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CHEMICAL APPROACHES TO BACTERIAL VACCINES. SYNTHESIS OF MYCOBACTERIAL OLIGOSACCHARIDE-PROTEIN CONJUGATES FOR USE AS SERODIAGNOSTICS AND IMMUNOGENS

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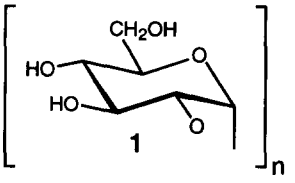
Abstract: Di- to penta-saccharide fragments (**2–5**) of Polysaccharide II (PS-II) of *Mycobacterium tuberculosis* were synthesized in spacer-linked form in a stepwise fashion using a new glycosyl donor featuring a *trans*-fused isopropylidene diol-protecting group. Covalent attachment of the oligosaccharides to proteins provides semi-synthetic antigens and immunogens which are being used to probe the role of PS-II as a possible mycobacterial antigen. Published by Elsevier Science Ltd

INTRODUCTION

Tuberculosis caused by *Mycobacterium tuberculosis* continues to be a major public health problem worldwide causing three million deaths annually, which exceeds the death-toll of any other single infectious pathogen.[1] While preventive public health measures appeared to control this disease, recent epidemiologic studies identify several problems.[2] First, multiple drug-resistant strains are emerging. Second, the direct transmission of the disease is sharply increasing and currently represents about one-third of the new cases.[3] This is especially alarming among children under four years of age.[4] Third, patients infected by the human immunodeficiency virus are increasingly susceptible to infection by *M. tuberculosis*. Prevention by vaccination could be an alternative to treatment with isoniazide, which is still the most successful therapeutic agent to treat tuberculosis. However, the efficacy of the Bacillus Calmette-Guérin (BCG) vaccine, which is the only vaccine available against tuberculosis, is controversial.[3] Most of the vaccine studies reported so far in this area focused on the immune responses to the soluble, secreted protein antigens of *M. tuberculosis*.[4]

BACKGROUND

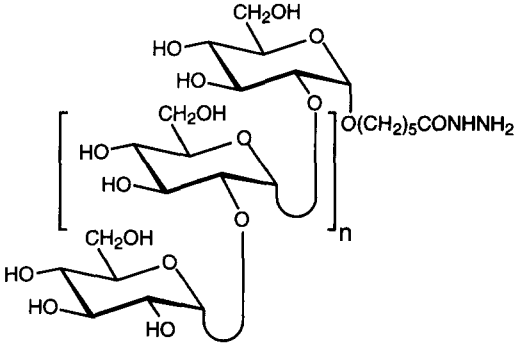
We are studying mycobacterial polysaccharides as protective antigens and we focus our attention on Polysaccharide II (**1**) of *M. tuberculosis*[5] that was proposed by Kent to be a linear polymer of D-glucose residues connected by α-(1→2) linkages.[6] Defined fragments of this unique polysaccharide may be used as diagnostics for the detection of antibodies directed against α-(1→2)-linked gluco-oligosaccharides (koji-oligosaccharides) and



also, as haptens, to induce anti-koji-oligosaccharide antibodies after their covalent attachment to immunogenic macromolecules. Earlier, we showed[7] that a stepwise strategy can be used successfully for the construction of oligosaccharide fragments corresponding to **1**, up to a pentasaccharide.

RESULTS

Here we describe an improved iterative synthesis of the koji-oligosaccharides **2–5** equipped with a hydrazido spacer. These compounds were assembled in a stepwise fashion using the direct strategy, whereby the initial glycosylation reaction is the attachment of the reducing-end unit to the linker moiety.* We also report the use of these compounds for the preparation of neoglycoproteins.

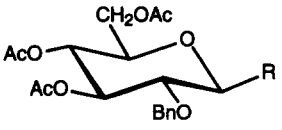


2 n = 0
3 n = 1
4 n = 2
5 n = 3

The glycosyl donors

Previously, we used 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-β-D-glucopyranosyl bromide[7] (**6**) and chloride[7] (**7**) as the building blocks that were coupled to the acceptor moiety by either non-classical halide-ion catalysis[8] or by silver salt-assisted reactions. The erratic yields in the glucosylation steps requiring the use of a large excess of the glycosyl donor for saccharides larger than a disaccharide prompted us to investigate alternative protecting group scenarios. Surprisingly, replacement of the *O*-acetyl protecting groups in the acceptor moiety by *O*-benzyls,

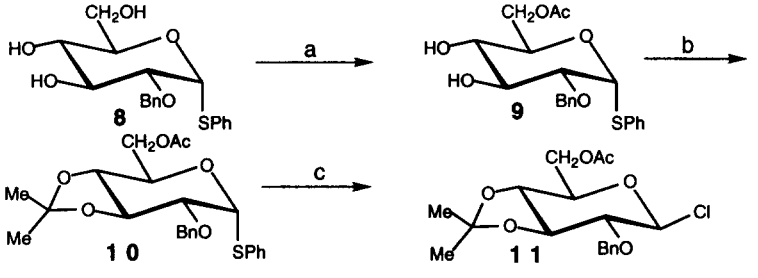
*An inverse strategy in which the target oligosaccharide is first assembled on a temporary aglycon is also possible. However, unavoidable losses during the replacement of the temporary aglycon by a leaving group followed by coupling with the spacer molecule make the inverse strategy prohibitive beyond a short oligosaccharide. Furthermore, the component saccharides can not be accessed in spacer-linked form.



