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Note

Synthesis of a tetrasaccharide related to the O-antigen from *Azospirillum lipoferum* SR65

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ABSTRACT

Concise synthesis of a tetrasaccharide repeating unit of the LPS isolated from *Azospirillum lipoferum* SR65 has been accomplished through suitable protecting group manipulations and stereoselective glycosylation starting from commercially available L-rhamnose and D-glucose. The target oligosaccharide in the form of its *p*-methoxyphenyl glycoside is suitable for further glycoconjugate formation via selective cleavage of the OMP glycoside. Plant growth-promoting bacteria (PGPB) of genus *Azospirillum* plays important roles in the growth and development of plants. The interaction between the roots of the plants and the microbes is governed by the cell surface carbohydrate polymers (CPS, LPS, etc.). The present synthetic-based study elucidates aspects of plant-microbe interaction and future biofertiliser design.

The Gram-negative nitrogen-fixing soil bacteria of genus *Azospirillum* are known as plant growth-promoting bacteria (PGPB) as they secrete many active substances that play vital roles in plant growth and development.[1] It is clear that these bacteria have a high adaptation potential and therefore are promising for application as biofertilizers. Cell surface carbohydrate polymers such as EPS, CPS and LPS play important roles for the survival of the bacteria in adverse environmental conditions as well as they regulate the interaction with the roots of plants.[1] Literature reports indicate that the LPS of the *Azospirillum* outer membrane are involved in the formation of bacterial association with the roots of cereals; for example, mutants defective in LPS synthesis are worse adsorbers to wheat roots[2] and worse colonizers to maize roots[3] compared to their non-defective counterparts. Although the bacteria of the genus *Azospirillum* have been used as model to study associative plant-microbe relationship, only a few strains are studied so far. To get a better understanding of these plant-microbe interactions, synthetic studies on the LPSs will be useful. Recently, Fedonenko et al.[4] reported the structure of the LPS isolated from *Azospirillum lipoferum* SR65. Herein we report the total synthesis of the tetrasaccharide repeating unit (Fig. 1, **1**) of the LPS in the form of its *p*-methoxyphenyl glycoside. The choice of *p*-methoxyphenyl glycoside will enable us to conjugate the synthetic oligosaccharide with suitable aglycon, when needed.

Synthesis of the tetrasaccharide (**1**) was started with the synthesis of suitably protected L-rhamnose and D-glucose synthons followed by step-wise glycosylation and de-protection. Therefore, known *p*-methoxyphenyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**2**)[5] was benzylated using BnBr in the presence of NaH[6] to afford the corresponding benzylated derivative **3** in 87% yield. Hydrolysis of the isopropylidene acetal using 80% AcOH at 80 °C[7] followed by selective benzylation of the 2-OH position[8] using phase transfer catalyst furnished the required acceptor, *p*-methoxyphenyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside (**5**) in

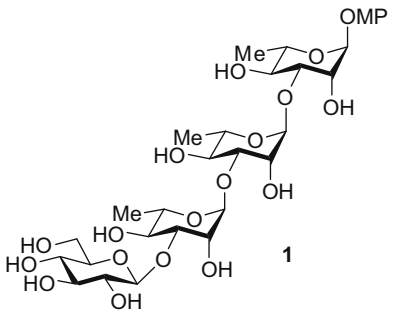

Figure 1. Structure of the target tetrasaccharide.

