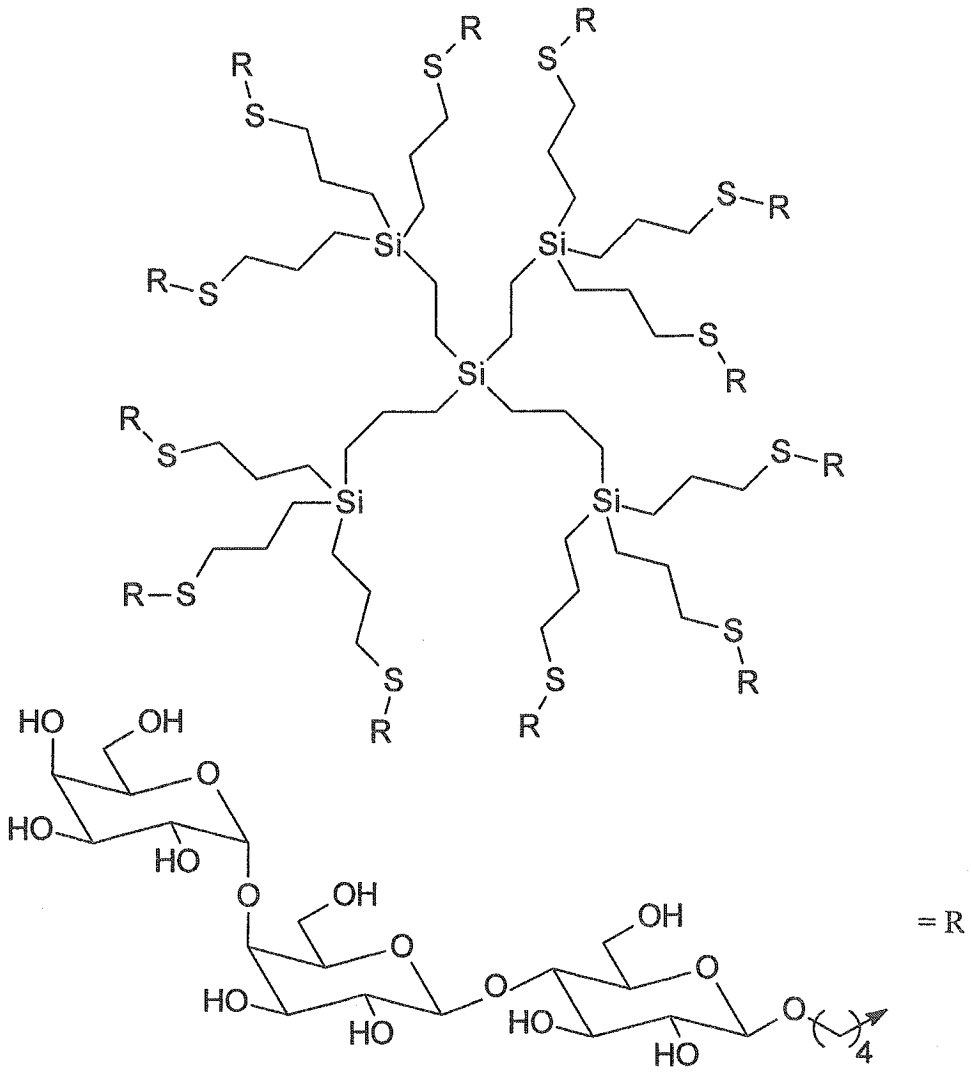
Scheme 1-13. Starfish molecule from Bundle's group.[22a]

Kuzuhara *et al.* adopted carbosilanes as cores to prepare several GB3-containing dendrimers (Scheme 1-15). The preliminary bioassays using cell culture showed some activities against Verotoxins.



Scheme 1-14. Toone's divalent glycopeptide containing Gb3.[22d]

Matsuoka *et al.*[22b] recently reported the synthesis of glycopolymers bearing Gb3 and isoGB3 with biological tests against Shiga toxins. It demonstrated that Gb3 and isoGb3 have different behaviours towards Shiga toxins and different toxins possess distinguishable activities to the biopolymers.

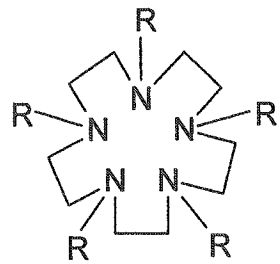


Scheme 1-15. Carbosilyl dendrimer bearing Gb3.[22b]

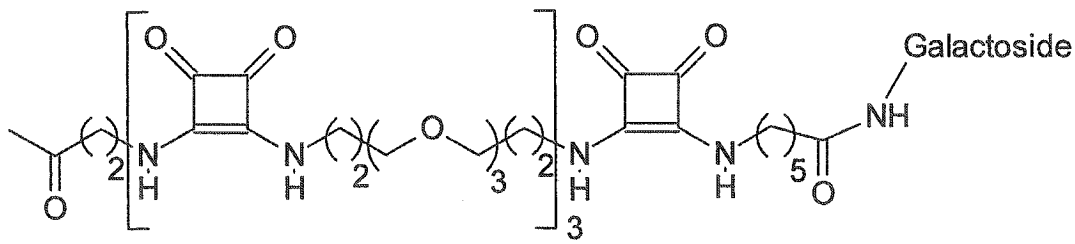
1.2.6. GM_1

GM1 (Galβ1-3GalNAcβ1-4Neu5Acα2-3Lac) is the carbohydrate epitope of some gangliosides existing on the cell surface of gastrointestinal lumen and specifically binds with enterotoxin from *E. coli*. Fan *et al.*[23c] using the so-called structure-based-design strategy, synthesised a pentavalent ligand to enterotoxin. In the synthesis, they adopted a structurally

constrained core and semi-rigid spacer, along with terminal galactose as the binding entity (Scheme 1-16). This molecule exhibited about 1-million fold enhancement in binding efficiency over galactoside with IC_{50} at the submicromolar level.



R =

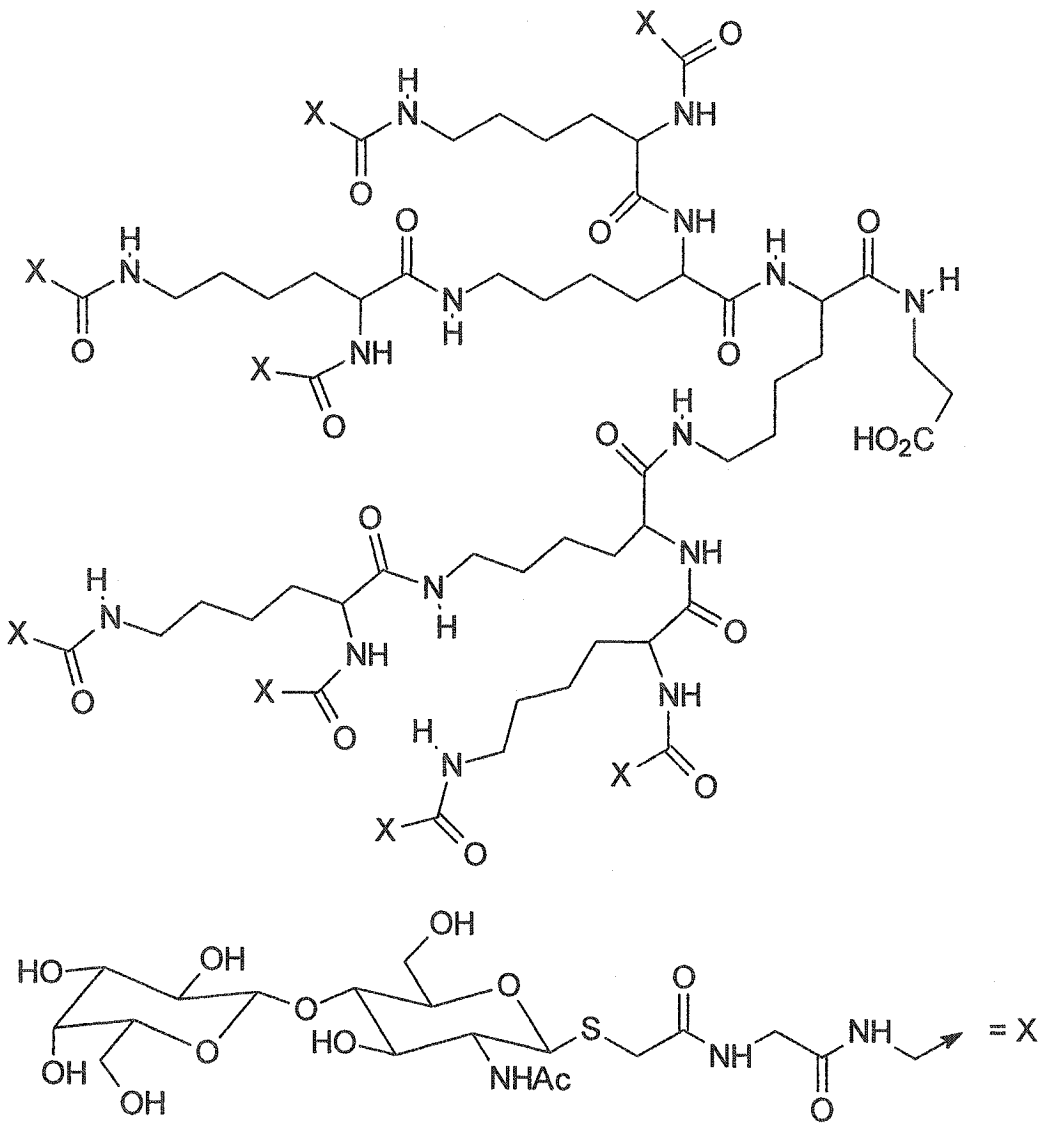


Scheme 1-16. Fan's glycodendrimer carrying GM1 entity.[23c]

1.2.7. *N*-Acetyllactosamine

N-Acetyllactosamine (LacNAc, Galβ1-4GlcNAc) is the typical sugar moiety of many glycoproteins and glycolipids involved in mouse colon cancer, thyroid disorders, etc. Roy *et al.*[17a] have reported the chemoenzymatic synthesis of lysine-based dendritic *N*-acetylactosamine (Scheme 1-17). Inhibitory binding assay against Wheat Germ Agglutinin

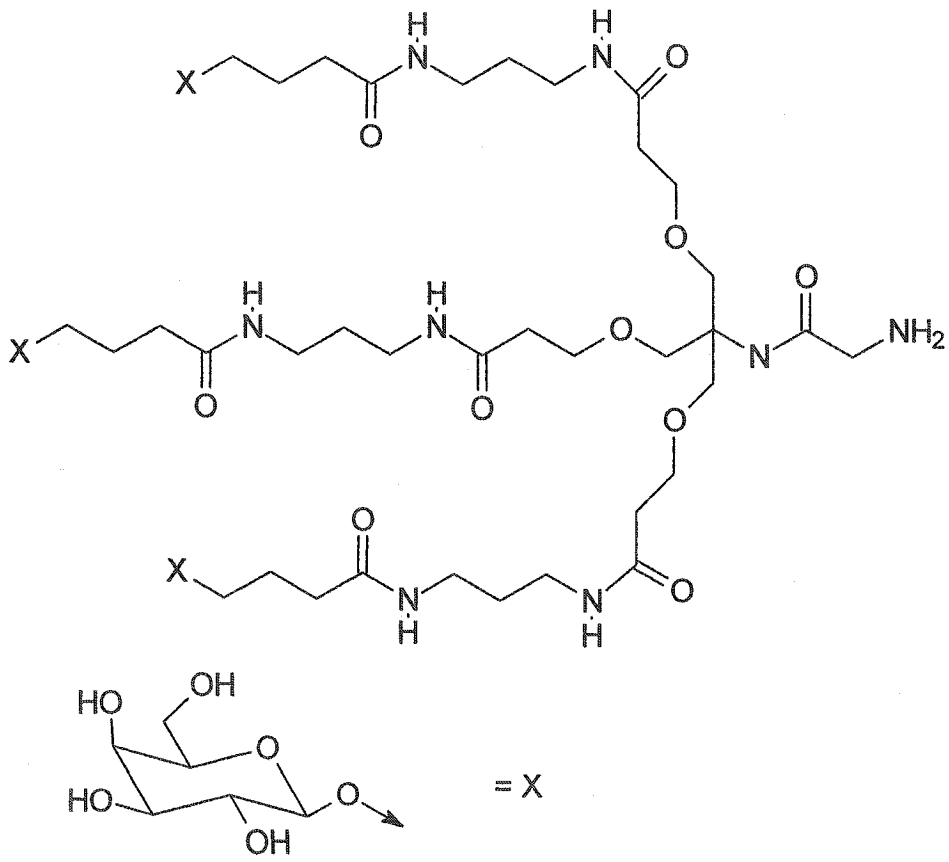
(WGA) demonstrated about 5-fold increase of binding affinity relative to monomeric lactosamine on per sugar basis.



Scheme 1-17. Roy's octameric lysine-based dendrimer bearing LacNAc.[17a]

1.2.8. Miscellaneous

Asialoglycoprotein receptor (ASGPr), a galactose-recognizing receptor, is abundantly expressed on parenchymal liver cell surface. Van Boom *et al.*[13f] have synthesized an analogue of bifunctional glycolipids, with a trivalent galactoside epitope on one side of the molecule and a drug on the other side (Scheme 1-18). In this case, based on the interaction between galactosides and ASPGPr, the galactoside serves as a site-specific carrier to convey the drug to the desired liver cells. The results have demonstrated an increase in the uptake efficiency of drugs.



Scheme 1-18. van Boom's trimeric galactocluster.[13f]

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3. Picture was taken from Wong, C.-H's homepage (the Scripps Research Institute).

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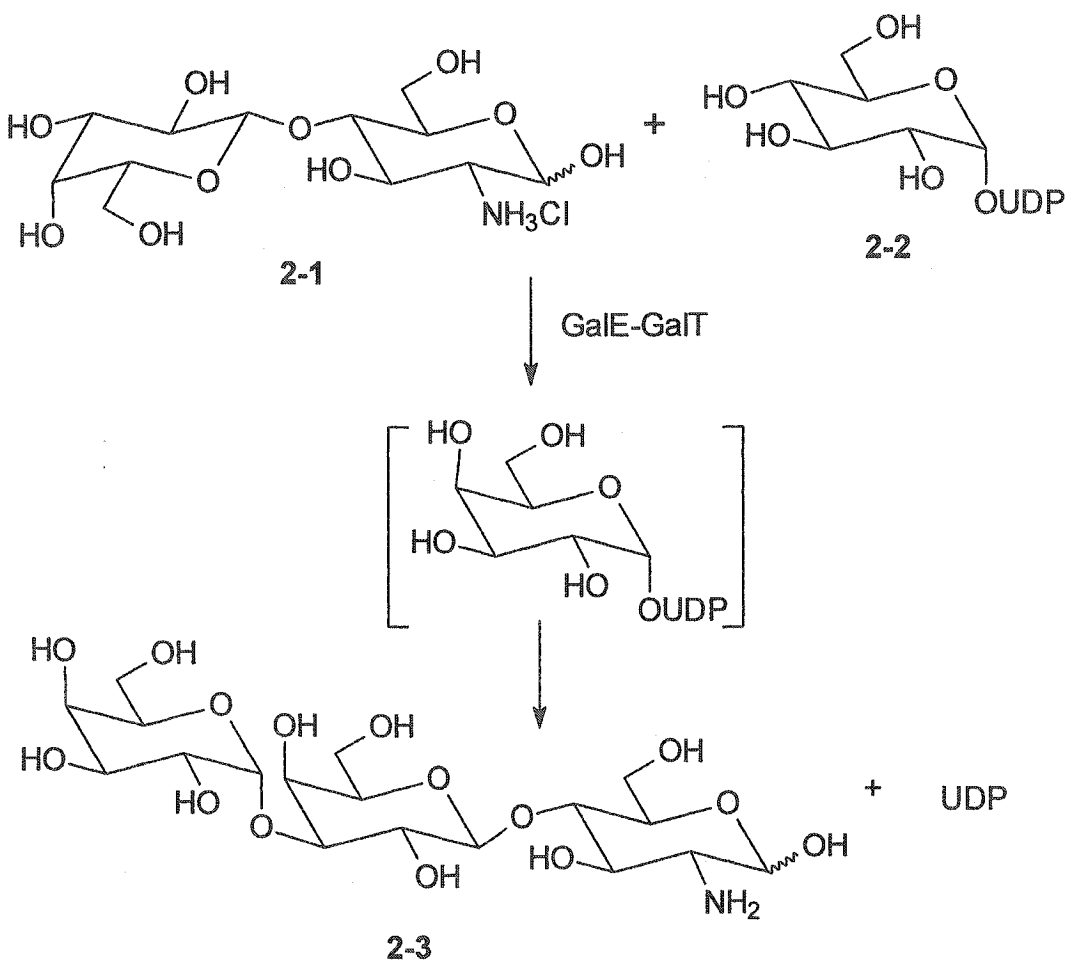
CHAPTER 2 SYNTHESIS OF GALILI ANTIGEN-CONTAINING PRECURSORS

2.1. Introduction

Galili antigens are cell surface carbohydrate motifs containing Galα1-3Galβ non-reducing ends responsible for hyperacute rejection following pig to human xenotransplantation.[1] The shortage of organ donors has caused a search for alternatives. Pig's livers have been considered as viable alternatives because their size, availability, and low risk of viral transmission are consistant with what is needed.[2] Unfortunately, most human sera possess abundant anti α-Gal antibodies (1-2% IgG, 3-8% IgM) as a result of prior exposure to bacterial or viral infections.[3] Thus, within minutes to hours of surgery and in spite of immunosuppressive treatments, the transplanted organs are rapidly rejected by most recipients. To overcome this problem, a possible strategy has been to develop antagonizing anti α-Gal inhibitors by infusing a large concentration of synthetic α-Gal oligosaccharides or by using affinity columns to deplete the patient's serum of α-Gal antibodies.[4] Polymeric α-Gal epitopes have also been designed to increase the antagonizing activity of simple, low affinity oligosaccharides.[5,6]

So far α-Gal epitopes have been synthesized using chemical or chemoenzymatic procedures. For instance, Wang's group [6c,7] reported the synthesis of the Galili antigen trisaccharide Galα1-3Galβ1-4GlcNNH$_2$ (**2-1**) using a chemoenzymatic method. The synthesis was started with lactosamine and UDP-glucose (**2-2**) (UDP-Glc) substrates, using an engineered enzyme (GalE-GalT) containing both uridine-5'-diphospho-galactose 4-epimerase (GalE) and α(1-3) galactosyltransferase (GalT) to catalyze the reaction. First,

UDP-Glc was epimerized to the glycosyl donor UDP-Gal, and then transformed into lactosamine as the receptor (Scheme 2-1). The glycosidation reaction was effected with excellent regio- and stereoselectivity and provided **2-3** in 64% yield.



Scheme 2-1. Wang's synthesis of α-Gal epitope **2-3**.

Rieben *et al.*[8] also reported a chemoenzymatic synthesis of α-Gal epitope Galα1-3Galβ1-3GlcNAc. In their work, they used disaccharide Galβ1-3GlcNAc and UDP-Gal as the substrates, a recombinant bovine α1-3-galactosyltransferase to effect the α1-3-

galactosylation to prepare the target compound in 60-90% yield. Galili *et al.* [9] also disclosed the synthesis of α-Gal epitope using α1-3-galactosyltransferases from various sources.

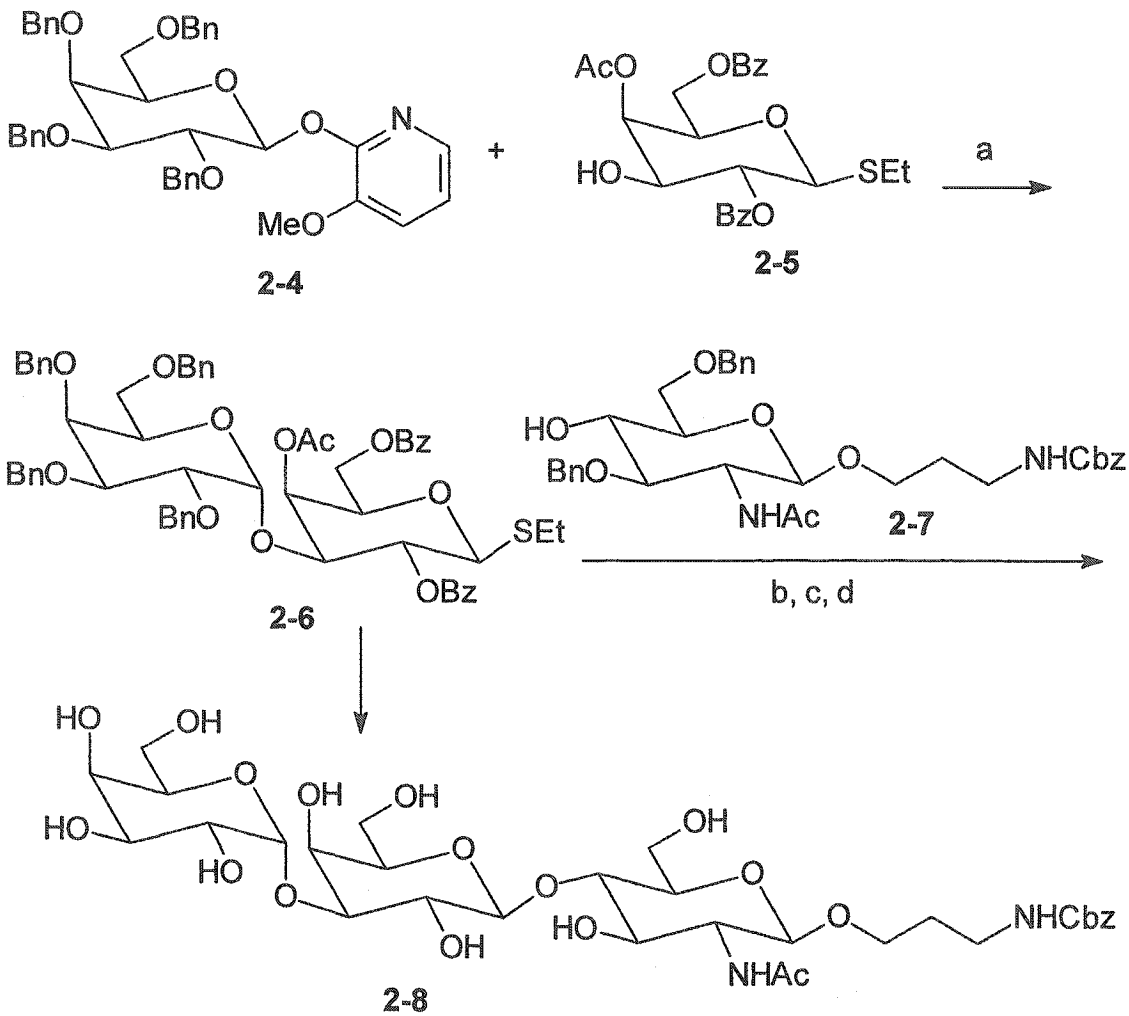
Alternatively, Hanessian *et al.* [10] applied chemical methods for the synthesis of α-Gal precursors (Scheme 2-2). In their synthesis, the α1-3-galactosylation was achieved by a protocol developed in their lab using $Cu(OTf)_2$ as a promoter to synthesize the disaccharide 2-6 in 75% yield. All the other transformations followed standard procedures. The following section describes our synthesis of an α–Gal precursor.

2.2. Results and Discussion

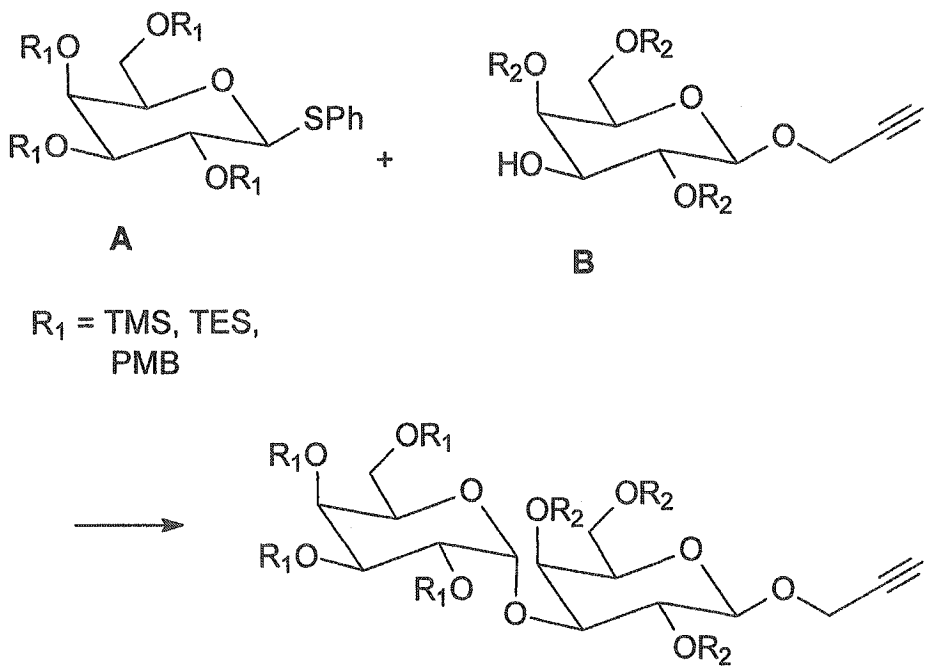
Recent investigations have shown that palladium-catalyzed cross-coupling of propynyl and aryl halides bearing saccharides provide an efficient entry into glycoclusters having high affinity.[11] The rationale for rigid-linkers is based on the findings that conformationally restricted ligands are exposed to a lesser entropic loss during binding to their homologous receptors.[12] To reach this goal for the Galili antigen, the plan was to synthesize 2-propynyl disaccharide **2-16** as the starting precursor (Scheme 2-5).

To achieve an α1-3 glycosidation, the key was to put a non-participating group at the C-2 position in the glycosyl donor **A** (Scheme 2-3). Originally, it was planed to use 2-propynyl galactoside **B** as the acceptor. To avoid hydrogenolysis process after glycosidation, trimethylsilyl (TMS) and triethylsilyl (TES) protecting groups were chosen to prepare persilylated thio galactosides as glycosyl donors **A**. Unfortunately, those silyl sugars were not stable enough to resist workup and/or purification on silica gel column. *T*-butyldimethylsilyl was too bulky a protecting group to fully derivatize the unsubstituted thio galactoside even on heating up to 90 °C in pyridine for 2 days. The use of *para*-methoxy

benzyl (PMB) as R_1 was also unsuccessful, since subsequent deprotection did not give a clean transformation.



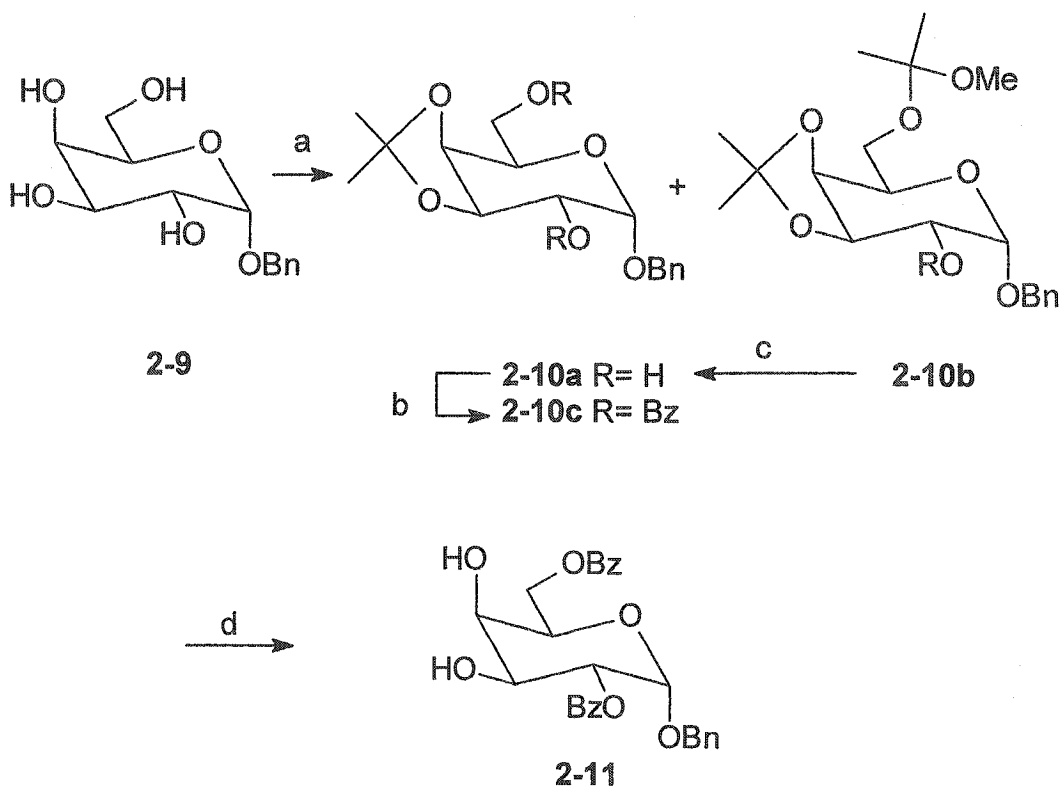
Scheme 2-2. Hanessian's synthesis of α-Gal epitope **2-8**. (a) Cu $(OTf)_2$, 75%; (b) NIS/TfOH, 45%; (c) NaOMe/MeOH, 95%; (d) H_2, 10 % Pd $(OH)_2$/C, 85%.



Scheme 2-3. Schematic diagram of α1-3 glycosidation.

Initial attempts to introduce the suitably protected 2-propynyl β-D-galactopyranoside as a glycosyl acceptor were also met with some difficulties due to the incompatibility of the alkyne moieties toward glycosylating reagents such as N-iodosuccinimide-triflic acid (NIS-TfOH). Therefore a feasible strategy was designed in which the required 2-propynyl aglycone was introduced at the very end of the disaccharide synthesis. Thus, benzyl 2,6-di-O-benzoyl-α-D-galactopyranoside acceptor **2-11** was synthesized using an established protocol (Scheme 2-4). Starting from the known[13] benzyl α-D-galactopyranoside **2-9**, 3,4-unprotected free galactosyl acceptor **2-11** was obtained in three steps starting with by regioselective acetalation of **2-9** with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid (PTSA) to provide the corresponding acetals **2-10a** and **2-10b**. Upon treatment with a catalytic amount of pyridinum *p*-toluenesulfonate (PPTS), **2-10b** was

hydrolyzed to **2-10a** and provided **2-10a** in 85% overall yield. Treatment of the resulting diol **2-10a** with benzoyl chloride (pyridine, 4-*N*,*N*-dimethylaminopyridine (DMAP)) gave **2-10c** (90%). Acetal hydrolysis in 60% aqueous acetic acid (80°C, 5 h) afforded galactosyl acceptor **2-11** (for NMR, see Fig. 2-1) in 95% yield.



Scheme 2-4. Synthesis of compound **2-11.** (a) 2,2-dimethoxypropane, PTSA, rt, 5 h, 85%; (b) BzCl, pyridine, DMAP, rt, 2 h, 90%; (c) CH_2Cl_2/MeOH, PPTS, rt, 2 h; (d) 60% AcOH, 80°C, 5 h, 95%.

Perbenzylated phenyl β-D-thiogalactosyl donor **2-13b** was prepared from tetraacetylated β-D-galactopyranoside **2-12**[14]

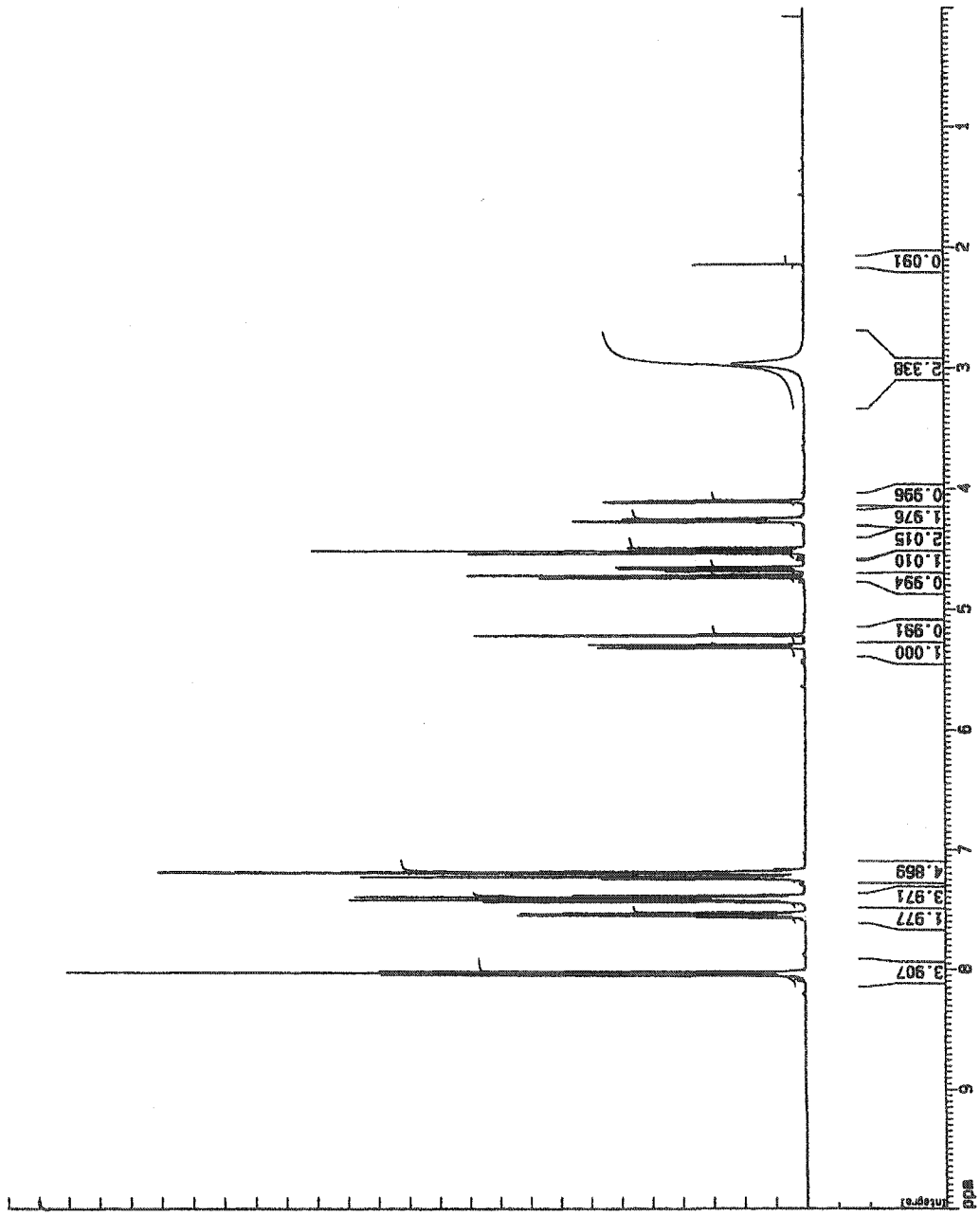
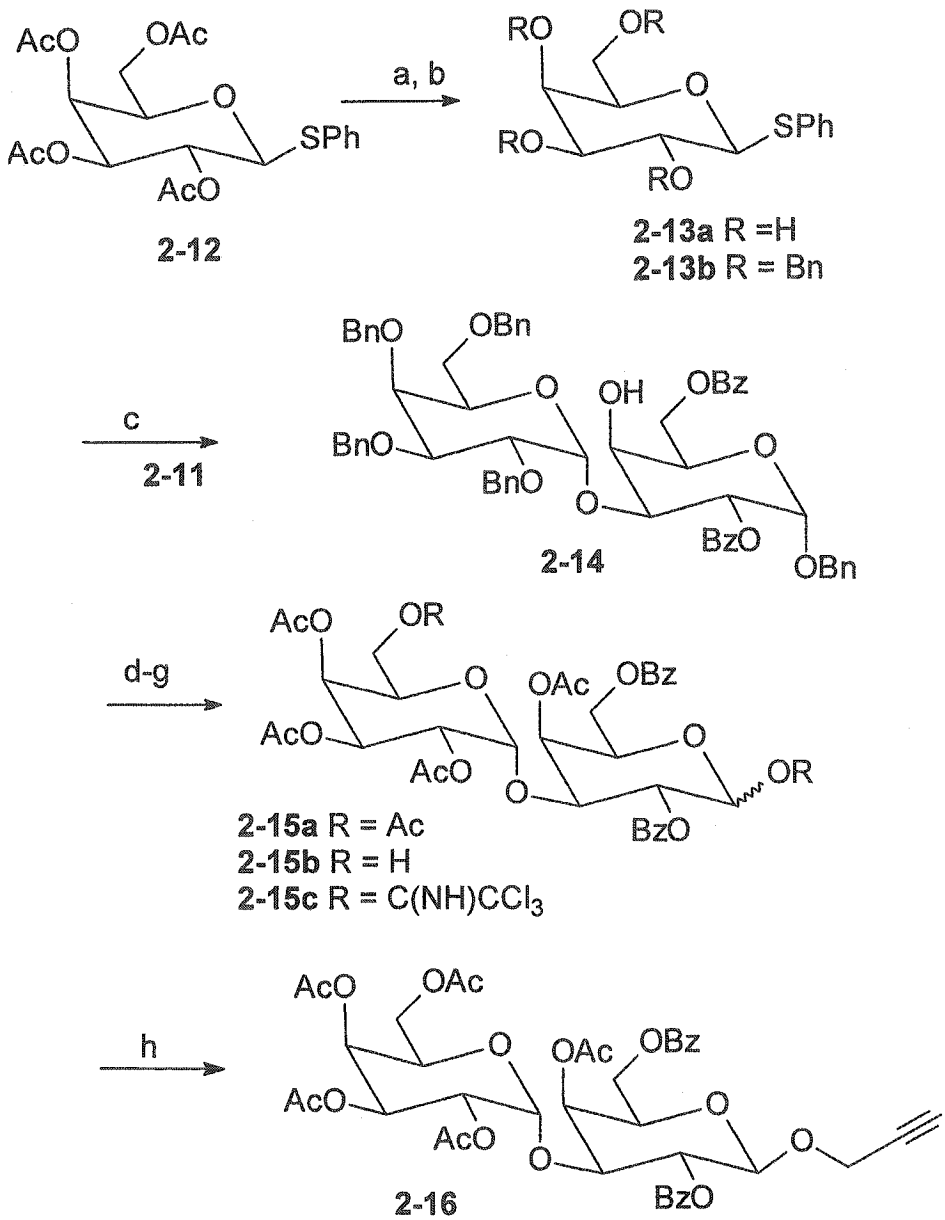


Fig. 2-1. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **2-11.**

obtained under phase transfer catalyzed conditions (PTC) previously developed in our group (Scheme 2-5).[15] Glycosidation of diol **2-11** with galactosyl donor **2-13b** using NIS/triflic acid as promoter gave disaccharide **2-14** as an α/β mixture (6:1, 76%) from which the pure α-anomer (δ H-1' 4.84 ppm, $J_{1',2'}$ 3.8 Hz) was isolated in 65% yield after careful separation using column chromatography. The size of the coupling constant $J_{1',2'}$ 3.8 Hz proved the α anomeric linkage.

The desired 2-propynyl disaccharide **2-16** was then readily synthesized through a sequence of straightforward manipulations. Benzyl glycoside **2-14** (for NMR, see Fig. 2-2) was hydrogenolyzed (H_2, Pd-C, MeOH, 95%) and peracetylated using acetyl chloride to give **2-15a** (pyridine, DMAP, 90%). The anomeric mixture was then regioselectively de-*O*-acetylated using hydrazinium acetate to provide the reducing disaccharide **2-15b** that was transformed into trichloroacetimidate **2-15c** (Cl_3CCN, DBU, CH_2Cl_2, 90%). Finally, treatment of **2-15c** with propargyl alcohol and triflic acid afforded β-galactoside **2-16** (for NMR, see Fig. 2-3) in 86% yield (H-1 4.90 ppm, $J_{1,2}$ 7.9 Hz). The size of the coupling constant $J_{1,2}$ of 7.9 Hz is typical for *trans*-1, -2 hydrogens which proved that the glycosidic linkage had the β anomeric configuration.



Scheme 2-5. Synthesis of α–Gal precursor **2-16.** (a) NaOMe, MeOH, quant.; (b) BnBr, NaH, DMF, rt, 2 h, 85%; (c) NIS, TfOH, CH_2Cl_2, 4Å MS, -40°C, 1 h, α/β (6:1), 76%; (d) H_2, Pd-C, MeOH, AcOH, rt, 2 d, 95%; (e) AcCl, pyridine, DMAP, rt, 4h, 90%; (f) H_2NNH_2-HOAc, CH_2Cl_2, rt, 2 h, 75%; (g) Cl_3CCN, DBU, CH_2Cl_2, rt, 5 h, 90%; (h) propargyl alcohol, CH_2Cl_2, TfOH, 4Å MS.

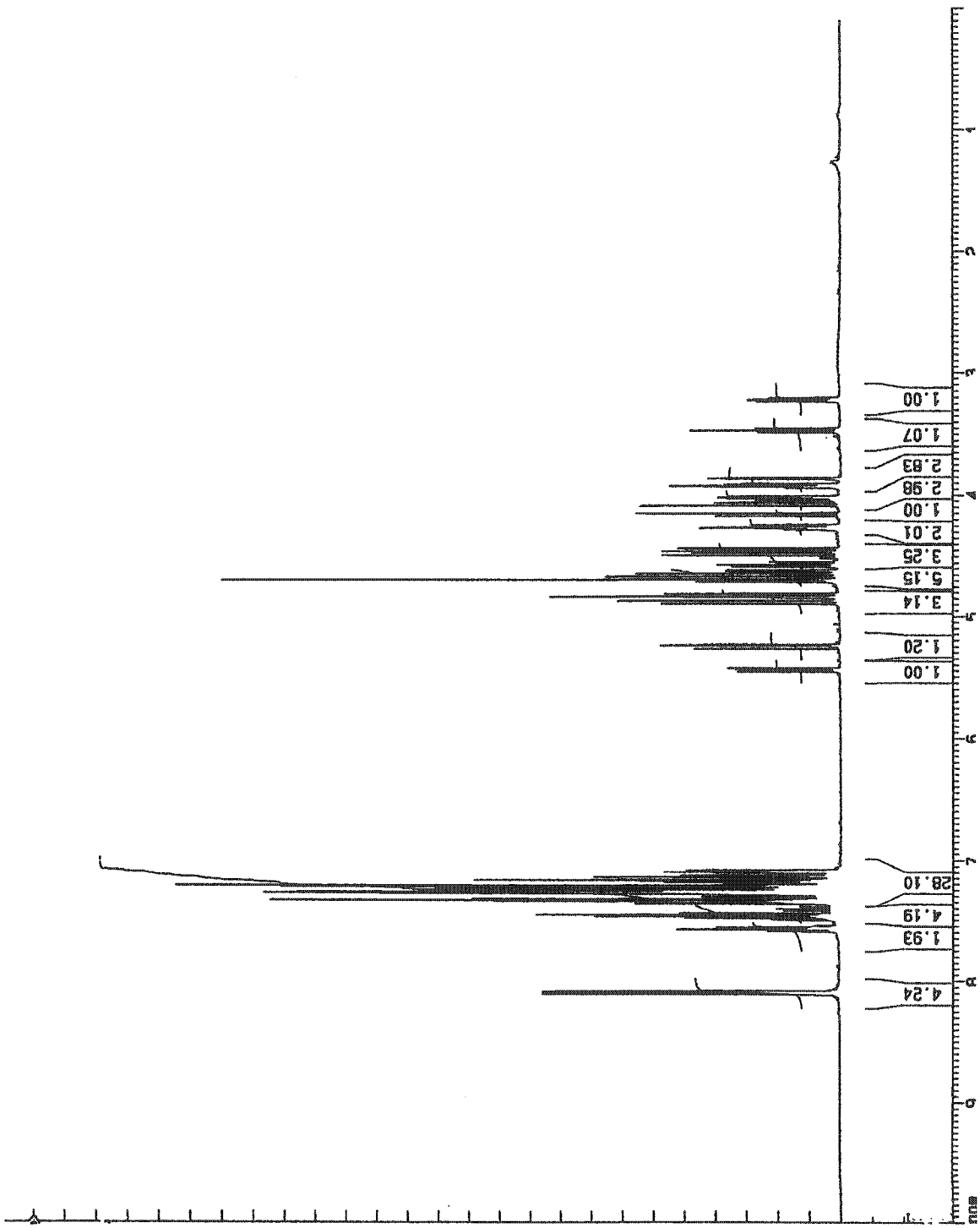


Fig. 2-2. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **2-14**.

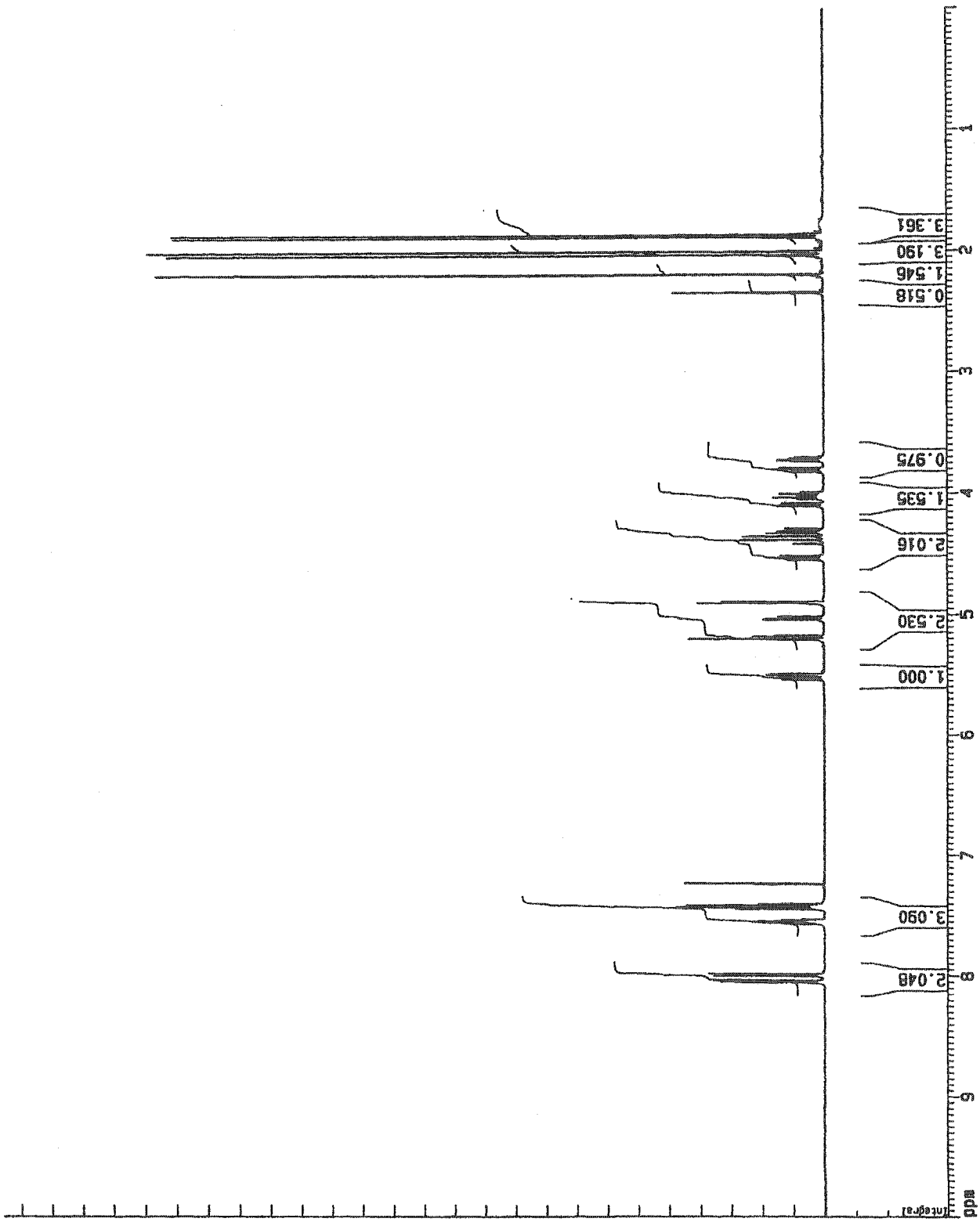
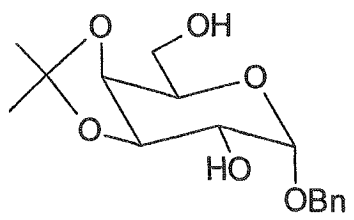


Fig. 2-3. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **2-16**.

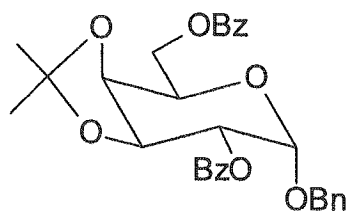
2.3. Experimental Section

Dichloromethane was stored over 4 A^{o} MS after drying over P_2O_5 and distillation. Propargyl alcohol was obtained from Fluka and *p*-iodobenzyl bromide was obtained from Karl Industries Inc. All the other reagents were purchased from Aldrich. Thin layer Chromatography was performed on Silica Gel F_{254} (Merck) precoated aluminium sheets and visualized with molybdenum solution and UV lamp. Column chromatography was run on Ultra Pure Silica Gel (SILICYCLE). Elemental analysis was measured on a CE-2500 instrument. Melting points were determined on a Gallenkamp melting point apparatus without temperature correction. Optical rotations were measured on a PERKIN-ELMER 241 polarimeter. All the NMR spectra (500 MHz for ^{1}H and 125.7 MHz for ^{13}C) were recorded on a Bruker AMX-500 spectrometer. The resonances were assigned based on ^{1}H, ^{13}C, ^{1}H-^{1}H COSY, DEPT, and HMQC experiments. Chemical shifts were referenced to $CDCH_3$ (δ_H 7.29 and δ_C 77.0 ppm). J values are given in Hz. ESI-MS analyses were carried out on a MICROMASS Quattro LC. FAB-MS spectra were recorded on KRATOS Concepts IIH with Cs^{+} beam. MALDI-TOF MS was acquired on a PerSeptive Biosystems Elite-STR (Framingham, MA, USA). In all cases, NaH is of 60% purity in mineral and was weighed on a balance and then washed twice with pentane before use.



2-10a

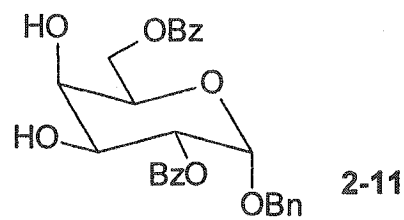
Benzyl 3,4-*O*-isopropylidene-α-D-galactopyranoside (2-10a). Benzyl α-D-galactopyranoside **2-9**[12] (246 mg, 1 mmol) was dissolved in acetone (5 mL) to which was added 2,2-dimethoxypropane (250 μL, 2 mmol) and a catalytic amount of *para*-toluenesulfonic acid. The mixture was stirred at room temperature for 5 h. After removal of the solvent under reduced pressure, the residue was purified by silica gel column chromatography using ethyl acetate : hexane (1.5:1) to give **2-10a** as a thick liquid in 85% yield (263 mg, 0.85 mmol); $[\alpha]_D$ +110° (*c* 3.2, $CHCl_3$); ^1H (500 MHz; $CDCl_3$) δ_H 7.28-7.36 (5 H, m, aromatic), 4.99 (1 H, d, *J* = 3.8, H-1), 4.77 (1 H, d, *J* = 11.8, PhCH_2), 4.57 (1 H, d, *J* = 11.8, PhCH_2), 4.26 (1 H, t, *J* = 6.2, H-4), 4.21 (1 H, dd, *J* = 3.3, 7.2, H-3), 4.07-4.09 (1 H, m, H-5), 3.89 (1 H, dd, *J* = 6.3, 11.8, H-6a), 3.82 (1 H, dd, *J* = 3.8, 7.2, H-2), 3.77 (1 H, dd, *J* = 6.3, 11.8, H-6b), 1.48 (3 H, s, CH_3), 1.32 (3 H, s, CH_3); ^{13}C (125 MHz; $CDCl_3$) δ_C 137.0, 128.5, 128.1, 128.0 and 109.8 (aromatic), 96.9 (C-1), 76.1 (C-4), 73.9 (C-3), 69.4 (PhCH_2), 69.3 (C-2), 68.4 (C-5), 62.7 (C-6), 27.5 and 25.8 (CH_3); *m/z* (FAB-MS) 349.2 (Found: $[M + K^+]$; $C_{16}H_{22}O_6$ requires $[M + K^+]$ 349.1).



2-10c

Benzyl 2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-α-D-galactopyranoside (2-10c). Compound **2-10a** (518 mg, 1 mmol) was dissolved in pyridine (5 mL) and a catalytic amount of DMAP (10 mg) was added followed by benzoyl chloride (280 μL, 2.2 mmol). The mixture was stirred at room temperature for 2 h. After removal of pyridine under

reduced pressure, the residue was dissolved into 50 mL of ether which was washed with water (3 x 30 mL). The organic layer was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography using ethyl acetate: hexane (1: 4) to provide **2-10b** as a liquid in 90% yield (528 mg, 0.9 mmol); $[\alpha]_D$ +90.2° (*c* 1, $CHCl_3$); ^1H (500 MHz; $CDCl_3$) δ 8.03-8.09 (4H, m, aromatic), 7.55-7.59 (6H, m, aromatic), 7.15-7.20 (5 H, m, aromatic), 5.20 (1H, dd, *J* = 3.7, 7.9, H-2), 5.17 (1 H, d, *J* = 3.7, H-1), 4.77 (1 H, d, *J* = 12.3, PhCH_2), 4.67 (1H, dd, *J* = 4.8, 11.8, H-6a), 4.63 (1H, dd, *J* = 7.5, 11.8, H-6b), 4.50 (1H, dd, *J* = 5.5, 7.9, H-3), 4.50 (1 H, d, *J* = 11.8, PhCH_2), 4.47-4.51 (1 H, m, H-5), 4.38 (1 H, dd, *J* = 2.6, 5.5, H-4), 1.56 (3 H, s, CH_3), 1.36 (3 H, s, CH_3); ^{13}C (125 MHz; $CDCl_3$) δ_C 166.3, 165.9 (Ph*C*O), 136.8, 133.1, 133.0, 130.0, 129.8, 129.7, 129.6, 128.4, 128.3, 128.2, 127.8, 127.5 and 110.1 (aromatic), 95.3 (C-1), 73.6 (C-2), 72.1 (C-4), 69.6 ($PhCH_2$), 66.1 (C-3 and C-5), 64.0 (C-6), 27.9 and 26.3 (CH_3); *m/z* (FAB-MS) 557.0 (Found: $[M + K^+]$. $C_{30}H_{30}O_8$ requires $[M + K^+]$ 557.0).



2-11

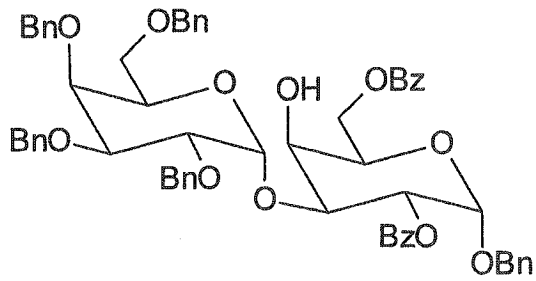
Benzyl 2,6-di-*O*-benzoyl-α-D-galactopyranoside (2-11). Benzyl 2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-α-D-galactopyranoside **2-10b** (518 mg, 1 mmol) was placed into a round-bottom flask with 30 mL of 60% aqueous acetic acid. The mixture was heated and stirred at 80 °C for 5 h. After removing the solvent under reduced pressure, the residue was purified by silica gel column chromatography using ethyl acetate: hexane (1.5:1) to yield 2-

11 as a white solid in 95% yield (454 mg, 0.95 mmol); $[\alpha]_D$ +111.6° (*c* 1, $CHCl_3$); ^1H (500 MHz; $CDCl_3$) δ 8.02-8.11 (4 H, m, aromatic), 7.53-7.58 (2 H, m, aromatic), 7.39-7.46 (4 H, m, aromatic), 7.16-7.29 (5 H, m, aromatic), 5.33 (1 H, dd, *J* = 3.8, 10.2, H-2), 5.21 (1 H, d, *J* = 3.8, H-1), 4.72 (1 H, d, *J* = 12.2, PhCH_2), 4.66 (1 H, dd, *J* = 6.0, 11.4, H-6a), 4.52 (1 H, d, *J* = 12.2, PhCH_2), 4.50 (1 H, dd, *J* = 7.0, 11.4, H-6b), 4.27 (1 H, d, *J* = 1.4, H-5), 4.26 (1 H, dd, *J* = 3.6, 10.2, H-3), 4.10 (1 H, dd, *J* = 1.0, 3.6, H-4), 2.97 (2 H, bs, -OH); ^{13}C (125 MHz; $CDCl_3$) δ_C 166.9 and 166.6 (PhCO_2), 137.0, 133.3, 133.2, 129.9, 129.6, 128.4, 128.3, 127.8 and 127.6 (aromatic), 95.7 (C-1), 72.1 (C-2), 69.6 (PhCH_2), 69.5 (C-4), 68.4 and 68.2 (C-3 and C-5), 63.5 (C-6); *m/z* (ESI-MS) 496.0 Found: [M + NH_4^+]. $C_{27}H_{30}NO_8$ requires [M + NH_4^+] 496.2.

Phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (2-13b). Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**2-12**)[14] (1 g, 2.27 mmol) was dissolved into methanol (25 mL) to which was added a catalytic amount of sodium methoxide. The solution was stirred overnight at room temperature. After neutralization of the reaction mixture with Amberlite (IR-120, H^+), the solution was filtered through a cotton plug. Methanol was evaporated under reduced pressure to provide **2-13a** in a quantitative yield and the product was used directly in the next step without further purification.

Compound **2-13a** was dissolved into DMF (30 ml) to which was added sodium hydride (440 mg, 11 mmol). After stirring at room temperature for 20 min, benzyl bromide (1.3 mL, 10.9 mmol) was added to the suspension during a period of 10 min. The mixture was further stirred at room temperature for 2 h. Excess sodium hydride was destroyed with several drops of methanol. The solution was diluted with ether (100 mL) and washed with water (3 x 100 mL). The organic phase was dried over anhydrous sodium sulfate and the

solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography using ethyl acetate: hexane (1:10) to provide **2-13b** as a white solid: mp 88.9-90.1 °C and a $[\alpha]_D$ 1° (*c* 1.0, $CHCl_3$), lit.[13] mp 88-89 °C; $[\alpha]_D$ 1° (*c* 1.0, $CHCl_3$).



2-14

Benzyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-2,6-di-*O*-benzoyl-α-D-galactopyranoside (2-14). Glycosyl donor **2-13b** (200 mg, 0.32 mmol) and glycosyl acceptor 2-11 (230 mg, 0.48 mmol) and 4 Å MS were put into a dry flask. After flushing the flask with dry nitrogen, 20 mL of dry CH_2Cl_2 was injected into it. The suspension was stirred for 30 min at –40 °C, then NIS (106.6 mg, 0.48 mmol) and a catalytic amount of triflic acid was added to the solution. One hour later, the mixture was neutralized with DIPEA and filtered over Celite. The filtrate was washed with dilute sodium thiosulfate solution and the organic phase was dried over anhydrous sodium sulfate. The solvent was evaporated on rotavapor. The residue was subjected to silica gel column chromatography using ethyl acetate: hexane (2:3) to afford **2-14** as a white foam in 65% yield (208 mg, 0.21 mmol); $[\alpha]_D$ +84.4 (*c* 1.8, $CHCl_3$); ^1H (500 MHz; $CDCl_3$) δ 8.08-8.11 (4 H, m, aromatic), 7.08-7.60 (31 H, m, aromatic), 5.45 (1 H, *J* = 3.8, 10.0, dd, H-2), 5.24 (1 H, d, *J* = 3.8, H-1), 4.88 (1 H, d, *J* = 11.5, PhCH_2), 4.84 (1 H, d, *J* = 3.8, H-1'), 4.82 (1H, d, *J* = 11.5, PhCH_2), 4.70 (1 H, d, *J* = 12.5, PhCH_2), 4.69 (2 H, s, PhCH_2), 4.65 (1 H, d, *J* = 11.5, PhCH_2), 4.63

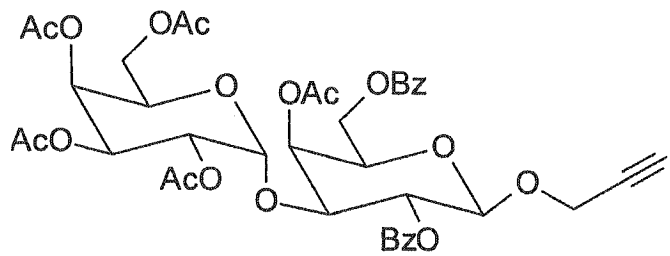
(1 H, dd, J = 6.3, 11.6, H-6a), 4.56 (1 H, dd, J = 7.6, 11.6, H-6b), 4.48 (1 H, d, J = 12.5, PhCH_2), 4.45 (1 H, d, J = 11.5, PhCH_2), 4.27 (1 H, t, J = 6.6, H-5), 4.25 (1 H, dd, J = 3.4, 10.0, H-3), 4.16 (1 H, d, J = 11.5, PhCH_2), 4.08 (1 H, d, J = 11.5, PhCH_2), 4.02 (1 H, dd, J = 3.8, 10.0, H-2'), 4.01 (1 H, m, H-4), 3.91-3.94 (2 H, m , H-3', H-5'), 3.86 (1 H, bd, J = 1.5, H-4'), 3.47 (1H, t, J = 8.6, H-6a'), 3.22 (1 H, dd, J = 5.5, 8.8, H-6b'); ^{13}C (125 MHz, $CDCl_3$) δ_C 166.3 and 165.8 (PhCO_2), 138.6, 138.3, 137.9, 137.5, 137.0, 136.5, 135.5, 133.0, 131.3, 130.1, 129.8, 129.6, 129.4, 128.7, 128.5, 128,4, 128.3, 128.2, 128.1, 128.0, 127.7, 127.6, 127.5, 127.4, and 127.3 (aromatic), 96.1 (C-1), 95.4 (C-1'), 79.5 (C-3' or C-5'), 75.6 (C-2'), 74.9 and 74.8 (PhCH_2), 74.5 (C-3), 74.4 (C-4'), 73.2 and 72.6 (PhCH_2), 69.8 (C-5' or C-3'), 69.6 (C-2), 69.3 (PhCH_2), 68.3 (C-6'), 67.8 (C-5), 66.2 (C-4), 64.5 (C-6); *m/z* (ESI-MS) 1018.1 Found: [M + NH_4^+]. $C_{61}H_{60}NO_{16}$ requires [M + NH_4^+] 1018.4.

2-Propynyl (2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-galactopyranoside (2-16). To compound **2-14** (1 g, 1 mmol) dissolved in methanol (30 mL) was added a catalytic amount of 10% Pd/C and 2 drops of acetic acid. The suspension was stirred to hydrogenolysis using H_2 gas at rt for 2 d. After the reaction was completed, the solution was filtered through a pad of celite. After removal of methanol under reduced pressure, the dry residue was dissolved into pyridine (10 mL) to which was added acetyl chloride (0.52 mL, 6 mmol) and a catalytic amount of DMAP (10 mg) at 0 °C. The mixture was stirred at room temperature for 4 h. Pyridine was then removed under reduced pressure. The residue was taken up in ether (60 mL) and the ether solution was washed with water (3 x 40 mL). The organic layer was dried over anhydrous sodium sulfate. After removal of ether under reduced pressure, the residue was purified by silica gel column

chromatography using ethyl acetate: hexane (1:1) to provide **2-15a** (α/β) as a white foam in 90% yield which was used in the next step without further purification.

Compound **2-15a** (800 mg, 1 mmol) was dissolved in dichloromethane (20 mL) to which was added hydrazinium acetate (135 mg, 1.5 mmol). The solution was stirred at room temperature for 2 h. After removal of the solvent under reduced pressure, the residue was subjected to silica gel column chromatography using ethyl acetate: hexane (1.5:1) to give **2-15b** as a white foam in 75% yield. Compound **2-15b** was obtained as an anomeric α/β mixture, which was used directly in the next step without further purification.

Compound **2-15b** (760 mg, 1 mmol), trichloroacetonitrile (0.15 mL, 1.5 mmol), and DBU (30 μL, 0.2 mmol) were added to 20 mL of dry dichloromethane. The solution was stirred at room temperature for 5 h. After removal of the solvent under reduced pressure, the residue was subject to silica gel column chromatography using ethyl acetate: hexane (1:1) to give **2-15c** as a white foam in 90% yield. Compound **2-15c** exists as an α/β mixture (~10/1 ratio), which was used directly in the next step without further purification.



2-16

Crude trichloroacetimidate **2-15c** (200 mg, 0.22 mmol), propargyl alcohol (25.8 μL, 0.44 mmol) and 4 A° MS were added into 20 mL of dry dichloromethane. The mixture was

stirred at room temperature for 30 min to which was added a catalytic amount of triflic acid. Four hours later the suspension was neutralized with DIPEA and filtered through Celite. After evaporation of the solvent, the residue was purified by silica gel column chromatography using ethyl acetate: hexane (1:1) to provide **2-16** as a white foam in 86 % yield (210 mg, 0.19 mmol); $[\alpha]_D$ +117.0° (*c* 1, $CHCl_3$); ^1H (500 MHz; $CDCl_3$) δ 7.98-8.06 (4H, m, aromatic), 7.53-7.57 (2H, m, aromatic), 7.40-7.45 (4H, m, aromatic), 5.52 (1H, dd, *J* = 7.9, 10.1, H-2), 5.50 (1H, bd, *J* = 2.4, H-4), 5.21 (1H, bd, *J* ~3.5 Hz, H-1'), 5.19 (1H, dd, *J* = 3.5, 10.5, H-2'), 5.03 (1H, dd, *J* = 7.3, 10.5, H-3'), 4.91 (1H, m, H-4'), 4.90 (1H, d, *J* = 7.9, H-1), 4.53 (1H, dd, *J* = 6.6, 11.3, H-6a), 4.41 (1H, dd, *J* = 2.4, 16.1, C*H*$_2$-CCH), 4.34 (1H, dd, *J* = 2.4, 16.1, C*H*$_2$-CCH), 4.32 (1H, dd, *J* = 6.7, 11.3, H-6b), 4.10 (1H, dd, *J* = 3.2, 10.1, H-3), 4.03 (1H, m, H-5), 4.00 (1H, m, H-5'), 3.80 (1H, dd, *J* = 6.8, 12.1, H-6a'), 3.72 (1H, dd, *J* = 6.6, 12.1, H-6b'), 3.45 (1H, t, *J* = 3.4, acetylenic), 2.20, 2.04, 2.00, 1.89 and 1.86 (each 3H, 5s, 5 C*H*$_3$CO$_2$); ^{13}C (125 MHz; $CDCl_3$) δ_C 170.2, 189.9, 169.8 and 169.3 (CH_3CO_2), 165.7 and 165.0 (Ph*C*O$_2$), 133.4 (2), 129.7, 129.6, 129.3, 129.2, 128.5 and 128.4 (aromatic), 98.7 (C-1), 95.7 (C-1'), 78.2 and 75.3 (acetylenic), 73.7 (C-3), 71.1 (C-5), 69.8 (C-2), 67.6 (C-4'), 66.9 (C-3' and C-5'), 66.5 (C-2'), 65.2 (C-4), 61.8 (C-6), 61.1 (C-6'), 55.9 (C-1''), 20.7, 20.5, 20.3 (3) (*C*H$_3$CO$_2$); *m/z* (FAB-HRMS) 1149.40 (Found: $[M + K^+]$. $C_{63}H_{66}O_{18}$ requires $[M + K^+]$ 1149.38).

2.4. References

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CHAPTER 3 SYNTHESIS OF α-GAL-CONTAINING CLUSTERS

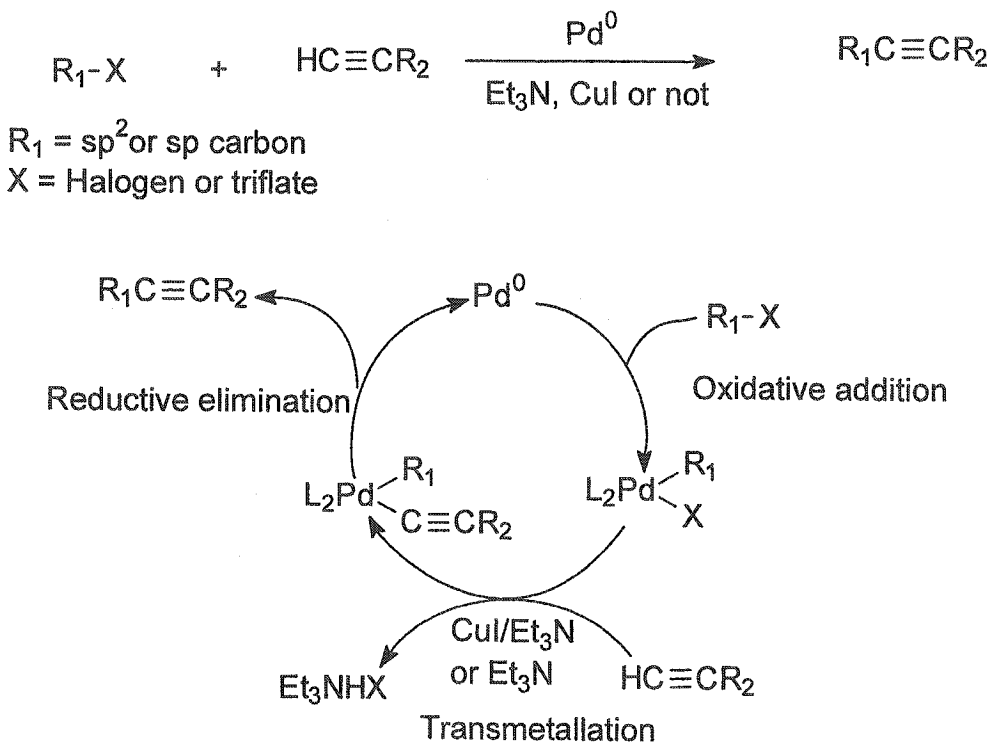
3.1. Introduction

As mentioned before, the interaction between monomeric carbohydrates and proteins is usually weak with Kds at milimolar levels and rarely reaching micromolar levels. To enhance the binding affinity, the "glycocluster effect"[1-3] has been extensively explored in the design and development of efficient multivalent carbohydrate ligands.[4] Indeed, there are numerous successful examples reported in the literature. So far it has been widely recognised that flexible linkers of sugar moieties may contribute to "entropic penalty" during binding, due to the loss of hydrating water and conformational changes.[3] Thus, it might be helpful for the compensation of entropic loss if one could incorporate a structurally restrained and hydrophobic residue into the design of glycoforms.

The use of Palladium-mediated organic reactions has evolved into a very fast growing field during the last two decades.[5] Among those reactions the Sonogashira reaction,[6] based on cross-coupling between vinyl or aryl halides and terminal alkynes using palladium(0) species offers a very efficient method for the construction of molecular rods and dendrimers with rigid structures.[7,8] From Scheme 3-1, it can be seen that the coupling reaction between an aryl iodide and a terminal alkynyl sugar provides a less flexible spacer for the terminal sugars.

Applications of the Sonogashira reaction toward the synthesis of rigid bivalent glycoclusters have been reported;[9-12] however its usage for the synthesis of high-order glycoclusters was limited. In order to obtain Galili antigens as rigid clusters that may have the potential to form stable cross-linked complexes with naturally occurred anti α-Gal

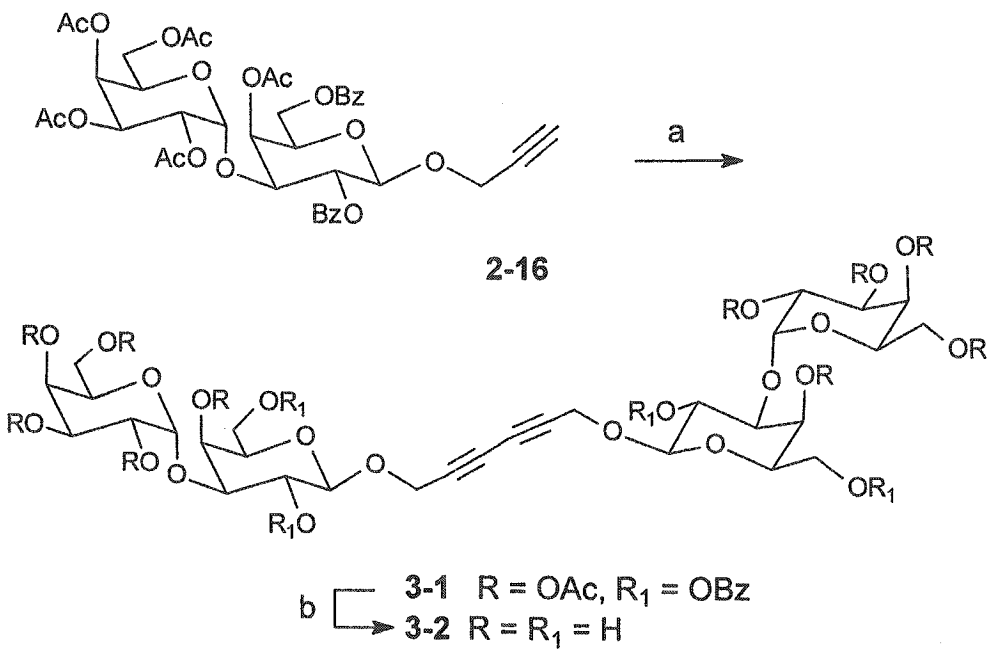
antibodies, a series of palladium-catalyzed transformations were then effected. The goal was to obtain multivalent α-Gal analogous to those previously prepared for galabiosides that have shown nanomolar inhibitory properties against various pathogens with carbohydrate binding lectins on their surfaces,[13] and to investigate the effect of molecular geometry and flexibility on the multivalent effect as well as the molecular mechanism of carbohydrate-protein recognition.



Scheme 3-1. Schematic diagram of catalytic cycle for the Sonogashira coupling.

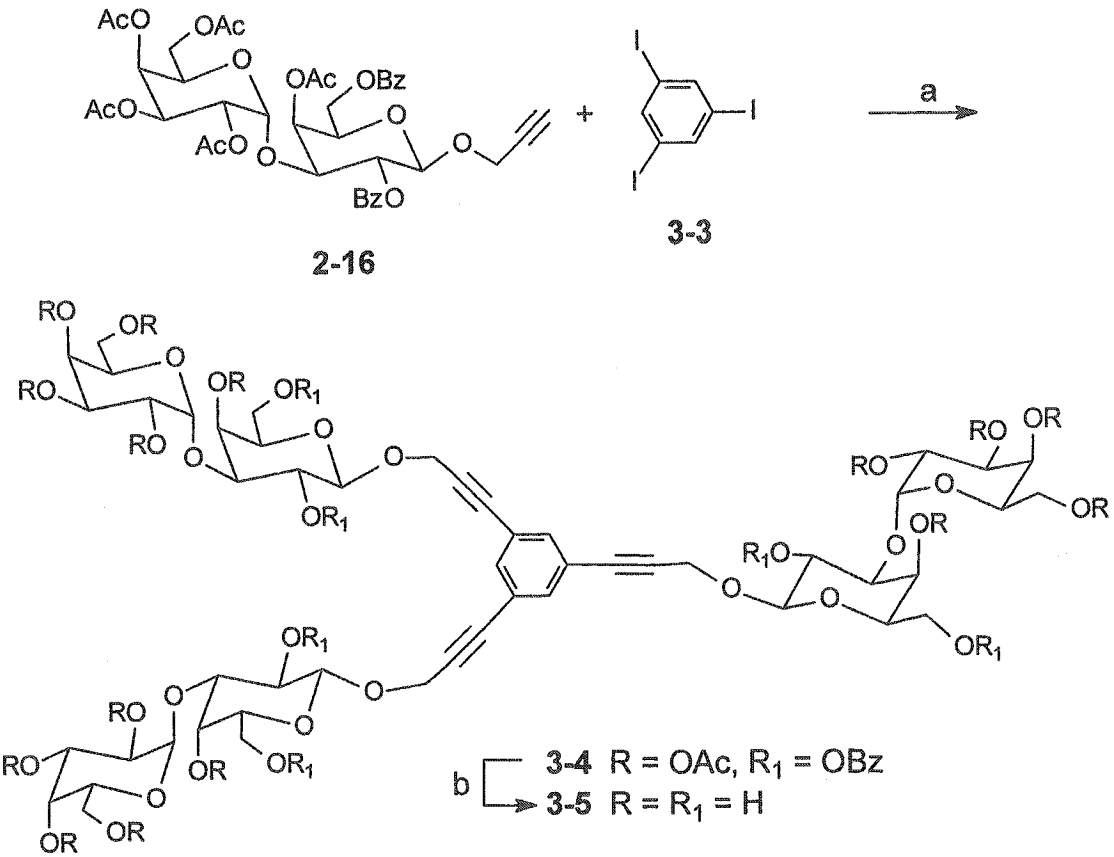
3.2. Results and Discussion

Dimer **3-1** was successfully prepared from 2-propynyl glycoside **2-16** by oxidative homocoupling using palladium-catalyzed conditions ($(Ph_3P)_2PdCl_2$, CuI, DMF-TEA (1:1), rt, 95%) (Scheme 3-2).[11] Interestingly, these conditions were found to be milder than the classical Glaser reaction[14] previously used in analogous circumstances.[10] Acetylenic dimer **3-1** (For NMR, see Fig. 3-1) was then subjected to Zemplèn transesterification (NaOMe, MeOH) to provide fully deprotected dimer **3-2** (for the NMR spectrum, see Fig. 3-2) in 95% yield.



Scheme 3-2. Synthesis of dimer **3-2.** (a) **2-16**, rt, 4 h, 86%; (b) $(Ph_3P)_2PdCl_2$, CuI, DMF:TEA (1:1), rt, 3 h, 95%.

Trimer **3-5** was then obtained using a Sonogashira reaction[15] similar to the one recently described for the synthesis of mannoside clusters.[11,12] Thus, palladium catalysts such as $(PPh_3)_2PdCl_2$, $(PPh_3)_4Pd$ and $Pd_2(dba)_3$ were all successfully applied toward the cross-coupling of 2-propynyl glycoside **2-16** and 1,3,5-triiodobenzene (**3-3**)[16] which provided trimer **3-4** in similiar yields (85% with $(PPh_3)_2PdCl_2$) (Scheme 3-3). As previously observed,[11] it is noteworthy to mention that CuI is not essential for this cross-coupling reaction. In fact, without CuI, the coupling reaction proceeds at elevated temperature (60°C) The reaction does occur at room temperature in the presence of CuI, albeit in lower yields. However in the absence of CuI, the oxidative homodimerization of the alkynyl moiety is abolished. These observations are particularly important when the desired cross-coupling products and the homodimers are difficult to separate. Finally, trimer **3-4** was fully deprotected to its corresponding analog **3-5** using NaOMe in methanol (95%).



Scheme 3-3. Synthesis of compound **3-5.** (a) $(Ph_3P)_2PdCl_2$, DMF:TEA (1:1), 60°C, 5 h, 85%; (b) NaOMe, MeOH, 24 h, rt, 95%.

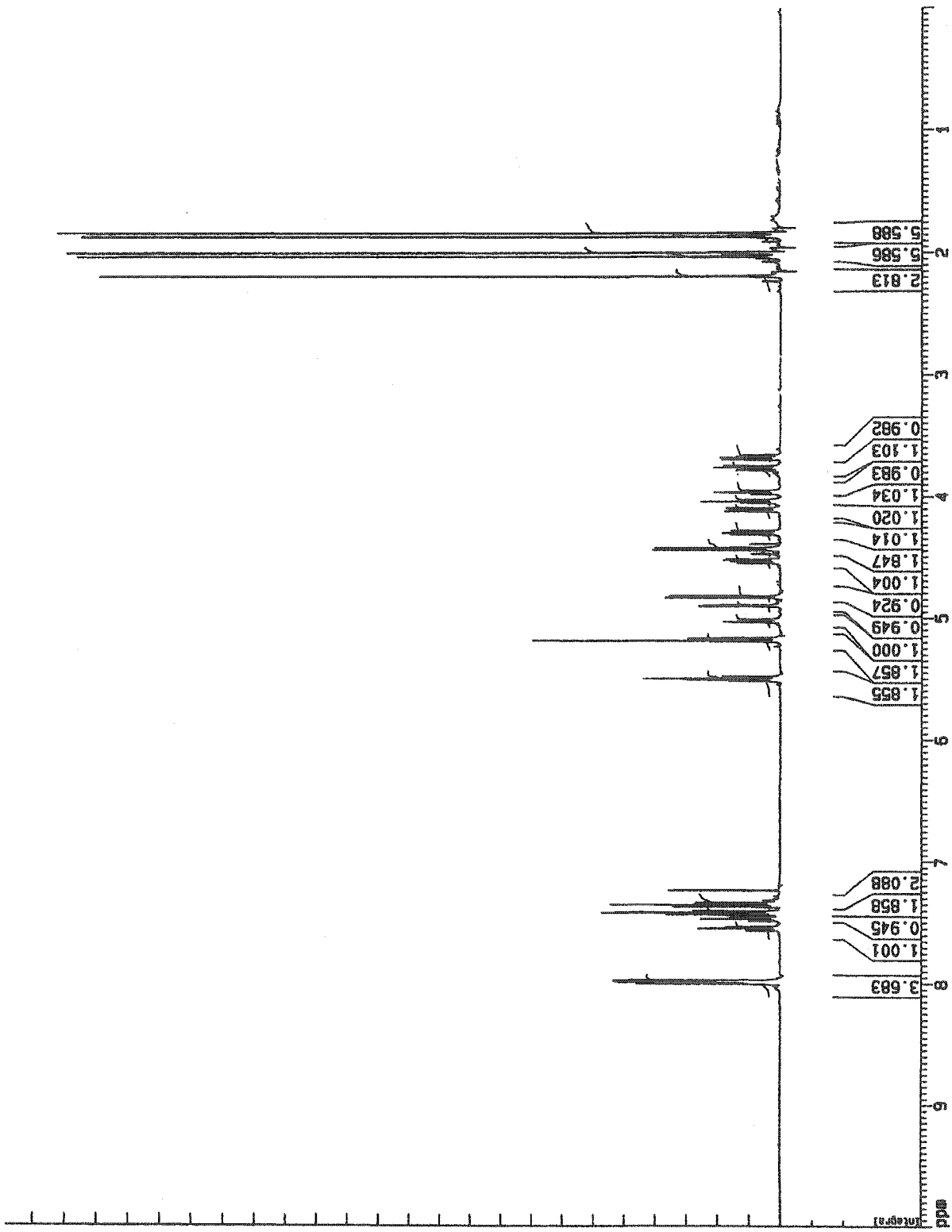


Fig. 3-1. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **3-1**.

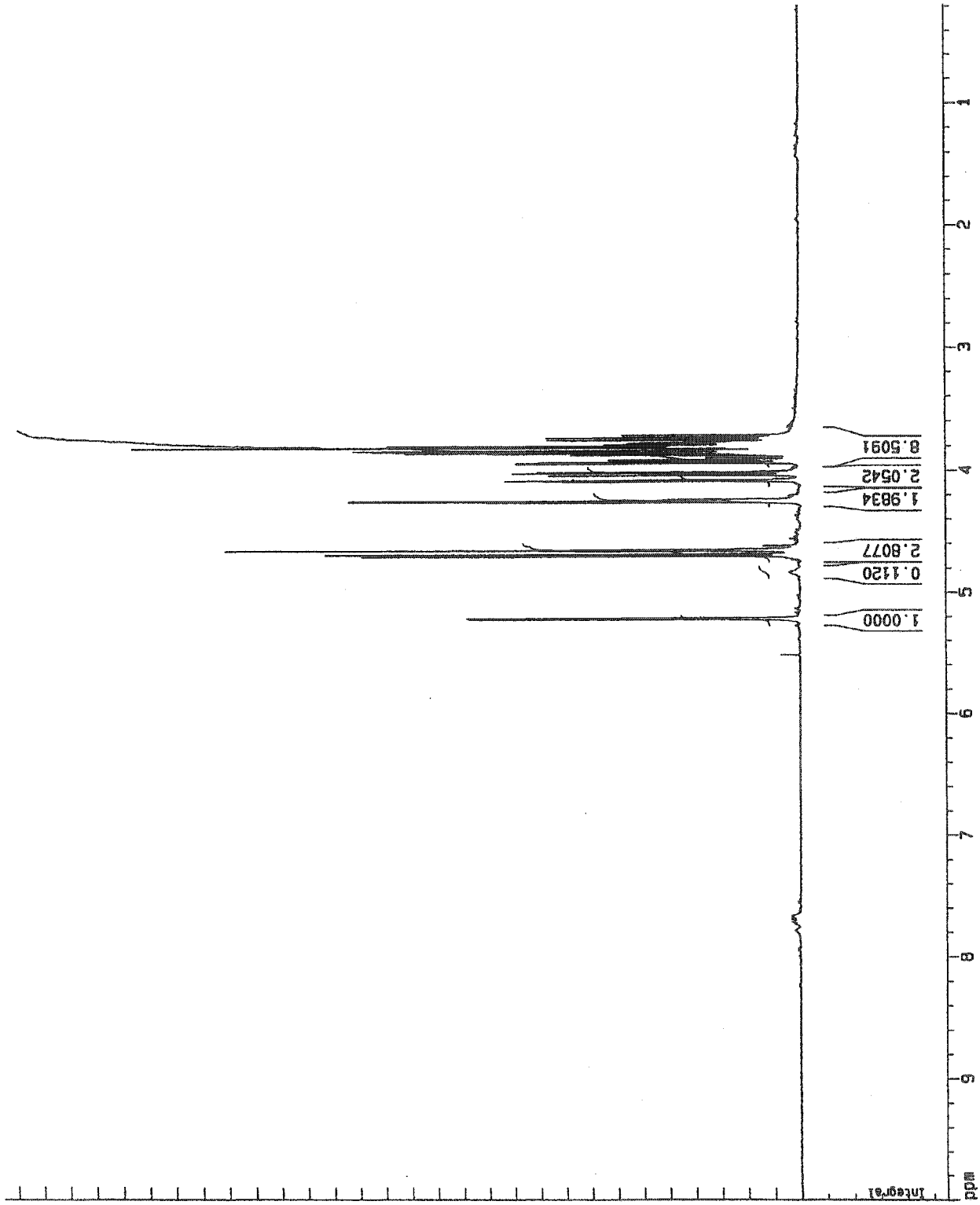
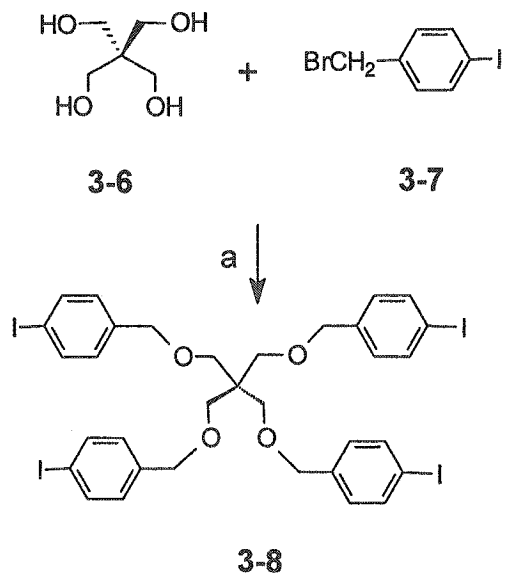


Fig. 3-2. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound 3-2.

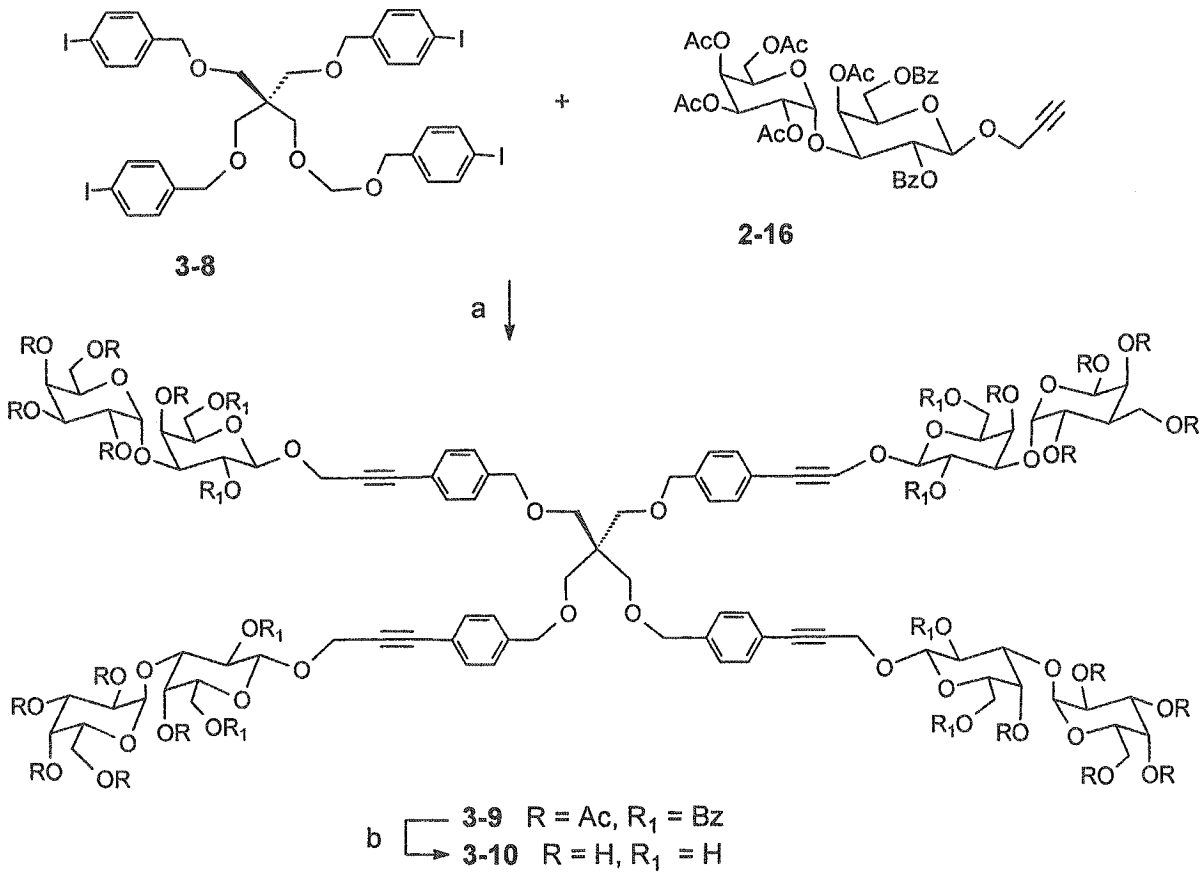
To provide sufficient inter-sugar distances required for efficient protein cross-linking, a tetrameric core was constructed using pentaerythritol (**3-6**) as a seed molecule. Tetra-*p*-iodobenzylated precursor **3-8** was prepared by a classical etherification procedure using *p*-iodobenzyl bromide **3-7**, tetrabutylammonium iodide, NaH, and DMF in moderate yield (47%) (Scheme 3-4). In spite of several efforts, the yield could not be improved further. However, for reasons not yet fully understood, **3-6** is known to be reluctant to undergo full derivatization under the conditions of Williamson etherification.[17] Moreover, *p*-iodobenzyl bromide is light sensitive and unstable in various solvents which may contribute to the low yield.



Scheme 3-4. Synthesis of compound **3-8.** (a) NaH, TBAI, DMF, rt, 5 h, 47%.

Using the cross-coupling conditions described above for the synthesis of trimer **3-4** without CuI, treatment of propynyl glycoside **3-16** with tetrakis *p*-iodobenzyl ether **3-8** ($(PPh_3)_2PdCl_2$), DMF-TEA (1:1), 60°C, 5 h) afforded fully protected tetramer **3-9** (See Fig.

3-3 for the NMR spectrum) (Scheme 3-5). Deprotetection (NaOMe, MeOH) of all ester protecting groups using the normal conditions in **3-9** gave tetramer **3-10** (95%).



Scheme 3-5. Synthesis of compound **3-10.** (a) $(Ph_3P)_2PdCl_2$, DMF:TEA (1:1), 60°C, 5 h, 85%; (b) NaOMe, MeOH, 24 h, rt, 95%.

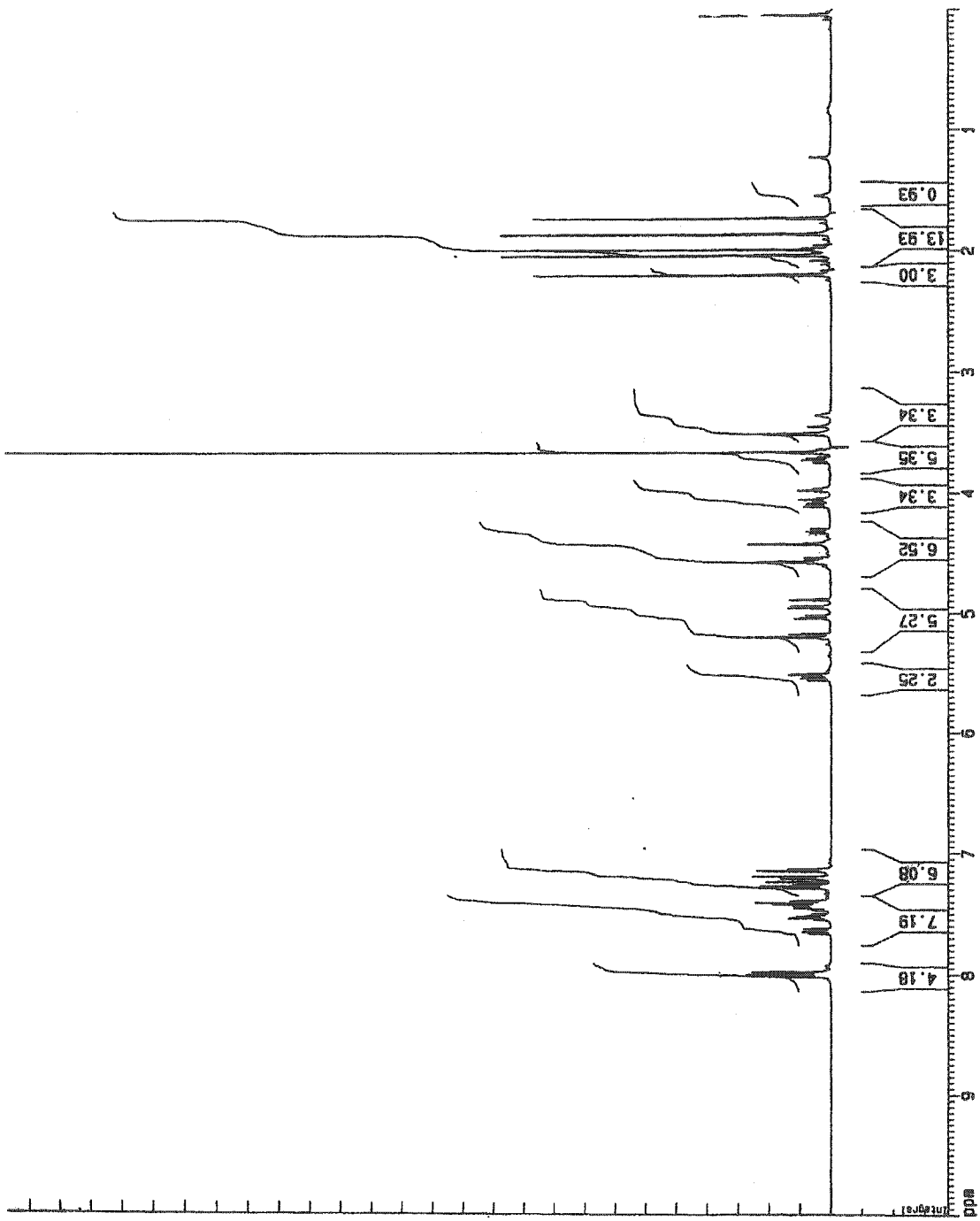
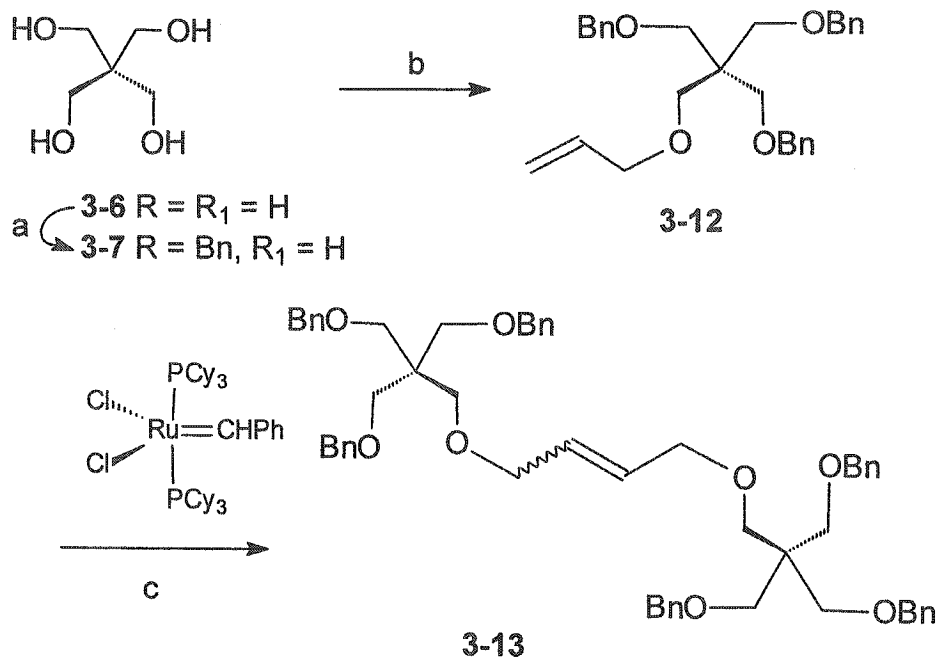


Fig. 3-3. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **3-9**.

Hexameric Cluster

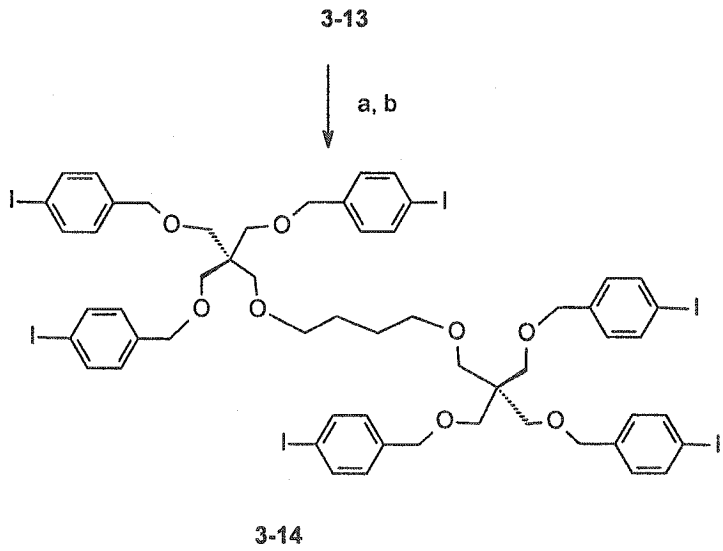
To prepare a hexameric cluster, a pentaerythritol (**3-6**) platform functionalised with a unique allyl ether group was adopted, and from which, olefin self-metathesis provided, as expected,[18] a hexameric cluster according to Scheme 3-6. Thus, substoichiometric benzylation of the tetraol **3-6** (3 eq. BnBr, NaH, DMF) provided tribenzyl ether **3-11** as a thick syrup in 65% yield after silica gel column chromatography. Allylation of the remaining hydroxyl group (allyl bromide, NaH, DMF, rt) gave the fully protected ether **3-12** in 90% yield. Treatment of olefin **3-12** with a catalytic amount of ruthenium carbenoid ($Cl_2Ru(Pcy_3)_2$=CHPh (Grubbs' catalyst), CH_2Cl_2, rt, 12 h) afforded homodimer **3-13** as an inseparable mixture of *trans/cis* stereoisomers (10:1) in 85% yield.



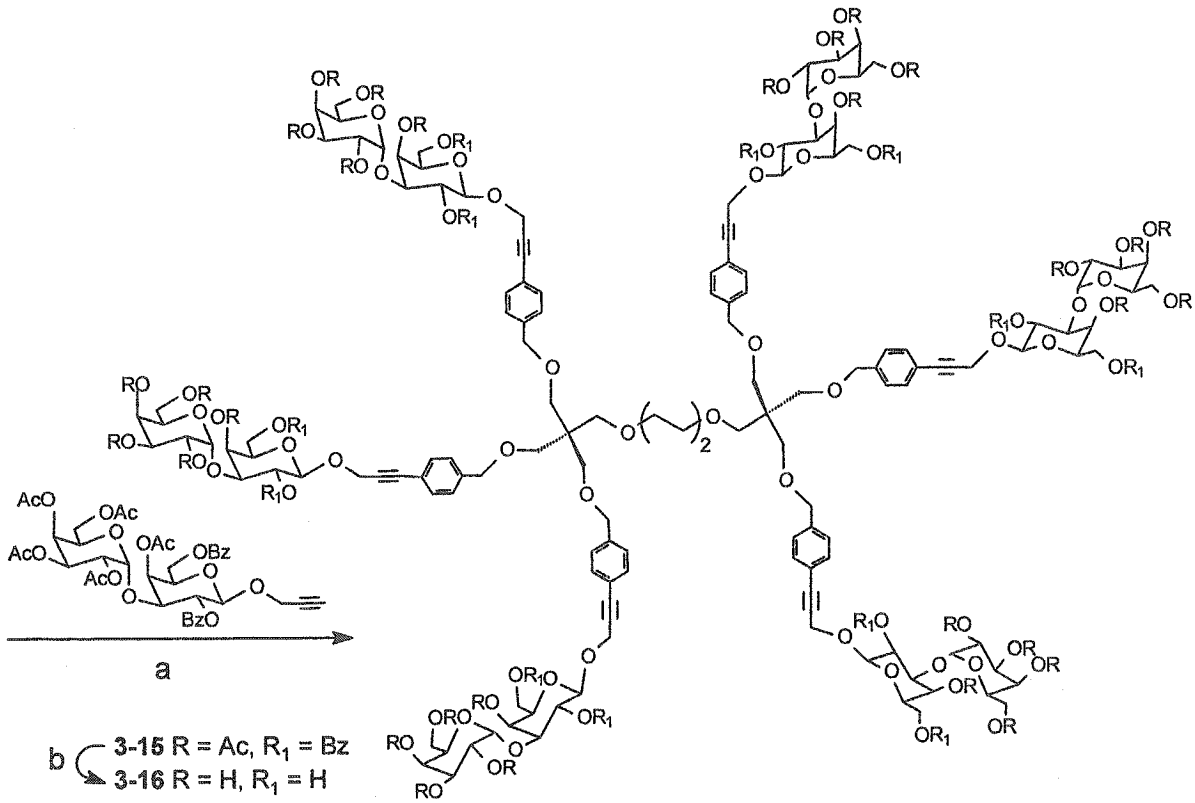
Scheme 3-6. Synthesis of compound **3-13.** (a) 3 eq. benzyl bromide, NaH, DMF, rt, 6 h, 65%; (b) allyl bromide, NaH, DMF, 6 h, 90%; (c) Grubbs' catalyst, CH_2Cl_2, rt, 12 h, 85%, *E/Z* = 10:1.

Interconversion of the perbenzylated ether **3-13** into *p*-iodobenzylated ether **3-14** with concomitant reduction of the alkene functionality was accomplished by complete hydrogenolysis (H_2, Pd/C, 90%) followed by *p*-iodobenzylation in 45% yield (*p*-$IPhCH_2Br$, TBAI, NaH, DMF, rt, 6 h) according to Scheme 3-7.

The final preparation of the hexameric perglycosylated cluster **3-16** is illustrated in Scheme 3-8. Sonogashira cross-coupling of the *para*-aryliodide **3-14** with known **2-16** under palladium-catalyzed conditions in the absence of copper co-catalyst provided fully protected hexamer **3-15** (see Fig. 3-4 for NMR) in 75% yield. Finally, complete deprotection of both acetyl and benzyl ester protecting groups of **3-15** was accomplished under catalytic transesterification condition to afford **3-16**. Interestingly, compound **3-16** was readily soluble in water.



Scheme 3-7. Synthesis of compound **3-14**. (a) H_2, Pd/C, methanol, 24 h, 90%; (b) *p*$IPHCH_2Br$, TBAI, NaH, DMF, rt, 6 h, 45%.



Scheme 3-8. Synthesis of compound **3-16.** (a) $(Ph_3P)_2PdCl_2$, TEA, DMF, 60 °C, 6 h, 75%; (b) NaOMe, MeOH, rt, 2 d, 95%.

PAMAM-based glycodendrimers

Startburst^{RT} PAMAM[19] is a family of synthetic polyamidoamines with primary amino groups on the spherical surface of the molecules. For example, the third generation of commercially available PAMAM has 32 amino groups (see Fig. 3-5). So far PAMAM has gained considerable attention as a new type of material for scaffolding, mostly in the development of glycodendrimers[19] and dendronized functional materials, such as

multivalent catalysts[21] and dendritic polymers.[22] Other interesting applications involve

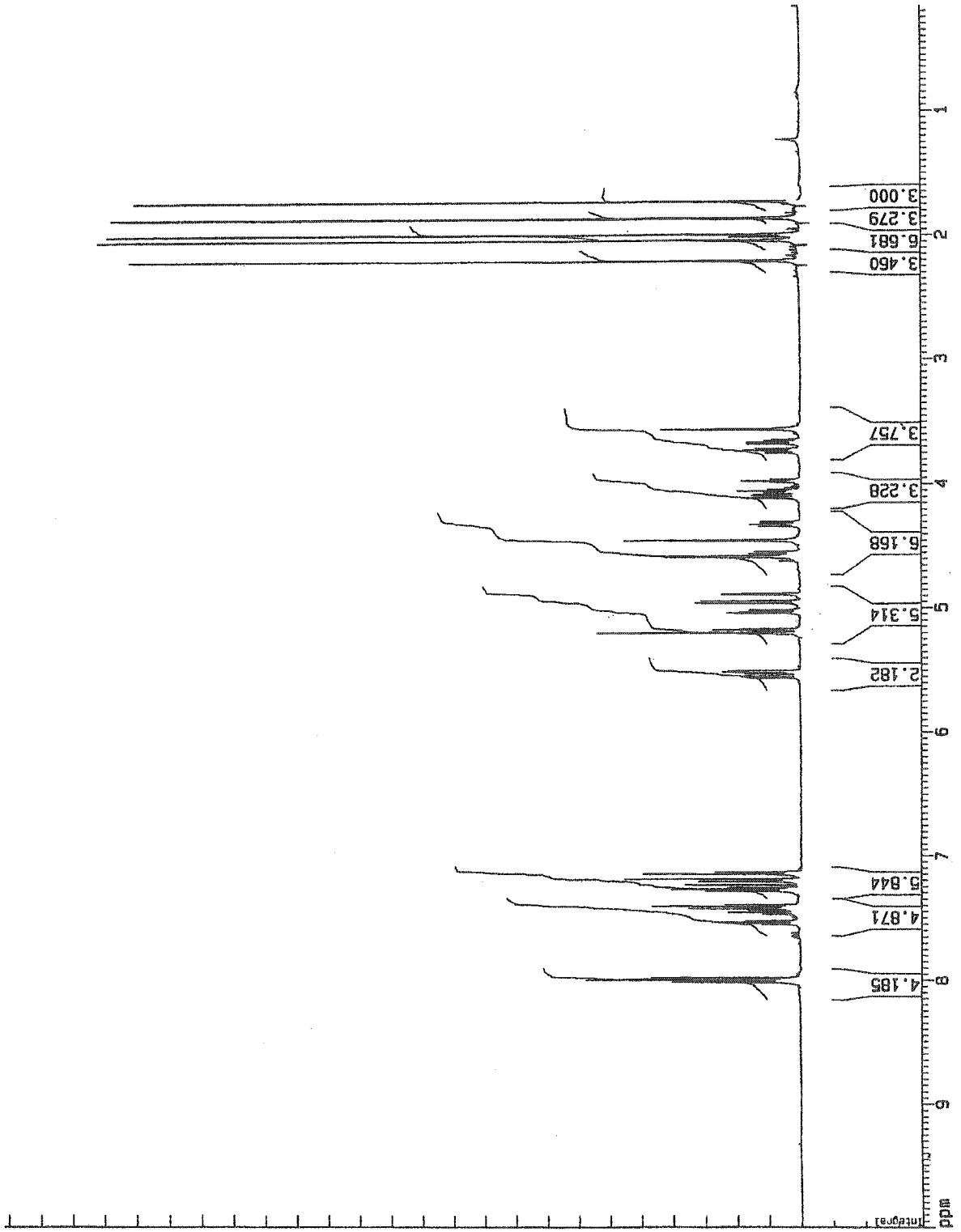


Fig. 3-4. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **3-15**.

drug delivery[23] and imaging.[24] Since PAMAMs are not immunogenic and are commercially available, we decided to synthesize PAMAM-based Galili antigens for the first time.

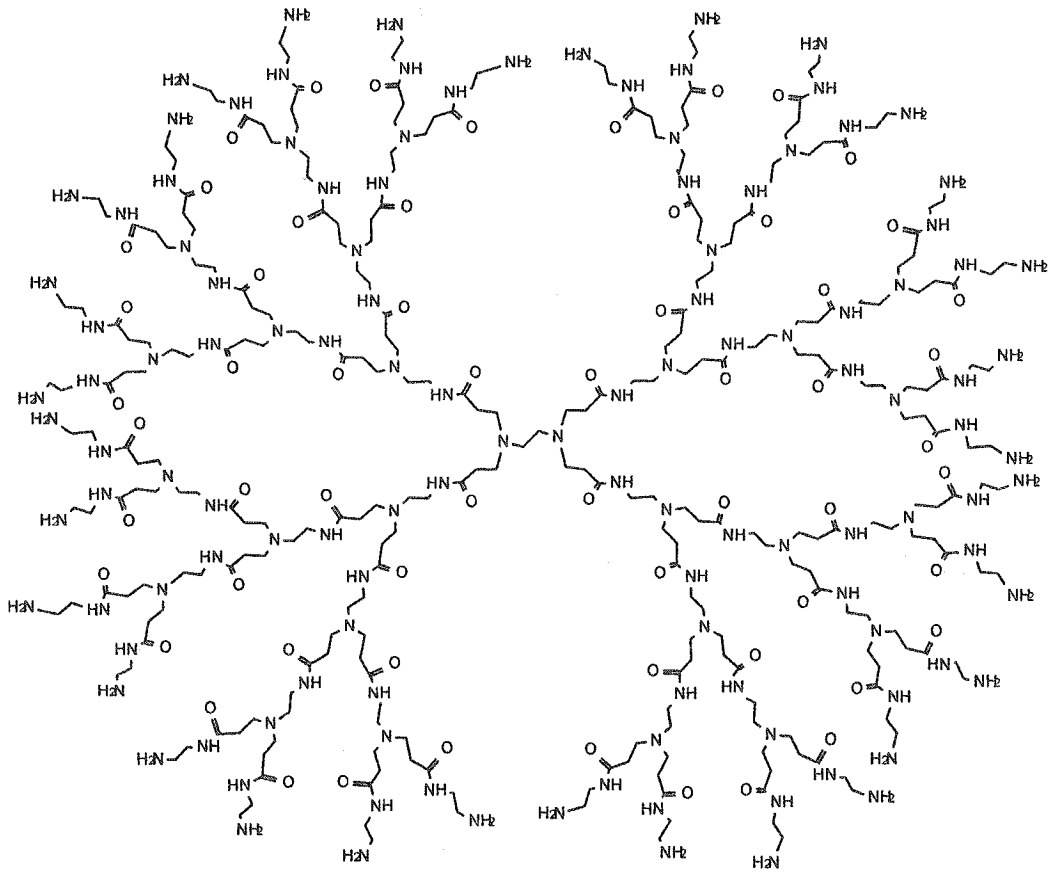
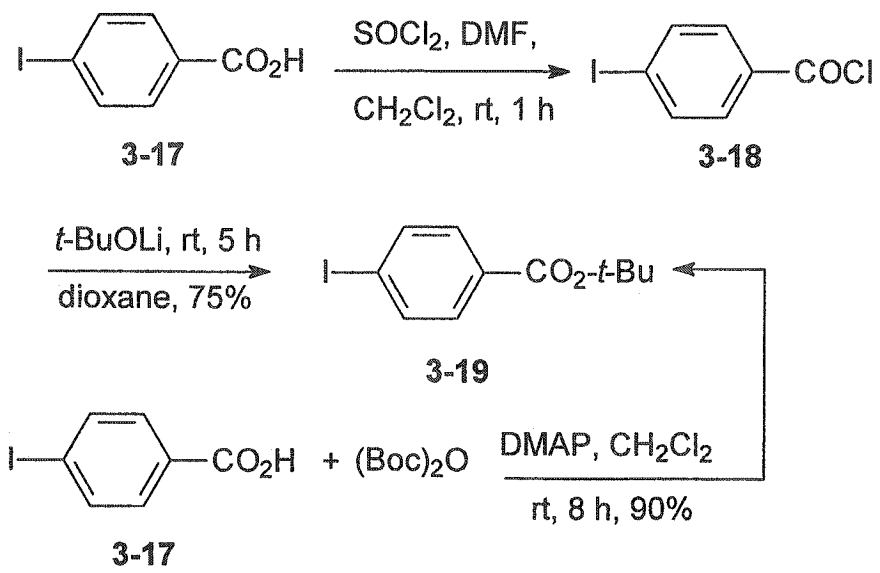


Fig. 3-5. Structure of PAMAM G3.

