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Concise synthesis of *Bacillus anthracis* exosporium tetrasaccharide via two-stage activation of allyl glycosyl donor strategy

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ABSTRACT

A highly efficient glycosylation protocol recently developed in our laboratory has been utilized in the short synthesis of the tetrasaccharide **1**, an antigen important to the development of carbohydrate-based diagnostic tools and vaccines against anthrax. The protocol employs allyl glycosides as building blocks and improves the overall synthetic efficiency.

Anthrax is a fatal infectious disease in humans and other mammals caused by spores of the bacterium *Bacillus anthracis*. The durable spores enter the host, germinate, grow, and kill the host within several days of infection. Due to their lethality, ready production, resistance to harsh conditions, and ease of spread, *B. anthracis* spores are considered as the foremost threat in biological warfare. Unfortunately, there are few medical remedies for bioterrorism preparedness against *B. anthracis* spores. The known pre-exposure prevention against anthrax is vaccination. Development of vaccines targeting spores could inactivate spores prior to their establishment of infectious loci and vegetative cell outgrowth, and thus prevent development of anthrax.

The exosporium is the outermost layer of a spore, the first point of contact of spores with host cells and the source of spore surface antigens. It can be the target of diagnostic and preventative procedures. In 2004, Turnbough and coworkers found multiple copies of a tetrasaccharide on BclA (*Bacillus* collagen-like protein of *anthracis*) of the *B. anthracis* exosporium.[1] This unique tetrasaccharide, that is, 4,6-di-deoxy-4-(3-hydroxy-3-methylbutamido)-2-*O*-methyl-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-l-rhamnopyranosyl-(1→2)-L-rhamnopyranose (**1**), is capped at the non-reducing end with a novel D-anthrose. Since its structure was published in 2004, synthesis of the tetrasaccharide **1** and related anthrose-containing oligosaccharides has become a high priority.[2] Immunological studies of the synthetic tetrasaccharides and their conjugates demonstrate that the tetrasaccharide **1** is a promising antigen for the development of carbohydrate-based diagnostic tools and vaccines against spores of the known virulent strains of *B. anthracis*.[2f,l,3,4] The potential biomedical applications of the tetrasaccharide highlights the need for rapid access to the material in a sufficient amount. Herein we report a highly efficient route to this tetrasaccharide from allyl glycoside building blocks.

We have been pursuing step economy-oriented carbohydrate synthesis in solution phase for rapid access to oligosaccharides of biochemical and biomedical significance. An efficient synthesis demands (1) efficient and effective glycosylation methods with minimal anomeric position manipulation of glycosyl donors and (2) tactically planned protecting group strategy to minimize selective protecting/deprotecting steps. Along this direction, we have recently demonstrated a simple and mild glycosylation reaction employing only allyl glycosides as building blocks. Thus, a prop-1-enyl glycosyl donor readily derived from an allyl glycoside can undergo a highly effective glycosylation reaction with an allyl glycosyl acceptor upon activation with NIS or NIS/TfOH at room temperature.[5,6] As prop-1-enyl glycosyl donors are directly isomerized from allyl glycosides, time-consuming anomeric group replacement and intermediate purification are avoided. The isomerization of the anomeric allyl group to the corresponding prop-1-enyl is typically of high efficiency with a variety of facile methods available in literature.[7] Mindful of the ease of anomeric allyl group placement and allyl to prop-1-enyl isomerization, and simple conditions of glycosylation with prop-1-enyl donors, we anticipate that the new protocol would be suitable for rapid oligosaccharide synthesis with high overall synthetic efficiency.

With the new glycosylation approach, the tetrasaccharide can be synthesized from simple allyl glycoside building blocks **2**–**4** as shown in the retrosynthesis (Scheme 1). The building blocks **2**

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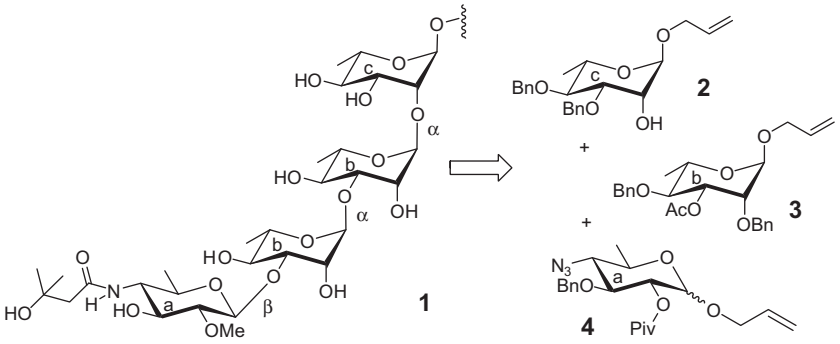