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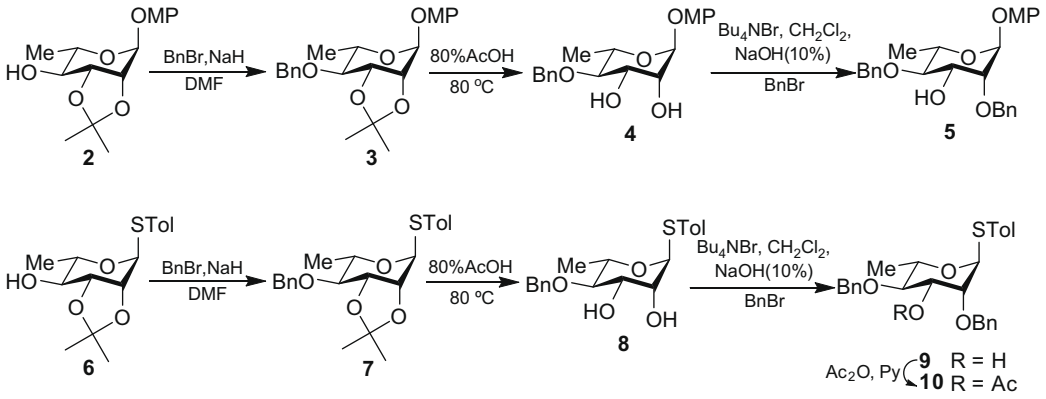
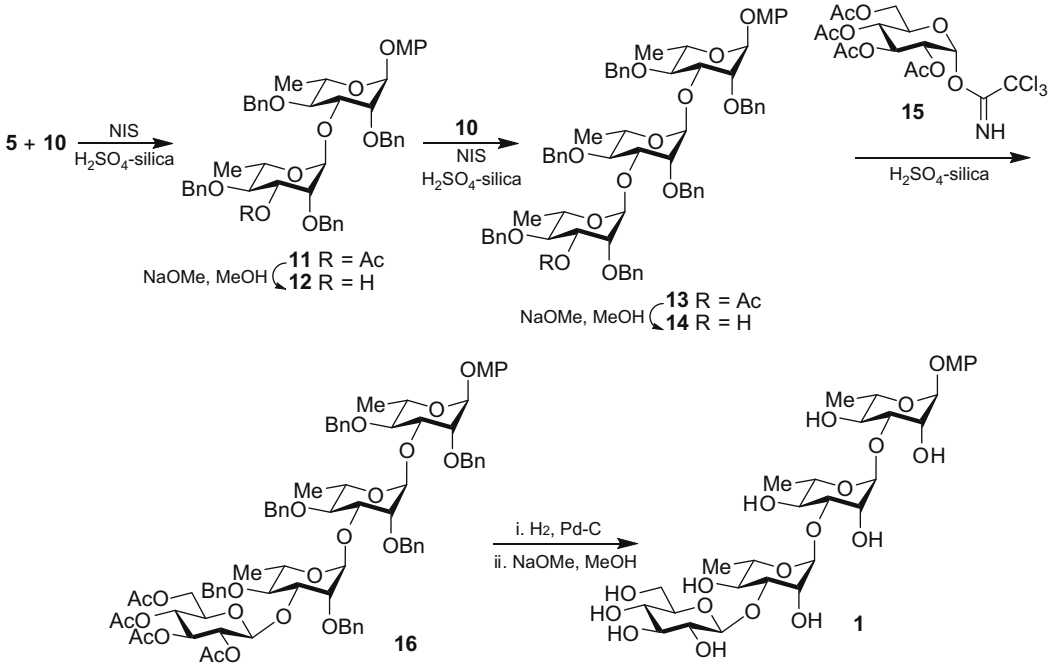


Scheme 1. Synthesis of the monosaccharide acceptor **5** and donor **10**.

78% yield over two steps. Subjected to the same benzylation-hydrolysis of the isopropylidene acetal and selective benzylation using phase transfer catalyst reaction sequence, known *p*-tolyl 2,3-*O*-isopropylidene-1-thio-α-L-rhamnopyranoside (**6**) afforded the protected derivative **9**. Compound **9** upon acetylation using Ac_2O in pyridine[9] furnished the required donor, *p*-tolyl 3-*O*-acetyl-2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**10**) in 94% yield (Scheme 1).

Glycosylation between rhamnosyl acceptor **5** and donor **10** was accomplished by using *N*-iodosuccinimide in conjunction with H_2SO_4-silica[10] to afford the disaccharide **11** in 87% yield. Use of H_2SO_4-silica as the activator of NIS was found to be beneficial over the use of traditional TfOH[11] or TMSOTf[12] as promoters. Glycosylation of acceptor **5** and donor **10** using TMSOTf and TfOH in conjunction with NIS resulted in 79% and 76% yield, respectively. The disaccharide **11** was reacted with NaOMe in MeOH to afford the disaccharide acceptor **12** in 89% yield. It was further glycosylated with rhamnosyl donor **10** following the same glycosylation strategy as mentioned above to furnish the trisaccharide **13** in 86% yield. NaOMe-catalyzed de-O-acetylation afforded the trisaccharide acceptor **14** in 94% yield. Up to this stage, activation of thioglycosides for the glycosylation reactions was satisfactory. But when we tried to couple the known *p*-tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside with the trisaccharide acceptor **14**, it failed to afford the desired tetrasaccharide. Only the corresponding hemiacetal of the donor and the unreacted trisaccharide acceptor were recovered. Even the use of TMSOTf instead of H_2SO_4-silica failed to produce the desired result. Anticipating the lesser reactivity of thioglycoside donor, we used the known 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate[13] and it gave the desired tetrasaccharide **16** in 78% yield upon activation of trichloroacetimidate by H_2SO_4-silica.[14] The protected tetrasaccharide subjected to catalytic hydrogenation using Pd–C followed by treatment with NaOMe in MeOH afforded the target tetrasaccharide **1** (Scheme 2).

In conclusion, synthesis of the tetrasaccharide repeating unit of the LPS isolated from *A. lipoferum* SR65 has been accomplished. Since the protecting group manipulation strategies and glycosylation steps were selective and high-yielding, the present synthetic strategy is capable of reasonably large-scale preparation. The syn-



Scheme 2. Synthesis of the tetrasaccharide **1**.