



# Facile and efficient synthesis of *N*-benzyl chitosan via a one-pot reductive amination utilizing 2-picoline borane

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## Abstract

In this paper, the use of 2-picoline borane (pic-BH<sub>3</sub>) as a reducing agent for the reductive amination of chitosan is reported for the first time. By optimizing the feed molar ratio of chitosan, benzaldehyde and pic-BH<sub>3</sub>, a high yield of isolated *N*-benzyl chitosan with a nearly perfect degree of substitution is successfully obtained. This material has rarely been obtained with previous methods. The newly developed synthetic method herein has many advantages, including being more facile, more efficient, and less harmful than conventional methods; thus, this method is applicable to other aldehydes or ketones, leading to wide varieties of *N*-modified chitosan in the future.

## Introduction

Chitin is the second most abundant natural polymer on earth, in which *N*-acetyl-D-glucosamines are connected through β-(1 → 4) linkages [1]. Chitosan, which is obtained from chitin via the deacetylation process, has long been regarded as a useful and green material because of its wide variety of applications, such as in catalysts [2], aerogels [3], antimicrobial films [4–6], semiconductors [7], fuel cells [8], supercapacitors [9], and zinc–air batteries [9]. Recently, the precise chemical modification of such green materials (i.e., chitosan) has become a highly important research topics for obtaining chitosan specimens with enhanced properties [10–17].

Among these reactions, reductive amination can introduce a wide variety of substitution groups into chitosan via stable N–C linkages. The reductive amination proceeds selectively at -NH<sub>2</sub> groups over -OH groups; however, the low degree of substitution and the use of a highly toxic chemical, sodium cyanoborohydride (NaBH<sub>3</sub>CN; the acute

oral toxicity (based on LD50 value) is Category 2 in the GHS classification), as a reducing agent have posed issues. For example, Tantayanon and collaborators [18] reported that reductive amination of chitosan with benzaldehyde using NaBH<sub>3</sub>CN under homogeneous conditions (or liquid-phase reactions) has provided *N*-benzyl chitosan with a very low degree of substitution (e.g., 18.5%).

Finding an alternative, less harmful, and more efficient method for the reductive amination of chitosan compared to the one currently in place is thus one of the most important issues for the chemical modification of chitosan.

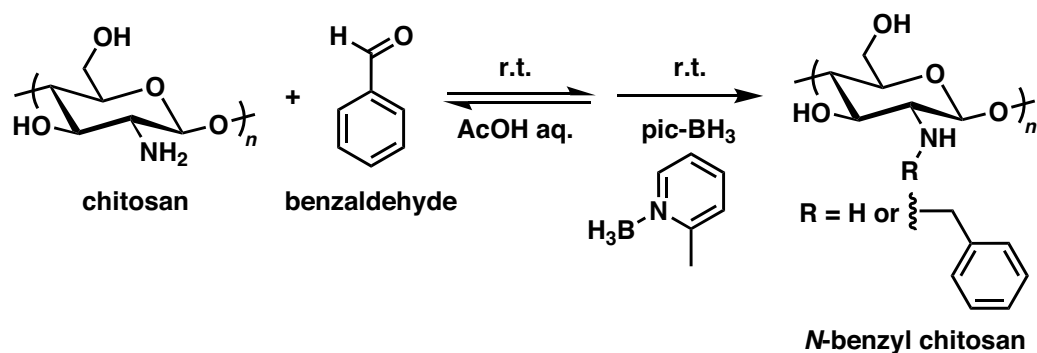
Recently, Dez and collaborators [19] reported that their three-step synthesis (i.e., chitosan aerogel bead preparation/Schiff base formation/reduction using NaBH<sub>3</sub>CN) is effective and that an *N*-benzyl chitosan derivative with a high degree of substitution (82%) can be obtained. This synthetic method is of great interest; however, it requires three individual synthetic steps and supercritical CO<sub>2</sub>, which is not available in some laboratories. Thus, there is still a need to develop a facile and efficient reductive amination method for accessing chitosan with relatively few toxic reagents.

