CHEMISTRY LETTERS, pp. 2063-2066, 1994.

Synthesis of Non-Natural Branched Polysaccharides. Regioselective Introduction of α-Mannoside Branches into Chitin

Keisuke KURITA,* Masayuki KOBAYASHI, Tetsuya MUNAKATA, Shigeru ISHII, and Shin-Ichiro NISHIMURA†

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

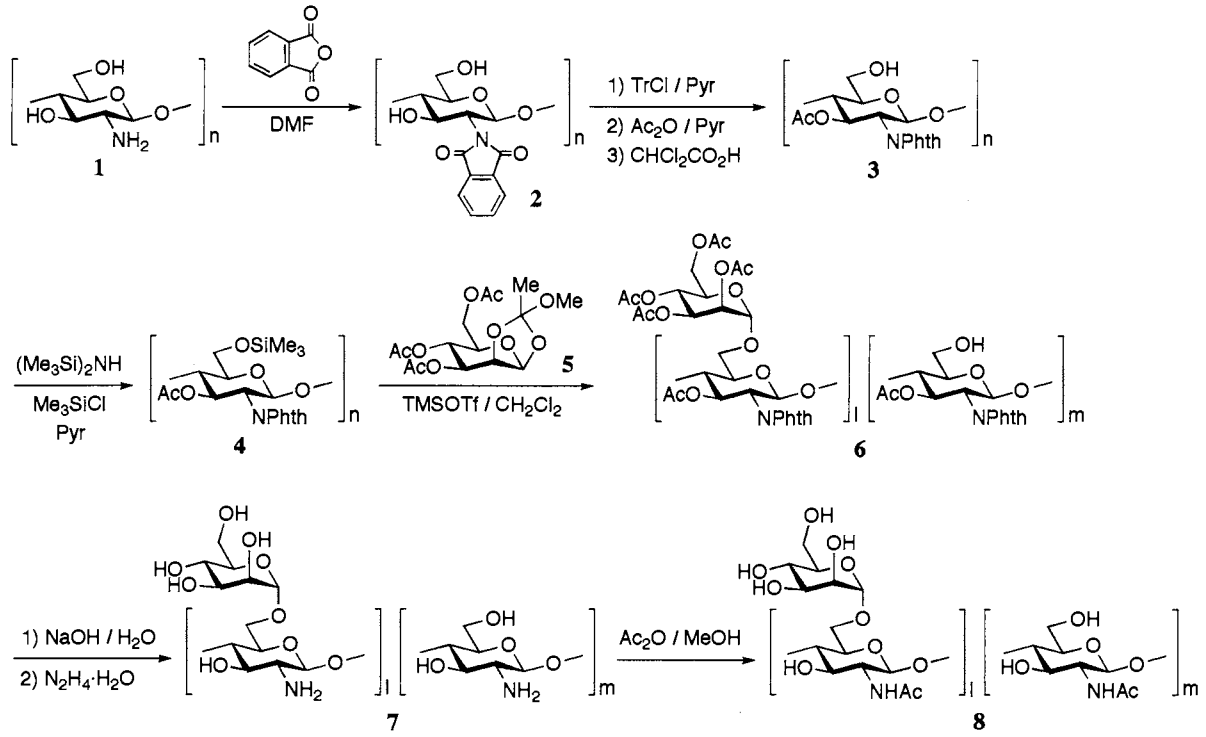
†Division of Biological Science, Graduate School of Science, Hokkaido University, Sapporo, Hokkaido 060

N-Phthaloyl-chitosan was converted into 3-*O*-acetyl-6-*O*-trimethylsilyl-*N*-phthaloyl-chitosan, which was then subjected to the glycosylation of a peracetylated mannose orthoester to form an α-(1→6) linkage. Subsequent deprotection followed by *N*-acetylation afforded chitins having mannose branches. The branched chitins were readily soluble in water and showed strong affinity for concanavalin A.

Increasing attention has been paid to polysaccharides, but in view of specific biological activity, solubility, and solution viscosity behavior, branched polysaccharides are particularly interesting. Some of such polysaccharides including lentinan[1,2] and shizophyllan[3] are, for example, significant because of the high immunostimulating activity. In order to develop favorable bioactivities or advanced functions characteristic of branched polysaccharides and also to discuss the relationship between the molecular structure and properties, preparation of non-natural branched polysaccharides is quite crucial. Regioselective introduction of sugar groups is, however, difficult owing to the multi-functionality and limited solubility of linear polysaccharides. Ring-opening copolymerization of 1,6-anhydro mono- and disaccharide derivatives[4] or polymerization of a 1,6-anhydro derivative followed by glucosylation may be convenient ways for preparing branched polysaccharides,[5] and analogs of pharmacologically interesting panaxan A were synthesized.

Of abundant polysaccharides, chitin is quite attractive owing to the inherent biological activities, but it is insoluble in organic solvents suitable for modification reactions, and this has caused serious difficulties in modifications. Some reducing sugars[6,7] and non-reducing sugars having an aldehyde group[8,9] were introduced into chitosan by reductive *N*-alkylation. Although the sugar groups were not attached by glycosidic linkages, the chitosan derivatives showed interesting solubility and rheological properties.