In the course of synthesis of PAMAM-based Galili antigens, it was planed to use the palladium-mediated Sonogashira reaction as the key step because of its proven simplicity and high efficiency. At the beginning it was attempted to couple PAMAM and *para*-iodobenzoic acid by amide coupling mediated by HATU in DMF. Unfortunately, after the reaction, the products precipitated out and were not soluble in any solvents including DMSO, presumably due to intramolecular hydrogen bonding or aromatic π-stacking effect.

To tackle this problem, the cross-coupling reaction between *para*-iodobenzoate and Galili-antigen precursor **2-16** was conducted first, and then the amide coupling with PAMAM was achieved.

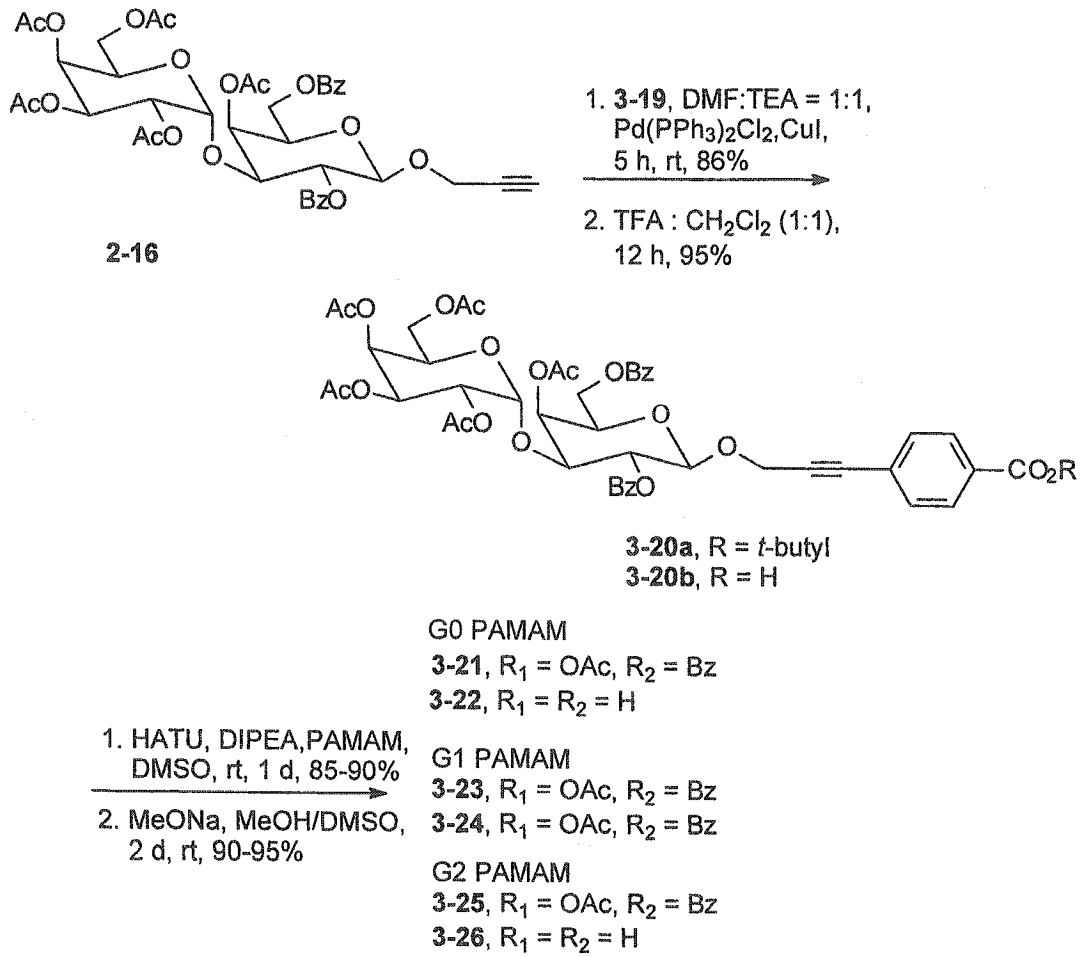
Two procedures were adopted herein to prepare *t*-butyl *p*-iodobenzoate (Scheme 3-9). First, *p*-iodobenzoic acid **3-17** was suspended in dichloromethane and reacted with thionyl chloride (catalyzed by a drop of DMF), to prepare iodobenzoyl chloride **3-18**. Then iodobenzoyl chloride was reacted with lithium *t*-butoxide in anhydrous 1,4-dioxane to afford the desired product **3-19** in 75% yield. The second protocol,[25] in which iodobenzoic acid reacted with Boc anhydride (Boc_2O) to generate the *t*-butyl ester **3-19** was more efficient. (90% yield, reaction catalyzed by 20 mol% DMAP).



Scheme 3-9. Synthesis of compound **3-19**.

The attachment between the Galili antigen precursor **2-16** and the ester **3-19** was performed using the normal coupling procedure with CuI as a co-catalyst at rt to provide **3-**

20a in 86% yield. After Boc deprotection, the substituted benzoic acid **3-20b** was ready for amide coupling with PAMAM. To avoid the solubility problem from the resulting products, DMSO was chosen as the solvent. The reaction was continued until ninhydrin tests (Kaiser reaction)[26] on aliquots negative which meant that all the primary amino groups had been derivatized. This reaction provided the fully protected products **3-21**, **3-23** and **3-25** in 85% to 90% yields. Finally, after Zemplèn deacetylation, the fully deprotected Galili antigen-containing glycodendrimers **3-22**, **3-24** and **3-26** were obtained in excellent yields (Scheme 3-10).



Scheme 3-10. Synthesis of PAMAM-based Galili antigens.

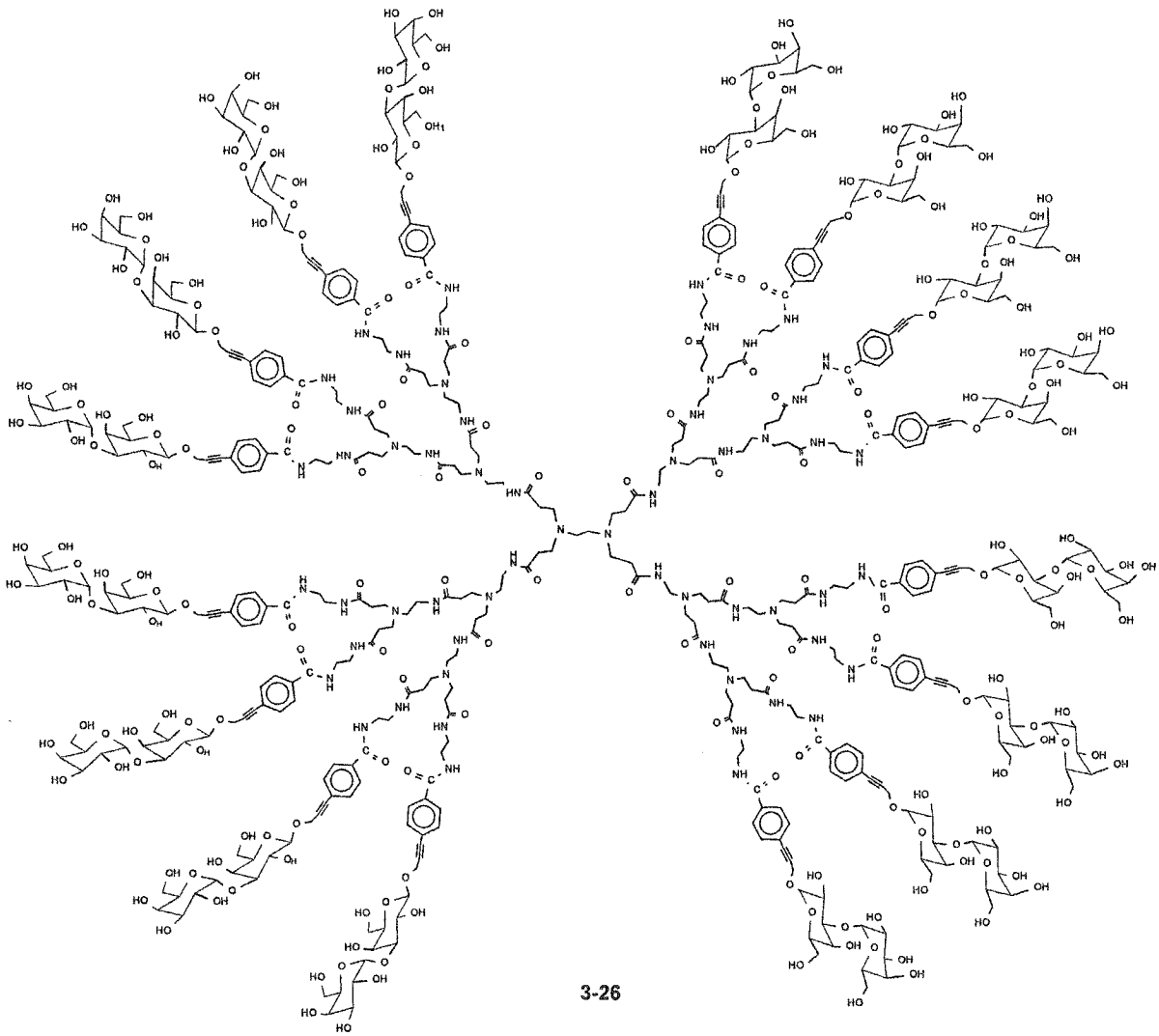


Fig. 3-6. Structure of compound **3-26**.

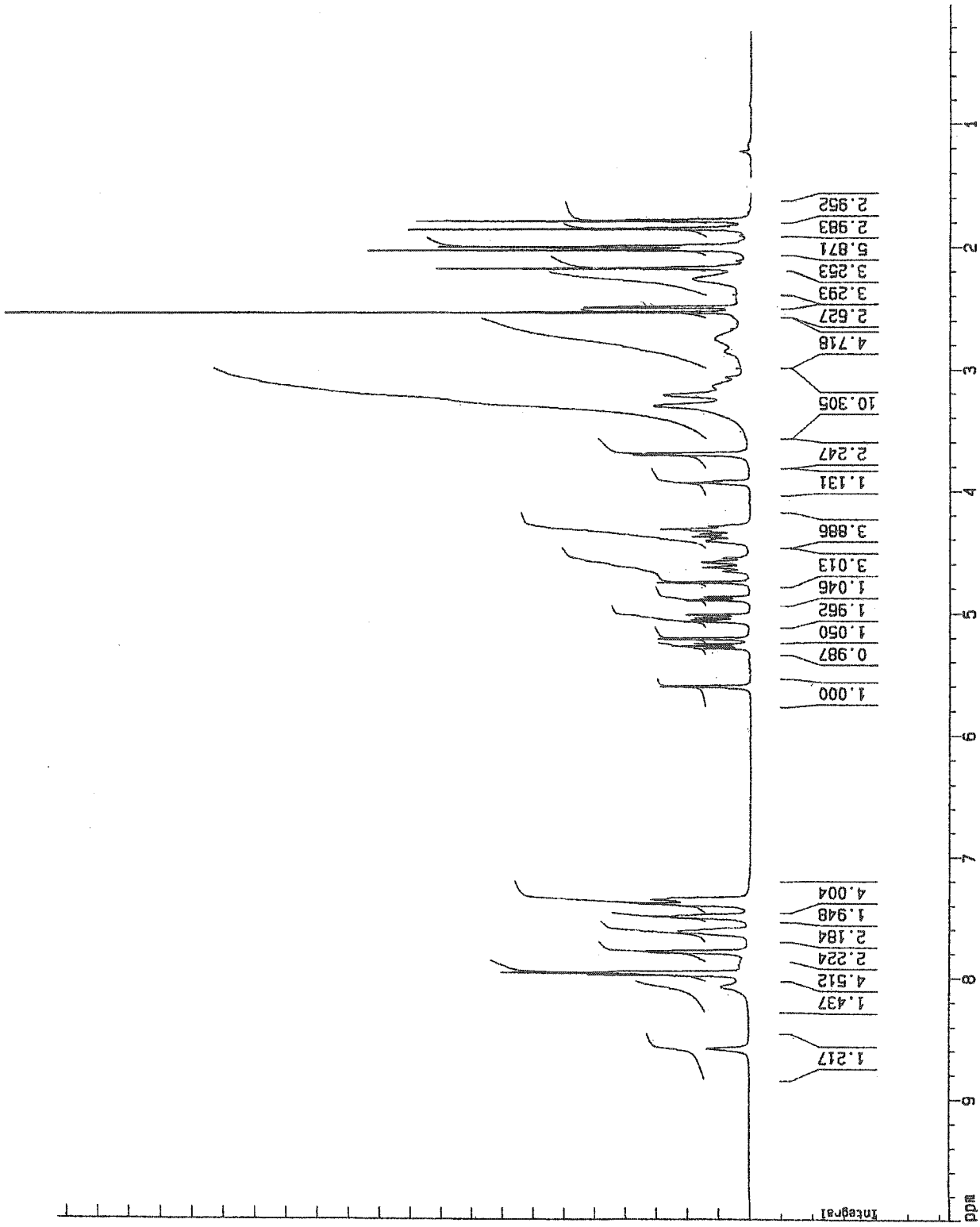


Fig. 3-7. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **3-23**.

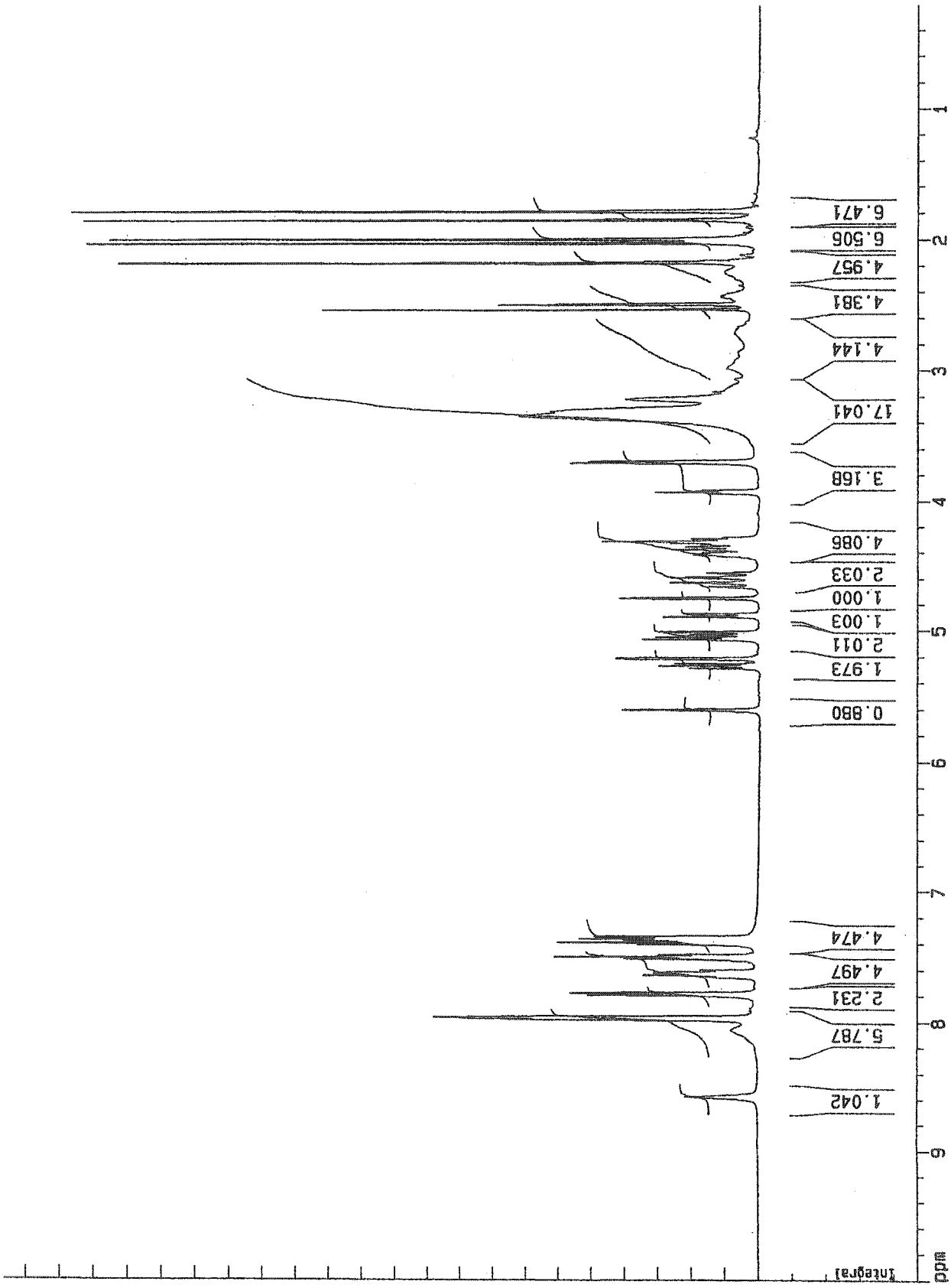


Fig. 3-8. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **3-25**.

3.3. Biochemical Assays

Table 3-1 Inhibition of binding of purified human IgG to Galα(1,3)-LacNAc-BSA by Galili clusters.

Inhibitor	**Dimer**	**Trimer**	**Tetra**	**Hexa**	**G0**	**G1**	**G2**
(mg/mL)	**3-2**	**3-5**	**3-10**	**3-16**	**3-22**	**3-24**	**3-26**
2.5	89	88	81	87	77	87	88
1.25	86	84	86	85	84	88	87
0.625	84	86	85	83	83	86	87
0.3125	81	82	80	78	75	84	82
0.15625	75	79	79	72	70	80	77
0.078125	65	71	71	60	59	71	67
0.03906	46	60	61	56	52	65	65
0.01953	39	46	46	40	39	53	51
IC_{50} μM (per hapten)	**122**	**60**	**48**	**60**	**60**	**24**	**32**
IC_{50} μM (per sugar residue)	**61**	**20**	**12**	**10**	**15**	**3**	**2**

Table 1 summarizes the ELISA results of our synthetic Galili clusters binding with Human IgG against a neoglycoprotein model Galα(1,3)-LacNAc-BSA. Based on the binding curves, IC_{50} values have been calculated as follows: IC_{50} (μM): dimer **3-2** 61; trimer **3-5** 20; tetramer **3-10** 12; hexamer **3-16** 11; PAMAM G0 **3-22** 15; G1 **3-24** 3; G2 **3-26** 2. From the results, it is clear that all the IC_{50}s are at micromolar levels. On a per molecule basis, G2 is the best antigen, while G1 is the best antigen on a per sugar residue basis.[27] Compared to the monomeric α-Gal (normally at milimolar levels), these Gilili antigens clearly demonstrate much enhanced binding affinity toward human IgG.

Table 3-2 Determination of the minimal inhibitory concentration (MIC) and the relative inhibitory capacity in haemagglutination.[c]

Probe:	**VAA (0.5 μg mL^{-1})**		**α-Gal-IgG (20 μg mL^{-1})**	
Inhibitor	MIC [μM]	relative potency	MIC [μM]	relative potency
galactose	25000	1	50000	1
lactose	6250	4	no inhibition up to 100 mM	
melibiose	12500	2	3000	16.7
asialofetuin	1.25	20000	no inhibition up to 83.4 μM	-
thyroglobulin	0.09	2.8x10^5	no inhibition up to 6.0 μM	-
MONO-1 (1)[a]	no inhibition up to 2930 μM		ND[b]	ND
MONO-2 (1)	no inhibition up to 380 μM		ND	ND
3-2 (2)	33.0	758 (379)	132	387 (195)
3-5 (3)	20.6	1214 (405]	41.3	1211 (404)
3-10 (4)	6.2	4032 (1008)	49.8	1004 (251)
3-16 (6)	4.0	6250 (1042)	31.9	1567 (261)
3-24 (8)	1.2	20833 (2604)	19.0	2632 (329)
3-26 (16)	1.3	19231 (1202)	18.2	2747 (172)

[a]The number in brackets denotes the number of α-Gal moieties per synthetic substance; [b]not determined; [c]see ref. 29 for the details for biochemical assays.

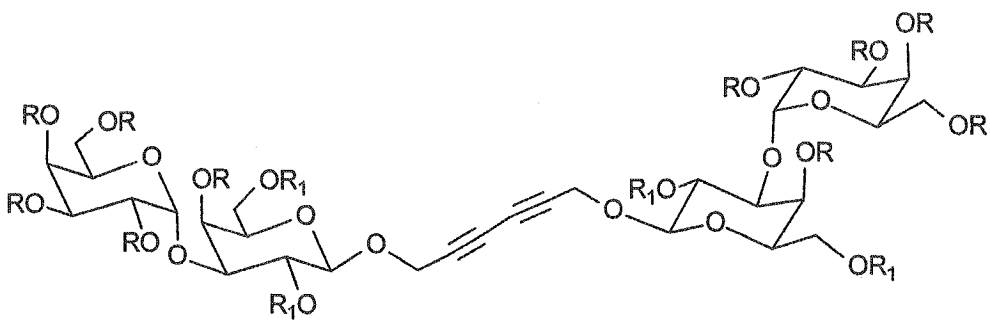
Viscum album agglutinin (VAA)[28] is a tetrameric plant lectin binding to galactosides. It does not discriminate between anomers. MICs showed that all the Galili

antigens were several hundred times to thousand times better than monomeric galactose on a per sugar residue. As for the dimeric IgG, the synthetic clusters were several hundred times better than galactose. Compared to the monomeric α-Gal-containing epitope melibiose, they still show more than 10-fold enhancement of binding affinity.

3.4. Conclusions

Analogues of multivalent Galili antigens from different cores have been prepared using Sonogashira coupling as key reactions. All clusters have demonstrated improved binding affinity toward human IgG compared melibios and may be potent ligands for human antibodies.

3.5. Experimental Section



3-1 R = OAc, R_1 = OBz

α-Gal homodimer (3-1). To a solution of compound **2-16** (79.8 mg, 0.1 mmol) in 5 mL of DMF: TEA (1:1) was added $Pd(PPh_3)_2Cl_2$ (3.6 mg, 5 mol%) and CuI (3.8 mg, 20 mol%). The solution was stirred under nitrogen at room temperature for 3 h. The solvent and

TEA were evaporated under reduced pressure. The resulting mixture was purified by silica gel column chromatography using ethyl acetate : hexane (1.5:1) to provide compound **3-1** as a white foam in 95% yield (151 mg, 0.95 mmol); $[\alpha]_D$ +115.6° (*c* 1, $CHCl_3$); ^1H (500 MHz; $CDCl_3$) δ 7.98-8.00 (8H, m, aromatic), 7.32-7.47 (12H, m, aromatic), 5.49-5.53 (4H, m, H-2, H-4), 5.19 (2H, d, $J < 1$, H-1'), 5.18 (2H, dd, J = 3.5, 10.5, H-2'), 5.02 (2H, dd, J = 3.3, 10.5, H-3'), 4.90 (2H, m, H-4'), 4.83 (2H, d, J = 7.9, H-1), 4.54 (2H, dd, J = 6.6, 11.3, H-6a), 4.46 (2H, d, J = 16.1, H-1a''), 4.41 (2H, d, J = 16.1, H-1b''), 4.30 (2H, dd, J = 6.6, 11.3, H-6b), 4.11 (2H, dd, J = 3.1, 10.1, H-3), 4.04 (2H, t, J = 6.7, H-5), 3.96 (2H, t, J = 7.1, H-5'), 3.77 (2H, dd, J = 7.1, 11.1, H-6a'), 3.68 (2H, dd, J = 6.6, 11.1, H-6b'), 2.19, 2.03, 2.00, 1.86 and 1.83 (each 6H, 5s, 10 CH_3CO_2); ^{13}C (125 MHz; $CDCl_3$) δ_C 170.2, 169.9, 169.8, 169.7, 169.3 (CH_3CO_2), 166.0, 165.1 ($PhCO_2$), 133.5, 133.4, 129.7, 129.3, 129.1, 128.5, 128.4 and 128.1 (aromatic), 99.2 (C-1), 93.4 (C-1'), 74.3 (acetylenic), 73.7 (C-3), 71.2 (C-5), 70.3 (acetylenic), 69.8 (C-2), 67.6 (C-4'), 66.9 (C-3' and C-5'), 66.5 (C-2'), 65.2 (C-4), 61.7 (C-6), 61.0 (C-6'), 56.5 (C-1''), 20.7, 20.5 and 20.4 (CH_3CO_2); *m/z* (FAB-HRMS) 1633.45. Found: $[M + K^+]$, $C_{78}H_{82}O_{36}$ requires $[M + K^+]$ 1633.42.

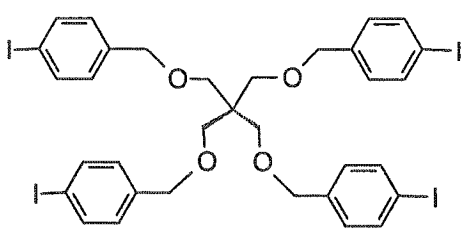
Fully deprotected α-Gal homodimer (3-2). α-Gal homodimer **3-1** (80 mg, 0.050 mmol) was dissolved into 30 mL of methanol. A catalytic amount of sodium methoxide was then added to the solution. The mixture was stirred at room temperature for 24 h. Sodium methoxide was neutralized with AMBERLITE IR-120 (H+). After careful filtration of the resin through a loose cotton plug, methanol was evaporated under reduced pressure. The dried residue was extracted with ether 3 x 10 mL to remove methyl benzoate to afford the deprotected glycocluster **3-2** as a white powder in 95% yield (36 mg, 0.047 mmol); $[\alpha]_D$ 0.0° (*c* 2.1, H_2O); ^1H (500 MHz; H_2O) δ 5.22 (2H, d, J = 3.9, H-1'), 4.69 (2H, d, J = 7.9, H-1),

4.65 (4H, bs, H-1''), 4.25 (4H, m, H-5 and H-5'), 4.08 (2H, m, H-4'), 4.02 (2H, dd, J = 3.4, 10.4, H-3'), 3.92 (2H, dd, J = 3.9, 10.4, H-2'), 3.84-3.94 (6H, m, H-3 and H-6 or H-3 and H-6'), 3.76-3.81 (6H, m, H-4 and H-6 or H-4 and H-6'), 3.72 (2H, dd, J = 7.9, 10.0, H-2); ^{13}C (125 MHz; D_2O) δ_C 100.6 (C-1), 94.8 (C-1'), 76.8 (C-3), 74.6 (C-2), 74.5 (acetylenic), 70.42 (C-5 or C-5'), 70.0 (acetylenic), 68.8 (C-3'), 68.7 (C-4'), 68.6 (C-4), 67.7 (C-2'), 64.3 (C-5 or C-5'), 60.5 and 60.4 (C-6 and C-6'), 56.5 (C-1''); *m/z* (FAB-MS) 797.1 Found: [M + K^+], $C_{30}H_{46}O_{22}$ requires [M + K^+] 797.2.

α-Gal trimer (3-4). To a solution of compound **2-16** (100 mg, 0.12 mmol) in 5 mL of DMF: TEA (1:1) was added $Pd(PPh_3)_2Cl_2$ (4.3 mg, 0.006 mmol (5 mol%)) and 1,3,5-triiodobenzene (**3-3**)[19] (15.8 mg, 0.035 mmol). The solution was stirred under nitrogen at 60 °C for 5 h. The solvent and TEA were evaporated under reduced pressure and the resulting mixture was purified by silica gel column chromatography using ethyl acetate: hexane (2:1)as eluent to provide compound **3-4** as a white foam in 85% yield (248 mg, 0.1 mmol); $[\alpha]_D$ +93.4° (*c* 1.8, $CHCl_3$); ^{1}H (500 MHz; $CDCl_3$) δ 7.98-8.00 (12H, m, aromatic), 7.32-7.47 (18H, m, aromatic), 5.53 (3H, dd, J = 8.0, 10.0, H-2), 5.46 (3H, d, J = 3.0, H-4), 5.19 (3H, d, J < 1, H-1'), 5.18 (3H, dd, J = 3.5, 10.5, H-2'), 5.04 (3H, dd, J = 3.3, 10.5, H-3'), 4.91 (3H, d, J = 8.0, H-1), 4.90 (3H, m, H-4'), 4.60 (6H, s, H-1''), 4.55 (3H, dd, J = 6.4, 11.3, H-6a), 4.32 (3H, dd, J = 6.6, 11.3, H-6b), 4.12 (3H, dd, J = 3.1, 10.0, H-3), 4.04 (3H, t, J = 6.7, H-5), 3.97 (3H, t, J = 6.9, H-5'), 3.74 (3H, dd, J = 7.1, 11.1, H-6a'), 3.68 (3H, dd, J = 6.6, 11.1, H-6b'), 2.19, 2.03, 2.00, 1.86 and 1.72 (each 9H, 5s, 15 CH_3CO_2); ^{13}C (125 MHz; $CDCl_3$) δ_C 170.2, 169.9, 169.8, 169.7 and 169.3 (CH_3CO_2), 166.0 and165.1 ($PhCO_2$), 134.7, 133.4, 133.3, 129.7, 129.6, 129.3, 129.1, 128.5, 128.4 and 122.1 (aromatic), 99.2 (C-1), 94.0 (C-1'), 85.4 and 84.5 (acetylenic), 73.9 (C-3), 71.1 (C-5), 70.1 (C-2), 67.6 (C-4'), 66.9 (C-

3'), 66.5 (C-2' and C-5'), 65.3 (C-4), 61.8 (C-6), 61.0 (C-6'), 56.5 (C-1''), 20.7, 20.6, 20.4 and 20.3 (CH_3CO_2); *m/z* (ESI-MS) 2485.1 [Found: $[M + NH_4^+]$, $C_{123}H_{130}NO_{54}$ requires $[M + NH_4^+]$ 2484.7.

Fully deprotected α-Gal trimer (3-5). α-Gal trimer **3-4** (90 mg, 0.036 mmol) was dissolved into 30 mL of methanol. A catalytic amount of sodium methoxide was added to the solution. The mixture was stirred at room temperature for 24 h. Sodium methoxide was neutralized with AMBERLITE IR-120 (H+) resin. After careful filtration of the resin through a loose cotton plug, methanol was removed under reduced pressure. The dried residue was extracted with ether 3 x 10 mL to remove methyl benzoate to provide the fully deprotected glycocluster **3-5** as a off-white foam in 95% yield (41 mg, 0.034 mmol); $[\alpha]_D$ 0.0° (*c* 1.5, H_2O); ^1H (500 MHz; D_2O) δ 7.67 (3H, s, aromatic), 5.23 (3H, d, *J* = 3.8, H-1'), 4.79 (6H, bs, H-1''), 4.75 (3H, d, *J* = 7.9, H-1), 4.27 (6H, m, H-5 and H-5'), 4.09 (3H, m, H-4'), 4.04 (3H, dd, *J* = 3.3, 10.4, H-3'), 3.94 (3H, dd, *J* = 3.9, 10.4, H-2'), 3.76-3.89 (21H, m, H-2, H-3, H-4, H-6 and H-6'); ^{13}C (125 MHz; D_2O) δ_C 134.4 and 122.2 (aromatic),100.8 (C-1), 94.9 (C-1'), 85.2 and 84.6 (acetylenic), 76.9 (C-3), 74.6 (C-2 or C-4), 72.3 (C-5 or C-5'), 70.4 (C-3'), 68.9 and 68.7(C-3', C-4' and C-4 or C-3', C-4' and C-2), 67.8 (C-2'), 64.4 (C-5 or C-5'), 60.5 (C-6 and C-6'), 56.7 (C-1''); *m/z* (ESI-MS) 1230.0 Found: $[M + NH_4^+]$, $C_{30}H_{50}NO_{22}$ requires $[M + NH_4^+]$ 1230.3.



3-8

Pentaerythritol tetrakis (*p*-iodobenzyl) ether (3-8). Pentaerythritol **3-6** (27.2 mg, 0.2 mmol) was dissolved into 10 mL of dry DMF, then a catalytic amount of tetrabutylammonium iodide and 46 mg of NaH (0.96 mmol, 1.2 eq./OH) were added to the solution. After one hour, *p*-iodobenzyl bromide **3-7** (286 mg, 0.96 mmol) was added. The mixture was stirred at room temperature for 5 h. Excess NaH was slowly quenched with cold water. The solution was washed with 3 x 20 mL of ether and the organic layerss were combined together and washed with 3 x 20 mL of water. After drying over anhydrous sodium sulfate the solvent was evaporated and the residue was carefully separated by silica gel column chromatography using hexane: ether (7:1) to give **3-8** as a white solid in 46% yield (92 mg, 0.09 mmol); mp 136-138 °C; ^{1}H (500 MHz; $CDCl_3$) δ 7.58 (8 H, d, J = 8.1, aromatic), 6.95 (8 H, d, J = 8.1, aromatic), 4.36 (8 H, s, PhCH_2), 3.45 (8 H, s, C(CH_2-)$_4$); ^{13}C (125 MHz; $CDCl_3$) δ_C 138.2, 137.3, 129.2 and 92,8 (aromatic), 72.5 (PhCH_2), 69.1 (C(CH_2-)$_4$), 45.5 (*C*(CH$_2$-)$_4$); *m/z* (FAB-MS) 1001.2 Found: [M + H$^+$], $C_{33}H_{33}O_4$ requires [M + H$^+$] 1001.0. Anal. Calcd for $C_{33}H_{32}O_4I_4$: C, 39.63; H, 3.22. Found: C, 39.83; H, 3.19.

α-Gal tetramer (3-9). To a solution of compound **2-16** (150 mg, 0.188 mmol) in 10 mL of DMF: TEA (1:1) were added $Pd(PPh_3)_2Cl_2$ (6.7 mg, 0.009 mmol (5 mol%)) and **3-8** (39.1 mg, 0.039 mmol). The solution was stirred under nitrogen at 60 °C for 5 h. The solvent and TEA were evaporated under reduced pressure. The resulting mixture was purified by silica gel column chromatography using ethyl acetate: hexane (2:1) to provide compound **3-9** as a white foam in 81% yield (560 mg, 0.15 mmol); $[\alpha]_D$ +97.5° (*c* 1, $CHCl_3$); ^{1}H (500 MHz; $CDCl_3$) δ 7.98-8.02 (16H, m, aromatic), 7.40-7.55 (16H, m, aromatic), 7.14-7.29 (20H, m, aromatic), 5.54 (4H, dd, J = 8.0, 10.1, H-2), 5.51 (4H, d, J = 2.8, H-4), 5.20 (4H, d, J < 1, H-1'), 5.18 (4H, dd, J = 3.5, 10.4, H-2'), 5.03 (4H, dd, J = 3.3, 10.4, H-3'), 4.90

(4H, d, J = 8.0, H-1), 4.88 (4H, m, H-4'), 4.58 (8H, s, H-1''), 4.55 (4H, dd, J = 6.4, 11.3, H-6a), 4.45 (8H, s, PhCH_2), 4.32 (4H, dd, J = 6.6, 11.3, H-6b), 4.10 (4H, dd, J = 3.1, 10.1, H-3), 4.04 (4H, t, J = 6.7, H-5), 3.96 (4H, t, J = 6.5, H-5'), 3.73 (4H, dd, J = 6.6, 11.2, H-6a'), 3.66 (4H, dd, J = 6.6, 11.2, H-6b'), 3.56 (8H, s, C(CH_2OR)$_4$), 2.20, 2.03, 1.99, 1.86 and 1.72 (each 12H, 5s, 20 CH_3CO$_2$); ^{13}C (125 MHz; $CDCl_3$) δ_C 170.2, 169.9, 169.8, 169.7, and 169.3 (CH_3CO_2), 166.0, 165.0 (PhCO_2), 139.5, 133.4, 133.3, 132.1, 132.0, 131.7, 129.7, 129.4, 129.3, 128.5, 128.4, 127.1, 127.0 and 121.1 (aromatic), 98.9 (C-1), 93.7 (C-1'), 86.7 and 83.5 (acetylenic), 73.7 (C-3), 72.8 (PhCH_2), 71.0 (C-5), 70.0 (C-2), 69.5 (C(CH_2OR)$_4$), 67.6 (C-4'), 66.9 (C-3'), 66.5 (C-2'), 66.4 (C-5'), 65.1 (C-4), 61.7 (C-6), 61.2 (C-6'), 56.8 (C-1''), 45.7 (C(CH$_2$OR)$_4$), 20.7, 20.4 and 20.3 (CH_3CO_2); *m/z* (MALDI-TOF MS) 3706.55 Found: [M + Na$^+$], $C_{189}H_{196}O_{78}$ requires [M + Na$^+$] 3706.68.

Fully deprotected α-Gal tetramer (3-10). α-Gal tetramer **3-9** (95 mg, 0.026 mmol) was dissolved into 40 mL of methanol to which a catalytic amount of sodium methoxide was added. The mixture was stirred at room temperature for 24 h. Sodium methoxide was neutralized with AMBERLITE IR-120 (H+) resin. After careful filtration of the resin through a loose cotton plug, methanol was removed under reduced pressure, the dried residue was extracted with ether 3 x 10 mL to remove methyl benzoate to afford the deprotected glycocluster **3-10** as a off-white foam in 95% yield (48 mg, 0.024 mmol); $[\alpha]_D$ 0.0° (*c* 1.8, H_2O); ^{1}H (500 MHz; D_2O) δ 7.30 (8H, bs, aryl), 6.98 (8H, bs, aryl), 5.21 (4H, bs, H-1'), 4.82 (8H, s, PhCH_2 or H-1''), 4.63 (8H, s, PhCH_2 or H-1''), 4.60 (4H, bs, H-1), 4.23 (8H, bs, H-5 and H-5'), 4.06 (4H, bs, H-4'), 4.01 (4H, bd, J = 10.8, H-3'), 3.93 (4H, dd, J = 2.9, 10.1, H-2'), 3.77-3.83 (28H, m, H-2, H-3, H-4, H-6 and H-6'); 3.42 (8H, s, C(CH_2OR)$_4$); ^{13}C (125 MHz; D_2O) δ_C138.5, 131.3, 126.8 and 120.6 (aromatic),101.1 (C-1),

95.0 (C-1'), 85.2 and 84.5 (acetylenic), 77.2, 70.3, 68.9, 68.7, 67.8, 64.2, 60.5, 60.2, 56.6 (C-4, C-5, C-6, C-2', C-3', C-4', C-5', C-6', Ph*C*H_2, C-1''); *m/z* (ESI-MS) 1958.4 Found: [M + NH_4^+; $C_{88}H_{120}NO_{48}$ requires [M + NH_4^+], 1958.6.

Pentaerythritol allyl tribenzyl ether (3-12). Pentaerythritol **3-6** (27.2 mg, 0.2 mmol) was dissolved into dry DMF (10 mL), to which were added a catalytic amount of tetrabutylammonium iodide (10 mg) and NaH (36 mg, 0.9 mmol). The mixture was stirred at room temperature for 30 minutes and then benzyl bromide (87 μL, 0.72 mmol) was added to it. The reaction was monitored by TLC and was completed in 6 hours. Excess NaH was quenched with methanol. The mixture was diluted with 50 mL of water and then washed with 3 x 30 mL of ether. The combined organic layers were washed with 3 x 30 mL of water and dried over anhydrous sodium sulfate. After evaporating the solvent under reduced pressure, the residue was separated by silica gel column chromatography using hexane : ether (4:1). After removal of the solvent, pentaerythritol tribenzyl ether **3-11** was obtained as a thick liquid in 65% yield.

To a solution of **3-11** (81.2 mg, 0.2 mmol) in dry DMF (10 mL) were added a catalytic amount of tetrabutylammonium iodide (10 mg) and NaH (12 mg, 0.3 mmol). The mixture was stirred at room temperature for 30 minutes and then allyl bromide (21 μL, 0.24 mmol) was added to it. After 6 hours, the reaction was stopped and excess NaH was destroyed with several drops of methanol. The mixture was diluted with 50 mL of water and then washed with 3 x 30 mL of ether. The combined organic layers were washed with 3 x 30 mL of water and dried over anhydrous sodium sulfate. After removal of ether under reduced pressure, the residue was separated by silica gel chromatography using hexane : ether (10:1). After evaporating the solvent, compound **3-12** was obtained as a thick liquid in 90% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 to 7.32 (m, 15H, aromatic), 5.83 to 5.90 (m, 1H, vinyl), 5.11 to 5.25 (m, 2H, vinyl), 4.48 (s, 6H, benzylic), 3.93 to 3.95 (m, 2H. allylic), 3.56 (s, 6H, $C(CH_2OR)_4$), 3.53 (s, 2H, $C(CH_2OR)_4$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.9 (aromatic), 135.2 (vinyl), 128.2, 127.3 and 127.2 (aromatic),116.1 (vinyl), 73.3 (benzylic), 72.3 (allylic), 69.3 ($C(CH_2OR)_4$), 45.6 ($C(CH_2OR)_4$); FAB-MS calcd for $C_{29}H_{34}O_4$ ($M + H^+$) 447.25; Found 447.32.

Compound 3-13. To a 0.06 M solution of **3-12** in dry dichloromethane was added Grubbs' catalyst (15 mol %). The mixture was stirred under nitrogen at room temperature for 12 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using ether and hexane (4:1) to provide **3-13** as an inseparable mixture (*E* : *Z* = 10:1) in 85 % yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 to 7.32 (m, 30H, aromatic), 5.68 (m, 2H, vinyl), 4.48 (s, 12H, benzylic), 3.91 (m, 4H. allylic), 3.56 (s, 12H, $C(CH_2OR)_4$), 3.53 (s, 4H, $C(CH_2OR)_4$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.9 (aromatic), 129.0 (vinyl), 128.2, 127.3 and 127.2 (aromatic), 73.3 (benzylic), 71.4 (allylic), 69.4 and 65.4 ($C(CH_2OR)_4$), 45.6 ($C(CH_2OR)_4$); ESI-MS (in formic acid) calcd for $C_{56}H_{64}O_8$($M + H^+$) 865.4; Found 865.2.

Compound 3-14. To a solution of **3-13** (86.5 mg, 0.1 mmol) in ethanol (50 mL) was added a catalytic amount of 10 % Pd/C. This compound was hydrogenolyzed at room temperature for 12 h and then it was filtered through a pad of celite. After evaporation of the solvent, a solid was obtained in 90 % yield. This fully deprotected compound (16.3 mg, 0.05 mmol) was dissolved into 10 mL of DMF and then to the solution were added a catalytic amount of tetrabutylammonium iodide (10 mg) and NaH (24 mg, 0.6 mmol). The mixture was stirred at room temperature for 30 minutes and then p-iodobenzyl bromide (134

mg, 0.45 mmol) was added to it. After 6 hours, excess NaH was quenched with several drops of methanol. The mixture was diluted with 40 mL of water and then washed with 3 x 30 mL of ether. The combined organic layers were washed with 3 x 30 mL of water and dried over anhydrous sodium sulfate. After evaporating the solvent under reduced pressure, the residue was separated by silica gel column chromatography using hexane : ethyl acetate (5:1). After removal of the solvent, a white solid was obtained **3-14** in 45% yield (36 mg, 0.022 mmol); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 (d, 12H, J = 8 Hz, aromatic), 6.96 (d, 12H, J = 8 Hz, aromatic), 4.39 (s, 12H, benzylic), 3.44 (s, 12H, $C(CH_2OR)_4$), 3.40 (s, 4H, $C(CH_2OR)_4$), 3.33 (bs, 4H, $-CH_2O-$),1.50 (bs, 4H, -CH2-); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.9 (aromatic), 137.7, 129.6 and 127.3 (aromatic), 72.9 (benzylic), 71.6 ($-CH_2O-$), 69.7 ($C(CH_2OR)_4$), 45.8 ($C(CH_2OR)_4$), 26.7 ($-CH_2-$); ESI-MS calcd for $C_{56}H_{60}O_8I_6$ ($M + NH_4^+$) 1639.4; Found 1639.2.

Compound 3-15. To a solution of **3-14** (16.2 mg, 0.01 mmol) in 5 mL of DMF/TEA (1:1) was added $Pd(PPh_3)_2Cl_2$ (2.2 mg, 0.003 mmol (5 mol %)) and compound **2-16** (30.1 mg, 0.078 mmol). The solution was stirred under nitrogen at 60 °C for 5 h. The solvent and TEA were evaporated under reduced pressure and the resulting mixture was purified by silica gel column chromatography using ethyl acetate-hexane (4:1) to provide compound **3-15** as a white solid in a yield of 75% (42.3 mg, 0.007 mmol); $[\alpha]_D$ 0.0° (c 1.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 to 8.02 (m, 24 H, aromatic), 7.49 to 7.55 (m, 12 H, aromatic), 7.39 to 7.47 (m, 12 H, aromatic), 7.13 to 7.28 (m, 36 H, aromatic), 5.56 (dd, J = 8.0, 10.0, 6 H, H-2), 5.45 (bd, J = 2.1, 4 H, H-4), 5.20 (d, J< 1, 6 H, H-1'), 5.19 (dd, J = 3.5, 10.4, 6 H, H-2'), 5.03 (dd, J = 3.3, 10.4, 6 H, H-3'), 4.95 (d, J = 8.0, 6 H, H-1), 4.89 (bd, J = 2.1, 6 H, H-4'), 4.58 (s, 12H, H-1''), 4.56 (dd, J = 6.2, 11.0, 6H, H-6a), 4.42 (s, 12H,

benzylic), 4.39 (dd, *J* = 7.0, 11.0, 6 H, H-6b), 4.10 (dd, *J* = 3.1, 10.0, 6H, H-3), 4.05 (t, *J* = 6.9, 6H, H-5), 3.97 (t, J = 6.6, 6H, H-5'), 3.73 (dd, J = 6.5, 11.2, 6H, H-6a'), 3.66 (s, 12H, 3.66 (dd, J = 6.5, 11.2, 6H, H-6b'), 3.52 (s, 12H, C(C*H*$_2$OR)$_4$), 3.46 (s, 4H, C(CH$_2$OR)$_4$), 3.36 (s, 4H, -CH$_2$O-), 2.20, 2.03, 1.99, 1.86 and 1.73 (5s, 90H, C*H*$_3$CO), 1.54 (bs, 4H, -C*H*$_2$-),); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.2, 169.8, 169,7 and 169.3 (CH$_3$*C*O), 166.0 and 165.0 (Ph*C*O), 139.6, 133.4, 133.3, 132.1, 132.0, 131.7, 129.7, 129.4, 129.3, 128.5, 128.4, 127.1, 126.9 and 121.0 (aromatic), 98.9 (C-1), 93.7 (C-1'), 86.7 and 83.5 (acetylenic), 73.7 (C-3), 72.8 (benzylic), 71.3 (-*C*H$_2$O-), 70.9 (C-5), 70.0 (C-2), 69.7 and 69.6 (C(*C*H$_2$OR)$_4$), 67.6 (C-4'), 66.9 (C-3'), 66.5 (C-2'), 66.4 (C-5'), 65.1 (C-4), 61.7 (C-6), 61.2 (C-6'), 56.8 (C-1''), 45.6 (*C*(CH$_2$O)$_4$), 26.4 (-*C*H$_2$-), 20.7, 20.5, 20.4 and 20.3 (*C*H$_3$CO); MALDI-TOF-MS calcd for $C_{290}H_{308}O_{116}$ (M + Na^+) 5668.8; Found 5668.5.

Fully deprotected α-Gal hexamer (3-16). α-Gal hexamer **3-15** (95 mg, 0.017 mmol) was dissolved into 40 mL of ethanol to which a catalytic amount of sodium methoxide was added. The mixture was stirred at room temperature for 48 h. Sodium methoxide was neutralized with AMBERLITE IR-120 (H+) resin. After careful filtration of the resin through a loose glass wool plug, methanol was remove under reduced pressure, the desired residue was extracted with ether 3 x 10 mL to removed methyl benzoate to afford the fully deprotected glycocluster **3-16** as an off-white foam in 95% yield (51 mg, 0.016 mmol); $[\alpha]_D$ 0.0° (*c* 1.5, H_2O); ^{1}H (500 MHz, D_2O) δ 7.34 (bs, 12H, aromatc), 7.06(bs, 12H, aromatic), 5.21 (bs, 6H, H-1'), 4.82 (bs, 12H, PhCH$_2$ or H-1''), 4.63 (bs, 12H, PhCH$_2$ or H-1''), 4.58 (bs, 6H, H-1), 4.23 (bs, 12H, H-5 and H-5'), 4.06 (bs, 6H, H-4'), 4.02 (bd, J = 10.8, 6H, H-3'), 3.94 (dd, J = 2.9, 10.1, 4H, H-2'), 3.63-3.91 (m, 42H, H-2, H-3, H-4, H-6 and H-6'); 3.42 (m, 20H, C(C*H*$_2$OR)$_4$, -C*H*$_2$O-), 1.52 (bs, 4H, -C*H*$_2$-); ^{13}C (125 MHz; D_2O)

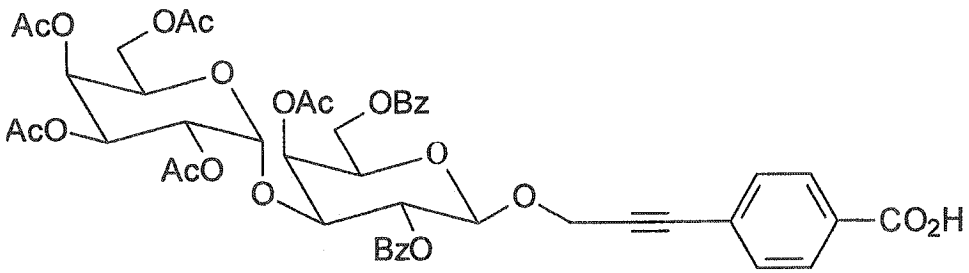
δ_C 138.6, 131.3, 126.7 and 120.6 (aromatic), 101.1 (C-1), 95.0 (C-1'), 85.1 and 84.4 (acetylenic), 77.2, 74.1, 71.9, 70.3, 68.7, 67.8 and 64.1 (C-4, C-5, C-2', C-3', C-4', C-5', Ph*C*H_2, -*C*H_2O-, C(*C*H_2OR)$_4$), 60.5 (C-6), 60.1 (C-6'), 56.6 (C-1''), 45.5 (*C*(CH$_2$O)$_4$), 25.1 (-*C*H_2-); MALDI-TOF MS calcd for $C_{146}H_{198}O_{74}$ (M + Na^+) 3158.2; Found 3158.3.

Compound 3-20. Compound **3-19** was prepared in two different approaches. Approach a: To a suspension of compound **3-17** (250 mg, 1 mmol) in thionyl chloride and dichloromethane (2 ml : 2 ml), was added one drop of DMF. The mixture was stirred at rt for 2 h till a homogeneous solution was formed. After removal of the solvent under reduced pressure, the residue was dissolved in dry dioxane (3 ml). Then to the solution was added *t*-BuOLi (1.1 mL of 1 M solution in dioxane) at rt. The mixture was stired at rt for 5 h. After neutralized to neutral using 1 M HCl, the solution was diluted with water (20 mL) and extracted with ether (3 x 10 mL). The ether layers were combined together, dried over sodium sulphate, evaporated under reduced pressure, and purified on silica gel column using haxane and ethyl acetate (4 : 1) to afford **3-19** in 75% yield (197 mg, 0.75 mmol). Approach b: To a suspension of compound **3-17** (250 mg, 1 mmol) in dichloromethane (10 ml) were added DMAP (20 mol%) and Boc anhydrade (2 mmol). The mixture was stirred at rt for 8 h. Workup following the above mentioned procedure provided **3-19** in 90% yield (237 mg, 0.9 mmol).

t-Butyl *p*-iodobenzoate **3-19** (30.5 mg, 0.1 mmol) was dissolved into a solution of 10 mL of DMF and TEA (1:1) to which were added Pd(PPh$_3$)$_2$Cl$_2$ (3.6 mg, 5 mol%), 2-propynyl (2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→3)-4-*O*-acetyl-2,6-di-O-benzoyl-β-D-galactopyranoside (**2-16**) (94.5 mg, 0.12 mmol) and CuI (3.8 mg, 0.02 mmol). The solution was stirred at rt for 5 h under a N_2 atmosphere. The solvent and triethylamine

were removed under reduced pressure. The residue was purified by silica gel column chromatography using hexane : ethyl acetate (3:2) to afford **2-20a** as a white foam in 86% yield. Then **3-20a** (91.8 mg, 0.1 mmol) was dissolved into TFA : CH_2Cl_2 (2.5 mL : 2.5 mL) and the mixture was stirred at rt for 12 h. After evaporation of the solvent under reduced pressure, a white solid **3-20b** was obtained in 95% yield.

For **3-20a**: $[\alpha]_D$ 100.5° (*c* 1.5, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.98-8.04 (m, 6H, aromatic), 7.50-7.58 (m, 2H, aromatic), 7.41 (m, 2H, aromatic), 7.31-7.38 (m, 4H, aromatic), 5.56 (1H, dd, *J* = 8.0, 10.1, H-2), 5.53 (1H, bd, *J* = 3.0, H-4), 5.22 (1H, d, *J* < 1, H-1'), 5.20 (1H, dd, *J* = 3.5, 10.5, H-2'), 5.05 (1H, dd, *J* = 3.3, 10.5, H-3),4.95 (1H, d, *J* = 7.9, H-1), 4.92 (1H, dd, *J* = 1.4, 3.2, H-4'), 4.62 (2H, *J* = 3.6, H-1''), 4.58 (1H, dd, *J* = 6.6, 11.4, H-6a), 4.33 (1H, dd, *J* = 6.8, 11.4, H-6b), 4.12 (1H, dd, *J* = 3.2, 10.1, H-3), 4.08 (1H, t, *J* = 6.7, H-5), 4.00 (1H, *J* = 6.7, H-5'), 3.78 (1H, dd, *J* = 6.9, 11.2, H-6a'), 3.70 (1H, dd, *J* = 6.7, 11.1, H-6b'), 2.22, 2.05, 2.01, 1.87 and 1.77 (each 3H, 5s, 5 CH_3CO_2), 1.58 (9H, s, -$C(CH_3)$); ^{13}C (125 MHz; $CDCl_3$) δ_C 170.2, 169.9, 169.8 and 169.5 (CH_3CO_2), 166.1 and 165.0 ($PhCO_2$), 133.5, 133.4, 131.8, 130.0, 129.7, 129.3, 129.1, 128.5 and 127.6 (aromatic), 99.1 (C-1), 93.8 (C-1'), 87.1 and 85.9 (acetylenic), 86.7 (-$C(CH_3)$), 73.8 (C-3), 71.1 (C-5), 70.0 (C-2), 67.6 (C-4), 66.9 (C-3'), 66.5 (C-2' and C-5'), 65.2 (C-4'), 61.9 (C-6), 61.1 (C-6'), 56.7 (C-1''), 28.8 (-$C(CH_3)$), 20.8, 20.7, 20.5 and 20.4 (CH_3CO_2); FAB-MS calcd for $C_{50}H_{54}O_{20}K$ ($M + K^+$): 1013.22; Found: 1013.15.



3-20b, R = H

For **3-20b**: $[\alpha]_D$ 95.2° (*c* 1.5, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.98-8.04 (m, 6H, aromatic), 7.50-7.58 (m, 2H, aromatic), 7.41 (m, 2H, aromatic), 7.31-7.38 (m, 4H, aromatic), 5.56 (1H, dd, *J* = 8.0, 10.1, H-2), 5.53 (1H, bd, *J* = 3.0, H-4), 5.22 (1H, d, *J* < 1, H-1'), 5.20 (1H, dd, *J* = 3.5, 10.5, H-2'), 5.05 (1H, dd, *J* = 3.3, 10.5, H-3'),4.95 (1H, d, *J* = 7.9, H-1), 4.92 (1H, dd, *J* = 1.4, 3.2, H-4'), 4.62 (2H, *J* = 3.6, H-1''), 4.58 (1H, dd, *J* = 6.6, 11.4, H-6a), 4.33 (1H, dd, *J* = 6.8, 11.4, H-6b), 4.12 (1H, dd, *J* = 3.2, 10.1, H-3), 4.08 (1H, t, *J* = 6.7, H-5), 4.00 (1H, *J* = 6.7, H-5'), 3.78 (1H, dd, *J* = 6.9, 11.2, H-6a'), 3.70 (1H, dd, *J* = 6.7, 11.1, H-6b'), 2.22, 2.05, 2.01, 1.87 and 1.77 (each 3H, 5s, 5 CH_3CO_2); ^{13}C (125 MHz; $CDCl_3$) δ_C 170.2, 169.9, 169.8 and 169.5 (CH_3CO_2), 166.1 and 165.0 ($PhCO_2$), 133.5, 133.4, 131.8, 130.0, 129.7, 129.3, 129.1, 128.5 and 127.6 (aromatic), 99.1 (C-1), 93.8 (C-1'), 87.1 and 85.9 (acetylenic), 73.8 (C-3), 71.1 (C-5), 70.0 (C-2), 67.6 (C-4), 66.9 (C-3'), 66.5 (C-2' and C-5'), 65.2 (C-4'), 61.9 (C-6), 61.1 (C-6'), 56.7 (C-1''), 20.8, 20.7, 20.5 and 20.4 (CH_3CO_2); FAB-HRMS calcd for $C_{46}H_{46}O_{20}K$ ($M + K^+$): 957.22; Found: 927.20.

