Nonnatural Branched Polysaccharides: Synthesis and Properties of Chitin and Chitosan Having α-Mannoside Branches

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ABSTRACT: Regioselective introduction of α-mannoside branches at C-6 of chitin and chitosan has been accomplished by a series of regioselective modification reactions starting from *N*-phthaloyl-chitosan as a key precursor. Glycosylation of the derived acceptor with reactive groups only at C-6 with an ortho ester of D-mannose proceeded smoothly in dichloromethane in the presence of trimethylsilyl trifluoromethanesulfonate, and the degree of branching was up to 0.6. Full deprotection gave chitosans with α-mannoside branches, which were subsequently transformed into the corresponding branched chitins by *N*-acetylation. The resulting branched polysaccharides showed a remarkable solubility in neutral water in sharp contrast to the insoluble linear chitin and chitosan. Concanavalin A exhibited a specific affinity for these products, which was ascribable to the presence of α-mannoside groups. Though nonnatural, the branched chitins were susceptible to lysozyme, and the enzymatic degradation was heavily dependent on the extent of branching. Furthermore, the branched chitosan exhibited considerable antimicrobial activity.

Introduction

Branched polysaccharides are found in nature and some are attracting increasingly more attention because of their unique biological activities and physicochemical properties. Branched glucans, such as lentinan[1] and schizophyllan[2] extracted from mushrooms, exhibit high antitumor activities and are used clinically. To shed light on the structure−properties relationship of branched polysaccharides and thereby to enable sophisticated molecular design, it is necessary to establish versatile procedures for preparing various tailored polysaccharides with sugar branches.

Despite the growing interest in branched polysaccharides, only limited numbers of papers have been published on their preparation. Ring-opening polymerization of 1,6-anhydro monosaccharides followed by branching[3] or polymerization of 1,6-anhydro disaccharides[4] gave synthetic branched or comblike polysaccharides. A more versatile approach would be the introduction of sugar branches into natural polysaccharides. Some sugar branches were introduced into amylose and cellulose with proper protecting groups.[5] However, it is generally difficult to introduce such branches at a specific position of linear polysaccharides such as cellulose and curdlan[6] because of the multifunctionality and limited solubility of the polysaccharides. Reducing sugars could be incorporated at the amino groups of chitosan by reductive alkylation,[7] but branching of amino polysaccharides with glycosidic linkages has not been exploited.

Our recent attention has been focused on the efficient and controlled modifications of chitin to prepare bioactive derivatives with well-defined structures. Although structurally similar to cellulose, chitin is an amino polysaccharide characterized by various specific bioactivities as well as biocompatibility, affinity for biospecies, and low toxicity.[8,9] Chitin is therefore expected to have a higher potential than cellulose as a specialty functional material. To explore the full potential of this abundant but unutilized biomass resource, special emphasis should be placed on the chemical modifications. Modifications of chitin are, however, difficult because of its lack of solubility, and the reactions under heterogeneous conditions are accompanied by various problems, including poor extents of reaction, structural ambiguity of the products, and partial degradation due to harsh reaction conditions.

Regioselective introduction of sugar groups into chitin, which is quite significant in view of preparing polysaccharide-based advanced functional materials, would become possible by controlled modification reactions of appropriate precursors. A recently developed derivative, *N*-phthaloyl-chitosan, is an organosoluble precursor that has enabled some modification reactions to proceed efficiently and regioselectively in solution.[10,11] Starting from this precursor, chitosan derivatives with liquid crystalline nature were prepared.[12] Glycosylation of chitin acceptors derived from *N*-phthaloyl-chitosan with some sugar donors would be promising to prepare chitin derivatives with sugar branches. An ortho ester of D-mannose was found to be suitable to introduce α-mannoside branches, and some preliminary results were already published.[13] In this paper, we report the results of detailed studies on the efficient preparation and characteristic properties of the nonnatural branched polysaccharides chitin and chitosan with α-mannoside branches at the C-6 positions.

Experimental Section

General. Chitin isolated from shrimp shells was treated with 40% aqueous sodium hydroxide at 110 °C for 4 h and washed with deionized water. The alkaline treatment and washing were repeated two more times.[14] Starting from 20.0

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g of chitin, 12.7 g (80% yield) of chitosan was obtained. The degree of deacetylation was 1.0 as determined by conductometric titration with a TOA conductivity meter CM-40S. The IR and UV−vis spectra were recorded on JASCO IRA-700 and JASCO Ubest-30 instruments, respectively. The NMR spectra were taken with a JEOL JNM-GX270, and 3-(trimethylsilyl)-propanesulfonic acid sodium salt was used as the internal reference when D_2O was used as a solvent. Elemental analysis was performed with a Yanaco MT-3 CHNcorder. The gel permeation chromatography (GPC) was carried out with a JASCO 880-PU connected to a Shodex RI detector SE−61 (column, TSK guard column + TSKgel Type GMPWXL no. PWMXE 0009 + TSKgel Type GMPWXL no. 0053; solvent, 0.1% aqueous lactic acid; flow rate 1.0 mL/min; standards, pullulan).

