Automated Synthesis of the Lewis Blood Group Oligosaccharides

By

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B.S., Chemistry
University of Virginia, 1999

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For the glory of God in science
Preface

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Blood Group Oligosaccharides

By

Kerry Routenberg Love

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ABSTRACT

Cell-surface carbohydrates are markers of specific cell types. These
oligosaccharides are involved in recognition, adhesion, and signal transduction events. Advances in molecular glycobiology rely heavily on straightforward access to structurally defined oligosaccharides, but traditional syntheses of complex carbohydrates have been very laborious. Development of a novel linker and monitoring of each glycosylation reaction during automated solid-phase oligosaccharide synthesis allowed for the rapid synthesis of three Lewis-type cell surface oligosaccharides. The assembly of the nonasaccharide adenocarcinoma marker Le\(^\text{a}\)Le\(^\text{b}\) from monosaccharide building blocks was achieved in just 23 hours, while the syntheses of the tumor markers Lewis X, a pentasaccharide, and Lewis Y, a hexasaccharide, required only 12 and 14 hours respectively. The automation of carbohydrate synthesis greatly accelerates access to molecules for biological study and vaccine development.

Thesis Supervisor: Peter H. Seeberger
Title: Professor of Chemistry
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<thead>
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<th>Abbr.</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>Ac</td>
<td>Acetyl</td>
</tr>
<tr>
<td>AcOH</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>All</td>
<td>Allyl</td>
</tr>
<tr>
<td>AgOTf</td>
<td>Silver Triflate</td>
</tr>
<tr>
<td>AZMB</td>
<td>2-(azidomethyl)benzoyl</td>
</tr>
<tr>
<td>BDMA</td>
<td>Benzaldehyde dimethyl acetal</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>Bu</td>
<td>Butyl</td>
</tr>
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<td>Bz</td>
<td>Benzoyl</td>
</tr>
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<tr>
<td>ClAc</td>
<td>Chloroacetyl</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-Diazobicyclo-undec-7-ene</td>
</tr>
<tr>
<td>DCA</td>
<td>Dichloroacetic acid</td>
</tr>
<tr>
<td>DIPC</td>
<td>Diisopropylcarbodiimide</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-(Dimethylamino)pyridine</td>
</tr>
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<td>DMDO</td>
<td>Dimethyldioxirane</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
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<td>Dimethoxytrityl</td>
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<td>DMTST</td>
<td>Dimethyl(methylthio)sulfonium triflate</td>
</tr>
<tr>
<td>ESI-MS</td>
<td>Electrospray ionization mass spectrometry</td>
</tr>
<tr>
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<td>Ethyl</td>
</tr>
<tr>
<td>EtOAc</td>
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<td>Equivalent(s)</td>
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<td>FAB-MS</td>
<td>Fast atom bombardment mass spectrometry</td>
</tr>
<tr>
<td>Fmoc</td>
<td>9-(fluorenyl)methoxycarbonate</td>
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<tr>
<td>gCOSY</td>
<td>¹H, ¹H Gradient correlation spectroscopy</td>
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<tr>
<td>Glu</td>
<td>Glucose</td>
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<td>h</td>
<td>Hour(s)</td>
</tr>
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<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution mass spectrometry</td>
</tr>
<tr>
<td>HSQC</td>
<td>Heteronuclear single quantum coherence spectroscopy</td>
</tr>
<tr>
<td>HMBC</td>
<td>Heteronuclear multiple bond correlation spectroscopy</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>Lev</td>
<td>Levulinoyl</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>Matrix-assisted laser desorption ionization – time of flight</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
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<td>Minute(s)</td>
</tr>
<tr>
<td>MHz</td>
<td>Megahertz</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>MS</td>
<td>Molecular sieves</td>
</tr>
<tr>
<td>NBS</td>
<td>N-Bromosuccinimide</td>
</tr>
<tr>
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<td>N-Iodosuccinimide</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>Obsd</td>
<td>Observed</td>
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<tr>
<td>Phth</td>
<td>Phthaloyl</td>
</tr>
<tr>
<td>Piv</td>
<td>Pivaloyl</td>
</tr>
<tr>
<td>PMB</td>
<td>para-Methoxybenzyl</td>
</tr>
<tr>
<td>PMP</td>
<td>para-Methoxyphenyl</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>s</td>
<td>Second(s)</td>
</tr>
<tr>
<td>TBAF</td>
<td>tetra-$n$-Butylammonium fluoride</td>
</tr>
<tr>
<td>TBDPS</td>
<td>tert-Butyldiphenylsilyl</td>
</tr>
<tr>
<td>TBS</td>
<td>tert-Butyldimethylsilyl</td>
</tr>
<tr>
<td>TBSOTf</td>
<td>tert-Butyldimethylsilyl trifluoromethanesulfonate</td>
</tr>
<tr>
<td>TCA</td>
<td>Trichloroacetyl</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>TES</td>
<td>Triethylsilane</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TFAA</td>
<td>Trifluoroacetic acid anhydride</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TIPS</td>
<td>Triisopropylsilyl</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin-layer chromatography</td>
</tr>
<tr>
<td>TMSOTf</td>
<td>Trimethylsilyl trifluoromethanesulfonate</td>
</tr>
<tr>
<td>Tol</td>
<td>Tolyl</td>
</tr>
<tr>
<td>Troc</td>
<td>2,2,2-Trichloroethoxycarbonyl</td>
</tr>
<tr>
<td>$p$-TsOH</td>
<td>para-Toluenesulfonic acid</td>
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</tbody>
</table>
Chapter 1
Introduction
1.1 Introduction

The diverse structures and functions of carbohydrates in nature are now widely recognized.\textsuperscript{1,2} Carbohydrates as part of glycoproteins, glycosphingolipids, \textit{N}-linked glycans and glycosylphosphatidinositol anchors are key components of cellular signaling processes essential for healthy communication between cells. In this manner, discrete oligosaccharides are important players in the inflammatory response,\textsuperscript{3} the immune response,\textsuperscript{4} and the maintenance of normal cellular function. Conversely, aberrant glycosylation can lead to a variety of disease states; oligosaccharides also serve as mammalian tumor markers,\textsuperscript{5} triggers of autoimmune disorders\textsuperscript{6} and sites for recognition and attachment during microbial infection of a human host.\textsuperscript{7}

While the field of glycobiology continues to make amazing strides in understanding the roles of carbohydrates, advances have been slow paced when compared to other biopolymers. The awareness of protein and nucleic acid biological function has resulted directly from the ready availability of synthetic materials; the syntheses of both biopolymers is routinely automated by non-specialists. Carbohydrate biology, however, has suffered from a lack of materials available for study, as purification of oligosaccharides from natural sources often yields heterogeneous samples. Unfortunately, the biosynthesis of carbohydrates is not easily exploited; glycoconjugate formation is neither template-driven, as for nucleic acids, nor under transcriptional control, as for proteins. Direct synthesis of carbohydrates has, until recently, required knowledge of a specialist in both synthetic planning and product purification.

Clearly, there is a need for oligosaccharides of defined structure to aid in elucidation of biological functions.\textsuperscript{8} Developments in carbohydrate synthesis impact biology directly as they increase both the rapidity of access and the quantities of procured materials. To this end, the work in this thesis is focused on the methodological developments in the syntheses of a family of carbohydrate structures important in signaling processes ranging in biological outcomes. The application of an automated methodology drawing on a novel linker system and monitoring of glycosylation reactions during the addition of each sugar unit brings complex oligosaccharide syntheses another step closer to that of peptides and nucleic acids.
1.2 The Biological Significance of the Lewis Blood Group Oligosaccharides

The roles carbohydrates play in biological processes are typically initiated by molecules existing as part of a glycoprotein or glycolipid on the cell surface. One class of glycolipids, glycosphingolipids (GSLs), is essential for cellular adhesion and recognition. The Lewis blood group antigens (Figure 1) are a group of six fucosylated, ceramide containing GSLs having widespread implications in both normal cellular adhesion processes like the inflammatory response, as well as adhesion associated with disease states, including microbial infections and metastatic cancers. The Lewis blood group oligosaccharides are composed of a reducing end lactose $\beta-(1\rightarrow3)$ linked to a central $N$-acetyl glucosamine unit. The structures differ in the arrangement of galactose and fucose residues about this core glucosamine.

![Lewis Blood Group Oligosaccharides Diagram]

**Figure 1.1** Lewis group oligosaccharides
1.2.1 The Lewis Oligosaccharides as Blood Group Determinants

The serological differences in human blood were first examined by Landsteiner at the turn of the 20th century.\textsuperscript{12} Extensive studies revealed that antigen specificity was inherited and that three specific alleles were involved at the ABO locus. Subsequent discovery of secretory substances having the same specificities as erythrocyte-derived molecules led to the suggestion that these were carbohydrate-containing complexes.\textsuperscript{13} These secretory substances were termed the Lewis blood group antigens, even though they are only adsorbed onto erythrocytes, and studies of these molecules ensued due to ready availability from tissues and their complex relationship with blood-group type.

\begin{itemize}
  \item O Type Antigen: R = H (H-Type II)
  \item A Type Antigen: R = \(\alpha\)-GalNAc
  \item B Type Antigen: R = \(\alpha\)-Gal
\end{itemize}

**Figure 1.2** Structure of ABO blood antigens

The ABO related antigens are the terminal carbohydrate portions of glycan chains located on the erythrocyte surface. Antigen biosynthesis proceeds via a stepwise addition of monosaccharide units by a particular set of glycosyl transferases.\textsuperscript{14} Kabat and coworkers confirmed the role of \(N\)-acetyl glucosamine as a precursor for fucosyl transferases during H-type antigen synthesis.\textsuperscript{15} The H-type II antigen causes agglutination of O-type erythrocytes and is the precursor to the A and B antigens, these antigens being the \(\alpha\)-(1\(\rightarrow\)3) addition of an \(N\)-acetyl galactosamine or galactose residue, respectively.\textsuperscript{16} Derivatization of the H-type II antigen to include an \(\alpha\)-(1\(\rightarrow\)3) fucose residue on the central \(N\)-acetyl glucosamine leads to the formation of Type II antigens, namely \(Le^x\) and \(Le^y\) antigens. The remaining Lewis oligosaccharides are termed Type I antigens and are accessed by \(\alpha\)-(1\(\rightarrow\)3) galactosylation of the \(N\)-acetyl glucosamine and decoration with fucose residues.
1.2.2 The Lewis Oligosaccharides and the Inflammatory Response

Lewis oligosaccharides are present as cell surface carbohydrates on healthy leukocytes.\textsuperscript{3} Leukocytes, white blood cells, are essential for the repair of tissue in the vicinity of an injury and subsequent defense against possible microbial infection. The recruitment of leukocytes to a site of injury is brought about in a series of signaling steps, beginning with the release of cytokines by damaged tissue. In response to the cytokine release, the endothelium releases two proteins to its surface, E and P-selectin. These selectins were shown to recognize carbohydrate ligands containing the Le\textsuperscript{x} and Le\textsuperscript{a} sequences, including sialylated and sulfated variants.\textsuperscript{3,17}

![Diagram of the inflammatory cascade](image)

Figure 1.3 The inflammatory cascade\textsuperscript{3}

Endothelial recognition of the leukocyte via selectin binding to Lewis sugars induces further adhesion. Leukocyte "rolling" occurs leading to binding events between leukocyte integrins and ICAM-1 (intercellular adhesion molecule-1), an endothelial protein. ICAM-1 was also shown to recognize sialyl Le\textsuperscript{x}.\textsuperscript{17} Finally, the leukocyte passes through the endothelium to the site of injury as a result of these strong protein-carbohydrate interactions.
1.2.3 Microbial and Tumor Associated Antigens

The Lewis antigens are present on the surface of the endothelium lining the gastrointestinal tract. Le\textsuperscript{a} and Le\textsuperscript{b} are the predominant antigen types displayed on these cells.\textsuperscript{18} In a manner analogous to the migration of leukocytes through the endothelium adjacent to a site of injury, bacteria can infect host organisms by recognition of the carbohydrate antigens and subsequent adhesion to the gastrointestinal lining.\textsuperscript{7} \textit{H. pylori} was shown to recognize the Le\textsuperscript{b} antigen in the gastric epithilium.\textsuperscript{19} The interaction between Le\textsuperscript{b} and the \textit{H. pylori} adhesin BabA is essential for bacterial adhesion and infection leading to a variety of diseases including gastric ulcers and gastric adenocarcinoma.\textsuperscript{19-21}

Lewis antigens, like many other glycosphingolipids,\textsuperscript{5} are tumor markers in various human cancers. Although GSLs are present in both cancerous and normal cellular tissue, their composition and density are altered in tumors.\textsuperscript{11} Human cancers, including adenocarcinoma,\textsuperscript{22,23} colorectal cancer,\textsuperscript{24} pancreatic cancer,\textsuperscript{25} and breast cancer,\textsuperscript{26} express novel cell surface structures that occur due to enhanced fucosylation and chain elongation of the Lewis antigens expressed on normal cells. Aberrant glycosylation in tumor cells helps to promote motility and adhesion necessary for tumor-cell invasion and progression.\textsuperscript{11}

Since GSLs are cell surface antigens, it was postulated that their altered composition in cancer states would directly affect cellular binding of antibodies and result in a varied immune response to that of healthy cells.\textsuperscript{4} Constructs comprised of GSLs and an immunocarrier protein was synthesized in efforts to exploit this altered antigenicity and utilize an immunological response for cancer therapy. One successful example uses the KH-1 adenocarcinoma antigen as the basis for treatment of colonic adenocarcinoma.\textsuperscript{27}
Figure 1.4 Tumor-associated carbohydrate antigen KH-1 (R = ceramide)

KH-1 is a branched oligosaccharide containing the full Le<sup>x</sup> pentasaccharide attached to the antigenic motif of Le<sup>y</sup> and is therefore also commonly referred to as the Le<sup>y</sup>-Le<sup>x</sup> nonasaccharide. Following the synthesis of the Le<sup>y</sup>-Le<sup>x</sup> nonasaccharide, Danishefsky and coworkers conjugated the oligosaccharide to the carrier protein keyhole limpet hemocyanin (KLH) for elicitation of an immune response. Immunization of mice with the GSL-protein construct was followed by an immune response that successfully targets two adenocarcinoma markers. Based on these initial results, a similar strategy is being pursued for the preparation of a vaccine for clinical evaluation in humans.

1.3 Chemical Synthesis of Carbohydrates

Following the first reported glycosylation reaction by Koenigs and Knorr in 1901, when a glycosyl bromide was condensed with methanol by activation with Ag<sub>2</sub>CO<sub>3</sub>, many new methods for the efficient construction of glycosidic bonds have been developed. Glycosylation reactions may be simplified as an example of nucleophilic substitution; a glycosyl acceptor presenting a nucleophilic hydroxyl group displaces a leaving group at the electrophonic anomic carbon of the glycosyl donor. While it is understood that glycosylation requires the formation of a putative oxocarbenium species susceptible to nucleophilic attack, the exact ion pairing of the activated donor preceding attack remains unclear. Each anomeric leaving group does not result in the same type of intermediate, as there are many stereochemical outcomes varying in yield between different glycosyl donors and a single glycosyl acceptor.
**Scheme 1.1** General glycosylation mechanism leading to the formation of α- and β-glycosides

Glycosylation reactions may result in one of two stereochemical outcomes; either an α-glycoside, resulting from acceptor attack of the bottom face of the glycosyl donor, or a β-glycoside, resulting from acceptor attack of the top face of the glycosyl donor, may be formed. This is a distinct challenge of carbohydrate chemistry, as coupling reactions in both peptide and nucleic acid synthesis produce no new stereogenic centers. Access to discrete materials requires stereoselective control, and therefore glycosylation stereochemistry is typically controlled by use of neighboring group participation. In this manner, a participating protective group, usually an ester or amide, is installed at the C-2 position of the glycosylating agent. During the formation of the oxocarbenium cation in glycosylation, this participating group acts as an electron-donating moiety, thereby blocking the bottom face of the intermediate leading to β-glycoside formation. Glycosylation may also be under "acceptor control" where the stereochemistry of glycosylation controlled by the conformation of the nucleophile.

**Scheme 1.2** Synthesis of β-glycosides via neighboring group participation from the C-2 protecting group (X = O or N)
1.3.1 Glycosylating Reagents

The past century of research efforts in synthetic carbohydrate chemistry have produced a selection of reagents for establishing glycosidic bonds. Today’s synthetic chemists may choose between glycosyl fluorides,\textsuperscript{33} thioglycosides,\textsuperscript{34} \textit{n}-pentenyl glycosides,\textsuperscript{35} 1,2-anhydrosugars,\textsuperscript{36} glycosyl trichloroacetimidates,\textsuperscript{37} glycosyl sulfoxides,\textsuperscript{38} glycosyl phosphites,\textsuperscript{39} and glycosyl phosphates.\textsuperscript{39} While each of these glycosylating agents still enjoys use, glycosyl trichloroacetimidates, thioglycosides, and glycosyl phosphates are the most widely used donors.

![Chemical structures](image)

**Figure 1.5** Glycosylating agents

Popularized by Schmidt and coworkers in the 1980s,\textsuperscript{37} glycosyl trichloroacetimidates are easily activated using catalytic Lewis acid conditions, thereby being compatible with a host of acid and base labile protecting groups. These glycosylating agents typically demonstrate rapid activity and high yields in glycoside formation. They also exhibit remarkable stability when stored under inert atmosphere at low temperatures. A potential drawback to this glycosyl donor is its lengthy synthesis, as preparation requires generation of an anomic lactol, access to which may require many protecting group manipulations.

Another class of popular glycosyl donors, thioglycosides, result from substitution of the anomic oxygen by an alkyl or aryl thiol group.\textsuperscript{34} These donors are stable for extended periods at room temperature reacting only when activated under mildly acidic or basic conditions. The anomic thiol group offers sufficient temporary protection of
the anomeric center, thus protecting group manipulations are minimized. Lengthy reaction times and toxic thiophilic reaction conditions for activation are potential drawbacks.

\textit{n}-Pentenyl glycosides,\textsuperscript{35} like thioglycosides, are a class of donors in which the anomeric leaving group functions well for temporary protection during donor differentiation. Activation of these donors is achieved in the presence of an iodonium species, typically using NIS, or with strongly acidic conditions, like triflic acid. Deprotection of completed glycosides may be achieved under reducing metal conditions leaving the \textit{n}-pentenyl moiety in tact for derivatization yielding a variety of spacer functionalities useful in preparation of \textit{neo}-glycoconjugates.\textsuperscript{40}

Recently, the Seeberger lab addressed the need for efficient glycosylating agents that could be prepared in a facile manner in differentially protected form. Using a one-pot procedure, glycosyl phosphates are readily prepared from glycals for the rapid installation of a variety of linkages.\textsuperscript{41} Using differentiated glycals grossly simplifies the protecting group manipulations required to achieve a differentially protected donor, as glycals have only three hydroxyl groups, each having unique reactivity, rather than the five hydroxyls of similar reactivity in most glycopyranoses. Being highly reactive donors, glycosyl phosphates do require storage at low temperatures to avoid decomposition, but activation does require stoichiometric amounts of a Lewis acid.

\textbf{1.3.2 Protecting Groups}

The orthogonal protection of hydroxyl groups poses a great challenge in the total synthesis of natural products and biologically important molecules. Differential protection of hydroxyl functionalities becomes increasingly important in the synthesis of complex, branched oligosaccharide structures, thus requiring multiple groups that can be removed selectively. A host of protecting groups including ethers, esters, and silyl ethers has been introduced to date.\textsuperscript{42} Typically, benzyl ethers are used to mask hydroxyl moieties during the entire course of the synthesis and are termed permanent protecting groups. Allyl ethers, silyl ethers, and a multitude of esters are commonly used for intermittent protection, masking a position for only a few synthetic steps. The choice of
protecting groups constitutes a central strategic consideration in planning a synthesis, particularly for oligosaccharide assembly.

Traditional modes of hydroxyl protection, such as esters or ethers, rely on varying degrees of basic or acidic conditions for their removal. Protective groups that may be removed using neutral or mildly basic conditions offer an additional dimension of orthogonality to existing groups. Protecting groups that may also serve as stereodirectors in glycosylation, namely C-2 participating groups, offer additional utility in oligosaccharide synthesis. To this end, Chapter 4 details the development of two novel protecting groups, the para-chlorophenyl carbonate and the 2-(azidomethyl)benzoate ester, that are stable to glycosylation conditions, are effective stereodirectors, and are removed under mild conditions, thereby offering a high degree of orthogonality with common esters.

1.3.3 Solid-Phase Oligosaccharide Synthesis

Due to the extensive methods required for purification of products at each stage of solution-phase oligosaccharide synthesis, solid-phase methods have been developed. In this manner, the first monosaccharide may be attached to a soluble or insoluble polymeric support for decoration with subsequent saccharide units to achieve the desired product. Use of excess reagents in this context can drive reactions to completion while allowing for facile separation of the resin by filtration in an insoluble solvent system.

1.3.3.1 Glycosylation Strategy

There is a choice between two possibilities when choosing a strategy for solid-phase; both donor-bound and acceptor-bound strategies are cited in the literature. In the donor-bound approach, the first sugar is bound to the resin via the non-reducing end of the saccharide unit, typically the C-6 position. The polymer-bound monosaccharide is then activated as a glycosylating agent in the presence of excess nucleophile. In this manner, a productive coupling will lead to inclusion of an additional sugar unit that may, in turn, be activated as a donor itself for elongation of the oligosaccharide chain.
Danishefsky and coworkers have relied on this strategy for their glycal assembly of oligosaccharides on solid support.\textsuperscript{44}

**Scheme 1.3 Strategies for solid-phase oligosaccharide synthesis**

Due to the highly reactive nature of most glycosyl donors, more side products result from donor decomposition than acceptor decomposition. In the donor-bound strategy, this decomposed product remains resin-bound for the remainder of the synthesis and is unable to participate in productive coupling reactions. The acceptor-bound strategy was therefore popularized with nearly all types of glycosyl donors as a means of avoiding the low yields often associated with donor-bound approaches. In this approach, the first sugar is coupled to the resin in a glycosylation reaction, thereby linking the growing oligosaccharide chain from the anomic position of the first oligosaccharide. Unveiling of a single hydroxyl group yields a resin-bound acceptor ready for exposure to
excess glycosylating agent in the presence of an activator. After each glycosylation, any
glycosyl donor-derived decomposition products may be washed away to prepare for the
next coupling reaction.

One final strategy is a combination of both the aforementioned solid-phase
methodologies. Boons and coworkers have demonstrated the utility of a two directional
approach in the generation of carbohydrate libraries.\textsuperscript{45} Using this method, a
monosaccharide, typically a thioglycoside, is attached to a polymeric support by the non-
reducing end and activated as a donor in the presence of a monosaccharide acceptor. The
polymer-bound sugar unit is then deprotected in order to generate a resin-bound acceptor
for further elaboration.

1.3.3.2 Monitoring of Solid-Phase Reactions

Choice of the acceptor bound approach enables another feature aiding in solid-
phase oligosaccharide synthesis: monitoring of solid-phase reactions. Solid-phase
peptide and oligonucleotide synthesis are routinely monitored by removal of a temporary
protecting group that forms a byproduct detectable by UV, such as 9-
fluorenylmethoxycarbonyl (Fmoc)\textsuperscript{46} and dimethoxytrityl (DMT)\textsuperscript{47} groups. Likewise in
peptide synthesis, detection of the residual amino group liberated in each step of chain
elongation by the Kaiser test enables evaluation of the preceding coupling and
deprotection steps.\textsuperscript{48} In carbohydrate synthesis, however, monitoring by analogous
methods would require detection of a hydroxyl moiety, a much greater challenge, or by
protecting group removal strategies similar to those for other biopolymers.

Several methods for monitoring solid-phase oligosaccharide synthesis now exist.
Spectroscopic means including high-resolution magic angle spinning NMR,\textsuperscript{49} gated
decoupling $^{13}$C enriched NMR,\textsuperscript{50} and $^{19}$F NMR techniques\textsuperscript{51} have provided means for
identifying resin-bound compounds, but are not practical for real-time monitoring during
a synthesis. Recently, Ito and coworkers developed a colorimetric, real-time detection of
glycosylation and deprotection efficiency based on the use of the chloroactetyl (ClAc)
group for temporary protection.\textsuperscript{52} Exposure of a ClAc protected glycoside to ($p$-
nitrobenzyl)pyridine produces an intense red color, enabling determination of incomplete
deprotection. Similarly, a Disperse Red conjugate was used to detect incomplete glycosylation.

![Chemical reaction diagram](image)

**Figure 1.6** Monitoring of solid-phase oligosaccharide chemistry

While these methods for solid-phase reaction monitoring do enable evaluation of glycosylation and deprotection efficiency in real time, both methods require resin sampling at these critical steps, where sampled resin is irrevocably changed by the chemical process used in monitoring. This poses problems for small scale and exploratory syntheses, as resin sampling over many steps could adversely affect the total amount of resin available at the end of a synthesis for product recovery. Another pitfall of resin sampling is the introduction of water into the reaction vessel. While peptide and oligonucleotide chemistries are moderately tolerant of humidity, carbohydrate chemistry requires a completely anhydrous environment for the construction of glycosidic linkages. The rigorous washing protocol required to purge a resin-bound sample of any water is too lengthy to be performed between each reaction step if one values a timely production of synthetic materials. Furthermore, this additional time is especially burdensome to an automated approach, where one seeks to minimize all manipulations in the interest of total synthesis time.

Chapter 3 demonstrates the use of an Fmoc monitoring method, bringing solid-phase carbohydrate synthesis a step closer to modern peptide assembly methods.
Removal of the Fmoc group from resin-bound glycosides enables determination of a quantitative yield for the coupling and deprotection steps associated with the addition of each monomer unit. As a result of employing this methodology, significant time required for typical trial-and-error efforts was saved in the syntheses of several complex, branched oligosaccharides. Troublesome steps were immediately identified and reaction conditions optimized averting a waste of monosaccharide building blocks and time in identification and purification of unknown oligomers at the end of the synthesis.

1.3.3.3 Solid-Phase Linkers

Another critical component of a solid-phase strategy is employment of a suitable linker for attachment of the first monomer unit to the solid support. This linker must be robust under conditions for both glycosylation and deprotection of temporary protecting groups. Likewise, the cleavage of the linker at the termination of the solid-phase synthesis must be rapid, high yielding, and produce a cleavage product that is either easily converted to the anomic lactol or that is useful in the construction of a neoglycoconjugate. Some of the most commonly used linker systems include silane-based linkers,\textsuperscript{44} acid-labile linkers,\textsuperscript{53} base-labile linkers,\textsuperscript{54} oxidatively removable linkers,\textsuperscript{55} hydrogenation cleavable linkers,\textsuperscript{56} and photo-cleavable linkers.\textsuperscript{57}

One notable example popularized by the Seeberger lab is the octenediol linker strategy.\textsuperscript{58} In this strategy, 4,5-Z-octenediols is linked as an ether to Merrifield’s resin and used as the first acceptor in glycosylation of the solid support. The double bond present in this linker is completely stable to acidic glycosylation conditions commonly used to activate glycosyl trichloroacetimidates and glycosyl phosphates. Thioglycosides, however, may not be used in concert with the octenediyl linker, as the double bond would also undergo attack in the presence of thiophilic reagents used for donor activation. Cleavage of the octenediyl may be achieved at the end of the synthesis via olefin metathesis with Grubbs’ catalyst under an ethylene atmosphere yielding the desired oligosaccharide as the corresponding anomic \(n\)-pentenyl glycoside. As discussed earlier, \(n\)-pentenyl glycosides are useful compounds owing to their potential as glycosyl donors and the ample methods available for derivatization of the terminal olefin.\textsuperscript{40}
1.3.4 Automated Solid-Phase Oligosaccharide Synthesis

The routine automated synthesis of peptides and oligonucleotides led to significant biological understanding of these biopolymers and allowed for the birth of new fields of study, namely proteomics and genomics. While it is evident that carbohydrates have considerable biological function, the impact of this realization has yet to be observed in glycobiology. Progress in understanding carbohydrate structure and function is severely hampered by the lack of automated methodology suitable for oligosaccharide construction by the chemical layperson.

Recently, two examples of automated carbohydrate synthesis have emerged. Wong and coworkers have reported the use of computer-assisted reactivity profiling to predetermine the outcome of a series of glycosylation reactions carried out in a one-pot synthesis. Using NMR data for each of over 100 protected thioglycosides, the OptiMer program selects the best reactants for a sequential solution-phase synthesis by activation of donors decreasing in activity, beginning at the non-reducing end of the desired oligosaccharide and working toward the reducing end. Using this methodology, the tumor-associated antigen Globo H was synthesized as a stepwise assembly of a hexasaccharide from a monosaccharide, a disaccharide, and a trisaccharide each activated

Figure 1.7 Solid-phase paradigm using resin-bound octane diol linker
in turn. While this approach does hasten the assembly process once donors have been made for a particular synthesis, each synthesis requires building blocks usable only in the construction of that carbohydrate motif. Additionally, manipulations carried out during the one-pot assembly are still done manually in the solution-phase.

Scheme 1.4 Optimer© synthesis of the Globo H antigen

A more general methodology has been demonstrated in the Seeberger lab. As in peptide and oligonucleotide assembly, this approach relies on the stepwise addition of monomer units, enabling the facile construction of long polymers and a variety of carbohydrate motifs without the construction of entirely new building blocks for each synthesis. Moreover, once the necessary glycosyl donors have been synthesized, the solid-phase synthesis is completely automated, including all coupling, deprotection and washing protocols. Automated assembly has enabled access to purified carbohydrate material within a few days, rather than several weeks or even months required to complete a manual solution-phase assembly.
Scheme 1.5 Automated solid-phase synthesis of a phytoalexin elicitor dodecasaccharide

The Seeberger lab initially demonstrated this technique in the synthesis of a phytoalexin elicitor dodecasaccharide.\textsuperscript{61} In this synthesis, monosaccharide and disaccharide building blocks were used alternately, allowing the branching observed in the final structure to be built into the disaccharide donor. The completion of this structure in less than 24 hours was the first reported example of automated oligosaccharide assembly. Since that initial synthesis, the Seeberger lab has reported the synthesis of several biologically important structures, including the Leishmania cap tetrasaccharide,\textsuperscript{62} the proteoglycan linkage region,\textsuperscript{63} the high mannose portion of a glycosphatidinositol anchor,\textsuperscript{64} and the $N$-linked core pentasaccharide.\textsuperscript{65} In the context of these syntheses, establishment of branching points during a synthesis has become routine and a method for capping unreacted oligomers to aid in final purification has been developed.\textsuperscript{66}

Improvements to this existing methodology are reported in Chapter 3, including the automated synthesis of several Lewis group oligosaccharides. The Lewis group oligosaccharides were ideal for automated methods development due to their diversity of glycosidic linkages and multiple points of branching. During the development of automated methods suitable for assembly of these complex, branched carbohydrates, new
protecting group and linker strategies were devised, as well as a real-time method for monitoring success of glycosylation and deprotection during the course of an automated synthesis.

1.4 Enzymatic Synthesis of Carbohydrates

Enzymatic approaches for the generation of carbohydrates for biological study have also been explored.\(^\text{67}\) Using glycosyl transferases, the enzymes that nature uses for carbohydrate biosynthesis, and glycosidases, those enzymes that typically hydrolyze glycosidic bonds, tedious routes for differential protection of intermediates are overcome, as enzymes are both stereo and regioselective. Additionally, enzymes can promote glycosyl bond formation both catalytically and under mild conditions.

While popular for the relatively few chemical manipulations required to access complex naturally occurring glycosides, enzymatic methods have several drawbacks. Some prominent examples are the availability and price of both the enzymes themselves and suitable substrates; many glycosyl transferases are not yet available and require expensive nucleotide-sugars for glycosylation. This necessity for high substrate specificity may be overcome by using glycosidases, which can utilize glycosides including various anomeric functionalities like halides or a \(p\)-nitrophenyl group.\(^\text{67}\) Additionally, yields are typically quite low, especially with glycosidases, and usually only allow procurement of minute quantities of material.

A noteworthy exception to the aforementioned drawbacks is the chemo-enzymatic synthesis of globotriose by Wang and coworkers.\(^\text{68}\) Using a cloned \(\alpha\)-(1\(\rightarrow\)4)-galactotransferase expressed in \(E.\ coli\), a highly efficient synthesis of globotriose and a library of \(\alpha\)-(1\(\rightarrow\)4)-galactosylated derivatives was accomplished. Chemosynthetic efforts yielded compounds for testing as potential antibacterial agents in quantities exceeding 200 mg.
1.5 Dissertation Objective

For the past five years, the Seeberger lab has been principally concerned with the development of new methodology for the synthesis of biologically significant carbohydrates by automated solid-phase synthesis. Glycomics may emerge as a significant field much as genomics and proteomics have in the recent past, owing to automated chemical processes for the layperson to generate synthetic materials. This thesis discusses the evolution of solution- and solid-phase syntheses of the Lewis group oligosaccharides as a model for synthetic methods development and improvement to existing automated technology.

Chapter 2 details the solution phase syntheses of two of the six Lewis group oligosaccharides: the H-type II and Le$^x$ pentasaccharides. These solution-phase syntheses served as models for future automated assembly, as discussed in Chapter 3. Completion of a series of three of these complex, branched oligosaccharides on an automated synthesizer required implementation of a new protecting group strategy, as well as development of a method for monitoring the productivity of reactions in real time. Improvement upon linker systems previously introduced in the Seeberger lab lead to the use of a new linker system for more rapid access to products at the end of an automated run. Finally, Chapter 4 introduces two novel modes of temporary protection for solution-phase oligosaccharide synthesis, the para-chlorophenyl carbonate and the 2-(azidomethyl)benzoate ester.
1.6 References

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Chapter 2
Solution-Phase Synthesis of the Type II Lewis Oligosaccharides
This chapter describes work performed in collaboration with Rodrigo Andrade. He prepared compounds 66-74.\textsuperscript{1}

\subsection*{2.1 Introduction}

The widespread biological implications of the Lewis antigens have rendered them targets of intense biological study. Since only small amounts of pure oligosaccharides can be obtained from natural sources due to microheterogeneity, chemical synthesis is the best way to procure appreciable amounts of material for biological investigations. The Lewis blood group antigens have served as a synthetic challenge prompting the development of new methods for oligosaccharide synthesis since the seminal work by Lemieux in the mid 1970s.\textsuperscript{2,3} Advances in oligosaccharide chemistry, including the development of novel glycosyl donors and a host of new protecting groups, have since eased the synthesis of these complex, branched carbohydrates.

\subsection*{2.2 Prior Syntheses of the Lewis Group Oligosaccharides}

Beginning with Lemieux and coworkers,\textsuperscript{2} attempts were made to synthesize the antigenic portions of the Lewis structures, namely the terminal tri- and tetrasaccharides of each of the six oligosaccharides. Lemieux’s approach in the construction of the Le\textsuperscript{a} trimer was likened to a natural product total synthesis; glycosyl bromides were used for the installation of the glycosidic linkages and extensive protecting group manipulations preceded each coupling step.\textsuperscript{2} Jaquinet and Sinay reported the synthesis of the H-type II trimer also using primarily glycosyl bromides as donors,\textsuperscript{4} but later introduced a fucosyl imidate as a means of installing the fucosidic linkage in Le\textsuperscript{a} and Le\textsuperscript{X} trisaccharides.\textsuperscript{5,6} These initial syntheses were streamlined and extended to produce all three of the Type II antigens.\textsuperscript{3} As in the earlier synthesis, the Le\textsuperscript{X}, Le\textsuperscript{Y}, and H-type II were built up from the reducing end glucosamine 2 (Scheme 2.1), but protecting group manipulations were minimized using a single lactosamine core disaccharide 3, from which allyl and p-nitro benzoyl protecting groups could be selectively removed for further elaboration with fucose residues.
Scheme 2.1 Lemieux’s synthesis of the H-type II trisaccharide 6

Subsequent syntheses of the trisaccharide antigens employed thioglycosides as glycosylating agents.\(^7\)\(^8\) Construction of a lactosamine core tetrascarhide 11 with thioglycosides, followed by fucosylation with bromo-sugar 5 yielded the dimeric Le\(^\times\) hexasaccharide 13 (Scheme 2.2).\(^8\) Hasegawa and coworkers reported the first synthesis of the sialyl Le\(^\times\) (SLe\(^\times\)) antigen in 1991, utilizing thioglycosides and DMTST activation for installation of glycosidic linkages.\(^9\) Based on this work, the dimeric structure was also completed.\(^10\) Thioglycosides also have been used in combination with selective protection in a one-pot, two-step glycosylation.\(^11\) The sequential activation of similar thiophenyl donors, based on the “armed/disarmed” glycosylation concept, resulted in the construction of the trimeric antigens in one-pot in good yield.
Scheme 2.2 Synthesis of dimeric Le\textsuperscript{x} hexasaccharide 13 using thioglycosides

In recent years, many laboratories have used the Lewis oligosaccharides as a means of showcasing novel glycosylating agents. Kahne and coworkers\textsuperscript{12} synthesized the Le\textsuperscript{a}, Le\textsuperscript{b}, and Le\textsuperscript{x} trisaccharides using a single set of glycosyl sulfoxide donors. Likewise, Wong has investigated glycosyl phosphites for use as glycosylating agents in synthesis of the Le\textsuperscript{x} trisaccharide.\textsuperscript{13} Following initial studies, the completion of the SLe\textsuperscript{x} structure using solely glycosyl phosphites is noteworthy, as sialyl phosphites were efficient sialyl donors.\textsuperscript{14}
Scheme 2.3 Glycal assembly of the Le\textsuperscript{b} hexasaccharide

The glycal assembly method, introduced by Danishefsky, was applied to synthesize the entire family of tri- and tetrasaccharide Lewis\textsuperscript{15} antigens, as well as SLe\textsuperscript{x}.\textsuperscript{16} Construction of the Le\textsuperscript{b} hexasaccharide begins (Scheme 2.3) similarly to earlier approaches; the antigenic tetrasaccharide 19 is built up from a lactosamine disaccharide precursor 16, and then difucosylated with fucosyl fluoride donor 18. The protected amine functionality of 20 is installed via iodo-sulfonamidation of tetrasaccharide 19, followed by coupling with lactal derivative 21. The global deprotection of all remaining groups on 22 is accomplished by desilylation and subsequent reduction using sodium-ammonia conditions to give the Le\textsuperscript{b} hexasaccharide.

Schmidt and coworkers have synthesized the Lewis antigens with trichloroacetimidate donors using a host of nitrogen protecting groups for construction of the core glucosamine building block, including azide,\textsuperscript{17,18} tetrachlorophthaloyl,\textsuperscript{19,20} dimethylmaleoyl (DMM),\textsuperscript{21,22} and trichloroethoxycarbonyl (Troc).\textsuperscript{23} Schmidt’s synthesis
of the dimeric \( \text{Le}^x \) octasaccharide\(^{24} \) is very similar to both Ogawa\(^{25} \) and Nicolaou’s\(^{26} \) approaches; all three groups have chosen a block coupling strategy of di- and trisaccharide glycosylating agents to maximize convergence using either glycosyl trichloroacetimidates or glycosyl fluorides (Scheme 2.4).

\[
\begin{align*}
\text{23} + \text{26} & \xrightarrow{\text{AgOTf, HCl}_{2} \text{CH}_{2} \text{Cl}_{2}} \text{27} \\
\text{23} & \xrightarrow{\text{R}^{1} = \text{Protected ceramide}} \text{24} \\
\text{25} & \xrightarrow{\text{Thiourea, 2,6-lutidine}} \text{26} \end{align*}
\]

**Scheme 2.4** Synthesis of a \( \text{Le}^x \) dimeric octasaccharide

Due to the extensive protecting group manipulations required, chemoenzymatic synthesis of the Lewis antigens has also been explored (Scheme 2.5). Chemical construction of poly-lactosamine backbone 34 by chemical glycosylation followed by enzymatic transfer of one sialic acid and three fucose residues yielded 35, a trimeric version of SLe\(^x\).\(^{27} \) As seen in this synthesis, glycosyl transferases have the ability to greatly simplify the creation of complex, branched oligosaccharides.
Scheme 2.5 Chemoenzymatic synthesis of trimeric sialyl \( \text{Le}^x \)

2.3 Synthetic Strategy

Our retrosynthetic analysis of the Lewis group followed a markedly different path from previous routes that had been striving for maximum convergence (vide supra). In keeping with our goal of automating solution phase protocols in a solid phase paradigm, five glycosyl donors served as building blocks for a sequential synthesis (Figure 2.1), adding one donor at a time to a growing oligosaccharide chain. Since these solution phase syntheses were used as studies for future automation, we were cognizant of constraints in
solid phase oligosaccharide chemistry in developing reaction conditions for the incorporation of glycosyl phosphate and trichloroacetimidate donors and in choosing modes of temporary protection.

2.4 Synthesis of a Protected H-Type II Pentasaccharide

We chose to begin our solution-phase studies with the synthesis of the H-type II pentasaccharide, due to its linear nature and comparable facility with the branched Lewis oligosaccharides. The \( n \)-pentenyl group was chosen as the reducing end protecting group for the duration of the synthesis. In this manner, the solution phase synthesis serves as a model for future solid-phase syntheses, since the octenediol linker connecting the anomeric position of the reducing sugar is cleaved to yield an \( n \)-pentenyl glycoside,\(^{28}\) aiding in both product and deletion sequence identification. A protecting group strategy was chosen employing benzyl ethers and pivaloyl esters as permanent protecting groups; several temporary protecting groups were tested during the course of the solution-phase studies. Glycosyl phosphates were used whenever possible, as their facility in preparation from glycals and efficient formation of a host of glycosidic linkages\(^{29}\) made them ideal as donors.

![Figure 2.1 Retrosynthesis of the H-type II pentasaccharide](image)

\( R \) = Amine protecting group

\( PG \) = Temporary protecting group

43
2.4.1 Synthesis of the Lactose Disaccharide

Two glycal-derived glycosyl phosphate donors were required to construct the lactose (Scheme 2.6). The first building block, glucosyl phosphate 39, was derived from readily available 3,6-di-O-benzyl glycal 36.\(^{30}\) Silylation of 36 yielded differentially protected glycal 37 ready for conversion to the corresponding glycosyl phosphate 39 via the one-pot procedure for phosphate preparation.\(^{31}\)

![Scheme 2.6 Synthesis of lactose disaccharides](image)

Galactose phosphate building block 44 was prepared in a manner analogous to that for glucose donor 39. Deprotection of per-acetylated galactal was followed by regioselective benzylation of the C-4 and C-6 hydroxyls using benzyl bromide and sodium hydride at 0°C.\(^{32}\) Although the yield of the desired galactal was low (34%), three other synthetically useful galactals (tri-O-benzyl galactal (21%), 3,6-di-O-benzyl galactal (11%), and 3,4-di-O-benzyl galactal (10%)) were also obtained from this procedure after
silica column chromatography. Protection of the 4,6-di-\(O\)-benzyl galactal as the silyl ether using TBSCI and imidazole afforded 42 and subsequent conversion to the glycosyl phosphate produced 44 in good yield.

A reducing end building block was prepared by coupling glucose phosphate 39 with 4-penten-1-ol under the agency of TBSOTf. Deprotection with TBAF yielded acceptor 46, ready for coupling with galactose phosphate 44. Union of acceptor 46 and galactosyl phosphate 44 furnished disaccharide 47 in good yield. Removal of the silyl ether in 47, however, proved difficult and could not be achieved using standard conditions (TBAF/THF). \(^1\)H NMR analysis of the crude product indicated the migration of the pivaloyl ester from the C-2 to the C-3 hydroxyl of galactose to form an inseparable mixture of disaccharides. A host of other deprotection conditions (TBAF/AcOH, 1% HCl/MeOH, sat. HCl/ether) failed to cleanly liberate disaccharide 47 from the C-3 silyl ether.

Reevaluation of the synthetic route in light of difficulties with protecting group removal proved silyl ethers incompatible with both the solution phase synthesis and subsequent solid phase automation. Levulinate esters, therefore, were chosen as the temporary protecting group in the synthesis of both building blocks for the lactose portion of the Lewis antigens. Levulinate esters are readily removed in the presence of other esters with hydrazine.

Glycosyl phosphates 40 and 45 were synthesized from glycals substituting the Lev group for the TBS (Scheme 2.6). Formation of disaccharide 48, followed by treatment with hydrazine\(^{33}\) yielded desired lactose disaccharide 49. With 49 in hand, our focus turned to the construction of the \(\beta-(1\rightarrow3)\) glucosamine linkage bridging the lactose and the antigenic portions of the Lewis oligosaccharides.

### 2.4.2 Development of a Glucosamine Building Block

The branched nature of the Lewis antigens around the central glucosamine residue mandates a diverse protecting group scheme for this monosaccharide building block. The choice of amine protecting group is essential since it dictates the overall protecting group strategy. Previous syntheses of the Lewis antigens often protected the amine in the form
of an azide,\textsuperscript{17} owing to the base stability of this group under conditions required for removal of esters used in temporary protection of hydroxyl groups. Following these examples, we initially chose 2-azido glucosamine donor 52 for installation of the β-(1→3) glucosamine linkage (Scheme 2.7).

\begin{center}
\includegraphics[width=\textwidth]{scheme2.7.png}
\end{center}

**Scheme 2.7** Synthesis of azide-protected glucosamine building block 52

The synthesis commenced with known compound 50\textsuperscript{34} by acetylation of the C-3 hydroxyl position and regioselective ring opening of the benzylidene using triethylsilane and trifluoroacetic acid,\textsuperscript{35} followed by protection of the C-4 hydroxyl to yield differentially protected monosaccharide 51. Removal of the anomeric silyl ether and reaction of the lactol with trichloroacetonitrile in the presence of catalytic DBU afforded 52. Reaction of donor 52 with a series of model compounds using BF\textsubscript{3}-OEt\textsubscript{2} activation at a range of temperatures was unsuccessful. Increased Lewis acidity of the activator had no effect on the outcome of the glycosylation reaction, but resulted in increased donor decomposition. The lack of success with the C-2 azido donor, while disappointing, was not surprising in light of the fact that ester protected 2-azido glucosamines are only weakly active glycosylating agents.\textsuperscript{36} Glycosyl donor reactivity can be increased by introduction of allyl and benzyl ethers as hydroxyl protecting groups, but this was impossible given the desired branching pattern.

\begin{center}
\includegraphics[width=\textwidth]{scheme2.8.png}
\end{center}

**Scheme 2.8** Synthesis of glycosyl oxazoline 54
Since most naturally occurring glucosamines are N-acetates, we next investigated glucosamine oxazolines as glycosyl donors for installation of the β-(1→3) linkage and the N-acetate in a single step. While oligosaccharide oxazolines are readily prepared using a variety of methods, their synthetic utility has been limited. Model donor 54 (Scheme 2.8), derived from per-acetylated glucosamine, was activated with triflic acid in the presence of several secondary alcohols as acceptors, but yielded no coupling products.

![Scheme 2.9 Synthesis of dimethylmaleoyl-protected glucosamine building block 59](image)

Given the failure of both C2 azido glucosamine building block 52 and glucosyl oxazoline 54 to yield coupling products, additional nitrogen protecting groups were investigated. Protection of the glucosamine nitrogen with a dimethylmaleoyl (DMM) group was recently reported to effectively mask the glucosamine nitrogen while ensuring trans glycosylation products. Installation of the DMM group using dimethyl maleic anhydride, followed by global acetylation produced 55 (Scheme 2.9). Further elaboration of 55 in a manner analogous to that used in preparing 52 afforded 58. Conversion of 58 to glycosyl trichloroacetimidate 59 proceeded in modest yield (65%). While 59 proved an acceptable glycosylating agent in initial model studies, unsatisfactory yields (38%, impure product) of trisaccharide 63 were obtained when coupling 59 with lactose acceptor 49 (Scheme 2.11). Additionally, poor yields were reported for the removal of DMM from complex oligosaccharides rendering the DMM group unattractive for amine protection in the context of this synthesis.
Scheme 2.10 Synthesis of phthalimide-protected glucosamine donor 62

Inadequate performance of donors employing the aforementioned base-stable amine protecting groups in coupling reactions with lactose acceptor 49 prompted us to consider the use of the phthaloyl group for amine protection. The phthaloyl group is commonly used for the protection of glucosamines in the context of oligosaccharide syntheses; phthaloyl is readily installed and functions well as a C-2 participating group to ensure β-selectivity of glycosylation reactions. Since the phthalamide group is not completely stable under basic conditions it was not selected initially in anticipation of problems during removal of ester protecting groups. In light of the development of a novel 2-(azidomethyl)benzoyl protecting group (vide infra) the use of base was no longer mandatory during the late stages of the synthesis and allowed for the use of phthaloyl as an amine protecting group. Glycosyl trichloroacetimidate building block 62 was prepared using standard protecting group manipulations via known n-pentenyl glycoside intermediates41 (Scheme 2.10). Glycosylation of lactose acceptor 49 employing glycosyl trichloroacetimidate 62 at –20°C afforded trisaccharide 64 in reasonable yield (65-87%) albeit an excess of donor 62 (3 equiv) was required. Removal of the C-4 levulinate ester using hydrazine acetate readily furnished trisaccharide 65 in 98% yield (Scheme 2.11).

Scheme 2.11 Synthesis of H-type II core trisaccharides
2.4.3 Completion of the H-Type II Pentasaccharide

Completion of the protected H-type II pentasaccharide required addition of a galactosyl donor capable of fashioning the β-(1→4) linkage between the galactose and glucosamine building blocks, followed by fucosylation of the C-2 position of this newly installed galactose moiety. The nature of the C-2 participating group was of crucial importance. Acetate, benzoate and pivaloyl esters were disqualified as participating groups since the basic conditions required for their removal were not compatible with the phthalimide and ester groups present in the existing trisaccharide. The need for a participating group that could be selectively removed in the presence of other esters prompted us to develop the 2-(azidomethyl)benzoyl (AZMB) group \emph{vide infra} for carbohydrate synthesis.

\begin{equation}
\text{Scheme 2.12 Synthesis of H-type II model trisaccharide 71}
\end{equation}

We initially focused on a simpler trisaccharide model system 71 (Scheme 2.12) that constitutes the antigenic trisaccharide portion of H-type II without the reducing end lactose. Given our success using the AZMB group as a C-2 participating group with a variety of glycosyl donors, we chose to equip the galactose phosphate building block with this protective group. Galactosyl phosphate 66 was prepared via a one-pot procedure from tri-O-benzyl galactal. Glycosylation of the C-4 position of glucosamine 67 using
galactose phosphate 66 proceeded in excellent yield to afford disaccharide 68 (Scheme 10). Removal of the C-2 AZMB group did not cause any unexpected problems and furnished disaccharide acceptor 69.

Fucosylation of the C-2 hydroxyl of the terminal galactose is the final step for completion of H-type II oligosaccharide. As in most glycoconjugates, Lewis group antigens contain exclusively α-(1,2-cis) linkages. This cis linkage poses a synthetic challenge since C-2 participating groups cannot be employed. The use of fucosyl phosphates to establish this difficult linkage was addressed in the context of the Lewis antigen synthesis.

Fucosylation using dibutyl per-benzylated fucose phosphate resulted as expected in an inseparable mixture of diastereomers upon reaction with a model acceptor. Similar results had been obtained previously with other fully benzyalted fucose donors such as fucosyl bromides, trichloroacetimidates, fluorides, thioglycosides, and n-pentenyl glycosides. The problem of stereocontrol during fucosylation was addressed by introduction of a C-4 ester protecting group following precedence with other glycosylating agents to create fucosyl phosphate 70. Using model disaccharide 69 as acceptor, fucosylation with 70 (1.6 equiv) under the agency of stoichiometric amounts of TBSOTf provided protected H-type II trisaccharide 71 in excellent yield with complete α-selectivity. It is interesting to note that the corresponding fucosyl trichloroacetimidate donor failed to ensure complete α-selectivity in glycosylation reactions.

![Scheme 2.13](image)

Scheme 2.13 Completion of the protected H-type II pentasaccharide 74

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After establishing the feasibility of all synthetic transformations for the construction of the antigenic portion of the oligosaccharide target on a trisaccharide model, we focused our attention on the final steps of the pentasaccharide assembly (Scheme 2.13). Galactosylation of trisaccharide acceptor 65 afforded tetrasaccharide 72 in 92% yield. Reduction of the azide using Staudinger conditions cleanly removed the AZMB protecting group in 72 to yield desired tetrasaccharide acceptor 73. Fucosylation of the tetrasaccharide 73 was carried out at low temperatures using phosphate 70 to yield 94% of the desired H-type II pentasaccharide 74 in fully protected form.

2.5 Refinement of H-Type II Synthesis

While the H-type II pentasaccharide was accomplished in solution, preliminary efforts at automated solid-phase synthesis following this paradigm yielded only milligram quantities of material following cleavage from the solid support. Poor solution-phase yields in glycosylation with glucosamine trichloroacetimidate 62 prompted a triple glycosylation cycle with a total of 30 equivalents donor over a three hour period. These harsh conditions required for glycosylation onto lactose acceptor 49 indicate further manipulation of the glucosamine building block is necessary to achieve all six Lewis antigens in solution and on solid support.

2.5.1 Construction and Use of a Modified Glucosamine Donor

Recently, the trichloroacetamide (TCA) group has been used as an amine protecting group in the synthesis of complex oligosaccharides.\textsuperscript{47-49} Its remarkable base stability and lack of steric bulk make it ideal for use in the synthesis of the Lewis antigens. Additionally, the TCA group is easily converted to the N-acetate using a hydrogen-halogen exchange reaction.\textsuperscript{47}
Scheme 2.14 Synthesis of trichloroacetimidate-protected glucosamine building block 80

Synthesis of glucosamine donor 80 begins with installation of the TCA onto glucosamine 75, followed by per-acetylation to furnish compound 76 (Scheme 2.14). Installation of an anomic silyl protecting group in two steps yielded 77. Compound 77 was then subjected to NaOMe/MeOH for the removal of all three remaining acetates and protected as the 4,6-benzylidene by addition of benzaldehyde dimethyl acetal and catalytic p-toluene sulfonic acid. Protection of the C-3 position of 78 as the benzoate ester, followed by regioselective opening of the benzylidene, and protection of the 4-OH position as a levulinyl ester yielded 79. Glycoside 79 was then converted to glycosyl trichloroacetimidate 80 via the anomic lactol in 82% yield over two steps.

Scheme 2.15 Synthesis of H-type II tetrasaccharide using glucosamine 84
Glycosyl trichloroacetimidate 80 was successfully coupled to lactose acceptor 1 in five minutes at -20°C using 1.8 equivalents donor and 0.1 equivalents TMSOTf for activation (Scheme 2.15). The resulting trisaccharide 81 was isolated in 83% yield with no observation of either donor or acceptor decomposition products. The levulinate ester of trisaccharide 81 was cleaved in 95% yield using a solution of hydrazine acetate in MeOH/CH₂Cl₂. Coupling of C-2 AZMB galactosyl phosphate 66 with deprotected trisaccharide 82 using two equivalents of donor gave 87% of the desired tetrasccharide 83, after careful separation from the highly stable α-galactosyl phosphate. Repeated attempts to remove the AZMB group from tetrasccharide 83 using the Staudinger conditions previously detailed (3 equiv. PBu₃, 5 equiv. water, in THF) led to isolation of product 84 in poor yields, typically less than 20%.

