Synthesis of a Hexasaccharide Repeating Unit from *Bacillus anthracis* Vegetative Cell Walls
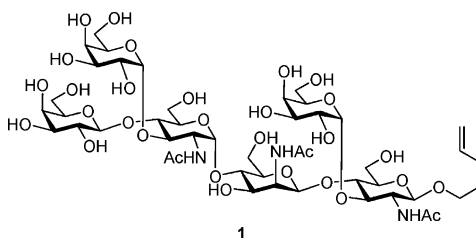
Matthias A. Oberli,[†] Pascal Bindschädler,[†] Daniel B. Werz,[§] and Peter H. Seeberger*

Laboratory for Organic Chemistry, Swiss Federal Institute of Technology (ETH) Zürich, 8093 Zürich, Switzerland

seeberger@org.chem.ethz.ch

Received December 17, 2007

ABSTRACT

1

The first synthesis of hexasaccharide 1 representing a repeat unit of a polysaccharide specific to the vegetative cell wall of *Bacillus anthracis* is reported. The synthetic hexasaccharide is equipped with an *n*-pentenyl handle at the reducing terminus to allow for further functionalization. Key transformations during the synthesis are the conversion of a glucose into a mannosazide residue, a (2+2) coupling, followed by double α-galactosylation to furnish the hexasaccharide, and global deprotection under Birch conditions.

Infections caused by the Gram-positive, spore-forming soil bacterium *Bacillus anthracis* result in very serious disease.[1,2] The durable form of the pathogen, the spores, is remarkably resistant to physical stress[3] and has been used as a biowarefare agent. Inhalation of spores that are ground into fine particles that penetrate the lungs of people will kill most victims if treatment does not commence within 24–48 h. The intentional release of anthrax spores through contaminated letters caused the death of five people and widespread panic among the U.S. population in autumn 2001. Following these events, intensified efforts to detect *B. anthracis* identified unique molecules on the surface of the bacteria.

A unique tetrasaccharide **2** (Figure 1) on the surface of *B. anthracis* spores was discovered[4] and synthesized shortly thereafter.[5] Antibodies against this carbohydrate are able to detect *B. anthracis* endospores in a highly selective manner.[6] Carbohydrate–protein conjugates are currently evaluated in challenge studies in animal experiments. Once inhaled, anthrax spores germinate to produce vegetative cells within 30 min. An effective anthrax-subunit vaccine would likely contain multiple antigens. Recently, another potential car-

[†] These authors contributed equally.

[§] Present address: Institute for Organic and Biomolecular Chemistry, University of Göttingen, D-37077 Göttingen, Germany.

(1) Mock, M.; Fouet, A. *Annu. Rev. Microbiol.* **2001**, *55*, 647–671.

(2) Sylvestre, P.; Couture-Tosi, E.; Mock, M. *Mol. Microbiol.* **2002**, *45*, 169–178.

(3) Nicholson, W. L.; Munakata, N.; Horneck, G.; Melosh, H. J.; Setlow, P. *Microbiol. Mol. Biol. Rev.* **2000**, *64*, 548–572.

(4) Daubenspeck, J. M.; Zeng, H.; Chen, P.; Dong, S.; Steichen, C. T.; Krishna, N. R.; Pritchard, D. G.; Turnbough, C. L., Jr. *J. Biol. Chem.* **2004**, *279*, 30945–30953.

(5) (a) Werz, D. B.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2005**, *44*, 6315–6318. (b) Werz, D. B.; Adibekian, A.; Seeberger, P. H. *Eur. J. Org. Chem.* **2007**, 1976–1982. (c) Adamo, R.; Saksena, R.; Kováč, P. *Carbohydr. Res.* **2005**, *340*, 2579–2582. (d) Adamo, R.; Saksena, R.; Kováč, P. *Helv. Chim. Acta* **2006**, *89*, 1075–1089. (e) Mehta, A. S.; Saile, E.; Zhong, W.; Buskas, T.; Carlson, R.; Kannenberg, E.; Reed, Y.; Quinn, C. P.; Boons, G.-J. *Chem. Eur. J.* **2006**, *12*, 9136–9149. (f) Crich, D.; Vinogradova, O. *J. Org. Chem.* **2007**, *72*, 6513–6520. (g) Guo, H.; O'Doherty, G. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 5206–5208.