3-*O*-Acetyl-2-*N*-phthaloyl-chitosan and 3-*O*-Acetyl-2-*N*-phthaloyl-6-trimethylsilyl-chitosan. Using a previously reported procedure,[10] chitosan was converted into 3-*O*-acetyl-2-*N*-phthaloyl-chitosan, which was then transformed into the C-6 trimethylsilylated derivative as follows.

3-*O*-Acetyl-2-*N*-phthaloyl-chitosan (1.00 g; 3 mmol of pyranose units) was dissolved in 10 mL of pyridine, and 4.85 g (30 mmol) of hexamethyldisilazane and 3.26 g (30 mmol) of chlorotrimethylsilane were added. The mixture was stirred at room temperature for 24 h and poured into ethanol to precipitate the silylated derivative. The derivative was filtered and dried to give 0.86 g (71%) of the product as a pale tan powdery material. IR (KBr): ν 1777 (imide C═O), 1750 (acetyl C═O), 1719 (imide C═O), 1226 (C−Si), 1150−1000 (pyranose), 846 (C−Si), and 721 cm^{-1} (arom). ^1H NMR ($CDCl_3$): δ 0.00 (s, 9H, Si−CH_3), 1.81 (s, 3H, CO−CH_3), 3.2−4.1 (m, 5H, pyranose H (C-2,4,5,6)), 5.36 (broad s, 1H, pyranose H (C-1)), 5.61 (broad s, 1H, pyranose H (C-3)), and 7.7−7.9 ppm (m, 4H, phthalimide H). ^{13}C NMR ($CDCl_3$): δ 0.0 (Si−CH_3), 20.6 (CO-CH_3), 55.9 (pyranose C-2), 60.8 (pyranose C-6), 70.2 (pyranose C-3), 75.2 (pyranose C-5), 76.2 (pyranose C-4), 96.0 (pyranose C-1), 123.8, 132.0, and 134.1 (phthalimide aromatic C), 167.9 (phthalimide C═O), and 170.2 ppm (acetyl C═O).

Anal. Calcd for $C_{19}H_{23}NO_7Si$: C, 56.28; H, 5.72; N, 3.45. Found: C, 56.03; H, 5.40; N, 3.61.

3,4,6-Tri-*O*-acetyl-β-D-mannopyranose 1,2-(Methyl orthoacetate). An ortho ester of D-mannose was prepared according to the reported method.[15] Starting from D-mannose, peracetylation, bromination at C-1, and ortho esterification with methanol gave the corresponding ortho ester, which was recrystallized from methanol/hexane to give colorless needles; mp 110 °C (lit.[15] 109−110 °C). The overall yield from D-mannose was 67%.

Glycosylation Reaction. To a solution of 0.50 g of the trimethylsilylated chitosan derivative in 20 mL of dichloromethane were added 1.34 g (3 equiv of pyranose units) of the mannose ortho ester and 0.02 mL (0.08 equiv of pyranose units) of trimethylsilyl trifluoromethanesulfonate (TMSOTf). The resulting light brown solution was stirred at room temperature for 24 h in nitrogen and poured into methanol. The precipitated product was washed thoroughly with methanol and dried to give 0.38 g of a light tan powdery material. The degree of substitution (ds) was 0.30 as calculated from the C/N ratio of the elemental analysis and was 0.32 as determined from the peak area ratio of acetyl/phthaloyl in the ^1H NMR spectrum. The yield was 71% on the basis of ds 0.30. IR (KBr): ν 1775 (imide C═O), 1745 (acetyl C═O), 1719 (imide C═O), 1150−1000 (pyranose), and 722 cm^{-1} (arom). ^1H NMR (DMSO-d_6): δ 1.5−2.1 (m, CO−CH_3), 3.2−5.5 (m, pyranose H), and 7.7−8.0 ppm (m, phthalimide H).

Anal. Calcd for $(C_{30}H_{33}NO_{16})_{0.30}(C_{16}H_{15}NO_7)_{0.70}\cdot 0.6H_2O$: C, 54.74; H, 4.91; N, 3.16. Found: C, 54.68; H, 5.08; N, 3.16.

Deprotection of the Branched Product. The branched product obtained (0.30 g) was added to 15 mL of hydrazine hydrate, and the light brown solution was stirred at 80 °C for 15 h in nitrogen. The solution was dialyzed in deionized water until neutral (3 days) and concentrated under reduced pressure. The resulting viscous solution was freeze-dried to give 113 mg (78%) of the fully deprotected product, branched chitosan, as an almost colorless powdery material. IR (KBr): ν 1636 (NH_2) and 1150−1000 cm^{-1} (pyranose).

