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Synthesis of O- and C-glycosides derived from β-(1,3)-D-glucans

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ABSTRACT

A series of β-(1,3)-D-glucans have been synthesized incorporating structural variations specifically on the reducing end of the oligomers. Both O- and C-glucosides derived from di- and trisaccharides have been obtained in good overall yields and with complete selectivity. Whereas the O-glycosides were obtained via a classical Koenigs–Knorr glycosylation, the corresponding C-glycosides were obtained through allylation of the anomeric carbon and further cross-metathesis reaction. Finally, the compounds were evaluated against two glycosidases and two *endo*-glucanases and no inhibitory activity was observed.

1. Introduction

Fungal cells possess a thick cell-wall mainly constituted by a linear chain of β-(1,3)-D-glucan linked to chitin via β-(1,4) linkage with ca. 3–4% of interchain β-(1,6) glucosidic bonds (Fig. 1).[1] Synthesis, degradation, and remodeling of polysaccharides forming the fungal cell wall are dynamic processes important for growth, budding, branching, or cell lysis.[2] The major component of the cell wall, β-(1,3)-D-glucan **1** units, are not present in human cells, and therefore the enzymes responsible for the synthesis and remodeling of fungal cell walls are suitable targets for the treatment of fungal infections. Among these enzymes β-(1,3)-D-glucan synthase (EC 2.4.1.34), that makes β-(1,3)-D-glucan from UDP-D-glucose, is one of the best drug targets.[3] Indeed, β-(1,3)-D-glucan synthase inhibitors have shown promising biological activities for the treatment of important fungal infections such as Candidiasis and Aspergillosis.[4]

In addition to β-(1,3)-D-glucan synthase, glucanases play an active role in the metabolism of β-(1,3)-D-glucans.[5] In this context, the activities of three endo-β-(1,3) glucanases that exclusively hydrolyze linear β-(1,3)-D-glucans have been recently studied.[6] Similarly, β-(1,3)-D-transglycosylases are also important enzymes in fungal cell wall assembly and rearrangement.[7] These enzymes are classified as GH72 family in the CAZy database and are named differently depending on the organism from which they come from.[8] These proteins have been also shown to be essential in organisms like *Aspergillus fumigatus* and *Schizosaccharomyces*

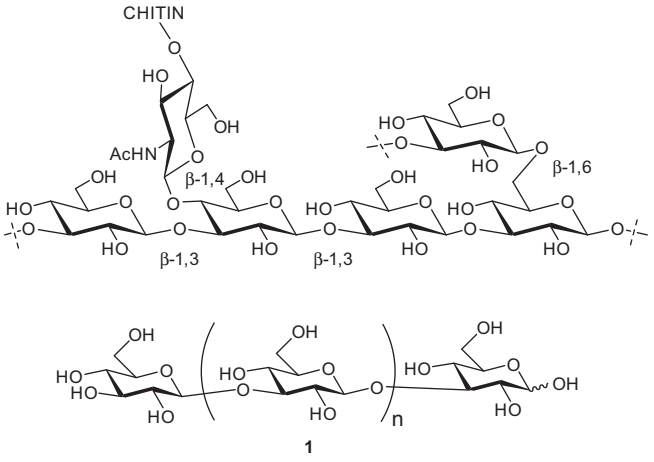

Figure 1. β-(1,3)-D-Glucans in the fungal cell wall.

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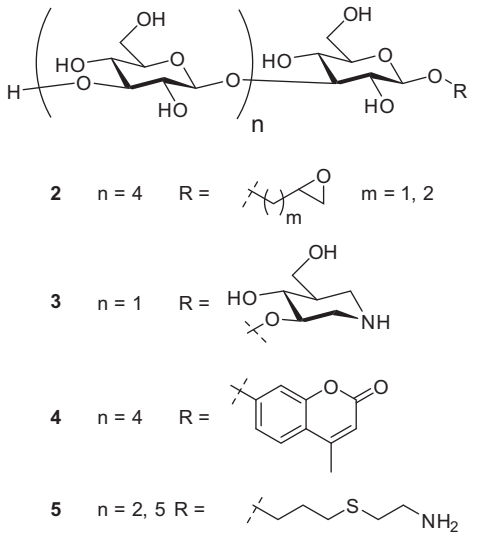


Figure 2. O-Glycoside analogues of β-(1,3)-D-glucans.

pombe suggesting that the development of inhibitors against them might be a new approach to tackle fungal diseases.[9]

