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De Novo Synthesis of the Bacterial 2-Amino-2,6-Dideoxy Sugar Building Blocks D-Fucosamine, D-Bacillosamine, and D-Xylo-6-deoxy-4-ketohexosamine

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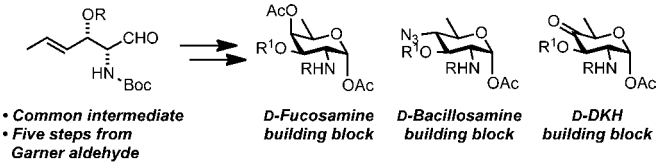
ABSTRACT



The cell-surface glycans on bacteria contain many monosaccharides that cannot be obtained by isolation from natural sources. Availability of differentially protected monosaccharides is therefore often limiting access to potential oligosaccharide vaccine antigens. D-Fucosamine, D-bacillosamine, and D-xylo-2,6-deoxy-4-ketohexosamine building blocks were prepared via a divergent *de novo* synthesis from L-Garner aldehyde. The route relies on a chelation-control assisted organometallic addition and an *anti*-selective dihydroxylation reaction.

Protein glycosylation is an important post-translational event that directly influences many proteins' functions.[1] Glycans on the surface of bacteria serve as a basis for the development of new therapeutics.[2] The ability of bacteria to synthesize complex oligosaccharides directly influences their ability to colonize human hosts and to cause disease.[3] Oligosaccharide antigens containing unique or unusual monosaccharides that are not found in the human body give rise to the development of novel vaccine candidates against bacterial infections.[4] However, access to pure and well-defined oligo- or polysaccharide antigens represents a major obstacle toward the development of carbohydrate vaccines.[5]

Vaccine candidates[6] against the highly pathogenic bacteria *Pseudomonas aeruginosa, Neisseria gonorrheae*, and *Streptococcus pneumoniae* would be useful in addressing a significant medical need. These bacteria express cell-surface glycans containing the unusual 2-amino-2,6-dideoxy sugars D-fucosamine (Fuc),[7] D-bacillosamine (Bac),[8] and D-xylo-6-deoxy-4-ketohexosamine (DKH).[9] Glycans containing these monosaccharide units are important for bacterial adherence and invasion (Figure 1).

Although many of these monosaccharide building blocks can be synthesized using classical carbohydrate routes, those methods often require lengthy synthetic

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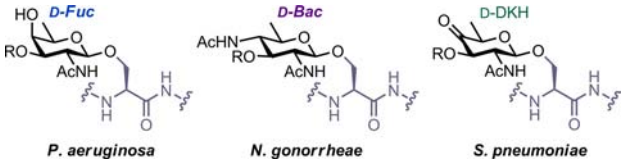
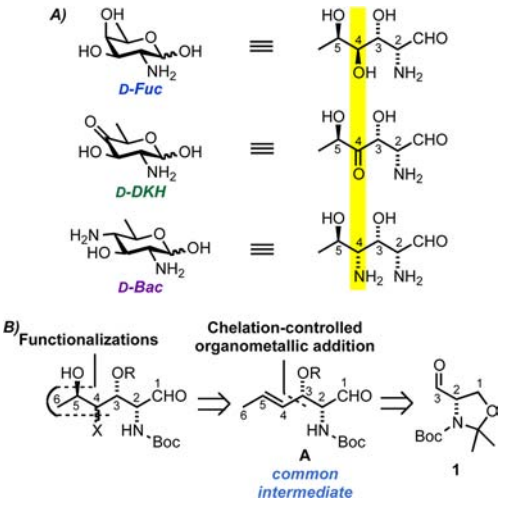


Figure 1. Rare 2-amino-2,6-dideoxy sugars found in bacteria.

sequences, extensive protecting group manipulations, and expensive starting materials.[10] Here, we demonstrate that our *de novo* synthetic approach[11] can be applied to a straightforward synthesis of orthogonally protected bacterial 2-amino-2,6-dideoxy monosaccharides that can be used in the assembly of oligosaccharide antigens.[12]

