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Synthesis of Sulfated Acetamido Group-Containing Copolyribose by Selective Ring-Opening Polymerization and Chemical Modifications

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Abstract : Synthesis and sulfation of an acetamido group-containing copolyribose was carried out by a five-step synthetic route. Firstly, selective ring-opening copolymerization of 1,4-anhydro-3-azido-2-*O*-*t*-butyldimethylsilyl (TBDMS)-3-deoxy- α -D-ribofuranose (A3ASR) and 1,4-anhydro-2,3-di-*O*-TBDMS- α -D-ribofuranose (ADSR) with $\text{BF}_3 \cdot \text{OEt}_2$ as a catalyst at low temperature produced an azido group-containing copolyribose, i.e., poly[3-azido-2-*O*-TBDMS-3-deoxy-(1 $\rightarrow$ 5)- α -ribofuranose-co-2,3-di-*O*-TBDMS-(1 $\rightarrow$ 5)- α -ribofuranose] [copoly (A3ASR)]. The number-average molecular weight ($\bar{M}_n$) of the azido group-containing copolyriboses was in a range of 1.17×10^4 to 2.96×10^4 . The specific rotation ($[\alpha]^{20}_D$) increased from $+117^\circ$ to $+204^\circ$ as the mole fractions (%) of azido group-containing units (A3ASR) in the copolymers decreased from 83% to 16%. The reactivity ratios of A3ASR ($r_1 = 0.42$) and ADSR ($r_2 = 1.16$) in the ring-opening copolymerizations were estimated according to the Kelen-Tudos method.

Then, the azido group in a copoly (3A3ASR-ADSR) ($\bar{M}_n$ of 1.11×10^4 and $[\alpha]^{20}_D$ of $+141.6^\circ$) was reduced into amino group with NaBH_4 in a mixture of THF-ethanol to afford an amino group-containing copolymer, i.e., poly[3-amino-2-*O*-TBDMS-3-deoxy-(1 $\rightarrow$ 5)- α -ribofuranose-co-2,3-di-*O*-TBDMS-(1 $\rightarrow$ 5)- α -ribofuranose] [copoly (AAMSR-ADSR)], and subsequent acetylation of the amino group with acetyl chloride gave an intermediate acetamido group-containing copolymer, i.e., poly[3-acetamido-2-*O*-TBDMS-3-deoxy-(1 $\rightarrow$ 5)- α -ribofuranose-co-2,3-di-*O*-TBDMS-(1 $\rightarrow$ 5)- α -ribofuranose] [copoly (A3AAdSR-ADSR)], and then de-*t*-butyldimethylsilylation of the copolymer produced a water-soluble acetamido group-containing copolyribose, i.e., poly[3-acetamido-3-deoxy-(1 $\rightarrow$ 5)- α -ribofuranose-co-(1 $\rightarrow$ 5)- α -ribofuranose] [copoly (A3AAdR-R)] with $\bar{M}_n$ of 8.63×10^3 and $[\alpha]^{20}_D$ of $+141.0^\circ$.

Finally, sulfation of the acetamido group-containing copolyribose [copoly (A3AAdR-R)] with a sulfur trioxide-pyridine complex in a mixture of dimethyl sulfoxide (DMSO) and pyridine produced a sulfated acetamido group-containing copolyribose [copoly (SA3AAdR-SR)] with $\bar{M}_n$ of 10.68×10^3 and $[\alpha]^{20}_D$ of $+84.9^\circ$. The structures of acetamido group-containing copolymer and its sulfate were determined by ^{13}C NMR. The degree of sulfation ($\text{DS} = 1.2$) was measured by elemental analysis.

1. Introduction

Ring-opening polymerization of anhydro sugars have provided a number of stereoregular polysaccharides [1-4] and functional polysaccharides possessing azido, amino, acetamido, sulfamido and sulfate groups [5-7]. Recently, both sulfated polysaccharide and acetamido group-containing polysaccharide have been attracted

much attention, since it was found that the sulfated polysaccharides exhibit high anti-HIV activity [8] and an acetamido group-containing natural polysaccharide chitin has a wide variety of biological activities [9-13].

