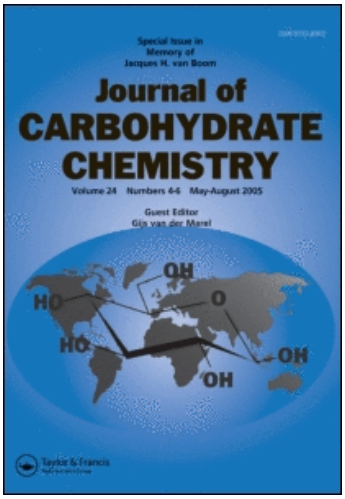


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# A Facile Method for the Preparation of 6-Deoxy Derivatives of Chitin

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# A FACILE METHOD FOR THE PREPARATION OF 6-DEOXY DERIVATIVES OF CHITIN[1]

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## ABSTRACT

A novel two-step method for the preparation of 6-deoxychitin from *N*-acetylchitosan (**1**) is described. Compound **1** was prepared by *N*-acetylation of chitosan, and was specifically brominated at C6 to afford 6-bromo-6-deoxychitin (**2**). Compound **2** was treated with $NaBH_4$ in dimethylsufoxide to give 6-deoxychitin (**3**). Repeated treatment of **3** with aq 40% NaOH at 100 ℃ for 10 h gave 6-deoxychitosan (**4**). Compound **3** was insoluble in aq 2% acetic acid, but **4** was soluble in aq 2% acetic acid. Compound **3** was hydrolyzed by chitinase from *Bacillus* sp. at a rate of 0.8 time that of **1**.

## INTRODUCTION

Chitin [(1->4)-*N*-acetyl-$\beta$-D-glucosaminan] and chitosan (*N*-deacetylated chitin) are aminopolysaccharides found widely in nature, which have various biological functions.[2] 6-Deoxychitin (**3**) and 6-deoxychitosan (**4**) have not yet been found in nature, but their chemical preparation from chitin is of significance from a viewpoint of creating novel functional polymers. The classical three-step method for the preparation of **3** from chitin was by 6-*p*-toluenesulfonylation (tosylation), iodination, followed by reduction.[2,3] We now report a facile two-step method for the preparation of **3** in relatively good yield by direct bromination of *N*-acetylchitosan (**1**), followed by reduction.

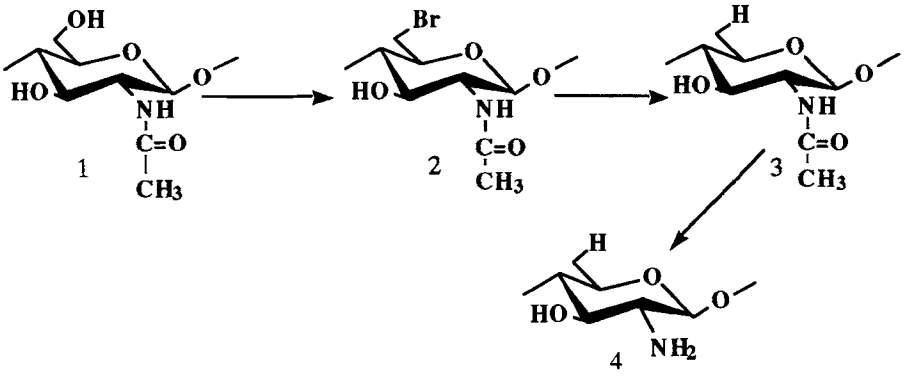
## RESULTS AND DISCUSSION

Chitosan was *N*-acetylated by treatment with acetic anhydride in aq acetic acid-MeOH to afford **1** (a regenerated chitin).[5] The hydroxyl group at C6 of **1** was directly brominated by treatment with a solution of *N*-bromosuccinimide (NBS) and triphenylphosphine (TPP) in 5% *N*,*N*-dimethylacetamide (DMA), to afford 6-bromo-6-deoxychitin (**2**, 0.67 for Br) in 89% yield. This procedure was originally used for the preparation of 6-bromo-6-deoxycellulose from cellulose.[6] Treatment of **2** with $NaBH_4$ afforded **3** (d.s. 0.60 for 6-deoxy) in 72% yield. Compound **3** was repeatedly treated with aq 40% NaOH at 100 ℃ for 10 h to afford **4** (Scheme 1). The yield of **3** was 24% higher than that of the previous three-step method.[4]