General Procedure for the Preparation of Fully Protected PAMAM-based Galili-Antigen Clusters.

A solution of PAMAM G(0) (Aldrich) (72 mg of 36.02 % (w/w) methanolic solution, 0.05 mmol) was co-evaporated with benzene twice under reduced pressure. The residue was dissolved into DMSO (5 mL) and to this solution were added HATU (70 mg, 0.21 mmol), DIPEA (29 mg, 0.21 mmol) and **3-20b** (193 mg, 0.21 mmol). The solution was stirred at room temperature for 1 d. The mixture was dialyzed against DMSO (MW cutoff 1200 Da), and then the solution was freeze-dried to give 4mer **3-21** as an off-white solid in 90% yield.

The same procedure was applied for the synthesis of **3-23** and **3-25** in yield of 87% and 85%, respectively. The MW cutoff of dialysis was 3500 Da.

Compound G0 3-21. $[\alpha]_D$ 95.2° (*c* 1.5, $CHCl_3$); ^1H NMR (500 MHz, DMSO-*d6*) δ 8.59 (bs, 4H, amide), 8.21 (bs, 4H, amide), 7.79-7.99 (m, 16H, aromatic), 7.80 (8H, d, *J* = 7.8, aromatic), 7.61-7.68 (8H, m, aromatic), 7.50-7.53 (m, 8H, aromatic), 7.36-7.42 (m, 16H, aromatic), 5.61 (4H, bd, *J* = 2.5, H-4), 5.27 (4H, dd, *J* = 8.9, 10.0, H-2), 5.22 (1H, d, *J* = 3.2, H-1'), 5.07 (1H, d, *J* = 8.0, H-1), 5.03 (1H, dd, *J* = 3.2, 11.1, H-2'), 4.89 (1H, dd, *J* = 3.1, 11.1, H-3'), 4.76 (1H, d, *J* < 1, H-4'), 4.65 (2H, *J* = 16.4, H-1a''), 4.58 (2H, *J* = 16.4, H-1b''), 4.42 (1H, dd, *J* = 6.6, 11.4, H-6a), 4.37 (1H, dd, *J* = 2.8, 10.4, H-3), 4.29-4.34 (12H, m, H-6b, H-5), 3.93 (1H, t, *J* = 6.6, H-5'), 3.70 (8H, d, *J* = 6.5, H-6'), 2.87-3.32 (26 H, m, -CH_2-, from PAMAM), 2.53 (8H, bs, -CH_2- from PAMAM), 2.17, 2.02, 2.00, 1.85 and 1.78 (each 12H, 5s, 5 CH_3CO_2); ^{13}C (125 MHz; DMSO-*d6*) δ_C 169.8, 169.7 169.6, 169.3 and 169.2 (CH_3CO_2), 165.5, 165.3 and 164.8 ($PhCO_2$), 133.4, 133.7, 133.5, 131.2, 129.3, 129.1, 128.9, 128.7, 128.6, 127.3 and 124.2 (aromatic), 98.7 (C-1), 92.6 (C-1'), 86.9 and 85.3 (acetylenic), 72.5 (C-3), 70.3 (C-5), 70.0 (C-2), 66.9(C-4'), 66.3 (C-3'), 66.8 (C-4 and C-5'), 65.2 (C-2'), 61.9 (C-6), 60.3 (C-6'), 56.4 (C-1''), 40.4, 39.9, 39.7, 38.0 (-CH_2- from

PAMAM), 20.5, 20.4, 20.2 and 20.1 (CH_3CO_2); ESI-MS calcd for $C_{206}H_{226}N_{10}O_{80}$ (M + $2H^+$): 2059.88; Found: 2159.10.

Compound G1 3-23. $[\alpha]_D$ 95.2 (*c* 1.5, $CHCl_3$); ^1H NMR (500 MHz, DMSO-*d6*) δ 8.59 (bs, 8H, amide), 8.06 (bs, 8H, amide), 7.96-7.98 (m, 16H, aromatic), 7.79 (8H, d, *J* = 8.1, aromatic), 7.60-7.66 (8H, m, aromatic), 7.49-7.52 (m, 8H, aromatic), 7.35-7.49 (m, 16H, aromatic), 5.61 (4H, bd, *J* = 2.1, H-4), 5.27 (4H, dd, *J* = 8.3, 9.8, H-2), 5.22 (1H, d, *J* = 3.1, H-1'), 5.06 (1H, d, *J* = 8.0, H-1), 5.03 (1H, dd, *J* = 3.1, 11.1, H-2'), 4.89 (1H, dd, *J* = 3.0, 11.1, H-3'), 4.76 (1H, d, *J* < 1, H-4'), 4.65 (2H, *J* = 16.3, H-1a''), 4.58 (2H, *J* = 16.3, H-1b''), 4.42 (1H, dd, *J* = 6.6, 11.4, H-6a), 4.37 (1H, m, H-3), 4.28-4.34 (12H, m, H-6b, H-5), 3.93 (1H, t, *J* = 6.8, H-5'), 3.70 (8H, d, *J* = 6.5, H-6'), 2.67-3.71 (68H, m, -C*H*$_2$- from PAMAM), 2.17-2.43 (32H, m, -C*H*$_2$- from PAMAM), 2.17, 2.02, 1.99, 1.84 and 1.78 (each 24H, 5s, 5 C*H*$_3$CO$_2$); ^{13}C (125 MHz; DMSO-*d6*) δ_C 169.8, 169.7, 169.6, 169.4 and 169.3 (CH_3CO_2), 165.6, 165.3 and 164.8 (PhCO_2), 133.4, 133.7, 133.5, 131.2, 129.3, 129.2, 128.9, 128.7, 128.6, 127.3 and 124.2 (aromatic), 98.7 (C-1), 92.7 (C-1'), 86.9 and 85.3 (acetylenic), 72.6 (C-3), 70.4 (C-5), 70.1 (C-2), 67.0(C-4'), 66.4 (C-3'), 66.9 (C-2' and C-5'), 65.2 (C-4), 61.9 (C-6), 60.3 (C-6'), 56.5 (C-1''), 49.4, 40.5, 39.0, 38.3 and 28.9 (-*C*H$_2$- from PAMAM), 20.6, 20.4, 20.2 and 20.1 (CH_3CO_2); ESI-MS calcd for $C_{430}H_{484}N_{26}O_{164}$ (M + $4H^+$): 2158.90; Found: 2159.40.

Compound G2 3-25. $[\alpha]_D$ 95.2° (*c* 1.5, $CHCl_3$); ^1H NMR (500 MHz, DMSO-*d6*) δ 8.59 (bs, 16H, amide), 8.08 (bs, 16H, amide), 7.96-7.98 (m, 16H, aromatic), 7.79 (8H, bd, *J* = 74, aromatic), 7.60-7.66 (8H, m, aromatic), 7.49-7.52 (m, 8H, aromatic), 7.34-7.49 (m, 16H, aromatic), 5.61 (4H, bs, H-4), 5.28 (4H, dd, *J* = 8.3, 9.8, H-2), 5.22 (1H, d, *J* = 2.7, H-1'), 5.06 (1H, d, *J* = 8.0, H-1), 5.03 (1H, dd, *J* = 2.7, 11.1, H-2'), 4.89 (1H, dd, *J* = 2.8,

11.1, H-3'), 4.76 (1H, bs, H-4'), 4.64 (2H, J = 16.5, H-1a''), 4.57 (2H, J = 16.5, H-1b''), 4.42 (1H, m, H-6a), 4.37 (1H, bd, J = 10.5, H-3), 4.30-4.34 (12H, m, H-6b, H-5), 3.93 (1H, t, J = 6.5, H-5'), 3.70 (8H, bd, J = 6.0, H-6'), 3.07-3.71 (112 H, m, -CH_2- from PAMAM), 2.45-2.99 (60 H, m, -CH_2- from PAMAM), 2.26 (56H, bs, -CH_2- from PAMAM), 2.17, 2.01, 1.99, 1.84 and 1.78 (each 12H, 5s, 5 CH_3CO_2); ^{13}C (125 MHz; DMSO-*d6*) δ_C 169.8, 169.7, 169.6, 169.3 and 169.2 (CH_3CO_2), 165.6, 165.3 and 164.8 (PhCO_2), 133.4, 133.7, 133.5, 131.2, 129.3, 129.2, 128.9, 128.7, 128.6, 127.3 and 124.2 (aromatic), 98.7 (C-1), 92.6 (C-1'), 86.9 and 85.3 (acetylenic), 72.5 (C-3), 70.3 (C-5), 70.1 (C-2), 67.0(C-4'), 66.3 (C-3'), 65.8 (C-2' and C-5'), 65.7 (C-4), 61.8 (C-6), 60.3 (C-6'), 56.4 (C-1''), 49.4, 40.4, 39,0 and 38.2 (-CH_2- from PAMAM), 20.5, 20.4and 20.1 (CH_3CO_2); MALDI-TOF MS calcd for $C_{878}H_{992}N_{58}O_{332}$ (M + 2Na$^+$): 8852.1; Found: 8851.5.

Preparation of 3-22, 3-24 and 3-26 using Zemplèn Reaction. General Procedure.

The fully protected glycocluster **3-21** (52 mg, 0.01 mmol) was dissolved into DMSO/methanol (4mL/1mL), to which was added a catalytic amount of sodium methoxide. The solution was stirred at room temperature for 48 h. After neutralization of sodium methoxide with diluted HCl, the solution was filtered through a cotton plug. After removal of methanol under reduced pressure, the resulting DMSO solution was dialyzed against DMSO/water (60/40) + 0.2% triethylamine (MW cutoff 1200 Da). Lyophilization afforded the fully deprotected cluster **3-22** as a white solid in 95%. For **3-24** and **3-26** the MW cutoff for dialysis tubing is 3500 Da.

Compound **3-24** and **3-26** were prepared in the same way in 91% and 90% yields, respectively.

Deprotected 4mer 3-22. $[\alpha]_D$ 0.0° (*c* 1.8, H_2O); ^1H (500 MHz; D_2O) δ_H 7.68 (4H, bs, aromatc), 7.52 (4H, bs, aromatic), 5.20 (4H, bs, H-1'), 4.81 (12H, m, H-1, H-1''), 4.22 (8H, bs, H-5 and H-5'), 4.04 (4H, bs, H-4'), 3.99 (4H, bd, *J* = 10.3, H-3'), 3.92 (4H, dd, *J* = 3.2, 10.3, H-2'), 3.73-3.83 (28H, m, H-2, H-3, H-4, H-6 and H-6'); 3.27-3.60 (72H, m, -C*H*$_2$- from PAMAM), 2.94-3.07 (64H, m, -C*H*$_2$- from PAMAM), 2.48-2.56 (32H, m, -C*H*$_2$- from PAMAM); ^{13}C (125 MHz; D_2O) δ_C 181.2, 172.9 and 168.6 (Ph*C*O-), 132.1, 131.2, 126.7 and 125.2 (aromatic),101.0 (C-1), 94.9 (C-1'), 86.4 and 85.4 (acetylenic), 77.0 (C-3), 74.5 (C-2), 70.4 (C-5 or C-5'), 68.8 and 68.7 (C-4, C-3' and C-4'), 67.7 (C-2'), 64.3 (C-5 or C-5'), 60.5 and 60.4 (C-6 and C-6'), 56.7 (C-1''), 50.7, 48.7, 41.1, 38.9, 38.3, 38.3, 36.3 and 28.5 (-*C*H$_2$- from PAMAM); ESI-MS calcd for $C_{110}H_{152}N_{10}O_{52}$ ($M + Na^+$): 2467.93; Found: 2467.56.

Deprotected 8mer 3-24. $[\alpha]_D$ 0.0° (*c* 1.8, H_2O); ^1H (500 MHz; D_2O) 7.367 (16H, bs, aromatc), δ_H 7.53 (16H, bs, aromatic), 5.20 (8H, bs, H-1'), 4.80 (24H, m, H-1, H-1''), 4.23 (16H, bs, H-5 and H-5'), 4.06 (8H, bs, H-4'), 3.99 (8H, bd, *J* = 10.4, H-3'), 3.92 (8H, dd, *J* = 3.6, 10.4, H-2'), 3.77-3.83 (56H, m, H-2, H-3, H-4, H-6 and H-6'); 3.27-3.60 (38H, m, -C*H*$_2$- from PAMAM), 2.94-3.07 (32H, m, -C*H*$_2$- from PAMAM), 2.48-2.56 (32H, m, -C*H*$_2$- from PAMAM); ^{13}C (125 MHz; D_2O) δ_C 181.2, 172.9 and 168.8 (Ph*C*O-), 132.8, 131.4, 126.7 and 125.0 (aromatic),101.0 (C-1), 94.9 (C-1'), 86.5 and 85.4 (acetylenic), 77.0 (C-3), 74.5 (C-2), 70.4 (C-5 or C-5'), 68.9 and 68.8 (C-4, C-3' and C-4'), 67.8 (C-2'), 64.3 (C-5 or C-5'), 60.5 and 60.4 (C-6 and C-6'), 56.7 (C-1''), 49.1, 38.8, 38.3, 38.3, 37.9 and 28.9 (-*C*H$_2$- from PAMAM); ESI-MS calcd for $C_{238}H_{336}N_{26}O_{108}$ ($M + 2Na^+$): 2666.05; Found: 2666.34.

Deprotected 16mer 3-26. $[\alpha]_D$ 0.0° (*c* 1.8, H_2O); ^1H (500 MHz; D_2O) δ_H 7.67 (32H, bs, aromatc), 7.51 (32H, bs, aromatic), 5.20 (16H, bs, H-1'), 4.80 (48H, m, H-1, H-1''),

4.23 (32H, bs, H-5 and H-5'), 4.04 (16H, bs, H-4'), 3.99 (16H, bd, J = 10.3, H-3'), 3.91 (16H, dd, J = 3.2, 10.3, H-2'), 3.73-3.83 (112H, m, H-2, H-3, H-4, H-6 and H-6'); 3.27-3.60 (72H, m, -CH_2- from PAMAM), 2.94-3.07 (64H, m, -CH_2- from PAMAM), 2.48-2.56 (32H, m, -CH_2- from PAMAM), ^{13}C (125 MHz; D_2O) δ_C 181.2, 172.9 and 168.6 (PhCO-), 132.9, 131.4, 126.7 and 125.0 (aromatic),101.0 (C-1), 94.9 (C-1'), 86.4 and 85.4 (acetylenic), 77.0 (C-3), 74.5 (C-2), 70.4 (C-5 or C-5'), 68.8 and 68.7 (C-4, C-3' and C-4'), 67.7 (C-2'), 64.3 (C-5 or C-5'), 60.5 and 60.4 (C-6 and C-6'), 56.7 (C-1''), 50.7, 48.7, 41.1, 38.9, 38.3, 38.3, 36.3 and 28.5 (-CH_2- from PAMAM); MALDI-TOF MS calcd for $C_{494}H_{704}N_{58}O_{220}$ (M + $2Na^+$): 5507.2; Found: 5506.8.

3.6. References

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CHAPTER 4 SYNTHESIS OF GALECTIN INHIBITORS

4.1. Introduction

The wide occurrence of β-D-galactopyranoside ligands on mammalian glycoproteins and glycolipids having only subtle differences in topologies and structures is a puzzling phenomena that has to be addressed in the design of potent selective inhibitors.[1] This fact is particularly important if the drug targeting to the C-type lectins, asialoglycoprotein receptors of hepatocytes or macrophages,[2] is not to be confounded by that to the soluble S-type lectins such as the galectin family.[3] This type of difficulty would often occur, for instance, the blockage of the apoptotic behavior of galectin-3 was the target receptor,[4] then their rapid clearance by the liver/macrophages would be obstacles to surmount. Moreover, the increasing body of evidence for the distinctive functions of the galectins themselves,[5] all binding to some undefined β-D-galactoside ligands, clearly underscore our need to understand multiple but selective binding interactions.[6] Given the success of multivalent glycans as powerful inhibitors of carbohydrate-protein interactions, the next strategy ought to address the selectivity issues. In fact, few recent studies point towards the rational understanding of multiple receptor-multivalent glycan arrays of interactions.[7,8] Trivalent Gal/GalNac clusters have been shown to markedly distinguish between C-type lectins and galectins.[9]

As mentioned before, galectins belong to a family of Ca-independent lectins with at least 10 members (Fig. 4-1), based on their structural differences and binding properties.[3-5] Of these galectin-1, a prototype homodimer, and galectin-3, a chimera-type monomer have been extensively studied.[4] Galectins perform a variety of biological functions such as

modulating cell adhesion, regulating cell growth,[10-12] and promoting or inhibiting cell apoptosis and metathesis[13-15] (See Fig. 4-2).[16] Since galectin-1 and galectin-3 are abundantly expressed in many tumor cells and are closely related to the development of tumors,[4] this property along with the high affinity of galectins towards terminal β-galactose-containing glycoforms[17,18] promoted us to synthesize galactose-based clusters as probes to study the biological behaviors of galectins.

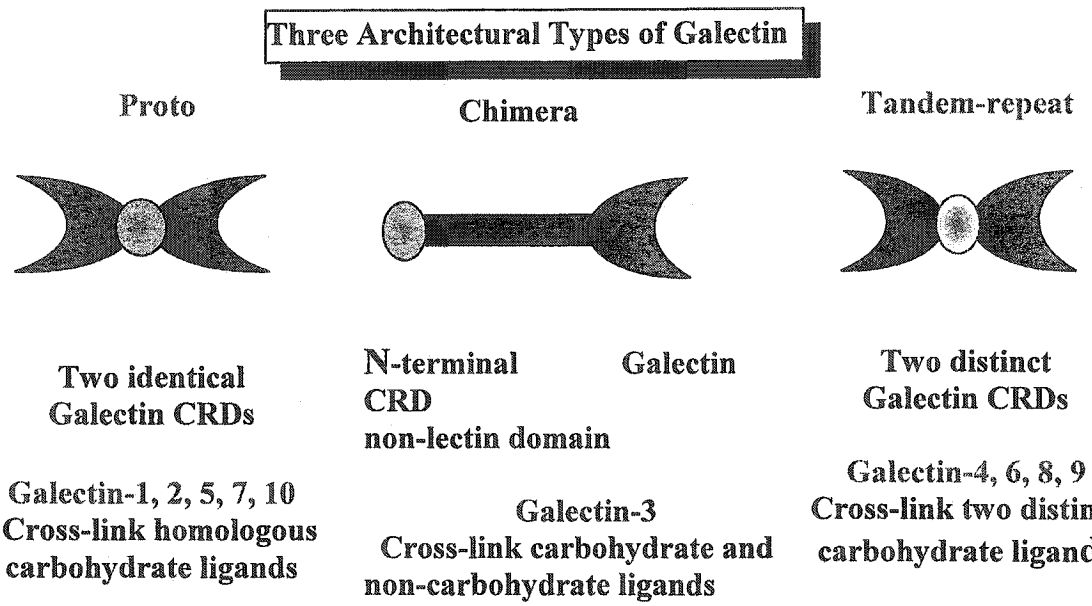
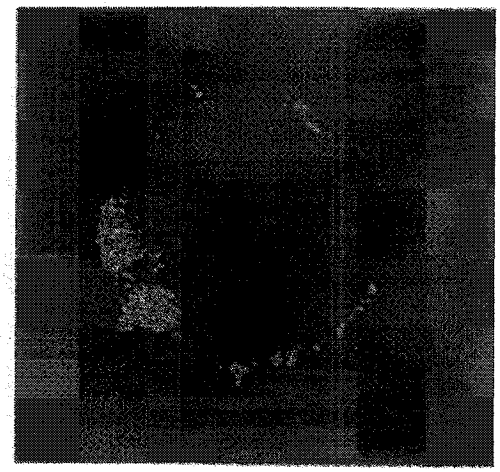


Fig. 4-1. Structures of Galectins.

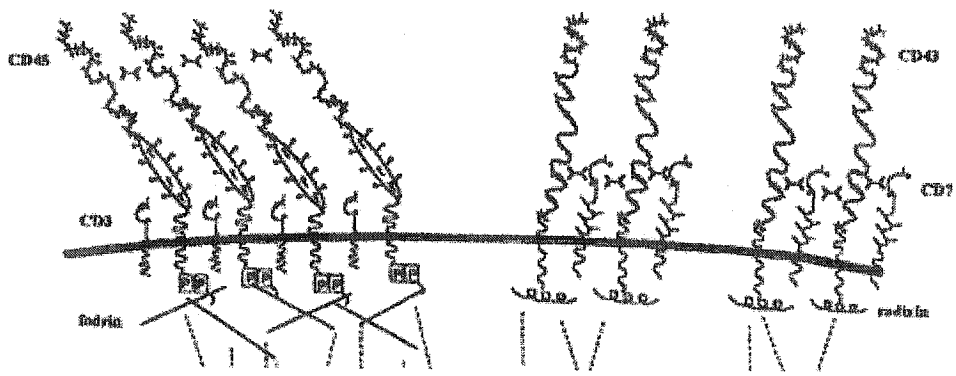
In this chapter, strategies towards the design and synthesis of galactose and lactose clusters having "rigidified" core structures will be discussed.[19] The rational for conformationally restrained clusters is based on the expectation that more rigid structures would be less entropically penalized upon binding to their counter receptors. Additionally, using organometallic cross-coupling chemistry allows for the introduction of more lipophilic

linkers, thus potentially limiting solvating water molecules. The use of palladium(0) catalyzed cross-coupling between 2-propynyl galactoside and lactoside and a wide range of aryl iodide scaffolds will be described. The biological activities of these synthetic glycoclusters will also be briefly illustrated.

Control: Random CD45CD43



Galectin-1 induces patches of CD45/CD3 (Red) and CD43/CD7 [MOLT-4 cells] (Green)



Taken from Baum *et al. Biochemistry*, **2001**, *40*, 3013.

Fig. 4-2. Schematic diagram of galectin-1 induced apoptosis of human T-cells.

4.2. Results and Discussion

4.2.1. Synthesis of β-D-Galactoside-Containing Glycoclusters

From the X-ray structure of the binding between an octasaccharide with galectin-1, it is clear that the terminal galactose plays the major role in binding, all the other sugars serve as a spacer (Fig. 4-3).[20] Thus, in this section, the synthesis of β-D-galactoside-containing glycoclusters will be addressed.

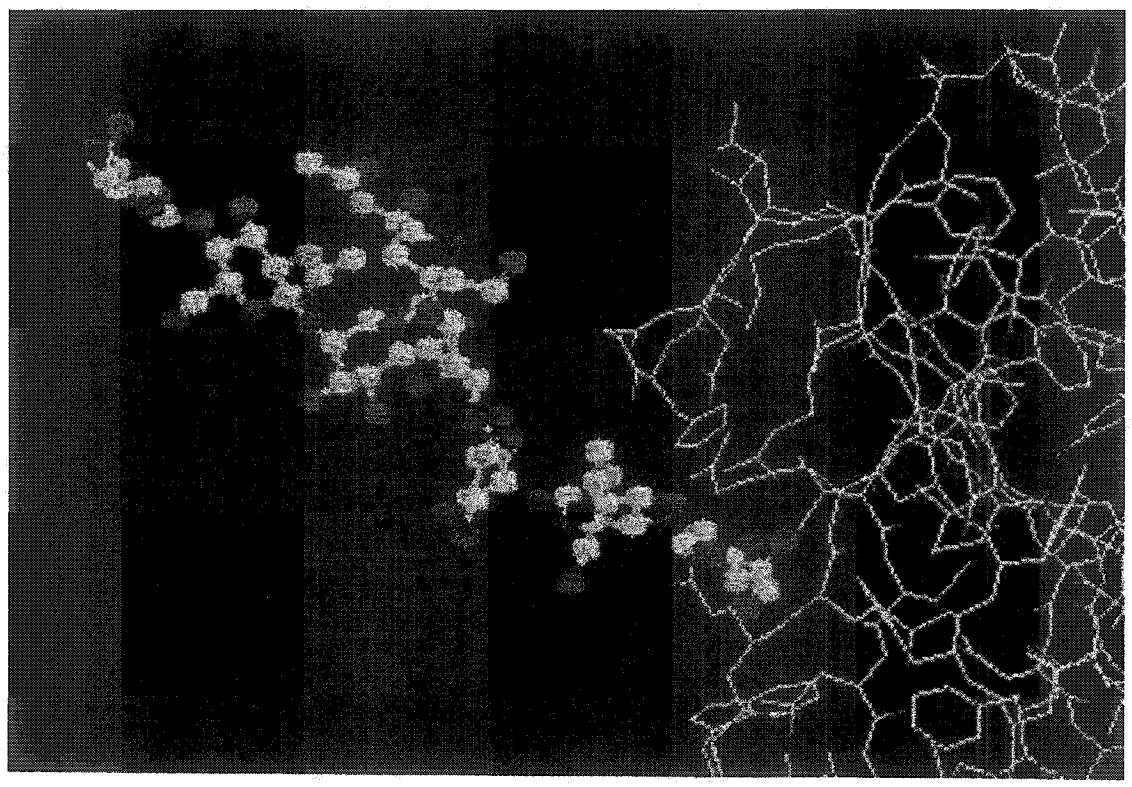
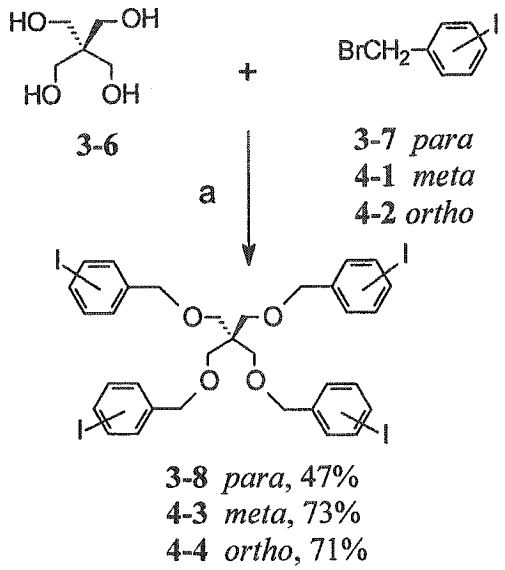


Fig. 4-3. Binding between an octasaccharide and galectin-1.[20]

Traditionally, the Sonogashira reaction is carried out using CuI as a co-catalyst.[21] However, it was observed that in the presence of catalytic amount of CuI, the cross-coupling reaction also catalyzed the oxidative homocoupling of terminal alkynes at room temperature,[22,23] while this latter reaction did not proceed or proceeded very slowly without CuI. To explore the role of CuI in the Sonogashira reaction in carbohydrate chemistry, the reaction was performed at high temperature and it was found that in the absence of CuI the formation of homodimers was greatly inhibited or abolished.[19,24,25]

Syntheses of pentaerythritol based cores. Due to its tetrahedral geometry pointing toward all directions, pentaerythritol **3-6** can provide reasonable inter-carbohydrate distances for the purpose of studying sugar-protein binding. Thus, pentaerythritol was chosen as the basic core for the preparation of fully iodobenzylated ethers. The rationale for the use of *para-*, *meta-* and *ortho-* iodobenzyl bromides **3-7**, **4-1** and **4-2** was based on the fact that after the Sonogashira reaction, the inter-sugar distances and relative orientations would be varied which in turn provided a panel for the exploration of sugar-protein recognition, analogous to that provided by the glycoforms seen in multi-antennary glycoproteins.

Treatment of pentaerythritol with NaH, iodobenzyl bromides (**4-1**, **4-2**), and a catalytic amount of tetrabutylammonium iodide in DMF, provided *meta-* and *ortho-* iodobenzyl precursors **4-3** and **4-4** in yields of 73% and 71%, respectively (Scheme 4-1). The yield for the formation of the *para-* derivative **3-8** was however moderate (47% yield)[26].

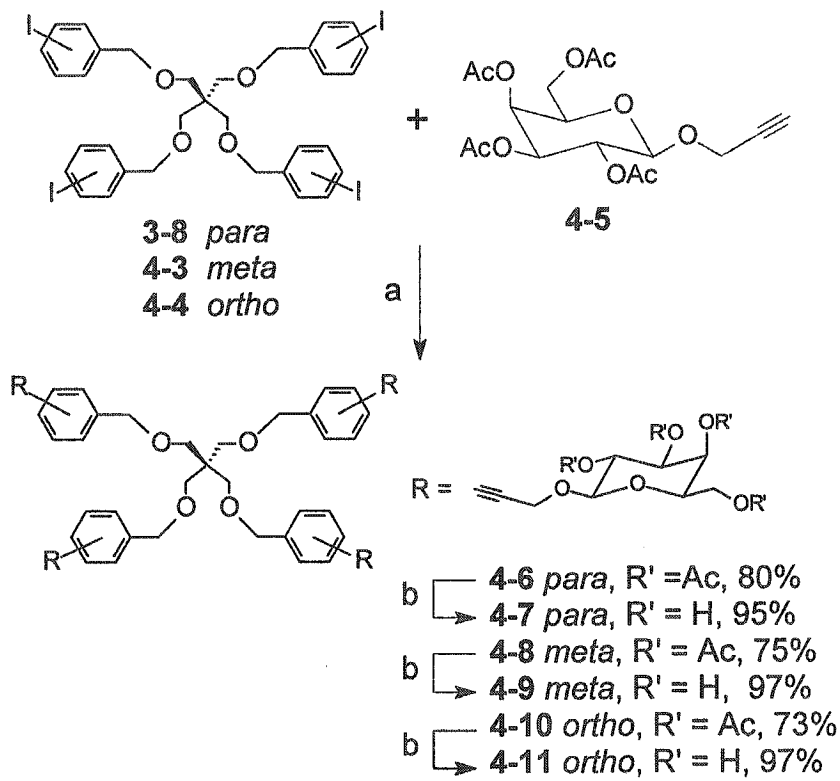


Scheme 4-1. Synthesis of pentaerythritol-based cores. (a) NaH, TBAI, DMF, rt, 5 h.

Syntheses of β-D-Gal-containing clusters. In this event, 2-propynyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside **4-5**[27] was used in the synthesis. The preparation of the tetrameric glycoclusters was effected using the Sonogashira reaction protocol described above without CuI. Treatment of **4-5** with iodobenzyl precusors **3-8**, **4-3**, or **4-4** provided their corresponding tetrameric glycoclusters **4-6** (see Fig. 4-5 for the NMR spectrum), **4-8** (see Fig. 4-6 for the NMR spectrum, and Fig. 4-4 for structure), and **4-10** in good yields (Scheme 4-2). Transesterification of **4-6**, **4-8**, and **4-10** under Zemplèn conditions (NaOMe, MeOH) resulted in the fully deprotected glycoclusters **4-7**, **4-9** (Fig. 4-4), and **4-11** in yields of 95%, 97%, and 97%, respectively.

The synthesis of the β-D-Gal trimer **4-12** was carried out in the same manner (Scheme 4-3). The cross coupling between 1,3,5-triiodobenzene **3-3**[28] and **4-5** provided a rigid structure, thus giving access to a good model for the study of the relationship between rigidity and binding behavior. Treatment of **3-3** with **4-5** catalyzed by $(PPh_3)_2PdCl_2$ in DMF

and triethylamine provided **4-12** in 85% yield. Complete deprotection of **4-12** with a catalytic amount of sodium methoxide in methanol as above generated **4-13** in 95% yield.



Scheme 4-2. Syntheses of tetrameric β-D-Gal-containing clusters. (a) $(PPh_3)_2PdCl_2$, DMF:TEA (1:1), 60 °C, 5 h; (b) NaOMe, MeOH, 24 h.

In summary, a convenient route towards the synthesis of glycoclusters with different geometries and shapes for the investigation of multiple carbohydrate-protein interactions using the Sonogashira reaction has been reported. Unfortunately, these compounds were found to be insufficiently soluble in water to justify further bilogical investigations after deprotection. The lack of water solubility of **4-7**, **4-9**, **4-11** and **4-13** is in striking contrast to

previously described glycoclusters bearing α-Gal epitope in which the 'extra' α-galactoside residue obviously conferred the desired solubility.

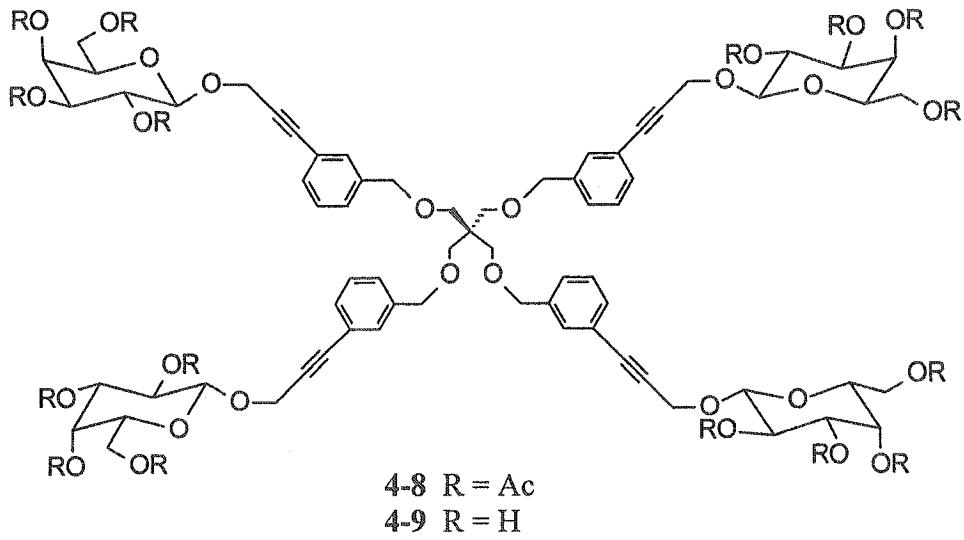
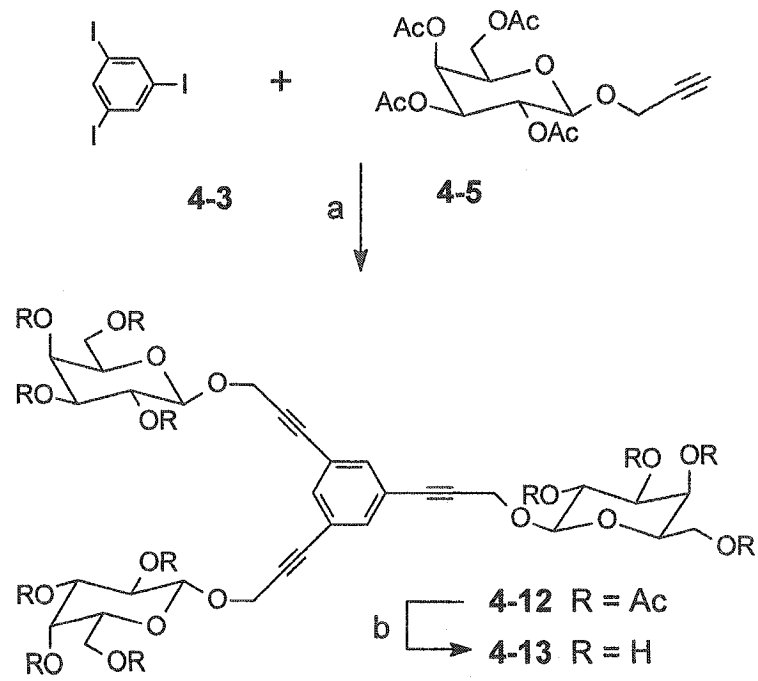


Fig. 4-4. Structure of compounds **4-8** and **4-9**.



Scheme 4-3. Synthesis of trimeric β-D-Gal-containing clusters. (a) $(PPh_3)_2PdCl_2$, DMF:TEA (1:1), 60 °C, 85%, 5 h; (b) NaOMe, MeOH, 24 h, 95%.

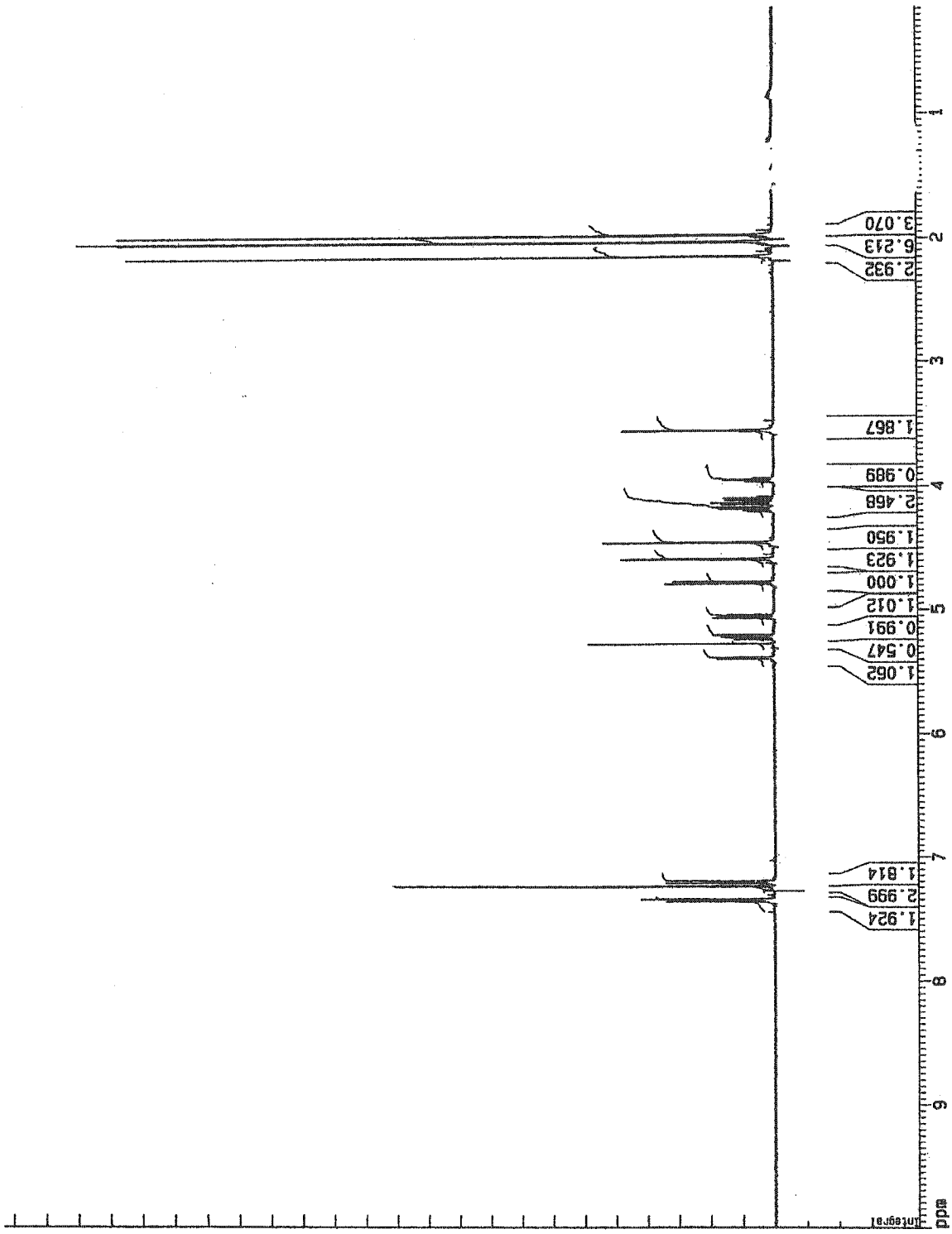


Fig. 4-5. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **4-6**.

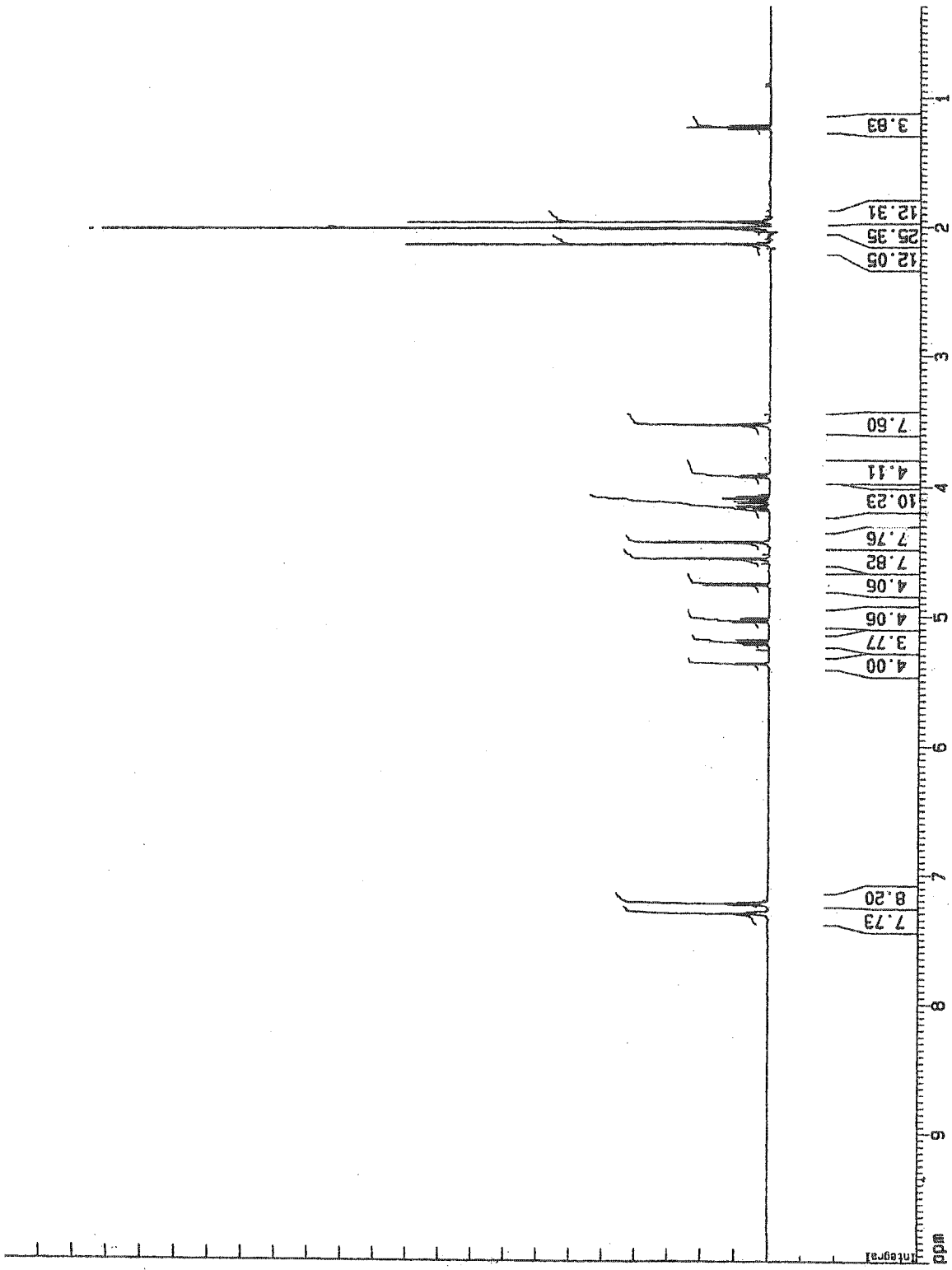


Fig. 4-6. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound 4-8.

4.2.2. Synthesis of Lactoside-Bearing Glycoclusters

Since the poor solubility of the galactose clusters was due to the fact that the sugar moieties could not compensate for the high hydrophobicity of the aryl-alkynyl domain, our attention was turned to lactose disaccharide (Galβ1-4Glcβ-OR). After all, a recent report also illustrated the preferred binding requirement of Galectin-1 toward lactose over single galactose, thus supporting the need for this investigation.

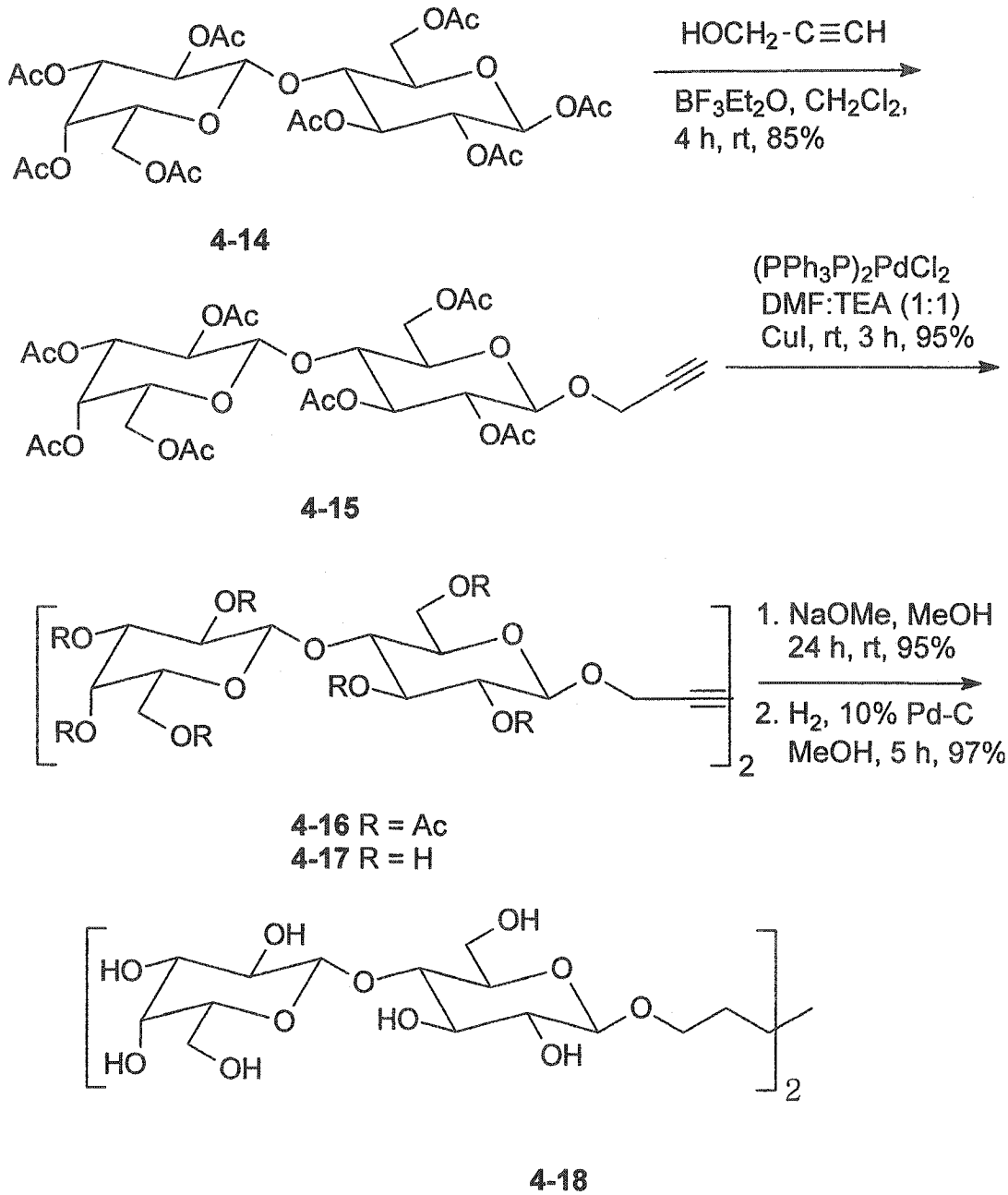
The synthesis of the required 2-propynyl lactoside peracetate **4-15** (see Fig. 4-7 for NMR) is described in Scheme 4-4. The procedure simply involved the treatment of lactose octaacetate **4-14** with propargyl alcohol and a Lewis acid, preferably boron trifluoride etherate, in dichloromethane at room temperature (85%)[29]. This protocol is applicable to a wide range of other glycosyl acetates.[25] Before attempting the cross-coupling reactions with various aryl iodides, lactoside **4-15** was treated under oxidative homocoupling condition to provide dimer **4-16** in 95% yield.[24,26] Although the homocoupling could be achieved under simpler but harsher conditions (Glazer[30] (Cu(I), O_2) or Eglinton[31] (Cu(II) processes), the reactions were carried out under milder Sonogashira conditions[32] using dichlorobis(triphenylphosphine)palladium(II) ($(Ph_3P)_2PdCl_2$), CuI in a one to one mixture of DMF and trimethylamine at room temperature. However, the choice of the catalysts was not critical since other palladium catalysts such as $(Ph_3P)_4Pd$ or $Pd_2(dba)_3$ were equally effective. Formation of homodimers is a typical side-reaction of the Sonogashira coupling, especially when cupper (I) salts are present. It provides, however, milder reaction conditions.

Complete de-*O*-acetylation of dimer **4-16** under Zemplèn conditions (NaOMe, MeOH) afforded water-soluble di-lactoside **4-17** in 95% yield. Hydrogenation of the

conjugated triple bonds with 10% palladium on charcoal provided dimer **4-18** in essentially quantitative yield. It is worth mentioning that although dimers such as **4-18** could be directly obtained by bis-glycosylation of 1,6-hexanediol, the yield is usually much lower. Because compound **4-16** was expected as a side-product from the next cross-coupling reactions, it served as a valuable comparative element.

The other cross-coupling reactions were all effected using the protocol described above for **4-16**. The simultaneous cross-coupling of 2-propynyl lactoside **4-15** with 1,4-diiodobenzene **4-19**, 1,3,5-triiodobenzene **3-3**, and to both pentaerythritol tetrakis(*meta*- and *para*-iodobenzyl) ether **4-3** and **3-8** provided a very efficient entry into this novel family of carbohydrate clusters **4-20** (dimer 90%) (see Fig. 4.8 for the NMR spectrum), **4-23** (trimer, 80%) (see Fig. 4-9 for the NMR spectrum), and tetramer **4-25** (*meta*) and **4-27** (*para*) (see Fig. 4-10 for the NMR spectrum), in 78 and 75% yields, respectively (Scheme 4.5-4.7).[33,34] As described above, complete de-*O*-acetylation of the clusters were uneventful and afforded completely water-soluble clusters **4-21**, **4-24**, **4-26** and **4-28** (see Fig. 4-11 for the NMR spectrum), in more than 90% yields. As expected, the solubility of these lactosylated clusters is in striking contrast to those observed for the corresponding β-D-galctoside clusters.[33]

While dimer **4-17** and **4-21** can be further hydrogenated, the benzylic nature of the remaining tetrakis ether **4-26** and **4-28** prevents such treatment since the pentaerythritol moieties would be cleaved. Thus, catalytic hydrogenation of dimesr **4-17** and **4-22** under standard conditions (H_2, 10% Pd-C, MeOH) afforded dimers **4-18** and **4-22** in excellent yields which possesses more flexible arms in comparison to the their original dimers.



Scheme 4-4. Synthesis of divalent lactoside using alkyne homocoupling.

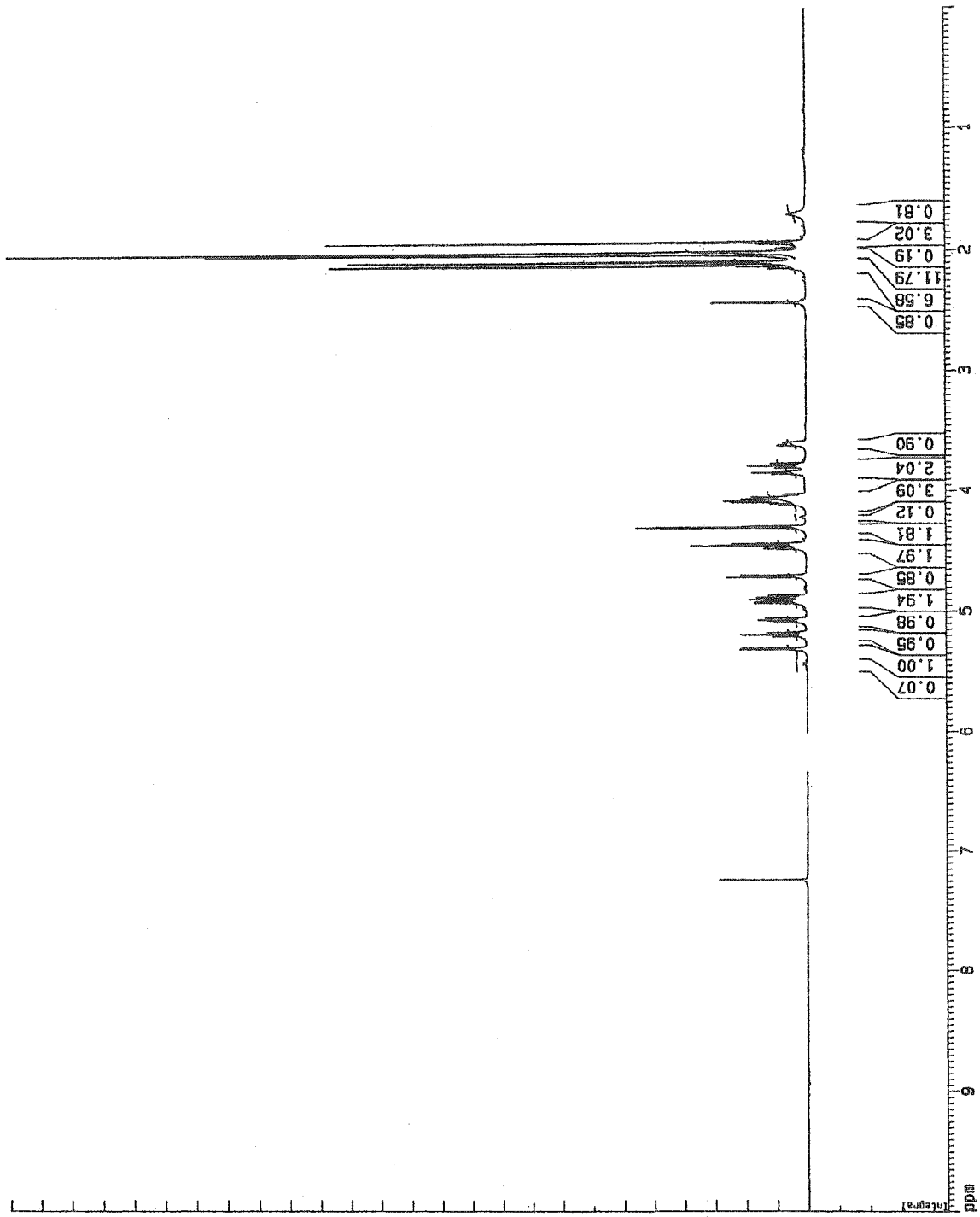


Fig. 4-7. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **4-15**.

