Clarification of the Transfer Reaction in the Synthesis of Manno-oligosaccharide

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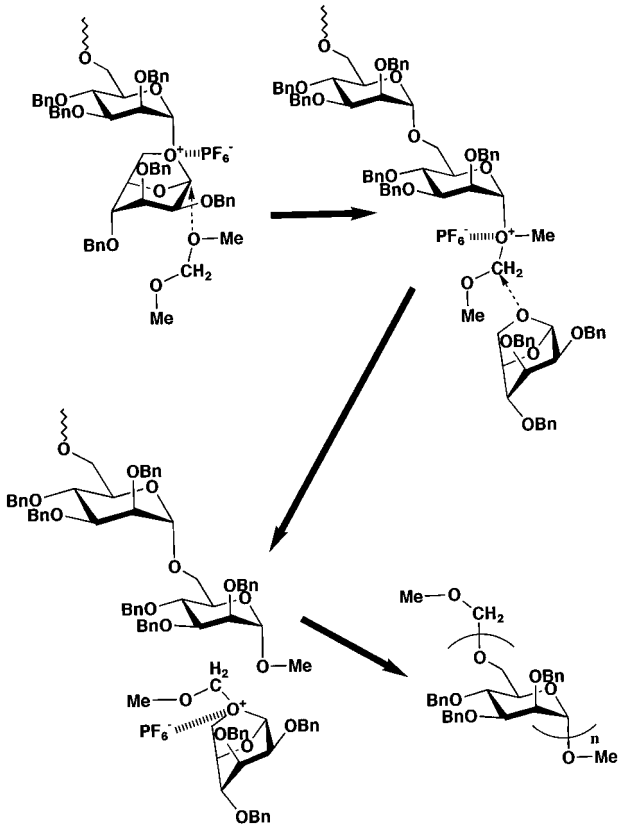
ABSTRACT: The cationic ring-opening reaction of 1,6-anhydro-2,3,4-tri-*O*-benzyl-*β*-D-mannopyranose was applied to the synthesis of α-(1→6)-linked manno-oligosaccharide which is a part of natural, high-mannose-type oligosaccharides. Dimethoxymethane was added into the reaction system as a transfer reagent in order to diminish the molecular weight of the products. It was confirmed by gel permeation chromatography measurements that additional dimethoxymethane distinctly lowered the molecular weight of the oligomerization products. The presence of the $-OCH_3$ group, which was transferred from dimethoxymethane, at the reducing terminal of the obtained oligosaccharide derivatives was confirmed by ^{13}C NMR measurements. In addition, methyl 2,3,4-tri-*O*-benzyl-6-*O*-methoxymethyl-α-D-mannopyranoside was synthesized as a model compound. From the comparison of the ^{13}C NMR spectrum of the reaction product with that of the model compound, $-O-CH_2-O-CH_3$ group binding to the nonreducing end was found to be present. These results showed the mechanism of the transfer reaction. Since another product was detected by using ^{13}C NMR spectroscopy, isolation of the products was carried out by silica gel column chromatography. It was shown by ^1H and ^{13}C NMR spectroscopies, COSY, HMQC, and HMBC that a 4,6-methylidene structure was also formed at the nonreducing end of the oligosaccharide derivatives.

Introduction

Recently, it has been shown that some oligosaccharides play important roles with respect to cell recognition. To elucidate the functions of oligosaccharides and their application to many fields such as medicinal drugs, the syntheses of oligosaccharides are important. The targets of such syntheses are not only natural oligosaccharides but also unnatural oligosaccharides, which may have great potential for higher biological activity than the natural compounds. Thus far, artificial oligosaccharides have been synthesized by organic and enzymatic procedures. In the studies of the organic procedure,[1] many glycosylation methods have been developed. And in the studies of the enzymatic procedure, some glycosyltransferases[2] and glycosidases[3] have been shown to be useful for syntheses of oligosaccharides. However, glycosylation involves a number of reaction steps, and enzymatic synthesis is limited to natural oligosaccharides. Therefore, a convenient synthetic method for an artificial homo-oligosaccharide is necessary. The cationic ring-opening polymerization of 1,6-anhydrosugar derivatives is an effective method to obtain artificial stereoregular polysaccharides with high molecular weight.[4] However, until now, this method has been used only for obtaining polysaccharides, and the application of this method to synthesis of oligosaccharide has not been undertaken.