Sulfated polysaccharides with high anti-HIV activity were synthesized by sulfation of natural polysaccharides and synthetic polysaccharides obtained by the ring-opening polymerization of

1,4-anhydro sugars[14-18]. Of the sulfated polysaccharides, curdlan sulfate had the most potent anti-HIV activity and low side-effects[10], and its Phase I/II test for an AIDS drug was carried out[19].

The acetamido group-containing natural polysaccharide chitin is considered as biomaterial in fields such as biomedicine, pharmacology, and biotechnology due to its biocompatibility, biodegradability, and biological activities[20].

However, there are also many problems in using chitin because of its water-insolubility. So, various studies have been conducted to make water-soluble derivatives of chitin and chitosan by chemical modification techniques[21].

In this field, two kinds of new synthetic acetamido group-containing polysaccharides were synthesized in our laboratory. One is an acetamido group-containing polyxylose, i.e., 3-acetamido-3-deoxy-(1→5)- α -D-xylofuranan (3AAdXF)[22], and the other is an acetamido group-containing polyribose, i.e., 3-acetamido-3-deoxy-(1→5)- α -D-ribofuranan (3AAdRF)[23]. Both acetamido group-containing polyxylose and polyribose had water solubility so that the characterization of these acetamido group-containing polysaccharides were carried out in aqueous solution smoothly. The structure was determined by NMR on sample solutions in D₂O, the specific rotation was measured in aqueous solution and the molecular weight was measured by gel permeation chromatography (GPC) using phosphate buffer solution as an eluent. Thus, the water solubility is very important for chemical and biological studies of acetamido group-containing polysaccharides.

In this paper, synthesis and sulfation of a new acetamido group-containing copolysaccharide, i.e., poly[3-acetamido-3-deoxy-(1→5)- α -ribofuranose-co-(1→5)- α -ribofuranose] [copoly(A3AAdF-R)] was investigated (Scheme 1).

Structures of acetamido group-containing copolyriboses and the sulfated copolyribose were determined by NMR and specific rotation. The degree of sulfation was measured by elemental analysis.

In addition, reactivity ratios in the ring-opening copolymerization of 1,4-anhydro-3-azido-2-*O*-TBDMS-3-deoxy- α -D-ribofuranose (A3ASR) and 1,4-anhydro-2,3-di-*O*-TBDMS- α -D-ribofuranose (ADSR) were

Scheme 1 Synthesis of sulfated acedamido group-containing copolyribose was carried out via five steps, (1)selective ring-opening copolymerization of 1,4-anhydro ribose derivatives (A3ASR and ADSR) into an azido group-containing copolyribose, and (2)reduction of the azido group into the amino group, (3)acetylation of the amino group into the acetamido group, and (4)*t*-butyldimethylsilylation, and (5)sulfation.

examined by using the Kelen-Tudos method[24,25].

2. Experimental

Materials and Measurements

Lithium azide was prepared by the reaction of sodium azide with lithium sulfate according to the

literature method[26]. Solvents were distilled before use. 500 MHz ^1H and 125 MHz ^{13}C NMR spectra were taken on sample solutions in CDCl_3 or D_2O by use of a JEOL Lambda 500 spectrometer. The 2-dimensional H-H COSY-FG and C-H HMQC-FG measurements were used to assign the ^1H and ^{13}C absorptions. Tetramethyl silane (TMS) and sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) were used as internal standards for organic solvent and deuterium oxide, respectively. Specific rotations were measured in chloroform or aqueous solution at 20°C by use of a Perkin Elmer 240 polarimeter. Molecular weights of polymers were determined by gel permeation chromatography using CHCl_3 or phosphate buffer solution ($\text{pH} = 7.0$) as eluent and standard polystyrenes or pullulans as references, respectively.

Monomers

1,4-Anhydro-2,3-di-*O*-TBDMS- α -D-ribofuranose (ADSR) was prepared by pyrolysis of D-ribose and following regioselective *t*-butyldimethylsilylation according to the method previously described[26].

1,4-Anhydro-3-azido-2-*O*-TBDMS-3-deoxy- α -D-ribofuranose (A3ASR) was synthesized by triflation and azidation of the 3-deoxy homolog according to the method in the literatures[6,23].