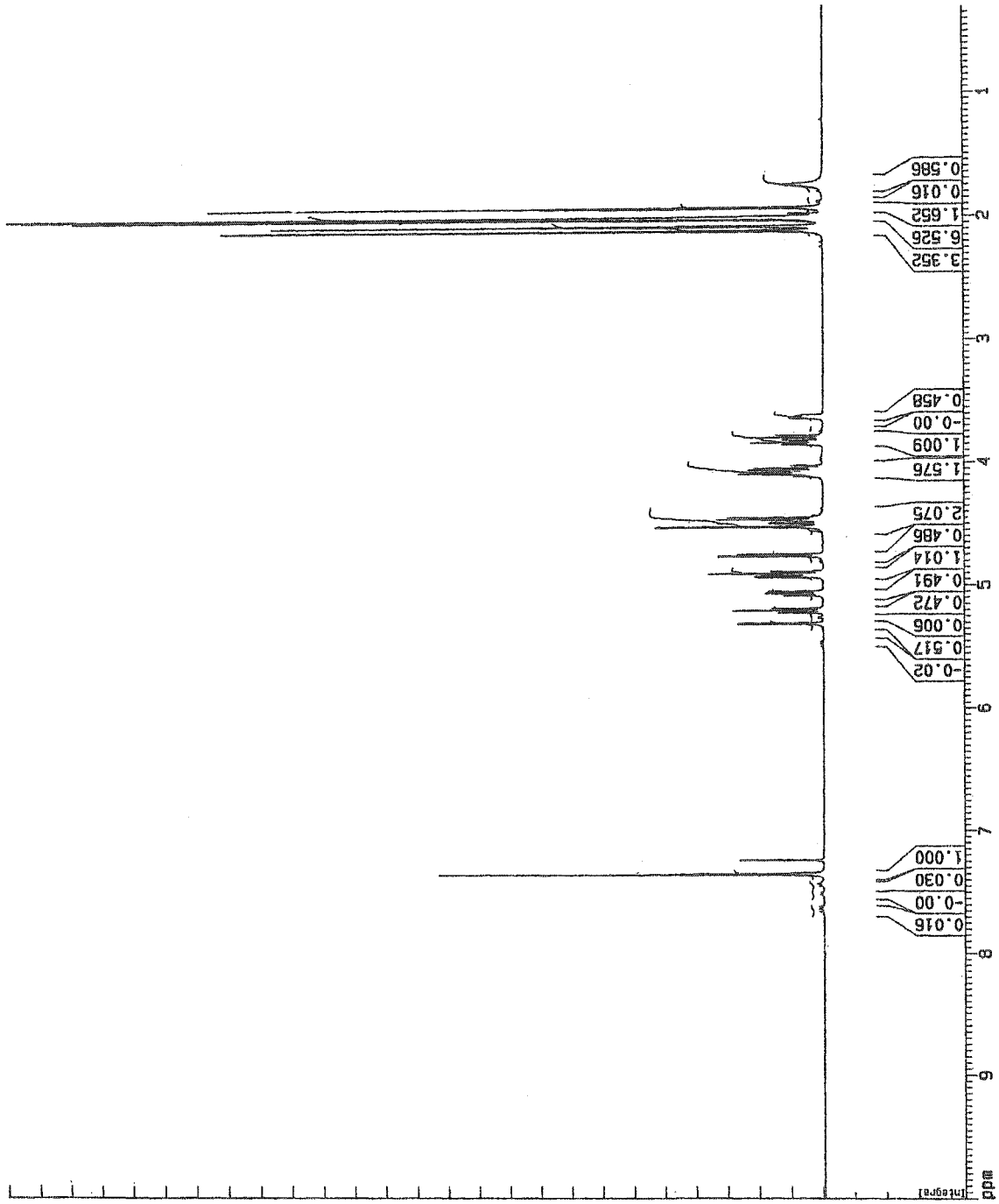
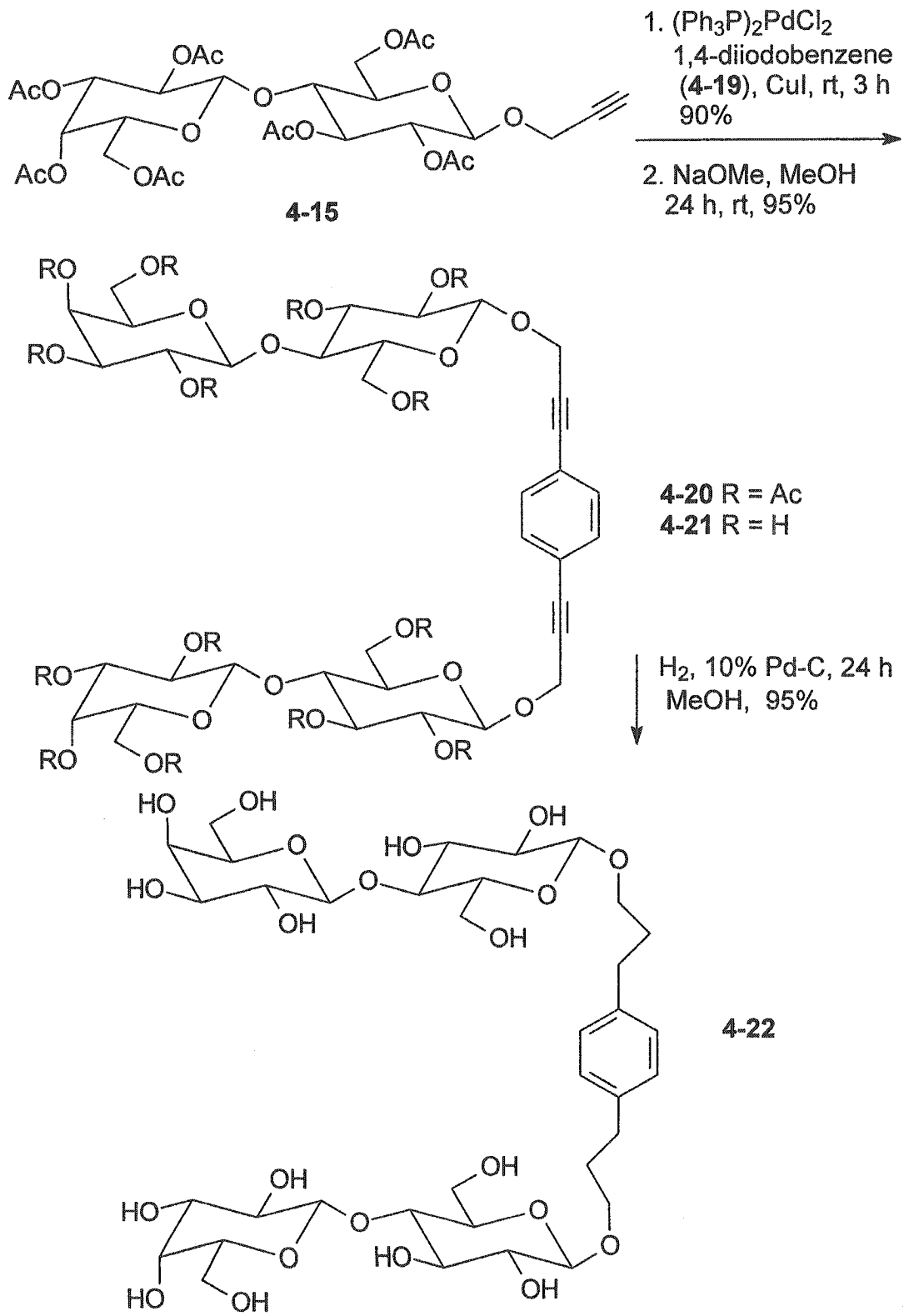
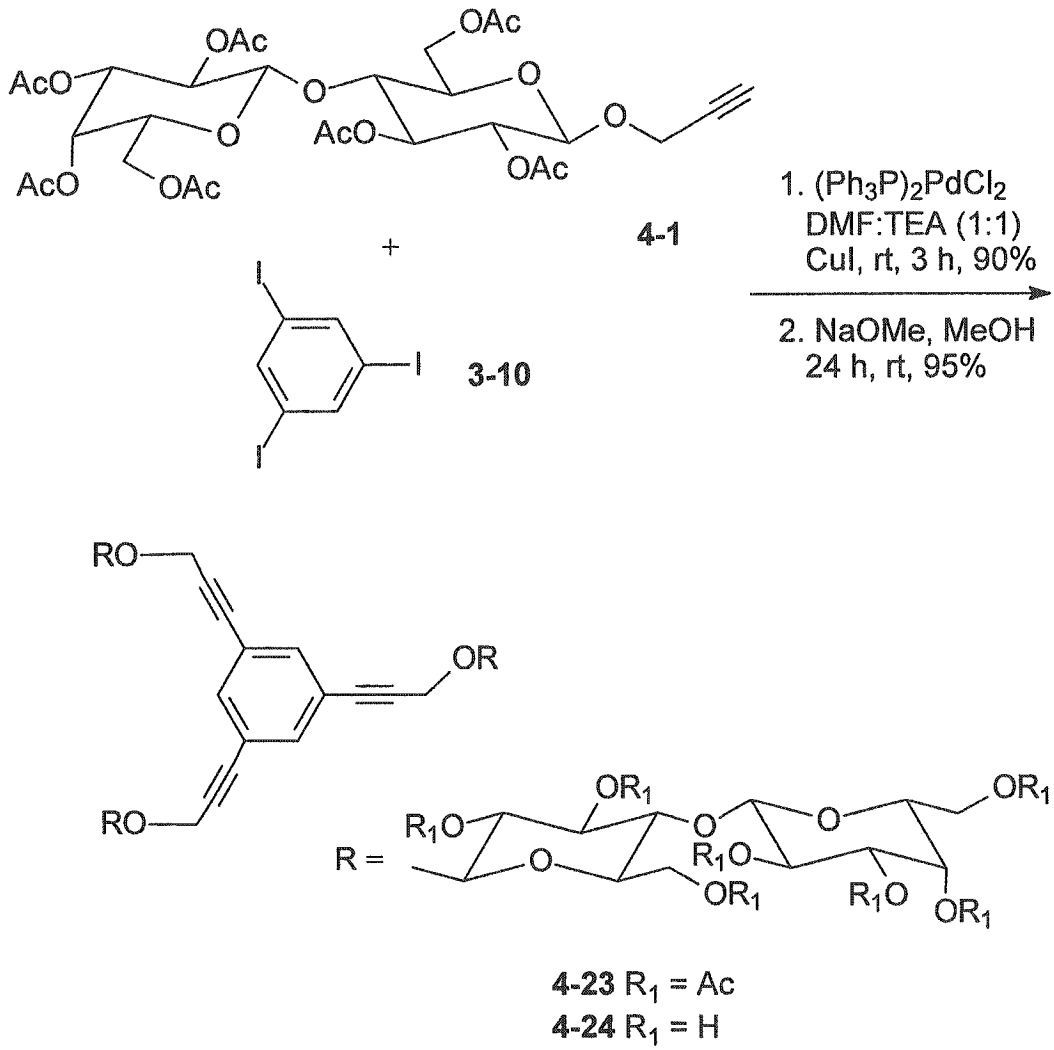


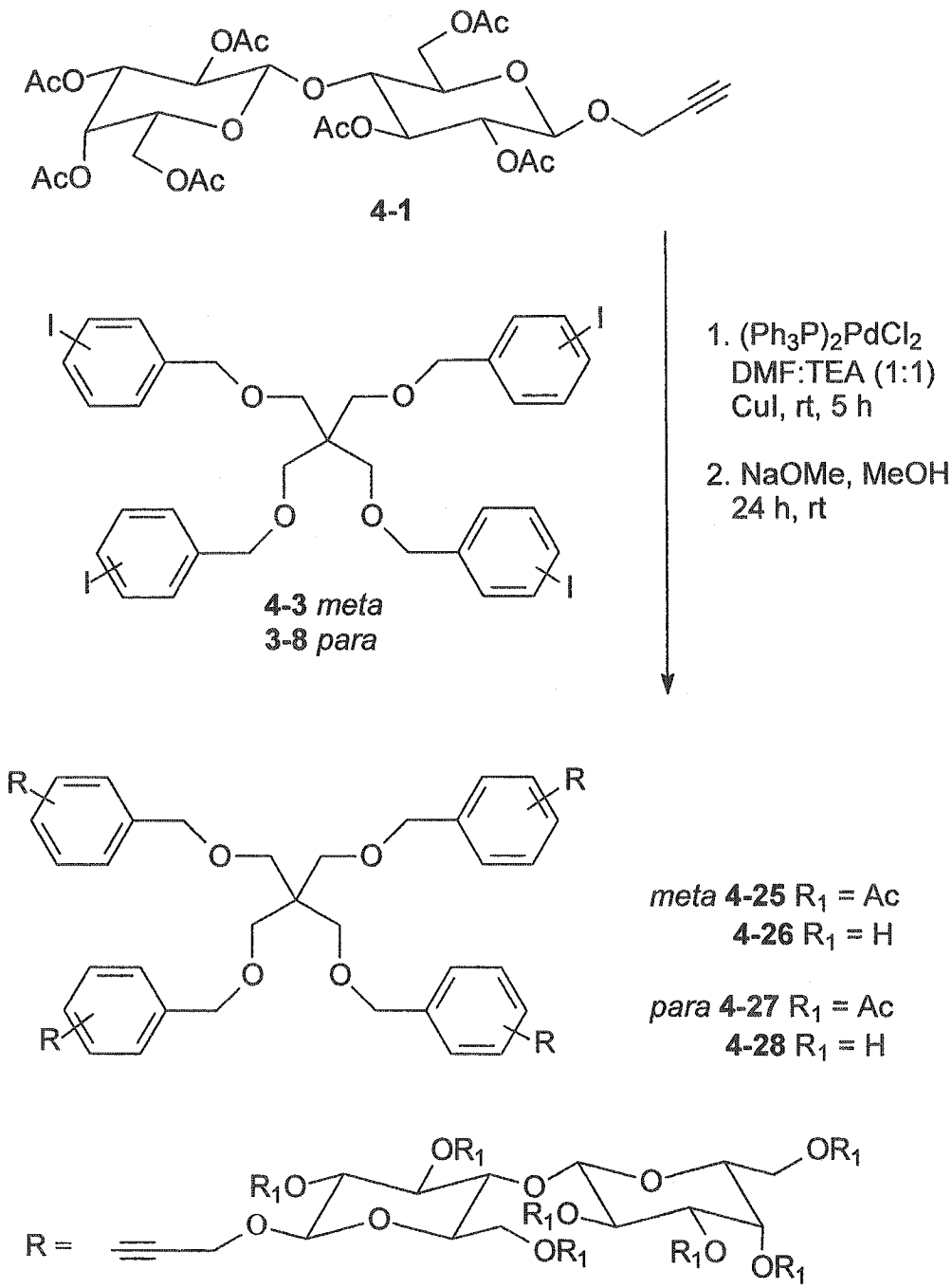
Fig. 4-8. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **4-20**.



Scheme 4-5. Synthesis of divalent lactosides using 1,4-diiodobenzene.



Scheme 4-6. Synthesis of trivalent lactoside.



Scheme 4-7. Synthesis of tetravalent lactosides.

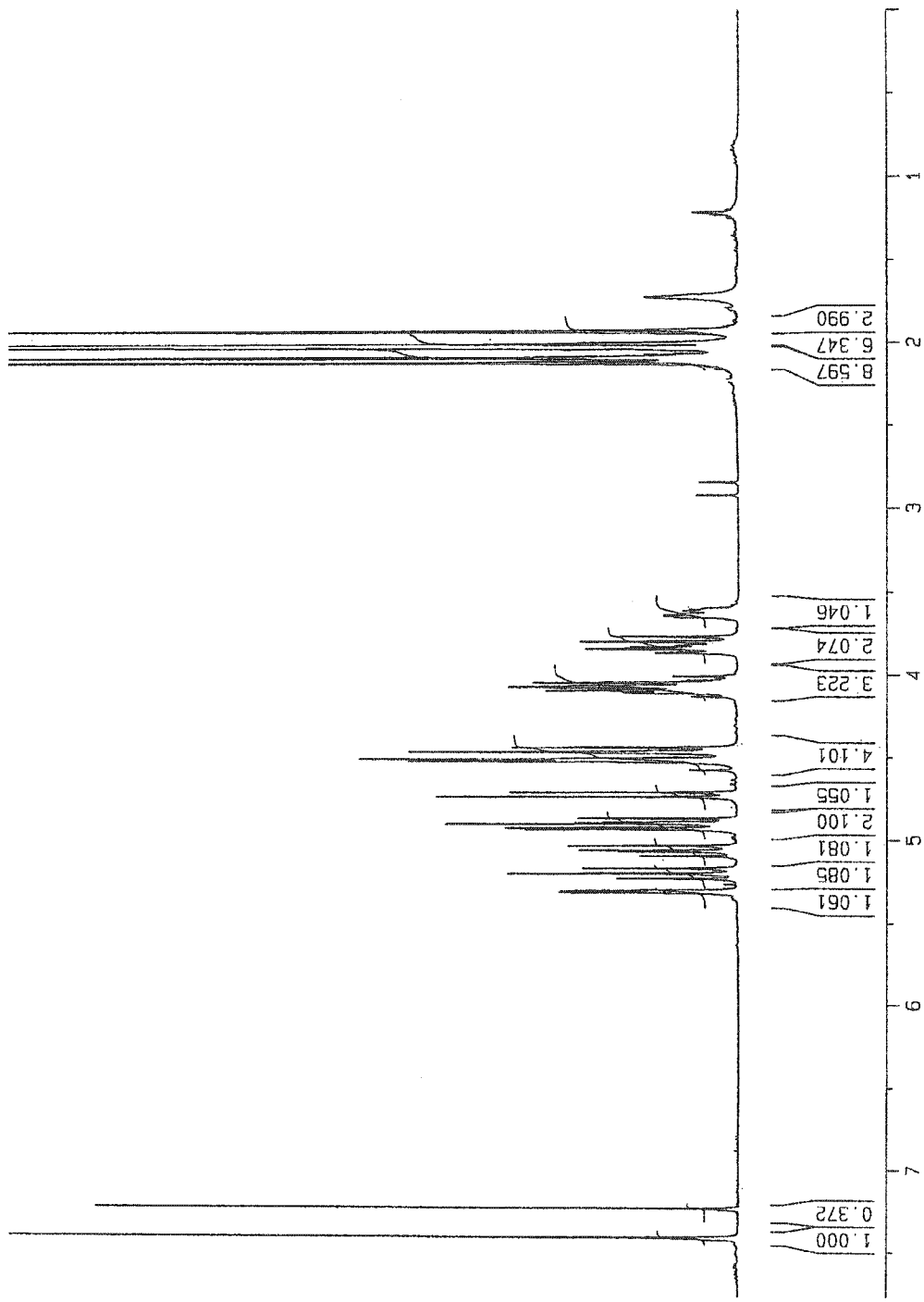


Fig. 4-9. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **4-23**.

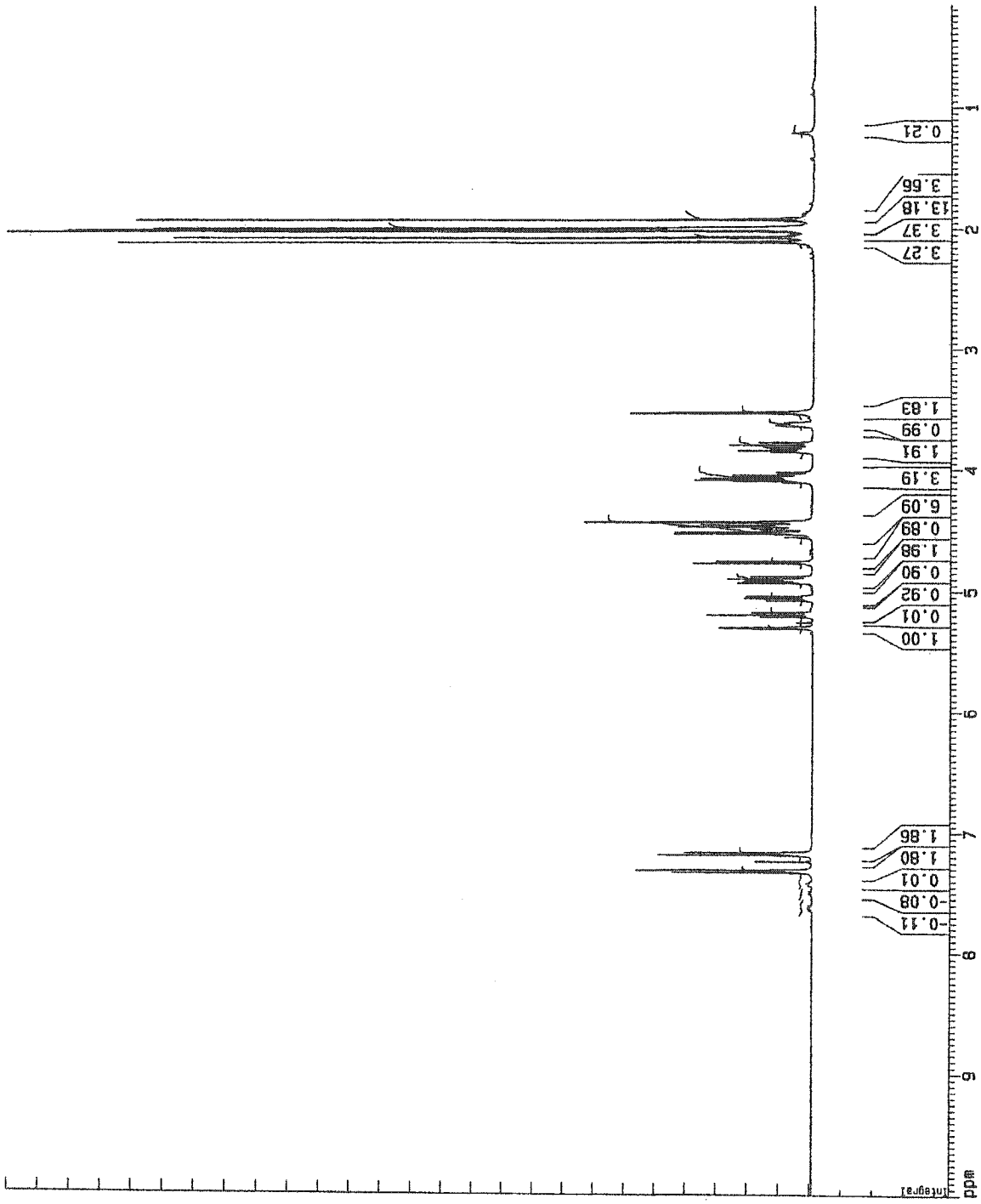


Fig. 4-10. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **4-27**.

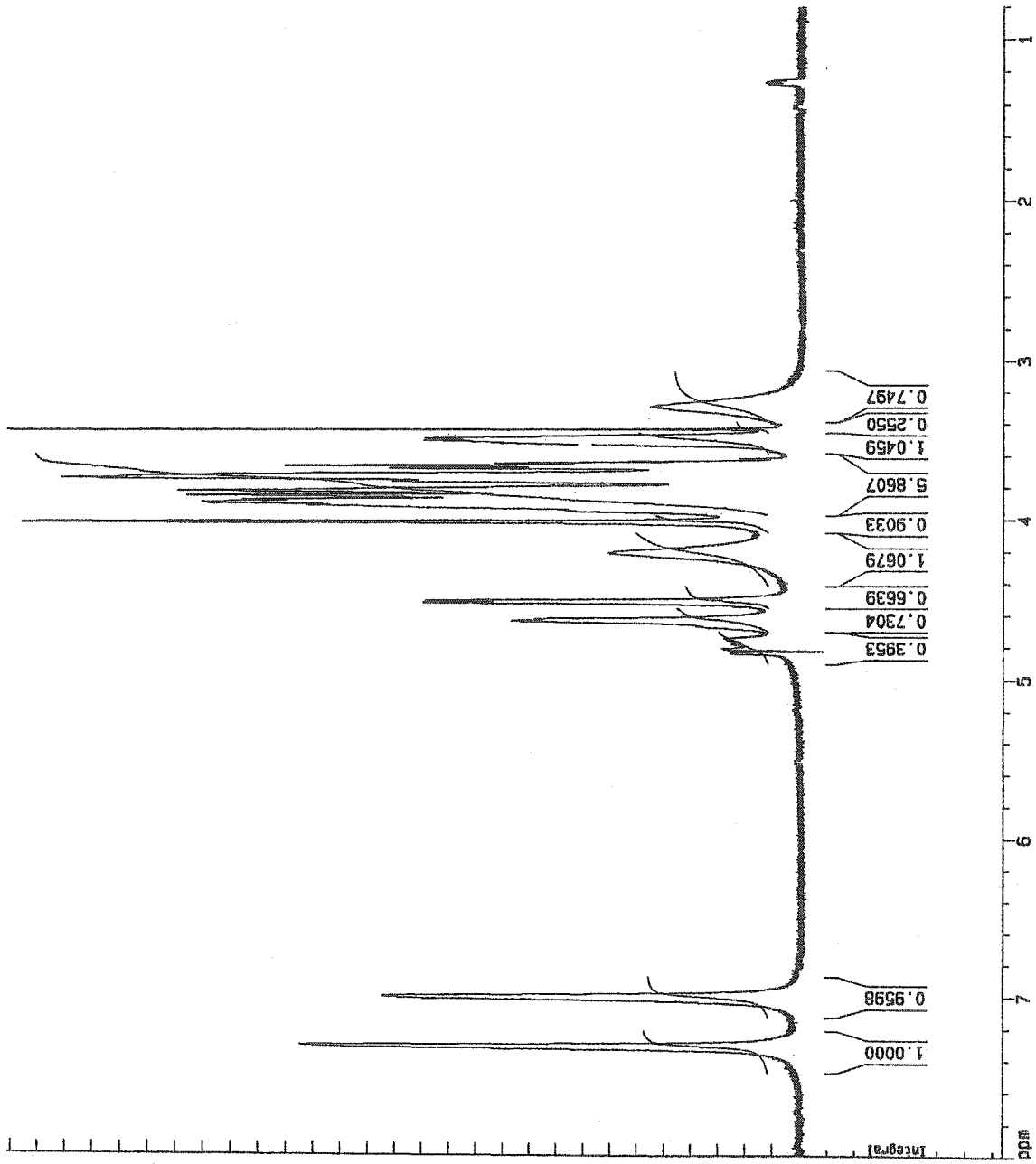


Fig. 4-11. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **4-28**.

4.3. Biochemical Assays

Initially, isothermal titration microcalorimetry (ICT) test[35] was carried out to measure the thermodynamic values (such as ΔG, ΔH and ΔS) from the binding between galectins and our synthetic lactose clusters. However, once the glycoclusters and galectins were mixed together, they cross-linked with each other and formed precipitates very rapidly, which did not allow successful determination of those thermodynamic factors. This is the first time researchers observed this phenomenon for galectin-3. Kinetic precipitation experiments demonstrated that galectin-3 cross-linked with lactosides **4-17**, **4-21**, **4-22**, **4-24**, **4-26**, **4-28** to precipitate out in about 200 seconds (Fig. 4-12). Interestingly, when the concentrations of galectin-3 and the two tetramers (*para* and *meta*) reached 1:1 ratio (50 mM : 50 mM), the yields of precipitates were 100% and 76% precipitation, respectively. In solution, the monomeric galectin-3 exists as two conformers, conformer 1 with a twisted "tail" and conformer 2 with a straight "tail" (Fig. 4-13). While conformer 2 is also in an equilibrium with a soluble tetramer, which upon exposure to the synthetic lactose cluster, would form a high molecular-weight cross-linked complex insoluble in solution. From molecular modeling, it is clear that the tetrameric cluster **4-28** takes the shape of a tetrahedron (Fig. 4-14) with the four sugar moieties pointing toward four different directions which forms a perfect 3-D lattice with the tetrameric galectin-3. Based on this result, it can be surmised that these synthetic lactose clusters may be useful probes in the study of the biological behaviors of galectin-3. They might also be a potential therapeutics for the treatment of tumor cells since galectin-3 is related to cell metastasis.

Kinetic precipitation experiments of Galectin-3 with Lactoside Clusters 2-4

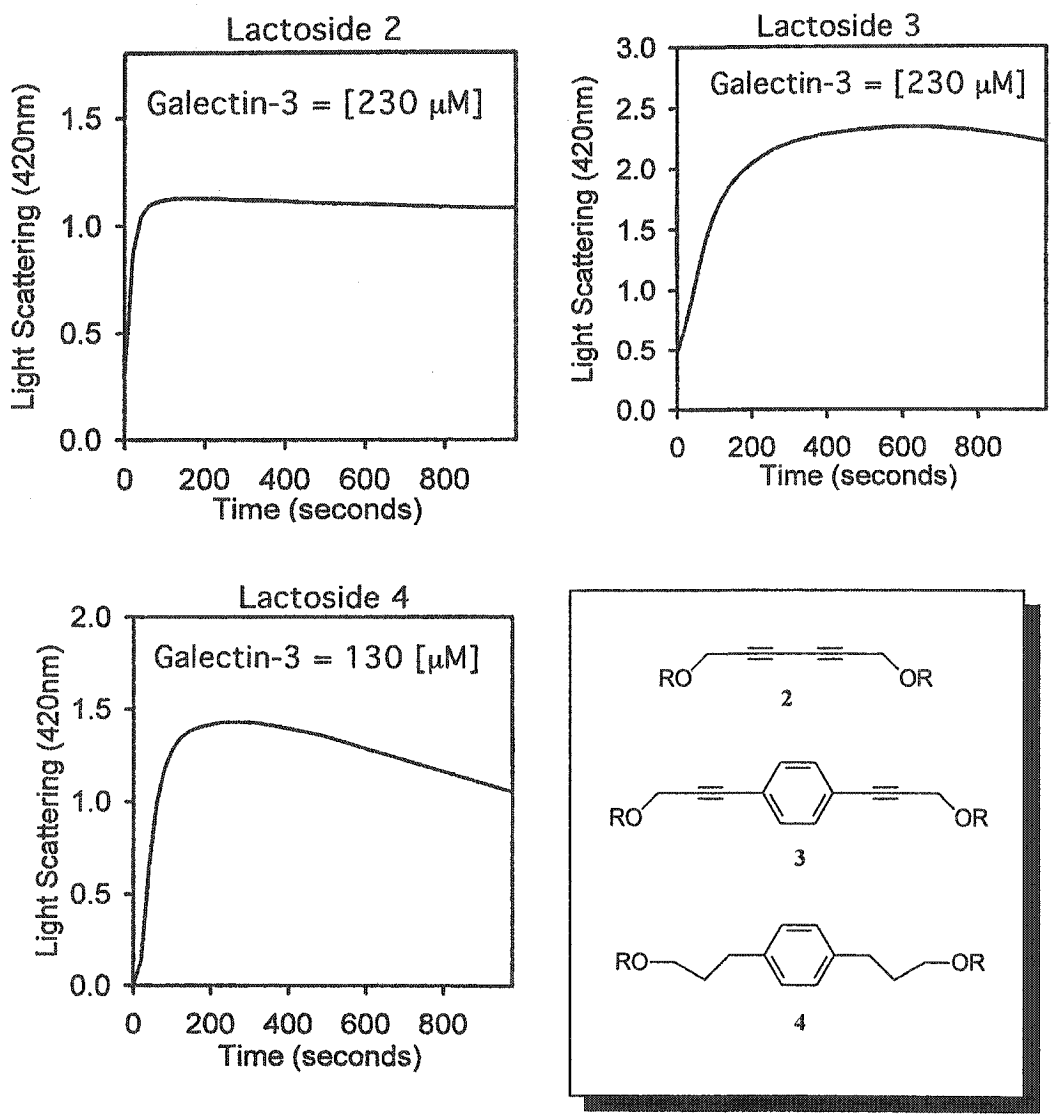


Fig. 4-12. Kinetic precipitation experiments of galectin-3 with synthetic lactosides.

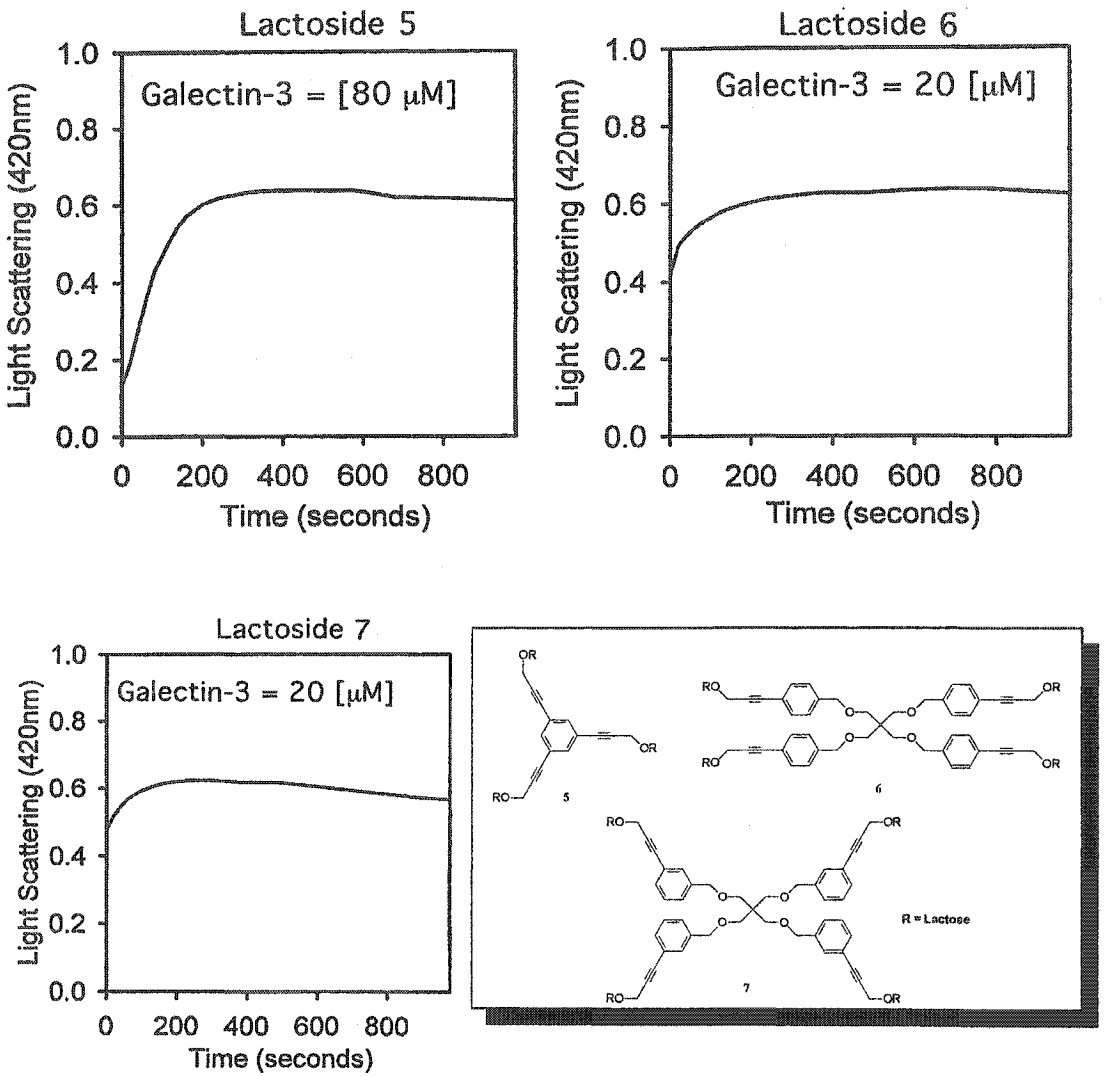


Fig. 4-12. (Continued).

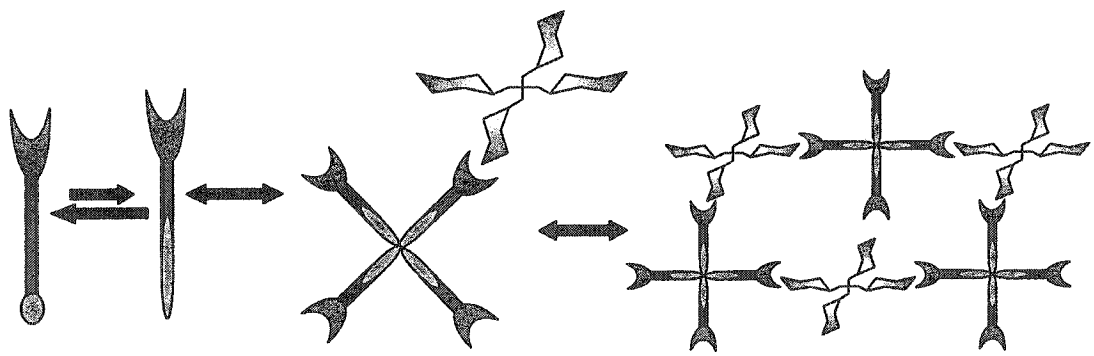


Fig. 4-13. Galectin-3 equilibria in the absence and presence of a multivalent carbohydrate ligand and its cross-linking with tetravalent lactosides.

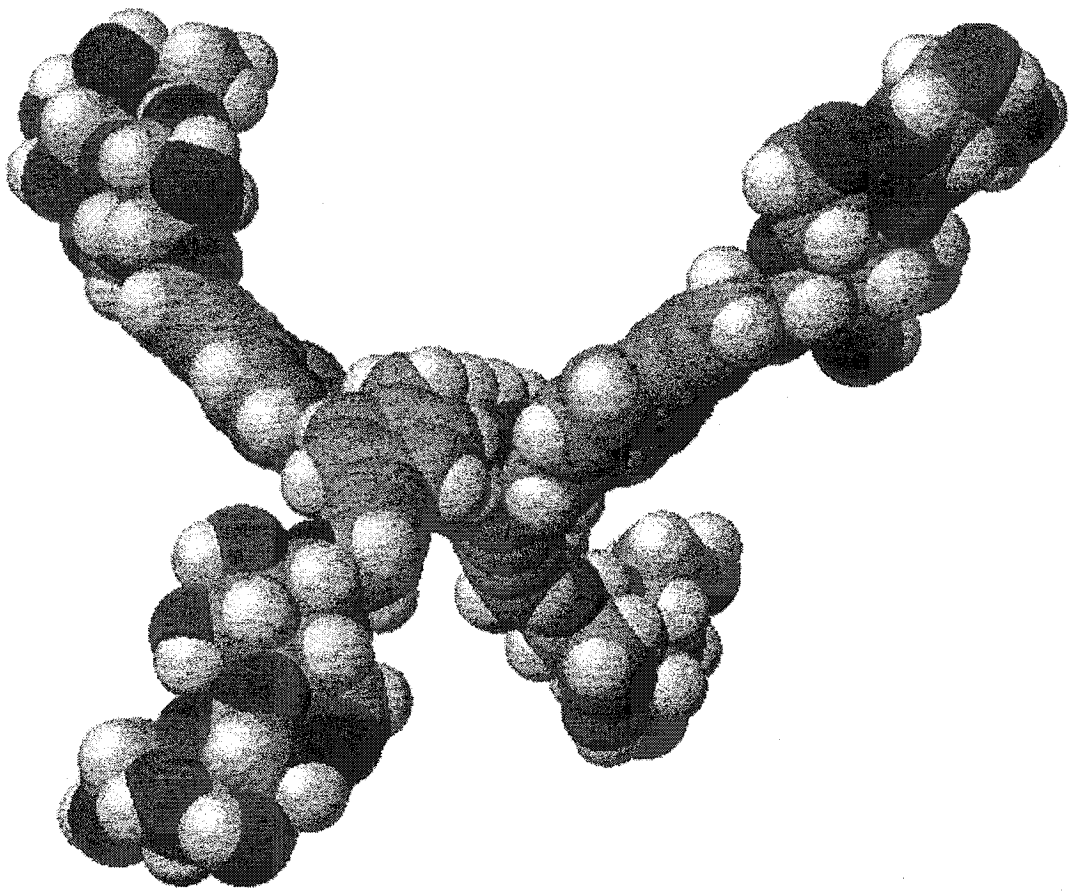


Fig. 4-14. 3D molecular modeling of tetrameric lactoside **4-28**.

Note: The structure (CPK model) represents one of its low energy (MM3) extended conformations. The lactose portion was extracted from the X-ray data of galectin 7 bound to it (PDB 4GAL). Illustrations were rendered from SymApps software (BioRad) for the stick model and with CAChe 6.0 software from Fujitsu for the CPK model.

Table 4-1 Determination of the IC_{50}-values and the relative inhibitory potency in a solid-phase assay with surface-immobilized lactosylated BSA and different types of sugar receptors in solution.[a]

	matrix	**0.2 µg LacBSA**		**0.25 µg LacBSA**		**0.05 µg LacBSA**	
	probe	VAA		galectin-3		IgG($\alpha^-\beta^+$)	
		(1.5 µg mL^{-1})		(20 µg mL^{-1})		(2 µg mL^{-1})	
type of inhibitor	lactose valency	IC_{50} (µM)	relative potency	IC_{50} (µM)	relative potency	IC_{50} (µM)	Relative potency
galactose	1	4000	0.5	5000	0.14	no inh. up to 200 mM	
lactose	1	2000	1	700	1	3000	1
4-17	2	653	3.1 (1.6)	163	4.3 (2.1)	2610	1.1 (0.55)
4-18	2	950	2.1 (1.1)	165	4.2 (2.1)	1058	2.8 (1.4)
4-21	2	4315	0.5 (0.25)	no inh.		431	7.0 (3.5)
4-22	2	1900	1.1 (0.55)	no inh.		570	5.3 (2.6)
4-24	3	825	2.4 (0.8)	11.6	60.3 (20.1)	1650	1.8 (0.6)
4-26	4	40	50 (12.5)	125	5.6 (1.4)	199	15.1 (3.8)
4-28	4	40	50 (12.5)	62.3	11.2 (2.8)	1992 µM = 31% inh.	

[a]See ref. 7 and 8 for the methods for biochemical assays.

Table 4.1 summarized the results of inhibition assay of lactoside analogue against lactosylated-BSA. For the binding with VAA, there was no significant increase in relative potency for the divalent **4-17**, **4-18**, **4-21**, **4-22** and trivalent **4-24** clusters. However the tetravalent clusters **4-26** and **4-28** showed 12.5-fold increase in binding efficiency. In the

assay with galectin-3, the triavalent **4-24** turned out to be the best ligand, and interestingly it did not show binding affinity toward Human IgG ($\alpha^-\beta^+$). This result suggests that if compound **4-24** could be used as a potential therapeutics targeting galectin-3, it will not interact efficiently against human IgG. The results also suggest that in this case, the flexibility of the spacers did not play significant roles comparing **4-17** with **4-18** and **4-21** and **4-22**.

4.4. Conclusions

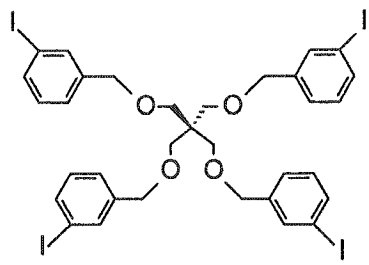
Analogues of lactoside clusters have been prepared, which have shown a cross-linking effect with galectin-3 for the first time. These clusters may be probes for the study of the biological behavior of galectin-3.

4.5. Experimental Section

4.5.1. Synthesis of Galactose-Based Clusters

Pentaerythritol tetrakis (iodobenzyl) ethers (4-3) and (4-4). Pentaerythritol **3-6** (27.2 mg, 0.2 mmol) was dissolved in dry DMF (10 mL), to which were added a catalytic amount of tetrabutylammonium iodide (10 mg) and NaH (46 mg, 0.96 mmol). The mixture was stirred at room temperature for 30 min and then iodobenzyl bromide (**4-1** or **4-2**) (286 mg, 0.96 mmol) was added to it. The reaction was monitored by TLC and was completed in 4 h. Excess NaH was quenched with several drops of methanol. The mixture was diluted with 50 mL of water and then washed with ether (3 x 30 mL). The combined organic layers were washed with water (3 x 30 mL) and dried over anhydrous sodium sulfate. After evaporating the solvent under reduced pressure, the residue was carefully separated by silica

gel chromatography using hexane : ether (10:1). After removal of the solvent, a white solid was obtained. Tetrameric iodobenzyl ethers **4-3** and **4-4** were obtained in 73% and 75% yields respectively.



4-3

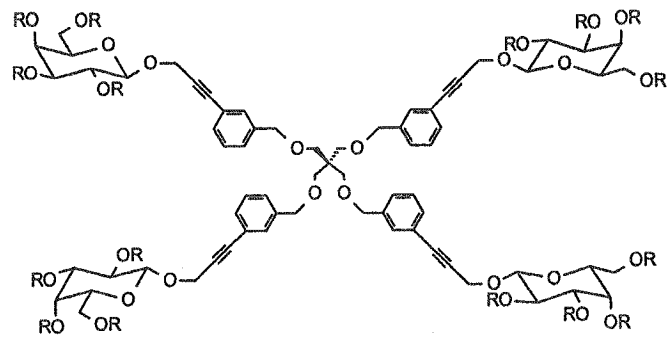
Pentaerythritol tetrakis (*m*-iodobenzyl) ether (4-3). Mp 85- 87 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (s, 4H, aromatic), 7.56 (d, 4H, J = 7.9 Hz, aromatic), 7.19 (d, 4H, J = 7.7 Hz, aromatic), 7.05 (t, 4H, J = 7.8 Hz, aromatic), 4.40 (s, 8H, PhCH_2), 3.49 (s, 8H, C(CH_2OR)$_4$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 141.2, 136.4, 136.1, 130.0, 126.4 and 94.3 (aromatic), 72.3 (PhCH_2), 69.1 (C(CH_2OR)$_4$), 45.5 (C(CH$_2$OR)$_4$); Anal. calcd for $C_{33}H_{32}O_4I_4$: C, 39.64; H, 3.22, found: C, 39.74; H, 3.27; FAB-MS calcd for $C_{33}H_{32}O_4I_4K$ (M + K^+) 1038.75; Found 1038.80.

Pentaerythritol tetrakis (*o*-iodobenzyl) ether (4-4). Mp: 109-111 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, 4H, J = 7.9 Hz, aromatic), 7.35 (d, 4H, J = 7.7Hz, aromatic), 7.21 (t, 4H, J = 7.7 Hz, aromatic), 6.91 (t, 4H, J = 7.7 Hz, aromatic), 4.46 (s, 8H, PhCH_2), 3.70 (s, 8H, C(CH_2OR)$_4$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 140.8, 138.8, 128.8, 128.5, 128.0 and 97.3 (aromatic), 77.0 (PhCH_2) , 69.6 (C(CH_2OR)$_4$), 45.9 (C(CH$_2$OR)$_4$); Anal calcd for $C_{33}H_{32}O_4I_4$: C, 39.63; H, 3.22; Found: C, 39.77; H, 3.30); FAB-MS calcd for $C_{33}H_{32}O_4I_4K$ (M + K^+) 1038.75; Found 1038.70.

Preparations of 4-6, 4-8, 4-9, and 4-12 using the Sonogashira coupling reaction. Pentaerythritol tetrakis (*p*-iodobenzyl) ether **3-8**[27] (100 mg, 0.1 mmol) was dissolved into a mixture of DMF and TEA (20 mL, 1:1) to which were added $(PPh_3)_2PdCl_2$ (3.6 mg, 5 mol%), and 2-propynyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside **4-5** (185 mg, 0.48 mmol). The solution was stirred at 60 °C for 6 h under nitrogen. The solvent and triethylamine were removed under reduced pressure. The residue was purified by silica gel column chromatography using hexane:ethyl acetate (1:1.5) to afford **4-6** as a white foam in 80% yield. Compounds **4-8** and **4-10** were prepared in 75% and 73% yields, respectively, following the same procedure.

The same procedure was adopted for the cross coupling between triiodobenzene **3-3** and 2-propynyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside **4-5** to generate **4-12** in 85% yield.

Compound 4-6. $[\alpha]_D$ 95.2° (*c* 1.5, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.35 (d, 8H, *J* = 7.7 Hz, aromatic), 7.20 (d, 8H, *J* = 7.7 Hz, aromatic), 5.38 (dd, 4H, *J* = 1.0, 3.5 Hz, H-4), 5.24 (dd, 4H, *J* = 8.0, 10.4 Hz, H-2), 5.05 (dd, 4H, *J* = 3.5, 10.4 Hz, H-3), 4.77 (d, 4H, *J* = 8.0 Hz, H-1), 4.58 (d, 8H, *J* = 1.4 Hz, H-1'), 4.45 (s, 8H, $PhCH_2$), 4.08 to 4.21 (m, 8H, H-6), 3.95 (m, 4H, H-5), 3.55 (s, 8H, $C(CH_2OR)_4$), 2.14, 2.03, 2.02 and 1.97 (4s, 48H, CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.4, 170.2, 170.1 and 169.5 (CH_3CO), 139.6, 131.7, 127.1 and 121.2 (aromatic), 99.0 (C-1), 86.9 and 83.5 (acetylenic), 72.9 (C-3), 70.9 ($PhCH_2$), 70.8(C-5), 69.6 ($C(CH_2OR)_4$), 68.7 (C-2), 67.0 (C-4), 61.2 (C-6), 56.9 (C-1'), 45.7 ($C(CH_2OR)_4$); Anal calcd for $C_{101}H_{116}O_{44}$: C, 59.52; H, 5.94; Found: C, 59.87; H, 5.80); FAB-MS calcd for $C_{101}H_{116}O_{44}K$ ($M+K^+$): 2071.66; Found 2071.45.



4-8 R = Ac

Compound 8. $[\alpha]_D$ 101.3° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.29 to 7.31 (m, 8H, aromatic), 7.21 to 7.24 (m, 8H, aromatic), 5.37 (dd, 4H, *J* = 1.0, 3.4 Hz, H-4), 5.21 (dd, 4H, *J* = 8.0, 10.4 Hz, H-2), 5.03 (dd, 4H, *J* = 3.4, 10.4 Hz, H-3), 4.75 (d, 4H, *J* = 8.0 Hz, H-1), 4.55 (d, 8H, *J* = 2.0 Hz, H-1'), 4.43 (s, 8H, PhCH_2), 4.07 to 4.18 (m, 8H, H-6), 3.91 (t, 4H, *J* = 6.7 Hz, H-5), 3.52 (s, 8H, C(CH_2OR)$_4$), 2.12 (12 H, s, CH_3CO), 2.00 (s, 24H, CH_3CO), 1.95 (s, 12H, CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 139.1, 130.8, 130.4, 128.4, 127.7 and 122.1(aromatic), 98.9 (C-1), 86.9 and 83.5 (acetylynic), 72.7 (C-3), 70.8 (PhCH_2), 70.8 (C-5), 69.3 (C(CH_2OR)$_4$), 68.6 (C-2), 67.0 (C-4), 61.2 (C-6), 56.8 (C-1'), 45.6 (*C*(CH_2OR)$_4$); Anal calcd for $C_{101}H_{116}O_{44}$: C, 59.52; H, 5.94; Found: C, 59.41; H, 5.79; HRMS-FAB calcd for $C_{101}H_{116}O_{44}K$ ($M + K^+$): 2071.6659; Found: 2071.7598.

Compound 4-10. $[\alpha]_D$ 97.0° (*c* 1.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.36 to 7.41 (m, 8H, aromatic), 7.14 to 7.20 (m, 8H, aromatic), 5.36 (bd, 4H, *J* = 3.3 Hz, H-4), 5.21 (dd, 4H, *J* = 8.0, 10.4 Hz, H-2), 5.03 (dd, 4H, *J* = 3.4, 10.4 Hz, H-3), 4.75 (d, 4H, *J* = 8.0 Hz, H-1), 4.64 (s, 8H, PhCH_2), 4.55 (s, 8H, H-1'), 4.07 to 4.17 (m, 8H, H-6), 3.91 (t, 4H, *J* = 6.7 Hz, H-5), 3.69 (s, 8H, C(CH_2OR)$_4$), 2.12, 1.98, 1.96 and 1.94 (4s, 48H, CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 140.8, 132.0, 128.8, 126.8, 126.5 and 119.7 (aromatic), 98.8 (C-1), 88.3 and 84.4 (acetylenic), 70.9 (PhCH_2 and C-3), 70.8 (C-5), 69.7 (C(CH_2OR)$_4$),

68.6 (C-2), 67.0 (C-4), 61.2 (C-6), 56.7 (C-1'), 45.8 ($C(CH_2OR)_4$); FAB-MS calcd for $C_{101}H_{116}O_{44}K$ ($M + K^+$): 2071.66; Found: 2071.45.

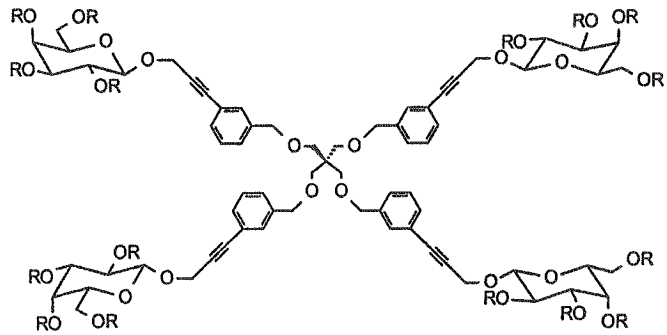
1,3,5-Tris [3'-O-(2'',3'',4'',6''-tetra-O-acetyl-β-D-galactopyranosyl)-prop-1'-enyl] benzene (4-12). $[\alpha]_D$ 105.4° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.43 (s, 3H, aromatic), 5.38 (dd, 3H, *J* = 1.0, 3.4 Hz, H-4), 5.20 (dd, 3H, *J* = 8.0, 10.5 Hz, H-2), 5.05(dd, 3H, *J* = 3.4, 10.5 Hz, H-3), 4.73 (d, 3H, *J* = 8.0 Hz, H-1), 4.56 (d, 6H, *J* = 3.2 Hz, H-1'), 4.09-4.12 (m, 6H, H-6), 3.94 (t, 3H, *J* = 6.2 Hz, H-5), 2.13, 2.02, 2.01 and 1.99 (4s, 36H, CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 134.7 (aromatic), 123.2 (aromatic), 99.0 (C-1), 85.2 and 84.8 (acetylenic), 70.8 (C-3 and C-5), 68.6 (C-2), 66.9 (C-4), 61.2 (C-6), 56.5 (C-1'); HRMS-FAB calcd for $C_{57}H_{66}O_{30}K$ ($M + K^+$): 1269.3276; Found: 1269.3567 ($M + K^+$).

Preparation of 4-7, 4-9, 4-11 and 4-13 using Zemplén Reaction. The fully protected glycocluster **4-6** (50 mg, 0.025 mmol) was dissolved into methanol (20 mL), to which was added a catalytic amount of sodium methoxide. The solution was stirred at room temperature for 24 h. After neutralization of sodium methoxide with Amberlite IR-120 (H+) resin, the solution was filtered through a cotton plug. Removal of methanol under reduced pressure afforded the fully deprotected cluster **4-7** as a white foam in 95%.

Compound **4-9** and **4-11** were generated in the same way in 97% and 97% yields respectively. The same reaction was applied to compound **4-12** to provide **4-13** as a white foam in 95% yield.

Compound 4-7. $[\alpha]_D$ 98.5° (*c* 1.5, DMSO); ^1H NMR (500 MHz, DMSO-*d*6) δ 7.38 (d, 8H, *J* = 7.9 Hz, aromatic), 7.25 (d, 8H, *J* = 7.9 Hz, aromatic), 4.61 (d, 4H, *J* = 15.8 Hz, H-1a'), 4.49 (d, 4H, *J* = 15.8 Hz, H-1b'), 4.44 (s, 8H, $PhCH_2$), 4.26 (d, 4H, *J* = 6.8 Hz, H-1), 3.63 (bd, 4H, *J* = 2.0 Hz, H-4), 3.52 (d, 8H, *J* = 6.1 Hz, H-6), 3.45 (s, 8H, $C(CH_2OR)_4$),

3.36 (m, 4H, H-5), 3.32 (m, 4H, H-3), 3.30 (m, 4H, H-2); ^{13}C NMR (125 MHz, DMSO-*d*6) δ_C 139.4, 131.3, 127.4 and 120.9 (aromatic), 101.8 (C-1), 85.81 (acetylenic), 75.4 (C-5), 73.4 (C-2), 72.5 (PhCH_2), 70.4 (C-3), 69.0 (C(CH_2OR)$_4$), 68.2 (C-4), 60.5 (C-6), 55.6 (C-1'), 49.5 (*C*(CH$_2$OR)$_4$); FAB-MS cald for $C_{69}H_{84}O_{28}K$ (M + K$^+$): 1399.48; Found: 1399.64.



4-9 R = H

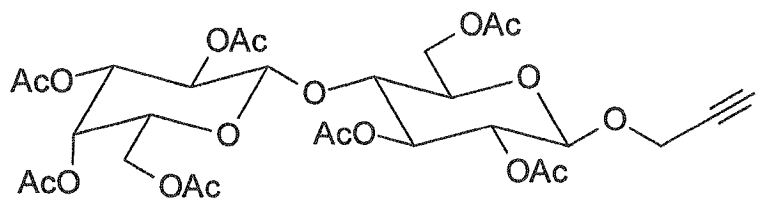
Compound 4-9. [α]$_D$ 101.2° (*c* 1, DMSO); ^{1}H NMR (500 MHz, DMSO-*d*6) δ 7.26 to 7.34 (m, 16H, aromatic), 4.59 (d, 4H, *J* = 15.6 Hz, H-1a'), 4.48 (dd, 4H, *J* = 15.6 Hz, H-1b'), 4.43 (s, 8H, PhCH_2), 4.26 (d, 4H, *J* = 7.0 Hz, H-1), 3.63 (bs, 4H, H-4), 3.51 (m, 8H, H-6), 3.44 (s, 8H, C(CH_2OR)$_4$), 3.30-3.36 (m, 12H, H-2, H-3 and H-5); ^{13}C NMR (125 MHz, DMSO-*d*6) δ_C 139.2, 130.4, 130.0, 128.7, 127.6 and 121.8 (aromatic), 101.8 (C-1), 85.9 and 85.4 (acetylenic), 75.4 (C-5), 73.4 and 70.4 (C-2 and C-3), 71.8 (PhCH$_2$), 68.8 (C(*C*H$_2$OR)$_4$), 68.2 (C-4), 60.5 (C-6), 55.2 (C-1'), 45.2 (*C*(CH$_2$OR)$_4$); FAB-MS calcd for $C_{69}H_{84}O_{28}K$ (M + K$^+$): 1399.48; Found: 1399.42.

Compound 4-11. [α]$_D$ 96.3° (*c* 1, DMSO); ^{1}H NMR (500 MHz, DMSO-*d*6) δ 7.23 to 7.42 (m, 16H, aromatic), 4.65 (m, 12H, PhCH_2 and H-1a'), 4.49 (d, 4H, *J* = 15.8 Hz, H-1b'), 4.27 (d, 4H, *J* = 6.7 Hz, H-1), 3.62 (bs, 4H, H-4), 3.59 (bs, 8H, C(CH_2OR)$_4$), 3.51 (m, 8H, H-6), 3.36 (m, 4H, H-5), 3.30 (m, 8H, H-2 and H-3); ^{13}C NMR (125 MHz, DMSO-*d*6)

δ_C 140.4, 131.8, 128.8, 127.2, 126.8 and 119.9 (aromatic), 101.7 (C-1), 90.5 and 82.9 (acetylenic), 75.4 (C-5), 73.4 (C-2 or C-3), 70.4 ($PhCH_2$, C-2 or C-3), 69.3 ($C(CH_2OR)_4$), 68.1 (C-4), 60.5 (C-6), 55.6 (C-1'), 45.4 ($C(CH_2OR)_4$); FAB-MS calcd for $C_{69}H_{84}O_{28}K$ ($M + K^+$): 1399.48; Found: 1399.43.

