LAKEHEAD UNIVERSITY

Synthesis of Alginate Neoglycolipids

by

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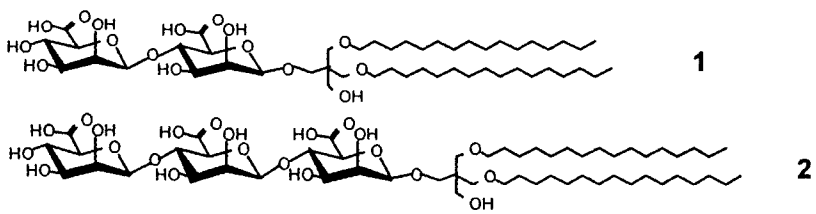
ABSTRACT

Innate immunity, one of two arms of the immune system, can provide more rapid defense mechanisms than adaptive immunity in human immune defense systems. The innate immune system recognizes invading pathogens by binding a variety of ligands to the family of Toll-like receptors (TLRs) which play important roles in linking innate and adaptive immunities in host immune defense.

Direct application of natural TLR ligands in the pharmaceutical industry is evidently limited due to their complicated components and high toxicity, and hence synthetic TLR ligands have emerged as competitive therapeutic agents involved in various diseases including infection, inflammation and cancers.

Alginates are unbranched and non-repeating linear polysaccharides comprising 1,4-linked β-D-mannuronic acid (M) and the C-5-epimer α-L-guluronic acid (G) with highly variable composition and sequential structure. They act as strong immune stimulants mediated by TLR2 and TLR4. β-1,4-D-mannuronic acid neoglycolipids (**1** and **2**), in this thesis, have been designed as potential ligands for TLR 2/4. The chemical synthesis of **1** and **2** have been described. Through diastereoselective β-glycosylation of the 4,6-*O*-benzylidene protected α-thiomannoside donor **9** with glycosylation acceptors **6**, **12** and **18** respectively, followed by a series of deprotection and protection steps, the protocol successfully provides β-1,4-*di*- and *tri*-D-mannosides. Finally, the TEMPO/BAIB mediated oxidation and global hydrogenlization steps lead us to the target molecules **1** and **2**:



1

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LIST OF ABBREVIATIONS

Ac	acetyl
anal.	analysis
aq.	aqueous
BAIB	[bis(acetoxy)iodo]benzene
Bn	benzyl
Bu	butyl
BSP	1-benzenesulfinyl piperidine
Bz	benzoyl
Calcd.	calculated
CSA	carnphorsulfonic acid
d	doublet
DCM	dichloromethane
DMAP	4-N,N-dimethyl pyridine
DMF	N,N-dimethyl formamide
DTBMP	2,6-di-tert-butyl-4-methyl pyridine
equiv.	equivalent
Et	ethyl
Fuc	Fucose
G	L-guluronic acid
Gal	galactose
D-GalNAc	2-(Acetyamido)-2-deoxy-D-galactose
Glc	glucose
GlcA	Glucuronic acid
D-GlcNAc	2-(Acetamido)-2-deoxy-D-glucose
hr(s)	hour(s)
HOAc	acetic acid
Hz	hertz
Ido	Idose
IdoA	Idouronic acid
i-PrOH	isopropanol
J	coupling constant
M	D-mannuronic acid
m	multiplet
Man	D-mannose
Me	methyl

MeOH	methanol
mg	milligram (s)
MHz	megahertz
min(s)	minute (s)
mol(s)	mole (s)
mmol(s)	millimole (s)
MS	mass spectrometry
Neu5Ac	N-Acetylneuraminic acid
NMR	nuclear magnetic resonance
Ph	phenyl
Py	pyridine
q	quartet
r.t.	room temperature
s	singlet
satd	saturated
t	triplet
TEMPO	2,2,6,6-tetramethyl -piperridinyloxy free radical
Tf	trifluoromethanesulfonyl
Tf_2O	trifluoromethanesulfonic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
TLR(s)	Toll like receptor(s)
TNF-α	Tumor necrosis factor-alpha
TMS	tetramethyl silane
Xyl	Xylose

1. INTRODUCTION

1.1. Glycobiological Background

1.1.1. Carbohydrates

Historically, the name carbohydrates came from the description of compounds with formula $C_n(H_2O)_n$ "carbon-hydrates". Today, the word "carbohydrate" no longer has limitations in "carbon & hydrates" because many carbohydrates are devoid of specific hydroxyl groups or have amino groups or other functional groups.

Carbohydrates were traditionally viewed as energy-storage materials (in the form of monosaccharides and polysaccharides such as starch), structural materials (the polysaccharides cellulose in plants and chitin in the exoskeletons of insects) and primary metabolites that were produced in photosynthesis and were destined for further conversion in nature.

Compared with two of three major polymers responsible for the storage of information and signal transduction processes in biological systems; nucleic acids and proteins, carbohydrates are the most complex and diverse class of biopolymers commonly found in nature as glycoconjugates. A wide array of available monosaccharides and diversified stereochemical linkages between each pair of carbohydrates results in tremendous complexity. Furthermore, the chain length of the oligosaccharides can also vary widely from monosaccharides up to branched oligosaccharides with more than 30 building blocks, or in the case of polysaccharides to several thousand building blocks.

There are ten monosaccharides (**Fig. 1.1**) found in mammalian systems which are covalently linked to other types of molecules at the anomeric position, and are given the generic name, glycoconjugates.[1]

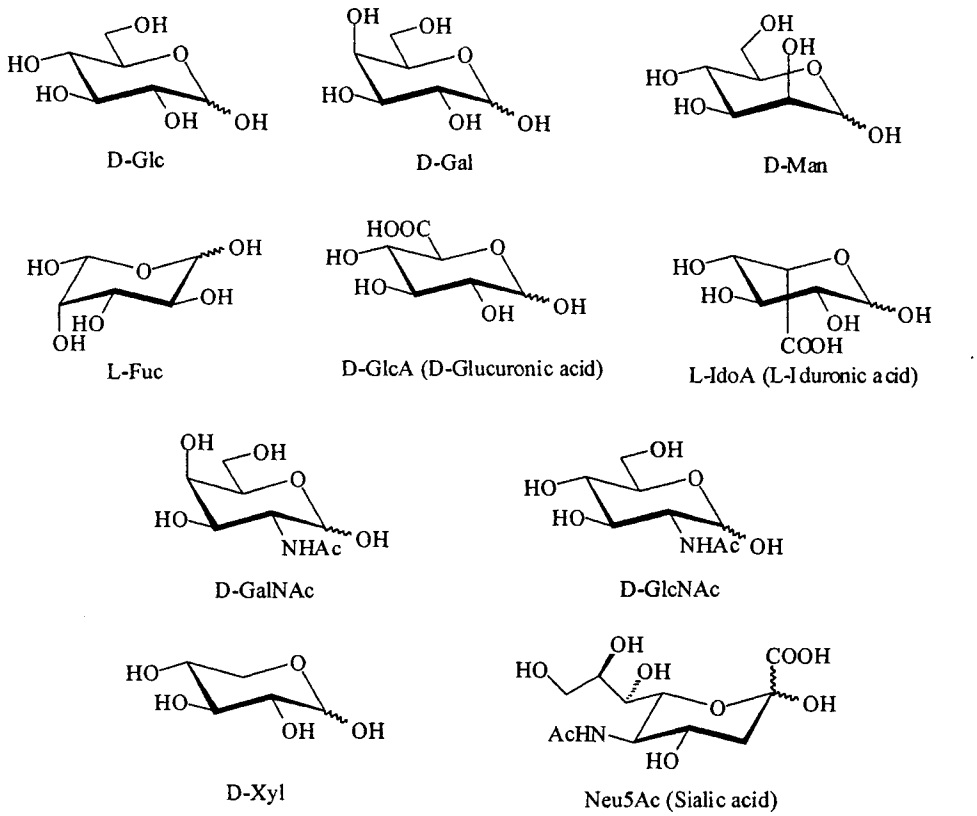


Fig. 1.1 Monosaccharides found in mammalian glycoconjugates

1.1.2. Biological Roles of Carbohydrates

Cells can recognize one another through pairs of structures on their surfaces such that a structure on one cell carries encoded biological information that the structure on the other cell can decipher. Nature's choice of carbohydrates as information carriers is very clever because oligosaccharides are polyols and a large diversity of structures is possible from a small number of monosaccharides.

The biological roles of oligosaccharides include functions such as structural, protective and stablizing roles for polypeptides and proteins, specific receptors for noxious agents, masking and decoys for protection from microorganisms and antibodies, specific receptors for symbiotic functions, on-off and tuning functions for the biological activity of proteins,

intercellular trafficking functions, regulating the clearance or turnover of proteins and whole cells, hormonal action, and cell-cell and cell-matrix recognition.[2]

Among these biological roles of oligosaccharides, the binding of bacteria, viruses, fungi, parasites and toxins to carbohydrate receptors are not beneficial to human.[3] Binding of pathogens to glycoconjugates often causes various inflammation, cancer or infection.

In addition, oligosaccharides are present as glycoconjugates in all cell walls mediating a variety of events such as inflammation, cell-cell recognition, immunological response, metastasis, and fertilization. Alterations in cell surface oligosaccharides have been found to be associated with many pathological conditions such as cancer, the growth of tumors and tuberculosis.

1.1.3. Glycolipids

The carbohydrate part of glycoconjugates is called the glycan and the non-carbohydrate part is called the aglycon. When the aglycon is a lipid, it is termed a glycolipid; the lipid part functions to anchor the sugar residue in the lipid bi-layer of eukaryotic cell membranes.

Glycolipids have been commonly viewed as carbohydrate-attached lipids. Their role is to provide energy and also serve as markers for cellular recognition. They extend from the lipid bilayer into the aqueous environment outside the cell where they act as a recognition site for specific chemicals and help to maintain the stability of the membrane. In an aqueous milieu, the polar heads of lipids tend to orient toward the polar, aqueous environment, while the hydrophobic tails tend to minimize their contact with water. The lipophilic tails of lipids (U) tend to cluster together, forming a lipid bi-layer (1) or a micelle (2) (**Fig. 1.2**). The polar heads (P) face the aqueous environment. The spherical micelles form at given points

above the critical micelle concentration in a polar milieu, while the lipids form a single layer on the liquid surface and are (sparingly) dispersed in the solution below the critical micelle concentration in a polar environment.

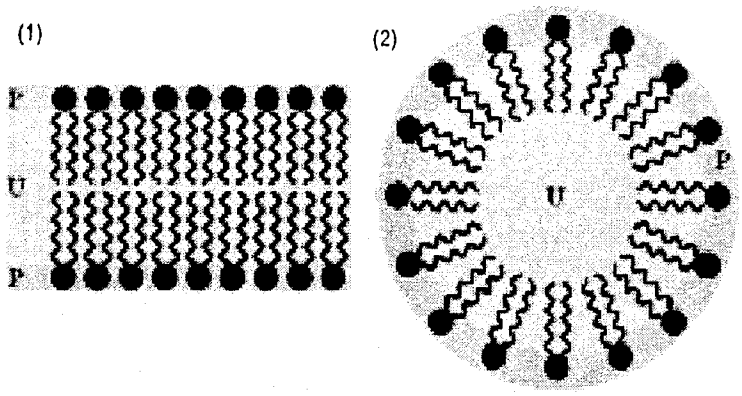


Fig. 1.2 Two orientations of lipids as (1) bilayer (2) micelle

Using both their hydrophilic head and hydrophobic tail, glycolipids can readily and spontaneously form a liposome structure in an aqueous environment (**Fig. 1.3**). Novel advances in liposomal formulation technology have resulted in combination of drugs or compounds in unique and stable liposomes. The liposomal formulation overcomes the problem of delivery of difficult-to formulate and water-insoluble drugs to intracellular targets to treat a variety of disease states. Through their adsorption, endocytosis, fusion of the cell with a vesicle and lipid exchange, liposome systems[4] as the popular drug delivery platform have various desired properties, including:

- Being nontoxic and bio-degradable.
- Being modified to release drugs slowly resulting in prolonged exposure and hence increasing the specific therapeutic efficacy of drugs.
- As a potential source of nutrition recognized by cancer cells and eventually destroying them.

- Providing an environment enclosed both hydrophilic and hydrophobic molecules.

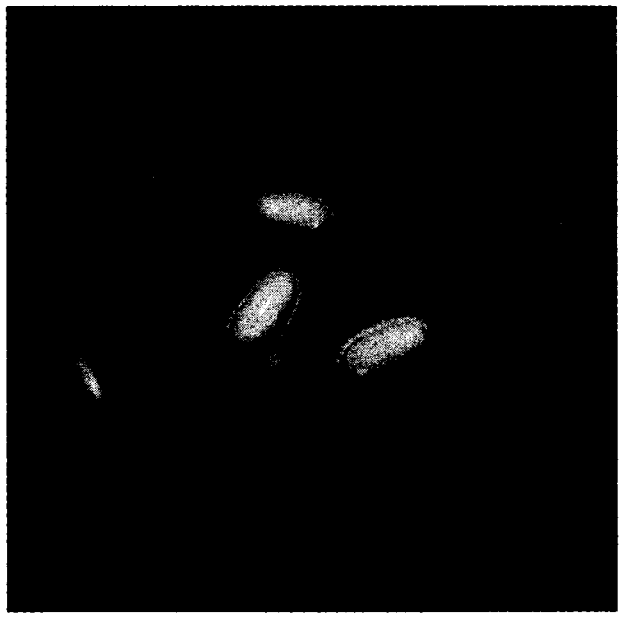


Fig. 1.3 Schematic representation of a liposome
(Image courtesy of Neopharm. Inc.)

1.1.4. Roles of Carbohydrates in Drug Design

An effective drug should first be a target-specific one which can be expected by its users to have high efficiency and having few, if any, side effects. This type of target specificity also means recognition when carbohydrates come in. A huge number of drugs contain carbohydrates as components in their molecular structure while other drugs, lacking carbohydrates covalently bound to their molecules, can also be guided by them. It has been widely realized that carbohydrates' value is to provide a guidance mechanism for sick cells and to enable drugs to arrive there with precision and act properly. As they should be, carbohydrates also provide a defense mechanism for sick or deadly cells, preventing drugs to act effectively.

1.2. Alginates

1.2.1. Structure of Alginate

Chemically, alginate is an unbranched, non-repeating and linear polysaccharide with homopolymeric blocks of (1-4)-linked β-D-mannuronic acid (M) and its C-5 epimer α-L-guluronic acid (G) residues, respectively, covalently linked together in different sequences or blocks (**Fig. 1.4**). The monomers can appear in homopolymeric blocks of consecutive G-residues (G-blocks), consecutive M-residues (M-blocks), alternating M and G-residues (MG-blocks) or randomly organized blocks. The relative amount of each block-type varies depending upon the different alginates sources from which they are isolated. Alginates from bacteria also display a certain degree of acetylation at the *O*-2 and *O*-3 positions of D-mannuronic acid residues. [5]

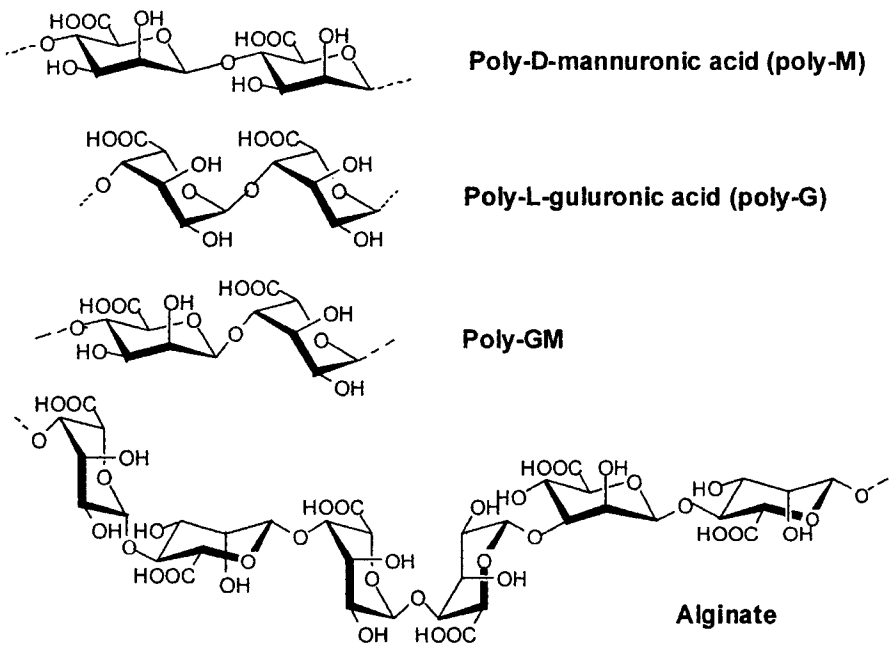


Fig. 1.4 Structure of poly-D-mannuronic acid, poly-L-guluronic acid, poly-GM and alginate

1.2.2. Natural Occurrence of Alginates

In nature, alginate is mainly found as the structural substance of marine brown seaweed in which alginate exists as the most abundant polysaccharide comprising up to 40% of their dry weight.[6] In addition, alginate has been found as a mucoid exopolysaccharide (MEP), so-called mucoid strain, produced by bacteria such as *Pseudomonas aeruginosa*, which is the predominant bacterial pathogen in chronic pulmonary infection of cystic fibrosis (CF) patients. Furthermore, alginate also exists as a capsular polysaccharide for the bacteria *Azotobactor vinelandii* and *Azotobacter chroococcum*.[7-11] The main difference between algal and bacterial alginate is that the mannuronate residues of bacterial rather than those of the seaweed polymers are *O*-acetylated to a variable extent at positions *O*-2 and *O*-3.[12]

1.2.3. Industrial Uses of Alginates

The block structure and extent of *O*-acetylation determine the physicochemical properties of different alginates.

MG-blocks form the most flexible chains and are more soluble at low pH than the other two block-types.[13] Alginates containing polyguluronate can form rigid gels in the presence of divalent cations (e.g.Ca^{2+}, Ba^{2+}, Sr^{2+}), whereas an absence of poly-G, in *P. aeruginosa* but not in vinelandii, produces relatively soft acidic gels at low pH in the presence of Ca^{2+}. The stability of the gel is mostly dependent on the content of G-blocks.[6, 14, 15] Moreover, extensive *O*-acetylation of bacterial alginates increases the water-binding capacity of the polysaccharide, which may enhance bacterial survival under desiccating conditions.[12]

Based on the characteristics above, alginates have various industrial uses as viscosifiers, stabilizers and gel-forming, film-forming or

water-binding agents ranging from textile printing, ceramics manufacturing, welding rods production and water treatment, to custard creams and restructured food production, as well as stabilizers and thickeners utilized in the variety of beverage, ice-creams, emulsions and sauces.[16] In addition, the pharmaceutical industry also uses alginates as wound dressings, dental impression materials and tablet binders or disintegrants.[16]

Furthermore, alginates have been biotechnologically utilized for the semi-permeable encapsulation of cells and enzymes. Through droplets of a mixture of cells or enzymes and Na-alginate into a solution with a gel-forming cation (Ca^{2+}), the gel-beads with a high amount of poly-G blocks exhibit high porosity, low shrinkage during gel-formation and low swelling after drying.[6] Hence, entrapment within spheres of a calcium alginate gel as a useful technique for immobilization of living cells has been wildly recognized.

1.2.4. Roles of Alginates in *Pseudomonas Aeruginosa*

Pseudomonas aeruginosa is a Gram-negative, aerobic, rod-shaped bacterium with unipolar motility.[17] An opportunistic human pathogen, *P. aeruginosa* is also an opportunistic pathogen of plants.[18] As an opportunistic pathogen of immunocompromised individuals, *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes blood infections.[19] One in ten hospital-acquired infections are from *Pseudomonas*. Cystic fibrosis patients are also predisposed to *P. aeruginosa* infection of the lungs.

Alginates, being the essential constituent in *P. aeruginosa*, have been found to have inherent and excellent performance in immune modulating and anti-tumor activities. It has been reported that alginates have the ability to form biofilms, cause chronic infection in the lungs of cystic fibrosis patients and act as a barrier to protect the infected cells from humoral and

cellular host defence systems as well as from the action of antibiotics.[20]

Co-administration of antibodies with alginate lyase, which degrades the exopolysaccharide produced by mucoid strains of *P. aeruginosa*, might benefit CF patients by increasing the efficacy of antibiotic in the respiratory tract.[21] The MEP-KLH (Keyhole Limpet Hemocyanin) conjugate vaccine, comes from using SMCC (Sulfosuccinimidyl 4-[*N*-maleimidomethyl] cyclohexane-1-carboxylate) as a linker and it can enhance high titers of MEP-specific opsonic antibodies in mice and rabbits.[22]

The alginate capsule produced by the human pathogen *P. aeruginosa*, mainly composed of 1-4-linked β-D-mannuronic acid polymers (poly-M). They have been found to immunostimulate strongly and share with lipopolysaccharide (LPS) the ability of stimulating human monocytes mediated by TLR2/4 to produce the cytokines tumor necrosis factor alpha (TNF-α) and interleukins (IL-1 and IL-6), through binding to membrane CD14.[13]

1.2.5. Biosynthesis of Alginates

The biosynthesis of alginate was first illucidated by Lin and Hassid in 1975 involved in detecting the enzyme activities necessary for the synthesis of mannuronan in a cell-free system from *fucus gardnerii*.[23] *A.vinelandii* and *P.aeruginosa* have been found to have the same biosynthesis pathway as follows: D-fructose-6-phosphate is firstly converted to sugar nucleotide GDP-D-mannose and then oxidized to GDP-D-mannuronic acid followed by being polymerized to mannuronan. The mannuronan can be further modified by an acetyl transferase and epimerized by a mannuronan C-5-epimerase to L-guluronic acid [24] (**Fig. 1.5**).

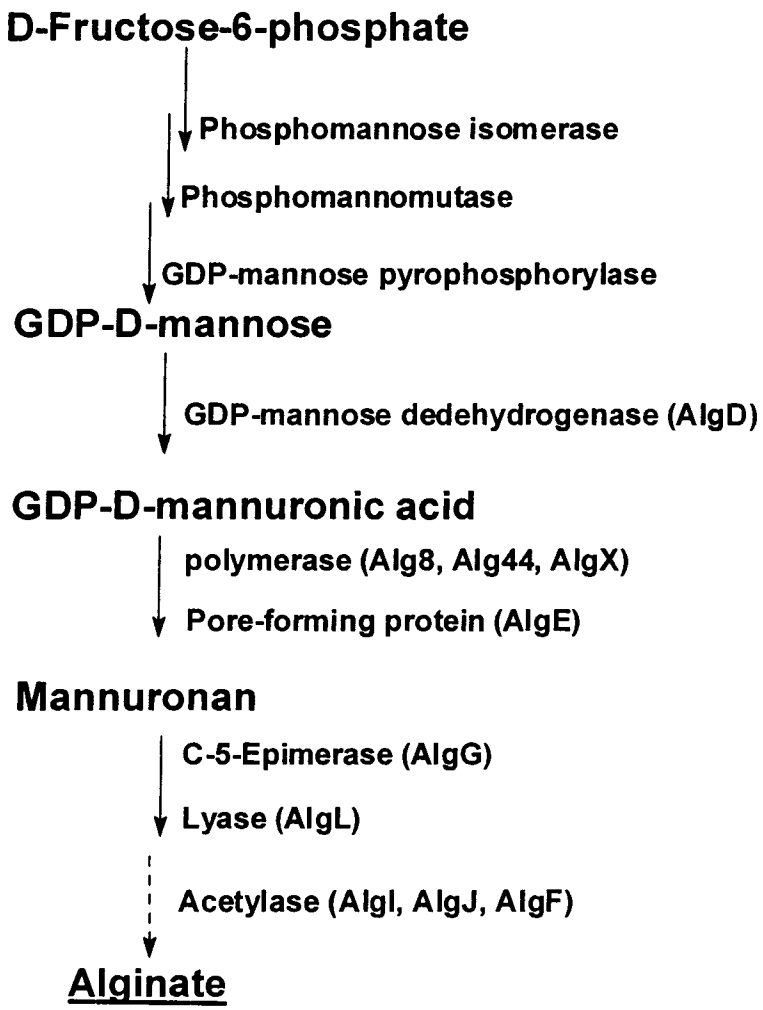


Fig. 1.5 Biosynthesis pathway of alginate

1.3. Toll-like Receptors

1.3.1. Discovery of TLR

The name TOLL originally came from a cell surface receptor governing dorsal/ventral orientation in the early drosophila larvae which was also found later to play a critical role in antifungal defense together with other antimicrobial peptides.[25, 26] In the 1990s, the first mammalian proteins structurally similar to Drosophila TOLL were discovered and later named human Toll-like receptor (TLR) 1 and 4. It has been revealed that 10 human and 9 murine transmembrane proteins belong to the mammalian TLR

family.[27]

Toll-like receptors (TLRs) are single-pass transmembrane integral membrane proteins (IMP) that span from the internal to the external surface of the biological membrane or lipid bilayer in which they are embedded.[28]

TOLL and TLR family proteins are characterized by the presentation of an extracellular domain with leucine-rich repeats and an intracytoplasmic region containing a TOLL/interleukin-1 receptor homology (TIR) domain. The TOLL/interleukin-1 receptor homology (TIR) domain is very important to both Drosophilia TOLL and mammalian TLR signaling, indicating that both of them share homologous signaling components. In fact, the homologous signaling components have been actually described as two systems: mammalian TLR and Drosophila TOLL, for each signaling step.[29]

1.3.2. Roles of PAMP and TLR in Host Defense

Pathogen-associated molecular patterns (PAMP), referring to the recognition of components on the pathogen that are not normally found in the host, have been described as a critical element in the initiation of an innate immune response against various pathogens.

Toll-like receptors have a crucial role in the detection of microbial infection in mammals and insects. In mammals, these receptors have evolved to recognize conserved products unique to microbial metabolism.[28] This specificity allows the Toll proteins to detect the presence of infection and to induce activation of inflammatory and antimicrobial innate immune responses.

TLRs can recognize pathogens and activate immune cell responses as a key part of the innate immune system and hence appear as recognition receptors with evolutionary conserved patterns. These receptors can be utilized to distinguish between self and non-self by adhesion of pathogen-related molecular models with no obvious structure similarity in host immune defense.[28] TLR2 and TLR4 have been found to mediate the

cytokine production induced by alginate polymers and alginate oligosaccharides in human immune system.[30]

In vertebrates, TLRs can help activate the adaptive immune system, linking innate and acquired immune responses. They function as a dimer and may depend on other co-receptors for full ligand sensitivity, such as in the case of TLR4's recognition of lipopolysaccharides (LPS), which requires MD-2, CD14 and LPS Binding Protein (LBP).[31]

Activation of TLRs and cascade signaling pathways lead to the expression of pro- and anti-inflammatory mediators which have great impact on human physiology.

The particular mechanism[32] has been described (**Fig. 1.6**) that, after infection, antigen-presenting cells (APC), like macrophages and dendritic cells (DC), express TLR on their surface, bind these PAMP and initiate a signaling pathway that stimulates the host defenses through the induction of reactive oxygen and nitrogen intermediates (ROI and RNI). TLR, bound with these PAMP, initiates adaptive immunity as it activates APC by inducing production of pro-inflammatory cytokines and up-regulating co-stimulatory molecules.

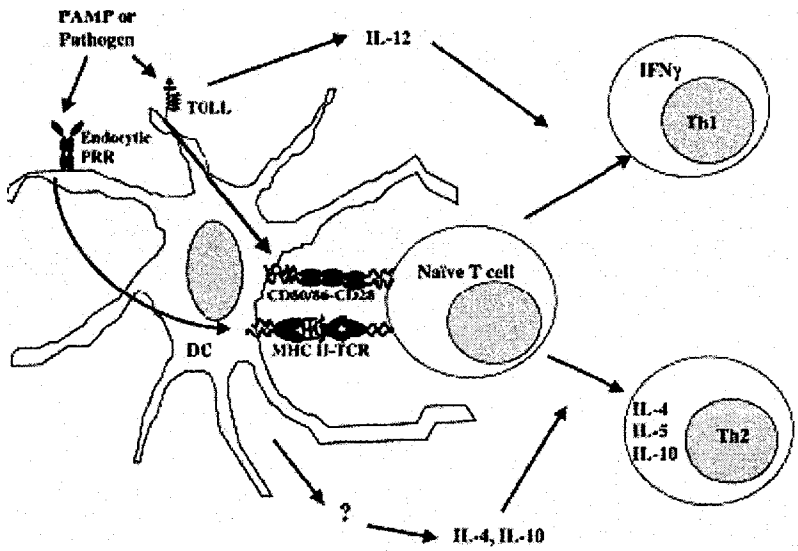


Fig. 1.6 Activation of adaptive immunity through TLR[32]

TLRs are crucial not only in the early phase of infection when innate immunity is important, but also link innate and adaptive immunity throughout the entire process of the host defense response.

1.3.3. Interaction of PAMP with TLRs

LPS, the exogenous and the best-characterized PAMP as well as the major component of the outer membrane of Gram-negative bacteria, have been found to induce a variety of immunostimulatory responses including the production of pro-inflammatory cytokines such as IL-12 and inflammatory effector substances.[32]

CD14, a glycosylphosphatidylinositol (GPI)-anchored protein expressed by mononuclear phagocytes, has been identified to bind to LPS but lacks an intracytoplasmic region, suggesting that it is unable to transduce a signal and hence TLR4, an additional membrane protein, has emerged as an important factor to transmit an LPS signal.[33] In addition to exogenous PAMP, TLR4 also has been revealed to bind to endogenous molecules such as heat shock protein (HSP) to induce an inflammatory response in normal mice.[25]

Gram-positive bacteria can also provoke immune responses similar to those generated by LPS. Based on its ability to form at least two distinct types of functional heterodimers with other TLR (TLR6 and TLR1), TLR2 can bind a variety of ligands including various glycolipids and peptidoglycan (PGN).[34]

1.3.4. Immune Stimulating Activities of Alginate Mediated by TLR 2/4

Defined uronic acid polymers have been reported to induce cytokine production from human monocytes and poly-M is widely considered as the most potent tumor necrosis factor (TNF) inducer in uronic acid tribe.[16, 30, 37]

It has been found that poly-M from alginates has immunomodulating properties. Accompanying the important roles of TLRs in signal transduction, poly-M isolated from mucoid strains produced by *P.aeruginosa* can share with

lipopolysaccharide the ability to stimulate cytokine production from human monocytes in a CD14-dependent co-receptor manner.[35]

Membrane CD14 together with either TLR2 or TLR4/MD-2 could mediate an immune response by poly-M. Meanwhile, the ability of cytokine production induced by monocytes has been found to increase depending upon the composition of poly-M residues.[36] Poly-G blocks have been reported not to be able to provoke cytokine production by monocytes and further reduce the level of cytokine production induced by poly-M blocks.[37]

Moreover, blocking antibodies to TLR2 and TLR4 have been found to partly inhibit the tumor necrosis factor production induced by poly-M in human monocytes, and further inhibition was obtained in combination with antibodies.[35] Alginate oligomers with molecular weights less than 2000 are also recognized by innate immune system through TLRs.[30]

Unfortunately, the underlying mechanism of these bioactivities is still not clear and further study of structure-activity relationship of alginate oligomers is required to provide insight into the TLR-mediated recognition mechanism.

1.4. Objective of Thesis Project

TLRs play essential roles in mammalian immunity. Recently, it was reported that alginate oligomers have immunomodulating properties by binding to TLR2/4. In addition, previous research has elicited that optimal cytokine stimulatory activity from uronic acid polymers requires a certain polymer length and mainly β−1,4 diequatorial glycosidic linkages. In various degrees of unsaturated oligomers mannuronate (M3-M9) and guluronate (G3-G9), G8 and M7 showed the most potential TNF-α-inducing activity even though G3 and M3, the smallest oligomers among PM and PG, also showed relatively high cytokine inducing activities compared with other larger oligomers.[30]

Insight in the mechanism of TLR-mediated recognition and signal transduction, leading to cytokine production, needs well-defined fragments of alginates and their functionalized derivatives. The chemical synthesis of alginate oligosaccharides has not been well studied. During the course of our investigation, the group of Van den Bos reported the successful synthesis of an alginate trisaccharide in which α-thio-monomannuronic acid derivatives were selected as glycosylation donors. [38] Different donors (α-thio-monomannosides) have been chosen in our synthesis of β-1,4-di- and tri-D-mannuronic acid glycolipids. In the following chapters, the details of chemical synthesis of **1** / **2** are described.

The main objective of the thesis is to synthesize *oligo*-β-1,4-D-mannuronic acid glycolipids (**1** & **2**) (**Fig. 1.7**) as potential TLR ligands which may show immune stimulatory properties.

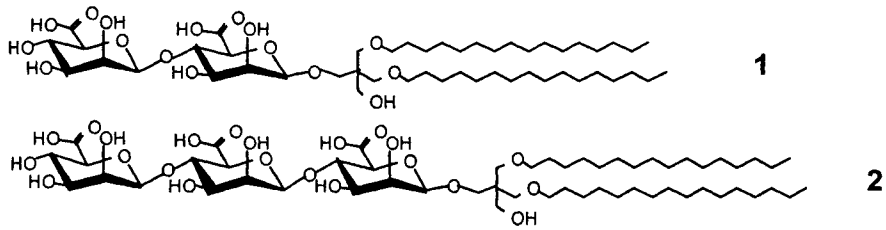


Fig. 1.7 Structure of β-1,4-D-mannuronic acid glycolipid **1** / **2**

2. Synthesis of β-1,4-D-mannuronic Acid Glycolipids

2.1. General Features of Glycosylation Reactions[44]

In glycosylation reaction, there are a number of important aspects that need to be considered. The following features will be taken into account in our synthetic strategy:

1. Monosaccharides have to be properly protected, by using protective groups, prior to the application of synthetic strategies aimed at oligosaccharide synthesis. The protection steps are generaly unavoidable and are a consequence of the polyhydroxylated nature of sugars.
2. Although hydroxyl groups on pyranose rings have different nucleophilicities that permit selective glycosylation to be accomplished with the sugar having several unprotected hydroxyls, this is not the general case in our glycosylation strategy.
3. Most commonly, for the glycosyl acceptor, it is necessary to follow a strategy in which all the hydroxyl groups are protected with the exception of the one to be glycosylated. For the glycosyl donor, besides the protection steps, it is essential to have a suitable activating group at the anomeric center, which in turn functions as a leaving group generating an oxocarbenium ion that is susceptible to nucleophilic attack by the acceptor.
4. For the formation of a 1,2-*trans* glycoside, it is common practice to take advantage of the protecting group at C-2.
5. Nucleophilic attack on the oxocarbenium ion (**Fig. 2.1**) leads to the formation of α or β glycosidic linkages and highlights the numerous variables such as temperature, solvent, polarity, catalyst and protecting groups, that should be taken into account when a stereoselective product is desired.

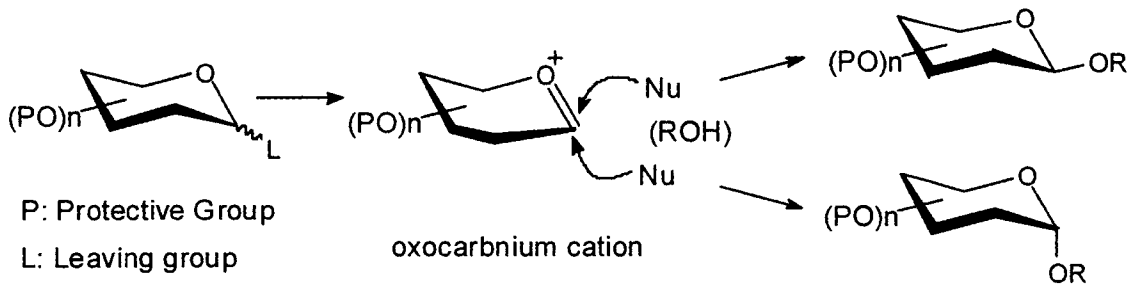


Fig. 2.1 Nucleophilic attacking on the oxocarbenium ion

6. Non participating groups and relatively non-polar solvents favor 1,2-*cis*-glycosylation products. A non-participating benzyl group has been chosen in the organic synthesis of target molecules.