2-Picoline borane (Pic-BH<sub>3</sub>) is a commercially available, thermally stable, and less toxic reducing agent (acute oral toxicity is Category 4) than NaBH<sub>3</sub>CN. Kikugawa and collaborators [20] reported that pic-BH<sub>3</sub> is a very effective reducing agent for the reductive amination of low-molecular-weight organic compounds. However, to the best of our knowledge, there have been no reports on the use of pic-BH<sub>3</sub> for the reductive amination of high-molecular-weight materials, such as chitosan.

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**Scheme 1** One-pot synthesis of *N*-benzyl chitosan utilizing pic-BH<sub>3</sub> as a reducing agent

**Table 1** Reductive amination of chitosan with benzaldehyde and NaBH<sub>4</sub>

Run	Feed molar ratio			Benzylation degree (%) <sup>a</sup>	Residual imine moiety (%) <sup>b</sup>	Yield (%) <sup>c</sup>
	-NH <sub>2</sub>	-CHO	NaBH <sub>4</sub>			
1	1	2	3	22	0.91	62
2	1	2	22.6	16	0	62
3	1	20	22.6	74	1.0	80

<sup>a</sup>Based on the deconvoluted integral ratio between protons **2** (*N*-benzyl chitosan) and **2'** (chitosan) in <sup>1</sup>H NMR spectra

<sup>b</sup>Based on the integral ratio between the proton ca. 9.8 ppm and the sum of the protons **2** (*N*-benzyl chitosan) and **2'** (chitosan) in the <sup>1</sup>H NMR spectra

<sup>c</sup>The 100% yield is based on the mass of 100% *N*-benzyl chitosan

In this paper, pic-BH<sub>3</sub> was utilized for the first time as a reducing agent for the reductive amination of chitosan. Consequently, *N*-benzyl chitosan with a nearly perfect degree of substitution was successfully obtained from a facile reaction procedure (only mixing all chemicals in the same beaker) within a short reaction time (<1 day) (Scheme 1). For comparison, the use of NaBH<sub>4</sub> as another reducing agent (sodium borohydride with Category 3 acute oral toxicity) was examined.

## Experimental procedures

### Materials

Commercial chitosan (degree of deacetylation = 75–85%, viscosity (1 wt.% in 1% acetic acid at 25 °C) = 200–800 cP) was obtained from Sigma–Aldrich (medium molecular weight grade). Commercial chitosan was treated twice in ca. 33 wt.% aqueous NaOH at 105 °C for 3 h under a nitrogen atmosphere to prepare chitosan (with a degree of deacetylation ≈ 99%) with a high degree of deacetylation, similar to previous literature [12]. Sodium borohydride (NaBH<sub>4</sub>; TCI),

2-picoline borane (pic-BH<sub>3</sub>; TCI), benzaldehyde (FUJIFILM Wako), acetic acid (AcOH; Kanto Chemical), sodium hydroxide (NaOH; Kanto Chemical), potassium carbonate (K<sub>2</sub>CO<sub>3</sub>; Kanto Chemical), trifluoroacetic acid (TFA; TCI), and deuterium oxide (D<sub>2</sub>O; Kanto Chemical) were of commercial grade and used as received.

