# Novel Direct Preparation of *n*-Butyl 2-Amino-2-deoxy-β-D-glucopyranoside from Chitosan and *n*-Butanol Using Biocatalyst

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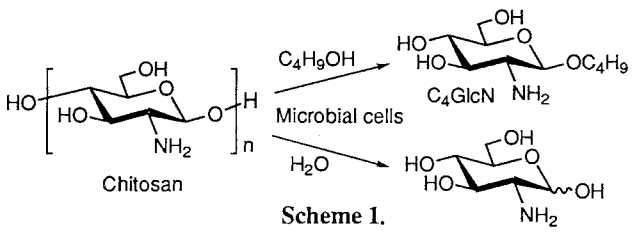
*n*-Butyl 2-amino-2-deoxy-β-D-glucopyranoside (C4GlcN) was prepared by the novel direct transglycosylation reaction of chitosan and *n*-butanol using the resting cells of *Penicillium funiculosum* KY616 as the enzyme source of exo-β-D-glucosaminidase. Using this strain, a versatile synthetic intermediate, C4GlcN, was prepared in a yield of 210 mg/g chitosan when chitosan and *n*-butanol were incubated at a temperature of 30 °C and pH 4 for 2 d.

The D-glucosamine (2-amino-2-deoxy-D-glucopyranose) is the monomeric component of chitosan readily produced by the deacetylation of naturally abundant chitin and has wide applications as the hydrophilic moiety of amphiphilic molecules having biological properties and molecular recognition abilities. Such amphiphilic molecules that form supramolecular assemblies involves long chain alkyl glycosides[1-3] and short or medium chain alkyl glycoside fatty acid esters.[4,5] Recently, the lipase-catalyzed 6-*O*-acylation of short chain alkyl glucosides having potentially important applications in detergents, food and feed, cosmetics and pharmaceuticals as surfactant and emulsifiers were reported.[4-6] As the short or medium chain alkyl glycoside is soluble in organic solvents or miscible with fatty acids, the 6-*O*-monoesterification of glucoside with a fatty acid may be efficiently carried out using lipase as a catalyst.[6] Based on these facts, short and medium chain alkyl 2-amino-2-deoxy-D-glucopyranosides may become versatile intermediates for the production of novel biologically active and environmentally benign amphiphiles. The direct preparation of such glycosides from chitosan and an alcohol using an enzyme may become an industrially feasible way. It is known that chitosan is hydrolyzed by exo-β-D-glucosaminidase to liberate D-glucosamine residues from the nonreducing terminal. The alkyl glycoside will be produced instead of glucosamine when exo-β-D-glucosaminidase has transglycosylation activity and an alcohol is present in place of water as shown in Scheme 1. However, the direct transglycosylation reaction of chitosan and an alcohol using microbes or enzymes to produce short and medium chain alkyl 2-amino-2-deoxy-D-glucopyranosides has not yet been reported. The enzymatic preparation of the alkyl glycoside by the transglycosylation reaction of polysaccharide and alcohol has, so far, been restricted to xylan.[7-10]

In this report, *n*-butyl 2-amino-2-deoxy-β-D-glucopyranoside (C4GlcN) was prepared by the direct transglycosylation reaction of chitosan[11] and *n*-butanol using the resting cells of *Penicillium funiculosum* KY616, as the enzyme source of exo-β-D-glucosaminidase.




**Scheme 1.**

The chitosan-assimilating microbes were isolated from soil using enrichment culture techniques with chitosan as the sole carbon source. Among them, the most active fungal strain, KY616, was selected as the chitosan-assimilating strain having a transglycosylation activity. The KY616 strain was identified as *Penicillium funiculosum*. The strain KY616 was grown in an inorganic medium (200 mL, initial pH 6.0) containing 0.5% chitosan[12] as the growing substrate in a shaking flask at 30 °C. After 4 d ($OD_{660}$=7), the cells were harvested by centrifugation (18000 g, 30 min, 4 °C), washed with saline to obtain 6 g of wet cells (160 mg for dry cells) for the preparation of C4GlcN. It was confirmed that the resting cells showed exo-β-D-glucosaminidase activity as measured using chitotriitol.[13]

