

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

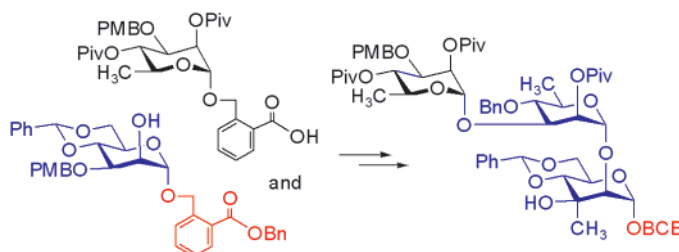
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ABSTRACT



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

(1) (a) Warren, J. R.; Marshall, B. J. *Lancet* **1983**, 321, 1273–1275. (b) Marshall, B. J.; Warren, J. R. *Lancet* **1984**, 323, 1311–1315.

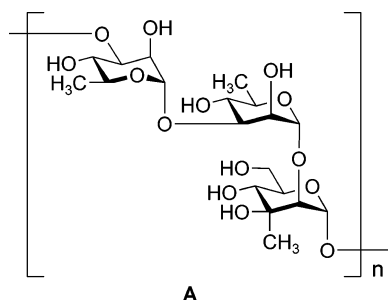
(2) (a) Dunn, B. E.; Cohen, H.; Blaser, M. J. *Clin. Microbiol. Rev.* **1997**, 10, 720–741. (b) Blaser, M. J. *Sci. Am.* **1996**, 274, 104–109. (c) Dubois, A. *Emerg. Infect. Dis.* **1995**, 1, 79–85.

(3) (a) Covacci, A.; Telford, J. L.; Giudice, G. D.; Parsonnet, J.; Rappuole, R. *Science*, **1999**, 284, 1328–1333. (b) Logan, R. P. H. *Lancet* **1994**, 344, 1078–1079.

(4) (a) Monteiro, M. A.; Chan, K. H. N.; Rasko, D. A.; Taylor, D. E.; Zheng, P. Y.; Appelmek, B. J.; Wirth, H. P.; Yang, M. Q.; Blaser, M. J.; Hynes, S. O.; Moran, A. P.; Perry, M. B. *J. Biol. Chem.* **1998**, 273, 11533–11543. (b) Aspinall, G. O.; Monteiro, M. A. *Biochemistry* **1996**, 35, 2498–2504. (c) Aspinall, G. O.; Monteiro, M. A.; Pang, H.; Walsh, E. J.; Moran, A. P. *Biochemistry* **1996**, 35, 2489–2497.

O-antigens expressing different Lewis antigens⁶ 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: $\rightarrow 3\text{-}\alpha\text{-L-Rhap-(1}\rightarrow 3\text{)-}\alpha\text{-D-Rhap-(1}\rightarrow 2\text{)-}\alpha\text{-D-Manp3CMe-(1}\rightarrow (\text{A})$.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.

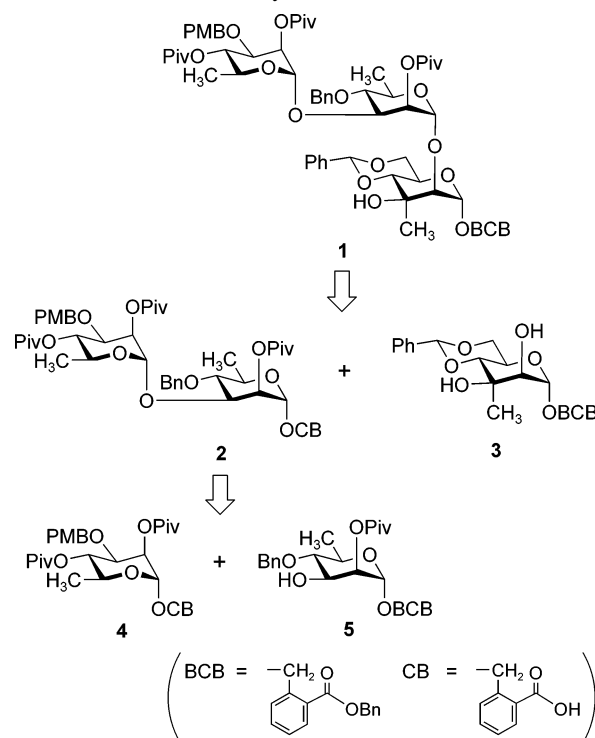
Due to its structural uniqueness and biomedical potential, the repeat unit **A** is an attractive synthetic target. Although a number of methods for the synthesis of Lewis antigens have been reported,⁹ the synthesis of the repeating unit **A** or compounds with the similar structure has not been reported. 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 -mannopyranosylation¹⁰ 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 methylide¹² to the C-3 carbonyl carbon of **8** afforded only the spiroepoxide **9**, which was reduced with LiAlH₄ 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-

(5) Raghavan, S.; Hjulstom, M.; Holgren, J.; Svennerholm, A.-M. *Infect. Immun.* **2002**, *70*, 6383–6388.