2.5.2 Evaluation of Protecting Group Incompatibilities

Unsatisfactory yields in AZMB removal from tetrasccharide 83 led to an investigation of possible incompatibilities between Staudinger conditions and the glucosamine building block. Subjection of per-acetylated glucosamine 76 to the aforementioned Staudinger reaction conditions resulted in quantitative re-isolation of the starting material; the TCA group was stable to the reducing conditions. Using model compounds 85 and 86, containing either TCA or phthalimide protected amines respectively, a series of conditions for AZMB removal were examined. Each model compound was subjected to a battery of conditions (Table 2.1) for the removal of the AZMB group. Phthalimide-containing model compound 86 appeared to out perform its TCA protected analog in all cases. Isolation of some decomposed material from the deprotection of compound 85 produced a ¹H NMR spectrum lacking the doublet between 7.2 and 6.5 ppm characteristic of the TCA protected amine. Further analysis by HRMS points to an internal O to P rearrangement of the intermediate iminophosphorane, rather than the expected reduction of this species by water (Figure 2.2). ⁵⁰
<table>
<thead>
<tr>
<th>Compound</th>
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<th>Yield</th>
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</thead>
<tbody>
<tr>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>5 equiv. PBu₃, 3 equiv. H₂O, THF</td>
<td>decomposition</td>
</tr>
<tr>
<td>85</td>
<td>5 equiv. PBu₃, 3 equiv. H₂O, MeCN</td>
<td>no reaction</td>
</tr>
<tr>
<td>85</td>
<td>5 equiv. PMePh₂, 3 equiv. H₂O, THF</td>
<td>&lt; 60% (crude)</td>
</tr>
<tr>
<td>85</td>
<td>5 equiv. PMePh₂, THF/H₂O 5:1</td>
<td>60%</td>
</tr>
<tr>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>5 equiv. PMePh₂, 3 equiv. H₂O, THF</td>
<td>70%</td>
</tr>
<tr>
<td>86</td>
<td>5 equiv. PBu₃, THF/H₂O 5:1</td>
<td>60%</td>
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<tr>
<td>86</td>
<td>5 equiv. PMePh₂, THF/H₂O 5:1</td>
<td>72%</td>
</tr>
</tbody>
</table>

Table 2.1 Removal of the AZMB group from model disaccharides

In the case of model 85, the amide oxygen donates electron density to the electrophilic iminophosphorane, which in turn causes a release of the amine protecting group and relocation to the core nitrogen of the previously existing azide. This mechanism would also explain the difference in isolated yields between the two model compounds, as the amide bond is more polarized in the case of the TCA protected amine in 85 leading to increased rearrangement rather than reduction of the iminophosphorane. Attempts to reduce this intramolecular side-reaction by both increasing the electron density of the phosphine and the concentration of water were unsuccessful.
2.5.3 Completion of Modified H-Type II Synthesis

The difficulties observed in AZMB removal from model compounds 85 and 86 translated into similar problems for removal of the AZMB from tetrasaccharide 83 (Scheme 2.16). Changing the phosphine from PBu₃ to the more electron rich PMePh₂ did, however, enable the isolation of tetrasaccharide 84 in 42% yield. While acceptable for solution-phase synthesis, this poor yield would be the demise of a solid-phase synthesis sequence. Additionally, a second product was isolated from this reaction; migration of the C-3 benzoyl ester of the glucosamine to the newly free C-2 hydroxyl of the neighboring galactose residue was observed. Benzoate migration confirms the close spatial proximity of these two rings, providing more support for the proposed iminophosphorane rearrangement mechanism. Benzoyl migration was remedied when reductions were performed in a 5:1 mixture of THF/water, yet no increase in yield of 84 was observed. Completion of the H-type II pentasaccharide by fucosylation of 84 with donor 70 gave a 75% yield of 85.
Scheme 2.16 Completion of the H-type II pentasaccharide

While ideal due to the neutral conditions for its removal, AZMB is not useful in cases when spatially juxtaposed with an amide-protected amine. Similar results of incompatibility due to internal rearrangement of the iminophosphorane may arise if placed in the vicinity of other electron rich or nucleophilic functional groups. The AZMB group, however, is still useful for orthogonal hydroxyl protection, given an awareness of its limitations.

2.6 New Strategies for the Synthesis of the H-type II Pentasaccharide

Incompatibilities between the C-2 AZMB group on the terminal galactose residue and amino protecting groups lead to the use of traditional esters for protection of the terminal galactose residue in the Lewis series. Galactosyl phosphate 91 was synthesized from tri-O-benzyl galactal in a one-pot conversion. Opening of a DMDO derived epoxide at the anomic position with dibutyl phosphate and subsequent protection of the C-2 position with benzyl chloride and DMAP produced glycosyl phosphate 91 in 73% yield over the three steps. A new glucosamine building block was constructed containing a benzyl group in place of the C-3 benzoate to avoid generation of a diol in the basic hydrolysis of tetrascaride 92 to form acceptor 93 (Scheme 2.17). Glucosamine 88 was synthesized via benzylation of intermediate 78 using silver carbonate and benzyl
bromide to prevent loss of the anomeric silyl group. Following benzyla
tion, the acetal was regioselectively opened to the C-6 position and the C-4 protected as the levulinate
ester. Conversion of 86 to the glucosamine trichloroacetimidate 87 via the anomeric
lactol and subsequent translation to the phosphate produced 88.

Scheme 2.17 Synthesis of glucosamine phosphate 88

Glycosylation of lactose acceptor 49 with glucosamine phosphate 88 yielded
trisaccharide 89 and after cleavage of the C-4 levulinate ester, acceptor 90 in 90% for the
two steps (Scheme 2.18). Trisaccharide nucleophile 90 was galactosylated in good yield
with galactosyl phosphate 91. Saponification of the benzoate ester on the terminal
galactose of 92 furnished glycosyl acceptor 93 ready for the final step in the H-type II
synthesis. Addition of fucose phosphate 70 and activation with TMSOTf produced the
desired pentasaccharide 94, but as a 2:1 α/β mixture of anomers. Attempts to obtain only
the desired α-fucoside using trichloroacetimidate donor 95 led to increased yield of
pentasaccharide 96, yet lowering the α/β ratio of anomers to 1:1. Clearly this
glycosylation constitutes an example of acceptor-controlled stereoselectivity, as both
fucosyl donors 70 and 95 contained esters able to transannularly donate to the carbenium
species and block the β-face from glycosylation. A further conformational examination
and modification of the acceptor is necessary to effectively control the stereochemistry of
glycosylation in this case.
Scheme 2.18 Synthesis of α/β mixtures of H-type II pentasaccharides

2.7 Synthesis of the Le\(^{3}\) and Le\(^{y}\) Oligosaccharides

Complications in the final stages of the H-type II pentasaccharide synthesis prompted us to move on to the syntheses of the remaining oligosaccharides in the type II series. Retrosynthesis of the Le\(^{x}\) pentasaccharide indicates a new glycosyl donor: glucosamine donor 98, which interchanges the levulinate and benzoate esters from the C-3 and C-4 positions in donor 80. Glycosylation to form branched Lewis oligosaccharides must occur first on the C-3 position of the glucosamine, then on the C-4 position, so as to achieve acceptable steric for favorable acceptor reactivity.\(^1\) It is for this reason that donor 98 was synthesized having a levulinate ester on the C-3 position, as it must be selectively removable in the presence of a benzoate ester.
Figure 2.3 Retrosynthesis of the Le\(^x\) pentasaccharide

As in the synthesis of donor 80, glucosamine trichloroacetimidate 98 is synthesized beginning with protection of the C-3 position of intermediate 78, in this case as the levulinate ester (Scheme 2.19). Regioselective benzylidene opening to the C-6 position is followed by benzylation of the C-4 position furnishing fully protected glucosamine 97. Anomeric silyl cleavage of 97 and treatment with trichloroacetonitrile and DBU generate trichloroacetimidate donor 98.

Scheme 2.19 Synthesis of glucosamine building block 98

Beginning with lactose acceptor 49, addition of glucosamine building block 98 and levulinate ester cleavage at the C-3 position using hydrazine acetate in a solution of methanolic dichloromethane rendered trisaccharide acceptor 100 (Scheme 2.20). Fucosylation of this trisaccharide with phosphate 70 afforded 101. Methoxide deprotection of the C-4 benzoate ester and galactosylation with donor 91 completed the Le\(^x\) pentasaccharide. Removal of the C-2 benzoate ester from the terminal galactose
residue was concomitant with a partial loss of pivaloyl esters from the fucose residue resulting in a poor yield of the pentasaccharide acceptor 104 necessary for construction of the Le\textsuperscript{y} hexasaccharide. Nonetheless, Le\textsuperscript{y} hexasaccharide 105 was procured following glycosylation of the pentasaccharide with excess fucosyl phosphate 70.

Scheme 2.20 Synthesis of Le\textsuperscript{x} pentasaccharide 103 and Le\textsuperscript{y} hexasaccharide 105

2.8 Conclusions

We have demonstrated the solution-phase synthesis of the Type II Lewis oligosaccharides, H-type II, Le\textsuperscript{x} and Le\textsuperscript{y}, keeping in mind the limitations of automated solid-support synthesis. Use of the novel AZMB protecting group allowed for completion of the H-type II pentasaccharide, but incompatibilities when juxtaposed with amides led us to discontinue its use in the context of the Lewis series. Re-evaluation of our synthetic route enabled the synthesis of the complex, branched Le\textsuperscript{x} and Le\textsuperscript{y} using
levulinate and benzoate esters as temporary protecting groups. Conditions used making these penta- and hexasaccharides are amenable to automated solid-phase oligosaccharide synthesis, thus these solution syntheses will provide the basis for studies in Lewis group automation.

2.9 Experimental Section

General Methods. All chemicals were reagent grade and used as supplied unless otherwise noted. Dichloromethane (CH₂Cl₂), tetrahydrofuran (THF), and toluene were purified by a JT Baker Cycle-Tainer Solvent Delivery System. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F₂₅₄ plates (0.25 mm). Compounds were visualized by dipping the plates in a cerium sulfate-ammonium molybdate solution followed by heating. Liquid column chromatography was performed using forced flow of the indicated solvent on Silicycle silica (230 - 400 mesh). ¹H NMR spectra were obtained using a Bruker-400 NMR spectrometer (400 MHz) or a Varian VXR-500 (500 MHz) and are reported in parts per million (δ) relative to CDCl₃ (7.27 ppm). Coupling constants (J) are reported in Hertz. ¹³C NMR spectra were obtained using a Bruker-400 NMR spectrometer (100 MHz) or a Varian VXR-500 (125 MHz) and are reported in δ relative to CDCl₃ (77.23 ppm) as an internal reference. ³¹P spectra were obtained using a Varian VXR-300 NMR spectrometer (120 MHz) or a Varian VXR-500 (200 MHz) and are reported in δ relative to H₃PO₄ (0.0 ppm) as an external reference. Optical rotations were measured at 24 °C.

4-O-tert-Butyldimethylsilyl-3,6-di-O-benzyl-glucal 37. A solution of 3,6-di-O-benzyl glucal (14.54 g, 44.7 mmol) in CH₂Cl₂ (50 mL) was cooled to 0°C and lutidine (10.41 mL, 89.4 mmol) was added. After 15 minutes, TBSOTf (10.77 mL, 46.9 mmol) was added dropwise and the mixture was stirred for 30 minutes. The mixture was quenched by addition of water (200 mL), extracted with hexanes (3 × 100 mL), dried over Na₂SO₄, filtered and concentrated in vacuo. The resulting residue was purified by flash silica gel
chromatography (20% EtOAc/hexanes) to yield 16.50 g (87%) of 37. Characterization data was consistent with previously reported data.  

3,6-Di-O-benzyl-4-O-levulinyl-glucal 38. Levulinic acid (1.0 g, 8.6 mmol) and DMAP (1.10 g, 9 mmol) were dissolved in 50 mL CH₂Cl₂ and cooled to 0°C. After 10 minutes, diisopropylcarbodiimide (DIPC) (1.3 mL, 8.2 mmol) was added with vigorous stirring. After five additional minutes, a solution of 3,6-di-O-benzyl glucal (2.67 g, 8.2 mmol) in 30 mL CH₂Cl₂ was added to the levulinic acid solution via cannula and the mixture was left to slowly warm to room temperature. After 15 hours, the reaction mixture was diluted with EtOAc and flushed through a plug of silica gel. The filtrate was concentrated and the residue was purified by flash silica gel chromatography (20% EtOAc/hexanes) to afford 3.17 g (91%) of 38. \([\alpha]_D^\circ = -11.03^\circ\) (c = 4.09, CH₂Cl₂); IR (thin film): 3030, 2869, 1740, 1718, 1648 cm⁻¹; \(^1\)H NMR (400 MHz, CDCl₃) δ 7.36-7.27 (m, 10 H), 6.47 (dd, J = 6.3, 1.1 Hz, 1 H), 5.30 (dd, J = 4.9, 4.8 Hz, 1 H), 4.90 (ddd, J = 6.3, 3.8, 0.7 Hz, 1 H), 4.60 (d, J = 1.1 Hz, 2 H), 4.54 (dd, J = 16.7, 12.0 Hz, 2 H), 4.32-4.27 (m, 1 H), 3.98-3.95 (m, 1 H), 3.76 (dd, J = 10.6, 6.9 Hz, 1 H), 3.68 (dd, J = 10.6, 4.2 Hz, 1 H), 2.75-2.71 (m, 2 H), 2.58-2.54 (m, 2 H), 2.18 (s, 3 H); \(^13\)C NMR (100 MHz, CDCl₃): δ 206.6, 171.9, 144.8, 138.4, 138.1, 128.6, 128.1, 127.9, 127.8, 127.8, 99.6, 75.3, 73.6, 70.6, 70.2, 68.4, 68.1, 38.0, 30.0, 28.2; ESI-MS: m/z (M + Na)⁺ calcd 447.1784, obsd 447.1770.

Dibutyl 4-O-t-butyldimethylsilyl-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranosyl phosphate 39. 4-O-t-Butyldimethylsilyl-3,6-di-O-benzyl-glucal 37 (1.00 g, 2.3 mmol) was azeotroped with toluene (3 × 5 mL), then dried under vacuum for 1 h. A solution of 2 in CH₂Cl₂ (12 mL) was cooled to 0°C and dimethyl dioxirane (34 mL of a 0.08 M solution in acetone, 2.7 mmol) was added. After 20 minutes, volatiles were removed under vacuum and the resulting residue was dried for 10 minutes. The residue was redissolved in CH₂Cl₂ (12 mL), cooled to -78°C, and a solution of dibutyl phosphate
(0.50 mL, 2.5 mmol) in CH₂Cl₂ (5 mL) was added dropwise over a period of 5 minutes. After 10 minutes, the reaction mixture was warmed to 0°C and DMAP (1.12 g, 9.2 mmol) and pivaloyl chloride (0.56 mL, 4.6 mmol) were added. The solution was left to slowly warm to room temperature, and after 2 hours was diluted with EtOAc (50 mL) and filtered through a plug of silica gel. The filtrate was concentrated and the residue was purified by flash silica gel chromatography (20% EtOAc/hexanes) to yield 1.35 g (78%) of 39 as a white solid. Characterization data was consistent with previously reported data.³¹

![Chemical structure](image)

**Dibutyl 3,6-di-O-benzyl-4-O-levulinyl-2-O-pivaloyl-β-D-glucopyranosyl phosphate 40.** 3,6-di-O-benzyl-4-O-levulinyl-glucal 38 (2.34 g, 5.5 mmol) was azeotroped with toluene (3 × 5 mL), then dried under vacuum for 1 h. A solution of 38 in CH₂Cl₂ (40 mL) was cooled to 0°C and dimethyl dioxirane (103 mL of a 0.08 M solution in acetone, 8.3 mmol) was added. After 20 minutes, volatiles were removed under vacuum and the resulting residue was dried for 10 minutes. The residue was redissolved in CH₂Cl₂ (40 mL), cooled to -78°C, and a solution of dibutyl phosphate (1.3 mL, 6.6 mmol) in CH₂Cl₂ (5 mL) was added dropwise over a period of 5 minutes. After 10 minutes, the reaction mixture was warmed to 0°C and DMAP (2.69 g, 22 mmol) and pivaloyl chloride (1.35 mL, 11 mmol) were added. The solution was left to slowly warm to room temperature, and after 2 hours was diluted with EtOAc (100 mL) and filtered through a plug of silica gel. The filtrate was concentrated and the residue was purified by flash silica gel chromatography (25% EtOAc/hexanes) to yield 3.518 g (89%) of 40 as a white solid. 

[α]D: +0.98° (c = 2.10, CH₂Cl₂); IR (thin film): 2962, 2874, 1745, 1719, 1029 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.35-7.22 (m, 10 H), 5.28 (dd, J = 7.8, 7.1 Hz, 1 H), 5.22-5.17 (m, 2 H), 4.65 (d, J = 11.3 Hz, 1 H), 4.57 (d, J = 11.3 Hz, 1 H), 4.49 (dd, J = 13.3, 11.8 Hz, 2 H), 4.07-3.98 (m, 4 H), 3.80-3.72 (m, 2 H), 3.63-3.56 (m, 2 H), 2.67-2.50 (m, 2 H), 2.44-2.36 (m, 1 H), 2.32-2.24 (m, 1 H), 2.12 (s, 3 H), 1.65-1.53 (m, 4 H), 1.42-1.32 (m, 4 H), 1.21 (s, 9 H), 0.92 (t, J = 7.4 Hz, 3 H), 0.87 (t, J = 7.4 Hz, 3 H); ¹³C NMR (100 MHz, CDCl₃): δ 206.3, 176.8, 171.6, 138.0, 137.9, 128.5, 128.5, 128.0, 127.8, 127.7,
96.7, 80.3, 74.1, 73.8, 73.7, 72.3, 70.3, 69.2, 68.3, 68.1, 39.0, 37.9, 32.3, 32.3, 32.2, 30.0, 28.0, 27.3, 18.8, 13.8, 13.7; $^{31}$P NMR (120 MHz, CDCl$_3$): δ -2.34; ESI-MS: m/z (M + Na)$^+$ calcld 757.3329, obsd 757.3320.

4,6-Di-O-benzyl galactal. Galactal (1.82 g, 14 mmol) was azeotroped with toluene (3 × 5 mL), then dried under vacuum for 30 minutes. A solution of galactal in 30 mL DMF was cooled to 0°C and NaH (1.23 g of a 60% suspension in oil, 30.8 mmol) was added. After 2 h, the suspension was treated with benzyl bromide (3.66 mL, 30.8 mmol) and left to stand for 3 h at 0°C. The reaction mixture was allowed to thaw and 50 mL toluene were added. The resulting mixture was washed with 30 mL water and extracted with 3 × 20 mL toluene. The organic layer was dried over Na$_2$SO$_4$, filtered and concentrated. The residue was purified by flash silica gel chromatography (30% BzOAc/hexanes) to yield 1.47 g 4,6-di-O-benzyl galactal (34%) as a white solid, along with 0.48 g 3,6-di-O-benzyl galactal (11%), 0.47 g 3,4-di-O-benzyl galactal (10%), and 1.17 g tri-O-benzyl galactal (21%). Characterization data was consistent with that previously reported.$^{32}$

3-O-t-Butyldimethylsilyl-4,6-di-O-benzyl-galactal 42. A solution of 4,6-di-O-benzyl galactal (400 mg, 1.2 mmol) in DMF (5 mL) was treated with TBS chloride (271 mg, 1.8 mmol) and imidazole (160 mg, 2.4 mmol). After 90 minutes, the mixture was diluted with water (20 mL) and extracted with ether (3 × 20 mL). The combined organic layers were washed once with saturated aqueous NaHCO$_3$ (30 mL) and once with water (30 mL), dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. The residue was purified by flash silica gel chromatography to afford 525 mg (quant. yield) of 42. [α]$_D$: -52.26° (c = 3.69, CH$_2$Cl$_2$); IR (thin film): 3067, 2927, 2856, 1649, 1101 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): δ 7.36-7.27 (m, 10 H), 6.32 (dd, J = 6.2, 1.3 Hz, 1 H), 4.93 (d, J = 11.9 Hz, 1 H), 4.69 (ddd, J = 6.1, 2.9, 1.2 Hz, 1 H), 4.60 (d, J = 11.9 Hz, 1 H), 4.51-4.48 (m, 2 H), 4.41 (d, J = 12.0 Hz, 1 H), 3.78-3.74 (m, 2 H), 3.56 (dd, J = 10.1, 4.6 Hz, 1 H), 0.92 (s, 9 H), 0.12 (s, 3 H), 0.11 (s, 3 H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 143.4, 138.7, 138.2, 128.6,
4,6-Di-O-benzyl-3-O-levulinyl-galactal 43. Levulinic acid (0.77 g, 6.66 mmol) and DMAP (0.856 g, 6.97 mmol) were dissolved in 25 mL CH₂Cl₂ and cooled to 0°C. After 10 minutes, DIPC (0.99 mL, 6.34 mmol) was added with vigorous stirring. After 5 additional minutes, a solution of 4,6-di-O-benzyl galactal (2.07 g, 6.34 mmol) in 25 mL CH₂Cl₂ was added to the levulinic acid solution via cannula and the mixture was left to slowly warm to room temperature. After 12 hours, the reaction mixture was diluted with EtOAc and flushed through a plug of silica gel. The filtrate was concentrated and the residue was purified by flash silica gel chromatography (40% EtOAc/hexanes) to afford 2.57 g (95%) of 43 as a white solid. \([\alpha]_D^{21.09^0} (c = 2.15, \text{CH}_2\text{Cl}_2)\); IR (thin film): 3030, 2916, 2870, 1719, 1645 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.36-7.27 (m, 10 H), 6.45 (dd, J = 6.2, 1.4 Hz, 1 H), 5.52-5.49 (m, 1 H), 4.78-4.74 (m, 2 H), 4.55 (d, J = 11.9 Hz, 2 H), 4.46 (d, J = 12.0 Hz, 1 H), 4.29-4.26 (m, 1 H), 4.03-4.01 (m, 1 H), 3.77 (dd, J = 10.3, 7.5 Hz, 1 H), 3.62 (dd, J = 10.3, 4.7 Hz, 1 H), 2.73-2.69 (m, 2 H), 2.58-2.55 (m, 2 H), 1.6 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃): δ 206.5, 172.5, 145.8, 144.4, 138.0, 137.9, 128.7, 128.6, 128.6, 128.5, 128.3, 128.2, 128.0, 127.9, 127.8, 98.6, 75.6, 73.5, 73.2, 70.7, 67.9, 65.7, 38.0, 30.0, 28.2; ESI-MS: m/z (M + Na)⁺ calcd 447.1784, obsd 447.1758.

Dibutyl 3-O-t-butyldimethylsilyl-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl phosphate 44. Galactal 42 (0.460 g, 1.04 mmol) was azeotroped with toluene (3 × 3 mL), then dried under vacuum for 1 h. A solution of 42 in CH₂Cl₂ (5 mL) was cooled to 0°C and dimethyl dioxirane (16 mL of a 0.08 M solution in acetone, 1.25 mmol) was added. After 20 minutes, volatiles were removed under vacuum and the resulting residue was dried for 10 minutes. The residue was redissolved in CH₂Cl₂ (6 mL), cooled to -
78°C, and a solution of dibutyl phosphate (0.23 mL, 1.14 mmol) in CH₂Cl₂ (2 mL) was added dropwise over a period of 5 minutes. After 10 minutes, the solution was warmed to 0°C and DMAP (0.508 g, 4.16 mmol) and pivaloyl chloride (0.25 mL, 2.08 mmol) were added. After 30 minutes the reaction mixture was diluted with a solution of 30% EtOAc/hexanes (25 mL) and filtered through a plug of silica gel. The filtrate was concentrated and the residue was purified by flash silica gel chromatography (15% EtOAc/hexanes) to yield 0.563 g (73%) of 44 as a white solid. Characterization data was consistent with previously reported data.⁴³

Dibutyl 4,6-di-O-benzyl-3-O-levulinyl-2-O-pivaloyl-β-D-galactopyranosyl phosphate 45. Galactal 43 (0.622 g, 1.46 mmol) was azeotroped with toluene (3 × 3 mL), then dried under vacuum for 1 h. A solution of 43 in CH₂Cl₂ (15 mL) was cooled to 0°C and dimethyl dioxirane (27 mL of a 0.08 M solution in acetone, 2.19 mmol) was added. After 20 minutes, volatiles were removed under vacuum and the resulting residue was dried for 10 minutes. The residue was redissolved in CH₂Cl₂ (40 mL), cooled to -78°C, and a solution of dibutyl phosphate (0.35 mL, 1.76 mmol) in CH₂Cl₂ (5 mL) was added dropwise over a period of 5 minutes. After 10 minutes, the solution was warmed to 0°C and DMAP (0.716 g, 5.86 mmol) and pivaloyl chloride (0.36 mL, 2.93 mmol) were added. After 30 minutes the reaction mixture was diluted with EtOAc (100 mL) and filtered through a plug of silica gel. The filtrate was concentrated and the residue was purified by flash silica gel chromatography (30% EtOAc/hexanes) to yield 0.984 g (94%) of 45 as a white solid. [α]D: +16.22° (c = 1.57, CH₂Cl₂); IR (thin film): 2962, 2873, 1742, 1720, 1028 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.36-7.26 (m, 10 H), 5.45 (dd, J = 10.4, 7.9 Hz, 1 H), 5.24 (dd, J = 7.8, 7.1 Hz, 1 H), 5.03 (dd, J = 10.5, 3.1 Hz, 1 H), 4.76 (d, J = 11.6 Hz, 1 H), 4.43 (dd, J = 18.6, 11.8 Hz, 2 H), 4.05-3.95 (m, 4 H), 3.76 (dd, J = 6.8, 6.8 Hz, 1 H), 3.59 (dd, J = 7.1, 2.8 Hz, 1 H), 2.70-2.63 (m, 2 H), 2.48-2.43 (m, 2 H), 2.15 (s, 3 H), 1.62-1.57 (m, 4 H), 1.39-1.31 (m, 4 H), 1.20 (s, 9 H), 0.91 (t, J = 7.5 Hz, 3 H), 0.88 (t, J = 7.4 Hz, 3 H); ¹³C NMR (100 MHz, CDCl₃): δ 206.2, 177.1, 172.1, 138.2, 137.7, 128.6, 128.5, 128.3, 128.0, 128.0, 127.9, 96.9, 75.3, 74.1, 73.9, 73.8, 73.6, 69.4, 68.2,
68.1, 68.0, 67.7, 39.0, 37.7, 32.3, 32.2, 32.1, 29.9, 27.9, 27.9, 27.2, 18.8, 13.8, 13.7; ^31^P NMR (120 MHz, CDCl₃): δ -2.45; ESI-MS: m/z (M + Na)^+ calcd 757.3329, obsd 757.3350.

*n*-Pentenyl 3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 46. Method A: Phosphate 39 (0.350 g, 0.47 mmol) was azeotroped with toluene (3 x 3 mL) and dried under vacuum for 1 h. The residue was dissolved in CH₂Cl₂ (5 mL), along with 4-penten-1-ol (58 µL, 0.56 mmol), and the solution was cooled to -78°C. TBSOTf (108 µL, 0.47 mmol) was added and after 30 minutes the reaction mixture was neutralized with Et₃N and concentrated in vacuo. The residue was purified by flash silica gel chromatography (25% EtOAc/hexanes) to yield 262 mg (89%) of *n*-pentenyl 4-*O*-t-butyldimethylsilyl-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside. [α]D: +4.77 (c = 0.73, CH₂Cl₂); IR (thin film): 2947, 2851, 1742, 1140, 1057 cm⁻¹; ^1^H NMR (400 MHz, CDCl₃): δ 7.35-7.21 (m, 10 H), 5.85-5.75 (m, 1 H), 5.09 (dd, J = 9.2, 8.0 Hz, 1 H), 5.01 (ddd, J = 18.7, 3.4, 1.6 Hz, 1 H), 4.97-4.94 (m, 1 H), 4.71-4.64 (m, 3 H), 4.54 (d, J = 12.3 Hz, 1 H), 4.43 (d, J = 7.9 Hz, 1 H), 3.88 (dt, J = 9.4, 6.5 Hz, 1 H), 3.78 (dd, J = 9.4, 6.5 Hz, 1 H), 3.65 (app t, J = 9.1 Hz, 1 H), 3.60-3.45 (m, 4 H), 2.13-2.07 (m, 2 H), 1.71-1.60 (m, 2 H), 1.13 (s, 9 H), 0.83 (s, 9 H), -0.04 (s, 3 H), -0.10 (s, 3 H); ^13^C NMR (100 MHz, CDCl₃): δ 177.0, 138.3, 138.3, 128.6, 128.3, 127.8, 127.4, 127.1, 115.0, 101.3, 83.5, 77.5, 74.7, 73.6, 71.3, 69.6, 69.1, 39.0, 30.3, 29.1, 27.3, 26.1, 18.2, -3.7, -4.7; ESI-MS: m/z (M + Na)^+ calcd 626.3639, obsd 626.3620.

A solution of *n*-pentenyl 4-*O*-t-butyldimethylsilyl-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside (1.75 g, 2.78 mmol) in THF (30 mL) was treated with TBAF (5.56 mL of a 1.0 M solution in THF, 5.56 mmol). After 30 minutes, the mixture was diluted with water (50 mL), extracted with EtOAc (3 x 50 mL), dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography (20% EtOAc/hexanes) to yield 1.26 g (89%) of 46. [α]D: -20.94° (c = 1.31, CH₂Cl₂); IR (thin film): 3492, 2922, 1733, 1130, 1071 cm⁻¹; ^1^H NMR (400 MHz, CDCl₃): δ 7.38-7.28 (m,
10 H), 5.83-5.74 (m, 1 H), 5.06-4.98 (m, 2 H), 4.96 (dd, J = 10.3, 1.7 Hz, 1 H), 4.74 (d, J = 11.4 Hz, 1 H), 4.69 (d, J = 11.4 Hz, 1 H), 4.63 (d, J = 12.1 Hz, 1 H), 4.58 (d, J = 12.0 Hz, 1 H), 4.40 (d, J = 8.0 Hz, 1 H), 3.85 (dt, J = 9.5, 6.5 Hz, 1 H), 3.80-3.70 (m, 3 H), 3.58-3.44 (m, 3 H), 2.69 (d, J = 1.3 Hz, 1 H), 2.12-2.06 (m, 2 H), 1.70-1.62 (m, 2 H), 1.22 (s, 9 H), 13C NMR (100 MHz, CDCl3): δ 177.0, 138.4, 138.3, 138.0, 128.7, 128.7, 128.0, 128.0, 127.8, 115.1, 101.5, 83.0, 74.6, 74.3, 73.9, 72.8, 72.1, 70.6, 69.3, 39.0, 30.3, 29.0, 27.4; ESI-MS: m/z (M + Na)+ calc 535.2666, obsd 535.2645.

Method B: Phosphate 40 (0.220 g, 0.306 mmol) was azeotroped with toluene (3 × 3 mL) and dried under vacuum for 1 h. The residue was dissolved in CH2Cl2 (5 mL), along with 4-penten-1-ol (29 mg, 0.337 mmol), and the solution was cooled to -78°C. TBSOTf (70 μL, 0.306 mmol) was added and after 10 minutes the reaction mixture was neutralized with Et3N and concentrated in vacuo. The residue was purified by flash silica gel chromatography (25% EtOAc/hexanes) to yield 160 mg (88%) of n-pentenyl 3,6-di-O-benzyl-4-O-levulinyl-2-O-pivaloyl-β-D-glucopyranoside. [α]D: -15.66° (c = 1.70, CH2Cl2), IR (thin film): 2975, 2879, 1743, 1722, 1151 cm⁻¹; 1H NMR (400 MHz, CDCl3): δ 7.34-7.21 (m, 10 H), 5.85-5.74 (m, 1 H), 5.12-5.07 (m, 2 H), 5.04-4.99 (m, 1 H), 4.98-4.95 (m, 1 H), 4.63 (d, J = 11.3 Hz, 1 H), 4.58-4.52 (m, 3 H), 4.43 (d, J = 7.9 Hz, 1 H), 3.88 (dt, J = 9.5, 6.4 Hz, 1 H), 3.76 (t, J = 9.4 Hz, 1 H), 3.65-3.57 (m, 3 H), 3.47 (dt, J = 9.5, 6.8 Hz, 1 H), 2.65-2.52 (m, 2 H), 2.41 (ddd, J = 17.3, 7.6, 6.0 Hz, 1 H), 2.29 (dt, J = 17.3, 6.4 Hz, 1 H), 2.12 (s, 3 H), 2.12-2.06 (m, 3 H), 1.71-1.63 (m, 2 H), 1.21 (s, 9 H), 13C NMR (100 MHz, CDCl3): δ 206.4, 176.8, 171.8, 138.3, 138.2, 128.5, 128.5, 128.0, 127.8, 127.8, 127.7, 115.1, 101.4, 80.6, 73.8, 73.8, 73.5, 72.5, 71.0, 69.8, 69.4, 39.0, 37.9, 30.2, 30.0, 29.0, 28.0, 27.4; ESI-MS: m/z (M + Na)+ calc 633.3034, obsd 633.3018.

A solution of hydrazine acetate (20 mg, 0.27 mmol) in MeOH (0.3 mL) was added to a solution of pentenyl 3,6-di-O-benzyl-4-O-levulinyl-2-O-pivaloyl-β-D-glucopyranoside (160 mg, 0.27 mmol) in CH2Cl2 (3 mL) and was stirred for 90 minutes at room temperature. The reaction mixture was diluted with CH2Cl2 (5 mL) and concentrated in vacuo. The crude product was purified by flash silica gel chromatography (25% EtOAc/hexanes) to afford 109 mg (81%) of 46.
\textit{n-Pentenyl 3-O-\textit{t}-butyldimethylsilyl-4,6-di-O-benzyl-2-O-pivaloyl-\textit{\beta}-D-galactopyranosyl-(1\rightarrow4)-3,6-di-O-benzyl-2-O-pivaloyl-\textit{\beta}-D-glucopyranoside 47.}

Monosaccharide 46 (170 mg, 0.42 mmol) and glycosyl phosphate 9 (336 mg, 0.46 mmol) were azeotroped with toluene (3 \times 3 mL), then dried under vacuum for 1 h. Dichloromethane (5 mL) was added, the solution was cooled -78°C, and TBSOTf (105 \muL, 0.46 mmol) was added. After 15 minutes, the mixture was neutralized with Et\textsubscript{3}N and concentrated in vacuo. The residue was purified by flash silica gel chromatography (5% EtOAc/hexanes) to yield 314 mg (74%) of 47. $[\alpha]_{\text{D}}^2 = -17.86^\circ$ (c = 1.36, CH\textsubscript{2}Cl\textsubscript{2}); IR (thin film): 3030, 2929, 2859, 1740, 1144 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): $\delta$ 7.35-7.22 (m, 15 H), 7.12-7.08 (m, 3 H), 7.01-6.97 (m, 2 H), 5.83-5.76 (m, 1 H), 5.36 (dd, $J = 9.6, 7.8$ Hz, 1 H), 5.07-4.94 (m, 5 H), 4.76 (d, $J = 12.1$ Hz, 1 H), 4.50 (d, $J = 12.1$ Hz, 1 H), 4.45 (d, $J = 10.8$ Hz, 1 H), 4.40 (d, $J = 11.0$ Hz, 1 H), 4.37-4.35 (m, 2 H), 4.32 (d, $J = 12.0$ Hz, 1 H), 4.20 (d, $J = 11.9$ Hz, 1 H), 4.05 (t, $J = 9.6$ Hz, 1 H), 3.86 (dt, $J = 9.5, 6.3$ Hz, 1 H), 3.76 (d, $J = 2.3$ Hz, 1 H), 3.72 (d, $J = 2.4$ Hz, 1 H), 3.67 (dd, $J = 9.8, 2.7$ Hz, 1 H), 3.57 (t, $J = 9.3$ Hz, 1 H), 3.47-3.35 (m, 4 H), 3.29 (dd, $J = 8.9, 5.1$ Hz, 1 H), 2.12-2.05 (m, 2 H), 1.70-1.62 (m, 2 H), 1.23 (s, 9 H), 1.17 (s, 9 H), 0.89 (s, 9 H), 0.18 (s, 3 H), 0.12 (s, 3 H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): $\delta$ 177.1, 176.9, 139.1, 139.0, 138.3, 138.2, 128.6, 128.4, 128.1, 128.0, 128.0, 127.8, 127.8, 127.5, 126.9, 115.0, 101.4, 99.8, 81.0, 75.5, 75.5, 74.8, 74.7, 74.0, 73.7, 73.6, 73.4, 73.1, 73.1, 72.3, 69.1, 68.5, 68.3, 39.1, 38.9, 30.2, 28.9, 27.8, 27.3, 26.0, 18.0, -3.1, -4.7; ESI-MS: m/z (M + Na)$^+$ calcd 1053.3974, obsd 1053.3981.

\textit{n-Pentenyl 4,6-di-O-benzyl-3-O-levulinyl-2-O-pivaloyl-\textit{\beta}-D-galactopyranosyl-(1\rightarrow4)-3,6-di-O-benzyl-2-O-pivaloyl-\textit{\beta}-D-glucopyranoside 48.} Monosaccharide 46 (109 mg, 0.22 mmol) and glycosyl phosphate 45 (190 mg, 0.26 mmol) were azeotroped with toluene (3 \times 3 mL), then dried under vacuum for 1 h. Dichloromethane (5 mL) was
added, the solution was cooled to -78°C, and TBSOTf (55 µL, 0.242 mmol) was added. After 30 minutes, the mixture was neutralized with Et₃N and concentrated in vacuo. The residue was purified by flash silica gel chromatography (25% EtOAc/hexanes) to yield 175 mg (90%) of 48. [α]₀D: -11.51° (c = 3.95, CH₂Cl₂); IR (thin film): 3027, 2977, 1745, 1723, 1138 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.38-7.24 (m, 13H), 7.23-7.19 (m, 4H), 7.16-7.13 (m, 1H), 7.09-7.06 (m, 2H), 5.85-5.74 (m, 1H), 5.35 (dd, J = 10.5, 7.9 Hz, 1H), 5.04-4.94 (m, 4H), 4.83 (dd, J = 10.5, 3.1 Hz, 1H), 4.74 (app t, J = 11.9 Hz, 2H), 4.52-4.44 (m, 4H), 4.36 (dd, J = 8.0 Hz, 1H), 4.30 (d, J = 11.8 Hz, 1H), 4.17 (d, J = 11.8 Hz, 1H), 4.04 (app t, J = 9.2 Hz, 1H), 3.92 (d, J = 2.9 Hz, 1H), 3.86 (dt, J = 9.6, 6.4 Hz, 1H), 3.72-3.70 (m, 2H), 3.61 (app t, J = 9.1 Hz, 1H), 3.49-3.41 (m, 2H), 3.37-3.26 (m, 3H), 2.69-2.65 (m, 2H), 2.45 (app t, J = 6.3 Hz, 2H), 2.15 (s, 3H), 2.13-2.06 (m, 2H), 1.68-1.63 (m, 2H), 1.18 (s, 18H); ¹³C NMR (100 MHz, CDCl₃): δ 206.3, 177.0, 176.8, 172.1, 138.9, 138.6, 138.3, 138.1, 138.0, 128.7, 128.6, 128.4, 128.2, 128.2, 128.0, 128.0, 127.9, 127.7, 127.7, 127.1, 115.0, 101.5, 99.7, 81.0, 75.3, 75.2, 74.6, 74.5, 74.3, 73.7, 73.5, 73.3, 72.4, 70.2, 69.1, 68.0, 67.7, 39.0, 38.9, 37.7, 30.2, 29.9, 28.9, 27.9, 27.3; ESI-MS: m/z (M + Na)⁺ calcd 1059.5076, obsd 1059.5043.

**n-Pentenyl 4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 49.** A solution of hydrazine acetate (14 mg, 0.19 mmol) in MeOH (0.3 mL) was added to a solution of 48 (175 mg, 0.17 mmol) in CH₂Cl₂ (3 mL) and the resulting solution was stirred for 90 minutes at room temperature. The reaction mixture was diluted with CH₂Cl₂ (5 mL) and concentrated in vacuo. The crude product was purified by flash silica gel chromatography (25% EtOAc/hexanes) to yield 156 mg (98%) of 49. [α]₀D: -32.05° (c = 0.40, CH₂Cl₂); IR (thin film): 3529, 2969, 1736, 1137, 1064 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.36-7.13 (m, 20H), 5.85-5.74 (m, 1H), 5.04-4.92 (m, 5H), 4.76 (d, J = 12.1 Hz, 1H), 4.62 (s, 2H), 4.52 (d, J = 10.8 Hz, 1H), 4.47 (d, J = 12.1 Hz, 1H), 4.41 (d, J = 8.0 Hz, 1H), 4.37 (dd, J = 8.0 Hz, 1H), 4.35 (d, J = 11.8 Hz, 1H), 4.25 (d, J = 11.7 Hz, 1H), 4.03 (dd, J = 9.4, 9.3 Hz, 1H), 3.86 (dt, J = 9.6, 6.4 Hz, 1H), 3.79 (dd, J = 8.5, 3.4 Hz, 2H), 3.74 (dd, J = 10.7, 1.6 Hz, 1H), 3.59 (dd, J =
9.2, 9.2 Hz, 1H), 3.47-3.31 (m, 6H), 2.17 (d, J = 10.5 Hz, 1H), 2.12-2.06 (m, 2H), 1.70-
1.62 (m, 2H), 1.20 (s, 9H), 1.17 (s, 9H). $^{13}$C NMR (100 MHz, CDCl$_3$): δ 176.8, 139.1,
138.3, 138.1, 137.9, 128.7, 128.7, 128.6, 128.2, 128.1, 128.0, 127.9, 127.6, 127.2, 115.0,
101.5, 99.7, 81.1, 76.7, 75.7, 75.5, 75.4, 74.5, 74.1, 73.8, 73.6, 73.3, 72.4, 69.1, 68.1,
67.8, 39.1, 38.9, 30.3, 29.0, 27.4, 27.3; ESI-MS: m/z (M + Na)$^+$ calcld 961.4714, obsd
961.4744.

$t$-Butyldimethylsilyl 3-O-acetyl-6-O-benzyl-2-deoxy-2-azido-4-O-levulinyl-$\beta$-D-
glucopyranoside 51. A solution of 50 (427 mg, 1.04 mmol) in CH$_2$Cl$_2$ (10 mL) was
treated with acetic anhydride (0.15 mL, 1.57 mmol) and DMAP (192 mg, 1.57 mmol).
After 30 minutes, the mixture was diluted with CH$_2$Cl$_2$ (50 mL) and washed with a
solution of saturated aqueous NaHCO$_3$ (50 mL), a 5% aqueous HCl solution (2 × 50 mL),
and water (50 mL). The organic layer was dried over Na$_2$SO$_4$, filtered, and concentrated
in vacuo. Purification of the residue by silica gel chromatography (25% EtOAc/hexanes)
yielded $t$-butyldimethylsilyl 3-O-acetyl-4,6-O-benzylidene-2-deoxy-2-azido-$\beta$-D-
glucopyranoside (425 mg, 90%). $[\alpha]_D$: -72.1° (c = 0.99, CH$_2$Cl$_2$); IR (thin film): 2111,
1751, 1370, 1222, 1099 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): δ 7.45-7.33 (m, 5H), 5.49 (s,
1H), 5.13 (dd, J = 9.9, 9.7 Hz, 1H), 4.72 (d, J = 7.6 Hz, 1H), 4.31 (dd, J = 10.5, 4.9 Hz,
1H), 3.80 (dd, J = 10.3, 10.2 Hz, 1H), 3.65 (dd, J = 9.5, 9.4 Hz, 1H), 3.49 (ddd, J = 9.6,
9.6, 4.9 Hz, 1H), 3.42 (dd, J = 10.1, 7.5 Hz, 1H), 2.14 (s, 3H), 0.95 (s, 9H), 0.19 (s, 3H),
0.18 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$): δ 169.9, 136.9, 129.3, 128.4, 126.3, 101.7,
97.8, 78.9, 71.1, 68.7, 67.4, 66.8, 25.8, 21.1, 18.2, -4.1, -4.9; FAB MS: m/z (M)$^+$ calcld
449.1982, obsd 449.1876.

Trifluoroacetic acid (36 μL, 0.47 mmol) was added dropwise to a solution of
$\beta$-butyldimethylsilyl 3-O-acetyl-4,6-O-benzylidene-2-deoxy-2-azido-$\beta$-D-glucopyranoside
(42 mg, 0.093 mmol) and triethylsilane (75 μL, 0.47 mmol) in CH$_2$Cl$_2$ (1 mL) at 0°C.
The reaction mixture was slowly warmed to ambient temperature over a period of 3 h,
quenched by addition of saturated aqueous NaHCO$_3$, and extracted with CH$_2$Cl$_2$ (3 × 10
mL). The combined organic extracts were dried over Na$_2$SO$_4$, filtered, and concentrated
in vacuo. The residue was purified by flash silica gel chromatography (20% EtOAc/hexanes) to furnish \( \tau \)-butyldimethylsilyl 3-O-acetyl-6-O-benzyl-2-deoxy-2-azido-\( \beta \)-D-glucopyranoside (38 mg, 91%). \([\alpha]_D\) = -21.6° (c = 1.00, CH\(_2\)Cl\(_2\)); IR (thin film): 3434, 2111, 1749, 1252, 1069 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)):\(\delta\) 7.40-7.28 (m, 5H), 4.80 (dd, \(J = 10.3, 9.1\) Hz, 1H), 4.61 (d, \(J = 7.7\) Hz, 1H), 4.61-4.57 (m, 2H), 3.75 (dd, \(J = 4.9, 1.6\) Hz, 1H), 3.69 (ddd, \(J = 9.3, 9.3, 3.5\) Hz, 1H), 3.53-3.45 (m, 1H), 3.36 (dd, \(J = 10.3, 7.7\) Hz, 1H), 3.00 (d, \(J = 3.7\) Hz, 1H), 2.18 (s, 3H), 0.95 (s, 9H), 0.18 (s, 3H), 0.17 (s, 3H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)):\(\delta\) 171.5, 137.7, 128.6, 128.0, 127.8, 97.3, 75.4, 74.4, 73.9, 71.1, 70.3, 66.1, 25.8, 21.3, 18.2, -4.1, -5.0; FAB MS: \(m/z\) (M\(^+\)) calcd 551.2138, obsd 451.2131.

Levulinic acid (137 mg, 1.19 mmol) and DMAP (152 mg, 1.24 mmol) were dissolved in 10 mL CH\(_2\)Cl\(_2\) and cooled to 0°C. After 10 minutes, DIPC (0.18 mL, 1.13 mmol) was added with vigorous stirring. After 5 additional minutes, a solution of \( \tau \)-butyldimethylsilyl 3-O-acetyl-6-O-benzyl-2-deoxy-2-azido-\( \beta \)-D-glucopyranoside (510 mg, 1.13 mmol) in 10 mL CH\(_2\)Cl\(_2\) was added to the levulinic acid solution via cannula and the mixture was left to slowly warm to room temperature. After 12 hours, the reaction mixture was diluted with EtOAc and flushed through a plug of silica gel. The filtrate was concentrated and the residue was purified by flash silica gel chromatography (40% EtOAc/hexanes) to afford 572 mg (92%) of 51. \([\alpha]_D\) = -1.29° (c = 1.31, CH\(_2\)Cl\(_2\)); IR (thin film): 2857, 2110, 1750, 1363, 1226 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)):\(\delta\) 7.30-7.22 (m, 5H), 4.97 (t, \(J = 9.4\) Hz, 1H), 4.93 (t, \(J = 9.5\) Hz, 1H), 4.59 (d, \(J = 7.7\) Hz, 1H), 4.47 (s, 2H), 3.60-3.56 (m, 1H), 3.52-3.50 (m, 2H), 3.36 (dd, \(J = 9.8, 7.8\) Hz, 1H), 2.62-2.57 (m, 2H), 2.38-2.32 (m, 2H), 2.09 (s, 3H), 2.07 (s, 3H), 0.90 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)):\(\delta\) 206.1, 171.6, 170.4, 138.1, 128.6, 128.4, 127.9, 127.8, 97.3, 73.6, 73.5, 72.2, 69.6, 69.2, 66.3, 37.7, 29.8, 27.9, 25.7, 21.1, 20.9, 18.1, -4.2, -5.1; ESI-MS: \(m/z\) (M + Na\(^+\)) calcd 549.2506, obsd 549.2524.

\[\text{3-O-Acetyl-6-O-benzyl-2-deoxy-2-azido-4-O-levulinyl-\( \beta \)-D-glucopyranosyl trichloroacetimidate 52.}\]

TBAF (1.34 mL of a 1.0 M solution in THF, 1.34 mmol) and
acetic acid (77 μL, 1.34 mmol) were added dropwise simultaneously to a solution of 51 (570 mg, 1.04 mmol) in THF (10 mL) at 0°C and the solution was left to slowly warm to room temperature. After 2 h, the mixture was diluted with water (50 mL) and extracted with CH₂Cl₂ (3 × 50 mL). The combined organic extracts were washed with saturated aqueous NaHCO₃ (2 × 50 mL), dried over Na₂SO₄, filtered, concentrated and used without further purification.

The residue was azeotroped with toluene (3 × 5 mL), then dried under vacuum for 30 minutes. A solution of 3-O-acetyl-6-O-benzyl-2-deoxy-2-azido-4-O-levulinyl-β-D-glucopyranoside in CH₂Cl₂ (10 mL) was treated with trichloroacetonitrile (1.25 mL, 12.48 mmol) and DBU (16 μL, 0.10 mmol). After 45 minutes, the reaction mixture was concentrated and purified by flash silica gel chromatography (40% EtOAc/hexanes) to afford 52 (438 mg, 73%). [α]D < 90.38° (c = 1.04, CH₂Cl₂); IR (thin film): 2923, 2112, 1751, 1222, 1152 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.81 (s, 1H), 7.35-7.27 (m, 5H), 6.50 (d, J = 3.5 Hz, 1H), 5.55 (dd, J = 10.4, 9.6 Hz, 1H), 5.33 (app t, J = 10.1 Hz, 1 H), 4.52 (d, J = 11.8 Hz, 1H), 4.48 (d, J = 11.8 Hz, 1H), 4.16-4.13 (m, 1H), 3.76 (dd, J = 10.6, 3.6 Hz, 1H), 3.61 (dd, J = 11.2, 2.6 Hz, 1H), 3.55 (dd, J = 11.1, 3.4 Hz, 1H), 2.71-2.67 (m, 2H), 2.45-2.38 (m, 2H), 2.17 (s, 3H), 2.15 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 206.3, 171.6, 170.4, 160.8, 137.8, 128.5, 128.2, 127.9, 94.5, 73.7, 71.6, 70.6, 68.5, 67.6, 60.9, 37.8, 29.9, 27.9, 21.0; ESI-MS: m/z (M + Na)⁺ calcd 578.0738, obsd 578.0733.

3,4,6-Tri-O-acetyl-β-D-glucopyranosyl-1,2-α-oxazoline 54. A solution of 53 (4.25 g, 10.9 mmol) in CH₂Cl₂ (100 mL) was treated with TMSOTf (2.17 mL, 12.0 mmol) then heated to 40°C. After 40 h, the mixture was cooled to room temperature, quenched by addition of triethylamine (3 mL), and concentrated in vacuo. The resulting residue was purified by flash silica gel chromatography (60% EtOAc/hexanes) to afford 3.15 g (88%) of 54 as a yellow oil. Characterization data was consistent with previously reported data.⁵¹
t-Butyldimethylsilyl 3,4,6-tri-O-acetyl-2-deoxy-2-dimethylmaleoyl-β-D-glucopyranoside 56. A solution of 55 (1.70 g, 3.64 mmol) in DMF was treated with hydrazine acetate (0.33 g, 4.37 mmol). After 4 h, the reaction mixture was diluted with EtOAc (60 mL) and washed with cold saturated aqueous NaHCO₃ (3 × 100 mL). The organic layer was dried over Na₂SO₄, filtered, and concentrated in vacuo. The crude product was purified by flash silica gel chromatography (50% EtOAc/hexanes) to yield 3,4,6-tri-O-acetyl-2-deoxy-2-dimethylmaleoyl-β-D-glucopyranoside (1.39 g, 90%). Characterization data was consistent with previously reported data.²¹

A solution of 3,4,6-tri-O-acetyl-2-deoxy-2-dimethylmaleoyl-β-D-glucopyranoside (314 mg, 0.74 mmol) in CH₂Cl₂ (5 mL) was treated with imidazole (101 mg, 1.48 mmol) and TBS chloride (134 mg, 0.89 mmol). After 90 minutes, the reaction mixture was diluted with water (20 mL) and extracted with CH₂Cl₂ (3 × 30 mL). The combined organic extracts were dried over Na₂SO₄, filtered, concentrated and purified by flash silica gel chromatography (50% EtOAc/hexanes) to afford 340 mg (85%) of 56. Characterization data was consistent with previously reported data.²¹

\[ \text{57} \]

t-Butyldimethylsilyl 4,6-O-benzylidene-2-deoxy-2-dimethylmaleoyl-β-D-glucopyranoside 57. To a solution of 56 (1.46 g, 2.70 mmol) in methanol (27 mL) was added sodium methoxide (60 μL of a 25% solution by weight, 0.27 mmol). After 2 h, the mixture was quenched by addition of amberlite acidic resin to pH 6, filtered, and concentrated to afford t-butyldimethylsilyl 2-deoxy-2-dimethylmaleoyl-β-D-glucopyranoside (1.15 g, quant.), which was used without further purification.