2.2. Structural and Synthetic Features of Target Molecules

Compound **1** / **2** (β-1,4-D-mannuronic acid glycolipid) have been designed as glycolipids mainly considering the following aspects:

1. Naked small carbohydrates molecule would be rapidly cleared away from the biological system,[39] while conjugation with lipids may stablize them and hence make them better drug agents than naked carbohydrates.
2. The concept of liposome formulation, a drug delivery platform to reduce toxicity and side effects as well as to slow down release speed, has been a concern and, therefore, two C_{16}-chains were introduced into molecule design in this thesis, since the 14- to 18-carbon lipid chains is considered to be more efficient in liposome formulation.[2]

Our approach to target molecules, β-1,4-D-mannuronic acid glycolipid **1** / **2** (**Fig. 2.2**), mainly focuses on the 1,4-linked 1,2-*cis* mannosyl relationship which is hard to achieve by normal stereocontrolled manner. Some

methods exist for the challenging β-D-mannoside linkage, but they are not efficient until the remarkable methodology developed by Crich's group recently. A similar method for the direct synthesis of β-D-mannosides has been utilized in our synthetic strategy involving in the diastereoselective β-glycosylation of 4,6-*O*-benzylidene protected α-thiomannoside donor with prepared acceptors ROH. A subsequent specific TEMPO / BAIB oxidation leads to target molecules **1** / **2** (**Fig. 2.2**).

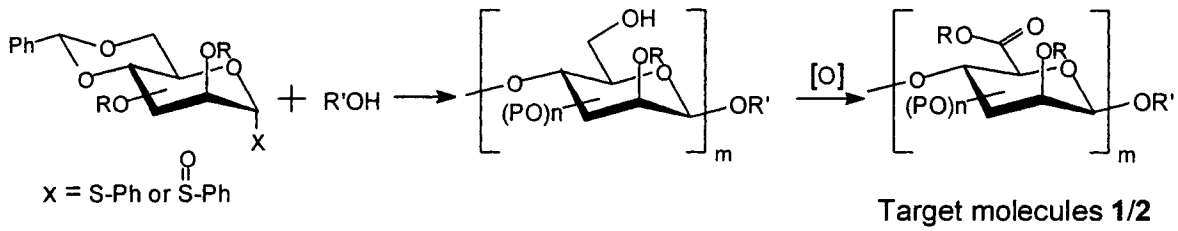


Fig. 2.2 Crich's methodology followed by an oxidation step leads to **1/2**

Several conformational characteristics feature β-glycosylation of D-mannosides in Crich's methodology, including:

1. The presence of the 4,6-*O*-benzylidene acetal protective group at 1-thio functionalized mannoside donor **9** has a profound influence on the stereoselectivity of mannosylation reactions conducted by BSP/Tf_2O/DTBMP activated thioglycoside coupling protocol. The trans fused configuration of 4,6-*O*-benzylidene acetal protective group restricts the molecules from ring flexibility and hence make it increasingly difficult to reach a half-chair transition state from a chair ground state. [40] The stereodirecting influence of 4,6-*O*-benzylidene protecting group arises from so called "torsionally disarming" effect on glycosyl cations (oxacarbenium ions), which effectively shifts all equilibria towards the covalent axial-α-mannosyl triflates and in turn promotes S_N2-like displacements with the incoming acceptor ROH to

reach β-glycosylation.[41]

2. The smaller and non-participating 2-*O*-benzyl ether protective group stimulates the greater β:α ratios for secondary mannosyl acceptors ROH, compared with the other bigger steric bulk of the *O*-2 protecting groups such as 2-*O*-TBDMS (*tert*-butyldimethylsilyl) and 2-*O*-TMS (trimethylsilyl).[42]

2.3. Synthesis of β-1,4-di-D-mannuronic Acid Glycolipid 1

2.3.1. Synthetic Strategy for β-1,4-di-D-mannuronic Acid Glycolipid 1

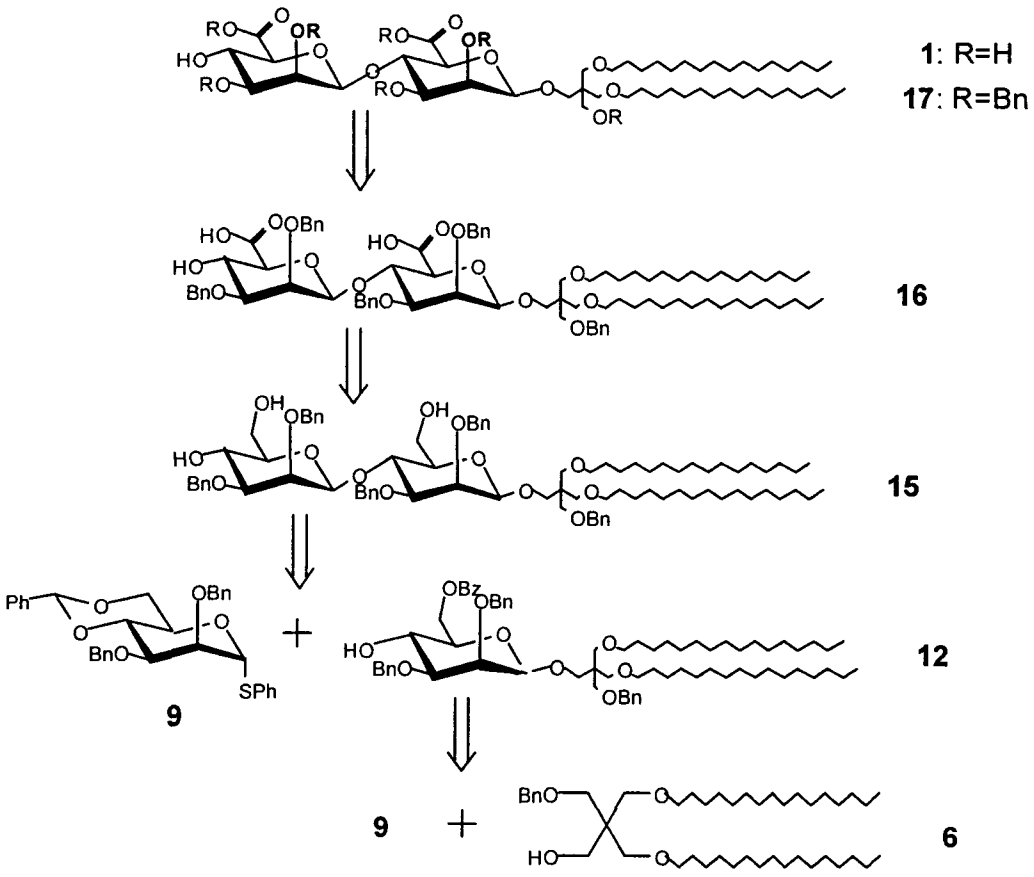
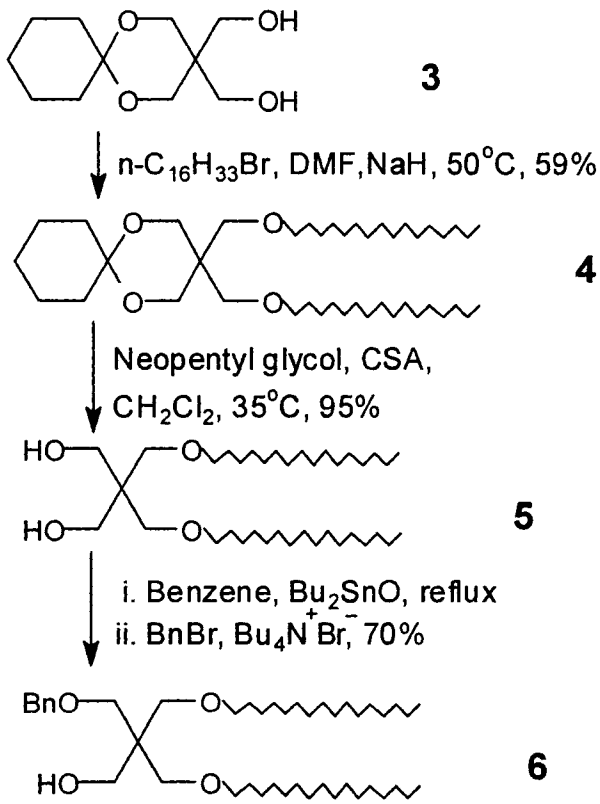


Fig. 2.3 Retrosynthetic analysis of glycolipid **1**

The strategy employed to produce the β-1,4-di-D-mannuronic acid moiety is to first synthesize the β-1,4-di-D-mannobiose unit, followed by an oxidation step to convert the mannose residues to the glycolipid **1** (**Fig. 2.3**). The 4,6-di-O-benzylidene protected methodology recently developed by Crich and co-workers offers an efficient method for the direct synthesis of β-D-mannosides. [42, 48] A similar strategy has been used to construct the β-1,4-di-D-mannobiose moiety.

2.3.2. Synthesis of Lipid Anchor 6

Lipidated and benzylated pentaerythritol derivative **6** was prepared as the lipid anchor acceptor according to **Scheme 1**.



Scheme 1 Synthesis of lipidated anchor **6**

The start point of the synthesis was a known compound **3**.[45] Two C_{16}-lipid chains were introduced to **3** through *O*-alkylation with 1-bromo –hexadecane and sodium hydride (deprotonating agent) in DMF for 48 hours to offer monoketal-ether **4** in 59% yield. In this reaction, DMF acts as polar aprotic solvent since protic solvents and apolar solvents tend to slow the reaction rate strongly in an S_N2 attack.

Trans-ketalization of **4** with neopentyl glycol in the presence of camphorsulfonic acid (CSA) in anhydrous dichloromethane (DCM) furnished O-alkyl diol **5** in 95% yield. Recrystallization was used here to purify **5** by using dichloromethane (DCM) as a suitable solvent.

Two other acidic hydrolysis reaction systems **a** (EtOAc / MeOH / H_2O = 10 : 1 : 0.2 and p-TsOH 0.1 eq. at r.t.) and **b** (EtOAc / Hexane / ethanediol = 4 : 2 : 1 and p-TsOH 0.1 eq. at r.t.) also have been employed but gave lower yields (5% and 61% respectively). The lower yields probably result from the low solubility of **4** in which two saturated C_{16}-n-alkyl chains were connected and the relatively difficult intermolecular reaction. Actually, the mono-hydroxyl intermediate product has been competitively obtained under the conditions of the **b** system.

Monobenzylation of **5** by reflux with dibutyltin oxide (Bu_2SnO), a deprotonating agent, in benzene at 90 ^{0}C for 16 hours and azeotropic removal of produced water, followed by addition of benzyl bromide and tetrabutylammonium bromide ($Bu_4N^+Br^-$) at the same temperature, provided compound **6** in 70% yield. A proposed mechanism (**Fig. 2.4**) of the monoderivatization of symmetric diol **5** using one of stannoxanes, dibutyltin oxide (Bu_2SnO), is shown. 1,2-Diol **5** is treated with Bu_2SnO with azeotropic removal of produced H_2O to afford the requisite tin acetal (**I**). The stannylidene then goes through selective monobenzylation [(**II**), (**III**) and (**IV**)] with BnBr in the presence of nucleophlic catalyst $Bu_4N^+Br^-$ to activate electrophilic substitution of the benzyl group of BnBr and finally provides desired compound **6**.

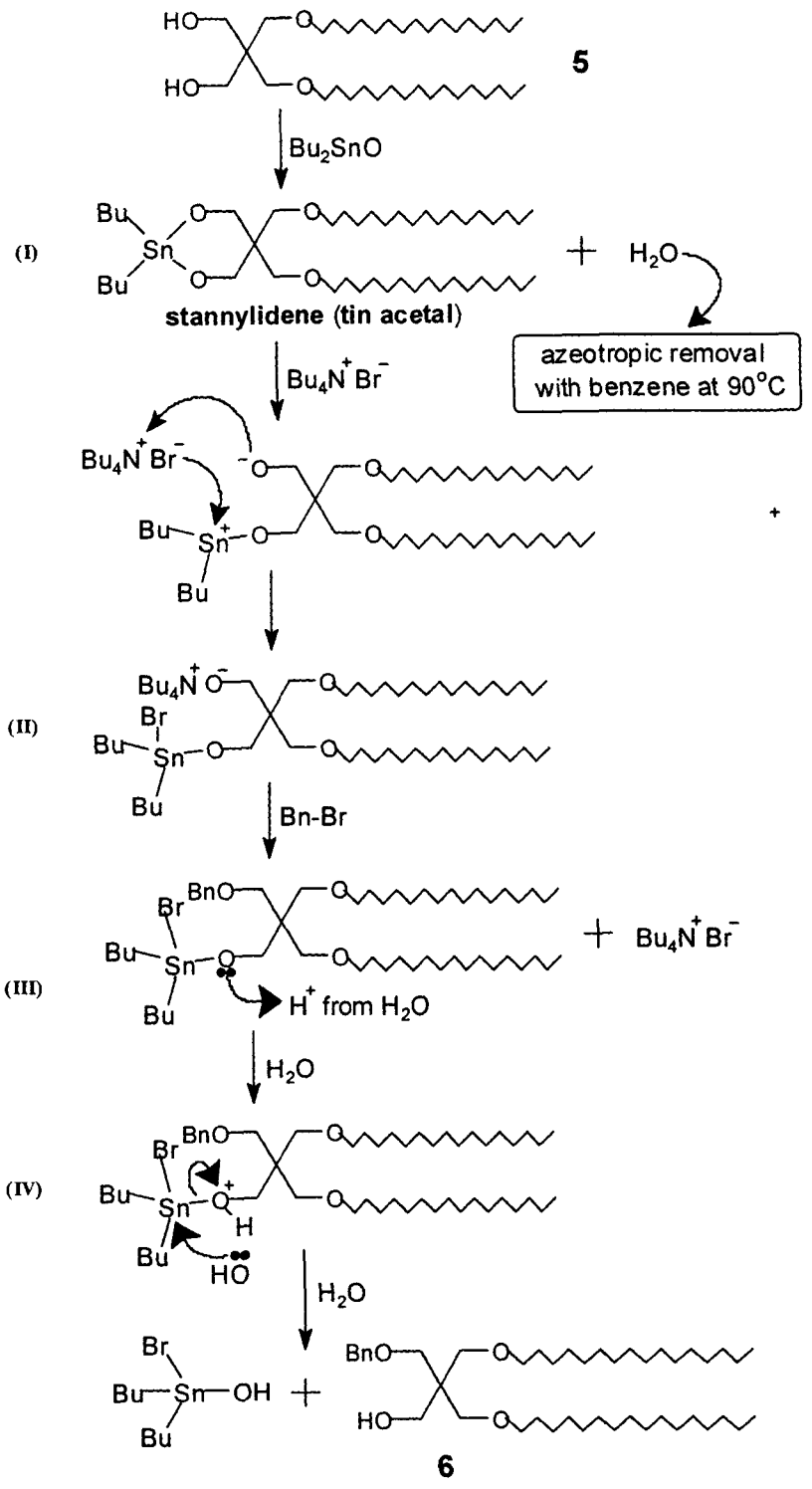
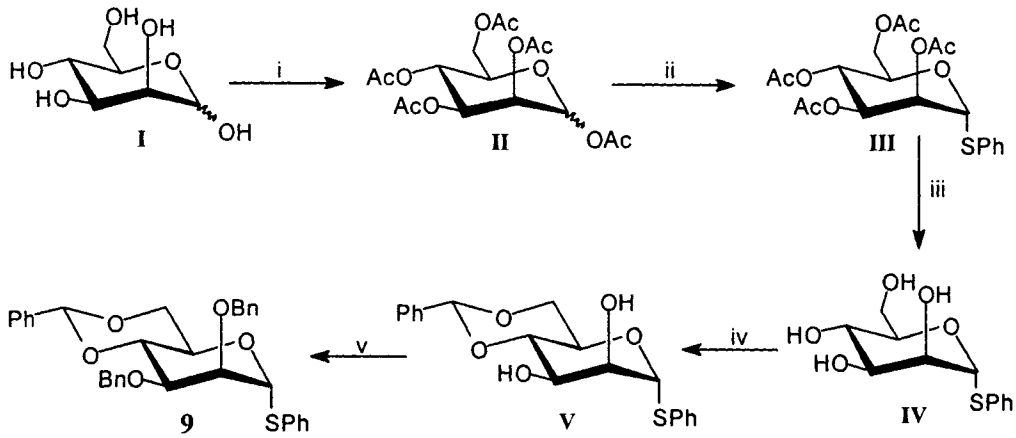


Fig. 2.4 Proposed monobenzylation mechanism of **5**

2.3.3. Synthesis of 4,6-O-benzylidene Protected α-Thiomannoside Donor 9



i. Pyridine, Acetic anhydride(Ac_2O), 0°c, 100 %.

ii.  , BF_3•$(EtO)_2$, DCM(anhydrous), r.t., 95 %

iii. MeONa, MeOH(anhydrous), PH=10, r.t., 100 %.

iv. $PhCH(OMe)_2$, p-TsOH, Dry DMF, rt, 51 %.

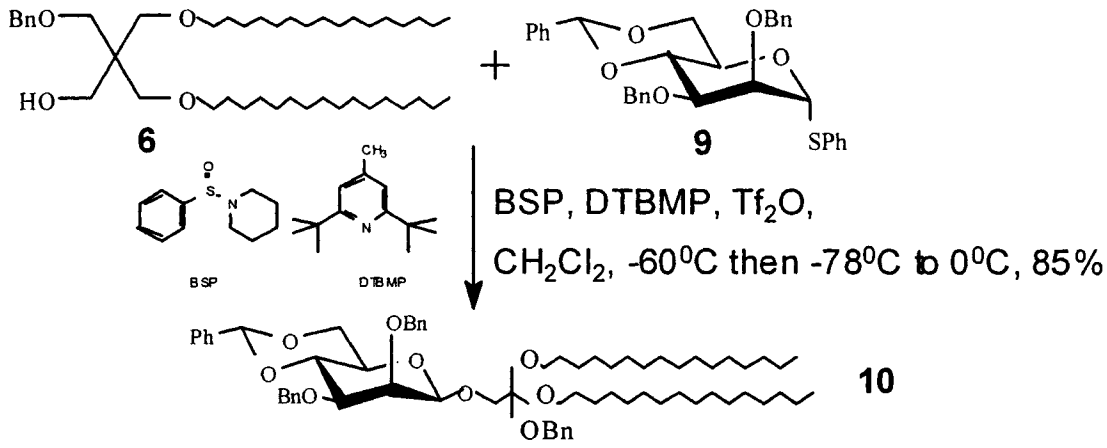
v. NaH, DMF, Bn-Br, r.t., 95 %

Scheme 2 Synthesis of mono-α-thiomannoside donor **9**

The synthesis of thiomannoside donor **9** reported by Crich and co-workers [46, 47] has been repeated (**Scheme 2**).

2.3.4. Synthesis of Monosaccharide 10

Crich and co-workers recently developed an efficient method for the direct synthesis of β-D-mannosides. [47, 48, 49, 50] A similar protocol has been employed here to produce mono-β-D-mannoside lipid **10** (**Scheme 3**).



Scheme 3 β-glycosylation of **9** with lipid anchor **6**

Therefore, by activating 4,6-*O*-benzylidene protected thiomannoside donor **9** with 1-benzenesulfinyl piperidine (BSP) and trifluoromethanesulfonic anhydride (Tf_2O) in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) at -60 ^{0}C, the protocol offers 1-α-mannosyl triflate intermediate. After the addition of two C_{16}-chains attached lipid anchor acceptor **6** at -78^{0}C, the protocol finally provides the desired β-mannoside **10** in 85% yield.

An appreciable rationale [51] (**Fig. 2.5**) shows that BSP is first converted to corresponding sulfonium **(I)** and then sulfonium triflate **(II)** respectively, with Tf_2O in the presence of a non-nucleophilic hindered base DTBMP as neutralizer. **(II)** rapidly expels a sulfinate ester **(III)** to offer oxacarbenium cation **(IV)** which is trapped in turn by the triflate anion to give glycosyl triflate **(V)**, and a following S_N2-like attack of **(V)** with acceptor **6** produces β-D-mannoside lipid **10**.

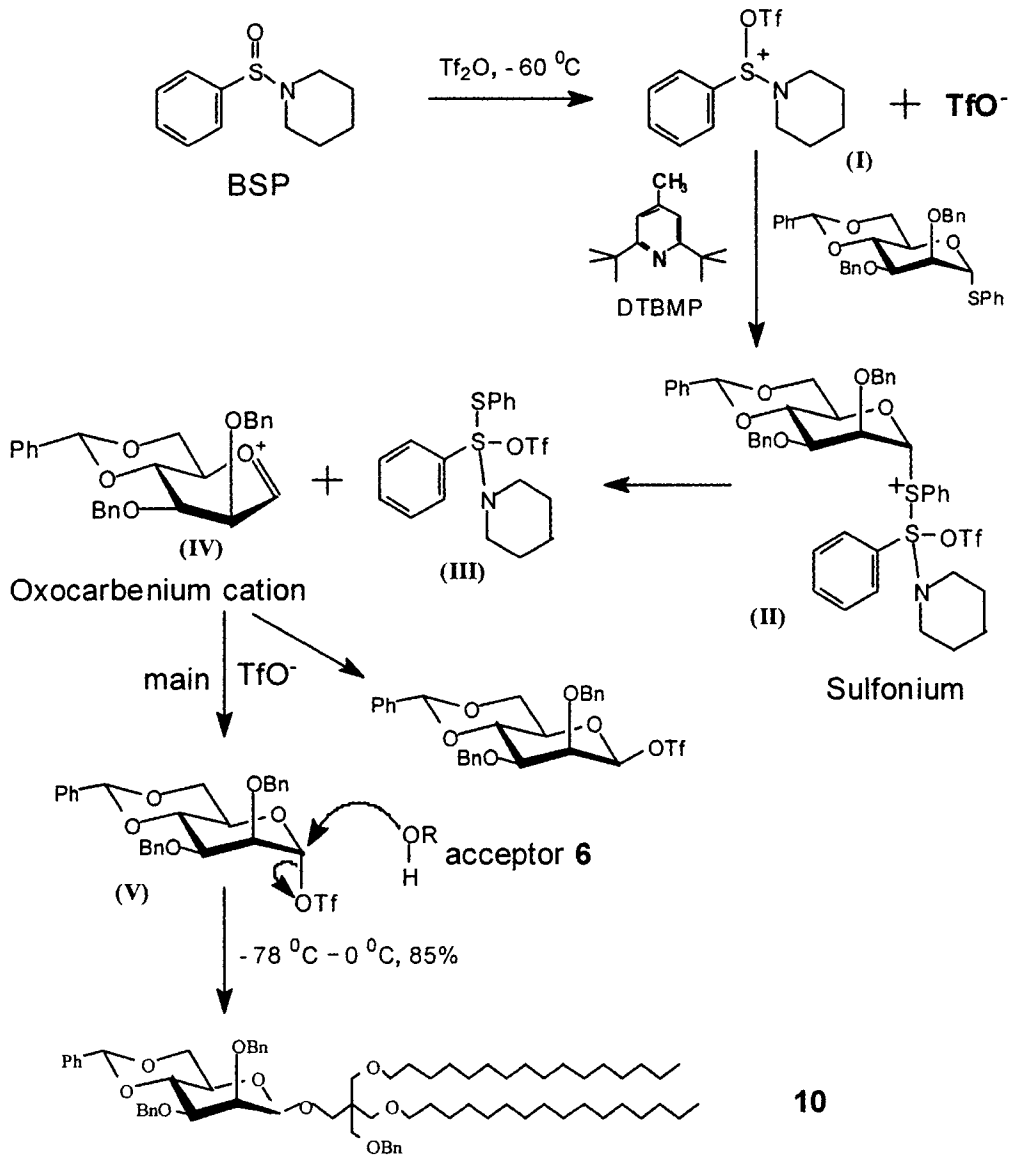
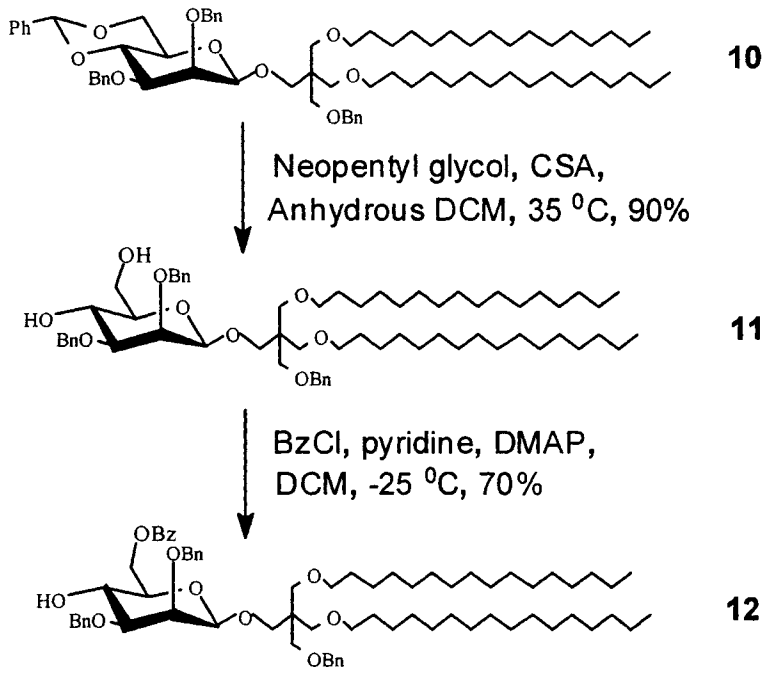


Fig. 2.5 Mechanism of β-glycosylation of thiomannoside donor **9** with acceptor **6**

The β-linkage has been confirmed by the value of $^{1}J_{C,H}$ coupling constant of the anomeric carbon in **10** ($^{1}J_{C,H}$ = 155.0 Hz) and the value of the unusual, somewhat upfield, H-5 chemical shift of δ 3.28 (ddd, 1H, J 10.0, 10.0, 4.5Hz,) in **10** with the 4,6-di-O-benzylidene protective group. The α-isomer is also formed in around 10% yield, but an analytically pure material has not been obtained.

2.3.5. Trans-ketalization and Regioselective Benzoylation of 10



Scheme 4 Deacetylation and regiobenzoylation of **10**

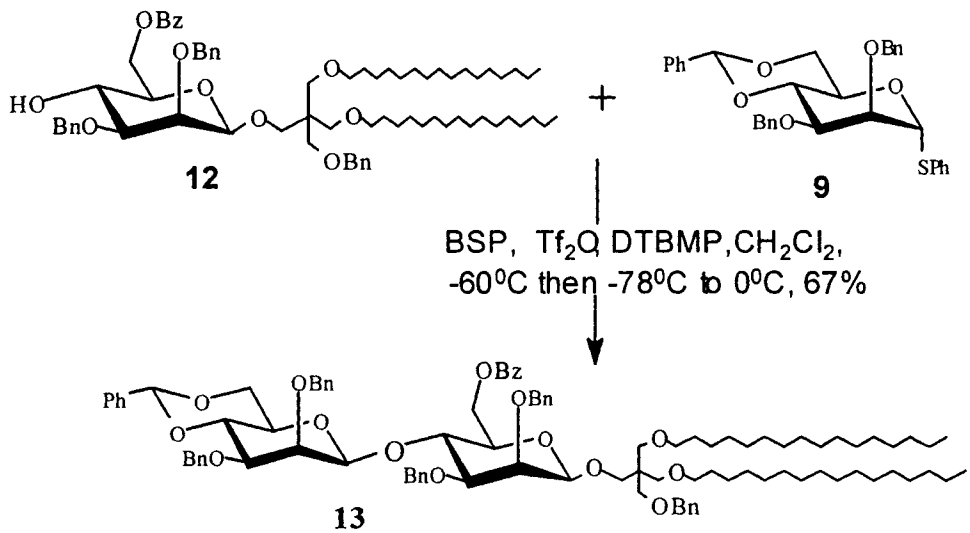
Removal of the ketal on **10** with neopentyl glycol in the presence of camphorsulfonic acid (CSA) in anhydrous dichloromethane (DCM) liberates 4,6-diol **11** in 90% yield.

Compound **11** is selectively converted to 6-*O*-benzoate **12** in 70% yield (**Scheme 4**), through regioselective benzoylation with benzoyl chloride in anhydrous pyridine in the presence of 4-N,N-dimethylaminopyridine (a nucleophilic catalyst).

2.3.6. Synthesis of Disaccharide 13

Activation of the prepared 4,6-*O*-benzylidene protected thiomannoside donor **9** with 1-benzenesulfinyl piperidine and trifluoromethanesulfonic anhydride (BSP/Tf_2O) at -60^{0}C, followed by the dropwise addition of

mono-β-D-mannoside acceptor **12** at -78 ^{0}C in the presence of hindered base 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) as neutralizing agent in anhydrous DCM provides the desired β-1,4-di-D-mannoside **13** in 67% yield (**Scheme 5**). The formed β-linkage in **13** is confirmed by the specific H-5 signal [^{1}H NMR (500 Hz, CDCl3): d 3.03 - 3.07 (ddd, J = 10.0, 10.0, 4.5 Hz, 1H, ***H-5b***)]. It is also confirmed by the $^1J_{C,H}$ coupling constant of two anomeric carbons and the CH carbon of the benzylidene group [^{13}C NMR (125 Hz, CDCl3): d 102.2 ($^1J_{C,H}$ = 155.5 Hz), 101.9 ($^1J_{C,H}$ =154.5 Hz), 101.3 ($^1J_{C,H}$ = 157.9 Hz)]. A minor product, probably the α-isomer of **13** is also detected, but an analytically pure sample has not been obtained.

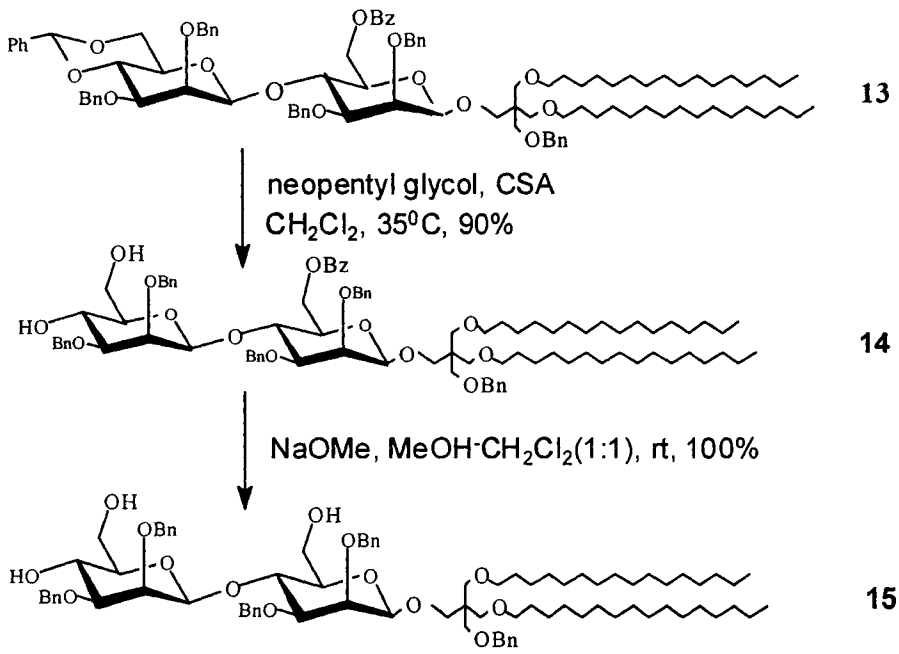


Scheme 5 β-Glycosylation to **13**

2.3.7. Removal of 4,6-di-*O*-benzylidene and Deacylation of 13

Using the same procedure as described earlier, removal of the 4,6-*O*-benzylidene protecting group of **13** is achieved through a trans-ketal reaction with neopentyl glycol in the presence of CSA in anhydrous DCM at 35 ^{0}C to offer diol **14** in 90% yield. Deacylation of **14** with a catalytic amount

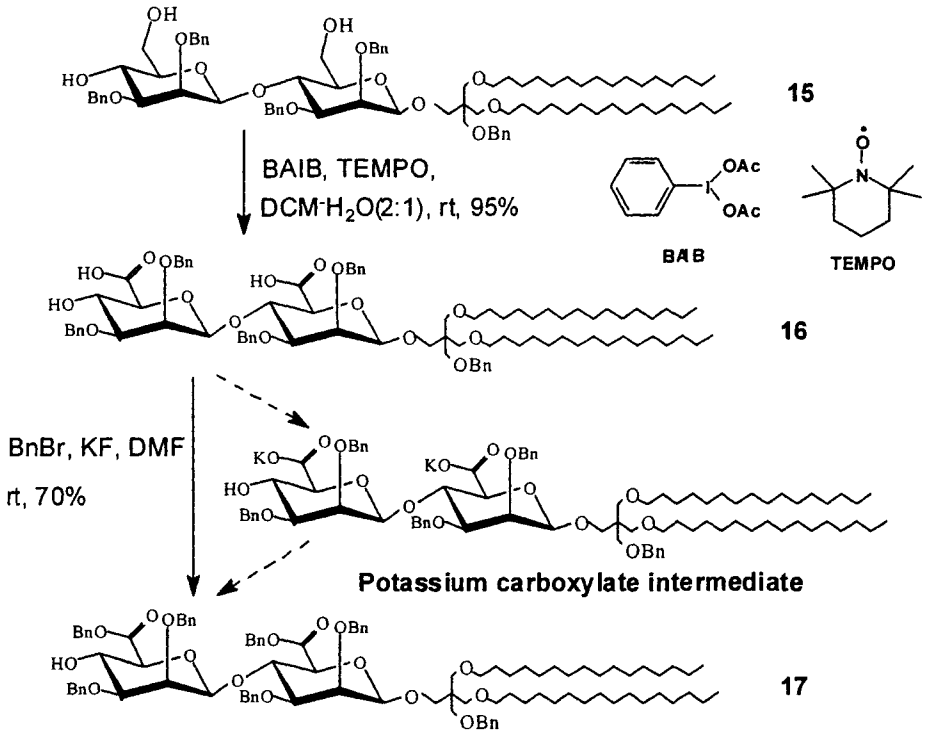
of sodium methoxide (NaOMe) in dichloromethane -methanol (1:1 v/v) under pH=9-10 at room temperature provides **15** in quantitative yield (**Scheme 6**).



Scheme 6 Deketalization and deacylation of **13**

2.3.8. Regioselective Oxidation of Disaccharide 15

Regioselective oxidation of the primary hydroxyl group (C-6) in **15** to the carboxylic acid is furnished by the combination of 2,2,6,6-tetramethyl -piperridinyloxy free radical (TEMPO) and acidic oxidant [bis(acetoxy)iodo]benzene (BAIB) in DCM-H_2O (2:1 v/v), where triol **15** is converted into its corresponding dicarboxylic acid **16** (**Scheme 7**) in 95% yield.



