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Finely selective protections and deprotections of multifunctional chitin and chitosan to synthesize key intermediates for regioselective chemical modifications

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ABSTRACT

Site-selective protection of chitin and chitosan has been studied in detail in order to distinguish the three kinds of functional groups, which would make possible finely controlled regiospecific structural modifications. To allow reliable chemical manipulations and to establish stable protection and facile deprotection at high selectivity, benzyl was evaluated for the C-3 protection in combination with other protective groups including triphenylmethyl (trityl) for C-6 and acetyl or phthaloyl for C-2. Chitin was first tritylated and then benzylated to give 3-*O*-benzyl-6-*O*-trityl-chitin, of which each of the trityl, benzyl, and acetyl groups could be removed selectively with dichloroacetic acid, hydrogen-Pd/C, and aqueous sodium hydroxide, respectively, affording three kinds of derivatives having a reactive group at C-6, C-3, or C-2. 2-*N*-Phthaloyl-chitosan was also tritylated at C-6 and benzylated at C-3; the resulting fully protected product was detritylated, debenzylated, or dephthaloylated, similarly giving rise to three kinds of precursors having a reactive group only at one position. The extents of all the substitution and removal reactions proved quantitative under appropriate conditions to give structurally well-defined derivatives. They exhibited improved solubility in organic solvents, indicating high potential of these derivatives as novel convenient intermediates for designing diversified molecular architectures through regiospecific chemical modifications.

1. Introduction

Because of the presence of amino groups, polysaccharides chitin and chitosan are expected to be particularly useful biopolymers in various fields (Domard, Guibal, & Vårum, 2007; Uragami, Kurita, & Fukamizo, 2001; Uragami & Tokura, 2006). Although they are abundant and easily accessible, and therefore attracting much attention, their utilization has been quite limited. This is primarily due to the difficulty in fabrication and structural transformation, which is ascribed to the lack of solubility in suitable solvents. The various distinctive biological and physicochemical functions of chitin and chitosan suggest the prospect of synthesizing intelligent polymeric materials having medicinal and pharmaceutical activities by appropriate chemical modifications (Kurita, 1997, 2001, 2006a, 2006b; Nishimura, Kohgo, Kurita, & Kuzuhara, 1991; Roberts, 1992).

For precise and well-controlled structural modifications of these polysaccharides to develop advanced materials with desirable bioactivities, it is necessary to clearly distinguish the three kinds of functional groups in their repeating units. In this respect, 2-*N*-phthaloyl-chitosan is the practical derivative currently used, since it is organosoluble and useful for the regioselective chemical modifications (Nishimura et al., 1991). Several kinds of sugar branches can be incorporated at the C-6 position to synthesize non-natural branched chitins and chitosans (Kurita, Akao, Kobayashi, Mori, & Nishiyama, 1997; Kurita, Shimada, Nishiyama, Shimojoh, & Nishimura, 1998; Kurita, Kojima, Nishiyama, & Shimojoh, 2000; Kurita, Akao, Yang, & Shimojoh, 2003). Many other substituents have also been introduced for various purposes using 2-*N*-phthaloyl-chitosan (Holappa et al., 2004, 2005; Kurita, Hayakawa, Nishiyama, & Harata, 2002; Nishimura et al., 1993, 1998; Nishiyama et al., 2000; Ouchi, Nishizawa, & Ohya, 1998; Yoksan, Akashi, Hiwatari, & Chirachanchai, 2003; Yoksan, Matsusaki, Akashi, & Chirachanchai, 2004).

In the above procedures, the C-3 hydroxy was protected by acetylation, but the resulting ester linkage is somewhat vulnerable and may undergo hydrolysis and/or acetyl migration under certain reaction conditions, which limits its utility as a precursor. To further expand the scope of structural modifications of chitin and chitosan, stable protection and facile deprotection of the C-3 hydroxy group are undoubtedly key issues. In view of the reliability for *O*-protection in chemical manipulations, benzyl would be a promising candidate. It has, however, not been used for chitin and

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Table 1
Benzylation of 6-*O*-trityl-chitin (**1**)[a].

Base	Base/pyranose[b]	BnCl/pyranose[b]	Repetition of reaction[c]	ds for Bn[d]	Yield (%)
NaOH	2	6	2	0.85	65
NaOH	6	12	2	0.80	72
NaH	2	6	1	1.00	75

[a] **1**, 0.30 g; DMSO, 4.5 mL; temp, rt; time, 24 h.
[b] Mole ratio.
[c] Number of repetition under the same reaction conditions.
[d] Degree of substitution calculated from the C/N of elemental analysis.

chitosan, and we have thus examined the possibility of benzyl as a protecting group for C-3 in terms of both protection and deprotection in the presence of other protective groups to synthesize versatile intermediates for site-selective chemical modifications at the three different fictional groups.