Selective Ring-Opening Copolymerization of A3ASR and ADSR

Typical procedure of copolymerization is as following. In a sealed glass ampoule, a methylene chloride solution (5 mL) of A3ASR (100 mg) and ADSR (100 mg) was polymerized with $\text{BF}_3 \cdot \text{OEt}_2$ (5 mol% to feed) as catalyst under high vacuum (10^{-5} – 10^{-6} mmHg) at -20°C for 1 h. After purification by dissolution - reprecipitation with chloroform - methanol system and freeze-drying from benzene, the copoly(A3ASR-ADSR) was obtained (40 mg in 20.0% yield) with $\overline{M}_n$ of 1.17×10^4 and specific rotation of $+130^\circ$ (Table 1. No 4).

Synthesis of Copoly(3AAdRF-RF)

An azido group-containing copolyribose, (0.30 g) (53% A3ASR unit; $\overline{M}_n = 11.1 \times 10^4$; $[\alpha]^{20}_D = +140.6^\circ$) was dissolved in a mixture of THF (5 mL) and ethanol (3 mL), and then sodium borohydride (1.0 g) was added, followed by stirring at 60°C for 6 h. The reduced copolymer was purified by dissolution - reprecipitation using THF - methanol system twice, and finally freeze-dried from benzene. The amino group-

containing copolymer [copoly(3AmSRF-DSRF)] was obtained. Yield was 0.28 g.

Copoly(A3AmSRF-ADSR) (0.28 g) was acetylated with acetyl chloride (2.0 mL) in a mixture of THF - pyridine (10/5 mL) at 20°C under nitrogen atmosphere for 3 h. After work-up, acetamido group-containing copolymer [copoly(A3AAdSR-ADSR)] (0.27 g) was obtained.

The TBDMS group in copoly(A3AAdSR-ADSR) (0.25g) was removed by reaction with tetra-*n*-butylammonium fluoride (10.0 mL) in THF (10 mL) at 20°C for 1 h. After desilylation, H_2O (10 mL) was added, and then THF was evaporated. The aqueous polymer solution was dialyzed with H_2O for 2 d. The acetamido group-containing copolymer [copoly(A3AAdR-AR)] (0.12 g) was obtained by freeze-drying from water.

Sulfation of Acetamido Group-Containing Copolyribose

Copoly(3AAdRF-RF) (0.10 g) was dissolved in a mixture of dimethyl sulfoxide (DMSO) (2 mL) and pyridine (1 mL), and then sulfur trioxide- pyridine complex (0.32 g) was added and stirred at 60° for 1 h. After the reaction mixture was cooled to room temperature, it was neutralized with NaHCO_3 solution (4 mL), and dialyzed with deionized water for 2 d. After freeze-drying from water, the sulfated copolyribose was obtained. Yield was 0.15 g. Elemental analysis: C 23.2%, H 4.05%, N 2.9%, S 15.1%).

3. Results and Discussions

Ring-Opening Copolymerization and Reactivity Ratios of A3ASR and ADSR

1,4-Anhydro-3-azido-2-*O*-TBDMS-3-deoxy- α -D-ribofuranose (A3ASR) and 1,4-anhydro-2,3-di-*O*-TBDMS- α -D-ribofuranose (ADSR) were polymerized with a Lewis acid $\text{BF}_3 \cdot \text{OEt}_2$ as a catalyst at low temperature to give a azido group-containing stereoregular copolyribose, i.e., poly[3-azido-2-*O*-TBDMS-3-deoxy- α -D-ribofuranose-co-2,3-di-*O*-TBDMS- α -D-ribofuranose] [copoly(A3ASR-ADSR)] [7]. The whole backbone of the azido group-containing copolymer, consisted of (1 $\rightarrow$ 5)- α -D-ribose-type units.

In previous work[22,23], the ring-opening polymerization of A3ASR or A2ASX with $\text{BF}_3 \cdot \text{OEt}_2$ catalyst was carried out at low temperatures for long time (10~24h) to afford the corresponding stereoregular

polyribose and polyxylose in high yields, respectively. On the other hand, since one of the purpose was to examine reactivity ratios of the two monomers in the ring-opening copolymerizations, some of the copolymerizations were carried out for relatively short time to acquire low yields (Table 1).