Scheme 7 Regioselective oxidation of **15**

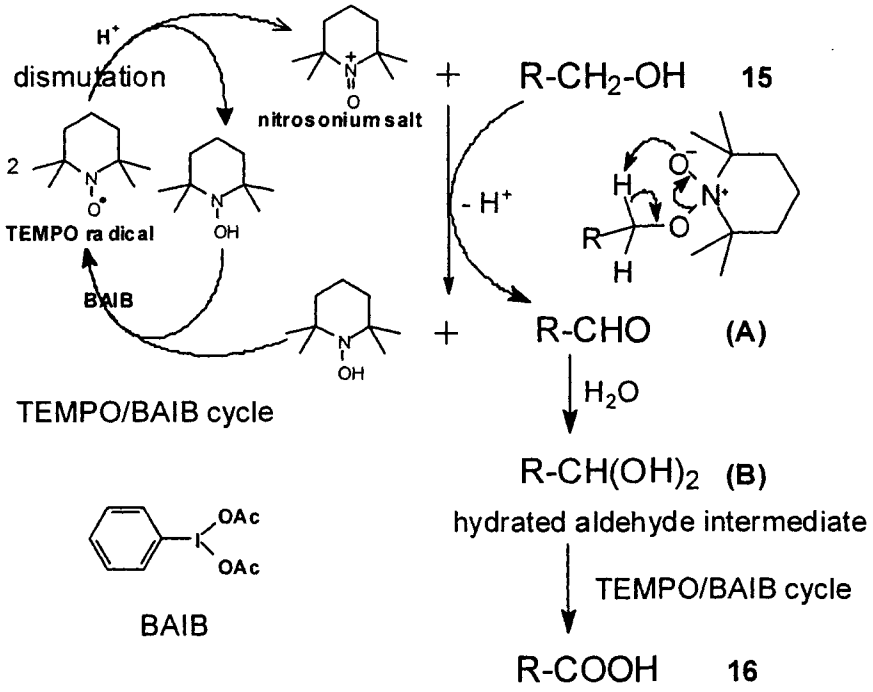


Fig. 2.6 Proposed oxidation mechanism of triol **15** with BAIB/TEMPO

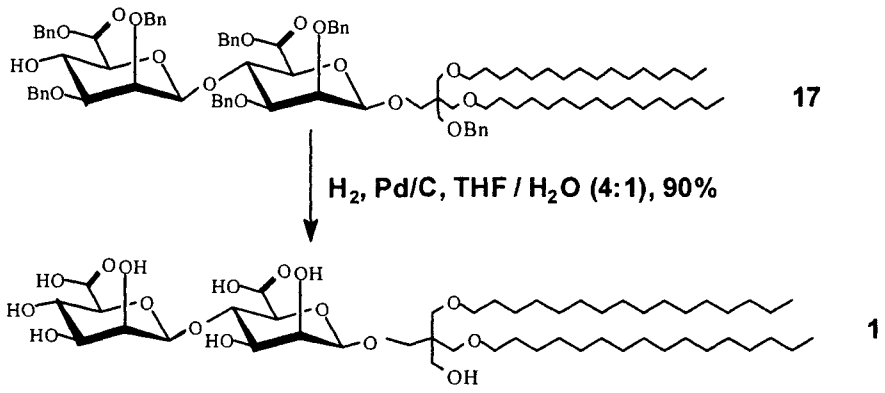
A proposed reaction pathway [53] (**Fig. 2.6**) for the oxidation of triol **15** has been illustrated here; after a ligand exchange around the iodine atom of BAIB, the developed acetic acid catalyzes the dismutation of the TEMPO radical to nitrosonium salt which quickly and selectively oxidizes two primary C-6 hydroxyl groups in triol **15** to the corresponding aldehyde. The nitrosonium salt itself is converted to hydroxylamine which goes through a TEMPO/BAIB catalytic cycle to regenerate TEMPO radical in the presence of BAIB. The produced aldehyde is converted to hydrated aldehyde intermediate in the presence of H_2O followed by another TEMPO/BAIB catalytic cycle to achieve desired dicarboxylic acid **16**.

Considering that it is hard to obtain analytically pure dicarboxylic acid **16** through silica gel chromatography because the carboxyl groups cause strong "drag tail", dicarboxylic acid **16** was converted to its benzyl ester **17**. Thus **16** was treated with benzyl bromide (BnBr) in the presence of potassium fluoride (KF as base) in DMF at room temperature to achieve benzyl ester **17** (**Scheme 7**) in 67% yield. The dicarboxylic acid **16** first reacts with basic potassium fluoride to produce the potassium carboxylate intermediate, a corresponding salt of **16**, which in turn goes through a S_N2 displacement toward BnBr. The polar aprotic solvent DMF was used to increase S_N2 attack, providing desired benzyl ester **17**.

2.3.9. Global Debenzylation to Target Molecule 1

Hydrogenolysis of **17** to remove the benzyl protecting group was carried out in tetrahydrofuran (THF) – water (4:1) under a hydrogen atmosphere in the presence of palladium on charcoal to furnish the target product β-1,4-di-D-mannuronic acid glycolipid **1** in 90% yield (**Scheme 8**). The structure of **1** has been confirmed by mass spectroscopy data {(MALDI-MS) calcd for $C_{49}H_{92}O_{16}$ 936.63$[M]^+$, found 959.63 $[M+Na]^+$,

981.62 [M-H+2Na]$^+$}. A well resolved NMR spectrum was not obtained due to its low solubility in most solvents such as MeOH, H_2O, THF and DMSO.



Scheme 8 Hydrogenolytic debenzylation of **17** to target **1**

2.4. Synthesis of β-1,4-tri-D-mannuronic acid glycolipid 2

2.4.1. Synthetic Strategy of β-1,4-di-D-mannuronic Acid Glycolipid 2

A similar synthetic strategy (**Fig. 2.7**) as synthesis of β-1,4-di-D-mannuronic acid glycolipid **1** was used here to produce target molecule **2**.

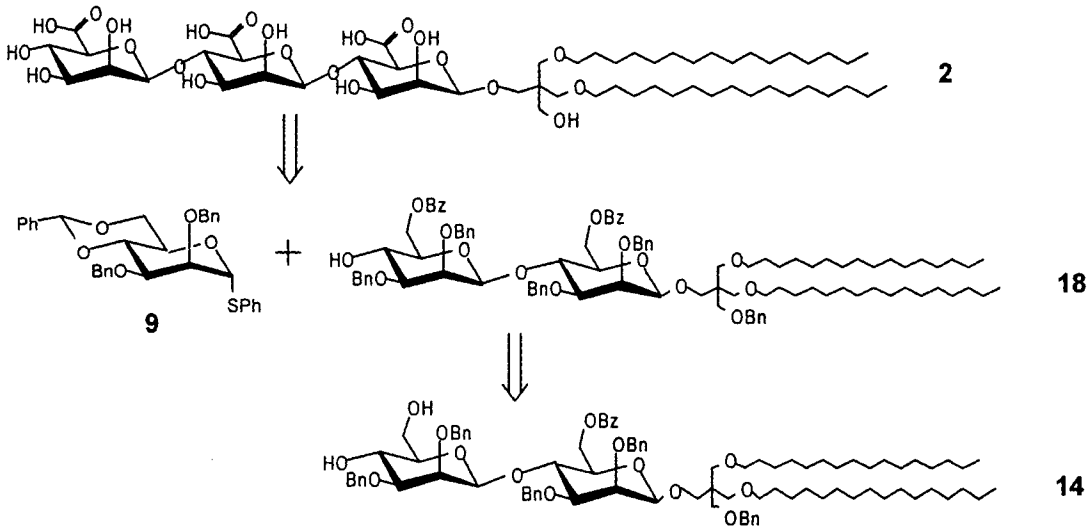
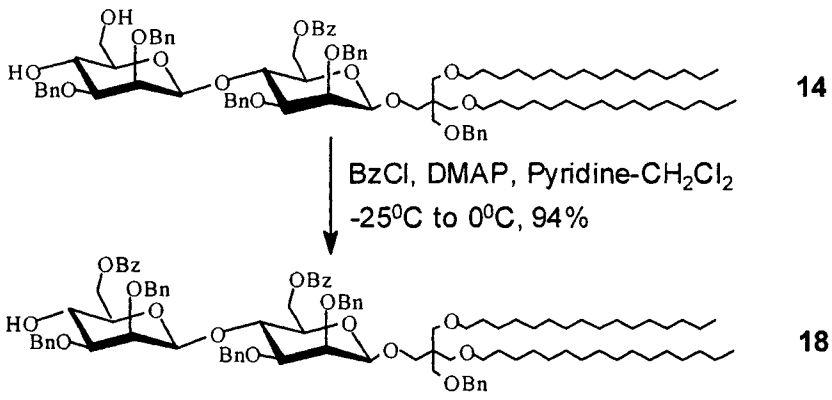


Fig. 2.7 Retrosynthetic analysis of β-1,4-*tri*-D-mannuronic acid glycolipid **2**

2.4.2. Regioselective Protection of Diol 14

Regioselective benzoylation of diol **14** with benzoyl chloride (BzCl) in anhydrous pyridine in the presence of 4-N,N-dimethylpyridine (as a nucleophilic catalyst) at -25^{0}C under N_2 atmosphere for 5 hours provides **18** in 94% yield (**Scheme 9**). This yield has been observed to be higher than that of benzoylation of monosaccharide **11** (70%) (**Scheme 4**) to a great extent but the reason is unclear.



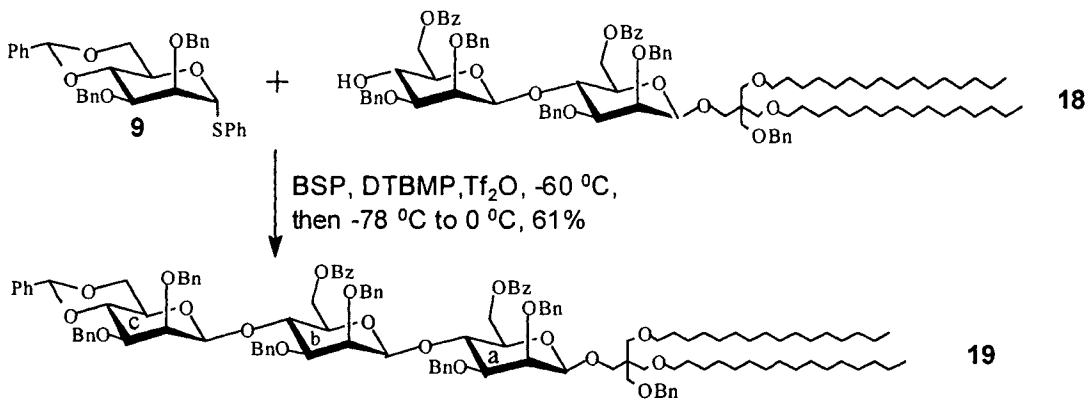
14

18

Scheme 9 Regioselective benzoylation of diol **14**

2.4.3. β-glycosylation of Thiomannoside Donor 9 with Acceptor 18

β-glycosylation of 4,6-O-benzylidene protected thiomannoside donor **9** with acceptor **18** has been employed to produce **19** (**Scheme 10**):



9

18

19

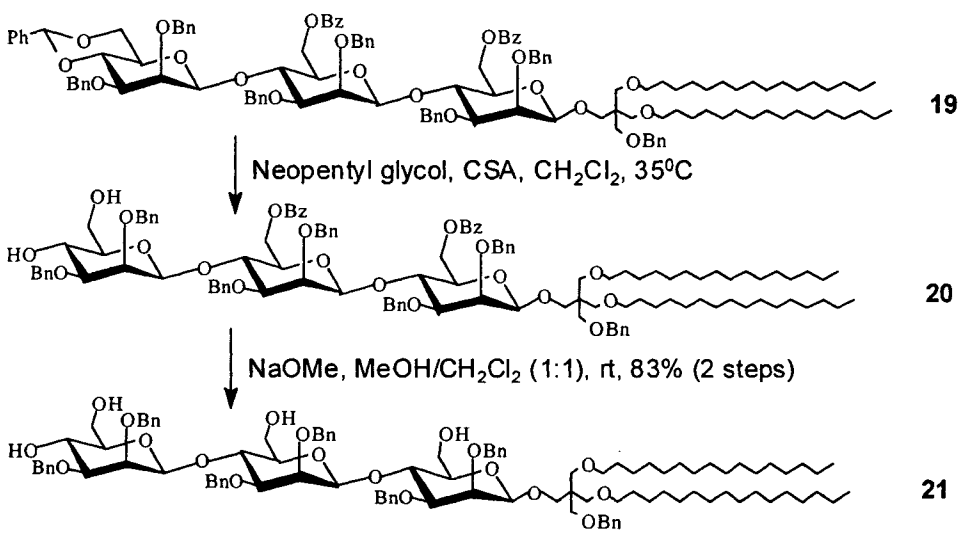
Scheme 10 β-Glycosylation of **9** with acceptor **18**

Activation of the α-thiomannoside donor **9** with 1-benzene sulfinyl piperidine and trifluoromethanesulfonic anhydride (BSP/Tf_2O) at -60°C, followed by the dropwise addition of mono-β-D-mannoside acceptor **18** at -78°C in the presence of the hindered base 2,6-di-*tert*-butyl-4-

methylpyridine (DTBMP) in anhydrous DCM provides the desired β-1,4-tri-D-mannoside **19** in 51% yield (the yield was modified to 61% after partial starting material was recovered). β-linkage in **19** is confirmed by the specific H-5 signal [^{1}H NMR (500 MHz, $CDCl_3$): δ 2.97 (ddd, 1H, $J_{4c,5c/5c,6c}$ 9.5Hz, $J_{5,6c'}$ 5Hz, *H-5c*)] and the $^1J_{C,H}$ coupling constant of three anomeric carbons and the CH carbon of the benzylidene group [^{13}C NMR (125 Hz,CDCl3): d 102.5 ($^1J_{C,H}$ = 156.5 Hz), 102.1($^1J_{C,H}$ =152.3Hz), 101.6 ($^1J_{C,H}$ = 151.6 Hz), 101.5 ($^1J_{C,H}$ = 153.1 Hz)].

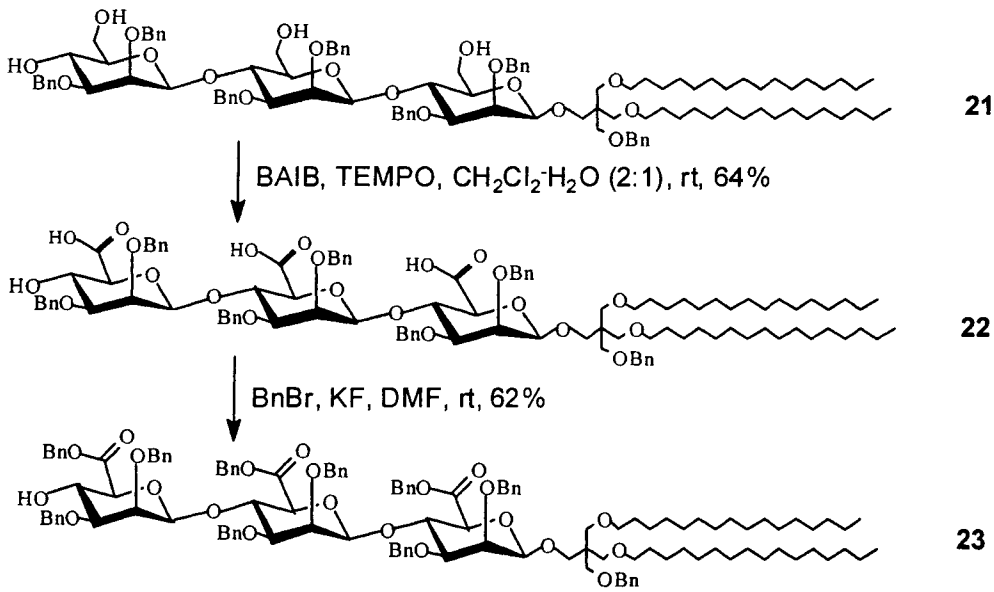
2.4.4. Preparation of Tetraol 21

Preparation of tetraol **21** is the same as that of triol **15**: removal of 4,6-di-O-benzylidene of **19** was carried out through a trans-ketal reaction with neopentyl glycol in the presence of camphorsulfonic acid (CSA) in anhydrous dichloromethane (DCM) at 35^{0}C to liberate 4,6-diol **20**. Deacylation of **20** with sodium methoxide (NaOMe) in dichloromethane-methanol (DCM : MeOH = 1:1 v/v) under pH=9 - 10 at room temperature provides **21** in 83% yield (2 steps) (**Scheme 11**).



Scheme 11 Deketalization and deacylation of **19**

2.4.5. Preparation of Tri-carboxylic Acid Ester 23



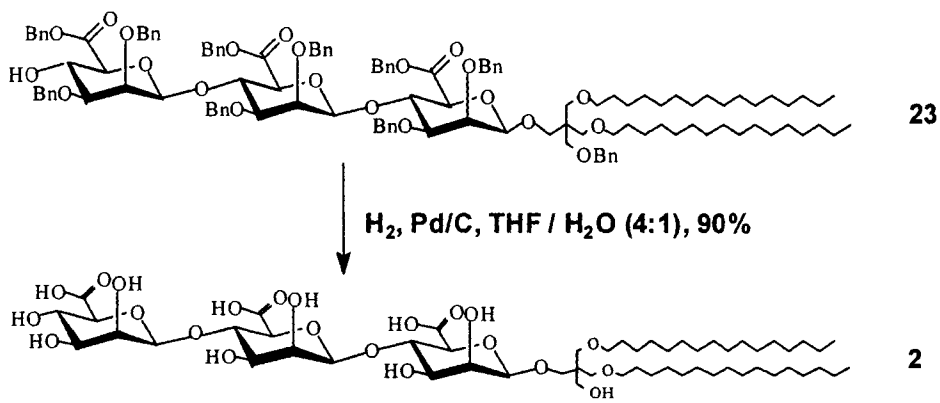
Scheme 12: Regioselective oxidation and benzylation of **21**

Regioselective oxidation of the primary hydroxyl (C-6) in **21** to its carboxylic acid is achieved by the combination of TEMPO free radical and BAIB in the presence of DCM-H_2O(2:1), where triol **21** is converted into its corresponding tricarboxylic acid **22** in 64% yield. The same consideration as described for **16**, benzyl esterification of **22** with benzyl bromide (BnBr) in the presence of potassium fluoride (KF) in DMF at room temperature, yields benzyl ester **23** in 64% yield (**Scheme 12**). β-linkage is confirmed by the specific $^1J_{C,H}$ coupling constant of the anomeric carbons in **23** [(^{13}C NMR (125.664 Hz,$CDCl_3$): d 102.3 ($^1J_{C,H}$ = 157.7 Hz), 102.5 ($^1J_{C,H}$ =155.6Hz), 103.2 ($^1J_{C,H}$ = 155.3 Hz)].

2.4.6. Global Debenzylation to Target Molecule 2

Hydrogenolysis of **23** has been carried out in tetrahydrofuran (THF) – H_2O (4:1 v/v) under hydrogen atmosphere in the presence of palladium on

charcoal to achieve the target product β-1,4-*tri*-D-mannuronic acid glycolipid **2** in 90% yield (**Scheme 13**). The structure of **2** has been confirmed by mass spectroscopy data {MALDI-MS) calcd for $C_{55}H_{100}O_{22}$ 1112.67[M]$^+$, found 1136.05 [M+Na]$^+$, 1158.06 [M-H+2Na]$^+$.



Scheme 13 Removal of benzyl protecting groups of **23**

2.5. Concluding Remarks

β-D-mannuronic acid oligomers have a 1,2-*cis* mannosyl relationship which makes it hard to obtain β-glycosylation in a commonly stereocontrolled manner. Van Den Bos and co-workers have recently presented a facile synthetic route[38] toward β-linked mannuronic acids, in which a carboxylic ester function on C-5 of the mannuronate donors have been used to sufficiently influence the electronic surrounding around the anomeric center and allow good to excellent β-selectivities.

In this thesis, Crich's methodology has been used to introduce β-1,4-D-mannoside linkage by application of diastereoselective β-glycosylation of 4,6-*O*-benzylidene protected thiomannoside donor bearing nonparticipating benzyl group at *O*-2 and *O*-3 positions with suitable acceptors, followed by a 2,2,6,6-tetramethylpiperidinyloxy free

radical (TEMPO) / [bis(acetoxyiodo] benzene (BAIB) regioselective oxidation step to achieve targets: β-1,4-*di* & *tri*-D-mannuronic acid oligomer moieties (**1** & **2**).

An efficient route for the synthesis of β-1,4-D-mannuronic acid glycolipids **1** and **2** designed as potential ligands for TLR2/4 has been described. The strategy presented here shall be applicable to the synthesis of larger *oligo*-β-1,4-D-mannuronic acids.

The initial biological evaluation of compounds 1 and 2 with respect to the immune stimulating and modulating properties is under way.

3. Experimental Section

3.1. General Methods

TLC was performed on Silica Gel 60Å-F_{254} (Silicycle) with detection by quenching of fluorescence (254 nm), by dipping into 15% solution of H_2SO_4 and/or mostaine [ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$ (20 mg) and Cerium (IV) sulfate $Ce(SO_4)_2 \cdot xH_2O$ in 10 % sulfuric acid (400 ml)] followed by charring at ~120 ^{0}C.

Column chromatography was performed on Silica Gel 60 (Silicycle, 40-63 μm).

Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 22±2 ^{0}C.

^{1}H NMR spectra were recorded at 499.9 MHz (Varian Unity Inova 500 MHz) in solutions of $CDCl_3$ [internal Me_4Si (TMS), δ 0 ppm). Coupling constants can be ascribed a resolution of ± 0.5 Hz. ^{13}C NMR spectra were recorded at 125 MHz on the same instruments in $CDCl_3$ (δ 77.26 ppm).

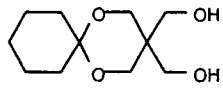
Matrix-assisted laser desorption / ionization (MALDI) mass spectra (MALDI-MS) were obtained from a Biflex-IV MALDI linear / reflector instrument at the University of Manitoba.

Elemental analyses were carried out on a CEC (SCP) 240-XA Analyzer instrument by Lakehead University Instrumentation Laboratory (LUIL).

All commercial reagents were used as supplied. Solvents for anhydrous reactions were dried according to literature procedures. Toluene was distilled over sodium; pyridine and dichloromethane were distilled over calcium hydride; methanol was distilled from magnesium turnings and a catalytic amount of iodine.

3.2. Synthetic Procedures and Structure Characteristics

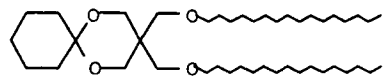
1,5-dioxaspiro[5,5]undecande-3,3-dimethanol (3).



3 was prepared according to the literature procedure.[54]

R_f 0.36 (CH_2Cl_2 / MeOH, 9 : 1).

1,5-dioxaspiro[5,5]undecande-3,3-dihexadecyloxymethyl ether (4).



To a completely dissolved solution of 1-bromo hexadecane (26.2 mL, 85.44 mmol, 2 eq.) in anhydrous DMF (60 mL) was carefully added NaH (2.6 g, 102.65 mmol, 2.4 eq.) and heated to 50 ^{0}C in an oil-bath followed by dropwise addition of **3** (9.25 g, 42.77 mmol, 1.0 eq.) which was predissolved in 20 mL anhydrous DMF. The mixture was then left overnight before being washed with ice-water (100 mL), taken up in EtOAc (3x120 mL), dried over Na_2SO_4, filtered and concentrated. The residue was further purified by chromatography on silica gel (eluent: hexane / ethyl acetate = 30 : 1 / 20 : 1 v/v) and concentrated to provide **4** (14.31g, 59%) as a white powder.

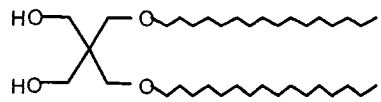
R_f0.41 (Hexane / EtOAc, 20 : 1 v/v).

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6 H, J 3.5 Hz, 2 CH_3), 1.26 (bs, 52 H, 2 $C_{13}H_{26}$), 1.40 (bs, 2 H, 1 CH_2), 1.52 (bs, 8 H, 4 CH_2), 1.75 (bs, 4 H, 2 CH_2), 3.38 (t, 8H, J 7.0 Hz, 4 CH_2O), 3.74 (s, 4H, 2 CH_2O).

^{13}C NMR (125 MHz, $CDCl_3$): δ 14.40, 22.83, 22.96, 25.98, 26.43, 29.64, 29.77, 29.82, 29.91, 29.93, 29.98, 32.19, 32.87, 39.23, 62.30, 70.61, 71.88, 98.21

Anal.Calcd for $C_{43}H_{84}O_4$: C, 77.65; H, 12.73. Found: C, 76.97; H, 12.33.

2,2- dihexadecyloxymethyl-1,3-propandiol (5).



To a solution of **4** (14.31 g, 21.52 mmol, 1.0 eq.) and neopentyl glycol (9 g, 86.1 mmol, 4.0 eq.) in anhydrous DCM (200 mL) at 35 ^{0}C, conducted under N_2 atmosphere, was added CSA (camphorsulfonic acid) (1.0 g, 4.3 mmol, 0.2 eq.) with vigorous stirring. The mixture was left overnight and quenched with saturated aqueous $NaHCO_3$, washed with ice-water (200 mL), taken up in DCM (3 x 200 mL), dried over Na_2SO_4 and followed by recrystallization in which the solution (800 mL) was first rotary evaporated under reduced pressure to 200 mL of solution, and then cooled to -30 ^{0}C to get a suspended white crystal which was quickly filtered and washed with cooled DCM (-30 ^{0}C). The same procedure was operated 3 times. The collected crystals were rotary evaporated under reduced pressure to provide compound **5** (12.1 g, 95%) as a white powder.

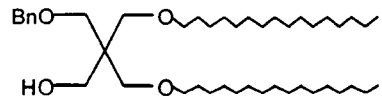
R_f 0.45 (Hexane / EtOAc, 2 : 1 v/v).

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6 H, J 3.5 Hz, 2 CH_3), 1.26 (bs, 52 H, 2 $C_{13}H_{26}$), 1.57 (bs, 4 H, 2 CH_2), 2.80 (t, 2 H, J 6.5 Hz, 2 OH), 3.42 (t, 4 H, J 6.5 Hz, 2 OCH_2), 3.51 (s, 4 H, 2 CH_2O), 3.64 (bs, 4 H, 2 C*H*$_2$OH).

^{13}C NMR (125 MHz, $CDCl_3$): δ 14.14, 22.71, 29.38, 29.45, 29.52, 29.61, 29.64, 29.68, 29.70, 29.72, 31.94, 31.98, 44.5, 65.48, 72.06, 73.23.

Anal. Calcd for $C_{37}H_{76}O_4$: C, 75.97; H, 13.09. Found: C, 75.85; H, 12.87.

2,2-dihexadecyloxymethyl-1-*O*-benzyl-3-propanol (6).



A solution of **5** (5.85 g, 10.0 mmol, 1.0 eq.) and dibutyltinoxide (2.49 g, 10.0 mmol, 1.0 eq.) in benzene (100 mL) was kept under reflux for 16 hours at 90 ^{0}C in an oil-bath with removal of produced water by a Dean-Stark trap, followed by addition of tetrabutylammonian bromide (3.22 g, 10.0 mmol, 1.0 eq.) and dropwise addition of benzylbromide (2.38 ml, 20.0 mmol, 2.0 eq.) which was pre-dissolved in 5ml benzene. The mixture was kept under reflux until TLC indicated a complete reaction and then quenched with saturated aqueous $NaHCO_3$ (20 mL). The residue was washed with ice-water (120 mL), extracted with EtOAc (3 x 120 mL), dried over Na_2SO_4, filtered, concentrated, purified by column chromatography on silica gel (eluent: hexane / ethyl acetate = 20 : 1 / 12 : 1 v/v) and concentrated to provide compound **6** (4.72 g, 70%) as a white powder.

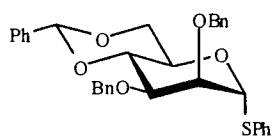
R_f 0.36 (Hexane/ EtOAc, 10 : 1 v/v).

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6 H, J 3.5 Hz, 2 CH_3), 1.25 (bs, 52 H, 2 $C_{13}H_{26}$), 1.52 (bs, 5 H, 1 OH and 2 CH_2), 3.38 (t, 4 H, 2 OCH_3), 3.47 (s, 4 H, 2 CH_2O), 3.52 (s, 2 H, CH_2OH), 3.73 (s, 2 H, CH_2OH), 4.50 (s, 2 H, 2 PhC*H*H), 7.27 - 7.35 (m, 5 H, Arom).

^{13}C NMR (125 MHz, $CDCl_3$): δ 14.38, 22.95, 26.42, 29.62, 29.74, 29.83, 29.89, 29.91, 29.93, 29.96, 32.19, 45.10, 66.84, 71.01, 71.88, 72.05, 73.71, 126.60, 127.71, 128.54 (5 CH Arom), 138.76 (1 C_q Ph).

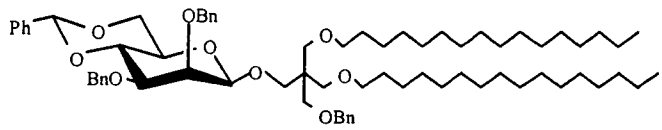
Anal. Calcd for **$C_{44}H_{82}O_4$**: C, 78.28; H, 12.24. Found: C, 77.92; H, 12.14.

Phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-thio-mannopyranoside (9).



9 was prepared according to the literature procedure.[47, 48]

(3-benzyloxy-2,2-dihexadecyloxymethyl)-propyl 2,3-*di*-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (10).



To a solution of **9** (1.63 g, 3.0 mmol, 1.5 eq.) in anhydrous DCM (50 mL) conducted under N_2 atmosphere were added 2 g of activated 4Å molecular sieves, 1-benzenesulfinyl piperidine (BSP) (0.69 g, 3.3 mmol, 1.65 eq.) and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) (1.26 g, 6.12 mmol, 3.0 eq.). The mixture was kept stirring for 1 hour at r.t.. Trifluoromethanesulfonic anhydride (Tf_2O) (0.56 ml, 3.3 mmol, 1.65 eq.) was added dropwise at -60 ^{0}C and 5 min later, a solution of **6** (1.35 g, 2.0 mmol, 1.0 eq.) which was pre-dissolved in 10 mL anhydrous DCM, was added dropwise at -78 ^{0}C. The mixture was kept vigorously stirring at -78 ^{0}C for 2 more hr and then warmed up to r.t. before being quenched with saturated aqueous $NaHCO_3$ (10 mL), washed with ice-water (80 ml), extracted with DCM (3 x 80 mL), dried over Na_2SO_4, filtered and concentrated. The residue was further purified by column chromatography on silica gel (eluent: hexane / ethyl acetate = 20 : 1 / 10 : 1 v/v) and concentrated to provide compound **10** (2.1 g, 85%) as a syrup.

R_f 0.35 (Hexane / EtOAc, 8 : 1 v/v).

$[\alpha]_D^{22}$ -15.7 (C 0.66, $CHCl_3$).

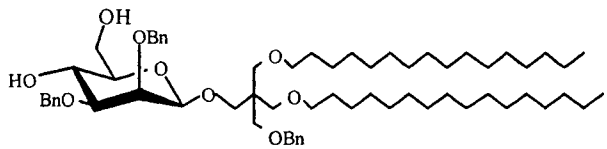
^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6 H, J 3.5 Hz, 2 CH_3), 1.26 (bs, 52 H, 2 $C_{13}H_{26}$), 1.51 (bs, 4 H, 2 CH_2), 3.28 (ddd, 1 H, $J_{5,6a} = J_{4,5}$ 10.0, $J_{5,6b}$ 4.5 Hz, H-5), 3.32 - 3.38 (m, 4 H), 3.39 - 3.54 (m, 8 H), 3.83 (d, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.92 (t, 1 H, $J_{5,6a} = J_{6a,6b}$ 10.0 Hz, H-6a), 3.99 (d, 1 H, $J_{3,4}$ 10.0 Hz, H-3), 4.19 (dd, 1 H, $J_{3,4} = J_{4,5}$ 10.0 Hz, H-4), 4.30 (dd, 1 H, $J_{6a,6b}$ 10.0, $J_{5,6b}$ 4.5 Hz, H-6b), 4.37 (s, 1 H, H-1), 4.41, 4.50, 4.57, 4.68, 4.79, 4.95 (6d, 1 H

each, J 12.0 Hz, 6 PhC*H*H), 5.60 (s, 1 H, CHPh), 7.21-7.50 (m, 20 H, 4 C_6H_5).

^{13}C NMR (125 MHz, $CDCl_3$): δ 14.36 (2 CH_3), 22.93 (2 CH_2), 26.49 (2 CH_2), 29.61, 29.77, 29.87, 29.90, 29.92, 29.95 (2 $C_{11}H_{22}$), 32.18 (2 CH_2), 45.46 (1 C_q), 67.76, 68.83, 69.81, 69.88, 69.90, 70.65, 71.80, 71.81, 72.62, 73.50, 74.81, 76.04, 78.28, 78.87, 101.55, 103.44 (C-1, CHPh, $^1J_{C,H}$ 154.6, 155.8 Hz), 126.26, 127.55, 127.57, 127.71, 127.72, 128.35, 128.43, 128.49, 128.75, 129.00 (20 CH Arom), 137.86, 138.61, 138.72, 139.07 (4 C_q Ph).

Anal. Calcd for **$C_{71}H_{108}O_9$**: C, 77.13; H, 9.85. Found: C, 76.93; H, 9.81.

(3-benzyloxy-2,2-dihexadecyloxymethyl)-propyl 2,3-*di*-*O*-benzyl-β-D –mannopyranoside (11).



To a solution of **10** (2.10 g, 1.92 mmol, 1.0 eq.) and neopentyl glycol (0.8 g, 7.68 mmol, 4.0 eq.) in anhydrous DCM (20 mL) at 35 ^0C, conducted under N_2 atmosphere, was added CSA (0.30 g, 1.15 mmol, 0.6 eq.) with vigorous stirring. The mixture was left overnight and TLC checked until a complete reaction followed by a work up: quenched with saturated aqueous $NaHCO_3$, washed with ice-water, extracted with EtOAC, dried over Na_2SO_4, filtered, concentrated, purified by column chromatography on silica gel (eluent: hexane / ethyl acetate = 5 : 1 / 2 : 1 v/v) and concentrated under vacuum to provide compound **11** (1.74 g, 90%) as a syrup.

R_f 0.36 (Hexane / EtOAc, 5 : 4 v/v).

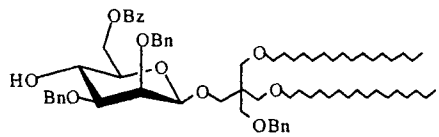
$[\alpha]_D^{22}$ -40.4 (C 0.37, $CHCl_3$).

^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6 H, J 3.5 Hz, 2 CH_3), 1.25 (bs, 52 H, 2 $C_{13}H_{26}$), 1.52 (bs, 4 H, 2 CH_2), 3.25 (dd, 1 H, $J_{3,4}$ 9.5, $J_{2,3}$ 3.0 Hz,

H-3), 3.27 - 3.30 (m, 1 H), 3.34 - 3.38(m, 4 H), 3.40, 3.41, 3.44, 3.46, 3.48 (5 d, 5 H, J 9.0 Hz, CC*H*HO), 3.51 (s, 1 H, OH-C-6), 3.54 (d, 1 H, J 9.0 Hz, CC*H*HO), 3.80 (dd, 1 H, $J_{6a,6b}$ 12.0, $J_{5,6}$ 6.0 Hz, H-6a), 3.85 (d, 1 H, H-1), 3.91 (t, 1 H, H-4), 3.92, 3.99 (2 d, 2 H, J 9.0 Hz, CC*H*HO), 4.24 (1 d, 1 H, J 13.0 Hz, 1 PhC*H*H), 4.39 (s, 1 H, OH-C-4), 4.44, 4.45, 4.52, 4.69, 4.91 (5 d, 5 H, J 13.0 Hz, 5 PhC*H*H), 7.23 - 7.39 (m, 15 H, 3 C_6H_5).

