Chemospecific Manipulations of a Rigid Polysaccharide: Syntheses of Novel Chitosan Derivatives with Excellent Solubility in Common Organic Solvents by Regioselective Chemical Modifications[1]

Shin-Ichiro Nishimura,* Osamu Kohgo, and Keisuke Kurita*

Department of Industrial Chemistry, Faculty of Engineering, Seikei University, Musashino-shi, Tokyo 180, Japan

Hiroyoshi Kuzuhara

RIKEN (The Institute of Physical and Chemical Research), Wako-shi, Saitama 351-01, Japan

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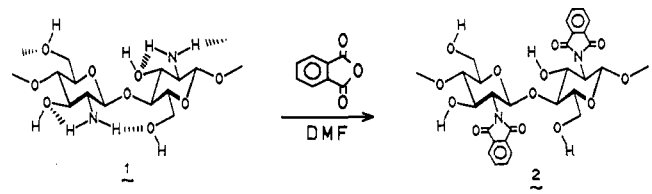
ABSTRACT: Efficient procedures for the preparations of soluble chitosan derivatives have been established on the basis of the regioselective chemical modifications. Selective and quantitative N-phthaloylation of chitosan proceeds smoothly by the reaction of chitosan with phthalic anhydride in *N,N*-dimethylformamide (DMF) at 130 °C. The resulting phthaloylchitosan exhibits much improved solubility in common organic solvents such as DMF, *N,N*-dimethylacetamide, dimethyl sulfoxide, and pyridine. The enhanced solubility of new types of chitosan derivatives under homogeneous reaction conditions. Facile conversions of phthaloylchitosan, a key starting material, into several 6-*O*-substituted derivatives are carried out by the reactions with bulky substituents such as triphenylmethyl (trityl) and (*p*-tolylsulfonyl)oxy (tosyloxy) groups. These reactions also proceed in homogeneous solution under mild conditions, and the degrees of substitution are estimated to be 1.0. Subsequent 3-*O*-acetylation of the secondary hydroxyl groups of 6-*O*-substituted materials gives rise to regioselectively modified chitosan derivatives showing much better solubility. Specific N-dephthaloylation or O-detritylation of 6-O-trityl derivatives affords versatile intermediates, which permit the introduction of additional functional groups regioselectively.

Introduction

Chitosan is a linear polymer of (1→4)-linked 2-amino-2-deoxy-D-glucopyranose easily derived from chitin by N-deacetylation. Recently much attention has been paid to chitosan as a potential polysaccharide resource owing to its specific structure and properties. Although several efforts have been reported to prepare functional derivatives of chitosan by chemical modifications,[2-5] only a few examples attained solubility in general organic solvents.[6,7] Since chitosan is soluble in aqueous solutions of some acids, selective N-alkylidenations[2,3] and N-acylations[4,5] have been attempted. Though several water-soluble[8,9] or highly swelling[5,10] derivatives were obtained, development of solubility in common organic solvents was difficult by these methods.

Solubility of derivatives of chitin and chitosan in organic solvents is a key and essential requirement for effecting fine molecular design leading to novel types of functional materials, since intractability of these aminopolysaccharides has made regioselective modifications almost impossible. Clearly, one of the reasons for the intractability of chitin and chitosan lies in the rigid crystalline structures,[11,12] and the acetamido or primary amino groups of (*N*-acetyl-)D-glucosamine residues have an important role in the formation of peculiar conformational features through intra- and/or intermolecular hydrogen bonding (Scheme I). Therefore, removal of the two hydrogen atoms of amino groups of chitosan and introduction of some hydrophobic nature by chemical modifications will cause destruction of its inherent crystalline structure, resulting in the improvement of solubility in general organic solvents. This has been preliminarily demonstrated by the partial N-phthaloylation reaction of water-soluble chitin with phthalic anhydride[7] and by the complete N,O-polyacylation of chitosan with an excess of long-chain acid chlorides.[6]

Scheme I



1 2

Our attention is now directed to basic studies on the regioselective and quantitative chemical modifications of chitosan using soluble derivatives for the purpose of creating finely designed biomedical materials such as polymeric drugs and biocompatible artificial organs with high specificity and wide applicability. In this study we report a facile and reproducible procedure for the preparation of complete N-phthaloylated chitosan (phthaloylchitosan) and the efficient transformation into a variety of soluble synthetic precursors. As we shall discuss, the synthetic strategy of novel chitosan derivatives described here will allow further regioselective and quantitative introduction of transformation into additional functional groups in homogeneous reaction systems.

