TECHNISCHE UNIVERSITÄT WIEN
Vienna University of Technology

DISSERTATION

Synthesis of *Acinetobacter* lipopolysaccharide ligands

ausgeführt zum Zwecke der Erlangung des akademischen Grades einer

Doktorin der technischen Wissenschaften

unter der Leitung von

Univ. Prof. Dipl.-Ing. Dr. techn. Johannes **Fröhlich** und Ass. Prof. Dipl.-Ing. Dr. techn. Christian **Hametner**

163 - Institut für Angewandte Synthesechemie – TU Wien

und Univ. Prof. Dipl.-Ing. Dr. techn. Paul **Kosma**

Department für Organische Chemie – Universität für Bodenkultur

eingereicht an der **Technischen Universität Wien**

Fakultät für Technische Chemie

von

Dipl.-Ing. Barbara **Pokorny**, BSc

0625512

Haidengasse 3/2/24, A-1230 Wien

Wien, am 10.07.2015

“There’s always a chance, Doctor, as long as one can think.”

Basil – The Great Mouse Detective (Walt Disney)

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Danksagung

In erster Linie möchte ich meinem Betreuer **Prof. Dr. Paul Kosma** danken, der mir die Möglichkeit gegeben hat, meine Dissertation in seiner Gruppe durchzuführen, der mir genügend Freiraum für eigene Ideen geben aber auch mit guter Führung ein effizientes Arbeiten ermöglicht hat. Des weitern bin ich dankbar für die Teilnahme an vielen interessanten Konferenzen und Fortbildungen, die wesentlich zu meiner fachlichen und persönlichen Entwicklung beigetragen haben, und für das stets offene Ohr für Probleme. Ich werde immer mit großer Freude an diese Zusammenarbeit zurückdenken!

Ebenfalls herzlich bedanken möchte ich mich bei **VR Dr. Johannes Fröhlich**, der sich zu der TU-seitigen Betreuung trotz eines dichten Terminkalenders bereit erklärt hat, und bei **Dr. ^{13}C. ^{1}H. (Christian Hametner)** für die rasche und sorgfältige Korrektur meiner Dissertation.

Ein großer Dank geht an meine ehemaligen Kollegen der Arbeitsgruppe, die ein ausgezeichnet ausgestattetes Labor hinterlassen haben und mir einen guten und effizienten Start ermöglicht haben. Ohne ihre Erfahrungen, die sie stets bereitwillig mit mir geteilt haben, wäre es ein steinigerer Weg geworden. Danke **Christian**, **Markus**, **Flo** und **Ralph**! Danke auch an **Carmen** und **Alessio** für die netten beruflichen und privaten Gespräche. Ebenso bedanken möchte ich mich bei meinen früheren Kollegen und Freunden an der TU Wien! Danke, **Gitti**, dass du mich ermutigt hast, mich für die Stelle zu bewerben! An **Markus** und **Theresa**: alles Gute für das Eheleben! **Alice** und **Binci**: danke für eine geniale Studienzeit! Ich hoffe, dass wir uns niemals aus den Augen verlieren!

Danke an **Maria Hobel**, die immer versucht hat, das Labor bestmöglich in Stand zu halten und sich um so viele Dinge gekümmert hat, was uns allen einen reibungslosen Arbeitsalltag ermöglicht hat. Danke auch an **Dr. Andi Hofinger** für die Instandhaltung und Messungen am NMR.

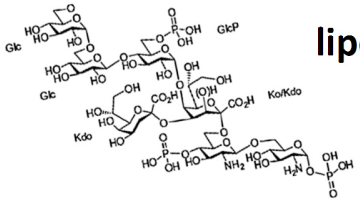
Danke auch an meine ehemaligen Kolleginnen (**Irene Pretl**, **Irene Weiner**, **Martina**, **Tanja**…) bei Boehringer Ingelheim, die an mich gedacht haben, wenn es Geräte abzugeben gab!

Den größten Dank möchte ich meiner Familie widmen. Obgleich sie nicht direkt in meine Arbeit involviert waren, haben mich meine Eltern (**Susanne** und **Werner Pokorny**) gelehrt, kritisch und hartnäckig, aber auch menschlich und aufrichtig zu sein. Ihre finanzielle und mentale Unterstützung war entscheidend für meinen Erfolg. Last but not least: Danke, für die bedingungslose Unterstützung durch meinen Lebensgefährten und besten Freund **Philipp Lehrer**, der mir viel Arbeit abgenommen und sich viel Jammerei mit Geduld angehört hat, und mit dem das Leben einfach Freude macht. Danke!

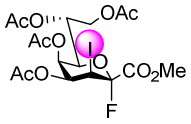
Gewidmet an Mama, Papa und Philipp

Highlights

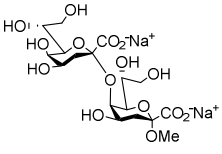
Synthesis of inner core fragments of *Acinetobacter haemolyticus* lipopolysaccharide (LPS)

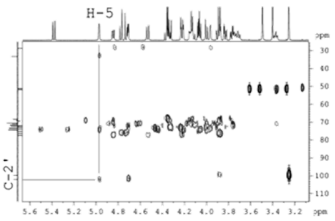


New α-selective 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) donor & synthesis of Chlamydiaceae antigens



First synthesis of an α-(2→5)-linked Kdo disaccharide - a structure found in *A. baumannii* LPS





Abstract

Lipopolysaccharide (LPS) constitutes the outer leaflet of the gram-negative bacterial cell wall and comprises three compartments: the lipid A, the inner and the outer core, and the O-antigen. During infection all three parts play an important role for the innate and adaptive immune systems of the respective host. In this regard, LPS is frequently recognized by C-type lectins triggering inherent immune response in hosts. These Ca^{2+}-dependent sugar binding proteins have a broad specificity for structural motifs that are ubiquitously found on the surface of pathogens. Among them, the mannose-binding lectin (MBL) recognizes structures that display vicinal diequatorial hydroxyl groups like in D-*manno*pyranose. While the interaction of MBL with an individual binding site is weak, high affinity relies on multivalent binding of appropriately arranged epitopes.

In contrast, an untypically strong interaction of the isolated inner core of *Acinetobacter haemolyticus* to murine and human mannose-binding lectin MBL-A has been observed. Within this thesis, the carbohydrate epitope responsible for this unusual specific interaction with a lectin should be revealed to give insight into the ongoing binding mechanisms in molecular detail. Chemical synthesis of selected di- to pentasaccharide fragments of the inner core provided structurally defined ligands for ensuing binding studies (ELISA). Therefore properly decorated glycosyl donors and acceptors were linked by high yielding and stereoselective chemical *O*-glycosylation. Subsequent modifications (phosphorylation, Smith degradation) should give further insight into the structural features essential for the high affinity. Stereoselective α-glycosylation of the rare sugar 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo), which is a principle component of the LPS core, posed the major challenge. Thus, a new potent Kdo donor with excellent glycosylation properties was introduced capitalizing on a temporary directing group which allowed for high yields and complete stereoselectivity. Based on these promising results the first synthetic protocol providing the challenging α-(2→5)-interconnected Kdo disaccharide was elaborated. This motif is a characteristic feature of several *Acinetobacter* strains including the highly pathogenic *A. baumannii*. In immunocompromised patients *Acinetobacter* causes severe nosocomial infections and due to long survival on abiotic surfaces and its multi-drug resistance it is becoming an increasing threat especially in intensive care units.

In general, revealing the binding mechanism of MBL-A to the *A. haemolyticus* inner core should contribute to a better understanding of the yet underexplored structural prerequisites of specific lectin-core interactions. A profound knowledge about the complex interplay of LPS and the immune system is necessary for future drug development.

Kurzfassung

Die äußere Zellwand Gram-negativer Bakterien wird von einer dichten Schicht an Lipopolysacchariden (LPS) ummantelt, welche drei Abschnitte umfassen: das Lipid A, die innere und äußere Kernregion, und das O-Antigen. Im Zuge einer Infektion sind alle diese Kompartimente in der Stimulation des angeborenen und erworbenen Immunsystems des jeweiligen Wirts involviert. Dabei wird LPS häufig von C-Typ Lektinen erkannt. Diese Ca^{2+}-abhängigen, Kohlenhydrat-bindenden Proteine verfügen über eine breite Spezifität für einfache Strukturmotive, die sich ubiquitär auf der Oberfläche von Pathogenen befinden. Beispielsweise erkennt das Mannose-bindende Lektin (MBL) vicinale, diäquatoriale Hydroxylgruppen, wie sie in D-Mannopyranose vorkommen. Während die Interaktion von MBL mit einer einzelnen solchen Struktur schwach und unspezifisch ist, wird eine multivalente Bindung dieser Motive, die in geeigneter Anordnung an der Bakterienoberfläche präsentiert werden, für eine hohe Bindungsaffinität benötigt.

Im Gegensatz dazu wurde eine ungewöhnlich starke Bindung der isolierten inneren Kernregion von *Acinetobacter haemolyticus* zu murinem und humanem Mannose-bindenden Lektinen MBL-A beobachtet. In Zuge der Dissertation sollte das Kohlenhydrat-Epitop, das für diese untypisch spezifische Interaktion mit dem Lektin verantwortlich ist, identifiziert werden, um Einsicht in den Bindungsmechanismus auf molekularer Ebene zu erhalten. Chemische Synthese von ausgewählten Di- bis Pentasaccharidfragmenten lieferte strukturell definierte Liganden für spätere Bindungsstudien (ELISA). Dazu wurden optimierte Glykosyldonoren und –akzeptoren effizient durch stereoselektive O-Glykosylierungen verbunden. Anschließende Modifikationen (Phosphorylierung, Abbau nach Smith) sollen weitere Einblicke darüber geben, welche Teilstrukturen essentiell für die hohe Affinität sind. Eine zentrale Rolle spielte dabei die Entwicklung eines neuen, α-selektiven Donors des seltenen Zuckers 3-Desoxy-D-*manno*-oct-2-ulosonsäure (Kdo), welcher ein prinzipieller Bestandteil der LPS Kernregion ist. Dieser Donor ermöglichte durch eine temporäre, dirigierende Gruppe hohe Ausbeuten mit absoluter Stereoselektivität. Darauf basierend wurde zusätzlich die erste Syntheseroute zu einem α-(2→5)-verknüpften Kdo Disaccharid ausgearbeitet, welches ein charakteristisches Motiv der Kernregion verschiedener *Acinetobacter* Stämme darstellt. In immunsupprimierten Patienten kann *Acinetobacter* schwere nosokomiale Infektionen auslösen. Aufgrund seiner Resistenz gegen Desinfektionsmittel und eine Vielzahl an Antibiotika stellt dieser Krankenhauskeim eine zunehmende Bedrohung – besonders in Intensivstationen – dar.

Generell sollen durch die Aufklärung des Bindungsmechanismus zwischen MBL-A und der inneren Kernregion von *A. haemolyticus* die bis jetzt mangelhaft untersuchten strukturellen Voraussetzungen für spezifische Wechselwirkungen zwischen Lektin und Kernregion geklärt werden. Ein fundiertes Wissen über das komplexe Zusammenspiel von LPS und unserem Immunsystem ist für die Entwicklung zukünftiger Behandlungsstrategien unerlässlich.

Aim of the thesis

The present work was aimed at the synthesis and modification of carbohydrate-fragments of the lipopolysaccharide (LPS) from the gram-negative bacterium *Acinetobacter*. These compounds serve as ligands for binding studies (ELISA, STD-NMR) with the objective to uncover the minimal binding epitope for an untypically strong interaction between the isolated inner core of *A. haemolyticus* LPS and several mannose-binding lectins. As these lectins contribute to the innate immune response, a closer investigation of the ongoing binding mechanisms might be of medical significance. From a synthetic point of view, these oligosaccharide fragments should be prepared by chemical O-glycosylation of properly protected monosaccharides in high yields and stereoselectivity. In particular, a reliable protocol for an appropriate glycosyl donor of 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) for efficient coupling reactions should be established. Furthermore, the closely related and clinically highly relevant *A. baumannii* expresses a thus far unique and complex LPS inner core structure that contains a rarely observed α-(2→5)-linked disaccharide of Kdo. A first synthetic access of such a disaccharide should be developed providing the basis for biological examination of this extraordinary linkage-type.

Structure of the thesis

This thesis was written as a cumulative work and performed in the framework of the Austrian Science Fund (FWF) project P24921-N28. An introductory part (Chapter 1) provides a general overview on both the biochemical background and the applied methods. The subsequent chapter “Results and Discussion” is split into three main parts. In a first section the context of the published manuscripts and paper drafts will be outlined (Chapter 2.2). The second part comprises the original manuscripts describing the synthesis of the various ligands (Chapter 2.4). In a last section, preliminary biological results obtained from the prepared oligosaccharide fragments will be presented (Chapter 2.5). In chapters 2.1 and 2.3 the author’s contributions and the references of the preceding chapters are listed, respectively. The results are briefly summarized in chapter 3 accompanied by a short outlook. For explicit illustration of the author’s contribution to each manuscript the reader may refer to chapter 4 “Statement of contribution”. Where applicable, “Supporting Information” including further synthetic detail and analytical data is attached as “Appendix” (Chapter 5).

Collaboration

PD Dr. Sven **Müller-Loennies,** Structural Biochemistry, Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany

This collaborator played an important role in the establishment of the project idea. The underlying biological results showing an uncommon strong binding affinity of MBL-A (and other immune-related lectins) to isolated *A. haemolyticus* lipopolysaccharide core were collected in this laboratory. Evaluation of the generated ligands by a type of Enzyme Linked Immunosorbent Assay (ELISA) has been performed in the collaborator's laboratory and one test series has been performed by the author of this thesis under the supervision of the collaborator.

1. Introduction

1.1. Bacterial lipopolysaccharide (LPS)

Bacteria belong to the prokaryotes and are divided into two major classes according to the fundamental differences of their cell envelope. They are distinguished by their behavior toward gram-staining, thus they are termed gram-positive or gram-negative. The gram-negative bacterial cell wall comprises a cytoplasmic (inner) membrane and an outer membrane embedding the periplasm, which contains a thin peptidoglycan layer (Figure 1, Figure 2). The outer membrane is composed of a mono-phospholipid layer to which densely packed lipopolysaccharides (LPS) anchor by non-covalent interactions.[1]

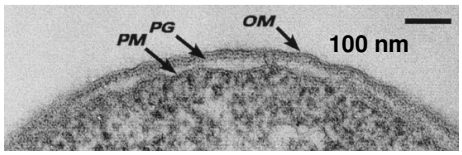


Figure 1: Embedded thin section of Escherichia coli strain K-12 cell envelope showing the plasma membrane (PM), the peptidoglycan (PG) and the fuzzy outer membrane (OM) decorated with lipopolysaccharides; adapted from [1].

LPS are amphiphilic glycolipids which serve as a barrier for different kinds of chemical, physical and biological stress and thus, are vital for most species.[2] Structurally, bacterial LPS is divided into three parts: (a) the lipid A; (b) the core region that consists of an inner and an outer core; and (c) the O-antigen (Figure 2). While lipid A is widely conserved within a genus, structural variability increases for the outer sections culminating in highly diverse O-antigen structures. It is worth to mention that bacteria may express LPS comprising all three compartments (smooth-type) or structures devoid of the O-antigen (rough-type).[2,3]

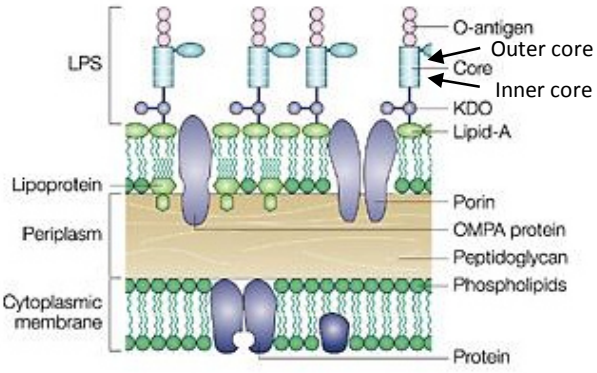


Figure 2: Schematic diagram of the gram-negative bacterial cell envelope; adapted from [4].

Many species further display capsular polysaccharide (CPS) or S-layers as additional cell wall components on the outermost sphere (not shown).[1] Several pathogens including *E. coli, Neisseria*, *Salmonella*, *Pseudomonas*, *Haemophilus influenzae* and *Acinetobacter* belong to the class of gram-negative bacteria.[3]

1.1.1. Lipid A

The structure of *E. coli* lipid A was elucidated as the first of its kind (Figure 3). It comprises a β-(1→6)-linked glucosamine (GlcN) disaccharide decorated with four O-/N-linked 3-(R)-hydroxymyristoyl (C-14) chains. The fatty acids on the distal GlcN residue are further acylated by one myristoyl (C-14) and one lauroyl (C-12) chain on their β-hydroxyl groups. In addition, the lipid A bears two phosphate groups: one α-linked at the reducing end; the other on the 4'-position of the distal GlcN. The fatty acids anchor to the phospholipid monolayer of the outer bacterial membrane. The negatively charged phosphate groups are capable of ionic interaction with divalent cations (Mg^{2+}, Ca^{2+}) forming a rigid cross-linked structure which contributes to the protective barrier function of LPS.[2]

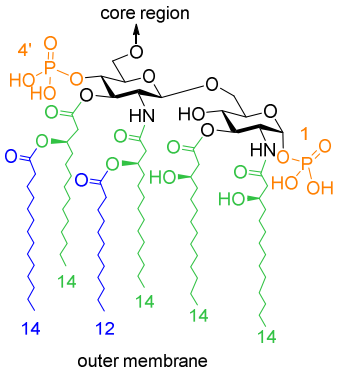


Figure 3: Chemical structure of lipid A found in *E. coli*.[2]

Different bacterial families, species or strains express slightly modified lipid A structures deviating from this general motif (Figure 4). The acylation pattern may vary with regard to the length (usually even-numbered from ten to 22 carbons), the number (three to seven fatty acids), the distribution between the two GlcN moieties (*e.g.* 3+3 vs. 4+2 for six fatty acids) and the hydroxylation. Long-chain, odd-numbered or unsaturated fatty acids as well as those branched with methyl substituents are less common. In view of the phosphorylation pattern, one of the two or both phosphates may be absent. The phosphates are often non-stoichiometrically substituted by polar groups like a second phosphate (resulting in a diphosphate), ethanolamine, 2-aminoethyl phosphate, 4-amino-4-deoxy-L-arabinose or galactosamine. Furthermore, the phosphates may be replaced by other acidic groups like galacturonic acid. In some cases, the GlcN residues of the carbohydrate backbone are replaced by 2,3-diamino-2,3-dideoxy-D-glucose (Glc2N3N). The backbone can be extended by 4'-substitution with D-mannose residues and the anomeric carbon of the reducing end may be oxidized giving rise to an open-chain carboxylic acid. All these variations are believed to account for adaption to the particular environmental or growth conditions, enhanced resistance towards cationic antimicrobials and evasion from the immune system.[2,5]

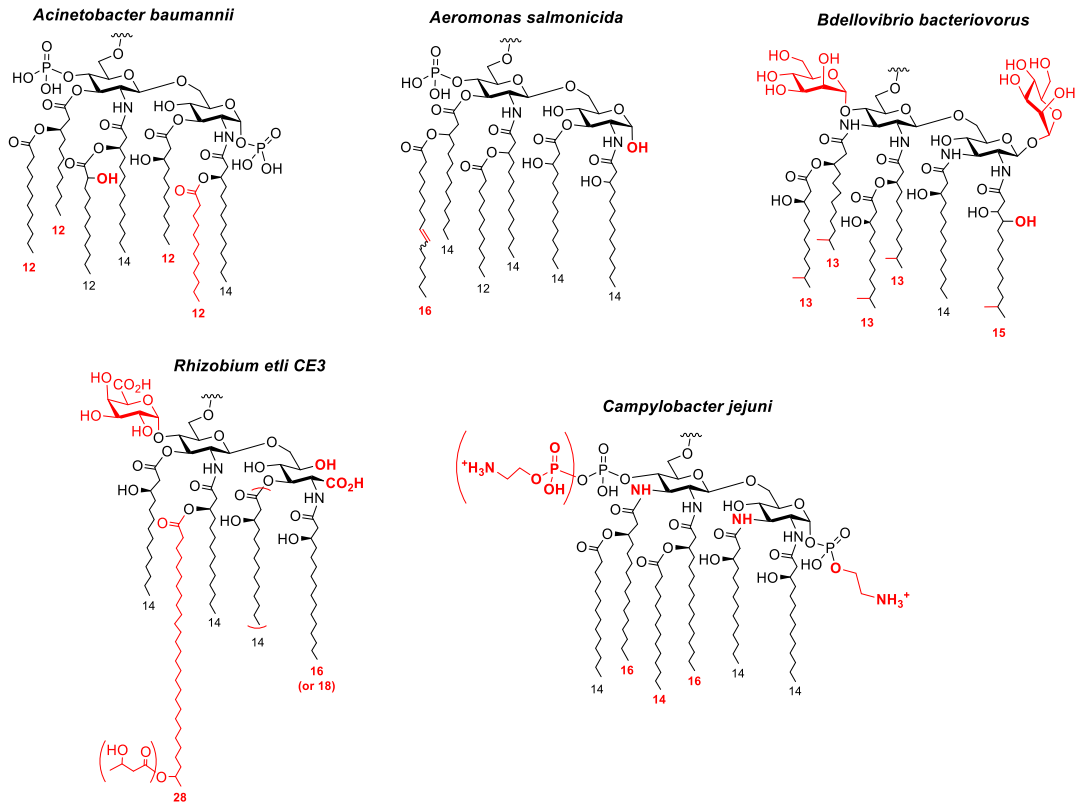


Figure 4: Lipid A of selected species showing selected structural variations[2,6–8]; non-stoichiometric modifications are shown in brackets.

Lipid A as the minimal entity of LPS was believed to be vital for bacterial growth and survival for a long time. Observed inability of bacteria to survive a knock-out of essential genes encoding early stages in the biosynthesis of lipid A supported this dogma. In contrast to this assumption, recent research has shown that some species like *Neisseria meningitidis* do not rely on lipid A expression. Thus, it has been proposed that lipid A is essential for most species due to the strong integration of lipid A/LPS-biosynthesis in the metabolism and that its suppression could have fatal consequences for other vital biosynthetic pathways. However, a significant decrease in virulence and a distinct susceptibility towards antimicrobials of LPS-deficient strains is indisputable.[9]

1.1.2. Core region

The core region comprises an oligosaccharide of up to 15 sugar residues and is covalently linked to the 6'-hydroxyl group of the distal GlcN of lipid A (Figure 3). It is divided into an inner (proximal to the lipid A) and an outer core (distal to lipid A). The inner fraction is typically more conserved between different strains within a genus. The 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) is the almost exclusive linkage unit forming an α-(2→6) connection to

lipid A (Figure 5). This first Kdo is often substituted by a second Kdo residue in an α-(2→4)-linkage.[10] In some species, either the lipid A-linked Kdo (*Acinetobacter*) or the distal Kdo (*Yersinia, Burkholderia*) may be replaced by its 3-oxy analogue D-*glycero*-D-*talo*-2-octulosonic acid[11] (Ko). This modification leads to significantly increased chemical stability of the glycosidic linkage.[10] In *Y. pestis* and *B. ambifaria* the modification with Ko results from the action of a O_2/Fe^{2+}/α-ketoglutarate dependent Kdo 3-hydroxylase using Kdo_2-lipid A as a substrate.[12,13]

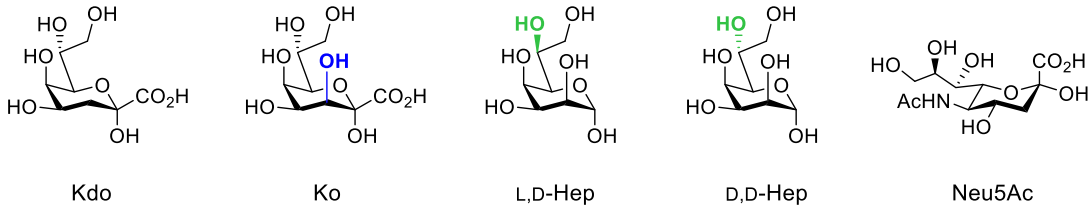


Figure 5: Structure of characteristic sugars of the LPS inner core and mammalian-associated N-acetylneuraminic acid, which is a frequent component of mammalian glycans (Neu5Ac); all monosaccharides are depicted in the α-pyranose-form.

In enteric bacteria, the proximal Kdo is usually α-(1→5)-glycosylated by an L-*glycero*-D-*manno*-heptose (L,D-Hep, see Figure 5). In general, L,D-Hep and its biosynthetic precursor D-*glycero*-D-*manno*-heptose (D,D-Hep) are frequent components of the inner core region, however, several bacterial LPS structures are devoid of any heptoses. In contrast, the outer core usually consists of prevalent hexoses like D-galactose (Gal), D-glucose (Glc) and derivatives thereof. This is not an ultimate characteristic as both Kdo and heptoses have been identified as constituents of the outer core in different strains.[5,10]

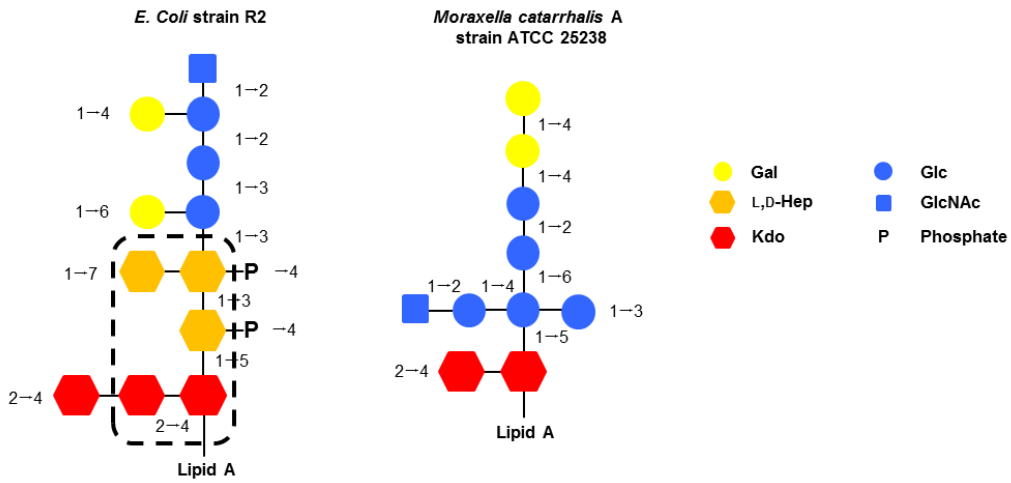


Figure 6: Two examples for inner core structures showing the common enterobacterial inner core comprising a L,D-Hep trisaccharide α-(1→5) linked to the Kdo disaccharide (black frame, left) and the α-(1→5)-linkage of Glc to the Kdo disaccharide in the Moraxella species (right); anomeric configurations not shown.[14]

In some species D-mannose (*Francisella, Legionella*) or D-glucose (Moraxella, *Acinetobacter*) is α-(1→5)-linked to the proximal Kdo instead of heptose (Figure 6). Most monosaccharide units are present in the pyranose form, but *e.g.* Kdo-furanose (Kdo*f*) was identified in *Aeromonas*. The connectivity between the monosaccharide units ranges from linear to heavily branched structures. Various (often non-stoichiometric) substituents like phosphate, pyrophosphate, phosphoethanolamine, both O- and N-acetyl groups, amino acids and uronic acids are responsible for a high microheterogeniety of the LPS core. Notably, the anionic phosphates as well as the carboxylic groups from Kdo interact with divalent cations with high-affinity. This is believed to result in a cross-linking between the lipopolysaccharide chains and the formation of a rigid and protective coat.[3,15] Interestingly, some species decorate the core with typical mammalian glycan structures like Neu5Ac (Figure 5). This leads to reduced antibody recognition and increased serum resistance of pathogens in the host and it is suggested that bacteria thereby try to evade the immune defense.[10]

1.1.3. O-Antigen

Regarding the three compartments of LPS, the O-antigen is the least conserved and shows high variability between strains of the same species. Structurally, repeating building blocks of two to eight sugars of various kind form a linear polysaccharide that may be irregularly substituted by smaller groups like: (a) O-/N-acetyl; (b) methyl groups on hydroxyl and amino groups or those esterifying carboxylic acids; (c) amino acids forming amides with hexuronic acids; and (d) phosphodiesters. Due to its specificity for individual strains it is commonly used for serotyping.[16]

1.1.4. Selected recognition mechanisms of LPS by the host's immune system

The LPS of pathogens is an important virulence factor initiating innate immune response. Although LPS is not secreted by bacteria, fragments may enter the hosts serum during cell division or processes like phagocytosis and complement action.[5] Small local concentrations of LPS are capable of stimulating fast inherited immune response and are protective for the host. However, larger quantities may result in a systemic and overwhelming response. Thereby, tissue damage and acute septic shock can lead to death that is why LPS is also called "endotoxin". In this regard, recognition of lipid A by a 1:1-complex of myeloid differentiation factor 2 (MD-2) and Toll-like receptor 4 (TLR4) is a dominant pathway of innate immune response (Figure 7). The Toll-like receptors (TLRs) belong to the inherent pattern recognition receptors (PRR) in hosts, which recognize pathogen-associated molecular pattern (PAMP) of invading pathogens and thus play a crucial role in innate immunity. During gram-negative bacterial infection lipid A is initially bound by the lipid A binding protein (LBP), and is further transmitted to the CD14 protein, that delivers lipid A to the transmembrane MD-2/TLR4 complex. The liaison with lipid A induces

dimerization with another MD-2/TLR4 complex triggering one of two possible signaling pathways: (a) the MyD88-dependent cascade; and (b) the TRIF- or MyD88-independent-pathway. Thereby, a complex concert of different components of immune response is initiated. On a molecular level, five fatty acids of *E. coli* lipid A stick into the hydrophobic pocket of MD-2 protein, while the sixth attracts the second MD-2/TLR4 complex. Additional ionic interaction of the C-1 phosphate with positively charged amino acid residues is further necessary to promote dimerization. Therefore, the agonistic or antagonistic properties of lipid A strongly depend on the acylation and phosphorylation pattern.[17,18] While some bacteria modulate their lipid A structure to induce decreased immune response and to survive during infection of the respective host, the weak agonist monophosphoryl lipid A from *E. coli* (MPLA) is applied as immunostimulating agent in vaccine formulations.[19] Notably, there are substantial differences between murine and human MD-2/TLR4 recognition emphasizing a species-specific mode of interaction. Recently, it became obvious that not only TLR4 but also TLR2 is involved in lipid A recognition especially during *Acinetobacter* infection.[17]

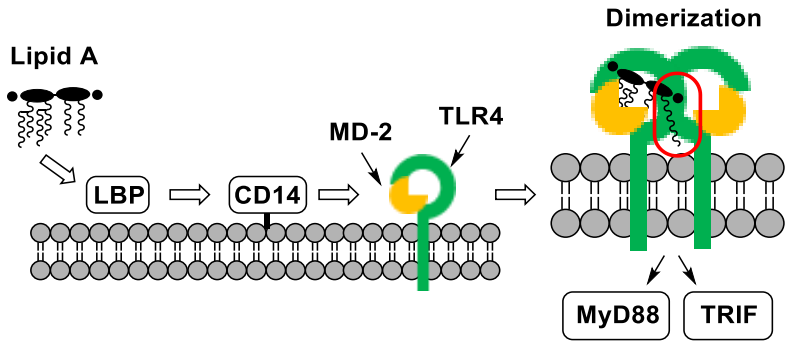


Figure 7: Recognition cascade of lipid A (*E. coli*) by the MD-2/TLR4 complex and activation pathways after dimerization; the red frame shows the participation of one fatty acid and the anomeric phosphate during dimer-formation.[17]

Another class of PRRs are the C-type lectin receptors (CLR, see chapter 1.3.) which recognize PAMPs including peptidoglycan, capsular polysaccharide and LPS. Via the "lectin pathway" they activate complement, which is a system of plasma proteins involved in both the innate and the adaptive immunity.[20] Some reports describing the recognition of the core region by CLR exist, however, the underlying binding epitopes and structural requirements for binding remain widely unclear.[21–24]

In addition, all three sections of LPS are antigenic, concluded from their ability to elicit antibody formation in hosts, but the O-antigen and the outer core are usually more exposed to the external surface. While monoclonal antibodies (mAb) directed against the O-antigen and outer core are often highly species- and strain-specific, mAbs targeting the inner core region may show cross-reactivity between different species.[25] For example, the mAb WN1 222-5A recognizes an inner core fragment occurring in different strains of *E. coli* as well as *Salmonella enterica*.[26]

In contrast, both cross-reactive and specific mAbs could be raised against the structurally conserved inner core in Chlamydiaceae. The untypical core comprises a few α-(2→4)- and α-(2→8)-interconnected Kdo units. Crystal structures of antigen in complex with the mAbs revealed, that mAbs with low specificity bound to only a Kdo monosaccharide while high specificity relied on extended binding epitopes.[27] In general, a profound and collective understanding of the various mechanisms of LPS interacting with the host's immune system is necessary for future drug development.

1.2. *Acinetobacter* spp.

The taxonomy of species from the genus *Acinetobacter* has underwent several changes, as the classification by geno- and phenotyping methods is not straightforward. In general, *Acinetobacter* spp. (family: *Moraxellaceae*, order: *γ-Proteobacteria*) are gram-negative, aerobic, non-fermenting, nonfastidious, nonmotile, catalase-positive and oxidase-negative bacteria that contain 39% to 47% of DNA G+C.[28] Most members of this genus are ubiquitously found in nature with their natural habitat in soil and water, but they have likewise been isolated from human skin, the intestinal tract, human feces, the oral cavity and vegetables.[28,29] *A. baumannii*, *A. nosocomialis* (former species 13TU) and *A. pittii* (former species 3) constitute the three major pathogenic species.[30,31]

1.2.1. *A. baumannii*

While *Acinetobacter* species rarely cause community-acquired diseases, nosocomial (hospital-acquired) infections pose a serious threat especially for immunocompromised people. In this regard, *A. baumannii* is the most prevalent species despite a low colonization rate on the human skin.[28] However, its low susceptibility to disinfection agents and desiccation lead to long survival both on biotic and abiotic surfaces and thus, contact with the opportunistic pathogen can hardly be avoided.[32] Ventilator-associated pneumonia, post-neurosurgical meningitis, bacteremia and occasional urinary tract infections caused by catheters are the most commonly observed clinical manifestations.[28] Furthermore, *A. baumannii* is multi-drug resistant against the majority of antibiotics, which has been attributed to various characteristics: (a) expression of a low level of porins resulting in limited membrane permeability; (b) the presence of effective efflux pumps; and (c) a notorious talent to set up mechanisms to evade antimicrobial treatment as a response to its exposure.[32,33] The different virulence factors include biofilm formation, siderophore-mediated iron acquisition and LPS.[28,34] Interestingly, it has been shown that *A. baumannii* strains are still viable and equally resistant to human serum in complete absence of LPS

including lipid A.[35]

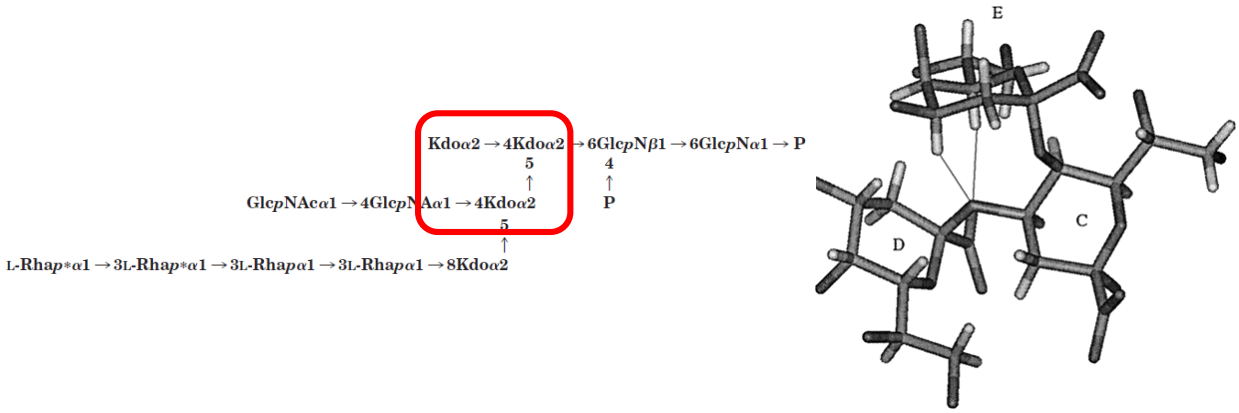


Figure 8: Part structure of the core region of *A. baumannii* LPS strain ATCC 17904 (left) and calculated conformation of the Kdo-trisaccharide in the red frame (right); adapted from [36].

Structural elucidation of *A. baumannii* (strain ATCC 17904) LPS revealed a novel type of core region comprising a unique Kdo-tetrasaccharide (Figure 8). The common enterobacterial α-Kdo(2→4)-α-Kdo(2→6)-lipid A motif is 5-O-substituted by another α-Kdo.[36] This untypical α-(2→5)-interconnection has so far only been identified in other *A. baumannii* strains[6,37], in *A. radioresistans* S13[38] and in *Campylobacter lari* strain ATCC 35221[39].

1.2.2. *A. haemolyticus*

A. haemolyticus is less accountable for nosocomial infections and the course of an eventual disease is usually benign.[31] However, its pathogenic potential has been shown recently *in vitro*, whereby *A. haemolyticus* strains induced hemolysis and were capable of mammalian cell detachment and lysis.[40]

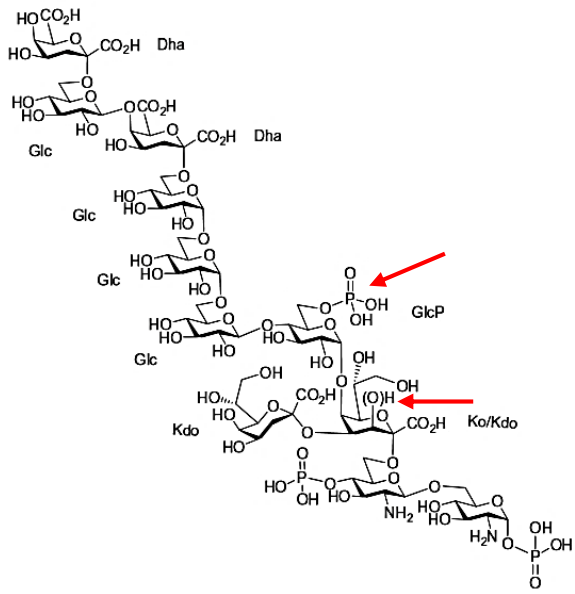


Figure 9: Structure of the inner core and the deacylated lipid A of *A. haemolyticus* strain ATCC 17906;[41] adapted from [42].

Its inner core comprises an α-(2→4)-linked Kdo disaccharide that is 5-O-substituted by an α-(1→5)-connected glucose residue – a typical structural motif in the Moraxellaceae family. Replacement of the lipid A-linked Kdo (ca. 20%) by its 3-oxy pendant Ko (ca. 80%) is a special feature of *Acinetobacter* and results in high resistance against acidic hydrolysis (see chapter 1.1.2). The innermost Glc carries a 6-O-phosphate as a non-stoichiometric substituent and is further elongated on its 4-position by the β-linked isomaltotriose trisaccharide. In the outer sphere the rare 3-deoxy-D-*lyxo*-2-heptuosaric acid (Dha) has been identified.[41]

1.3. C-type lectins

Lectins are carbohydrate-binding (glyco)proteins that are found in bacteria, viruses, fungi, protista, plants and animals. They play an important role in cell-cell interactions responsible for symbiosis, host colonization and immune response during bacterial infections.[43] The C-type lectins are Ca^{2+}-dependent and comprise both soluble (collectins) and membrane-bound (macrophage mannose receptor) proteins. Like the Toll-like receptors (TLR), C-type lectins belong to the class of PRR recognizing PAMPs during bacterial infection.[20] The innate immune response involves three classes of collectins: (a) the mannose-binding lectin (MBL); and the two lung surfactant proteins (b) SP-A and (c) SP-D.[44] Only the MBL will be treated in further detail due to its relevance for the thesis.

1.3.1. Mannose-binding lectins (MBL)

The common structure of the MBL comprises four compartments (Figure 10a): (a) an N-terminal cysteine-rich peptide chain; (b) a collagenous-domain; (c) the “neck” region formed by a small α-helical coiled-coil; and (d) the carbohydrate recognition domain (CRD). Three homologous protein chains are arranged in a triple helix, and three of these bunches form a bouquet-like supramolecular structure.[20,44]

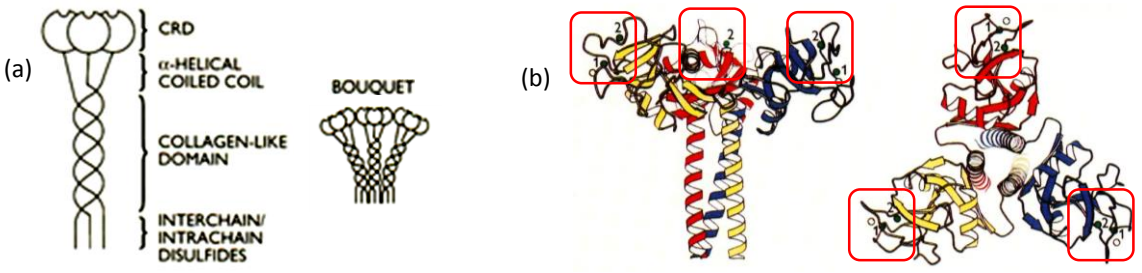


Figure 10: (a) Structure of MBL and (b) ribbon-diagram of the MBL from the side (left) and from the top (right) with two Ca^{+2} binding sites on each CRD (red frames); adapted from [44].

Carbohydrate recognition is localized at the CRD and is selective for "mannose-type" structures. The binding motif consists of the two vicinal equatorially oriented C-3 and C-4 hydroxyl groups in D-mannose (Figure 11), but sugars like GlcNAc or L-fucose are similarly recognized. Each oxygen of the two hydroxyl groups shares one electron lone pair with the essential Ca^{2+}-ion and the residual lone pairs form hydrogen bonds to asparagine of the CRD pocket. The hydrogens of the hydroxyl groups interact with acidic amino acid side chains. In contrast, MBL exhibits no selectivity for sialic acid or galactose, which are frequently expressed as terminal units of mammalian glycans. The broad specificity for MBL-binding results in high dissociation constants (ca. 2 mM) of a single carbohydrate-lectin interaction.[44]

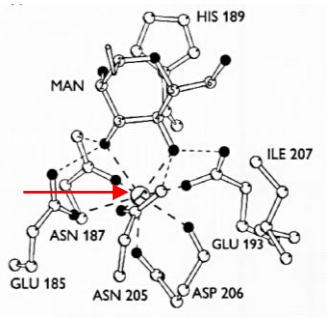


Figure 11: Binding interactions between D-mannose and the amino acids within the CRD in the presence of Ca^{2+} (red arrow); adapted from [44].

Multivalent binding of appropriately displayed "mannose-type" motifs on the bacterial surface potently enhances this weak interaction leading to high affinity. Thereby, human MBL is capable of discriminating the body's own cells (low expression of "mannose-type" units) from non-self structures. In this regard, both gram-negative and -positive bacteria, viruses, fungi and protozoa are recognized by different PAMPs including peptidoglycan, lipoteichoic acids and LPS. On a molecular level, binding to MBL is believed to result in a conformational change triggering complement activation, cytokine release and phagocytosis amongst others. Furthermore, MBL binds to damage-associated molecular pattern (DAMP) of "altered-self" cell structures and assists clearance of apoptotic cells. Recently, other pathways of innate immunity involving MBL have been revealed. For example, MBL seems to be a co-receptor for TLR2/6-signaling similar to the LBP that delivers LPS to CD14 initiating the TLR4/MD-2 cascade (see chapter 1.1.1).[20]

1.4. Chemical *O*-glycosylation of carbohydrates

Carbohydrates comprise a substance class that is highly abundant in nature with monosaccharides as their common building blocks. The simplest monosaccharides exhibit the chemical formula $C_n(H_2O)_n$ (Figure 12a). Depending on the position of the carbonyl group they are classified as either aldoses or ketoses, and the number of carbon-atoms decides on their assignment to *e.g.* pentoses or hexoses. As an example, various hexoses like D-mannose, D-galactose or D-glucose only differ in their configuration of the chiral centers forming a group of diastereoisomers. The position of the chiral center most distant from the carbonyl group in the Fischer projection determines whether the D- or L-form is present. Usually, monosaccharides are present in their cyclic form resulting from an intramolecular hemiacetal formation (Figure 12b). Depending on the ring size these structures are called furanoses (5-membered) or pyranoses (6-membered), respectively. Due to the ring formation, a new stereogenic center – the **anomeric center** – arises. The configuration at the anomeric center is designated as "α" or "β" depending on the stereochemical relationship to the most distant stereogenic center (Figure 12c). If they are in a *cis*-orientation, the α-anomer is present, if they are *trans*-related, it is called the β-anomer. There are several conformations for the six-membered rings, however, the chair conformation $^4C_1$ is usually the most proper description. For D-sugars in the $^4C_1$-conformation the α-isomer bears an axial, the β-isomer an equatorial anomeric substituent.[45]

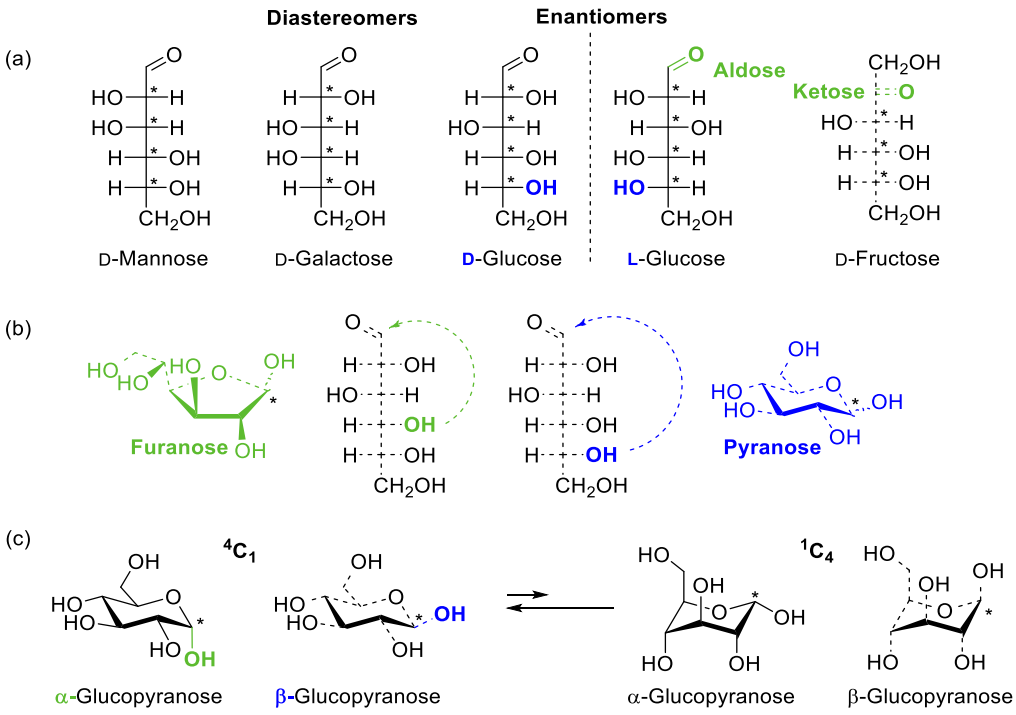


Figure 12: Monosaccharides: (a) Fischer projection of selected hexoses, D/L-descriptors and the difference between aldose and ketose; (b) hemiacetal formation generating furanose- and pyranose-rings; (c) two pyranose chair-conformers $^4C_1$ and $^1C_4$ and the α- and β-forms thereof.[45]

If possible, a ring substituent will try to orient in an equatorial position to avoid unfavourable 1,3-diaxial Van-der-Waals repulsion. However, at the anomeric center the axial substituent (α-anomer in the $^4C_1$-conformation) is thermodynamically preferred. This effect is called the **anomeric effect** and is explained by dipole-dipole-interaction or molecular orbital theory.

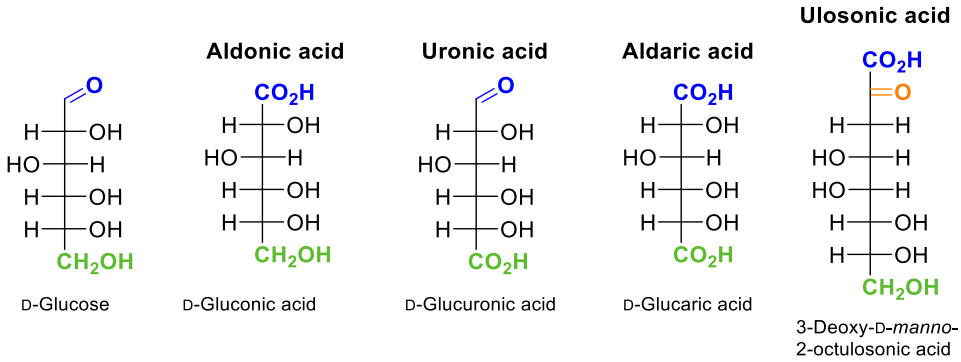


Figure 13: Sugar acids of the *gluco*-configuration and the ulosonic acid Kdo.

Oxidation of the aldehyde or the primary hydroxyl group affords different sugar acids (Figure 13): (a) oxidation of the aldehyde group (aldonic acids); (b) oxidation of the primary hydroxyl group (uronic acids); (c) oxidation of both the aldehyde and the primary hydroxyl group (aldaric acids); (d) oxidation of C-1 in a 2-ketose (ulosonic acids).

1.4.1. Principle of O-glycosylation

If a carbohydrate moiety is linked *via* its anomeric center to an alcohol functionality, an O-glycosidic bond is formed. The hydroxyl group can derive from a simple alkanol (*e.g.* methanol, ethanol, 2-propanol), a complex natural product or another monosaccharide. In case of the latter, a disaccharide is formed and consecutive elongation provides oligo- and polysaccharides. The chemical O-glycosylation of a glycosyl acceptor with a glycosyl donor is still the predominant method to synthesize defined oligosaccharide structures (Figure 14). Thereby, one saccharide unit is designed as the donor moiety, carrying a dormant leaving group (L) at the anomeric center. This group is activated by treatment with a suitable promotor, generating a reactive species that possesses enough electrophilic character to be attacked by a weakly nucleophilic hydroxyl group of the glycosyl acceptor. This attack is usually irreversible and may occur from the top or the bottom face, respectively. The direction of the incoming nucleophile dictates the anomeric configuration of the glycosidic linkage.[45]

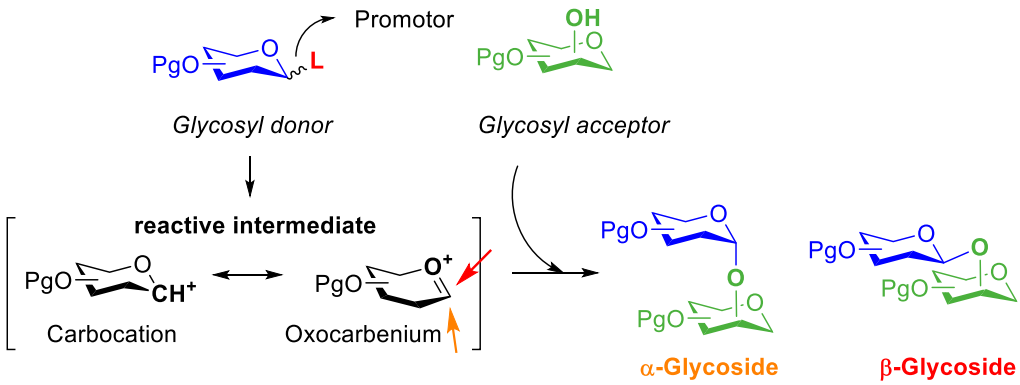


Figure 14: General mechanism of chemical O-glycosylation; L = leaving group; Pg = protecting group.[45]

The major problems encountered during chemical O-glycosylation are: (a) control over α/β-selectivity; (b) the need to match the reactivity of glycosyl donor and acceptor for productive glycoside formation; (c) the high propensity of activated donors to react with other nucleophiles like water necessitating water- (and air-) free conditions; (d) loss of donor due to other side reactions[46] (*e.g.* elimination, rearrangement of imidate donors).[47] While the latter two issues are usually overcome by a sacrificial excess of donor, stereoselectivity and reactivity adjustments are often not precisely predictable and have to be controlled by variation of protecting groups, leaving groups and reaction conditions. Rational design for high stereoselectivities is mainly complicated by the lack of knowledge of the exact reaction mechanism. There is some debate about the reactive intermediates after leaving group activation and their influence on the α/β-ratio. Basically, O-glycosylation can be considered as a substitution reaction. If the leaving group L of a glycosyl donor is activated, the nucleophile may attack in an S_N2 reaction prior to dissociation of L (Figure 15). Proceeding with stereo-inversion, the outcome is dependent on the configuration of the applied donor. If the leaving group is cleaved before the attack of the alcohol, a solvent-separated ion pair (SSIP) or oxocarbenium ion is formed, which will react in an S_N1-reaction without stereo-selectivity. Although examples are known where the reaction may follow one of these two extremes, most cases are supposed to follow either an "S_N1-like" or "S_N2-like" route. Depending on the equilibrium between the activated donor, the oxocarbenium ion (SSIP) and an intermediate contact ion pair (CIP), in which the leaving group is still blocking one of the two faces, a mixture of α/β-anomers will be obtained.[48] Notably, this type of O-glycosylation proceeds under kinetic control and is irreversible. High α-selectivity is often rationalized by the anomeric effect. However, although the axial anomeric substituent is thermodynamically more stable, this trend should not have an influence on the stereochemical outcome of a kinetically controlled reaction.[49] General strategies to influence the stereoselectivity will be discussed in the course of the following chapters.

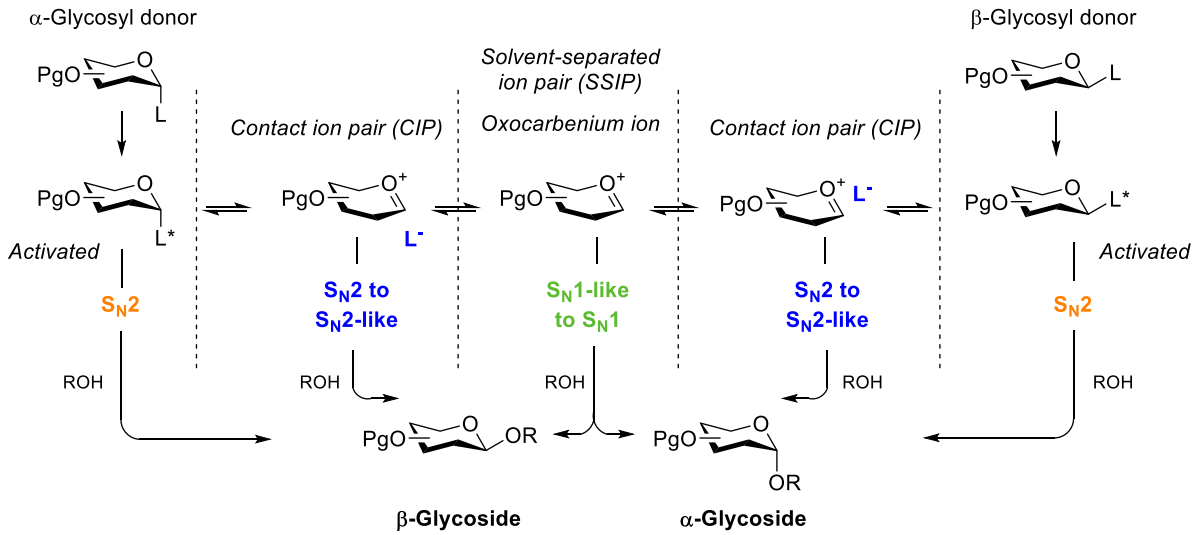


Figure 15: Detailed mechanism for chemical O-glycosylation: L = leaving group; Pg = protecting group; ROH = alcohol.[48]

Fischer glycosylation

In contrast to the kinetically controlled O-glycosylation presented above, the Fischer glycosylation capitalizes on thermodynamic control, whereby high α-selectivities may be encountered according to the anomeric effect. A hemiacetal is converted in a large excess of alcohol (solvent) under acid catalysis and (usually) reflux conditions.[50,51]

1.4.2. Leaving groups

Choosing a proper leaving group is a crucial step when attempting a glycosylation reaction. Factors like stability, reactivity, methods for their activation and the possibility to influence the anomeric selectivity during glycoside formation have to be considered.[47] In the following section a brief and incomprehensive overview of the most important classes of glycosyl donors is presented mainly focusing on aspects relevant for the thesis.

Halides

Historically, glycosyl halides were the first donors applied in stereoselective O-glycosylation. The reactivity increases in the following order: F < Cl < Br < I (Figure 16). In spite of their high reactivity, properly protected glycosyl iodides have gained interest as they allow for stereocontrolled S_N2-reactions in anionic glycosylation protocols. In contrast, bromide donors are more versatile in their use. Since the application of the Koenigs-Knorr method for the first stereoselective glycoside formation at the beginning of the last century, the basic protocol has been developed into a powerful tool in modern synthesis. Glycosyl chlorides exhibit higher stability being

beneficial for long-term storage, but the concomitant decrease in reactivity may completely suppress glycoside formation. In contrast, fluoride donors feature high stability while activation is possible with mild fluorophilic promotors. Notably, bromide and fluoride are among the major leaving groups applied in modern glycosylation reactions.[52]

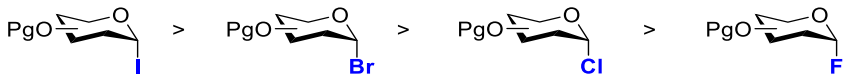


Figure 16: Glycosyl halides and their order of reactivity in glycosylation reactions.[52]

For a long time glycosyl fluorides were believed to be too stable to serve as convenient donors due to the large dissociation energy of the C-F bond (558 kJ/mol). Since the opposite observation that they can be easily activated by $SnCl_2$, which is a mild Lewis acid, fluoride donors have evolved to one of the major donor types due to several advantages: (a) mild activation with a panoply of promotor systems that is often orthogonal to other leaving groups; (b) versatile preparation methods from different starting compounds; (c) usually high stability allowing for storage and chromatographic purification on silica; (d) the possibility for ^{19}F NMR analysis. The fluorophilic rather than the acidic properties of the promotor are important for activation. Prominent methods rely on $SnCl_2$-AgX (X = OTf, ClO_4), Cp_2MCl_2/AgX (M = Zr, Hf; X = OTf, ClO_4), $BF_3{\cdot}Et_2O$ or protic acids (TfOH). Glycosyl fluorides are usually prepared starting either from hemiacetals (DAST, Selectfluor/Me_2S), from glycosyl esters like acetates (HF, HF·pyridine), from other halides (AgF, KHF_2) or from thioglycosides (DAST/NBS). In addition, glycosyl fluorides are capable of glycosylating silyl protected hydroxyl groups. No free acid is generated, thus rendering base addition unnecessary. In several cases an increase in reaction rate has been observed for silyl-protected acceptors in comparison to the naked hydroxyl group. However, this is not a general rule.[52]

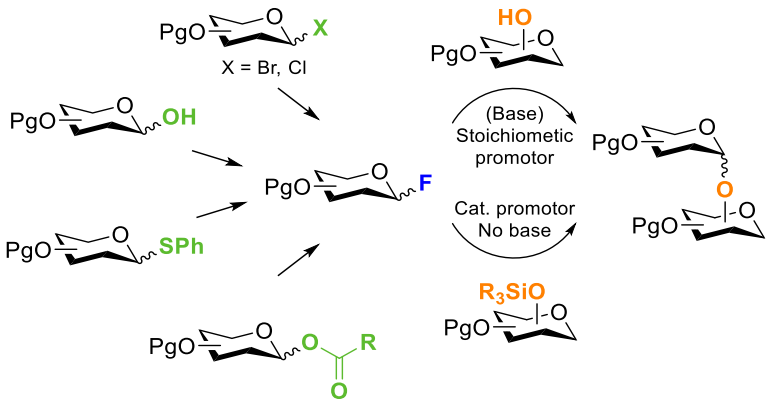


Figure 17: Preparation and application of fluoride donors.[52]

Thioglycosides

Thioglycosides have experienced tremendous popularity due to their stability towards methods used for the introduction and cleavage of a wide range of protecting groups. Thus, the thioalkyl or –aryl group, respectively, may serve as convenient anomeric blocking group during installation of a proper protecting group pattern (Figure 18). Subsequently, the leaving group can be activated by thiophilic reagents (*e.g.* NIS/TfOH). Alternatively, the thioglycoside may be converted into various types of donors either directly or via an intermediate hemiacetal.[53]

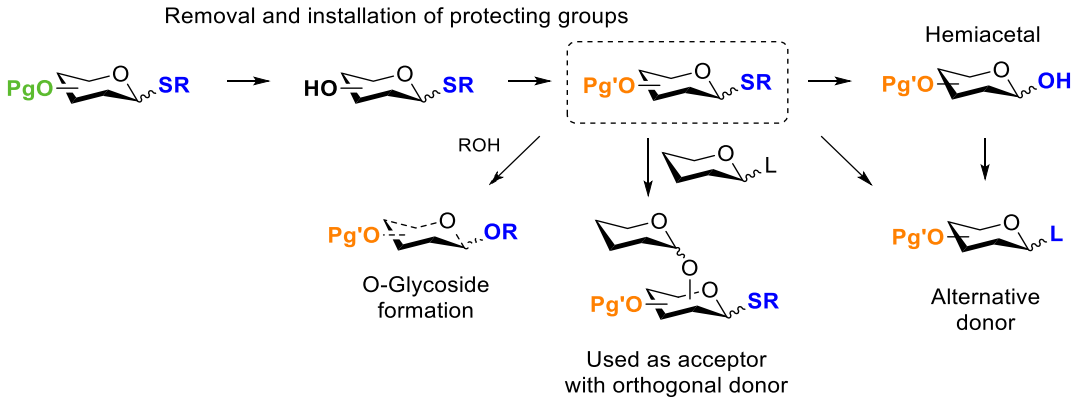


Figure 18: Protecting group manipulation and potential applications of thioglycosides.[53]

Imidates

Another important class comprises the glycosyl imidates invented by Schmidt and co-workers.[54] Trichloroacetimidates are highly reactive and due the possibility to activate them with only a catalytic amount of Lewis acid (TMSOTf, $BF_3{\cdot}Et_2O$) they are often advantageous over glycosyl halides or thioglycosides (Figure 19a). They are easily prepared by a base-catalyzed reaction of the respective hemiacetal with trichloroacetonitrile. Their major drawback is the potential rearrangement to form unreactive amidates during glycosylation. Thus, the advanced N-substituted trifluoroacetimidoyl donors are often applied, which exhibit decreased reactivity, what makes them, however, easier to handle (Figure 19b). In contrast to the trichloroacetimidates, these donors have to be prepared from the corresponding imidoyl chloride.[55]

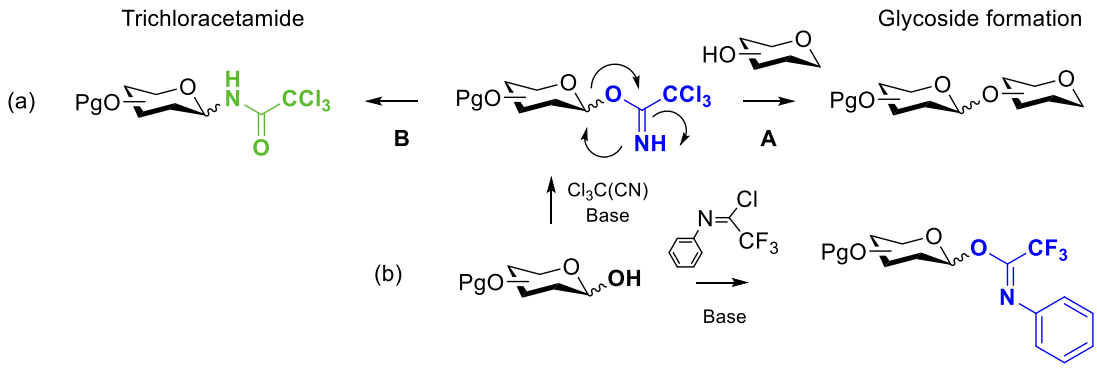


Figure 19: Preparation and use of trichloroacetimidates (a): productive disaccharide formation (route A) and side reaction yielding unreactive trichloroacetamides (route B); preparation of refined N-phenyl trifluoroacetimidoyl donors (b).

1.4.3. Protecting groups

The hydroxyl groups at the carbohydrate ring differ in their reactivities (Figure 20). The anomeric hydroxyl group is usually the most reactive and has to be protected prior to manipulation of the remaining groups. The primary hydroxyl group will react preferentially over an equatorially oriented secondary OH, which is in turn more reactive than an axially oriented one. In addition, other functional groups (amines, carboxylic acids) may be present. The sequential introduction of suitable protecting groups capitalizes on these slight differences in reactivity.

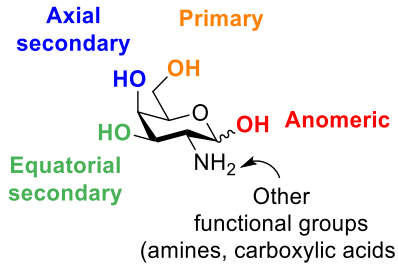


Figure 20: Hydroxyl and other functional groups within a monosaccharide with different reactivity.[45]

The blocking groups not only allow for regioselective glycosylation, but also enhance solubility in the typically applied aprotic solvents, influence the reactivity and contribute to the stereochemical outcome.[45]

Anchimeric assistance – 1,2-*trans* glycosides

Ester-type protecting groups including O-/N-acetyl, -chloroacetyl, -benzoyl and -pivaloyl as well as N-phthalimidoyl, in position next to the anomeric center can interact with the reactive intermediate after leaving group activation forming a cyclic dioxolenium ion (Figure 21). Thereby, one face is blocked and the nucleophile attacks from the opposite side forming a 1,2-*trans*-glycoside. Depending on the configuration of the sugar, α- (*e.g.* D-mannose) or β- (*e.g.* D-glucose) glycosides are selectively obtained capitalizing on this **anchimeric effect**. However, whether neighboring group participation has an impact during the course of glycosylation further depends on the leaving group and other reaction conditions. In a side reaction, the nucleophile can also attack on

the dioxolenium ring forming an orthoester, which may be rearranged to the glycoside in a stereoselective way.[45] Further development of this concept gave rise to new protecting groups aiming at: (a) the suppression of orthoester formation by sterical hindrance; (b) milder and orthogonal cleavage methods; (c) the combination of a participating and still electronically-arming group and (d) the potential application of chiral auxiliary groups to address both 1,2-*trans* and 1,2-*cis* glycosides by simply varying the chiral center. Notably, it has been shown that remote protecting groups may significantly influence the stereochemical outcome by either electronic effects or actual interaction with the reactive intermediate.[56] Furthermore, temporary directing groups are conveniently applied in the stereoselective glycosylation of 2-deoxy sugars (see below).

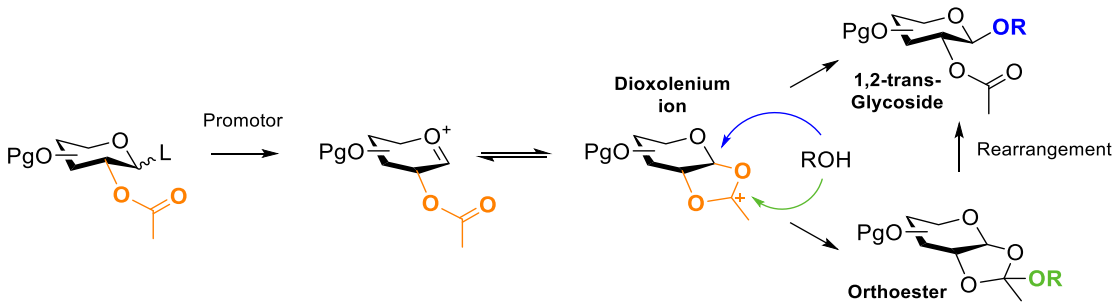


Figure 21: The anchimeric effect – a participating group on C-2 directs the nucleophile to attack from the *trans*-side.[45]

1,2-*cis* Glycosides

Non-participating groups including ethers, acetals or silyl groups, are typically installed in vicinity to the anomeric center if 1,2-*cis*-glycosides are aspired. To obtain the desired stereoisomer selectively, the influence of other parameters may be exploited: (a) solvent effects (see chapter 1.4.4); (b) temperature (see chapter 1.4.4); (c) the influence of protecting groups on the conformation and reactivity of the glycosyl donor and acceptor; (d) tethering of acceptor to the donor, which is transferred from the respective face upon leaving group activating in an intramolecular fashion; (e) *in-situ* anomerization of glycosyl halides by X^- providing a more reactive β-halide; (f) sterical shielding by blocking groups; (g) long-range participation of remote protecting groups (see above); (h) hydrogen-bond mediated aglycon delivery (HAD) by *e.g.* picolinyl groups; (i) direction by the coordination of metals; and (j) use of other additives.[57]

Benzylidene protection

The use of 4,6-O-benzylidene protected donors in combination with triflate-based promotors is a prominent glycosylation method to obtain high stereoselectivities. While the D-*manno*-configured compounds give mostly β-glycosides (which are otherwise difficult to address), high α-selectivitiy may be achieved in the D-*gluco*-series. For

D-mannose, there is a general agreement about the formation of an intermediate α-triflate - based on NMR spectroscopic evidence - which is in fast equilibrium with a contact ion pair (CIP, see Figure 22). The nucleophile is believed to attack on the CIP in an S_N2-like replacement, in which the bottom-face is shielded by the proximal triflate, and thus, the β-attack is preferred. Partly contradictory mechanistic investigations indicate that the benzylidene group contributes by both torsional and electronic effects to this outcome. Concerning the first, the rigidity of the acetal restricts the development of the common conformations (half-chair H, boat B) of the intermediary oxocarbenium ion rendering its formation unlikely. Similarly, the benzylidene group locks the C5-C6 bond in a *trans-gauche* (*tg*) relation, forcing the C5-O5 and the C6-O6 bonds into an anti-periplanar alignment, whereby the electron-withdrawing effect is maximized and thus, the formation of the oxocarbenium ion is disfavored. Irrespective of the exact contribution of both factors, destabilization of the oxocarbenium ion favors formation of the α-triflate which governs the stereochemical outcome. Notably, this trend does not follow a general rule as other factors (leaving group, participating and remote protecting groups, solvent) may dominate the glycosylation pathway. In the *gluco*-series, the ongoing mechanisms are less well understood. One explanation – supported by primary kinetic isotope effects – assumes rapid interconversion between the α- and the less stable β-triflate, and stereoselctive S_N2-type displacement of the latter.[48,56,58–60]

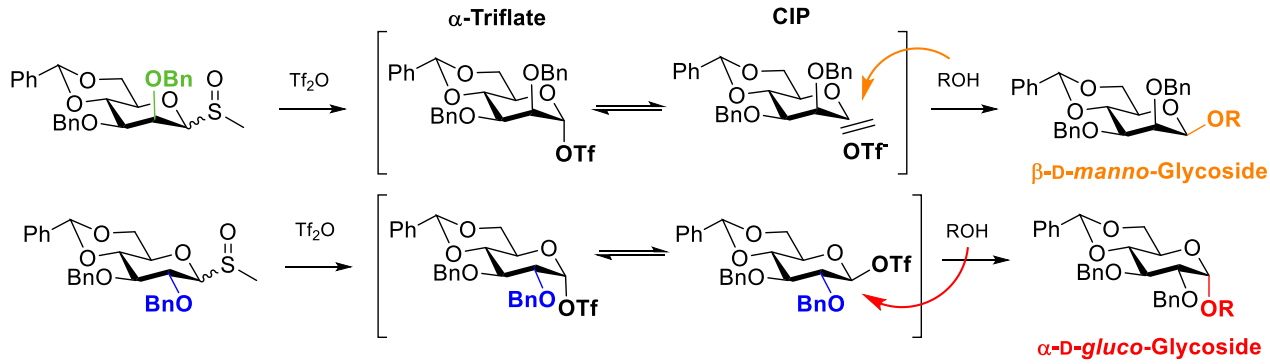


Figure 22: Effect of 4,6-O-benzylidene group on the stereochemistry in the *manno*- (top) and *gluco*- (bottom) series.[48,58]

In addition to its stereochemical influence, the benzylidene group provides high potential for further modifications. Beside removal by hydrolysis or catalytic hydrogenation and oxidative opening generating benzoyl structures, its regioselective reductive opening has become a major synthetic tool in carbohydrate chemistry.[61] The 4,6-O-benzylidene group in hexoses is activated by an acid followed by attack of a reducing agent resulting in a benzyl-protected and a free hydroxyl group. High selectivity for either the 4-O-benzyl or the 6-O-benzyl isomer depends on an appropriate combination of a Broensted or Lewis acid (LA), the reducing agent, the solvent and the temperature. Various reagents have been reported for the regioselective opening, amongst which the most

prominent are: (a) $LiAlH_4/AlCl_3$ providing 4-O-benzyl ethers (Liptak's method); (b) $NaCNBH_3$/HCl in diethyl ether (the original Garegg protocol); (c) the modified Garegg method using $BH_3{\cdot}NMe_3/AlCl_3$ providing either 4-O-benzyl (toluene) or the 6-O-benzyl (THF) isomers in a solvent-dependent manner; (d) the use of $BH_3{\cdot}THF$ as a hydride source in combination with different LA like TMSOTf or Bu_2BOTf; and (e) the application of Et_3SiH as an alternative reducing agent. Previously it was assumed that the stereoselectivty is a result of steric factors that dictate the attack of the acid (H^+ vs. LA). However, further mechanistic investigations for the opening based on boron-containing reducing agents have revealed a different picture.

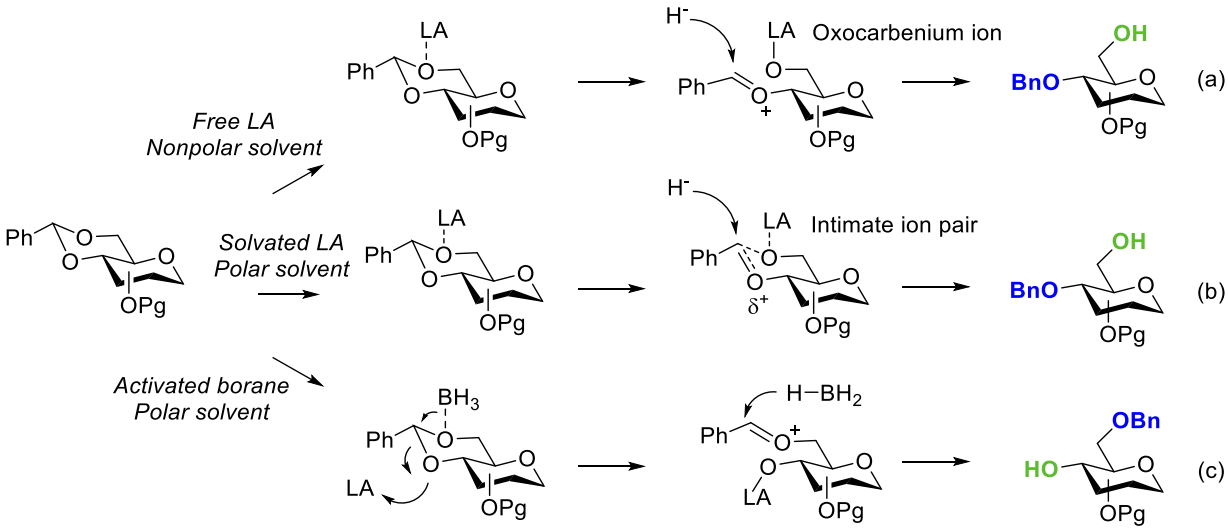


Figure 23: Mechanistic proposal for the reductive ring-opening of 4,6-O-benzylidene protected hexopyranosides involving boron-based reducing agents.[62]

In general, three pathways for boron-based hydride sources have been proposed depending on the reaction conditions (Figure 23): (a) in non-polar solvents (toluene) the LA is non-solvated and thus highly electrophilic; it is attacked by the more nucleophilic O-6, an oxocarbenium ion is formed which is attacked by a hydride from the reducing agent giving the 4-O-benzyl protected product; (b) in polar solvents (THF) the LA is solvated and thus less reactive; after coordination to the more nucleophilic O-6 the reducing agent attacks on an intimate ion pair giving again the 4-O-benzyl isomer; (c) in contrast, activated borane species (*e.g.* $BH_3{\cdot}NMe_3$) may react with the solvated LA (polar solvents) forming a naked BH_3 (and *e.g.* $LA{\cdot}NMe_3$), which is more reactive towards the 6-oxygen than the LA; with the primary oxygen occupied, the LA coordinates to the 4-oxygen generating an oxocarbenium ion which is reduced by the borane; thereby, the reversed regioselectivity is accessible. Notably, free hydroxyl groups may coordinate the borane species leading to a different outcome. If Et_3SiH is employed as the hydride source, 6-O-benzyl protected products are usually obtained with high selectivity. An exception of this trend is the combination with the strong LA $PhBCl_2$ giving access to the 4-O-benzyl isomer. [62]

Glycosylation of 2-deoxy sugars

The lack of a C-2 substituent in 2-deoxy glycosyl donors prohibits reliable control over the α/β-ratio (Figure 24a) and makes the resulting glycosidic linkage more susceptible to hydrolysis. Typically, the α-anomer is formed preferentially and β-selectivity is difficult to address, however, this usually depends on the protecting groups and the applied reaction conditions in a widely unpredictable manner. The most prominent leaving groups include acetates, fluorides, trichloroacetimidates and thioglycosides, while glycosyl chlorides and bromides are often sensitive to hydrolysis. For reliable control of the stereochemistry a temporary directing group can be installed at the C-2 position (Figure 24b). Prominent assisting groups include halides (iodine, bromine), alkylthio and alkylseleno substituents, which direct the incoming nucleophile to form 1,2-*trans* products. Depending on the desired anomeric configuration, the directing group has to be introduced at C-2 in an axial or equatorial configuration, respectively.[63] In a subsequent step the directing group is reductively removed, and treatment with tributyltin hydride/azobisisobutyronitrile (AIBN) or catalytic hydrogenation are the most prominent procedures.

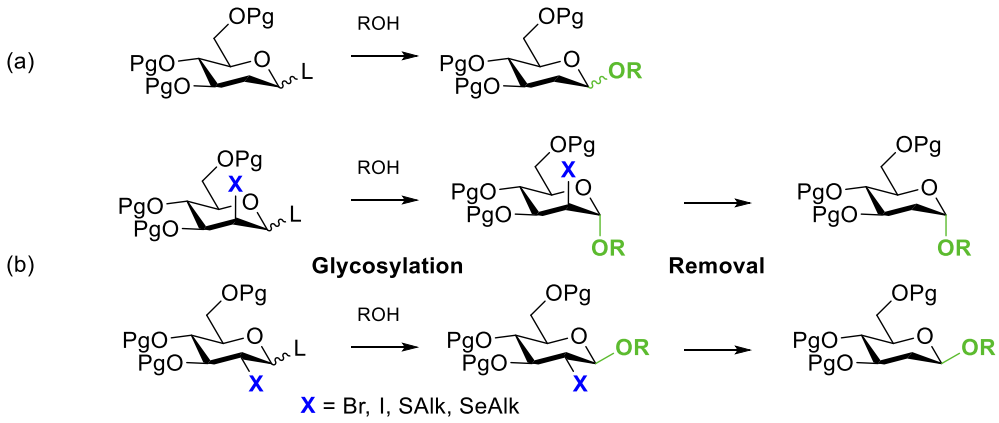


Figure 24: Direct (a) and indirect (b) glycosylation methods for 2-deoxy sugars and the stereochemical outcome.

The mechanism of the directing effect of these assisting groups is subject to some debate. The intermediate (oxocarbenium ion) may interact with the directing group forming a cyclic species (episulfonium ions, iodonium), which forces the nucleophile to attack from the opposite side (Figure 25). However, frequently observed parallel formation of the 1,2-*cis* isomer is contradictory to this assumption. Theoretical calculations for the episulfonium ion indicated that this cyclic intermediate is unfavored and less reactive compared to the oxocarbenium ion. Experimentally, however, it is difficult to distinguish between the two species. Thus, although the presence of a cyclic episulfonium ion is possible, it may not be the reactive intermediate. Alternatively, favorable axial orientation (hyperconjugation) of the large substituent in the oxocarbenium ion may direct the nucleophile to

attack from the *trans*-side due to stereoelectronic effects.[63,64]

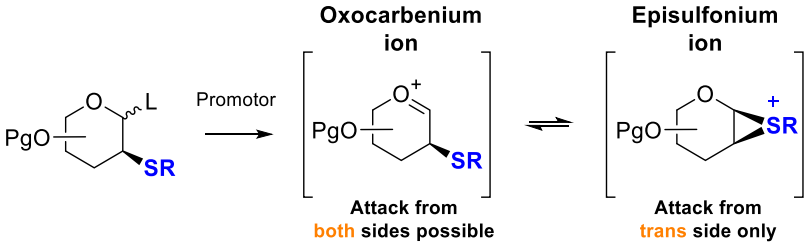


Figure 25: Plausible intermediates during glycosylation with a 2-alkylthio-2-deoxy sugar donor.[64]

1.4.4. Parameters

Solvent

As a rule of thumb, in polar solvents the formation of β-glycosides is preferred due to charge separation between the endocyclic oxygen and the β-oriented glycosidic oxygen. To enhance the α-selectivity chlorinated solvents (CH_2Cl_2, $ClCH_2CH_2Cl$) or toluene may be suitable. However, more pronounced effects are seen for ether-type (Et_2O, THF, 1,4-dioxane) and nitrile (MeCN, EtCN) solvents. Ethers are believed to interact with the intermediate oxocarbenium to form an equatorially oriented oxonium ion due to electronic interaction with O-5 (Figure 26). Thus, the nucleophile attacks from the bottom face providing the α-anomer. In contrast, the axial nitrilium ion is preferred allowing for a stereoselective formation of the β-glycoside. A prerequisite for these effects is the absence of a participating neighboring group.[45,57]

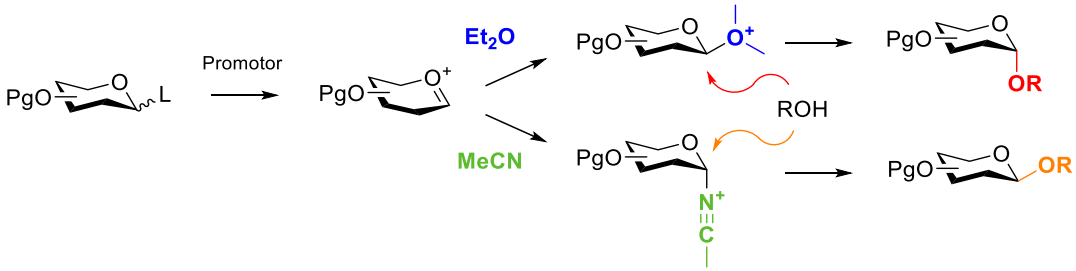


Figure 26: Solvent effects of ether-type and nitrile solvents and their influence on the anomeric selectivity.[45]

Temperature

In kinetically controlled glycosidation, preferential formation of the β-anomer is usually observed at lower temperature, while the α-selectivity increases at higher temperature. This has been rationalized by the fact, that

the β-product is the kinetic product and the α-anomer exhibits higher thermodynamic stability according to the anomeric effect (see chapter 1.4).[57] However, it has been argued that the irreversibility of the kinetically controlled glycoside formation excludes an influence of the anomeric effect.[49]

1.5. 3-Deoxy-D-*manno*-2-octulosonic acid (Kdo)

Kdo is an eight-carbon 2-keto sugar with a carboxylic acid at C-1 (Figure 27). The cyclic furanose or pyranose structures are formed by nucleophilic attack of the 5- or 6-hydroxyl group at the 2-ketone, respectively, generating the anomeric center at C-2. The 3-position lacks any substituent, thus, Kdo belongs to the group of deoxy-sugars (see chapter 1.4.1). [65] For the prevalent pyranose form, carbons 7 and 8 comprise an exocyclic side chain, which aligns in a sterically favored equatorial orientation in the preferred $^5C_2$ chair conformation unless torsionally restricting protecting groups (*e.g.* 4,5-O-isopropylidene) promote a conformational change. The axial glycosidic linkage is termed as α-connection, an equatorial substituent gives rise to the β-isomer.[65]

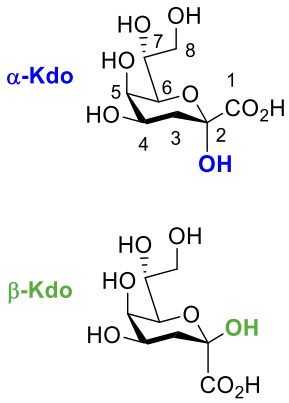


Figure 27: Structure of α- and β-Kdo.[65]

Kdo is an essential component of bacterial LPS. It connects the core oligosaccharide in a conserved α-(2→6) linkage to the lipid A (see chapter 1.1.2).[10,66,67] Kdo was further identified in the capsular polysaccharide of several bacterial strains (*e.g. E. coli* serotype K12)[68] and related cell-wall structures of root-nodule bacteria[69,70], wherein the β-anomer is prevalent. In eukaryotes Kdo is a component of polysaccharides in plants.[71]

1.5.1. Chemical synthesis of Kdo

As Kdo is naturally not abundant, different chemical and (semi-)enzymatic preparation protocols have been developed. Syntheses mainly rely on a [5+3]-approach starting from the available five-carbon sugar D-arabinose and a non-carbohydrate three-carbon reagent, or on a [6+2]-route capitalizing on D-mannose and a two-carbon synthon (Figure 28). Miscellaneous strategies including radical and enzymatic approaches as well as *de novo* syntheses from non-carbohydrate precursors – despite their value for the exploration of synthetic scope – are rarely used for large-scale applications.[72]

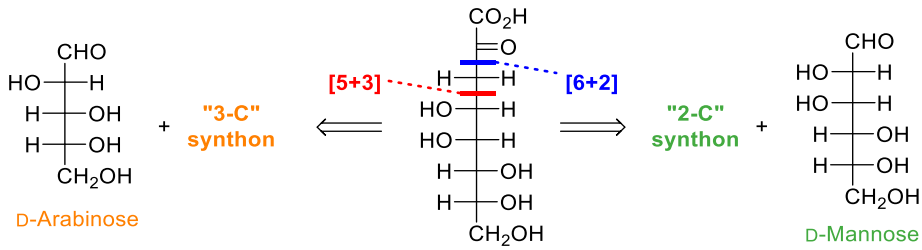


Figure 28: The two most common strategies for Kdo synthesis.[72]

Still, large-scale preparation of ammonium Kdo by the revised [5+3]-Cornforth protocol is one of the most important methods (Figure 29). An aldol reaction between D-arabinose and the enolate of oxaloacetic acid under alkaline conditions gives a diastereomeric mixture of C-3 and C-4 epimers with preference for the desired "*manno*"-configured product. The carboxylic acid on carbon-3 is subsequently removed by a Ni^{2+} catalyzed decarboxylation under slightly acidic conditions resulting in a mixture of Kdo and its *gluco*-isomer. The two epimers can be separated by anion exchange chromatography (AEC) using a gradient of ammonium hydrogen carbonate. Crystallization provides ammonium Kdo serving as a general starting material for diverse Kdo moieties.[65,72,73] The overall yields of the Cornforth method are usually moderate (45-60%) but are outweighed by its shortness and the inexpensive reagents. Very recently, an optimized three-step [6+2]-synthesis capitalizing on a C2-Wittig elongation of a D-mannose substrate was reported to provide protected Kdo ethyl ester in 75 to 80 % yield in a one-pot procedure.[74]

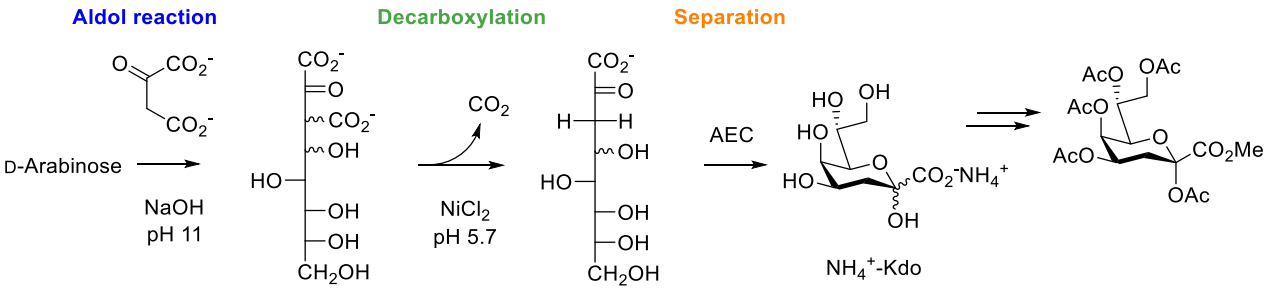


Figure 29: The Cornforth method for the preparation of ammonium Kdo – a starting material for Kdo-peracetate.

1.5.2. Glycosylation chemistry of Kdo

Glycosyl acceptors

Kdo provides hydroxyl groups of significantly different reactivities and, thus, sequential introduction of blocking groups to prepare glycosyl acceptors is generally feasible.[75] After protection of the carboxyl group (typically as a methyl, ethyl or benzyl ester) and the anomeric hydroxyl as a stable glycoside (*e.g.* methyl, allyl and thioglycosides or functional spacer groups), the exocyclic 7,8-O-side chain can be converted – in favor of the endocyclic 4,5-diol – with moderate to good selectivity if cyclic protecting groups are applied (Figure 30). These cyclic structures include the disarming carbonate as well as the activating tetraisopropyldisiloxane (TIPDS) and isopropylidene groups. For selective glycosylation on the 4-hydroxyl group, protection of the vicinal 5-position is usually not required as its axial orientation and sterical hindrance by the side chain significantly decrease its nucleophilicity.[72,76,77] In contrast, the 5-OH group can be selectively addressed only after protection of the 4-position. This is typically achieved by stannylene acetal activation of the 4,5-*cis*-diol and the subsequent regioselective reaction with an electrophile like alkyl or acyl halides (Figure 30). Usually, an equatorial hydroxyl group reacts in favor of an axial one and the vicinity to a deoxy-position further promotes a preferential attack. Thus, the 4-hydroxyl group can usually be protected with high selectivity, which can be further promoted by addition of a nucleophile like tetrabutylammonium iodide. Notably, 7,8-O-protection with the bulky TIPDS has been described to be essential to disfavor intramolecular 1,5-lactone formation. For formation of the stannylene acetal the diol is treated with dibutyltin oxide in dry MeOH, or using toluene for azeotropic removal of released water.[78]

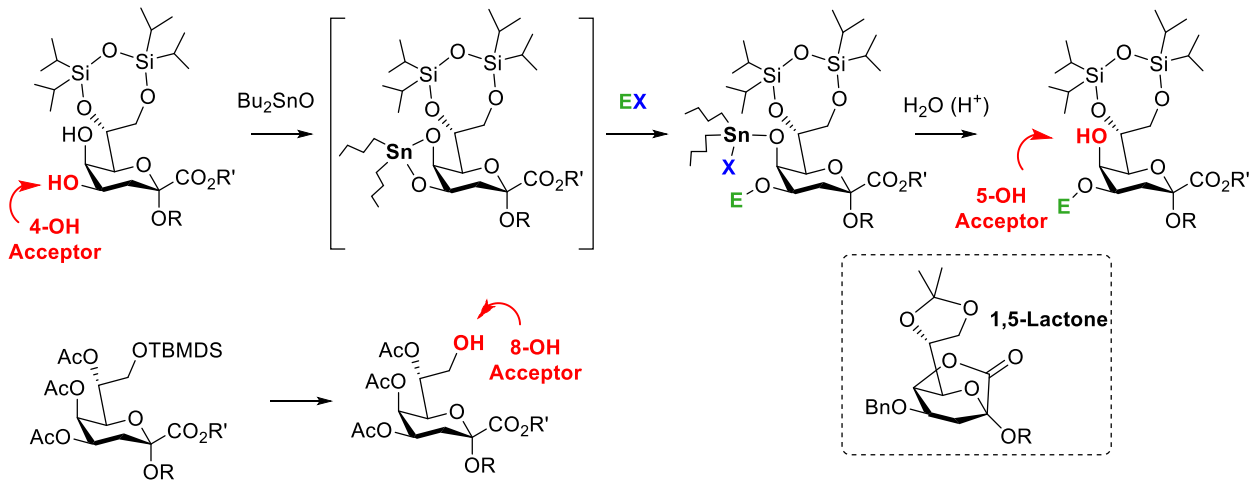


Figure 30: Preparation strategies for 4-, 5- and 8-OH acceptors of Kdo and the structure of a 1,5-lactone side product.

For 8-OH acceptors the primary hydroxyl group can be first blocked by selective introduction of a silyl group (TBDMS), followed by global acetylation and subsequent removal of the silane (Figure 30). However, this procedure suffers from acetyl migration during both the silyl deprotection and ensuing glycosylation and conditions have to be carefully chosen.[76,77]

General aspects of glycosylation with Kdo donors

The use of Kdo glycosyl donors poses a challenge due to several factors: (a) the deactivating carboxylic acid next to the anomeric centers leads to intrinsically low reactivity; (b) the lack of a substituent on position-3 hinders control over the α/β-ratio; (c) also, the elimination side reaction leading to glycal esters is promoted by the C-1 ester group and the 3-deoxy position; this side reaction increases with higher temperatures and, thus, the reaction conditions are often limited; (d) the susceptibility to hydrolysis under acidic conditions has to be considered when choosing protecting and leaving groups.[75] While an excess of precious donor is usually applied to compensate for the losses due to elimination reaction, the reactivity and stereoselectivity are influenced by an appropriate protecting group pattern and the reaction conditions (solvent, temperature) which have to be optimized for each individual case.

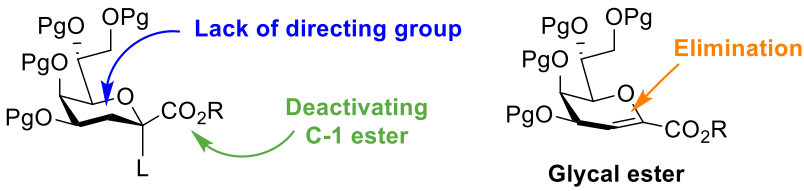


Figure 31: General problems encountered using Kdo glycosyl donors.

Anomeric configuration

The diastereomeric α- and β-Kdo products can be distinguished by NMR spectroscopic methods. Two characteristics of the α-pyranose form of acetylated-protected Kdo have been determined empirically and are used for assignment: (a) the H-4 signal is significantly shifted (ca. 0.4 to 0.7 ppm) to lower field compared to the β-isomer resulting in a chemical shift above 5.0 ppm; (b) the diastereotopic H-3*ax* and H-3*eq* signals are usually barely separated in α-Kdo, while a large difference in their chemical shift indicates the β-configuration.[65,79] However, especially the latter feature is not always unambiguous. More general, the two isomers differ in their heteronuclear coupling constant $^{3}J_{H3ax,C1}$ between the axial H-3 and the 1-carbon in proton-coupled ^{13}C-NMR spectra. Equatorial orientation of the C-1 ester in the α-Kdo results in a small coupling constant *J* smaller than 1

to 2 Hz, while the antiperiplanar arrangement in the β-anomer gives a *J* value of about 4 Hz.[65,79]

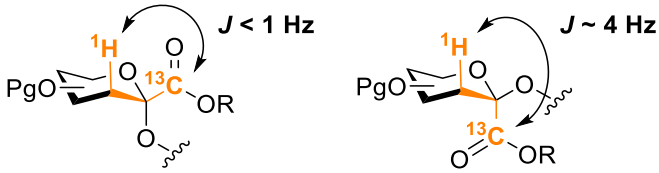


Figure 32: Heteronuclear coupling constants between axial H-3 and C-1 in α- (left) and β- (right) Kdo.[65,79]

2. Results and Discussion

2.1. Overview of contributions

 # 1 Pokorny, B.; Müller-Loennies, S.; Kosma, P. *Carbohydr. Res*. **2014**, *391*, 66-81.

"Synthesis of α-D-glucosyl substituted methyl glycosides of 3-deoxy-α-D-*manno*- and D-*glycero*-α-D-*talo*-oct-2-ulosonic acid (Kdo/Ko) corresponding to inner core fragments of *Acinetobacter* lipopolysaccharide"

 # 2 Pokorny, B.; Kosma, P. *Chem. Eur. J.* **2015**, *21*, 305-313.

"Synthesis of Chlamydia Lipopolysaccharide Haptens through the use of α-Specific 3-Iodo-Kdo Fluoride Glycosyl Donors"

 # 3 Pokorny, B.; Kosma, P. *manuscript draft*.

"Synthesis of branched oligosaccharides comprising the conserved Kdo (3-deoxy-D-manno-2-octulosonic acid) disaccharide substituted with α-(1→5) D-glucose "

 # 4 Pokorny, B.; Kosma, P. *Chemistry Open*, *accepted manuscript*, **2015**. (doi: 10.1002/open.201500126)

"Scope and limitations of 3-iodo-Kdo fluoride based glycosylation chemistry using N-acetyl glucosamine acceptors"

 # 5 Pokorny, B.; Kosma, P. *Org. Lett.* **2015**, *17*, 110-113.

"First and Stereoselective Synthesis of an α-(2→5)-Linked Disaccharide of 3-Deoxy-D-*manno*-oct-2-ulosonic Acid (Kdo)"

2.2. Context of contributions

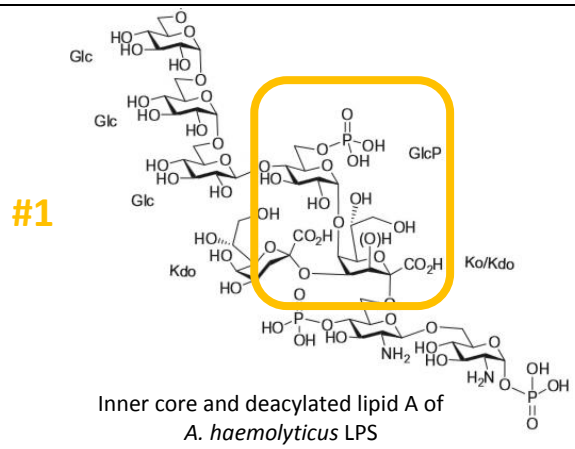
Synthesis of inner core fragments of *Acinetobacter haemolyticus* lipopolysaccharide (LPS)

Biological background

About 30 years ago, a mouse serum protein of 28 kDa was detected to bind to the LPS core region of several bacterial strains. Unaware of its actual structure and function, this protein was termed "serum factor".[80] Inhibition of the factor binding to LPS was observed for the di- and trisaccharides α-Hep(1→5)Kdo and α-Hep(1→3)-α-Hep(1→5)-Kdo, respectively, which are common motifs of the enterobacterial inner core (see chapter 1.1.2).[80] An ensuing investigation revealed a cross-reactivity of this factor with structurally divergent lipoteichoic acids, which are integral components of the gram-positive bacterial cell wall.[81] Interestingly, this factor exhibited high affinity for the LPS of *Acinetobacter haemolyticus* NCTC 10305 (former *A. calcoaceticus*) comprising a heptose-deficient inner core with Glc as the only non-charged carbohydrate moiety.[82,83] Recently, this serum factor was identified to be the mannose-binding lectin MBL-A.[84] This untypically strong binding of the *A. haemolyticus* inner core to the MBL, which usually depends on multivalent interaction for potent binding (see chapter 1.3.1), poses the question about the ongoing mechanisms on a molecular level. While the structure of the *A. haemolyticus* LPS inner core has previously been elucidated[41] (see chapter 1.2.2), this thesis shall contribute to the identification of the minimal epitope responsible for this uncommon interaction. Therefore, truncated fragments of the *A. haemolyticus* inner core have been synthesized by chemical O-glycosylation of properly protected glycosyl donors and acceptors that gave access to chemically defined structures and offered high flexibility for further modifications. The binding affinity of the deprotected compounds was subsequently evaluated by a competitive-ELISA study using murine MBL-A.

Chemical synthesis of di- to pentasaccharides

Di- to pentasaccharide fragments have been prepared in three series and the results are distributed between manuscripts **#1**, **#3** and **#4**. Paper **#2** deals with the development of a glycosylation protocol capitalizing on a new α-selective Kdo donor, on which the synthetic routes in manuscripts **#3** and **#4** relied.



Inner core and deacylated lipid A of
A. haemolyticus LPS

Contribution #1 presents the synthesis of ligands based on the α-Glc(1→5)-α-Kdo disaccharide (Figure 33). Therefore, a properly protected glucosyl donor was needed for α-selective glycosylation. The known α-directing effect of a torsionally disarming 4,6-O-benzylidene group in the *gluco*-series was successfully applied to obtain the α-anomer exclusively and in high yields (the published yield in manuscript #1 could later be increased to 94% on larger scale). Subsequently, the 4',6'-O-benzylidene, the 7,8-O-TIPDS and 4-O-PMB (*para*-methoxybenzyl) groups could be orthogonally cleaved permitting ensuing modifications.

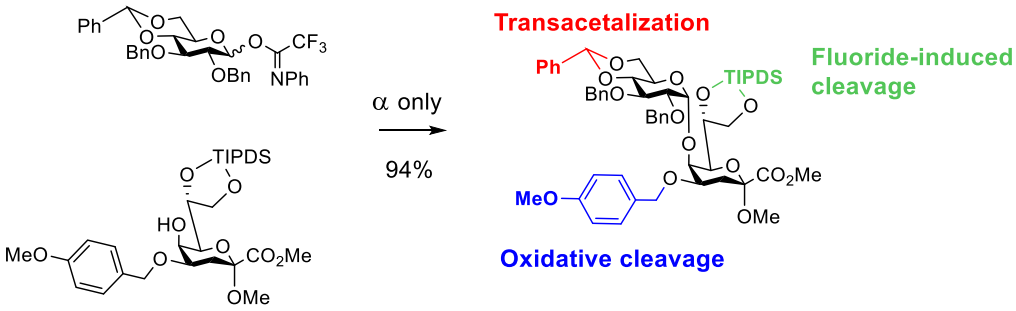


Figure 33: Key-step glycosylation in manuscript #1 and the orthogonal deprotection strategy.

Phosphorylation

The inner core of *A. haemolyticus* is non-stoichiometrically phosphorylated at the primary hydroxyl group of the innermost Glc residue. Whether this modification plays a significant role in the strong interaction with MBL-A should be revealed by comparison of binding affinities obtained for the phosphorylated and the non-phosphorylated ligands **I** and **II** (Figure 35). Reductive opening of the 4',6'-O-benzylidene group to generate a free 6-O-hydroxyl group selectively, afforded a moderate yield only (43%). Thus, a method for regioselective phosphorylation of the 4,6-diol obtained after complete removal of the acetal was developed. A common method to introduce protected phosphates capitalizes on a **phosphoramidite** reagent to form an intermediate phosphite

triester under acid catalysis (tetrazole; Figure 34, right).[61,85] The intermediate P(III) species is subsequently oxidized with *meta*-chloroperbenzoic acid (*m*CPBA) affording the protected phosphate. The acid is believed to weaken the stable P-N bond upon interaction with the lone pair of the amino group, which allows for nucleophilic attack of the alcohol. This procedure provided the desired 6-O-phosphate as the major product (67%) together with its 4-O-regioisomer (10%). Clean and quantitative removal of the benzyl groups was achieved by catalytic hydrogenation during global deprotection. In contrast, nucleophilic substitution of the diphenyl phosphoryl **chloride** in the presence of the acid scavenger 4-(*N,N*-dimethylamino)pyridine (DMAP) proceeded with complete regioselectivity and gave excellent yields (96%; Figure 34, left). However, the phenyl protecting groups suffered from nucleophilic displacement by fluoride[86] during ensuing silyl deprotection and side product formation during final deblocking with PtO_2/H_2. Thus, despite decreased regioselectivity the phosphoramidite route provided the more convenient access to the phosphorylated ligands.

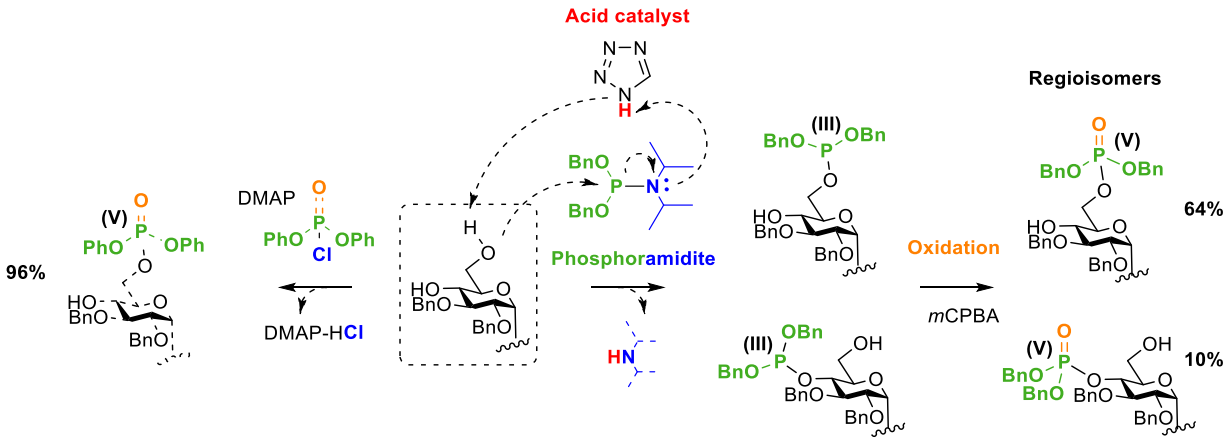


Figure 34: Two strategies to form the phosphate triesters: using the P(V)-chloride and DMAP (left); the phosphoramidite method and subsequent oxidation of the intermediate phosphite triester (right).

Smith degradation

Previously, Smith degradation of the isolated inner core did not decrease its high binding affinity to MBL-A.[84] The Smith reaction is a periodate oxidation that cleaves the covalent bond between two vicinal diol groups providing two reactive aldehyde groups, which are subsequently reduced to the alcohol oxidation level.[87,88] Following this procedure, the exocyclic side-chain of Kdo was cleaved between carbon-7 and carbon-8, and subsequent reduction afforded disaccharides **III** and **IV** containing the non-natural 3-deoxy-D-*lyxo*-2-heptulosonic acid (Figure 35). Comparative binding studies of ligands **I** and **III** (or **II** and **IV**) should evaluate the contribution of the 7,8-O-side chain of Kdo to the interaction with MBL-A.

The 3-oxy analoge: Ko

Glycosylation of a suitably protected D-*glycero*-α-D-*talo*-2-octulosonic acid (Ko, see chapter 1.1.2) acceptor with the previously applied Glc donor (Figure 33) provided the α-(1→5)-connected disaccharide **V** (Figure 35) after global deprotection. Notably, the exocyclic chain of Ko was blocked by an acetonide, which was not fully compatible with the acidic glycosylation conditions, but could be selectively generated from the 4,5:7,8-di-O-acetonide under equilibrating conditions in wet acetone. Thus, the easy and short preparation of the acceptor outweighed the low glycosylation yield (42%).

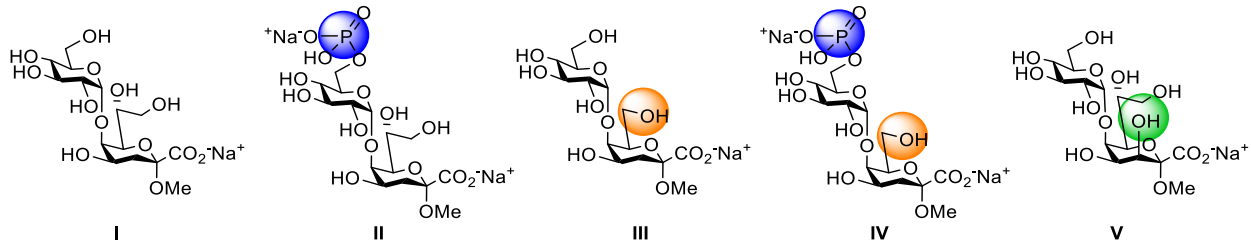


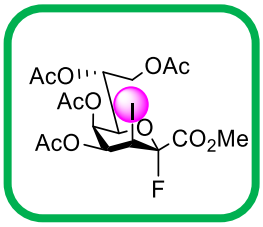
Figure 35: Disaccharide ligands (I) to (V) obtained after removal of blocking groups and ester saponification (#1).

Preliminary results (see chapter 2.5) revealed weak but similar binding affinities of the Kdo-containing disaccharides **I** to **IV** (Figure 35) to murine MLB-A. The α-Glc(1→5)-α-Ko disaccharide **V** with the additional 3-hydroxyl group was not recognized by the lectin. Although a first result for Ko-based structures has been obtained, this aspect is the topic of another thesis and thus, the remaining work focused exclusively on compounds comprising the 3-deoxy sugar Kdo.

The need for a new Kdo donor

As the Glc-Kdo disaccharides **I** to **IV** (Figure 35) were bound by the murine MBL-A with significantly weaker affinity than the isolated inner core fraction, this disaccharide should be further elongated by the α-(2→4)-linked side-chain Kdo and additional glucose residues. However, to introduce the second Kdo, a glycosyl donor of the octulosonic acid was needed. To avoid the usual problems encountered for Kdo donors (see chapter 1.5.2), a new donor capitalizing on a temporary directing group for α-selective glycosylation was introduced.

#2



New α-selective 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) donor

& synthesis of Chlamydiaceae antigens

Manuscript **#2** presents the new Kdo donor and its application in the synthesis of Chlamydiaceae inner core antigens. These structures comprise several α-(2→4) and α-(2→8)-interconnected Kdo di- to tetrasaccharides. Linking two Kdo residues poses a challenge as both the donor and acceptor residues are electronically disarmed by the C-1 ester group. Thus, the synthesis of these compounds constituted an excellent possibility for the evaluation of the new donor.

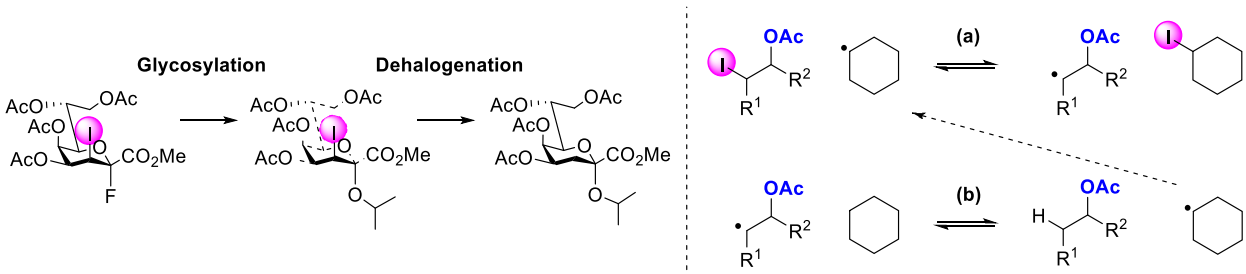


Figure 36: General strategy for the 3-iodo-Kdo donor (left) and the proposed mechanism[89] for the hydrogen atom transfer reaction using cyclohexane and lauroyl peroxide (right).

The new protocol combined the α-directing effect of a temporary axially oriented 3-iodo-substituent with the fluoride leaving group (Figure 36, left). Previously reported Kdo fluoride donors exhibited good stereoselectivity and high stability (see references in manuscript **#2**). The 3-iodo group should further enhance the preference for the α-anomer (see chapter 1.4.3). For all reactions presented in manuscript **#2**, the donor provided the desired α-linkage exclusively. Additionally, the elimination side reaction towards the glycal ester could be significantly reduced (usually below 10%) rendering a large excess of donor unnecessary. For subsequent removal of the iodo-substituent, a hydrogen atom transfer using cyclohexane in a radical chain reaction was applied. The method is described to be suitable for secondary iodide groups with electron-withdrawing groups like acetates in close proximity. The formation of a cyclohexane radical (CH·) by treatment with catalytic amounts of lauroyl peroxide is driven by the subsequent hydrogen atom transfer. The CH· may abstract the iodine substituent forming an unstabilized radical due to the vicinal acetate group (Figure 36, right: equilibrium a). In a rate determining step this radical abstracts a hydrogen atom from cyclohexane sustaining the radical chain reaction (Figure 36, right:

equilibrium b). In the case of a primary iodide substituent, equilibrium (b) would be driven even more to the product side, but reaction (a) would be extremely disfavored leading to an endothermic reaction in total. This method was capable of removing the C-3-iodine group in near-quantitative yields and, thus, the classical method using the noxious combination of tributyltin hydride/AIBN could be avoided.

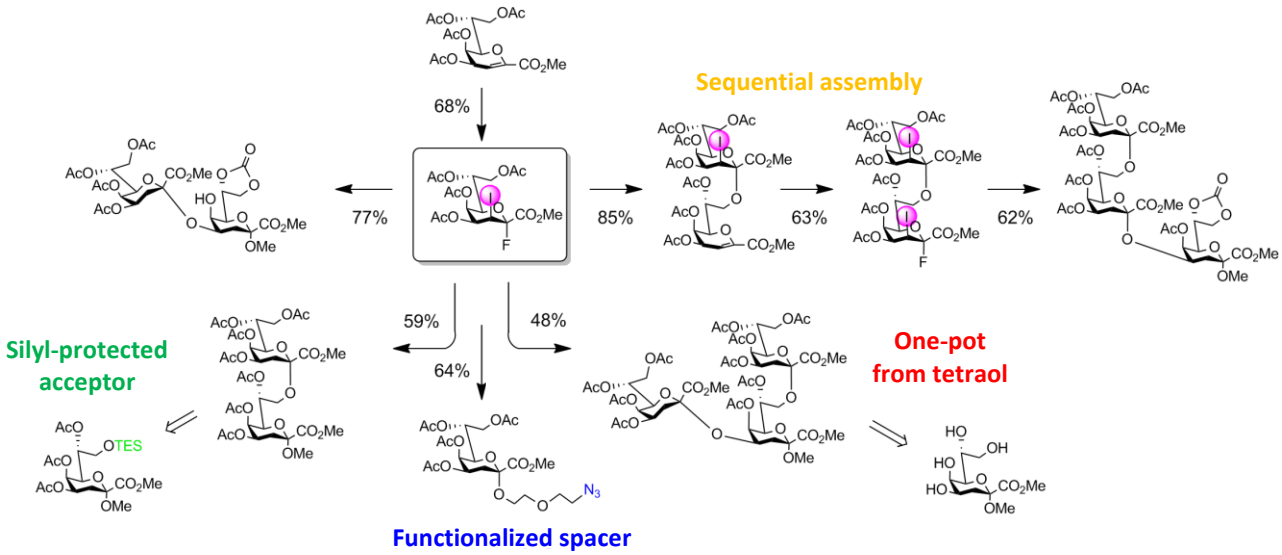
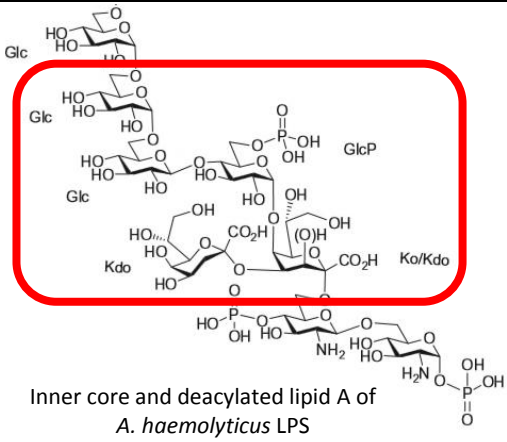


Figure 37: Scope of the new Kdo donor in the synthesis of Chlamydiaceae antigens (#2); TES = Triethylsilyl.

Manuscript **#2** presents the application of the optimized protocol in the synthesis of the following *Chlamydia*-related oligosaccharides (Figure 37): (a) the α-(2→4)-linked Kdo disaccharide (the general enterobacterial inner core motif); (b) the α-(2→8)-linked Kdo disaccharide whereby a 8-O-silyl protected Kdo acceptor could be directly converted; (c) a branched trisaccharide exhibiting both the α-(2→4)- and the α-(2→8)-linkages; notably, this trisaccharide could be prepared in a one-pot fashion starting from an unprotected Kdo methyl glycoside as acceptor; and (d) the linear Chlamydia-specific α-Kdo(2→8)-α-Kdo(2→4)-α-Kdo epitope. The application of a glycal acceptor afforded a disaccharide which could be similarly transformed into a glycosyl donor allowing for sequential assembly of Kdo moieties towards linear oligosaccharides. Notably, the method was compatible with a functional spacer bearing an azide for further reactions.

#3



Inner core and deacylated lipid A of
A. haemolyticus LPS

Capitalizing on the new Kdo donor, manuscript **#3** focuses on the synthesis of extended oligosaccharides from the *A. haemolyticus* inner core. It is mainly concerned with the synthesis of the branched trisaccharide shown in Figure 38. Two routes have been envisaged using (a) the α-Glc(1→5)-α-Kdo disaccharide acceptor (from manuscript **#1**) with the new Kdo donor or linking (b) a properly protected Glc donor to an α-Kdo(2→4)-α-Kdo (Kdo_2) disaccharide acceptor. As no trisaccharide could be obtained from route (a), a Kdo_2 acceptor was prepared in high yields and stereoselectively following a slightly modified procedure from manuscript **#2**.

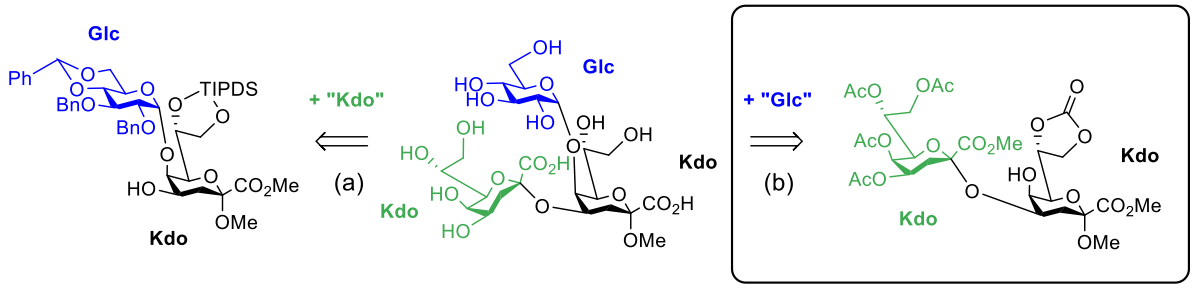
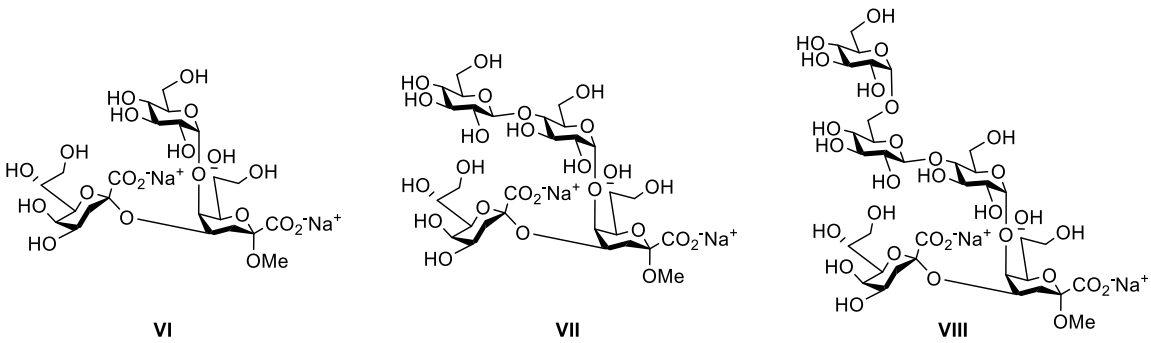


Figure 38: Two alternative approaches towards the trisaccharide: (a) using the Glc-Kdo disaccharide acceptor from paper #1; (b) using a Kdo-disaccharide acceptor (after modified procedure) from paper #2.

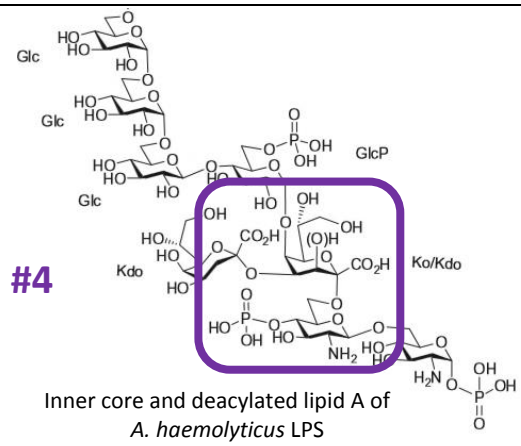
In a first experiment the Glc donor from manuscript **#1** (Figure 33), which exhibited excellent α-selectivity in previous reactions, gave only a moderate selectivity and low yields for this glycosylation step. However, both the α-selectivity (α:β 4:1) and the yield (91%) could be significantly improved by further optimization capitalizing on the α-directing effect of ether-type solvents (see chapter 1.4.4) and on accurate reaction control.



VI **VII** **VIII**

Figure 39: Extended ligands (VI) to (VIII) obtained after removal of blocking groups and ester saponification (#3).

The benzylidene group was then reductively opened in a regioselective way to give a 6-O-benzyl protected trisaccharide acceptor (see chapter 1.4.3). Further elongation with Glc and isomaltose donors in a β-(1→4)-connection, followed by deprotection afforded oligosaccharides **VII** and **VIII** (Figure 39).



Inner core and deacylated lipid A of
A. haemolyticus LPS

Manuscript **#4** deals with the synthesis of the α-Kdo(2→6)-GlcN disaccharide – the common core-lipid A linkage – using the stereoselective 3-iodo-Kdo fluoride donor (**#2**) and an orthogonally protected GlcNAc acceptor allowing for subsequent 4-O-phosphate installation. Two main problems were encountered (Figure 40): (a) the methyl **β**-glycoside acceptor resulted in concomitant furanosyl and pyranosyl oxazoline formation during glycosylation; and (b) in the presence of an **allyl** aglycon the 3-iodo-Kdo donor completely degraded to the glycal ester.

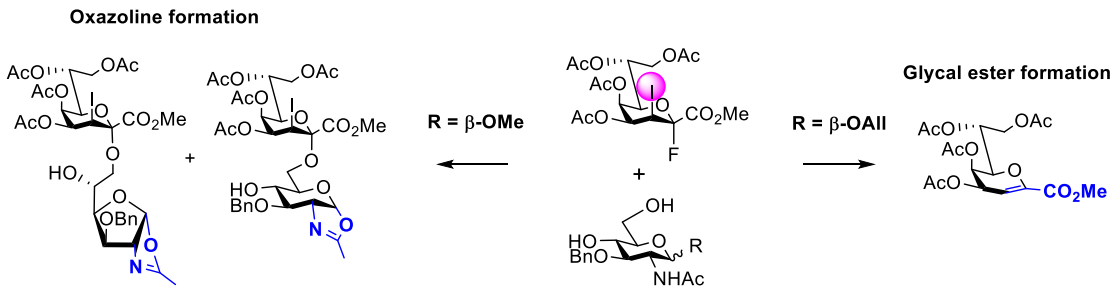


Figure 40: Side reactions using the 3-iodo-Kdo donor with different GlcNAc acceptors (#4).

The incompatibility of the new 3-iodo-Kdo donor (**#2**) and the allyl group was further examined by NMR studies, showing that nucleophilic olefins (1-octene, cyclohexene) completely suppressed glycoside formation with 2-propanol and led to fast degradation to the glycal ester (Figure 41). It was proposed that migration of iodine from the cyclic iodonium ion – a plausible intermediate of the activated 3-iodo Kdo donor (see chapter 1.4.3) – to an olefin (Route b) competes with glycoside formation (Route a). Previous examinations from Roush *et al*. revealed that halonium ions tend to migrate between olefins preferring those with high electron density while steric factors were less important (for references see manuscript **#4**). The iodonium ion of the activated donor originates from an α,β-unsaturated ester, thus it is reasonable that the iodine migrates to more electrophilic olefins including the allyl group. Consistent with these assumptions electrophilic olefins like methyl crotonate or a Kdo glycal acceptor (manuscript **#2**) did not interfere with the glycosylation.

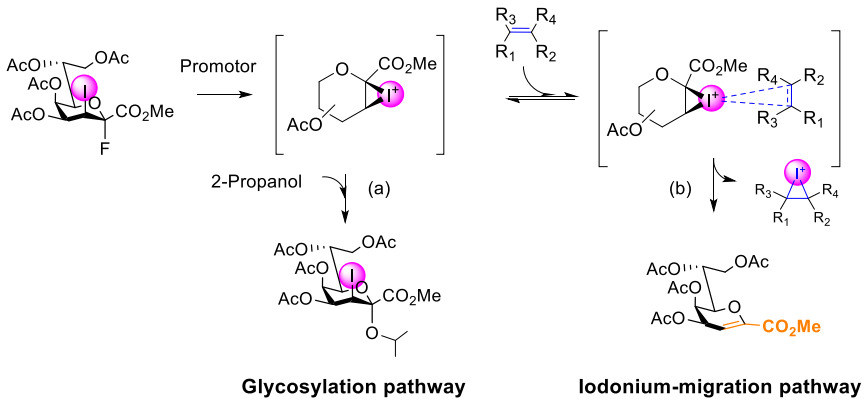


Figure 41: Proposed mechanism to explain the exclusive formation of the glycal ester in the presence of an allyl aglycone; adapted from manuscript #4.

In contrast, an appropriately protected methyl **α**-glycoside acceptor gave the desired α-linkage exclusively and in a high yield. Orthogonal deprotection allowed for the introduction of a dibenzyl phosphate capitalizing on the

phosphoramidite method. The 3- and 4-hydroxyl groups of GlcNAc comprise the common mannose-type recognition motif of MBL (see chapter 1.3.1), which may not be accessible in the presence of the natural 4-O-phosphate group of lipid A. Therefore, both the non-phosphorylated and the 4-O-phosphorylated ligands **IX** and **X** have been prepared to compare their binding affinities.

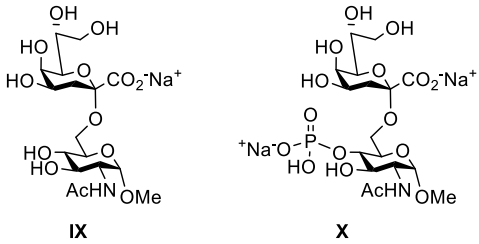


Figure 42: Ligands (IX) to (X) obtained after removal of blocking groups and ester saponification (#4).

The ligands from manuscripts #1, **#3** and **#4** have been evaluated by inhibition-ELISA studies. Preliminary results for the murine MBL-A are summarized in chapter 2.5.

First synthesis of an α-(2→5)-linked Kdo disaccharide – a structure found in *A. baumannii* LPS

Driven by the excellent glycosylation properties obtained for the 3-iodo-2-fluoride Kdo donor (**#2**) the even more challenging α-(2→5)-interconnection of two Kdo residues was addressed as target. Due to very low reactivity of the 5-hydroxyl group (see chapter 1.5.2), this linkage-type has previously not been obtained by synthetic methods. Paper **#5** presents the coupling of a highly reactive benzyl protected 3-iodo-2-fluoride donor to a benzyl protected Kdo 5-O-acceptor providing the first described synthetic access to the α-(2→5)-linked disaccharide found in *A. baumannii* strains (see chapter 1.2.1).

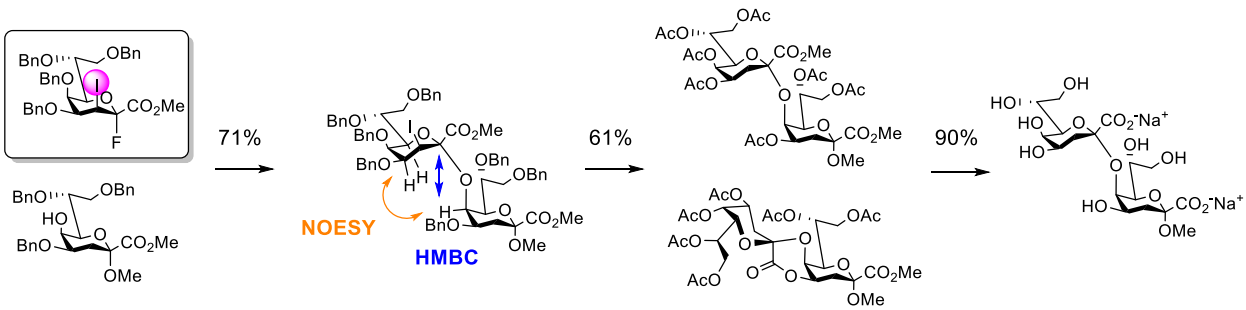


Figure 43: Synthesis of the α-(2→5)-linked Kdo disaccharide (#5).

In contrast to the disarmed acetyl protected donor (manuscript **#2**), several difficulties were encountered for this highly activated donor: (a) optimized conditions were necessary to suppress epimerization of the C-3-iodo substituent during donor preparation, which resulted in an inseparable mixture of epimers; (b) also during glycosylation epimerization occurred which could be avoided by using toluene as a solvent under strict temperature control; (c) the protected disaccharide was susceptible to glycosidic bond hydrolysis necessitating an appropriate work-up procedure and proper handling of the isolated disaccharide; (d) dehalogenation relied on Pd-catalyzed hydrogenolysis leading to some concomitant removal of benzyl groups, thus, ensuing deprotection was performed in one step; (e) chromatographic purification after acetylation provided an only partially separable mixture of disaccharide and inter-residue lactone. Solutions to all these issues and analytical detail for the new α-(2→5)-interconnected Kdo disaccharide are presented in manuscript **#5**.

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2.4. Original works

Manuscript #1

Pokorny, B.; Müller-Loennies, S.; Kosma, P.* *Carbohydr. Res*. **2014**, *391*, 66-81.

"Synthesis of α-D-glucosyl substituted methyl glycosides of 3-deoxy-α-D-*manno*- and D-*glycero*-α-D-*talo*-oct-2-ulosonic acid (Kdo/Ko) corresponding to inner core fragments of *Acinetobacter* lipopolysaccharide"

doi:10.1016/j.carres.2014.03.004

Carbohydrate Research 391 (2014) 66–81

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Synthesis of α-D-glucosyl substituted methyl glycosides of 3-deoxy-α-D-*manno*- and D-*glycero*-α-D-*talo*-oct-2-ulosonic acid (Kdo/Ko) corresponding to inner core fragments of *Acinetobacter* lipopolysaccharide

Barbara Pokorny [a], Sven Müller-Loennies [b], Paul Kosma [a,*]

[a] Department of Chemistry, University of Natural Resources and Life Sciences, Muthgasse 18, A-1190 Vienna, Austria
[b] Research Center Borstel, Parkallee 22, D-23845, Germany

ARTICLE INFO

Article history:
Received 20 January 2014
Received in revised form 26 February 2014
Accepted 5 March 2014
Available online 12 March 2014

Keywords:
Lipopolysaccharide
Kdo
Ko
Oligosaccharide synthesis
Acinetobacter

ABSTRACT

The α-D-glucopyranosyl-(1→5)-substituted methyl glycosides of 3-deoxy-α-D-*manno*-oct-2-ulosonic acid (Kdo), 3-deoxy-α-D-*lyxo*-hept-2-ulosonic acid (Kdh), and D-*glycero*-α-D-*talo*-oct-2-ulosonic acid (Ko) were prepared using orthogonally protected glycosyl acceptor derivatives via glycosylation with a torsionally disarmed 4,6-*O*-benzylidene protected trifluoroacetimidate glucosyl donor followed by global deprotection. The related 6-*O*-phosphoryl-α-D-glucopyranosyl-(1→5)-substituted Kdo and Kdh derivatives were derived from a benzylidene-protected glucosyl intermediate using phosphoramidite and phosphoryl chloride-based phosphorylation steps, respectively. The deprotected disaccharides serve as ligands to study lectin binding of *Acinetobacter* lipopolysaccharide core oligosaccharides.

1. Introduction

Bacteria of the genus *Acinetobacter* have increasingly been implicated in nosocomial infections which are difficult to eradicate due to resistance against major antimicrobial drugs.[1] Lipopolysaccharide (LPS)—located in the outer leaflet of the bacterial cell membrane—is a major virulence factor contributing to bacterial evasion of adaptive and innate immune responses.[2] A general but not exclusive architecture of LPS comprises a bisphosphorylated acylated diglucosamine backbone, termed lipid A, a core region and an antigenic polysaccharide which is the main target of specific antibodies allowing the distinction of O-serotypes and which is therefore called O-polysaccharide or O-antigen.[3] The first sugar connecting the core region and the lipid A is—in general—3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) but it may be partially replaced by the isosteric, acid-stable 3-hydroxy-derivative D-*glycero*-D-*talo*-oct-2-ulosonic acid (Ko).[4–7] In LPS from *Acinetobacter haemolyticus* NCTC 10305 in particular, a large fraction (~80%) contains Ko instead of Kdo (~20%), providing the linkage to the lipid A. In addition, the core oligosaccharide contains several α- and β-configured glucosyl as well as two 3-deoxy-D-*lyxo*-hept-2-ulosaric acid (Dha) residues (Fig. 1).

Previously, a 28 kDa murine serum protein has been described which binds to the inner core region of LPS from this *Acinetobacter* strain but also to oligosaccharides containing L-*glycero*-D-*manno*-heptosyl-Kdo units.[8–10] This "serum factor" has only recently been recognized as mannose binding lectin-A (MBL-A). Most notably this MBL binds to isolated LPS oligosaccharides with an unusual high affinity for the individual binding site (with ELISA IC_{50} values in the mid to low nanomolar range).[11] Extending our previous studies detailing the interaction of the core region with antibodies and lectins[12–15], we have set out to prepare a first series of fragments of the *Acinetobacter haemolyticus* NCTC 10305 inner core region in order to define the binding epitope at the molecular level. Since Smith degradation of the isolated LPS core resulted in ligands which were still bound by the lectin with similar affinity[5], non-natural disaccharides containing 3-deoxy-D-*lyxo*-hept-2-ulosonic acid (Kdh) have been prepared in addition. The synthetic oligosaccharides serve as ligands in forthcoming binding and STD NMR studies with C-type lectins such as human lung surfactant protein D and mannose-binding lectins.

2. Results and discussion

2.1. Preparation of the disaccharides α-Glc-(1→5)-α-Kdo/Kdh

The glycosides were designed as methyl (Me) glycosides since the single ^{1}H NMR signal of the Me group serves as a suitable

* Corresponding author. Tel.: +43 1 47654 6055; fax: +43 1 47654 6059.
E-mail address: paul.kosma@boku.ac.at (P. Kosma).

http://dx.doi.org/10.1016/j.carres.2014.03.004
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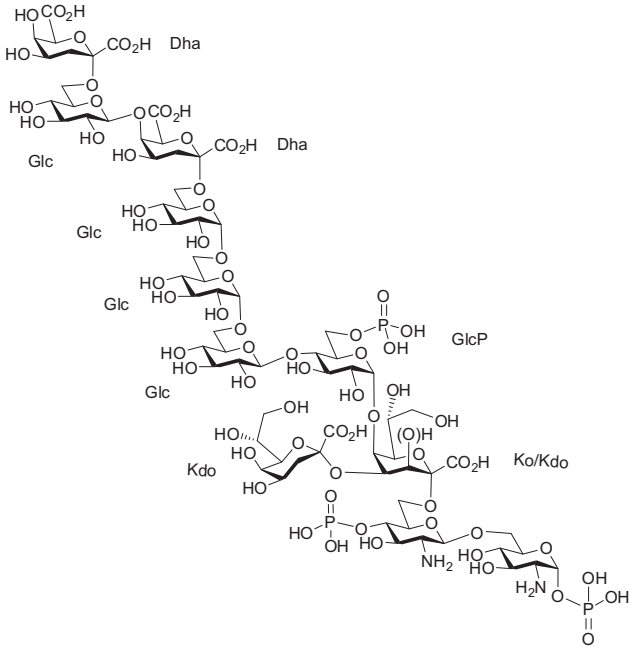
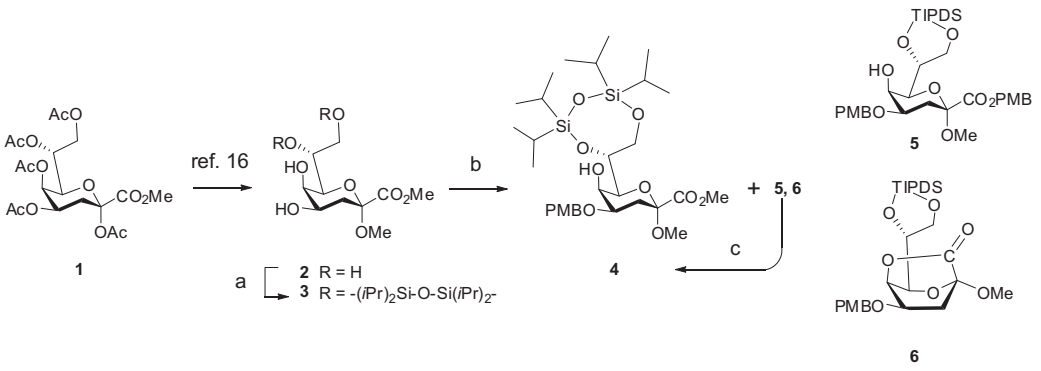


Figure 1. Structure of the deacylated inner core LPS fraction from *Acinetobacter haemolyticus* NCTC 10305.[6]

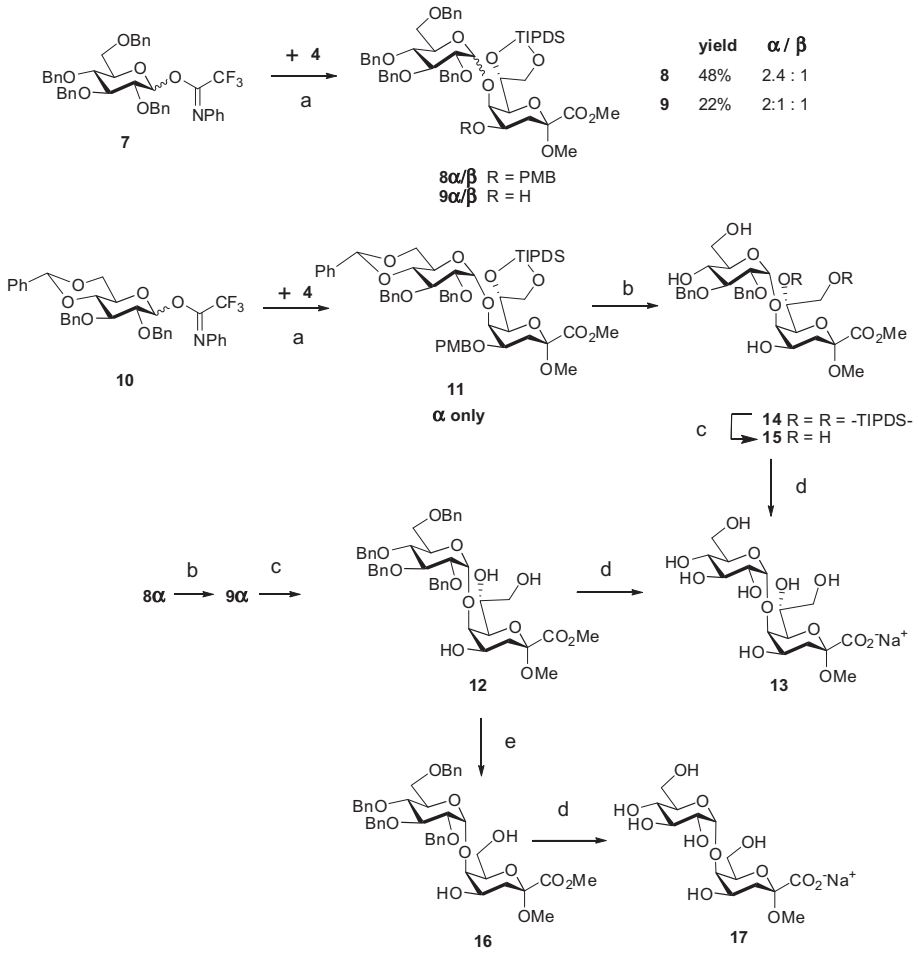
reference for integration of STD effects in binding studies. Thus the previously reported Me α-Kdo glycoside **2**[16] (easily available via the scalable reaction of the peracetate **1** with MeOH, catalyzed by Dowex (H^+) ion-exchange resin) was employed for the preparation of the Kdo glycosyl acceptor derivative. The *exo*cyclic 7,8-*O* positions of **2** were protected by a 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDS) group in order to utilize its arming effect and to provide orthogonality of fluoride-induced silyl deprotection.[17] Thus, by using imidazole and $TIPDSCl_2$ in DMF at −40 °C the bis-silyl ether derivative **3** was obtained in 68% yield (Scheme 1). In order to generate an option for eventual attachment of the lateral Kdo unit (Fig. 1) and to also activate the 5-OH group for subsequent glycosylation, a *p*-methoxybenzyl group (PMB) was installed at position 4. The diol derivative **3** was first reacted with dibutyltin oxide to give the intermediate stannylene derivative followed by treatment with PMB-chloride/tetrabutyl ammonium iodide/DMF in toluene. As previously reported, the activated stannylene acetal also induced formation of the corresponding PMB ester **5** and the 1.5-lactone **6** as by-products.[18] Treatment of the mixture with sodium methoxide, however, eventually afforded the methyl ester derivative **4** in 70% yield.