Anal. Calcd for **$C_{64}H_{104}O_9$ • 1/2H_2O**: C, 74.89; H, 10.31. Found: C, 75.07; H, 10.13.

(3-benzyloxy-2,2-dihexadecyloxymethyl)-propyl 2,3-*di*-*O*-benzyl-6-*O*-benzoyl-β-D-mannopyranoside (12).



To a solution of **11** (480.0 mg, 0.48 mmol, 1.0 eq.) and 4-(dimethylamino) -pyridine (4-DMAP) (7.6 mg, 0.048 mmol, 0.1 eq.) in 5 mL pyridine at -25 ^{0}C was added benzoylcholoride (0.061 ml, 0.53 mmol, 1.1 eq.) which was pre-dissolved in 1 mL anhydrous DCM. The mixture was kept stirring for 2 hours at -25 ^{0}C and allowed to warm up to r.t. before a TLC check indicated a complete reaction. The mixture was then diluted in 100 mL EtOAc followed by washing with 30 ml 4 M HCL before being quenched with saturated aqueous $NaHCO_3$, washed with ice-water (150 mLl), extracted with EtOAC (3x150 ml), dried over Na_2SO_4, filtered, concentrated, purified by column chromatography on silica gel (eluent: hexane / ethyl acetate = 10 : 1 / 4 : 1 v/v) and concentrated under vacuum to provide compound **12** (370 mg, 70%) as a syrup.

R_f 0.63 (Hexane / EtOAc, 2 : 1 v/v).

$[\alpha]_D^{22}$ -35.0 (C 0.38, $CHCl_3$).

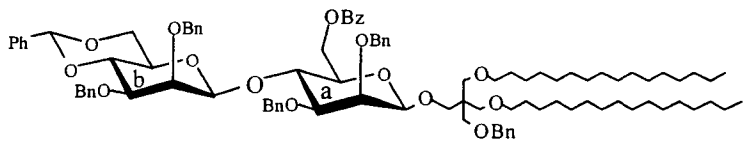
^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6 H, J 3.5 Hz, 2 CH_3), 1.26 (bs, 52 H, 2 $C_{13}H_{26}$), 1.49 (bs, 4 H, 2 CH_2), 2.90 (bs, 1H, 1 OH), 3.29 – 3.35 (m,

4 H), 3.40 - 3.55 (m, 6 H), 3.85 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-2), 4.02, 4.04 (2 d, 2 H, J 9.5 Hz), 4.35 (d, 1 H, J 12.0 Hz, 1 PhC*H*H), 4.39 (s, 1 H, H-1), 4.41, 4.48 (2 d, 2 H, J 12.0 Hz, 2 PhC*H*H), 4.57 (ddd, 1 H, $J_{3,4/4,5}$ 12.0, J 6.0 Hz, H-4), 4.67, 4.69, 4.93 (3 d, 3 H, J 12.0, 3 PhC*H*H), 7.20 - 7.48 (m, 18 H, $4C_6H_5$), 8.05 (d, 2 H, $J_{2,6} = J_{3,5}$ 8.0 Hz, 2 H_{ortho} in Bz).

^{13}C NMR (125 MHz, $CDCl_3$): δ 14.30 (2 CH_3), 22.85 (2 CH_2), 26.38 (2 CH_2), 29.52, 29.68, 29.77, 29.82, 29.85, 29.87 (2 $C_{11}H_{22}$), 32.09 (2 CH_2), 45.35 (1 C_q), 64.35, 66.95, 69.77, 69.84, 69.86, 70.18, 71.35, 71.69, 71.71, 73.39, 73.53, 74.45, 81.56, 73.94, 102.86 (C-1, $J_{C,H}$ 154.6 Hz), 126.99, 127.40, 127.47, 127.85, 127.96, 128.19, 128.27, 128.32, 128.34, 128.58, 129.96 (20 CH Arom), 132.91 (1 C_q in PhCOO), 137.89, 138.93, 138.99 (3 C_q in $PhCH_2$), 166.82 (PhCOO).

Anal. Calcd for $\mathbf{C_{71}H_{108}O_{10}}$: C, 76.03; H, 9.71. Found: C, 75.85; H, 9.49.

(3-benzyloxy-2,2-dihexadecyloxymethyl)-propyl (2,3-*di*-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1–4)-2,3-*di*-*O*-benzyl-6-*O*-benzoyl-β-D-mannopyranoside (13).



To a solution of **9** (245.0 mg, 0.41 mmol, 1.5 eq.) in anhydrous DCM (10 mL), conducted under N_2 atmosphere, were added 4Å molecular sieves, 1-benzenesulfinyl piperidine (BSP) (102.7 mg, 0.45 mmol, 1.65 eq.) and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) (186.6 mg, 0.83 mmol, 3.0 eq.). The mixture was kept stirring for 1 hour at r.t.. Trifluoromethanesulfonic anhydride (Tf_2O) (0.08 ml, 0.45 mmol, 1.65 eq.) was added dropwise at -60 ^0C and 5 min later, a solution of **12** (330.0 mg, 0.27 mmol, 1.0 eq.), pre-dissolved in anhydrous DCM (5 ml), was added dropwise at -78 ^0C,. The mixture was kept vigorously stirring at -78 ^0C for 2 more hr and allowed to warm up to r.t. before being quenched with saturated aqueous $NaHCO_3$,

washed with ice-water(15 mL), extracted with DCM (3 x 15 mL), dried over Na_2SO_4, filtered, concentrated, purified by column chromatography on silica gel (eluent: hexane / ethyl acetate = 10 : 1 / 5 : 1 v/v) and concentrated under vacuum to provide compound **13** (291.0 mg, 67%) as a syrup.

R_f 0.64 (Hexane / EtOAc, 5 : 2 v/v).

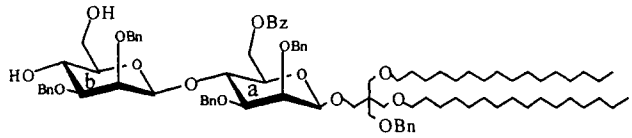
$[\alpha]_D^{22}$ -26.8 (C 0.40, $CHCl_3$).

^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6 H, J 3.5 Hz, 2 CH_3), 1.25 (bs, 52 H, 2 $C_{13}H_{26}$), 1.46 (bs, 4 H, 2 CH_2), 3.05 (ddd, 1 H, $J_{4,5} = J_{5,6b}$ 10.0, $J_{5,6b'}$ 4.5 Hz, H-5b), 3.28-3.32 (m, 4 H), 3.36-3.43 (m, 4 H), 3.45-3.52 (m, 4 H), 3.49 (dd, 1 H, $J_{4,5}$ 10.5, $J_{5,6'}$ 4.5 Hz, H-5a), 3.54, 3.59 (2 dd, 2 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.5 Hz, H-3a & H-3b), 3.67 (dd, 1 H, J 10.0 Hz, H-6b), 3.83, 3.93 (2 d, 2 H, $J_{2,3}$ 3.0 Hz, H-2a & H-2b), 4.00 (d, 1 H, $J_{6b,6b'}$ 10.0 Hz, H-6b'), 4.10, 4.16 (2 t, 2 H, $J_{3,4} = J_{4,5}$ 9.0 Hz, H-4a & H-4b), 4.38 (d, 1 H, J 12.5 Hz, PhC*H*H), 4.42 (s, 1 H, H-1), 4.43 (d, 1 H, J 12.5 Hz, PhC*H*H),4.50 (2 d, 2 H, $J_{5a,6a}$ 4.5 Hz, 2H-6a), 4.55, 4.57 (2 d, 2 H, J 12.5 Hz, 2 PhC*H*H), 4.60 (s, 1 H, H-1), 4.66, 4.67, 4.69, 4.88, 4.83, 4.89 (6 d, 6 H, J 12.5 Hz, 6 PhC*H*H), 5.51 (s, 1 H, PhCH), 7.23-7.53 (m, 32 H, CH Arom), 7.52 (t, 1 H, $J_{3,4} = J_{4,5}$ 7.5 Hz, H-4 in Bz), 8.03 (d, 2 H, $J_{2,3} = J_{5,6}$ 8.0 Hz, H-2 & H-6 in Bz).

^{13}C NMR (125 MHz, $CDCl_3$): δ 14.40 (2 CH_3), 22.95 (2 CH_2), 26.45 (2 CH_2), 29.63, 29.79, 29.89, 29.92, 29.95, 29.97 (2 $C_{11}H_{22}$), 32.18 (2 CH_2), 45.43 (1 C_q), 64.09, 67.63, 68.74, 69.86, 69.89, 70.23, 71.81, 71.83, 72.10, 72.56, 73.47, 73.54, 73.98, 74.78, 75.39, 76.16, 77.32, 77.47, 78.55, 78.67, 80.03, 101.57, 102.14, 102.48 (C-1a, C-1b, CHPh, $J_{C,H}$ 157.5, 153.7, 155.4 Hz),126.32, 127.40, 127.49, 127.52, 127.68, 127.72, 127.75, 128.02, 128.31, 128.37, 128.43, 128.54, 128.55, 128.63, 129.05, 130.01, 130.17 (35 CH Arom), 133.24 (1 C_q in PhCOO), 137.79, 138.54, 138.77, 138.82, 139.10, 139.12 (5 C_q in $PhCH_2$ &1 C_q in PhCH),166.55 (PhCOO).

Anal. Calcd for **$C_{98}H_{134}O_{15} \cdot H_2O$**: C, 74.96; H, 8.72. Found: C, 74.98; H, 9.07.

(3-benzyloxy-2,2-dihexadecyloxymethyl)-propyl (2,3-*di*-*O*-benzyl-β-D -mannopyranosyl)-(1–4)-2,3-*di*-*O*-benzyl-6-*O*-benzoyl-β-D-mannopyran oside (14).



To a solution of **13** (105 mg, 0.07 mmol, 1.0 eq.), neopentyl glycol (30 mg, 0.28 mmol, 4.0 eq.) and anhydrous DCM (20 ml) at 35 ^{0}C, conducted under N_2 atmosphere, was added CSA (10 mg, 0.042 mmol, 0.6 eq.) with vigorous stirring. The mixture was left overnight and TLC checked until a complete reaction followed by a work up: quenched with saturated aqueous $NaHCO_3$, washed with ice-water (20 ml), extracted with EtOAC (3 x 20 mL), dried over Na_2SO_4, filtered, concentrated, purified by column chromatography on silica gel (eluent: hexane / ethyl acetate = 2 : 1 / 5 : 4 v/v) and concentrated under vacuum to provide compound **11** (84.5 mg, 82%) as a syrup.

R_f 0.38 (Hexane / EtOAc, 5 : 3 v/v).

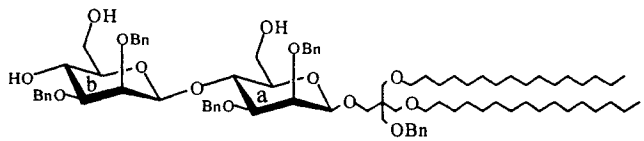
$[\alpha]_D^{22}$ -39.0 (C 0.34, $CHCl_3$).

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6 H, J 3.5 Hz, 2 CH_3),1.25 (bs, 52 H, 2 $C_{13}H_{26}$), 1.48 (bs, 4 H, 2 CH_2), 3.07 (m,1 H, H-5b), 3.20 (dd, 1 H, $J_{3,4}$ 9.5, $J_{2,3}$ 3.5 Hz, H-3), 3.30 - 3.34 (m, 4 H), 3.38 - 3.42 (m, 4 H), 3.44 - 3.56 (m, 4 H), 3.63 (m, 1 H, H-5a), 3.65, 3.72 (2 dd, 2 H, $J_{6b,6b'}$ 12.5, $J_{5,6b} = J_{5,6b'}$ 3.5 Hz, H-6b' & H-6b), 3.86 (d, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.88 (t, 1 H, J 9.5 Hz, H-4b'), 3.92 (d, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 4.00 (d, 1 H, J 9.5 Hz), 4.05 (s, 1 H, H-1b), 4.23 (t, 1 H, J 9.5Hz, H-4b), 4.31, 4.40 (2 d, 2 H, J 12.5 Hz, 2 PhC*H*H), 4.46 (dd, 1 H, $J_{6a,6a'}$ 12.0 Hz, H-6a), 4.47, 4.48, 4.55 (3 d, 3 H, J 12.5 Hz, 3 PhC*H*H), 4.63 (dd, 1 H, $J_{6a,6a'}$ 12.0 Hz, H-6a'), 4.68, 4.72, 4.76, 4.83, 4.92 (5 d, 5 H, J 12.5 Hz, 5 PhC*H*H), 7.22 - 7.55 (m, 27 H, CH-Arom), 7.54 (t, 1 H, $J_{3,4} = J_{4,5}$ 7.5 Hz, H-4 in Bz), 8.04 - 8.06 (d, 2 H, $J_{2,3} = J_{5,6}$ 8.5 Hz, H-2 & H-6 in Bz).

^{13}C NMR (125 MHz, $CDCl_3$): δ 14.36 (2 CH_3), 22.93 (2 CH_2), 26.43 (2 CH_2), 29.59, 29.75, 29.85, 29.88, 29.91, 29.93 (2 $C_{11}H_{22}$), 32.15 (2 CH_2), 45.44 (1 C_q), 61.42, 62.65, 64.17, 67.07, 69.75, 69.80, 70.14, 71.37, 71.74, 71.76, 72.35, 73.39, 73.58, 74.10, 74.44, 74.60, 75.18, 75.74, 75.94, 79.55, 81.94, 101.41, 102.57 (C-1a, C-1b, $J_{C,H}$ 155.7, 157.2 Hz), 127.49, 127.52, 127.58, 127.66, 127.76, 127.85, 128.00, 128.16, 128.29, 128.36, 128.41, 128.42, 128.56, 128.71, 128.75, 129.98, 130.06 (30 CH Arom), 133.42 (1 C_q in PhCOO), 137.80, 138.48, 138.74, 138.99, 139.11 (5 C_q in $PhCH_2$), 166.66 (PhCOO).

Anal. Calcd for **$C_{91}H_{130}O_{15} \cdot H_2O$**: C, 74.66; H, 8.95. Found: C, 74.46; H, 8.74.

(3-benzyloxy-2,2-dihexadecyloxymethyl)-propyl (2,3-*di*-*O*-benzyl-β-D -mannopyranosyl)-(1–4)-2,3-*di*-*O*-benzyl-β-D-mannopyranoside (15).



To a solution of **14** (370.0 mg) dissolved in MeOH : DCM=2:1 (30 mL) at r.t. was added MeONa dropwise until the pH of the solution reached 10. The mixture was kept stirring for 17 hr followed by TLC check indicating a complete reaction and then Resin-RCOOH was added until the pH=7, followed by filtration and concentration under vacuum to provide compound **15** (319 mg, 92%) as a syrup.

R_f 0.21 (Hexane / EtOAc, 1 : 1 v/v).

$[\alpha]_D^{22}$ -42.0 (C 0.29, $CHCl_3$).

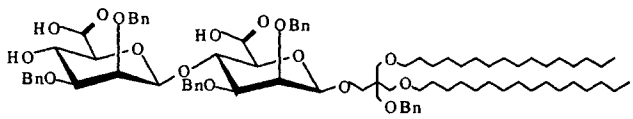
^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6 H, J 3.5 Hz, 2 CH_3), 1.25 (bs, 52 H, 2 $C_{13}H_{26}$), 1.51 (bs, 4 H, 2 CH_2), 2.24 (b, 3 H, 3 OH), 3.19 (m, 1 H,), 3.29 - 3.51 (m, 13 H), 3.70 (d, 1 H, J 12.5 Hz), 3.73 (d, 1 H, J 13.5 Hz), 3.81, (s, 1 H), 3.84 (d, 1 H, J 12.0 Hz), 3.87 (dd, 1 H, J 10.0 Hz), 3.91 (s, 1 H), 3.96 (d, 1 H, J 9.5 Hz), 4.11, 4.14 (2 t, 2 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4a & H-4b),

4.37 (s, 1 H, H-1a or H-1b), 4.38, 4.49, 4.43, 4.50, 4.56 (5 d, 5 H, J 12.5 Hz, 5 PhC*H*H), 4.59 (s, 1 H, H-1a or H-1b), 4.67, 4.73, 4.85, 4.78, 4.88 (5 d, 5 H, J 12.5 Hz, 5 PhC*H*H), 7.24 - 7.34 (m, 25 H, 25 CH Arom).

^{13}C NMR (125 MHz, $CDCl_3$): δ 14.37 (2 CH_3), 22.93 (2 CH_2), 26.47 (2 CH_2), 29.61, 29.77, 29.87, 29.90, 29.93, 29.95 (2 $C_{11}H_{22}$), 32.16 (2 CH_2), 45.55 (1 C_q), 62.09, 63.01, 67.56, 69.68, 69.72, 71.54, 71.89, 72.43, 73.02, 73.52, 74.20, 74.49, 74.52, 74.83, 75.37, 75.71, 75.76, 76.64, 80.29, 82.20, 101.60, 102.56 (C-1a, C-1b), 127.21, 127.43, 127.52, 127.61, 127.63, 127.72, 127.73, 127.78, 127.80, 127.92, 128.19, 128.23, 128.36, 128.38, 128.46, 128.47, 128.54, 128.78 (25 CH Arom), 137.88, 138.68, 138.75 (2), 139.06 (5 C_q in $PhCH_2$).

Anal. Calcd for **$C_{84}H_{126}O_{14} \cdot 1/2H_2O$**: C, 73.70; H, 9.35. Found: C, 73.65; H, 9.16.

(3-benzyloxy-2,2-dihexadecyloxymethyl)-propyl (2,3-*di*-*O*-benzyl-β-D -mannopyranosyluronate)-(1–4)-(2,3-*di*-*O*-benzyl-β-D-mannopyranosid) uronic acid (16).



To a flask charged with **15** (300.0 mg, 0.22 mmol, 1.0 eq.), 2,2,6,6-tetramethyl -piperridinyloxy free radical (TEMPO) (21.0 mg, 0.132 mmol, 0.6 eq.) and [bis(acetoxy)iodo]benzene (BAIB) (425.0 mg, 1.32 mmol, 6.0 eq.) at r.t., was added 4 mL DCM and 2 mL H_2O with vigorous stirring. The reaction mixture was stirred for 2 hr until a TLC check indicated complete conversion of the starting material to a lower running spot, followed by a work up: being quenched with aqueous $Na_2S_2O_3$ (10% in H_2O), washed with ice-water (10 mL), extracted with DCM (3 x 10 mL), dried through Na_2SO_4, filtered, concentrated, purified by column chromatography with silica gel (eluent: H / E / MeOH / HOAc = 3 : 1 : 0.1 : 0.2 v/v) and

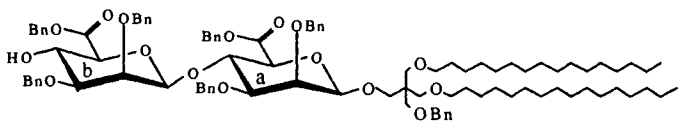
concentrated under vacuum to afford pure compound **16** (291mg, 95%) as a syrup.

R_f 0.32 (Hexane / EtOAc / MeOH / HOAc, 3 : 1 : 0.1 : 0.2 v/v).

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6 H, J 3.5 Hz, 2 CH_3), 1.25 (bs, 52 H, 2 $C_{13}H_{26}$), 1.48 (bs, 4 H, 2 CH_2), 3.31 - 3.47 (2 m, 13 H), 3.58, 3.72 (2 d, 2 H, J 9.5 Hz, H-5a & H-5b), 3.79 (d, 1 H, $J_{2,3}$ 3.0 Hz, H-2b), 3.91, 3.94 (2 d, 2 H, J 9.0 Hz) , 3.97 (d, 1 H, $J_{2,3}$ 3.0 Hz, H-2a), 4.19, 4.34 (2 t, 2 H, $J_{3,4}$ = $J_{4,5}$ 9.0 Hz, H-4a & H-4b), 4.41, 4.47, 4.48 (3 d, 3 H, J 12.5 Hz, 3 C*H*H in OBn), 4.49 (s, 1 H, H-1b), 4.53, 4.58, 4.64, 4.66, 4.73 (5 d, 5 H, J 12.5 Hz, 5 C*H*H in OBn), 4.77 (s, 1 H, H-1a), 4.78, 4.80 (2 d, 2 H, J 12.5 Hz, 2 C*H*H in OBn), 7.12 - 7.38 (m, 25 H, 5 C_6H_5), 10.20 (s, 2 H, 2 HOOC)

^{13}C NMR (125 MHz, $CDCl_3$): δ14.32 (2 CH_3), 22.89 (2 CH_2), 26.40 (2 CH_2), 29.65, 29.73, 29.83, 29.85, 29.89, 29.91 (2 $C_{11}H_{22}$), 32.12 (2 CH_2), 45.67 (1 C_q), 68.53, 69.11, 69.25, 70.35, 71.94, 71.97, 72.54, 72.79, 73.21, 73.53, 74.11, 74.29, 74.56, 74.98, 75.06, 76.21, 77.93, 80.62, 100.97, 101.99 (C-1a, C-1b, $J_{C,H}$ 153.4, 156.7 Hz), 127.61, 127.64, 127.68, 127.82, 128.00, 128.03, 128.23, 128.33, 128.45, 128.53, 128.55, 128.57, 137.46, 138.07, 138.25, 138.71, 138.79 (5 C_q in PhCH), 172.02, 176.93 (2 HOOC).

(3-benzyloxy-2,2-dihexadecyloxymethyl)-propyl (benzyl 2,3-*di*-*O*-benzyl-β-D-mannopyranosyluronate)-(1–4)-(benzyl 2,3-*di*-*O*-benzyl-β-D-mannopyranosid)uronate (17).



To a solution of **16** (266.0 mg, 0.19 mmol, 1.0 eq.) and potassium fluoride (220.0 mg, 3.8 mmol, 20.0 eq.) in distilled DMF (5 ml) at r.t. was added dropwise benzylbromide (0.23 mL, 1.9 mmol, 10.0 eq.) which was pre-dissolved in 1 mL DMF with vigorous stirring. The mixture was kept stirring overnight until TLC check indicated complete reaction. DMF was

removed by rotary evaporation under reduced pressure. To the residue was added 25 mL H_2O and 25 ml DCM, extracted with DCM (3 x 25 mL), dried over Na_2SO_4, filtered, concentrated, purified by column chromatography with silica gel (eluent: Hexane / Ethyl acetate = 5 : 1 / 7 : 2 v/v) and concentrated under vacuum to provide pure compound **17** (202.0 mg, 67%) as a syrup.

R_f 0.55 (Hexane / EtOAc / MeOH / HOAc, 4 : 1 : 0.1 : 0.2 v/v).

$[\alpha]_D^{22}$ -76.5 (C 0.11, $CHCl_3$).

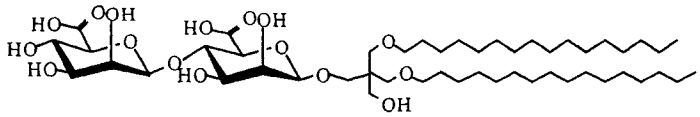
^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6 H, J 3.5 Hz, 2 CH_3), 1.25 (bs, 52 H, 2 $C_{13}H_{26}$), 1.48 (bs, 4 H, 2 CH_2), 2.77 (d, 1 H, J 2.5 Hz, 1 OH), 3.11 (dd, 1 H, $J_{3,4}$ 9.5, $J_{2,3}$ 3.0 Hz, H-3b), 3.28 – 3.48 (m, 13 H), 3.69, 3.70 (2 d, 2 H, $J_{2,3}$ 3.0 Hz, H-2a & H-2b), 3.84 (d, 1 H, J 9.0 Hz), 4.00 (d, 1 H, J 9.0 Hz), 4.15 (ddd, 1 H, $J_{4,5} = J_{3,4}$ 9.5, $J_{OH,4}$ 2.5 Hz, H-4b), 4.33 (s, 1 H, H-1b), 4.39 (d, 1 H, J 12.5 Hz, PhC*H*H), 4.40 (d, 1 H, J 9.5 Hz), 4.43 (d, 1 H, J 12.5 Hz, PhC*H*H), 4.45 (d, 2 H, J 12.5 Hz, 2 PhC*H*H), 4.46 (s, 1 H, H-1a), 4.49, 4.57, 4.64, 4.68, 4.71, 4.85, 5.00, 5.01, 5.10, 5.21 (10 d, 10 H, J 12.5 Hz, 10 PhC**H**H), 7.14 -7.36 (m, 35 H, 7 C_6H_5),

^{13}C NMR (125 MHz, $CDCl_3$): δ14.34 (2 CH_3), 22.94 (2 CH_2), 26.46 (2 CH_2), 29.63, 29.79, 29.89, 29.92, 29.95, 29.97 (2 $C_{11}H_{22}$), 32.16 (2 CH_2), 45.46 (1 C_q), 61.86, 62.86, 64.06, 64.23, 67.35, 68.23, 68.58, 68.72, 69.53, 69.81, 70.73, 71.83, 72.53, 73.46, 73.47, 73.81, 74.41, 74.85, 75.18, 75.27, 80.52,102.35,103.20 (C-1a, C-1b, $J_{C,H}$153.9, 152.4 Hz), 127.50, 127.54, 127.56, 127.66, 127.83, 127.97, 128.03, 128.29, 128.39, 128.42, 128.46, 128.54, 128.58, 128.59, 18.67, 128.71, 128.75, 128.88, 135.31, 135.52 (2 C_q in COOBn), 138.29, 138.76, 138.92, 139.06, 139.08 (5 C_q in $PhCH_2$), 168.35, 168.36 (2 BnOOC).

Anal. Calcd for **$C_{98}H_{134}O_{16}$**: C, 75.06; H, 8.61. Found: C, 74.79; H, 8.67.

(3-benzyloxy-2,2-dihexadecyloxymethyl)-propyl(β-D-mannopyranosyl

uronate)-(1–4)-(β-D-manno pyranosid)uronic acid (1).



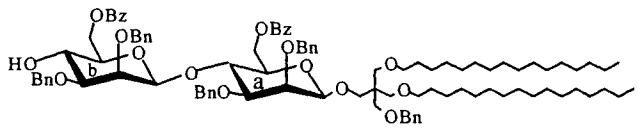
To a solution of **17** (117.0 mg, 0.075 mmol), dissolved in 60 mL freshly distilled THF, was carefully added palladium charcoal powder (Pd/C) (400 mg). The solution was H_2 saturated through for 2 mins. The reaction mixture was kept stirring for 2 hr at r.t. under H_2 atmosphere established with a H_2 balloon, followed by addition of 15 mL H_2O and 0.1 mL Et_3N and kept stirring overnight before a TLC check indicated complete conversion of the starting material to a lower single running spot. The reaction mixture was filtered and the filtrate [catalyst (Pd/C)] was washed with distilled THF-MeOH (1:1 v/v) several times. The final filtrate was concentrated under vaccum and followed by a freeze-drying from fresh 1,4-dioxane to provide a white powder **1** (46.0 mg, 64%).

R_f 0.52 ($CHCl_3$ / MeOH / H_2O / HOAc, 4 : 2 : 0.4 : 0.4 v/v).

$[\alpha]_D^{22}$ -116.0 (C 0.05, MeOH / $CHCl_3$ = 2 : 1).

(**MALDI-MS**) calcd for $C_{49}H_{92}O_{16}$ 936.63$[M]^+$, found 959.63 $[M+Na]^+$, 981.62 $[M-H+2Na]^+$.

(3-benzyloxy-2,2-dihexadecyloxymethyl)-propyl (2,3-*di*-*O*-benzyl-6-*O*-benzoyl-β-D-mannopyranosyl)-(1–4)-2,3-*di*-*O*-benzyl-6-*O*-benzoyl-β-D-mannopyranoside (18).



To a solution of **14** (310.0 mg, 0.21 mmol, 1.0 eq.) and 4-(dimethylamino)-pyridine (4-DMAP) (0.5 mg, 0.04 mmol, 0.2 eq.) in 5 mL dried pyridine at -25 ^{0}C was added benzoylcholoride (0.08 mL, 0.69 mmol, 3.3 eq.) which was pre-dissolved in 1 mL anhydrous DCM. The mixture was

kept stirring for 2 hours at -25 ^{0}C until a TLC check indicating a complete reaction. Mixture was allowed to warm up to r.t. and then diluted in 20 mL EtOAC, followed by washing with 10 mL 4M HCL before being quenched with saturated aqueous $NaHCO_3$, washed with ice-water (30 mL), extracted with EtOAC (3 x 30 mL), dried over Na_2SO_4, filtered, concentrated, purified by column chromatography on silica gel (eluent: hexane / ethyl acetate = 8 : 1 / 5 : 1 v/v) and concentrated under vacuum to provide compound **18** (310 mg, 94%) as a syrup.

R_f 0.72 (Hexane / EtOAc, 5 : 3 v/v).

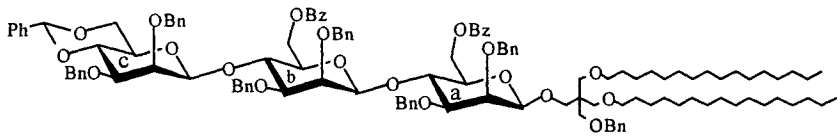
$[\alpha]_D^{22}$ -58.7 (C 0.29, $CHCl_3$).

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, 6 H, J 3.5 Hz, 2 CH_3), 1.26 (bs, 52 H, 2 $C_{13}H_{26}$), 1.46 (bs, 4 H, 2 CH_2), 3.20 - 3.46 (m, 13 H), 3.59 (ddd, 1 H, $J_{3,4} = J_{4,5}$ 9.5, $J_{OH,4}$ 3.5 Hz, H-4b), 3.68 (d, 1 H, J 9.5 Hz), 3.80, 3.92 (2 d, 2 H, $J_{2,3}$ 3.0 Hz, H-2a & H-2b), 3.96 – 4.01 (m, 2 H), 4.07 (d, 1 H, J 12.5 Hz), 4.24 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4a), 4.33 (d, 1 H, J 12.5 Hz, PhC*H*H), 4.36 (s, 1 H, H-1b), 4.37(d, 1 H, J 12.5 Hz, PhC*H*H), 4.43 (d, 2 H, J 3.5 Hz), 4.44 (d, 1 H, J 12.5 Hz, PhC*H*H), 4.51, 4.57 (2d, 2 H, J 12.5 Hz, 2 PhC*H*H), 4.58 (d, 2 H, J 3.5 Hz), 4.60 (s, 1 H, H-1a), 4.64, 4.68, 4.86, 4.75, 4.84 (5 d, 5 H, J 12.5 Hz, 5 PhC*H*H), 7.19 - 7.39 (m, 29 H, 7 C_6H_5), 7.44, 7.51 (2 t, 2 H, $J_{3,4} = J_{4,5}$ 7.5 Hz, 2 H-4 in Bz), 7.95, 8.04 (2 d, 2 H each, $J_{2,3} = J_{5,6}$ 7.5 Hz, 2 H-2 & H-6 in Bz).

^{13}C NMR (125 MHz, $CDCl_3$): δ14.35 (2 CH_3), 22.92 (2 CH_2), 26.41 (2 CH_2), 29.59, 29.75, 29.85, 29.88, 29.91, 29.93 (2 $C_{11}H_{22}$), 32.15 (2 CH_2), 45.41 (1 C_q), 61.64, 63.99, 64.22, 66.38, 69.84, 69.87, 70.14, 71.45, 71.79, 71.81, 72.01, 73.45, 73.57, 73.89, 74.49, 74.54, 74.65, 74.79, 75.64, 79.27, 81.67, 101.54, 102.40 (C-1a, C-1b, $J_{C,H}$ 156.4, 158.2 Hz), 127.47, 127.48, 127.62, 127.75, 127.94, 128.05, 128.07, 128.17, 128.29, 128.31, 128.40, 128.46, 128.52, 128.61, 128.75 (35 CH Arom),133.12, 133.24 (2 C_q in PhCOO), 137.75, 138.60, 138.99, 139.05, 139.10 (5 C_q in $PhCH_2$), 166.57, 166.92 (2 PhCOO).

Anal. Calcd for $C_{98}H_{134}O_{16}$: C, 75.06; H, 8.61. Found: C, 74.94; H, 9.16.

(3-benzyloxy-2,2-dihexadecyloxymethyl)-propyl (2,3-*di*-*O*-benzyl-4,6 -*O*-benzylidene-β-D-mannopyranosyl)-(1–4)-(2,3-*di*-*O*-benzyl-6-*O*-benz oyl-β-D-mannopyranosyl)-(1–4)-2,3-*di*-*O*-benzyl-6-*O*-benzoyl-β-D-mann opyranoside (19).



To a solution of **9** (70.3 mg, 0.13 mmol, 1.8 eq.) in anhydrous DCM (10 mL), conducted under N_2 atmosphere, were added 4Å molecular sieves, 1-benzenesulfinyl piperidine (BSP) (29.3 mg, 0.14 mmol, 2.0 eq.) and 2,6-di-*tert*-butyl-4 methylpyridine (DTBMP) (55.4 mg, 0.27 mmol, 3.6 eq.). The mixture was kept stirring for 0.5 hour at r.t.. Trifluoromethanesulfonic anhydride (Tf_2O) (0.08 ml, 0.45 mmol, 1.65 eq.) was added dropwise at -60 ^{0}C and 5 min later, a solution of **18** (110.0 mg, 0.07 mmol, 1.0 eq.) which was pre-dissolved in anhydrous DCM (3 ml), was added dropwise at -78 ^{0}C. The mixture was kept at -78 ^{0}C with vigorously stirring for 2 more hr and then allowed to be warmed up to r.t. before being quenched with saturated aqueous $NaHCO_3$, washed with ice-water (15 mL), extracted with DCM (3 x 15 mL), dried over Na_2SO_4, filtered, concentrated, purified by column chromatography on silica gel (eluent: hexane / ethyl acetate = 5 : 1 v/v) and concentrated under vacuum to provide a compound **19** (71.7 mg, 51%) as a syrup. After 11 mg starting material **18** was recovered, the yield was modified to 61%.

R_f 0.26 (Hexane / EtOAc / DCM, 5 : 0.7 : 0.5 v/v).

$[\alpha]_D^{22}$ -23.6 (C 0.41, MeOH / $CHCl_3$ = 2 : 1).