Scheme 1. Retrosynthesis of tetrasaccharide **1.**

and **3** were obtained from commercially available L-rhamnose in six steps.[5b]

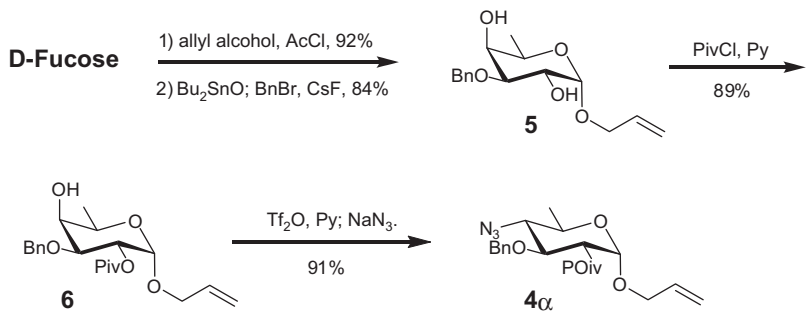
The building block **4α** was prepared from commercially available D-fucose in five steps (Scheme 2). Thus, installation of the anomeric allyl group followed by selective benzylation at 3-OH provided the allyl fucoside **5** in 76% yield in two steps.[8] Subsequent placement of a *O*-2 pivalolyl group generated **6** in 89% yield. The axial 4-OH of **6** was then converted to an equatorial azido group via a triflate intermediate in 91% yield.[9]

With the building blocks in hand, we began to assemble the tetrasaccharide (Scheme 3). The allyl glycoside **3** was first isomerized to the corresponding prop-1-enyl glycosyl donor with hydrogen-activated $[Ir(COD)(PMePh_2)_2]PF_6$.[5,10] Upon activation of the vinyl glycoside with NIS/TfOH in the presence of the acceptor **2**, the desired disaccharide **7** was obtained in 82% yield after alkali workup. The β anomer of **7** was not produced in a detectable amount. However, when the same procedure was repeated with the donor **3** and the acceptor **7**, the trisaccharide **8** was obtained as a mixture of two anomers ($\alpha/\beta = 7:1$) in 84% yield after alkali workup. These two anomers were separated by column chromatography. The anomeric stereochemistry of the newly constructed glycosidic bonds in **7** and **8** was confirmed by the $^1J_{C1-H1}$ coupling constants.[11,12] The iterative process continued with the donor **4** and the acceptor **8α** to provide the tetrasaccharide **10** after the O-2 pivalolyl group of **9** was replaced by a methyl group. With the neighboring directing effect of the O-2 pivalolyl group, stereoselective construction of the new glycosidic bond was accomplished based on ^1H NMR analysis.

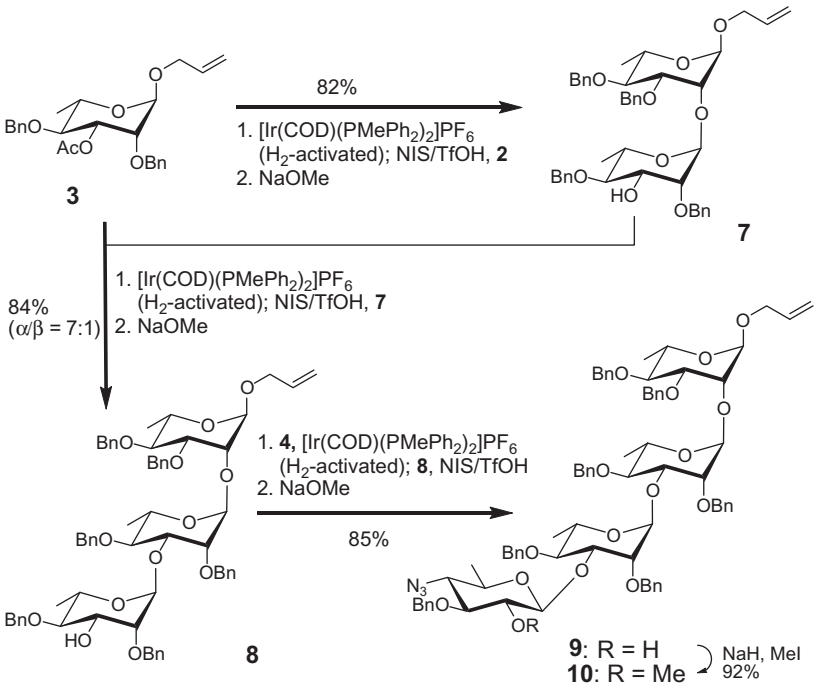
Alternatively, synthesis of the tetrasaccharide was attempted via a [2+2] route (Scheme 4). Thus, the disaccharide **11** was prepared in 66% yield from the allyl glycosyl donor **4** and the acceptor **12**, derived from **3** by removing the O-3 acetyl group. The reducing end expansion with the donor **11** and the acceptor **7** led to the tetrasaccharide **10** in 59% yield along with a trace amount of the β anomer. It is unclear why the yields of producing **10** and **11** were lower than those of **8** and **9**, respectively.