***N*−Acetylation of Branched Chitosan.** The branched chitosan (100 mg) was dispersed in 10 mL of methanol, and 0.3 mL of acetic anhydride was added. The mixture was stirred at room temperature for 24 h, and 20 mL of water was added. The mixture was dialyzed in deionized water and freeze-dried to give 105 mg (87%) of the product, branched chitin, as an off-white powdery material. IR (KBr): ν 1647 (amide I), 1556 (amide II), and 1150−1000 cm^{-1} (pyranose). ^{13}C NMR (D_2O): δ 24.8 (CH_3), 57.7 (C-2), 62.7 (C-6′), 63.7 (C-6), 69.5 (C-4′), 72.8 (C-3′), 72.9 (C-2′), 75.8 (C-3), 76.0 (C-5), 77.2 (C-5′), 81.8 (C-4), 103.0 (C-1′), 103.9 (C-1), and 177.2 ppm (C═O).

Anal. Calcd for $(C_{14}H_{23}NO_{10})_{0.30}(C_8H_{13}NO_5)_{0.70}\cdot 0.3H_2O$: C, 45.76; H, 6.50; N, 5.44. Found: C, 46.02; H, 6.44; N, 5.17.

In case a small peak was observed at 19.6 ppm due to *O*-acetyl groups in the ^{13}C NMR spectrum, the product was treated with a small amount of sodium methoxide in dry methanol at room temperature overnight. Water was added to the mixture, and the solution was dialyzed. The solution was concentrated under reduced pressure and freeze-dried to give the product with no *O*-acetyl groups.

Viscosity Measurement. A branched chitin was dissolved in deionized water to give solutions of 1, 5, and 10% concentrations, and the viscosities were measured with a rotational viscometer (Tokyo Keiki Type E) with a cone geometry.

Interaction with Concanavalin A. In a UV cell was placed 2 mL of a 1-mg/mL aqueous solution of a branched chitin. An aqueous solution of concanavalin A (0.1 mg/mL) was added in 0.2-mL steps and, after letting the mixture stand at room temperature for 5 min, the absorbance at 311 nm was measured.

Enzymatic Degradation. Branched chitins were treated with lysozyme from egg white in pH 4.50 acetate buffer at 37 °C, and the amount of the resulting reducing ends was determined with ferricyanide as reported previously.[16]

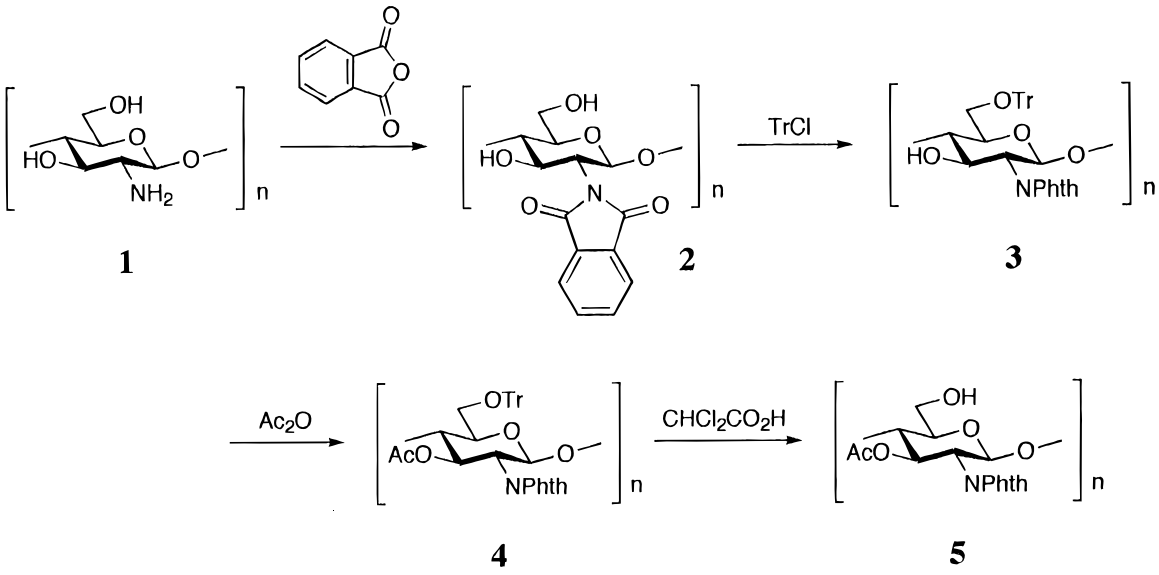
Antimicrobial Activity. Bactericidal effect was measured by essentially the same method as that described in a previous paper.[17] Precultures (50 μL) of bacteria were mixed with 10 mL of a 50 or 5 ppm solution of a branched chitosan dissolved in 0.45% L-lactic acid buffer solution (pH 5.8). The mixtures were left standing at room temperature for 1 min with occasional stirring. After appropriate dilution, they were cultured aerobically at 25 °C for 48 h, except *S. mutans*, which was cultured anaerobically at 37 °C for 24 h. Nutrient agar (NA) media were used for *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa*, and yeast malt (YM) and brain heart infusion (BHI) agar media were used for *C. albicans* and *S. mutans*, respectively. The bactericidal activity was expressed as a suppression percentage of the growth; that is, a percentage of the decreased number of colony forming units to the number of colony forming units of the control.

