Tetrasaccharide Synthesis

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De Novo Asymmetric Synthesis of the Anthrax Tetrasaccharide by a Palladium-Catalyzed Glycosylation Reaction**

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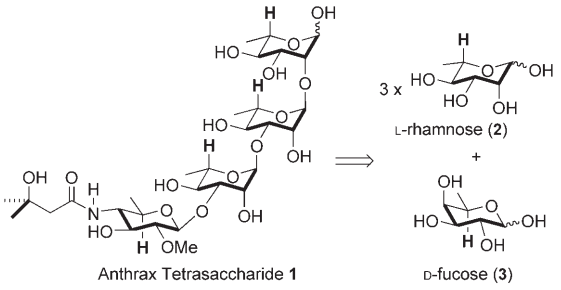
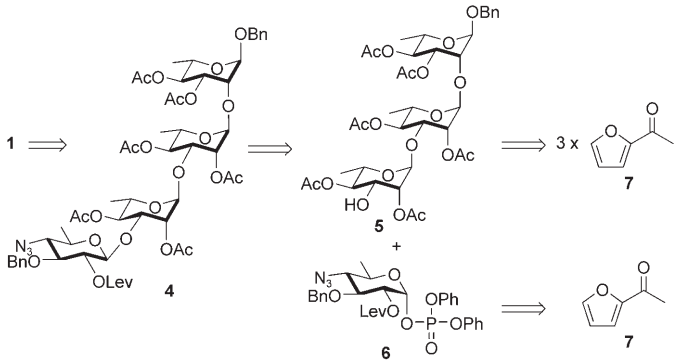
Anthrax is a zoonotic disease caused by the spore-forming bacterium *Bacillus anthracis*.[1] *Bacillus anthracis* belongs to the family *Bacillaceae*, which consists of a diverse group of bacteria, all of which form endospores.[2] *Bacillus anthracis* is the most important member of this genus and is considered to be a potent agent for biological warfare. Its protective polypeptide capsule consists of poly-D-glutamic acid, which inhibits phagocytosis.[3] Recently, a tetrasaccharide made up of three L-rhamnose sugars and a rare sugar, D-anthrose, was isolated from the capsule (Scheme 1).[4] The uniqueness of the anthrose sugar and the resistance of carbohydrate structures to evolutionary change make the anthrax tetrasaccharide **1** an interesting target for anthrax detection and vaccine development.[5] Thus, synthetic access to this tetrasaccharide is desired.



Scheme 1. Anthrax tetrasaccharide **1**.

Recently, two carbohydrate-based approaches to the anthrax tetrasaccharide and one to a related trisaccharide have been reported.[6] In these routes the stereochemistry is derived from the known but less common sugar L-rhamnose (**2**) and the rare D-fucose (**3**). In contrast to these traditional approaches, we have been investigating de novo asymmetric approaches to mono, di-, and oligosaccharides.[7] Herein we describe our successful application of this methodology for the de novo asymmetric synthesis of **1**.[8]

In our retrosynthetic analysis we envisioned **1** as being prepared from tetrasaccharide **4**, which in turn could be prepared by a glycosylation of trisaccharide **5** with phosphate **6** (Scheme 2). At the outset, we hoped to use our de novo approach to prepare both of these fragments (**5** and **6**) from the achiral acetylfuran **7**, which is significantly cheaper than either L-rhamnose (**2**) or D-fucose (**3**).[9]



Scheme 2. Retrosynthesis of anthrax tetrasaccharide **1**. Bn = benzyl, LevOH = levulinic acid

