# Towards the synthesis of a *Yersinia pestis* cell wall polysaccharide: enantioselective synthesis of an L-*glycero*-D-*manno*-heptose building block†

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**A short and enantioselective *de novo* synthesis of an L-*glycero*-D-*manno*-heptose building block for the total synthesis of a *Yersinia pestis* cell wall polysaccharide is described.**

The plague is re-emerging as cases have been reported in more than 20 countries in Africa and Asia.[1] *Yersinia pestis* is the causative agent of the plague, an infection of rodents accidentally transmitted to humans by the bite of infected fleas. The disease is manifested in bubonic and pneumonic forms and constitutes a potential bioterrorism threat. The need for vaccines against *Yersinia pestis* is becoming urgent.

As a first step toward a synthetic carbohydrate vaccine we are pursuing the synthesis of the core pentasaccharide of the cell wall lipopolysaccharide (LPS) extracted from different bacterial strains of *Yersinia pestis* (Fig. 1).[2] The trisaccharide part is entirely composed of α-linked L-*glycero*-D-*manno*-heptoses and is synthetically most challenging. Several syntheses of L-*glycero*-D-*manno*-heptose[3] based on traditional carbohydrate chemistry required protection and deprotection steps to access the desired protecting group pattern. A *de novo* approach presents an attractive alternative to access the building block.[4] Here, we report an enantioselective synthesis of differentially protected L-*glycero*-D-*manno*-heptoses. The retrosynthetic strategy relies on the enantioselective construction of the seven carbon backbone using two aldol reactions (Scheme 1). The heptose hemiacetal presented in a linear form can be accessed by a Mukaiyama-type aldol reaction between C5-aldehyde **3** and silyl enolether **4**.[5] The aldehyde can further be dissected through a second retroaldol step into C3-ketone **5** and C2-aldehyde **6**. We established a proline-catalyzed aldol reaction as a highly enantioselective and readily scalable transformation.[6]

The synthesis commenced with the *anti*-selective L-proline catalyzed aldol reaction followed by TBS-protection of the aldol product to obtain ketone **7** in multi-gram quantities (Scheme 2).[6] Reduction of **7** with L-selectride resulted in 1,3-migration of the TBS-group to yield regioisomer **8** in 79% yield. The free hydroxyl was subsequently protected as *para*-bromobenzyl (PBB) ether, a protecting group orthogonal to benzyl ethers.[7] The temporary silyl group was then exchanged for a benzyl group to afford bisacetal **11**.

Treatment with CSA and methanol resulted in the selective deprotection of the ketal. The resulting primary hydroxyl was protected with TBDPS-Cl and the secondary hydroxyl of **12** was masked as TBS-ether to afford fully protected dimethylacetal **13**. The dimethylacetal was smoothly hydrolyzed using a catalytic amount of *para*-toluenesulfonic acid in acetone to yield aldehyde **3**. A second aldol reaction was performed using silyl enolether **4** and $MgBr_2 \cdot OEt_2$ as chelating activator.[4c,d] Under these conditions the aldol product **14** was formed in moderate yield but excellent 2,3-*anti*-3,4-*syn*-selectivity, as determined by analysis of coupling constants at a later stage of the synthesis.[11] Treatment with trifluoroacetic acid resulted in cleavage of the TBS-group and concomitant lactonization to alcohol **15** as the major product.

Lactone **15** was efficiently reduced using lithium tri-*tert*-butoxyaluminium hydride,[8] however, partial migration of the silyl group from C2 to C3 was observed. Both regioisomers **17** and **18** were separated by flash chromatography and were used for the synthesis of two different building blocks as depicted in Scheme 3. Diol **17** was diacetylated to diester **19** and the

