# Synthesis of Cellulose-Type Polyriboses and Their Branched Sulfates with Anti-AIDS Virus Activity by Selective Ring-Opening Copolymerization of 1,4-Anhydro-α-D-ribopyranose Derivatives

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ABSTRACT: Copolymerizations of 1,4-anhydro-2,3-*O*-benzylidene- and 1,4-anhydro-2,3-bis-*O*-(*tert*-butyldimethylsilyl)-α-D-ribopyranoses in various feeds were carried out with antimony pentachloride as catalyst at 0 °C to give, 1,4-β-linked ribopyranan copolymers. Next the copolymers were subjected to a branching reaction by D- and L-glucose ethyl orthoacetates followed by the selective removal of silyl groups to afford branched ribopyranans. These were sulfated with piperidine-*N*-sulfonic acid to form branched ribopyranan sulfates having potent anti-AIDS virus activities, $EC_{50}$ = 0.3–0.9 μg/mL (a standard curdlan sulfate, $EC_{50}$ = 0.43 μg/mL). Stereoregular ribopyranan sulfates and sulfated poly(ribose)s composed of 1,4-β-pyranosidic and 1,5-α-furanoside structures were also synthesized to examine the relationship between polymer structure and anti-AIDS virus activity. The structural analysis of the ribopyranans was performed by high resolution $^{13}C$ NMR spectroscopy.

## Introduction

Ring-opening polymerization of anhydro sugars has provided various stereoregular polysaccharide derivatives which were deprotected to give biologically active polysaccharides.[1] Schuerch first synthesized a stereoregular polysaccharide by cationic ring-opening polymerization of 1,6-anhydro sugars.[2] Developing Schuerch's method to selective ring-opening polymerization of 1,4-anhydroriboses, we also found a method to prepare cellulose-type (1→4)-β-ribopyranan and furanan-type (1→5)-α-ribofuranan.[3]

Previously, (1→5)-α-ribofuranans having branchings, which were obtained by selective ring-opening polymerization followed by a branching reaction, were converted into sulfated and branched ribofuranans with high anti-AIDS (acquired immunodeficiency syndrome) virus activity.[4] However, they also showed high anticoagulant activities[5] which are undesirable byproducts with anti-AIDS virus activity.

(1→4)-β-Ribopyranans have been obtained from 1,4-anhydro-2,3-*O*-benzylidene-α-D-ribopyranose (ABRP) with antimony pentachloride as catalyst. We reported recently that ring-opening copolymerization of ABRP and 1,4-anhydro-2,3-bis-*O*-(*tert*-butyldimethylsilyl)-α-D-ribopyranose (ADSR) in various feeds leads completely to 1,4-β-linked stereoregular copolymers consisting of both benzylidenated and silylated ribopyranosidic units, even though the ring-opening polymerization of ADSR gave no 1,4-β-linked polymer.[6] With use of selective desilylation from the copolymers and a subsequent branching reaction they can be converted into (1→4)-β-ribopyranans having branches.

On the other hand, of pyranose-type polysaccharides, such sulfated polysaccharides as curdlan sulfate,[7,8] lentinan sulfate,[9] and mannan sulfate[10] exhibited high anti-AIDS virus activities but low anticoagulant activities. These biological activities are assumed to be desirable for an AIDS drug. Accordingly, it is important to synthesize polysaccharides with high anti-AIDS virus activities but with other biological activities being low.

In this study, we wish to report the synthesis of branched (1→4)-β-ribopyranans by ring-opening copolymerization followed by a branching reaction. In addition, it is revealed that the sulfated branched (1→4)-β-ribopyranans exhibited potent anti-AIDS virus activities.

## Experimental Section

**Measurement.** The $^1H$ (270-MHz) and $^{13}C$ (67.8-MHz) NMR spectra were recorded on a JEOL GX-270 spectrometer. Specific rotation measurements were carried out on polymer solutions in $CHCl_3$ or $H_2O$ at 25 °C with a Perkin-Elmer 241 polarimeter. The molecular weight of polymers was determined by organic phase GPC (column, Toso TSK-gel, G2000H, G3000H, G4000H, G5000H, 7.6 mm × 600 mm × 4 mm; eluent, THF) using polystyrene standards and by aqueous phase GPC (column Toso TSK-gel, G2000SW, G3000SW, G4000SW, 7.6 mm × 600 mm × 3 mm; eluent, 66.7 mmol of phosphate buffer, pH = 6.86) using pullulan standards.