2. Experimental

2.1. General procedures

IR spectra were taken on a Shimadzu FTIR-8900 spectrometer. ^{1}H NMR spectra were recorded with a JEOL JNM-LA400D FT-NMR in deuterated dimethyl sulfoxide (DMSO-d_6) at 90 °C. Elemental analysis was conducted with a Perkin-Elmer 2400 II instrument. Conductometric titration was carried out with a DKK·TOA CM-20J. HPLC was performed with a Waters 486 equipped with a Waters Controller 800, SIM Chromatocorder 21, and a Bondasphere column (5 μm, C18, 100A) with a mobile phase of acetonitrile/water (4/1). Chemicals were of reagent grade and used after drying. Dimethyl sulfoxide (DMSO) and *N*,*N*-dimethylformamide (DMF) were dried with calcium hydride and molecular sieves, respectively, and distilled. Pyridine was refluxed with potassium hydroxide and distilled. All the solvents were stored over molecular sieves.

2.2. Structurally uniform chitin and chitosan

Squid chitin with a degree of acetylation of 0.8–0.9 was *N*-acetylated with acetic anhydride in methanol, and a small amount of *O*-acetyl groups were selectively removed by treating with potassium hydroxide/methanol to give white powdery chitin with a degree of *N*-acetylation of 1.00 as determined by conductometric titration (Kurita, Ishii, Tomita, Nishimura, & Shimoda, 1994).

Pulverized shrimp chitin was deacetylated repeatedly with 40% aqueous sodium hydroxide to give fully deacetylated chitosan as a white powder. Conductometric titration indicated the degree of deacetylation to be 1.00 (Nishimura, Matsuoka, & Kurita, 1990).

2.3. 6-*O*-Trityl-chitin

Trimethylsilylated chitin with a degree of substitution (ds) 2.00 was treated with chlorotriphenylmethane to introduce triphenylmethyl (trityl) group at C-6 according to the method reported previously (Kurita, Sugita, Kodaira, Hirakawa, & Yang, 2005). The ds was 1.00 for the trityl as confirmed by spectroscopy and elemental analysis. IR (KBr): ν 3408 (OH and NH), 3057 (arom), 1670 (amide I), 1522 (amide II), 1150–1000 (pyranose), and 748 and 706 cm^{-1} (arom).

Anal. Calcd for $C_{27}H_{27}NO_5 \cdot 1.4H_2O$: C, 68.89; H, 6.38; N, 2.98. Found: C, 68.72; H, 6.31; N, 2.92

2.3.1. Benzylation of 6-*O*-trityl-chitin

6-*O*-Trityl-chitin (0.30 g, 0.67 mmol) obtained above was dissolved in 4.5 mL of DMSO, and 0.03 g (1.34 mmol) of sodium hydride was added. After stirring the mixture in nitrogen at room temperature for 2 h, 0.51 g (4.04 mmol) of benzyl chloride (BnCl) was added dropwise. The mixture was stirred at room temperature for 24 h, and the resulting solution was poured into 30 mL of methanol to precipitate the product. It was washed with water and methanol and dried to give 0.27 g (75%) of 3-*O*-benzyl-6-*O*-trityl-chitin as a pale tan powdery material. IR (KBr): ν 3420 (NH), 3058 (arom), 1682 (amide I), 1150–1000 (pyranose), and 746 and 706 cm^{-1} (arom).

Anal. Calcd for $C_{34}H_{33}NO_5 \cdot 0.5H_2O$: C, 74.98; H, 6.29; N, 2.57. Found: C, 75.04; H, 6.39; N, 2.53.

2.3.2. Detritylation of 3-*O*-benzyl-6-*O*-trityl-chitin

To 3 mL of a dichloroacetic acid/DMSO (1/1) mixed solvent was added 30 mg (0.056 mmol) of 3-*O*-benzyl-6-*O*-trityl-chitin, and the mixture was stirred at room temperature for 1 h. It was poured into 10 mL of acetonitrile/water (4/1), and the precipitate was washed with water and methanol. After drying, 13 mg (79%) of 3-*O*-benzyl-chitin was obtained as a pale tan powdery material. IR (KBr): ν 3406 (OH and NH), 3059 (arom), 1653 (amide I), 1523 (amide II), 1150–1000 (pyranose), and 768, 748, and 702 cm^{-1} (arom).

Anal. Calcd for $C_{15}H_{19}NO_5 \cdot 0.4H_2O$: C, 59.95; H, 6.64; N, 4.66. Found: C, 59.94; H, 6.47; N, 4.60.

2.3.3. Deacetylation of 3-*O*-benzyl-6-*O*-trityl-chitin

A mixture of 20 mg (0.037 mmol) of 3-*O*-benzyl-6-*O*-trityl-chitin in 10 mL of 12 mol/L sodium hydroxide was stirred at 80 °C for 8 h. After cooling to room temperature, the precipitate was washed by repeated decantations with water until neutral, filtered, and dried to give 15 mg (81%) of 3-*O*-benzyl-6-*O*-trityl-chitosan. IR (KBr): ν 3411 (NH_2), 3059 (arom), 1599 (NH_2), 1150–1000 (pyranose), and 746 and 700 cm^{-1} (arom).