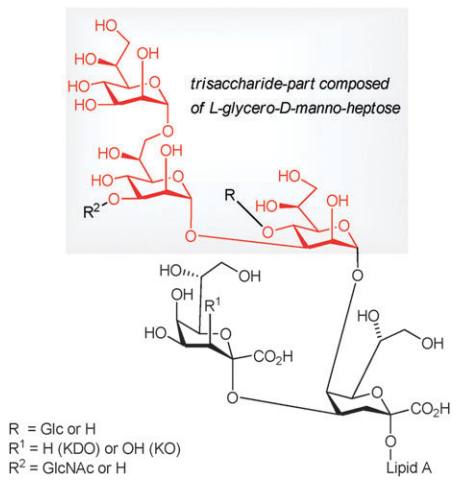


**Fig. 1** Structure of the *Yersinia pestis* core pentasaccharide.

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† Electronic supplementary information (ESI) available: General information and procedures (S1–S12) and copies of NMR spectra (S13–S49). See DOI: 10.1039/c000784f

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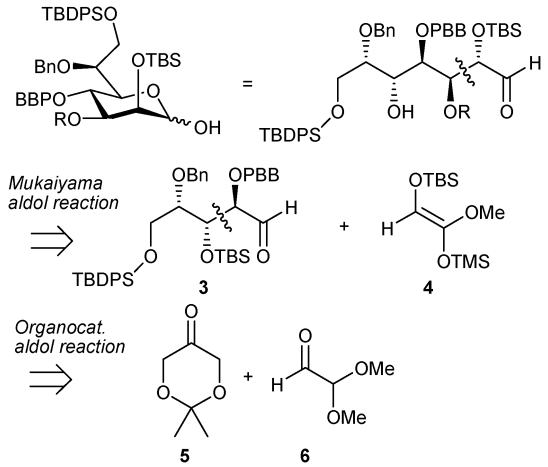
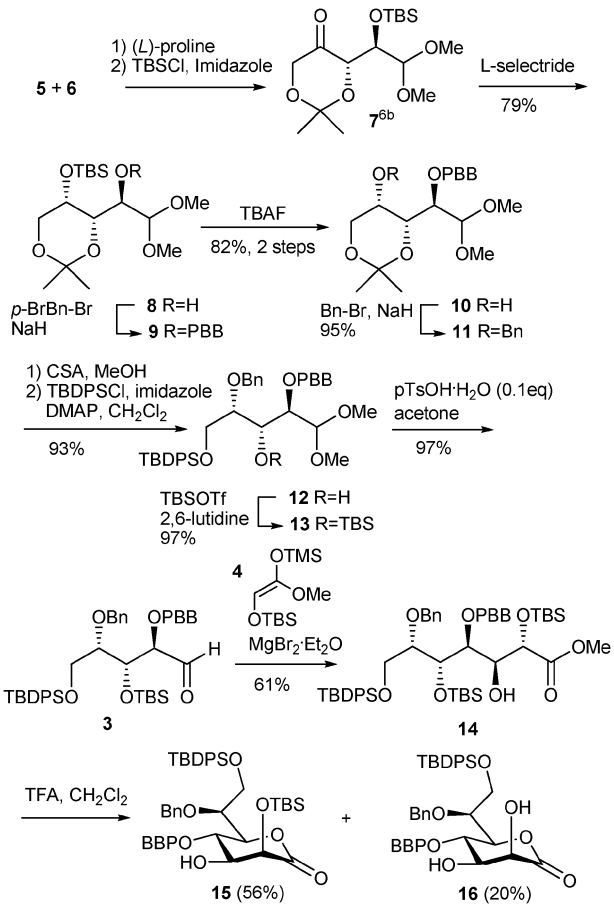
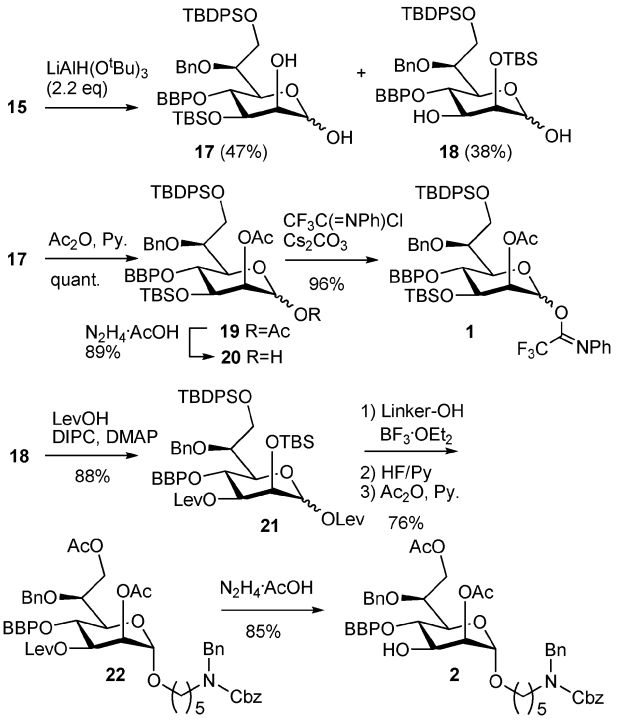



