Chemoenzymatic synthesis of an unnatural Manβ1,4GlcNAc library using a glycoside phosphorylase

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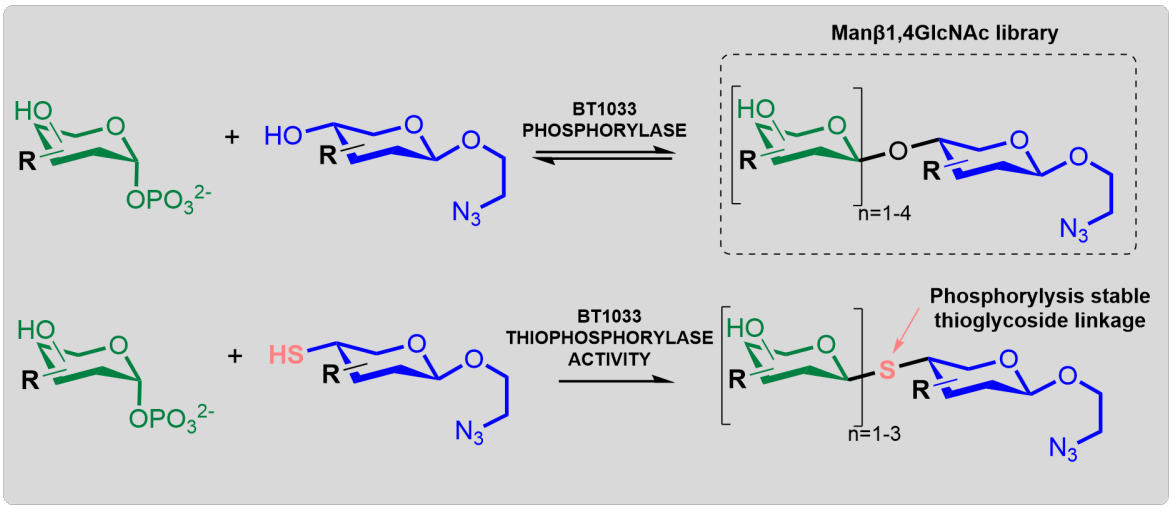
β-Mannosides are ubiquitous in nature, with diverse roles in many biological processes such as energy storage and cell wall biosynthesis. Notably Manβ1,4GlcNAc, a constituent of the core *N*-glycan in eukaryotes, was recently identified as a novel STING immune pathway activator, highlighting its potential for use in immunotherapy.[1] Yet, despite their biological significance, the synthesis of β-mannosidic linkages remains one of the major challenges in glycoscience. Here we present a chemoenzymatic strategy that affords a series of novel unnatural Manβ1,4GlcNAc analogues using the β-1,4-D-mannosyl-*N*-acetyl-D-glucosamine phosphorylase, BT1033. We incorporate unnatural functionality into the enzymatic building blocks through chemical synthesis and show that when fluorine is present in the GlcNAc acceptor, this facilitates further extension of Manβ1,4GlcNAc with Man producing longer β-mannan like glycans. We also pioneer a "reverse thiophosphorylase" enzymatic activity, favouring the synthesis of longer glycans by catalysing the formation of a phosphorylysis-stable thioglycoside linkage, an approach that may be generally applicable to other glycoside phosphorylases.



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