In this study, we aimed at synthesis of an α-(1→6)-linked manno-oligosaccharide which is an example of asparagine-linked, high-mannose-type oligosaccharides. Dimethoxymethane was used as a transfer reagent to diminish the molecular weight of the reaction product (Scheme 1). The reaction mechanism of the transfer reaction is discussed based on ^{13}C NMR spectra of the reaction products and the synthesized model compound.

Scheme 1. Expected Transfer Reaction Mechanism



Results and Discussion

Effect of Dimethoxymethane on the Molecular Weight of Products. At first, oligomerization was carried out with 5 mol % of PF_5 (catalyst) and 10 mol % of dimethoxymethane in methylene chloride at −60 °C for 1 h. When dimethoxymethane was not added to the reaction system, the molecular weight of the product was several hundred thousand. On the other hand, in the presence of dimethoxymethane, most of the products were monosaccharide derivatives. From these results,

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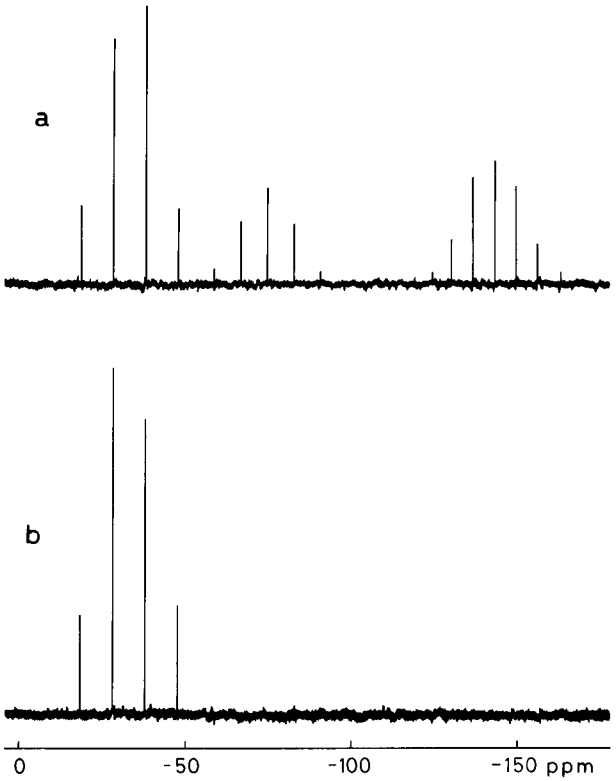


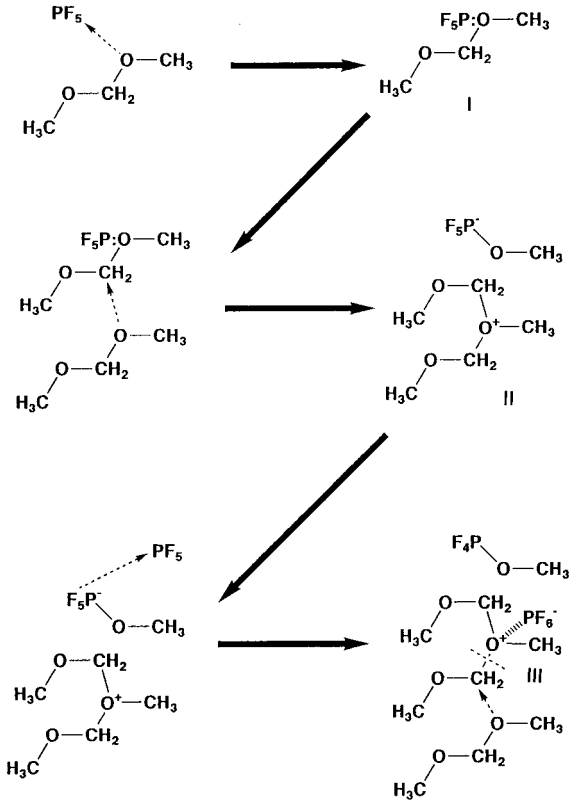
Figure 1. ^{31}P NMR spectra of PF_5 in CD_2Cl_2 at −60 °C: (a) without and (b) with dimethoxymethane.

