Preparation of Nonnatural Branched Chitin and Chitosan

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N-Acetyl-D-glucosamine and D-glucosamine branches were introduced into chitin and chitosan, respectively, by a series of regioselective modification reactions based on *N*-phthaloyl-chitosan. Unlike insoluble chitin and chitosan, the resulting nonnatural branched amino polysaccharides showed a remarkable water solubility. Moreover, the branched chitosan exhibited significant antimicrobial activity.

Branched polysaccharides are attracting much attention owing to the characteristic physicochemical properties and biological activities. Lentinan[1] and schizophyllan[2] are particularly important because of their considerable antitumor activity. It is therefore worthwhile to establish procedures for preparing tailored branched polysaccharides; this will make possible discussing the structure-properties relationship to develop various advanced functions.

Ring-opening polymerization of 1,6-anhydro monosaccharides followed by branching[3] or polymerization of 1,6-anhydro disaccharides[4] gave synthetic comb-like polysaccharides. A more versatile approach may be the introduction of sugar branches into natural polysaccharides. It is, however, generally difficult to introduce such branches at a specific position of linear polysaccharides such as cellulose and curdlan.[5] Some sugar branches were introduced at C-6 of amylose and cellulose having proper protecting groups.[6]

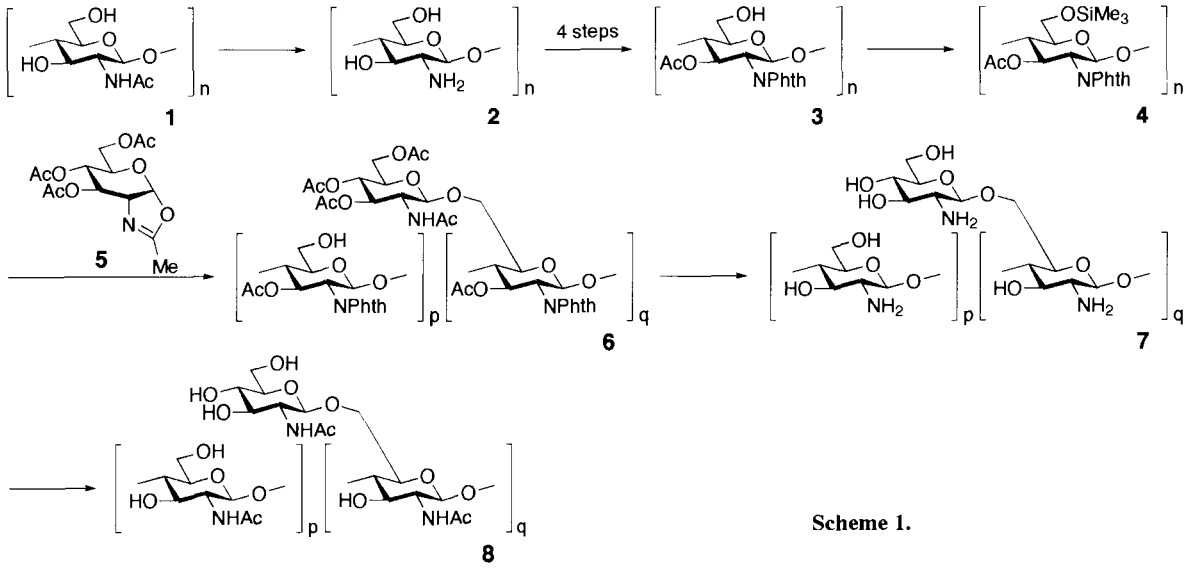
As a linear trunk polysaccharide, chitin is considered to be particularly useful for glycosylation owing to the presence of three different kinds of functional groups. Furthermore, chitin (**1**) and the deacetylated form, chitosan (**2**), are characterized by various specific bioactivities as well as low toxicity. In order to develop advanced functions based on these abundant amino polysaccharides, controlled and regioselective modifications are crucial. In this regard, *N*-phthaloyl-chitosan has been suggested to be a suitable starting material that enables efficient modification reactions in solution under mild conditions.[7] α-Mannoside branches could thus be introduced by the reaction with an orthoester of D-mannose.[8]

Of various sugars to be introduced into chitin and chitosan, *N*-acetyl-D-glucosamine and D-glucosamine are especially interesting, since the resulting polysaccharides have the same sugar units in the main chains and branches. The products are nonnatural branched chitin and chitosan and would be significant in view of elucidating the influence of branches on the properties and thereby controlling their bioactivities. Here we report the regioselective introduction of glucosamine units at the C-6 positions and some properties of the resulting chitin and chitosan derivatives.

A chitosan-based acceptor (**3**) was prepared from fully deacetylated chitosan (**2**)[9] according to the procedure previously reported.[7] All the transformations were quantitative in terms of the degree of substitution (ds) (Scheme 1). As the donor for glycosylation, an oxazoline (**5**) was prepared from peracetylated D-glucosamine as reported.[10]

The glycosylation of **3** with **5** was expected to result in the formation of peracetylated *N*-acetyl-D-glucosamine branches with the β-glycosidic linkage. The reaction was first attempted in DMF solution, since **3** was soluble only in polar organic solvents, in the presence of (+)-10-camphorsulfonic acid as the catalyst at 80 °C. However, **3** was recovered quantitatively.

