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Chemo-enzymatic synthesis of allyl penta-*N*-acetyl-chitopentaose

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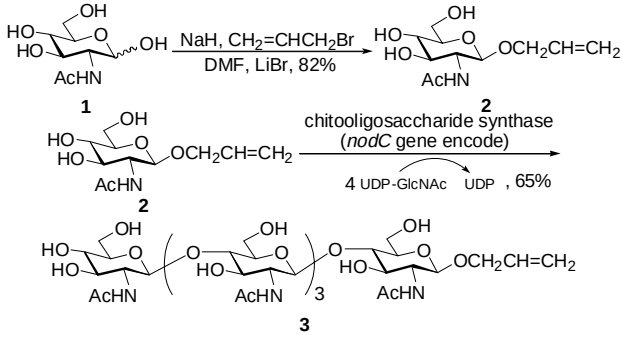
Abstract—Cell density cultivation of recombinant *Escherichia coli* strains harboring the *nodC* gene (encoding chitooligosaccharide synthase) from *Azorhizobium caulinodans* has been previously described as a practical method for the preparation of gram-scale quantities of penta-*N*-acetyl-chitopentaose. We have now extended this method to the production of allylated derivative of penta-*N*-acetyl-chitopentaose by using allyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**2**) as the initial acceptor for the synthesis of target pentaoside in vivo.

It has already been shown that *Escherichia coli* cells overproducing the recombinant proteins NodC (i.e., *N*-acetylglucosaminyltransferase) accumulated high intracellular concentrations of penta-*N*-acetyl-chitopentaose.[1] It has furthermore recently been proved that the synthesis of chitin oligosaccharides by NodC proceeds by the addition of GlcNAc residues from the donor UDP-GlcNAc to O-4 of the nonreducing-terminal residue of the growing chain.[2] The enzyme is able to use free GlcNAc as acceptor. The fact that allyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**2**) as starting sugar can be used with recombinant *E. coli* strains expressing NodC gene in vitro has already been described.[3] We herein describe the synthesis of allyl penta-*N*-acetyl-chitopentaose by using **2** as acceptor and a batch culture of *E. coli* strain BL21 containing a plasmid carrying the cloned *nodC* gene from *Azorhizobium caulinodans*.

2-Acetamido-2-deoxy-D-glucose **1** and LiBr (2 equiv) were suspended in DMF (1 mL per mmol of **1**) and successively treated with sodium hydride (1.3 equiv) and allyl bromide (3 equiv) at room temperature for 3 h. The crude product was directly purified by flash chromatography (4:1, EtOAc/MeOH), and the β-allyl glycoside **2**[4] was obtained in 82% yield (Scheme 1). Addition of LiBr



Scheme 1. The synthetic route of **3**.

can notably improve the stereoselectivity of the glycosylation of **1**. This one-step glycoside synthesis compares well with the standard peracetylation, selective anomeric deprotection, O-alkylation at C1, and deacetylation steps.[5]

Cell density cultures were carried out as previously described[1] in 2 or 10 L bioreactors containing an initial culture volume of 1 or 7 L, respectively. Compound **2** was added to culture system. The *E. coli* strain BL21(DE3) containing a plasmid carrying the cloned *nodC* gene from *A. caulinodans* strain E1R[6] was used as the source of NodC protein. The culture time lasted 48 h.

After centrifugation of the culture broth, chitooligosaccharide was recovered exclusively in the pellet containing

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the bacterial cells. After disruption of the cells by boiling, cell debris was removed by centrifugation and the chitooligosaccharide was purified by activated charcoal adsorption and aq ethanol (55%, v/v) elution. The crude product was further purified by size exclusion chromatography on Biogel P2. The NMR and MS data confirmed the identification of the obtained sample as target pentaoside **3** (for details, see Supporting information). The yield was 65%. Therefore, **2** containing the hydrophobic allyl group linked to C-1 is acceptor for NodC in vivo. The most obvious explanation for this phenomenon is that the allylated derivative **2** of GlcNAc does not influence the binding affinity of NodC for the oligosaccharide intermediate and therefore leads to an elongation with additional GlcNAc units.

As seen from the above-mentioned synthetic route, it demonstrates the possibility of using recombinant technology for the production of high-value substituted oligosaccharides in vivo by using the derivatives of monosaccharides as the initial acceptors that are involved in the introduction of the substitutions.

Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.12.055.

References and notes

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