Results and Discussion

Facile and Complete N-Phthaloylation of Chitosan. Protection by phthaloyl groups was chosen as the most suitable protection for the amino groups of chitosan from the point of view of our assumption for solubilization of a rigid aminopolysaccharide. N-Phthaloylation reaction of chitosan (degree of deacetylation = 100%) with 3-fold excess phthalic anhydride proceeded smoothly in dimethylformamide (DMF) at 130 °C without any basic reagent, and no side reactions with hydroxyl groups at the C-3 and C-6 positions were observed. Under typical

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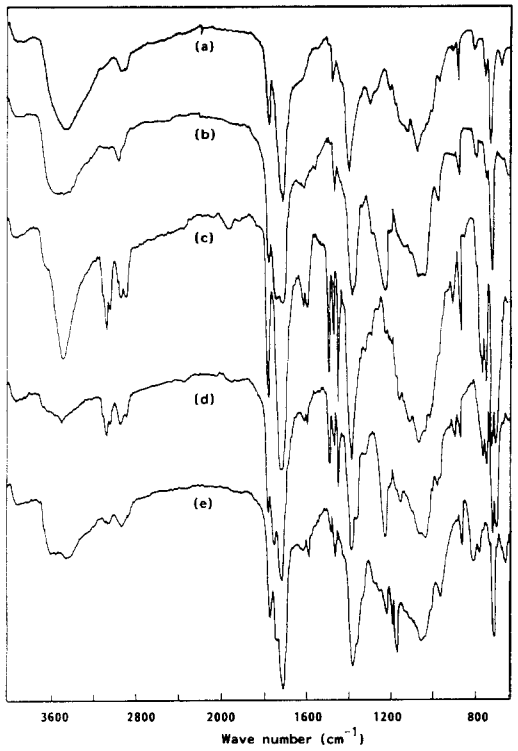


Figure 1. IR spectra of (a) **2**, (b) **3**, (c) **4**, (d) **5**, and (e) **6** (KBr pellets).

Table I
Solubility Data of Chitosan Derivatives[a]

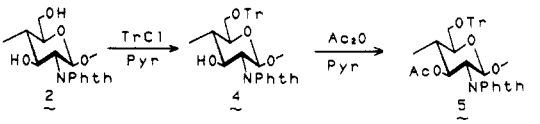
	solvent					
compd	DMF	DMAc	DMSO	pyridine	$CHCl_3$	CH_2Cl_2
2	+	+	+	+	-	-
3	+	+	+	+	+	+
4	+	+	+	+	±	+
5	+	+	+	+	+	+
6	±	±	±	+	-	-
7	-	+	-	+	-	-
8	+	+	+	+	-	-

[a] (-) insoluble; (+) partially soluble or swelled; (+) soluble.

reaction conditions, the reaction mixture became a clear and viscous solution after 5–7 h. As anticipated, the use of phthaloyl protection[13] of the primary amino groups of chitosan (1) gave a derivative having a much improved solubility in organic solvents such as DMF, dimethylacetamide (DMAc), dimethyl sulfoxide (DMSO), and pyridine. The yield was quantitative.

The characteristic absorptions due to phthalimido groups at 1770 and 1710 cm^{-1} were observed in the IR spectrum (Figure 1), and the analytical data suggested that the degree of substitution was almost 1.0 per D-glucosamine residue. For further structural elucidation of **2**, complete O-acetylation of **2** was examined by treatment with acetic anhydride and pyridine. Acetylation of **2** proceeded smoothly also in homogeneous solution, and the product was purified by chromatography on a Sephadex LH-20. 3,6-O-Acetylated derivative **3** showed characteristic absorptions of *O*-acetyl groups at 1745 and 1230 cm^{-1} (Figure 1). Since peracetate **3** showed remarkable solubility, in general, of organic solvents such as chloroform and dichloromethane in addition to polar solvents (Table I), a more definitive analysis of the structure of compound **3** was made possible with carbon and proton nuclear magnetic resonance spectroscopies. The presence of phthalimido and *O*-acetyl groups was shown in the ^{13}C NMR spectrum of **3** in chloroform-*d*. The signals due to ring carbons of hexosamine residues were also reasonably