A solution of t-butyldimethylsilyl 2-deoxy-2-dimethylmaleoyl-β-D-glucopyranoside (1.15 g, 2.70 mmol) in DMF (20 mL) was treated with benzaldehyde dimethyl acetal (0.81 mL, 5.40 mmol) and tetrafluoroboric acid (0.20 mL, 2.70 mmol). After 5 h, the reaction mixture was diluted with water (50 mL) and extracted with ether (3 × 100 mL).
The combined organic extracts were washed with saturated aqueous NaHCO₃ (2 × 100 mL) and water (100 mL), dried over Na₂SO₄, filtered and concentrated. The residue was purified by flash silica gel chromatography (40% EtOAc/hexanes) to afford 57 (1.24 g, 91%). Characterization data was consistent with previously reported data.²¹

$$\text{AcO} \quad \text{O} \quad \text{O} \quad \text{H}$$
$$\text{LevO} \quad \text{OTBS} \quad \text{NDMM}$$

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*t-Butyldimethylsilyl 4-O-acteyl-6-O-benzy1-2-deoxy-2-dimethylmaleoyl-3-O-levuliny1-β-D-glucopyranoside* 58. Levulinic acid (48 mg, 2.13 mmol) and DMAP (272 mg, 2.23 mmol) were dissolved in 10 mL CH₂Cl₂ and cooled to 0°C. After 10 minutes, DIPC (0.32 mL, 2.03 mmol) was added with vigorous stirring. After 5 additional minutes, a solution of 57 (1.017 g, 2.03 mmol) in 10 mL CH₂Cl₂ was added to the levulinic acid solution via cannula and the mixture was left to slowly warm to room temperature. After 12 hours, the reaction mixture was diluted with EtOAc and flushed through a plug of silica gel. The filtrate was concentrated and the residue was purified by flash silica gel chromatography (25% EtOAc/hexanes) to afford 1.126 g (92%) of *t*-butyldimethylsilyl 4,6-O-benzyldiene-2-deoxy-2-dimethylmaleoyl-3-O-levulinyl-β-D-glucopyranoside. [α]D²⁵: -31.63° (c = 1.30, CH₂Cl₂); IR (thin film): 2927, 1749, 1707, 1405, 1103 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.47-7.44 (m, 2H), 7.38-7.35 (m, 3H), 5.80 (dd, J = 10.5, 8.9 Hz, 1H), 5.51 (s, 1H), 5.45 (d, J = 8.0 Hz, 1H), 4.34 (dd, J = 10.6, 4.6 Hz, 1H), 4.02 (dd, J = 10.5, 8.0 Hz, 1H), 3.83 (app t, J = 9.7 Hz, 1H), 3.74-3.66 (m, 2H), 2.72-2.65 (m, 1H), 2.59-2.40 (m, 3H), 2.07 (s, 3H), 1.97 (s, 6H), 0.78 (s, 9H), 0.07 (s, 3H), -0.02 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 206.1, 171.9, 137.5, 137.2, 129.3, 128.4, 126.5, 101.8, 94.0, 79.7, 69.7, 68.9, 66.4, 57.2, 38.0, 29.9, 28.1, 25.5, 17.8, 9.0, -4.0, -5.4; ESI-MS: m/z (M+Na)⁺ calcd 587.7334, obsd 587.7350.

A solution of *t*-butyldimethylsilyl 4,6-O-benzyldiene-2-deoxy-2-dimethylmaleoyl-3-O-levulinyl-β-D-glucopyranoside (1.005 g, 1.67 mmol) in CH₂Cl₂ (17 mL) at 0°C was treated with triethylsilane (1.59 mL, 10 mmol). After 10 minutes, trifluoroacetic acid (0.77 mL, 10 mmol) was added dropwise and the mixture was left to slowly warm to room temperature. After 3 h, the mixture was diluted with CH₂Cl₂, washed with
saturated aqueous NaHCO₃ (3 × 75 mL), dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was purified by flash silica gel chromatography (40% EtOAc/hexanes) to afford t-butyldimethylsilyl 6-O-benzyl-2-deoxy-2-dimethylmaleoyl-3-O-levulinyl-β-D-glucopyranoside (0.700 g, 70%). [α]D: -9.65° (c = 1.72, CH₂Cl₂); IR (thin film): 2955, 2927, 2856, 1705, 1396 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.36-7.28 (m, 5H), 5.59 (dd, J = 11.0, 8.6 Hz, 1H), 5.36 (d, J = 8.0 Hz, 1H), 4.62 (app d, J = 1.0 Hz, 2H), 3.97 (dd, J = 11.0, 8.1 Hz, 1H), 3.81-3.67 (m, 4H), 2.73-2.69 (m, 2H), 2.49-2.46 (m, 2H), 2.12 (s, 3H), 1.95 (s, 6H), 0.77 (s, 9H), 0.08 (s, 3H), -0.01 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 207.2, 173.0, 138.1, 137.4, 128.6, 127.9, 127.8, 93.4, 74.4, 74.3, 74.4, 73.9, 73.8, 71.6, 70.3, 56.3, 38.3, 29.9, 28.3, 25.5, 17.8, 9.0, -4.0, -5.4; ESI-MS: m/z (M+Na)⁺ calcld 589.7493, obsd 589.7484.

A solution of t-butyldimethylsilyl 6-O-benzyl-2-deoxy-2-dimethylmaleoyl-3-O-levulinyl-β-D-glucopyranoside (411 mg, 0.68 mmol) in CH₂Cl₂ (7 mL) was treated with acetic anhydride (80 μL, 0.82 mmol) and DMAP (100 mg, 0.82 mmol). After 30 minutes, the mixture was diluted with CH₂Cl₂ (20 mL) and washed with a solution of saturated aqueous NaHCO₃ (2 × 50 mL) and water (50 mL). The organic layer was dried over Na₂SO₄, filtered, and concentrated in vacuo. Purification of the residue by silica gel chromatography (40% EtOAc/hexanes) yielded 58 (440 mg, quant.). [α]D: +13.49° (c = 4.07, CH₂Cl₂); IR (thin film): 2927, 2856, 1747, 1705, 1396 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 7.36-7.26 (m, 5H), 5.69 (dd, J = 10.9, 9.1 Hz, 1H), 5.37 (d, J = 8.1 Hz, 1H), 5.07 (dd, J = 10.1, 9.3 Hz, 1H), 4.58 (d, J = 12.1 Hz, 1H), 4.52 (d, J = 12.1 Hz, 1H), 4.0 (dd, J = 10.9, 8.1 Hz, 1H), 3.78 (dt, J = 10.0, 4.2 Hz, 1H), 3.57 (d, J = 4.2 Hz, 1H), 2.65-2.62 (m, 2H), 2.40-2.36 (m, 2H), 2.09 (s, 3H), 1.96 (s, 3H), 1.94 (s, 6H), 0.77 (s, 9H), 0.09 (s, 3H), -0.01 (s, 3H); ¹³C NMR (75 MHz, CDCl₃): δ 206.2, 171.8, 170.1, 138.1, 137.4, 128.5, 127.9, 127.8, 93.3, 74.6, 73.4, 70.9, 70.0, 69.3, 56.5, 37.8, 29.7, 28.1, 25.5, 20.9, 17.8, -4.0, -5.5; ESI-MS: m/z (M + Na)⁺ calcld 631.2183, obsd 631.2155.
4-O-Acetyl-6-O-benzyl-2-deoxy-2-dimethylmaleoyl-3-O-levulinyl-β-D-glucopyranosyl trichloroacetimidate 59. TBAF (0.74 mL of a 1.0 M solution in THF, 0.74 mmol) and acetic acid (40 µL, 0.74 mmol) were added dropwise simultaneously to a solution of 58 (367 mg, 0.57 mmol) in THF (6 mL) at 0°C and the solution was left to slowly warm to room temperature. After 90 minutes, the mixture was diluted with water (50 mL) and extracted with CH₂Cl₂ (3 × 50 mL). The combined organic extracts were washed with saturated aqueous NaHCO₃ (2 × 50 mL), dried over Na₂SO₄, filtered, concentrated to yield 286 mg (94%) 4-O-acetyl-6-O-benzyl-2-deoxy-2-dimethylmaleoyl-3-O-levulinyl-β-D-glucopyranoside, which was used without further purification.

The residue was azeotroped with toluene (3 × 3 mL), then dried under vacuum for 2 h. A solution of 4-O-acetyl-6-O-benzyl-2-deoxy-2-dimethylmaleoyl-3-O-levulinyl-β-D-glucopyranoside in CH₂Cl₂ (6 mL) was treated with trichloroacetonitrile (0.63 mL, 6.30 mmol) and DBU (7 µL, 0.052 mmol). After 2 h, the reaction mixture was concentrated and purified by flash silica gel chromatography (25% EtOAc/hexanes) to afford 59 (247 mg, 70%). [α]D: +40.59° (c = 2.70, CH₂Cl₂); IR (thin film): 3304, 2922, 1751, 1715, 1404 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.67 (s, 1H), 7.36-7.27 (m, 5H), 6.45 (d, J = 8.9 Hz, 1H), 5.78 (dd, J = 10.7, 9.2 Hz, 1H), 5.24 (app t, J = 10.0 Hz, 1H), 4.61 (d, J = 12.1 Hz, 1H), 4.52 (d, J = 12.1 Hz, 1H), 4.37 (dd, J = 10.8, 8.9 Hz, 1H), 3.97 (dd, J = 10.1, 4.4, 2.9 Hz, 1H), 3.66 (dd, J = 11.2, 5.4 Hz, 1H), 2.65 (app t, J = 5.8 Hz, 2H), 2.39 (app t, J = 6.5 Hz, 2H), 2.10 (s, 3H), 1.94 (s, 3H), 1.93 (s, 6H); ¹³C NMR (100 MHz, CDCl₃): δ 206.1, 171.8, 169.9, 160.8, 137.8, 137.6, 128.6, 128.2, 127.9, 93.8, 74.4, 73.6, 70.7, 69.2, 68.1, 53.4, 37.8, 29.7, 28.1, 20.9, 9.0; ESI-MS: m/z (M + Na)⁺ calcd 660.1044, obsd 660.1056.
**n-Pentenyln-3-O-benzoyl-6-O-benzyl-4-O-levulinoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside 61.** To a stirred solution of n-pentenyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside 60 (2.0 g, 4.02 mmol) and DMAP (738 mg, 6.04 mmol) in 20 mL CH₂Cl₂ was added benzoyl chloride (583 μL, 5.02 mmol) at room temperature under N₂. The reaction mixture was stirred for 2 h and partitioned between EtOAc and water. The aqueous phase was extracted once with EtOAc and the combined organic phases were washed with 5% HCl, brine, saturated NaHCO₃, brine and dried over Na₂SO₄. Upon filtration and concentration, the crude product was purified by flash silica gel column chromatography (20% EtOAc/hexanes) to afford 2.20 g (91%) of n-pentenyl 3-O-benzoyl-4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside. [α]ᵦ: +13.6° (c = 1.99, CH₂Cl₂); IR (thin film): 2941, 1776, 1720, 1387, 1255 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.89 (d, J = 8.0 Hz, 1H), 7.82-7.59 (m, 4H), 7.49-7.42 (m, 5H), 7.33-7.29 (m, 5H), 6.25 (dd, J = 9.0, 10.2 Hz, 1H), 5.60 (m, 1H), 5.59 (s, 1H), 5.54 (d, J = 8.5 Hz, 1H), 4.79 (d, J = 8.5 Hz, 1H), 4.75 (d, J = 11.5 Hz, 1H), 4.54 (dd, J = 8.5, 10.5 Hz, 1H), 4.46 (dd, J = 4.5, 10.2 Hz, 1H), 3.94 (m, 1H), 3.91 (m, 1H), 3.88 (m, 1H), 3.53 (m, 1H), 1.98-1.83 (m, 2H), 1.63-1.49 (m, 2H); ¹³C NMR (125 MHz, CDCl₃): δ 165.6, 137.5, 136.9, 134.2, 133.1, 129.7, 129.2, 129.0, 128.3, 128.2, 126.2, 123.5, 114.8, 101.5, 98.8, 79.7, 70.1, 69.4, 68.7, 66.3, 55.3, 29.7, 28.5; FAB-MS: m/z (M+Na)+ calcd 592.1942, obsd 592.1919.

To a stirred solution of n-pentenyl 3-O-benzoyl-4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (3.3 g, 5.79 mmol) and triethylsilane (4.65 mL, 29 mmol) in 25 mL CH₂Cl₂ at 0°C under N₂ was added trifluoroacetic acid (2.25 mL, 29 mmol). The reaction mixture was allowed to warm to room temperature over 30 min and stirred for another 2 h. The reaction mixture was diluted with EtOAc, washed with saturated NaHCO₃, brine and dried over Na₂SO₄. Upon filtration and concentration, the crude product was purified by flash silica gel column chromatography (30% → 50% EtOAc/hexanes) to afford 2.92 g (88%) of n-pentenyl 3-O-benzoyl-6-O-benzyl-2-deoxy-
2-pthalimido-β-D-glucopyranoside. [α]D: +60.6° (c = 2.68, CH2Cl2); IR (thin film): 3487, 2940, 1717, 1387, 1268 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.88 (d, J = 8.5 Hz, 1H), 7.83-7.68 (m, 4H), 7.63 (m, 1H), 7.46 (m, 1H), 7.39-7.30 (m, 7H), 5.95 (dd, J = 8.5, 10.7 Hz, 1H), 5.61 (m, 1H), 5.47 (d, J = 8.0 Hz, 1H), 4.78 (m, 2H), 4.68 (dd, J = 8.5, 12.5 Hz, 2H), 4.46 (dd, J = 8.5, 10.5 Hz, 1H), 3.96-3.86 (m, 5H), 3.52 (m, 3H), 3.43 (d, J = 4.0 Hz, 1H), 1.97-1.83 (m, 2H), 1.64-1.50 (m, 2H); ¹³C NMR (125 MHz, CDCl₃): δ 166.9, 137.9, 137.8, 134.2, 133.4, 131.4, 129.9, 129.0, 128.5, 128.4, 127.8, 123.6, 114.8, 98.1, 74.8, 74.5, 73.8, 71.6, 70.1, 69.1, 54.6, 29.9, 58.6; FAB-MS: m/z (M+Na)⁺ calc 594.2098, obsd 594.2114.

To a stirred solution of n-pentenyl 3-O-benzoyl-6-O-benzyl-2-deoxy-2-pthalimido-β-D-glucopyranoside (2.85 g, 4.98 mmol) and DMAP (3.65 g, 29.88 mmol) in 25 mL CH₂Cl₂ at room temperature under N₂ was added Lev₂O (5.4 mL, 28.95 mmol). The reaction mixture was stirred in the dark for 45 min, diluted with EtOAc, washed with 5% HCl, saturated NaHCO₃, brine and dried over Na₂SO₄. Upon filtration and concentration, the crude product was purified by flash silica gel column chromatography (50% EtOAc/hexanes) to afford 61 (3.08 g, 92%). [α]D: +70.8° (c = 1.84, CH₂Cl₂); IR (thin film): 2943, 1718, 1387, 1267, 1145 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.83 (d, J = 7.5 Hz, 1H), 7.82-7.61 (m, 4H), 7.47 (m, 1H), 7.37-7.31 (m, 7H), 7.28 (m, 1H), 6.05 (dd, J = 9.0, 11.0 Hz, 1H), 5.59 (m, 1H), 5.44 (d, J = 8.0 Hz, 1H), 5.37 (t, J = 9.0 Hz, 1H), 4.77 (m, 1H), 4.72 (m, 1H), 4.60 (dd, J = 11.5, 15.0 Hz, 2H), 4.78 (dd, J = 8.5, 11.0 Hz, 1H), 3.94 (m, 1H), 3.88 (m, 1H), 3.74 (m, 1H), 3.67 (m, 1H), 3.48 (m, 1H), 2.59-2.25 (m, 4H), 1.99 (s, 3H), 1.97-1.83 (m, 2H), 1.62-1.48 (m, 2H); ¹³C NMR (125 MHz, CDCl₃): δ 205.9, 171.4, 165.8, 138.0, 137.7, 134.2, 133.3, 129.9, 128.8, 128.4, 127.9, 127.7, 123.5, 114.8, 98.1, 73.6, 73.4, 71.5, 70.0, 69.2, 68.9, 54.8, 37.8, 29.8, 29.5, 28.5, 27.9; FAB-MS: m/z (M+Na)⁺ calc 692.2466, obsd 692.2458.

![Chemical Structure](image)

**3-O-Benzoyl-6-O-benzyl-4-O-levulinoyl-2-deoxy-2-pthalimido-β-D-glucopyranosyl trichloroacetimidate 62.** To a stirred solution of 61 (1.90 g, 2.83 mmol) in 50 mL
CH₃CN/H₂O (8:1) was added NBS (1.51 g, 8.51 mmol) and the reaction mixture was stirred for 5 h at room temperature. After addition of Na₂S₂O₃ (7 mL of a saturated aqueous solution), the reaction mixture was partitioned between EtOAc and water and the aqueous phase was extracted once with EtOAc. The combined organic phases were washed with saturated NaHCO₃, brine and dried over Na₂SO₄. Upon filtration and concentration, the crude product was purified by flash silica gel column chromatography (40% EtOAc/hexanes) to afford 3-O-benzoyl-6-O-benzyl-4-O-levulinoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (1.10 g, 66% yield), which was used immediately in the next step.

To a stirred solution of 3-O-benzoyl-6-O-benzyl-4-O-levulinoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (1.10 g, 1.89 mmol) and trichloroacetonitrile (1.89 mL, 18.91 mmol) in 20 mL CH₂Cl₂ under N₂ was added DBU (60 μL, 0.38 mmol). The reaction mixture was stirred at room temperature for 2 h and then concentrated. The crude product was purified by flash silica gel column chromatography (30%-40% EtOAc/hexanes with 1% triethylamine) to afford 62 (620 mg, 45% yield). [α]D: +88.2° (c = 2.42, CH₂Cl₂); IR (thin film): 2921, 1779, 1720, 1681, 1386 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 8.68 (s, 1H), 7.85 (d, J = 7.5 Hz, 1H), 7.82-7.64 (m, 4H), 7.51 (m, 1H), 7.38-7.29 (m, 8H), 6.72 (d, J = 9.0 Hz, 1H), 6.17 (dd, J = 9.0, 10.5 Hz, 1H), 5.53 (t, J = 9.5 Hz, 1H), 4.80 (dd, J = 9.0, 10.7 Hz, 1H), 4.62 (d, J = 12.0 Hz, 1H), 4.59 (d, J = 12.0 Hz, 1H), 4.14 (m, 1H), 3.79 (dd, J = 3.0, 11.2 Hz, 1H), 3.71 (dd, J = 5.0, 11.2 Hz, 1H), 2.59-2.28 (m, 4H), 2.00 (s, 3H); ¹³C NMR (125 MHz, CDCl₃): δ 206.0, 171.4, 165.8, 160.7, 138.0, 134.4, 133.5, 131.4, 130.0, 128.8, 128.5, 128.2, 127.8, 123.8, 93.8, 74.5, 73.6, 71.2, 69.4, 68.2, 68.2, 53.9, 37.9, 29.6, 28.0; FAB-MS: m/z (M+Na)⁺ calcd 767.0936, obsd 767.0932.

n-Pentenyl 3-O-benzoyl-6-O-benzyl-4-O-levulinoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 64. To a stirred solution of 49 (77
mg, 0.082 mmol) and 62 (180 mg, 0.248 mmol) in 2.5 mL CH₂Cl₂ under N₂ at -30°C was added TMSOTf (8 µL, 0.044 mmol). The reaction was stirred at -30°C for 5 min and warmed to -15°C over the course of 10 min. Triethylamine (15 µL) was added, the solution was concentrated, and the crude product purified by flash silica column chromatography (30%–40% EtOAc/hexanes) to afford 64 (107 mg, 87% yield). [α]D: -5.7° (c = 2.16, CH₂Cl₂); IR (thin film): 2869, 1730, 1387, 1267, 1144 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.79 (d, J = 7.0 Hz, 1H), 7.78-7.59 (m, 4H), 7.47 (t, J = 7.0 Hz, 1H), 7.38-7.21 (m, 26H), 7.07 (t, J = 8.0 Hz, 1H), 6.97 (t, J = 8.0 Hz, 1H), 6.14 (dd, J = 9.0, 10.7 Hz, 1H), 5.77 (m, 1H), 5.40 (d, J = 8.0 Hz, 1H), 5.31 (t, J = 9.5 Hz, 1H), 5.10 (dd, J = 5.0, 11.5 Hz, 1H), 5.03 (d, J = 11.0 Hz, 1H), 4.97 (m, 1H), 4.94 (m, 1H), 4.92 (m, 1H), 4.87 (d, J = 10.5 Hz, 1H), 4.70 (d, J = 12.0 Hz, 1H), 4.57-4.42 (m, 2H), 4.43 (d, J = 12.0 Hz, 1H), 4.28 (m, 2H), 4.17 (d, J = 11.5 Hz, 1H), 4.00 (t, J = 9.5 Hz, 2H), 3.94 (m, 1H), 3.86 (dd, J = 3.0, 10.0 Hz, 1H), 3.81 (m, 1H), 3.74 (dd, J = 7.5, 10.5 Hz, 1H), 3.67 (m, 2H), 3.46-3.38 (m, 3H), 3.33-3.27 (m, 3H), 2.62-2.23 (m, 4H), 2.03 (s, 3H), 2.12-2.01 (m, 2H), 1.65-1.59 (m, 2H), 1.14 (s, 9H), 1.09 (s, 9H); ¹³C NMR (125 MHz, CDCl₃): δ 206.0, 176.6, 176.5, 171.5, 165.6, 139.1, 138.7, 138.3, 138.2, 137.9, 137.8, 134.0, 133.4, 130.0, 129.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.6, 127.3, 126.9, 123.7, 114.8, 101.3, 99.4, 98.1, 80.9, 77.6, 76.8, 75.3, 74.6, 74.3, 73.7, 73.5, 72.3, 70.6, 70.2, 69.0, 68.9, 68.4, 68.2, 55.3, 38.8, 38.7, 37.8, 30.1, 29.6, 28.8, 27.9, 27.2, 27.1; FAB-MS: m/z (M+Na)⁺ calc 1544.6551, obsd 1544.6523.

**n-Penteny1 3-O-benzoyl-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 65.** To a stirred solution of 64 (54 mg, 0.036 mmol) in 2 mL CH₂Cl₂ at room temperature under N₂ was added a solution of hydrazine acetate (90 µL, 0.40 M) in MeOH. The reaction mixture was stirred at room temperature for 3 h, concentrated, and the crude product was purified by flash silica column chromatography (50% EtOAc/hexanes) to afford 65 (49 mg, 98% yield). [α]D: -24.4° (c = 1.20, CH₂Cl₂);
IR (thin film) 3481, 2868, 1726, 1386, 1272 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$): δ 7.87 (d, $J = 7.0$ Hz, 1H), 7.82-7.59 (m, 4H), 7.48 (t, $J = 7.0$ Hz, 1H), 7.42-7.20 (m, 26H), 7.08 (t, $J = 8.0$ Hz, 1H), 6.96 (t, $J = 8.0$ Hz, 1H), 5.99 (dd, $J = 8.5, 11.0$ Hz, 1H), 5.78 (m, 1H), 5.37 (d, $J = 8.5$ Hz, 1H), 5.12 (dd, $J = 8.0, 10.0$ Hz, 1H), 5.07 (d, $J = 11.0$ Hz, 1H), 5.01 (m, 1H), 4.95 (m, 1H), 4.87 (d, $J = 10.5$ Hz, 1H), 4.73 (d, $J = 12.0$ Hz, 1H), 4.64 (d, $J = 12.0$ Hz, 1H), 4.62 (d, $J = 12.0$ Hz, 1H), 4.50 (d, $J = 11.0$ Hz, 1H), 4.48 (m, 2H), 4.34-4.27 (m, 4H), 4.23 (d, $J = 12.0$ Hz, 1H), 4.03 (t, $J = 9.0$ Hz, 1H), 3.99-3.81 (m, 7H), 3.70 (m, 2H), 3.41-3.39 (m, 2H), 3.35-3.29 (m, 3H), 2.13-2.02 (m, 2H), 1.78 (bs, 1H), 1.70-1.59 (m, 2H), 1.16 (s, 9H), 1.13 (s, 9H); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 176.7, 176.6, 167.1, 139.2, 138.8, 138.2, 138.0, 137.7, 134.0, 133.6, 130.0, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.3, 126.9, 123.8, 114.9, 101.3, 99.5, 98.0, 81.0, 77.4, 75.4, 75.2, 75.0, 74.7, 74.4, 74.0, 73.9, 73.8, 73.7, 73.6, 72.5, 72.3, 71.9, 70.0, 68.9, 68.4, 68.3, 55.0, 38.9, 38.8, 30.1, 28.9, 27.3, 27.2, 27.1; FAB-MS: m/z (M+Na)$^+$ calcd 1446.6183, obsd 1446.6122.

Dibutyl 2-O-(2-(azidomethyl)benzoyl)-3,4,6-tri-O-benzyl-β-D-galactopyranoside phosphate 66. To a stirred solution of tri-O-benzyl-D-galactal (505 mg, 1.214 mmol) in 10 mL CH$_2$Cl$_2$ at 0°C under N$_2$ was added a solution of dimethylidioxirane in acetone (23 mL, 0.08 M). The reaction mixture was stirred for 15 min at 0°C, and the volatiles were removed in vacuo. After 1 h under vacuum at 0°C, 10 mL CH$_2$Cl$_2$ were added and the reaction mixture was cooled to -78°C and stirred for 10 min. To the reaction vessel was added a solution of dibutyl phosphate (277 μL, 1.396 mmol) in CH$_2$Cl$_2$ (2 mL) dropwise over 5 min and the reaction mixture was warmed to 0°C over 5 min. After stirring for 10 min at 0°C, DMAP (445 mg, 3.642 mmol) was added followed by a solution of AZMB chloride (475 mg, 2.428 mmol) in CH$_2$Cl$_2$ (2 mL) dropwise over 5 min. The reaction was warmed to room temperature and stirred for 3 h. The reaction mixture was diluted with hexanes to precipitate DMAP salts, filtered and washed several times with 40% EtOAc/hexanes. The crude product was concentrated and purified by flash silica column chromatography (40% EtOAc/hexanes with 1% triethylamine) to afford of 66 (603 mg,
62% yield. \([\alpha]_D^1 +24.2^\circ\) (c = 3.46, CH\textsubscript{2}Cl\textsubscript{2}); IR (thin film) 2960, 2102, 1729, 1259, 1082 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\textsubscript{3}): \(\delta\) 8.02 (d, \(J = 7.7\) Hz, 1H), 7.61-7.54 (m, 3H), 7.19-7.04 (m, 16H), 5.72 (dd, \(J = 8.0, 10.0\) Hz, 1H), 5.35 (t, \(J = 8.0\) Hz, 1H), 5.02 (d, \(J = 11.5\) Hz, 1H), 4.83 (d, \(J = 15.5\) Hz, 1H), 4.79 (d, \(J = 15.0\) Hz, 1H), 4.68 (d, \(J = 8.5\) Hz, 1H), 4.66 (d, \(J = 8.5\) Hz, 1H), 4.47 (d, \(J = 11.5\) Hz, 1H), 4.46 (s, 2H), 4.08 (d, \(J = 1.5\) Hz, 1H), 4.07-3.99 (m, 2H), 3.81-3.62 (m, 6H), 1.62-1.56 (m, 2H), 1.38-1.29 (m, 4H), 1.10-1.02 (m, 2H), 0.89 (t, \(J = 7.5\) Hz, 1H), 0.69 (t, \(J = 7.5\) Hz, 1H); \(^13\)C NMR (125 MHz, CDCl\textsubscript{3}): \(\delta\) 165.1, 138.4, 138.1, 137.7, 137.4, 133.1, 131.4, 129.5, 128.6, 128.4, 128.3, 128.1, 128.0, 127.8, 127.7, 97.0, 79.7, 74.8, 74.4, 73.6, 72.2, 72.0, 68.0, 67.9, 32.1, 32.0, 31.9, 31.8, 18.6, 18.4, 13.7, 13.4; \(^31\)P NMR (200 MHz, CDCl\textsubscript{3}): \(\delta\) -2.26; FAB-MS: m/z (M\(^+\)) calcd 824.3282, obsd 824.3264.

**n-Pentenyl 2-O-(2-(azidomethyl)benzoyl)-3,4,6-tri-O-benzyl-\(\beta\)-D-galactopyranosyl-(1→4)-3-O-benzyl-6-O-benzyl-2-deoxy-2-phthalimido-\(\beta\)-D-glucopyranoside 68.** To a solution n-pentenyl glycoside 67 (135 mg, 0.236 mmol) and galactosyl phosphate 66 (190 mg, 0.238 mmol) in 2.5 mL CH\textsubscript{2}Cl\textsubscript{2} under N\textsubscript{2} at -60\(^\circ\)C was added TMSOTf (43 \(\mu\)L, 0.238 mmol). The reaction was stirred at this temperature for 5 min and allowed to warm to -50\(^\circ\)C over 5 min. Triethylamine (60 \(\mu\)L) was added, the solution was concentrated, and the crude product purified by flash silica column chromatography (5% \(\rightarrow\) 10% EtOAc/toluene) to afford 68 (265 mg, 96% yield). \([\alpha]_D^1 +28.4^\circ\) (c = 2.06, CH\textsubscript{2}Cl\textsubscript{2}); IR (thin film): 2870, 2102, 1775, 1727, 1388 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\textsubscript{3}): \(\delta\) 7.82 (d, \(J = 7.0\) Hz, 1H), 7.80 (d, \(J = 7.5\) Hz, 1H), 7.73-7.61 (m, 4H), 7.56 (q, \(J = 6.5\) Hz, 1H), 7.41 (t, \(J = 7.5\) Hz, 1H), 7.42-7.08 (m, 26H), 6.01 (dd, \(J = 9.0, 10.7\) Hz, 1H), 5.58 (m, 1H), 5.46 (dd, \(J = 7.5, 10.2\) Hz, 1H), 5.32 (d, \(J = 8.5\) Hz, 1H), 4.87 (d, \(J = 15.5\) Hz, 1H), 4.86 (d, \(J = 11.0\) Hz, 1H), 4.78 (d, \(J = 15.5\) Hz, 1H), 4.75 (m, 1H), 4.71 (dd, \(J = 2.0, 3.2\) Hz, 1H), 4.57 (d, \(J = 8.0\) Hz, 1H), 4.52 (d, \(J = 12.5\) Hz, 1H), 4.47 (d, \(J = 12.0\) Hz, 1H), 4.42 (d, \(J = 11.0\) Hz, 1H), 4.36 (dd, \(J = 8.5, 11.0\) Hz, 1H), 4.31 (d, \(J = 12.0\) Hz, 1H), 4.25 (d, \(J = 12.5\) Hz, 1H), 4.11 (t, \(J = 8.5\) Hz, 1H), 4.07 (s, 2H), 3.85 (d, \(J = 2.5\) Hz, 1H), 3.78 (m,
1H), 3.80-3.76 (m, 2H), 3.56 (d, J = 9.5 Hz, 1H), 3.41 (m, 2H), 3.10 (dd, J = 4.5, 9.0 Hz, 1H), 3.00 (t, J = 8.5 Hz, 1H), 2.92 (dd, J = 4.5, 8.5 Hz, 1H), 1.89-1.81 (m, 2H), 1.58-1.42 (m, 2H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) 165.3, 164.8, 138.8, 138.5, 138.4, 138.0, 137.9, 137.8, 132.9, 132.8, 131.0, 130.2, 129.9, 129.4, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 114.8, 110.1, 98.2, 80.1, 76.3, 74.7, 74.5, 74.4, 73.3, 72.8, 72.4, 71.7, 71.3, 69.2, 67.9, 67.1, 55.1, 53.1, 30.0, 28.6; FAB-MS: \(m/z\) (M+Na)\(^+\) calcd 1185.4468, obsd 1185.4457.

\[ \text{[Image]} \]

\(n\)-Pentenyl 3,4,6-tri-\(\text{O}\)-benzyl-\(\beta\)-\(D\)-galactopyranosyl-(1→4)-3-\(\text{O}\)-benzoyl-6-\(\text{O}\)-benzyl-2-deoxy-2-phthalimido-\(\beta\)-\(D\)-glucopyranoside 69.\) A solution 68 (95 mg, 0.081 mmol) in THF (1 mL) was treated with water (7 \(\mu\)L, 0.407 mmol), and Pb\(\text{O}_\text{S}\) (60 \(\mu\)L, 0.244 mmol). After 30 minutes, the reaction mixture was diluted with CH\(_2\)Cl\(_2\) (20 mL) and washed with saturated aqueous NaHCO\(_3\) (20 mL) and water (2 \(\times\) 20 mL). The organic layer was dried over Na\(_2\)SO\(_4\), filtered and concentrated. Crude residue was purified by flash silica gel column chromatography (30% EtOAc/hexanes) to yield 69 (76 mg, 92%). \([\alpha]_D^\text{20}\) +30.6° (c = 1.34, CH\(_2\)Cl\(_2\)); IR (thin film): 3526, 2874, 1716, 1387, 1097 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.79 (d, J = 7.0 Hz, 1H), 7.69-7.57 (m, 4H), 7.37-7.07 (m, 24H), 6.04 (dd, J = 9.0, 11.0 Hz, 1H), 5.55 (m, 1H), 5.34 (d, J = 8.5 Hz, 1H), 4.73 (d, J = 10.0 Hz, 1H), 4.70 (d, J = 12.0 Hz, 1H), 4.67 (d, J = 12.0 Hz, 1H), 4.61 (s, 1H), 4.57 (d, J = 12.5 Hz, 1H), 4.51 (d, J = 12.0 Hz, 1H), 4.29 (d, J = 7.0 Hz, 1H), 4.14 (t, J = 9.0 Hz, 1H), 4.01 (dd, J = 3.5, 13.7 Hz, 1H), 3.98 (s, 2H), 3.84-3.77 (m, 3H), 3.68 (d, J = 2.5 Hz, 1H), 3.42 (m, 1H), 3.16 (dd, J = 3.0, 11.0 Hz, 1H), 3.01 (s, 1H), 2.98 (d, J = 8.5 Hz, 1H), 2.89 (dd, J = 4.5, 9.5 Hz, 1H), 2.66 (dd, J = 4.0, 8.0 Hz, 1H), 1.90-1.79 (m, 2H), 1.58-1.43 (m, 2H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) 165.3, 138.9, 138.4, 138.0, 137.9, 133.0, 129.9, 129.8, 128.6, 128.5, 128.3, 128.1, 127.9, 127.8, 127.7, 127.6, 127.3, 123.6, 114.8, 104.2, 98.4, 81.6, 77.4, 74.4, 73.6, 73.2, 72.8, 72.6, 72.2, 72.0, 71.8, 69.2, 68.5, 67.1, 55.1, 30.0, 28.6; FAB-MS: \(m/z\) (M+Na)\(^+\) calcd 1026.4035, obsd 1026.4020.
\[ \text{n-Pentenyl} \quad 2-O\text{-benzyl-3,4-di-O-pivaloyl-\text{\textalpha}-L-fucopyranosyl-(1\to2)-3,4,6-tri-O-benzyl-\text{\textbeta}-D-galactopyranosyl-(1\to4)-3-O-benzoyl-6-O-benzyl-2-deoxy-2-phthalimido-\text{\textbeta}-D-glucopyranoside 71.} \to \text{To a stirred solution of disaccharide 69 (90 mg, 0.089 mmol) and fucosyl phosphate 70 (85 mg, 0.138 mmol) in 2.5 mL CH}_2\text{Cl}_2 \text{ under N}_2 \text{ at -55°C was added TBSOTf (32 \mu L, 0.138 mmol). After 10 minutes, triethylamine (60 \mu L) was added, the solution was concentrated, and the crude product purified by flash silica column chromatography (20% EtOAc/hexanes) to afford 71 (117 mg, 93\%). [\alpha]_D^2: -27.8° (c = 1.51, CH}_2\text{Cl}_2); \text{IR (thin film): 2973, 1734, 1161, 1096, 735 cm}^{-1}; \text{^1H NMR (500 MHz, CDCl}_3): \delta 7.79 (d, J = 7.0 Hz, 1H), 7.78-7.62 (m, 4H), 7.43 (d, J = 7.5 Hz, 1H), 7.38-7.12 (m, 25H), 7.04 (m, 2H), 6.93 (d, J = 7.0 Hz, 1H), 5.94 (dd, J = 9.0, 11.0 Hz, 1H), 5.63 (m, 1H), 5.61 (d, J = 3.5 Hz, 1H), 5.38 (dd, J = 3.0, 11.2 Hz, 1H), 5.32 (d, J = 3.0 Hz, 1H), 4.86 (d, J = 12.0 Hz, 1H), 4.81 (m, 2H), 4.77 (m, 1H), 4.74 (q, J = 7.0 Hz, 1H), 4.69 (d, J = 12.0 Hz, 1H), 4.61 (dd, J = 4.0, 12.0 Hz, 1H), 4.48 (d, J = 11.0 Hz, 1H), 4.47 (d, J = 11.5 Hz, 1H), 4.45 (d, J = 12.0 Hz, 1H), 4.36 (d, J = 7.5 Hz, 1H), 4.33 (d, J = 9.5 Hz, 1H), 4.28 (d, J = 12.0 Hz, 1H), 4.25 (d, J = 12.0 Hz, 1H), 4.23 (d, J = 11.5 Hz, 1H), 4.20 (d, J = 12.0 Hz, 1H), 4.13 (dd, J = 2.0, 11.0 Hz, 1H), 4.04 (d, J = 10.0 Hz, 1H), 3.98 (dd, J = 8.0, 11.0 Hz, 1H), 3.91 (m, 1H), 3.81 (d, J = 3.5 Hz, 1H), 3.79 (d, J = 3.0 Hz, 1H), 3.67 (d, J = 10.5 Hz, 1H), 3.56 (m, 1H), 3.41 (dd, J = 2.5, 9.7 Hz, 1H), 3.23 (dd, J = 5.0, 8.7 Hz, 1H), 3.11 (dd, J = 4.5, 8.7 Hz, 1H), 2.79 (t, J = 8.5 Hz, 1H), 1.98-1.86 (m, 2H), 1.65-1.52 (m, 2H), 1.31 (d, J = 7.0 Hz, 3H), 1.20 (s, 9H), 1.18 (s, 9H); \text{^13C NMR (125 MHz, CDCl}_3): \delta 177.7, 177.6, 165.5, 138.9, 138.3, 138.0, 137.9, 137.7, 134.1, 132.3, 130.5, 130.0, 128.6, 128.5, 128.1, 128.0, 127.9, 127.6, 127.5, 127.4, 127.3, 127.2, 126.2, 123.6, 114.8, 100.0, 98.5, 97.1, 83.5, 75.2, 74.3, 73.5, 73.4, 73.3, 72.7, 72.4, 71.9, 71.5, 71.4, 70.7, 70.2, 69.3, 67.5, 65.0, 54.9, 39.0, 38.9, 30.0, 28.6, 27.4, 27.3, 15.4; \text{FAB-MS: m/z (M+Na)}^+ \text{calced 1430.6234, obsd 1430.6278.} \]
n-Pentenyl 2-O-(2-(azidomethyl)benzoyl)-3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-3-O-benzoyl-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 72. To a stirred solution of 65 (31 mg, 0.022 mmol) and galactosyl phosphate 66 (66 mg, 0.127 mmol) in 2.5 mL CH₂Cl₂ under N₂ at -60°C was added TMSOTf (23 μL, 0.127 mmol). The reaction was stirred at -60°C for 5 min and allowed to warm to -50°C over 5 min. Triethylamine (30 μL) was added, the solution was concentrated, and the crude product purified by flash silica column chromatography (10% EtOAc/toluene) to afford 72 (41.2 mg, 92% yield). [α]D₉⁻11.5° (c = 1.95, CH₂Cl₂);
IR (thin film): 2870, 2102, 1733, 1077 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.83 (d, J = 7.5 Hz, 1H), 7.78 (d, J = 7.5 Hz, 1H), 7.62-7.58 (m, 6H), 7.42-7.02 (m, 44H), 6.92 (t, J = 7.0 Hz, 1H), 6.10 (t, J = 9.0 Hz, 1H), 5.77 (m, 1H), 5.51 (t, J = 9.5 Hz, 1H), 5.34 (d, J = 8.5 Hz, 1H), 5.07 (t, J = 8.0 Hz, 1H), 5.02 (d, J = 11.5 Hz, 1H), 4.96 (d, J = 11.0 Hz, 1H), 4.92 (d, J = 15.0 Hz, 1H), 4.90 (d, J = 17.5 Hz, 1H), 4.83 (m, 2H), 4.76 (d, J = 15.0 Hz, 1H), 4.69 (d, J = 12.0 Hz, 1H), 4.60 (d, J = 8.0 Hz, 1H), 4.57 (d, J = 12.5 Hz, 1H), 4.44 (m, 3H), 4.36 (t, J = 7.5 Hz, 1H), 4.32-4.27 (m, 4H), 4.21-4.05 (m, 4H), 4.05 (s, 2H), 3.98 (t, J = 9.0 Hz, 1H), 3.57 (d, J = 10.5 Hz, 1H), 3.44-3.36 (m, 5H), 3.32-3.26 (m, 3H), 3.08 (m, 1H), 3.02 (t, J = 8.5 Hz, 1H), 2.92 (m, 1H), 2.12-2.01 (m, 2H), 1.68-1.59 (m, 2H), 1.10 (s, 9H); ¹³C NMR (125 MHz, CDCl₃): δ 176.7, 176.6, 165.0, 164.9, 139.2, 138.8, 138.7, 138.4, 138.3, 138.0, 138.0, 137.9, 137.7, 133.9, 133.0, 132.9, 131.0, 130.0, 129.8, 129.6, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.8, 127.6, 127.6, 127.5, 127.5, 126.9, 114.9, 101.3, 101.2, 99.5, 97.9, 81.0, 80.0, 76.6, 75.5, 75.1, 74.7, 74.6, 74.5, 74.4, 73.7, 73.6, 73.5, 73.3, 72.8, 72.4, 72.3, 71.5, 71.5, 71.1, 68.9, 68.3, 67.8, 67.0, 55.6, 53.1, 38.9, 38.8, 30.2, 28.9, 27.3, 27.2, 25.7; FAB-MS: m/z (M+Na)⁺ calcd 2037.8552, obsd 2037.8478.
$n$-Pentenyl 3,4,6-tri-$O$-benzyl-$\beta$-$D$-galactopyranosyl-(1$\rightarrow$4)-3-$O$-benzoyl-6-$O$-benzyl-2-deoxy-2-phthalimido-$\beta$-$D$-glucopyranosyl-(1$\rightarrow$3)-4,6-di-$O$-benzyl-2-$O$-pivaloyl-$\beta$-$D$-galactopyranosyl-(1$\rightarrow$4)-3,6-di-$O$-benzyl-2-$O$-pivaloyl-$\beta$-$D$-glucopyranoside 73. A solution of tetrascarharide 72 (45 mg, 0.022 mmol) in THF (1 mL) was treated with water (4 $\mu$L, 0.225 mmol), and PBU$_3$ (30 $\mu$L, 0.112 mmol). After 30 minutes, the reaction mixture was diluted with CH$_2$Cl$_2$ (20 mL) and washed with saturated aqueous NaHCO$_3$ (20 mL) and water ($2 \times 20$ mL). The organic layer was dried over Na$_2$SO$_4$, filtered and concentrated. Crude residue was purified by flash silica gel column chromatography (30% EtOAc/hexanes) affording 73 (40 mg, 96%). $[\alpha]_D^{-1}=-18.1^\circ$ (c 1.35, CH$_2$Cl$_2$); IR (thin film): 3387, 2868, 2360, 1733, 1070 cm$^{-1}$; $^1$H NMR 500 MHz, CDCl$_3$: $\delta$ 7.79 (d, $J=8.5$ Hz, 1H), 7.67-7.58 (m, 4H), 7.41-7.13 (m, 41H), 7.11 (d, $J=7.0$ Hz, 1H), 7.07 (t, $J=7.5$ Hz, 1H), 6.93 (t, $J=7.5$ Hz, 1H), 6.55 (dd, $J=9.0$, 11.0 Hz, 1H), 5.77 (m, 1H), 5.39 (d, $J=8.0$ Hz, 1H), 5.11 (t, $J=10.0$ Hz, 1H), 5.04 (d, $J=10.5$ Hz, 1H), 4.99 (d, $J=18.5$ Hz, 1H), 4.93 (d, $J=12.5$ Hz, 1H), 4.90 (d, $J=9.5$ Hz, 1H), 4.86 (d, $J=10.5$ Hz, 1H), 4.74 (d, $J=8.0$ Hz, 1H), 4.72 (d, $J=8.0$ Hz, 1H), 4.64 (d, $J=9.0$ Hz, 1H), 4.60 (d, $J=9.0$ Hz, 1H), 4.52 (d, $J=11.5$ Hz, 1H), 4.47 (d, $J=13.0$ Hz, 1H), 4.44 (d, $J=12.5$ Hz, 1H), 4.39 (d, $J=12.0$ Hz, 1H), 4.37 (d, $J=8.5$ Hz, 1H), 4.33-4.27 (m, 6H), 4.18 (d, $J=12.0$ Hz, 1H), 4.17 (d, $J=7.0$ Hz, 1H), 4.06-3.99 (m, 6H), 3.88-3.80 (m, 4H), 3.79 (d, $J=1.5$ Hz, 1H), 3.68 (s, 2H), 3.46-3.38 (m, 3H), 3.36-3.22 (m, 2H), 3.21 (dd, $J=2.5$, 9.7 Hz, 1H), 2.97 (d, $J=8.0$ Hz, 1H), 2.79 (s, 1H), 2.73 (q, $J=4.5$ Hz, 1H), 2.10-2.01 (m, 2H), 1.67-1.59 (m, 3H), 1.14 (s, 9H), 1.10 (s, 9H); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 176.8, 176.7, 165.1, 139.3, 139.0, 138.9, 138.3, 138.1, 137.9, 137.8, 133.0, 130.0, 129.9, 128.7, 128.6, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.5, 127.4, 126.9, 114.9, 104.0, 101.4, 99.6, 98.2, 81.8, 81.1, 75.6, 75.3, 74.8, 74.6, 74.5, 74.4, 73.8, 73.7, 73.6, 73.3, 73.0, 72.5, 72.4, 72.1, 72.0, 71.8, 71.7, 69.0, 68.4, 67.1, 55.6, 39.0, 38.9, 30.2, 29.0, 27.3, 27.2; FAB-MS: $m/z$ (M+Na)$^+$ calcd 1878.8120, obsd 1878.8152.
n-Pentenyl 2-O-benzyl-3,4-di-O-pivaloyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-3-O-benzoyl-6-O-benzyl-2-deoxy-2-phthalamido-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 74. To a stirred solution of tetrasaccharide acceptor 73 (12.1 mg, 0.0065 mmol) and fucosyl phosphate 70 (39.7 mg, 0.0645 mmol) in 1.5 mL CH₂Cl₂ under N₂ at -55°C was added TBSOTf (15 µL, 0.0645 mmol). The reaction was stirred at this temperature for 10 min. Triethylamine (60 µL) was added, the solution was concentrated, and the crude product purified by flash silica column chromatography (10% EtOAc/toluene) to afford 74 (13.9 mg, 94%). [α]D: -32.7° (c = 1.16, CH₂Cl₂); IR (thin film): 2930, 2869, 1732, 1387, 1268 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.81-7.57 (m, 4H), 7.71 (d, J = 8.5 Hz, 1H), 7.35-6.90 (m, 49H), 6.02 (dd, J = 9.0, 11.0 Hz, 1H), 5.77 (m, 1H), 5.60 (d, J = 3.5 Hz, 1H, H₆-1), 5.39 (d, J = 8.5 Hz, 1H, H₆-1), 5.36 (dd, J = 3.0, 10.5 Hz, 1H), 5.28 (d, J = 3.0 Hz, 1H), 5.15 (dd, J = 7.5, 10.2 Hz, 1H), 5.09 (d, J = 11.0 Hz, 1H), 5.05-4.99 (m, 2H), 4.91 (d, J = 8.0 Hz, 1H), 4.87 (d, J = 11.0 Hz, 1H), 4.72 (d, J = 12.0 Hz, 1H), 4.71 (d, J = 14.0 Hz, 1H), 4.68 (d, J = 12.0 Hz, 1H), 4.59 (d, J = 12.5 Hz, 1H), 4.57 (d, J = 12.5 Hz, 1H), 4.52 (d, J = 12.5 Hz, 1H), 4.48-4.41 (m, 3H), 4.38 (d, J = 7.5 Hz, 1H, H₆-1), 4.35 (d, J = 8.0 Hz, 1H, H₆-1), 4.33-4.30 (m, 4H), 4.29 (d, J = 7.5 Hz, 1H, H₆-1), 4.20 (d, J = 11.5 Hz, 1H), 4.18-4.11 (m, 4H), 4.03-3.96 (m, 5H), 3.83-3.78 (m, 3H), 3.74-3.65 (m, 4H), 3.49 (t, J = 6.5 Hz, 1H), 3.46-3.38 (m, 4H), 3.32-3.28 (m, 3H), 3.24 (dd, J = 4.5, 9.0 Hz, 1H), 3.05 (dd, J = 5.0, 9.0 Hz, 1H), 2.68 (t, J = 9.0 Hz, 1H), 2.14-2.01 (m, 2H), 1.66-1.59 (m, 2H), 1.27 (d, J = 6.5 Hz, 3H), 1.18 (s, 9H), 1.16 (s, 9H), 1.14 (s, 9H), 1.09 (s, 9H); ¹³C NMR (125 MHz, CDCl₃): δ 177.8, 177.6, 176.7, 176.6, 165.2, 139.4, 138.9, 138.8, 138.4, 138.3, 138.2, 138.0, 137.7, 134.0, 132.3, 130.4, 130.0, 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.4, 127.3, 126.9, 126.3, 123.8, 114.9, 101.3 (C₆-1), 100.2 (C₆-1), 99.7 (C₅-1), 98.5 (C₄-1), 97.1 (C₄-1), 83.6, 81.1, 77.6, 77.4,
75.6, 75.3, 75.2, 74.5, 74.3, 73.7, 73.6, 73.5, 73.4, 73.2, 72.7, 72.5, 72.4, 72.2, 71.8, 71.5, 71.4, 70.8, 70.4, 70.3, 68.9, 68.6, 68.4, 67.7, 67.4, 65.2, 55.4, 39.2, 38.9, 38.8, 30.2, 29.9, 28.9, 27.4, 27.3, 27.2, 15.5; ESI-MS: m/z (M+2Na)$^{2+}$ calcd 1153.0106, obsd 1153.0107.

Acetyl 3,4,6-tri-<i>O</i>-acetyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranoside 76. A solution of acetyl 3,4,6-tri-<i>O</i>-acetyl-2-deoxy-2-azido-β-D-glucopyranoside (4.02 g, 10.8 mmol) in THF (50 mL) was treated with water (0.77 mL, 43.0 mmol), followed by tri-<i>n</i>-butyl phosphine (3.50 mL, 14.0 mmol). Nitrogen gas was observed to evolve from the reaction mixture and the solution became yellow. After 1 hour, the mixture was concentrated in vacuo and azeotroped with toluene (3 × 5 mL). The resulting yellow residue was dissolved in CH$_2$Cl$_2$ (50 mL) and cooled to 0 °C. To this solution was added triethylamine (3.00 mL, 21.5 mmol) followed by trichloroacetylchloride (1.56 mL, 14.00 mmol). The reaction mixture became dark and turned black. After 15 min, the solution was poured into a solution of sat. aqueous NaHCO$_3$ (100 mL) and extracted with CH$_2$Cl$_2$ (3 × 50 mL). The combined organic extracts were dried over Na$_2$SO$_4$, filtered and concentrated to a brown residue. The crude residue was purified by flash silica gel chromatography (30% EtOAc/hexanes) to yield 3.60 g (69%) of 76. Characterization data was consistent with previously reported data.$^{47}$

<i>t</i>-Butyldimethylsilyl 3,4,6-tri-<i>O</i>-acetyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranoside 77. A solution of 76 (2.32 g, 4.7 mmol) in <i>N</i>,<i>N</i>-dimethylformamide (DMF) (50 mL) was treated with hydrazine acetate (0.53 g, 7.1 mmol). After 3 h, the reaction mixture was diluted with 100 mL ethyl acetate and washed with sat. aqueous NaHCO$_3$, brine, and water (50 mL each). The organic layer was dried over Na$_2$SO$_4$, filtered, and the filtrate concentrated to a give 1.76 g (83%) of 3,4,6-tri-<i>O</i>-acetyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranoside as a white solid, which was used without further purification.
A solution of 3,4,6-tri-O-acetyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranoside (6.58 g, 14.6 mmol) in DMF (50 mL) was treated with imidazole (2.00 g, 29.2 mmol) and t-butyldimethylsilyl chloride (2.64 g, 17.5 mmol). After 3 h, the reaction mixture was diluted with water (100 mL) and the solution was extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with water, sat. aqueous NaHCO₃, brine, 5% aqueous HCl, and again with water (100 mL each). The organic extracts were dried over Na₂SO₄, filtered, and concentrated. The crude residue was purified by flash silica gel chromatography (40% EtOAc/hexanes) to yield 7.81 g (95%) of 77 as a clear oil. Characterization data was consistent with previously reported data.⁴⁷

![Chemical Structure](image)

**t-Butyldimethylsilyl 4,6-O-benzylidene-2-deoxy-2-trichloroacetimido-β-D-glucopyranoside 78.** A solution of 77 (4.04 g, 7.20 mmol) in methanol (50 mL) was treated with sodium methoxide (164 μL of a 25% solution by weight, 0.72 mmol) at room temperature. After 2 h, the reaction mixture was diluted with methanol (50 mL) and the pH lowered to 6 by addition of Dowex acidic resin. The solution was filtered and the filtrate concentrated in vacuo to yield 3.50 g (quant. yield) of t-butyldimethylsilyl 2-deoxy-2-trichloroacetimido-β-D-glucopyranoside as a white foam, which was used without further purification.

A solution of t-butyldimethylsilyl 2-deoxy-2-trichloroacetimido-β-D-glucopyranoside (3.50 g, 7.20 mmol) in acetonitrile (50 mL) was treated with benzaldehyde dimethyl acetal (2.93 mL, 19.4 mmol) and p-toluenesulfonic acid (34 mg, 0.18 mmol). After 1 h, the reaction mixture was diluted with ethyl acetate (100 mL) and washed with sat. aqueous NaHCO₃, brine, and water (100 mL each). The organic layer was dried over Na₂SO₄, filtered and concentrated in vacuo. The crude residue was purified by flash silica gel chromatography (5–40% EtOAc/hexanes) to yield 3.50 g (93%) of 78 as a clear oil. Characterization data was consistent with previously reported data.⁴⁷

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t-Butyldimethylsilyl 3-O-benzoyl-6-O-benzyl-2-deoxy-4-O-levulinyl-2-trichloroacetimido-β-D-glucopyranoside 79. A solution of 78 (1.58 g, 3.00 mmol) in CH₂Cl₂ (30 mL) was treated with DMAP (0.73 g, 4.50 mmol), followed by benzoyl chloride (0.52 mL, 4.50 mmol) After 4 h, the reaction mixture diluted with CH₂Cl₂ (100 mL) and was washed with a 5% aqueous HCl solution and water (50 mL each), dried over Na₂SO₄, filtered and concentrated to a yellow oil. The crude residue was purified by flash silica gel chromatography (5% MeOH/CH₂Cl₂) to yield t-butyldimethylsilyl 3-O-benzoyl-4,6-O-benzylidene-2-deoxy-2-trichloroacetimido-β-D-glucopyranoside (1.73 g, 92%). [α]D: -65.53° (c = 1.92, CH₂Cl₂); IR (thin film): 3354, 2930, 2859, 1704, 1100 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.02-7.99 (m, 1H), 7.71 (d, J = 9.8 Hz, 1H), 7.61-7.56 (m, 1H), 7.47-7.41 (m, 4H), 7.34-7.31 (m, 3H), 5.85 (d, J = 11.2 Hz, 1H), 5.54 (s, 1H), 4.92 (d, J = 7.9 Hz, 1H), 4.38-4.31 (m, 1H), 3.95-3.87 (m, 2H), 3.71 (t, J = 10.1 Hz, 1H), 3.55 (dt, J = 9.6, 5.0 Hz, 1H), 0.89 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 167.8, 162.6, 137.3, 130.2, 129.0, 128.7, 128.3, 126.1, 101.1, 96.6, 92.5, 79.3, 72.7, 68.5, 66.4, 58.3, 25.7, 17.9, -3.8, -5.3; ESI-MS: m/z (M + Na)⁺ calc 652.1062, obsd 652.1064.