(6) Tamborrini, M.; Werz, D. B.; Frey, J.; Pluschke, G.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2006**, *45*, 6581–6582.
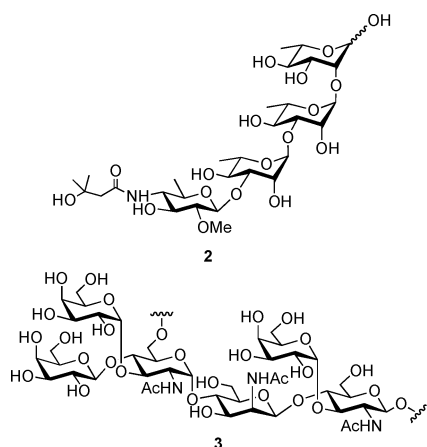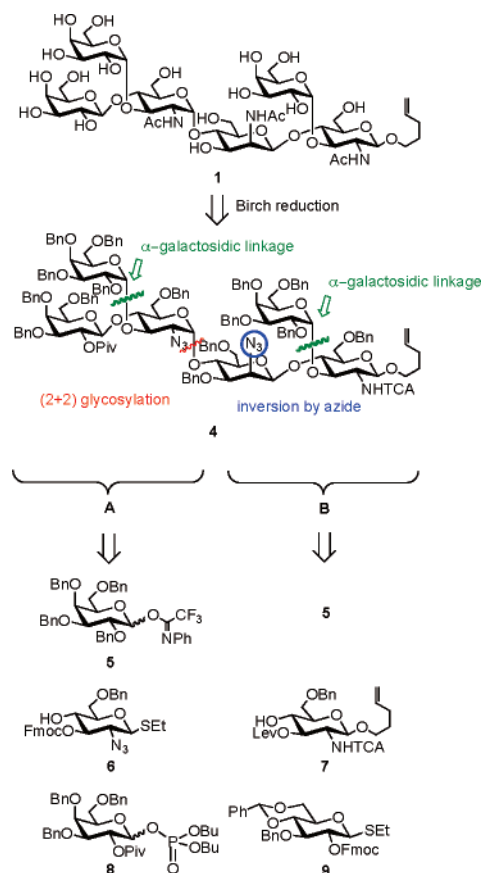
Figure 1. Carbohydrate structures from *Bacillus anthracis*: The spore surface tetrasaccharide **2** and the repeating hexasaccharide **3** of vegetative cell walls.

bohydrate antigen, the major cell wall polysaccharide **3**, was isolated from vegetative cells and structurally characterized (Figure 1).[7] This polysaccharide is also species-specific and differs even from that of the closely related *B. cereus* strains. The terminal repeating unit has not been described.[7] However, any hexameric repeating unit itself constitutes an attractive target for potential vaccine development and pathogen detection.[8]

Here, we describe the first total synthesis of the hexasaccharide repeating unit **1** containing a terminal *n*-pentenyl glycoside for subsequent functionalization and attachment to a carrier protein.

The doubly branched hexasaccharide **1** reveals several challenging linkages such as two α-galactosidic linkages, an α-linkage to a glucosamine residue and a β-linkage to a mannosamine unit. Our retrosynthetic analysis of fully protected hexasaccharide **4** (Figure 2) dissects the target molecule into two terminal α-galactose building blocks **5**[9] and two disaccharide parts **A** and **B**. For part **A** two building blocks **6** and **8** are identified. The *N*-acetyl group of the glucosamine of part **A** is masked as an azide to ensure α-selectivity in the (2+2) glycosylation. The azide containing building block **6** is derived from a precursor used in carbohydrate antigen synthesis.[10] Building block **8** is well-known for the successful construction of terminal β-galactose units.[11] Trichloroacetyl-protected glucosamine **7** is used for part **B**, and contains already the pentenyl handle that allows for further transformations, into either an aldehyde[6,12] or a thiol.[12a,13] The β-mannosamine linkage is introduced via inversion of the C2-hydroxyl of glucose following construction of the β-glucosidic bond with **9**.