C4GlcN was prepared by the transglycosylation reaction of chitosan and *n*-butanol using the resting cells as the enzyme source. A typical preparation procedure is as follows. A mixture of 5 mL of 0.5% chitosan (pH 4.0),[12] 1 g of resting cells, 3 mL *n*-butanol and 2 mL water was incubated in a shake tube with stirring at 30 °C for 48 h. The yield of C4GlcN in the incubation mixture was directly analyzed by HPLC[14] using an authentic standard. The product was purified by column chromatography and its structure was analyzed. That is, after the reaction, the unreacted chitosan was filtered, and the filtrate was evaporated, which was then purified by column chromatography [($SiO_2$, $CHCl_3/CH_3OH/NH_4OH$ = 9/4/1 (v/v)]. The isolated product was analyzed using HPLC, elemental analysis, IR, $^1H$ NMR and $^{13}C$ NMR spectroscopy.[15]

It was found that the resting cells of *P. funiculosum* KY616 were capable of the transglycosylation reaction, and the C4GlcN was produced by the direct reaction of chitosan and *n*-butanol in a yield of 210 mg/g chitosan. However, no C4GlcN was produced using the monomeric D-glucosamine or N-acetyl-D-glucosamine in place of chitosan. The transglycosylation reaction between chitosan and *n*-butanol using the resting cells of *P. funiculosum* KY616 was analyzed with respect to reaction time, temperature, pH, butanol concentration, chitosan concentration and the degree of deacetylation of the chitosan.

The time course of the yield of C4GlcN at 30 °C is shown in Figure 1a. A mixture of 5 mL of 0.5% chitosan (pH 4.0), 1 g of resting cells, 3 mL *n*-butanol and 2 mL water was incubated with stirring at 30 °C. The yield of C4GlcN was periodically analyzed by HPLC. It was found that the yield of C4GlcN gradually increased with incubation time and after a 4-d incubation, the yield of C4GlcN reached 210 mg/g chitosan. Under the same conditions at 30 °C except that the pH of the reaction medium was varied from 2 to 7, the yield of C4GlcN was maximized at pH 4.0. The relationship between reaction temperature and yield of C4GlcN was analyzed and the results

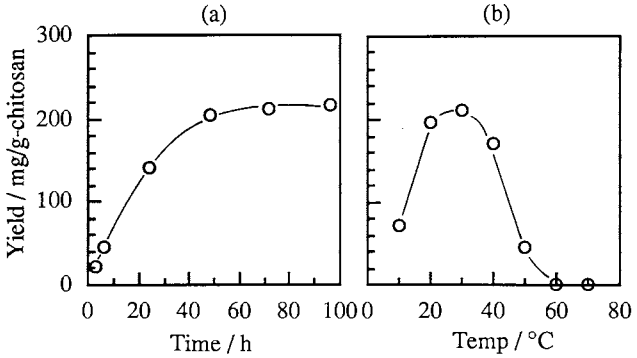



**Figure 1.** Yield of $C_4$GlcN by the transglycosylation reaction of 0.25% chitosan and 30 vol% *n*-butanol using the resting cells at pH 4. Effects of (a) time at 30 °C and (b) temperature after 48 h.

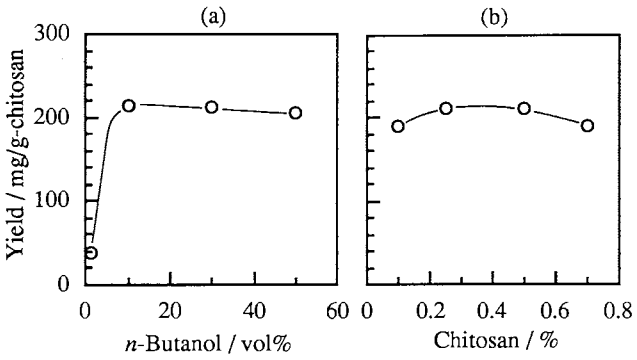



**Figure 2.** Yield of $C_4$GlcN by the transglycosylation reaction of chitosan and butanol at pH 4 using the resting cells at 30 °C for 48 h. Effects of (a) *n*-butanol concentration using 0.25% chitosan and (b) chitosan concentration using 30 vol% butanol.