the possibilities of coordination of dimethoxymethane to PF_5 and of inhibition of the reaction were assumed. Therefore, ^{31}P NMR measurements of PF_5 with and without dimethoxymethane in CD_2Cl_2 were examined. The spectra are shown in Figure 1. When PF_5 was dissolved in CD_2Cl_2, only a quartet peak appeared at −33.1 ppm. However, when both PF_5 and dimethoxymethane were dissolved, additional quintet and septet peaks appeared at −74.38 and −142.70 ppm, respectively. On the basis of the results described by Uryu et al.,[5] quartet, quintet, and septet peaks were assigned to PF_3O which was produced by the reaction of PF_5 with Pyrex,[6] PF_4OCH_3, and PF_6^-, respectively. From the ^{31}P NMR data, it can be considered that the reaction which occurred in the NMR sample tube should be as shown in Scheme 2. Dimethoxymethane reacts with PF_5, and then another dimethoxymethane molecule attacks nucleophilically to the dimethoxymethane−PF_5 complex (**I**) to give the ion pair (**II**). After the reaction of **II** with another PF_5 molecule, species **III** is reacted with dimethoxymethane by nucleophilic S_N2 attack. It can be concluded that while dimethoxymethane did not inhibit the reaction, the rate of ATBM addition reaction must be slow.

Therefore, the reaction temperature was raised to −40 °C, and the reaction time was extended to 54 h. In this manner the oligosaccharide derivatives were confirmed by a GPC measurement, which showed the generation of oligomers. When the concentration of dimethoxymethane was changed, the obtained GPC chromatogram (Figure 2) showed that a larger amount of dimethoxymethane addition lowered the molecular weight of products.

Proof of Dimethoxymethane Functioning as a Chain Transfer Reagent. So far, it has been shown that additional dimethoxymethane lowered the molecular weight of reaction products, and dimethoxymethane

Scheme 2. Mechanism of Reaction of PF_5 with Dimethoxymethane[a]



[a] PF_4OMe and PF_6^- were considered to be obtained by this reaction mechanism; i.e., the electron pair of oxygen atoms of dimethoxymethane attacked PF_5, and then another dimethoxymethane molecule attacked the carbon atom of the PF_5− dimethoxymethane complex. The obtained $MeOPF_5^-$ attacked another PF_5 molecule, and then PF_4OMe and PF_6^- were produced.

did not inhibit the reaction. For the application of this method to effective syntheses of oligosaccharides, it is necessary to prove that dimethoxymethane acts as a transfer reagent as we expected.

Scheme 1 shows the expected transfer reaction mechanism. If this transfer reaction occurs, the reaction products should have a methyl group and a methoxymethyl group at the reducing end and at the nonreducing end, respectively. In the ^{13}C NMR spectrum of the reaction product (Figure 3a), the peak at 54.6 ppm shows the presence of a methyl group at the reducing terminal. Even when ethanol instead of methanol was used for quenching, the ^{13}C NMR spectrum of the products (Figure 3b) showed the absorption of the methyl group at the reducing end at 54.6 ppm. This result showed that the methyl group of dimethoxymethane was transferred to the reducing end of the reaction products.

However, the reaction shown in Scheme 3 may have occurred. Even if ATBM could not react with the dimethoxymethane-derived oxonium ion, the oligosaccharide with a methyl group at the reducing end may have been produced by the reaction with methanol or ethanol. Therefore, to prove that dimethoxymethane acts as a transfer reagent, the presence of a methoxymethyl group at the nonreducing end of the products has to be shown. Since direct assignment of the complicated ^{13}C NMR spectrum of the product was difficult because of the mixture of oligosaccharides with various sizes, methyl 2,3,4-tri-*O*-benzyl-6-*O*-methoxymethyl-α-D-man-