Anal. Calcd for $C_{32}H_{31}NO_4 \cdot 0.5H_2O$: C, 76.47; H, 6.42; N, 2.79. Found: C, 76.35; H, 6.41; N, 2.78.

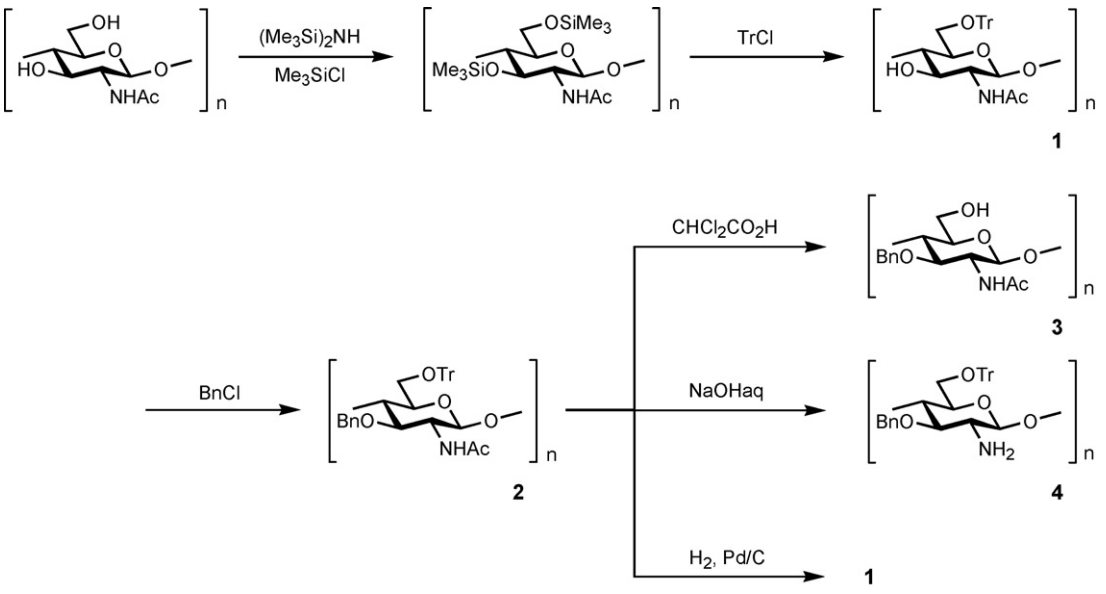
2.3.4. Debenzylation of 3-*O*-benzyl-6-*O*-trityl-chitin

To a solution of 3-*O*-benzyl-6-*O*-trityl-chitin (30 mg, 0.056 mmol) in 6 mL of DMSO/acetic acid (10:1) was added 30 mg of 5% Pd/C. The mixture was stirred under an atmosphere of hydrogen at 70 °C for 24 h and filtered with Celite 545. The filtrate was concentrated under reduced pressure. The product was precipitated in water, washed with water and methanol, and dried to give 20 mg (80%) of 6-*O*-trityl-chitin. The spectral data were identical with those of the authentic sample.

Anal. Calcd for $C_{27}H_{27}NO_5 \cdot 0.4H_2O$: C, 71.63; H, 6.19; N, 3.09. Found: C, 71.70; H, 6.05; N, 3.08.

2.4. 2-*N*-Phthaloyl-6-*O*-trityl-chitosan

Chitosan was subjected to *N*-phthaloylation with phthalic anhydride in DMF/water (Kurita, Ikeda, Yoshida, Shimojoh, & Harata, 2002) followed by tritylation with chlorotriphenylmethane in pyridine as reported (Nishimura et al., 1991). IR (KBr): ν 3472 (OH), 3058 (arom), 1775 and 1716 (imide C=O), 1150–1000 (pyranose), and 743 and 700 cm^{-1} (arom).



Scheme 1.

Anal. Calcd for $C_{33}H_{27}NO_6 \cdot 1.1H_2O$: C, 68.89; H, 6.38; N, 2.98. Found: C, 68.72; H, 6.31; N, 2.92.

