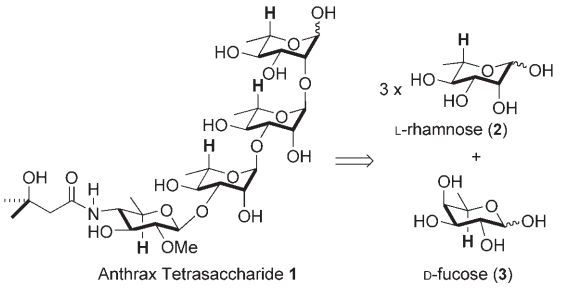
Tetrasaccharide Synthesis

DOI: 10.1002/ange.200701354

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

*Haibing Guo and George A. O'Doherty**

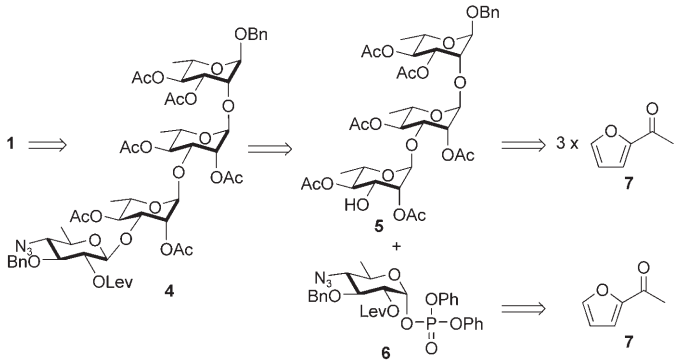
Anthrax is a zoonotic disease caused by the spore-forming bacterium *Bacillus anthracis*.[1] *Bacillus anthracis* belongs to the family *Bacillaceae*, which consists of a diverse group of bacteria, all of which form endospores.[2] *Bacillus anthracis* is the most important member of this genus and is 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

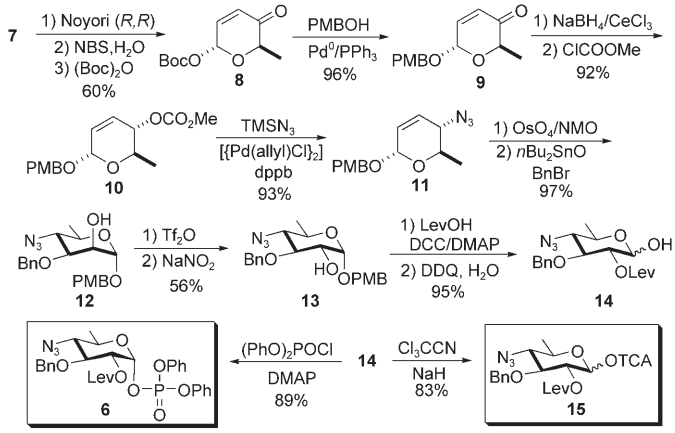
[*] H. Guo, Prof. G. A. O'Doherty
Department of Chemistry
West Virginia University
217 Clark Hall, P.O. 6045
Morgantown, WV 26506 (USA)
Fax: (+1) 304-293-4904
E-mail: george.odoherty@mail.wvu.edu
Homepage: http://www.as.wvu.edu/~odoherty/site/

[**] We thank the NIH (GM63150) and NSF (CHE-0415469) for their generous support of our research program. Funding for a 600-MHz NMR spectrometer by the NSF-EPSCoR (grant 0314742) is also gratefully acknowledged.

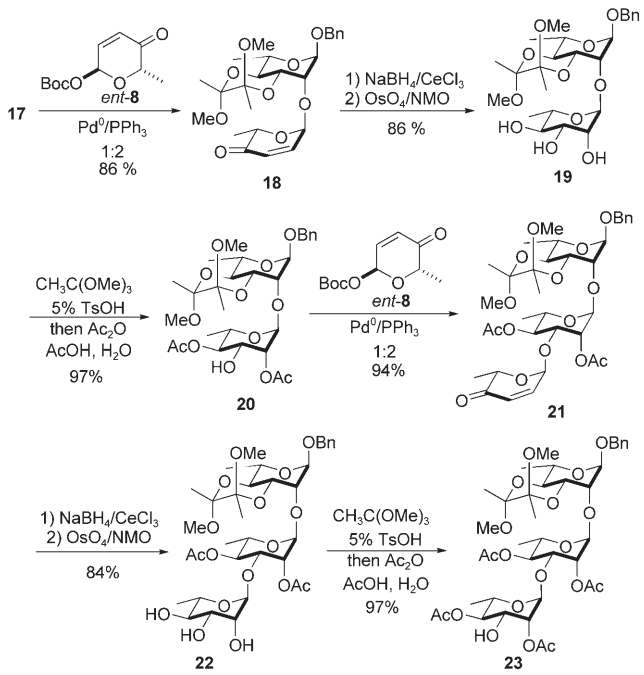
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