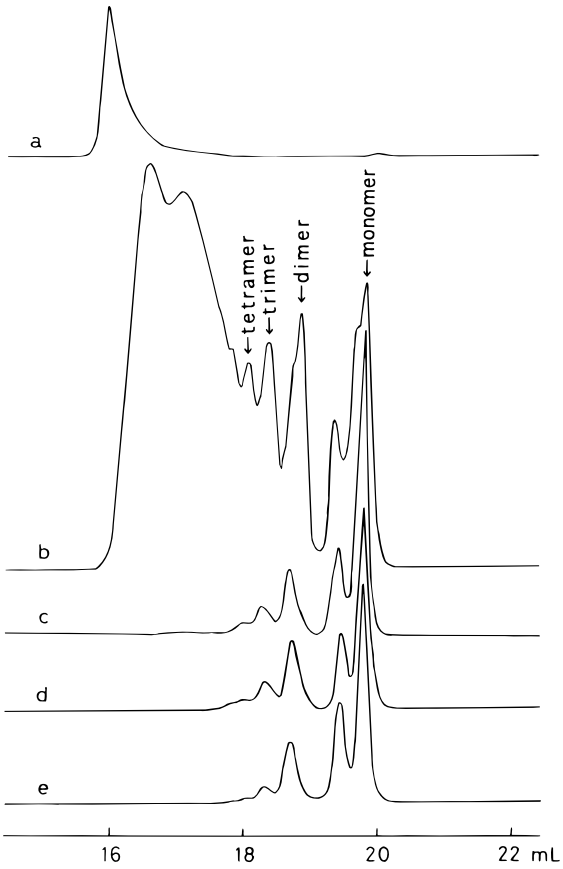


Figure 2. GPC chromatogram of products obtained from the reactions which were carried out with 10 mol % of PF_5 and (a) 0 mol %, (b) 10 mol %, (c) 20 mol %, (d) 30 mol %, and (e) 40 mol % of dimethoxymethane in methylene chloride at −40 °C for 54 h.

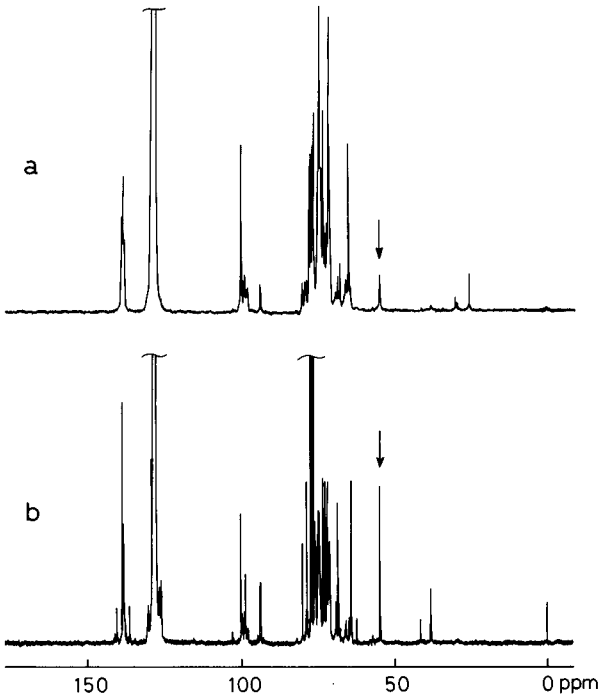


Figure 3. ^{13}C NMR spectra of the reaction products: (a) methanol quenching and (b) ethanol quenching.

nopyranoside was synthesized as a model compound. The peak assignment of the ^{13}C NMR spectrum of the model compound shown in Figure 4a was carried out by comparing with the spectrum of methyl 2,3,4,6-tetra-

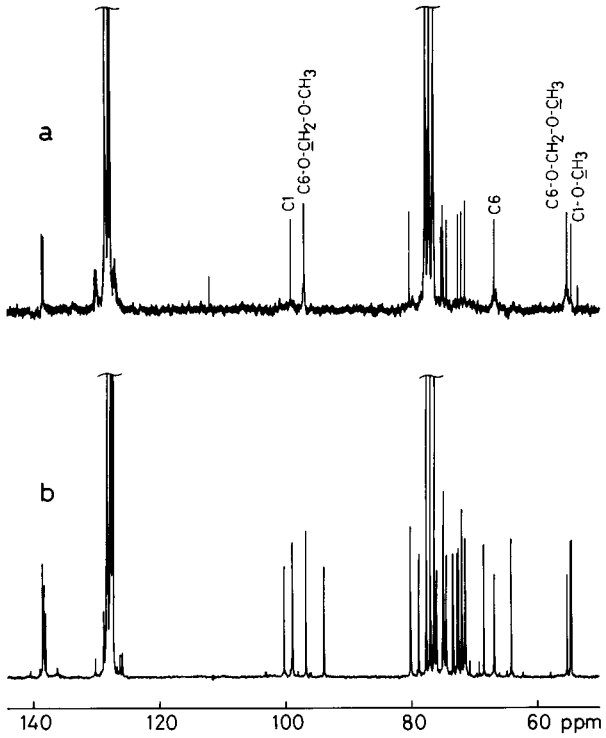
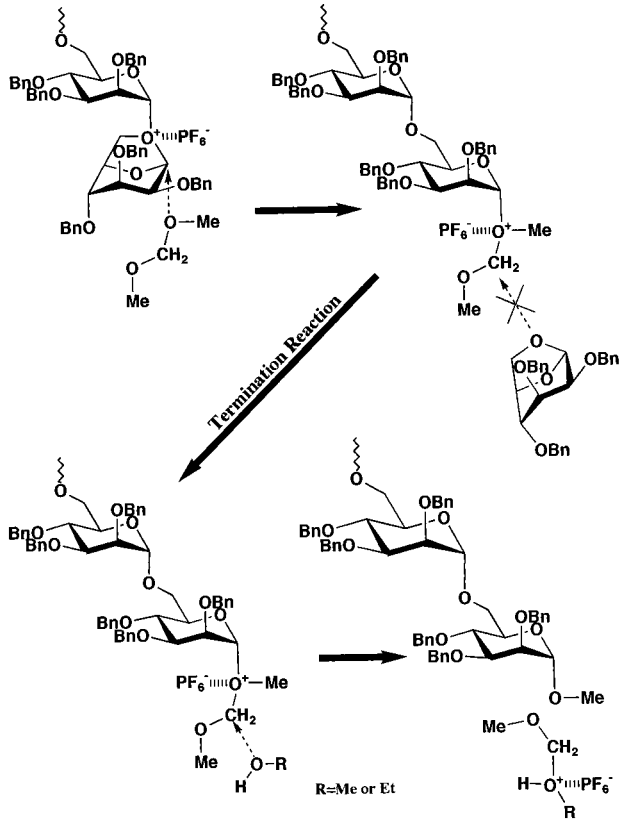