Our recent attention has been focused on the evaluation of *N*-phthaloyl-chitosan as a versatile key intermediate for regioselective and quantitative chemical modifications, since it is soluble in common organic solvents and easily deprotected to regenerate the free amino groups leading to effective discrimination of the three kinds of functional groups.[10-12] *N*-Phthaloyl-chitosan is thus expected to be a suitable precursor to prepare non-natural branched polysaccharides with various advanced functions depending on the kinds of sugar groups and branching positions. It has thus been examined as a starting material for the introduction of mannose branches at the C-6 positions.



Scheme 1.

In order to make possible regioselective substitution at C-6 of chitin, *N*-phthaloyl-chitosan (**2**) prepared from fully deacetylated chitosan (**1**) was transformed into the derivative (**3**) having free hydroxyl groups at C-6 and protecting groups at C-2 and C-3[11] as shown in Scheme I. **3** was subjected to the reaction with a peracetylated mannose orthoester, 3,4,6-tri-*O*-acetyl-1,2-*O*-methylorthoacetyl-α-D-mannopyranose (**5**), in various solvents under heterogeneous conditions. The attempts were, however, unsuccessful as judged from the dark colors and low substitution degrees of the products.

The glycosidation reaction may proceed much more efficiently in solution in suitable solvents such as dichloromethane, and thus the C-6 hydroxyl groups were trimethylsilylated. The reaction was effected with a mixture of trimethylsilazane and trimethylsilyl chloride in pyridine solution at room temperature to afford 3-*O*-acetyl-6-*O*-trimethylsilyl-*N*-phthaloyl-chitosan (**4**). **4** showed much improved solubility as expected and was soluble in low boiling solvents such as dichloromethane and chloroform.

A series of transformations from **1** to **4** proceeded in solution under mild conditions, and perfect discrimination of the three kinds of functional groups of chitosan was accomplished to enable regioselective substitutions. Moreover, the reactions were quantitative, and every substitution degree was 1.0 as convinced by the IR spectra and the thoroughly satisfactory results of elemental analysis.

The reaction of **4** with **5** was quite efficient in dichloromethane solution in the presence of TMSOTf at room temperature to form α-(1→6) linkages. The resulting product (**6**) was isolated by pouring the solution into ethanol as a white powdery material. The degree of substitution, as determined from the C/N ratio of the elemental analysis, could be regulated by the amount of the orthoester and reached 0.52 with a 10-fold excess

of the orthoester in 72 h reaction. The IR spectrum showed strong ester bands as a result of the incorporation of the peracetylated mannose branches.

6 was then treated with 1 mol/L aqueous sodium hydroxide at 50 °C for deprotection. The IR spectra, however, showed weak bands due to the phthaloyl groups, and thus complete dephthaloylation was achieved with hydrazine at 100 °C. The resulting product (**7**) was the chitosan derivative having mannose branches, and the IR spectrum was quite similar to that of chitosan.