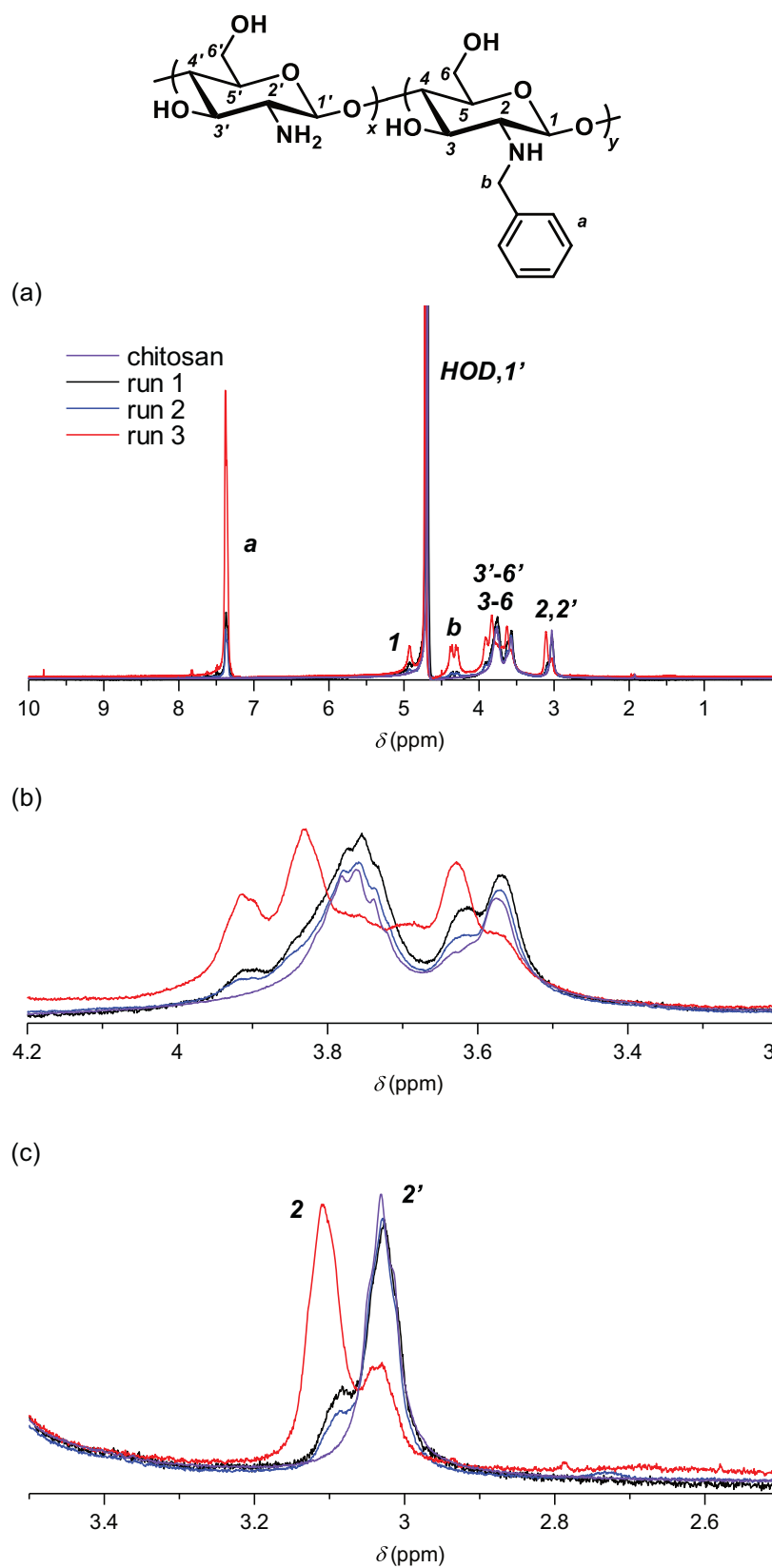
### Measurement

Nuclear magnetic resonance (NMR) spectra were obtained with an AVANCE III HD spectrometer (500 MHz, Bruker) in 1% TFA containing D<sub>2</sub>O as a solvent. Viscosity was measured using a Ubbelohde capillary viscometer (No. 1) at 23 °C. Aqueous polymer solutions (*c* = 1 g L<sup>-1</sup>) in 0.5 M AcOH and 0.1 M NaNO<sub>3</sub> were prepared. Immediately before the measurements, the solutions were filtered through a 1.0-μm GF filter. The flow times of the solutions and a blank solvent (0.5 M AcOH and 0.1 M NaNO<sub>3</sub>) were recorded three times, and the average values were calculated.