Table 1 Ring-Opening Copolymerization of A3ASR and ADSR into Copoly (A3ASR-ADSR)^a

No.	Comonomers		Copolymer					
	A3ASR ^b	ADSR ^d	Yield	A3ASR ^e	$\bar{M}_n^f$	$[\alpha]^{20}_D^g$		
	mg	molL% ^c	mg	mg	%	molL%	$\times 10^4$	deg.
1	300	100	0	160	80.5	100	1.93	+266
2	150	79	50	20	10.0	83	1.06	+204
3	190	70	72	37	12.4	58		+141
4	100	57	100	40	20.0	38	1.17	+130
5	82	30	252	255	76.6	24		+128
6	50	31	150	150	75.0	23	2.96	+127
7	550	20	288	328	98.8	16		+117
8	0	0	400	38	92.0	0	11.8	+98

^aSolvent, CH₂Cl₂, (c=1.0%); BF₃·OEt₂ catalyst, 5 mol % to monomer; -20°C, 1 h. ^bFraction of first monomer (A3ASR) in feed.

^cMole fraction of A3ASR in monomer feed. ^dFraction of second monomer (ADSR) in feed. ^eMole fraction (%) of A3ASR units in copolymer was measured by 125 MHz ¹³C NMR. ^fDetermined in CHCl₃ by GPC. ^gMeasured in CHCl₃ (c=1.0%) at 20°C.

When A3ASR and ADSR were copolymerized with BF₃·OEt₂ as a catalyst at low temperature for 1 h, the copolymers were obtained in 10.0 ~ 98.8% yields with number average molecular weight in the range of 1.06×10^4 to 11.8×10^4 , and specific rotations from +98 to +266°.

The mole fractions of 3AzSRF unit in the copolymers were measured by 125 MHz ¹³C NMR spectroscopy according to the integrated area of the C2 peaks at 77.2 ppm for 3AzSRF and C2' peaks at 73.6 ppm for ADSR units (Figure 1).

The mole fractions of A3AAdSR units in these copolymers increased from 16% to 83% with increasing mole fraction of the A3ASR monomer (M1) in the monomer feed from 20% to 79%.

Mole fractions of the A3ASR and ADSR units in the copolymer were determined by using the C2 and C2' absorptions of the respective monomeric units, because

Fig. 1 125 MHz ¹³C NMR spectra of azido group-containing copolyriboses with various mole fractions (%) of azido group-containing units (A3ASR).

these peaks did not overlap together in the ¹³C NMR spectra.

An extended Kelen-Tudos method[24,25], was used to estimate the reactivity ratio (r_1) of A3ASR (M1) and that (r_2) of ADSR (M2). The Kelen-Tudos method is expressed by the following equation (1):

$$\eta = (r_1 + r_2/\alpha) \xi - r_2/\alpha \quad (1)$$

where

$$\eta = G/(\alpha + F)$$

$$\xi = F/(\alpha + F)$$

$$\alpha = (F_{\min} F_{\max})^{1/2}$$

$$F = Y/Z^2$$

$$G = (Y - 1)/Z$$

$$Z = \log(1 - \xi_1)/\log(1 - \xi_2)$$

where

$$\xi_2 = \omega(\mu + X)/(\mu + Y)$$

$$\xi_1 = \xi_2(Y/X)$$

where X and Y represent the molar ratio of monomer 1 to monomer 2 in comonomer feed and the resulting copolymer, respectively. The parameter ω corresponds to the conversion of the copolymerization, and μ is the

ratio of the monomer 2 molecular weight to that of monomer 1. the Kelen-Tudos parameters for the ring-opening polymerizations of A3ASR and ADSR are listed in Table 2, and the extended Kelen-Tudos plot is shown in Figure 2.