Previously, an α-(1→5)-linked glucosyl residue had been coupled to Kdo using an acetylated 2-*O*-benzyl thioglycoside donor under promotion with DMTST in 85% yield.[19] The use of the known[20] perbenzylated *N*-phenyl trifluoroacetimidate donor (NPTFA) **7α/β** for the TMSOTf-catalyzed glycosylation of **4**, however, resulted in decreased anomeric selectivity and a temperature dependent outcome.[21,22] Glycosylation of acceptor **4** promoted by 10% TMSOTf at −5 °C in CH_2Cl_2 afforded the α-(1→5)-linked disaccharide **8α** as the major anomer (α/β ratio 2.4:1) accompanied by formation of the alcohols **9α** and **9β** (resulting from cleavage of the acid-labile PMB group). By lowering the temperature to −20 °C and reducing the amount of promoter (5% TMSOTf) the PMB cleavage could be largely suppressed. However, under these conditions, the undesired **8β** was formed preferentially (α/β ratio 1:1.4). The change of the solvent to CH_2Cl_2/Et_2O (9:1) resulted in a sluggish reaction with slightly enhanced α/β ratio (1.7:1), albeit in poor yield. Presumably, the formation of an intermediate α-anomeric triflate at a lower temperature leads to an increased contribution of an S_N2-type glycosylation pathway.[23] Alternatively, the torsionally disarmed 4,6-*O*-benzylidene trifluoroacetimidate donor **10** was prepared from its hemiacetal precursor[24] as a separable anomeric mixture.[25] The glycosylation of acceptor **4** with donor **10** in CH_2Cl_2 at −5 °C provided the disaccharide **11** in 80% yield as the α-anomer only, irrespective of the anomeric configuration of the donor (Scheme 2). Due to milder reaction conditions (lower temperature and promoter concentration) PMB cleavage could be completely suppressed without affecting the stereochemical outcome. The PMB group of the α-linked disaccharide derivative **8α** was selectively removed by treatment with trifluoroacetic acid (TFA) affording **9α**, followed by cleavage of the silyl ether group in order to generate the triol derivative **12**. Treatment of **9α** with tetrabutylammonium fluoride (TBAF) produced compound **12** in near quantitative yield, which was then fully deprotected by catalytic hydrogenation followed by alkaline hydrolysis of the methyl ester group to furnish disaccharide **13** as sodium salt in 90% yield. Global deprotection of the benzylidene protected disaccharide **11** provided disaccharide **13** in an excellent overall yield of 65% (based on acceptor **4**). TFA-treatment of **11** simultaneously cleaved the benzylidene and the PMB group, respectively, to give triol **14** in 88% yield, followed by TIPDS removal and ensuing full deprotection of the resulting derivative **15** to give disaccharide **13**.

In addition, the side-chain shortened analog was prepared by oxidative cleavage of the *exo*cyclic diol of **12** with sodium *meta*periodate generating the heptulosonic glycoside **16**. The intermediate aldehyde formed upon oxidation could be analyzed by NMR, but proved to be rather labile upon attempted purification on silica gel. The ensuing borohydride reduction was accompanied by concomitant ester reduction. The reaction was therefore not allowed to run until completion, but was stopped when ester reduction



Scheme 1. Reagents and conditions: (a) $TIPDSCl_2$, 1*H*-imidazole, DMF, −40 °C, 68%; (b) Bu_2SnO, toluene, reflux, then DMF, PMBCl, Bu_4NI, toluene, 60 °C; mixture of **4**, **5** and **6**; then (c) 0.1 M NaOMe, MeOH, rt, 70% for **4**.



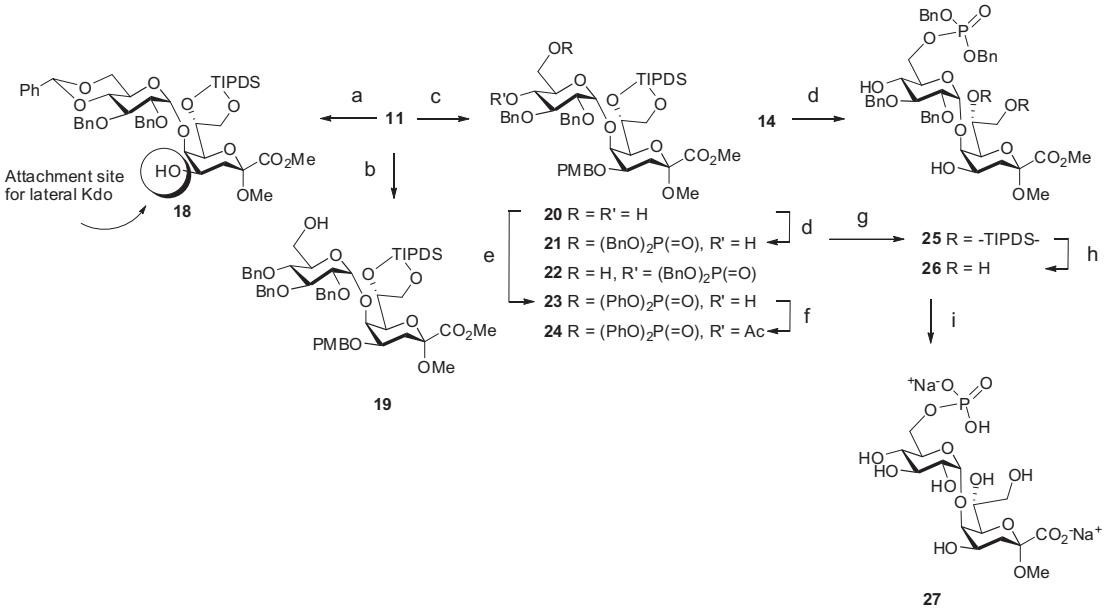
Scheme 2. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, molecular sieves 4 Å, −5 °C, 34% for **8α**, 14% for **8β**, 15% for **9α**, 7% for **9β**, 80% for **11**; (b) 99% TFA, CH_2Cl_2, 0 °C, 83% for **9α**, 88% for **14**; (c) TBAF, THF, rt, 98% for **12**, 97% for **15**; (d) H_2 (1 atm), 10% Pd–C, MeOH, then 0.01 M aq NaOH, rt, 90% for **12**→**13**, 95% for **15**→**13**, 79% for **16**→**17**; (e) $NaIO_4$–SiO_2, CH_2Cl_2, −10 °C, then $NaBH_4$, MeOH, 0 °C, 34%.

was first detected on TLC. Global deprotection of **16** eventually afforded disaccharide **17** in 79% yield.

2.2. Preparation of the disaccharides α-Glc6*P*-(1→5)-α-Kdo/Kdh

Selective cleavage of the PMB-group of the benzylidene-protected disaccharide derivative **11** via DDQ-promoted oxidation afforded the glycosyl acceptor **18** (suitable for attachment of the lateral Kdo unit) in 83% yield (Scheme 3). The orthogonal protecting group pattern also allows for the selective introduction of the 6-phosphoester group as well as for additional glucosyl extension at position 4 of the glucosyl unit upon reductive opening of the benzylidene group (cf. Fig. 1). Reductive opening of the benzylidene toward the 6-OH derivative **19**, however, met with difficulties when applying various combinations of Lewis acids and hydride sources ($CoCl_2/BH_3$.THF, TMSCl/$NaCNBH_3$, TMSOTf/BH_3, Bu_2BOTf/BH_3·THF). Lack of reactivity, loss of the PMB group or cleavage of the benzylidene group with formation of additional by-products were observed under these conditions. A modest conversion of compound **11** into the 4-*O*-benzyl ether **19** could eventually be accomplished in the presence of $PhBCl_2/Et_3SiH$ in 43% yield. Thus, it was envisaged to introduce the 6-*O*-phosphate group at the 4,6-diol intermediate, with the additional option to utilize the remaining 4-hydroxyl group as an acceptor site for future extension by glucosyl residues. Hence, the benzylidene group of **11** was selectively removed—without cleavage of the PMB group—using *p*-toluenesulfonic acid in dry MeOH to afford the diol **20** in 91% yield. Short reaction times at 40 °C gave better and reproducible yields compared to prolonged reaction times at ambient temperature. Phosphorylation of the diol **20** was first approached using the phosphoramidite chemistry. Thus, treatment of **20** with dibenzyl *N*,*N*-diisopropylphosphoramidite/1*H*-tetrazole in the presence of ground molecular sieves (4 Å) in CH_2Cl_2 followed by oxidation with *m*CPBA gave the 6-*O*-phosphotriester derivative **21** in 67% yield and the corresponding 4-*O*-regioisomer **22** (10%). The phosphoramidite protocol also proved to be appropriate for the phosphorylation of the triol **14** and furnished the 6-*O*-phosphotriester derivative **25** in 63% yield. The assignment of the phosphate substitution at *O*-6 was based on the downfield shift of the ^1H NMR signals of the H-6 protons and the ^{13}C–^{31}P coupling interaction leading to splitting of ^{13}C NMR signals of C-6 and C-5, respectively. To increase the selectivity in the phosphorylation step, a more reactive phosphoryl halide was used at a low temperature. Indeed, reaction of the diol **20** with diphenyl phosphoryl chloride at 0 °C in the presence of 4-*N*,*N*-dimethylaminopyridine afforded the corresponding 6-*O*-phosphotriester derivative **23** in 96% yield.

TFA-treatment of the dibenzylphosphate derivative **21** proved to be selective for the removal of the PMB group and gave the diol derivative **25** in 88% yield. Subsequent reaction of **25** with TBAF afforded the tetraol derivative **26** (92%), which was subjected to hydrogenation on 10% Pd–C followed by alkaline hydrolysis to

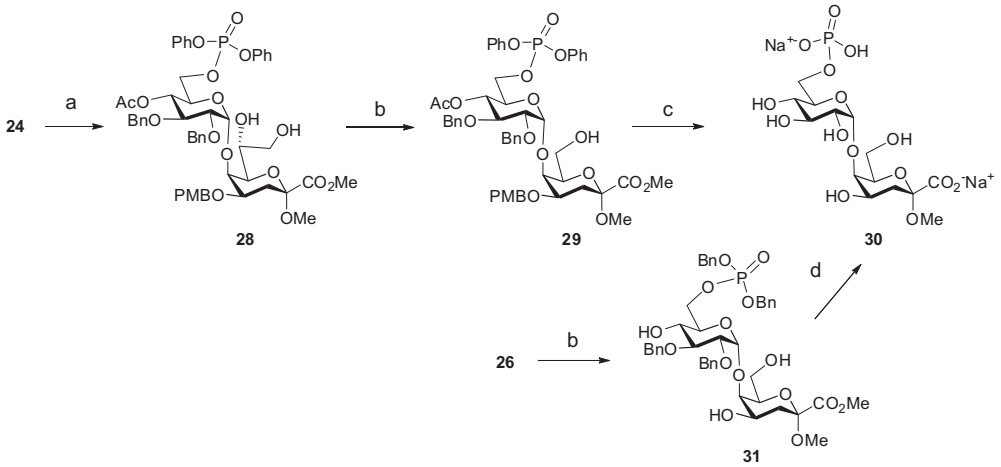


Scheme 3. Reagents and conditions: (a) DDQ, CH_2Cl_2/MeOH (3:1), rt, 83%; (b) $PhBCl_2$, Et_3SiH, CH_2Cl_2, −70 °C, 43%; (c) *p*TosOH, MeOH, 40 °C, 91%; (d) $(BnO)_2PN(iPr)_2$, 1*H*-tetrazole, CH_2Cl_2, −5 °C/0 °C, molecular sieves 4 Å, then *m*CPBA, 67% for **21**, 10% for **22**, 63% for **25**; (e) $(PhO)_2P(=O)Cl$, DMAP, CH_2Cl_2, molecular sieves 4 Å, 0 °C, 96%; (f) Ac_2O, pyr., DMAP, 0 °C, 96%; (g) TFA, CH_2Cl_2, 0 °C, 88%; (h) 1 M TBAF, THF, rt, 92%; (i) H_2 (1 atm), 10% Pd–C, MeOH, then 0.01 M aq NaOH, rt, 98%.

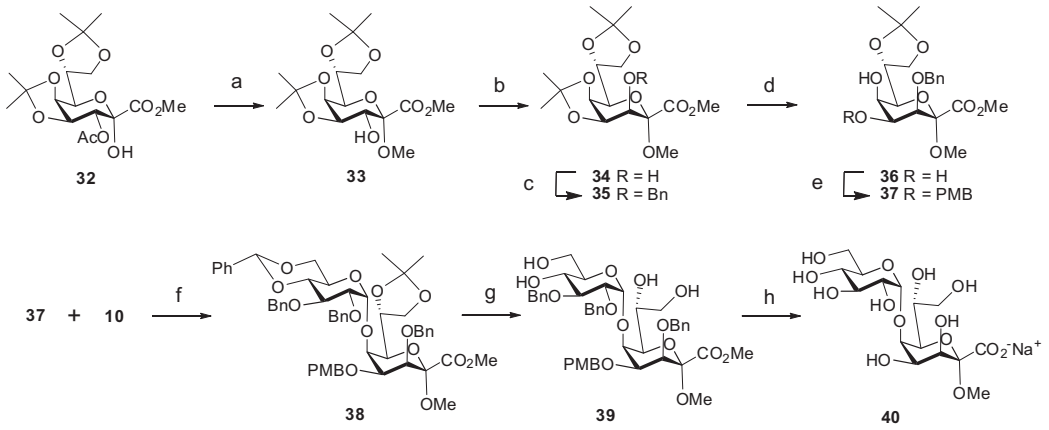
furnish the target disaccharide phosphate **27** as sodium salt in 98% yield. Deprotection of the silyl group of diphenylphosphate **23**, however, required a modified protocol, since treatment with TBAF resulted in fluoride-induced nucleophilic displacement of the phenoxy groups on the phosphoester.[26] The 4-OH group of **23** was thus acetylated to give compound **24** in order to prevent intramolecular phosphate migration or cyclization, respectively. The silyl ether groups of **24** were then removed by the action of triethylamine trihydrogen fluoride (TREAT), which afforded **28** in 82% yield with only minor (~10%) phosphate cleavage (Scheme 4). Compound **28** was used for the preparation of the side-chain-shortened analog **29** by periodate oxidation (36%) followed by successive hydrogenolysis on Pd-C and PtO_2, and saponification to afford the heptulosonic glycoside **30** in 52% yield. The poor yield was due to formation of an unidentified side product, which had to be separated on a HILIC column. Alternatively, dibenzylphosphate **26** was subjected to the oxidative degradation protocol affording **31** (47%), which reacted cleanly to target compound **30** upon hydrogenolysis with Pd–C and subsequent saponification.

2.3. Preparation of the disaccharide α-Glc-(1→5)-α-Ko

The previously reported intermediate **32** was employed for the preparation of a suitably protected methyl glycoside of D-*glycero*-α-D-*talo*-oct-2-ulopyranosylonic acid (Ko).[27] The epimeric 3-*O*-acetate **32** was subjected to base-induced anomeric methylation followed by de-*O*-acetylation to produce **33**. Ensuing Dess–Martin periodinane oxidation and reduction with ammonia–borane complex gave the alcohol **34** in 65% overall yield (Scheme 5). Reaction of **34** with benzyl bromide/NaH in DMF afforded the 3-*O*-benzyl derivative **35** in 94% yield. The reaction had to be performed in high dilution at 0 °C in order to prevent formation of the



Scheme 4. Reagents and conditions: (a) TREAT, CH_2Cl_2, rt, 82%; (b) $NaIO_4$–SiO_2, CH_2Cl_2, 0 °C → rt, then $NaBH_4$, MeOH, 0 °C, 36% for **29**, 47% for **31**; (c) H_2 (1 atm), 10% Pd–C, MeOH, then H_2 (1 atm), PtO_2, MeOH, then 0.01 M aq NaOH, rt, 52%; (d) H_2 (1 atm), 10% Pd–C, MeOH, then 0.01 M aq NaOH, rt, 87%.



Scheme 5. Reagents and conditions: (a) NaH, MeI, DMF, 0°C, then NaOMe, rt, 86%; (b) Dess–Martin reagent, CH_2Cl_2, rt, then $NH_3{\cdot}BH_3$, MeOH, 0 °C, 75%; (c) NaH, BnBr, DMF, 0 °C, 94%; (d) *p*TosOH, wet acetone, rt, 71%; (e) Bu_2SnO, toluene, reflux, then DMF, PMBCl, Bu_4NI, toluene, 60 °C, 81%; (f) TMSOTf, CH_2Cl_2, molecular sieves 4 Å, −30 °C, 42%; (g) *p*TosOH, MeOH, 0 °C → rt, 70%; (h) H_2 (1 atm), 10% Pd–C, MeOH, then 0.01 M aq NaOH, rt, 92%.

corresponding benzyl ester derivative. Next, the 4,5-*O*-isopropylidene ketal was selectively cleaved by the action of *p*-toluenesulfonic acid in wet acetone under equilibrating conditions.

The use of a defined amount of water was critical in order to prevent additional loss of the 7,8-*O*-acetonide. This way diol **36** was obtained in 71% yield ready for further processing into suitable glycosyl acceptor derivatives. Similar to the corresponding Kdo acceptor **4**, the 4-*O*-PMB group was installed via the respective stannylidene acetal intermediate and ensuing treatment with PMBCl/Bu_4NI/DMF in toluene affording the orthogonally protected acceptor **37** in 81% yield. Coupling of **37** with the benzylidene-protected NPTFA donor **10** promoted by TMSOTf in CH_2Cl_2 at −5 °C afforded the α-linked disaccharide **38** in 42% yield. The reduced yield of the coupling step was due to the concomitant cleavage of the acetonide during the glycosylation reaction. Deprotection of **38** was achieved by treatment with *p*-toluenesulfonic acid hydrate in methanol for 24 h which furnished the tetraol **39** in 70% yield. Hydrogenation of **39** with 10% Pd–C in methanol and final purification on Bio-Gel PD10 afforded the glucosyl-Ko disaccharide **40** as sodium salt in 92% yield. ^{13}C NMR data of the deprotected target disaccharides **13**, **17**, **27**, **30**, and **40** were fully assigned and confirmed the respective structures (Table 1).

2.4. Conclusions and outlook

A benzylidene-protected glucosyl NPTFA donor proved as an efficient and α-selective glycosyl donor for the glycosylation of OH-5 of orthogonally protected Kdo and Ko glycosides and allowed for the regioselective introduction of the 6-*O*-phosphoryl group. Oxidative cleavage of the *exo*cyclic side chain of Kdo provided the Kdh containing fragments. Global deprotection gave the disaccharide ligands related to the inner core region of *Acinetobacter* LPS which are suited to perform various binding assays to elucidate structural details of the interaction with MBL which are serum components and important in innate first-line immune reactions such as complement activation. The synthesis of larger fragments is currently in progress.

3. Experimental

3.1. General

All purchased chemicals were used without further purification unless stated otherwise. Solvents were dried over activated

Table 1
^{13}C NMR chemical shifts (δ) of disaccharide derivatives **13**, **17**, **27**, **30**, and **40**

Atom position	Compound				
	13	**17**	**27**	**30**	**40**
OCH_3	51.42	51.31	51.42	51.31	51.64
Kdo/Kdh/Ko					
1	176.03	175.93	176.06	175.95	174.16
2	101.29	100.98	101.30	100.99	103.26
3	35.47	35.36	35.22	35.16	72.76
4	66.60	66.45	66.42	66.31	66.95
5	75.95	76.73	75.48	76.46	77.19
6	72.35	73.90	72.35	73.95	71.75
7	69.24	61.67	69.01	61.63	68.96
8	63.94	—	64.07	—	63.87
Glc					
1	100.71	100.67	100.61	100.60	100.98
2	72.80	72.74	72.99	72.79	72.49
3	73.70	73.60	73.23	73.30	73.88
4	70.05	70.03	69.50	69.61	69.86
5	72.51	72.45	72.25	71.84	72.82
			$J_{C,P}$ 7.5 Hz	$J_{C,P}$ 7.7 Hz	
6	60.77	60.73	62.93	63.73	60.77
			$J_{C,P}$ 4.1 Hz	$J_{C,P}$ 4.5 Hz	

3 Å (acetone) or 4 Å (CH_2Cl_2, DMF, pyridine, toluene) molecular sieves. THF was distilled on 4 Å molecular sieves shortly before use. Dry MeOH (secco solv) was purchased from Merck. Cation exchange resin DOWEX 50 H^+ was regenerated by consecutive washing with HCl (3 M), water, and dry MeOH. Aqueous solutions of salts were saturated unless stated otherwise. Concentration of organic solutions was performed under reduced pressure <40 °C. Optical rotations were measured with a Perkin–Elmer 243 B polarimeter. $[\alpha]_D^{20}$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Thin layer chromatography was performed on Merck precoated plates: generally on 5 × 10 cm, layer thickness 0.25 mm, silica gel $60F_{254}$; alternatively on HPTLC plates with 2.5 cm concentration zone (Merck). Spots were detected by dipping reagent (anisaldehyde–H_2SO_4). For column chromatography silica gel (0.040–0.063 mm) was used. HP-column chromatography was performed on pre-packed columns (YMC-Pack SIL-06, 0.005 mm, 250 × 10 mm and 250 × 20 mm). Size exclusion chromatography was performed on Bio-Gel® P-2 Gel extra fine <45 μm (wet) (Bio-Rad, 45–90 μm) or on pre-packed PD-10 columns (GE Healthcare, Sephadex™ G-25 M). NMR spectra were recorded with a Bruker Avance III 600 instrument (600.22 MHz for ^1H, 150.93 MHz for ^{13}C and 242.97 MHz for ^{31}P) using standard Bruker NMR software. ^1H spectra were referenced to 7.26 ($CDCl_3$), 5.32 (CD_2Cl_2), 3.31 (MeOD), and 0.00 (D_2O, external calibration to 2,2-dimethyl-2-silapentane-5-sulfonic acid) ppm unless stated otherwise. ^{13}C spectra were referenced to 77.00 ($CDCl_3$), 53.84 (CD_2Cl_2), 49.00 (MeOD), and 67.40 (D_2O, external calibration to 1,4-dioxane) ppm. ^{31}P spectra in D_2O were referenced to external *ortho*-phosphoric acid (0.00 ppm). ESI-MS data were obtained on a Waters Micromass Q-TOF Ultima Global instrument.

3.2. Methyl [methyl 3-deoxy-7,8-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-*manno*-oct-2-ulopyranosid]onate (3)

A cooled mixture of 1*H*-imidazole (0.22 g, 3.228 mmol) and TIPDSCl$_2$ (0.46 mL, 1.412 mmol) in dry DMF (7.4 mL) was added dropwise to a solution of **2** (0.36 g, 1.345 mmol) in dry DMF (8.6 mL) at −40 °C. After 2 h at −40 °C another portion of 1*H*-imidazole (22 mg, 0.323 mmol) and TIPDSCl$_2$ (46 μL, 0.141 mmol) in dry DMF (0.74 mL) was slowly added and after 30 min excessive reagent was scavenged by addition of dry MeOH (2 mL). The mixture was allowed to warm up to ambient temperature, solid $NaHCO_3$ (0.4 g) was added, and the suspension was concentrated. The residue was partitioned between EtOAc and aq $NaHCO_3$, the aqueous phase was extracted with EtOAc (2 × 10 mL) and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residual oil was purified by column chromatography (toluene/EtOAc 4:1 → 1:1) affording **3** (0.46 g, 68%) as a colorless amorphous solid: $[\alpha]_D^{20}$ +36.4 (*c* 0.85, $CHCl_3$); R_f 0.38 (toluene/EtOAc 1:1); ^1H NMR ($CDCl_3$): δ 4.30–4.26 (m, 1H, H-7), 4.18 (dd, 1H, $J_{8a,8b}$ 12.1, $J_{8a,7}$ 1.7 Hz, H-8a), 4.06–4.04 (m, 1H, H-5), 4.03–3.98 (m, 1H, H-4), 3.84 (dd, 1H, $J_{8b,7}$ 7.0 Hz, H-8b), 3.78 (s, 3H, CO_2CH_3), 3.50 (dd, $J_{6,7}$ 8.0, $J_{6,5}$ 1.0 Hz, H-6), 3.22 (s, 3 H, OCH_3), 2.15 (dd, 1H, $J_{3eq,3ax}$ 13.0, $J_{3eq,4}$ 5.1 Hz, H-3*eq*), 1.86 (dd, 1H, $J_{3ax,4}$ 11.5 Hz, H-3*ax*), 1.12–0.93 (m, 28 H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 168.53 (s, C-1), 99.36 (s, C-2), 74.08 (d, C-7), 71.35 (d, C-6), 66.81 (t, C-8), 66.67 (d, C-5), 66.17 (d, C-4), 52.52 (q, CO_2CH_3), 51.17 (q, OCH_3), 35.10 (t, C-3), 17.40, 17.35, 17.33, 17.23, 17.21 [q, 8C, Si-CH-$(CH_3)_2$], 13.31, 12.77, 12.48 [d, 4C, Si-CH-$(CH_3)_2$]; ESI-TOF HRMS: *m*/*z* = 531.2409; calcd for $C_{22}H_{44}O_9Si_2Na^+$: 531.2416.

3.3. Methyl [methyl 3-deoxy-4-*O*-(4-methoxybenzyl)-7,8-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-*manno*-oct-2-ulopyranosid]onate (4)

A mixture of **3** (91 mg, 0.179 mmol) and dibutyltin oxide (49 mg, 0.197 mmol) in dry toluene (4.0 mL) was heated to reflux on a Dean–Stark apparatus for 2 h. To the cooled solution dry DMF (166 μL, 2.146 mmol), 4-methoxybenzyl chloride (73 μL, 0.537 mmol) and tetrabutylammonium iodide (73 mg, 0.197 mmol) were added successively. After 16 h at 60 °C the solution was allowed to cool to ambient temperature. The solution was diluted with EtOAc and consecutively washed with HCl (1 M), aq $NaHCO_3$, aq $Na_2S_2O_3$ (50 g/L) and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated. The residual oil was taken up in dry MeOH (4.0 mL) and treated with 0.1 M NaOMe in dry MeOH (0.179 mmol, 1.8 mL) at 0 °C. After 1 h at ambient temperature the solution was made neutral by adding DOWEX 50 H^+ resin, the suspension was filtered, and the filtrate was concentrated. Column chromatography of the residue (toluene/EtOAc 19:1 → 9:1) provided **4** (79 mg, 70%) as a colorless oil: $[\alpha]_D^{20}$ +24.5 (*c* 1.07, $CHCl_3$); R_f 0.66 (toluene/EtOAc 3:1); ^1H NMR ($CDCl_3$): δ 7.27–7.24 (m, 2H, Ar), 6.89–6.86 (m, 2H, Ar), 4.56 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.50 (d, 1H, *J* 11.4 Hz, CH*H*Ph), 4.36–4.32 (m, 1H, H-7), 4.25 (dd, 1H, $J_{8a,8b}$ 11.9, $J_{8a,7}$ 1.6 Hz, H-8a), 4.14–4.12 (m, 1H, H-5), 3.84 (ddd, 1H, $J_{4,3ax}$ 11.5, $J_{4,3eq}$ 5.2, $J_{4,5}$ 2.9, H-4), 3.80–3.75 (m, 7H, H-8b, PhOCH_3, CO_2CH_3), 3.38 (dd, 1H, $J_{6,7}$ 8.6, $J_{6,5}$ 1.0 Hz, H-6), 3.20 (s, 3H, OCH_3), 2.21 (bs, 1H, O*H*), 2.18 (dd, 1H, $J_{3eq,3ax}$ 12.8, H-3*eq*), 2.01 (dd, 1H, H-3*ax*), 1.18–0.91 (m, 28H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 168.60 (s, C=O), 159.38, 130.03 (s, 2C, Ar), 129.28, 113.96 (d, 4C, Ar), 99.36 (s, C-2), 72.97 (d, 2C, C-4, C-7), 71.88 (d, C-6), 69.99 (t, CH_2Ph), 67.16 (t, C-8), 63.77 (d, C-5), 55.26 (q, PhOCH_3), 52.50 (q, CO_2CH_3), 50.99 (q, OCH_3), 32.09 (t, C-3), 17.47, 17.41, 17.37, 17.27 [q, 8C, Si-CH-$(CH_3)_2$], 13.29, 12.82, 12.54, 12.49 [d, 4C, Si-CH-$(CH_3)_2$]; ESI-TOF HRMS: *m*/*z* = 651.2995; calcd for $C_{30}H_{52}O_{10}Si_2Na^+$: 651.2991.

Data for **5**: colorless oil; $[\alpha]_D^{20}$ +18.2 (*c* 0.72, $CHCl_3$), R_f 0.76 (toluene/EtOAc 3:1); ^1H NMR ($CDCl_3$): δ 7.32–7.29 (m, 2H, Ar), 7.26–7.22 (m, 2H, Ar), 6.89–6.85 (m, 4H, Ar), 5.15 (s, 2H, CH_2Ph), 4.53 (d, 1H, *J* 11.6 Hz, C*H*HPh), 4.49 (d, 1H, *J* 11.4 Hz, CH*H*Ph), 4.33 (ddd, 1H, $J_{7,6}$ 8.6, $J_{7,8b}$ 7.2, $J_{7,8a}$ 1.5 Hz, H-7), 4.24 (dd, 1H, $J_{8a,8b}$ 11.8 Hz, H-8a), 4.13–4.11 (m, 1H, H-5), 3.84–3.79 (m, 7H, H-4, 2 × PhOCH_3), 3.76 (dd, 1H, H-8b), 3.37 (d, 1H, H-6), 3.13 (s, 3H, OCH_3), 2.21 (d, 1H, $J_{OH,5}$ 2.6 Hz, O*H*), 2.15 (dd, 1H, $J_{3eq,3ax}$ 12.9, $J_{3eq,4}$ 4.9 Hz, H-3*eq*), 1.97 (dd, 1H, $J_{3ax,4}$ 11.7 Hz, H-3*ax*), 1.10–0.90 (m, 28H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 167.77 (s, C=O), 159.74, 159.37 (s, 2C, Ar), 130.26 (d, 2C, Ar), 130.03 (s, Ar), 129.29 (d, 2C, Ar), 127.57 (s, Ar), 113.94 (d, 4C, Ar), 99.18 (s, C-2), 73.06 (d, 2C, C-4, C-7), 71.84 (d, C-6), 69.98 (t, CH_2Ph), 67.10 (t, C-8), 66.98 (t, CH_2Ph), 63.75 (d, C-5), 55.26 (q, 2C, 2 × PhOCH_3), 50.94 (q, OCH_3), 31.99 (t, C-3), 17.47, 17.40, 17.38, 17.27 [q, 8C, Si-CH-$(CH_3)_2$], 13.29, 12.81, 12.54, 12.51 [d, 4C, Si-CH-$(CH_3)_2$]; ESI-TOF HRMS: *m*/*z* = 757.3414; calcd for $C_{37}H_{58}O_{11}Si_2Na^+$: 757.3410.

Data for **6**: colorless amorphous solid; $[\alpha]_D^{20}$ −13.8 (*c* 0.82, $CHCl_3$); R_f 0.59 (toluene/EtOAc 9:1); ^1H NMR ($CDCl_3$): δ 7.26–7.23 (m, 2H, Ar), 6.89–6.86 (m, 2H, Ar), 5.00–4.99 (m, 1H, H-5), 4.54 (d, 1H, *J* 11.9 Hz, C*H*HPh), 4.49 (d, 1H, *J* 11.8 Hz, CH*H*Ph), 4.16 (dd, 1H, $J_{8a,8b}$ 12.1, $J_{8a,7}$ 1.7 Hz, H-8a), 3.93 (ddd, 1H, $J_{7,6}$ 9.5, $J_{7,8b}$ 7.6 Hz, H-7), 3.89 (app. td, 1H, $J_{4,3ax}$ 8.8, $J_{4,3eq} = J_{4,5}$ 2.2 Hz, H-4), 3.80 (s, 3H, PhOCH_3), 3.69 (dd, 1H, H-8b), 3.58 (s, 3H, OCH_3), 3.57 (d, 1H, H-6), 2.50 (dd, 1H, $J_{3ax,3eq}$ 14.7 Hz, H-3*ax*), 2.00–1.97 (m, 1H, H-3*eq*), 1.12–0.91 (m, 28H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 168.24 (s, C=O), 159.54 (s, Ar), 129.36 (d, 2C, Ar), 128.92 (s, Ar), 114.03 (d, 2C, Ar), 94.91 (s, C-2), 73.37 (d, C-6), 72.71, 72.68 (d, 2C, C-5, C-7), 71.93 (d, C-4), 70.05 (t, CH_2Ph), 66.64 (t, C-8), 55.25 (q, PhOCH_3), 52.71 (q, OCH_3), 38.72 (t, C-3), 17.40, 17.35, 17.32,

17.27, 17.20, 17.18 [q, 8C, Si-CH-(CH_3)$_2$], 13.20, 12.64, 12.45, 12.39 [d, 4C, Si-CH-(CH_3)$_2$].

3.4. 2,3,4,6-Tetra-*O*-benzyl-α-D-*gluco*pyranosyl-(1→5)-methyl [methyl 3-deoxy-4-*O*-(4-methoxybenzyl)-7,8-*O*-(1,1,3,3-tetra isopropyldisiloxane-1,3-diyl)-α-D-*manno*-oct-2-ulopyranosid] onate (8α), 2,3,4,6-tetra-*O*-benzyl-β-D-*gluco*pyranosyl-(1→5)-methyl [methyl 3-deoxy-4-*O*-(4-methoxybenzyl)-7,8-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-*manno*-oct-2-ulopyranosid]onate (8β), 2,3,4,6-tetra-*O*-benzyl-α-D-*gluco*pyranosyl-(1→5)-methyl [methyl 3-deoxy-7,8-*O*-(1,1,3,3-tetraisopropyl disiloxane-1,3-diyl)-α-D-*manno*-oct-2-ulopyranosid]onate (9α), and 2,3,4,6-tetra-*O*-benzyl-β-D-*gluco*pyranosyl-(1→5)-methyl [methyl 3-deoxy-7,8-*O*-(1,1,3,3-tetraisopropyl disiloxane-1,3-diyl)-α-D-*manno*-oct-2-ulopyranosid] onate (9β)

A solution of glycosyl acceptor **4** (50 mg, 0.080 mmol) in dry CH_2Cl_2 (0.6 mL) containing 4 Å molecular sieves (75 mg) was stirred for 20 min followed by addition of glycosyl donor **7**[22] (85 mg, 0.120 mmol) in dry CH_2Cl_2 (0.6 mL) under Ar. TMSOTf (1.4 μL, 0.008 mmol) in dry CH_2Cl_2 (50 μL) was added dropwise to the cold (−5 °C) mixture and after 5 min the reaction was quenched by adding a solution of Et_3N (23 μL, 0.160 mmol) in dry CH_2Cl_2 (0.3 mL). Filtration of the suspension over Celite®, concentration of the filtrate, and purification of the residue by HP-column chromatography (*n*-hexane/EtOAc 9:1 → 2:1) provided target compound **8α** (31 mg, 34%) together with **8β** (13 mg, 14%) and disaccharide alcohols **9α** (14 mg, 15%) and **9β** (6 mg, 7%) as colorless syrups.

Data for **8α**: $[\alpha]_D^{20}$ +68.4 (*c* 0.53, $CHCl_3$); R_f 0.62 (*n*-hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$): δ 7.36–7.20 (m, 20H, Ar), 7.10–7.07 (m, 2H, Ar), 6.85–6.81 (m, 2H, Ar), 5.28 (d, 1H, $J_{1',2'}$ 3.7 Hz, H-1′), 4.94 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.86 (d, 1H, *J* 11.7 Hz, C*H*HPh), 4.83 (d, 1H, *J* 11.1 Hz, CH*H*Ph), 4.75 (d, 1H, *J* 10.7 Hz, C*H*HPh), 4.68 (d, 1H, *J* 12.0 Hz, CH*H*Ph), 4.54 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.51 (d, 1H, *J* 12.3 Hz, C*H*HPh), 4.50–4.45 (m, 2H, H-7, CH*H*Ph), 4.41 (d, 1H, *J* 11.0 Hz, CH*H*Ph), 4.27 (d, 1H, $J_{5,4}$ 2.2 Hz, H-5), 4.24–4.19 (m, 2H, H-5′, CH*H*Ph), 4.06 (app. t, 1H, $J_{3',2'} = J_{3',4'}$ 9.5 Hz, H-3′), 4.01 (dd, 1H, $J_{8a,8b}$ 13.1, $J_{8a,7}$ 1.9 Hz, H-8a), 3.86–3.80 (m, 4H, H-4, CO_2CH_3), 3.79 (s, 3H, PhOCH_3), 3.72 (app. t, 1H, $J_{4',5'}$ 9.4 Hz, H-4′), 3.64–3.60 (m, 3H, H-2′, H-6, H-8b), 3.40 (dd, 1H, $J_{6'a,6'b}$ 10.7, $J_{6'a,5'}$ 2.1 Hz, H-6′a), 3.29 (s, 3H, OCH_3), 3.12 (dd, 1H, $J_{6'b,5'}$ 1.9 Hz, H-6′b), 2.21 (dd, 1H, $J_{3eq,3ax}$ 12.5, $J_{3eq,4}$ 4.4 Hz, H-3*eq*), 2.16 (app. t, 1H, $J_{3ax,4}$ 12.2 Hz, H-3*ax*), 1.06–0.87 (m, 28H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 168.80 (s, C=O), 159.07, 138.86, 138.78, 138.57, 138.11, 130.52 (s, 6C, Ar), 129.01, 128.25, 128.23, 128.21, 128.09, 127.98, 127.85, 127.82, 127.50, 127.48, 127.36, 127.00, 126.77, 113.70 (d, 24C, Ar), 99.13 (s, C-2), 98.66 (d, C-1′), 81.82 (d, C-3′), 80.19 (d, C-2′), 77.95 (d, C-4′), 75.10 (t, CH_2Ph), 74.78 (t, CH_2Ph), 73.62 (d, C-4), 73.29 (t, CH_2Ph), 72.53 (t, CH_2Ph), 71.82 (d, C-5), 71.75 (d, C-7), 71.39 (d, C-6), 70.23 (d, C-5′), 69.94 (t, CH_2Ph), 67.79 (t, C-6′), 63.00 (t, C-8), 55.22 (q, PhOCH_3), 52.34 (q, CO_2CH_3), 51.12 (q, OCH_3), 33.21 (t, C-3), 17.60, 17.54, 17.50, 17.49, 17.29, 17.17, 17.09 [q, 8C, Si-CH-(CH_3)$_2$], 14.18, 13.55, 12.99, 12.75 [d, 4C, Si-CH-(CH_3)$_2$]; ESI-TOF HRMS: *m*/*z* = 1168.5848; calcd for $C_{64}H_{86}O_{15}Si_2NH_4^+$: 1168.5844.

Data for **8β**: $[\alpha]_D^{20}$ +14.4 (*c* 0.94, $CHCl_3$); R_f 0.60 (*n*-hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$): δ 7.37–7.34 (m, 2H, Ar), 7.29–7.22 (m, 16H, Ar), 7.18–7.13 (m, 4H, Ar), 6.83–6.80 (m, 2H, Ar), 5.31 (d, 1H, $J_{1',2'}$ 7.8 Hz, H-1′), 5.08 (d, 1H, *J* 11.6 Hz, C*H*HPh), 4.89 (d, 1H, *J* 10.9 Hz, C*H*HPh), 4.81 (d, 1H, *J* 11.0 Hz, C*H*HPh), 4.78–4.76 (m, 1H, H-5), 4.74–4.71 (m, 2H, 2 × CH*H*Ph), 4.62 (d, 1H, *J* 11.8 Hz, C*H*HPh), 4.52–4.45 (m, 4H, 1 × C*H*HPh, 3 × CH*H*Ph), 4.35–4.31 (m, 1H, H-7), 4.27 (dd, 1H, $J_{8a,8b}$ 12.0, $J_{8a,7}$ 2.0 Hz, H-8a), 3.94 (ddd, 1H, $J_{4,3ax}$ 11.7, $J_{4,3eq}$ 4.6, $J_{4,5}$ 1.8 Hz, H-4), 3.81 (dd, 1H, $J_{8b,7}$ 6.4 Hz, H-8b), 3.78 (s, 3H, PhOCH_3), 3.73–3.69 (m, 1H, H-6′a), 3.65–3.55 (m, 2H, H-3′, H-6′b), 3.49 (s, 3H, CO_2CH_3), 3.46–3.39 (m, 3H, H-4′, H-5′, H-6), 3.34 (dd, 1H, $J_{2',3'}$ 9.1 Hz, H-2′), 3.20 (s, 3H, OCH_3), 2.26 (app. t, 1H, $J_{3ax,3eq}$ 12.7 Hz, H-3*ax*), 2.20 (dd, 1H, H-3*eq*), 1.11–0.87 (m, 28H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 168.52 (s, C=O), 159.00, 138.95, 138.67, 138.22, 138.19, 130.32 (s, 6C, Ar), 128.66, 128.35, 128.29, 128.25, 128.15, 128.01, 127.96, 127.89, 127.68, 127.56, 127.51, 127.45, 127.19, 113.79 (d, 24C, Ar), 100.49 (d, C-1′), 99.36 (s, C-2), 84.73 (d, C-3′), 82.31 (d, C-2′), 78.36 (d, C-4′), 75.81 (d, C-4), 75.75 (t, CH_2Ph), 74.94 (t, CH_2Ph), 74.57 (d, C-6), 74.09 (t, CH_2Ph), 73.28 (d, C-5′), 73.24 (t, CH_2Ph), 72.69 (d, C-7), 69.86, 69.83 (t, 2C, CH_2Ph, C6′), 66.57 (t, C-8), 64.98 (d, C-5), 55.24 (q, PhOCH_3), 52.19 (q, CO_2CH_3), 50.94 (q, OCH_3), 33.30 (t, C-3), 17.81, 17.79, 17.57, 17.48, 17.42, 17.34, 17.28 [q, 8C, Si-CH-(CH_3)$_2$], 13.38, 12.79, 12.74 [d, 4C, Si-CH-(CH_3)$_2$]; ESI-TOF HRMS: *m*/*z* = 1168.5854; calcd for $C_{64}H_{86}O_{15}Si_2NH_4^+$: 1168.5844.

Data for **9α**: $[\alpha]_D^{20}$ +34.3 (*c* 0.64, $CHCl_3$); R_f 0.39 (*n*-hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$): δ 7.33–7.24 (m, 18H, Ar), 7.13–7.10 (m, 2H, Ar), 5.04 (d, 1H, $J_{1',2'}$ 3.4 Hz, H-1′), 4.93 (d, 1H, *J* 11.0 Hz, C*H*HPh), 4.82 (d, 1H, *J* 11.0 Hz, CH*H*Ph), 4.79 (d, 1H, *J* 10.7 Hz, C*H*HPh), 4.75 (s, 2H, CH_2Ph), 4.54 (d, 1H, *J* 12.2 Hz, C*H*HPh), 4.52 (m, 1H, H-7), 4.45 (d, 1H, *J* 12.2 Hz, CH*H*Ph), 4.44 (d, 1H, *J* 10.5 Hz, CH*H*Ph), 4.16 (dd, 1H, $J_{8a,8b}$ 12.9, $J_{8a,7}$ 2.9 Hz, H-8a), 4.09–3.99 (m, 5H, H-3′, H-5′, H-4, H-5, H-8b), 3.79–3.77 (m, 4H, H-6, CO_2CH_3), 3.61–3.56 (m, 2H, H-6′a, H-6′b), 3.54 (dd, 1H, $J_{2',3'}$ 9.6 Hz, H-2′), 3.48 (dd, 1H, *J* 9.9, *J* 8.9 Hz, H-4′), 3.44 (d, 1H, $J_{OH,4}$ 9.6 Hz, O*H*), 3.31 (s, 3H, OCH_3), 2.13 (dd, 1H, $J_{3eq,3ax}$ 12.8, $J_{3eq,4}$ 4.4 Hz, H-3*eq*), 1.86 (app. t, 1H, $J_{3ax,4}$ 12.5 Hz, H-3*ax*), 1.06–0.82 (m, 28H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 168.57 (s, C=O), 138.67, 138.30, 137.96, 137.69 (s, 4C, Ar), 128.39, 128.38, 128.35, 138.33, 127.99, 127.89, 127.87, 127.80, 127.73, 127.58, 127.53, 127.45 (d, 20C, Ar), 99.46 (d, C-1′), 99.03 (s, C-2), 81.42 (d, C-3′), 78.74 (d, C-2′), 78.60 (d, C-5), 77.87 (d, C-4′), 75.37 (t, CH_2Ph), 75.08 (t, CH_2Ph), 73.50 (t, CH_2Ph), 73.08 (d, C-7), 72.56 (t, CH_2Ph), 72.05 (d, C-6), 71.39 (d, C-5′), 68.67 (t, C-6′), 66.79 (d, C-4), 63.87 (t, C-8), 52.37 (q, CO_2CH_3), 51.19 (q, OCH_3), 36.13 (t, C-3), 17.63, 17.54, 17.53, 17.50, 17.35, 17.22, 17.14 [q, 8C, Si-CH-(CH_3)$_2$], 13.98, 13.60, 12.96, 12.77 [d, 4C, Si-CH-(CH_3)$_2$]; ESI-TOF HRMS: *m*/*z* = 1048.5273; calcd for $C_{56}H_{78}O_{14}Si_2NH_4^+$: 1048.5268.

Data for **9β**: $[\alpha]_D^{20}$ +19.7 (*c* 0.85, $CHCl_3$); R_f 0.27 (*n*-hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$): δ 7.33–7.23 (m, 18H, Ar), 7.17–7.13 (m, 2H, Ar), 5.00 (d, 1H, *J* 11.0 Hz, C*H*HPh), 4.90 (d, 1H, *J* 10.9 Hz, C*H*HPh), 4.83 (d, 1H, *J* 10.7 Hz, CH*H*Ph), 4.78 (2d, 2H, *J* 10.7 Hz, C*H*HPh, CH*H*Ph), 4.70 (d, 1H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.62 (d, 1H, *J* 11.9 Hz, C*H*HPh), 4.57 (d, 1H, *J* 10.7 Hz, CH*H*Ph), 4.41 (d, 1H, *J* 11.9 Hz, CH*H*Ph), 4.38 (app. dt, 1H, $J_{7,6} = J_{7,8a}$ 8.7, $J_{7,8b}$ 2.5 Hz, H-7), 4.17–4.09 (m, 3H, H-5, H-8a, H-8b), 3.90 (app. ddt, 1H, $J_{4,3ax} = J_{4,OH}$ 11.6, $J_{4,3eq}$ 4.8, $J_{4,5}$ 2.6 Hz, H-4), 3.81 (dd, 1H, $J_{6'a,6'b}$ 10.9, $J_{6'a,5'}$ 3.2 Hz, H-6′a), 3.80 (app. t, 1H, $J_{4',3'} = J_{4',5'}$ 9.4 Hz, H-4′), 3.78 (s, 3H, CO_2CH_3), 3.72–3.68 (m, 3H, H-3′, H-6′b, O*H*), 3.63 (d, 1H, H-6), 3.55 (dd, 1H, $J_{2',3'}$ 9.1 Hz, H-2′), 3.41–3.37 (m, 1H, H-5′), 3.26 (s, 3H, OCH_3), 1.82 (dd, 1H, $J_{3eq,3ax}$ 12.7 Hz, H-3*eq*), 1.64 (app. t, 1H, H-3*ax*), 1.08–0.85 (m, 28H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 168.73 (s, C=O), 138.15, 137.16 (s, 4C, Ar), 128.47, 128.44, 128.39, 128.34, 127.96, 127.94, 127.8, 127.71, 127.69, 127.67, 127.60 (d, 20C, Ar), 103.86 (d, C-1′), 99.21 (s, C-2), 85.57 (d, C-3′), 82.12 (d, C-2′), 78.06 (d, C-4′), 77.23 (d, C-5), 75.53 (t, CH_2Ph), 75.50 (t, CH_2Ph), 75.13 (d, C-5′), 74.90 (t, CH_2Ph), 73.43 (t, CH_2Ph), 72.73 (d, C-7), 71.12 (d, C-6), 68.75 (t, C-6′), 67.43 (d, C-4), 63.88 (t, C-8), 52.33 (q, CO_2CH_3), 51.20 (q, OCH_3), 35.88 (t, C-3), 17.83, 17.74, 17.54, 17.47, 17.37, 17.34, 17.21, 17.15 [q, 8C, Si-CH-(CH_3)$_2$], 13.77, 13.67, 12.95, 12.84 [d, 4C, Si-CH-(CH_3)$_2$]; ESI-TOF HRMS: *m*/*z* = 1053.4818; calcd for $C_{56}H_{78}O_{14}Si_2Na^+$: 1053.4822.

Alternatively compound **9α** was obtained by acid treatment of **8α**:

A solution of **8α** (17.6 mg, 0.015 mmol) in CH_2Cl_2 (14.2 mL) was treated with 99% TFA (3.6 mL) at 0 °C for 10 min. The solution was

concentrated and coevaporated with toluene. Subsequent column chromatography of the residue (*n*-hexane/EtOAc 5:1) yielded **9α** (13.0 mg, 83%) as a colorless oil.

3.5. 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α,β-D-*gluco*pyranosyl (*N*-phenyl)trifluoroacetimidate (10α/β)

2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α,β-D-glucopyranose[28] (128 mg, 0.285 mmol) was dissolved in a mixture of dry CH_2Cl_2 (2.0 mL) and dry acetone (0.9 mL) followed by successive addition of K_2CO_3 (79 mg, 0.570 mmol) and 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (92 μL, 0.570 mmol). The heterogenous mixture was stirred at ambient temperature for 16 h, filtered through a pad of Celite®, and rinsed with CH_2Cl_2. After addition of one drop of Et_3N the filtrate was concentrated and the crude residue was purified by chromatography (*n*-hexane/EtOAc 10:1) affording an anomeric mixture ($\alpha/\beta \sim 1{:}8$) of **10** (169 mg, 96%).

Data for **10β**: colorless amorphous solid; $[\alpha]_D^{20}$ +49.1 (*c* 2.57, $CHCl_3$); R_f 0.75 (*n*-hexane/EtOAc 4:1); ^{1}H NMR (CD_2Cl_2): δ 7.54–7.46 (m, 2H, Ar), 7.42–7.27 (m, 15H, Ar), 7.17–7.13 (m, 1H, Ar), 6.88–6.75 (m, 2H, Ar), 5.83 (bs, 1H, H-1), 5.60 (s, 1H, C*H*Ph), 4.92 (d, 1H, *J* 11.4 Hz, C*H*HPh), 4.84 (d, 1H, *J* 11.0 Hz, C*H*HPh), 4.81 (2d, 2H, *J* 11.1 Hz, 2 × CH*H*Ph), 4.38–4.29 (m, 1H, H-6a), 3.88–3.65 (m, 4H, H-2, H-3, H-4, H-6b), 3.45 (bs, 1H, H-5); ^{13}C NMR (CD_2Cl_2): δ 143.78, 138.94, 138.41, 137.84 (s, 4C, Ar), 129.37, 129.20, 128.75, 128.67, 128.59, 128.49, 128.41, 128.27, 128.06, 126.49, 124.88, 119.63 (d, 20C, Ar), 101.69 (d, C*H*Ph), 81.50, 81.13, 81.11 (d, 3C, C-2, C-3, C-4), 75.71, 75.21 (t, 2C, 2 × *C*H$_2$Ph), 68.91 (t, C-6), 67.06 (d, C-5).

Data for **10α**: colorless oil; $[\alpha]_D^{20}$ +43.0 (*c* 1.92, $CHCl_3$); R_f 0.61 (*n*-hexane/EtOAc, 4:1); ^{1}H NMR (CD_2Cl_2): δ 7.53–7.49 (m, 2H, Ar), 4.42–7.27 (m, 15H, Ar), 7.15–7.11 (m, 1H, Ar), 6.80–6.74 (m, 2H, Ar), 6.45 (bs, 1H, H-1), 5.61 (s, 1H, C*H*Ph), 4.94 (d, *J* 11.4 Hz, C*H*HPh), 4.85 (d, *J* 11.3 Hz, CH*H*Ph), 4.84 (d, *J* 11.8 Hz, C*H*HPh), 4.76 (d, *J* 11.9 Hz, CH*H*Ph), 4.36 (dd, 1H, $J_{6a,6b}$ 10.2, $J_{6a,5}$ 4.8 Hz, H-6a), 4.09 (app. t, 1H, $J_{3,2} = J_{3,4}$ 9.5 Hz, H-3), 4.03–3.96 (m, 1H, H-5), 3.81–3.70 (m, 3H, H-2, H-4, H-6b); ^{13}C NMR (CD_2Cl_2): δ 144.06, 139.23, 138.46, 137.89 (s, 4C, Ar), 129.37, 129.20, 128.83, 128.63, 128.60, 128.30, 128.27, 128.12, 127.96, 126.52, 124.69, 119.75 (d, 20C, Ar), 101.82 (d, C*H*Ph), 81.77 (d, C-4), 79.14 (d, C-2), 78.62 (d, C-3), 75.50, 74.29 (t, 2C, 2 × *C*H$_2$Ph), 69.07 (t, C-6), 65.52 (d, C-5).

3.6. 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-*gluco*pyranosyl-(1→5)-methyl [methyl 3-deoxy-4-*O*-(4-methoxybenzyl)-7,8-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-*manno*-oct-2-ulopyranosid]onate (11)

A solution of donor **10α/β** (74 mg, 0.119 mmol) in dry CH_2Cl_2 (1.0 mL) was added to dry acceptor **4** (50 mg, 0.080 mmol) under Ar followed by addition of 4 Å molecular sieves. After stirring for 2.5 h at ambient temperature a solution of TMSOTf (0.4 μL, 0.002 mmol) in dry CH_2Cl_2 (0.1 mL) was added dropwise at −5 °C. The reaction was quenched after 15 min by addition of Et_3N (23 μL, 0.159 mmol) in dry CH_2Cl_2 (0.7 mL). The mixture was allowed to warm to ambient temperature and was filtered through a pad of Celite®. The filtrate was concentrated and the residue was purified by chromatography (*n*-hexane/EtOAc 8:1→3:1, with 0.1% TEA) to give **11** (67 mg, 80%) as a coloress oil: $[\alpha]_D^{20}$ +59.7 (*c* 0.53, $CHCl_3$); R_f 0.63 (*n*-hexane/EtOAc 3:1), ^{1}H NMR ($CDCl_3$): δ 7.51–7.47 (m, 2H, Ar), 7.42–7.21 (m, 15H, Ar), 6.88–6.84 (m, 2H, Ar), 5.51 (s, 1H, C*H*Ph), 5.28 (d, 1H, $J_{1',2'}$ 3.8 Hz, H-1′), 5.08 (d, 1H, *J* 11.8 Hz, C*H*HPh), 4.96 (d, 1H, *J* 11.3 Hz, C*H*HPh), 4.75 (d, 1H, *J* 11.0 Hz, CH*H*Ph), 4.66 (d, 1H, *J* 11.5 Hz, CH*H*Ph), 4.57 (s, 2H, C*H*$_2$Ph), 4.42–4.39 (m, 1H, H-7), 4.35 (app. dt, 1H, $J_{5',4'} = J_{5',6'b}$ 9.9, $J_{5',6'a}$ 5.1 Hz, H-5′), 4.25 (d, 1H, $J_{5,4}$ 2.3 Hz, H-5), 4.19 (app. t, 1H, $J_{3',2'} = J_{3',4'}$ 9.5 Hz, H-3′), 3.96 (dd, 1H, $J_{8a,8b}$ 13.2, $J_{8a,7}$ 1.6 Hz, H-8a), 3.89 (dd, 1H, $J_{6'a,6'b}$ 10.1 Hz, H-6′a), 3.85 (ddd, 1H, $J_{4,3ax}$ 11.6, $J_{4,3eq}$ 4.7, H-4), 3.81 (s, 3H, CO_2CH_3), 3.77 (s, 3H, PhOCH_3), 3.67 (dd, 1H, H-2′), 3.64–3.59 (m, 3H, H-4′, H-6, H-8b), 3.55 (app. t, 1H, $J_{6'b,5'}$ 10.2 Hz, H-6′b), 3.28 (s, 3H, OCH_3), 2.23 (dd, 1H, $J_{3eq,3ax}$ 12.5, H-3*eq*), 2.17 (app. t, 1H, H-3*ax*), 1.14–0.77 (m, 28H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 168.76 (s, C═O), 159.08, 138.93, 138.78, 137.79, 130.34 (s, 5C, Ar), 129.01, 128.69, 128.23, 128.10, 128.05, 127.91, 127.43, 126.96, 126.08, 113.85 (d, 19C, Ar), 100.99 (d, C*H*Ph), 99.24 (d, C-1′), 99.16 (s, C-2), 82.96 (d, C-4′), 79.28 (d, C-2′), 78.04 (d, C-3′), 74.70 (t, *C*H$_2$Ph), 73.09 (d, C-4), 71.99 (t, *C*H$_2$Ph), 71.65 (d, C-5), 71.54 (d, C-7), 71.23 (d, C-6), 70.11 (t, *C*H$_2$Ph), 69.08 (t, C-6′), 62.79 (t, C-8), 62.37 (d, C-5′), 55.19 (q, PhOCH_3), 52.34 (q, CO_2CH_3), 51.06 (q, OCH_3), 33.20 (t, C-3), 17.55, 17.49, 17.47, 17.44, 17.27, 17.16, 17.07 [q, 8C, Si-CH-(*C*H$_3$)$_2$], 14.22, 13.44, 12.99, 12.79 [d, 4C, Si-*C*H-(CH$_3$)$_2$]; ESI-TOF HRMS: *m*/*z* = 1076.5210; calcd for $C_{57}H_{78}O_{15}Si_2NH_4^+$: 1076.5218.

3.7. 2,3,4,6-*O*-benzyl-α-D-*gluco*pyranosyl-(1→5)-methyl (methyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (12)

Compound **9α** (16.0 mg, 0.016 mmol) was dissolved in dry THF (3.3 mL) and was treated with TBAF (1 M in THF, 23 μL, 0.023 mmol) at ambient temperature for 20 min. After addition of dry MeOH (1.7 mL) the solvent was removed in vacuo. Purification of the residue by chromatography (toluene/EtOAc 1:1) afforded **12** (12.0 mg, 98%) as a colorless oil; $[\alpha]_D^{20}$ +36.0 (*c* 0.53, $CHCl_3$); R_f 0.18 (toluene/EtOAc 1:1); ^{1}H NMR ($CDCl_3$): δ 7.39–7.24 (m, 18H, Ar), 7.18–7.15 (m, 2H, Ar), 4.96 (d, 1H, *J* 11.1 Hz, C*H*HPh), 4.93 (d, 1H, *J* 11.1 Hz, CH*H*Ph), 4.89 (d, 1H, *J* 11.6 Hz, C*H*HPh), 4.83 (d, 1H, *J* 10.7 Hz, C*H*HPh), 4.73 (d, 1H, $J_{1',2'}$ 3.7 Hz, H-1′), 4.64 (d, 1H, *J* 11.8 Hz, CH*H*Ph), 4.52 (d, 1H, *J* 12.3 Hz, C*H*HPh), 4.49 (d, 1H, *J* 10.7 Hz, CH*H*Ph), 4.45 (d, 1H, *J* 12.1 Hz, CH*H*Ph), 4.28 (d, 1H, *J* 5.6 Hz, O*H*), 4.07 (app. t, 1H, $J_{3',2'} = J_{3',4'}$ 9.5 Hz, H-3′), 4.03–3.95 (m, 4H, H-5′, H-4, H-5, H-7), 3.86–3.81 (m, 1H, H-8a), 3.79 (s, 3H, CO_2CH_3), 3.68 (dd, 1H, $J_{6,7}$ 8.9, $J_{6,5}$ 1.6 Hz, H-6), 3.66–3.61 (m, 1H, H-8b), 3.60 (dd, 1H, $J_{6'a,6'b}$ 10.4, $J_{6'a,5'}$ 2.2 Hz, H-6′a), 3.58 (dd, 1H, H-2′), 3.55 (dd, 1H, $J_{6'b,5'}$ 5.6 Hz, H-6′b), 3.54 (d, 1H, *J* 12.5 Hz, O*H*), 3.51 (app. t, 1H, $J_{4',5'}$ 9.6 Hz, H-4′), 3.18 (s, 3H, OCH_3), 2.16–2.11 (m, 1H, H-3*eq*), 2.08–2.00 (m, 1H, O*H*), 1.68 (app. t, 1H, $J_{3ax,3eq} = J_{3ax,4}$ 12.3 Hz, H-3*ax*); ^{13}C NMR ($CDCl_3$): δ 168.20 (s, C═O), 138.15, 137.55, 137.48, 136.60 (s, 4C, Ar), 128.85, 128.72, 128.61, 128.54, 128.53, 128.40, 128.03, 128.01, 127.89, 127.86, 127.83 (d, 20C, Ar), 100.65 (d, C-1′), 98.99 (s, C-2), 81.83 (d, C-3′), 80.06 (d, C-5), 78.83 (d, C-2′), 78.31 (d, C-4′), 75.77 (t, *C*H$_2$Ph), 75.37 (t, *C*H$_2$Ph), 74.96 (t, *C*H$_2$Ph), 73.51 (t, *C*H$_2$Ph), 71.98 (d, C-6), 71.75 (d, C-5′), 69.06 (d, C-7), 68.44 (t, C-6′), 65.85 (d, C-4), 64.32 (t, C-8), 52.46 (q, CO_2CH_3), 51.02 (q, OCH_3), 36.12 (t, C-3); ESI-TOF HRMS: *m*/*z* = 811.3299; calcd for $C_{44}H_{52}O_{13}Na^+$: 811.3300.

3.8. α-D-*Gluco*pyranosyl-(1→5)-sodium (methyl 3-deoxy-α-D-*manno*-2-oct-2-ulopyranosid)onate (13)

Compound **12** (11.2 mg, 0.014 mmol) was dissolved in dry MeOH (0.5 mL). The atmosphere was exchanged to argon by alternating evacuation and flushing with argon. Then, palladium on active charcoal (10%, 1 mg) was added to the flask followed by successive exchange of the atmosphere to argon and hydrogen using the same method described before. The mixture was stirred intensively for 3 h, diluted with MeOH, and passed through a 0.45 μm syringe filter. Concentration of the filtrate afforded debenzylated methyl ester which was saponified with aq NaOH (0.01 M, 2.0 mL) at ambient temperature for 1.5 h. The solution was neutralized by addition of freshly regenerated DOWEX 50 H^+, the ion-exchange resin was filtered off, and the filtrate was lyophilized. Purification by SEC (Bio-Gel P2 gel, 5% aq EtOH) and freeze-drying

of pooled fractions provided **13** (5.6 mg, 90%) as a colorless amorphous solid; $[\alpha]_D^{20}$ +99.9 (*c* 0.56, D_2O); ^{1}H NMR (D_2O): 5.11 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1′), 4.12–4.03 (m, 4H, H-4, H-5, H-7, H-5′), 3.89 (dd, 1H, $J_{8a,8b}$ 11.8, $J_{8a,7}$ 2.8 Hz, H-8a), 3.79–3.68 (m, 3H, H-3′, H-6′a, H-6′b), 3.62 (dd, 1H, $J_{8b,7}$ 6.1 Hz, H-8b), 3.56–3.54 (m, 1H, H-6), 3.49 (dd, 1H, $J_{2',3'}$ 10.0 Hz, H-2′), 3.41 (dd, 1H, *J* 10.0, *J* 9.0 Hz, H-4′), 3.11 (s, 3H, OCH_3), 1.99–1.95 (m, 1H, H-3*eq*), 1.82 (app. t, 1H, $J_{3ax,3eq} = J_{3ax,4}$ 12.5 Hz, H-3*ax*); ^{13}C NMR data: see Table 1; ESI-TOF HRMS: *m*/*z* = 413.1296; calcd for $C_{15}H_{25}O_{13}^-$: 413.1301.

3.9. 2,3-Di-*O*-benzyl-α-D-*gluco*pyranosyl-(1→5)-methyl [methyl 3-deoxy-7,8-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-*manno*-oct-2-ulopyranosid]onate (14)

Compound **11** (50.0 mg, 0.047 mmol) was treated with trifluoroacetic acid (99%, 1.0 mL) in CH_2Cl_2 (8.0 mL) at 0 °C for 10 min. The solution was concentrated, coevaporated with toluene, and the crude product was purified by chromatography (*n*-hexane/EtOAc 3:1, then 1:2) yielding **14** (40.2 mg, 88%) as a colorless oil; $[\alpha]_D^{20}$ +79.6 (*c* 0.52, $CHCl_3$); R_f 0.24 (*n*-hexane/EtOAc 1:2); ^{1}H NMR ($CDCl_3$): δ 7.36–7.26 (m, 10H, Ar), 5.09 (d, 1H, $J_{1',2'}$ 3.4 Hz, H-1′), 4.91 (d, 1H, *J* 11.6 Hz, C*H*HPh), 4.79 (d, 1H, *J* 12.4 Hz, C*H*HPh), 4.75 (d, 1H, *J* 12.5 Hz, CH*H*Ph), 4.71 (d, 1H, *J* 11.5 Hz, CH*H*Ph), 4.44 (app. td, 1H, $J_{7,6}$ 8.3, $J_{7,8a} = J_{7,8b}$ 2.3 Hz, H-7), 4.14 (dd, 1H, $J_{8a,8b}$ 12.8 Hz, H-8a), 4.10–4.05 (m, 2H, H-4, H-5), 4.03–3.97 (m, 2H, H-5′, H-8b), 3.85 (app. t, 1H, $J_{3',2'} = J_{3',4'}$ 8.6 Hz, H-3′), 3.81–3.76 (m, 5H, CO_2CH_3, H-6, H-6′a), 3.73 (dd, 1H, $J_{6'b,6'a}$ 11.7, $J_{6'b,5'}$ 5.6 Hz, H-6′b), 3.53 (dd, 1H, H-2′), 3.49 (app. t, 1H, $J_{4',5'}$ 8.7 Hz, H-4′), 3.31 (s, 3H, OCH_3), 2.17 (m, 1H, H-3*eq*), 1.90 (app. t, 1H, $J_{3ax,3eq} = J_{3ax,4}$ 12.2 Hz, H-3*ax*), 1.07–0.81 (m, 28H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 168.62 (s, C=O), 138.38, 138.02 (s, 2C, Ar), 128.58, 128.41, 127.96, 127.91, 127.67, 127.42 (d, 10C, Ar), 99.07 (s, C-2), 98.90 (d, C-1′), 80.20 (d, C-3′), 78.39 (d, C-2′), 77.62 (d, C-5), 74.73 (t, CH_2Ph), 73.12 (d, C-5′), 72.91 (d, C-7), 72.55 (t, CH_2-Ph), 71.79 (d, C-6), 69.91 (d, C-4′), 66.60 (d, C-4), 63.70 (t, C-8), 62.19 (t, C-6′), 52.42 (q, CO_2CH_3), 51.20 (q, OCH_3), 36.15 (t, C-3), 17.56, 17.52, 17.50, 17.44, 17.31, 17.20, 17.12 [q, 8C, Si-CH-(CH_3)$_2$], 14.00, 13.57, 12.93, 12.77 [d, 4C, Si-C*H*-(CH$_3$)$_2$]; ESI-TOF HRMS: *m*/*z* = 873.3880; calcd for $C_{42}H_{66}O_{14}Si_2Na^+$: 873.3883.

3.10. 2,3-Di-*O*-benzyl-α-D-*gluco*pyranosyl-(1→5)-methyl (methyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (15)

TBAF (1 M in THF, 28 μL, 0.028 mmol) was added to a solution of **14** (15.9 mg, 0.019 mmol) in dry THF (1.0 mL) at ambient temperature and stirred for 5 min. Dry MeOH (2.0 mL) was added followed by concentration. The residue was purified by chromatography (EtOAc, then EtOAc/methanol 19:1) to provide **15** (11.0 mg, 97%) as a colorless oil; $[\alpha]_D^{20}$ +50.6 (*c* 0.52, $CHCl_3$); R_f 0.22 (EtOAc); ^{1}H NMR ($CDCl_3$): δ 7.41–7.30 (m, 10H, Ar), 4.98 (d, 1H, *J* 11.4 Hz, C*H*HPh), 4.91 (d, 1H, *J* 11.6 Hz, C*H*HPh), 4.85 (d, 1H, *J* 11.4 Hz, CH*H*Ph), 4.77 (d, 1H, $J_{1',2'}$ 3.6 Hz, H-1′), 4.66 (d, 1H, *J* 11.5 Hz, CH*H*Ph), 4.20 (d, 1H, *J* 5.8 Hz, O*H*), 4.06–3.97 (m, 3H, H-4, H-5, H-7), 3.92 (app. t, 1H, $J_{3',2'} = J_{3',4'}$ 9.3 Hz, H-3′), 3.86–3.78 (m, 6H, CO_2CH_3, H-5′, H-6′a, H-8a), 3.75–3.68 (m, 2H, H-6′b, H-6), 3.66–3.61 (m, 1 H, H-8b), 3.57 (app. dt, 1H, $J_{4',5'}$ 9.3, $J_{4',OH}$ 3.2 Hz, H-4′), 3.54 (dd, 1H, H-2′), 3.44 (d, 1H, *J* 11.7 Hz, O*H*), 3.19 (s, 3H, OCH_3), 2.63 (d, 1H, $J_{OH,4'}$ 3.7 Hz, O*H*), 2.20 (dd, 1H, $J_{3eq,3ax}$ 12.6, $J_{3eq,4}$ 4.3 Hz, H-3*eq*), 2.14–2.07 (m, 2H, 2 × O*H*), 1.73–1.65 (m, 1H, H-3*ax*); ^{13}C NMR ($CDCl_3$): δ 168.40 (s, C=O), 138.17, 136.48 (s, 2C, Ar), 128.89, 128.80, 128.73, 128.63, 128.11, 127.90 (d, 10C, Ar), 100.72 (d, C-1′), 99.04 (s, C-2), 81.33 (d, C-3′), 79.78 (d, C-5), 78.97 (d, C-2′), 75.60 (t, CH_2Ph), 74.86 (t, CH_2Ph), 73.01 (d, C-5′), 71.90 (d, C-6), 70.69 (d, C-4′), 69.06 (d, C-7), 65.87 (d, C-4), 64.19 (t, C-8), 62.01 (t, C-6′), 52.62 (q, CO_2CH_3), 51.10 (q, OCH_3), 36.20 (t, C-3); ESI-TOF HRMS: *m*/*z* = 626.2804; calcd for $C_{30}H_{40}O_{13}NH_4^+$: 626.2807.

*Deprotection of **15**:* A solution of **15** (5.6 mg, 0.009 mmol) in dry MeOH (1.0 mL) was hydrogenated for 4 h with 10% Pd–C (1 mg) as described for **13**. The suspension was diluted with MeOH and passed through a 0.45 μm syringe filter. Concentration of the filtrate afforded the debenzylated methyl ester which was treated with 0.01 M aq NaOH (1.0 mL) at ambient temperature for 12 h. The solution was made neutral by addition of DOWEX 50 H^+ resin. The ion-exchange resin was filtered off and the filtrate was lyophilized. Purification of the residue on Bio-Gel PD10 column (H_2O) and freeze-drying of pooled fractions provided **13** (3.8 mg, 95%) as a colorless amorphous solid.

3.11. 2,3,4,6-Tetra-*O*-benzyl-α-D-*gluco*pyranosyl-(1→5)-methyl (methyl 3-deoxy-α-D-*lyxo*-2-hept-2-ulopyranosid)onate (16)

A solution of **12** (32.0 mg, 0.041 mmol) in dry CH_2Cl_2 (3.5 mL) was cooled to −10 °C. Sodium *meta*periodate on silica (15 w%, 116 mg, 0.082 mmol) was added and the suspension was stirred for 1 h at −10 °C with exclusion of light. The excess of reagent was destroyed by addition of ethylene glycol (3 w% in water, 84 μL, 0.041 mmol). The mixture was diluted with $CHCl_3$ and extracted with distilled water. The aqueous phase was further extracted with CH_2Cl_2 (3 × 5 mL) and the combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. The crude material was dissolved in dry MeOH (1.0 mL), cooled to 0 °C, and treated with sodium borohydride (2.5 mg, 0.065 mmol) for 1 h. Another portion of sodium borohydride (1.0 mg, 0.026 mmol) was added at 0 °C and after 5 min the solution was diluted with EtOAc and aq NH_4Cl. The aqueous phase was extracted with EtOAc (3 × 5 mL) and the combined organic layers were dried (Na_2SO_4). Concentration of the solution gave a crude product which was purified by HP-column chromatography (*n*-hexane/EtOAc 4:1 → 3:2) to afford **16** (10.4 mg, 34%) as a colorless oil; $[\alpha]_D^{20}$ +40.4 (*c* 0.96, $CHCl_3$); R_f 0.54 (toluene/EtOAc 1:2); ^{1}H NMR ($CDCl_3$): δ 7.39–7.26 (m, 18H, Ar), 7.18–7.15 (m, 2H, Ar), 4.95–4.91 (m, 2H, CH_2Ph), 4.85 (d, 1H, *J* 11.8 Hz, C*H*HPh), 4.84 (d, 1H, *J* 10.6 Hz, C*H*HPh), 4.74 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1′), 4.64 (d, 1H, *J* 11.9 Hz, CH*H*Ph), 4.52 (d, 1H, *J* 11.8 Hz, C*H*HPh), 4.49 (d, 1H, *J* 10.7 Hz, CH*H*Ph), 4.45 (d, 1H, *J* 12.4 Hz, CH*H*Ph), 4.06 (app. t, 1H, $J_{3',2'} = J_{3',4'}$ 9.4 Hz, H-3′), 4.04–3.98 (m, 2H, H-5′, H-4), 3.92–3.86 (m, 2H, H-5, H-7a), 3.86–3.83 (m, 1H, H-6), 3.82–3.78 (m, 4H, H-7b, CO_2CH_3), 3.60 (dd, 1H, $J_{6'a,6'b}$ 10.3, $J_{6'a,5'}$ 2.1 Hz, H-6′a), 3.58–3.54 (m, 2H, H-2′, H-6′b), 3.54–3.48 (m, 2H, H-4′, O*H*), 3.21 (s, 3H, OCH_3), 2.15 (dd, 1H, $J_{3eq,3ax}$ 12.2, $J_{3eq,4}$ 4.5 Hz, H-3*eq*), 1.71 (app. t, 1H, $J_{3ax,4}$ 12.5 Hz, H-3*ax*); ^{13}C NMR ($CDCl_3$): δ 168.28 (s, C=O), 138.25, 137.63, 137.53, 136.83 (s, 4C, Ar), 128.82, 128.62, 128.51, 128.40, 128.02, 127.99, 127.88, 127.83, 127.80 (d, 20C, Ar), 100.47 (d, C-1′), 99.03 (s, C-2), 81.74 (d, C-3′), 79.80 (d, C-5), 79.22 (d, C-2′), 78.11 (d, C-4′), 75.80 (t, CH_2Ph), 75.36 (t, CH_2Ph), 74.77 (t, CH_2Ph), 73.49 (t, CH_2Ph), 71.77 (d, C-5′), 71.59 (d, C-6), 68.49 (t, C-6′), 65.95 (d, C-4), 60.64 (t, C-7), 52.50 (q, CO_2CH_3), 51.01 (q, OCH_3), 36.27 (t, C-3); ESI-TOF HRMS: *m*/*z* = 781.3198; calcd for $C_{43}H_{50}O_{12}Na^+$: 781.3194.

3.12. α-D-*Gluco*pyranosyl-(1→5)-sodium (methyl 3-deoxy-α-D-*lyxo*-hept-2-ulopyranosid)onate (17)

A solution of **16** (8.0 mg, 0.011 mmol) in dry MeOH (0.5 mL) was hydrogenated for 4 h with 10% Pd–C (1 mg) as described for **13**. Another portion of catalyst (1 mg) was added and stirring under H_2 was continued for 19 h. The suspension was diluted with MeOH and passed through a 0.45 μm syringe filter. Concentration of the filtrate afforded the debenzylated methyl ester which was treated with 0.01 M aq NaOH (1.5 mL) at ambient temperature

for 1 h. The solution was made neutral by addition of DOWEX 50 H^+ resin. The ion-exchange resin was filtered off and the filtrate was lyophilized. Purification of the residue on Bio-Gel P2 (5% aq EtOH) and freeze-drying of pooled fractions provided **17** (3.4 mg, 79%) as a colorless amorphous solid; $[\alpha]_D^{20}$ +170.7 (*c* 0.28, D_2O); ^{1}H NMR (D_2O): 4.89 (d, 1H, $J_{1',2'}$ 3.8 Hz, H-1′), 4.10 (ddd, 1H, $J_{3ax,4}$ 12.3, $J_{3eq,4}$ 4.8, $J_{5,4}$ 3.0 Hz, H-4), 4.06 (ddd, 1H, $J_{6'a,5'}$ 3.5, $J_{6'b,5'}$ 2.6, $J_{4',5'}$ 10.3 Hz, H-5′), 3.96 (dd, 1H, $J_{7a,7b}$ 11.4, $J_{7a,6}$ 8.2 Hz, H-7a), 3.88 (br d, 1H, H-5), 3.81 (dd, 1H, $J_{7b,6}$ 4.7 Hz, H-7b), 3.76-3.68 (m, 4H, H-6, H-3′, H-6′a, H-6′b), 3.48 (dd, 1H, $J_{2',3'}$ 10.0 Hz, H-2′), 3.41 (app. t, 1H, $J_{4',3'}$ 9.1 Hz, H-4′), 3.14 (s, 3H, OCH_3), 2.01–1.99 (m, 1H, H-3*eq*), 1.82 (app. t, 1H, $J_{3ax,3eq}$ 12.6 Hz, H-3*ax*); ^{13}C NMR data: see Table 1; ESI-TOF HRMS: *m*/*z* = 383.1193; calcd for $C_{14}H_{23}O_{12}^-$: 383.1195.

3.13. 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-*gluco*pyranosyl-(1→5)-methyl [methyl 3-deoxy-7,8-*O*-(1,1,3,3-tetraisopropyl disiloxane-1,3-diyl)-α-D-*manno*-oct-2-ulopyranosid] onate (18)

2,3-Dichloro-4,5-dicyano-*p*-benzoquinone (26.2 mg, 0.116 mmol) was added in four portions during 1 h to a solution of **11** (15.3 mg, 0.014 mmol) in dry CH_2Cl_2 (0.9 mL) and dry MeOH (0.3 mL). After complete addition the mixture was stirred at ambient temperature for 1.5 h, diluted with $CHCl_3$, and washed with aq $NaHCO_3$. The aqueous phase was extracted with CH_2Cl_2 (2 × 5 mL) and the combined organic phases were washed with aq $NaHCO_3$, dried ($MgSO_4$), filtered, and concentrated. To remove aromatic impurities the crude product was dissolved in toluene and subjected to chromatography (toluene→toluene/EtOAc 9:1) to afford **18** (11.2 mg, 83%) as a colorless oil; $[\alpha]_D^{20}$ +52.2 (*c* 0.54, $CHCl_3$); R_f 0.30 (*n*-hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$): δ 7.49–7.47 (m, 2H, Ar), 7.39–7.24 (m, 13H, Ar), 5.54 (s, 1H, C*H*Ph), 5.11 (d, 1H, $J_{1',2'}$ 3.9 Hz, H-1′), 4.93 (d, 1H, *J* 11.2 Hz, C*H*HPh), 4.90 (d, 1H, *J* 12.2 Hz, C*H*HPh), 4.79 (d, 1H, *J* 11.2 Hz, CH*H*Ph), 4.75 (d, 1H, *J* 12.5 Hz, CH*H*Ph), 4.47 (app. dt, 1H, $J_{7,6} = J_{7,8a}$ 8.7, $J_{7,8b}$ 2.1 Hz, H-7), 4.24 (dd, 1H, $J_{6'a,6'b}$ 10.4, $J_{6'a,5'}$ 4.9 Hz, H-6′a), 4.15–4.07 (m, 5H, H-3′, H-5′, H-4, H-5, H-8a), 3.90 (dd, 1H, $J_{8b,8a}$ 12.9 Hz, H-8b), 3.80 (s, 3H, CO_2CH_3), 3.75 (d, 1H, H-6), 3.69 (app. t, 1H, $J_{6'b,5'}$ 10.4 Hz, H-6′b), 3.65 (app. t, 1H, $J_{4',3'} = J_{4',5'}$ 9.5 Hz, H-4′), 3.62 (dd, 1H, $J_{2',3'}$ 9.0 Hz, H-2′), 3.32 (s, 3H, OCH_3), 2.85 (bs, 1H, O*H*), 2.20–2.16 (m, 1H, H-3*eq*), 1.92 (app. t, 1H, $J_{3ax,3eq} = J_{3ax,4}$ 12.2 Hz, H-3*ax*), 1.05–0.81 (m, 28H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 168.53 (s, C=O), 138.59, 138.39, 137.28 (s, 3C, Ar), 128.90, 128.27, 128.22, 127.96, 127.56, 127.46, 127.41, 125.97 (d, 15C, Ar), 101.13 (d, C*H*Ph), 100.05 (d, C-1′), 99.07 (s, C-2), 82.09 (d, C-4′), 78.26 (d, C-2′), 78.18 (d, C-3′), 77.25 (d, C-5), 74.84 (t, *C*H_2Ph), 72.57 (t, *C*H_2Ph), 72.32 (d, C-7), 71.49 (d, C-6), 68.75 (t, C-6′), 66.50 (d, C-4), 63.37 (d, C-5′), 63.33 (t, C-8), 52.46 (q, CO_2CH_3), 51.22 (q, OCH_3), 36.28 (t, C-3), 17.62, 17.53, 17.52, 17.49, 17.32, 17.18, 17.11 [q, 8C, Si-CH-(*C*H_3)$_2$], 14.12, 13.58, 12.96, 12.81 [d, 4C, Si-*C*H-(CH$_3$)$_2$]; ESI-TOF HRMS: *m*/*z* = 961.4190; calcd for $C_{49}H_{70}O_{14}Si_2Na^+$: 961.4196.

3.14. 2,3,4-Tri-*O*-benzyl-α-D-*gluco*pyranosyl-(1→5)-methyl [methyl 3-deoxy-4-*O*-(4-methoxybenzyl)-7,8-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-*manno*-oct-2-ulopyranosid]onate (19)

A suspension of compound **11** (20.0 mg, 0.019 mmol) and 4 Å molecular sieves (70 mg) in dry CH_2Cl_2 (0.8 mL) was cooled to −70 °C followed by consecutive addition of triethylsilane (9.8 μL, 0.061 mmol) and dichlorophenylborane (7.2 μL, 0.055 mmol). After 1 h Et_3N (44 μL) and dry MeOH (44 μL) were added to the cold solution. The mixture was diluted with $CHCl_3$, allowed to warm up to ambient temperature and washed with aq $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL), the combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (*n*-hexane/EtOAc 9:1 → 3:1) to give **19** (8.6 mg, 43%) as a colorless oil; $[\alpha]_D^{20}$ +87.6 (*c* 0.78, $CHCl_3$); R_f 0.31 (*n*-hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$): δ 7.36–7.21 (m, 17H, Ar), 6.84–6.81 (m, 2H, Ar), 5.25 (d, 1H, $J_{1',2'}$ 3.7 Hz, H-1′), 4.93 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.88–4.82 (m, 3H, C*H*HPh, CH_2Ph), 4.68 (d, 1H, *J* 12.0 Hz, CH*H*Ph), 4.63 (d, 1H, *J* 11.0 Hz, CH*H*Ph), 4.51 (d, 1H, *J* 11.6 Hz, C*H*HPh), 4.49 (d, 1H, *J* 11.8 Hz, CH*H*Ph), 4.40 (app. td, 1H, $J_{7,6}$ 9.3, $J_{7,8a} = J_{7,8b}$ 1.9 Hz, H-7), 4.23–4.21 (m, 1H, H-5), 4.20 (app. td, 1H, $J_{5',4'}$ 10.1, $J_{5',6'a} = J_{5',6'b}$ 2.8 Hz, H-5′), 4.09 (app. t, 1H, $J_{3',2'} = J_{3',4'}$ 9.4 Hz, H-3′), 4.01 (dd, 1H, $J_{8a,8b}$ 13.0 Hz, H-8a), 3.83 (ddd, 1H, $J_{4,3ax}$ 11.8, $J_{4,3eq}$ 4.6, $J_{4,5}$ 2.4 Hz, H-4), 3.81 (s, 3H, CO_2CH_3), 3.77 (s, 3H, PhOCH_3), 3.67 (dd, 1H, H-8b), 3.61 (d, 1H, H-6), 3.57–3.51 (m, 2H, H-2′, H-4′), 3.45–3.41 (m, 2H, H-6′a, H-6′b), 3.27 (s, 3H, OCH_3), 2.22 (dd, 1H, $J_{3eq,3ax}$ 12.5, H-3*eq*), 2.15 (app. t, 1H, H-3*ax*), 1.57–1.53 (m, 1H, O*H*), 1.04–0.78 (m, 28H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 168.79 (s, C=O), 159.14, 138.77, 138.72, 138.42, 130.22 (s, 5C, Ar), 129.05, 128.40, 128.29, 128.14, 127.98, 127.86, 127.73, 127.44, 127.09, 126.82, 113.77 (d, 19C, Ar), 99.15 (s, C-2), 98.09 (d, C-1′), 81.52 (d, C-3′), 80.24 (d, C-4′), 77.79 (d, C-2′), 75.12 (t, *C*H_2Ph), 74.84 (t, *C*H_2Ph), 73.22 (d, C-4), 72.52 (t, *C*H_2Ph), 71.86 (d, C-7), 71.57 (d, C-5), 71.37 (d, C-6), 70.73 (d, C-5′), 69.81 (t, *C*H_2Ph), 63.02 (t, C-8), 61.43 (t, C-6′), 55.23 (q, PhOCH_3), 52.38 (q, CO_2CH_3), 51.10 (q, OCH_3), 33.02 (t, C-3), 17.53, 17.50, 17.49, 17.46, 17.28, 17.17, 17.08 [q, 8C, Si-CH-(*C*H_3)$_2$], 14.17, 13.59, 12.98, 12.78 [d, 4C, Si-*C*H-(CH$_3$)$_2$]; ESI-TOF HRMS: *m*/*z* = 1078.5373; calcd for $C_{57}H_{80}O_{15}Si_2NH_4^+$: 1078.5374.

3.15. 2,3-Di-*O*-benzyl-α-D-*gluco*pyranosyl-(1→5)-methyl [methyl 3-deoxy-4-*O*-(4-methoxybenzyl)-7,8-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-*manno*-oct-2-ulopyranosid]onate (20)

A solution of **11** (59 mg, 0.056 mmol) and *p*-toluenesulfonic acid monohydrate (1 mg, 0.006 mmol) in dry MeOH (2.5 mL) was stirred at 40 °C for 2 h. The solution was allowed to cool to ambient temperature followed by treatment with solid $NaHCO_3$ (25 mg) for 2 min. After removal of the solvent, the residue was partitioned between EtOAc and aq $NaHCO_3$, the aqueous layer was washed with EtOAc (2 × 10 mL) and the combined organic phases were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (*n*-hexane/EtOAc 7:3) providing **20** (49 mg, 91%) as a colorless oil; $[\alpha]_D^{20}$ +86.0 (*c* 0.44, $CHCl_3$); R_f 0.70 (*n*-hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$): δ 7.38–7.35 (m, 2H, Ar), 7.34–7.22 (m, 10H, Ar), 6.87–6.84 (m, 2H, Ar), 5.27 (d, 1H, $J_{1',2'}$ 3.4 Hz, H-1′), 4.89 (d, 1H, *J* 11.9 Hz, C*H*HPh), 4.88 (d, 1H, *J* 11.8 Hz, C*H*HPh), 4.74 (d, 1H, *J* 11.3 Hz, CH*H*Ph), 4.71 (d, 1H, *J* 12.0 Hz, CH*H*Ph), 4.52 (s, 2H, CH_2Ph), 4.40–4.37 (m, 1H, H-7), 4.25–4.23 (m, 1H, H-5), 4.14 (app. td, 1H, $J_{5',4'}$ 9.9, $J_{5',6'a} = J_{5',6'b}$ 3.8 Hz, H-5′), 4.02 (dd, 1H, $J_{8a,8b}$ 13.1, $J_{8a,7}$ 1.9 Hz, H-8a), 3.91 (app. t, 1H, $J_{3',2'} = J_{3',4'}$ 9.5 Hz, H-3′), 3.86–3.82 (m, 4H, H-4, CO_2CH_3), 3.80 (s, 3H, PhOCH_3), 3.69 (dd, 1H, $J_{8b,7}$ 2.1 Hz, H-8b), 3.63 (d, 1H, $J_{6,7}$ 9.6 Hz, H-6), 3.57 (app. dt, 1H, $J_{4',OH}$ 2.8 Hz, H-4′), 3.53–3.45 (m, 3H, H-2′, H-6′a, H-6′b), 3.28 (s, 3H, OCH_3), 2.25–2.20 (m, 2H, H-3*eq*, O*H*), 2.15 (app. t, 1H, $J_{3ax,3eq} = J_{3ax,4}$ 12.1 Hz, H-3*ax*), 1.67–1.63 (m, 1H, O*H*), 1.05–0.79 (m, 28H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 168.84 (s, C=O), 159.20, 138.70, 138.51, 130.20 (s, 4C, Ar), 129.05, 128.50, 128.21, 127.99, 127.75, 127.22, 126.89, 113.81 (d, 14C, Ar), 99.18 (s, C-2), 98.06 (d, C-1′), 80.72 (d, C-3′), 79.87 (d, C-2′), 74.68 (t, *C*H_2Ph), 73.19 (d, C-4), 72.30 (t, *C*H_2Ph), 71.96 (d, C-7), 71.50 (d, C-5), 71.38 (d, C-6), 70.94 (d, C-4′), 70.76 (d, C-5′), 69.84 (t, *C*H_2Ph), 63.07 (t, C-8), 62.44 (t, C-6′), 55.26 (q, PhOCH_3), 52.40 (q, CO_2CH_3), 51.14 (q, OCH_3), 33.11 (t, C-3), 17.51, 17.48, 17.44, 17.27, 17.17, 17.08 [q, 8C, Si-CH-(*C*H_3)$_2$], 14.14, 13.61, 12.97, 12.78 [d, 4C,

Si-CH-$(CH_3)_2$]; ESI-TOF HRMS: m/z = 988.4917; calcd for $C_{50}H_{74}O_{15}Si_2NH_4^+$: 988.4905.

3.16. 2,3-Di-*O*-benzyl-6-*O*-(dibenzylphosphoryl)-α-D-*gluco*pyranosyl-(1→5)-methyl [methyl 3-deoxy-4-*O*-(4-methoxybenzyl)-7,8-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-*manno*-oct-2-ulopyranosid]onate (21) and 2,3-di-*O*-benzyl-4-*O*-(dibenzyl phosphoryl)-α-D-*gluco*pyranosyl-(1→5)-methyl [methyl 3-deoxy-4-*O*-(4-methoxybenzyl)-7,8-*O*-(1,1,3,3-tetra isopropyldisiloxane-1,3-diyl)-α-D-*manno*-oct-2-ulopyranosid] onate (22)

A solution of **20** (47.0 mg, 0.048 mmol) in dry CH_2Cl_2 (3.0 mL) was flushed with Ar before 1*H*-tetrazole (6.8 mg, 0.097 mmol) and 4 Å molecular sieves (50 mg) were added. The suspension was stirred for 30 min at ambient temperature and cooled to −5 °C. Dibenzyl *N*,*N*-diisopropylphosphoramidite (15.9 μL, 0.048 mmol) in dry CH_2Cl_2 (1.0 mL) was added dropwise at −5 °C. After 30 min another portion of dibenzyl *N*,*N*-diisopropylphosphoramidite (4.0 μL, 0.012 mmol) in dry CH_2Cl_2 (0.25 mL) was added at −5 °C and after 15 min the solution was treated with *m*-chloroperbenzoic acid (70 w%, 23.9 mg, 0.097 mmol) for 5 min. The reaction mixture was partitioned between aq $NaHCO_3$ and CH_2Cl_2, the aqueous phase was extracted with $CHCl_3$ (2 × 10 mL) and the combined organic phases were dried ($MgSO_4$), filtered, and concentrated. Purification by HP-column chromatography (*n*-hexane/EtOAc 3:1 → 3:2) provided **21** (39.6 mg, 67%) and **22** (5.8 mg, 10%) as colorless oils.

Data for **21**: $[\alpha]_D^{20}$ +96.7 (*c* 0.32, $CHCl_3$); R_f 0.19 (*n*-hexane/EtOAc 2:1, HPTLC); ^{1}H NMR ($CDCl_3$): δ 7.37–7.19 (m, 22H, Ar), 6.84–6.81 (d, 2H, Ar), 5.21 (d, 1H, $J_{1',2'}$ 3.6 Hz, H-1′), 5.03–4.89 (m, 6H, 2 × C*H*HPh, 2 × POC*H*HPh, 2 × POCH*H*Ph), 4.80 (d, 1H, *J* 11.2 Hz, CH*H*Ph), 4.64 (d, 1H, *J* 11.9 Hz, CH*H*Ph), 4.47 (d, 1H, *J* 11.4 Hz, C*H*HPh), 4.44 (d, 1H, *J* 11.4 Hz, CH*H*Ph), 4.42 (app. td, 1H, $J_{7,6}$ 9.8, $J_{7,8a} = J_{7,8b}$ 1.7 Hz, H-7), 4.22–4.20 (m, 1H, H-5), 4.17–4.14 (m, 1H, H-5′), 4.00–3.92 (m, 3H, H-3′, H-6′a, H-8a), 3.85–3.81 (m, 4H, H4, CO_2CH_3), 3.70 (s, 3H, PhOCH_3), 3.66–3.53 (m, 4H, H-4′, H-6′b, H-6, H-8b), 3.49 (dd, 1H, $J_{2',3'}$ 9.7 Hz, H-2′), 3.45–3.41 (m, 1H, O*H*), 3.28 (s, 3H, OCH_3), 2.23 (dd, 1H, $J_{3eq,3ax}$ 12.5, $J_{3eq,4}$ 4.1 Hz, H-3*eq*), 2.11 (app. t, 1H, $J_{3ax,4}$ 12.2 Hz, H-3*ax*), 1.02–0.72 (m, 28H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 168.79 (s, C═O), 159.20, 138.95, 138.77 (s, 3C, Ar), 135.72 (s, $J_{C,P}$ 6.7 Hz, Ar), 135.62 (s, $J_{C,P}$ 7.3 Hz, Ar), 130.14 (s, Ar), 129.22, 128.52, 128.49, 128.27, 128.07, 127.96, 127.93, 127.88, 127.44, 127.01, 126.81, 113.79 (d, 24C, Ar), 99.11 (s, C-2), 98.76 (d, C-1′), 80.47 (d, C-3′), 79.46 (d, C-2′), 74.89 (t, CH_2-Ph), 73.32 (d, C-4), 72.22 (t, CH_2Ph), 71.94 (d, C-5), 71.58 (d, C-7), 71.30 (d, C-6), 70.24 (d, $J_{C,P}$ 4.8 Hz, C-5′), 69.96 (t, CH_2Ph), 69.64 (d, C-4′), 69.47 (t, $J_{C,P}$ 5.4 Hz, POCH_2Ph), 69.32 (t, $J_{C,P}$ 5.8 Hz, POCH_2-Ph), 66.00 (t, $J_{C,P}$ 5.4 Hz, C-6′), 62.83 (t, C-8), 55.14 (q, PhOCH_3), 52.37 (q, CO_2CH_3), 51.10 (q, OCH_3), 33.02 (t, C-3), 17.54, 17.48, 17.44, 17.26, 17.14, 17.06 [q, 8C, Si-CH-(CH_3)$_2$], 14.16, 13.40, 12.98, 12.74 [d, 4C, Si-C*H*-$(CH_3)_2$]; ^{31}P NMR ($CDCl_3$): δ 0.81; ESI-TOF HRMS: m/z = 1253.5072; calcd for $C_{64}H_{87}O_{18}PSi_2Na^+$: 1253.5061.

Data for **22**: colorless oil; $[\alpha]_D^{20}$ +72.4 (*c* 0.53, $CHCl_3$); R_f 0.26 (*n*-hexane/EtOAc 2:1, HPTLC); ^{1}H NMR ($CDCl_3$): δ 7.35–7.08 (m, 22H, Ar), 6.84–6.81 (m, 2H, Ar), 5.30 (d, 1H, $J_{1',2'}$ 3.8 Hz, H-1′), 5.00 (dd, 1H, *J* 11.8, $J_{H,P}$ 7.9 Hz, POC*H*HPh), 4.94–4.87 (m, 3H, C*H*HPh, POCH_2Ph), 4.84 (dd, 1H, *J* 11.9, $J_{H,P}$ 9.8 Hz, POCH*H*Ph), 4.81 (d, 1H, *J* 12.2 Hz, C*H*HPh), 4.79 (d, 1H, *J* 11.9 Hz, CH*H*Ph), 4.65 (d, 1H, *J* 11.9 Hz, CH*H*Ph), 4.54 (d, 1H, *J* 11.9 Hz, C*H*HPh), 4.50 (d, 1H, *J* 11.8 Hz, CH*H*Ph), 4.44 (app. q, 1H, $J_{4',3'} = J_{4',5'} = J_{4',P}$ 9.5 Hz, H-4′), 4.37–4.34 (m, 1H, H-7), 4.27–4.25 (m, 1H, H-5), 4.24–4.20 (m, 1H, H-5′), 4.07 (app. t, 1H, $J_{3',2'}$ 9.5 Hz, H-3′), 3.97 (dd, 1H, $J_{8a,8b}$ 13.1, $J_{8a,7}$ 1.7 Hz, H-8a), 3.86–3.78 (m, 5H, CO_2CH_3, H-4, O*H*), 3.74 (s, 3H, PhOCH_3), 3.62–3.56 (m, 4H, H-2′, H-6′a, H-6, H-8b), 3.46–3.41 (m, 1H, H-6′b), 3.28 (s, 3H, OCH_3), 2.23 (dd, 1H, $J_{3eq,3ax}$ 12.4, $J_{3eq,4}$ 4.5 Hz, H-3*eq*), 2.14 (app. t, $J_{3ax,4}$ 12.2 Hz, H-3*ax*), 1.03–0.75 (m, 28H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 168.91 (s, C═O), 159.17, 138.38 (s, 3C, Ar), 135.65 (s, $J_{C,P}$ 6.4 Hz, Ar), 135.42 (s, $J_{C,P}$ 7.5 Hz, Ar), 130.08 (s, Ar), 128.93, 128.58, 128.55, 128.43, 128.39, 128.14, 128.10, 127.97, 127.87, 127.81, 127.32, 127.13, 126.77, 113.83 (d, 24C, Ar), 99.12 (s, C-2), 97.92 (d, C-1′), 79.85 (d, C-2′), 79.12 (d, $J_{C,P}$ 4.2 Hz, C-3′), 75.04 (d, $J_{C,P}$ 5.8 Hz, C-4′), 74.85 (t, CH_2Ph), 73.08 (d, C-4), 72.71 (t, CH_2Ph), 71.73 (d, C-7), 71.32 (d, C-5), 71.20 (d, C-6), 70.65 (d, $J_{C,P}$ 3.3 Hz, C-5′), 69.91, 69.86 (t, 2C, CH_2Ph, POCH_2Ph), 69.55 (t, $J_{C,P}$ 5.5 Hz, POCH_2Ph), 62.90 (t, C-8), 60.24 (t, C-6′), 55.19 (q, PhOCH_3), 52.37 (q, CO_2CH_3), 51.14 (q, OCH_3), 33.27 (t, C-3), 17.56, 17.49, 17.27, 17.15, 17.07 [q, 8C, Si-CH-(CH_3)$_2$], 14.19, 13.51, 12.98, 12.77 [d, 4C, Si-C*H*-$(CH_3)_2$]; ^{31}P NMR ($CDCl_3$): δ 0.67; ESI-TOF HRMS: m/z = 1253.5067; calcd for $C_{64}H_{87}O_{18}PSi_2Na^+$: 1253.5061.

3.17. 2,3-Di-*O*-benzyl-6-*O*-(diphenylphosphoryl)-α-D-*gluco* pyranosyl-(1→5)-methyl [methyl 3-deoxy-4-*O*-(4-methoxy benzyl)-7,8-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-*manno*-oct-2-ulopyranosid]onate (23)

A suspension of compound **20** (34.0 mg, 0.035 mmol) and 4-*N*,*N*-dimethylaminopyridine (8.6 mg, 0.070 mmol) in dry CH_2Cl_2 (1.0 mL) containing 4 Å molecular sieves (50 mg) was stirred for 30 min at 0 °C. Diphenyl phosphoryl chloride (7.6 μL, 0.037 mmol) dissolved in dry CH_2Cl_2 (0.1 mL) was added dropwise to the cold solution. After stirring for 20 min the reactive species was destroyed with dry MeOH (0.1 mL). The solution was stirred for 10 min, filtered over a pad of Celite®, rinsed with CH_2Cl_2, and concentrated. The residue was purified by chromatography (*n*-hexane/EtOAc 5:2) to provide **23** (40.6 mg, 96%) as a colorless oil; $[\alpha]_D^{20}$ +76.5 (*c* 0.81, $CHCl_3$); R_f 0.42 (*n*-hexane/EtOAc 3:2); ^{1}H NMR ($CDCl_3$): δ 7.36–7.21 (m, 16H, Ar), 7.19–7.11 (m, 6H, Ar), 6.85–6.82 (m, 2H, Ar), 5.21 (d, 1H, $J_{1',2'}$ 3.7 Hz, H-1′), 4.92 (d, 1H, *J* 11.9 Hz, C*H*HPh), 4.82 (d, 1H, *J* 11.2 Hz, C*H*HPh), 4.77 (d, 1H, *J* 11.3 Hz, CH*H*Ph), 4.64 (d, 1H, *J* 12.0 Hz, CH*H*Ph), 4.49 (d, 1H, *J* 11.2 Hz, C*H*HPh), 4.46 (d, 1H, *J* 11.2 Hz, CH*H*Ph), 4.42–4.39 (m, 1H, H-7), 4.22 (d, 1H, $J_{5,4}$ 2.3 Hz, H-5), 4.20–4.16 (m, 1H, H-5′), 4.13–4.08 (m, 1H, H-6′a), 3.99 (dd, 1H, $J_{8a,8b}$ 13.1, $J_{8a,7}$ 1.6 Hz, H-8a), 3.91 (app. t, 1H, $J_{3',2'} = J_{3',4'}$ 9.4 Hz, H-3′), 3.85 (ddd, 1H, $J_{4,3ax}$ 11.9, $J_{4,3eq}$ 4.5, H-4), 3.83 (s, 3H, CO_2CH_3), 3.76–3.71 (m, 1H, H-6′b), 3.70 (s, 3H, PhOCH_3), 3.64–3.61 (m, 2H, H-6, H-8b), 3.52 (app. t, 1H, $J_{4',5'}$ 9.7 Hz, H-4′), 3.39 (dd, 1H, H-2′), 3.29 (s, 3H, OCH_3), 2.25 (dd, 1H, $J_{3eq,3ax}$ 12.4 Hz, H-3*eq*), 2.11 (app. t, 1H, H-3*ax*), 1.04–0.75 (m, 28 H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 168.78 (s, C═O), 159.24 (s, Ar), 150.51 (s, $J_{C,P}$ 7.0 Hz, Ar), 150.38 (s, $J_{C,P}$ 7.3 Hz, Ar), 138.93, 138.73, 130.11 (s, 3C, Ar), 129.68, 129.21, 128.28, 128.09, 127.84, 127.46, 127.04, 126.82, 125.43, 125.29 (d, 18C, Ar), 120.25 (d, $J_{C,P}$ 4.6 Hz, 2C, Ar), 120.05 (d, $J_{C,P}$ 5.2 Hz, 2C, Ar), 113.82 (d, 2C, Ar), 99.12 (s, C-2), 98.59 (d, C-1′), 80.30 (d, C-3′), 79.36 (d, C-2′), 74.58 (t, CH_2Ph), 73.55 (d, C-4), 72.11 (t, CH_2Ph), 71.86 (d, C-5), 71.64 (d, C-7), 71.30 (d, C-6), 70.09 (t, CH_2Ph), 70.03 (d, $J_{C,P}$ 4.9 Hz, C-5′), 69.30 (d, C-4′), 67.25 (t, $J_{C,P}$ 5.9 Hz, C-6′), 62.87 (t, C-8), 55.13 (q, PhOCH_3), 52.37 (q, CO_2CH_3), 51.12 (q, OCH_3), 33.00 (t, C-3), 17.54, 17.50, 17.48, 17.46, 17.27, 17.16, 17.07 [q, 8C, Si-CH-(CH_3)$_2$], 14.17, 13.47, 12.99, 12.76 [d, 4C, Si-C*H*-$(CH_3)_2$]; ^{31}P NMR ($CDCl_3$): δ—10.34; ESI-TOF HRMS: m/z = 1225.4760; calcd for $C_{62}H_{83}O_{18}PSi_2Na^+$: 1225.4748.

3.18. 4-*O*-Acetyl-2,3-di-*O*-benzyl-6-*O*-(diphenylphosphoryl)-α-D-*gluco*pyranosyl-(1→5)-methyl [methyl 3-deoxy-4-*O*-(4-methoxybenzyl)-7,8-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-*manno*-oct-2-ulopyranosid]onate (24)

Distilled acetic anhydride (9.4 μL, 0.100 mmol) and 4-*N*,*N*-dimethylaminopyridine (0.3 mg, 0.003 mmol) were added

successively at 0 °C to a solution of **23** (30.0 mg, 0.025 mmol) in dry pyridine (1.0 mL). After stirring for 2 h at 0 °C the reaction was quenched with dry MeOH (100 μL). Concentration of the solution and coevaporation with toluene gave a residue which was purified by chromatography (*n*-hexane/EtOAc 2:1) to furnish **24** (29.8 mg, 96%) as a colorless oil; $[\alpha]_D^{20}$ +72.8 (*c* 0.47, $CHCl_3$); R_f 0.25 (*n*-hexane/EtOAc 2:1); ^{1}H NMR ($CDCl_3$): δ 7.35–7.12 (m, 22H, Ar), 6.82–6.79 (m, 2H, Ar), 5.26 (d, 1H, $J_{1',2'}$ 3.7 Hz, H-1′), 5.07 (app. t, 1H, $J_{4',3'} = J_{4',5'}$ 9.9 Hz, H-4′), 4.90 (d, 1H, *J* 11.7 Hz, C*H*HPh), 4.73 (d, 1H, *J* 11.3 Hz, C*H*HPh), 4.64 (d, 1H, *J* 11.8 Hz, CH*H*Ph), 4.60 (d, 1H, *J* 11.3 Hz, CH*H*Ph), 4.50 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.47 (d, 1H, *J* 11.5 Hz, CH*H*Ph), 4.42–4.38 (m, 1H, H-5′), 4.38–4.34 (m, 1H, H-7), 4.25 (d, 1H, $J_{5,4}$ 2.2 Hz, H-5), 4.03 (app. t, 1H, $J_{3',2'}$ 9.7 Hz, H-3′), 4.01 (dd, 1H, $J_{8a,8b}$ 13.0, $J_{8a,7}$ 1.6 Hz, H-8a), 3.97 (ddd, 1H, $J_{6'a,6'b}$ 11.3, $J_{6'a,P}$ 5.4, $J_{6'a,5'}$ 2.3 Hz, H-6′a), 3.88–3.83 (m, 4H, CO_2CH_3, H-4), 3.74–3.69 (m, 4H, H-6′b, $PhOCH_3$), 3.67 (dd, 1H, $J_{8b,7}$ 1.9 Hz, H-8b), 3.64 (d, 1H, $J_{6,7}$ 9.4 Hz, H-6), 3.50 (dd, 1H, H-2′), 3.29 (s, 3H, OCH_3), 2.27 (dd, 1H, $J_{3eq,3ax}$ 12.5, $J_{3eq,4}$ 4.6 Hz, H-3*eq*), 2.13 (app. t, 1H, $J_{3ax,4}$ 12.2 Hz, H-3*ax*), 1.86 (s, 3H, $COCH_3$), 1.03–0.78 (m, 28H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 169.18 (s, $COCH_3$), 168.94 (s, C-1), 159.23 (s, Ar), 150.70 (s, $J_{C,P}$ 7.2 Hz, Ar), 150.54 (s, $J_{C,P}$ 7.4 Hz, Ar), 138.51, 138.34, 129.97 (s, 3C, Ar), 129.62, 129.57, 129.28, 128.27, 128.16, 128.05, 127.53, 127.16, 126.76, 125.11, 125.02 (d, 18C, Ar), 120.21 (d, $J_{C,P}$ 5.1 Hz, 2C, Ar), 120.11 (d, $J_{C,P}$ 5.1 Hz, 2C, Ar), 113.82 (d, 2C, Ar), 99.15 (s, C-2), 97.84 (d, C-1′), 79.60 (d, C-2′), 78.36 (d, C-3′), 74.19 (t, CH_2Ph), 72.99 (d, C-4), 72.59 (t, CH_2Ph), 71.73 (d, C-7), 71.19 (d, C-5), 71.17 (d, C-6), 69.95 (t, CH_2Ph), 69.25 (d, C-4′), 67.91 (d, $J_{C,P}$ 8.1 Hz, C-5′), 66.64 (t, $J_{C,P}$ 5.5 Hz, C-6′), 62.91 (t, C-8), 55.18 (q, $PhOCH_3$), 52.42 (q, CO_2CH_3), 51.16 (q, OCH_3), 33.11 (t, C-3), 20.83 (q, $COCH_3$), 17.52, 17.48, 17.45, 17.26, 17.15, 17.07 [q, 8C, Si-CH-$(CH_3)_2$], 14.21, 13.55, 12.96, 12.79 [d, 4C, Si-CH-$(CH_3)_2$]; ^{31}P NMR ($CDCl_3$): δ–12.14; ESI-TOF HRMS: *m*/*z* = 1262.5295; calcd for $C_{64}H_{85}O_{19}PSi_2NH_4^+$: 1262.5299.

3.19. 2,3-Di-*O*-benzyl-6-*O*-(dibenzylphosphoryl)-α-D-*gluco*pyranosyl-(1→5)-methyl [methyl 3-deoxy-7,8-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-*manno*-oct-2-ulopyranosid]onate (25)

A solution of dibenzyl *N*,*N*-diisopropylphosphoramidite (22.0 μL, 0.066 mmol) in dry CH_2Cl_2 (1.0 mL) was added under Ar in two portions to an ice-cold solution of **14** (28.4 mg, 0.033 mmol) and 1*H*-tetrazole (7.9 mg, 0.113 mmol) in dry CH_2Cl_2 (2.1 mL) and stirred for 30 min at 0 °C. *m*-Chloroperbenzoic acid (70 w%, 28.8 mg, 0.117 mmol) was added, and the solution was stirred for 15 min. The solution was diluted with CH_2Cl_2 and extracted with aq $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL) and the combined organic phases were dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (1:1 *n*-hexane/EtOAc containing 0.1% MeOH and 0.1% Et_3N) to give **25** (23.4 mg, 63%) as a colorless oil: $[\alpha]_D^{20}$ +56.0 (*c* 0.72, $CHCl_3$); R_f 0.49 (*n*-hexane/EtOAc 1:2); ^{1}H NMR ($CDCl_3$): δ 7.35–7.26 (m, 20H, Ar), 5.10 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.04–4.96 (m, 4H, 2 × $POCH_2Ph$), 4.87 (d, 1H, *J* 11.4 Hz, C*H*HPh), 4.80 (d, 1H, *J* 12.2 Hz, C*H*HPh), 4.78 (d, 1H, *J* 11.5 Hz, CH*H*Ph), 4.74 (d, 1H, *J* 12.4 Hz, CH*H*Ph), 4.45–4.42 (m, 1H, H-7), 4.25–4.20 (m, 1H, H-6′a), 4.16–4.04 (m, 5H, H-5′, H-6′b, H-4, H-5, H-8a), 3.96 (dd, 1H, $J_{8b,8a}$ 12.8, $J_{8b,7}$ 1.9 Hz, H-8b), 3.87 (app. t, 1H, $J_{3',2'} = J_{3',4'}$ 9.0 Hz, H-3′), 3.79 (s, 3H, CO_2CH_3), 3.75 (d, 1H, $J_{6,7}$ 8.3 Hz, H-6), 3.50–3.45 (m, 2H, H-2′, H-4′), 3.31 (s, 3H, OCH_3), 2.98 (bs, 1H, O*H*), 2.14 (dd, 1H, $J_{3eq,3ax}$ 12.8, $J_{3eq,4}$ 4.9 Hz, H-3*eq*), 1.87 (app. t, $J_{3ax,4}$ 12.3 Hz, H-3*ax*), 1.04–0.80 (m, 28 H, TIPDS); ^{13}C NMR ($CDCl_3$): δ 168.63 (s, C═O), 138.56, 138.23, (s, 2C, Ar), 135.66 (s, $J_{C,P}$ 6.5 Hz, 2C, Ar), 128.58, 128.55, 128.48, 128.35, 128.02, 127.99, 127.97, 127.78, 127.56, 127.32 (d, 20C, Ar), 99.04 (s, C-2), 99.01 (d, C-1′), 80.05 (d, C-3′), 78.41 (d, C-2′), 77.30 (d, C-5), 74.93 (t, CH_2Ph), 72.76 (d, C-7), 72.49 (t, CH_2Ph), 71.69, 71.63 (d, 2C, C-5′, C-6), 69.60 (t, $J_{C,P}$ 6.1 Hz, $POCH_2Ph$), 69.56 (t, $J_{C,P}$ 7.0 Hz, $POCH_2Ph$), 69.50 (d, C-4′), 66.61 (t, $J_{C,P}$ 7.7 Hz, C-6′), 66.54 (d, C-4), 63.62 (t, C-8), 52.39 (q, CO_2CH_3), 51.18 (q, OCH_3), 35.93 (t, C-3), 17.57, 17.53, 17.50, 17.47, 17.31, 17.20, 17.12 [q, 8C, Si-CH-$(CH_3)_2$], 14.03, 13.52, 12.95, 12.78 [d, 4C, Si-CH-$(CH_3)_2$]; ^{31}P NMR ($CDCl_3$): δ–0.08; ESI-TOF HRMS: *m*/*z* = 1133.4487; calcd for $C_{56}H_{79}O_{17}PSi_2Na^+$: 1133.4486.

Alternatively, a solution of **21** (39.9 mg, 0.032 mmol) in CH_2Cl_2 (2.0 mL) was treated with trifluoroacetic acid (99%, 0.25 mL) at 0 °C for 10 min. Dilution with toluene and azeotropic distillation afforded a crude product which was immediately purified by chromatography (*n*-hexane/EtOAc 1:1) providing **25** (31.8 mg, 88%) as a colorless oil.

3.20. 2,3-Di-*O*-benzyl-6-*O*-(dibenzylphosphoryl)-α-D-*gluco* pyranosyl-(1→5)-methyl (methyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate (26)

A solution of **25** (6.4 mg, 0.006 mmol) in dry THF (1.2 mL) was treated with TBAF (1 M in THF, 9 μL, 0.009 mmol) at ambient temperature for 15 min. Addition of dry MeOH (1 mL) and concentration provided a crude product which was purified by chromatography (EtOAc) yielding **26** (4.6 mg, 92%) as a colorless oil; $[\alpha]_D^{20}$ +96.0 (*c* 0.37, MeOH); R_f 0.34 (EtOAc); ^{1}H NMR (MeOD): δ 7.40–7.25 (m, 20H, Ar), 5.18 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.07–5.04 (m, 4H, 2 × $POCH_2Ph$), 4.91 (d, 1H, *J* 11.2 Hz, C*H*HPh), 4.84–4.82 (m, 1H, CH*H*Ph), 4.75 (d, 1H, *J* 11.6 Hz, C*H*HPh), 4.72 (d, 1H, *J* 11.7 Hz, CH*H*Ph), 4.30–4.27 (m, 1H, H-6′a), 4.26–4.18 (m, 2H, H-5′, H-6′b), 4.10–4.02 (m, 3H, H-4, H-5, H-7), 3.86 (dd, 1H, $J_{3',2'}$ 9.6, $J_{3',4'}$ 9.0 Hz, H-3′), 3.74 (s, 3H, CO_2CH_3), 3.70 (dd, 1H, $J_{8a,8b}$ 11.2, $J_{8a,7}$ 2.9 Hz, H-8a), 3.67 (dd, 1H, $J_{6,7}$ 9.3, $J_{6,5}$ 1.1 Hz, H-6), 3.64 (dd, 1H, $J_{8b,7}$ 4.4 Hz, H-8b), 3.51 (app. t, 1H, $J_{4',5'}$ 9.3 Hz, H-4′), 3.43 (dd, 1H, H-2′), 3.21 (s, 3H, OCH_3), 2.06–2.01 (m, 1H, H-3*eq*), 1.96 (app. t, 1H, $J_{3ax,3eq} = J_{3ax,4}$ 12.3 Hz, H-3*ax*); ^{13}C NMR (MeOD): δ 170.39 (s, C═O), 140.30, 139.34 (s, 2C, Ar), 135.82 (s, $J_{C,P}$ 6.6 Hz, Ar), 135.81 (s, $J_{C,P}$ 6.6 Hz, Ar), 129.67, 129.66, 129.43, 129.38, 129.24, 129.17, 129.09, 129.07, 128.87, 128.52 (d, 20C, Ar), 100.44 (s, C-2), 100.10 (d, C-1′), 82.37 (d, C-3′), 81.07 (d, C-2′), 77.55 (d, C-5), 76.26 (t, CH_2Ph), 74.63 (t, CH_2Ph), 73.18 (d, C-6), 72.47 (d, $J_{C,P}$ 7.3 Hz, C-5′), 71.18 (d, C-4′), 70.82 (t, $J_{C,P}$ 5.9 Hz, 2C, 2 × $POCH_2Ph$), 69.98 (d, C-7), 68.23 (t, $J_{C,P}$ 5.5 Hz, C-6′), 67.25 (d, C-4), 64.13 (t, C-8), 52.94 (q, CO_2CH_3), 51.55 (q, OCH_3), 36.47 (t, C-3); ^{31}P NMR (MeOD): δ–1.20; ESI-TOF HRMS: *m*/*z* = 891.2966; calcd for $C_{44}H_{53}O_{16}PNa^+$: 891.2963.

3.21. 6-*O*-Phosphono-α-D-*gluco*pyranosyl-(1→5)-methyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosidonic acid (sodium salt) (27)

A solution of **26** (8.2 mg, 0.009 mmol) in dry MeOH (1.0 mL) was hydrogenated for 1 h in the presence of 10% Pd–C (1 mg) as described for **13.** The suspension was diluted with MeOH and passed through a 0.45 μm syringe filter. The filtrate was made neutral by adding 0.1 M NaOMe (0.1 M in MeOH, 200 μL). Concentration of the filtrate afforded the debenzylated methyl ester which was saponified with 0.01 M aq NaOH (1.5 mL) at ambient temperature for 1 h. The solution was made neutral by addition of DOWEX 50 H^+ resin. The ion exchange resin was filtered off and the filtrate was lyophilized. Purification of the residue on a Bio-Gel PD10 column (H_2O) and freeze-drying of pooled fractions provided **27** (5.2 mg, 98%) as a colorless amorphous solid; $[\alpha]_D^{20}$ +97.5 (*c* 0.49, H_2O); ^{1}H NMR (D_2O): δ 5.11 (d, 1H, $J_{1',2'}$ 4.1 Hz, H-1′), 4.15–4.06 (m, 3H, H-4, H-7, H-5′), 4.05–4.00 (m, 2H, H-5, H-6′a), 3.90 (dd, 1H, $J_{8a,8b}$ 11.8, $J_{8a,7}$ 2.8 Hz, H-8a), 3.78–3.68 (ddd, 1H, $J_{6'b,6'a}$ 12.1, *J*

4.7, J 1.9 Hz, H-6′b), 3.73 (app. t, 1H, $J_{3',2'} = J_{3',4'}$ 9.6 Hz, H-3′), 3.62 (dd, 1H, $J_{8b,7}$ 6.1 Hz, H-8b), 3.59 (app. t, 1H, $J_{4',5'}$ 9.7 Hz, H-4′), 3.55–3.52 (m, 2H, H-6, H-2′), 3.11 (s, 3H, OCH_3), 1.96 (ddd, 1H, $J_{3eq,3ax}$ 12.9, $J_{3eq,4}$ 4.9, $J_{3eq,5}$ 0.7 Hz, H-3*eq*), 1.82 (app. t, 1H, $J_{3ax,4}$ 12.5 Hz, H-3*ax*); ^{13}C NMR data: see Table 1; ^{31}P NMR (D_2O): δ 4.65; ESI-TOF HRMS: m/z = 493.0966; calcd for $C_{15}H_{26}O_{16}P^-$: 493.0964.

3.22. 4-*O*-Acetyl-2,3-di-*O*-benzyl-6-*O*-(diphenylphosphoryl)-α-D-*gluco*pyranosyl-(1→5)-methyl [methyl 3-deoxy-4-*O*-(4-methoxybenzyl)-α-D-*manno*-oct-2-ulopyranosid]onate (28)

A solution of **24** (17.9 mg, 0.014 mmol) in dry CH_2Cl_2 was treated with triethylamine trihydrofluoride (117 μL, 0.719 mmol) for 4 h at ambient temperature. Another portion of triethylamine trihydrofluoride (117 μL, 0.719 mmol) was added and stirring was continued for 12 h. The colorless solution was added dropwise into ice-cold aq $NaHCO_3$ and extracted with CH_2Cl_2 (4 × 10 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The residue was purified by chromatography (toluene/EtOAc 1:1) which afforded **28** (11.8 mg, 82%) as a colorless oil; $[\alpha]_D^{20}$ +51.6 (*c* 1.13, $CHCl_3$); R_f 0.24 (toluene/EtOAc 1:1); ^{1}H NMR ($CDCl_3$, ref. to TMS at 0.00 ppm): δ 7.37–7.10 (m, 22H, Ar), 6.84–6.80 (m, 2H, Ar), 5.05 (app. t, 1H, $J_{4',3'} = J_{4',5'}$ 9.5 Hz, H-4′), 5.04 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1′), 4.83 (d, 1H, J 11.8 Hz, C*H*HPh), 4.82 (d, 1H, J 11.5 Hz, C*H*HPh), 4.69 (d, 1H, J 11.5 Hz, CH*H*Ph), 4.61 (d, 1H, J 11.7 Hz, CH*H*Ph), 4.44 (d, 1H, J 11.9 Hz, C*H*HPh), 4.37 (d, 1H, J 11.8 Hz, CH*H*Ph), 4.19–4.15 (m, 2H, H-5′, H-6′a), 4.12 (bs, 1H, H-5), 4.09–4.03 (m, 2H, H-6′b, H-7), 4.00 (app. t, 1H, $J_{3',2'}$ 9.5 Hz, H-3′), 3.85 (ddd, 1H, $J_{4,3ax}$ 11.9, $J_{4,3eq}$ 4.5, $J_{4,5}$ 2.4 Hz, H-4), 3.79 (dd, 1H, $J_{8a,8b}$ 11.1, $J_{8a,7}$ 3.4 Hz, H-8a), 3.76 (s, 3H, CO_2CH_3), 3.73 (s, 3H, PhOCH_3), 3.63 (dd, 1H, $J_{8b,7}$ 4.9 Hz, H-8b), 3.59 (d, 1H, $J_{6,7}$ 9.2 Hz, H-6), 3.52 (dd, 1H, H-2′), 3.18 (s, 3H, OCH_3), 2.19 (dd, 1H, $J_{3eq,3ax}$ 12.7 Hz, H-3*eq*), 1.98 (app. t, 1H, H-3*ax*), 1.92 (s, 3H, COCH_3); ^{13}C NMR ($CDCl_3$): δ 169.44 (s, *C*OCH$_3$), 168.46 (s, C-1), 159.13 (s, Ar), 150.57 (s, $J_{C,P}$ 6.9 Hz, Ar), 150.45 (s, $J_{C,P}$ 6.5 Hz, Ar), 138.04, 136.99, 130.15 (s, 3C, Ar), 129.69, 129.64, 128.85, 128.75, 128.52, 128.49, 128.01, 127.83, 125.22, 125.14 (d, 18C, Ar), 120.16 (d, $J_{C,P}$ 4.6 Hz, 2C, Ar), 120.10 (d, $J_{C,P}$ 4.9 Hz, 2C, Ar), 113.85 (d, 2C, Ar), 99.15 (s, C-2), 98.49 (d, C-1′), 80.60 (d, C-2′), 79.04 (d, C-3′), 75.20 (t, *C*H$_2$Ph), 74.74 (t, *C*H$_2$Ph), 73.98 (d, C-5), 72.95 (d, C-4), 72.39 (d, C-6), 69.89 (t, *C*H$_2$Ph), 69.82 (d, C-4′), 69.02 (d, $J_{C,P}$ 8.3 Hz, C-5′), 68.59 (d, C-7), 67.07 (t, $J_{C,P}$ 5.5 Hz, C-6′), 63.96 (t, C-8), 55.19 (q, PhO*C*H$_3$), 52.55 (q, CO_2CH_3), 51.01 (q, O*C*H$_3$), 32.66 (t, C-3), 20.84 (q, CO*C*H$_3$); ^{31}P NMR ($CDCl_3$): δ–11.95; ESI-TOF HRMS: m/z = 1020.3773; calcd for $C_{52}H_{59}O_{18}PNH_4^+$: 1020.3777.

3.23. 4-*O*-Acetyl-2,3-di-*O*-benzyl-6-*O*-(diphenylphosphoryl)-α-D-*gluco*pyranosyl-(1→5)-methyl [methyl 3-deoxy-4-*O*-(4-methoxybenzyl)-α-D-*lyxo*-hept-2-ulopyranosid]onate (29)

A solution of **28** (27.0 mg, 0.027 mmol) in dry CH_2Cl_2 (3.5 mL) was treated with sodium periodinate on silica (15 w%, 77 mg, 0.054 mmol) for 1.5 h at −10 °C under light protection. Excessive reagent was destroyed with ethylene glycol (3 w% in water, 56 μL, 0.027 mmol). The mixture was diluted with $CHCl_3$ and water, the phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were dried ($MgSO_4$), filtered, and concentrated. The residue was treated with sodium borohydride (3.1 mg, 0.081 mmol) in dry MeOH (3.5 mL) at −5 °C for 30 min. The mixture was partitioned between EtOAc and aq NH_4Cl, the aqueous phase was extracted with EtOAc (3 × 5 mL) and the combined organic phases were dried ($MgSO_4$). Filtration and concentration afforded a crude product which was purified by HP-column chromatography (toluene/EtOAc 2:1) providing **29** (9.5 mg, 36%) as a colorless oil: $[\alpha]_D^{20}$ +48.0 (*c* 0.42, $CHCl_3$); R_f 0.32 (toluene/EtOAc 1:1); ^{1}H NMR ($CDCl_3$, ref. to TMS at 0.00 ppm): δ 7.36–7.19 (m, 16H, Ar), 7.18–7.10 (m, 6H, Ar), 6.84–6.81 (m, 2H, Ar), 5.03 (app. t, 1H, $J_{4',3'} = J_{4',5'}$ 9.8 Hz, H-4′), 4.98 (d, 1H, $J_{1',2'}$ 3.4 Hz, H-1′), 4.83 (d, 1H, J 11.5 Hz, C*H*HPh), 4.79 (d, 1H, J 11.5 Hz, C*H*HPh), 4.66 (d, 1H, J 11.5 Hz, CH*H*Ph), 4.61 (d, 1H, J 11.8 Hz, CH*H*Ph), 4.47 (d, 1H, J 11.8 Hz, C*H*HPh), 4.38 (d, 1H, J 11.6 Hz, CH*H*Ph), 4.22–4.18 (m, 1H, H-5′), 4.15 (ddd, $J_{6'a,6'b}$ 11.1, $J_{6'a,P}$ 6.1, $J_{6'a,5'}$ 2.4 Hz, H-6′a), 4.03–3.96 (m, 3H, H-3′, H-6′b, H-5), 3.90–3.84 (m, 2H, H-4, H-7a), 3.82–3.76 (m, 4H, H-7b, CO_2CH_3), 3.74–3.70 (m, 4H, H-6, PhOCH_3), 3.49 (dd, 1H, $J_{2',3'}$ 9.6, H-2′), 3.20 (s, 3H, OCH_3), 2.99 (bs, 1H, O*H*), 2.23 (dd, 1H, $J_{3eq,3ax}$ 12.7, $J_{3eq,4}$ 4.3 Hz, H-3*eq*), 2.02 (app. t, 1H, $J_{3ax,4}$ 12.2 Hz, H-3*ax*), 1.90 (s, 3H, COCH_3); ^{13}C NMR ($CDCl_3$): δ 169.41 (s, *C*OCH$_3$), 168.56 (s, C-1), 159.16 (s, Ar), 150.60 (s, $J_{C,P}$ 7.5 Hz, Ar), 150.47 (s, $J_{C,P}$ 6.6 Hz, Ar), 138.15, 137.11, 130.14 (s, 3C, Ar), 129.68, 129.63, 128.92, 128.69, 128.47, 128.45, 128.40, 127.95, 127.75, 125.20, 125.12 (d, 18C, Ar), 120.15 (d, 2C, $J_{C,P}$ 5.1 Hz, Ar), 120.09 (d, 2C, $J_{C,P}$ 5.3 Hz, Ar), 113.85 (d, 2C, Ar), 99.15 (s, C-2), 98.22 (d, C-1′), 80.54 (d, C-2′), 78.95 (d, C-3′), 75.20 (t, *C*H$_2$Ph), 74.56 (t, *C*H$_2$Ph), 73.59 (d, C-5), 73.09 (d, C-4), 72.70 (d, C-6), 69.99 (t, *C*H$_2$Ph), 69.66 (d, C-4′), 68.97 (d, $J_{C,P}$ 7.8 Hz, C-5′), 67.04 (t, $J_{C,P}$ 5.5 Hz, C-6′), 60.68 (t, C-7), 55.19 (q, PhO*C*H$_3$), 52.60 (q, CO_2CH_3), 51.01 (q, OCH$_3$), 32.71 (t, C-3), 20.81 (q, COCH$_3$); ^{31}P NMR ($CDCl_3$): δ −11.96; ESI-TOF HRMS m/z: 990.3668; calcd for $C_{51}H_{57}O_{17}PNH_4^+$: 990.3672.

3.24. 6-*O*-Phosphono-α-D-*gluco*pyranosyl-(1→5)-(methyl 3-deoxy-α-D-*lyxo*-hept-2-ulopyranosid)onic acid (sodium salt) (30)

A suspension of compound **29** (8.0 mg, 0.008 mmol) in dry MeOH was hydrogenated for 36 h and processed as described for **13**. The solution obtained upon removal of the catalyst was concentrated. The residue was dried and dissolved in dry MeOH (1.0 mL). PtO_2 (1 mg) was added under an Ar atmosphere and hydrogenation was continued for 4 h at rt. The suspension was diluted with MeOH and passed through a 0.45 μm syringe filter. The filtrate was concentrated, and the residue was stirred with 0.01 M aq. NaOH (3.0 mL) at ambient temperature for 3 h. The solution was neutralized by addition of DOWEX 50 H^+ resin, the ion-exchange resin was filtered off and the filtrate was lyophilized. Purification of the residue by HILIC (SeQuant ZIC®-HILIC, 5 μm, 250 × 10 mm pre-packed from Merck, 5:1 → 2:3 acetonitrile/water) followed by desalting on a PD10 column (H_2O) afforded **30** (2.2 mg, 52%) as an amorphous colorless solid: $[\alpha]_D^{20}$ +69.2 (*c* 0.39, D_2O); ^{1}H NMR (D_2O): δ 4.88 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1′), 4.19–4.15 (m, 1H, H-5′), 4.12 (ddd, 1H, $J_{4,3ax}$ 12.3, $J_{4,3eq}$ 4.8, $J_{4,5}$ 3.0 Hz, H-4), 4.05 (ddd, 1H, $J_{6'a,6'b}$ 11.6, J 6.2, J 3.2 Hz, H-6′a), 3.96 (dd, 1H, $J_{7a,7b}$ 11.5, $J_{7a,6}$ 8.3 Hz, H-7a), 3.89–3.85 (m, 2H, H-5, H-6′b), 3.81 (dd, 1H, $J_{7b,6}$ 4.4 Hz, H-7b), 3.75 (br dd, 1H, H-6), 3.72 (app. t, 1H, $J_{3',2'} = J_{3',4'}$ 9.7 Hz, H-3′), 3.53 (app. t, 1H, $J_{4',5'}$ 9.7 Hz, H-4′), 3.51 (dd, 1H, H-2′), 3.14 (s, 3H, OCH_3), 2.01 (dd, 1H, $J_{3eq,3ax}$ 13.0, H-3*eq*), 1.83 (app. t, 1H, H-3*ax*); ^{13}C NMR data: see Table 1; ^{31}P NMR (D_2O): δ 2.40; ESI-TOF HRMS: m/z = 463.0857; calcd for $C_{14}H_{24}O_{15}P^-$: 463.0858.

3.25. 2,3-Di-*O*-benzyl-6-*O*-(dibenzylphosphoryl)-α-D-*gluco*pyranosyl-(1→5)-methyl (methyl 3-deoxy-α-D-*lyxo*-hept-2-ulopyranosid)onate (31)

Sodium *meta*periodinate on silica (15 w%, 70 mg, 0.049 mmol) was added to a solution of **26** (21.4 mg, 0.025 mmol) in dry CH_2Cl_2 (3.0 mL) at 0 °C under light protection. The suspension was allowed to warm up to ambient temperature during 1.5 h. Excessive reagent was destroyed by addition of aq ethylene glycol (3 w%, 51 μL, 0.025 mmol). The product was partitioned between

$CHCl_3$ and brine, the aqueous phase extracted with CH_2Cl_2 (2×5 mL), and the combined organic layers were dried ($MgSO_4$) and concentrated. The crude aldehyde was dissolved in dry MeOH (3.0 mL) and treated with sodium borohydride (2.8 mg, 0.074 mmol) at 0 °C for 10 min. The solution was extracted with CH_2Cl_2 (3×10 mL) and aq NH_4Cl, and the organic phase was dried ($MgSO_4$), and concentrated. The crude material was purified by HP-column chromatography (*n*-hexane/EtOAc 1:4 → 0:1) to yield **31** (9.6 mg, 47%) as a colorless oil; $[\alpha]_D^{20}$ +29.2 (*c* 0.99, $CHCl_3$); R_f 0.59 (EtOAc); ^{1}H NMR ($CDCl_3$): δ 7.42–7.28 (m, 20H, Ar), 5.03–4.95 (m, 4H, $2 \times$ POC*H*$_2$Ph), 4.93 (d, 1H, *J* 11.6 Hz, C*H*HPh), 4.88 (d, 1H, *J* 11.3 Hz, CH*H*Ph), 4.85 (d, 1H, *J* 11.7 Hz, C*H*HPh), 4.81 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1′), 4.65 (d, 1H, *J* 11.9 Hz, CH*H*Ph), 4.23 (ddd, 1H, $J_{6'a,6'b}$ 11.8, *J* 8.1, *J* 5.0 Hz, H-6′a), 4.09 (ddd, 1H, *J* 9.2, *J* 2.1 Hz, H-6′b), 4.06–4.00 (m, 1H, H-4), 3.94–3.76 (m, 9H, H-3′, H-5′, H-5, H-6, H-7a, H-7b, CO_2CH_3), 3.52–3.36 (m, 4H, H-2′, H-4′, $2 \times$ O*H*), 3.21 (s, 3H, OC*H*$_3$), 3.21–3.18 (m, 1H, O*H*), 2.19 (ddd, 1H, $J_{3eq,3ax}$ 12.5, $J_{3eq,4}$ 4.6, $J_{3eq,5}$ 0.6 Hz, H-3*eq*), 1.72–1.62 (m, 1H, H-3*ax*); ^{13}C NMR ($CDCl_3$): δ 168.34 (s, C=O), 138.35, 136.76 (s, 2C, Ar), 135.53 (s, $J_{C,P}$ 6.5 Hz, Ar), 135.51 (s, $J_{C,P}$ 6.8 Hz, Ar), 128.85, 128.67, 128.63, 128.60, 128.52, 128.03, 128.02, 127.99 (d, 20C, Ar), 100.37 (d, C-1′), 99.02 (s, C-2), 80.50 (d, C-3′), 79.22 (d, C-5), 79.06 (d, C-2′), 75.61 (t, *C*H$_2$Ph), 74.77 (t, *C*H$_2$Ph), 72.03 (d, $J_{C,P}$ 5.7 Hz, C-5′), 71.66 (d, C-6), 69.87 (d, C-4′), 69.75 (t, $J_{C,P}$ 5.4 Hz, POC*H*$_2$Ph), 69.69 (t, $J_{C,P}$ 5.6 Hz, POC*H*$_2$Ph), 66.27 (t, $J_{C,P}$ 5.1 Hz, C-6′), 66.03 (d, C-4), 60.59 (t, C-7), 52.58 (q, CO_2CH_3), 51.06 (q, OCH_3), 36.32 (t, C-3); ^{31}P NMR ($CDCl_3$): δ 0.04; ESI-TOF HRMS: m/z = 861.2855; calcd for $C_{43}H_{51}O_{15}PNa^+$: 861.2858.

*Deprotection of **31**:* A suspension of compound **31** (7.4 mg, 0.009 mmol) and 10% Pd–C (1 mg) in dry MeOH (1.0 mL) was hydrogenated for 1 h at room temperature as described for **13**. The suspension was diluted with MeOH, passed through a 0.45 μm syringe filter and the filtrate was made neutral by addition of 0.1 M NaOMe in MeOH (100 μL). Concentration afforded the debenzylated methyl ester which was saponified with 0.01 M NaOH (1.5 mL) at ambient temperature for 8 h. The solution was neutralized by addition of DOWEX 50 H^+ ion-exchange resin. The resin was filtered off and the filtrate was lyophilized. Purification of the residue on BioGel PD10 (H_2O) and freeze-drying of pooled fractions provided **30** (3.9 mg, 87%) as a colorless amorphous solid.

3.26. Methyl (methyl 4,5;7,8-di-*O*-isopropylidene-D-*glycero*-α-D-*talo*-oct-2-ulopyranosid)onate (34)

Sodium hydride (0.13 g, 3.28 mmol) was added in small portions to an ice-cold solution of methyl (3-*O*-acetyl-4,5;7,8-di-*O*-isopropylidene-D-*glycero*-α-D-*galacto*-oct-2-ulopyranosyl)onate **32** (0.85 g, 2.19 mmol) and iodomethane (0.16 mL, 2.63 mmol) in dry DMF (10 mL). After vigorous stirring at 0 °C for 45 min, cleavage of the 3-*O*-acetyl group was induced by addition of dry MeOH (15 mL) forming NaOMe in situ. After 15 h at ambient temperature the solution was partitioned between EtOAc and aq NH_4Cl. The aqueous phase was extracted with EtOAc (3×50 mL) and the combined organic phases were dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by chromatography (toluene/EtOAc 2:1) affording **33** (680 mg, 86%) as a colorless oil with minor impurities.

A solution of **33** (0.67 g, 1.85 mmol) in dry CH_2Cl_2 (15 mL) was treated with Dess-Martin periodinane (1.57 g, 3.70 mmol) at ambient temperature for 17 h. The mixture was dissolved in Et_2O (150 mL) and aq $NaHCO_3$ (50 mL) containing $Na_2S_2O_3$ (5 g) and was stirred for 30 min. The organic phase was washed with aq $NaHCO_3$, dried ($MgSO_4$), filtered, and concentrated. The residue was dissolved in dry MeOH (15 mL) and treated with borane ammonia complex (0.08 g, 2.50 mmol) at 0 °C for 15 min. After dilution with dry MeOH the solvent was removed. The residue was partitioned between CH_2Cl_2 and aq NH_4Cl and the aqueous layer was extracted with CH_2Cl_2 (2×50 mL). The combined organic phases were dried ($MgSO_4$), filtered, and concentrated. Chromatography of the residue (2:1 toluene/EtOAc) gave **34** (0.50 g, 75% based on **33**) as a colorless oil; $[\alpha]_D^{20}$ +57.0 (*c* 1.19, $CHCl_3$); R_f 0.40 (toluene/EtOAc 1:1); ^{1}H NMR ($CDCl_3$): δ 4.46 (dd, 1H, $J_{4,5}$ 6.7, $J_{4,3}$ 4.2 Hz, H-4), 4.44 (ddd, 1H $J_{7,6}$ 7.8, $J_{7,8a}$ 6.1, $J_{7,8b}$ 4.6 Hz, H-7), 4.33 (dd, 1H, $J_{5,6}$ 2.6 Hz, H-5), 4.16 (dd, 1H, $J_{8a,8b}$ 8.8, H-8a), 4.10 (dd, 1H, H-8b), 3.92 (dd, 1H, $J_{3,OH}$ 7.8, H-3), 3.82 (s, 3H, CO_2CH_3), 3.72 (dd, 1H, H-6), 3.25 (s, 3 H, OCH_3), 3.01 (d, 1H, O*H*), 1.51 [s, 3H, C(C*H*$_3$)], 1.44 [s, 3H, C(C*H*$_3$)], 1.38 [s, 6H, $2 \times$ C(C*H*$_3$)]; ^{13}C NMR ($CDCl_3$): δ 167.98 (s, C=O), 109.95 [s, *C*(CH$_3$)$_2$], 109.41 [s, *C*(CH$_3$)$_2$], 99.92 (s, C-2), 73.90 (d, C-7), 72.40 (d, C-4), 71.44 (d, C-5), 69.75 (d, C-6), 68.84 (d, C-3), 66.77 (t, C-8), 52.62 (q, CO_2CH_3), 51.22 (q, OCH_3), 26.90, 25.37, 25.33, 25.18 [q, 4C, $4 \times$ C(*C*H$_3$)]; ESI-TOF HRMS: m/z = 385.1464; calcd for $C_{16}H_{26}O_9Na^+$: 385.1469.