The three target molecules bear striking elements of similarity (Scheme 1A). Three of the four stereocenters (*C*2, *C*3, and *C*5) display identical stereochemical substitution patterns. The *C*4 position differs in oxidation state or stereochemical substitution. A divergent strategy is

Scheme 1. Structural and Retrosynthetic Analysis

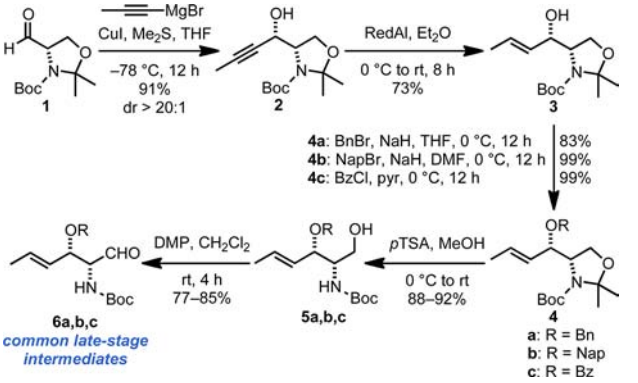


synthetically desirable to access all targets via selective functionalization of a common late-stage intermediate.

The retrosynthetic analysis (Scheme 1B) revealed intermediate **A** as a suitable precursor for further elaboration. The carbon skeleton of this intermediate would be accessed by organometallic addition to commercially available L-Garner aldehyde **1**.[13]

The synthesis commenced with the chelation-controlled addition[14] of propynyl magnesium bromide to **1** (Scheme 2).[15] Subsequent *E*-selective alkyne reduction was accomplished using Red-Al in Et_2O. With gram quantities of **3** in hand, a sequence of *O*-protection, acid-catalyzed acetonide deprotection and Dess-Martin oxidation[16] yielded the desired intermediates **6a**–**c**, in five steps from commercially available starting materials and only two chromatographic purifications. Three different hydroxyl protecting groups, namely benzyl ether, naphthyl ether, and benzoate ester, were installed to access a series of orthogonally protected derivatives (Scheme 2).

Scheme 2. Synthesis of Common Intermediates **6a**–**c**



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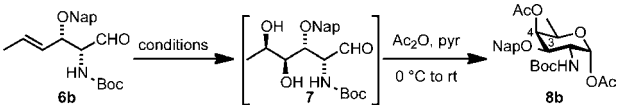
With the late-stage intermediates **6a**−**c** in hand, the elaboration to the target monosaccharides was established. In order to obtain D-fucosamines, a *syn*-dihydroxylation *anti* to the allylic ether moiety had to be achieved. Exposure of **6b** to Upjohn oxidative conditions gave, after peracetylation, D-fucosamine building block **8b** in 81% yield and 5:1 dr (*C*3−*C*4 *anti*/*syn*) whereby the diastereomers are separable by column chromatography (Table 1, entry 1).[17] Decreasing the reaction temperature to 0 °C (entry 2) or using a different Os(VIII) source (entry 3) did not improve the selectivity. The use of Sharpless AD conditions led to quantitative recovery of unreacted starting material (entry 4). A $^3J_{H3-H4}$ of 3.5 Hz ensured the formation of the desired *syn* cyclic product **8b**.

