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## Precision Synthesis of Non-natural Heteropolysaccharides by Enzymatic Polymerization

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## Abstract

Phosphorylase catalyzes enzymatic polymerization of  $\alpha$ -D-glucose 1-phosphate (Glc-1-P) as a monomer using a maltooligosaccharide as a primer to produce amylose with liberating inorganic phosphate [1, 2]. It was previously reported that  $\alpha$ -D-glucosamine 1-phosphate (GlcN-1-P) and  $\alpha$ -D-mannose 1-phosphate (Man-1-P) could be recognized as analogue substrates of Glc-1-P by potato phosphorylase in enzymatic  $\alpha$ -glycosylations to give oligosaccharides having each residue at the nonreducing end. Because it is known that thermostable phosphorylase differs in recognition ability of substrates from potato phosphorylase, we found that the enzymatic polymerization of GlcN-1-P occurred when thermostable phosphorylase-catalyzed reaction was examined under the conditions of removal of inorganic phosphate as an ammonium magnesium phosphate precipitate in ammonia buffer including magnesium ion [3, 4]. On the basis of these backgrounds, in this study, we investigated the thermostable phosphorylase-catalyzed enzymatic copolymerization of Glc-1-P with Man-1-P to produce a non-natural  $\alpha$  (1 $\rightarrow$ 4)-linked mannoglucan. The reaction was conducted using the maltotriose primer at 40 °C for 7 days in ammonia buffer including magnesium ion. The <sup>1</sup>H NMR and MALDI-TOF mass spectra of the isolated product supported the structure of mannoglucan (Figure). The molecular weights and Glc/Man unit ratios were depended on the monomer/primer feed ratios.



Figure. <sup>1</sup>H NMR spectrum of isolated product in D<sub>2</sub>O

## References

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