6 R=Br
7 R=Cl

which are known to increase the nucleophilicity of the acceptor OH groups, did not improve this situation.[9] The steric demands of the aromatic rings may offer a plausible explanation for this finding.[10] Eventually, we designed the cyclic acetal-protected β chloride **11**[11] as the key building block in which the steric requirement of the protecting groups at O-3 and O-4 is at a minimum. Moreover, we hoped, that the acetal moiety may cause the pyranose ring to adapt a conformation that is more favorable than the normal $^4C_1$ chair conformation for the glycosylation. The precursor was the thioglucoside[7] **8** which was regioselectively acetylated at O-6 with a limited amount of acetyl chloride to give **9**[11] (Scheme 1.). Reaction of the *trans*-diequatorial diol **9** with 2-methoxypropene afforded the crystalline thioglucoside **10**[11] containing a cyclic acetal moiety.[12] We have previously presented evidence that treatment of a 1-thio α-glucopyranoside having a benzyl protecting group at O-2 with chlorine affords the β chloride exclusively.[7] Indeed, chlorination of **10** proceeded stereoselectively to give chloride **11*** in a quantitative yield (^{1}H NMR).[13]

Scheme 1.



Reagents and conditions: (a) 1.2 equiv of AcCl, 1.5 equiv of s-collidine, CH_2Cl_2, 23 °C, 24 h, 68%; (b) $CH_3C(OCH_3)=CH_2$ (excess), CSA (cat), CH_2Cl_2, 23 °C, 1 h, 88%; (c) Cl_2 (excess) in CCl_4, CH_2Cl_2, 23 °C, 1 h, quant.

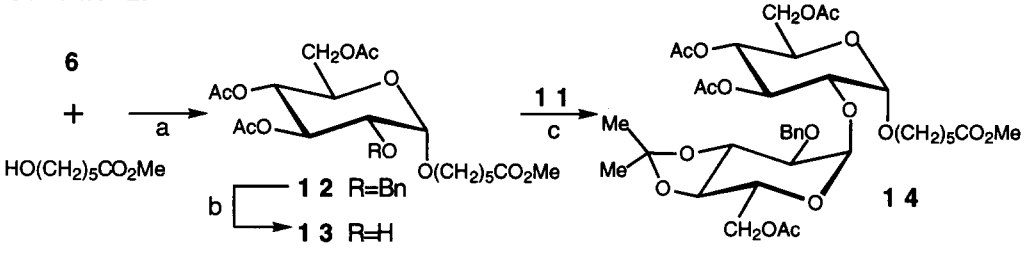
Assembly of the oligosaccharides

The β bromide[7] **6** was chosen as the reducing-end unit and was coupled with the heterobifunctional spacer 5-methoxycarbonylpentanol[15] under the conditions of non-classical halide-ion catalysis[8] to afford **12**[11] in 79% yield. Hydrogenolysis yielded the alcohol **13**[11] that was glucosylated with the chloride **11** to give the disaccharide **14**.[11] Subsequent iterations of the hydrogenolytic debenzylation and the glycosylation step with **11** afforded the protected tri- to penta-saccharides **15–20**. We note that removal of the *O*-benzyl group from the intermediates was performed in the presence of a base to avoid the loss of the labile isopropylidene group. The glycosylation steps

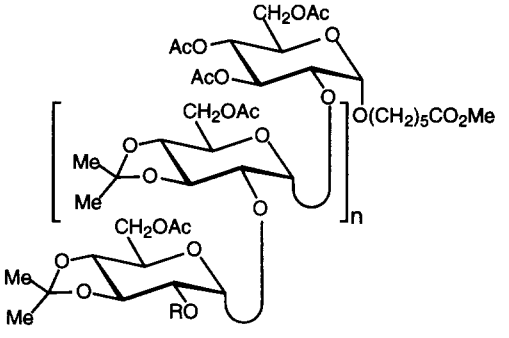
* Compound **11** appears to be the first glycosyl donor with a *trans* isopropylidene acetal moiety on a vicinal diol system.[14]

used the donor **11** in a 1.5 to 2.5-fold molar excess and proceeded in 75–89% isolated yields of the target anomer, thus representing a marked improvement over the previous protocol.[7] The blocking group scheme also allowed efficient removal of the protecting groups. For example, hydrogenolytic removal of the benzyl groups in EtOH/AcOH was accompanied by complete cleavage of the isopropylidene groups. The acetyl protecting groups were removed by the Zemplén protocol[16] which left the terminal methoxycarbonyl moiety unaffected. The target hydrazides **2–5**[11] were obtained from the methyl ester precursors using hydrazine hydrate in ethanol in 75–85% yields.

