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Synthesis of the tetrasaccharide side chain of the major glycoprotein of the *Bacillus anthracis* exosporium☆

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Abstract—An α-glycoside of the tetrasaccharide sequence β-Ant-(1 → 3)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p* whose aglycon allows conjugation to suitable carriers was synthesized. The NMR characteristics of the compound are virtually identical with those of the α-anomer of the tetrasaccharide isolated from the major glycoprotein of the *Bacillus anthracis* exosporium. Thus, the correct structure of the natural product has been proven by chemical synthesis.

Bacillus anthracis is the causative agent of anthrax. Because of concerns regarding the use of some of its forms as a biological weapon, there is a worldwide effort to develop a potent vaccine for the disease. Since *B. anthracis* is a spore-forming pathogen, a strategy toward a vaccine for anthrax could be based on targeting spores with antibodies that are specific for the tetrasaccharide β-Ant-(1 → 3)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-L-Rha*p* (**1**), which was recently discovered[2] as the oligosaccharide side chain of the collagen-like region of the major glycoprotein of the pathogen's exosporium. Within our work toward a conjugate vaccine for anthrax we have prepared and here report a synthesis (Scheme 1) of sequence **1** in the form of the α-glycoside **20** whose aglycone can be further transformed to allow conjugation to suitable carriers.

The initial glycosyl acceptor **4**[13] was synthesized by reaction of the known[3] thioglycoside **2** with methyl 6-hydroxyhexanoate[4] (→**3**) followed by deacetylation.

Extension of the oligosaccharide chain to give rhamnotrioside **9** was effected by glycosylation of **4** with the disaccharide building block **8**. To prepare **8**, the known[5] thioglycoside **6** was treated with glycosyl chloride **7**, which was obtained by treatment of **5**[5] with chlorine.[6]

Subsequent deacetylation of **9** gave the trisaccharide glycosyl acceptor **10**.

Synthesis of **20** required formation of 1,2-*trans* glycosidic linkage, which could be problematic with a glycosyl donor bearing a non-participating group at *O*-2, such as the 2-*O*-methyl group in anthrose. Therefore, the versatile 2-*O*-bromoacetylated glycosyl donor **14** (Scheme 2) was prepared. In addition to being a participating group, the bromoacetyl function[7,8] can be selectively removed in the presence of other acyl groups. Thus, donor **14** can be used in alternative syntheses of **1** involving acyl-protected intermediates. Compound **14** was obtained as shown in Scheme 2. Accordingly, methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-glucopyranoside[9] (**11**) was bromoacetylated,[10] and the formed **12** was acetolyzed to give **13**. The anomeric mixture of acetates thus obtained was treated with EtSH under Lewis acid catalysis to give **14**. Condensation of the trirhamnoside glycosyl acceptor **10** with **14** gave the fully protected tetrasaccharide **15**, which was sequentially de-*O*-bromoacetylated[10] (→**16**), methylated[11] (→**17**), and treated with H_2S,[12] to selectively reduce the azido group to amino function, to afford **18**. Treatment of **18** with 3-hydroxy-3-methylbutyric acid in the presence of HATU {*N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide} gave butanamido derivative **19**, which was fully deprotected by hydrogenolytic debenzylation, to give the target tetrasaccharide **20** equipped with a spacer that makes it amenable for conjugation to proteins or other suitable carriers. NMR data observed for **20** in DMSO-d_6 (Tables 1 and 2) are virtually identical to those reported[2] for the solution of α-anomer