Results and Discussion

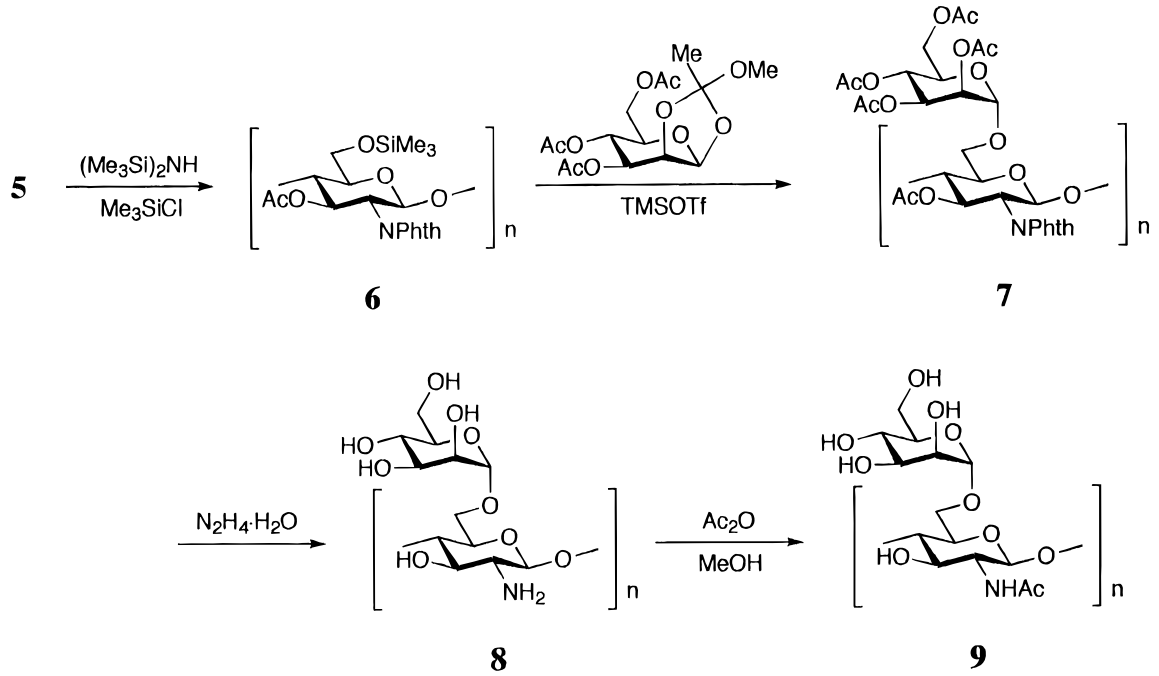
Preparation of Acceptors and a Donor for Glycosylation. To achieve regioselective introduction of α-mannoside branches into chitin, an ortho ester of D-mannose, 3,4,6-tri-*O*-acetyl-β-D-mannopyranose 1,2-(methyl orthoacetate), was used. The C-1 of the ortho ester is attacked by the C-6 oxygen of a chitosan acceptor, giving rise to a branched derivative.

Soluble chitosan acceptors with reactive groups only at C-6 are necessary for efficient glycosylation, and such a derivative was prepared by a series of modification reactions starting from chitosan according to Scheme 1. Fully deacetylated chitosan (**1**) was first phthaloylated to give *N*-phthaloyl-chitosan (**2**). Phthaloylation often takes place at hydroxy groups to some extent in addition to the amino groups, depending on the reaction conditions, as pointed out by Gross et al.[12] *O*-Phthaloyl groups were, however, replaced by the trityl groups in

Scheme 1



Scheme 2



the subsequent tritylation to give 2-*N*-phthaloyl-6-*O*-trityl-chitosan (**3**), as confirmed by the elemental analysis. The IR and ^{1}H NMR spectra also indicated the absence of *O*-phthaloyl groups; for example, bands due to free carboxyl groups at 2700−2500 cm^{-1} and peaks due to aromatic rings of *O*-phthaloyl groups at 7.3−7.4 ppm disappeared on tritylation.