3.27. Methyl (methyl 3-*O*-benzyl-4,5;7,8-di-*O*-isopropylidene-D-*glycero*-α-D-*talo*-oct-2-ulopyranosid)onate (35)

A solution of **34** (184 mg, 0.508 mmol) in dry DMF (8.5 mL) was treated with sodium hydride (41 mg, 1.016 mmol) and stirred for 5 min at 0 °C. Benzyl bromide (121 μL, 1.016 mmol) was added dropwise and the solution was stirred for 40 min at 0 °C. Dry MeOH (1.1 mL) was added to the cold mixture and after 5 min the solution was neutralized with DOWEX 50 H^+ resin. The resin was filtered off and washed thoroughly with EtOAc. The filtrate was washed successively with aq $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated. The crude product was purified by chromatography (toluene/EtOAc 5:1) providing **35** (217 mg, 94%) as a colorless oil: $[\alpha]_D^{20}$ −18.5 (*c* 1.04, $CHCl_3$); R_f 0.29 (toluene/EtOAc 4:1); ^{1}H NMR ($CDCl_3$): δ 7.37–7.26 (m, 5H, Ar), 4.80 (s, 2H, C*H*$_2$Ph), 4.42 (dd, 1H, $J_{4,5}$ 7.7, $J_{4,3}$ 3.6 Hz, H-4), 4.40 (ddd, 1H, $J_{7,6}$ 7.3, $J_{7,8a}$ 6.3, $J_{7,8b}$ 4.9 Hz, H-7), 4.30 (dd, 1H, $J_{5,6}$ 2.2 Hz, H-5), 4.12 (dd, 1H, $J_{8a,8b}$ 8.9, Hz, H-8a), 4.02 (dd, 1H, H-8b), 3.77 (s, 3H, CO_2CH_3), 3.61 (d, 1H, H-3), 3.55 (dd, 1H, H-6), 3.30 (s, 3H, OC*H*$_3$), 1.54 [s, 3H, C(C*H*$_3$)], 1.41 [s, 3H, C(C*H*$_3$)], 1.36 [s, 3H, C(C*H*$_3$)], 1.34 [s, 3H, C(C*H*$_3$)]; ^{13}C NMR ($CDCl_3$): δ 167.04 (s, C=O), 137.62 (s, Ar), 128.43, 128.30, 127.72 (d, 5C, Ar), 110.60 [s, *C*(CH$_3$)$_2$], 109.11 [s, *C*(CH$_3$)$_2$], 101.90 (s, C-2), 75.91 (d, C-3), 73.90 (d, C-4), 73.62 (t, *C*H$_2$Ph), 73.42 (d, C-7), 72.38 (d, C-5), 70.06 (d, C-6), 66.78 (t, C-8), 52.36 (q, CO_2CH_3), 50.83 (q, OCH_3), 26.84, 25.28, 25.26, 25.07 [q, 4C, $4 \times$ C(*C*H$_3$)]; ESI-TOF HRMS: m/z = 470.2389; calcd for $C_{23}H_{32}O_9NH_4^+$: 470.2385.

3.28. Methyl (methyl 3-*O*-benzyl-7,8-*O*-isopropylidene-D-*glycero*-α-D-*talo*-oct-2-ulopyranosid)onate (36)

A solution of **35** (101 mg, 0.223 mmol), *p*-toluenesulfonic acid monohydrate (42 mg, 0.223 mmol) and distilled water (84 μL, 4.460 mmol) in dry acetone (3.0 mL) was stirred at ambient temperature for 15 min. Et_3N (160 μL) was added, the solution was stirred for 30 min, and concentrated. The crude product was purified by chromatography (toluene/EtOAc 2:1) affording **36** (65 mg, 71%) as a colorless amorphous solid; $[\alpha]_D^{20}$ +36.8 (*c* 0.80, $CHCl_3$); R_f 0.41 (toluene/EtOAc 1:1); ^{1}H NMR ($CDCl_3$): δ 7.35–7.27 (m, 3H, Ar), 7.24–7.21 (m, 2H, Ar), 4.81 (d, 1H, *J* 10.9 Hz, C*H*HPh), 4.56 (d, 1H, *J* 11.0 Hz, CH*H*Ph), 4.48 (ddd, 1H, $J_{7,6}$ 7.9, $J_{7,8a}$ 6.3, $J_{7,8b}$ 5.1 Hz, H-7), 4.18 (dd, 1H, $J_{8a,8b}$ 8.8 Hz, H-8a), 4.08 (dd, 1H, $J_{3,4}$ 3.3, $J_{3,5}$ 1.2 Hz, H-3), 4.05 (dd, 1H, H-8b), 3.96 (app. td, 1H, $J_{4,OH}$ 9.8, $J_{4,5}$ 3.4 Hz, H-4), 3.93–3.89 (m, 1H, H-5), 3.71 (s, 3H, CO_2CH_3), 3.55 (dd, 1H, $J_{6,5}$ 1.2 Hz, H-6), 3.22 (s, 3H, OC*H*$_3$), 3.06 (d, 1H, $J_{OH,5}$ 11.8 Hz, O*H*), 2.92 (d, 1H, $J_{OH,4}$ 9.8 Hz, O*H*), 1.42 [s, 3H, C(C*H*$_3$)], 1.37 [s, 3H, C(C*H*$_3$)]; ^{13}C NMR ($CDCl_3$): δ 167.36 (s, C=O), 136.93 (s, Ar), 128.55, 128.15, 127.78 (d, 5C, Ar), 109.19 [s, *C*(CH$_3$)$_2$], 100.70 (s, C-2), 79.86 (d, C-3), 76.55 (t, *C*H$_2$Ph), 73.35 (d, C-7),

73.28 (d, C-6), 68.69 (d, C-5), 66.96 (d, C-4), 66.77 (t, C-8), 52.44 (q, CO_2CH_3), 51.09 (q, OCH_3), 26.73, 25.31 [q, 2C, 2 × C(CH_3)]; ESI-TOF HRMS: m/z = 435.1624; calcd for $C_{20}H_{28}O_9Na^+$: 435.1626.

3.29. Methyl [methyl 3-*O*-benzyl-7,8-*O*-isopropylidene-4-*O*-(4-methoxybenzyl)-D-*glycero*-α-D-*talo*-oct-2-ulopyranosid]onate (37)

A mixture of **36** (64 mg, 0.155 mmol) and dibutyltin oxide (42 mg, 0.171 mmol) in dry toluene (3.5 mL) was heated to reflux using a Dean-Stark apparatus for 3 h. The solution was allowed to cool to ambient temperature followed by consecutive addition of dry DMF (144 μL, 1.862 mmol), 4-methoxybenzyl chloride (105 μL, 0.776 mmol), and tetrabutylammonium iodide (63 mg, 0.171 mmol). After stirring at 60 °C for 16 h the mixture was diluted with EtOAc and washed successively with HCl (1 M), aq $NaHCO_3$, aq $Na_2S_2O_3$ (50 g/L), and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated. Chromatography of the residue (toluene/EtOAc 5:1) gave **37** (67 mg, 81%) as a colorless oil; $[\alpha]_D^{20}$ +28.0 (c 0.90, $CHCl_3$); R_f 0.51 (toluene/EtOAc 2:1); ^{1}H NMR ($CDCl_3$): δ 7.35–7.24 (m, 5H, Ar), 7.22–7.20 (m, 2H, Ar), 6.91–6.88 (m, 2H, Ar), 4.97 (d, 1H, J 10.7 Hz, C*H*HPh), 4.81 (d, 1H, J 11.3 Hz, C*H*HPh), 4.56–4.51 (m, 1H, H-7), 4.52 (d, 1H, J 11.2 Hz, CH*H*Ph), 4.49 (d, 1H, J 10.7 Hz, CH*H*Ph), 4.19 (dd, 1H, $J_{8a,8b}$ 8.8, $J_{8a,7}$ 6.3 Hz, H-8a), 4.17–4.14 (m, 2H, H-3, H-5), 4.04 (dd, 1H, $J_{8b,7}$ 5.4 Hz, H-8b), 3.82–3.80 (m, 1H, H-4), 3.81 (s, 3H, PhOCH_3), 3.68 (s, 3H, CO_2CH_3), 3.68 (d, 1H, $J_{OH,5}$ 10.1 Hz, O*H*), 3.46 (dd, 1H, $J_{6,7}$ 7.7, $J_{6,5}$ 1.3 Hz, H-6), 3.19 (s, 3 H, OCH_3), 1.43 [s, 3H, C(CH_3)], 1.37 [s, 3H, C(CH_3)]; ^{13}C NMR ($CDCl_3$): δ 167.47 (s, C=O), 159.27, 137.22, 130.03 (s, 3C, Ar), 129.20, 128.36, 128.19, 127.95, 113.86 (d, 9C, Ar), 109.07 [s, $C(CH_3)_2$], 100.79 (s, C-2), 78.20 (d, C-3), 75.99 (t, CH_2Ph), 74.04 (d, C-6), 73.62, 73.60 (d, 2C, C-4, C-7), 69.36 (t, CH_2Ph), 66.87 (t, C-8), 65.84 (d, C-5), 55.26 (q, PhOCH_3), 52.44 (q, CO_2CH_3), 51.07 (q, OCH_3), 26.68, 25.40 [q, 2C, 2 × C(CH_3)]; ESI-TOF HRMS: m/z = 550.2641; calcd for $C_{28}H_{36}O_{10}NH_4^+$: 550.2647.

3.30. 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-*gluco*pyranosyl-(1→5)-methyl [methyl 3-*O*-benzyl-7,8-*O*-isopropylidene-4-*O*-(4-methoxybenzyl)-D-*glycero*-α-D-*talo*-oct-2-ulopyranosid]onate (38)

A suspension of predried compounds **37** (34.6 mg, 0.065 mmol) and **10** (52.3 mg, 0.084 mmol) in dry CH_2Cl_2 (1.0 mL) containing 4 Å molecular sieves was stirred at ambient temperature for 1 h. At −30 °C TMSOTf (0.6 μL, 0.004 mmol) in dry CH_2Cl_2 (0.2 mL) was added in two portions within an interval of 1 h. The mixture was kept at −30 °C for 10 min after complete addition. The promoter was destroyed by addition of Et_3N (19 μL, 0.130 mmol) in dry CH_2Cl_2 (0.3 mL). The suspension was allowed to warm up to ambient temperature, filtered over a pad of Celite®, rinsed with CH_2Cl_2, and the filtrate was concentrated. Product **38** (26.0 mg, 42%) was isolated by chromatography (n-hexane/EtOAc 3:1) as a colorless oil; $[\alpha]_D^{20}$ +27.8 (c 0.68, $CHCl_3$); R_f 0.45 (n-hexane/EtOAc 3:2, HPTLC); ^{1}H NMR ($CDCl_3$): δ 7.41–7.32 (m, 9H, Ar), 7.31–7.20 (m, 10H, Ar), 7.08–7.03 (m, 3H, Ar), 6.91–6.88 (m, 2H, Ar), 5.43 (s, 1H, C*H*Ph), 5.20 (d, 1H, $J_{1',2'}$ 3.8 Hz, H-1′), 5.18 (d, 1H, J 11.9 Hz, C*H*HPh), 4.91 (d, 1H, J 11.6 Hz, C*H*HPh), 4.78 (ddd, 1H, $J_{7,6}$ 9.6, $J_{7,8b}$ 6.1, $J_{7,8a}$ 3.5 Hz, H-7), 4.73 (d, 1H, J 12.0 Hz, C*H*HPh), 4.69 (d, 1H, J 11.8 Hz, CH*H*Ph), 4.62 (d, 1H, J 11.6 Hz, CH*H*Ph), 4.54 (d, 1H, J 11.3 Hz, CH*H*Ph), 4.49 (app. dt, 1H, $J_{5',6'b} = J_{5',4'}$ 9.9, $J_{5',6'a}$ 5.1 Hz, H-5′), 4.48 (d, 1H, J 11.7 Hz, C*H*HPh), 4.20 (d, 1H, J 11.2 Hz, CH*H*Ph), 4.13–4.12 (m, 2H, H-3, H-5), 3.93 (dd, 1H, $J_{8a,8b}$ 8.9 Hz, H-8a), 3.91 (dd, 1H, $J_{6'a,6'b}$ 10.0 Hz, H-6′a), 3.88 (app. t, 1H, $J_{3',2'} = J_{3',4'}$ 9.4 Hz, H-3′), 3.84 (dd, 1H, H-8b), 3.78 (s, 3H, PhOCH_3), 3.78–3.77 (m, 1H, H-4), 3.70 (s, 3H, CO_2CH_3), 3.57 (dd, 1H, H-2′), 3.51 (app. t, 1H, H-6′b), 3.48 (app. t, 1H, H-4′), 3.38 (dd, 1H, $J_{6,5}$ 1.1 Hz, H-6), 3.18 (s, 3H, OCH_3), 1.36 [s, 3H, C(CH_3)], 1.23 [s, 3H, C(CH_3)]; ^{13}C NMR ($CDCl_3$): δ 167.72 (s, C=O), 159.33, 139.12, 138.75, 138.62, 137.97, 129.74 (s, 6C, Ar), 129.11, 128.56, 128.13, 128.11, 128.08, 127.94, 127.83, 127.75, 127.31, 127.29, 127.22, 127.02, 126.16, 114.05 (d, 24C, Ar), 109.19 [s, $C(CH_3)_2$], 101.59 (s, C-2), 101.02 (d, C*H*Ph), 99.98 (d, C-1′), 83.01 (d, C-4′), 78.87 (d, C-2′), 77.55 (d, C-3′), 76.96 (d, C-3), 75.38 (t, CH_2Ph), 75.22 (d, C-4), 74.64 (t, CH_2Ph), 73.83 (d, C-6), 72.79 (d, C-5), 71.96 (d, C-7), 71.75 (t, CH_2Ph), 70.93 (t, CH_2Ph), 69.16 (t, C-6′), 67.16 (t, C-8), 62.61 (d, C-5′), 55.23 (q, PhOCH_3), 52.36 (q, CO_2CH_3), 51.11 (q, OCH_3), 27.37, 25.34 [q, 2C, 2 × C(CH_3)]; ESI-TOF HRMS: m/z = 985.3979; calcd for $C_{55}H_{62}O_{15}Na^+$: 985.3981.

3.31. 2,3-Di-*O*-benzyl-α-D-*gluco*pyranosyl-(1→5)-methyl [methyl 3-*O*-benzyl-4-*O*-(4-methoxybenzyl)-D-*glycero*-α-D-*talo*-oct-2-ulopyranosid]onate (39)

A suspension of **38** (26.4 mg, 0.027 mmol) and p-toluenesulfonic acid monohydrate (0.9 mg, 0.005 mmol) in dry MeOH (1.5 mL) was kept at 0 °C for 1 h followed by stirring at ambient temperature for 24 h. Et_3N (10 μL) was added to the mixture, stirring was continued for 10 min, and the solution was concentrated. Chromatography of the residue afforded **39** (16.0 mg, 70%) as a colorless oil; $[\alpha]_D^{20}$ +53.8 (c 0.71, $CHCl_3$); R_f 0.16 (n-hexane/EtOAc 1:4); ^{1}H NMR ($CDCl_3$): δ 7.39–7.27 (m, 16H, Ar), 7.25–7.21 (m, 1H, Ar), 6.91–6.89 (m, 2H, Ar), 5.01 (d, 1H, J 12.0 Hz, C*H*HPh), 4.92 (d, 1H, J 11.5 Hz, C*H*HPh), 4.81 (d, 1H, J 11.5 Hz, C*H*HPh), 4.80 (d, 1H, $J_{1',2'}$ 3.4 Hz, H-1′), 4.79 (d, 1H, J 11.0 Hz, CH*H*Ph), 4.61 (d, 1H, J 12.4 Hz, C*H*HPh), 4.59 (d, 1H, J 11.9 Hz, CH*H*Ph), 4.54 (d, 1H, J 11.8 Hz, CH*H*Ph), 4.52 (d, 1H, J 11.9 Hz, CH*H*Ph), 4.20–4.15 (m, 1H, H-7), 4.12 (d, 1H, $J_{OH,7}$ 6.0 Hz, O*H*), 4.02–3.99 (m, 2H, H-3, H-5), 3.83–3.79 (m, 4H, PhOCH_3, H-5′), 3.78–3.72 (m, 3H, H-3′, H-4, H-8a), 3.68 (s, 3H, CO_2CH_3), 3.66–3.62 (m, 1H, H-8b), 3.61 (dd, 1H, $J_{6,7}$ 9.2, $J_{6,5}$ 1.7 Hz, H-6), 3.44 (dd, 1H, $J_{2',3'}$ 9.6 Hz, H-2′), 3.33–3.28 (m, 1H, H-4′), 3.26–3.19 (m, 2H, H-6′a, H-6′b), 3.16 (s, 3H, OCH_3), 1.85 (bt, 1H, $J_{OH,8a} = J_{OH,8b}$ 6.5 Hz, O*H*), 1.80 (bt, 1H, $J_{OH,6'a} = J_{OH,6'b}$ 6.0 Hz, O*H*), 0.88 (d, 1H, $J_{OH,4'}$ 7.5 Hz, O*H*); ^{13}C NMR ($CDCl_3$): δ 167.81 (s, C=O), 159.47, 139.09, 138.59, 137.05, 129.53 (s, 5C, Ar), 129.27, 128.72, 128.71, 128.51, 128.48, 128.32, 127.99, 127.74, 127.35, 126.29, 113.99 (d, 19C, Ar), 101.25 (d, C-1′), 101.10 (s, C-2), 82.07 (d, C-3′), 80.31 (d, C-2′), 76.56, 75.82 (d, 2C, C-3, C-5), 75.26 (t, CH_2Ph), 75.07 (d, C-4), 75.00 (t, CH_2Ph), 74.39 (t, CH_2Ph), 72.55 (d, C-4′), 71.79 (d, C-6), 71.61 (d, C-5′), 70.85 (t, CH_2Ph), 68.05 (d, C-7), 64.12 (t, C-8), 62.45 (t, C-6′), 55.27 (q, PhOCH_3), 52.46 (q, CO_2CH_3), 51.04 (q, OCH_3); ESI-TOF HRMS: m/z = 857.3351; calcd for $C_{45}H_{54}O_{15}Na^+$: 857.3355.

3.32. α-D-*Gluco*pyranosyl-(1→5)-sodium (methyl D-*glycero*-α-D-*talo*-oct-2-ulopyranosid)onate (40)

A suspension of **39** (5.9 mg, 0.007 mmol) in dry MeOH (1 mL) was hydrogenated for 4 h with 10% Pd–C (1 mg) as described for **13.** Fresh catalyst (1 mg) was added and stirring was continued under H_2 for 14 h. The suspension was diluted with MeOH and passed through a 0.45 μm syringe filter. The filtrate was concentrated and the residue was treated with 0.01 M aq. NaOH (2 mL) at ambient temperature for 3 h. The solution was neutralized by addition of DOWEX 50 H^+ resin. The ion-exchange resin was filtered off and the filtrate was lyophilized. Purification of the residue on a PD10 column (H_2O) and freeze-drying of pooled fractions gave **40** (2.9 mg, 92%) as a colorless amorphous solid; $[\alpha]_D^{20}$ +128.1 (c 0.25, H_2O); ^{1}H NMR (D_2O): δ 5.13 (d, 1H, $J_{1',2'}$ 3.9 Hz, H-1′), 4.21–4.19 (m, 1H, H-5), 4.15–4.10 (m, 2H, H-5′, H-7), 4.01 (app. t, 1H, $J_{4,3} = J_{4,5}$ 3.3 Hz, H-4), 3.95 (dd, 1H, $J_{8a,8b}$ 11.8, $J_{8a,7}$ 2.8 Hz, H-8a), 3.85 (dd, 1H, $J_{3,5}$ 0.9 Hz, H-3), 3.81 (dd, 1H, $J_{6'a,6'b}$ 12.5, $J_{6'a,5'}$ 3.6 Hz, H-6′a), 3.75 (dd, 1H, $J_{6'b,5'}$ 2.4 Hz, H-6′b), 3.69 (dd, 1H, $J_{8b,7}$

5.9 Hz, H-8b), 3.64 (dd, 1H, $J_{6,7}$ 9.9, $J_{6,5}$ 1.0 Hz, H-6), 3.59 (dd, 1H, $J_{3',2'}$ 10.1, $J_{3',4'}$ 9.2 Hz, H-3′), 3.52 (dd, 1H, H-2′), 3.44 (dd, 1H, $J_{4',5'}$ 10.2 Hz, H-4′), 3.15 (s, 3H, OCH_3); ^{13}C NMR data: see Table 1; ESI-TOF HRMS: m/z = 453.1210; calcd for $C_{15}H_{26}O_{14}Na^+$: 453.1215.

Acknowledgments

The authors thank the Austrian Science Fund FWF for financial support (Grant P 24921) and Dr. Andreas Hofinger for recording NMR spectra.

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Manuscript #2

Pokorny, B.; Kosma, P.* *Chem. Eur. J.* **2015**, *21*, 305-313.

"Synthesis of Chlamydia Lipopolysaccharide Haptens through the use of α-Specific 3-Iodo-Kdo Fluoride Glycosyl Donors"

doi:10.1002/chem.201405424

ChemPubSoc Europe

DOI: 10.1002/chem.201405424

CHEMISTRY A European Journal Full Paper

Glycosylation

Synthesis of Chlamydia Lipopolysaccharide Haptens through the use of α-Specific 3-Iodo-Kdo Fluoride Glycosyl Donors**

Barbara Pokorny and Paul Kosma*[a]

Abstract: A scalable approach towards high-yielding and (stereo)selective glycosyl donors of the 2-ulosonic acid Kdo (3-deoxy-D-*manno*-oct-2-ulosonic acid) is a fundamental requirement for the development of vaccines against Gram-negative bacteria. Herein, we disclose a short synthetic route to 3-iodo Kdo fluoride donors from Kdo glycal esters that enable efficient α-specific glycosylations and significantly suppress the elimination side reaction. The potency of these donors is demonstrated in a straightforward, six-step synthesis of a branched Chlamydia-related Kdo-trisaccharide ligand without the need for protecting groups at the Kdo glycosyl acceptor. The approach was further extended to include sequential iteration of the basic concept to produce the linear Chlamydia-specific α-Kdo-(2→8)-α-Kdo-(2→4)-α-Kdo trisaccharide in a good overall yield.

Introduction

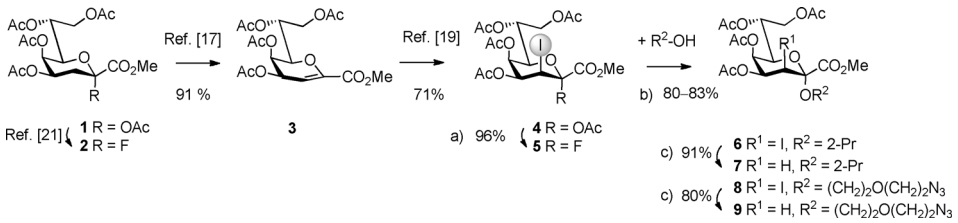
Lipopolysaccharides (LPS) are amphiphilic glycolipids located in the outer leaflet of the cell membrane of Gram-negative bacteria, and are of high biomedical relevance due to their interaction with components of the innate and adaptive immune system of their respective hosts.[1] In structural terms, enterobacterial LPS displays a tripartite architecture composed of an endotoxically active diglucosamine unit (Lipid A) followed by a core region, and the O-antigenic polysaccharide chain.[2] 3-Deoxy-α-D-*manno*-oct-2-ulosonic acid (α-Kdo) serves as the common linkage sugar to Lipid A and constitutes the structurally conserved α-Kdo-(2→4)-α-Kdo disaccharide core domain. This basic Kdo unit is extended by additional Kdo residues in the LPS of *Chlamydiaceae*, a biomedically important obligate intracellular parasite.[3] The genus *Chlamydia trachomatis* is a leading cause of sexually transmitted diseases, eventually resulting in infertility in women, but it is also responsible for conjunctivitis and secondary blindness.[4] A second genus harboring the species *Chlamydophila psittaci* and *Chlamydophila pneumoniae* is involved in pulmonary infections and the latter species has additionally been linked to chronic infections such as arthritis and atherosclerosis.[5] All Chlamydiae share a truncated lipopolysaccharide wherein the Kdo part forms an immunodominant, family-specific trisaccharide epitope of the sequence α-Kdo-(2→8)-α-Kdo-(2→4)-α-Kdo.[6] In addition to this group-specific antigen, a second linear trisaccharide α-Kdo-(2→4)-α-Kdo-(2→4)-α-Kdo and the branched tetrasaccharide α-Kdo-(2→4)[α-Kdo-(2→8)]-α-Kdo-(2→4)-α-Kdo constitute further antigenic epitopes in the *Chl. psittaci*.[7] A similarly branched Kdo trisaccharide has also been identified in the core region of *Acinetobacter lwoffii* F78 LPS.[8] Recently, it has been shown that these Kdo residues are involved in important binding interactions with proteins, and insight into the recognition of Kdo saccharides by monoclonal antibodies and Toll-like receptor-4 (TLR-4) has been achieved at the molecular level.[9] Thus, the chemical synthesis of Kdo glycosides is an attractive goal in the field of (bio)organic chemistry to provide immunoreagents and antigens for the development of synthetic carbohydrate-based vaccines and diagnostics.[10]

Glycosylation reactions of 3-deoxy-2-ulosonic acid donors are challenging because of the lack of a stereodirecting neighboring group at C-3, the deactivation of the anomeric center by the adjacent carboxyl group, the high propensity of the glycosyl donors towards elimination reactions—leading to glycal ester derivatives (e.g., **3**; Scheme 1)—and the acid-sensitivity of the ketosidic linkage.[11] To enhance the stereoselectivity and coupling yields of glycoside formation, different approaches have been reported that have mainly been based on optimization of dedicated protecting and leaving groups. Thus, electron-donating ("arming") protecting groups such as silyl ether, benzyl, or isopropylidene groups have mostly been used to counteract the deactivating effect of the carboxyl group, whereas halides, thioalkyl, thioaryl, phosphites, and trihaloacetimidates served as leaving groups.[12] In particular, suitably protected Kdo-fluoride donors have been reported to afford good to moderate α/β-stereoselectivity in the outcome of glycosylation reactions.[13] Nevertheless, in most cases, a large excess of donor is required to compensate for losses due to the elimination side reaction, and the formation of anomeric mixtures eventually requires separation of the undesired anomer (and the glycal ester) by extensive chromatography.

[a] *B. Pokorny, Prof. P. Kosma*
Department of Chemistry
University of Natural Resources and Life Sciences-Vienna
Muthgasse 18, 1190 Vienna (Austria)
E-mail: paul.kosma@boku.ac.at

[**] *Kdo = 3-Deoxy-α-D-*manno*-oct-2-ulosonic acid.*

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201405424.



Scheme 1. Synthesis of stereodirecting 3-iodo-Kdo fluoride donor **5** and Kdo glycosides. a) HF-pyridine, CH_2Cl_2; b) $BF_3{\cdot}Et_2O$, CH_2Cl_2, MS 3 Å; c) lauroyl peroxide, cyclohexane.

To improve the selectivity in the glycosylation reaction in favor of the axial α-Kdo glycoside, temporary auxiliary groups such as 3-iodo, 3-thio, and 3-selenylphenyl groups have previously been introduced through the use of addition reactions to Kdo glycal esters.[14] These groups provide anchimeric assistance but have to be removed after the coupling step. Recently, direct iodonium-ion induced activation of Kdo glycal esters to give glycosides has also been reported. Activation of a 4,5-*O*-isopropylidene-protected Kdo glycal ester by *N*-iodosuccinimide (NIS)/TMSOTf preferentially afforded β-linked Kdo oligosaccharides.[15] Activation towards α-configured Kdo spacer glycosides has been accomplished by using the acetylated glycal ester **3** in the presence of an excess of triflic acid.[14a] Recently, iodoalkoxylation of a perbenzylated Kdo glycal ester was employed for sequential assembly of α-(2→8)-linked Kdo-oligosaccharides.[16] However, highly activated primary hydroxyl groups of perbenzylated open-chain glycosyl acceptors were necessary for this method, which was thus limited to the (2→8)-linkage, and stoichiometric amounts of triflic acid as promoter were needed to activate the benzylated glycal derivatives.

Despite these achievements, a versatile and α-selective Kdo donor that is suitable for efficient and scalable synthesis is still needed. Herein, we disclose a short route to novel 3-iodo-Kdo fluoride donors and demonstrate their efficient application in Chlamydia LPS ligand assembly.

Results and Discussion

The acetyl-protected glycal methyl ester **3**,[17] which can be prepared in three steps in multigram amounts from ammonium Kdo via the peracetylated Kdo methyl ester **1**,[18] was subjected to iodoacetoxylation with *N*-iodosuccinimide (NIS) in acetic acid to give the known 2,3-*trans*-diaxial derivative **4**.[19] In consideration of the stability of anomeric Kdo fluorides,[13,20] conversion of the 3-iodo derivative **4** into donor **5** was carried out. Previously, Kdo fluorides such as **2** have mainly been prepared by reaction of a Kdo 2-*O*-hemiketal (which is prone to 2,3-elimination) with diethylaminosulfur trifluoride (DAST).[13,20] However, a direct replacement of the anomeric acetate by using the easily scalable reaction of **4** with HF-pyridine complex afforded α-fluoride **5** (96%) neatly after flash-chromatography (Scheme 1).[21] Fluoride **5** is bench-top stable for several months and was obtained as a single anomer, as seen from the ^{19}F NMR spectrum (Figure 1). The α-anomeric configuration was consistent with the ^{13}C NMR chemical shift data for C-4 and C-6 (see the Supporting Information) and the value of the vicinal $^{19}F–^{1}H$ coupling constant ($^3J(F_{ax},H3_{eq}) = 5.4$ Hz).[22]

To evaluate its glycosyl donor properties, model glycosylation reactions of **5** with 2-propanol (2 equiv) in dichloromethane, promoted by $BF_3{\cdot}Et_2O$ were performed and compared to a similar glycosylation using the peracetylated Kdo-fluoride donor **2**, lacking a stereodirecting group.[20d,21] The latter glycosylation afforded a moderate yield (48%) of an α/β-glycoside mixture (4.8:1) and generated large amounts of glycal ester **3** (31%). By contrast, application of the new donor **5** revealed an α-specific outcome, giving the 2,3-*trans*-ketoside **6** in high yield (83%) accompanied by the formation of only very minor amounts of **3** (5%); for further details see the Supporting Information.

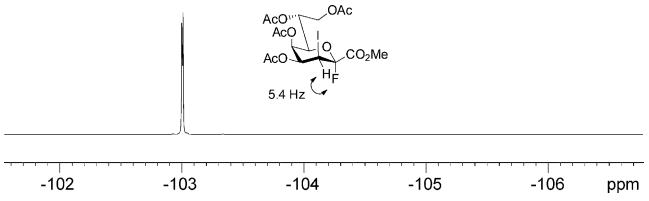
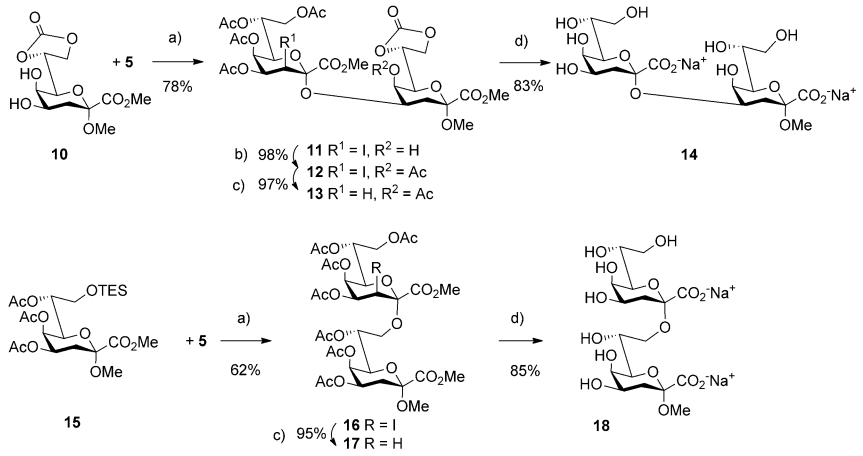


Figure 1. Proton-coupled ^{19}F NMR spectrum of fluoride donor **5**.

For the subsequent dehalogenation of **6**, free-radical chain reduction using AIBN/tributyltin hydride gave moderate yields after extensive purification to remove the tin reagent. Hydrogenation over different Pd catalysts gave irreproducible yields because of concomitant epimerization of the 3-iodo-substituent among other side reactions. Eventually, however, the 3-iodo-substituent was cleanly removed through hydrogen atom transfer from cyclohexane[23] induced by lauroyl peroxide to furnish the Kdo α-glycoside **7** (Scheme 1) in near-quantitative yield. The α-anomeric configuration was then confirmed on the basis of the chemical shift difference between the axial and equatorial protons at C-3, the low-field shift (>5 ppm) observed for H-4 in 4-*O*-acetylated Kdo derivatives as well as ^{13}C NMR chemical shifts for C-4 and C-6, which are shifted to higher field when compared with the β-anomers.[18]

We further evaluated the practicality of the dehalogenation method for Kdo-glycosides containing functionalized linkers, which are needed for conversion into neoglycoconjugates and for the preparation of glycoarrays. By following the described procedure, ω-azido-3-iodo-glycoside **8** was obtained as the α-anomer exclusively and in excellent yields (83% based on the amount of donor). Notably, the azide moiety remained unaffected during subsequent dehalogenation by hydrogen atom transfer, giving the Kdo-spacer glycoside **9** in comparable yields.

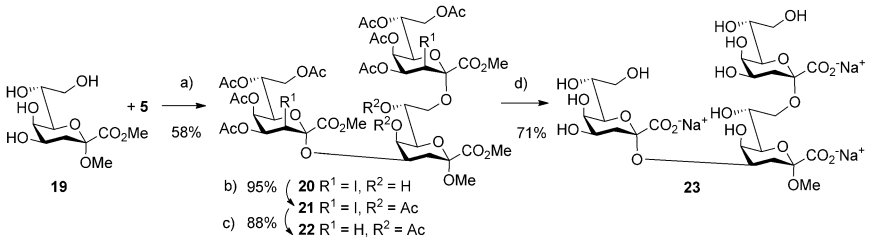
To assess the glycosylation potential of the 3-iodo fluoride donor **5**, the structurally conserved α-(2→4)-linkage of the enterobacterial Kdo region was then assembled in a highly efficient approach. Coupling of **10** with one equivalent only of donor **5** proceeded smoothly and regioselectively and furnished the disaccharide **11** in 78% yield without formation of the β-anomeric product and with only minor levels of elimination (4% of **3**) and donor hydrolysis (5%) (Scheme 2). Subsequent dehalogenation never reached completion because of the poor solubility of the 5-OH disaccharide derivative **11** in cyclohexane, and separation from unreacted starting material could only be achieved by using HP-chromatography. To secure smooth dehalogenation, disaccharide **11** was therefore acetylated (98%) to give fully protected **12** prior to dehalogenation towards **13** (97%). Global deprotection through sodium-methoxide catalyzed transesterification and alkaline hydrolysis of the methyl ester group afforded the known disaccharide methyl glycoside **14**.[24]



Scheme 2. Synthesis of 2→4 and 2→8 linked Kdo disaccharides **14** and **18**. a) BF_3·Et_2O, CH_2Cl_2, MS 3 Å; b) Ac_2O, pyridine, DMAP; c) lauroyl peroxide, cyclohexane; d) 0.1 M NaOMe, MeOH, then 0.1 M aq. NaOH.

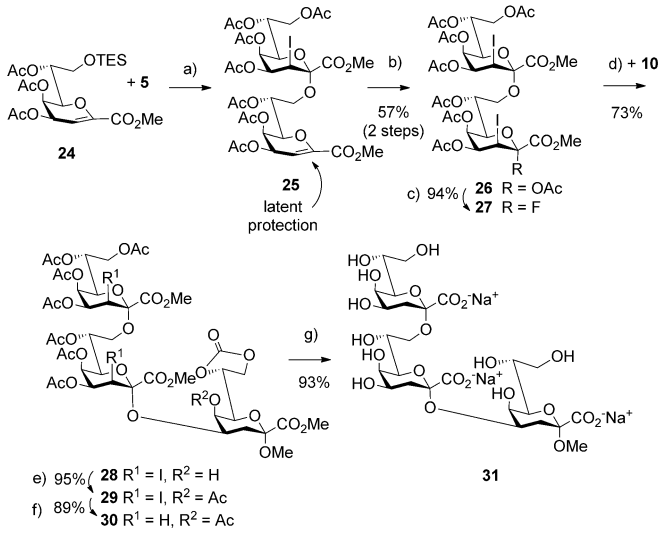
To further extend the scope of Kdo-glycoside synthesis beyond the common enterobacterial α-(2→4)-linked Kdo disaccharide, the Chlamydia-specific[6] α-(2→8)-disaccharide **18** was then prepared along similar lines by capitalizing on a glycodesilylation approach.[25] Coupling of the 8-*O*-triethylsilyl (TES)-protected derivative **15** with 1.2 equivalents of donor **5** proceeded smoothly to give the α-(2→8)-linked disaccharide **16** in 62% yield. Again, no β-isomer was observed. The moderate yield was due to concomitant migration of the 7-*O*-acetyl protecting group towards the primary 8-OH group, forming a regioisomer that was not glycosylated under these reaction conditions. The glycosylation of the corresponding 8-OH alcohol (derived from **15** by F^- treatment) gave a similar yield (64%), whereas the incorporation of bulkier silyl protecting groups (8-*O*-TBDMS, 7,8-*O*-TIPDS) substantially lowered the isolated yield.

The extraordinary glycosylation properties of **5** prompted us to apply the donor for the one-pot synthesis of a branched Kdo trisaccharide, which corresponds to an LPS antigen of *Chlamydophila psittaci* and serves as a species-specific marker.[26] Indeed, glycosylation of unprotected tetraol **19** with 2.4 equivalents of donor **5** proceeded in a remarkably regioselective reaction, despite being heterogeneous, which allowed the target trisaccharide **20** to be isolated in 58% yield (Scheme 3). The branched α-(2→4)[α-(2→7)]-linked Kdo trisaccharide was isolated in 7% yield as a minor by-product. Processing of **20** as described for the disaccharides gave **21** and **22**, which was then deblocked to afford the branched Kdo trisaccharide **23**.



Scheme 3. One-pot glycosylation towards the branched *Chl. psittaci* specific Kdo trisaccharide **23**. a) BF_3·Et_2O, CH_2Cl_2, MS 3 Å; b) Ac_2O, pyridine, DMAP; c) lauroyl peroxide, cyclohexane; d) 0.1 M NaOMe, MeOH, then 0.1 M aq. NaOH.

This basic concept was further extended to iterative oligosaccharide-synthesis, which was demonstrated for the linear Kdo trisaccharide **31**, representing the family-specific Chlamydia epitope (Scheme 4). After applying the 8-*O*-silyl protected glycal ester **24** as glycosyl acceptor, the resulting disaccharide **25** was treated likewise by iodoacetoxylation, and reaction with HF-pyridine afforded the bis-iodo disaccharide donor **27**. Thus, the glycal moiety as in **25** serves as a latent protecting group for the anomeric position, which may subsequently be activated. Interestingly, BF_3·Et_2O was not capable of promoting the subsequent coupling step with glycosyl acceptor **10**, however, TfOH-catalyzed (0.15 equiv) coupling provided trisaccharide **28** stereospecifically in good yield (73%). Acetylation and dehalogenation of **28** followed by global deprotection of **30** afforded the target trisaccharide **31**. The ^1H and ^{13}C NMR data of the deprotected di- and trisaccharides **14**, **18**, **23**, and **31** are in full agreement with published data of chlamydial oligosaccharides.[27]



Scheme 4. Synthesis of the family-specific Chlamydia Kdo trisaccharide **31**. a) BF_3·Et_2O, CH_2Cl_2, MS 3 Å; b) NIS, AcOH; c) HF-pyridine, CH_2Cl_2; d) TfOH, CH_2Cl_2, MS 5 Å; e) Ac_2O, pyridine, DMAP; f) lauroyl peroxide, cyclohexane; g) 0.1 M NaOMe, MeOH, then 0.1 M aq. NaOH.

Conclusions

The easily accessible 3-iodo-2-fluoride Kdo donors enable a very efficient and α-specific glycosylation of different Kdo glycosyl acceptors to generate biomedically important LPS fragments. In addition to the exquisite α-selectivity, the axial iodo-substituent substantially suppressed elimination and consequent formation of the glycal side-product (Table 1). Sequential iteration of this basic concept may be combined with variable protecting group patterns, thus considerably expanding the scope of the presented work towards other biomedically important Kdo-antigens.

Table 1. Summary of glycosylation reactions using 3-iodo-Kdo fluoride donors.

Acceptor	Donor	Equiv	Product	Yield [%]	α/β ratio	Glycal [%][a]
2-PrOH[b]	**5**	1.0	**6**	83	α only	7
	**5**	1.0	**8**	80	α only	trace
10	**5**	1.0	**11**	78	α only	4
15	**5**	1.2	**16**	62	α only	7
19	**5**	2.4	**20**	58[c]	α only	11
24	**5**	1.2	**25**	85[d]	α only	n.d.
10	**27**	1.2	**28**	73[e]	α only	n.d.

[a] Relative to reacted donor. [b] 2 Equivalents were used. [c] In addition, the 2→4/7 linked regioisomer was isolated in 7% yield. [d] Purification afforded a mixture of hydrolyzed donor and **25**, therefore, the yield was determined from ^{1}H NMR data. [e] Promoted by TfOH.

Experimental Section

General methods

All purchased chemicals were used without further purification unless stated otherwise. Solvents (CH_2Cl_2, DMF, pyridine, cyclohexane) were dried over activated 4 Å molecular sieves. 2-Propanol for glycosylation was dried over 5 Å molecular sieves for 24 h. THF was directly distilled on 4 Å molecular sieves shortly before use. Anhydrous MeOH (SeccoSolv) was purchased from Merck. Cation exchange resin DOWEX 50 H^+ was regenerated by consecutive washing with HCl (3 M), water, and anhydrous MeOH. Aqueous solutions of salts were saturated unless stated otherwise. Concentration of organic solutions was performed under reduced pressure at temperatures below 40 °C. Optical rotations were measured with a PerkinElmer 243 B Polarimeter. $[\alpha]_D^{20}$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Thin-layer chromatography was performed on Merck precoated plates: generally on 5×10 cm, layer thickness 0.25 mm, Silica Gel $60F_{254}$; alternatively on HP-TLC plates with 2.5 cm concentration zone (Merck). Spots were detected by dipping reagent (anisaldehyde-H_2SO_4). For column chromatography, silica gel (0.040–0.063 mm) was used. HP-column chromatography was performed on pre-packed columns (YMC-Pack SIL-06, 0.005 mm, 250×10 mm and 250×20 mm). Size-exclusion chromatography was performed on Bio-Gel P-2 Gel extra fine <45 μm (wet) (1×30 cm) or on pre-packed PD-10 columns (GE Healthcare, Sephadex G-25M). NMR spectra were recorded with a Bruker Avance III 600 instrument (600.22 MHz for ^{1}H, 150.93 MHz for ^{13}C and 564.77 MHz for ^{19}F) using standard Bruker NMR software. ^{1}H NMR spectra were referenced to 7.26 ppm ($CDCl_3$) and 0.00 ppm (D_2O, external calibration to 2,2-dimethyl-2-silapentane-5-sulfonic acid) unless stated otherwise. ^{13}C NMR spectra were referenced to 77.00 ppm ($CDCl_3$) and 67.40 ppm (D_2O, external calibration to 1,4-dioxane) ppm. ^{19}F NMR spectra were indirectly referenced according to IUPAC recommendations.[28] ESI-MS data were obtained with a Waters Micromass Q-TOF Ultima Global instrument.

General procedure for glycosylation

Under an atmosphere of argon, a mixture of donor, glycosyl acceptor, and ground 3 Å molecular sieves (ca. 50 mg mL^{-1} CH_2Cl_2) in anhydrous CH_2Cl_2 was stirred at ambient temperature for 2 h. At 0 °C, BF_3·Et_2O (2 equiv/donor) was added dropwise and after complete addition, the ice bath was removed immediately. Typically, the solution turned pink after a couple of minutes. When full conversion was determined by TLC, aq. $NaHCO_3$ and CH_2Cl_2 were added. The aqueous phase was washed with $CHCl_3$ and the combined organic phases were washed successively with aq. sodium thiosulfate (5 w%) and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by column chromatography.

General procedure for dehalogenation

A solution/suspension of the iodo-compound in anhydrous cyclohexane was degassed by bubbling argon through the mixture followed by heating to reflux under inert gas for 15 min. Lauroyl peroxide was added to the warm solution in one portion and heating to reflux was continued until complete conversion was detected by (HP-) TLC. The solvent was removed in vacuo and the residue was purified by column chromatography.

General procedure for global deprotection

The acetylated saccharide was treated with a catalytic amount of NaOMe (0.1 M in MeOH) in anhydrous MeOH until cleavage of the majority of acetyl groups had been detected by TLC. Ion-exchange resin DOWEX 50 (H^+) was added until the solution became neutral, the resin was then filtered off and the filtrate was concentrated. The residue was dissolved in water and treated with aq NaOH (0.1 M) at ambient temperature. Neutralization of the solution with DOWEX 50 (H^+) ion-exchange resin, filtration, and lyophilization of the filtrate afforded the product, which was purified by gel chromatography to remove residual sodium acetate.

Synthesis

For experimental data for compounds **6**–**18** and **32**–**33**, and NMR spectra of compounds **5**–**18**, **20**–**24**, and **26**–**33**, refer to Supporting Information.

Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate fluoride (5): A solution of **4**[19] (0.75 g, 1.275 mmol) in anhydrous CH_2Cl_2 (13.0 mL) was treated with hydrogen fluoride pyridine (ca. 70% HF, ca. 30% pyridine; 4.0 mL) at 0 °C for 2.5 h in a sealed Teflon flask. Ice-cold water (10 mL) was added, the phases were mixed thoroughly and separated. The aqueous phase was once again extracted with CH_2Cl_2 and the combined organic extracts were washed with aq. $NaHCO_3$, dried ($MgSO_4$), filtered, and concentrated. The crude product was quickly purified by flash chromatography (*n*-hexane/EtOAc, 1:1) to afford **5** (0.67 g, 96%) as a colorless oil; $[\alpha]_D^{20} = +18.8$ (*c* 0.76, MeOH); R_f 0.51 (toluene/EtOAc, 2:1); ^{1}H NMR ($CDCl_3$): $\delta = 5.47$ (ddd, $J(5,4) = 3.5$, $J(5,6) = 1.9$, $J(5,3) = 0.8$ Hz, 1H; H-5), 5.37–5.34 (m, 1H; H-7), 4.97 (dd, $J(4,3) = 5.1$ Hz, 1H; H-4), 4.64 (app dt, 1H; H-3), 4.52 (dd, $J(8a,8b) = 12.4$, $J(8a,7) = 2.3$ Hz, 1H; H-8a), 4.49 (dd, $J(6,7) = 9.8$ Hz, 1H; H-6), 4.23 (dd, $J(8b,7) = 4.2$ Hz, 1H; H-8b), 3.88 (s, 3H; CO_2CH_3), 2.12, 2.07, 2.05, and 1.98 ppm (4×s, each 3H; $COCH_3$); ^{13}C NMR ($CDCl_3$): $\delta = 170.42$, 169.90, 169.36, and 169.17 (4×s, *C*OCH_3), 163.29 (ds, $J(1,F) = 28.2$ Hz; C-1), 108.92 (ds, $J(2,F) = 231.1$ Hz, C-2), 70.15 (dd, $J(6,F) = 4.5$ Hz; C-6), 67.11 (d, C-7), 64.56 (d, C-4), 62.76 (d, C-5), 61.84 (t, C-8), 53.49 (q, CO_2CH_3), 20.79, 20.66, 20.61, and 20.51 (4× q, $COCH_3$), 18.02 ppm (dd, $J(3,F) = 31.3$ Hz; C-3); ^{19}F NMR ($CDCl_3$): $\delta = -103.00$ ppm (d, $J(F,H\text{-}3) = 5.4$ Hz); HRMS (ESI-TOF): *m/z* calcd for $C_{17}H_{22}FIO_{11}NH_4^+$: 566.0529; found: 566.0533.

Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→4)[methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→8)]-methyl (methyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (20): A mixture of predried **19**[18] (16.3 mg, 0.061 mmol) and **5** (80.6 mg, 0.147 mmol) in anhydrous CH_2Cl_2 (4.0 mL) was treated with $BF_3{\cdot}Et_2O$ (120.7 μL, 0.588 mmol) according to the general procedure for glycosylation. After 1 h, the suspension was subjected to aqueous work-up and the crude product was purified by HP-column chromatography (toluene/EtOAc, 2:1→1:1) to give glycal **3**[17] (6.8 mg, 11%), the slower migrating (2→4/7)-regioisomer trisaccharide (5.4 mg, 7%), and finally the slowest migrating trisaccharide **20** (47.0 mg, 58%).

(2→4/7)-Trisaccharide: Colorless oil; R_f 0.39 (toluene/EtOAc, 1:1, HP-TLC); ^{1}H NMR ($CDCl_3$): $\delta = 5.46$–5.44 (m, 2H; H-5′, H-5″), 5.38–5.34 (m, 2H; H-7′, H-7″), 5.04 (dd, $J(4'',3'') = 4.6$, $J(4'',5'') = 3.7$ Hz, 1H; H-4″), 4.97 (dd, $J(4',3') = 4.8$, $J(4',5') = 3.6$ Hz, 1H; H-4′), 4.74 (dd, $J(8''a,8''b) = 12.5$, $J(8''a,7'') = 2.9$ Hz, 1H; H-8″a), 4.71 (dd, $J(8'a,8'b) = 12.5$, $J(8'a,7') = 2.8$ Hz, 1H; H-8′a), 4.69 (dd, $J(6'',7'') = 9.2$, $J(6'',5'') = 2.2$ Hz, 1H; H-6″), 4.55 (dd, $J(3',5') = 0.8$ Hz, 1H; H-3′), 4.47 (dd, $J(3'',4'') = 0.9$ Hz, 1H; H-3″), 4.26–4.22 (m, 2H; H-6′, H-8′b), 4.17–4.11 (m, 2H; H-4, H-8″b), 4.00–3.97 (m, 1H; H-8a), 3.94–3.88 (m, 8H; H-7, H-8b, 2×CO_2CH_3), 3.80 (s, 3H; CO_2CH_3), 3.72 (dd, $J(6,7) = 5.2$, $J(6,5) = 1.2$ Hz, 1H; H-6), 3.63–3.61 (m, 1H; H-5), 3.24 (s, 3H; OCH_3), 2.64 (br s, 1H; C8-OH), 2.49 (br s, 1H; C5-O*H*), 2.14–2.11 (m, 11H; H-3_{ax}, H-3_{eq}, 3×$COCH_3$), 2.10, 2.07, 2.06, 1.98, and 1.979 ppm (5×s, each 3H; $COCH_3$).

Compound 20: Colorless amorphous solid; $[\alpha]_D^{20} = +63.1$ (*c* 0.55, $CHCl_3$); R_f 0.38 (toluene/EtOAc, 1:1, HP-TLC); ^{1}H NMR ($CDCl_3$): $\delta = 5.46$–5.44 (m, 1H; H-5′), 5.39–5.32 (m, 3H; H-7′, H-5″, H-7″), 4.98–4.94 (m, 2H; H-4′, H-4″), 4.75 (dd, $J(8'a,8'b) = 12.5$, $J(8'a,7') = 2.7$ Hz, 1H; H-8′a), 4.60 (dd, $J(8''a,8''b) = 12.2$, $J(8''a,7'') = 2.2$ Hz, 1H; H-8″a), 4.55–4.52 (m, 2H; H-3′, H-3″), 4.33 (dd, $J(6'',7'') = 9.7$, $J(6'',5'') = 1.8$ Hz, 1H; H-6″), 4.26–4.21 (m, 2H; H-6′, H-8″b), 4.17 (ddd, $J(4,3_{ax}) = 11.2$, $J(4,3_{eq}) = 5.6$, $J(4,5) = 2.9$ Hz, 1H; H-4), 4.13–4.07 (m, 2H; H-7, H-8′b), 3.87 (s, 6H; 2×CO_2CH_3), 3.82 (dd, $J(8a,8b) = 10.6$, $J(8a,7) = 5.4$ Hz, 1H; H-8a), 3.78 (s, 3H; CO_2CH_3), 3.76–3.74 (m, 1H; H-5), 3.53 (dd, $J(8b,7) = 3.0$ Hz, 1H; H-8b), 3.49 (d, $J(6,7) = 8.2$ Hz, 1H; H-6), 3.20 (s, 3H; OCH_3), 2.87 (d, $J(OH,7) = 7.5$ Hz, 1H; OH), 2.52 (br s, 1H; OH), 2.17–2.08 (m, 11H; H-3_{ax}, H-3_{eq}, 3×$COCH_3$), 2.07, 2.06, 2.05, 1.98, and 1.97 ppm (5×s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): $\delta = 170.89$, 170.67, 170.12, 170.00, 169.47, 169.43, 169.38 and 169.29 (8×s, *C*OCH_3), 167.55, 166.96, and 166.78 (3×s, C-1, C-1′, C-1″), 102.10 and 100.42 (2×s, C-2′, C-2″), 98.90 (s, C-2), 70.33 (d, C-6), 70.13 (d, C-4), 69.23 (d, C-6′), 68.52 (d, C-6″), 68.34 (d, C-7), 67.73 (d, C-7′), 67.47 (d, C-7″), 67.12 (t, C-8), 65.19 and 65.09 (2×d, H-4′, H-4″), 63.22 (d, C-5), 63.21 (d, C-5′), 63.15 (d, C-5″), 62.10 (t, C-8″), 61.33 (t, C-8′), 53.25, 53.24, and 52.52 (3×q, CO_2CH_3), 50.81 (q, OCH_3), 32.77 (t, C-3), 22.34 and 21.41 (2×d, C-3′, C-3″), 20.91, 20.90, 20.78, 20.75, 20.71, 20.59, and 20.51 ppm (7×q, 8C, 8×$COCH_3$); HRMS (ESI-TOF): *m/z* calcd for $C_{44}H_{60}I_2O_{30}NH_4^+$: 1340.1597; found: 1340.1596.

Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→4)[methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→8)]-methyl (methyl 5,7-di-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (21): A solution of **20** (42.9 mg, 0.032 mmol) in anhydrous pyridine (5.0 mL) was treated with acetic anhydride (0.25 mL) and 4-(*N*,*N*-dimethylamino)pyridine (0.4 mg, 0.003 mmol) at 0 °C for 2.5 h. Excess reagent was destroyed by slow addition of anhydrous MeOH (0.5 mL) and the mixture was stirred for 10 min followed by removal of the solvent by coevaporation with toluene. Column chromatography (toluene/EtOAc, 1:1) of the residue provided **21** (43.2 mg, 95%) as a colorless oil; $[\alpha]_D^{20} = +47.1$ (*c* 0.74, $CHCl_3$); R_f 0.37 (toluene/EtOAc, 1:1); ^{1}H NMR ($CDCl_3$): $\delta = 5.40$–5.38 (m, 1H; H-5′), 5.36–5.31 (m, 3H; H-5″, H-7′, H-7″), 5.19–5.16 (m, 1H; H-5), 4.92 (dd, $J(4',3') = 4.6$, $J(4',5') = 3.7$ Hz, 1H, H-4′), 4.89 (dd, $J(4'',3'') = 4.8$, $J(4'',5'') = 3.7$ Hz, 1H; H-4″), 4.84 (ddd, $J(7,6) = 9.8$, $J(7,8a) = 3.8$, $J(7,8b) = 2.4$ Hz, 1H; H-7), 4.75 (dd, $J(8'a,8'b) = 12.4$, $J(8'a,7) = 2.8$ Hz, 1H; H-8′a), 4.64 (dd, $J(8''a,8''b) = 12.3$, $J(8''a,7) = 2.4$ Hz, 1H; H-8″a), 4.54–4.53 (m, 1H; H-3″), 4.41–4.39 (m, 1H; H-3′), 4.27–4.23 (m, 2H; H-4, H-6′), 4.19 (dd, $J(8''b,7'') = 3.8$ Hz, 1H; H-8″b), 4.14–4.10 (m, 2H; H-6″, H-8′b), 3.99 (dd, $J(6,5) = 1.2$ Hz, 1H; H-6), 3.89–3.86 (m, 4H; H-8a, CO_2CH_3), 3.83 (s, 3H; CO_2CH_3), 3.79 (s, 3H, CO_2CH_3), 3.68 (dd, $J(8b,8a) = 11.9$ Hz, 1H; H-8b), 3.28 (s, 3H; OCH_3), 2.27–2.23 (m, 1H; H-3_{eq}), 2.15–2.09 (m, 10H; H-3_{ax}, 3× $COCH_3$), 2.09, 2.07, and 2.066 (3×s, each 3H; CO*CH*$_3$), 2.04 (s, 6H; 2×$COCH_3$), 1.97 and 1.96 ppm (2×s, each 3H; $COCH_3$); ^{13}C NMR ($CDCl_3$): $\delta = 170.58$, 170.38, 170.09, 170.05, 169.80, 169.74, 169.43, 169.41, 169.39, and 169.25 (10×s, *C*OCH_3), 167.10, 166.15, and

165.47 (3×s, C-1, C-1′, C-1″), 102.10, 101.46, and 98.88 (3×s, C-2, C-2′, C-2″), 69.84 and 69.29 (2×d, C-4, C-6′), 68.61 (d, C-6″), 68.43 (d, C-7), 68.37 (d, C-6), 67.92 and 67.56 (2×d, C-7′, C-7″), 65.14 and 65.12 (2×d, C-5, C-4″), 64.99 (d, C-4′), 64.03 (t, C-8), 63.24 (d, C-5′), 63.01 (d, C-5″), 61.84 (t, C-8″), 61.46 (t, C-8′), 53.05, 52.90 and 52.60 (3×q, CO_2CH_3), 51.38 (q, OCH_3), 33.42 (t, C-3), 22.39 (d, C-3′), 21.36 (d, C-3″), 20.94, 20.88, 20.83, 20.76, 20.72, 20.65, 20.57, and 20.52 ppm (8×q, 10C, $COCH_3$); HRMS (ESI-TOF): *m/z* calcd for $C_{48}H_{64}I_2O_{32}Na^+$: 1429.1362; found 1429.1357.

Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)[methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)]-methyl (methyl 5,7-di-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (22): According to the general procedure for dehalogenation, **21** (38.2 mg, 0.027 mmol) was treated with lauroyl peroxide (6.5 mg, 0.016 mmol) in anhydrous cyclohexane (4.0 mL) for 4 h. Removal of the solvent and subsequent column chromatography (toluene/EtOAc 1:1) afforded **22** (27.6 mg, 88%) as a colorless oil; $[\alpha]_D^{20} = +79.4$ (*c* 0.91, $CHCl_3$); R_f 0.19 (toluene/EtOAc, 1:1; HP-TLC); ^{1}H NMR ($CDCl_3$): $\delta = 5.36–5.34$ (m, 1H; H-5′), 5.30–5.28 (m, 1H; H-5″), 5.21–5.13 (m, 5H; H-4′, H-4″, H-5, H-7′, H-7″), 5.00 (ddd, $J(7,6) = 9.8$, $J(7,8b) = 4.5$, $J(7,8a) = 2.4$ Hz, 1H; H-7), 4.66 (dd, $J(8'a,8'b) = 12.2$, $J(8'a,7') = 2.7$ Hz, 1H; H-8′a), 4.63 (ddd, $J(4,3_{ax}) = 12.0$, $J(4,3_{eq}) = 5.0$, $J(4,5) = 3.0$ Hz, 1H; H-4), 4.58 (dd, $J(8''a,8''b) = 12.4$, $J(8''a,7'') = 2.2$ Hz, 1H; H-8″a), 4.15–4.11 (m, 2H; H-6′, H-8″b), 4.09 (dd, $J(8'b,7') = 3.5$ Hz, 1H; H-8′b), 4.01 (dd, $J(6'',7'') = 9.6$, $J(6'',5'') = 1.4$ Hz, 1H; H-6″), 3.98 (dd, $J(6,5) = 1.2$ Hz, 1H; H-6), 3.87–3.83 (m, 4H; H-8a, CO_2CH_3), 3.80 (s, 3H; CO_2CH_3), 3.79 (s, 3H; CO_2CH_3), 3.73 (dd, $J(8b,8a) = 11.8$, 1H; H-8b), 3.28 (s, 3H; OCH_3), 2.24–2.20 (m, 1H; H-3″$_{eq}$), 2.18–2.00 (m, 23H; H-3$_{ax}$, H-3$_{eq}$, H-3′$_{ax}$, H-3′$_{eq}$, H-3″$_{ax}$, 6×$COCH_3$), 1.99, 1.98, 1.96, and 1.955 ppm (4×s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): $\delta = 170.89$, 170.49, 170.32, 170.28, 170.06, 169.83, 169.65, 169.63 and 169.62 (9×s, 10C, $COCH_3$), 167.47, 167.24, and 167.02 (3×s, C-1, C-1′, C-1″), 99.08, 98.94, and 97.38 (3×s, C-2, C-2′, C-2″), 69.32 (d, C-6′), 68.79 (d, C-6″), 68.48 and 68.43 (2×d, C-6, C-7), 67.78 (d, C-7′), 67.65 (d, C-7″), 66.33 (d, C-4′), 66.11 (d, 2C, C-4, C-4″), 65.03 (d, C-5), 64.33 (d, C-5′), 64.13 (d, C-5″), 62.39 (t, C-8), 61.89 (t, C-8″), 61.64 (t, C-8′), 52.70, 52.67, and 52.47 (3×q, CO_2CH_3), 51.19 (q, OCH_3), 33.96 (t, C-3), 31.44 (t, 2C, C-3′, C-3″), 20.76, 20.73, 20.71, 20.66, 20.64, 20.59, and 20.53 ppm (7×q, 10C, $COCH_3$); HRMS (ESI-TOF): *m/z* calcd for $C_{48}H_{66}O_{32}NH_4^+$: 1172.3875; found: 1172.3875.

Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)[sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)]-sodium (methyl α-D-*manno*-oct-2-ulopyranosid)onate (23): According to the general procedure for global deprotection, a solution of **22** (20.5 mg, 0.018 mmol) in anhydrous MeOH (2.0 mL) was treated with NaOMe (0.1 M in MeOH; 105 μL, 0.012 mmol), which was added in three portions within 24 h. Upon complete addition, stirring was continued for 8 h. After work-up as described above, the residue was dissolved in water (2.0 mL) and treated with aq. NaOH (0.1 M, 1.0 mL) for 3 h at RT. The solution was subjected to standard work-up und the crude product was desalted by successive purification on a PD10 (H_2O) and BioGel-P2 (H_2O/EtOH, 19:1) column followed by lyophilization of pooled fractions affording **23** (9.6 mg, 71%) as an amorphous colorless solid; $[\alpha]_D^{20} = +80.1$ (*c* 0.87, D_2O); ^{1}H NMR (D_2O, ca. pH 6.5): $\delta = 4.05–4.00$ (m, 4H; H-4, H-4′, H-4″, H-5), 3.98–3.82 (m, 7H; H-5′, H-5″, H-7, H-7′, H-7″, H-8′a, H-8″a), 3.69–3.55 (m, 5H; H-6′, H-6″, H-8a, H-8′b, H-8″b), 3.52–3.46 (m, 2H; H-6, H-8b), 3.09 (s, 3H; OCH_3), 2.08 (dd, $J(3''_{eq},3''_{ax}) = 13.4$, $J(3''_{eq},4'') = 4.5$ Hz, 1H; H-3″$_{eq}$), 2.01 (dd, $J(3'_{eq},3'_{ax}) = 13.2$, $J(3'_{eq},4') = 4.8$ Hz, 1H; H-3′$_{eq}$), 1.93–1.89 (m, 1H; H-3$_{eq}$), 1.84 (dd, $J(3_{ax},3_{eq}) = 12.9$, $J(3_{ax},4) = 11.6$ Hz, 1H; H-3$_{ax}$), 1.75 (dd, $J = 12.9$, $J = 12.2$ Hz, 1H; H-3′$_{ax}$), 1.72 ppm (dd, $J(3''_{ax},3''_{eq}) = 13.2$, $J(3''_{ax},4'') = 12.3$ Hz, 1H; H-3″$_{ax}$); ^{13}C NMR (D_2O, ca. pH 6.5): $\delta = 176.48$, 176.44, and 175.92 (3×s, C-1, C-1′, C-1″), 101.30, 101.22, and 100.43 (3×s, C-2, C-2′, C-2″), 73.04 (d, C-6), 72.34 and 72.26 (2×d, C-6′, C-6″), 70.77 and 70.10 (2×d, C-7′, C-7″), 69.70 (d, C-7), 68.69 (d, C-4), 67.15 and 67.10 (2×d, C-5′, C-5″), 66.85 and 66.68 (2×d, C-4′, C-4″), 65.98 (t, C-8), 65.23 (d, C-5), 63.83 and 63.81 (2×t, C-8′, C-8″), 51.40 (q, OCH_3), 35.36 and 34.87 ppm (2×t, C-3′, C-3″), 34.03 (t, C-3); HRMS (ESI-TOF): *m/z* calcd for $C_{25}H_{40}O_{22}Na^+$: 715.1903; found: 715.1901.

Methyl (2,6-anhydro-4,5,7-tri-*O*-acetyl-3-deoxy-8-*O*-triethylsilyl-D-*manno*-oct-2-enos)onate (24): Glycal **34** was prepared from **3** by standard transesterification catalyzed by sodium methoxide and was used without purification after neutralization and solvent evaporation. A suspension of glycal **34** (94 mg, 0.401 mmol) and 1,4-diazabicyclo[2.2.2]octane (77 mg, 0.682 mmol) in anhydrous MeCN (10.0 mL) was treated with chlorotriethylsilane (74 μL, 0.442 mmol) at ambient temperature for 1 h. After addition of two more portions of chlorotriethylsilane (14.8 μL, 0.088 mmol) within 30 min, the mixture was concentrated. The residue was partitioned between EtOAc and half-saturated aq $NaHCO_3$. The aqueous phase was extracted with EtOAc (3 times) and the combined organic phases were dried ($MgSO_4$). After filtration, the solvent was removed and the residual oil was dried under high vacuum. The residue was dissolved in anhydrous pyridine (8.0 mL) and treated with acetic anhydride (0.4 mL) in the presence of 4-(*N*,*N*-dimethylamino)pyridine (5 mg) for 4 h at ambient temperature. Excess reagent was destroyed at 0 °C by dropwise addition of anhydrous MeOH (2.0 mL) and, after stirring for 5 min, the solvent was removed by repeated coevaporation with toluene. The crude product was purified by column chromatography (toluene/EtOAc, 15:1) to provide **24** (129 mg, 62%) as a colorless oil; $[\alpha]_D^{20} = -18.1$ (*c* 1.40, $CHCl_3$); R_f 0.39 (toluene/EtOAc, 9:1); ^{1}H NMR ($CDCl_3$): $\delta = 5.87$ (app t, 1H; H-3), 5.71 (ddd, $J(5,4) = 4.6$, $J(5,3) = 2.2$, $J(5,6) = 1.4$ Hz, 1H; H-5), 5.47 (ddd, $J(4,3) = 2.0$, $J(4,6) = 1.1$ Hz, 1H; H-4), 5.09 (ddd, $J(7,6) = 9.6$, $J(7,8a) = 3.1$, $J(7,8b) = 2.1$ Hz, 1H; H-7), 4.47–4.44 (m, 1H; H-6), 3.96 (dd, $J(8a,8b) = 11.6$ Hz, 1H; H-8a), 3.86 (dd, $J(8a,8b) = 11.6$, $J(7,8b) = 2.1$ Hz, 1H; H-8b), 3.81 (s, 3H; CO_2CH_3), 2.06, 2.05, and 2.02 (3×s, each 3H, $COCH_3$), 0.93 [t, $J = 8.0$ Hz, 9H; $Si(CH_2CH_3)_3$], 0.59 ppm [q, *J* 8.0 Hz, 6H; $Si(CH_2CH_3)_3$]; ^{13}C NMR ($CDCl_3$): $\delta = 170.34$, 170.01, and 169.83 (3×s, $COCH_3$), 161.68 (s, C-1), 144.85 (s, C-2), 107.42 (d, C-3), 72.69 (d, C-6), 69.97 (d, C-7), 64.93 (d, C-5), 61.44 (d, C-4), 60.73 (t, C-8), 52.35 (q, CO_2CH_3), 20.83, 20.62, and 20.55 (3×q, $COCH_3$), 6.60 [q, 3C, $Si(CH_2CH_3)_3$], 4.22 ppm [t, 3C, $Si(CH_2CH_3)_3$]; HRMS (ESI-TOF): *m/z* calcd for $C_{21}H_{34}O_{10}SiNa^+$: 497.1813; found: 497.1821.

Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→8)-methyl (2,6-anhydro-4,5,7-tri-*O*-acetyl-3-deoxy-8-*O*-triethylsilyl-D-*manno*-oct-2-enos)onate (25) and methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→8)-methyl (2,4,5,7-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate (26): A solution of **24** (200 mg, 0.421 mmol) and **5** (277 mg, 0.506 mmol) in anhydrous CH_2Cl_2 (8.0 mL) was treated with $BF_3{\cdot}Et_2O$ (173 μL, 0.843 mmol) according to the general procedure for glycosylation. After 2 h, complete conversion was determined by TLC and the mixture was subjected to aqueous work-up. Subsequent column chromatography (toluene/EtOAc, 2:1) afforded a mixture of hydrolyzed donor and **25** (yield determined by NMR spectroscopic analysis ca. 320 mg, ca. 85%, see the Supporting Information Figure S2) as a yellow oil, which was used directly in the next

step. A solution of this mixture in glacial acetic acid (25 mL) was treated with *N*-iodosuccinimide at 65 °C for 46 h. The cold solution was slowly poured onto ice-cold saturated $NaHCO_3$ solution and diluted with chloroform. The phases were separated, the aqueous phases was once again extracted with chloroform and the combined organic phases were successively washed with aq $NaHCO_3$, aq $Na_2S_2O_3$ (5 w%) and brine. After drying ($MgSO_4$), filtration, and concentration, the crude product was purified by HP-column chromatography (toluene/EtOAc, 5:2) to give **26** (256 mg, 57% over two steps) as a colorless oil; $[\alpha]_D^{20} = +34.9$ (*c* 0.80, $CHCl_3$); R_f 0.34 (toluene/EtOAc, 3:2); ^{1}H NMR ($CDCl_3$): $\delta = 5.48–5.46$ (m, 1H; H-5), 5.41–5.37 (m, 2H; H-5′, H-7′), 5.07 (app td, $J(7,6) = 9.8$ Hz, 1H; H-7), 5.05 (dd, $J(4,3) = 4.9$, $J(4,5) = 3.7$ Hz, 1H; H-4), 4.76 (dd, $J(4',3') = 4.9$, $J(4',5') = 3.7$ Hz, 1H, H-4′), 4.63–4.60 (m, 2H; H-3′, H-8′a), 4.51 (dd, $J(3,5) = 0.9$ Hz, 1H; H-3), 4.30 (dd, $J(6,5) = 2.1$ Hz, 1H; H-6), 4.22 (dd, $J(8'b,8'a) = 12.4$, $J(8'b,7') = 4.9$ Hz, 1H; H-8′b), 4.20 (dd, $J(6',7') = 9.6$, $J(6',5') = 2.0$ Hz, 1H; H-6′), 3.96 (dd, $J(8a,8b) = 11.9$, $J(8a,7) = 2.3$ Hz, 1H; H-8a), 3.83 (s, 3H; CO_2CH_3), 3.79 (s, 3H; CO_2CH_3), 3.48 (dd, $J(8b,7) = 2.7$ Hz, 1H; H-8b), 2.19, 2.13, 2.13, 2.12, 2.09, 2.06, 2.03, and 2.00 ppm (8×s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): $\delta = 170.88$, 170.11, 169.87, 169.66, 169.64, 169.28, 169.22, and 168.21 (8×s, $COCH_3$), 166.60 and 165.31 (2×s, C-1, C-1′), 102.49 and 98.89 (2×s, C-2, C-2′), 68.74 (d, C-6), 68.67 (d, C-6′), 67.92 (d, C-7), 67.60 (d, C-7′), 65.47 (d, C-4′), 64.98 (d, C-4), 63.54 (t, C-8), 63.26 (d, C-5′), 63.03 (d, C-5), 62.36 (t, C-8′), 53.11 and 53.07 (2×q, CO_2CH_3), 22.10 (d, C-3′), 20.95, 20.85, 20.81, 20.80, 20.77, 20.68, 20.65, and 20.52 ppm (9C, C-3, 8×$COCH_3$); HRMS (ESI-TOF): *m/z* calcd for $C_{34}H_{44}I_2O_{23}NH_4^+$: 1092.0701; found: 1092.0708.

Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→8)-methyl (4,5,7-tri-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate fluoride (27): A solution of **26** (125 mg, 0.116 mmol) in anhydrous CH_2Cl_2 (3.1 mL) was treated with hydrogen fluoride/pyridine (ca. 70% HF, ca. 30% pyridine; 0.8 mL) at 0 °C for 1 h in a sealed Teflon flask. Ice-cold water was added, the phases were mixed thoroughly and separated. The aqueous phase was once again extracted with CH_2Cl_2 and the combined organic extracts were washed with aq $NaHCO_3$, dried ($MgSO_4$), filtered, and concentrated. The crude product was quickly purified by flash chromatography (*n*-hexane/EtOAc 1:1) affording **27** (113 mg, 94%) as a colorless oil; $[\alpha]_D^{20} = +34.5$ (*c* 0.58, $CHCl_3$); R_f 0.38 (*n*-hexane/EtOAc, 1:1); ^{1}H NMR ($CDCl_3$): $\delta =$ 5.50–5.48 (m, 1H, H-5), 5.39 (ddd, $J(7',6') = 9.8$, $J(7',8'b) = 4.8$, $J(7',8'a) = 2.3$ Hz, 1H; H-7′), 5.31–5.30 (m, 1H; H-5′), 5.22–5.19 (m, 1H; H-7), 4.99 (dd, $J(4,3) = 5.0$, $J(4,5) = 3.6$ Hz, 1H; H-4), 4.90 (dd, $J(4',3') = 4.8$, $J(4',5') = 3.7$ Hz, 1H; H-4′), 4.74–4.69 (m, 2H; H-3, H-8′a), 4.66 (dd, $J(6,7) = 9.9$, $J(6,5) = 1.8$ Hz, 1H; H-6), 4.58 (dd, $J(3',5') = 0.8$ Hz, 1H; H-3′), 4.23 (dd, $J(6',5') = 1.9$ Hz, 1H; H-6′), 4.21 (dd, $J(8'b,8'a) = 12.4$ Hz, 1H; H-8′b), 4.01 (dd, $J(8a,8b) = 11.2$, $J(8a,7) = 3.5$ Hz, 1H; H-8a), 3.89 (s, 3H; CO_2CH_3), 3.83 (s, 3H; CO_2CH_3), 3.43 (dd, $J(8b,7) = 1.6$ Hz, 1H; H-8b), 2.14, 2.12, 2.11, 2.09, 2.05, 2.05, and 1.99 ppm (7×s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): $\delta = 170.61$, 170.15, 169.77, 169.51, 169.49, 169.12 and 169.02 (7×s, $COCH_3$), 165.93 (s, C-1′), 162.84 (ds, $J(1,F) = 27.8$ Hz; C-1), 109.03 (ds, $J(2,F) = 230.2$ Hz; C-2), 101.66 (s, C-2′), 69.74 (dd, $J(6,F) = 4.9$ Hz; C-6), 68.60 (d, C-6′), 67.62 (d, C-7′), 67.47 (d, C-7), 65.30 (d, C-4′), 64.56 (d, C-4), 64.01 (t, C-8), 63.08 (d, C-5′), 62.77 (d, C-5), 62.13 (t, C-8′), 53.41 (q, CO_2CH_3), 53.03 (q, CO_2CH_3), 21.37 (d, C-3′), 20.90, 20.78, 20.74, 20.67, 20.58 and 20.51 (6×q, 7C, $COCH_3$), 18.32 ppm (dd, $J(3,F) = 30.6$ Hz; C-3); ^{19}F NMR ($CDCl_3$): $\delta = -103.32$ ppm; HRMS (ESI-TOF): *m/z* calcd for $C_{32}H_{41}FI_2O_{21}NH_4^+$: 1052.0552; found: 1052.0570.

Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→8)-methyl (4,5,7-tri-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→4)-methyl (methyl 7,8-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid) onate (28): A suspension of **10** (11.4 mg, 0.039 mmol) and **27** (48.4 mg, 0.047 mmol) in anhydrous CH_2Cl_2 (2.0 mL) containing 5 Å ground molecular sieves (ca. 50 mg) was stirred at ambient temperature for 2 h under an atmosphere of argon. The mixture was then cooled to 0 °C and trifluoromethanesulfonic acid (1.1 μL, 0.006 mmol) in anhydrous CH_2Cl_2 (0.2 mL) was added dropwise. After stirring at ambient temperature for 1 h, aq. $NaHCO_3$ and CH_2Cl_2 were added, the phases were separated and the aqueous phase was once again extracted with CH_2Cl_2. The combined organic phases were washed consecutively with aq. $NaHCO_3$, aq. $Na_2S_2O_3$ (5w%), and brine, dried ($MgSO_4$), filtered, and concentrated. The crude mixture was purified by column chromatography (*n*-hexane/EtOAc, 1:1→1:2) to provide **28** (37.2 mg, 73%) as a colorless amorphous solid; $[\alpha]_D^{20} = +53.8$ (*c* 0.67, $CHCl_3$); R_f 0.22 (toluene/EtOAc, 1:1; HP-TLC); ^{1}H NMR ($CDCl_3$): $\delta = 5.43–5.36$ (m, 4H; H-5′, H-5″, H-7′, H-7″), 4.92 (ddd, $J(7,8b) = 8.3$, $J(7,8a) = 6.9$, $J(7,6) = 4.0$ Hz, 1H; H-7), 4.89 (dd, $J(4'',3'') = 4.7$, $J(4'',5'') = 3.5$ Hz, 1H; H-4″), 4.86 (dd, $J(4',3') = 4.9$, $J(4',5') = 3.4$ Hz, 1H; H-4′), 4.74–4.70 (m, 2H; H-8a, H-8″a), 4.55 (app t, $J(8b,8a) = 9.0$ Hz, 1H; H-8b), 4.52–4.49 (m, 2H; H-3′, H-3″), 4.24–4.20 (m, 2H; H-6″, H-8″b), 4.18 (ddd, $J(4,3_{ax}) = 11.8$, $J(4,3_{eq}) = 5.2$, $J(4,5) = 3.1$ Hz, 1H; H-4), 4.06 (dd, $J(6',7') = 7.6$, $J(6',5') = 2.0$ Hz, 1H; H-6′), 3.88–3.85 (m, 7H; H-6, 2×CO_2CH_3), 3.80 (s, 3H; CO_2CH_3), 3.79–3.74 (m, 2H; H-8′a, H-8′b), 3.68–3.66 (m, 1H; H-5), 3.27 (s, 3H; OCH_3), 2.45–2.43 (m, 1H; OH), 2.14 (s, 3H; $COCH_3$), 2.12 (s, 3H; $COCH_3$), 2.10 (app t, 1H; H-3_{ax}), 2.10, 2.09, 2.06, 2.05, and 1.98 (5×s, each 3H, $COCH_3$), 1.95 ppm (dd, $J(3_{eq},3_{ax}) =$ 12.5 Hz, 1H; H-3_{eq}); ^{13}C NMR ($CDCl_3$): $\delta = 170.39$, 170.10, 169.91, 169.48, 169.36, 169.31 and 169.27 (7×s, $COCH_3$), 167.14, 166.94, and 165.44 (3×s, C-1, C-1′, C-1″), 154.60 [s, O(*C*=O)O], 101.25 and 100.54 (2×s, C-2′, C-2″), 98.87 (s, C-2), 75.69 (d, C-7), 71.03 (d, C-6′), 70.46 (d, C-6), 69.34 (d, C-4), 68.56 (d, C-7′), 68.41 (d, C-6″), 67.62 (d, C-7″), 66.11 (t, C-8), 65.27 (d, C-4″), 64.99 (d, C-4′), 64.36 (d, C-5), 64.31 (t, C-8′), 63.12 (d, C-5′), 63.03 (d, C-5″), 62.06 (t, C-8″), 53.43, 53.01, and 52.86 (3×q, CO_2CH_3), 51.31 (q, OCH_3), 32.77 (t, C-3), 21.56 and 21.24 (2×d, C-3′, C-3″), 20.92, 20.86, 20.77, 20.73, 20.72, 20.51, and 20.39 ppm (7×q, $COCH_3$); HRMS (ESI-TOF): *m/z* calcd for $C_{43}H_{56}I_2O_{30}NH_4^+$: 1324.1284; found: 1324.1275.

Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→8)-methyl (4,5,7-tri-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→4)-methyl (methyl 5-*O*-acetyl-7,8-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (29): A solution of **28** (36.4 mg, 0.028 mmol) in anhydrous pyridine (2.0 mL) was treated with acetic anhydride (0.1 mL) and 4-(*N*,*N*-dimethylamino)-pyridine (0.5 mg, 0.004 mmol) at ambient temperature for 12 h. Excess reagent was destroyed by slow addition of anhydrous MeOH (1 mL) at 0 °C and, after 10 min, the solvent was removed by coevaporation with toluene (3×). The residue was purified by column chromatography (*n*-hexane/EtOAc, 2:3) to give **29** (35.5 mg, 95%) as a colorless amorphous solid; $[\alpha]_D^{20} = +61.9$ (*c* 0.62, $CHCl_3$); R_f 0.44 (*n*-hexane/EtOAc 1:2); ^{1}H NMR ($CDCl_3$): $\delta = 5.41–5.35$ (m, 4H; H-5′, H-5″, H-7′, H-7″), 5.05–5.02 (m, 1H; H-5), 4.90 (dd, $J(4'',3'') = 4.7$, $J(4'',5'') = 3.6$ Hz, 1H; H-4″), 4.86 (dd, $J(4',3') = 4.6$, $J(4',5') = 3.7$ Hz, 1H; H-4′), 4.80 (ddd, $J(7,8b) = 8.3$, $J(7,8a) = 6.1$, $J(7,6) = 3.6$ Hz, 1H; H-7), 4.72 (dd, $J(8''a,8''b) = 12.4$, $J(8''a,7'') = 2.4$ Hz, 1H; H-8″a), 4.57 (dd, $J(8a,8b) =$ 8.4 Hz, 1H; H-8a), 4.52–4.50 (m, 1H; H-3″), 4.37–4.32 (m, 2H; H-3′, H-8b), 4.27–4.21 (m, 3H; H-4, H-6″, H-8″b), 4.05 (dd, $J(6',7') = 7.7$, $J(6',5') = 2.1$ Hz, 1H; H-6′), 3.98 (dd, $J(6,5) = 1.0$ Hz, 1H; H-6), 3.87,

3.83, and 3.81 (3×s, each 3H; CO_2CH_3), 3.79–3.76 (m, 2H; H-8′a, H-8′b), 3.27 (s, 3H; OCH_3), 2.16–2.11 (m, 1H; H-3_{ax}), 2.15, 2.13, 2.11, 2.09, and 2.089 (5×s, each 3H; $COCH_3$), 2.06–2.01 (m, 1H; H-3_{eq}), 2.04, 2.036, and 1.97 ppm (3×s, each 3H; $COCH_3$); ^{13}C NMR ($CDCl_3$): δ = 170.35, 170.14, 170.06, 170.02, 169.41, 169.35, 169.33, and 169.26 (8×s, *C*OCH_3), 166.95, 165.47, and 165.44 (3×s, C-1, C-1′, C-1″), 154.09 [s, O(C=O)O], 101.28 and 101.12 (2×s, C-2′, C-2″), 98.85 (s, C-2), 74.97 (d, C-7), 71.19 (d, C-6′), 70.37 (d, C-6), 68.86 (d, C-7′), 68.44 and 68.31 (2×d, C-4, C-6″), 67.75 (d, C-7″), 66.40 (d, C-5), 65.32 (d, C-4″), 64.90 (d, C-4′), 64.66 and 64.54 (2×t, C-8, C-8′), 63.30 (d, C-5′), 63.18 (d, C-5″), 61.92 (t, C-8″), 53.29, 53.05, and 52.94 (3×q, CO_2CH_3), 51.56 (q, OCH_3), 34.10 (t, C-3), 21.75 (d, C-3′), 21.48 (d, C-3″), 20.97, 20.89, 20.84, 20.74, 20.54, and 20.41 ppm (6×q, 8C, $COCH_3$); HRMS (ESI-TOF): *m/z* calcd for $C_{45}H_{58}I_2O_{31}NH_4^+$: 1366.1390; found: 1366.1338.

Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-methyl (4,5,7-tri-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-methyl (methyl 5-*O*-acetyl-7,8-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (30): By following the general procedure for dehalogenation, **29** (26.6 mg, 0.020 mmol) was treated with lauroyl peroxide (4.7 mg, 0.012 mmol) in anhydrous cyclohexane (4.0 mL) for 24 h. Evaporation of the solvent and purification of the residue by column chromatography (*n*-hexane/EtOAc, 1:2) afforded **30** (19.2 mg, 89%) as an amorphous white solid; $[\alpha]_D^{20} = +82.3$ (*c* 0.43, $CHCl_3$); R_f 0.24 (toluene/EtOAc, 1:2, HP-TLC); ^{1}H NMR ($CDCl_3$): δ = 5.35–5.30 (m, 3H; H-5′, H-5″, H-7′), 5.26–5.21 (m, 2H; H-5, H-7″), 5.14–5.09 (m, 2H; H-4′, H-4″), 4.76 (ddd, *J*(7,8b) = 8.3, *J*(7,8a) = 6.4, *J*(7,6) = 4.3 Hz, 1H; H-7), 4.64 (dd, *J*(8″a,8″b) = 12.3, *J*(8″a,7″) = 2.4 Hz, 1H; H-8″a), 4.62 (dd, *J*(8a,8b) = 8.5 Hz, 1H; H-8a), 4.55 (ddd, *J*(4,3_{ax}) = 11.8, *J*(4,3_{eq}) = 5.0, *J*(4,5) = 2.9 Hz, 1H; H-4), 4.39 (app t, 1H; H-8b), 4.21 (dd, *J*(8″b,7″) = 4.9 Hz, 1H; H-8″b), 4.08 (dd, *J*(6″,7″) = 9.8, *J*(6″,5″) = 1.4 Hz, 1H; H-6″), 4.05 (dd, *J*(6,5) = 1.4 Hz, 1H; H-6), 3.94 (dd, *J*(8′a,8′b) = 10.6, *J*(8′a,7′) = 2.9 Hz, 1H; H-8′a), 3.89 (dd, *J*(6′,7′) = 8.1, *J*(6′,5′) = 1.4 Hz, 1H; H-6′), 3.84, 3.83, and 3.80 (3×s, each 3H, CO_2CH_3), 3.66 (dd, *J*(8′b,7′) = 9.5 Hz, 1H; H-8′b), 3.27 (s, 3H, OCH_3), 2.14–1.94 ppm (m, 30H; 8×$COCH_3$, H-3_{ax}, H-3_{eq}, H-$3'_{ax}$, H-$3'_{eq}$, H-$3''_{ax}$, H-$3''_{eq}$); ^{13}C NMR ($CDCl_3$): δ = 170.39, 170.31, 169.99, 169.96, 169.88, 169.64, and 169.54 (7×s, 8C, *C*OCH_3), 167.14, 167.03, and 166.94 (3×s, C-1, C-1′, C-1″), 154.16 [s, O(C=O)O], 99.12, 98.31, and 97.75 (3×s, C-2, C-2′, C-2″), 74.87 (d, C-7), 71.37 (d, C-6′), 70.38 (d, C-6), 68.84 (d, C-7′), 68.47 (d, C-6″), 67.86 (d, C-7″), 66.63 (d, C-5), 66.34, and 66.20 (2×d, C-4′, C-4″), 65.93 (d, C-4), 65.21 (t, C-8), 64.24 (d, C-5′), 64.21 (d, C-5″), 62.79 (t, C-8′), 62.03 (t, C-8″), 53.05, 52.83, and 52.69 (3×q, CO_2CH_3), 51.35 (q, OCH_3), 34.16 (t, C-3), 31.54, and 31.35 (2×t, C-3′, C-3″), 20.72, 20.67, 20.61, and 20.54 ppm (4×q, 8C, $COCH_3$); HRMS (ESI-TOF): *m/z* calcd for $C_{45}H_{60}O_{31}NH_4^+$: 1114.3457; found: 1114.3440.

Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-sodium (methyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (31): According to the general procedure for global deprotection, a solution of **30** (10.2 mg, 0.009 mmol) in anhydrous MeOH (2.0 mL) was treated with NaOMe (0.1 M in MeOH; 157 μL, 0.015 mmol), which was added in three portions within 24 h. After complete addition, stirring was continued for 4 h. After the described work-up, the residue was dissolved in water (2.0 mL) and treated with aq. NaOH (0.1 M, 1.0 mL). After 3 h the mixture was subjected to standard work-up und the crude product was desalted by purification on a PD10 column (H_2O) followed by lyophilization of the pooled fractions to afford **31** (6.5 mg, 93%) as an amorphous colorless solid; $[\alpha]_D^{20} = +82.6$ (*c* 0.64, D_2O); ^{1}H NMR (D_2O, ca. pH 6.5): δ = 4.12–4.06 (m, 2H; H-7′, H-4″), 4.03–3.99 (m, 4H; H-4′, H-5, H-5′, H-5″), 3.97–3.84 (m, 5H; H-4, H-7, H-7″, H-8a, H-8″a), 3.70–3.64 (m, 3H; H-6″, H-8′a, H-8″b), 3.62–3.55 (m, 2H; H-6′, H-8b), 3.47 (dd, *J*(8′b,8′a) = 10.2, *J*(8′b,7′) = 8.3 Hz, 1H; H-8′b), 3.44 (dd, *J*(6,7) = 8.8, *J*(6,5) = 1.0 Hz, 1H; H-6), 3.09 (s, 3H; OCH_3), 2.06 (ddd, *J*($3'_{eq}$,$3'_{ax}$) = 13.3, *J*($3'_{eq}$,4′) = 4.8, *J*($3'_{eq}$,5′) = 0.8 Hz, 1H; H-$3'_{eq}$), 2.03–1.99 (m, 1H; H-$3''_{eq}$), 1.90–1.71 ppm (m, 4H; H-3_{eq}, H-3_{ax}, H-$3'_{ax}$, H-$3''_{ax}$); ^{13}C NMR (D_2O, ca. pH 6.5): δ = 177.00, 176.14, and 175.91 (3×s, C-1, C-1′, C-1″), 101.32, 100.76, and 100.42 (3×s, C-2, C-2′, C-2″), 73.10 (d, C-6′), 72.09 and 72.01 (2×d, C-6, C-6″), 70.47, 70.45, and 70.26 (3×d, C-7, C-7′, C-7″), 69.76 (d, C-4), 67.55 and 67.27 (2×d, C-5′, C-5″), 66.85 and 66.68 (2×d, C-4′, C-4″), 65.05 (d, C-5), 65.00 (t, C-8′), 64.02 (2×t, C-8, C-8″), 51.40 (q, OCH_3), 35.31 and 35.09 (2×t, C-3′, C-3″), 34.02 ppm (t, C-3); HRMS (ESI-TOF): *m/z* calcd for $C_{25}H_{40}O_{22}Na^+$: 715.1903; found: 715.1938.

Acknowledgements

This work was supported by the Austrian Science Fund, FWF (grant P 24921).

Keywords: carbohydrates · diastereoselectivity · glycosylation · immunochemistry · oligosaccharides

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Received: September 26, 2014
Published online on October 29, 2014

Manuscript #3

Pokorny, B.; Kosma, P.* *manuscript draft*.

"Synthesis of branched oligosaccharides comprising the conserved Kdo (3-deoxy-D-manno-2-octulosonic acid) disaccharide substituted with α-(1→5) D-glucose"

Synthesis of branched oligosaccharides comprising the conserved Kdo (3-deoxy-D-manno-2-octulosonic acid) disaccharide substituted with α-(1→5) D-glucose

Barbara Pokorny, Paul Kosma*
Department of Chemistry, University of Natural Resources and Life Sciences,
Muthgasse 18, A-1190 Vienna, Austria

Abstract

The heptose-deficient inner core of the lipopolysaccharide of several pathogenic strains of the Moraxellaceae (*Moraxella*, *Acinetobacter*) family and *Bartonella henselae* comprises an α-(1→5)-linked glucose attached to the proximal unit of the conserved α-(2→4)-interconnected Kdo disaccharide. In the course of our project to synthesize inner core fragments of the *Acinetobacter haemolyticus* LPS to reveal the carbohydrate epitope in an untypically strong lectin-inner core interaction, we have developed a method to synthesize the branched α-Kdo(2→4)[α-Glc(1→5)]-α-Kdo motif with good stereoselectivities and yields. Subsequent regioselective deblocking provided a trisaccharide acceptor giving access to the tetra- and pentasaccharide structures of the *A. haemolyticus* inner core.

Keywords: oligosaccharide synthesis, *Acinetobacter,* glycosylation

* Corresponding author. Phone: *43-1-47654-6055. Fax: *43-1-47654-6059. *E-mail address*: paul.kosma@boku.ac.at

1. Introduction

The gram-negative bacterial cell wall is frequently covered by densely packed lipopolysaccharide (LPS) molecules which are involved in immune response of the respective host during pathogenesis.[1,2] Structurally, these glycolipids comprise a small endotoxically active lipid anchor (lipid A) and an extended oligosaccharide fraction.[3] In the outermost sphere the strain-specific O-antigen polysaccharide is located, which is, however, missing in rough-type mutants.[4] The core region constitutes the connection between these two compartments and is further divided into an inner and an outer core.[5] Rare sugars like heptoses or octulosonic acids and limited variability within a genus are hallmarks for the inner core domain, while the less conserved outer core is composed of common hexoses like D-galactose and D-glucose (Glc). The 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) commonly connects the inner core to lipid A, but it can be non-stoichiometrically replaced by its 3-oxy analogue D-*glycero*-D-*talo*-2-octulosonic

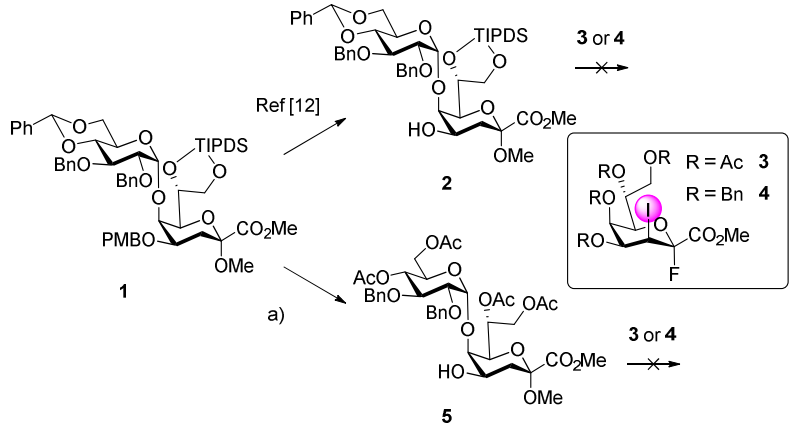
acid (Ko) in *Acinetobacter*.[6,7] In various species this first core unit is extended by a second α-(2→4)-linked Kdo moiety.[5] For the majority of species this Kdo_2 disaccharide is 5-O-substituted on the proximal Kdo by L-*glycero*-D-*manno*-heptose, but D-mannose, D-galactosamine and Kdo itself were identified as branching entities in others. In the Moraxellaceae family the branching sugar is often an α-(1→5)-linked Glc reside. The inner core of *M. catarrhalis* predicates on this trisaccharide motif and was shown to elicit cross-reactive antibodies recognizing different serotypes.[8] In addition, *Bartonella henselae* – the causative agent of the cat-scratch disease - expresses only this truncated trisaccharide linked to lipid A.[9] Furthermore, in *A. haemolyticus* this basic pattern is elongated at the glucose moiety by a β-(1→4)-linked isomaltotriose.[10] The observation of an untypically high binding affinity of this isolated inner core to different collectins[11] initiated the project to synthesize fragments thereof to identify the responsible carbohydrate epitope.[12]

Previously, stereoselective α-(1→5)-coupling of Glc to Kdo monosaccharide acceptors was successfully performed by Oscarson et al.[13] and us[12]. We capitalized on an orthogonal blocking group at position-4 of the octulosonic acid as introduction of the lateral Kdo was foreseen at a later stage. In contrast, Ichiyanagi et al. recently reported on the convenient preparation of 5-O-substituted Kdo_2 trisaccharides applying a Kdo_2 acceptor for heptose, D-mannose and 2-azido-2-deoxy-D-galactose (as a precursor for D-galactosamine) donors, however, no results for glucose have been reported.[14] Thus, a protocol towards the α-Kdo(2→4)[α-Glc(1→5)]-α-Kdo motif is needed and in the present work we present a high-yielding method to access the branched trisaccharide with reasonable stereoselectivity.

2. Results and discussion

2.1. Using an α-Glc(1→5)Kdo acceptor

Previously, we have prepared the α-Glc(1→5)Kdo disaccharide **1** with excellent stereoselectivity and in a high yield.[12] An orthogonal protecting group pattern allowed for selective cleavage of the *p*-methoxybenzyl group (PMB) affording the 4-OH acceptor **2**. Starting from **2** we envisaged to introduce the lateral Kdo moiety capitalizing on one of the respective 3-iodo-2-fluoride Kdo donors **3** and **4**, which we recently reported to yield only the α-anomer without significant elimination side reaction in a range of glycosylations.[15] The acetyl protected donor **3** was capable of regioselective coupling to a 4,5-diol Kdo acceptor yielding the α-(2→4)-interconnected Kdo disaccharide as a single isomer.[15a] However, its glycosylating ability was abolished when base (triethylamine, *sym*-collidine) was added to capture hydrogen fluoride. Due to incompatibility of the 7,8-O-siloxane and the 4,6-O-acetal groups in acceptor **2** towards glycosylation conditions lacking a HF-scavenger, these protecting groups were changed for acetyl groups. However, no trisaccharide formation was detected for acceptor **5** upon reaction with acetyl-protected donor **3** under $BF_3.Et_2O$ promotion.

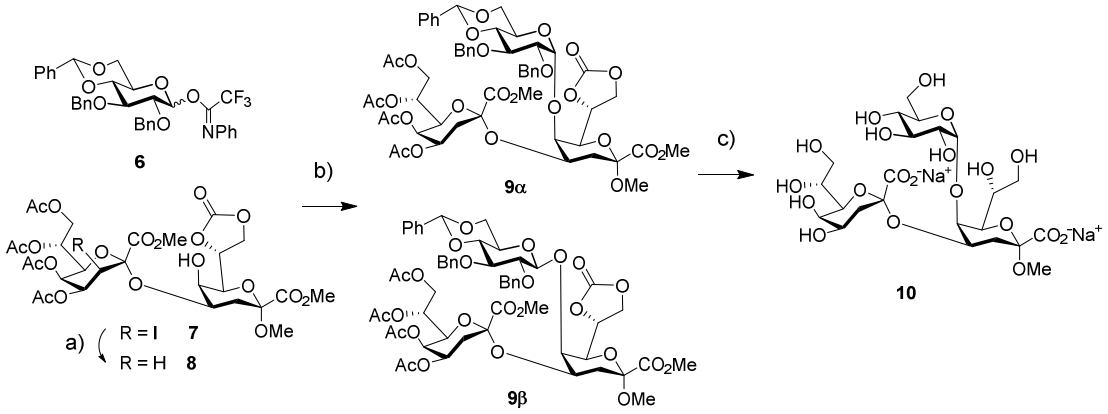


Scheme 1: Reagents and conditions: a) *p*TosOH, MeOH, 40 °C, 2 h; then TBAF, THF, 0 °C, 20 min; then Ac_2O, DMAP, pyridine, r.t., 14 h; then DDQ, CH_2Cl_2/MeOH (3:1), r.t., 3.5 h, 40% (4 steps).

In contrast, benzylated donor **4** readily glycosylated 2-propanol under $BF_3.Et_2O$ promotion in the presence of triethylamine, although higher temperatures were necessary compared to conditions devoid of base.[15b] While orthogonally protected acceptor **2** degraded upon attempted coupling with benzylated donor **4** in absence of base, addition of trimethylamine suppressed any trisaccharide formation. In a last attempt we intended to capitalize on the high reactivity of **4** and the compatibility of **5** towards base-free conditions. Despite the laborious blocking group exchange, no trisaccharide could be isolated.

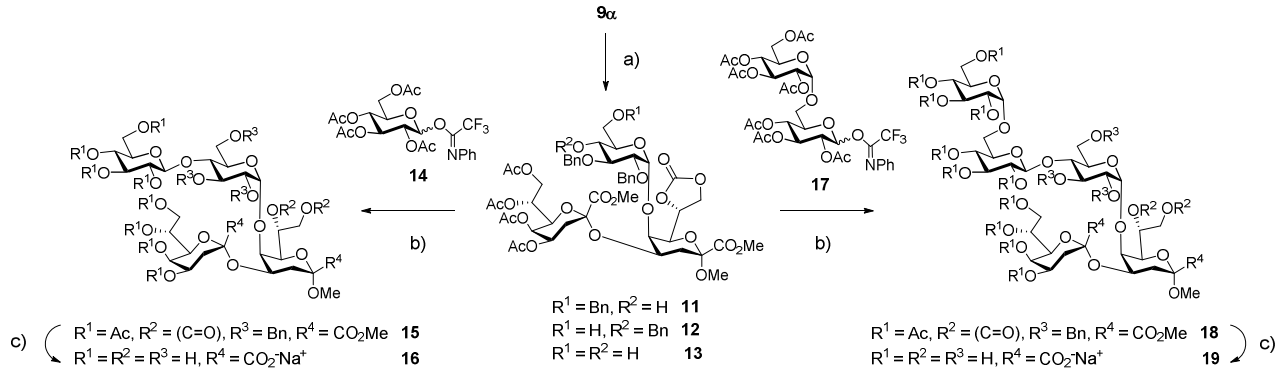
2.2. Using an α-Kdo(2→4)Kdo acceptor

To nevertheless benefit from the typically high stereoselectivities encountered for the 3-iodo donors **3** or **4** we decided to link a suitably protected glucose donor to a preassembled α-Kdo(2→4)Kdo disaccharide acceptor. Thus, one could capitalize on the previously elaborated route towards Kdo_2 **7**, which was obtained in high yields and without any β-formation.[18a] Recently, Ichiyanagi *et al.* successfully substituted a Kdo_2 acceptor with heptose and mannose trichloroacetimidates exploiting the trans-directing effect of simple C-2 ester groups.[17] We were confident, that an appropriately decorated glucose donor would be similarly capable to form this linkage, and stereocontrol would rely on optimized remote protecting groups and reaction conditions. Therefore, the 5-OH acceptor **8** was obtained from iodo-prescursor **7** by hydrogen atom transfer[16] in cyclohexane catalyzed by lauroyl peroxide. Previously, dehalogenation depended on fully blocked compounds to guarantee sufficient solubility in cyclohexane for smooth conversion. However, using a mixture of cyclohexane and 1,2-dichloroethane[16b] allowed for direct and clean dehalogenation of disaccharide **7** providing acceptor **8** in high yields (92%).



Scheme 2: Reagents and conditions: a) Lauroyl peroxide, cyclohexane/$ClCH_2CH_2Cl$ (7.2:1), reflux, 2h, 92%; b) TfOH, 5 Å molecular sieves, CH_2Cl_2/Et_2O (2:1), r.t., 3h, 91% (α/β 4:1, isolated **9α**: 67%, **9β**: 16%); c) Pd-C (10%), H_2 (1 atm), MeOH, r.t., 4 h; then NaOMe, MeOH, r.t., 17 h; then aq. NaOH, 0 °C → r.t., 4h, 94%.

Indeed, coupling of 5-OH acceptor **8** with the 4,6-O-benzylidene protected donor **6**[12] under TMSOTf catalysis and in CH_2CH_2 in the presence of ground 4 Å molecular sieves (MS) provided traces of both α- and β-trisaccharides **9α** and **9β**. Notably, by changing the solvent to diethyl ether, the α/β-ratio could be increased significantly. The observation that glycoside formation ceased after a short initial phase lead to the assumption that TfOH formed by partial hydrolysis of TMSOTf may be the active promotor, which would be rapidly captured by the 4 Å MS. Indeed, applying TfOH as activator in the presence of 5 Å MS improved the total yield to 31%. This still disappointing outcome was attributable to donor degradation that proceeded faster than glycoside formation. Eventually, controlled addition of donor **6** to a stirred mixture of acceptor **8** and promotor in diethyl ether containing molecular sieves (5 Å) afforded a mixture of anomers **9α** and **9β** in a total yield of 91% (α:β = 4:1) which could be conveniently separated on a HPLC column (isolated yield of α-product **9α**: 67%). Interestingly, suspected lactone formation[17] between C-1' and the free 5-hydroxyl group was observed to a low extent. Sequential removal of benzyl (Pd/C, H_2) and acetyl (NaOMe) groups from trisaccharide **9α** and ensuing methyl ester saponification under alkaline conditions (NaOH) yielded trisaccharide **10** (94%).



Scheme 3: Reagents and conditions: a) Triethylsilane, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C, 1h, **11**: 72%, **12**: 5%, **13**: 2% ; b) TfOH, 5 Å molecular sieves, CH_2Cl_2, 0 °C, 15 min (for →**15**)/45 min (for →**18**), **15**: 46%, **18**: 71%; c) Pd-C (10%), H_2 (1 atm), MeOH, r.t., 3 h (for →**16**)/4 h (for →**19**); then NaOMe, MeOH, r.t., 14 h (for →**16**)/20 h (for →**19**) ; then aq. NaOH, 0 °C → r.t., 3h, **16**: 99%, **19**: 90%.

Alternatively, regioselective opening of the benzylidene group in compound **9α** to provide the 6-OBn protected **11** was foreseen**,** which would serve as an acceptor for the extended glucose-rich core structure of *A. haemolyticus*. Thus, treatment of trisaccharide **9α** with triethylsilane/$BF_3.Et_2O$ in dry dichloromethane afforded the desired 4-OH **11** as the major product (72%) together with its 6-OH regioisomer **12** (5%) and traces of diol **13** (2%). Using the acetyl protected glucose donor **14**[18] under TfOH promotion tetrasaccharide **15** was obtained, however, the yield (46%) suffered due to several purification steps. Using the same strategy with the isomaltose donor **17**, the pentasaccharide **18** was quickly accessible in a 3+2 approach in reasonable yields (71%). Notably, for both glycosylation steps no α-anomers were detected and the side products mainly originated from the degraded donors. Global deprotection and ester saponification afforded the oligosaccharide ligands **16** (99%) and **19** (90%) in excellent yields.

2.3. Conclusions and outlook

In summary, a convenient method to prepare the α-Kdo(2→4)[α-Glc(1→5)]-α-Kdo trisaccharide capitalizing on a Kdo_2 disaccharide acceptor was established. Good α-selectivity relied on diethyl ether as a solvent and high yields were obtained by slowly adding the donor to a pre-stirred mixture of the Kdo_2 and TfOH as a promotor. The 4,6-O-benzylidene blocking group of the donor not only enhanced the α-selectivity but also gave access to extended oligosaccharides after regioselective opening. Thus, three fragments of the *A. haemolyticus* inner core were obtained which will serve as ligands in binding studies with the respective collectins.

3. Experimental

3.1. General

All purchased chemicals were used without further purification unless stated otherwise. The promotor $BF_3{\cdot}Et_2O$ was used as a solution in diethyl ether (≥46% according to the manufacturer). Solvents were dried over activated 3 Å (acetone, Et_2O) or 4 Å (CH_2Cl_2, $ClCH_2CH_2Cl$, cyclohexane, *N,N*-dimethylformamide, pyridine) molecular sieves. Dry MeOH (Merck) and dry THF (Sigma-Aldrich) were purchased. Cation exchange resin DOWEX 50 H^+

was regenerated by consecutive washing with HCl (3 M), water and dry MeOH. Aqueous solutions of salts were saturated unless stated otherwise. Concentration of organic solutions was performed under reduced pressure < 40 °C. Optical rotations were measured with a Perkin-Elmer 243 B Polarimeter. $[\alpha]_D^{20}$ values are given in units of 10^{-1}deg cm^2g^{-1}. Thin layer chromatography was performed on Merck precoated plates: generally on 5 x 10 cm, layer thickness 0.25 mm, Silica Gel $60F_{254}$; alternatively on HPTLC plates with 2.5 cm concentration zone (Merck). Spots were detected by dipping reagent (anisaldehyde-H_2SO_4). For column chromatography silica gel (0.040 – 0.063 mm) was used. HP-column chromatography was performed on pre-packed columns (YMC-Pack SIL-06, 0.005 mm, 250x10 mm and 250x20 mm). Desalting after ester saponification was performed on pre-packed PD-10 columns (GE Healthcare, Sephadex™ G-25 M). NMR spectra were recorded with a Bruker Avance III 600 instrument (600.22 MHz for ^{1}H, 150.93 MHz for ^{13}C) using standard Bruker NMR software. ^{1}H spectra were referenced to 7.26 ($CDCl_3$) and 0.00 (D_2O, external calibration to 2,2-dimethyl-2-silapentane-5-sulfonic acid) ppm unless stated otherwise. ^{13}C spectra were referenced to 77.00 ($CDCl_3$) and 67.40 (D_2O, external calibration to 1,4-dioxane) ppm. ESI-MS data were obtained on a Waters Micromass Q-TOF Ultima Global instrument.

3.2. 4,6-Di-*O*-acetyl-2,3-di-*O*-benzyl-α-D-*gluco*pyranosyl-(1→5)-methyl [methyl 7,8-di-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid]onate (5).

A solution of disaccharide **1**[12] (100 mg, 0.094 mmol) in dry MeOH (4.0 mL) containing para-toluenesulfonic acid-monohydrate (1.6 mg, 0.008 mmol) was heated to 40 °C for 2 h under an atmosphere of argon. The cooled solution was treated with solid $NaHCO_3$ (ca. 50 mg) for 5 min, the solvent was removed under reduced pressure and the residue was partitioned between EtOAc and aq. $NaHCO_3$. The organic phase was washed with brine, dried ($MgSO_4$), filtered and the residue obtained from concentration of the filtrate was dissolved in dry THF (6.0 mL) and treated with tetrabutylammonium fluoride (1M in THF, 0.14 mL, 0.142 mmol) at 0 °C for 20 min. After addition of dry MeOH (1.0 mL) the solvent was evaporated, the residue was filtered over a short pad of silica and rinsed with EtOAc. Concentration of the filtrate afforded a crude product, which was acetylated using acetic anhydride (0.8 mL), 4-(N,N-dimethylamino)pyridine (5 mg) and dry pyridine (4.0 mL). After 14 h at ambient temperature excessive reagent was destroyed with dry MeOH (1.0 mL) at 0 °C (10 min) followed by removal of solvent by codistillation with toluene (3x). Chromatography of the crude product provided the PMB-protected intermediate (52 mg, 61%) as a colorless oil.

This intermediate (41 mg, 0.046 mmol) was dissolved in dry CH_2Cl_2 (2.1 mL) and dry MeOH (0.7 mL) under an atmosphere of argon and treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (164 mg, 0.672 mmol) in eight portions within 2.5 h. After complete addition the mixture was stirred for 1 h, diluted with $CHCl_3$ and washed with aq. $NaHCO_3$. The aqueous phase was once more extracted with $CHCl_3$ and the combined organic phases were washed with aq. $NaHCO_3$ (2x), dried ($MgSO_4$) and filtered. Contentration of the filtrate and purification of the residue by chromatography (toluene/EtOAc 9:1 to remove excessive reagent, then 2:1 to elute product) afforded 4-

OH acceptor **5** (23 mg, 65%, total yield: 40%) as a colorless oil: $[\alpha]_D^{20}$ + 41.9 (*c* 0.56, $CHCl_3$); R_f 0.18 (*n*-hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$): δ 7.36 - 7.27 (m, 10H, Ar), 5.43 (app dt, 1H, $J_{7,6}$ ~ 5.7, $J_{7,8a}$ 2.1 Hz, H-7), 4.89 - 4.84 (m, 3H, H-1′, H-4′, C*H*HPh), 4.82 (dd, 1H, $J_{8a,8b}$ 12.5 Hz, H-8a), 4.75 - 4.66 (m, 3H, C*H*HPh, 2 x CH*H*Ph), 4.29 (dd, 1H, $J_{8b,7}$ 5.3 Hz, H-8b), 4.12 - 4.08 (m, 1H, 5′), 4.07 - 4.00 (m, 3H, H-4, H-6′a, H-6′b), 3.98 - 3.96 (m, 1H, H-5), 3.96 - 3.91 (m, 2H, H-3′, H-6), 3.83 (s, 3H, CO_2CH_3), 3.56 (dd, 1H, $J_{2',3'}$ 9.5, $J_{2',1'}$ 3.5 Hz, H-2′), 3.37 (d, 1H, $J_{OH,4}$ 11.5 Hz, O*H*), 3.27 (s, 3H, OCH_3), 2.20 - 2.16 (m, 1H, H-3*eq*), 2.03, 2.01, 1.95 and 1.94 (4 s, each 3H, $COCH_3$), 1.80 (app t, 1H, $J_{3ax,3eq}$ ~ $J_{3ax,4}$ 12.5 Hz, H-3*ax*); ^{13}C NMR ($CDCl_3$): δ 170.53, 170.43, 169.95 and 169.76 (4 s, 4C, $COCH_3$), 168.02 (s, C-1), 138.39 and 137.85 (2 s, 2C, Ar), 128.56, 128.39, 128.00, 127.95, 127.67 and 127.63 (6 d, 10C, Ar), 99.73 (d, C-1′), 99.04 (s, C-2), 79.42 (d, C-5), 78.79 (d, C-3′), 78.30 (d, C-2′), 75.17 (t, CH_2Ph), 73.73 (t, CH_2Ph), 71.54 (d, C-6), 71.13 (d, C-7), 69.90 and 69.51 (2 d, 2C, C-4′, C-5′), 66.19 (d, C-4), 62.84 (t, C-6′), 62.25 (t, C-8), 52.62 (q, CO_2CH_3), 51.04 (q, OCH_3), 36.08 (t, C-3′), 21.02, 20.76, 20.70 and 20.63 (4 q, 4C, $COCH_3$); ESI-TOF HRMS: *m/z* = 799.2784; calcd for $C_{38}H_{48}O_{17}Na^+$: 799.2784.

3.3. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-methyl (methyl 7,8-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (8).

A suspension of disaccharide **7**[15a] (0.71 g, 0.87 mmol) in a mixture of dry $ClCH_2CH_2Cl$ (9.0 mL) and dry cyclohexane (65.0 mL) was degassed with argon and heated to reflux for 15 min. Lauroyl peroxide (0.12 g, 0.30 mmol) was added to the warm solution and after 2 h at reflux the solvent was removed under reduced pressure. The residue was subjected to chromatography (*n*-hexane/EtOAc 1:2) to provide dehalogenated compound **8** (0.56 g, 92%) as a colorless amorphous solid: $[\alpha]_D^{20}$ + 73.1 (*c* 0.63, $CHCl_3$); R_f 0.31 (toluene/EtOAc 2:3); ^{1}H NMR ($CDCl_3$): δ 5.40 - 5.38 (m, 1H, H-5′), 5.26 - 5.19 (m, 2H, H-4′, H-7′), 4.91 (ddd, 1H, $J_{7,8b}$ 8.2, $J_{7,8a}$ 6.9 Hz, $J_{7,6}$ 3.7, H-7), 4.75 (dd, 1H, $J_{8a,8b}$ 8.9, H-8a), 4.65 (dd, 1H, $J_{8'a,8'b}$ 12.5, $J_{8'a,7'}$ 2.7 Hz, H-8′a), 4.54 (app t, 1H, H-8b), 4.25 (ddd, 1H, $J_{4,3ax}$ 11.2, $J_{4,3eq}$ 5.7, $J_{4,5}$ 3.2 Hz, H-4), 4.11 (dd, 1H, $J_{6',7'}$ 9.6, $J_{6',5'}$ 1.3 Hz, H-6'), 4.08 (dd, 1H, $J_{8'b,7'}$ 3.5 Hz, H-8′b), 3.89 - 3.87 (m, 1H, H-6), 3.84 (s, 3H, CO_2CH_3), 3.81 (s, 3H, CO_2CH_3), 3.69 - 3.67 (m, 1H, H-5), 3.22 (s, 3H, OCH_3), 2.46 (bs, 1H, O*H*), 2.20 (dd, 1H, $J_{3'eq,3'ax}$ ~ $J_{3'eq,4'}$ 12.7, H-3′*eq*), 2.13 (s, 3H, $COCH_3$), 2.12 - 2.03 (m, 6H, H-3*ax*, H-3′*ax*, H-3*eq*, $COCH_3$), 2.00 and 1.98 (2 s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 170.98, 170.20, 169.88 ans 169.61 (4 s, 4C, $COCH_3$), 168.87 and 167.58 (2 s, 2C, C-1, C-1′), 154.66 [s, O*C*(=O)O], 99.10 and 98.08 (2 s, 2C, C-2, C-2′), 76.03 (d, C-7), 70.38 (d, C-6), 69.61 (d, C-6′), 67.64 and 67.45 (2 d, 2C, C-4, C-7′), 66.10 (t, C-8), 65.88 (d, C-4′), 64.73 (d, C-5), 64.18 (d, C-5′), 61.58 (t, C-8′), 53.43 and 52.71 (2 q, 2C, CO_2CH_3), 51.14 (q, OCH_3), 33.00 and 32.29 (2 t, 2C, C-3, C-3′), 20.70, 20.60, 20.58 and 20.56 (4 q, 4C, $COCH_3$); ESI-TOF HRMS: *m/z* = 712.2298; calcd for $C_{28}H_{38}O_{20}NH_4^+$: 712.2295.

3.4. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-*gluco*pyranosyl-(1→5)]-methyl (methyl 7,8-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-

ulopyranosid)onate (9α) and **methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-*gluco*pyranosyl-(1→5)]-methyl (methyl 7,8-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (9β)**

A solution of disaccharide acceptor **8** (77 mg, 0.111 mmol) in dry Et_2O (1.5 mL) containing ground 5 Å molecular sieves (75 mg) was stirred at ambient temperature for 2 h under an atmosphere of argon. Diluted triflic acid (0.49 µL, 5.55 µmol) in dry CH_2Cl_2 (0.1 mL) was added and after 5 min a separately prepared solution of donor **6**[12] (344 mg, 0.555 mmol) in dry CH_2Cl_2 (3.0 mL) was added to this mixture using a syringe pump (1 mL/h). After complete addition the promotor was destroyed with triethylamine (32 µL, 0.222 mmol) and the mixture was filtered over Celite. The filtrate was concentrated and the residual solid was purified by chromatography (*n*-hexane/EtOAc 1:1) affording a mixture of the anomers **9α** and **9β** (114 mg, 91%, α:β = 4:1 according to ^{1}H NMR). The compounds were separated on an HPLC column (toluene/EtOAc 3:1 → 2:1) providing the desired α-anomer **9α** (83 mg, 67%) and the slower migrating β-isomer **9β** (20 mg, 16%) as colorless oils.

9α: $[\alpha]_D^{20}$ + 111.4 (*c* 0.82, $CHCl_3$); R_f 0.44 (toluene/EtOAc 2:1, HP-TLC); ^{1}H NMR ($CDCl_3$): δ 7.51 - 7.28 (m, 15H, Ar), 5.58 (s, 1H, PhC*H*), 5.35 - 5.33 (m, 1H, H-5′), 5.27 (ddd, 1H, $J_{4',3'ax}$ 12.5, $J_{4',3'eq}$ 4.8, $J_{4',5'}$ 3.0 Hz, H-4′), 5.19 (ddd, 1H, $J_{7',6'}$ 9.6, $J_{7',8'b}$ 3.9, $J_{7',8'a}$ 2.8 Hz, H-7′), 5.07 (d, 1H, $J_{1'',2''}$ 3.8 Hz, H-1″), 5.00 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.92 (d, 1H, *J* 10.6 Hz, C*H*HPh), 4.85 (d, 1H, *J* 11.5 Hz, CH*H*Ph), 4.67 (dd, 1H, $J_{8'a,8'b}$ 12.4 Hz, H-8′a), 4.66 - 4.62 (m, 1H, H-7), 4.59 (d, 1H, *J* 10.9 Hz, CH*H*Ph), 4.44 (dd, 1H, $J_{8a,8b}$ 8.6, $J_{8a,7}$ 5.8 Hz, H-8a), 4.30 (dd, 1H, $J_{6''a,6''b}$ 10.4, $J_{6''a,5''}$ 4.8 Hz, H-6″a), 4.20 (dd, 1H, $J_{6',5'}$ 1.5 Hz, H-6′), 4.16 (ddd, 1H, $J_{4,3ax}$ 11.8, $J_{4,3eq}$ 4.7, $J_{4,5}$ 2.4 Hz, H-4), 4.06 (app t, 1H, $J_{8b,7}$ ~ 8.4 Hz, H-8b), 4.01 (dd, 1H, H-8′b), 3.93 - 3.88 (m, 2H, H-3″, H-5″), 3.84 (s, 3H, CO_2CH_3), 3.83 - 3.82 (m, 1H, H-5), 3.79 (s, 3H, CO_2CH_3), 3.75 - 3.69 (m, 2H, H-6, H-6″b), 3.66 (app t, 1H, $J_{4'',3''}$ ~ $J_{4'',5''}$ 9.3 Hz, H-4″), 3.59 (dd, 1H, H-2″), 3.16 (s, 3H, OCH_3), 2.21 - 2.15 (m, 2H, H-3*eq*, H-3′*eq*), 2.10 (app. t, 1H, $J_{3ax,3eq}$ ~ 12.1 Hz, H-3*ax*), 2.09 and 2.07 (2 s, each 3H, $COCH_3$), 2.07 (app t, 1H, $J_{3'ax,3'eq}$ 12.7 Hz, H-3′*ax*), 1.99 and 1.95 (2 s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 170.85, 170.27, 169.69 and 169.60 (4 s, 4C, $COCH_3$), 168.06 and 167.35 (2 s, 2C, C-1, C-1′), 154.57 [s, O*C*(=O)O], 138.24, 137.70 and 137.28 (3 s, 3C, Ar), 128.95, 128.64, 128.46, 128.33, 128.25, 128.23, 128.20, 127.93 and 125.99 (9 d, 15C, Ar), 101.28 (d, Ph*C*H), 100.56 (d, C-1″), 99.34 and 98.92 (2 s, C-2, C-2′), 82.63 (d, C-4″), 79.86 (d, C-2″), 77.86 (d, C-3″), 76.00 (d, C-5), 75.16 (d, C-7), 75.01 (t, CH_2Ph), 74.81 (t, CH_2Ph), 72.03 (d, C-6), 69.50 (d, C-4), 69.44 (d, C-6′), 68.95 (t, C-6″), 67.72 (d, C-7′), 65.95 (t, C-8), 65.92 (d, C-4′), 64.37 (d, C-5′), 63.59 (d, C-5″), 61.58 (t, C-8′), 53.11 and 52.69 (2 q, 2C, CO_2CH_3), 51.23 (q, OCH_3), 33.85 (t, C-3), 32.40 (t, C-3′), 20.70, 20.63 and 20.58 (3 q, 4C, 4 $COCH_3$); ESI-TOF HRMS: *m/z* = 1147.3625; calcd for $C_{55}H_{64}O_{25}Na^+$: 1147.3629.

9β: $[\alpha]_D^{20}$ + 49.8 (*c* 0.82, $CHCl_3$); R_f 0.23 (toluene/EtOAc 2:1, HP-TLC); ^{1}H NMR ($CDCl_3$): δ 7.49 - 7.24 (m, 15H, Ar), 5.56 (s, 1H, PhC*H*), 5.35 - 5.33 (m, 1H, H-5′), 5.23 (ddd, 1H, $J_{4',3'ax}$ 12.3, $J_{4',3'eq}$ 4.9, $J_{4',5'}$ 3.0 Hz, H-4′), 5.18 (ddd, 1H, $J_{7',6'}$ 9.2, $J_{7',8'b}$ 4.3, $J_{7',8'a}$ 2.8 Hz, H-7′), 4.94 (d, 1H, $J_{1'',2''}$ 7.7 Hz, H-1″), 4.90 (d, 1H, *J* 11.5 Hz, C*H*HPh),

4.89 (d, 1H, *J* 11.3 Hz, C*H*HPh), 4.83 - 4.74 (m, 4H, 2 x CH*H*Ph, H-7, H-8a), 4.62 (dd, 1H, $J_{8'a,8'b}$ 12.5 Hz, H-8′a), 4.44 (app t, 1H, $J_{8b,8a} \sim J_{8b,7}$ 8.1 Hz, H-8b), 4.37 (dd, 1H, $J_{6''a,6''b}$ 10.4, $J_{6''a,5''}$ 5.0 Hz, H-6″a), 4.29 (ddd, 1H, $J_{4,3ax}$ 11.7, $J_{4,3eq}$ 5.2, $J_{4,5}$ 2.7 Hz, H-4), 4.11 - 4.09 (m, 1H, H-5), 4.04 - 4.00 (m, 2H, H-6′, H-8′b), 3.96 - 3.94 (m, 1H, H-6), 3.85 (s, 3H, CO_2CH_3), 3.81 (app t, 1H, $J_{3'',2''} \sim J_{3'',4''}$ 8.9 Hz, H-3″), 3.74 (s, 3H, CO_2CH_3), 3.74 (app t, 1H, $J_{6''b,5''}$ 10.4 Hz, H-6″b), 3.60 (app t, 1H, $J_{4'',5''}$ 9.2 Hz, H-4″), 3.46 (app dt, 1H, H-5″), 3.31 (app t, 1H, H-2″), 3.24 (s, 3H, OCH_3), 2.23 - 2.19 (m, 1H, H-3′*eq*), 2.16 (app t, 1H, $J_{3ax,3eq}$ ~ 12.2 Hz, H-3*ax*), 2.10 - 2.04 (m, 8H, 2 x $COCH_3$, H-3*eq*, H-3′*ax*), 2.00 and 1.98 (2 s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 170.93, 170.23, 169.64 and 169.57 (4 s, 4C, $COCH_3$), 168.18 and 167.52 (2 s, 2C, C-1, C-1′), 154.64 [s, O*C*(=O)O], 138.47, 138.03 and 137.13 (3 s, 3C, Ar), 129.02, 128.36, 128.25, 127.91, 127.76, 127.69, 127.58 and 125.96 (8 d, 15C, Ar), 102.92 (d, C-1″), 101.22 (d, Ph*C*H), 99.16 and 99.04 (2 s, 2C, C-2, C-2′), 81.67 (d, C-4″), 81.32 (d, C-2″), 80.72 (d, C-3″), 76.72 (d, C-7), 75.14 (t, CH_2Ph), 74.50 (t, CH_2Ph), 71.06 (d, C-6), 69.97 (d, C-5), 69.78 (d, C-6′), 68.88 (d, C-4), 68.67 (t, C-6″), 67.76 (d, C-7′), 66.11 (d, C-4′), 65.95 (d, C-5″), 65.51 (t, C-8), 64.23 (d, C-5′), 61.86 (t, C-8′), 53.16 and 52.68 (2 q, 2C, CO_2CH_3), 51.32 (q, OCH_3), 34.12 (t, C-3), 32.40 (t, C-3′), 20.78, 20.64 and 20.62 (3 q, 4C, $COCH_3$); ESI-TOF HRMS: *m/z* = 1142.4088; calcd for $C_{55}H_{64}O_{25}NH_4^+$: 1142.4075.

3.5. Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-[α-D-glucopyranosyl-(1→5)]-sodium (methyl α-D-*manno*-oct-2-ulopyranosid)onate (10)

Trisaccharide **9α** (11.3 mg, 0.010 mmol) was dissolved in dry MeOH (1.0 mL), the atmosphere was exchanged to argon and palladium on active charcoal (10%, 1 mg) was added. The atmosphere was exchanged successively to argon and hydrogen (1 atm). After 4 h the catalyst was filtered off, rinsed with MeOH and the filtrate was concentrated. The dried residue was dissolved in anhydrous MeOH (1.0 mL) and treated with sodium methoxide (0.1 N in MeOH, 60 µL, 0.006 mmol) for 17 h. Ion exchange resin DOWEX 50 (H^+ form) was added until the solution reacted neutral. The resin was filtered off immediately, rinsed with MeOH and the filtrate was concentrated. A solution of the obtained residue in H_2O (1.0 mL) was treated with aqueous sodium hydroxide (0.1 N, 1.0 mL) at 0 °C and after 4 h at ambient temperature the mixture was neutralized with DOWEX 50 (H^+-form). Filtration of the resin and freeze-drying of the filtrate afforded a salt-containing product, which was desalted on a PD10 SEC column (H_2O). Lyophilisation of the pooled fractions yielded trisaccharide **10** (6.4 mg, 94%) as a colorless amorphous solid: $[\alpha]_D^{20}$ + 114.9 (*c* 0.59, H_2O); ^{1}H NMR (D_2O, pH ~ 7.0): δ 5.24 (d, 1H, $J_{1'',2''}$ 3.6 Hz, H-1″), 4.19 - 4.17 (m, 1H, H-5), 4.08 - 4.03 (m, 2H, H-5″, H-7), 3.97 - 3.91 (m, 3H, H-4, H-5′, H-7′), 3.91 (ddd, 1H, $J_{4',5'}$ 3.0 Hz, H-4′), 3.89-3.86 (m, 2H, H-8a, H-8′a), 3.85 (dd, 1H, $J_{6''a,6''b}$ 12.2, $J_{6''a,5''}$ 2.6 Hz, H-6″), 3.78 (app t, 1H, $J_{3'',4''}$ 9.2 Hz, H-3″), 3.665 (br d, 1H, $J_{6',7'}$ 7.5 Hz, H-6′), 3.66 (dd, 1H, $J_{8'a,8'b}$ 11.8, $J_{7',8'b}$ 7.2 Hz, H-8′b), 3.58 (dd, 1H, $J_{8b,8a}$ 11.8, $J_{8b,7}$ 6.5 Hz, H-8b), 3.51 (app. t, 1H, $J_{5'',4''}$ 9.7 Hz, H-4″), 3.50 (br d, 1H, $J_{6,7}$ 9.5 Hz, H-6), 3.46 (dd, 1H, $J_{2'',3''}$ 9.9 Hz, H-2″), 3.07 (s, 3H, OCH_3), 2.05 (dd, 1H, $J_{3'eq,3'ax}$ 13.2, $J_{3'eq,4'}$ 4.7 Hz, H-3′*eq*), 2.01 (dd, 1H, $J_{3eq,3ax}$ 12.6, $J_{3eq,4}$ 4.8 Hz, H-3*eq*), 1.94 (app t, 1H, $J_{3ax,4}$ 12.3 Hz, H-3*ax*), 1.75 (app t, 1H, $J_{3'ax,4'}$ 12.7 Hz, H-3′*ax*); ^{13}C NMR

(D_2O, pH ~ 7.0): δ 176.05 (s, 2C, C-1, C-1′), 102.87 (s, C-2′), 101.35 (s, C-2), 100.31 (d, C-1″), 74.47 (d, C-5), 73.29 and 73.22 (d, C-3″, C-6), 72.88 (d, C-2″), 72.64 (d, C-5″), 72.50 (d, C-6′), 72.12 (d, C-4), 71.52 (d, C-7′), 69.76 (d, C-4″), 69.26 (d, C-7), 67.68 (d, C-5′), 66.67 (d, C-4′), 63.98 (t, C-8), 63.58 (t, C-8′), 60.69 (t, C-6″), 51.37 (q, O*C*H_3), 35.42 (t, C-3′), 35.24 (t, C-3); ESI-TOF HRMS: *m/z* = 657.1838; calcd for $C_{23}H_{38}O_{20}Na^+$: 657.1849.

3.6. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-[2,3,6-tri-*O*-benzyl-α-D-*gluco*pyranosyl-(1→5)]-methyl (methyl 7,8-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (11) and **methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-[2,3,4-tri-*O*-benzyl-α-D-*gluco*pyranosyl-(1→5)]-methyl (methyl 7,8-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (12)** and **methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-[2,3-di-*O*-benzyl-α-D-*gluco*pyranosyl-(1→5)]-methyl (methyl 7,8-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (13)**

A cooled (0 °C) solution of compound **9α** (188 mg, 0.167 mmol) in dry CH_2Cl_2 (6.0 mL) was treated with triethylsilane (400 µL, 2.506 mmol) and $BF_3{\cdot}Et_2O$ (41 µL, 0.334 mmol) successively. After 1 h at 0 °C the mixture was diluted with CH_2Cl_2 and washed with aq. $NaHCO_3$. The aqueous phase was once again extracted with CH_2Cl_2 and the combined organic phases were washed with water, dried ($MgSO_4$) and filtered. The filtrate was concentrated and the residue was purified on an HPLC column (n-hexane/EtOAc 3:2 → 1:2) providing some recovered starting material **9α** (7 mg, 3%), the desired 6-OBn compound **11** (135 mg, 72%), the 4-OBn isomer **12** (10 mg, 5%) and the diol **13** (3 mg, 2%) successively.

11: Colorless oil; $[\alpha]_D^{20}$ + 102.6 (*c* 1.12, $CHCl_3$); R_f 0.38 (*n*-hexane/EtOAc 1:1, HP-TLC); ^{1}H NMR ($CDCl_3$): δ 7.41 - 7.27 (m, 15H, Ar), 5.34 - 5.33 (m, 1H, H-5′), 5.23 (ddd, 1H, $J_{4',3'ax}$ 12.6, $J_{4',3'eq}$ 5.0, $J_{4',5'}$ 2.8 Hz, H-4′), 5.20 (ddd, 1H, $J_{7',6'}$ 9.4, $J_{7',8'b}$ 4.4, $J_{7',8'a}$ 2.8 Hz, H-7′), 5.10 (d, 1H, $J_{1'',2''}$ 3.6 Hz, H-1″), 4.97 (d, 1H, *J* 11.4 Hz, C*H*HPh), 4.87 (d, 1H, *J* 11.4 Hz, CH*H*Ph), 4.82 (d, 1H, *J* 10.8 Hz, C*H*HPh), 4.77 – 4.73 (m, 1H, H-7), 4.62 (dd, 1H, $J_{8'a,8'b}$ 12.1, $J_{8'a,7'}$ 2.6 Hz, H-8′a), 4.59 (d, 1H, *J* 10.8 Hz, CH*H*Ph), 4.55 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.48 (d, 1H, *J* 11.5 Hz, CH*H*Ph), 4.49 - 4.46 (m, 1H, H-8a), 4.22 - 4.18 (m, 2H, H-4, H-8b), 4.10 (dd, 1H, $J_{6',5'}$ 1.7 Hz, H-6′), 4.06 (dd, 1H, H-8′b), 3.86 - 3.85 (m, 1H, H-5), 3.84 - 3.81 (m, 4H, H-5″, CO_2CH_3), 3.80 (s, 3H, CO_2CH_3), 3.76 (dd, 1H, $J_{6''a,6''b}$ 10.1, $J_{6''a,5''}$ 3.8 Hz, H-6″a), 3.74 - 3.67 (m, 3H, H-3″, H-4″, H-6), 3.58 (dd, 1H, $J_{6''b,5''}$ 4.5 Hz, H-6″b), 3.53 (dd, 1H, $J_{2'',3''}$ 9.2 Hz, H-2″), 3.18 (s, 3H, OCH_3), 2.62 (d, 1H, $J_{OH,4''}$ 2.2 Hz, O*H*), 2.24 - 2.20 (m, 1H, H-3′*eq*), 2.12 (app t, 1H, $J_{3ax,3eq} \sim J_{3ax,4}$ 12.2 Hz, H-3*ax*), 2.09 and 2.08 (2 s, each 3H, $COCH_3$), 2.09 – 2.01 (m, 2H, H-3′*ax*, H-3*eq*), 1.99 and 1.91 (2 s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 170.93, 170.30, 169.71 and 169.63 (4 s, 4C, $COCH_3$), 168.36 and 167.46 (2 s, 2C, C-1, C-1′), 154.49 [s, O(*C*=O)O], 138.50, 137.90 and 137.74 (3 s, 3C, Ar), 128.65, 128.58, 128.41, 128.24, 128.00, 127.95, 127.92, 127.76 and 127.62 (9 d, 15C, Ar), 99.76 (d, C-1″), 99.12 and 99.04 (2 s, 2C, C-2, C-2′), 80.95 (d, C-3″), 80.12 (d, C-2″), 75.79 (d, C-5), 75.14 (t, CH_2Ph), 74.97 (d, C-7),

74.15 (t, *C*H$_2$Ph), 73.45 (t, *C*H$_2$Ph), 72.22 (d, 2C, C-4″, C-6), 70.70 (d, C-5″), 70.10 (t, C-6″), 69.62 (d, C-6′), 68.70 (d, C-4), 67.82 (d, C-7′), 66.21 (t, C-8), 66.06 (d, C-4′), 64.29 (d, C-5′), 61.94 (t, C-8′), 53.12 (q, CO$_2$*C*H$_3$), 52.64 (q, CO$_2$*C*H$_3$), 51.20 (q, O*C*H$_3$), 34.18 (t, C-3), 31.96 (t, C-3′), 20.69, 20.62 and 20.59 (3 q, 4C, CO*C*H$_3$); ESI-TOF HRMS: *m/z* = 1144.4239; calcd for $C_{55}H_{66}O_{25}NH_4^+$: 1144.4231.

12: Colorless oil; $[\alpha]_D^{20}$ + 110.8 (*c* 0.89, CHCl$_3$); R$_f$ 0.30 (n-hexane/EtOAc 1:1, HP-TLC); ^{1}H NMR (CDCl$_3$): δ 7.41 - 7.27 (m, 15H, Ar), 5.36 - 5.32 (m, 2H, H-4′, H-5′), 5.19 (ddd, 1H, $J_{7',6'}$ 9.4, $J_{7',8'b}$ 4.1, $J_{7',8'a}$ 2.8 Hz, H-7′), 5.09 (d, 1H, $J_{1'',2''}$ 3.6 Hz, H-1″), 4.97 (d, 1H, *J* 11.2 Hz, C*H*HPh), 4.94 (d, 1H, *J* 11.2 Hz, CH*H*Ph), 4.88 (d, 1H, *J* 10.7 Hz, C*H*HPh), 4.83 (d, 1H, *J* 10.8 Hz, C*H*HPh), 4.72 (d, 1H, *J* 10.6 Hz, CH*H*Ph), 4.66 (dd, 1H, $J_{8'a,8'b}$ 12.3 Hz, H-8′a), 4.61 - 4.54 (m, 3H, H-7, H-8a, CH*H*Ph), 4.32 (app t, 1H, $J_{8b,\,8a} \sim J_{8b,7}$ 7.6 Hz, H-8b), 4.18 (dd, 1H, $J_{6',5'}$ 1.3 Hz, H-6′), 4.12 (ddd, 1H, $J_{4,3ax}$ 12.0, $J_{4,3eq}$ 4.6, $J_{4,5}$ 2.5 Hz, H-4), 4.02 (dd, 1H, H-8′b), 3.86 (s, 3H, CO$_2$C*H$_3$*), 3.86 - 3.84 (m, 1H, H-5), 3.83 - 3.79 (m, 3H, H-3″, H-6″a, H-6″b), 3.78 (s, 3H, CO$_2$C*H$_3$*), 3.75 - 3.72 (m, 2H, H-5″, H-6), 3.67 (dd, 1H, *J* 9.8, *J* 9.1 Hz, H-4″), 3.54 (dd, 1H, $J_{2'',3''}$ 9.6 Hz, H-2″), 3.16 (s, 3H, OC*H$_3$*), 2.90 (app t, 1H, *J* 6.6 Hz, O*H*), 2.28 - 2.24 (m, 1H, H-3′*eq*), 2.16 -2.12 (m, 1H, H-3*eq*), 2.08 and 2.06 (2 s, each 3H, COC*H$_3$*), 2.07 - 2.01 (m, 2H, H-3*ax*, H-3′*ax*), 2.00 and 1.99 (2 s, each 3H, COC*H$_3$*); ^{13}C NMR (CDCl$_3$): δ 170.79, 170.52, 170.16 and 169.68 (4 s, 4C, *C*OCH$_3$), 168.27 and 167.44 (2 s, 2C, C-1, C-1′), 154.65 [s, O(*C*=O)O], 138.40, 137.91 and 137.70 (3 s, 3C, Ar), 128.70, 128.56, 128.51, 128.38, 128.19, 128.13, 127.99 and 127.83 (8 d, 15C, Ar), 99.89 (d, C-1″), 99.67 and 98.93 (2 s, 2C, C-2, C-2′), 81.50 (d, C-3″), 80.56 (d, C-2″), 77.72 (d, C-4″), 76.15 (d, C-5), 75.93 (d, C-7), 75.38 (t, *C*H$_2$Ph), 75.35 (t, *C*H$_2$Ph), 74.47 (t, *C*H$_2$Ph), 72.57 (d, C-5″), 71.84 (d, C-6), 70.05 (d, C-4), 69.65 (d, C-6′), 67.80 (d, C-7′), 66.41 (d, C-4′), 65.88 (t, C-8), 64.36 (d, C-5′), 61.63 (t, C-8′), 61.07 (t, C-6″), 53.17 (q, CO$_2$*C*H$_3$), 52.67 (q, CO$_2$*C*H$_3$), 51.20 (q, O*C*H$_3$), 33.81 and 32.53 (2 t, 2C, C-3, C-3′), 20.86, 20.63 and 20.59 (3 q, 4C, CO*C*H$_3$); ESI-TOF HRMS: *m/z* = 1149.3771; calcd for $C_{55}H_{66}O_{25}Na^+$: 1149.3785.