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☆ See Ref. 1.
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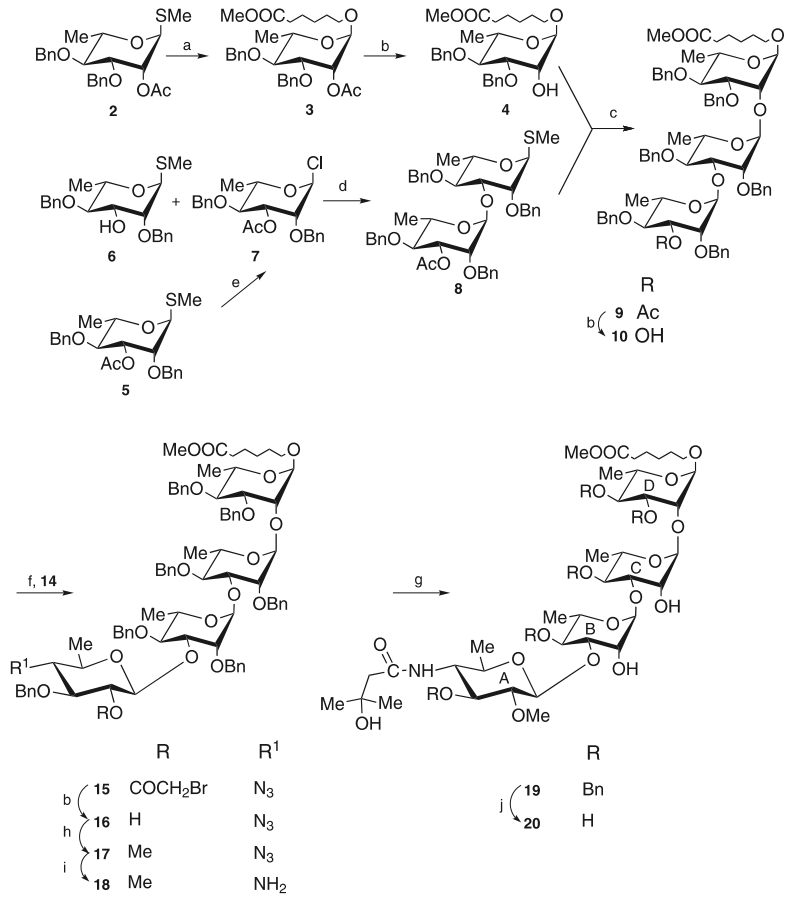
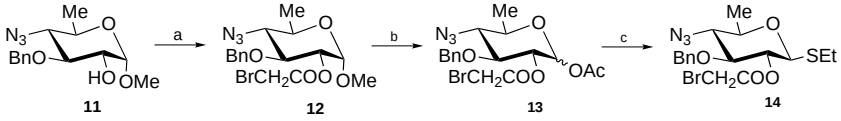


Scheme 1. Reagents and conditions: (a) NIS, AgOTf, methyl 6-hydroxyhexanoate, CH_2Cl_2, rt, 1 h; (b) NaOMe, MeOH, rt, 2 h; (c) NIS, AgOTf, CH_2Cl_2, rt, 1 h; (d) AgOTf, 2,6-di-*t*-Bu-Me-pyridine, CH_2Cl_2, rt, 1 h; (e) Cl_2, CH_2Cl_2, rt, 5 min; (f) NIS, AgOTf, CH_2Cl_2, rt, 1 h; (g) 3-hydroxy-3-methylbutyric acid, HATU, CH_2Cl_2, rt, 1 h; (h) MeI, Ag_2O, Me_2S, 1,2-dimethoxyethane, rt, 24 h; (i) H_2S, 3:1 pyridine–water, rt, 24 h; (j) H_2, Pd/C, MeOH, rt, 24 h.



Scheme 2. Reagents and conditions: (a) $BrCH_2COBr$, TMU, CH_2Cl_2, 0 °C → rt, 24 h; (b) Ac_2O, H_2SO_4, AcOH, rt, 1 h; (c) EtSH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt, 2 h.

Table 1. Comparison of ^1H chemical shifts[a] for tetrasaccharide **20** with data reported in Ref. 1 for **1**

Ring	H-1	H-2	H-3	H-4	H-5	H-6
A	4.577	2.845	3.281	3.402	3.275	1.074
	4.582	2.850	3.301	3.401	3.278	1.082
B	4.857	3.872	3.745	3.402	3.636	1.132
	4.876	3.880	3.760	3.404	3.655	1.142
C	4.842	3.775	3.609	3.325	3.487	1.101
	4.848	3.783	3.592	3.339	3.504	1.103
D	4.566	3.634	3.530	3.155	3.373	1.134
	4.870	3.601	3.615	3.144	3.592	1.114
	NH	CH_2	CH_3	OCH_3		
Other[b]	7.706	2.204	1.155, 1.143	3.519		
	7.754	2.211	1.160, 1.148	3.526		

[a] Spectra taken at 600 MHz for a solution in DMSO-d_6 at 25 °C. Data in the second row for each ring were taken from Ref. 1.

[b] δ_{OH} (disappear on deuteration): 4.995, 4.945, 4.885, 4.875, 4.831, 4.779, 4.718, 4.534. Not reported in Ref. 1.

of **1** in the same solvent. The minute differences in chemical shifts are due to the presence of the linker substituent at *O*-1 of the D-rhamnose residue. This confirms, by chemical synthesis, the structure assigned to the material isolated from the natural source.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.10.056.

