LINEAR POLYSACCHARIDES AS PRECURSORS FOR GLUCAN-CHITIN HYBRID MATERIALS

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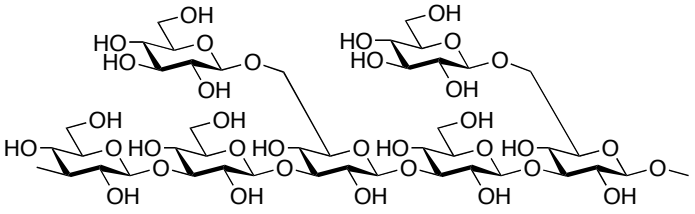
Abstract

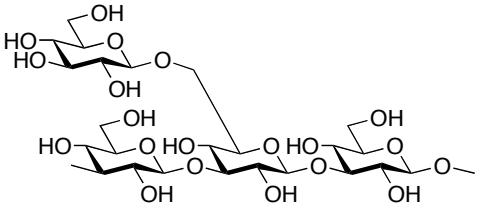
Synthesis of glucan-chitin hybrid materials composed of linear glucans and *N*-acetylglucosamine branches was undertaken in the course of our studies on the branched chitins and chitosans having various sugar branches. Curdlan was transformed into a derivative having a reactive group at C-6 and protective groups at C-2 and C-4, using appropriate protection-deprotection techniques. Glycosylation reaction of the resulting acceptor with an oxazoline prepared from glucosamine proceeded efficiently to give a branched product. The degree of substitution could be controlled easily by the amount of oxazoline. Deprotection of the product by transesterification resulted in the formation of a curdlan having *N*-acetylglucosamine branches at the C-6 position. Similar modification reactions could be conducted with cellulose, giving rise to branched celluloses.

Introduction

Among a wide variety of polysaccharides occurring in nature, some branched glucans isolated from mushrooms are attracting increasingly more attention because of their immunoadjuvant property. Lentinan from *Lentinula edodes* and schizophyllan from *Schizophyllum commune* are typical examples [1-3]. The main chains of lentinan and schizophyllan are a polymer of β-(1→3)-linked D-glucopyranose, curdlan, and they have β-(1→6)-linked glucopyranosyl branches: two branches per five repeating units and one branch per three repeating units, respectively, as shown in Scheme 1. The unique properties of these mushroom polysaccharides will be attributable to the presence of sugar branches [4].



Lentinan



Schizophyllan

Scheme 1: Branched polysaccharides in mushrooms.

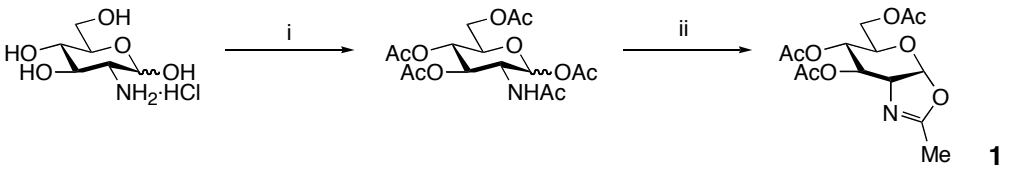
It is therefore significant to synthesize nonnatural branched polysaccharides starting from common linear polysaccharides to diversify the structures. Cellulose acetate, for example, was used for glycosylation with glucose orthoesters [5,6], but the substitution is not regioselective. In view of the bioactivity of mushroom polysaccharides and the distinctive biological and physicochemical properties of chitin [7-14], it is considered of interest to synthesize nonnatural branched polysaccharides having *N*-acetylglucosamine branches at the C-6 position of glucans as analogues of the mushroom polysaccharides.

As discussed in our previous papers on the controlled introduction of substituents into chitin and chitosan, it is crucial to protect the multifunctional polysaccharides precisely to prepare well-defined derivatives. Phthaloylation is a particularly useful protection reaction for chitosan, and the resulting *N*-phthaloyl-chitosan is convenient for various substitutions under mild conditions. Some *N*-phthaloyl-chitosan derivatives having only one reactive group at a specified position in the repeating unit have enabled regioselective modifications. Curdlan and cellulose are linear glucans and have three kinds of hydroxy groups in each repeating unit. Protection should thus be designed to discriminate these hydroxy functionalities. The derived acceptors having a reactive group at C-6 would allow glycosylation with donors such as an oxazoline prepared from D-glucosamine to introduce amino sugar branches.

Materials and Methods

General

Chitosan with a degree of deacetylation 1.0 was obtained by repeated deacetylation of chitin. Curdlan was used as received. Cellulose was prepared by deacetylation of acetyl-cellulose. An oxazoline (**1**) was synthesized from D-glucosamine by peracetylation with acetic anhydride at room temperature followed by cyclization with trimethylsilyl trifluoromethanesulfonate at 50 °C (Scheme 2) [15].



Scheme 2: Synthesis of an oxazoline from glucosamine: (i) acetic anhydride; (ii) trimethylsilyl trifluoromethanesulfonate.

