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4,6-Dimethoxy-1,3,5-triazine oligoxyloglucans: Novel one-step preparable substrates for studying action of endo-β-1,4-glucanase III from *Trichoderma reesei*

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ABSTRACT

Two kinds of 4,6-dimethoxy-1,3,5-triazine (DMT) oligoxyloglucans, DMT-β-XXXG and DMT-β-XLLG, have been synthesized via one-step procedure starting from the corresponding unprotected oligoxyloglucans in water. The resulting DMT derivatives were found to be hydrolyzed by endo-β-1,4-D-glucanase III from *Trichoderma reesei* (EGIII) and utilized as substrates for determination of the kinetic parameters of EGIII. The present DMT-method would be a convenient analytical tool for studying the action of glycosyl hydrolases due to the extremely simple synthetic process of DMT-glycosides without using protecting groups.
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The determination of kinetic parameters for endo-glycanases, a hydrolytic enzyme of polysaccharides, is extremely important for evaluating their hydrolysis patterns in the field of food science and woody biomass utilization. There is a drawback to employ naturally occurring polysaccharides as substrates for studying the action of endo-type glycanases that the structure and the molecular weight of these substrates change during an enzymatic hydrolysis reaction. Therefore, the development of an artificial sugar substrate with a definite leaving group at the reducing end has long been a hot topic in polysaccharide chemistry.

Nitrophenyl glycosides are known to be useful compounds for kinetic studies of glycosidases, because the liberated nitrophenol derivatives show yellowish colors in aqueous solutions (pH >7.5).[1] However, the synthesis of nitrophenyl glycosides normally requires multi-step reactions including the usage of strong acids or bases as well as the protection and deprotection of the hydroxyl groups (Scheme 1(A)).[2] For example, in order to activate the anomeric center, severe acidic reaction conditions by using hydrogen bromide/acetic acid is necessary, and protecting groups like acetyl groups must be removed under a basic conditions by using sodium methoxide at the final stage of the synthesis.[3,4]

An enzymatic approach to the production of *p*-nitrophenyl (*p*NP) oligosaccharides has been reported. A substrate for human amylase in serum, *p*NP maltopentaoside, can be prepared by connecting a maltotetraose moiety to *p*NP glucoside by using an amylase as catalyst under mild reaction conditions.[5] However, these methods are highly restricted to the synthesis of specific oligosaccharides, its applicability in principle being dominated by the class of the enzyme catalyst employed. A novel substrate having other leaving group than nitrophenyl has, therefore, strongly been demanded in order to carry out kinetic analyses of glycosidases, particularly in case of using substrates whose glycon parts possess acid or base-labile functions.

Recently, we have found that 4,6-dimethoxy-1,3,5-triazin-2-yl β-lactoside (DMT-β-Lac), prepared by the reaction of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl morpholinium chloride (DMT-MM)[6] and lactose, was hydrolyzed by a cellulase, giving rise to lactose and 2-hydroxy-4,6-dimethoxy-1,3,5-triazine.[7] The synthesis of DMT-β-Lac could be achieved in water without requiring any protection–deprotection process as well as any severe reaction conditions (Scheme 1(B)). These results prompted us to investigate a kinetic study on the enzymatic hydrolysis of DMT-oligosaccharides derived from naturally occurring xyloglucan (*Tamarindus* seeds).

It is well known that endo-β-1,4-glucanase III from *Trichoderma reesei* (EGIII) is able to hydrolyze naturally occurring xyloglucan into the oligoxyloglucan (XXXG or XLLG).[8] In order to know substrate recognition of EGIII in more detail, kinetic analysis using oligoxyloglucans carrying a chromophore as their aglycon moiety is ideal. In this paper, we demonstrated a kinetic analysis of EGIII