Compound 4-13. $[\alpha]_D$ 92.4° (*c* 1.5, DMSO); ^1H NMR (500 MHz, DMSO-*d*6) δ 7.51 (s, 3H, aromatic), 4.60(d, 3H, *J* = 16.0 Hz, H-1a'), 4.50 (d, 3H, *J* = 16.0 Hz, H-1b'), 4.27 (d, 3H, *J* = 7.2 Hz, H-1), 3.62 (bs, 3H, H-4), 3.51 (d, 6H, *J* = 6.1 Hz, H-6), 3.37 (t, 3H, *J* = 6.0 Hz, H-5), 3.30-3.31 (m, 6H, H-2 and H-3); ^{13}C NMR (125 MHz, DMSO-*d*6) δ_C 134.1 (aromatic), 123.3 (aromatic), 101.7 (C-1), 87.8, and 83.5 (acetylenic), 75.4 (C-5), 73.2 and 70.4 (C-2 and C-3), 68.2 (C-4), 60.5 (C-6), 55.4 (C-1'); FAB-MS calcd for $C_{33}H_{42}O_{18}K$ ($M + K^+$): 765.20; Found: 765.26.

4.5.2. Synthesis of Lactose-Based Clusters



4-15

2-Propynyl (2,3,4,6-*O*-acetyl-β-D-galactopyranosyl)-(1-4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (4-15). Lactosyl octaacetate (**4-14**) (1.36 g, 2 mmol) was dissolved into 30 mL of dichloromethane to which were added freshly distilled propargyl alcohol (0.24 mL, 4 mmol) and 1 g of 4 A° MS. The mixture was stirred at rt for 30 min. Then BF_3-Et_2O (0.62 mL, 5 mmol) was added to the flask and the mixture was stirred at rt for 4 h. After

completion of the reaction, sodium carbonate (0.6 g) was introduced into the flask and the mixture was stirred for 30 more min. The mixture was then filtered through a pad of celite and washed with 50 mL of dichloromethane. The filtrate was washed with 2 x 50 mL of water and the organic layer was dried over anhydrous sulfate. After removal of the solvent under reduced pressure, the residue was purified on silica gel column using hexane and ethylacetate (2:3) as eluent to provide the title **4-15** in 85% yield (1.14 g, 1.7 mmol); $[\alpha]_D$ -12.5° (*c* 1.5, $CHCl_3$), ^1H NMR (500 MHz, $CDCl_3$) δ 5.31 (1H, bd, *J* = 2.4, H-4'), 5.19 (1H, t, *J* = 9.3, H-3), 5.07 (1H, dd, *J* = 8.0, 10.4, H-2'), 4.91 (1H, dd, *J* = 3.4, 10.4, H-3'), 4.88 (1H, dd, *J* = 8.1, 9.4, H-2), 4.71 (1H, d, *J* = 8.1, H-1), 4.44-4.48 (2H, m, H-6a and H-1'), 4.30 (1H, d, *J* = 2.4, H-1''), 4.02-4.11 (3H, m, H-6b and H-6'), 3.84 (1H, t, *J* = 6.5, H-5'), 3.78 (1H, t, *J* = 9.6, H-4), 3.59-3.61 (1H, m, H-5), 2.43 (1H, t, *J* = 2.3, H-3''), 2.18, 2.09, 2.03, 2.02, 2.01, 1.93 (21H, 6s, CH_3CO); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 170.3, 170.1, 170.0, 169.7, 169.6 and 169.0 (CH_3CO), 101.0 (C-1'), 97.8 (C-1), 78.0 (alkynyl), 76.1 (C-4), 75.4 (alkynyl), 72.7 (C-5), 72.6 (C-3), 71.3 (C-2), 70.9 (C-3'), 70.7 (C-5'), 69.1 (C-2'), 66.6 (C-4'), 61.8 (C-6), 60.8 (C-6'), 55.8 (C-1''), 20.8, 20.7, 20.6, 20.5 and 20.4 (CH_3CO); ESI-MS calcd for $C_{29}H_{38}O_{18}$ + (NH_4^+): 692.2; Found: 692.2.

1,6-Bis-[2,3,4,6-*O*-acetyl-β-D-galactopyranosyl)-(1-4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy]-2,4-hexadyne (4-16). 2-Propynyl (2,3,4,6-O-acetyl-β-D-galactopyranosyl)-(1-4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**4-15**) (67.4 mg, 0.10 mmol) was dissolved into a solution of 10 mL of DMF and TEA (1:1), to which were added $Pd(PPh_3)_2Cl_2$ (3.6 mg, 5 mol%) and CuI (3.8 mg, 20 mmol%). The solution was stirred at rt for 3 h. The solvent and triethylamine were removed under reduced pressure. The residue was purified by silica gel column chromatography using hexane:ethyl acetate (2:3) to afford

4-16 as a white solid in 95% yield (156 mg, 0.095 mmol); $[\alpha]_D$ -22.1° (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 5.31 (2H, bd, *J* = 3.4, H-4'), 5.19 (2H, t, *J* = 9.3, H-3), 5.07 (2H, dd, *J* = 8.0, 10.3, H-2'), 4.91 (2H, dd, *J* = 3.4, 10.3, H-3'), 4.88 (2H, dd, *J* = 8.0, 9.4, H-2), 4.66 (2H, d, *J* = 8.0, H-1), 4.49 (2H, dd, *J* = 1.6, 12.0, H-6a), 4.45 (2H, d, *J* = 8.0 H-1'), 4.39 (2H, s, H-1''), 4.02-4.11 (6H, m, H-6b and H-6'), 3.84 (2H, t, *J* = 7.0, H-5'), 3.79 (2H, t, *J* = 9.5, H-4), 3.61-3.64 (2H, m, H-5), 2.12, 2.10, 2.03, 2.02, 2.01, 1.93 (42H, 6s, CH_3CO); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 170.3, 170.1, 170.0, 169.7, 169.6 and 169.0 (CH_3CO), 101.0 (C-1'), 98.2 (C-1), 76.0 (C-4), 74.2 (alkynyl), 72.8 (C-5), 72.6 (C-3), 71.2 (C-2), 70.9 (C-3'), 70.8 (alkynyl), 70.7 (C-5'), 69.0 (C-2'), 66.6 (C-4'), 61.7 (C-6), 60.8 (C-6'), 56.3 (C-1''), 20.8, 20.7, 20.6, 20.5 and 20.4 (CH_3CO); ESI-MS calcd for $C_{58}H_{74}O_{36}$ + (NH_4^+): 1364.4; Found: 1364.3.

De-*O*-acetylation to Prepare 4-17, 4-21, 4-24, 4-26, 4-28 under Zemplèn Reactions. General Procedure. The fully protected homodimer **4-16** (68 mg, 0.05 mmol) was suspended in methanol (15 mL), to which was added a catalytic amount of sodium methoxide. The solution was stirred at room temperature for 24 h. After neutralization of sodium methoxide with Amberlite (120 H+) resin, the solution was filtered through a cotton plug. Removal of methanol under reduced pressure afforded compound **4-17** as a white foam in 95% yield. Compound **4-21, 4-24, 4-26** and **4-28** were prepared in the same manner in 95%, 92%, 90% and 91% yields, respectively.

1,6-Bis-[(β-D-galactopyranosyl)-(1-4)-β-D-glucopyranosyloxy]-2,4-hexadiyne (4-17). $[\alpha]_D$ -20.8° (*c* 1.0, H_2O); ^1H NMR (500 MHz, D_2O) δ 4.70 (2H, *J* = 8.0, d, H-1), 4.63 (4H, s, H-1''), 4.49 (2H, d, *J* = 7.8, H-1'), 4.03 (2H, dd, *J* = 1.7, 11.8, H-6a), 3.98 (2H, d, *J* = 3.2, H-4'), 3.96 (2H, dd, *J* = 6.9, 12.0, dd, H-1a''), 3.81-3.88 (8H, m, H-5', H-6b, H-6,),

3.67-3.78 (8H, m,H-3, H-3', H-4, H-5), 3.59 (2H, dd, J = 7.8, 9.9, H-2'), 3.40 (2H, t, J = 8.3, H-2); ^{13}C NMR (125.7 MHz, D_2O) δ_C 102.5 (C-1'), 100.1 (C-1), 77.8, 74.9 (C-5'), 74.4 (C-5), 73.9, 72.4, 72.1, 72.0, 70.5 (C-2'), 69.9 (alkynyl), 68.1 (C-4'), 60.5 (C-6'), 59.5 (C-6), 56.6 (C-1''); ESI-MS calcd for $C_{30}H_{46}O_{22}$ + (Na^+): 781.1; Found: 781.2.

1,6-Bis-[(β-D-galactopyranosyl)-(1-4)-β-D-glucopyranosyloxy]-2,4-hexane (4-18). The fully deprotected homodimer **4-17** (39 mg, 0.05 mmol) was dissolved into methanol (10 mL), to which was added a catalytic amount of 10% Pd-C. The mixture was stirred at rt for 5 h. After filtration through a pad of celite, the filtrate was evaporated under reduced pressure to provide compound **4-18** in 97% yield (38 mg, 0.05 mmol); $[\alpha]_D$ -21.5° (*c* 1.0, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 4.53 (2H, J = 8.0, d, H-1), 4.50 (2H, J = 7.8, d, H-1'), 4.02 (2H, d, J = 12.0, H-6a), 3.97 (2H, bd, J = 3.2, H-4'), 3.96 (2H, dd, J = 6.9, 12.0, H-1a''), 3.63-3.90 (18H, m, H-3, H-3', H-4, H-5, H-5', H-6b, H-6, H-1b''), 3.59 (2H, dd, J = 7.8, 9.9, H-2'), 3.45 (2H, t, J = 8.3, H-2), 1.69 (4H, m, H-2''), 1.44 (4H, bs, H-3''); ^{13}C NMR (125.7 MHz, D_2O) δ_C 102.5 (C-1'), 101.5 (C-1), 78.0, 74.9, 74.3, 74.0, 72.4, 72.1, 71.3, 70.5, 70.3, 70.1 (C-2), 69.9, 68.1 (C-2'), 67.9 (C-1''), 60.5 (C-6'), 59.7 (C-6), 28.1 (C-2''), 24.3(C-3''); ESI-MS calcd for $C_{30}H_{54}O_{22}$ + (NH_4^+): 784.1; Found: 784.2.

General Procedure for the Sonogashira Reactions

1,4-diiodobenzene **4-19** (16.6 mg, 0.05 mmol) was dissolved into a solution of 10 mL of DMF and TEA (1:1), to which were added $Pd(PPh_3)_2Cl_2$ (3.6 mg, 5 mol%), lactoside **4-15** (80.9 mg, 0.12 mmol) and CuI (3.8 mg, 20 mmol%). Under nitrogen, the solution was stirred at rt for 5 h. The solvent and triethylamine were removed under reduced pressure. The residue was purified by silica gel column chromatography using hexane and ethyl acetate (2:3) to provide **4-20** as a white solid in 90% yield. The same procedure was applied

for the synthesis of compounds **4-23**, **4-25** and **4-27** in yields of 81%, 78% and 75%, respectively.

1,4-Phenylene-di-2-propyne-3,1-diyl bis-[(2,3,4,6-*O*-acetyl-β-D-galactopyranosyl)-(1-4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside] (4-20). $[\alpha]_D$ -31.0° (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.35 (4H, s, aromatic), 5.31 (2H, bd, *J* = 2.9, H-4'), 5.21 (2H, t, *J* = 9.2, H-3), 5.07 (2H, dd, *J* = 7.9, 10.3, H-2'), 4.91 (2H, dd, *J* = 3.5, 10.3, H-3'), 4.90 (2H, dd, *J* = 7.9, 9.4, H-2), 4.76 (2H, d, *J* = 7.9, H-1), 4.54 (2H, d, *J* = 16.1, H-1a''), 4.50 (2H, d, *J* = 16.1, H-1b''), 4.44-4.48 (4H, m, H-6a and H-1'), 4.02-4.11 (6H, m, H-6b and H-6'), 3.84 (2H, t, *J* = 6.8, H-5'), 3.80 (2H, t, *J* = 9.6, H-4), 3.61-3.64 (2H, m, H-5), 2.11, 2.08, 2.03, 2.02, 2.01, 2.00, 1.99 and 1.93 (42H, 7s, CH_3CO); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 170.3, 170.2, 170.1, 170.0, 169.7, 169.6 and 169.0 (CH_3CO), 131.6 and 122.5 (aromatic), 101.0 (C-1'), 98.2 (C-1), 86.4 and 85.4 (alkynyl), 76.0 (C-4), 72.8 (C-5), 72.7 (C-3), 71.4 (C-2), 70.9 (C-3'), 70.7 (C-5'), 69.1 (C-2'), 66.6 (C-4'), 61.8 (C-6), 60.8 (C-6'), 56.7 (C-1''), 20.8, 20.7, 20.6, 20.5 and 20.4 (CH_3CO); ESI-MS calcd for $C_{64}H_{78}O_{36}$ + (Na^+): 1445.3; Found: 1445.2.

1,4-Phenylene-di-2-propyne-1,1-diyl bis-[(β-D-galactopyranosyl)-(1-4)-β-D-glucopyranoside] (4-21) was obtained under Zemplèn conditions described above for **4-17.** $[\alpha]_D$ -7.0° (*c* 1.0, DMSO); ^1H NMR (500 MHz, DMSO) δ 7.30 (4H, s, aromatic), 4.64 (2H, d, *J* = 15.9, H-1a''), 4.53 (2H, d, *J* = 15.9, H-1b''), 4.38 (2H, d, *J* = 7.9, H-1), 4.17 (2H, d, *J* = 7.1, H-1'), 3.26-3.79 (22H, m, H-2', H-3, H-3', H-4, H-4', H-5, H-5', H-6, H-6'), 3.03-3.08 (2H, t, *J* = 8.3, H-2); ^{13}C NMR (125.7 MHz, DMSO) δ 131.7 and 122.2 (aromatic), 103.8 (C-1'), 100.9 (C-1), 87.7 and 85.0 (alkynyl), 80.6, 75.5, 75.0, 74.9, 73.2, 73.0, 70.5,

68.1, 60.5 (C-6'), 60.3 (C-6), 55.8 (C-1''); ESI-MS calcd for $C_{36}H_{50}O_{22}$ + (NH_4^+): 852.3; Found: 852.2.

1,4-Bis-[[[(β-D-galactopyranosyl)-(1-4)-β-D-glucopyranosyl]oxy]propyl]benzene (4-22). The fully deprotected homodimer **4-21** (42 mg, 0.05 mmol) was dissolved into methanol (10 mL), to which was added a catalytic amount of 10% Pd-C. The mixture was stirred at rt for 5 h. After filtration through a pad of celite, the filtrate was evaporated under reduced pressure to provide compound **4-22** in 95% yield (42 mg, 0.05 mmol); $[\alpha]_D$ -6.0° (*c* 1.0, H_2O); ^1H NMR (500 MHz, D_2O) δ 7.30 (4H, s, aromatic), 4.50 (4H, d, *J* = 7.8, H-1 and H-1'), 3.94-4.06 (6H, m, H-4', H-1a'', H-6a), 3.58-3.86 (24H, m, H-2', H-3, H-3', H-4, H-4', H-5, H-5', H-6b, H-6', H-1''), 3.37 (2H, t, *J* = 8.3, H-2), 2.74 (4H, t, *J* = 7.4, H-3''), 1.97 (4H, t, *J* = 6.8, H-2'') ; ^{13}C NMR (125.7 MHz, D_2O) δ_C 139.3 and 128.2 (aromatic), 102.5 (C-1'), 102.4 (C-1), 78.0, 74.9, 74.3, 74.0, 72.4, 72.1, 70.5, 69.2 (C-1''), 68.1, 59.7 (C-6'), 59.4 (C-6), 30.3 and 30.1 (C-2'' and C-3''); ESI-MS calcd for $C_{36}H_{58}O_{22}$ + (Na^+): 865.3; Found: 865.2.

Trimer 4-23. $[\alpha]_D$ -22.0° (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.42 (3H, s, aromatic), 5.31 (3H, dd, *J* = 0.9, 3.4, H-4'), 5.20 (3H, t, *J* = 9.3, H-3), 5.07 (3H, dd, *J* = 7.9, 10.4, H-2'), 4.91 (3H, dd, *J* = 3.4, 10.4, H-3'), 4.90 (3H, dd, *J* = 7.9, 9.4, H-2), 4.73 (3H, d, *J* = 7.9, H-1), 4.55 (3H, d, *J* = 16.1, H-1a''), 4.50 (3H, d, *J* = 16.1, H-1b''), 4.44-4.48 (6H, m, H-6a and H-1'), 4.02-4.11 (9H, m, H-6b and H-6'), 3.85 (3H, t, *J* = 7.1, H-5'), 3.80 (3H, t, *J* = 9.2, H-4), 3.61-3.66 (3H, m, H-5), 2.11, 2.08, 2.03, 2.02, 2.01, 2.00, 1.99 and 1.93 (63H, 7s, CH_3CO); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 170.7, 170.5, 170.4, 170.1, 170.0, 169.4 (CH_3CO), 135.1 and 123.5 (aromatic), 101.4 (C-1'), 98.6 (C-1), 85.5 and 85.3 (alkynyl), 76.4 (C-4), 73.1 (C-5), 73.0 (C-3), 71.7 (C-2), 71.3 (C-3'), 71.0 (C-5'), 69.4 (C-

2'), 66.9 (C-4'), 62.1 (C-6), 61.1 (C-6'), 56.9 (C-1''), 21.2, 21.1, 21.0 and 20.8 (CH_3CO); ESI-MS calcd for $C_{93}H_{114}O_{54} + (NH_4^+)$: 2112.6; Found: 2112.4.

Deprotected trimer 4-24 was obtained as described above for **4-17.** $[\alpha]_D$ -24.8° (*c* 1.0, D_2O); ^1H NMR (500 MHz, D_2O) δ 7.62 (3H, s, aromatic), 4.73-4.78 (9H, m, H-1, H-1''), 4.53 (3H, *J* = 7.8, d, H-1'), 4.03 (3H, bd, *J* = 11.2, H-6a), 4.00 (3H, bd, *J* = 3.2, H-4'), 3.73-3.92 (24H, m, H-3, H-3', H-4, H-4', H-5', H-6b, H-6'), 3.67 (3H, m, H-5), 3.63 (3H, dd, *J* = 7.8 and 9.5, H-2'), 3.47 (3H, m, H-2); ^{13}C NMR (125.7 MHz, D_2O) δ_C 134.4 and 122.3 (aromatic), 102.5 (C-1'), 100.3 (C-1), 85.4 and 84.6 (alkynyl), 77.8, 74.9, 74.4, 73.9, 72.2 (C-2), 72.1, 70.5 (C-2'), 68.1 (C-4'), 60.1 (C-6'), 59.6 (C-6), 56.7 (C-1''); ESI-MS calcd for $C_{51}H_{72}O_{33} + (NH_4^+)$: 1212.4; Found: 1212.4.

Tetramer (*meta*) 4-25 was obtained by the Sonogashira coupling of **4-15** and tetraiodide **4-1.** $[\alpha]_D$ -24.5° (*c* 1.5, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.17-7.41 (16H, m, aromatic), 5.29 (4H, bd, *J* = 3.1, H-4'), 5.18 (4H, t, *J* = 9.3, H-3), 5.05 (4H, dd, *J* = 7.9, 10.4, H-2'), 4.91 (4H, dd, *J* = 3.2, 10.3, H-3'), 4.90 (4H, dd, *J* = 7.9, 9.4, H-2), 4.73 (4H, d, *J* = 7.9, H-1), 4.51 (4H, d, *J* = 16.0, H-1a''), 4.50 (4H, d, *J* = 16.0, H-1b''), 4.44-4.48 (8H, m, H-6a and H-1'), 4.40 (8H, s, benzyl), 4.02-4.11 (12H, m, H-6b and H-6'), 3.83 (4H, t, *J* = 6.7, H-5'), 3.78 (4H, t, *J* = 9.6, H-4), 3.61-3.64 (4H, m, H-5), 3.49 (8H, s, $C(CH_2OR)_4$), 2.10, 2.05, 2.00, 1.99, 1.98, 1.96 and 1.91 (84H, 7s, CH_3CO); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 170.2, 170.0, 169.9, 169.7, 169.6 and 168.9 (CH_3CO), 139.0, 130.7, 130.3, 128.3, 127.6 and 121.9(aromatic), 100.9 (C-1'), 98.1 (C-1), 86.9 and 83.3 (alkynyl), 76.0 (C-4), 72.7 (C-5 and C-3), 72.6 (benzylic), 71.4 and 70.9 (C-3' and C-2), 70.6 (C-5'), 69.2 ($C(CH_2OR)_4$), 69.0 (C-2'), 66.5 (C-4'), 61.8 (C-6), 60.7 (C-6'), 56.7 (C-1''), 45.6

($C(CH_2OR)_4$), 20.7, 20.6, 20.5 and 20.4 (CH_3CO); ESI-MS calcd for $C_{149}H_{180}O_{76} + (2NH_4^+)$: 1610.5; Found: 1610.5.

Deprotected tetramer (*meta*) 4-26 was obtained as described above for **4-17.** $[\alpha]_D$ -7.2° (*c* 1.0, D_2O); ^1H NMR (500 MHz, D_2O at 60°C) δ 7.47-7.78 (16H, m, aromatic), 4.98-5.10 (12H, m, H-1, H-1''), 4.92 (4H, d, *J* = 7.4, H-1'), 4.65 (8H, bs, $PhCH_2$), 4.44 (4H, bs, H-4'), 4.06-4.36 (36H, m, H-2', H-3, H-3', H-4, H-5', H-6, H-6'), 3.93 (4H, bs, H-5), 3.88 (4H, 8, H-2), 3.47 (4H, m, H-2), 3.29 (8H, bs, $C(CH_2OR)_4$); ^{13}C NMR (125.7 MHz, D_2O) δ_C 138.7, 131.0, 130.2, 128.5, 127.6 and 121.9 (aromatic), 103.1 (C-1'), 101.1 (C-1), 86.8 and 85.0 (alkynyl), 78.7, 75.3, 74.8 (C-5), 74.5, 72.8, 72.3 ($PhCH_2$), 71.0, 68.8 ($C(CH_2OR)_4$), 68.6 (C-4'), 61.0 (C-6'), 60.4 (C-6), 57.2 (C-1''), 45.9 ($C(CH_2OR)_4$); ESI-MS calcd for $C_{93}H_{124}O_{48} + (Na^+)$: 2031.7; Found: 2031.4.

Tetramer (*para*) 4-27 was obtained by the Sonogashira coupling of 4-15 and tetraiodide 3-8. $[\alpha]_D$ -22.7° (*c* 1.2, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.32 (8H, d, *J* = 8.1, aromatic), 7.19 (8H, d, *J* = 8.1, aromatic), 5.30 (4H, bd, *J* = 2.9, H-4'), 5.19 (4H, t, *J* = 9.3, H-3), 5.06 (4H, dd, *J* = 7.8, 10.4, H-2'), 4.91 (4H, dd, *J* = 3.4, 10.4, H-3'), 4.89 (4H, dd, *J* = 7.9, 9.4, H-2), 4.76 (4H, d, *J* = 7.9, H-1), 4.53 (4H, d, *J* = 16.0, H-1a''), 4.50 (4H, d, *J* = 16.0, H-1b''), 4.48 (4H, m, H-6a), 4.46 (4H, *J* = 7.8, d, H-1'), 4.40 (8H, s, benzyl), 4.02-4.11 (12H, m, H-6b and H-6'), 3.84 (4H, t, *J* = 6.7, H-5'), 3.80 (4H, t, *J* = 9.6, H-4), 3.60-3.65 (4H, m, H-5), 3.52 (8H, s, $C(CH_2OR)_4$), 2.10, 2.07, 2.01, 2.00, 1.99, 1.98 and 1.91 (84H, 7s, CH_3CO); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 170.2, 170.0, 169.9, 169.7, 169.6 and 168.9 (CH_3CO), 139.5, 131.6, 127.0 and 121.0 (aromatic), 100.9 (C-1'), 98.1 (C-1), 86.9 and 83.3 (alkynyl), 76.0 (C-4), 72.7 (C-5 and C-3), 72.6 (benzylic), 71.4 and 70.9 (C-3' and C-2), 70.6 (C-5'), 69.2 ($C(CH_2OR)_4$), 69.1 (C-2'), 66.6 (C-4'), 61.8 (C-6), 60.7 (C-6'), 56.8

(C-1''), 45.6 ($C(CH_2OR)_4$), 20.7, 20.6, 20.5 and 20.4 (CH_3CO); ESI-MS calcd for $C_{149}H_{180}O_{76} + (2NH_4^+)$: 1610.6; Found: 1610.7.

Deprotected tetramer 4-28 was obtained as described above for **4-17**. $[\alpha]_D$ -60.0° (*c* 0.5, D_2O); ^1H NMR (500 MHz, D_2O) δ 7.31 (8H, bs, aromatic), 7.00 (8H, bs, aromatic), 4.62-4.78 (12H, m, H-1, H-1''), 4.52 (4H, *J* = 7.1, d, H-1'), 4.01 ((8H, bs, PhCH_2), 3.63-3.91 (44H, m, H-2', H-3, H-3', H-4, H-4', H-5, H-5', H-6, H-6'), 3.47 (4H, m, H-2), 3.29 (8H, bs, C(CH_2OR)$_4$); ^{13}C NMR (125.7 MHz, D_2O) δ_C 138.5, 131.3, 126.8 and 120.6 (aromatic), 102.5 (C-1'), 100.6 (C-1), 86.3 and 84.5 (alkynyl), 77.8, 74.9, 74.2, 74.0, 72.2, 71.7 (PhCH_2), 70.5 (C-2'), 68.1, 65.5, 60.6 (C-6'), 59.7 (C-6), 56.7 (C-1''), 45.9 ($C(CH_2OR)_4$); ESI-MS calcd for $C_{93}H_{124}O_{48} + (Na^+)$: 2026.7; Found: 2026.9.

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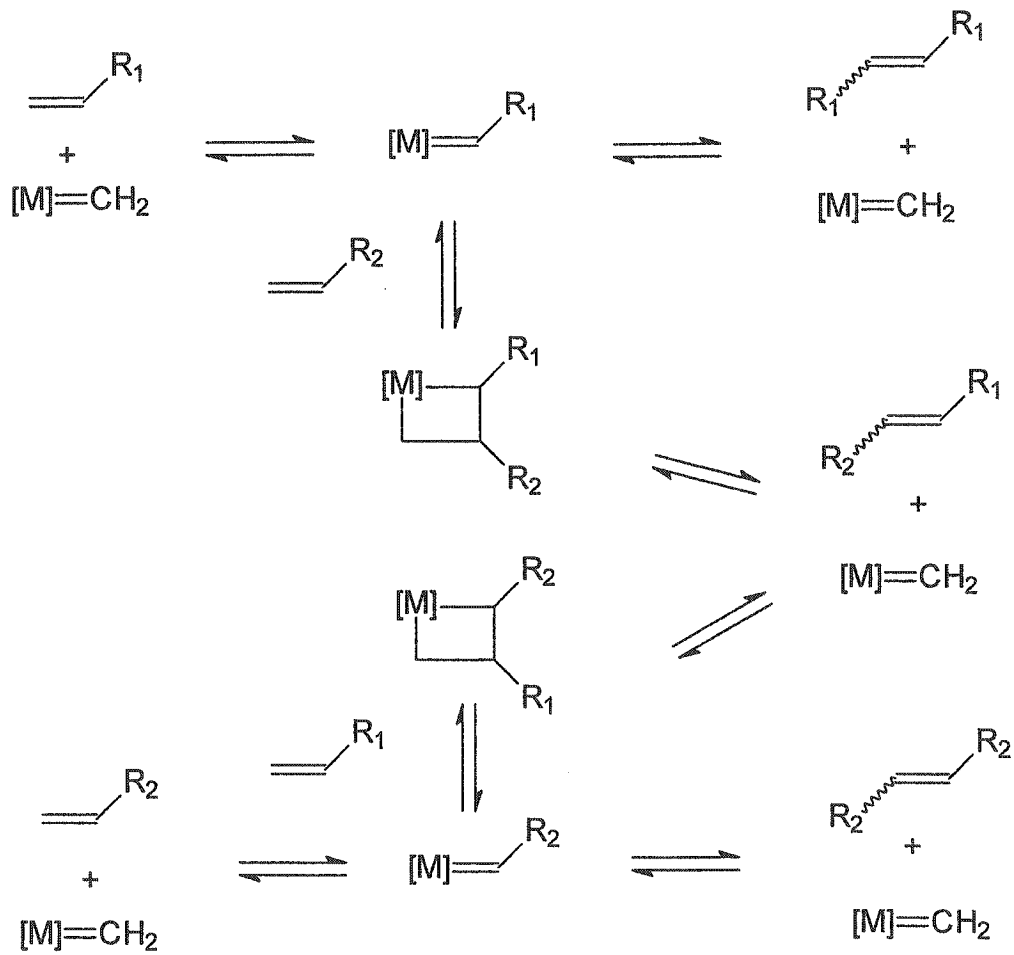
CHAPTER 5 SYNTHESIS OF 'MOLECULAR ASTERISKS' VIA SEQUENTIAL CROSS-METATHESIS, SONOGASHIRA AND CYCLOTRIMERIZATION REACTIONS

5.1. Introduction

Olefin metathesis has attracted much attention from synthetic chemists in recent years (Scheme 5-1 for mechanism).[1-2] The importance of this carbon-carbon bond construction method is evident from the huge number of publications that have appeared within a short span of time.[3] Although ring-closing metathesis has been successfully used for the synthesis of a variety of carbo- and hetero-cyclic products, and has proven to be the key step in several natural products syntheses, the utility of cross-metathesis reaction is somewhat more limited.[4] Cross-metathesis has not found widespread application due to the fact that general conditions which give rise to high yields and stereoselectivity has not yet been discovered and this procedure gives rise to large amount of self-metathesis by-products. With the development of new, well-defined metal alkylidenes such as Schrock's molybdenum catalyst,[5] $PhMe_2CCH{=}Mo{=}N\text{-}(2,6\text{-}i Pr_2C_6H_3)[OCMe(CF_3)_2]_2$ and Grubbs' ruthenium catalyst,[6] $Cl_2(PCy_3)_2Ru{=}CHPh$, some examples of selective cross-metathesis reactions have recently appeared in the literature.[7] The use of metathesis reactions in carbohydrate chemistry has also grown considerably.[8] Schrock's and Grubbs' catalysts have been used in carbohydrate-related ring-closing metathesis,[9] cross-metathesis[10] and ring-opening[11] metathesis reactions. Recently, our group has reported that allyl *O*-glycosides can be easily homodimerized in the presence of these catalysts in excellent yields.[12] We have also reported an interesting application of cross-metathesis reaction in the synthesis of *C*-

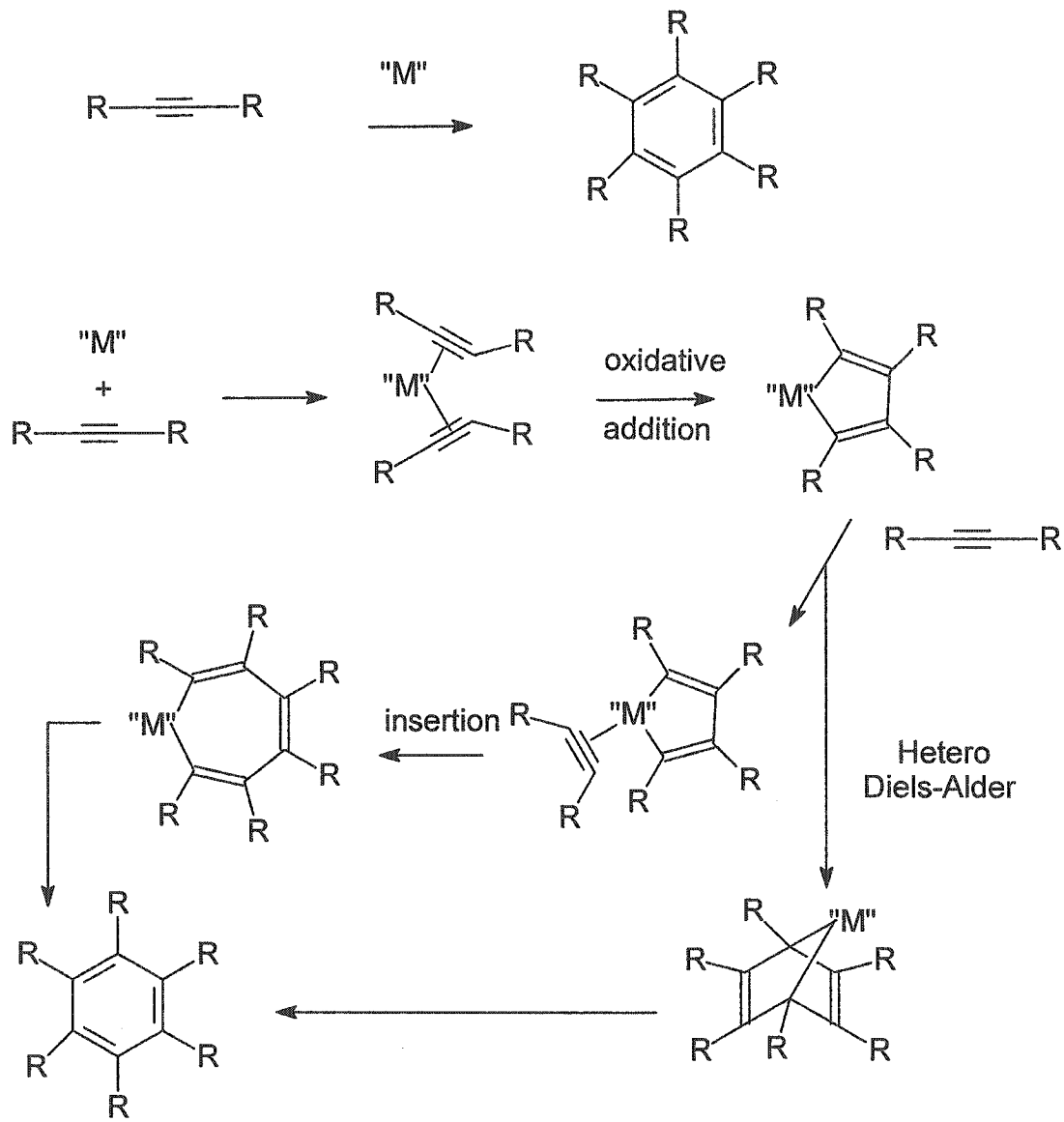
linked pseudodisaccharides between two different sugar moieties using a head to tail condensation procedure.[13]



Scheme 5-1. Reaction mechanism of olefin metathesis.[3a]

Alkyne cyclotrimerization has been proven to be a powerful tool for the synthesis of clusters with a rigid aromatic core to control the geometry of the molecules and with a tailorable spacer. So far many efficient protocols have been developed in this field, especially, some transition metals and their complexes such as Ru,[14] Co,[15] Pd,[16] Ti,[17] Ta[18]

and Ni,[19] have demonstrated impressive utility in this transformation. Commonly, terminal alkynes give two regisomeric cycloadducts: the 1,2,4-trisubtituted and the 1,3,5-trisubstituted benzene, and the former is usually preferred. Disubstituted symmetric alkynes give rise to hexameric clusters. Transition metal-catalyzed cyclotrimerizations follow the same mechanism (Scheme 5-2).[20] Due to the interesting structure of the resulting products and the effectiveness of this strategy, cyclotrimerization has attracted our attention in the synthesis of structurally unique glycoclusters.



Scheme 5-2. Mechanism of transition metal-mediated cyclotrimerization.[20]

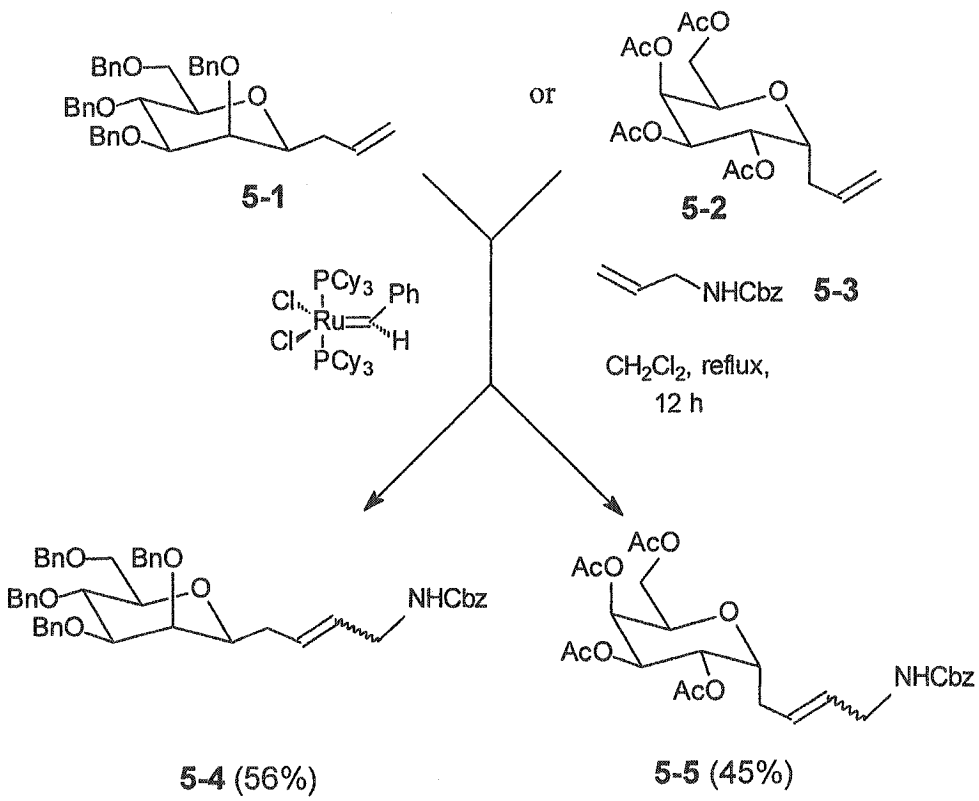
In view of the important role of multivalent carbohydrate derivatives in glycobiology, our group recently reported the synthesis of carbohydrate-containing clusters using various transition metals. We have shown the application of Grubbs' catalyst for the mild cyclotrimerization of terminal alkynes to prepare aryl cluster glycosides.[14b]

Symmetrical alkynes have been converted into 'molecular asterisks-like' hexakis glycosides by cyclotrimerization with dicobalt octacarbonyl.[15c] Recently, *C*-glycosides have been shown to be very important in carbohydrate, enzymatic, and metabolic chemistry because of the stability of the *C*-linkage.[21] In order to synthesize metabolically stable *C*-glycoside clusters with long spacers, we required a convenient cross-metathesis route to prepare carbohydrate derivatives having functionalized aglycons. These extended glycosides can then be further transformed into 'molecular asterisks' using Grubbs' catalyst or dicobalt octacarbonyl. Herein we report the cross-metathesis reaction of a *C*-galactoside with a Cbz-protected allylamine. The product was further transformed into a 'molecular asterisk' using Sonogashira coupling followed by a cyclotrimerization reaction. Thus, a sequence of transition metal catalyzed transformations has led to the synthesis of interesting *C*-linked glycoclusters.

5.2. Results and Discussion

The required starting material, perbenzylated β-*C*-allyl mannopyranoside (**5-1**) for the cross-metathesis reaction was prepared from commercially available 2,3,4,6-tetra-O-benzyl-D-mannopyranose in a few steps by a known procedure.[22] Treatment of **5-1** and N-(benzyloxycarbonyl)allylamine (**5-3**)[23] with 20 mol% of Grubbs' catalyst in refluxing dichloromethane under nitrogen provided a smooth conversion of the starting material into the cross-metathesis product **5-4** in 56% yield as a 3:1 mixture of *E* and *Z* isomers. The remainder of the starting material **5-1** was isolated along with a trace amount of a self-metathesis product. The stereoisomeric mixture was separated by silica gel column chromatography.

Once the reaction conditions were optimized for *N*-(benzyloxycarbonyl)allylamine, the cross-metathesis reaction was also applied to peracetylated allyl α-D-galactopyranoside (**5-2**)[24] which gave **5-5** in 45% as a single *trans* isomer (Scheme 5-3).

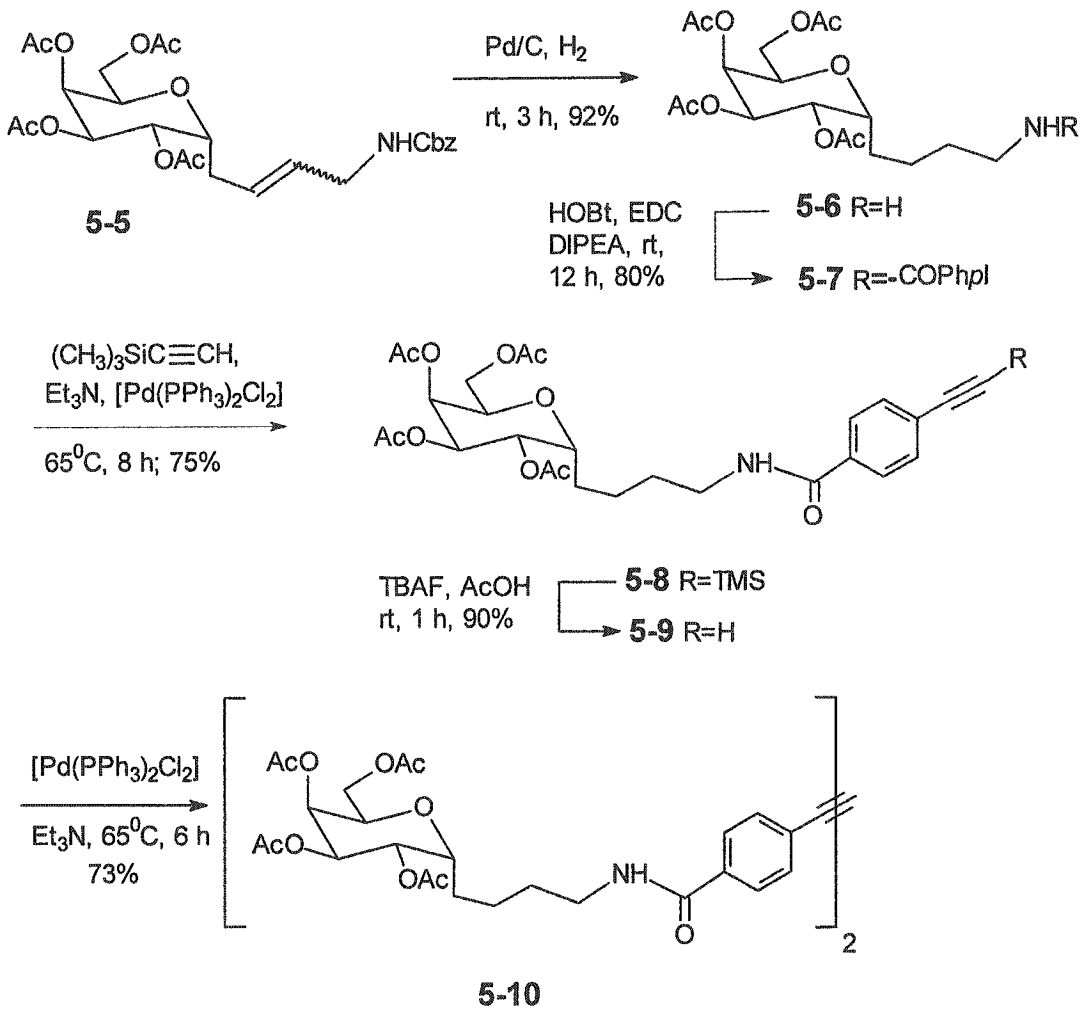


Scheme 5-3. Cross-metathesis of C-allyl glycosides with Cbz-protected allylamine.

In our continuing efforts towards the synthesis of multivalent neoglycoconjugates, it was deemed of interest to synthesize an aryl glycoside cluster, which has been named 'molecular asterisk' with a long spacer arm. As oligosaccharide mimetics, such molecules may find biological utility as 'cluster-type ligands' and may help to elucidate the binding specificity of multiple carbohydrate-protein interactions.[25] The synthesis of such a

'molecular asterisk' was initiated with allyl α-C-galactopyranoside derivative (**5-5**) which was converted into amine **5-6** (10% Pd/C, H_2) in 92% yield. Amine **5-6** was coupled with *p*-iodobenzoic acid to afford aryliodide 5-7 in 80% yield after chromatography (EDC, HOBt, DIPEA, rt, 12 h). The Sonogashira reaction between **5-7** and (trimethylsilyl)acetylene gave **5-8** [$Pd(PPh_3)_2Cl_2$, Et_3N, 65 ^oC, 8 h], which upon treatment with tetrabutylammonium fluoride gave the terminal alkyne **5-9** in 65% overall yield.[26] With compound **5-9**, the next Sonogashira reaction was carried out with **5-7** to afford compound **5-10** [$Pd(PPh_3)_2Cl_2$, Et_3N, 65^0C, 6h] in 73% yield (Scheme 5-4).

With the key intermediate **5-10** in hand, the stage was set for the crucial cyclotrimerization reaction. Unfortunately, the cyclotrimerization of compound **5-10** with Grubbs' catalyst according to our recently published conditions was not successful,[15c] perhaps due to steric hindrance. However, the desired cyclotrimerized product **5-11** was isolated in 53% yield when alkyne **5-10** was treated with the more conventional dicobalt octacarbonyl ($Co_2(CO)_8$) in dioxane at 60 ^oC for 12 h (Scheme 5-5).[15d]

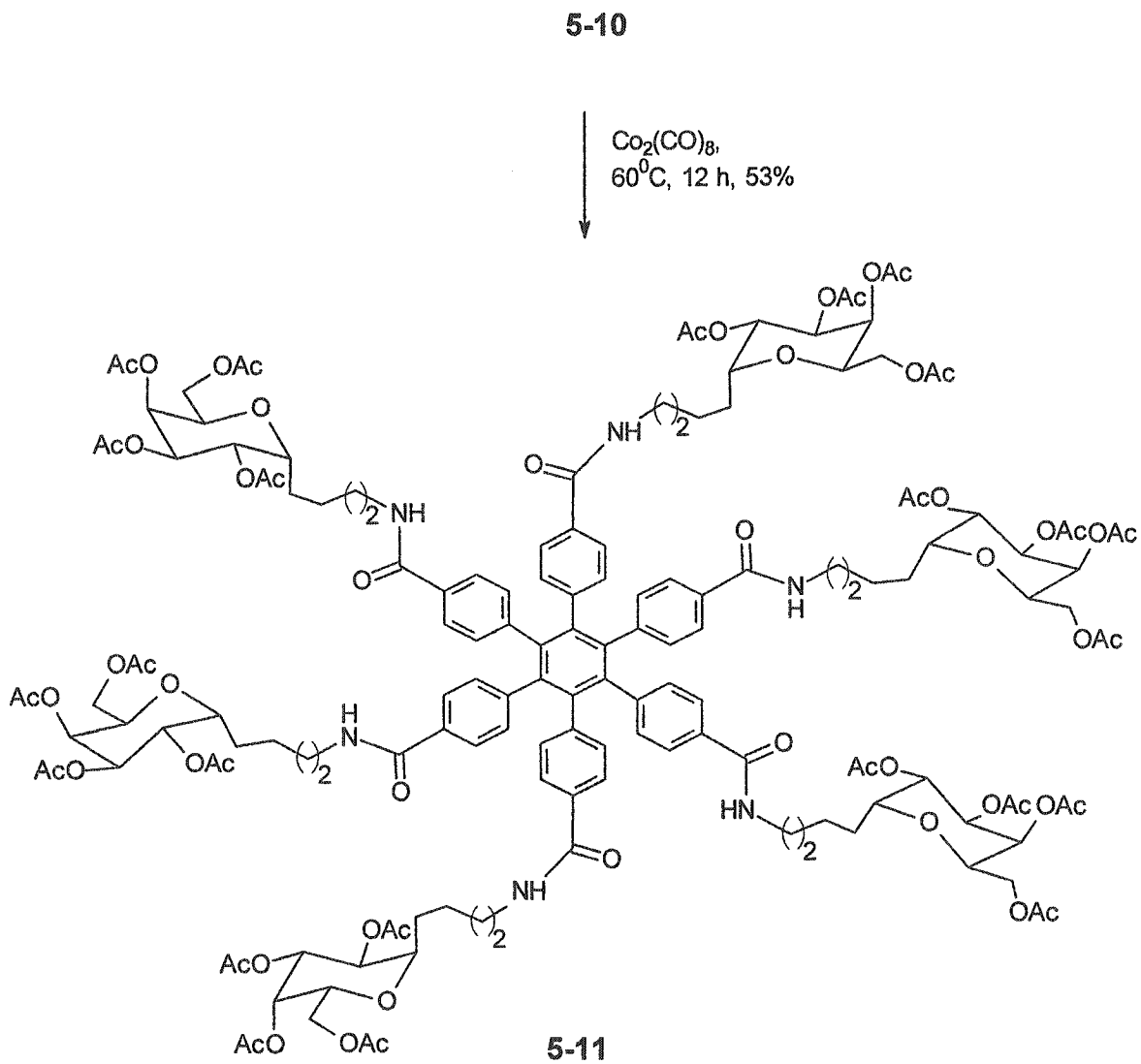


Scheme 5-4. Synthesis of disubstituted acetylene.

5.3. Conclusions

In conclusion, the potential use of cross-metathesis reaction for the introduction of functional groups on the aglycon moiety of *C*-linked glycosides has been demonstrated. It has also been reported the utility of the cross-metathesis reaction towards the synthesis of a novel *C*-linked glycopeptidomimetic for the first time. This cross-metathesis protocol is now widely used by several other research groups.[27] Finally, an aryl glycoside cluster, a so called 'molecular asterisk' has been synthesized from one of these extended glycosides by

sequential Sonogashira and cyclotrimerization reactions in good overall yield. Further studies for the cluster effects of this 'molecular asterisk' promise useful work ahead.



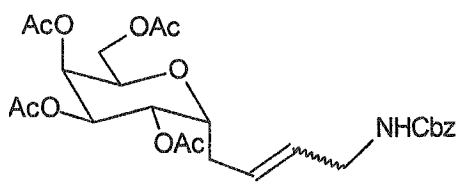
Scheme 5-5. Synthesis of compound **5-11** by cyclotrimerization.

5.4. Experimental Section

General procedure for cross-metathesis reaction

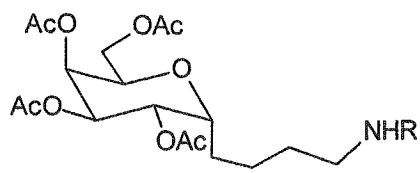
To a 0.06 M solution of C-allyl glycoside in dry dichloromethane was added the appropriate terminal alkene and Grubbs' catalyst (20 mol%), and the reaction mixture was heated at reflux under nitrogen for 12 h. The solution was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography using ethyl acetate and hexane as eluent.

***N*-(Benzyloxycarbonyl)-4-(2,3,4,6-tetra-*O*-benzyl-β-D-mannopyranosyl)-1-but-2-enyl amine (5-4).** Compound **5-4** was isolated as a syrup of pure E isomer; $[\alpha]^{25}_{D}$ +2.2° (*c* 2.75, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.22-2.25 (m, 1H, H-1'a), 2.41-2.46 (m, 1H, H-1'b), 3.27 (t, *J* = 6.8 Hz, 1H, H), 3.42-3.46 (m, 1H, H-5), 3.60 (dd, *J* = 2.6, 9.5 Hz, 1H, H-3), 3.65-3.76 (m, 5H, H-2, H-4', H-6), 3.89 (t, *J* = 9.5 Hz, 1H, H-4), 4.50-4.80 (m, 3 x C*H*$_2$Ph, NH, 7H), 4.87-5.02 (2 d, *J* = 11.8 Hz, C*H*$_2$Ph, 2H), 5.09 (s, 2 H, OC*H*$_2$), 5.38- 5.39 (m, 2H, H-2', H-3'), 7.15-7.39 (m, 25 H, Ar*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 34.0, 42.9, 66.6, 69.7, 72.5, 73.4, 74.1, 74.4, 75.2, 75.4, 78.1, 79.8, 85.4, 127.4, 127.5, 127.6, 127.8, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 138.3, 138.4, 156.1; Anal. Calcd for C46H49NO7: C, 75.90; H, 6.78; N, 1.92. Found C, 75.60; H, 6.92; N, 1.92.



5-5

***N*-(Benzyloxycarbonyl)-4-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-1-but-2-enyl amine (5-5).** Compound **5-5** was isolated as a syrup of pure E isomer; $[\alpha]^{25}_{D}$ +59.4° (*c* 1.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.99, 2.00, 2.04, 2.08 (4s, 12H, OAc), 2.16-2.21 (m, 1H, H-1'a), 2.37-2.42 (m, 1H, H-1'b), 3.75-3.76 (m, 2H, H-4'), 3.97-4.05 (m, 2H, H-5, H-6a), 4.20 (ddd, J = 4.6, 5.2, 14.9 Hz, 1H, H-1), 4.25-4.29 (m, 1H, H-6b), 4.90 (br s, 1H, NH), 5.07 (s, 2H, OCH_2), 5.17 (dd, J = 2.9, 8.9 Hz, 1H, H-3), 5.20 (dd, J = 4.6, 8.9 Hz, 1H, H-2), 5.37 (t, J = 2.9 Hz, 1H, H-4), 5.53-5.54 (m, 2H, H-2', H-3'), 7.27-7.33 (m, 5H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 20.5, 20.6, 20.7, 29.5, 42.8, 61.6, 66.6, 67.3, 67.8, 68.3, 68.6, 71.2, 127.4, 128.0, 128.4, 129.3, 136.5, 156.1, 169.7, 169.8, 169.9, 170.5; HRMS (FAB): *m/z* calcd for $C_{26}H_{34}NO_{11}$ ($M + H^+$): 536.2132; Found: 536.2168.



R=-COPhpI

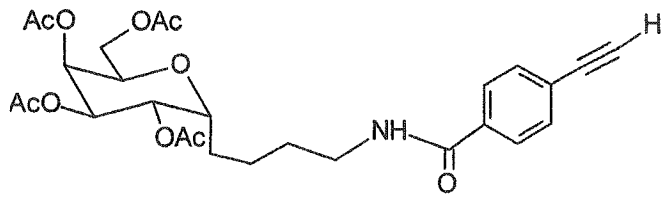
5-7

Synthesis of compound 5-7. To a solution of compound **5-5** (90 mg, 0.172 mmol) in CH_3OH (10 mL) was added 10 mg of Pd/C (10%). The reaction mixture was stirred under H_2 (1 atm) for 3 h at room temperature and then filtered through Celite. Removal of the solvent in vacuo afforded compound **5-6** (62 mg, 92% yield) as a syrup. To a solution of compound **5-6** (60 mg, 0.154 mmol) in DMF (5 mL), were added *p*-iodobenzoic acid (46 mg, 0.185 mmol), HOBt (25 mg, 0.185 mmol), EDC (35 mg, 0.185 mg) and DIPEA (54 μL, 0.309 mmol). The mixture was stirred at room temperature for 12 h and then solvent was

evaporated under reduced presure. The remaining residue was purified by column chromatography (silica gel, hexane/ethyl acetate, 1:1.5) to obtain compound **5-7** as a syrup (78 mg) in 80% yield; $[\alpha]^{25}_{D}$ +54.5° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.40 – 1.73 (m, 6H, H-1', H-2', H-3'), 1.96, 1.98, 2.03, 2.08 (s, 12H, C*H*$_3$CO$_2$-), 3.36–3.43 (m, 2H, H–4'), 3.98 – 4.02 (m, 2H, H-5, H-6a), 4.14-4.17 (dt, *J* = 11, 3.9Hz, 1H, H-1), 4.23 (dd, *J* = 9.1, 13.2 Hz, 1H, H-6b), 5.15 (dd, *J* = 3.2, 9.1Hz, 1H, H-3), 5.20 (dd, *J* = 9.1, 3.9 Hz, 1H, H-2), 5.36 (t, *J* = 3.2 Hz, 1H, H-4), 6.35 (t, *J* = 5.3 Hz, 1H, NH)), 7.45 (d, *J* = 8.3 Hz, 2H, ArH), 7.72 (d, *J* = 8.3 Hz, 2H, ArH); ^{13}C NMR (125 MHZ, $CDCl_3$) δ_C 20.6, 20,71 (CH$_3$CO-), 20.7 (C-2'), 25.35 (C-1'), 29.1 (C-3'), 39.8 (C-4'), 61.3 (C-6), 67.4 (C-4), 67.9 (C-3), 68.2 (C-5), 68.5 (C-2), 71.7 (C-1), 98.2, 128.5, 134.0, 137.6 (ArH), 166.6, 169.8, 170, 170.5 (C=O); HRMS (FAB): *m/z* calcd for $C_{25}H_{32}NO_{10}K$ (M + K^+): 672.0707; Found: 672.1090.