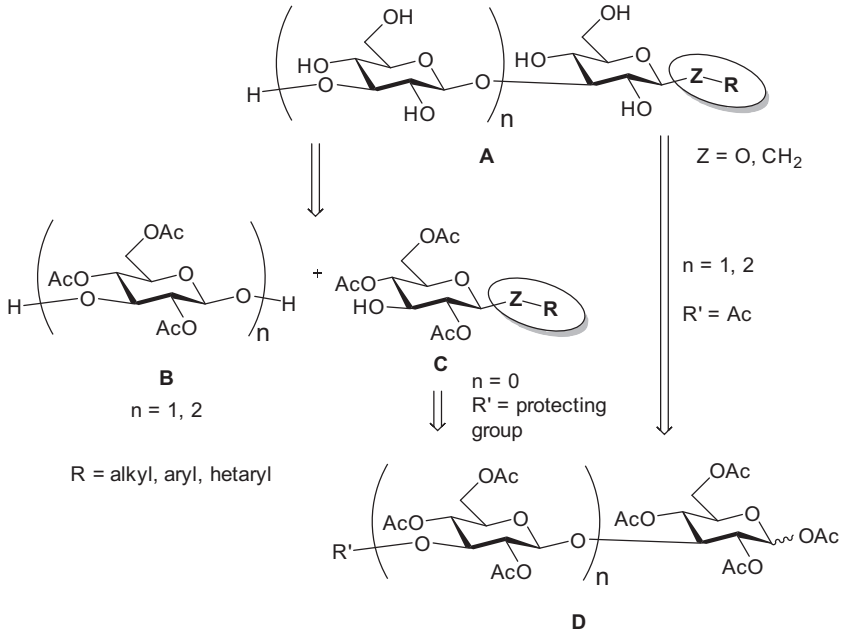
Other important aspects of β-(1,3)-D-glucans include the immunological and pharmacological effects of natural and synthetic analogues of this polysaccharide.[10] The synthesis of epoxyalkyl β-(1,3)-D-glucans **2** has attracted some attention owing to their implication in a variety of biological events including phagocytosis, hydrogen peroxide and citokine synthesis,[11] scavenging ability toward superoxide anion,[12] and as elicitors.[13] Stick and co-workers prepared β-(1,3)-di- and trisaccharides like **3** containing an isofagomine unit, which showed to be potent inhibitors of a β-(1,3)-glucan *endo*-hydrolase.[14] The 1-*O*-methylumbelliferyl β-(1,3)-D-pentaglucoside **4** has shown to be very stable toward fungal glucosidases with respect to the natural pentasaccharide (**1**, $n = 3$).[15] Very recently, Constantino and co-workers reported the synthesis of the β-(1,3)-glucan hexasaccharide **5** as a vaccine candidate against *Candida albicans* (see Fig. 2).[16]

In this paper we report an efficient synthesis of a variety of O- and C-glycosides related to laminaribiose and laminaritriose of general formula **A** (Scheme 1). The glycosides contain hydrophobic units at the reducing end of the oligosaccharides. The presence of a hydrophobic pocket in GH+1 subsite is well-known and it is admitted that additional mimicking of the transition state is required for inhibiting these enzymes.[17] However, in the case of compounds **A** the presence of additional carbohydrate units linked by 1,3-bonds can help to the recognition by the enzyme thus facilitating the interaction of the hydrophobic residue. Modified β-(1,3)-D-glucans **A** could also provide crucial information regarding the mechanism of action of the above mentioned enzymes, particularly transglycosylases which also have a hydrophobic pocket suitable of interacting with hydrophobic results.[8] Biological tests with a series of glycosidases and *endo*-glucanases are also reported.

2. Results and discussion

In principle, two approaches are possible for the synthesis of 1-O- and 1-C-glycosides **A**. Glycosylation of **B** with a suitable derivative **C**, in which the desired unit has been incorporated into the anomeric center could be considered a convergent approach (Scheme 1). Compound **C** should be prepared by the corresponding O- and C-glycosylation procedures from a D-glucose derivative **D** ($n = 0$, R′ = protecting group). This route, however, involves the regioselective protection of the first glucose unit at position 3 and two glycosylation reactions. On the other hand, the direct incorporation, by means of convenient O- and C-glycosylation reactions, of the hydrophobic residues at the anomeric center could be made in a straightforward way from peracetylated laminaribiose **D** ($n = 1$, R′ = Ac) and laminaritriose **D** ($n = 2$, R′ = Ac).

Even though both laminaribiose (**1**, $n = 0$) and laminaritriose (**1**, $n = 1$) are commercially available, the requirement of multigram quantities makes necessary to consider their preparation as peracetylated derivatives in multigram scale. The synthesis of laminaribiose and its peracetylated derivative has been reported through a typical Koenigs–Knorr condensation of two glucose units adequately functionalized.[18] Different protection with acetyl and benzyl groups in the two glucose moieties is also possible.[19] A large scale synthesis of laminaribiose has been optimized starting from



Scheme 1. Retrosynthetic analysis.