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Synthesis of a New Polymer Containing a Blood-Group Antigenic Oligosaccharide Chain

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ABSTRACT: A new polymerizable monomer having a nonreducing-terminal trisaccharide of an H type antigenic oligosaccharide was synthesized chemically, that is, 5-[[[2-(acryloyloxy)ethyl]amino]carbonyl]-pentyl 2-(acetoamido)-4,6-*O*-benzylidene-2-deoxy-3-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**3**), and was copolymerized with acrylamide or methyl acrylate. Copolymerization of **3** with acrylamide was carried out in dimethylformamide with AIBN as initiator, and copolymerization with methyl acrylate was carried out in benzene with the same initiator. The copolymer composition which was calculated by ^{1}H NMR spectroscopy was controlled by the monomer ratio in the feed. **3** was introduced into the copolymer in a content of 0.005–0.54. The number-average molecular weight of the copolymer was in the range of (1.8–8.4) × 10^4. Benzyl and benzylidene groups, which were the protective groups of the copolymers, were completely removed by catalytic hydrogenation over palladium–carbon in the mixed solvent.

Introduction

Among various kinds of human blood groups, the blood group of erythrocytes was first investigated. ABO blood-group substances are widespread in the human body and are represented by two blood factors (A and B) and four blood types (A, B, AB, and O). It is well-known that the ABO blood-group substances have antigenic oligosaccharide chains in sphingoglycolipids.

The progress in sugar-chain analysis has clarified the chemical structures of blood-group antigens. According to a hypothesis about the biosynthesis of blood-group antigens proposed in 1959,[1] fucosyl transferase prepared by the phenotypic expression of the H gene catalyzes the transfer of L-fucose from GDP-fucose to the galactose residue at the nonreducing end, producing an H type antigen. Then *N*-acetyl-D-galactosamine and D-galactose were transferred to the H type antigen by genetically determined A enzyme and B enzyme, respectively, to form the A type and the B type antigens, respectively. Recently, Hakomori et al. isolated the gene which codes for the A enzyme.[2] Furthermore, they analyzed the genetic codes which control the ABO blood-group antigens and clarified the sequencial differences in DNA and their meanings.[3]

On the other hand, syntheses of the polymers having saccharide chains which would exhibit the biological functions have been reported. Lactose-containing polystyrene[4] and polypeptide[5] are useful for the culture substrate of hepatocytes with asialoglycoprotein receptors.[6] As models of novel synthetic glycoconjugates, new poly(*n*-pentene)s having pendant *N*,*N*′-diacetylchitobiose and *N*-acetyllactosamine were prepared by polymerization.[7]

The polymer having a blood-group antigenic oligosaccharide chain might be used as blood-group-compatible materials; for example, the polymer containing A type oligosaccharide might be suitable to group A blood.

In this investigation, a vinyl monomer having a chemically synthesized H type oligosaccharide derivative was copolymerized with commercial comonomers in order to introduce a biological function of the blood-group antigen into the polymer materials. Deprotection of the obtained polymer is also reported.

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Table I
Copolymerization of 5-[[[2-(Acryloyloxy)ethyl]amino]carbonyl]pentyl 2-(Acetamido)-4,6-*O*-benzylidene-2-deoxy-3-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (3) with Acrylamide (AAm) or Methyl Acrylate (MA)[a]

no.	comonomer	mole fraction of 3 in feed	solvent	time, day	yield, %	mole fraction of 3 in copolymer[b]	$10^{-4}\bar{M}_n$[c]
1	AAm	0.200	DMF	2	49	0.32	7.0
2	AAm	0.020	DMF	1	59	0.02[d]	8.4[d]
3	AAm	0.007	DMF	1	67	0.005	nd
4	MA	0.200	benzene	2	10	0.54	1.8
5	MA	0.100	benzene	2	32	0.17	1.9
6	MA	0.040	benzene	2	26	0.06	3.4

[a] Monomer, 70–200 mg; solvent, 2 mL; AIBN, 1 wt %; temp, 70 °C. [b] Determined by ^{1}H NMR spectroscopy. [c] Approximated by GPC. [d] Determined after deprotection.

Experimental Section

5-[[[2-(Acryloyloxy)ethyl]amino]carbonyl]pentyl 2-(Acetoamido)-4,6-*O*-benzylidene-2-deoxy-3-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (3). 5-(Methoxycarbonyl)pentyl 2-(acetoamido)-4,6-*O*-benzylidene-2-deoxy-3-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (1;[8] 0.54 g, 0.42 mmol) was dissolved in a mixture of ethanolamine (52 mL), chloroform (25 mL), and triethylamine (0.25 mL). After refluxing for 5 h, the solution was washed with water, dried on sodium acetate, and evaporated to dryness. The syrupy product was chromatographed on a preparative TLC plate of silica gel, with chloroform–methanol (19:1, v/v) as eluent, and was freeze-dried from benzene to afford powdery 5-[[(2-hydroxyethyl)amino]carbonyl]pentyl 2-(acetoamido)-4,6-*O*-benzylidene-2-deoxy-3-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (2; 0.44 g).

