8-2005

Synthesis of a C-Linked Methyl β-Disaccharide as a Building Block of Hyaluronic Acid Oligosaccharide Mimetics

Kpakpo Ambroise Akue
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a dissertation written by Kpakpo Ambroise Akue entitled "Synthesis of a C-Linked Methyl β-Disaccharide as a Building Block of Hyaluronic Acid Oligosaccharide Mimetics." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Chemistry.

David C. Baker, Major Professor

We have read this dissertation and recommend its acceptance:

Richard Pagni, Ziling Xue, Marianne Breinig

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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and recommend its acceptance:

Richard Pagni

Ziling Xue

Marianne Breinig

Accepted for the Council:

Anne Mayhew

Vice Chancellor and Dean of Graduate Studies

(Original signatures are on file with official student records.)

SYNTHESIS OF A C-LINKED METHYL β-DISACCHARIDE AS A BUILDING BLOCK OF HYALURONIC ACID OLIGOSACCHARIDE MIMETICS

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Kpakpo Ambroise Akue

August 2005

DEDICATION

This dissertation is dedicated to my family:

To Kaila B. Akue, Adolphe A. Akue, Georgette A. Akue, Olga A. Gozo, Andrew T. Gozo, Olivia D. Gozo, Hughes A. Akue, Emily A. Akue, Gaudy Akue, Annette Akue, Vincent Akue, Clement Akue, Mathias Akue, Euloge A. Akue, Emmanuel K. Akue, Eric K. Akue, Randy Mitchell, Joyce Mitchell, Mandy Mitchell, Jeff Mitchell, Wonder Mitchell, Morgan Mitchell, Joshua Mitchell and Abigail Mitchell

ACKNOWLEDGEMENTS

I would start by expressing my gratitude to Dr. David C. Baker, my research advisor, for his sound guidance, patience and unwavering encouragement. I sincerely appreciate the opportunity that I have had to work under his guidance, for all he taught me about being a synthetic chemist, about determination and perseverance. I also thank Dr. Richard M Pagni, Dr. Zilling Ben Xue, and Dr. Marianne Breinig for their willingness to serve on my committee and for their priceless advice, especially Dr. Zilling Ben Xue for believing in me from the very beginning. I am indebted to Dr. Karen T. Welch who run the 2D NMR spectra, Dr. Benji S. Prebyl who acquired some of the mass spectra, to Dr. Sean K. Hamilton, Dr. Qiang Yang, Conrad Kaczmarek, Samson Francis, Riyam Kafri, Medhanit Batha and Julio Guitterrez who gave me valuable advice, support, encouragement as well as their precious friendship. I am also grateful to several past and present members of the Baker group especially, Chao Wang, Dr Mai Li, Dr. Zhong-Xu Ren, Dr. Sarah Hendrix, Dr. David White, Tony Condo, Dr. Neil Whittermore for their much appreciated assistance and amity. I give thanks to the University of Tennessee, Knoxville particularly the Department of Chemistry and the National Institutes of Health (NIH) for supporting me financially. Finally and most importantly I'm grateful for my family: my wife for her patience, love, understanding, and help through though times, to my parents for their considerable and unfailing support, to my siblings and their spouses for their assistance and encouragements, to my uncles, aunts, cousins, in-laws for their

tremendous moral support, and to my niece whose smile and enthusiasm for life motivate me in down times.

ABSTRACT

Hyaluronic acid is a viscous high MW polysaccharide that is synthesized in the plasma membrane. It is found in the connective tissue space, in the synovial fluid of movable joints and in the vitreous humor of the eye. Its chemical structure is composed of a repeating disaccharide unit in which D-glucuronic acid is linked to the 3-position of *N*-acetyl-D-glucosamine. It appears to play an important role in the metastasis of cancer cells since cancer cell's CD44 receptors adhere to it and migrate to host cells. Our goal was to synthesize small HA-oligosaccharides that would behave as CD44 metastasis receptor antagonists. However, HA is degraded in the liver, in the lymph nodes, as well as in local tissues by acid hydrolases. Several lines of evidences have established that replacing the glycosidic –O– linkages with $–CH_2–$ linkages provides resistance to enzymatic cleavage. Since synthesizing an all $–CH_2–$ linked oligosaccharide is an arduous task and virtually intractable, it was decided to design hydrolase-resistant HA mimics by substituting the interglycosidic oxygen atom with the CH_2-group at strategical positions. In this dissertation is described the synthesis of a $–CH_2–$ linked disaccharide to a methyl β-analog related to HA that can serve as a building block for the synthesis of long-chain, hydrolase-resistant oligosaccharide mimics. The *C*-disaccharide was acquired from coupling a glycosyl tin derivative with an aldehyde stemming from methyl β-D-glucopyranoside.

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ABBREVIATIONS AND ACRONYM

Ac	Acetyl
AcOH	Acetic acid
ADMA	Anisaldehyde dimethyl acetal
AIBN	2,2'-Azobisisobutyronitrile
All	Ally
Bn	Benzyl
n-Bu_3SnH	Tri-*n*-butyltin hydride
CAN	Ceric ammonium nitrate
CD	Clusters of differentiation
COSY	Correlated spectroscopy
m-CPBA	*m*-Chloroperoxybenzoic acid
DIBAL-H	Diisobutylaluminum hydride
DMF	Dimethylformaldehyde
DMSO	Dimethyl sulfoxide
ESIMS	Electrospray-ionization mass spectroscopy
GAG	Glycosaminoglycan
GlcA	D-Glucuronic acid
GlcNA	*N*-Acetyl-D-glucosamine
HA	Hyaluronic acid
HSQC	Heteronuclear single-quantum coherence

MS	Molecular sieves
NMR	Nuclear magnetic resonance
Pet Ether	Petroleum ether
PMB	*p*-Methoxybenzyl
Pyr	Pyridine
TEA	Triethylamine
TEMPO	2,2,6,6–tetramethyl–1–piperidinyloxy
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TOCSY	Totally correlated spectroscopy
TsOH	*p*-Toluenesulfonic acid
UDP-GlcA	Uridine-5'-diphosphoglucuronic acid
UDP-GlcNA	Uridine-5'-diphospho-*N*-glucosamine

I. INTRODUCTION

Hyluronan (HA, also referred to as hyaluronic acid) is a natural high molecular mass polysaccharide, ranging from about 10^5 to 10^7 Da. Its structure reveals an acidic glycosaminoglycan (GAG) made entirely of a repeating disaccharide unit, in which D-glucuronic acid is linked to the 3-position of *N*-acetyl-D-glucosamine, *i.e*. $[\beta\text{-D-GlcNAc-}(1\rightarrow4)\ \beta\text{-D-GlcA-}(1\rightarrow3)]_n$ (Figure 1).[1,2] It is the simplest of the GAGs, the only one not covalently linked to a core protein, not synthesized by way of the Golgi pathway, and the only non-sulfated GAG.[3-6] HA exists in vivo as a polyanion,[7] encompassing a large volume of water that expands extacellular space, hydrates tissues, and in the dermis is responsible for skin moisture. HA regulates water balance and osmotic pressure, and acts as an ion-exchange resin. HA is found in the highest concentrations in typical connective tissues such as umbilical cord, synovial fluid, skin, and vitreous humor of the eye where it acts as a lubricant, a shock absorber, and as a structural molecule.[8] Notable amounts are also present in lung, kidney, brain, and muscle but very little is found in the liver. The lowest concentration is found in blood serum.[7] HA is synthesized in the plasma membrane[7,9] by a single enzyme, a dual-headed transferase that utilizes alternately the two uridine-5'-diphosphosugar substrates (UDP-glucuronic acid and UDP-*N*-acetylglucosamine).[10,11] These add sugar units from nucleotide precursors to the chain by an unknown mechanism on the cytoplasmic aspect of the

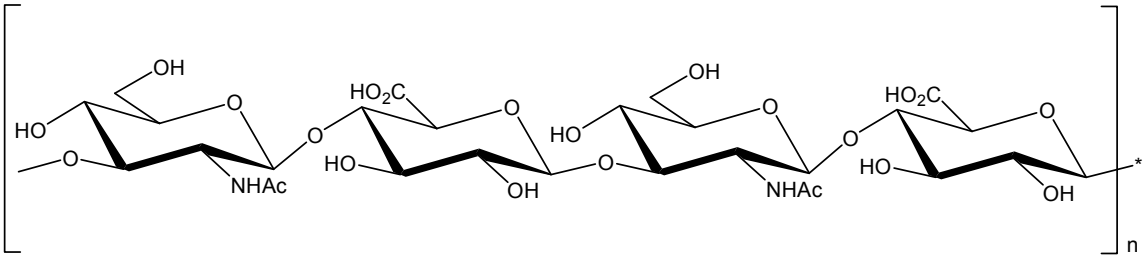


Figure 1. Hyaluronic Acid (HA).

membrane and translocate the growing chain to the pericellular space. The growth of the chain occurs at the reducing end.[7] There are three synthase genes in the mammalian genome, coding for Has-1, Has-2, and Has-3 that differentially regulate the size of the polymer.[3] Newly synthesized hyaluronan is extruded directly onto the cell surface; it is either retained there by sustained attachment to the synthase or by interactions with receptors, or it is released into the pericellular and extracellular matrices.[12] Hyluronan participates in fundamental processes such as embryological development and morphogenesis, wound healing,[13,14] repair and regeneration, and inflammation. [15-17] HA levels increase in response to severe stress, and in tumor progression and invasion. It has been implicated in the development and progression of cancer.[18,19]

A. Biological Background

The presence of HA is vital to cell division and migration during normal development. HA has also been shown to have a role in immune cell adhesion and activation, intracellular signaling, cellular growth and migration. Although these properties contribute to normal tissue development and function, in cancer HA may promote tumor growth and metastasis. Numerous studies performed over the past three decades have demonstrated a correlation between levels of hyaluronan production and malignancy in several types of tumor, both in animal models and in human patients.[19-22] In the case of human breast, ovarian, and colon carcinomas, a high level of hyaluronan associated with cancer cells

themselves or with the tumor stroma is a reliable prognostic indicator of patient morbidity.[23-25] Furthermore, studies on histological sections from various tumors, using a specific hyaluronan affinity probe, have indicated that virtually all human epithelial tumors are surrounded by a connective tissue matrix (stroma) enriched in hyaluronan.[19] The recurrence rate of colon carcinoma after an operation increases from 20% to 80% with increasing levels of hyaluronan associated with the carcinoma cells.[23] Likewise, elevated levels of hyaluronan and hyaluronidase in the urine form a clinically reliable marker for the presence and grade of bladder cancer.[26]

The ancient Greeks called the disease cancer because they likened its gross appearance to the outwardly extended claws of the crab.[27] Indeed, the observation that cancers extend into adjacent host tissues, invade blood vessels, and metastasize has been known since the early 19th century. The spread of malignant tumors culminating in the establishment of one or more secondary tumors at a remote site is called metastasis.[28] Metastasis is the major cause of morbidity and death for cancer patients since they succumb to multiple secondary tumor growths and not necessarily to the primary tumor.[29,30] Tumor invasion by melanoma B16F10, for instance, consists of seven steps: The process begins with a transforming event (1), which allows local growth (2) and invasion of malignant cells. These ultimately break through the basement membrane and invade blood vessels (3). Once in the blood the malignant cells are carried to the heart (4). Lodgment of the malignant cells in the lungs ensues (5), but exactly how the lodgment occurs is unclear. Following lodgment, the

malignant cells extravasate (6) with enzymatic dissolution of the basement membrane,[31] and under appropriate conditions the cells divide to form visible metastatic lesions (7). [32] Several types of malignant solid tumors contain elevated levels of hyaluronan.[33] The ability of a rabbit carcinoma to invade surrounding tissue has been shown to correlate with its ability to induce formation of hyaluronate in situ,[34] and a highly invasive bladder carcinoma was found to express high levels of hyaluronate binding activity.[34] There is growing evidence that hyaluronan–CD44 interactions may enhance tumor growth and metastasis.[35]

CD44, a multifunctional transmembrane glycoprotein, is currently thought to be the principal cell-surface receptor for hyaluronan.[36-39] Interaction between cell-surface CD44 and HA is proposed to mediate, at least in part, a variety of cellular functions, including cell migration[40], cell–matrix adhesion,[34,41-43] cell–cell adhesion,[44] lymphocyte activation,[45,46] and hematopoiesis.[47,48]

CD44 is highly polymorphic due to alternative splicing of at least 10 exons encoding a segment of the membrane proximal extracellular domain[49-51] and variable N- and O-linked glycosylation.[52-55] Multiple distinct CD44 isoforms are generated by incorporation of different combinations of the variable exons, which provide new oligosaccharide attachment sites resulting in potentially significant glycosylation changes.[54-57] Multiple studies have indicated that malignant cells undoubtedly use these isoforms to facilitate migration, thus spreading cancerous tumors. These facts have inspired the conception of a blueprint for the interactions between HA and cell-surface receptors like CD44 in metastasis. It is described as follows: Migrating cancer cells exude growth factors that incite

neighboring cells to increase production of extracellular HA. Migrating cancer cells then make use of their CD44 receptors to bind to and migrate along the newly synthesized HA tracks into new tissues, consequently extending malignancy to new tissues (Figure 2). This current design could lead to a novel therapeutic approach, based on the introduction of a CD44 receptor antagonist that would adeptly prevent a malignant cell from binding to HA. Unlike several conventional strategies that consist of cytotoxic agents that target the cell cycle, this approach would be acutely beneficial in cases where the tumor is highly metastatic or the primary tumor is untreatable.

Almost all patients with advanced cancer will receive chemotherapy at some point during the treatment of their disease. The principal limitations of chemotherapy in treating cancer include: treatment-related toxicities, inability to selectively target tumor tissue, and the development of resistance to the cancer-killing effects of chemotherapy. These lead to the need to design new treatment strategies that include the use of antiangiogenesis, anti-invasion and antimetastasis agents, both separately and particularly in conjunction with established antitumor agents.

B. Biological Evaluation of HA Oligosaccharides as Antimetastasis Agents

Several studies[58] have led us to conclude that the early steps in the metastatic process, from the time the cancer cells enter the bloodstream until

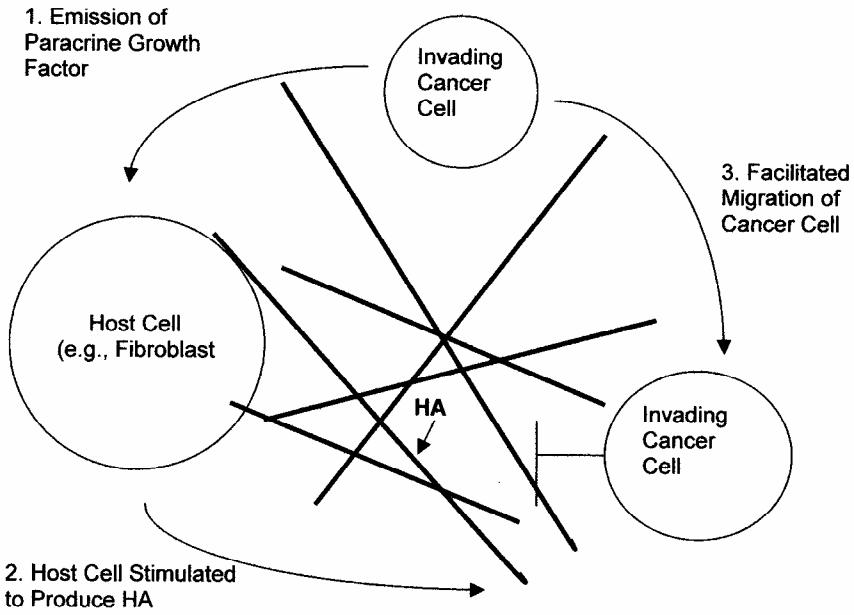


Figure 2. Blueprint for the interactions between HA and CD44 in cancer metastasis.

they extravasate into secondary organs, are completed very efficiently. By contrast, subsequent steps in the metastatic process are completed inefficiently, with only a small subset of cancer cells in a secondary site initiating cell division to form micrometastases, and only a small proportion of these micrometastases progressively growing into macroscopic metastasis. Therefore targeting a very inefficient biological process might be easier than targeting an efficient one. Furthermore, metastasis can rise long after the apparently successful treatment of a primary tumor. In breast cancer or melanoma, for example, metastases have been known to occur decades after primary treatment.[59,60]

Any therapy that prevents the progression of metastases to form clinically damaging tumors has the potential to benefit patients. Based on this premise, on several studies that support the blueprint for the interactions between HA and cell-surface receptors like CD44 in metastasis mentioned earlier (Figure 2), and on Toole's[41,61] studies where treatment with HA oligosacchsrides suppressed the growth of tumors both in vitro and in vivo, suggesting that HA obtains new activities and functions after depolymerization, we propose to use HA oligosaccharides as CD44 metastasis receptor antagonists. This could be achieved by introducing small HA oligosaccharide molecules that might out compete the extracellular matrix HA for the limited number of CD44 receptor binding sites, resulting in cancer cells no longer attaching themselves to and traveling along HA tracks into new tissues (Figure 3). If this therapeutic approach were successful, it would involve either surgical removal or radiation on the primary tumor, then treatment with a CD44 antagonist that would prevent

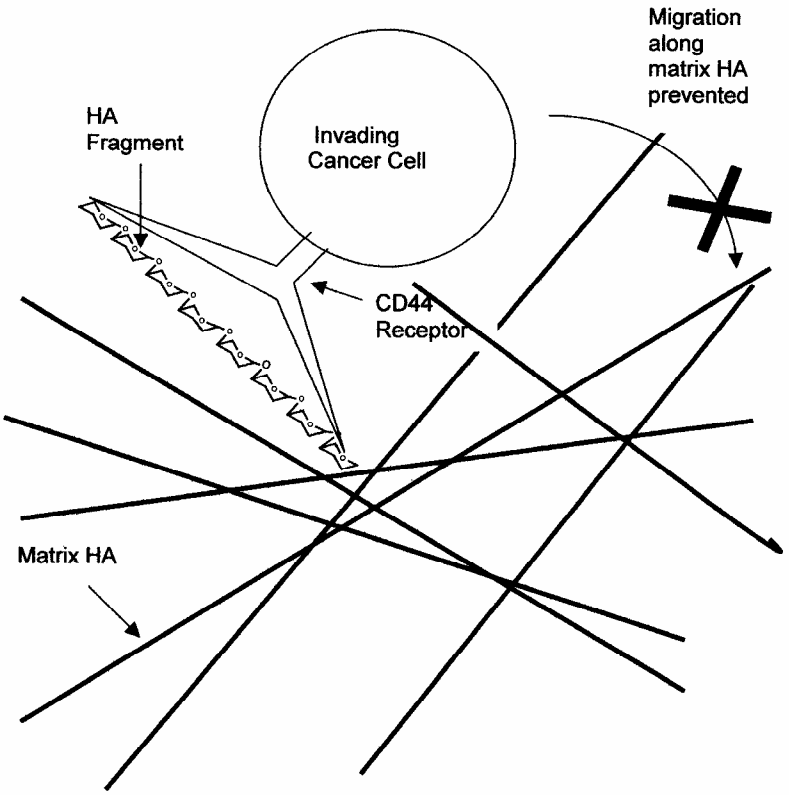


Figure 3. Theoretical prevention of metastasis through a CD44 receptor antagonist.

metastasis particularly in highly metastatic tumors. Besides, in cases where the primary tumor is untreatable, the antimetastasis approach could prolong patient lives. This idea involving non-HA type compounds such as ligand-mimicking drugs that efficiently block cancer-cell adhesion has been suggested for patients at high risk of metastasis.[62,63]

To test our hypothesis, a set of experiments was conducted by our collaborators, Dr. Richard Cysyk and co-worker[64], that can be summarized as follows: Malignant B16F10 melanoma cells were incubated in vitro (10^5 cell count) with a mixture of HA oligosaccharides (1 mg/mL) obtained from the enzymatic degradation of HA by hyaluronate lyase (EC 4.2.2.1) and injected in vitro into the tail vein of mice. In a control set of experiments, the mice were injected with only B16F10 melanoma cells (Figure 4). After ten days, the lungs of the mice, along with those of the control group were removed and examined for metastatic colonies. We expected a reduction in the metastatic sites on the lungs of the mice that were treated with B16F10 melanoma cells, premixed with HA oligosaccharides. Surprisingly, there was an almost total absence of metastatic lesions on these lungs, whereas the lungs of the control group displayed numerous tumors (Figure 5). To ascertain the genuineness of the results, the experiments were repeated using a variety of controls with identical results. In the sample pre-mixed with HA oligosaccharides, the viability of the B16F10 melanoma cells was checked as well as various other consequential factors. A check for toxicity, using double and triple the amount of HA oligosaccharides (up to 3 mg/mL), did not uncover any toxicity. These

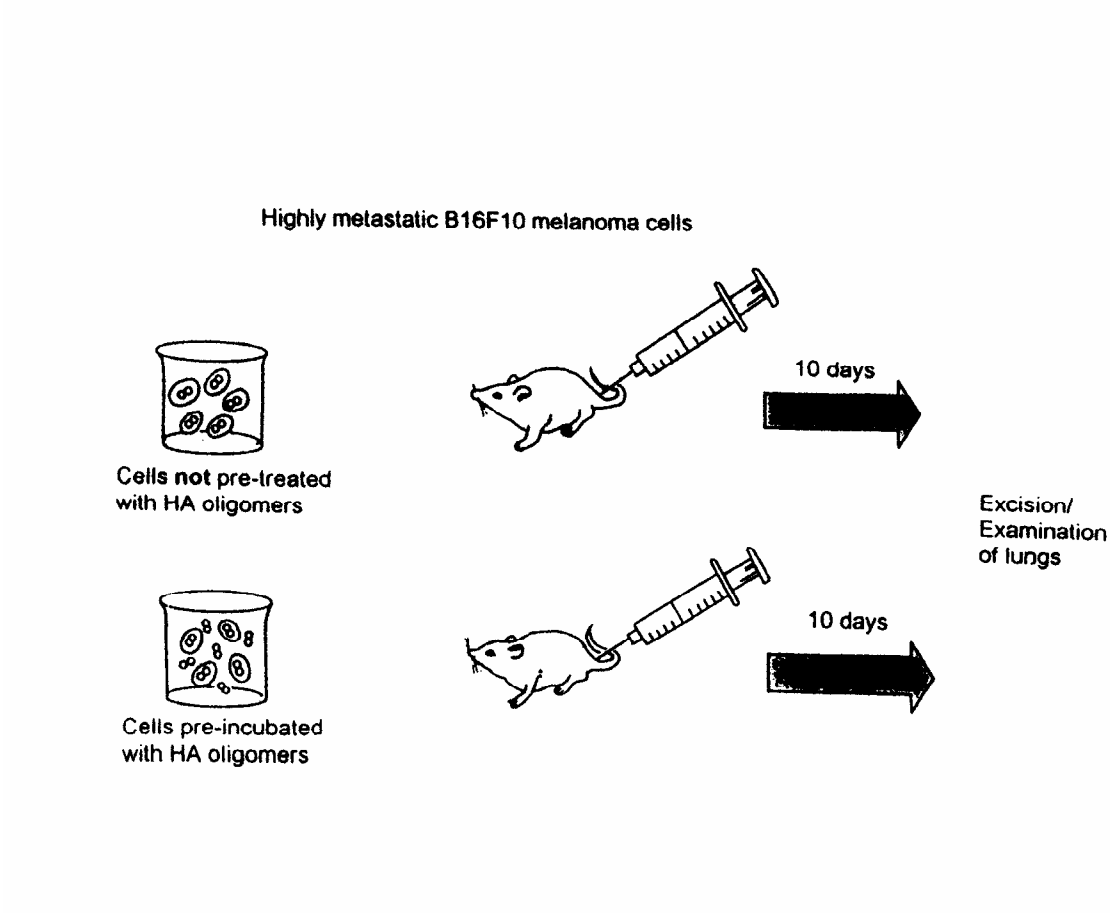


Figure 4. Mice injected with B16F10 melanoma cells.

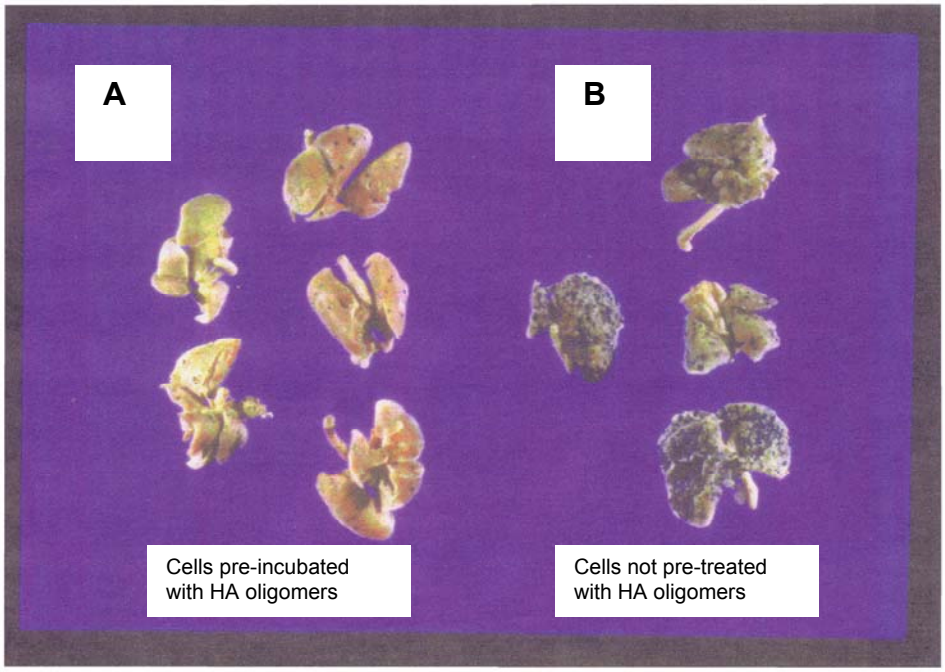


Figure 5. Excised mouse lungs showing prevention of B16F10 melanoma metastasis by HA oligosaccharides.

experiments confirm that small HA oligosaccharides show a powerful antimetastatic effect on B16F10 melanoma. While it is still premature to conclude that HA oligosaccharides were acting as CD44 receptor antagonists; the results provide strong evidence that a small molecule can behave as an antimetastatic probe, which is vital to our proposed research. Preliminary investigations show activity starting with the 8-mer (DP = 8).

C. Design of Hydrolase-Resistant Oligosaccharides Related to Hyaluronic Acid

To ensure proper cellular activity, HA levels in the body are finely regulated to control HA biosynthesis and degradation. A rapid turnover rate facilitates the use of HA and its products in many physiological regulatory mechanisms.[65] It has been estimated that about a third of hyaluronan in the human body is removed, and replaced each day. In many instances, removal is achieved by endocytic uptake, either within the tissue where it is made or in lymph nodes and the liver.[7] The catabolic rate of hyaluronan greatly varies between tissues.[66] Hyaluronan undergoes fragmentation in the presence of reactive oxygen species (free oxygen radicals), which can enhance hyaluronan turnover.[67,68] The molecular size of HA appears to be closely related to its biological activity.[69-73] Although HA usually exists as a high molecular mass polymer (in excess of 10 kDa) as a component of the extracellular matrix under physiological conditions,[74] HA of a much lower molecular mass, as a result of

enzymatic degradation, is detected in association with certain pathological conditions, such as inflammation[75] and tumors.[76-79] Furthermore, during tumor progression, HA that has been degraded into oligosaccharides has been reported in urine, blood or tumor tissue specimens of the patients with tumors.[80] There is strong evidence to indicate that HA is degraded in the local tissues where it is produced, in the lymph and in the liver.[81,82] Hyaluronidase is the enzyme responsible for HA degradation in bacteria and in mammalian systems.[83-88] The hyaluronidases fall into three classes[89] based on analyses of their reaction products: (1) Bacterial hyaluronidases (EC 4.2.99.1) are endo-β-acetyl-hexosaminidases that function as eliminases yielding disaccharides. (2) Endo-β-glucuronidase types of hyaluronidase (EC 3.2.1.36) found in leeches, crustaceans[90] and some parasites, generate tetra- and hexasaccharide end-products. (3) The mammalian types of hyaluronidase (EC 3.2.1.35) are endo-β-acetyl-hexosaminidases, but function as hydrolases, with tetrasaccharides as the predominant end-products.[3] The enzyme hyaluronidase acts together with two exoglycosidases (β-glucoronidase (E.C. 3.2.1.31) and β-*N*-acetyl glucosaminidase (E.C. 3.2.1.30)) and removes sugars sequentially from the non-reducing end of the polymer[91] (Figure 6). A considerable amount of evidence indicates that substituting the glycosidic −O− linkages with $-CH_2-$ linkages render oligosaccharide impervious to enzymatic or chemical hydrolysis.[92-96] It is thought that O to CH_2 substitution should lead to an alteration in both the size and electronic properties around the glycosidic linkage, eliminating the exoanomeric effect, thus allowing additional conformations around the

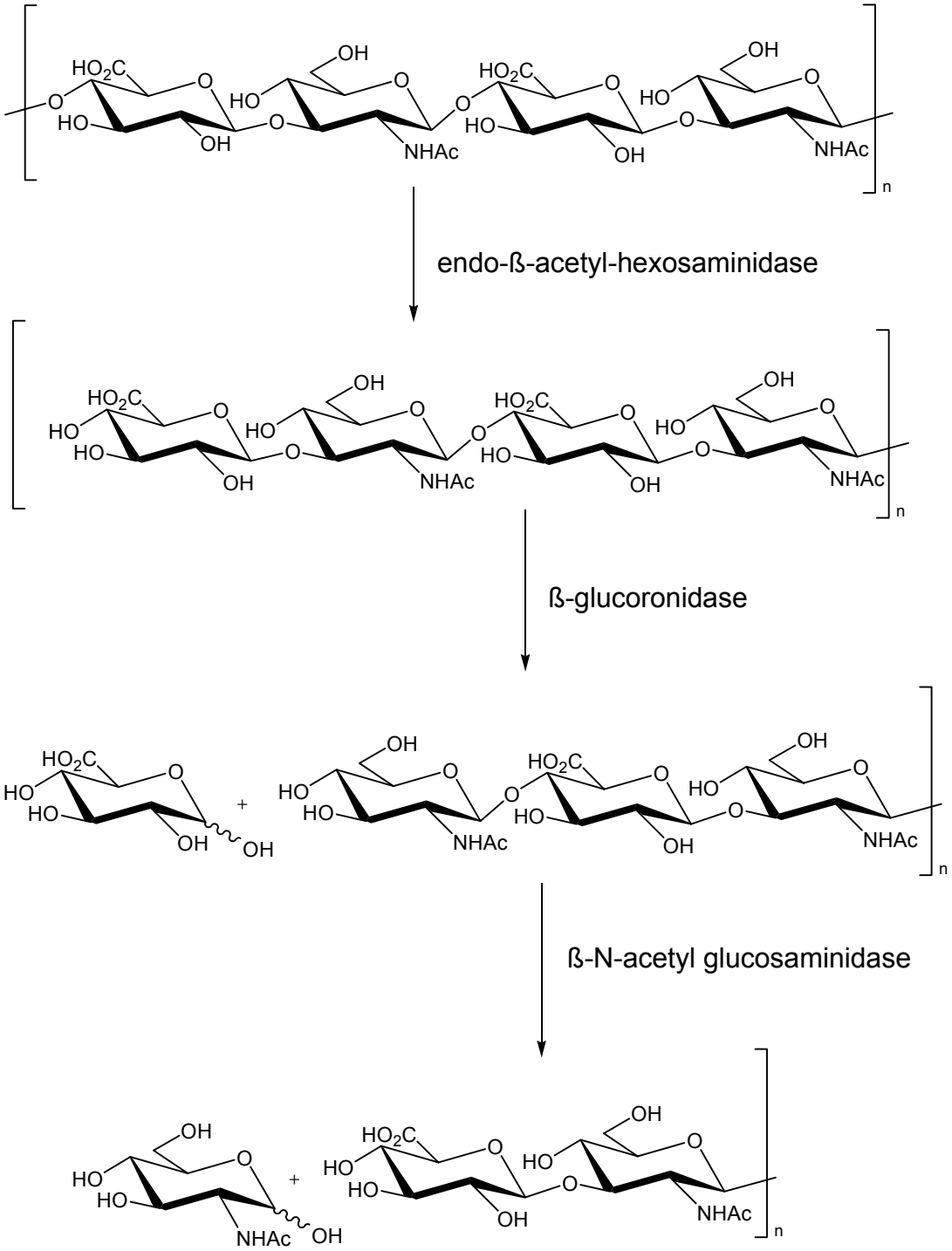


Figure 6. Enzymic degradation of HA.

interglycosidic linkage.[97] However, Sinaÿ et al. have shown that a mixed synthetic *C,O*-pentasaccharide[98,99] displays an antifactor Xa activity similar to that of the corresponding *O*-pentasaccharide. Kishi's group has demonstrated that *C*-trisaccharide analogs of the H-type II human blood group antigenic determinant are similar in both solution conformation and biological activity to their *O*-trisaccharide counterparts.[100,101] Furthermore, Sinaÿ et al. have reported the same results for *C*-disaccharides, *C*-trisaccharides, and *C*-tetrasacharides.[102,103] In addition, conformational similarity between $-CH_2-$ and −O− linked disaccharides has been proven.[103] Most of the published syntheses of linear *C*-saccharides have focused on the (1→6) coupling. The Sinaÿ group has achieved the synthesis of a *C*-β-(1→6)-tetrasaccharide by the addition of a C-6 acetylide anion to anomeric lactone followed by stereoselective reduction[102]. Dondoni et al. relied upon a iterative Wittig-based approach to make a *C*-β-(1→6)-pentasaccharide.[104,105] The *C*-β-(1→2)-β-(1→4)-trisaccharide synthesis by the Kishi group[100] remains the highest order of its kind[106,107] so far, because the level of difficulty increases with the length of the chain. Synthesizing an all $-CH_2-$ linked oligosaccharide is an overwhelming endeavor; therefore, we proposed to synthesize a "mixed" oligosaccharide, in which the −O− linkages susceptible to hydrolytic degradation are replaced with $-CH_2-$ in order to make the oligosaccharides hydrolytically stable. This "mixed" oligosaccharide can easily be built through a confluent block synthesis approach where multiple units of a single C-linked disaccharide (GlcNAc-GlcA) are brought together, via O-linked coupling, n times to produce oligosaccharide of a specified length

(Figure 7).