2.4.1. Benzylation of 2-N-phthaloyl-6-O-trityl-chitosan

A mixture of 50 mg (2.1 mmol) of sodium hydride and 4.5 mL of DMSO was heated at 70 °C in nitrogen for 1 h to give an almost homogeneous solution and cooled to room temperature. A portion (1.2 equiv.) of the solution was added to 0.30 g (0.56 mmol) of 2-*N*-phthaloyl-6-*O*-trityl-chitosan dissolved in 4.5 mL of DMSO. The mixture was stirred at room temperature for 2 h, and 0.43 g (3.40 mmol) of benzyl chloride was added dropwise. After stirring the mixture at room temperature for 24 h, the resulting solution was poured into 30 mL of methanol to precipitate the product, which was washed with water and methanol and dried to give 0.27 g (77%) of 3-*O*-benzyl-2-*N*-phthaloyl-6-*O*-trityl-chitosan as a pale tan powdery material. IR (KBr): ν 3059 (arom), 1778 and 1719 (imide C=O), 1150–1000 (pyranose), and 746 and 704 cm^{-1} (arom). ^1H NMR (DMSO-d_6): δ 3.5–5.2 (pyranose), 6.8–7.3 (phenyl), and 7.5–7.9 ppm (phthaloyl).

Anal. Calcd for $C_{40}H_{33}NO_6 \cdot 0.2H_2O$: C, 76.59; H, 5.37; N, 2.23. Found: C, 76.78; H, 5.21; N, 2.25.

2.4.2. Detritylation of 3-O-benzyl-2-N-phthaloyl-6-O-trityl-chitosan

To 2 mL of dichloroacetic acid/DMSO (2/3) was added 20 mg (0.032 mmol) of 3-*O*-benzyl-2-*N*-phthaloyl-6-*O*-trityl-chitosan, and the solution was stirred at room temperature for 1 h. The product was precipitated in acetonitrile/water (4:1), washed with water and methanol, and dried to give 3-*O*-benzyl-2-*N*-phthaloyl-chitosan. The yield of a white powdery material was 9 mg (74%). IR (KBr): ν 3481 (OH and NH), 3059 (arom), 1778 and 1719 (imide C=O), 1150–1000 (pyranose), and 746 and 712 cm^{-1} (arom). ^1H NMR (DMSO-d_6): δ 3.5–5.2 (pyranose) and 7.2–7.9 ppm (phenyl and phthaloyl).

Anal. Calcd for $C_{21}H_{19}NO_6 \cdot 0.3H_2O$: C, 65.21; H, 5.11; N, 3.62. Found: C, 65.28; H, 4.99; N, 3.56.

2.4.3. Dephthaloylation of 3-O-benzyl-2-N-phthaloyl-6-O-trityl-chitosan

3-*O*-Benzyl-2-*N*-phthaloyl-6-*O*-trityl-chitosan (30 mg, 0.048 mmol) was added to 10 mL of hydrazine monohydrate, and the mixture was heated at 90 °C for 24 h. The product was washed with water by decantation until neutral, filtered, and dried to give 21 mg (88%) of 3-*O*-benzyl-6-*O*-trityl-chitosan as a white powder. IR (KBr): ν 3450 (NH_2), 3057 (arom), 1595 (NH_2), 1150–1000 (pyranose), and 748 and 704 cm^{-1} (arom). ^1H NMR (DMSO-d_6): δ 3.5–5.2 (pyranose) and 7.2–7.9 ppm (phenyl and phthaloyl).

Anal. Calcd for $C_{33}H_{31}NO_4 \cdot 0.8H_2O$: C, 76.22; H, 6.32; N, 2.69. Found: C, 76.05; H, 6.24; N, 2.73.

2.4.4. Debenzylation of 3-O-benzyl-2-N-phthaloyl-6-O-trityl-chitosan

3-*O*-Benzyl-2-*N*-phthaloyl-6-*O*-trityl-chitosan (80 mg, 0.128 mmol) was dissolved in 4 mL of DMSO/acetic acid (10/1), and 80 mg of 5% Pd/C was added. The mixture was heated in a hydrogen atmosphere with stirring at 80 °C for 24 h. It was filtered with Celite 545, and the filtrate was concentrated under reduced pressure. The viscous solution was poured into 30 mL of ethanol, and the precipitate was collected by centrifugation. It was washed with water and methanol, and dried to give 65 mg (95%) of 2-*N*-phthaloyl-6-*O*-trityl-chitosan as a white powder. IR (KBr): ν 3425 (OH), 3059 (arom), 1776 and 1717 (imide C=O), 1150–1000 (pyranose), and 746 and 704 cm^{-1} (arom). ^1H NMR (DMSO-d_6): δ 3.5–5.2 (pyranose), 6.92 (phenyl), and 7.5–7.7 ppm (phthaloyl).

Anal. Calcd for $C_{33}H_{27}NO_6 \cdot 0.5H_2O$: C, 73.05; H, 5.20; N, 2.58. Found: C, 73.02; H, 5.07; N, 2.57.

3. Results and discussion

In order to discuss the influence of reaction conditions on the structures of products and to synthesize well-defined derivatives, structurally uniform chitin and chitosan should be used as starting materials. Because of the presence of some free amino groups in isolated chitin, acetylation was conducted to give fully *N*-acetylated chitin. Fully *N*-deacetylated chitosan was prepared by repeated alkaline treatments, from which *N*-phthaloyl-chitosan was derived.