Our synthesis of the anthrose portion of the tetrasaccharide commenced with the Noyori reduction of the acetylfuran **7** to install the D stereochemistry (Scheme 3). Subsequent Achmatowicz rearrangement (NBS/H_2O) and diastereoselective ($\alpha/\beta = 3:1$) Boc protection ($(Boc)_2O$/DMAP) provided pyranone **8** in 60% overall yield.[10] Exposure of the pyranone **8** and *p*-methoxybenzyl alcohol to our palladium glycosylation conditions (0.5% Pd^0/1% PPh_3) produced PMB-pyranone **9** in excellent yield (96%) as a single diastereomer. Luche reduction ($NaBH_4/CeCl_3$) of pyranone **9** followed by methyl carbonate formation ($ClCO_2CH_3$/DMAP) produced allylic carbonate **10** in 92% yield for the two steps.[11] The methyl carbonate group of **10** was regio- and stereoselectively replaced with an azide group by a Pd allylation ($TMSN_3$, $[\{(allyl)PdCl\}_2]$, dppb) to afford allylic azide **11** (93%).[12] Dihydroxylation of **11** under Upjohn conditions (OsO_4/NMO) installed the *manno* stereochemistry, and regioselective protection ($BnBr/Bu_2SnO$) provided benzyl ether **12** (97%).[13] Finally the axial hydroxy group at C2 in **12** was converted to give *gluco* stereochemistry by an S_N2 displacement. Thus, alcohol **12** was treated with triflic anhydride and inverted to give the equatorial alcohol **13** with $NaNO_2$ (56%).[14] Acylation of **13** (LevOH/DCC/DMAP) and

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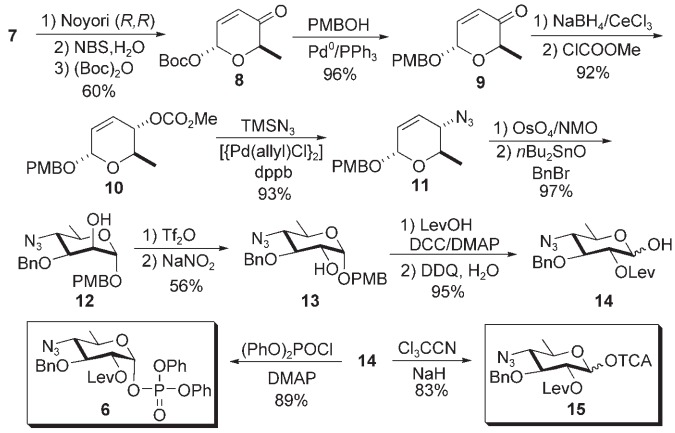
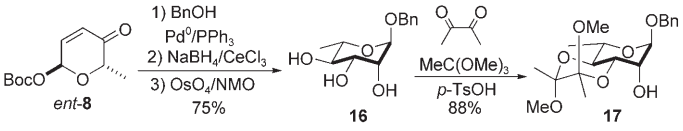
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.



Scheme 3. Synthesis of anthrose monosaccharide **6** and **15**. Noyori (*R*,*R*) = (*R*)-Ru(η⁶-mesitylene)-(*R*,*R*)-*N*-(4-toluenesulfonyl)-1,2-diphenylethylenediamine, NBS = 1-bromo-2,5-pyrollidinedione, PMBOH = *p*-methoxybenzyl alcohol, $(Boc)_2O$ = di-*tert*-butyl dicarbonate, $TMSN_3$ = trimethylsilyl azide, dppb = 1,4-bis(diphenylphosphino)-butane, NMO = *N*-methyl morpholine-*N*-oxide, Tf_2O = trifluoromethylsulfonic anhydride, DCC = *N*,*N*′-dicyclohexylcarbodiimide, DMAP = 4-dimethylaminopyridine, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, TCA = trichloroacetic imidate.

removal of the PMB group provided the anomeric alcohol **14** (89%). Finally two anthrose precursors were prepared from **14**, phosphate **6** (89%) and imidate **15** (83%).