Acetylation of **3** followed by detritylation gave a chitosan acceptor (**5**). The transformations from **3** to **5** proceeded quantitatively in terms of the ds as supported by the IR spectra and satisfactory results of elemental analysis.[10]

Glycosylation Reaction. The acceptor **5** was soluble only in polar organic solvents and subjected to the reaction with the mannose ortho ester in pyridine solution or in chloroform or chlorobenzene suspension under various conditions. The extents of reaction were, however, nil or low, and the mixtures generally assumed dark colors, indicating the decomposition of **5** and/or the ortho ester particularly at elevated temperatures.

The glycosylation reaction should be much more efficient if carried out in solution in rather nonpolar solvents, and thus **5** was converted into the trimethylsilylated derivative (**6**) to enhance the solubility (Scheme 2). IR spectroscopy as well as elemental analysis supported the fully substituted structure as shown in Figure 1; a broad band at 3450 cm^{-1} due to hydroxy groups of **5** disappeared completely in the spectrum of **6**. The acceptor **6** was soluble even in low boiling solvents such as dichloromethane, chloroform, 1,2-dichloroethane, and tetrahydrofuran. As anticipated, the reaction of **6** with the ortho ester proceeded quite smoothly in dichloromethane solution at room temperature in the presence of TMSOTf as the catalyst. The resulting branched derivative (**7**) was isolated in methanol as a light tan powdery material.

The ds values calculated from the C/N value of the elemental analysis were in close agreement with those

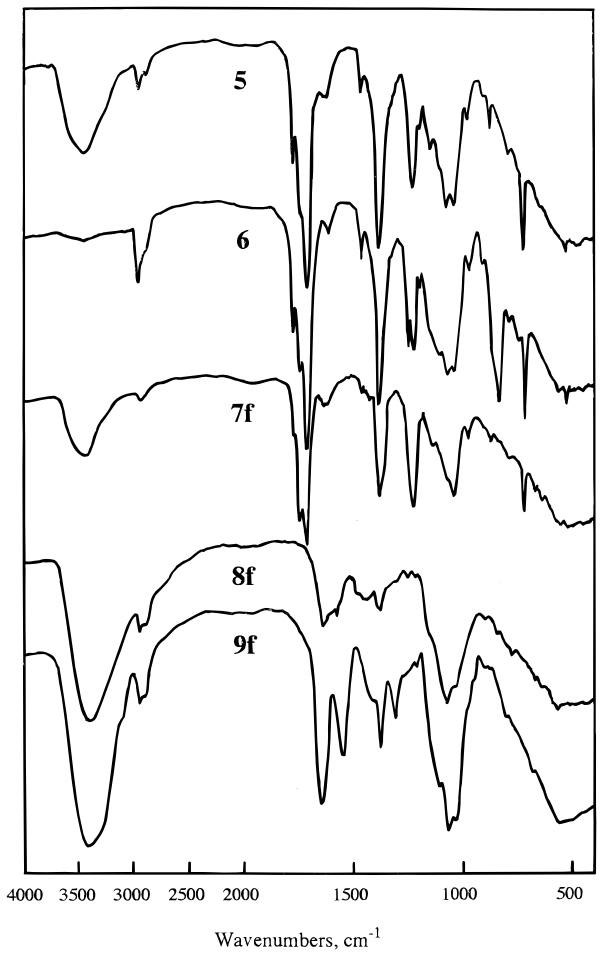


Figure 1. IR spectra of chitosan acceptors **5** and **6**, and the derived branched products **7f**, **8f**, and **9f** (KBr method).

Table 1. Glycosylation of 6 with the Mannose Ortho Ester to Form 7

product	ortho ester/ pyranose[a]	time (h)	ds		yield (%)[d]
			EA[b]	NMR[c]	
7a	1	24	0.15	0.22	70
7b	2	24	0.22	0.25	68
7c	3	24	0.30	0.32	71
7	3	48		0.33	62*
7e	5	24		0.36	50*
7f	5	48		0.42	65*
7g	10	24	0.58		74
7h	10	48	0.59		72
7	10	72	0.52		72

[a] Mole ratio. [b] Degree of substitution calculated from the C/N value of elemental analysis. [c] Degree of substitution calculated from the peak area ratio of Ac/Phth in ^{1}H NMR. [d] Calculated on the basis of the ds value determined by elemental analysis (* by NMR).

determined from the ratio of the peak area of acetyl to that of phthaloyl in ^{1}H NMR, as listed in Table 1. With increasing the amount of the ortho ester, the ds value increased, and with 10 equiv of the ortho ester, ds was raised to 0.59. Prolonged reactions up to 72 h did not improve the ds. The yields were not affected much by the reaction conditions and generally were ~70%.