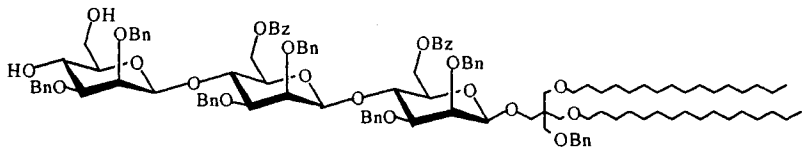
^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6 H, J 3.5 Hz, 2 CH_3),1.24 (bs, 52 H, 2 $C_{13}H_{26}$), 1.46 (bs, 4 H, 2 CH_2), 2.97 (ddd, 1 H, $J_{4c,5c}$ = $J_{5c,6c}$ 9.5, $J_{5,6c'}$ 5.0 Hz, H-5c), 3.26 - 3.47 (m, 14 H), 3.54 - 3.61 (m, 4 H), 3.78, 3.86, 3.89 (3

d, 3 H, $J_{2,3}$ 3.0 Hz, H-2a, H-2b & H-2c), 3.89 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4b), 3.97 (d, 1 H, J 9.5 Hz), 3.98, 4.31 (2 d, 2 H, $J_{3,4}$ 9.5 Hz, H-3a & H-3b), 4.04 (dd, 1 H, J 10.0 Hz), 4.16 – 4.21 (m, 2 H), 4.30 (d, 1 H, J 12.5 Hz, 1 CH_2 in OBn), 4.36 (s, 1 H, H-1c), 4.37, 4.44 (2 d, 2 H, J 12.5 Hz, 2 C*H*H in OBn), 4.47 (s, 1 H, H-1b), 4.50, 4.54 (2d, 2 H, J 12.5 Hz, 2 C*H*H in OBn), 4.55 (d, 2 H, J 4.0 Hz), 4.56 (d, 1 H, J 12.5 Hz, 1 C*H*H in OBn), 4.63 (d, 1 H, J 12.5 Hz, 1 C*H*H in OBn), 4.64 (d, 2 H, J 4.0 Hz), 4.65, 4.69, 4.78 (3 d, 3 H, J 12.5 Hz, 3 C*H*H in OBn), 4.78 (s, 1 H, H-1a), 4.82, 4.83, 4.87 (3 d, 3 H, J 12.5 Hz, 3 C*H*H in OBn), 5.48 (s, 1 H, CHPh), 7.04 - 7.43 (m, 39 H, 9 C_6H_5), 7.47, 7.51 (2 t, 2 H, $J_{3,4} = J_{4,5}$ 7.5 Hz, 2 H-4 in Bz), 7.94, 8.04 (2 d, 2 H each, $J_{2,3} = J_{5,6}$ 7.5 Hz, 2 H-2 & H-6 in Bz).

^{13}C NMR (125 MHz, $CDCl_3$): δ14.38 (2 CH_3), 22.93 (2 CH_2), 26.42 (2 CH_2), 29.62, 29.78, 29.88, 29.91, 29.94, 29.96 (2 $C_{11}H_{22}$), 32.15 (2 CH_2), 45.41 (1 C_q), 63.68, 64.13, 67.57, 68.66, 69.85, 70.18, 71.80, 71.81, 72.10, 72.15, 72.46, 73.45, 73.48, 73.55, 73.95, 74.57, 74.81, 75.36, 75.53, 75.89, 75.99, 77.20, 78.44, 78.56, 80.15, 101.51, 101.57, 102.11, 102.49 (C-1a, C-1b, C-1c, PhCH, $^1J_{C,H}$ 153.1, 151.6, 152.3, 156.5 Hz), 126.3, 127.42, 127.44, 127.47, 127.50, 127.66, 127.68, 127.71, 127.85, 128.06, 128.26, 128.30, 128.33, 128.41, 128.48, 128.51, 128.63, 129.89, 130.00 (10 Arom), 133.26, 133.29 (2 C_q in PhCOO), 137.81, 138.56, 138.65, 138.78, 138.79, 139.11, 139.12, 139.19 (7 C_q in $PhCH_2$ & 1 C_q in PhCH), 166.43, 166.52 (2 PhCOO).

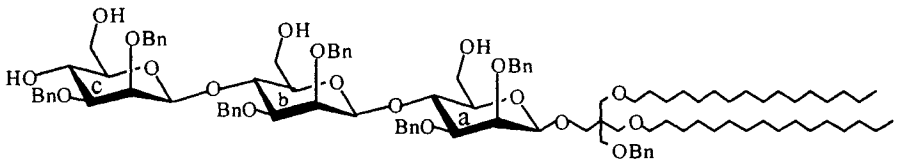
Anal. Calcd for $\mathbf{C_{125}H_{160}O_{21}}$: C, 75.12; H, 8.07. Found: C, 75.38; H, 8.22.

(3-benzyloxy-2,2-dihexadecyloxymethyl)-propyl(2,3-*di*-*O*-benzyl-β-D-mannopyranosyl)-(1–4)-(2,3-*di*-*O*-benzyl-6-*O*-benzoyl-β-D-mannopyranosyl)-(1–4)-2,3-*di*-*O*-benzyl-6-*O*-benzoyl-β-D-mannopyranoside (20).



To a solution of **19** (220.0 mg, 0.11 mmol, 1.0 eq.) and neopentyl glycol (75.0 mg, 0.72 mmol, 6.5 eq.) in anhydrous DCM (10 mL) at 35 ^{0}C, conducted under N_2 atmosphere, was added CSA (37.5 mg, 0.16 mmol, 1.5 eq.) with vigorous stirring. The mixture was left overnight until a TLC check indicated a complete reaction, followed by a work up: being quenched with saturated aqueous $NaHCO_3$, washed with ice-water (15 mL), extracted with EtOAC (3 x 15 mL), dried over Na_2SO_4, filtered and concentrated. The unpurified residue was directly used in synthesis of **21**.

(3-benzyloxy-2,2-dihexadecyloxymethyl)-propyl(2,3-*di*-*O*-benzyl-β-D-mannopyranosyl)-(1–4)-(2,3-*di*-*O*-benzyl-β-D-mannopyranosyl)-(1–4)-2,3-*di*-*O*-benzyl-β-D-mannopyranoside (21).



The unpurified **20** (400 mg) was dissolved in solution of MeOH / DCM = 2 : 1 (30 ml) and MeONa was added dropwise until the pH of solution reached 10. The mxture is kept stirring for 17 hr followed by a TLC check indicated a complete reaction and then Resin-RCOOH was added until the pH=7, followed by filtration, purified by column chromatography on silica gel (eluent: H : E : DCM = 1 : 1 : 0.5 v/v) and concentrated under vacuum to provide compound **21** [155.0 mg, 83% (2 steps)] as a syrup.

R_f 0.38 (Hexane / EtOAc / DCM, 1 : 2 : 0.5 v/v).

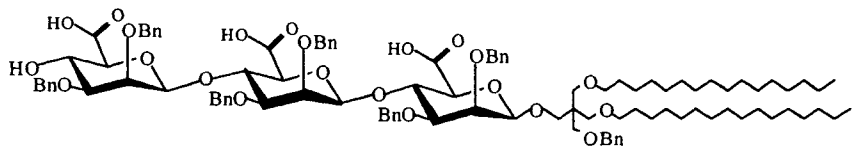
^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6 H, J 3.5 Hz, 2 CH_3),1.25 (bs, 52 H, 2 $C_{13}H_{26}$), 1.51 (bs, 4 H, 2 CH_2), 2.30 (b, 4 H, 4 OH), 3.17 - 3.21 (m, 2 H), 3.24 - 3.28 (2 dd, 2 H, J 3.0 Hz), 3.34 - 3.48 (m, 12 H), 3.50 (d, 2 H, J 9.5

Hz), 3.62 - 3.66 (m, 2 H), 3.70, 3.72 (2 d, 2 H, J 4.0 Hz), 3.80 - 3.84 (m, 3 H), 3.86, 3.88 (2 d, 2 H, J 3.0 Hz), 3.96 (d, 1 H, J 9.5 Hz), 4.08, 4.13 (2 dd, 2 H, 2 H-4), 4.34 (d, 1 H, J 12.5 Hz, 1 PhC*H*H), 4.37 (s, 1 H, H-1c), 4.44, 4.49 (2 d, 2 H, J 12.5 Hz, 2 PhC*H*H), 4.50 (s, 1 H, H-1b), 4.50, 4.54, 4.55 (3 d, 3 H, J 12.5 Hz, 3 PhC*H*H), 4.59 (s, 1 H, H-1a), 4.73, 4.74, 4.75, 4.79, 4.80, 4.83, 4.87, 4.89 (8 d, 8 H, J 12.5 Hz, 8 PhC*H*H], 7.24 - 7.40 (m, 35 H, 7 C_6H_5).

^{13}C NMR (125 MHz, $CDCl_3$): δ 14.38 (2 CH_3), 22.93 (2 CH_2), 26.42 (2 CH_2), 29.62, 29.78, 29.88, 29.91, 29.95, 29.96 (2 $C_{11}H_{22}$), 32.15 (2 CH_2), 45.41 (1 C_q), 62.11, 62.23, 63.03, 67.56, 69.69, 69.73, 70.21, 71.55, 71.91, 72.45, 73.03, 73.54, 74.22, 74.50, 74.55, 74.84, 75.39, 75.70, 75.73, 75.78, 76.65, 80.13, 80.30, 82.22, 101.20, 101.61, 102.58 (C-1a, C-1b, C-1c, $J_{C,H}$ 155.0, 156.7, 158.3 Hz), 127.23, 127.45, 127.53, 127.63, 127.65, 127.73, 127.75, 127.79, 127.81, 127.94, 128.20, 128.25, 128.37, 128.40, 128.47, 128.49, 128.55, 128.56, 128.80, 137.88, 138.72, 138.78, 138.82, 138.85, 138.90, 139.06 (7 C_q in Bn).

Anal. Calcd for **$C_{104}H_{148}O_{19}$ • 1/2H_2O**: C, 72.99; H, 8.48. Found: C, 72.88; H, 8.51.

(3-benzyloxy-2,2-dihexadecyloxymethyl)-propyl(2,3-*di*-*O*-benzyl-β-D-mannopyranosyluronate)-(1–4)-(2,3-*di*-*O*-benzyl-β-D-mannopyranosyluronate)-(1–4)-(2,3-*di*-*O*-benzyl-β-D-mannopyranosid)uronic acid (22).

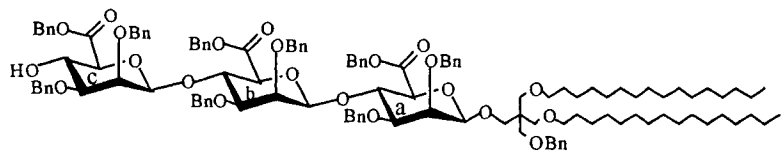


To a flask charged with **21** (155.0 mg, 0.09 mmol, 1.0 eq.), 2,2,6,6-tetramethyl -piperridinyloxy free radical (TEMPO) (11.5 mg, 0.074 mmol, 0.82 eq.) and [bis(acetoxy)iodo]benzene (BAIB) (232.0 mg, 0.72 mmol, 8.2 eq.) was added 4 ml DCM and 2 ml H_2O with vigorous stirring. The reaction mixture was kept stirring for 4 hr until a TLC check indicated

complete conversion of the starting material to a lower running spot. The mixture was quenched with aqueous $Na_2S_2O_3$ (10% in H_2O), washed with ice-water (10 ml), extracted with DCM (3x10 ml), dried through Na_2SO_4, filtered, concentrated, purified by column chromatography on silica gel (eluent: Hexane / EtOAc / MeOH / HOAc = 6 : 1 : 0.1 : 0.2 v/v) and concentrated under vacuum to afford pure compound **22** (101.0 mg, 63%) as a syrup.

R_f 0.28 (Hexane / EtOAc / MeOH / HOAc, 3 : 1 : 0.1 : 0.2 v/v).

(3-benzyloxy-2,2-dihexadecyloxymethyl)-propyl (benzyl 2,3-*di*-*O*-benzyl-β-D-mannopyranosyluronate)-(1–4)-(benzyl 2,3-*di*-*O*-benzyl-β-*D*-mannopyranosyluronate)-(1–4)-(benzyl 2,3-*di*-*O*-benzyl-β-D-mannopyranosid) urinate (23).



To a solution of **22** (101.0 mg, 0.06 mmol, 1.0 eq.) and potassium fluoride (158.0 mg, 2.7 mmol, 45.0 eq.) in distilled DMF (8 mL) at r.t. was added dropwise benzylbromide (0.19 mL, 1.6 mmol, 27.0 eq.) which was pre-dissolved in 1 mL DMF with vigorous stirring. The mixture was kept stirring overnight until a TLC check indicated complete reaction. DMF was removed by rotary evaporation under reduced pressure. 10 mL H_2O and 10 mL DCM was added in reaction mixture. The mixture was taken up with DCM (3 x 20 mL), dried through Na_2SO_4, filtered, concentrated, purified by column chromatography on silica gel (eluent: hexane / ethyl acetate = 7 : 1 v/v) and concentrated under vacuum to provide pure compound **17** (74.0 mg, 62%) as a syrup.

R_f 0.70 (Hexane / EtOAc / MeOH / HOAc, 3 : 1 : 0.1 : 0.2 v/v).

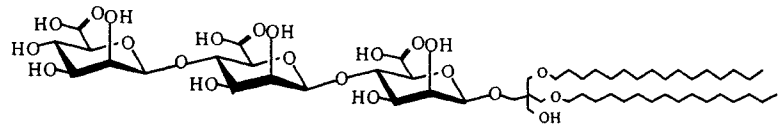
$[\alpha]_D^{22}$ -39.6 (C 0.66, MeOH / $CHCl_3$ = 2 : 1).

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 6 H, J 3.5 Hz, 2 CH_3),1.25 (bs, 52 H, 2 $C_{13}H_{26}$), 1.48 (bs, 4 H, 2 CH_2), 2.74 (d, 1 H, OH), 3.03, 3.22 (dd, 2 H, $J_{3,4}$ 9.5 Hz, $J_{2,3}$ 3.0 Hz, H-3b & H-3c), 3.21 - 3.42 (m, 11 H), 3.47 (dd, 2 H, J 3.0, J 9.5 Hz), 3.40 (d, 1 H, J 9.5 Hz), 3.61 (d, 1 H, J 3.5 Hz, H-2c), 3.62 (d, 1 H, J 9.5 Hz), 3.68 (d, 2 H, $J_{2,3}$ 3.0 Hz, 2 H-2), 3.75 (d, 1 H, J 9.5 Hz), 4.14 (ddd, 1 H, $J_{3c,4c} = J_{4c,5c}$ 9.5 Hz, $J_{4c,OH}$ 2.5 Hz, H-4c), 4.26 (dd, 1 H, J 9.5 Hz, H-4b), 4.28, 4.31 (2 s, 2 H, H-1b & H-1c), 4.34 (t, 1 H, J 9.5 Hz, H-4a), 4.38, 4.39, 4.40, 4.41 (4 d, 4 H, J 12.5 Hz, 4 PhC*H*H), 4.42 (s, 1 H, H-1a), 4.43, 4.45, 4.46, 4.57, 4.67, 4.69, 4.71, 4.72, 4.75, 4.79, 4.85, 4.92, 4.98, 5.03, 5.07, 5.16 (16 d, 16 H, J 12.5 Hz, 16 PhC*H*H), 7.12 - 7.35 (m, 50 H, 10 C_6H_5).

^{13}C NMR (125 MHz, $CDCl_3$): δ 14.39 (2 CH_3), 22.94 (2 CH_2), 26.47 (2 CH_2), 29.62, 29.80, 29.89, 29.93, 29.95, 29.97 (2 $C_{11}H_{22}$), 32.18 (2 CH_2), 45.46 (1 C_q), 66.97, 67.06, 67.14, 68.12, 69.68, 69.72, 69.75, 70.66, 71.51, 71.71, 71.72, 72.51, 72.72, 73.36, 73.84, 74.62, 74.69, 74.93, 74.96, 75.08, 75.17, 76.11, 77.25, 77.29, 79.75, 77.36, 79.50, 80.24, 102.25, 102.45, 103.21 (C-1a, C-1b, C-1c, $J_{C,H}$ 157.7, 155.6, 155.3 Hz), 127.49, 127.53, 127.56, 127.64, 127.82, 127.96, 128.02, 128.19, 128.29, 128.34, 128.40, 128.53, 128.64, 128.66, 128.70, 128.74, 128.78, 128.83, 128.86 (10 Arom], 135.39, 135.48, 135.49 (3 C_q in BnOOC), 138.26, 138.81, 139.10(2), 139.15, 139.20, 139.21 (7 C_q in $PhCH_2O$), 168.12, 168.21, 169.27 (3 BnOOC).

Anal. Calcd for **$C_{125}H_{160}O_{22} \cdot 1/2H_2O$** : C, 74.18; H, 8.01. Found: C, 74.12; H, 7.89.

(3-benzyloxy-2,2-dihexadecyloxymethyl)-propyl(β-D-mannopyranosyl uronate)-(1–4)-(β-D-mannopyranosyluronate)-(1–4)-(β-D-mannopyranosid)uronic acid (2).



To a solution of **23** (14.0 mg), dissolved in 32 mL freshly distilled THF, was carefully added palladium charcoal powder (Pd/C) (48.0 mg) and the solution was H_2 bubbled through for 2 mins. The reaction mixture was kept stirring for 2 hr under H_2 atmosphere established with a H_2 balloon at r.t., followed by addition of 6 ml H_2O and kept stirring overnight before a TLC check indicated complete conversion of the starting material to a lower single running spot. The reaction mixture was filtered and the filtrate [catalyst (Pd/C)] was washed with distilled THF-MeOH (1:1 v/v) several times. The final filtrate was concentrated under vaccum and followed by a freeze-drying from fresh 1,4-dioxane to provide a white powder **2** (7 mg, 90%).

R_f 0.38 (iPrOH / H_2O / $NH_3 \cdot H_2O$, 7 : 2 : 0.1 v/v).

$[\alpha]_D^{22}$ +47.3 (C 0.08, MeOH / $CHCl_3$ = 2 : 1).

MALDI-MS calcd for $\mathbf{C_{55}H_{100}O_{22}}$ 1112.67 $[M]^+$, found 1136.05 $[M+Na]^+$, 1158.06 $[M-H+2Na]^+$.

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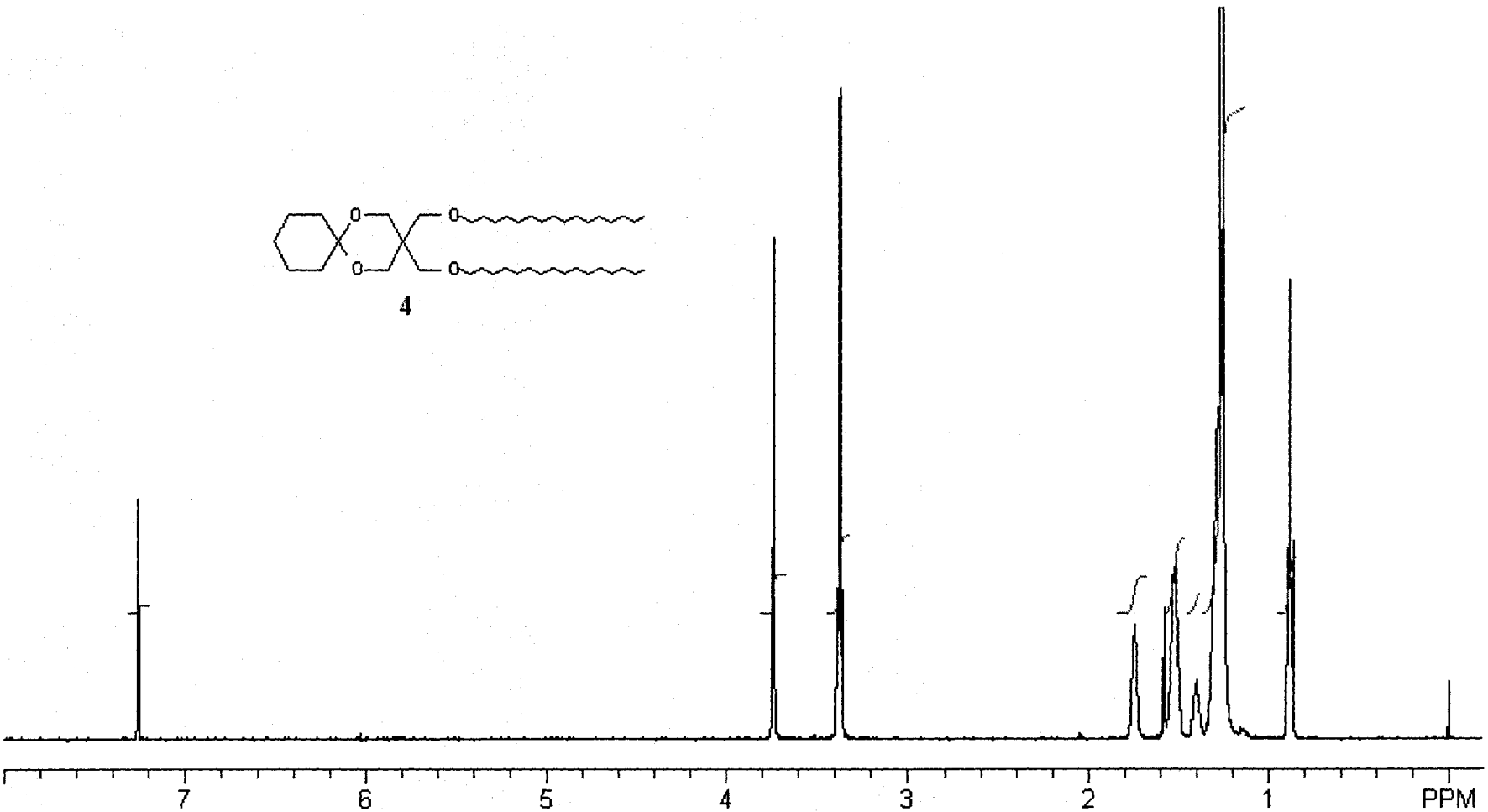
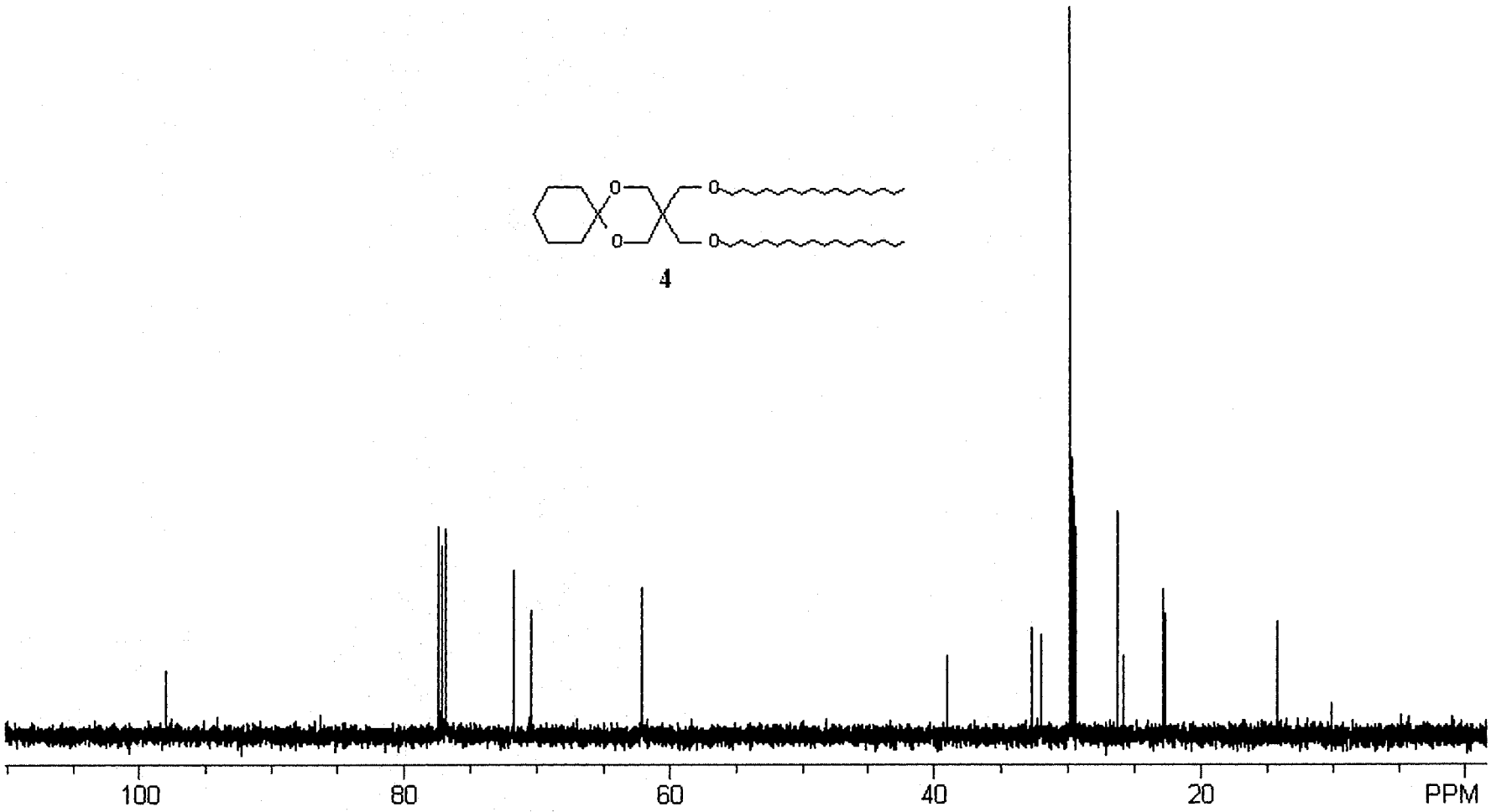
5. APPENDIX

^{1}H, ^{13}C and MS Spectra of Corresponding Compounds



4



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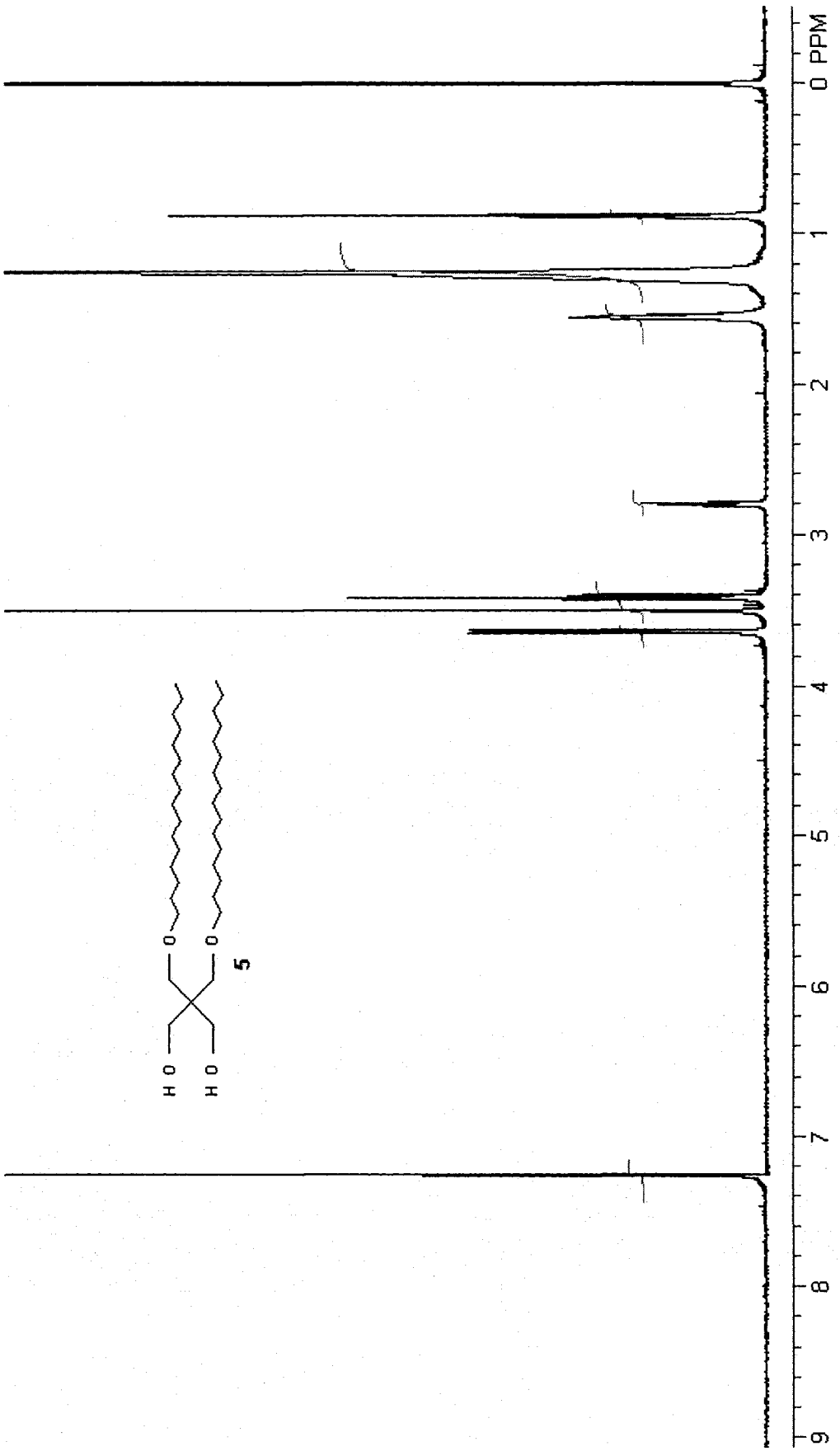