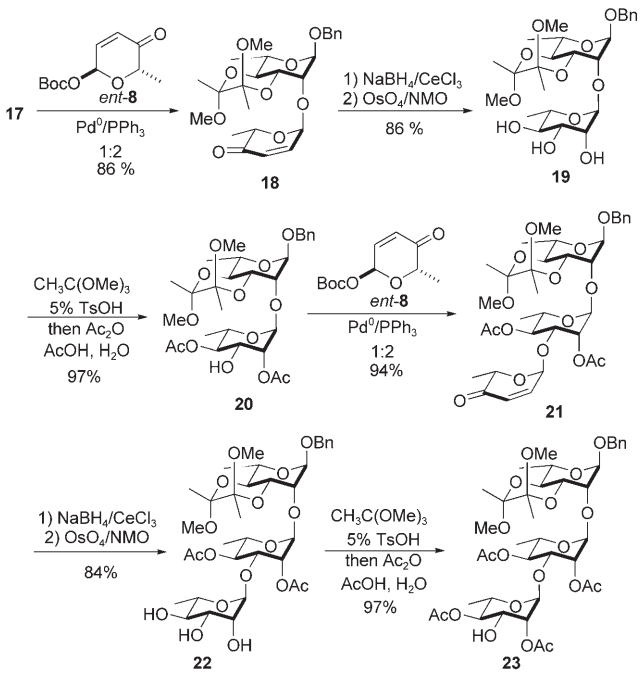
With the D-anthrose monosaccharide in hand, we turned to the synthesis of the tris-L-*rhamno* trisaccharide **5**, which required an L-*rhamno* sugar with an unprotected C2 hydroxy group (**17**, Scheme 4). Analogously, the L-pyranone *ent*-**8** was



Scheme 4. Pd^0-catalyzed glycosylation synthesis of **17**. *p*-TsOH = *p*-toluenesulfonic acid.

prepared in three steps from acetylfuran **7** by simply switching to the (*S*,*S*)-Noyori catalyst. By using our Pd-glycosylation procedure (BnOH, 0.25% Pd^0/0.5% Ph_3P), we protected the anomeric position of pyranone *ent*-**8** as a benzyl ether (90% yield). A post-glycosylation Luche reduction and dihydroxylation installed the *rhamno* triol **16** (75%, overall yield).[15] Finally the equatorial hydroxy groups of **16** at C3 and C4 were selectively protected using the Ley spiroketal procedure yielding **17** (66% from *ent*-**8**) which has a free axial hydroxy group at C2 for subsequent glycosylation.[16]

Palladium-catalyzed glycosylation of the axial hydroxy group at C2 in **17** with pyranone *ent*-**8** provided the pyranone **18** in 86% yield (Scheme 5). Once again a Luche reduction and Upjohn dihydroxylation diastereoselectively produced the *rhamno* triol **19** (86%, two steps). The triol **19** was treated with trimethyl orthoacetate and catalytic *p*-toluenesulfonic acid to form a cyclic orthoester intermediate, which subsequently underwent acetylation at C4 and regioselective hydrolytic opening to afford the 2,4-diacetate **20** in 97%

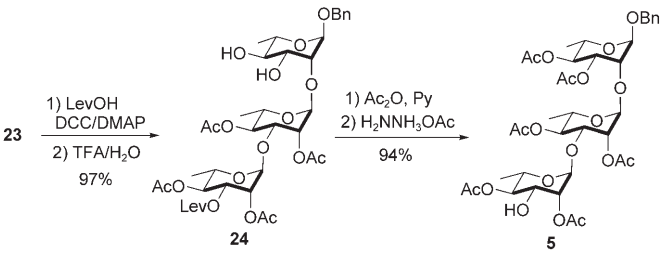


Scheme 5. Synthesis of trisaccharide **23**.

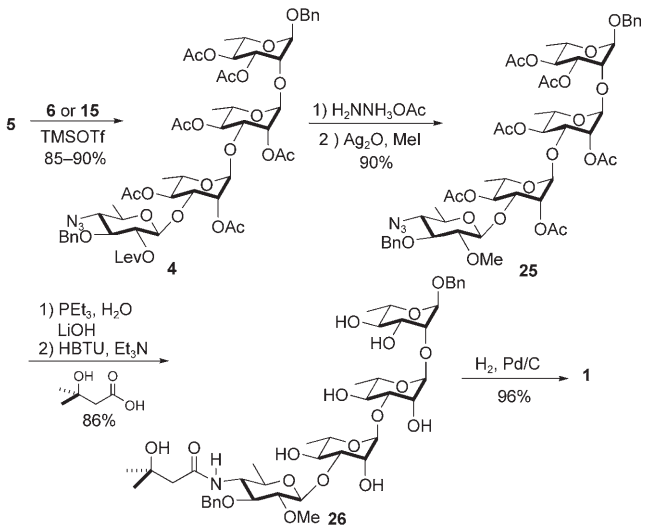
yield.[17] In an analogous fashion the final *rhamno* sugar in **23** was installed at the C3 hydroxy group of **20** (Pd glycosylation (**20** + *ent*-**8**→**21**), Luche reduction and dihydroxylation, 79% yield of **22**) and by orthoester chemistry the C2/C4 hydroxy groups of **22** were selectively acylated ($MeC(OMe)_3$/TsOH; Ac_2O; AcOH/H_2O, 97%) to form diacetate **23** in good overall yield (77% for four steps).[17]