Protection and Deprotection

Phthaloylation of chitosan was carried out with phthalic anhydride in dimethylformamide to give *N*-phthaloylated chitosan whose hydroxy groups were partially phthaloylated [16], or in dimethylformamide/water to afford regioselectively *N*-phthaloylated chitosan [17].

Chlorotriphenylmethane was added to a solution of curdlan in pyridine, and the mixture was heated at 80 °C to give 6-*O*-triphenylmethyl-curdlan with a degree of substitution 1.0. The product was treated with phenyl isocyanate in pyridine at 100 °C for full protection. The resulting 2,4-di-*O*-phenylcarbamoyl-6-*O*-triphenylmethyl-curdlan was stirred in dichloroacetic acid at room temperature, and the detriphenylmethylated derivative was isolated in water. The degree of substitution of 2,4-di-*O*-phenylcarbamoyl-curdlan was 2.0.

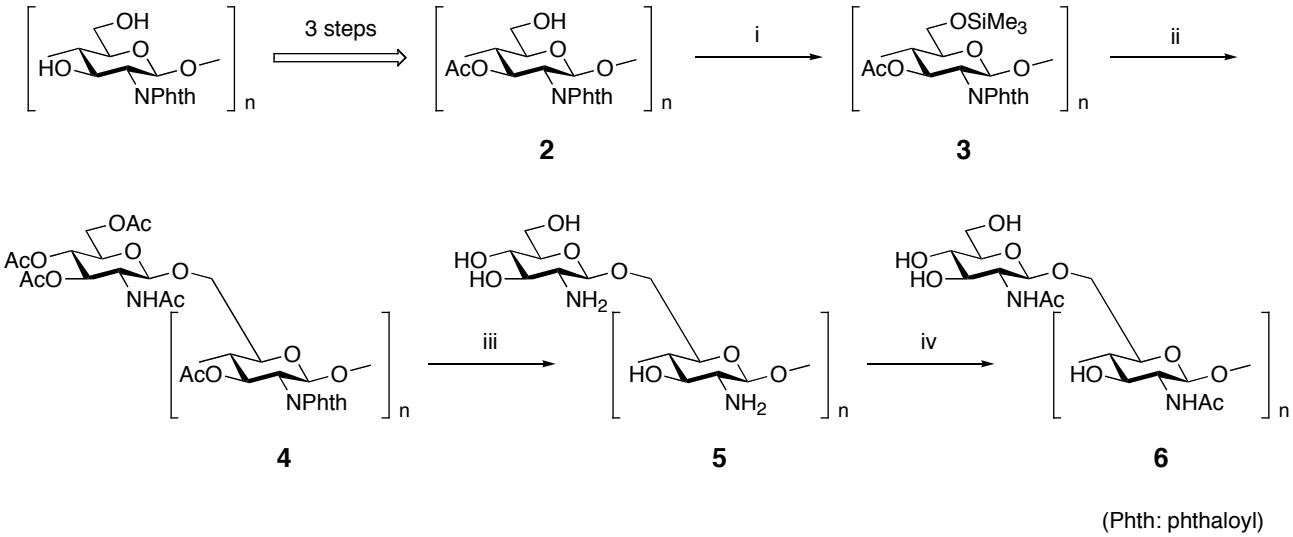
Glycosylation

Glycosylation of 2,4-di-*O*-phenylcarbamoyl-curdlan with glucosamine-derived oxazoline **1** was conducted in 1,2-dichloroethane in the presence of 10-camphorsulfonic acid at 80 °C. After the reaction, the branched product was isolated in methanol/water.

The glycosylated product was de-*O*-acetylated and de-*O*-phenylcarbamoylated by transesterification with sodium methoxide in a mixed solvent of methanol and dioxane at 60 °C. The final product was obtained after dialysis.