3.1. Benzylation of 6-O-trityl-chitin

6-*O*-Trityl-chitin (**1**) prepared by the tritylation of 3,6-*O*-bistrimethylsilyl-chitin was benzylated in the presence of pulverized sodium hydroxide in DMSO under various conditions. The substitution was, however, not quantitative, even after repeat-

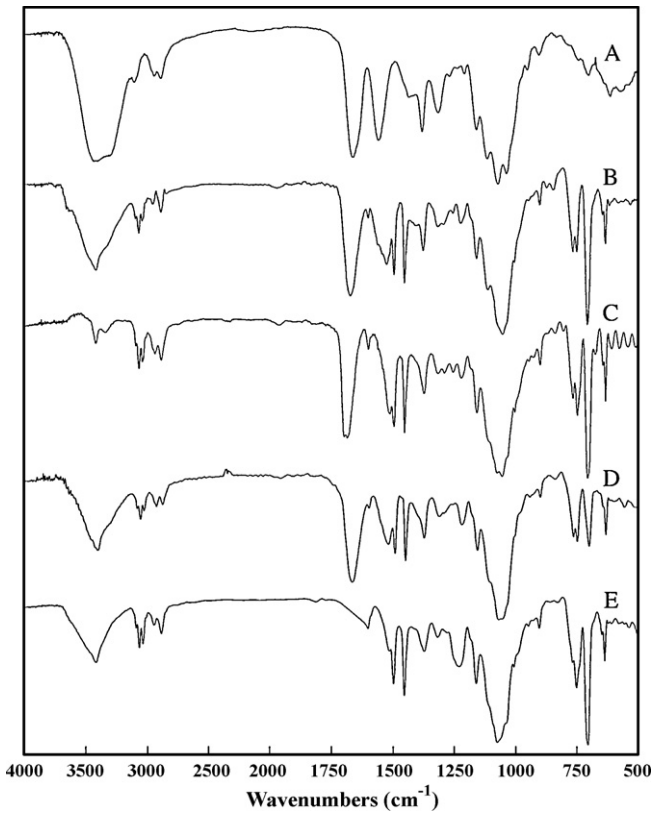


Fig. 1. IR spectra of chitin derivatives (KBr): A, chitin; B, 6-*O*-trityl-chitin (**1**); C, 3-*O*-benzyl-6-*O*-trityl-chitin (**2**); D, 3-*O*-benzyl-chitin (**3**); E, 3-*O*-benzyl-6-*O*-trityl-chitosan (**4**).

ing the reaction two times with excess base and benzyl chloride (Table 1), probably because of the high hygroscopicity of the base in the powder form. With sodium hydride, the reaction proceeded smoothly, and the ds reached 1.0 (Scheme 1, Table 1). In the IR spectrum of the product, 3-*O*-benzyl-6-*O*-trityl-chitin (**2**), bands due to the hydroxy disappeared, and strong aromatic bands were observed (Fig. 1).

3.2. Detritylation of 3-O-benzyl-6-O-trityl-chitin (*2*)

O-Trityl is usually removed with acid, and dichloroacetic acid was suited for the deprotection of 3-*O*-acetyl-2-*N*-phthaloyl-6-*O*-trityl-chitosan to afford a precursor for C-6 modifications (Nishimura et al., 1991). The acid is, however, rather strong and may possibly interfere with the benzyl of **2**. As expected, the HPLC analysis of the supernatant of the reaction mixture indicated the presence of both trityl and benzyl alcohols as shown in Fig. 2. On dilution of dichloroacetic acid with DMSO, the peak due to benzyl alcohol became small, and a 1/1 mixture did not cause debenzylation. Elemental analysis also revealed debenzylation in addition to detritylation at high acid concentrations, and the 1/1 mixture proved appropriate for selective full detritylation to give 3-*O*-benzyl-chitin (**3**) (Table 2, Scheme 1). The IR spectrum in Fig. 1 showed hydroxy bands, and the bands due to aromatic became weak.

3.3. Deacetylation of 3-O-benzyl-6-O-trityl-chitin (*2*)

To transform **2** into the derivative having free amino groups, it was treated with aqueous alkali to remove the acetyl. The hydrolysis of the amide group was, however, rather sluggish, and strong amide bands remained in the IR spectra under mild conditions.

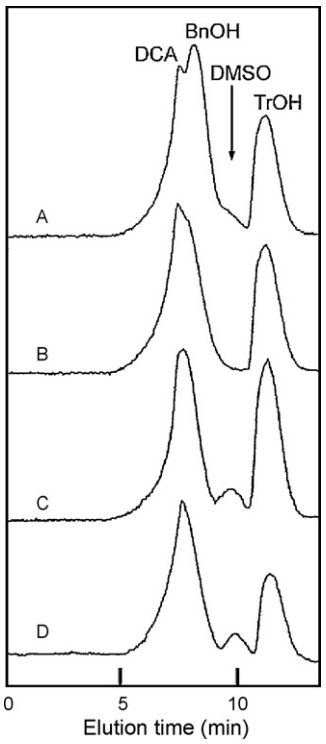


Fig. 2. HPLC profiles of the reaction mixtures of **2** with dichloroacetic acid (DCA)/DMSO (A, 10/0; B, 7/3; C, 5/5; D, 3/7).