InterScience

© 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



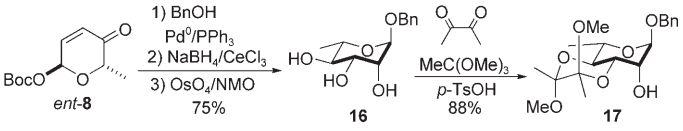
Scheme 3. Synthesis of anthrose monosaccharide **6** and **15**. Noyori (*R*,*R*) = (*R*)-Ru(η⁶-mesitylene)-(*R*,*R*)-*N*-(4-toluenesulfonyl)-1,2-diphenylethylenediamine, NBS = 1-bromo-2,5-pyrollidinedione, PMBOH = *p*-methoxybenzyl alcohol, $(Boc)_2O$ = di-*tert*-butyl dicarbonate, $TMSN_3$ = trimethylsilyl 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.



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

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



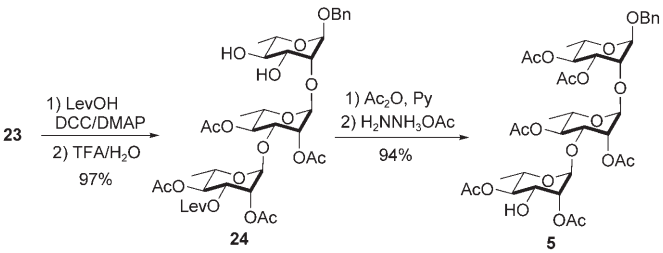
Scheme 4. Pd^0-catalyzed glycosylation synthesis of **17**. *p*-TsOH = *p*-toluenesulfonic acid.

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% 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]

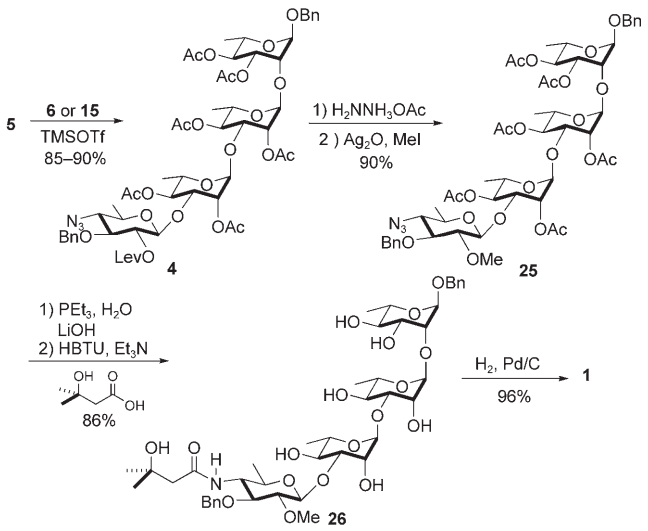
Unfortunately, our attempts at glycosylation of **23** with either the phosphate **6** or imidate **15** failed. Instead only hydrolysis of the spiroketal protecting group was observed. Thus, we decided to prepare the more acid-stable trisaccharide **5**, which could be prepared in four steps from **23** (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.

 © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



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.

Received: March 28, 2007
Published online: May 30, 2007

Keywords: anthrax tetrasaccharide · carbohydrates · glycosylation · natural products · palladium