Unfortunately, our attempts at glycosylation of **23** with either the phosphate **6** or imidate **15** failed. Instead only hydrolysis of the spiroketal protecting group was observed. Thus, we decided to prepare the more acid-stable trisaccharide **5**, which could be prepared in four steps from **23** (Scheme 6). Acylation of **23** (LevOH/DCC/DMAP), followed by removal of the spiroketal (TFA/H_2O) provided diol **24**. The two hydroxy groups were acetylated (Ac_2O/Py) and the levulinate group was selectively deprotected (H_2NNH_3OAc) to produce trisaccharide **5** (91% from **23**).

Our return to the final glycosylation step with the acid-stable *rhamno* trisaccharide **5** was more successful leading to the synthesis of the anthrax tetrasaccharide **1** (Scheme 7). In contrast to **23**, exposure of **5** to either imidate **15** or phosphate **6** and catalytic amounts of TMSOTf produced tetrasaccharide **4** in good yields (85% for **15** and 90% for **6**).[18] The anthrose



Scheme 6. Synthesis of trisaccharide **5**. TFA = trifluoroacetic acid, Py = pyridine.



Scheme 7. Completion of the synthesis of anthrax tetrasaccharide **1**. TMSOTf = trimethylsilyl trifluoromethanesulfonate, HBTU = *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium-hexafluorophosphate

methyl ether was installed in **25** by selective levulinate hydrolysis (H_2NNH_3OAc, 96%) and silver(I) oxide promoted methylation (Ag_2O in MeI, 94%).[19] A one-pot global deprotection of the acetate in **25** along with azide reduction afforded a primary amine (PEt_3/LiOH/H_2O, 95%), which was selectively coupled with 3-hydroxy-3-methylbutanoic acid (HBTU/Et_3N, 90%) to give amide **26**. Finally the natural product **1** was prepared by hydrogenolysis of both benzyl groups (H_2, Pd/C) in good yield (96%).[20]

In summary, a de novo asymmetric synthesis of the natural product anthrax tetrasaccharide **1** has been developed in 25 steps (longest linear, 39 total steps) 13% overall yield from achiral acetylfuran **7**. This highly stereocontrolled route provides sufficient quantities of **1** for further studies. While this route is longer than the Seeberger approach in terms of longest linear sequence (20 steps and 7% overall yield from D-fucose (**3**)), it is shorter in terms of total steps (41 total steps). Thus we demonstrate the practicality of de novo approaches for oligosaccharide synthesis.[8] Further application of this approach to the preparation of an anthrax vaccine and detection device is ongoing.

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[9] Sigma-Aldrich sells acetylfuran **7** for $\$0.09\,g^{-1}$, L-rhamnose (**2**) for $\$5\,g^{-1}$ and D-fucose (**3**) for $\$71\,g^{-1}$, see: www.sigmaaldrich.com.
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[15] While in principle **16** can be prepared by Fischer glycosylation, in practice for moderately expensive sugars, like L-rhamnose, a four-step protocol is preferred: 1) peracylation, 2) selective hydrolysis of the anomeric position with HBr, 3) Ag- or Hg-promoted glycosylation of the anomeric bromide, and 4) LiOH global ester hydrolysis]; see Ref. [6].
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[19] Significantly lower yields were observed when cosolvents were used. Similarly low yields were also observed by Boons et al., see Ref. [6d].
[20] Since the spectral data for synthetic **1** matches that of the isolated material, this constitutes the first synthesis of **1**; see Ref. [6].