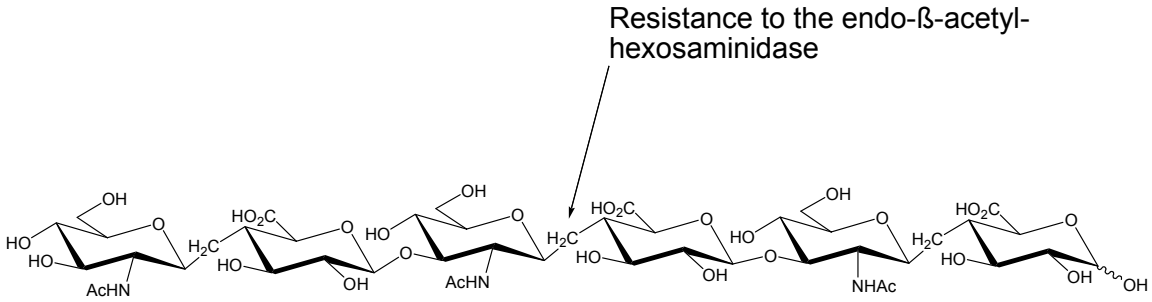


Figure 7. Hydrolase-resistant hexasaccharide.

II. STATEMENT OF THE PROBLEM

A. Objectives

This dissertation will focus mainly on the design and the synthesis of a C-linked methyl β-disaccharide unit that would serve as a building block of the higher order C-/O- linked HA oligosaccharide mimics. This disaccharide unit turns out to be very challenging to make; however, the small size of the group (methyl) at the anomeric position and its β position render it very valuable for NMR (nuclear magnetic resonance) spectroscopy and molecular modeling studies.

B. Retrosynthetic Analysis

Our ultimate goal is the synthesis of a "mixed" oligosaccharide that will be assembled from a single disaccharide intermediate, GlcNAc−X−GlcA (where X= $-CH_2-$) that is strategically protected so as to serve as acceptor having the 3'-hydroxy group free or as a C-1 glycosyl donor (Figure 8). The coupling of these two disaccharides will be achieved via Schmidt's trichloroacetimidate glycosylation. In addition, the GlcA moiety will be selectively protected in order to allow oxidation of the 6−OH to the carboxylic acid. The C-disaccharide building block presented two sites of disconnection. The first is between C-1 of the GlcNAc residue and the CH_2 group (Figure 9), and the second is between the CH_2 group and C-4 of the GlcA unit (Figure 9). Our group's previous work[108] has

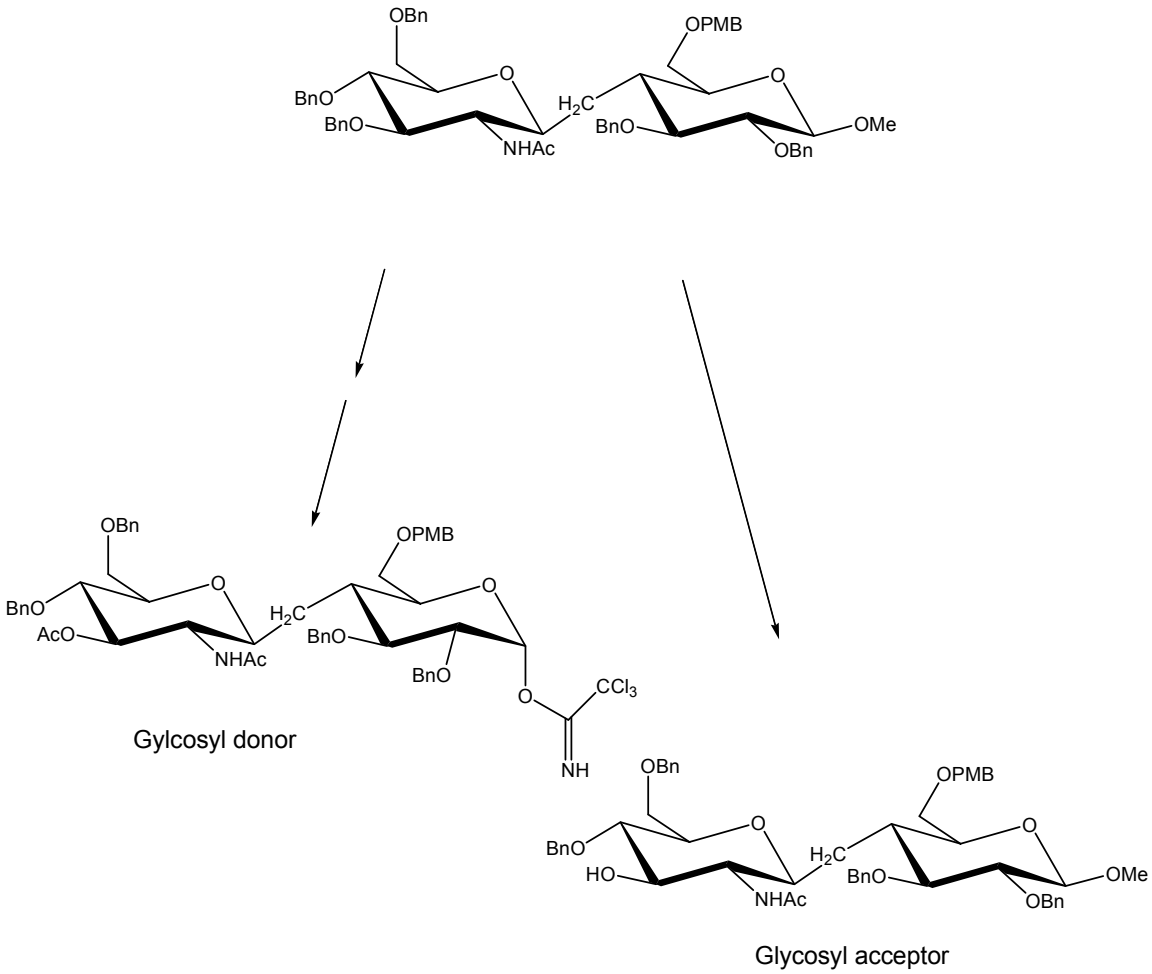


Figure 8. Building blocks for C-linked oligosaccharide.

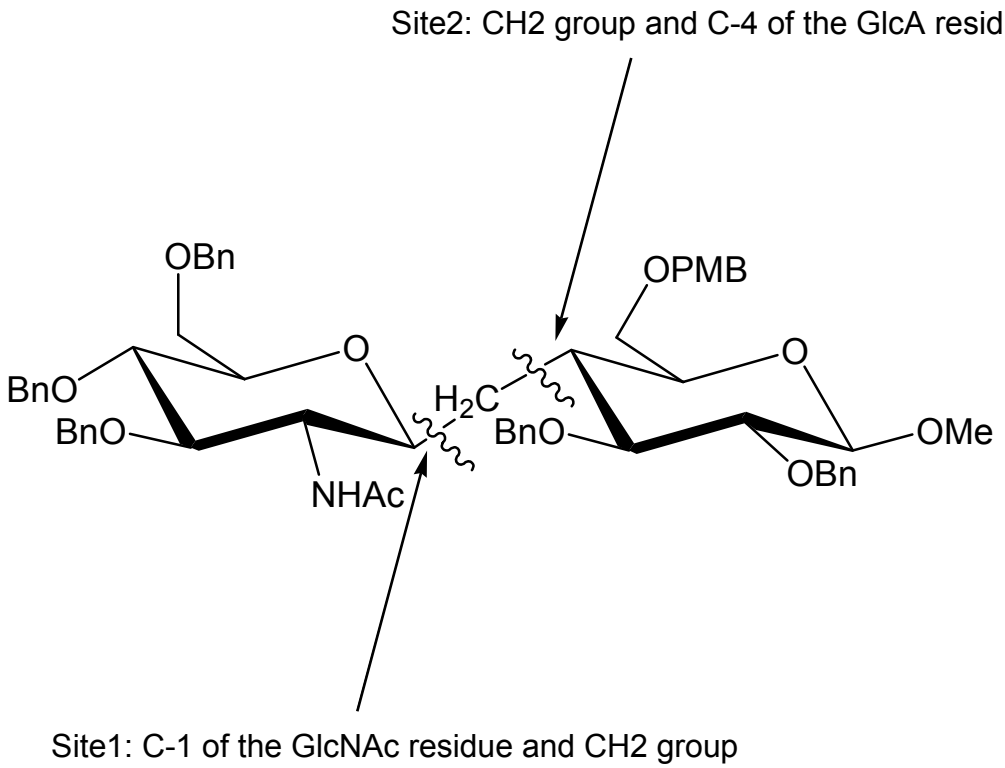
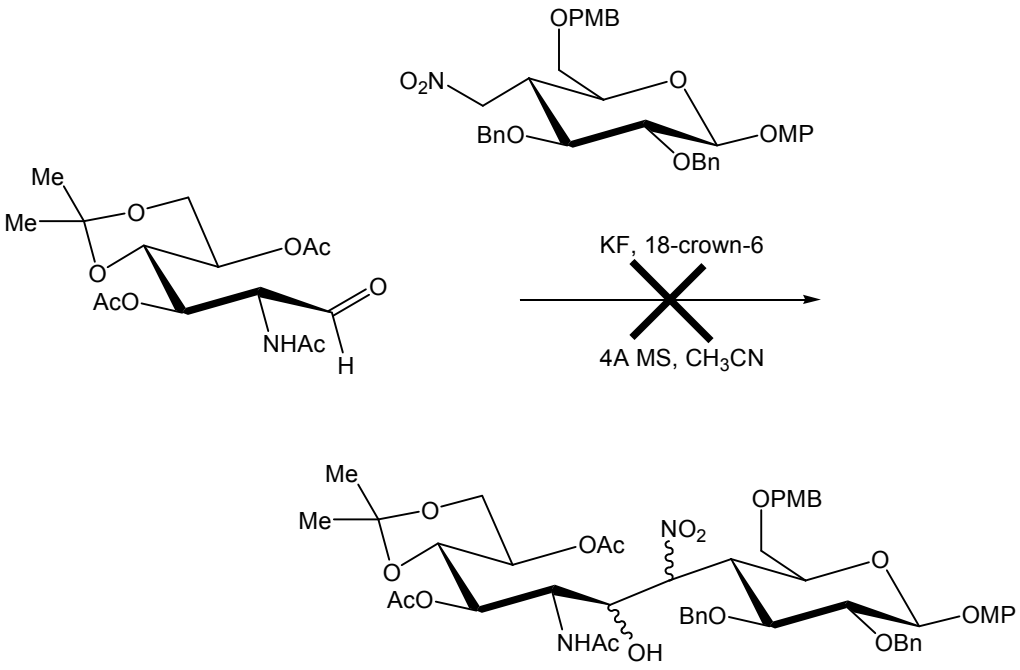


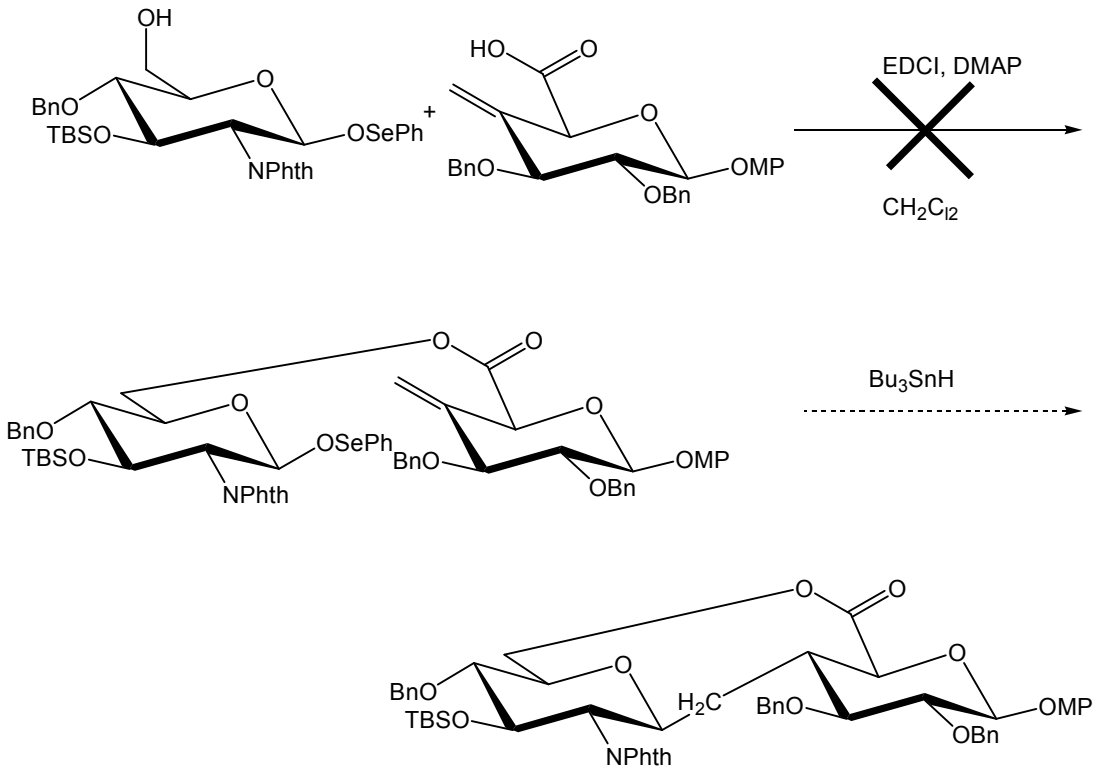
Figure 9. Two sites of disconnection of the C-disaccharide HA analog.

unsuccessfully attempted the coupling of these two units from these two sites using Martin's nitrosugar approach[109,110](Scheme 1), which is extensively covered in Ken Price's dissertation,[111] and the tether approach that involved intramolecular radical coupling via the condensation of phenylseleno amino sugar and 4−*C*−methylene−β−D−xylo−hexopyranosiduronic acid[112,113] (Scheme 2).

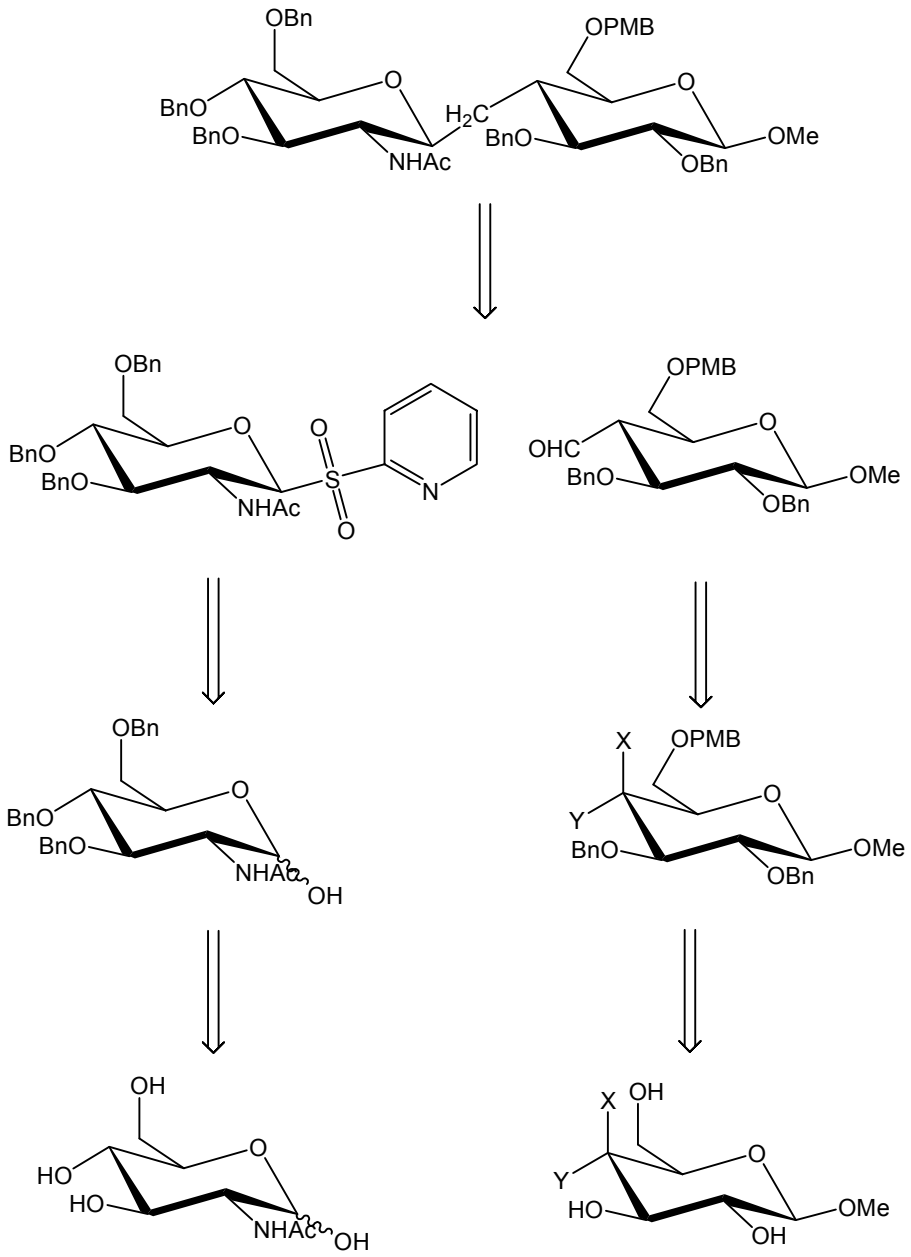
After investigation into a variety of options, Skrydstrup's approach seemed to make our goal more attainable. Skrydstrup and co-workers[114-117] have produced a *C*-α-disaccharide, that contains an *N*-acetylglucosamine subunit at the nonreducing end, through samarium diiodide coupling of an α-D-glucosyl-2-pyridysulfone with a simple aldehyde. It was theorized that the β isomer of the 2−pyridylsulfone would give predominately the *C*-β-disaccharide. The procedure would involve coupling a strategically protected β−2−pyridylsulfone with an adequately protected aldehyde (Scheme 3). I was strongly encouraged when my co-worker Qiang Yang using the Skrydstrup approach succeeded in obtaining predominantly *C*-β-disaccharide of a different analog.[118] However, my attempt at coupling was not as successful. The yield was poor and I had more of the α isomer than the β. At this stage, an alternative approach was needed and was found in the coupling of Kessler's dianion,[119-121] made from treating a glycosyl tin derivative with methyllithium, then butyllithium, and with a strategically protected aldehyde (Scheme 4). This approach presents several advantages. First, the yield of this reaction was much improved from the Skrydstrup approach.



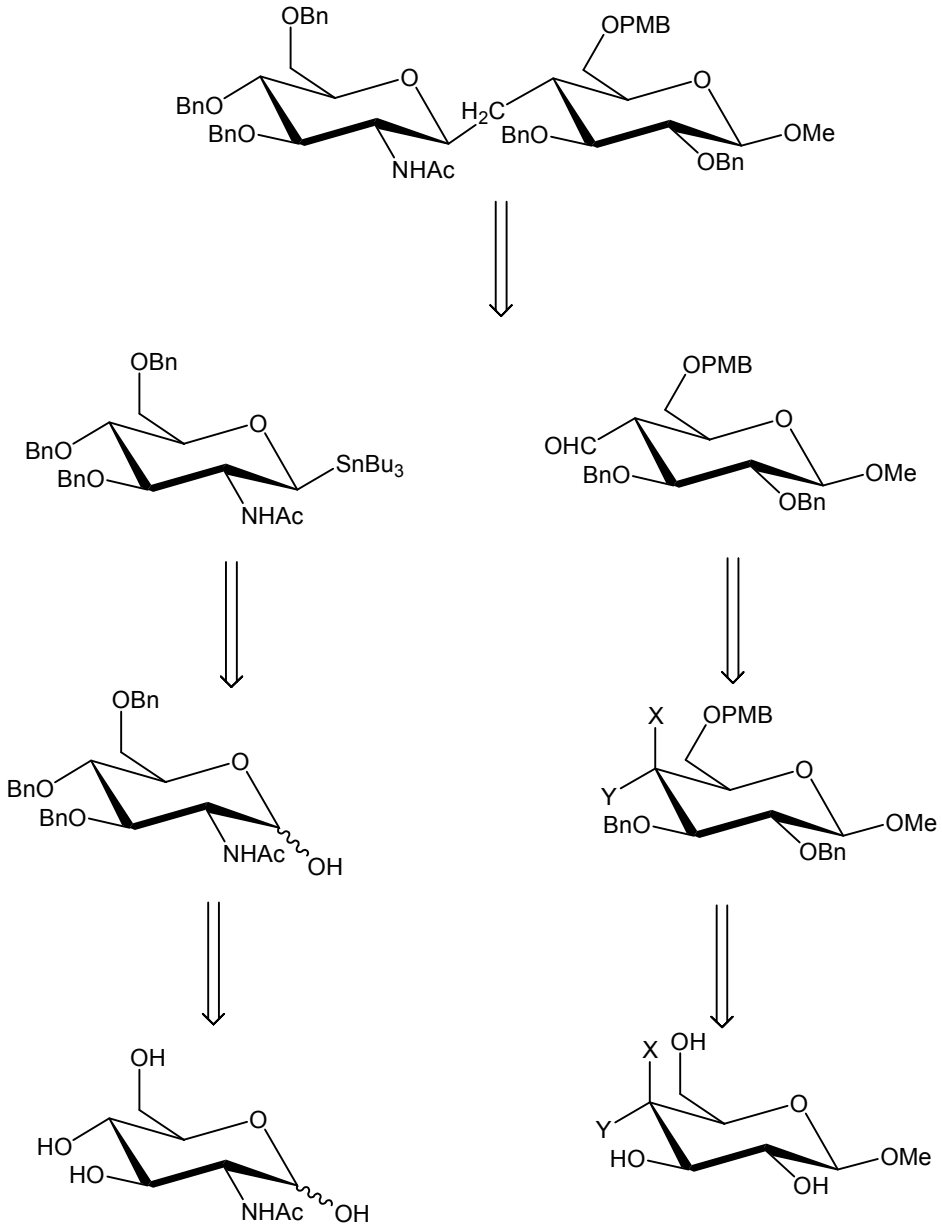
Scheme 1. Martin's nitrosugar approach.



Scheme 2. Tether approach.



Scheme 3. Retrosynthetic analysis for β-D-glucosyl-2-pyridysulfone and aldehyde.



X= OH, Y= H

X= H, Y= OH

Scheme 4. Retrosynthetic analysis for glycosyl tin derivative and aldehyde.

Second, since the reaction is ionic, the stereochemical outcome can be predicted and only the desired β anomer was obtained. This approach successfully used by my co-worker Sean K. Hamilton[122] and would become the blueprint for all *C*-β-disaccharide analogs synthesized in our group.

C. Significance

The synthesis of the *C*-disacharide mimetic related to HA provides at least two formidable challenges. The first one is the generation of the desired β stereochemistry. Although there are some examples in the literature, most of those methods give a mixture of α and β products where the α isomer predominates. The second is the selective oxidation of the 6−OH group to the carboxylic acid. The protecting groups have to be selected so as to allow only the deprotection of the 6−OH group for the oxidation, and the oxidation has to occur mildly enough so as not to limit the choice of protecting groups. In addition, the synthesis of the methyl *C*-β-disaccharide turned out to be a formidable task due to the low yield of reactions and expensive starting material. However, it was needed for comparative NMR and molecular modeling studies with the O-linked disaccharide synthesized by my co-worker, Bin Jiang.[108]

III. RESULTS AND DISCUSSION

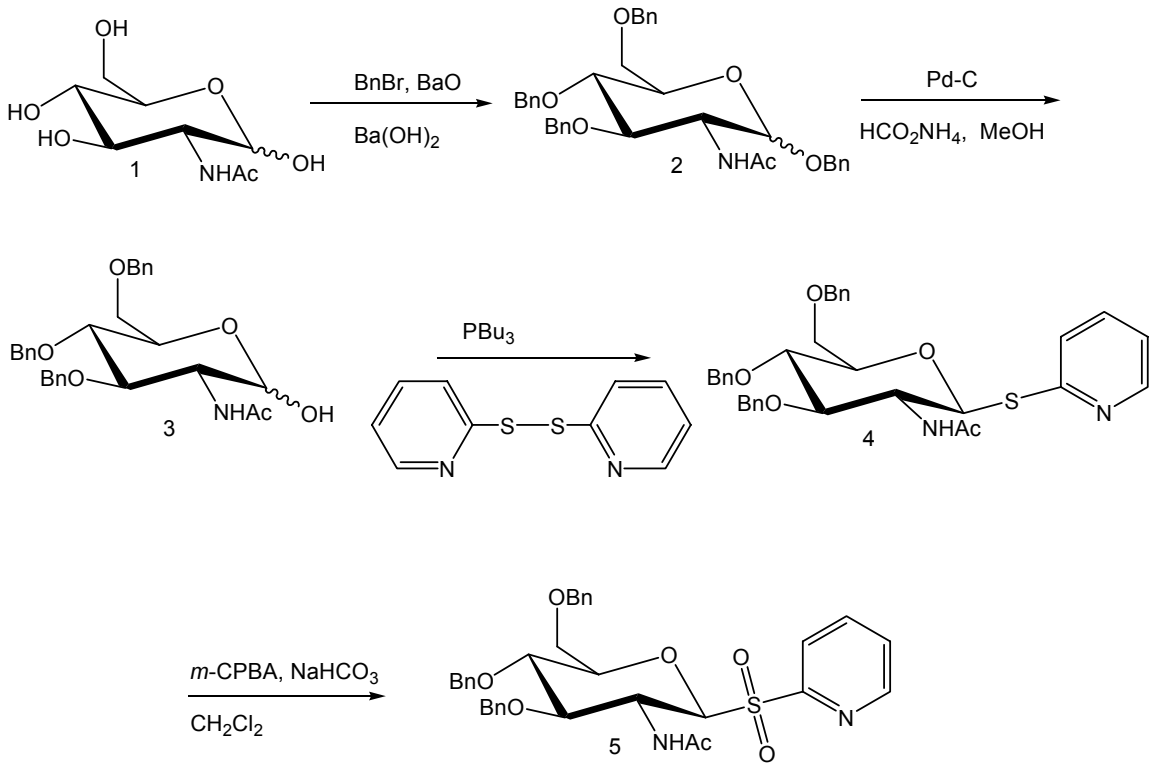
A. The Skrydstrup Approach

1. Synthesis of the Pyridyl Sulfone of *N*−Acetyl−glucosamine 5 (GlcNAc Component)

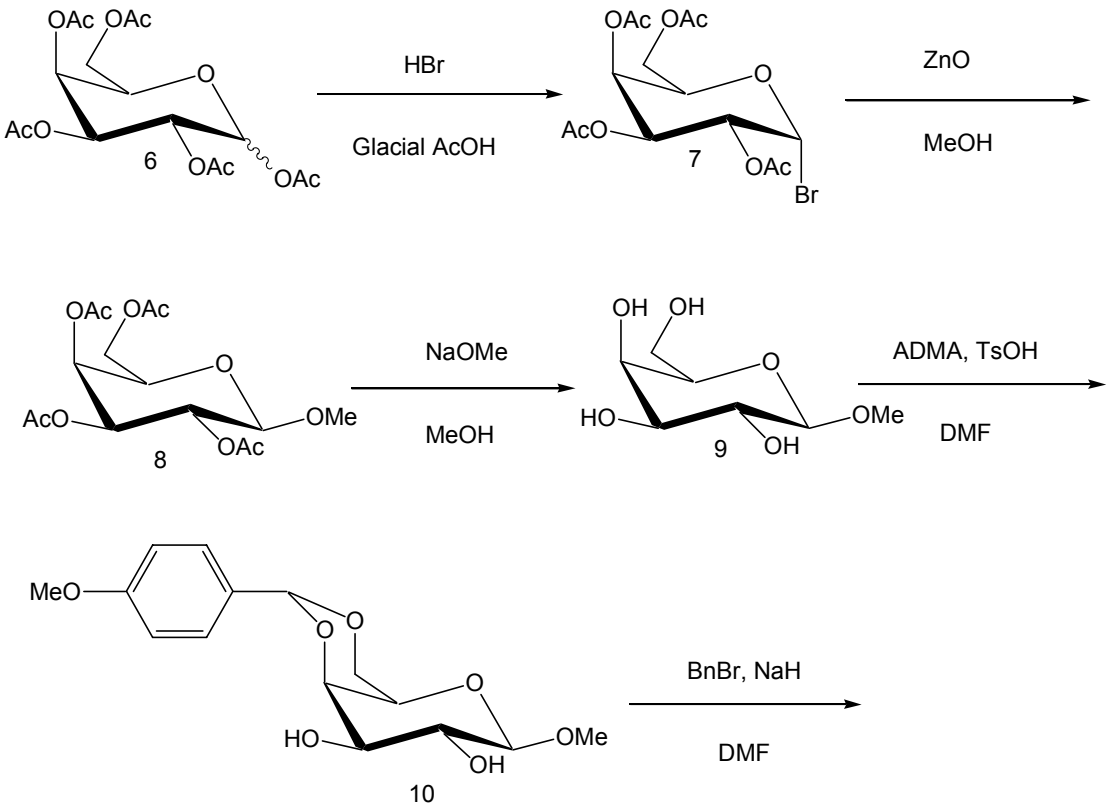
The synthesis of compound **5** (Scheme 5) starts with commercially available 2-acetamido-2-deoxy-D-glucose (**1**) which was benzylated according to the Harrison and Fletcher procedure[123,124] in the presence of barium oxide (BaO) and barium hydroxide ($Ba(OH)_2$) to give benzyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**2**). Hydrolysis of the anomeric benzyloxy group was achieved using a catalytic amount of a palladium-on-carbon (Pd−C) hydrogen-transfer system in a yield of 78%. The 2-acetamido-2-deoxy-D-glucopyranose derivative **3** was treated with tri-*n*-butylphosphine (PBu_3) and 2,2'-dipyridyl disulfide in CH_2Cl_2[117,125] to give the expected and chromatographically separable pyridyl sulfides α and β as a 1:3 anomeric mixture in high yield. The β−pyridyl sulfide **4** was then treated with *m*-chloroperoxybenzoic acid (*m*-CPBA) and sodium carbonate (Na_2CO_3) in dichloromethane (CH_2Cl_2) at 0 ^oC to give pyridyl sulfone **5** in a good yield of 80%.