(7) Choudhury, B.; Leoff, C.; Saile, E.; Wilkins, P.; Quinn, C. P.; Kannenberg, E. L.; Carlson, R. W. *J. Biol. Chem.* **2006**, *281*, 27932−27941.

(8) Verez-Bencomo, V.; et al. *Science* **2004**, *305*, 522−525.

(9) (a) Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Org. Lett.* **2003**, *5*, 987−989. (b) Tanaka, H.; Iwata, Y.; Takahashi, D.; Adachi, M.; Takahashi, T. *J. Am. Chem. Soc.* **2005**, *127*, 1630−1631.

(10) (a) Garegg, P. J.; Konradsson, P.; Oscarson, S.; Ruda, K. *Tetrahedron* **1997**, *53*, 17727−17734. (b) Turek, D.; Sundgren, A.; Lahmann, M.; Oscarson, S. *Org. Biomol. Chem.* **2006**, *4*, 1236−1241.

Figure 2. Retrosynthetic analysis.

The key carbohydrate subunits **6**,[10] **7**,[14] and **9**[15] were readily prepared from known intermediates by using standard protecting group transformations (Scheme 1). With these monosaccharide building blocks in hand, the synthesis commenced with the union of glucosamine *n*-pentenyl glucoside **7** and glucose thioglycoside **9** to form a β(1→4) glycosidic linkage.

The challenging β-mannosamine linkage was thus installed by creating the *trans*-glucosidic linkage, readily prepared with the help of the participating fluorenylmethoxycarbonate

(11) (a) Plante, O. J.; Andrade, R. B.; Seeberger, P. H. *Org. Lett.* **1999**, *1*, 211−214. (b) Hewitt, M. C.; Seeberger, P. H. *Org. Lett.* **2001**, *3*, 3699−3702. (c) Wang, C.-C.; Lee, J.-C.; Luo, S.-Y.; Fan, H.-F.; Pai, C.-L.; Yang, W.-C.; Lu, L.-D.; Hung, S.-C. *Angew. Chem., Int. Ed.* **2002**, *41*, 2360−2362. (d) Werz, D. B.; Ranzinger, R.; Herget, S.; Adibekian, A.; von der Lieth, C.-W.; Seeberger, P. H. *ACS Chem. Biol.* **2007**, *2*, 685−691.
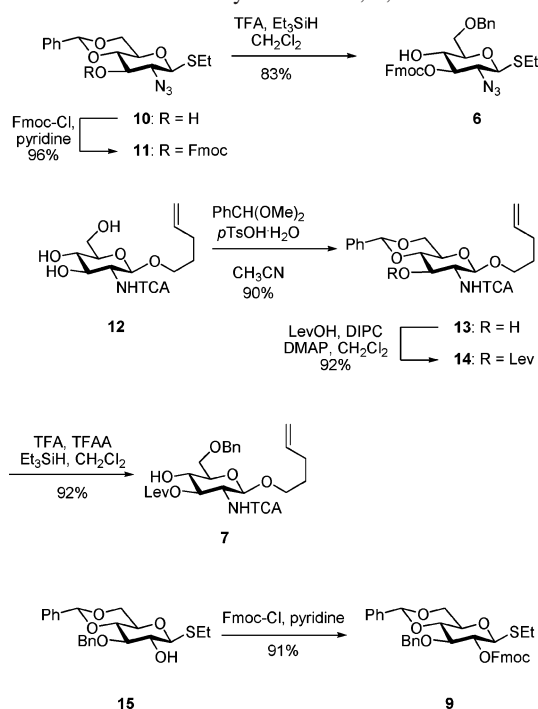
(12) (a) Buskas, T.; Söderberg, E.; Konradsson, P.; Fraser-Reid, B. *J. Org. Chem.* **2000**, *65*, 958−963. (b) Wang, Z.-G.; Williams, L. J.; Zhang, X.-F.; Zatorski, A.; Kudryashov, V.; Ragupathi, G.; Spassova, M.; Bornmann, W.; Slovin, S. F.; Scher, H. I.; Livingston, P. O.; Lloyd, K. O.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2719−2724.