Table 2
Detritylation of 3-*O*-benzyl-6-*O*-trityl-chitin (**2**)[a].

Dichloroacetic acid/DMSO (v/v)	ds[b]		Yield (%)
	Tr	Bn	
10/0	0.00	0.27	88
7/3	0.00	0.75	92
6/4	0.00	0.92	87
5/5	0.00	1.00	91
4/6	0.07	1.00	92
3/7	–	–	–[c]

[a] **2**, 30 mg; dichloroacetic acid/DMSO, 3 mL; temp, rt; time 1 h.
[b] Degree of substitution calculated from the C/N of elemental analysis.
[c] Aromatic bands in the IR spectrum were strong, and the peak due to trityl alcohol in the HPLC profile of the reaction mixture was small (Fig. 2).

The reaction was thus performed in 12 mol/L sodium hydroxide to give a fully deacetylated product, 3-*O*-benzyl-6-*O*-trityl-chitosan (**4**) (Table 3, Scheme 1). As evident in Fig. 1, amide I and II bands disappeared completely in the IR spectrum, and aromatic bands remained strong.

Table 3
Deacetylation of 3-*O*-benzyl-6-*O*-trityl-chitin (**2**)[a].

NaOHaq (mol/L)	Temperature (°C)	Time (h)	ds for Ac[b]	Yield (%)
6	60	8	–	–[c]
6	70	5	–	–[c]
6	70	8	0.17	80
6	80	8	0.19	91
12	80	5	0.02	81
12	80	8	0.00	86

[a] **2**, 20 mg; NaOHaq, 10 mL.
[b] Degree of substitution calculated from the C/N of elemental analysis.
[c] Strong amide bands were observed in the IR spectra.

Table 4
Debenzylation of 3-*O*-benzyl-6-*O*-trityl-chitin (**2**)[a].

Temperature (°C)	ds[b]		Yield (%)
	Bn	Tr	
50	0.16	1.00	70
60	0.08	1.00	63
70	0.00	1.00	80

[a] **2**, 30 mg; Pd/C (5%), 0.030 g; solvent (DMSO/AcOH (10/1)), 6 mL; H_2, 1 atm; temp, rt; time 24 h.
[b] Degree of substitution calculated from the C/N of elemental analysis.

3.4. Debenzylation of 3-O-benzyl-6-O-trityl-chitin (*2*)

Removal of the benzyl was then examined to demonstrate facile regeneration of the C-3 hydroxy after desired modification reactions based on **2**. Catalytic hydrogenation of **2** was carried out at various temperatures, and as listed in Table 4, complete debenzylation was possible at 70 °C without any interference with the other substituents (Scheme 1). The spectroscopy data were identical with those of the authentic sample, and the elemental analysis also supported the structure of **1**.

3.5. Benzylation of 2-N-phthaloyl-6-O-trityl-chitosan (*6*)

2-*N*-Phthaloyl-chitosan (**5**) and the derived 2-*N*-phthaloyl-6-*O*-trityl-chitosan (**6**) are key precursors for controlled modifications, and the C-3 hydroxy of **6** can be protected by acetylation (Nishimura et al., 1991). In order to explore for an alkali-resistive and yet readily removable protective group for C-3, benzyl was chosen as a candidate. Benzylation of **6** was first attempted with powdered sodium hydroxide, but the reaction proceeded only to low extents, as evidenced by strong hydroxy bands in the IR spectra, as in the benzylation of **1**. Sodium hydride appeared to be superior as a base, but even after two repeated reactions, the ds was 0.72 (Table 5).

Benzylation was then conducted according to the methylsulfinyl carbanion method (Corey & Chaykovsky, 1962; Greenwald, Chaykovsky, & Corey, 1963; Sjöberg, 1966). As included in Table 5, full substitution was achieved to give 3-*O*-benzyl-2-*N*-phthaloyl-6-*O*-trityl-chitosan (**7**). Complete disappearance of the hydroxy bands in the IR spectrum (Fig. 3), as well as the elemental analysis, concluded the full substitution (Scheme 2).