In the IR spectrum, absorption bands at 1745 and 1230 cm^{-1} due to ester groups became strong as a result of the incorporation of peracetylated mannoside branches, as illustrated in Figure 1. Characteristic peaks were observed in the ^{1}H NMR spectrum at 1.5−2.1 ppm for the acetyl methyl, 3.2−5.5 ppm for the pyranose, and 7.7−8.0 ppm for the phthaloyl. Elemental analysis also supported the structure.[18]

Table 2. Molecular Weight Characteristics of Chitosan 1 and Branched Chitosans 8[a]

compound	M_w	M_n	M_w/M_n
1	206 000	61 000	3.38
8b	32 000	21 000	1.52
8d	43 000	26 000	1.65
8f	43 000	24 000	1.79

[a] Determined by GPC with pullulan standards.

Transformation of 7 into Branched Chitosan and Chitin. The branched product **7** was then deprotected to form chitosan with α-mannoside branches (**8**). Treatment with aqueous sodium hydroxide for deacetylation and then with hydrazine hydrate for dephthaloylation gave the deprotected product. However, this procedure often caused a substantial reduction in the yield and incomplete removal of the phthaloyl groups. The reaction was thus examined under various conditions, and one-step deprotection of the acetyl and phthaloyl groups with hydrazine hydrate was confirmed to be suitable. After dialysis and freeze-drying, **8** was obtained as an almost colorless powdery material.

To convert **8** into the corresponding chitin derivative (**9**), **8** was *N*-acetylated with acetic anhydride in methanol. In the ^{13}C NMR spectrum in D_2O, however, a small peak was sometimes observed at 19.6 ppm that was probably due to a low degree of *O*-acetylation. The product was thus subjected to an ester exchange reaction with methoxide in methanol. The ^{13}C NMR spectrum of **9** showed peaks due to the *N*-acetyl groups at 24.8 and 177.3 ppm. Peaks at 57.6−103.9 ppm were reasonably assigned to the chitin backbone (C-1 to C-6) and mannose branches (C-1′ to C-6′). Conductometric titration of **9** also confirmed the absence of free amino groups.

The IR spectra of **8** and **9** were quite similar to those of chitosan and chitin, respectively, as shown by typical examples in Figure 1. However, because of the incorporation of mannoside branches, bands due to the pyranose rings at 1000−1150 cm^{-1} became evident.

Properties of Branched Chitosan and Chitin. *1. Molecular Weight.* GPC allows rough estimation of the molecular weights of the branched products with pullulan standards. The molecular weight of chitosan was thus compared with those of **8** derived from **7b**, **7d**, and **7f**. As summarized in Table 2, the molecular weights of **8b**, **8d**, and **8f** were lower than that of chitosan. This result implies a possibility of low extents of degradation during the course of modifications, most likely in the deprotection step, although quantitative comparison is difficult because of the difference in the structures. It should also be noted that the polydispersity value (M_w/M_n) became small on branching chitosan probably as a result of repeated purifications including reprecipitation and dialysis in the preparation process. Typical GPC profiles are shown in Figure 2.

2. Solubility. The protected products **7** exhibited a remarkable solubility and were even soluble in low-boiling organic solvents in addition to polar solvents, as summarized in Table 3. Both the deprotected derivatives, **8** and **9**, were readily soluble in neutral water, which is in sharp contrast to the insoluble linear chitosan and chitin. Compounds **8** and **9** also showed a high affinity for organic solvents and swelled considerably even in common solvents.

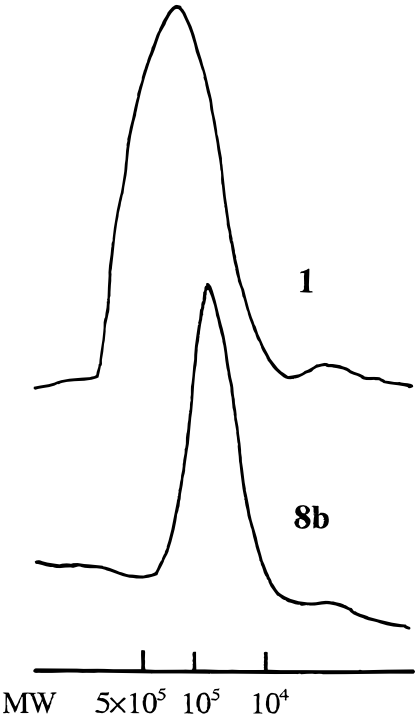


Figure 2. GPC profiles of chitosan **1** and branched chitosan **8b** with pullulan standards.

