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Synthesis and plant growth regulation activity of α-D-Man*p*NAc-(1→2)-[α-L-Rha*p*-(1→3)-]α-L-Rha*p*-(1→4)-β-D-Glu*p*NAc-(1→3)-α-L-Rha*p*, the repeating unit of O-antigen of *Rhizobium trifolii 4s*

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ABSTRACT

The synthesis of a pentasaccharide **2** containing acetamido-2-deoxy-D-glucose and acetamido-2-deoxy-D-mannose related to the cell wall polysaccharide of *Rhizobium trifolii 4s* has been achieved by a [2+3] approach from commercially available L-rhamnose, D-glucose, and D-glucosamine as the starting materials. The target molecule was equipped with a *p*-methoxylphenyl handle at the reducing terminus to allow for further glycoconjugate formation via selective cleavage of this group. The bioassay suggested that the synthetic pentasaccharide **2** can stimulate the growth of wheat coleoptile similarly to indole-3-acetic acid (IAA), and promote the wheat seedling development before winter by seed treatment at a concentration of 20 mg/L.

1. Introduction

Overuse of chemical fertilizer to maintain good harvest has posed a threat to our environment and also the country's food security in the past decades. In fact, some plants may not need so much chemical fertilizer, as they and their specific symbiotic bacteria of genus *Rhizobium* can combine nitrogen gas with other elements to form useful nitrogen compounds.[1] The use of chemical fertilizer will be greatly reduced if plants have a good use of biological nitrogen fixation. The lipopolysaccharides (LPSs) of *Rhizobium* are crucial to the bacteroid development and nodule occupancy. Bacteria having defects in LPSs structure also have defects in nodule invasion in plant symbiosis.[2–4] Presenting at the distal part of LPSs, the O-antigenic polysaccharides (OPSs) are in direct contact with the environment, and are closely involved in the process of exchanging signals between rhizobia and legumes.[5] Mutants with LPSs that lack their OPSs or have modified core components either are defective in the formation of infection threads or have the nodules unoccupied.[6–9] With the aim of investigating the biological roles of these OPSs, synthetic studies on the OPSs will be useful. Wang et. al. reported that the structure of OPS of *Rhizobium trifolii 4s* was constituted of a pentasaccharide repeating unit composed of L-rhamnose, *N*-acetyl-D-glucosamine, and *N*-acetyl-D-mannosamine in 3:1:1 molar proportion (Fig. 1, **1**).[10]

To obtain new oligosaccharide resources and discover novel bioactive substances, we reported the first total synthesis of the pentasaccharide repeating unit (Fig. 1, **2**). Moreover, the biological effect of the synthesized pentasaccharide on stimulating plant growth has been determined.

2. Results and discussion

The design of the synthesis of the pentasaccharide **2** is outlined in Scheme 1. Retrosynthetic analysis indicated that the pentasaccharide **2** could be achieved through a convergent strategy involving [2+3] glycosylation of a disaccharide acceptor **20** and a trisaccharide donor **11**. The trisaccharide **11** then could be constructed from the α-(1→2)-linked disaccharide acceptor **8** and the rhamnosyl donor **5**, while the disaccharide acceptor **20** could be built from the rhamnosyl acceptor **13**[11,12] and the glucosaminyl donor **12**.[13]

A number of suitably functionalized monosaccharide intermediates **4**,[14] **5**,[15] **6**,[11,12] **12**,[13] and **13**[11,12] were prepared from the commercially available reducing sugars, such as L-rhamnose, D-glucose, and D-glucosamine, using the previously reported reaction conditions. Compound **4**[14] was prepared in 77% yield from compound **3**[16] using a two-step sequence involving selective de-1-O-aetylation followed by trichloroacetimidate formation (Scheme 2).

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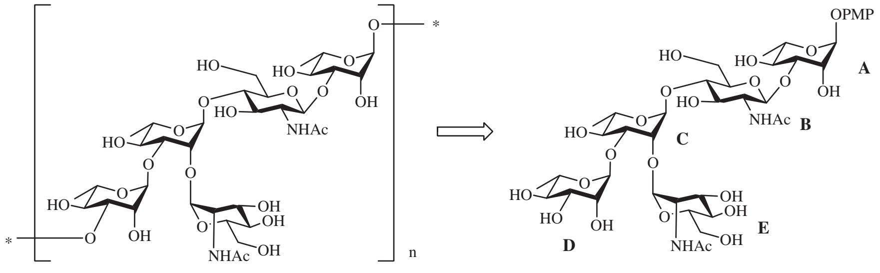


1 **2**

Figure 1. O-Antigenic chain repeating unit of LPSs (**1**) from *Rhizobium trifoki 4s* and the synthesized oligosaccharide **2**.