We demonstrated that the tetrasaccharide skeleton **10** was constructed in 17 steps from commercially available free monosaccharides, a synthesis shorter than previously reported work (typically taking over 30 steps to reach similar complexity from commercially available starting materials).[2] The anomeric allyl group of the tetrasaccharide **10** can be functionalized through ozonolysis,[13] olefin metathesis,[14] or thiol-ene click chemistry[15] for future immobilization or conjugation before or after[2a,j] the subsequent sequence of global deprotection, reduction of the azido group and amide formation. In this work we explored a different approach. Thus, from the tetrasaccharide **10**, the anomeric allyl group was converted to the (3-hydroxyl)propyl group under hydroboration conditions to provide **13** in 87% yield (Scheme 5). The subsequent one step global deprotection of **13** removed benzyl protecting groups and reduced the azido group to the amino group through palladium catalyzed hydrogenolysis. A standard amide formation reaction with HATU and the butanoic acid[2a,j] achieved the final product in 72% yield over two steps. The obtained tetrasaccharide equipped with a functionalized anomeric group provided another option for further elaboration.

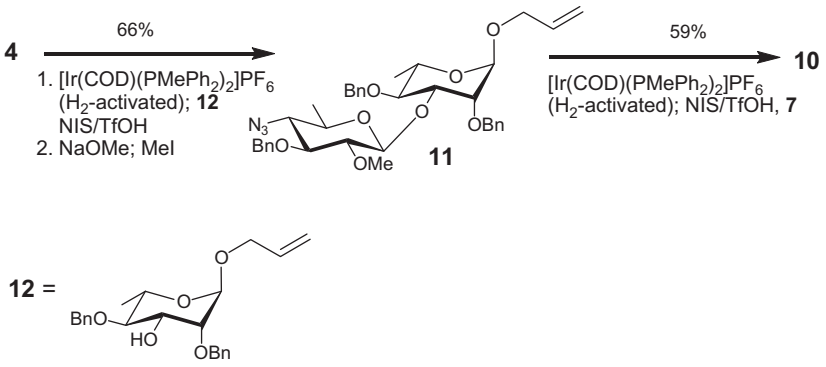
In summary, a concise synthesis of the important tetrasaccharide target was achieved by utilizing the effective and efficient glycosylation method recently developed in our laboratory.[5] This method employed allyl glycosides as building blocks. The anomeric allyl protecting group of glycosyl donors was first converted to the more reactive prop-1-enyl leaving group. Without purification, the prop-1-enyl intermediate was activated in the presence of an allyl glycosyl acceptor for glycosidic bond construction. This procedure avoided time-consuming anomeric group removal/installation and intermediate purification. Moreover, iterative use of allyl



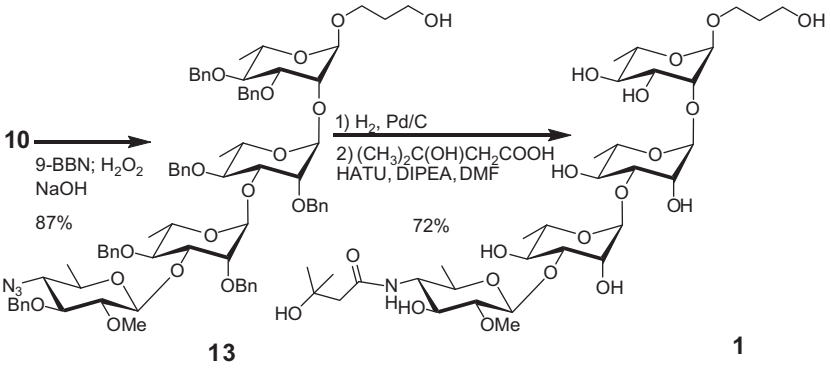
Scheme 2. Synthesis of building block **4.**



Scheme 3. Glycosidic bond construction.



Scheme 4. Synthesis of the tetrasaccharide via a [2+2] route.



Scheme 5. Synthesis of the tetrasaccharide.

glycosides for oligosaccharide synthesis does not require any armed/disarmed property tuning, significantly simplifying building block preparation, and improving the overall synthetic efficiency.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.05.089.

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