Scheme II



assigned as six singlet peaks (55.2–96.8 ppm). The ratio of methyl protons (1.70–1.95 ppm) and aromatic protons (7.6–7.9 ppm), as calculated by integration in the ^1H NMR spectrum, suggested that the degree of O-acetylation was 2.0 per D-glucosamine residue. The analytical data were also found to be in good agreement with the theoretical ones.

Selective Modifications of Hydroxyl Groups. The enhanced solubility of **2** prompted us to examine subsequent selective protections of the remaining hydroxyl groups at C-3 and C-6 positions. The next stage of regioselective modification should be focused on discrimination between the primary and secondary hydroxyl groups. Regioselective ether protection of primary hydroxyl groups in the presence of secondary or tertiary ones can be easily accomplished by reaction with chlorotriphenylmethane.[14] Since trityl ethers are readily deprotected by heating in aqueous acetic acid or by treatment with stronger acids without heating, trityl protection, even in case of polysaccharide chemistry, seems to be one of the most efficient temporary protections as established in the synthetic studies on mono- and oligosaccharide derivatives.

6-O-Tritylation of **2** was carried out in pyridine with 3-fold excess chlorotriphenylmethane at 80 °C under a nitrogen atmosphere (Scheme II). The reaction proceeded in homogeneous solution to give derivative **4** in quantitative yield. The structural elucidation of **4** was made unambiguously with the IR spectrum (Figure 1) and satisfactory results of elemental analysis. Characteristic absorptions at 690, 710, and 750 cm^{-1} due to monosubstituted phenyl groups are observed in the spectrum. Compound **4** was also superior in solubility in organic solvents to the starting phthaloylchitosan **2** (Table I). Because of the protection of the primary hydroxyl groups and high solubility, derivative **4** will facilitate further selective modifications of the secondary hydroxyl groups at the C-3 position. In fact, the acetylation of **4** with acetic anhydride and pyridine proceeded efficiently in solution to afford acetyl derivative **5** in high yield. In the IR spectrum of the product, characteristic absorptions at 1735 and 1240 cm^{-1} due to *O*-acetyl groups appeared and a decrease in the absorption at 3475 cm^{-1} due to hydroxyl groups was observed in the spectrum. The analytical data of **5** also suggested that the degree of acetylation was almost 1.0 per hexosamine residue.

As an extension of the above synthetic strategy, we also prepared the 6-*O*-tosyl derivative of chitosan from phthaloylchitosan. The (*p*-tolylsulfonyl)oxy group is one of the most effective leaving groups widely used in carbohydrate chemistry. Since primary hydroxyl groups are more rapidly esterified than secondary ones, regioselective reaction is generally possible; this has been demonstrated by direct tolylsulfonylation of chitin using the interfacial reaction technique.[15] Treatment of **2** with 10-fold excess *p*-toluenesulfonyl chloride in pyridine solution and subsequent acetylation afforded compound **6** in high yield (Scheme III). The IR spectrum of **6** showed a characteristic absorption at 1180 cm^{-1} due to tosyl groups in addition to the absorptions at 1740 and 1230 cm^{-1} due to acetyl groups (Figure 1). A variety of novel chitosan derivatives are preparable from this key reactive intermediate through displacement of the leaving group at the

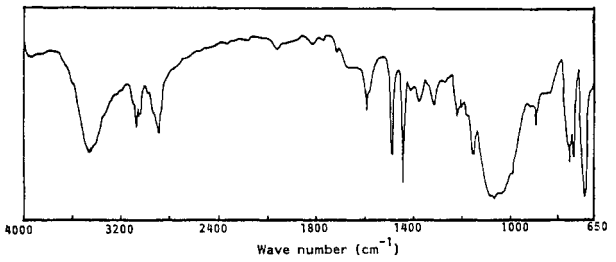
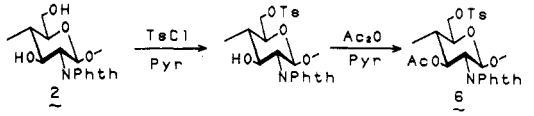
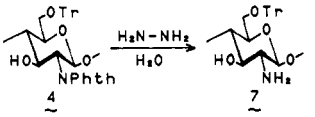


Figure 2. IR spectrum of derivative 7.