**Scheme 1** Retrosynthesis of L-*glycero*-D-*manno*-heptose.




**Scheme 2** Synthesis of lactones **15** and **16**.

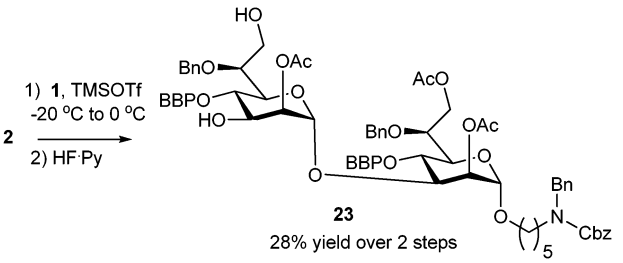
anomeric acetate was selectively removed using hydrazine acetate. The resulting hemiacetal was efficiently converted into *N*-phenyl-trifluoroacetimidate glycoside **1** in 85% overall yield over three steps. Levulinoylation of both hydroxyls on diol **18** yielded diketone **21** and the anomeric levulinoate ester was replaced by *N*-(benzyl)benzyloxycarbonyl-5-aminopentan-1-ol under the agency of boron trifluoride etherate. The protected amine linker was previously described to be robust towards various glycosylation and deprotection conditions as well as readily removable by hydrogenation at the end of the synthesis.[4c,9]




**Scheme 3** Synthesis of building blocks **1** and **2**.

The resulting crude product was treated with hydrogen fluoride/pyridine and was diacetylated to produce **22**. Finally, the levulinoyl protective group of the C3 position was selectively removed using hydrazine acetate to afford acceptor **2**. The diagnostic coupling constants[10] helped to confirm the *manno*-configuration on α-glycoside **22** and **2**.[11] To demonstrate the synthetic utility of the heptose building blocks **1** and **2** in glycosylations we synthesized disaccharide **23**. Using a catalytic amount of TMSOTf as promotor, glycosylating agent **1** was coupled with acceptor **2**. The resulting crude disaccharide was treated with hydrogen fluoride/pyridine to remove both silyl groups to afford the desired disaccharide **23** as depicted in Scheme 4.[12]

In conclusion, we describe a short, enantioselective route to L-*glycero*-D-*manno*-heptose building blocks. Compared to known synthetic routes to these sugars, our synthesis furnishes building blocks that are orthogonally protected, and suitable for assembly of a variety of different L-*glycero*-D-*manno*-heptose-containing oligosaccharides. In addition, this route will allow for the construction of other naturally occurring heptose epimers, by altering the stereochemical course of the aldol reactions and of the ketone reduction. These building blocks are currently employed in the total synthesis of *Yersinia pestis* core pentasaccharide.




**Scheme 4** Synthesis of disaccharide **23**.

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11 Characteristic coupling constants between H-3 and H-4 ($J$ = 9.7 Hz) as well as between H-2 and H-3 ($J$ = 2.8 Hz) of compound **22** served to confirm the *manno*-configuration of **22**. The coupling constant between anomeric C-1 and H-1 ($^1J_{C-H}$ = 169.7 Hz) of compound **2** also clearly showed that the anomeric configuration of **2** is alpha.

12 Compound **23** displays broadened signals in the $^1H$ and $^{13}C$ NMR spectra due to the slow rotation of the benzyl carbamate on the linker. An analytical sample of **23** was subjected to Zemplén conditions followed by hydrogenolysis to obtain the fully deprotected linker-equipped disaccharide. Purity was assessed by HPLC and $^1H$ NMR spectroscopy (see Supporting Information).


 