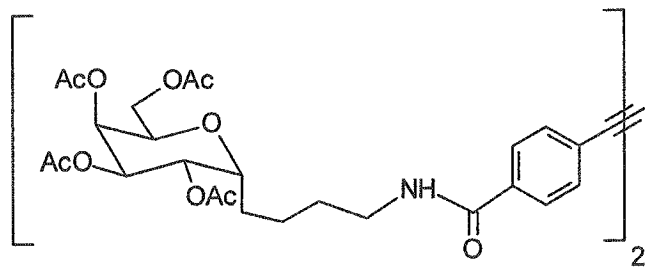
Synthesis of 5-8 by the Sonogashira coupling reaction between trimethylsilylacetylene and 5-7. To a solution of compound **5-7** (60 mg, 0.096 mmol) and trimethylsilylacetylene (27 μL, 0.192 mmol) in DMF : TEA (1:1, 3 mL) was added $Pd(PPh_3)_2Cl_2$ (3.4 mg, 5 mol%) and stirred under nitrogen atmosphere at 65 °C for 8 h. The solvent and TEA were removed in vacuo and the residue was purified by silica gel column chromatography (hexane : ethyl acetate, 1:1) to provide compound **5-8** as a syrup (43 mg) in 75% yield; $[\alpha]^{25}_{D}$ + 51 (*c* = 1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.22 (s, 9H, -SiMe$_3$), 1.41 – 1.75 (m, 6H, H-1', H-2', H-3'), 1.97, 2.00, 2.04, 2.09 (s, 12H, C*H*$_3$CO$_2$-), 3.38–3.47 (m, 2H, H–4'), 3.99 – 4.08 (m, 2H, H-5, H-6a), 4.14-4.17 (m, 1H, H-1), 4.23 (m, 1H, H-6b), 5.15 (dd, *J* = 3.2, 9.1Hz, 1H, H-3), 5.20 (dd, *J* = 9.1, 3.9 Hz, 1H, H-2), 5.37 (t, *J* = 3.2 Hz, 1H, H-4), 6.29 (t, *J* = 5.4 Hz, 1H, NH), 7.47 (d, *J* = 8.5 Hz, 2H, ArH), 7.68 (d, *J* = 8.5 Hz, 2H, ArH); ^{13}C NMR (50 MHZ, $CDCl_3$) δ_C 0.5, 21.3, 21.4, 23.27, 25.9, 29.8, 40.4, 62.0,

68.1, 68.5, 68.8, 69.0, 72.4, 97.5,104.6, 126.9, 127.3, 132.6, 134.7, 167.3, 170.5, 170.7, 171.3; HRMS (FAB): *m/z* calcd for $C_{30}H_{41}NO_{10}SiK$ ($M + K^+$): 642.21; Found: 642.21.



5-9

Desilylation of compound 5-8 to give 5-9. To a solution of compound **5-8** (60 mg, 0.1 mmol) in THF (3 mL), were added tetrabutylammonium fluoride (0.3 mL, 3 eq) and 2 drops of acetic acid. The reaction mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (hexane : ethylacetate, 1:1) to give compound **5-9** as a syrup (50 mg) in 90% yield; $[\alpha]^{25}_D$ + 55° (*c* 0.4, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.35 – 1.70 (m, 6H, H-1', H-2', H-3'), 1.97, 2.00, 2.04, 2.09 (s, 12H, CH_3CO_2-), 3.17(s, 1H, acetylenic), 3.36–3.43 (m, 2H, H–4'), 3.97 – 4.03 (m, 2H, H-5, H-6a), 4.14-4.23 (m, 2H, H-1, H-6b), 5.18-5.21 (m, 2H, H-2, H-3), 5.37 (t, *J* = 3.2 Hz, 1H, H-4), 6.30 (t, *J* = 5.3 Hz, 1H, NH)), 7.50 (d, *J* = 8.5 Hz, 2H, ArH), 7.70 (d, *J* = 8.5 Hz, 2H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ_C 21.3, 21.4, 23.3, 26.0, 29.8, 40.4, 61.9, 68.1, 68.5, 68.8, 69.0, 72.4, 80.1, 83.3, 125.8, 127.4, 132.8, 135.2, 170.5, 170.7, 171.3; HRMS (FAB): *m/z* calcd for $C_{27}H_{33}NO_{10}K$ ($M + K^+$): 570.17; Found: 570.20.



5-10

Synthesis of compound 5-10 by the coupling reaction between 5-7 and 5-9. A solution of compound **5-7** (40 mg, 0.070 mmol), compound **5-9** (57 mg, 0.09 1mmol), and $Pd(PPh_3)_2Cl_2$ (2.5 mg, 5 mol%) in DMF:TEA (1 ml : 3 mL) was stirred under nitrogen at 65 °C for 6 h. After completion of the reaction, the solvent and triethylamine were removed in vacuo and purified by silica gel column chromatography (hexane : ethyl acetate, 1:2) to give compound **5-10** as a syrup (53 mg, 73% yield); $[\alpha]^{25}_D$ + 63.8° (*c* 1,$CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41-1.80 (m, 12H, H-1', H-2', H-3'), 1.98, 2.00, 2.04, 2.09 (s, 24H, C*H*$_3CO_2$-), 3.38–3.46 (m, 2H, H–4'), 3.99 – 4.05 (m, 4H, H-5, H-6a), 4.14-4.24 (m, 4H, H-1, H-6b), 5.18-5.21 (m, 4H, H-2, H-3), 5.38 (t, *J* = 2.8 Hz, 2H, H-4), 6.36 (t, *J* = 5.3 Hz, 2H, NH)), 7.55 (d, *J* = 8.6 Hz, 4H, ArH), 7.74 (d, *J* = 8.6 Hz, 4H, ArH); ^{13}C NMR (50MHZ, $CDCl_3$) δ_C 21.3, 21.4, 23.3, 26.0, 29.8, 40.5, 62.0, 68.1, 68.5, 68.7, 69.0, 70.4, 78.3, 126.5, 127.6, 132.4, 134.9, 167.3, 170.5, 170.7, 171.3; HRMS (FAB): *m/z* calcd for $C_{52}H_{64}NO_{20}K$ ($M + K^+$): 1075.3690; Found: 1075.3658.

Cyclotrimerization of compound 5-10 to give 5-11. A solution of compound **5-10** (40 mg, 0.039 mmol) and $Co_2(CO)_8$ (1.3 mg, 10 mol%) in dry 1,4-dioxane (3 mL) was stired under nitrogen at 60 °C for 12 h. The solvent was removed under reduced pressure and the remaining residue was purified by silica gel column chromatography (hexane : ethyl acetate, 1:5) to give compound **5-11** as a syrup (21mg, 53% yield); $[\alpha]^{25}_D$ 0° (*c* 1, $CHCl_3$); ^{1}H NMR

(500 MHz, $CDCl_3$) δ 1.41-1.80 (m, 36H, H-1', H-2', H-3'), 2.00, 2.01, 2.06, 2.10 (s, 72H, CH_3CO_2-), 3.46 (brs, 6H, H–4'), 4.03 (brs, 12H, H-5, H-6a), 4.18-4.33 (m, 12H, H-1, H-6b), 5.19-5.22 (brd, 12H, H-2, H-3), 5.39 (brs, 6H, H-4), 6.23 (bs, 6H, NH)), 7.58 (brs, 12H, ArH), 7.75 (brs, 12H, ArH); ^{13}C NMR (125MHZ, $CDCl_3$) δ_C 21.3, 21.4, 23.3, 26.0, 29.8, 40.5, 62.0, 68.1, 68.5, 68.7, 69.0, 70.4, 78.3, 126.5, 127.6, 132.4, 134.9, 167.3, 170.5, 170.7, 171.3; ESI-MS: *m/z* calcd for $C_{156}H_{192}N_6O_{60}$ (M + 3 H^+): 1037.4; Found 1037.2.

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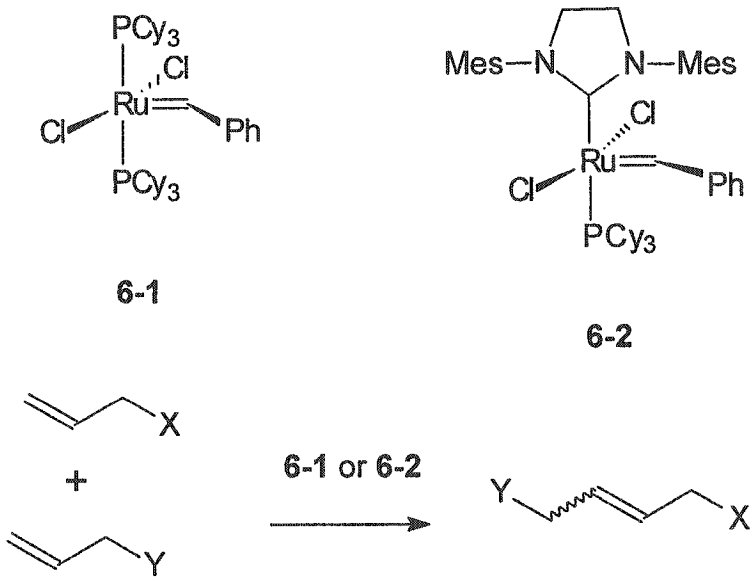
CHAPTER 6 EFFICIENT RUTHENIUM CARBENOID CATALYZED CROSS-METATHESIS OF ALLYL HALIDES WITH OLEFINS

6.1. Introduction

Functionalized allyl halides are very valuable synthetic synthons. They are widely used as *N*-, *O*-, *S*-, and *C*-alkylating reagents in organic synthesis and in the chemical industry.[1] However the synthesis of this kind of building blocks is not straightforward and may suffer from harsh conditions, long synthetic sequences, and low overall yields.[2] Thus, it is necessary to develop more efficient methods for their preparation.

The past decade has witnessed a great development in olefin metathesis. The discovery of ruthenium-carbene catalysts **6-1** and **6-2** has attracted a great deal of attention.[3] In particular, catalyst **6-2,** with its high stability, broad functional group tolerance, and excellent stereoselectivity, has gained widespread application.[4] Thus, one can readily foresee that cross-metathesis (Scheme 6-1) of allyl halides with alkenes would provide a promising method for the preparation of substituted allyl halides, which would serve as a handle for further functionalization. So far only a few examples of cross-metathesis involving allyl halides have been reported.[4d,5] Unfortunately the reactions using catalyst **6-1**[5] could not be reproduced in our hands. Herein efficient syntheses of olefin-containing sugars, or unnatural amino acids, and precursors thereof, by using catalyst **6-2**-mediated cross-metathesis reactions will be reported.

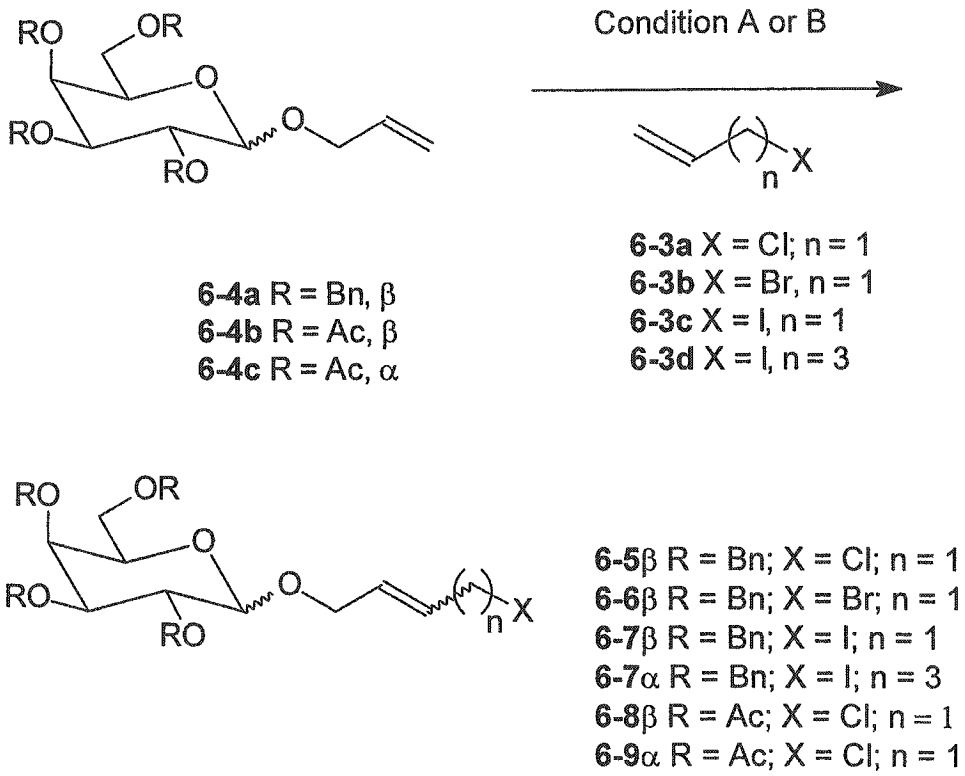


Scheme 6-1. Schematic diagram of olefin cross-metathesis.

6.2. Results and Discussion

It has been widely reported that catalyst **6-2** has demonstrated much higher *E/Z* selectivity compared to **6-1**. To investigate the suitability of catalyst **6-2** for cross-metathesis of allyl halides, the known perbenzylated *O*-allyl galactopyranoside **6-4a**[6,7] was subjected to reactions with different allyl halides under varying conditions (Scheme 6-2). First, the cross-metathesis of **6-4a** with allyl chloride in CH_2Cl_2 was performed under N_2 at reflux for 6 h to obtain compound **6-5** (see Fig. 6-1 for NMR spectrum) in 75% yield (Table 6-1) with a greater than 20:1 *E/Z* ratio. Since the NMR signals for vinyl protons were obscured, the *E/Z* ratio was determined on the basis of ^{13}C NMR spectroscopy. For the *E* isomer, the allylic chlorine-substituted carbon appeared at around δ 44 ppm, while the *Z* isomer appeared upfield by about 5 ppm (δ 38 ppm) due to the γ effect.[3d,8] From the IR spectrum, the *E*

isomer showed a medium to strong absorption band at about 968 cm^{-1} and the *Z* isomer did not show absorption in this range.[2b] The yields for allyl iodides were much lower than that of corresponding allyl chlorides (entry 1 to 5), presumably due to the increased coordinating ability of iodide toward ruthenium or else by oxidative insertion.



Scheme 6-2. Cross-metathesis of allyl galactosides with vinyl halides.

This chelating effect was also hypothesized by Grubbs *et al.* with neighboring carbonyl groups.[4a] However, catalyst **6-2** greatly dampened this chelating effect with improved yield and *E/Z* selectivity compared to catalyst **6-1**. Interestingly, when the iodide positioning was moved away, such as in entry 6 (see Fig. 6-2. for NMR spectrum) with 5-iodo-1-pentene, cross-metathesis provided **6-7a** (see Fig. 6-3 for NMR spectrum) in 64%

yield even when catalyst **6-1** was used. Attempts to vary the reaction conditions with allyl iodide **6-3c** provided no improvement in the reaction yields (<10%) when the catalysts were added in portions or the reactions were run at room temperature.

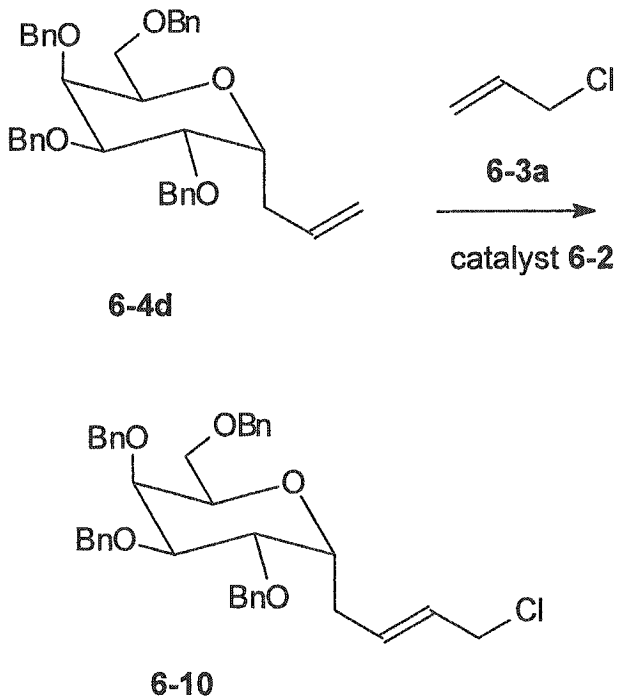
Table 6-1 Results of cross-metathesis of allyl halides with terminal alkenes

entry	Olefin	allyl halide	cond.[a]	product.	yield (E/Z)
1	**6-4a**	**6-3a**	B	**6-5**	75% (>20:1)
2	**6-4a**	**6-3b**	B	**6- 6**	56% (>20:1)
3	**6-4a**	**6-3c**	B	**6-7**	20% (N/D)[b]
4	**6-4a**	**6-3a**	A	**6-5**	51% (4:1)
5	**6-4a**	**6-3b**	A	**6-6**	20% (4:1)[b]
6	**6-4a**	**6-3d**	A	**6-7a**	64% (~5:1)[b,c]
7	**6-4b**	**6-3a**	B	**6-8**	65% (17:1)
8	**6-4c**	**6-3a**	B	**6-9**	55% (15:1)
9	**6-4d**[d]	**6-3a**	B	**6-10**	72% (>20:1)

[a]Method A: N_2, CH_2Cl_2, 20 mol% **1**, reflux, 6 h; method B: N_2, CH_2Cl_2, 10 mol% **2**, reflux, 6 h. [b]yields estimated on the basis of 1H NMR data of crude reaction mixtures. [c] Prepared from 5-iodo-1-pentene. See scheme 6-2.

Cross-metathesis of galactosides **6-4b**,[7] **6-4c**,[9] and **6-4d**[10] (Table 6-1) gave similar yields and stereoselectivities. These results indicated that the nature of sugar protecting groups together with the anomeric configurations did not play a significant role in terms of stereo and electronic effects. In all cases using catalyst **6-2**, less than 5% of homodimers were observed that could be recycled if desired.

It is worth mentioning that C-linked galactoside **6-10** (see Fig. 6-6 for NMR) (Scheme 6-3), obtained from cross-metathesis of **6-4d** and allyl chloride, is a very useful alkylating agent that could not be prepared easily through other routes. Presently, C-linked glycosides have gained tremendous attention because of their biological stability. Thus, **6-10** can easily be further functionalized to other C-linked glycomimetics.[11]



Scheme 6-3. Synthesis of compound **6-10** by cross-metathesis.

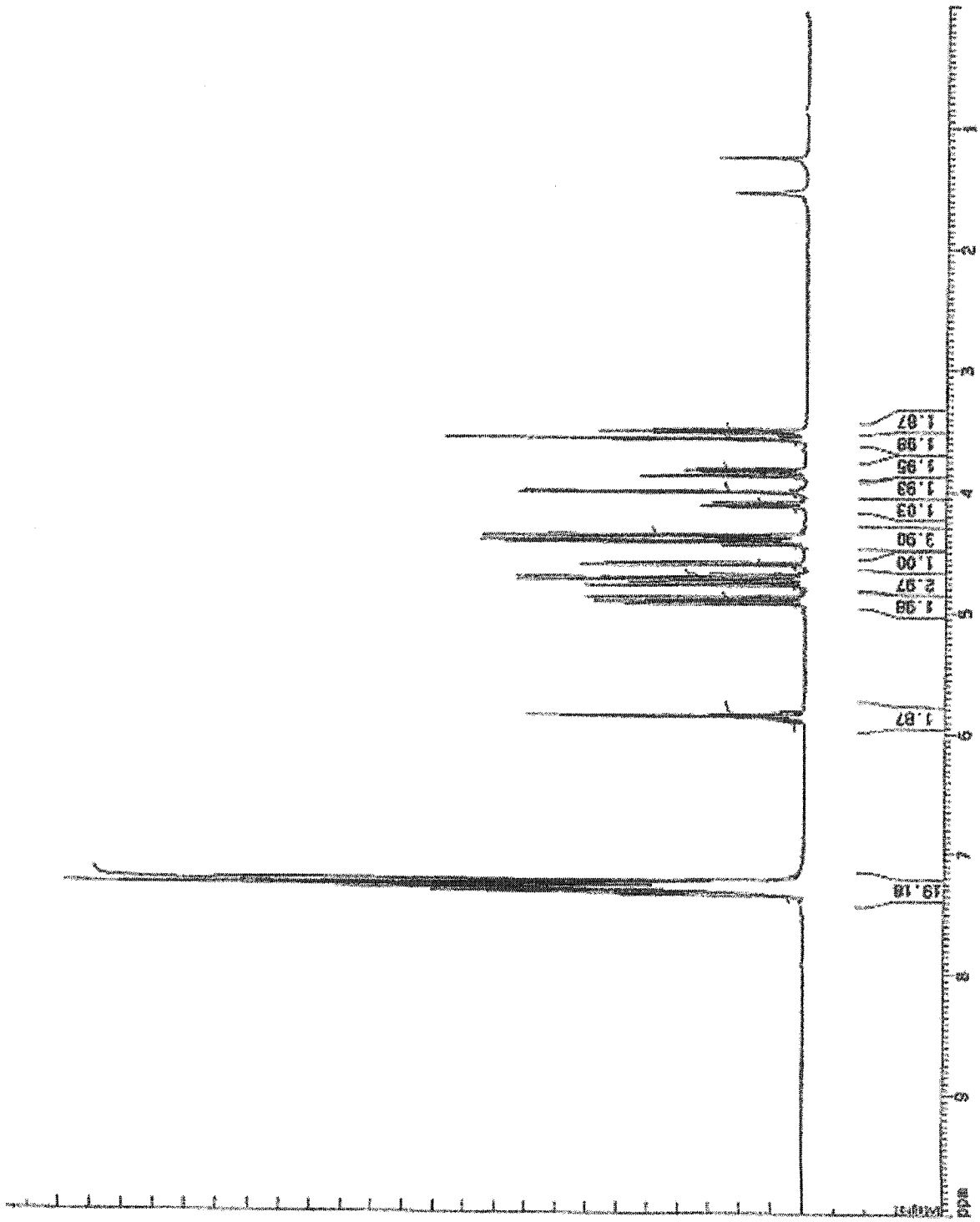


Fig. 6-1. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **6-5**.

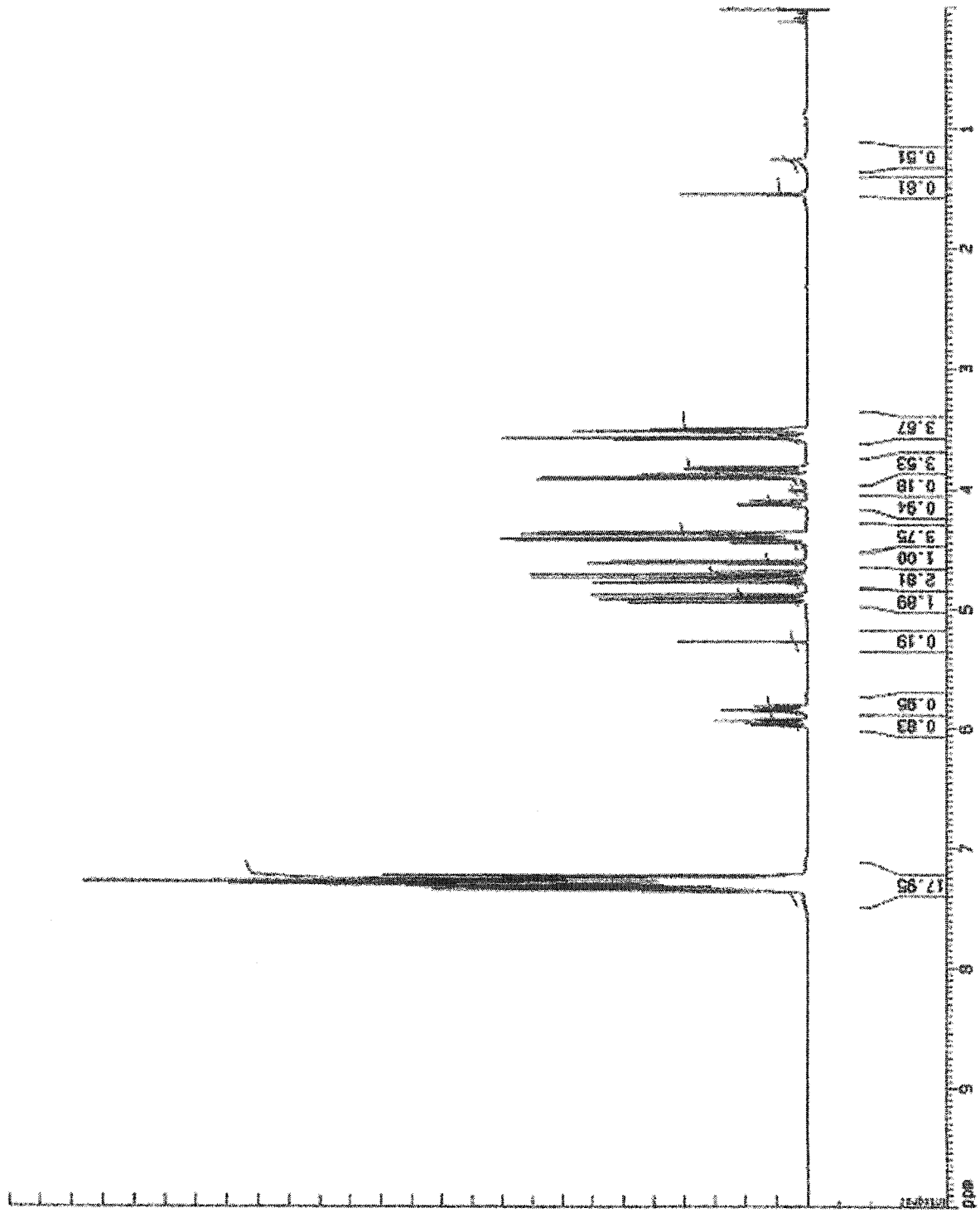


Fig. 6-2. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **6-6**.

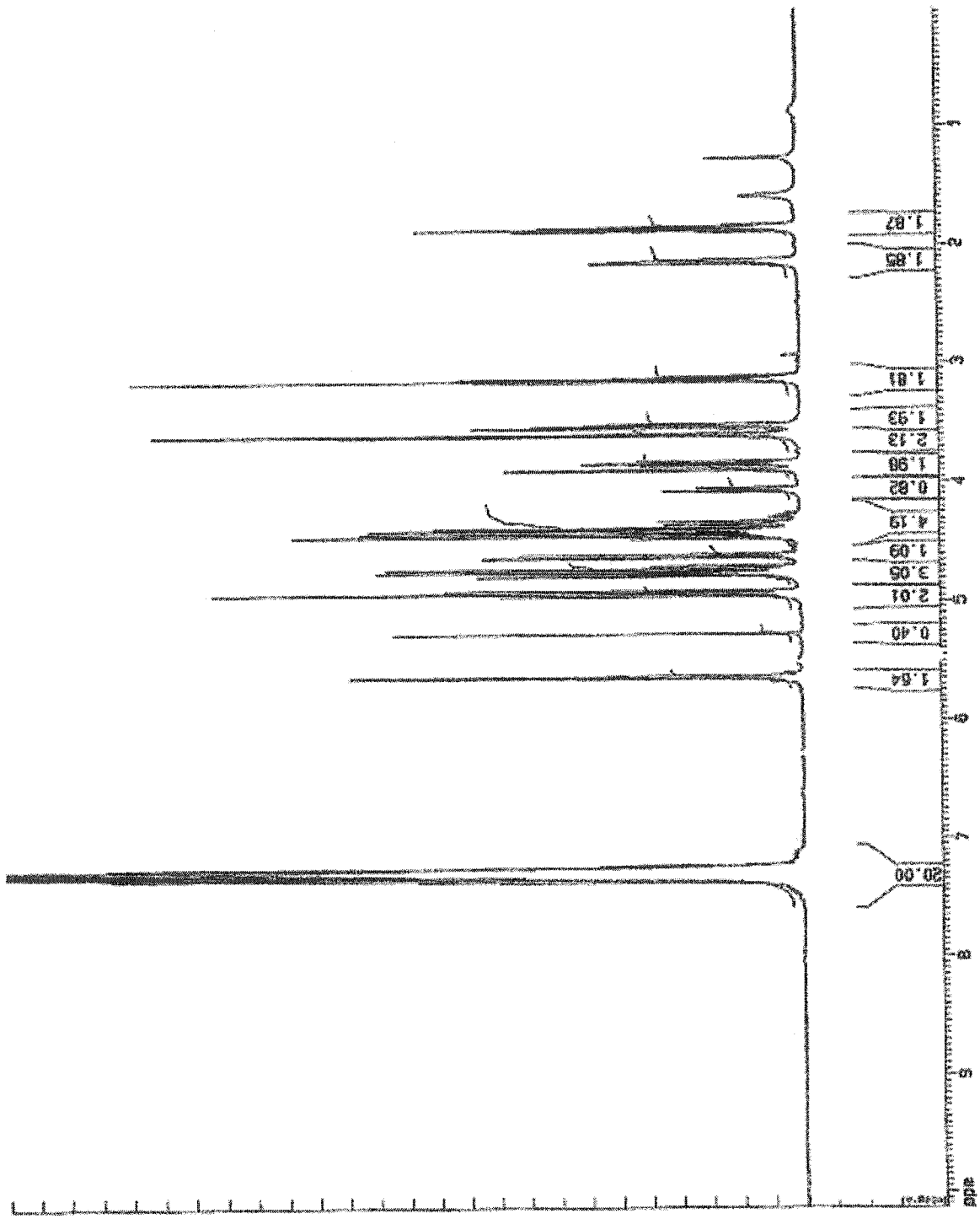


Fig. 6-3. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **6-7**.

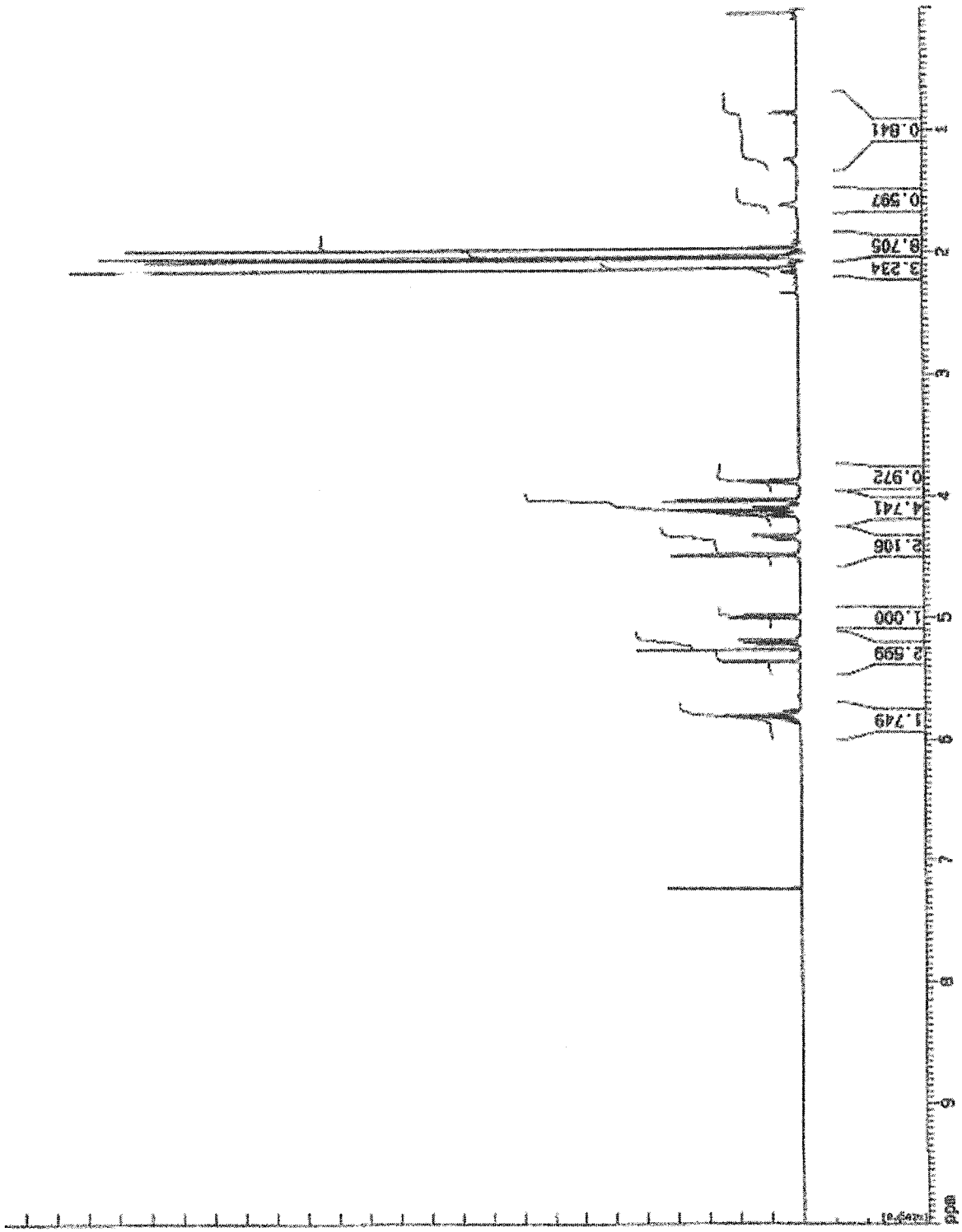


Fig. 6-4. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **6-8**.

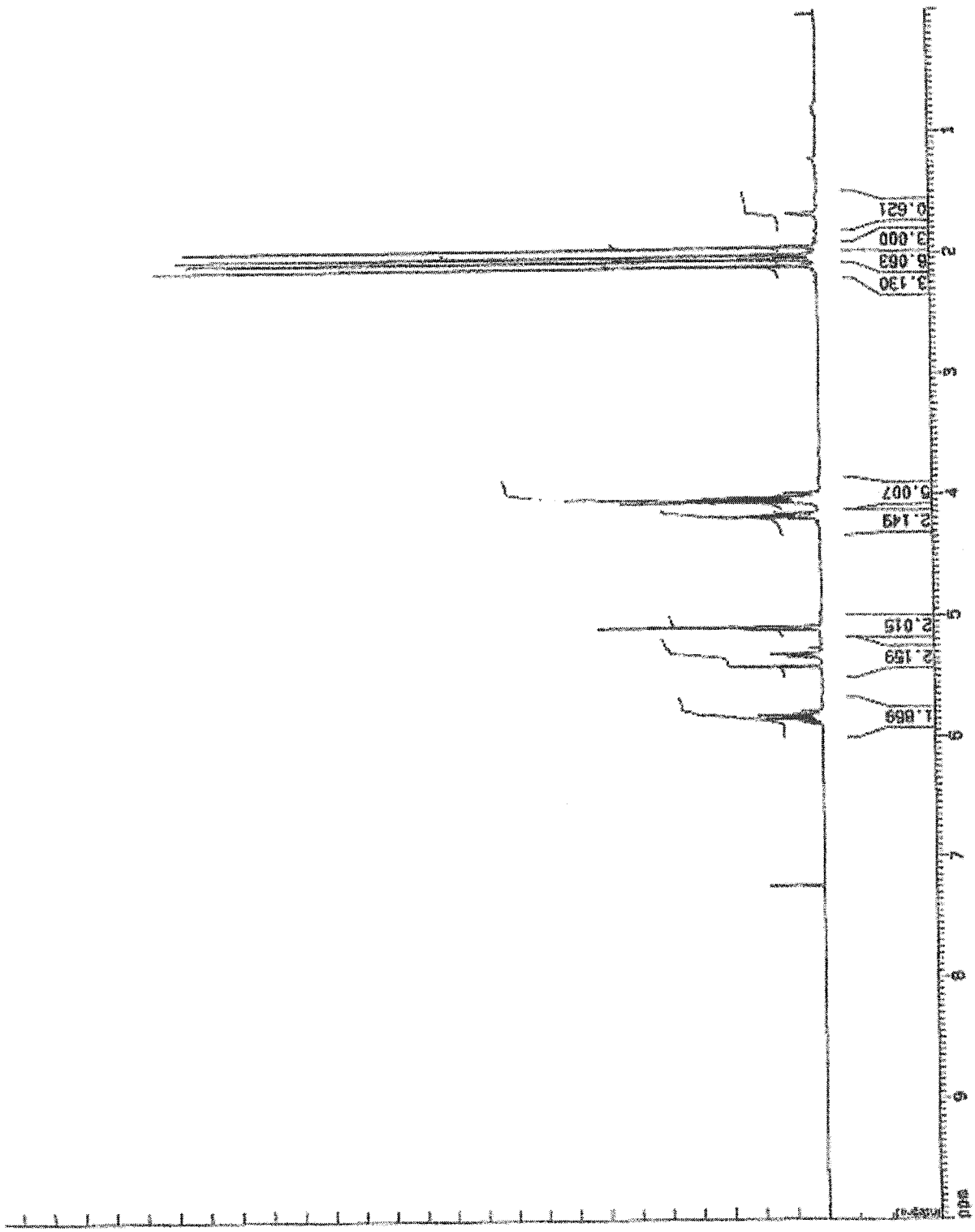


Fig. 6-5. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **6-9**.

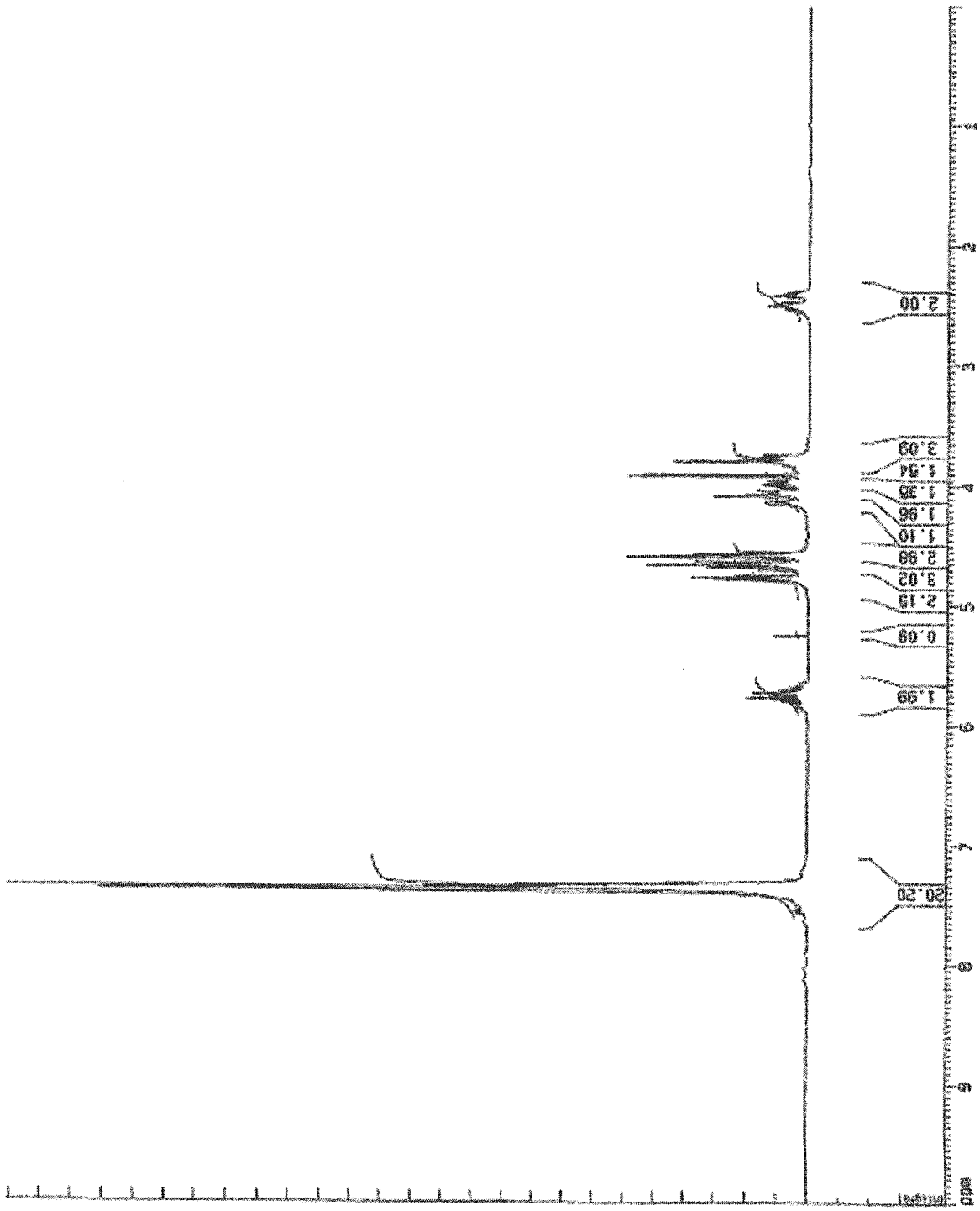
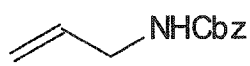
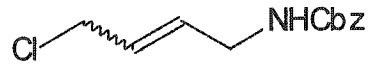
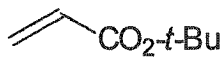
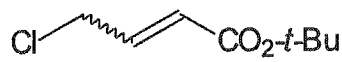
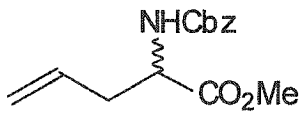
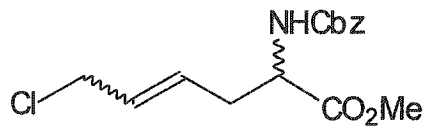


Fig. 6-6. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **6-10**.

Table 6-2 Summary of cross-metathesis of non-carbohydrates substrates with allyl halides.[a]

olefin	product	yield (E/Z)
 **6-11a**	 **6-12a**	65% (>20:1)
 **6-11b**	 **6-12b**	67% (>20:1)
 **6-11c**	 **6-12c**	70% (>20:1)

[a] Conditions: 10 mol% **6-2**, CH_2Cl_2, N_2, reflux, 6 h.

To further investigate the scope of this procedure, three noncarbohydrate substrates were chosen (Table 6-2). Under the above conditions with catalyst **6-2**, compound **6-11a** reacted with allyl chloride to obtain product **6-12a** in good yield and with excellent stereoselectivity. The second entry is an interesting one, since both the electron-deficient *t*-butyl acrylate (**6-11b**) and allyl chloride (**6-3a**) were poor reacting partners toward catalyst **6-1**. However, with catalyst **6-2**, the reaction provided a reasonable yield and remarkable stereoselectivity with the *trans* isomer being the only detectable stereoisomer observed. Compound **6-12c**, a cross-metathesis product from allyl halide and allylglycine derivative, is

an interesting compound, which can be further functionalized for the synthesis of peptidomimetics.

To provide an application of these allyl halides, compound **6-5** was selected for *O*-alkylation (Scheme 6-4). In this case, 1,4-dihydroxymethyl benzene **6-13** was treated with NaH in DMF and then reacted with **6-5** to provide **6-14** in 55% yield at room temperature.

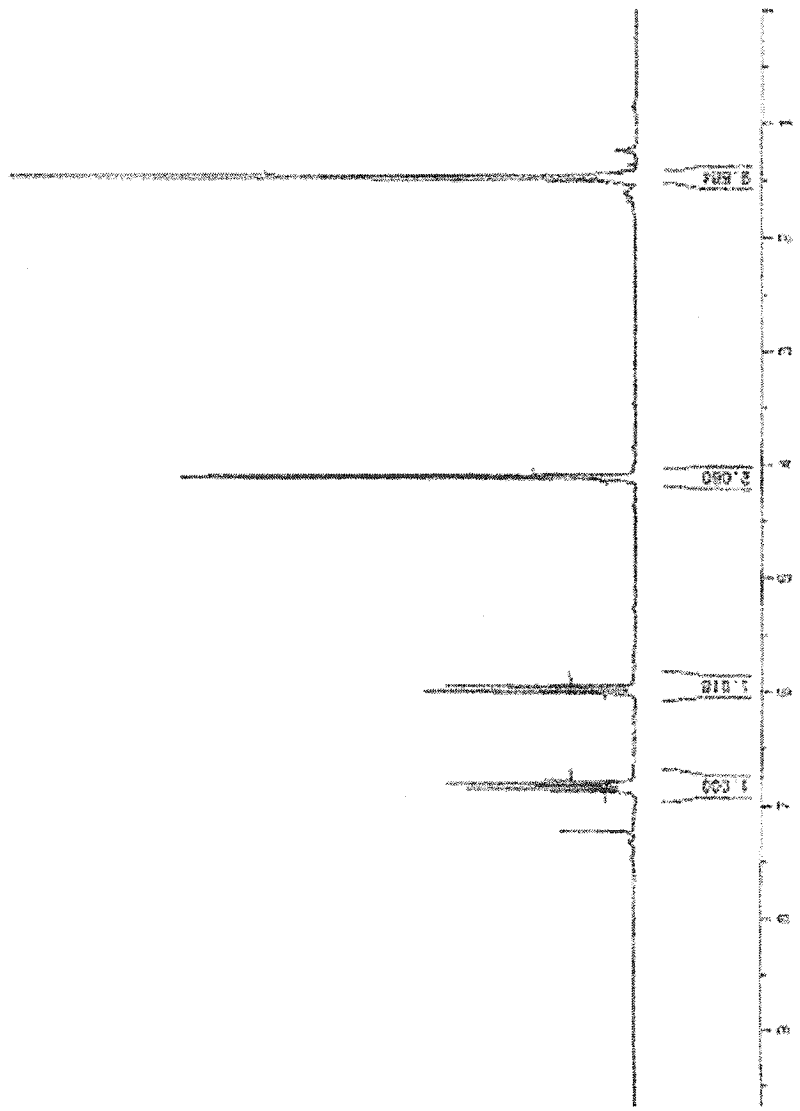
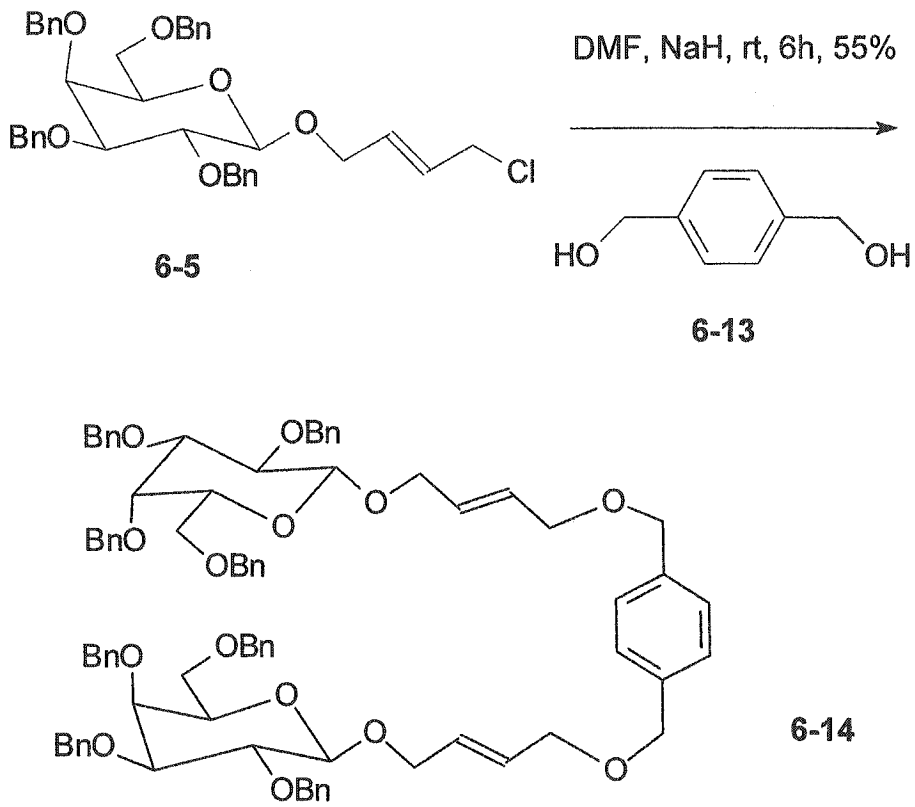


Fig. 6-7. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **6-12b**.

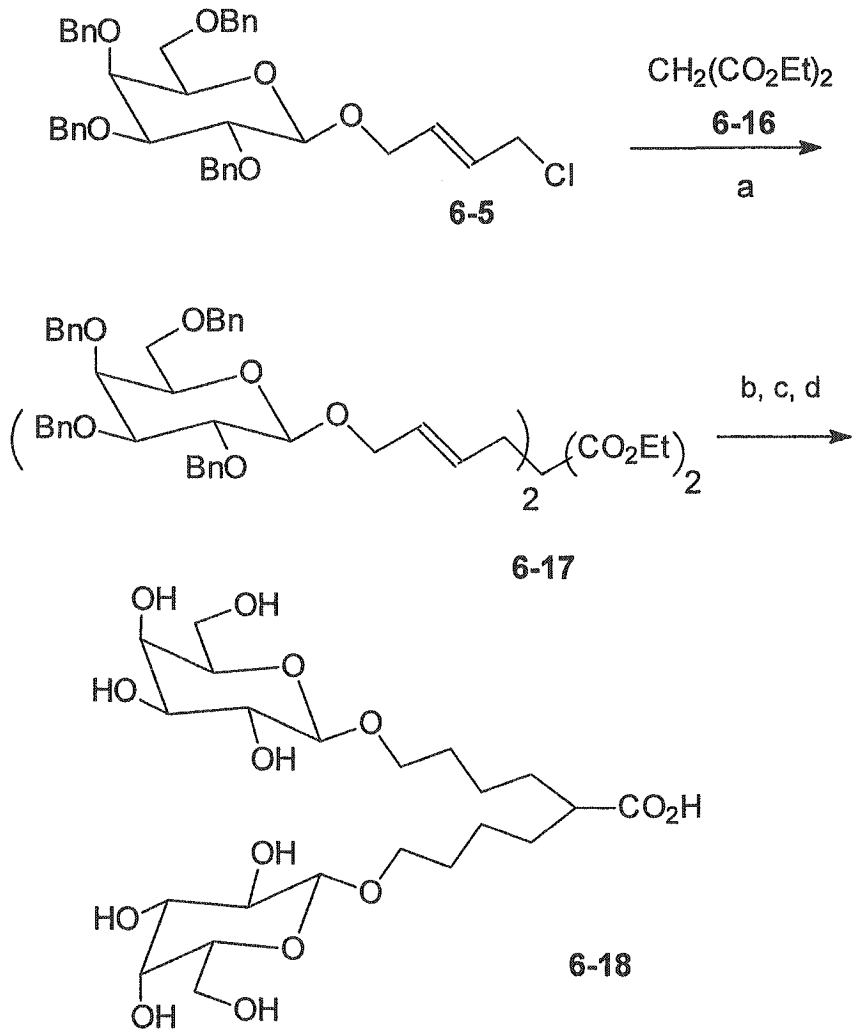
In the second case, the C-alkylating utility of **6-5** was tested (Scheme 6-5). After treatment with NaH in dry THF, diethyl malonate **6-16** reacted smoothly with **6-5** to provide the double-alkylated malonate ester **6-17** (see Fig. 6-8 for NMR) in an excellent yield at room temperature. The monodecarboxylation of **6-17** proved to be problematic. Due to the presence of the carbohydrate moiety, common procedures involving DMSO at high temperature failed.[12,13]



Scheme 6-4. Synthesis of compound **6-14**.

Eventually, ester **6-17** was hydrolyzed first by refluxing in 2M KOH/MeOH for 1 d and then decarboxylated in acetonitrile.[14] Interestingly, acetonitrile had to be degassed and

the catalyst CuCl could not be used at more than 25 mol %.[15] Finally, hydrogenolysis provided **6-18** in good yield. This fully deprotected **6-18** can be a useful building block for the synthesis of high-order glycodendrimers.



Scheme 6-5. Synthesis of compound **6-18**. (a) THF, NaH, rt, 4 h, 90%; (b) 2 M KOH, MeOH, reflux, 1 d, N_2, 85%; (c) 20 mol% CuCl, 24 h, CH_3CN, N_2, 82%; (d) 10% Pd-C, ethanol, 24 h, 95%.

As mentioned before, *C*-linked carbohydrates are resistant to hydrolysis under physiological conditions and are very useful as starting material for the synthesis of or as component of biologically stable glycomimetics. Herein methyl 3,5-dihydroxybenzoate **6-19** was treated with potassium carbohydrate and crown ether in DMF, and then reacted with the *C*-linked galactoside **6-10** to provide the double alkylated benzoate **6-20** (see Fig. 6-9 for NMR spectrum) in excellent yield (Scheme 6-6). Then **6-20** was subjected to hydrolysis, followed by hydrogenolysis to give the fully deprotected compound **6-21** (see Fig. 6-10 for NMR spectrum) in good yield, which can be used for the preparation of glycoclusters.

Compound **6-20** could also be hydrolyzed in KOH/MeOH and then coupled with N-hydroxysuccinimide, mediated by EDC to prepare the corresponding NHS ester **6-22** in excellent yield. Treatment of **6-22** with diamine **6-23**[16] in THF easily generated the glycocluster **6-24** (see Fig. 6-11 for NMR spectrum) with four copies of *C*-linked galactosides. This further proved the usefulness of the substituted allyl halides.

In conclusion, cross-metathesis of allyl halides and monosubstituted olefins mediated by catalyst **6-2** has been studied and was shown to occur in moderate to good yields and with excellent *E/Z* selectivity. The utility of these compounds has been also explored.

