CHEMISTRY LETTERS, pp. 685-686, 1993.

A Novel Method for Synthesis of Cellooligosaccharide Derivatives by Using Enzyme Catalyst

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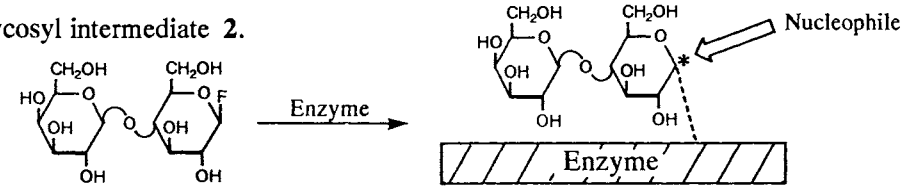
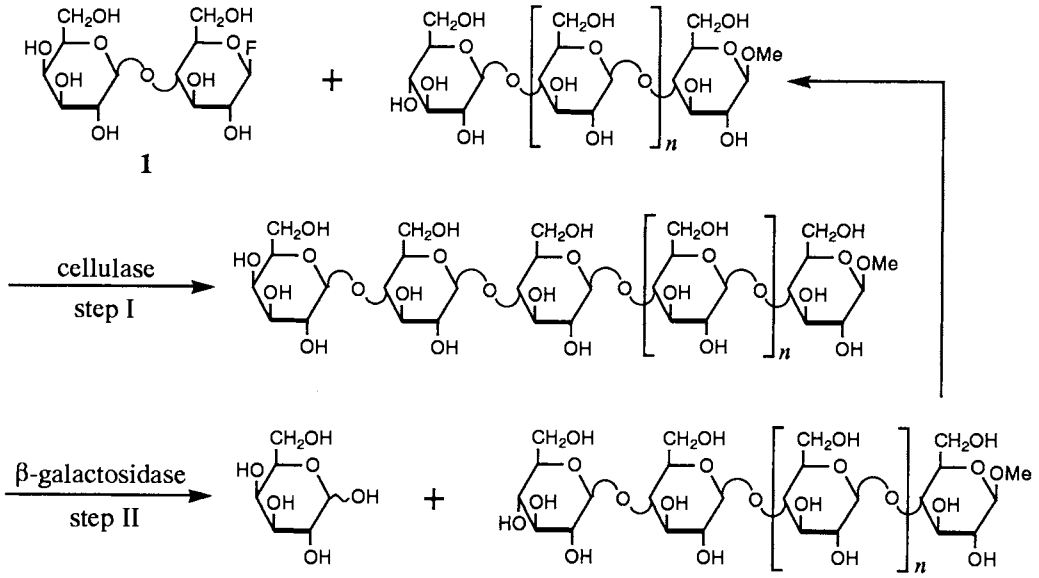
A new methodology for regio and stereoselective synthesis of cellooligosaccharide derivatives has been developed by utilizing the following two enzymatic reactions: A cellulase-catalyzed stereoselective lactosylation of an alkyl cellobioside giving rise to a new oligosaccharide having a D-galactose unit at the non-reducing end and a β-galactosidase-catalyzed regioselective cleavage of the terminal D-galactose unit of the product.

Invention of new synthetic tools for highly selective glycosidic bond formation is one of the most vivid topics in glycotechnology. Several requirements concerning the structure of a glycosyl donor, a glycosyl acceptor, as well as a suitable selection of catalyst must be fulfilled. Recently, we have reported the first preparation of "synthetic cellulose" via non-biosynthetic path by the enzymatic polymerization[1] of β-D-cellobiosyl fluoride (Glc-Glc-F) as substrate for cellulase.[2] In the course of these studies, we found that β-D-lactosyl fluoride (Gal-Glc-F) **1**, the 4' epimer of Glc-Glc-F, is hydrolyzed by the cellulase catalysis to D-lactose (Nucleophile=H_2O), indicating that **1** is also recognized by the active site of cellulase and capable to form a reactive glycosyl intermediate **2**.