13: Colorless oil; R$_f$ 0.06 (n-hexane/EtOAc); ^{1}H NMR (CDCl$_3$): δ 7.40 - 7.31 (m, 10H, Ar), 5.35 - 5.33 (m, 1H, H-5′), 5.31 (ddd, 1H, $J_{4',3'ax}$ 12.1, $J_{4',3'eq}$ 4.7, $J_{4',5'}$ 3.0 Hz, H-4′), 5.20 (ddd, 1H, $J_{7',6'}$ 9.4, $J_{7',8'b}$ 4.2, $J_{7',8'a}$ 2.8 Hz, H-7′), 5.13 (d, 1H, $J_{1'',2''}$ 3.5 Hz, H-1″), 5.02 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.83 (d, 2H, *J* 11.3 Hz, C*H*HPh, CH*H*Ph), 4.68 (ddd, 1H, $J_{7,8b}$ 8.3, $J_{7,8a}$ 5.7, $J_{7,6}$ 3.9 Hz, H-7), 4.65 (dd, 1H, $J_{8'a,8'b}$ 12.4 Hz, H-8′a), 4.60 (d, 1H, *J* 10.9 Hz, CH*H*Ph), 4.57 (dd, 1H, $J_{8a,8b}$ 8.4 Hz, H-8a), 4.32 (app t, 1H, H-8b), 4.18 - 4.14 (m, 2H, H-4, H-6′), 4.04 (dd, 1H, H-8′b), 3.88 - 3.87 (m, 1H, H-5), 3.86 (s, 3H, CO$_2$C*H$_3$*), 3.80 (s, 3H, CO$_2$C*H$_3$*), 3.79 - 3.76 (m, 2H, H-6″a, H-6″b), 3.75 - 3.66 (m, 4H, H-3″, H-4″, H-5″, H-6), 3.56 - 3.51 (m, 1H, H-2″), 3.18 (s, 3H, OC*H$_3$*), 2.85 (b s, 1H, O*H*), 2.44 (b s, 1H, O*H*), 2.27 (dd, 1H, $J_{3'eq,3'ax}$ 12.7 Hz, H-3′*eq*), 2.14 - 2.10 (m, 2H, H-3*ax*, H-3*eq*), 2.083 and 2.077 (2 s, each 3H, COC*H$_3$*), 2.05 (app t, 1H, H-3′*ax*), 2.00 and 1.98 (2 s, each 3H, COC*H$_3$*); ^{13}C NMR (CDCl$_3$): δ 170.83, 170.53, 170.15 and 169.68 (4 s, 4C, *C*OCH$_3$), 168.30 and 167.46 (2s, 2C, C-1, C-1′), 154.54 [s, O(C=O)O], 138.41 and 137.62 (2s, 2C, Ar), 128.73, 128.69, 128.39, 128.06, 128.03 and 127.81 (6 d, 10C, Ar), 99.71 (d, C-1″), 99.54 and 99.02 (2 s, 2C, C-2, C-2′), 81.21 (d, C-3″), 80.43 (d, C-2″), 75.85 (d, C-5), 75.41 (d, C-7), 75.12 (t, *C*H$_2$Ph), 74.17 (t, *C*H$_2$Ph), 72.41 (d, C-5″), 72.03 (d, C-6), 70.50 (d, C-4″), 69.76 and 69.66 (2 d, 2C, C-4, C-6′), 67.77 (d, C-7′), 66.40 (d, C-4′), 66.09 (t, C-8), 64.34 (d, C-5′), 61.88 (t, C-6″), 61.74 (t, C-8′), 53.20 (q, CO$_2$*C*H$_3$), 52.69 (q,

CO_2CH_3), 51.24 (q, OCH_3), 33.94 (t, C-3), 32.40 (t, C-3′), 20.86, 20.64 and 20.59 (3 q, 4C, COCH_3); ESI-TOF HRMS: *m/z* = 1059.3303; calcd for $C_{48}H_{60}O_{25}Na^+$: 1059.3316.

3.7. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-[2,3,4,6-tetra-*O*-acetyl-β-D-*gluco*pyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-*gluco*pyranosyl-(1→5)]-methyl (methyl 7,8-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (15)

A solution of acceptor **11** (24.8 mg, 0.022 mmol) and donor **14**[18] (22.9 mg, 0.044 mmol) in dry CH_2Cl_2 (1.0 mL) containing 5 Å molecular sieves (50 mg) was stirred at ambient temperature for 1 h. The cooled (0 °C) mixture was treated with TfOH (0.10 µL, 1.1 µmol) in dry CH_2Cl_2 (20 µL) and stirred for 15 min at 0°C. The promotor was destroyed with triethylamine (15 µL, 0.110 mmol), the mixture was filtered over Celite, rinsed with CH_2Cl_2 and the filtrate was concentrated. The residue was subjected to HP-chromatography (toluene/EtOAc 2:1 → 1:2) affording impure product fractions which were further purified by HPLC (*n*-hexane/EtOAc 2:1 → 1:2) to obtain pure **15** (14.7 mg, 46%) as a colorless oil: $[\alpha]_D^{20}$ + 93.5 (*c* 0.50, $CHCl_3$); R_f 0.30 (toluene/EtOAc 1:1, HP-TLC); ^{1}H NMR ($CDCl_3$): δ 7.44 - 7.18 (m, 15H, Ar), 5.36 - 5.34 (m, 1H, H-5′), 5.26 (ddd, 1H, $J_{4',3'ax}$ 12.5, $J_{4',3'eq}$ 5.0, $J_{4',5'}$ 2.9 Hz, H-4′), 5.21 (ddd, 1H, $J_{7',6'}$ 9.2, $J_{7',8'b}$ 4.1, $J_{7',8'a}$ 2.7 Hz, H-7′), 5.07 (d, 1H, $J_{1'',2''}$ 3.8 Hz, H-1″), 5.05 (d, 1H, *J* 11.5 Hz, C*H*HPh), 5.01 (app t, 1H, $J_{4''',3'''} \sim J_{4''',5'''}$ 9.5 Hz, H-4‴), 4.94 (app. t, 1H, $J_{3''',2'''}$ 9.2 Hz, H-3‴), 4.92 - 4.89 (m, 1H, H-2‴), 4.75 - 4.71 (m, 3H, 2 x C*H*HPh, CH*H*Ph), 4.68 (app td, 1H, $J_{7,8b}$ 8.1, $J_{7,6} \sim J_{7,8a}$ 5.0 Hz, H-7), 4.61 (dd, 1H, $J_{8'a,8'b}$ 12.4, $J_{8'a,7'}$ 2.8 Hz, H-8′a), 4.50 (d, 1H, *J* 10.9 Hz, CH*H*Ph), 4.48 (d, 1H, $J_{1''',2'''}$ 7.7 Hz, H-1‴), 4.45 (dd, 1H, $J_{8a,8b}$ 8.5, $J_{8a,7}$ 5.5 Hz, H-8a), 4.31 (d, 1H, *J* 11.6 Hz, CH*H*Ph), 4.20 (ddd, 1H, *J* 9.7, *J* 7.0, $J_{4,5}$ 2.5 Hz, H-4), 4.14 (dd, 1H, $J_{6''a,5''}$ 4.3, $J_{6''a,6''b}$ 12.5 Hz, H-6″a), 4.12 (br, d, 1H, $J_{6',7'}$ 9.2 Hz, H-6′), 4.11 (app t, 1H, H-8b), 4.07 (dd, 1H, H-8′b), 3.94 (app t, 1H, $J_{4'',3''} \sim J_{4'',5''}$ 9.5 Hz, H-4″), 3.90 (dd, 1H, $J_{6'''b,6'''a}$ 12.2, $J_{6''',5'''}$ 2.3 Hz, H-6‴b), 3.85 - 3.84 (m, 1H, H-5), 3.82 (s, 3H, CO_2CH_3), 3.81 (dd, 1H, $J_{6''a,6''b}$ 10.5, $J_{6''a,5''}$ 2.5 Hz, H-6″a), 3.81 (s, 3H, CO_2CH_3), 3.68 (app t, 1H, H-3″), 3.67 - 3.65 (m, 2H, H-5″, H-6), 3.53 (dd, 1H, $J_{6''b,5''}$ 1.1 Hz, H-6″b), 3.48 (dd, 1H, $J_{2'',3''}$ 9.8 Hz, H-2″), 3.29 (ddd, 1H, $J_{5''',6'''a}$ 4.2 Hz, H-5‴), 3.19 (s, 3H, OCH_3), 2.25 (dd, 1H, $J_{3'eq,3'ax}$ 13.0 Hz, H-3′*eq*), 2.10 (app t, 1H, H-3′*ax*), 2.095 and 2.08 (2 s, each 3H, COCH_3), 2.04 – 2.01 (m, 2 H, H-3*ax*, H-3*eq*), 2.01, 1.99, 1.98, 1.97, 1.94 and 1.90 (6 s, each 3H, COCH_3); ^{13}C NMR ($CDCl_3$): δ 170.92, 170.59, 170.33, 170.16, 169.91, 169.62, 169.34 and 169.10 (8 s, 8C, *C*OCH_3), 168.36 and 167.56 (2 s, 2C, C-1, C-1′), 154.57 [s, O(*C*=O)O], 139.00, 137.81 and 137.76 (3 s, 3C, Ar), 128.73, 128.54, 128.31, 128.23, 128.18, 127.56 and 127.48 (7 d, 15C, Ar), 100.10 (d, C-1″), 99.97 (d, C-1‴), 99.07 and 98.97 (2 s, 2C, C-2, C-2′), 79.68 (d, C-2″), 79.62 (d, C-3″), 77.0 (d, C-4″, overlapped by residual solvent peak), 76.16 (d, C-5), 75.06 (d, C-7), 75.03 (t, *C*H_2Ph), 74.52 (t, *C*H_2Ph), 73.49 (t, *C*H_2Ph), 73.19 (d, C-3‴), 72.26 (d, C-6), 71.90 (d, C-2‴), 71.46 (d, C-5‴), 71.35 (d, C-5″), 69.66 (d, C-6′), 68.55 (d, C-4), 68.21 (d, C-4‴), 67.85 (d, C-7′), 67.75 (t, C-6″), 66.06 (t, C-8), 66.03 (d, C-4′), 64.25 (d, C-5′), 61.92 (t, C-8′), 61.66 (t, C-6‴), 53.17 and 52.65 (2 q, 2C, CO_2CH_3), 51.22 (q, OCH_3), 34.28 (t, C-3), 31.91 (t, C-3′), 20.72, 20.63, 20.59 and 20.56 (4 q, 8C, COCH_3); ESI-TOF HRMS: *m/z* = 1479.4740; calcd for $C_{69}H_{84}O_{34}Na^+$: 1479.4736.

3.8. Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-[β-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→5)]-sodium (methyl α-D-*manno*-oct-2-ulopyranosid)onate (16)

According to the procedure for compound **10**, tetrasaccharide **15** (14.0 mg, 0.010 mmol) was debenzylated in dry MeOH (1.0 mL) over palladium on active charcoal (10%, 1 mg) under an atmosphere of hydrogen (1 atm) for 3 h, and deacetylated by treatment with sodium methoxide (0.1 N in MeOH, 24 µL, 2.4 µmol) in dry MeOH (1.0 mL) for 14 h. For ester saponification a solution of the deblocked intermediate in H_2O (2 mL) and aq. sodium hydroxide (0.1 N, 0.5 mL) was stirred for 2 h at 0 °C and 1 h at ambient temperature followed by neutralization. The residual solid obtained from freeze-drying of the filtrate, was desalted on a PD10 SEC column (H_2O) and lyophilisation of the pooled fractions provided compound **16** (8.0 mg, 99%) as a colorless amorphous solid: $[\alpha]_D^{20}$ + 91.4 (*c* 0.56, H_2O); ^{1}H NMR (D_2O, pH ~ 7.0): δ 5.25 (d, 1H, $J_{1'',2''}$ 3.5 Hz, H-1″), 4.49 (d, 1H, $J_{1''',2'''}$ 7.7 Hz, H-1‴), 4.25 - 4.20 (m, 2H, H-5, H-5″), 4.03 (ddd, 1H, $J_{7,6}$ 9.4, $J_{7,8b}$ 6.4, $J_{7,8a}$ 2.9 Hz, H-7), 3.97 - 3.84 (m, 10H, H-3″, H-4, H-4′, H-5′, H-6″a, H-6″b, H-6‴a, H-7′, H-8a, H-8′a), 3.71 (app t, 1H, $J_{4'',3''} \sim J_{4'',5''}$ 9.7 Hz, H-4″), 3.70 (dd, 1H, $J_{6'''b,6'''a}$ 12.1, $J_{6'''b,5'''}$ 6.1 Hz, H-6‴b), 3.67 (d, 1H, $J_{6',7'}$ 8.2 Hz, H-6′), 3.66 (dd, 1H, $J_{8'b,8'a}$ 11.6, $J_{8'b,7'}$ 7.3 Hz, H-8′b), 3.59 (dd, 1H, $J_{8b,8a}$ 11.9, H-8b), 3.52 (dd, 1H, $J_{2'',3''}$ 10.3 Hz, H-2″), 3.51 (d, $J_{6,7}$ 10.3 Hz, H-6), 3.49 - 3.44 (m, 2H, H-3‴, H-5‴), 3.38 (app t, 1H, $J_{4''',3'''} \sim J_{4''',5'''}$ 9.5 Hz, H-4‴), 3.28 (dd, 1H, $J_{2''',3'''}$ 9.3 Hz, H-2‴), 3.08 (s, 3H, OC*H*$_3$), 2.07 - 1.99 (m, 2H, H-3*eq*, H-3′*eq*), 1.95 (app t, 1H, $J_{3ax,3eq} \sim J_{3ax,4}$ 12.3 Hz, H-3*ax*), 1.76 (app t, $J_{3'ax,3'eq} \sim J_{3'ax,4'}$ 12.5 Hz, H-3′*ax*); ^{13}C NMR (D_2O, pH ~ 7.0): δ 176.03 (s, C-1′), 175.94 (s, C-1), 103.36 (d, C-1‴), 102.82 (s, C-2′), 101.35 (s, C-2), 99.72 (d, C-1″), 79.07 (d, C-4″), 76.78 (d, C-5‴), 76.33 (d, C-3‴), 73.94 (d, 2C, C-2‴, C-5), 73.11 (d, C-6), 72.60 (d, C-2″), 72.55 (d, C-6′), 72.04 and 71.90 (2 d, 2C, C-3″, C-4), 71.50 (d, C-7′), 71.25 (d, C-5″), 70.33 (d, C-4‴), 69.35 (d, C-7), 67.66 (d, C-5′), 66.73 (d, C-4′), 64.00 (t, C-8), 63.62 (t, C-8′), 61.46 (t, C-6‴), 60.29 (t, C-6″), 51.38 (q, O*C*H$_3$), 35.48 (t, C-3′), 35.21 (t, C-3); ESI-TOF HRMS: *m/z* = 819.2375; calcd for $C_{29}H_{48}O_{25}Na^+$: 819.2377.

3.9. 2,3,4,6-Tetra-*O*-acetyl-α-D-*gluco*pyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-α,β-D-*gluco*pyranosyl-(*N*-phenyl)trifluoroacetimidate (17)

A solution of isomaltose (100 mg, 0.292 mmol) in dry pyridine (2.0 mL) was treated with acetic anhydride (0.8 mL) and 4-(*N,N*-dimethylamino)pyridine (5 mg) at 0 °C and after 20 h at ambient temperature, excessive reagent was destroyed with dry MeOH (2.0 mL) at 0 °C. After 10 min the solvent was coevaporated with toluene (3x) and the residual oil was purified by chromatography (n-hexane/EtOAc 1:1 → 1:2) affording an α/β-mixture (1:1) of peracetylated isomaltose (198 mg, quantitative yield). The dried intermediate (101 mg, 0.149 mmol) was dissolved in dry DMF (2.0 mL) and treated with hydrazine acetate (27 mg, 0.298 mmol) at 40 °C for 2 h under an atmosphere of argon. The mixture was diluted with EtOAc, washed with cold brine and the organic layer was dried ($MgSO_4$)

and filtered. Concentration of the filtrate provided a crude product which was purified by chromatography (*n*-hexane/EtOAc 1:2) to obtain an α/β-mixture (1:0.3) of the hemiacetal (85 mg, 90%): ^{1}H NMR ($CDCl_3$): δ 5.53 (dd, 1H, $J_{3\alpha,2\alpha}$ 10.1, $J_{3\alpha,4\alpha}$ 9.4 Hz, H-3α), 5.46 (app t, 1H, $J_{3'\beta,2'\beta} \sim J_{3'\beta,4'\beta}$ 9.8 Hz, H-3′β), 5.45 (app t, 1H, $J_{3'\alpha,2'\alpha} \sim J_{3'\alpha,4'\alpha}$ 9.8 Hz, H-3′α), 5.40 (app t, 1H, $J_{1\alpha,2\alpha} \sim J_{1\alpha,OH}$ 3.5 Hz, H-1α), 5.23 (app t, 1H, $J_{3\beta,2\beta} \sim J_{3\beta,4\beta}$ Hz, H-3β), 5.14 (d, 1H, $J_{1'\beta,2'\beta}$ 3.8 Hz, H-1′β), 5.13 (d, 1H, $J_{1'\alpha,2'\alpha}$ 3.8 Hz, H-1′α), 5.02 - 4.97 (m, 3H, H-4′α, H-4β, H-4α), 4.94 (dd, 1H, $J_{4\alpha,5\alpha}$ 10.3 Hz, H-4α), 4.85 - 4.78 (m, 4H, H-2α, H-2′α, H-2β, H-2′β), 4.73 (app t, 1H, *J* 8.0 Hz, H-1β), 4.24 (ddd, 1H, $J_{5\alpha,6a\alpha}$ 6.5, $J_{5\alpha,6b\alpha}$ 2.5 Hz, H-5α), 4.21 - 4.05 (m, 7H, H-5′α, H-5′β, H-6′aα, H-6′aβ, H-6′bα, H-6′bβ, O*H*β), 4.02 - 3.99 (m, 1H, O*H*α), 3.74 - 3.57 (m, 5H, H-5β, H-6aα, H-6aβ, H-6bα, H-6bβ), 2.10 to 2.00 (10 s, 14 x COC*H*$_3$); ESI-TOF HRMS: *m/z* = 659.1794; calcd for $C_{26}H_{36}O_{18}Na^+$: 659.1798.

This mixture (70 mg, 0.110 mmol) was dissolved in dry CH_2Cl_2 (1.8 mL) and dry acetone (1.0 mL) and potassium carbonate (30 mg, 0.220 mmol) and 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (35 µL, 0.220 mmol) were added successively under an atmosphere of argon. After 12 h the mixture was filtered through a pad of Celite, rinsed with CH_2Cl_2 and from the filtrate the solvent was removed. Swift chromatographic purification (n-hexane/EtOAc 1:1) provided **17** (80 mg, 90%) which was directly used for the subsequent glycosylation reaction.

3.10. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-[2,3,4,6-tetra-O-acetyl-α-D-*gluco*pyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-*gluco*pyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-*gluco*pyranosyl-(1→5)]-methyl (methyl 7,8-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (18)

A solution of acceptor **11** (20.0 mg, 0.018 mmol) and donor **17** (28.7 mg, 0.035 mmol) in dry CH_2Cl_2 (1.0 mL) containing 5 Å molecular sieves (50 mg) was stirred at ambient temperature for 1 h. The cooled (0 °C) mixture was treated with TfOH (0.04 µL, 4.4 µmol) in dry CH_2Cl_2 (10 µL) and stirred for 30 min at 0°C. An additional portion of TfOH (0.16 µL, 17.6 µmol) was added (0 °C) and after 15 min triethylamine (12.3 µL, 0.089 mmol) was added. The mixture was filtered over Celite, rinsed with CH_2Cl_2 and the filtrate was concentrated. The residue was subjected to HP-chromatography (toluene/EtOAc 2:1 → 1:2) affording impure product fractions which were further purified by HPLC (*n*-hexane/EtOAc 1:1 → 1:2) to obtain pure **18** (22.0 mg, 71%) as a colorless oil: $[\alpha]_D^{20}$ + 105.8 (*c* 1.10, $CHCl_3$); R_f 0.16 (n-hexane/EtOAc 1:1, HP-TLC); ^{1}H NMR ($CDCl_3$): δ 7.44 - 7.25 (m, 15H, Ar), 5.38 (app t, 1H, $J_{3'''',2''''} \sim J_{3'''',4''''}$ 9.7 Hz, H-3′′′′), 5.34 - 5.33 (m, 1H, H-5′), 5.24 (ddd, 1H, $J_{4',3'ax}$ 12.6, $J_{4',3'eq}$ 4.9, $J_{4',5'}$ 2.9 Hz, H-4′), 5.20 (ddd, 1H, $J_{7',6'}$ 9.3, $J_{7',8'b}$ 4.3, $J_{7',8'a}$ 2.8 Hz, H-7′), 5.03 (d, 1H, $J_{1'',2''}$ 3.9 Hz, H-1′′), 5.02 (app t, 1H, $J_{4'''',5''''}$ 9.7 Hz, H-4′′′′), 5.00 (app t, 1H, $J_{3''',2'''} \sim J_{3''',4'''}$ 9.3 Hz, H-3′′′), 4.98 (d, 1H, $J_{1'''',2''''}$ 3.4 Hz, H-1′′′′), 4.92 (app t, 1H, $J_{4''',5'''}$ 9.5 Hz, H-4′′′), 4.90 - 4.87 (m, 2H, H-2′′′, C*H*HPh), 4.83 - 4.80 (m, 2H, H-2′′′′, C*H*HPh), 4.79 (d, 1H, *J* 11.7 Hz, CH*H*Ph), 4.69 (d, 1H, *J* 11.7 Hz, C*H*HPh), 4.67 (ddd, 1H, $J_{7,8b}$ 8.3, $J_{7,8a}$ 5.4, $J_{7,6}$ 4.6 Hz, H-7), 4.60 (dd, 1H, $J_{8'a,8'b}$ 12.3 Hz, H-8′a), 4.55 (d, 1H, *J* 11.0 Hz, CH*H*Ph), 4.51 (d, 1H, $J_{1''',2'''}$ 8.0 Hz, H-1′′′), 4.42 (dd, 1H, $J_{8a,8b}$ 8.6 Hz, H-8a), 4.29 (d, 1H, *J* 11.8 Hz, CH*H*Ph), 4.21 (dd, 1H, $J_{6''''a,6''''b}$ 12.3, $J_{6''''a,5''''}$ 4.0 Hz, H-6′′′′a), 4.19 (ddd, 1H, $J_{4,3ax}$ 11.3, $J_{4,3eq}$ 5.5, $J_{4,5}$ 2.5 Hz, H-4), 4.11 (dd, 1H, $J_{6',5'}$ 1.4 Hz, H-6′), 4.08 - 4.04 (m, 2H, H-8b, H-8′b), 3.96 (dd,

1H, $J_{6'''b,5'''}$ 2.3 Hz, H-6‴b), 3.94 (app t, 1H, $J_{4'',3''} \sim J_{4'',5''}$ 9.5 Hz, H-4″), 3.88 (ddd, 1H, H-5‴′), 3.84 - 3.83 (m, 1H, H-5), 3.82 (s, 3H, CO_2CH_3), 3.80 (s, 3H, CO_2CH_3), 3.78 (dd, 1H, $J_{6''a,6''b}$ 10.8, $J_{6''a,5''}$ 2.8 Hz, H-6″a), 3.67 - 3.63 (m, 3H, H-3″, H-5″, H-6), 3.49 (dd, 1H, $J_{6''b,5''}$ 1.5 Hz, H-6″b), 3.46 (dd, 1H, $J_{2'',3''}$ 9.7, H-2″), 3.42 (dd, 1H, $J_{6'''a,6'''b}$ 11.6, $J_{6'''a,5'''}$ 5.4 Hz, H-6‴a), 3.35 (dd, 1H, $J_{6'''b,5'''}$ 4.2 Hz, H-6‴b), 3.30 - 3.26 (m, 1H, H-5‴), 3.18 (s, 3H, OC*H*$_3$), 2.22 - 2.18 (m, 1H, H-3′*eq*), 2.11 (app t, 1H, $J_{3'ax,3'eq}$ 13.1 Hz, H-3′*ax*), 2.07 - 1.99 (m, 2H, H-3*eq*, H-3*ax*), 2.09, 2.08, 2.06, 2.02, 2.01, 2.00, 1.99, 1.98, 1.96 and 1.89 (10 s, 33H, COC*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 170.89, 170.48, 170.31, 170.14, 169.94, 169.91, 169.90, 169.60, 169.50, 169.45 and 169.16 (11 s, 11C, *C*OCH$_3$), 168.38 and 167.55 (2 s, 2C, C-1, C-1′), 154.58 [s, O(*C*=O)O], 138.81, 137.86 and 137.83 (3 s, 3C, Ar), 128.71, 128.60, 128.33, 128.24, 128.19, 128.14, 127.99 and 127.53 (8 d, 15C, Ar), 100.15 (d, C-1″), 99.76 (d, C-1‴), 99.09 and 98.95 (2 s, 2C, C-2, C-2′), 96.12 (d, C-1‴′), 79.71 (d, C-2″), 79.33 (d, C-3″), 76.88 (d, C-4″), 76.07 (d, C-5), 75.09 (d, C-7), 75.01 (t, *C*H$_2$Ph), 74.58 (t, *C*H$_2$Ph), 73.53 (t, *C*H$_2$Ph), 73.24 (d, C-3‴), 72.51 (d, C-5‴), 72.27 (d, C-6), 71.89 (d, C-2‴), 71.36 (d, C-5″), 70.45 (d, C-2‴′), 70.01 (d, C-3‴′), 69.80 (d, C-4‴), 69.64 (d, C-6′), 68.60 (d, C-4), 68.28 (d, C-4‴′), 68.12 (t, C-6‴), 68.04 (t, C-6″), 67.84 (d, C-7′), 67.64 (d, C-5‴′), 66.03 (t, C-8), 66.01 (d, C-4′), 64.23 (d, C-5′), 61.92 (t, C-8′), 61.67 (t, C-6‴′), 53.14 (q, CO_2CH_3), 52.60 (q, CO_2CH_3), 51.19 (q, OCH$_3$), 34.23 (t, C-3), 31.90 (t, C-3′), 20.68, 20.64, 20.62, 20.59, 20.56 and 20.55 (6 q, 11C, COCH$_3$); ESI-TOF HRMS: *m/z* = 1762.6029; calcd for $C_{81}H_{100}O_{42}NH_4^+$: 1762.6027.

3.11. Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-[α-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→5)]-sodium (methyl α-D-*manno*-oct-2-ulopyranosid)onate (19)

According to the procedure for compound **10**, pentasaccharide **18** (12.0 mg, 0.007 mmol) was debenzylated in dry MeOH (1.0 mL) over palladium on active charcoal (10%, 1 mg) under an atmosphere of hydrogen (1 atm) for 4 h, and deacetylated by treatment with sodium methoxide (0.1 N in MeOH, 100 µL, 10.0 µmol; 3 portions) in dry MeOH (1.0 mL) for 20 h. For ester saponification a solution of the deblocked intermediate in H_2O (2 mL) and aq. sodium hydroxide (0.1 N, 1.0 mL) was stirred for 3 h at 0 °C followed by neutralization. The residual solid after freeze-drying of the filtrate was desalted on a PD10 column (H_2O) and lyophilisation of the pooled fractions provided compound **19** (6.2 mg, 90%) as a colorless amorphous solid: $[\alpha]_D^{20}$ + 100.8 (*c* 0.43, H_2O); ^{1}H NMR (D_2O, pH ~ 7.0): δ 5.25 (d, 1H, $J_{1'',2''}$ 3.7 Hz, H-1″), 4.94 (d, 1H, $J_{1'''',2''''}$ 3.8 Hz, H-1‴′), 4.52 (d, 1H, $J_{1''',2'''}$ 8.0 Hz, H-1‴), 4.24 (app td, 1H, $J_{5'',4''}$ 10.1, $J_{5'',6''a} \sim J_{5'',6''b}$ 2.4 Hz, H-5″), 4.21 (d, 1H, $J_{5,6}$ 2.2 Hz, H-5), 4.03 (ddd, 1H, $J_{7,6}$ 9.4, $J_{7,8b}$ 6.3, $J_{7,8a}$ 3.0 Hz, H-7), 3.97 - 3.75 (m, 13H, H-3″, H-3‴′, H-4, H-4′, H-5′, H-6″a, H-6″b, H-6‴a, H-6‴b, H-6‴′a, H-7′, H-8a, H-8′a), 3.73 - 3.63 (m, 6H, H-4″, H-5‴, H-5‴′, H-6′, H-6‴′b, H-8′b), 3.58 (dd, 1H, $J_{8'b,8'a}$ 11.9 Hz, H-8b), 3.54 - 3.44 (m, 5H, H-2″, H-2‴′, H-3‴, H-4‴, H-6), 3.37 (app t, 1H, $J_{4'''',3''''} \sim J_{4'''',5''''}$ 9.5 Hz, H-4‴′), 3.29 (app t, $J_{2''',3'''} \sim$ 8.7 Hz, H-2‴), 3.07 (s, 3H, OC*H*$_3$), 2.06 - 1.99 (m, 2H, H-3*eq*, H-3′*eq*), 1.94 (app t, 1H, $J_{3ax,3eq} \sim J_{3ax,4}$ 12.1 Hz, H-3*ax*), 1.76 (app t, 1H, $J_{3'ax,3'eq} \sim J_{3'ax,4'}$ 12.5 Hz, H-3′*ax*); ^{13}C NMR (D_2O, pH ~ 7.0): δ 176.05 and 175.92 (2

s, 2C, C-1, C-1′), 103.69 (d, C-1‴), 102.84 (s, C-2′), 101.34 (s,C-2), 99.61 (d, C-1″), 98.79 (d, C-1⁗), 79.98 (d, C4″), 76.40 (d, C-3‴), 74.97 (d, C-5‴), 73.81 (d, C-2‴), 73.78 (d, C-5), 73.62 (d, C-3⁗), 73.10 (d, C-6), 72.71 and 72.55 (2d, 2C, C-5⁗, C-6′), 72.45 (d, C-2′), 72.32 (d, C-2⁗), 72.04 and 72.03 (2 d, 2C, C-3″, C-4), 71.52 (d, C-7′), 71.06 (d, C-5″), 70.49 (d, 2C, C-4‴, C-4⁗), 69.29 (d, C-7), 67.67 (d, C-5′), 66.75 (d, C-4′), 66.74 (t, C-6‴), 64.02 (t, C-8), 63.63 (t, C-8′), 61.39 (t, C-6⁗), 60.30 (t, C-6″), 51.37 (q, O*C*H$_3$), 35.50 (t, C-3′), 35.19 (t, C-3); ESI-TOF HRMS: *m/z* = 981.2906; calcd for $C_{35}H_{58}O_{30}Na^+$: 981.2095.

Acknowledgments

The authors thank the Austrian Science Fund FWF for financial support (grant P 24921).

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Manuscript #4

Pokorny, B.; Kosma, P.* *Chemistry Open*, *accepted manuscript* (**2015**).

"Scope and limitations of 3-iodo-Kdo fluoride based glycosylation chemistry using N-acetyl glucosamine acceptors"

doi:10.1002/open.201500126

Scope and limitations of 3-iodo-Kdo fluoride based glycosylation chemistry using *N*-acetyl glucosamine acceptors

Barbara Pokorny and Paul Kosma*[a]

Abstract: The ketosidic linkage of 3-deoxy-D-*manno*-octulosonic acid (Kdo) to lipid A constitutes a general structural feature of the bacterial lipopolysaccharide core. Glycosylation reactions of Kdo donors, however, are challenging due to the absence of a directing group at C-3 and elimination reactions resulting in low yields and anomeric selectivities of the glycosides. While 3-iodo-Kdo fluoride donors showed excellent glycosyl donor properties for the assembly of Kdo oligomers, glycosylation of *N*-acetyl-glucosamine derivatives was not straightforward. Specifically, oxazoline formation of a β-anomeric methyl glycoside as well as iodonium ion transfer to an allylic aglycon was found. In addition, dehalogenation of the directing group by hydrogen atom transfer proved to be incompatible with free hydroxyl groups next to benzyl groups. By contrast, glycosylation of a suitably protected methyl 2-acetamido-2-deoxy-α-D-glucopyranoside derivative and subsequent deiodination proceeded in excellent yields and α-specificity, and allowed for subsequent 4-O-phosphorylation. This way, the disaccharides α-Kdo-(2→6)-α-GlcNAcOMe and α-Kdo-(2→6)-α-GlcNAcOMe-4-phosphate were obtained in good overall yields.

Introduction

The eight-carbon sugar 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) is a biomedically important constituent of bacterial polysaccharides occurring in capsular polysaccharides (CPS), O-antigens and the core region of lipopolysaccharides (LPS). Whereas Kdo has been found in both anomeric configurations in CPS, the LPS of many Gram-negative bacteria harbors a structurally conserved α-(2→4)-linked Kdo-disaccharide which connects the endotoxically active lipid A part to the core region and the O-antigenic polymer.[1] Lipid A is composed of a bisphosphorylated β-(1→6)-linked N-/O-acylated glucosamine disaccharide which plays a decisive role in the immune response of host cells when infected by Gram-negative bacteria.[2] Kdo is present in an acid-labile ketosidic linkage to position 6 of the distal glucosamine unit. Whereas the acylated, bisphosphorylated diglucosamine unit provides the main interactions in the complex with Toll-like receptor 4 / myelodifferentiation factor MD-2, the Kdo and adjacent heptose and outer core sugars provide additional binding epitopes for receptor interactions and recognition by antibodies and lectins.[3]

[a] Dipl.-Ing. Barbara Pokorny, Prof. Dr. Paul Kosma
Department of Chemistry
University of Natural Resources and Life Sciences-Vienna
Muthgasse 18, A-1190 Vienna, Austria
E-mail: paul.kosma@boku.ac.at

Supporting information for this article is given via a link at the end of the document.

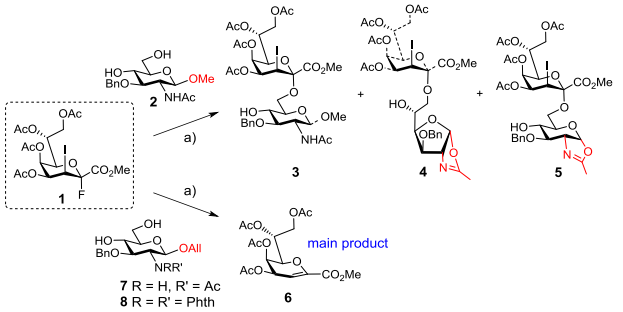
Thus, the chemical synthesis of relevant Kdo-lipid A fragments constitutes an important target and has successfully been pursued by several groups.[4] Coupling of Kdo donors, however, is far from trivial and an elaborate optimization of protecting and anomeric leaving groups for each glycosylation step is often crucial for good results.[5,6] Inherent challenges in glycosidation reactions of 3-deoxy-2-ulosonic acid glycosyl donors pertain to the absence of a stereodirecting group at C-3 resulting in low anomeric selectivities. Furthermore, competing elimination reactions are common and are promoted by the deactivating C-1 ester group. In our previous work we have presented a convenient approach towards the α-specific and regioselective synthesis of Chlamydia-related Kdo oligomers using 3-iodo-Kdo fluoride donor **1** for the coupling step followed by deiodination of the stereodirecting auxiliary group.[7] Gratifyingly, the elimination reaction could also be largely suppressed and the protocol was successfully expanded to include the formation of the sterically demanding α-(2→5)-linkage of Kdo units.[8] In continuation of these applications, we have set out to investigate the suitability of donor **1** for the glycosylation of glucosamine acceptors, specifically addressing regioselective glycosylation at position 6 with the option for subsequent phosphorylation at position 4 in order to generate the common phosphoester substitution at the distal glucosamine unit of lipid A.[2,9]

Results and Discussion

As glycosyl acceptors, *N*-acetyl-protected monosaccharide derivatives were selected, since glycosylation reactions of the primary alcohol of *N*-acetyl-, *N*-acyl-, *N*-benzyloxycarbonyl- and *N*-trichloroethoxycarbonyl-protected glucosamine derivatives with various Kdo donors led to comparable yields and anomeric selectivities.[10] In a first trial experiment, the easily accessible *N*-acetyl-β-D-glucosamine methyl glycoside derivative **2**[11] was coupled with the previously described peracetylated 3-iodo-Kdo fluoride donor **1**[7] under $BF_3{\cdot}Et_2O$-promotion in CH_2Cl_2 (Scheme 1). Provided, that a regioselective reaction at position 6 would be achieved, the 4-OH group should remain accessible for subsequent phosphate introduction, thus minimizing protecting group manipulations.

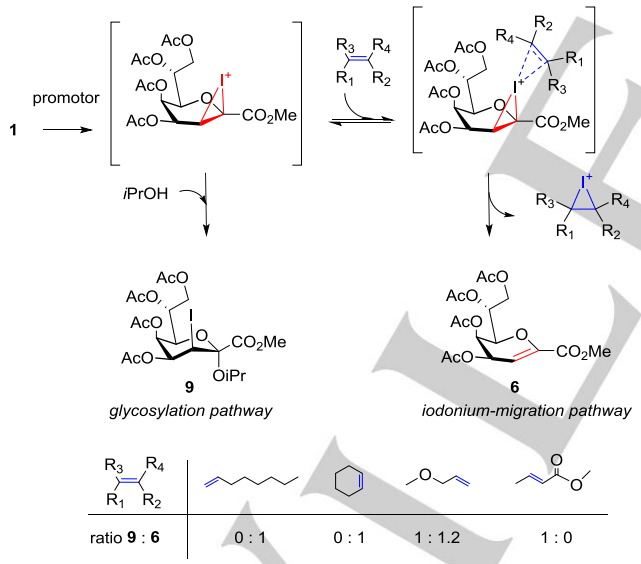
Disappointingly, disaccharide **3** was isolated in poor yield (32%). As expected, only traces of elimination product **6**[12] were found and formation of the corresponding β-linked Kdo-GlcNAc disaccharide was not observed. As the main disaccharide side products furanose **4** (11%) and pyranose **5** oxazolines (3%) were identified. The structure of the furanose ring form of **4** was proven by an HMBC correlation between H-1 and C-4. When donor **1** was reacted with allyl glycoside **7** (see Supporting Information) under similar glycosylation conditions, a complex mixture of degradation products was obtained. Interestingly, a

pronounced elimination of donor **1** giving glycal ester **6** was observed on TLC.



Scheme 1. Reagents and conditions: a) $BF_3{\cdot}Et_2O$, 3 Å molecular sieves, CH_2Cl_2, 0 °C → r.t, 1.5 h, **3**: 32%, **4**:11%, **5**: 3%.

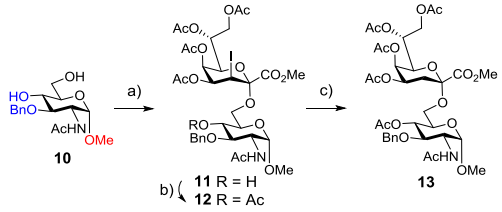
In a preliminary experiment, the same behavior was also noted for the *N*-phthalimido protected β-allyl glycosyl acceptor **8** (see Supporting Info). This outcome prompted us to investigate the reaction mechanism in more detail, since this high propensity of 3-iodo fluoride donor **1** towards elimination had previously not been observed.[7] We hypothesized that fast and irreversible migration of an intermediary iodonium ion[13,14] to the allylic double bond leads to formation of the glycal ester **6** and outcompetes glycoside formation (Scheme 2).



Scheme 2. Crossover experiments of intermediary iodonium ion in the presence of different olefins and ratio of obtained product mixture.

We expected that a transfer of iodonium ion generated from the activated donor to the allyl aglycon could be rationalized by the distinct difference in electron-density of the allyl aglycon when compared to the rather electron-deficient α,β-unsaturated ester **6**. To investigate this hypothesis, the competitive behavior of different olefins in a coupling reaction of donor **1** with 2-propanol in CH_2Cl_2 under $BF_3{\cdot}Et_2O$-promotion was screened (for experimental detail see Supporting Information). The reaction was performed and directly monitored in an NMR tube. The consumption of the respective olefin was determined by comparing representative ^{1}H NMR signals before and after donor activation in relation to the residual solvent peak. The decrease of olefin matched the amount of glycal **6** formed in the mixture. Immediate and intensive discoloration of the solutions upon activation indicated the instability of the resulting alkyl halide structures under the applied conditions that prevented identification of the iodo species by NMR. In fact the unsubstituted olefins 1-octene and cyclohexene suppressed formation of the known 2-propyl glycoside **9**[7] and donor **1** degraded completely to glycal **6** within a few seconds. Next we used allyl methyl ether resembling the aglycon of acceptors **7** and **8**. Parallel formation of glycoside **9** and glycal **6** (final composition of the mixture was based on relative ^{1}H NMR integral values: **9**:**6** = 1:1.2) over a period of 1 h indicated that both pathways (Scheme 2) are operative. In contrast, in the presence of α,β-unsaturated methyl crotonate, glycoside **9** was obtained without any formation of elimination product **6**. The observation that this electrophilic double bond does not inhibit the glycosylation reaction was in agreement with our previous result that donor **1** readily glycosylates glycal ester acceptors.[7] In summary, electronic rather than steric effects seem to dictate the halonium-migration - a trend that is conclusively reflected by the different results obtained for the two terminal olefins 1-octene and allyl methyl ether. This may further explain why 2-iodo-2-deoxy sugar donors, that – in comparison to ulosonic acids like Kdo - lack a deactivating C-1 ester group next to the anomeric center, are not affected by a competitive iodonium-ion migration in the presence of allyl groups.[15] Thus, the allyl group turned out to be an unsuitable protecting or aglyconic group for the 3-iodo-Kdo fluoride donor **1** under these conditions.

Next, the α-anomeric methyl glycoside **10** (see Supporting Info) was subjected to glycosylation (Scheme 3). In contrast to the outcome of the reaction with β-anomeric glycoside **2**, the resulting disaccharide **11** was not susceptible to oxazoline formation under the applied conditions. The coupling reaction with sparingly soluble acceptor **10** provided disaccharide **11** in moderate yield (ca. 55%) together with an inseparable impurity (ca. 5% based on the ^{1}H NMR data). First, we believed in the presence of the 4-*O*-regioisomer as only one *m/z* value was observed for the mixture. However, the expected low-field shifted ^{13}C NMR signal of C-4 and high-field shifted signal of C-6, respectively, were not detected. Instead, signals of C-3 and the benzylic carbon were shifted to lower field (see Supporting Information) in comparison to the major compound.



Scheme 3. Reagents and conditions: a) **1**, $BF_3{\cdot}Et_2O$, 3 Å molecular sieves, CH_2Cl_2, 0 °C → r.t., 1 h, 55%; b) Ac_2O, 4-(*N,N*-dimethylamino)pyridine, pyridine, r.t., 5 h, 59%; c) lauroyl peroxide, cyclohexane/1,2-dichloroethane (8:1), reflux, 2 h, 75%.

For further analysis, **11** was submitted to the previously optimized dehalogenation procedure capitalizing on a hydrogen atom transfer reaction using lauroyl peroxide in cyclohexane (Scheme 3).[16] Free hydroxyl groups were usually tolerated by this method in previous experiments, although decreased solubility of only partially protected substrates required prolonged reaction times.[7] Notably, the free 4-hydroxyl group vicinal to the benzyl group led to a significantly decreased yield. Among different side products, an acid labile 3,4-*O*-benzylidene protected disaccharide could be identified. Similar observations have been described by the Bols group, who later capitalized on this benzylidene formation and its ensuing cleavage as a regioselective deprotection method for benzyl groups vicinal to free OH groups.[17] In agreement with their observation that free hydroxyl groups were essential for this reaction type[17a]- involving a radical process[17b]- benzyl groups did not interfere with the hydrogen atom transfer reaction when using fully protected carbohydrate moieties. Further on, the disaccharide **11** containing the unknown entity was *O*-acetylated (**12**) prior to dehalogenation that provided disaccharide **13** in 75% yield. Neither of these steps allowed separation of the minor unit by HPLC. Again, the only significant differences were seen for the low-field NMR shifts of C-3 and the benzylic carbon of the minor species. Thus, we surmised the presence of two stable rotamers in the disaccharide product.

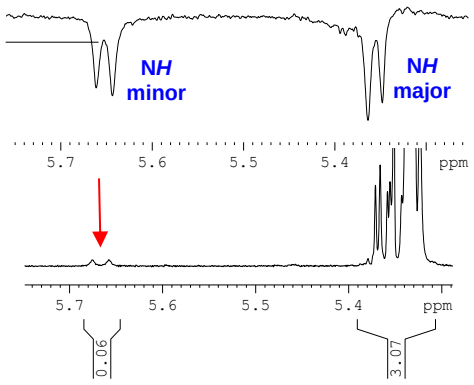
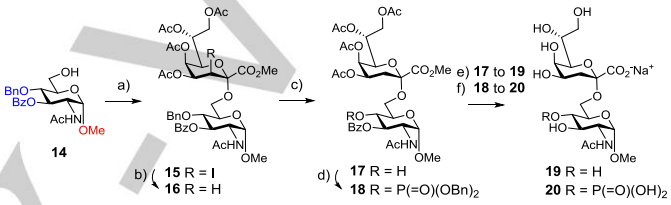


Figure 1. Evidence for the presence of two rotamers in disaccharide **13**; top: 1D NOE-difference spectrum after selective pulse at 5.64 ppm (N*H* signal of minor compound); bottom: expansion plot of ^{1}H-NMR of **13** showing the NH signals.

This was eventually proven by a method proposed by Ley's group wherein 1D-NOE-difference spectra are used to distinguish between equilibrating rotamers and non-equilibrating isomers.[18] By selective excitation of the N*H* signal of the minor entity a parallel attenuation of the distant and heavily overlapped N*H* of the major species was observed (Fig. 1). This phenomenon only occurs for the equilibrating rotamers but not for chemically distinct isomers.

To increase the limited solubility of the acceptor and to avoid problems at the dehalogenation step, the protecting group pattern of the GlcNAc acceptor was revised and the 3-*O*-benzoyl-4-*O*-benzyl-protected acceptor **14** (see Supporting Info) was coupled with 3-iodo fluoride donor **1** following the general protocol (Scheme 4). This way, disaccharide **15** was obtained in a high yield (89%) as the α-anomer only[19], and was dehalogenated (87% of **16**) by hydrogen atom transfer.[20] Debenzylation by catalytic hydrogenation (Pd/C, H_2) afforded 4-OH disaccharide **17** (96%). Subsequent phosphorylation capitalizing on the two-step procedure using dibenzyl *N,N*-diisopropylphosphoramidite/1*H*-tetrazole followed by oxidation with *m*CPBA yielded 4-O-phosphotriester **18** (70%).[21]



Scheme 4. Reagents and conditions: a) **1**, $BF_3{\cdot}Et_2O$, 3 Å molecular sieves, CH_2Cl_2, 0 °C → r.t., 1 h, 89%; b) lauroyl peroxide, cyclohexane/1,2-dichloroethane (8:1), reflux, 2 h, 87%; c) Pd/C (10%), H_2, MeOH, r.t., 3 h, 96%; d) dibenzyl *N,N*-diisopropylphosphoramidite, 1*H*-tetrazole, 4 Å molecular sieves, CH_2Cl_2, 0 °C, 70 min, then *m*-chloroperbenzoic acid, 0 °C, 1 h, 70%; e) NaOMe, MeOH, r.t., 3 h, then aq. NaOH, 0 °C, 5 h, 97%; f) Pd/C (10%), H_2, MeOH, r.t., 30 min, then NaOMe, MeOH, r.t., 17 h, then aq. NaOH, 0 °C, 5 h, 99%.

Compound **17** was subjected to transesterification with methanol under Zemplén conditions (the formed methyl benzoate had to be removed by repeated extraction between water and *n*-hexane), followed by saponification of the methyl ester to give disaccharide **19** in 97% yield. For global deprotection of **18**, the phosphotriester was first debenzylated affording the free phosphate, which was no longer prone to migration/hydrolysis under basic conditions. Deprotection of the acyl groups and ester hydrolysis furnished the deblocked disaccharide **20** as sodium salt in near theoretical yield.

Conclusions

In conclusion, 3-iodo-Kdo fluoride donor **1** was suitable for stereo- and regioselective glycosylation of a 6-OH GlcNAc acceptor, but high yields relied on an appropriate protecting group pattern. The acceptor had to be designed as the α-OMe glycoside to avoid oxazoline formation. Ensuing dehalogenation by hydrogen atom transfer reaction did not tolerate free hydroxyl

groups in close proximity to the benzyl protecting group. Furthermore, the 3-iodo group was incompatible with the presence of nucleophilic olefins due to putative iodonium ion migration resulting in glycal ester **6**. Within these boundary conditions, however, the α-specific glycosylation, dehalogenation and global deprotection proceeded in high overall yields.

Experimental Section

General

All purchased chemicals were used without further purification unless stated otherwise. Solvents (acetonitrile, CH_2Cl_2, cyclohexane, 1,2-dichloroethane, *N,N*-dimethylformamide) were dried over activated 4 Å molecular sieves. Dry MeOH (secco solv) was purchased from Merck. Cation exchange resin DOWEX 50 H^+ was regenerated by consecutive washing with HCl (3 M), water and dry MeOH. The promotor $BF_3{\cdot}Et_2O$ was used as a solution in diethyl ether (≥46% according to the manufacturer). Concentration of organic solutions was performed under reduced pressure < 40 °C. Optical rotations were measured with a Perkin-Elmer 243 B Polarimeter. $[\alpha]_D^{20}$ values are given in units of 10^{-1}deg cm^2g^{-1}. Thin layer chromatography was performed on Merck precoated plates: generally on 5 x 10 cm, layer thickness 0.25 mm, Silica Gel $60F_{254}$; alternatively on HP-TLC plates with 2.5 cm concentration zone (Merck). Spots were detected by dipping reagent (anisaldehyde-H_2SO_4). For column chromatography silica gel (0.040 – 0.063 mm) was used. HP-column chromatography was performed on a pre-packed column (YMC-Pack SIL-06, 0.005 mm, 250 x 10 mm). Size exclusion chromatography for desalting was performed on pre-packed PD-10 columns (GE Healthcare, Sephadex™ G-25 M). NMR spectra were recorded with a Bruker Avance III 600 instrument (600.22 MHz for ^1H, 150.93 MHz for ^{13}C and 242.97 MHz for ^{31}P) using standard Bruker NMR software. ^1H spectra were referenced to 7.26 ($CDCl_3$) and 0.00 (D_2O, external calibration to 2,2-dimethyl-2-silapentane-5-sulfonic acid) ppm unless stated otherwise. ^{13}C spectra were referenced to 77.00 ($CDCl_3$) and 67.40 (D_2O, external calibration to 1,4-dioxane) ppm. ^{31}P spectra in D_2O were referenced to external *ortho*-phosphoric acid (0.00 ppm). ESI-MS data were obtained on a Waters Micromass Q-TOF Ultima Global instrument or on a Thermo ScientificExactive Plus Orbitrap instrument.

Synthesis

Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→6)-methyl 2-acetamido-3-*O*-benzoyl-4-*O*-benzyl-2-deoxy-α-D-glucopyranoside (15)

A suspension of 3-iodo-Kdo donor **1** (76.6 mg, 0.140 mmol) and acceptor **14** (50.0 mg, 0.116 mmol) in dry CH_2Cl_2 (4.0 mL) containing ground 3 Å molecular sieves (200 mg) was stirred for 1 h at ambient temperature. The cooled mixture (0 °C) was treated with $BF_3{\cdot}Et_2O$ (44 µL, 0.349 mmol) and kept at ambient temperature for 1 h. After dilution with CH_2Cl_2 the organic phase was washed successively with satd. $NaHCO_3$, sodium thiosulfate (5 w%) and brine. The solution was dried ($MgSO_4$), filtered and concentrated and the crude product was purified by chromatography (toluene/EtOAc 1:2) affording disaccharide **15** (99.2 mg, 89%) as a colorless oil; R_f 0.31 (toluene/EtOAc 1:2, HP-TLC); $[\alpha]_D^{20}$ = + 68.2 (*c* = 1.28 , $CHCl_3$); ^1H NMR ($CDCl_3$): δ = 8.03 - 8.00 (m, 2H, Ar), 7.58 - 7.55 (m, 1H, Ar), 7.46 - 7.42 (m, 2H, Ar), 7.25 - 7.08 (m, 5H, Ar), 5.82 (d, 1H, $J_{NH,2}$ 9.5 Hz, N*H*), 5.55 (dd, 1H, $J_{3,2}$ 10.8, $J_{3,4}$ 9.1 Hz, H-3), 5.38 - 5.36 (m, 1H, H-5'), 5.34 (ddd, 1H, $J_{7',6'}$ 9.5, $J_{7',8'b}$ 4.1, $J_{7',8'a}$ 2.6 Hz, H-7'), 5.05 (dd, 1H, $J_{4',3'}$ 4.7, $J_{4',5'}$ 3.7 Hz, H-4'), 4.72 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.67 (dd, 1H, $J_{8'a,8'b}$ 12.3 Hz, H-8'a), 4.64 (d, 1H, *J* 11.2 Hz, C*H*HPh), 4.51 (d, 1H, H-3'), 4.44 (d, 1H, *J* 11.2 Hz, CH*H*Ph), 4.36 (ddd, 1H, H-2), 4.32 (dd, 1H, $J_{6',5'}$ 1.8 Hz, H-6'), 4.16 (dd, 1H, H-8'b), 3.93 – 3.89 (m, 1H, H-5), 3.74 (s, 3H, CO_2CH_3), 3.61 - 3.52 (m, 3H, H-4, H-6a, H-6b), 3.42 (s, 3H, OCH_3), 2.11, 2.05, 2.03, 1.99 and 1.83 ppm (5 s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ = 170.3, 170.1, 170.0, 169.5 and 169.3 (5 s, 5C, $COCH_3$), 166.8 (s, 1C, *C*OPh), 165.6 (s, C-1'), 137.0 (s, 1C, Ar), 133.4 (d, 1C, Ar), 129.7 (d, 2C, Ar), 129.3 (s, 1C, Ar), 128.5 (d, 2C, Ar), 128.4 (d, 2C, Ar), 128.0 (d, 1C, Ar), 127.9 (d, 2C, Ar), 101.4 (s, C-2'), 98.0 (d, C-1), 76.3 (d, C-4), 74.9 (t, CH_2Ph), 74.2 (d, C-3), 69.8 (d, C-5), 68.3 (d, C-6'), 67.7 (d, C-7'), 65.2 (d, C-4'), 65.1 (t, C-6), 63.4 (d, C-5'), 61.8 (t, C-8'), 55.1 (q, OCH_3), 52.8 (q, CO_2CH_3), 52.3 (d, C-2), 23.1 (q, $COCH_3$), 21.8, 20.9, 20.8, 20.7 and 20.6 ppm (4 q, 1 d, 5C, 4x $COCH_3$, C-3'); HRMS (ESI-TOF): *m/z* calcd for $C_{40}H_{48}INO_{18}Na^+$: 980.1808 [M+Na^+]; found: 980.1808.

Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→6)-methyl 2-acetamido-3-*O*-benzoyl-4-*O*-benzyl-2-deoxy-α-D-glucopyranoside (16)

Disaccharide **15** (99.0 mg, 0.103 mmol) was dissolved in dry cyclohexane (4.0 mL) and dry 1,2-dichloroethane (0.5 mL) and the solution was degassed with argon. After heating to reflux for 15 min, lauroyl peroxide (14.4 mg, 0.036 mmol) was added and reflux was continued for 2 h. The mixture was concentrated in *vacuo* and the residue was subjected to chromatography (*n*-hexane/EtOAc 1:9) yielding dehalogenated compound **16** (75.0 mg, 87%) as a colorless oil; R_f 0.36 (CH_2Cl_2/EtOAc 1:1, HP-TLC); $[\alpha]_D^{20}$ = + 82.2 (*c* = 0.66, $CHCl_3$); ^1H NMR ($CDCl_3$): δ = 8.02 - 8.00 (m, 2H, Ar), 7.58 - 7.54 (m, 1H, Ar), 7.46 - 7.42 (m, 2H, Ar), 7.22 - 7.12 (m, 5H, Ar), 5.81 (d, 1H, $J_{NH,2}$ 9.5 Hz, N*H*), 5.55 (dd, 1H, $J_{3,2}$ 10.8, $J_{3,4}$ 9.0 Hz, H-3), 5.37 - 5.33 (m, 2H, H-4', H-5'), 5.21 (ddd, 1H, $J_{7',6'}$ 9.6, $J_{7',8'b}$ 4.6, $J_{7',8'a}$ 2.5 Hz, H-7'), 4.72 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 4.65 (d, 1H, *J* 11.0 Hz, C*H*HPh), 4.59 (dd, 1H, $J_{8'a,8'b}$ 12.3 Hz, H-8'a), 4.49 (d, 1H, *J* 11.0 Hz, CH*H*Ph), 4.37 (ddd, 1H, H-2), 4.18 (dd, 1H, $J_{6',5'}$ 1.1 Hz, H-6'), 4.10 (dd, 1H, H-8'b), 3.92 - 3.88 (m, 1H, H-5), 3.76 (dd, 1H, $J_{6a,6b}$ 10.6, $J_{6a,5}$ 1.7 Hz, H-6a), 3.72 (s, 3H, CO_2CH_3), 3.61 (app t, 1H, $J_{4,5}$ ~ 9.7 Hz, H-4), 3.57 (dd, 1H, $J_{6b,5}$ 7.2 Hz, H-6b), 3.43 (s, 3H, OCH_3), 2.24 - 2.20 (m, 1H, H-3'*eq*), 2.10 (app t, 1H, $J_{3'ax,3'eq}$ ~ $J_{3'ax,4'}$ 12.3 Hz, H-3'*ax*), 2.08, 2.023, 2.020, 1.98 and 1.83 ppm (5 s, each 3H, $COCH_3$); ^{13}C NMR: δ = 170.5, 170.4, 170.0, 169.9, 169.7, 167.2 and 166.9 (7 s, 7C, 5x $COCH_3$, *C*OPh, C-1'), 137.2 (s, 1C, Ar), 133.4 (d, 1C, Ar), 129.8 (d, 2C, Ar), 129.4 (s, 1C, Ar), 128.5 (d, 2C, Ar), 128.4 (d, 2C, Ar), 127.91 (d, 1C, Ar), 127.87 (d, 2C, Ar), 98.5 (s, C-2'), 98.1 (d, C-1), 76.6 (d, C-4), 74.9 (t, CH_2Ph), 74.3 (d, C-3), 69.9 (d, C-5), 68.6 (d, C-6'), 67.7 (d, C-7'), 66.3 (d, C-4'), 64.5 (d, C-5'), 63.2 (t, C-6), 62.2 (t, C-8'), 55.2 (q, OCH_3), 52.6 (q, CO_2CH_3), 52.4 (d, C-2), 31.8 (t, C-3'), 23.1, 20.8, 20.73, 20.69 and 20.6 ppm (5 q, 5C, $COCH_3$); HRMS (ESI-TOF): *m/z* calcd for $C_{40}H_{49}NO_{18}Na^+$: 854.2842 [M+Na^+]; found: 854.2847.

Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→6)-methyl 2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (17)

Compound **16** (70.0 mg, 0.084 mmol) was dissolved in dry MeOH (3.0 mL). The atmosphere was exchanged to argon by alternating evacuation and flushing with argon. Pd/C (10%, 3 mg) was added followed by successive exchange of atmosphere to argon and hydrogen. After hydrogenation for 3 h, the mixture was filtered via a syringe filter, rinsed with MeOH and the filtrate was concentrated providing compound **17** (60.0 mg, 96%) as a colorless oil, which was used without purification; R_f 0.43 (EtOAc); $[\alpha]_D^{20}$ = + 99.3 (*c* = 0.78, $CHCl_3$); ^1H NMR ($CDCl_3$): δ = 8.05 - 8.03 (m, 2H, Ar), 7.60 - 7.56 (m, 1H, Ar), 7.47 - 7.43 (m, 2H, Ar), 5.78 (d, 1H, $J_{NH,2}$ 9.8 Hz, N*H*), 5.37 - 5.32 (m, 2H, H-4', H-5'), 5.24 (dd, 1H, $J_{3,2}$ 10.7, $J_{3,4}$ 9.2 Hz, H-3), 5.23 (ddd, 1H, $J_{7',6'}$ 9.3, $J_{7',8'b}$ 4.8,

$J_{7',8'a}$ 2.6 Hz, H-7'), 4.75 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.59 (dd, 1H, $J_{8'a,8'b}$ 12.1 Hz, H-8'a), 4.45 (ddd, 1H, H-2), 4.28 (dd, 1H, $J_{6',5'}$ 1.3 Hz, H-6'), 4.15 (dd, 1H, H-8'b), 3.89 - 3.84 (m, 1H, H-5), 3.82 (s, 3H, CO_2CH_3), 3.79 - 3.73 (m, 3H, H-4, H-6a, H-6b), 3.45 (s, 3H, OCH_3), 3.10 (d, 1H, *J* 3.8 Hz, O*H*), 2.21 - 2.17 (m, 1H, H-3'*eq*), 2.12 (app t, 1H, $J_{3'ax,3'eq}$ ~ $J_{3'ax,4'}$ 12.5 Hz, H-3'*ax*), 2.085, 2.077, 2.00, 1.97 and 1.86 ppm (5 s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ = 170.6, 170.4, 169.91, 169.89, 169.8, 168.2 and 167.6 (7 s, 7C, 5x $COCH_3$, *C*OPh, C-1'), 133.6 (d, 1C, Ar), 130.1 (d, 2C, Ar), 129.1 (s, 1C, Ar), 128.5 (d, 2C, Ar), 98.7 (s, C-2'), 98.3 (d, C-1), 75.5 (d, C-3), 70.4 (d, C-5), 70.0 (d, C-4), 68.7 (d, C-6'), 67.8 (d, C-7'), 66.3 (d, C-4'), 64.5 (d, C-5'), 63.5 (t, C-6), 62.3 (t, C-8'), 55.3 (q, OCH_3), 52.9 (q, CO_2CH_3), 51.4 (d, C-2), 31.9 (t, C-3'), 23.2, 20.8, 20.73, 20.68 and 20.6 ppm (5 q, 5C, $COCH_3$); HRMS (ESI-TOF): *m/z* calcd for $C_{33}H_{43}NO_{18}Na^+$: 764.2372 [M+Na$^+$]; found: 764.2372.

Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→6)-methyl 2-acetamido-3-*O*-benzoyl-2-deoxy-4-*O*-(dibenzylphosphoryl)-α-D-glucopyranoside (18)

A solution of compound **17** (19.5 mg, 0.026 mmol) in dry CH_2Cl_2 (1.0 mL) was degassed with argon. Under argon atmosphere 1*H*-tetrazole (5.5 mg, 0.079 mmol) was added followed by ground 4 Å molecular sieves (50 mg). After 30 min the suspension was cooled to 0 °C and treated dropwise with dibenzyl *N,N*-diisopropylphosphoramidite (17.3 µL, 0.053 mmol, in 3 portions within 70 min). *m*-Chloroperbenzoic acid (70 w%, 9.7 mg, 0.039 mmol) was added at 0 °C and the mixture was stirred for 1 h. The mixture was diluted with CH_2Cl_2 and satd. $NaHCO_3$, the aqueous phase was extracted with CH_2Cl_2 (3x) and the combined organic layers were dried ($MgSO_4$). Filtration, concentration of the organic phase and chromatography of the residue (toluene/EtOAc 1:4) provided phosphorylated compound **18** with a minor impurity which was separated by HP-column chromatography (*n*-hexane/EtOAc 1:2→1:4) affording **18** (18.3 mg, 70%) as a colorless oil; R_f 0.41 (*n*-hexane/EtOAc 1:4); $[\alpha]_D^{20}$ = + 64.8 (*c* = 0.78, $CHCl_3$); ^{1}H NMR ($CDCl_3$, ref. to 0.00, TMS): δ = 8.03 - 8.01 (m, 2H, Ar), 7.54 -7.51 (m, 1H, Ar), 7.39 - 7.35 (m, 2H, Ar), 7.29 - 7.13 (m, 8H, Ar), 6.91 - 6.89 (m, 2H, Ar), 5.79 (d, 1H, $J_{NH,2}$ 9.4 Hz, N*H*), 5.56 (dd, 1H, $J_{3,2}$ 10.9, $J_{3,4}$ 9.1 Hz, H-3), 5.41 (ddd, 1H, $J_{4',3'ax}$ 12.2, $J_{4',3'eq}$ 5.1, $J_{4',5'}$ 3.1 Hz, H-4'), 5.37 - 5.35 (m, 1H, H-5'), 5.21 (ddd, 1H, $J_{7',6'}$ 9.2, $J_{7',8'b}$ 5.0, $J_{7',8'a}$ 2.4 Hz, H-7'), 4.89 (dd, 1H, ^2J 11.8, $J_{H,P}$ 7.2 Hz, C*H*HPh), 4.78 (dd, 1H, ^2J 12.2, $J_{H,P}$ 7.9 Hz, C*H*HPh), 4.77 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.74 (dd, 1H, ^2J 11.7, $J_{H,P}$ 7.5 Hz, CH*H*Ph), 4.67 (dd, 1H, $J_{8'a,8'b}$ 12.2 Hz, H-8'a), 4.56 (dd, 1H, ^2J 11.8, $J_{H,P}$ 10.0 Hz, CH*H*Ph), 4.51 (app q, 1H, $J_{4,5}$ ~ $J_{4,P}$ 9.3 Hz, H-4), 4.41 (ddd, 1H, H-2), 4.34 (dd, 1H, $J_{6',7'}$ 1.4 Hz, H-6'), 4.09 (dd, 1H, H-8'b), 4.05 - 4.00 (m, 2H, H-5, H-6a), 3.74 (dd, 1H, $J_{6b,6a}$ 11.1, $J_{6b,5}$ 7.9 Hz, H-6b), 3.68 (s, 3H, CO_2CH_3), 3.48 (s, 3H, OCH_3), 2.22 - 2.18 (m, 1H, H-3'*eq*), 2.12 (app t, 1H, $J_{3'ax,3'eq}$ ~ 12.5 Hz, H-3'*ax*), 2.091, 2.086, 2.01, 1.96 and 1.84 ppm (5 s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ = 170.5, 170.4, 169.9, 169.83, 169.80, 167.2 and 166.9 (7 s, 7C, 5 x $COCH_3$, *C*OPh, C-1'), 135.5 (ds, 1C, *J* 7.2 Hz, Ar), 135.2 (ds, 1C, *J* 6.5 Hz, Ar), 133.4 and 130.0 (2 d, 3C, Ar), 129.1 (s, 1C, Ar), 128.44, 128.39, 128.32, 128.30, 127.69 and 127.66 (6 d, 12C, Ar), 98.5 (s, C-2'), 97.8 (d, C-1), 73.8 (dd, *J* 5.9 Hz, C-4), 72.1 (dd, *J* 2.0 Hz, C-3), 69.53 (d, C-5), 69.47 (dt, *J* 5.8 Hz, *C*H_2Ph), 69.4 (dt, *J* 5.9 Hz, *C*H_2Ph), 68.8 (d, C-6'), 68.1 (d, C-7'), 66.3 (d, C-4'), 64.7 (d, C-5'), 62.7 (t, C-6), 62.1 (t, C-8'), 55.4 (q, OCH_3), 52.6 (q, CO_2CH_3), 52.2 (d, C-2), 31.9 (t, C-3'), 23.1, 20.8, 20.73 and 20.66 ppm (4 s, 5C, $COCH_3$); ^{31}P NMR ($CDCl_3$): δ = -2.01 ppm; HRMS (ESI-TOF): *m/z* calcd for $C_{47}H_{56}NO_{21}PNa^+$: 1024.2975 [M+Na$^+$]; found: 1024.2976;

Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→6)-methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (19)

A solution of **17** (8.2 mg, 0.011 mmol) in dry MeOH was treated with sodium methoxide (0.1 M in MeOH, 22 µL, 2.2 µmol) at ambient temperature for 3 h. Ion-exchange resin DOWEX 50 (H^+-form) was added until the mixture reacted neutral. The resin was filtered off and the filtrate was concentrated. To remove methyl benzoate, water (3 mL) and *n*-hexane (3 mL) were added to the residual solid, the mixture was ultrasonicated for 10 sec and the organic layer was removed. Fresh *n*-hexane (3 mL) was added and the procedure was repeated. After two more extractions the aqueous phase was concentrated in *vacuo*, and the residue was stirred in aq NaOH (0.01 M, 3.0 mL) at 0 °C for 3 h. Additional aq NaOH (0.1 M, 0.5 mL) was added and after 2 h at 0 °C the mixture was neutralized with DOWEX 50 (H^+-form). The resin was filtered off, washed with water and the filtrate was freeze-dried. The crude product was desalted on a PD10 SEC column (H_2O). Freeze-drying of pooled fractions yielded sodium salt **19** (5.1 mg, 97 %) as a colorless amorphous solid; $[\alpha]_D^{20}$ = + 102.8 (*c* = 0.26, H_2O); ^{1}H NMR (D_2O, pH 7): δ = 4.71 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1, overlapped by residual solvent peak), 4.04 (ddd, 1H, $J_{4',3'ax}$ 12.1, $J_{4',3'eq}$ 5.0, $J_{4',5'}$ 2.9 Hz, H-4'), 3.99 - 3.97 (m, 1H, H-5'), 3.92 - 3.87 (m, 3H, H-2, H-7', H-8'a), 3.74 (ddd, 1H, $J_{5,4}$ 10.1, $J_{5,6a}$ 4.5, $J_{5,6b}$ 3.4 Hz, H-5), 3.68 - 3.54 (m, 5H, H-3, H-6a, H-6b, H-6', H-8'b), 3.44 (dd, 1H, $J_{4,3}$ 9.4 Hz, H-4), 3.34 (s, 3H, OCH_3), 2.05 (dd, 1H, $J_{3'eq,3'ax}$ 13.1 Hz, H-3'*eq*), 1.99 (s, 3H, $COCH_3$) and 1.76 ppm (app t, 1H, H-3'*ax*); ^{13}C NMR (D_2O, pH 7): δ = 175.8 and 175.3 (2 s, 2C, C-1', *C*OCH_3), 100.7 (s, C-2'), 98.8 (d, C-1), 72.3 and 72.2 (2 d, 2C, C-3, C-6'), 71.2 (d, C-4), 71.0 (d, C-5), 70.3 (d, C-7'), 67.1 and 66.9 (2 d, 2C, C-4', C-5'), 64.2 (t, C-8'), 62.7 (t, C-6), 56.0 (q, OCH_3), 54.4 (d, C-2), 34.9 (t, C-3') and 22.7 ppm (q, $COCH_3$); HRMS (ESI-TOF): *m/z* calcd for $C_{17}H_{28}NO_{13}^-$: 454.1555 [M-H]; found: 454.1564.

3-Deoxy-α-D-*manno*-oct-2-ulopyranosylonic acid-(2→6)-methyl 2-acetamido-2-deoxy-4-phosphono-α-D-glucopyranoside sodium salt (20)

Phosphotriester **18** (11.0 mg, 0.011 mmol) was dissolved in dry MeOH (2.0 mL). The atmosphere was exchanged to argon by alternating evacuation and flushing with argon. Pd/C (10%, 1 mg) was added followed by successive exchange of atmosphere to argon and hydrogen. After hydrogenation for 30 min the mixture was filtered via a syringe filter into a flask containing sodium methoxide (0.1 M in MeOH, 110 µL, 0.011 mmol) in dry MeOH (2.0 mL). After rinsing the filter with MeOH the filtrate was neutralized by further addition of sodium methoxide (0.1 M in MeOH). The solvent was removed until a volume of 3 mL of MeOH remained: The pH was adjusted to 8 with sodium methoxide and the mixture was stirred for 3 h at room temperature. After increasing the pH to 9, stirring was continued for 14 h. Excessive base was neutralized by adding DOWEX 50 (H^+-form) resin. The resin was filtered off, rinsed with MeOH and the filtrate was concentrated. Removal of methyl benzoate, ester saponification and desalting were performed as described for compound **19** providing phosphate **20** (6.3 mg, 99 %) as a colorless amorphous solid; $[\alpha]_D^{20}$ = + 91.6 (*c* = 0.63, H_2O); ^{1}H NMR (D_2O, pH 7): δ = 4.71 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1, overlapped by residual solvent peak), 4.06 (ddd, 1H, $J_{4',3'ax}$ 12.0, $J_{4',3'eq}$ 5.2, $J_{4',5'}$ 3.0 Hz, H-4'), 3.99 - 3.98 (m, 1H, H-5'), 3.92 - 3.76 (m, 7H, H-2, H-3, H-4, H-5, H-6', H-7', H-8'a), 3.70 - 3.63 (m, 2H, H-6a, H-8'b), 3.57 - 3.53 (m, 1H, H-6b), 3.34 (s, 3H, OCH_3), 2.03 (dd, 1H, $J_{3'eq,3'ax}$ 13.1 Hz, H-3'*eq*), 1.98 (s, 3H, $COCH_3$) and 1.74 ppm (app t, 1H, H-3'*ax*); ^{13}C NMR (D_2O, pH 7): δ = 176.0 and 175.1 (2 s, 2C, C-1', *C*OCH_3), 100.6 (s, C-2'), 98.1 (d, C-1), 74.3 (dd, *J* 5.2 Hz, C-4), 72.2 (d, 2C, C-3, C-6'), 70.5 (d, C-7'), 70.3 (dd, *J* 8.7 Hz, C-5), 67.2 (d, C-5'), 66.9 (d, C-4'), 64.2 (t, C-8'), 63.2 (t, C-6), 55.9 (q, OCH_3), 54.1 (d, C-2), 35.0 (t, C-3') and 22.7 ppm (q, $COCH_3$); ^{31}P NMR (D_2O, pH 7): δ = -3.30 ppm; HRMS (ESI-TOF): *m/z* calcd for $C_{17}H_{29}NO_{16}P^-$: 534.1219 [M-H]; found: 534.1229.

Acknowledgements

The authors thank the Austrian Science Fund (FWF) for financial support (grant P 24921).

Keywords: Glycosylation • Kdo • Iodonium • Lipopolysaccharide • Hydrogen transfer

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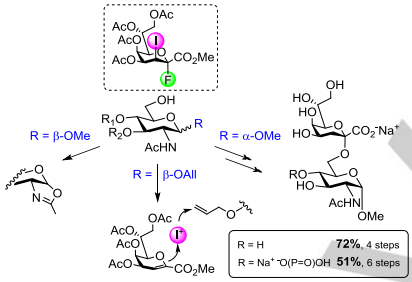

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Pokorny, B.; Kosma, P.* *Org. Lett.* **2015**, *17*, 110-113.

"First and Stereoselective Synthesis of an α-(2→5)-Linked Disaccharide of 3-Deoxy-D-*manno*-oct-2-ulosonic Acid (Kdo)"

doi:10.1021/ol5033128

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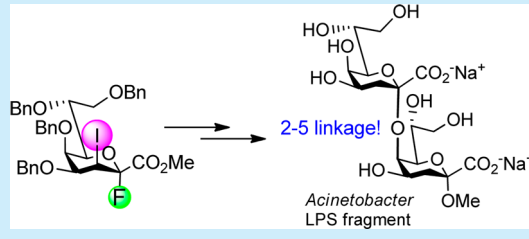
First and Stereoselective Synthesis of an α-(2→5)-Linked Disaccharide of 3-Deoxy-D-*manno*-oct-2-ulosonic Acid (Kdo)

Barbara Pokorny and Paul Kosma*

Department of Chemistry, University of Natural Resources and Life Sciences-Vienna, Muthgasse 18, A-1190 Vienna, Austria

Supporting Information

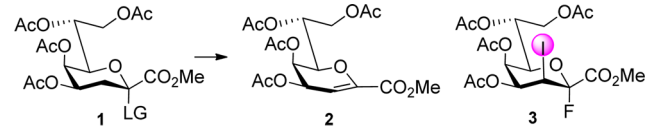
ABSTRACT: Resistance of bacterial pathogens toward antibiotics has revived interest in lipopolysaccharide (LPS) motifs as potential therapeutic targets. The LPS of several pathogenic *Acinetobacter* strains comprises a 4,5-branched Kdo trisaccharide containing an uncommon (2→5)-linkage. In this contribution the first stereoselective glycosylation method for obtaining an α-Kdo-(2→5)-α-Kdo disaccharide in good yield is highlighted. The synthetic approach used for accessing this linkage type will allow for future studies of the immunoreactivity associated with this unique bacterial Kdo inner core structure.



Lipopolysaccharides (LPS) are found in the outer membrane of the Gram-negative bacterial cell wall and comprise the conserved Lipid A which is noncovalently anchored to the cell wall via sugar-bound fatty acids. This lipophilic part is further linked to the core region followed by a species-specific polysaccharide, termed *O*-antigen.[1] The common bacterial sugar 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) is a fundamental component of the LPS core domain, being present in a structurally conserved α-(2→4)-linked Kdo disaccharide unit.[2] This basic disaccharide is elongated at position 5 of the proximal Kdo unit by various additional core sugars such as L-*glycero*-D-*manno*-heptose, glucose or mannose, resulting in branched structures.[3] Investigations of LPS from different *Acinetobacter* strains, however, have revealed the presence of branched Kdo tetra- and trisaccharides containing an unusual α-(2→5) inter-Kdo linkage. In particular, several *A. baumannii* strains as well as the *A. radioresistans* strain S13 have been found to harbor an α-Kdo-(2→5)[α-Kdo-(2→4)]α-Kdo trisaccharide fragment in the inner core region.[4a−d] An α-(2→5)-linked Kdo disaccharide was also detected in *Campylobacter lari* strain ATCC 35221.[4e] *Acinetobacter* strains are known to be responsible for severe nosocomial infections which are difficult to treat due to a pronounced resistance of the bacteria against antimicrobial drugs.[5]

While various routes toward (2→4)-, (2→8)-, and also (2→7)- interconnected Kdo disaccharides have been reported,[6−8] a synthetic access toward the (2→5)-disaccharide via glycosylation has not been achieved thus far. The chemical glycosylation of Kdo glycosyl acceptors at position 5 is hampered by low reactivity due to the axial orientation of the C-5 hydroxyl group, the steric hindrance exerted by the side chain unit, and the deactivating effect of the C-1 ester group. Likewise, Kdo donors (e.g. **1**, Scheme 1) usually suffer from considerable degradation during glycosylation reactions since they are prone to elimination reactions that produce glycal esters such as **2**.[2c,9] The elimination is also observed for conformationally constrained Kdo donors equipped with a 4,5-isopropylidene group.[6c,d] In addition, Kdo donors show low anomeric selectivity and reduced reactivity for glycoside formation due to the deactivating effect of the carboxylic group adjacent to the anomeric center. The challenge met in the construction of a Kdo-(2→5)-Kdo linkage is reflected in the complete regioselectivity observed during coupling of Kdo donors with 4,5-unprotected Kdo diol acceptors, leading to *O*-4 substituted products only.[6,10] Also, previous attempts in the author's laboratory using a peracetylated Kdo phosphite donor and 4-*O*-protected Kdo acceptor derivatives did not produce any disaccharides.[11] Notably, however, glycosylations of the 5-OH group of 4-*O*-protected Kdo substrates with aldopyranosyl donors (e.g., heptosyl, glucosyl, mannosyl donors) have been successfully implemented, illustrating the difficulty in forming a ketosidic bond to the sterically hindered 5-OH group of a Kdo acceptor.[12]

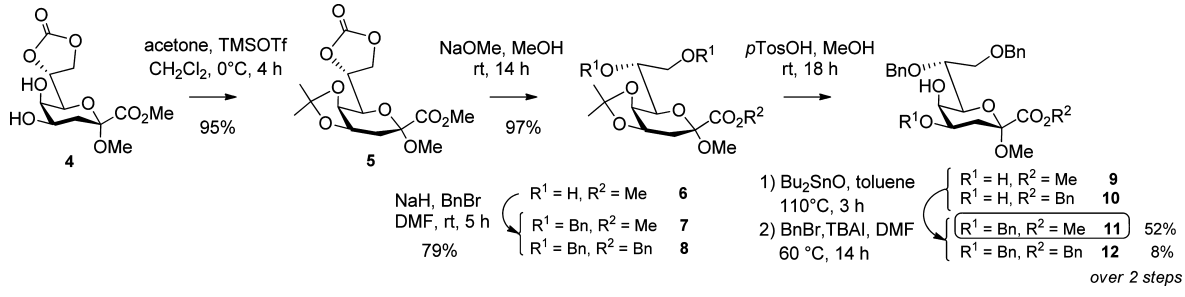
Scheme 1. Kdo Donors (LG = Leaving Group) and Glycal Ester Formation during Glycosylation



Recently, we have developed the acetylated 3-iodo-Kdo fluoride donor **3**[13] with a stereodirecting group at position 3. This donor has proven itself capable of α-stereoselective glycosylation reactions to provide several α-Kdo homomers related to *Chlamydia* LPS in high yield. Additionally, the elimination side reaction toward glycal **2** could be significantly suppressed and we surmised that these properties might provide access to the α-(2→5) Kdo disaccharide motif. For the design of an appropriate Kdo glycosyl acceptor, three

Received: November 14, 2014
Published: December 12, 2014

Scheme 2. Synthesis of the 4,7,8-Tri-*O*-benzyl Kdo Acceptor (11)

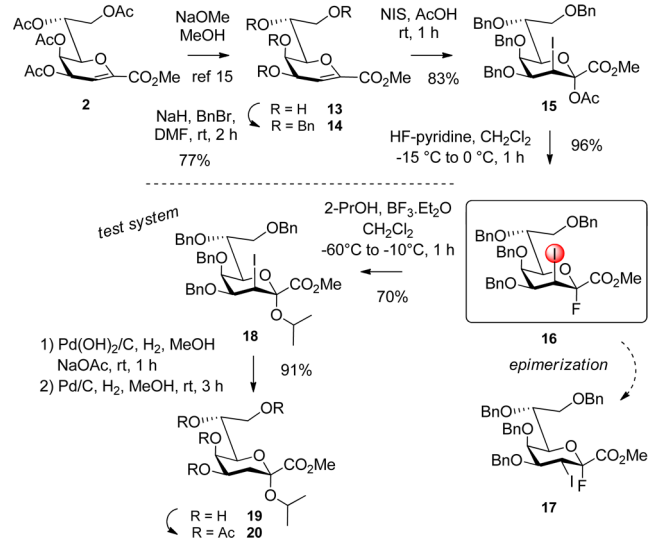


prerequisites for protecting groups were foreseen: (a) activating groups at remote positions to compensate for the intrinsically low reactivity of HO-5, (b) exclusion of silyl groups that would eventually be cleaved by fluoride species formed during the glycosylation step, and (c) stability toward Bronsted acids. The latter requirement resulted from a previous observation that addition of triethylamine abolished the glycosylation capability of donor **3** (thus, Bronsted acid formed after HF cleavage seems to be the active species involved in the coupling step). Since the disaccharide should serve as a ligand in STD-NMR and crystallographic studies, a methyl aglycon was selected.[13] Thus, the 4,7,8-tri-*O*-benzyl protected Kdo glycosyl acceptor derivative **11** was prepared starting from the previously described 7,8-*O*-carbonyl derivative **4**[13] (Scheme 2).

Reaction of **4** with acetone in the presence of TMSOTf yielded the 4,5-*O*-isopropylidene derivative **5** in 95% yield after silica gel filtration. Treatment of **5** with sodium methoxide in anhydrous methanol liberated the 7-OH and 8-OH groups (97% of **6**[14]), which were subsequently *O*-benzylated using sodium hydride and BnBr in dry DMF. Thus, a mixture of the methyl ester **7** and the benzyl ester **8** was obtained in a total yield of 79%. Cleavage of the 4,5-*O*-acetonide by treatment of the mixture of **7** and **8** with *para*-toluenesulfonic acid in methanol yielded a mixture of the two esters **9** and **10** after filtration through silica gel. Treatment of the mixture with dibutyl tin oxide and subsequent conversion of the tin acetals with BnBr in the presence of tetra-*n*-butylammonium iodide (TBAI) in DMF afforded a 52% yield of methyl ester **11** and 8% of benzyl ester **12** (over 2 steps) after chromatography. The latter product was finally quantitatively transformed into the methyl ester **11** by treatment with stoichiometric amounts of sodium methoxide in MeOH.

The glycosylation reaction of acceptor **11** with the acetylated donor **3** in dry CH_2Cl_2 containing ground molecular sieves (3 Å) and 2 equiv of $BF_3{\cdot}Et_2O$ did not afford the disaccharide. Instead, unreacted acceptor **11** was isolated, while donor **3** degraded very slowly over a period of several hours. Thus, in order to enhance the reactivity of the glycosyl donor, the armed analogue **16** (Scheme 3) was prepared. De-*O*-acetylation of glycal ester **2**[15] and benzylation of the resulting tetraol **13** using NaH/BnBr in dry DMF provided **14**[7c] in 77% isolated yield. Notably, destruction of excessive NaH for the workup was performed, after dilution with anhydrous CH_2Cl_2 at 0 °C, by addition of anhydrous MeOH followed by quick extraction and purification. Thus, degradation of the reactive glycal **14** and ester saponification could be avoided. Iodoacetoxylation using *N*-iodosuccinimide (NIS) in acetic acid was performed at ambient temperature and afforded the 2,3-*trans*-diaxial product **15** in 83% yield.[16] Direct conversion of the anomeric acetate, with an optimized amount of hydrogen fluoride pyridine

Scheme 3. Preparation of Fluoride Donor (16) and Model Glycosylation



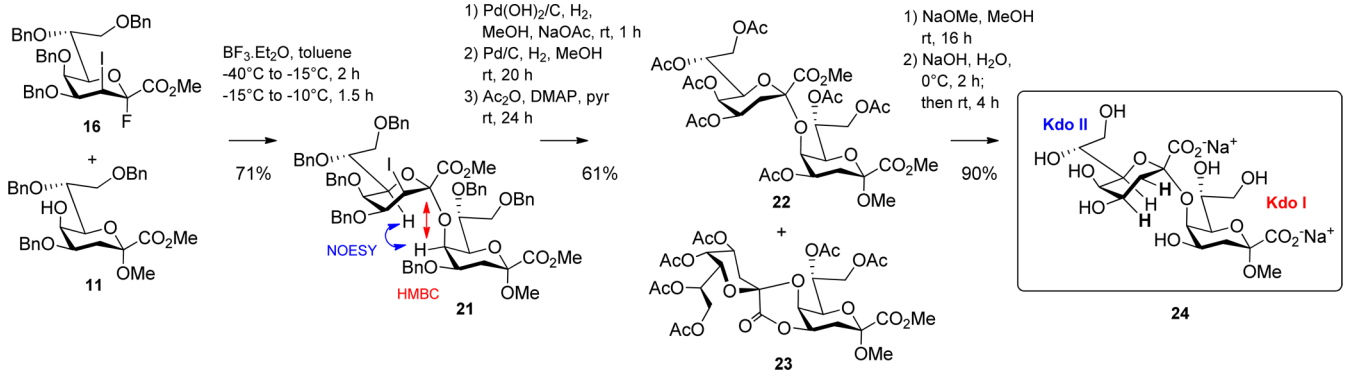
complex at −5−0 °C, afforded the α-fluoride **16** ($J_{H\text{-}3eq,F\text{-}ax}$ 5.1 Hz) selectively and in excellent yield (96%).[7b] Increased amounts of HF-pyridine and temperatures above 0 °C, however, resulted in epimerization of the 3-iodo substituent, and an inseparable mixture of the desired D-*glycero*-D-*talo* donor **16** and the D-*glycero*-D-*galacto*-epimer **17** was obtained.

To evaluate its glycosylation properties, donor **16** was converted in 70% yield into the 2-propyl glycoside **18** using 2 equiv of 2-propanol under $BF_3{\cdot}Et_2O$-promotion in CH_2Cl_2. TLC-analysis of the glycosylation mixture indicated a pronounced reactivity of **16**, which was already activated at −60 °C. In comparison, no reaction of acetylated donor **3** with 2-propanol had been observed below ambient temperature.[13] Dehalogenation was performed by catalytic hydrogenation using $Pd(OH)_2/C$ (20%) in the presence of NaOAc in MeOH; it was followed by workup (extraction with sodium thiosulfate and water to remove iodo impurities). The ensuing debenzylation over Pd/C (10%) gave **19** in 91% yield (2 steps). Notably, dehalogenation also led to partial cleavage of the benzyl groups. *O*-Acetylation of **19** afforded the known glycoside **20**, and comparison with published data confirmed the assignment of the α-anomeric configuration.[13]

When applying these optimized glycosylation conditions to the coupling of donor **16** with 4,7,8-tri-*O*-benzyl acceptor **11**, trace amounts of product **21** could be isolated (Scheme 4). Furthermore, large amounts of unreacted acceptor **11** and hydrolyzed donor were present. Surprisingly, ^{1}H NMR analysis revealed the presence of a second 2→5-linked disaccharide

dx.doi.org/10.1021/ol5033128 | Org. Lett. 2015, 17, 110−113

Scheme 4. Glycosylation and Deprotection towards the α-(2→5) Kdo Disaccharide (24)



bearing an equatorially oriented 3-iodo substituent resulting from epimerization. Optimization of the glycosylation conditions, however, allowed for suppression of the 3-iodo-epimerization when toluene was used as the solvent. Still, the isolated yield was quite low (~30%). On the one hand, at low temperature (−40 °C), incomplete conversion of acceptor **11** was observed, while at elevated temperatures (0 °C) extensive degradation of the product occurred. Stability tests of the isolated product **21** revealed that it was unstable toward BF_3·Et_2O at temperatures above −10 °C. However, working at high dilution and keeping the temperature between −40 °C to −10 °C (warming up to −10 °C was, however, essential) afforded **21** in a good yield (71%) as the α-anomer only. In addition, only very minor formation of the glycal ester **14** was observed and no β-anomeric product was detected. For workup, BF_3·Et_2O had to be neutralized by dropwise addition of diluted triethylamine at low temperature followed by quick extraction and purification to secure a high isolated yield. This procedure was reproducible without the need for a large excess of donor **16** (only 1.5 equiv were used).

Broad signal shapes in the ^{1}H NMR spectrum (600 MHz, in $CDCl_3$) of **21** complicated the analysis and assignments. This line broadening suggests a high steric load and a rigid structure of **21**. Thus, the sample was subjected to high-temperature measurements[17] in d_8-toluene to confirm the presence of the (2→5) linkage. Indeed, the HMBC spectrum showed a cross-correlation between H-5 and C-2′ (Figure 1) and the assignment was further supported by a NOESY signal between H-5 and H-6′ (see Supporting Information).

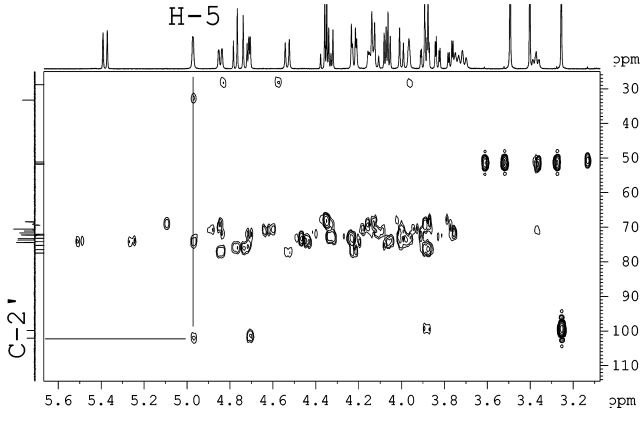


Figure 1. HMBC spectrum (d_8-tol, 323 K) of disaccharide **21**.

The dehalogenation/debenzylation sequence of **21** using $Pd(OH)_2$/C-NaOAc and Pd/C in MeOH under an atmosphere of H_2 yielded an impure product, which was *O*-acetylated and purified by normal-phase HPLC providing a mixture of **22** together with the 1′→4 lactone **23** in 61% yield (3 steps).

NMR characterization of **22** supported the assignment of the α-anomeric configuration as indicated by the characteristic chemical shifts of H-3′*ax* and H-3′*eq* and the low-field shift of H-4′.[18] Analysis of the side products revealed that partial cleavage of the glycosidic bond had occurred. This was further supported by LC-MS studies showing that glycoside hydrolysis (and lactone formation) had already occurred during the treatment with $Pd(OH)_2$/C-NaOAc. Deacetylation of the ~10:1 mixture of **22** and **23** using sodium methoxide and subsequent methyl ester hydrolysis with aq NaOH afforded **24** containing a trace impurity. To obtain the pure target disaccharide, the mixture of **22** and **23** was partially separated by normal-phase HPLC, providing a pure fraction of **22** (1.2 mg from 9.9 mg of a 10:1 mixture of **22**/**23**, 12%), which was globally deprotected to afford the α-(2→5)-linked Kdo disaccharide **24**.

The NMR spectroscopic data for **24** (recorded in D_2O) were in close agreement with the related Kdo units present in the branched Kdo-tetrasaccharide.[4a−d] The proton signals of H-4′ and H-6′ of Kdo II (see Scheme 4) were substantially shifted to lower field (4.26 and 3.90 ppm, respectively), presumably due to the deshielding effect of the O-4 group of Kdo I. Similarly, the equatorial proton H-3′*eq* at the distal Kdo II unit was also shifted to lower field (2.24 ppm), which is most likely attributable to the fixed orientation of the lone pairs at O-5 of the glycosidic bond. These effects have been published for the branched Kdo tetrasaccharide isolated from *A. baumannii* strain NCTC 10303.[4a] Since these features are also seen in the α-(2→5)-linked disaccharide, the additional, lateral α-(2→4)-linked Kdo unit has thus only a limited impact on the NMR characteristics of the α-(2→5)-unit (except for ^{13}C NMR shift differences at C-4 and C-5 for the branched Kdo moiety, respectively). Carbon-5 of Kdo I revealed a significant shift to low field (74.2 ppm), confirming the site of attachment of the second Kdo unit (which was again proven by an HMBC correlation from H-5 to carbon C-2′).

In conclusion, we have developed a stereoselective route toward α-(2→5)-interconnected Kdo residues using an armed 3-iodo-Kdo fluoride donor displaying high reactivity and α-anomeric stereoselectivity. The NMR data for the deprotected disaccharide corresponding to fragments of *Acinetobacter* LPS

dx.doi.org/10.1021/ol5033128 | *Org. Lett.* 2015, 17, 110−113

favorably match the assignments made for the native oligosaccharides.

■ ASSOCIATED CONTENT

Supporting Information

Detailed synthetic procedures for **5**−**12**, **14**−**19**, and **21**−**24**; characterization data; and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: paul.kosma@boku.ac.at.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support of this work by the Austrian Science Fund FWF (Grant No. P-24921) and high-temperature NMR measurements by Dr. Andreas Hofinger (Department of Chemistry, University of Natural Resources and Life Sciences-Vienna) are gratefully acknowledged.

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dx.doi.org/10.1021/ol5033128 | *Org. Lett.* 2015, 17, 110−113

2.5. Preliminary biological data from ELISA studies

The synthesized *A. haemolyticus* inner core ligands were subjected to a modified competitive-ELISA protocol to examine their binding affinities towards the murine MBL-A. Therefore, D-Mannose (Man) - conjugated to bovine serum albumin (BSA) - is immobilized on an ELISA plate and treated with the lectin, which is weakly bound to Man in an unspecific way (Figure 44). After removal of excessive lectin in a subsequent washing step, the bound MBL-A is detected by a specific antibody (AB), which itself is recognized by a horseradish peroxidase (HRPO)-labeled AB. The HRPO catalyzes the oxidation of the substrate 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) providing a stabilized, green-colored radical. Thus, intensive staining indicates the presence of MBL-A, which is quantified by measuring the optical density (OD) at a suitable wavelength.

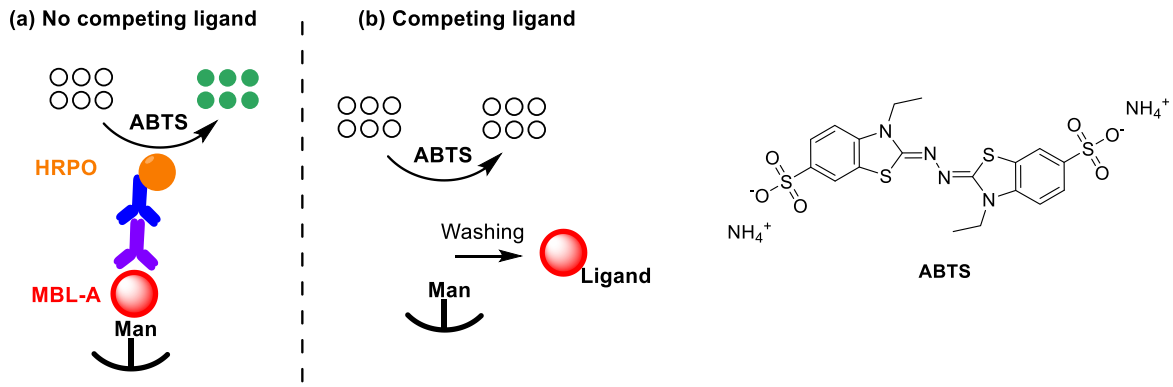


Figure 44: Principle of the competitive-ELISA studies: a) In the absence of a competing ligand the HRPO-labeled antibody catalyzes the reaction of ABTS to form a colored radical; b) In the presence of a competing ligand the MBL-A binds to the ligand and is removed from the plate.

The synthetic ligands were added prior to the detection cascade to evaluate their ability to compete with the immobilized Man for the lectin. Ligands with high affinity exceed the weak interaction with Man and the lectin is removed together with the ligand in the ensuing washing step. Consequently, the decreased concentration of MBL-A results in reduced amounts of HRPO and to diminished OD values. In summary, a ligand that induces a decline in the OD is capable of interacting with the MBL-A. The lower the required concentration to observe a decrease in OD, the higher the affinity of the ligand.

The results for selected ligands and for the isolated inner core fraction are shown in Figure 45.[1] The α-Glc(1→5)-Kdo disaccharide **I** is capable of inhibiting the interaction of the lectin with the immobilized Man at high concentrations (ca. 1 mM). A very similar trend was detected for the 6'-O-phosphorylated analogue **II** indicating that the phosphate is not directly involved in binding. Although a slight decrease in affinities was observed for the disaccharides containing the non-natural heptulosonic acid **III** and **IV**[2] (after Smith degradation), the ligands are still recognized by the MBL-A. Thus, only a minor contribution of the exocyclic Kdo side-chain to the binding is assumed. However, the isolated inner core of *A. haemolyticus* inhibits the Man-lectin interaction already at much lower concentrations. Thus, the actual epitope for high affinity binding could not be revealed yet. Notably, for all other synthetic ligands – including the disaccharide containing the 3-oxy pendant Ko **V** (see chapter 2.2), the extended structures of the Glc-Kdo disaccharide **VI** to **VIII**, and the Kdo-GlcNAc structures **IX** and **X** – no inhibition was detected at concentrations smaller than 1 mM.

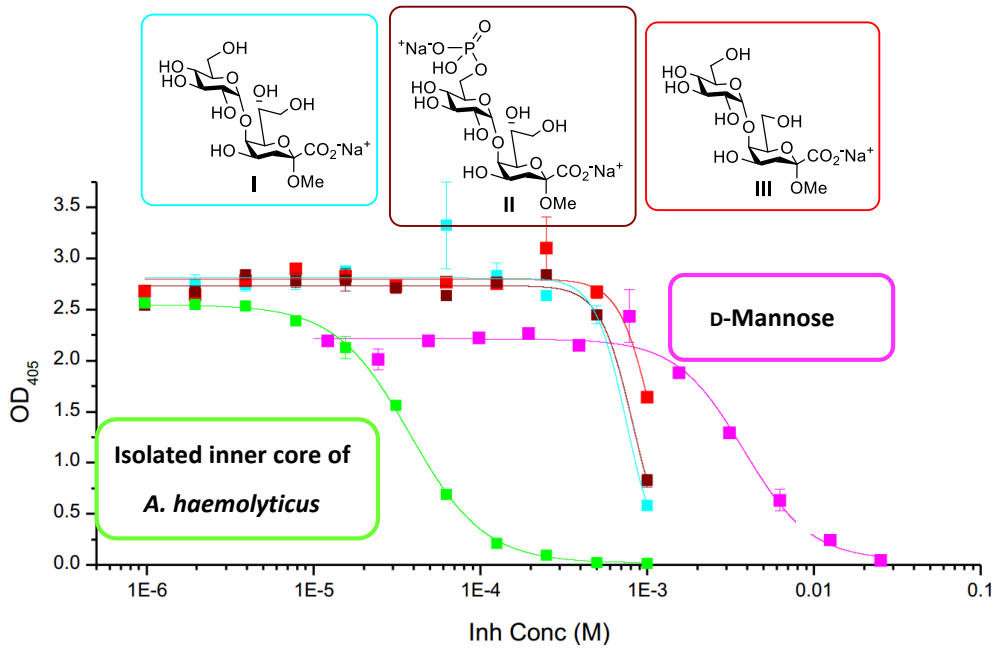


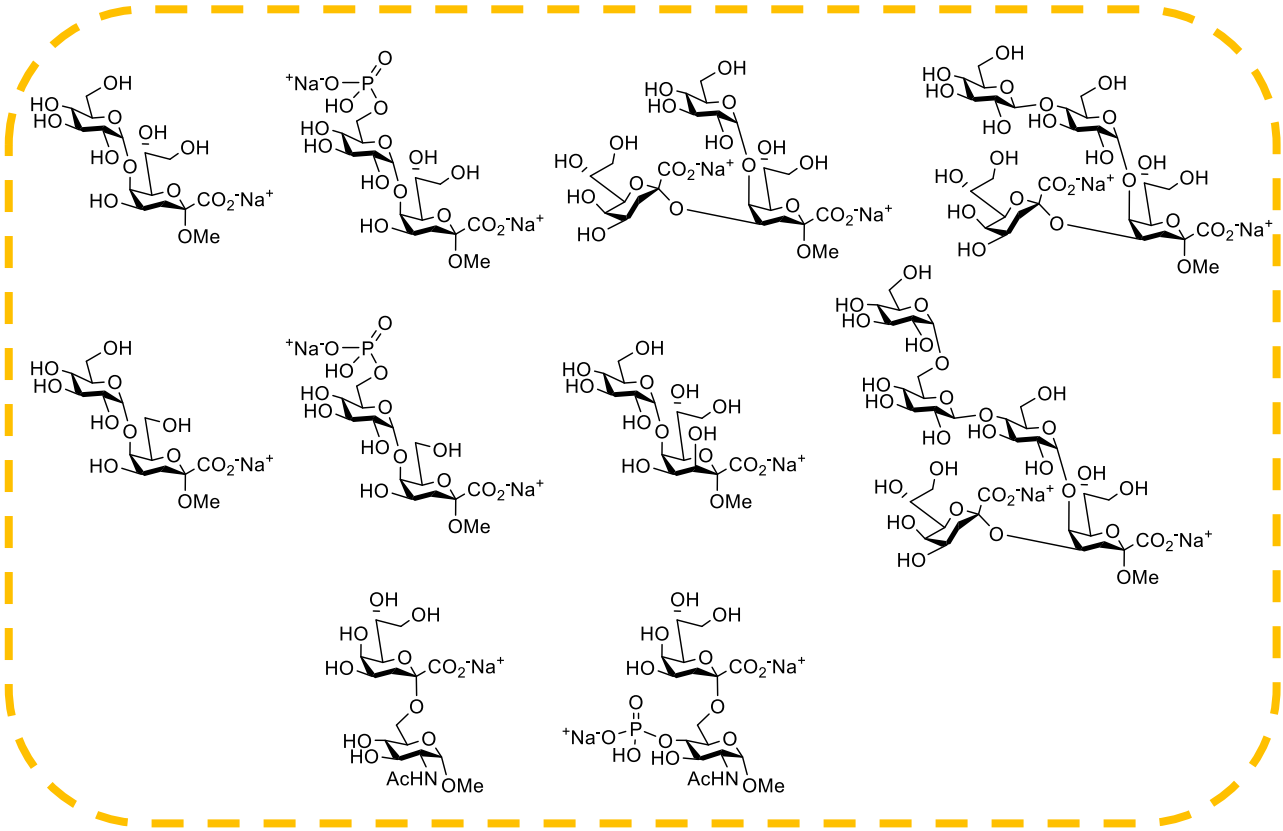
Figure 45: Results for the competitive-ELISA of selected ligands using murine MBL-A; x-axis = Concentration of ligand; y-axis = Optical Density at 405 nm wavelength.

[1] In addition, unbound Man was measured to serve as a reference and to assure the reproducibility of the assay.

[2] The 6'-O-phosphorylated disaccharide comprising the heptulosonic acid **IV** exhibits a similar behavior to its non-phosphorylated analogue (not shown in Figure 45).

3. Summary and Outlook

In the course of this thesis 9 di- to pentasaccharide fragments from the *A. haemolyticus* inner core, containing the rare sugar 3-deoxy-D-*manno*-2-octulosonic acid (Kdo), have been prepared by chemical O-glycosylation of properly protected monosaccharide precursor. High stereoselectivities and yields were obtained for all linkages, capitalizing on optimized combinations of blocking group pattern, leaving group and reaction conditions. Orthogonal protecting group strategies allowed for subsequent modifications like phosphorylation (natural) or Smith-degradation (non-natural), and oligosaccharide synthesis by sequential assembly of monosaccharide units. As a highlight, a new Kdo donor has been introduced, which showed excellent α-selectivites and significantly reduced the elimination side reaction. It capitalizes on a temporary 3-iodo directing group, which could be subsequently removed by a tin-free dehalogenation protocol affording near-quantitative yields.

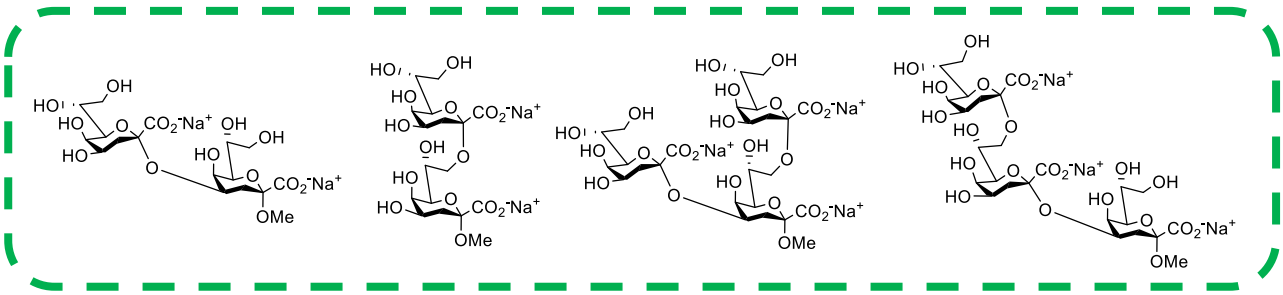


In addition, one ligand containing D-*glycero*-D-*talo*-2-octulosonic acid (Ko) - the 3-oxy pendant of Kdo - has been synthesized. Preliminary biological results for the binding to murine MBL-A indicated, that only the α-Glc(1→5)-α-Kdo disaccharide and modified structures (phosphorylated, side-chain truncated after Smith degradation) thereof are bound during the essay. Elongated structures of this basic core and the disaccharide containing Ko as

a substitute for Kdo as well as the general lipid A-core connection motif α-Kdo(2→6)-α-GlcNAc gave negative results.

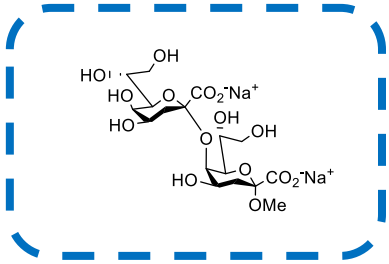
The binding affinities of the synthesized ligands recognized by MBL-A are, however, significantly lower than observed for the isolated inner core of *A. haemolyticus*. Thus, the question about the minimal binding epitope could not yet be solved. In a parallel thesis, further structures containing Ko will be prepared. As the lipopolysaccharide fraction isolated from a bacterial strain is a heterogeneous mixture, from which only the main structure is usually accessible and thus elucidated, the binding site may reside on a not yet identified lipopolysaccharide structure present in a minor fraction. Further purification and analysis of the biological material will be necessary to narrow down the possible binding epitopes. Identification of the binding specificities of the MBL, however, would give valuable insight into the yet underexplored MBL-core interactions on a molecular level.

In addition, the properties of the new 3-iodo-2-fluoride Kdo donor have been evaluated by synthesizing four interconnected Kdo di- and trisaccharides ranging from the classical enterobacterial α-(2→4)-linked inner core motif to various antigens from *Chlamydia*-related inner core structures. Thereby, the donor was capable of direct glycosylation of a silyl protected acceptor and provided access to a branched Kdo-trisaccharide in one step with good regioselectivity starting from an unprotected Kdo glycoside. Sequential iteration of the basic concept expands the scope to further produce linear oligosaccharide. Due to the excellent glycosylation properties the previously described synthetic routes towards the final structures could be significantly improved.



Finally, a more reactive analogue of the described donor has been prepared and successfully applied in the challenging (2→5)-linkage of two Kdo moieties. Again, only the α-anomer was obtained, but high yields depended on careful optimization of the reaction conditions. Notably, this is the first reported synthetic access to this linkage-type found in *Acinetobacter baumannii* strains – a human pathogen which is responsible for severe nosocomial infections. This ligand and prospective structures capitalizing on the elaborated glycosylation

protocol, may help to explore the biosynthesis of the unusual core structure of *A. baumannii* and a potential interaction with the host's immune system. A profound understanding of these mechanisms is necessary for the development of new strategies fighting against these multi-drug resistant bacteria.



4. Statement of contribution

Manuscript #1

Synthesis and **structure elucidation** of all compounds has been performed by the author of the thesis. Preparation of the manuscript was shared among all authors.

Manuscript #2

Synthesis and **structure elucidation** of all compounds has been performed by the author of the thesis. Preparation of the manuscript was shared among all authors.

Manuscript #3

Synthesis and **structure elucidation** of all compounds has been performed by the author of the thesis. Preparation of the manuscript was shared among all authors.

Manuscript #4

Synthesis and **structure elucidation** of all compounds, as well as **mechanistic examinations** by kinetic NMR experiments have been performed by the author of the thesis. Preparation of the manuscript was shared among all authors.

Manuscript #5

Synthesis and **structure elucidation** of all compounds has been performed by the author of the thesis. Preparation of the manuscript was shared among all authors.

5. Appendix

Manuscript #2

Supporting Information

doi:10.1002/chem.201405424

CHEMISTRY
A European Journal

Supporting Information

Synthesis of Chlamydia Lipopolysaccharide Haptens through the use of α-Specific 3-Iodo-Kdo Fluoride Glycosyl Donors**

Barbara Pokorny and Paul Kosma*[a]

chem_201405424_sm_miscellaneous_information.pdf

Supporting information

Table of contents

1. General

All purchased chemicals were used without further purification unless stated otherwise. Solvents were dried over activated 4 Å (CH_2Cl_2, DMF, pyridine, cyclohexane) molecular sieves. 2-Propanol for glycosylation was dried over 5 Å molecular sieves for 24 h. THF was directly distilled on 4 Å molecular sieves shortly before use. Dry MeOH (secco solv) was purchased from Merck. Cation exchange resin DOWEX 50 H^+ was regenerated by consecutive washing with HCl (3 M), water and dry MeOH. Aqueous solutions of salts were saturated unless stated otherwise. Concentration of organic solutions was performed under reduced pressure < 40 °C. Optical rotations were measured with a Perkin-Elmer 243 B Polarimeter. $[\alpha]_D^{20}$ values are given in units of 10^{-1}deg cm^{2}g^{-1}. Thin layer chromatography was performed on Merck precoated plates: generally on 5 x 10 cm, layer thickness 0.25 mm, Silica Gel $60F_{254}$; alternatively on HP-TLC plates with 2.5 cm concentration zone (Merck). Spots were detected by dipping reagent (anisaldehyde-H_2SO_4). For column chromatography silica gel (0.040 – 0.063 mm) was used. HP-column chromatography was performed on pre-packed columns (YMC-Pack SIL-06, 0.005 mm, 250x10 mm and 250x20 mm). Size exclusion chromatography was performed on Bio-Gel® P-2 Gel extra fine < 45 µm (wet) (1 x 30 cm) or on pre-packed PD-10 columns (GE Healthcare, Sephadex™ G-25 M). NMR spectra were recorded with a Bruker Avance III 600 instrument (600.22 MHz for ^{1}H, 150.93 MHz for ^{13}C and 564.77 MHz for ^{19}F) using standard Bruker NMR software. ^{1}H NMR spectra were referenced to 7.26 ($CDCl_3$) and 0.00 (D_2O, external calibration to 2,2-dimethyl-2-silapentane-5-sulfonic acid) ppm unless stated otherwise. ^{13}C NMR spectra were referenced to 77.00 ($CDCl_3$) and 67.40 (D_2O, external calibration to 1,4-dioxane) ppm. ^{19}F NMR spectra were indirectly referenced according to IUPAC recommendations[S1]. ESI-MS data were obtained on a Waters Micromass Q-TOF Ultima Global instrument.

2. Assignment of NMR signals

The ^{1}H and ^{13}C signals were assigned with the aid of COSY, HSQC and HMBC spectra and compared to published data for known compounds. The monosaccharides were numbered along the carbohydrate backbone according to IUPAC. The branched trisaccharides have been labeled according to Fig.S1.

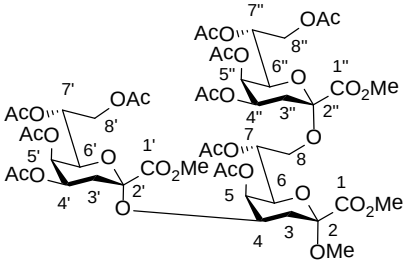
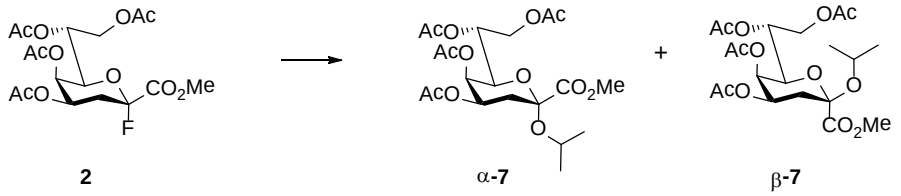


Figure S1: NMR-signal assignment of a branched trisaccharide

3. Synthesis and characterization of compounds

3.1 Model glycosylations

3.1.1 Model glycosylation without directing group



Under an atmosphere of argon a solution of **2**[S2] (33.3 mg, 0.079 mmol) and dry 2-propanol (12.1 µL, 0.158 mmol) in dry CH_2Cl_2 (1.0 mL) was stirred for 1 h at ambient temperature in the presence of ground 3 Å molecular sieves (50 mg). At 0 °C $BF_3.Et_2O$ (32.4 µL, 0.158 mmol) was added dropwise and after stirring for 6 h at 0 °C the mixture was diluted with CH_2Cl_2 and aq $NaHCO_3$. The phases were separated and the organic extract was dried ($MgSO_4$), filtered and concentrated. HP-column chromatography (n-hexane/EtOAc 3:1→1:1) provided a mixture of **α/β-7** (17.5 mg, 48%, α/β 4.8:1) and the slower migrating glycal **3**[S3] (9.7 mg, 31%).

Methyl (2-propyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-β-D-*manno*-oct-2-ulopyranosid)onate (β-7)

Colorless oil; $[\alpha]_D^{20}$ + 53.8 (*c* 0.58, $CHCl_3$); R_f 0.75 (*n*-hexane/EtOAc 1:1, HP-TLC); ^{1}H NMR ($CDCl_3$): δ 5.28 - 5.27 (m, 1H, H-5), 5.16 (ddd, 1H, $J_{7,6}$ 9.4, $J_{7,8b}$ 4.3, $J_{7,8a}$ 2.5 Hz, H-7), 4.85 (ddd, 1H, $J_{4,3ax}$ 13.3, $J_{4,3eq}$ 4.5, $J_{4,5}$ 3.1 Hz, H-4), 4.38 (dd, 1H, $J_{8a,8b}$ 12.3 Hz, H-8a), 4.35 (dd, 1H, H-8b), 4.15 (dd, 1H, $J_{6,5}$ 1.4 Hz, H-6), 4.06 [m, 1H, *J* 6.2 Hz, C*H*(CH_3)$_2$], 3.80 (s, 3H, CO_2CH_3), 2.35 (ddd, 1H, $J_{3eq,3ax}$ 12.5, $J_{3eq,5}$ 1.0 Hz, H-3*eq*), 2.12 (s, 3H, COCH_3), 2.10 (s, 3H, COCH_3), 2.09 (app t, 1H, H-3*ax*), 2.01 (s, 3H, COCH_3), 1.98 (s, 3H, COCH_3), 1.23 [d, 3H, *J* 6.2 Hz, CH(CH_3)], 1.09 [d, 3H, *J* 6.2 Hz, CH(CH_3)]; ^{13}C NMR ($CDCl_3$): δ 170.73, 170.51, 169.95 and 169.85 (4 x s, *C*OCH$_3$), 168.81 (s, C-1), 99.61 (s, C-2), 70.54 (d, C-6), 68.67 [d, *C*H(CH_3)$_2$], 68.16 (d, C-7), 67.14 (d, C-4), 64.06 (d, C-5), 62.50 (t, C-8), 52.57 (q, CO_2CH_3), 32.81 (t, C-3), 24.46 [q, 1C, CH(*C*H$_3$)], 23.19 [q, 1C, CH(*C*H$_3$)], 20.79, 20.73, 20.70 and 20.66 (4 x q, COCH$_3$); ESI-TOF HRMS: *m/z* = 485.1624; calcd for $C_{20}H_{30}O_{12}Na^+$: 485.1629.

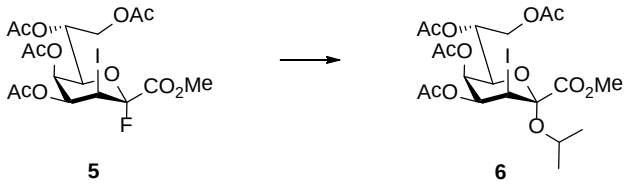
Methyl (2-propyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (α-7)

Colorless oil; $[\alpha]_D^{20}$ + 94.3 (*c* 0.93, $CHCl_3$); R_f 0.72 (*n*-hexane/EtOAc 1:1, HP-TLC); ^{1}H NMR ($CDCl_3$): δ 5.36 - 5.34 (m, 1H, H-5), 5.31 (ddd, 1H, $J_{4,3ax}$ 12.4, $J_{4,3eq}$ 4.9, $J_{4,5}$ 3.0 Hz, H-4), 5.20 (ddd, 1H, $J_{7,6}$ 9.8, $J_{7,8b}$ 3.6, $J_{7,8a}$ 2.6 Hz, H-7), 4.62 (dd, 1H, $J_{8a,8b}$ 12.3 Hz, H-8a), 4.18 (dd, 1H, $J_{6,5}$ 1.7 Hz, H-6), 4.16 (dd, 1H, H-8b), 4.01 [m, 1H, *J* 6.1 Hz, C*H*(CH_3)$_2$], 3.79 (s, 3H, CO_2CH_3), 2.22 (ddd, 1H, $J_{3eq,3ax}$ 12.8, $J_{3eq,5}$ 1.0 Hz, H-3*eq*), 2.05 (s, 6H, 2 x COCH_3), 1.99 (app t, 1H, H-3*ax*), 1.98 (s, 3H, COCH_3), 1.96 (s, 3H, COCH_3), 1.21 [d, 3H, *J* 6.1 Hz, CH(CH_3)], 1.04 [d, 3H, *J* 6.1 Hz, CH(CH_3)]; ^{13}C NMR ($CDCl_3$): δ 170.47, 170.38, 169.96 and 169.65 (4 x s, *C*OCH$_3$), 168.34 (s, C-1), 97.96 (s, C-2), 68.40 (d, C-6), 67.86 (d, C-7), 67.08 [d, *C*H(CH_3)$_2$], 66.55 (d, C-4), 64.50 (d, C-

5), 61.96 (t, C-8), 52.49 (q, CO_2CH_3), 32.65 (t, C-3), 23.80 [q, 1C, CH(CH_3)], 22.46 [q, 1C, CH(CH_3)], 20.75, 20.73 and 20.62 (3 x q, 4C, COCH_3); ESI-TOF HRMS: *m/z* = 485.1628; calcd for $C_{20}H_{30}O_{12}Na^+$: 485.1629.

The anomeric configuration was assigned by comparison with published data[S4].

3.1.2 Model glycosylation with directing 3-iodo-group

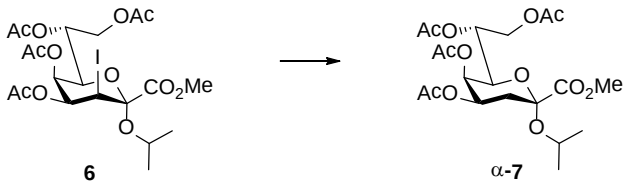


Glycosyl donor **5** (274 mg, 0.500 mmol) and dry 2-propanol (77 µL, 1.000 mmol) were treated with $BF_3.Et_2O$ (205 µL, 1.000 mmol) in dry CH_2Cl_2 (8.0 mL) for 1 h according to the general procedure for glycosylation (see main paper). After aqueous work-up column chromatography (*n*-hexane/EtOAc 2:1) afforded **6** (244 mg, 83%) and the slower migrating glycal **3**[S3] (13.3 mg, 7%).

Methyl (2-propyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosid)onate (6)

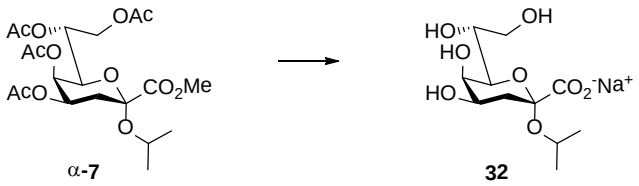
Colorless oil; $[\alpha]_D^{20}$ + 41.8 (*c* 0.55, $CHCl_3$); R_f 0.32 (*n*-hexane/EtOAc 2:1, HP-TLC); ^{1}H NMR ($CDCl_3$): δ 5.42 - 5.41 (m, 1H, H-5), 5.36 (ddd, 1H, $J_{7,6}$ 9.7, $J_{7,8b}$ 3.6, $J_{7,8a}$ 2.5 Hz, H-7), 5.04 (dd, 1H, $J_{4,3}$ 4.7, $J_{4,5}$ 3.8 Hz, H-4), 4.70 (dd, 1H, $J_{8a,8b}$ 12.5 Hz, H-8a), 4.47 (dd, 1H, $J_{3,5}$ 0.9 Hz, H-3), 4.32 (dd, 1H, $J_{6,5}$ 2.2 Hz, H-6), 4.20 (dd, 1H, H-8b), 3.95 [m, 1H, *J* 6.2 Hz, C*H*(CH_3)$_2$], 3.83 (s, 3H, CO_2CH_3), 2.10 (s, 3H, COCH_3), 2.05 (s, 6H, 2 x COCH_3), 1.96 (s, 3H, COCH_3), 1.27 [d, 3H, *J* 6.2 Hz, CH(CH_3)], 1.06 [d, 3H, *J* 6.1 Hz, CH(CH_3)]; ^{13}C NMR ($CDCl_3$): δ 170.35, 170.16, 169.44 and 169.41 (4 x s, *C*OCH_3), 166.66 (s, C-1), 101.09 (s, C-2), 69.67 [d, *C*H(CH_3)$_2$], 68.22 (d, C-6), 67.84 (d, C-7), 65.46 (d, C-4), 63.37 (d, C-5), 61.67 (t, C-8), 52.63 (q, CO_2CH_3), 23.88, 23.81 [2d/q, 2C, C-3, CH(CH_3)], 22.27 [q, 1C, CH(CH_3)], 20.93, 20.82, 20.73 and 20.55 (4 x q, COCH_3); ESI-TOF HRMS: *m/z* = 606.1044; calcd for $C_{20}H_{29}IO_{12}NH_4^+$: 606.1042.

3.1.3. Dehalogenation



Dehalogenation of **6**: A solution of **6** (24.8 mg, 0.042 mmol) in dry cyclohexane (2.0 mL) was treated with lauroyl peroxide (5.0 mg, 0.013 mmol) for 2 h according to the general procedure for dehalogenation (see main paper). Pure **α-7** (17.7 mg, 91%) was isolated by column chromatography (toluene/EtOAc 4:1).

3.1.4. Global deprotection

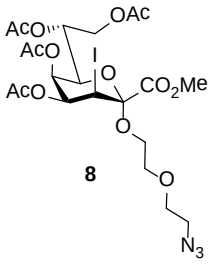


Sodium (2-propyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (32)

According to the general procedure for global deprotection (see main paper): A solution of **α-7** (12.8 mg, 0.0228 mmol) in dry MeOH (2.0 mL) was treated with NaOMe (0.1 M in MeOH, 22.4 µL, 0.002 mmol) for 19 h at rt. After isolation of the crude product the residue was dissolved in water (2 mL) and treated with aq NaOH (0.1 M, 1.0 mL) for 3 h. The crude product was desalted on a PD10-column and lyophilisation of pooled fractions provided **32** (8.3 mg, 99%) as a colorless amorphous solid; $[\alpha]_D^{20}$ + 50.9 (*c* 0.83, D_2O); ^{1}H NMR (D_2O, pH ~ 6.0): δ 4.02 – 3.97 (m, 2H, H-4, H-5), 3.96 – 3.89 [m, 3H, H-7, H-8a, C*H*(CH_3)$_2$], 3.66 – 3.60 (m, 2H, H-6, H-8b), 2.09 – 2.04 (m, 1H, H-3*eq*), 1.71 (dd, 1H, $J_{3ax,3eq}$ 13.1, $J_{3ax,4}$ 11.5 Hz, H-3*ax*), 1.13 [d, 3H, *J* 6.3 Hz, CH(CH_3)], 1.10 [d, 3H, *J* 6.3 Hz, CH(CH_3)]; ^{13}C NMR (D_2O, pH ~ 6.0): δ 176.45 (s, C-1), 100.00 (s, C-2), 72.68 (d, C-6), 70.66 (d, C-7), 68.07 [d, *C*H(CH_3)$_2$], 67.09 (d, C-5), 66.89 (d, C-4), 63.93 (t, C-8), 35.61 (t, C-3), 24.03 [q, 1C, CH(*C*H_3)$_2$], 22.57 [q, 1C, CH(*C*H_3)$_2$]; ESI-TOF HRMS: *m/z* = 303.1048; calcd for $C_{11}H_{20}O_8Na^+$: 303.1050.

3.2. Synthesis of Kdo spacer-glycosides

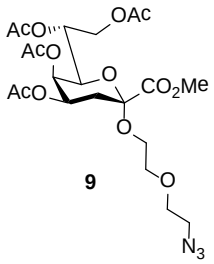
3.2.1. Methyl [2-(2-azidoethoxy)ethan-1-yl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl]onate (8)



Glycosyl donor **5** (25.5 mg, 0.047 mmol) and 2-(2-azidoethoxy)ethanol (7.3 mg, 0.056 mmol) were treated with BF_3.Et_2O (19.1 µL, 0.093 mmol) in dry CH_2Cl_2 (1.0 mL) for 1 h according to the general procedure for glycosylation. After aqueous work-up column chromatography (*n*-hexane/EtOAc 2:1) afforded a mixture of **8** and remaining 2-(2-azidoethoxy)ethanol, which was further purified by column chromatography (toluene/EtOAc 2:1) yielding pure **8** (24.5 mg, 80%) as a colorless oil; $[\alpha]_D^{20}$ + 43.3 (*c* 0.68, $CHCl_3$); R_f 0.42 (*n*-hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$): δ 5.41 - 5.37 (m, 2H, H-5, H-7), 5.07 (dd, 1H, $J_{4,3}$ 4.9, $J_{4,5}$ 3.5 Hz, H-4), 4.63 (dd, 1H, $J_{8a,8b}$ 12.4, $J_{8a,7}$ 2.3 Hz, H-8a), 4.52 (dd, 1H, $J_{3,5}$ 0.8 Hz, H-3), 4.41 (dd, 1H, $J_{6,7}$ 9.7, $J_{6,5}$ 1.8 Hz, H-6), 4.23 (dd, 1H, $J_{8b,7}$ 4.6 Hz, H-8b), 3.84 (s, 3H, CO_2CH_3), 3.75 (ddd, 1H, *J* 10.4, *J* 7.3, *J* 3.3 Hz, OCH_2), 3.70 - 3.60 (m,

4H, 2 x OCH_2), 3.46 - 3.35 (m, 3H, OCH_2, NCH_2), 2.11, 2.06, 2.05 and 1.97 (4s, each 3H, 4 x COCH_3); ^{13}C NMR ($CDCl_3$): δ 170.45, 170.14, 169.55 and 169.32 (4 x s, *C*OCH$_3$), 166.08 (s, C-1), 101.40 (s, C-2), 70.22 (t, 1C, O*C*H$_2$), 69.48 (t, 1C, O*C*H$_2$), 68.07 (d, C-6), 67.51 (d, C-7), 65.39 (d, C-4), 65.08 (t, 1C, O*C*H$_2$), 63.33 (d, C-5), 62.22 (t, C-8), 53.00 (q, CO_2CH_3), 50.81 (t, 1C, NCH_2), 22.24 (d, C-3), 20.91, 20.79, 20.62 and 20.56 (4 x s, CO*C*H$_3$); ESI-TOF HRMS: *m/z* = 682.0719; calcd for $C_{21}H_{30}IN_3O_{13}Na^+$: 682.0716.

3.2.2. Methyl [2-(2-azidoethoxy)ethan-1-yl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl]onate (9)

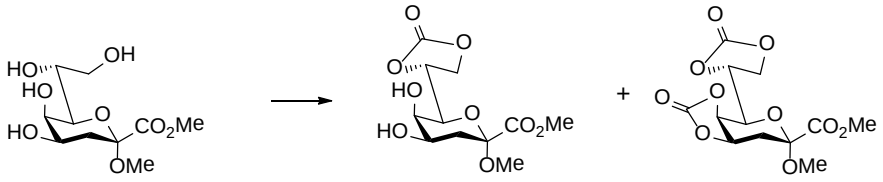


9

A suspension[S5] of **8** (16.6 mg, 0.025 mmol) in dry cyclohexane (3.0 mL) was treated with lauroyl peroxide (6.0 mg, 0.015 mmol) for 3 h according to the general procedure for dehalogenation. Pure **9** (11.2 mg, 83%) was obtained by column chromatography (*n*-hexane/EtOAc 1:1) as a colorless oil; $[\alpha]_D^{20}$ + 73.3 (*c* 1.06, $CHCl_3$); R_f 0.40 (*n*-hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$): 5.39 - 5.35 (m, 2H, H-4, H-5), 5.25 (ddd, 1H, $J_{7,6}$ 9.8, $J_{7,8b}$ 4.7, $J_{7,8a}$ 2.4 Hz, H-7), 4.57 (dd, 1H, $J_{8a,8b}$ 12.3 Hz, H-8a), 4.25 (dd, 1H, $J_{6,5}$ 1.0 Hz, H-6), 4.17 (dd, 1H, H-8b), 3.81 (s, 3H, CO_2CH_3), 3.71 - 3.63 (m, 5H, OCH_2), 3.57 - 3.53 (m, 1H, OCH_2), 3.46 - 3.39 (m, 2H, NCH_2), 2.20 - 2.16 (m, 1H, H-3*eq*), 2.12 - 2.07 (m, 1H, H-3*ax*), 2.09, 2.06, 2.00 and 1.97 (4 x s, each 3H, COCH_3); ^{13}C NMR ($CDCl_3$): δ 170.55, 170.42, 169.89 and 169.77 (4 x s, *C*OCH$_3$), 167.56 (s, C-1), 98.64 (s, C-2), 70.14, 69.84 (2t, 2C, O*C*H$_2$), 68.41 (d, C-6), 67.63 (d, C-7), 66.38 (d, C-4), 64.44 (d, C-5), 63.29 (t, 1C, O*C*H$_2$), 62.43 (t, C-8), 52.72 (q, CO_2CH_3), 50.84 (t, 1C, N*C*H$_2$), 31.94 (t, C-3), 20.76 and 20.65 (2 x q, 4C, CO*C*H$_3$); ESI-TOF HRMS: *m/z* = 556.1740; calcd for $C_{21}H_{31}N_3O_{13}Na^+$: 556.1749.

3.3. Synthesis of glycosyl acceptors

3.3.1. Methyl (methyl 7,8-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (10) and methyl (methyl 4,5;7,8-di-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (33)



19 **10** **33**

Carefully pre-dried **19**[S6] (294 mg, 1.104 mmol) was dissolved in dry THF under an atmosphere of argon. Freshly distilled *sym*-collidine (732 µL, 5.521 mmol) was added and the solution was cooled to -25 °C. To this

mixture a separately prepared solution of diphosgene (133 µL, 1.104 mmol) in dry THF (1.5 mL) was added dropwise and the reaction mixture was stirred at -20 °C for 30 min. The reaction was stopped by dropwise addition of dry MeOH (1.0 mL) at -20 °C. After concentration the residual colorless solid was partitionated between EtOAc and aq HCl (1 M). The aqueous phase was reextracted with EtOAc 5-times and the combined organic phases were dried ($MgSO_4$), filtered and concentrated. The crude mixture was separated by column chromatography (EtOAc/MeOH 1:0 → 9:1) yielding **10** (215 mg, 67%) and **33** (106 mg, 30%).

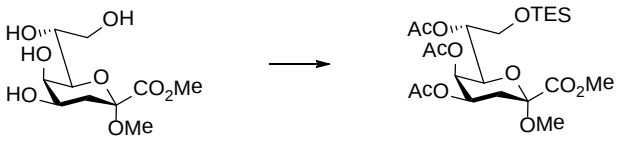
33: Colorless amorphous solid; $[\alpha]_D^{20}$ + 43.6 (*c* 0.24, $CHCl_3$); R_f 0.65 (EtOAc); ^{1}H NMR ($CDCl_3$): δ 5.06 (app td, 1H, $J_{4,5}$ 8.4, H-4), 5.01 (ddd, 1H, $J_{7,8a}$ 8.1, $J_{7,8b}$ 6.3, $J_{7,6}$ 5.1 Hz, H-7), 4.85 (dd, 1H, $J_{5,6}$ 1.8 Hz, H-5), 4.67 (app t, H-8a), 4.63 (dd, 1H, $J_{8b,8a}$ 9.0, H-8b), 4.12 (dd, 1H, H-6), 3.85 (s, 3H, CO_2CH_3), 3.29 (s, 3H, OCH_3), 2.83 (dd, 1H, $J_{3a,3b}$ 16.3, $J_{3a,4}$ 4.0 Hz, H-3a), 2.10 (dd, 1H, $J_{3b,4}$ 3.5 Hz, H-3b); ^{13}C NMR ($CDCl_3$): δ 167.79 (s, C-1), 153.82 [s, O*C*(=O)O], 152.52 [s, O*C*(=O)O], 98.03 (s, C-2), 73.63 (d, C-7), 71.82 (d, C-5), 71.07 (d, C-4), 68.75 (d, C-6), 65.89 (t, C-8), 53.32 (q, CO_2CH_3), 51.60 (q, OCH_3), 32.71 (t, C-3); ESI-TOF HRMS: *m/z* = 336.0925; calcd for $C_{12}H_{14}O_{10}NH_4^+$: 336.0925.

10: Colorless foam; $[\alpha]_D^{20}$ + 65.6 (*c* 0.74, $CHCl_3$); R_f 0.36 (EtOAc); ^{1}H NMR ($CDCl_3$): δ 5.00 (ddd, 1H, $J_{7,8b}$ 8.3, $J_{7,8a}$ 6.7, $J_{7,6}$ 4.3 Hz, H-7), 4.73 (dd, 1H, $J_{8a,8b}$ 8.9 Hz, H-8a), 4.59 (app t, 1H, H-8b), 4.13 (ddd, 1H, $J_{4,3ax}$ 11.6, $J_{4,3eq}$ 5.0, $J_{4,5}$ 3.2 Hz, H-4), 3.93 - 3.90 (m, 2H, H-5, H-6), 3.81 (s, 3H, CO_2CH_3), 3.23 (s, 3H, OCH_3), 2.10 (dd, 1H, $J_{3eq,3ax}$ 12.8 Hz, H-3*eq*), 1.90 (dd, 1H, H-3*ax*); ^{13}C NMR ($CDCl_3$): δ 168.42 (s, C-1), 155.08 [s, O*C*(=O)O], 99.36 (s, C-2), 75.89 (d, C-7), 71.13 (d, C-6), 66.80 (d, C-5), 66.53 (t, C-8), 65.02 (d, C-4), 52.88 (q, CO_2CH_3), 51.34 (q, OCH_3), 34.23 (t, C-3); ESI-TOF HRMS: *m/z* = 310.1140; calcd for $C_{11}H_{16}O_9NH_4^+$: 310.1133.

Recycling of starting material from 33:

A suspension of dicarbonate **33** (89 mg, 0.280 mmol) in dry MeOH (2 mL) was treated with NaOMe (0.1 M in MeOH, 112 µL, 0.011 mmol) for 18 h at ambient temperature. Another portion of NaOMe (0.1 M in MeOH, 112 µL, 0.0111 mmol) was added and after 5 h the solution was neutralized by addition of ion exchange resin DOWEX 50 (H^+). Filtration and concentration of the filtrate afforded **19** in quantitative yield in sufficient purity.

3.3.2. Methyl (methyl 4,5,7-tri-*O*-acetyl-3-deoxy-8-*O*-triethylsilyl-α-D-*manno*-oct-2-ulopyranosid)onate (15)



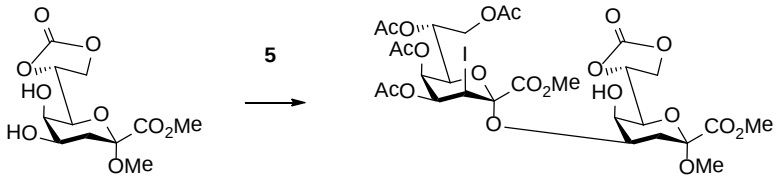
19 **15**

Dried **19**[S6] (89 mg, 0.334 mmol) and dried diazabicyclo[2.2.2]octane (64 mg, 0.567 mmol) were dissolved in dry MeCN. Chlorotriethylsilane (59 µL, 0.350 mmol) was added and the mixture was stirred at ambient temperature for 2 h. After concentration of the mixture the residue was dried under high vacuum, dissolved in dry pyridine (6.0 mL) and treated with acetic anhydride (0.2 mL) and 4-(*N*,*N*-dimethylamino)-pyridine (2 mg,

0.017 mmol) at ambient temperature for 4 h. Excessive reagent was destroyed at 0 °C by slow addition of dry MeOH (1 mL) and after stirring for 10 min the solvent was coevaporated with toluene (3 x). Column chromatography of the residue (toluene/EtOAc 9:1) provided **15** (107 mg, 60%, 2 steps) as a colorless oil; $[\alpha]_D^{20}$ + 57.4 (*c* 1.07, $CHCl_3$); R_f 0.60 (toluene/EtOAc 2:1); ^{1}H NMR ($CDCl_3$): δ 5.37 - 5.33 (m, 2H, H-4, H-5), 5.02 (app td, 1H, $J_{7,6}$ 9.8 Hz, H-7), 4.24 (dd, 1H, $J_{6,5}$ 1.0 Hz, H-6), 3.96 (dd, 1H, $J_{8a,8b}$ 11.7, $J_{8a,7}$ 2.7 Hz, H-8a), 3.81 (s, 3H, CO_2CH_3), 3.78 (dd, 1H, $J_{8b,7}$ 2.0 Hz, H-8b), 3.28 (s, 3H, OCH_3), 2.15 - 2.11 (m, 1H, H-3*eq*), 2.07 (s, 3H, $COCH_3$), 2.07 - 2.01 (m, 1H, H-3*ax*), 2.00 and 1.96 (2 x s, each 3H, $COCH_3$), 0.92 [t, 9H, *J* 8.0 Hz, $Si(CH_2CH_3)_3$], 0.55 [q, 6H, *J* 8.0 Hz, $Si(CH_2CH_3)_3$]; ^{13}C NMR ($CDCl_3$): δ 170.50, 169.98 and 169.88 (3 x s, $COCH_3$), 167.93 (s, C-1), 98.90 (s, C-2), 70.31 (d, C-7), 67.15 (d, C-6), 66.56 (d, C-4), 64.83 (d, C-5), 60.83 (t, C-8), 52.60 (q, CO_2CH_3), 51.23 (q, OCH_3), 32.09 (t, C-3), 20.80, 20.78 and 20.70 (3 x s, $COCH_3$), 6.60 [q, 3C, $Si(CH_2CH_3)_3$], 4.28 (t, 3C, $Si(CH_2CH_3)_3$]; ESI-TOF HRMS: *m/z* = 529.2072; calcd for $C_{22}H_{38}O_{11}SiNa^+$: 529.2076.

3.4. α-Kdo disaccharides

3.4.1. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→4)-methyl (methyl 7,8-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (11)

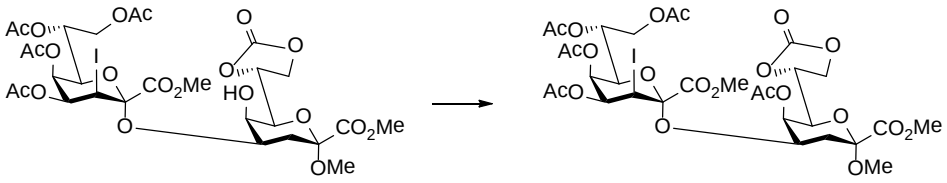


10 **11**

A mixture of **10** (105 mg, 0.359 mmol) and **5** (197 mg, 0.359 mmol) in dry CH_2Cl_2 (8.0 mL) was treated with $BF_3.Et_2O$ (148 µL, 0.719 mmol) according to the general procedure for glycosylation. After 1 h complete conversion was detected by TLC and the reaction mixture was subjected to aqueous work-up. The crude product was purified by column chromatography (toluene/EtOAC 2:1 → 1:1) affording glycal **3**[S3] (5.9 mg, 4%) and the slower migrating disaccharide **11** (229 mg, 78%).