### Reductive amination of chitosan with benzaldehyde

The following synthetic procedure for run 6 (Table 2) is a typical example. In a 200 mL beaker, chitosan (0.2 g, -NH<sub>2</sub> group of ca. 1.24 mmol) was dissolved in a 1% AcOH aqueous solution (14 mL, AcOH of 2.45 mmol), followed by the addition of 1 M NaOH (0.52 g, NaOH of 0.5 mmol). After the solution became homogeneous, benzaldehyde (0.2632 g, 2.48 mmol) was added dropwise. The solution was stirred at room temperature for 1 h, after which pic-BH<sub>3</sub> (purity of 86.7%, 0.4589 g, 3.72 mmol) was slowly added. After stirring at room temperature for 19 h, ca. 150 mL of water and 1 M K<sub>2</sub>CO<sub>3</sub> (5 mL, 5 mmol of K<sub>2</sub>CO<sub>3</sub>) were added to the mixture. The mixture was filtered, and the obtained solid was washed with methanol and water. The solid was dried in a vacuum oven at 50 °C, providing a white solid (0.271 g) in 87% yield.

**Fig. 1**  $^1\text{H}$  NMR spectra of *N*-benzyl chitosan in runs 1–3 (a) from 0 to 10 ppm, (b) from 3.2 to 4.2 ppm, and (c) from 2.5 to 3.5 ppm at room temperature in 1% TFA containing  $\text{D}_2\text{O}$  as a solvent. The chitosan data are included for comparison



**Table 2** Reductive amination of chitosan with benzaldehyde and pic-BH<sub>3</sub>

Run	Feed molar ratio			Benzylation degree (%) <sup>a</sup>	Residual imine moiety (%) <sup>b</sup>	Yield (%) <sup>c</sup>
	-NH <sub>2</sub>	-CHO	pic-BH <sub>3</sub>			
4	1	1	1	46	0	63
4 <sup>d</sup>	1	1	1	49	0	54
5	1	2	2	80	0.22	70
6	1	2	3	100 <sup>e</sup>	0.15	87

<sup>a</sup>Based on the deconvoluted integral ratio between protons **2** (*N*-benzyl chitosan) and **2'** (chitosan) in <sup>1</sup>H NMR spectra

<sup>b</sup>Based on the integral ratio between the proton (ca. 9.8 ppm) and the sum of the protons **2** (*N*-benzyl chitosan) and **2'** (chitosan) in the <sup>1</sup>H NMR spectra

<sup>c</sup>The 100% yield is based on the mass of 100% *N*-benzyl chitosan

<sup>d</sup>Low-molecular-weight chitosan (with a degree of deacetylation ≈99%) was utilized. This was prepared from commercial chitosan (degree of deacetylation = 75–85%, viscosity (1 wt.% in 1% acetic acid at 25 °C) = 20–300 cP, Sigma–Aldrich (low molecular weight grade)). The deacetylation process was conducted in the same manner as that for medium-molecular-weight chitosan.

<sup>e</sup>Deconvolution was not possible for the signals of **2** (*N*-benzyl chitosan) and **2'** (chitosan)

## Results and discussion

As described in the experimental procedures, chitosan with a high degree of deacetylation (ca. 99%) was prepared and utilized in this paper. The reductive amination of chitosan was conducted in a one-pot manner. The dissolution of chitosan in 1% acetic acid (AcOH), the addition of benzaldehyde (imine formation), and the addition of a reducing agent (imine reduction) were conducted in the same beaker.