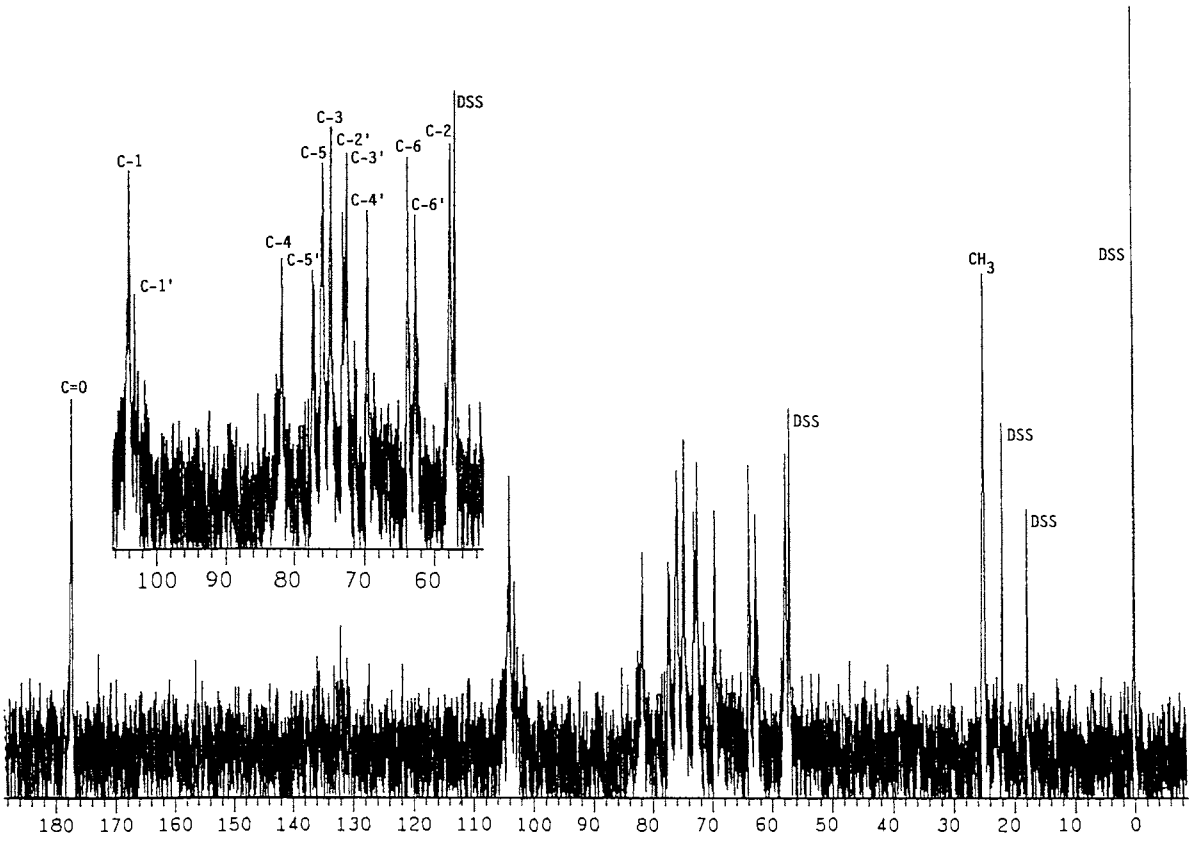


Fig. 1. ^{13}C NMR spectrum of **8** in D_2O (internal reference, DSS).

On selective *N*-acetylation of **7** with acetic anhydride in methanol at room temperature, the chitosan backbone was transformed into the chitin backbone to give branched chitin **8**. The IR spectrum was quite similar to that of chitin, but the bands at 1000-1150 cm^{-1} due to the sugar groups became more evident. The ^{13}C NMR spectrum of **8** in D_2O (Fig. 1) shows the acetyl methyl peak at 24.8 ppm and acetyl carbonyl peak at 177.3 ppm, the internal reference being DSS, 3-(trimethylsilyl)propanesulfonic acid sodium salt.. The peaks observed in the range of 57.6 to 103.9 ppm are ascribable to both the chitin backbone (C-1 to C-6) and mannose branches (C-1' to C-6').

The resulting chitosan and chitin derivatives having mannose branches, **7** and **8**, were readily soluble in water and swelled highly in common organic solvents in sharp contrast to insoluble chitin and chitosan. Unlike other soluble chitin derivatives including water-soluble chitin,[13] these branched polysaccharides showed strong affinity for concanavalin A owing to the presence of the α-mannoside groups as confirmed by turbidity

on mixing aqueous solutions of **7** or **8** and concanavalin A even at a low concentration of 0.2 mg/mL each at room temperature. At concentrations higher than 0.3 mg/mL, precipitation took place on standing for a few minutes.

N-Phthaloyl-chitosan **2** has thus proved to be the first organosoluble starting material to enable regioselective and quantitative modifications of chitin, and this key precursor would be quite useful to diversify the molecular design for polysaccharide-based advanced materials including various types of non-natural as well as natural branched polysaccharides.

Partial financial support by Towa Shokuhin Kenkyu Shinkoukai is greatly acknowledged.

References

1) G. Chihara, Y. Maeda, J. Hamuro, T.Sasaki, and F. Fukuoka, *Nature*, **222**, 687 (1969).
2) G. Chihara, Y. Maeda, and J. Hamuro, *Int. J. Tissus. React.*, **4** (3), 207 (1982).
3) M. Mitani, T. Ariga, T. Matsuo, T. Asano, and G. Saito, *Int. J. Immunopharmacol.*, **2**, 174 (1980).
4) H. Kuzuhara, Y. Ichikawa, N. Sakairi, T. Uryu, and T. Yoshida, XIVth Int. Carbohydr. Symp. (Stockholm), Abstr., 201 (1988).
5) K. Hatanaka, S.-O. Song, A. Maruyama, T. Akaike, A. Kobayashi, and H. Kuzuhara, *J. Carbohydr. Chem.*, **11**, 1027 (1992).
6) L. D. Hall and M. Yalpani, *J. Chem. Soc., Chem. Commun.*, **1980**, 1153.
7) M. Yalpani and L. D. Hall, *Macromolecules*, **17**, 272 (1984).
8) L. D. Hall and K. R. Holme, *J. Chem. Soc., Chem. Commun.*, **1986**, 217.
9) K. R. Holme and L. D. Hall, *Macromolecules*, **24**, 3828 (1991).
10) S. Nishimura, O. Kohgo, K. Kurita, C. Vittavatvong, and H. Kuzuhara, *Chem. Lett.*, **1990**, 243.
11) S. Nishimura, O. Kohgo, K. Kurita, and H. Kuzuhara, *Macromolecules*, **24**, 4745 (1991).
12) S. Nishimura, Y. Miura, L. Ren, M. Sato, A. Yamagishi, N. Nishi, S. Tokura, K. Kurita, and S. Ishii, *Chem. Lett.*, **1993**, 1623.
13) K. Kurita, T. Sannan, and Y. Iwakura, *Makromol. Chem.*, **178**, 3197 (1977).

(Received August 12, 1994)