To a solution of (2; 0.58 g, 0.44 mmol) and triethylamine (0.1 mL) in dichloromethane (18 mL) cooled on an ice-water bath was added acryloyl chloride (0.1 mL, 1.24 mmol), and the mixture was stirred under cooling for 1 h. After confirming the disappearance of 2 with TLC in methylene chloride–methanol (19:1, v/v) (product (3), R_F 0.30; 2, R_F 0.20), the reaction mixture was neutralized with aqueous sodium hydrogen carbonate, washed with water, dried on sodium sulfate, and evaporated to dryness. The syrupy product was chromatographed on a preparative TLC plate of silica gel, with chloroform–methanol (19:1, v/v) as eluent, and was freeze-dried from benzene to afford powdery 5-[[[2-(acryloyloxy)ethyl]amino]carbonyl]pentyl 2-(acetoamido)-4,6-*O*-benzylidene-2-deoxy-3-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (3; 0.34 g). $[\alpha]_D$ −57.9° (*c* 0.86, $CHCl_3$).

Copolymerization. Copolymerization of 3 with acrylamide was carried out in DMF with AIBN as initiator at 70 °C under high vacuum. For the copolymerization of 3 with methyl acrylate, benzene was used as solvent. Copolymerizations were terminated by the addition of methanol. The copolymer was precipitated twice by adding methanol into a benzene, water, or DMF–water (1:1, v/v) solution, and it was finally dried in vacuo or freeze-dried from benzene.

Deprotection of Copoly(3–acrylamide). A solution of copoly(3–acrylamide) (no. 2 in Table I; 100 mg) in DMF and water (3:1, v/v; 10 mL) was hydrogenated in the presence of 10% Pd–C (100 mg) and a small amount of HCl at 40 °C for 24 h and filtered with Celite. The filtrate was concentrated, and the residual product was purified by repeated dissolution–reprecipitation using a water–methanol system. The obtained OH-free copolymer was freeze-dried from water.

General Procedures. NMR spectra were recorded on a JEOL EX-270 spectrometer in $CDCl_3$ and D_2O using TMS and DSS as internal standards, respectively. GPC was carried out on 1% solutions of the polymer in tetrahydrofuran with a Shimadzu liquid chromatograph (Model LC-9A; columns GPC-803 and GPC-804). The number-average molecular weights calculated by GPC were based on a polystyrene calibration curve. The number-average molecular weights of water-soluble polysaccharides were determined by aqueous phase GPC (columns: Asahipak GS-510 (Asahi Chemical Industry)) using standard pullulan as reference. Optical rotations were recorded with a Jasco Model 370 polarimeter at room temperature in chloroform or water using a 0.5-dm cell.

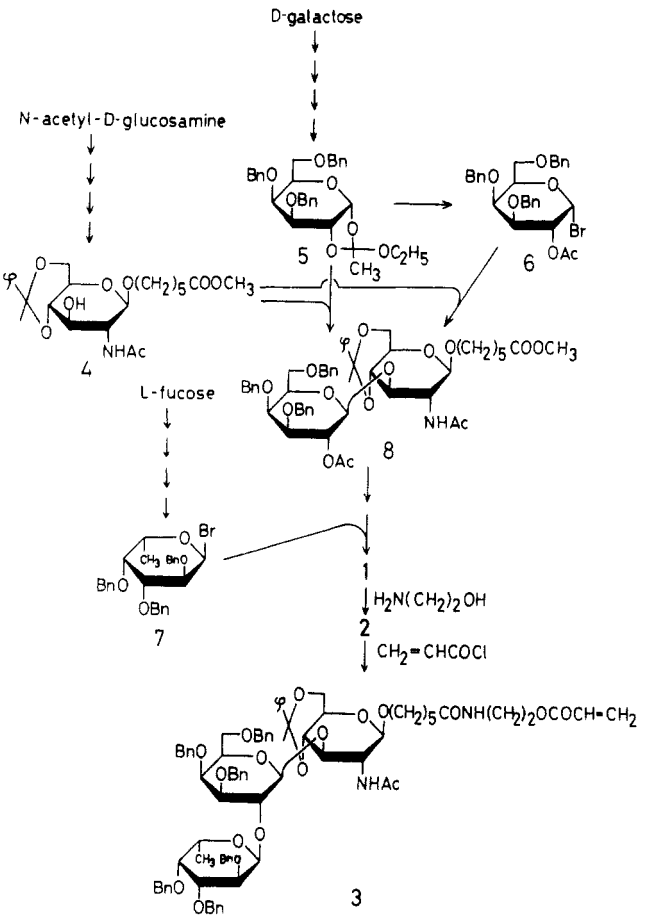

Figure 1. Synthetic scheme of the monomer having an antigenic oligosaccharide.