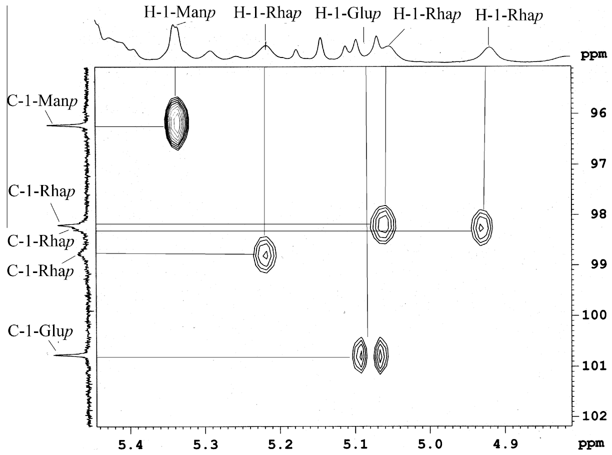
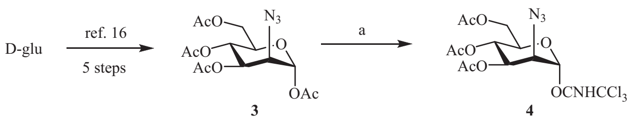
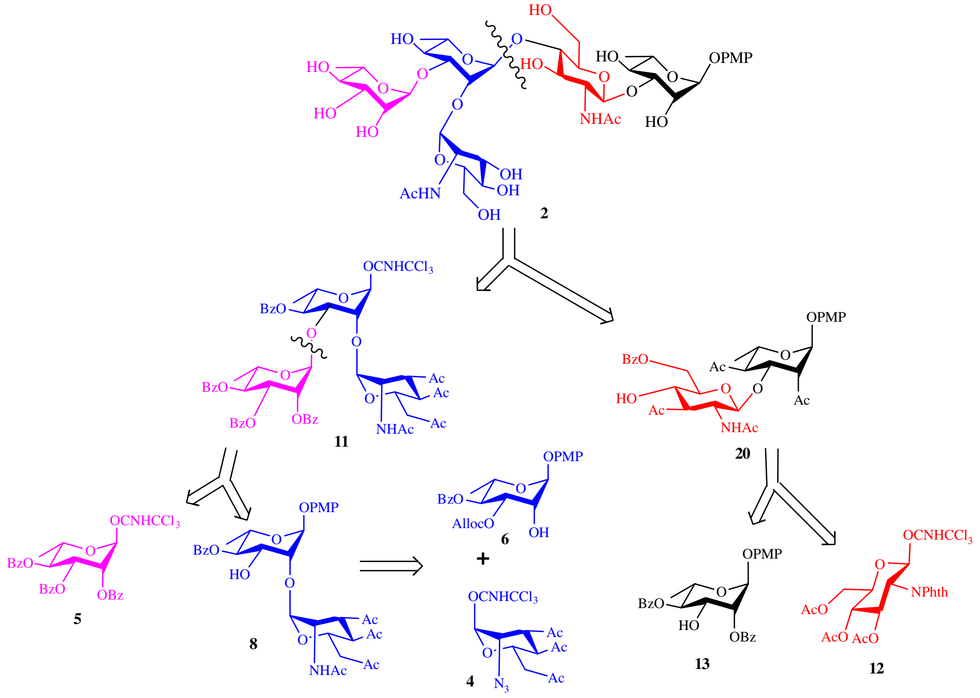


Figure 2. Partial HSQC spectra of the fully protected pentasaccharide **21**.



Scheme 2. Synthesis of key synthon **4**. Reagents and conditions: (a) $BnNH_2$, THF, rt, 12 h; then CCl_3CN, DBU, CH_2Cl_2, rt, 0.5 h, 77% over two steps for **4**.

Synthesis of trisaccharide **11** was shown in Scheme 3. Glycosylation between rhamnosyl acceptor **6** and 2-acetamido-2-deoxy-α-D-mannosyl donor **4** was accomplished by using TMSOTf as the catalyst in the presence of 4 Å molecular sieves to afford the disaccharide **7** in 86% yield. Deallyloxycarbonylation of **7** was successfully achieved in MeOH–THF[17] in the presence of CH_3COONH_4, $Pd[P(C_6H_5)_3]_4$, and $NaBH_4$, within 4 min without affecting any of the other protecting groups, giving the desired acceptor **8** in 92% yield. Formation of compound **8** was supported by its spectral analysis [signals at 5.45 ppm (d, J = 1.4 Hz, H-1) and 5.06 ppm (d, J = 1.4 Hz, H-1′) in the ^{1}H NMR, and 97.4 and 96.3 ppm (2× C-1) in the ^{13}C NMR spectra]. The coupling reaction between trichloroacetimidate **5** and acceptor **8** by using TMSOTf as the catalyst smoothly yielded trisaccharide **9**. The formation of compound **9** was confirmed by its ^{13}C NMR spectrum [signals at 98.7, 96.3 and 95.8 ppm (3× C-1) corresponding to the three anomeric carbons]. Hydrogenolysis of compound **9** followed by N-acetylation



Scheme 1. A retrosynthetic strategy for the synthesis of the target pentasaccharide **2**.