(13) (a) Yamada, A.; Hatano, K.; Koyama, T.; Matsuoka, K.; Esumi, Y.; Terunuma, D. *Carbohydr. Res.* **2006**, *341*, 467−473. (b) Isobe, H.; Cho, K.; Solin, N.; Werz, D. B.; Seeberger, P. H.; Nakamura, E. *Org. Lett.* **2007**, *9*, 4611−4614.

(14) Melean, L. G.; Love, K. R.; Seeberger, P. H. *Carbohydr. Res.* **2002**, *337*, 1893−1916.
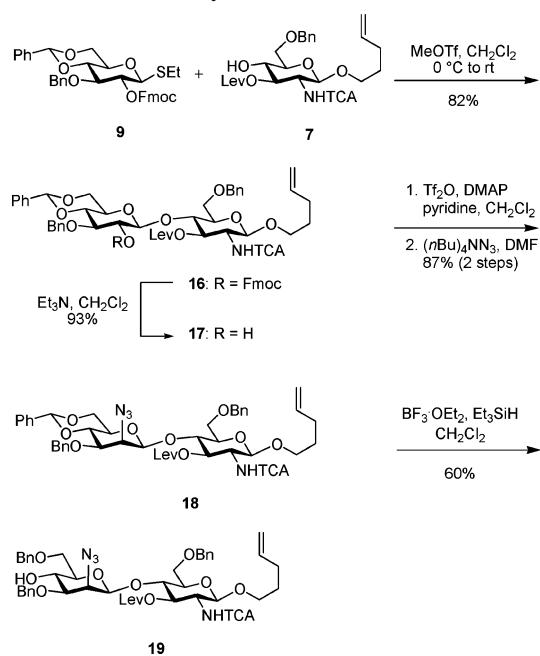
(15) (a) Zuurmond, H. M.; van der Klein, P. A. M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1992**, *33*, 2063−2066. (b) Veselý, J.; Rohlenová, A.; Džoganová, M.; Trnka, T.; Tišlerová, I.; Šaman, D.; Ledvina, M. *Synthesis* **2006**, 699−705.

Scheme 1. Synthesis of 6, 7, and 9

10: R = H
11: R = Fmoc

Fmoc-Cl, pyridine 96%

12

13: R = H
14: R = Lev

LevOH, DIPC DMAP, CH₂Cl₂ 92%

7
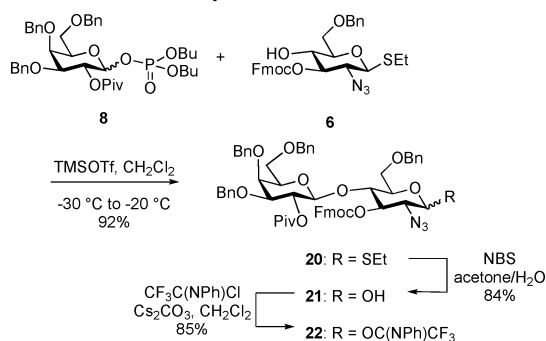
15 → **9**

Fmoc-Cl, pyridine 91%

(Fmoc) group in C2.[16] The Fmoc group was subsequently cleaved and the C2 stereocenter was inverted via displacement of the triflate by tetrabutylammonium azide to furnish disaccharide **18** that contains the mannosamine motif.[15b,17] Benzylidene protection of the C4 and C6 hydroxyls of the glucose unit proved to be essential for the successful inversion. Selective benzylidene ring opening yielded disaccharide acceptor **19** ready for glycosylation (Scheme 2).