Table 1. Dihydroxylation of **6b**



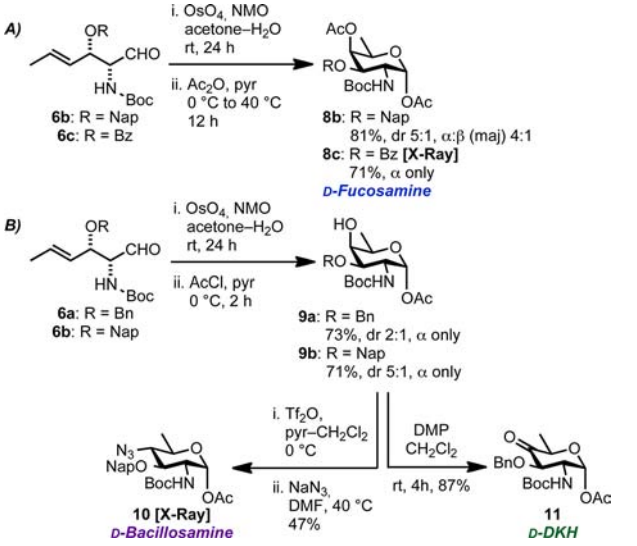
entry	reaction conditions	dr	yield (%)[a]
1	cat. OsO_4, NMO, acetone−H_2O, rt	5[b]:1	81
2	cat. OsO_4, NMO, acetone−H_2O, 0 °C	5[b]:1	78
3	cat. $K_2(OH)_2OsO_4$, NMO, acetone−H_2O, rt	4[b]:1	85
4[c]	AD-mix(α or β), *t*-BuOH−H_2O, rt	–	–

[a] Yield for both diastereomers. [b] α:β 4:1. [c] Addition of $MeSO_2NH_2$ was also evaluated.

When the reaction was performed using benzoylated aldehyde **6c**, compound **8c** was formed in 71% as the only detectable diastereomer (Scheme 3A). Monosaccharide **8c** was crystallized from hot *n*-hexane/EtOAc and the stereochemical assignment was confirmed by X-ray analysis. To access D-bacillosamine derivatives, an equatorial nitrogen moiety had to be introduced at *C*4. Dihydroxylation of aldehyde **6b**, and selective anomeric acetylation, gave sugar **9b**. After activation of the *C*4 hydroxyl using Tf_2O and triethylamine, followed by azide displacement with sodium azide, the desired building block **10** was obtained over seven steps from **1** (Scheme 3B). Crystallization of **10** from boiling *n*-hexane/CH_2Cl_2 provided good quality crystals for X-ray analysis that confirmed the absolute configuration. Finally, treatment of monosaccharide **9a**[18] that was obtained in analogy to **9b**, with Dess-Martin Periodinane (DMP), gave the desired D-2,6-DKH building block **11** in 87% yield and in seven steps from commercially available starting materials. Observation of an *NOE* enhancement between *H*3−*H*5 confirmed the stereochemical arrangement of **11** using NMR spectroscopy (Scheme 3C).

For preparative purposes, the synthesis of **8b** from alcohol **5b** was carried out on gram scale with only one chromatographic purification.[19]

Scheme 3. Building Blocks Synthesis



In order to present these monosaccharides on glycan microarrays or on carrier proteins, the introduction of an alkyl-amine spacer in the anomeric position is highly desirable.[20] Placement of a *C*3 napthyl ether provides for further glycosylation at this position that typically serves as a connection to the next sugar. Building block **8b** is therefore ideal in terms of orthogonality and chemical synthesis. Hence we decided to test its ability to undergo anomeric functionalization and to effect glycosylation at the *C*3-OH. Due to the presence of *N*-acetylated D-fucosamine residues in *P. aeruginosa* O-linked glycans the strategic *N*-protecting group pattern needed to be evaluated. The direct use of building block **8b** under glycosylating conditions proved difficult. When $BF_3 \cdot Et_2O$ at −78 °C

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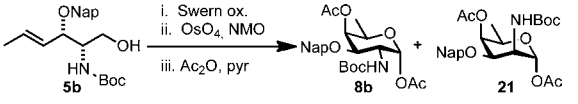
(18) The peracetylated diastereomers of **9a** could not by separated by column chromatography.

(19) Oxidation of **5b** under Swern conditions resulted in partial epimerization at *C*2, and monosaccharide **21** was isolated.



For a synthetic discussion of epimerizable *N*-protected α-amino aldehydes, see: Myers, A. G.; Zhong, B.; Movassaghi, M.; Kung, D. V.; Lanman, B. A.; Kwon, S. *Tetrahedron Lett.* **2000**, *41*, 1359.