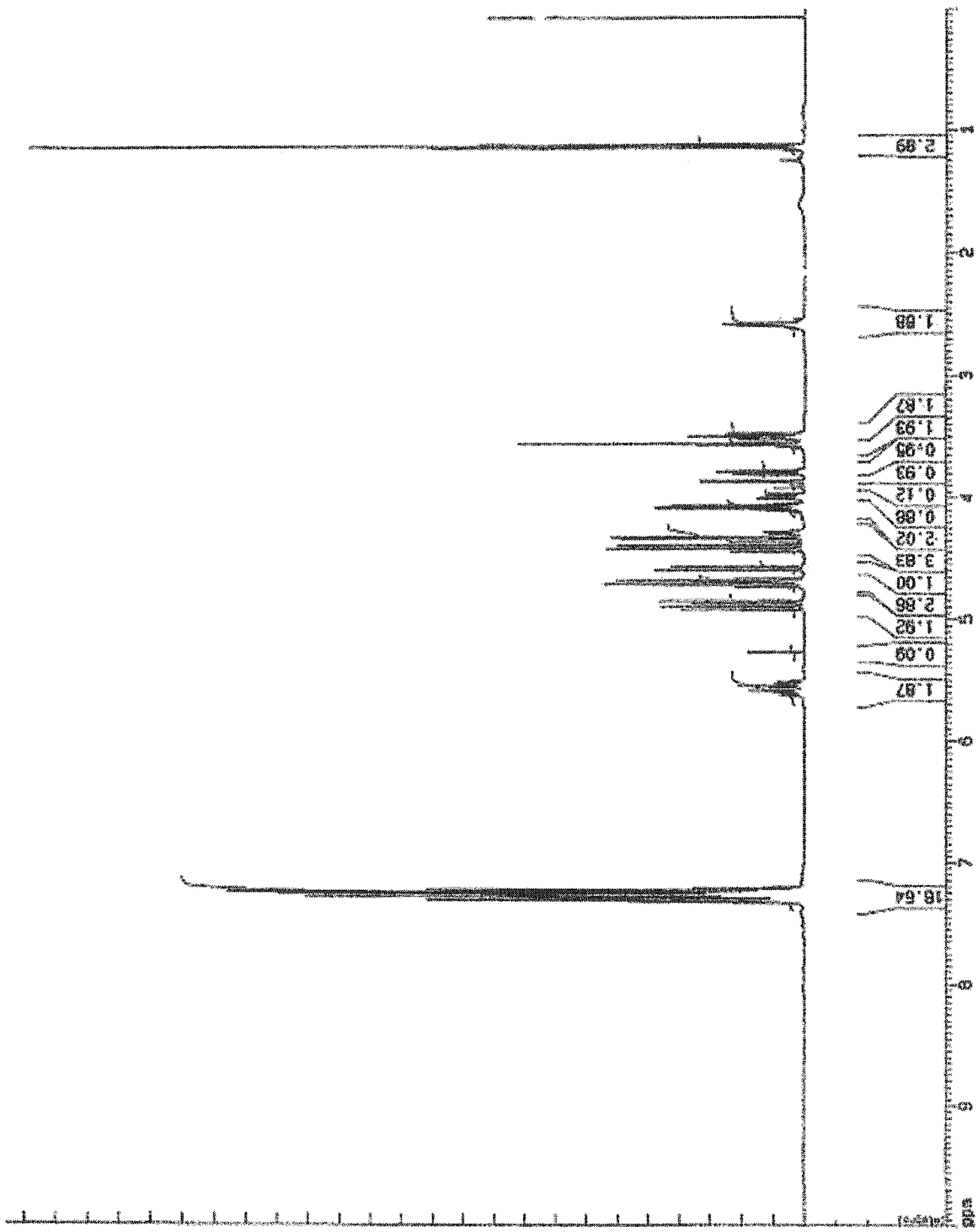
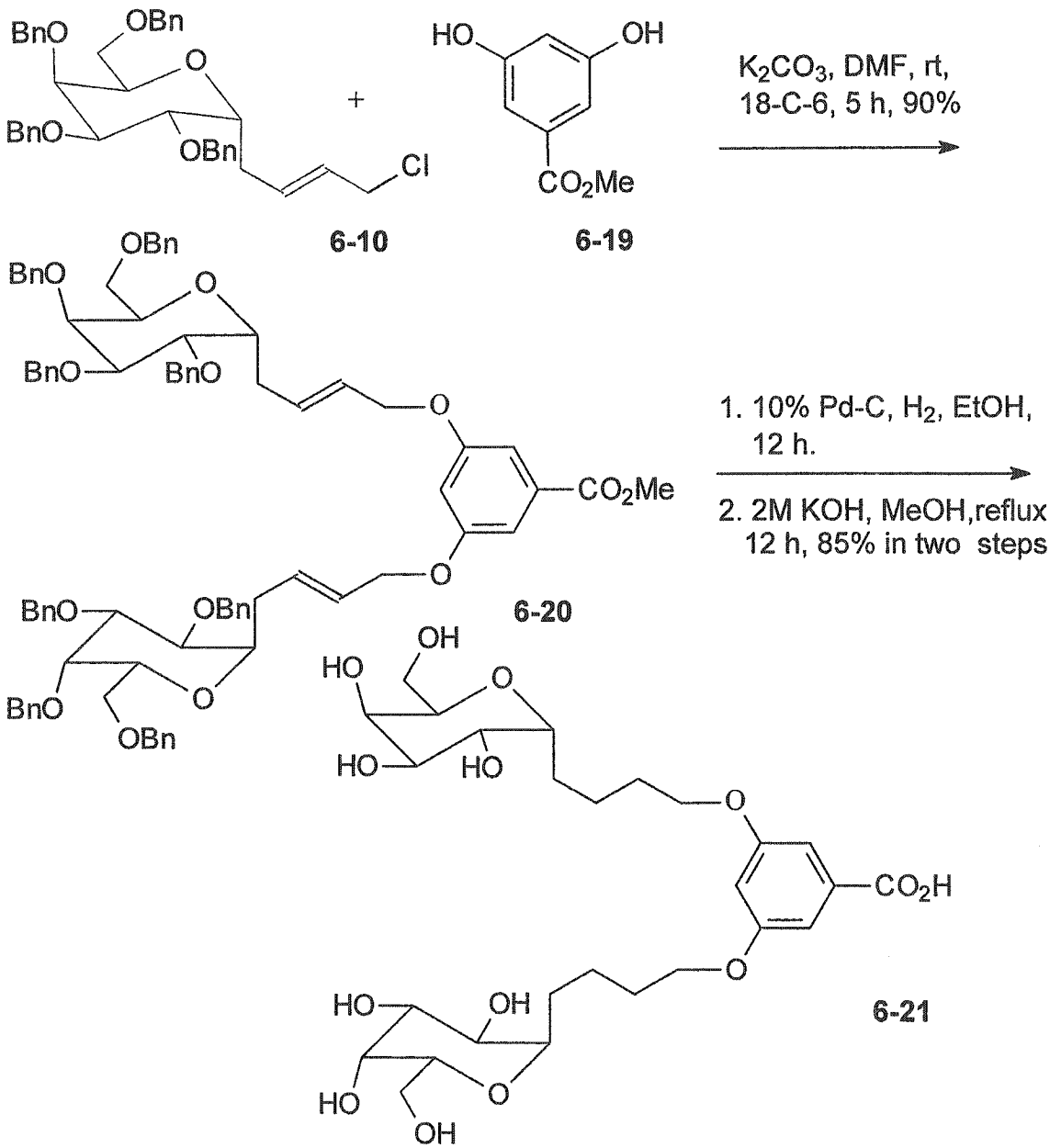
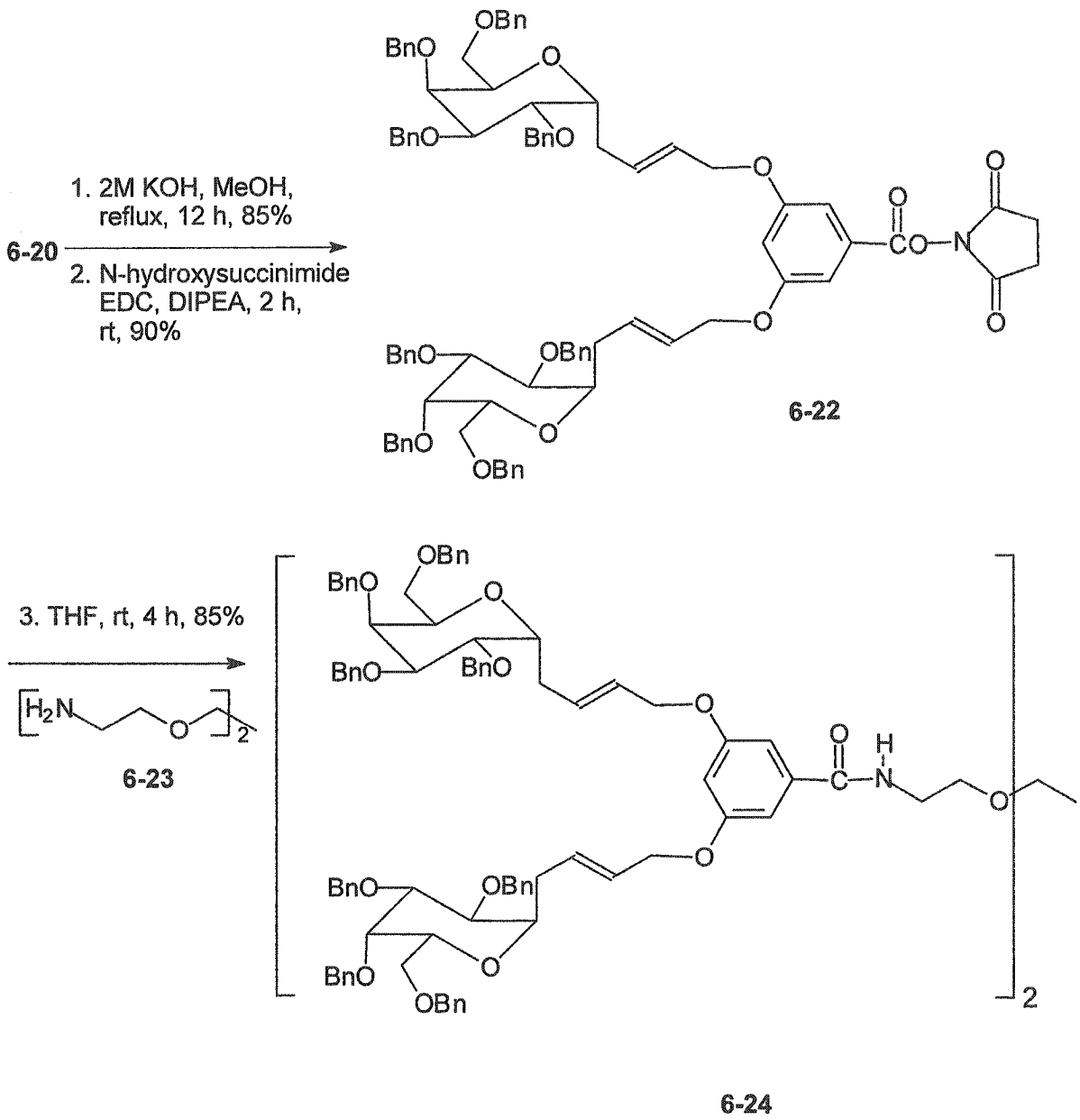


Fig. 6-8. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **6-17**.



Scheme 6-6. Synthesis of compound **6-21**.



Scheme 6-7. Synthesis of compound **6-24**.

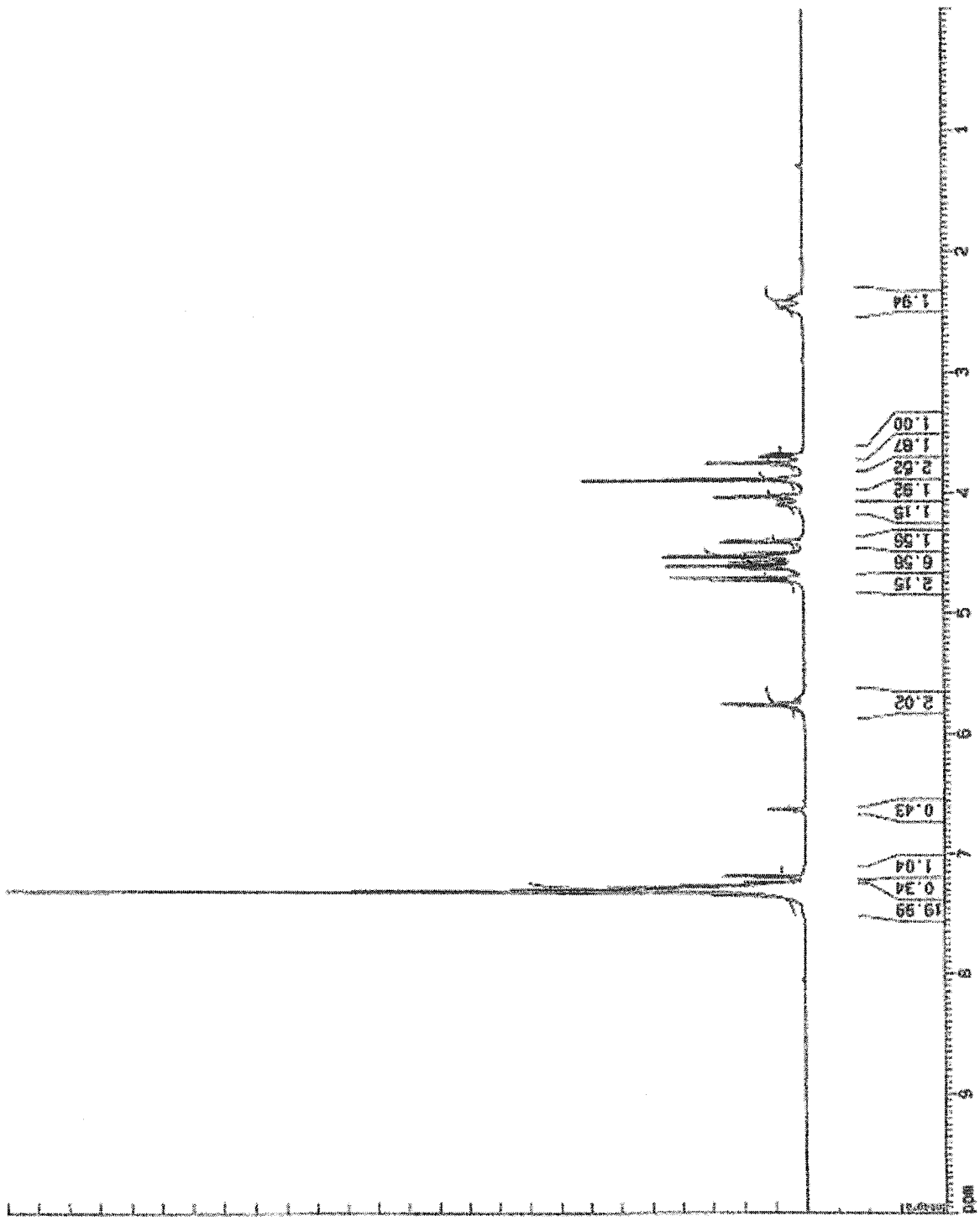


Fig. 6-9. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **6-20**.

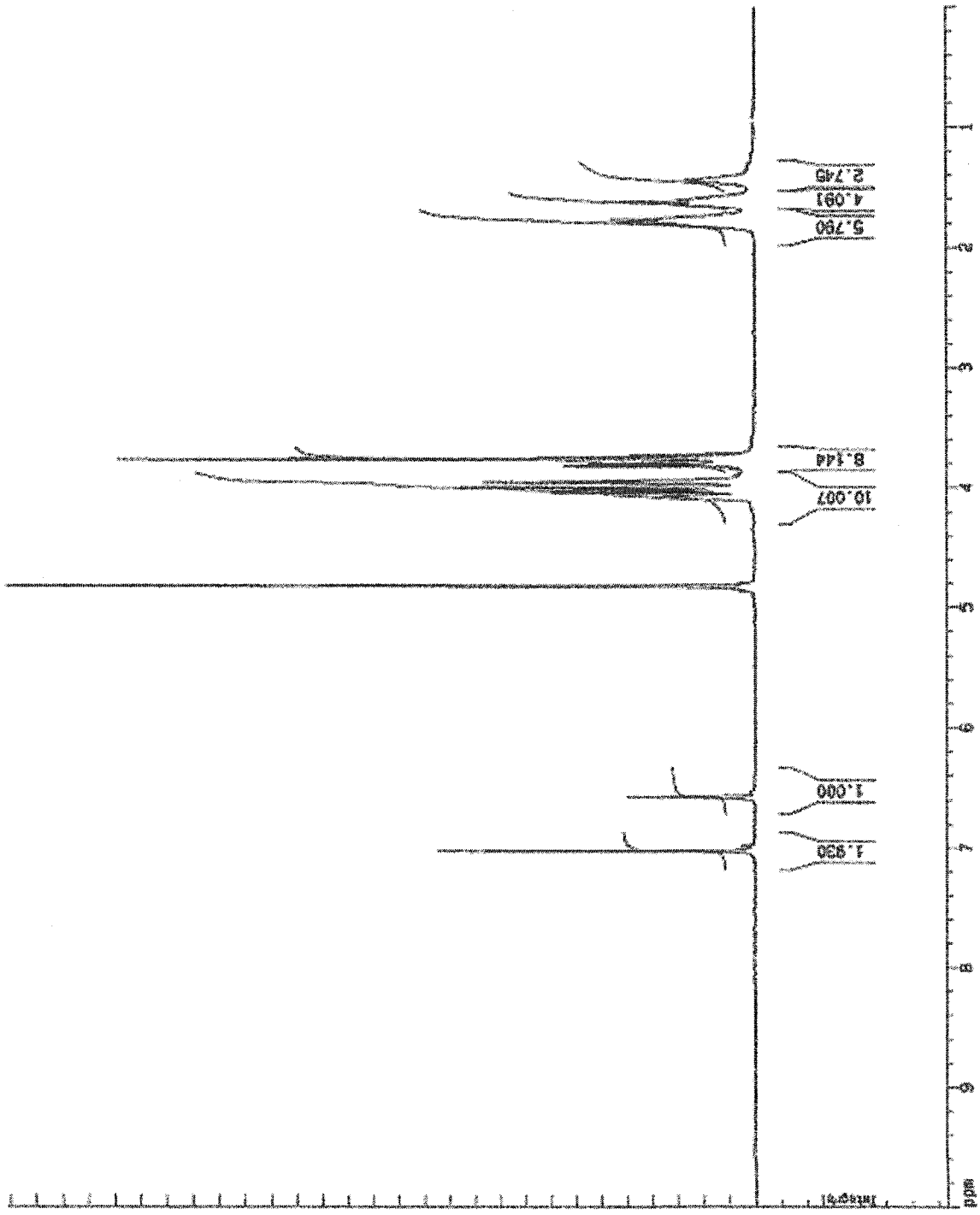


Fig. 6-10. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **6-21**.

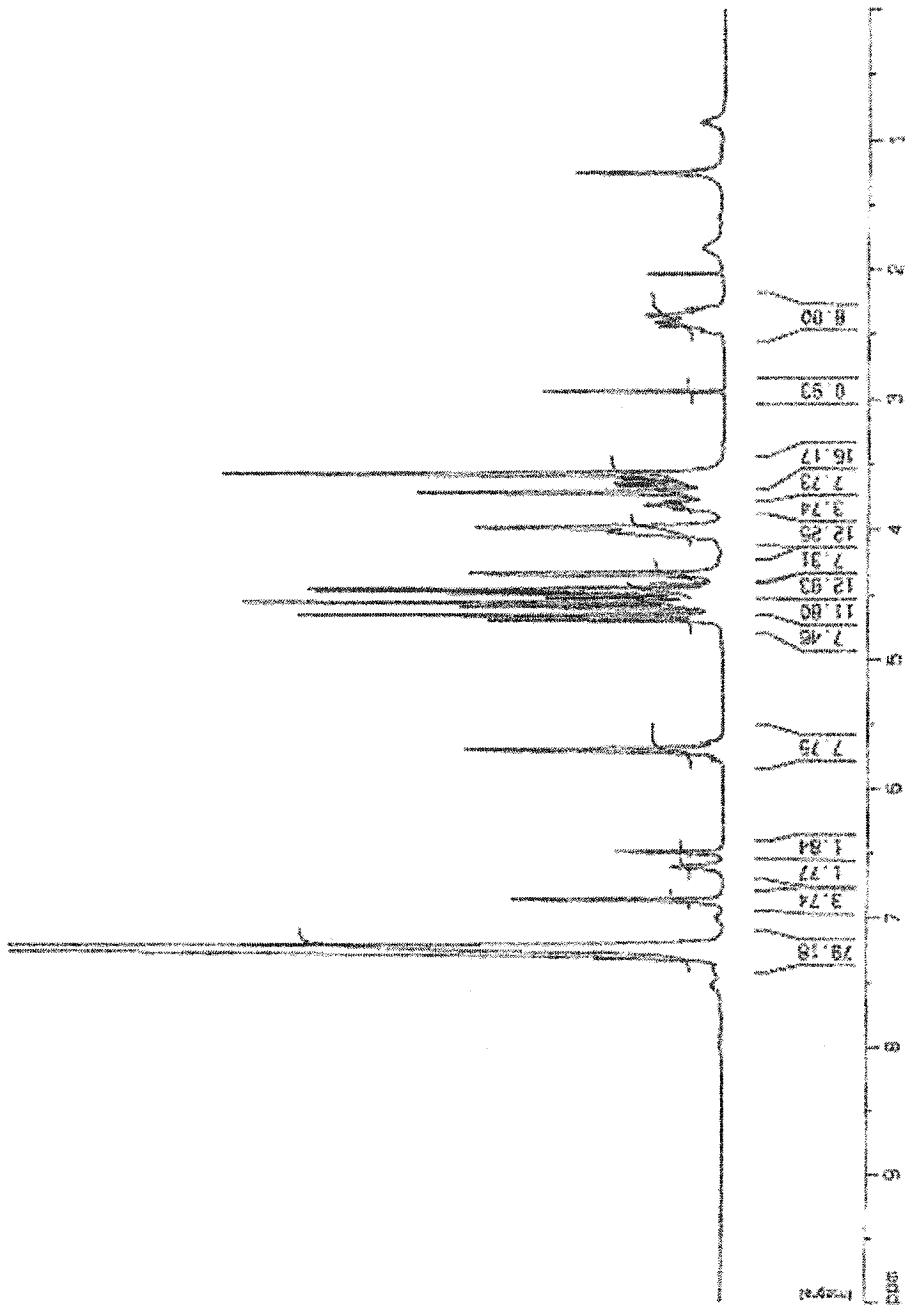
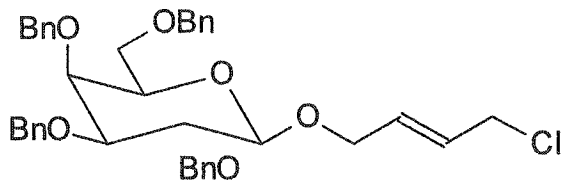


Fig. 6-11. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **6-24**.

6.3. Experimental Section

General Procedure for the Cross-Metathesis Reactions. Typically, a terminal alkene substrate (0.2 mmol) was dissolved into dry methylene chloride (3.3 mL) to make a solution of 0.06 M. To the solution were added Grubbs' catalyst **6-1** or **6-2** (20 mol % for **6-1** and 10 mol % for **6-2**) and an allyl halide (0.5 mmol, 2.5 eq.), and then the mixture was refluxed under nitrogen for 6 h. The solvent was evaporated *in vacuo* and the residue was purified by silica gel chromatography using ethyl acetate and hexane.

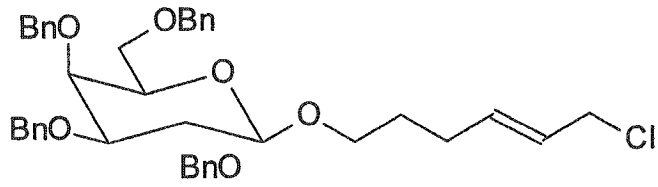


6-5

4'-Chloro-but-2'-ene-1'-yl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside (6-5). Once the reaction was completed, the mixture was purified by silica gel column chromatography using ethyl acetate and hexane (20:80) to obtain a thick syrup with a *E/Z* ratio >20:1. The *trans* isomer **6-5** was isolated from the mixture as a single isomer; for *trans* isomer, $[\alpha]^{20}_{D}$ = - 8.0° (*c* 2.0, $CHCl_3$); FTIR (film) 968 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.24-7.36 (m, 20H, aromatic), 5.82-5.91 (m, 2H, H-2' and H-3'), 4.93 (d, *J* = 11.6, 1H, benzylic), 4.89 (d, *J* = 10.9, 1H, benzylic), 4.77 (d, *J* = 10.9, 1H, benzylic), 4.74 (d, *J* = 11.8, 1H, benzylic), 4.70 (d, *J* = 11.8, 1H, benzylic), 4.60 (d, *J* = 11.6, 1H, benzylic), 4.40-4.45 (m, 2H, benzylic and H-1a'), 4.38 (d, *J* = 7.5, 1H, H-1), 4.37 (d, *J* = 7.6, 1H, benzylic), 4.19 (dd, *J* = 4.9, 13.7, 1H, H-1b'), 4.05 (d, *J* = 5.7, 2H, H-4'), 3.88 (bd, *J* = 2.7, 1H, H-4), 3.83 (dd, *J* = 7.5, 9.7, 1H, H-2), 3.58 (m, 2H, H-6), 3.49-3.52 (m, 2H, H-3 and H-5); ^{13}C NMR

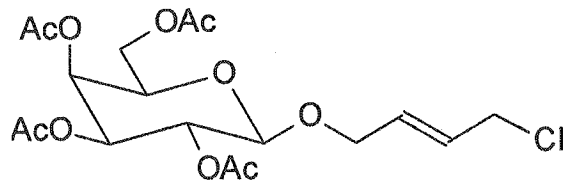
(125.7 MHz, $CDCl_3$) δ_C 138.7, 138.6, 138.5 and 137.9 (aromatic), 130.7 (C-2'), 128.4, 128.3, 128.2 and 128.1(aromatic), 127.9 (C-3'), 127.8, 127.7, 127.6 and 127.5 (aromatic), 103.1 (C-1), 82.2 (C-5 or C-3), 79.6 (C-2), 75.3, 74.5 and 73.5 (benzylic), 73.4 (C-3 or C-5 and C-4), 73.1 (benzylic), 68.8 (C-1'), 68.6 (C-6), 44.3 (C-4'); ESI-MS: calcd for $C_{38}H_{41}ClO_6 + NH_4^+$: 646.8; Found: 646.6.

4'-Bromo-but-2'-ene-1'-yl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside (6-6). The reaction mixture was purified by silica gel column chromatography using ethyl acetate and hexane (20:80) to obtain a thick syrup with a *E/Z* ratio >20:1. The *trans* isomer **6-6** was isolated from the mixture; for *trans* isomer, $[\alpha]^{20}_D$ = -15.8 ° (c 3.0, $CHCl_3$); FTIR (film) 970 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.22-7.36 (m, 20H, aromatic), 5.94-5.98 (m, 1H, H-3'), 5.81-5.86 (m, 1H, H-2'), 4.93 (d, *J* = 11.6, 1H, benzylic), 4.89 (d, *J* = 11.4, 1H, benzylic), 4.77 (d, *J* = 11.4, 1H, benzylic), 4.75 (d, *J* = 11.8, 1H, benzylic), 4.70 (d, *J* = 11.8, 1H, benzylic), 4.61 (d, *J* = 11.6, 1H, benzylic), 4.40-4.45 (m, 2H, benzylic and H-1a'), 4.38 (d, *J* = 7.5, 1H, H-1), 4.37 (d, *J* = 7.6, 1H, benzylic), 4.12 (dd, *J* = 5.0, 13.4, 1H, H-1b'), 3.91 (d, *J* = 7.4, 2H, H-4'), 3.88 (bd, *J* = 2.7, 1H, H-4), 3.83 (dd, *J* = 7.5, 9.7, 1H, H-2), 3.56 (m, 2H, H-6), 3.50-3.52 (m, 2H, H-3 and H-5); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 138.7, 138.6, 138.5 and 137.9 (aromatic), 131.1 (C-2'), 128.5, 128.4, 128.3, 128.2 and 128.1(aromatic), 127.9 (C-3'), 127.8, 127.7, 127.6 and 127.5 (aromatic), 103.1 (C-1), 82.2 (C-3 or C-5), 79.5 (C-2), 75.3, 74.5 and 73.5 (benzylic), 73.4 (C-3 or C-5 and C-4), 73.0 (benzylic), 68.8 (C-1'), 68.6 (C-6), 31.9 (C-4'); ESI-MS: calcd for $C_{38}H_{41}BrO_6 + NH_4^+$: 691.2; Found: 691.3.



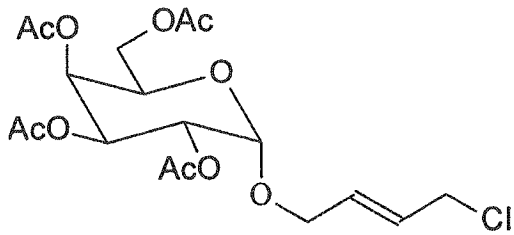
6-7a

6'-Iodo-hex-2'-enc-1'-yl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside (6-7a). The reaction mixture was purified by silica gel column chromatography using ethyl acetate and hexane (20:80) to obtain an inseparable mixture with a *E/Z* ratio ~5:1; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26-7.37 (m, 20H, aromatic), 5.63-5.95 (m, 2H, H-2' and H-3'), 4.94 (d, *J* = 11.6, 1H, benzylic), 4.92 (d, *J* = 11.1, 1H, benzylic), 4.69-4.79 (m, 3H, benzylic), 4.62 (d, *J* = 11.7, 1H, benzylic), 4.45 (d, *J* = 11.8, 1H, benzylic), 4.41 (d, *J* = 11.8, 1H, benzylic), 4.39 (d, *J* = 7.9, 1H, H-1), 4.32-4.35 (m, 1H, H-1a'), 4.03-4.07 (m, 1H, H-1b'), 3.88 (bd, *J* = 2.5, 1H, H-4), 3.85 (dd, *J* = 7.7, 9.6, 1H, H-2), 3.50-3.60 (m, 4H, H-6, H-3 and H-5); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 138.9, 138.7, 138.6, 138.0, 131.7, 131.2, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.5 (aromatic and vinylic), 102.9 (C-1), 82.3 (C-3 or C-5), 79.6 (C-2), 75.2, 74.5 and 73.7 (benzylic), 73.6 and 73.5 (C-3 or C-5 and C-4), 73.1 (benzylic), 69.7 (C-1', *trans*), 69.0 (C-6), 64.8 (C-1', *cis*), 32.8 (C-4', *trans*), 32.6 (C-5'), 28.3 (C-4', *cis*), 6.1 (C-6'); ESI-MS: calcd for $C_{40}H_{45}IO_6 + NH_4^+$: 766.2; Found: 766.1.



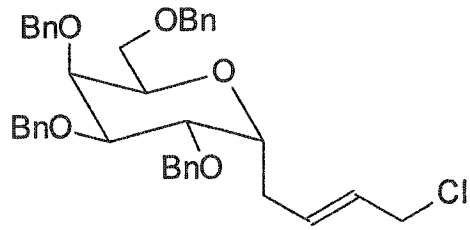
6-8

4'-Chloro-but-2'-ene-1'-yl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (6-8). The reaction mixture was purified by silica gel column chromatography using ethyl acetate and hexane (30:70) to obtain a thick syrup with a *E/Z* ratio 17:1. The *trans* isomer **6-8** was isolated from the mixture; for *trans* isomer, $[\alpha]^{20}_{D}$ = -16.6 ° (*c* 2.5, $CHCl_3$); FTIR (film) 968 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.76-5.86 (m, 2H, H-2' and H-3'), 5.36 (bd, *J* = 3.4, 1H, H-4), 5.20 (dd, *J* = 7.9, 10.4, 1H, H-2), 5.00 (dd, *J* = 3.4, 10.4, 1H, H-3), 4.78 (d, *J* = 7.9, 1H, H-1), 4.34 (dd, *J* = 4.7, 13.6, 1H, H-1a'), 4.08-4.17 (m, 3H, H-6, H-1b'), 4.03 (d, *J* = 5.7, 2H, H-4'), 3.87 (t, *J* = 6.6, 1H, H-5), 2.12, 2.04, 2.02 and 1.95 (4 s, 12H, CH_3CO); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 170.3, 170.1, 170.0 and 169.3 (CH_3CO), 129.6 and 128.6 (C-2' and C-3'), 100.3 (C-1), 70.8 (C-5), 70.7 (C-3), 68.8 (C-1'), 68.4 (C-2), 67.0 (C-4), 61.2 (C-6), 44.0 (C-4'), 20.7, 20.6 and 20.5 (CH_3CO); ESI-MS: calcd for $C_{18}H_{25}ClO_{10} + NH_4^+$: 454.7; Found: 454.5.



6-9

4'-Chlorobut-2'-ene-1'-yl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (6-9). The reaction mixture was purified by silica gel column chromatography using ethyl acetate and hexane (20:80) to obtain a thick syrup with a *E/Z* ratio 15:1. The *trans* isomer **6-9** was isolated from the mixture; for *trans* isomer, $[\alpha]^{20}_{D}$ 12 ° (*c* 2.5, $CHCl_3$); FTIR (film) 970 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.78-5.89 (m, 2H, H-2' and H-3'), 5.42 (bd, *J* = 1.2, 1H, H-4), 5.31 (dd, *J* = 3.3 and 10.4, 1H, H-3), 5.09-5.12 (m, 2H, H-1 and H-2), 4.15-4.19 (m, 2H, H-1a' and H-5), 3.98-4.08 (m, 5H, H-1b', H-4' and H-6), 2.10, 2.04, 2.01 and 1.95 (4 s, 12H, CH_3CO); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 170.3, 170.2, 170.1 and 169.8 (CH_3CO), 129.6 and 129.0 (C-2' and C-3'), 95.6 (C-1), 70.0 (C-2 and C-4), 67.5 (C-3), 67.2 (C-1'), 66.4 (C-5), 61.6 (C-6), 43.8 (C-4'), 20.7, 20.6 and 20.5 (CH_3CO); ESI-MS: calcd for $C_{18}H_{25}ClO_{10}$ + (NH_4^+): 454.7; Found: 454.6.



6-10

4'-Chloro-1'-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-but-2'-ene (6-10). The reaction mixture was purified by silica gel column chromatography using ethyl acetate and hexane (20:80) to obtain a thick syrup with a *E/Z* ratio >20:1. The *trans* isomer **6-10** was isolated from the mixture; for *trans* isomer, $[\alpha]^{20}_{D}$ + 42.6° (*c* 3.0, $CHCl_3$); FTIR (film) 969 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.24-7.44 (m, 20H, aromatic), 5.67-5.85 (m, 2H, H-2' and H-3'), 4.76-4.80 (m, *2*H, benzylic), 4.58-4.71 (m, 6H, benzylic), 4.14 (bs, 1H, H-5), 4.09 (m, 1H, H-1), 4.05 (m, 1H, H-4), 3.95-4.01 (m, 1H, H-6a), 3.91-4.01 (d, *J* = 7.2, 2H, H-4'), 3.80-3.88 (m, 2H, H-2 and H-3), 3.76 (dd, *J* = 4.2, 10.5, 1H, H-6b), 2.47-2.55 (m, 1H, H-1'a), 2.37-2.43 (m, 1H, H-1'b); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 138.4, 138.3, 138.2 and 138.0 (aromatic), 132.3 (C-2'), 128.3, 128.2 and 128.1(aromatic), 127.8 (C-3'), 127.7, 127.6, 127.5, 127.4 and 127.3 (aromatic), 76.4 and 76.2 (C-2 and C-3), 74.0 (C-1), 73.2, 73.0, 72.9 and 72,8 (benzylic), 72.5 (C-5), 70.2 (C-4), 67.0 (C-6), 44.3 (C-4'). 30.6 (C-1'); ESI-MS: calcd for $C_{38}H_{41}ClO_5 + (NH_4^+)$: 630.8; Found: 630.8.

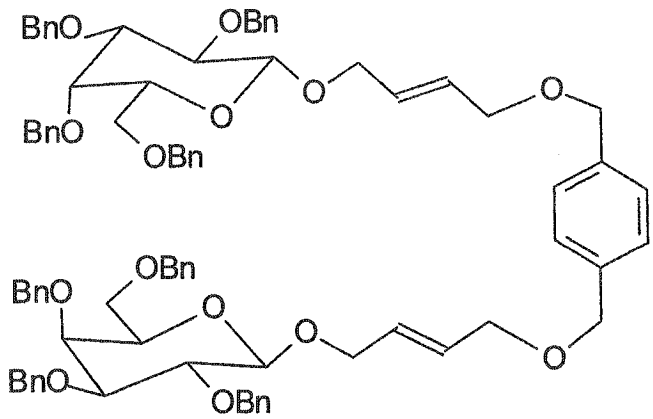
4-Chloro-*N*-carbobenzyloxy-but-2-enamine (6-12a). After reaction, the solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using ethyl acetate and hexane (10:90) to obtain a colourless liquid. The *trans* isomer was isolated from the mixture as a single isomer.; for *trans* isomer, ^1H NMR

(300 MHz, $CDCl_3$) δ 7.27-7.38 (m, 5H, aromatic), 5.74-5.85 (m, 2H, H-2 and H-3), 5.09 (s, 2H, benzylic), 4.88 (bs, 1H, amide), 4.02(d, 2H, J = 5.0 Hz, H-1), 3.82 (d, J = 5.2 Hz, 2H, H-4), 4.41-4.48 (m, 1H, H-2), 3.95 (bd, J = 5.8, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 156.5 (carbonyl from Cbz), 136.7 (aromatic), 131.1 (vinyl), 128.5, 128.2 and 128.1 (aromatic), 127.5 (vinylic), 67.2 (benzylic), 44.6 (C-4), 42.4 (C-1); EI-MS: calcd for $C_{15}H_{18}ClNO_4$ $[M-Cl]^+$: 276.12; Found: 276.10.

***t*-Butyl 3-chloro-but-2-enoate (6-12b).** After reaction, the solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using ethyl acetate and hexane (10:90) to obtain a colourless liquid. The *trans* isomer was isolated from the mixture as a single isomer; for *trans* isomer, ^1H NMR (300 MHz, $CDCl_3$) δ 6.84 (dt, J = 6.3, 6.2, 15.3, 1H, H-3), 5.98 (bd, J = 15.3, 1H, H-2), 4.11 (d, J = 6.2, 2H, H-4), 1.46 (s, 9H, *t*-butyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 165.3 (-CO_2-*t*Bu), 140.8 (C-3), 126.3 (C-2), 81.3 (C-4), 43.0 ($C(CH_3)_3$), 28.4 ($C(CH_3)_3$); EI-MS: calcd for $C_8H_{13}ClO_2$ $[M-CH_3]^+$: 161.56; Found: 161.50.

Methyl *N*-carbobenzyloxyallylglycine (6-12c). After reaction, the solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using ethyl acetate and hexane (10:90) to obtain a colourless liquid. The *trans* isomer was isolated from the mixture as a single isomer; for *trans* isomer, ^1H NMR (300 MHz, $CDCl_3$) δ 7.29-7.34 (m, 5H, aromatic), 5.57-5.71 (m, 2H, H-4 and H-5), 5.34 (bd, J = 7.9, 1H, amide), 5.09 (s, 2H, benzylic), 4.41-4.48 (m, 1H, H-2), 3.95 (bd, J = 5.8, 2H, H-6), 3.73 (s, 3H, -OCH_3), 2.43-2.63 (m, 2H, H-3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 172.3 (-CO_2Me), 156.0 (carbonyl from Cbz), 136.5 (aromatic), 130.9 (vinyl), 129.1 (aromatic), 128.9 (aromatic and vinylic), 128.6 and 128.5 (aromatic), 67.0 (benzylic), 53.2 (-

OCH_3), 52.5 (C-2), 44.4 (C-6), 35.1 (C-3); EI-MS: calcd for $C_{12}H_{14}ClNO_2$ $[M-Cl]^+$: 204.10; Found: 204.11.



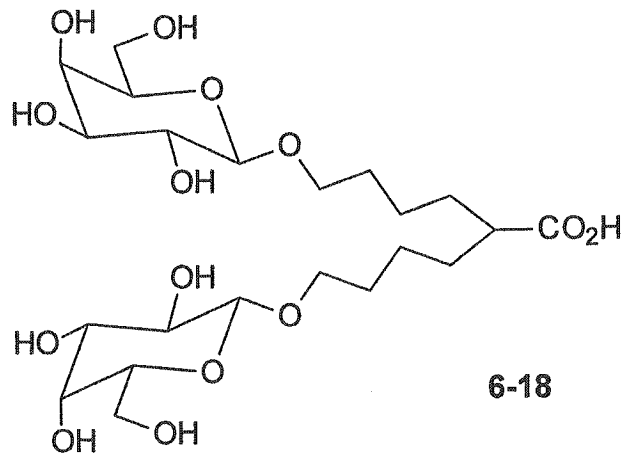
6-14

Compound 6-14. To a solution of 1,4-dihydroxymethyl benzene (**6-13**) (13.8 mg, 0.1 mmol) in DMF (5 mL), was added NaH (12 mg, 0.3 mmol) and a catalytic amount of tetrabutyl ammonium iodide. After stirring for 20 min, compound **6-5** (150 mg, 0.24 mmol) was added to the mixture. The mixture was stirred at room temperature for 5 h. After quenching the excess NaH with methanol, the mixture was diluted with 50 mL of water and extracted with 3 x 30 mL of ether. The organic layers were combined together and dried over anhydrous sodium sulphate. Removal of the solvent under reduced pressure and purification by silica gel chromatography using ethyl acetate and hexane (20:80) afforded **6-14** as a thick syrup in 55% yield (218 mg, 0.16 mmol); $[\alpha]^{20}_D$ - 10 ° (*c* 1.5 , $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.22-7.35 (m, 44H, aromatic), 5.75-5.88 (m, 4H, H-2' and H-3'), 4.93 (d, *J* = 11.7, 2H, benzylic), 4.89 (d, *J* = 10.7, 2H, benzylic), 4.76 (d, *J* = 10.7, 2H, benzylic), 4.74 (d, *J* = 11.8, 2H, benzylic), 4.69 (d, *J* = 11.8, 2H, benzylic), 4.60 (d, *J* = 11.7, 2H, benzylic), 4.46 (s, 4H, benzylic), 4.39-4.45 (m, 4H, benzylic, H-1 and H-1a'), 4.37 (d, *J*

= 7.6, 2H, benzylic), 4.12 (dd, J = 4.7, 13.9, 2H, H-1b'), 3.99 (d, J = 4.1, 4H, H-4'), 3.88 (bd, J = 2.7, 2H, H-4), 3.83 (dd, J = 7.7, 9.8, 2H, H-2), 3.58 (m, 4H, H-6), 3.49-3.52 (m, 4H, H-3 and H-5); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 138.7, 138.6, 138.5, 137.9, 137.6 and 134.4 (aromatic), 129.3 and 129.0 (C-2' and C-3'), 128.4, 128.3, 128.2, 128.1, 127.8, 127.7, 127.6 and 127.5 (aromatic), 103.0 (C-1), 82.2 (C-5), 79.6 (C-2), 75.2 and 74.5 (benzylic), 73.5 (C-3 and C-4), 73.4, 73.0 and 71.8 (benzylic), 70.0 (C-4'), 69.2 (C-1'), 68.8 (C-6); ESI-MS: calcd for $C_{84}H_{90}O_{14}$ + (NH_4^+): 1340.4; Found: 1340.3.

Compound 6-17. To a solution of diethyl malonate (**6-16**) (16 mg, 0.1 mmol) in dry THF (5 mol) was added NaH (12 mg, 0.3 mmol). The mixture was stirred for 20 min and then compound **6-5** (150 mg, 0.2 mmol) was added to it. After stirred for 4 h, metanol was added to the solution to quench the excess NaH. After removal of the solvent under reduced pressure, the residue was purified by silica gel column chromatography using ethyl acetate and hexane (1/3) to provide compound **6-17** as a thick syrup in a yield of 90% (120 mg, 0.09 mmol); $[\alpha]^{20}_D$ - 11.5° (*c* 2.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22-7.34 (m, 40H, aromatic), 5.58-5.63 (m, 2H, H-3'), 5.51-5.59 (m, 2H, H-2'), 4.91 (d, J = 11.7, 2H, benzylic), 4.87 (d, J = 11.0, 2H, benzylic), 4.73 (d, J = 11.7, 2H, benzylic), 4.71 (d, J = 11.0, 2H, benzylic), 4.70 (d, J = 11.7, 2H, benzylic), 4.59 (d, J = 11.7, 2H, benzylic), 4.43 (d, J = 11.8, 2H, benzylic), 4.39 (d, J = 11.8, 2H, benzylic), 4.34 (d, J = 7.8, 2H, H-1), 4.29 (dd, J = 4.8, 12.3, 2H, H-1a'), 4.08 (q, J = 7.1, 4H, $R_2C(CO_2CH_2CH_3)_2$), 3.99 (dd, J = 6.2, 12.3, 2H, H-1b'), 3.88 (bd, J = 2.3, 2H, H-4), 3.79 (dd, J = 7.8, 9.7, 2H, H-2), 3.58 (m, 4H, H-6), 3.51 (bd, J = 1.0, 2H, H-5), 3.49 (dd, J = 3.0, 9.7, 2H, H-3), 2.54-2.62 (m, 4H, H-4'), 1.14 (t, J = 7.1, 6H, $R_2C(CO_2CH_2CH_3)_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 170.54 (malonate ester), 138.7, 138.6, 138.5 and 137.9 (aromatic), 130.5 (C-2'), 128.4, 128.3, 128.2, 128.1, 127.8,

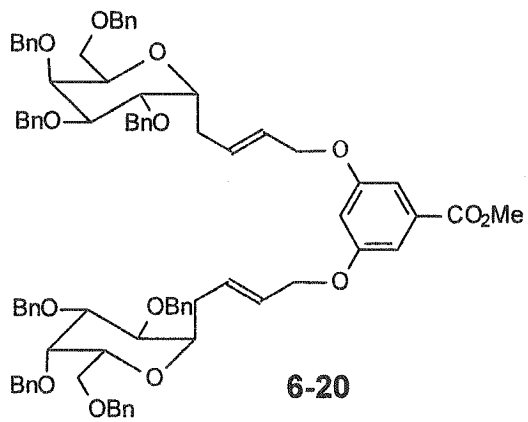
127.7, 127.6 and 127.5 (aromatic), 127.4 (C-3'), 102.6 (C-1), 82.2 (C-3), 79.5 (C-2), 75.1 and 74.8 (benzylic), 73.6 (C-4), 73.5 (benzylic), 73.3 (C-5), 73.1 (benzylic), 68.8 (C-1'), 68.6(C-6), 61.2 ($R_2C(CO_2CH_2CH_3)_2$), 57.5 ($R_2C(CO_2CH_2CH_3)_2$), 35.4 (C-4'), 14.1 ($R_2C(CO_2CH_2CH_3)_2$); ESI-MS: calcd for $C_{83}H_{92}O_{16} + NH_4^+$: 1362.7; Found: 1362.6.



6-18

Compound 6-18. Compound **6-17** (134 mg, 0.1 mol) was suspended in a solution of 2 M KOH and methanol (1 mol : 3 mol) and the mixture was refluxed under nitrogen for 24 h. The solution was then neutralized with 5% HCl to pH 2. After dilution with 50 mL of water the mixture was extracted with 3 x 30 mL of methylene chloride. The organic layers were combined together and dried over anhydrous sodium sulphate. Evaporation of the solvent under reduced pressure afforded the hydrolysed product as a syrup in 85% yield (0.085 mmol). The hydrolysed compound (64 mg, 0.05 mmol) was dissolved into 5 mL of acetonitrile and sonicated for 15 min to degas the solution. Then CuCl (1 mg, 20 mol%) was added to the solution and the mixture was refluxed under nitrogen for 24 h. After evaporation of the solvent, 10 drops of 5% HCl was added to the residue. The mixture was diluted with 50 mL of water and extracted with 3 x 30 mL of dichloromethane to give the

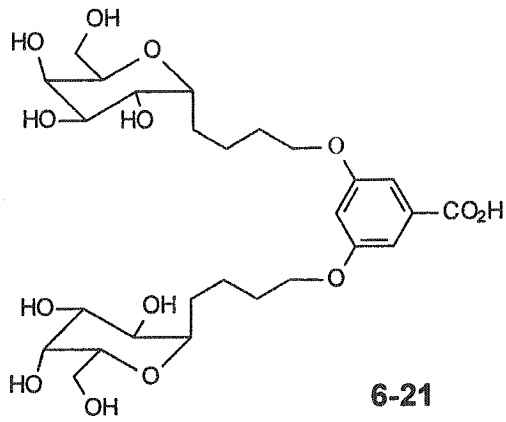
monodecarboxylated product as a thick syrup in 82% yield. Without further purification, the crude product was subjected to hydrogenolysis with 10 % Pd/C in ethanol at room temperature for 8 h. The mixture was filtered through a pad of celite and the solvent was evaporated under reduced pressure. The crude mixture was purified by Biogel P-2 size exclusion chromatography to obtain the fully deprotected compound **6-18** as an amorphous solid in 90% yield (44mg, 0.085 mmol); $[\alpha]^{20}_{D}$ = - 15° (*c* 2.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.43 (d, *J* = 7.9, 2H, H-1), 3.92-3.99 (m, 4H, 1a' and H-4), 3.75-3.85 (m, 4H, H-6), 3.71-3.74 (m, 4H, H-1b' and H-5), 3.68 (dd, *J* = 3.6, 10.0, 2H, H-3), 3.54 (dd, *J* = 7.9, 10.0, 2H, H-2), 2.29 (m, 1H, H-5'), 1.63-1.73 (m, 4H, H-2'), 1.44-1.57 (m, 4H, H-4'), 1.37-1.41 (m, 4H, H-3'); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 162.7 (-*C*O_2H), 102.3 (C-1), 74.6 (C-5), 72.4 (C-3), 70.3 (C-2), 69.9 (C-1'), 68.2 (C-4), 60.4 (C-6), 48.1 (R_2*C*HCO_2H), 31.9 (C-2'), 28.3 (C-4'), 23.0 (C-3'); ESI-MS: calcd for $C_{22}H_{40}O_{14}$ + (Na^+): 551.2; Found: 551.1.



6-20

Methyl 3,5-di-(1'-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-but-2'-enyl-4'-oxy)benzoate (6-20). To a solution of methyl 3,5-dihydroxybenzoate **6-19** (16.8 mg, 0.1 mmol) in DMF (5 mL), were added potassium carbonate (41.5 mg, 0.3 mmol) and 18-C-6

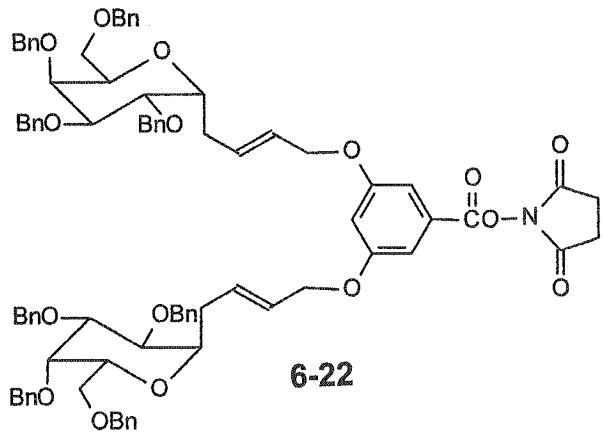
crown ether (53 mg, 0.2 mmol). After stirred for 20 min, compound **6-10** (153 mg, 0.25 mmol) was added to the mixture. The reaction mixture was then stirred at room temperature for 3 h. After dilution with 50 mL of ether the mixture was washed with 3 x 30 mL of water. The ether solution was dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography using ethyl acetate and hexane (25:75) to give compound **6-20** as a thick syrup in 90% yield (118 mg, 0.09 mmol); $[\alpha]^{20}_{D}$ + 36.0° (*c* 3.0 , $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24-7.39 (m, 40H, aromatic), 7.19 (d, *J* = 2.3, 2H, aromatic), 6.64 (t, *J* = 2.3, 1H, aromatic), 5.67-5.85 (m, 4H, H-2' and H-3'), 4.48-4.73 (m, 16H, benzylic), 4.40 (d, *J* = 4.4, 4H, H-4'), 4.14 (m, 2H, H-5), 4.03 (m, 4H, H-1 and H-4), 3.86-3.91 (m, 5H, H-6a and -CO_2CH_3), 3.75 (m, 4H, H-2 and H-3), 3.69 (dd, *J* = 4.5, 10.7, 2H, H-6b), 2.47-2.51 (m, 2H, H-1'a), 2.37-2.40 (m, 2H, H-1'b); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 167.2 (-CO_2Me), 160.0, 138.9, 138.4, 138.1 and 138.0 (aromatic), 131.7 (C-2'), 128.3, 128.2, 128.1, 127.9, 127.7, 127.6, 127.5 and 127.4 (aromatic), 126.6 (C-3'), 107.7 and 106.9 aromatic), 76.5 and 76.3 (C-2 and C-3), 74.2 (C-1), 73.1 and 73.0 (benzylic), 72.6 (C-5), 70.5 (C-4), 68.7 (C-4'), 67.2 (C-6), 52.2 (-CO_2CH_3), 30.9 (C-1'); ESI-MS: calcd for $C_{84}H_{88}O_{14}$ + (NH_4^+): 1338.6; Found: 1338.5.



6-21

3,5-di-(1'-α-D-galactopyranosyl)-butyl-4'-oxy)benzoic acid (6-21). To a suspension of compound **6-20** (133 mg, 0.1 mmol) in ethanol (20 mL) was added a catalytic amount of 10 % Pd-C. This mixture was stirred at room temperature for 12 h under H_2, and then it was filtered through a pad of celite. After evaporation of the solvent, a thick syrup was obtained. Without further purification, the crude product was hydrolyzed using 1 mL of 2M KOH in 2 mL of methanol at room temperature for 12 h. The mixture was neutralized with Amberlite ir-120 (H+) resin and the solvent was removed under reduced pressure. The residue was purified by Biogel P-2 size exclusion chromatography to provide compound **6-21** as a thick syrup in 85% (50 mg, 0.085 mmol) yield in two steps; $[\alpha]^{20}{}_{D}$ 35.0° (*c* 2.0 , H_2O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.04 (d, *J* = 2.0, 2H, aromatic), 6.58 (t, *J* = 2.0, 1H, aromatic), 4.06-4.10 (m, 2H, H-1), 4.03 (dd, *J* = 3.8, 9.8, 2H, H-2), 3.99 (d, *J* = 3.4, 2H, H-4), 3.95 (t, *J* = 6.3, 4H, H-4'), 3.81 (dd, *J* = 3.4, 9.8, 2H, H-3), 3.71-3.78 (m, 6H, H-6 and H-5), 1.72-1.84 (m, 6H, H-3' and H-1a'), 1.57-1.67 (m, 4H, H-1b' and H-2a'), 1.38-1.47 (m, 2H, H-2b'); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 169.0 (-CO_2H), 158.9, 131.0, 107.8 and 106.2 (aromatic), 74.8 (C-1), 71.1 (C-5), 69.4 (C-3), 68.8 (C-4), 68.1 (C-4'), 67.9 (C-3'), 60.4 (C-

6), 27.7 (C-3'), 22.8 (C-1'), 21.0 (C-2'); ESI-MS: calcd for $C_{27}H_{42}O_{14}$ + (Na^+): 613.2; Found: 613.1.



6-22

Compound 6-22. Compound **6-20** (100 mg, 0.076 mmol) was suspended in a solution of methanol and 2M KOH (4mL/1mL) and the mixture was refluxed for 12 h. After evaporating the solvent in vacuo, 50 mL of water was added to the residue and the pH was adjusted to 1 using 10% HCl solution. The solution was extracted with 3 x 30 mL of dichlomethane. The organic layers were combined together and dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure provided the substituted benzoic acid as a thick syrup in 85% yield. Without further purification, the crude acid (65 mg, 0.05 mmol) was subjected to coupling reaction with N-hydroxysuccinimide (11.5 mg, 0.1mmol), EDC (19 mg, 0.1 mmol), and one drop of DIPEA in dioxane. Th reaction was run at rt for 2 h. After removal of the solvent, the residue was purified on silica gel column using hexane and ethylacetate (65/35) to afford compound **6-22** as a solid in 90% yield (95 mg, 0.068 mmol); $[\alpha]^{20}_D$ = + 40.0° (*c* 3.0 , $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22-7.30 (m, 42H, aromatic), 6.69 (t, *J* = 2.3, 1H, aromatic), 5.65-5.80 (m, 4H, H-2' and H-3'), 4.47-

4.72 (m, 16H, benzylic), 4.36 (d, J = 4.8, 4H, H-4'), 4.14 (m, 2H, H-5), 4.03 (m, 4H, H-1 and H-4), 3.86-3.91 (m, 2H, H-6a), 3.69-3.75 (m, 6H, H-2, H-3 and H-6b), 2.80 (bs, 4H, -CH_2- from NHS), 2.31-2.51 (m, 4H, H-1'); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ_C 169.7 (-CO_2R), 162.2 (-CO_2R), 160.3, 138.9, 138.8 and 138.5 (aromatic), 132.4 (C-2'), 128.8, 128.7, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 126.9, 126.8 (aromatic and C-3'), 107.7 and 106.9 (aromatic), 76.3, 74.2, 73.2 and 73.0 (benzylic), 72.6, 70.5, 68.7 (C-4'), 67.2 (C-6), 30.9 (C-1'), 25.6 (-CH_2- from NHS); ESI-MS: calcd for $C_{87}H_{90}NO_{16}$ + (K^+): 1443.5; Found: 1443.2.

Compound 6-24. To a solution of compound **6-22** (100 mg, 0.069 mmol) in THF (5 mL), was added diamine **6-3** (4.3 mg, 0.0288 mmol). The solution was stirred at rt for 4 h and monitored by ninhydrin test and TLC till it was completed. The solvent was evaporated to dryness under reduced pressure. The residue was purified on silica gel column using hexane and ethyl acetate (40/60) to afford compound **6-24** as a solid in 85% yield (159 mg, 0.059 mmol); $[\alpha]^{20}_D$ + 42.0° (*c* 2.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19-7.33 (m, 80H, aromatic), 6.88 (bs, 4H, aromatic), 6.50 (bs, 2H, aromatic), 5.65-5.80 (m, 8H, H-2' and H-3'), 4.43-4.71 (m, 32H, benzylic), 4.35 (d, J = 3.3, 8H, H-4'), 3.99-4.04 (m, 12H, H-1, H-4 and H-5), 3.81 (m, 2H, H-6a), 3.58-3.72 (m, 24H, H-2, H-3 and H-6b and –CH_2- from the spacer), 2.29-2.50 (m, 8H, H-1'); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ_C 167.7 (-CO_2R), 160.2, 138.9, 138.8, 138.7 and 138.5 (aromatic), 132.2 (C-2'), 128.8, 128.7, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.0, 126.8 (aromatic and C-3'), 106.1 and 105.0 (aromatic), 76.3, 74.2, 73.2 and 73.0 (benzylic), 72.5, 70.2, 70.2 and 69.8 (-CH_2- from the spacer), 68.8 (C-4'), 67.2 (C-6), 39.7 (-$NHCH_2$- from the spacer) 30.8 (C-1'); ESI-MS: calcd for $C_{172}H_{184}N_2O_{28}$ + ($2K^+$): 1401.6; Found: 1401.6.

6.4. References

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CLAIMS TO ORIGINAL RESEARCH

1. Synthesis of α-Gal precursor and the preparation of propargyl α-Gal monomer.
2. Synthesis of multivalent α-Gal clusters using the palladium-mediated Sonogashira reaction as the key steps.
3. ELISA tests of these synthetic α-Gal clusters against human anti α-Gal antibodies.
4. Synthesis of analogues of galacto- and lactoclusters using the Sonogashira reaction in key steps.
5. Biochemical tests demonstrated that these synthetic lactoclusters cross-linked with galectin-3 for the first time.
6. Synthesis of a hexameric galactopeptidomemtics using a sequence of olefin cross-metathesis, Sanogashira reaction, and cyclotrimerization.
7. Preparation of extended glycoallyl halides as *O*- and *C*-alkylating reagents for the synthesis of high order glycoclusters using the 2nd generation Grubbs catalyst.
8. Synthesis of unnatural amino acids and their precursors using the 2nd generation Grubbs catalyst.

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