11: colorless amorphous solid; $[\alpha]_D^{20}$ + 70.9 (*c* 0.61, $CHCl_3$); R_f 0.30 (toluene/EtOAc 1:1); ^{1}H NMR ($CDCl_3$): δ 5.45 - 5.43 (m, 1H, H-5′), 5.33 (app td, 1H, $J_{7',6'}$ 9.3, H-7′), 4.91 (dd, 1H, $J_{4',3'}$ 5.0, $J_{4',5'}$ 3.5 Hz, H-4′), 4.90 (ddd, 1H, $J_{7,8b}$ 8.4, $J_{7,8a}$ 6.7, $J_{7,6}$ 4.1 Hz, H-7), 4.73 (dd, 1H, $J_{8'a,8'b}$ 12.2, $J_{8'a,7'}$ 3.0 Hz, H-8′a), 4.71 (dd, 1H, $J_{8a,8b}$ 9.0 Hz, H-8a), 4.54 (app t, 1H, H-8b), 4.51 (dd, 1H, $J_{3',5'}$ 0.9 Hz, H-3′), 4.22 - 4.18 (m, 2H, H-4, H-6′), 4.10 (dd, 1H, $J_{8'b,7'}$ 3.4 Hz, H-8′b), 3.88 (s, 3H, CO_2CH_3), 3.88 - 3.86 (dd, 1H, $J_{6,5}$ 2.0 Hz, H-6), 3.80 (s, 3H, CO_2CH_3), 3.58 - 3.55 (m, 1H, H-5), 3.21 (s, 3H, OCH_3), 2.47 (b s, 1H, O*H*), 2.16 - 2.12 (m, 2H, H-3*ax*, H-3*eq*), 2.11, 2.09, 2.06 and 1.97 (4 x s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 170.91, 169.92, 169.38 and 169.36 (4 x s, $COCH_3$), 167.40 (s, 2C, C-1, C-1′), 154.55 [s, O*C*(=O)O], 100.60 (s, C-2′), 98.99 (s, C-2), 75.76 (d, C-7), 70.28 (d, C-6), 69.46, 69.18 (2d, 2C, C-4, C-6′), 67.58 (d, C-7′), 66.10 (t, C-8), 64.96 (d, C-4′), 64.11 (d, C-5), 63.09 (d, C-5′), 61.32 (t, C-8′), 53.51 and 52.77 (2 x q, CO_2CH_3), 51.13 (q, OCH_3), 32.85 (t, C-3), 21.97 (d, C-3′), 20.88, 20.75, 20.51 and 20.48 (4 x q, $COCH_3$); ESI-TOF HRMS: *m/z* = 838.1258; calcd for $C_{28}H_{37}IO_{20}NH_4^+$: 838.1261.

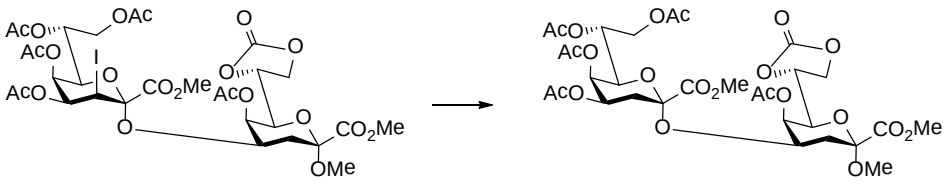
3.4.2. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→4)-methyl (methyl 5-*O*-acetyl-7,8-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (12)



11 **12**

A solution of **11** (32.1 mg, 0.039 mmol) in dry pyridine (2.0 mL) was treated with acetic anhydride (0.1 mL) and 4-(*N*,*N*-dimethylamino)pyridine (0.5 mg, 0.004 mmol) at 0 °C for 2 h. Excessive reagent was destroyed by slow addition of dry MeOH (1 mL) at 0 °C and after 10 min the solvent was removed by coevaporation with toluene (3 x). The residue was purified by column chromatography (toluene/EtOAc 1:1) yielding **12** (32.9 mg, 98%) as a colorless amorphous solid; $[\alpha]_D^{20}$ + 68.5 (*c* 0.55, $CHCl_3$); R_f 0.34 (toluene/EtOAc 1:1); ^{1}H NMR ($CDCl_3$): δ 5.42 - 5.40 (m, 1H, H-5′), 5.34 (ddd, 1H, $J_{7',6'}$ 9.2, $J_{7',8'b}$ 3.8, $J_{7',8'a}$ 3.0 Hz, H-7′), 5.03 - 5.01 (m, 1H, H-5), 4.92 (dd, 1H, $J_{4',3'}$ 4.4, $J_{4',5'}$ 3.8 Hz, H-4′), 4.81 (ddd, 1H, $J_{7,8b}$ 8.4, $J_{7,8a}$ 6.1, $J_{7,6}$ 3.4 Hz, H-7), 4.75 (dd, 1H, $J_{8'a,8'b}$ 12.5 Hz, H-8′a), 4.57 (dd, 1H, $J_{8a,8b}$ 8.4 Hz, H-8a), 4.39 (dd, 1H, $J_{3',5'}$ 0.6 Hz, H-3′), 4.34 (app t, 1H, H-8b), 4.25 (ddd, 1H, $J_{4,3ax}$ 11.6, $J_{4,3eq}$ 5.2, $J_{4,5}$ 3.2 Hz, H-4), 4.21 (dd, 1H, $J_{6',5'}$ 2.1 Hz, H-6′), 4.12 (dd, 1H, H-8′b), 3.99 (dd, 1H, $J_{6,5}$ 0.9 Hz, H-6), 3.86 and 3.82 (2 x s, each 3H, CO_2CH_3), 3.24 (s, 3H, OCH_3), 2.24 (dd, 1H, $J_{3eq,3ax}$ 12.8 Hz, H-3*eq*), 2.17 (s, 3H, $COCH_3$), 2.15 (app t, 1H, H-3*ax*), 2.11, 2.10, 2.06 and 1.97 (4 x s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 170.72, 170.10, 170.01, 169.44 and 169.38 (5 x s, $COCH_3$), 167.15 and 165.68 (2 x s, C-1, C-1′), 154.04 [s, O*C*(=O)O], 101.26 (s, C-2′), 98.97 (s, C-2), 75.07 (d, C-7), 70.38 (d, C-6), 69.46 (d, C-6′), 68.63 (d, C-4), 67.79 (d, C-7′), 66.39 (d, C-5), 64.95 (d, C-4′), 64.55 (t, C-8), 63.14 (d, C-5′), 61.45 (t, C-8′), 53.38 and 52.85 (2 x q, CO_2CH_3), 51.46 (q, OCH_3), 34.01 (t, C-3), 22.08 (d, C-3′), 20.92, 20.78, 20.76, 20.58 and 20.50 (5 x q, $COCH_3$); ESI-TOF HRMS: *m/z* = 880.1362; calcd for $C_{30}H_{39}IO_{21}NH_4^+$: 880.1367.

3.4.3. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-methyl (methyl 5-*O*-acetyl-7,8-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (13)

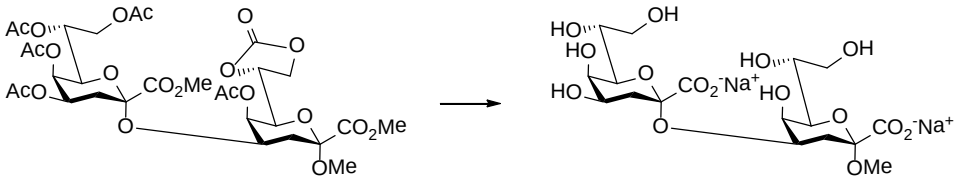


12 **13**

A solution of **12** (27.3 mg, 0.032 mmol) and lauroyl peroxide (7.6 mg, 0.019 mmol) in dry cyclohexane (4 mL) was treated according to the general procedure for dehalogenation. After 4 h another portion of lauroyl peroxide (7.6 mg, 0.019 mmol) was added and after 24 h at reflux temperature full conversion was detected by TLC. After removal of the solvent, column chromatography (toluene/EtOAc 1:1) provided **13** (22.6 mg, 97%) as a colorless oil; $[\alpha]_D^{20}$ + 89.6 (*c* 0.80, $CHCl_3$); R_f 0.30 (toluene/EtOAc 1:1, HP-TLC); ^{1}H NMR ($CDCl_3$): δ 5.36 - 5.34 (m, 1H, H-5′), 5.22 - 5.20 (m, 1H, H-5), 5.20 - 5.15 (m, 2H, H-4′, H-7′), 4.75 (ddd, 1H, $J_{7,8b}$ 8.3, $J_{7,8a}$ 6.5, $J_{7,6}$ 4.1 Hz,

H-7), 4.66 - 4.60 (m, 3H, H-4, H-8a, H-8′a), 4.38 (app t, 1H, $J_{8b,8a}$ 8.5 Hz, H-8b), 4.11 (dd, 1H, $J_{6',7'}$ 9.4, $J_{6',5'}$ 1.4 Hz, H-6'), 4.09 (dd, 1H, $J_{8'b,8'a}$ 12.5, $J_{8'b,7'}$ 3.6 Hz, H-8′b), 4.03 (dd, 1H, $J_{6,5}$ 1.2 Hz, H-6), 3.85 and 3.83 (2 x s, each 3H, CO_2CH_3), 3.24 (s, 3H, OCH_3), 2.17 (app t, 1H, $J_{3'ax,3'eq} = J_{3'ax,4'}$ 12.8 Hz, H-3′*ax*), 2.15 - 2.11 (m, 7H, H-3*eq*, 2 x $COCH_3$), 2.08 - 2.01 (m, 5H, H-3*ax*, H-3′*eq*, $COCH_3$), 1.99 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 170.92, 170.27, 170.08, 169.97 and 169.57 (5 x s, $COCH_3$), 167.38 and 167.11 (2 x s, C-1, C-1′), 154.07 [s, $OC(=O)O$], 99.20 and 97.63 (2 x s, C-2, C-2′), 74.97 (d, C-7), 70.31 (d, C-6), 69.56 (d, C-6′), 67.69 (d, C-7′), 66.57 (d, C-5), 66.24 (d, C-4′), 65.79 (d, C-4), 65.12 (t, C-8), 64.26 (d, C-5′), 61.71 (t, C-8′), 53.12 and 52.76 (2 x q, CO_2CH_3), 51.38 (q, OCH_3), 34.23 (t, C-3′), 31.46 (t, C-3), 20.77, 20.62 and 20.59 (3 x s, 5C, $COCH_3$); ESI-TOF HRMS: *m/z* = 759.1993; calcd for $C_{30}H_{40}O_{21}Na^+$: 759.1954.

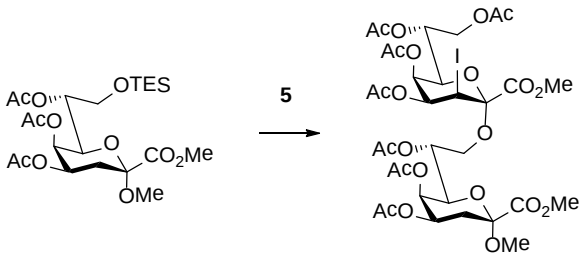
3.4.4. Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-sodium (methyl α-D-*manno*-oct-2-ulopyranosid)onate (14)



13 **14**

According to the general procedure for global deprotection: A solution of **13** (10.6 mg, 0.014 mmol) in dry MeOH (1.0 mL) was treated with NaOMe (0.1 M in MeOH; 20 µL, 0.002 mmol) for 8 h and after addition of further NaOMe (0.1 M in MeOH; 20 µL, 0.002 mmol) stirring was continued for 14 h. After the described work-up the residue was dissolved in water (2.0 mL) and treated with aq NaOH (0.1 M, 1.0 mL). After 3 h it was subjected to standard work-up und the crude product was desalted by successive purification on a PD10 (H_2O) and BioGel-P2 column (H_2O/EtOH 19:1) followed by lyophilisation of pooled fractions affording **14** (6.2 mg, 83%) as an amorphous colorless solid. $[\alpha]_D^{20}$ + 80.2 (*c* 0.51, D_2O); ^{1}H NMR (D_2O, pH ~ 6.5)[S7]: δ 4.00 - 3.96 (m, 3H, H-4, H-4′, H-5), 3.93 - 3.92 (m, 1H, H-5′), 3.89 - 3.80 (m, 4H, H-7, H-7′, H-8a, H-8′a), 3.64 - 3.60 (m, 1H, H-8′b), 3.55 - 3.50 (m, 2H, H-6′, H-8b), 3.41 (dd, 1H, $J_{6,7}$ 8.6, $J_{6,5}$ 0.8 Hz, H-6), 3.03 (s, 3H, OCH_3), 2.03 (ddd, 1H, $J_{3'eq,3'ax}$ 13.2, $J_{3'eq,4'}$ 5.0, $J_{3'eq,5'}$ 0.8 Hz, H-3′*eq*), 1.88 - 1.84 (m, 1H, H-3*eq*), 1.82 - 1.76 (app t, 1H, H-3*ax*), 1.67 (dd, 1H, $J_{3'ax,4'}$ 12.1 Hz, H-3′*ax*); ^{13}C NMR (D_2O, pH ~ 6.5): δ 176.67 and 176.07 (2 x s, C-1′, C-1), 101.34 and 100.36 (2 x s, C-2, C-2′), 73.09 (d, C-6′), 72.15 (d, C-6), 70.76 (d, C-7′), 70.24 (d, C-7), 69.64 (d, C-4), 67.17 (d, C-5′), 66.72 (d, C-4′), 65.17 (d, C-5), 63.91 and 63.86 (2 x t, C-8, C-8′), 51.41 (q, OCH_3), 35.38 (t, C-3′), 34.05 (t, C-3); ESI-TOF HRMS: *m/z* = 495.1327; calcd for $C_{17}H_{28}O_{15}Na^+$: 495.1320.

3.4.5. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→8)-methyl (methyl 4,5,7-tri-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (16)

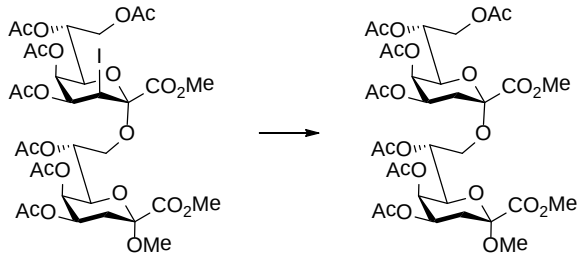


15 **16**

A suspension of **15** (27.1 mg, 0.053 mmol) and **5** (35.2 mg, 0.064 mmol) in dry CH_2Cl_2 (2.0 mL) was treated with $BF_3.Et_2O$ (22.0 µL, 0.107 mmol) according to the general procedure for glycosylation. After 2 h complete conversion was determined by TLC and the mixture was subjected to aqueous work-up. Subsequent HP-column chromatography (toluene/EtOAc 3:1 → 1:1) afforded glycal **3**[S3] (1.8 mg, 7%) and the slower migrating disaccharide **16** (30.3 mg, 62%).

16: colorless oil; $[\alpha]_D^{20}$ + 48.8 (*c* 0.46, $CHCl_3$); R_f 0.22 (toluene/EtOAc 1:1); ^{1}H NMR ($CDCl_3$): δ 5.38 - 5.33 (m, 3H, H-5, H-5′, H-7′), 5.31 (ddd, 1H, $J_{4,3ax}$ 12.3, $J_{4,3eq}$ 5.0, $J_{4,5}$ 3.1 Hz, H-4), 5.11 (ddd, 1H, $J_{7,6}$ 9.6, $J_{7,8a}$ 4.8, $J_{7,8b}$ 2.4 Hz, H-7), 4.92 (dd, 1H, $J_{4',3'}$ 4.7, $J_{4',5'}$ 3.8 Hz, H-4′), 4.64 (dd, 1H, $J_{8'a,8'b}$ 12.4, $J_{8'a,7'}$ 2.3 Hz, H-8′a), 4.52 (d, 1H, H-3′), 4.23 (dd, 1H, $J_{8'b,7'}$ 3.6 Hz, H-8′b), 4.17 (dd, 1H, $J_{6',7'}$ 9.7, $J_{6',5'}$ 2.0 Hz, H-6′), 4.12 (dd, 1H, $J_{6,5}$ 1.3 Hz, H-6), 3.87 (dd, 1H, $J_{8a,8b}$ 11.6 Hz, H-8a), 3.84 (s, 3H, CO_2CH_3), 3.81 (s, 3H, CO_2CH_3), 3.67 (dd, 1H, H-8b), 3.31 (s, 3H, OCH_3), 2.20 - 2.17 (m, 1H, H-3*eq*), 2.11 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 2.09 - 2.04 (m, 10H, 3 x $COCH_3$, H-3*ax*), 1.98 (s, 3H, $COCH_3$), 1.97 (s, 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 170.40, 170.35, 170.11, 169.85, 169.68, 169.43 and 169.24 (7 x s, $COCH_3$), 167.35 and 165.92 (2 x s, C-1, C-1'), 101.89 (s, C-2′), 99.10 (s, C-2), 68.50 (d, C-6′), 68.36 and 68.35 (2 x d, C-6, C-7), 67.58 (d, C-7′), 66.30 (d, C-4), 65.16 (d, C-4′), 64.44 (d, C-5), 64.40 (t, C-8), 63.09 (d, C-5′), 61.82 (t, C-8′), 53.05 and 52.63 (2 x q, CO_2CH_3), 51.48 (q, OCH_3), 31.55 (t, C-3), 21.38 (d, C-3′), 20.88, 20.74, 20.71, 20.67, 20.65 and 20.58 (6 x q, 7C, $COCH_3$); ESI-TOF HRMS: *m/z* = 938.1783; calcd for $C_{33}H_{45}IO_{22}NH_4^+$: 938.1785.

3.4.6. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate]-(2→8)-methyl (methyl 4,5,7-tri-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (17)

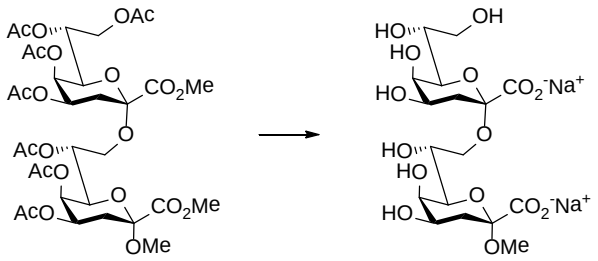


16 **17**

Following the general procedure for dehalogenation: **16** (29.0 mg, 0.032 mmol) was treated with lauroyl peroxide (3.8 mg, 0.009 mmol) in dry cyclohexane (2.0 mL) for 2 h. Evaporation of the solvent and purification by column chromatography (toluene/EtOAc 2:1) provided **17** (23.7 mg, 95%) as a colorless oil; $[\alpha]_D^{20}$ + 68.8 (*c* 0.88, $CHCl_3$); R_f 0.38 (toluene/EtOAc 1:1, HP-TLC); ^{1}H NMR ($CDCl_3$): δ 5.36 - 5.34 (m, 1H, H-5), 5.33 - 5.28

(m, 2H, H-4, H-5′), 5.22 - 5.15 (m, 2H, H-4′, H-7′), 5.13 (ddd, 1H, $J_{7,6}$ 9.7, $J_{7,8b}$ 4.5, $J_{7,8a}$ 2.4 Hz, H-7), 4.56 (dd, 1H, $J_{8'a,8'b}$ 12.4, $J_{8'a,7'}$ 2.3 Hz, H-8′a), 4.16 (dd, 1H, $J_{8'b,7'}$ 4.0 Hz, H-8′b), 4.14 (dd, 1H, $J_{6,5}$ 1.6 Hz, H-6), 4.04 (dd, 1H, $J_{6',7'}$ 9.7, $J_{6',5'}$ 1.4 Hz, H-6′), 3.85 (dd, 1H, $J_{8a,8b}$ 11.5, H-8a), 3.81 (s, 3H, CO_2CH_3), 3.79 (s, 3H, CO_2CH_3), 3.75 (dd, 1H, H-8b), 3.33 (s, 3H, OCH_3), 2.23 - 2.15 (m, 2H, H-3*eq*, H-3′*eq*), 2.11 - 2.03 (m, 14H, 4 x $COCH_3$, H-3*ax*, H-3′*ax*), 2.00, 1.96 and 1.95 (3 x s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 170.49, 170.41, 170.35, 169.88, 169.81, 169.75 and 169.65 (7 x s, $COCH_3$), 167.47 and 167.20 (2 x s, C-1, C-1′), 99.07 and 98.96 (2 x s, C-2, C-2′), 68.85 (d, C-6′), 68.36 and 68.32 (2 x d, C-6, C-7), 67.63 (d, C-7′), 66.37 and 66.12 (2 x d, C-4, C-4′), 64.51 (d, C-5), 64.16 (d, C-5′), 62.47 (t, C-8), 62.02 (t, C-8′), 52.73 and 52.61 (2 x q, CO_2CH_3), 51.43 (q, OCH_3), 31.66 and 31.48 (2 x t, C-3, C-3′), 20.74, 20.73, 20.67 and 20.61 (4 x q, 7C, $COCH_3$); ESI-TOF HRMS: *m/z* = 812.2819; calcd for $C_{33}H_{46}O_{22}NH_4^+$: 812.2819.

3.4.7. Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate]-(2→8)-sodium (methyl α-D-*manno*-oct-2-ulopyranosid)onate (18)



17 **18**

According to the general procedure for global deprotection: a solution of **17** (23.0 mg, 0.029 mmol) in dry MeOH (4.0 mL) was treated with NaOMe (0.1 M in MeOH; 41 µL, 0.004 mmol) for 24 h. After the described work-up the residue was dissolved in water (2.0 mL) and treated with aq NaOH (0.1 M, 2.0 mL). After 2 h it was subjected to standard work-up and the crude product was desalted by successive purification on a PD10 (H_2O) and BioGel-P2 (H_2O/EtOH 19:1) column followed by lyophilisation of pooled fractions affording **18** (12.7 mg, 85%) as an amorphous colorless solid; $[\alpha]_D^{20}$ + 80.9 (*c* 0.73, D_2O); ^{1}H NMR (D_2O, pH ~ 6.5): δ 3.98 - 3.89 (m, 5H, H-4, H-4′, H-5, H-5′, H-7), 3.84 - 3.78 (m, 2H, H-7′, H-8′a), 3.58 - 3.48 (m, 5H, H-6, H-6′, H-8a, H-8b, H-8′b), 3.06 (s, 3H, OCH_3), 1.96 (ddd, 1H, $J_{3eq,3ax}$ 13.2, $J_{3eq,4}$ 5.4, $J_{3eq,5}$ 0.7 Hz, H-3*eq*), 1.93 - 1.88 (m, 1H, H-3′*eq*), 1.74 - 1.65 (m, 2H, H-3*ax*, H-3′*ax*); ^{13}C NMR (D_2O, pH ~ 6.5): δ 176.62 and 176.10 (2 x s, C-1, C-1′), 101.36 and 101.34 (2 x s, C-2, C-2′), 72.46 and 72.21 (2 x d, C-6, C-6′), 70.12 (d, C-7′), 68.80 (d, C-7), 67.19 and 67.13 (2 x d, C-5, C-5′), 66.94 and 66.85 (2 x d, C-4, C-4′), 65.89 (t, C-8), 63.88 (t, C-8′), 51.47 (q, OCH_3), 34.89 (t, 2C, C-3, C-3′) ; ESI-TOF HRMS: *m/z* = 495.1336; calcd for $C_{17}H_{28}O_{15}Na^+$: 495.1320.

3.5. [1]H-NMR for calculation of molecular ratio of compound 25 and hydroylsed donor

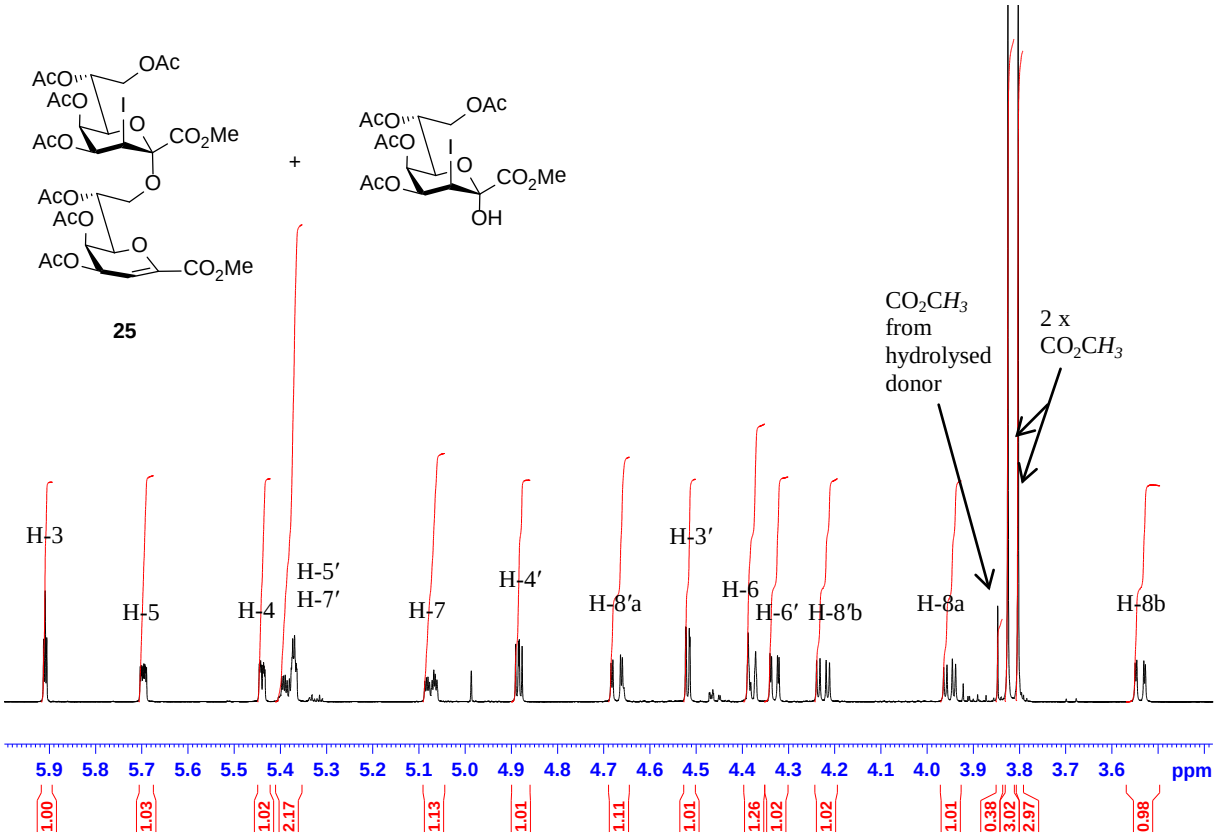


Figure S2: [1]H-NMR of **25** containing hydrolysed donor as minor impurity: The yield was calculated using the CO_2CH_3-signals to determine the molecular ratio

4. References for SI

[S1] R. K. Harris, E. D. Becker, S. M. C. De Menezes, R. Goodfellow, P. Granger, *Pure Appl. Chem.* **2001**, *73*, 1795-1818.

[S2] D. Solomon, M. Fridman, J. Zhang, T. Baasov, *Org. Lett.* **2001**, *3*, 4311-4314.

[S3] A. Claesson, K. Luthman, *Acta Chem. Scand. B* **1982**, *36*, 719-720.

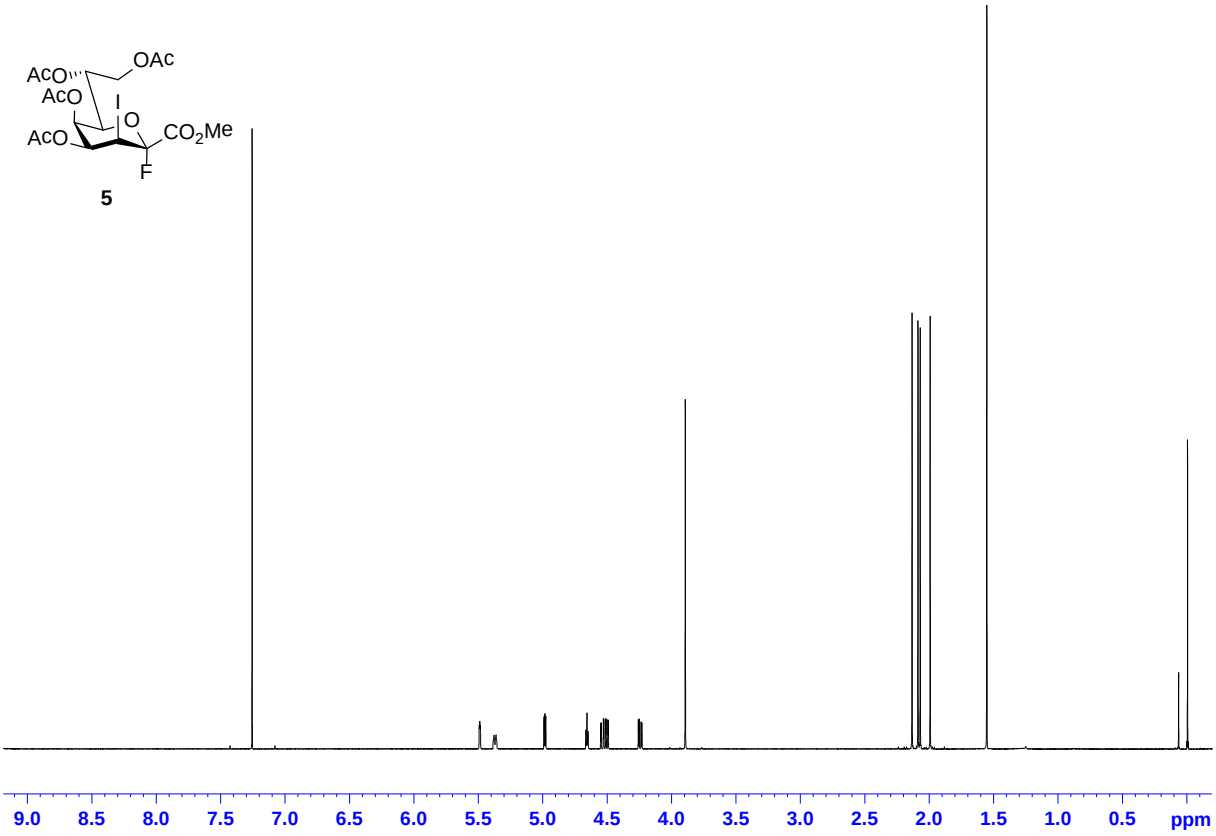
[S4] F. M. Unger, D. Stix, G. Schulz, *Carbohydr. Res.* **1980**, *80*, 191-195; P. Kosma, M. Strobl, G. Allmaier, E. Schmid, H. Brade, *Carbohydr. Res.* **1994**, 254, 105-132.

[S5] Dissolves in boiling cyclohexane.

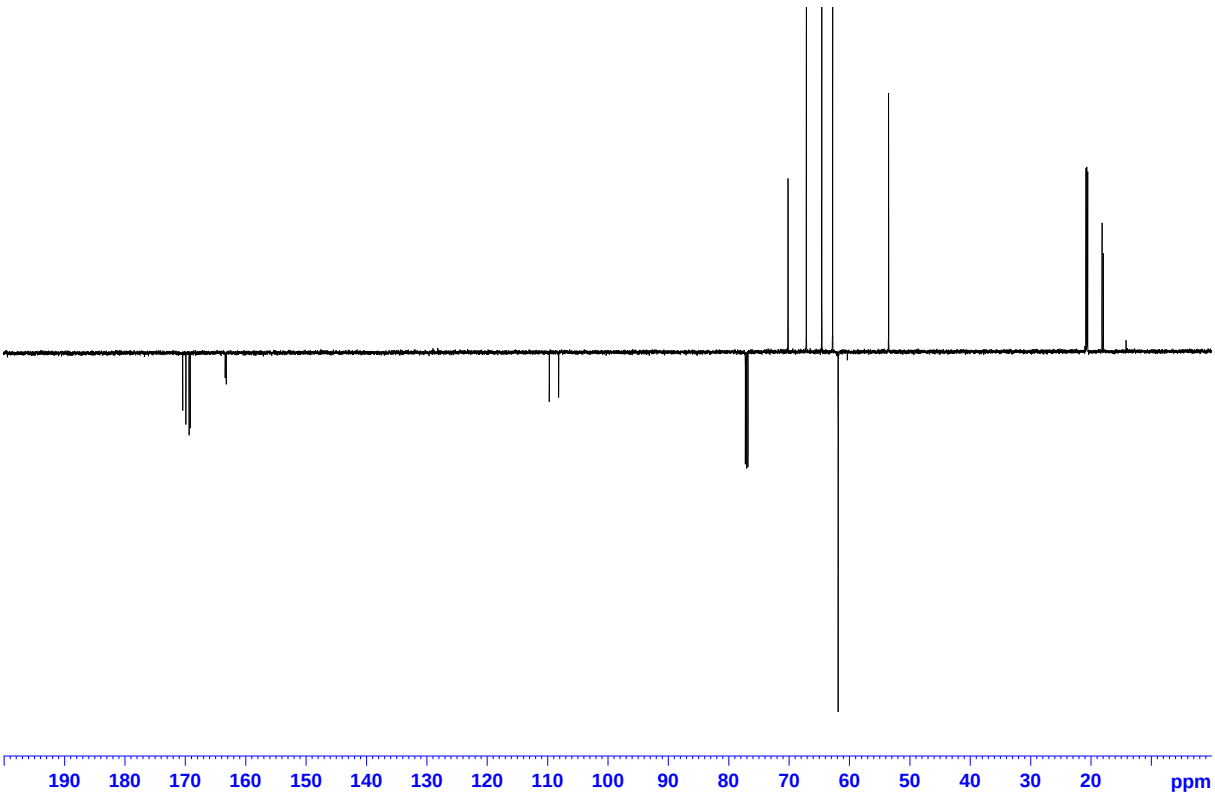
[S6] Tetraol **19** was prepared from Kdo-peracetate **1** by standard transesterification catalyzed by sodium methoxide and was used without purification after neutralization and solvent evaporation.

[S7] Spectroscopic data is in accordance with published data: H. Brade, U. Zähringer, E. T. Rietschel, R. Christian, G. Schulz, F. M. Unger, *Carbohydr. Res.* **1984**, *134*, 157-166.

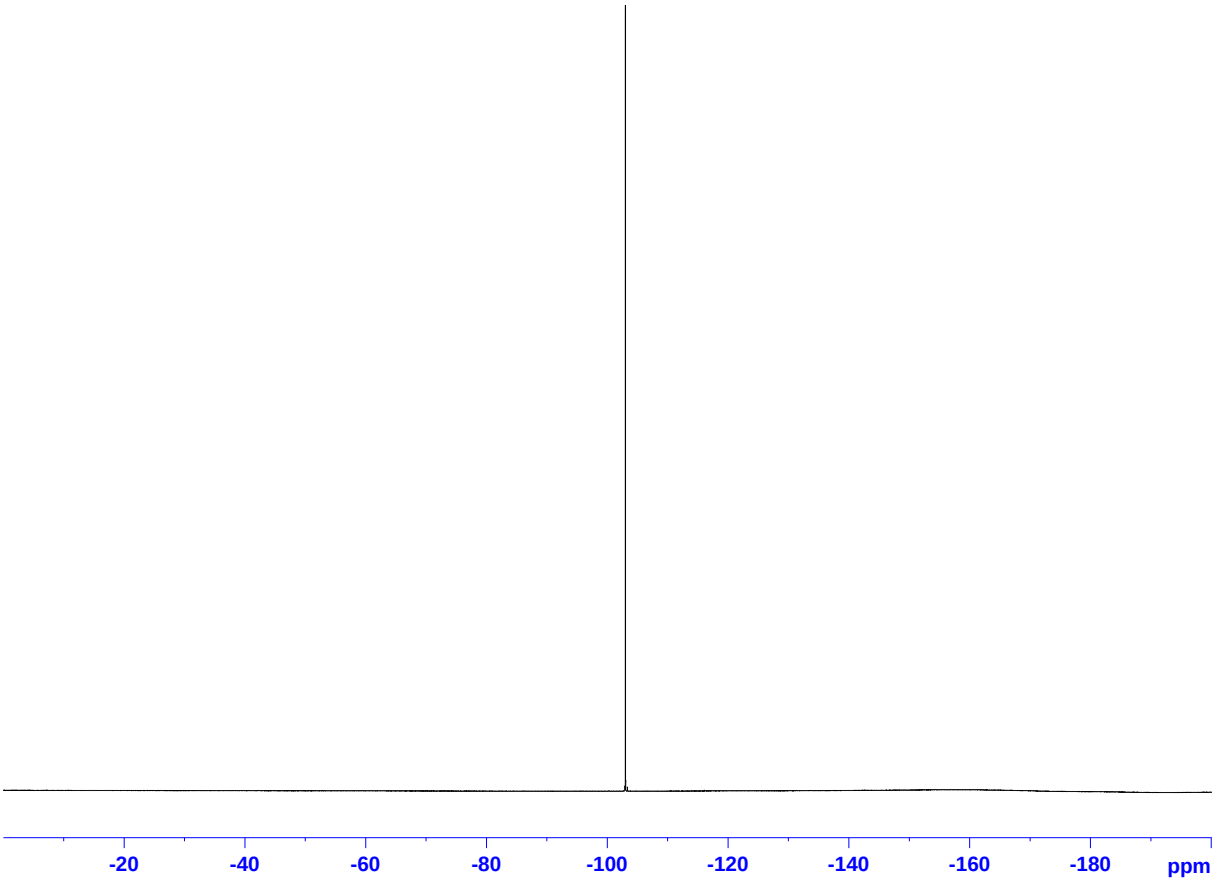
5. NMR spectra of compounds 5 - 18, 20 - 24 and 26 - 33



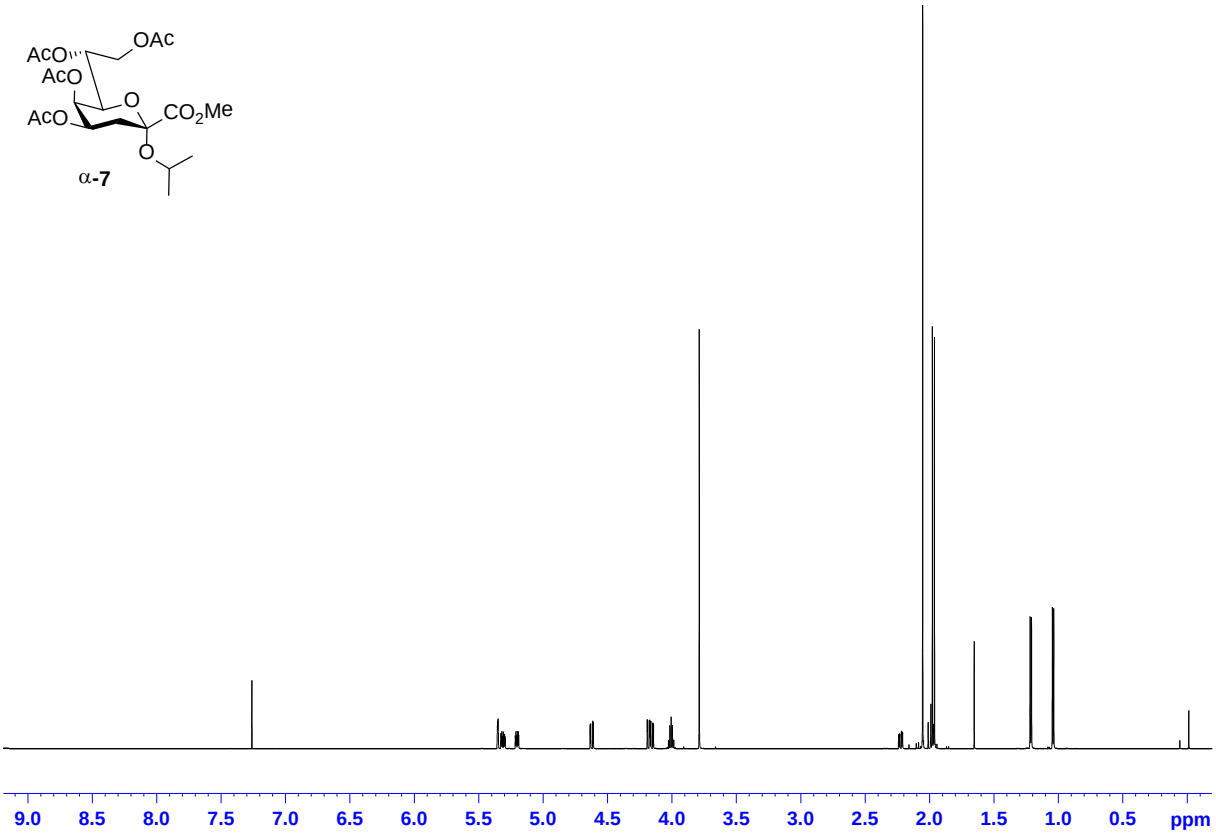
^{1}H-NMR (600 MHz, $CDCl_3$)



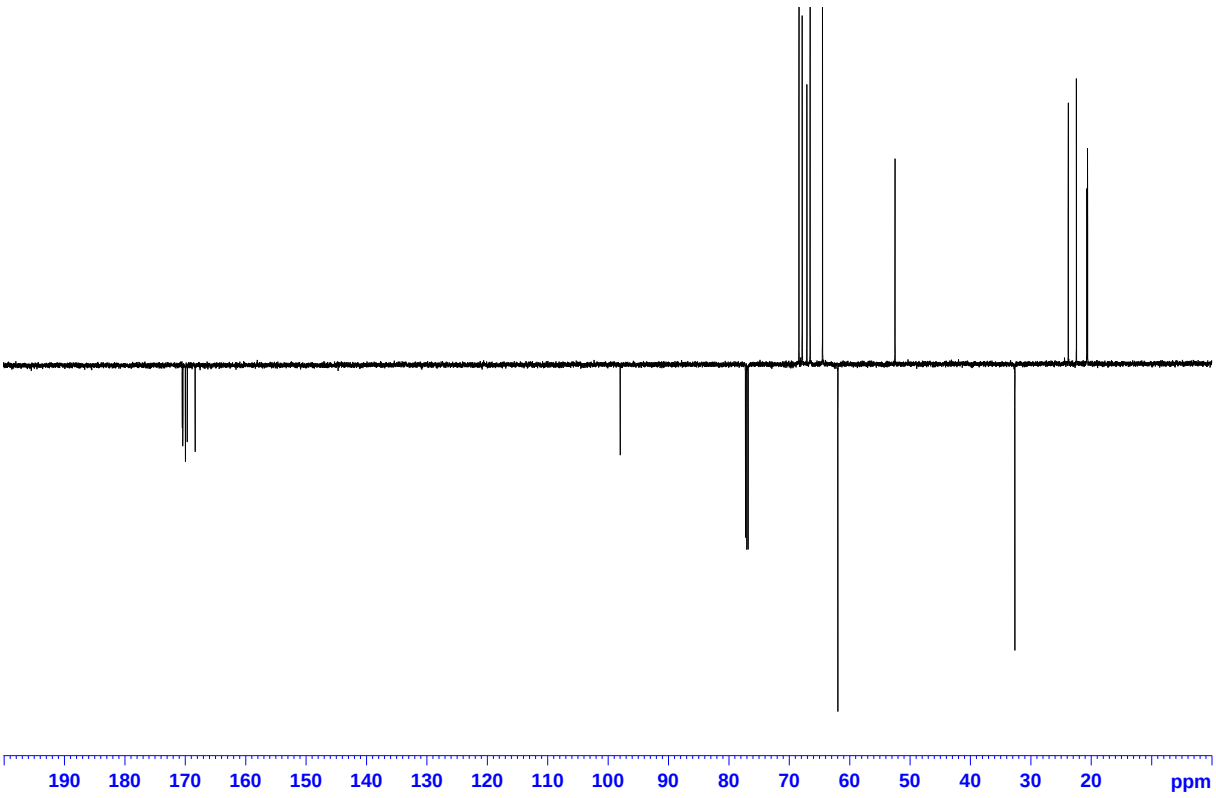
^{13}C-NMR (150 MHz, $CDCl_3$)



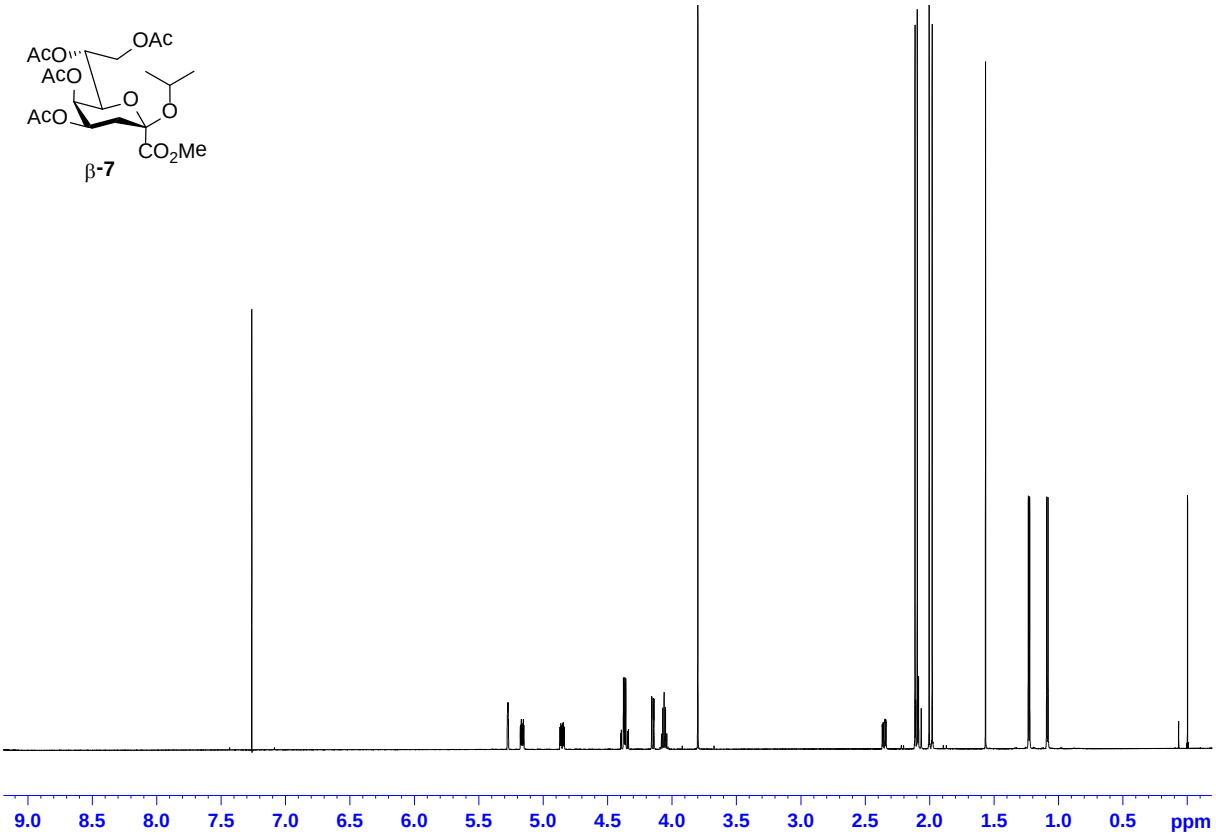
^{19}F-NMR (565 MHz, $CDCl_3$)



^{1}H-NMR (600 MHz, $CDCl_3$)



^{13}C-NMR (150 MHz, $CDCl_3$)



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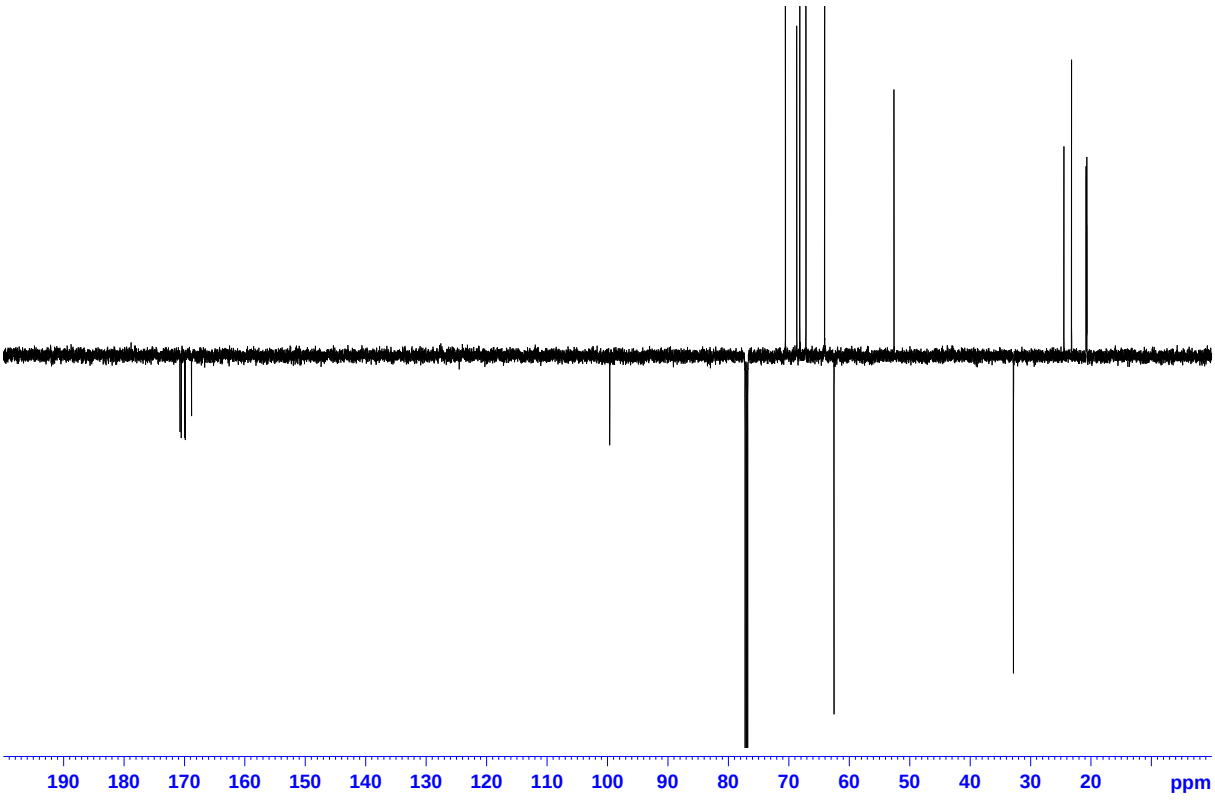

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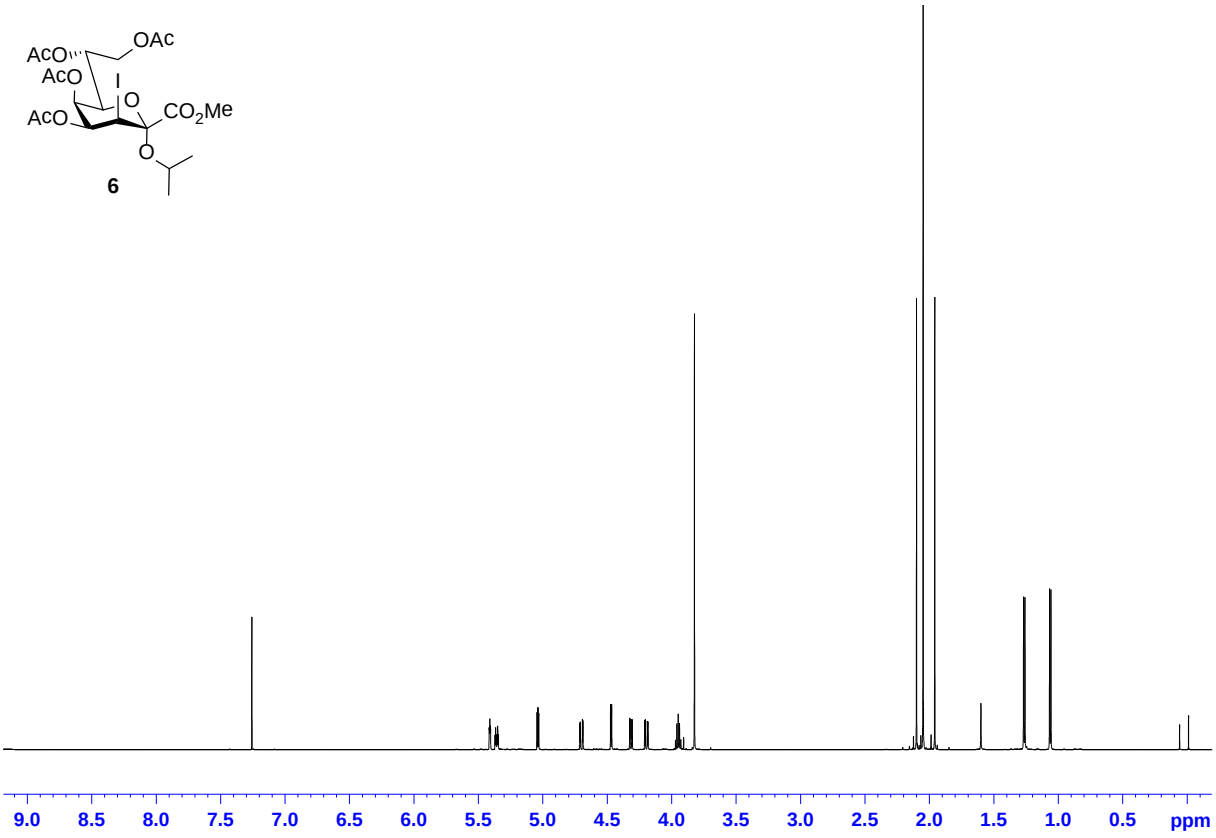
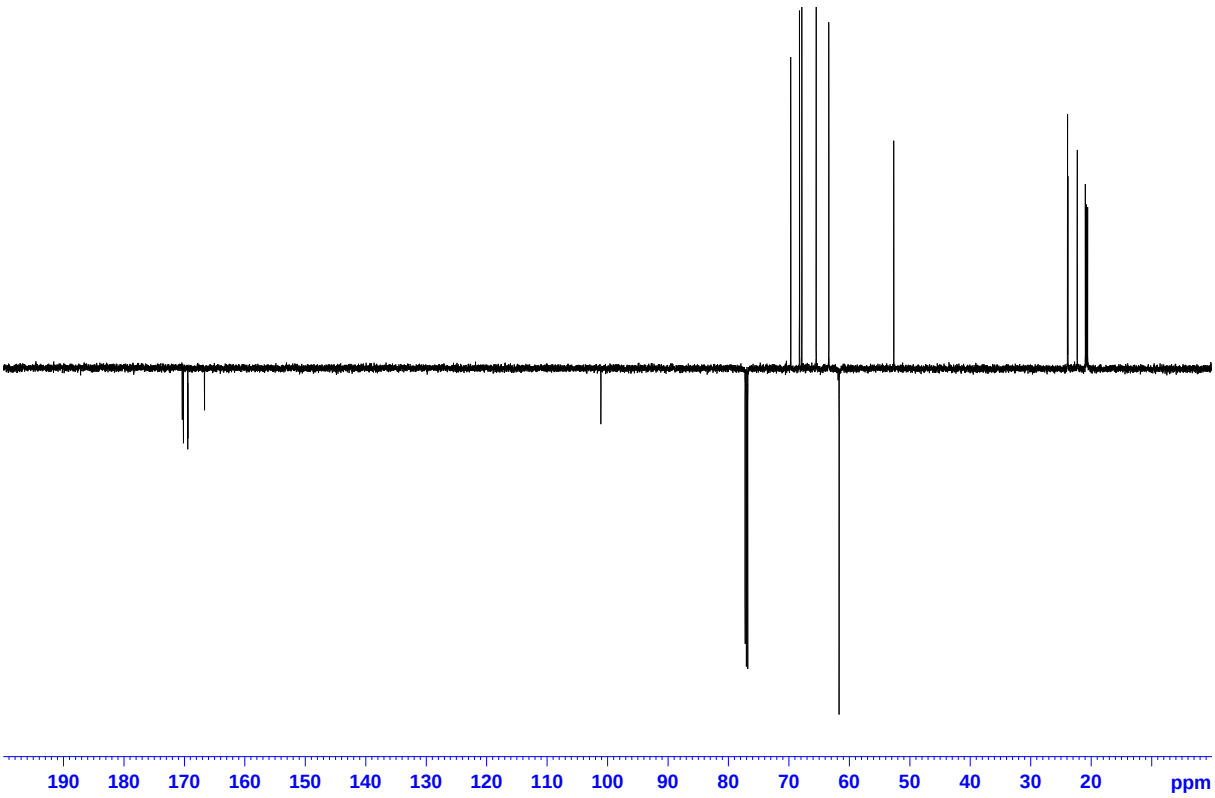
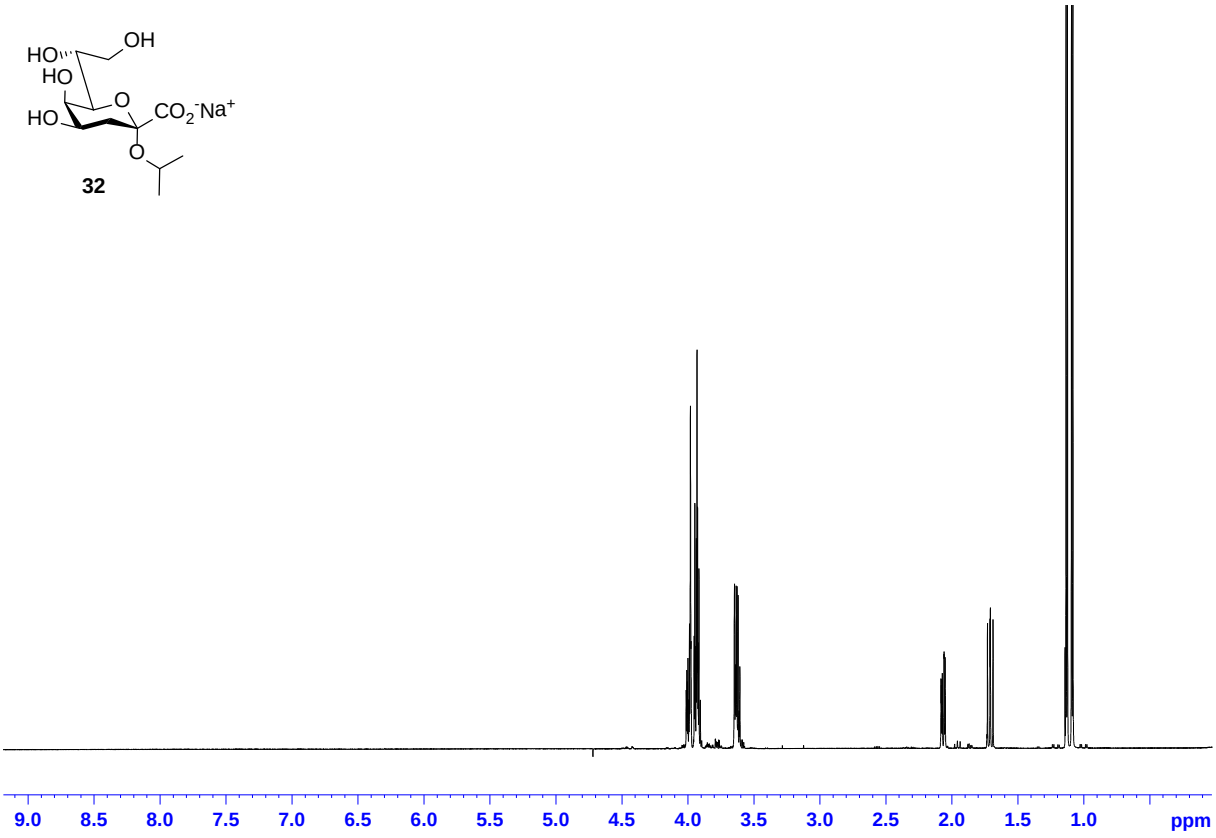


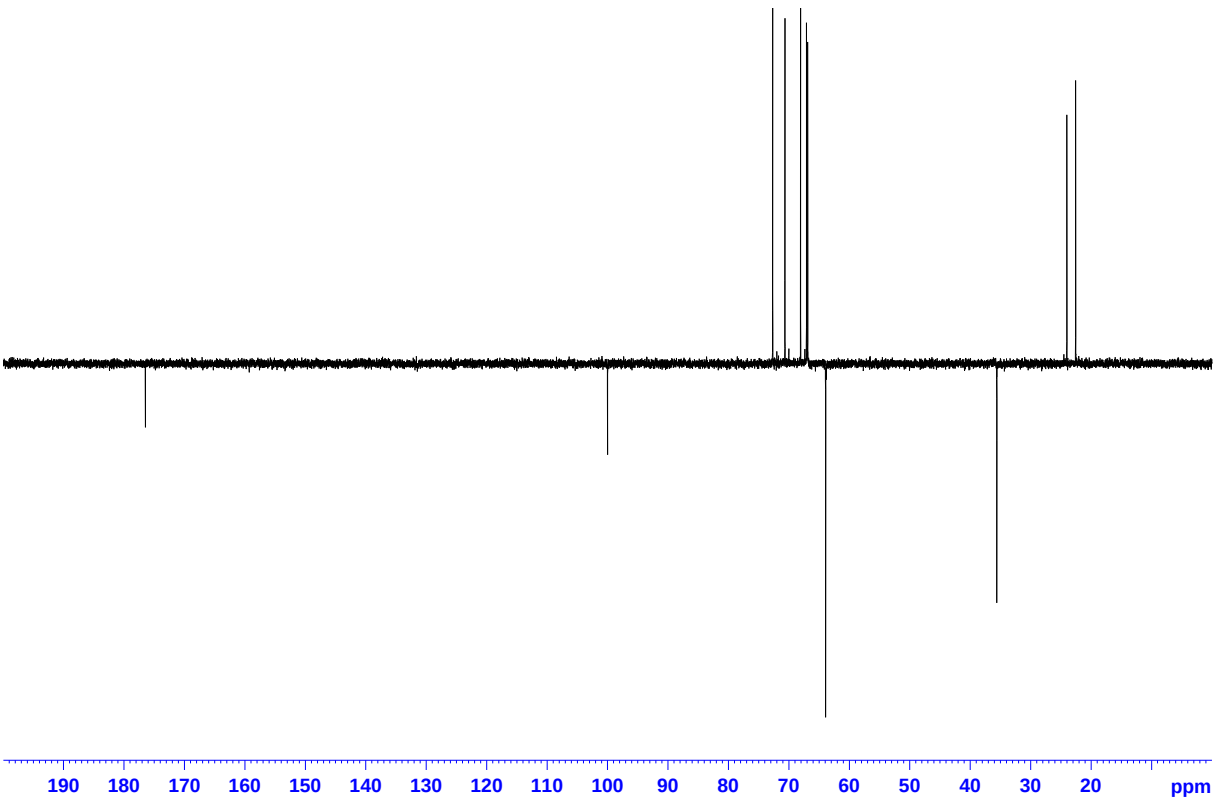
^{1}H-NMR (600 MHz, $CDCl_3$)



^{13}C-NMR (150 MHz, $CDCl_3$)



^{1}H-NMR (600 MHz, D_2O)



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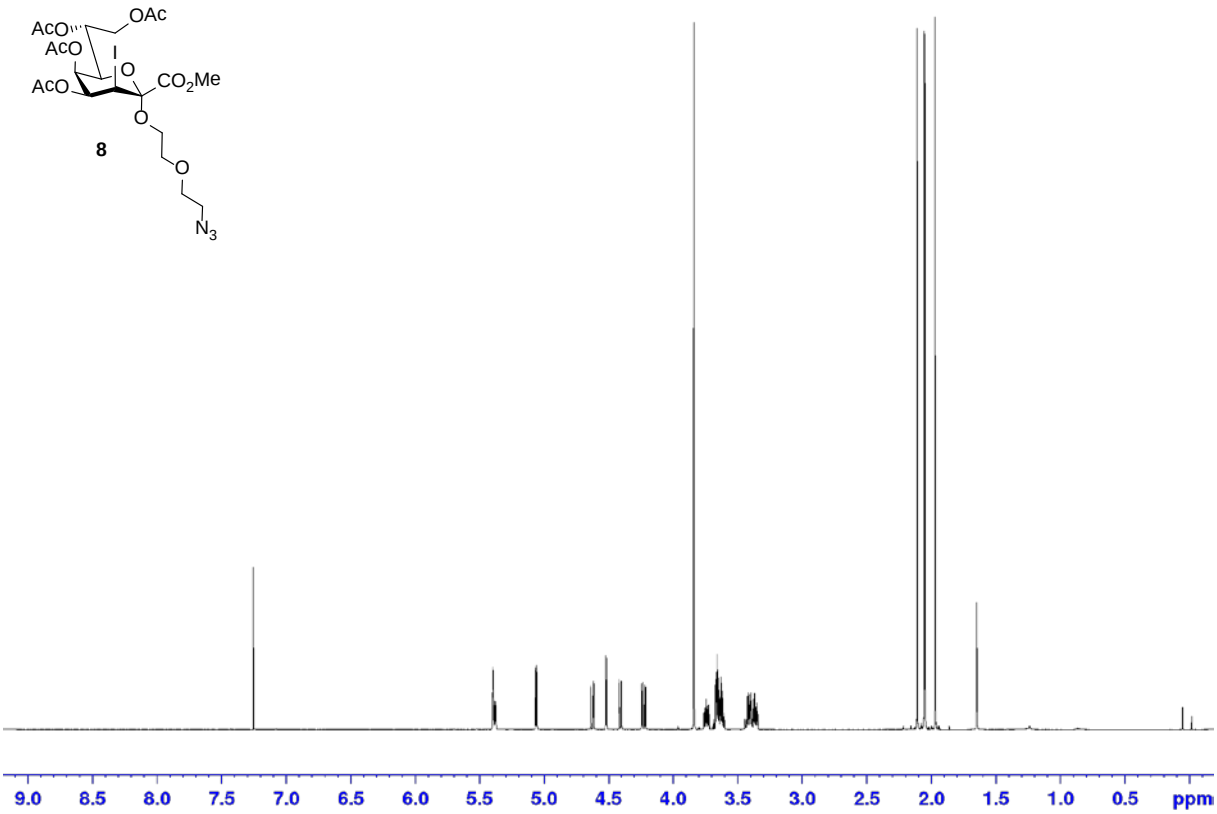
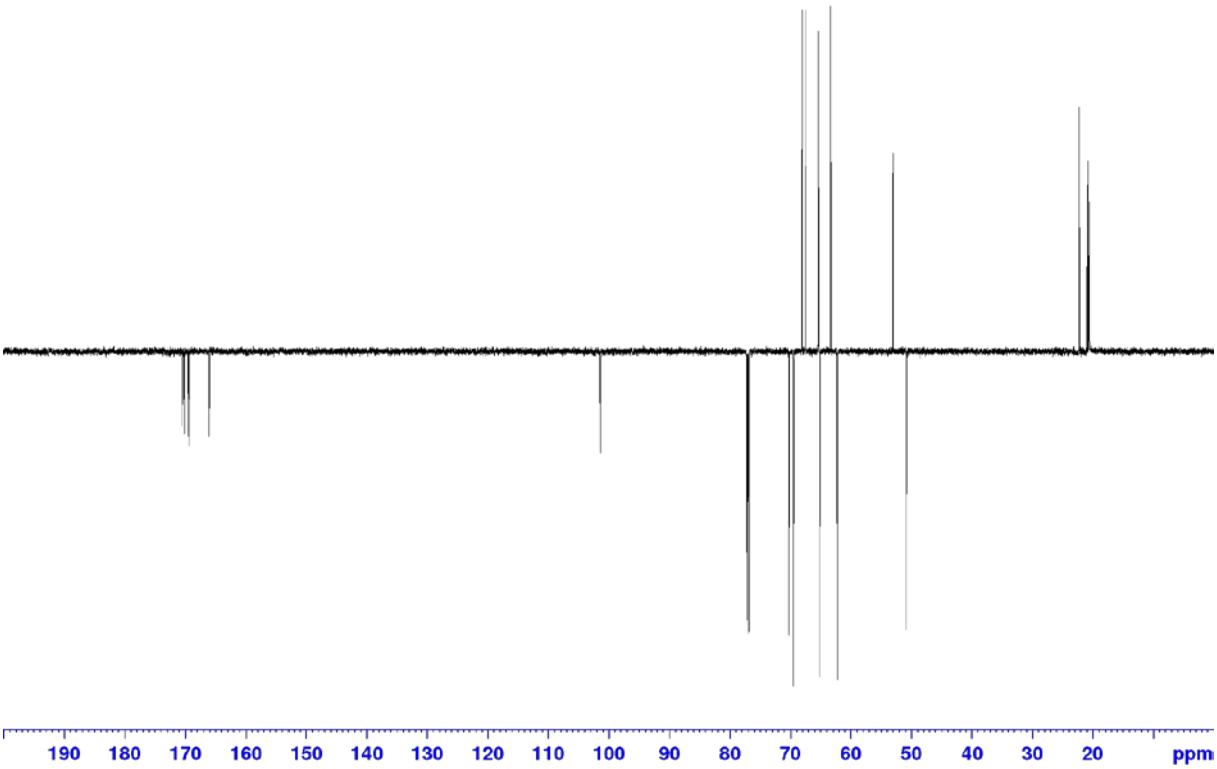


^{1}H-NMR (600 MHz, $CDCl_3$)



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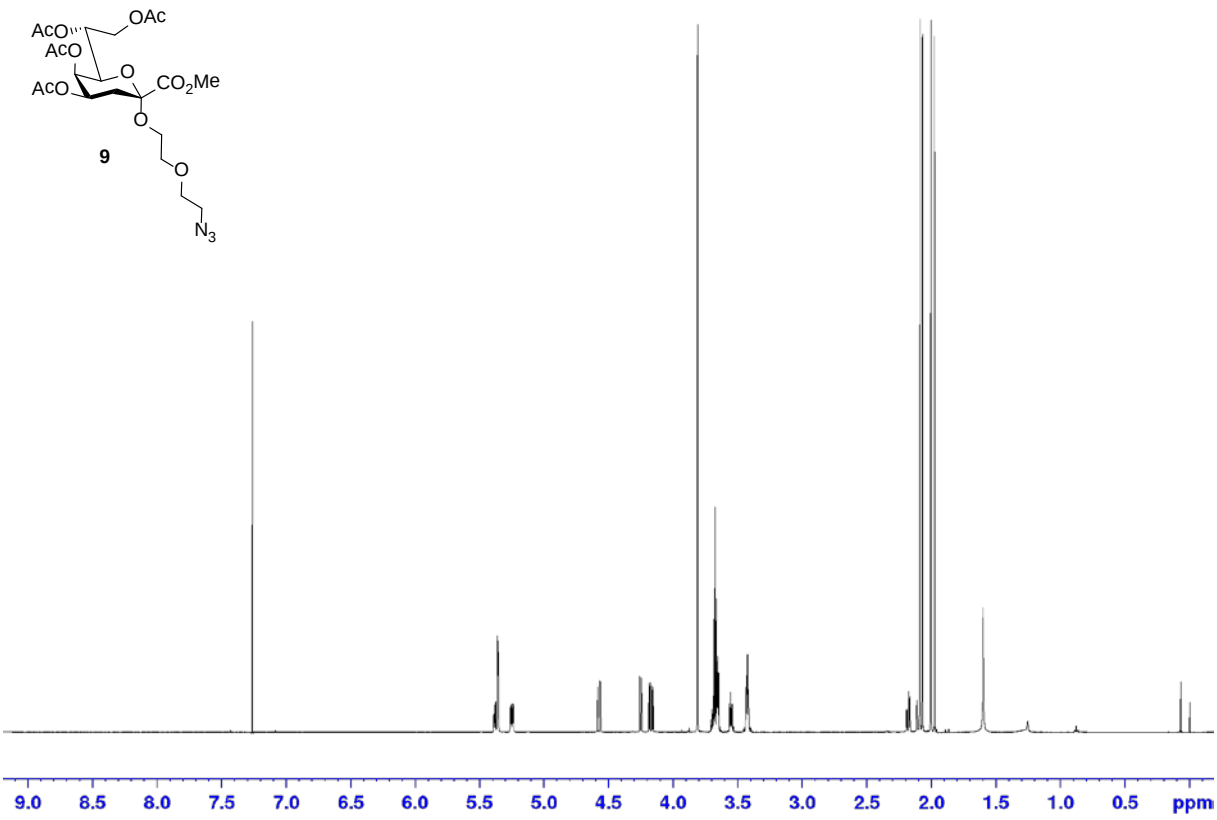

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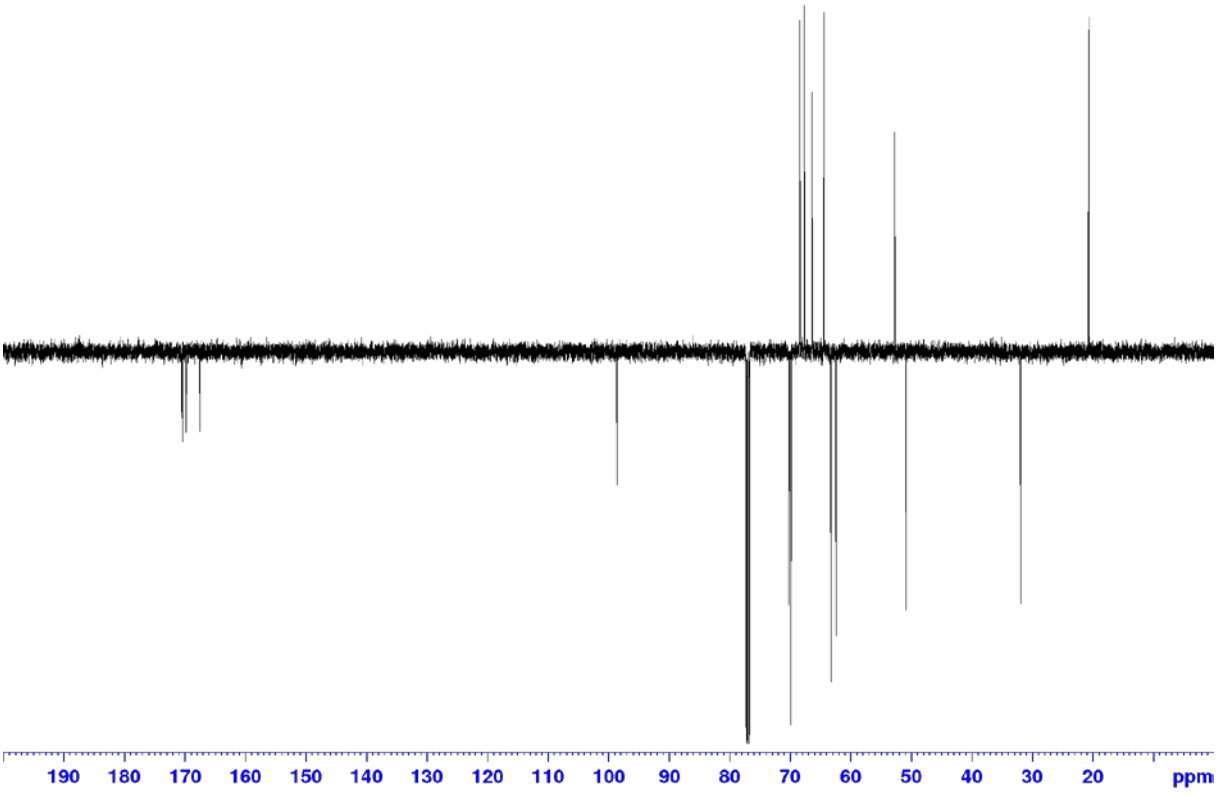

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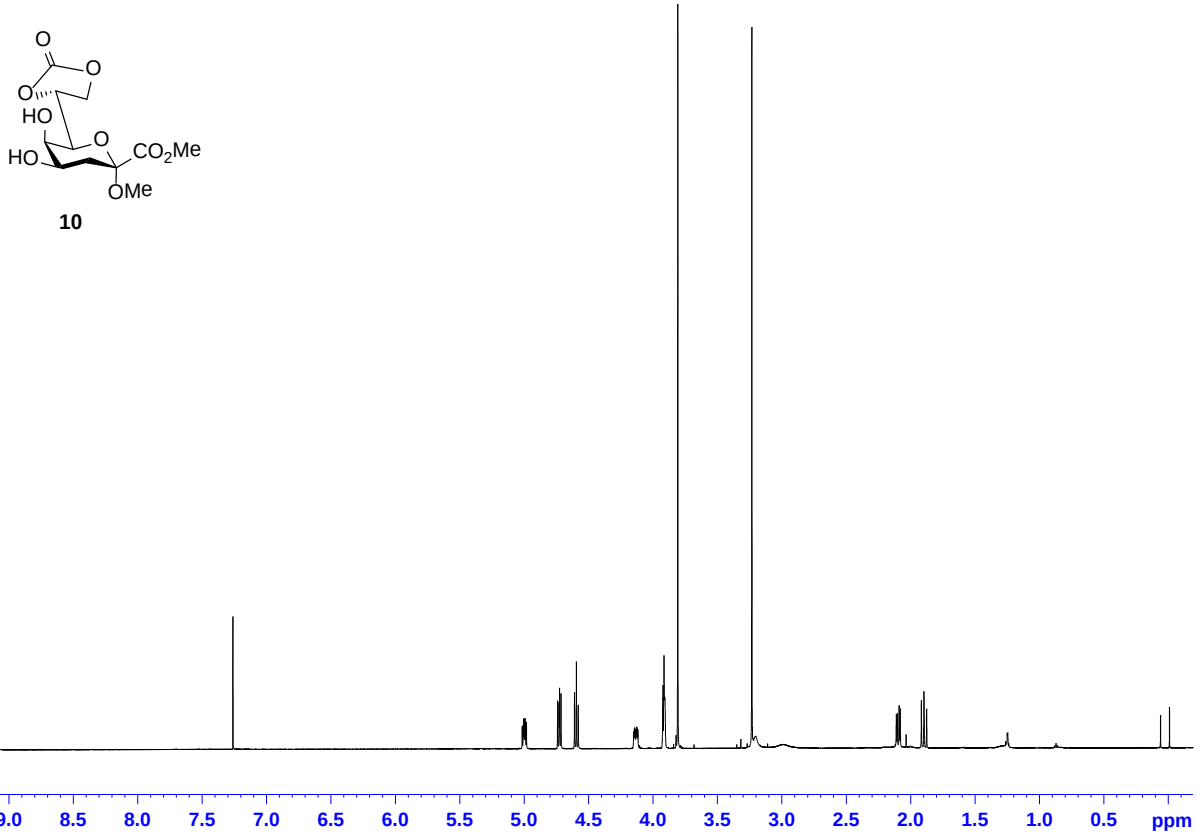

10

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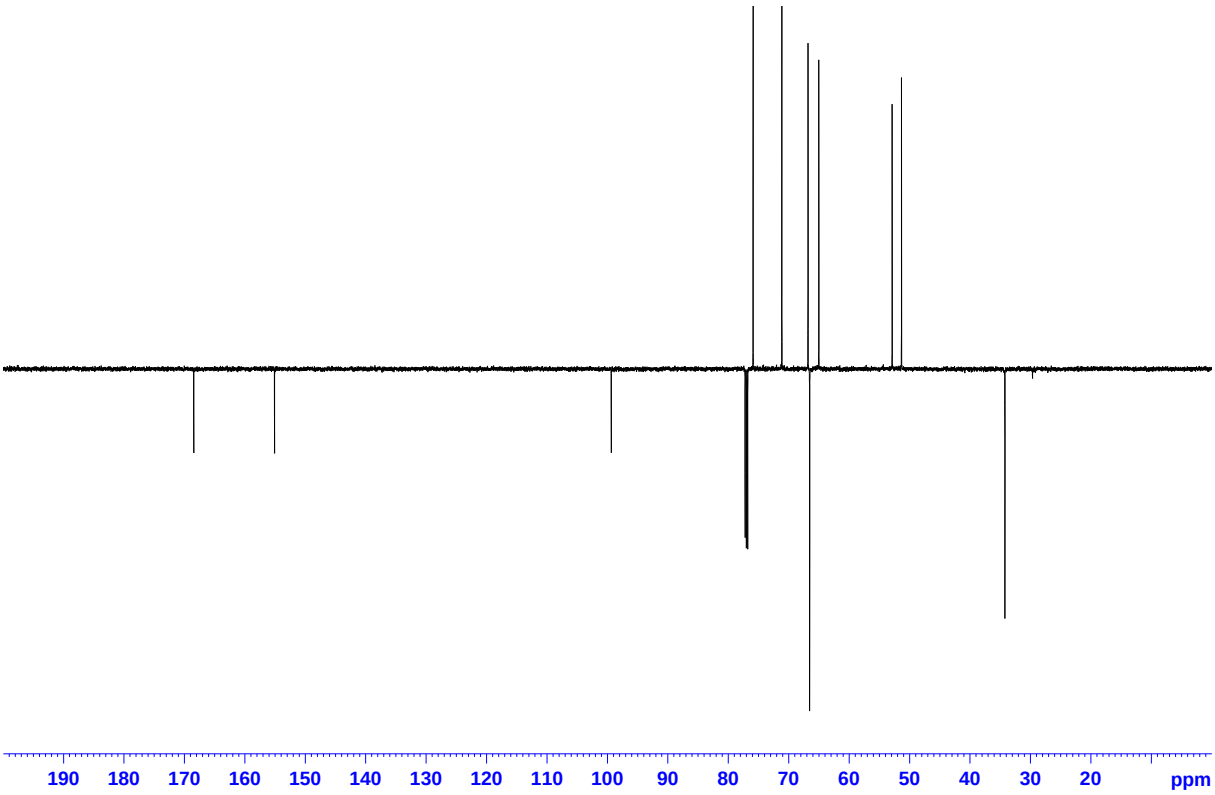

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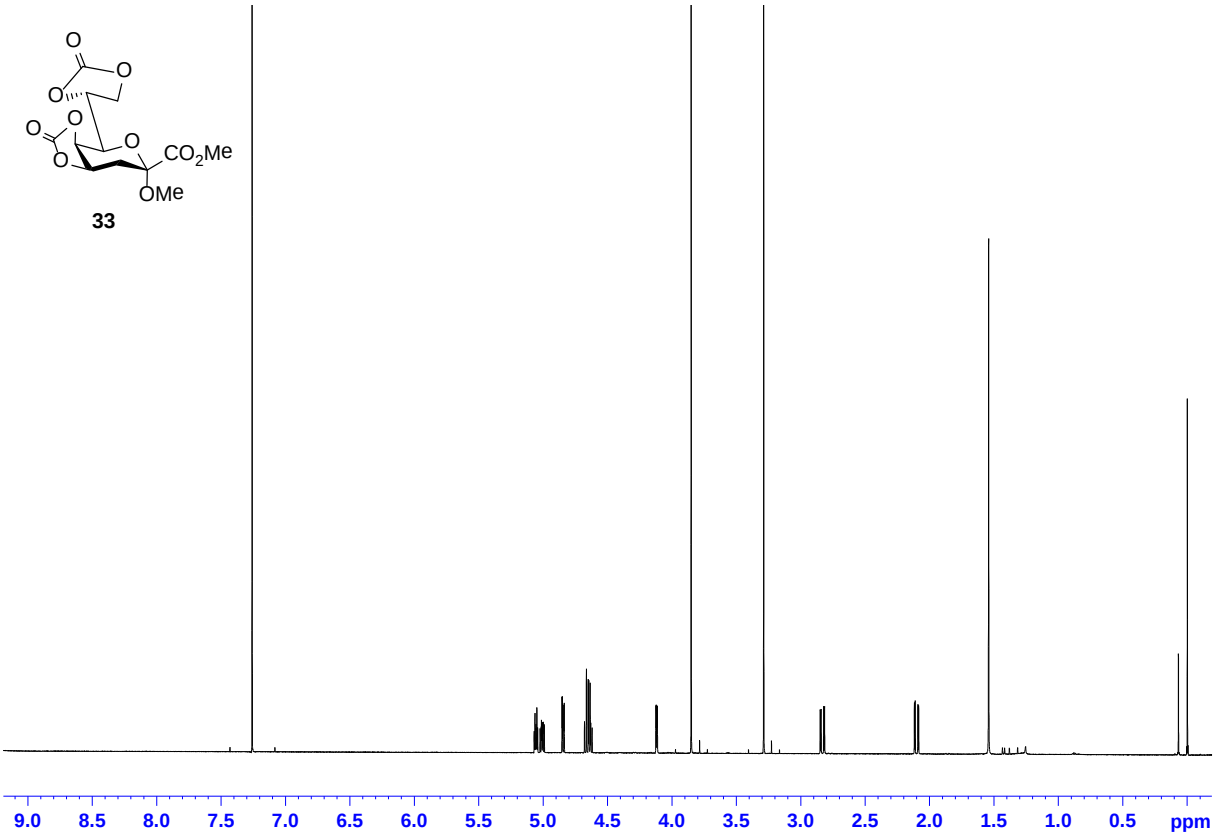

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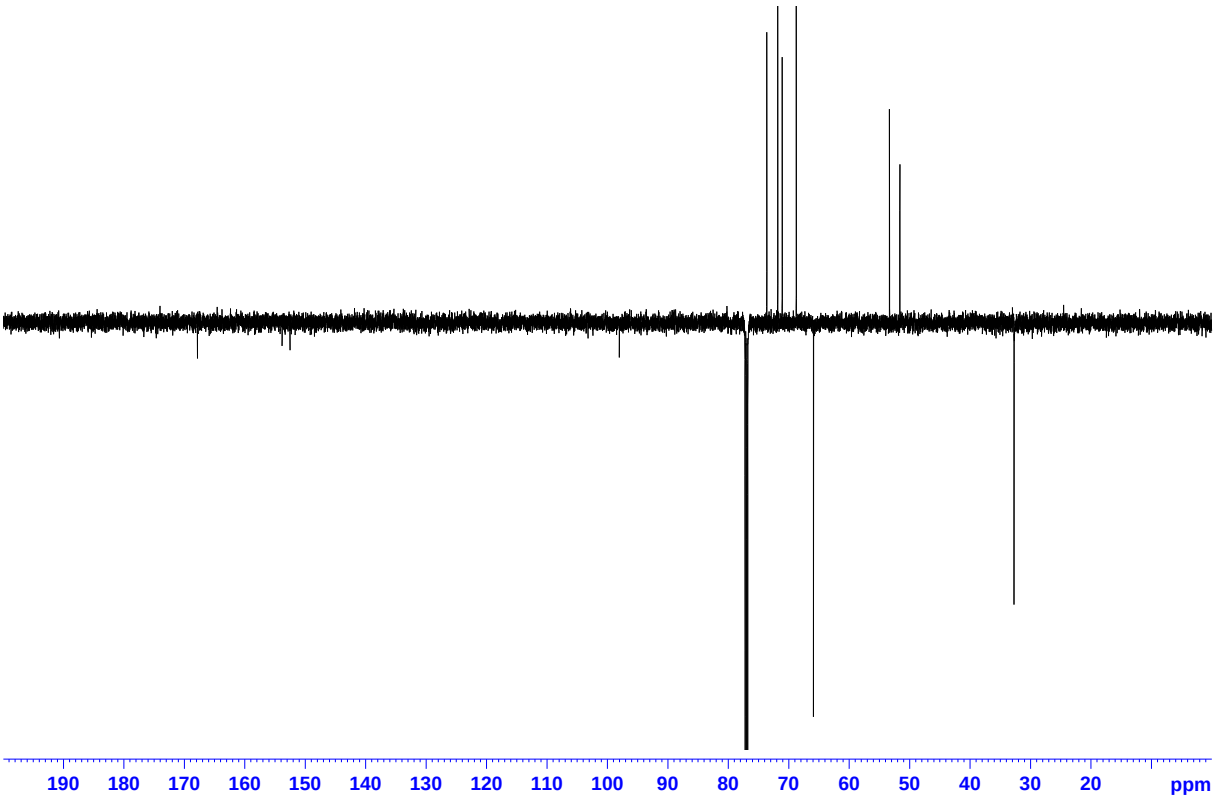

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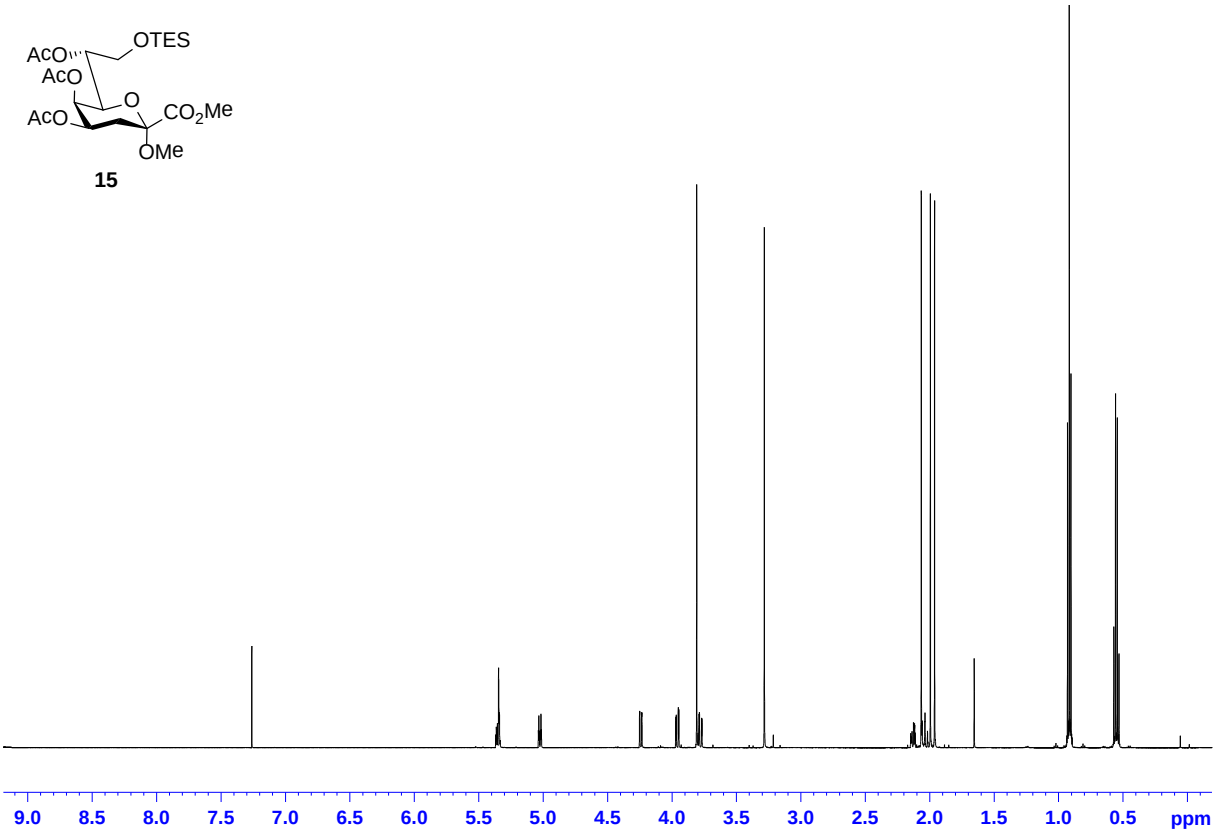

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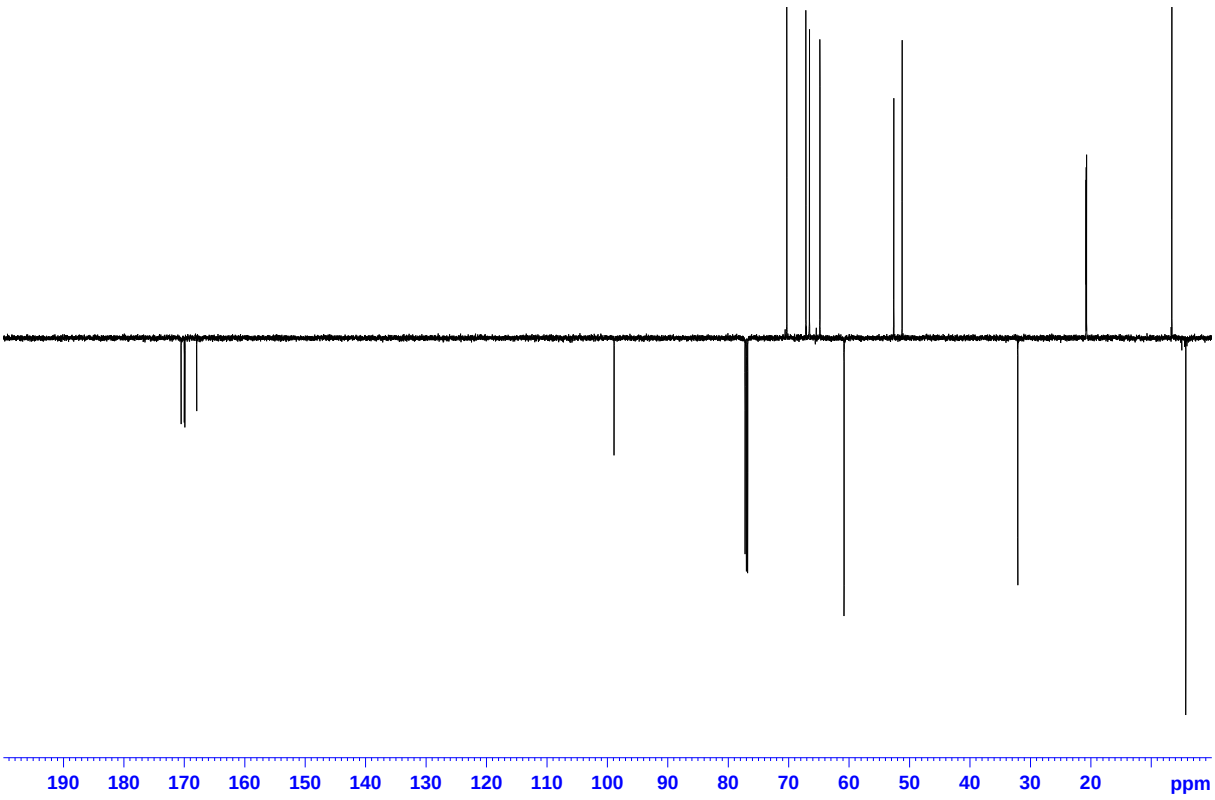

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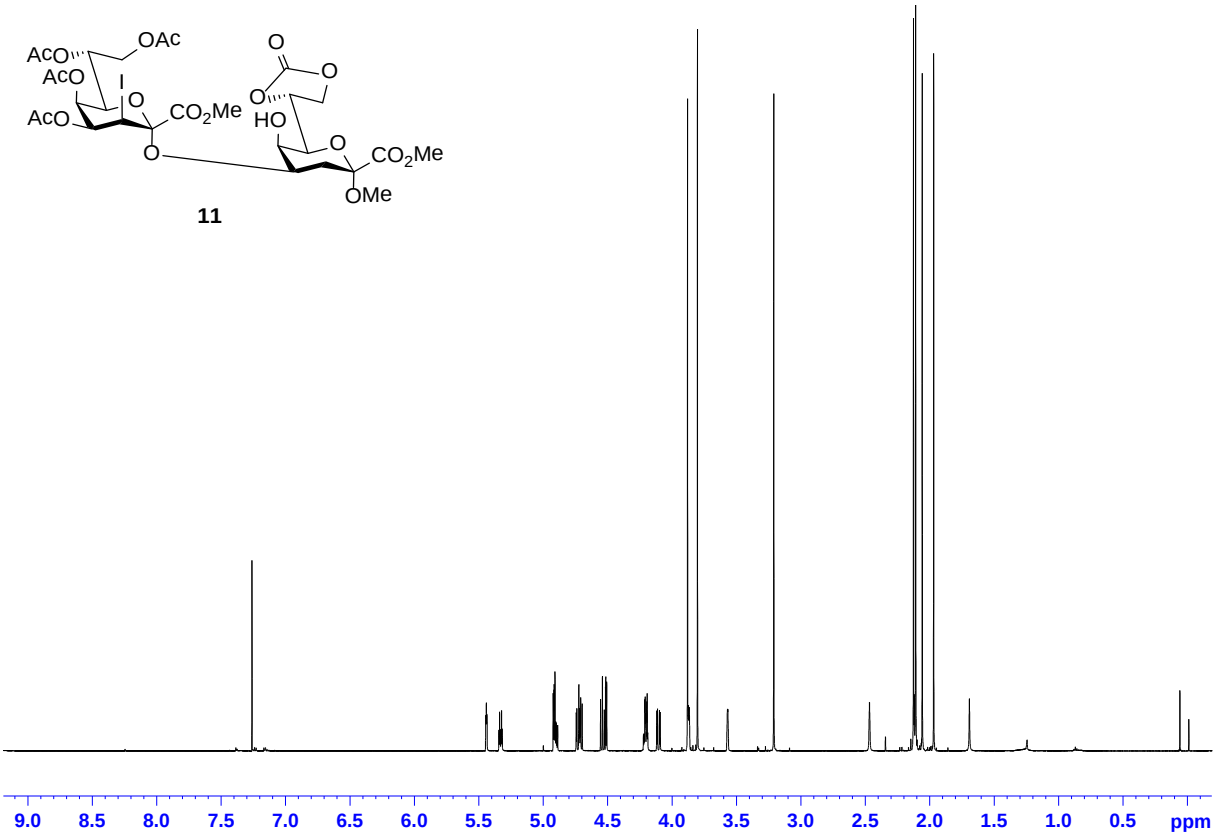

11

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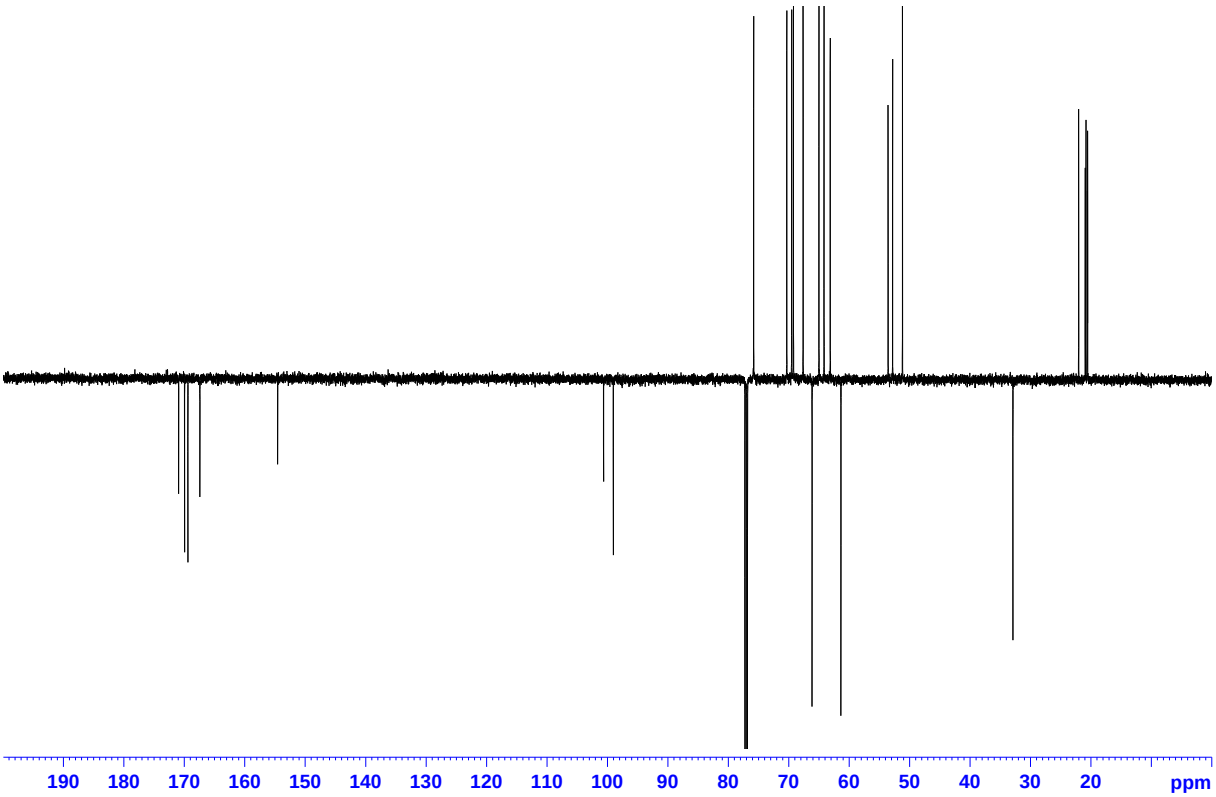

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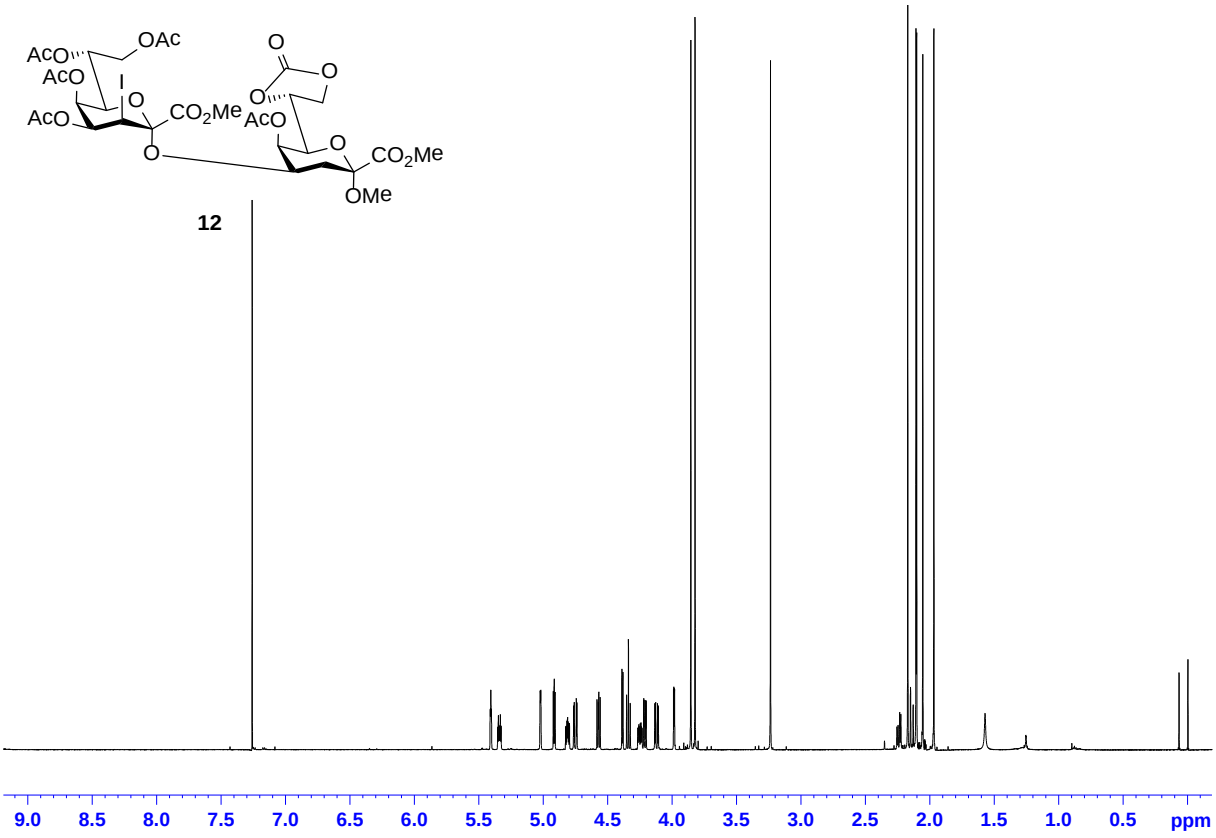

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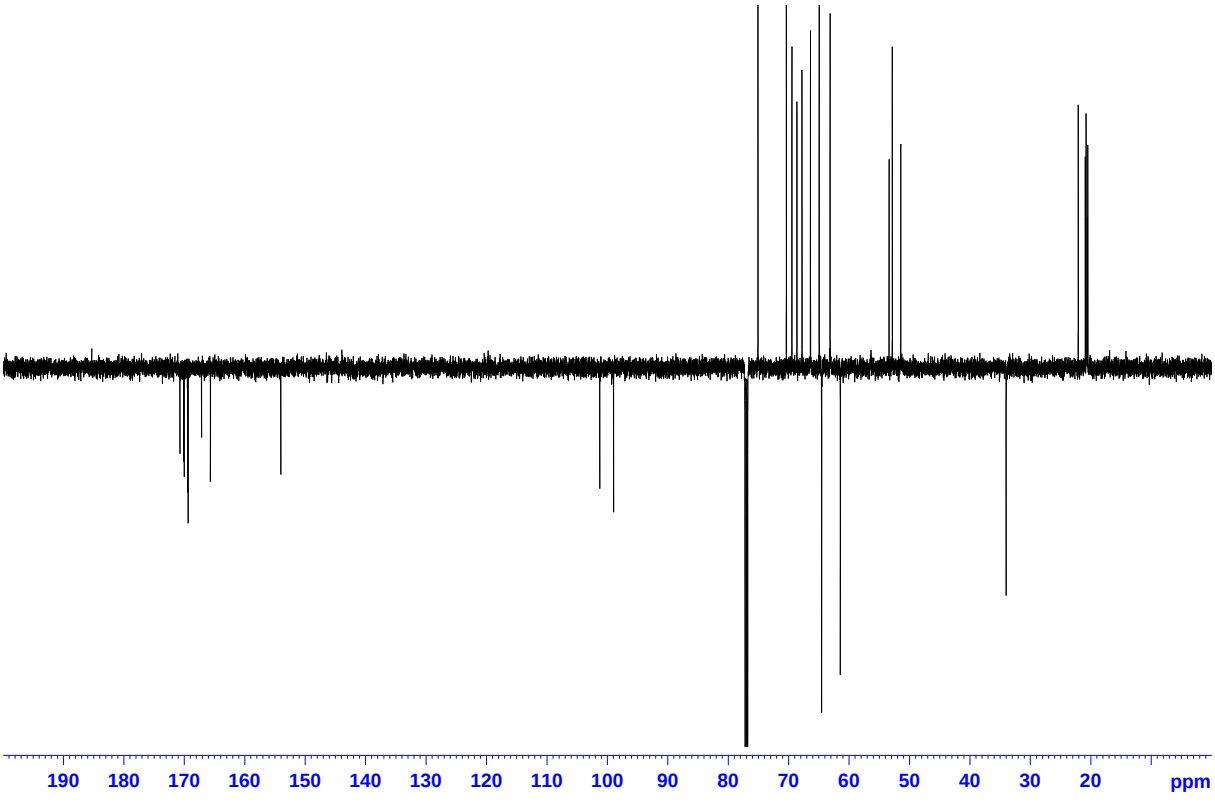

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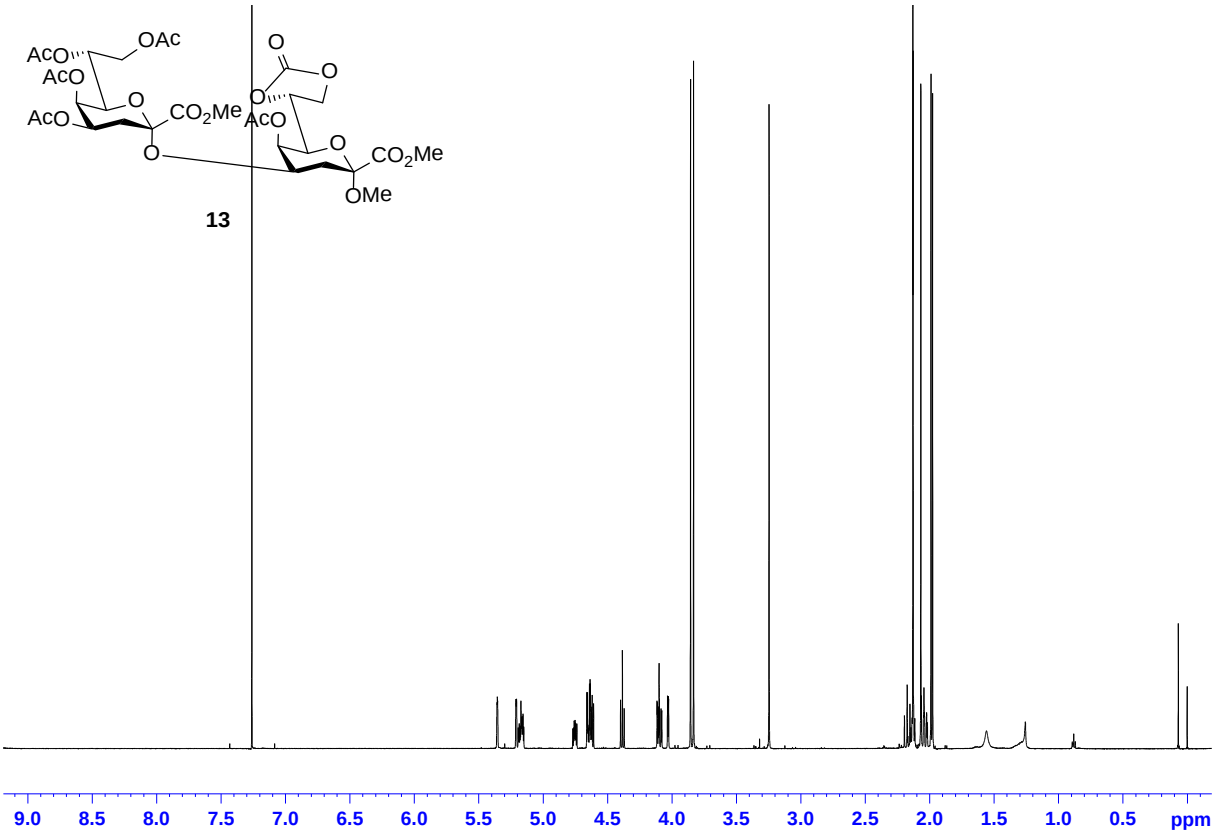

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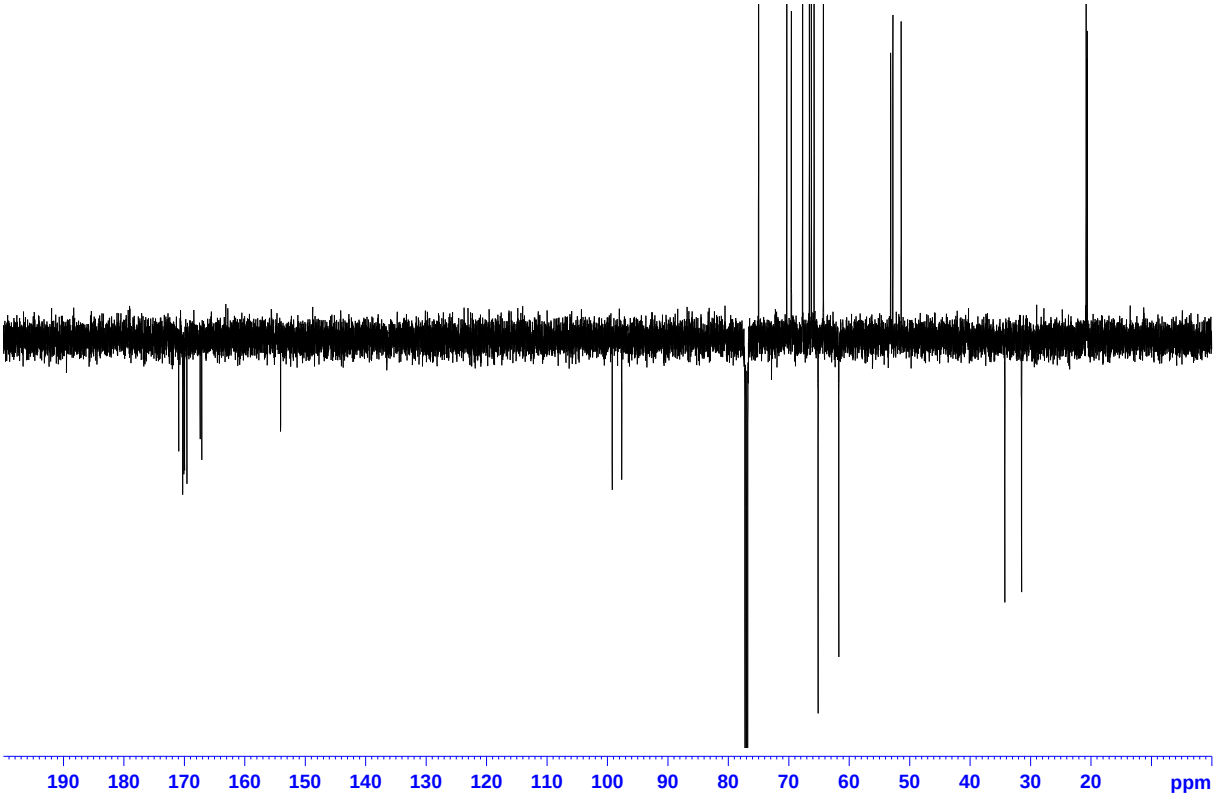

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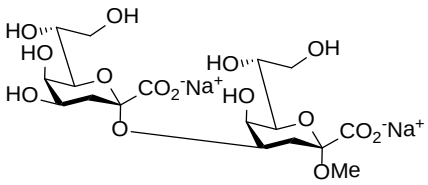

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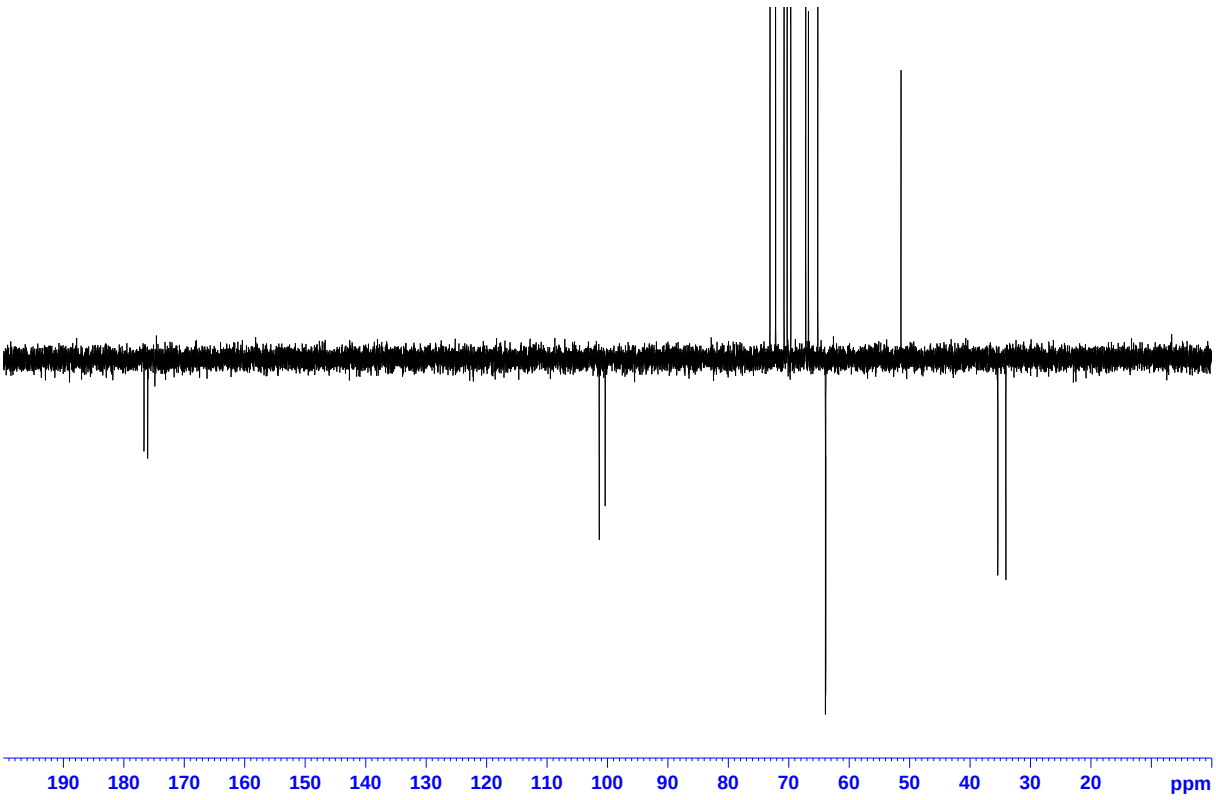

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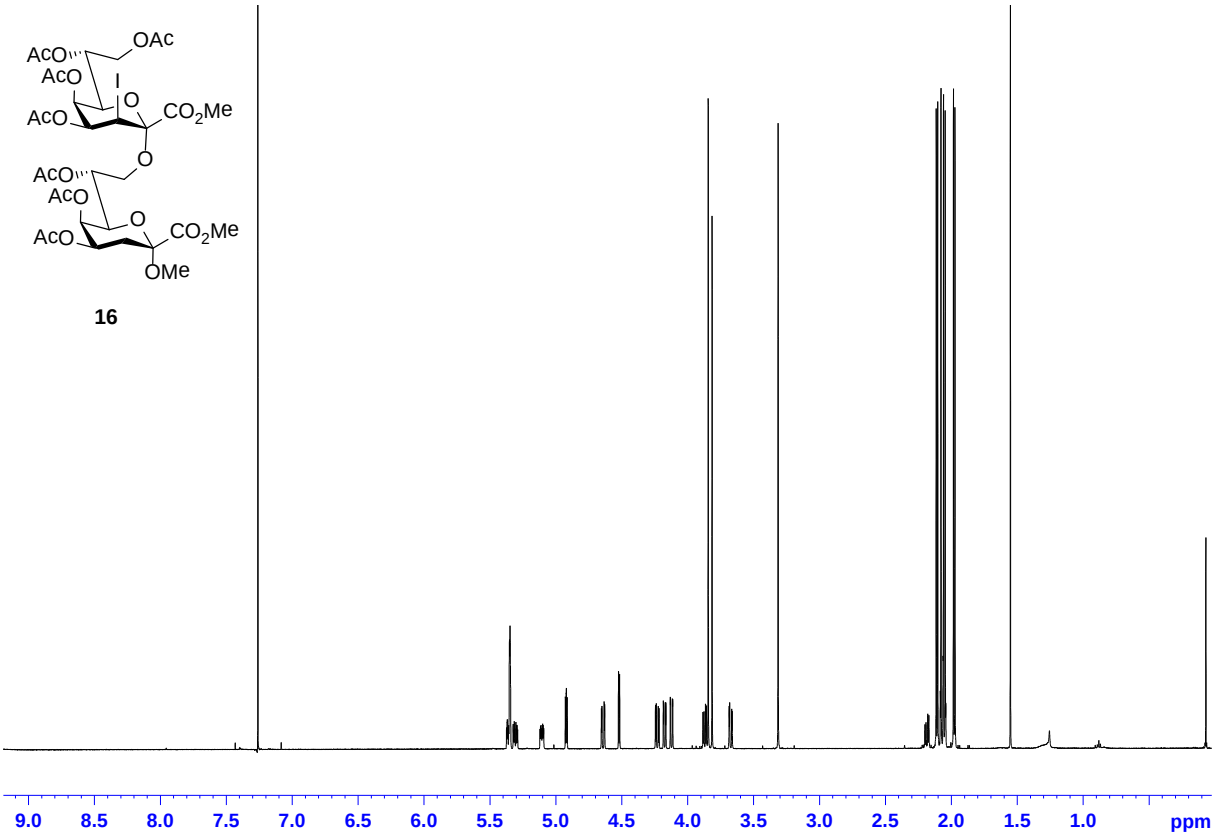

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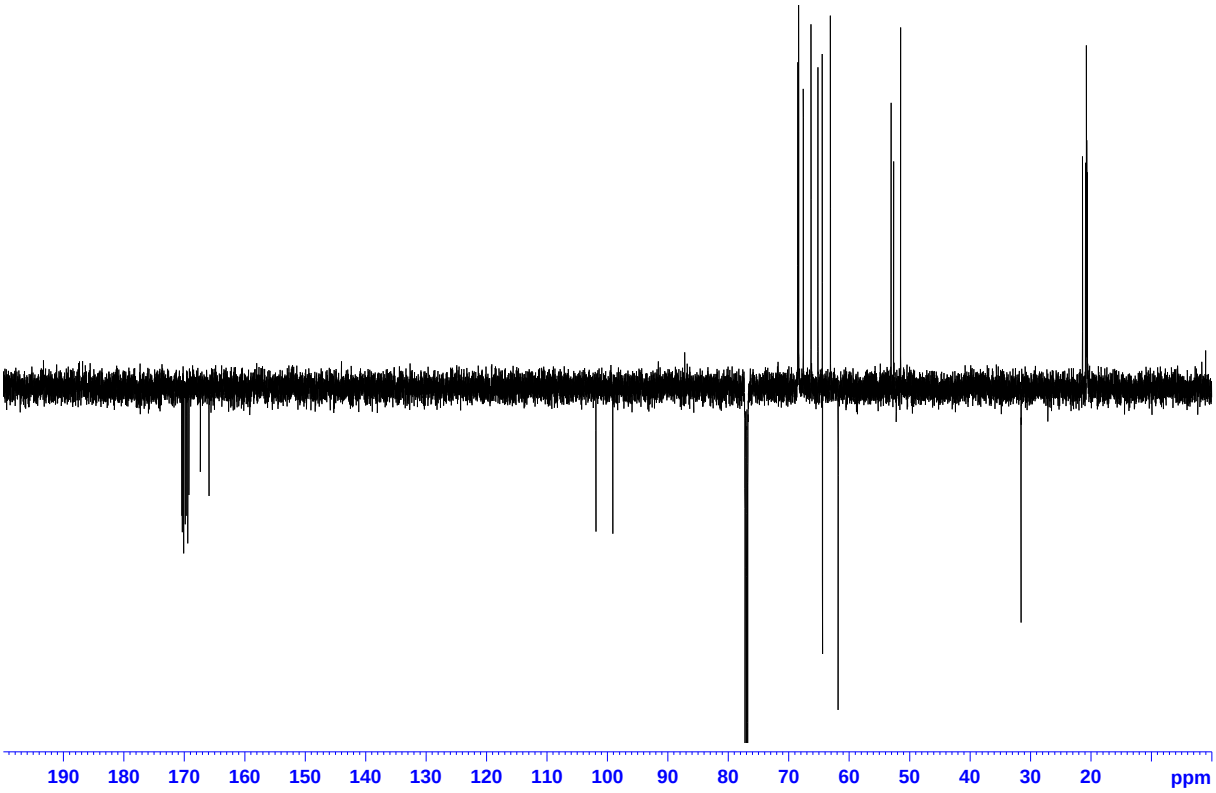

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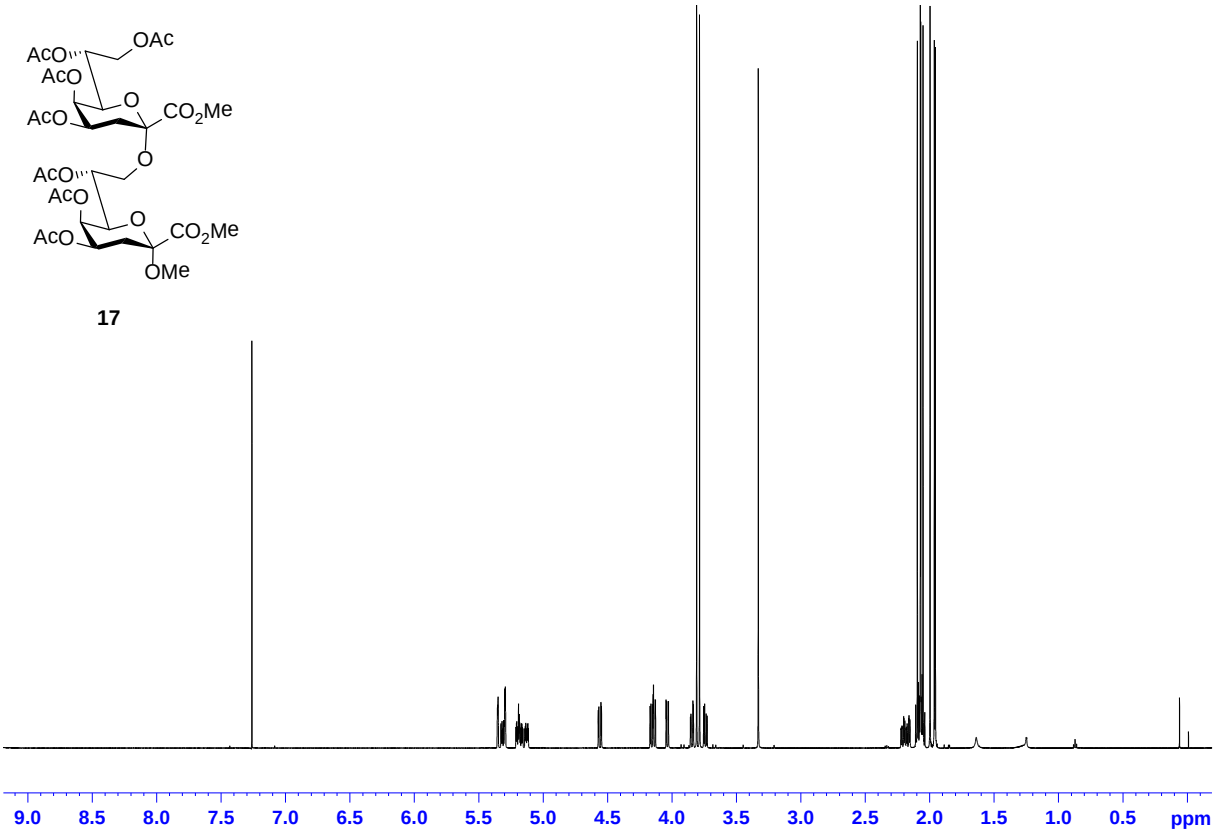

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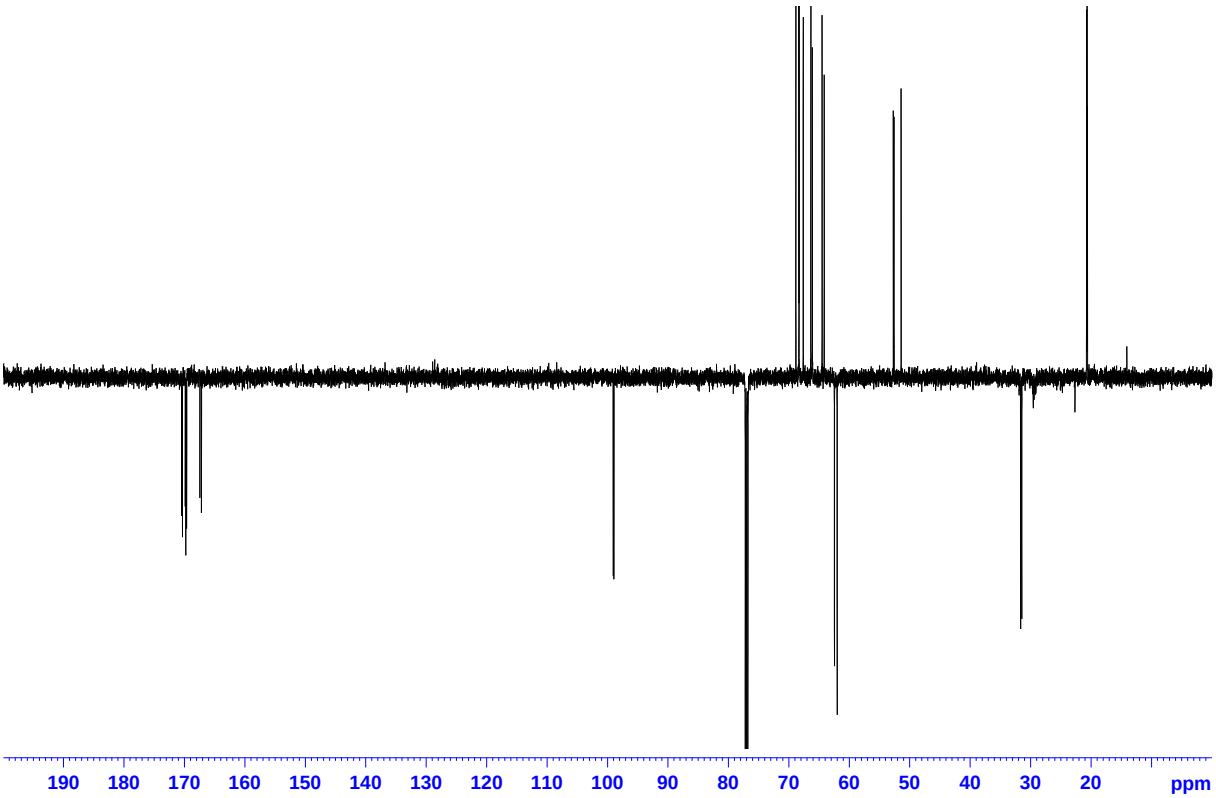

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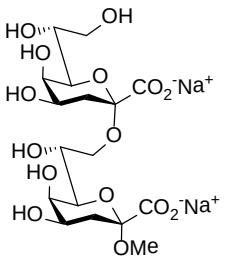

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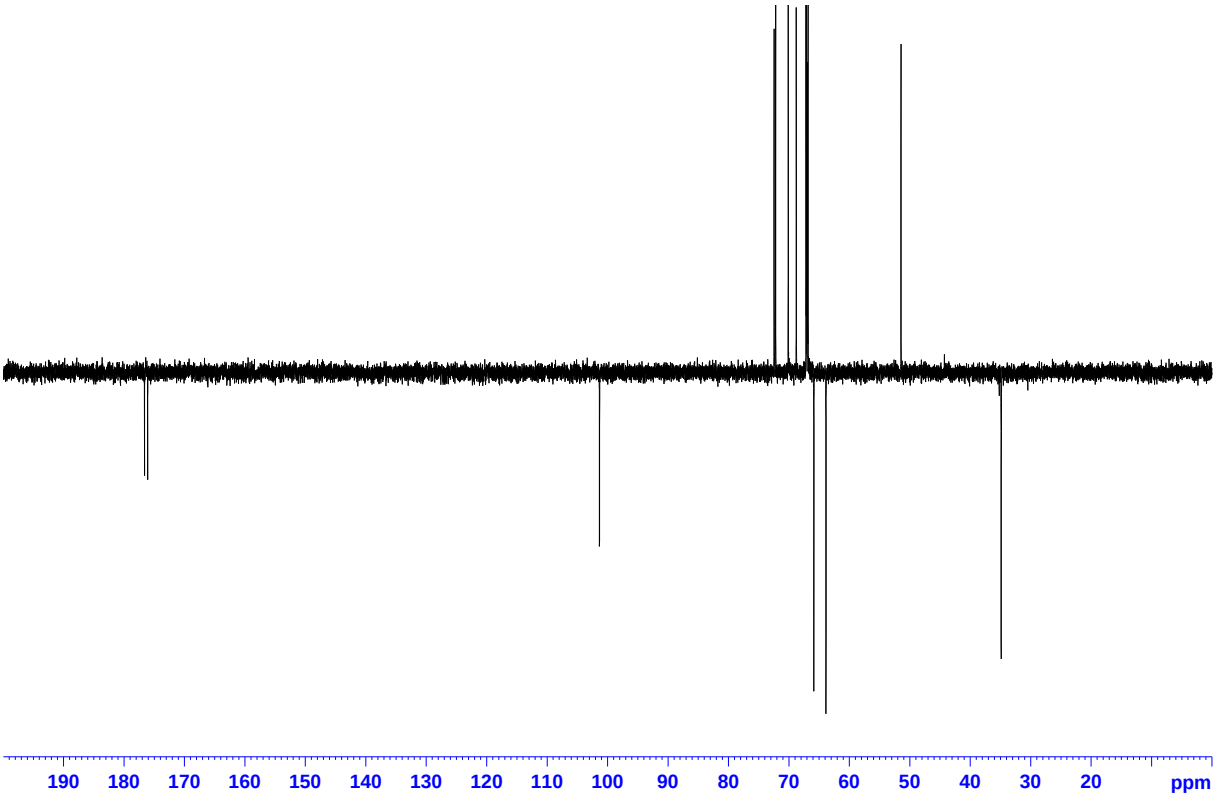

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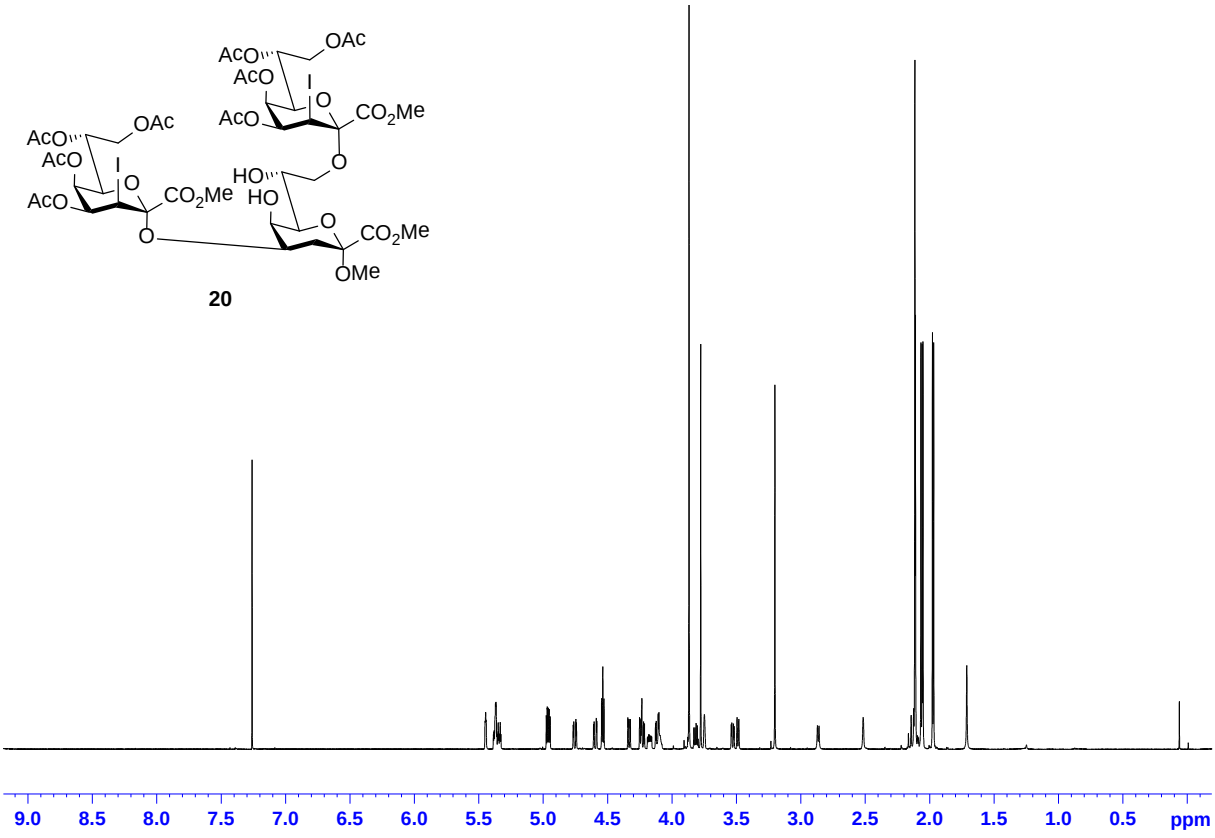

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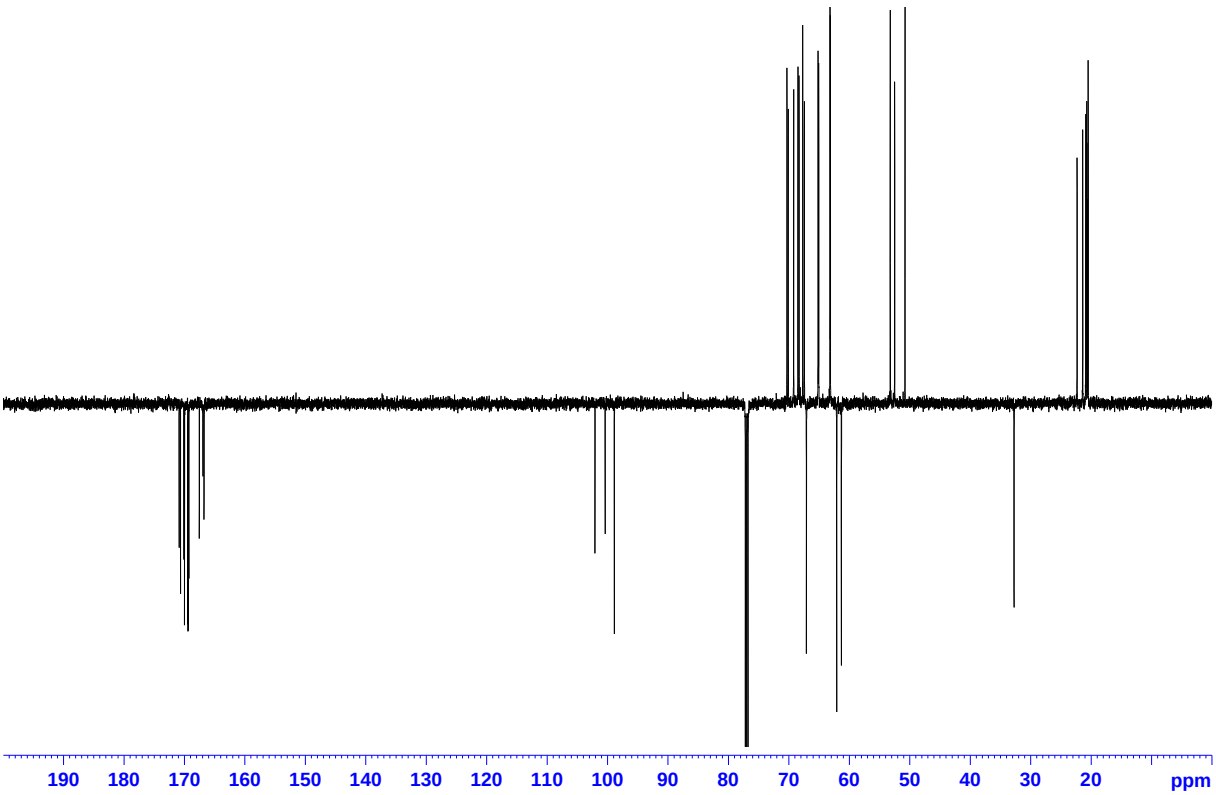

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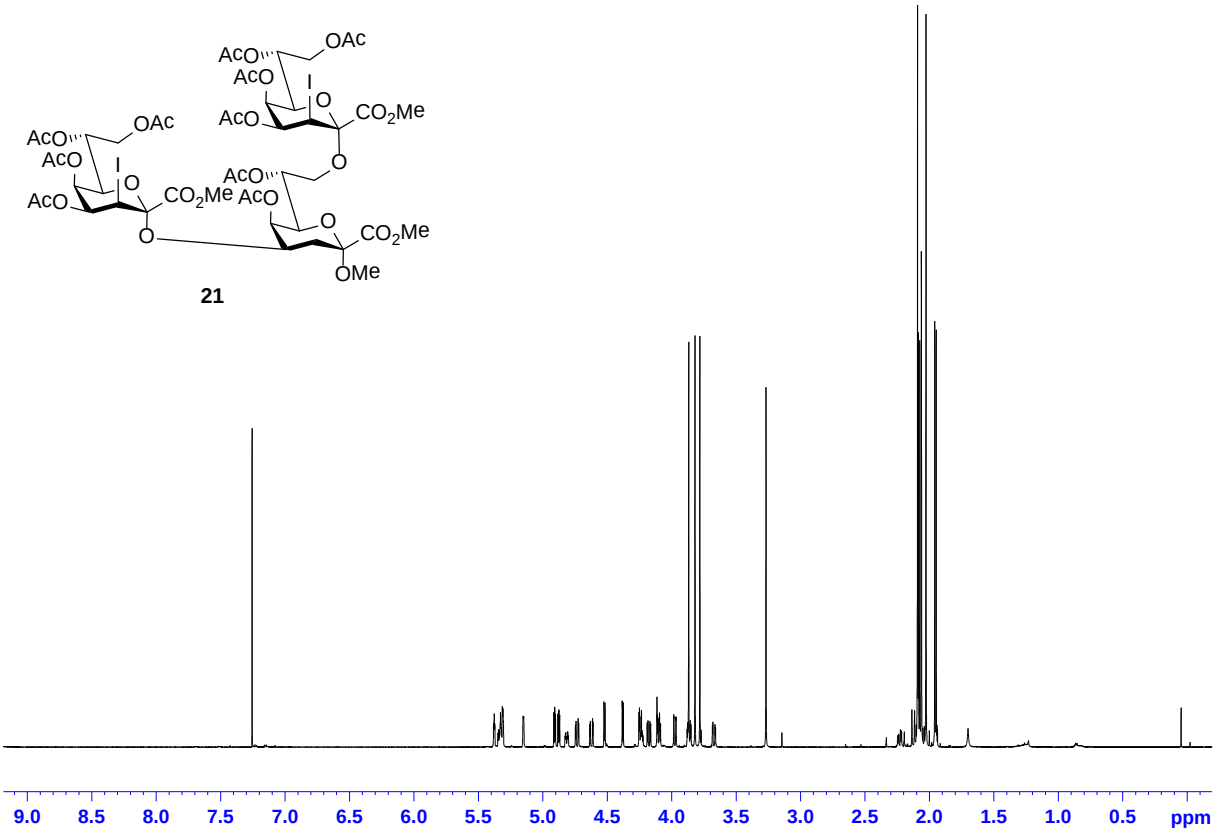