Results and Discussion

Synthesis of H Type 1 Having a Polymerizable Vinyl Group. Blood-group antigenic oligosaccharide (H type 1) was synthesized according to the literature.[8] The reaction pathway is shown in Figure 1.

5-(Methoxycarbonyl)pentanol was glycosylated with 2,3,4,6-tetra-*O*-acetyl-α-D-glucosaminyl chloride using mercuric cyanide to give a β-D-glucosaminide, which was then converted to 5-(methoxycarbonyl)pentyl 2-(acetoamido)-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (4) by deacetylation and benzylidenation of the β-D-glucosaminide. Two kinds of galactosylation of 4 were attempted by the orthoester method using 3,4,6-tri-*O*-benzyl-α-D-galactopyranose 1,2-(ethylorthoacetate) (5) and the Koenigs–Knorr reaction using 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl bromide (6). The disaccharide derivative of 5-(methoxycarbonyl)pentyl 2-(acetoamido)-

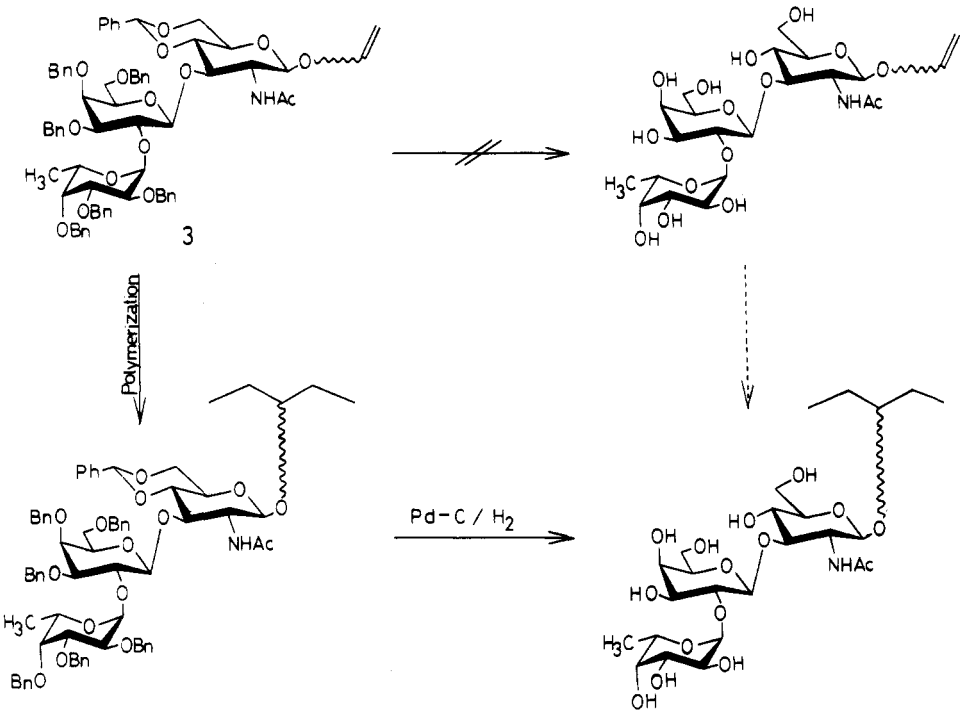

Figure 2. Deprotection of the oligosaccharide portion.

4,6-*O*-benzylidene-2-deoxy-3-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**8**) was obtained in each procedure. **8** was deacetylated with sodium methoxide in methanol and then was fucosylated with **7** to give a blood-group antigenic oligosaccharide (H type 1) derivative (**1**).

Introduction of a polymerizable vinyl group into the blood-group antigenic oligosaccharide was attempted by an ester–amide exchange reaction between **1** and *p*-vinylbenzylamine [*p*-(aminomethyl)styrene]. However, the reaction failed under various conditions. Then **1** was reacted with 2-aminoethanol to give 5-[(2-hydroxyethyl)-carbonyl]pentyl 2-(acetoamido)-4,6-*O*-benzylidene-2-deoxy-3-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**2**). The target vinyl compound was synthesized by the reaction of **2** with acryloyl chloride.