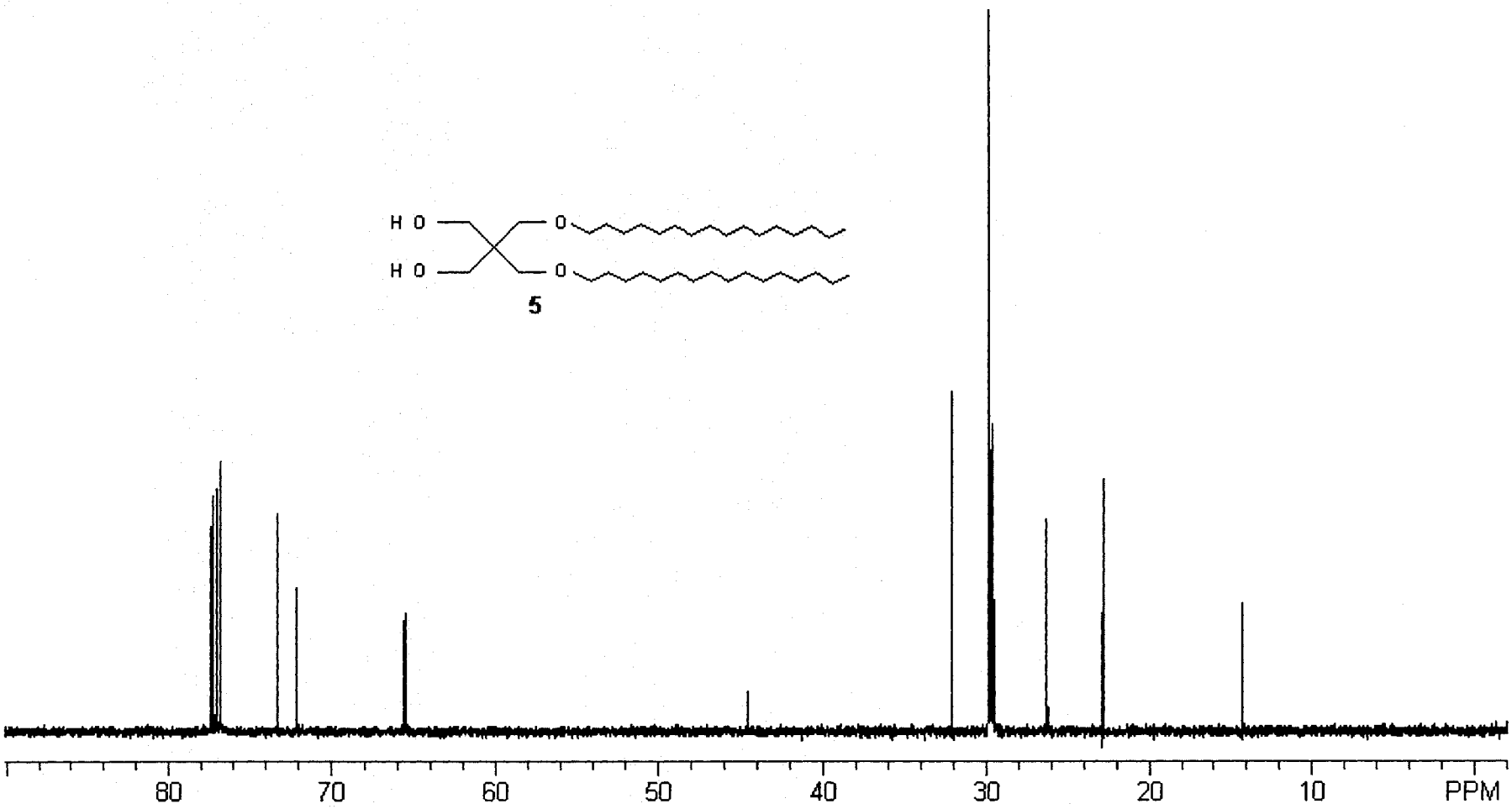

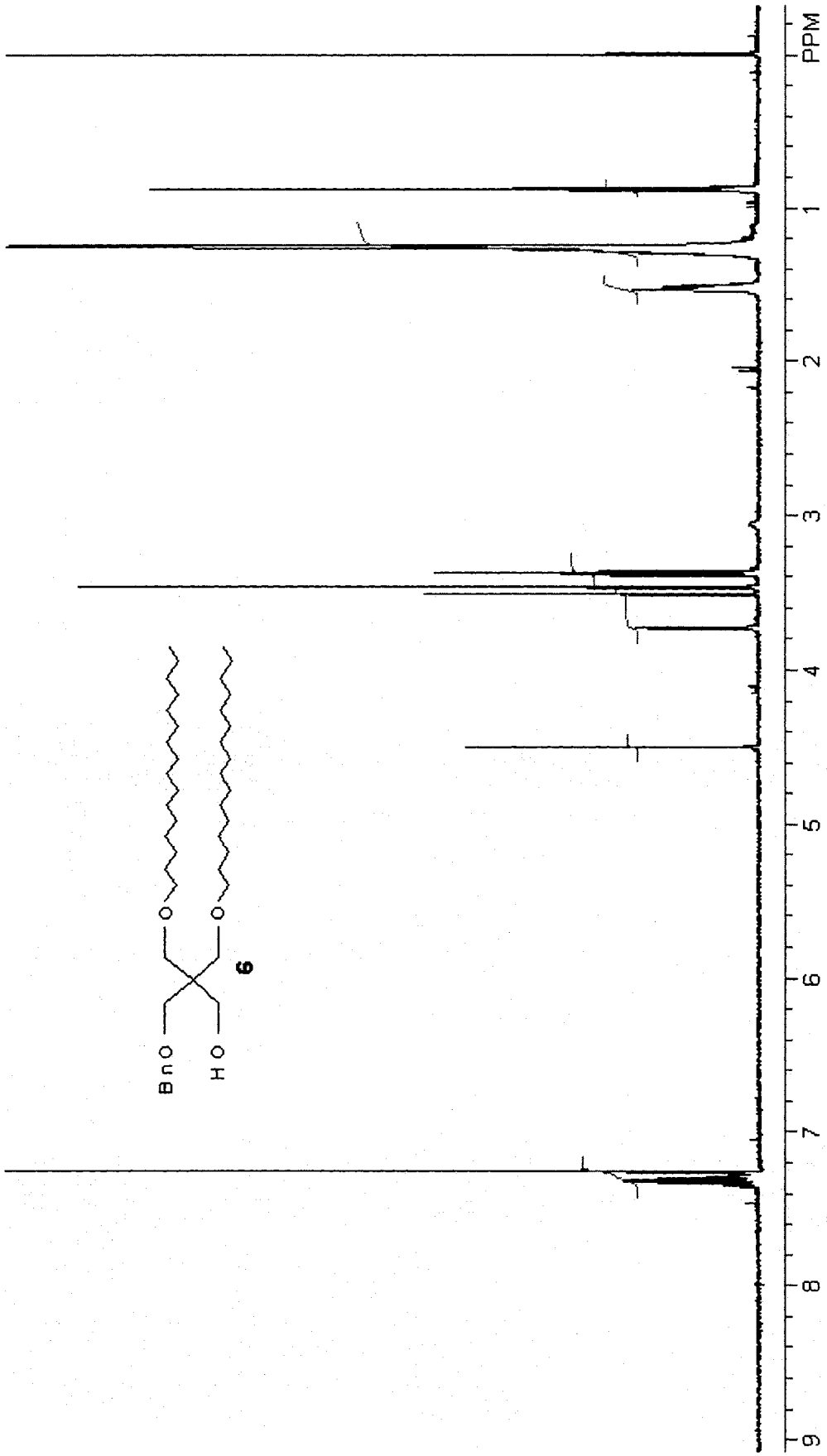

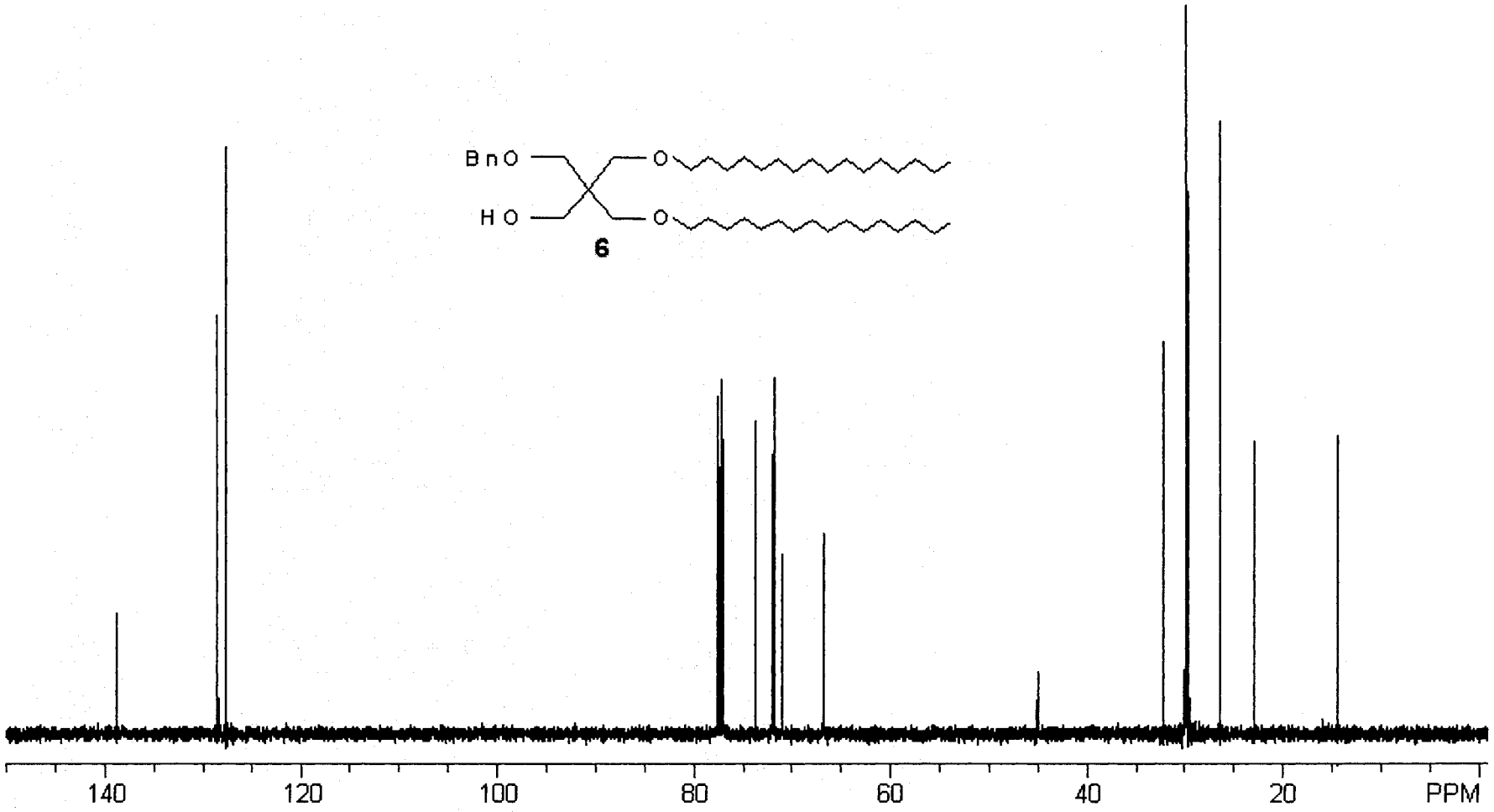

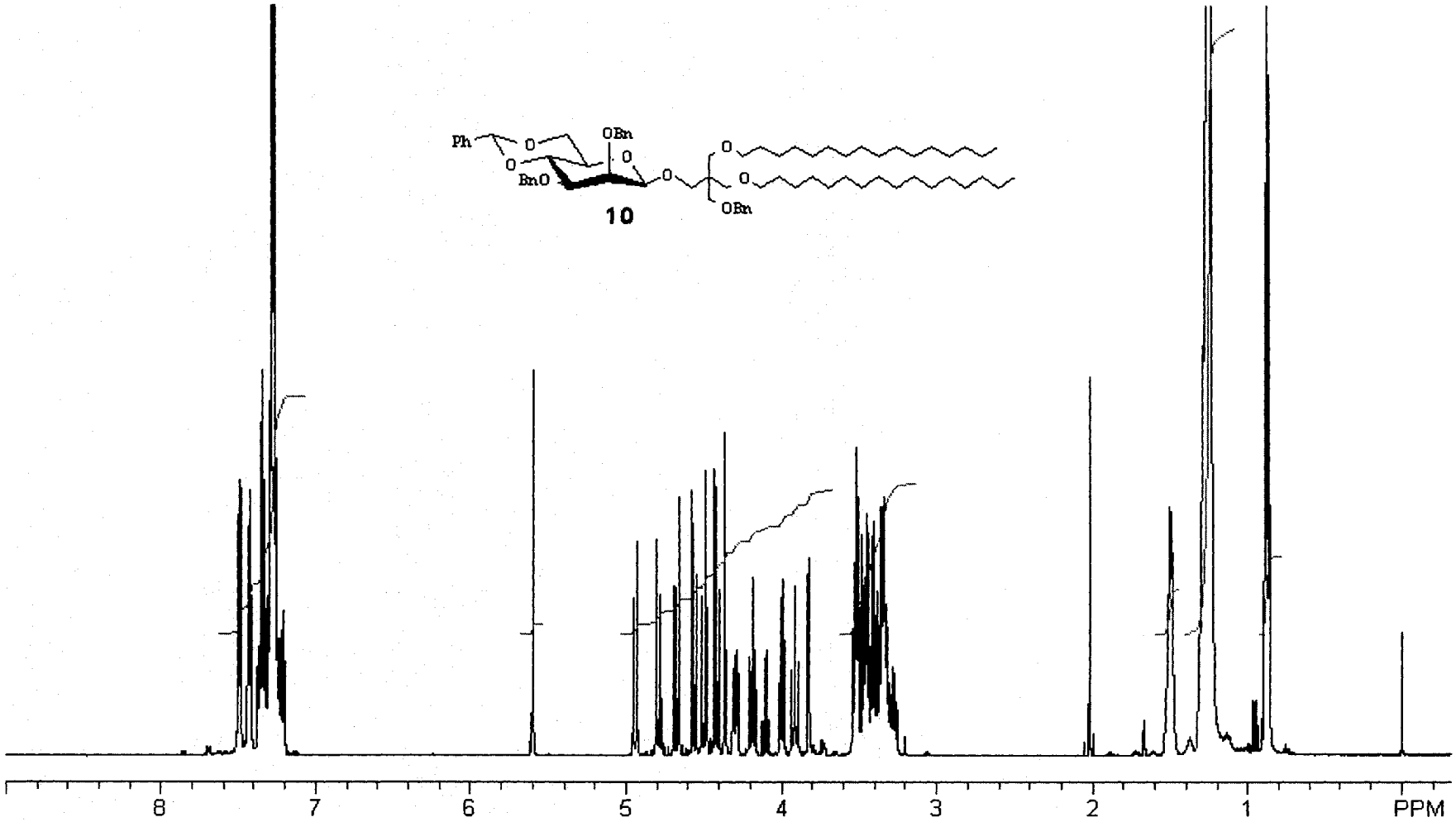

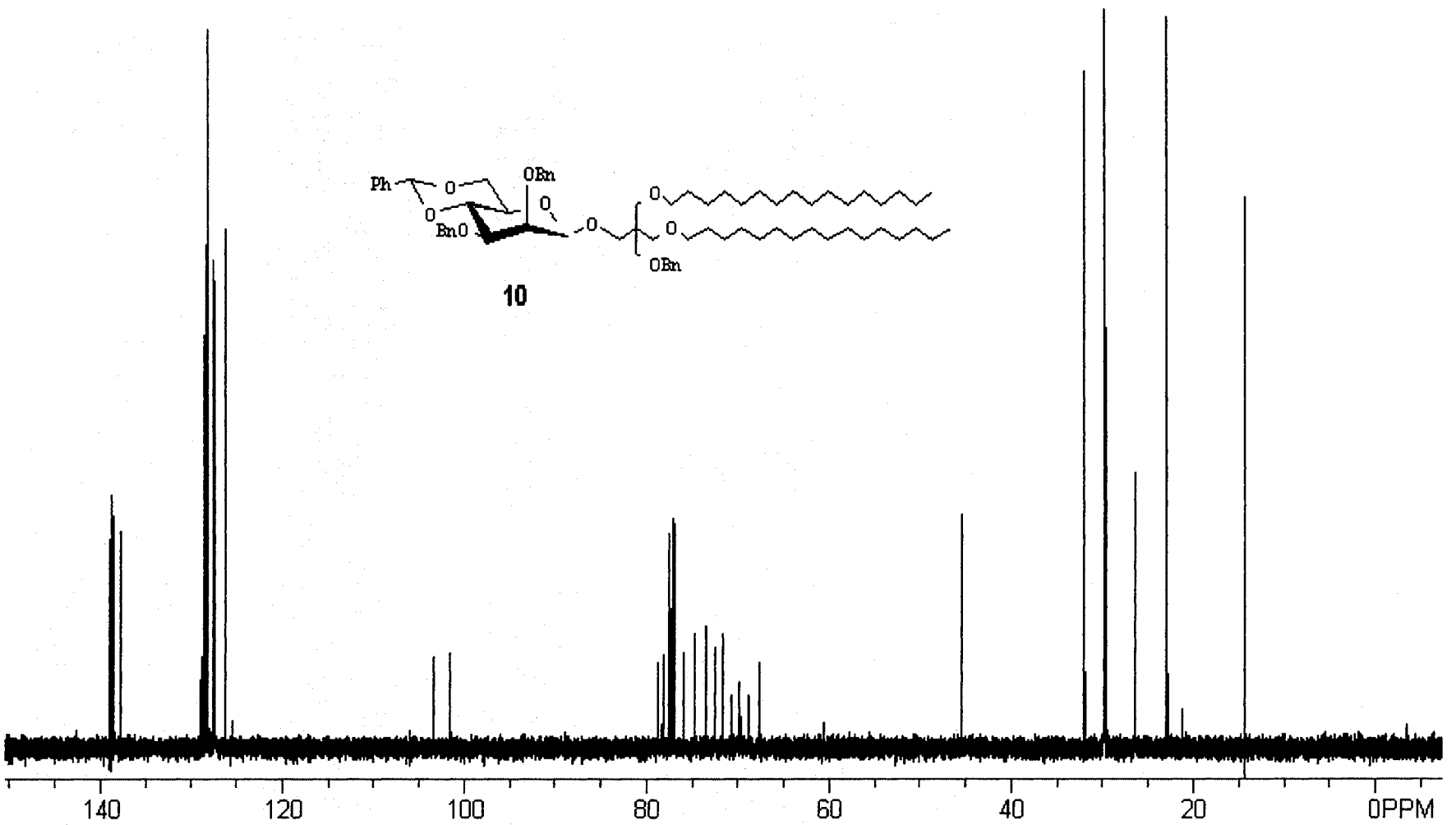

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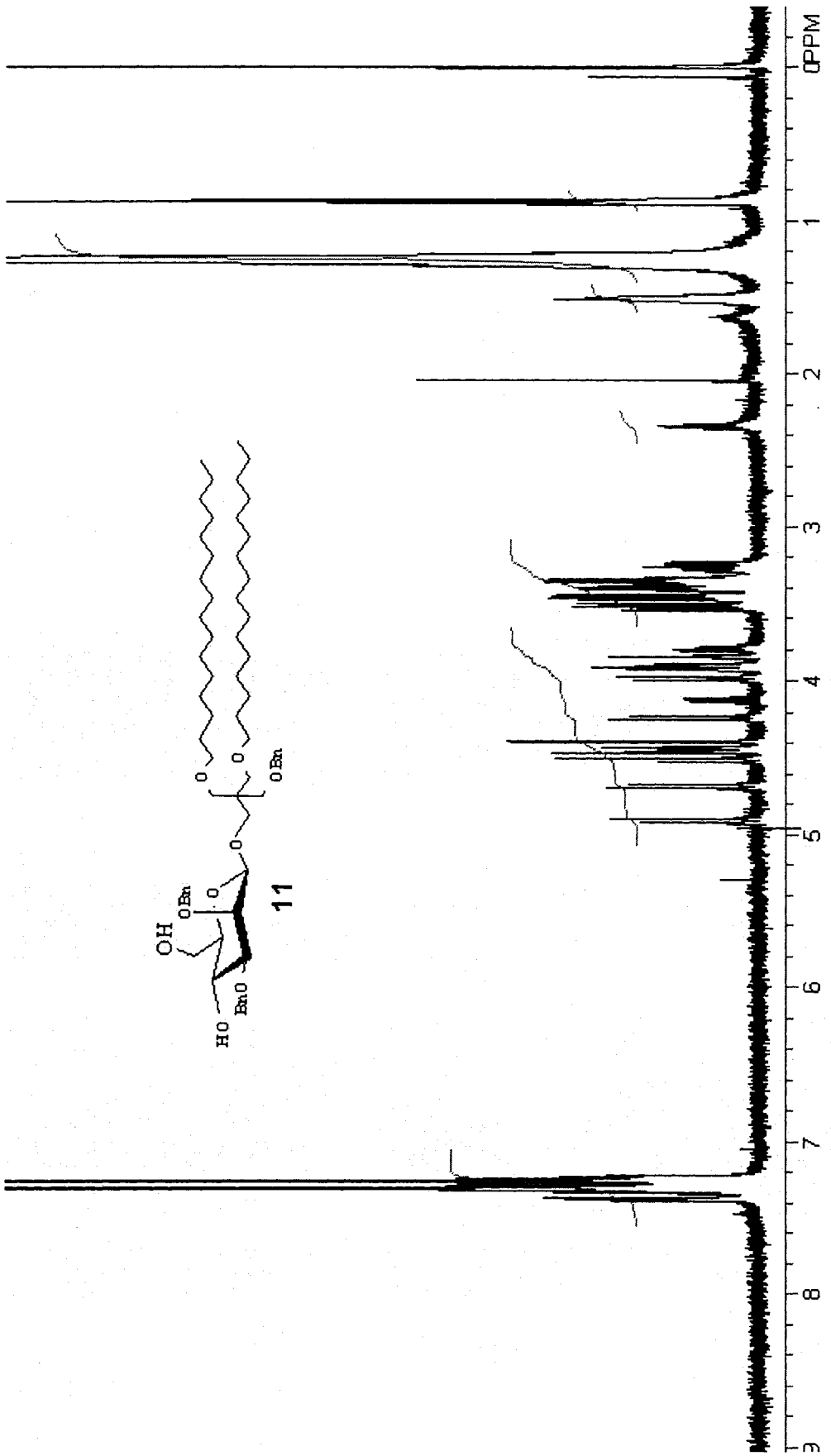
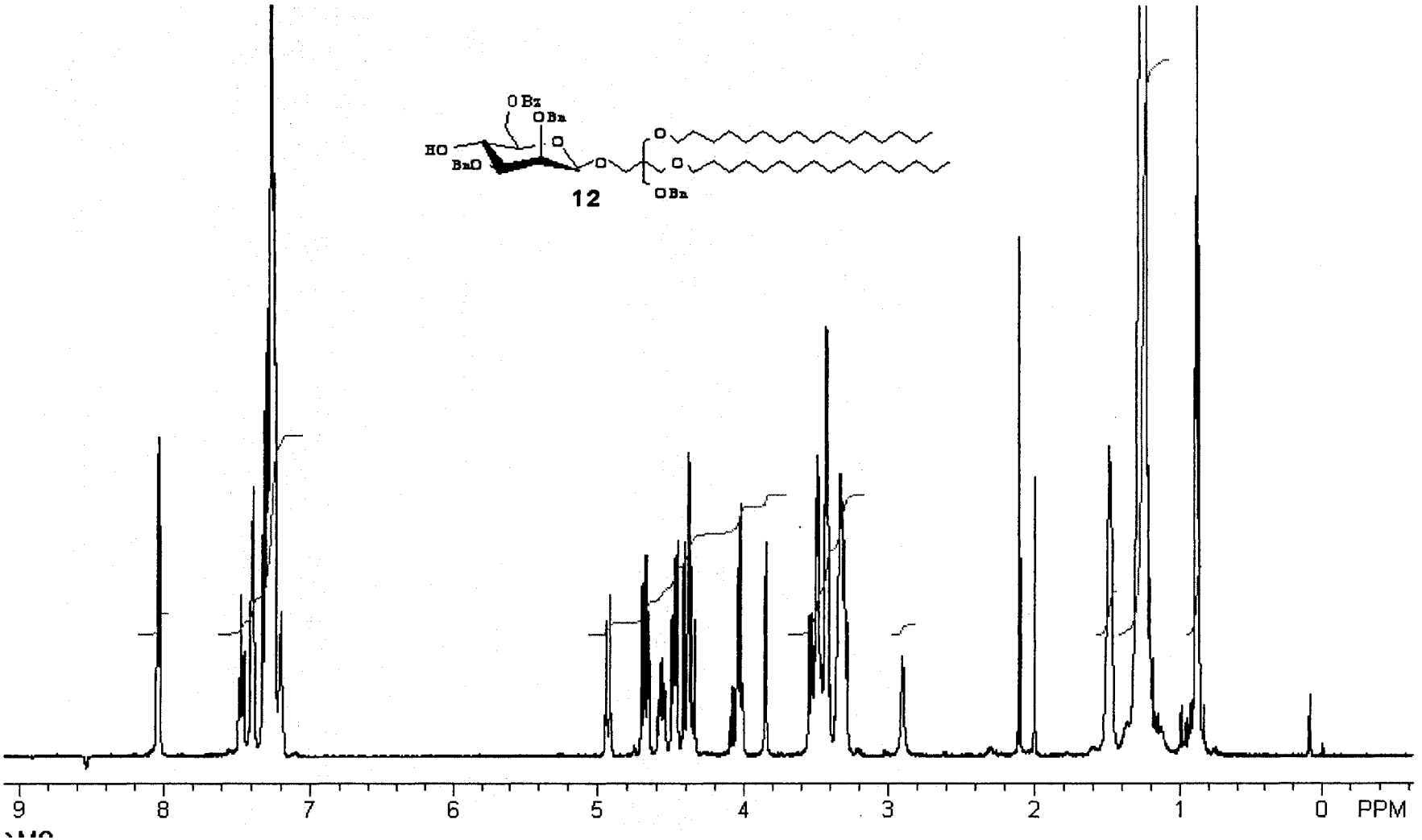
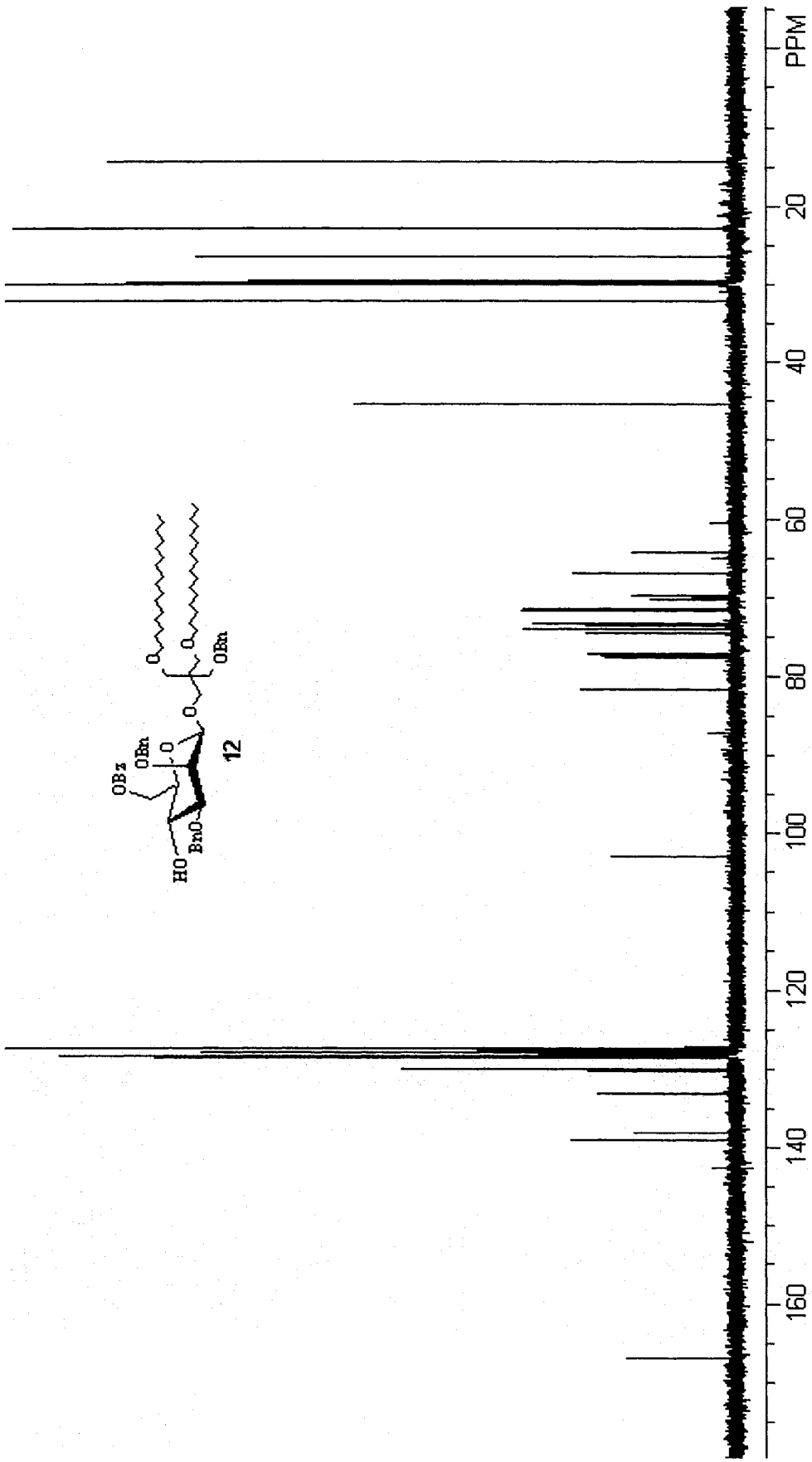
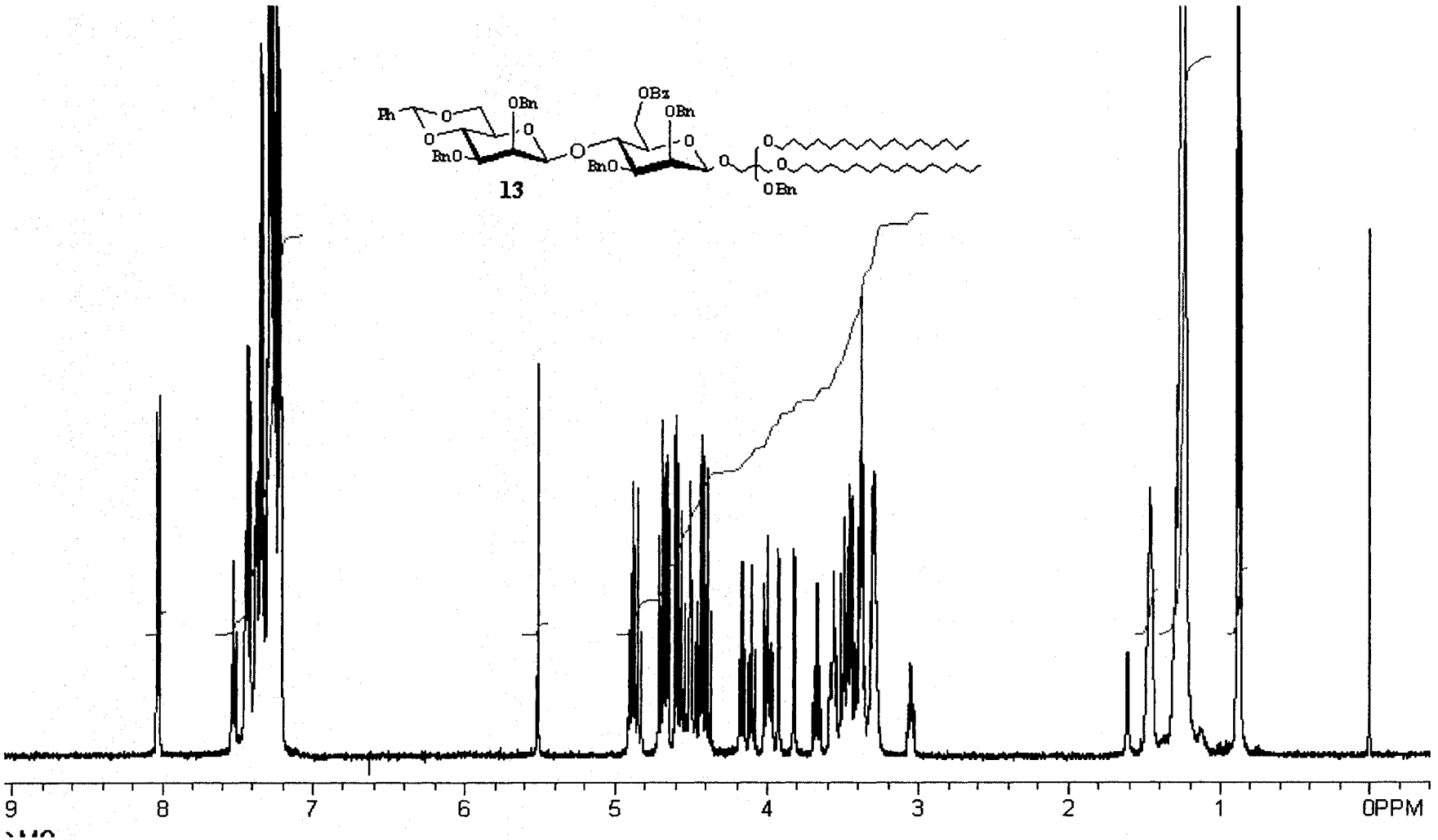
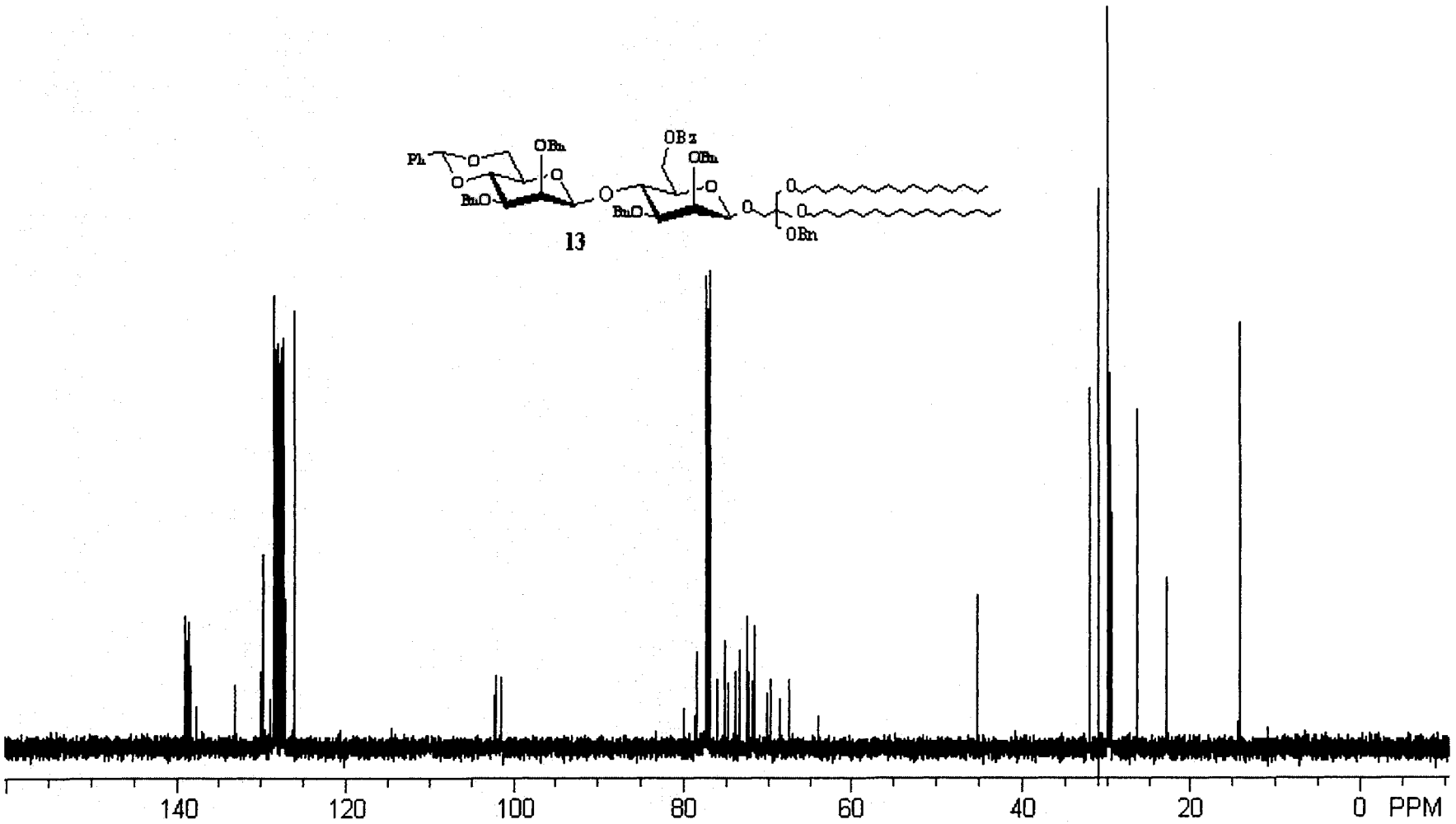
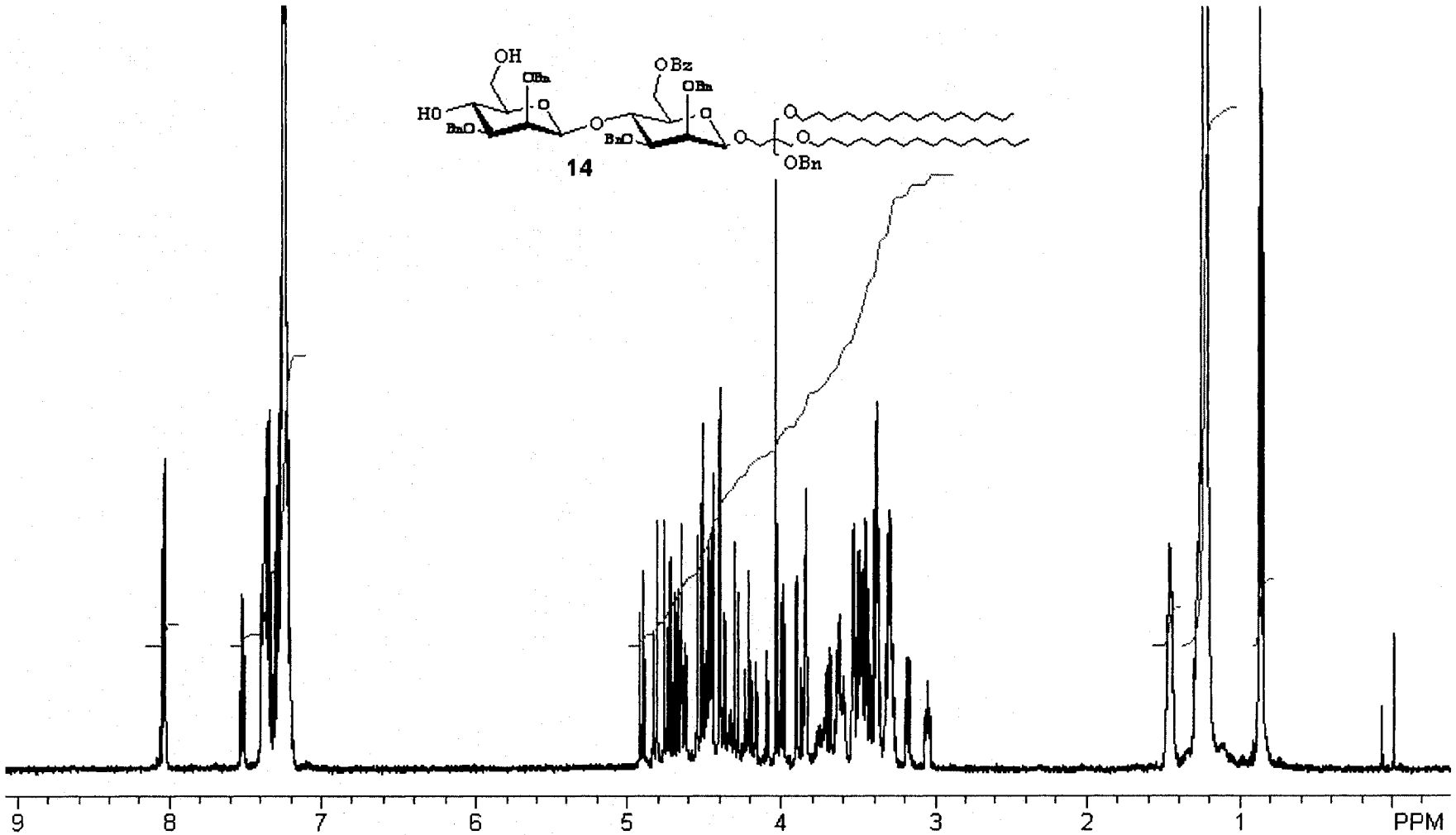