A solution of dry t-butyldimethylsilyl 3-O-benzoyl-4,6-O-benzylidene-2-deoxy-2-trichloroacetimido-β-D-glucopyranoside (1.54 g, 2.40 mmol) in CH₂Cl₂ (25 mL) was treated with triethylsilane (1.92 mL, 12.0 mmol) and cooled to 0 °C. After 10 min, trifluoroacetic acid (0.92 mL, 12.0 mmol) was added slowly dropwise to the solution. The reaction mixture was left to slowly warm to room temperature. After 2 ½ h, the solution was diluted with CH₂Cl₂ (50 mL) and washed with sat. aqueous NaHCO₃ (2 × 50 mL) and with water (50 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated. The crude residue was purified by flash silica gel chromatography (30 % EtOAc/hexanes) to give t-butyldimethylsilyl 3-O-benzoyl-6-O-benzyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranoside (1.40 g, 93%) as a white solid. [α]D: -18.42° (c = 6.90, CH₂Cl₂); IR (thin film): 3488, 3347, 2928, 2863, 1701 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.02-7.99 (m, 2H), 7.59-7.55 (m, 1H), 7.44-7.39 (m, 3H), 7.37-7.30 (m, 4H),
Levulinic acid (238 mg, 2.06 mmol) and DMAP (240 mg, 1.96 mmol) were dissolved in 10 mL CH₂Cl₂ and cooled to 0°C. After 10 minutes, DIPC (0.34 mL, 2.16 mmol) was added with vigorous stirring. After 5 additional minutes, a solution of tert-butyldimethylsilyl 3-O-benzoyl-6-O-benzyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranoside (1.24 g, 1.96 mmol) in 10 mL CH₂Cl₂ was added to the levulinic acid solution via cannula and the mixture was left to slowly warm to room temperature. After 12 hours, the reaction mixture was diluted with EtOAc and flushed through a plug of silica gel. The filtrate was concentrated and the residue was purified by flash silica gel chromatography (25→40% EtOAc/hexanes) to afford 1.16 g (81%) of 79 as a clear oil. \([\alpha]_D^{20}: -18.05^\circ\) (c = 0.82, CH₂Cl₂); IR (thin film): 3353, 2929, 2858, 1748, 1719, 1703 cm⁻¹; \(^1\)H NMR (400 MHz, CDCl₃): δ 7.96-7.94 (m, 2H), 7.58-7.53 (m, 1H), 7.40 (app t, \(J = 7.9\) Hz, 1H), 7.33-7.26 (m, 6H), 5.71 (app t, \(J = 9.8\) Hz, 1H), 5.33 (t, \(J = 9.8\) Hz, 1H), 5.00 (d, \(J = 7.9\) Hz, 1H), 4.56 (s, 2H), 4.27-4.20 (m, 1H), 3.93-3.88 (m, 1H), 3.69 (dd, \(J = 10.8, 2.7\) Hz, 1H), 3.64 (dd, \(J = 10.7, 6.0\) Hz, 1H), 2.47-2.38 (m, 3H), 2.30-2.25 (m, 1H), 1.94 (s, 3H), 0.90 (s, 9H), 0.16 (s, 3H), 0.10 (s, 3H); \(^13\)C NMR (100 MHz, CDCl₃): δ 205.9, 171.8, 167.3, 162.2, 138.3, 133.9, 130.2, 128.8, 128.6, 128.4, 127.8, 127.7, 96.1, 92.4, 73.8, 73.6, 72.9, 69.4, 58.1, 37.8, 29.6, 27.9, 25.7, 18.0, -3.9, -5.2; ESI-MS: \(m/z\) (M + Na)^+ calcd 752.1587, obsd 752.1579.

3-O-benzoyl-6-O-benzyl-2-deoxy-4-O-levulinyl-2-trichloroacetimido-β-D-glucopyranosyl trichloroacetimidate 80. A solution of 79 (390 mg, 0.536 mmol) in THF (6 mL) was cooled to 0°C. To this solution were added tetrabutylammonium fluoride (TBAF) (563 µL of a 1.0 M solution in THF) and acetic acid (32 µL, 0.563
mmol) simultaneously dropwise. After 90 min, the solution was diluted with water (50 mL) and extracted with CH₂Cl₂ (3 × 50 mL). The combined organic extracts were dried over Na₂SO₄, filtered, and concentrated to yield 3-O-benzoyl-6-O-benzyl-2-deoxy-4-O-levuliny1-2-trichloroacetimidoo-β-D-glucopyranoside (328 mg, quant. yield) as a yellow foam, which was used without further purification. [α]D: +16.70° (c = 3.21, CH₂Cl₂); IR (thin film): 3414, 2919, 1745, 1721, 1272 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.97-7.95 (m, 2H), 7.58-7.54 (m, 1H), 7.46-7.41 (m, 2H), 7.37-7.30 (m, 5H), 7.20 (d, J = 9.0 Hz, 1H), 5.66 (dd, J = 10.7, 9.7 Hz, 1H), 5.42 (br s, 1H), 5.31 (t, J = 9.9 Hz, 1H), 4.60 (d, J = 11.9 Hz, 1H), 4.56 (d, J = 11.9 Hz, 1H), 4.38-4.29 (m, 2H), 4.18 (br s, 1H), 3.63 (dd, J = 4.7, 3.6 Hz, 2H), 2.65-2.39 (m, 3H), 2.34-2.27 (m, 1H), 2.04 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 206.3, 171.7, 167.1, 162.1, 137.6, 133.8, 130.2, 129.0, 128.7, 128.6, 128.4, 128.1, 92.1, 91.2, 73.9, 71.2, 69.1, 68.8, 68.8, 54.6, 38.0, 29.8, 28.0; ESI-MS: m/z (M + Na)⁺ calcd 638.0722, obsd 638.0726.

3-O-benzoyl-6-O-benzyl-2-deoxy-4-O-levuliny1-2-trichloroacetimidoo-β-D-glucopyranoside (286 mg, 0.465 mmol) was azeotroped with toluene (3 × 3 mL), then dried under vacuum for 1 h. The residue was dissolved in CH₂Cl₂ (5 mL) and 1 mL of trichloroacetoniitrile was added, followed by DBU (7 μL, 0.0465 mmol). After 10 min, the solution was concentrated in vacuo and the crude residue was purified by flash silica gel chromatography (20% EtOAc/hexanes) to give 80 (291 mg, 82 %) as a yellow foam. [α]D: +9.55° (c = 1.21, CH₂Cl₂); IR (thin film): 3346, 1922, 1722, 1712, 1272 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.82 (s, 1H), 7.98-7.96 (m, 2H), 7.62-7.58 (m, 1H), 7.45 (app t, J = 7.9 Hz, 2H), 7.38-7.27 (m, 6H), 6.61 (d, J = 3.4 Hz, 1H), 5.73-5.65 (m, 2H), 4.58 (d, J = 11.9 Hz, 1H), 4.55-4.51 (m, 2H), 4.23-4.19 (m, 1H), 3.72 (dd, J = 11.2, 2.6 Hz, 1H), 3.66 (dd, J = 11.2, 3.6 Hz, 1H), 2.63-2.55 (m, 2H), 2.40-2.31 (m, 2H), 2.01 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 212.2, 206.1, 199.1, 188.7, 186.4, 171.5, 167.7, 162.3, 160.3, 137.8, 134.1, 130.2, 128.8, 128.6, 128.6, 128.3, 128.0, 94.1, 91.9, 90.9, 78.0, 73.8, 72.1, 71.1, 67.7, 67.5, 54.6, 38.0, 29.7, 27.9; ESI-MS: m/z (M + Na)⁺ calcd 780.9818, obsd 780.9810.
*n*-Pentenyl 3-O-benzoyl-6-O-benzyl-4-O-levulinyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 81. Lactose acceptor 49 (60 mg, 0.064 mmol) and glucosamine imidate 80 (98 mg, 0.129 mmol) were coevaporated in toluene (3 × 1 mL), then dried under vacuum for 1 h. Dichloromethane was added (2 mL) and the solution was cooled to -20°C. TMSOTf (1 μL, 0.0064 mmol) was added and the reaction mixture turned pink. The reaction was stirred at -20°C for 30 min, until the lactose was nearly consumed. Triethylamine was added to quench the mixture and the solution was slowly warmed to room temperature. The solution was concentrated, and the crude product purified by flash silica column chromatography (25% EtOAc/hexanes) to afford 81 (82 mg, 83% yield). [α]_D^25 -31.46° (c = 0.48 CH₂Cl₂); IR (thin film): 2924, 1741, 1726, 1273, 1068 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.94 (app d, J = 8.4 Hz, 2H), 7.56 (app t, J = 7.4 Hz, 1H), 7.41 (app t, J = 7.8 Hz, 2H), 7.32-7.23 (m, 20H), 7.15-7.10 (m, 3H), 7.03 (app t, J = 7.1 Hz, 2H), 6.75 (d, J = 9.0 Hz, 1H), 5.82-5.73 (m, 1H), 5.38-5.28 (m, 3H), 5.02-4.92 (m, 5H), 4.74 (d, J = 12.0 Hz, 1H), 4.67 (d, J = 8.2 Hz, 1H), 4.57 (d, J = 17.9 Hz, 1H), 4.54 (d, J = 18.0 Hz, 1H), 4.50 (d, J = 11.2 Hz, 1H), 4.45 (d, J = 12.0 Hz, 1H), 4.44 (d, J = 10.6 Hz, 1H), 4.35-4.29 (m, 3H), 4.20-4.16 (m, 2H), 4.00 (t, J = 9.1 Hz, 1H), 3.95-3.90 (m, 2H), 3.84 (dt, J = 9.5, 6.4 Hz, 1H), 3.77-3.65 (m, 5H), 3.54 (t, J = 9.0 Hz, 1H), 3.49-3.39 (m, 3H), 3.35-3.29 (m, 2H), 2.62-2.41 (m, 3H), 2.35-2.28 (m, 1H), 2.12-2.01 (m, 2H), 2.02 (s, 3H), 1.67-1.60 (m, 2H), 1.25 (s, 9H), 1.16 (s, 9H); ¹³C NMR (100 MHz, CDCl₃): δ 206.1, 176.8, 176.8, 171.6, 166.9, 162.4, 139.0, 138.9, 138.3, 138.2, 138.1, 137.8, 133.9, 130.2, 129.0, 128.7, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.6, 127.1, 115.0, 101.4, 100.3, 99.6, 92.1, 91.3, 81.0, 77.4, 76.4, 75.5, 75.4, 74.8, 74.7, 74.3, 73.8, 73.8, 73.8, 73.5, 72.6, 72.4, 71.1, 69.4, 69.2, 69.1, 69.0, 68.7, 68.2, 56.5, 39.1, 38.9, 38.0, 30.2, 29.7, 29.0, 27.9, 27.8, 27.3; ESI-MS: m/z (M + Na)^+ calcd 1558.5433, obsd 1558.5476.
n-Pentenyl 3-O-benzyol-6-O-benzyl-2-deoxy-2-trichloroacetimido-\(\beta\)-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-\(\beta\)-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-\(\beta\)-D-glucopyranoside 82. A solution of hydrazine acetate (5 mg, 0.070 mmol) in MeOH (0.1 mL) was added to a solution of 81 (98 mg, 0.064 mmol) in CH₂Cl₂ (1 mL) and the resulting solution was stirred overnight at room temperature. The reaction mixture was diluted with CH₂Cl₂ (5 mL) and concentrated in vacuo. The crude product was purified by flash silica gel chromatography (20% EtOAc/hexanes) to yield 87 mg (95%) of 82. \([\alpha]_D^{20} = -20.89^\circ\) (c = 1.25, CHCl₃); IR (thin film): 3672, 3621, 3015, 2964, 1733 cm⁻¹; \(^1\)H NMR (400 MHz, CDCl₃): δ 8.02 (app d, J = 8.4 Hz, 2H), 7.57 (app t, J = 7.4 Hz, 1H), 7.43 (app t, J = 7.9 Hz, 1H), 7.36-7.23 (m, 20H), 7.15-7.10 (m, 3H), 7.02 (app t, J = 7.5 Hz, 2H), 6.76 (d, J = 9.1 Hz, 1H), 5.82-5.73 (m, 1H), 5.30 (app t, J = 9.0 Hz, 1H), 5.18 (dd, J = 10.9, 9.0 Hz, 1H), 5.02-4.91 (m, 5H), 4.76 (d, J = 12.0 Hz, 1H), 4.67-4.58 (m, 3H), 4.49-4.43 (m, 3H), 4.37-4.28 (m, 3H), 4.24-4.17 (m, 2H), 4.05-3.97 (m, 2H), 3.93-3.83 (m, 6H), 3.71 (d, J = 2.4 Hz, 2H), 3.66-3.61 (m, 1H), 3.54 (t, J = 9.2 Hz, 1H), 3.50-3.40 (m, 3H), 3.35-3.30 (m, 2H), 3.17 (d, J = 2.8 Hz, 1H), 2.11-2.04 (m, 2H), 1.69-1.63 (m, 2H), 1.25 (s, 9H), 1.16 (s, 9H); \(^{13}\)C NMR (100 MHz, CDCl₃): δ 177.0, 176.9, 167.7, 162.6, 139.0, 138.8, 138.3, 138.1, 137.4, 134.0, 130.2, 128.9, 128.7, 128.6, 128.6, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.9, 127.6, 127.1, 115.0, 101.4, 100.2, 99.6, 92.1, 81.0, 77.4, 76.5, 75.7, 75.6, 75.4, 75.0, 74.8, 74.6, 74.0, 73.8, 73.7, 72.4, 71.2, 70.2, 69.1, 68.2, 55.9, 39.1, 38.9, 31.8, 30.2, 28.9, 27.8, 27.3, 22.9, 14.4; ESI-MS: m/z (M + Na)⁰ calcd 1460.5065, obsd 1460.5040.

\[ n\text{-Pentenyl} 2-O-(2-(azidomethyl)benzoyl)-3,4,6-tri-O-benzyl-\(\beta\)-D-galactopyranosyl-(1→4)-3-O-benzyol-6-O-benzyl-2-deoxy-2-trichloroacetimido-\(\beta\)-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-\(\beta\)-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2- \]
**O-pivaloyl-β-D-glucopyranoside 83.** Trisaccharide acceptor 82 (48 mg, 0.033 mmol) and galactosyl phosphate 66 (53 mg, 0.066 mmol) were coevaporated in toluene (3 × 2 mL), then dried under vacuum for 2 h. Dichloromethane (1.5 mL) was added and the solution was cooled to −78°C. TMSOTf (10 μL, 0.59 mmol) was added and the reaction mixture was stirred for 30 min at −78°C. The reaction mixture was quenched by addition of TEA and slowly warmed to room temperature. The solution was concentrated and the crude residue was purified by flash silica gel chromatography (10% EtOAc/hexanes) to yield 54 mg (87%) of 83. [α]D: -5.59° (c = 1.68, CHCl₃); IR (thin film): 3015, 2923, 2103, 1728, 1077 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.97 (app d, J = 7.2 Hz, 2H), 7.86 (app d, J = 7.7 Hz, 1H), 7.61-7.53 (m, 3H), 7.49 (app t, J = 7.5 Hz, 1H), 7.37-7.07 (m, 41H), 7.00 (app t, J = 7.2 Hz, 2H), 6.76 (d, J = 9.3 Hz, 1H), 5.82-5.73 (m, 1H), 5.50 (dd, J = 10.1, 8.0 Hz, 1H), 5.31 (dd, J = 10.8, 8.7 Hz, 1H), 5.26 (dd, J = 10.1, 8.0 Hz, 1H), 5.04-4.90 (m, 6H), 4.87 (d, J = 11.6 Hz, 1H), 4.71 (d, J = 11.5 Hz, 2H), 4.66-4.57 (m, 3H), 4.53-4.40 (m, 6H), 4.35-4.25 (m, 5H), 4.23-4.19 (m, 1H), 4.18-4.13 (m, 5H), 3.98 (dd, J = 9.4 Hz, 1H), 3.92 (d, J = 2.1 Hz, 1H), 3.85-3.78 (m, 3H), 3.66 (d, J = 2.7 Hz, 2H), 3.60 (dd, J = 11.7, 4.3 Hz, 1 H), 3.55-3.50 (m, 3H), 3.47 (dd, J = 10.2, 2.6, Hz, 1H), 3.43-3.38 (m, 3H), 3.32-3.24 (m, 3H), 3.16 (dd, J = 9.0, 4.4 Hz, 1H), 3.01 (dd, J = 8.3, 4.3 Hz, 1H), 2.11-2.04 (m, 2H), 1.68-1.62 (m, 2H), 1.22 (s, 9H), 1.15 (s, 9H); ¹³C NMR (100 MHz, CDCl₃): δ 176.8, 176.6, 165.0, 162.5, 139.1, 138.7, 138.3, 138.2, 138.0, 137.9, 137.7, 133.2, 131.2, 130.1, 130.0, 129.7, 128.7, 128.7, 128.6, 128.5, 128.5, 128.4, 128.3, 128.1, 128.1, 128.0, 127.9, 127.8, 127.6, 127.5, 127.0, 115.0, 99.7, 92.2, 81.1, 80.1, 77.4, 76.6, 75.5, 74.7, 73.8, 73.7, 73.5, 73.3, 72.5, 71.5, 69.1, 68.4, 67.2, 56.4, 53.2, 39.1, 38.9, 30.3, 29.9, 29.0, 27.8, 27.4, 11.2; ESI-MS: m/z (M + Na)⁺ calcd 2051.7434, obsd 2051.7404.

**n-Pentenyl 3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-3-O-benzoyl-6-O-benzyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-**
**glucopyranoside 84.** Method A: A solution of 83 (55 mg, 0.027 mmol) in THF (2 mL) was treated with water (5 μL, 0.27 mmol), followed by tri-\textit{n}-butyl phosphine (34 μL, 0.135 mmol) and stirred at room temperature. After 1 h, the reaction mixture was diluted with dichloromethane (10 mL) and washed with sat. aqueous NaHCO₃, brine, and water (20 mL each). The organic layer was dried over Na₂SO₄, filtered and concentrated. The crude residue was purified by flash silica gel chromatography (20% EtOAc/hexanes) to yield 7 mg (15%) of 84. [α]D: -33.08° (c = 1.10, CH₂Cl₂); IR (thin film): 3672, 3621, 3015, 2964, 1733 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.97 (app d, J = 7.1 Hz, 2H), 7.49 (app t, J = 7.5 Hz, 2H), 7.37-7.06 (m, 39H), 7.03 (app t, J = 7.5 Hz, 2H), 6.75 (d, J = 9.3 Hz, 1H), 5.82-5.74 (m, 1H), 5.36-5.28 (m, 2H), 5.05-4.90 (m, 7H), 4.74 (d, J = 11.8 Hz, 2H), 4.66-4.42 (m, 14H), 4.38-4.29 (m, 5H), 4.24-4.16 (m, 4H), 4.06 (s, 2H), 4.02-3.97 (m, 3H), 3.90-3.78 (m, 8H), 3.58-3.52 (m, 2H), 3.46-3.40 (m, 4H), 3.34-3.31 (m, 2H), 3.25 (dd, J = 7.1, 2.7 Hz, 1H), 3.20 (t, J = 9.1 Hz, 1H), 3.05 (dd, J = 8.6, 4.4 Hz, 1H), 2.74 (dd, J = 8.5, 4.5 Hz, 1H), 2.11-2.06 (m, 2H), 1.69-1.62 (m, 2H), 1.23 (s, 9H), 1.16 (s, 9H); ¹³C NMR (125 MHz, CDCl₃): δ 177.1, 176.8, 176.7, 166.5, 166.5, 164.4, 162.5, 139.1, 139.0, 139.0, 138.9, 138.3, 138.3, 138.2, 137.9, 137.8, 137.7, 133.5, 130.0, 129.8, 129.7, 129.7, 128.6, 128.6, 128.6, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.7, 127.5, 127.5, 127.1, 127.0, 115.0, 104.4, 101.4, 101.1, 100.6, 99.7, 99.5, 81.9, 81.0, 77.9, 77.4, 76.6, 76.6, 76.4, 75.6, 75.5, 75.5, 75.4, 75.4, 74.9, 74.9, 74.7, 74.6, 74.0, 73.9, 73.9, 73.7, 73.7, 73.4, 73.4, 72.5, 72.2, 72.2, 71.8, 71.8, 69.0, 68.5, 68.4, 68.3, 67.3, 66.5, 56.3, 55.3, 39.2, 39.1, 38.9, 30.2, 29.0, 27.8, 27.4, 27.1; ESI-MS: m/z (M + 2Na)²⁺ calcd 957.8452, obsd 957.8425.

Method B: A solution of 83 (219 mg, 0.107 mmol) in THF (3 mL) was treated with water (20 μL, 1.07 mmol), followed by methylidiphenyl phosphine (120 μL, 0.650 mmol) and stirred at room temperature. After 2 h, the reaction mixture was diluted with water (20 mL) and extracted with dichloromethane (3 × 20 mL). The combined organic extracts were dried over Na₂SO₄, filtered and concentrated. The crude residue was purified by flash silica gel chromatography (25→40% EtOAc/hexanes) to yield 81 mg (42%) of 84.

Tetrasaccharide acceptor 84 (17 mg, 0.0095 mmol) and fucosyl phosphate 70 (12 mg, 0.019 mmol) were coevaporated in toluene (3 x 1 mL), then dried under vacuum for 45 min. Dichloromethane (1 mL) was added and the solution was cooled to -78°C, then TMSOTf (3 μL, 0.0019 mmol) was added. The solution was warmed to -25°C over 50 min, then quenched by addition of TEA and allowed to warm to room temperature. The solution was concentrated and the crude residue was purified by flash silica gel chromatography (10% EtOAc/hexanes) to yield 16 mg (crude, 75%) of 85. [α]D: -53.45° (c = 1.56, CH2Cl2); IR (thin film): 3378, 3029, 2978, 2875, 1735 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.88 (app d, J = 7.2 Hz, 2H), 7.36-7.01 (m, 46H), 6.95 (app d, J = 6.5 Hz, 2H), 6.79 (d, J = 9.2 Hz, 1H), 5.84-5.74 (m, 1H), 5.62 (d, J = 3.4 Hz, 1H), 5.38-5.29 (m, 3H), 5.25-5.23 (m, 1H), 5.12 (dd, J = 10.9, 9.3 Hz, 1H), 4.98-4.89 (m, 5H), 4.77-4.57 (m, 8H), 4.53-4.43 (m, 6H), 4.41-4.26 (m, 10H), 4.22-4.16 (m, 2H), 4.12-4.08 (m, 1H), 4.06-3.94 (m, 5H), 3.87-3.78 (m, 4H), 3.75 (m, 1H), 3.71 (dd, J = 11.0, 4.5 Hz, 1H), 3.61-3.38 (m, 7H), 3.38-3.35 (m, 1H), 3.31 (dd, J = 9.1, 5.4 Hz, 2H), 3.24 (dd, J = 8.8, 4.9 Hz, 1H), 3.03 (t, J = 8.8 Hz, 1H), 2.14-2.05 (m, 2H), 1.71-1.63 (m, 2H), 1.26 (s, 9H), 1.18 (s, 9H), 1.16 (s, 9H), 1.14 (s, 9H), 1.11 (d, J = 6.5 Hz, 3H), ¹³C NMR (125 MHz, CDCl₃): δ 177.9, 177.7, 177.6, 177.0, 176.9, 167.0, 162.5, 139.1, 139.0, 138.9, 138.4, 138.3, 138.2, 138.0, 138.0, 137.8, 137.6, 132.9, 130.2, 130.1, 128.8, 128.7, 128.7, 128.6, 128.6, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 127.5, 127.4, 127.0, 126.5, 114.5, 101.4, 100.7, 100.7, 99.8, 97.7, 97.1, 92.3, 92.0, 84.0, 81.1, 78.4, 77.9, 77.4, 76.6, 76.2, 76.2, 75.6, 75.0, 74.9, 74.6, 73.9, 73.9, 73.7, 73.7, 73.6, 73.6, 73.4, 73.2, 73.1, 73.0, 73.0, 72.9, 72.5, 72.2, 71.8, 71.6, 71.3, 71.1, 70.5, 70.4, 70.3, 69.7, 69.0, 68.7, 68.5, 67.7, 67.5.
65.5, 65.1, 56.2, 39.3, 39.3, 39.2, 39.0, 38.9, 30.3, 29.1, 27.8, 27.5, 27.5, 27.4, 27.4, 16.4, 16.2, 15.8; ESI-MS: m/z (M + Na)^+ calcd 2296.9206, obsd 2296.9187.

**t-Butyldimethylsilyl 3,6-di-O-benzyl-2-deoxy-4-O-levulinyl-2-trichloroacetimido-β-D-glucopyranoside 86.** To a stirred solution of 78 (1.03g, 2.01 mmol) in CH₂Cl₂ (10 mL) was added 1 g activated 4 Å molecular sieves, benzyl bromide (0.60 mL, 5.02 mmol), and Ag₂O (1.40 g, 6.03 mmol). The flask was shielded from light and the solution stirred for 2 h. The solution was filtered through a pad of silica and the filtrate was concentrated *in vacuo*. The resulting residue was purified by flash silica gel chromatography (5→10% EtOAc/hexanes) to yield 1.01g (82%) of *tert*-butyldimethylsilyl 3-O-benzyl-4,6-O-benzylidene-2-deoxy-2-trichloroacetylaminom-α-D-glucopyranoside as a white solid. [α]_D: -21.03° (c = 0.64, CH₂Cl₂); IR (thin film): 3321, 2929, 2857, 1692, 1085 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.52-7.49 (m, 2H), 7.43-7.38 (m, 3H), 7.31-7.28 (m, 5H), 6.87 (d, J = 8.7 Hz, 1H), 5.60 (s, 1H), 5.18 (d, J = 7.9 Hz, 1H), 4.91 (d, J = 11.2 Hz, 1H), 4.70 (d, J = 11.2, 1H), 4.33 (dd, J = 5.0, 10.5 Hz, 1H), 4.25 (dd, J = 9.0, 10.2 Hz, 1H), 3.83 (t, J = 10.3 Hz, 1H), 3.77 (t, J = 9.2 Hz, 1H), 3.58-3.47 (m, 2H), 0.89 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 161.9, 138.1, 137.4, 129.3, 128.6, 128.5, 128.1, 126.2, 101.5, 95.2, 83.0, 75.8, 74.9, 68.9, 66.4, 61.2, 25.8, 18.0, -4.0, -5.0; ESI FT-MS: m/z (M+Na)^+ calcd 638.1270, obsd 638.1250.

A solution of 3-O-benzyl-4,6-O-benzylidene-2-deoxy-2-trichloroacetylaminom-α-D-glucopyranoside (0.62g, 1.0 mmol) in CH₂Cl₂ (10 mL) was cooled to 0°C and triethylsilane (0.96 mL, 6.0 mmol) was added. After 10 minutes trifluoroacetic acid anhydride (0.14 mL, 1.0 mmol) was added, followed by trifluoroacetic acid (0.39 mL, 5.0 mmol) dropwise over 10 minutes. The solution was allowed to warm slowly to room temperature over 2 h before dilution with EtOAc (100 mL) and washing with sat. aqueous NaHCO₃ (3 × 100 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated. The crude residue was purified by flash silica gel chromatography (10→25→40% EtOAc/hexanes) to yield *t*-butyldimethylsilyl 3,6-di-O-benzyl-2-deoxy-2-
trichloroacetimido-\(\beta\)-D-glucopyranoside (0.52g, 82%). \([\alpha]_D^0\): -10.28° (c = 0.92, CH\(_2\)Cl\(_2\)); IR (thin film): 3331, 3032, 2929, 1692, 1528 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.39-7.28 (m, 1OH), 6.89 (d, \(J = 7.8\) Hz, 1H), 5.09 (d, \(J = 7.9\) Hz, 1H), 4.79 (d, \(J = 11.2\) Hz, 1H), 4.76 (d, \(J = 11.2\), 1H), 4.62 (d, \(J = 12.0\) Hz, 1H), 4.58 (d, \(J = 12.0\) Hz, 1H), 3.99 (dd, \(J = 10.5, 8.5\) Hz, 1H), 3.80-3.72 (m, 3H), 3.57-3.53 (m, 2H), 3.47 (dt, \(J = 10.5, 7.9\) Hz, 1H), 0.89 (s, 9H), 0.13 (s, 3H), 0.10 (s, 3H); \(^13\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 161.8, 138.3, 137.8, 128.8, 128.7, 128.3, 128.2, 128.1, 127.9, 94.7, 92.7, 79.7, 77.9, 77.4, 74.7, 73.8, 73.8, 70.9, 60.5, 25.8, 18.1, -3.9, -5.0; ESI-MS: \(m/z\) (M+Na)\(^+\) calc 640.1426, obsd 640.1402.

A solution of \(\alpha\)-butyldimethylsilyl 3,6-di-O-benzyl-2-deoxy-2-trichloroacetimido-\(\beta\)-D-glucopyranoside (0.50g, 0.81 mmol) in CH\(_2\)Cl\(_2\) was cooled to 0°C and levulinic acid (86 \(\mu\)L, 0.85 mmol) and DMAP (0.11 g, 0.89 mmol) were added. After 10 minutes, DIPC (126 \(\mu\)L, 0.81 mmol) was added and the solution was left to warm to room temperature overnight. After 12 h the solution was concentrated to a yellow oil. The crude residue was purified by flash silica gel chromatography (10→25% EtOAc/Hexanes) to afford 0.52g (86%) of 86 as a clear oil. \([\alpha]_D^0\): +9.33° (c = 4.78, CH\(_2\)Cl\(_2\)); IR (thin film): 3333, 3033, 2929, 1747, 1719, 1694 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.31-7.20 (m, 1OH), 7.08 (d, \(J = 7.7\) Hz, 1H), 5.15 (d, \(J = 7.8\) Hz, 1H), 5.06 (app t, \(J = 9.2\) Hz, 1H), 4.63 (d, \(J = 11.1\), 1H), 4.57 (d, \(J = 11.1\) Hz, 1H), 4.50 (s, 2H), 4.25 (dd, \(J = 10.4, 9.1\) Hz, 1H), 3.68-3.63 (m, 1H), 3.57-3.44 (m, 3H), 2.58-2.53 (m, 2H), 2.41-2.24 (m, 2H), 2.09 (s, 3H), 0.87 (s, 9H), 0.12 (s, 3H), 0.09 (s, 3H); \(^13\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 206.4, 171.8, 161.9, 138.2, 137.9, 128.5, 128.4, 128.2, 128.0, 127.9, 127.8, 127.7, 94.3, 92.6, 77.4, 74.1, 73.6, 73.4, 72.0, 69.7, 61.8, 37.8, 29.9, 28.0, 25.8, 18.0, -4.0, -5.1; ESI-MS \(m/z\) (M+Na)\(^+\) calc 738.1794, obsd 738.1767.

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3,6-di-O-benzyl-2-deoxy-4-O-levulinyl-2-trichloroacetimido-\(\beta\)-D-glucopyranosyl

trichloroacetimidate 87. A solution of 86 (500 mg, 0.697 mmol) in THF (7 mL) was cooled to 0°C. To this solution were added TBAF (1.39 mL of a 1.0 M solution in THF)
and acetic acid (80 µL, 1.39 mmol) simultaneously dropwise. After 3 h, the solution was
diluted with water (50 mL) and extracted with CH₂Cl₂ (3 x 50 mL). The combined
organic extracts were washed once with sat. aqueous NaHCO₃ (75 mL), dried over
Na₂SO₄, filtered, and concentrated to yield 3,6-di-O-benzyl-2-deoxy-4-O-levulinyl-2-
trichloroacetimido-β-D-glucopyranoside (415 mg), which was used crude without further
purification.

3,6-di-O-benzyl-2-deoxy-4-O-levulinyl-2-trichloroacetimido-β-D-
glucopyranoside (415 mg, 0.688 mmol) was dissolved in CH₂Cl₂ (5 mL) and
trichloroacetonitrile (2 mL) and DBU was added (10 µL, 0.0688 mmol). After 2 h, the
solution was concentrated in vacuo and the crude brown residue was purified by flash
silica gel chromatography (25% EtOAc/hexanes) to give 87 (448 mg, 86 % for the two
steps) as a yellow foam. [α]D: +73.23° (c = 2.28, CH₂Cl₂); IR (thin film): 3416, 3339,
1749, 1718, 1677 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.76, (s, 1H), 7.36-7.28 (m, 10H),
6.56 (d, J = 8.4 Hz, 1H), 6.49 (d, J = 3.5 Hz, 1H), 5.44 (app t, J = 9.7 Hz, 1H), 4.70 (d, J
= 11.3 Hz, 1H), 4.66 (d, J = 11.3 Hz, 1H), 4.54 (d, J = 11.8 Hz, 1H), 4.50 (d, J = 11.8 Hz,
1H), 4.48-4.43 (m, 1H), 4.10-4.02 (m, 2H), 3.64 (dd, J = 11.0, 3.2 Hz, 1H), 3.59 (dd, J =
11.0, 4.1 Hz, 1H), 2.70-2.66 (m, 2H), 2.46 (app t, J = 6.3 Hz, 2H), 2.15 (s, 3H); ¹³C NMR
(100 MHz, CDCl₃): δ 206.4, 171.5, 161.9, 160.1, 137.9, 137.3, 128.9, 128.5, 128.3,
128.2, 127.9, 94.5, 92.5, 91.1, 75.9, 73.7, 73.2, 72.3, 70.2, 68.4, 53.6, 37.9, 30.0,
28.0; ESI-MS: m/z (M+Na)+ calcd 767.0031, obsd 767.0068.

Dibutyl 3,6-di-O-benzyl-2-deoxy-4-O-levulinyl-2-trichloroacetimido-β-D-
glucopyranosyl phosphate 88. 87 (428 mg, 0.573 mmol) was azeotroped with toluene
(3 x 3 mL) and then was dried under vacuum for 1 h. The dried residue was dissolved in
toluene (6 mL) and cooled to 0°C. Dibutyl phosphate (125 µL, 0.603 mmol) was added
dropwise to the solution over 5 minutes and the solution was left to slowly warm to room
temperature. After 2 h, the solution was concentrated and the crude residue was purified
by flash silica gel chromatography (40% EtOAc/hexanes) furnishing 88 (642 mg, 95%)
as a white foam. \([\alpha]_D^0: +56.13^\circ\) (c = 2.01, CH\(_2\)Cl\(_2\)); IR (thin film): 3329, 3245, 2961, 1748, 1718, 1697 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 8.13\) (d, \(J = 8.1\) Hz, 1H), 7.34-7.23 (m, 10H), 5.56 (t, \(J = 7.8\) Hz, 1H), 5.16 (app t, \(J = 9.3\) Hz, 1H), 4.71 (d, \(J = 11.2\) Hz, 1H), 4.58 (d, \(J = 11.2\) Hz, 1H), 4.51 (d, \(J = 12.8\) Hz, 1H), 4.48 (d, \(J = 12.4\) Hz, 1H), 4.21-3.99 (m, 6H), 3.75-3.72 (m, 1H), 3.60 (dd, \(J = 10.8, 3.0\) Hz, 1H), 3.55 (d, \(J = 10.8, 5.6\) Hz, 1H), 2.65 (ddd, \(J = 18.5, 7.9, 6.0\) Hz, 1H), 2.53 (dt, \(J = 18.5, 6.1\) Hz, 1H), 2.34 (ddd, \(J = 17.1, 7.9, 5.7\) Hz, 1H), 2.21 (dt, \(J = 17.3, 6.2\) Hz, 1H), 2.13 (s, 3H), 1.68-1.55 (m, 4H), 1.44-1.30 (m, 4H), 0.92 (t, \(J = 7.4\) Hz, 3H), 0.88 (t, \(J = 7.3\) Hz, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 206.3, 171.7, 162.5, 138.1, 137.9, 128.5, 128.2, 127.9, 127.9, 127.8, 96.5, 92.8, 78.6, 74.4, 73.8, 73.7, 70.4, 69.2, 68.9, 68.8, 68.6, 68.5, 57.1, 37.9, 32.3, 32.2, 32.0, 30.0, 27.9, 18.9, 18.8, 13.9, 13.8; \(^{31}\)P NMR (120 MHz, CDCl\(_3\)) \(\delta -1.989\); ESI-MS: \(m/z\) (M+N\(^+\)) \(\text{calcd} 816.1850\), \(\text{obsd} 816.1868\).

**Dibutyl 2-O-benzoyl-3,4,6-tri-O-benzyl-\(\beta\)-D-galactopyranosyl phosphate 91.** Tri-\(\beta\)-benzyl-D-galactal (995 mg, 2.39 mmol) was azeotroped with toluene (3×3 mL) and then dried under vacuum for 1 h. Dichloromethane (20 mL) was added and the solution was cooled to 0°C before a solution of dimethyldioxirane in acetone (50 mL, 0.08 M) was added. The reaction mixture was stirred for 15 min at 0°C, then the volatiles were removed *in vacuo*. The residue was dried under vacuum 5 minutes at 0°C, then 20 mL CH\(_2\)Cl\(_2\) were added and the reaction mixture was cooled to -78°C and stirred for 10 min. To the reaction vessel was added a solution of dibutyl phosphate (570 \(\mu\)L, 2.87 mmol) in CH\(_2\)Cl\(_2\) (10 mL) via cannula and the reaction mixture was warmed to 0°C after 5 min. DMAP (1.17 g, 9.56 mmol) and benzoyl chloride (0.55 mL, 4.78 mmol) were added and the solution was left to warm to room temperature over 1 h. The reaction mixture was diluted with a solution of 25% EtOAc/hexanes (50 mL), filtered and concentrated. The crude product was purified by flash silica column chromatography (25→40% EtOAc/hexanes) to afford of 91 (1.28 mg, 73% yield). \([\alpha]_D^0: +39.88^\circ\) (c =2.13, CH\(_2\)Cl\(_2\)); IR (thin film) 3337, 2961, 1732, 1268, 1028 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 8.04\).
(app d, J = 7.1 Hz, 2H), 7.59 (app t, J = 7.5 Hz, 1H), 7.45 (app t, J = 7.9 Hz, 2H), 7.39-7.15 (m, 15H), 5.74 (dd, J = 10.1, 8.1 Hz, 1H), 5.32 (app t, J = 7.6 Hz, 1H), 5.01 (d, J = 11.5 Hz, 1H), 4.67-4.64 (m, 2H), 4.49 (d, J = 12.4 Hz, 1H), 4.46 (s, 2H), 4.05-3.95 (m, 3H), 3.78-3.60 (m, 6H), 1.59-1.54 (m, 2H), 1.35-1.22 (m, 4H), 1.03-0.97 (m, 2H), 0.87 (t, J = 7.4 Hz, 3H), 0.67 (t, J = 7.3 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 165.5, 138.5, 137.9, 137.5, 133.4, 130.1, 130.0, 128.7, 128.6, 128.5, 128.1, 128.0, 128.0, 127.9, 97.3, 79.5, 74.9, 74.5, 73.8, 72.5, 72.0, 71.8, 71.7, 68.1, 68.1, 68.0, 68.0, 67.9, 32.2, 32.2, 32.0, 31.9, 187, 18.4, 13.8, 13.6; $^{31}$P NMR (120 MHz, CDCl$_3$): δ -2.04; ESI-MS: m/z (M+Na)$^+$ calc 769.3112, obsd 769.3116.

**n-Pentenyl 3,6-di-O-benzyl-4-O-levulinyl-2-deoxy-2-trichloroacetimidob-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-D-glucopyranoside 89.** Lactose acceptor 49 (66 mg, 0.070 mmol) and glucosamine phosphate 88 (167 mg, 0.211 mmol) were coevaporated in toluene (3 × 1 mL), then dried under vacuum for 45 min. Dichloromethane was added (2 mL) and the solution was cooled to -78°C. TMSOTf (39 μL, 0.211 mmol) was added and the reaction was stirred at -78°C for 30 min, until the lactose was nearly consumed. Triethylamine (200 μL) was added to quench the mixture and the solution was slowly warmed to room temperature. The solution was concentrated, and the crude product purified by flash silica column chromatography (25% EtOAc/hexanes) to afford 89 (121 mg, 95% yield). [α]$_D^2$: -8.75° (c = 0.91 CH$_2$Cl$_2$); IR (thin film): 3362, 3031, 2963, 1742, 1718 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): δ 7.37-7.22 (m, 25H), 7.17 (app d, J = 7.0 Hz, 2H), 7.11 (app t, J = 7.3 Hz, 1H), 7.03 (app t, J = 7.6 Hz, 2H), 6.65 (d, J = 7.9 Hz, 1H), 5.82-5.74 (m, 1H), 5.30 (dd, J = 9.7, 7.8 Hz, 1H), 5.12 (t, J = 8.9 Hz, 1H), 5.03-4.93 (m, 4H), 4.88 (d, J = 11.5 Hz, 1H), 4.81 (d, J = 7.9 Hz, 1H), 4.72 (d, J = 12.0 Hz, 1H), 4.63 (d, J = 11.1 Hz, 1H), 4.57 (d, J = 11.1 Hz, 1H), 4.52 (s, 2H), 4.49-4.44 (m, 2H), 4.35-4.30 (m, 3H), 4.20 (d, J = 11.7 Hz, 1H), 4.09 (app t, J = 10.2 Hz, 1H), 4.03 (t, J = 8.8 Hz, 1H), 3.96-3.91 (m, 2H, 3.84 (dt, J = 9.3, 6.2 Hz, 1H), 3.69-3.50 (m, 8H), 3.46-
3.31 (m, 5H), 2.71-2.55 (m, 2H), 2.45 (dd, J = 17.4, 7.7, 5.6 Hz, 1H), 2.32 (dt, J = 17.4, 6.4 Hz, 1H), 2.15 (s, 3H), 2.12-2.05 (m, 2H), 1.69-1.62 (m, 2H), 1.22 (s, 9H), 1.17 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 206.4, 176.8, 176.7, 171.8, 162.1, 139.2, 138.9, 138.3, 138.2, 138.1, 137.9, 137.7, 128.7, 128.6, 128.6, 128.8, 128.3, 128.3, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.6, 127.1, 115.0, 101.4, 99.6, 98.8, 92.5, 81.3, 81.0, 77.4, 76.5, 75.6, 75.4, 74.7, 74.6, 74.0, 73.8, 73.7, 73.7, 73.6, 73.3, 72.4, 71.7, 69.5, 69.1, 68.4, 68.2, 58.5, 39.0, 38.9, 37.9, 30.3, 30.3, 29.0, 28.0, 27.6, 27.4; ESI-MS: m/z (M + Na)$^+$ calcd 1544.5640, obsd 1544.5669.

**n-Pentenyl 3,6-di-O-benzyl-2-deoxy-2-trichloroacetimidophenol-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 90.** A solution of hydrazine acetate (8 mg, 0.105 mmol) in MeOH (0.2 mL) was added to a solution of 89 (121 mg, 0.070 mmol) in CH$_2$Cl$_2$ (2 mL) and the resulting solution was stirred overnight at room temperature. After 12 h, the reaction mixture was diluted with CH$_2$Cl$_2$ (5 mL) and concentrated *in vacuo*. The crude product was purified by flash silica gel chromatography (25% EtOAc/hexanes) to yield 103 mg (95%) of 90. [α]$_D$ = -27.83° (c = 1.00, CH$_2$Cl$_2$); IR (thin film): 3424, 2870, 1741, 1720, 1065 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): δ 7.38-7.21 (m, 25H), 7.17-7.10 (m, 3H), 7.03 (app t, J = 7.5 Hz, 1H), 6.65 (d, J = 7.6 Hz, 1H), 5.82-5.74 (m, 1H), 5.30 (dd, J = 9.9, 7.9 Hz, 1H), 5.03-4.90 (m, 5H), 4.78 (d, J = 11.3 Hz, 1H), 4.75-4.72 (m, 2H), 4.66-4.61 (m, 2H), 4.57 (d, J = 12.0 Hz, 1H), 4.49-4.45 (m, 3H), 4.36-4.33 (m, 2H), 4.30 (d, J = 11.7 Hz, 1H), 4.19 (d, J = 11.7 Hz, 1H), 4.04 (app t, J = 9.4 Hz, 1H), 3.92 (dd, J = 10.0, 3.1 Hz, 1H), 3.87-3.65 (m, 10H), 3.55 (app t, J = 9.0 Hz, 1H), 3.54-3.39 (m, 4H), 3.36-3.30 (m, 2H), 2.76 (br s, 1H), 2.11-2.05 (m, 2H), 1.69-1.61 (m, 2H), 1.20 (s, 9H), 1.17 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 177.0, 176.8, 162.3, 139.1, 138.9, 138.3, 138.2, 138.0, 137.5, 128.8, 128.6, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.5, 127.3, 115.0, 101.4, 99.5, 99.2, 92.7, 81.0, 80.5, 77.4, 76.6, 76.4, 75.5, 75.5, 74.7, 74.6, 74.5, 74.4, 74.0, 73.8, 73.7, 73.6, 73.4, 72.4, 70.5, 69.8, 69.1, 68.4,
n-Pentenyl 2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 92. Trisaccharide acceptor 90 (29 mg, 0.020 mmol) and galactosyl phosphate 91 (30 mg, 0.041 mmol) were coevaporated in toluene (3 × 1 mL), then dried under vacuum for 1 h. Dichloromethane (1.3 mL) was added and the solution was cooled to −65°C. TMSOTf (7 μL, 0.041 mmol) was added and the reaction mixture was stirred for 20 min at −65°C. The reaction mixture was quenched by addition of TEA and slowly warmed to room temperature. The solution was concentrated and the crude residue was purified by flash silica gel chromatography (10→25% EtOAc/hexanes) to yield 37 mg (93%) of tetrasaccharide 92. [α]D: -1.63° (c = 0.97, CHCl₃); IR (thin film): 3367, 3031, 2870, 1734, 1095 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.96 (app d, J = 7.2 Hz, 2H), 7.54 (app t, J = 7.3 Hz, 1H), 7.44 (app t, J = 7.8 Hz, 2H), 7.36-7.08 (m, 43H), 7.02 (app t, J = 7.5 Hz, 2H), 6.71 (d, J = 8.6 Hz, 1H), 5.82-5.74 (m, 1H), 5.60 (dd, J = 10.0, 8.0 Hz, 1H), 5.28 (dd, J = 10.1, 7.9 Hz, 1H), 5.02-4.92 (m, 6H), 4.89 (d, J = 11.0 Hz, 1H), 4.72-4.55 (m, 7H), 4.51-4.39 (m, 6H), 4.36-4.23 (m, 6H), 4.12 (d, J = 11.8 Hz, 1H), 4.02-3.92 (m, 3H), 3.86-3.79 (m, 3H), 3.72-3.64 (m, 4H), 3.60-3.62 (m, 8H), 3.32-3.29 (m, 5H), 3.27-3.25 (m, 1H), 2.11-2.04 (m, 2H), 1.68-1.61 (m, 2H), 1.18 (s, 9H), 1.16 (s, 9H); ¹³C NMR (100 MHz, CDCl₃): δ 176.8, 176.7, 165.0, 162.0, 139.1, 139.0, 138.8, 138.3, 138.3, 138.2, 138.0, 138.0, 137.8, 133.5, 130.0, 130.0, 128.7, 128.7, 128.6, 128.6, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.4, 127.0, 115.0, 101.4, 101.0, 99.8, 92.6, 81.1, 79.7, 78.7, 77.9, 77.4, 76.6, 75.6, 75.5, 75.3, 74.9, 74.8, 74.7, 74.1, 73.8, 73.7, 73.7, 73.6, 73.6, 73.5, 72.7, 72.6, 72.6, 72.4, 71.5, 69.1, 68.4, 68.3, 68.2, 68.0, 57.1, 39.0, 38.9, 30.2, 29.0, 28.3, 27.7, 27.3; ESI-MS: m/z (M + Na)⁺ calcd 1980.7678, obsd 1980.7646.
n-Pentenyl 3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-3-O-benzoyl-6-O-benzyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 93. A solution of tetrasaccharide 92 (49 mg, 0.025 mmol) in methanol (1.5 mL) was treated with sodium methoxide (30 μL of a 25% by weight solution, 0.126 mmol) at room temperature. After 48 h, the reaction mixture was diluted with methanol (10 mL) acidified to pH 6 by addition of amberlite acidic gel resin. The solution was filtered and the filtrate concentrated. The crude residue was purified by flash silica gel chromatography (25% EtOAc/hexanes) to yield 40 mg (86%) of 93. [α]D: -72.39° (c = 1.09, CH2Cl2); IR (thin film): 3429, 2918, 2870, 1740, 1063 cm⁻¹; 1H NMR (400 MHz, CDCl₃): δ 7.40-7.09 (m, 43H), 7.02 (app t, J = 7.5 Hz, 2H), 6.64 (d, J = 8.3 Hz, 1H), 5.82-5.74 (m, 1H), 5.31 (dd, J = 9.9, 8.0 Hz, 1H), 5.02-4.85 (m, 7H), 4.73-4.70 (m, 3H), 4.63-4.53 (m, 6H), 4.49-4.44 (m, 4H), 4.34-4.28 (m, 4H), 4.24 (d, J = 11.7 Hz, 1H), 4.17 (d, J = 11.6 Hz, 1H), 4.11 (t, J = 8.5 Hz, 1H), 4.02 (t, J = 9.3 Hz, 1H), 3.98-3.93 (m, 1H), 3.91-3.80 (m, 7H), 3.73-3.69 (m, 3H), 3.60-3.53 (m, 2H), 3.51-3.24 (m, 11H), 2.78 (br s, 1H), 2.11-2.04 (m, 2H), 1.69-1.61 (m, 2H), 1.19 (s, 9H), 1.17 (s, 9H); 13C NMR (125 MHz, CDCl₃): δ 176.8, 176.8, 162.0, 139.1, 139.0, 138.9, 138.3, 138.2, 138.2, 138.0, 137.8, 128.8, 128.7, 128.6, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.7, 127.5, 127.1, 115.0, 103.4, 101.4, 99.7, 99.4, 92.6, 82.1, 81.1, 79.8, 79.1, 77.4, 76.7, 76.6, 76.4, 75.5, 75.5, 75.3, 74.8, 74.7, 74.2, 73.8, 73.8, 73.7, 73.6, 73.5, 72.4, 72.3, 72.0, 69.1, 68.4, 68.0, 68.1, 58.0, 39.2, 39.1, 38.9, 30.3, 29.9, 29.0, 27.7, 27.4; ESI-MS: m/z (M + Na)⁺ calcd 1878.7516, obsd 1878.7564.
n-Pentenyl 2-O-benzyl-3,4-di-O-pivaloyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetimidod-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 94. Tetrasaccharide acceptor 93 (40 mg, 0.0217 mmol) and fucosyl phosphate 70 (27 mg, 0.0434 mmol) were coevaporated in toluene (3 × 1 mL), then dried under vacuum for 1 h. Dichloromethane (1.5 mL) was added and the solution was cooled to −78°C, then TMSOTf (8 μL, 0.0434 mmol) was added. After 20 minutes, then quenched by addition of TEA and allowed to warm to room temperature. The solution was concentrated and the crude residue was purified by flash silica gel chromatography (17→25→40% EtOAc/hexanes) to yield 34 mg (2:1 α/β, 70%) of 94. 1H NMR (400 MHz, CDCl3): δ 7.41 (app d, J = 7.1 Hz, 1H), 7.36–7.12 (m, 44H), 7.10–6.95 (m, 5H), 6.69 (app t, J = 7.7 Hz, 1H), 5.82–5.74 (m, 1H), 5.70 (d, J = 3.4 Hz, 0.6H), 5.38–5.31 (m, 2H), 5.25 (d, J = 2.2 Hz, 0.6H), 5.19 (d, J = 3.0 Hz, 0.4 H), 5.07–4.89 (m, 8H), 4.82–4.79 (m, 3H), 4.74–4.62 (m, 4H), 4.58–4.26 (m, 16H), 4.18–4.12 (m, 2H), 4.06–3.63 (m, 12H), 3.58–3.29 (m, 13H), 2.11–2.05 (m, 2H), 1.69–1.62 (m, 2H), 1.23 (s, 3H), 1.21 (s, 5H), 1.18 (s, 6H), 1.17 (s, 4H), 1.17 (s, 5H), 1.16 (s, 4H), 1.16 (s, 6H), 1.10 (s, 3H).

n-Pentenyl 2-O-benzyl-3,4-di-O-acetyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-trichloroacetimidod-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 96. Tetrasaccharide acceptor 93 (13 mg, 0.007 mmol) and fucosyl trichloroacetimidate 95 (8 mg, 0.0175 mmol) were coevaporated in toluene (3 × 1 mL), then dried under vacuum for 1 h. Dichloromethane
(1 mL) was added and the solution was cooled to -40°C, then TMSOTf (1 μL, 0.0018 mmol) was added. After 20 minutes the reaction mixture warmed to -10°C, then was quenched by addition of TEA and allowed to warm to room temperature. The solution was concentrated and the crude residue was purified by size exclusion gel chromatography using Bio-Rad S-X3 beads (10% EtOAc/toluene) to yield 16 mg (1:1 α/β, 95%) of 96. 1H NMR (400 MHz, CDCl₃): δ 7.46-6.98 (m, 45H), 6.68 (d, J = 7.9 Hz, 1H), 5.82-5.74 (m, 1H), 5.73 (d, J = 3.6 Hz, 1H), 5.37-5.31 (m, 1H), 5.24 (d, J = 2.3 Hz, 1H), 5.02-4.90 (m, 7H), 4.82-4.77 (m, 3H), 4.74-4.69 (m, 3H), 4.63-4.60 (m, 1H), 4.55-4.42 (m, 10H), 4.38-4.23 (m, 8H), 4.17-4.12 (m, 2H), 4.12-3.98 (m, 3H), 3.96-3.91 (m, 3H), 3.87-3.79 (m, 4H), 3.75-3.68 (m, 2H), 3.57-3.34 (m, 13H), 3.29 (dd, J = 9.1, 5.5 Hz, 1H), 2.12-2.04 (m, 2H), 2.11 (s, 3H), 1.97 (s, 3H), 1.68-1.61 (m, 2H), 1.20 (s, 9H), 1.16 (s, 9H), 1.13 (d, J = 6.6 Hz, 3H).

\[ \text{tu-Butyldimethylsilyl} \quad 4-O\text{-benzoyl-6-O-benzyl-2-deoxy-3-O-levulinyl-2-trichloroacetimido-}\beta-D\text{-glucopyranoside 97.} \]

Levulinic acid (410 mg, 3.70 mmol) and DMAP (452 mg, 3.53 mmol) were dissolved in 15 mL CH₂Cl₂ and cooled to 0°C. After 10 minutes, DIPC (0.53 mL, 3.36 mmol) was added with vigorous stirring. After 5 additional minutes, a solution of 78 (1.76 g, 3.36 mmol) in 10 mL CH₂Cl₂ was added to the levulinic acid solution via cannula and the mixture was left to slowly warm to room temperature. After 12 hours, the reaction mixture was diluted with EtOAc and flushed through a plug of silica gel. The filtrate was concentrated and the residue was purified by flash silica gel chromatography (10 → 25% EtOAc/hexanes) to afford 1.76 g (84%) of tu-butyldimethylsilyl 4,6-O-benzylidene-2-deoxy-3-O-levulinyl-2-trichloroacetimido-β-D-glucopyranoside as a clear oil. Characterization data consistent with published data.⁵²

A solution of dry tu-butyldimethylsilyl 4,6-O-benzylidene-2-deoxy-3-O-levulinyl-2-trichloroacetimido-β-D-glucopyranoside (1.76 g, 2.82 mmol) in CH₂Cl₂ (20 mL) was treated with triethylsilane (2.70 mL, 16.9 mmol) and cooled to 0 °C. After 10 min, trifluoroacetic acid anhydride (0.4 mL, 2.82 mmol) and trifluoroacetic acid (1.08 mL, 14.1 mmol) was added slowly dropwise to the solution. The reaction mixture was left to
slowly warm to room temperature and after 1 h was diluted with CH$_2$Cl$_2$ (50 mL) and washed with sat. aqueous NaHCO$_3$ (2 × 100 mL) and with water (100 mL). The organic layer was dried over Na$_2$SO$_4$, filtered and concentrated. The crude residue was purified by flash silica gel chromatography (40 % EtOAc/hexanes) to give $t$-butyldimethylsilyl 6-O-benzyl-2-deoxy-3-O-levulinyl-2-trichloroacetimidido-$\beta$-D-glucopyranoside (1.47 g, 83%). Characterization was consistent with published data.$^{52}$

A solution of $t$-butyldimethylsilyl 6-O-benzyl-2-deoxy-3-O-levulinyl-2-trichloroacetimidido-$\beta$-D-glucopyranoside (1.47 g, 2.35 mmol) in CH$_2$Cl$_2$ (20 mL) was treated with DMAP (0.574 g, 4.70 mmol), followed by benzoyl chloride (0.40 mL, 3.52 mmol). After 12 h, the reaction mixture was diluted with 100 mL of a 25% EtOAc/hexanes solution and filtered through a pad of silica. The filtrate was concentrated and the crude residue was purified by flash silica gel chromatography (25%) to EtOAc/hexanes yield 97 (1.72 g, quant. yield) as a white solid. Characterization data was consistent with published data.$^{52}$

![Diagram of 4-O-benzoyl-6-O-benzyl-2-deoxy-3-O-levulinyl-2-trichloroacetimidato-$\beta$-D-glucopyranosyl trichloroacetimidate](image)

4-O-benzoyl-6-O-benzyl-2-deoxy-3-O-levulinyl-2-trichloroacetimidato-$\beta$-D-glucopyranosyl trichloroacetimidate 98. A solution of 97 (1.72 g, 2.35 mmol) in THF (20 mL) was cooled to 0°C. To this solution were added TBAF (2.6 mL of a 1.0 M solution in THF) and acetic acid (150 µL, 2.60 mmol) simultaneously dropwise. After 90 min, the solution was diluted with water (70 mL) and extracted with CH$_2$Cl$_2$ (3 × 50 mL). The combined organic extracts were washed once with sat. aqueous NaHCO$_3$ (100 mL), dried over Na$_2$SO$_4$, filtered, and concentrated to yield 4-O-benzoyl-6-O-benzyl-2-deoxy-3-O-levulinyl-2-trichloroacetimidido-$\beta$-D-glucopyranoside (1.32 g, 91%) as a yellow foam, which was used without further purification.