Table 1 presents a summary of the results of the reductive amination of chitosan with benzaldehyde and NaBH<sub>4</sub>. The molecular structure of the obtained *N*-benzyl chitosan was characterized by <sup>1</sup>H NMR spectroscopy at room temperature in 1% TFA containing D<sub>2</sub>O as a solvent (Fig. 1). The <sup>1</sup>H-<sup>1</sup>H COSY spectra (Supplementary Figs. S1 and S2) helped with the assignment of all the <sup>1</sup>H NMR signals in Fig. 1. Notably, the integral ratios of signals derived from the side chain (i.e., protons **a** and **b** of benzyl groups) and a part of the main chain (i.e., protons **1** and **2** of *N*-benzyl chitosan) clearly increased as the degree of substitution or degree of benzylation increased. In fact, the referential unsubstituted chitosan (purple line in Fig. 1) did not have any signals in these areas and had a main chain proton **2'** (ca. 3.03 ppm) instead of the proton **2** (ca. 3.10 ppm) that appeared in *N*-benzyl chitosan. This assignment was in good accordance with the previous literature by Kurita and Isogai [12]. In this paper, the degree of benzylation was calculated based on the integral ratio between protons **2** (*N*-benzyl chitosan) and **2'** (chitosan) in the <sup>1</sup>H NMR spectra. Furthermore, the degree of incomplete reductive amination

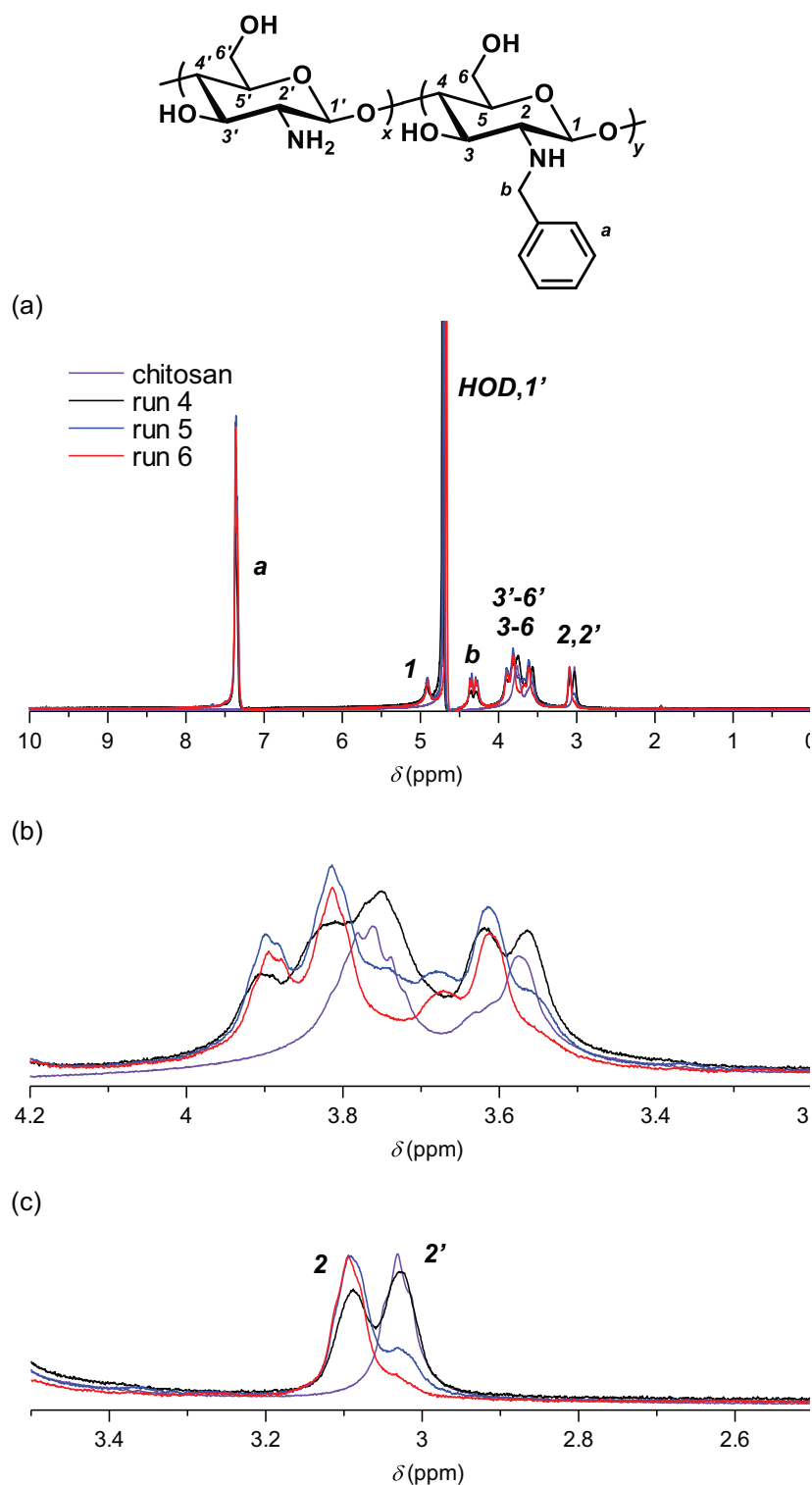
(or residual imine moiety) was quantified based on the <sup>1</sup>H NMR spectra.

