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Synthesis of the β anomer of the spacer-equipped tetrasaccharide side chain of the major glycoprotein of the *Bacillus anthracis* exosporium☆

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Abstract—The β glycoside of the tetrasaccharide sequence β-Ant-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→2)-L-Rha*p*, whose aglycon allows conjugation to proteins, was synthesized for the first time. A stepwise synthetic approach was applied with thioglycosides as glycosyl donors, and the β anomer of the compound was obtained equipped with a spacer group whose further transformation allows conjugation to suitable carriers. To synthesize the β-anthrosyl linkage with high stereoselectivity, a linker-equipped rhamnotriose derivative was glycosylated with ethyl 4-azido-3-*O*-benzyl-2-*O*-bromoacetyl-4,6-dideoxy-1-thio-β-D-glucopyranoside. Further functionalization of the tetrasaccharide thus obtained, followed by deprotection, gave the target substance.

Keywords: Anthrax; Anthrose; Exosporium glycoprotein; Thioglycoside; Oligosaccharide

Bacillus anthracis is the etiological cause of anthrax.[1] In view of current concerns regarding the use of some form of the bacterium as a biological weapon, development of a potent vaccine for anthrax is a pressing issue worldwide. *B. anthracis* is a spore-forming pathogen, and a vaccine for anthrax could be based on targeting spores with neutralizing antibodies that are specific for one of their surface components. The tetrasaccharide β-Ant-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→2)-L-Rha*p* was recently established[2] as the oligosaccharide side chain of the collagen-like region of the major glycoprotein of the *B. anthracis* exosporium (Fig. 1). In connection with developing a conjugate vaccine for anthrax following the above-outlined strategy, we have recently synthesized anthrose, as well as intermediates that could be used to prepare its glycosyl donors.[3] With the plan to generate immunogens for antibodies specific for the carbohydrate component of the anthrax exosporium

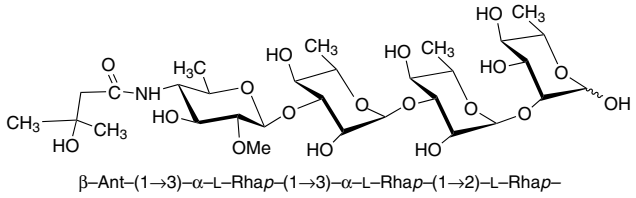
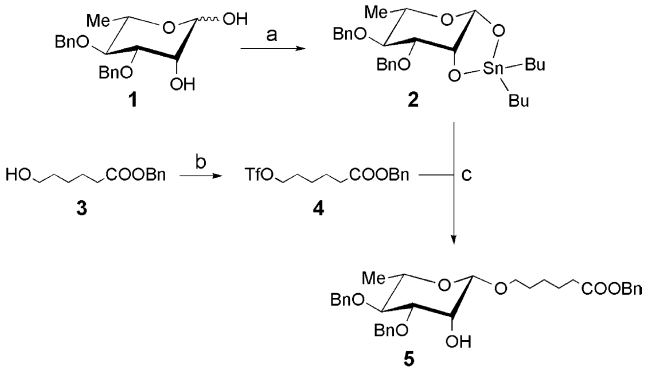


Figure 1. Structure of the tetrasaccharide side chain of the collagen-like glycoprotein of *Bacillus anthrasis* exosporium.



Scheme 1. Reagents and conditions: (a) Bu_2SnO, toluene; (b) Tf_2O, K_2CO_3, CH_2Cl_2; (c) CsF, MeCN.