4-O-benzoyl-6-O-benzyl-2-deoxy-3-O-levulinyl-2-trichloroacetimidido-$\beta$-D-glucopyranoside (1.32 g, 2.15 mmol) was azeotroped with toluene (3 × 3 mL), then dried under vacuum for 1 h. The residue was dissolved in CH$_2$Cl$_2$ (10 mL) and trichloroacetonitrile (10 mL) and DBU was added (32 µL, 0.215 mmol). After 10 min,
the solution was concentrated in vacuo and the crude residue was purified by flash silica gel chromatography (10→15→25% EtOAc/hexanes) to give 98 (1.08, 66%) as a yellow foam. Characterization data was consistent with published data.\textsuperscript{52}

\begin{center}
\includegraphics[width=0.5\textwidth]{n-Pentenyl_4-O-benzoyl-6-O-benzyl-3-O-levulinyl-2-deoxy-2-trichloracetimido-beta-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-beta-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-beta-D-glucopyranoside_99.png}
\end{center}

\textit{n}-Pentenyl 4\textit{O}-benzoyl-6\textit{O}-benzyl-3\textit{O}-levulinyl-2-deoxy-2-trichloracetimido-\beta-\textit{D}-glucopyranosyl-(1→3)-4,6-di\textit{O}-benzyl-2\textit{O}-pivaloyl-\β-\textit{D}-galactopyranosyl-(1→4)-3,6-di\textit{O}-benzyl-2\textit{O}-pivaloyl-\β-\textit{D}-glucopyranoside 99. Lactose acceptor 49 (20 mg, 0.0212 mmol) and glucosamine imidate 98 (32 mg, 0.0424 mmol) were coevaporated in toluene (3 × 1 mL), then dried under vacuum for 2 h. Dichloromethane was added (1.5 mL) and the solution was cooled to −30°C. TMSOTf (1 μL, 0.0064 mmol) was added and reaction was allowed to warm to 0°C over 30 min. The reaction mixture was quenched with TEA and the solution was slowly warmed to room temperature. The solution was concentrated, and the crude product purified by flash silica column chromatography (25% EtOAc/hexanes) to afford 99 (27 mg, 80%). [α]_D:\textsuperscript{20} -28.42° (c = 1.19, CH\textsubscript{2}Cl\textsubscript{2}); IR (thin film): 3346, 3031, 2971, 1735, 1066 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): δ 7.97 (app t, J = 7.1 Hz, 2H), 7.60 (app t, J = 7.4 Hz, 1H), 7.46 (app t, J = 7.9 Hz, 2H), 7.34-7.10 (m, 23H), 7.04 (app t, J = 7.5 Hz, 1H), 6.69 (d, J = 8.7 Hz, 1H), 5.82-5.74 (m, 1H), 5.39-5.28 (m, 3H), 5.03-4.93 (m, 5H), 4.73 (d, J = 4.7 Hz, 1H), 4.71 (s, 1H), 4.53-4.43 (m, 5H), 4.35 (d, J = 8.0 Hz, 2H), 4.32 (d, J = 11.9 Hz, 1H), 4.20 (d, J = 11.7 Hz, 1H), 4.07-4.00 (m, 2H), 3.94-3.91 (m, 2H), 3.86-3.79 (m, 2H), 3.70-3.63 (m, 4H), 3.56 (t, J = 9.0 Hz, 1H), 3.48-3.40 (m, 3H), 3.36-3.31 (m, 2H), 2.57 (app t, J = 6.5 Hz, 2H), 2.43 (app t, J = 6.5 Hz, 2H), 2.13-2.03 (m, 2H), 1.69-1.63 (m, 2H), 1.25 (s, 9H), 1.17 (s, 9H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): δ 205.8, 176.9, 176.8, 172.7, 165.5, 162.4, 139.0, 139.0, 138.3, 138.2, 138.2, 137.5, 133.8, 130.1, 129.2, 128.7, 128.7, 128.6, 128.6, 128.6, 128.3, 128.1, 128.0, 127.9, 127.6, 127.1, 115.0, 101.4, 100.0, 99.6, 92.3, 81.0, 77.4, 76.5, 75.6, 74.8, 74.6, 74.5, 73.9, 73.8, 73.5, 72.4, 72.1, 69.9, 69.4, 69.1, 68.4, 68.3, 56.7, 39.1, 38.9, 38.0, 30.3, 29.6, 29.0, 28.2, 27.8, 27.4; ESI-MS: m/z (M + Na)+ calcd 1558.5433, obsd 1558.5437.
n-Pentenyl 4-O-benzoyl-6-O-benzyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 100. A solution of hydrazine acetate (5 mg, 0.070 mmol) in MeOH (0.2 mL) was added to a solution of 99 (76 mg, 0.050 mmol) in CH₂Cl₂ (2 mL) and the resulting solution was stirred overnight at room temperature. The reaction mixture was diluted with CH₂Cl₂ (5 mL) and concentrated in vacuo. The crude product was purified by flash silica gel chromatography (30% EtOAc/hexanes) to yield 69 mg (97%) of 100. [α]D: -16.84° (c = 0.40, CH₂Cl₂); IR (thin film): 3367, 2918, 1734, 1718, 1059 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.05 (app t, J = 7.2 Hz, 2H), 7.63 (app t, J = 7.4 Hz, 1H), 7.48 (app t, J = 7.9 Hz, 3H), 7.38-7.04 (m, 25H), 5.89-5.77 (m, 1H), 5.34-5.30 (m, 1H), 5.21 (t, J = 9.4 Hz, 1H), 5.06-4.96 (m, 4H), 4.89 (d, J = 11.4 Hz, 1H), 4.77 (d, J = 12.1 Hz, 1H), 4.65 (d, J = 8.1 Hz, 1H), 4.58-4.48 (m, 6H), 4.43-4.35 (m, 3H), 4.26 (d, J = 11.7 Hz, 1H), 4.10-3.97 (m, 5H), 3.87 (dt, J = 9.6, 6.2 Hz, 1H), 3.80-3.57 (m, 8H), 3.52-3.36 (m, 5H), 2.25-2.05 (m, 2H), 1.72-1.67 (m, 2H), 1.26 (s, 9H), 1.20 (s, 9H); ¹³C NMR (100 MHz, CDCl₃): δ 177.7, 176.8, 171.4, 166.3, 164.3, 162.5, 154.9, 138.9, 138.8, 138.3, 138.1, 137.8, 137.7, 137.5, 136.8, 133.7, 133.5, 130.1, 129.4, 129.3, 129.0, 128.9, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.9, 127.7, 127.1, 121.0, 115.0, 102.5, 101.4, 99.3, 99.1, 98.2, 98.0, 92.5, 92.2, 80.9, 77.4, 77.0, 76.7, 76.1, 75.6, 75.4, 74.7, 74.5, 74.4, 74.0, 73.9, 73.8, 73.7, 73.7, 73.5, 73.4, 73.0, 72.3, 71.7, 71.6, 71.2, 69.8, 69.3, 69.1, 68.2, 68.0, 67.3, 60.6, 59.9, 39.1, 38.9, 30.2, 29.5, 28.9, 28.3, 27.6, 27.3, 27.1, 22.6, 21.3, 14.4, 14.2; ESI-MS: m/z (M + Na)⁺ calcd 1460.5065, obsd 1460.5060.

n-Pentenyl 2-O-benzyl-3,4-di-O-pivaloyl-α-L-fucopyranosyl-(1→3)-4-O-benzoyl-6-O-benzyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-
2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 101. Trisaccharide acceptor 100 (66 mg, 0.045 mmol) and fucosyl phosphate 70 (56 mg, 0.091 mmol) were coevaporated in toluene (3 × 2 mL), then dried under vacuum for 2 h. Dichloromethane (2 mL) was added and the solution was cooled to -60°C. TMSOTf (10 μL, 0.59 mmol) was added and the reaction mixture was allowed to warm to -30°C over 30 min. The reaction mixture was quenched by addition of TEA and slowly warmed to room temperature. The solution was concentrated and the crude residue was purified by flash silica gel chromatography (10→20% EtOAc/hexanes) to yield 62 mg (83%) of 101. [α]D: -27.64° (c = 1.25, CH2Cl2); IR (thin film): 3351, 3031, 2973, 1736, 1094 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.92 (app d, J = 7.5 Hz, 2H), 7.58 (app t, J = 7.4 Hz, 1H), 7.43 (app t, J = 7.9 Hz, 2H), 7.37-7.10 (m, 33H), 7.04 (app t, J = 7.1 Hz, 2H), 6.78 (d, J = 7.2 Hz, 1H), 5.82-5.73 (m, 1H), 5.34-5.30 (m, 1H), 5.25 (app t, J = 9.6 Hz, 1H), 5.21 (dd, J = 10.7, 3.1 Hz, 1H), 5.10-5.08 (m, 1H), 5.02-4.90 (m, 6H), 4.79 (d, J = 3.4 Hz, 1H), 4.72 (d, J = 12.3 Hz, 1H), 4.69 (d, J = 11.9 Hz, 1H), 4.57 (d, J = 11.5 Hz, 1H), 4.50-4.42 (m, 5H), 4.34-4.29 (m, 2H), 4.21 (app t, J = 8.7 Hz, 1H), 4.19 (d, J = 11.8 Hz, 1H), 4.05-3.93 (m, 4H), 3.83 (dt, J = 9.6, 6.3 Hz, 1H), 3.79-3.74 (m, 1H), 3.70-3.53 (m, 8H), 3.47-3.30 (m, 5H), 2.11-2.04 (m, 2H), 1.68-1.62 (m, 2H), 1.23 (s, 9H), 1.17 (s, 9H), 1.11 (s, 18H), 0.66 (d, J = 6.5 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 177.3, 177.3 176.8, 176.2, 165.5, 162.1, 139.3, 139.0, 138.3, 138.3, 138.3, 138.1, 137.6, 133.4, 130.0, 129.9, 128.8, 128.8, 128.6, 128.6, 128.5, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 127.1, 115.0, 101.4, 99.9, 99.7, 99.3, 92.4, 81.1, 78.1, 77.4, 75.5, 74.8, 74.6, 74.0, 73.9, 73.8, 73.7, 73.7, 73.6, 72.8, 72.5, 71.2, 70.7, 69.9, 69.0, 68.4, 66.4, 59.0, 39.1, 39.0, 38.9, 38.8, 30.3, 30.9, 27.6, 27.4, 27.4, 15.4; ESI-MS: m/z (M + Na)⁺ calcd 1864.7264, obsd 1864.7322.

n-Pentenyl 2-O-benzyl-3,4-di-O-pivaloyl-α-L-fucopyranosyl-(1→3)-6-O-benzyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-
glucopyranoside 102. A solution of 101 (49 mg, 0.027 mmol) in methanol (1.5 mL) was treated with sodium methoxide (30 µL of a 25% solution by weight, 5 equiv.) at room temperature. After 90 minutes the solution was diluted with 10 mL methanol and acidified to pH 6 by addition of amberlite acidic resin. The solution was filtered and the filtrate concentrated. The crude residue was filtered through a plug of silica with a 25% EtOAc/Hexanes solution and concentrated to furnish 39 mg of the tetrasaccharide 102 (85%). \[^{13}\text{C}]\delta: -36.59^\circ (c = 0.60, \text{CH}_2\text{Cl}_2); \text{IR (thin film): 3431, 3031, 2973, 1735, 1066 cm}^{-1};^1\text{H NMR (400 MHz, CDCl}_3): \delta 7.38-7.21 (m, 25H), 7.16 (app d, \text{J} = 7.1 \text{ Hz, 2H}), 7.11 (app t, \text{J} = 7.3 \text{ Hz, 1H}), 7.02 (app t, \text{J} = 7.6 \text{ Hz, 2H}), 6.69 (d, \text{J} = 7.7 \text{ Hz, 1H}), 5.84-5.74 (m, 1H), 5.33-5.27 (m, 3H), 5.03-4.93 (m, 6H), 4.75-4.69 (m, 3H), 4.62 (d, \text{J} = 12.1 \text{ Hz, 1H}), 4.59-4.44 (m, 5H), 4.40-4.29 (m, 4H), 4.18 (d, \text{J} = 11.8 \text{ Hz, 1H}), 4.11 (br s, 1H), 4.03-3.91 (m, 3H), 3.86-3.72 (m, 6H), 3.68-3.51 (m, 7H), 3.48-3.39 (m, 3H), 3.35-3.30 (m, 2H), 2.11-2.04 (m, 2H), 1.69-1.63 (m, 2H), 1.22 (s, 9H), 1.20 (s, 9H), 1.17 (s, 18H), 1.07 (d, \text{J} = 6.5 \text{ Hz, 3H});^{13}\text{C NMR (100 MHz, CDCl}_3): \delta 177.4, 177.3, 176.8, 176.5, 162.1, 139.2, 138.9, 138.3, 138.3, 138.1, 138.0, 137.7, 129.8, 128.8, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.0, 128.0, 127.9, 127.9, 127.8, 127.5, 127.0, 115.0, 101.4, 99.8, 99.5, 98.5, 92.6, 91.9, 82.8, 81.1, 77.9, 77.4, 76.6, 75.5, 75.5, 75.2, 74.7, 73.8, 73.7, 73.6, 73.6, 73.3, 72.4, 71.0, 70.6, 69.8, 69.6, 69.0, 68.3, 67.0, 57.0, 39.2, 39.0, 38.9, 30.2, 29.5, 29.0, 28.3, 27.6, 27.3, 22.7, 16.1, 14.2; ESI-MS: m/z (M + Na)\(^+\) calcd 1760.7002, obsd 1760.7031.

\[n\text{-Pentenyl} \ 2\text{-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl(1→4)-[2-O-benzyl-3,4-di-O-pivaloyl-α-L-fucopyranosyl-(1→3)]-6-O-benzyl-2-deoxy-2-trichloroacetimidato-β-D-galactopyranosyl(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranoside} \ 103.

Tetrasaccharide acceptor 102 (48 mg, 0.028 mmol) and galactosyl phosphate 91 (40 mg, 0.055 mmol) were coevaporated in toluene (3 × 2 mL), then dried under vacuum for 2 h. Dichloromethane (2 mL) was added and the solution was cooled to −60°C. TMSOTf (10
µL, 0.55 mmol) was added and the reaction mixture was allowed to warm to −20°C over 30 min. The reaction mixture was quenched by addition of TEA and slowly warmed to room temperature. The solution was concentrated and the crude residue was purified by flash silica gel chromatography (10→20% EtOAc/hexanes) to yield 65 mg (98%) of 103. \([\alpha]_D: -11.72^\circ \ (c = 0.30, \text{CH}_2\text{Cl}_2); \text{IR (thin film): 3421, 3031, 2971, 1734, 1718 cm}^{-1}; \text{H NMR (400 MHz, CDCl}_3\text{):} \delta 7.90 (dd, J = 7.7, 1.3 Hz, 2H), 7.46-7.12 (m, 46H), 7.04 (app t, J = 7.0 Hz, 2H), 6.86 (d, J = 8.3 Hz, 1H), 5.83-5.74 (m, 1H), 5.48 (dd, J = 10.0, 8.0 Hz, 1H), 5.32 (d, J = 10.4 Hz, 1H), 5.30 (d, J = 10.7 Hz, 1H), 5.24-5.19 (m, 2H), 5.03-4.95 (m, 4H), 4.85 (d, J = 11.5 Hz, 1H), 4.83 (d, J = 11.5 Hz, 1H), 4.74 (d, J = 12.0 Hz, 1H), 4.68-4.59 (m, 5H), 4.56-4.54 (m, 2H), 4.48-4.33 (m, 13H), 4.22-4.19 (m, 2H), 4.07 (d, J = 11.8 Hz, 1H), 4.04 (d, J = 2.6 Hz, 1H), 4.00 (t, J = 9.4 Hz, 1H), 3.96-3.91 (m, 2H), 3.87-3.81 (m, 4H), 3.72 (d, J = 2.6 Hz, 2H), 3.65-3.40 (m, 9H), 3.37-3.34 (m, 2H), 3.29-3.20 (m, 3H), 2.12-2.05 (m, 2H), 1.69-1.61 (m, 2H), 1.20 (s, 9H), 1.19 (s, 9H), 1.17 (s, 9H), 1.11 (s, 9H), 0.98 (d, J = 6.5 Hz, 3H); \(^{13}\text{C NMR (100 MHz, CDCl}_3\text{):} \delta 177.4, 177.1, 176.9, 176.7, 165.5, 161.7, 139.2, 139.1, 138.3, 138.3, 137.9, 137.9, 137.6, 137.6, 130.0, 130.0, 129.9, 129.9, 129.2, 129.1, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.2, 128.2, 128.1, 128.0, 127.8, 127.7, 127.4, 127.0, 115.0, 101.4, 100.1, 100.0, 99.9, 96.5, 92.3, 91.2, 81.1, 79.5, 78.1, 77.9, 77.4, 76.1, 75.7, 74.9, 74.7, 74.5, 73.9, 73.8, 73.6, 73.6, 73.3, 73.1, 72.8, 72.5, 72.4, 72.3, 71.9, 71.4, 71.4, 71.0, 70.3, 69.8, 69.0, 68.7, 68.4, 67.8, 65.5, 58.2, 39.2, 39.0, 38.9, 38.8, 30.2, 29.5, 29.0, 28.3, 27.7, 27.4, 27.4, 27.3, 22.7, 15.7, 14.4, 14.2; \text{ESI-MS: m/z (M + Na)}^+ \text{ calcld 2296.9200, obsd 2296.9263. }

\text{n-Pentenyl 2-O-benzyl-3,4-di-O-pivaloyl-\alpha-L-fucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-\beta-D-galactopyranosyl(1→4)-[2-O-benzyl-3,4-di-O-pivaloyl-\alpha-L-fucopyranosyl-(1→3)]-6-O-benzyl-2-deoxy-2-trichloroacetimido-\beta-D-glucopyranosyl(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-\beta-D-galactopyranosyl(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-\beta-D-glucopyranoside 105. A solution of 103 (55 mg,}
0.024 mmol) in methanol (1.5 mL) was treated with sodium methoxide (28 μL of a 25% solution by weight, 10 equiv.) at room temperature. After 2.5 h the solution was diluted with 10 mL methanol and acidified to pH 6 by addition of Dowex resin. The solution was filtered and the filtrate was concentrated and purified by column to give 25 mg of pentasaccharide 104.

Pentasaccharide acceptor 104 (20 mg, 0.0092 mmol) and fucosyl phosphate 70 (12 mg, 0.018 mmol) were coevaporated in toluene (3 × 1 mL), then dried under vacuum for 2 h. Dichloromethane (1 mL) was added and the solution was cooled to −50°C. TMSOTf (5 μL, 0.018 mmol) was added and the reaction mixture was allowed to warm to −20°C over 30 min. The reaction mixture was quenched by addition of TEA and slowly warmed to room temperature. The solution was concentrated and the crude residue was purified by flash silica gel chromatography (5→10→17→25% EtOAc/hexanes) to yield 22 mg (95%) of hexasaccharide 105. [α]D: -51.33° (c = 1.38, CH2Cl2); IR (thin film): 3487, 3050, 2937, 1741, 1720 cm⁻¹; 1H NMR (500 MHz, CDCl₃): δ 7.36-7.01 (m, 46H), 6.99 (app t, J = 7.7 Hz, 2H), 6.90 (app d, J = 6.6 Hz, 2H), 6.60 (d, J = 7.6 Hz, 1H), 5.81-5.75 (m, 1H), 5.66 (d, J = 3.5 Hz, 1H), 5.37-5.27 (m, 4H), 5.24 (d, J = 3.6 Hz, 1H), 5.19 (dd, J = 10.7, 3.3 Hz, 1H), 5.05 (d, J = 7.3 Hz, 1H), 5.01-4.98 (m, 1H), 4.97-4.93 (m, 3H), 4.91-4.85 (m, 2H), 4.75-4.62 (m, 7H), 4.60 (d, J = 7.8 Hz, 1H), 4.55 (d, J = 12.1 Hz, 1H), 4.51 (d, J = 12.2 Hz, 1H), 4.48-4.36 (m, 6H), 4.34-4.31 (m, 2H), 4.29-4.25 (m, 2H), 4.21-4.16 (m, 2H), 4.14-4.11 (m, 1H), 4.08-4.01 (m, 2H), 3.98-3.95 (m, 1H), 3.92-3.86 (m, 3H), 3.83-3.79 (m, 2H), 3.74-3.67 (m, 3H), 3.55 (t, J = 8.8 Hz, 1H), 3.45-3.40 (m, 3H), 3.36-3.25 (m, 4H), 2.09-2.04 (m, 2H), 1.67-1.61 (m, 2H), 1.21 (s, 9H), 1.20 (s, 9H), 1.17 (s, 9H), 1.16 (s, 9H), 1.15 (s, 18H), 1.11 (d, J = 6.5 Hz, 3H), 0.99 (d, J = 6.5 Hz, 3H); 13C NMR (125 MHz, CDCl₃): δ 177.9, 177.5, 177.2, 177.0, 176.8, 176.4, 161.4, 139.2, 139.1, 138.8, 138.4, 138.4, 138.3, 138.3, 138.2, 138.1, 137.7, 130.0, 129.6, 128.7, 128.6, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.4, 127.3, 127.2, 127.0, 126.4, 114.9, 101.4, 99.9, 99.9, 99.7, 97.5, 84.1, 81.2, 75.6, 75.6, 75.5, 75.3, 75.1, 74.7, 74.5, 74.3, 73.9, 73.9, 73.8, 73.7, 73.7, 73.6, 73.4, 73.4, 73.3, 73.3, 73.1, 73.0, 72.6, 72.2, 71.9, 71.5, 71.2, 70.8, 70.2, 69.0, 68.4, 67.9, 65.4, 65.3, 65.3, 39.2,
39.1, 39.0, 38.9, 38.8, 30.3, 29.0, 27.6, 27.5, 27.4, 27.4, 27.3, 15.8, 15.8; ESI-MS: m/z (M + Na)^+ calcd 2597.1137, obsd 2597.1087.
2.10 References


Chapter 3
Automated Solid-Phase Synthesis of the
Type II Lewis Oligosaccharides
3.1 Introduction

In Chapter 2, we demonstrated the solution-phase syntheses of the Type II Lewis oligosaccharides. Despite our success in procuring complex penta- and hexasaccharides via high yielding coupling and deprotection reactions in solution, each of these syntheses was extremely tedious and time consuming due to the purification regiment required at every step. Our initial work on automated solid-phase oligosaccharide assembly demonstrated that relatively simple carbohydrates could be prepared on a machine that executes a coupling cycle, including steps for glycosylation and deprotection.¹ Further fundamental advances required to expand this methodology to include structures containing a variety of residues and linkages and to simplify the synthetic process for the Lewis oligosaccharides are presented in this chapter.

3.2 Previous Solid-Phase Syntheses of the Lewis Group Oligosaccharides

Following the multitude of solution-phase syntheses of the Lewis group oligosaccharides (vide supra), several manual solid-phase syntheses of these molecules have arisen. Solid-phase assembly enables reactions to be driven to completion using an excess of reagents while retaining facile product purification; unreacted reagents are simply washed away at the end of each step. Solid-phase assembly also speeds the generation of oligosaccharide libraries by allowing traditional combinatorial methods, for example the split-and-mix approach, to be used.²

Danishefsky and coworkers utilized a donor-bound approach with glycals as the key building blocks in the synthesis of the Le⁰ blood group determinant (Scheme 3.1).³ Construction of glycal 3 by activation of resin-bound 1 in the presence of 2, followed by fucosylation with glycosyl fluoride 4 produced tetrasaccharide 5. A translation of solution-phase sulfonamidoglycosylation protocols to resin-bound 5 proved impossible, however, conversion of 5 to thioethyl donor 6 and subsequent activation toward 7 produced resin-bound 8, the fully protected Le⁰ pentasaccharide.
Scheme 3.1 Solid-phase synthesis of Le\textsuperscript{b} via glycal assembly

Zhu and Boons have utilized soluble polymeric supports for the synthesis of the entire family of Type II Lewis oligosaccharides (Scheme 3.2).\textsuperscript{4} Soluble polymers closely resemble solution-phase conditions, thereby allowing a reduction of the excess of glycosylating agent typically needed to drive solid-phase reactions to completion and accelerating the rate of reactions on the solid-support. Using a soluble polyethylene glycol methyl ether (MPEG) polymer, Troc protected glycoside 9 is attached to the support, followed by galactosylation with 10. Selective removal of the Lev or Fmoc groups from 11 allows for decoration of either the terminal galactose of 13 to form resin-bound H-type II trimer 17 or the glucosamine of 12 to form resin-bound Le\textsuperscript{x} trimer 16 following fucosylation with 15. Removal of both the Lev and Fmoc groups and subsequent fucosylation of 14 produces bound Le\textsuperscript{y} tetrasaccharide 18. Completion of the final penta- and hexasaccharides is carried out by solution-phase glycosylation of a
lactose acceptor using trichloracetimidates derived from solid-phase syntheses.\textsuperscript{4} This methodology was extended to the synthesis of a Le\textsuperscript{X}-Le\textsuperscript{X} hexasaccharide.\textsuperscript{5}

Scheme 3.2 Synthesis of the Type-II Lewis oligosaccharides on a soluble MPEG support

The Lewis oligosaccharides have also been constructed via chemo-enzymatic transformations of polymer-bound oligosaccharides.\textsuperscript{6,7} In this manner, the sialyl Le\textsuperscript{X} tetrasaccharide was constructed by glycosylation of a sepharose matrix with a glucosamine thioglycoside, followed by a sequential enzymatic addition of monosaccharides for the completion of the oligosaccharide.\textsuperscript{7} Solid-support synthesis of
the Lewis oligosaccharides has also been utilized for the development of a gated $^{13}$C NMR methodology for monitoring of solid-phase glycosylation chemistry.$^8$

3.3 Automated Solid-Phase Synthesis of Oligosaccharides

Previous efforts in the Seeberger lab led to the development of a general methodology for the automation of carbohydrate synthesis on the solid-support.$^1$ Based on results with manual solid-phase synthesis, a strategy was devised employing the acceptor bound glycosylation method using glycosyl trichloroacetimidate and glycosyl phosphate donors. Building blocks were temporarily protected as acetate and levulinate esters owing to facility in removing these esters on the solid-support. Likewise, these protecting groups had been used in model solution-phase studies and were known to be stable under the acidic conditions required for glycosylation.

Merrifield's resin (1% crosslinked) was investigated as the solid-support and worked well in concert with the 4,5-Z-octenediol linker, as Grubbs' conditions employed in product cleavage via cross metathesis were compatible with the resin. Functionalized resin was loaded into a reaction vessel, which was installed onto a modified *Applied Biosystems* (ABI) Model 433A peptide synthesizer. Reaction cycles typically used for peptide synthesis were adapted for carbohydrate synthesis. Likewise, reagent bottles were equipped with solutions for oligosaccharide coupling and deprotection. Design and use of a jacketed reaction vessel connected to a circulating chiller allowed glycosylation reactions to be carried out at low temperatures, markedly improving coupling yields compared to room temperature glycosylation.
Figure 3.1 Schematic design of an automated oligosaccharide synthesizer

The utilization of this instrument to make a series of repeating unit carbohydrate polymers, including poly α-(1→2)-D-mannosides,\(^1\) poly α-(1→2)-L-rhamnosides,\(^9\) and β-glucans\(^1\) fostered development of a productive coupling cycle. Inclusion of each monosaccharide building block on the solid-support required a double glycosylation with 5 equivalents of glycosylating agent in each reaction, followed by several washing steps. Removal of the temporary protecting group for unveiling the resin-bound acceptor was similarly accomplished; a double deprotection step was pursued by a rigorous washing protocol in preparation for the next glycosylation.
Table 3.1 Model coupling cycle used with glycosyl phosphate donors

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<th>Reagent</th>
<th>Time (min)</th>
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<td>5 equiv. donor and 5 equiv. TMSOTf</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>Wash</td>
<td>Dichloromethane</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>Couple</td>
<td>5 equiv. donor and 5 equiv. TMSOTf</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>Wash</td>
<td>1:9 Methanol/Dichloromethane</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Wash</td>
<td>Tetrahydrofuran</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Wash</td>
<td>3:2 Pyridine/Acetic Acid</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>Deprotection</td>
<td>3 X 20 equiv. Hydrazine in 3:2 Pyridine/Acetic Acid</td>
<td>30</td>
</tr>
<tr>
<td>8</td>
<td>Wash</td>
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<td>Wash</td>
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</table>

3.4 Initial Attempts at Lewis Oligosaccharide Automation

Give our results in the synthesis of the H-type II pentasaccharide in solution (Scheme 3.3), we began our automated solid-phase studies with this structure. Samples from the solution-phase synthesis served to aid in the identification and characterization of products and deletion sequences. Our studies commenced with the automated synthesis of the lactose disaccharide. Using 5 equivalents of building blocks 19 and 21, the lactose was constructed as a series of double glycosylations for 15 minutes each at -15°C, followed by double deprotections of the levulinic esters at 0°C. After five hours, the synthesis was complete and the disaccharide was cleaved from the resin using Grubbs' catalyst in an ethylene atmosphere. Cross metathesis yielded lactose 23 as the n-pentenyl glycoside and no other products were observed by either HPLC or NMR analysis.
Scheme 3.3 Solution synthesis of the H-type II pentasaccharide 31

Successful automation of lactose 23 allowed for investigations in the automated synthesis of core trisaccharide 25. Again, 5 equivalents of each of the three donors 19, 21, and 24, were employed in double glycosylations. Following cleavage from the solid-support, the only oligosaccharide observable by $^1$H NMR was lactose 23. Repetition of this experiment yielded a mixture of products; both lactose 23 and trimer 25 were detected, but 23 constituted over 90% of the isolated product by HPLC analysis. Modification of glycosylation conditions during the automated cycle of donor 24 was
therefore investigated. The first variation in conditions was to lower the temperature of the vessel during glycosylation from -15°C to -25°C, maintaining the equivalents of glycosylating agent and reaction time. No increase in the yield of trimer 25 versus lactose 23 was observed; glycosylation times were increased from 15 minutes to 1 hour for each of the two glycosylations with donor 24, maintaining the temperature at -20°C during the glycosylations. HPLC analysis of the crude product revealed that significantly more trimer 25 was formed in this run: 57% trimer 25 and 34% lactose 23. Finally, the equivalents of donor 24 were increased from 5 to 10 for each glycosylation and the longer reaction time was retained. These conditions yielded 25 as the major product formed; HPLC analysis revealed 60% of the trimer in the crude product.

Encouraged by these results, we turned to the synthesis of the complete H-type II pentasaccharide. Using building blocks 19, 21, and 24, previously used in the synthesis of the core trisaccharide 25, and adding galactose phosphate 27 and fucose phosphate 30, we constructed the pentasaccharide in an automated fashion in 16 hours. Each glycosylation reaction was conducted using the standard coupling conditions of 5 equivalents glycosylating agent for a 15 minute coupling at -15°C, with the exception of glucosamine trichloroacetimidate 24 wherein the modified conditions described above were used. Additionally, we used a triple glycosylation for the installation of this building block to ensure complete glycosylation. Analysis of the crude product achieved after cleavage from the resin with Grubbs’ catalyst showed the H-type II pentasaccharide 31 as the major product. The chief contaminant observed was the protected tetrasaccharide 28, indicating a need for optimization of the Staudinger conditions for AZMB removal on solid-support.

While pentasaccharide 31 was achieved in an automated fashion, the large excess of glycosyl trichloroacetimidate 24 combined with the lengthy reaction times required for installation of this building block prompted development and implementation of an improved glucosamine donor, first in solution and then on solid-support. Utilization of TCA protected glucosamines improved the reactivity of glucosamine donors toward lactose acceptor 23, but further complications in solution-phase AZMB removal in the presence of TCA protected amines stalled automated efforts.
Scheme 3.4 Solution synthesis of Le\(^x\) pentasaccharide 38

Successful completion of the solution synthesis of the Le\(^x\) pentasaccharide (Scheme 3.4) provided proof of principle and standards necessary to deconvolute an automated run. The solid-phase synthesis of 38 utilized standard automated conditions: 5 equivalents of each monomer in a double glycosylation at -15\(^\circ\)C followed by a double deprotection of levulinate or benzoate esters at +15\(^\circ\)C. After 11 hours, the automation of 38 was complete, however cleavage from the solid-support yielded amounts of product detectable only by mass spectroscopy. Clearly the solution-phase strategy was unsuccessful on the solid-support, but problem steps could not be identified without considerable effort by synthesizing each deletion sequence.
3.5 Development of a Monitoring Method for Solid-Phase Glycosylation Reactions

Following our unsuccessful attempt at the automated synthesis of Le^x pentasaccharide 38, we realized the potential impact solid-phase reaction monitoring would have on our carbohydrate syntheses. The ability to identify problematic steps before the final cleavage and purification of automation derived products would enable us to quickly make changes in coupling cycles or identify and replace unreactive donors in our efforts to improve automated assembly. Furthermore, glycosyl donors following unproductive steps would be spared, thereby reducing the overall amounts of donor required to optimize a particular synthesis.

Taking a cue from peptide synthesis, we chose the 9-fluorenymethoxycarbonyl (Fmoc) group for temporary protection of hydroxyl groups because it is completely stable to the acidic glycosylation conditions, it is readily cleaved by mildly basic amines,\textsuperscript{11} and it facilitates monitoring of protecting group removal by UV-Vis spectroscopy.\textsuperscript{12} Analysis of Fmoc deprotection provided a qualitative assay for the efficiency of each glycosylation and deprotection cycle curing automated assembly. While real time analysis is common in automated peptide and oligonucleotide synthesis, analogous monitoring of solid-phase glycosylation reactions had not been demonstrated using methods compatible with automated carbohydrate synthesis.\textsuperscript{13}

The use of temporary protecting groups that require only mildly basic conditions for removal allowed for the improvement of another feature of the prior automated synthesis: the lengthy cleavage of the product from the solid support following assembly. Condensation of the previously employed octenediol linker\textsuperscript{14} with carboxy-terminated polystyrene resin resulted in an ester linkage, which is rapidly cleaved at the end of the synthesis with strong base. The internal double bond remains a functional handle for further chemistry\textsuperscript{14} and can be cross-linked to a variety of olefins via metathesis.
Scheme 3.5 Automated solid-phase synthesis of disaccharide 41

The reliability of Fmoc monitoring and development of a deprotection protocol was tested in the construction of disaccharide 41 (Scheme 3.5). Glucose phosphate 40 was used in double couplings with support-bound acceptor 39 at -15°C using a solution of trimethylsilyltriflate for activation. Following glycosylation, Fmoc cleavage was achieved by three exposures to piperidine (20% in DMF). Following each exposure, the solution from the reaction vessel was collected for UV analysis, enabling determination of amount of Fmoc protected oligomer bound to the solid support at each step. Additional exposures proved unnecessary, as UV data indicated complete Fmoc cleavage after three exposures. Using this data, the efficiency of the coupling and Fmoc removal steps together was resolved qualitatively, thus allowing for termination or correction of syntheses that indicated unproductive coupling steps.

3.6 Automated Synthesis of the Le\(^x\), Le\(^y\), and Tumor-Associated Antigen Le\(^y\)-Le\(^x\)

Initially we identified the monosaccharide building blocks found in Le\(^x\) pentasaccharide 42, Le\(^y\) hexasaccharide 43, and Le\(^y\)-Le\(^x\) nonasaccharide 44 (Figure 3.2). Five monomers would be sufficient for the construction of the three target structures that each contain five different glycosidic bonds. Our strategy for the synthesis of these branched carbohydrates differs markedly from previous routes that had been striving for
maximum convergence. Typically, a series of block couplings specific to each target were used, combining di- and trisaccharide portions of the molecule. The \( \text{Le}^\text{y}-\text{Le}^\text{x} \) nonasaccharide has thereby twice been constructed in heroic solution-phase total syntheses.\(^{15,16}\)

![Chemical structures](image)

**Figure 3.2** Retrosynthesis of \( \text{Le}^\text{x} \) pentasaccharide 42, \( \text{Le}^\text{y} \) pentasaccharide 43, and \( \text{Le}^\text{y}-\text{Le}^\text{x} \) nonasaccharide 44 indicates monosaccharide building blocks 45-49

Each monosaccharide unit must contain appropriate temporary protecting groups to establish the required connections in the target and be synthetically readily accessible. Additionally, we were interested in demonstrating the utility of monitoring the progress of solid-phase assembly in real time. The monitoring of coupling success during automated oligopeptide and oligonucleotide synthesis has enabled the synthesis of lengthy oligomers of these biopolymers.

For each monosaccharide, a protecting group pattern that allowed for selective unveiling of a single hydroxyl group was selected. Differentially protected monosaccharide building blocks incorporating Fmoc as a temporary protecting group were rapidly prepared from glycals using a one-pot procedure.\(^{17}\) Glycosyl phosphates 45,
were synthesized for the placement of glucose and galactose residues. Glucosamine phosphate 47 was selected based on solution-phase studies. Fucosyl phosphate 48 showed excellent selectivity in creating the difficult $\alpha$-(1→2-$cis$) and $\alpha$-(1→3-$cis$) fucose linkages common to the Lewis antigens.\textsuperscript{10,17} To account for branching connections via both the C3 and C4 positions of the glucosamine units in targets 42-44, an additional temporary protecting group was installed in monomer 47. The levulinoyl ester was selected, as it is easily removed with hydrazine and completely orthogonal to Fmoc.

<table>
<thead>
<tr>
<th>Step</th>
<th>Function</th>
<th>Reagent</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Couple</td>
<td>5 equiv. donor and 5 equiv. TMSOTf</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>Wash</td>
<td>Dichloromethane</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>Couple</td>
<td>5 equiv. donor and 5 equiv. TMSOTf</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>Wash</td>
<td>$N,N$-dimethylformamide (DMF)</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>Deprotection</td>
<td>3 X 175 equiv. piperidine in DMF</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>or 5 X 10 equiv. hydrazine in DMF</td>
<td>80</td>
</tr>
<tr>
<td>6</td>
<td>Wash</td>
<td>$N,N$-dimethylformamide (DMF)</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>Wash</td>
<td>0.2 M acetic acid in tetrahydrofuran</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>Wash</td>
<td>Tetrahydrofuran</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>Wash</td>
<td>Dichloromethane</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 3.2 General coupling cycle used with glycosyl phosphates for construction of 42, 43, and 44

In order to accommodate the real time monitoring and further increase coupling and deprotection efficiency, new coupling cycles for use on the automated oligosaccharide synthesizer were devised (Table 3.2). The glycosylation and Fmoc deprotection protocols developed in the synthesis of dimer 41 were used, along with new conditions for Lev ester removal. Removal of the C4 levulinate group from the glucosamine residue of a resin-bound trisaccharide initially proved to be difficult and required modification of the initial conditions to improve deprotection efficiencies. The levulinoyl group was removed by three washes with a solution of hydrazine (10% in DMF). Before and after each deprotection, a series of washing steps were conducted to
adequately swell the resin or purge it from any remaining base in preparation for the next glycosylation cycle.

Scheme 3.6 Automated synthesis of pentasaccharide 42, hexasaccharide 43 and nonasaccharide 44 on a 25 mmol scale: 25 mmol resin for each synthesis (45 mg, 0.56 mmol/g loading). a, 5 equiv. donor 45, 5 equiv. TMSOTf repeated two times for 15 min each. b, 2.2 mL of a 20% piperidine in DMF solution repeated three times for 10 min. each. c, 5 equiv. donor 46, 5 equiv. TMSOTf repeated two times for 15 min each. d, 3.5 equiv. donor 47, 3.5 equiv. TMSOTf repeated three times for 15 min each. e, 5 equiv. donor 48, 5 equiv. TMSOTf repeated two times for 15 min each. f, 2.5 mL of a 10% N2H4 in DMF solution repeated 5 times for 15 min. each. g, 5 equiv. donor 49, 5 equiv. TMSOTf repeated two times for 15 min each. h, 10 equiv. NaOMe in MeOH/CH2Cl2 (1:4) repeated 4 times for 90 min. each.

Protected Le\textsuperscript{x} pentasaccharide 42 was constructed in just 12 hours (Scheme 3.6) utilizing the reported coupling cycles (Table 3.2). Initial glycosylation of linker 39 with glucose monomer 45 was followed by removal of Fmoc and iterative addition of monomers 46-49 to complete the resin-bound pentasaccharide. Cleavage from the support was achieved by repeated treatment with a solution of sodium methoxide over a 6 hour period. Pentasaccharide 42 was isolated in a 12.6% yield after HPLC purification.

Given our success in synthesizing Le\textsuperscript{x}, the reaction sequence was modified to include a coupling cycle for the addition of a fucose residue to the terminal galactose. In this manner, protected Le\textsuperscript{y} hexasaccharide 43 was completed in 14 hours in an isolated
yield of 9.9%. The assembly of pentamer 42 and hexamer 43 not only provided access to biologically relevant oligosaccharides, but also demonstrated the preparation of important portions of the Le\textsuperscript{y}-Le\textsuperscript{x} nonasaccharide 44.

For the synthesis of the Le\textsuperscript{y}-Le\textsuperscript{x} nonasaccharide, the first four monosaccharides of 44 were installed in an identical fashion to the assembly of Le\textsuperscript{x} 42. Galactose building block 46 enabled the formation of the additional glucosamine β(1→3) galactose linkage. The assembly of 44 was completed by addition of glucosamine 47 followed by the final three residues of the Le\textsuperscript{y} hexamer. The solid-phase synthesis of nonasaccharide 44 was finished after 23 hours; cleavage from the solid support and HPLC purification produced 44 as the major product in 6.5% isolated yield.

3.7 Conclusions

Two innovations provided the foundation for the rapid access to these complex oligosaccharides of biological importance: monitoring of monosaccharide installation and a novel linker system. Fmoc analysis proved instrumental in the development of the coupling cycles for all syntheses. Unproductive steps were easily identified and glycosylation or deprotection conditions were modified accordingly, working toward successful coupling cycles. Facile removal of the oligosaccharide from the resin using a base labile linker enabled swift analysis of the final products. Fully protected oligosaccharides Le\textsuperscript{x} 42, Le\textsuperscript{y} 43, and the Le\textsuperscript{y}-Le\textsuperscript{x} dimer 44 were all produced in overall yields comparable or better than previous solution-phase syntheses, but in a fraction of the time previously required. It should be noted that each of these syntheses were completed without initial solution-phase exploration of the synthetic route.

Automation of oligosaccharide assembly has the potential to revolutionize the biological understanding of complex oligosaccharides as non-specialists gain access to a variety of structures. Automated assembly of the Le\textsuperscript{y}-Le\textsuperscript{x} tumor-marker and Le\textsuperscript{x} and Le\textsuperscript{y} blood group antigens via a stepwise monosaccharide approach serves as a first example of our ability to make a host of structures of biological significance. Adaptation of coupling cycles to include Fmoc protection and linker strategies to enable base cleavage brings current automated methods a step closer to existing protocols for automated
synthesis of peptides. Additional research to expand the diversity of structures accessible via automation, to increase overall yields, and decrease assembly times is ongoing.

3.8 Experimental Section

General Methods. All chemicals were reagent grade and used as supplied unless otherwise noted. Dichloromethane (CH$_2$Cl$_2$), tetrahydrofuran (THF), and toluene were purified by a JT Baker Cycle-Tainer Solvent Delivery System. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F$_{254}$ plates (0.25 mm). Compounds were visualized by dipping the plates in a cerium sulfate-ammonium molybdate solution followed by heating. Liquid column chromatography was performed using forced flow of the indicated solvent on Silicycle silica (230 - 400 mesh). $^1$H NMR spectra were obtained using a Bruker-400 NMR spectrometer (400 MHz) or a Varian VXR-500 (500 MHz) and are reported in parts per million (δ) relative to CDCl$_3$ (7.27 ppm). Coupling constants (J) are reported in Hertz. $^{13}$C NMR spectra were obtained using a Bruker-400 NMR spectrometer (100 MHz) or a Varian VXR-500 (125 MHz) and are reported in δ relative to CDCl$_3$ (77.23 ppm) as an internal reference. $^{31}$P spectra were obtained using a Varian VXR-300 NMR spectrometer (120 MHz) or a Varian VXR-500 (200 MHz) and are reported in δ relative to H$_3$PO$_4$ (0.0 ppm) as an external reference. Optical rotations were measured at 24°C.

Glycosylations Using Glycosyl Phosphates: General Procedure A. Resin-bound acceptor (1 equiv.) was treated with glycosyl phosphate (5 equiv.) as a solution in CH$_2$Cl$_2$, (2 mL). After cooling to -15°C, TMSOTf (5 equiv., 1.1 mL of a 0.125 M solution in CH$_2$Cl$_2$) was added and the reaction mixture was agitated (10 s vortex, 50 s rest) for 20 min. The reaction vessel was drained and the resin was washed with CH$_2$Cl$_2$ (6 x 3 mL). A second quantity of the same glycosyl phosphate was again added to the reaction vessel as a solution in CH$_2$Cl$_2$, followed by activator, and the mixture agitated an additional 20 minutes. The vessel was drained and the resin was washed again with CH$_2$Cl$_2$ (6 x 3 mL).
Glycosylations Using Glycosyl Trichloroacetimidates: General Procedure B. Resin-bound acceptor (1 equiv.) was treated with glycosyl trichloroacetimidate (5 equiv.) as a solution in CH$_2$Cl$_2$ (2 mL). After cooling to -15°C, TMSOTf (0.5 equiv, 0.3 mL of a 0.125 M solution in CH$_2$Cl$_2$) was added and the reaction mixture was agitated (10 s vortex, 50 s rest) for 20 min. The reaction vessel was drained and the resin was washed with CH$_2$Cl$_2$ (6 × 3 mL). A second quantity of the same glycosyl imidate was again added to the reaction vessel as a solution in CH$_2$Cl$_2$, followed by activator, and the mixture agitated an additional 20 minutes. The vessel was drained and the resin was washed again with CH$_2$Cl$_2$ (6 × 3 mL).

Cleavage of Levulinate Esters: General Procedure C. The resin-bound saccharide was washed with an acetic acid-methanol solution (0.2 M in THF, 6 × 3 mL). A solution of hydrazine monohydrate (0.5 M in 3:2 pyridine/acetic acid, 40 equiv.) was added and the reaction mixture agitated (10 s vortex, 50 s rest) for 15 min at 15°C. The reaction vessel was drained and the deprotection was repeated, followed by washing the resin with THF (6 × 3.5 mL), acetic acid-methanol (0.2 M in THF, 6 × 3 mL), THF (6 × 3.5 mL), and CH$_2$Cl$_2$ (6 × 3 mL).

Cleavage of 2-(Azidomethyl)benzoyl (AZMB) Esters: General Procedure D. The resin-bound saccharide was washed with THF (6 × 3.5 mL). A solution tri-$n$-butyl phosphate (0.15 M P(Bu)$_3$, 0.25 M H$_2$O in THF, 40 equiv.) was added and the solution was agitated (10 s vortex, 50 s rest) for 15 min at 15°C. The reaction vessel was drained and the deprotection was repeated, followed by washing the resin with THF (6 × 3.5 mL), acetic acid-methanol (0.2 M in THF, 6 × 3 mL), THF (6 × 3.5 mL), and CH$_2$Cl$_2$ (6 × 3 mL).

Cleavage of Benzoyl Esters: General Procedure E. A solution of sodium methoxide (0.75 M in methanol, 100 equiv.) was added and the solution was agitated (10 s vortex, 50 s rest) for 15 min at 25°C. The reaction vessel was drained and the deprotection was
repeated, followed by washing the resin with acetic acid-methanol (0.2 M in THF, 6 × 3 mL), THF (6 × 3.5 mL), and CH₂Cl₂ (6 × 3 mL).

**Cleavage of Saccharides from Octenediol Resin: General Procedure F.** The resin-bound saccharide was vacuum dried upon completion of its automated assembly over P₂O₅ for 12 h. The resin-bound saccharide was then transferred to a 50 mL solid-phase flask and CH₂Cl₂ (3 mL) was added before agitation commenced. After 5 minutes shaking, the flask was purged with ethylene before adding Grubbs’ catalyst (5 mg, 20 mol %). After 36 to 48 h agitation under an ethylene atmosphere, the reaction mixture was quenched with triethylamine (0.1 mL, 160 equiv.) and the solution was filtered through a plug of silica gel. The silica was thoroughly washed with EtOAc and dichloromethane and the filtrates were washed with a saturated aqueous solution of tris-hydroxy methyl phosphine (3 × 20 mL) to remove catalyst impurities. The organic phase was dried over Na₂SO₄, filtered and the solution was concentrated. The crude saccharide was then purified by either flash silica gel chromatography or high-pressure liquid chromatography (HPLC) to yield the desired saccharides as n-pentenyl glycosides.

![Structural formula](image)

**n-Pentenyl 4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 23.** General Procedure A using octenediol functionalized polystyrene resin (70 mg, 0.36 mmol/g, 25 μmol) and glucosyl phosphate 19 (90 mg per glycosylation, 125 μmol) was followed by General Procedure C. This cycle was repeated using galactosyl phosphate 21 (90 mg per glycosylation, 125 μmol) in General Procedure A. The completion of the automated assembly of the saccharide was followed by General Procedure F, resulting in 20 mg crude saccharide. The crude saccharide was purified by flash silica gel chromatography (40% EtOAc/hexanes) to yield 11 mg (47%) of lactose 23. Characterization data was consistent with reported solution-phase data.
**n-Pentenyl 3-O-benzoyl-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 25.** General Procedure A using octenediol functionalized polystyrene resin (117 mg, 0.36 mmol/g, 25 µmol) and glucosyl phosphate 19 (90 mg per glycosylation, 125 µmol) was followed by General Procedure C. This cycle was repeated using galactosyl phosphate 21 (90 mg per glycosylation, 125 µmol) in General Procedure A. General Procedure B was then modified to include glycosylation with 10 equiv. of glycosyl trichloroacetimidate 24 (200 mg per glycosylation, 250 µmol) at -25°C. Each glycosylation module using donor 24 was allowed to agitate for 1 h, rather than the typical 15 minutes. Addition of donor 24 was followed by General Procedure C. The completion of the automated assembly of the saccharide was followed by General Procedure F, resulting in 24 mg crude saccharide. The crude saccharide was purified by flash silica gel chromatography (30% EtOAc/hexanes) to yield 5 mg (14%) of trisaccharide 25. Characterization data was consistent with reported solution-phase data.

