Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.


# Supplementary Information

# Synthesis of chitin and chitosan stereoisomers by thermostable α-glucan phosphorylase-catalyzed enzymatic polymerization of α-D-glucosamine 1-phosphate

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## Experimental Section

### 1. Enzymatic polymerization for MALDI-TOF MS measurement

A mixture of GlcN-1-P (12.7 mg, 0.0490 mmol) and $Glc_3$ (2.4 mg, 4.76 μmol) in 0.5 M ammonia buffer (pH 8.6, 100 μL, 0.050 mmol) dissolving $MgCl_2$ (4.8 mg, 0.0505 mmol) was incubated in the presence of thermostable α-glucan phosphorylase (20 U) at 40 $^{o}$C for 7 days. After the precipitate was removed by centrifugation, supernatant was maintained at 100 $^{o}$C for 1 h in order to deactivate the enzyme. The deactivated enzyme was removed by centrifugation and the supernatant was lyophilized and subjected to MALDI-TOF MS measurement.

MALDI-TOF MS (Fig. 1b):

| | Found: *m/z* | Calcd: *m/z* |
|---|---|---|
| $[GlcN\text{-}Glc_3+Na]^+$ | 688.5511 | 688.2276 |
| $[GlcN_2\text{-}Glc_3+Na]^+$ | 849.5557 | 849.2964 |
| $[GlcN_3\text{-}Glc_3+Na]^+$ | 1010.5670 | 1010.3652 |
| $[GlcN_4\text{-}Glc_3+Na]^+$ | 1171.5513 | 1171.4340 |
| $[GlcN_5\text{-}Glc_3+Na]^+$ | 1332.5306 | 1332.5028 |
| $[GlcN_6\text{-}Glc_3+Na]^+$ | 1493.4929 | 1493.5716 |
| $[GlcN_7\text{-}Glc_3+Na]^+$ | 1654.4647 | 1654.6404 |
| $[GlcN_8\text{-}Glc_3+Na]^+$ | 1815.4103 | 1815.7092 |
| $[GlcN_9\text{-}Glc_3+Na]^+$ | 1976.3684 | 1976.7780 |
| $[GlcN_{10}\text{-}Glc_3+Na]^+$ | 2138.3005 | 2137.8468 |
| $[GlcN_{11}\text{-}Glc_3+Na]^+$ | 2298.2569 | 2298.9156 |
| $[GlcN_{12}\text{-}Glc_3+Na]^+$ | 2460.1899 | 2459.9844 |

**2. Glucoamylase treatment for MALDI-TOF MS measurement**

Glucoamylase (20 U) was added to a solution of the above enzymatic polymerization product in water (1.0 mL) and the mixture was incubated at 40 $^{o}$C for 1 h. The reaction mixture was then maintained at 100 $^{o}$C for 1 h in order to deactivate the enzyme. The deactivated enzyme was removed by centrifugation and the supernatant was lyophilized and subjected to MALDI-TOF MS measurement.

MALDI-TOF MS (Fig. S1):

| | Found: *m/z* | Calcd: *m/z* |
|---|---|---|
| $[GlcN\text{-}Glc_3+Na]^+$ | 688.3587 | 688.2276 |
| $[GlcN_2\text{-}Glc_3+Na]^+$ | 849.3421 | 849.2964 |
| $[GlcN_3\text{-}Glc_3+Na]^+$ | 1010.2971 | 1010.3652 |
| $[GlcN_4\text{-}Glc_3+Na]^+$ | 1171.2575 | 1171.4340 |
| $[GlcN_5\text{-}Glc_3+Na]^+$ | 1332.1765 | 1332.5028 |
| $[GlcN_6\text{-}Glc_3+Na]^+$ | 1493.0937 | 1493.5716 |
| $[GlcN_7\text{-}Glc_3+Na]^+$ | 1653.9938 | 1654.6404 |
| $[GlcN_8\text{-}Glc_3+Na]^+$ | 1814.9132 | 1815.7092 |
| $[GlcN_9\text{-}Glc_3+Na]^+$ | 1976.8174 | 1976.7780 |
| $[GlcN_{10}\text{-}Glc_3+Na]^+$ | 2136.7214 | 2137.8468 |
| $[GlcN_{11}\text{-}Glc_3+Na]^+$ | 2298.6161 | 2298.9156 |
| $[GlcN_{12}\text{-}Glc_3+Na]^+$ | 2459.5253 | 2459.9844 |

