Syntheses of Polysaccharides Having Various Contents of Carboxyl Groups by Ring-Opening Copolymerization

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ABSTRACT: Polysaccharides having various contents of carboxyl group precursors were synthesized by ring-opening copolymerization of 1,6-anhydro-2,4-di-*O*-benzyl-3-*O*-1-(methoxycarbonyl)ethyl- β -D-glucopyranose (**1**) with tri-*O*-benzyl-levoglucosan (**2**) with phosphorus pentafluoride as catalyst in methylene chloride at -60°C . The monomer reactivity ratios calculated by Kelen-Tüdös method were $r_1 = 0.77$ and $r_2 = 0.71$. Birch reduction with lithium metal was quite effective for the deprotection of both methyl ester and benzyl ether groups of the polymer.

KEY WORDS Ring-Opening Polymerization / Artificial Polysaccharide / Acidic Polysaccharide / Copolymerization / Monomer Reactivity Ratios /

Many natural acidic polysaccharides are known to have varieties of interesting characters. For example, heparin is a well-known natural polysaccharide with high concentration of sulfate and carboxyl groups, and it shows anticoagulant activity,^{1,2} anti-HIV activity,³ and still more, several other physiological or pharmaceutical activities.⁴ Then for the purpose of easily obtaining a potential replacement for heparin, a lot of studies have been carried out to prepare heparin-like polymers, and to define the structural requirements for its possible activities.^{5–9}

Also, several polysaccharide polycarboxylates are found. Interestingly almost all of them show gel-forming properties.¹⁰ And alginic acid, which is polyuronic acid extracted from brown seaweed, is reported to show considerable antitumor activity.^{11,12} Although those polycarboxylates are of interest in industrial and pharmaceutical standpoints, there are few investigations that deal with the relationships between their characters and the contents of carboxyl groups in the polysac-

charide molecules.

We aimed, in this study, at novel syntheses of artificial polysaccharides having various contents of carboxyl groups, in order to elucidate the relationships above and to produce novel functional polymers. Various carboxymethylated polysaccharides with different degrees of substitutions have been reported with interesting properties.^{7,13,14} However since sugar hydroxyls have similar reactivities, such chemical modifications of polysaccharides are usually non-regioselective. Previously we reported the synthesis of the stereoregular polysaccharide regioselectively modified by the carboxyl function precursor, by means of polymerization technique.¹⁵ In this investigation, we report the copolymerization of 1,6-anhydro-2,4-di-*O*-benzyl-3-*O*-1-(methoxycarbonyl)ethyl- β -D-glucopyranose (**1**) with 1,6-anhydro-2,3,4-tri-*O*-benzyl- β -D-glucopyranose (**2**) to obtain polysaccharides with different contents of carboxyl group precursors. And we also discuss the calculated monomer reactivity ratios. In addition, attempt to deprotect

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the obtained polysaccharides was also reported.

EXPERIMENTAL

General Methods

NMR spectra were recorded at room temperature on a JEOL EX-270 spectrometer in CDCl_3 and D_2O using tetramethylsilane (TMS) and 4,4-dimethyl-4-silapentanesulfonate (DSS) as internal standard, respectively. IR spectra were recorded on a JASCO IR-700 infrared spectrophotometer using KBr disks. GPC was carried out using tetrahydrofuran as eluent with Shimadzu LC-9A liquid chromatograph (GPC-802, 803, 804 columns). TLC was carried out on a Merck silica gel 60 F_{254} coated plates, using ether–benzene (2:8 v/v) mixture as developing agent.

Monomers

1,6-Anhydro-2,4-di-*O*-benzyl-3-*O*-1-(methoxycarbonyl)ethyl- β -D-glucopyranose (**1**) and 1,6-anhydro-2,3,4-tri-*O*-benzyl- β -D-glucopyranose (**2**), were synthesized from D-glucose, according to the literature^{15–17} with some improvements.