Figure 4. ^{13}C NMR spectra: (a) the model compound and (b) the reaction product.

Scheme 3. Mechanism of the Possible Reaction Which Produces the Oligosaccharide with a Methyl Group Transferred from Dimethoxymethane at the Reducing End, Even When the Quenching Is Done by the Addition of Ethanol



O-benzyl-α-D-mannopyranoside. The peaks of C6−O−$\underline{C}H_2$−O−CH_3, C6−O−CH_2−O−$\underline{C}H_3$, and C1−O−$\underline{C}H_3$ appeared at 96.7, 55.3, and 54.6 ppm, respectively. The comparison of the ^{13}C NMR spectra of the model

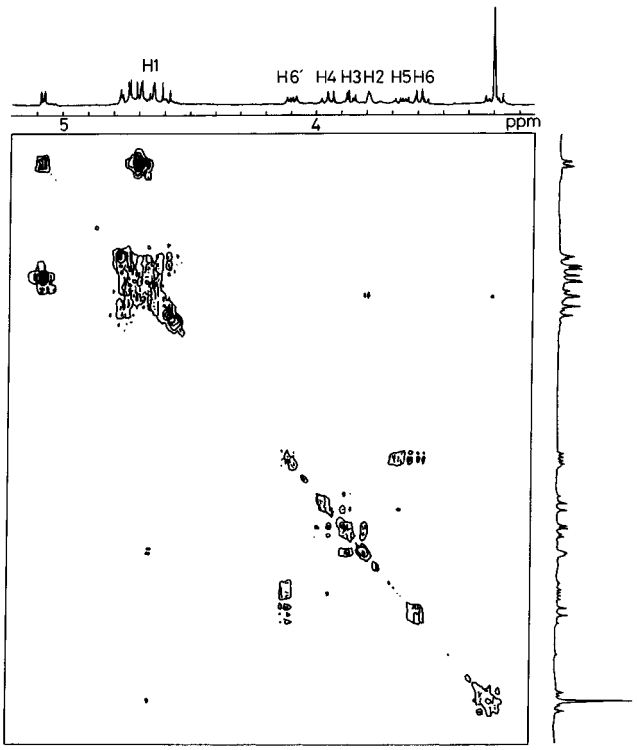


Figure 5. COSY map of the compound (fraction 2).

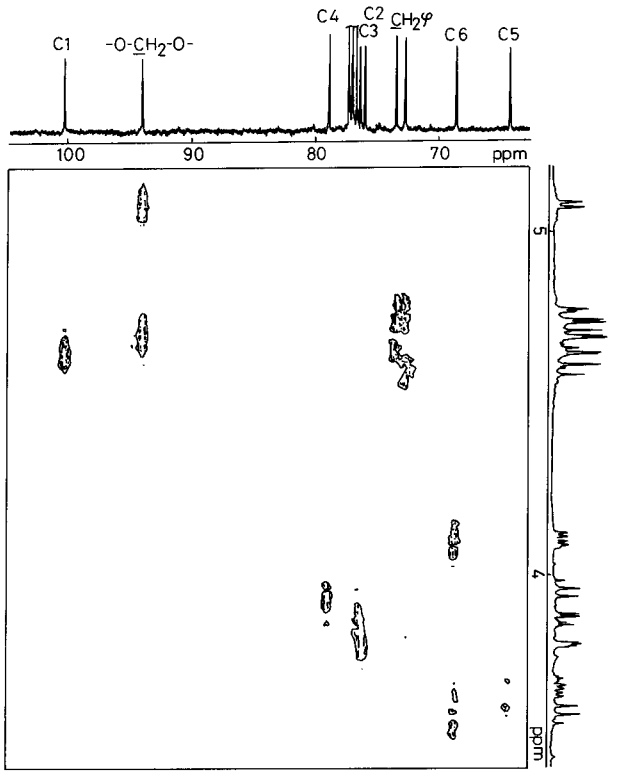


Figure 6. HMQC map of the compound (fraction 2).