The degree of bromination of **1** by treatment with NBS and TPP in 5% LiBr-DMA solution under the present condition was up to 0.67 per GlcNAc. The selective bromination at the C6 position was confirmed from chemical shift data in the $^{13}C$ NMR spectrum. This result is in agreement with the bromination of cellulose under the same conditions.[6] In the $^{13}C$ NMR spectrum (20% DCl) of **3**, the 6-methyl group (d.s. 0.60) was detected at $\delta$ 18.9 ppm, although its distribution in the chitin molecule is unknown (Fig.1). The methyl signal was also detected in the acid hydrolysate of **3** at $\delta$ 19.1-19.3 ppm in the $^{13}C$ NMR spectrum (10% DCl) and at $\delta$ 1.27-1.36 ppm in the $^{1}H$ NMR spectrum (10% DCl). In the $^{13}C$ NMR spectrum (20%DCl-$D_2O$) of the *N*-acetylated product, the C6 methyl signal appeared at $\delta$ 18.9 ppm, *N*-acetylmethyl signals at $\delta$ 24.0 and 25.0, and *N*-acetyl C=O signals at $\delta$ 177.0 and 180.0 ppm, respectively. Appearance of two signals for each *N*-acetylmethyl and *N*-acetyl C=O signal are due to presence of both *N*-acetyl-D-glucosamine and 6-deoxy-*N*-acetyl-D-glucosamine residues in **3**.

The hydrolysis rate of **3** (d.s. 0.6 for 6-deoxy) by chitinase from *Bacillus* sp. was at 78% of **1**, indicating that chitinase reaction was a little inhibited by introducing a methyl group at C6 of chitin. However, the distribution for 6-methyl group in **3** and the structure of enzymatic reaction products are unknown.

For the *N*-deacetylation of **3** in concd alkaline solution, more vigorous conditions were required than for chitin,[7] resulting in considerable degradation. This indicates that *N*-deacetylation is inhibited by the introduction of a C6 methyl group in the chitin molecule.




**Scheme 1**

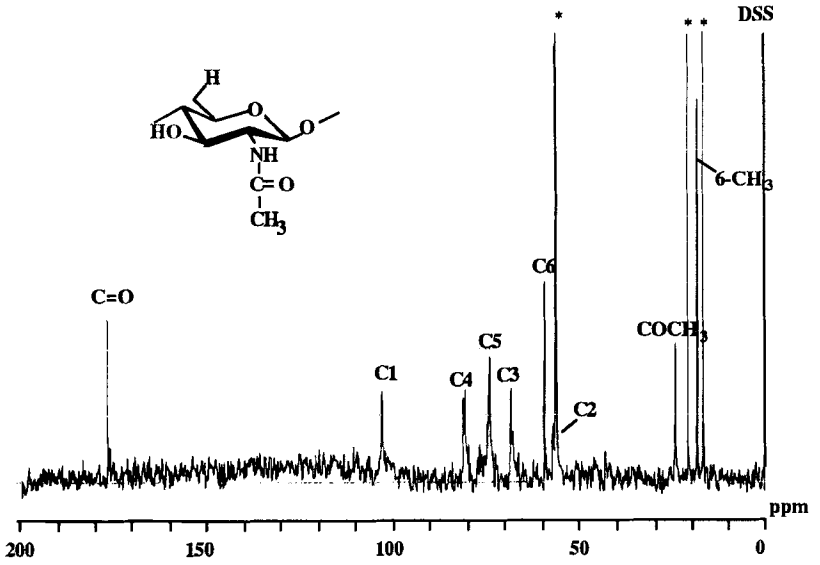



**Figure 1.** $^{13}C$ NMR spectrum of **3** in 20% DCl-$D_2O$. Sodium 2, 2-dimethyl-2-silapentane 5-sulfonate(DSS) was used as an internal signal standard, $\delta = 0$.

