Vaccines

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Total Synthesis of Antigen *Bacillus Anthracis* Tetrasaccharide—Creation of an Anthrax Vaccine Candidate**

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A few letters containing a seemingly inconspicuous white powder killed four innocent people, instilled fear in most Americans for several weeks, and brought the US Postal Service to the brink of collapse shortly after the events of September 11, 2001. The white powder was identified as dormant spores of the Gram-positive soil bacterium *Bacillus anthracis*,[1,2] which are highly resistant to extreme temperatures, radiation, harsh chemicals, desiccation, and physical damage. These properties allow them to persist in the soil for many years.[3] These spores cause anthrax, a serious infection of herbivores and cattle, but infects humans only rarely, except when specially prepared and dispensed as biowarfare agents. If the spores are inhaled, the host is usually killed within days. Three polypeptides that comprise the anthrax toxin play a major role in all stages of infection, from germination to the induction of vascular collapse leading to host death.[4]

Bacillus anthracis, like most bacteria, bears unique oligosaccharides on the surface of the spore for interaction with the host. Specific oligosaccharide antigens can be used to design an antibacterial vaccine for the induction of an immune response.[5] Carbohydrates are evolutionarily more stable than proteins and have been exploited in a series of commonly employed vaccines.[6] Synthetic oligosaccharide vaccines have shown very encouraging results against cancer,[7] malaria,[8] and *Haemophilus influenzae* type b[9] to name just a few.

The structure of tetrasaccharide **1**, which is found on the surface of the exosporium glycoprotein BC1A of *Bacillus anthracis* was elucidated in 2004 (Figure 1).[10] A unique characteristic of this antigen is the nonreducing terminal sugar, the so-called anthrose, which is not even found in closely related species.[10] Tetrasaccharide **1** is therefore a very attractive target for vaccine development and the elucidation

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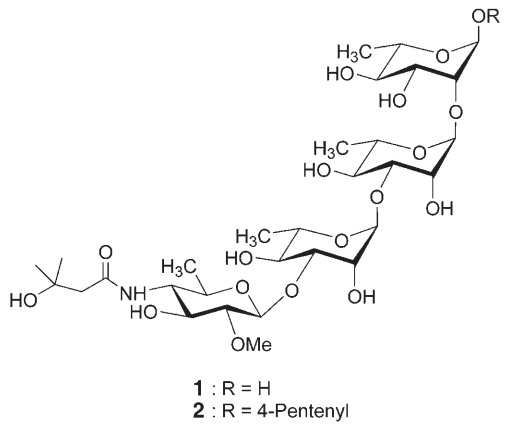
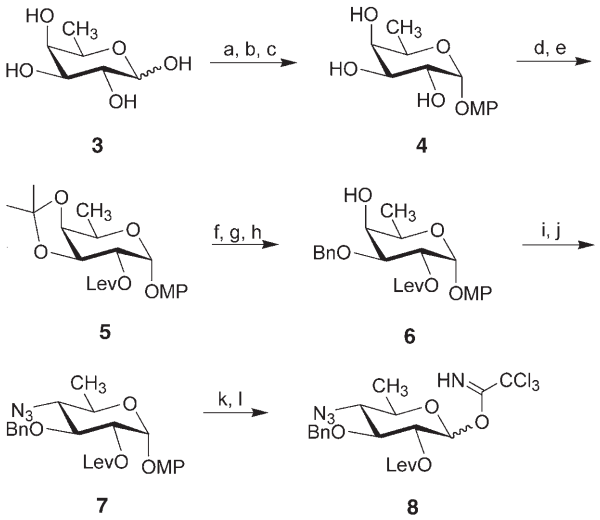


Figure 1. Structure of the terminal tetrasaccharide **1** of the major surface glycoprotein of *Bacillus anthracis* and analogue **2** ready for conjugation.