3.6. Detritylation of 3-O-benzyl-2-N-phthaloyl-6-O-trityl-chitosan (*7*)

To synthesize a precursor having a reactive group at C-6, 3-*O*-benzyl-2-*N*-phthaloyl-chitosan (**8**), detritylation of **7** was studied with dichloroacetic, trifluoroacetic, and acetic acids. As in the detritylation of **2**, dichloroacetic acid removed the benzyl in addition to the trityl as summarized in Table 6. However, a mixture of about equal volumes of the acid and DMSO removed the trityl while retaining the benzyl (Scheme 2). Trifluoroacetic acid was similarly effective. Selective detritylation in these solvents was also confirmed by HPLC as in the case of **2** mentioned above. Bands ascribable to the hydroxy were observed in the IR spectrum of the

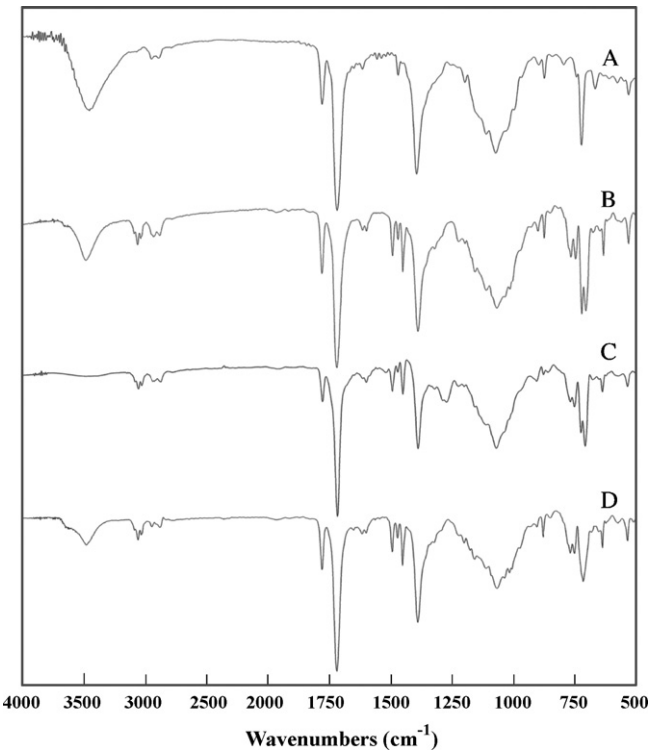


Fig. 3. IR spectra of chitosan derivatives (KBr): A, 2-*N*-phthaloyl-chitosan (**5**); B, 2-*N*-phthaloyl-6-*O*-trityl-chitosan (**6**); C, 3-*O*-benzyl-2-*N*-phthaloyl-6-*O*-trityl-chitosan (**7**); D, 3-*O*-benzyl-2-*N*-phthaloyl-chitosan (**8**).

Table 6
Detritylation of 3-*O*-benzyl-2-*N*-phthaloyl-6-*O*-trityl-chitosan (**7**)[a].

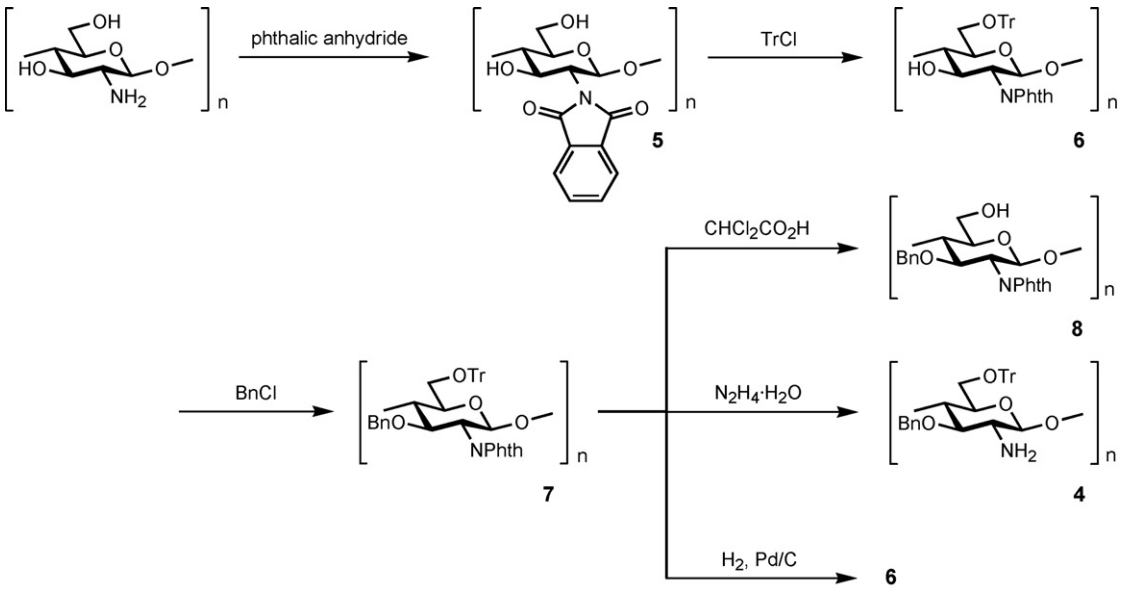
Acid/DMSO (v/v)	Time (h)	ds[b]		Yield (%)
		Tr	Bn	
Dichloroacetic acid (10/0)	1	0.00	0.46	56
Dichloroacetic acid (7/3)	1	0.00	0.70	70
Dichloroacetic acid (6/4)	1	0.00	0.86	76
Dichloroacetic acid (5/5)	1	0.00	0.97	74
Dichloroacetic acid (4/6)	1	0.00	1.00	83
Dichloroacetic acid (3/7)	1	0.10	1.00	69
Trifluoroacetic acid (5/5)	1	0.00	0.92	67
Trifluoroacetic acid (4/6)	1	0.03	1.00	80
Acetic acid (4/6)	24	–	–	–[c]