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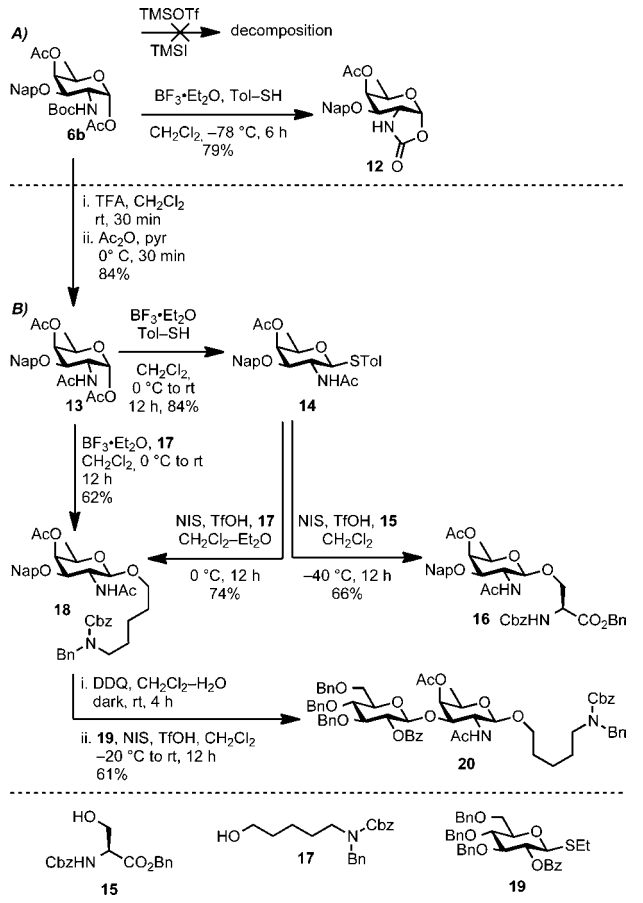
Scheme 4. Fuctionalization of Fucosamines **6b** (A) and **13** (B)



was used, the intramolecular cyclization of the Boc-group on the oxocarbonium ion could not be prevented and bicyclic carbamate **12** was isolated in 79% yield (Scheme 4A).

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At this point a sequence of *N*-protecting group modification and anomeric functionalization was attempted (Scheme 4B). Direct *N*-Boc deprotection/*N*-acetylation afforded **13** in 84% yield. In this case, glycosylation with thiocresol ($BF_3 \cdot Et_2O$) resulted in the clean formation of thioglycoside **14** in 84% yield (Scheme 4B). The $^3J_{H1\text{-}H2}$ of 10.3 Hz matched the selective formation of the expected β-anomer. Building block **14** proved to be suitable for further modification, and after activation with NIS and TfOH, the anomeric position was successfully functionalized with L-serine derivative **15** or linker **17** to give glycosylated amino acid **16** or linker-functionalized saccharide **18** as β-anomers ($^3J_{H1\text{-}H2} \approx 8.3$ Hz). Direct glycosylation of **17** using donor **13** was also possible. At this point, the *C*3 Nap ether was cleaved under oxidative conditions and the corresponding alcohol was glycosylated with differentially protected glucose building block **19**.[21] The desired β-disaccharide **20** was formed in 61% yield over two steps (β-anomer, $^3J_{H1\text{-}H2}$ of 7.8 Hz).

In conclusion, we report the first *de novo* syntheses of the rare bacterial 2-amino-2,6-dideoxy sugars D-fucosamine, D-bacillosamine, and D-xylo-2,6-DKH. Our method allows for the divergent and scalable synthesis of the fully functionalized building blocks in six to seven steps from commercially available starting materials. The use of these rare sugar building blocks in the synthesis of complex bacterial glycans is currently under investigation.

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Supporting Information Available. Experimental details and characterization data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.