Results and Discussion

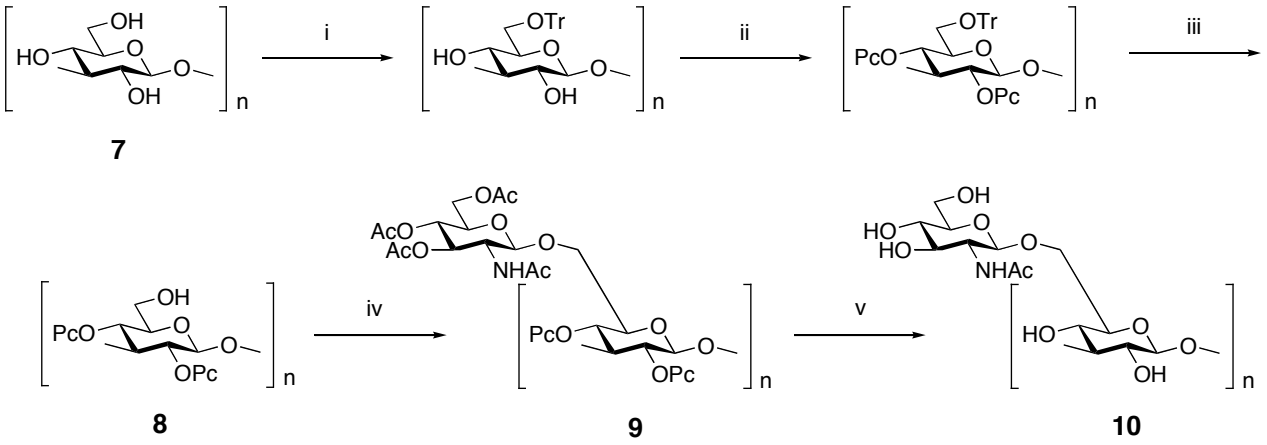
An example of regioselective branching of chitin based on 2-*N*-phthaloyl-chitosan is illustrated in Scheme 3. Both 3-*O*-acetyl-2-*N*-phthaloyl-chitosan (**2**) and its 6-*O*-trimethylsilyl derivative (**3**) were suitable acceptors for the glycosylation with glucosamine-derived oxazoline **1** to introduce amino sugar branches at C-6. Deprotection of the glycosylated products (**4**) with hydrazine gave branched chitosans (**5**), and the subsequent *N*-acetylation resulted in the formation of branched chitins (**6**) [18].



Scheme 3: Synthesis of branched chitosan and chitin: (i) hexamethyldisilazane, chlorotrimethylsilane; (ii) oxazoline **1**; (iii) hydrazine monohydrate; (iv) acetic anhydride.

Curdlan (**7**) was triphenylmethylated with chlorotriphenylmethane and then phenylcarbamoylated with phenyl isocyanate. Subsequent detriphenylmethylation in dichloroacetic acid yielded a derivative having a free hydroxy group at C-6 (**8**) (Scheme 4). All these transformations proceeded in solution and were quantitative in terms of the substitution degrees. The intermediates as well as **8** were identified by spectroscopies and elemental analysis.

Curdlan acceptor **8** was treated with glucosamine donor **1** in 1,2-dichloroethane at 80 °C, 10-camphorsulfonic acid being used as the promoter, giving rise to the branched products (**9**). The degree of glycosylation could be controlled by the amount of **1**, and some typical results are summarized in Table 1. As evidenced in the table, the glycosylation of **8** proceeded easily compared to those of chitosan acceptors **2** and **3**. The degree of substitution reached 0.73 in the reaction with five-fold excess **1**, and it would become higher under appropriate conditions. Deprotection of **9** with methoxide/methanol removed *O*-acetyl and *O*-phenylcarbamoyl groups to produce *N*-acetylglucosamine-branched curdlans (**10**).



Scheme 4: Synthesis of branched curdlans: (i) chlorotriphenylmethane; (ii) phenyl isocyanate; (iii) dichloroacetic acid; (iv) oxazoline **1**; (v) sodium/methanol.

Table 1: Glycosylation reaction of curdlan acceptor **8** with oxazoline donor **1**

Oxazoline **1**/Pyranose of **8** (mol/mol)	Reaction conditions		DS[a]
	Temperature (°C)	Time (h)	
0.3	80	24	0.11
0.5	80	1	0.22
0.5	80	6	0.38
0.5	80	24	0.27
1	80	24	0.51
3	80	24	0.68
5	80	24	0.73

[a]Degree of substitution per pyranose unit determined from the acetyl/phenyl peak area ratio in ^{1}H NMR in DMSO-d_6.

The protected products **9** and deprotected products **10** showed remarkable solubility in sharp contrast to the original linear polysaccharide. Branched curdlans **10** were soluble in both water and common organic solvents such as methanol and pyridine.

In a similar manner, regioselective branching of cellulose, a β-(1→4) linked glucan, was examined. The C-6 hydroxy group of cellulose was protected by triphenylmethylation, and the remaining C-2 and C-3 hydroxy groups were phenylcarbamoylated. Detriphenylmethylation of the fully protected cellulose followed by trimethylsilylation gave a derivative having a reactive group at C-6. Glycosylation of the resulting acceptor with **1** was conducted to afford branched celluloses, but the reactivity of the cellulose acceptor appeared to be somewhat lower than that of curdlan acceptor **8**, judging from the degrees of substitution of the branched products obtained under similar reaction conditions.

Conclusion

In addition to the amino polysaccharides chitin and chitosan, linear glucans such as curdlan and cellulose have proved promising starting materials for regioselective chemical modifications to synthesize novel types of derivatives with well-defined structures, provided protection-deprotection techniques are applied properly. The resulting nonnatural polysaccharides having branches at a specific position are expected to exhibit interesting properties including some biological activities

and would be useful for discussing the structure-property relationship of polysaccharide-based functional materials.

Acknowledgements

This work was partially supported by "High-Tech Research Center" Project for Private Universities: matching fund subsidy from MEXT, 2004-2008.

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