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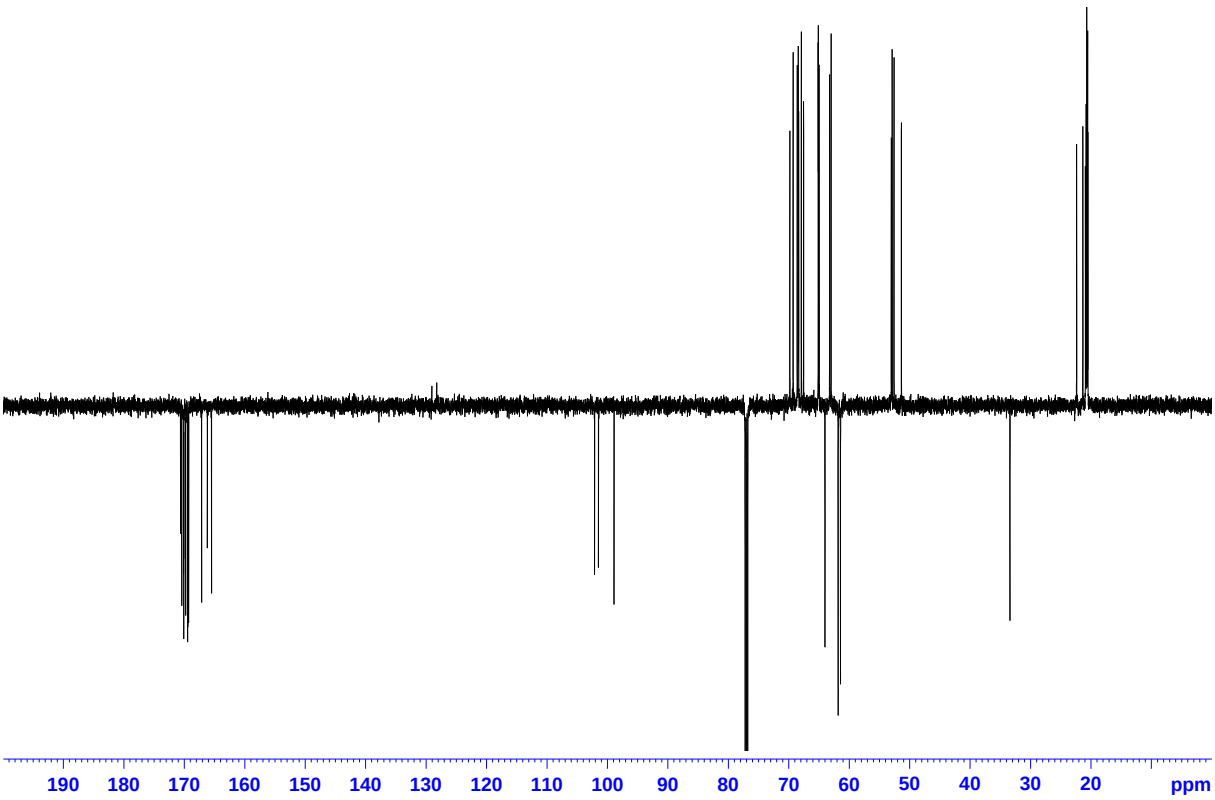

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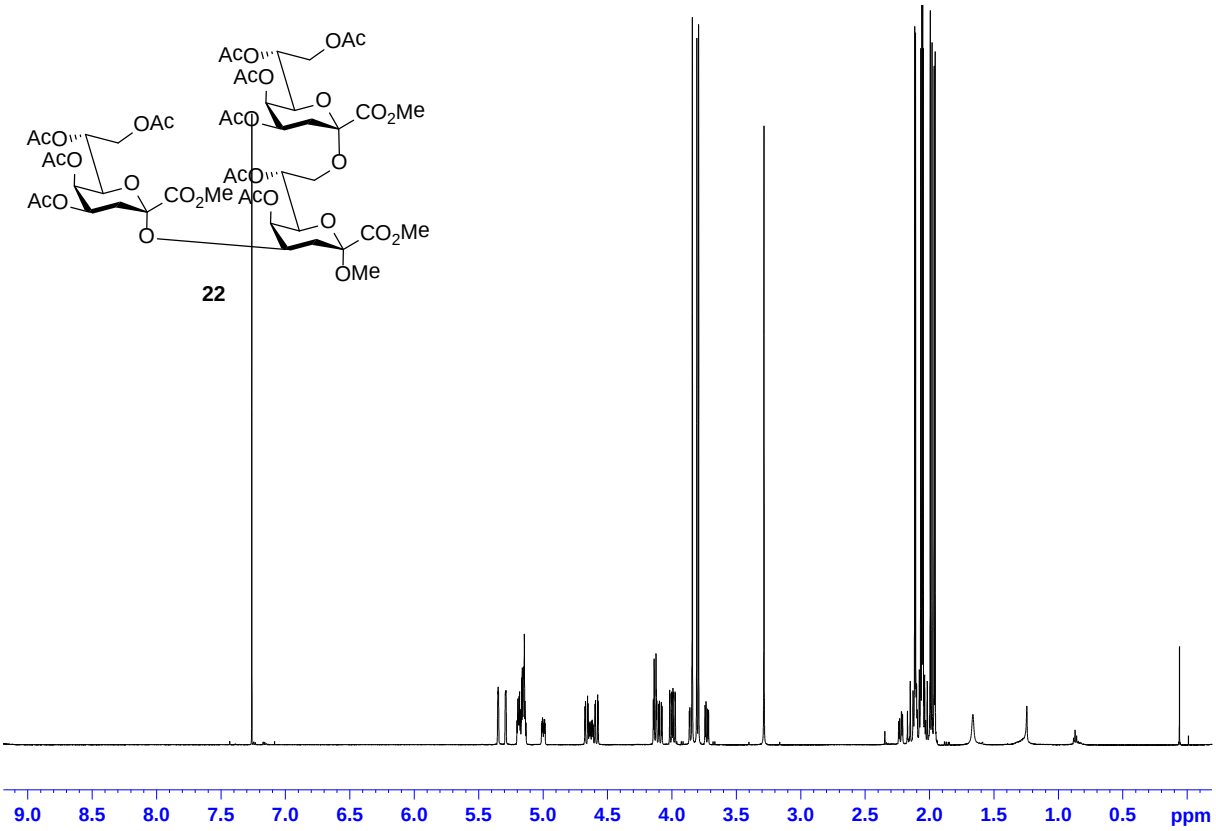

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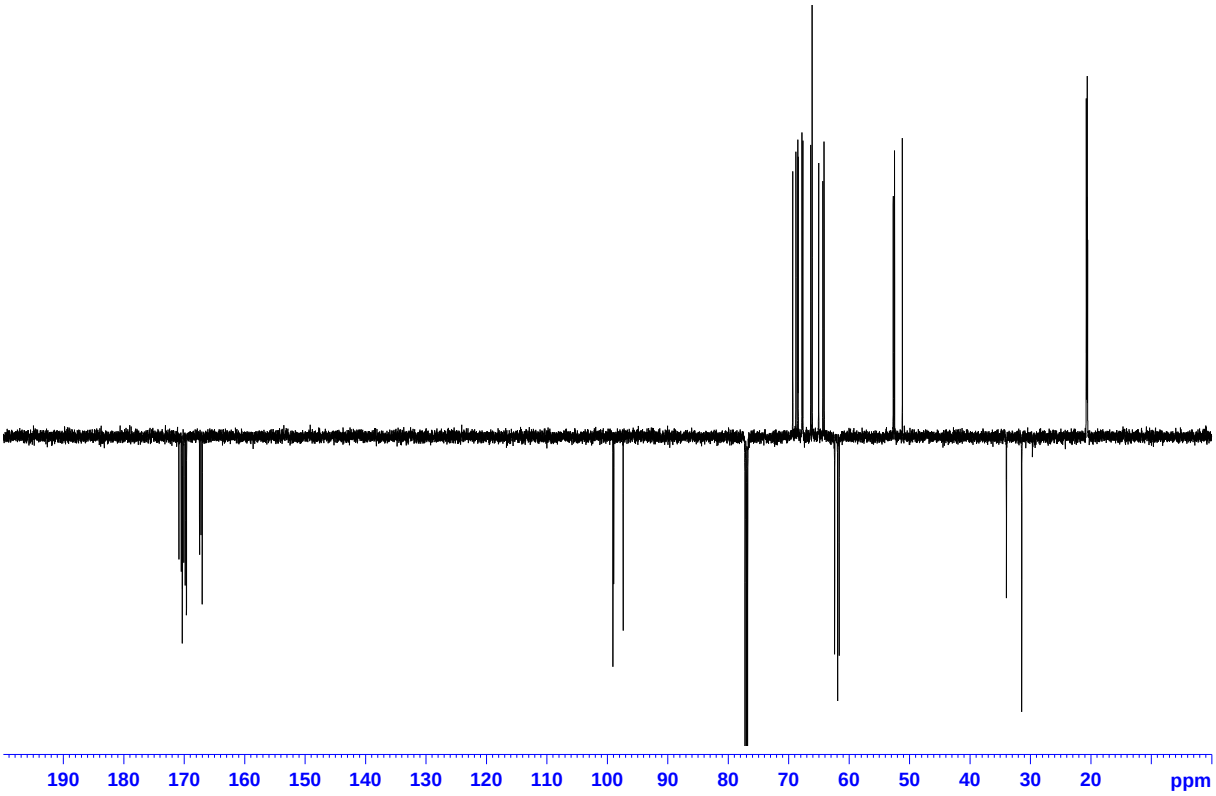

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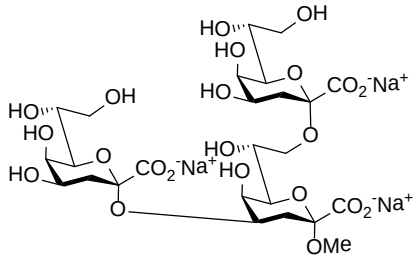

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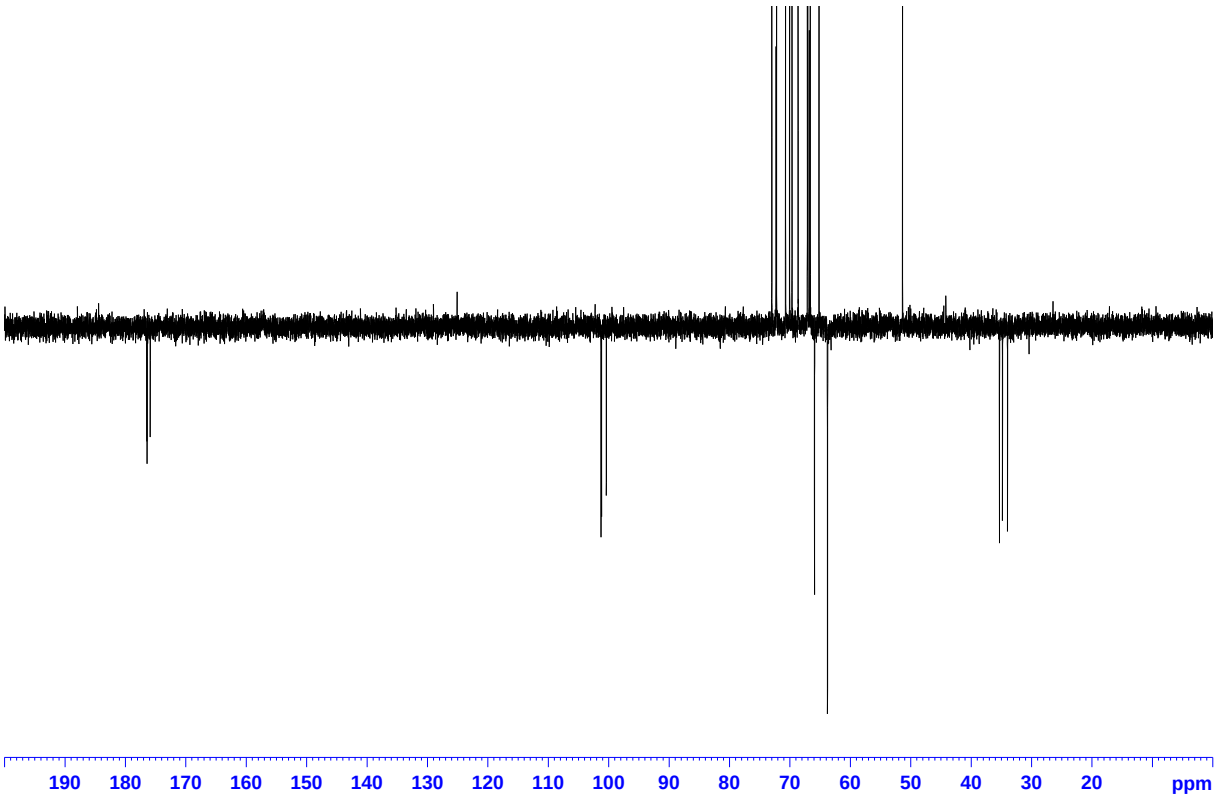

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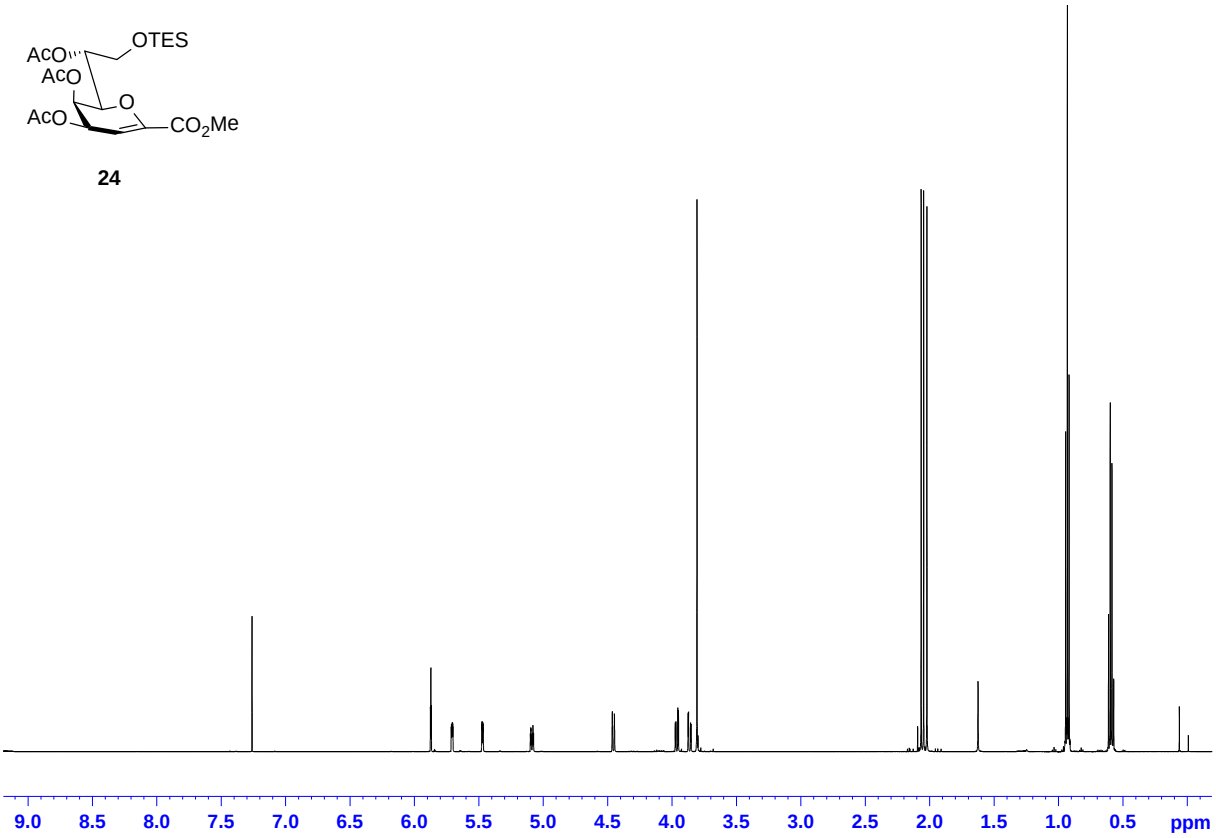

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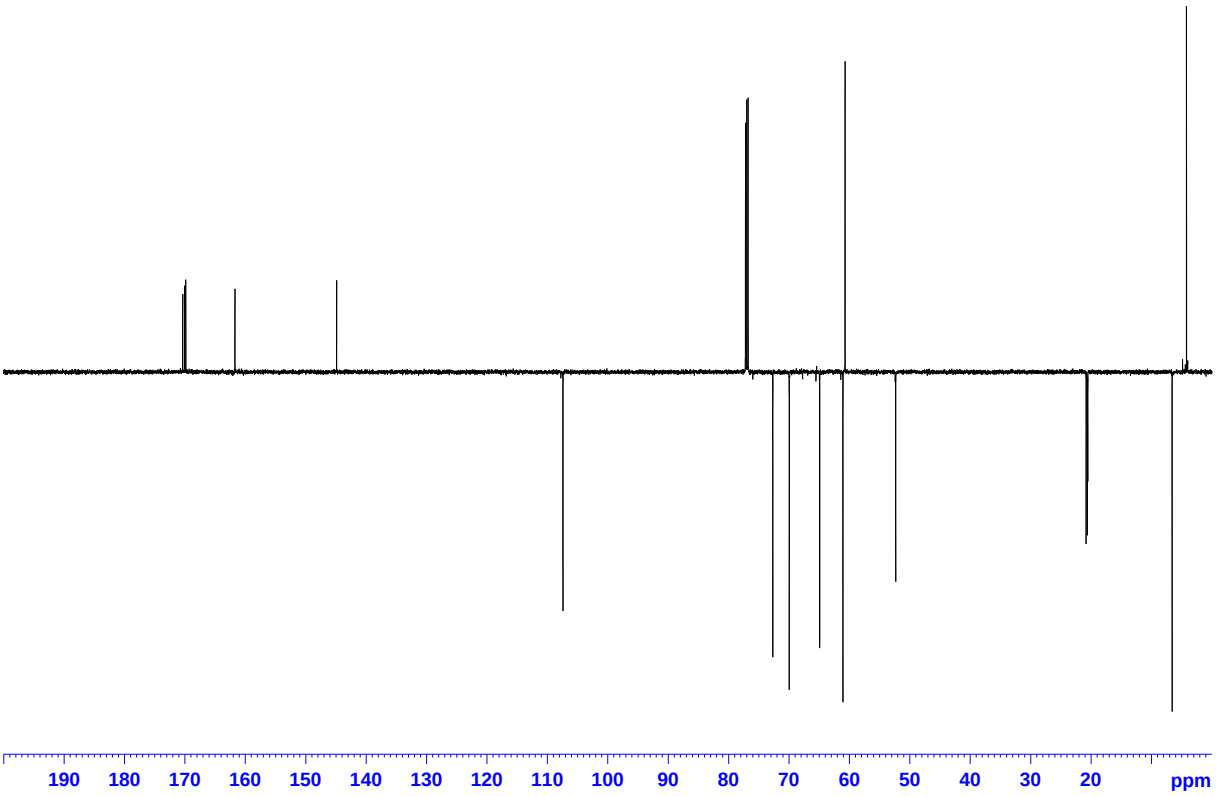

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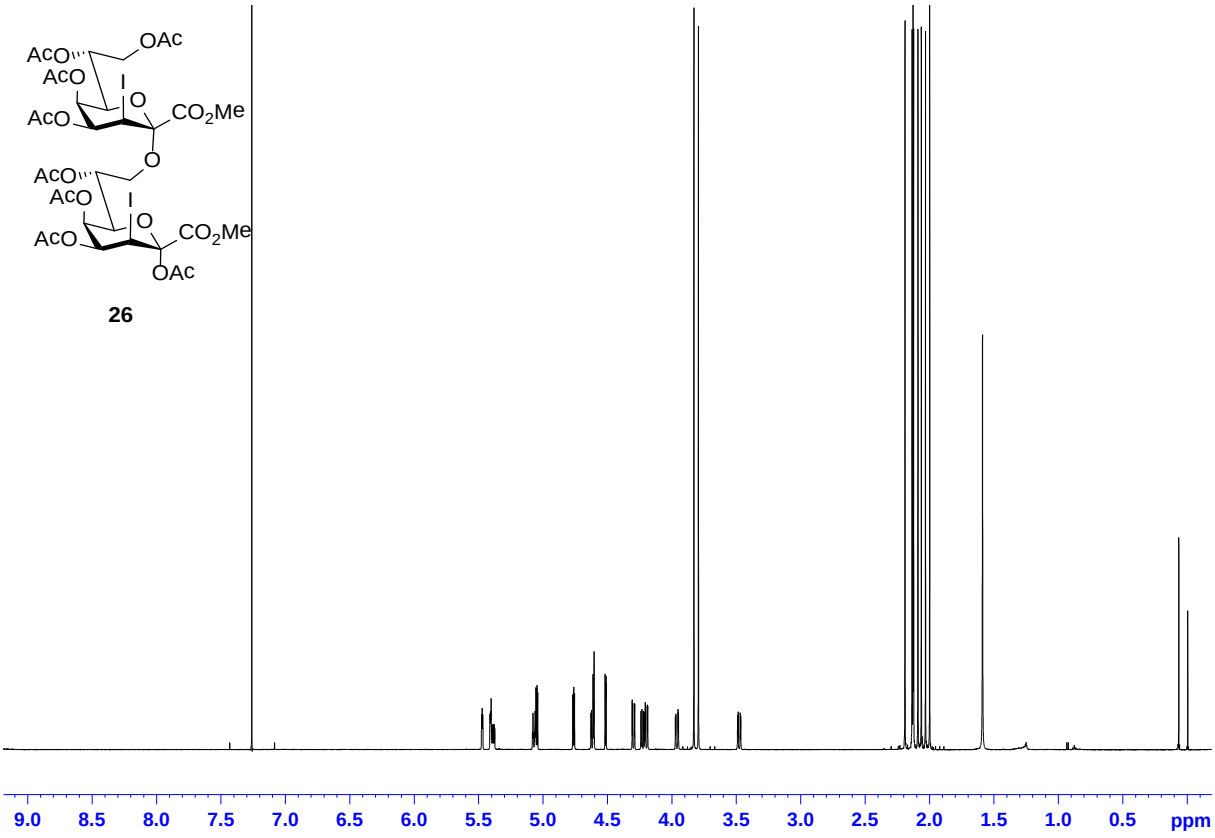

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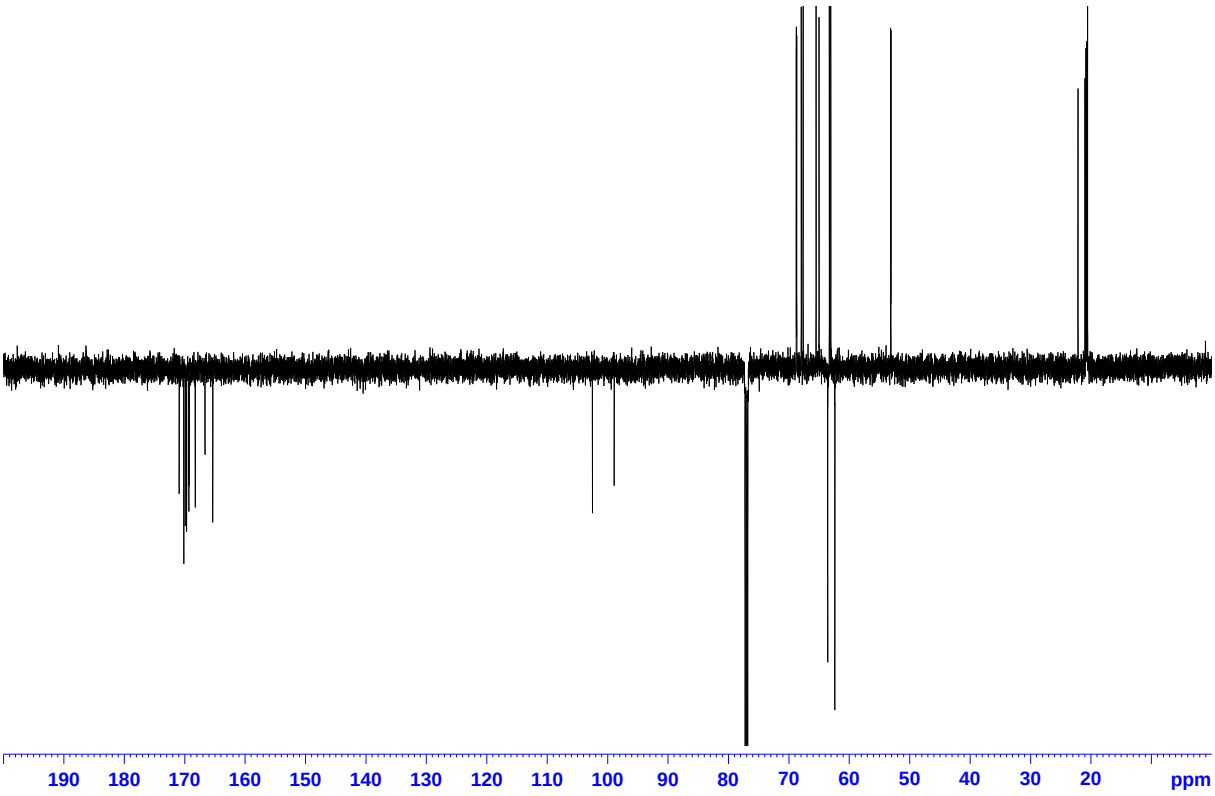

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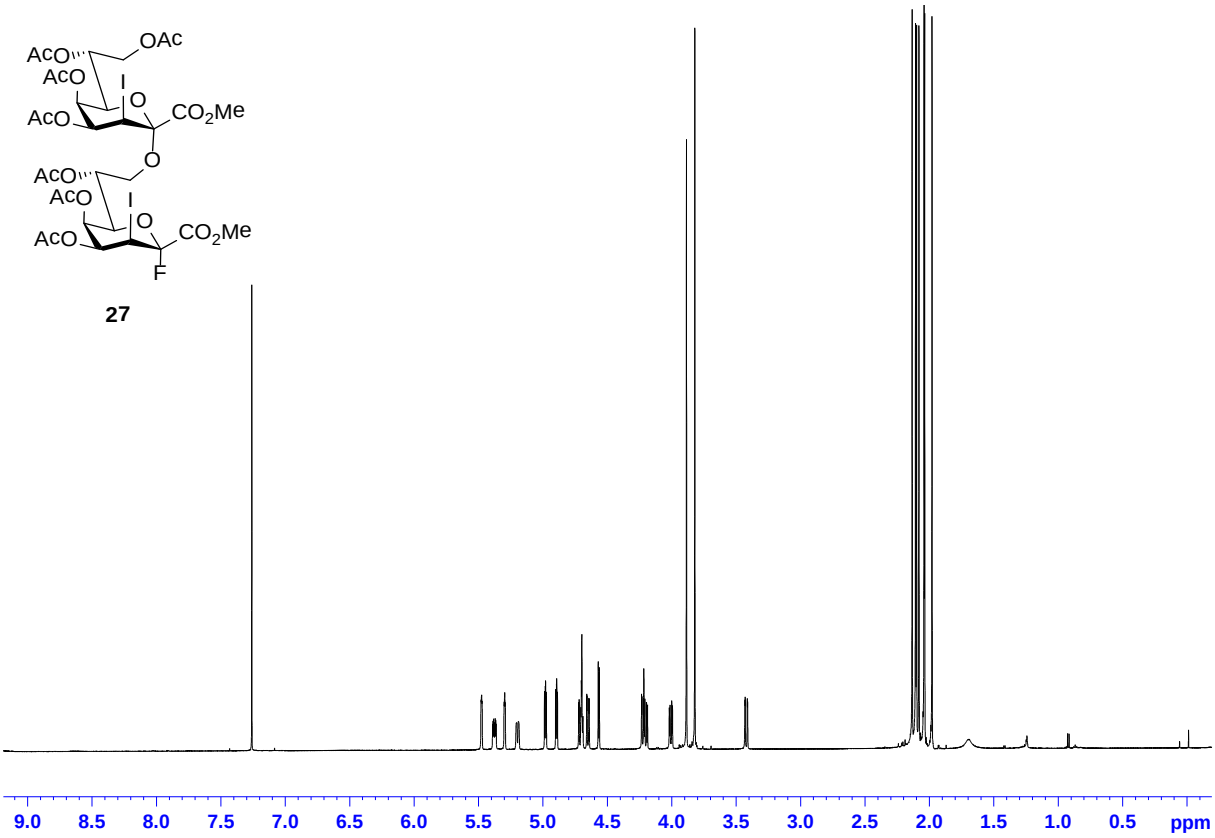

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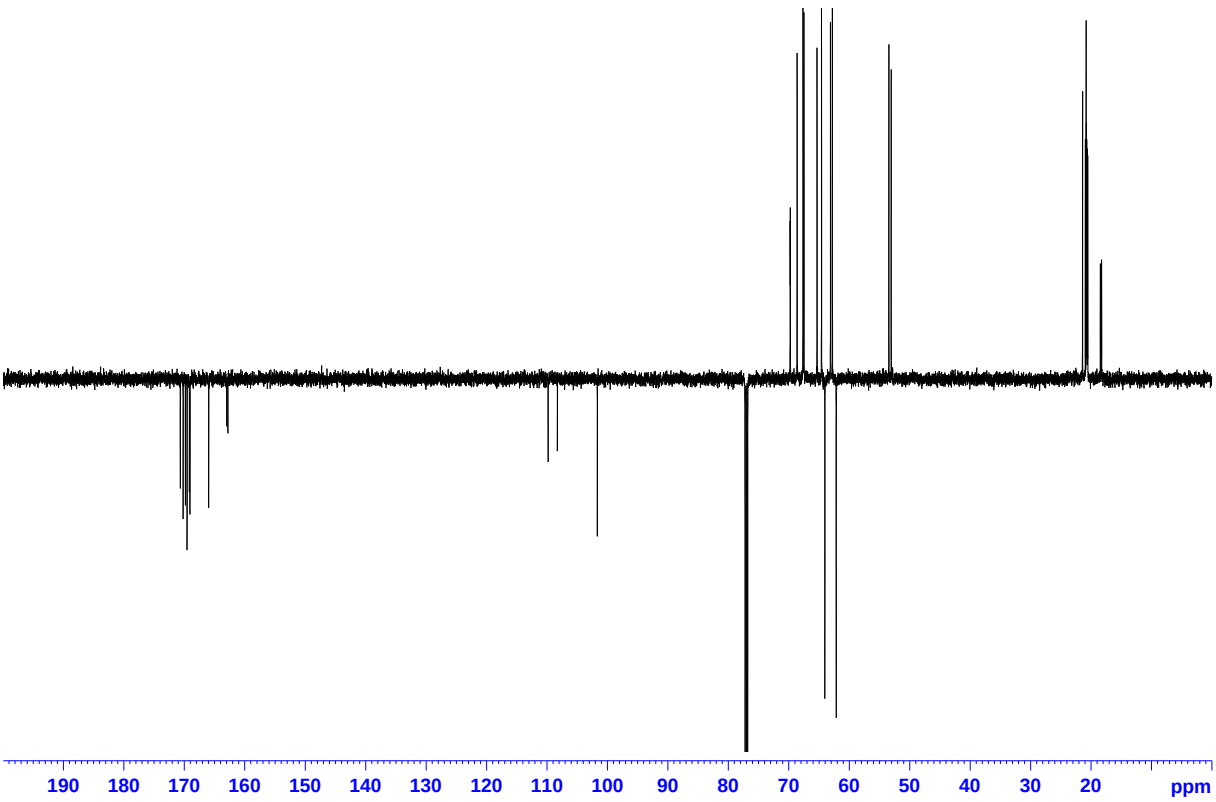

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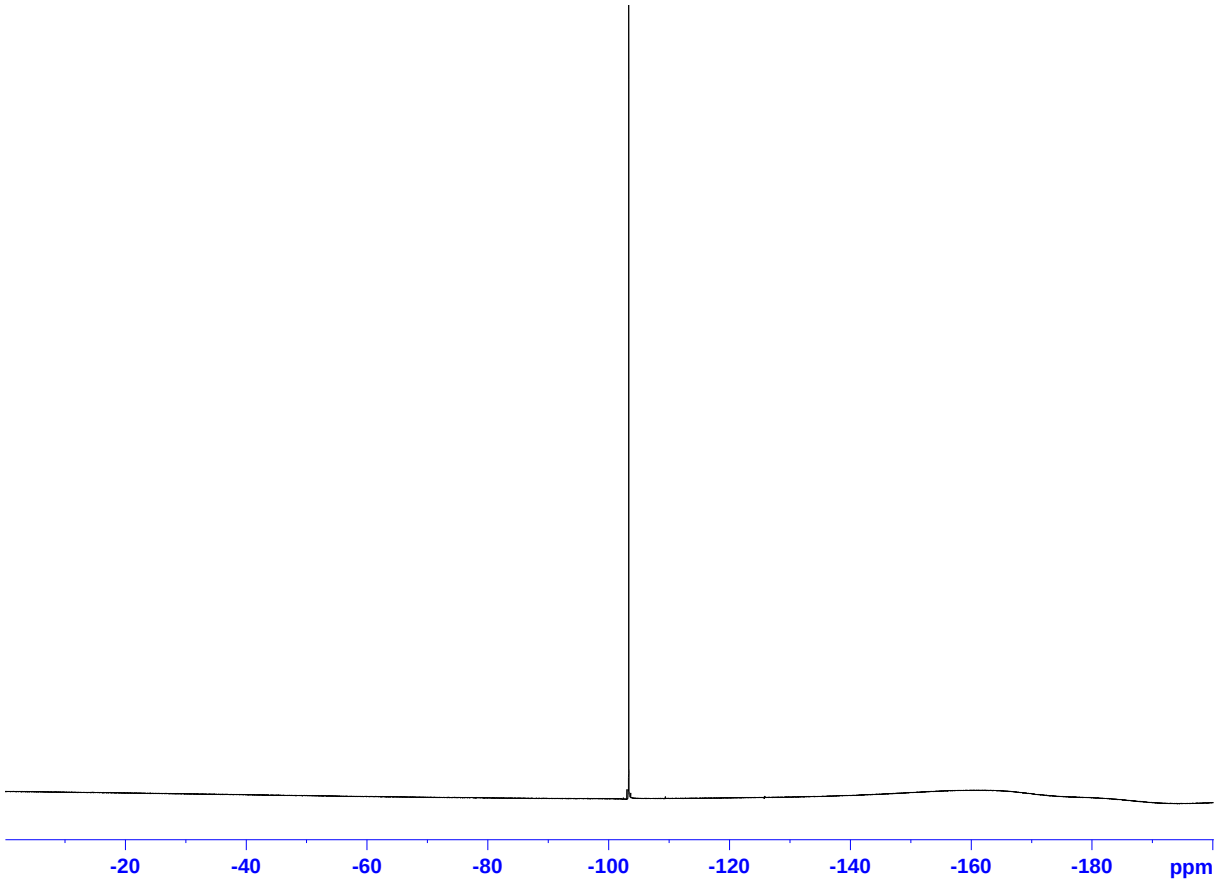
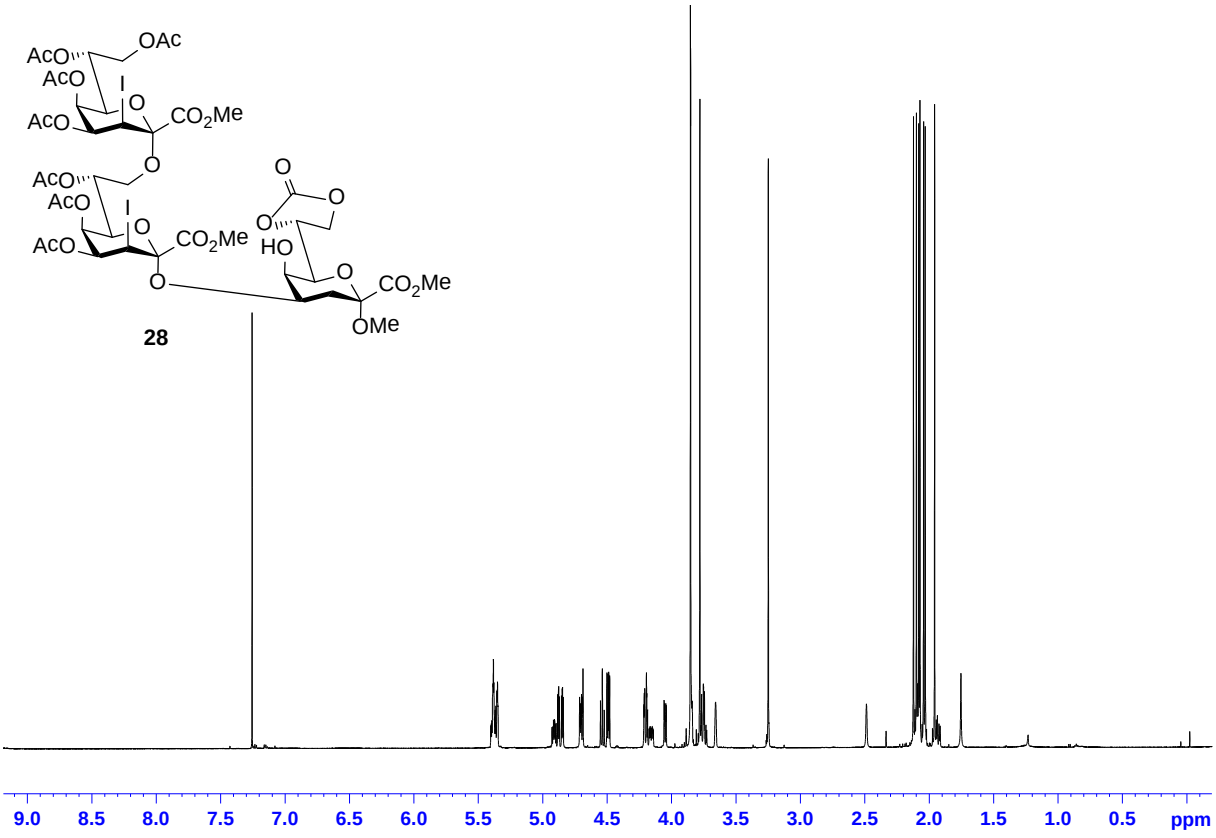


^{19}F-NMR (565 MHz, $CDCl_3$)



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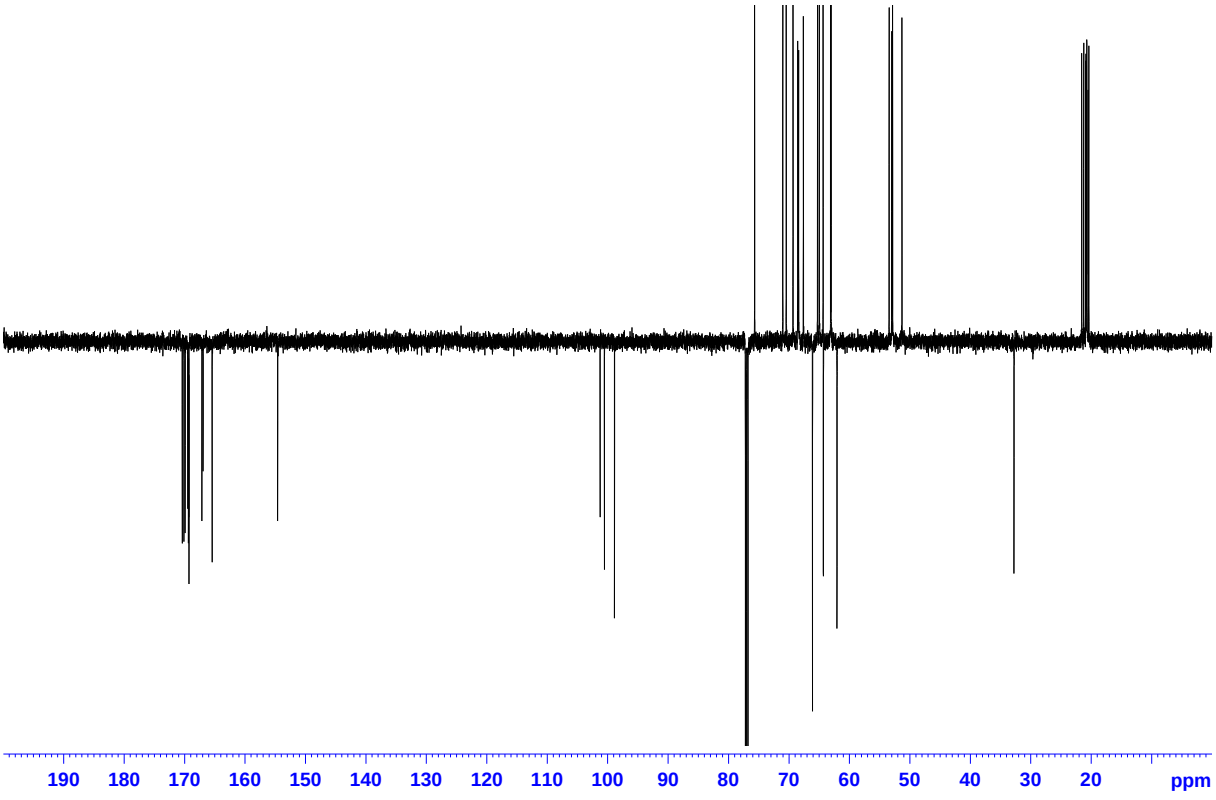

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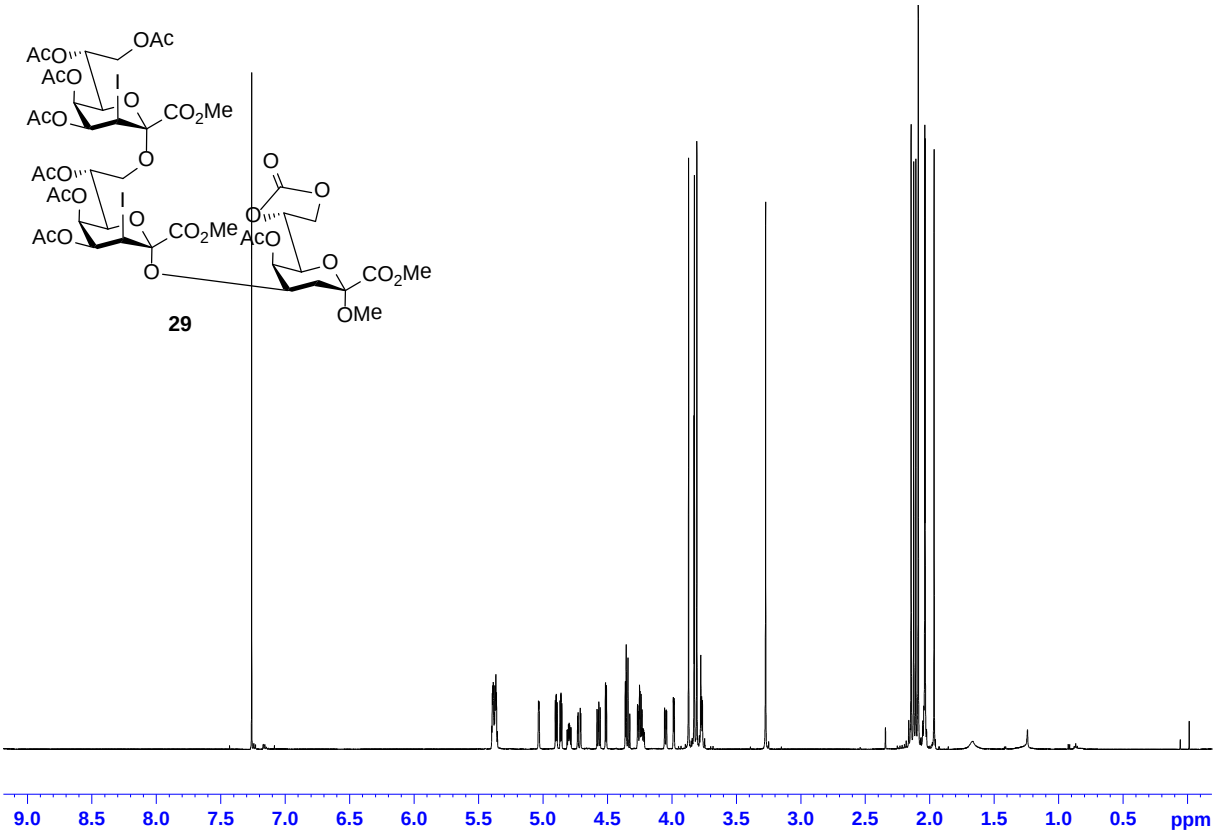

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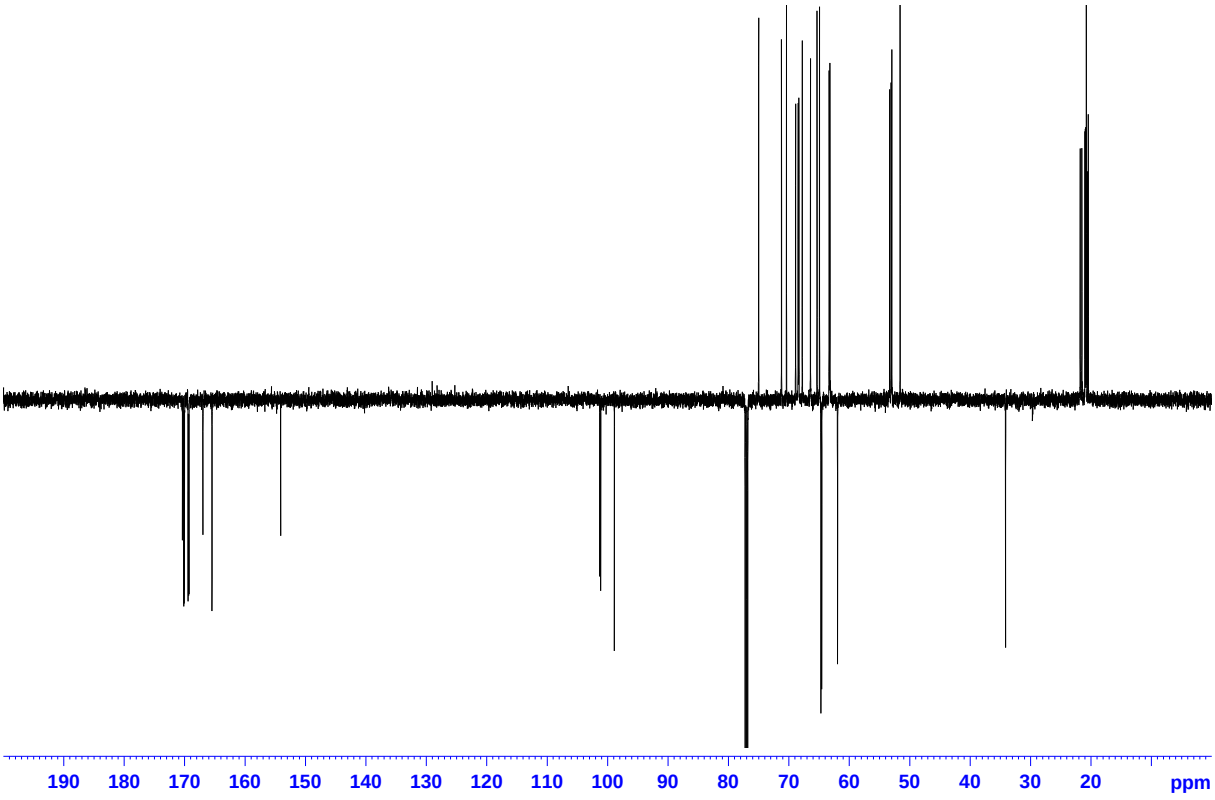

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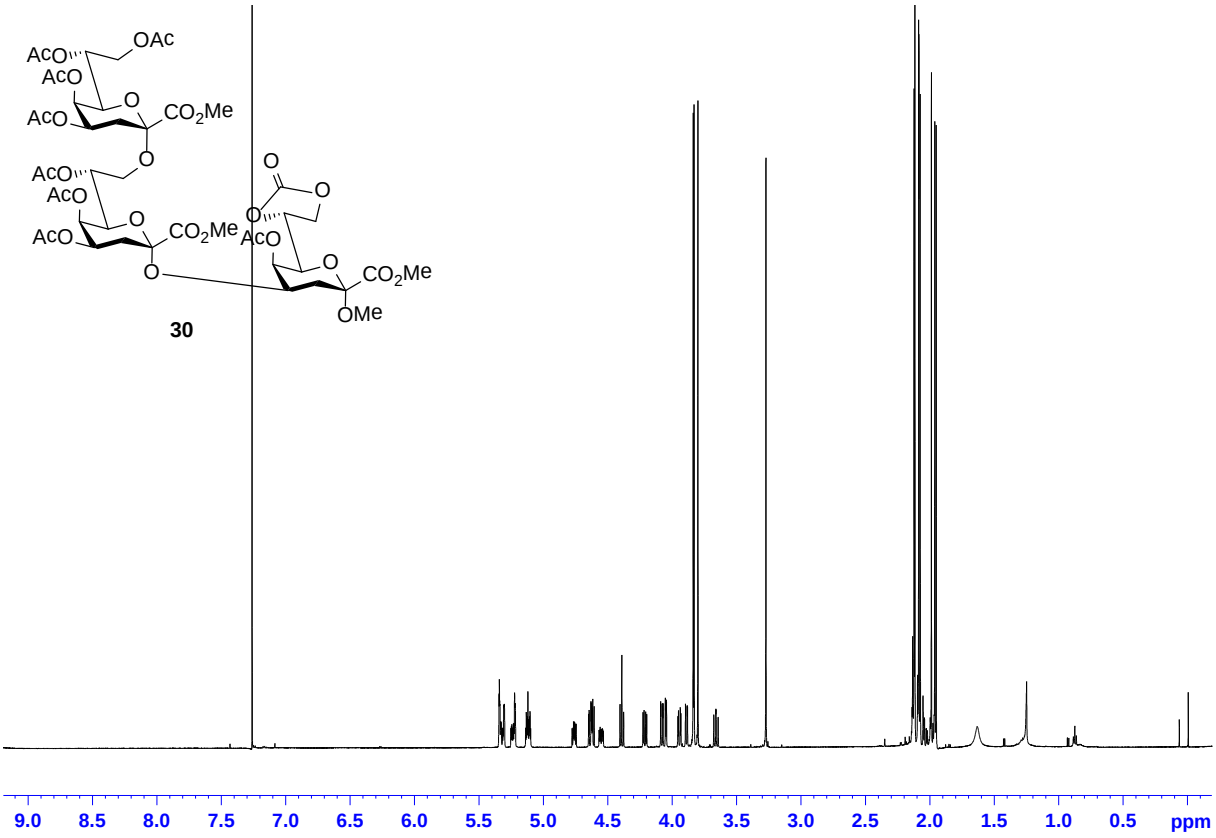

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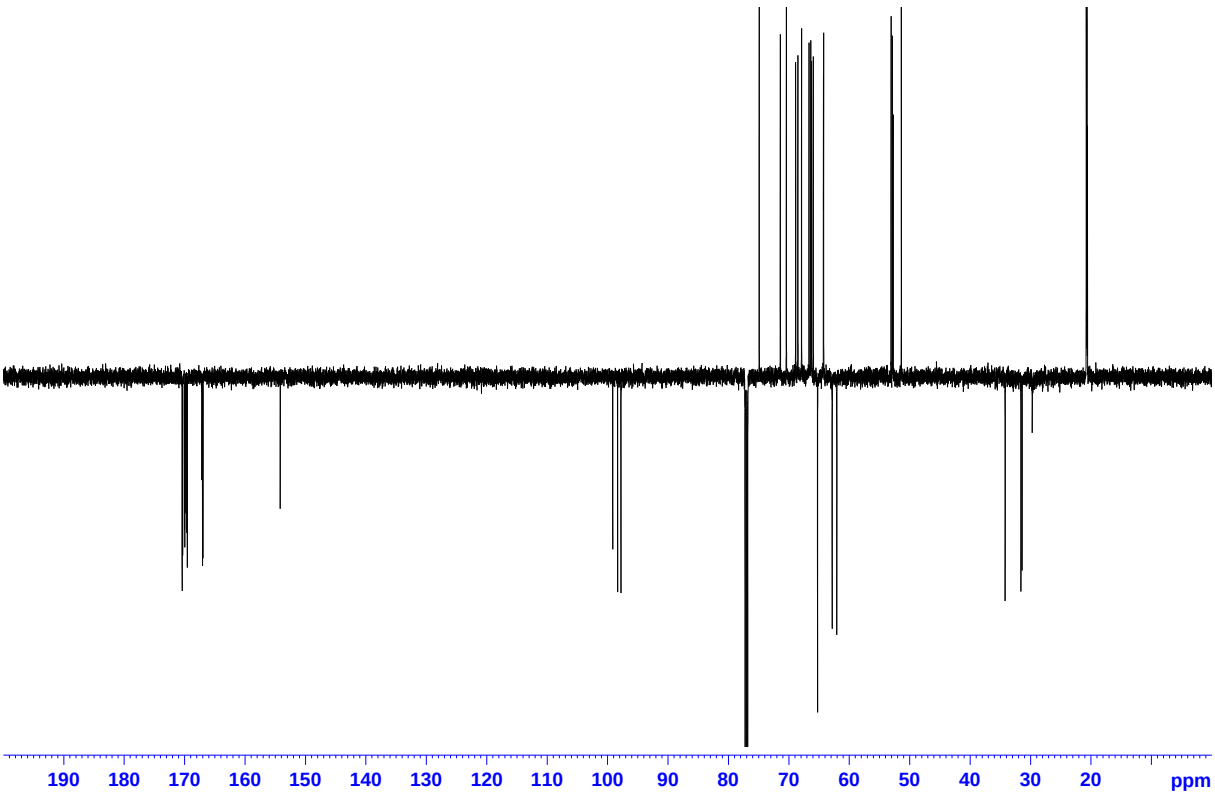

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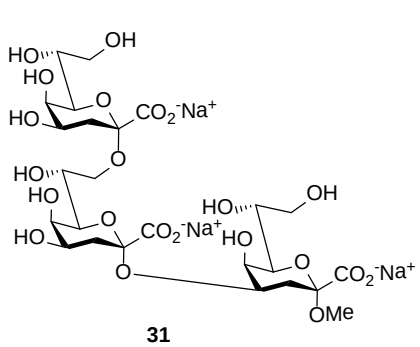

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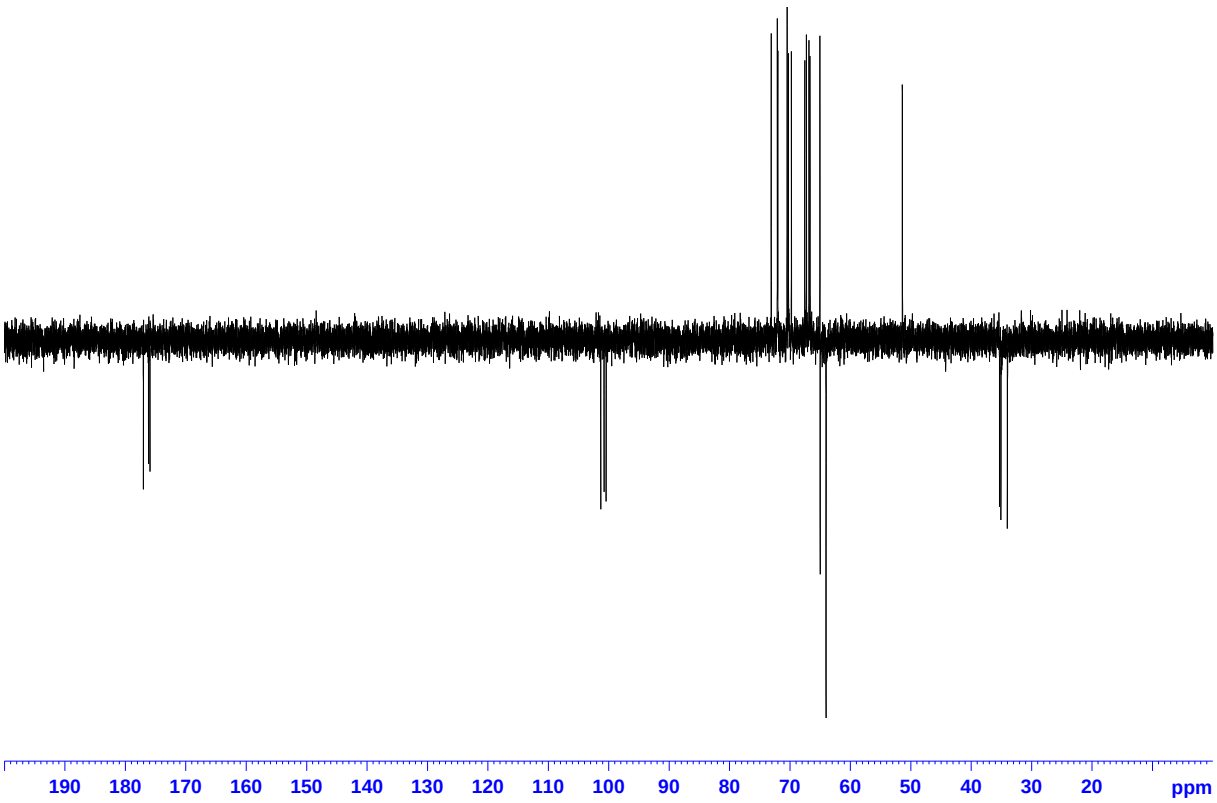
^{1}H-NMR (600 MHz, D_2O)



^{13}C-NMR (150 MHz, D_2O)

Manuscript #4

Supporting Information

doi:10.1002/open.201500126

Supporting information

Scope and limitations of 3-iodo-Kdo fluoride based glycosylation chemistry using *N*-acetyl glucosamine acceptors

Barbara Pokorny, Paul Kosma*

Department of Chemistry, University of Natural Resources and Life Sciences-Vienna, Muthgasse 18, A-1190 Vienna, Austria

Email: paul.kosma@boku.ac.at

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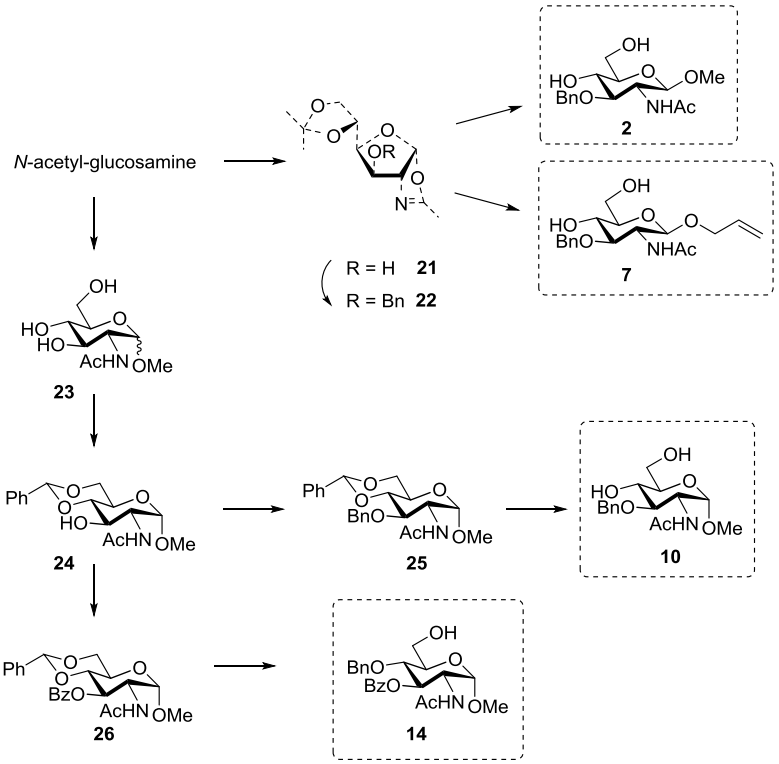
2. Synthesis of glucosamine acceptors



2.1. Methyl 2-acetamido-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside (2)

Starting from *N*-acetyl glucosamine, acceptor **2** was prepared in three steps according to literature.[S1]

2.2. Allyl 2-acetamido-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside (7)

The 3-*O*-benzylated oxazoline **22**[S1b] (0.51 g, 1.52 mmol) was reacted with allyl alcohol (17 mL) in the presence of camphorsulfonic acid (0.10 g, 0.42 mmol) similar to the literature procedure for compound **2**. The crude product was purified by two consecutive chromatography steps ($CHCl_3$:MeOH 95:5; EtOAc:EtOH 95:5) affording pure β-allyl glycoside **7**[S2] (0.21 g, 37%): ^{1}H NMR (CD_3OD) = δ 7.34 - 7.23 (m, 5H, Ar), 5.88 (dddd, 1H, *J* 17.3, *J* 10.6, *J* 5.7, *J* 4.9 Hz, CH_2-C*H*=CH_2), 5.29 - 5.25 (m, 1H, CH_2-CH=C*H*H), 5.15 - 5.12 (m, 1H, CH_2-CH=CH*H*), 4.86 (d, 1H, *J* 11.3 Hz, C*H*HPh, overlapped by water peak), 4.65 (d, 1H, *J* 11.6 Hz, CH*H*Ph), 4.48 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), 4.33 (tdd, 1H, *J* 13.3, *J* 4.9, *J* 1.6 Hz, C*H*H-CH=CH_2), 4.07 (tdd, 1H, *J* 13.3, *J* 5.8, *J* 1.6 Hz, CH*H*-CH=CH_2), 3.89 (dd, 1H, $J_{6a,6b}$ 11.9, $J_{6a,5}$ 2.3 Hz, H-6a), 3.81 - 3.75 (m, 1H, H-2), 3.69 (dd, 1H, $J_{6b,5}$ 6.1 Hz, H-6b), 3.53 - 3.48 (m, 2H, H-3, H-4), 3.32 - 3.27 (m, 1H, H-5), 1.88 ppm (s, 3H, COCH_3).

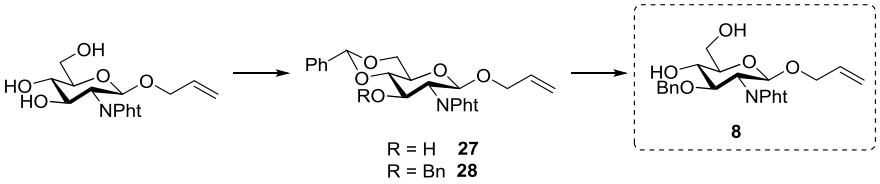
2.3. Methyl 2-acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (10)

A mixture of *N*-acetyl-D-glucosamine (5.00 g, 22.6 mmol) in dry MeOH (50 mL) containing ion exchange resin DOWEX 50 (H^+ form, 5 g) was heated to reflux for 18 h. The cooled mixture was filtered and rinsed with MeOH. The filtrate was concentrated providing an α/β-mixture of **23** (5.25 g, 97%, α:β = 10:1). According to literature[S3] this mixture was used to prepare **25**, and the β-anomer was removed by crystallization of the intermediate **24**. The benzylidene group of compound **25** was cleaved according to literature[S4]. The ^{1}H NMR spectrum of **10** was in agreement with data in the literature[S3].

2.4. Methyl 2-acetamido-3-*O*-benzoyl-4-*O*-benzyl-2-deoxy-α-D-glucopyranoside (14)

Compound **24**[S3] (175 mg, 0.54 mmol) was dissolved in dry pyridine (3.5 mL) and treated with benzoyl chloride (187 µL, 1.62 mmol) at 0 °C. After stirring for 16 h at ambient temperature excessive reagent was destroyed by slow addition of dry MeOH (2 mL, at 0 °C). The mixture was coevaporated with toluene (2x) and the residue was purified by chromatography (toluene/EtOAc 1:1) yielding **26**[S5] (165 mg, 71%). A solution of compound **26** (61 mg, 0.143 mmol) in dry CH_2Cl_2 (3.0 mL) containing 4 Å ground molecular sieves (150 mg) was stirred at ambient temperature for 1 h. To the cooled (-78 °C) mixture triethylsilane (114 µL, 0.714 mmol) and dichlorophenylborane (93 µL, 0.714 mmol) were added dropwise.[S6] After 30 min triethylamine (0.35 mL) and dry MeOH (0.35 mL) were added consecutively. The mixture was partitioned between chloroform and satd. $NaHCO_3$, the aqueous phase was further extracted (2x) with chloroform. The combined organic phases were dried ($MgSO_4$), filtered and the filtrate was concentrated. The residue was purified by two consecutive chromatographic separations (EtOAc:EtOH 9:1, then $CHCl_3$:MeOH 100:2) which gave acceptor **14** (43 mg, 70%); the analytical data was in agreement with published data.[S7]

2.5. Allyl 3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (8)



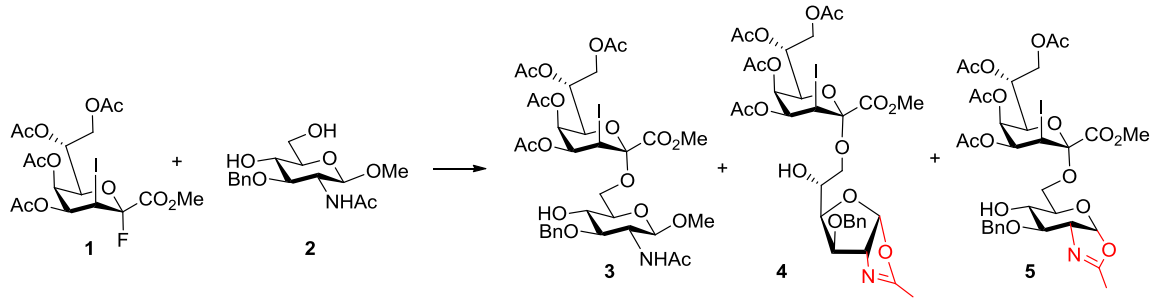
A solution of allyl 2-deoxy-2-phthalimido-β-glucopyranoside (140 mg, 0.40 mmol), benzaldehyde dimethyl acetal (72 µL 0.48 mmol) and *p*-toluenesulfonic acid monohydrate (4 mg, 0.024 mmol) in dry acetonitrile (6.0 mL) was stirred at ambient temperature for 1 h. After addition of triethylamine (12 µL) the volatile components were removed in *vacuo* and the residue was purified by chromatography (toluene/EtOAc 40:1 →7:1) affording **27**[S8] (162 mg, 92%): ^{1}H NMR ($CDCl_3$) = δ 7.88 - 7.34 (m, 9H, Ar), 5.72 - 5.65 (m, 1H, CH_2-C*H*=CH_2), 5.56 (s, 1H, C*H*Ph), 5.29 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.15 - 5.11 (m, 1H, CH_2-CH=C*H*H), 5.06 - 5.03 (m, 1H, CH_2-CH=CH*H*), 4.65 - 4.60 (m, 1H, H-3), 4.40 - 4.36 (m, 1H, H-6a), 4.30 - 4.24 (m, 2H, H-2, C*H*H-CH=CH_2), 4.05 - 4.00 (m, 1H, CH*H*-CH=CH_2), 3.83 (app t, 1H, $J_{6b,6a}$ ~ $J_{6b,5}$ 10.0 Hz, H-6b), 3.65 - 3.58 (m, 2H, H-4, H-5) and 2.68 ppm (d, 1H, *J* 4.0 Hz, O*H*).

A solution of compound **27** (346 mg, 0.79 mmol) in dry DMF (10.0 mL) was treated with sodium hydride (60% in mineral oil, 63 mg, 1.58 mmol) portionwise and was stirred for 30 min after complete addition. Next, benzyl bromide (376 µL, 3.16 mmol) was added and stirring was continued for and 1 h at room temperature. Excessive reagent was destroyed by addition of dry MeOH (2 mL). The mixture was partitioned between satd. ammonium chloride and diethyl ether and the aqueous phase was extracted with diethyl ether twice. The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by chromatography (toluene/EtOAc 20:1) providing **28**[S9] (294 mg, 71%): ^{1}H NMR ($CDCl_3$) = δ 7.90 - 7.36 (m, 9H, Ar), 7.02 - 6.86 (m, 5H, Ar), 5.69 - 5.63 (m, 1H, CH_2-C*H*=CH_2), 5.62 (s, 1H, C*H*Ph), 5.25 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), 5.13 - 5.09 (m, 1H, CH_2-CH=C*H*H), 5.03 - 5.00 (m, 1H, CH_2-CH=CH*H*), 4.80 (d, 1H, *J* 12.4 Hz, C*H*HPh), 4.51 (d, 1H, *J* 12.3 Hz, CH*H*Ph), 4.44 (dd, 1H, $J_{3,2}$ 10.2, $J_{3,4}$ 8.8 Hz, H-3), 4.41 (dd, 1H, $J_{6a,6b}$ 10.1, $J_{6a,5}$ 4.6 Hz, H-6a), 4.28 - 4.23 (m, 2H, H-2, C*H*H-CH=CH_2), 4.02 - 3.98 (m, 1H, CH*H*-CH=CH_2), 3.87 (app t, 1H, $J_{6b,5}$ ~ 9.8 Hz, H-6b), 3.83 (app t, 1H, $J_{4,5}$ ~ 9.3 Hz, H-4) and 3.65 ppm (app td, 1H, H-5).

Compound **28** (290 mg, 0.55 mmol) was dissolved in 80% AcOH (10 mL) and heated to 60 °C for 2.5 h. The mixture was concentrated and purified by chromatography (toluene/EtOAc 1:1) yielding **8**[S10] (230 mg, 95%): ^{1}H NMR ($CDCl_3$) = δ 7.86 - 7.64 (m, 4H, Ar), 7.09 - 6.93 (m, 5H, Ar), 5.67 (dddd, 1H, *J* 17.2, *J* 10.4, *J* 6.2, *J* 5.2 Hz, CH_2-C*H*=CH_2), 5.21 (d, 1H, $J_{1,2}$ 8.7 Hz, H-1), 5.12 - 5.07 (m, 1H, CH_2-CH=C*H*H), 5.03 - 5.00 (m, 1H, CH_2-CH=CH*H*), 4.70 (d, 1H, *J* 12.4 Hz, C*H*HPh), 4.54 (d, 1H, *J* 12.2 Hz, CH*H*Ph), 4.28 (dd, 1H, $J_{3,2}$ 10.7, $J_{3,4}$ 8.6 Hz, H-3), 4.24 - 4.21 (m, 1H, C*H*H-CH=CH_2), 4.18 (dd, 1H, H-2), 4.02 - 3.98 (m, 1H, CH*H*-CH=CH_2), 3.95 (dd, 1H, $J_{6a,6b}$ 11.8, $J_{6a,5}$ 3.6 Hz, H-6a), 3.88 (dd, 1H, $J_{6b,5}$ 4.1 Hz, H-6b), 3.82 (app td, 1H, $J_{4,5}$ 9.7, $J_{4,OH}$ 3.1 Hz, H-4), 3.53 (ddd, 1H, H-5) and 2.85 ppm (d, 1H, *J* 3.7 Hz, O*H*).

3. Synthetic details for compounds (3), (4), (5), (11), (12) and (13)

3.1. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→6)-methyl 2-acetamido-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside (3) and 2-methyl [methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→6)-3-*O*-benzyl-1,2-dideoxy-α-D-glucofurano]-[2,1-d]-2-oxazoline (4) and [methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→6)-3-*O*-benzyl-1,2-dideoxy-α-D-glucopyrano]-[2,1-d]-2-oxazoline (5).



A suspension of glycosyl acceptor **2** (28.3 mg, 0.087 mmol) and 3-iodo donor **1** (52.5 mg, 0.096 mmol) in dry CH_2Cl_2 (4.0 mL) containing ground 3 Å molecular sieves (200 mg) was stirred at ambient temperature for 2h. $BF_3{\cdot}Et_2O$ (35.7 µL, 0.278 mmol) was added at 0 °C and the mixture kept at ambient temperature for 90 min. After addition of satd. aq. $NaHCO_3$ and CH_2Cl_2, the mixture was extracted, the aqueous phase once again treated with CH_2Cl_2 and the combined organic layers were washed successively with sodium thiosulfate (5 w%) and brine. The organic layer was dried ($MgSO_4$), filtered and concentrated. The crude product was purified by chromatography (toluene/EtOAc 2:1→0:1) affording a mixture of several disaccharide compounds, which were further separated by HP-chromatography (EtOAc) which afforded disaccharide **3** (23.5 mg, 32%), furano-oxazoline **4** (7.6 mg, 11%) and pyrano-oxazoline **5** (2.1 mg, 3 %) as colourless oils.

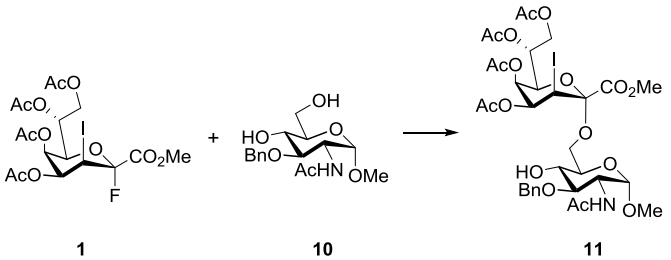
3: $[\alpha]_D^{20}$ +37.2 (*c* = 0.72, $CHCl_3$); R_f 0.44 (EtOAc, HP-TLC); ^{1}H NMR ($CDCl_3$) = δ 7.38 - 7.29 (m, 5H, Ar), 5.66 (d, 1H, $J_{NH,2}$ 7.9 Hz, N*H*), 5.38 - 5.35 (m, 2H, H-5', H-7'), 5.02 (dd, 1H, $J_{4',3'}$ 4.7, $J_{4',5'}$ 3.7 Hz, H-4'), 4.73 (d, 1H, *J* 11.9 Hz, C*H*HPh), 4.71 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), 4.68 (d, 1H, *J* 11.7 Hz, CH*H*Ph), 4.64 (dd, 1H, $J_{8'a,8'b}$ 12.2, $J_{8'a,7'}$ 2.3 Hz, H-8'a), 4.50 (dd, 1H, $J_{3',5'}$ 0.7 Hz, H-3'), 4.44 (dd, 1H, $J_{6',7'}$ 9.7, $J_{6',5'}$ 1.8 Hz, H-6'), 4.19 (dd, 1H, $J_{8'b,7'}$ 4.5 Hz, H-8'b), 3.95 (dd, 1H, $J_{3,2}$ 10.2, $J_{3,4}$ 8.7 Hz, H-3), 3.84 (s, 3H, CO_2CH_3), 3.68 (dd, 1H, $J_{6a,6b}$ 10.1, $J_{6a,5}$ 6.9 Hz, H-6a), 3.58 (dd, 1H, $J_{6b,5}$ 2.5 Hz, H-6b), 3.51 (ddd, 1H, $J_{5,4}$ 9.6 Hz, H-5), 3.47 (s, 3H, OCH_3), 3.41 (app dt, 1H, $J_{4,OH}$ 3.1 Hz, H-4), 3.30 (app td, 1H, H-2), 2.59 (d, 1H, O*H*), 2.11, 2.05, 2.04, 1.96 and 1.94 ppm (5 s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$) = δ 170.69, 170.65, 170.2, 169.5 and 169.4 (5 s, 5C, $COCH_3$), 166.1 (s, C-1'), 138.1 (s, 1C, Ar), 128.7 (d, 2C, Ar), 128.2 (d, 1C, Ar), 128.1 (d, 2C, Ar), 101.3 (s, C-2'), 100.9 (d, C-1), 80.7 (d, C-3), 74.2 (t,

CH_2Ph), 73.9 (d, C-5), 71.4 (d, C-4), 68.1 (d, C-6'), 67.7 (d, C-7'), 65.5 (t, C-6), 65.4 (d, C-4'), 63.3 (d, C-5'), 62.0 (t, C-8'), 57.1 (d, C-2), 56.9 (q, OCH_3), 53.1 (q, CO_2CH_3), 23.6 (q, COCH_3), 21.9 (d, C-3'), 20.90, 20.86, 20.7 and 20.6 ppm (4 q, 4C, COCH_3); HRMS (ESI-TOF): *m/z* calcd for $C_{33}H_{44}INO_{17}Na^+$: 876.1546 [M+Na^+]; found: = 876.1532.

4: R_f 0.42 (EtOAc, HP-TLC); ^{1}H NMR ($CDCl_3$) = δ 7.40 - 7.30 (m, 5H, Ar), 6.13 (d, 1H, $J_{1,2}$ 5.2 Hz, H-1), 5.41 – 5.36 (m, 2H, H-5', H-7'), 4.98 (dd, 1H, $J_{4',3'}$ 4.8, $J_{4',5'}$ 3.6 Hz, H-4'), 4.76 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.63 - 4.56 (m, 3H, H-2, H-8'a, CH*H*Ph), 4.50 (dd, 1H, $J_{3',5'}$ 0.6 Hz, H-3'), 4.43 (dd, 1H, $J_{6',7'}$ 9.8, $J_{6',5'}$ 2.1 Hz, H-6'), 4.23 (dd, 1H, $J_{8'b,8'a}$ 12.5, $J_{8'b,7'}$ 4.4 Hz, H-8'b), 4.14 - 4.09 (m, 2H, H-3, H-5), 3.82 (s, 3H, CO_2CH_3), 3.77 (dd, 1H, $J_{4,5}$ 8.6, $J_{4,3}$ 3.2 Hz, H-4), 3.73 (dd, 1H, $J_{6a,6b}$ 10.0, $J_{6a,5}$ 5.5 Hz, H-6a), 3.42 (dd, 1H, $J_{6b,5}$ 2.6 Hz, H-6b), 2.33 (b d, 1H, *J* 6.8 Hz, O*H*), 2.12 (s, 3H, COCH_3), 2.06 (s, 3H, COCH_3), 2.04 (d, 3H, *J* 1.4 Hz, oxazoline-CH_3), 2.01 (s, 3H, COCH_3), 1.98 ppm (s, 3H, COCH_3); ^{13}C NMR ($CDCl_3$) = δ 170.6, 170.2, 169.6 and 169.2, 167.3 [s, N=*C*(CH_3)O], 166.2 (s, C-1'), 137.1 (s, 1C, Ar), 128.8 (d, 2C, Ar), 128.3 (d, 1C, Ar), 127.9 (d, 2C, Ar), 106.9 (d, C-1), 101.5 (s, C-2'), 81.2 (d, C-3), 79.6 (d, C-4), 74.9 (d, C-2), 71.9 (t, *C*H_2Ph), 68.2 (d, C-6'), 67.7 (d, C-7'), 67.6 (t, C-6), 67.1 (d, C-5), 65.3 (d, C-4'), 63.3 (d, C-5'), 62.0 (t, C-8'), 53.2 (q, CO_2CH_3), 21.8, 20.9, 20.8, 20.7 and 20.6 (4 q, 1d, 5C, C-3', 4 x COCH_3), 14.0 (q, oxazoline-*C*H_3) ppm; HRMS (ESI-TOF): *m/z* calcd for $C_{32}H_{40}INO_{16}H^+$: 822.1465 [M+H^+]; found: 822.1458.

5: R_f 0.38 (EtOAc, HP-TLC); ^{1}H NMR ($CDCl_3$) = δ 7.38 - 7.28 (m, 5H, Ar), 5.96 (d, 1H, $J_{1,2}$ 7.2 Hz, H-1), 5.45 - 5.43 (m, 1H, H-5'), 5.36 (ddd, 1H, $J_{7',6'}$ 9.5, $J_{7',8'b}$ 4.3, $J_{7',8'a}$ 2.4 Hz, H-7'), 5.03 (dd, 1H, $J_{4',3'}$ 4.7, $J_{4',5'}$ 3.9 Hz, H-4'), 4.78 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.65 (d, 1H, *J* 11.8 Hz, CH*H*Ph), 4.64 (dd, 1H, $J_{8'a,8'b}$ 12.3 Hz, H-8'a), 4.54 (b d, 1H, H-3'), 4.42 (dd, 1H, $J_{6',5'}$ 2.0 Hz, H-6'), 4.21 (dd, 1H, H-8'b), 4.12 - 4.08 (m, 1H, H-2), 3.85 (s, 3H, CO_2CH_3), 3.75 (dd, 1H, $J_{6a,6b}$ 10.0, $J_{6a,5}$ 5.9 Hz, H-6a), 3.71 (app t, 1H, $J_{3,2}$ = $J_{3,4}$ 4.4 Hz, H-3), 3.66 - 3.62 (m, 1H, H-4), 3.49 (ddd, 1H, $J_{5,4}$ 8.8, $J_{5,6b}$ 2.7 Hz, H-5), 3.45 (dd, 1H, H-6b), 2.31 - 2.25 (m, 1H, O*H*), 2.12 (s, 3H, COCH_3), 2.08 (d, 3H, *J* 1.6 Hz, oxazoline-CH_3), 2.06 (s, 3H, COCH_3), 1.99 (s, 3H, COCH_3), 1.97 ppm (s, 3H, COCH_3); ^{13}C NMR ($CDCl_3$) = δ 170.7, 170.2, 169.6 and 169.3 (4 s, 4C, *C*OCH_3), 166.8 [s, N=*C*(CH_3)-O], 166.0 (s, C-1'), 137.5 (s, 1C, Ar), 128.6 (d, 2C, Ar), 128.04 (d, 1C, Ar), 127.96 (d, 2C, Ar), 101.5 (s, C-2'), 101.1 (d, C-1), 80.4 (d, C-3), 72.2 (d, C-5), 72.0 (t, *C*H_2Ph), 68.3 and 68.2 (2 d, 2C, C-4, C-6'), 67.8 (d, C-7'), 66.3 (d, C-2), 66.1 (t, C-6), 65.4 (d, C-4'), 63.4 (d, C-5'), 61.9 (t, C-8'), 53.1 (q, CO_2CH_3), 21.9, 20.9, 20.8, 20.7 and 20.6 (4 q, 1 d, 5C, C-3', 4 x COCH_3), 14.3 (q, oxazoline-*C*H_3) ppm; HRMS (ESI-TOF): *m/z* calcd for $C_{32}H_{40}INO_{16}H^+$: 822.1465 [M+H^+]; found: 822.1483.

3.2. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→6)-methyl 2-acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (11)



1 **10** **11**

A solution of donor **1** (40 mg, 0.074 mmol) in dry CH_2Cl_2 (3.0 mL) was added to acceptor **10** (20 mg, 0.061 mmol) and the suspension was stirred at ambient temperature for 1 h in the presence of 3 Å ground molecular sieves (150 mg). BF_3.Et_2O (23 µL, 0.184 mmol) was added at 0 °C. After 1 h at room temperature satd. aq. $NaHCO_3$ solution was added and the mixture was repeatedly extracted with

CH_2Cl_2. The combined organic layers were washed successively with thiosulfate (5 w%) and brine, dried ($MgSO_4$), filtered and the filtrate was concentrated. The crude product was purified by chromatography (SiO_2, toluene/EtOAc 1:2 → 0:1) yielding disaccharide **11** (29 mg, 55%) as a colorless oil: R_f 0.31 (toluene/EtOAc 1:4); ^{1}H NMR ($CDCl_3$) = δ 7.38 - 7.29 (m, 5H, Ar), 5.50 (d, 1H, *J* 9.7 Hz, N*H*), 5.40 - 5.38 (m, 1H, H-5'), 5.34 (ddd, 1H, $J_{7',6'}$ 9.4, $J_{7',8'b}$ 4.5, $J_{7',8'a}$ 2.6 Hz, H-7'), 5.05 (dd, 1H, $J_{4',3'}$ 4.8, $J_{4',5'}$ 3.6 Hz, H-4'), 4.71 (d, 1H, *J* 11.8 Hz, C*H*HPh), 4.67 - 4.64 (m, 3H, H-1, H-8'a, CH*H*Ph), 4.73 (b d, 1H, H-3'), 4.42 (dd, 1H, $J_{6'5'}$ 2.0 Hz, H-6'), 4.22 (app td, 1H, $J_{2,3}$ 9.8, $J_{2,1}$ 4.0 Hz, H-2), 4.19 (dd, 1H, $J_{8'b,8'a}$ 12.5 Hz, H-8'b), 3.85 (s, 3H, CO_2CH_3), 3.74 (ddd, 1H, $J_{5,4}$ 9.6, $J_{5,6a}$ 7.1, $J_{5,6b}$ 2.2 Hz, H-5), 3.67 (dd, 1H, $J_{6a,6b}$ 10.0 Hz, H-6a), 3.56 (dd, 1H, H-6b), 3.53 (app t, 1H, $J_{3,4}$ 9.4 Hz, H-3), 3.48 (app dt, 1H, $J_{4,OH}$ 2.7 Hz, H-4) 3.38 (s, 3H, OCH_3), 2.54 (d, 1H, O*H*), 2.12, 2.06, 2.04, 1.98 and 1.92 ppm (5 s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$) = δ 170.5, 170.2, 169.8, 169.6, 169.3 and 166.1 (6 s, 6C, 5x $COCH_3$, C-1'), 138.1 (s, 1C, Ar), 128.7, 128.11 and 128.08 (3 d, 5C, Ar), 101.3 (s, C-2'), 98.6 (d, C-1), 80.5 (d, C-3), 74.0 (t, CH_2Ph), 70.7 (d, C-4), 70.1 (d, C-5), 68.3 (d, C-6'), 67.9 (d, C-7'), 65.5 (t, C-6), 65.3 (d, C-4'), 63.4 (d, C-5'), 62.0 (t, C-8'), 55.1 (q, OCH_3), 53.1 (q, CO_2CH_3), 51.9 (d, C-2), 23.4, 21.9, 20.9, 20.81, 20.75 and 20.6 ppm (5 q, 1 d, 6C, 5 x $COCH_3$, C-3'); LC-MS[S11]: m/z found: 854.45 [$M+H^+$]; 876.45 [$M+Na^+$].

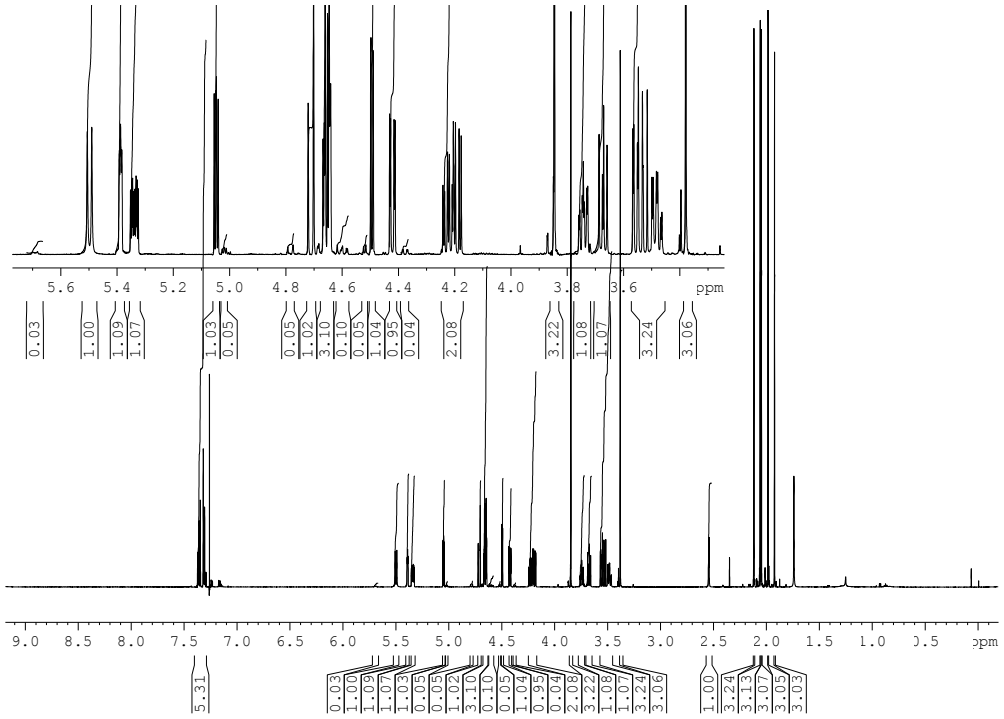


Fig. S1: ^{1}H NMR ($CDCl_3$, 600 MHz) of compound (11).

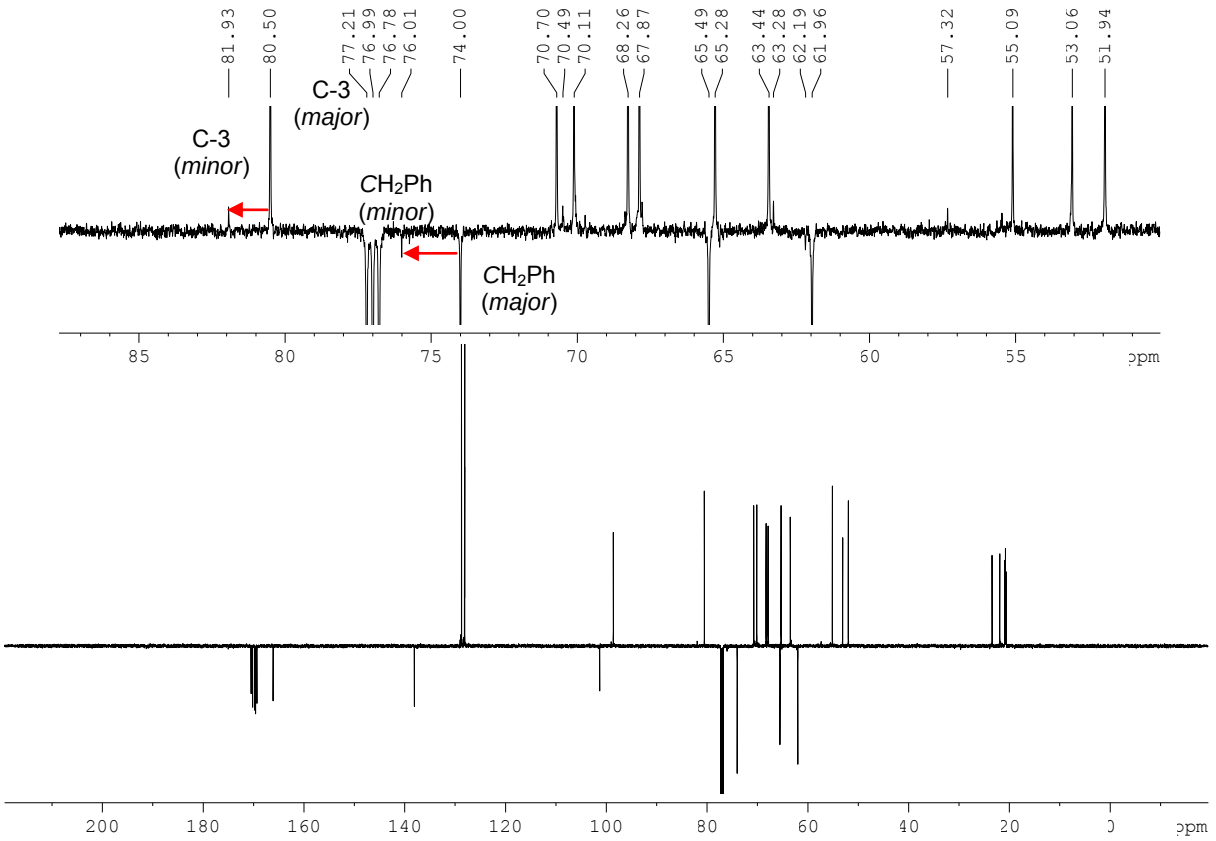
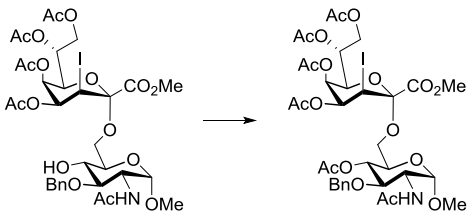


Fig. S2: ^{13}C NMR ($CDCl_3$, 150 MHz) of compound (11).

3.3. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→6)-methyl 2-acetamido-4-*O*-acetyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (12)



A solution of compound **11** in dry pyridine (3 mL) was treated with 4-(*N,N*-dimethylamino)pyridine (1 mg) and acetic anhydride (0.3 mL) for 5 h at room temperature. The mixture was cooled (0 °C) and dry MeOH (1 mL) was added slowly. After 5 min the mixture was coevaporated with toluene (3 x) and the residue was purified by chromatography (toluene/EtOAc 1:3) followed by HPLC (toluene/EtOAc 1:2) which afforded disaccharide **12** (13 mg, 59%) as a colorless oil: R_f 0.30 (CH_2Cl_2/EtOAc 1:1, HP-TLC); ^{1}H NMR ($CDCl_3$) = δ 7.35 - 7.23 (m, 5H, Ar), 5.39 - 5.37 (m, 1H, 5'), 5.33 - 5.30 (m, 2H, H-7', N*H*), 5.08 (dd, 1H, $J_{4',3'}$ 4.7, $J_{4',5'}$ 3.7 Hz, H-4'), 4.90 (dd, 1H, $J_{4,3}$ 10.5, $J_{4,5}$ 9.2 Hz, H-4), 4.74 - 4.70 (m, 2H, H-1, H-8'a), 4.62 (d, 1H, *J* 11.6 Hz, C*H*HPh), 4.52 - 4.49 (m, 2H, H-3', CH*H*Ph), 4.37 (dd, 1H, $J_{6',7'}$ 9.4, $J_{6',5'}$ 2.3 Hz, H-6'), 4.26 (ddd, 1H, $J_{2,NH}$ 10.6, $J_{2,3}$ 9.1, $J_{2,1}$ 3.6 Hz, H-2), 4.15 (dd, 1H, $J_{8'b,8'a}$ 12.6, $J_{8'b,7'}$ 4.2 Hz, H-8'b), 3.86 (ddd, 1H, $J_{5,6a}$ 8.2, $J_{5,6b}$ 1.7 Hz, H-5), 3.84 (s, 3H, CO_2CH_3), 3.71 (dd, 1H, H-3), 3.64 (dd, 1H, $J_{6a,6b}$ 10.4 Hz, H-6a), 3.40 (s, 3H, OCH_3), 3.25 (dd, 1H, H-6b), 2.12, 2.11, 2.04, 2.02, 1.99 and 1.87 ppm (6 s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$) = δ 170.4, 170.1, 169.7, 169.6, 169.5, 169.3 and 166.1 (7 s, 7C, 6x $COCH_3$, C-1'), 137.8 (s, 1C, Ar), 128.5, 128.1 and 128.0 (3 d, 5C, Ar), 101.3 (s, C-2'), 98.2 (d, C-1), 77.5 (d, C-3), 73.1 (t, CH_2Ph), 70.5 (d, C-4), 68.9 (d, C-5), 68.4 (d, C-6'), 67.9 (d, C-

7'), 65.2 (d, C-4'), 64.9 (t, C-6), 63.5 (d, C-5'), 61.8 (t, C-8'), 55.3 (d, OCH_3), 52.9 (q, CO_2CH_3), 51.9 (d, C-2), 23.3, 22.0, 20.89, 20.86, 20.8, 20.7 and 20.6 ppm (6 q, 1 d, 7C, 6 x $COCH_3$, C-3'); LC-MS[S11]: m/z found: 896.50 [$M+H^+$]; 918.50 [$M+Na^+$].

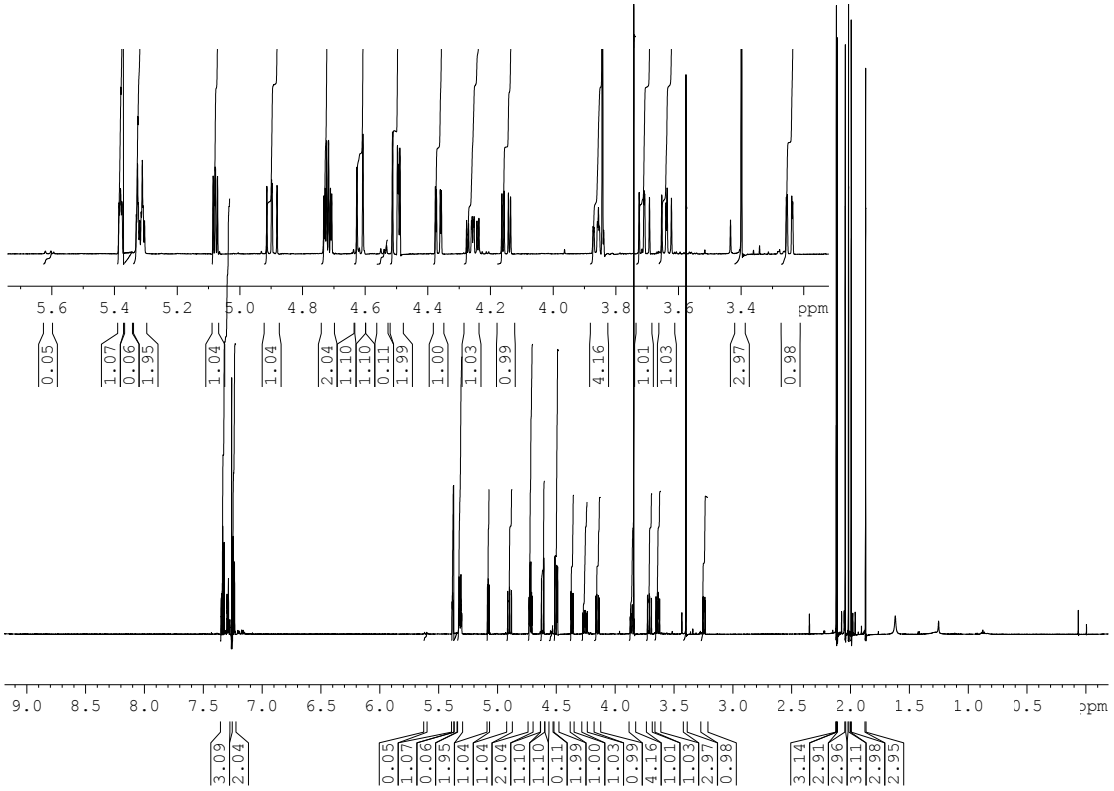


Fig. S3: ^{1}H NMR ($CDCl_3$, 600 MHz) of compound (12).

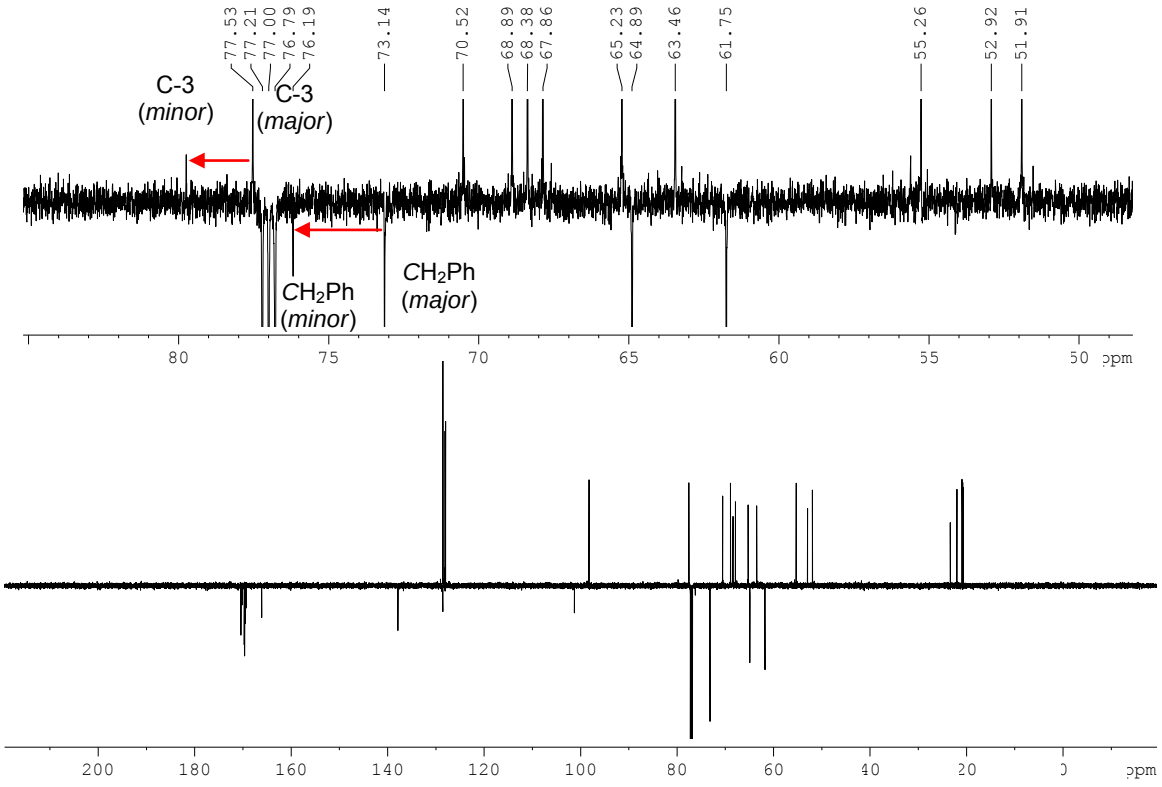
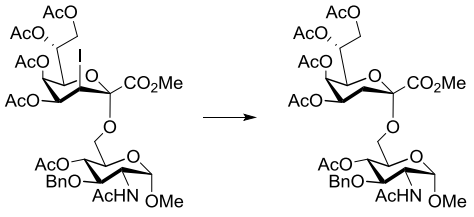


Fig. S4: ^{13}C NMR ($CDCl_3$, 150 MHz) of compound (12).

3.4. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→6)-methyl 2-acetamido-4-*O*-acetyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (13)



Compound **12** (12.0 mg, 0.013 mmol) was suspended in dry cyclohexane (4.0 mL) and dry 1,2-dichloroethane (0.5 mL). After degassing with argon the mixture was refluxed for 15 min followed by addition of lauroyl peroxide (1.9 mg, 0.005 mmol). Refluxing for 2 h and solvent evaporation afforded a crude product which was separated by chromatography (toluene/EtOAc 1:4 → 0.1) and HPLC (EtOAc) providing disaccharide **13** (7.7 mg, 75%) as a colorless oil: R_f 0.28 (CH_2Cl_2/EtOAc 1:1, HP-TLC); ^{1}H NMR ($CDCl_3$) = δ 7.35 - 7.24 (m, 5H, Ar), 5.38 - 5.31 (m, 3H, N*H*, H-4', H-5'), 5.19 (ddd, 1H, $J_{7',6'}$ 9.3, $J_{7',8'b}$ 4.7, $J_{7',8'a}$ 2.4 Hz, H-7'), 4.91 (dd, 1H, $J_{4,5}$ 10.4, $J_{4,3}$ 9.1 Hz, H-4), 4.72 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.65 (dd, 1H, $J_{8'a,8'b}$ 12.4 Hz, H-8'a), 4.63 (d, 1H, *J* 11.7 Hz, C*H*HPh), 4.51 (d, 1H, *J* 11.6 Hz, CH*H*Ph), 4.29 - 4.24 (m, 2H, H-2, H-6'), 4.06 (dd, 1H, H-8'b), 3.88 (ddd, 1H, $J_{5,6a}$ 8.2, $J_{5,6b}$ 2.1 Hz, H-5), 3.80 (s, 3H, CO_2CH_3), 3.72 (dd, 1H, $J_{3,2}$ 10.7 Hz, H-3), 3.57 (dd, 1H, $J_{6a,6b}$ 10.6 Hz, H-6a), 3.49 (dd, 1H, H-6b), 3.41 (s, 3H, OCH_3), 2.13 - 2.07 (m, 8H, H-3'*eq*, H-3'*ax*, 2 x $COCH_3$), 2.03, 2.02, 1.96 and 1.88 ppm (4 s, each 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$) = δ 170.5, 170.4, 169.83, 169.82, 169.7, 169.6 and 167.4 (7 s, 7C, 6 x $COCH_3$, C-1'), 137.9 (s, 1C, Ar), 128.5, 128.1 and 127.9 (3 d, 5C, Ar), 98.4 (s, C-2'), 98.2 (d, C-1), 77.6 (d, C-3), 73.1 (t, CH_2Ph), 70.8 (d, C-4), 69.1 (d, C-5), 68.8 (d, C-6'), 68.0 (d, C-7'), 66.2 (d, C-4'), 64.6 (d, C-5'), 63.2 (t, C-6), 62.0 (t, C-8'), 55.3 (q, OCH_3), 52.7 (q, CO_2CH_3), 51.9 (d, C-2), 32.0 (t, C-3'), 23.4, 20.9, 20.77, 20.75, 20.72 and 20.66 ppm (6 q, 6C, $COCH_3$); LC-MS[S11]: m/z found: 770.55 [M+H$^+$]; 792.55 [M+Na$^+$].

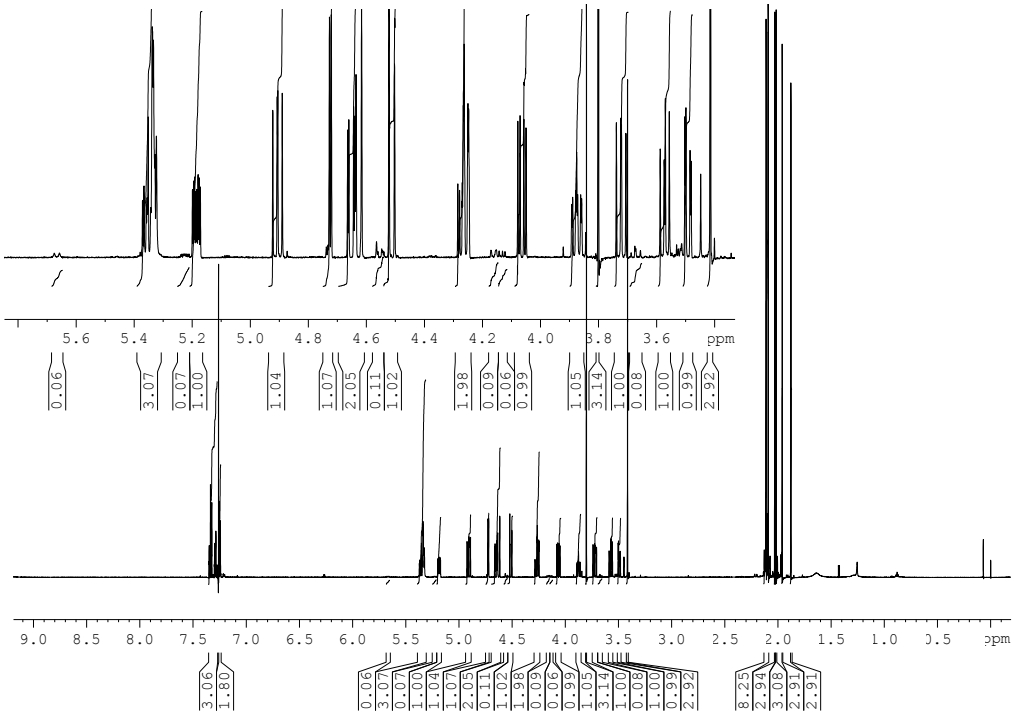


Fig S5: ^{1}H NMR ($CDCl_3$, 600 MHz) of compound (13).

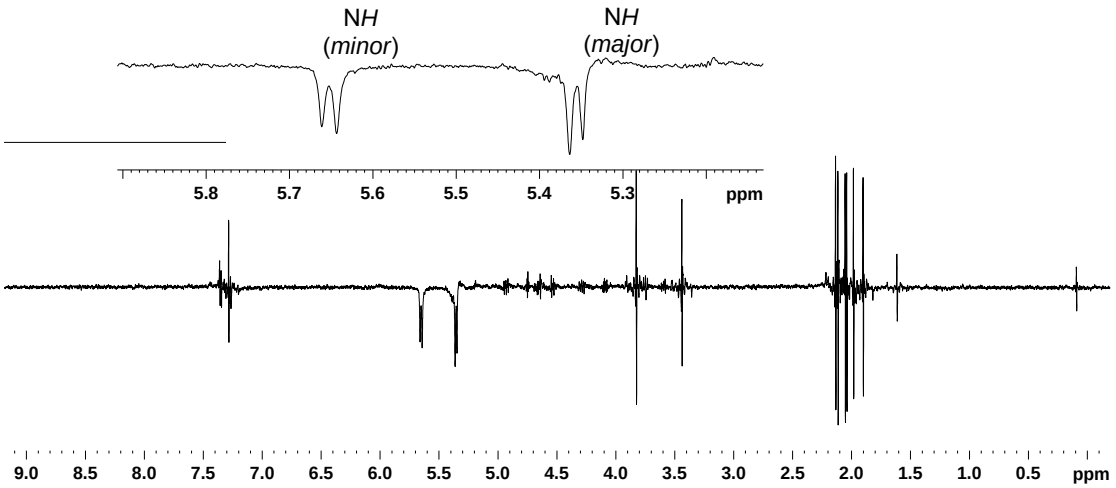


Fig S6: 1D-NOE-difference spectrum ($CDCl_3$, 600 MHz) of compound (13); selective pulse @ 5.64 ppm (N*H* of minor rotamer).

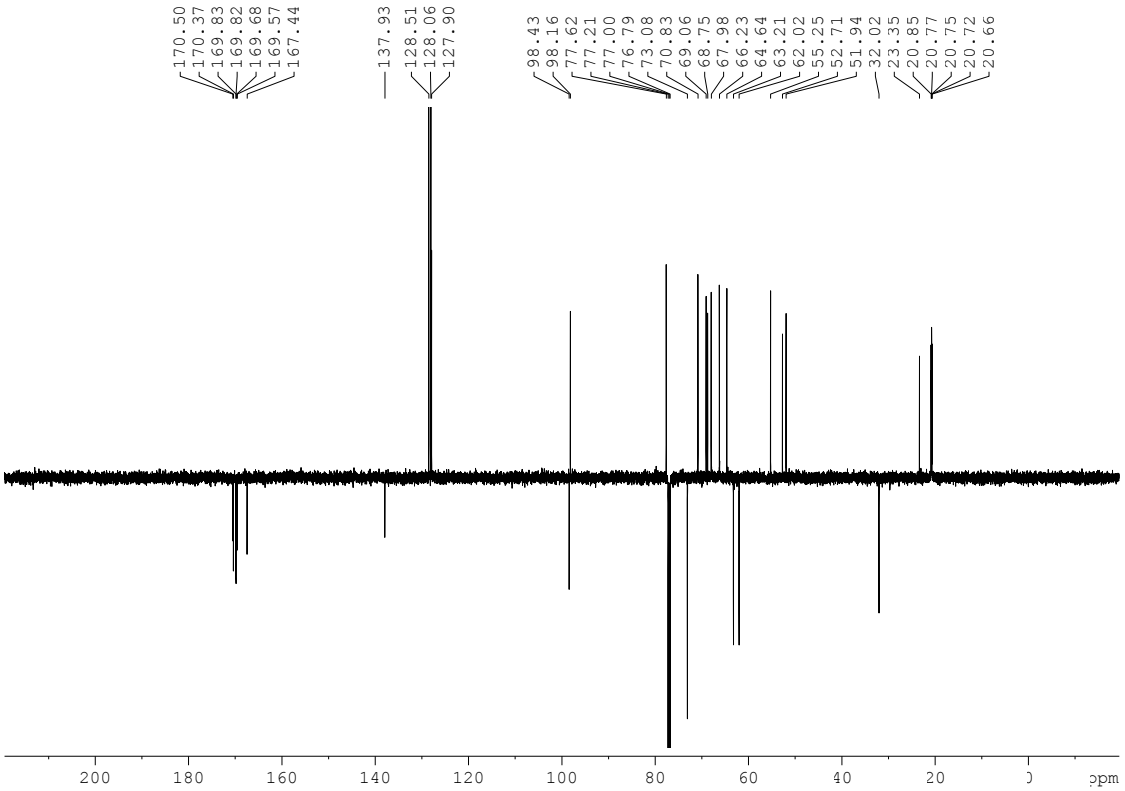


Fig S7: ^{13}C NMR ($CDCl_3$, 150 MHz) of compound (13).

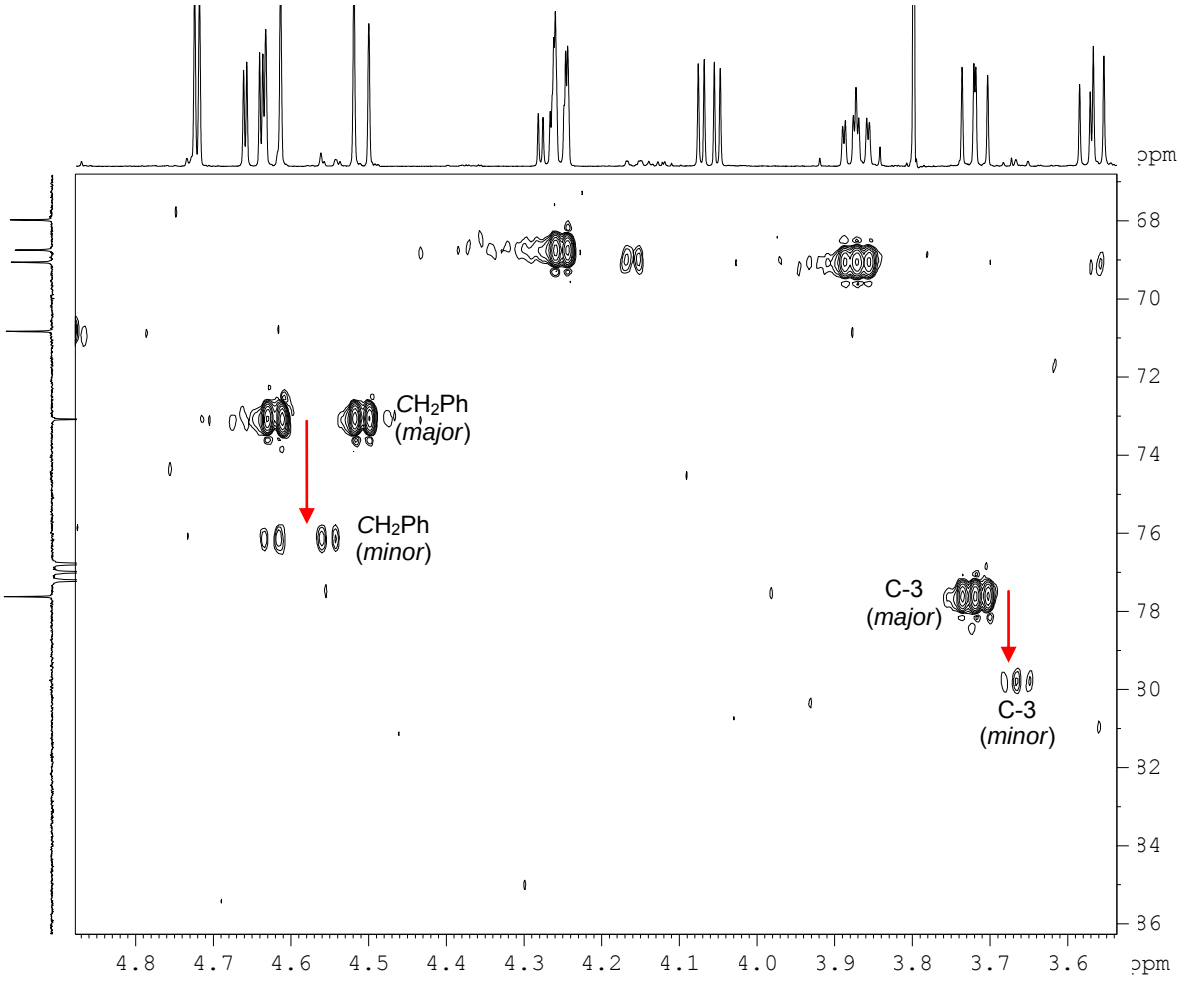
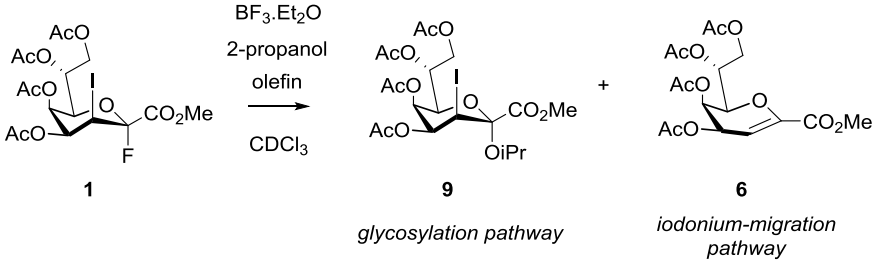


Fig S8: HSQC spectrum ($CDCl_3$, 600 MHz) of compound (13).

4. Competitive NMR studies on iodonium ion migration

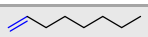
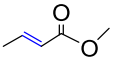
4.1. Experimental procedure



The 3-iodo donor **1** (10 mg, 0.018 mmol) was dissolved in dry $CDCl_3$ (kept over 4 Å molecular sieves overnight) in an NMR tube and 2-propanol (2.8 µL, 0.036 mmol) and the respective olefin (~ 2 eq., see Table 1) were added. From this solution a ^1H NMR spectrum was recorded as a reference. Then, the mixture was treated with $BF_3{\cdot}Et_2O$ (7.5 µL, 0.036 mmol), agitated and a ^1H spectrum was recorded

immediately. In the presence of 1-octene (entry I) and cyclohexene (entry II) complete consumption of donor **1** was already detected in the first spectrum (reaction time: < 1min). For allyl methyl ether (entry III) ^{1}H spectra were collected over a period of 1 h with variable time intervals in between. The integrals of representative signals for donor **1**, glycal ester **6** and glycoside **9** were collected in relation to the residual solvent peak. The integral of the donor signal in the reference spectrum was defined as 100%, the percentages of the collected integral values were calculated and plotted over time in a diagram (see Fig. S14). For methyl crotonate (entry IV) no effect of the olefin on the glycosylation was observed.

Table S1. Reaction conditions and changes of NMR-integration values

Entry	Olefin	Ratio 6:9	Time	Eq. olefin	Δ-Integral Olefin	Integral Glycal
I		0 : 1	< 1 min	2.3	-0.94	0.92
II		0 : 1	< 1 min	3.0	-0.92	1.02
III		1 : 1.2	60 min	1.5	-0.52	0.46
IV		1 : 0	60 min	2.7	+0.05	0.00

To perform a negative control, the same procedure was repeated in the absence of donor **1** (for entries I to III). The ^{1}H-NMR spectra were collected before and after $BF_3.Et_2O$ addition (see below). In all cases the signals of 2-propanol were significantly shifted, the olefin signals remained unchanged or were slightly shifted, respectively. However, no degradation or consumption of the respective olefin was observed.

4.2. 1-Octene (entry I)

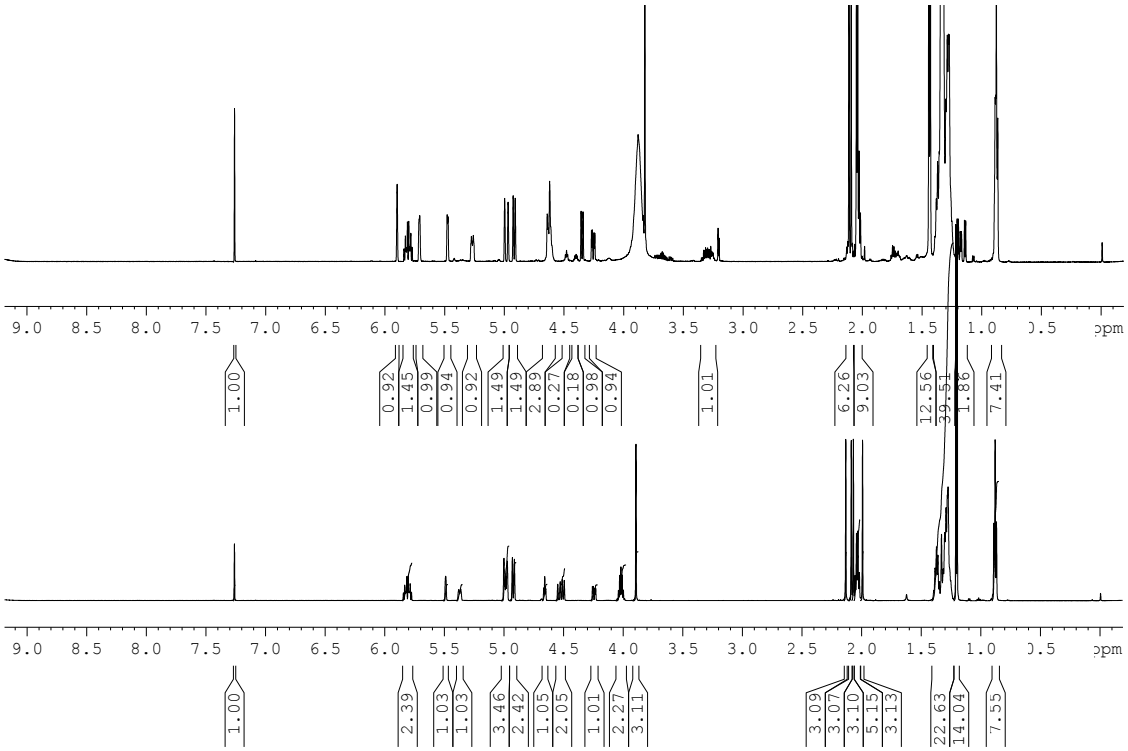


Fig. S9: Comparison of ^{1}H NMR spectra before (bottom) and after (top) donor activation (reaction time < 1min).

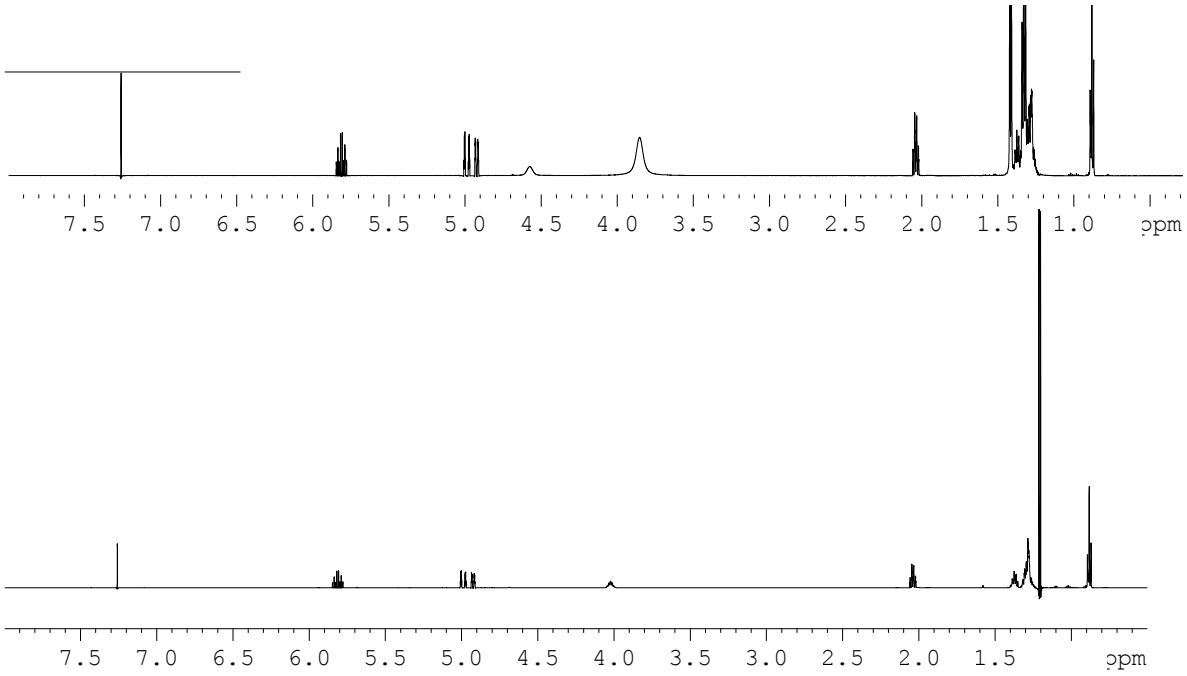


Fig. S10: Negative control: Comparison of a solution without donor (1) before (bottom) and after (top) promotor addition.

4.3. Cyclohexene (entry II)

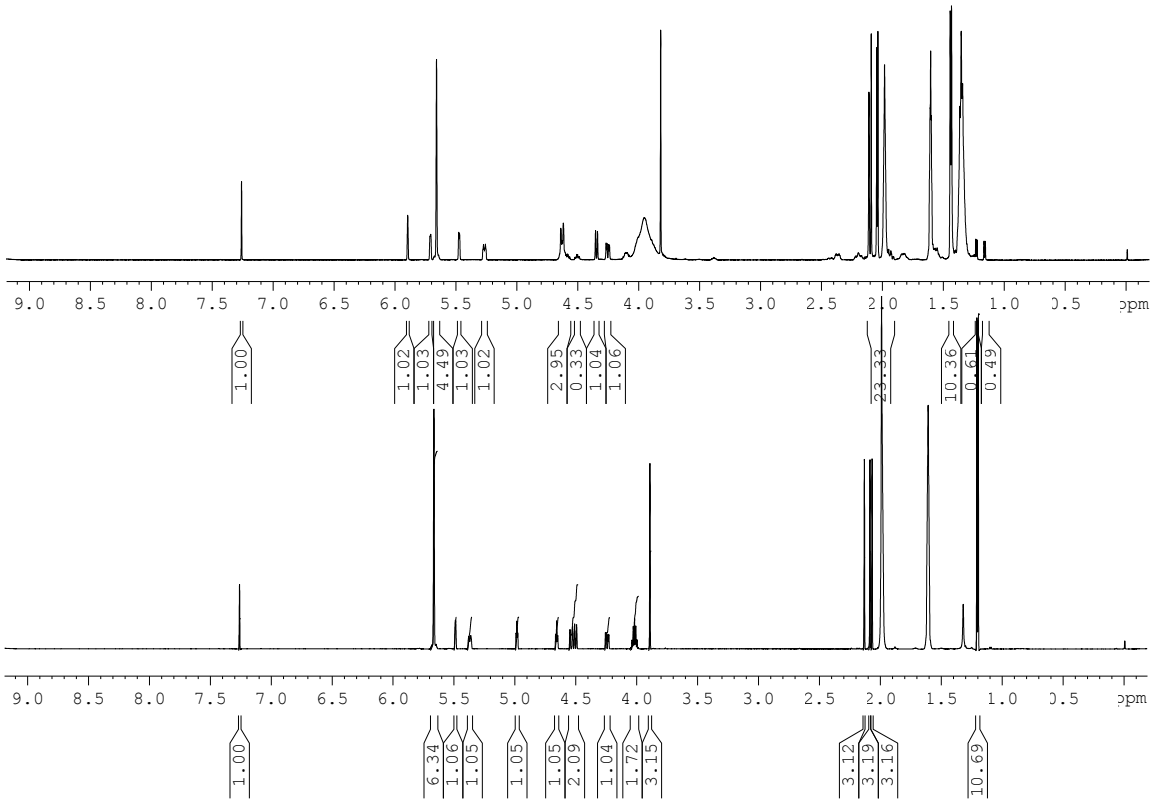


Fig. S11: Comparison of ^{1}H NMR spectra before (bottom) and after (top) donor activation (reaction time < 1min).

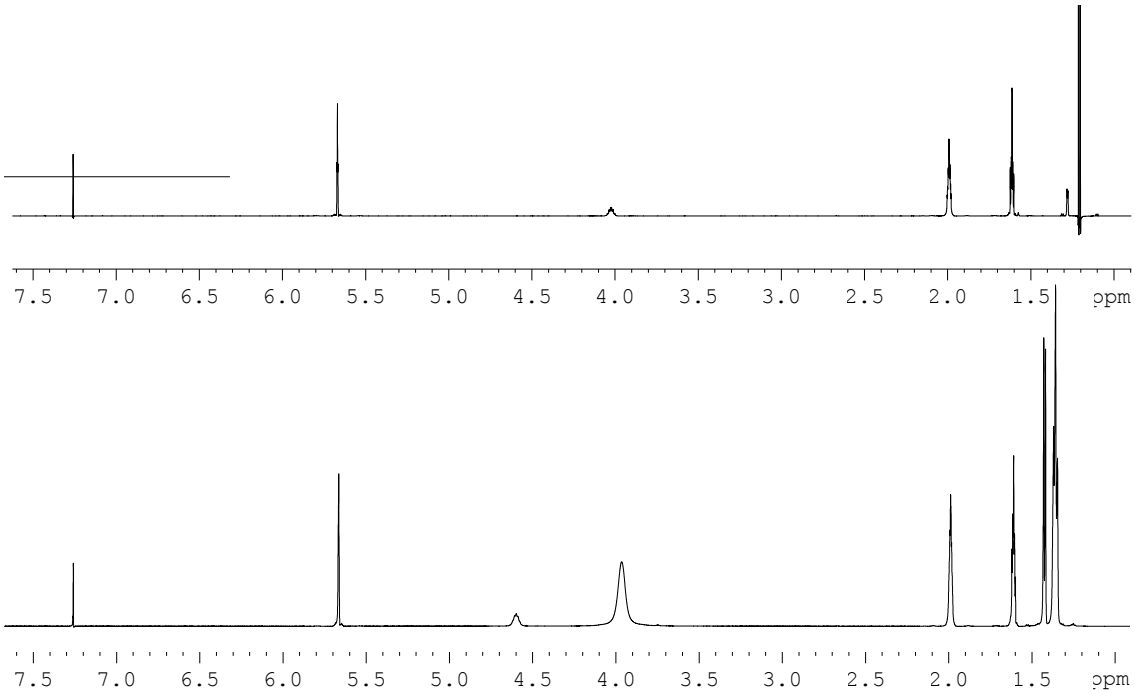


Fig. S12: Negative control: Comparison of a solution without donor (1) before (bottom) and after (top) promotor addition.

4.4. Allyl methyl ether (entry III)

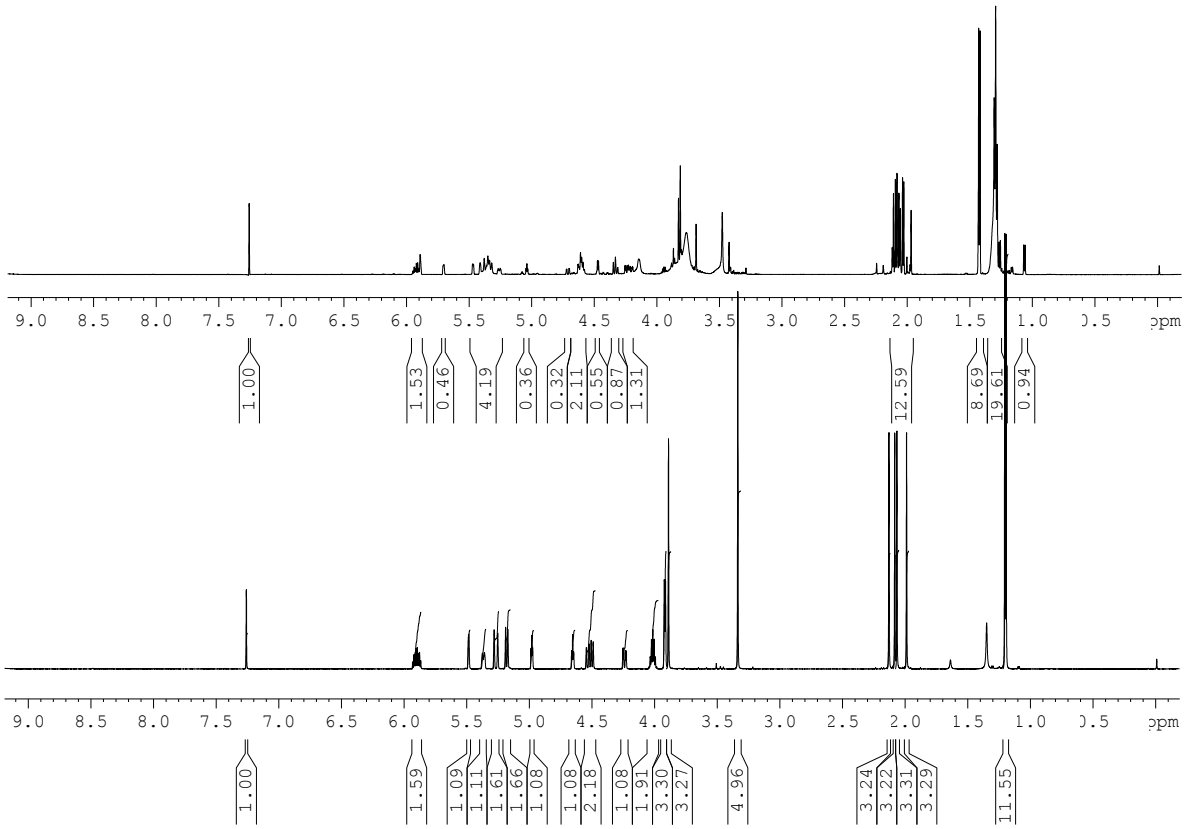


Fig. S13: Comparison of ^{1}H NMR spectra before (bottom) and after (top) donor activation (reaction time = 1h).

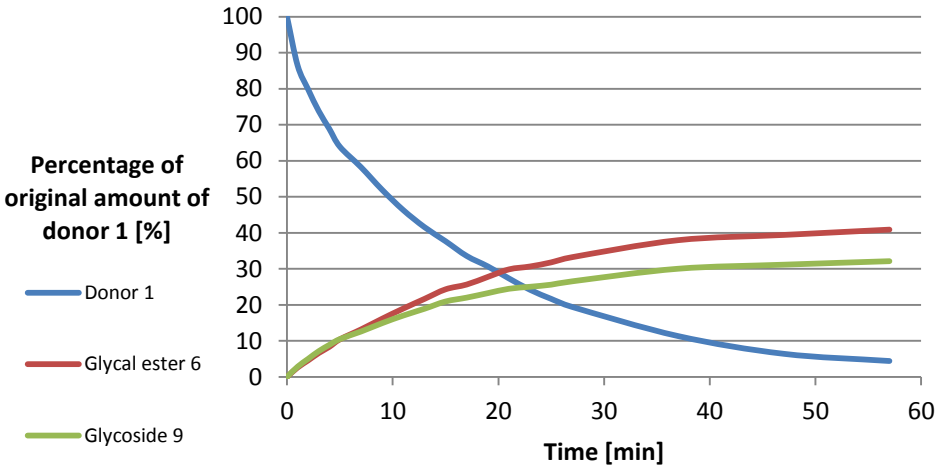


Fig. S14: The decrease of donor **1** correlates with the parallel formation of glycal ester **6** and glycoside **9**. This shows, that both pathways are active. The total amount of glycal **6** and glycoside **9** did not reach 100% due to some residual donor (~ 4%) and formation of hydrolysed donor.

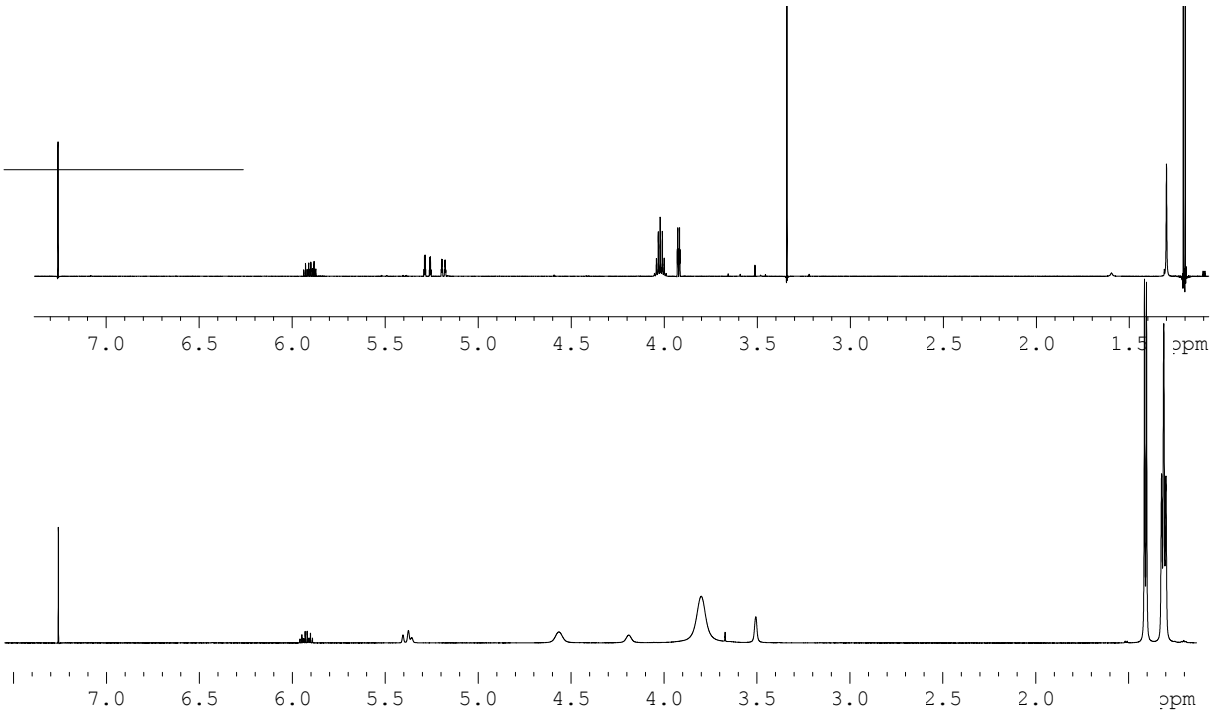


Fig. S15: Negative control: Comparison of a solution without donor 1 before (bottom) and after (top) promotor addition.