compound and the reaction products are shown in Figure 4. The latter were obtained with 190 mol % dimethoxymethane, to enhance the peaks which are assigned to terminal groups by diminishing the molecular weight to monosaccharide derivatives. From this comparison, the presence of a methyl group at the reducing end and a methoxymethyl group at the nonreducing end of the reaction products was shown. In addition, all the peaks which appeared in the spectrum of the model compound were also observed in the spectrum of the products. Therefore, it was indicated that a transfer reaction by dimethoxymethane took place as expected.

Elucidation of All Reactions Which Occurred in the Presence of Dimethoxymethane. So far, it was clear that dimethoxymethane undoubtedly acted as a transfer reagent in the cationic ring-opening reaction of 1,6-anhydro-2,3,4-tri-*O*-benzyl-*β*-D-mannopyranose as expected. However, some unassignable peaks were observed in the ^{13}C NMR spectrum of the products. For the purpose of application of this method to effective syntheses of oligosaccharides, it seemed necessary to elucidate the mechanism of all the reactions which occurred. Column chromatography on silica gel gave two main compounds. One (fraction 1) was confirmed to be the same as the model compound (methyl 2,3,4-tri-*O*-benzyl-6-*O*-methoxymethyl-α-D-mannopyranoside) by comparing their ^{13}C NMR spectra. On the other hand, assignment of ^{1}H NMR spectrum of the other compound (fraction 2) was carried out with $^{1}H-^{1}H$ COSY (Figure 5); then, assignment of the ^{13}C NMR spectrum was carried out with HMQC (Figure 6). From the ^{13}C NMR spectrum, it was suggested that one benzyl group binding to C2, C3, or C4 must be eliminated because only two peaks, which can be assigned to the methylene carbon of the benzyl group, appeared. From these results, three possible structures of this compound were considered. These are methyl 3,4-di-*O*-benzyl-2,6-*O*-methylidene-α-D-mannopyranoside, methyl 2,4-di-*O*-benzyl-3,6-*O*-methylidene-α-D-mannopyranoside, and methyl 2,3-di-*O*-benzyl-4,6-*O*-methylidene-α-D-mannopyranoside. HMBC, which can observe the long-range correlation between the protons of the methylidene group and the neighboring carbon to the methylidene group, was measured. Figure 7 shows the HMBC map of this compound. Since it was clear that the protons of the methylidene group correlated with C4 and C6, this compound was identified as methyl 2,3-di-*O*-benzyl-