In Figure 2, the ξ versus μ plot showed a linear line within an experimental error, and the intercept at $\xi = 0$ and $\xi = 1$ of the ξ versus μ plot gave $r_1 = r_{A3ASR} = 0.42$ and $r_2 = r_{ADSR} = 1.16$. This indicated that the disilylated monomer (ADSR) had higher reactivity than that of monosilylated azido monomer (A3ASR). This result was in good agreement with the experimental fact obtained from the ring-opening homo-polymerization of A3ASR and ADSR. Usually, the relative polymerizability of anhydro sugars can be deduced from the homopolymerization behaviors[1,2]. And, it was revealed that A3ASR and ADSR were

Table 2 Kelen-Tudos Parameters for Ring-Opening Copolymerization of A3ASR and ADSR^a

No	f_{A3ASR}^b	F_{A3ASR}^c	X	Y	F	G	ξ	η
1	0.79	0.83	3.76	4.88	2.897	2.990	0.718	0.741
2	0.70	0.58	2.33	1.38	3.934	0.642	0.775	0.126
3	0.57	0.38	1.33	0.61	2.900	-0.850	0.718	-0.179
4	0.30	0.24	0.43	0.32	0.578	-0.913	1.336	-0.531
5	0.31	0.23	0.45	0.30	0.675	-1.050	0.372	-0.578
6	0.20	0.16	0.25	0.19	0.330	-1.066	0.224	-0.725

^aConditions: $BF_3 \cdot OEt_2/CH_2Cl_2$, $-20^\circ C$, 1 h. ^bMole fraction of A3ASR in monomer feed. ^cMole fraction of 3AzSRF in copolymer.

Fig. 2 Extended Kelen-Tudos plot of $\eta = (r_{A3ASR} + r_{ADSR}/\alpha)$ against $\xi = r_{ADSR}/\alpha$.

independently polymerized under the same conditions, the rate of polymerization and the conversion of the former were much lower than those of the latter.

Synthesis of Copoly(A3AAdR-R)

An acetamido group-containing copolyribose [copoly (A3AAdR-AR)] was synthesized by the synthetic route shown in Scheme 1. First, reduction of the azido group in copoly (A3ASR-ADSR) with sodium borohydride gave an amino group-containing copolyribose [copoly (A3AmSR-ADSR)]. In the ^{13}C NMR spectrum (Figure 3A), the absorption of C3 carbon binding to the amino group shifted to 54.0 ppm from the original chemical shift of C3 binding to the azido group at 61.9 ppm.

Acetylation of the amino group in copoly (A3AmSR-ADSR) with acetyl chloride afforded an acetamido group-containing intermediate copolymer

Fig. 3 125 MHz ^{13}C NMR spectra of poly[3-amino-2-*O*-TBDMS-3-deoxy-(1→5)- α -ribofuranose-co-2,3-di-*O*-TBDMS-(1→5)- α -ribofuranose] (A), poly[3-acetamido-2-*O*-TBDMS-3-deoxy-(1→5)- α -ribofuranose-co-2,3-di-*O*-TBDMS-(1→5)- α -ribofuranose] (B), poly[3-acetamido-3-deoxy-(1→5)- α -ribofuranose-co-(1→5)- α -ribofuranose] (C), and sulfated acetamido group-containing copolymer (D).

[copoly (A3AAdSR-ADSR)]. The ^{13}C NMR spectrum of copoly (A3AAdSR-ADSR) showed the absorption of methyl group and carboxyl group of the acetamido group at 25.6 and 170.2 ppm, respectively (Figure 3B).

Finally, the de-*t*-butyldimethylsilylation of copoly (A3AAdSR-ADSR) with tetra-*n*-butylammonium fluoride produced the resulting acetamido group-containing copolyribose. By comparison of ^{13}C NMR spectrum of acetamido group-containing copolyribose (Figure 3C) with that of the 3-acetamido-3-deoxy-(1 $\rightarrow$ 5)- α -D-ribofuranan[23] and that of (1 $\rightarrow$ 5)- α -D-ribofuranan[27], it was found that the acetamido group-containing copolyribose is a poly [3-acetamido-3-deoxy-(1 $\rightarrow$ 5)- α -ribofuranose-co-(1 $\rightarrow$ 5)- α -ribofuranose] [copoly (A3AAdR-AR)]. The absorption of C1 in A3AAdR unit and that of C1' in R unit appeared overlapping around 105.0 ppm, and absorption peaks due to the methyl and carboxyl group in the acetamido group appeared at 25.6 and 170.7 ppm, respectively.