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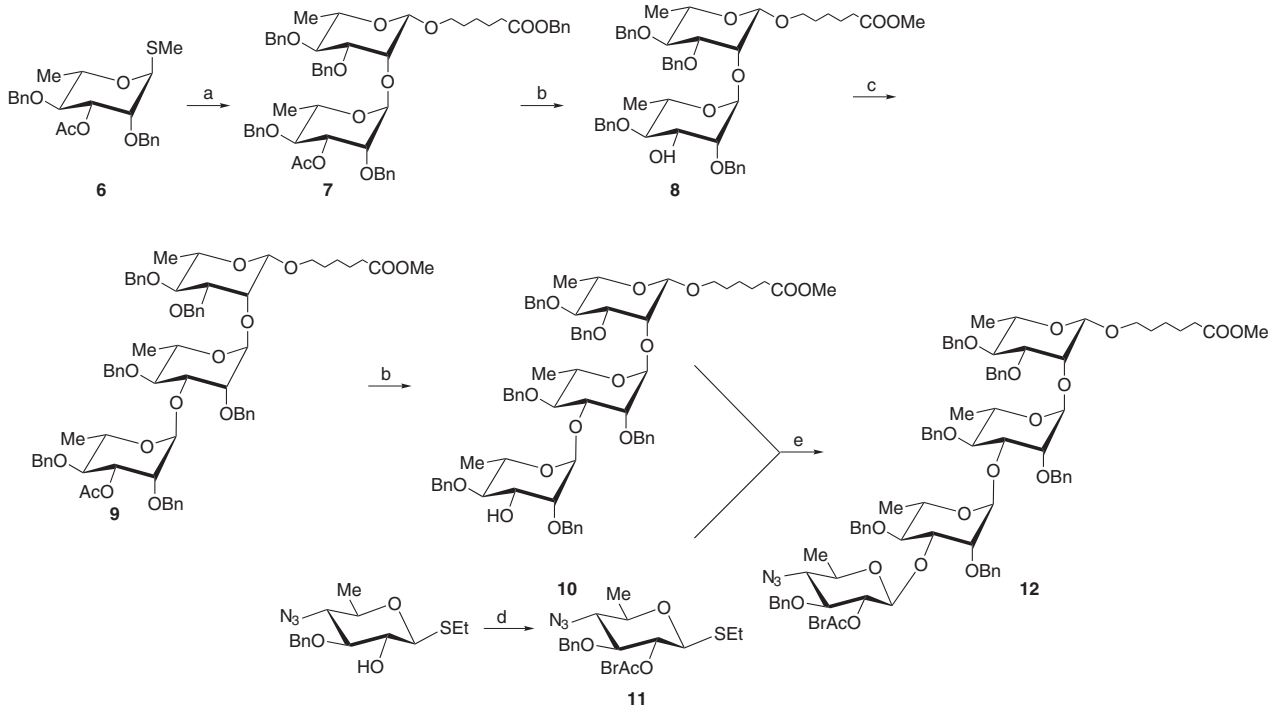


Scheme 2. Reagents and conditions: (a) **5**, NIS, AgOTf, CH_2Cl_2; (b) NaOMe, MeOH; (c) **6**, NIS, AgOTf, CH_2Cl_2; (d) $BrCH_2COBr$, TMU, CH_2Cl_2; (e) NIS, AgOTf, CH_2Cl_2.

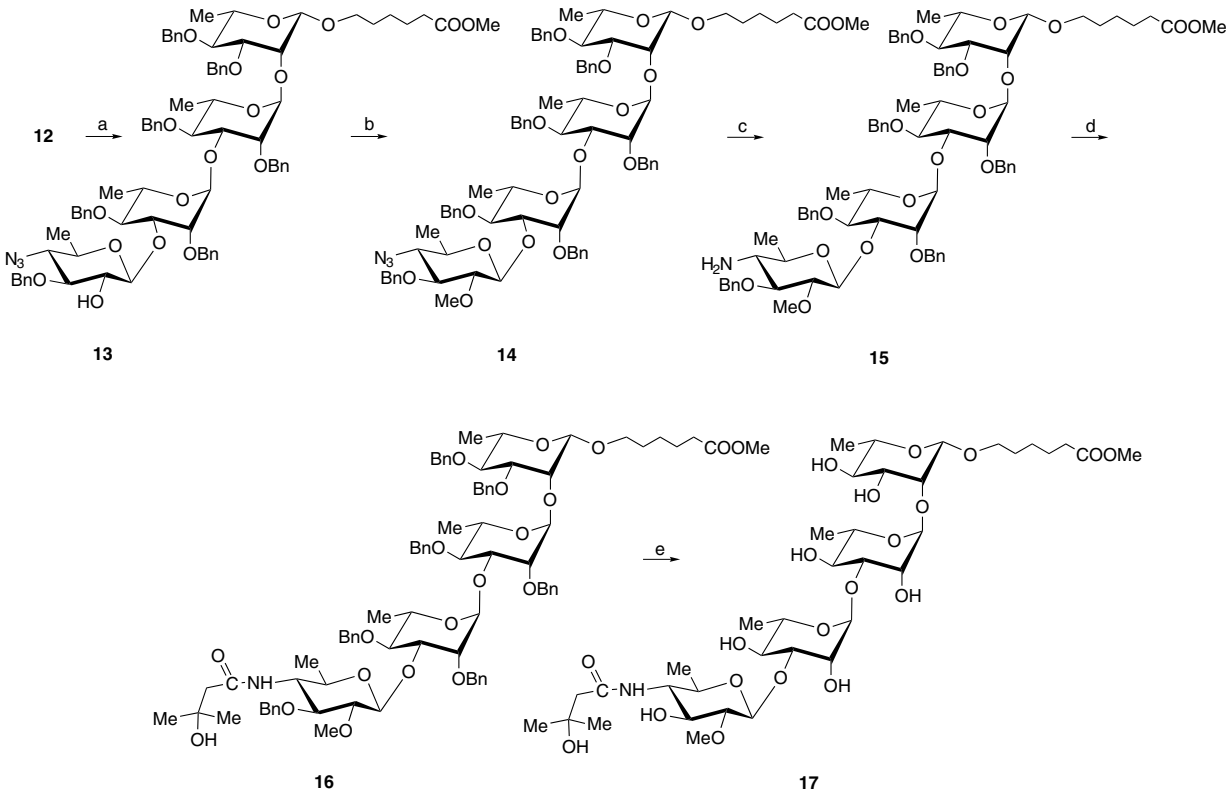
glycoprotein, we have now prepared and here report the first synthesis of tetrasaccharide glycoside **17**, whose aglycone can be transformed to allow conjugation to suitable carriers.