Scheme 2. Synthesis of Disaccharide 19

9 + **7**

MeOTf, CH₂Cl₂ 0 °C to rt 82%

16: R = Fmoc
17: R = H

Et₃N, CH₂Cl₂ 93%

1. Tf₂O, DMAP pyridine, CH₂Cl₂
2. (nBu)₄NN₃, DMF 87% (2 steps)
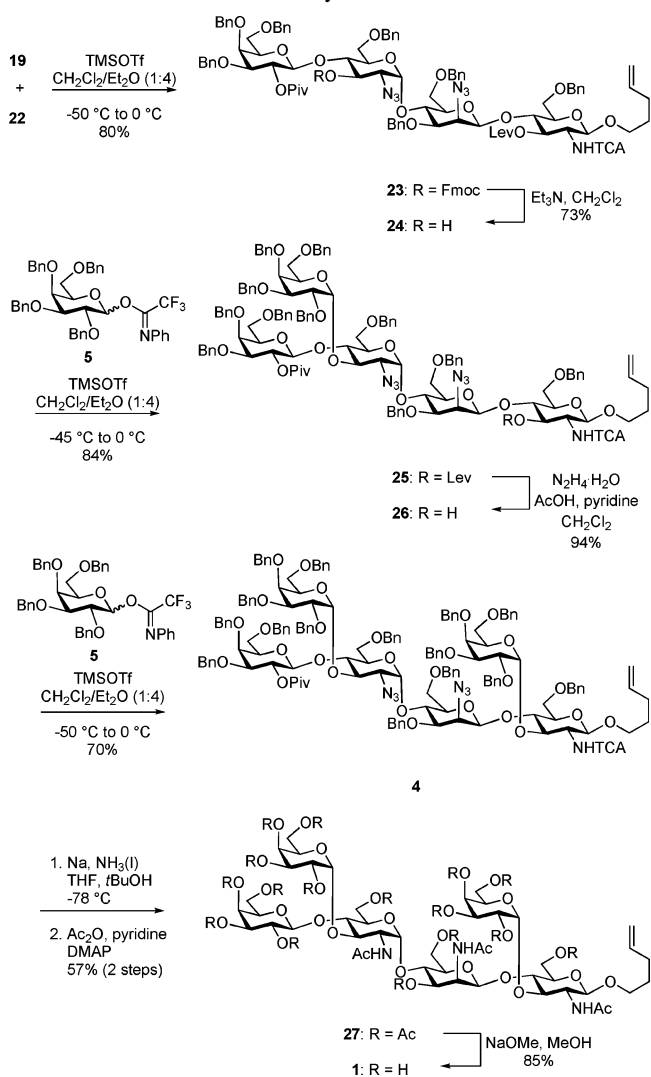
18

BF₃·OEt₂, Et₃SiH CH₂Cl₂ 60%

19

Differentially protected lactosamine building block **22** was prepared from galactose phosphate **8** (Scheme 3). The

Scheme 3. Synthesis of Disaccharide 22

8 + **6**

TMSOTf, CH₂Cl₂ -30 °C to -20 °C 92%

20: R = SEt
21: R = OH

NBS acetone/H₂O 84%

CF₃C(NPh)Cl Cs₂CO₃, CH₂Cl₂ 85%

22: R = OC(NPh)CF₃

pivaloyl (Piv) group as neighboring-participating group in C2 ensured the selective creation of the β-linkage. Experiments to use the thioglycoside **20** for direct glycosylation with **19** proved unsuccessful. Thus, we converted the

