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Automated Solid-Phase Synthesis of a Branched *Leishmania* Cap Tetrasaccharide

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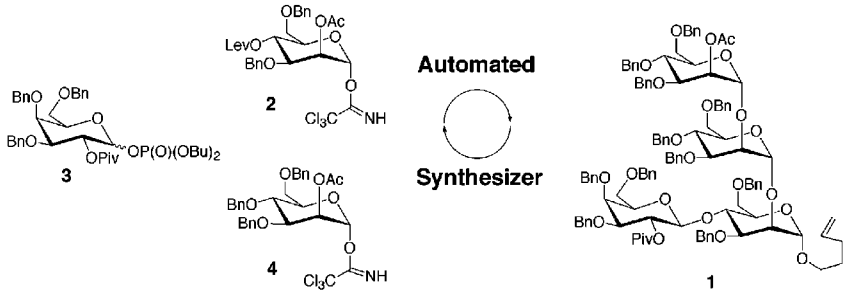
ABSTRACT



Described is the first automated solid-phase synthesis of a branched oligosaccharide by stepwise assembly from monosaccharides. Cap tetrasaccharide 1, found as part of the cell surface lipophosphoglycan (LPG) of the protozoan parasite *Leishmania,* was readily prepared using glycosyl phosphate and glycosyl trichloroacetimidate building blocks.

Oligopeptides[1] and oligonucleotides[2] are routinely prepared by automated solid-phase synthesis. Oligosaccharides constitute the third major class of repeating biopolymers found in nature and have been much more difficult to prepare. Automated solid-phase synthesis and one-pot solution-phase synthesis approaches for the preparation of these molecules have only very recently been reported.[3,4] Unlike peptides and oligonucleotides, oligosaccharides are commonly encountered in highly branched form. In the first automated oligosaccharide synthesis the branching points were installed by incorporation of disaccharide building blocks to maintain the linear mode of assembly.[3] The common occurrence of branched motifs in carbohydrates of biochemical and biomedical significance prompted us to address this challenge.

Leishmaniasis is a tropical disease that afflicts over 12 million people worldwide[5] and is on the verge of becoming endemic in the U.S.[6,7] Lipophosphoglycans (LPG),[8] which are ubiquitous on the cell surface of *Leishmania* parasites, are composed of a glycosylphosphatidylinositol (GPI) anchor, a repeating phosphorylated disaccharide, and different cap oligosaccharides (Figure 1). The branched tetrasaccharide at the terminus of the LPG constitutes an attractive vaccine target and has been previously synthesized in solution[9] and on solid support.[9c] While the initial immunological results using the synthetic carbohydrate vaccine are promising,[10] more rapid access to the carbohydrate portion of potential vaccines would greatly facilitate such ventures.

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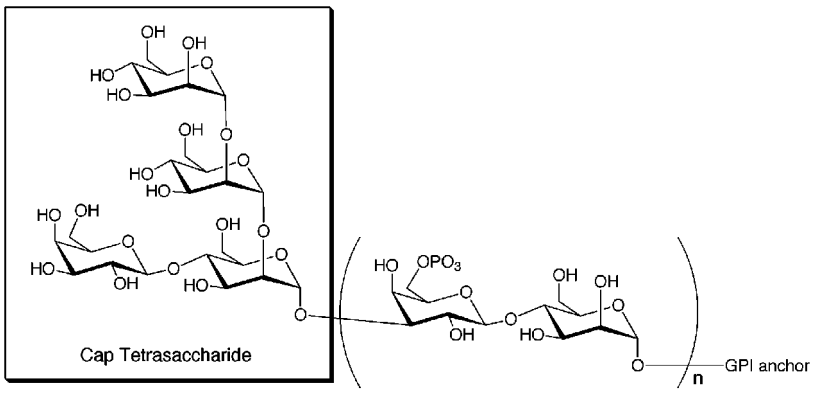


Figure 1. Leishmania LPG.

Here we report the first automated solid-phase synthesis of *Leishmania* tetrasaccharide **1** from monosaccharide building blocks. This automated synthesis utilized both glycosyl phosphates and glycosyl trichloroacetimidate building blocks and different ester protecting groups to accomplish branching.

Examination of the tetrasaccharide revealed that it can be accessed using two suitably protected mannose building blocks and one galactose monomer (Figure 2). Mannosyl

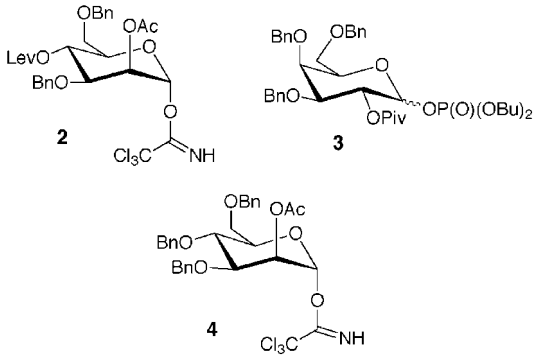


Figure 2. Monosaccharide building blocks required for automated synthesis.