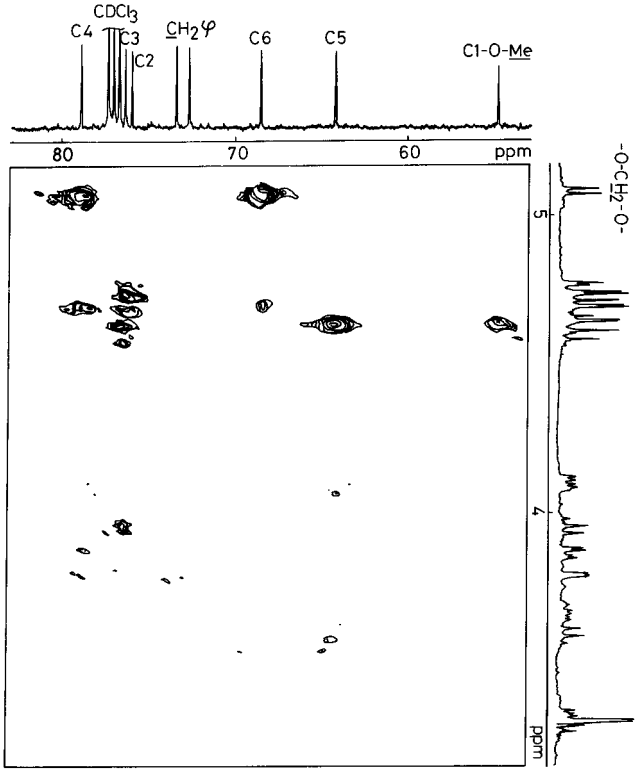
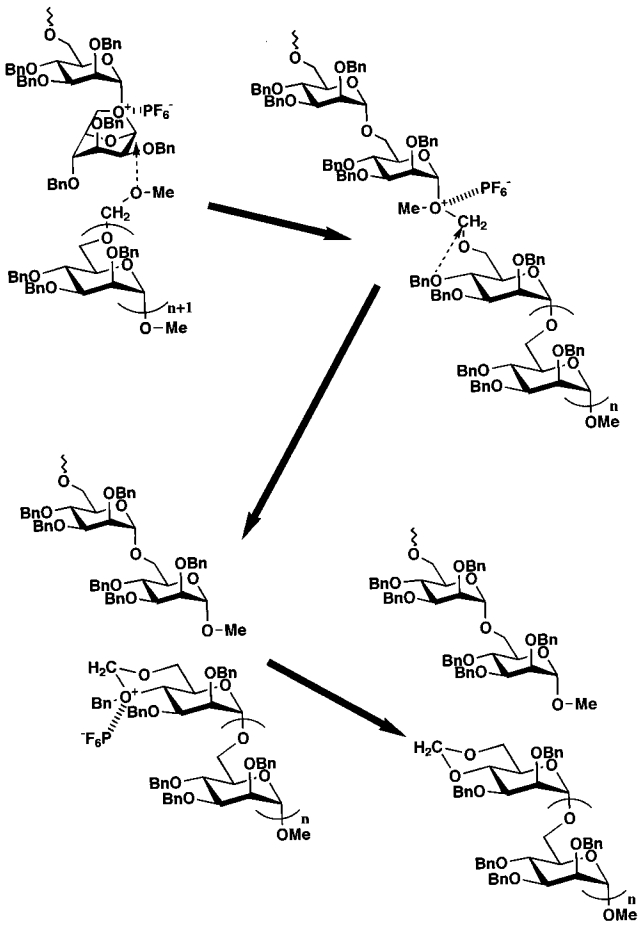


Figure 7. HMBC map of the compound (fraction 2).

Scheme 4. Expected Reaction Mechanism of 4,6-*O*-Methylidene Formation at the Nonreducing End of Oligosaccharide



4,6-*O*-methylidene-α-D-mannopyranoside. The reaction mechanism of 4,6-*O*-methylidene formation was considered as shown in Scheme 4. It can be suggested that the methoxymethyl group at the nonreducing terminal formed by the first transfer reaction (Scheme 1) attacked the cationic active site, then an electron pair of oxygen atom between the benzyl group and the C4 carbon attacked the neighboring methylene carbon to the oxonium ion, and finally, the benzyl cation was eliminated. Therefore, from the analyses carried out in this study, it was indicated that oligosaccharide derivatives with two types of nonreducing end structures were obtained: one was the 6-*O*-methoxymethyl type and the other was the 4,6-*O*-methylidene type.

Experimental Section

General Procedures. NMR spectra were recorded on JEOL EX-270, Varian Gemini-200, and JEOL EX-400 spectrometers. ^{13}C and ^{31}P measurements were carried out in $CDCl_3$ and CD_2Cl_2 using TMS (internal standard) and H_3PO_4 (external standard), respectively. Gel permeation chromatography (GPC) was carried out in tetrahydrofuran with a Shimadzu liquid chromatograph (Model LC-9A; columns GPC-802, GPC-803, and GPC-804) and in chloroform with a Tosoh liquid chromatograph (Model CCPD; columns G3000HXL, G2000HXL, and G1000HXL).

Materials. Commercial dimethoxymethane (Tokyo Chemical Industry Co., Ltd.) was dried on calcium chloride, calcium hydride, and sodium. After distillation, it was finally redistilled in the vacuum system. 1,6-Anhydro-2,3,4-tri-*O*-benzyl-β-D-mannopyranose (ATBM) was synthesized according to the literature.[7]

Reaction. ATBM was placed in the reaction ampule which had been connected to a vacuum line. After ATBM was dried for several hours, dried dichloromethane solvent and dimethoxymethane were distilled into the reaction ampule under high vacuum and then ATBM was dissolved completely. Phosphorus pentafluoride was condensed in the reaction ampule by cooling in a liquid-nitrogen bath. The ampule was sealed off from the vacuum line and the reaction was started by shaking the ampule for 10 min in an ethanol bath kept at a constant temperature. In another reaction, dimethoxymethane was added as a solution in dichloromethane after the reaction started. After the reaction was allowed to stand for a fixed time, methanol or ethanol was added for quenching. The product solution in chloroform was neutralized with aqueous sodium hydrogen carbonate, washed with water, and dried on anhydrous sodium sulfate. After evaporation of solvents, products were freeze-dried from benzene solution.