Table 2. Comparison of ^{13}C chemical shifts[a] for tetrasaccharide **20** with data reported in Ref. 1 of **1**

Ring	C-1	C-2	C-3	C-4	C-5	C-6
A	103.50; $J_{C,H}$ 160.7	84.36	72.77	56.42	70.34	18.18
	103.4; $J_{C,H}$ 161	84.3	72.7	56.4	70.3	18.1
B	101.71; $J_{C,H}$ 171.3	69.67	79.48	71.65	68.31	17.73
	101.6; $J_{C,H}$ 171	69.6	79.4	71.6	68.2	17.6
C	101.76; $J_{C,H}$ 170.6	69.97	76.28	71.37	69.04	17.81
	101.6; $J_{C,H}$ 170	69.9	76.2	71.4	68.8	17.9
D	98.62; $J_{C,H}$ 167.6	76.66	70.56	72.22	68.58	18.02
	92.8; $J_{C,H}$ 166	77.7	70.1	72.6	67.7	17.9
	CO	CH_2	CH_3	C_{quat}	OCH_3	
Other	171.45	48.67	29.58, 29.48	68.71	60.16	
	171.4	48.6	29.5, 29.4	Not reported	59.9	

[a] Spectra taken at 150 MHz for a solution in DMSO-d_6 at 25 °C. Data in the second row for each ring were taken from Ref. 1.