### 3. *N*-Acetylation for MALDI-TOF MS measurement

To a solution of the above glucoamylase-treated product (18.9 mg) and $Na_2CO_3$ (5.4 mg, 0.0510 mmol) in water (1.0 mL) was added acetic anhydride (0.0049 mL, 0.0520 mmol) and the mixture was stirred at room temperature for 10 min. After the reaction mixture was treated successively with cation- and anion-exchange resins (Amberlite IR-120(plus) (H) and Amberlite IRA-400J Cl, respectively) for 5 min each, the solution was filtered, lyophilized, and subjected to MALDI-TOF MS measurement.

MALDI-TOF MS (Fig. 1c):

| | Found: *m/z* | Calcd: *m/z* |
|---|---|---|
| $[GlcNAc\text{-}Glc_3+Na]^+$ | 730.5252 | 730.2382 |
| $[GlcNAc_2\text{-}Glc_3+Na]^+$ | 933.5286 | 933.3176 |
| $[GlcNAc_3\text{-}Glc_3+Na]^+$ | 1136.5005 | 1136.3970 |
| $[GlcNAc_4\text{-}Glc_3+Na]^+$ | 1339.4472 | 1339.4764 |
| $[GlcNAc_5\text{-}Glc_3+Na]^+$ | 1542.3622 | 1542.5558 |
| $[GlcNAc_6\text{-}Glc_3+Na]^+$ | 1745.2684 | 1745.6352 |
| $[GlcNAc_7\text{-}Glc_3+Na]^+$ | 1948.1881 | 1948.7146 |
| $[GlcNAc_8\text{-}Glc_3+Na]^+$ | 2152.0883 | 2151.7940 |
| $[GlcNAc_9\text{-}Glc_3+Na]^+$ | 2353.9885 | 2354.8734 |
| $[GlcNAc_{10}\text{-}Glc_3+Na]^+$ | 2557.83001 | 2557.9528 |

**4. Synthesis of standard amylose samples**

Solutions of Glc-1-P (96.0, 128, and 150 mg) with $Glc_3$ primer (5.5 mg, 0.0109 mmol) in 2.0 mol/L acetate buffer (pH 6.2, 5.0 mL) was incubated in the presence of thermostable α-glucan phosphorylase (40 U) at 40 $^{o}$C for 15 h (feed ratios of Glc-1-P/$Glc_3$ = 30, 40, and 50). After the reaction mixtures were then maintained at 100 $^{o}$C for 1 h in order to deactivate the enzyme, the deactivated enzyme was removed by centrifugation. Then, the supernatants were dialyzed against water (molecular cut off; 1000) and lyophilized to give enzymatically synthesized standard amylose samples. The degrees of polymerization of the amylose samples were determined by UV-Vis analysis of the complexes with iodine as follows (K. Kobayashi, S. Kamiya, and N. Enomoto, *Macromolecules*, 1996, **29**, 8670–8676). A standard iodine-iodide solution was prepared by dissolving potassium iodide (26.3 mg, 0.158 mmol) and iodine (26.3 mg, 0.207 mmol) in water (50 mL). The amylose sample (1.0 mg) was dissolved in DMSO (0.20 mL) and the standard iodine-iodide solution (1.0 mL) was added to the solution. After the resulting solution was diluted with water (10 mL), the violet solution was then characterized by UV-vis spectroscopy to determine the degree of polymerization (DP) values as follows; 53.0 for 30 equiv., 55.7 for 40 equiv., and 59.6 for 50 equiv. (Glc-1-P/$Glc_3$). Then, the amylose samples were used as the standards for GPC measurement.