(6) (a) Lozniewski, A.; Haristoy, X.; Rasko, D. A.; Hatier, R.; Plenat, F.; Taylor, D. E.; Angioi-Duprez, K. *Infect. Immun.* **2003**, *71*, 2902–2906. (b) Appelmek, B. J.; Simmons-Smit, I.; Negrini, R.; Moran, A. P.; Aspinall, G. O.; Forte, J. G.; De Vries, T.; Quan, H.; Verboom, T.; Maaskant, J. J.; Ghiara, P.; Kuipers, E. J.; Bloemena, E.; Tadema, T. M.; Townsend, R. R.; Tyagarajan, K.; Crothers, J. M.; Monteiro, M. A.; Savio, A.; Graaff, J. D. *Infect. Immun.* **1996**, *64*, 2031–2040.

(7) (a) Keenan, J. I.; Rijpkema, S. G.; Durrani, Z.; Roake, J. A. *FEMS Immun. Med. Microbiol.* **2003**, *36*, 199–205. (b) Keenan, J.; Oliaro, J.; Domigan, N.; Potter, H.; Aitken, G.; Allardyce, R.; Roake, J. *Infect. Immun.* **2000**, *68*, 3337–3343.

(8) Kocharova, N. A.; Knirel, Y. A.; Widmalm, G.; Jansson, P.-E.; Moran, A. P. *Biochemistry* **2000**, *39*, 4755–4760.

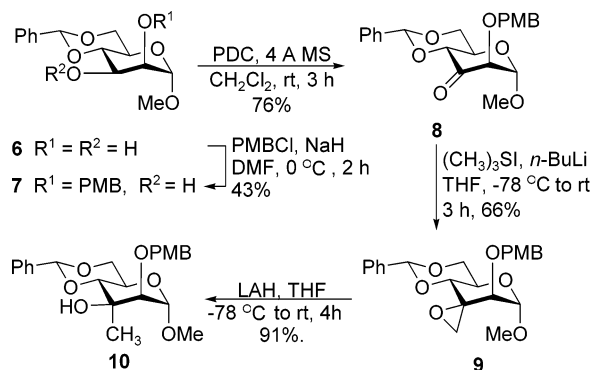
(9) (a) Vankar, Y. D.; Schmidt, R. R. *Chem. Soc. Rev.* **2000**, *29*, 201–216. (b) Brocke, C.; Kunz, H. *Bioorg. Med. Chem.* **2002**, *10*, 3085–3112.

(10) (a) Kim, K. S.; Kim, J. H.; Lee, Y. Joo; Lee, Y. Jun; Park, J. J. *Am. Chem. Soc.* **2001**, *123*, 8477–8481. (b) Kim, K. S.; Park, J.; Lee, Y. J.; Seo, Y. S. *Angew. Chem., Int. Ed.* **2003**, *42*, 459–462.

(11) Kim, K. S.; Kang, S. S.; Seo, Y. S.; Kim, H. J.; Lee, Y. J.; Jeong, K.-S. *Synlett* **2003**, 1311–1314.

(12) Corey, E. J.; Chaykovsky, M. J. *J. Am. Chem. Soc.* **1965**, *87*, 1353–1364.

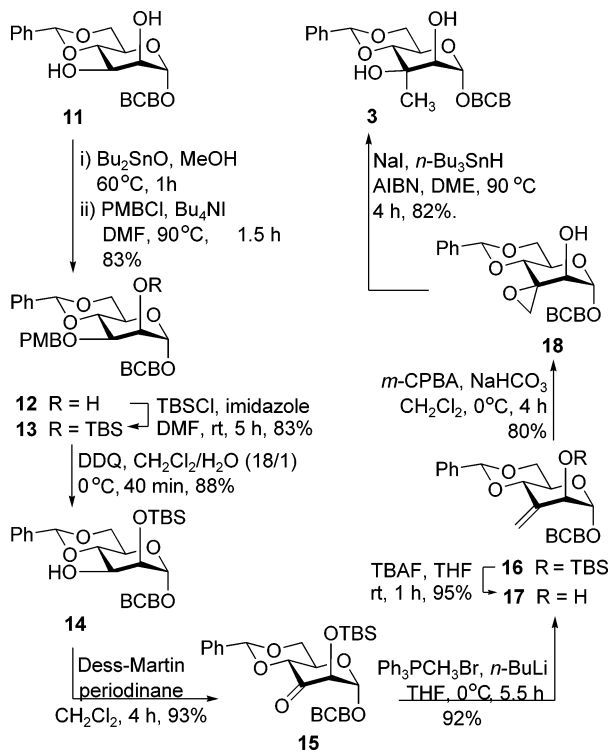
Scheme 2. Model Study for the Synthesis of 3-C-Methylmannoside