1 **2**

In this communication we wish to propose a novel strategy directing a regio and stereoselective construction of cellooligosaccharide moiety by the combination of two enzymatic processes as shown in the general scheme. The first step involves a highly stereoselective β-lactosylation of an alkyl cellobioside (n=0) catalyzed by cellulase using **1** as glycosyl donor (step I). As the reaction proceeded, the resulting condensation product of an oligosaccharide precipitated from the reaction system due to the poor solubility of the product toward the solvent (methanol/acetate buffer=4/1), which was caused by the two saccharide units elongation by the lactosylation. The filtration of the precipitate gave the corresponding tetrasaccharide (Gal-Glc-Glc-Glc-OMe) with more than 90% purity. The second step is a regioselective cleavage of the glycosidic bond between the terminal galactose unit and the adjacent glucose unit by the action of β-galactosidase affording the corresponding cellotrioside (step II). The resulting cellotrisaccharide derivative was further subjected to the condensation with **1** (n=1) followed by the enzymatic degalactosylation process leading to the formation of methyl β-D-cellotetraoside.



A typical procedure for preparation of methyl β-D-cellotrioside is as follows: The substrate **1** (213 mg, 0.62 mmol) and methyl β-D-cellobioside (221 mg, 0.62 mmol) were dissolved in a mixture of 0.05 M acetate buffer (pH 5, 3.0 mL) and methanol (20 mL). A acetate buffer (1.8 mL) solution of cellulase (11 mg, 17 units, from *Trichoderma viride*) was added and the mixture was stirred at 30 °C for 6 h. The white precipitate was filtered and dried in vacuo (36% yield). To a solution of the resulting tetrasaccharide[3] in 0.05 M phosphate buffer (pH 7.3) was added a phosphate buffer solution of β-galactosidase (from *E. coli*) and the reaction mixture was shaken at room temperature for 30 min. The resulting product of methyl β-D-cellotrioside was purified by a preparative HPLC (48% yield).

Concerning the synthesis of cellooligosaccharides, several oligomers up to a cellooctamer derivative have been synthesized starting from allyl 2,3,6-tri-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-β-D-glucoside by utilizing the imidate method; however, elimination of the protecting groups to the corresponding cellooctamer has not been achieved yet.[4] The present new methodology which involves the one glucose unit elongation process enables us to prepare cellooligosaccharide derivatives having a definite degree of polymerization in a regio and stereoselective manner without using any protecting group.[5]

References

1) S. Kobayashi, S. Shoda, and H. Uyama, *Adv. Polym. Sci.*, in press; S. Kobayashi, K. Kashiwa, J. Shimada, T. Kawasaki, and S. Shoda, *Makromol. Chem. Macromol. Symp.*, **54/55**,509 (1992).

2) S. Kobayashi, K. Kashiwa, T. Kawasaki, and S. Shoda, *J. Am. Chem. Soc.*, **113**, 3079 (1991).

3) ^{1}H NMR (D_2O): δ 4.56 (d, 2H, J=7.8 Hz, two anomeric protons are overlapped), 4.47 (d, 1H, J=7.6 Hz), 4.42 (d, 1H, J=7.9 Hz), 3.60 (s, 3H, -OC$\underline{H}_3$). ^{13}C NMR (D_2O): δ 103.9, 103.8, 103.2 (anomeric carbons, two signals are overlapped at 103.2 ppm), 58.1 (-O$\underline{C}H_3$).

4) F. Nakatsubo, T. Takano, T. Kawada, and K. Murakami, "Toward the Synthesis of Cellulose," ed by J. F. Kennedy, G. O. Phillips, and P. A. Williams, Ellis Horwood, Sussex (1989), p. 201.

5) For comprehensive results, S. Shoda, T. Kawasaki, K. Obata, and S. Kobayashi, *Carbohydr. Res.*, to be submitted.

(Received December 21, 1992)