**n-Pentenyl 2-O-benzyl-3,4-di-O-pivaloyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-3-O-benzoyl-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 31.** General Procedure A using octenediol functionalized polystyrene resin (124 mg, 0.36 mmol/g, 25 µmol) and glucosyl phosphate 19 (90 mg per glycosylation, 125 µmol) was followed by General Procedure C. This cycle was repeated using galactosyl phosphate 21 (90 mg per glycosylation, 125 µmol) in General Procedure A. General Procedure B was then modified to include a triple glycosylation with 10 equiv. of glycosyl trichloroacetimidate 24 (200 mg per glycosylation, 250 µmol) at -20°C. Each
glycosylation module using donor 24 was allowed to agitate for 1 h, rather than the typical 15 minutes. Addition of donor 24 was followed by General Procedure C. General Procedure A using galactosyl phosphate 27 (100 mg per glycosylation, 125 μmol) was followed by General Procedure D. Finally, General Procedure A using fucosyl phosphate 30 (80 mg per glycosylation, 125 μmol) accomplished the resin-bound pentasaccharide. The completion of the automated assembly of the saccharide was followed by General Procedure F, resulting in 30 mg crude saccharide. The crude saccharide was purified by flash silica gel chromatography (25% EtOAc/hexanes) to yield 7 mg (9%) of pentasaccharide 31. Characterization data was consistent with reported solution-phase data.

\[ n\text{-Pentenyl 2-O-benzoyl-3,4,6-tri-O-benzyl-}\beta\text{-D-galactopyranosyl(1→4)-[2-O-benzyl-3,4-di-O-pivaloyl-}\alpha\text{-L-fucopyranosyl-(1→3)]-6-O-benzyl-2-deoxy-2-trichloroacetimidob-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-}\beta\text{-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-}\beta\text{-D-glucopyranoside 38.} \]

General Procedure A using octenediol functionalized polystyrene resin (63 mg, 0.4 mmol/g, 25 μmol) and glucosyl phosphate 19 (90 mg per glycosylation, 125 μmol) was followed by General Procedure C. This cycle was repeated using galactosyl phosphate 21 (90 mg per glycosylation, 125 μmol) in General Procedure A. General Procedure B using glucosamine trichloroacetimidate 32 (95 mg per glycosylation, 125 μmol) was followed by General Procedure C. General Procedure A using fucosyl phosphate 30 (77 mg per glycosylation, 125 μmol) was followed by General Procedure E. Finally, General Procedure A using galactosyl phosphate 37 (91 mg per glycosylation, 125 μmol) accomplished the resin-bound pentasaccharide. The completion of the automated assembly of the saccharide was followed by General Procedure F, resulting in 20 mg crude saccharide. The crude saccharide was purified by flash silica gel chromatography.
(10→20% EtOAc/hexanes) to yield <1 mg of pentasaccharide 38 detectable only by ESI-MS. ESI-MS data was consistent with reported solution-phase data.

Functionalization of Resin for Synthesis of Resin-Bound Acceptor 39. Carboxypolystyrene resin (1.14 g containing 1.26 mmol functionalized sites, Novabiochem, 100-200 mesh, 1% crosslinked with divinylbenzene, 1.10 mmol/g loading) was transferred to a 100 mL solid-phase flask. In a separate flask, 1.64 g (3.77 mmol) of a mono-dimethoxytritylated Z-oc-t-4-ene-1,8-diol was dissolved in CH₂Cl₂ (10 mL) and added via cannula to the solid-phase flask. Additional CH₂Cl₂ (10 mL) was added to the flask before agitation commenced. After 10 minutes of shaking, 0.62 g (5.04 mmol) 4,4-dimethylaminopyridine (DMAP) and 0.74 mL (0.64 g, 5.04 mmol) diisopropylcarbodiimide were added and the resulting mixture shaken overnight.

After 18 h, 2 mL methanol were added to the slurry and the mixture left to shake again overnight. After an additional 18 h, the solution was drained from the reaction vessel and the resin was thoroughly washed with CH₂Cl₂, THF, 10% methanol in THF, and CH₂Cl₂ (3 × 50 mL each with 5 min shaking per wash). The resin was vacuum dried in a desiccator over P₂O₅ for 12 h yielding 1.22 g functionalized resin.

Dimethoxytrityl Assay for Determination of Resin Loading. A solution of 3% trichloroacetic acid was made in a 50 mL volumetric flask (1.50 g TCA diluted to 50 mL by addition of CH₂Cl₂). 18.5 mg linker functionalized resin were weighed into a 10 mL volumetric flask and diluted to 10 mL by addition of the 3% TCA in CH₂Cl₂ solution. From this solution, a 200 μL aliquot was diluted to 10 mL by addition of CH₂Cl₂ and absorbance of this solution was read at 504 nm. The average absorbance of this solution over 5 scans was 1.5313, which corresponds to a resin loading of 0.544 mmol/g. Likewise, a 100 μL aliquot was diluted to 10 mL by addition of CH₂Cl₂ and absorbance of this solution was read at 504 nm. The average absorbance of this solution over 5 scans was 0.8154, which corresponds to a resin loading 0.579 mmol/g. The average resin loading of the prepared linker-bound resin was thus determined to be 0.56 mmol/g.
Resin-bound Linker Deprotection (39). The remainder of the functionalized resin was washed with a solution of 3% dichloroacetic acid in CH₂Cl₂ (4 × 50 mL). The resin was then washed with CH₂Cl₂, 1% TEA in CH₂Cl₂, THF, and CH₂Cl₂ (4 × 50 mL each with 5 min shaking per wash). The resin was vacuum dried in a desiccator over P₂O₅ for 12 h yielding 1.11 g of 39.

1,5-Anhydro-3,4-di-O-benzyl-2-deoxy-6-O-(9-fluorenylmethoxycarbonyl)-D-arabinohex-1-enitol. 1,5-Anhydro-3,4-di-O-benzyl-2-deoxy-D-arabinohex-1-enitol (0.60 g, 1.84 mmol) was dissolved in pyridine (20 mL) and 9-fluorenyl methylchloroformate (0.95 g, 3.68 mmol) was added. After 1 h the solution was diluted with ethyl acetate (60 mL) and washed with 5% aqueous HCl (2 × 100 mL), saturated aqueous NaHCO₃, (100 mL), and water (100 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated. The resulting crude residue was purified by flash silica gel chromatography (10→25% EtOAc/Hexanes) to afford 0.88 g (87%) product as a yellow foam. [α]D: -1.38° (c = 1.42, CH₂Cl₂); IR (thin film): 3060, 2947, 1745, 1253, 1233 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.78 (app d, J = 7.5 Hz, 2 H), 7.63 (app d, J = 7.3 Hz, 2 H), 7.45-7.27 (m, 14 H), 6.44 (dd, J = 5.9, 0.9 Hz, 1 H), 4.96 (dd, J = 6.2, 2.8 Hz, 1 H), 4.89 (d, J = 11.2 Hz, 1 H), 4.71 (d, J = 11.2 Hz, 1 H), 4.68 (d, J = 11.5 Hz, 1 H), 4.58 (d, J = 11.5 Hz, 1 H), 4.51 (app d, J = 4.4 Hz, 2 H), 4.43-4.40 (m, 2 H), 4.30-4.18 (m, 3 H), 3.84 (dd, J = 8.4, 5.9 Hz, 1 H); ¹³C NMR (100 MHz, CDCl₃): δ 144.6, 143.6, 141.5, 138.0, 128.7, 128.2, 128.1, 128.0, 127.4, 125.4, 120.3, 111.2, 100.2, 75.2, 75.1, 74.1, 73.8, 70.7, 70.2, 66.4, 46.9; ESI-MS: m/z (M + Na)⁺ calcld 571.2091, obsd 571.2086.

Dibutyl 3,4-di-O-benzyl-6-O-(9-fluorenylmethoxycarbonyl)-2-O-pivaloyl-β-D-glucopyranosyl phosphate 40. 1,5-Anhydro-3,4-di-O-benzyl-2-deoxy-6-O-(9-fluorenylmethoxycarbonyl)-D-arabinohex-1-enitol (590 mg, 1.08 mmol) was azeotroped
with toluene (3 × 3 mL) and then dried under vacuum for 90 minutes. Dichloromethane (10 mL) was added and the solution was cooled to 0°C before adding dimethyldioxirane (20 mL of a 0.08 M solution in acetone, 1.61 mmol). After 30 minutes, volatiles were removed under vacuum and the resulting residue was dried for 10 minutes. The residue was redissolved in dichloromethane (10 mL) and the solution cooled to -78°C before dibutyl phosphate (255 µL, 1.29 mmol) was added dropwise over 30 minutes. After 45 additional minutes, the reaction mixture was warmed to 0°C and DMAP (525 mg, 4.30 mmol) and pivaloyl chloride (264 µL, 2.15 mmol) were added. After 90 minutes, the reaction mixture was diluted with a solution of 25% EtOAc/Hexanes (50 mL) and the resulting suspension was flushed through a one inch plug of silica gel. The filtrate was concentrated and the crude residue was purified by flash silica gel chromatography to provide 0.79 g (87%) of phosphate 40 as a clear oil. \([\alpha]_D: +2.59^\circ\) (c = 1.59, CH₂Cl₂); IR (thin film): 3056, 2954, 2862, 1739, 1256 cm⁻¹; \(^1\)H NMR (400 MHz, CDCl₃): δ 7.77 (app d, J = 7.5 Hz, 2 H), 7.61 (app d, J = 6.8 Hz, 2 H), 7.44-7.24 (m, 14 H), 5.28 (t, J = 7.8 Hz, 1 H), 5.22-5.16 (m, 1 H), 4.85-4.73 (m, 3 H), 4.60 (d, J = 10.9 Hz, 1 H), 4.51 (d, J = 11.5 Hz, 1 H), 4.45-4.23 (m, 4 H), 4.11-3.96 (m, 4 H), 3.78-3.74 (m, 3 H), 1.68-1.56 (m, 4 H), 1.45-1.29 (m, 4 H), 1.22 (s, 9 H), 0.92 (t, J = 7.2 Hz, 3 H), 0.87 (t, J = 7.5 Hz, 3 H); \(^{13}\)C NMR (100 MHz, CDCl₃): δ 177.0, 155.1, 143.5, 143.4, 141.5, 137.9, 137.5, 128.7, 128.6, 128.3, 128.1, 128.0, 127.6, 127.4, 125.4, 120.2, 96.6, 82.9, 76.9, 75.4, 75.3, 73.8, 72.9, 70.3, 68.3, 68.2, 68.1, 68.1, 66.1, 46.9, 39.1, 32.2, 27.3, 18.8, 13.8; ESI-MS: m/z (M + Na)⁺ calcd 881.3427, obsd 881.3419.

**Cleavage of N-(9-Fluorenylethoxy)carbonate (Fmoc) Esters: General Procedure G.** The resin-bound saccharide was washed with DMF (6 × 2.5 mL). A solution of 20% piperidine in DMF (2.2 mL) was added and the reaction mixture agitated (10 s vortex, 50 s rest) for 10 min at 15°C. The reaction vessel was drained to an auxiliary waste container and the deprotection was repeated an additional two times. After the final deprotection, the resin was washed with DMF (6 × 2.5 mL), acetic acid–methanol (0.2 M in THF, 6 × 3 mL), THF (6 × 3.5 mL), and CH₂Cl₂ (6 × 3 mL).
Auxiliary waste samples collected during Fmoc deprotection cycles were analyzed by UV. Each sample was diluted 100 fold (100 µL aliquot diluted to 10 mL by addition of methanol) and the absorbance read at 301 nm. The concentration of the dibenzofulvene-piperidine addition product, a byproduct of the deprotection, was determined using Beer's law: \( A = \varepsilon bc \), where the molar extinction coefficient (\( \varepsilon \)) of the species observed is 7800 L cm\(^{-1}\) mol\(^{-1}\). This data was then correlated to the coupling efficiency of the previous glycosylation step assuming complete deprotection efficiency of the Fmoc group.

**Modified Cleavage of Levulinate Esters: General Procedure H.** The resin-bound saccharide was washed with DMF (6 x 2.5 mL). A solution of hydrazine (10% in DMF, 10 equiv.) was added and the reaction mixture agitated (10 s vortex, 50 s rest) for 15 min at 25°C. The reaction vessel was drained and the deprotection was repeated an additional four times. After the final deprotection, the resin was washed with DMF (6 x 2.5 mL), acetic acid-methanol (0.2 M in THF, 6 x 3 mL), THF (6 x 3.5 mL), and CH\(_2\)Cl\(_2\) (6 x 3 mL).

**Cleavage of Saccharides from the Resin: General Procedure I.** The resin-bound saccharide was vacuum dried upon completion of its automated assembly over P\(_2\)O\(_5\) for 12 h. The resin-bound saccharide was then transferred to a 50 mL solid-phase flask and CH\(_2\)Cl\(_2\) (3.5 mL) was added before agitation commenced. After 5 min of shaking, the flask was charged with methanol (0.5 mL) and agitated an additional 5 min before sodium methoxide (10 equiv.) was added. The flask was shaken for 90 minutes and then the reaction solution was drained into another flask. The resin was then washed with methanol and CH\(_2\)Cl\(_2\) (5 mL each shaking 5 min per wash). The washing solutions were each drained into the same flask as the reaction solution. This deprotection and washing procedure was repeated an additional 3 times. All reaction and washing solutions were combined and acidified to pH 6 by addition of acidic resin. The resin was filtered off and the filtrate concentrated to yield the crude saccharide product. Crude residues were analyzed and purified by HPLC to yield the desired Z-oct-4-ene-8-ol saccharide.
Z-oct-4-ene-8-ol 3,4-di-O-benzyl-2-O-pivaloyl-\(\beta\)-D-glucopyranosyl-(1→6)-3,4-di-O-benzyl-2-O-pivaloyl-\(\beta\)-D-glucopyranoside 41. General Procedure A using functionalized polystyrene resin 39 (50 mg, 0.56 mmol/g, 27 \(\mu\)mol) and glucosyl phosphate 40 (100 mg per glycosylation, 0.125 \(\mu\)mol) was followed by General Procedure G. This cycle was repeated again using glucosyl phosphate 40. The completion of the automated assembly of the disaccharide was followed by General Procedure I, resulting in 24 mg crude saccharide. The crude saccharide was purified by flash silica gel chromatography (25→40% EtOAc/Hexanes) to furnish 6 mg (25% isolated yield) of 41. \([\alpha]_D\): -1.21° (c = 0.65, CH\(_2\)Cl\(_2\)); IR (thin film): 3491, 2968, 1735, 1140, 1079 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.37-7.26 (m, 20H), 5.43-5.34 (m, 2H), 5.08-4.99 (m, 2H), 4.84-4.67 (m, 6H), 4.63 (d, \(J = 11.2\) Hz, 1H), 4.59 (d, \(J = 11.2\) Hz, 1H), 4.53 (d, \(J = 7.8\) Hz, 1H), 4.34 (d, \(J = 8.1\) Hz, 1H), 3.94-3.76 (m, 4H), 3.74-3.67 (m, 7H), 3.53-3.51 (m, 2H), 3.47-3.39 (m, 2H), 2.16-2.04 (m, 4H), 1.65-1.63 (m, 4H), 1.20 (s, 9H), 1.18 (s, 9H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 177.1, 176.9, 138.2, 138.1, 138.0, 130.2, 129.6, 128.7, 128.6, 128.3, 128.2, 127.1, 127.6, 127.6, 101.4, 101.2, 83.3, 78.1, 77.4, 75.8, 75.6, 75.2, 75.2, 75.0, 74.4, 73.1, 69.4, 68.5, 62.4, 62.1, 39.0, 32.7, 29.4, 27.3, 23.9, 23.8; ESI-MS: \(m/z\) (M + Na\(^+\)) calcd 1019.5127, obsd 1019.5111.

1,5-Anhydro-3,6-di-O-benzyl-2-deoxy-4-O-(9-fluorenylmethoxycarbonyl)-\(\alpha\)-arabinohex-1-enitol. 1,5-Anhydro-3,6-di-O-benzyl-2-deoxy-\(\alpha\)-arabinohex-1-enitol (1.54 g, 4.73 mmol) was dissolved in pyridine (50 mL) and 9-fluorenylmethylchloroformate (2.45 g, 9.46 mmol) was added. After 2 h the solution was concentrated in vacuo and the residue was dissolved in EtOAc (100 mL). The solution was washed with 5%aq. HCl, sat. aq. NaHCO\(_3\), and water (2 × 100 mL each). The organic layer was dried over Na\(_2\)SO\(_4\), filtered and concentrated. The resulting residue was purified by flash silica gel
chromatography (0→5→10→25% EtOAc/Hex) to afford 2.05 g (79%) product as a yellow oil. \([\alpha]_D: -6.68^o (c = 2.71, \text{CH}_2\text{Cl}_2); \text{IR (thin film): 3065, 3031, 2868, 1749, 1260 cm}^{-1}; \text{^1H NMR (400 MHz, CDCl}_3): \delta 7.80 (d, J = 7.5 Hz, 2 H), 7.63 (d, J = 7.3 Hz, 2 H), 7.44 (t, J = 7.5 Hz, 2 H), 7.36-7.27 (m, 13 H), 6.52 (dd, J = 6.3, 1.0 Hz, 1 H), 5.22 (t, J = 5.4 Hz, 1 H), 4.95 (dd, J = 6.2, 3.4 Hz, 1 H), 4.69-4.61 (m, 2 H), 4.57 (s, 2 H), 4.46-4.40 (m, 3 H), 4.26 (t, J = 7.3 Hz, 1 H), 4.15-4.13 (m, 1 H), 3.81 (dd, J = 10.6, 6.5 Hz, 1 H), 3.75 (dd, J = 10.5, 4.5 Hz, 1 H); \text{^13C NMR (100 MHz, CDCl}_3): \delta 154.5, 144.9, 143.4, 143.4, 141.5, 138.2, 137.9, 128.6, 127.9, 127.9, 127.4, 125.3, 120.3, 99.4, 75.0, 73.6, 72.3, 70.9, 70.3, 70.3, 68.1, 46.9; \text{ESI-MS: m/z (M + Na)^+ calcd 571.2091, obsd 571.2106.}

![Chemical Structure](image)

**Dibutyl 3,6-di-O-benzyl-4-O-(9-fluorenylmethoxycarbonyl)-2-O-pivaloyl-β-D-glucopyranosyl phosphate 45.** 1,5-Anhydro-3,6-di-O-benzyl-2-deoxy-4-O-(9-fluorenylmethoxycarbonyl)-D-arabino-hex-1-enitol (0.75 g, 1.37 mmol) was azeotroped with toluene (3 × 3 mL) and then dried under vacuum for 3 h. A solution of the glucal in \text{CH}_2\text{Cl}_2 (15 mL) was cooled to 0°C and dimethyl dioxirane (26 mL of a 0.08 M solution in acetone, 2.05 mmol) was added. After 20 min, volatiles were removed under vacuum and the resulting residue was dried for 5 min. The residue was redissolved in \text{CH}_2\text{Cl}_2 (15 mL) and the solution was cooled to –78°C before dibutyl phosphate (0.33 mL, 1.64 mmol) was added dropwise over 10 min. After 10 additional minutes, the reaction mixture was warmed to 0°C and DMAP (0.67 g, 5.48 mmol) and pivaloyl chloride (0.34 mL, 2.74 mmol) were added. After 1 h the reaction mixture was diluted with a 25% EtOAc/Hexanes solution (50 mL) and flushed though a one inch plug of silica gel. The filtrate was concentrated and the residue was purified by flash silica gel chromatography (25→40% EtOAc/Hexanes) to furnish 0.87 g (76%) of 45 as a clear oil. \([\alpha]_D: +10.00^o (c = 0.83, \text{CH}_2\text{Cl}_2); \text{IR (thin film): 2961, 2873, 1754, 1259, 1028 cm}^{-1}; \text{^1H NMR (400 MHz, CDCl}_3): \delta 7.76 (dt, J = 7.5, 1.0 Hz, 2 H), 7.56 (d, J = 7.4 Hz, 1 H), 7.52 (d, J = 7.3 Hz, 1 H), 7.40 (t, J = 7.5 Hz, 2 H), 7.31-7.16 (m, 13 H), 5.29 (appt, J = 7.8 Hz, 1 H), 5.20 (dd, J = 9.3, 8.0 Hz, 1H), 5.05 (t, J = 9.6 Hz, 1 H), 4.65 (d, J = 11.2 Hz, 1 H), 4.62 (d, J = 12.0 Hz, 1 H).
Hz, 1 H), 4.49 (d, J = 12.0 Hz, 1 H), 4.29-4.27 (m, 2 H), 4.12-3.97 (m, 6 H), 3.85-3.81 (m, 2 H), 3.68-3.62 (m, 2 H), 1.67-1.55 (m, 4 H), 1.43-1.30 (m, 4 H), 1.21 (s, 9 H), 0.92 (t, J = 7.4 Hz, 3 H), 0.89 (t, J = 7.4 Hz, 3 H); 13C NMR (100 MHz, CDCl3): δ 176.8, 154.3, 143.4, 143.3, 141.5, 137.8, 137.6, 128.5, 128.5, 128.2, 128.1, 127.9, 127.8, 127.5, 127.4, 125.3, 125.2, 120.3, 96.7, 96.6, 80.2, 74.7, 74.4, 73.7, 73.7, 70.4, 69.2, 68.3, 68.2, 46.8, 39.0, 32.3, 32.2, 32.2, 27.3, 18.8, 18.8, 13.8; 31P NMR (120 MHz, CDCl3): δ 1.24 (s); ESI-MS: m/z (M + Na)+ calcld 881.3636, obsd 881.3620.

1,5-Anhydro-4,6-di-O-benzyl-2-deoxy-3-O-(9-fluorenylmethoxycarbonyl)-d-lyxo-hex-1-enitol. 1,5-Anhydro-4,6-di-O-benzyl-2-deoxy-3-d-lyxo-hex-1-enitol (0.63 g, 1.93 mmol) was dissolved in pyridine (25 mL) and 9-fluorenyl methylchloroformate (1.00 g, 3.85 mmol) was added. After 2 h the solution was concentrated in vacuo and the residue was dissolved in EtOAc (100 mL). The solution was washed with 5%aq. HCl, sat. aq. NaHCO3, and water (2 × 100 mL each). The organic layer was dried over Na2SO4, filtered and concentrated. The resulting residue was purified by flash silica gel chromatography (0→5→10→25% EtOAc/Hexanes) to afford 0.90 g (85%) product as a yellow oil. [α]D: -27.68° (c = 0.69, CH2Cl2); IR (thin film): 3031, 2871, 1743, 1261, 1236 cm⁻¹; 1H NMR (400 MHz, CDCl3): δ 7.77 (dd, J = 7.6, 3.3 Hz, 2 H), 7.61 (dd, J = 6.9, 4.6 Hz, 2 H), 7.42-7.26 (m, 15 H), 6.47 (dd, J = 6.2, 1.2 Hz, 1 H), 5.40-5.38 (m, 1 H), 4.84 (ddd, J = 6.1, 3.5, 0.7 Hz, 1 H), 4.76 (d, J = 11.8 Hz, 1 H), 4.59-4.49 (m, 3 H), 4.42-4.35 (m, 2 H), 4.32-4.30 (m, 1 H), 4.25 (t, J = 7.4 Hz, 1 H), 4.06 (t, J = 3.4 Hz, 1 H), 3.85 (dd, J = 10.3, 7.7 Hz, 1 H), 3.72 (dd, J = 10.4 , 4.5 Hz, 1 H); 13C NMR (100 MHz, CDCl3): δ 155.0, 146.2, 143.5, 143.5, 141.5, 138.1, 137.9, 128.6, 128.6, 128.3, 128.1, 128.0, 127.4, 125.4, 120.3, 98.1, 75.6, 73.4, 70.8, 70.0, 67.9, 46.9; ESI-MS: m/z (M + Na)+ calcld 571.2091, obsd 571.2088.
Dibutyl 4,6-di-O-benzyl-3-O-(9-fluorenlymethoxycarbonyl)-2-O-pivaloyl-β-D-galactopyranosyl phosphate 46. 1,5-Anhydro-4,6-di-O-benzyl-2-deoxy-3-O-(9-fluorenlymethoxycarbonyl)-D-lyxo-hex-1-enitol (0.86 g, 1.57 mmol) was azeotroped with toluene (3 × 3 mL) and then dried under vacuum for 1 h. A solution of the galactal in CH₂Cl₂ (15 mL) was cooled to 0°C and dimethyl dioxirane (30 mL of a 0.08 M solution in acetone, 2.35 mmol) was added. After 30 min, volatiles were removed under vacuum and the resulting residue was dried for 5 min. The residue was redissolved in CH₂Cl₂ (20 mL) and the solution was cooled to −78°C before dibutyl phosphate (0.37 mL, 1.88 mmol) was added dropwise over 10 min. After 10 additional minutes, the reaction mixture was warmed to 0°C and DMAP (0.77 g, 6.28 mmol) and pivaloyl chloride (0.39 mL, 3.14 mmol) were added. After 2 h the reaction mixture was diluted with a 25% EtOAc/Hexanes solution (50 mL) and flushed through a one inch plug of silica gel. The filtrate was concentrated and the residue was purified by flash silica gel chromatography (25% EtOAc/Hexanes) to furnish 1.13 g (85%) of 46 as a clear oil. [α]₀°D: +13.59° (c = 2.20, CH₂Cl₂); IR (thin film): 3031, 2962, 2934, 1747, 1266 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.74 (dd, J = 7.5, 4.5 Hz, 2 H), 7.59 (d, J = 7.5 Hz, 2 H), 7.42-7.23 (m, 15 H), 5.51 (dd, J = 10.5, 7.9 Hz, 1 H), 5.25 (t, J = 7.3 Hz, 1H), 4.87 (dd, J = 10.5, 3.0 Hz, 1 H), 4.72 (d, J = 11.3 Hz, 1 H), 4.52-4.33 (m, 5 H), 4.21 (t, J = 7.2 Hz, 1 H), 4.08-3.95 (m, 5 H), 3.86 (t, J = 6.6 Hz, 1 H), 3.70-3.61 (m, 2 H), 1.65-1.57 (m, 4 H), 1.41-1.33 (m, 4 H), 1.19 (s, 9 H), 0.92 (t, J = 7.4 Hz, 3 H), 0.89 (t, J = 7.4 Hz, 3 H); ¹³C NMR (100 MHz, CDCl₃): δ 176.9, 154.6, 143.3, 143.1, 141.5, 141.4, 137.8, 137.7, 128.7, 128.5, 128.5, 128.1, 128.1, 128.0, 128.0, 127.4, 125.3, 120.3, 96.9, 75.4, 73.9, 73.7, 73.4, 70.4, 69.2, 68.2, 68.2, 68.1, 68.0, 67.6, 46.8, 39.0, 32.3, 32.2, 32.2, 27.2, 18.8, 13.8, 13.7; ³¹P NMR (120 MHz, CDCl₃): δ 1.11 (s); ESI-MS: m/z (M + Na)⁺ calcd 881.3636, obsd 881.3666.

tert-Butyldimethylsilyl 4,6-O-benzylidine-2-deoxy-3-O-(9-fluorenlymethoxycarbonyl)-2-trichloroacetimidob-β-D-glucopyranoside.
Butyldimethylsilyl 4,6-O-benzylidene-2-deoxy-2-trichloroacetimido-β-D-glucopyranoside (2.58 g, 5.04 mmol) was dissolved in pyridine (50 mL) and 9-fluorenyl methylchloroformate (2.45 g, 9.46 mmol) was added. After 1 h the solution was concentrated in vacuo and the residue was dissolved in EtOAc (150 mL). The solution was washed with 5% aq. HCl, sat. aq. NaHCO₃, and water (2 × 100 mL each). The aqueous layers were each back extracted with EtOAc (75 mL) and the combined organic layers were dried over Na₂SO₄, filtered and concentrated. The resulting residue was purified by flash silica gel chromatography (0→5→10→25% EtOAc/Hexanes) to afford 3.40 g (92%) tert-butyldimethylsilyl 4,6-O-benzylidene-2-deoxy-3-O-(9-fluorenylethoxycarbonyl)-2-trichloroacetimido-β-D-glucopyranoside as a white foam. [α]D: -36.48° (c = 0.88, CH₂Cl₂); IR (thin film): 3362, 2956, 2930, 1724, 1275 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.76 (d, J = 7.6 Hz, 2 H), 7.56 (t, J = 7.2 Hz, 2 H), 7.49-7.46 (m, 2 H), 7.42-7.34 (m, 6 H), 7.29 (td, J = 7.4, 0.9 Hz, 1 H), 7.22 (td, J = 7.5, 0.8 Hz, 1 H), 7.10 (d, J = 9.3 Hz, 1 H), 5.56 (s, 1 H), 5.29 (t, J = 10.0 Hz, 1H), 4.83 (d, J = 7.8 Hz, 1 H), 4.37-4.22 (m, 3 H), 4.14-4.05 (m, 2 H), 3.83 (t, J = 9.5 Hz, 1 H), 3.76 (t, J = 10.2 Hz, 1 H), 3.46 (td, J = 9.7, 4.9 Hz, 1 H), 0.82 (s, 9 H), 0.05 (s, 3 H), -0.03 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃): δ 162.1, 155.5, 143.2, 143.0, 141.4, 141.3, 137.1, 129.2, 128.4, 128.2, 128.2, 127.5, 127.4, 126.3, 125.4, 120.3, 101.5, 96.5, 92.6, 78.7, 75.4, 71.1, 65.5, 66.4, 58.6, 46.5, 25.6, 17.9,-3.9, -5.3; ESI-MS: m/z (M + Na)⁺ calcd 770.1481, obsd 770.1453.

tert-Butyldimethylsilyl 6-O-benzyl-2-deoxy-3-O-(9-fluorenylethoxycarbonyl)-4-O-levulinyl-2-trichloroacetimido-β-D-glucopyranoside. A solution of tert-butyldimethylsilyl 4,6-O-benzylidene-2-deoxy-3-O-(9-fluorenylethoxycarbonyl)-2-trichloroacetimido-β-D-glucopyranoside (2.62 g, 3.56 mmol) in CH₂Cl₂ (20 mL) was cooled to 0°C and treated with triethylsilane (3.40 mL, 21.35 mmol). After 10 min, trifluoroacetic anhydride (0.50 mL, 3.56 mmol) was added, followed by trifluoroacetic acid (1.37 mL, 17.8 mmol). The solution was slowly warmed to room temperature and after 1 h the reaction was quenched by addition of sat. aq NaHCO₃ (100 mL). The
mixture was extracted with EtOAc (100 mL) and the organic layer was washed with sat. 
aq NaHCO₃ (3 × 100 mL), dried over Na₂SO₄, filtered and concentrated. The residue 
was purified by flash silica gel chromatography (10→25% EtOAc/Hexanes) to yield 2.18 
g (83%) tert-butylidimethylsilyl 6-O-benzyl-2-deoxy-3-O-(9-fluorenylmethoxycarbonyl)- 
2-trichloroacetimidido-β-D-glucopyranoside as a white foam.

A solution of levulinic acid (0.21 mL, 2.09 mmol) was treated with DMAP (0.27 
g, 2.19 mmol) and cooled to 0°C. After 30 min, diisopropylcarbodiimide (DIPC) (0.31 
mL, 1.99 mmol) was added. After an additional 30 minutes a solution of tert- 
butylidimethylsilyl 6-O-benzyl-2-deoxy-3-O-(9-fluorenylmethoxycarbonyl)- 
2-trichloroacetimidido-β-D-glucopyranoside in CH₂Cl₂ (10 mL) was added via cannula. The 
reaction mixture was shielded from light with foil and allowed to slowly warm to ambient 
temperature over 4 h. A solution of 25% EtOAc/Hexanes (100 mL) was added and the 
reaction mixture was flushed through a one inch pad of silica gel. The filtrate was 
concentrated and the residue purified by flash silica gel chromatography (20% 
EtOAc/Hexanes) to afford 1.12 g (67%) tert-butylidimethylsilyl 6-O-benzyl-2-deoxy-3-O- 
(9-fluorenylmethoxycarbonyl)-4-O-levulinyl-2-trichloroacetimidido-β-D-glucopyranoside 
as a clear oil. [α]D: -1.78° (c = 2.64, CH₂Cl₂); IR (thin film): 3361, 2929, 1753, 1721, 
1276 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.76 (d, J = 7.4 Hz, 2 H), 7.59 (dd, J = 7.4, 1.9 
Hz, 2 H), 7.42-7.27 (m, 10 H), 6.79 (d, J = 8.6 Hz, 1 H), 5.28-5.18 (m, 2 H), 5.01 (d, J = 
7.9 Hz, 1H), 4.56 (s, 2 H), 4.44 (dd, J = 8.8, 6.0 Hz, 1 H), 4.31-4.24 (m, 2 H), 3.94-3.89 
(m, 1 H), 3.76-3.73 (m, 1 H), 3.64-3.62 (m, 2 H), 2.63 (t, J = 6.2 Hz, 2 H), 2.42 (t, J = 6.6 
Hz, 2 H), 2.07 (s, 3 H), 0.89 (s, 9 H), 0.16 (s, 3 H), 0.12 (s, 3 H); ¹³C NMR (100 MHz, 
CDCl₃): δ 206.1, 171.6, 162.0, 155.3, 143.4, 143.2, 141.4, 141.2, 138.1, 128.7, 128.5, 
128.1, 127.8, 127.5, 125.5, 125.4, 120.2, 120.1, 95.5, 92.5, 76.0, 73.7, 73.5, 71.1, 
69.5, 69.4, 58.4, 46.5, 37.8, 29.7, 27.9, 25.7, 17.9,-3.9, -5.2; ESI-MS: m/z (M + Na)+ 

6-O-Benzyl-2-deoxy-3-O-(9-fluorenylmethoxycarbonyl)-4-O-levulinyl-2- 
trichloroacetimidido-α-D-glucopyranosyl trichloroacetimide. A solution of tert-
butyldimethylsilyl 6-O-benzyl-2-deoxy-3-O-(9-fluorenylmethoxycarbonyl)-4-O-levulinyl-2-trichloroacetimido-β-D-glucopyranoside (0.80 g) in THF (10 mL) was transferred to a 30 mL plastic bottle and HF pyridine (0.80 mL) was added. After 9 h the reaction mixture was diluted with 100 mL water and extracted with CH₂Cl₂ (3 × 100 mL). The combined organic extracts were washed with sat. aq. NaHCO₃ (250 mL), dried over Na₂SO₄, filtered and concentrated. The resulting residue was used without further purification.

A solution of 6-O-benzyl-2-deoxy-3-O-(9-fluorenylmethoxycarbonyl)-4-O-levulinyl-2-trichloroacetimido-β-D-glucopyranoside (0.65 g, 0.88 mmol) in CH₂Cl₂ (5 mL) and CCl₃CN (5 mL) was treated with sodium hydride (4 mg of a 60% suspension in oil). Gas evolved from the reaction mixture and the solution became yellow. After 20 minutes, the reaction was quenched by addition of silica gel and the solution was concentrated. The crude product was purified by flash silica gel chromatography (25→40% EtOAc/Hexanes) to furnish 0.71 g (92%) 6-O-benzyl-2-deoxy-3-O-(9-fluorenylmethoxycarbonyl)-4-O-levulinyl-2-trichloroacetimido-α-D-glucopyranosyl trichloroacetimidate as a white foam. [α]D: +43.84° (c = 2.16, CH₂Cl₂); IR (thin film): 3416, 3342, 1755, 1721, 1264 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.83 (s, 1 H), 7.77 (d, J = 7.5 Hz, 2 H), 7.58 (d, J = 7.5 Hz, 2 H), 7.42 (td, J = 7.4, 2.4 Hz, 2 H), 7.38-7.28 (m, 8 H), 7.15 (d, J = 8.3 Hz, 1 H), 6.57 (d, J = 3.5 Hz, 1 H), 5.55 (t, J = 9.9 Hz, 1H), 5.37 (t, J = 10.8 Hz, 1H), 4.58-4.52 (m, 3 H), 4.46 (dd, J = 10.0, 7.4 Hz, 1 H), 4.34 (dd, J = 10.0, 7.7 Hz, 1 H), 4.27 (appt, J = 7.5 Hz, 1 H), 4.18 (dt, J = 10.1, 3.2 Hz, 1 H), 3.70 (dd, J = 11.2, 2.7 Hz, 1 H), 3.64 (dd, J = 11.2, 3.8 Hz, 1 H), 2.71-2.67 (m, 2 H), 2.46-2.43 (m, 2 H), 2.07 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃): δ 206.2, 171.2, 162.2, 155.8, 143.3, 143.1, 141.4, 141.3, 138.7, 128.5, 128.2, 128.2, 127.9, 127.5, 127.4, 125.4, 120.3, 120.2, 93.9, 91.9, 90.7, 74.5, 73.8, 72.0, 71.2, 67.8, 67.6, 54.3, 46.6, 37.9, 29.8, 27.9; ESI-MS: m/z (M + Na)⁺ calcd 899.0237, obsd 899.0245.

Dibutyl 6-O-benzyl-2-deoxy-3-O-(9-fluorenylmethoxycarbonyl)-4-O-levulinyl-2-trichloroacetimido-β-D-glucopyranosyl phosphate 47. A solution of 6-O-benzyl-2-
deoxy-3-O-(9-fluorenlymethoxycarbonyl)-4-O-levulinyl-2-trichloroacetimido-α-D-glucopyranosyl trichloroacetimide (1.49 g, 1.69 mmol) in toluene (15 mL) was cooled to 0°C before treating dropwise with dibutyl phosphate (0.40 mL, 2.03 mmol). After 1 h, the solution was warmed to room temperature and concentrated under vacuum. The crude residue was purified by flash silica gel chromatography (40% EtOAc/Hexanes) to yield 1.35 g (86%) of 47 as a waxy solid. [α]D: +7.02° (c = 0.47, CH2Cl2); IR (thin film): 3343, 3252, 2962, 1758, 1721 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.08 (d, J = 9.4 Hz, 1 H), 7.74 (d, J = 7.6 Hz, 2 H), 7.58 (d, J = 7.5 Hz, 2 H), 7.40-7.25 (m, 10 H), 5.62 (t, J = 8.0 Hz, 1 H), 5.34 (t, J = 9.5 Hz, 1H), 5.28 (t, J = 9.5 Hz, 1H), 4.56-4.49 (m, 2 H), 4.47-4.34 (m, 2 H), 4.26-4.20 (m, 2 H), 4.13-3.97 (m, 4 H), 3.87-3.83 (m, 1 H), 3.65 (dd, J = 10.9, 2.8 Hz, 1 H), 3.59 (dd, J = 10.9, 5.1 Hz, 1 H), 4.34 (dd, J = 10.0, 7.7 Hz, 1 H), 4.27 (appt, J = 7.5 Hz, 1 H), 4.18 (dt, J = 10.1, 3.2 Hz, 1 H), 2.60-2.55 (m, 2 H), 2.31 (t, J = 6.1 Hz, 2 H), 2.03 (s, 3 H), 1.64-1.56 (m, 4 H), 1.41-1.31 (m, 4 H), 0.89 (appq, J = 14.3, 7.2 Hz, 6 H); ¹³C NMR (100 MHz, CDCl₃): δ 206.0, 171.5, 162.2, 155.0, 143.6, 143.3, 141.4, 141.2, 137.9, 128.5, 128.0, 127.9, 127.8, 127.5, 127.3, 125.6, 125.5, 120.1, 120.1, 96.4, 92.6, 76.9, 76.2, 74.0, 73.7, 71.1, 68.9, 68.6, 68.5, 56.0, 46.6, 37.8, 32.2, 32.1, 29.8, 27.8, 18.7, 13.8; ³¹P NMR (120 MHz, CDCl₃): δ 0.79 (s); ESI-MS: m/z (M + Na)⁺ calcd 948.2056, obsd 948.2082.

![Diagram](https://example.com/diagram.png)

Dibutyl 3,4,6-tri-O-benzyl-2-O-(9-fluorenlymethoxycarbonyl)-β-D-galactopyranosyl phosphate 49. 1,5-Anhydro-3,4,6-tri-O-benzyl-2-deoxy-β-D-lyxo-hex-1-enitol (1.11 g, 2.67 mmol) was azeotroped with toluene (3 × 3 mL) and then dried under vacuum for 30 min. A solution of the galactal in CH₂Cl₂ (25 mL) was cooled to 0°C and dimethyl dioxirane (50 mL of a 0.08 M solution in acetone, 4.00 mmol) was added. After 15 min, volatiles were removed under vacuum and the resulting residue was dried for 5 min. The residue was redissolved in CH₂Cl₂ (20 mL) and the solution was cooled to −78°C before dibutyl phosphate (0.64 mL, 3.21 mmol) was added dropwise over 10 min. After 10 additional minutes, the reaction mixture was warmed to 0°C and DMAP (1.30 g, 10.68 mmol) and 9-fluorenly methylchloroformate (1.38 g, 5.34 mmol) were added. After 90
min the reaction mixture was diluted with a 25% EtOAc/Hexanes solution (100 mL) and flushed through a one inch plug of silica gel. The filtrate was concentrated and the residue was purified by flash silica gel chromatography (25→40% EtOAc/Hexanes) to furnish 1.11 g (49%) of 49 as a clear oil. [α]D: +15.00° (c = 1.06, CH2Cl2); IR (thin film): 3241, 2960, 1759, 1256, 1028 cm⁻¹; 1H NMR (400 MHz, CDCl3): δ 7.77 (dd, J = 7.5, 2.6 Hz, 2 H), 7.62 (dd, J = 7.5, 0.7 Hz, 2 H), 7.42-7.22 (m, 20 H), 5.33-5.23 (m, 2 H), 4.97 (d, J = 11.5 Hz, 1H), 4.70 (d, J = 12.1 Hz, 1H), 4.61 (d, J = 11.5 Hz, 1H), 4.60 (d, J = 12.1 Hz, 1H), 4.49-4.44 (m, 3 H), 4.29 (dd, J = 10.1, 7.6 Hz, 1 H), 4.22 (appt, J = 7.4 Hz, 1 H), 4.05-3.88 (m, 4 H), 3.73-3.71 (m, 1 H), 3.68-3.64 (m, 2 H), 3.60 (dd, J = 8.8, 3.3 Hz, 1 H), 1.63-1.56 (m, 2 H), 1.48-1.43 (m, 2 H), 1.39-1.31 (m, 2 H), 1.27-1.19 (m, 2 H), 0.87 (t, J = 7.4 Hz, 3 H), 0.78 (t, J = 7.4 Hz, 3 H), 13C NMR (100 MHz, CDCl3): δ 254.6, 143.6, 143.4, 141.5, 138.4, 137.8, 137.7, 128.7, 128.5, 128.4, 128.1, 127.9, 127.4, 125.5, 125.4, 120.2, 80.0, 74.9, 74.4, 72.7, 72.6, 70.5, 68.1, 68.0, 46.9, 32.3, 32.2, 18.8, 18.7, 13.8, 13.7; 31P NMR (120 MHz, CDCl3): δ 1.25 (s); ESI-MS: m/z (M + Na)+ calcd 887.3531, obsd 887.3528.

Automated Assembly of a Protected Le³ Pentasaccharide (42): Z-oct-4-ene-8-ol 3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-[2-O-benzyl-3,4-di-O-pivaloyl-α-L-fucopyranosyl-(1→3)],[6-O-benzyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranosyl-(1→3)-]4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranoside. General Procedure A using functionalized polystyrene resin 39 (51 mg, 0.56 mmol/g, 28 μmol) and glucosyl phosphate 45 (100 mg per glycosylation, 125 μmol) was followed by General Procedure F. This cycle was repeated using galactosyl phosphate 46 (100 mg per glycosylation, 125 μmol) in General Procedure A. General Procedure A was then modified to include a triple glycosylation with glucosamine phosphate 47 (80 mg per glycosylation, 88 μmol) and followed again by General Procedure G. General Procedure A using fucosyl
phosphate 48 (80 mg per glycosylation, 125 μmol), was followed by General Procedure H. Finally, General Procedure A using galactosyl phosphate 49 (110 mg per glycosylation, 125 μmol) was followed by General Procedure G. The completion of the automated assembly of the saccharide was followed by General Procedure I, resulting in 29 mg crude saccharide and a 51% yield (HPLC peak area) of the crude protected pentasaccharide. The crude saccharide was separated into 2 fractions, each of which was purified by preparative HPLC using a silica gel column (15–40% EtOAc/Hexanes over 20 min) to afford 8 mg (12.6% isolated yield) of pure 42. 1H NMR (400 MHz, CDCl3): δ 7.40-7.17 (m, 39 H), 7.13-7.08 (m, 4 H), 7.00 (app t, J = 7.6 Hz, 2 H), 6.64 (d, J = 8.3 Hz, 1 H), 5.41-5.22 (m, 7 H), 4.96-4.91 (m, 3 H), 4.77-4.56 (m, 8 H), 4.50-4.39 (m, 8 H), 4.33-4.29 (m, 2 H), 4.25 (app t, J = 12.1 Hz, 1 H), 4.16-4.11 (m, 2 H), 4.01-3.92 (m, 5 H), 3.86-3.70 (m, 6 H), 3.68-3.59 (m, 6 H), 3.56 (t, J = 9.0 Hz, 1 H), 3.43-3.24 (m, 6 H), 3.21 (dd, J = 9.7, 2.6 Hz, 1 H), 2.45 (br s, 1 H), 2.13-2.04 (m, 4 H), 1.62-1.56 (m, 4 H), 1.19 (s, 9 H), 1.18 (s, 9 H), 1.16 (s, 9 H), 1.12 (s, 9 H), 0.80 (d, J = 6.5 Hz, 3 H); ESI-MS: m/z (M + Na)+ calc 2250.9357, obsd 2250.9304.

Automated Assembly of a Protected Leα Hexasaccharide (43): Z-oct-4-ene-8-ol 2-O-benzyl-3,4-di-O-pivaloyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-[2-O-benzyl-3,4-di-O-pivaloyl-α-L-fucopyranosyl-(1→3)]-6-O-benzyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside. General Procedure A using functionalized polystyrene resin 39 (48 mg, 0.56 mmol/g, 27 μmol) and glucosyl phosphate 45 (100 mg per glycosylation, 125 μmol) was followed by General Procedure G. This cycle was repeated using galactosyl phosphate 46 (100 mg per glycosylation, 125 μmol) in General Procedure A. General Procedure A was then modified to include a triple glycosylation with glucosamine.
phosphate 47 (80 mg per glycosylation, 88 μmol) and followed again by General Procedure G. General Procedure A using fucosyl phosphate 48 (80 mg per glycosylation, 125 μmol), was followed by General Procedure H. General Procedure A using galactosyl phosphate 49 (110 mg per glycosylation, 125 μmol) was followed by General Procedure G, and then finished by addition of fucosyl phosphate 48 (80 mg per glycosylation, 125 μmol) using General Procedure A. The completion of the automated assembly of the saccharide was followed by General Procedure I, resulting in 33 mg crude saccharide and a 59% yield (HPLC peak area) of the crude protected hexasaccharide. The crude saccharide was purified by size exclusion chromatography using Bio-Rad Bio-Beads (S-X1) in toluene. Fractions containing the desired hexasaccharide 43 were further purified by preparative HPLC using a C18 reverse phase column (10→50% EtOAc/MeCN over 20 min) to afford 7 mg (9.9% isolated yield) of pure 43. \(^1\)H NMR (400 MHz, CDCl\(_3\)): δ 7.42-7.05 (m, 46 H), 6.98 (app t, \(J = 7.6\) Hz, 2 H), 6.88 (app d, \(J = 6.7\) Hz, 2 H), 6.56 (d, \(J = 7.6\) Hz, 1 H), 5.66 (d, \(J = 3.5\) Hz, 1 H), 5.40-5.26 (m, 10 H), 5.22 (d, \(J = 3.6\) Hz, 1 H), 5.17 (d, \(J = 2.0\) Hz, 1 H), 5.07-5.03 (m, 2 H), 4.96-4.91 (m, 2 H), 4.87 (d, \(J = 11.2\) Hz, 1 H), 4.72-4.60 (m, 11 H), 4.55 (d, \(J = 12.1\) Hz, 1 H), 4.50 (d, \(J = 12.0\) Hz, 1 H), 4.45-4.34 (m, 8 H), 4.32-4.28 (m, 2 H), 4.25-4.19 (m, 2 H), 4.16 (d, \(J = 12.2\) Hz, 1 H), 4.12-4.08 (m, 1 H), 4.07-3.96 (m, 3 H), 3.90-3.86 (m, 2 H), 3.84-3.76 (m, 2 H), 3.73-3.66 (m, 2 H), 3.62-3.60 (m, 2 H), 3.55 (t, \(J = 9.0\) Hz, 1 H), 3.46-3.38 (m, 3 H), 3.35-3.24 (m, 4 H), 2.12-2.06 (m, 4 H), 1.72 (br t, 1 H), 1.62-1.55 (m, 4 H), 1.21 (s, 9 H), 1.20 (s, 9 H), 1.15 (s, 9 H), 1.15-1.14 (m, 27 H), 1.00 (d, \(J = 6.4\) Hz, 3 H), 0.89-0.85 (m, 3 H); ESI-MS: m/z (M+Na\(^+\)) calc 2655.1556, obsd 2655.1486.

\[\text{Automated Assembly of a Protected Le³ – Le³ Nonasaccharide (3): Z-oct-4-ene-8-ol 2-O-benzyl-3,4-di-O-pivaloyl-\(\alpha\)-L-fucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-\(\beta\)-D-galactopyranosyl-(1→4)-[2-O-benzyl-3,4-O-di-pivaloyl-\(\alpha\)-L-fucopyranosyl-(1→3)]-6-}\]
O-benzyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-[2-O-benzyl-3,4-di-O-pivaloyl-α-L-fucopyranosyl-(1→3)]-6-O-benzyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranosyl-(1→3)-4,6-di-O-benzyl-2-O-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside. General Procedure A using functionalized polystyrene resin 39 (51 mg, 0.56 mmol/g, 28 µmol) and glucosyl phosphate 45 (100 mg per glycosylation, 125 µmol) was followed by General Procedure G. This cycle was repeated using galactosyl phosphate 46 (100 mg per glycosylation, 125 µmol) in General Procedure A. General Procedure A was then modified to include a triple glycosylation with glucosamine phosphate 47 (80 mg per glycosylation, 88 µmol) and followed again by General Procedure G. General Procedure A using fucosyl phosphate 48 (80 mg per glycosylation, 125 µmol), was followed by General Procedure H. General Procedure A, using galactosyl phosphate 46 (100 mg per glycosylation, 125 µmol), was followed by General Procedure G. General Procedure A was then modified again to include a triple glycosylation with glucosamine phosphate 47 (80 mg per glycosylation, 88 µmol) and followed by General Procedure G. General Procedure A using fucosyl phosphate 48 (80 mg per glycosylation, 125 µmol), was followed by General Procedure H. General Procedure A using galactosyl phosphate 49 (110 mg per glycosylation, 125 µmol) was followed by General Procedure G, and then finished by addition of fucosyl phosphate 48 (80 mg per glycosylation, 125 µmol) using General Procedure A. The completion of the automated assembly of the saccharide was followed by General Procedure I, resulting in 42 mg crude saccharide and a 50% yield (HPLC peak area) of the crude protected nonasaccharide. The crude saccharide was purified by size exclusion chromatography using Bio-Rad Bio-Beads (S-X1) in toluene. Fractions containing the desired nonasaccharide 44 were further purified by preparative HPLC using a C18 reverse phase column (10→50% EtOAc/MeCN over 20 min) to afford 7 mg (6.5% isolated yield) of pure 44. 1H NMR (400 MHz, CDCl3): δ 7.38-7.01 (m, 69 H), 6.88 (app d, J = 6.8 Hz, 2 H), 6.52 (d, J = 7.5 Hz, 1 H), 5.66 (d, J = 3.4 Hz, 1 H), 5.43-5.34 (m, 5 H), 5.27-5.24 (m, 2 H), 5.20-5.19 (m, 2 H), 5.13-5.06 (m, 5 H), 5.03 (d, J = 10.9 Hz, 1 H), 4.96 (dd, J = 9.2, 8.1 Hz, 1 H), 4.78-4.33 (m, 23 H), 4.30-4.23 (m, 3 H),
4.21-4.15 (m, 2 H), 4.13-4.08 (m, 1 H), 4.07-3.86 (m, 13 H), 3.85-3.57 (m, 20 H), 3.55-3.47 (m, 3 H), 3.45-3.36 (m, 5 H), 3.33-3.29 (m, 2 H), 3.19 (dd, J = 10.1, 6.1 Hz, 1 H), 3.12 (dd, J = 9.7, 5.9 Hz, 1 H), 2.12-2.04 (m, 4 H), 1.63-1.55 (m, 4 H), 1.22 (s, 9 H), 1.23 (s, 9 H), 1.19 (s, 9 H), 1.16-1.10 (m, 39 H), 1.07 (s, 9 H), 1.01 (d, J = 6.4 Hz, 3 H), 0.76 (d, J = 6.4 Hz, 3 H); ESI-MS: m/z (M + 2Na)^2+ calcd 1951.7892, obsd 1951.7727.
3.9 References


Chapter 4
Development of Novel Protecting Groups
This chapter describes work performed in collaboration with Rodrigo Andrade. He prepared compounds 39-42.\textsuperscript{1}

4.1 Introduction

In Chapters 2 and 3, we described new methods for the synthesis of the Type II Lewis oligosaccharides both in solution and on solid-support. Esters and carbonates were used for temporary protection of hydroxyl groups, whereas benzyl groups and more robust pivaloyl esters were used for permanent protection. Complexities and branching found in the Lewis oligosaccharides required a carefully planned protecting group strategy utilizing multiple removable groups. The development and implementation of new protecting groups that are stable during glycosylation reactions and can be selectively removed in high yields under conditions orthogonal to other protecting groups are of utmost importance in carbohydrate synthesis. Two novel hydroxyl protecting groups, the para-chlorophenyl carbonate (CPC) and the 2-(azidomethyl)benzoate ester (AZMB), are described in this chapter.

4.2 Synthetic Strategy

Both the para-chlorophenyl carbonate and the 2-(azidomethyl)benzoate ester were developed with the synthesis of the Lewis oligosaccharides in mind. In synthesizing this family of branched penta- and hexasaccharides our linear strategy forced development of a glucosamine building block with two orthogonally removable groups and a galactose building block with a removable C-2 participating group. While traditional esters provided us with a protecting group capable of stereodirection in glycosylation, we also needed an additional orthogonal group. Temporary protecting groups also must be useful in solid-phase syntheses, as well as in solution-phase syntheses; deprotections must be rapid, high yielding and using conditions compatible with the solid-support.

A survey of the protecting groups used in previous syntheses of the Lewis antigens highlighted a variety of esters and silyl ethers (\textit{vide supra}). Preliminary results
in solid-phase oligosaccharide synthesis indicated silyl ethers were often too bulky for rapid removal from the solid-support, particularly in sterically hindered cases like the glucosamine C-4 position. Modification of traditional esters to afford groups either less robust under basic conditions or removable under neutral conditions allowed glycosyl donors to retain electronic properties important for donor activity while adding a stereodirecting capability.

