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# Controllable iterative β-glucosylation from UDP-glucose by Bacillus cereus glycosyltransferase GT1: application for the synthesis of disaccharide-modified xenobiotics

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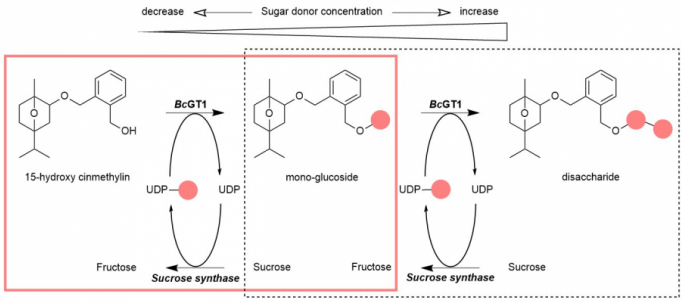
## PURPOSE OF THE ABSTRACT

Glycosylation in natural product metabolism and xenobiotic detoxification often leads to disaccharide-modified metabolites. The chemical synthesis of such glycosides typically separates the glycosylation steps in space and time. The option to perform the two-step glycosylation in one pot, and catalyzed by a single permissive enzyme, is interesting for a facile access to disaccharide-modified products. Here, we reveal the glycosyltransferase GT1 from Bacillus cereus (BcGT1) for iterative O-β-glucosylation from uridine 5'-diphosphate (UDP)-glucose to form a β-linked disaccharide of different metabolites, including C15-hydroxylated cinmethylin (15HCM). 15HCM is a phase I detoxification intermediate of the agricultural herbicide cinmethylin (CM), which is a benzyl ether derivative of the natural terpene 1,4-cineole. Screening studies of glycosyltransferase for β-D-mono-glucosylation of 15HCM from UDP-glucose revealed the product mixtures of the monosaccharide- and disaccharide-modified 15HCM formed from BcGT1 reaction. The disaccharide-modified products of 15HCM were determined in detailed product characterization with NMR and MS analysis. Combined with the comprehensive analysis of time courses for the enzymatic glucosylation of 15HCM, we identify thermodynamic and kinetic requirements for the selective formation of the disaccharide compared to the monosaccharide-modified 15HCM. Glycosylation reactions on methylumbelliferone and 4-nitrophenol involve reversible glycosyl transfer from and to UDP as well as UDP-glucose hydrolysis, both catalyzed by BcGT1. Collectively, this study delineates the iterative β-D-glucosylation of aglycones by BcGT1 and demonstrates applicability for the programmable one-pot synthesis of disaccharide-modified 15HCM.

## FIGURES




### FIGURE 1

Controllable iterative glycosylation of 15-hydroxy cinmethylin by BcGT1

### FIGURE 2


## KEYWORDS

Iterative glycosylation | Disaccharide modification | Leloir glycosyltransferases | Xenobiotics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