2. Synthesis of Adehyde 15 (GlcA Component)

Because methyl β-D-galactopyranoside is rather expensive and the pure compound was not required, our synthesis commenced with the more affordable α,β-D-galactose pentaacetate (scheme 6). The anomeric carbon on

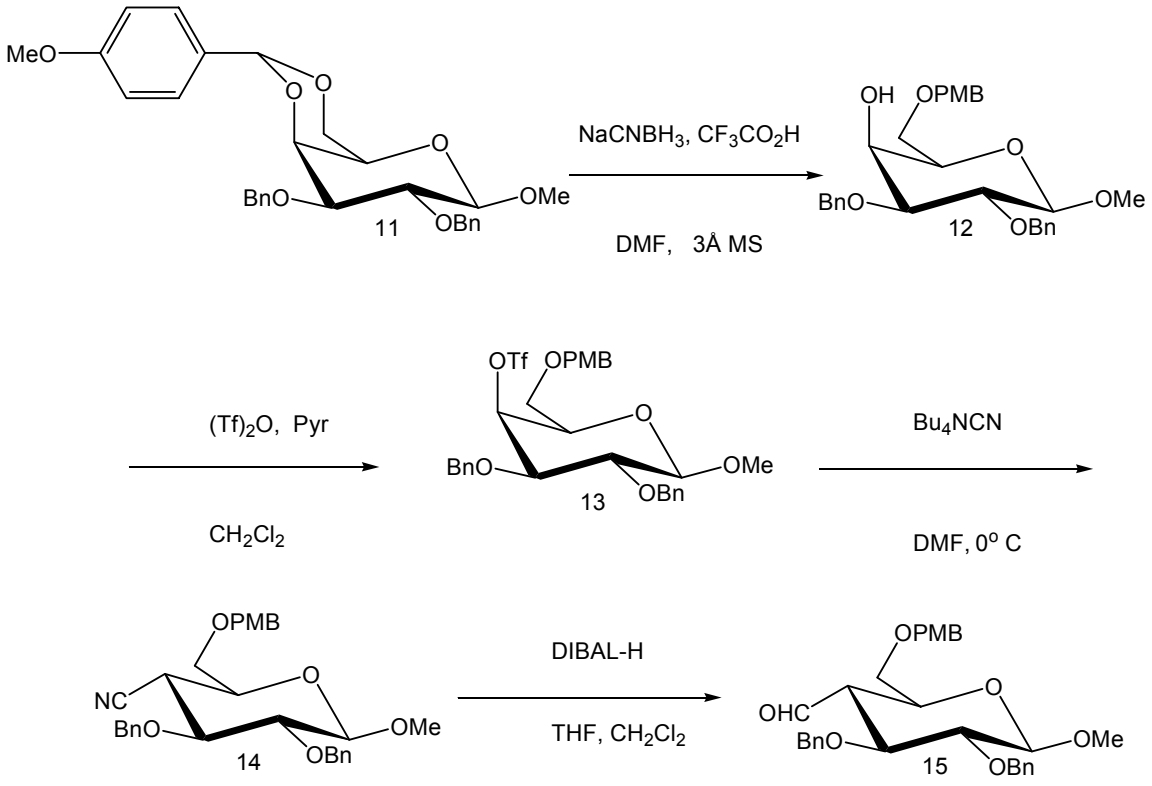


Scheme 5. Synthesis of GlcNAc component **5**.



Scheme 6. Synthesis of Glc aldehyde **15**.

α,β-D-galactose pentaacetate was brominated with hydrobromic acid, (33 wt% solution in glacial acetic acid). The glycosyl bromide **7** obtained from recrystallization in dry ether was dried and treated with zinc oxide (ZnO) in methanol[126] at room temperature over 6 hours to give exclusively methyl tetra-*O*-acetyl-β-D-galactopyranoside (**8**) in a 60% yield. Deacetylation using sodium methoxide in methanol afforded methyl β-D-galactopyranoside (**9**) in an overall yield of 40%. Following Samuelson and Johansson's procedure,[127,128] galactose derivative **12** was available from methyl β-D-galactopyranoside (**9**) in three steps in 46% overall yield. Thus a solution of 4-methoxybenzaldehyde dimethyl acetal (ADMA), a catalytic amount of *p*-toluenesulfonic acid (TsOH), and methyl β-D-galactopyranoside (**9**) in dry *N*,*N*−dimethylformamide (DMF) was placed on a rotatory evaporator at 45 ^{o}C for 2 hours to give galactoside **10**. Benzylation of the free hydroxyl groups of compound 1**0** using benzyl bromide and sodium hydride easily led to the formation of the fully protected galactose derivative **11**. Regioselective reductive ring opening of acetal **11**(scheme 7) via sodium cyanoborohydride ($NaCNBH_3$), trifluoroacetic acid (CF_3CO_2H), and 3Å molecular sieves (MS) in dry DMF afforded compound **12**. The galacto-triflate **13** was prepared by reaction of galactose derivative **12**, pyridine (Pyr) and trifluoromethanesulfonic anhydride (Tf_2O) in CH_2Cl_2 over a period of 3 hours. The triflate **13** was obtained as an oil, which when treated with dry tetrabutylammonium cyanide (NH_4CN) in dry DMF, gave the gluco-configured nitrile **14** in a yield of 32%, along with 30% of the elimination product and 2% of an isonitrile product. Reduction of the nitrile compound **14** with



Scheme 7. Synthesis of Glc aldehyde **15** (cont'd).

diisobutylaluminium hydride (DIBALH)[129] yielded 74% of the aldehyde **15**.

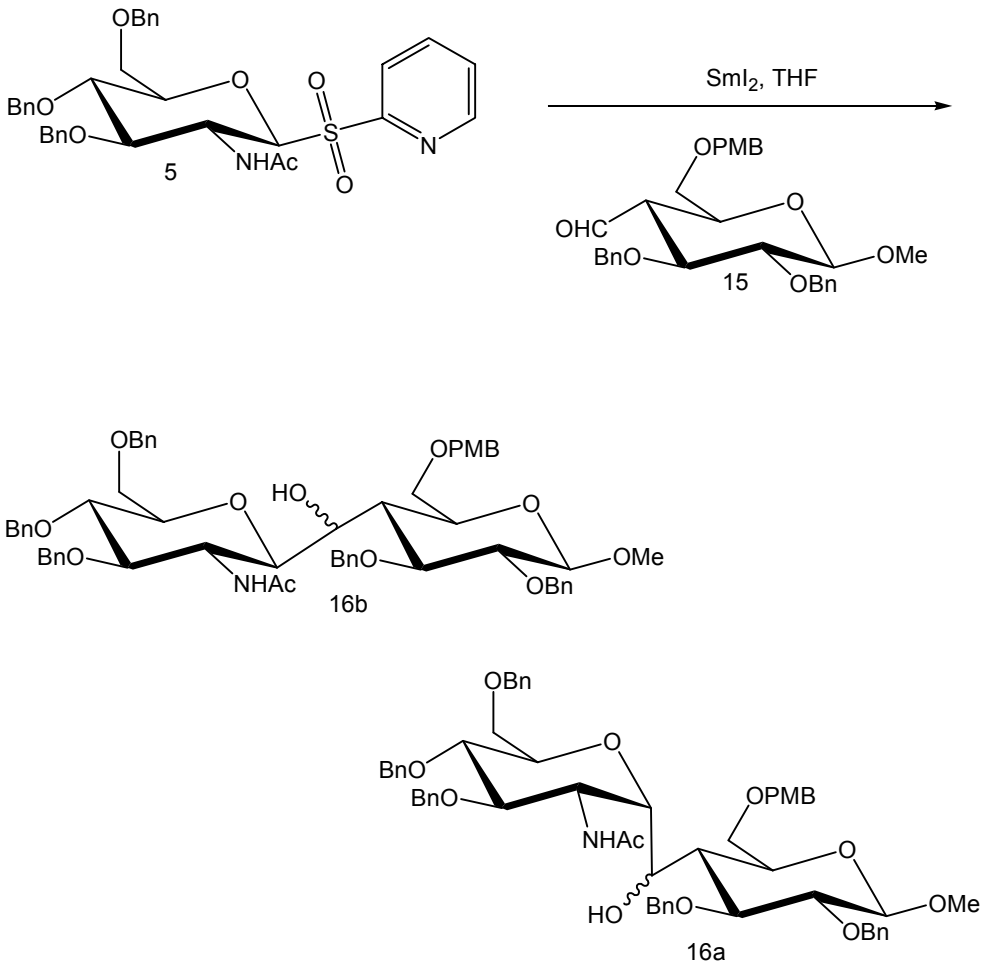
3. Samarium Diiodide-Promoted Coupling Reaction

With pyridyl sulfone **5** and aldehyde **15** in hand, the stage was now set for the samarium diiode-promoted coupling[115,117] of compound **5** with aldehyde **15** (Scheme 8)**.** Upon addition of a solution of samarium diiodide (2.2 equiv) to a tetrahydrofuran (THF) solution of compound **5** and compound **15,** an instantaneous reaction ensued, affording a 4:1 anomeric mixture of α and β disaccharide **16**. However, the yield of the reaction was very low (9%). Although the α/β anomeric mixture of disaccharide **16** was easily separated, and the α anomer can be converted to the β anomer, the very low yield of the reaction and the predominance of the α anomer over the β led us to investigate an alternative approach to making disaccharide **16b**.

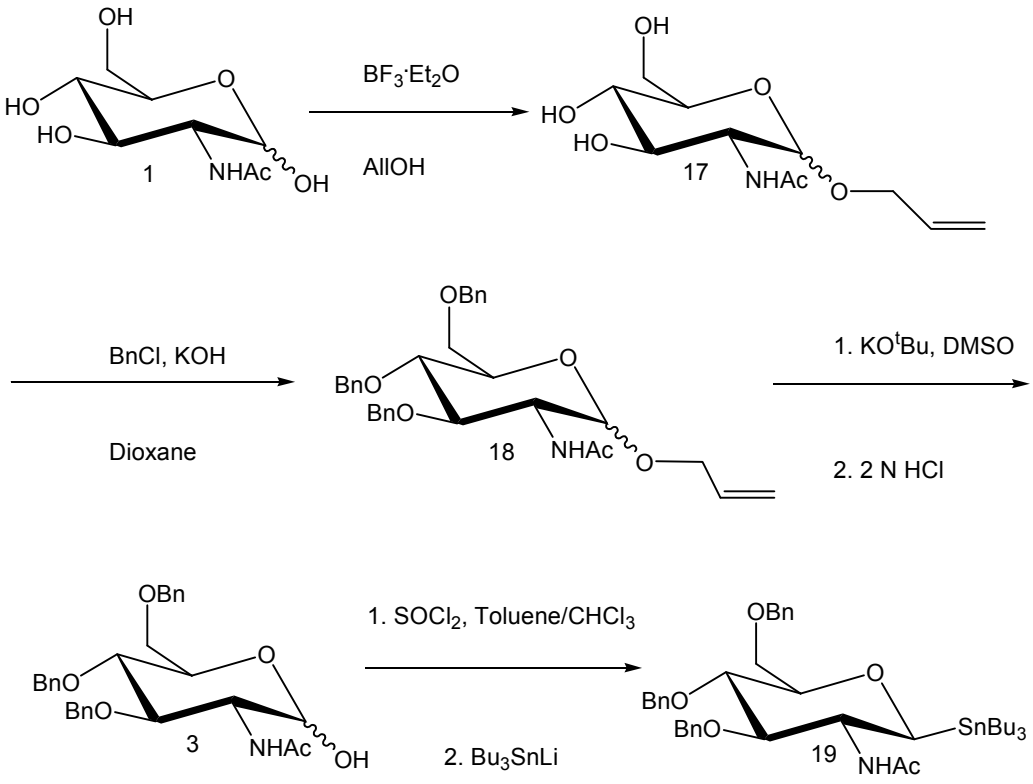
B. Kessler's Dianion Approach

1. Synthesis of Glycosyl Tin Compound 19 (GlcNAc Component)

2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (**3**) was synthesized in four steps (Scheme 9) from commercially available 2−acetamido−2−deoxy−D−glucose (**1**) by a modified procedure of Warren and co-workers.[130,131] That is, a suspension of glycosyl derivative **1,** allyl alcohol



Scheme 8. Skrydstrup approach (samarium diiodide-promoted coupling reaction).



Scheme 9. Synthesis of GlcNAc component **19**.

and boron trifluoride etherate ($BF_3{\cdot}Et_2O$) was refluxed for 2 hours to give the allyl glucoside **17**. Benzylation of the crude product **17** using benzyl chloride (BnCl) and powdered potassium hydroxide (KOH) in refluxing dioxane afforded glucose derivative **18**. Hydrolysis at the anomeric carbon is accomplished in two steps: first, isomerization of the double bond on the allyl group with potassium tert-butoxide (KO^tBu) in anhydrous dimethyl sulfoxide (DMSO), and second, removal of the resulting propenyl group with 2 M hydrochloric acid in a refluxing mixture of acetone and water. The resulting product **3** obtained by recrystallization from methanol, was chlorinated with thionyl chloride in dry CH_2Cl_2. The resulting glycosyl chloride was then treated with lithium tributyltin, which was produced by reacting lithium metal with tributyltin chloride, to give glycosyl tin derivative **19** in an overall yield of 46%.

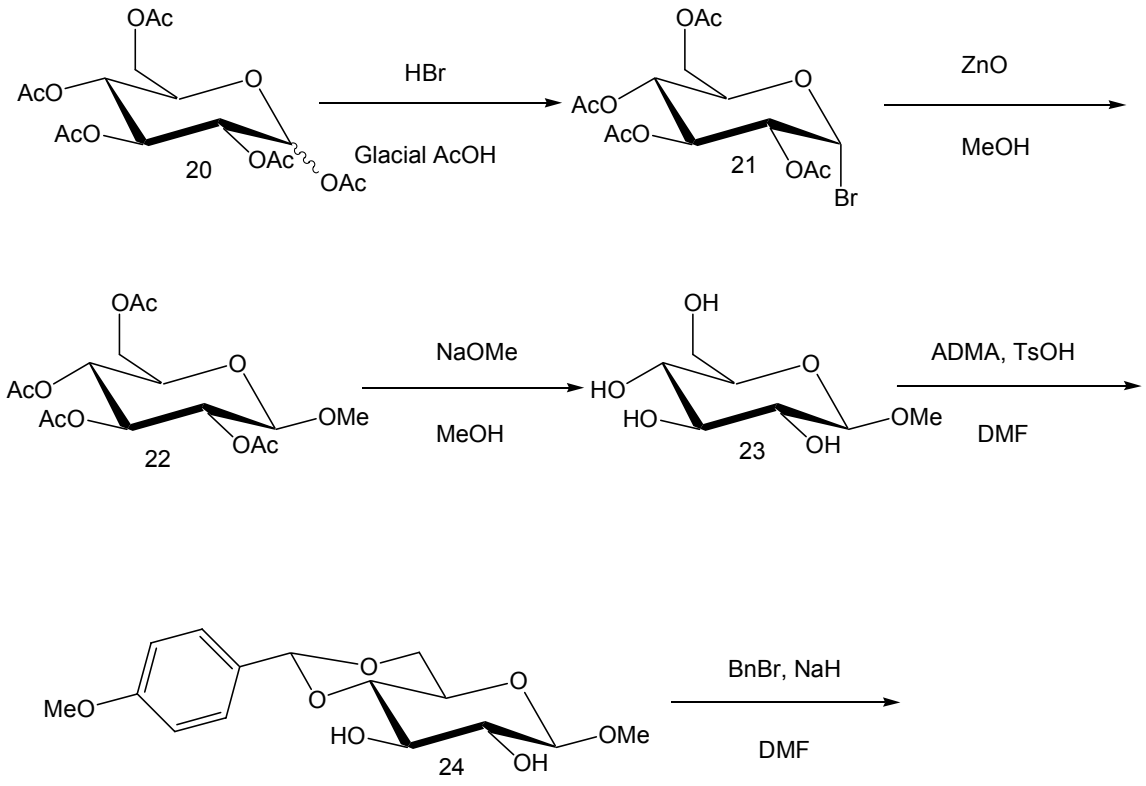
2. Synthesis of Aldehyde 15 (GlcA Component)

Earlier aldehyde **15** was synthesized from α,β-D-galactose pentaacetate (**6**) via methyl β-D-galactopyranoside (**9**)**;** however, because the low yield of recrystallization of glycosyl bromide **7** from dry ether and especially the low yield of the nitrile-forming reaction to give the gluco-configured nitrile **14** (32% versus 82% in the literature) and 30% of elimination byproduct, an alternative route was investigated. Although the success of this approach was uncertain, it was noticed that the glycosyl bromide **21** recrystallized readily from dry ether, already improving the overall yield of making methyl β-D-glucopyranoside (**23**), which could lead to the galacto-configured nitrile **28**. The nitrile of the D-gluco configuration could then be reached via isomarization of the C-4 bond. It was

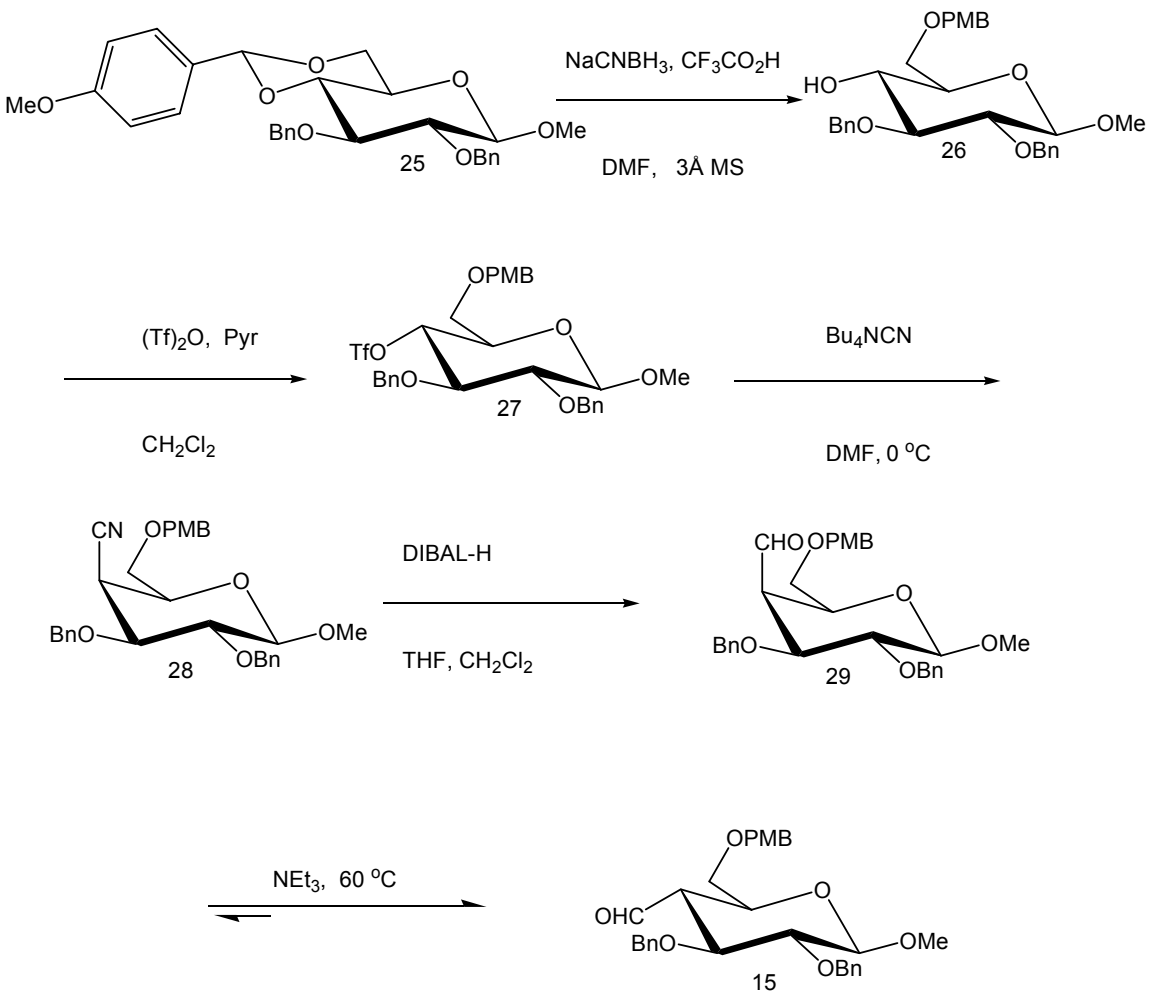
encouraging to find that Schmidt and Preuss[132] have successfully isomerized the more stable gluco-aldehyde from the galacto-aldehyde in 80% yield. Consequently, the new synthesis for aldehyde **15** starts with α,β-D-glucose pentaacetate (Scheme 10), and as described above methyl β-D-glucopyranoside (**23**) was obtained in three steps in 60% overall yield (1. HBr, glacial AcOH 2. ZnO, MeOH 3. NaOMe, MeOH). The gluco-triflate **27** was arrived at in four steps from methyl β-D-glucopyranoside (**23**) (1. ADMA, TsOH, DMF 2. BnBr, NaH, DMF 3. $NaCNBH_3$, CF_3CO_2H, 3A MS, DMF 4. $(Tf)_2O$, Pyr, CH_2Cl_2). To our astonishment when gluco-triflate **27** (Scheme 11) was treated with dry tetrabutylammonium cyanide (NH_4CN) in dry DMF, it gave the galacto-configured nitrile **28** in a yield of 64% along with barely 5% elimination product and 1% of isonitrile product. Reduction of the nitrile compound **28** with diisobutylaluminium hydride (DIBAL-H) yielded 82% of galacto-aldehyde **29**. Triethylamine (Et_3N) treatment of aldehyde **29** overnight at 60 ^oC led to the thermodynamically more stable gluco-aldehyde **15** in a 71% yield after separation of the two aldehydes by flash chromatography. This new route afforded us an improved yield by about 20%.

3. Dianion-Promoted Coupling Reaction

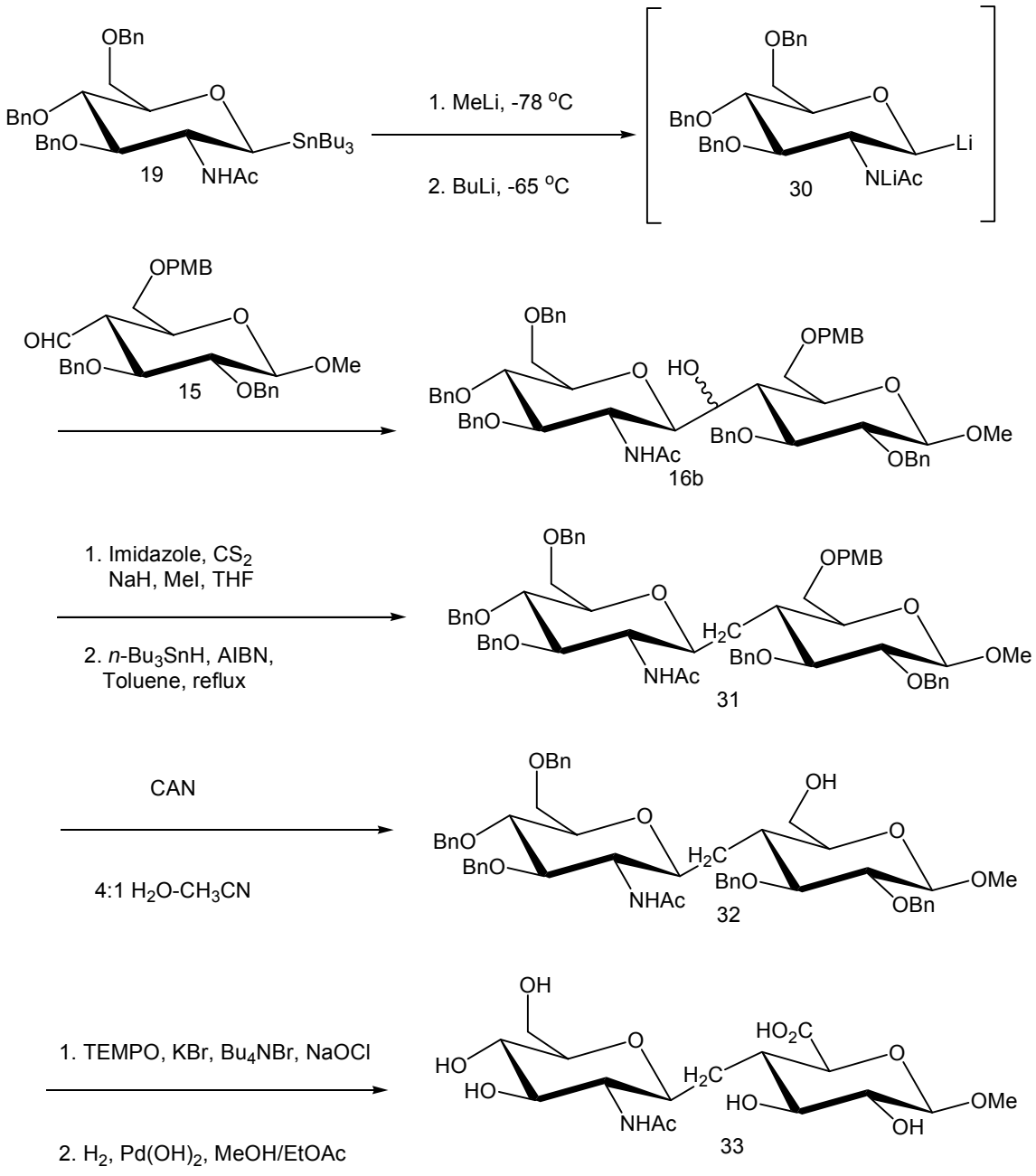
With glycosyl tin compound **19** and aldehyde **15** in hand**,** coupling was achieved (Scheme 12) using a modified procedure of Kessler and co-workers[120]. Glycosyl tin compound **19** was deprotonated at –78 ^oC with methyllithium (MeLi)



Scheme 10. Synthesis of Glc aldehyde **15** via aldehyde **29**.



Scheme 11. Synthesis of aldehyde **15** via aldehyde **29** (cont'd).



Scheme 12. Kessler's dianion promoted-coupling.

in dry THF, then transmetallated at –65 ^{o}C by butyllithium (BuLi) to give the intermediate **30,** indicated by a deep red color of the solution. Subsequent addition of aldehyde **15** provided the required disaccharide **16b**, which was identified by mass spectrometry (Figure 10). The C-(1→4) linkage was confirmed to be β by 1D and 2D (gCOSY, HSQC) NMR spectroscopy with its coupling constant of $J_{1,2} = J_{2,3} = 10.2$ Hz (Figure 11).

Alcohol **16b** was then treated, according to the Barton and McCombie procedure[133,134], with an excess of sodium hydride (NaH) in THF containing a trace of imidazole as catalyst. An excess of carbon disulfide (CS_2) was added after one hour. The dithiocarbonate salt that resulted from the addition of CS_2 was alkylated with excess of iodomethane (MeI) after one more hour. Treatment of the resulting thioester with an excess of tributylstannane (*n*-Bu_3SnH) and azoisobutyronitrile (AIBN) in refluxing toluene gave the deoxygenated disaccharide **31.** Compound **31** was treated with ceric ammonium nitrate (CAN),[135,136] which selectively removed the *p*-methoxybenzyl group to give alcohol **32**. The deprotected hydroxyl 6−OH group on alcohol **32** was then easily oxidized to the corresponding carboxylic acid in the presence of a catalytic amount of 2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPO), potassium bromide, and tetrabutylammonium bromide under aqueous organic two-phase conditions.[137-139] Hydrogenolytic debenzylation of the carboxylic acid was conducted using Pearlman's catalyst, palladium hydroxide-on-carbon

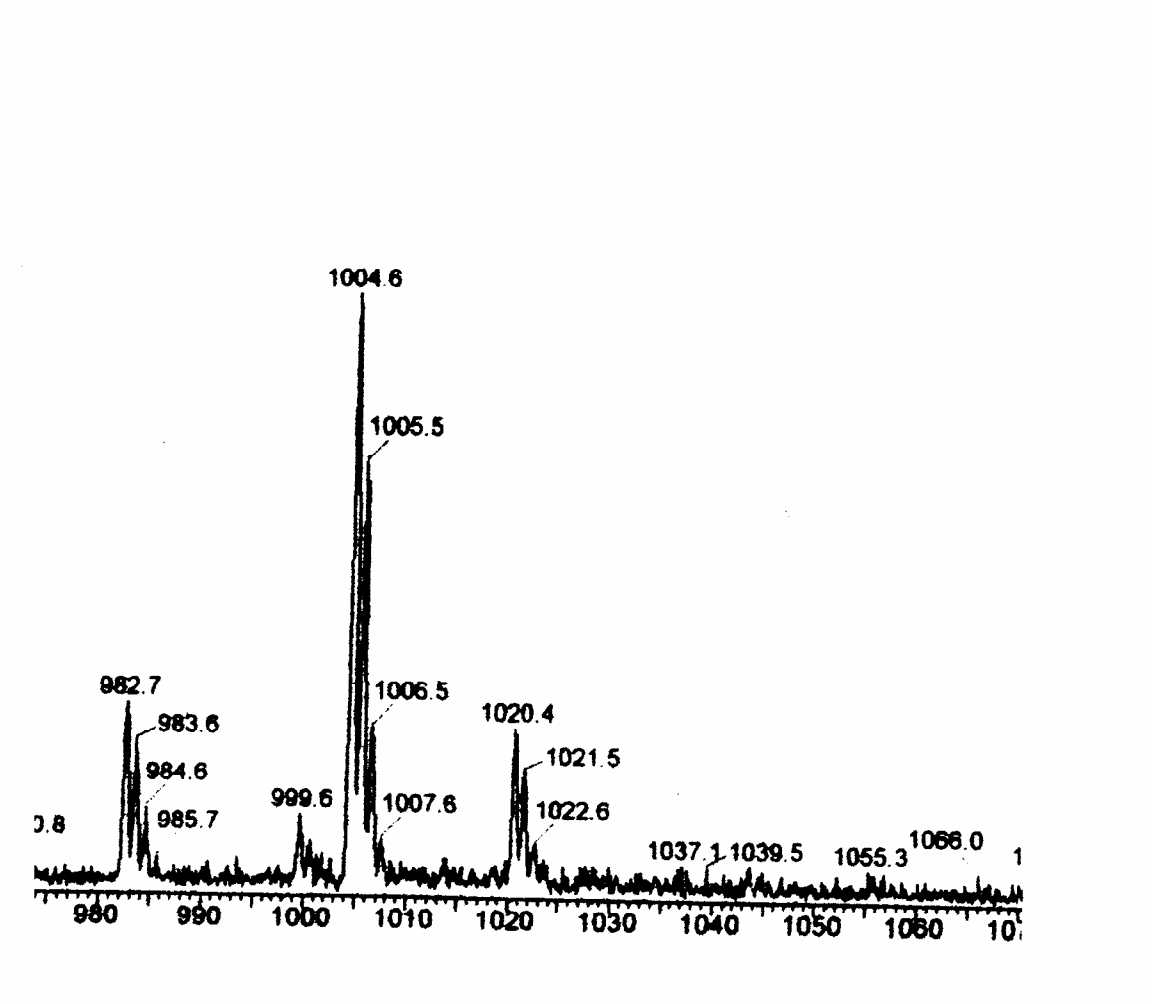


Figure 10. Mass spectrum of disaccharide **16b**.