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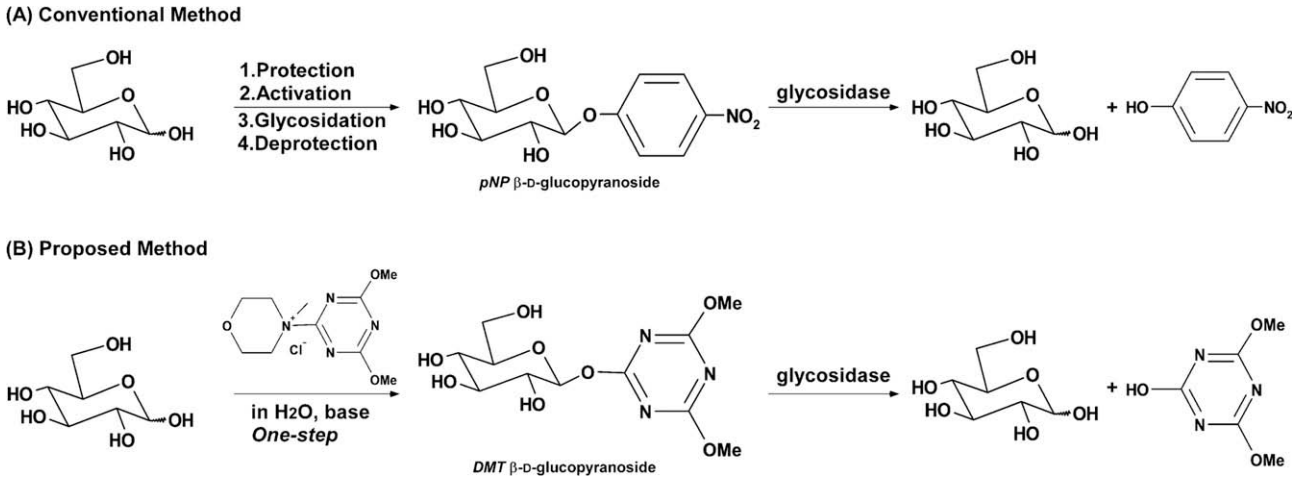
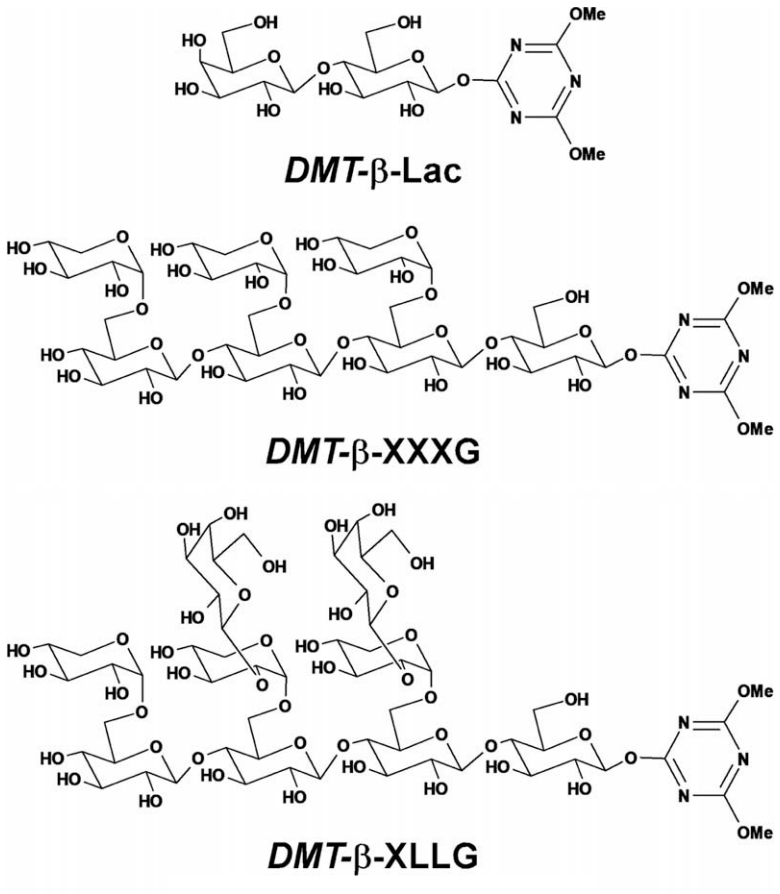


Scheme 1. Comparison of chromogenic substrates: conventional *p*NP-glycoside versus one-step preparable substrate DMT-glycoside.



Scheme 2. DMT-glycosides used in this study. The backbone structure of naturally occurring xyloglucans from *Tamarindus* seeds is a mixture of at least two oligosaccharide units denoted by XXXG and XLLG, where a capital G represents an unsubstituted glucopyranose residue, a capital X represents a glucopyranose residue substituted with a xylopyranose through an α-1,6-glycosidic bond, and a capital L represents a glucopyranose residue substituted with a galactopyranose β(1–2)xylopyranose through an α-1,6-glycosidic bond.

by using two kinds of DMT-oligoxyloglucans, DMT-β-XXXG and DMT-β-XLLG, as novel sugar substrates (Scheme 2). The results of enzyme kinetics employing DMT-β-Lac have also been discussed.

The substrates, DMT-β-Lac, DMT-β-XXXG, and DMT-β-XLLG, were directly prepared starting from lactose,[7] XXXG,[9] and XLLG,[9] respectively, by the action of DMT-MM in the presence of 2,6-lutidine in water (yields: 61%, 44%, 36%, respectively). The enzyme EGIII was expressed and purified according to the previous report.[10] The reagent DMT-MM was synthesized from 2-chloro-4,6-dimethoxy 1,3,5-triazine and *N*-methylmorpholine.[6] Deuterium oxide was obtained by Acros.

NMR spectra (^{1}H, ^{13}C) were recorded on a Bruker DRX500 spectrometer. The samples were dissolved in D_2O, and acetone was used as an internal standard. HPLC analysis was carried out using

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