In runs 1–3 in Table 1, the reactions were conducted in a similar manner, except for the feed molar ratio of the -NH<sub>2</sub> group in chitosan, the -CHO group of benzaldehyde, and NaBH<sub>4</sub>. First, the run 1 condition resulted in a degree of benzylation of 22%, despite the excess amounts of benzaldehyde and NaBH<sub>4</sub> (molar ratio of -NH<sub>2</sub>:-CHO:-NaBH<sub>4</sub> = 1:2:3). The low degree of benzylation was in good agreement with that reported in a previous paper under similar conditions by Smagghe and collaborators [21] (molar ratio of -NH<sub>2</sub>:-CHO:NaBH<sub>4</sub> = 1:1:1.5, degree of benzylation of 15%). In run 2, a further increase in the molar ratio of NaBH<sub>4</sub> (molar ratio of -NH<sub>2</sub>:-CHO:-NaBH<sub>4</sub> = 1:2:22.6) slightly decreased the degree of benzylation (from 22 to 16%). Conversely, in run 3, an increase in the molar ratio of benzaldehyde (molar ratio of -NH<sub>2</sub>:-CHO:NaBH<sub>4</sub> = 1:20:22.6) drastically increased the degree of benzylation to 74%. Overall, the degree of benzylation of chitosan using NaBH<sub>4</sub> via reductive amination could be improved by greatly increasing the feed molar ratios of benzaldehyde and NaBH<sub>4</sub>. However, the efficiency was overly low, which was not acceptable in the context of green chemistry. One of the reasons for the low efficiency of NaBH<sub>4</sub> was its low stability in acidic aqueous solution. The reason for the importance of the stability was that the reaction between the -NH<sub>2</sub> and -CHO groups was reversible (Scheme 1), and the imine formation did not occur sufficiently when an unstable reductant, such as NaBH<sub>4</sub>, was used in the reductive amination (Table 1).

Pic-BH<sub>3</sub> is known to be more stable than NaBH<sub>4</sub> in acidic aqueous solution. Thus, it was expected that the use of pic-BH<sub>3</sub> as a reducing agent would improve the reductive amination efficiency of chitosan. Table 2 presents a summary of the results of the reductive amination of chitosan with benzaldehyde and pic-BH<sub>3</sub>, and Fig. 2 shows the <sup>1</sup>H NMR spectra for the obtained *N*-benzyl chitosan in runs 4–6.