4.5. Methyl crotonate (entry IV)

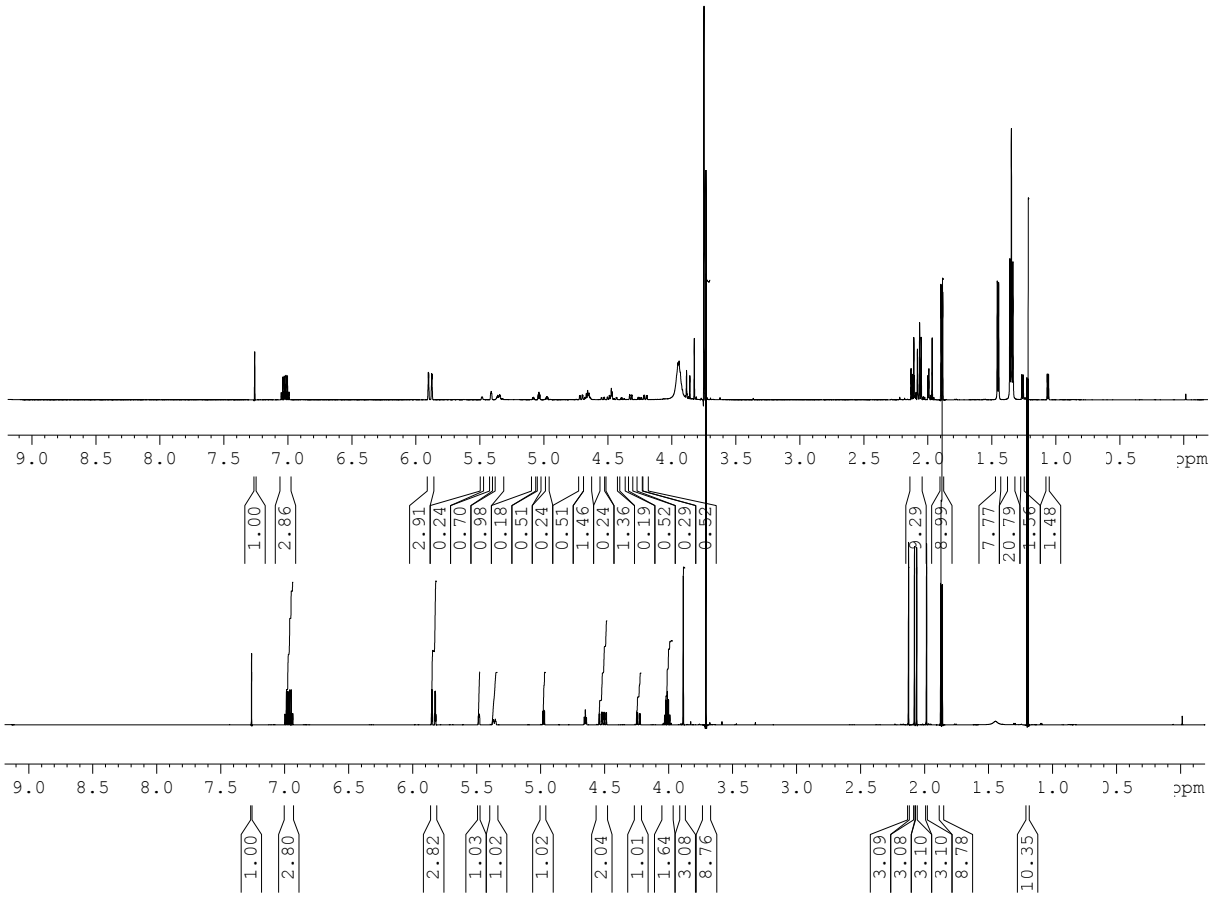
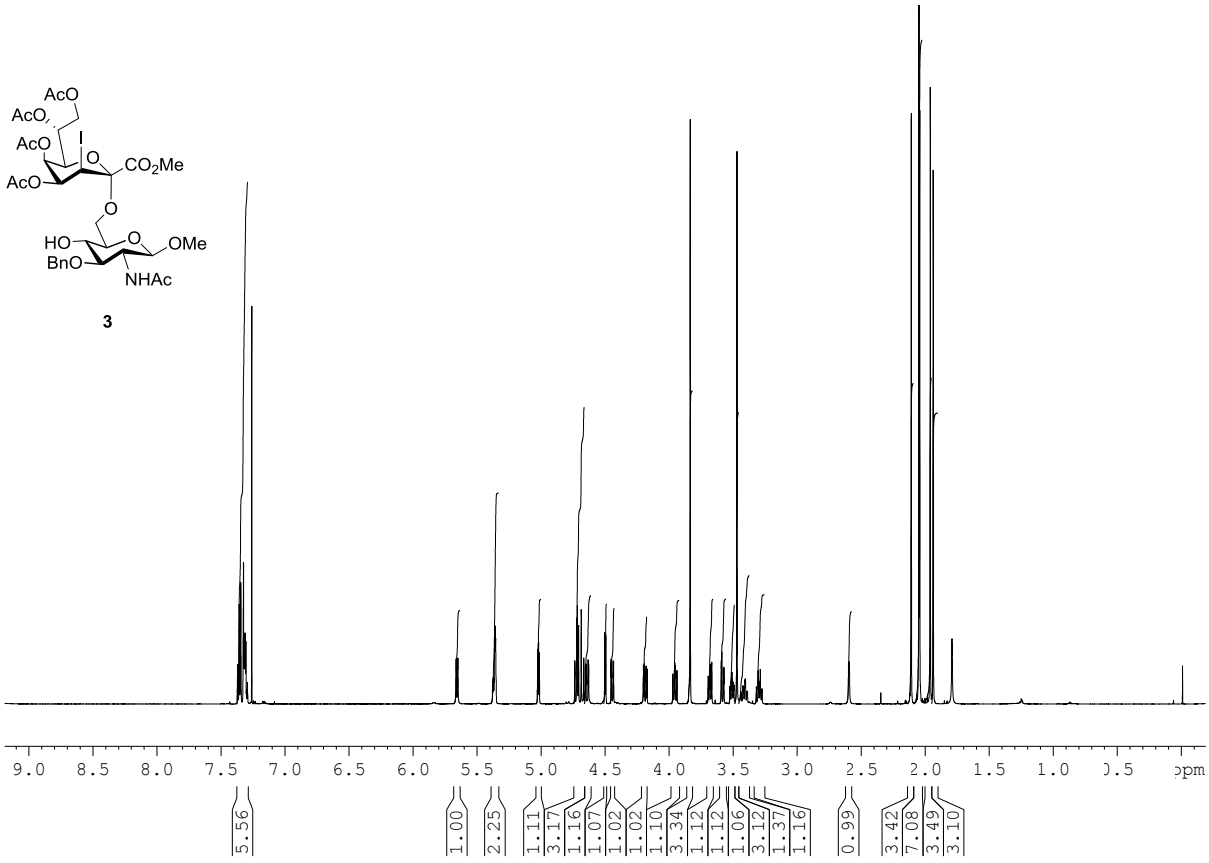
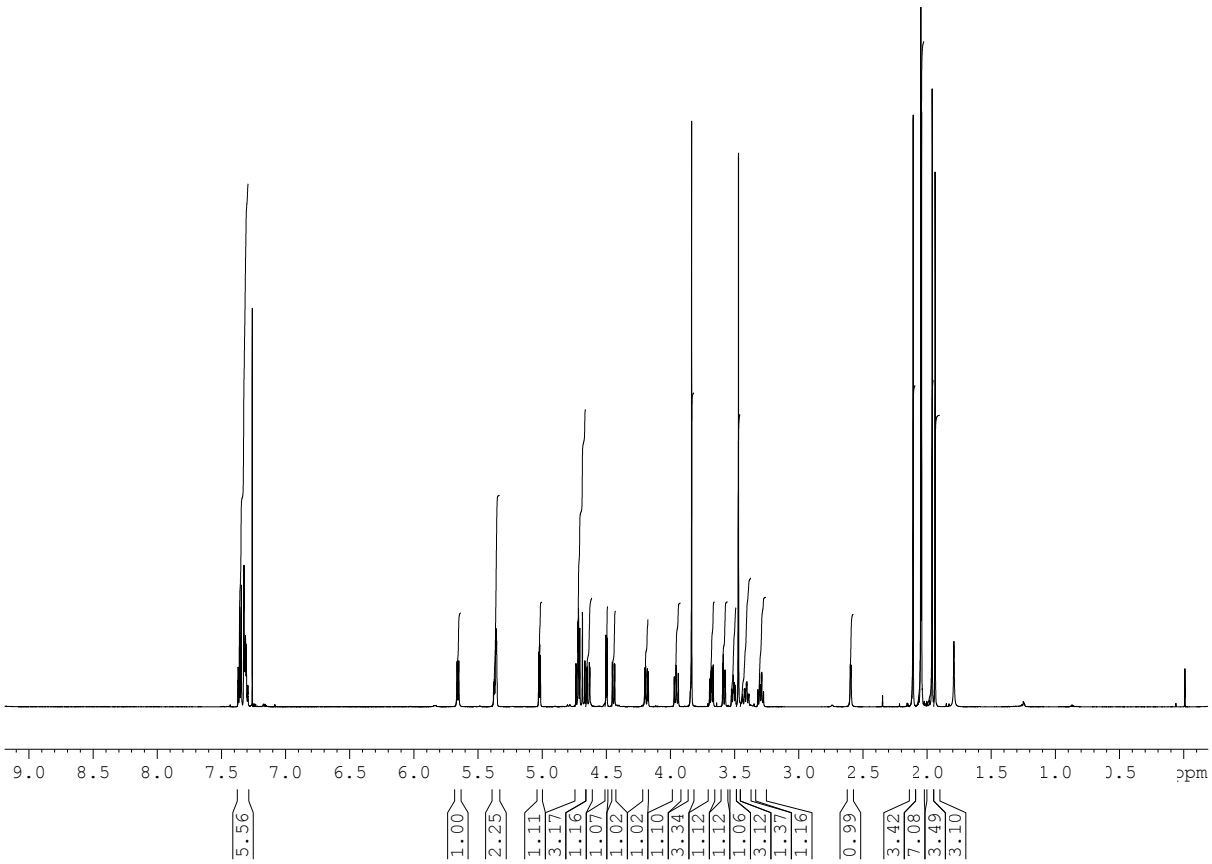


Fig. S16: Comparison of ^{1}H NMR spectra before (bottom) and after (top) donor activation (reaction time = 1 h).

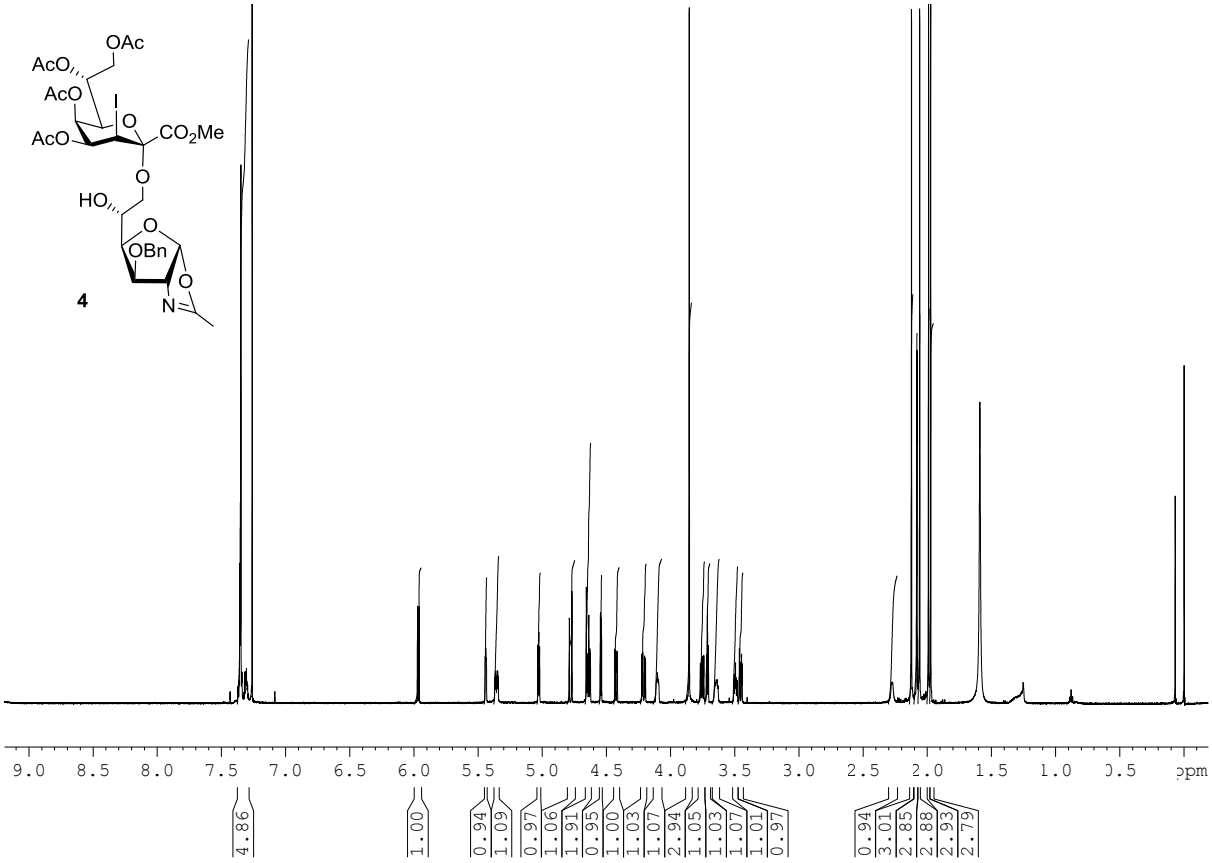
5. NMR spectra of compounds (3) – (5) and (15) – (20)



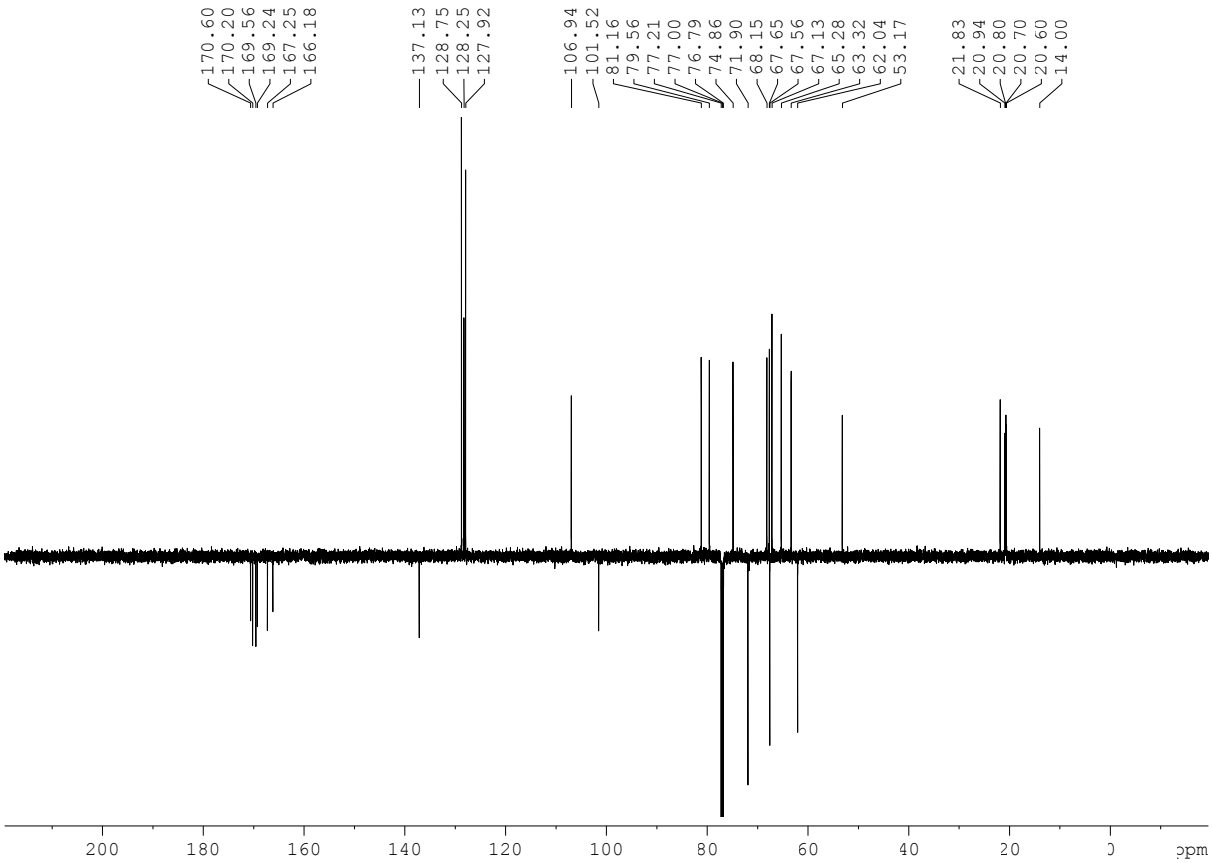
^{1}H NMR ($CDCl_3$, 600 MHz)



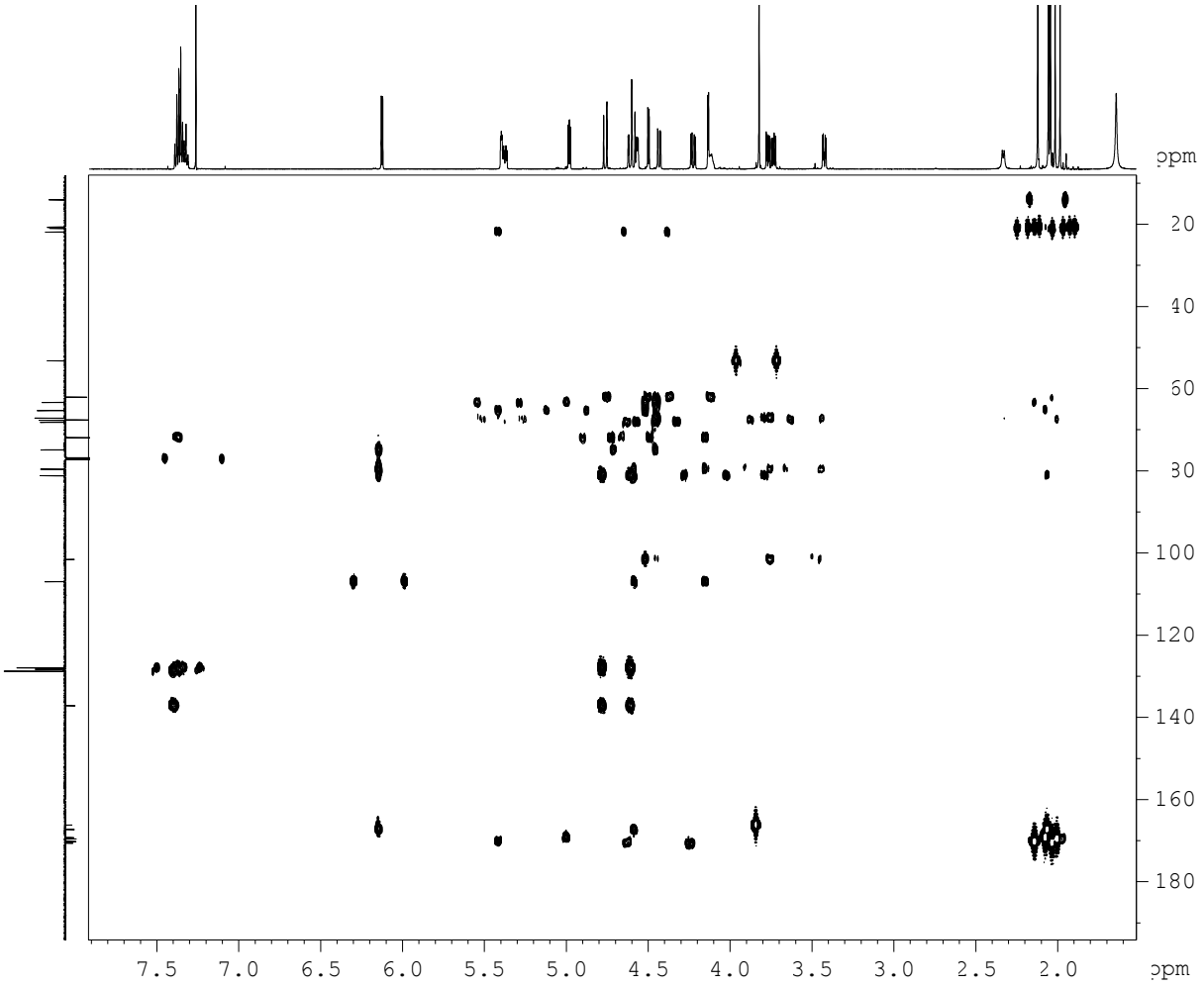
^{13}C NMR ($CDCl_3$, 150 MHz)



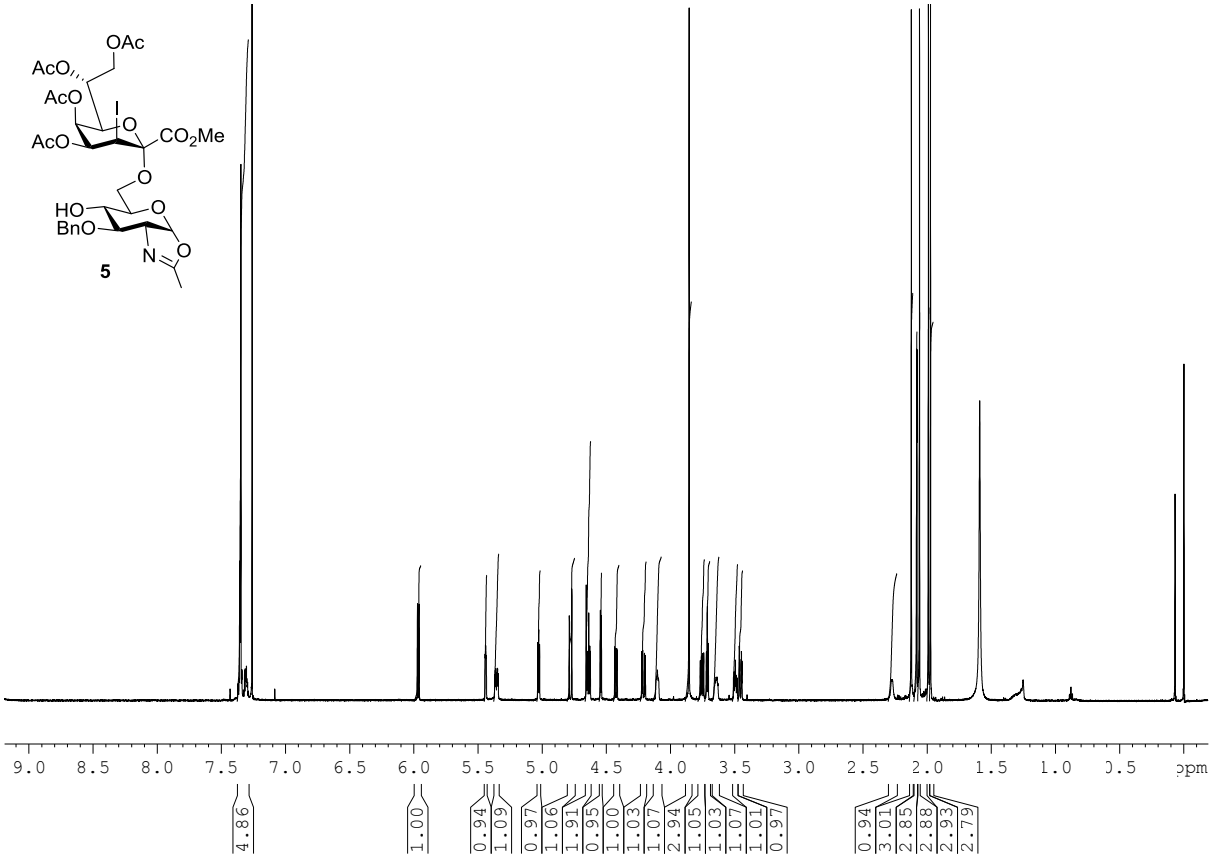
^{1}H NMR ($CDCl_3$, 600 MHz)



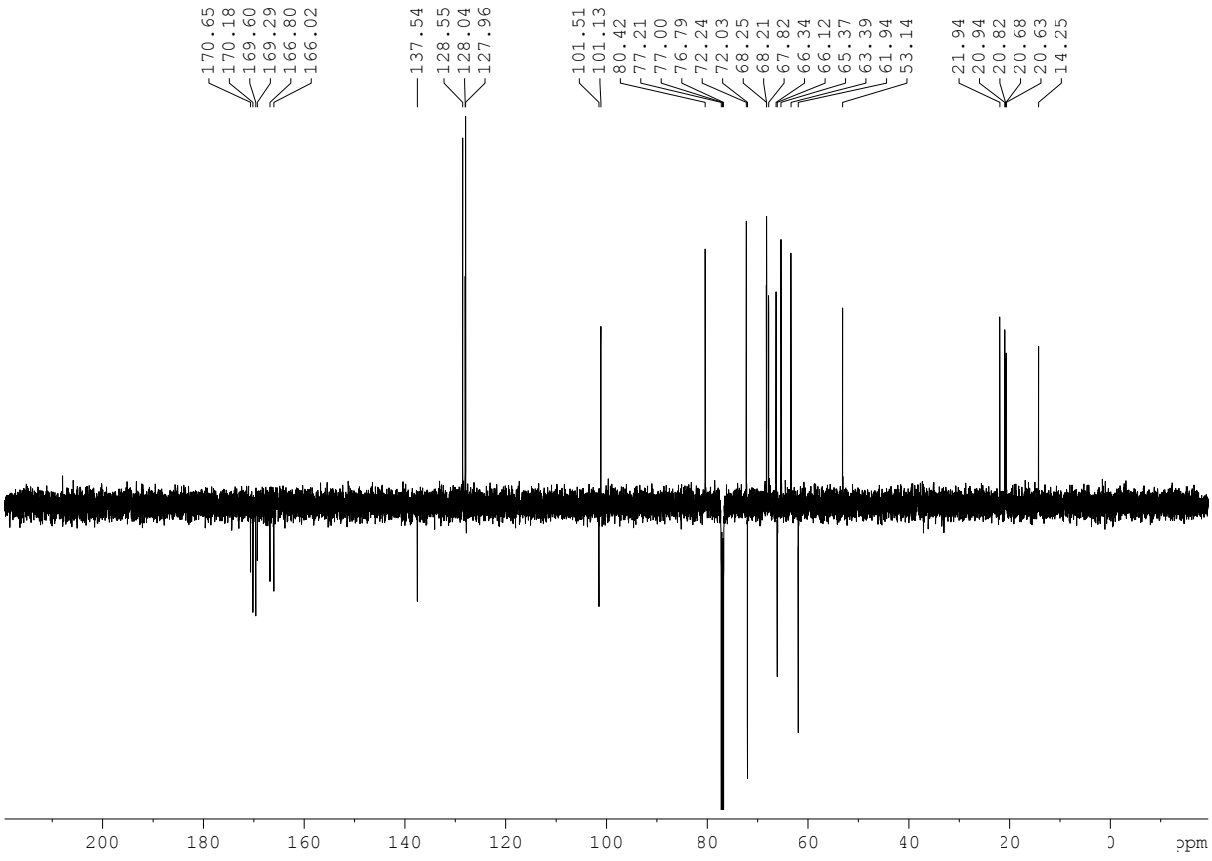
^{13}C NMR ($CDCl_3$, 150 MHz)



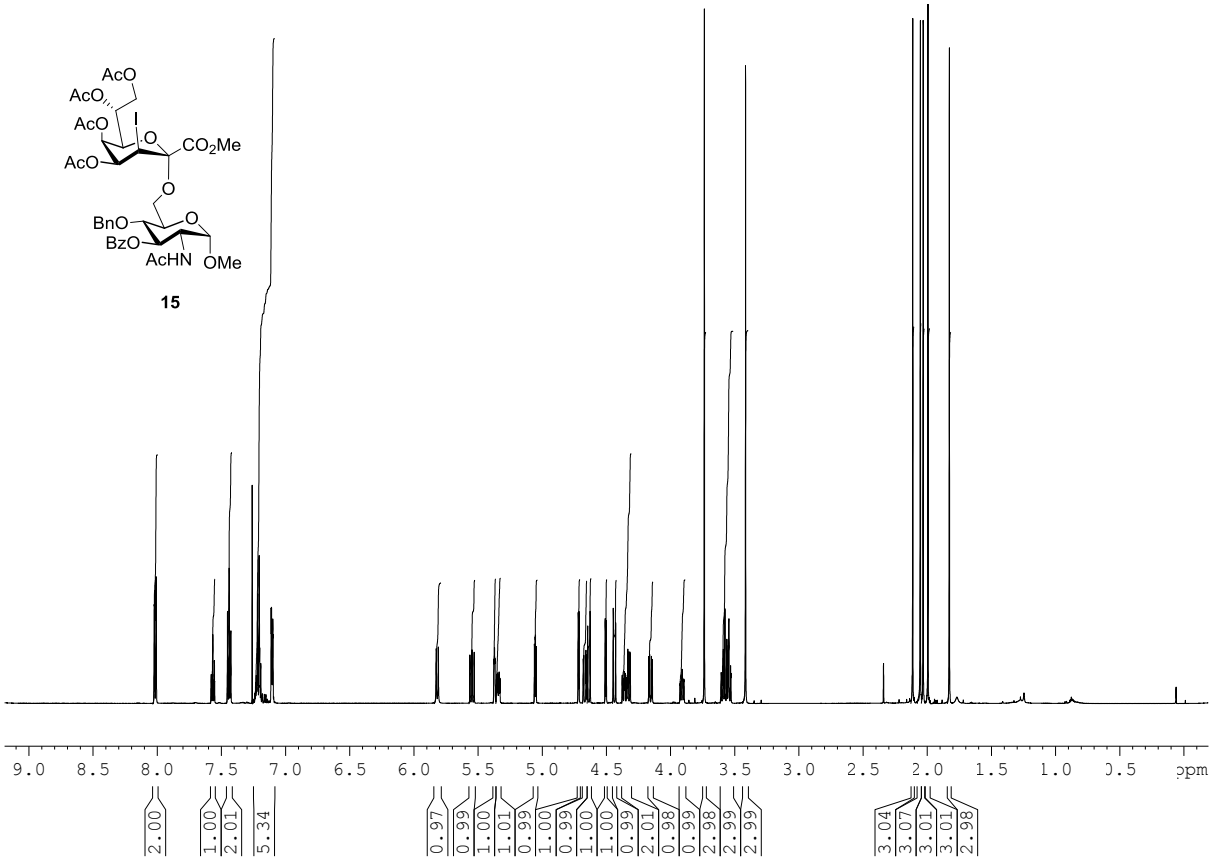
HMBC ($CDCl_3$, 600 MHz)



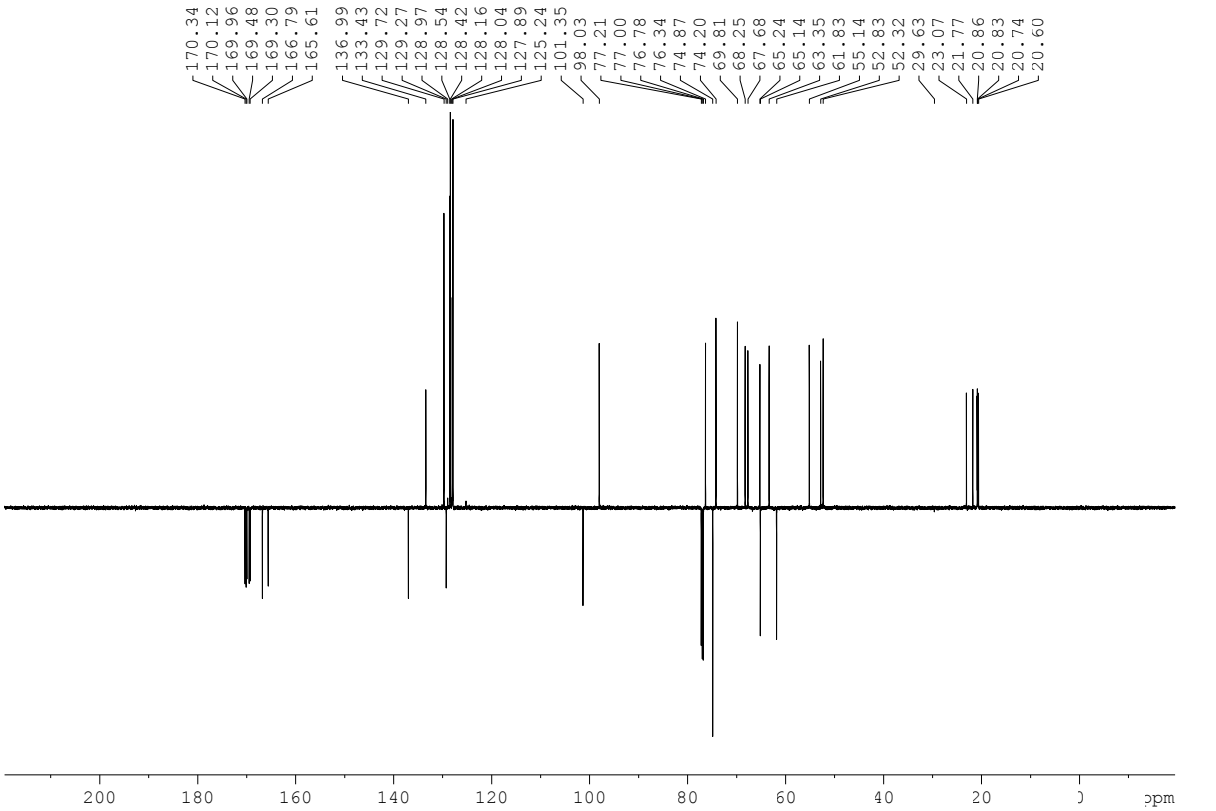
^{1}H NMR ($CDCl_3$, 600 MHz)



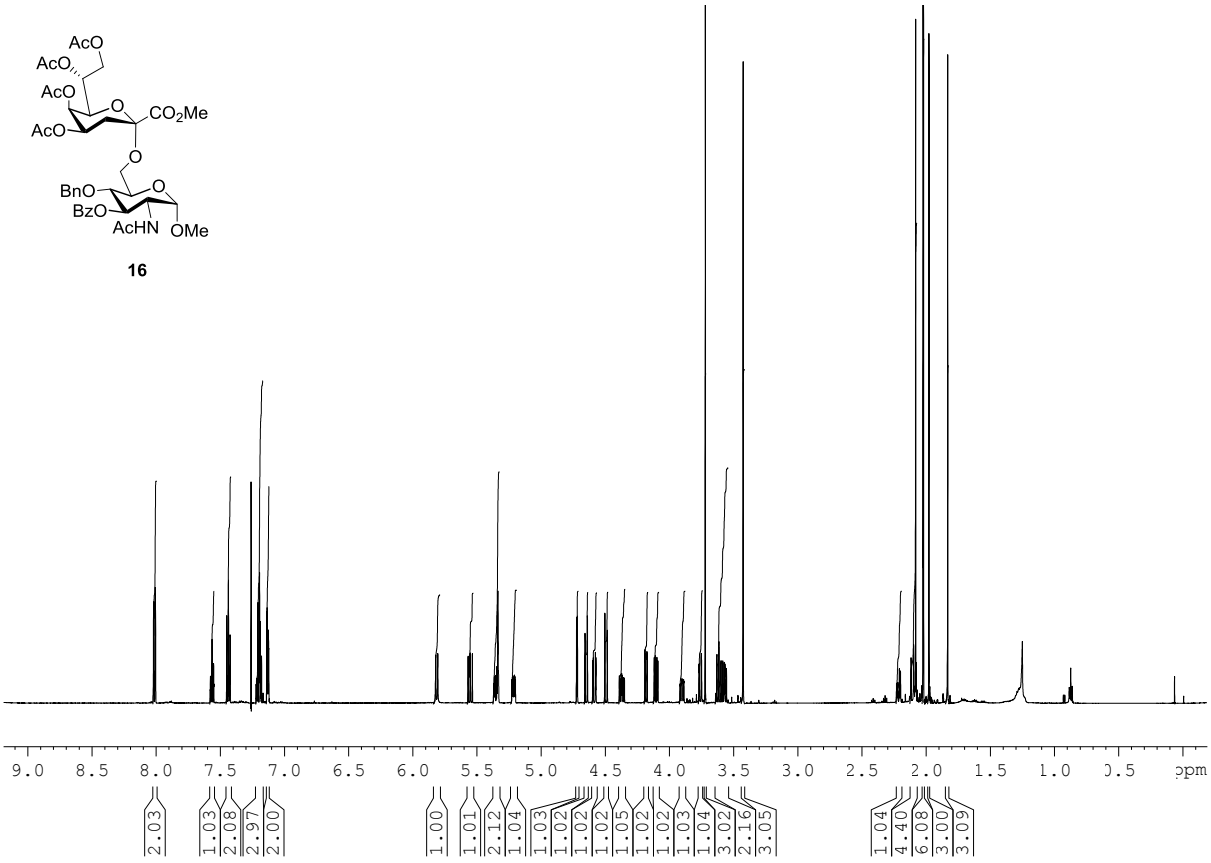
^{13}C NMR ($CDCl_3$, 150 MHz)



^{1}H NMR ($CDCl_3$, 600 MHz)

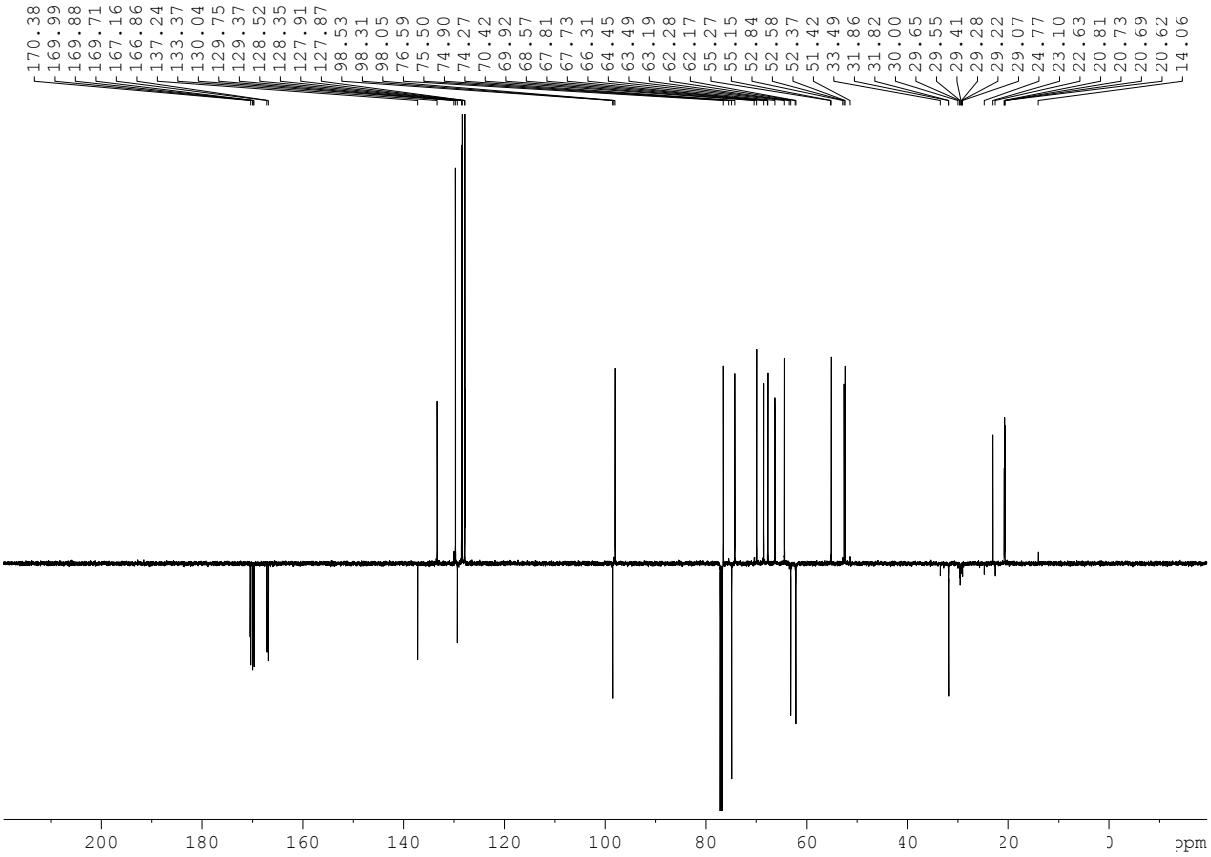


^{13}C NMR ($CDCl_3$, 150 MHz)

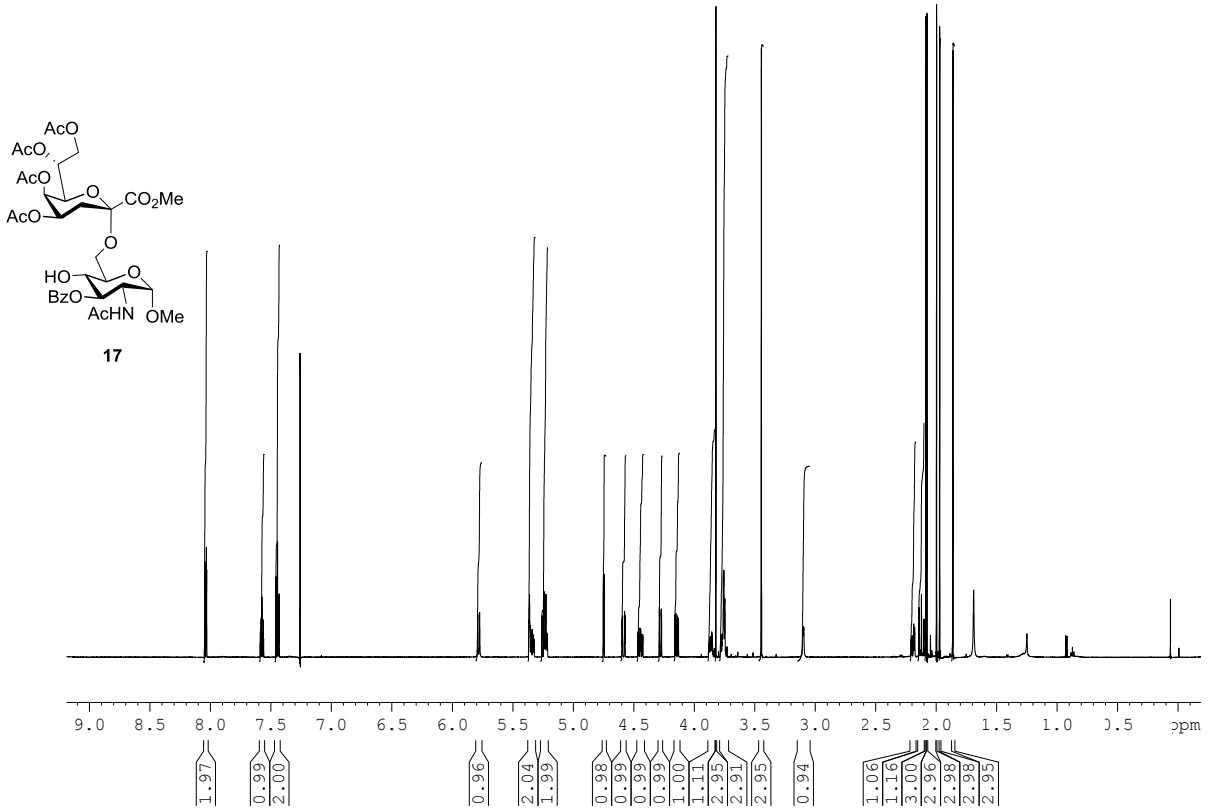


16

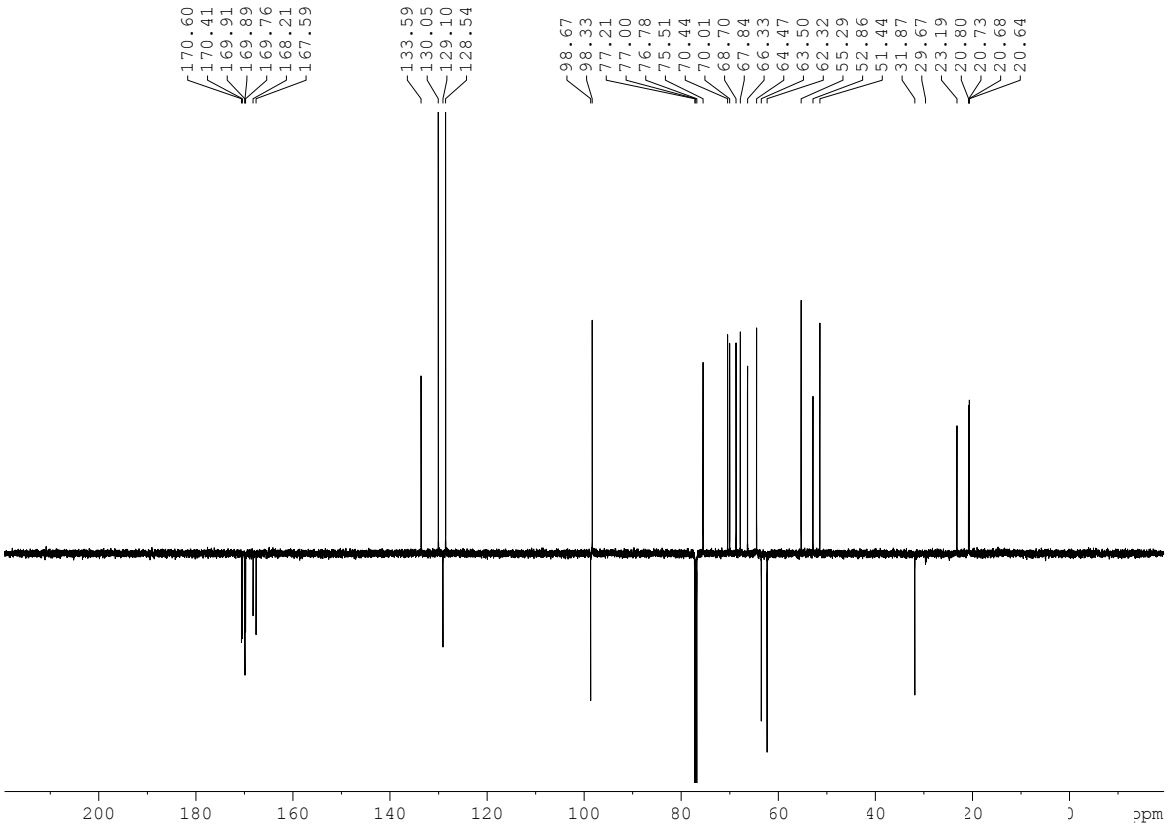
^{1}H NMR ($CDCl_3$, 600 MHz)



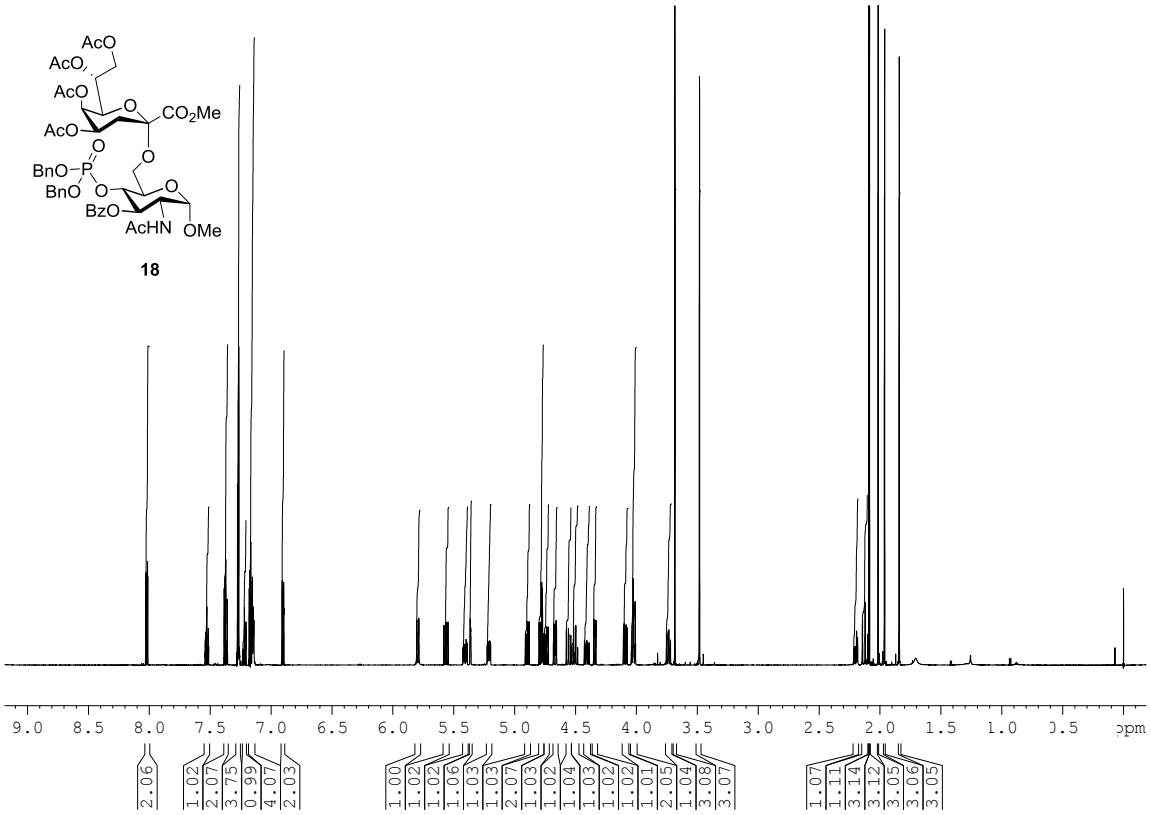
^{13}C NMR ($CDCl_3$, 150 MHz)



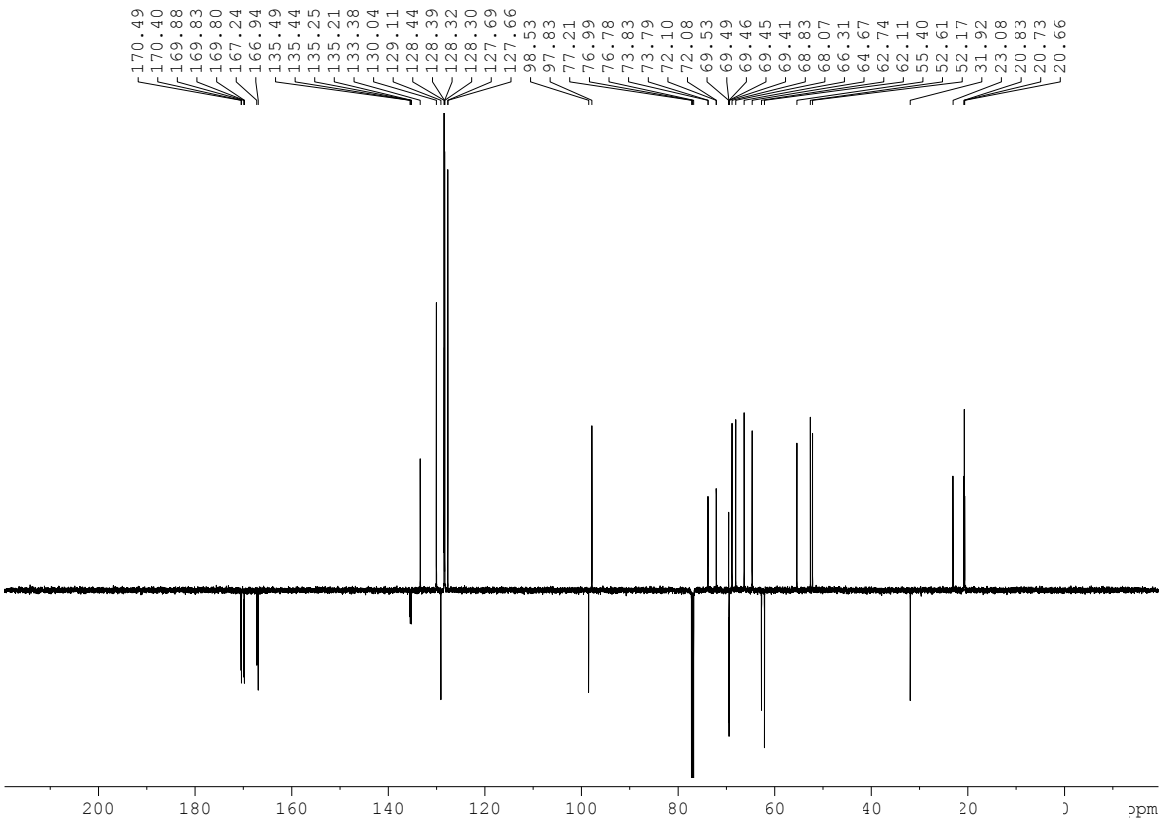
^{1}H NMR ($CDCl_3$, 600 MHz)



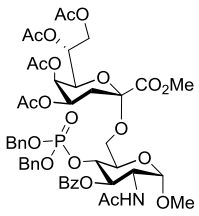
^{13}C NMR ($CDCl_3$, 150 MHz)



^{1}H NMR ($CDCl_3$, 600 MHz)

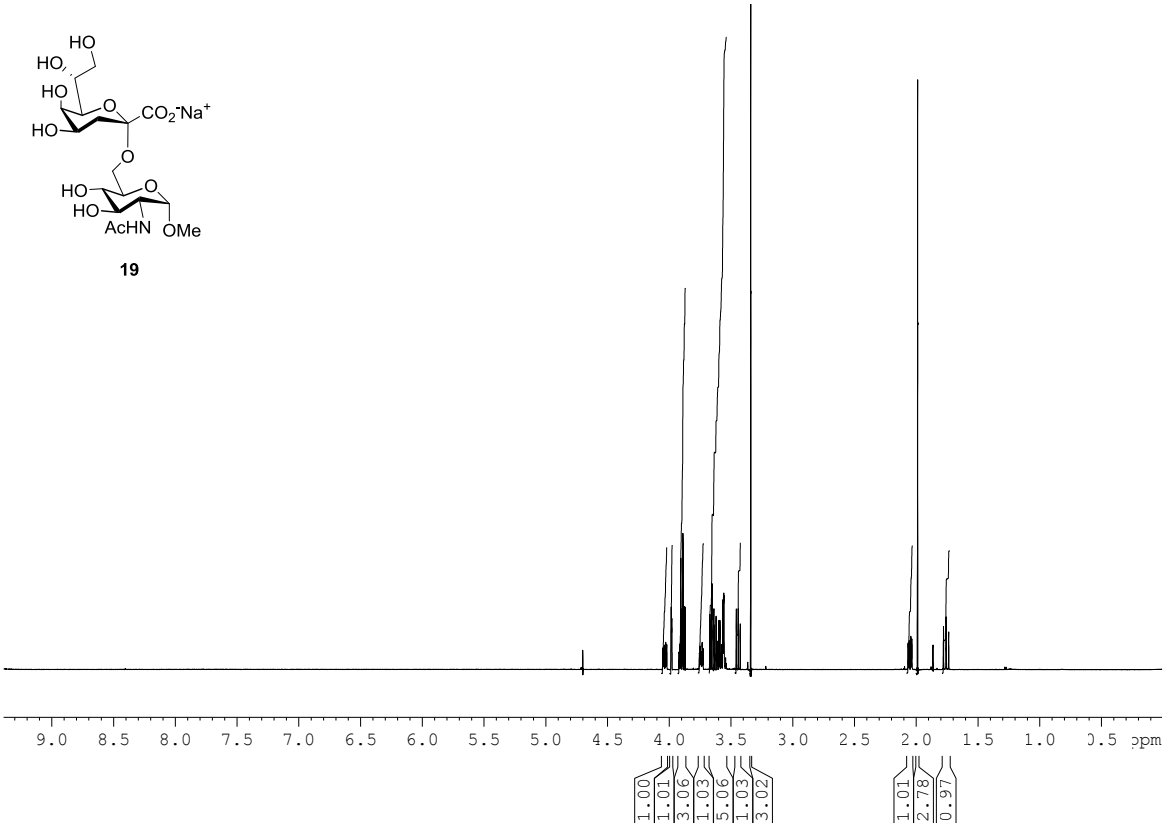


^{13}C NMR ($CDCl_3$, 150 MHz)



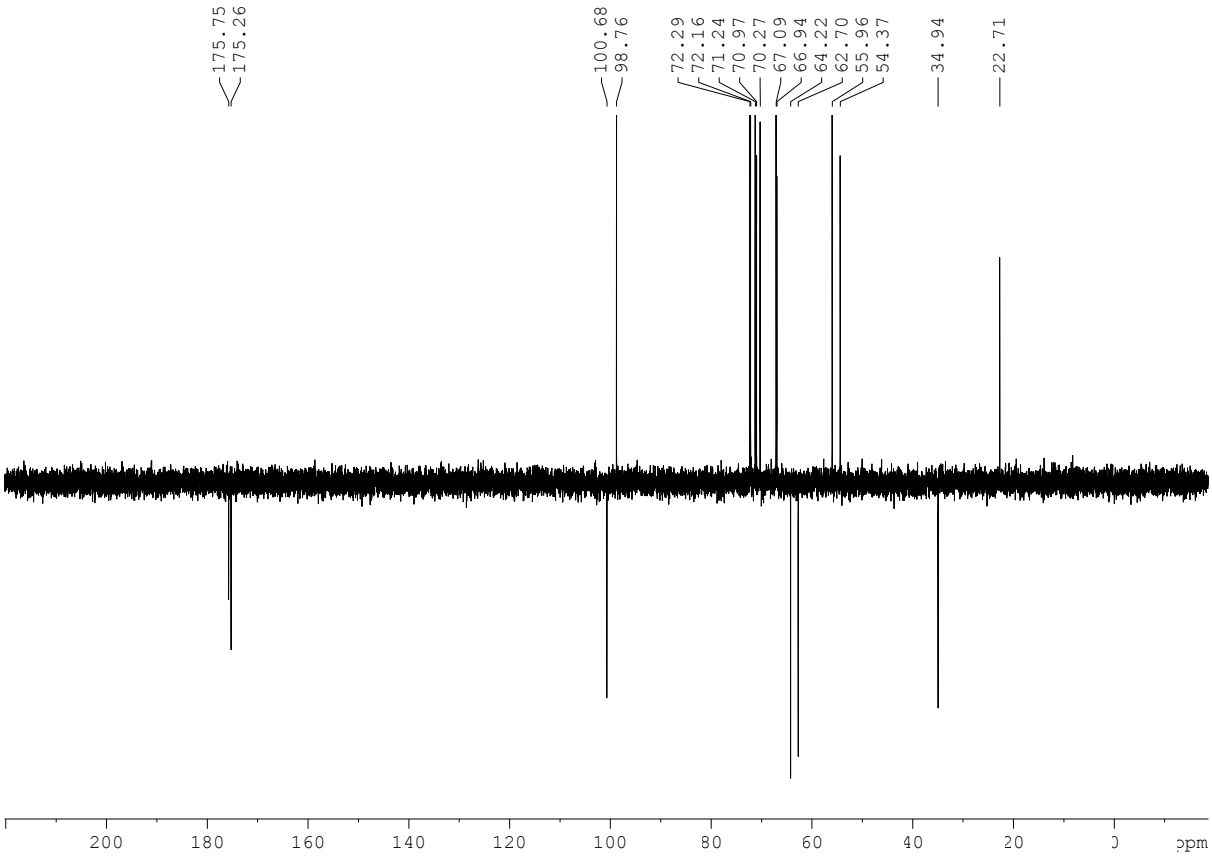
18

^{31}P NMR ($CDCl_3$, 243 MHz)

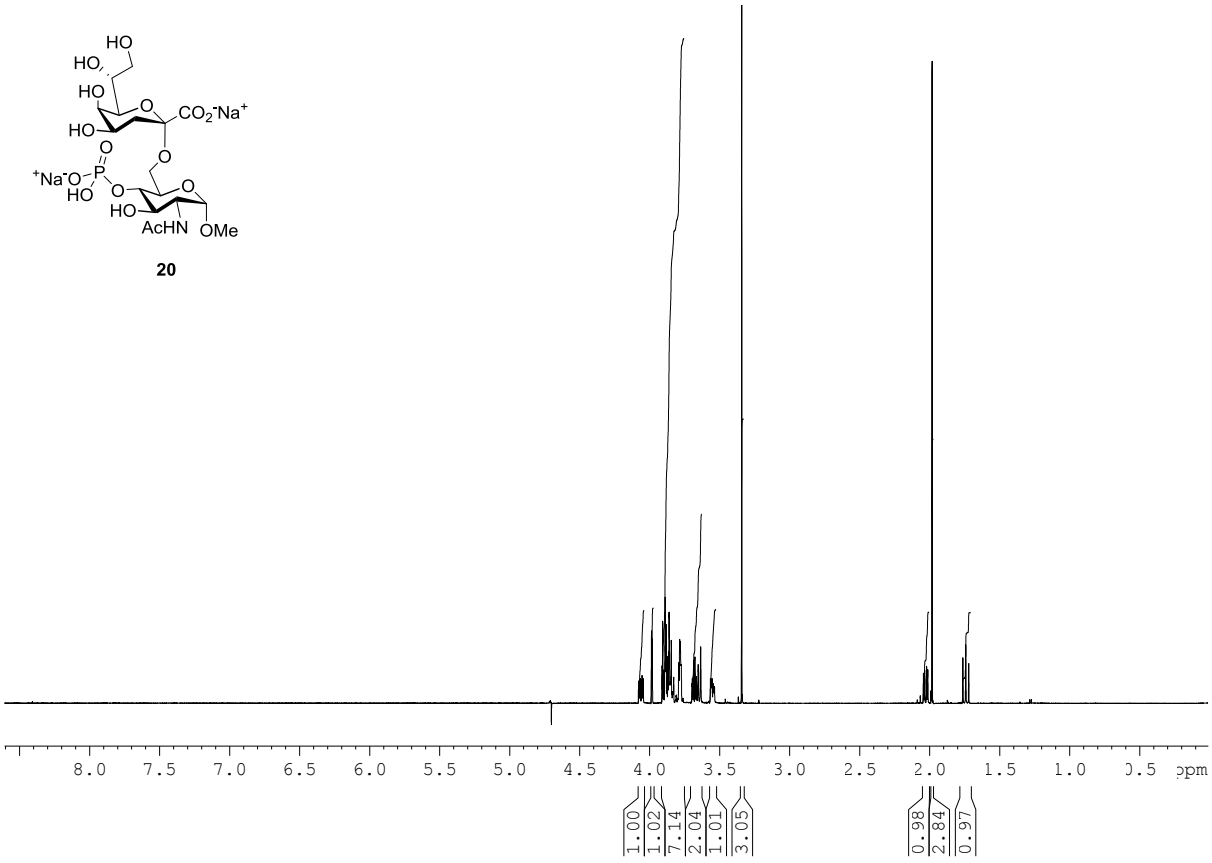


19

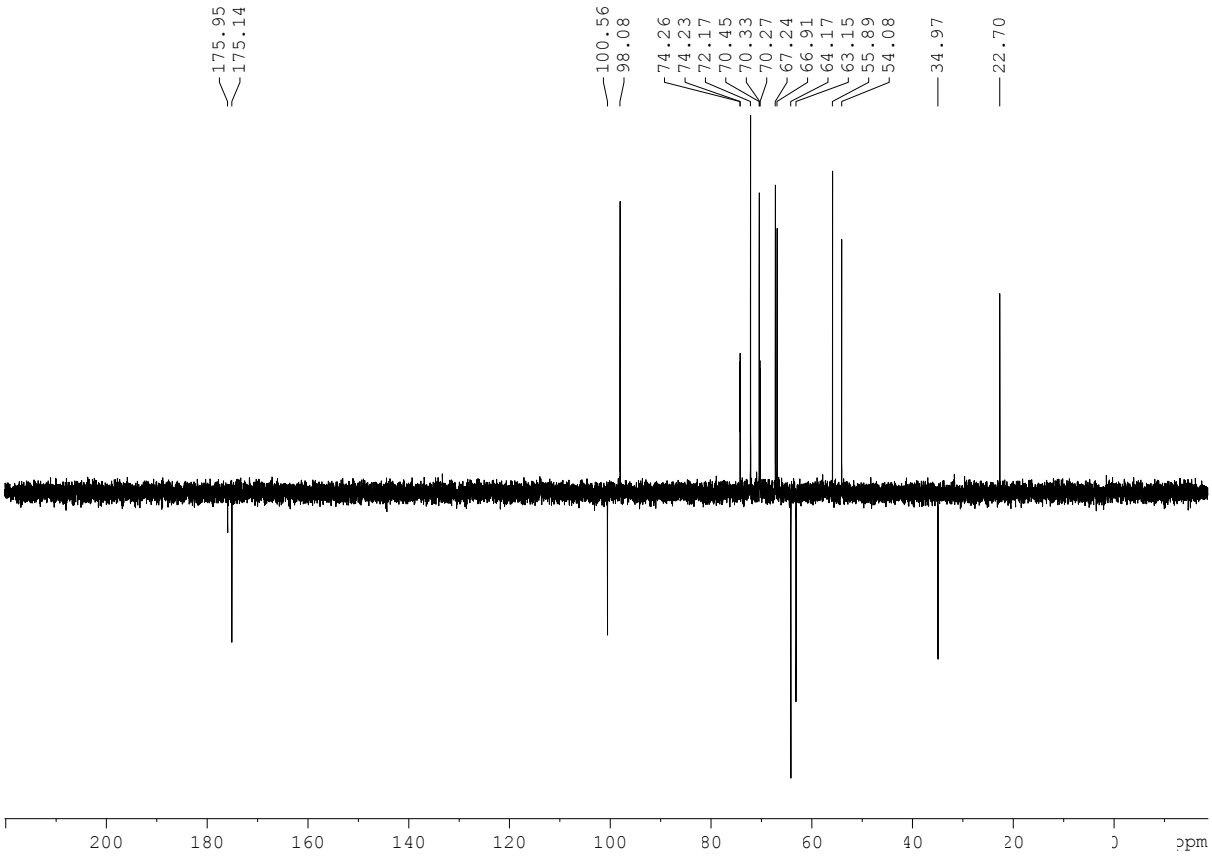
^{1}H NMR (D_2O, 600 MHz, pH ~ 7.0)



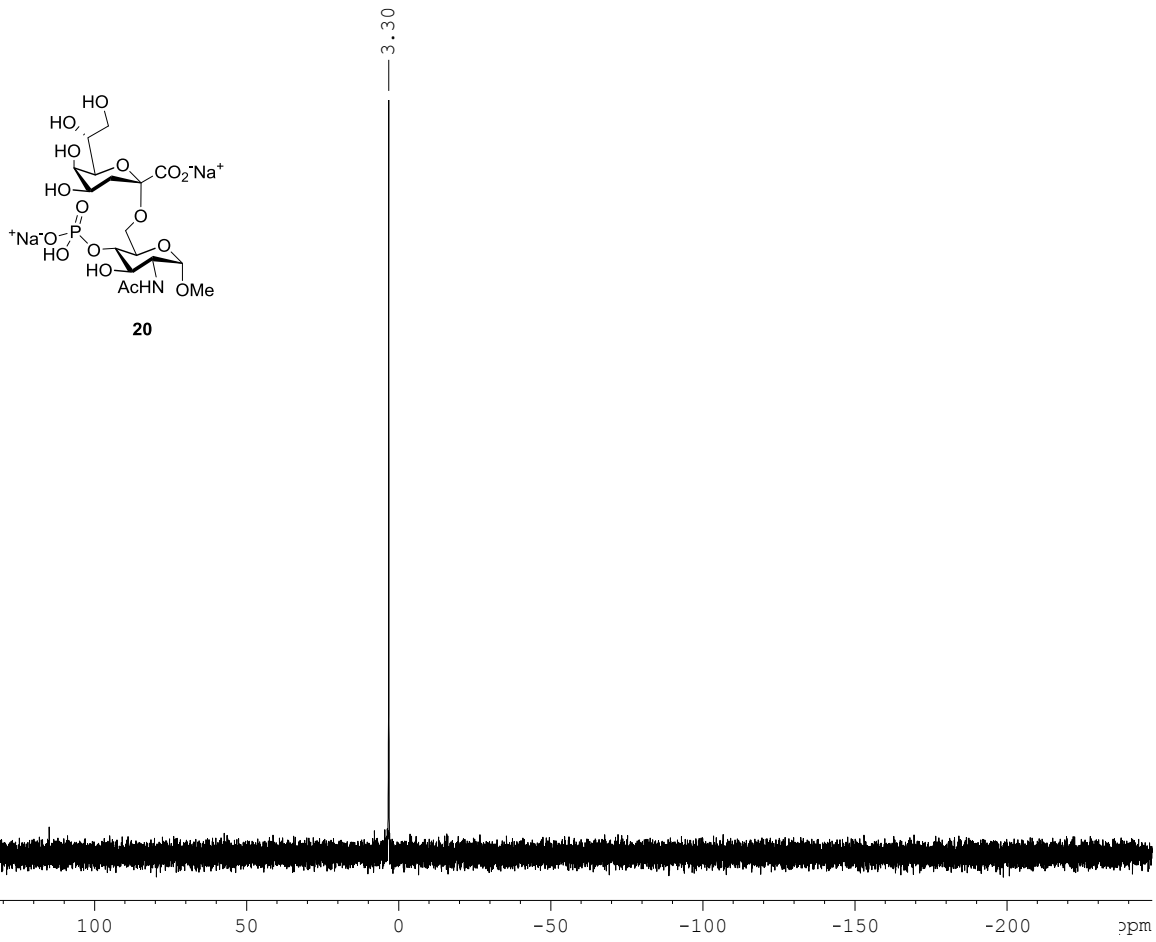
^{13}C NMR (D_2O, 150 MHz, pH ~ 7.0)



^{1}H NMR (D_2O, 600 MHz, pH ~ 7.0)



^{13}C NMR (D_2O, 150 MHz, pH ~ 7.0)



20

^{31}P NMR (D_2O, 243 MHz, pH ~ 7.0)

6. References

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[S9] Jpn. Kokai Tokkyo Koho JP 60 51, 702 [85 51, 702]; Chem. Abstr. 104, 34298j (**1985**)

[S10] T. Ogawa, T. Kitajima, T. Nukada, *Carbohydr. Res*. **1983**, *123*, C5-C7.

[S11] LC-MS data were obtained on a Shimadzu LC-MS-2020 quadrupole (ESI source) instrument.

Manuscript #5

Supporting Information

doi:10.1021/ol5033128

Supporting information

First and Stereoselective Synthesis of an α-(2→5)-Linked Disaccharide of 3-Deoxy-D-*manno*-oct-2-ulosonic Acid (Kdo)

Barbara Pokorny, Paul Kosma*

Department of Chemistry, University of Natural Resources and Life Sciences-Vienna, Muthgasse 18, A-1190 Vienna, Austria

Email: paul.kosma@boku.ac.at

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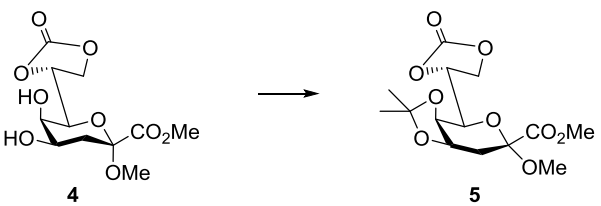
1. General

All purchased chemicals were used without further purification unless stated otherwise. Solvents were dried over activated 4 Å (CH_2Cl_2, DMF, pyridine, toluene, acetone) molecular sieves. 2-Propanol for glycosylation was dried over 5 Å molecular sieves for 24 h. Anhydrous MeOH (secco solv) was purchased from Merck. Cation exchange resin DOWEX 50 H^+ was regenerated by consecutive washing with HCl (3 M), water and anhydrous MeOH. Aqueous solutions of salts were saturated unless stated otherwise. Concentration of organic solutions was performed under reduced pressure < 40 °C. Optical rotations were measured with a Perkin-Elmer 243 B Polarimeter. $[\alpha]_D^{20}$ values are given in units of 10^{-1}deg cm^2g^{-1}. Thin layer chromatography was performed on Merck precoated plates: generally on 5 x 10 cm, layer thickness 0.25 mm, Silica Gel $60F_{254}$; alternatively on HP-TLC plates with 2.5 cm concentration zone (Merck). Spots were detected by dipping reagent (anisaldehyde-H_2SO_4). For column chromatography silica gel (0.040 – 0.063 mm) was used. HP-column chromatography was performed on a pre-packed column (YMC-Pack SIL-06, 0.005 mm, 250x20 mm). Size exclusion chromatography was performed on Bio-Gel® P-2 Gel extra fine < 45 µm (wet) (1 x 30 cm) and on pre-packed PD-10 columns (GE Healthcare, Sephadex™ G-25 M). NMR spectra were recorded with a Bruker Avance III 600 instrument (600.22 MHz for ^1H, 150.93 MHz for ^{13}C and 564.77 MHz for ^{19}F) at 300.15 K using standard Bruker NMR software unless stated otherwise. Alternatively, spectra were recorded with a Bruker DPX 300 instrument (300.13 MHz for ^1H, 75.47 MHz for ^{13}C). ^1H NMR spectra were referenced to 7.26 ($CDCl_3$), 3.31 (MeOD), 2.08 (d_8-toluene) and 0.00 (D_2O, external calibration to 2,2-dimethyl-2-silapentane-5-sulfonic acid) ppm. ^{13}C NMR spectra were referenced to 77.00 ($CDCl_3$), 49.00 (MeOD), 20.43 (d_8-toluene) and 67.40 (D_2O, external calibration to 1,4-dioxane) ppm. ^{19}F NMR spectra were indirectly referenced according to IUPAC recommendations[S1]. ESI-MS data were obtained on a Waters Micromass Q-TOF Ultima Global instrument.

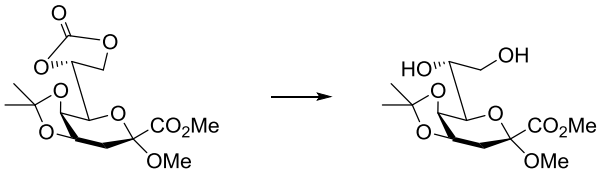
2. Synthesis and characterization of compounds

2.1 Methyl (methyl 7,8-*O*-carbonyl-3-deoxy-4,5-*O*-isopropylidene-α-D-*manno*-oct-2-ulopyranosid)onate (5)



A solution of the known 7,8-O-carbonyl derivative **4**[S2] (0.37 g, 1.27 mmol) in anhydrous CH_2Cl_2 (32 mL) and anhydrous acetone (0.65 mL) was treated with TMSOTf (0.29 mL, 1.58 mmol) at 0 °C under an atmosphere of argon. After 4 h at 0 °C triethylamine (0.4 mL) was added dropwise and after complete addition the solution was stirred for 10 min. The mixture was diluted with CH_2Cl_2 and washed with aq. $NaHCO_3$. The organic phase was dried ($MgSO_4$), filtered and concentrated. Filtration of the residue over silica gel (EtOAc) afforded **5** (0.40 g, 95%) as a colorless oil; $[\alpha]_D^{20}$ + 71.5 (*c* 0.99, $CHCl_3$); R_f 0.51 (toluene/EtOAc 1:2); ^{1}H NMR ($CDCl_3$): δ 4.94 (ddd, 1H, $J_{7,8b}$ 8.3, $J_{7,8a}$ 7.0, $J_{7,6}$ 3.3 Hz, H-7), 4.80 (dd, 1H, $J_{8a,8b}$ 9.0 Hz, H-8a), 4.55 - 4.50 (m, 2H, H-4, H-8b), 4.21 (dd, 1H, $J_{5,4}$ 7.6, $J_{5,6}$ 2.0 Hz, H-5), 3.99 (dd, 1H, H-6), 3.78 (s, 3H, CO_2CH_3), 3.21 (s, 3H, OCH_3), 2.74 (dd, 1H, $J_{3a,3b}$ 15.5, $J_{3a,4}$ 4.4 Hz, H-3a), 1.88 (dd, 1H, $J_{3b,4}$ 3.4 Hz, H-3b), 1.39 [s, 3H, $C(CH_3)$], 1.26 [s, 3H, $C(CH_3)$]; ^{13}C NMR ($CDCl_3$): δ 168.69 (s, C-1), 154.67 [s, O(*C*=O)O], 109.95 [s, $C(CH_3)_2$], 97.78 (s, C-2), 75.53 (d, C-7), 71.63 (d, C-5), 70.00 (d, C-4), 69.61 (d, C-6), 65.86 (t, C-8), 52.45 (q, CO_2CH_3), 50.85 (q, OCH_3), 32.79 (t, C-3), 25.25 [q, $C(CH_3)$], 24.57 [q, $C(CH_3)$]; ESI-TOF HRMS: *m/z* = 350.1464; calcd for $C_{14}H_{20}O_9NH_4^+$: 350.1446.

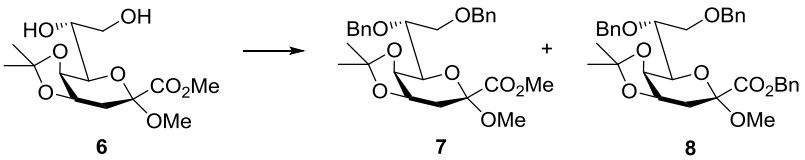
2.2 Methyl (methyl 3-deoxy-4,5-*O*-isopropylidene-α-D-*manno*-oct-2-ulopyranosid)onate (6)



5 **6**

A solution of 0.1 M methanolic sodium methoxide (4.0 mL, 0.40 mmol) was added dropwise to a solution of **5** (0.40 g, 1.20 mmol) in anhydrous MeOH (15 mL) and the mixture was stirred 14 h at ambient temperature. The solution was treated with ion exchange resin DOWEX 50 (H^+ form) to give a neutral pH. The resin was filtered off, and concentration of the filtrate afforded pure **6**[S3] (0.36 g, 97%) as a colorless oil; $[\alpha]_D^{20}$ + 64.5 (*c* 1.16, $CHCl_3$); R_f 0.29 (toluene/EtOAc 1:2); ^{1}H NMR (MeOD): δ 4.51 (ddd, 1H, $J_{4,5}$ 7.6, $J_{4,3a}$ 4.1, $J_{4,3b}$ 2.9 Hz, H-4), 4.38 (dd, 1H, $J_{5,6}$ 1.8 Hz, H-5), 3.91 (ddd, 1H, $J_{7,6}$ 8.9, $J_{7,8b}$ 5.5, $J_{7,8a}$ 2.9 Hz, H-7), 3.84 (dd, 1H, $J_{8a,8b}$ 11.3 Hz, H-8a), 3.75 (s, 3H, CO_2CH_3), 3.65 (dd, 1H, H-8b), 3.62 (dd, 1H, H-6), 3.18 (s, 3H, OCH_3), 2.61 (dd, 1H, $J_{3a,3b}$ 15.2 Hz, H-3a), 1.90 (dd, 1H, H-3b), 1.37 [s, 3H, $C(CH_3)$], 1.30 [s, 3H, $C(CH_3)$]; ^{13}C NMR (MeOD, 75 MHz): δ 171.10 (s, C-1), 110.13 [s, $C(CH_3)_2$], 99.29 (s, C-2), 73.12 (d, C-5), 71.67 (d, C-6), 71.51 (d, C-4), 70.94 (d, C-7), 64.67 (t, C-8), 52.78 (q, CO_2CH_3), 50.97 (q, OCH_3), 33.79 (t, C-3), 26.09 [q, $C(CH_3)$], 25.12 [q, $C(CH_3)$]; ESI-TOF HRMS: *m/z* = 324.1655; calcd for $C_{13}H_{22}O_8NH_4^+$: 324.1653.

2.3 Methyl (methyl 7,8-di-*O*-benzyl-3-deoxy-4,5-*O*-isopropylidene-α-D-*manno*-oct-2-ulopyranosid)onate (7) and benzyl (methyl 7,8-di-*O*-benzyl-3-deoxy-4,5-*O*-isopropylidene-α-D-*manno*-oct-2-ulopyranosid)onate (8)



NaH (0.18 g, 4.60 mmol) was added in small portions at 0 °C to a solution of **6** (0.35 g, 1.15 mmol) in anhydrous DMF (14 mL). After complete addition the mixture was stirred at 0 °C for 30 min. Then, BnBr (0.55 mL, 4.60 mmol) was added dropwise and after 3 h at ambient temperature the mixture was cooled to 0 °C and diluted with anhydrous CH_2Cl_2. Anhydrous MeOH (1.0 mL) was added dropwise and after completed addition the mixture was immediately subjected to extraction with CH_2Cl_2 and aq. $NaHCO_3$. The organic phase was dried ($MgSO_4$), filtered and concentrated. Column chromatography (SiO_2, toluene/EtOAc 15:1) afforded partly separated[S4] methyl ester **7** and benzyl ester **8** (0.33 g; **7**: 54%; **8**: 5%) as colorless oils. Additionally, a mixture of different mono-benzylated compounds could be isolated (~18%).

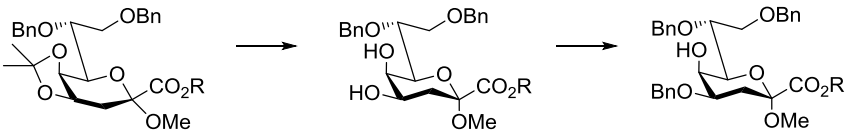
To improve the yield, **6** (22.0 mg, 0.072 mmol) was dissolved in anhydrous DMF (1.0 mL) and treated with NaH (17 mg, 0.431 mmol) at ambient temperature for 30 min. After dropwise addition of BnBr (51 µL, 0.431 mmol), the mixture was stirred for 5 h before it was cooled to 0 °C and diluted with anhydrous CH_2Cl_2. MeOH (0.1 mL) was added dropwise followed by immediate extraction with CH_2Cl_2 and aq. $NaHCO_3$. The organic phase was further washed with brine, dried ($MgSO_4$), filtered and concentrated. Column chromatography (SiO_2, toluene/EtOAc 15:1) provided benzyl ester **8** (13.3 mg, 33%) and methyl ester **7** (16.1 mg, 46%) as a mixture. Thus, by increasing the amount of NaH and BnBr from 2 eq. to 3 eq./ free hydroxyl group and performing deprotonation with NaH at ambient temperature instead of 0 °C monobenzylation could be avoided and the yield was increased from 59% to 79%.

8: $[\alpha]_D^{20}$ + 31.7 (*c* 0.74, $CHCl_3$); R_f 0.57 (toluene/EtOAc 5:1); ^{1}H NMR ($CDCl_3$): δ 7.41 - 7.25 (m, 15H, Ar), 5.25 (d, 1H, *J* 12.4 Hz, C*H*HPh), 5.20 (d, 1H, *J* 12.4 Hz, CH*H*Ph), 4.75 (d, 1H, *J* 11.1 Hz, C*H*HPh), 4.65 (d, 1H, *J* 11.1 Hz, CH*H*Ph), 4.59 (d, 1H, *J* 12.1 Hz,C*H*HPh), 4.55 (d, 1H, *J* 12.1 Hz, CH*H*Ph), 4.48 (ddd, 1H, $J_{4,5}$ 7.0, $J_{4,3a}$ 5.5, $J_{4,3b}$ 4.1 Hz, H-4), 4.40 (dd, 1H, $J_{5,6}$ 2.0 Hz, H-5), 4.00 (ddd, 1H, $J_{7,6}$ 9.4, $J_{7,8b}$ 4.2, $J_{7,8a}$ 2.1 Hz, H-7), 3.91 (dd, 1H, H-6), 3.82 (dd, 1H, $J_{8a,8b}$ 10.5 Hz, H-8a), 3.73 (dd, 1H, H-8b), 3.09 (s, 3H, OCH_3), 2.50 (dd, 1H, $J_{3a,3b}$ 14.8 Hz, H-3a), 1.97 (dd, 1H, H-3b), 1.39 [s, 3H, C(CH_3)], 1.32 [s, 3H, C(CH_3)]; ^{13}C NMR ($CDCl_3$): δ 168.17 (s, C-1), 138.60, 138.44 and 135.54 (3 x s, 3C, Ar), 128.46, 128.35, 128.27, 128.20, 128.07, 127.57, 127.54 and 127.43 (8 x d, 15C, Ar), 108.96 [s, *C*$(CH_3)_2$], 98.20 (s, C-2), 76.65 (d, C-7), 73.39 (t, *C*H_2Ph), 72.97 (t, *C*H_2Ph), 71.38 (d, C-5), 70.08 (d, C-4), 69.30 (t, C-8), 68.74 (d, C-6), 66.99 (t, *C*H_2Ph), 50.61 (q, O*C*H_3), 33.43 (t, C-3),

26.35 [q, C(*C*H$_3$)], 25.43 [q, C(*C*H$_3$)]; ESI-TOF HRMS: *m/z* = 585.2457; calcd for $C_{33}H_{38}O_8Na^+$: 585.2459.

7: $[\alpha]_D^{20}$ + 33.0 (*c* 0.84, $CHCl_3$); R_f 0.45 (toluene/EtOAc 5:1); ^{1}H NMR ($CDCl_3$): δ 7.37 - 7.25 (m, 10H, Ar), 4.75 (d, 1H, *J* 11.2 Hz, C*H*HPh), 4.65 (d, 1H, *J* 11.2 Hz, CH*H*Ph), 4.61 (d, 1H, *J* 12.2 Hz, C*H*HPh), 4.57 (d, 1H, *J* 12.2 Hz, CH*H*Ph), 4.48 (ddd, 1H, $J_{4,5}$ 7.1, $J_{4,3a}$ 5.3, $J_{4,3b}$ 3.9 Hz, H-4), 4.41 (dd, 1H, $J_{5,6}$ 1.9 Hz, H-5), 4.00 (ddd, 1H, $J_{7,6}$ 9.6, $J_{7,8b}$ 4.3, $J_{7,8a}$ 2.0 Hz, H-7), 3.91 (dd, 1H, H-6), 3.82 (dd, 1H, $J_{8a,8b}$ 10.4 Hz, H-8a), 3.78 (s, 3H, CO_2CH_3), 3.74 (dd, 1H, H-8b), 3.15 (s, 3H, OCH_3), 2.50 (dd, 1H, $J_{3a,3b}$ 14.8 Hz, H-3a), 1.95 (dd, 1H, H-3b), 1.44 [s, 3H, C(C*H*$_3$)], 1.33 [s, 3H, C(C*H*$_3$)]; ^{13}C NMR ($CDCl_3$): δ 169.04 (s, C-1), 138.57 and 138.43 (2 x s, 2C, Ar), 128.27, 128.21, 128.09, 127.60, 127.56 and 127.46 (6 x d, 10C, Ar), 108.95 [s, *C*(CH$_3$)$_2$], 98.32 (s, C-2), 76.60 (d, C-7), 73.42 (t, *C*H$_2$Ph), 72.99 (t, *C*H$_2$Ph), 71.39 (d, C-5), 70.05 (d, C-4), 69.26 (t, C-8), 68.81 (d, C-6), 52.35 (q, CO_2CH_3), 50.67 (q, OCH_3), 33.52 (t, C-3), 26.28 [q, C(*C*H$_3$)], 25.36 [q, C(*C*H$_3$)]; ESI-TOF HRMS: *m/z* = 509.2143; calcd for $C_{27}H_{34}O_8Na^+$: 509.2146.

2.4 Methyl (methyl 4,7,8-tri-*O*-benzyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (11) and benzyl (methyl 4,7,8-tri-*O*-benzyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (12)

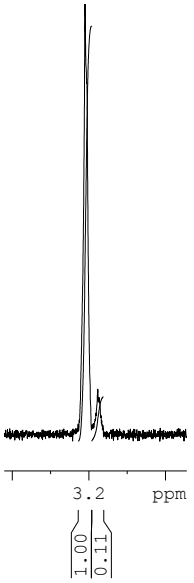


R = Me **7**
R = Bn **8**

R = Me **9**
R = Bn **10**

R = Me **11**
R = Bn **12**

The 9:1 (ratio derived by ^{1}H NMR) mixture of **7** and **8** (0.37 g, 0.756 mmol) was dissolved in anhydrous MeOH (15 mL) and treated with *p*-toluenesulfonic acid (0.03 g, 0.15 mmol) at ambient temperature for 18 h. Triethylamine (0.3 mL) was added dropwise and after 10 min the solvent was removed *in vacuo*. Silica gel filtration of the residue using 5:1 toluene/EtOAc (to elute traces of remaining starting material) followed by 9:1 EtOAc/EtOH provided a mixture of **9** and **10** (0.31 g, 90%) as a colorless oil.



A solution of this mixture (0.31 g, 0.680 mmol) in anhydrous toluene (10 mL) containing dibutyltin oxide (0.19 g, 0.75 mmol) was refluxed in a Dean-Stark apparatus for 3 h. The solution was allowed to cool to room temperature before BnBr (0.40 mL, 3.39 mmol), tetra-*n*-butylammonium iodide (0.28 g,

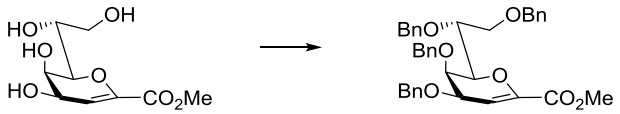
0.75 mmol) and anhydrous DMF (1.05 mL, 13.55 mmol) were added successively and the mixture was kept at 60 °C for 14 h. The mixture was cooled to rt, then diluted with EtOAc and washed sequentially with 1 M aq. HCl, aq. $NaHCO_3$, aq. $Na_2S_2O_3$ (5 w/w%) and brine. The organic layer was dried ($MgSO_4$), filtered over Celite and concentrated. The crude mixture was purified by column chromatography (SiO_2, toluene/EtOAc 9:1→5:1) yielding **12** (0.04 g, 8%) as the faster migrating compound followed by **11** (0.21 g, 52%). Yields were calculated for 2 steps and were based on the relative amounts of **7** and **8** in the mixture.

To obtain the methyl ester **11** from benzyl ester **12**, the latter compound (22 mg, 0.036 mmol) was treated with a 0.1 M methanolic sodium methoxide (0.43 mL, 0.043 mmol) in anhydrous MeOH (2 mL) under argon for 2 h. Neutralization of the solution by addition of ion exchange resin DOWEX 50 (H^+ form), filtration and concentration of the filtrate gave a residue which was subjected to elution on silica gel (toluene/EtOAc 5:1) affording the methyl ester **11** (19 mg, 98%)

12: colorless oil; $[\alpha]_D^{20}$ + 35.5 (*c* 0.63, $CHCl_3$); R_f 0.20 (toluene/EtOAc 9:1); ^{1}H NMR ($CDCl_3$): δ 7.38 - 7.26 (m, 20H, Ar), 5.25 (d, 1H, *J* 12.4 Hz, C*H*HPh), 5.20 (d, 1H, *J* 12.4 Hz, CH*H*Ph), 4.73 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.68 (d, 1H, *J* 11.5 Hz, CH*H*Ph), 4.58 (s, 2H, CH_2Ph), 4.56 (d, 1H, *J* 12.3 Hz, C*H*HPh), 4.51 (d, 1H, *J* 12.3 Hz, CH*H*Ph), 4.23 - 4.20 (m, 1H, H-5), 4.02 (ddd, 1H, $J_{7,6}$ 8.6, $J_{7,8b}$ 3.8, $J_{7,8a}$ 3.1 Hz, H-7), 3.88 (ddd, 1H, $J_{4,3ax}$ 11.7, $J_{4,3eq}$ 5.0, $J_{4,5}$ 2.8 Hz, H-4), 3.80 (dd, 1H, $J_{6,5}$ 0.9 Hz, H-6), 3.78 (dd, 1H, $J_{8a,8b}$ 10.6 Hz, H-8a), 3.72 (dd, 1H, H-8b), 3.14 (s, 3H, OCH_3), 2.38 - 2.35 (m, 1H, O*H*), 2.23 - 2.18 (m, 1H, H-3*eq*), 2.04 (dd, 1H, $J_{3ax,3eq}$ 12.8 Hz, H-3*ax*); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 167.86 (s, C-1), 138.34, 138.22, 137.93 and 135.40 (4 x s, 4C, Ar), 128.57, 128.50, 128.38, 128.33, 128.30, 128.19, 128.07, 127.84, 127.69, 127.64 and 127.54 (11 x d, 20C, Ar), 99.15 (s, C-2), 76.15 (d, C-7), 73.37 (t, *C*H_2Ph), 73.28 (d, C-4), 73.03 (t, *C*H_2Ph), 70.32 (t, *C*H_2Ph), 70.22 (d, C-6), 68.58 (t, C-8), 67.08 (t, *C*H_2Ph), 63.93 (d, C-5), 50.94 (q, O*C*H_3), 32.08 (t, C-3); ESI-TOF HRMS: *m/z* = 635.2619; calcd for $C_{37}H_{40}O_8Na^+$: 635.2615.

11: colorless oil; $[\alpha]_D^{20}$ + 39.4 (*c* 1.14, $CHCl_3$); R_f 0.10 (toluene/EtOAc 9:1); ^{1}H NMR ($CDCl_3$): δ 7.37 - 7.26 (m, 15H, Ar), 4.74 (d, 1H, *J* 11.3 Hz, C*H*HPh), 4.67 (d, 1H, *J* 11.3 Hz, CH*H*Ph), 4.60 (d, 1H, *J* 11.9 Hz, C*H*HPh), 4.59 (d, 1H, *J* 12.1 Hz, C*H*HPh), 4.58 (d, 1H, *J* 11.9 Hz, CH*H*Ph), 4.53 (d, 1H, *J* 12.1 Hz, CH*H*Ph), 4.24 - 4.22 (m, 1H, H-5), 4.01 (ddd, 1H, $J_{7,6}$ 8.9, $J_{7,8b}$ 3.8, $J_{7,8a}$ 2.8 Hz, H-7), 3.89 (ddd, 1H, $J_{4,3ax}$ 11.6, $J_{4,3eq}$ 5.0, $J_{4,5}$ 2.8 Hz, H-4), 3.81 (d, 1H, H-6), 3.79 (s, 3H, CO_2CH_3), 3.78 (dd, 1H, $J_{8a,8b}$ 10.5 Hz H-8a), 3.72 (dd, 1H, H-8b), 3.18 (s, 3H, OCH_3), 2.33 - 2.31 (m, 1H, O*H*), 2.21 - 2.17 (m, 1H, H-3*eq*), 2.03 (dd, 1H, $J_{3ax,3eq}$ 12.7 Hz, H-3*ax*); ^{13}C NMR ($CDCl_3$): δ 168.71 (s, C-1), 138.38, 138.26 and 137.95 (3 x s, 3C, Ar), 128.53, 128.36, 128.32, 128.10, 127.87, 127.72, 127.68 and 127.56 (8 x d, 15C, Ar), 99.31 (s, C-2), 76.05 (d, C-7), 73.39 (t, *C*H_2Ph), 73.18 (d, C-4), 73.01 (t, *C*H_2Ph), 70.31 (t, *C*H_2Ph), 70.23 (t, C-6), 68.55 (t, C-8), 63.98 (d, C-5), 52.53 (q, CO_2CH_3), 50.99 (q, O*C*H_3), 32.22 (t, C-3); ESI-TOF HRMS: *m/z* = 554.2744; calcd for $C_{31}H_{36}O_8NH_4^+$: 554.2748.

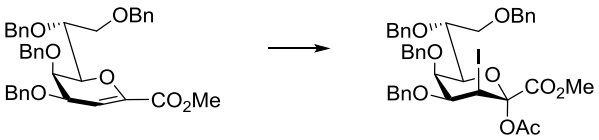
2.5. Methyl 2,6-anhydro-4,5,7,8-tetra-*O*-benzyl-3-deoxy-D-*manno*-oct-2-enosonate (14)



13 **14**

A solution of glycal **13**[S2] (0.30 g, 1.30 mmol) in anhydrous DMF (14.0 mL) was treated with NaH (60% dispersion in mineral oil; 0.42 g, 10.38 mmol) at 0 °C for 30 min. Benzyl bromide (1.23 mL, 10.38 mmol) was added dropwise and stirring at ambient temperature was continued for 2 h. At 0 °C the mixture was diluted with anhydrous CH_2Cl_2 (15 mL) and treated with anhydrous MeOH (1 mL). The reaction mixture was swiftly partitioned between CH_2Cl_2 and ice-cold 1 M aq. HCl, the aqueous phase was re-extracted with CH_2Cl_2 and the combined organic extracts were washed with aq. $NaHCO_3$ and brine successively. Drying ($MgSO_4$), filtration and concentration of the filtrate afforded a crude product which was purified by column chromatography (SiO_2, toluene/EtOAc 50:1 → 10:1) yielding **14** (0.60 g, 77%) as a colorless oil: $[\alpha]_D^{20}$ - 32.2 (*c* 1.02, $CHCl_3$); R_f 0.81 (toluene/EtOAc 3:1); ^{1}H NMR ($CDCl_3$): δ 7.37 - 7.20 (m, 20H, Ar), 6.10 (app. t, 1H, $J_{3,4} = J_{3,5}$ 2.1 Hz, H-3), 5.03 (d, 1H, *J* 11.6 Hz, C*H*HPh), 4.71 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.66 (d, 1H, *J* 11.3 Hz, C*H*HPh), 4.63 (d, 1H, *J* 12.0 Hz, CH*H*Ph), 4.58 (s, 2H, CH_2Ph), 4.55 (d, 1H, *J* 11.6 Hz, CH*H*Ph), 4.41 - 4.39 (m, 1H, H-4), 4.28 (d, 1H, *J* 11.3 Hz, CH*H*Ph), 4.26 - 4.25 (m, 1H, H-5), 4.10 – 4.07 (m, 1H, H-6), 4.01 (ddd, 1H, $J_{7,6}$ 9.3, $J_{7,8b}$ 3.8, $J_{7,8a}$ 2.1 Hz, H-7), 3.91 (dd, 1H, $J_{8a,8b}$ 10.6 Hz, H-8a), 3.77 - 3.74 (m, 4H, H-8b, CO_2CH_3); ^{13}C NMR ($CDCl_3$): δ 162.48 (s, C-1), 143.88 (s, C-2), 138.84, 138.41, 138.11 and 137.85 (4 x s, 4C, Ar), 128.48, 128.36, 128.30, 128.18, 127.91, 127.78, 127.70, 127.64, 127.49 and 127.36 (10 x d, 20C, Ar), 109.64 (d, C-3), 76.12 (d, C-6), 75.40 (d, C-7), 74.28 (t, *C*H_2Ph), 73.99 (d, C-4), 73.46 (t, *C*H_2Ph), 71.79 (t, *C*H_2Ph), 71.01 (t, *C*H_2Ph), 68.51 (d, C-5), 67.97 (t, C-8), 52.09 (q, CO_2CH_3); ESI-TOF HRMS: *m/z* = 612.2956; calcd for $C_{37}H_{38}O_7NH_4^+$: 612.2956.

2.6. Methyl 2-*O*-acetyl-4,5,7,8-tetra-*O*-benzyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosonate (15)

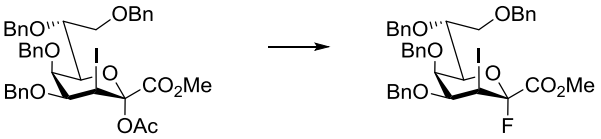


14 **15**

A solution of **14** (0.45 g, 0.755 mmol) in glacial acetic acid (30.0 mL) was treated with N-iodosuccinimide (0.48 g, 2.114 mmol) at ambient temperature for 1 h. The solution was poured onto ice-cold aq. sat. $NaHCO_3$ solution. After gas evolution had ceased, the aqueous phase was extracted with $CHCl_3$ (2 x). The combined organic phases were washed successively with aq. $NaHCO_3$, aq.

$Na_2S_2O_3$ (5 w/w%) and brine. The organic phase was dried (Na_2SO_4), filtered and concentrated. The crude product was purified by column chromatography to give the diaxial product **15** (0.49 g, 83%) as a colorless oil; $[\alpha]_D^{20}$ + 2.4 (*c* 0.54, $CHCl_3$); R_f 0.37 (*n*-hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$): δ 7.44 - 7.41 (m, 2H, Ar), 7.40 - 7.36 (m, 4H, Ar), 7.34 - 7.20 (m, 12H, Ar), 7.17 - 7.13 (m, 2H, Ar), 5.29 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.79 (d, 1H, *J* 11.6 Hz, C*H*HPh), 4.56 (dd, 1H, $J_{3,4}$ 4.5, $J_{3,5}$ 0.9 Hz, H-3), 4.53 (d, 1H, *J* 11.9 Hz, C*H*HPh), 4.50 - 4.45 (m, 3H, 1 x C*H*HPh, 2 x CH*H*Ph), 4.35 (d, 1H, *J* 12.2 Hz, CH*H*Ph), 4.27 - 4.23 (m, 2H, CH*H*Ph, H-5), 4.15 - 4.11 (m, 1H, H-6), 4.02 - 3.99 (m, 1H, H-7), 3.87 (s, 3H, CO_2CH_3), 3.70 (dd, 1H, $J_{8a,8b}$ 10.6, $J_{8a,7}$ 2.0 Hz, H-8a), 3.62 - 3.58 (m, 2H, H-4, H-8b), 1.81 (s, 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 167.39 (s, $COCH_3$), 166.72 (s, C-1), 138.95, 138.57, 138.08 and 137.22 (4 x s, 4C, Ar), 128.53, 128.29, 128.23, 128.06, 127.94, 127.76, 127.67, 127.65, 127.61, 127.42 and 127.19 (11 x d, 20C, Ar), 99.24 (s, C-2), 75.12 (d, C-7), 74.08 (t, CH_2Ph), 73.78 (d, C-4), 73.00 (t, CH_2Ph), 71.99 (d, C-5), 71.72 (d, C-6), 71.61 (t, CH_2Ph), 70.29 (t, CH_2Ph), 66.27 (t, C-8), 52.98 (q, CO_2CH_3), 23.79 (d, C-3), 20.04 (q, $COCH_3$); ESI-TOF HRMS: *m/z* = 798.2134; calcd for $C_{39}H_{41}IO_9NH_4^+$: 798.2134.

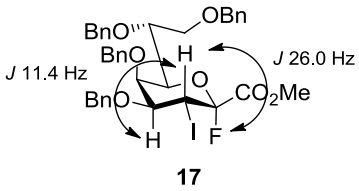
2.7. Methyl (4,5,7,8-tetra-*O*-benzyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate fluoride (16)



A solution of **15** (1.07 g, 1.37 mmol) in anhydrous CH_2Cl_2 (10.0 mL) was treated with hydrogen fluoride pyridine (ca. 70% HF, ca. 30% pyridine; 0.65 mL) at -15 °C and allowed to warm up to 0 °C over period of 1 h in a sealed Teflon flask. Ice-cold water (10 mL) was added, the phases were mixed thoroughly followed by separation. The aqueous phase was once again extracted with CH_2Cl_2 and the combined organic extracts were washed with aq. sat. $NaHCO_3$ solution, dried ($MgSO_4$), filtered and concentrated. The crude product was swiftly purified by flash chromatography (SiO_2, *n*-hexane/EtOAc 3:1) affording **16** (0.97 g, 96%) as a colorless oil; $[\alpha]_D^{20}$ - 9.5 (*c* 0.47, $CHCl_3$); R_f 0.54 (toluene/EtOAc 9:1); ^{1}H NMR ($CDCl_3$): δ 7.44 - 7.20 (m, 18H, Ar), 7.18 - 7.15 (m, 2H, Ar), 5.30 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.78 (d, 1H, *J* 11.6 Hz, C*H*HPh), 4.70 (app. t, 1H, H-3), 4.62 (d, 1H, *J* 11.3 Hz, C*H*HPh), 4.57 (d, 1H, *J* 12.3 Hz, C*H*HPh), 4.50 (2d, 2H, *J* 11.9 Hz, 2 x CH*H*Ph), 4.40 (d, 1H, *J* 11.6 Hz, CH*H*Ph), 4.31 (dd, 1H, $J_{6,7}$ 9.5, $J_{6,5}$ 1.8 Hz, H-6), 4.28 - 4.25 (m, 2H, CH*H*Ph, H-5), 4.09 - 4.06 (m, 1H, H-7), 3.87 (s, 3H, CO_2CH_3), 3.80 (dd, 1H, $J_{8a,8b}$ 10.8, $J_{8a,7}$ 2.0 Hz, H-8a), 3.68 (dd, 1H, $J_{8b,7}$ 3.6 Hz, H-8b), 3.59 (dd, 1H, $J_{4,3}$ 4.5, $J_{4,5}$ 2.8 Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 164.37 (ds, $J_{C\text{-}1,F}$ 28.9 Hz, C-1), 138.84, 138.35, 138.23 and 137.07 (4 x s, 4C, Ar), 128.56, 128.24, 128.20, 128.04, 128.01, 127.69, 127.64, 127.62, 127.55, 127.50, 127.40 and 127.15 (12 x d, 20C, Ar), 109.64 (ds, $J_{C\text{-}2,F}$ 227.3 Hz, C-2),

75.48 (d, C-7), 74.06 (t, *C*H_2Ph), 73.39 (t, *C*H_2Ph), 73.13 (d, C-4), 72.99 (dd, $J_{C\text{-}6,F}$ 3.7 Hz, C-6), 72.01 (t, *C*H_2Ph), 71.92 (d, C-5), 70.44 (t, *C*H_2Ph), 67.70 (t, C-8), 53.18 (q, CO_2CH_3), 20.79 (dd, $J_{C\text{-}3,F}$ 31.8 Hz, C-3); ^{19}F NMR ($CDCl_3$): δ -100.43 (d, $J_{F,H\text{-}3}$ 5.6 Hz); ESI-TOF HRMS: *m/z* = 763.1535; calcd for $C_{37}H_{38}FIO_7Na^+$: 763.1538.

2.7.1. Epimerization



17

Figure S1: Structure of **17** with coupling constants $J_{H3ax\text{-}H4}$ and $J_{H3ax\text{-}Fax}$

When **15** was treated with a 10-fold amount of hydrogen fluoride pyridine (as described under 2.7) between 0 °C and ambient temperature, a ~2:1 mixture of **16** and the 3-iodo-epimer **17** (Fig. S1) was obtained after chromatography in significantly lower yield (~70%). The presence of two signals (-100.4 ppm for **16** and -124.1 ppm for **17**) in the ^{19}F spectrum (Fig. S2) revealed two fluorinated species. The ^{1}H NMR spectrum (Fig. S3) showed a signal at 4.85 ppm of the minor compound split into a dd with $J_{H\text{-}3ax,F\text{-}ax}$ ~ 26.0 Hz and $J_{H\text{-}3ax,4}$ ~ 11.4 Hz. The large coupling constant confirmed the *trans*-diaxial relationship between H-3 and the anomeric fluoride, which was further supported by the large coupling between H-3 and the axial H-4. The carbon signal of C-3 at 26.6 ppm was identified by an HSQC experiment and confirmed the position of the iodo substituent.

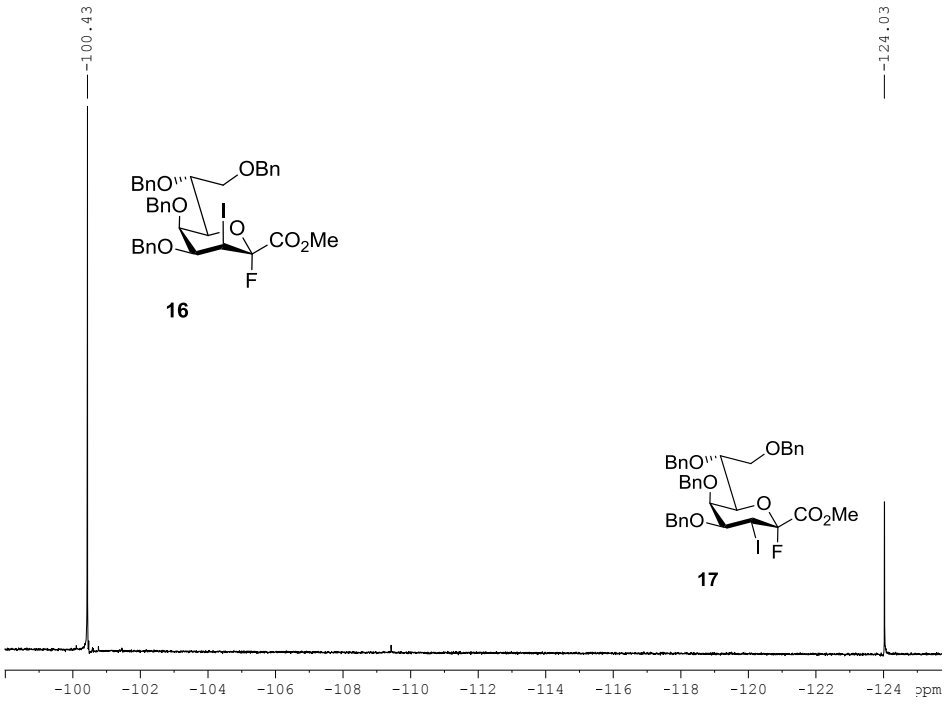


Figure S2: ^{19}F NMR ($CDCl_3$, 565 MHz) of a mixture of donor **16** and its 3-iodo-epimer **17**

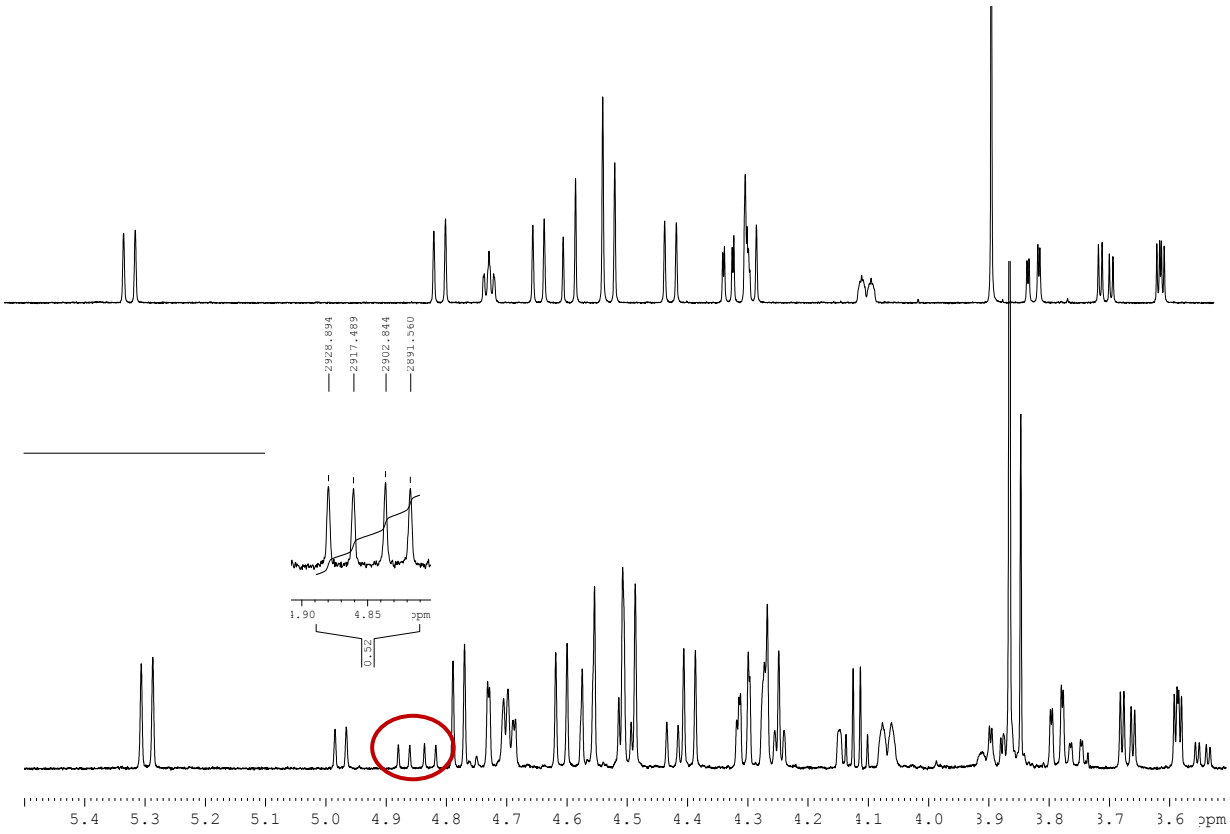
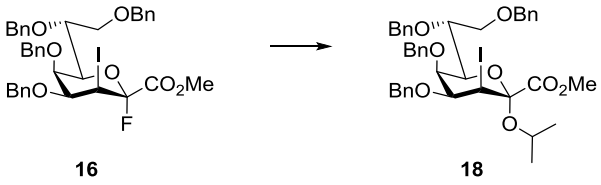


Figure S3: ^{1}H NMR spectra ($CDCl_3$, 600 MHz) of pure **16** (top) and a mixture of **16** and **17** (bottom)

2.8. Model reaction of fluoride donor (16) with 2-propanol



2.8.1. $BF_3.Et_2O$ as promotor

A mixture of pre-dried fluoride donor **16** (21.4 mg, 0.029 mmol) and anhydrous 2-propanol (4.5 µL, 0.058 mmol) in anhydrous CH_2Cl_2 (0.7 mL) containing ground molecular sieves (3 Å, 50 mg) was stirred at ambient temperature for 1 h. After cooling to -60 °C, $BF_3.Et_2O$ (11.9 µL, 0.058 mmol, ~ 46% BF_3 basis) was added dropwise and the mixture was kept at -60 °C for 15 min. The mixture was warmed up to -40 °C quickly and kept at this temperature again for 15 min. The procedure was repeated at a temperature of -20 °C and -10 °C. After 15 min at each temperature a sample was taken, which

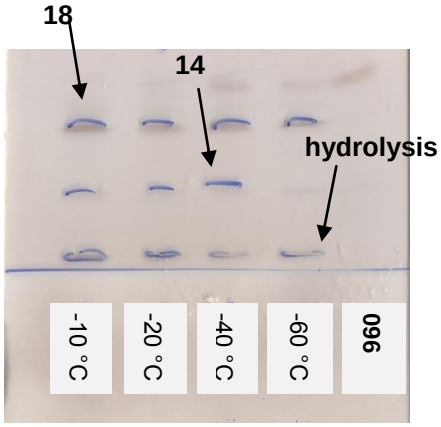


Figure S4: TLC analysis of temperature-dependent activation of donor **16**

was immediately added to a cold solution of triethylamine in CH_2Cl_2. TLC analysis after each step showed, that the donor had been converted already at -60 °C (Fig. S4). Thus, a much higher reactivity was observed than for the acetylated pendant described recently.[S2] The reaction mixture was diluted with CH_2Cl_2 and washed with aq. sat. $NaHCO_3$ solution, aq. $Na_2S_2O_3$ (5 w/w%) solution and water successively. The organic layer was dried ($MgSO_4$), filtered and concentrated. The crude product was purified by column chromatography (SiO_2, toluene/EtOAc 40:1) affording **18** (15.9 mg, 70%) in sufficient purity for further reactions.

2.8.2. $Cp_2ZrCl_2/AgClO_4$ as promotor

To examine the reactivity of donor **16**, it was converted under milder conditions ($Cp_2ZrCl_2/AgClO_4$). Under an atmosphere of argon a suspension of **16** (15.0 mg, 0.020 mmol) and 2-propanol (3.1 µL, 0.041 mmol) in anhydrous CH_2Cl_2 (1.0 mL) containing ground molecular sieves (4 Å, 50 mg) was stirred at ambient temperature for 1 h. Then, $AgClO_4$ (9.1 mg, 0.041) and Cp_2ZrCl_2 (11.8 mg, 0.041 mmol) were added sequentially at 0 °C and the mixture was stirred at ambient temperature for 24 h. After addition of aq. $NaHCO_3$ (5 mL) the mixture was filtered over a pad of Celite and rinsed with CH_2Cl_2. The filtrate was subjected to phase separation and the organic phase was thoroughly washed successively with aq. $Na_2S_2O_3$ (5 w/w%) and water. The organic phase was dried ($MgSO_4$), filtered and concentrated. Purification by column chromatography (SiO_2, toluene/EtOAc 50:1) provided **18** (11.7 mg, 74%).

2.8.3. Addition of base

The addition of triethylamine as base (1.5 eq/ eq donor **16**) during glycosylation drastically reduced the reactivity of the donor. With $BF_3.Et_2O$ as promotor, the temperature had to be raised to 0°C to get conversion. With $Cp_2ZrCl_2/AgClO_4$ as promotor couple no reaction took place in the presence of triethylamine even after 2 d.

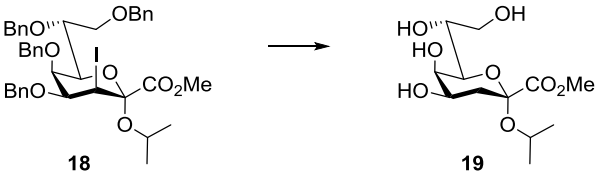
2.8.4. Methyl (propan-2-yl 4,5,7,8-tetra-*O*-benzyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosid)onate (18)

Colorless oil; $[\alpha]_D^{20}$ - 7.8 (*c* 0.78, $CHCl_3$); R_f 0.29 (toluene/EtOAc 50:1, HP-TLC); ^{1}H NMR ($CDCl_3$): δ 7.43 - 7.41 (m, 2H, Ar), 7.39 - 7.18 (m, 16H, Ar), 7.16 - 7.14 (m, 2H, Ar), 5.35 (d, 1H, *J* 11.6 Hz, C*H*HPh), 4.78 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.60 (dd, 1H, $J_{3,4}$ 4.2, $J_{3,5}$ 1.1 Hz, H-3), 4.55 (d, 1H, *J* 12.1 Hz, C*H*HPh), 4.53 (d, 1H, *J* 11.9 Hz, C*H*HPh), 4.47 (d, 1H, *J* 11.4 Hz, CH*H*Ph), 4.46 (d, 1H, *J* 12.1 Hz, CH*H*Ph), 4.37 (d, 1H, *J* 11.7 Hz, CH*H*Ph), 4.25 (d, 1H, *J* 11.1 Hz, CH*H*Ph), 4.20 - 4.19 (m, 1H, H-5), 4.11 (app. td, 1H, $J_{7,6}$ 9.3, $J_{7,8a} \sim J_{7,8b}$ 2.5 Hz, H-7), 4.08 (dd, 1H, $J_{6,5}$ 2.0 Hz, H-6), 4.00 [hept, 1H, *J* 6.1 Hz, C*H*$(CH_3)_2$], 3.83 (s, 3H, CO_2CH_3), 3.81 - 3.77 (m, 2H, H-8a, H-8b), 3.66 (dd, 1H, $J_{4,5}$ 3.0 Hz, H-4), 1.04 [d, 3H, *J* 6.1 Hz, CH(C*H*$_3$)], 0.97 [d, 3H, *J* 6.1 Hz, CH(C*H*$_3$)]; ^{13}C NMR ($CDCl_3$): δ 167.94 (s, C-1), 139.29, 138.41, 138.33 and 137.70 (4 x s, 4C, Ar), 128.42, 128.25, 128.24, 127.96, 127.75, 127.72, 127.67, 127.62, 127.55, 127.51, 127.48 and 126.94 (12 x d, 20C, Ar), 100.65 (s, C-2),

76.10 (d, C-7), 74.19 (d, C-4), 74.02 (t, *C*H_2Ph), 73.35 (t, *C*H_2Ph), 72.71 (d, C-5), 72.04 (t, *C*H_2Ph), 71.15 (d, C-6), 70.23 (t, *C*H_2Ph), 67.99 [d, *C*H(CH_3)$_2$], 67.76 (t, C-8), 52.36 (q, CO_2*C*H_3), 26.39 (d, C-3), 23.74 [q, 1C, CH(*C*H_3)], 21.80 [q, 1C, CH(*C*H_3)]; ESI-TOF HRMS: *m/z* = 803.2052; calcd for $C_{40}H_{45}IO_8Na^+$: 803.2051.

2.8.5. Methyl (propan-2-yl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (19)

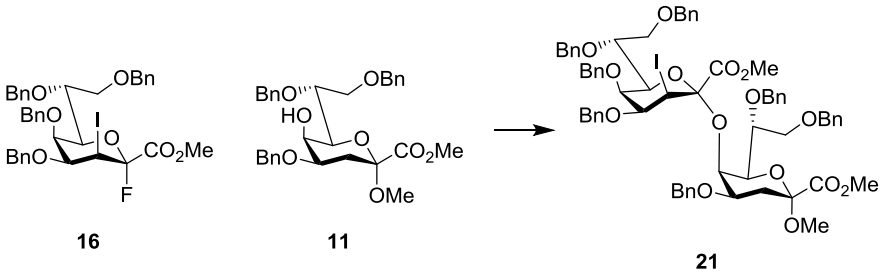
Dehalogenation and deprotection:



Iodo compound **18** (18.4 mg, 0.024 mmol) was dissolved in anhydrous MeOH (2.0 mL) and sodium acetate (3.9 mg, 0.047 mmol) was added to the stirred solution. The atmosphere was exchanged to argon, $Pd(OH)_2$ on carbon (20%, 3 mg) was added and the atmosphere was successively exchanged to argon and hydrogen. After hydrogenation for 1 h at ambient temperature the mixture was filtered *via* a syringe filter, rinsed with MeOH and the filtrate was concentrated. The yellow residue was dissolved in CH_2Cl_2 and washed with aq. $Na_2S_2O_3$ (5 w/w%, 2 x) and water (2 x). The organic phase was dried ($MgSO_4$), filtered and concentrated. The crude product was directly debenzylated in anhydrous MeOH (2.0 mL) over Pd on carbon (10%, 3 mg) at ambient temperature for 3 h. Filtration and concentration of the filtrate afforded **19** (6.3 mg, 91%). Colorless oil; $[\alpha]_D^{20}$ + 56.2 (*c* 0.62, MeOH); R_f 0.25 (EtOAc/EtOH 19:1); ^{1}H NMR (MeOD): δ 4.02 - 3.91 [m, 4H, C*H*(CH_3)$_2$, H-4, H-5, H-7], 3.85 (dd, 1H, $J_{8a,8b}$ 11.3, $J_{8a,7}$ 4.1 Hz, H-8a), 3.76 (s, 3H, CO_2CH_3), 3.68 (dd, 1H, $J_{6,7}$ 8.2, $J_{6,5}$ 1.2 Hz, H-6), 3.63 (dd, 1H, $J_{8b,7}$ 5.8 Hz, H-8b), 2.06 (ddd, 1H, $J_{3eq,3ax}$ 12.8, $J_{3eq,4}$ 4.9, $J_{3eq,5}$ 0.9 Hz, H-3*eq*), 1.87 (dd, 1H, $J_{3ax,4}$ 11.9 Hz, H-3*ax*), 1.17 [d, 3H, *J* 6.3 Hz, CH(CH_3)], 1.02 [d, 3H, *J* 6.3 Hz, CH(CH_3)]; ^{13}C NMR (MeOD): δ 171.36 (s, C-1), 99.34 (s, C-2), 74.07 (d, C-6), 71.23 (d, C-7), 67.77, 67.65, 67.30 [3 x d, 3C, C-4, C-5, *C*H(CH_3)$_2$], 64.76 (t, C-8), 52.79 (q, CO_2*C*H_3), 36.13 (t, C-3), 24.20 [q, CH(*C*H_3)], 22.88 [q, CH(*C*H_3)]; ESI-TOF HRMS: *m/z* = 317.1234; calcd for $C_{12}H_{22}O_8Na^+$: 317.1207.

For determination of the anomeric configuration of **19**, latter compound (ca. 6 mg) was dissolved in anhydrous pyridine (2 mL) and treated with acetic anhydride (0.2 mL) at ambient temperature over night. Excessive reagent was destroyed at 0 °C by addition of anhydrous MeOH (1 mL) and after 10 min the mixture was coevporated with toluene (3x). Column chromatography (SiO_2, *n*-hexane/EtOAc 1:1) of the crude residue afforded the known methyl (propan-2-yl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate[S2] verifying the α-configuration of **19**.

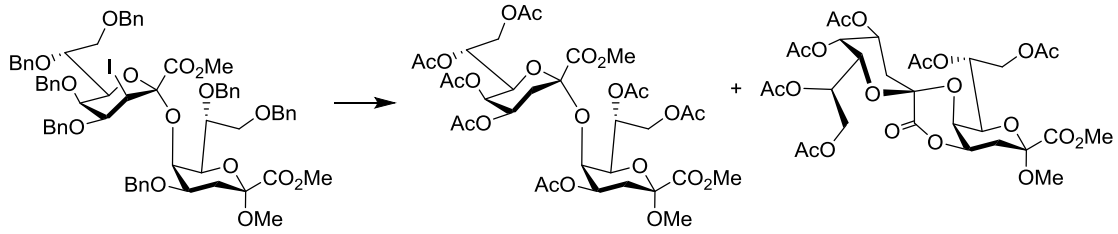
2.9. Methyl (4,5,7,8-tetra-*O*-benzyl-3-deoxy-3-iodo-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate-(2→5)-methyl (methyl 4,7,8-tri-*O*-benzyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (21)



A mixture of pre-dried starting materials **11** (38.0 mg, 0.071 mmol) and **16** (78.7 mg, 0.106 mmol) in anhydrous toluene (7.6 mL) containing ground molecular sieves (3 Å, 350 mg) was stirred at ambient temperature for 2 h. The suspension was cooled to -40 °C (MeCN/dry ice) and $BF_3.Et_2O$ (29.1 µL, 0.142 mmol, ~ 46% BF_3 basis) was added dropwise. The mixture was allowed to warm to -15 °C over 2 h and was kept between -20 °C and -10 °C for additional 1.5 h (Note: for TLC analysis, samples were immediately treated with triethylamine in CH_2Cl_2 to avoid degradation). The mixture was cooled to -30 °C and a solution of triethylamine (0.1 mL) in anhydrous toluene (0.9 mL) was slowly added. During addition, the color of the solution turned orange and finally to a pale yellow. After complete addition the mixture was stirred at -30 °C for further 5 min. Then, the solution was poured onto an ice-cold aq. sat. $NaHCO_3$ solution (10 mL) and was quickly extracted with EtOAc (40 mL). The aqueous phase was once again washed with EtOAc (10 mL) and the combined organic phases were washed successively with ice-cold aq. $Na_2S_2O_3$ (5 w/w%) solution and brine. Drying ($MgSO_4$), filtration and swift concentration afforded a crude product which was purified by column chromatography (SiO_2, *n*-hexane/EtOAc 3:1) yielding **21** (63.0 mg, 71%) as a colorless oil; $[\alpha]_D^{20}$ + 16.3 (*c* 0.60, $CHCl_3$); R_f 0.32 (*n*-hexane/EtOAc 4:1, HP-TLC); ^{1}H NMR (d_8-toluene, 323.15 K): δ 7.64 - 7.61 (m, 2H, Ar), 7.41 - 7.38 (m, 2H, Ar), 7.31 - 7.27 (m, 4H, Ar), 7.24 - 7.19 (m, 8H, Ar), 7.17 - 6.99 (m, 19H, Ar), 5.38 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.98 - 4.96 (m, 1H, H-5), 4.84 (dd, $J_{6',7'}$ 9.1, $J_{6',5'}$ 1.7 Hz, H-6′), 4.77 (d, 1H, *J* 10.9 Hz, C*H*HPh), 4.73 (d, 1H, *J* 10.9 Hz, CH*H*Ph), 4.71 (dd, 1H, $J_{3',4'}$ 4.2, $J_{3',5'}$ 0.7 Hz, H-3′), 4.53 (d, 1H, *J* 11.1 Hz, C*H*HPh), 4.37 (d, 1J, *J* 11.8 Hz, C*H*HPh), 4.34 (d, 1H, *J* 11.8 Hz, CH*H*Ph), 4.33 (d, 1H, *J* 12.0 Hz, CH*H*Ph), 4.22 (d, 1H, *J* 11.1 Hz, CH*H*Ph), 4.22 (d, 1H, *J* 11.6 Hz, C*H*HPh), 4.17 - 4.10 (m, 3H, H-7′, CH_2Ph), 4.07 (d, 1H, *J* 10.7 Hz, C*H*HPh), 4.06 (d, 1H, *J* 11.6 Hz, CH*H*Ph), 4.00 (d, 1H, *J* 10.7 Hz, CH*H*Ph), 3.98 - 3.96 (m, 1H, H-5′), 3.92 - 3.87 (m, 3H, H-4′, H-6, H-8a), 3.83 (dd, 1H, $J_{8b,8a}$ 10.4, $J_{8b,7}$ 2.9 Hz, H-8b), 3.77 (dd, 1H, $J_{8'a,8'b}$ 10.4, $J_{8'a,7'}$ 3.5 Hz, H-8′a), 3.76 - 3.69 (m, 2H, H-7, H-8′b), 3.49 (s, 3H, CO_2CH_3), 3.40 (s, 3H, CO_2CH_3), 3.39 - 3.35 (m, 1H, H-4), 3.26 (s, 3H, OCH_3), 2.25 - 2.22 (m, 2H, H-3*ax*, H-3*eq*); ^{13}C NMR (d_8-toluene, 323.15 K)[S5]: δ 168.39 (s, C-1), 167.87 (s, C-1′), 140.39, 139.96, 139.69, 139.06, 138.82 and 138.60 (6 x s, Ar), 129.25, 128.97, 128.64, 128.62, 128.56, 128.54, 128.48, 128.44, 128.31, 127.96, 127.95, 127.71, 127.68, 127.53, 127.11 and 125.47 (16 x d, Ar), 102.12 (s, C-2′), 99.84 (s, C-2), 77.48 (d, C-7′), 76.49 (d, C-

7), 75.24 (d, C-4), 74.43 (t, *C*H_2Ph), 74.21 and 73.21 (2 x d, C-4′, C-5′), 73.72 (t, *C*H_2Ph), 73.27 (t, *C*H_2Ph), 72.35 and 72.05 (2 x d, C-6, C-6′), 72.28 (t, *C*H_2Ph), 71.67 (t, *C*H_2Ph), 71.10 (t, *C*H_2Ph), 70.58 (t, *C*H_2Ph), 69.41 (d, C-5), 69.15 (t, C-8′), 68.45 (t, C-8), 51.82 (q, CO_2CH_3), 51.72 (q, CO_2CH_3), 51.16 (q, O*C*H_3), 33.28 (t, C-3), 28.80 (d, C-3′); ESI-TOF HRMS: *m/z* = 1274.4332; calcd for $C_{68}H_{73}IO_{15}NH_4^+$: 1274.4332.

2.10. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→5)-methyl (methyl 4,7,8-tri-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (22) and [4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosylono-(1'-4)-lactone]-(2→5)-methyl (methyl 4,7,8-tri-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (23)



21 **22** **23**

Disaccharide **21** (63.0 mg, 0.050 mmol) was dissolved in anhydrous MeOH (5 mL) and sodium acetate (41.0 mg, 0.501 mmol) was added in one portion. The atmosphere was exchanged to argon by consecutive evacuation and flushing with inert gas. Then, $Pd(OH)_2/C$ (20%, 63 mg) was added to the solution followed by exchange of atmosphere to argon and finally to hydrogen. After 1 h at ambient temperature the suspension was filtered via a syringe filter and rinsed with anhydrous MeOH. Swift concentration afforded a yellow residue which was partitioned between ice-cold aq. sat. $NaHCO_3$ solution (10 mL) and EtOAc (30 mL). The aqueous phase was once again extracted with EtOAc (10 mL) and the combined organic phases were washed successively with aq. $Na_2S_2O_3$ (5 w/w%, 2x) solution and brine (2 x). Drying ($MgSO_4$), filtration and concentration provided a colourless oil which was dissolved in anhydrous MeOH (5 mL) and treated with hydrogen on Pd/C (10%, 10 mg) after exchange of atmosphere as described above. After 20 h at ambient temperature the catalyst was filtered off via a syringe filter and rinsed with anhydrous MeOH. The filtrate was concentrated, the residue taken up in anhydrous pyridine (2 mL), cooled to 0°C and treated with acetic anhydride (0.4 mL) and 4-(*N*,*N*-dimethylamino)-pyridine (3 mg). After 24 h at ambient temperature excessive reagent was destroyed by dropwise addition of anhydrous MeOH (2 mL) at 0 °C and the solution was kept at this temperature for 5 min. Coevaporation with toluene (3 x) provided a crude product which was purified by HP-column chromatography (SiO_2, toluene/EtOAc 2:1) providing a mixture of **23** and **22** (3.5 mg of **23**, 10%; 20.0 mg of mixture in a ratio of **22** : **23** of 10 : 1, 51%; total: 61%, see Fig. S5).

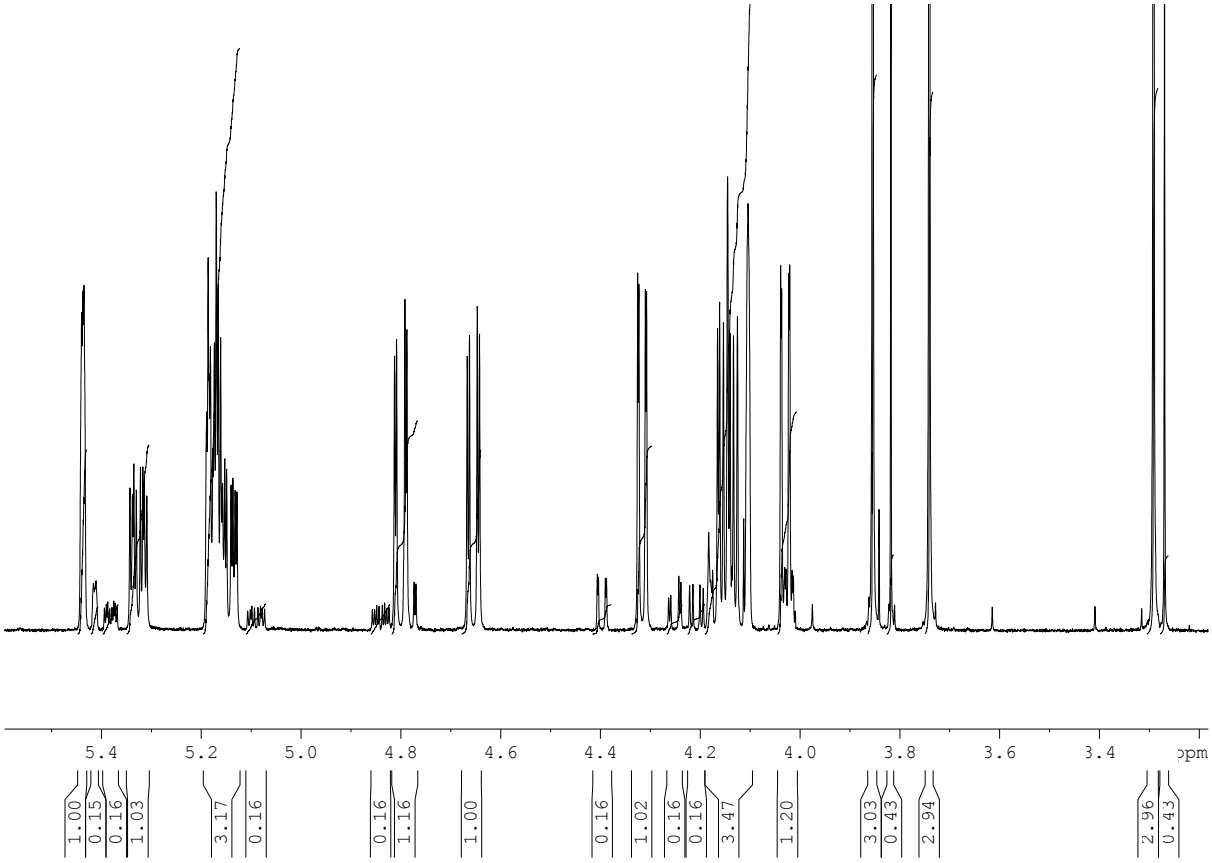


Figure S5: ^{1}H NMR ($CDCl_3$) of mixture of **22** and lactone **23**

Partial separation of this mixture (9.9 mg, 10:1 ratio of **22**:**23**) was obtained by normal-phase HP chromatography (*n*-hexane/EtOAc 2:1) giving a small fraction of pure **22**[S6] (1.2 mg, 12%) for spectroscopic analysis. ^{1}H NMR analysis of the minor compound (Fig. S6) strongly suggested the expected formation of an interresidue lactone, based on the absence of one methyl ester signal and the presence of six acetyl groups only. The presence of the 1'-4 lactone was furthermore evident from the ^{13}C NMR signals, which showed a pronounced low-field shift of C-4 (ca. +5 ppm) as well as a high-field shift (ca. -6 ppm) of C-5 in comparison to the C-4 and C-5 signals of **22**. Thus, the lactone was formed between O-4 of the proximal Kdo and C-1' forming a six-membered ring. This assignment was further supported by the fact that saponification of the mixture yielded an identical product.

22: ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.45 - 5.43 (m, 1H, H-5'), 5.33 (ddd, 1H, *J* 12.6, J 4.6, J 3.0 Hz, H-4'), 5.20 - 5.13 (m, 3H, H-4, H-7, H-7'), 4.80 (dd, 1H, *J* 12.5, J 2.3 Hz, H-8a), 4.66 (dd, 1H, *J* 12.0, J 2.7 Hz, H-8'a), 4.32 (dd, 1H, *J* 9.3, J 1.3 Hz, H-6'), 4.16 (dd, 1H, *J* 12.3, J 2.6 Hz, H-8b), 4.14 (dd, 1H, *J* 12.0, J 4.7 Hz, H-8'b), 4.12 - 4.10 (m, 1H, H-5), 4.03 (dd, 1H, *J* 9.8, J 1.1 Hz, H-6), 3.86 (s, 3H, CO_2CH_3), 3.74 (s, 3H, CO_2CH_3), 3.29 (s, 3H, OCH_3), 2.29 (ddd, 1H, *J* 12.9, J 4.7, J 0.8 Hz, H-3'*eq*), 2.14 (s, 3H, $COCH_3$), 2.13 (app t, 1H, *J* 12.5 Hz, H-3*ax*), 2.09 (s, 3H, $COCH_3$), 2.09 – 2.05 (m, 1H, H-3*eq*), 2.064 (s, 3H, $COCH_3$), 2.055 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.99 (app t, 1H, *J* 12.8 Hz, H-3'*ax*), 1.99 (s, 3H, $COCH_3$); ^{13}C NMR (150 MHz, $CDCl_3$): δ

170.91, 170.63, 170.42, 170.34, 170.14, 170.08 and 169.78 (7 x s, 7C, *C*OCH$_3$), 167.49 and 166.60 (2 x s, 2C, C-1, C-1'), 100.88 (s, C-2'), 98.76 (s, C-2), 70.80 (d, C-5), 69.39 (d, 2C, C-6, C-6'), 68.26, 68.21 and 68.14 (3 x d, 3C, C-4, C-7, C-7'), 66.12 (d, C-4'), 64.56 (d, C-5'), 62.26 (t, C-8'), 61.37 (d, C-8), 52.76 and 52.65 (2 x q, 2C, CO$_2$*C*H$_3$), 51.15 (q, O*C*H$_3$), 33.70 (t, C-3'), 32.37 (t, C-3), 21.38, 21.11, 20.85, 20.77, 20.73, 20.69 and 20.63 (7 x q, 7C, CO*C*H$_3$).

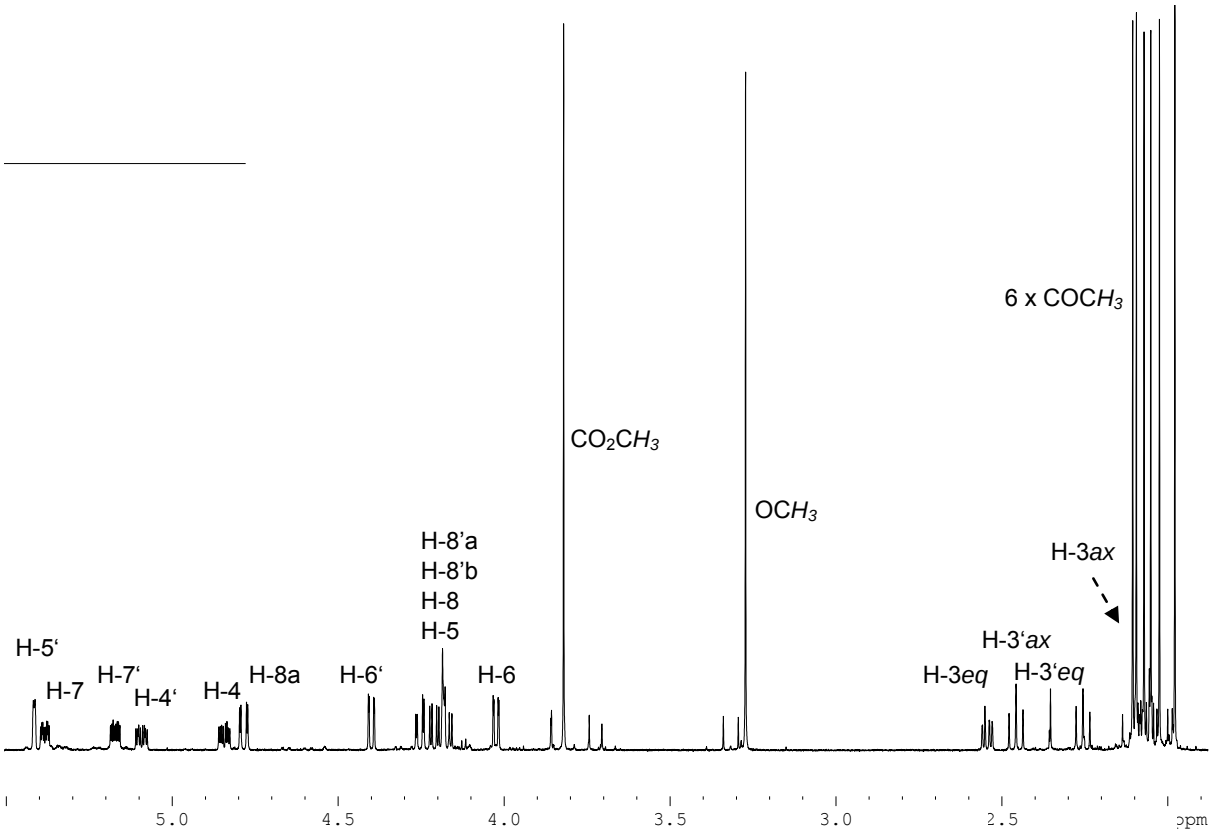
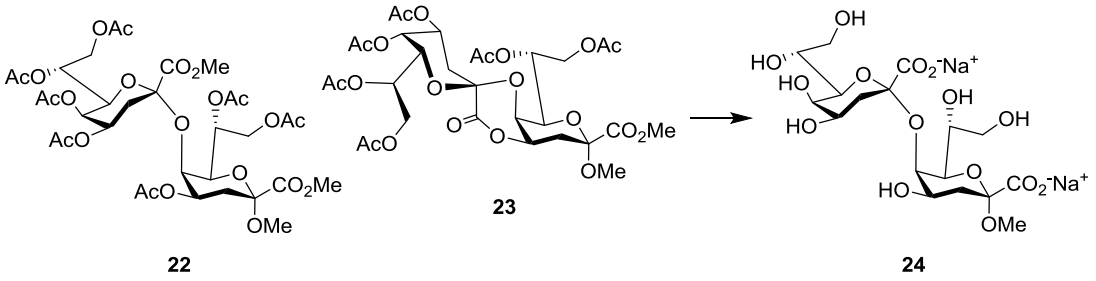


Figure S6: ^{1}H NMR (CDCl$_3$) of lactone **23**

2.11. Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→5)-sodium (methyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (24)



A solution of **22** and **23** (9.4 mg, 0.012 mmol) in anhydrous MeOH (1.0 mL) was treated with 0.1 M sodium methoxide (60 µL) in anhydrous) at ambient temperature for 16 h followed by neutralization using ion exchange resin DOWEX 50 (H^+ form). The resin was filtered off, rinsed with anhydrous

MeOH and the solvent was removed under reduced pressure. The residue was dissolved in water (1.0 mL) and was treated with 0.1 M aq. NaOH (1.0 mL) at 0 °C for 2 h and at ambient temperature for 4 h. The solution was made neutral by adding DOWEX 50 resin (H^+ form), followed by filtration and freeze-drying of the filtrate. The residue was desalted on a PD-10 column (H_2O) to afford **24** as a colorless amorphous solid (5.6 mg, ca. 90%; minor impurity present; Fig. S7).