4.3 Development and Utilization of the para-Chlorophenyl Carbonate Ester as a Hydroxyl Protecting Group

Carbonate esters are relatively stable under acidic and neutral conditions, but are typically quite labile when treated with weak base. It is this base lability that renders carbonates orthogonal to many ester protective groups. Carbonate protection of 5'-hydroxyl groups in oligonucleotide synthesis was explored to avoid depurination associated with the removal of dimethoxytrityl 5'-hydroxyl blocking groups by acid hydrolysis.² The removal of p-nitrophenyl carbonate protecting groups from nucleosides was found to proceed rapidly under mildly basic conditions using imidazole.³ Since the preparation of p-nitrophenyl protected nucleoside building blocks was extremely slow (up to 5 days!), other carbonates that could be more readily introduced were investigated for temporary protection of hydroxyl groups. Oligonucleotides of up to 20 residues were prepared by the phosphoramide method on solid-phase using 9-fluorenylmethoxycarbonyl (Fmoc)² and 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) protected nucleosides.⁴ Both Fmoc and npeoc groups are extremely base labile and served well as transient protecting groups in reiterative syntheses. The npeoc group found additional application in the synthesis of nucleotide-vitamin conjugates.⁵

Most recently, carbonates have been successfully used as 5'-hydroxyl protecting groups in the solid-phase synthesis of a 16-mer of RNA.⁶ Peroxyanions facilitated the reiterative removal of the p-chlorophenyl carbonate group in less than 10 minutes. A buffered peroxyanion solution was used to avoid oxidative ring cleavage without a loss in yield or increased deprotection times.⁷
To date, carbonates have been less frequently applied in oligosaccharide synthesis. Alkyl ethyl carbonate was employed to protect glucose and was subsequently cleaved with a $\text{K}_2\text{CO}_3$ solution (1%) in 15 h. The Fmoc group, although extremely base labile, has been used as a transient protecting group in the solid-phase synthesis of a dodecasaccharide phytoalexin elicitor. Coupling of an Fmoc-protected thioglycoside donor resulted in concomitant loss of the carbonate during work-up with triethylamine. An alternative work-up procedure, relying on dilution of the coupling reaction mixture rather than quenching with triethylamine, avoided loss of Fmoc, but was accompanied by lower yields. The incapacity of Fmoc to withstand a variety of glycosylation conditions makes it less than ideal as a protective group in solution-phase carbohydrate chemistry. Cyclic carbonates have been used to protect the C3 and C4 hydroxyl groups of galactose simultaneously and conformationally constrain the molecule to favor the formation of $\beta$-galactosidic linkages. Cyclic carbonates withstand a variety of glycosylation conditions and their removal is effected by treatment with strong base (NaOMe).

4.3.1 Protection of Monosaccharides and Compatibility Studies with the para-Chlorophenyl Carbonate Ester

Hydroxyl protection by reaction with $p$-chlorophenylchloroformate in the presence of DMAP readily furnished CPC protected monosaccharides 4-7, 12 and 14 in yields exceeding 85% (Scheme 4.1). Differentially protected monosaccharides 4-7, 12 and 14 (Table 4.1) were used to explore the compatibility of the CPC group with other modes of protection such as esters (4, 5, 14), silyl ethers (12), $p$-methoxybenzyl (PMB) ethers, and allyl ethers (6, 7). Using 4-7, a method for the rapid removal of CPC was investigated.

\[
\begin{align*}
\text{R-OH} & \xrightarrow{\text{CH}_2\text{Cl}_2/\text{Pyridine 5:1, DMAP, rt}} \quad 2 \\
\text{Cl} & \quad \text{Cl} \\
\text{O} & \quad \text{O} \\
1 & \quad 2
\end{align*}
\]

\[
\begin{align*}
\text{LiOH, HOOH} & \xrightarrow{\text{THF/H}_2\text{O 3:1, 0\degree C}} \quad 3 \\
\text{Cl} & \quad \text{Cl} \\
\text{O} & \quad \text{O} \\
1 & \quad 1
\end{align*}
\]

Scheme 4.1 General protection and deprotection of alcohols using $p$-chlorophenyl chloroformate

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Initially, a mildly basic peroxide deprotection solution, as used in RNA synthesis, was explored. These mild conditions, however, proved unsuccessful in the removal of the CPC group, likely due to a pH too low for generation of the nucleophilic peroxyanion species responsible for carbonate cleavage. A more strongly basic lithium hydroperoxide solution, often used in the recovery of carboximide chiral auxiliaries, readily achieved CPC removal. Exposure of the monosaccharides to a solution of lithium hydroperoxide in THF/water (3:1) at 0°C rapidly furnished deprotected monosaccharides 8-11 in 90% yield. Both benzoyl and pivaloyl esters endured conditions used to effect CPC removal in monosaccharides 4 and 5. Furthermore, the CPC group was stable under conditions that facilitated the removal of the allyl, PMB, levulinyl (Lev) and triisopropylsilyl (TIPS) groups (Table 4.1).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPO&lt;sub&gt;Bn&lt;/sub&gt; &lt;br&gt; 4 R = Bz &lt;br&gt; 5 R = Piv &lt;br&gt; 6 R = All &lt;br&gt; 7 R = PMB &lt;br&gt; 12 TIPS&lt;sub&gt;Bn&lt;/sub&gt; &lt;br&gt; 13 TIPS&lt;sub&gt;Bn&lt;/sub&gt;</td>
<td>HO&lt;sub&gt;Bn&lt;/sub&gt; &lt;br&gt; 8 R = Bz &lt;br&gt; 9 R = Piv &lt;br&gt; 10 R = All &lt;br&gt; 11 R = PMB &lt;br&gt; 15 CPO&lt;sub&gt;Bn&lt;/sub&gt;</td>
<td>89 &lt;br&gt; 90 &lt;br&gt; 90 &lt;br&gt; 88 &lt;br&gt; 91</td>
</tr>
<tr>
<td>CPO&lt;sub&gt;Bn&lt;/sub&gt; &lt;br&gt; 6 R = All &lt;br&gt; 7 R = PMB &lt;br&gt; 14 R = Lev &lt;br&gt; 12 TIPS&lt;sub&gt;Bn&lt;/sub&gt;</td>
<td>HO&lt;sub&gt;Bn&lt;/sub&gt; &lt;br&gt; 16</td>
<td>&gt;99&lt;sup&gt;b&lt;/sup&gt; &lt;br&gt; 91&lt;sup&gt;c&lt;/sup&gt;</td>
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Table 4.1 Deprotection and functional group compatibility of CPC protected monosaccharides. Reaction conditions: a 6 (1 eq.), Pd(II)chloride (2.5 eq.), and sodium acetate (7 eq.) in acetic acid (90% eq.) at 70°C for 1.5 h; b 7 (1 eq.) and TFA (15 eq.) at room temperature for 1 h; c 14 (1 eq.) and hydrazine acetate (1 eq.) in MeOH/CH<sub>2</sub>Cl<sub>2</sub> at room temperature for 40 min.
4.3.2 Glycosylation with \textit{para}-Chlorophenyl Carbonate Protected Glycosides

Having succeeded in introduction and removal of the CPC group, we sought to employ a CPC protected sugar in glycoside construction. Additionally, the ability of CPC to act as a C-2 participating group in directing the stereochemistry in formation of the glycosidic bond was explored. Commonly used participating groups, such as pivaloyl esters, are often difficult to remove, thus an easily cleavable participating group would greatly facilitate the synthesis of C-2 branched oligosaccharides. Both the tri-$O$-benzyl protected phosphate 18 and the thioethyl glycoside donor 23 containing C-2 CPC protection were synthesized (Scheme 4.2). Attempts to prepare the corresponding trichloroacetimidate donor, as anticipated, did not meet with success due to the instability of carbonate esters to DBU or $K_2CO_3$.\(^4\) Glycosylation of diisopropylidene galactose with glucosyl phosphate 18 yielded 92\% of the desired disaccharide 20.\(^{14}\) Likewise, thioglycoside 23 was used to render disaccharide 20 in 72\% yield.\(^{15}\) In both couplings, exclusively the $\beta$-glycosidic linkage was obtained by virtue of CPC participation (Scheme 4.2). The C-2 CPC carbonate in disaccharide 20 was cleaved within 10 minutes to yield disaccharide 21 in 87\% yield using the standard deprotection protocol.

![Scheme 4.2 Synthesis and use of CPC protected glycosylating agents 18 and 23](image)
4.4 Development and Utilization of the 2-(Azidomethyl)benzoyl Ester as a Hydroxyl Protecting Group

Esters and carbonates, like the CPC group, require varying degrees of basicity to effect removal, therefore protecting groups using neutral or mildly basic conditions for cleavage are most likely to be orthogonal. Protecting groups removed by “assisted cleavage”, in which a latent functionality is unmasked to facilitate an indirect removal of the group, often make use of temperate cleavage conditions.\textsuperscript{16} The 4-azidobutyryl ester in 24 introduced by Kusumoto and coworkers\textsuperscript{17} is removed using mild reductive conditions to transform the γ-azide to an amino group promoting an intramolecular γ-lactamization of intermediate 25 (Scheme 4.3). Recovery of acceptor 26 following cyclization and expulsion of the hydroxyl from 25 was slow; rotational freedom allowed by the aliphatic hydrocarbon required several hours of reflux conditions for complete protecting group removal.

![Scheme 4.3 Mechanism of “assisted cleavage” of the 4-azidobutyryl group](image)

With the 4-azidobutyryl ester in mind, the 2-(azidomethyl)benzoyl group (AZMB)\textsuperscript{18,19} was developed by replacing the saturated carbon chain with an aromatic ring. As demonstrated by Ziegler,\textsuperscript{20} substitution of the butyl group with a phenyl group both increases the rigidity of the system allowing for swift removal of the corresponding lactam and provides the bulk necessary to prevent orthoester formation when used as a C-2 stereodirector in glycosylation.
4.4.1 Synthesis, Protection of Monosaccharides, and Compatibility Studies with the 2-(Azidomethyl)benzoate Ester

Synthesis of the 2-(azidomethyl)benzoyl chloride (AZMB-Cl) commenced with bromination of commercially available methyl 2-methylbenzoate with NBS in the presence of benzoyl peroxide to afford 29 (Scheme 4.4). Displacement of the bromide with sodium azide, followed by saponification yielded 2-azidomethyl benzoic acid 30. Conversion of the 30 to AZMB-Cl 31 was achieved by treatment with thionyl chloride. Installation of the AZMB group was achieved quantitatively by reaction of the free hydroxyl group with the AZMB-Cl and a stoichiometric amount of DMAP. Removal of AZMB is equally facile under Staudinger conditions for reduction of the azide (tributyl phosphine and water).

![Scheme 4.4 Synthesis of 2-(azidomethyl)benzoyl chloride](image)

The compatibility of the AZMB group with other commonly employed used protecting groups for oligosaccharide synthesis was examined (Table 4.2). Differentially protected monosaccharides 32-35 were used to explore orthogonality with other modes of protection including esters, silyl ethers, PMB ethers, and allyl ethers. AZMB was not affected by conditions used for the removal of any of these groups. Similarly, all groups remained intact during AZMB removal from a model monosaccharide. Cleavage of AZMB in the presence of phthaloyl and trichloroethoxycarbonyl (Troc) amino protecting groups afforded high yields of deprotected monosaccharides 41 and 42. The possibility of AZMB removal in the presence of acetate, levinulate, and phthaloyl groups was particularly notable since these protecting groups were used in preparation of the H-type II pentasaccharide.
Table 4.2 Compatibility of AZMB with commonly used hydroxyl and amine protecting groups. Reaction conditions: \( \textsuperscript{a} \) 33(1 equiv.) and hydrazine acetate (1 equiv.) in MeOH/CH\(_2\)Cl\(_2\) at RT, 40 min; \( \textsuperscript{b} \) 34 (1 equiv.) Pd(II)chloride (2.5 equiv.) and sodium acetate (7 equiv.) in acetic acid (90% eq.) at 70°C for 1.5 h; \( \textsuperscript{c} \) 35 (1 equiv.) and TFA (15 equiv.) at RT for 1 h; \( \textsuperscript{d} \) 38 (0.1 M) in a 1:3:3 TFA/THF/H\(_2\)O solution.

4.4.2 2-(Azidomethyl)benzoate Protected Glycosides as Glycosylating Agents

In addition to its utility as a temporary protecting group, we intended to demonstrate the ability of the AZMB group to function as a stereodirecting entity during glycosylation reactions utilizing different glycosyl donors (Scheme 4.5). Glycosyl phosphate 45, thioglycoside 47, and glycosyl trichloroacetimidate 49 each containing a C-2 AZMB group were synthesized. The glycosylating agents were activated following standard protocols\(\textsuperscript{21-23}\) to fashion disaccharides 46 and 52. The AZMB group proved stable to activation conditions and exclusively β-linked glycosidic products were
obtained. Cleavage of AZMB from disaccharide 46 was carried out to highlight its utility as a removable C-2 participating group.

Scheme 4.5 Synthesis and use of AZMB protected donors 45, 47, and 49

4.4.3 Application of the 2-(Azidomethyl)benzoate Group to the Synthesis of the H-Type II Pentasaccharide

As described in Chapter 2, the synthesis of a protected H-type II pentasaccharide required installation of a $\alpha$-(1→2) linkage in the final glycosylation step. This in turn warranted a glycosylating agent capable of fashioning the $\beta$-(1→4) linkage between the galactose and glucosamine building blocks containing a removable participating group. Installation of the AZMB group in the C-2 position of donor 52 allowed for complete stereocontrol in glycosylation of core trisaccharide 51 (Scheme 4.6), as well as
completion of H-type II structure 56 following its facile removal and subsequent fucosylation of acceptor 54.

Scheme 4.6 Completion of H-type II pentasaccharide 56 using AZMB protected donor 52

4.5 Conclusions

We have developed two new hydroxyl protecting groups suitable for use as removable C-2 participating entities in complex carbohydrate synthesis. While both groups display orthogonality with commonly used modes of temporary protection, including silyl and para-methoxybenzyl ethers, pivaloyl and benzoate esters, the AZMB group is superior to the CPC group in that its removal is compatible with all esters. This versatility combined with speedy removal in solution made the AZMB group ideal in solution-phase studies for the synthesis of the Lewis oligosaccharides on solid-support.

4.6 Experimental Section

General Methods. All chemicals were reagent grade and used as supplied unless otherwise noted. Dichloromethane (CH$_2$Cl$_2$), tetrahydrofuran (THF), and toluene were purified by a JT Baker Cycle-Tainer Solvent Delivery System. Analytical thin-layer
chromatography was performed on E. Merck silica gel 60 F$_{254}$ plates (0.25 mm). Compounds were visualized by dipping the plates in a cerium sulfate-ammonium molybdate solution followed by heating. Liquid column chromatography was performed using forced flow of the indicated solvent on Silicycle silica (230 - 400 mesh). $^1$H NMR spectra were obtained using a Bruker-400 NMR spectrometer (400 MHz) or a Varian VXR-500 (500 MHz) and are reported in parts per million ($\delta$) relative to CDCl$_3$ (7.27 ppm). Coupling constants ($J$) are reported in Hertz. $^{13}$C NMR spectra were obtained using a Bruker-400 NMR spectrometer (100 MHz) or a Varian VXR-500 (125 MHz) and are reported in $\delta$ relative to CDCl$_3$ (77.23 ppm) as an internal reference. $^{31}$P spectra were obtained using a Varian VXR-300 NMR spectrometer (120 MHz) or a Varian VXR-500 (200 MHz) and are reported in $\delta$ relative to H$_3$PO$_4$ (0.0 ppm) as an external reference. Optical rotations were measured at 24 °C.

**Protection of Alcohols with CPC: General Procedure A.** A solution of the alcohol (1.0 equiv) in a 5:1 dichloromethane/pyridine mixture (10 mL/mmol alcohol) was stirred at room temperature and DMAP (2.0 equiv) was added, followed by $p$-chlorophenylchloroformate (2.0 equiv). After 1 hour, the reaction mixture was diluted with diethyl ether and washed with HCl (5% aq.), brine, and sat. aq. NaHCO$_3$. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The crude product was purified by flash silica column chromatography.

**Deprotection of CPC Carbonates: General Procedure B.** A solution of the carbonate in a THF/water (3:1) mixture (20 mL/mmol carbonate) was cooled to 0°C. After 10 minutes, hydrogen peroxide (4 equiv) was added followed by LiOH•H$_2$O (2 equiv). Excess peroxides were quenched after 1 hour by dilution with a 1.5 M Na$_2$S$_2$O$_3$ solution. The resulting mixture was extracted with dichloromethane. The organic layer was washed once with sat. aq. NaHCO$_3$, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The crude product was purified by flash silica column chromatography.
Protection of Alcohols with AZMB: General Procedure C. A solution of the alcohol (1.0 equiv) in CH₂Cl₂ (10 mL/mmol alcohol) was stirred at room temperature and DMAP (2 equiv) was added, followed by a solution of the 2-(methylazido)benzoyl (AZMB) chloride 31 (1.5 equiv) in CH₂Cl₂ (1 mL). After 10 minutes, the reaction mixture was diluted with CH₂Cl₂ and washed twice with saturated aqueous NaHCO₃, and once with water. The organic layer was dried (Na₂SO₄), filtered, and concentrated in vacuo. The crude product was purified by flash silica gel column chromatography.

Cleavage of AZMB Esters: General Procedure D. Water (5 equiv) was added to a solution of the ester in THF (10 mL / mmol ester), followed by tributyl phosphine (3 equiv). After 30 minutes, the reaction mixture was diluted with CH₂Cl₂ and washed once with saturated aqueous NaHCO₃, and twice with water. The organic layer was dried (Na₂SO₄), filtered, and concentrated in vacuo. The crude product was purified by flash silica gel column chromatography.

Methyl 2-O-benzoyl-3,4-di-O-benzyl-6-para-chlorophenylcarbonyl-β-D-glucopyranoside 4. General procedure A using 2-O-benzoyl-3,4-di-O-benzyl-β-D-glucopyranoside (0.135 g, 0.28 mmol), DMAP (0.068 g, 0.56 mmol), and p-chlorophenylchloroformate (0.04 mL, 0.31 mmol) gave 150 mg (85%) of 4 as a white solid after purification by flash silica column chromatography (5% ethyl acetate/hexanes). [α]D = +36.31° (c = 2.2, CH₂Cl₂); IR (thin film): 3030, 2915, 1765, 1726, 1265 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.15 (dd, J = 8.3, 1.1 Hz, 2H), 7.71 (ddd, J = 8.3, 8.3, 1.1 Hz, 1H), 7.57 (dd, J = 7.7, 7.7 Hz, 2H), 7.48-7.37 (m, 12H), 7.25-7.22 (m, 2H), 5.41 (dd, J = 9.1, 8.0 Hz, 1H), 5.02 (d, J = 11.0 Hz, 1H), 4.85 (dd, J = 11.2, 11.2 Hz, 2H), 4.76 (d, J = 11.0 Hz, 1H), 4.69 (dd, J = 11.4, 11.4 Hz, 1H), 4.62 (d, J = 7.8 Hz, 1H), 4.49 (dd, J = 11.5, 4.8 Hz, 1H), 4.00 (dd, J = 8.7, 8.7 Hz, 1H), 3.88-3.79 (m, 2H), 6.60 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 165.4, 153.4, 149.7, 137.7, 137.6, 133.4, 131.7, 130.0, 129.9, 129.8, 128.8, 128.6, 128.5, 128.4, 128.2, 127.9, 122.6, 102.0, 82.9, 75.3, 73.7, 73.1, 67.3, 57.1; El-MS m/z (M+Na)⁺: calcd 656.0841, obsd 656.1744.
Methyl 3,4-di-O-benzyl-6-O-para-chlorophenylcarbonyl-2-O-pivaloyl-β-D-glucopyranoside 5. General procedure A using methyl 3,4-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside (0.119 g, 0.26 mmol), DMAP (0.064 g, 0.52 mmol), and p-chlorophenylchloroformate (0.054 mL, 0.39 mmol) gave 136 mg (85%) of 5 as a white solid after purification by flash silica column chromatography (10% ethyl acetate/hexanes). $[\alpha]_D = -2.39^\circ$ (c = 2.52, CH$_2$Cl$_2$); IR (thin film): 3030, 2966, 1756, 1737, 1219 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.34-7.23 (m, 12H), 7.11-7.08 (m, 2H), 5.05 (dd, $J = 8.9, 8.0$ Hz, 1H), 4.84 (d, $J = 11.0$ Hz, 1H), 4.72 (dd, $J = 13.1, 11.3$ Hz, 2H), 4.58 (d, $J = 11.0$ Hz, 1H), 4.53 (dd, $J = 11.5, 1.9$ Hz, 1H), 4.32 (dd, $J = 11.5, 4.0$ Hz, 2H), 3.74 (dd, $J = 8.9, 8.9$ Hz, 1H), 3.69-3.59 (m, 2H), 3.47 (s, 3H), 1.19 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 177.1, 153.4, 149.7, 138.1, 137.7, 131.7, 129.8, 128.8, 128.6, 128.3, 127.9, 127.7, 122.6, 102.4, 83.3, 75.3, 75.2, 73.1, 73.1, 67.3, 57.2, 39.0, 27.3; EI-MS $m/z$ (M+Na)$^+$: calcd 636.0968, obsd 636.2056.

Methyl 2-O-allyl-3,4-di-O-benzyl-6-O-para-chlorophenylcarbonyl-β-D-glucopyranoside 6. General procedure A using methyl 2-O-allyl-3,4-di-O-benzyl-β-D-glucopyranoside (86 mg, 0.207 mmol), DMAP (50 mg, 0.41 mmol), and p-chlorophenylchloroformate (0.057 mL, 0.41 mmol) gave 111 mg (95%) of 6 after purification by flash silica column chromatography (20% ethyl acetate/hexanes). $[\alpha]_D = +2.92^\circ$ (c = 1.25, CH$_2$Cl$_2$); IR (thin film): 3029, 2911, 1764, 1248, 1218 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.40-7.28 (m, 12H), 7.14-7.11 (m, 2H), 5.96 (dddd, $J = 21.8, 10.4, 5.5, 1.1$ Hz, 1H), 5.32 (dd, $J = 17.2, 1.7$ Hz, 1H), 5.20 (dd, $J = 10.4, 1.7$ Hz, 1H), 5.00 (d, $J = 10.9$ Hz, 1H), 4.93 (d, $J = 10.9$ Hz, 1H), 4.82 (d, $J = 10.9$ Hz, 1H), 4.62 (d, $J = 10.9$ Hz, 1H), 4.53 (dd, $J = 11.5, 1.7$ Hz, 1H), 4.46-4.40 (m, 1H), 4.35 (dd, $J = 11.5$ Hz, 4.9 Hz, 1H), 4.32 (d, $J = 7.8$ Hz, 1H), 4.26-4.19 (m, 1H), 3.66 (dd, $J = 8.8, 8.8$ Hz, 1H), 3.57 (s, 3H), 3.35 (dd, $J = 8.9, 7.8$ Hz, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 153.4, 149.7, ...
138.6, 137.9, 135.1, 131.6, 129.7, 128.8, 128.7, 128.3, 128.2, 128.1, 127.9, 122.5, 117.3, 104.7, 84.7, 82.0, 77.3, 75.9, 73.9, 72.8, 67.6, 57.4; EI-MS m/z (M+Na)⁺: calcd 592.0428, obsd 592.1816.

7 Methyl 3,4-di-O-benzyl-2-para-methoxybenzyl-6-O-para-chlorophenyl carbonyl-β-D-glucopyranoside 7. General procedure A using methyl 3,4-di-O-benzyl-2-para-methoxybenzyl-β-D-glucopyranoside (127 mg, 0.258 mmol), DMAP (63 mg, 0.516 mmol), and p-chlorophenylchloroformate (0.07 mL, 0.516 mmol) gave 151 mg (90%) of 7 as a white solid after purification by flash silica column chromatography (5% ethyl acetate/hexanes). [α]D = +26.68° (c = 1.0, CH2Cl2); IR (thin film): 2907, 1764, 1248, 1217, 1088 cm⁻¹; 1H NMR (400 MHz, CDCl₃): δ 7.36-7.26 (m, 15H), 7.14-7.10 (m, 2H), 6.87-6.84 (m, 2H), 4.98 (d, J = 11.0 Hz, 1H), 4.90 (d, J = 10.9 Hz, 1H), 4.86 (d, J = 10.6 Hz, 1H), 4.80 (d, J = 11.0 Hz, 1H), 4.66 (d, J = 10.6 Hz, 1H), 4.60 (d, J = 10.9 Hz, 1H), 4.53 (dd, J = 11.4, 1.4 Hz, 1H), 4.37-4.33 (m, 2H), 3.81 (s, 3H), 3.68 (dd, J = 8.9, 8.8 Hz, 1H), 3.60 (s, 3H), 3.60-3.56 (m, 2H), 3.45 (dd, J = 9.1, 7.9 Hz, 1H); 13C NMR (100 MHz, CDCl₃): δ 159.5, 153.4, 149.7, 138.6, 137.8, 131.6, 130.7, 130.1, 129.7, 128.8, 128.6, 128.3, 128.2, 128.0, 127.9, 122.6, 114.0, 104.9, 84.7, 82.0, 77.4, 75.8, 75.3, 74.7, 72.8, 67.6, 57.5, 55.5; EI-MS m/z (M+Na)⁺: calcd 672.1268, obsd 672.2057.

14 Methyl 3,4-di-O-benzyl-6-O-para-chlorophenylcarbonyl-2-O-levulinoyl-β-D-glucopyranoside 14. General procedure A using methyl 3,4-di-O-benzyl-2-O-levulinoyl-β-D-glucopyranoside (41 mg, 0.087 mmol), DMAP (21 mg, 0.174 mmol), and p-chlorophenylchloroformate (0.017 mL, 0.174 mmol) gave 51 mg (91%) of 14 as a white solid after purification by flash silica column chromatography (10% ethyl acetate/hexanes). [α]D = +4.16° (c = 0.61, CH2Cl2); IR (thin film): 2922, 1766, 1720, 1218, 1088 cm⁻¹; 1H NMR (400 MHz, CDCl₃): δ 7.37-7.28 (m, 12H), 7.14-7.11 (m, 2H), 5.01 (dd, J = 9.1, 7.9 Hz, 1H), 4.88 (d, J = 10.9 Hz, 1H), 4.77 (dd, J = 19.8, 11.4 Hz, 2H),
4.62 (d, J = 11.0 Hz, 1H), 4.55 (dd, J = 11.5, 1.8 Hz, 1H), 4.36-4.32 (m, 2H), 3.74 (dd, J = 9.0, 8.5 Hz, 1H), 3.68-3.60 (m, 2H), 3.49 (s, 3H), 2.74-2.70 (m, 2H), 2.59-2.46 (m, 2H), 2.17 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 206.5, 171.7, 153.4, 138.2, 137.6, 131.7, 129.8, 128.8, 128.7, 128.4, 128.1, 128.0, 122.6, 101.9, 83.0, 75.4, 75.2, 73.5, 73.0, 67.3, 57.2, 38.0, 30.1, 28.1; EI-MS m/z (M+Na)$^+$ calcld 650.0798, obsd 650.1843.

Methyl 2-O-benzoyl-3,4-di-O-benzyl-β-D-glucopyranoside 8. General procedure B using 4 (44 mg, 0.0695 mmol), hydrogen peroxide (0.016 mL, 0.28 mmol) and LiOH·H$_2$O (6 mg, 0.14 mmol) gave 30 mg (89%) of 8 as a white solid after purification by flash silica column chromatography (10% ethyl acetate/hexanes). Characterization data was consistent with literature data.$^{24}$

Methyl 3,4-di-O-benzyl-2-O-pivaloyl-β-D-glucopyranoside 9. General procedure B using 5 (103 mg, 0.168 mmol), hydrogen peroxide (0.04 mL, 0.67 mmol) and LiOH·H$_2$O (14 mg, 0.33 mmol) gave 69 mg (90%) of 9 as a white solid after purification by flash silica column chromatography (10% ethyl acetate/hexanes). [α]$_D$ = -27.64° (c = 0.5, CH$_2$Cl$_2$); IR (thin film): 3031, 2964, 1738, 1136, 1081 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): δ 7.33-7.23 (m, 10H), 4.99 (dd, J = 8.9, 8.2 Hz, 1H), 4.79 (d, J = 10.9 Hz, 1H), 4.74 (d, J = 11.1 Hz, 1H), 4.68 (d, J = 11.0 Hz, 1H), 4.61 (d, J = 10.9 Hz, 1H), 4.31 (dd, J = 7.9, 7.0 Hz, 1H), 3.88 (ddd, J = 12.1, 5.6, 2.6 Hz, 1H), 3.75-3.63 (m, 3H), 3.46 (s, 3H), 3.39 (m, 1H), 1.93 (dd, J = 7.8, 7.8 Hz, 1H), 1.17 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 177.2, 138.2, 138.0, 128.7, 128.6, 128.3, 128.2, 127.9, 127.7, 102.6, 83.2, 75.5, 75.3, 75.2, 73.3, 62.1, 57.4, 39.0, 27.3; EI-MS m/z (M+Na)$^+$: calcld 481.5416, obsd 481.2197.

Methyl 2-O-allyl-3,4-di-O-benzyl-β-D-glucopyranoside 10. Method A: General procedure B using 6 (107 mg, 0.19 mmol), hydrogen peroxide (0.043 mL, 0.76 mmol)
and LiOH·H₂O (16 mg, 0.38 mmol) gave 71 mg (90%) of 10 as a white solid after purification by flash silica column chromatography (20% ethyl acetate/hexanes). Characterization data was consistent with literature data.²⁴

Method B: General Procedure D using 34 (53 mg, 0.092 mmol), water (8 µL, 0.46 mmol), and tributyl phosphine (70 µL, 0.285 mmol) after purification by flash silica gel column chromatography (20% EtOAc/hexanes) yielded 10 (34 mg, 90%). Characterization data were consistent with previously reported data.²⁴

Methyl 3,4-di-O-benzyl-2-para-methoxybenzyl-β-D-glucopyranoside 11. Method A: General Procedure B using 7 (40 mg, 0.062 mmol), hydrogen peroxide (0.007 mL, 0.246 mmol) and LiOH·H₂O (0.007 mL, 0.123 mmol) gave 27 mg (88%) of 11 as a white solid after purification by flash silica column chromatography (30% ethyl acetate/hexanes). [α]D = +10.61° (c = 1.45, CH₂Cl₂); IR (thin film): 3030, 2910, 1514, 1248, 1070 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.36-7.26 (m, 13H), 6.87-6.83 (m, 2H), 4.94 (d, J = 11.0 Hz, 1H), 4.87 (d, J = 11.3 Hz, 1H), 4.84 (d, J = 11.2 Hz, 1H), 4.81 (d, J = 10.9 Hz, 1H), 4.66 (dd, J = 10.7, 3.7 Hz, 2H), 4.35 (d, J = 7.8 Hz, 1H), 3.89 (ddd, J = 11.7, 5.6, 2.7 Hz, 1H), 3.81 (s, 3H), 3.73 (ddd, J = 12.2, 7.8, 4.4 Hz, 1H), 3.66 (dd, J = 9.0, 9.0 Hz, 1H), 3.40 (s, 3H), 3.57 (dd, J = 9.2, 9.2 Hz, 1H), 3.39 (dd, J = 9.1, 7.8 Hz, 1H), 3.39-3.35 (m, 1H), 1.93 (dd, J = 7.6, 5.9 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃): δ 159.5, 138.7, 138.2, 130.7, 130.0, 128.7, 128.6, 128.3, 128.2, 128.1, 127.9, 114.0, 105.1, 84.6, 82.2, 77.1, 75.9, 75.3, 75.2, 74.7, 62.2, 57.6, 55.5; EI-MS m/z (M+Na)⁺: calcd 517.5746, obsd 517.2172.

Method B: General Procedure D using 35 (51 mg, 0.078 mmol), water (7 µL, 0.39 mmol), and tributyl phosphine (60 µL, 0.234 mmol) after purification by flash silica gel column chromatography (20% EtOAc/hexanes) afforded 11 (35 mg, 91%). Characterization data were consistent with previously reported data.²⁵
Methyl 3,4-di-O-benzyl-6-O-para-chlorophenylcarbonyl-β-D-glucopyranoside 15.

Method A. A solution of 6 (79 mg, 0.14 mmol) in acetic acid (90% aq., 5 mL) was treated with Pd(II)chloride (62 mg, 0.35 mmol), and sodium acetate (114 mg, 0.84 mmol). The reaction mixture was warmed to 70°C and stirring for 1.5 hours. The reaction mixture was allowed to cool to room temperature, diluted with ethyl acetate (25 mL), and washed repeatedly with sat. aq. NaHCO₃ (3 × 30 mL), brine (30 mL), and water (30 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to yield a yellow residue. The residue was purified by flash silica column chromatography (20% ethyl acetate/hexanes) to yield 15 (70 mg, 94.5%). \([\alpha]_D = +4.12^\circ\) (c = 0.68, CH₂Cl₂); IR (thin film): 3053, 2916, 1756, 1265, 738 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.37-7.23 (m, 12H), 7.09-7.05 (m, 2H), 4.93 (d, \(J = 11.2\) Hz, 1H), 4.89 (d, \(J = 10.9\) Hz, 1H), 4.84 (d, \(J = 11.2\) Hz, 1H), 4.59 (d, \(J = 10.9\) Hz, 1H), 4.49 (dd, \(J = 11.5, 1.7\) Hz, 1H), 4.32 (dd, \(J = 11.4, 4.6\) Hz, 1H), 4.18 (d, \(J = 7.6\) Hz, 1H), 3.61-3.46 (m, 7H), 2.35 (d, \(J = 2.1\) Hz, 1H); ¹³C NMR (100 MHz, CDCl₃): δ 153.4, 149.7, 138.6, 137.8, 131.7, 129.8, 128.8, 128.7, 128.3, 128.2, 128.1, 122.6, 103.8, 84.5, 75.4, 75.4, 74.8, 73.2, 67.5, 57.6, 29.9; EI-MS m/z (M+Na)⁺: calcd 551.4780, obsd 551.1443.

Method B: To a solution of 7 (49 mg, 0.076 mmol) in dichloromethane (1 mL) 15 eq. trifluoroacetic acid (0.09 mL, 1.14 mmol) were added. The reaction mixture was quenched by addition of sat. aq. NaHCO₃ (20 mL) and extracted with ethyl acetate (3 × 20 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by flash silica column chromatography (30% ethyl acetate/hexanes) to yield 41 mg of 15 (quant. yield) as a white solid. Characterization data were consistent with that previously reported for 15.

Method C. A solution of hydrazine acetate (9 mg, 0.115 mmol) in methanol (0.2 mL) was added to a solution of 14 (72 mg, 0.115 mmol) in dichloromethane (2 mL) and the resulting solution was stirred for 40 minutes at room temperature. The reaction mixture was diluted with dichloromethane (20 mL) and concentrated in vacuo. The crude product was purified by flash silica column chromatography (40%ethyl acetate/hexanes)
to give 55 mg (91%) of 15 as a white solid. Characterization data were consistent with that previously reported for 15.

Methyl 3,4-di-O-benzyl-2-O-para-chlorophenylcarbonyl-6-O-triisopropylsilyl-β-D-glucopyranoside 12. General procedure A using methyl 3,4-di-O-benzyl-6-O-triisopropylsilyl-β-D-glucopyranoside (133 mg, 0.25 mmol), DMAP (61 mg, 0.5 mmol), and p-chlorophenylchloroformate (0.07 mL, 0.5 mmol) gave 165 mg (96%) of 12 as a white solid after purification by flash silica column chromatography (5% ethyl acetate/hexanes). [α]_D = -24.11° (c = 1.33, CH₂Cl₂); IR (thin film): 3031, 2942, 1773, 1253, 1066 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.38-7.29 (m, 12H), 7.05-7.01 (m, 2H), 4.92 (d, J = 11.3 Hz, 1H), 4.86 (d, J = 10.8 Hz, 1H), 4.82-4.75 (m, 3H), 4.41 (d, J = 8.0 Hz, 1H), 4.03 (dd, J = 11.3, 1.8 Hz, 1H), 3.98 (dd, J = 11.3, 3.6 Hz, 1H), 3.83 (dd, J = 9.1, 9.1 Hz, 1H), 3.77 (dd, J = 9.1, 9.1 Hz, 1H), 3.50 (s, 3H), 3.36 (ddd, J = 9.2, 3.4, 1.9 Hz), 1.16-1.06 (m, 21H); ¹³C NMR (100 MHz, CDCl₃): δ 149.8, 138.3, 138.2, 131.5, 129.6, 128.8, 128.7, 128.6, 128.3, 128.2, 128.0, 127.9, 122.5, 101.3, 83.0, 78.4, 77.7, 77.4, 76.4, 75.7, 75.3, 62.2, 56.5, 18.2, 18.1, 12.2; EI-MS m/z (M+Na)^+: calcd 708.3220, obsd 708.3216.

Methyl 3,4-di-O-benzyl-6-O-triisopropylsilyl-β-D-glucopyranoside 13. Method A: General procedure B using 12 (80 mg, 0.12 mmol), hydrogen peroxide (0.027 mL, 0.47 mmol), and LiOH·H₂O (10 mg, 0.235 mmol) gave 57 mg (91%) of 13 as a clear oil after purification by flash column silica chromatography (20% ethyl acetate/hexanes). [α]_D = -6.00° (c = 2.22, CH₂Cl₂); IR (thin film): 3440, 3031, 2865, 1114, 1057 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.42-7.28 (m, 10H), 4.91 (d, J = 3.0 Hz, 2H), 4.89 (d, J = 10.9 Hz, 1H), 4.74 (d, J = 10.8 Hz, 1H), 4.2 (d, J = 7.6 Hz, 1H), 4.02 (dd, J = 11.2, 1.9 Hz, 1H), 3.96 (d, J = 11.2, 4.0 Hz, 1H), 3.70 (dd, J = 9.1, 9.1 Hz, 1H), 3.61 (dd, J = 9.0, 9.0 Hz, 1H), 3.53 (s, 3H), 3.53-3.48 (m, 1H), 3.35 (ddd, J = 9.5, 3.9, 1.9 Hz, 1H), 2.45 (s, 1H), 177
1.16-1.07 (m, 21 H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 138.8, 138.5, 128.7, 128.7, 128.2, 128.0, 127.9, 103.5, 84.7, 76.4, 75.4, 75.2, 74.8, 62.5, 56.8, 18.2, 18.1, 13.5, 12.2; El-MS m/z (M+Na)$^+$: calcd 553.7702, obsd 553.7709.

Method B: General Procedure D using 43 (55 mg, 0.11 mmol), water (7 μL, 0.415 mmol), and tributyl phosphine (60 μL, 0.248 mmol) after purification by flash silica gel column chromatography (15% EtOAc/hexanes) yielded 44 (36 mg, 86%). Characterization data were consistent with previously reported data.²⁵

Methyl 3,4-di-O-benzyl-2-O-para-chlorophenylcarbonyl-β-D-glucopyranoside 16. A solution of 12 (84 mg, 0.123 mmol) in THF (3 mL) was treated with tetrabutylammonium fluoride (0.245 mL of a 1.0 M solution in THF) and acetic acid (7 μL, 0.123 mmol) at room temperature. Additional TBAF (0.12 mL, 0.12 mmol) was added after 12 hours. The reaction mixture was diluted with water (20 mL) and extracted with diethyl ether (3 × 30 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by flash silica column chromatography (30% ethyl acetate/hexanes) to yield 40 mg (60%) of 16 as a white solid. [α]$_D$ = -28.12° (c = 2.36, CH$_2$Cl$_2$); IR (thin film): 3456, 3031, 2876, 1770, 1255 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): δ 7.39-7.30 (m, 12H), 7.04-7.00 (m, 2H), 4.92 (d, J = 11.3 Hz, 1H), 4.87 (d, J = 10.9 Hz, 1H), 4.83-4.78 (m, 2H), 4.45 (d, J = 8.0 Hz, 1H), 3.93 (ddd, J = 12.1, 5.4, 2.5 Hz, 1H), 3.82-3.71 (m, 3H), 3.55 (s, 3H), 3.44 (ddd, J = 9.4, 4.2, 2.6 Hz, 1H), 1.94 (dd, J = 8.0, 5.6 Hz, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 152.8, 149.7, 138.1, 137.8, 131.6, 129.7, 128.8, 128.7, 128.3, 128.1, 127.8, 122.5, 101.7, 82.8, 78.3, 75.6, 75.4, 61.8, 57.4; El-MS m/z (M+Na)$^+$: calcd 551.9780, obsd 551.7002.

Dibutyl 3,4,6-tri-O-benzyl-2-O-para-chlorophenylcarbonyl-β-D-glucopyranosyl phosphate 18. 3,4,6-tri-O-benzyl glucal (0.454 g, 1.09 mmol) was azeotroped in toluene (3 × 5mL), then dried under vacuum for 1 hour. A solution of the glucal in
dichloromethane (10 mL) was cooled to 0°C and dimethyldioxirane (20 mL of a 0.08 M solution in acetone, 1.6 mmols) was added. After 20 minutes, volatiles were removed under vacuum and the resulting residue was dried for 10 minutes. The residue was redissolved in dichloromethane (10 mL) and cooled to -78°C. A solution of dibutyl phosphate (0.26 mL, 1.3 mmol) in dichloromethane (3 mL) was added dropwise to the reaction mixture over a period of 5 minutes. After 10 minutes, the reaction mixture was warmed to 0°C and DMAP (0.533 g, 4.36 mmol) and p-chlorophenylchloroformate (0.3 mL, 2.18 mmol) were added. The reaction mixture was diluted after 30 minutes with ethyl acetate (30 mL) and filtered. The filtrate was concentrated in vacuo and the crude product was purified by flash silica column chromatography (20% ethyl acetate/hexanes) to yield 0.663 g (76.4%) of 18 as a white solid. $[\alpha]_D = -1.82^\circ$ (c = 2.35, CH$_2$Cl$_2$); IR (thin film): 2960, 1770, 1251, 1218, 1088 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.38-7.29 (m, 15H), 7.23-7.20 (m, 2H), 7.04-7.01 (m, 2H), 5.32 (dd, $J = 7.9, 7.2$ Hz, 1H), 4.97 (dd, $J = 8.7, 8.7$ Hz, 1H), 4.92 (d, $J = 11.3$ Hz, 1H), 4.82 (dd, $J = 14.5, 11.3$ Hz, 2H), 4.63 (dd, $J = 11.3, 2.2$ Hz, 2H), 4.54 (d, $J = 12.0$ Hz, 1H), 4.14-4.04 (m, 4H), 3.85 (dd, $J = 12.5, 9.1$ Hz, 2H), 3.77 (dd, $J = 7.3, 2.0$ Hz, 1H), 3.66 (m, 1H), 1.67-1.59 (m, 4H), 1.43-1.33 (m, 4H), 0.93 (t, $J = 7.4$ Hz, 3H), 0.88 (t, $J = 7.4$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 152.6, 149.6, 137.9, 137.8, 137.7, 131.6, 129.6, 129.3, 128.6, 128.6, 128.3, 128.1, 128.0, 127.9, 127.8, 122.6, 122.3, 116.9, 96.3, 96.2, 82.5, 78.2, 78.1, 75.77, 75.6, 75.3, 73.7, 68.3, 68.2, 68.1, 32.2, 32.2, 32.1, 32.1, 18.7, 18.7, 13.7, 13.6; $^{32}$P (300 MHz, CDCl$_3$): $\delta$ -2.271; EI-MS m/z (M+Na)$^+$: calcd 820.3416, obsd 820.2686.

**Thioethyl 3,4,6-tri-O-benzyl-2-O-para-chlorophenylcarbonyl-β-D-glucopyranoside 23.** General procedure A using thioethyl 3,4,6-tri-O-benzyl-β-D-glucopyranoside (0.286 g, 0.58 mmol), DMAP (0.142 g, 1.16 mmol), and p-chlorophenylchloroformate (0.16 mL, 1.16 mmol) gave 0.339 g (90%) of 23 as a white solid after purification by flash silica column chromatography (10% ethyl acetate/hexanes). $[\alpha]_D = -12.9^\circ$ (c = 2.89, CH$_2$Cl$_2$); IR (thin film): 3029, 2868, 1768, 1252, 1216 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.38-7.29 (m, 15H), 7.22-7.18 (m, 2H), 7.06-7.01 (m, 2H), 4.93-4.87 (m, 2H), 4.82 (dd, $J =$
11.0, 7.5 Hz, 2H), 4.65-4.55 (m, 3H), 4.49 (d, J = 10.0 Hz, 1H), 3.82-3.72 (m, 3H), 3.54 (ddd, J = 9.4, 4.4, 1.9 Hz, 1H), 2.84-2.70 (m, 2H), 1.31 (t, J = 3.6 Hz, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 152.9, 149.7, 138.2, 137.9, 131.7, 129.7, 128.7, 128.6, 128.2, 128.1, 128.0, 127.9, 127.8, 122.6, 84.5, 83.3, 79.7, 77.9, 75.7, 75.4, 73.7, 68.9, 24.2, 15.2; EI-MS m/z (M+Na): calcd 672.1906, obsd 672.1882.

3,4,6-tri-O-benzyl-2-para-chlorophenyl carbonyl-\(\beta\)-D-glucopyranosyl-(1\(\rightarrow\)6)-1,2,3,4-diisopropylidene-\(\alpha\)-D-galactopyranoside 20. Method A: 18 (143 mg, 0.18 mmols) and 1,2,3,4-diisopropylidene-\(\alpha\)-D-galactopyranoside (31 mg, 0.12 mmol) were co-evaporated in toluene, then dried under vacuum for 2 hours. Dichloromethane (3 mL) was added and the solution was cooled to \(-78^\circ\)C and TBSOTf (0.041 mL, 0.18 mmol) was added. After 30 minutes, the reaction mixture was neutralized with triethylamine and concentrated in vacuo. The residue was purified by flash silica column chromatography (10% ethyl acetate/hexanes) to yield 94 mg (92%) of 20 as a clear oil. \([\alpha]_D = -32.5^\circ\) (c = 5.8, CH\(_2\)Cl\(_2\)); IR (thin film): 2985, 1773, 1255, 1217, 1069 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.40-7.28 (m, 15H), 7.20-7.17 (m, 2H), 7.15-7.11 (m, 2H), 5.54 (d, J = 4.9 Hz, 1H), 4.91-4.86 (m, 2H), 4.81, (dd, J = 11.0, 8.7 Hz, 2H), 4.66 (d, J = 12.2 Hz, 1H), 4.61-4.57 (m, 3H), 4.55 (d, J = 6.2 Hz, 1H), 4.31 (dd, J = 4.9, 2.4 Hz, 1H), 4.23 (dd, J = 7.9, 1.8 Hz, 1H), 4.13 (dd, J = 10.8, 3.7 Hz, 1H), 4.02 (ddd, J = 7.0, 3.7, 1.8 Hz, 1H), 3.79-3.75 (m, 3H), 3.70 (dd, J = 10.8, 7.3 Hz, 1H), 3.51 (m, 1H), 1.45 (s, 6H), 1.32 (s, 6H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 152.7, 149.9, 138.3, 138.2, 138.0, 131.4, 129.5, 128.6, 128.5, 128.2, 128.1, 128.0, 127.9, 127.8, 122.9, 109.6, 108.9, 101.2, 96.4, 82.9, 78.3, 77.9, 75.5, 75.3, 73.8, 71.6, 70.8, 70.7, 69.5, 68.5, 67.8, 26.2, 26.1, 25.3, 24.6; EI-MS m/z (M+Na): calcd 870.3456, obsd 870.2943.

Method B: 1,2,3,4-diisopropylidene-\(\alpha\)-D-galactopyranoside (37 mg, 0.14 mmol) and 23 (77.4 mg, 0.12 mmol) were co-evaporated in toluene, then dried under vacuum for 1 hour. Residues were dissolved in dichloromethane (2 mL) and activated, 4Å powdered molecular sieves (220 mg) were added to the solution. The reaction mixture was cooled
to 0°C and after 10 minutes, methyl triflate (0.05 mL, 0.48 mmol) was added. After 5 hours, the reaction mixture was diluted with dichloromethane (10 mL), quenched with triethylamine (1 mL), filtered through a plug of silica gel, and the filtrate was concentrated in vacuo. The crude product was purified by flash silica column chromatography (10% ethyl acetate/hexanes) to yield 73 mg (72%) of 20 as a clear oil. Characterization data was consistent with that previously reported for 20.

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\begin{align*}
\text{Bn} &\quad \text{O} &\quad \text{OBn} \\
\text{Bn} &\quad \text{O} &\quad \text{OH} \\
\text{21}
\end{align*}
\]

3,4,6-Tri-O-benzyl-β-D-glucopyranosyl-(1→6)-1,2,3,4-diisopropylidene-α-D-galactopyranoside 21. Method A: General procedure B using 20 (73 mg, 0.086 mmol), hydrogen peroxide (0.02 mL, 0.76 mmol) and LiOH·H₂O (7 mg, 0.17 mmols) gave 52 mg (87%) of 21 after purification by flash silica column chromatography (20% ethyl acetate/hexanes). [α]_D = -36.12° (c = 4.32, CH₂Cl₂); IR (thin film): 3067, 2900, 1382, 1105, 1058 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.41-7.39 (m, 2H), 7.36-7.26 (m, 11H), 7.17-7.14 (m, 2H), 5.57 (d, J = 5.0 Hz, 1H), 5.05 (d, J = 11.2 Hz, 1H), 4.85 (d, 10.8 Hz, 1H), 4.82 (d, J = 11.2 Hz, 1H), 4.63 (dd, J = 2.5, 2.5 Hz, 1H), 4.60 (s, 1H), 4.54 (d, J = 12.1 Hz, 1H), 4.51 (d, J = 10.7 Hz, 1H), 4.37-4.33 (m, 2H), 4.23 (dd, J = 7.9, 1.8 Hz, 1H), 4.12 (dd, J = 11.0, 3.2 Hz, 1H), 4.04 (dd, J = 8.02, 2.9, 2.0 Hz, 1H), 3.77-3.71 (m, 3H), 3.64-3.61 (m, 3H), 3.50-3.48 (m, 1H), 3.23 (s, 1H), 1.55 (s, 3H), 1.47 (s, 3H), 1.35 (s, 3H), 1.34 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 139.0, 138.4, 138.3, 128.6, 128.2, 128.1, 127.9, 127.8, 127.7, 109.7, 109.0, 104.2, 96.5, 84.8, 77.4, 75.2, 75.0, 73.7, 71.4, 70.9, 70.6, 69.6, 69.0, 68.0, 26.2, 26.1, 25.1, 24.6; EI-MS m/z (M+Na)+: calcd 714.7854, obsd 715.3089.

Method B: General Procedure D using disaccharide 46 (121 mg, 0.142 mmol), water (13 µL, 0.71 mmol), and tributyl phosphine (100 µL, 0.426 mmol) yielded 21 (86 mg, 88%) after purification by flash silica gel column chromatography (15% EtOAc/hexanes). Characterization data were consistent with previously reported data.²⁵
2-(Azidomethyl) benzoic acid 30. To a stirred solution of methyl 2-methyl benzoate (4.29 g, 28.56 mmol) in 100 mL CCl₄ was added NBS (5.1 g, 28.65) and a catalytic amount of benzoyl peroxide (55 mg, 0.227 mmol). The reaction mixture was refluxed for 24 h and allowed to cool to room temperature. Filtration and concentration afforded crude bromide 29, which was taken up in 80 mL anhydrous EtOH before NaN₃ (1.86 g, 28.65 mmol) was added and the reaction mixture was stirred for 48 h at room temperature under N₂. The reaction was quenched with brine and extracted twice with EtOAc. The combined organic phases were dried over Na₂SO₄, filtered and concentrated. The crude product was purified by flash silica column chromatography (2%→5%→10% EtOAc/hexanes) to afford 4.58 g (84% for 2 steps) of 2-(azidomethyl)methyl benzoate. IR (thin film): 2952, 2109, 1739, 1434, 1263 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 8.01 (d, J = 7.0 Hz, 1H), 7.51 (t, J = 7.5 Hz, 1H), 7.47 (d, J = 7.5 Hz, 1H), 7.38 (t, J = 7.5 Hz, 1H), 4.79 (s, 2H), 3.84 (s, 3H); ¹³C NMR (125 MHz, CDCl₃): δ 167.1, 137.3, 132.7, 131.6, 129.7, 128.7, 128.1, 53.11, 52.21; FAB-MS: m/z (M+Na)⁺ calcd 214.0587, obsd 214.0591.

To a stirred solution of 2-(azidomethyl)methyl benzoate (15.0 g, 78.45 mmol) in 192 mL THF/H₂O (10:1) was added LiOH•H₂O (16.2 g, 41.96). The reaction mixture was stirred at room temperature for 60 h, diluted with H₂O, and extracted twice with CH₂Cl₂. The aqueous phase was acidified with 2 N HCl and the precipitated acid was extracted three times with CH₂Cl₂. The combined organic phases were washed with brine, dried over Na₂SO₄, filtered and concentrated to afford 30 (13.5 g, 97%) in analytically pure form. Characterization data were consistent with that previously reported.¹⁷

2-(Azidomethyl)benzoyl chloride 31. 2-(Azidomethyl) benzoic acid 30 (10.45 g, 59 mmol) was dissolved in CHCl₃ (100 mL) and thionyl chloride (12.9 mL, 177 mmol) was
added. The reaction mixture was heated to reflux for 5 h, cooled and concentrated *in vacuo* using a base trap. The resulting residue was coevaporated with toluene (3 × 15 mL) to yield 11.15 g of 31 (97%). IR (thin film): 3071, 2962, 2105, 1769, 1201 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.33 (dd, J = 8.0, 1.2 Hz, 1 H), 7.70 (td, J = 7.6, 1.3 Hz, 1 H), 7.61 (dd, J = 7.6, 0.5 Hz, 1 H), 7.54 (td, J = 7.9, 1.3 Hz, 1 H), 4.76 (s, 2 H); ¹³C NMR (100 MHz, CDCl₃): δ 138.5, 135.2, 134.7, 131.7, 130.0, 129.7, 128.8, 53.2; ESI-MS: m/z = (M + Na)⁺ calcd 195.0200, obsd 195.0217.