Polymerization. Copolymerizations of sugar monomer (**3**) with acrylamide and methyl acrylate were carried out in DMF and benzene, respectively, with AIBN as initiator at 70 °C under high vacuum. The results are summarized in Table I. Copolymer composition, i.e., the introduction rate of oligosaccharide, was determined by ^{1}H NMR spectroscopy. Absorptions at 7.0–7.4 ppm are due to the aromatic protons of benzyl and benzylidene groups in an oligosaccharide unit, and those at higher magnetic field are due to the nonaromatic protons of both sugar monomer and comonomer units. Accordingly, the fraction of sugar monomer unit was calculated by using the relative intensities of the absorptions in the two regions. Copolymers with a wide range of compositions were obtained from the various monomer ratios in the feed.

In the copolymerization of **3** with acrylamide, the increasing mole fraction of acrylamide in the feed (nos. 2 and 3 in Table I) caused precipitation of the copolymer in the polymerization system. Copolymer no. 2 in Table I was insoluble in either DMF or water but was soluble in the mixed solvent of DMF and water (3:1), suggesting that there may be a spatial distance between the polymer main chain and the oligosaccharide portions, which may be solubilized by water and DMF, respectively. The copolymer having a low introduction rate of oligosaccharide (no. 3 in Table I) was soluble in water.

In the copolymerization of **3** with methyl acrylate, the mole fraction of **3** in the copolymer was more than the mole fraction of **3** in the feed, indicating that **3** was more reactive than methyl acrylate. Because increasing the mole fraction of **3** in the feed tended to decrease the copolymer yield and the molecular weight of the copolymer and the number of monomeric units of **3** per one copolymer chain was independent of the monomer ratio in the feed, it was estimated that the active species of **3** is liable to terminate the polymerization. Therefore, it was concluded that the monomer reactivity of **3** was higher than methyl acrylate and the active end of **3** was more unstable.

Debenzylation of Copoly(3-acrylamide). In this investigation, polymerizable monomer (**3**) has benzyl ether and benzylidene acetal groups as protective groups. In general, the benzyl group is often used for sugar synthesis because of its stability in both acidic and basic conditions. On the other hand, debenzylation of the reaction product was sometimes difficult. For example, debenzylation of the synthetic polysaccharide derivatives is carried out only by Birch reduction (sodium in liquid ammonia), and other methods are not effective. However, Birch reduction was not useful for the debenzylation of the copolymer of **3** with acrylamide, because the ester linkage of the copolymer was cleaved under the reaction conditions.

Deprotection of the monomer (**3**) was attempted first (Figure 2). However, not any methods (Birch reduction, catalytic hydrogenation, trimethylsilyl iodide, etc.) were effective, since the carbon–carbon double bonds and/or ester linkages were quite sensitive under the reaction conditions. Deprotection of the copolymer of **3** with acrylamide was then attempted by catalytic hydrogenation.

The catalytic hydrogenation of the copolymer (no. 2 in Table I) was carried out in the presence of 10% Pd–C and a small amount of HCl in a mixed solvent of DMF and water (3:1, v/v) at 40 °C for 24 h. In the ^{1}H NMR spectrum of the obtained copolymer in D_2O (Figure 3), absorptions of aromatic protons disappeared, indicating the perfect

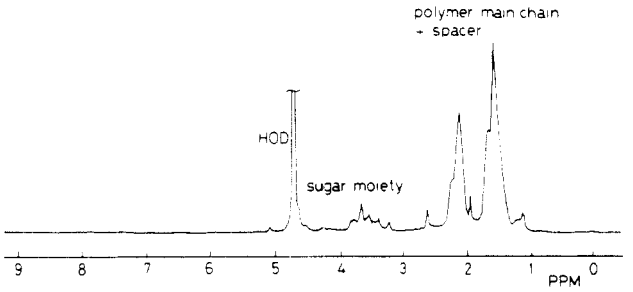

Figure 3. ^{1}H NMR spectrum of the deprotected copoly(3-acrylamide).

debenzylation and debenzylidenation of the copolymer. The mole fraction of **3** in the copolymer calculated from the relative intensity of the absorptions was 0.02 which was the same as the mole fraction of **3** in the monomer composition, indicating that the cleavage of the oligosaccharide portion scarcely occurred during deprotection.

In general, debenzylation of the polymer by catalytic hydrogenation does not occur in the individual solvent. Debenzylation of the polymer by hydrogenation was first accomplished using mixed solvents. However, it may be a problem in that the combination of the solvents must be changed depending upon the carbohydrate content and the kind of main chain.

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