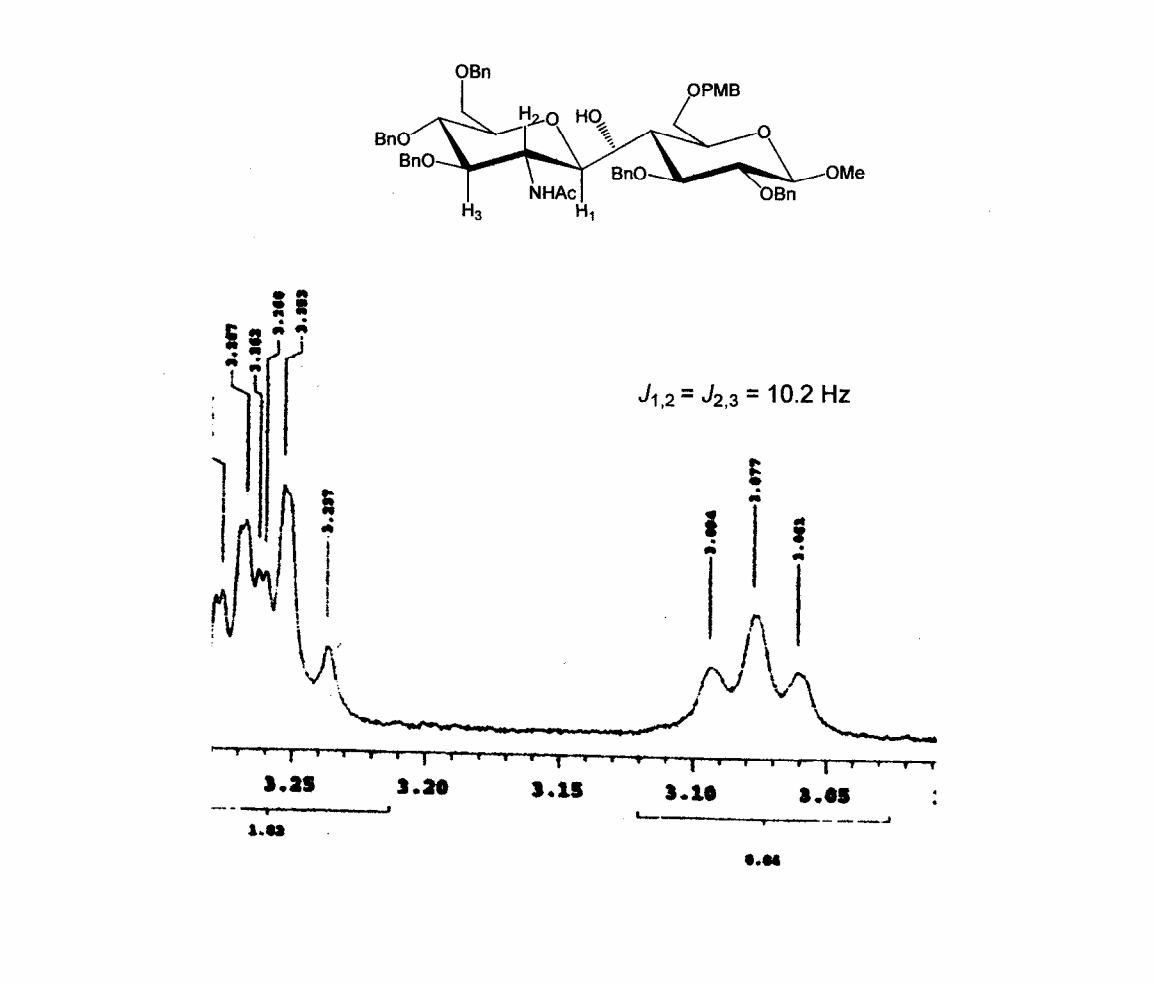


Figure 11. Partial ^{1}H NMR spectrum of disaccharide **16b**.

($Pd(OH)_2/C$) in 4:1methanol–ethyl acetate (MeOH–EtOAc) over 3 days under an atmosphere of hydrogen (H_2)[137,140] to finally give the elusive dissacharide **33,** which was characterized by mass spectrometry (Figure 12).

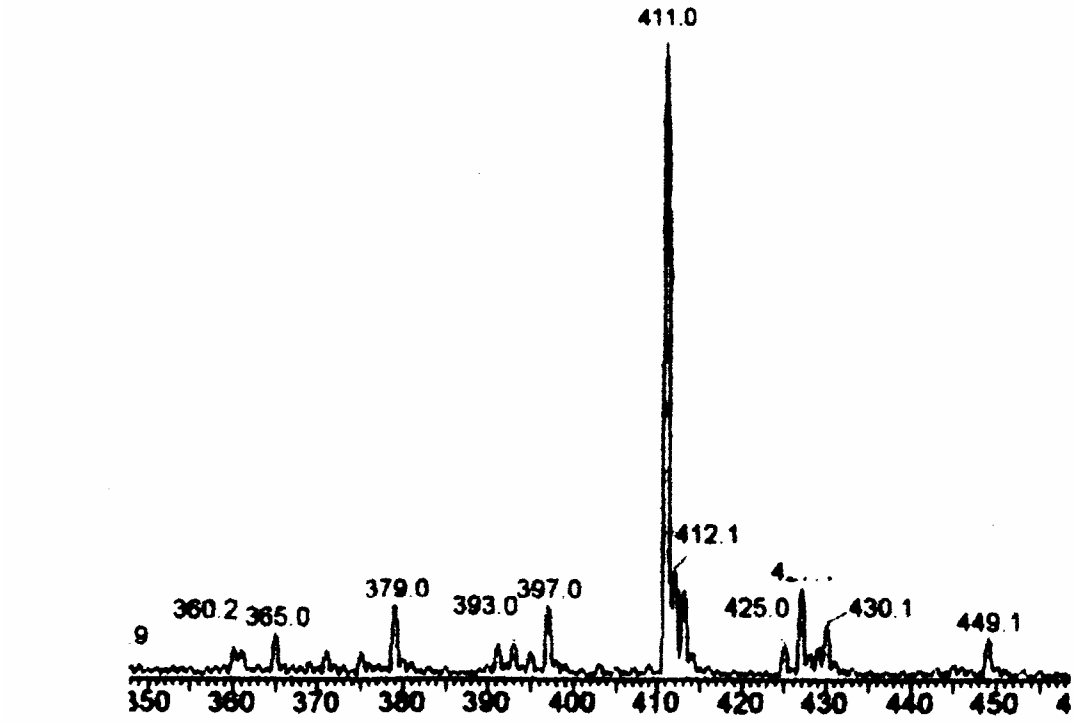


Figure 12. Mass spectrum of disaccharide **33**.

IV. CONCLUSIONS

The synthesis of the *C*-disaccharide mimetic of hyaluronic acid was accomplished first via samarium diiodide-promoted coupling then by an adaptation of Kessler's dianion-promoted coupling methology. The samarium diiodide approach gave predominantly the α-*C*-disaccharide over the β anomer in a rather poor yield, the Kessler dianion approach, however, gave almost only the desired β-*C*-disaccharide in a rather good yield for a coupling reaction. This latter approach relied on the coupling of an adequately protected glycosyl tin derivative that acts as the glycosyl acceptor and the GlcNAc component with a strategically protected aldehyde that acts as the glycosyl donor and GlcA component of the synthesized disaccharide unit. This approach has also been used by my co-worker, Dr. Zhong-Xu Ren, in the synthesis of a C−/O−linked HA tetrasaccharide mimetic where the two disaccharide units built were strategically protected in order to act either as C−1 glycosyl donor (activated as trichloroacetimidate) or as glycosyl acceptor with the 3'−OH free.[118] Thus this strategy should enable us to assemble C−/O−linked oligosaccharides of the eight-mer and beyond.

The disaccharide achieved here will be used for NMR spectroscopy and molecular modeling studies, and the techniques used will add to the vast knowledge acquired by my group in the synthesis of HA-related oligosaccharides that can potentially act as antimetastatic agents.

V. EXPERIMENTAL

General methods.— ^{1}H and ^{13}C NMR spectra were recorded at 25 ^{o}C on a Varian Mercury 300, or a Varian Inova 600 instrument at resonance frequencies of 300 MHz or 600 MHz and 75 MHz or 150 MHz, respectively. Chemical shift are reported in ppm relative to tetramethylsilane (TMS) the internal standard for ^{1}H and $CDCl_3$ for ^{13}C, unless otherwise indicated. Multiplicities are first-order values in Hz: s, singlet; bs broad singlet; d, doublet; dd, doublet of doublet; t, triplet; dt, doublet of triplet; ddd doublet of doublet of doublet; m, multiplet. All two-dimensional experiments (gCOSY, HSQC, HMBC and TOCSY) were recorded on a Varian Inova 600. NMR spectra of compounds are provided in the Appendix. All reactions were monitored by thin-layer chromatography (TLC) on aluminum-backed plates coated with E. Merck silica gel and detection by 254 nm UV light and/or by charring with *p*-anisaldehyde–sulfuric acid. Column chromatography was performed on 60 Å (63−200 μm) and 40 Å (40−60 μm) silica gel. Melting points were determined with a Thomas–Hoover melting point instrument equipped with a Cole–Palmer model 8520-50 Digi-Sense digital thermocouple thermometer. Mass spectra were acquired on a Micromass VG Quattro II electrospray instrument. All organic and inorganic reagents were ACS grade and were used without further purification. All solvents were freshly distilled by standard methods with suitable drying agents. All moisture-sensitive reactions were carried out under nitrogen or argon. Organic extracts were dried with anhyd. magnesium sulfate ($MgSO_4$).

Benzyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranoside (2)

To a solution of 2-acetamido-2-deoxy-D-glucose **1** (10.0 g, 45.2 mmol) and benzyl bromide (91.4 mL, 768.5 mmol) in *N*,*N*-dimethylformaldehyde (DMF, 200 mL), stirred at 0 ^{o}C, were added portionwise barium oxide (BaO, 65.15 g, 424.9 mmol) and then barium hydroxide [$Ba(OH)_2$, 25.7 g, 81.4 mmol]. The mixture was stirred at 0 ^{o}C for 5 h, and then for 18 h at room temperature. Dichloromethane (CH_2Cl_2, 200 mL) was added, and the solution was filtered. The organic phase was washed with water, dried and concentrated in vacuo. Recrystallization from MeOH afforded white crystals (75% yield) of **2;** mp 160–168 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.81 (s, 3H, CH_3), 3.5–3.8 (m, 6H), 4.04 (dd, 1H, *J* = 9.6, 8.2), 4.51–4.92 (m, 6H), 4.76–4.92 (m, 5H), 5.45 (d, 1H, *J* = 8.2), 7.10–7.35 (m, 20). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.48, 56.36, 69.03, 70.63, 73.43, 74.45, 74.82, 78.45,74.75, 74.80, 74.92, 75.10 80.47, 99.27,127.95, 128.09, 128.12, 128.20, 128.27, 128.33, 128.70, 128.77, 128.80, 137.87, 138.30, 138.46, 138.71, 170.54. NMR spectral data matched that of the known compound.[124]

2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (3)

Benzyl glycoside **2** (0.86 g, 1.95 mmol), palladium-on-carbon [Pd–C, 0.70 g, 0.66 mmol], and ammonium formate [HCO_2NH_4, 0.3 g, 4.8 mmol] were stirred in dry MeOH (30 mL) at 70 ^{o}C. The reaction was monitored by TLC (19:1 CH_2Cl_2–MeOH), and after 16 min, the mixture was filtered, and the catalyst was washed

with hot MeOH. The solution was concentrated in vacuo. Recrystallization from MeOH gave white crystals of tri-*O*-benzyl glycoside **3** in a 78% yield; mp 218–219 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.82 (s, 3H, CH_3), 3.65 (m, 3H), 3.76 (m, 2H), 4.02 (m, 1H), 4.12 (m, 1H), 4.54 (m, 5H), 4.8 (m, 2H), 5.2 (t, 1H, *J* = 3.6 Hz), 5.35 (d, 1H, *J* = 9 Hz), 7.16– 7.37 (m, 15H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.8, 53.4, 69.1, 71.1, 73.8, 75.2, 75.4, 79.0, 79.7, 92.4, 128.1, 128.3, 128.4, 128.7, 128.8, 128.83, 128.9, 138.1, 38.2, 138.7, 170.4. NMR spectral data matched that of the known compound.[124]

2-Acetamido-3,4,6-tri-*O*-benzyl-2deoxy-β-D-glucopyranosyl 2-pyridyl sulfide (4)

Tributylphosphine [Bu_3P, 1.2 mL, 4.68 mmol] was added to a stirred solution of benzyl glycoside **3** (2.1 g, 3.6 mmol) and 2,2‘-dipyridyl disulfide (0.73 mL, 3.3 mmol) in CH_2Cl_2 (60 mL). After being stirred for 1 h, the solution was evaporated to dryness in vacuo. Purification of the residue by flash chromatography (7:2.8:0.2 hexanes–CH_3Cl–MeOH) provided pyridyl sulfide **4** in a yield of 73% (α/β 1/3). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.77 (s, 3H, CH_3), 3.53–3.84 (m, 5H), 4.13 (td, 1H, *J* = 10.2, 8.2 Hz), 4.45–4.60 (m, 3), 4.71–4.86 (m, 3H), 5.56 (d, 1H, *J* = 10.2 Hz), 6.06 (d, 1H, *J* = 8.7 Hz), 7.00 (dt, 1H, *J* = 6.0, 1.2 Hz), 7.18–7.31 (m, 17H), 7.43 (t, 1H, *J* = 7.8 Hz), 8.37 (d, 1H, *J* = 4.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.37, 54.70, 68.80, 73.22, 74.68, 74.75, 78.14, 79.56, 82.70, 83.25, 120.45, 123.64, 127.45, 127.67, 127.73, 127.86, 128.13, 128.21, 128.27, 128.34,

128.39, 136.55, 137.90, 138.02, 138.18, 149.18, 157.28, 170.26. ESIMS (positive-ion): *m/z* = 585.1 ($M + H^+$), 607.1 ($M + Na^+$), 623.2 ($M + K^+$).

2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl 2-pyridyl sulfone (5)

m-Chloroperoxybenzoic acid (*m*-CPBA) of 85% purity (0.523 g, 2.58 mmol) was added to a stirred mixture of sulfide **4** (0.7 g, 1.2 mmol) and sodium bicarbonate ($NaHCO_3$,0.71 g, 8.4 mmol) in CH_2Cl_2 (10 mL) at 0 ^oC. After 2.5 h TLC (1:1 $CHCl_3$–EtOAc) showed the reaction was completed. The mixture was diluted with CH_2Cl_2 and then washed consecutively with a 50% satd $Na_2S_2O_3$, satd $NaCO_3$, and brine. The organic phase was dried over $MgSO_4$ and concentrated to dryness in vacuo. Flash chromatography (3:1, 2:1, 1:1 $CHCl_3$–EtOAc) gave sulfone **5.** ^1H NMR (300 MHz, $CDCl_3$): δ 1.91 (s, 3H, CH_3), 3.46–3.62 (m, 4H), 3.90–4.00 (m, 1H), 4.17–4.28 (m, 2H), 4.46 (t, 1H, *J* = 9.0 Hz), 4.54 (d, 1H, *J* = 11.1 Hz), 4.67–4.84 (m, 3H), 5.51 (d, 1H, 10.2 Hz), 6.32 (d, 1H, *J* = 5.1 Hz), 7.10–7.39 (m, 16H), 7.77 (dt, 1H, *J* = 7.8, 3.6 Hz), 8.05 (d, 1H, *J* = 7.8 Hz), 8.66 (d, 1H, *J* = 3.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.50, 52.47, 68.41, 73.03, 74.72, 75.39, 77.87, 79.68, 80.97, 86.13, 124.54, 127.34, 127.41, 127.44, 127.72, 127.87, 128.18, 128.31,128.36, 137.55, 137.79, 137.99, 138.14, 150.17, 154.96, 171.40. ESIMS (positive-ion): *m/z* = 617.1 ($M + H^+$), 639.1 ($M + Na^+$), 655.0 ($M + K^+$).

Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (8)

Hydrobromic acid (9.5 mL of a 33 wt% solution in HOAc) was added to α,β-D-galactose pentaacetate (10.7 g, 27.4 mmol), and the solution was stirred for 2 h (TLC 2:1 petroleum ether–EtOAc). The reaction mixture was evaporated and co-evaporated with dry toluene to give the corresponding glycosyl bromide. The glycosyl bromide was crystallized from dry ether and dried over night. The dried tetra-*O*-acetyl-α-D-glucopuranosyl bromide (17.1 g, 41.7 mmol) was then dissolved in dry MeOH (500 mL) with zinc oxide [ZnO, 2.5 g, 30.9 mmol] at 0 °C and allowed to warm to room temperature for 7 h (TLC 2:1 Petroleum ether–EtoAc). The reaction mixture was filtered, and the filtrate was concentrated. The residue was dissolved in chloroform (50 mL), washed with water (15 mL x 3), dried over anhyd. $MgSO_4$ and concentrated. The crude product crystallized from MeOH as needles in a yield of 60% (α/β 0/100); mp 84–85 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.99 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), 3.52 (s, 3H, CH_3), 3.92 (dt, 1H, *J* = 6.7, 1.00), 4.10–4.25 (m, 2H), 4.41 (d, 1H, *J* = 8.0), 5.02 (dd, 1H, *J* = 10.2, 3.6), 5.21 (dd, 1H, *J* = 10.2, 8.0), 5.4 (dd, 1H, *J* = 3.6, 0.6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.88, 20.99, 21.03, 21.04, 57.34, 62.13, 68.61, 71.46, 72.01, 73.10, 101.87, 169.69, 170.58, 170.75, 171.03. NMR spectral data matched that of the known compound.[126]

Methyl β-D-galactopyranoside (9)

To a solution of methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**8)** (7.1 g, 19.6 mmol) in dry CH_2Cl_2 (10 mL) and MeOH (50 mL) was added sodium methoxide (25% in MeOH). The reaction was allowed to stir at room temperature for 5 h, after which time it was neutralized with Dowex 50 x 2-100 (H^+ form), filtered, and concentrated. The crude product was crystallized from ethanol (EtOH) as a white amorphous solid in high yield of 70%.); mp 169–171 ^oC ^1H NMR (300 MHz, $CDCl_3$): δ 3.27 (dt, 1H, *J* = 8.1, 1.8 Hz), 3.35 (s, 3H, CH_3), 3.42 (dd, 1H, *J* = 10, 3.6 Hz), 3.45–3.5 (m, 1H), 3.53–3.58 (m, 2H), 3.69 (dd, 1H, *J* = 3.6, 1.8 Hz), 4.09 (d, 1H, *J* = 7.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 57.18, 61.00, 68.67, 70.72, 72.78, 75.15, 103.82. NMR spectral data matched that of the known compound.[126,141]

Methyl 4,6-*O*-(4-methoxybenzylidene)-β-D-galactopyranoside (10)

A solution of methyl β-D-galactopyranoside (**9**) (10.0 g, 51.5 mmol), 4-methoxybenzaldehyde dimethyl acetal (17.6 mL, 103.1 mmol), and *p*-toluenesulfonic acid monohydrate (0.09 g, 0.51 mmol) in anhydrous DMF (50 mL) was heated at 50 °C on a rotary evaporator under water aspirator pressure (~ 22 mm Hg) for 2 h. The temperature was then increased to 70 °C, and the mixture was concentrated in volume to 20 mL. This remaining solution was poured into stirred slurry of ice (25 g), satd aq soduim bicarbonate (50 mL), and diethyl ether (50 mL). The white precipitate that formed was filtered, washed with

hexanes (50 mL x 3), water (50 mL x 2) to give a white solid in 84% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.51 (bs, 2H), 3.45 (m, 1H), 3.56 (s, 3H, CH_3), 3.60–3.76 (m, 2H), 3.78 (s, 3H, CH_3), 4.05 (d, 1H, *J* = 12.6 Hz), 4.21 (d, 2H, *J* = 7.0 Hz), 4.32 (d, 1H, *J* = 12.6 Hz), 5.48 (s, 1H), 6.83– 6.90 (m, 2H), 7.39– 7.44 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.51, 57.46, 66.85, 69.31, 72.02, 72.92, 75.44, 101.50, 103.95, 113.75, 127.96, 130.14, 160.43. ESIMS (positive-ion): *m/z* = 313.1 ($M + H^+$), 335.0 ($M + Na^+$), 351.1 ($M + K^+$).

Methyl 2,3-di-*O*-benzyl-4,6-*O*-(4-methoxybenzylidene)-β-D-galactopyranoside (11)

Sodium hydride [60%, 13.8 g , 346.0 mmol] was washed with anhyd hexanes (3 x 30 mL), then dispersed in anhyd DMF (300 mL) and cooled to 0 °C. A solution of methyl 4,6-*O*-(4-methoxybenzylidene)-β-D-galactopyranoside (**10**) (26.9 g, 86 mmol) in anhyd DMF (78 mL) was added dropwise to the NaH slurry. After 15 min benzyl bromide [BnBr, 25.0 mL, 210.0 mmol] was added dropwise over a 15 min period. The reaction was allowed to warm to room temperature 3 h; later TLC (4:1 toluene–EtOAc) showed completion. The reaction was quenched with MeOH (74 mL), diluted with EtOAc (1000 mL), and washed with H_2O (3 x 390 mL). The combined aq phases were extracted with ether (800 mL). The combined organic phases were dried ($MgSO_4$) and concentrated in vacuo to give 43.3 g of an off-white residue, which was recrystalized from EtOH–acetone in a yield of 72%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.30 (m, 1H), 3.53–3.57 (m, 1H),

3.58 (s, 3H, CH_3), 3.79 (s, 3H, CH_3), 3.85 (dd, 1H, *J* = 10, 5.4 Hz), 3.99 (dd, 1H, *J* = 12.6, 1.2 Hz), 4.07–4.09 (m, 1H), 4.27–4.32 (m, 2H), 4.75–4.80 (m, 3H), 4.90 (d, 1H, *J* = 10.8 Hz), 5.45 (s, 1H), 6.84–6.95 (m, 2H), 7.24–7.52 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.26, 57.07, 66.30, 69.13, 71.89, 73.84, 75.19, 78.41, 79.04, 101.24, 104.65, 113.39, 127.48, 127.61, 127.70, 127.81, 128.00, 128.24, 128.29, 130.41, 138.36,138.83, 159.98. ESIMS (positive-ion): *m/z* = 493.2 (M + H^+), 515.2 (M + Na^+), 531.2 (M + K^+).

Methyl 2,3 di-*O*-benzyl-6-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (12)

A solution of trifluoroacetic acid [CF_3CO_2H, 15.6 mL, 203 mmol] in anhyd DMF (120 mL) over 3A MS at 0 °C was added to a slurry of methyl 2,3-di-*O*-benzyl-4,6-O-4-methoxybenzylidene-β-D-galactopyranoside (**11**) (10.0 g 20.3 mmol), sodium cyanoborohydride ($NaBH_3CN$, 6.4 g, 101.5 mmol) and crushed 3 Á MS (10.0 g) in anhyd DMF (160 mL) at room temperature. After 7 h the mixture was filtered through Celite into iced satd aq $NaHCO_3$ (280 mL). The aq phase was extracted with CH_2Cl_2 (160 mL x 5). The combined organic phases were washed with satd aq $NaHCO_3$ (280 mL), H_2O (280 mL), and satd aq NaCl (280 mL), dried with MgSO4, and concentrated in vacuo to give a white solid. This was purified by flash column chromatography (7:1 toluene–EtOAc) to yield 76% of colorless oil, which slowly solidified to a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.35 (ddd, 1H, *J* = 5,4.6, 1.2 Hz), 3.42–3.58 (m, 2H), 3.55 (s, 3H, CH_3), 3.69–3.84 (m, 3H), 3.79 (s, 3H, CH_3), 4.28 (d, 1H, *J*= 7.5 Hz), 4.60 (d, 1H, *J* =11.5 Hz), 4.7–4.96 (m, 3H), 6.82–6.89 (m, 2H), 7.21–7.42 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$):

δ 55.24, 57.02, 61.99, 72.11, 73.35, 73.52, 74.36, 75.15, 79.73, 82.19, 105.03, 113.79, 127.53, 127.60, 127.67, 128.06, 128.27, 128.42, 130.25, 138.39, 138.73, 159.42. ESIMS (positive-ion): *m/z* = 495.2 ($M + H^+$), 517.2 ($M + Na^+$), 533.1 ($M + K^+$).

Methyl 2,3 di-*O*-benzyl-4-*C*-cyano-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (14)

To a solution of methyl 2,3-di-*O*-benzyl-4,6-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (2.7 g, 5.5 mmol) in dry CH_2Cl_2 (15 mL) and dry pyridine (1.4 mL, 16.4 mmol) at –10 °C (ice–acetone) was added dropwise trifluoromethanesulfonic anhydride Tf_2O (1.1 mL, 6.6 mmol). The reaction was allowed to warm to room temperature within 3 h. TLC (3:2 hexanes–EtOAc) showed complete consumption of the starting material. The orange solution was passed through a plug of silica gel (3:1 hexanes–EtOAc) to yield the crude trifate as a pale-yellow syrup that was used as follows without further purification.

To the triflate in dry DMF (15 mL) at –45 °C was added tetrabutylammonium cyanide Bu_4NCN [5 mL, 1.8 M soln in THF, 1.5 equiv]

(CAUTION! Bu_4NCN is highly toxic: all manipulations were carried out with gloves and in a fume hood). The stirring was continued for 10 min at 0 °C and allowed to warm to room tenperature. An hour later TLC (3:2 hexanes–EtOAc) showed complete consumption of the triflate. Cold 1 M aq HCl was added, and the organic layer was washed with satd NH_4Cl solution and H_2O (5 mL x 3). The

organic layer was dried over anhydr $MgSO_4$, then filtered and concentrated to orange syrup. Flash column chromatography (8:1 hexanes–EtOAc) gave a yellow syrup in a yield of 32%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.97 (t, 1H, *J* = 11 Hz), 3.13 (t, 1H, *J* = 8.0 Hz), 3.55 (s, 3H, CH_3), 3.64–3.82 (m, 4H), 3.80 (s, 3H, CH_3), 4.27 (d, 1H, *J* = 7.8 Hz), 4.51 (d, 1H, *J* = 12 Hz), 4.57 (d, 1H, *J* = 11.4 Hz), 4.69 (d, 1H, *J* = 10.8 Hz), 4.84–4.93 (m, 3H), 6.82–6.91 (m, 2H), 7.28–7.38 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 36.07, 55.24, 57.18, 69.18, 72.17, 73.44, 74.93, 75.84, 79.28, 81.73, 104.73, 113.81, 117.71, 127.82, 128.03, 128.06, 128.30, 128.41, 129.36, 129.57, 137.3, 138.02, 159.31. ESIMS (positive-ion): *m/z* = 526.2 (M+ Na^+), 542.2 (M+ K^+).

Methyl 2,3 di-*O*-benzyl-4-deoxy-4-*C*-formyl-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (15)

To a solution of methyl 2,3-di-*O*-benzyl-4-*C*-cyano-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside **14** (1.2 g, 2.4 mmol) at –78 °C was added diisobutylaluminum hydride (DIBAL-H, 14.4 mL, 1 M soln in toluene, 6 equiv), and the reaction was allowed warm to room temperature overnight. TLC (3:2 hexane–EtOAc) showed complete consumption of the starting material. The clear solution was cooled to –10 °C, stirred vigorously, and treated dropwise with 1:1 MeOH–THF (5 mL) and 1 N HCl (5 mL) stirred for 10 min at room temperature, diluted with Et_2O (70 mL), washed with aq 1 N HCl (15 mL x 2) and H_2O (10 mL x 3). The organic layer was dried with $MgSO_4$ and concentrated to give crude pale yellow syrup. Flash column chromatography (5:1 Hexanes–

EtOAc) gave a yellow syrup in a yield of 74%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.98 (dt, 1H, *J* = 10.5, 2.4 Hz), 3.45 (t, 1H, *J* = 8.1 Hz), 3.57–3.67 (m, 1H), 3.57 (s, 3H, CH_3), 3.74–3.8 (m, 1H), 3.81 (s, 3H, CH_3), 3.96 (t, 1H, *J* = 10.5 Hz), 4.32 (d, 1H, *J* = 7.8 Hz), 4.40–4.58 (m, 3H), 4.70–4.87 (m, 3H), 4.95 (d, 1H, *J* = 11 Hz), 6.87–6.90 (m, 2H), 7.22–7.37 (m, 12H), 9.68 (d, 1H, *J* = 2.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.50, 57.36, 57.76, 70.5, 72.07, 73.36, 74.98, 75.37, 78.97, 83.03, 104.97, 114.07, 127.77, 127.97, 128.06, 128.29, 128.31, 128.62, 128.65, 129.70, 129.86, 138.19, 138.62, 159.57, 200.57. ESIMS (positive-ion): *m/z* = 529.1 ($M + Na^+$), 545.1 ($M + K^+$).

2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (3)

A suspension of 2-acetamido-2-deoxy-D-glucose (10.0 g, 45.2 mmol), allyl alcohol (200 mL), and boron trifluoride·diethyl etherate ($BF_3{\cdot}Et_2O$, 1.3 mL) was reflux for 2 h (TLC 4:1 MeCN–H_2O). The hot solution was filtered to remove impurities, which might have been present in the starting material. The filtrate was evaporated, and the solid residue was dried in vacuo overnight. The crude product was suspended in dioxane (500 mL), benzyl chloride BnCl (40 mL) and powdered potassium hydroxide (KOH, 30 g) were added, and the mixture was heated under reflux for 4 h (TLC 1:1 hexane–EtOAc). After evaporation, the orange residue was extracted with CHCl3–H2O, the organic phase was dried ($MgSO_4$) and evaporated, and the residue was co-evaporated with toluene and dried in vacuo overnight. The crude product was suspended in anhydr dimethyl sulfoxide (DMSO, 60 mL) and treated with potassium *tert*-butoxide (KO^tBu, 30 g).

While the mixture was stirred for 3 hours at 100°C (TLC1:1 hexane–EtOAc). The solultion turned dark brown. After being cooled, the solution was diluted with H_2O (250 mL), and the brown precipitate was filtered off, dissolved in acetone (125 mL), and treated with 2 M HCl (200 mL) under reflux for 30 min. H_2O (1 L) was added and the pale-brown precipitate was filtered off. Recrystallization from MeOH afforded colourless needles in a 40% yield; mp 214–216 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.82 (s, 3H, CH_3), 3.65 (m, 3H), 3.76 (m, 2H), 4.02 (m, 1H), 4.12 (m, 1H), 4.54 (m, 5H), 4.8 (m, 2H), 5.2 (t, 1H, *J* = 3.6 Hz), 5.35 (d, 1H, *J* = 9 Hz), 7.16– 7.37 (m, 15H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.8, 53.4, 69.1, 71.1, 73.8, 75.2, 75.4, 79.0, 79.7, 92.4, 128.1, 128.3, 128.4, 128.7, 128.8, 128.83, 128.9, 138.1, 38.2, 138.7, 170.4. NMR spectral data matched that of the known compound.[119,120]

(2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl) tributylstannane (19)

(Tributylstannyl)lithium was prepared by the addition of tributyltin chloride (Bu_3SnCl, 5.2 mL, 19.0 mmol) to pieces of Li (0.34 g, 48.8 mmol) in THF (20 mL). After 10 to 30 min, heat evolved and LiCl precipitated, then the solution slowly turned dark green. After 2 h the dark green solution was stored in the refrigerator overnight. Prior to use the dark-green reagent was removed with a syringe from the residual Li.