Methyl 2,3,4-Tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-mannopyranoside. Methyl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-mannopyranoside was synthesized according to the literature.[8] 25 g (0.13 mol) of methyl α-D-mannopyranoside and 43 g (0.15 mol) of triphenylmethyl chloride were dissolved in 250 mL of dimethylformamide. Then 20 mL of triethylamine and 1.6 g of (dimethylamino)pyridine were also added, and the reaction mixture was stirred for 2 days. Then, 31 g of sodium hydride which had been activated by washing with *n*-hexane was added. After the reaction was stirred for 1 h, 125 g of benzyl bromide was added dropwise. The reaction mixture was vigorously stirred for 2 days, and then methanol was added to stop the reaction. The product was extracted with chloroform, washed with water, dried on anhydrous sodium sulfate, and concentrated to a syrup. The obtained compound was purified by chromatography on a silica gel column (*n*-hexane: ethyl acetate = 4:1 as eluent) and was crystallized from *n*-hexane: yield 34 g (49%); ^1H NMR (270 MHz, $CDCl_3$) δ 3.23−3.29 (m, 1H and H6), 3.38 (s, 3H, C1−OCH_3), 3.49−3.53 (m, 1H and H6′), 3.73−4.04 (m, 4H, H2, 3, 4, and 5), 4.24−4.86 (m, 7H, H1 and −OCH_2Ph), 6.87−7.52 (m, 30H, −OCH$_2$*Ph*, −OC*Ph*$_3$).

Methyl 2,3,4-Tri-*O*-benzyl-α-D-mannopyranoside. Detriphenylmethylation was carried out as described.[9] A 34 g sample of methyl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-mannopyranoside was dissolved in 300 mL of 80% aqueous acetic acid by heating to 80 °C, and the mixture was stirred for 1 week. Then, the mixture was neutralized with aqueous sodium bicarbonate which was cooled with ice. The organic phase was extracted with ethyl acetate, washed with water, and dried on anhydrous sodium sulfate. After the salts were removed by filtration, the solution was concentrated. The product was purified by column chromatography of silica gel (20% ethyl acetate in *n*-hexane).

Methyl 2,3,4-Tri-*O*-benzyl-6-*O*-methoxymethyl-α-D-mannopyranoside. The reaction was carried out under nitrogen atmosphere. A 1.13 g sample of methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (syrup) was dissolved in 25 mL of DMF. Then, 0.2 g of sodium hydride (oil dispersion) was washed with *n*-hexane and added to the DMF solution. After overnight stirring, 0.38 mL of chloromethylmethyl ether, which was dissolved in 5 mL of DMF, was added dropwise to the reaction mixture with a syringe. After overnight stirring, the mixture was heated (60 °C), and 2 g of sodium hydride and 21 mL of chloromethylmethyl ether were added. After 4 days of stirring, the reaction was quenched by the addition of methanol. The mixture was extracted with chloroform, and the chloroform solution was washed with water, dried on anhydrous sodium sulfate, and concentrated to obtain a syrup. The residue was purified by chromatography twice on a silica gel column (20% ethyl acetate in *n*-hexane, and 10% ethyl acetate in chloroform): yield 212 mg (17%); ^1H NMR (200 MHz, $CDCl_3$) δ 3.32 (s, 3H, C1−OCH_3), 3.38 (s, 3H, C6−OCH$_2$OCH_3), 3.67−3.99 (m, 6H, H2, 3, 4, 5, 6, and 6′), 4.60−4.99 (m, 9H, H1, C6−OCH_2OCH$_3$, and −OCH_2Ph), 7.26−7.37 (m, 15H, −OCH$_2$*Ph*); ^{13}C NMR (50 MHz, $CDCl_3$) δ 54.6 (C1−O*C*H$_3$), 55.3 (C6−OCH$_2$O*C*H$_3$), 66.8 (C6), 71.3, 74.3, 74.8 and 80.1 (C2, 3, 4, and

5), 71.9, 72.5, and 75.0 ($-OCH_2Ph$), 96.7 (C6$-OCH_2OCH_3$), 98.9 (C1), 127.4−138.4 ($-OCH_2Ph$).

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