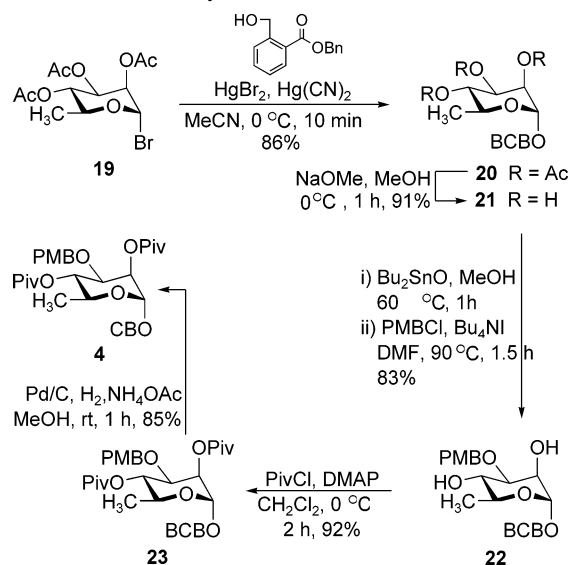
O-benzylidenemannoside **11**^{10a} (Scheme 3). Treatment of **11** with Bu_2SnO in refluxing methanol followed by reaction of the resulting crude *O*-stannylene acetal¹³ with PMB chloride in the presence of Bu_4NI in DMF afforded the C-3 PMB ether **12** in 78% yield for the two steps with complete 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 periodinane¹⁴ 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 DME¹⁵ 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 triacetyl-L-rhamnosyl bromide **19** into BCB triacetyl-L-rhamnoside **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 $60^\circ C$ 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

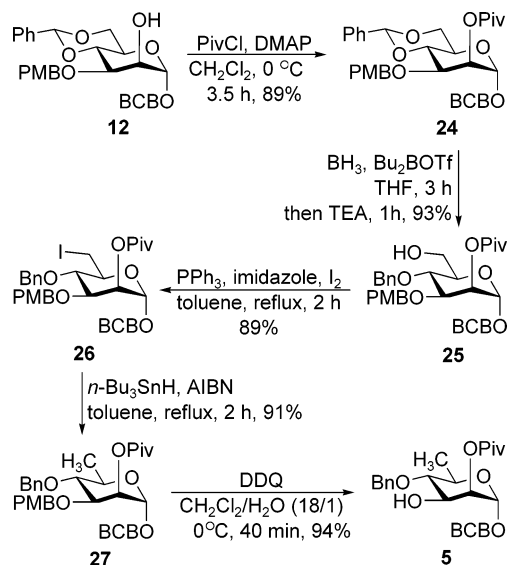
(13) For a review on stannylene acetals of carbohydrates, see: Grindley, T. B. *Adv. Carbohydr. Chem. Biochem.* **1998**, *53*, 17–142.

(14) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155–4156.

(15) Bonini, C.; Fabro, R. D. *Tetrahedron Lett.* **1988**, *29*, 819–822.

(16) Jiang, L.; Chan, T.-H. *Tetrahedron Lett.* **1998**, *39*, 355–358.

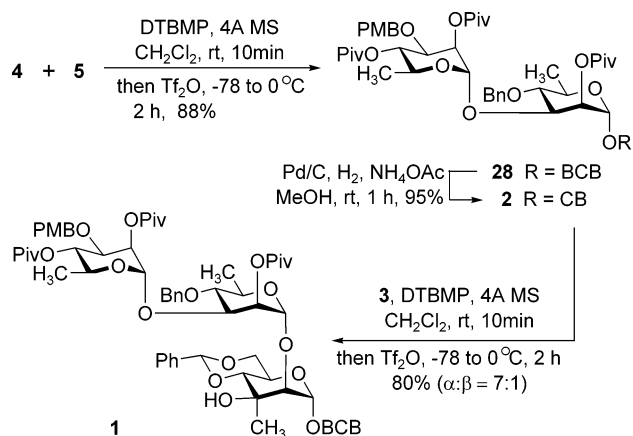
Scheme 5. Synthesis of BCB D-Rhamnoside **5**



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 $-78\text{ }^\circ\text{C}$ followed by warming to $0\text{ }^\circ\text{C}$; 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 $-78\text{ }^\circ\text{C}$. Warming the reaction mixture to $0\text{ }^\circ\text{C}$, 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

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



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: $^1J_{C1'-H1'} = 171.1\text{ Hz}$ in compound **2** and $^1J_{C1'-H1'} = 170.8\text{ Hz}$ 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.

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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.