At 20 °C and under argon, glucosyl derivative **3** (3.0 g, 6.1 mmol) was suspended in toluene (10 mL) and $CHCl_3$ (10 mL) and treated with freshly distilled thionyl chloride $SOCl_2$ (22.5 mL, 0.3 mmol) for 30 min. The solution was evaporated and co-evaporated with $CHCl_3$ (50 mL) to give the corresponding α-D-glucopyranosyl chloride as a yellow crystalline moisture-sensitive solid. This solid was dissolved in THF (50 mL) and added within 15 min to the solution of Bu_3SnLi at –78 °C. The mixture warmed to 20 °C, diluted with H_2O, and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phase was dried ($MgSO_4$) and evaporated to give a yellow oily compound. The yellow oil was purified by flash chromatography (4:1, 3:1 hexanes–EtOAc) and gave tin glucosyl derivative **19** in a 78% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.78–1.00 (m, 15H, CH_2, CH_3), 1.2–1.36 (m, 6H, CH_2), 1.77 (s, 3H, CH_3), 3.25 (m, 1H), 3.38 (t, 1H, *J* = 9.2 Hz), 3.44 (d, 1H, *J* = 11.6 Hz), 3.62 (t, 1H, *J* = 9.2 Hz), 3.68 (s, 2H), 4.15 (dt, 1H, *J* = 9.4, 11.6 Hz), 4.52– 4.67 (m, 4H), 4.8– 4.93 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 9.2, 13.7, 27.3, 29.1, 54.7, 69.4, 73.4 74.6, 74.9, 79.3, 83.4, 85.5, 127.3, 127.7, 127.9, 128.3, 128.4, 138.3, 138.6, 169.4. NMR Spectra data matched that of the known compound.[119,120]

Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (22)

The procedure described for preparation of methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**8**) was adopted. Recrystallisation from MeOH gave the tetra-*O*-acetyl glucopyranoside (**22**) as white needles in a 79% yield; mp 94–96 °C. ^{1}H

NMR (300 MHz, $CDCl_3$): δ 1.98 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 3.49 (s, 3H, CH_3), 3.48–3.50 (m, 1H), 4.13 (dd, 1H, 10.5, 1.8), 4.26 (dd, 1H, *J* = 10.5, 1.8 Hz), 4.41 (d, 1H, *J* = 8.1 Hz), 4.97 (t, 1H, *J* = 9.6 Hz), 4.97 (dd, 1H, *J* = 9.6, 8.1 Hz), 5.19 (dd, 9.6, 7.8). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.79, 20.83, 20.85, 20.94, 57.29, 62.08, 68.56, 71.41, 71.96, 73.05, 101.82, 169.64, 170.32, 170.53, 170.95. NMR spectral data matched that of the known compound.[126]

Methyl β-D- glucopyranoside (23)

The procedure described for the preparation of methyl β-D-glucopyranoside (**9**) was adopted. Recrystallisation from absolute EtOH gave the methyl β-D-glucopyranoside (**23**) as a white amorphous solid in a % yield; mp 104–106 ^oC ^1H NMR (300 MHz, $CDCl_3$): δ 3.1–3.26 (m, 1H), 3.18 (s, 3H, CH_3), 3.32 (dd, 1H, J=9.9, 7.8 Hz), 3.39–3.46 (m, 2H), 3.53 (dd, 1H, *J* = 12.3, 5.4 Hz), 3.63 (dd, 1H, *J* = 12.3, 1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 54.97, 60.49, 69.50, 69.50, 71.18, 71.54, 73.02, 73.05, 103.84. NMR spectral data matched that of the known compound.[126,141]

Methyl 4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside (24)

The procedure described for the preparation of methyl 4,6-*O*-(4-methoxybenzylidene)-β-D-galactopyranoside (**10**) was adopted. Methyl 4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside (**24**) was precipitated out as a white

solid in 85% yield and was washed with hexanes, then water: mp 176–178 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.71 (s, 1H), 2.84 (s, 1H), 3.37 (ddd, 1H, *J* = 10, 9.5,5.0 Hz), 3.40– 3.54 (m, 2H), 3.5 (s, 3H), 3.67–3.76 (m, 2H), 3.73 (s, 3H), 4.25 (d, 1H, *J* = 7.5 Hz), 4.27 (dd, 1H, *J* = 10.5, 5.0 Hz), 5.42 (s, 1H), 6.82– 6.84 (d, 2H, *J* = 8.7 Hz), 7.34– 7.36 (d, 2H, *J* = 8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.24, 57.40, 66.28, 68.55, 73.10, 74.40, 80.46, 101.75, 104.08, 113.65, 127.56, 129.40, 160.19. NMR spectral data matched that of the known compound.[127]

Methyl 2,3-di-O-benzyl-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside (25)

The procedure described for the preparation of methyl 2,3-di-*O*-benzyl-4,6-*O*-(4-methoxybenzylidene-β-D-galactopyranoside (**11**) was adopted. Recrystallisation from EtOH–acetone gave white crystals of methyl 2,3-di-*O*-benzyl-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside (**25**) in a yield of 76%: mp 150–151 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.41 (td, 1H, *J* = 10.0, 5.0 Hz), 3.45 (dd, 1H, *J* = 9.0, 7.5 Hz), 3.59 (s, 3H), 3.67 (dd, 1H, *J* = 10.5, 9.0 Hz), 3.77 (m, 2H), 3.82 (s, 3H), 4.35 (dd, *J* = 10.5, 5.0 Hz), 4.42 (d, 1H, J=7.5 Hz), 4.82 (m, 2H), 4.85 (m, 2H), 5.54 (s, 1H), 6.90–6.92 (m, 2H), 7.24–7.26 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 57.18, 57.33, 65.89, 68.65, 74.96, 75.15, 80.74, 81.38, 82.11, 101.02, 105.12, 113.50, 127.24, 127.50, 127.57, 127.92, 127.95, 128.19, 128.24,129.77, 138.37, 138.47, 159.93. NMR spectral data matched that of the known compound.[127]

Methyl 2,3 di-*O*-benzyl-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (26)

The procedure described for the preparation of methyl 2,3-di-*O*-benzyl-4,6-*O*-(4-methoxybenzyl)-β-d-galactopyranoside (12) was adopted. Flash chromatography (7:1 toluene–EtOAc) yielded 76% of a colorless oil that slowly solidified to a white solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.33–3.38 (m, 1H), 3.34 (dd, 1H, *J* = 9.0, 9.0 Hz), 3.50 (s, 3H, CH_3), 3.50–3.54 (m, 1H), 3.65 (m, 2H), 3.74 (s, 3H, CH_3), 4.26 (d, 1H, *J* = 7.5 Hz), 4.46 (d, 2H, *J* = 11.8 Hz), 4.75 (d, 2H, *J* = 11.3 Hz), 4.77 (d, 2H, *J* = 11.5 Hz), 6.79–6.83 (m, 2H), 7.18–7.31 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.14, 56.98, 69.90, 71.62, 73.21, 73.92, 74.55, 75.13, 81.67, 83.91, 104.64, 113.73, 127.54, 127.67, 127.83, 127.98, 128.25, 128.39, 129.26, 129.90, 138.42, 138.59, 159.19. NMR spectral data matched that of the known compound.[127]

Methyl 2,3 di-*O*-benzyl-4-*C*-cyano-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (28)

The procedure described for the preparation of methyl 2,3 di-*O*-benzyl-4-*C*-cyano-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (**14**) was adopted. Flash chromatography (8:1 hexanes–EtOAc) gave methyl 2,3 di-*O*-benzyl-4-*C*-cyano-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (**28**) as a yellow syrup in a 64% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.01 (t, 1H, *J* = 5.1 Hz), 3.57–3.83 (m, 5H), 3.59 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 4.31 (d, 1H, *J* = 7.8 Hz), 4.52–4.59 (m, 2H), 4.63–4.79 (m, 2H), 4.90–4.95 (m, 2H), 6.90–6.93 (m, 2H),

7.29–7.38 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 34.36, 55.52, 57.46, 69.47, 72.46, 73.72, 75.22, 76.12, 78.57, 80.02, 105.02, 113.71, 117.81, 127.79, 128.12, 128.24, 128.33, 128.36, 128.49, 128.60, 128.71, 129.66, 129.78, 129.87, 137.62, 138.33, 159.61. ESIMS (positive-ion): *m/z* = 526.2 ($M+Na^+$), 542.2 ($M+K^+$).

Methyl 2,3 di-*O*-benzyl-4-deoxy-4-*C*-formyl-6-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (29)

The procedure described for the preparation of methyl 2,3 di-*O*-benzyl-4-deoxy-4-*C*-formyl-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (**15)** was adopted. Flash chromatography (5:1 hexanes–EtOAc) gave methyl 2,3 di-*O*-benzyl-4-deoxy-4-*C*-formyl-6-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (**29)** as a yellow syrup in 76% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.90 (td, 1H, *J* = 5.1, 2.5 Hz), 3.31–3.46 (m, 2H), 3.46 (m, 3H, CH_3), 3.62–3.70 (m, 2H), 3.70 (s, 3H, CH_3), 4.27 (d, 1H, *J* =7.8 Hz), 4.29–4.50 (m, 3H), 4.63 (d, 1H, *J* = 11.4 Hz), 4.67 (d, 1H, *J* = 11.4 Hz), 4.82 (d, 1H, *J* = 12.6 Hz), 6.79 (m, 2H), 6.76–4.83 (m, 12H), 9.82 (d, 1H, *J* = 5.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 53.63, 55.20, 69.53, 71.84, 72.46, 73.26,75.13, 79.09, 80.01, 105.37, 113.68, 127.51, 127.61, 127.69, 127.87, 128.15, 128.20, 129.35, 137.96, 138.11, 159.32, 200.58. ESIMS (positive-ion): *m/z* = 529.1 ($M + Na^+$), 545.1 ($M + K^+$).

Methyl 2,3 di-*O*-benzyl-4-deoxy-4-*C*-formyl-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (15)

Methyl 2,3 di-*O*-benzyl-4-deoxy-4-*C*-formyl-6-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (**29**) was dissolved in dry Et_3N (10 mL) and heated for 48 h at 45 °C. The reaction solution was concentrated and flash chromatography (11:1 hexanes–EtOAc) gave predominantly methyl 2,3 di-*O*-benzyl-4-deoxy-4-*C*-formyl-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (**15**) as a colorless syrup in 71% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.98 (td, 1H, *J* = 10.5, 2.4 Hz), 3.45 (t, 1H, *J* = 8.1 Hz), 3.57–3.67 (m, 1H), 3.57 (s, 3H, CH_3), 3.74–3.8 (m, 1H), 3.81 (s, 3H, CH_3), 3.96 (t, 1H, *J* = 10.5 Hz), 4.32 (d, 1H, *J* = 7.8 Hz), 4.40–4.58 (m, 3H), 4.70–4.87 (m, 3H), 4.95 (d, 1H, *J* = 11 Hz), 6.87–6.90 (m, 2H), 7.22–7.37 (m, 12H), 9.68 (d, 1H, *J* = 2.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.50, 57.36, 57.76, 70.5, 72.07, 73.36, 74.98, 75.37, 78.97, 83.03, 104.97, 114.07, 127.77, 127.97, 128.06, 128.29, 128.31, 128.62, 128.65, 129.70, 129.86, 138.19, 138.62, 159.57, 200.57. ESIMS (positive-ion): *m/z* = 529.1 ($M + Na^+$), 545.1 ($M + K^+$).

Methyl 4-*C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranosylhydroxymethyl)-2,3-di-*O*-benzyl-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (16b)

Under argon at –78 °C, methyllithium (MeLi, 0.5 mL, 1.4 equiv of a 1.6 M solution in hexanes) was added within 10 min to a solution of (2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)tributylstannane (**19**) (420 mg, 0.556 mmol)

in THF (10 mL). The mixture was warmed to –65 ° C, and buthyllithium (BuLi, 0.3 mL, 1.0 equiv of a 1.6 M solution in hexanes) was added within 5 min (the solution turned deep red). The subsequent addition of methyl 2,3-di-*O*-benzyl-4-deoxy-4-*C*-formyl-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (**15**) (429 mg, 0.24 mmol) changed the color of the solution to pale yellow. After 1 h, the reaction was stopped with satd aq NH_4Cl solution, and the mixture was left to warm up to room temperature, then diluted with CH_2Cl_2. The organic layer was washed with H_2O (5 mL x 2) and dried over anhydr $MgSO_4$, then filtered and concentrated to yellow syrup. Flash chromatography (4-1, 3-1 hexane–EtOAc) gave a pale yellow solid in 42% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.54 (s, 3H, CH_3), 2.26 (t, 1H, *J* = 9.0 Hz), 3.07 (t, 1H, *J* = 10.2 Hz), 3.23–3.39 (m, 1H), 3.51 (s, 3H, CH_3), 3.63–3.72 (m, 5H), 3.78 (s, 3H, CH_3), 3.86–3.92 (m, 6H) 3.99 (bs, 1H), 4.84–4.09 (m, 4H), 4.45–4.56 (m, 5H), 4.67–4.69 (m, 2H), 4.76 (d, 1H, *J* = 7.2 Hz), 4.89 (d, 1H, *J* = 12 Hz), 5.07 (d, 1H, *J* = 11.4 Hz), 6.84–6.85 (m, 2H), 7.23–7.37 (m, 27H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.37, 45.71, 52.07, 55.44, 56.90, 68.37, 69.79, 71.14, 73.14, 73.63, 74.09, 74.25, 74.91, 78.42, 78.82, 79.21, 83.66, 104.67, 113.84, 127.81, 127.94, 128.05, 128.10, 128.19, 128.29, 128.38, 128.42, 128.58, 128.66, 128.79, 128.86, 129.62, 130.96, 137.80, 138.23, 138.56, 138.75, 158.90, 170.39. ESIMS (positive-ion): *m/z* = 982.7 ($M + H^+$), 1004.6 ($M + Na^+$), 1020.4 ($M + K^+$).

Methyl 4-*C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranosylmethyl)-2,3-di-*O*-benzyl-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (31)

A mixture of methyl 4-*C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranosylhydroxymethyl)-2,3-di-*O*-benzyl-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (**16b**) (242 mg, 0.25 mmol) and imidazole (1.7 mg), NaH dispersion (80%, 43.3 mg) and dry THF (10 ml) was stirred for 1 h at room temperature. CS_2 (0.25 mg, 4.2 mmol) was added with stirring that continued for 1h. Iodomethane (MeI, 0.07 mL, 1.10 mmol) was added with stirring. TLC of the reaction mixture after an additional h showed complete consumption of the starting alcohol. The mixture was diluted with CH_2Cl_2 and poured into H_2O. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (30 mL x 3). The combined organic layers were dried over anhydr $MgSO_4$ and concentrated to yellow syrup. Flash chromatography (2:1 petroleum ether–EtOAc) gave the thiocarbonyl compound as a pale-yellow syrup.

The thiocarbonyl compound (169 mg, 0.16 mmol) and 2,2'-azobisisobutyronitrile AIBN (1.3 mg) in anhydrous toluene (10 mL) was added to a refluxing solution of *n*-Bu_3SnH (0.23 mL, 0.91 mmol) in anhyd. toluene (5 mL) under argon. Heating was stopped after 40 min, and the reaction was allowed to cool to room temperature. Toluene was added, and the solvent was evaporated. Flash chromatography of the crude material gave methyl 4-*C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranosylmethyl)-2,3-di-*O*-benzyl-4-deoxy-6-*O*-(4-

methoxybenzyl)-β-D-glucopyranoside (**31**) as a colorless syrup in a 78% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85–0.90 (m, 1H), 1.58 (s, 3H, CH_3), 1.81 (m, 1H), 2.26 (t, 1H, *J* = 9.6 Hz), 3.09 (t, 1H, *J* = 10.2 Hz), 3.20–3.23 (m, 1H), 3.32–3.34 (m, 1H), 3.44–3.88 (m, 11H), 3.51 (s, 3H, CH_3), 3.76 (s, 3H, CH_3), 4.25 (d, 1H, *J* = 7.5 Hz), 3.39–4.53 (m, 7H), 4.61-4.76 (m, 3H), 5.16 (d, 1H, *J* = 9 Hz), 6.82–6.86 (m, 2H), 7.22–7.33 (m, 25H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.37, 29.95, 45.75, 52.10, 55.45, 56.90, 68.38, 69.81, 71.17, 73.15, 73.65, 73.90, 74.12, 74.26, 74.91, 78.27, 78.44, 78.84, 79.23, 83.61, 83.67, 104.69,113.87, 127.82, 127.92, 127.95, 128.06, 128.11, 128.21, 128.30, 128.39, 128.43, 128.60, 128.67, 128.80, 128.87, 129.64, 130.97, 138.25, 138.38, 138.58, 138.61, 138.78, 159.21, 170.67. ESIMS (positive-ion): *m/z* = 966.4 ($M + H^+$), 988.4 ($M + Na^+$), 1004.4 ($M + K^+$).

Methyl 4-*C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranosylmethyl)-2,3-di-*O*-benzyl-β-D-glucopyranoside (32)

Ceric ammonium nitrate (CAN, 0.18 g, 0.33 mmol) was added at once to a solution of compound methyl 4-*C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranosylmethyl)-2,3-di-*O*-benzyl-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (**31**) (64 mg, 0.069 mmol) in 4:1 CH_3CN–H_2O (2 mL). After the yellow solution had stirred for 5 min, CH_2Cl_2 (5 mL), H_2O (1 mL), and brine (2 mL) were added. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (5 mL x 3). The combined organic layer was dried with

$MgSO_4$ and evaporated to give a pale-yellow syrup. Flash chromatography (1:1 Petroleum ether-EtOAc) gave the intermediate alcohol (6-OH) **32** in 69% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86–0.89 (m, 1H), 1.50 (s, 3H, CH_3), 2.00–2.02 (m, 1H), 2.26–2.29 (m, 1H), 3.22–3.8 (m, 11H), 3.44 (s, 3H, CH_3), 4.14 (d, 1H, *J* = 7.2 Hz), 4.41–4.59 (m, 7H), 4.64 (d, 1H, *J* = 11 Hz), 4.78–4.87 (m, 4H), 4.93 (d, 1H, *J* = 10.8 Hz), 7.16-7.37 (m, 25H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.13, 29.94, 46.29, 51.55, 56.82, 64.86, 66.59, 69.69, 73.56, 73.76, 74.22, 74.52, 74.91, 75.29, 78.14, 78.97, 79.24, 79.33, 82.05, 83.98, 105.02, 127.50, 127.94, 127.99, 128.06, 128.35, 128.43, 128.49, 128.67, 128.82, 129.04, 137.81, 138.01, 138.47, 138.63, 139.18, 139.42, 172.52. ESIMS (positive-ion): *m/z* = 846.3 (M + H^+), 868.4 (M + Na^+), 884.4 (M + K^+).

Methyl 4-*C*-(2-acetamido-2-deoxy-β-D-glucopyranosylmethyl)-4-deoxy-β-D-glucopyranosiduronic acid (33)

To a solution of the alcohol (34.2 mg, 0.04 mmol) in CH_2Cl_2 (1 mL) containing 2,2,6,6-tetramethyl-1-piperidinyloxy [TEMPO, 0.07 mg, 0.40 μmol] was added a solution of saturd aq $NaHCO_3$ (0.08 mL) containing potassium KBr (0.45 mg, 3.80 μmol) and Bu_4NBr (0.6 mg, 1.9 μmol). The mixture was cooled to 0 °C and a solution of NaOCl (1,3 M, 0.1 mL), $NaHCO_3$ (0.045 mL) and brine (0.09 mL) were added dropwise over 45 min TLC (12:1:1 $CHCl_3$–CH_3OH–AcOH). The two layers were separated, and the organic extract was washed with H_2O (2 mL x 3). The combined aq extracts were acidified with 4 M HCl and extracted with EtOAc (5 x 15 mL), dried over $MgSO_4$, concentrated and dried overnight.

To a solution of the acid in 4:1 MeOH–EtOAc, 28 mg of $Pd(OH)_2$-on-carbon and a drop of HOAc were added under H_2. The mixture was allowed to stir for 3 days and then filtered through Celite. The filtrate was evaporated to give an oily compound. Flash chromatography on LH-20 and HP-20 provided compound **33** in a 34% overall yield. ESIMS (positive-ion): *m/z* = 411.0 ($M + D^+$).

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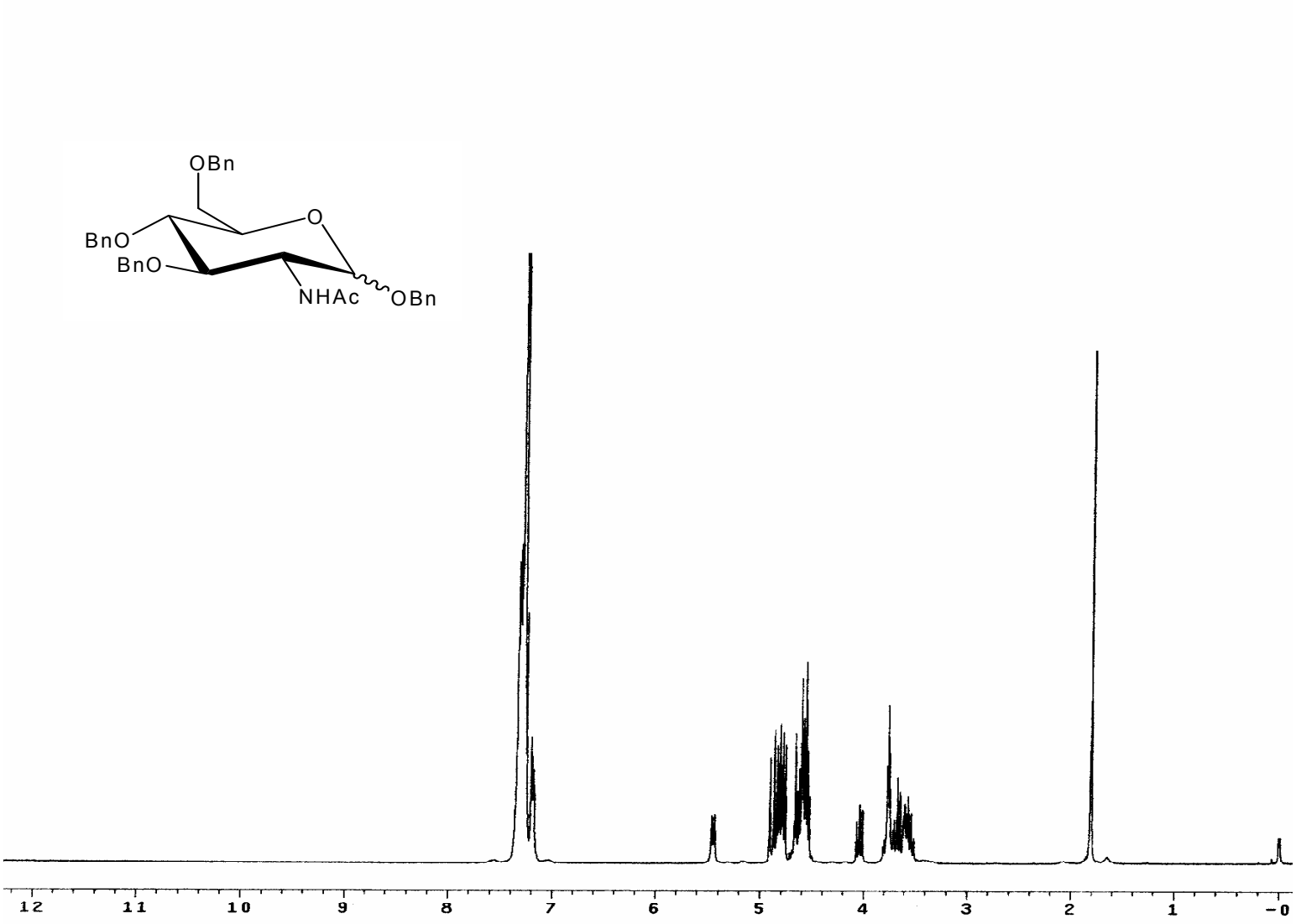
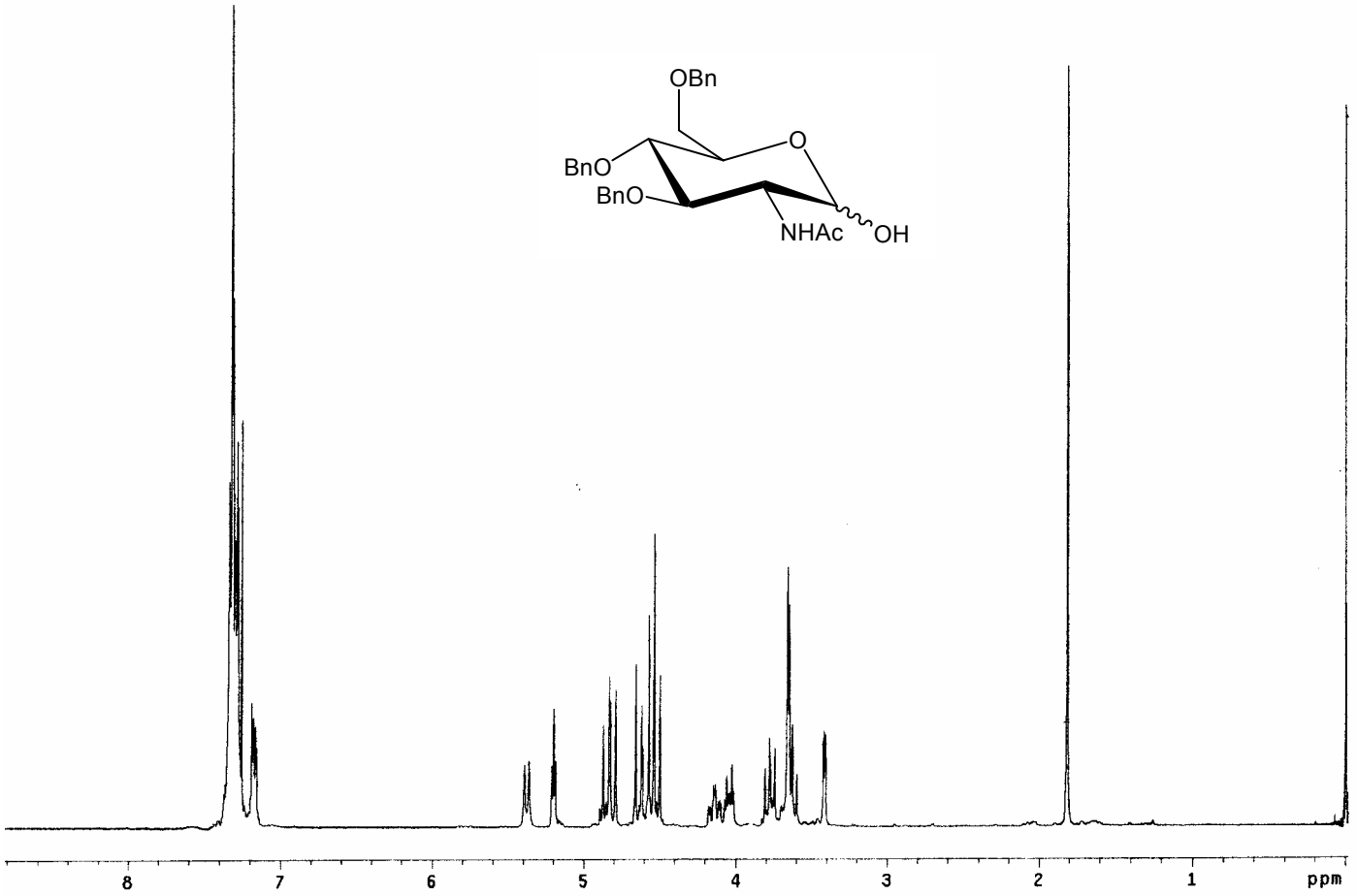
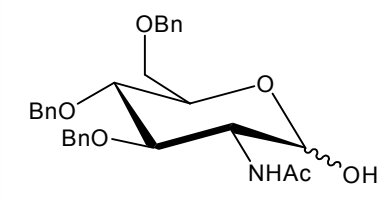
APPENDIX

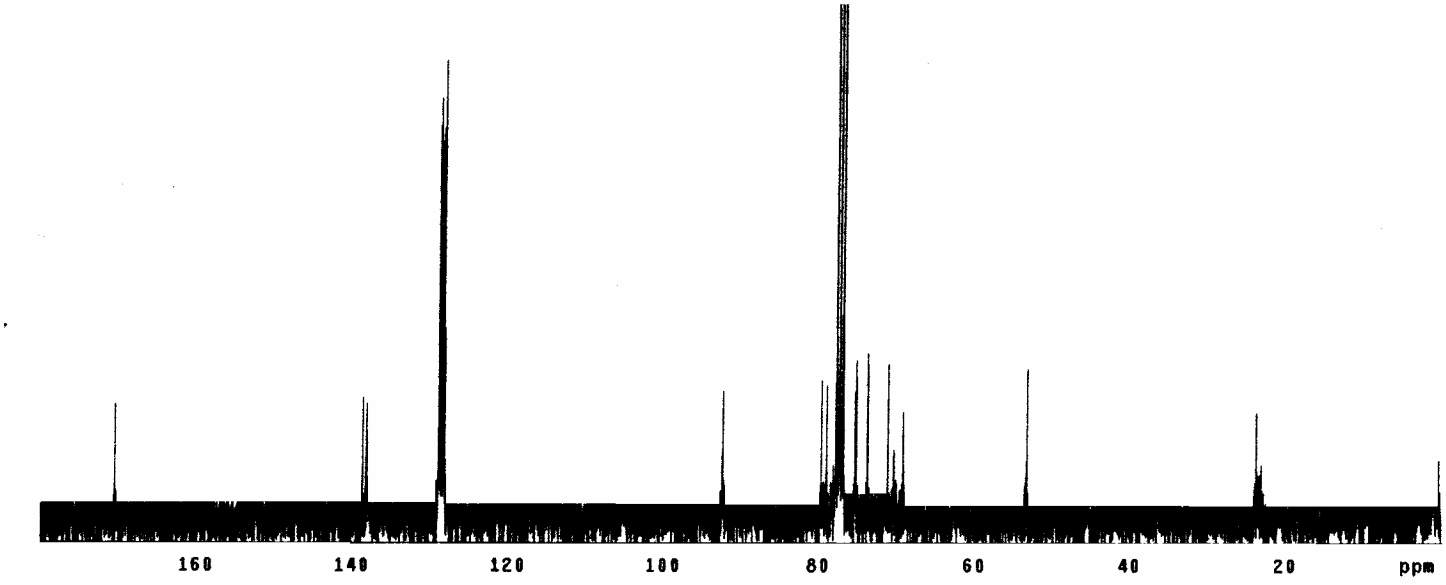


^{1}H NMR (300 MHz, $CDCl_3$) Spectrum for Benzyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranoside **(2)**

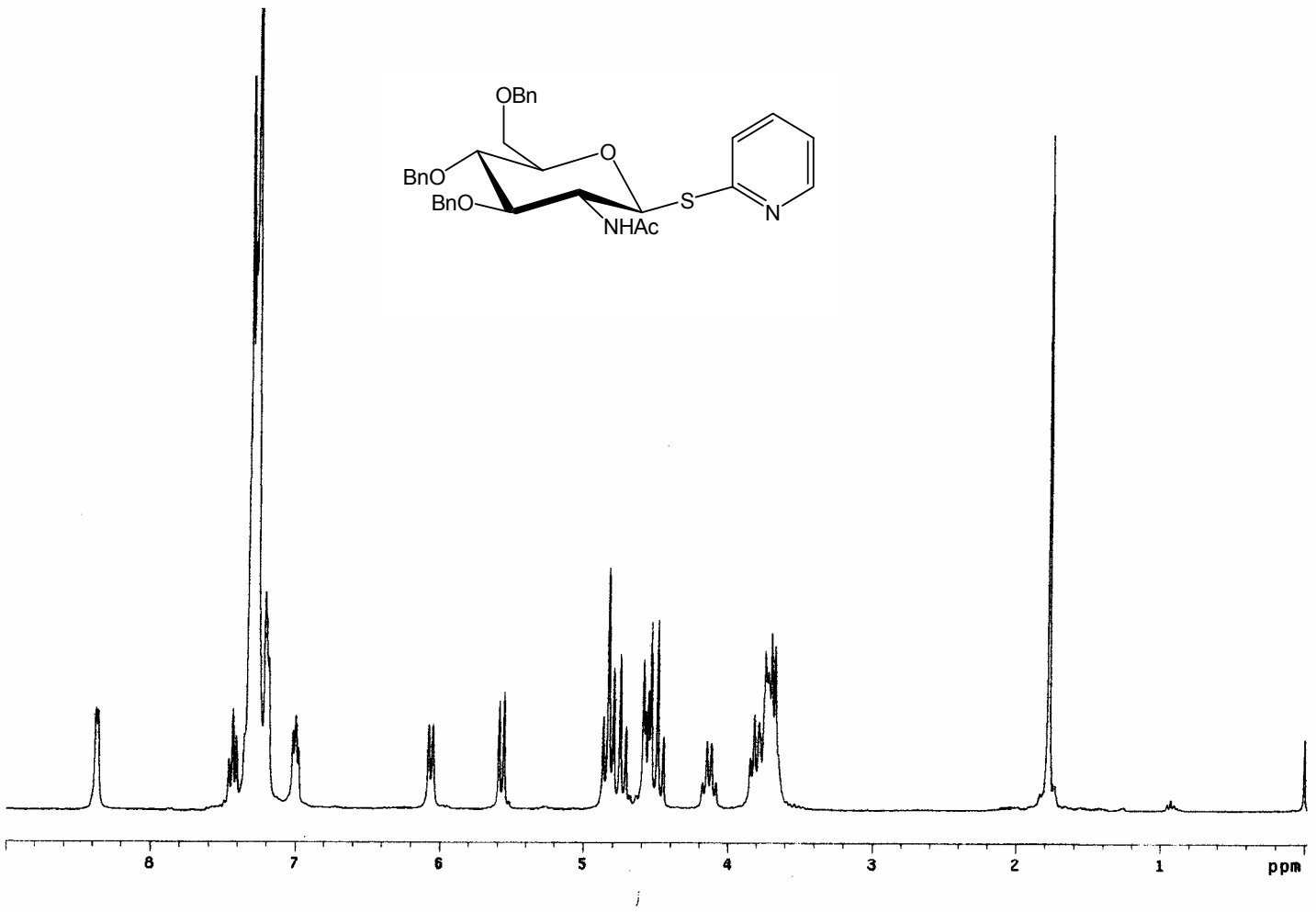


^{1}H NMR (300 MHz, $CDCl_3$) Spectrum for 2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose **(3)**