Table 3. Solubility of Chitin and the Derivatives[a]

compound	DMSO	DMF	pyridine	$CHCl_3$	acetone	MeOH	H_2O
chitin	−	−	−	−	−	−	−
chitosan	−	−	−	−	−	−	−
7	+	+	+	+	+	±	−
8	±	±	±	±	±	±	+
9	±	±	±	±	±	±	+

[a] DMSO, dimethyl sulfoxide; DMF, *N*,*N*-dimethylformamide; +, soluble; ±, swelled; −, insoluble.

Table 4. Viscosities of Branched Chitin 9f

concentration[a] (%)	viscosity ($\times 10^{-3}$ Pa·s)				
	30 °C	35 °C	40 °C	45 °C	50 °C
1	1.09	1.04	0.96	0.89	0.83
5	3.17	2.87	2.18	1.93	1.78
10	9.74	8.35	7.18	6.76	6.22

[a] In deionized water.

3. Solution Viscosity. Branched polysaccharides are interesting in view of the possible unique viscosity behavior as suggested by the chitosan derivatives having sugar groups at the amino groups.[19] Shear stresses of aqueous solutions of **9f** derived from **7f** with 1, 5, and 10% concentrations were thus measured at varying shear rates up to 400 s^{-1} at temperatures ranging from 30 to 50 °C. The shear stresses increased linearly with shear rates, and the solutions behaved as Newtonian liquids under these conditions. As summarized in Table 4, the viscosities calculated from these measurements were high even at low concentrations.

4. Interaction with Concanavalin A. Because of the introduced α-mannoside branches, **8** and **9** were expected to show affinity for concanavalin A, a lectin with a specificity for α-mannoside groups. On mixing an aqueous solution of **8** or **9** with an aqueous solution of concanavalin A, a turbid mixture resulted even when the concentrations were as low as 0.1 mg/mL. The turbidity became obvious at higher concentrations, and precipitation was observed after a few minutes at room temperature. The presence of such branches is essential for the interaction with the lectin, and no aggregation occurred with the linear water-soluble chitin derivative with 50% deacetylation.[14,20]

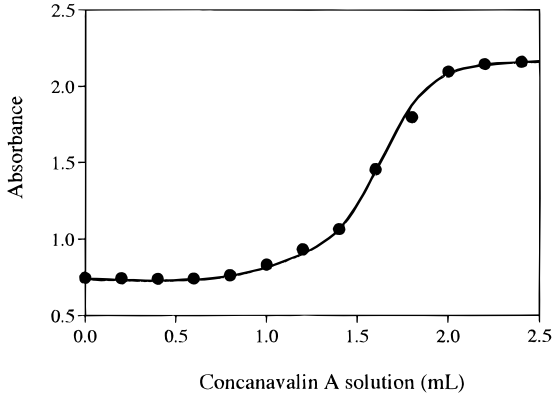


Figure 3. Aggregation of branched chitin **9f** (1 mg/mL, 2 mL) by concanavalin A (0.1 mg/mL).

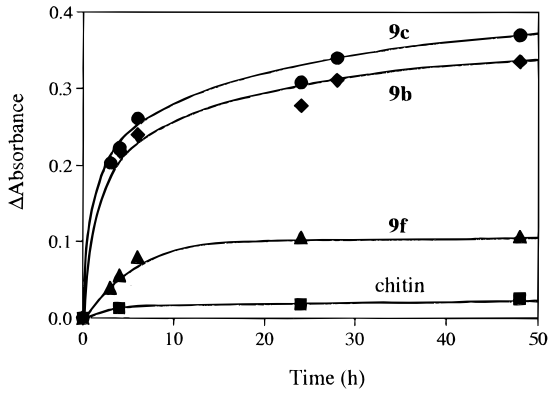


Figure 4. Enzymatic degradation of chitin and branched chitins **9b**, **9c**, and **9f** with lysozyme.

Figure 3 shows the change in the absorbance on addition of a solution of the lectin (0.1 mg/mL) to 2 mL of a **9f** solution (1 mg/mL). The absorbance decreased slightly in the initial stage due to dilution of **9f**, but started to increase at 0.8 mL of the lectin solution, indicating the onset of the formation of an insoluble aggregate. The concentrations of the lectin and **9f** were 28.6 and 714 μg/mL, respectively, at this point. With an increase in the amount of lectin, the turbidity increased markedly and then leveled off. This aggregation behavior supports efficient recognition of the α-mannoside branches of **9f** by concanavalin A.