Scheme 2.



Reagents and conditions: (a) 10 equiv of $HO(CH_2)_5COOMe$, 1.4 equiv of Et^iPr_2N, CH_2Cl_2, 23 °C, 24 h, 79%; (b) H_2/Pd-C, EtOH, AcOH, 23 °C, 24 h, 92%; (c) 1.5 equiv of **11**, 3.75 equiv of CF_3SO_2OAg, 3.25 equiv of 2,6-di-*tert*-butyl-4-methylpyridine, CH_2Cl_2, 4 A molecular sieves, 0 °C, 30 min, 89%.



15 n=0 R=H **18** n=2 R=Bn
16 n=1 R=Bn **19** n=2 R=H
17 n=1 R=H **20** n=3 R=Bn

Attachment of the oligosaccharides to protein carriers

The hydrazinocarbonyl moiety is a versatile anchoring device that allows attachment either to the protein's carboxyl groups using a water-soluble carbodiimide[17] or to its amino groups, through a highly reactive acyl azide intermediate.[18] We used both protocols and found that while the carbodiimide procedure gave lower levels of incorporation, it allowed an almost complete recovery of the unbound hapten. Typically, oligosaccharide-human and bovine serum albumin conjugates containing less than 5% saccharide (by weight) were obtained with the carbodiimide method using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide as the condensing agent at pH 5. The

modified acyl azide procedure according to Pinto and Bundle[19] afforded neoglycoproteins containing up to 10 oligosaccharide chains.

In summary, we have demonstrated that the five-membered cyclic acetal-protected glucopyranosyl donor **11** can be used successfully in an iterative manner to assemble extended, α-1,2-linked gluco-oligosaccharides in heterobifunctional spacer-equipped form. The use of a *trans*-fused isopropylidene group on the vicinal 3,4-diol system in **11** proved advantageous over conventional blocking group schemes and also allowed mild deprotection at these sites. Neoglycoproteins containing oligosaccharides **2–5** have been synthesized and characterized. Immunochemical experiments with the synthetic koji-oligosaccharides to probe the role of Polysaccharide II of *M. tuberculosis* as a possible mycobacterial carbohydrate antigen are the subject of active investigation and will be reported elsewhere.[20]

ACKNOWLEDGMENT

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Hz, anomeric protons), δ_C 176.5 (*C*=O), 96.2, 95.6, 93.7 (3C) (anomeric carbons); **9** δ_H 6.15 (d, *J* 5.1 Hz, H-1), 2.06 (s, C*H*$_3$CO), δ_C 171.6 (*C*=O), 86.4 (C-1), 20.8 (*C*H$_3$CO); **10** δ_H 5.67 (d, *J* 4.9 Hz, H-1), 2.06 (s, C*H*$_3$CO), 1.48, 1.46 [2 s, (C*H*$_3$)$_2$C], δ_C 170.5 (*C*=O), 111.5 [*C*(CH$_3$)$_2$], 87.2 (C-1), 26.8, 26.6 [(*C*H$_3$)$_2$C], 20.7 (*C*H$_3$CO); **11** δ_H 7.50-7.06 (m, 5 H, aromatic), 5.32 (d, 1 H, $J_{1,2}$ 6.1 Hz, H-1), 3.82 (dd, $J_{2,3}$ 9.2 Hz, H-2), 2.10 (s, C*H*$_3$CO), 1.465, 1.460 [2 s, (C*H*$_3$)$_2$C]; **12** δ_H 7.40-7.25 (aromatic), 5.43 (dd, 1 H, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 9.6 Hz, H-3), 4.96 (dd, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 4.76 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 2.07, 2.02, 2.00 (3 s, 3 C*H*$_3$CO), δ_C 96.8 (C-1), 51.5 (*C*H$_3$O); **13** δ_H 5.22 (dd, 1 H, $J_{2,3}$ 9.6 Hz, $J_{3,4}$ 9.8 Hz, H-3), 5.01 (dd, 1 H, $J_{4,5}$ 10.0 Hz, H-4), 4.91 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 2.09, 2.08, 2.04 (3 s, 3 C*H*$_3$CO), δ_C 98.2 (C-1), 51.7 (*C*H$_3$O); **14** δ_H 7.36-7.26 (aromatic), 5.44 (t, 1 H, H-3$_A$), 4.98, 4.94 (2 d, 2 H, *J* 3.2-3.9 Hz, anomeric protons), 3.66 (C*H*$_3$O), 2.11-2.02 (m, 12 H, 4 C*H*$_3$CO), δ_C 170.5-169.8 (*C*=O), 138.0-127.8 (aromatic), 111.1 [*C*(CH$_3$)$_2$], 98.1, 96.4 (anomeric carbons), 51.5 (*C*H$_3$O), 26.9 and 26.4 [(*C*H$_3$)$_2$C].

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