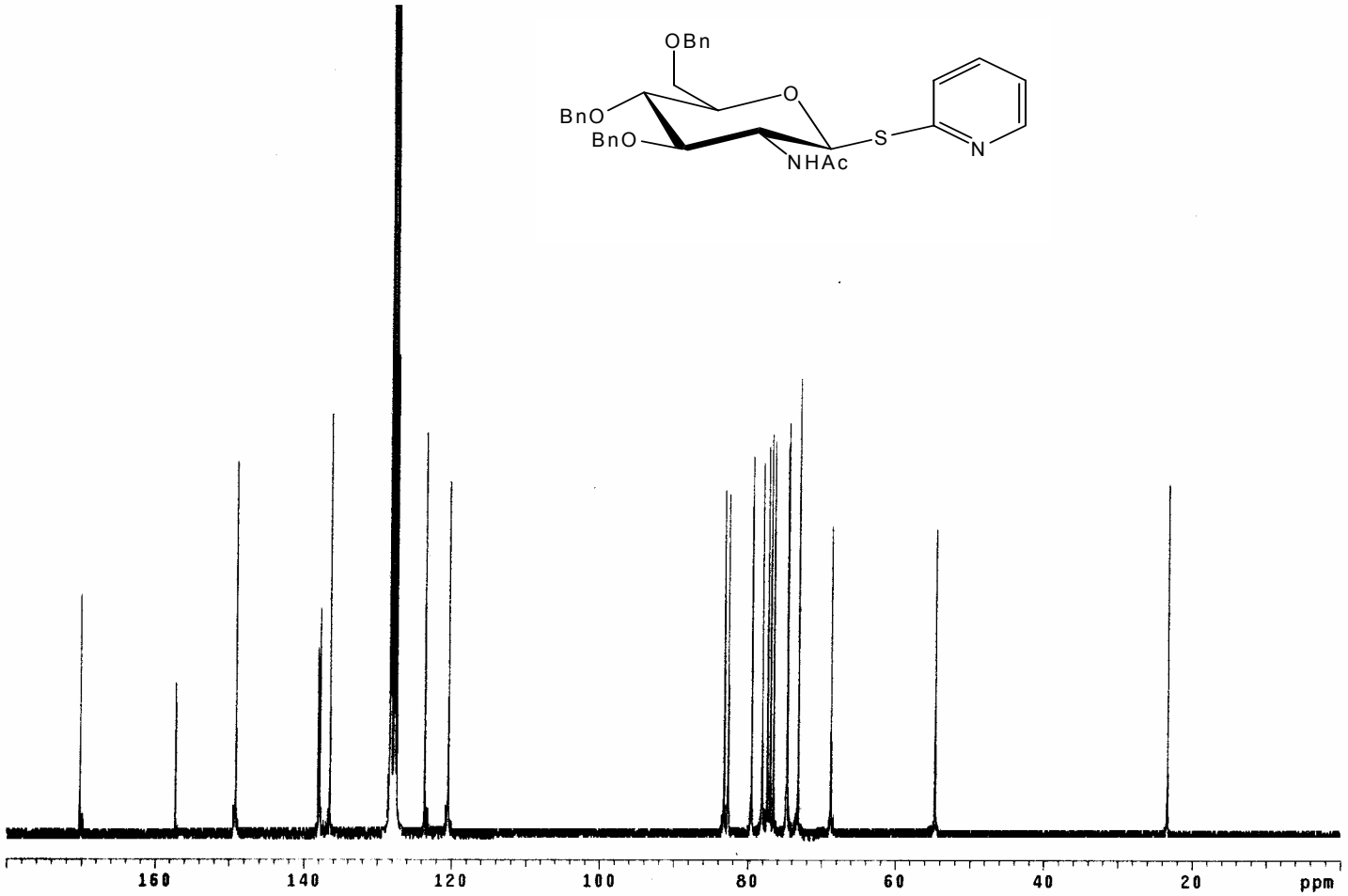




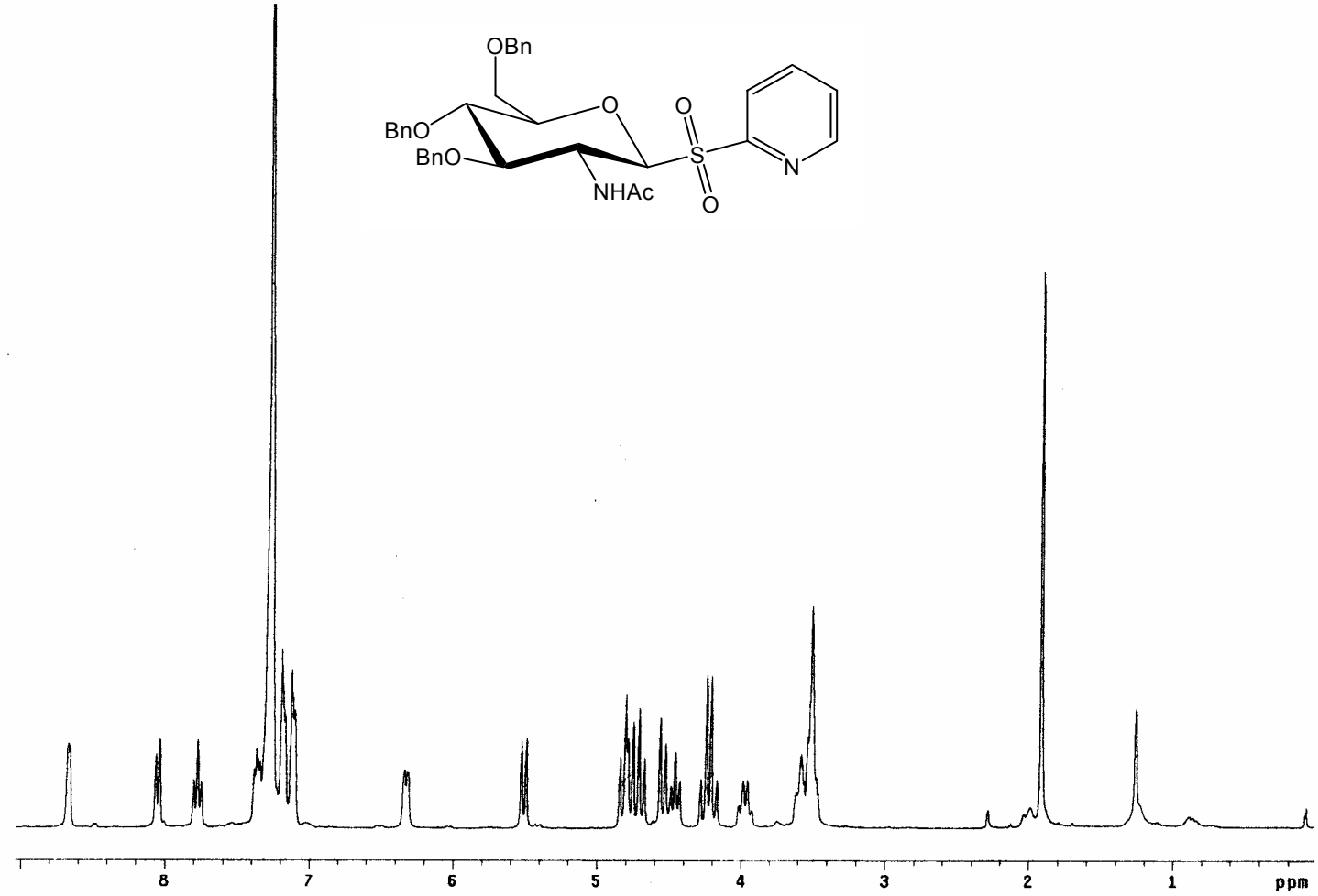
^{13}C NMR (75 MHz, $CDCl_3$) spectrum for 2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose **(3)**



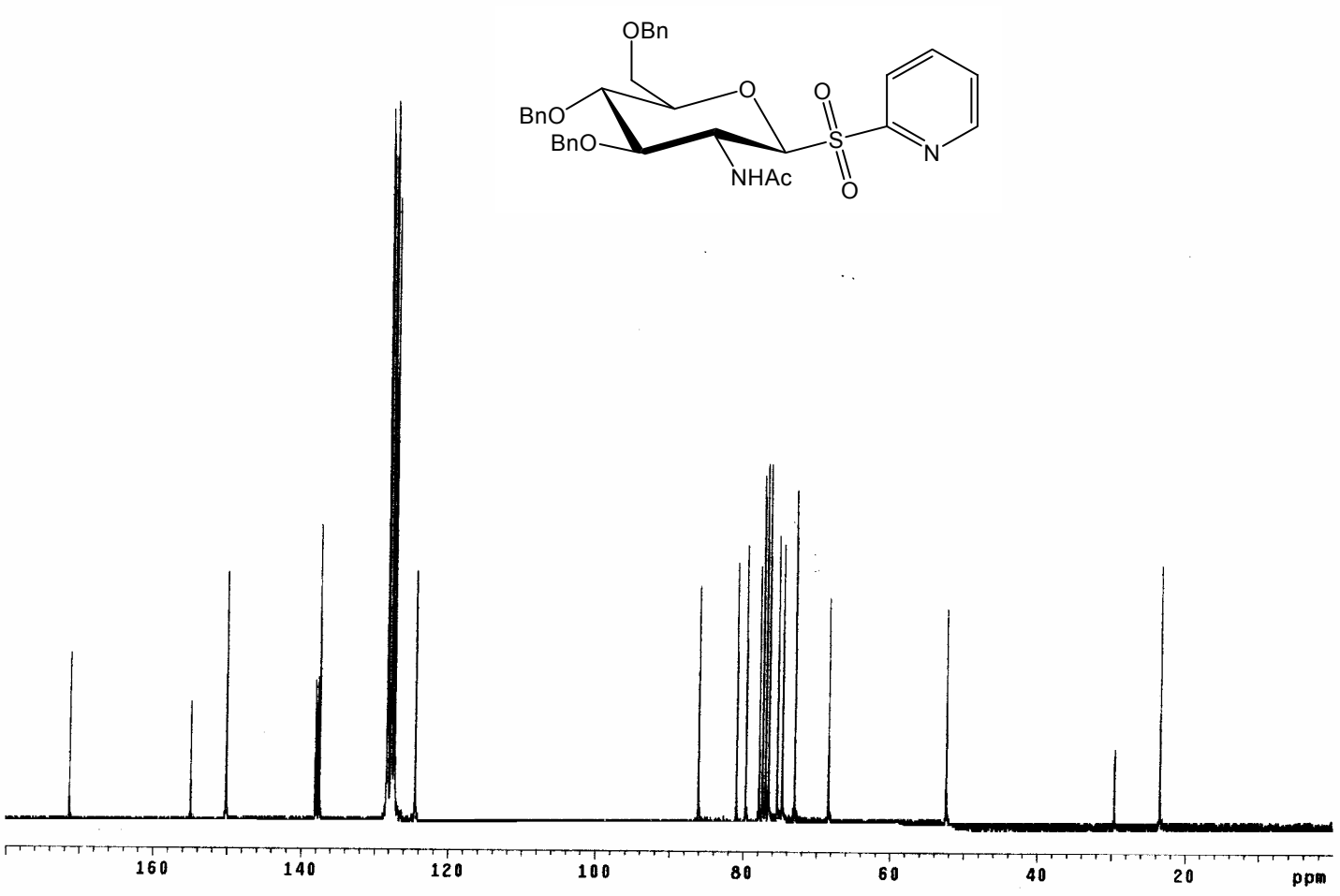
^{1}H NMR (300 MHz, $CDCl_3$) Spectrum for 2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl 2-pyridyl sulfide **(4)**



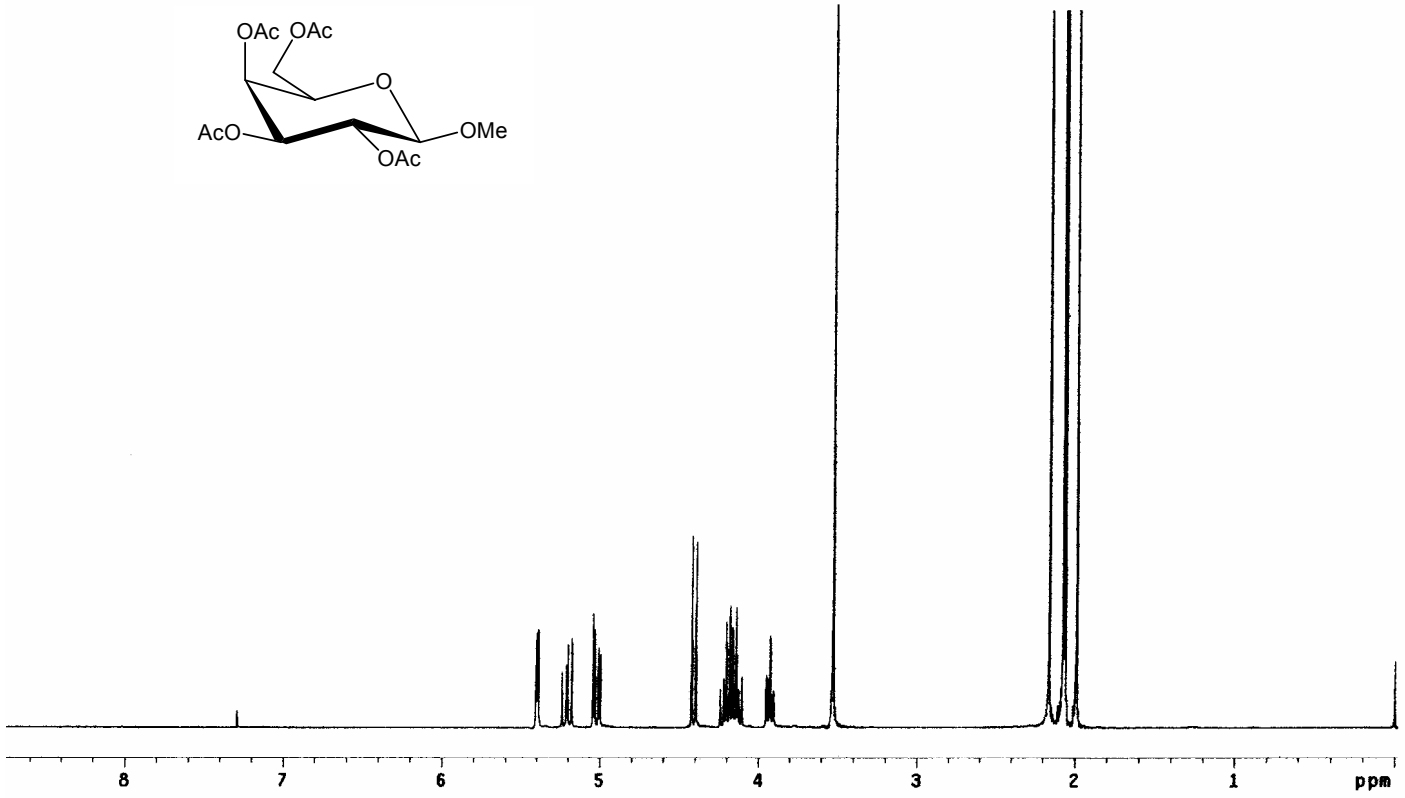
^{13}C NMR (300 MHz, $CDCl_3$) spectrum for 2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl 2-pyridyl sulfide **(4)**



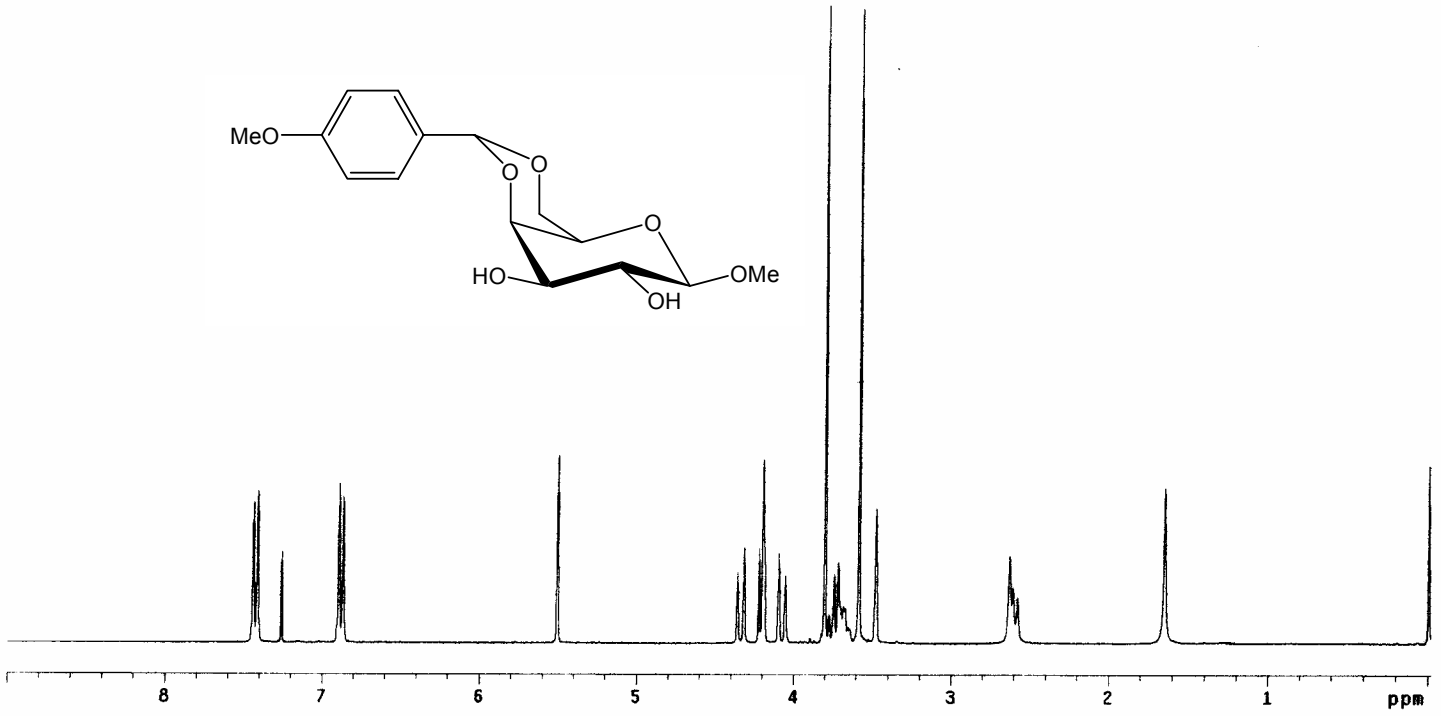
^{1}H NMR (300 MHz, $CDCl_3$) Spectrum for 2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl 2-pyridyl sulfone **(5)**



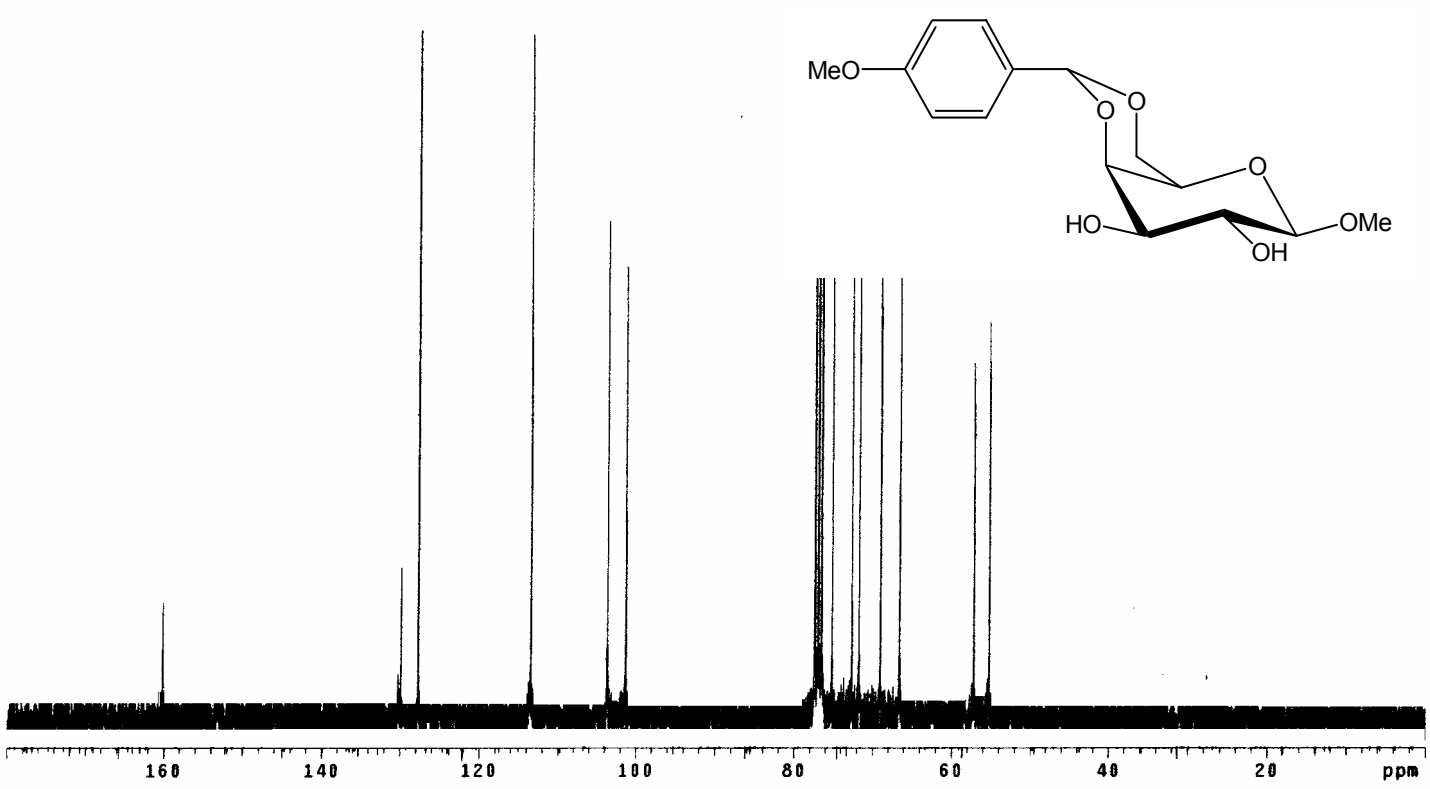
^{13}C NMR (300 MHz, $CDCl_3$) spectrum for 2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl 2-pyridyl sulfone **(5)**



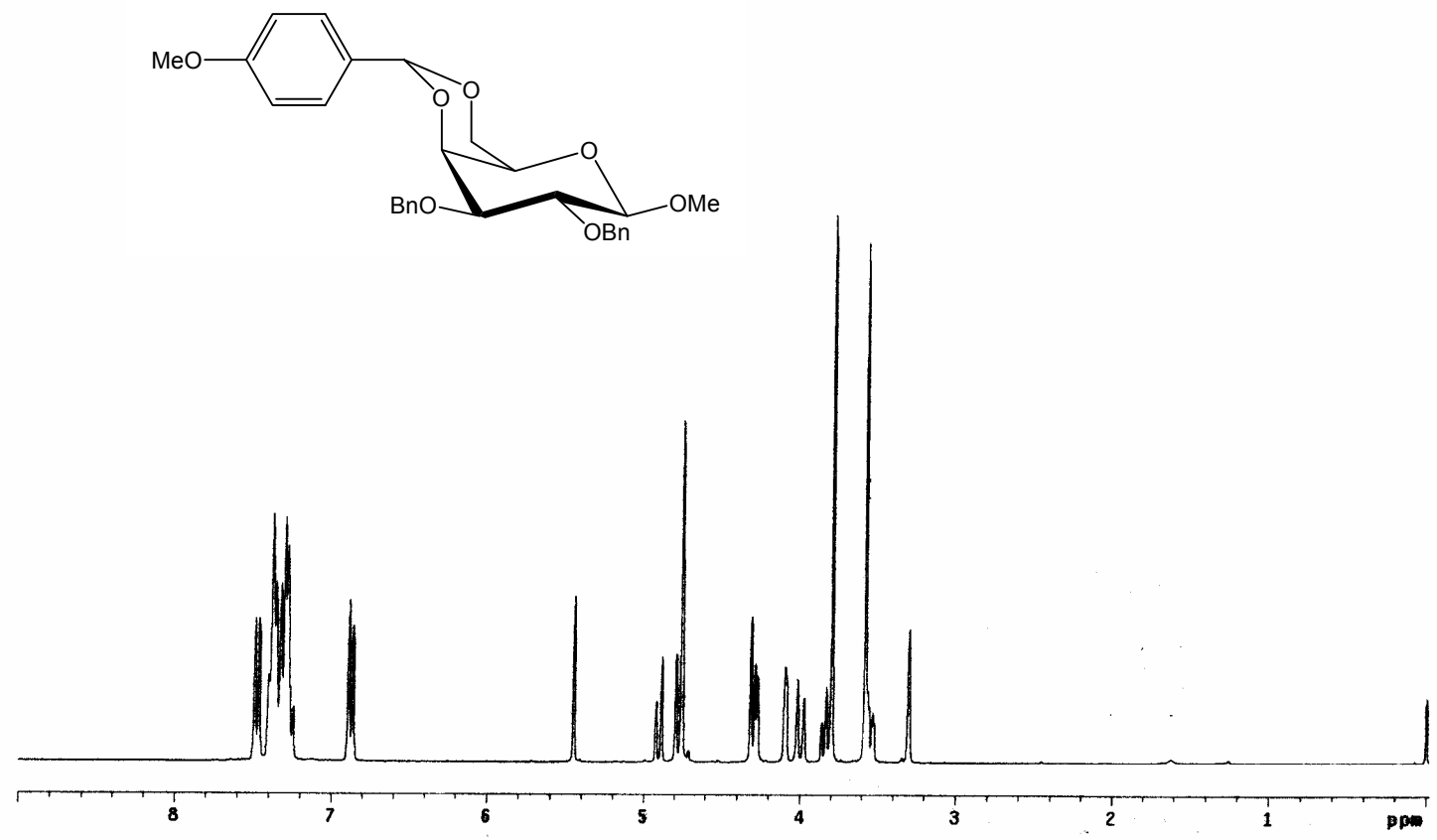
^{1}H NMR (300 MHz, $CDCl_3$) Spectrum for Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside **(8)**



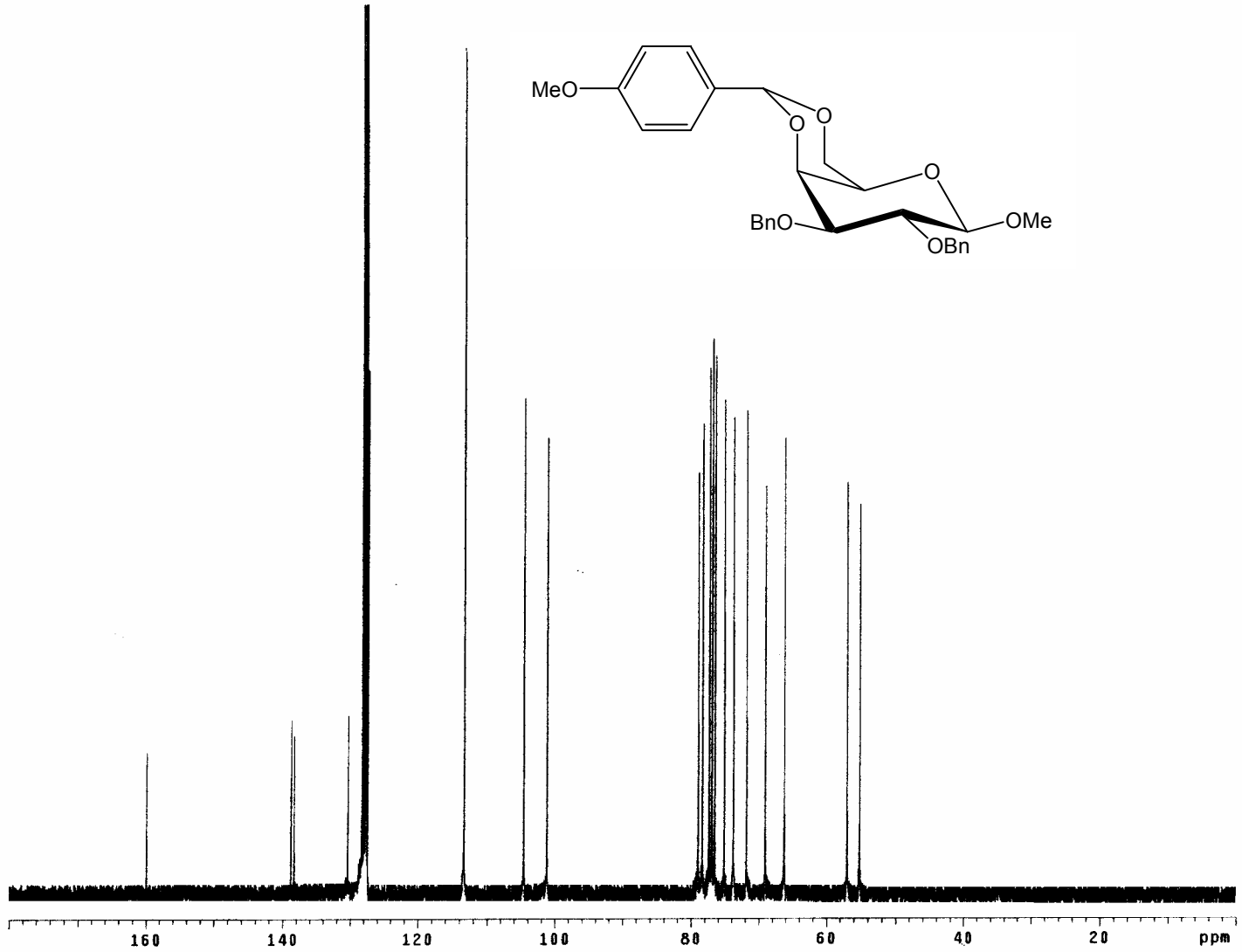
^{1}H NMR (300 MHz, $CDCl_3$) Spectrum for Methyl 4,6-*O*-(4-methoxybenzylidene)-β-D-galactopyranoside **(10)**