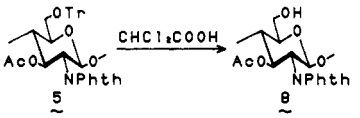
Scheme III



Scheme IV



Scheme V



C-6 positions with nucleophiles including azide, halides, hydride, and so on.

Selective Deprotection of Chitosan Derivatives. One of the most important and desirable characteristics for a protecting group is that it should be removed easily and in high yield at appropriate reaction stages. Selective deprotections of compounds 4 and 5 containing two different types of protective groups were thus examined.

Removal of the phthaloyl group from 4 was efficiently carried out by treatment with hydrazine hydrate at 100 °C (Scheme IV). The resulting 2-amino-3-hydroxyl derivative 7 showed a characteristic absorption at 1600 cm^{-1} due to primary amino groups in addition to those due to trityl groups, and absorptions at 1770 and 1710 cm^{-1} due to phthalimido groups disappeared completely (Figure 2). The selective and quantitative dephthaloylation of 4 was also supported by the satisfactory result of elemental analysis. Further modifications of 7 will accelerate systematic preparations of new types of bioactive polysaccharides such as anticoagulant or antimetastatic reagents,[16,17] and immunomodulators,[18] by selective introduction of sulfate groups at the amino groups and/or hydroxyl groups.

Unexpectedly, the trityl ether of 5 was rather resistant on heating in aqueous acetic acid was readily cleaved by treatment with dichloroacetic acid at room temperature for 30 min, giving 3-*O*-acetyl-2-phthalimido derivative 8 in quantitative yield (Scheme V). The chemical structure of 8 was elucidated by the IR spectrum (Figure 3) and elemental analysis. Obviously, disappearance of absorptions due to monosubstituted phenyl groups was observed without appreciable change in absorptions due to 3-*O*-acetyl and phthalimido groups in the spectrum, and the quantitative removal of trityl ether was also suggested by the satisfactory result of elemental analysis. The versatility of this derivative having primary hydroxyl groups at the C-6 positions will be demonstrated as a key glycosyl acceptor for coupling reactions with some glycosyl donors, which would afford novel branched type derivatives.[9]

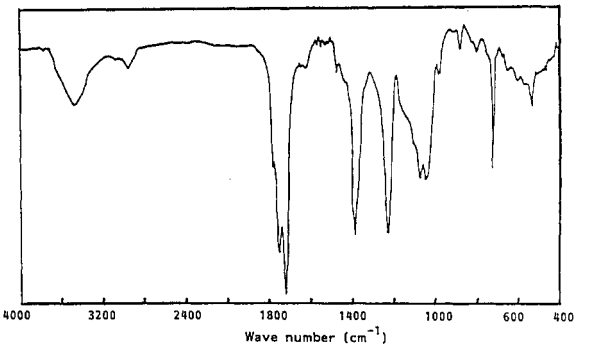


Figure 3. IR spectrum of derivative 8.

Solubility of Novel Chitosan Derivatives. In contrast to other known chitosan derivatives, all of the products synthesized here showed excellent solubility in common organic solvents (see Table I). The high solubility of derivative 2 in pyridine allowed further reactions at considerably high concentrations above 5%. Derivatives 3 and 5 were soluble even in low-boiling solvents such as chloroform and dichloromethane. Transparent thin films could be obtained by solution casting from these solvents.

In conclusion, we have demonstrated the availability of regioselective chemical modifications in homogeneous solution under mild reaction conditions for efficient transformations of chitosan into a variety of soluble derivatives by using completely N-protected derivative 2. The synthetic strategy described here has numerous advantages for the preparation of new types of chitosan derivatives with potential applications to biomedical systems. The versatile intermediates 2, 4, and 6–8 will provide finely designed materials by further selective modifications.