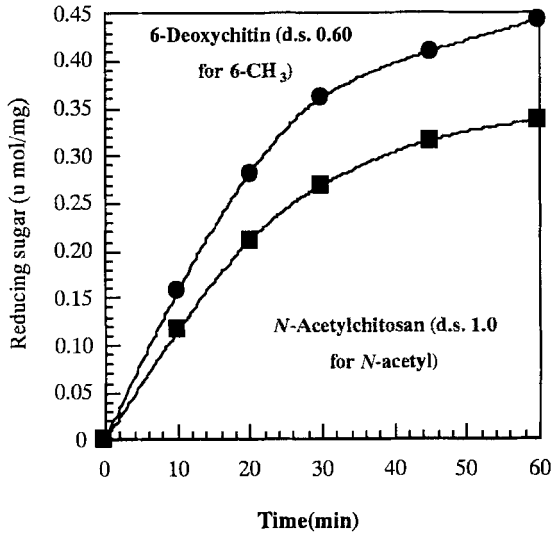



**Figure 2.** Time course of the enzymatic hydrolysis of **1** and **3** by chitinase from *Bacillus* sp.



## EXPERIMENTAL

**General Procedures.** $^{13}C$ CPMAS NMR spectra were recorded on a Chemagnetics CMX 360 NMR spectrometer, $^{13}C$ NMR spectra (DMSO) on a Jeol JNM-GX 270 FT-NMR spectrometer, FT-IR spectra (KBr) on a Jasco FT-IR 5300 spectrometer, and specific rotations on a Horiba SEPA-200 polarimeter. Elemental analysis were performed at the Elemental Analysis Center of Kyoto University, Kyoto.

***N*-Acetylchitosan (1).** Chitosan was *N*-acetylated in aq 2% acetic acid-methanol to give **1** (95% yield, d.s. 1.0 for NAc). FT-IR (KBr) 1660 and 1554 $cm^{-1}$ (C=O and NH of NAc). $^{13}C$ CPMAS NMR $\delta$ 174.3 (C=O of Ac), 104.3 (C1), 75.5 (C3), 83.7 (C4), 74.0 (C5), 60.7 (C6), 55.7 (C2), 23.4 ppm ($CH_3$ of Ac).[8]

**6-Bromo-6-deoxychitin (2).** The following treatments were performed in anhydrous conditions under nitrogen gas. LiBr (8 g) was dissolved in DMA by stirring at 75 ℃ for 1 h to give 5% LiBr-DMA solution, to which **1** (0.4 g) was added. The mixture was stirred at 75 ℃ 24 h to give a clear viscous solution. Under cooling in an ice bath, a solution of NBS (3.5 g, 10 mol/GlcNAc) and TPP (5.2 g, 10 mol/GlcNAc) dissolved in

DMA (10 mL) was added, and the total volume was adjusted to 80 mL by addition of DMA. The mixture solution was stirred at room temperature for 15 min and then at 75 ℃ for 1.5 h. The reaction mixture was poured dropwise into acetone (800 mL) to give a precipitate, and the precipitate was collected by centrifugation and washed thoroughly with acetone. The precipitate was suspended in 1 M $Na_2CO_3$ solution, stirred at room temperature overnight, collected by filtration, washed successively with water, ethanol and ether, and dried to give a slightly yellow product [ **2**, 0.44 g (90% yield), $[\alpha]^{20}_D$ -40° (*c* 1, fromic acid), d.s. 0.67 for Br]. The product was soluble in 5% LiBr-DMA and swollen in DMSO and pyridine. FT-IR (KBr) 1658 and 1531 (C=O and NH of NAc), and 680 $cm^{-1}$ (C-Br).[9]

Anal. Calcd for $[C_8H_{12}O_4N(Br)_{0.67}(OH)_{0.33}]_n$: C, 38.67; H, 5.20; N, 5.60; Br, 19.09. Found: C, 38.48; H, 5.05; N, 5.61; Br, 19.24.