The content of acetamido groups in the acetamido group-containing copolymer was 48.3% and 48.7% by ^1H NMR and ^{13}C NMR, respectively. In addition, more than 90% of the R units and 100% of A3AAdR units were composed of 1,5- α -D-ribofuranosidic units. Thus, the acetamido group-containing copolymer had a high α -stereoregular structure with high positive specific rotation of +141°.

The stereoregular acetamido group-containing copolyribose with $\overline{M}_n$ of 8.6×10^3 was soluble in water as was the acetamido group-containing homopolyribose. According to this synthetic route, water-soluble acetamido group-containing ribofuranans with various degrees of N-acetylation can be prepared.

Sulfation of Copoly (A3AAdR-R)

An acetamido group-containing synthetic polysaccharide was sulfated with sulfur trioxide-pyridine complex in a mixture of pyridine and dimethyl sulfoxide (DMSO) (Scheme 1). The characterization (Table 3) was carried out by elemental analysis, specific rotation, and NMR.

The sulfated acetamido group-containing copolyribose [copoly (SA3AAdR-SR)] with $\overline{M}_n$ of 10.7×10^3 ($\overline{DP} = 56$), was obtained by sulfating the starting acetamido group-containing copolyribose with

Table 3 Characterization of Acetamido and Sulfate Group-Containing Copolyribose

Copolymer	$\overline{M}_n^b$ $\times 10^3$	$\overline{DP}^c$	$[\alpha]^{20}_{\text{D}}^d$ deg.	CH_3CONH^e mol%	DS' of (SA3AAdR/SR)/2
copoly (A3AAdR-R)	8.6	56	+141	48.3	—
copoly (SA3AAdR-SR)	10.7	49	+85	49.4	1.2

^a Poly [3-acetamido-3-deoxy-(1 $\rightarrow$ 5)- α -ribofuranose-co-(1 $\rightarrow$ 5)- α -ribofuranose] and its sulfate. ^b Determined by GPC. ^c Degree of polymerization. ^d Measured in H_2O ($c = 1.0\%$) at 20°C. ^e Mole fraction (%) of the A3AAdR units in copolymer was measured by ^1H NMR. ^f Degree of sulfation was measured by elemental analysis.

$\overline{M}_n$ of 8.6×10^3 ($\overline{DP} = 49$). During synthesis of sulfated acetamido group-containing polyribose, the degree of polymerization decreased mainly in the reduction of the azido group into the amino group, while it decreased to a small extent during the sulfation.

In the ^{13}C NMR spectrum (Figure 3D) of sulfate acetamido group-containing copolyribose, the absorption peaks of methyl and carboxyl group in the acetamido residue appeared at 25.6 ppm and 170.7 ppm, respectively. Absorption peaks due to sulfated group-binding carbons, C2 in SA3AAdR unit, and C2' and C3' in SAR units, overlapped in the range of 76.3 to 76.5 ppm. In the ^{13}C NMR spectrum of sulfated (1 $\rightarrow$ 5)- α -D-ribofuranan, the absorption peaks of C2' and C3' overlapped around 76.3 ppm[27]. Before sulfation, the absorption of C2 in A3AAdR unit, and that of C2' and C3' in R unit appeared at 72.6, 73.6 and 72.5 ppm, respectively. In addition, each absorption appeared as a sharp peak, indicating that the sulfated acetamido group-containing copolyribose had high degrees of sulfation and that no de-acetylation took place. By elemental analysis, the degree of sulfation (DS) per glucosidic unit was determined to be 1.2. After sulfation, the degree of acetylation (DA) of copoly [SA3AAdR-SR] was 49.4% by ^1H NMR, which was almost the same as the DA (48.7%) of the starting copoly [A3AAdR-R].

In conclusion, the synthesis and sulfation of a water-soluble acetamido group-containing copolyribose was completed by the ring-opening polymerization of a 1,4-anhydro ribose derivatives and by subsequent chemical modifications, which including reduction of the azido group into amino group, acetylation of the amino group into acetamido group,

removal of the OH-protective t-butyldimethylsilyl group, and sulfation of the free hydroxy groups by starting from azido and t-butyldimethylsilyl group-containing copolyribose.

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