are shown in Figure 1b. It was found that the yield of $C_4$GlcN was increased with increasing temperature up to 30 °C. The yield of $C_4$GlcN then decreased probably ascribed to the deactivation of the enzyme. The best reaction temperature which gave the highest yield of $C_4$GlcN was 30 °C.

The effect of butanol concentration on the yield of $C_4$GlcN was analyzed and the results are shown in Figure 2a. The yield of $C_4$GlcN was quickly increased to a maximum value with increasing alcohol concentration from 0.2 to 10%. The yield was then slightly decreased. This slight decrease in yield of $C_4$GlcN will be due to the deactivation of the enzyme by the high alcohol concentration.

The chitosan concentration between 0.1 and 0.7% in the reaction mixture showed no significant influence on the yield of $C_4$GlcN as shown in Figure 2b. However, a further increase in the concentration of chitosan increased the viscosity of the reaction mixture which disturbed the efficient reaction.

It was found that the resting cells could catalyze the direct transglycosylaltion reaction of chitosan with various alcohols, such as methanol, ethanol, *n*-propanol, *iso*-propanol *n*-butanol and *sec*-butanol.

It was also found that the yield of $C_4$GlcN was dependent on the degree of deacetylation of chitosan as shown in Figure 3.

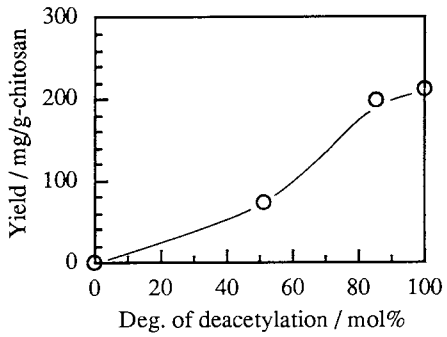



**Figure 3.** Yield of $C_4$GlcN by the transglycosylation reaction of 0.25% chitosan having varying degree of deacetylation and 30 vol% *n*-butanol at pH 4 using the resting cells at 30 °C for 48 h.

That is, the yield of $C_4$GlcN was increased with increasing degree of deacetylation of chitosan, and the maximum yield was obtained when 100% deacetylated chitosan was used. This means that the N-acetylated D-glucosamine moiety was not accepted as the substrate of the exo-β-D-glucosaminidase. Details of the exo-β-D-glucosaminidase of *P. funiculosum* KY616 are now under study.

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## References and Notes

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14 $C_4$GlcN was analyzed using high-performance liquid chromatography (HPLC) with a refractive index detector and a commercial HPLC column (Asahipak GS-220, Asahi Chemical Industry Co., Ltd., Tokyo, Japan) and 0.05 M $NaNO_3$ was used as the eluent.
15 Typical analytical results are shown for $C_4$GlcN: $^1$H NMR (270 MHz: $D_2O$): δ=0.95(3H, t, *J*=8.0), 1.41(2H, m), 1.65(2H, m), 2.87(1H, dd, *J*=9.6), 3.47(1H, m, *J*=2.0), 3.53(1H, m), 3.58(1H, m), 3.72 (1H, dt), 3.78(1H, dd, *J*=5.0). 3.97 (1H, dd, *J*=12.0), 3.97 (1H, m), 4.62(1H, d, *J*=8.2), $^{13}$C NMR(67.5 MHz: $CDCl_3$): δ=103.7(β-anomeric C1 of the pyranose ring), 57.4(C2), 76.5(C3), 71.3(C4), 77.6(C5), 61.8(C6), 70.8($OCH_2$), 19.4, 31.8($CH_2$), 14.0($CH_3$).