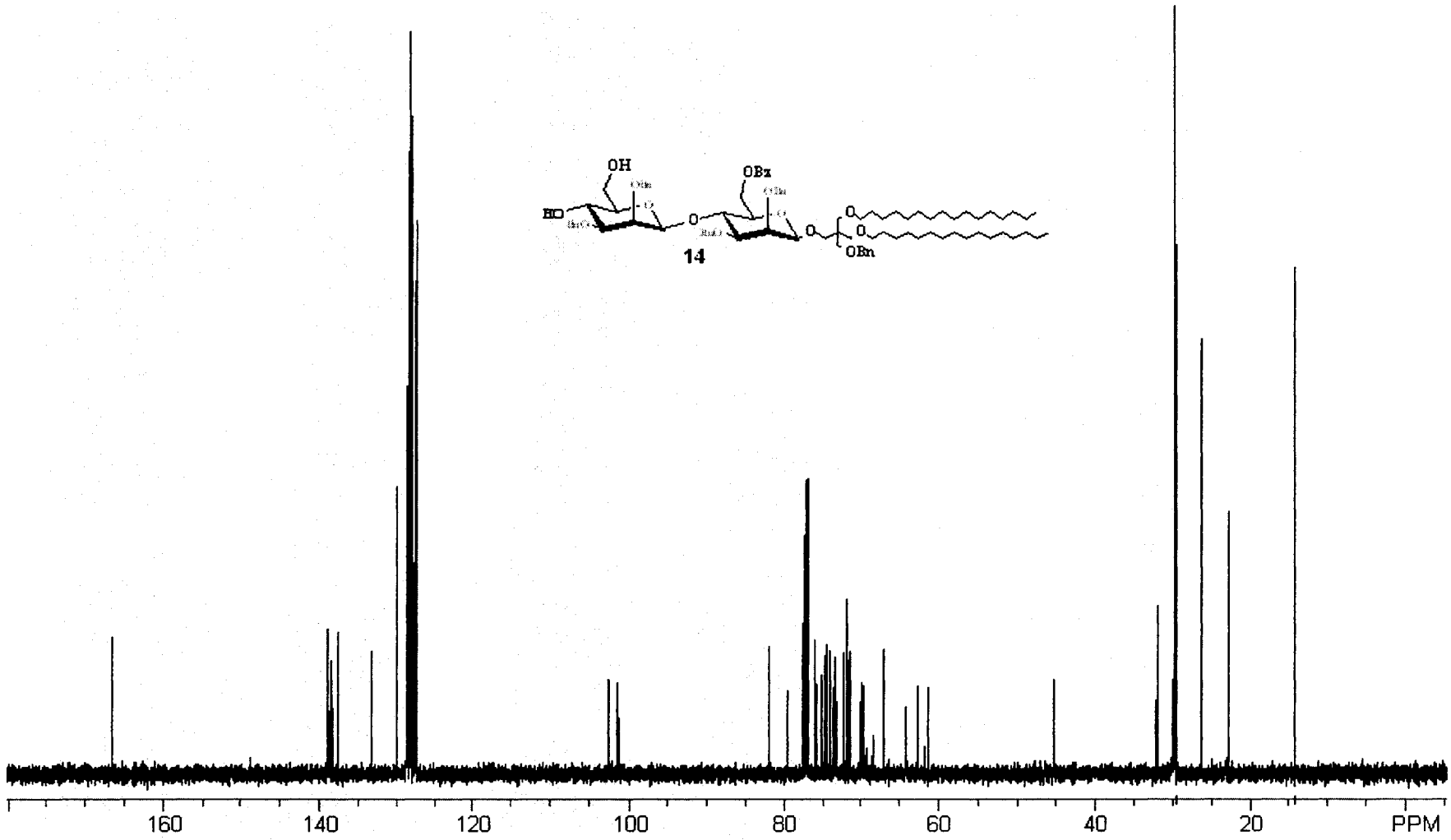


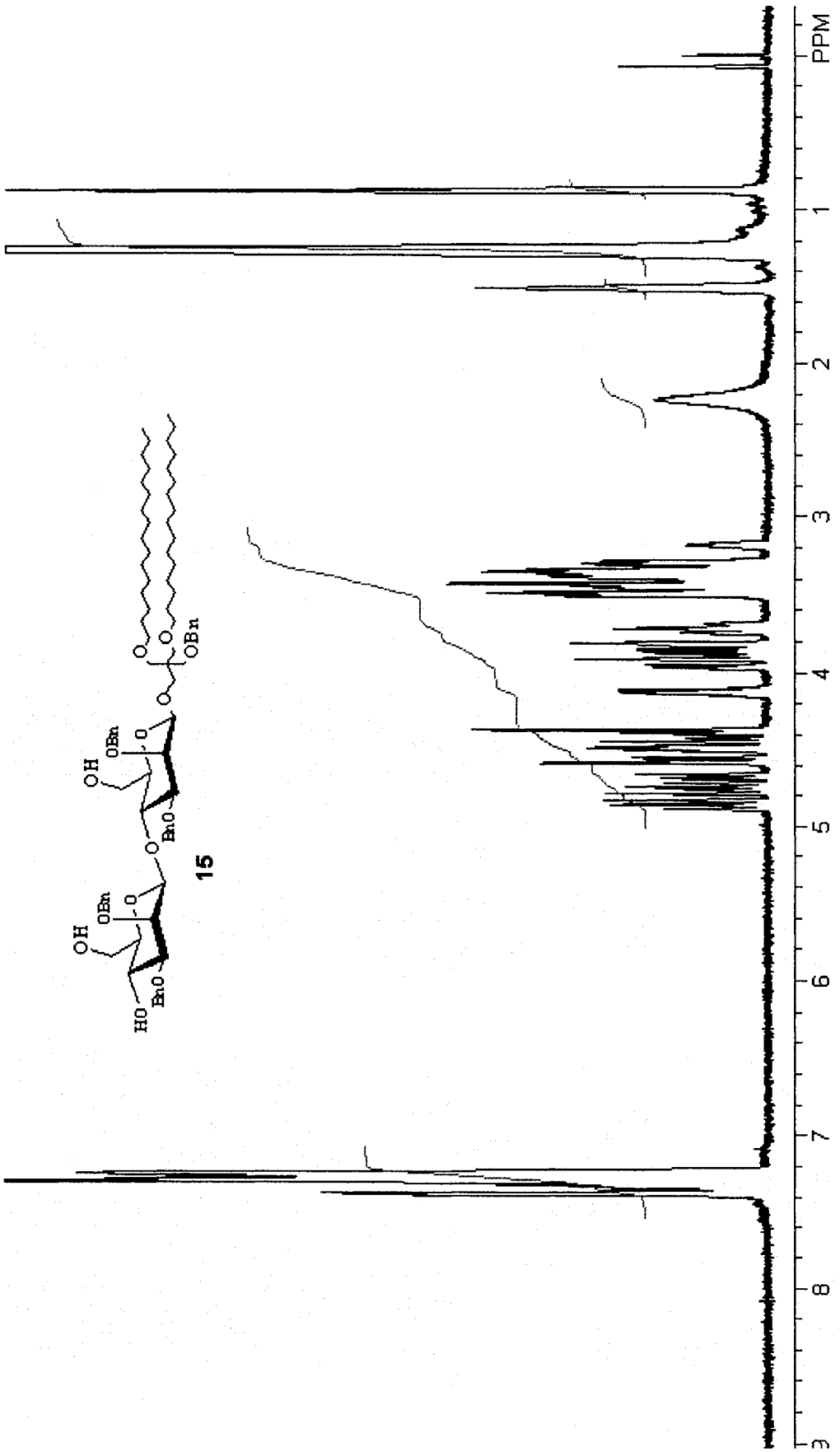


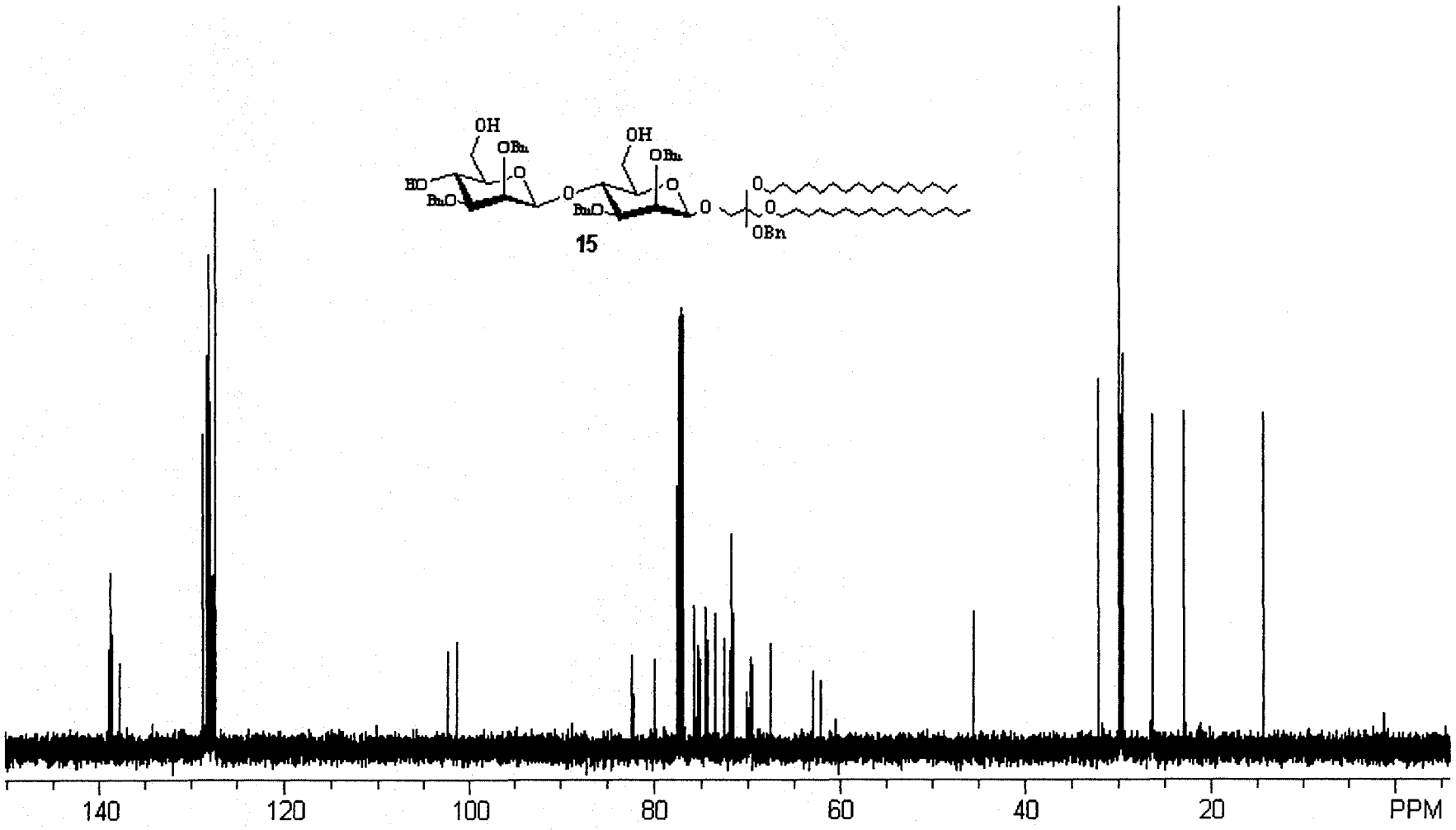


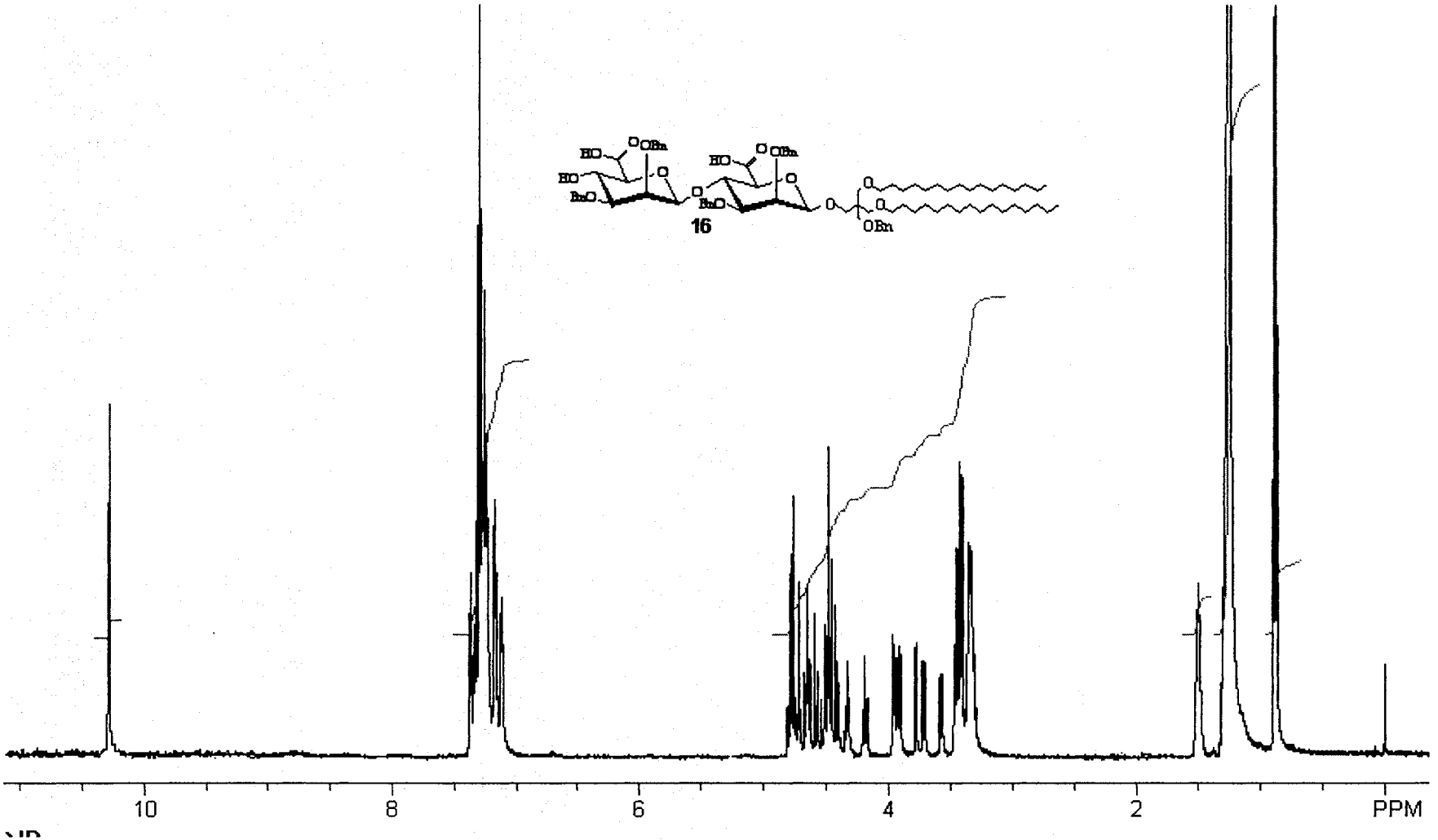


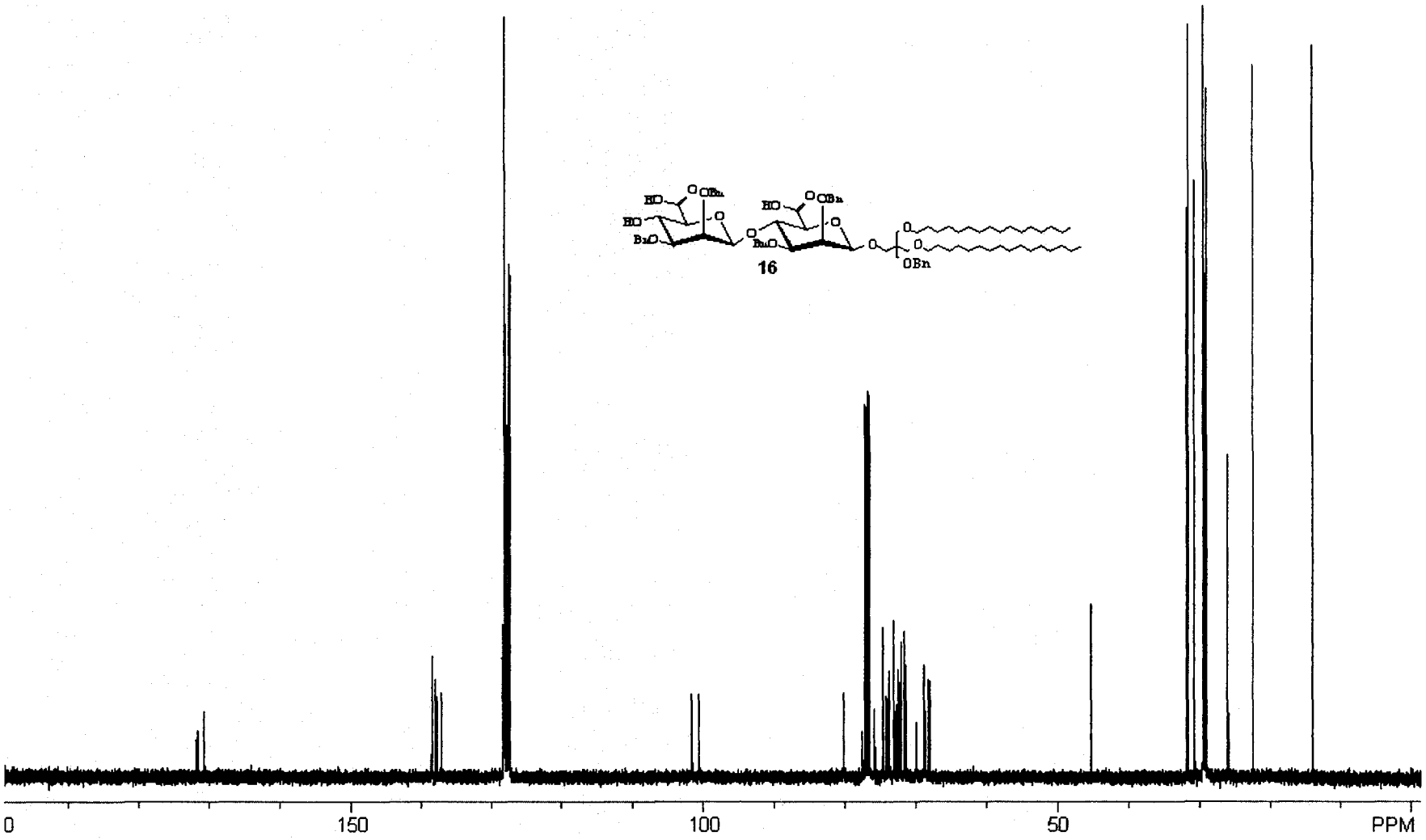


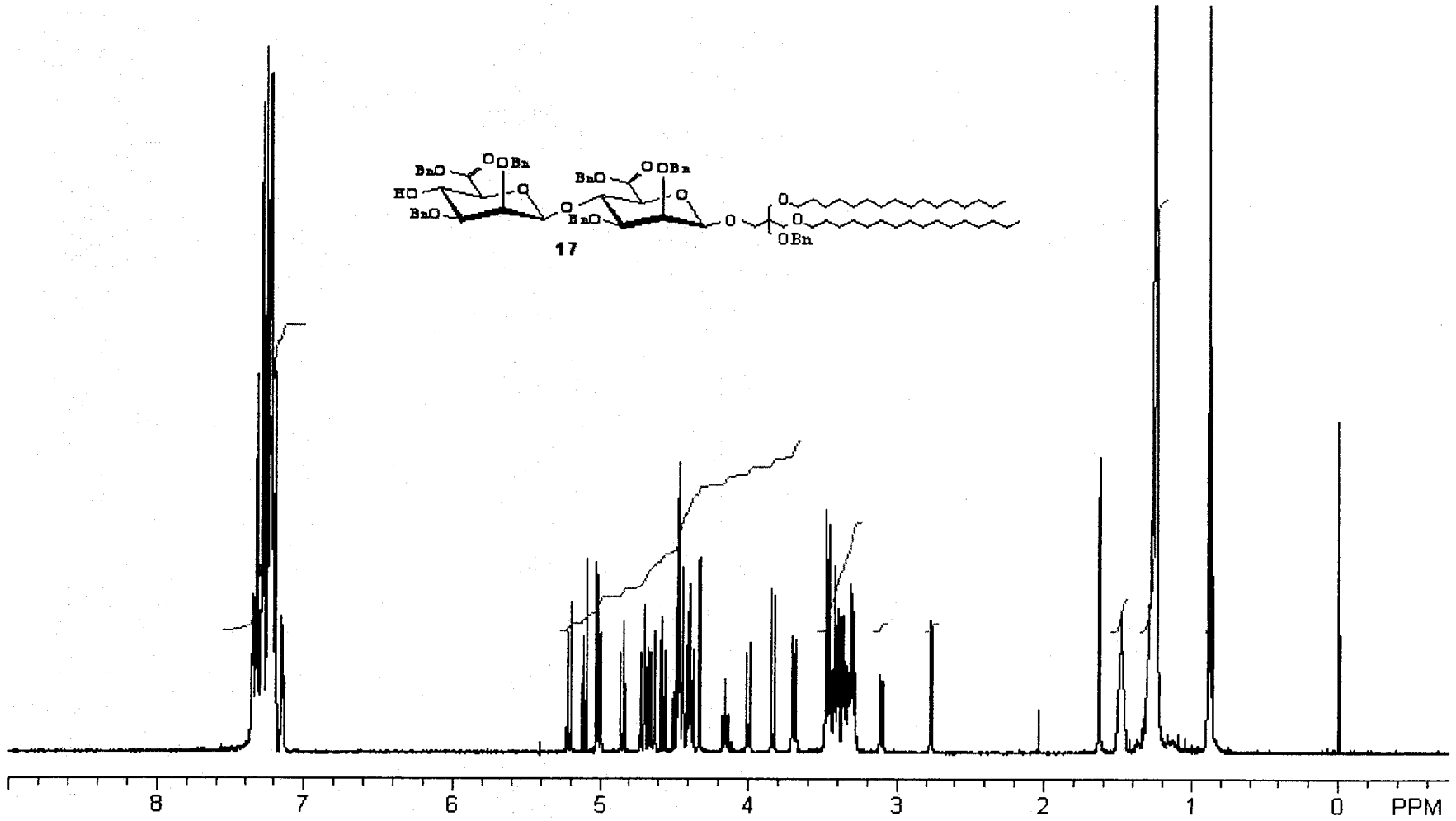


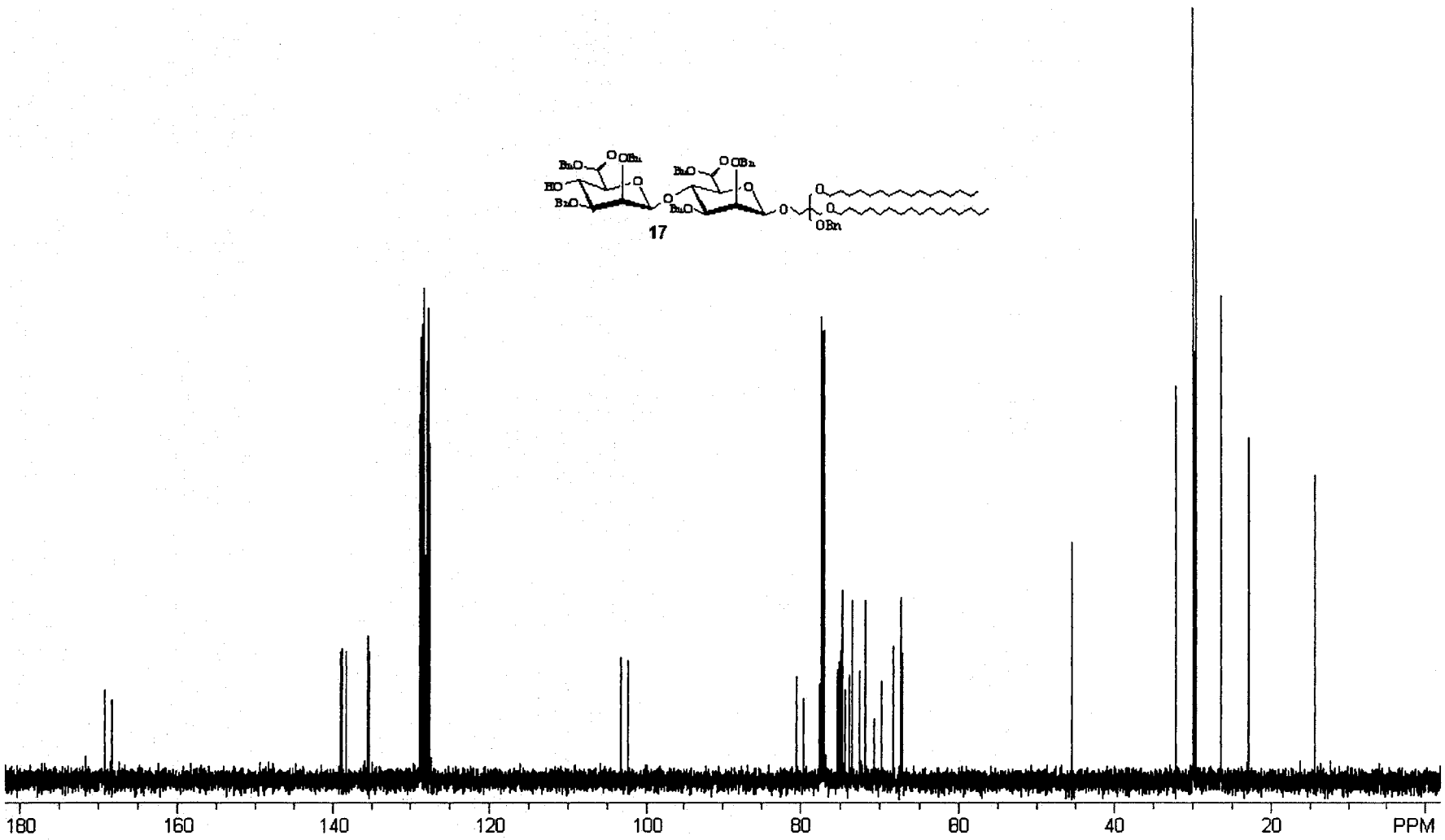


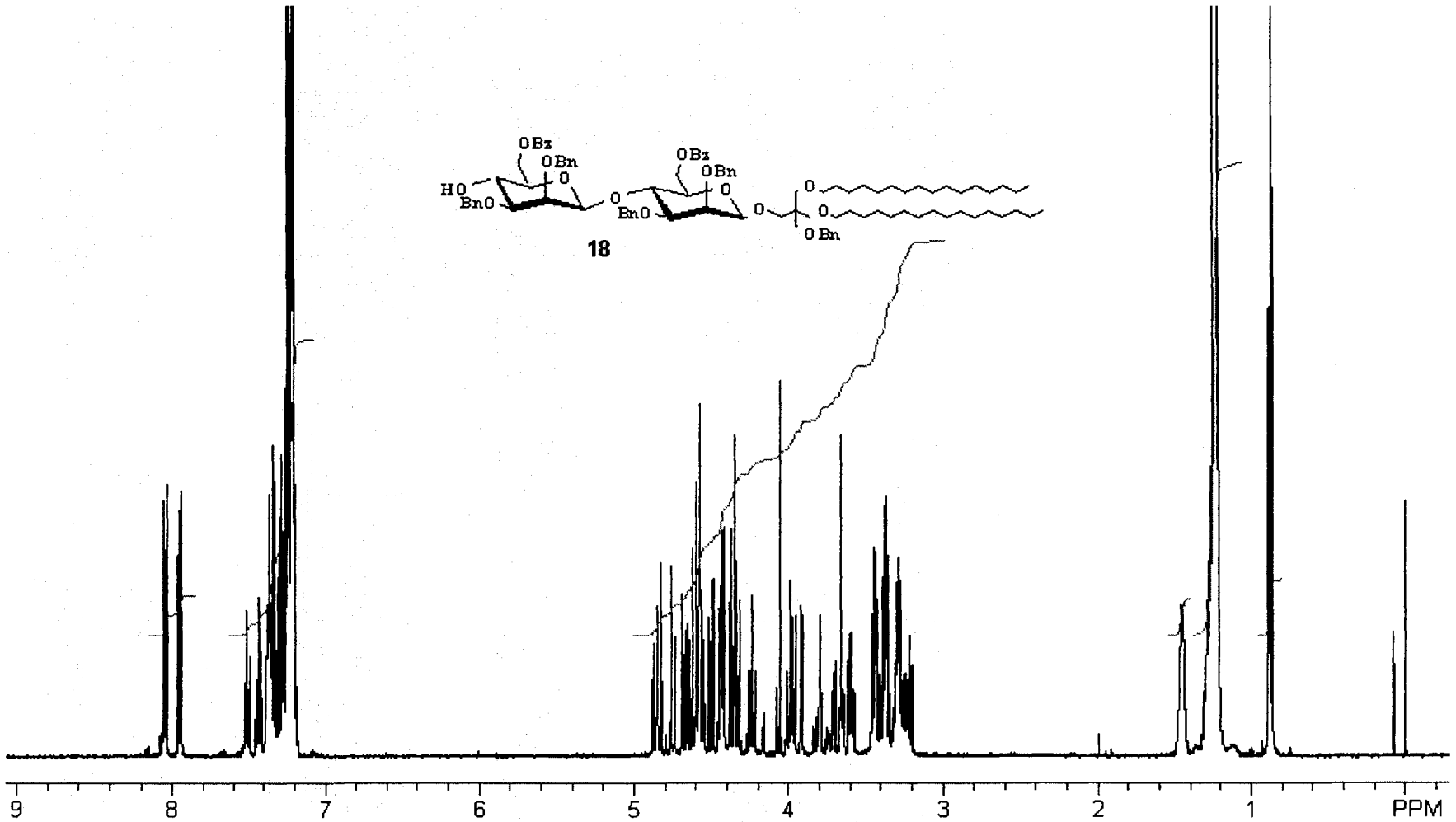


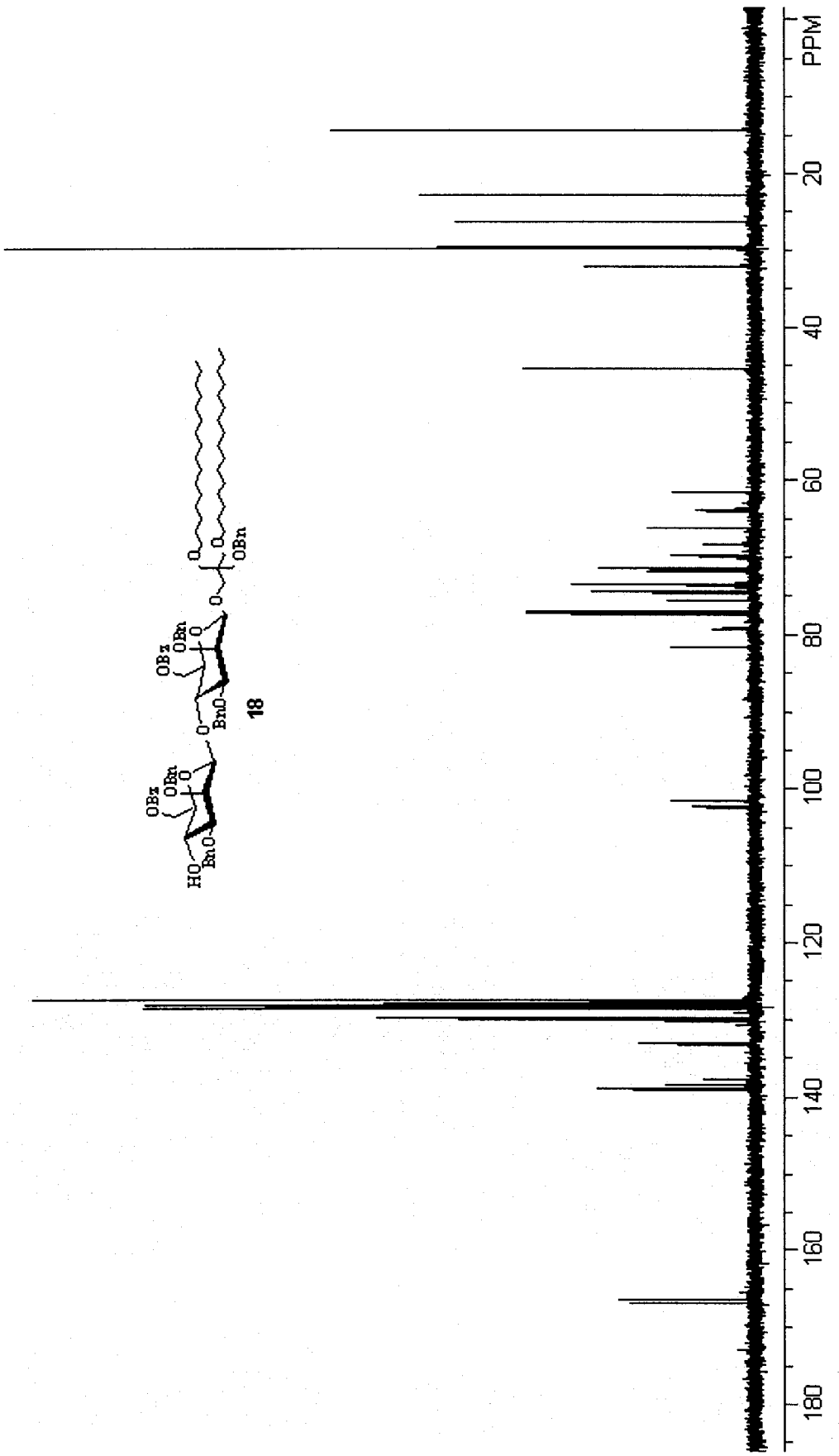


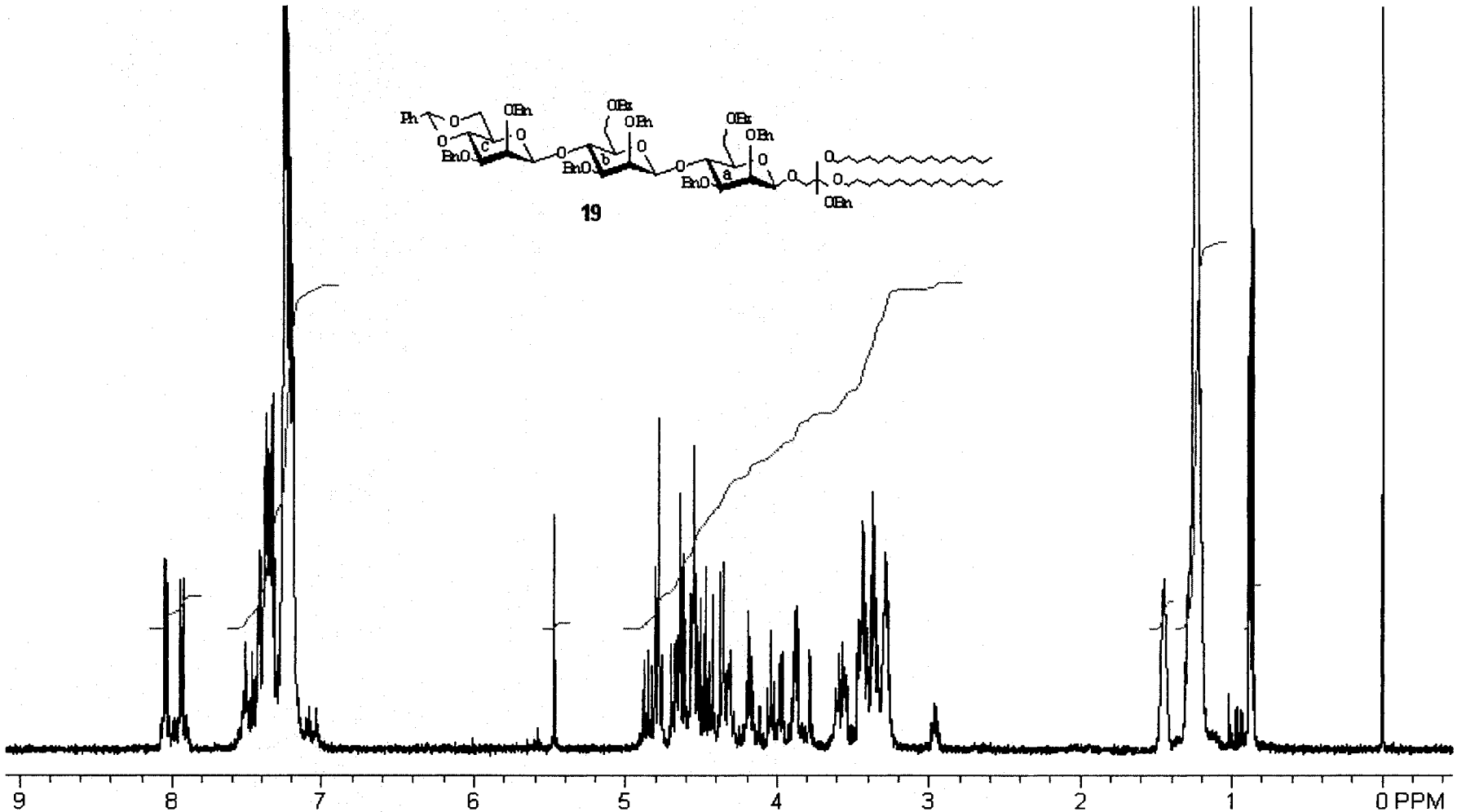


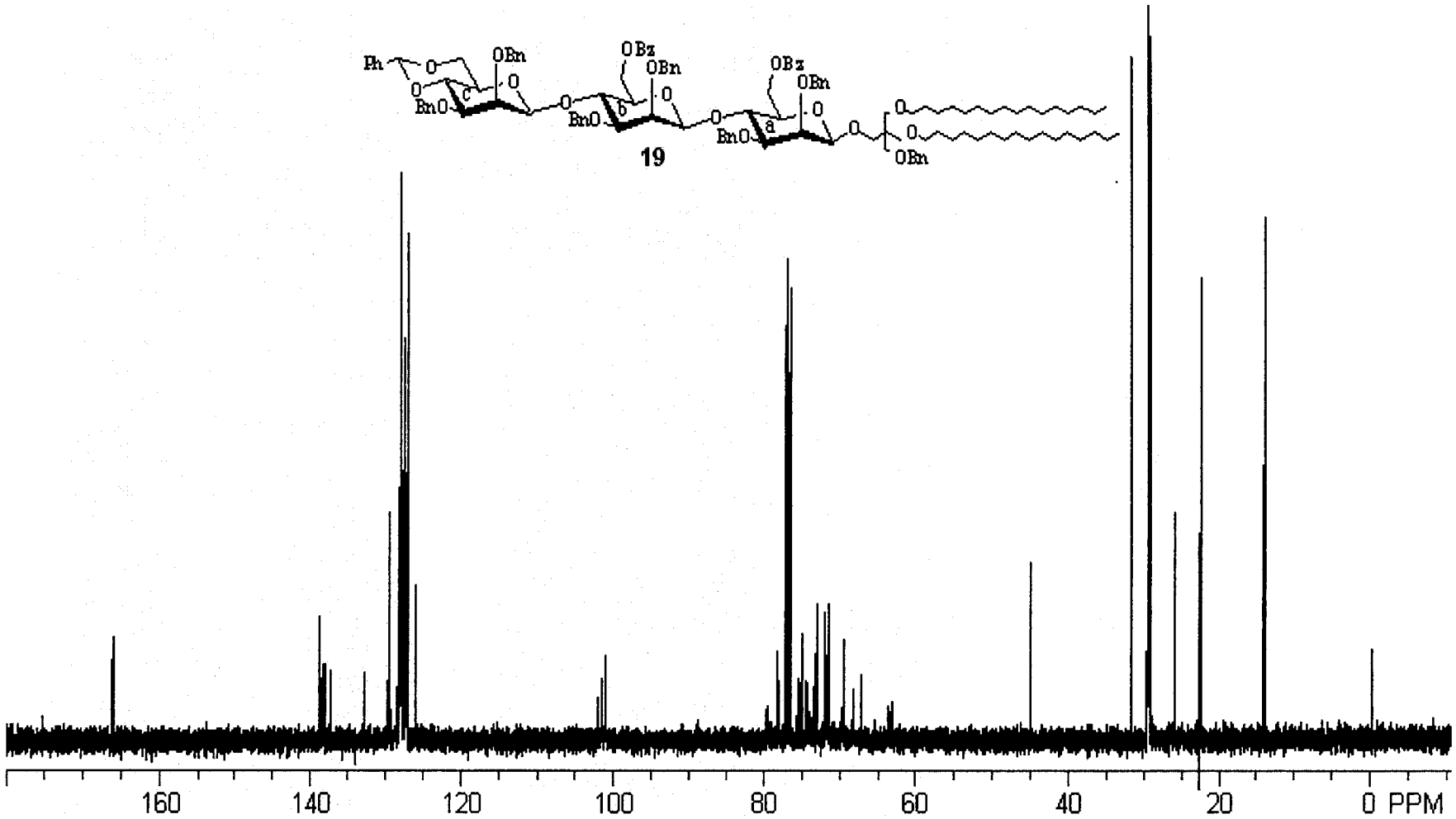


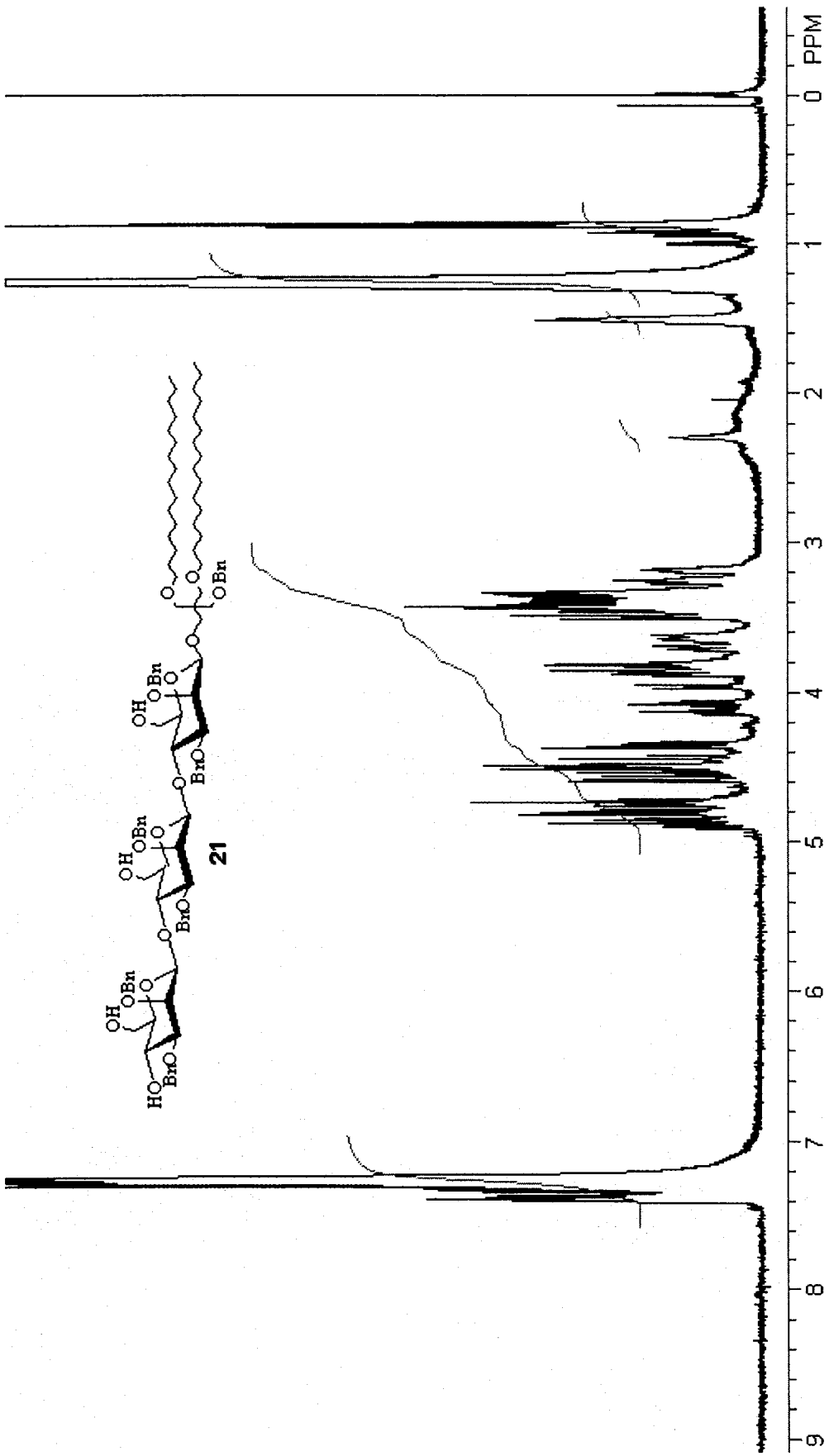


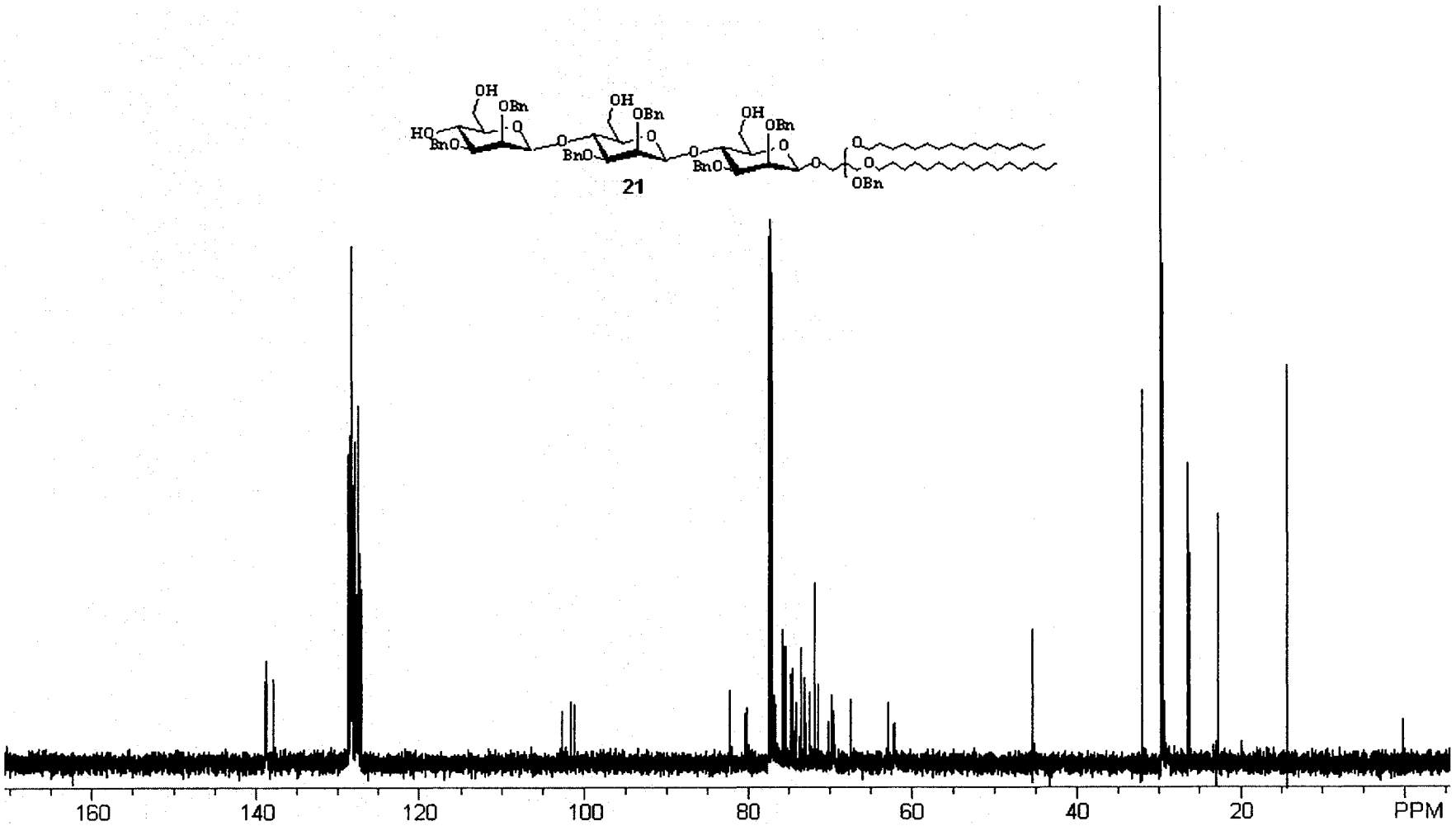


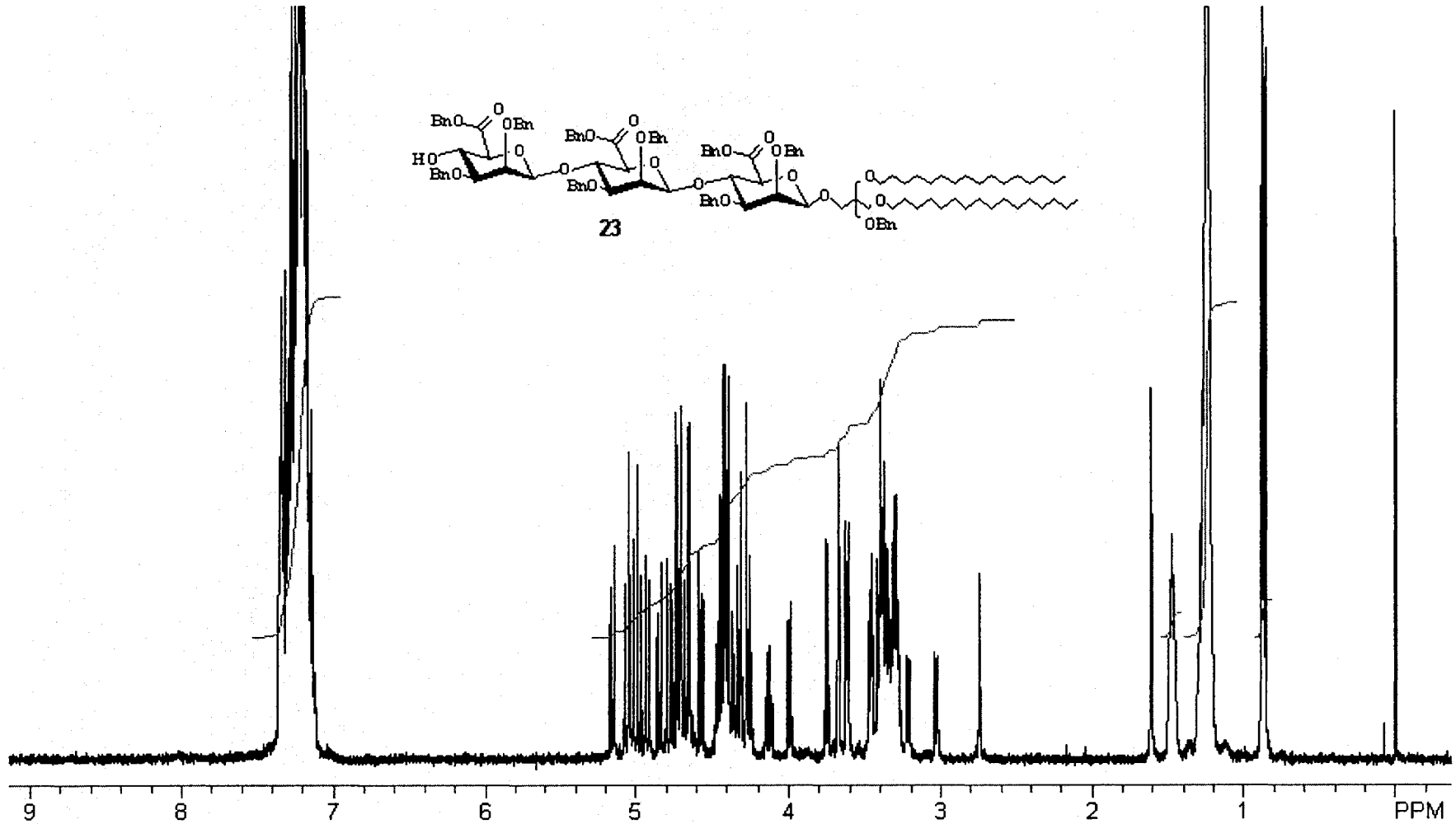


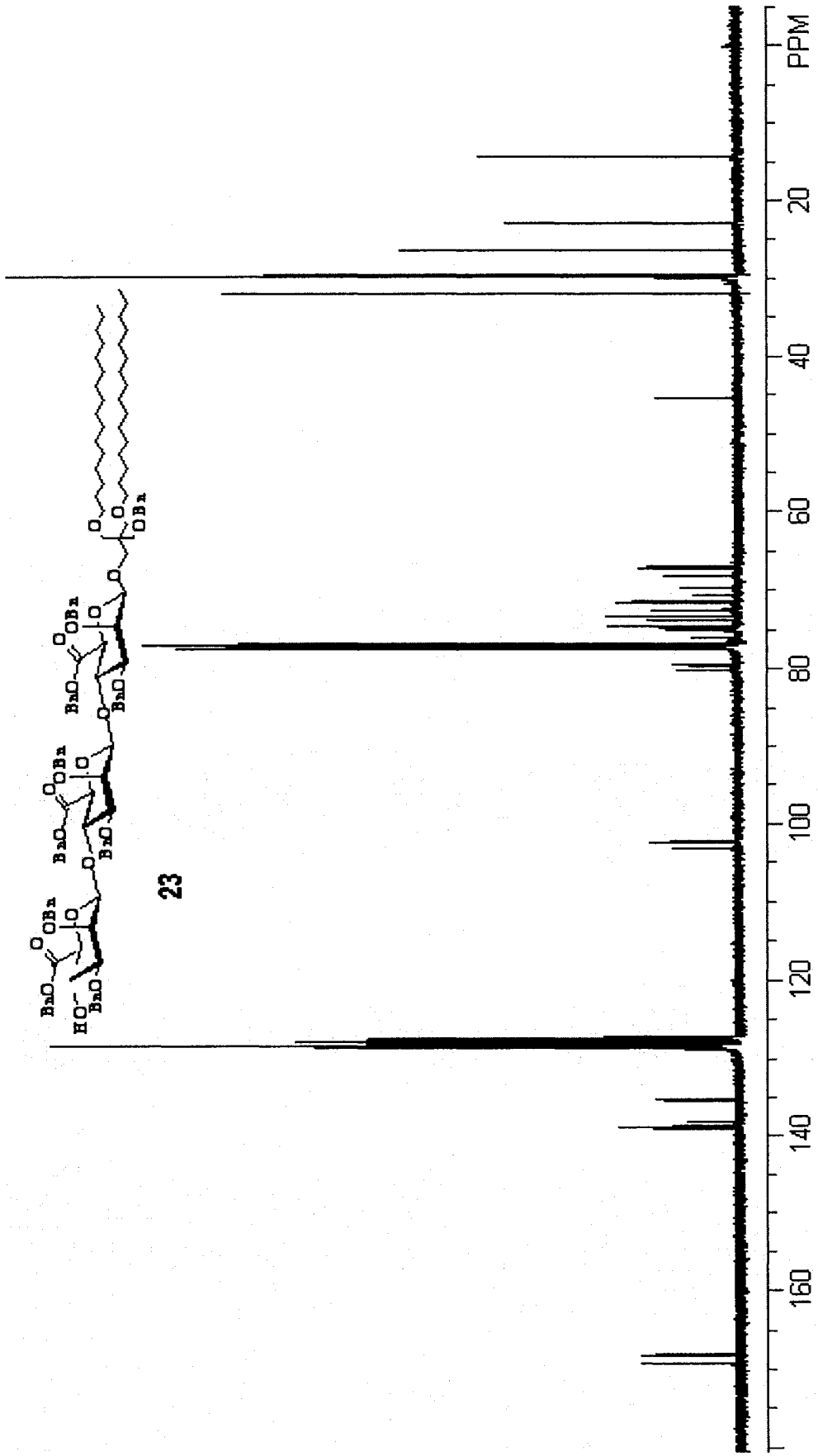




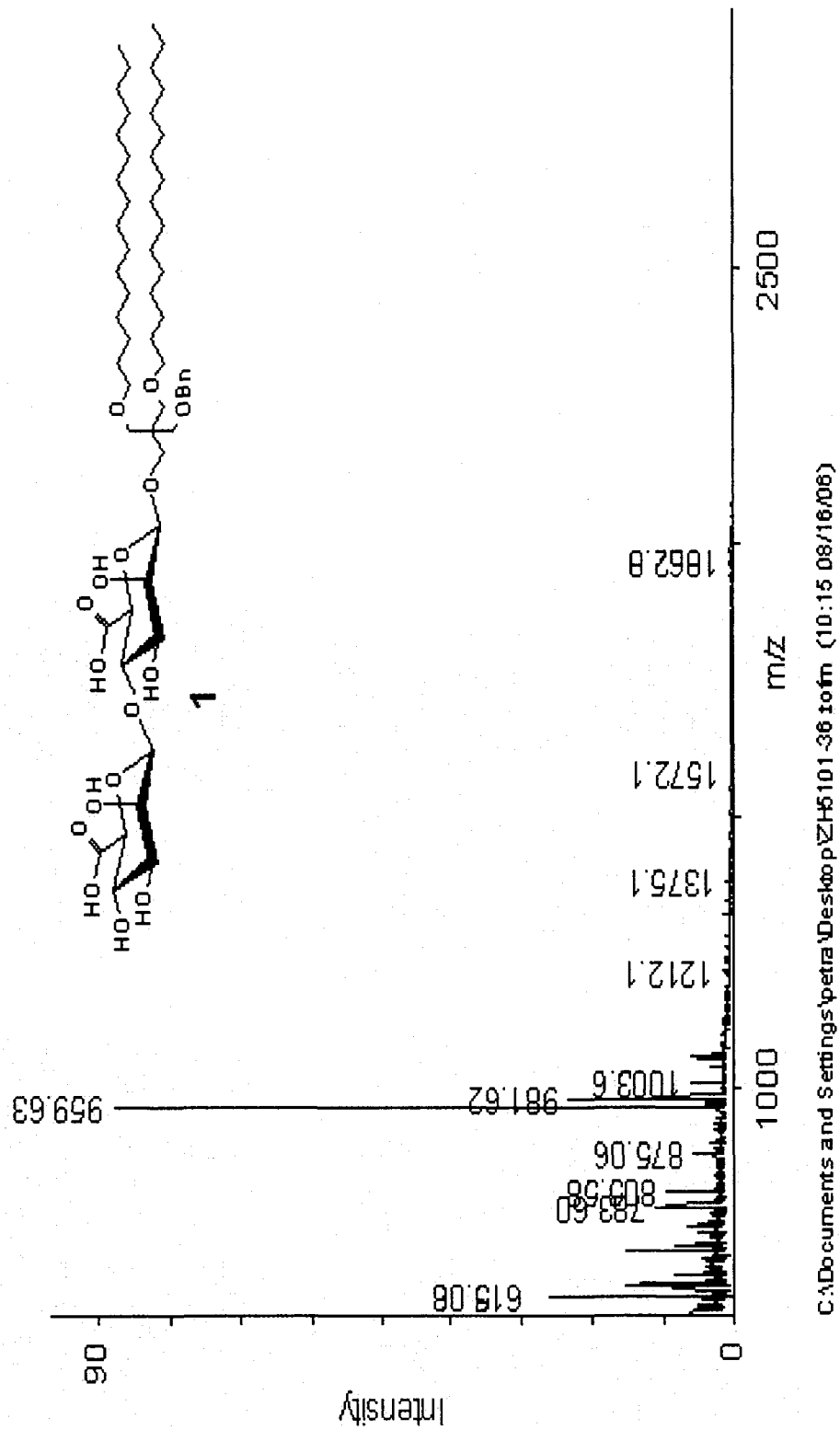