[a] **7**, 20 mg; acid/DMSO, 2 mL; temp, rt.
[b] Degree of substitution calculated from the C/N of elemental analysis.
[c] No appreciable change was observed in the IR spectrum.

Table 5
Benzylation of 2-*N*-phthaloyl-6-*O*-trityl-chitosan (**6**)[a].

Method[b]	Base	Base/pyranone[c]	BnCl/pyranose[c]	Repetition of reaction[d]	ds[e]	Yield (%)
A	NaOH	6	12	1	–	–[f]
A	NaH	2	6	1	–	–[f]
A	NaH	6	12	1	–	–[f]
A	NaH	1.2	6	2	0.72	65
B	NaH/DMSO	1.2	6	1	1.00	76

[a] **6**, 0.30 g; DMSO, 4.5 mL; temp, rt; time, 24 h.
[b] Base (method A) or NaH/DMSO (methylsulfinyl carbanion; method B) was added to a solution of the chitosan derivative in DMSO.
[c] Mole ratio.
[d] Number of repetition under the same reaction conditions.
[e] Degree of substitution calculated from the C/N of elemental analysis.
[f] Strong hydroxy bands were observed in the IR spectra.



Scheme 2.

Table 7
Dephthaloylation of 3-*O*-benzyl-2-*N*-phthaloyl-6-*O*-trityl-chitosan (**7**)[a].

Temperature (°C)	ds for Phth[b]	Yield (%)
80	–	–[c]
90	0.04	83
100	0.00	95

[a] **7**, 30 mg; hydrazine monohydrate, 10 mL; time, 24 h.
[b] Degree of substitution calculated from the C/N of elemental analysis.
[c] Weak imide bands were observed in the IR spectrum.

product (Fig. 3). Acetic acid was, however, too weak an acid for detritylation.

3.7. Dephthaloylation of 3-O-benzyl-2-N-phthaloyl-6-O-trityl-chitosan (7)

Reactive free amino groups were expected to regenerate by dephthaloylation of **7** to give a chitosan derivative having protective groups at both C-3 and C-6. Derivative **7** was thus heated in hydrazine at 90 °C to give an almost dephthaloylated product. The full hydrazinolysis was possible at 100 °C (Table 7, Scheme 2) resulting in the formation of **4**, identical with the compound prepared from **2**. In the IR spectrum, strong bands at 1778 and 1719 cm^{-1} characteristic of imide disappeared completely.

3.8. Debenzylation of 3-O-benzyl-2-N-phthaloyl-6-O-trityl-chitosan (7)

Catalytic hydrogenation of **7** also proceeded thoroughly at 80 °C with Pd/C to remove the benzyl as in the case of **2**, resulting in the formation of **6** (Table 8, Scheme 2). The structure of the product was supported by comparing the spectral data with those of the authentic sample and by elemental analysis.

Table 8
Debenzylation of 3-*O*-benzyl-2-*N*-phthaloyl-6-*O*-trityl-chitosan (**7**)[a].

Temperature (°C)	ds for Bn[b]	Yield (%)
60	0.11	97
70	0.04	96
80	0.00	95

[a] **7**, 80 mg; 5% Pd/C, 80 mg; solvent (DMSO/AcOH (10/1)), 4 mL; H_2, 1 atm; temp, 80 °C; time, 24 h.
[b] Degree of substitution calculated from the C/N of elemental analysis.

3.9. Solubility of the derivatives

Qualitative solubilities of the products were examined in excess organic solvents at room temperature. Though chitin and chitosan were insoluble in common solvents, protected derivatives were soluble in common aprotic polar organic solvents such as DMSO, DMF, and pyridine, except **4** that was only partially soluble in these solvents.

4. Conclusions

Properly protected derivatives of chitin and chitosan are indispensable to attain finely controlled chemical manipulations for designing complicated and yet well-defined molecular environments. The results described here revealed the efficient synthesis of site-selectively and quantitatively protected derivatives of chitin and chitosan having only one reactive group in the repeating units. The perfect discrimination of the different functional groups and improved solubility are significant in the chemical aspects of these almost unutilized biomass resources, and the derivatives will be of practical utility as convenient and versatile key intermediates for the regioselective chemical modifications under mild conditions.

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