Copolymerization

Copolymerization was carried out under high vacuum at -60°C with phosphorus pentafluoride as initiator in anhydrous methylene chloride, and terminated by the addition of methanol. Obtained copolymer was purified by reprecipitation using chloroform-methanol system, and lyophilized from benzene solution.

Hydrolysis of Ester Linkage

To a suspension of 500 mg of potassium *tert*-butoxide in the mixture of water (3 ml) and tetrahydrofuran (THF) (6 ml) was added THF solution of 300 mg of copolymer. The reaction mixture was stirred at room temperature for 24 h. The polymer solution was washed with methylene chloride, and the solution was dialyzed for 4 days with water. The polymer

was freeze-dried from water.

Birch Reduction

10 ml of dimethoxyethane solution or suspension of the polymer (0.1 to 0.5 g) was added dropwise to about 50 ml of liquid ammonia containing sodium or lithium (8 times as much as benzyl groups) at -60°C . After 1.5 h ammonium chloride was added until the blue color completely disappeared, and then about 30 ml of distilled water was added. After evaporation of ammonia, the debenzylated polymer suspension was washed several times with methylene chloride, and the solution was dialyzed for 4 days with water. The polymer was freeze-dried from water.

RESULTS AND DISCUSSION

For the first step, in order to obtain some informations about the copolymerization mechanism, copolymerizations of **1** with **2** were carried out in various ratios of monomer feed, and the approximate monomer reactivity ratios were evaluated. Copolymerization was carried out with PF_5 catalyst in anhydrous methylene chloride, and terminated at around 10% conversion, judging from the mobility of the polymerization solution. Mole fraction of each monomeric unit in the obtained copolymer was determined from ^1H NMR integrated data. The results are summarized in Table I. The proportion of the **1** unit incorporated into the copolymer was almost equal to that in the feed. However, as the mole fraction of **1** in the feed increased, the slower rate of the propagation was obviously observed.

From the data shown in Table I, the monomer reactivity ratios were evaluated by the Kelen–Tüdös method.^{18,19} The calculated values were $r_1=0.77$ and $r_2=0.71$. These values indicate that each monomer has nearly the same homopolymerizability and tends to react to an active end of the other one. It was assumed that these results were attributable to the higher nucleophilicity of **1** and the

Scheme 1.

hindering substituent introduced into **1** (conclusive data were not obtained). The higher nucleophilicity of **1** was estimated by ^1H NMR data, in which chemical shift of H-1 proton of **1** appears at higher magnetic field than that of **2**. That is, **1**, which is presumed to have higher nucleophilicity than **2**, may react to an active end preferentially, while, when it reacts to the cation derived from **1**, the steric hindrance or the electronic repulsion between the both methoxycarbonyl groups may occur. Therefore, **2** reacts preferentially to an active end derived from **1**. This assumption may also account for the observed slower homopolymerization rate of **1**.

Secondly, for the purpose of obtaining copolymers for physicochemical or biological assay, syntheses of copolymers with higher yields were attempted. Copolymerization reaction was carried out for longer time, and lower catalyst concentration was used. The results are shown in Table II. The yields of the copolymers were relatively high, and the proportion of the monomeric units in the copolymers were nearly the same to the data shown in Table I. The number-average molecular

Table I. Copolymerization of 1,6-anhydro-2,4-di-O-benzyl-3-O-1-(methoxycarbonyl)ethyl-β-D-glucopyranose (**1**) with 1,6-anhydro-2,3,4-tri-O-benzyl-β-D-glucopyranose (**2**)^a

Exptl. no.	Feed		Mole fraction of 1 in feed %	Time min	Yield %	Mole fraction of 1 in copolymer ^b %
	1	2				
	mg	mg				
31	0	504.7	0	1	68.0	0
32	50	454.2	10	<1	3.2	14.5
33	100	403.7	20	<1	3.8	23.2
30	150	353.3	30	1	7.0	33.3
25	200	302.8	40	2	18.5	37.8
22	250	252.3	50	2	13.9	48.8
23	300	201.9	60	3	12.0	59.0
27	350	151.4	70	4	6.6	70.4
28	400	101.0	80	7	9.0	75.3
29	450	50.5	90	9	8.2	89.7
26	500	0	100	9	11.4	100

^a Total monomers, 1.17 mmol; initiator, phosphorus pentafluoride, 5 mol% to total monomers; solvent, methylene chloride, 1 ml; temperature, -60°C . ^b Determined by ^1H NMR spectroscopy.