Consequently, even stoichiometric conditions (namely, a feed molar ratio of -NH<sub>2</sub>:-CHO:pic-BH<sub>3</sub> = 1:1:1) resulted in a high degree of benzylation of 46% (Table 2, run 4). During the reaction, pic-BH<sub>3</sub> probably avoided decomposition for a longer time than NaBH<sub>4</sub>, sufficiently shifting the equilibrium toward imine formation and resulting in highly efficient reductive amination. Using relatively low-molecular-weight chitosan (see run 4' in Table 2 and Supplementary Fig. S3 for details) did not greatly affect the degree of benzylation. However, increasing the feed molar ratios of both benzaldehyde and pic-BH<sub>3</sub> were very effective (runs 5 and 6). In particular, run 6 provided *N*-benzyl chitosan with a nearly perfect degree of benzylation and a high isolated yield. The negligible amount of residual imine moiety would provide evidence of the high reactivity of pic-

**Fig. 2**  $^1\text{H}$  NMR spectra of *N*-benzyl chitosan in runs 4–6 (a) from 0 to 10 ppm, (b) from 3.2 to 4.2 ppm, and (c) from 2.5 to 3.5 ppm at room temperature in 1% TFA containing  $\text{D}_2\text{O}$  as a solvent. The chitosan data are included for comparison



$\text{BH}_3$ . Notably, the integral ratios of all the  $^1\text{H}$  NMR signals were in good accordance with the molecular structures of *N*-benzyl chitosan specimens with degrees of benzylation of 100% (red line in Fig. 2). The greater efficiency of pic- $\text{BH}_3$  compared with that of  $\text{NaBH}_3\text{CN}$  [18, 19] could be due to the greater nucleophilicity of the B-H bond in pic- $\text{BH}_3$

because the cyano group in  $\text{NaBH}_3\text{CN}$  weakened the nucleophilicity of the B-H bond as a hydride source.

To determine the molecular weight, viscosity measurements ( $c = 1 \text{ g L}^{-1}$  in 0.5 M AcOH and 0.1 M  $\text{NaNO}_3$ ) were conducted at 23 °C. The specific viscosity of chitosan was 0.95, whereas that of *N*-benzyl chitosan (run 6) was 0.37.

Similarly, Gorochovceva and collaborators [22] reported that after performing  $\text{NaBH}_3\text{CN}$ -mediated reductive amination, functionalized chitosan exhibited a much lower viscosity (less than one in tenth) than the original chitosan, although the molecular weight was sufficiently high (before deacetylation:  $M_r$  of 400,000; after reductive amination:  $M_n$  of 450,000;  $M_w$  of 830,000).

The new synthetic method designed herein (i.e., the reductive amination of chitosan utilizing  $\text{pic-BH}_3$ ) had significant advantages, such as being more facile, more efficient, and less harmful than the conventional method; thus, this method could be one of the first choices for enhancing the sustainability of the reductive amination of chitosan in the future. Since one of the concerns of this method was its atom economy being lower than that of  $\text{NaBH}_4$ , finding an efficient recycling method for  $\text{pic-BH}_3$  could be considered an important research topic. Furthermore, the application of this new method for functional polymer synthesis is currently underway, such as by synthesizing ion-conductive polymers for many applications.

## Conclusions

For the first time, this paper revealed that  $\text{pic-BH}_3$  is a very effective reducing agent for the reductive amination of chitosan. Therefore, *N*-benzyl chitosan with a degree of benzylation of ca. 100% can be obtained, which has rarely been achieved by other synthetic methods. Compared with conventional methods, the new synthetic method described herein has several advantages (e.g., less harm, a more concise reaction procedure, and a shorter reaction time).  $\text{Pic-BH}_3$  can be applied to other aldehydes or ketones, leading to a wide variety of *N*-modified chitosan specimens with desirable degrees of substitution in the future. Since the precise control of the degree of substitution strongly affects the polymer properties, the knowledge herein can accelerate the development of new *N*-modified chitosan materials for each application.

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## Compliance with ethical standards

**Conflict of interest** The author declares no competing interests.

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