Experimental Section

General Procedures. Chitosan was prepared by repeating N-deacetylation of chitin from shrimp shells.[19] The degree of deacetylation was 1.00 (100%) as determined by elemental analysis and conductometric titration. Unless otherwise stated, all commercially available solvents and reagents were used without further purification. DMF was distilled under reduced pressure from calcium hydride (CaH_2) and stored over molecular sieves (3 Å). Pyridine was stored over molecular sieves (3 Å) for several days before use. IR spectra were recorded on a Jasco IR-700. ^1H NMR and proton-decoupled carbon NMR spectra were recorded at 270 and 67.8 MHz, respectively, with a JEOL JNM-GX 270 spectrometer, using tetramethylsilane (TMS) as the internal standard and chloroform-*d* as the solvent. Optical rotations were determined with a Jasco DIP-370 digital polarimeter at 23 °C. Conductometric titration was done with a Toa CM-40S conductivity meter. Elemental analyses were performed with a Yanaco MT-3 CHNcorder on the samples extensively (ca. 24 h) dried in vacuo [50 °C (0.1 Torr)] over phosphorus pentoxide (P_2O_5).

(1→4)-2-Deoxy-2-phthalimido-β-D-glucopyranan (2). A mixture of chitosan (1; 5.00 g, 31.0 mmol) and phthalic anhydride (13.8 g, 93.1 mmol) in DMF (100 mL) was heated with stirring at 130 °C under a nitrogen atmosphere. After 5–7 h, the mixture became a clear and viscous solution. The precipitate obtained by pouring the solution into ice–water was collected by filtration, successively washed completely by Soxhlet's extraction with ethanol, and dried over P_2O_5, to give 2 (8.7 g, 95.8%); $[\alpha]_D$ +15.5° (*c* 0.30, DMF).

Anal. Calcd for $C_{14}H_{13}O_6N \cdot H_2O$: C, 54.37; H, 4.89; N, 4.53. Found: C, 54.07; H, 4.39; N, 3.90.

(1→4)-3,6-Di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranan (3). To a solution of 2 (500 mg, 1.72 mmol) in pyridine (20 mL) was added acetic anhydride (10 mL), and the mixture was stirred for 32 h at room temperature. It was poured into ice–water (300 mL). The precipitate was collected, washed successively with ethanol and ether, and dried over P_2O_5 to afford

white powdery 3 (586 mg, 88.2%). The resulting product was subsequently purified by chromatography on a Sephadex LH-20 with 95:5 (v/v) chloroform–ethanol as eluant to give analytically pure material; $[\alpha]_D$ −8.4° (c 0.30, $CHCl_3$); ^1H NMR δ ($CDCl_3$) 1.70–1.95 (each br s, 6 H, 2 $-COCH_3$), 7.6–7.9 (m, 4 H, aromatic); ^{13}C NMR δ ($CDCl_3$) 20.1 and 20.4 ($-COCH_3$), 55.2 (C-2), 61.9 (C-6), 69.9, 72.4, and 74.8 (C-3, -4, and -5), 96.8 (C-1), 123.8, 131.4, and 134.4 (-phthalimide), 167.7 and 169.9 (C=O).

Anal. Calcd for $C_{18}H_{17}O_8N \cdot 0.5H_2O$: C, 56.25; H, 4.72; N, 3.64. Found: C, 56.36; H, 4.51; N, 3.68.

(1→4)-2-Deoxy-2-phthalimido-6-*O*-(triphenylmethyl)-β-D-glucopyranan (4). To a solution of 2 (5.00 g, 17.2 mmol) in pyridine (75 mL) was added chlorotriphenylmethane (47.9 g, 0.172 mol), and the mixture was stirred for 24 h at 90 °C under a nitrogen atmosphere. The homogeneous solution was cooled to room temperature and evaporated to viscous syrup. The white solid obtained by pouring the residue into ethanol (300 mL) was collected by filtration, washed with ethanol and ether, and dried over P_2O_5 to give 4 (9.07 g, 99%); $[\alpha]_D$ −2.9° (c 0.30, DMF).