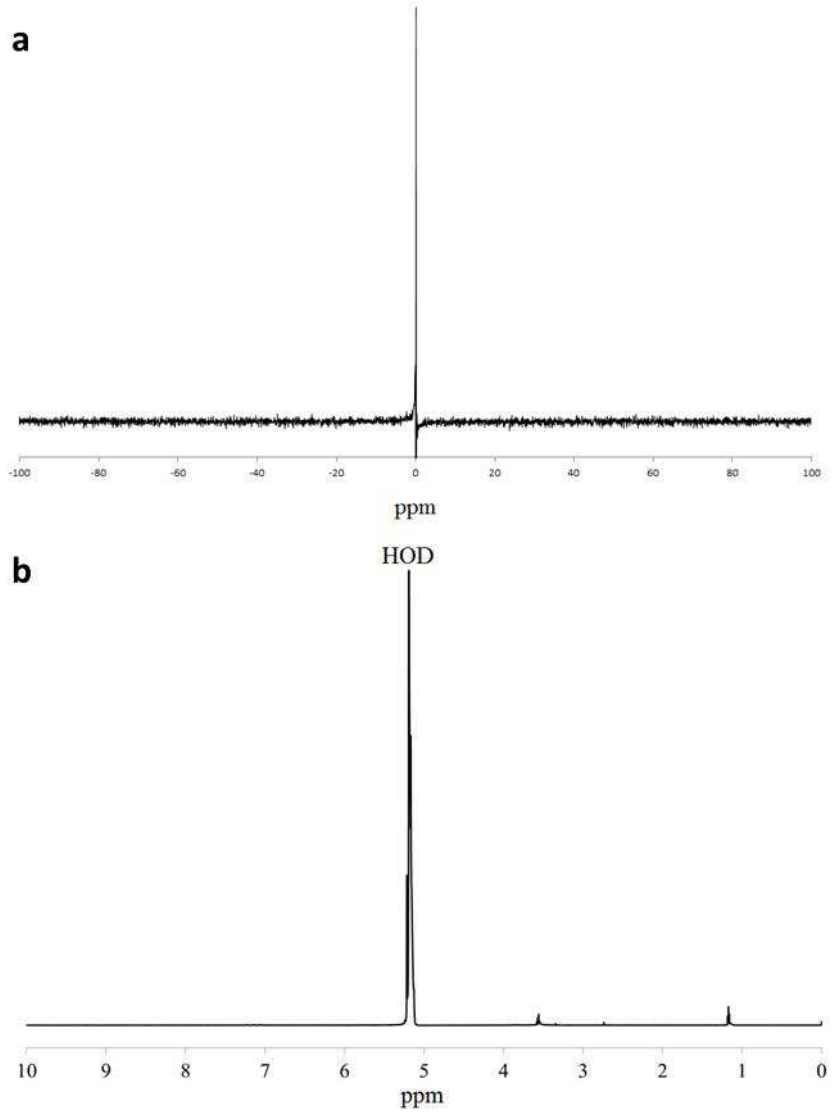


**Fig. S1** $^{31}P$ and $^{1}H$ NMR spectra (**a** and **b**, respectively) of precipitate from reaction mixture in enzymatic polymerization (GlcN-1-P/$Glc_3$ = 10:1) in $DCl/D_2O$.