**6-Deoxychitin (3).** Compound **2** (0.35 g) was swollen in DMSO (10 mL) under nitrogen gas, $NaBH_4$ (0.5 g) was added at room temperature, and the mixture was stirred at 80 ℃ for 8 h. The reaction mixture was poured into ethanol (150 mL), and excess $NaBH_4$ was degraded by addition of aq acetic acid to give a white precipitate. The precipitate was collected by filtration, washed with ethanol, and dried to give a white solid. The solid was treated with acetic anhydride (0.1 mL) in methanol (50 mL) at room temperature for 3 h to give a white product [ **3**, 0.19 g (72% yield ), d.s. 0.6 for $CH_3$-C6]. Compound **3** was soluble in formic acid, but insoluble in water and in common organic solvents. FT-IR (KBr) 1660 and 1554 $cm^{-1}$ (C=O and NH of NAc); $^{13}C$ NMR (20% DCl in $D_2O$) $\delta$ 177.0 (C=O of NAc), 103.5 (C1), 81.7 (C4), 75.0 (C5), 68.8 (C3), 60.0 (C6), 56.3 (C2), 24.0 ($CH_3$ of NAc), and 18.9 ppm (6-methyl).

Anal. Calcd for $[C_8H_{12}O_4N(H)_{0.60}(OH)_{0.40} \cdot 0.36\ H_2O]_n$: C, 45.10; H, 6.05; N, 6.37. Found: C, 44.86; H, 5.92; N, 6.52.

***O*-Acetylation of 3.** Compound **3** (0.5 g) was suspended in pyridine (20 mL), and 2,4-dimethylpyridine (0.2 g) and acetic anhydride (2 mL) were added. The mixture was stirred at room teperature overnight to give a brown solution, and the solution was added dropwise to ice-water. The precipitate produced was collected by centrifugation, washed with water and ethanol, and dried to give a brown product (*O*-acetyl 6-deoxy-

chitin) in 0.42 g yield. The product was soluble in DMSO. FT-IR (KBr) 1748 and 1228 (C=O and C-O of OAc), and 1660 and 1554 $cm^{-1}$ (C=O and NH of NAc); $^{13}C$ NMR (DMSO-$d_6$) $\delta$ 169.3-171.8 (C=O of NAc and OAc), 100.5 (C1), 80.8 (C4), 71.5 (C5), 70.3 (C3), 54.3 (C6), 52.5 (C2), 20.2-22.4 ($CH_3$ of NAc and OAc), and 17.2 ppm (6-methyl).

**Acid hydrolysis of 3.** Compound **3** (0.1 g) was hydrolyzed in aq 6N HCl (2 mL) at 100 ℃ for 15 h, and the hydrolysate was diluted with water, decolorized with activated carbon, filtered, concentrated in vacuo, and dried over solid NaOH in a desiccator to give hydrolyzed product as a syrup. The product was *N*-acetylated with acetic anhydride in methanol [10] to give *N*-acetylated product as a syrup.

**Chitinase hydrolysis of 3.** Compounds **1** and **3** (each 10 mg, >200 mesh) were each stirred in 1.8 mL of 0.2M acetate buffer solution (pH 5.0) at 40 ℃ for 1 h. Chitinase (2.0 mg) from *Bacillus* sp. dissolved in 0.2 mL of the same buffer solution was added. The resulting solution was incubated at 40 ℃ for 10, 20, 30, 45 and 60 min,[11] and the reducing sugar formed was analyzed by a modified method[12] of Schales and Schales.

**6-Deoxychitosan (4).** Compound **3** (0.20 g) was treated in an aq 40% NaOH solution in the presence of $NaBH_4$ (0.7 mol/GlcNAc) at 100 ℃ for 10 h. After cooling to room temperature, the reaction mixture was diluted with ice water, and the precipitate was collected by centrifugation, washed thoroughly with water and ethanol and dried. The treatment was repeated at least two times until disappearance of *N*-acetyl absorptions at 1650 and 1550 $cm^{-1}$ in the FT-IR spectra to afford **4** in 0.08 g yield. The product was soluble in aq 2% acetic acid. FT-IR (KBr) 1604 $cm^{-1}$ ($NH_2$).



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