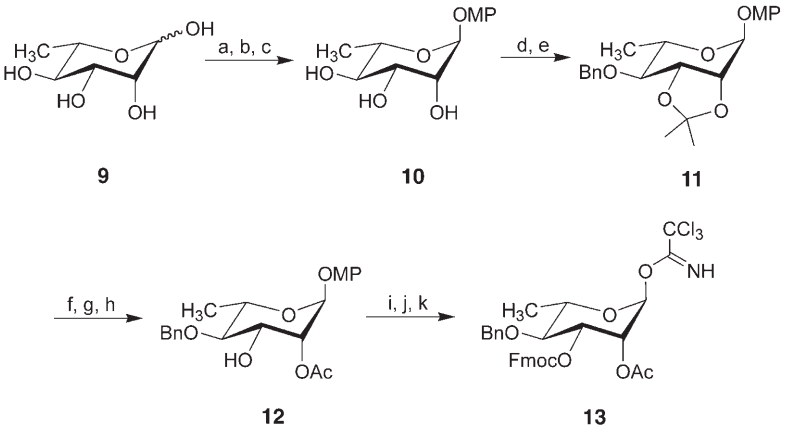


Scheme 1. Synthesis of anthrose building block **8**. Reagents and conditions: a) Ac_2O, pyridine, 12 h, quant.; b) MPOH, $BF_3{\cdot}OEt_2$, acetone, 0 °C→25 °C, 12 h, 71 %; c) NaOMe, MeOH, 6 h, quant.; d) 2,2-dimethoxypropane, $BF_3{\cdot}OEt_2$, acetone, 0 °C→25 °C, 12 h, 96 %; e) LevOH, DMAP, DIPC, CH_2Cl_2, 0 °C, 3 h, 92 %; f) HCl (pH 3), MeOH, 50 °C, 18 h, 85 %; g) $n$$Bu_2SnO$, toluene, Dean–Stark apparatus, reflux, 2 h; h) BnBr, TBAI, toluene, reflux, 3 h, 95 % (two steps); i) Tf_2O, pyridine, 0 °C, 90 min; j) NaN_3, DMF, 25 °C, 10 h, 80 % (two steps); k) CAN, H_2O/CH_3CN, 25 °C, 1 h; l) Cl_3CCN, NaH, CH_2Cl_2, 25 °C, 45 min, 78 % (two steps). MPOH = *para*-methoxyphenol, LevOH = levulinic acid, DMAP = 4-dimethylaminopyridine, DIPC = diisopropyl carbodiimide, Bn = benzyl, TBAI = tetrabutylammonium iodide, CAN = cerium ammonium nitrate.

of a highly specific immune response against *Bacillus anthracis*.

Herein, we describe the first total synthesis of tetrasaccharide **2** through a convergent [2+2] approach that facilitates access to analogues and shorter sequences. The terminal pentenyl group can serve as a point of attachment during conjugation to a carrier protein in vaccine development. A straightforward synthesis of the unique monosaccharide anthrose is part of this total synthesis.

Synthesis of the terminal anthrose[10] started from commercially available D-fucose (**3**) (Scheme 1). Acetylation of **3**, followed by immediate protection of the anomeric center with *para*-methoxyphenol (MPOH) and subsequent cleavage of the acetates furnished **4**. A levulinoyl group proved to be the best choice to protect the C2 hydroxy group during installation of the β(1→3) glycosidic linkage in anticipation of its selective removal prior to methylation of *O*2. Thus, reaction of **4** with 2,2-dimethoxypropane and introduction of the levulinic ester at C2 furnished **5**. Removal of the isopropylidene and tin-mediated selective benzylation of the hydroxy group at C3 afforded **6**. The configuration of C4 was inverted by reaction of the hydroxy group with triflic anhydride to install a triflate, which was displaced by sodium azide in an S_N2-type fashion to give **7**.[11] Removal of the anomeric *p*-methoxyphenyl group with wet cerium ammonium nitrate was followed by the formation of the anthrose trichloroacetimidate **8** by treatment with trichloroacetonitrile and a catalytic amount of sodium hydride. A completely different, more lengthy approach to the synthesis of an anthrose monosaccharide was reported recently.[12]