It has been proposed[2] that the tetrasaccharide may be attached to the exosporium glycoprotein through an *N*-acetyl-D-galactosamine linker, but the type of glycosidic linkage providing that attachment to the GalNAc linker is unknown. To insure production of antibodies homologous to the naturally occurring tetrasaccharide, conjugates from both α- and β-linked tetrasaccharides will have to be prepared and tested for their immunogenicity. Results of such studies may provide clues regarding the mode of linkage of the tetrasaccharide in the natural exosporium, as well as information about specificity and cross-reactivity of antibodies formed. Described below is the stepwise construction of tetrasaccharide glycoside **17** in which the spacer is attached through a β-rhamnosyl linkage. The initial glycosyl acceptor **5**[†] [52% from **1**, $[\alpha]_D$ +11 (*c* 0.6, $CHCl_3$)] was synthesized from triflate **4** {*m*/*z* 354 ($[M]^+$)} of benzyl 6-hydroxyhexanoate[4] **3** and the stannylidene acetal **2** of 3,4-di-*O*-benzyl rhamnose[5] (Scheme 1). Using the benzyl ester allowed monitoring the formation and purification of the triflate by UV light. The formation of the β-rhamnopyranosyl linkage[6,7] in the formation of **5** manifested[8] itself in the proton-coupled ^{13}C NMR spectrum ($J_{C\text{-}1,H\text{-}1}$ 156.4 Hz).

Stepwise extension of the oligosaccharide chain was effected (Scheme 2) by glycosylation with methyl 3-*O*-acetyl-2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside **6**,[9] first of **5** [→**7**, 82%, $[\alpha]_D$ +38.5 (*c* 0.8, $CHCl_3$)], and then of the product of deacetylation of **7**, alcohol **8** [94%, $[\alpha]_D$ +13 (*c* 0.6 $CHCl_3$)], to give rhamnotrioside **9** [92%, $[\alpha]_D$ +22 (*c* 1.2, $CHCl_3$)]. Positioning anthrose at the upstream terminus of the desired tetrasaccharide **17** required formation of a β-glucosidic linkage. Because of the presence of a 2-*O*-methyl group in **17**, formation of such a linkage in a highly stereoselective manner could be problematic using a glycosyl donor derived from a synthon having a nonparticipating group at O-2, such as the OMe group in anthrose. Therefore, glycosyl acceptor **10**, obtained [96%, $[\alpha]_D$ +14 (*c* 0.4, $CHCl_3$)] by deacetylation of **9**, was glycosylated with the versatile glucosyl donor **11** [mp 82–82.5 °C, $[\alpha]_D$ +60 (*c* 0.5, $CHCl_3$)], obtained (~70%) by bromoacetylation[10] of ethyl 4-azido-3-*O*-benzyl-4,6-dideoxy-1-thio-β-D-glucopyranoside,[3] to give the fully protected tetrasaccharide **12** [66%, $[\alpha]_D$ +18 (*c* 1, $CHCl_3$)]; $J_{C\text{-}1^{I},H\text{-}1^{I}}$ 152.3 Hz, $J_{C\text{-}1^{II},H\text{-}1^{II}}$ 169.8 Hz, $J_{C\text{-}1^{III},H\text{-}1^{III}}$ 171.2 Hz, $J_{C\text{-}1^{IV},H\text{-}1^{IV}}$ 160.9 Hz.