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13. All reactions were monitored by TLC on silica gel coated glass slides, and the structure of products was verified by MS and NMR spectra. All new compounds produced correct analytical figures by combustion analysis, except for compounds **7**, **13**, **17**, and **20**. Copies of NMR spectra of the four compounds are available as Supplementary data.
Compound **3**: 83%; $[\alpha]_D$ −6° (*c* 1, $CHCl_3$); $\delta_{H\text{-}1}$ 5.35, $J_{1,2}$ 1.8 Hz; $\delta_{C\text{-}1}$ 97.60.
Compound **4**: 96%; $[\alpha]_D$ −39° (*c* 0.9, $CHCl_3$); $\delta_{H\text{-}1}$ 4.78, $J_{1,2}$ 1.6 Hz; $\delta_{C\text{-}1}$ 98.83.
Compound **7**: 88%; $\delta_{H\text{-}1}$ 5.98, $J_{1,2}$ 1.6 Hz; $\delta_{C\text{-}1}$ 90.97.
Compound **8**: 63%; $[\alpha]_D$ −42° (*c* 0.4, $CHCl_3$); $\delta_{H\text{-}1^C}$ 5.10, $J_{1,2}$ 1.6 Hz, $\delta_{H\text{-}1^D}$ 4.67, $J_{1,2}$ not determined due to overlap of signals; $\delta_{C\text{-}1^C}$ 99.16, $\delta_{C\text{-}1^D}$ 98.79.
Compound **9**: 94%; $[\alpha]_D$ −4.6° (*c* 0.9, $CHCl_3$); $\delta_{H\text{-}1^B}$ 5.16, $J_{1,2}$ 1.7 Hz; $\delta_{H\text{-}1^C}$ 5.11, $J_{1,2}$ 1.9 Hz, $\delta_{H\text{-}1^D}$ 4.68, $J_{1,2}$ 1.8 Hz, $\delta_{C\text{-}1^B}$ 99.25, $\delta_{C\text{-}1^C}$ 98.98, $\delta_{C\text{-}1^D}$ 98.87.
Compound **10**: 85%; $[\alpha]_D$ −6.6° (*c* 0.6, $CHCl_3$); $\delta_{H\text{-}1^B}$ 5.21 (s), δ_{H-1^C} 5.11, $J_{1,2}$ 1.9 Hz, $\delta_{H\text{-}1^D}$ 4.70, $J_{1,2}$ 1.8 Hz; $\delta_{C\text{-}1^B}$ 98.34,$\delta_{C\text{-}1^C}$ 98.93, $\delta_{C\text{-}1^D}$ 98.82.
Compound **12**: 90%; $[\alpha]_D$ +204° (*c* 0.5, $CHCl_3$); $\delta_{H\text{-}1}$ 4.86, $J_{1,2}$ ~3.7 Hz; $\delta_{C\text{-}1}$ 96.41.
Compound **13**: 93%; $\delta_{H\text{-}1\alpha}$ 6.24, $J_{1,2}$ 3.7 Hz, $\delta_{H\text{-}1\beta}$ 5.58, $J_{1,2}$ 8.4 Hz; $\delta_{C\text{-}1\alpha}$ 89.04, $\delta_{C\text{-}1\beta}$ 91.41.
Compound **14**: β anomer, 62%; mp 82.0–82.5 °C (from EtOH); $[\alpha]_D$ +60° (*c* 0.5, $CHCl_3$); $\delta_{H\text{-}1}$ 4.34, $J_{1,2}$ 10.0 Hz; $\delta_{C\text{-}1}$ 82.82; the corresponding α-anomer, mp 31–32 °C (from hexane), $[\alpha]_D$ +263° (*c* 0.9, $CHCl_3$); ($\delta_{H\text{-}1}$ 5.57, $J_{1,2}$ 5.4 Hz; $\delta_{C\text{-}1}$ 81.27) was formed in 23%, total yield of thioglycosidation, 85%.
Compound **15**: 84%; $[\alpha]_D$ +2.3° (*c* 0.7, $CHCl_3$); $\delta_{H\text{-}1^A}$ ~4.69, $J_{1,2}$ ~8.0 Hz, $\delta_{H\text{-}1^B}$ 5.09 (s); $\delta_{H\text{-}1^C}$ 5.10, $J_{1,2}$ 1.9 Hz; $\delta_{H\text{-}1^D}$ ~4.66, $J_{1,2}$ not determined due to overlap; $\delta_{C\text{-}1^A}$ 100.62, $\delta_{C\text{-}1^B}$ 100.22, $\delta_{C\text{-}1^C}$ 98.75, $\delta_{C\text{-}1^D}$ 98.87.
Compound **16**: 93%; [a]$_D$ +10.4° (*c* 0.5, $CHCl_3$); $\delta_{H\text{-}1^A}$ 4.39, $J_{1,2}$ 7.7 Hz, $\delta_{H\text{-}1^B}$ 5.08 (s), $\delta_{H\text{-}1^C}$ 5.11, $J_{1,2}$ 2.0 Hz,$\delta_{H\text{-}1^D}$ 4.67, $J_{1,2}$ 1.7 Hz; $\delta_{C\text{-}1^A}$ 103.70, $\delta_{C\text{-}1^B}$ 99.17, $\delta_{C\text{-}1^C}$ 98.70, $\delta_{C\text{-}1^D}$ 98.89.
Compound **17**: 96%; $[\alpha]_D$ +2° (*c* 1, $CHCl_3$); $\delta_{H\text{-}1^A}$ 4.61, $J_{1,2}$ 8.0 Hz, $\delta_{H\text{-}1^B}$ 5.11 (s); $\delta_{H\text{-}1^C}$ 5.08, $J_{1,2}$ 1.8 Hz; $\delta_{H\text{-}1^D}$ 4.66, $J_{1,2}$ 1.8 Hz; $\delta_{C\text{-}1^A}$ 103.52, $\delta_{C\text{-}1^B}$ 100.26, $\delta_{C\text{-}1^C}$ 98.96, $\delta_{C\text{-}1^D}$ 98.90.
Compound **18**: 88%; $\delta_{H\text{-}1^A}$ 4.70, $J_{1,2}$ 7.7 Hz, $\delta_{H\text{-}1^B}$ 5.12 (s), $\delta_{H\text{-}1^C}$ 5.08, $J_{1,2}$ 1.9 Hz, $\delta_{H\text{-}1^D}$ ~4.66, $J_{1,2}$ not determined due to overlap; $\delta_{C\text{-}1^A}$ 103.82, $\delta_{C\text{-}1^B}$ 100.37, $\delta_{C\text{-}1^C}$ 99.03, $\delta_{C\text{-}1^D}$ 98.89.
Compound **19**: 97%; [a]$_D$ −26° (*c* 0.5, $CHCl_3$); $\delta_{H\text{-}1^A}$ 4.62, $J_{1,2}$ 8.0 Hz, $\delta_{H\text{-}1^B}$ 5.09 (s), $\delta_{H\text{-}1^C}$ 5.07, $J_{1,2}$ 2.0 Hz, $\delta_{H\text{-}1^D}$ ~4.66, ~1.9 Hz;$\delta_{C\text{-}1^A}$ 103.69, $\delta_{C\text{-}1^B}$ 100.34, $\delta_{C\text{-}1^C}$ 98.94, $\delta_{C\text{-}1^D}$ 98.86.
Compound **20**: 83%; $[\alpha]_D$ −52.6° (*c* 0.5, H_2O).