Scheme 2. Synthesis of rhamnose building block **13**. Reagents and conditions: a) Ac_2O, pyridine, 12 h, quant.; b) MPOH, $BF_3{\cdot}OEt_2$, acetone, 0 °C→25 °C, 12 h, 80 %; c) NaOMe, MeOH, 12 h, 96 %; d) 2,2-dimethoxypropane, $BF_3{\cdot}OEt_2$, acetone, 0 °C→25 °C, 12 h, 84 %; e) NaH, BnBr, DMF, 0 °C→25 °C, 4 h, quant.; f) HCl (pH 3), MeOH, 50 °C, 89 %; g) 1,1,1-triethoxyethane, *p*-TsOH (cat.), DMF, 50 °C, 50 min; h) AcOH/H_2O (4/1, *v*/*v*), 10 °C, 10 min, 98 % (two steps); i) FmocCl, pyridine, 25 °C, 2 h, 88 %; j) CAN, H_2O/CH_3CN, 25 °C, 1 h, 76 %; k) Cl_3CCN, NaH, CH_2Cl_2, 25 °C, 1 h, 94 %. TsOH = *para*-toluenesulfonic acid, DMF = *N*,*N*-dimethylformamide, Fmoc = fluorenylmethoxycarbonyl.

Rhamnose building block **13**, which is equipped with a robust participating group at C2 to ensure α selectivity and a readily removable temporary protecting group (Fmoc) at 3-OH was synthesized next (Scheme 2). First, the anomeric center was protected with a *para*-methoxyphenol group under the conditions described above to give **10**.[13] Formation of the *cis*-fused acetal and subsequent benzylation afforded **11**. The transformation of the acetal into the corresponding

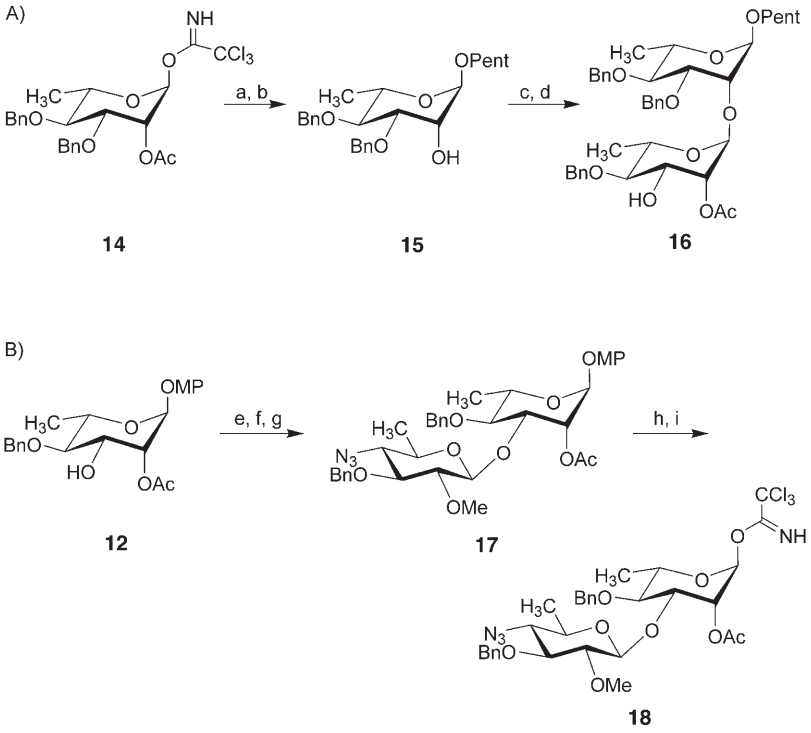
orthoester and ring opening resulted in the kinetically preferred axial acetate in **12**. The remaining hydroxyl function was protected with an Fmoc group, and the *para*-methoxyphenyl glycoside was cleaved. Subsequent reaction with trichloroacetonitrile in the presence of traces of sodium hydride afforded building block **13**.

The assembly of the tetrasaccharide through a [2+2] approach commenced with the reaction of known building block **14**[14] with 4-penten-1-ol (Scheme 3). The pentenyl moiety serves at a later stage as a handle for conjugation to a carrier protein in the preparation of the vaccine candidate. Cleavage of the acetate at C2, further glycosylation with **13**, and subsequent removal of Fmoc yielded disaccharide **16**. During the cleavage of the Fmoc group, minor acetate migration (≈10%) from the 2-OH to the 3-OH group was observed, but the undesired product was easily removed by column chromatography.