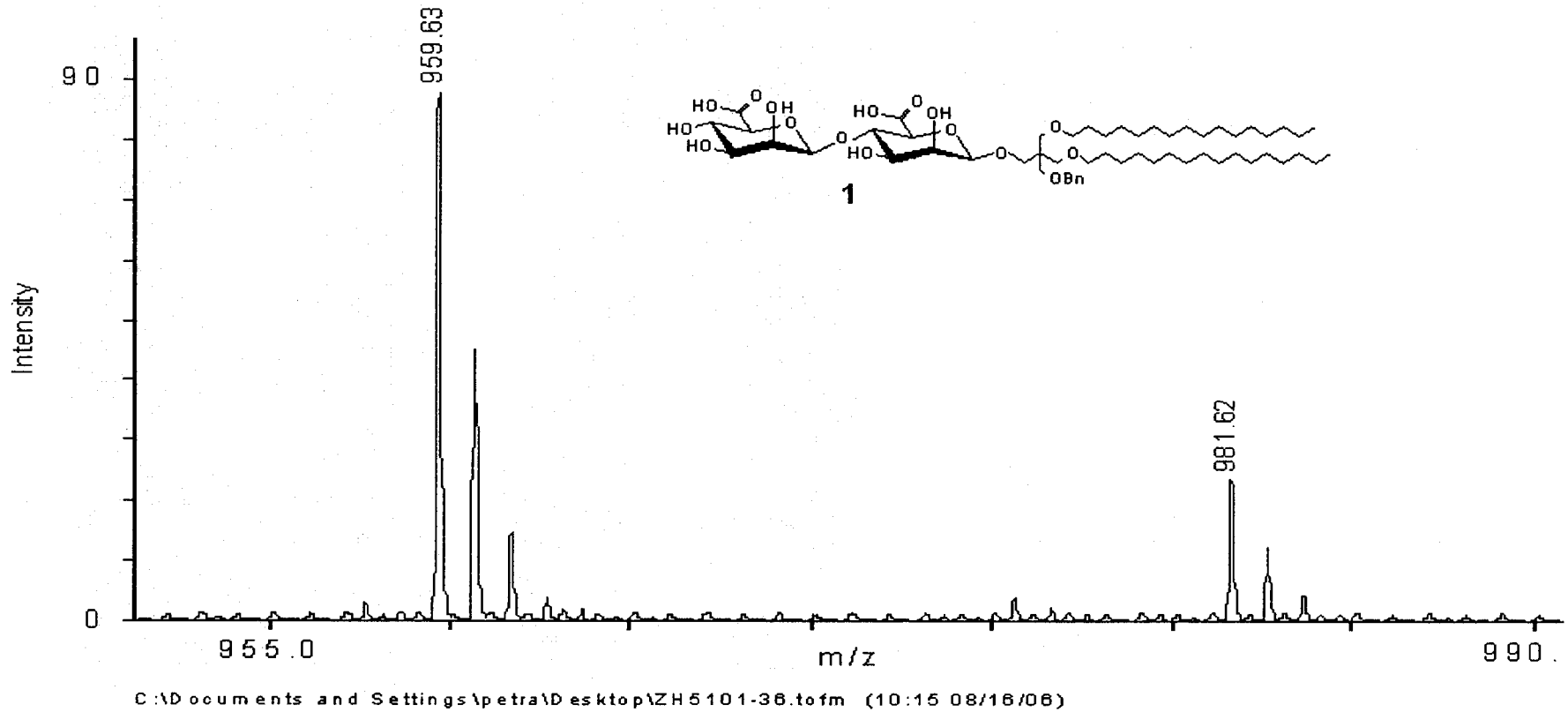




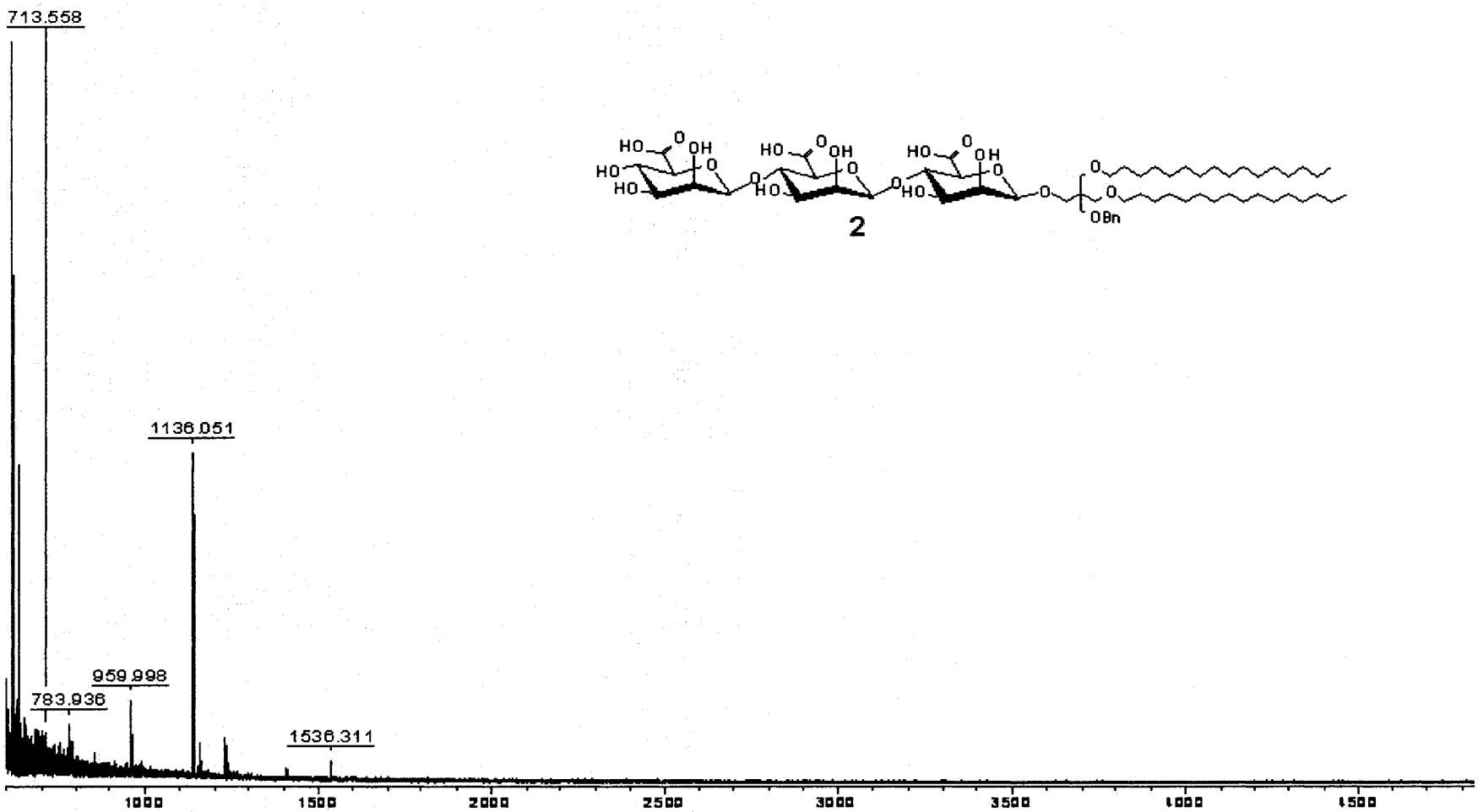
Positive mode MALDI-MS : calcd for $C_{49}H_{92}O_{16}$ 936.64$[M]^+$, found 959.63 $[M+Na]^+$, 981.62 $[M-H+2Na]^+$



Positive mode MALDI-MS : calcd for $C_{49}H_{92}O_{16}$ 936.64$[M]^+$, found 959.63 $[M+Na]^+$, 981.62 $[M-H+2Na]^+$



Positive mode MALDI-MS : calcd for $C_{55}H_{100}O_{22}$ 1112.67$[M]^+$, found 1136.05 $[M+Na]^+$, 1158.06 $[M-H+2Na]^+$



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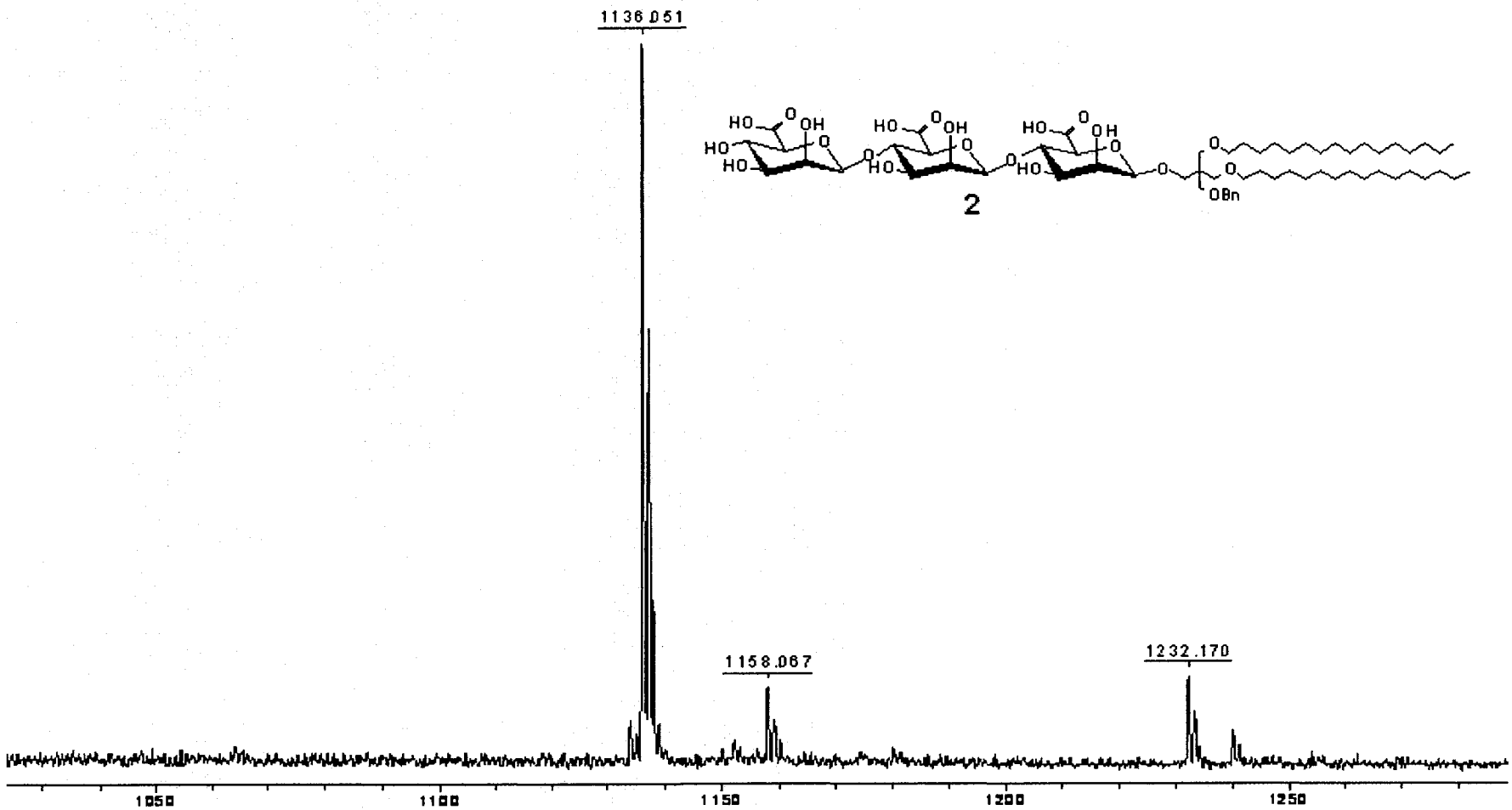
Positive mode MALDI-MS : calcd for $C_{55}H_{100}O_{22}$ 1112.67$[M]^+$, found 1136.051 $[M+Na]^+$, 1158.067 $[M-H+2Na]^+$



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