Table II. Syntheses of copolymers^a

Exptl. no.	Feed		Mole fraction of 1 in feed %	Solvent ^b ml	Initiator ^c mol%	Time h	Yield %	Mole fraction of 1 in copolymer ^b %	$M_n^d \times 10^{-4}$
	1	2							
	mg	mg							
17	0	1000.0	0	4.0	3	2	56.0	0	26.2
36	300	706.5	30	2.5	3	3	51.6	34.6	46.7
34	500	504.7	50	2.5	3	4	33.6	47.6	42.4
35	800	201.9	80	2.5	3	10	40.8	77.8	22.5

^a Temperature, -60°C . ^b Methylene chloride. ^c Phosphorus pentafluoride. ^d Determined by GPC, polystyrene standard.

weights of the copolymers were fairly high in the range of 2.3×10^5 to 4.7×10^5 .

^1H NMR spectra of the copolymers were compared with that of a homopolymer mixture of poly(**1**) and poly(**2**). The spectrum of the homopolymer mixture gave consistently sharp signals, whereas those of copolymers gave some undivided broad signals owing to the chain sequence. This result indicated that both monomers were incorporated into the copolymer chains, and the copolymer was not the mixture of the homopolymers. The GPC profiles and the TLC patterns of the copolymers also supported the same conclusion. In ^{13}C NMR spectra of copolymers, there appeared no signal derived from β -anomeric resonance. And in the IR spectra, the existence of the carbonyl group (1740 cm^{-1}) and the pyranose units having α -configuration (type **2a** absorption, 844 cm^{-1})²⁰ was indicated. These results suggested that the obtained copolymers were highly stereoregular α -linked copolysaccharides. The syntheses of the artificial polysaccharides with different contents of carboxyl group precursors were successful.

The hydrolysis of poly(**1**) with potassium *tert*-butoxide gave the product which was water soluble. However, after dialysis and concentration, the obtained polymer became insoluble. In IR spectra, the absorption of carbonyl group was shifted from 1740 cm^{-1} (before reaction) to 1600 cm^{-1} (after reaction), indicating that the methyl ester was hydrolyzed to give

Figure 1. ^{13}C NMR Spectra of Poly **1** (A) and reduced polysaccharide (B).

potassium carboxylate. Since this hydrolysis was carried out under alkaline condition, main chain scission of the polysaccharide might not occur. After Birch reduction of the hydrolyzed polymer, the obtained polymer was soluble in water. However, after dialysis and freeze-drying, it became insoluble again. The IR spectrum of the Birch reduced polymer indicated that the benzyl groups were removed.

When poly(**1**) was reduced with sodium in liquid ammonia to prepare a free polysac-

charide having carboxyl and hydroxyl groups in one step, the resulting polymer was found to have lost a considerable amount of carbonyl groups. Thus, lithium metal was used for Birch reduction. After the reduction, the obtained polysaccharide was water soluble even after dialysis and freeze-drying. Figure 1(A) shows ^{13}C NMR spectrum of the poly(1) and Figure 1(B) shows that of reduced polysaccharide. After the reduction, the absorptions of aromatic groups were hardly observed and the peak of carboxyl group was shifted from 174 ppm to 182 ppm, indicating that benzyl groups were practically removed and ester groups were hydrolyzed to carboxylate groups. The obtained polysaccharide has free carboxyl and hydroxyl groups. The number-average molecular weights were 7.50×10^4 for the protected polysaccharide and 1.04×10^4 for the derotected polysaccharide, respectively, indicating that main chain scission took place during the deprotection.

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