[1] a) P. S. Brachman, A. F. Kaufmann in *Bacterial Infections of Humans* (Eds.: A. S. Evans, P. S. Brachman), Plenum Medical Book Company, New York, **1998**, pp. 95–111.
[2] a) M. Mock, A. Fouet, *Annu. Rev. Microbiol.* **2001**, *55*, 647–671; b) P. Sylvestre, E. Couture-Tosi, M. Mock, *Mol. Microbiol.* **2002**, *45*, 169–178.
[3] a) W. L. Nicholson, N. Munakata, G. Horneck, H. J. Melosh, P. Setlow, *Microbiol. Mol. Biol. Rev.* **2000**, *64*, 548–572; b) M. Moayeri, S. H. Leppla, *Curr. Opin. Microbiol.* **2004**, *7*, 19–24.
[4] J. M. Daubenspeck, H. Zeng, P. Chen, S. Dong, C. T. Steichen, N. R. Krishna, D. G. Pritchard, C. L. Turnbough, Jr., *J. Biol. Chem.* **2004**, *279*, 30945–30953.
[5] M. Tamborrini, D. B. Werz, J. Frey, G. Pluschke, P. H. Seeberger, *Angew. Chem.* **2006**, *118*, 6731–6732; *Angew. Chem. Int. Ed.* **2006**, *45*, 6581–6582.
[6] There have been two syntheses of the anthrax tetrasaccharide with an anomeric protecting group at the reducing end. a) D. B. Werz, P. H. Seeberger, *Angew. Chem.* **2005**, *117*, 6474–6476; *Angew. Chem. Int. Ed.* **2005**, *44*, 6315–6318; b) R. Adamo, R. Saksena, P. Kovac, *Carbohydr. Res.* **2005**, *340*, 2579–2582; c) R. Saksena, R. Adamo, P. Kovac, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 615–617. Of the two syntheses that in Ref. [6a] is the shorter. For the related trisaccharide, see: d) A. S. Mehta, E. Saile, W. Zhong, T. Buskas, R. Carlson, E. Kannenberg, Y. Reed, C. P. Quinn, G.-J. Boons, *Chem. Eur. J.* **2006**, *12*, 9136–9149.
[7] a) R. S. Babu, M. Zhou, G. A. O'Doherty, *J. Am. Chem. Soc.* **2004**, *126*, 3428–3429; b) M. Zhou, G. A. O'Doherty, *Org. Lett.* **2006**, *8*, 4339–4342.
[8] While there are many uses of the term "de novo" in carbohydrate chemistry, we use the term de novo asymmetric synthesis to refer to the use of catalysis for the asymmetric synthesis of carbohydrates from achiral compounds (e.g., this de novo route derives its asymmetry from enantiodivergent Noyori reductions of acetylfuran **7**).
[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.
[10] a) M. Li, J. G. Scott, G. A. O'Doherty, *Tetrahedron Lett.* **2004**, *45*, 1005–1009; b) R. S. Babu, G. A. O'Doherty, *J. Carbohydr. Chem.* **2005**, *24*, 169–177; c) H. Guo, G. A. O'Doherty, *Org. Lett.* **2005**, *7*, 3921–3924.
[11] a) J. L. Luche, *J. Am. Chem. Soc.* **1978**, *100*, 2226–2227; b) M. H. Haukaas, G. A. O'Doherty, *Org. Lett.* **2001**, *3*, 401–404.
[12] a) R. N. de Oliveira, L. Cottier, D. Sinou, R. M. Srivastava, *Tetrahedron* **2005**, *61*, 8271–8281; b) H. Guo, G. A. O'Doherty, *Org. Lett.* **2006**, *8*, 1609–1612.
[13] V. VanRheenen, R. C. Kelly, D. Y. Cha, *Tetrahedron Lett.* **1976**, *17*, 1973–1976.
[14] a) R. Saksena, R. Adamo, P. Kovac, *Carbohydr. Res.* **2005**, *340*, 1591–1600; b) D. W. C. Jones, R. J. Nash, E. A. Bell, J. M. Williams, *Tetrahedron Lett.* **1985**, *26*, 3125–3126.
[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].
[16] a) S. V. Ley, M. Woods, A. Zanotti-Gerosa, *Synthesis* **1992**, 52–54; b) S. V. Ley, R. Leslie, P. D. Tiffin, M. Woods, *Tetrahedron Lett.* **1992**, *33*, 4767–4770.
[17] See Ref. [7b] and a) J. F. King, A. D. Allbutt, *Can. J. Chem.* **1970**, *48*, 1754–1769; b) Y. E. Tsvetkov, A. S. Shashkov, Y. A. Knirel, U. Zahringer, *Carbohydr. Res.* **2001**, *335*, 221–243.
[18] While both reactions worked well, we had a preference for the phosphate glycosylation (**5** + **6**), see: a) O. J. Plante, E. R. Palmacci, R. B. Andrade, P. H. Seeberger, *J. Am. Chem. Soc.* **2001**, *123*, 9545–9554; b) R. R. Schmidt, W. Kinzy, *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21–123.
[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].

 © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim