ORGANIC LETTERS

2004
Vol. 6, No. 22
3901−3904

Synthesis of the Trisaccharide Repeating Unit of the Atypical *O*-Antigen Polysaccharide from Danish *Helicobacter pylori* Strains Employing the 2′-Carboxybenzyl Glycoside

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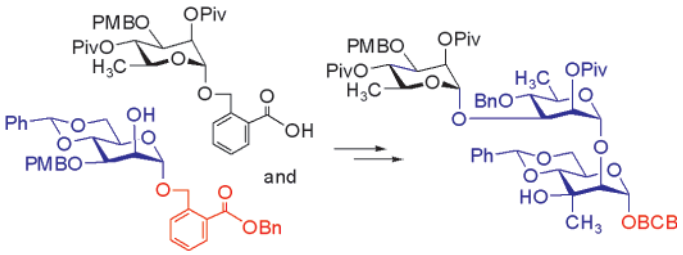
Received July 15, 2004

ABSTRACT



Synthesis of the unique trisaccharide repeating unit of the *O*-polysaccharide of the lipopolysaccharide from Danish *Helicobacter pylori* strains has been accomplished. Key steps include the coupling of three monosaccharide moieties by glycosylations employing the 2′-carboxybenzyl glycoside method. Also presented is a method for the synthesis of the novel branched sugar, 3-*C*-methyl-D-mannose, which is one of three monosaccharide components.

Since the first isolation of *Helicobacter pylori*,[1] extensive studies have led to recognition of this bacterium as the major cause of chronic gastritis and gastric and duodenal ulcers.[2] Moreover, persistent infection with *H. pylori* is strongly associated with the risk of development of gastric cancer.[2] *H. pylori* is estimated to infect over one-half of the world's population and thus has been classified as a category 1 (definite) human carcinogen.[3] Like the cell envelope of other gram-negative bacteria, that of *H. pylori* contains lipopolysaccharides (LPS). The *O*-antigen polysaccharide is a part of the LPS, which interacts with the bacterial microenvironment and the infected host. Structural studies on the LPS of a number of *H. pylori* strains have shown that the *O*-antigen polysaccharide exhibits mimicry of Lewis blood group antigens by expression of the corresponding determinants in the *O*-chain or at the nonreducing end of the *O*-chain polysaccharide.[4] Consequently, a number of studies have been performed to account for the pathogenic relevance of Lewis antigen mimicry by *H. pylori*.[5] There have also been immunization studies with inactivated *H. pylori* whole-cell vaccines having homologous and heterologous LPS

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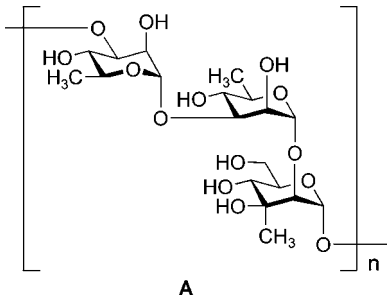
10.1021/ol048648u CCC: $27.50
Published on Web 09/29/2004

O-antigens expressing different Lewis antigens[6] and with *H. pylori* outer membrane vesicles, which are enriched with LPS.[7] Recently, however, an atypical *O*-antigen polysaccharide of LPS was isolated from Danish *H. Pylori* strains D1, D3, and D6, and the following repeat trisaccharide structure was established: →3)-α-L-Rhap-(1→3)-α-D-Rhap-(1→2)-α-D-Manp3CMe-(1→ (**A**).[8] An unusual feature of this *O*-polysaccharide is the occurrence of the novel branched sugar, 3-*C*-methyl-D-mannose, which has not been found in Nature before. The simultaneous occurrence of L- and D-rhamnose is also unusual.

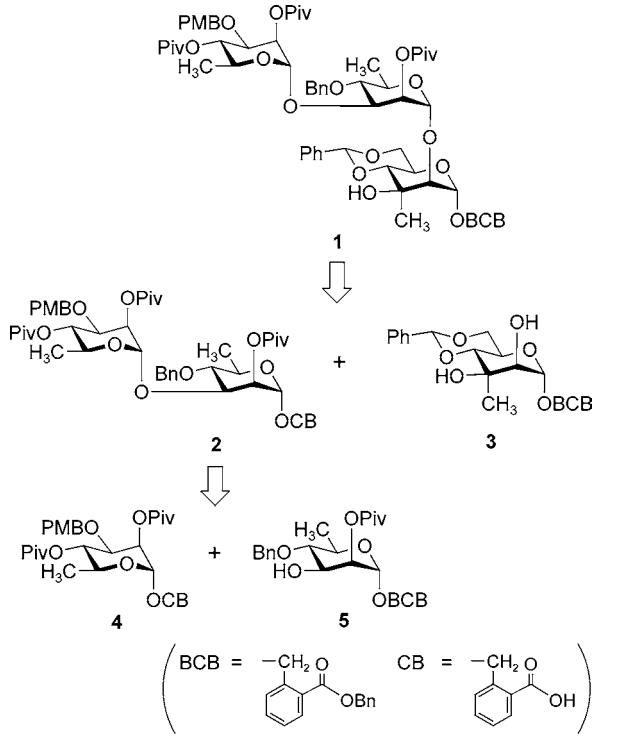
Due to its structural uniqueness and biomedical potential, the repeat unit **A** is an attractive synthetic target. Although a number of methods for the synthesis of Lewis antigens have been reported,[9] the synthesis of the repeating unit **A** or compounds with the similar structure has not been reported. Herein we report the synthesis of trisaccharide **1** in a suitably protected form of the repeating unit **A**.



Key problems needing to be addressed in the synthesis of **1** are the synthesis of the unique 3-*C*-methyl-D-mannose moiety and the coupling of three monosaccharide moieties by efficient glycosylation methods. We have recently developed a glycosylation method that employs a new type of glycosyl donor, 2′-carboxybenzyl (CB) glycosides, and have used these extensively for the stereoselective β-mannopyranosylation[10] and 2-deoxyglycosylation of various acceptors. We have also used this method in the synthesis of a tetrasaccharide.[11]

The CB glycosylation method would be here used for the coupling of all three monosaccharide components of **1**. The

Scheme 1. Retrosynthesis of Trisaccharide **1**



protective groups in the target trisaccharide **1** were chosen after consideration of the future synthesis of a hexasaccharide or a nonasaccharide by dimerization or trimerization of **1**. Thus, the latent 2′-(benzyloxycarbonyl)benzyl (BCB) group[10a] at C-1 in the reducing end of trisaccharide **1** would be readily converted into the active CB group to give the trisaccharide donor, while the *p*-methoxybenzyl (PMB) group at C-3 in the nonreducing end of **1** would be selectively cleaved to provide the trisaccharide acceptor. Retrosynthesis of **1** leads to disaccharide donor **2** and acceptor **3**, and further analysis of **2** provides L-rhamnosyl donor **4** and D-rhamnosyl acceptor **5** as shown in Scheme 1.

Before commencing the synthesis of the BCB α-D-mannopyranoside **3**, we carried out a model study on elaboration of the 3-*C*-methyl group in the mannoside with simpler methyl α-D-mannopyranoside as shown in Scheme 2. The model study began with a selective protection of the C-2 axial hydroxyl group of methyl 4,6-*O*-benzylidene-D-mannoside **6** with PMBCl and oxidation of the resulting PMB ether **7** with PDC to obtain ketosugar **8**. Axial attack of dimethylsulfonium methylide[12] to the C-3 carbonyl carbon of **8** afforded only the spiroepoxide **9**, which was reduced with $LiAlH_4$ to desired 3-*C*-methylmannoside **10**. The stereochemistry at C-3 of **10** was confirmed by NOE experiments and by comparison with the corresponding 3-*C*-methylaltroside, the C-3 epimer of **10**, which was obtained by direct addition of methyllithium to the ketosugar **8**.

The sequence developed in the model study with methyl 4,6-*O*-benzylidenemannoside **6** was then applied to BCB 4,6-

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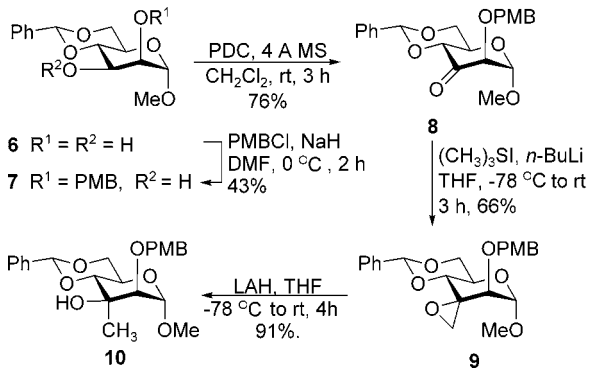
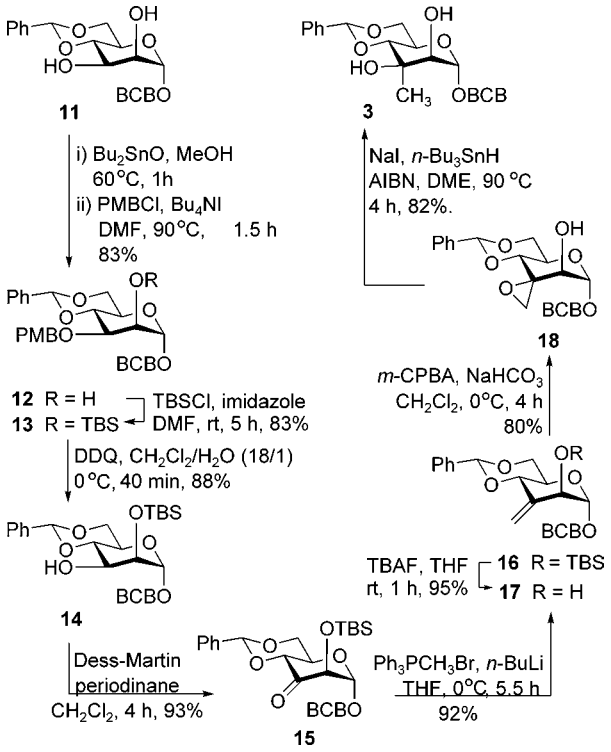
Scheme 2. Model Study for the Synthesis of 3-*C*-Methylmannoside



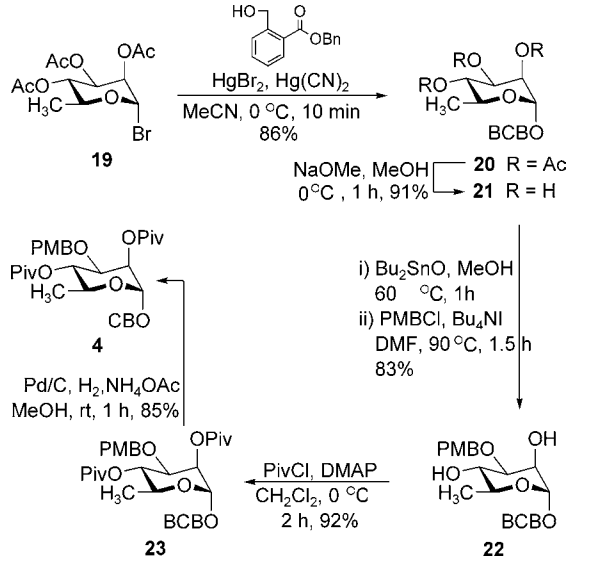
O-benzylidenemannoside **11**[10a] (Scheme 3). Treatment of **11** with Bu_2SnO in refluxing methanol followed by reaction of the resulting crude *O*-stannylene acetal[13] with PMB chloride in the presence of Bu_4NI in DMF afforded the C-3 PMB ether **12** in 78% yield for the two steps with complete regioselectivity. Reaction of **12** with TBS chloride gave the fully protected sugar **13**, and deprotection of the PMB group of **13** with DDQ provided the C-3 hydroxyl sugar **14** in high yield. Oxidation of **14** with Dess−Martin periodinane[14] gave ketosugar **15** in 93% yield.

However, unlike the model study with the ketosugar **8**, reaction of the keto sugar **15** with dimethylsulfonium methylide did not proceed, possibly due to steric congestion between the incoming sulfur ylide and the bulky C-1 axial

Scheme 3. Synthesis of BCB 3-*C*-Methylmannoside **3**



Scheme 4. Synthesis of CB L-Rhamnoside **4**



OBCB group. Reaction of the same sulfur ylide with other ketosugars having different C-2 protective groups such as PMB and acetyl groups did not occur. Ketosugar **15**, therefore, was converted into methylene sugar **16** in 92% yield by Wittig reaction with triphenylphosphonium methylide. The TBS group of **16** was removed by Bu_4NF, and epoxidation of the resulting hydroxy methylene sugar **17** afforded spiroepoxide **18** in 80% yield with complete stereoselectivity. Chemoselective reduction of spiroepoxy sugar **18** with Bu_3SnH in the presence of NaI and AIBN under reflux in DME[15] afforded the desired BCB 3-*C*-methylmannoside **3**. The stereochemistry at C-3 of compound **3** was confirmed on the basis of NOE experiments. Thus, NOE was observed between the methyl protons at C-3 and the proton at C-5 in compound **3**.

Synthesis of the L-rhamnose moiety **4** started with conversion of triacetylrhamnosyl bromide **19** into BCB triacetylrhamnoside **20** by coupling of **19** with benzyl 2-(hydroxymethyl)benzoate[10a] in the presence of Hg(II) salts (Scheme 4). Sodium methoxide converted triacetate **20** into triol **21** in 91% yield without affecting the BCB moiety. Compound **21** was treated with Bu_2SnO in methanol at 60 °C and subsequently reacted with PMB chloride in the presence of Bu_4NI in DMF to afford exclusively the C-3 PMB ether **22** in 83% yield. Pivaloylation of the diol **22** and hydrogenolysis of resultant BCB glycoside **23** in the presence of ammonium acetate gave the desired CB L-rhamnoside **4** in 85% yield.

The D-rhamnose unit **5** was prepared starting from the BCB mannoside **12** (Scheme 5). Pivaloylation of **12** followed by reductive cleavage of the benzylidene group in the resulting pivaloate **24** with borane in the presence of Bu_2BOTf[16] afforded the C-4 benzyl ether **25** having a free

(13) For a review on stannylene acetals of carbohydrates, see: Grindley, T. B. *Adv. Carbohydr. Chem. Biochem.* **1998**, *53*, 17−142.
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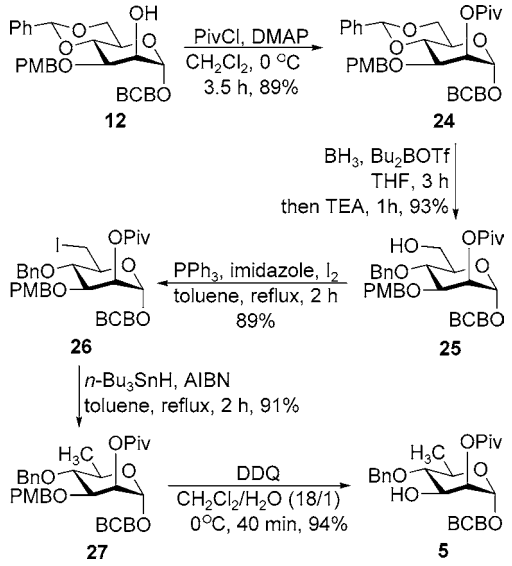
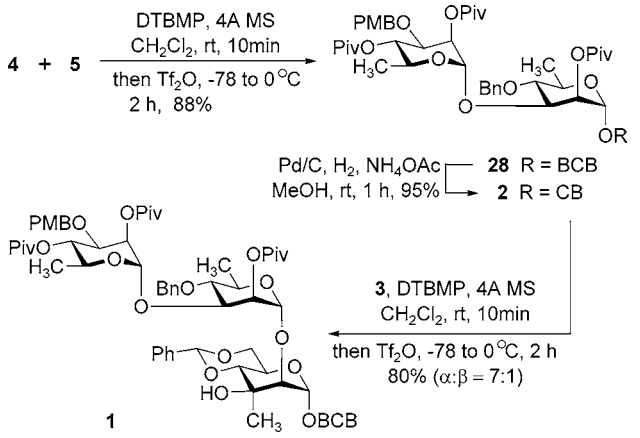
Scheme 5. Synthesis of BCB D-Rhamnoside **5**



Scheme 6. Completion of the Synthesis of Trisaccharide **1**



hydroxyl group at C-6 in high yield. Iodination of the primary alcohol **25** with I_2, Ph_3P, and imidazole provided the iodide **26** without difficulty. Reduction of the iodide **26** with Bu_3SnH in the presence of AIBN and subsequent removal of PMB group of the rhamnoside **27** with DDQ gave the desired BCB D-rhamnoside **5** in high yield.

Coupling of CB L-rhamnoside **4** and BCB D-rhamnoside **5** was achieved by the addition of Tf_2O to the mixture of **4** and **5** in the presence of DTBMP (2,6-di-*tert*-butyl-4-methylpyridine) and 4 Å MS in CH_2Cl_2 at −78 °C followed by warming to 0 °C; the desired α-disaccharide **28** was obtained in 88% yield (Scheme 6). Selective hydrogenolysis of the BCB disaccharide **28** in the presence of NH_4OAc in methanol afforded the CB disaccharide **2** in 95% yield. Finally, glycosylation with the CB disaccharide donor **2** and BCB 3-*C*-methylmannoside acceptor **3** was carried out by the addition of Tf_2O to a solution of **2** and **3** in CH_2Cl_2 in the presence of DTBMP at −78 °C. Warming the reaction mixture to 0 °C, quenching with $NaHCO_3$, and purification by chromatography afforded the target α-trisaccharide **1** along with its *β*-anomer in a 7:1 ratio in 80% yield. The stereochemistries at newly generated anomeric centers of disaccharides **28** and **2** and trisaccharide **1** were unequivocally determined on the basis of their ^1H and ^{13}C NMR spectral data, especially one-bond C1′−H1′ coupling constants: $^1J_{C1'-H1'} = 171.1$ Hz in compound **2** and $^1J_{C1'-H1'} = 170.8$ Hz[17] in compound **1**.

In summary, we have described the synthesis of the suitably protected trisaccharide repeating unit **1** of an unusual *O*-antigen polysaccharide of the lipopolysaccharide from Danish *H. pylori* strains. The 2′-carboxybenzyl glycosides method proved to be effective in the coupling of three monosaccharide components of the trisaccharide, **3**−**5**. We also presented the first synthesis of a novel branched sugar, 3-*C*-methyl-D-mannose.

Acknowledgment. This work was supported by a grant form the Korea Science and Engineering Foundation through Center for Bioactive Molecular Hybrids (CBMH).

Supporting Information Available: Synthetic procedures and spectral/analytical data of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) For C−H coupling constants in pyranoses, see: Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293−297.