**6-O-(2-(Azidomethyl)benzoyl)-3,4-di-O-benzyl-glucal.** General Procedure C using 3,4-di-O-benzyl glucal (1.171 g, 3.59 mmol), DMAP (0.525 g, 4.30 mmol), and AZMB chloride (0.843 g, 4.30 mmol) yielded 6-(2-(azidomethyl)benzoyl)-3,4-di-O-benzyl glucal (1.58 g, 91%) after purification by flash silica gel column chromatography (30% EtOAc/hexanes). [α]D: +24.77° (c = 2.87, CH₂Cl₂); IR (thin film) 3067, 2868, 2099, 1716, 1259 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.99 (app d, J = 7.8 Hz, 1 H), 7.57 (app t, J = 7.6 Hz, 1 H), 7.50 (app d, J = 7.6 Hz, 1 H); 7.41-7.24 (m, 11 H), 6.44, (d, J = 6.1 Hz, 1 H), 4.96 (dd, J = 6.1, 2.7 Hz, 1 H), 4.89 (d, J = 11.3 Hz, 1 H), 4.83 (d, J = 14.6 Hz, 1 H), 4.78 (d, J = 14.6 Hz, 1 H), 4.70 (dd, J = 11.4, 1.8 Hz, 2 H), 4.63 (d, J = 3.8, Hz, 2 H), 4.58 (d, J = 11.6 Hz, 1 H), 4.29-4.23 (m, 2 H), 3.89 (dd, J = 8.5, 6.0 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃): δ 166.4, 144.6, 138.1, 137.8, 137.7, 133.1, 131.4, 129.8, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 100.2, 75.6, 75.1, 74.0, 73.8, 70.8, 63.4, 53.2; ESI-MS: m/z (M + Na)⁺ calcd 508.1843, obsd 508.1820.

**32**

**Methyl 2-O-acetyl-6-O-(2-(azidomethyl)benzoyl)-3,4-di-O-benzyl-β-D-glucopyranoside** 32. 6-(2-(Azidomethyl)benzoyl)-O-3,4-di-O-benzyl-β-D-glucopyranoside 43 (117 mg, 0.22 mmol) was dissolved in CH₂Cl₂ (2 mL) and DMAP (29 mg, 0.26 mmol) and acetic anhydride (25 µL, 0.26 mmol) were added. After one hour at room temperature, the reaction mixture was diluted with CH₂Cl₂ (20 mL) and washed with saturated aqueous NaHCO₃ (20 mL) and water (20 mL). The organic layer
was dried over Na₂SO₄, filtered, and concentrated. The crude product was purified by flash silica gel column chromatography (30% EtOAc/hexanes) to yield 120 mg of 32 (95%). [α]D: + 50.52° (c = 3.62, CH₂Cl₂); IR (thin film): 3031, 2884, 2102, 1747, 1719 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.99 (app d, J = 7.8 Hz, 1 H), 7.57 (app t, J = 7.5 Hz, 1 H), 7.49 (app d, J = 7.5 Hz, 1 H), 7.42 (app t, J = 7.6 Hz, 1 H), 7.35-7.23 (m, 10 H), 5.04-5.00 (m, 1 H), 4.86-4.74 (m, 4 H), 4.71 (d, J = 11.3 Hz, 1 H), 4.63-4.55 (m, 2 H), 4.45 (dd, J = 11.9, 4.4 Hz, 1 H), 4.34 (d, J = 7.9 Hz, 1 H), 3.75-3.7 (m, 3 H), 3.46 (s, 3 H), 2.00 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃): δ 169.9, 166.4, 138.0, 137.6, 137.5, 133.2, 131.5, 130.0, 128.7, 128.7, 128.4, 128.4, 128.3, 128.1, 102.0, 83.2, 77.8, 75.4, 73.2, 63.5, 57.0, 53.2, 21.2; ESI-MS: m/z (M + Na)⁺ calcd 598.2160, obsd 598.2168.

Methyl 6-O-(2-(azidomethyl)benzoyl)-3,4-di-O-benzyl-2-levulinoyl-β-D-glucopyranoside 33. Levulinic acid (33 μL, 0.32 mmol) and DMAP (42 mg, 0.34 mmol) were dissolved in CH₂Cl₂ (1.5 mL) and cooled to 0 °C. After 10 minutes, DIPC (48 μL, 0.31 mmol) was added with vigorous stirring. After an additional 10 minutes, a solution of 43 (163 mg, 0.31 mmol) in CH₂Cl₂ (1.5 mL) was added to the levulinic acid solution via cannula and the mixture was left to slowly warm to room temperature. After 8 hours, the reaction mixture was diluted with EtOAc (10 mL) and filtered through a plug of silica gel. The filtrate was concentrated and the residue purified by flash silica gel column chromatography (30% EtOAc/hexanes) to afford 33 (182 mg, 93%). [α]D: + 4.54° (c = 2.78, CH₂Cl₂); IR (thin film): 3030, 2916, 2103, 1747, 1718 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.95 (dd, J = 7.8, 1.2 Hz, 1 H), 7.52 (td, J = 7.5, 1.3 Hz, 1 H), 7.44 (app d, J = 7.1 Hz, 1 H), 7.36 (td, J = 7.8, 1.2 Hz, 1 H), 7.30-7.19 (m, 10 H), 4.99-4.95 (m, 1 H), 4.81-4.69 (m, 5 H), 4.58-4.53 (m, 2 H), 4.40 (dd, J = 11.9, 4.4 Hz, 1 H), 4.30 (d, J = 7.9 Hz, 1 H), 3.71-3.62 (m, 3 H), 3.42 (s, 3 H), 2.68-2.64 (m, 2 H), 2.51-2.44 (m, 2 H), 2.12 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃): δ 206.5, 171.8, 166.4, 138.1, 137.6, 137.5, 133.1, 130.0, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.0, 102.0, 83.1,
Methyl 2-O-allyl-6-O-(2-(azidomethyl)benzoyl)-3,4-di-O-benzyl-β-D-glucopyranoside 34. General Procedure C using methyl 2-O-allyl-3,4-di-O-benzyl-β-D-glucopyranoside (97 mg, 0.234 mmol), DMAP (86 mg, 0.702 mmol), and AZMB chloride (92 mg, 0.468) after purification by flash silica gel column chromatography (25% EtOAc/hexanes) yielded 34 (122 mg, 91%). [α]D +7.48° (c = 0.76, CH2Cl2); IR (thin film): 2913, 2103, 1720, 1259, 1070 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.01 (dd, J = 7.8, 1.3 Hz, 1 H), 7.58 (td, J = 7.6, 1.3 Hz, 1 H), 7.51 (dd, J = 7.6, 0.7 Hz, 1 H), 7.43-7.24 (m, 11 H), 6.03-5.94 (m, 1 H), 5.33 (ddd, J = 17.2, 3.2, 1.6 Hz, 1 H), 5.21 (ddd, J = 10.4, 2.8, 1.2 Hz, 1H), 5.00 (d, J = 10.7 Hz, 1 H), 4.92 (d, J = 10.8 Hz, 1 H), 4.86-4.77 (m, 3 H), 4.64-4.61 (m, 2 H), 4.48-4.41 (m, 2 H), 4.32 (d, J = 7.8 Hz, 1 H), 4.26-4.20 (m, 1 H), 3.70-3.65 (m, 1 H), 3.65-3.64 (m, 1 H), 3.56 (s, 3 H), 3.37 (dd, J = 8.9, 7.8 Hz, 1 H); ¹³C NMR (100 MHz, CDCl₃): δ 166.4, 138.5, 137.8, 137.6, 135.1, 133.1, 131.5, 129.9, 128.7, 128.6, 128.3, 128.3, 128.2, 128.0, 84.8, 82.1, 77.6, 76.1, 75.3, 73.9, 73.0, 63.9, 57.4, 53.2; ESI-MS: m/z (M + Na)⁺ calcd 596.2367, obsd 596.2333.

Methyl 6-O-(2-(azidomethyl)benzoyl)-3,4-di-O-benzyl-2-O-p-methoxybenzyl-β-D-glucopyranoside 35. General Procedure C using methyl 3,4-di-O-benzyl-2-O-p-methoxybenzyl-β-D-glucopyranoside (107 mg, 0.216 mmol), DMAP (79 mg, 0.648 mmol), and AZMB chloride (85 mg, 0.432) after purification by flash silica gel column chromatography (25% EtOAc/hexanes) yielded 35 (128 mg, 91%). [α]D +35.73° (c = 0.6, CH₂Cl₂); IR (thin film): 2909, 2102, 1718, 1250, 1068 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.01 (dd, J = 7.8, 1.2 Hz, 1 H), 7.58 (td, J = 7.6, 1.3 Hz, 1 H), 7.52-7.50 (m, 1 H), 7.41 (td, J = 7.7, 1.3, Hz, 1 H), 7.34-7.24 (m, 12 H), 6.88-6.85 (m, 2 H), 4.98 (d, J = 10.8 Hz, 1 H), 4.91-4.77 (m, 5 H), 4.67 (d, J = 10.6 Hz, 1H), 4.64-4.59 (m, 2 H), 4.46
(dd, J = 11.8, 4.4 Hz, 1 H), 4.37 (d, J = 7.8 Hz, 1 H), 3.81 (s, 3 H), 3.73-3.69 (m, 1 H), 3.64-3.60 (m, 2 H), 3.59 (s, 3 H), 3.47 (dd, J = 8.8, 7.8 Hz, 1 H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 166.5, 159.5, 138.6, 137.8, 137.7, 133.1, 131.5, 130.7, 130.1, 129.9, 128.7, 128.7, 128.3, 128.2, 128.2, 128.0, 114.0, 105.8, 84.8, 82.1, 77.8, 76.1, 75.4, 74.7, 73.0, 64.0, 57.5, 55.5, 53.3; ESI-MS: $m/z$ (M + Na)$^+$ calcd 676.2629, obsd 676.2629.

Methyl 2-O-acetyl-3,4-di-O-benzyl-$\beta$-D-glucopyranoside 36. General Procedure D using methyl 2-O-acetyl-6-O-(2-(azidomethyl)benzoyl)-3,4-O-benzyl-$\beta$-D-glucopyranoside 32 (68 mg, 0.118 mmol), water (10 $\mu$L, 0.59 mmol), and tributyl phosphate (90 $\mu$L, 0.355 mmol) afforded 36 (45 mg, 92%) after purification by flash silica gel column chromatography (25% EtOAc/hexanes). Characterization data were consistent with that previously reported.$^{24}$

Methyl 3,4-di-O-benzyl-2-levulinyl-$\beta$-D-glucopyranoside 37. General Procedure D using 33 (60 mg, 0.095 mmol), water (9 $\mu$L, 0.475 mmol), and tributyl phosphate (70 $\mu$L, 0.285 mmol) after purification by flash silica gel column chromatography (30% EtOAc/hexanes) yielded 37 (45 mg, 96%). Characterization data were consistent with previously reported data.$^{25}$

Methyl 2-O-(2-(azidomethyl)benzoyl)-3,4-di-O-benzyl-6-O-tri-isopropylsilyl-$\beta$-D-glucopyranoside 38. General Procedure C using methyl 3,4-di-O-benzyl-2-O-tri-isopropylsilyl-$\beta$-D-glucopyranoside (122 mg, 0.23 mmol), DMAP (84 mg, 0.69 mmol), and AZMB chloride (90 mg, 0.46 mmol) after purification by flash silica gel column chromatography (10% EtOAc/hexanes) yielded 38 (130 mg, 82%). $\left[\alpha\right]_D^{\text{28}}$ +12.31° (c = 0.72, CH$_2$Cl$_2$); IR (thin film): 2941, 2865, 2102, 1728, 1075 cm$^{-1}$; $^1$H NMR (400 MHz,
CDCl₃): δ 7.89 (dd, J = 7.9, 1.0 Hz, 1 H), 7.58 (td, J = 7.1, 1.2, Hz, 1 H), 7.54-7.52 (m, 1 H), 7.40-7.31 (m, 7 H), 7.18-7.14 (m, 4 H), 5.22 (dd, J = 9.0, 8.2 Hz, 1 H), 4.88-4.74 (m, 5 H), 4.69 (d, J = 13.1 Hz, 1H), 4.61 (d, J = 27.1, 1 H), 4.46 (d, J = 8.0 Hz, 1 H), 4.04-4.02 (m, 2 H), 3.89 (app t, J = 9.3 Hz, 1 H), 3.82 (app t, J = 9.2 Hz, 1 H), 3.46 (s, 3 H), 3.41-3.38 (m, 1 H), 1.17-1.07 (m, 21 H); ¹³C NMR (100 MHz, CDCl₃): δ 165.6, 138.3, 137.8, 132.9, 129.6, 128.7, 128.5, 128.3, 128.1, 127.9, 101.7, 82.9, 77.9, 76.4, 75.4, 75.3, 73.3, 73.9, 56.4, 53.1, 18.2, 18.2, 12.2; ESI-MS: m/z (M + Na)⁺ calcd 712.3388, obsd 712.3370.

![Chemical Structure](image)

**n-Pentenyl 4-O-(2-(azidomethyl)benzoyl)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside 39.** General Procedure C using n-pentenyl 3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (375 mg, 0.672 mmol), DMAP (205 mg, 1.68 mmol), and AZMB chloride (197 mg, 1.00 mmol) after purification by flash silica gel column chromatography (40% EtOAc/hexanes) afforded 39 (445 mg, 93%). \([\alpha]_D^\circ: +19.8° (c = 3.07, CH₂Cl₂);\) IR (thin film) 2874, 1714, 1389, 1253, 1134 cm⁻¹, ¹H NMR (500 MHz, CDCl₃): δ 7.98 (d, J = 8.0 Hz, 1H), 7.90-7.68 (m, 4H), 7.60 (t, J = 7.5 Hz, 1H), 7.53 (d, J = 8.0 Hz, 1H), 7.41 (t, J = 7.5 Hz, 1H), 7.29-7.22 (m, 6H), 6.96 (m, 2H), 6.87 (m, 2H), 5.61 (m, 1H), 5.46 (dd, J = 9.0, 10.0 Hz, 1H), 5.25 (d, J = 8.5 Hz, 1H), 4.78 (d, J = 15.0 Hz, 1H), 4.76 (m, 2H), 4.70 (d, J = 15.0 Hz, 1H), 4.64 (d, J = 9.0 Hz, 1H), 4.62 (d, J = 8.5 Hz, 1H), 4.59 (d, J = 12.0 Hz, 1H), 4.55 (d, J = 12.0 Hz, 1H), 4.39 (d, J = 10.5 Hz, 1H), 4.34 (d, J = 12.0 Hz, 1H), 3.97 (m, 1H), 3.88 (m, 1H), 3.74 (m, 2H), 3.47 (m, 1H), 1.98-1.81 (m, 2H), 1.62-1.47 (m, 2H); ¹³C NMR (125 MHz, CDCl₃): δ 165.2, 138.1, 137.9, 137.8, 137.6, 134.0, 133.2, 131.0, 129.7, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.5, 114.8, 98.4, 73.9, 73.7, 73.6, 73.1, 69.9, 69.1, 55.7, 53.0, 29.9, 28.6; FAB-MS: m/z (M+Na)⁺ calcd 739.2738, obsd 739.2710.
**40**

*n*-Pentenyl 4-(2-(azidomethyl)benzoyl)-3,6-di-\(\mathcal{O}\)-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonyl)-\(\beta\)-D-glucopyranoside 40. General Procedure C using *n*-pentenyl 3,6-di-\(\mathcal{O}\)-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonyl)-\(\beta\)-D-glucopyranoside (455 mg, 0.754 mmol), DMAP (230 mg, 1.89 mmol) and AZMB chloride (221 mg, 1.13 mmol) after purification by flash silica gel column chromatography (50% EtOAc/hexanes) yielded 40 (445 mg, 93%). \([\alpha]_D^{\text{D}} -2.1^\circ\) (c = 3.34, CH\(_2\)Cl\(_2\)); IR (thin film): 3316, 2952, 2103, 1718, 1545 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 8.00 (d, \(J = 7.5\) Hz, 1H), 7.68 (dt, \(J = 1.5, 8.0\) Hz, 1H), 7.60 (d, \(J = 7.7\) Hz, 1H), 7.47 (t, \(J = 7.5\) Hz, 1H), 7.34-7.25 (m, 11H), 5.91 (m, 1H), 5.57 (bd, \(J = 5.5\) Hz, 1H), 5.45 (t, \(J = 9.5\) Hz, 2H), 5.10 (d, \(J = 10.5\) Hz, 1H), 5.08 (m, 1H), 4.87 (d, \(J = 12.0\) Hz, 1H), 4.84 (d, \(J = 8.0\) Hz, 1H), 4.82 (d, \(J = 12.0\) Hz, 1H), 4.77 (d, \(J = 11.0\) Hz, 1H), 4.75 (d, \(J = 8.0\) Hz, 1H), 4.72 (s, 1H), 4.69 (d, \(J = 11.5\) Hz, 1H), 4.62 (d, \(J = 12.0\) Hz, 1H), 4.60 (d, \(J = 12.0\) Hz, 1H), 4.42 (m, 1H), 4.05 (m, 1H), 3.93 (t, \(J = 4.5\) Hz, 1H), 3.79 (m, 2H), 3.65 (m, 1H), 3.51 (m, \(J = 8.0\) Hz, 1H), 2.26-2.22 (m, 2H), 1.87-1.79 (m, 2H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) 165.2, 154.0, 138.1, 138.0, 137.8, 137.7, 133.1, 131.1, 129.6, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 115.1, 99.9, 95.5, 78.0, 74.4, 74.1, 73.7, 73.4, 72.3, 69.8, 66.4, 58.2, 53.0, 30.1, 28.8; FAB-MS: \(m/z\) (M+Na\(^+\) calcd 783.1726, obsd 783.1704.

**41**

*n*-Pentenyl 3,6-di-\(\mathcal{O}\)-benzyl-2-deoxy-2-phthalimido-\(\beta\)-D-glucopyranoside 41. General Procedure D using 39 (100 mg, 0.14 mmol), water (13 \(\mu\)L, 0.70 mmol), and tributyl phosphine (105 \(\mu\)L, 0.42 mmol) after purification by flash silica gel column chromatography (40% EtOAc/hexanes) yielded 41 (73 mg, 94%). Characterization data were consistent with previously reported data.\(^{26}\)
**n-Pentenyl 3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonyl)-β-D-glucopyranoside 42.** Method A: To a stirred solution of 40 (830 mg, 1.49 mmol) in 15 mL anhydrous EtOH was added ethylenediamine (4.97 mL, 74.4 mmol). The reaction mixture was heated to 80°C and stirred for 6 h. After cooling to room temperature, the volatiles were removed in vacuo. The crude product was purified by flash silica column chromatography (5% MeOH/CH₂Cl₂) and taken up in 30 mL diethyl ether/saturated NaHCO₃ (1:1 v/v). To this stirred solution was added TrocCl (225 µL, 1.64 mmol) and the reaction mixture was stirred for 1 h. The layers were separated, and the aqueous phase was extracted twice with diethyl ether. The combined organic phases were washed with saturated NaHCO₃, brine and dried over Na₂SO₄. Upon filtration and concentration, the crude product was purified by flash silica gel column chromatography (30% EtOAc/hexanes) to afford 42 (789 mg, 88% yield for 2 steps). [α]₀\text{D}: -11.8° (c = 1.44, CH₂Cl₂); IR (thin film): 3432, 2943, 1710, 1549, 1243 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.24-7.39 (m, 10H), 5.80 (m, 1H), 5.40 (d, J = 8.5 Hz, 1H), 5.03 (d, J = 17.2 Hz, 1H), 4.98 (d, J = 10.0 Hz, 1H), 4.79 (dd, J = 11.5 Hz, 2H), 4.74 (s, 2H), 4.60 (dd, J = 12.5 Hz, 2H), 3.87 (m, 1H), 3.76 (m, 3H), 3.71 (t, J = 8.5 Hz, 1H), 3.47 (m, 2H), 3.39 (m, 1H), 3.10 (s, 1H), 2.17-2.05 (m, 2H), 1.76-1.62 (m, 2H); ¹³C NMR (125 MHz, CDCl₃): δ 154.1, 138.3, 138.1, 137.8, 128.6, 128.5, 128.1, 128.0, 127.9, 127.8, 115.0, 100.4, 95.6, 80.4, 76.1, 74.4, 74.1, 73.9, 73.7, 72.8, 70.4, 69.1, 57.3, 30.0, 28.7; FAB-MS: m/z (M+Na)⁺ calcd 624.1293, obsd 624.1290.

Method B: General Procedure D using 40 (123 mg, 0.16 mmol), water (15 µL, 0.80 mmol), and tributyl phosphine (121 µL, 0.48 mmol) yielded 42 (93 mg, 95%) after purification by flash silica gel column chromatography (20% EtOAc/hexanes).
Methyl 6-O-(2-(azidomethyl)benzoyl)-3,4-di-O-benzyl-β-D-glucopyranoside 43.

Method A: 6-(2-(Azidomethyl)benzoyl)-3,4-di-O-benzyl-glucal (0.548 g, 1.13 mmol) was azeotroped with toluene (3 × 3 mL), then dried under vacuum for 1 h. A solution of the glucal in CH₂Cl₂ (10 mL) was cooled to 0°C and dimethyl dioxirane (30 mL of a 0.08 M solution in acetone, 2.26 mmol) was added. After 20 minutes, volatiles were removed under vacuum and the resulting residue was dried for 10 minutes. The residue was redissolved in CH₂Cl₂ (10 mL) and methanol (10 mL) and left to slowly warm to room temperature. After 10 hours, the solution was concentrated in vacuo and the residue was purified by flash silica column chromatography (30% EtOAc/hexanes) to afford 43 (0.54 g, 90%). [α]D +20.62° (c = 1.55, CH₂Cl₂); IR (thin film): 3474, 2910, 1718, 1064 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.98 (dd, J = 7.8, 1.2 Hz, 1 H), 7.55 (td, J = 7.5, 1.4 Hz, 1 H), 7.48 (app d, J = 7.0 Hz, 1 H), 7.41-7.23 (m, 11 H), 4.96 (d, J = 11.1 Hz, 1 H), 4.90 (d, J = 10.8 Hz, 1H), 4.87 (d, J = 11.1 Hz, 1H), 4.81 (d, J = 14.6 Hz, 1 H), 4.75 (d, J = 14.5 Hz, 1 H), 4.61 (app d, J = 12.4 Hz, 2 H), 4.44 (dd, J = 11.7, 4.4 Hz, 1 H), 4.22 (d, J = 7.7 Hz, 1 H), 3.66-3.61 (m, 2 H), 3.54 (s, 3 H), 2.41 (s, 1 H); ¹³C NMR (100 MHz, CDCl₃): δ 171.6, 166.5, 138.5, 137.7, 137.6, 133.1, 131.5, 130.0, 129.8, 128.8, 128.7, 128.6, 128.3, 128.3, 128.2, 128.1, 103.9, 100.5, 84.6, 80.4, 75.6, 75.4, 74.9, 73.3, 64.2, 63.9, 57.5, 53.2; ESI-MS: m/z (M + Na)+ calcd 556.2054, obsd 556.2049.

Method B: A solution of hydrazine acetate (7 mg, 0.09 mmol) in MeOH (0.1 mL) was added to a solution of 33 (52 mg, 0.082 mmol) in CH₂Cl₂ (1 mL) and the resulting solution was stirred for 90 minutes at room temperature. The reaction mixture was diluted with CH₂Cl₂ (5 mL) and concentrated in vacuo. The crude product was purified by flash silica gel chromatography (30% EtOAc/hexanes) to yield 43 (44 mg, 99%).

Method C: A solution of 34 (61 mg, 0.106 mmol) in AcOH (90% aq., 2.5 mL) was treated with PdCl₂ (47 mg, 0.266 mmol), and NaOAc (87 mg, 0.636 mmol). The reaction mixture was warmed to 70°C and stirred for 3 h. The reaction mixture was then allowed to cool to room temperature, diluted with EtOAc (25 mL), and washed repeatedly with saturated aqueous NaHCO₃ (4 × 30 mL). The organic layer was dried
(Na₂SO₄), filtered and concentrated in vacuo. The residue was purified by flash silica gel chromatography (30 % EtOAc/hexanes) to yield 43 (48 mg, 85%).

Method D: To a solution of 35 (55 mg, 0.084 mmol) in CH₂Cl₂ (1 mL) was added trifluoroacetic acid (0.13 mL, 1.68 mmol, 20 equiv.). The reaction mixture was quenched by addition of saturated aqueous NaHCO₃ (20 mL) and extracted with EtOAc (3 × 20 mL). The organic layer was dried (Na₂SO₄), filtered, and concentrated in vacuo. The crude product was purified by flash silica gel column chromatography (20% EtOAc/hexanes) to yield 43 (40 mg, 89%).

**Methyl 2-O-(2-(azidomethyl)benzoyl)-3,4-di-O-benzyl-β-D-glucopyranoside 44.** A solution of 38 (75 mg, 0.11 mmol) was made in 2.33 ml of a 1:3:3 mixture of trifluoroacetic acid/THF/water. After 1 h an additional 0.10 mL TFA was added. After 10 more minutes, the reaction mixture was diluted with EtOAc (20 mL) and washed with saturated aqueous NaHCO₃ (3 × 20 mL). The organic layer was dried (Na₂SO₄), filtered and concentrated in vacuo. The residue was purified by flash silica gel chromatography (15% EtOAc/hexanes) to yield 44 (50 mg, 90%). [α]D +14.18° (c = 3.50, CH₂Cl₂), IR (thin film): 3444, 2877, 2102, 1726, 1076 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.90 (dd, J = 7.9, 1.2 Hz, 1 H), 7.59 (td, J = 7.2, 1.1 Hz, 1 H), 7.55-7.53 (m, 1 H), 7.42-7.32 (m, 6 H), 7.16 (m, 5 H), 5.23 (dd, J = 9.3, 9.1 Hz, 1 H), 4.89 (d, J = 10.9 Hz, 1 H), 4.83 (d, J = 11.2 Hz, 1H), 4.77-4.66 (m, 4 H), 4.52 (d, J = 8.0 Hz, 1H), 3.97-3.93 (m, 1 H), 3.88-3.76 (m, 3 H), 3.51 (s, 3 H), 3.51-3.48 (m, 1 H), 2.02 (m, 1 H); ¹³C NMR (100 MHz, CDCl₃): δ 226.0, 165.4, 137.9, 137.9, 137.8, 133.0, 131.0, 129.7, 128.7, 128.7, 128.5, 128.3, 128.3, 128.1, 128.0, 127.9, 102.1, 82.7, 77.9, 75.6, 75.3, 75.3, 73.8, 61.1, 57.2, 53.0; ESI-MS: m/z (M + Na)⁺ calcd 556.2054, obsd 556.2026.

**Dibutyl 2-O-(2-(azidomethyl)benzoyl)-3,4,6-tri-O-benzyl-β-D-glucopyranosyl phosphate 45.** 3,4,6-Tri-O-benzyl glucal (402 mg, 0.965 mmol) was azeotroped with
toluene (3 × 5 mL), then dried under vacuum for 1 h. A solution of the glucal in CH$_2$Cl$_2$ (10 mL) was cooled to 0°C and dimethyl dioxirane (20 mL of a 0.08 M solution in acetone, 1.44 mmol) was added. After 20 minutes, volatiles were removed under vacuum and the resulting residue was dried for 10 minutes. The residue was redissolved in CH$_2$Cl$_2$ (10 mL) and cooled to -78°C, before a solution of dibutyl phosphate (0.23 mL, 1.16 mmol) in CH$_2$Cl$_2$ (5 mL) was added dropwise over a period of 5 minutes. After 10 minutes, the solution was warmed to 0°C and DMAP (472 mg, 3.86 mmol) and AZMB chloride (377 mg, 1.93 mmol) were added. After 10 minutes the reaction mixture was diluted with EtOAc (100 mL) and filtered through a plug of silica gel. The filtrate was concentrated and the residue was purified by flash silica gel chromatography (20% EtOAc/hexanes) to yield 45 (547 mg, 71%) as a white solid. [α]$_D$ +17.26° (c = 1.04, CH$_2$Cl$_2$); IR (thin film): 2961, 2102, 1729, 1076, 1028 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): δ 8.01 (dd, $J = 7.9$, 1.1 Hz, 1 H), 7.59 (td, $J = 7.8$, 1.3 Hz, 1 H), 7.55-7.53 (m, 1 H), 7.41-7.30 (m, 9 H), 7.23-7.21 (m, 2 H), 7.15-7.10 (m, 5 H), 5.42-5.33 (m, 2 H), 4.86-4.79 (m, 3 H), 4.73 (d, $J = 15.1$ Hz, 1 H), 4.65-4.62 (m, 3 H), 4.55 (d, $J = 12.0$ Hz, 1 H), 4.09-4.03 (m, 2 H), 3.94-3.89 (m, 1 H), 3.83-3.72 (m, 4 H), 3.70-3.65 (m, 1 H), 1.63-1.58 (m, 2 H), 1.39-1.27 (m, 4 H), 1.08-1.03 (m, 2 H), 0.89 (t, $J = 7.4$ Hz, 3 H), 0.69 (t, $J = 7.4$ Hz, 3 H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 164.9, 138.4, 138.0, 137.9, 137.7, 133.3, 131.4, 129.4, 128.7, 128.6, 128.4, 128.2, 128.1, 127.9, 96.7, 82.3, 77.6, 75.9, 75.3, 75.3, 73.7, 73.5, 73.4, 68.3, 68.2, 68.1, 53.1, 32.2, 32.1, 32.0, 31.9, 18.7, 18.4, 13.7, 13.5; $^{31}$P NMR (120 MHz, CDCl$_3$): -2.21; ESI-MS: m/z (M + Na)$^+$ calcd 824.3283, obsd 824.3299.

Thioethyl 2-O-(2-(azidomethyl)benzoyl)-3,4,6-tri-O-benzyl-β-D-glucopyranoside 47.

General Procedure C using thioethyl 3,4,6-tri-O-benzyl-β-D-glucopyranoside (187 mg, 0.38 mmol), DMAP (139 mg, 1.14 mmol), and AZMB chloride (148 mg, 0.76) after purification by flash silica gel column chromatography (15% EtOAc/hexanes) yielded 47 (210 mg, 85%). [α]$_D$ +4.03° (c = 1.04, CH$_2$Cl$_2$); IR (thin film): 3030, 2868, 2101, 1724, 1074 cm$^{-1}$, $^1$H NMR (400 MHz, CDCl$_3$): δ 7.92-7.89 (m, 1 H), 7.59-7.52 (m, 2 H), 7.41-
7.27 (m, 9 H), 7.22-7.14 (m, 7 H), 5.33-5.29 (m, 1 H), 4.85-4.79 (m, 3 H), 4.71 (d, \( J = 14.9 \) Hz, 1 H), 4.67-4.53 (m, 5 H), 3.84-3.75 (m, 4 H), 3.60-3.57 (m, 1 H), 2.78-2.72 (m, 2 H), 1.27 (t, \( J = 7.5 \) Hz, 3 H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 165.5, 138.3, 138.0, 137.8, 133.0, 131.1, 129.6, 128.7, 128.7, 128.6, 128.5, 128.2, 128.1, 128.0, 127.9, 127.8, 84.5, 83.5, 79.8, 78.2, 75.4, 75.3, 73.7, 72.5, 69.0, 53.0, 24.0, 15.1; ESI-MS: \( m/z \) (M + Na\(^+\)) calcd 676.2452, obsd 676.2459.

**2-O-(2-(Azidomethyl)benzoyl)-3,4,6-tri-O-benzyl-\( \beta \)-D-glucopyranosyl-(1→6)-1,2;3,4-diisopropylidene-\( \alpha \)-D-galactopyranoside 46.** Method A: Phosphate 45 (75 mg, 0.094 mmol) and 1,2,3,4-diisopropylidene-\( \alpha \)-D-galactopyranoside 19 (21 mg, 0.079 mmol) were coevaporated in toluene (3 x 1 mL), then dried under vacuum for 1 h. After addition of CH\(_2\)Cl\(_2\) (1 mL), the solution was cooled -78\(^\circ\)C, and TBSOTf (20 \( \mu \)L, 0.087 mmol) was added. After 10 minutes, the mixture was neutralized with \( \text{Et}_3\text{N} \) and concentrated in vacuo. The residue was purified by flash silica gel chromatography (10% EtOAc/hexanes) to yield 46 (75 mg, 94%). \([\alpha]_D^{22} = -22.76^\circ \) (c = 1.96, CH\(_2\)Cl\(_2\)); IR (thin film): 3030, 2933, 2102, 1728, 1073 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 7.96 (dd, \( J = 7.6, 1.1 \) Hz, 1 H), 7.59-7.53 (m, 2 H), 7.40-7.29 (m, 9 H), 7.22-7.13 (m, 7 H), 5.39 (d, \( J = 5.0 \) Hz, 1 H), 5.31-5.27 (m, 1 H), 4.85-4.77 (m, 4 H), 4.70-4.65 (m, 3 H), 4.62-4.58 (m, 2 H), 4.49 (dd, \( J = 8.4, 2.4 \) Hz, 1 H), 4.22 (dd, \( J = 5.0, 2.4 \) Hz, 1 H), 4.16 (dd, \( J = 7.9, 1.8 \) Hz, 1 H), 4.07 (dd, \( J = 11.1, 4.0 \) Hz, 1 H), 3.90-3.88 (m, 1 H), 3.84-3.82 (m, 2 H), 3.80-3.79 (m, 2 H), 3.71 (dd, \( J = 11.1, 7.2 \) Hz, 1 H), 3.58-3.56 (m, 1 H), 1.27 (s, 3 H), 1.21 (s, 3 H), 1.20 (s, 3 H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 165.5, 138.3, 138.1, 138.0, 132.7, 131.5, 129.0, 128.8, 128.6, 128.4, 128.2, 128.0, 127.8, 109.4, 108.6, 101.5, 96.3, 82.9, 78.2, 75.4, 75.2, 73.8, 73.7, 71.3, 70.7, 70.5, 68.8, 67.6, 53.2, 26.1, 25.8, 25.6, 24.4; ESI-MS: \( m/z \) (M + Na\(^+\)) calcd 874.3521, obsd 874.3496.

Method B: Thioglycoside 47 (85 mg, 0.13 mmol) and galactopyranoside 19 (41 mg, 0.16 mmol) were coevaporated in toluene (3 x 1 mL), then dried under vacuum for 1
h. The residue was dissolved in CH$_2$Cl$_2$ (1 mL) and activated powdered molecular sieves (4 Å, 200 mg) were added to the solution. The reaction mixture was cooled to 0°C and NIS (36 mg, 0.16 mmol) was added, followed by triflic acid (2 µL, 0.016). After 10 minutes, the reaction mixture was neutralized by addition of Et$_3$N, filtered through a plug of silica, and concentrated in vacuo. The crude product was purified by flash silica gel column chromatography (25% EtOAc/hexanes) to yield 46 (97 mg, 88%).

![Chemical Structure](image)

3,4,6-Tri-O-acetyl-2-O-(2-(azidomethyl)benzoyl)-α-D-galactopyranosyl trichloroacetimidate 49. General Procedure C using 1,3,4,6-tetra-O-acetyl-α-D-galactopyranoside$^{26}$ (0.816 g, 2.16 mmol), DMAP (0.792 mg, 6.48 mmol), and AZMB chloride (0.849 g, 4.33) after purification by flash silica gel column chromatography (30% EtOAc/hexanes) afforded 1.087 g (94%) of acetyl 3,4,6-O-acetyl-2-O-(2-(azidomethyl)benzoyl)-α-D-galactopyranoside. [α]$_D$ +82.47° (c = 5.42, CH$_2$Cl$_2$); IR (thin film): 2975, 2104, 1754, 1743, 1732 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): δ 7.84 (dd, $J$ = 7.8, 1.2 Hz, 1 H), 7.59 (td, $J$ = 7.6, 1.3 Hz, 1 H), 7.50 (app d, $J$ = 7.6 Hz, 1 H), 7.41 (td, $J$ = 7.6, 1.1 Hz, 1 H), 6.55 (d, $J$ = 2.0 Hz, 1 H), 5.56-5.54 (m, 3 H), 4.82 (d, $J$ = 14.4 Hz, 1 H), 4.66 (d, $J$ = 14.5 Hz, 1 H), 4.40 (dd, $J$ = 7.3, 6.7 Hz, 1 H), 4.14 (dd, $J$ = 6.7, 4.7 Hz, 2 H), 2.20 (s, 3 H): 2.13 (s, 3 H), 2.06 (s, 3 H), 2.00 (s, 3 H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 170.6, 170.4, 169.0, 165.6, 137.7, 133.5, 131.1, 130.3, 128.5, 127.9, 89.8, 68.9, 67.7, 67.6, 61.4, 53.0, 21.1, 20.9, 20.8; ESI-MS: m/z (M + Na)$^+$ calc 530.1381, obsd 530.1365.

A solution of 1,3,4,6-tetra-O-acetyl-2-O-(2-(azidomethyl)benzoyl)-α-D-galactopyranoside (0.38 g, 0.71 mmol) in THF (10 mL) was cooled to 0°C and treated with benzylamine (0.12 mL, 1.06 mmol). The reaction mixture was allowed to warm slowly to room temperature and after 24 h the reaction mixture was diluted with water and extracted with CH$_2$Cl$_2$ (3 × 50 mL). The combined organic extracts were dried over Na$_2$SO$_4$, filtered and concentrated. The crude product was purified by flash silica gel column chromatography (30% EtOAc/hexanes) to yield 3,4,6-tri-O-acetyl-2-O-(2-
(azidomethyl)benzoyl)-α-D-galactopyranoside (0.241 g, 72%). \([\alpha]_D^0 +64.72^\circ\) (c = 3.13, CH₂Cl₂); IR (thin film): 3435, 2969, 2104, 1748, 1236 cm⁻¹; \(^1\)H NMR (400 MHz, CDCl₃): \(\delta\) 8.02 (dd, \(J = 7.8, 1.2\) Hz, 1 H), 7.61-7.57 (m, 1 H), 7.49-7.42 (m, 2 H), 5.67 (m, 1 H), 5.62 (dd, \(J = 10.8, 3.4\) Hz, 1 H), 5.53 (dd, \(J = 3.3, 1.1\) Hz, 1 H), 5.41 (dd, \(J = 10.7, 3.5\) Hz, 1 H), 4.76 (dd, \(J = 21.8, 14.1\) Hz, 2 H), 4.55-4.53 (m, 1 H), 4.21-4.11 (m, 2 H), 3.45 (s, 1 H), 2.19 (s, 3 H), 2.07 (s, 3 H), 1.97 (s, 3 H); \(^1^3\)C NMR (100 MHz, CDCl₃): \(\delta\) 170.8, 170.5, 170.4, 137.3, 133.5, 131.9, 130.5, 128.8, 128.3, 90.9, 69.5, 68.5, 67.4, 66.6, 62.0, 53.5, 20.9; ESI-MS: \(m/z\) (M + Na)\(^+\) calcd 488.1267, obsd 488.1275.

3,4,6-Tri-O-acetyl-2-O-(2-(azidomethyl)benzoyl)-α-D-galactopyranoside (194 mg, 0.413 mmol) was azeotroped with toluene (3 x 3 mL), then dried under vacuum for 1 h. CH₂Cl₂ was added (2 mL) along with trichloroacetimide (2 mL), followed by DBU (6 μL, 0.04 mmol). After 1 h, the reaction mixture was diluted with CH₂Cl₂ (5 mL), and concentrated in vacuo. The crude product was purified by flash silica gel column chromatography (20% EtOAc/hexanes) to yield 49 (221 mg, 87%). \([\alpha]_D^0 +82.39^\circ\) (c = 7.89, CH₂Cl₂); IR (thin film): 3338, 2970, 2105, 1754, 1677 cm⁻¹; \(^1\)H NMR (400 MHz, CDCl₃): \(\delta\) 8.64 (s, 1 H), 7.94 (dd, \(J = 7.9, 1.2\) Hz, 1 H), 7.58 (td, \(J = 7.6, 1.3\) Hz, 1 H), 7.50 (dd, \(J = 7.0, 0.6\) Hz, 1 H), 7.37 (td, \(J = 7.7, 1.2\) Hz, 1 H), 6.75 (s, 1 H); 5.63-5.62 (m, 3 H), 4.84 (d, \(J = 14.6\) Hz, 1 H), 4.75 (d, \(J = 14.6\) Hz, 1 H), 4.54-4.50 (m, 1 H), 4.21 (dd, \(J = 11.3, 6.6\) Hz, 1 H), 4.12 (dd, \(J = 11.3, 6.7\) Hz, 1 H), 2.21 (s, 3 H), 2.04 (s, 3 H), 1.99 (s, 3 H); \(^1^3\)C NMR (100 MHz, CDCl₃): \(\delta\) 170.5, 170.3, 170.2, 165.5, 160.8, 138.1, 133.6, 131.5, 130.1, 128.4, 127.4, 93.7, 69.3, 67.7, 61.4, 53.1, 20.8; ESI-MS: \(m/z\) (M + Na)\(^+\) calcd 631.0372, obsd 631.0394.

\[\begin{align*}
\text{AcO} & \quad \text{OAc} \\
\text{AcO} & \quad \text{AZMBO} \\
\text{0} & \\
\end{align*}\]

2-O-(2-(Azidomethyl)benzoyl)-3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→6)-1,2,3,4-diisopropylidene-α-D-galactopyranoside 50. Glycosyl trichloroacetiminate 49 (62 mg, 0.1 mmol) and galactopyranoside 19 (31 mg, 0.12 mmol) were coevaporated in toluene (3 x 3 mL), then dried under vacuum for 1 h. Dichloromethane (1 mL) was
added, the solution was cooled 0°C, and TMSOTf (2 µL, 0.01 mmol) was added. After 20 minutes, the reaction mixture was diluted with CH₂Cl₂ (5 mL), and concentrated in vacuo. The crude product was purified by flash silica gel column chromatography (20% EtOAc/hexanes) to yield 50 (64 mg, 90%). [α]D -23.37° (c = 4.79, CH₂Cl₂); IR (thin film): 2987, 2937, 2104, 1748, 1243 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.94 (dd, J = 7.8, 1.2 Hz, 1 H), 7.56 (td, J = 7.6, 1.3 Hz, 1 H), 7.52-7.49 (m, 1 H), 7.39 (td, J = 7.7, 1.3 Hz, 1 H), 5.50-5.44 (m, 2 H), 5.35 (d, J = 5.0 Hz, 1 H), 5.21 (dd, J = 10.5, 3.4 Hz, 1 H), 4.85 (d, J = 14.8 Hz, 1 H), 4.76 (d, J = 8.0 Hz, 1 H), 4.75 (d, J = 14.8 Hz, 1 H), 4.49 (dd, J = 7.9, 2.4 Hz, 1 H), 4.42-4.18 (m, 2 H), 4.13 (dd, J = 7.9, 1.8 Hz, 1 H), 4.03 (dd, J = 11.3, 3.5 Hz, 1 H), 3.39-3.97 (m, 1 H), 3.90-3.88 (m, 1 H), 3.73 (dd, J = 11.3, 7.5 Hz, 1 H), 2.18 (s, 3 H), 2.06 (s, 3 H), 1.94 (s, 3 H), 1.42 (s, 3 H), 1.28 (s, 3 H), 1.20 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃): δ 170.6, 170.5, 170.4, 165.4, 137.8, 133.0, 131.5, 129.5, 128.5, 128.2, 109.5, 108.7, 101.8, 71.4, 71.0, 70.9, 70.8, 70.4, 69.6, 69.2, 67.7, 67.4, 61.4, 53.2, 26.1, 25.8, 25.0, 24.4, 20.9, 20.8; ESI-MS: m/z (M + Na)⁺ calcd 730.2430, obsd 730.2436.
4.7 References


Chapter 5
Summary and Future Directions
5.1 Thesis Summary

Elucidation of the biological roles of complex carbohydrates, such as the Lewis blood group oligosaccharides, has led to utilization of carbohydrates as therapeutic agents for treatment of parasitic infections,¹ auto-immune disorders,² and as cancer vaccines.³ Medicinal use of carbohydrates requires access to discrete, isomerically pure oligosaccharides to demonstrate specificity. Many methods exist for the generation of these biopolymers, including enzymatic degradation of naturally occurring oligosaccharides,⁴ chemo-enzymatic synthesis,⁵ and chemical synthesis,⁶ but it is still difficult for the layperson to gain easy access to complex saccharides.

Carbohydrates persist as the last of the three major biopolymers to have no available general assembly method. The first oligosaccharide synthesizer⁷ broke new ground the future of complex carbohydrate synthesis, as a large phytoalexin elicitor dodecasaccharide was completed in less than a day. While the potential impact of oligosaccharide automation was recognized, application toward the syntheses of varied structures containing many different linkages and multiple points of branching was not initially realized. Automation of the Lewis oligosaccharides, being a class of compounds exhibiting an intricacy in structure indicative of the most complex naturally occurring sugars, demonstrates the generality and capability of automated assembly.

Solution-phase syntheses serve as models for future automated syntheses; modular construction of compounds as n-pentenyl glycosides aided in identification and characterization of compounds achieved by automated assembly. Utilization of glycosyl phosphates and trichloroacetimidates in a stepwise approach allowed for the construction of three of the Lewis oligosaccharides (Chapter 2); the H-type II pentasaccharide, Le⁸ pentasaccharide and Le⁹ hexasaccharide were all completed in high-yielding solution-phase syntheses. In the course of the H-type II synthesis, the utility and shortcomings of the 2-(azidomethyl)benzoate ester were demonstrated. Development of a glucosamine building block employing a trichloroacetimidate group for amino protection improved coupling yields onto a lactose acceptor and allowed for completion of the Le⁸ and Le⁹ structures.
Application of solution-phase syntheses to the solid-support indicated steps requiring modification in solution. Difficulties in the inclusion of an \(N\)-phthaloyl protected glucosamine building block in the synthesis of a core trisaccharide structure prompted development of a more reactive glycosyl donor. Likewise, difficulties in solid-phase 2-(azidomethyl)benzoate ester removal provoked a reassessment of the overall protecting group strategy and resulted in a return to traditional esters for temporary protection in the solution-phase syntheses of Le\(^x\) and Le\(^y\).

An initial solid-phase automated synthesis of the Le\(^x\) pentasaccharide met with little success and highlighted a need for rapid identification of problematic steps during the course of an automated run. Chapter 3 describes the development of a coupling cycle incorporating the Fmoc group for temporary protection and quantitative monitoring of each glycosylation and deprotection sequence by UV absorbance. Recognition of incomplete glycosylation allowed us to retrieve remaining donors and rapidly modify monosaccharide coupling or protecting group cleavage protocols to enhance production of resin-bound oligosaccharides. Employment of Fmoc for intermittent protection, which requires only mildly basic conditions for cleavage, allowed improvement of another facet of prior solid-phase syntheses. An ester-type linkage replaced the previously used ether linkage connecting the 4,5-Z-octenediol to the solid-support. Cleavage of the resin-bound saccharide following completion of the automated synthesis with sodium methoxide greatly accelerated product recovery, as complete cleavage was achieved in a few hours compared with 1 to 2 days required for cross-metathesis using Grubbs’ catalyst.

Application of Fmoc monitoring methods and the novel linker system to the synthesis of Le\(^x\) produced the branched pentasaccharide with excellent stepwise yields (90-95%) in under 12 hours. Extension of this synthesis by one building block completed the Le\(^y\) hexasaccharide and the dimeric Le\(^y\)-Le\(^x\) nonasaccharide was completed in less than a day using the same monosaccharide building blocks. This is a significant achievement in the chemical synthesis of carbohydrates; only a few years ago the Le\(^y\)-Le\(^x\) nonasaccharide required more than one year to complete. Using a stepwise monosaccharide approach allows lengthy structures to be synthesized using an identical
synthetic strategy to that for smaller oligomers. The emphasis in carbohydrate synthesis can now shift from donor development to automated cycle development.

As noted in both the solution and solid-phase syntheses of the Lewis oligosaccharides, a large part of the synthetic strategy in the chemical synthesis of carbohydrates revolves around the choice of protecting groups used during a synthesis. Seeking greater diversity in protecting groups, the para-chlorophenyl carbonate and the 2-(azidomethyl)benzoate ester were developed (Chapter 4). Both groups demonstrated orthogonality to a variety of traditionally used protecting groups; the 2-(azidomethyl)benzoate ester, however, was superior by virtue of being cleaved under non-basic, reducing conditions that left all other esters intact. Using the 2-(azidomethyl)benzoate ester, the solution-phase synthesis of the H-type II pentasaccharide was completed.

5.2 Future Directions

While the syntheses presented in this thesis represent the most complex carbohydrates accessed by automation to date, there are still considerable advances to be made before this methodology is generally useful for the chemical layperson. Monitoring of coupling cycles brings our automated assembly another step closer to peptide and oligonucleotide assembly, but currently only the Fmoc group has proven robust enough under glycosylation conditions to provide visual indication of coupling efficiency. Highly branched carbohydrates, the Lewis antigens included, often require several orthogonal protecting groups for installation of branching. Development of additional groups orthogonal to Fmoc bearing UV-active reporting groups would enhance current syntheses since every step could be monitored for efficiency. Efforts are underway within the Seeberger lab to develop such additional groups.

Current automated efforts allow for the procurement of fully protected oligosaccharides using 10 total equivalents of a building block for installation of each monosaccharide. In the synthesis of longer oligomers, this can translate to using gram quantities of donors to achieve milligram quantities of final products. While the time saved in automated syntheses is worthwhile when operating on an exploratory scale (25
μmolar), the time consumed in acquiring ample amounts of donors for larger scale syntheses is no longer reasonable. Coupling cycles must be developed that utilize less overall equivalents of donor or donor syntheses must be simplified to enable mass quantities to be procured.

Following the completion of an automated run and cleavage of the saccharide from the solid-support, protecting groups must still be removed by solution-phase chemistries. Given the complex pattern of protecting groups required for the chemical synthesis of oligosaccharides, protecting group removal to yield a fully deprotected compound is not always straightforward. In the Lewis group series, cleavage of the $N$-trichloroacetamide and pivaloyl esters has proved difficult. Protecting groups that are easily removed at the end of a synthesis, either during product cleavage from the solid-support or just afterward, need to be developed to ensure success of this methodology in the hands of a non-specialist. Improvement in automated coupling cycles, monitorable protecting groups, donor procurement, and product deprotection and isolation will grant access to complex carbohydrates for studies that will expand our understanding of the roles oligosaccharides play in nature.
5.3 References


Appendix A

Selected Spectral Data from Chapter 2
Current Data Parameters
NAME: May21-2001-11
CHRM: 10
PROCD: 1

F2 - Acquisition Parameters
Data: 20010521
Time: 14:43
TR: 6500
SOLVENT: CDC13
NS: 10
DS: 2
DH: 8278.146 Hz
TDB: 0.126314 Hz
TD: 3.75564243 sec
BG: 143.7
D1: 0.40000000 sec
D2: 0.00000000 sec

----------- CHANNEL 1 -----------
MUC1: 4H
PL: 7.95 ussec
PL: 0.00 ussec
SF: 400.1324710 MHz

F2 - Processing Parameters
SF: 32768
DF: 0.1300051 MHz
MOM: EN
DD: 0
LB: 0.30 Hz
BB: 0
PC: 1.00

1D NMR plot parameters
CF: 26.00 cm
F: 7.938 ppm
F1: 3200.40 Hz
F2: -0.026 ppm
F2: -10.87 Hz
NV: 0.40124 ppm/cm
HCM: 160.54851 Hz/cm
Appendix B
Selected Spectral Data from Chapter 3
Current Data Parameters
NAME: Nesy1-2002-1k1
EXPNO: 20
PROCNO: 1

F2 - Acquisition Parameters
Data_ 20000031
Time: 9.10
INSTRUM: spect
PROBHD: 5mm 800 90-1
PULPROG: zg50
TD: 65536
SOLVENT: CDC13
NS: 16
DS: 2
SNH: 8278.145 Hz
FTDRES: 0.128314 Hz
AQ: 3.0584243 sec
R6: 161.3
DN: 60.400 usec
DE: 6.00 usec
TE: 360.0 K
d1: 1.0000000000 usec

--------------- CHANNEL 1 --------------
NUC1: 1H
F1: 7.90 usec
PL: 0.00 dB
SF01: 400.1324710 MHz

F2 - Processing parameters
SI: 2768
SF: 400.13000065 MHz
WDM: EH
SSB: 0
LB: 0.30 Hz
GB: 0
PC: 1.00

1D NMR plot parameters
G4: 20.00 cm
F1P: 8.040 ppm
F1: 3217.04 Hz
F2P: 0.338 ppm
F2: 15.30 Hz
PPM: 0.4509 ppm/cm
H2CM: 160.08745 Hz/cm
HPLC Trace of crude Lewis X (1) in a gradient run (15 to 40% ethyl acetate/hexanes over 20 min) on a silica gel analytical column. Lewis X retention time is 18.95 min.
HFLC Trace of crude Lewis Y (A) in a gradient run (10 to 50% ethyl acetate/acetonitrile over 20 min) on a C18 analytical column. Lewis Y retention time is 10.71 min.
HPLC Trace of crude Le\textsuperscript{+}-Le\textsuperscript{-} (3) in a gradient run (10 to 50% ethyl acetate/acetonitrile over 20 min) on a C18 analytical column. Le\textsuperscript{+}-Le\textsuperscript{-} retention time is 13.64 min.
Appendix C

Selected Spectral Data from Chapter 4
Current Data Parameters
NAME Oct31-2000-111
EXPNO 10
PROCNO 1

F2 - Acquisition Parameters
Date 20001031
Time 14:14
INSTRUM spect
PROBB 50m 800 89-1
PULPROG zg30
TD 65236
SOLVENT CDCl3
NS 16
DS 2
SN 8279, 146 Hz
FRES 0.125314 Hz
AD 3.9554243 sec
AB 574.7
DM 60.400 usec
DE 0.00 usec
TE 300.0 K
D1 1.00000000 sec

-------- CHANNEL 11 --------
MCD 1H
P1 7.00 usec
PL1 0.00 GS
SF01 400.1324710 MHz

F2 - Processing parameters
SI 32768
SF 400.13000005 Hz
WM EM
SSB 0
LB 0.30 Hz
DB 0
PC 1.00

10 NMR plot parameters
C1 26.00 cm
F1P 0.261 ppm
F1 3305.34 Hz
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### F2 - Acquisition Parameters

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Kerry Routenberg Love

Education
Massachusetts Inst. of Technology Cambridge, MA
- Ph.D. Candidate in Organic Chemistry, Expected February 2004

University of Virginia Charlottesville, VA
- B.S in Chemistry with High Distinction, May 1999
- Thesis: “Synthesis of Lysophosphatidic Acid Analogos”

Honors
2001-2002 NIH Biotechnology Training Grant Recipient
2000 NDSEG Fellowship Honorable Mention

Research Experience
Graduate Research Fellow, Mass. Inst. of Technology Cambridge, MA
Advisor: Prof. Peter H. Seeberger January 2000 - present
- Developed a linear strategy for the synthesis of the Lewis group antigens and adapted it to automation using a fluorescence-based method for monitoring coupling efficiency and a novel linker for solid-phase synthesis.

NSF Undergraduate Research Fellow, Columbia Univ. New York, NY
Advisor: Prof. Nick Turro June – August 1998
- Synthesized chiral faujasite zeolites and analyzed stereodirecting ability in photolysis of organic substrates by chiral GC.

NSF Undergraduate Res. Fellow, Univ. of Virginia Charlottesville, VA
Advisor: Prof. Timothy L. MacDonald Winter 1997 – Spring 1999
- Synthesized analogs of Lysophosphatidic acid, a G-coupled protein receptor agonist, containing a rigid fatty acid moiety for a structure-activity analysis of the lipid segment.

Publications