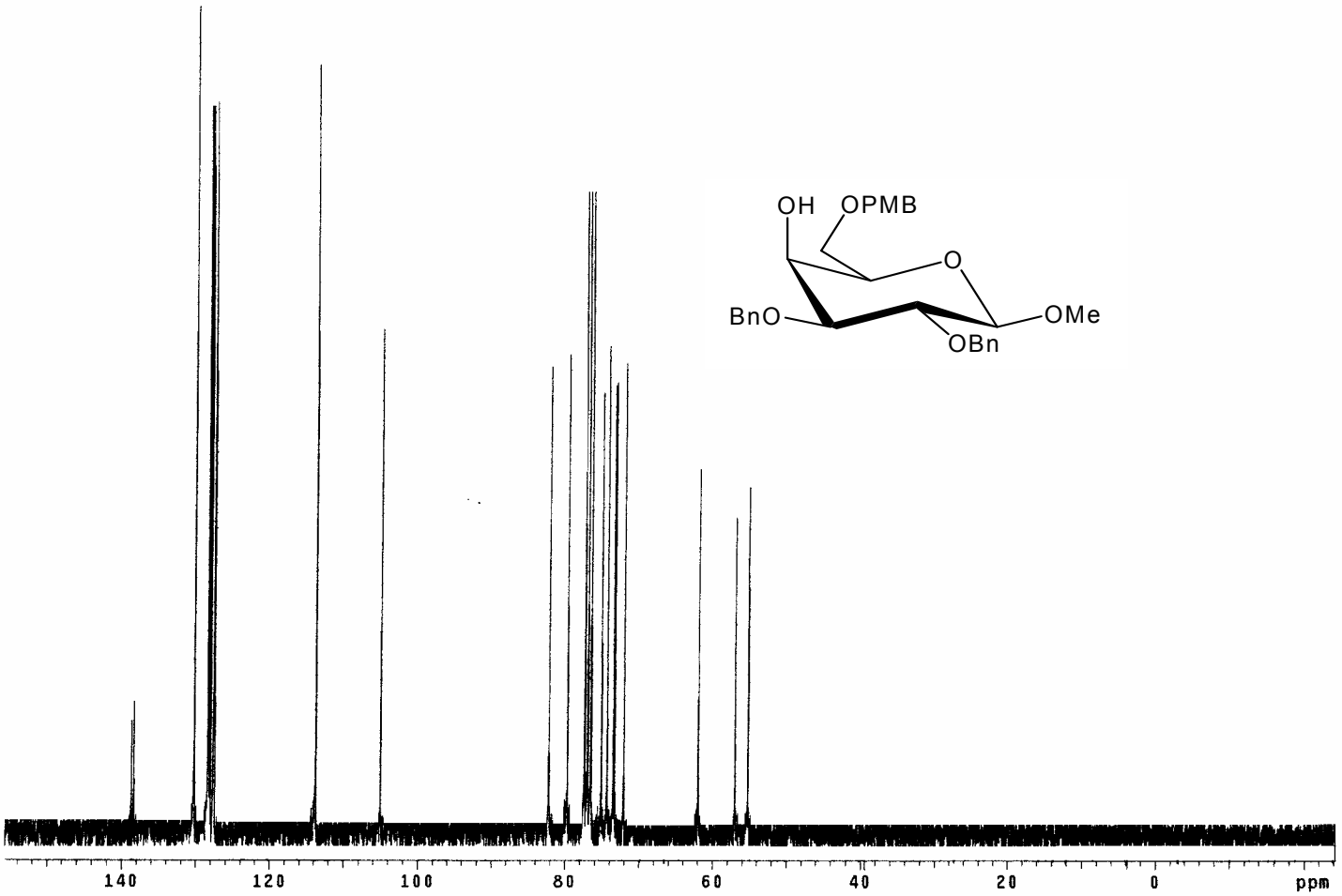
^{13}C NMR (75 MHz, $CDCl_3$) spectrum for Methyl 4,6-*O*-(4-methoxybenzylidene)-β-D-galactopyranoside **(10)**



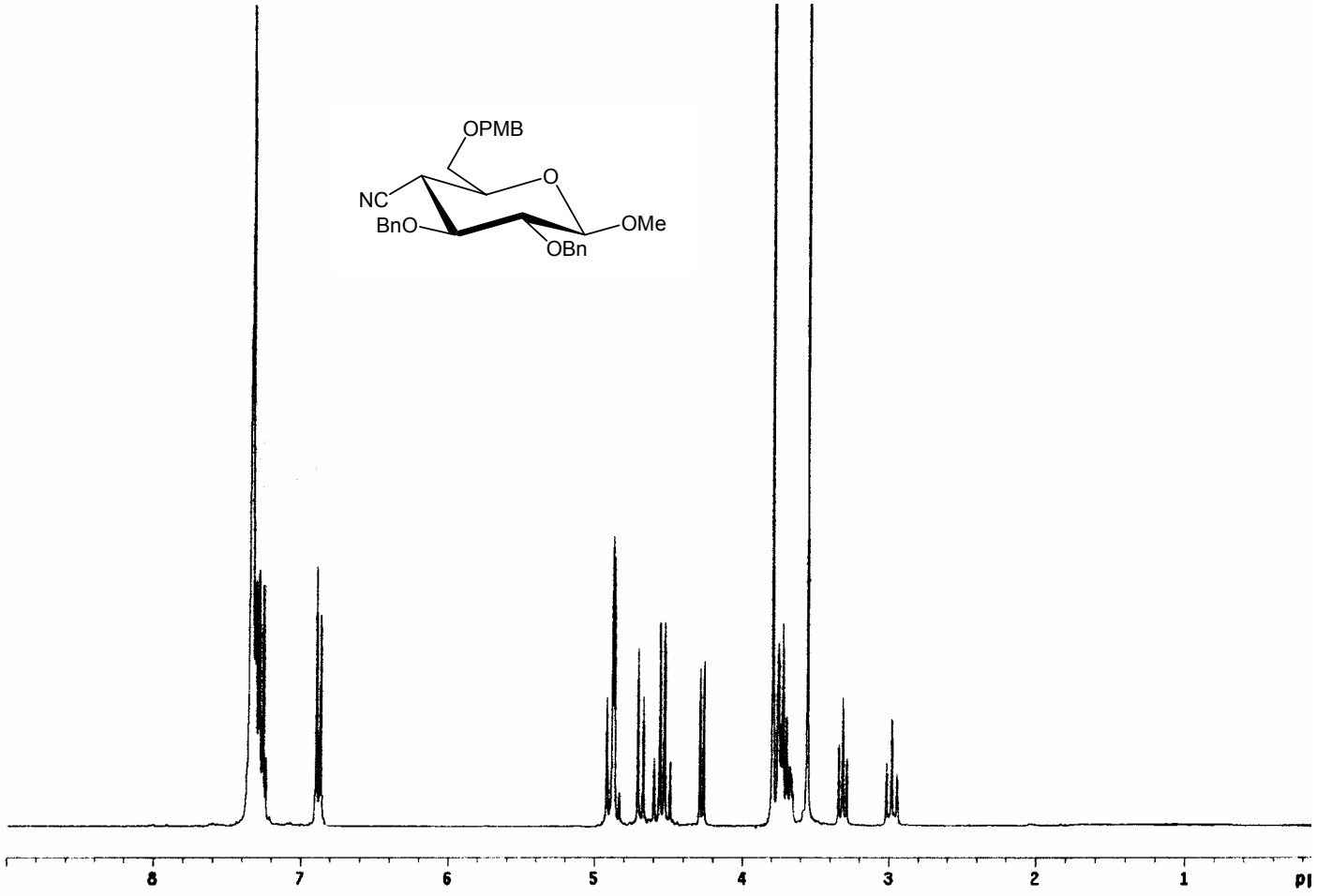
^{1}H NMR (300 MHz, $CDCl_3$) Spectrum for Methyl 2,3-di-*O*-benzyl-4,6-*O*-(4-methoxybenzylidene-β-D-galactopyranoside **(11)**



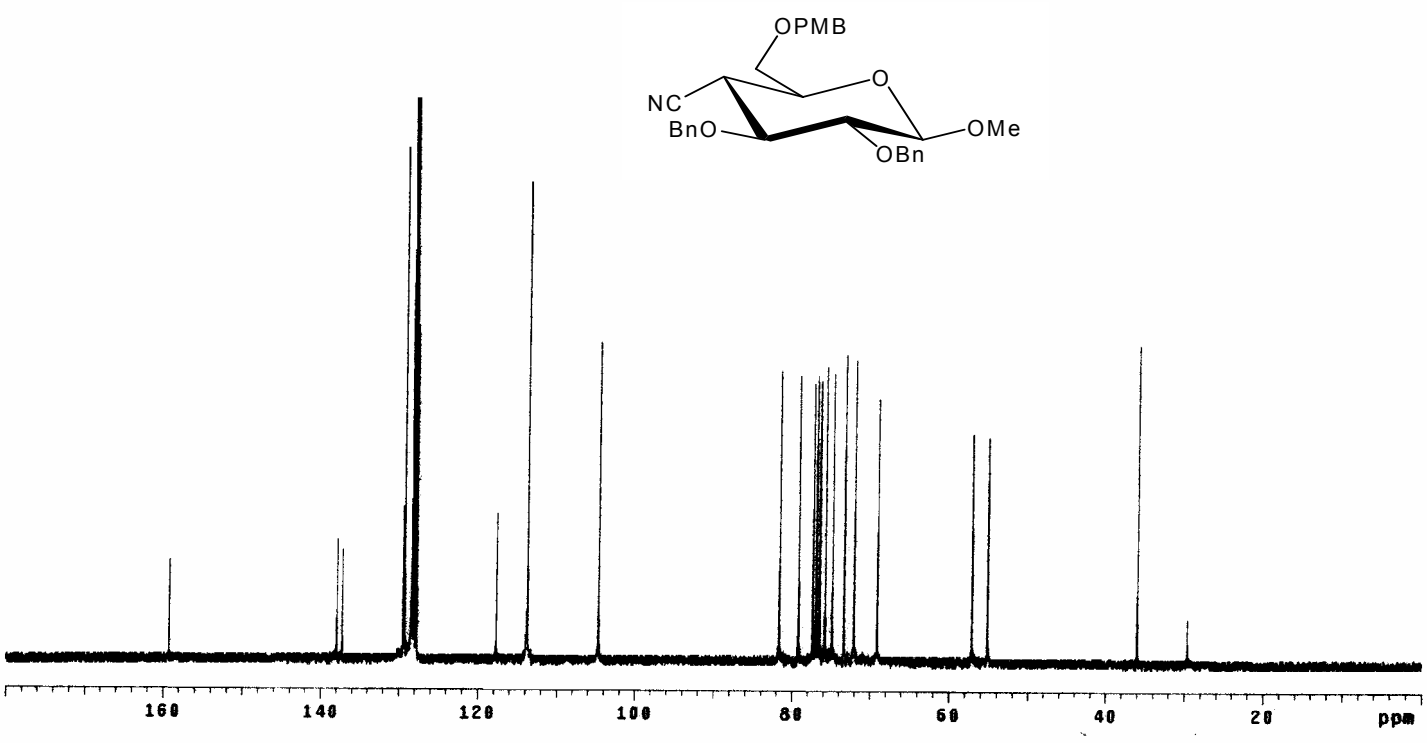
^{13}C NMR (300 MHz, $CDCl_3$) spectrum for Methyl 2,3-di-*O*-benzyl-4,6-*O*-(4-methoxybenzylidene-β-D-galactopyranoside **(11)**



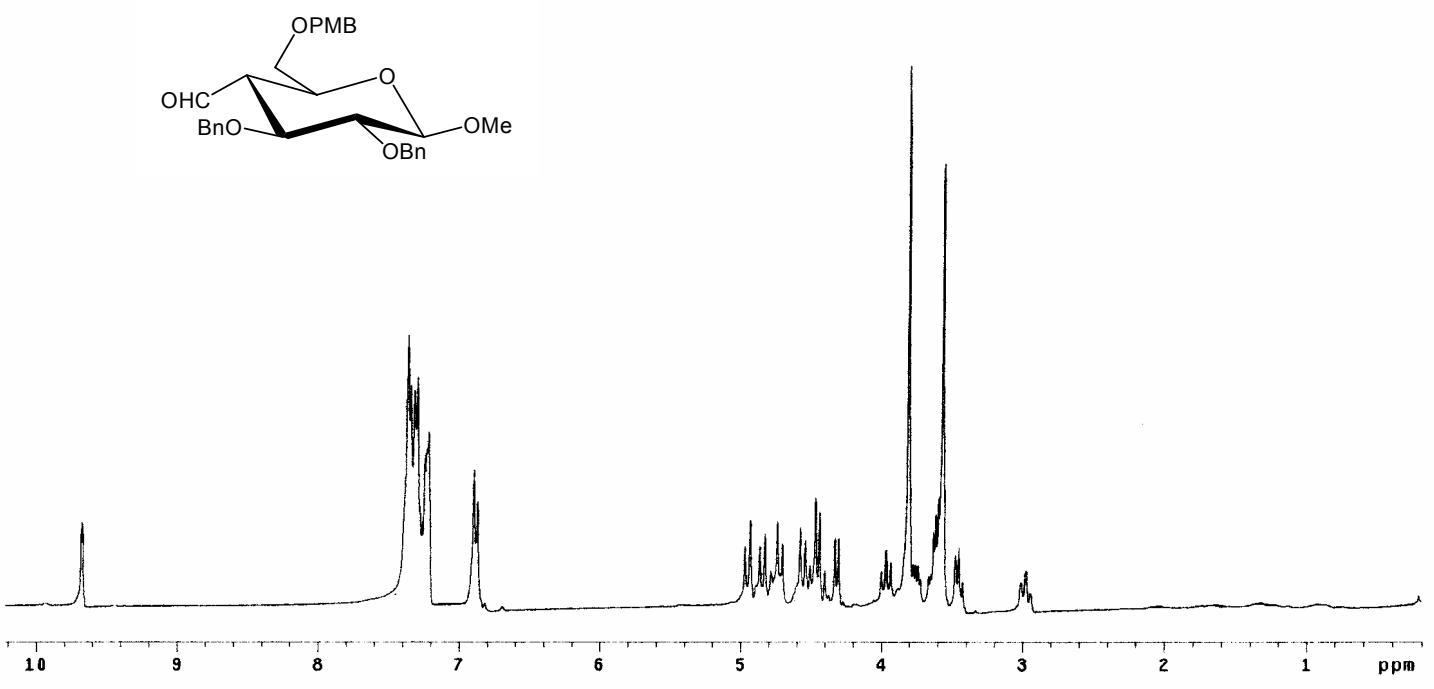
^{13}C NMR (300 MHz, $CDCl_3$) spectrum for Methyl 2,3 di-*O*-benzyl-4,6-*O*-(4-methoxybenzyl)-β-D-galactopyranoside **(12)**



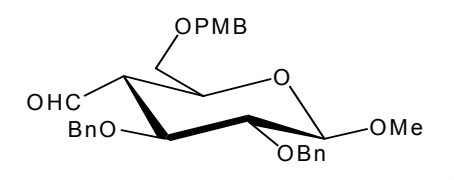
^{1}H NMR (300 MHz, $CDCl_3$) Spectrum for Methyl 2,3-di-*O*-benzyl-4-*C*-cyano-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside **(14)**

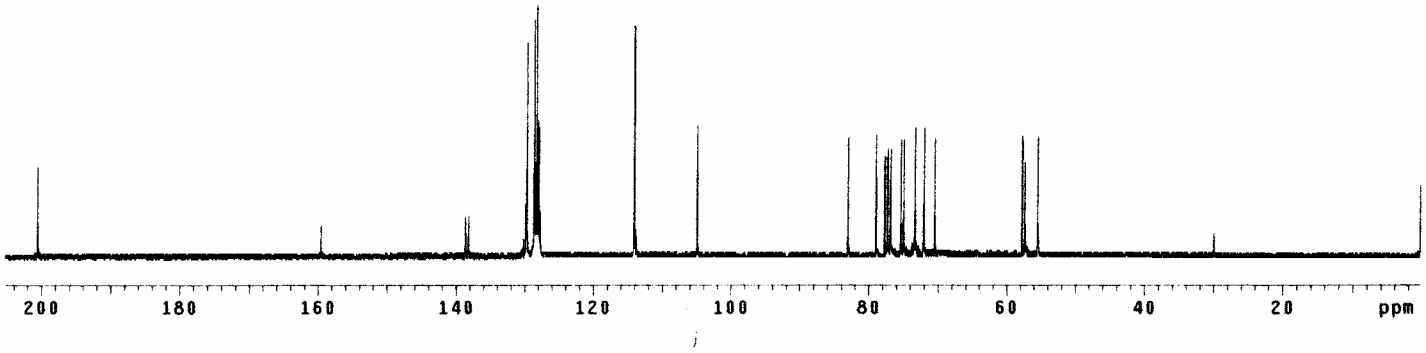


^{13}C NMR (75 MHz, $CDCl_3$) spectrum for Methyl 2,3-di-*O*-benzyl-4-*C*-cyano-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside **(14)**

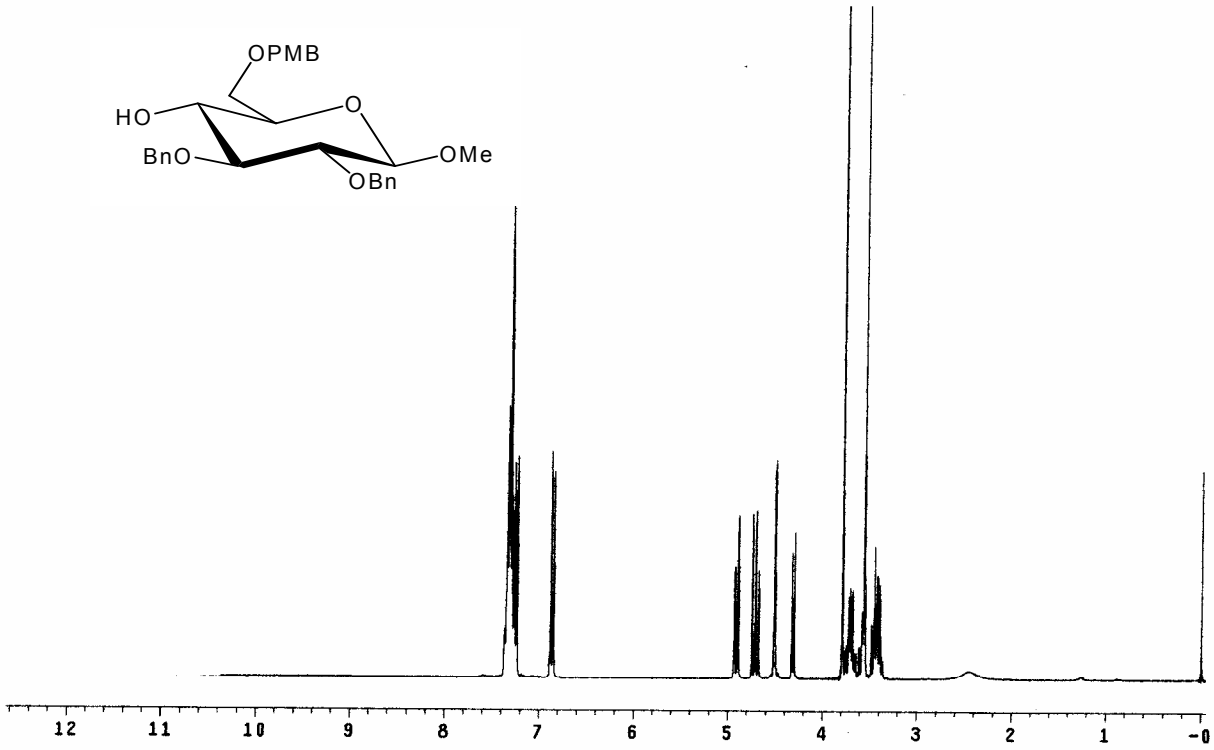


^{1}H NMR (300 MHz, $CDCl_3$) Spectrum for Methyl 2,3 di-*O*-benzyl-4-deoxy-4-*C*-formyl-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside **(15)**

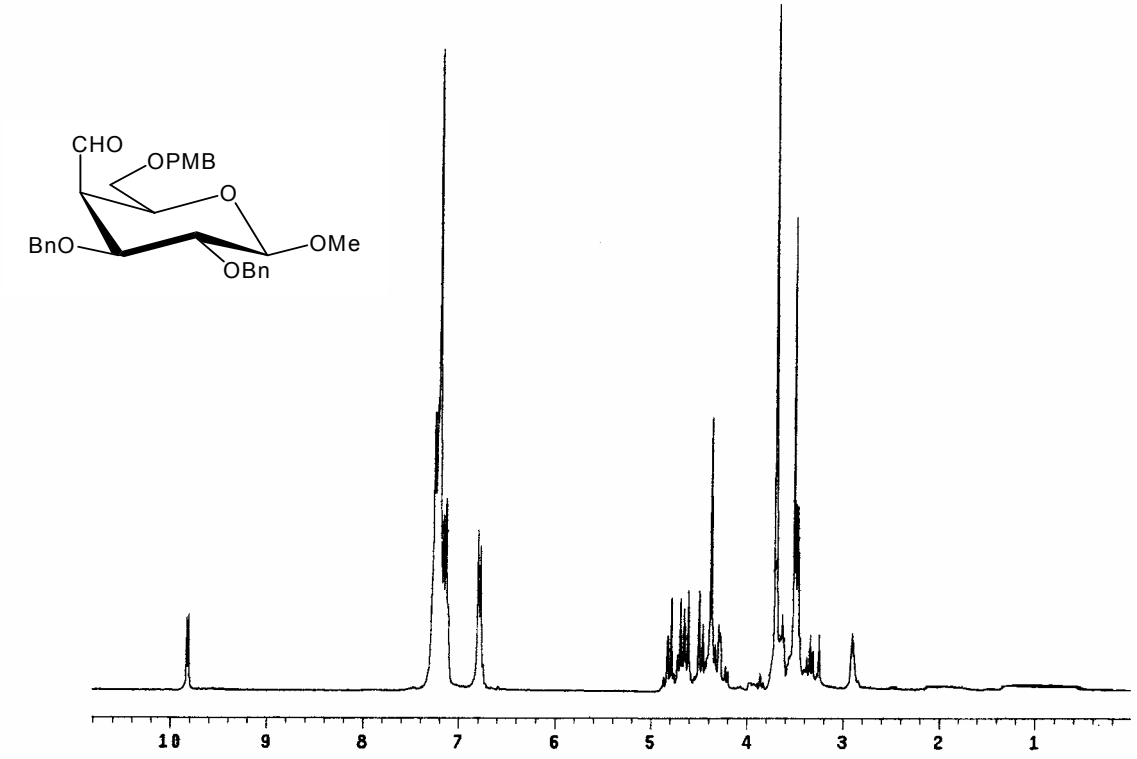




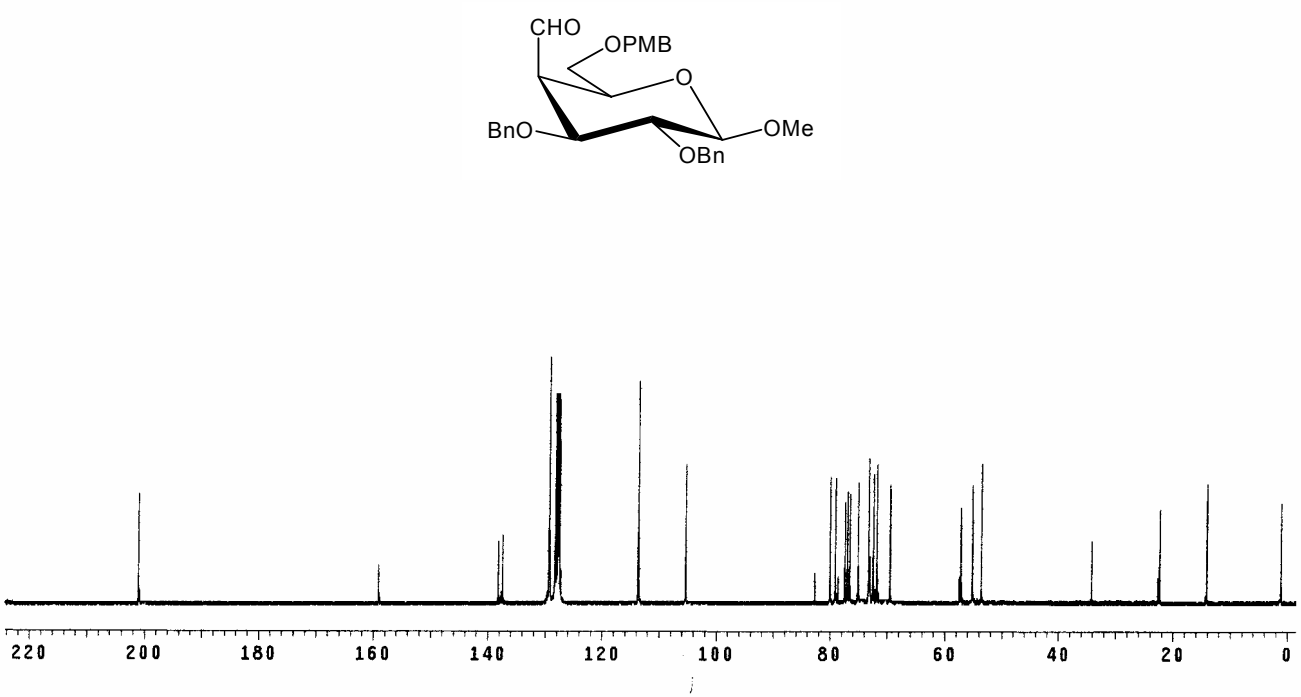
^{13}C NMR (75 MHz, $CDCl_3$) spectrum for Methyl 2,3 di-*O*-benzyl-4-deoxy-4-*C*-formyl-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside **(15)**



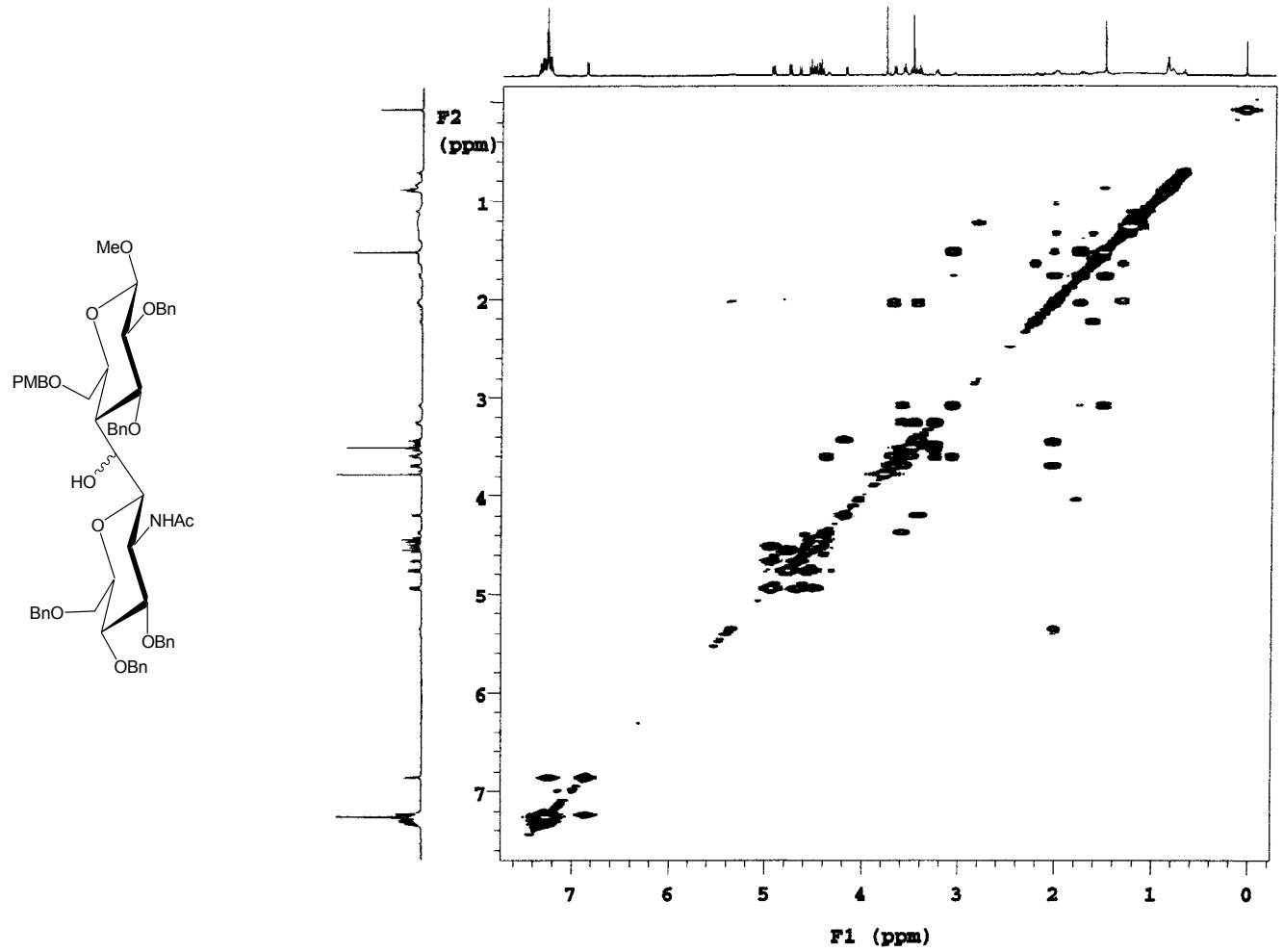
^{1}H NMR (300 MHz, $CDCl_3$) Spectrum for Methyl 2,3 di-*O*-benzyl-4,6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside **(26)**



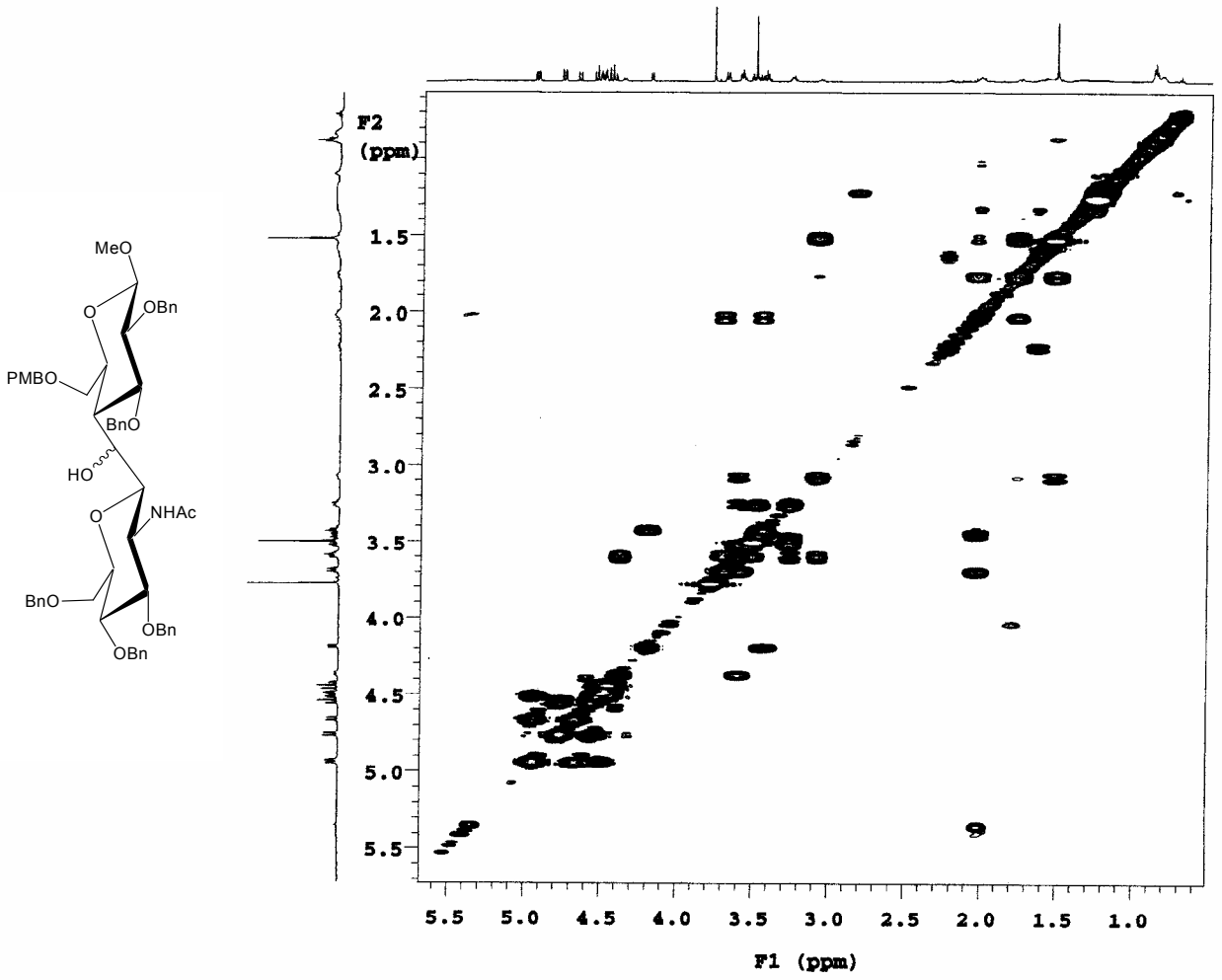
^{1}H NMR (300 MHz, $CDCl_3$) Spectrum for Methyl 2,3 di-*O*-benzyl-4-deoxy-4-*C*-formyl-6-*O*-(4-methoxybenzyl)-β-D-galactopyranoside **(29)**