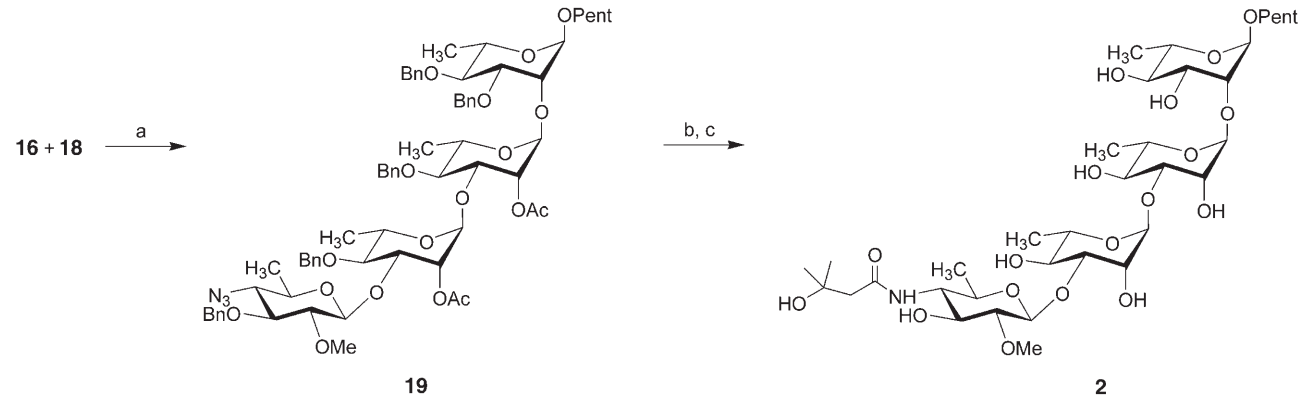
The second disaccharide (Scheme 3B) was assembled by glycosylation of rhamnose **12**, an intermediate in the synthesis of building block **13**, with the anthrose unit **8**. The levulinoyl group, which ensured β selectivity, was replaced by the final methoxy substituent at C2. The methylation in the presence of acetate proved to be challenging. Even powerful methylating agents such as methyl triflate and diazomethane failed to facilitate the transformation. Satisfying yields were only possible with MeI/Ag_2O in the presence of catalytic amounts of dimethyl sulfide. The commonly used maneuver to convert the methoxyphenyl glycoside into the corresponding trichloroacetimidate furnished disaccharide unit **18**.

To complete the total synthesis, the two disaccharide units **16** and **18** were coupled to afford tetrasaccharide **19** (Scheme 4). Sodium in liquid ammonia removed all permanent protecting groups and transformed the azide moiety into an amine, thus achieving global deprotection. The formation of the amide with 3-hydroxy-3-methylbutanoic acid under peptide-coupling conditions[15] led to tetrasaccharide **2**, whose structure was confirmed by comprehensive spectroscopic analysis and comparison with the reported analytical data for **1**.

In conclusion, we have reported a convergent total synthesis of a *Bacillus anthracis* tetrasaccharide antigen ready for conjugation



Scheme 3. Syntheses of disaccharide building blocks **16** (A) and **18** (B). Reagents and conditions: a) 4-pentenol, TMSOTf, CH_2Cl_2, −20°C, 45 min, 79%; b) NaOMe, MeOH, 4 h, 96%; c) **13**, TMSOTf, CH_2Cl_2, 0°C, 1 h, 91%; d) piperidine, DMF, 25°C, 30 min, 89%; e) **8**, TMSOTf, CH_2Cl_2, 0°C, 1 h, 90%; f) hydrazinium acetate, CH_2Cl_2, MeOH, 25°C, 12 h, quant.; g) MeI, Ag_2O, THF, Me_2S (cat.), 25°C, 8 h, 73%; h) CAN, H_2O/CH_3CN, 25°C, 1 h; i) Cl_3CCN, NaH, CH_2Cl_2, 25°C, 1 h 95% (two steps). TMSOTf = trimethylsilyl trifluromethanesulfonate.



Scheme 4. Completion of the total synthesis. Reagents and conditions: a) TMSOTf, CH_2Cl_2, 0°C, 70 min, 73%; b) Na/NH_3(l), THF, −78°C, 60%; c) 3-hydroxy-3-methylbutanoic acid, HATU, DIPEA, DMF, 25°C, 2 h, 75%. HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate, DIPEA = diisopropylethylamine.

to carrier proteins. Immunological studies as well as the preparation of derivatives are currently under investigation.

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