5. Enzymatic Degradation. Chitin is a biodegradable linear polysaccharide, but the introduction of sugar branches may interfere with the biodegradation. The branched chitins **9** (**9b**, **9c**, and **9f** derived from **7b**, **7c**, and **7f**, respectively) with different ds values were thus subjected to lysozyme degradation to discuss the influence of such branches. The degradation was followed by the amount of the resulting reducing ends determined with ferricyanide, and the amount of consumed ferricyanide (ΔAbsorbance) was plotted against time as a measure of hydrolysis. As evident in Figure 4, the original linear chitin was degraded slowly, but **9** proved to be degraded much more facilely. It is, however, difficult to compare the susceptibilities of chitin and **9** directly, because the branched products **9** are soluble in the media to allow the reactions in homogeneous solution whereas chitin is not.

Table 5. Antimicrobial Activity of a Branched Chitosan and Chitosan

	suppression of the growth[a]		
	8f		chitosan[b]
bacteria	$c = 50$ ppm	$c = 5$ ppm	$c = 5$ ppm
Bacillus subtilis IAM1069	90 ± 2.6	26 ± 5.4	62 ± 5.0
Staphylococcus aureus IAM1011	39 ± 2.3	17 ± 1.7	81 ± 4.7
Escherichia coli IF014249	26 ± 3.1		21 ± 1.4
Pseudomonas aeruginosa IAM1095	>99	93 ± 7.9	83 ± 5.5
Streptococcus mutans GS5	36 ± 7.9		51 ± 5.1
Candida albicans TIMM0613	0		72 ± 2.7

[a] Percentage of the colony forming units decreased by treating with a 50 or 5 ppm solution of the branched chitosan or chitosan. [b] Molecular weight was 110 000 as determined by viscometry.[17]

The susceptibility of **9** to lysozyme was found to be dependent on the extent of branching; when the ds became high, the susceptibility reduced probably owing to the bulky nature of the branches. This suggests that the biodegradability can be effectively regulated by the extent of branching.

6. Antimicrobial Activity. As one of the most important properties linked directly to the possible applications, antimicrobial activity of the branched chitosan is interesting and was therefore examined in comparison with that of chitosan. Some bacteria and a yeast were treated with **8f**, and the activities were evaluated in terms of the suppression percentage of the colony formation. As listed in Table 5, **8f** showed a considerable bactericidal activity even at such low concentrations as 50 and 5 ppm. Although **8f** was generally somewhat less active than chitosan, it is interesting to note that **8f** exhibited a higher activity against *Pseudomonas sp.* Furthermore, because the branched chitosans are soluble in neutral water unlike linear chitosan, they would have advantages as a new type of water-soluble antimicrobial agent.

Conclusions

Although controlled modification reactions of chitin and chitosan have been generally difficult because of the limited solubility and multifunctionality, *N*-phthaloyl-chitosan has proved to be a superb precursor for regioselective introduction of sugar branches. The resulting branched chitosan and chitin are interesting from the viewpoint of their remarkable solubility and distinctive bioactivities. They may thus have a high potential as water-soluble amino polysaccharides for practical applications in various fields including medicine, cosmetics, and food. This synthetic approach would be beneficial for diversification of the molecular design for developing polysaccharide-based advanced materials.

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References and Notes

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(18) **7a**, Calcd for $(C_{30}H_{33}NO_{16})_{0.15}(C_{16}H_{15}NO_7)_{0.85}\cdot 1.4H_2O$: C, 53.28; H, 5.06; N, 3.43. Found: C, 53.21; H, 5.16; N, 3.43. **7b**, Calcd for $(C_{30}H_{33}NO_{16})_{0.22}(C_{16}H_{15}NO_7)_{0.78}\cdot 1.6H_2O$: C, 52.71; H, 5.13; N, 3.22. Found: C, 52.79; H, 5.06; N, 3.24. **7c**, Calcd for $(C_{30}H_{33}NO_{16})_{0.30}(C_{16}H_{15}NO_7)_{0.70}\cdot 0.6H_2O$: C, 54.74; H, 4.91; N, 3.16. Found: C, 54.68; H, 5.08; N, 3.16. **7g**, Calcd for $(C_{30}H_{33}NO_{16})_{0.58}(C_{16}H_{15}NO_7)_{0.42}\cdot 1.2H_2O$: C, 53.03; H, 5.14; N, 2.56. Found: C, 53.01; H, 5.13; N, 2.57. **7h**, Calcd for $(C_{30}H_{33}NO_{16})_{0.59}(C_{16}H_{15}NO_7)_{0.41}\cdot 0.9H_2O$: C, 53.47; H, 5.08; N, 2.57. Found: C, 53.53; H, 5.01; N, 2.57. **7i**, Calcd for $(C_{30}H_{33}NO_{16})_{0.52}(C_{16}H_{15}NO_7)_{0.48}\cdot 0.9H_2O$: C, 53.70; H, 5.06; N, 2.69. Found: C, 53.64; H, 5.00; N, 2.68.
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