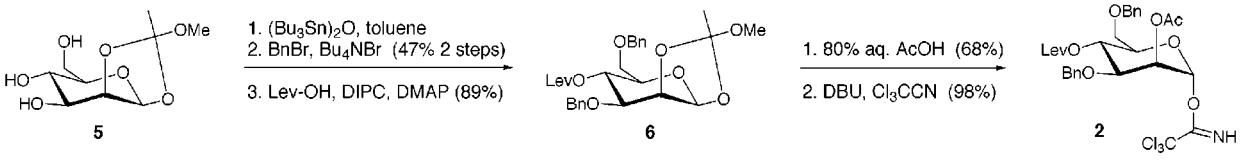
trichloroacetimidate **2** would provide the core mannose unit with branching points in the 2- and 4-positions. Glycosyl phosphate **3**[11] would supply the galactose moiety, and mannosyl trichloroacetimidate **4**[12] would be used twice to build the 1→2-linked mannose region.

After the three required monosaccharide building blocks had been identified, preparation of core mannose unit **2** commenced from known ortho ester triol **5** (Scheme 1).[13] Selective 3,6-di-benzylation of **5**[14] was followed by protection of the remaining 4-hydroxyl group as a levulinate ester to afford **6** in 89% yield. Treatment of the ortho ester with aqeuous acetic acid furnished a mixture of anomeric lactols, before reaction with DBU and trichloroacetonitrile provided differentially protected mannosyl trichloroacetimidate **2**.

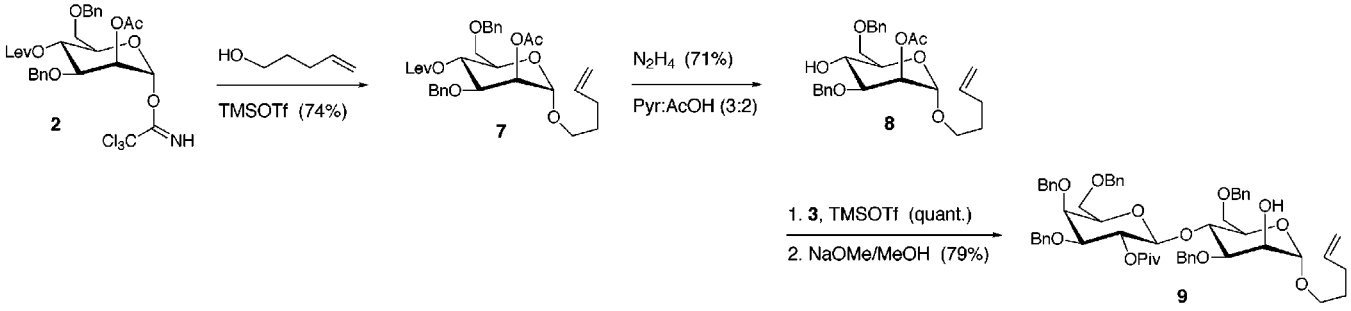
A key aspect of the synthesis was the ability to introduce branching from the central mannose unit by selective protecting group manipulations. To examine this issue, a solution-phase model study was conducted (Scheme 2). Reaction of glycosyl donor **2** with pentenyl alcohol upon activation with catalytic TMSOTf generated *n*-pentenyl glycoside (NPG)[15] **7**. NPGs are also the products of automated oligosaccharide syntheses on polymer support using our octenediol linker[16] (vida supra). Removal of the 4-levulinate group by treatment with hydrazine in the presence of the 2-acetate ester proceeded in 71% yield to furnish the 4-hydroxyl-containing monosaccharide **8**. Installation of the challenging β-1→4 linkage between galactose and mannose was readily accomplished by reaction with galactose phosphate **3**. Treatment with sodium methoxide to remove the axial acetate moiety in the presence of a 2′-pivaloyl group provided disaccharide acceptor **9** in 79% yield over two steps. These results confirmed the validity of our protecting group strategy en route to the automated synthesis of **1**.