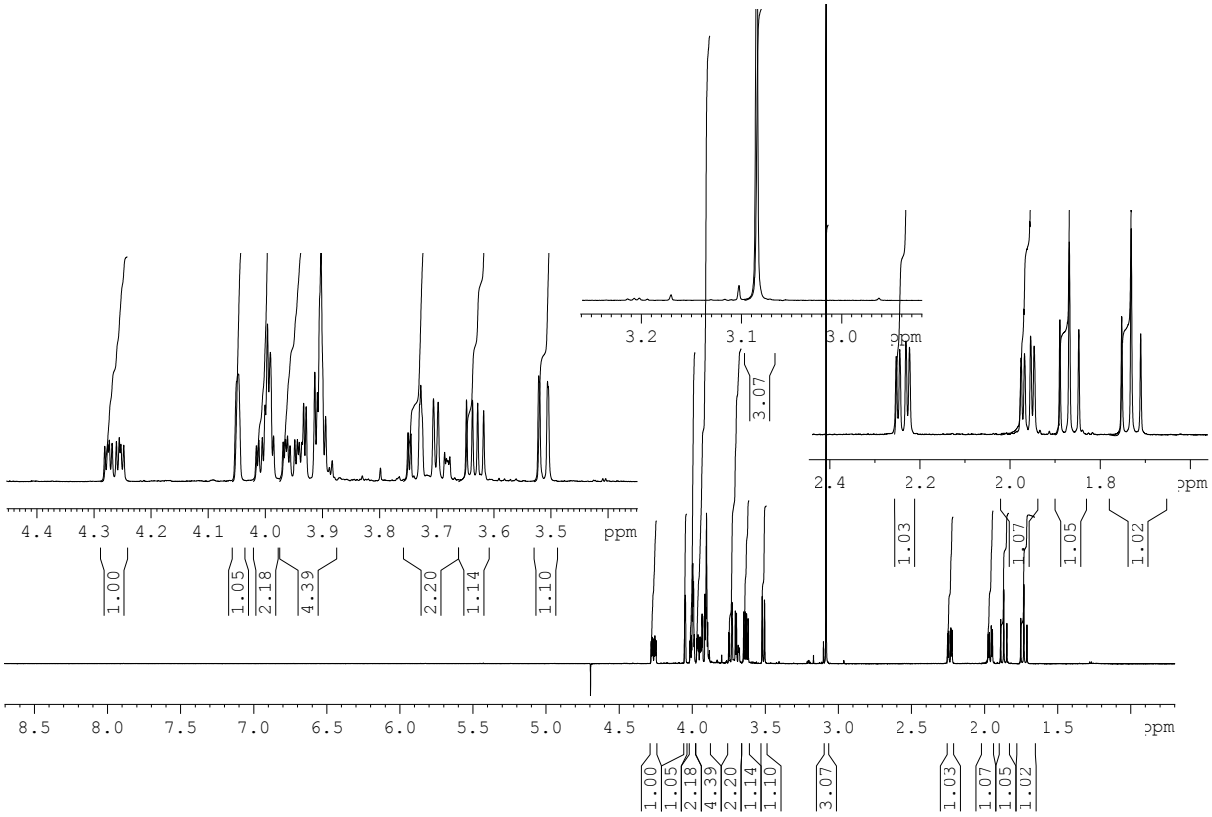


Figure S7: ^{1}H NMR (D_2O, pH ~ 7) of disaccharide **24** with minor impurity

2.12. Preparation of an analytically pure sample of (24)

A small fraction of pure **22** (ca. 1.2 mg) was deacetylated and saponified according to the described method under 2.11. to give the disaccharide **24** cleanly as colorless amorphous solid; $[\alpha]_D^{20}$ + 70.4 (*c* 0.66, H_2O); ^{1}H NMR (D_2O, pH ~ 7): δ 4.26 (ddd, 1H, $J_{4',3'ax}$ 12.2, $J_{4',3'eq}$ 4.7, $J_{4',5'}$ 3.0 Hz, H-4'), 4.06 - 4.04 (m, 1H, H-5), 4.02 - 3.98 (m, 2H, H-5', H-7), 3.95 (ddd, 1H, $J_{4,3ax}$ 12.2, $J_{4,3eq}$ 4.2, $J_{4,5}$ 2.5 Hz, H-4), 3.92 (dd, 1H, $J_{8a,8b}$ 11.7, $J_{8a,7}$ 2.7 Hz, H-8a), 3.91 - 3.88 (m, 2H, H-6', H-7'), 3.75 - 3.72 (m, 1H, H-8'a), 3.71 - 3.67 (m, 1H, H-8'b), 3.63 (dd, 1H, $J_{8b,7}$ 6.1 Hz, H-8b), 3.51 (dd, 1H, $J_{6,7}$ 9.2, $J_{6,5}$ 0.8 Hz, H-6), 3.08 (s, 3H, OC*H*$_3$), 2.24 (dd, 1H, $J_{3'eq,3'ax}$ ~ 12.9 Hz, H-3'*eq*), 1.96 (dd, 1H, $J_{3eq,3ax}$ ~ 12.8 Hz, H-3*eq*), 1.87 (app t, 1H, H-3'*ax*), 1.73 (app t, 1H, H-3*ax*); ^{13}C NMR (D_2O, pH ~ 7): δ 175.89 (s, C-1), 174.73 (s, C-1'), 102.92 (s, C-2'), 101.26 (s, C-2), 74.21 (d, C-5), 72.94 (d, C-6), 72.70 (d, C-6'), 71.46 (d, C-7'), 70.13 (d, C-7), 67.63 (d, C-5'), 66.59 (d, C-4), 66.31 (d, C-4'), 63.36 (t, C-8), 63.22 (t,

C-8'), 51.38 (q, O*C*H_3), 36.61 (t, C-3'), 35.87 (t, C-3); ESI-TOF HRMS: *m/z* = 495.1305; calcd for $C_{17}H_{28}O_{15}Na^+$: 495.1320.

3. References for SI

[S1] Harris, R. K.; Becker, E. D.; De Menezes, S. M. C.; Goodfellow, R.; Granger, P. *Pure Appl. Chem*. **2001**, *73*, 1795-1818.

[S2] Pokorny, B.; Kosma, P. *Chem. Eur. J.* **2014**, in press (doi: 10.1002/chem.201405424)

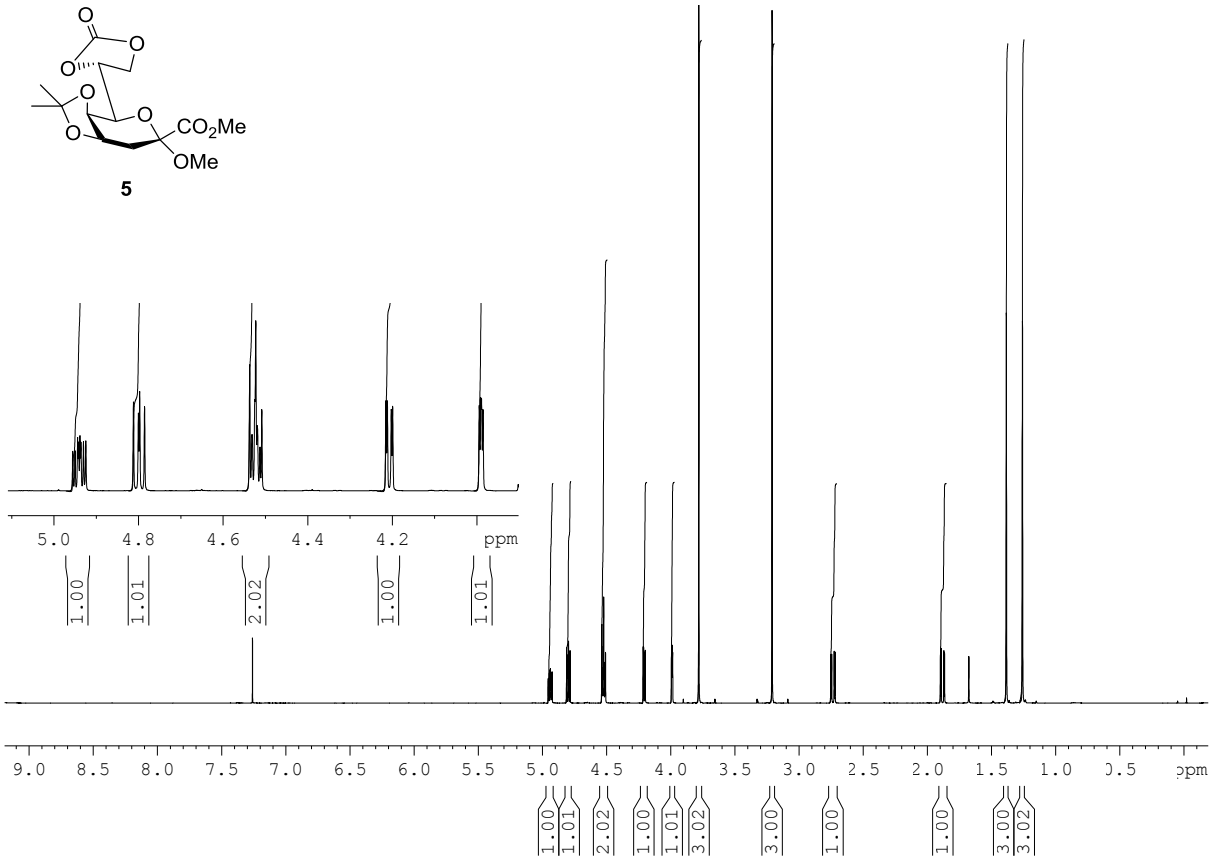
[S3] Charon, D.; Auzanneau, F.-I.; Mérinne, C.; Szabo, L. *Tetrahedron Lett.* **1987**, *28*, 1393-1396; published NMR data in $CDCl_3$; measured: $[\alpha]_D^{20}$ + 64.5 (*c* 1.16, $CHCl_3$), lit: +56 (c 1, $CHCl_3$).

[S4] Pure fractions were used for characterization of **8** and **7**. The yield was calculated from the ratio determined by ^{1}H NMR. For further reactions **8** and **7** were combined as the conversion from **9** towards **11** was known to also yield some benzyl ester **12** via partial transesterification. Thus, separation of benzyl and methyl ester was performed after the last step of the acceptor synthesis (**11** and **12**).

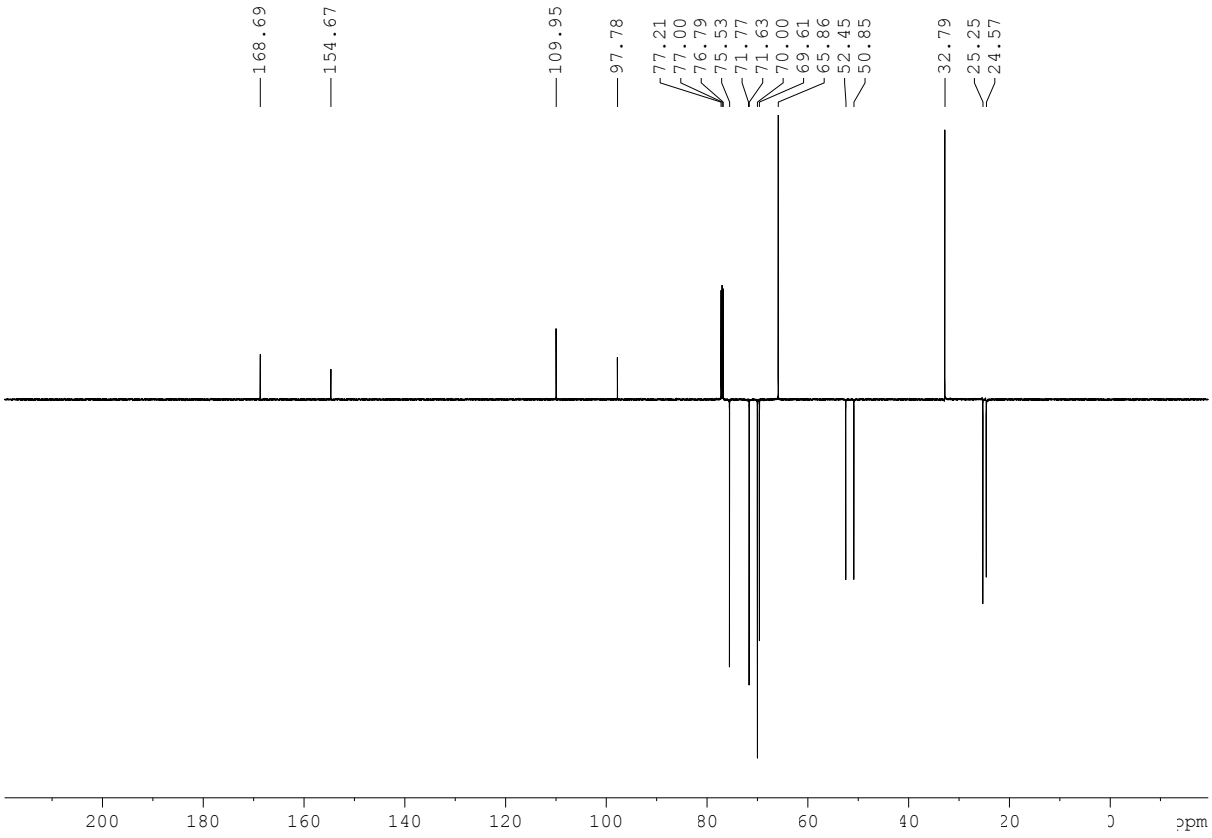
[S5] Product signals might be overlapping with d_8-tol

[S6] Colorless oil; ^{1}H NMR ($CDCl_3$): δ 5.45 - 5.43 (m, 1H, H-5'), 5.33 (ddd, 1H, *J* 12.6, J 4.6, J 3.0 Hz, H-4'), 5.20 - 5.13 (m, 3H, H-4, H-7, H-7'), 4.80 (dd, 1H, *J* 12.5, J 2.3 Hz, H-8a), 4.66 (dd, 1H, *J* 12.0, J 2.7 Hz, H-8'a), 4.32 (dd, 1H, *J* 9.3, J 1.3 Hz, H-6'), 4.16 (dd, 1H, *J* 12.3, J 2.6 Hz, H-8b), 4.14 (dd, 1H, *J* 12.0, J 4.7 Hz, H-8'b), 4.12 - 4.10 (m, 1H, H-5), 4.03 (dd, 1H, *J* 9.8, J 1.1 Hz, H-6), 3.86 (s, 3H, CO_2CH_3), 3.74 (s, 3H, CO_2CH_3), 3.29 (s, 3H, OCH_3), 2.29 (ddd, 1H, *J* 12.9, J 4.7, J 0.8 Hz, H-3'*eq*), 2.14 (s, 3H, $COCH_3$), 2.13 (app t, 1H, *J* 12.5 Hz, H-3*ax*), 2.09 (s, 3H, $COCH_3$), 2.09 – 2.05 (m, 1H, H-3*eq*), 2.064 (s, 3H, $COCH_3$), 2.055 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.99 (app t, 1H, *J* 12.8 Hz, H-3'*ax*), 1.99 (s, 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 170.91, 170.63, 170.42, 170.34, 170.14, 170.08 and 169.78 (7 x s, 7C, $COCH_3$), 167.49 and 166.60 (2 x s, 2C, C-1, C-1'), 100.88 (s, C-2'), 98.76 (s, C-2), 70.80 (d, C-5), 69.39 (d, 2C, C-6, C-6'), 68.26, 68.21 and 68.14 (3 x d, 3C, C-4, C-7, C-7'), 66.12 (d, C-4'), 64.56 (d, C-5'), 62.26 (t, C-8'), 61.37 (d, C-8), 52.76 and 52.65 (2 x q, 2C, CO_2CH_3), 51.15 (q, OCH_3), 33.70 (t, C-3'), 32.37 (t, C-3), 21.38, 21.11, 20.85, 20.77, 20.73, 20.69 and 20.63 (7 x q, 7C, $COCH_3$).

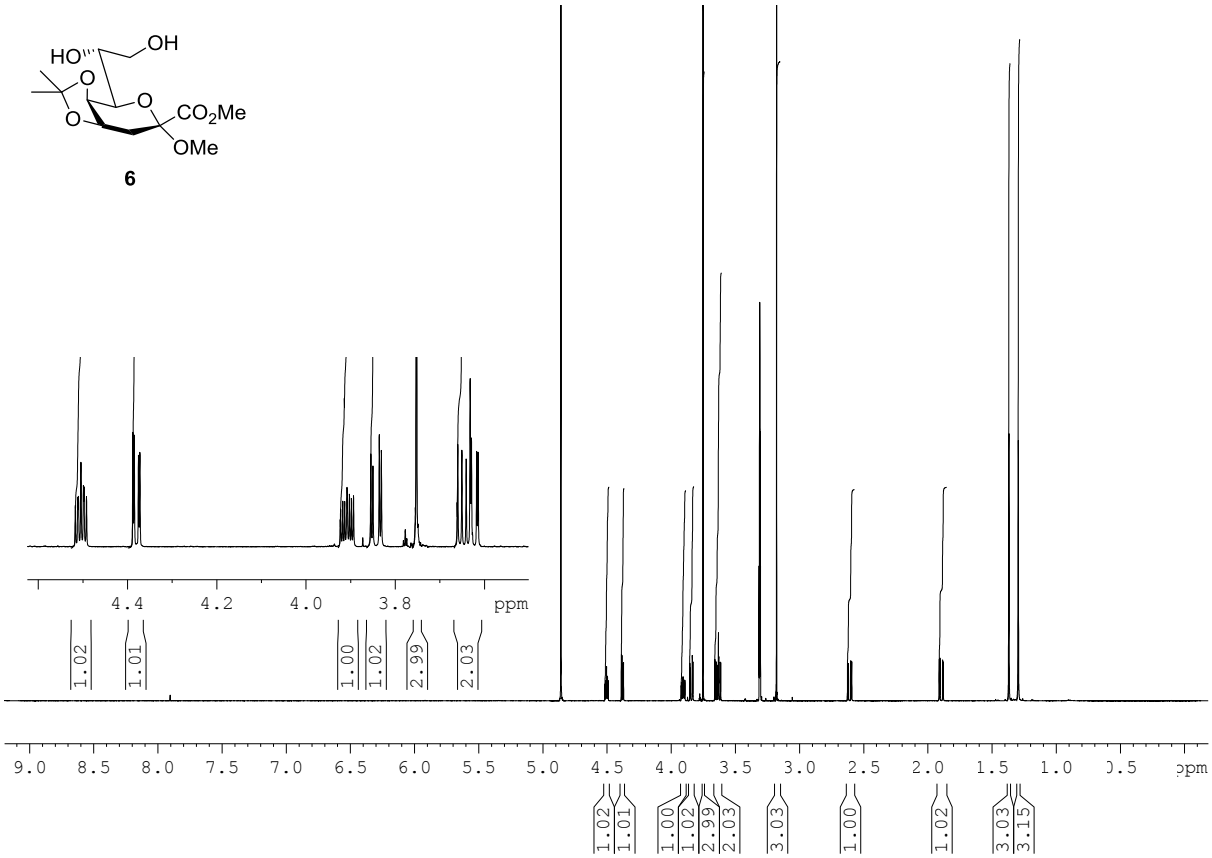
4. NMR spectra of compounds 5-8, 11-12, 14-16, 18, 19, 21, 22 and 24



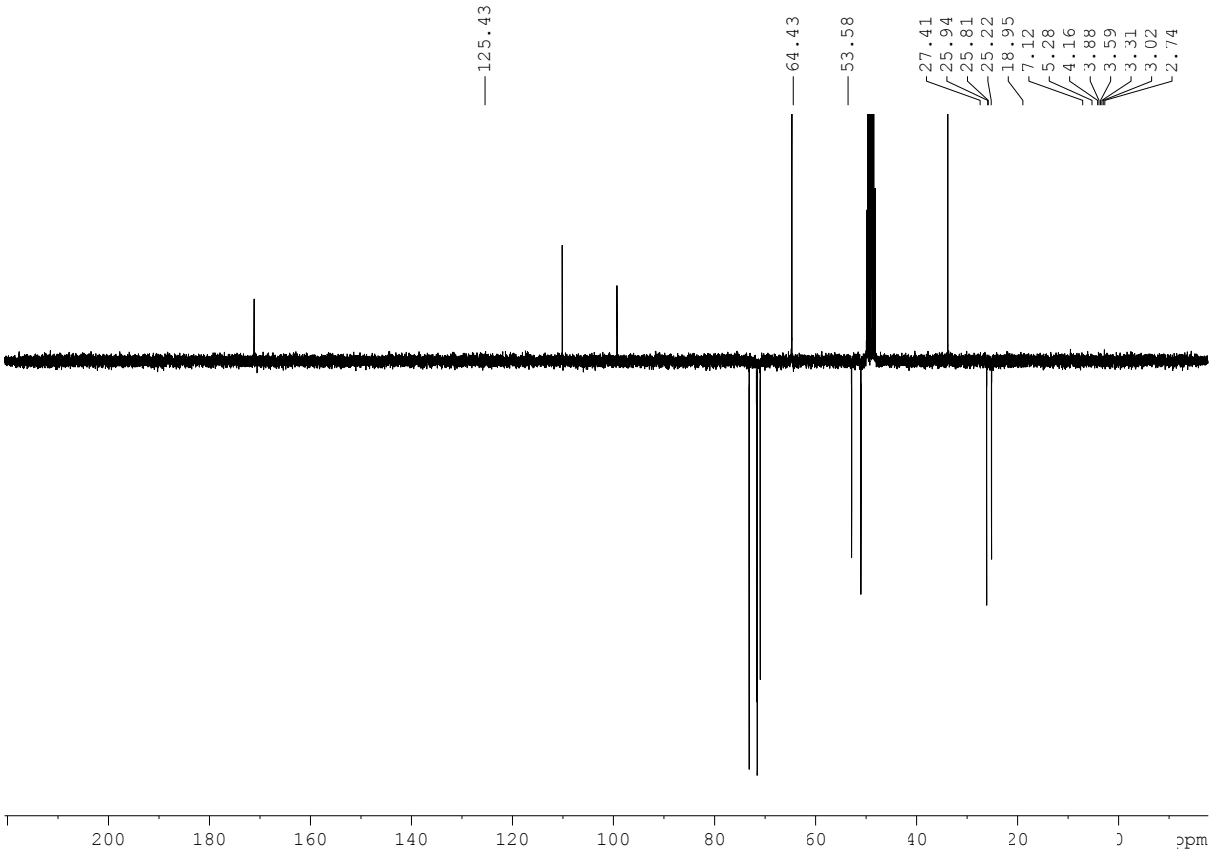
^{1}H NMR ($CDCl_3$, 600 MHz)



^{13}C NMR ($CDCl_3$, 150 MHz)



^{1}H NMR (MeOD, 600 MHz)



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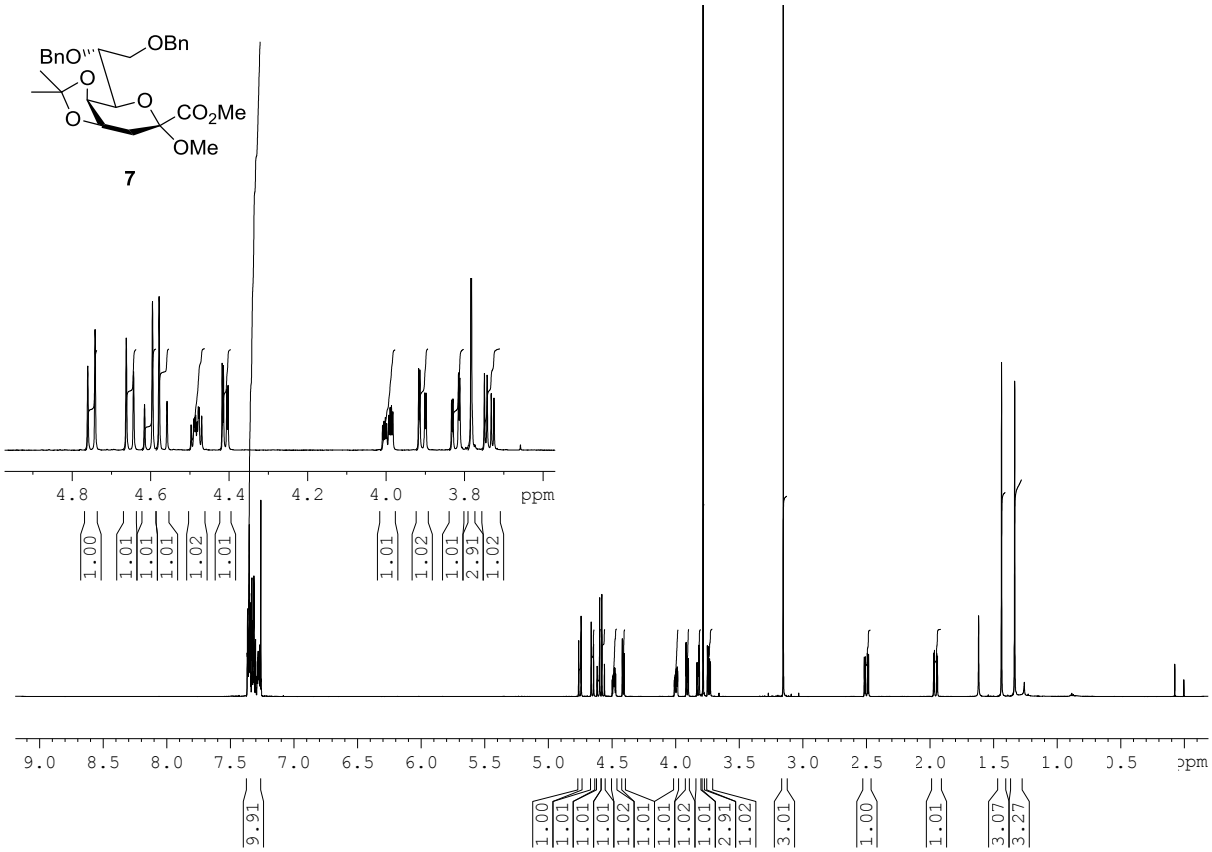
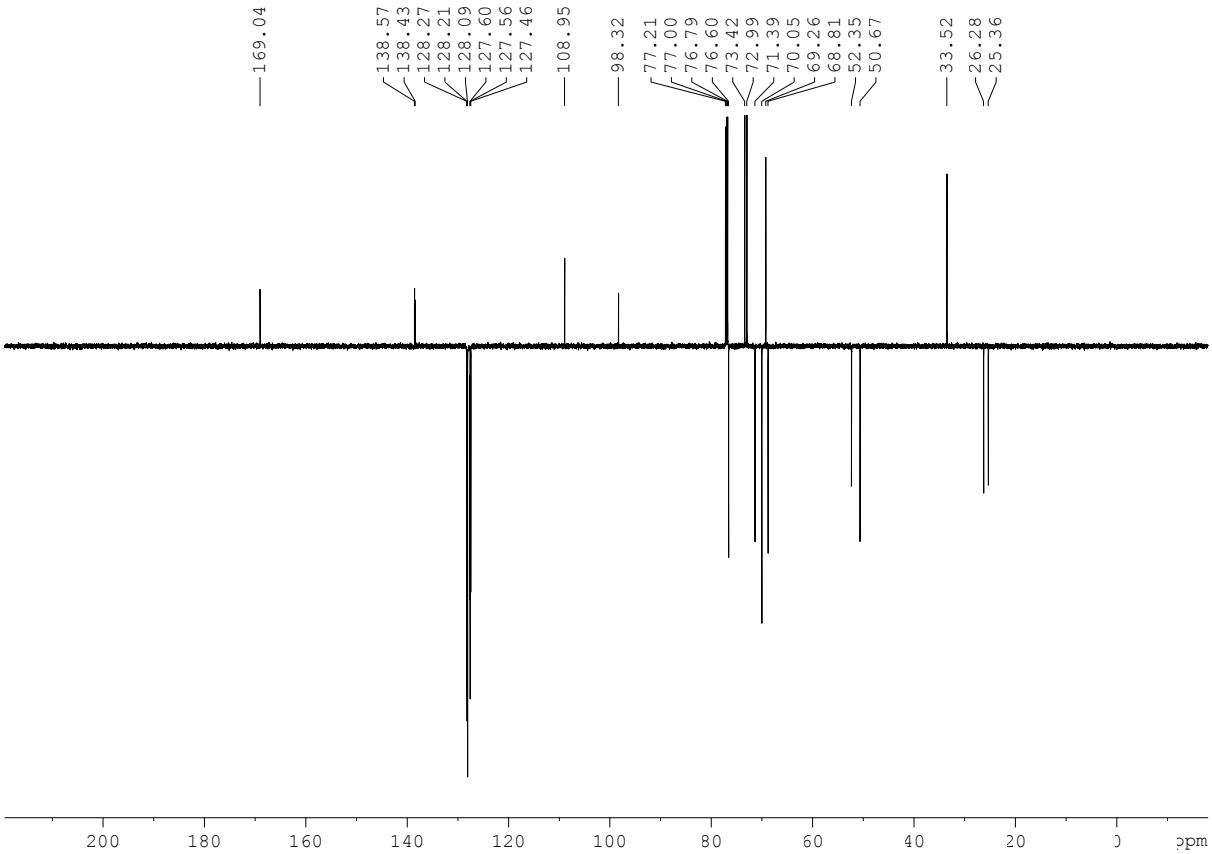
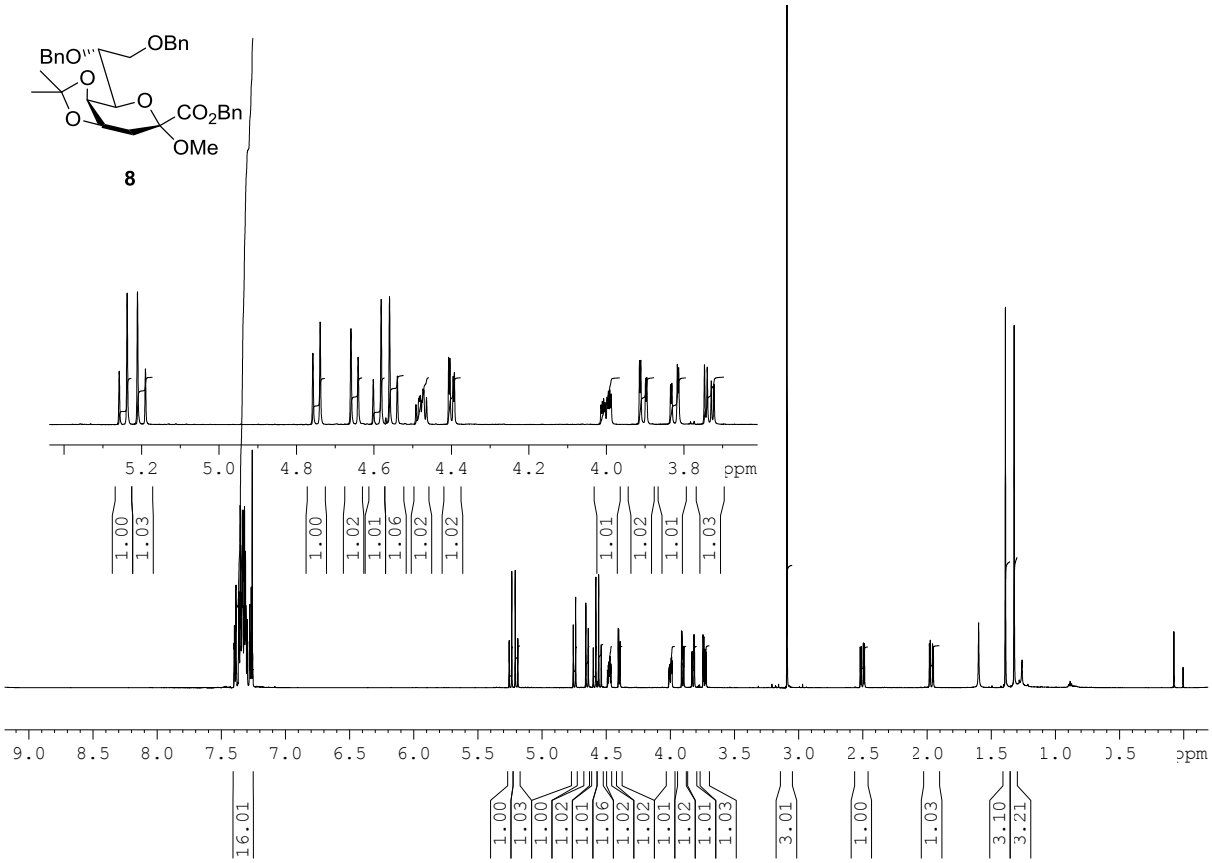


^{1}H NMR ($CDCl_3$, 600 MHz)



^{13}C NMR ($CDCl_3$, 150 MHz)



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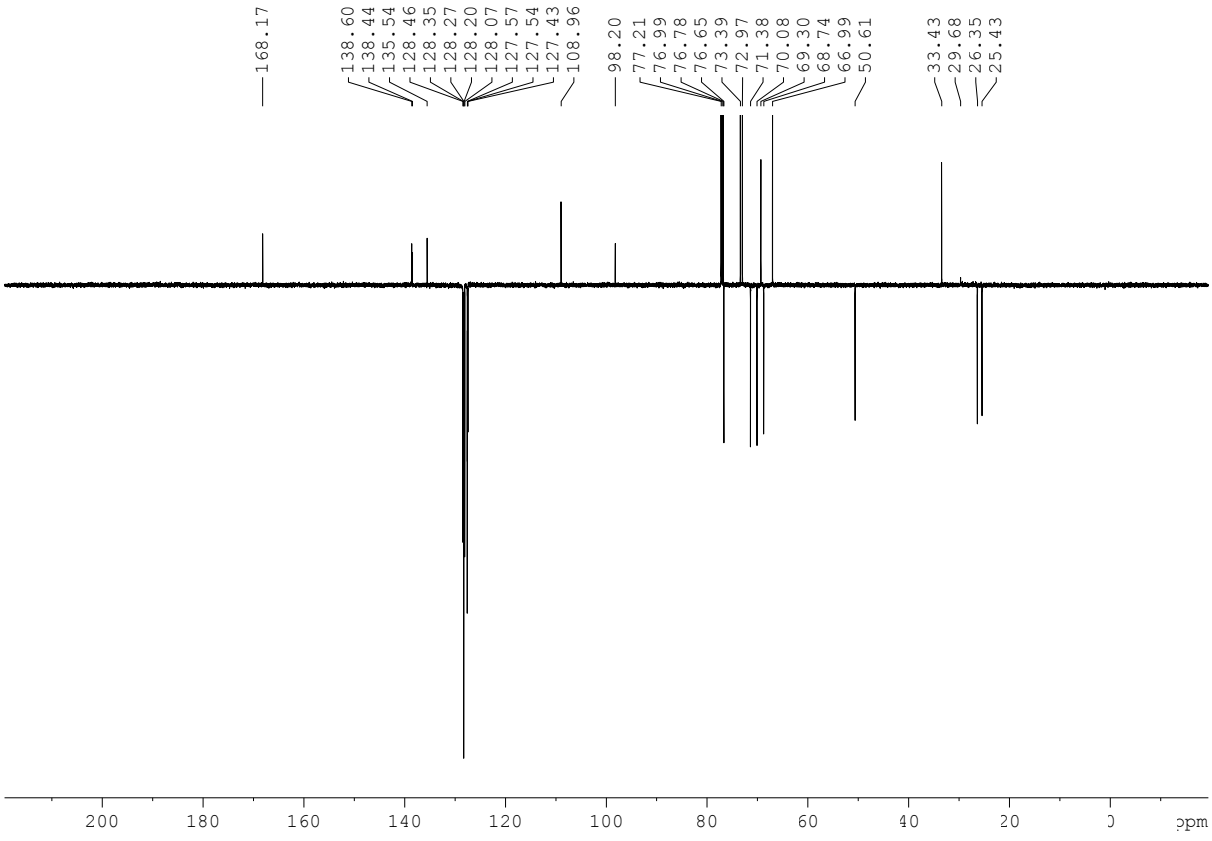
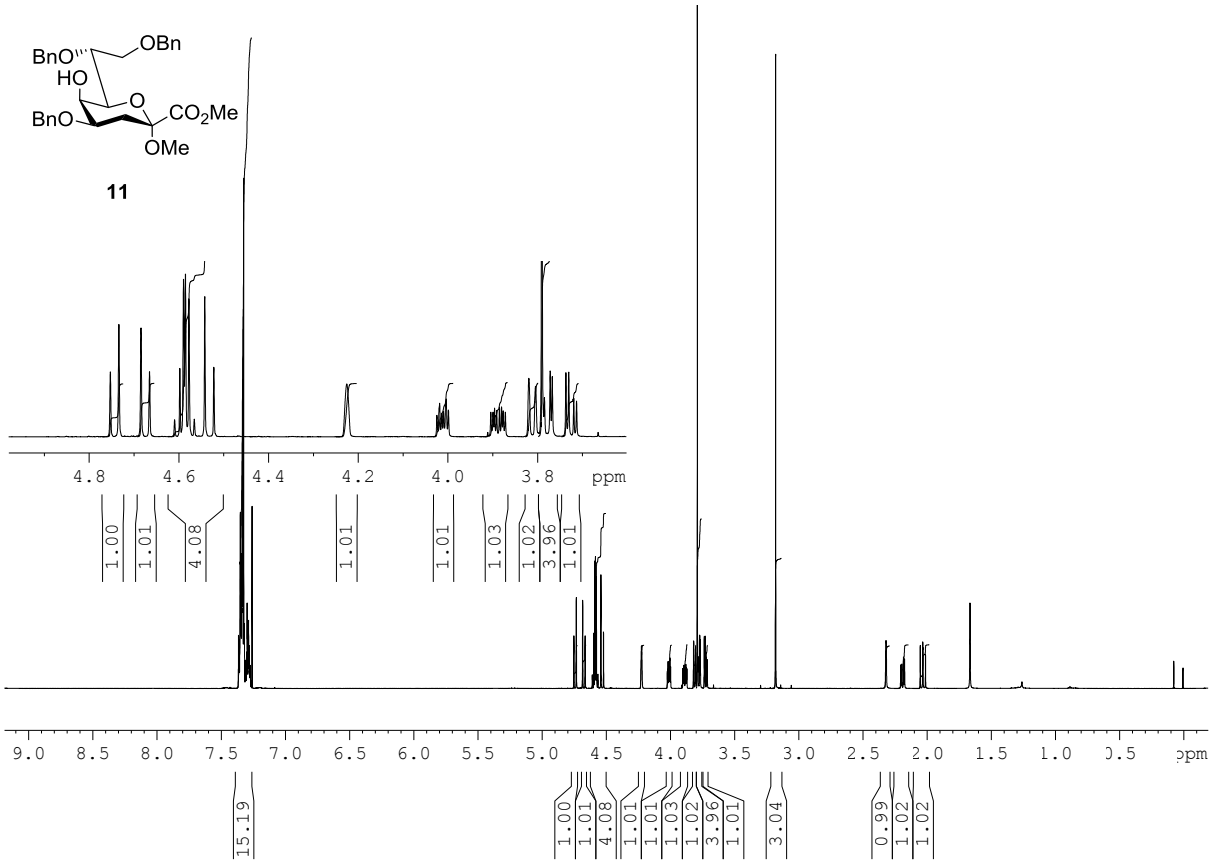
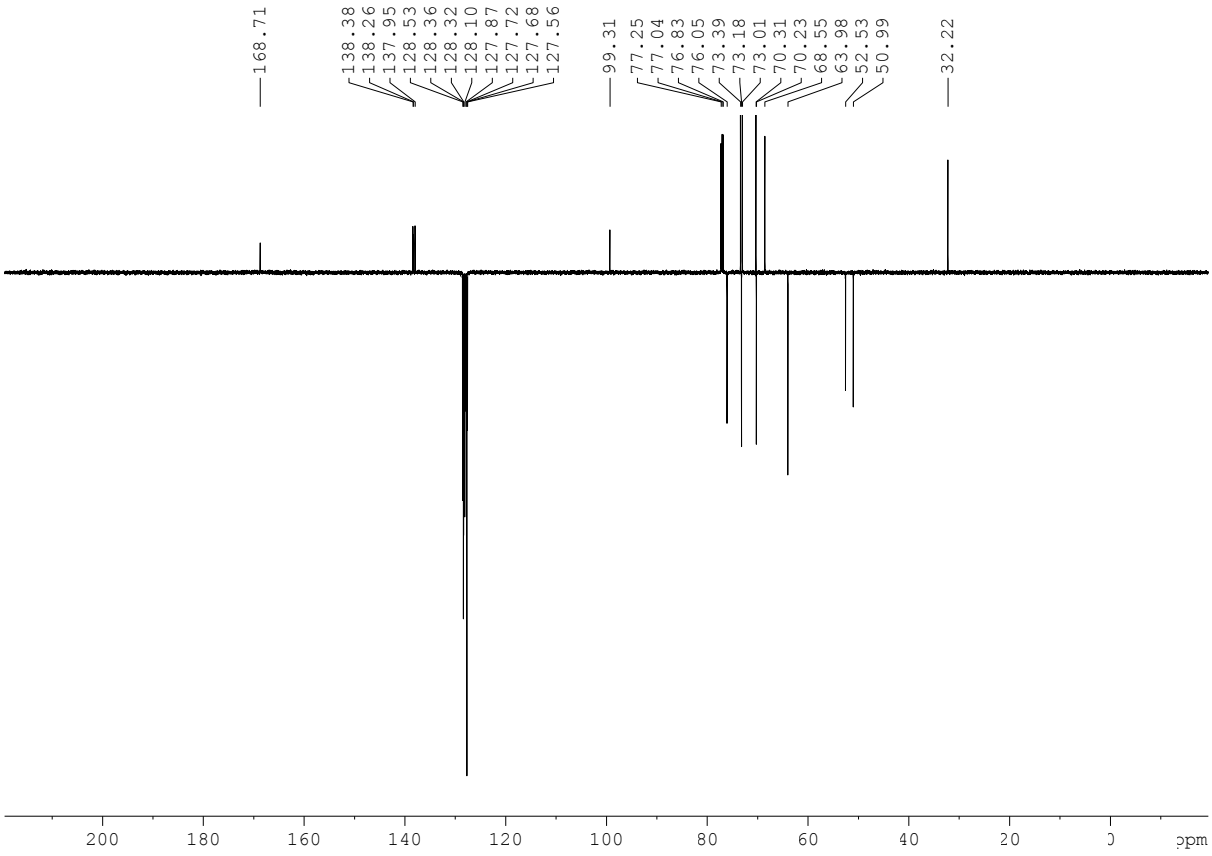


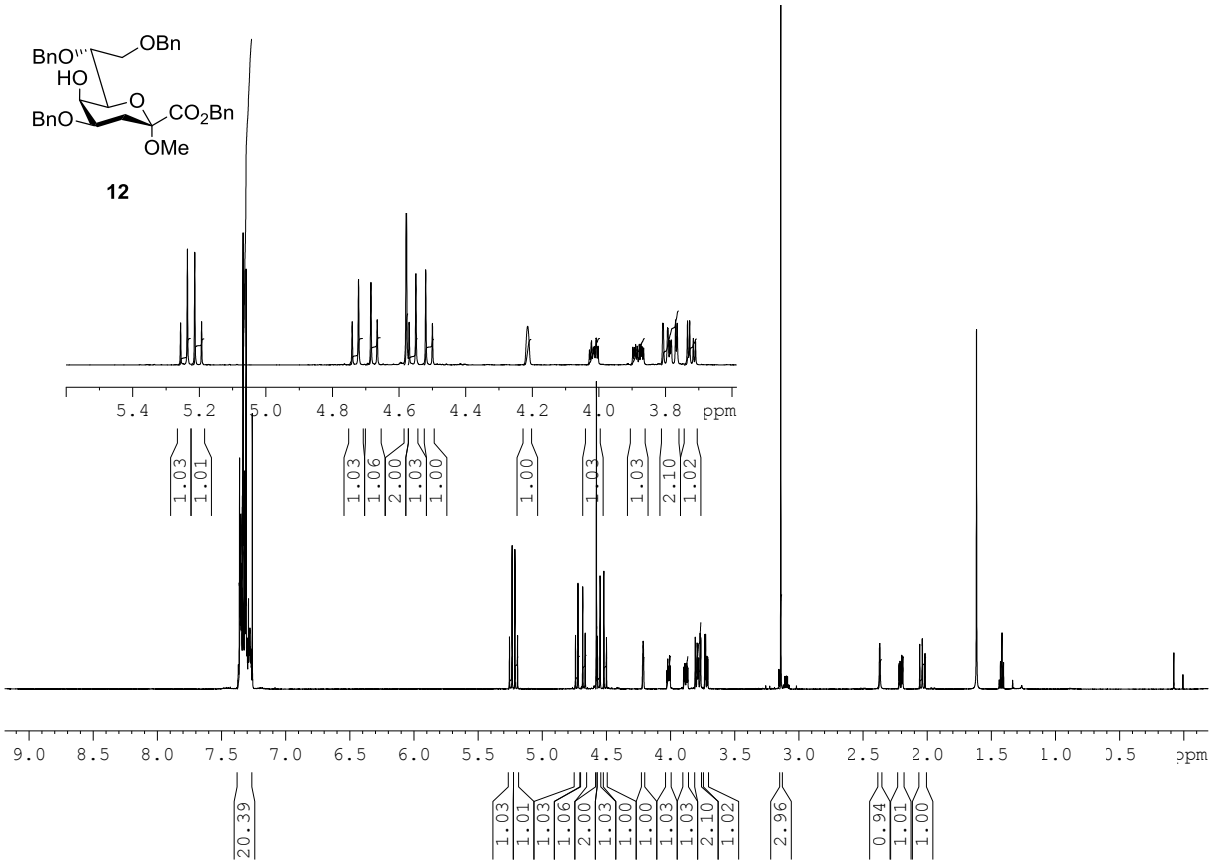
^{13}C NMR ($CDCl_3$, 150 MHz)



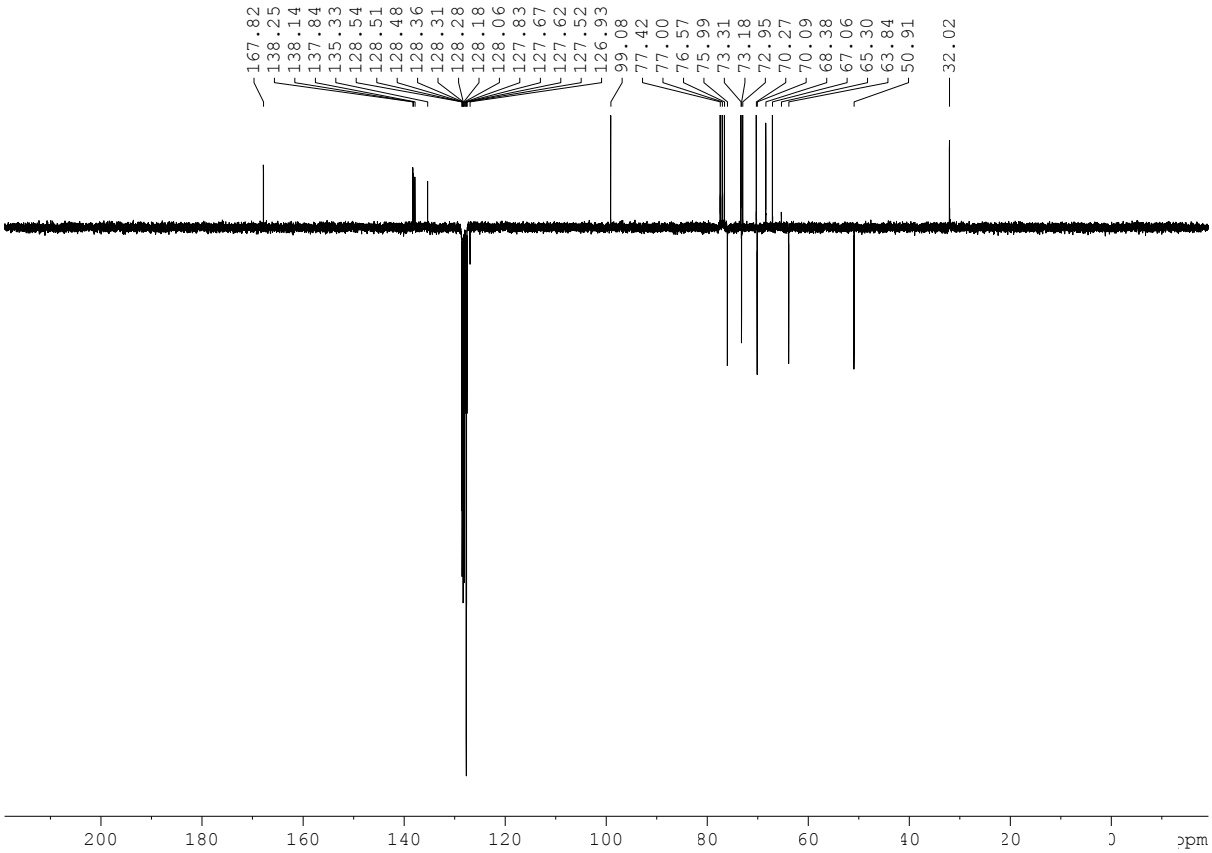
^{1}H NMR ($CDCl_3$, 600 MHz)



^{13}C NMR ($CDCl_3$, 150 MHz)



^{1}H NMR ($CDCl_3$, 600 MHz)



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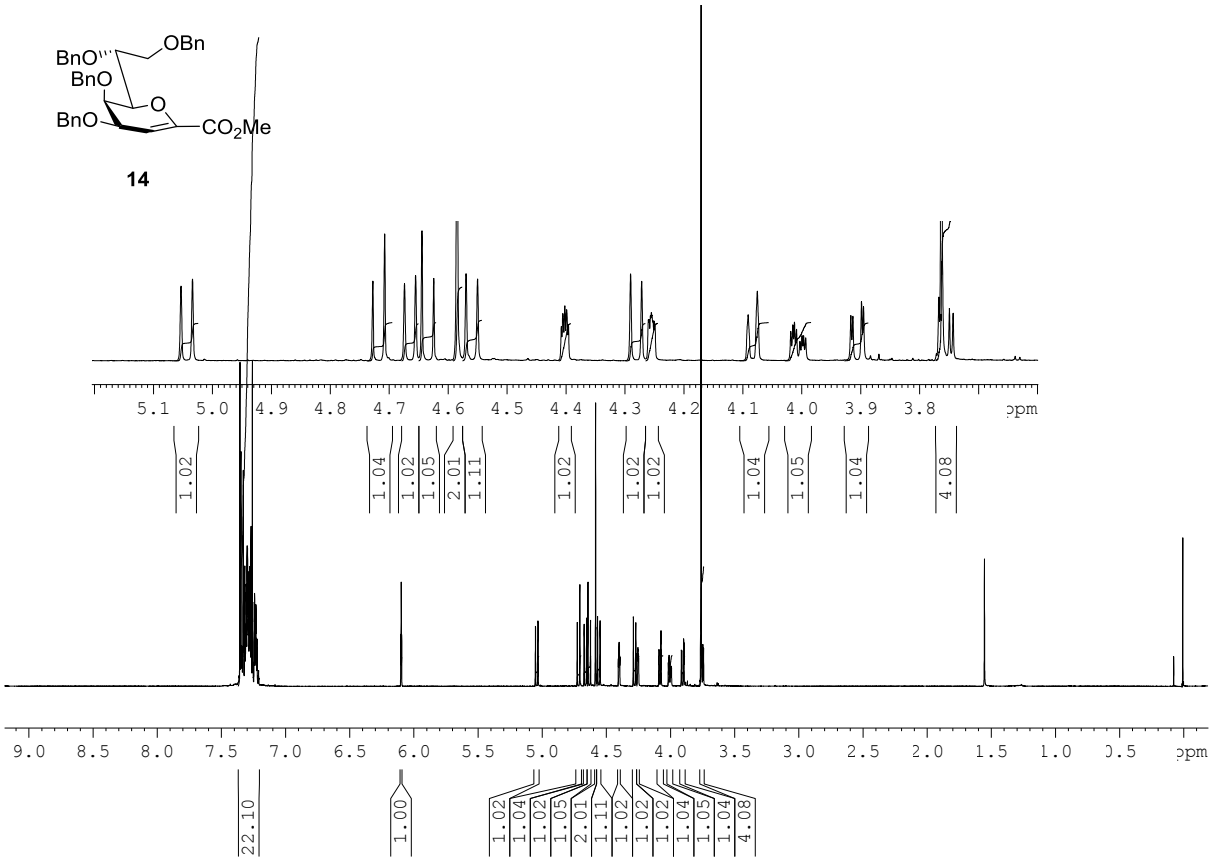
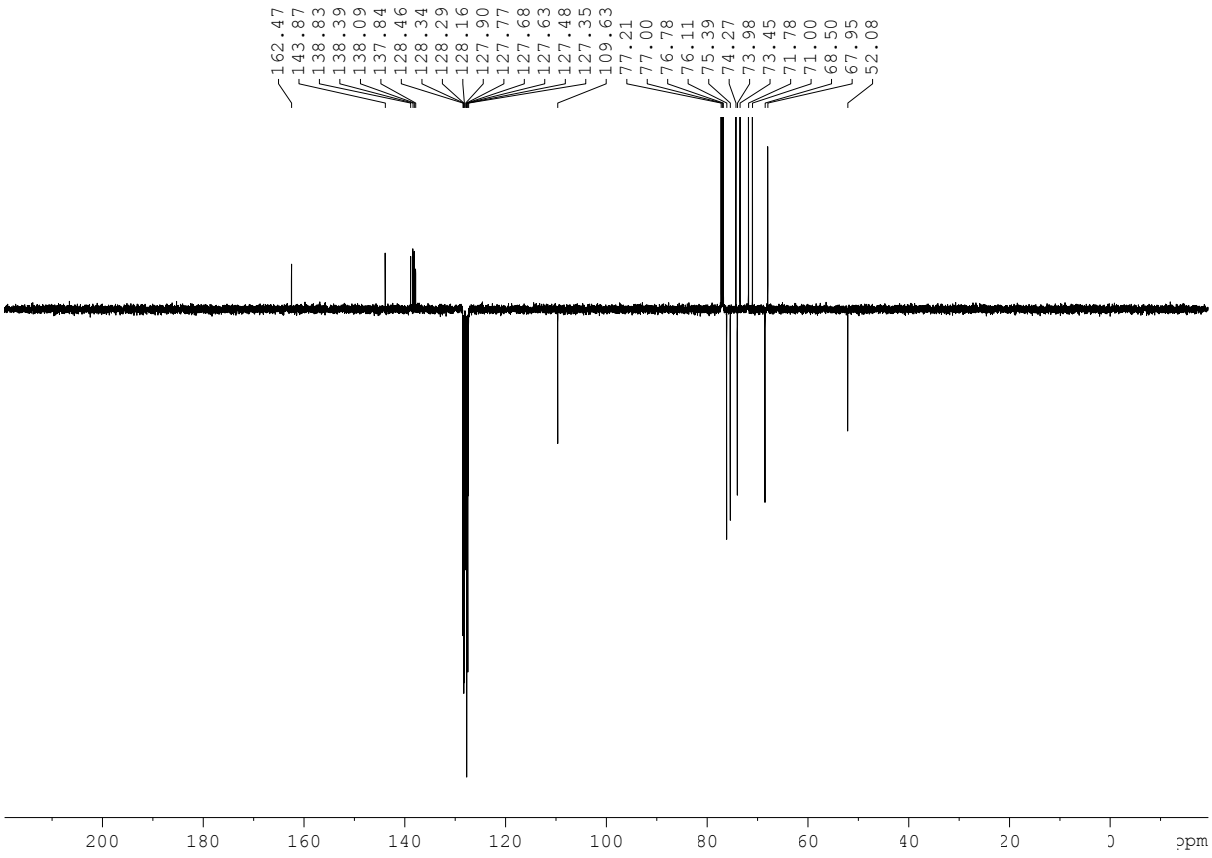
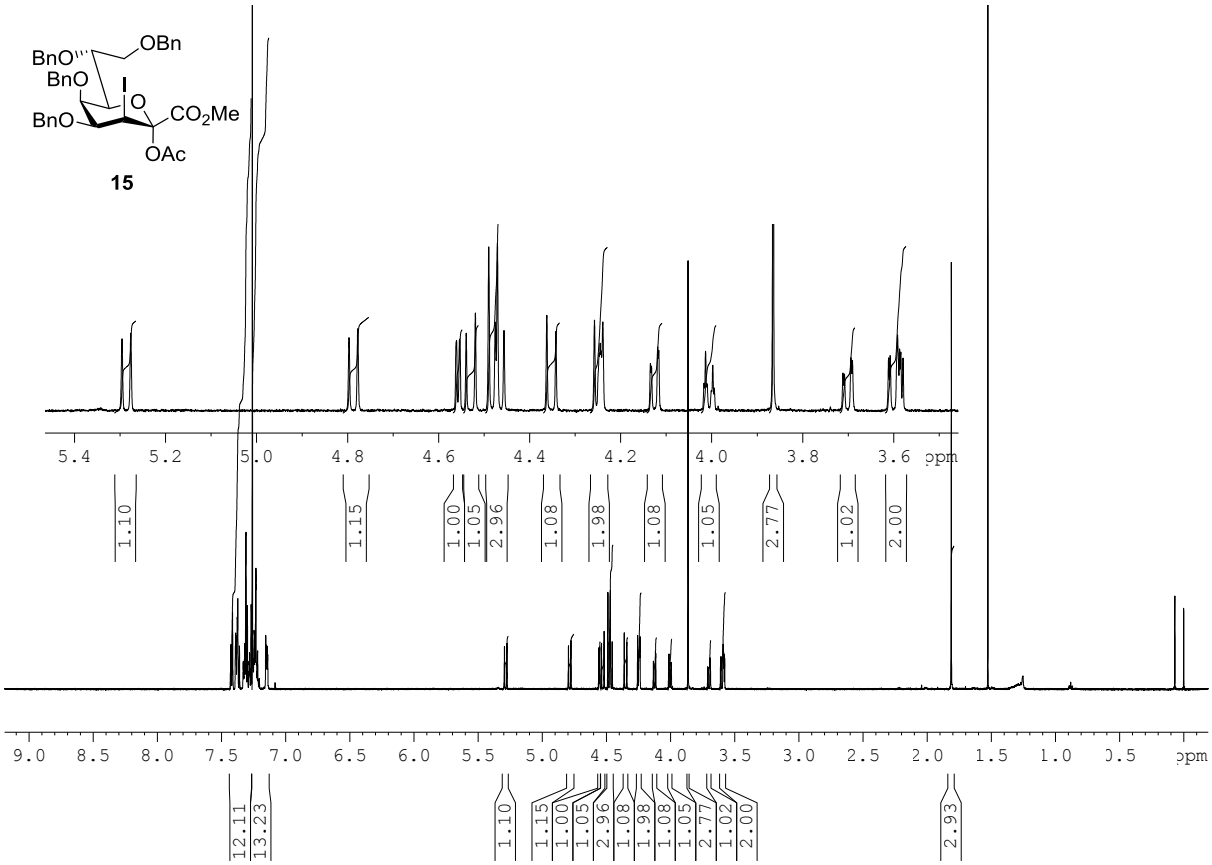


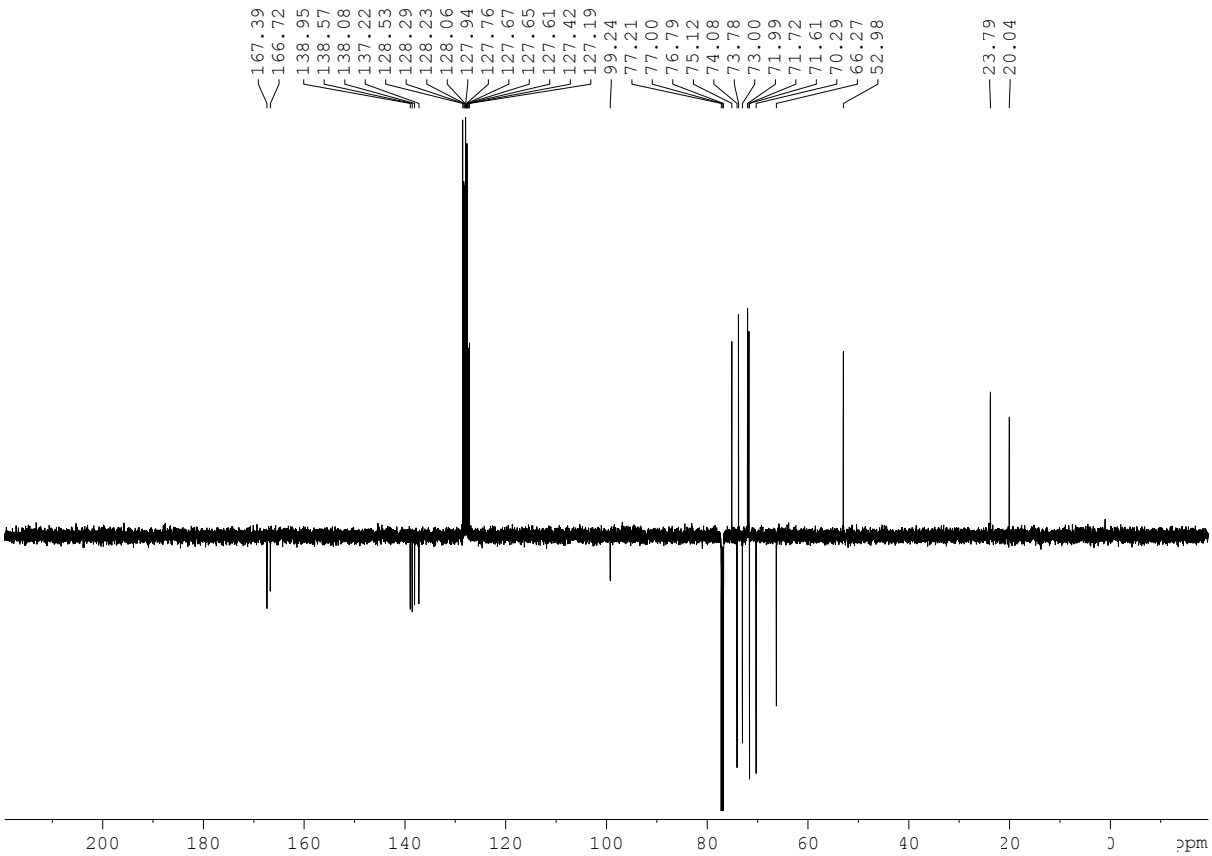
^{1}H NMR ($CDCl_3$, 600 MHz)



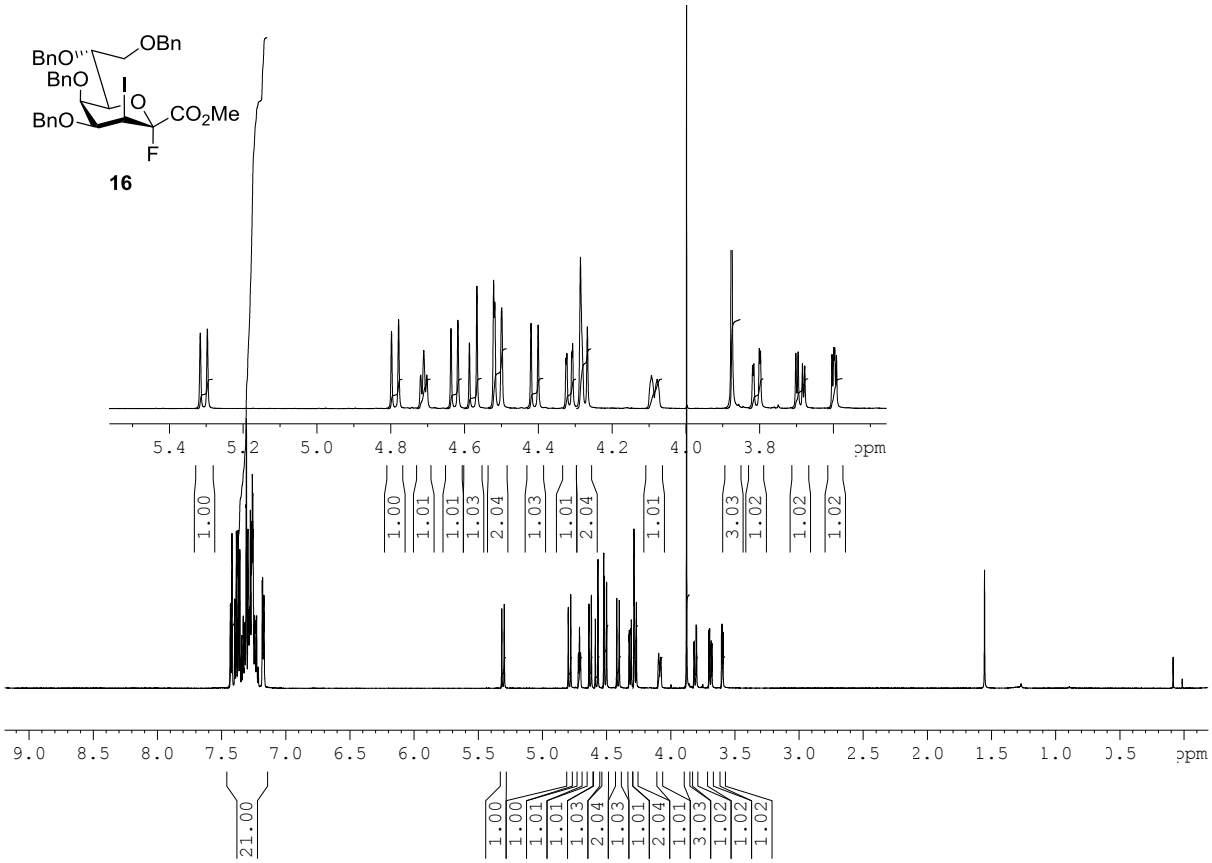
^{13}C NMR ($CDCl_3$, 150 MHz)



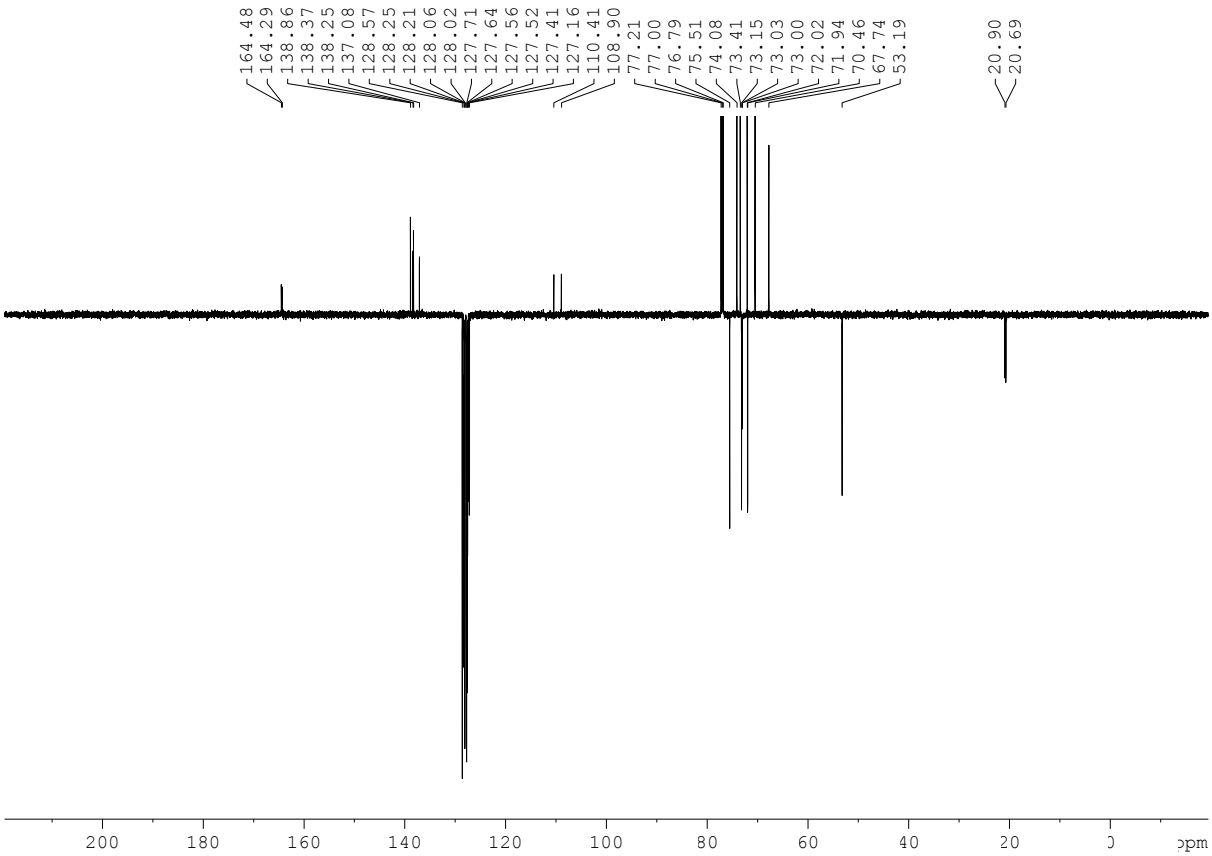
^{1}H NMR ($CDCl_3$, 600 MHz)



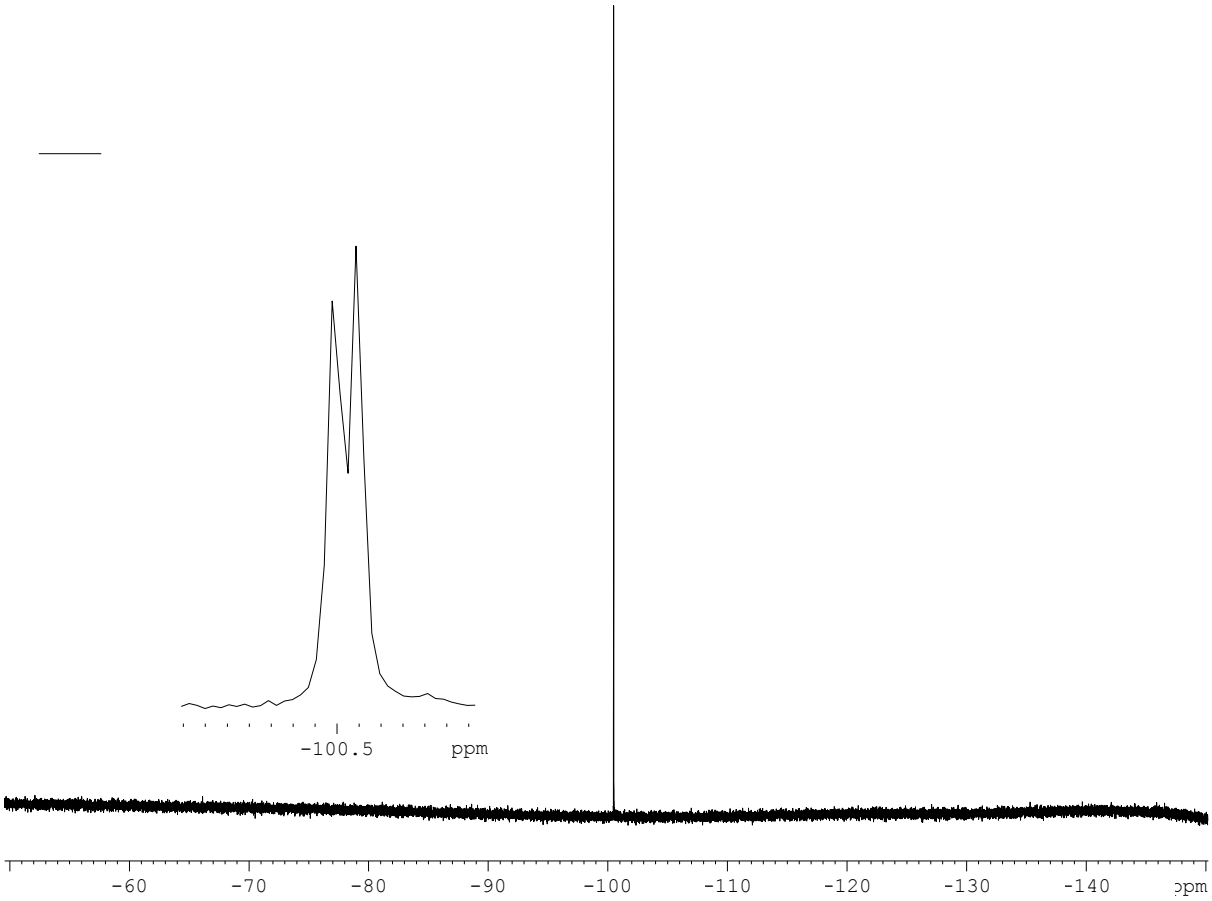
^{13}C NMR ($CDCl_3$, 150 MHz)



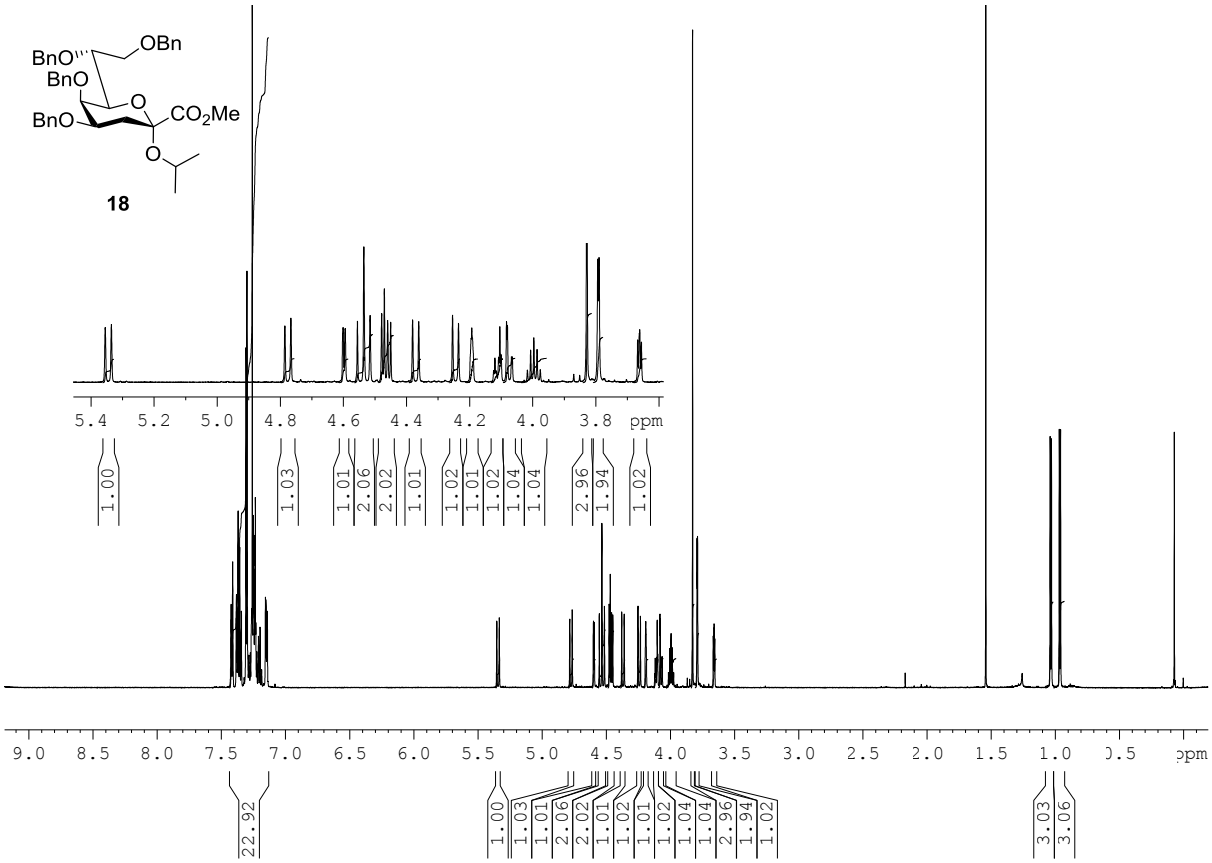
^{1}H NMR ($CDCl_3$, 600 MHz)



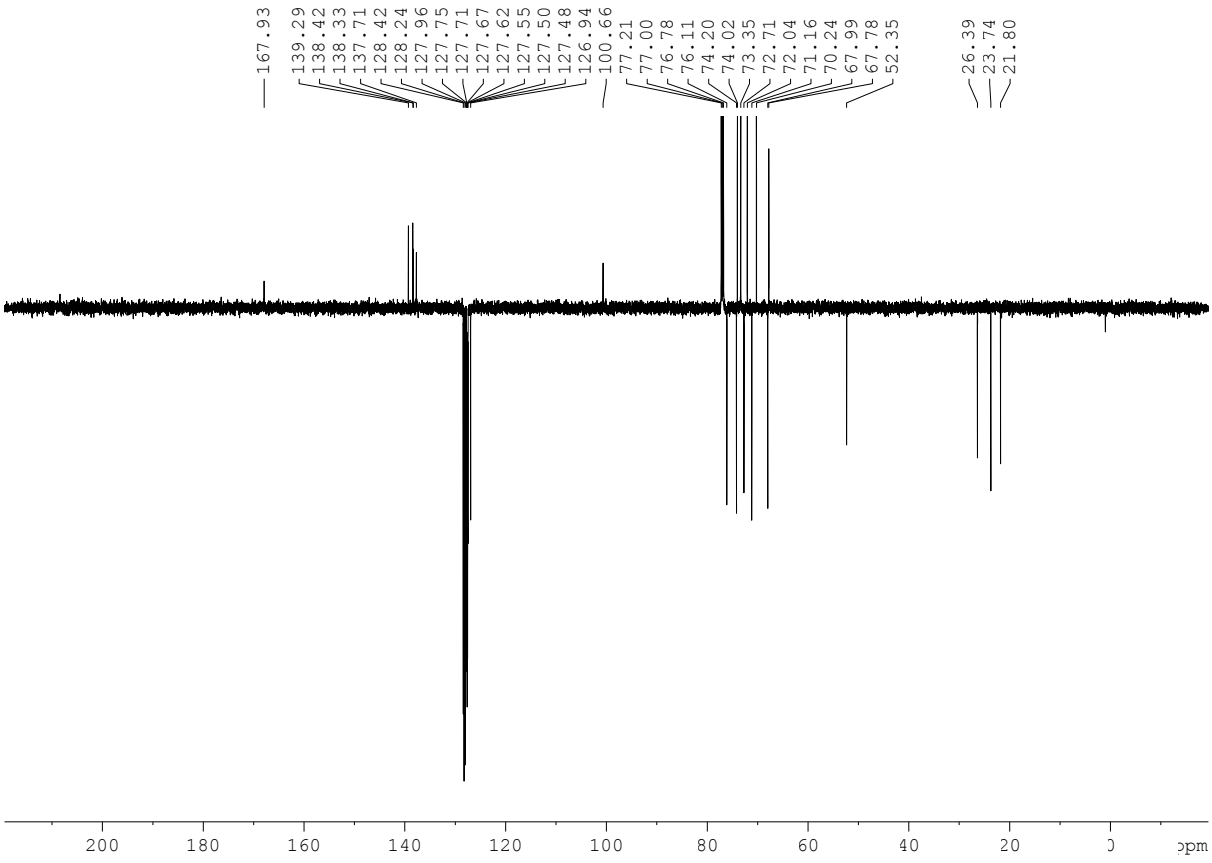
^{13}C NMR ($CDCl_3$, 150 MHz)



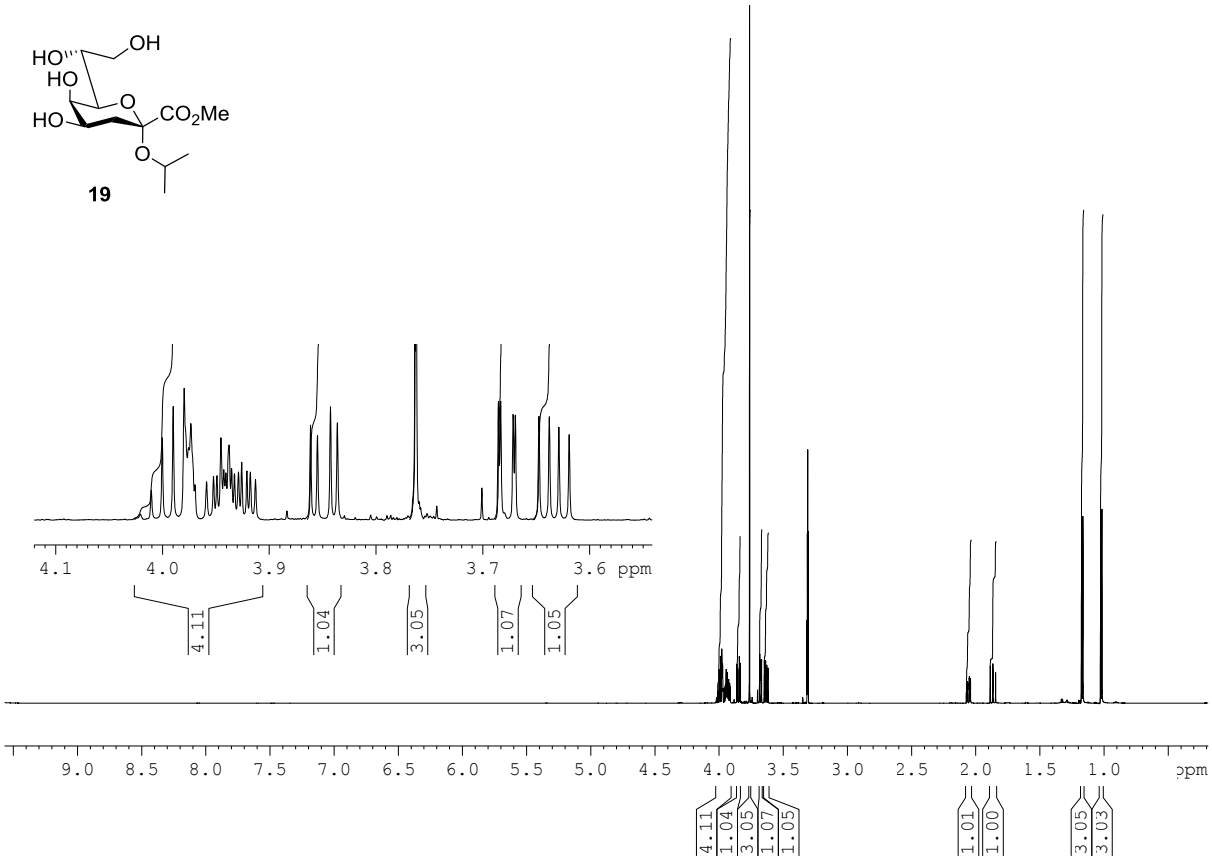
^{19}F NMR ($CDCl_3$, 565 MHz)



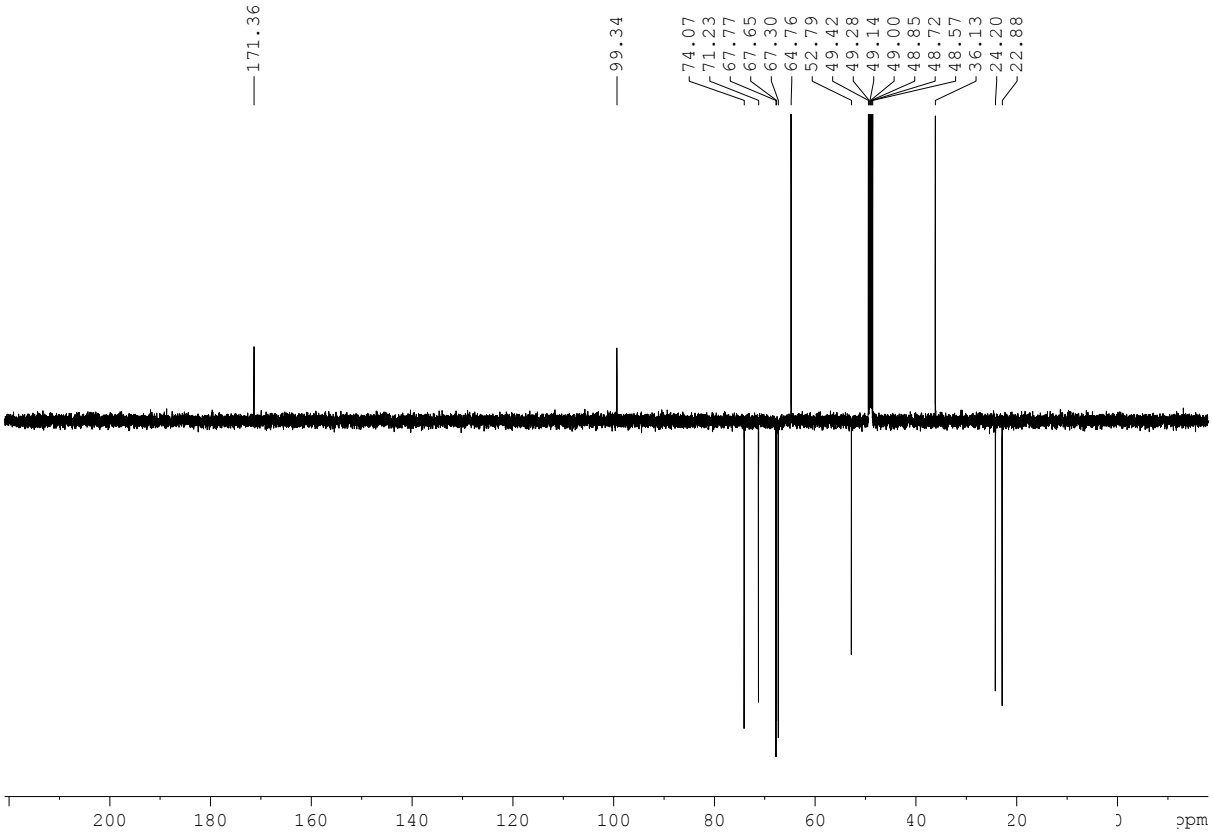
^{1}H NMR ($CDCl_3$, 600 MHz)



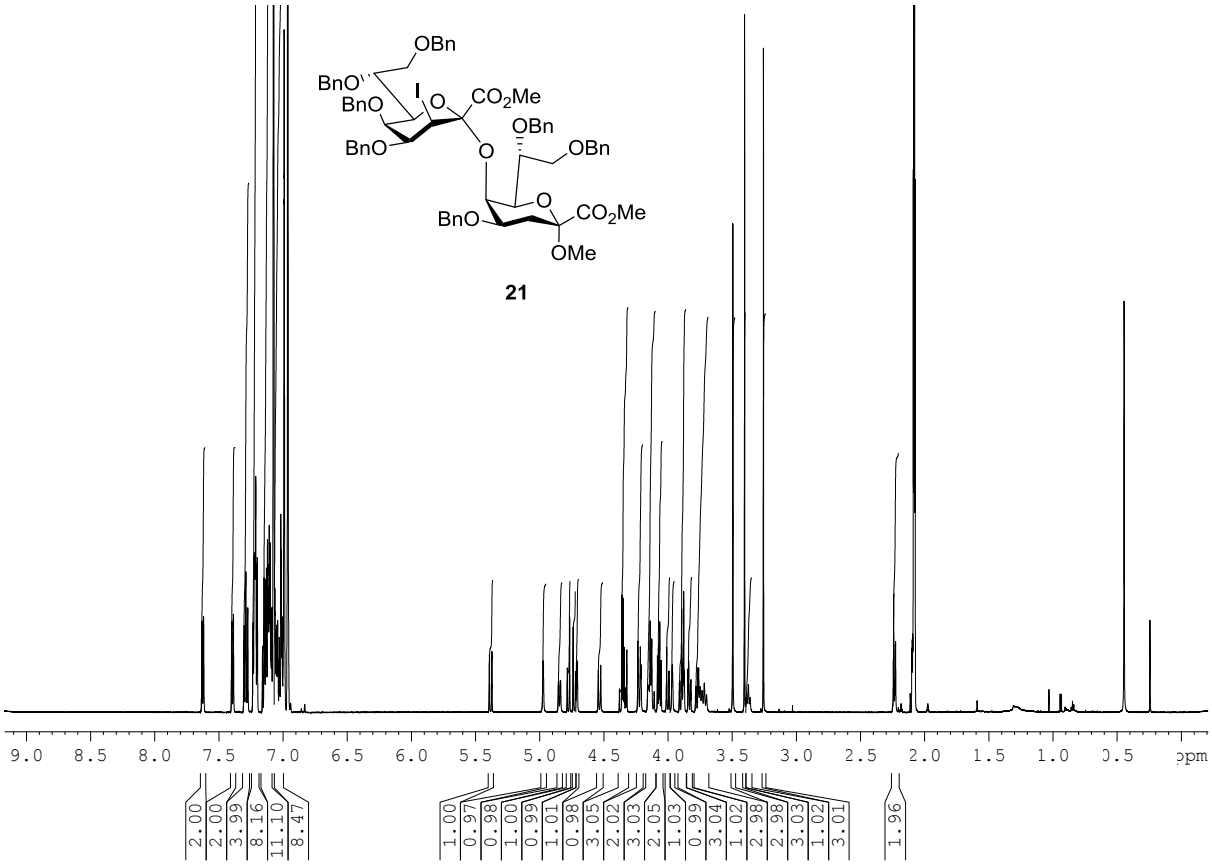
^{13}C NMR ($CDCl_3$, 150 MHz)



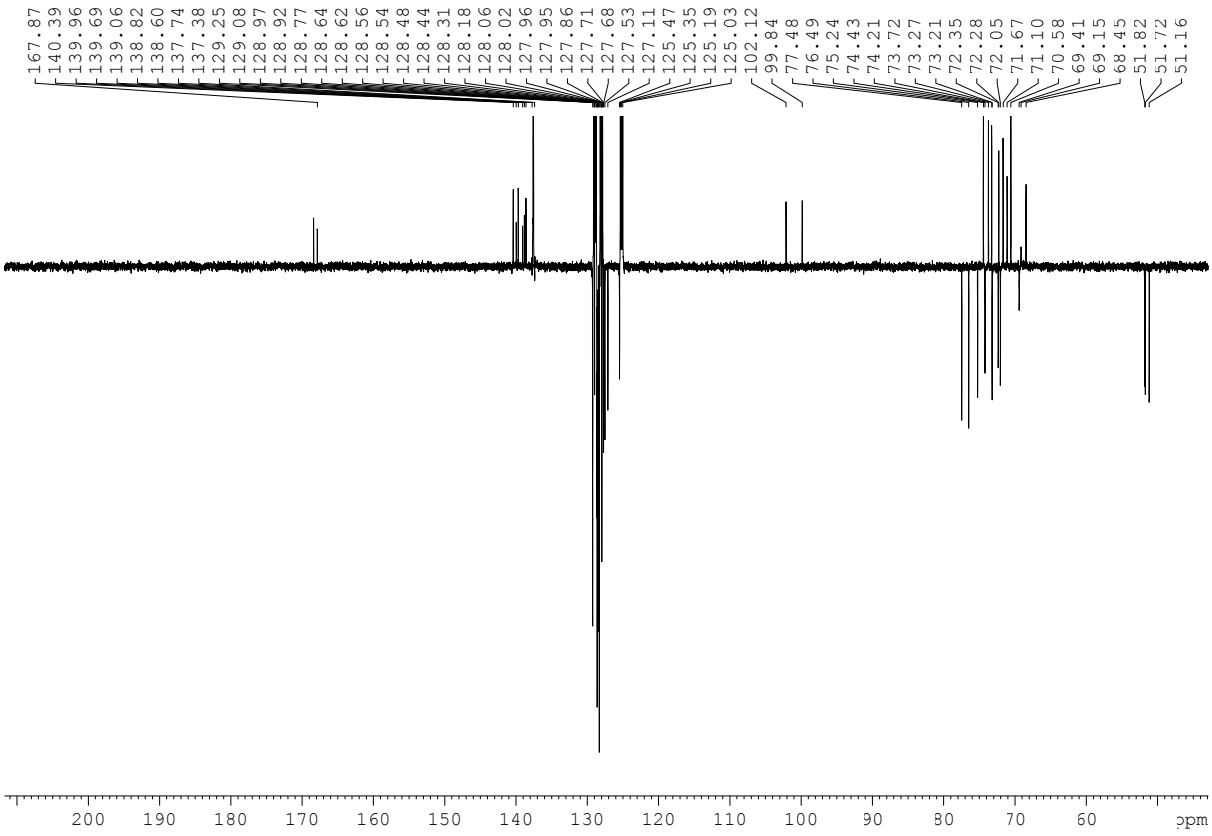
^{1}H NMR (MeOD, 600 MHz)



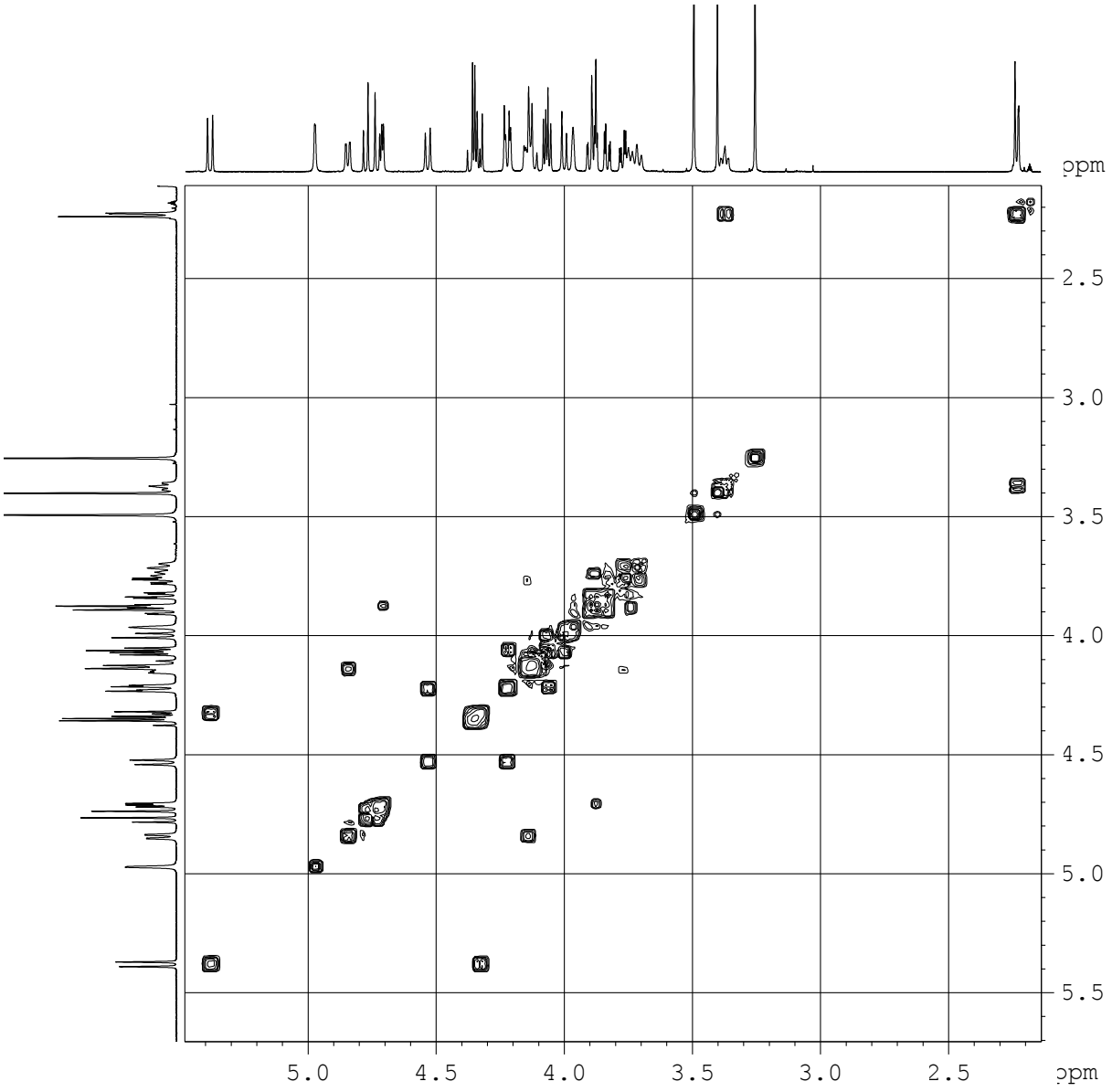
^{13}C NMR (MeOD, 150 MHz)



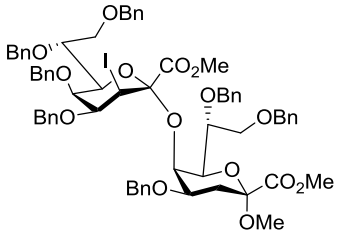
^{1}H NMR (d_8-toluene, 600 MHz, 323.15 K)



^{13}C NMR (d_8-toluene, 150 MHz, 323.15 K)



^{1}H-^{1}H COSY (d_8-toluene, 323.15 K)



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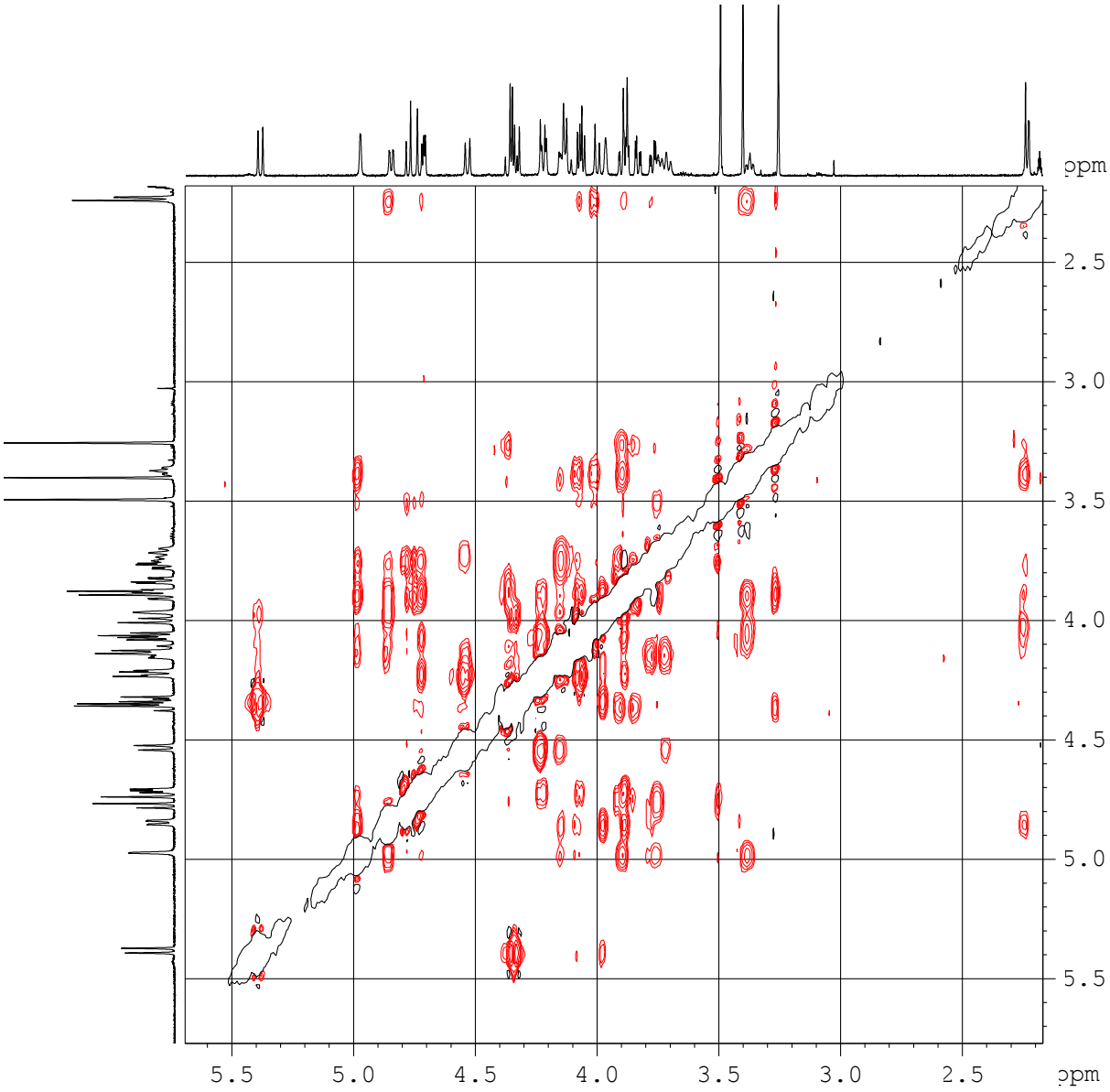
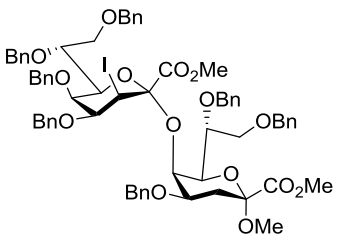


^{1}H-^{1}H NOESY (d_8-toluene, 323.15 K)



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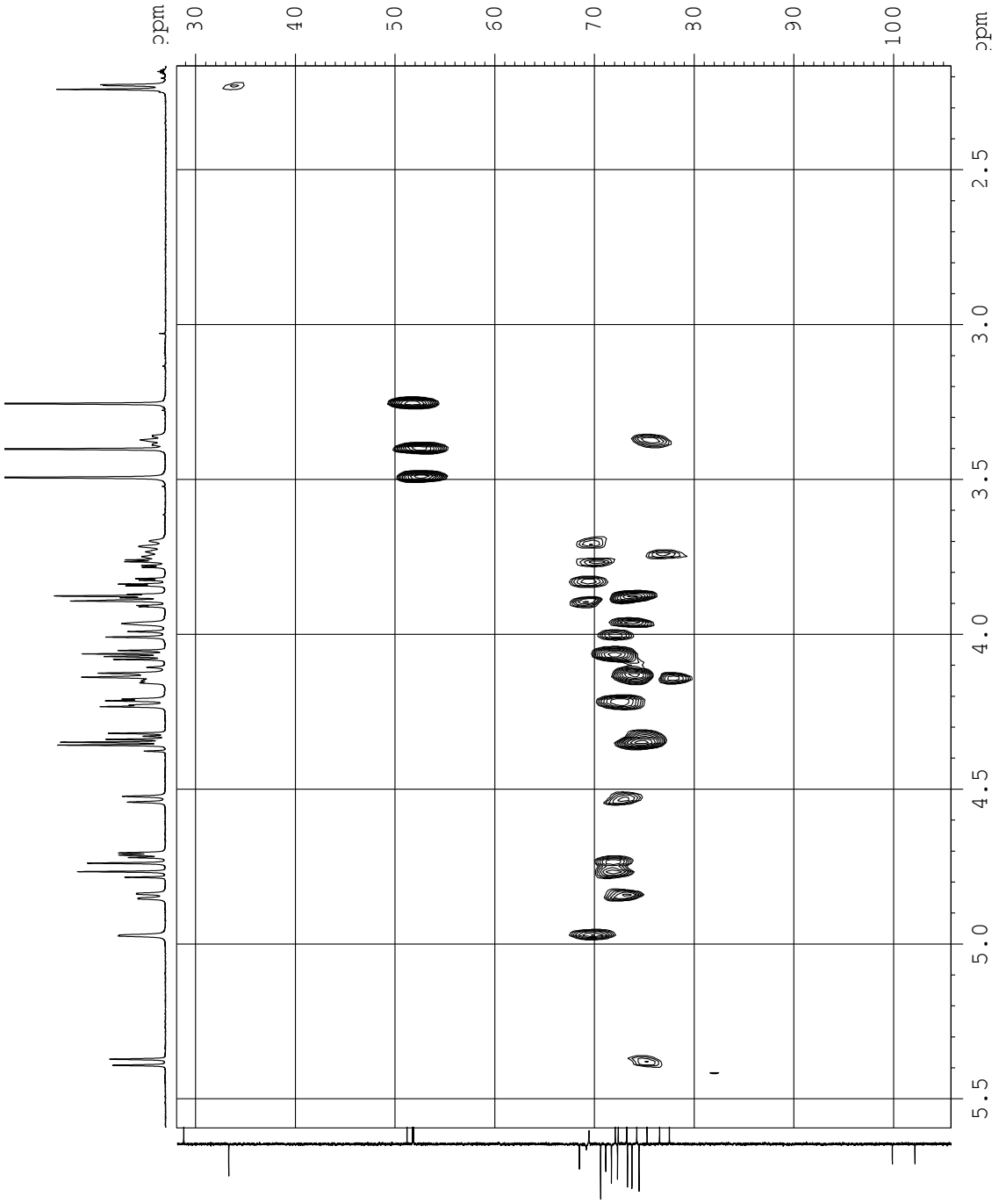
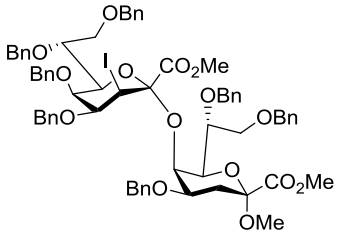


^{1}H-^{13}C HSQC (d_8-toluene, 323.15 K)



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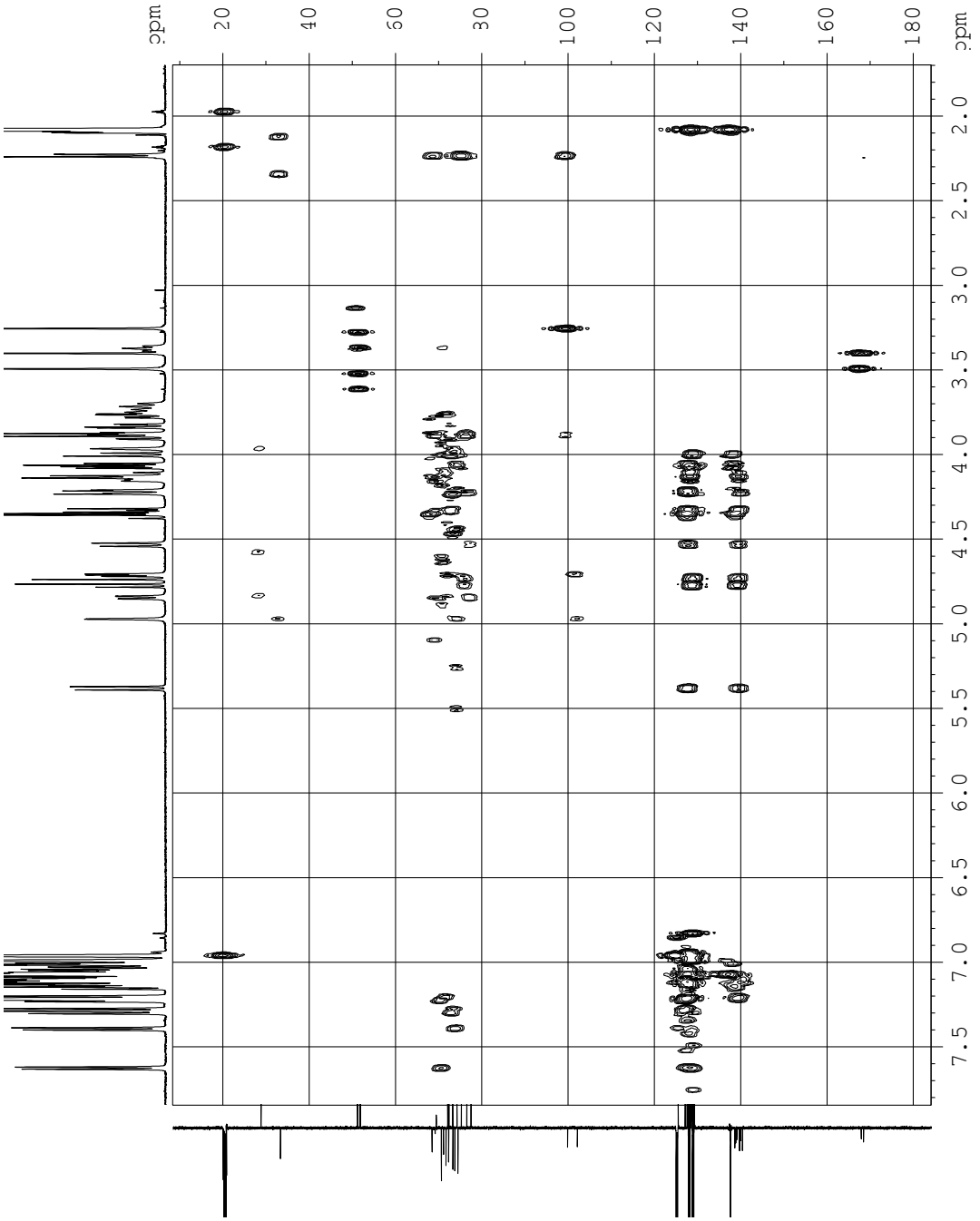
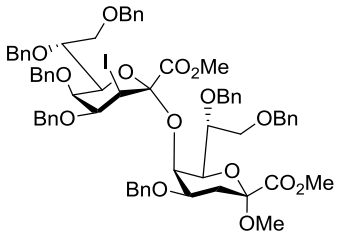


^{1}H-^{13}C HMBC (d_8-toluene, 323.15 K)



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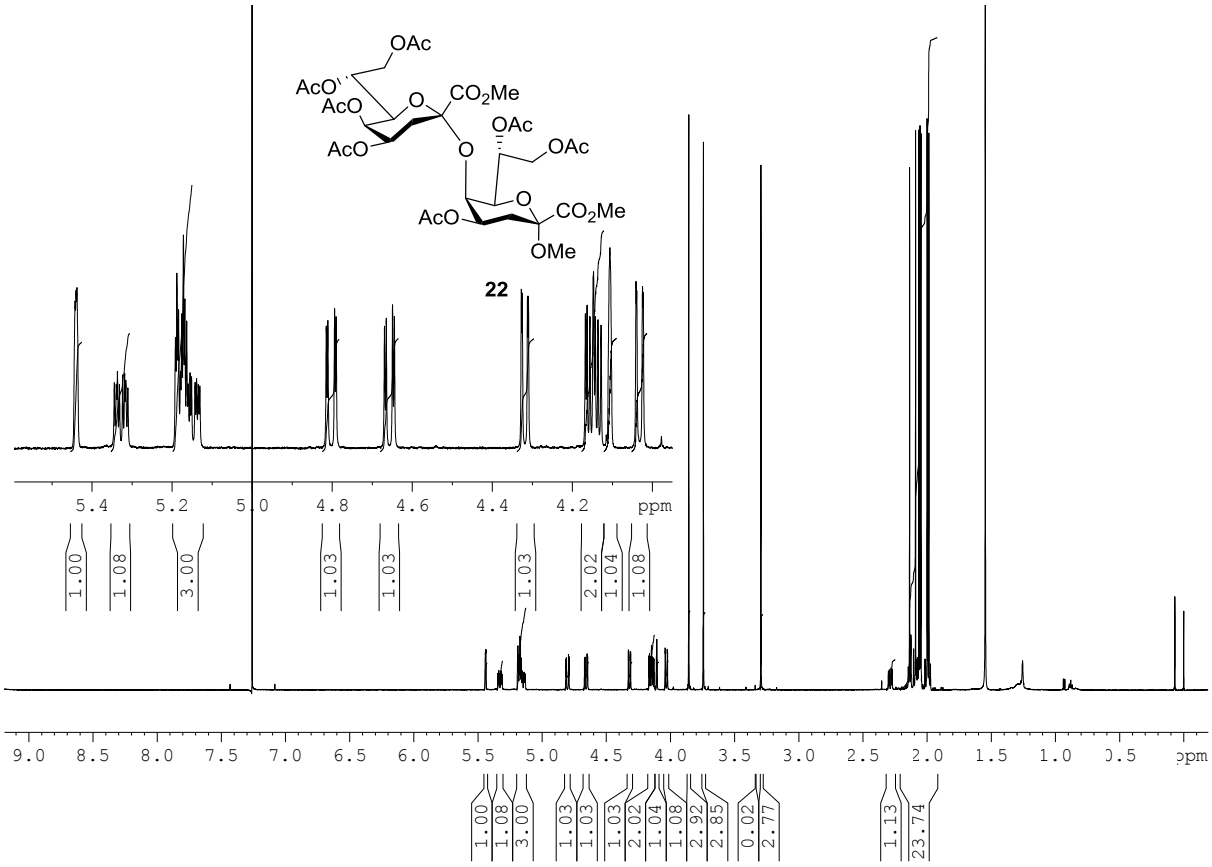
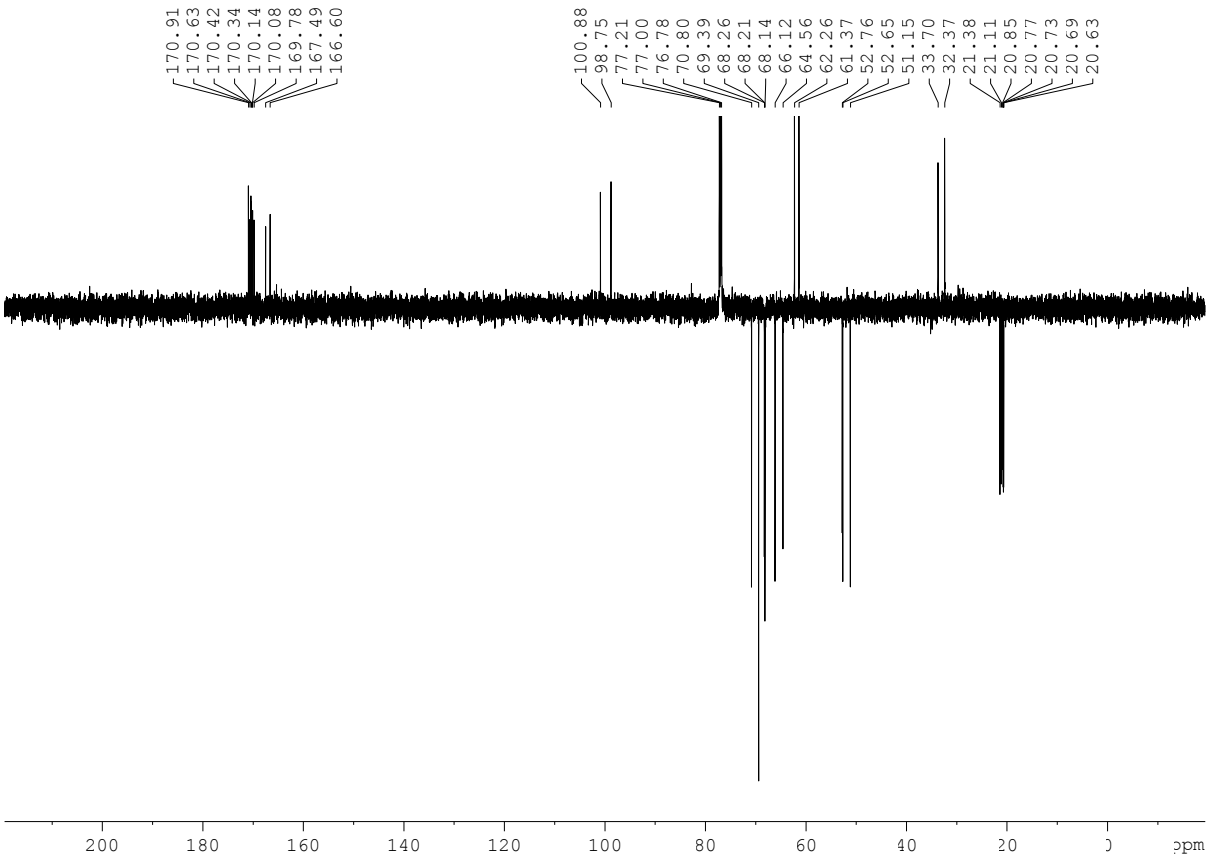
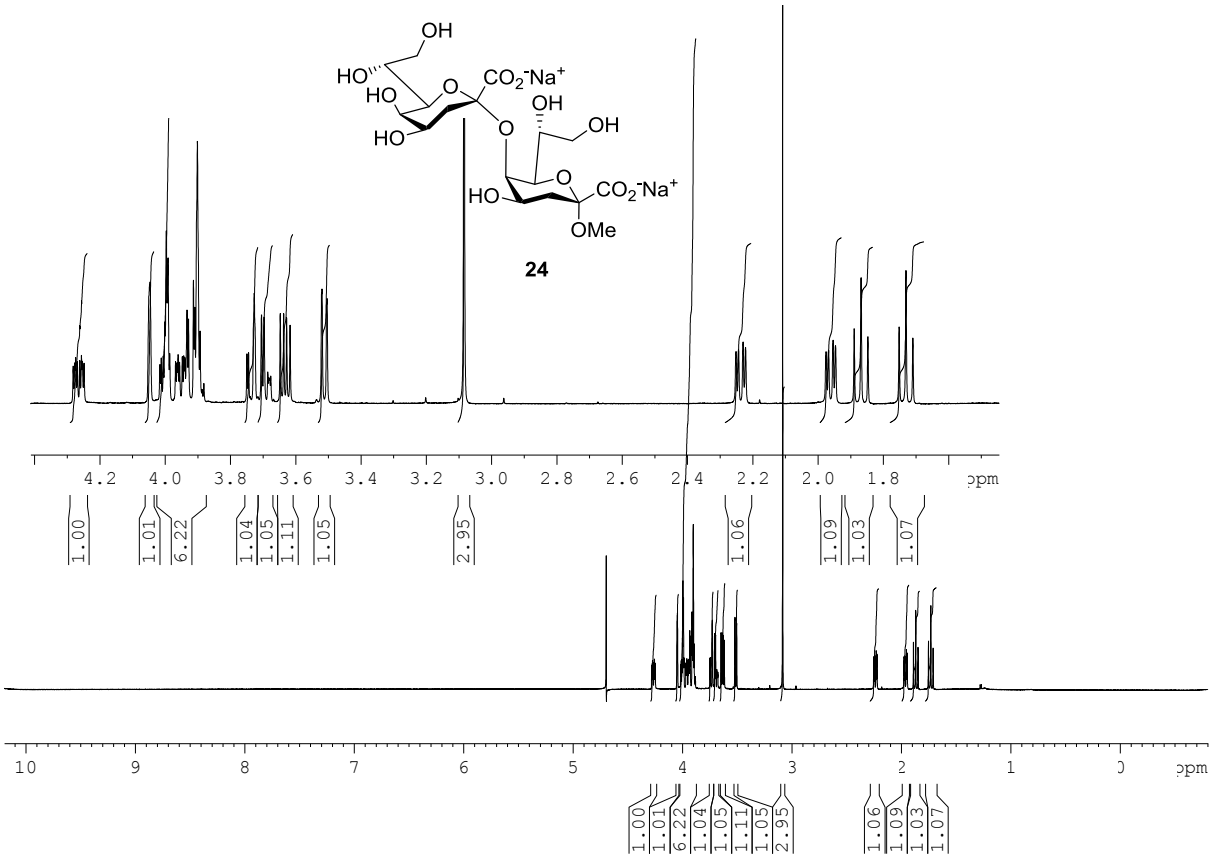


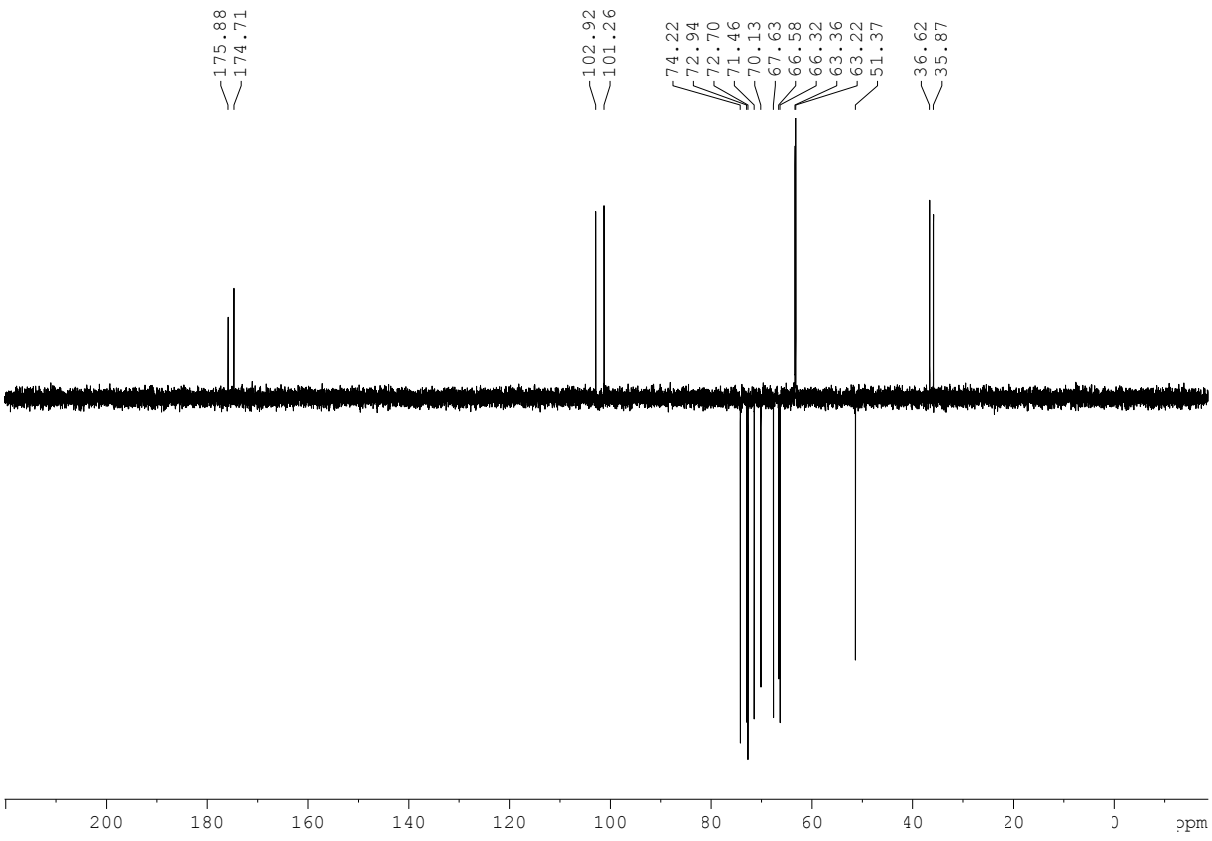
^{1}H NMR ($CDCl_3$, 600 MHz)



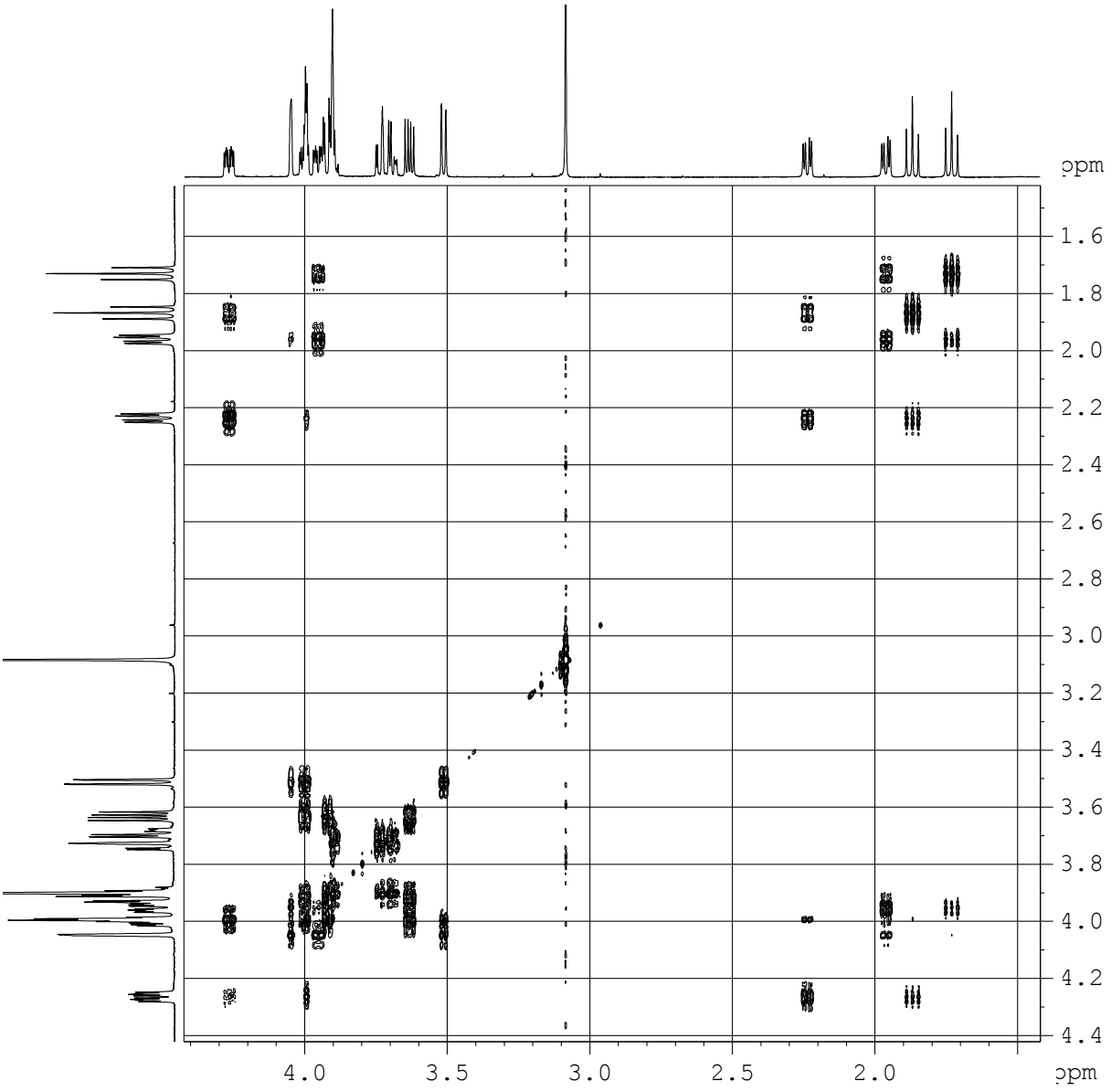
^{13}C NMR ($CDCl_3$, 150 MHz)



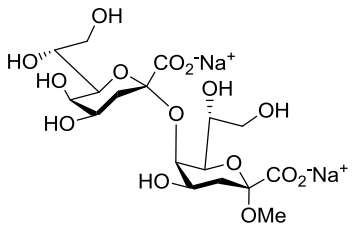
^{1}H NMR (D_2O, 600 MHz, pH ~ 7.0)



^{13}C NMR (D_2O, 150 MHz, pH ~ 7.0)



^{1}H-^{1}H COSY (D_2O, pH ~ 7.0)



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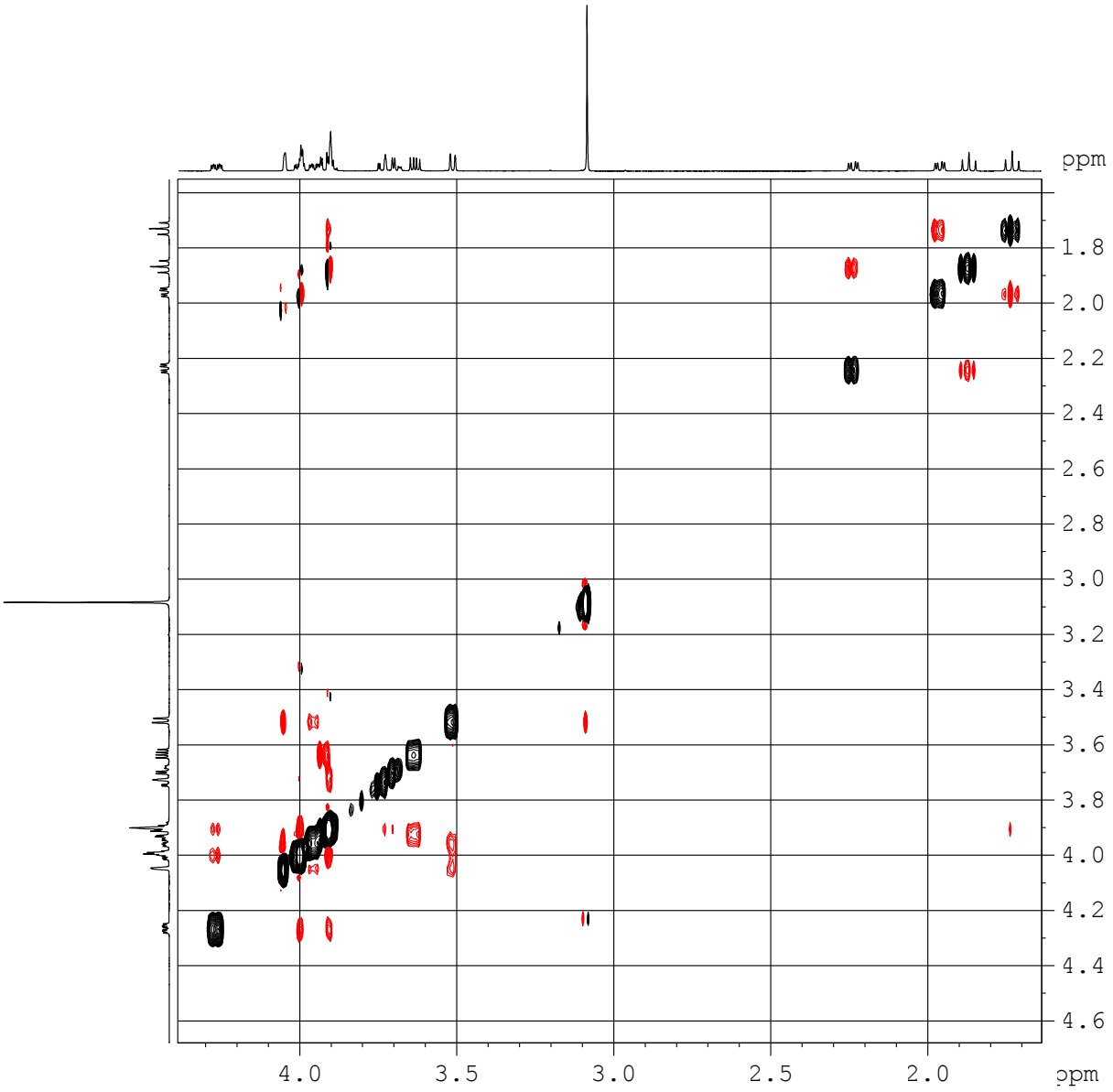
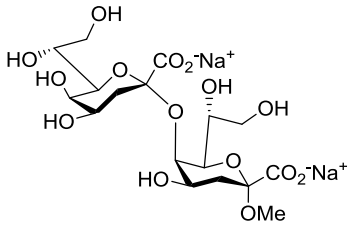


^{1}H-^{1}H NOESY (D_2O, pH ~ 7.0)



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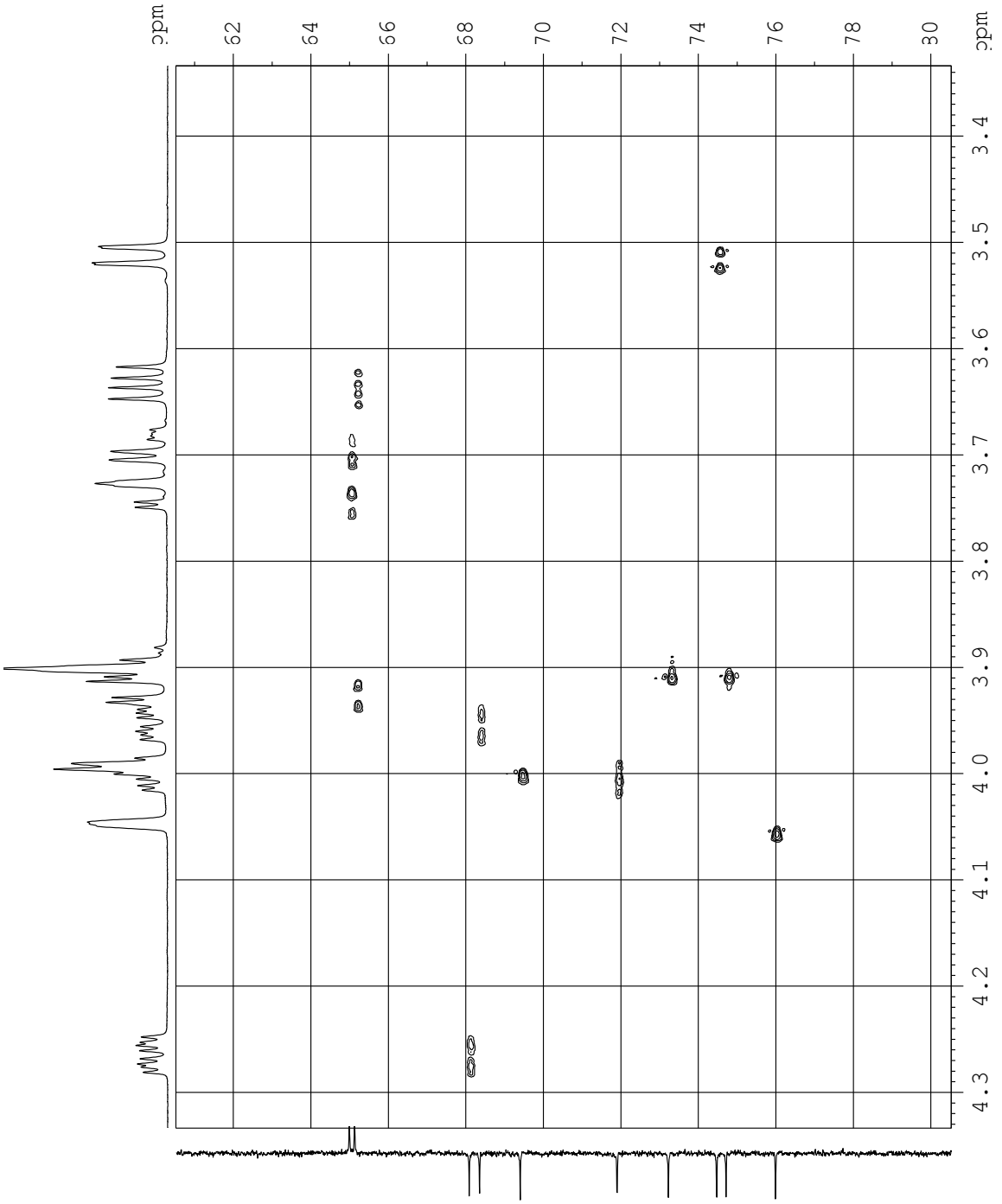
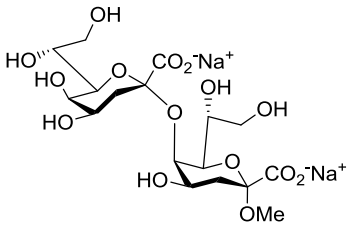


^{1}H-^{13}C HSQC (D_2O, pH ~ 7.0)



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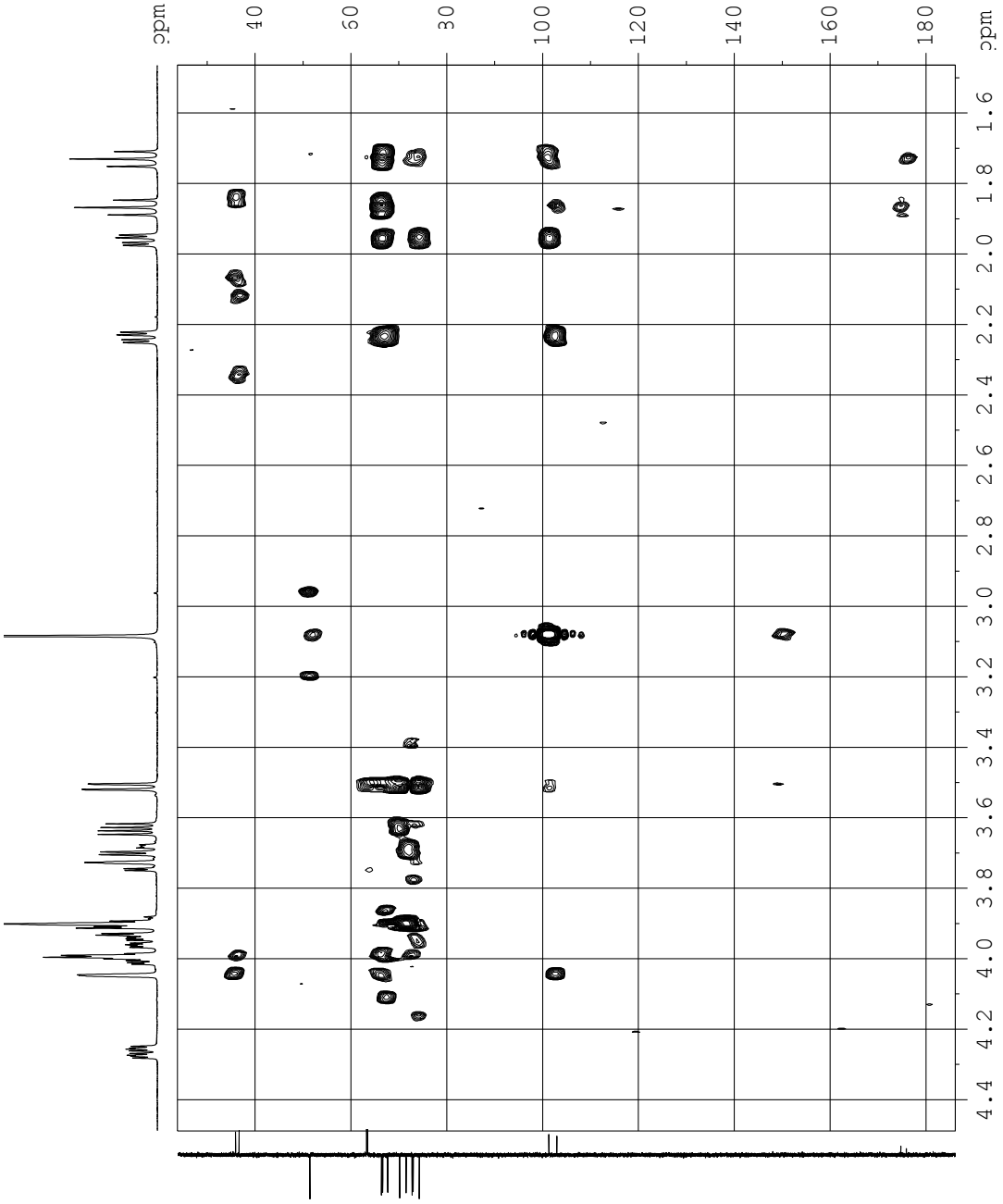
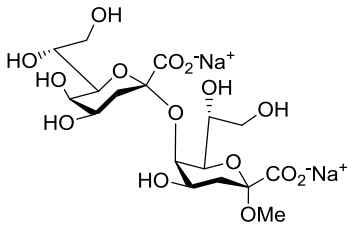


^{1}H-^{13}C HMBC (D_2O, pH ~ 7.0)



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Abbreviations

Ac	Acetyl
AEC	Anion exchange chromatography
AIBN	Azobisisobutyronitrile
Alk	Alkyl
Bn	Benzyl
CIP	Contact ion pair
Cp	Cyclopentadienyl
CPS	Capsular polysaccharide
CRD	Carbohydrate recognition domain
D,D-Hep	D-*glycero*-D-*manno*-heptose
DAMP	Damage-associated molecular pattern
DAST	Diethylaminosulfur trifluoride
Dha	3-Deoxy-D-*lyxo*-2-heptulosaric acid
Et	Ethyl
EtCN	Propionitrile
Gal	D-Galactose
Glc	D-Glucose
Glc2N3N	2,3-Diamino-2,3-dideoxy-D-glucose
GlcN	2-Amino-2-deoxy-D-glucose
GlcNAc	*N*-Acetyl-D-glucosamine
HAD	Hydrogen-bond mediated aglycon delivery
Kdo	3-Deoxy-D-*manno*-2-octulosonic acid
Ko	D-*Glycero*-D-*talo*-2-octulosonic acid
L	Leaving group
L,D-Hep	L-*glycero*-D-*manno*-heptose
LA	Lewis acid
LPS	Lipopolysaccharide
mAb	Monoclonal antibody
MBL	Mannose-binding lectin
MD-2	Myeloid differentiation factor 2
Me	Methyl
MeCN	Acetonitrile
MPLA	Mono-phosphoryl lipid A
NBS	N-Bromosuccinimide
Neu5Ac	N-Acetyl-neuraminic acid
NIS	N-Iodosuccinimide
OM	Outer membrane
PAMP	Pathogen-associated molecular pattern
Pg	Protecting group
PG	Peptidoglycan
Ph	Phenyl
PM	Plasma membrane
PMB	*para*-Methoxybenzyl
PRR	Pathogen recognition receptor
SP	Surfactant protein
spp.	Species
SSIP	Solvent-separated ion pair
TBDMS	*tert*-Butyldimethylsilyl
TES	Triethylsilyl
Tf	Triflate
tg	*trans-gauche*
THF	Tetrahydrofuran
TIPDS	Tetraisopropyldisiloxane
TLR	Toll-like receptor
TMS	Trimethylsilyl

6. Curriculum Vitae

BARBARA POKORNY, DIPL.-ING.

Haidengasse 3/2/24, A-1230 Vienna | **Phone:** +43 664 4456332

Date of birth: June 10, 1988 (Matzendorf-Hölles) | **Nationality**: Austria

barbara.pokorny@boku.ac.at

WORKING EXPERIENCE

PROJECT ASSISTANT – PhD position

University of Natural Resources and Life Sciences – Vienna

since NOV 2012

LECTURER – Organic chemistry lab courses

University of Natural Resources and Life Sciences – Vienna

2013 – 2015

INTERNSHIPS IN R&D – Synthesis of small molecules for oncological research

Boehringer Ingelheim – Vienna

2011 (AUG – NOV); 2012 (JULY – SEPT)

TUTOR – Organic synthesis and analytical chemistry lab courses

Workshops for young women (WIT project – Women in Technology)

Vienna University of Technology

2009 – 2012

PART-TIME EMPLOYMENT IN "REPAIR CENTER" –

Administration using SAP software and soldering of printed circuits for test systems

Frequentis AG – Vienna

2008 – 2010

HIGHER EDUCATION

VIENNA UNIVERSITY OF TECHNOLOGY

Doctoral program since 2013

Master's program "Technical Chemistry – Synthesis" 2010 – 2013

„Synthese von substituierten EnIn-Systemen und deren Anwendung zur Oberflächenmodifikation von Silicium Targets"

Passed with distinction

Bachelor's program "Technical Chemistry" 2006 – 2010

Passed with distinction

SCIENTIFIC CONTRIBUTION

CONFERENCES

COST spring training school Bangor (UK) 2015 (brief communication), 249th ACS National meeting Denver (CO, USA) 2015 (poster presentation), 5th Euchems in Istanbul 2014 (oral presentation), 13th Bratislava symposium on saccharides in Slovakia 2014 (oral presentation), 17th Eurocarb in Tel-Aviv 2013 (poster), 14th BDSHC in Slovakia 2011 (poster), 24th ECHC in Vienna 2010 (poster) among others;

PUBLICATIONS

Pokorny, B.; Kosma, P. *Chemistry Open* **2015**, *accepted manuscript*.

Pokorny, B.; Kosma, P. *Org. Lett.* **2015**, *17*, 110-113.

Pokorny, B.; Kosma, P. *Chem. Eur. J.* **2015**, *21*, 305-313.

Pokorny, B.; Müller-Loennies, S.; Kosma, P. *Carbohydr. Res.* **2014**, *391*, 66-81.

AWARDS AND GRANTS

"**Buchanan Award**" for best brief communication – COST meeting 2015

"**Leistungsstipendium**" (merit grant) – Vienna University of Technology

ADDITIONAL SKILLS

Languages: German (native), English (fluent), French (basic)

Economy: Graduation of "European Business Competence License – Level A"

Supervision of several bachelor theses

Lab responsibilities: NMR, LC-ESI-MS, preparative MPLC/HPLC, chemical database

Training: Seminar "Konflikte konstruktiv führen und gestalten";

HPLC troubleshooting seminar, NMR summer school

HOBBIES

Mountain bike, tennis, badminton, soccer, hiking, volleyball, meeting friends, guitar, cooking