The acceptor **3** was then transformed into the trimethylsilylated derivative (**4**), which showed much higher



Scheme 1.

solubility. The glycosylation reaction thus could be conducted in 1,2-dichloroethane solution efficiently at 80 °C, giving rise to the branched derivative (**6**). It was isolated in methanol as a pale tan powder,[11] and the ds was determined by ^{1}H-NMR. As shown in Table 1, the ds was 0.20 when the reaction was performed with equimolar amounts of reactants. With increases in the amount of the oxazoline and reaction time, the ds increased.

Table 1. Glycosylation reaction of chitosan derivatives (**3** and **4**) with an oxazoline of glucosamine (**5**)[a]

Oxazoline / Pyranose[b]	Temp/ °C	Time/ h	ds[c] from **3**[d]	ds[c] from **4**[e]
1	80	24	0	0.20
3	80	24	—	0.33
3	80	48	—	0.40
5	80	24	—	0.35

[a]Catalyst, (+)-10-camphorsulfonic acid (0.17 equivalent to pyranose). [b]Mole ratio. [c]Degree of substitution determined from the peak ratio of Ac/Phth in ^{1}H-NMR in $CDCl_3$. [d]In DMF. [e]In 1,2-dichloroethane.

Subsequent deprotection gave the corresponding chitosan derivative (**7**). The protected product **6** with ds 0.33 was, for example, deacetylated with 1 M sodium hydroxide at room temperature overnight and dephthaloylated with hydrazine hydrate at 90 °C for 24 h. After dialysis and freeze drying, **7** was obtained as a pale brown powdery material. The overall yield from **6** was 60%. The IR spectra of **7** was quite similar to that of chitosan, and a band due to free amino groups was observed at 1650 cm^{-1}.

Branched chitosan **7** was then N-acetylated with acetic anhydride in methanol at room temperature to give branched chitin (**8**) as a pale brown powder in 80% yield. The IR spectrum of **8** was almost identical with that of chitin; characteristic absorption bands appeared at 1650 (amide I) and 1559 cm^{-1} (amide II).

The resulting **6**, **7**, and **8** exhibited remarkable solubility in sharp contrast to the insoluble chitin and chitosan. The protected product **6** was soluble even in low boiling organic solvents such as acetone and dichloromethane. The deprotected derivatives, **7** and **8**, were readily soluble in neutral water. The solubilities were apparently high, but the solutions became so thick at a concentration above 5% that they showed only poor flows. The high viscosity suggested that the degradation of the main chain would not have occurred extensively during the multi-step modification process under mild conditions. Although both **7** and **8** were quite hydrophilic, **8** exhibited particularly high hygroscopicity. The weight increase of **8** in 93% relative humidity was 40% whereas that of chitin was only 17%. They also swelled considerably in organic solvents.

Antimicrobial activity of **7** was evaluated preliminarily according to the previously reported procedure,[12] and **7** was found to be somewhat more active (15% and 10%) than linear chitosan in the growth suppression against *Staphylococcus aureus* and *Streptococcus mutans*, respectively. This implies the high potential of the branched amino polysaccharides as a new type of water-soluble antimicrobial agents.

Consequently, branched amino polysaccharides have proved to be prepared through controlled modifications of chitosan and exhibit high solubility in water unlike the linear amino polysaccharides. This is the first example of branched amino polysaccharides, and they would be important in view of possible bioactivities including distinctive antimicrobial and pharmacological activities. Details of the preparation and characteristics of these branched polysaccharides will be reported in the near future.

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11 Spectral and analytical data for **6** with ds 0.33 are as follows. IR (KBr): ν 1775 and 1721 (phth C=O), 1750 (Ac C=O), and 1150-1000 cm^{-1} (pyranose). ^{1}H-NMR ($CDCl_3$): δ 1.7-2.1 (m, CH_3), 3.2-5.6 (m, CH and CH_2), and 8.1 ppm (broad s, arom H). ^{13}C-NMR ($CDCl_3$): δ 21.04 ($OCO\underline{C}H_3$), 25.00 ($NHCO\underline{C}H_3$), 55.60 (C-2,2'), 60.90 (C-6), 62.35 (C-6'), 75.19 (C-3,3'), 96.27 (C-1,1'), 123.36, 131.18, and 134.17 (phth arom C), 167.76 ($O\underline{C}OCH_3$), and 170.08 ppm ($NH\underline{C}OCH_3$ and phth C=O). Anal. Calcd for $(C_{30}H_{34}N_2O_{15})_{0.33}(C_{16}H_{15}NO_7)_{0.67}\cdot 0.7H_2O$: C, 54.48; H, 5.02; N, 4.09. Found: C, 54.69; H, 4.97; N, 4.01.
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