Scheme 4. Assembly of Hexasaccharide 1

19 + **22**

TMSOTf CH₂Cl₂/Et₂O (1:4) -50 °C to 0 °C 80%

23: R = Fmoc
24: R = H

Et₃N, CH₂Cl₂ 73%

5

TMSOTf CH₂Cl₂/Et₂O (1:4) -45 °C to 0 °C 84%

25: R = Lev
26: R = H

N₂H₄·H₂O AcOH, pyridine CH₂Cl₂ 94%

5

TMSOTf CH₂Cl₂/Et₂O (1:4) -50 °C to 0 °C 70%

4

1. Na, NH₃(l) THF, tBuOH -78 °C
2. Ac₂O, pyridine DMAP 57% (2 steps)

27: R = Ac
1: R = H

NaOMe, MeOH 85%

thioacetal via the hemiacetal **21** into glycosyl *N*-phenyltri-fluoroacetimidate **22**. This leaving group is a very attractive alternative[18] to the commonly used glycosyl trichloroacetimi-dates.[19] Glycosylation of disaccharide **19** with *N*-phenyltri-fluoroacetimidate **22** in a mixture of dichloromethane and diethyl ether afforded selectively tetrasaccharide **23** with the corresponding α-glucosaminic linkage (Scheme 4).

To complete the synthesis, tetrasaccharide **23** was treated with triethylamine to remove selectively the Fmoc group[20] and afforded tetrasaccharide acceptor **24**. This acceptor was glycosylated with galactose building block **5** to yield **25**. Pentasaccharide **25** was treated with hydrazine monohydrate to remove the levulinoyl ester. The final glycosylation with galactose building block **5** afforded hexasaccharide **4**. Extensive spectroscopic investigations were performed to identify the three diagnostic α-linkages, thereby confirming the correct stereochemistry. Sodium in liquid ammonia removed all permanent protecting groups and transformed the azide moieties into amines. To afford NHAc groups the completely deprotected carbohydrate was acetylated with a mixture of acetic anhydride, pyridine, and DMAP. The outcome of the Birch deprotection was shown to be highly dependent on the neutralizing conditions: Using Amberlite

acidic resin we obtained low yields (15%) presumably since the fully deprotected hexasaccharide stuck to the resin. Employing acetic acid instead to neutralize resulted in greatly improved yield (57%). Fragments from destructive fragmen-tation could be observed as well. Saponification of **27** finally yielded the desired target compound **1**. Comparison of key regions in the NMR spectra of **1** and **3** confirmed the structural assignment of the isolated polysaccharide repeating unit.[21]

In conclusion, we have reported a convergent total synthesis of a *Bacillus anthracis* hexasaccharide repeating unit of the major vegetative cell wall polysaccharide. The target molecule was functionalized with a pentenyl handle at the reducing terminus for conjugation to carrier proteins or for attachment to microarrays. Our approach also allows for facile synthesis of modifications and shorter sequences. Immunological studies as well as the preparation of derivates are under investigation. Results will be reported in due course.

Supporting Information Available: Experimental pro-cedures, spectroscopic data for all new compounds, and a full citation for ref 8. This material is available free of charge via the Internet at http://pubs.acs.org.

OL7030262

(16) (a) Grathwohl, M.; Schmidt, R. R. *Synthesis* **2001**, 2263−2272. (b) Love, K. R.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2004**, *43*, 602−605.

(17) (a) David, S.; Malleron, A.; Dini, C. *Carbohydr. Res.* **1989**, *188*, 193−200. (b) Classon, B.; Garegg, P. J.; Oscarson, S.; Tidén, A.-K. *Carbohydr. Res.* **1991**, *216*, 187−196.

(18) Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405−2407.

(19) Schmidt, R. R.; Michel, J. *Angew. Chem.*, *Int. Ed. Engl.* **1980**, *19*, 731−732.

(20) (a) Zhu, T.; Boons, G.-J. *Tetrahedron*: *Asymmetry* **2000**, *11*, 199−205. (b) Roussel, F.; Takhi, M.; Schmidt, R. R. *J. Org. Chem.* **2001**, *66*, 8540−8548.

(21) For detailed comparison of **1** and **3** see the Supporting Information.