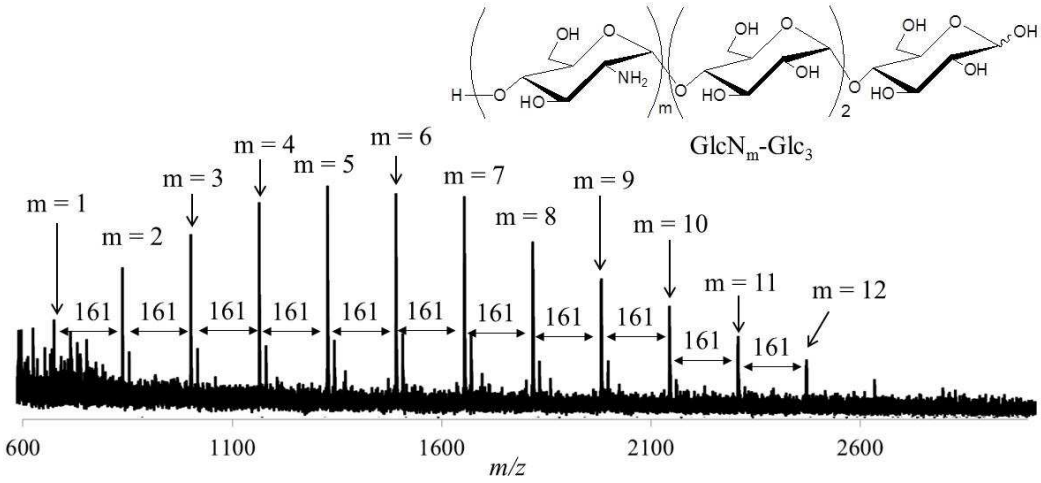


**Fig. S2** MALDI-TOF MS of glucoamylase-treated materials from enzymatic polymerization products (GlcN-1-P/$Glc_3$ = 10:1).

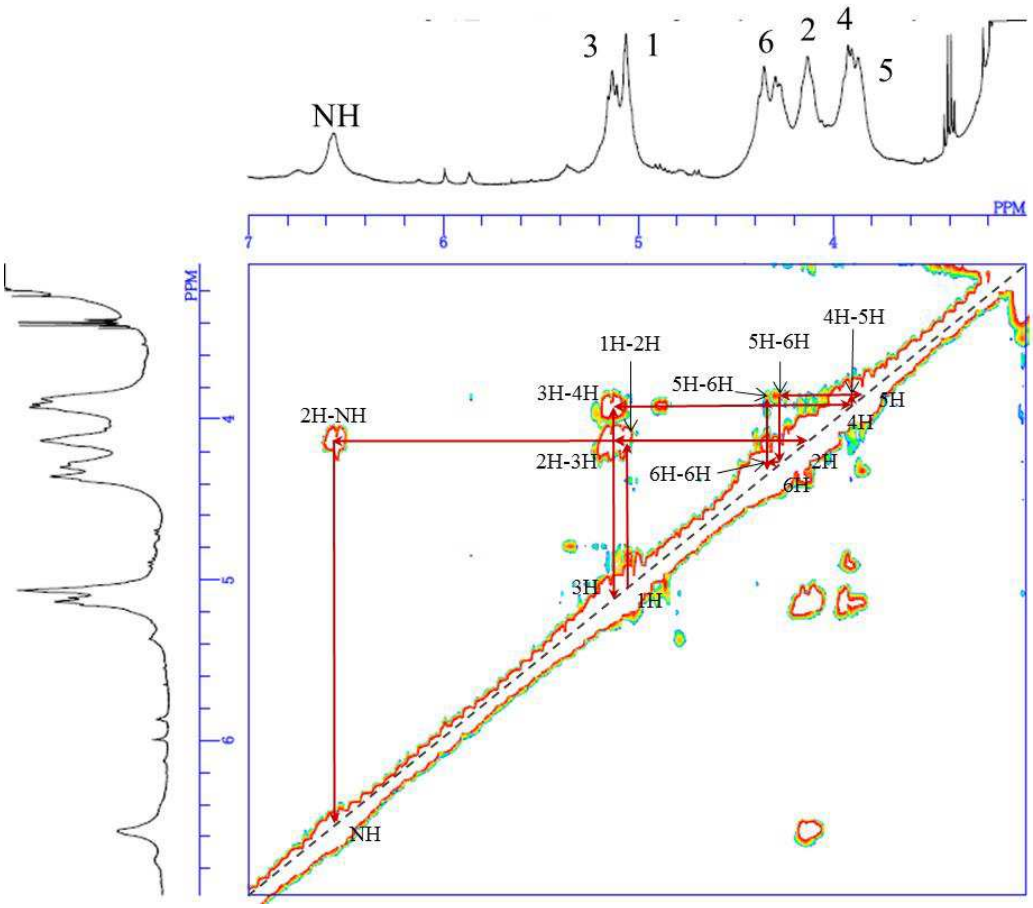


**Fig. S3** $^{1}$H-$^{1}$H COSY NMR spectrum of *O*-acetylated aminopolysaccharide (run 3) in DMSO-$d_6$.