With the three monosaccharide building blocks **2**, **3**, and **4** in hand, the automated synthesis was carried out on a

Scheme 1. Synthesis of Mannosyl Trichloroacetimidate **2**



Scheme 2. Synthesis of Disaccharide Acceptor **9**



modified ABI 433A peptide synthesizer[3] using octenediol functionalized Merrifields resin **10** (Scheme 3). Each coupling cycle (Table 1) relied on double glycosylations to ensure high coupling efficiencies and a single deprotection event. Coupling of **2** to resin **10** using catalytic TMSOTf was followed by removal of the levulinate ester upon exposure to hydrazine. Formation of the central β-(1→4) linkage was achieved by reaction of the support-bound alcohol with phosphate **3** using stoichometric amounts of TMSOTf. The 2′-pivaloyl ester ensured complete stereocontrol during this coupling. Selective removal of the acetate ester from the support-bound disaccharide with sodium methoxide preceded coupling with mannosyl trichloroacetimidate **4** using catalytic TMSOTf. Repetition of acetate cleavage and mannosylation with **4** completed the assembly of resin-bound tetrasaccharide **1**. The total time for assembly of the carbohydrate skeleton of **1** on the automated synthesizer was 9 h.

Table 1. Conditions and Reagents for the Automated Synthesis of **1**

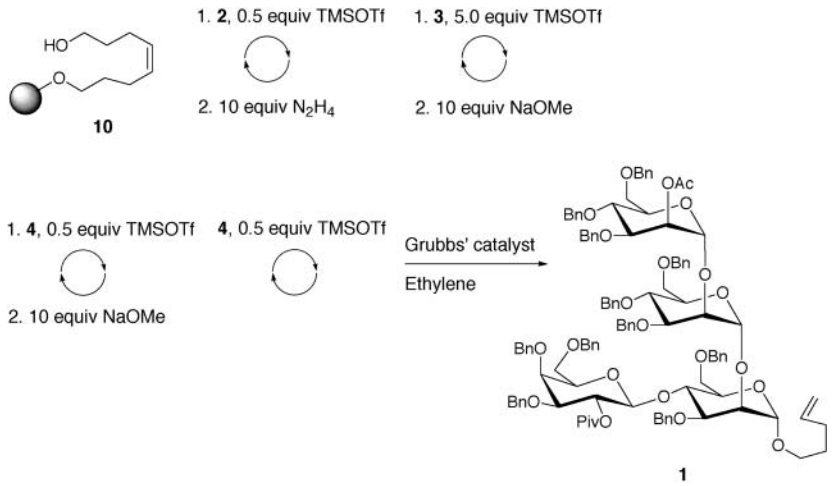
function	reagent	time (min)
glycosylation	5 equiv of donor and TMSOTf[a]	20
wash	dichloromethane	9
glycosylation	5 equiv of donor and TMSOTf[a]	20
wash	dichloromethane	9
deprotection	2 × 10 equiv of NaOMe or N_2H_4	60
wash	0.2 M AcOH/0.2 M MeOH/THF	9
wash	tetrahydrofuran	9
wash	dichloromethane	9

[a] Using 0.5 equiv for building blocks **2** and **4**, 5.0 equiv for building block **3**.

Cleavage of the octenediol linker using Grubbs catalyst[17] in an atmosphere of ethylene provided crude *n*-pentenyl glycoside tetrasaccharide **1**. HPLC analysis of the crude reaction products showed three major peaks (Figure 3): Styrene, the trisaccharide (n-1) deletion sequence (19% relative peak area), and the desired tetrasaccharide **1** (50% relative peak area). Comparison with pure standards of these compounds from solution-phase studies[9c] and ESI mass spectrometry was used to identify tetrasaccharide **1**. The desired product was purified by preparative HPLC and analyzed by NMR.

This first automated synthesis of a branched oligosaccharide was demonstrated on the example of a *Leishmania* cap tetrasaccharide. The desired tetrasaccharide was obtained in less than 4 days starting from the monosaccharide building

Scheme 3. Automated Synthesis of Tetrasaccharide **1**



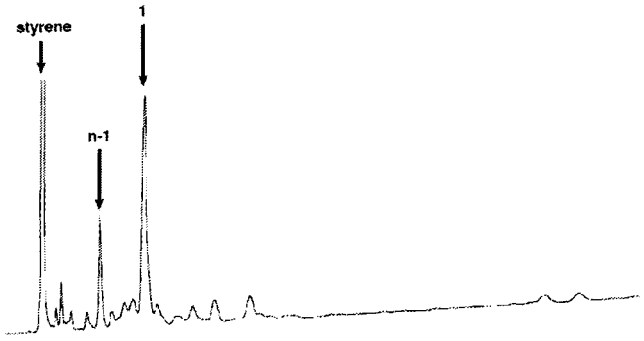


Figure 3. HPLC trace of crude materials obtained after automated synthesis. Conditions: 15−20% EtOAc/hexanes (30 min).

blocks, and showed that acetate and levulinate esters could be used in concert to assemble branched structures. The construction of complex carbohydrate motifs is now limited, in theory, only by the orthogonality of protecting groups available for organic synthesis on solid support. Work in this area is in progress and will be reported in due course.

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Supporting Information Available: Spectral data for all new compounds and intermediates in automated synthesis is included. This material is available free of charge via the Internet at http://pubs.acs.org.

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