Transformation of **12** into the target tetrasaccharide **17** was accomplished as shown in Scheme 3. Accordingly, after debromoacetylation [→**13**, 91%, $[\alpha]_D$ +40 (*c* 0.4, $CHCl_3$)], successive methylation with MeI and Ag_2O in the presence[11] of Me_2S [→**14**, 70%, $[\alpha]_D$ +29

[†] All new compounds produced correct analytical figures by combustion analysis, except **4**, **12**, **15**, **16**, and **17**. Copies of NMR spectra of the foregoing five compounds are available as supplementary material.



Scheme 3. Reagents and conditions: (a) NaOMe, MeOH; (b) MeI, Me_2S, Ag_2O, CH_2Cl_2; (c) H_2S, Py, H_2O; (d) 3-hydroxy-3-methylbutyric acid, HATU, EDA, CH_2Cl_2; (e) H_2, Pd/C, MeOH/AcOH.

(*c* 0.4, $CHCl_3$)], and selective reduction of the azido function with H_2S[12] gave amine **15** (70%), whose treatment 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 **16** (73%). Finally, hydrogenolytic debenzylation over 5% palladium-on-charcoal catalyst gave the target tetrasaccharide **17** [82%, $[\alpha]_D$ −38 (*c* 0.7, MeOH)], equipped with a spacer that makes it amenable to conjugation to proteins or other suitable carriers. NMR data observed for **17** (in D_2O with acetone as internal standard[13]) are in Table 1. Work toward the α-linked analog of **17** and to neoglycoconjugates from **17** is in progress.

Table 1. ^1H and ^{13}C chemical shifts (δ) for tetrasaccharide **17**[a]

Residue[b,c]	H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6 C-6
I	4.641	4.002	3.691	3.40	3.40	~1.301
	102.336	79.615	76.053	75.150[d]	74.979[d]	19.455[e]
II	5.005	4.186	3.902	3.508	4.213	1.249
	103.995	72.781	80.864	74.099	71.547	19.492
III	5.028	4.285	4.002	3.620	3.876	~1.315
	104.929	72.606	82.392	73.912	72.047	19.424[e]
IV	4.470	3.135	3.543	3.636	3.561	1.223
	106.431	86.030	75.620	59.345	73.536	19.086

[a] Measured at 600 MHz (^1H) and 150 MHz (^{13}C) for solutions in D_2O at 20 °C.

[b] Sugar residues in the tetrasaccharide are serially numbered, beginning with the rhamnose residue bearing the aglycone, and are identified by a Roman numeral superscript. Nuclei associated with the *N*-acyl substitutent and those with the linker aglycone are denoted with a single and double prime, respectively.

[c] $\delta_{H\text{-}1''a}$ ~3.84; δ_{COOMe} 3.680; $\delta_{H\text{-}1''b}$ ~3.67; $\delta_{OMe\text{-}2}$ 3.632; $\delta_{CH_2'}$ 2.459; $\delta_{H\text{-}5''}$ 2.400; $\delta_{H\text{-}2'',4''}$ 1.612; $\delta_{H\text{-}3''}$ 1.422; $\delta_{Me'}$ ~1.309, ~1.300. δ_{CO} 180.399, 176.830; $\delta_{C\text{-}3'(quart)}$ 72.990; $\delta_{C\text{-}1''}$ 72.486; $\delta_{OMe\text{-}2}$ 62.811; δ_{COOMe} 54.855; $\delta_{C\text{-}2'}$ 51.687; $\delta_{C\text{-}5''}$ 36.393; $\delta_{C\text{-}4''}$ 31.451; $\delta_{Me''}$ 31.040, 30.855; $\delta_{C\text{-}3''}$ 27.728; $\delta_{C\text{-}2''}$ 26.858.

[d] The assignment can be reversed.

[e] The assignment can be reversed.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2005.09.015.

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