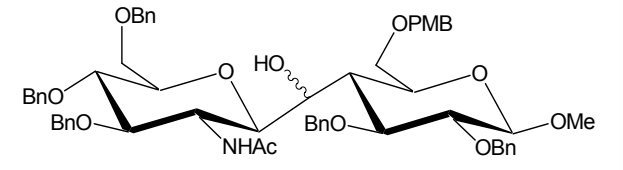
^{13}C NMR (75 MHz, $CDCl_3$) spectrum for Methyl 2,3 di-*O*-benzyl-4-deoxy-4-*C*-formyl-6-*O*-(4-methoxybenzyl)-β-D-galactopyranoside **(29)**

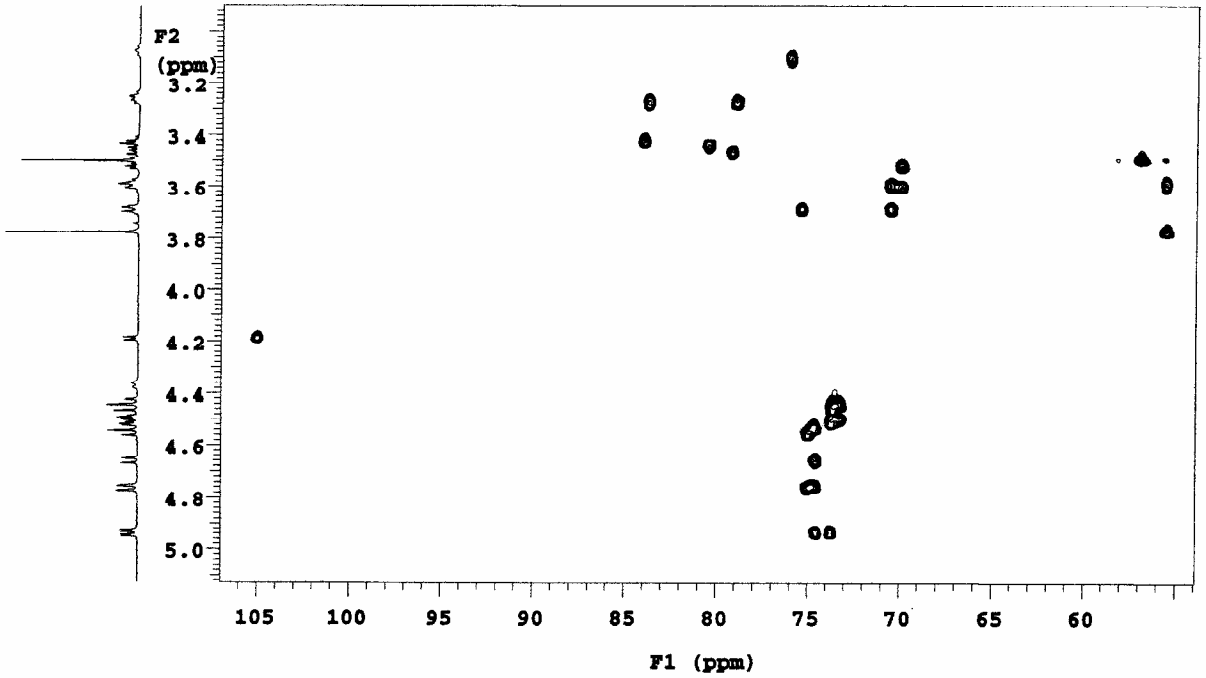


gCOSY (600 MHz, $CDCl_3$) full spectrum for Methyl 4-*C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranosylhydroxymethyl)-2,3-di-*O*-benzyl-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside **(16b)**

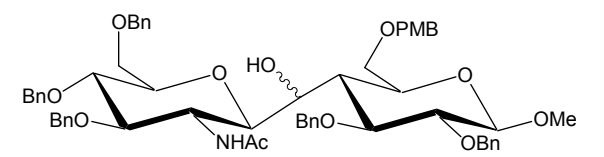


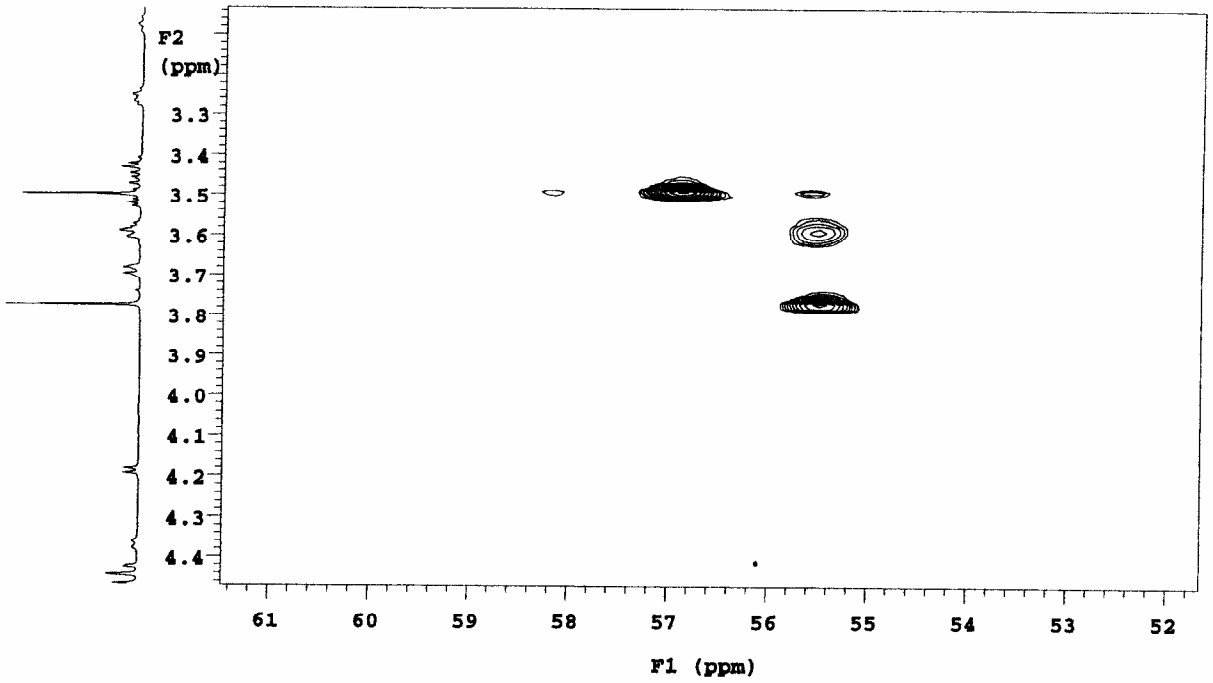
gCOSY (600 MHz, $CDCl_3$) partial spectrum for Methyl 4-*C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranosylhydroxymethyl)-2,3-di-*O*-benzyl-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside **(16b)**



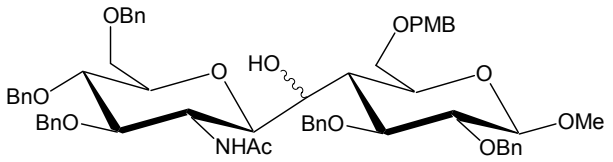


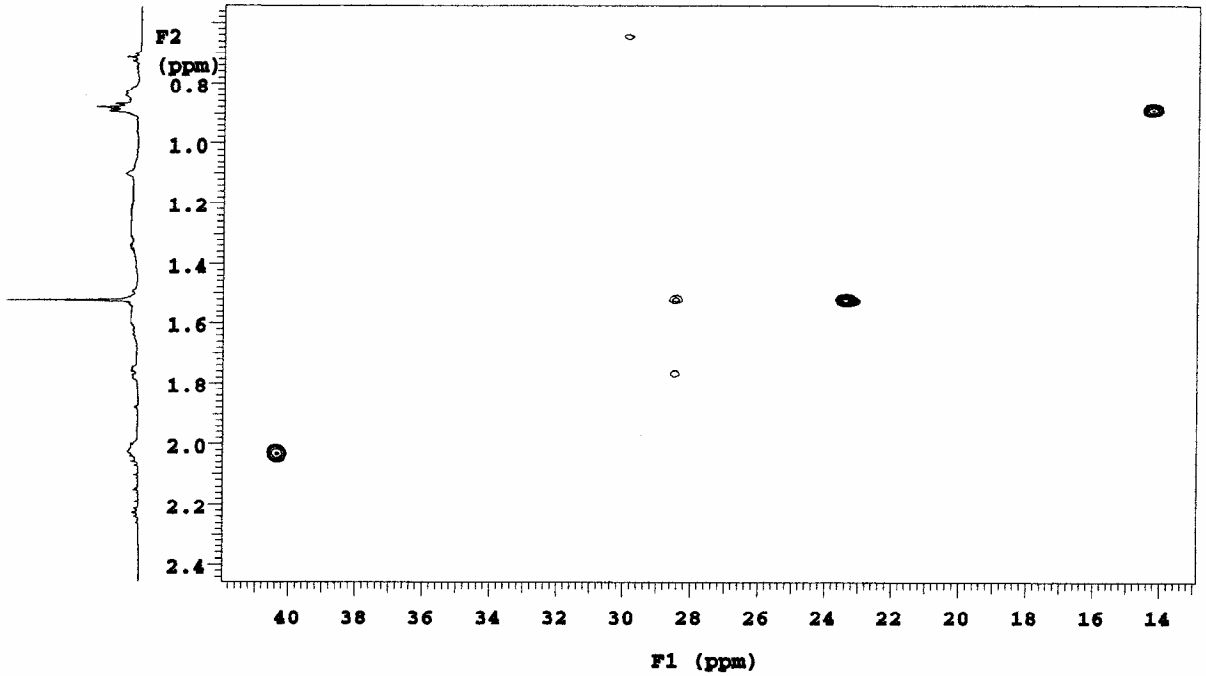
HSQC (600 MHz, $CDCl_3$) partial spectrum for Methyl 4-*C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranosylhydroxymethyl)-2,3-di-*O*-benzyl-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside **(16b)**



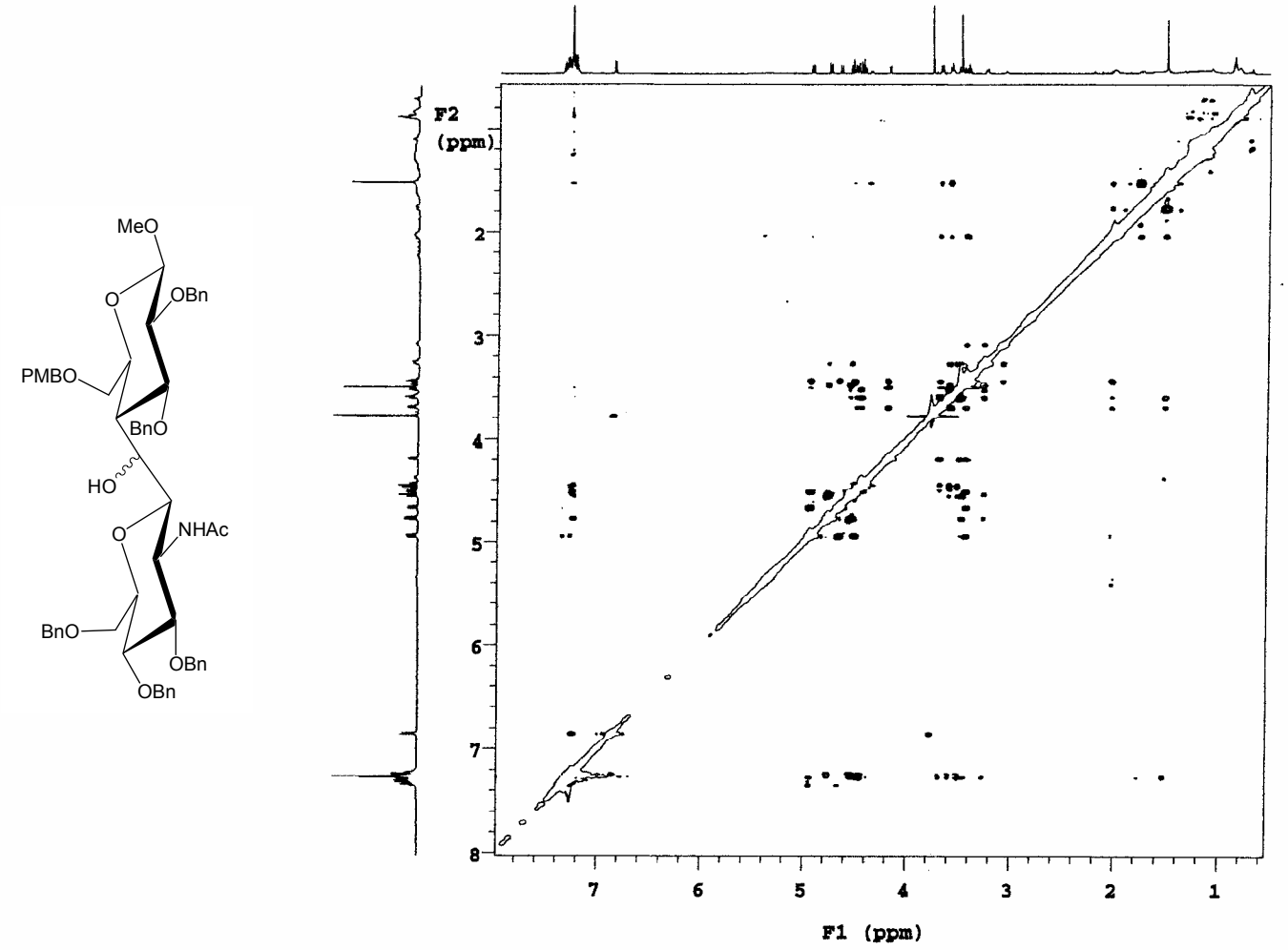


HSQC (600 MHz, $CDCl_3$) partial spectrum for Methyl 4-*C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranosylhydroxymethyl)-2,3-di-*O*-benzyl-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside **(16b)**

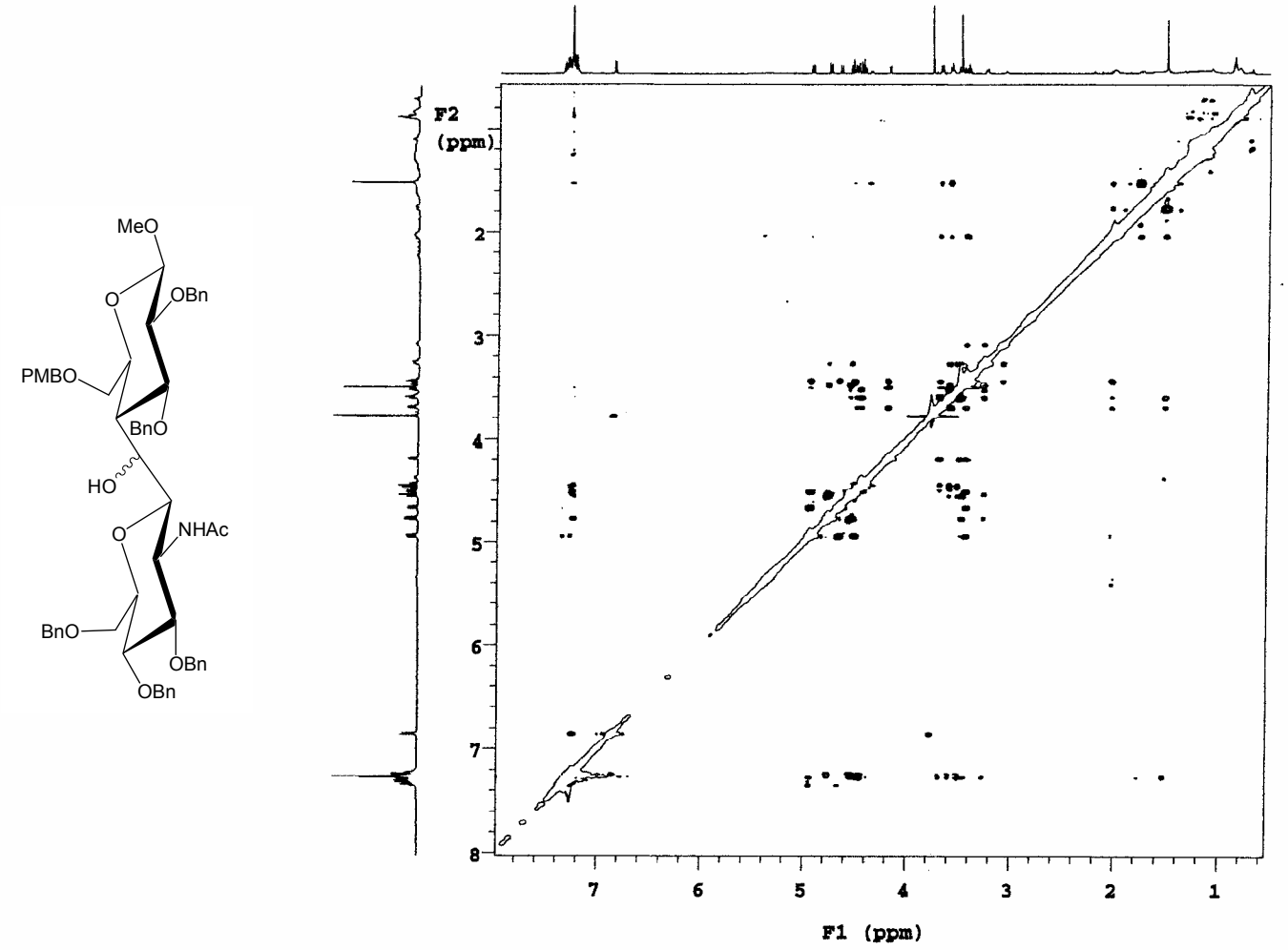




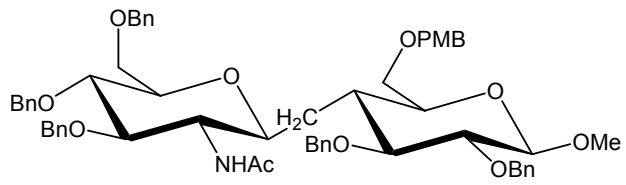
HSQC (600 MHz, $CDCl_3$) partial spectrum for Methyl 4-*C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranosylhydroxymethyl)-2,3-di-*O*-benzyl-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside **(16b)**

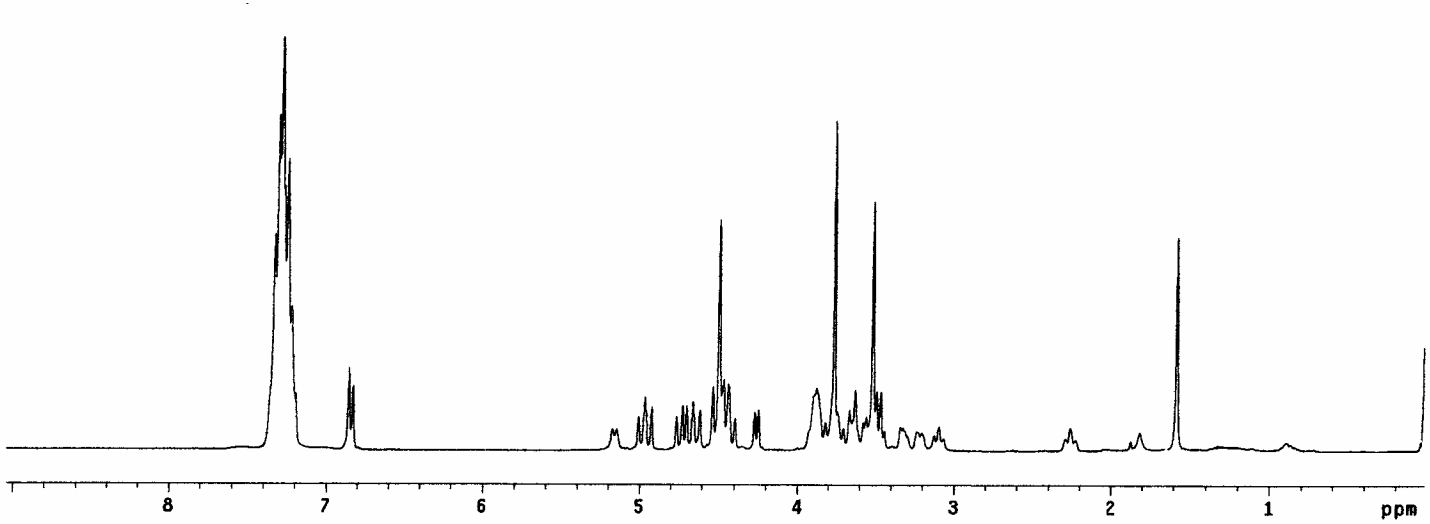


ROESY (600 MHz, $CDCl_3$) full spectrum for Methyl 4-*C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranosylhydroxymethyl)-2,3-di-*O*-benzyl-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside **(16b)**

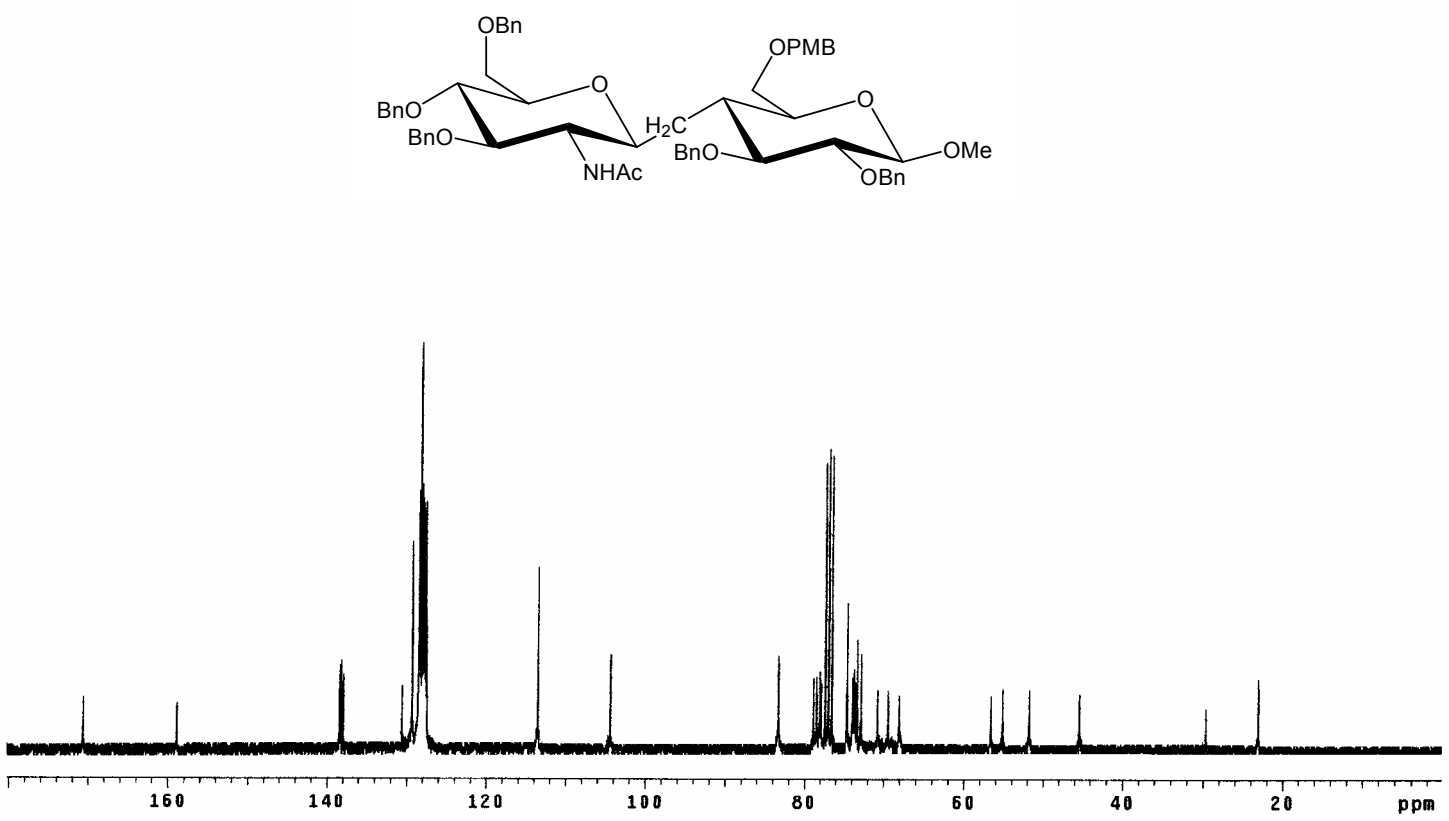


ROESY (600 MHz, $CDCl_3$) full spectrum for Methyl 4-*C*-(2-acetamido-2-deoxy-3,4,6- tri-*O*-benzyl-β-D-glucopyranosylhydroxymethyl)-2,3-di-*O*-benzyl-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside **(16b)**

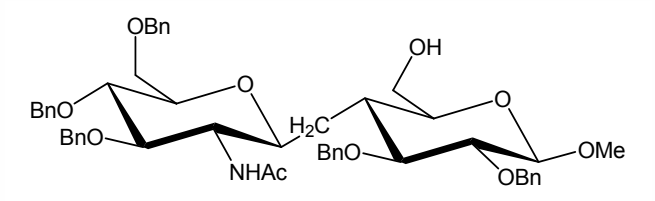


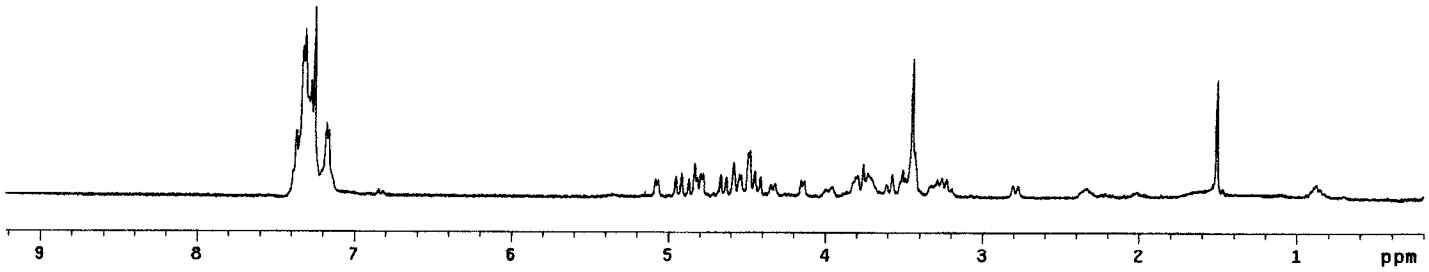


^{1}H NMR (300 MHz, $CDCl_3$) Spectrum for Methyl 4-*C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranosylmethyl)-2,3-di-*O*-benzyl-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside **(31)**

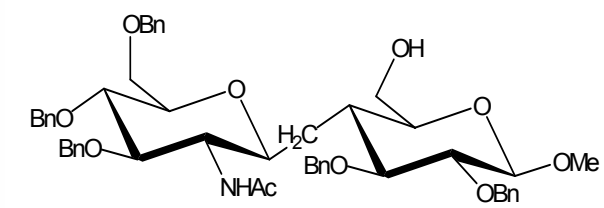


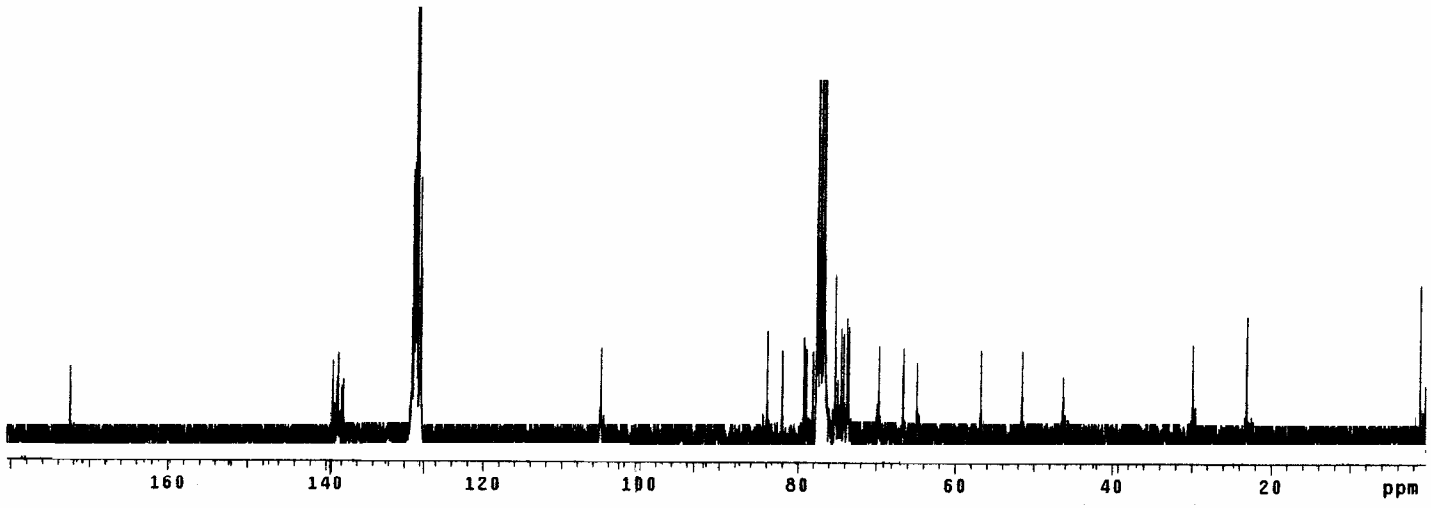
^{13}C NMR (75 MHz, $CDCl_3$) spectrum for Methyl 4-*C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranosylmethyl)-2,3-di-*O*-benzyl-4-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside **(31)**





^{1}H NMR (300 MHz, $CDCl_3$) Spectrum for Methyl 4-*C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranosylmethyl)-2,3-di-*O*-benzyl-β-D-glucopyranoside **(32)**





^{13}C NMR (75 MHz, $CDCl_3$) spectrum for Methyl 4-*C*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranosylmethyl)-2,3-di-*O*-benzyl-β-D-glucopyranoside **(32)**

VITA

Kpakpo Ambroise Akué was born in Aného, Togo. After completing his early education in Abidjan, Ivory Coast, he returned to Lomé, Togo, where he completed his Bachelor's of Science Degree in Chemistry at the Université du Benin in 1997. Then he migrated to the United State of American, joined the research group of Dr. David C. Baker in 1999 and obtained his Doctor of Philosophy Degree at the University of Tennessee in 2005. He has accepted a postdoctoral position in the Research and Development Department of PharmAgra Labs Inc in Asheville, North Carolina.