Anal. Calcd for $C_{33}H_{27}O_6N$: C, 74.28; H, 5.10; N, 2.63. Found: C, 74.03; H, 5.57; N, 2.41.

(1→4)-3-*O*-Acetyl-2-deoxy-2-phthalimido-6-*O*-(triphenylmethyl)-β-D-glucopyran (5). A mixture of 4 (198 mg, 0.371 mg) and acetic anhydride (5 mL) in pyridine (10 mL) was stirred at room temperature for 15 h. The resulting wine-colored solution was poured into ice–water, and the pale yellow precipitation was collected by filtration. It was washed successively with ethanol and ether and dried over P_2O_5 to give 5 (191 mg, 89.3%); $[\alpha]_D$ −16.9° (c 0.30, $CHCl_3$).

Anal. Calcd for $C_{35}H_{29}O_7N \cdot 1.5H_2O$: C, 69.76; H, 5.53; N, 2.32. Found: C, 69.63; H, 4.16; N, 2.73.

(1→4)-3-*O*-Acetyl-2-deoxy-2-phthalimido-6-*O*-(*p*-tolylsulfonyl)-β-D-glucopyranan (6). To a cooled solution of 2 (1.00 g, 3.43 mmol) in pyridine (20 mL) was gradually added *p*-tolylsulfonyl chloride (6.50 g, 34.3 mmol), and the mixture was stirred for 17 h at room temperature. The precipitate obtained by pouring the viscous solution into ice–water (300 mL) was collected by filtration, washed with ethanol and ether, and dried to give a crude 6-*O*-tolyl derivative (1.33 g). The product was used for the subsequent 3-O-acetylation without further purification.

A mixture of the tosyl derivative (1.33 g) and acetic anhydride (10 mL) in pyridine (20 mL) was stirred for 17 h at room temperature, and the mixture was poured into ice–water (300 mL). The resulting precipitate was filtered, successively washed with ethanol and ether, and dried over P_2O_5 to yield powdery 6 (0.91 g, 77.4% from 2); $[\alpha]_D$ −17.3° (c 0.30, pyridine).

Anal. Calcd for $C_{23}H_{21}O_9NS \cdot 0.5H_2O$: C, 55.64; H, 4.34; N, 2.87. Found: C, 55.14; H, 4.16; N, 3.27.

(1→4)-2-Amino-2-deoxy-6-*O*-(triphenylmethyl)-β-D-glucopyranan (7). A mixture of 4 (3.70 g, 6.93 mmol), hydrazine monohydrate (20 mL), and water (40 mL) was heated with stirring for 15 h at 100 °C under a nitrogen atmosphere. After cooling, the mixture was diluted with water (50 mL) and evaporated with a rotary evaporator; this procedure was repeated three times. The residue was resuspended in deionized water, and the white precipitate was filtered. It was washed with ethanol and ether and dried over P_2O_5 to afford 7 (2.79 g, 84%); $[\alpha]_D$ −10.6° (c 0.30, DMAc). Compound 7 was ninhydrin positive.

Anal. Calcd for $C_{25}H_{25}O_4N \cdot H_2O$: C, 71.24; H, 6.46; N, 3.32. Found: C, 71.08; H, 6.22; N, 3.67.

(1→4)-3-*O*-Acetyl-2-deoxy-2-phthalimido-β-D-glucopyranan (8). Compound 5 (129 mg, 0.218 mmol) was suspended in dichloroacetic acid (5 mL) and stirred at room temperature. After 20–30 min, the reaction mixture became a clear viscous solution. The precipitate obtained by pouring the solution into a cold mixture of water (30 mL) and ethanol (10 mL) was quickly collected by filtration, washed with 1:1 (v/v) ethanol–ether (200 mL), and dried over P_2O_5 to yield 8 (74 mg, 98%); $[\alpha]_D$ −13.3° (c 0.30, DMAc).

Anal. Calcd for $C_{16}H_{15}O_8N$: C, 55.02; H, 4.33; N, 4.01. Found: C, 55.19; H, 4.35; N, 3.46.

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