**Monomers.** 1,4-Anhydro-α-D-ribopyranose was obtained by vacuum pyrolysis of D-ribose.[11] 1,4-Anhydro-2,3-*O*-benzylidene-α-D-ribopyranose (ABRP) was prepared by protection of 1,4-anhydro-α-D-ribopyranose with a benzylidene group.[12] To a DMF solution (60 mL) of 1,4-anhydro-α-D-ribopyranose (10 g) was added 18 g of dimethoxytoluene and 30 mg of *p*-toluenesulfonic acid. The mixture was stirred for 3 h at 60 °C and then poured into 5% sodium bicarbonate solution (300 mL). The organic layer was extracted with dichloromethane, washed with water, dried with anhydrous sodium sulfate, and evaporated to give crude ABRP, which was purified by successive recrystallization from ethanol three times and finally from *n*-butyl chloride–petroleum ether. Yield: 12.0 g (71%), as a mixture of syn and

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**Scheme 1. Synthesis of Branched Ribopyranan Sulfates**

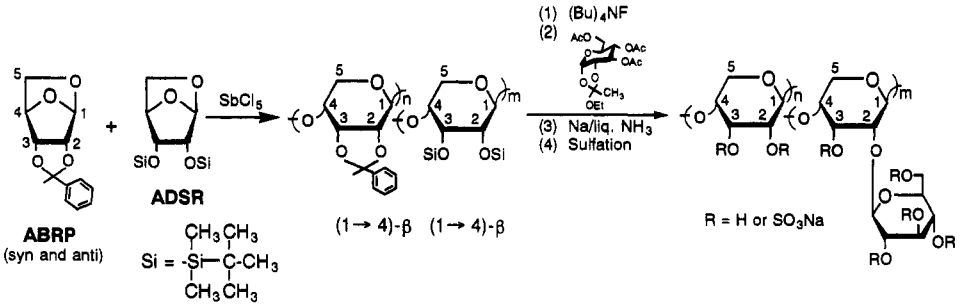


**Table 1. Copolymerization of 1,4-Anhydro-2,3-*O*-benzylidene-α-D-ribopyranose (ABRP) with 1,4-Anhydro-2,3-bis-*O*-(*tert*-butyldimethylsilyl)-α-D-ribopyranose (ADSR)**

| | | | | | | | | | stereoregularity, %[e] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | amt of ADSR feed | | | | | | | ABRP | | ADSR | |
| run[a] | amt of ABRP feed, g | g | mol % | time, h | yield, % | $[\alpha]_D^{25}$,[b] deg | $10^{-4}\bar{M}_n$[c] | amt of ADSR unit in polymer,[d] mol % | 1,4-β | 1,5-α | 1,4-β | 1,5-α |
| 1 | 0.50 | 0 | 0 | 3 | 62.7 | −60.5 | 4.9 | 0 | 100 | 0 | 0 | 0 |
| 2 | 0.40 | 0.10 | 13.2 | 5 | 58.3 | −58.6 | 1.6 | 11 | 100 | 0 | 100 | 0 |
| 3 | 0.35 | 0.15 | 20.7 | 24 | 50.3 | −48.2 | 1.3 | 15 | 100 | 0 | 100 | 0 |
| 4 | 0.30 | 0.20 | 28.6 | 24 | 66.0 | −17.3 | 1.4 | 33 | 92 | 8 | 59 | 41 |
| 5 | 0.25 | 0.25 | 37.9 | 24 | 67.4 | −3.0 | 1.2 | 44 | 86 | 14 | 51 | 46 |
| 6 | 0.20 | 0.30 | 47.8 | 24 | 74.9 | +30.1 | 1.5 | 61 | 81 | 19 | 46 | 54 |
| 7 | 0.15 | 0.35 | 59.0 | 24 | 76.9 | +37.5 | 2.0 | 68 | 82 | 18 | 34 | 66 |
| 8 | 0.10 | 0.40 | 71.0 | 24 | 82.6 | +66.4 | 2.7 | 81 | 71 | 29 | 29 | 71 |
| 9 | 0.05 | 0.45 | 83.6 | 4 | 68.5 | +74.4 | 1.6 | 91 | 52 | 48 | 33 | 67 |
| 10 | 0 | 0.50 | 100 | 1.5 | 51.3 | +64.5 | 5.9 | 100 | 0 | 0 | 15 | 85 |

[a] Conditions: solvent, $CH_2Cl_2$; catalyst, $SbCl_5$ (2.0–2.5 mol %); temperature, −40 °C. [b] Measured in $CHCl_3$ (*c* 1%). [c] Determined by GPC. [d] Calculated from the $^1H$ NMR spectrum. [e] Calculated from the $^{13}C$ NMR spectrum.

**Table 2. Effect of Temperature on Copolymerization of 1,4-Anhydro-2,3-*O*-benzylidene-α-D-ribopyranose (ABRP) with 1,4-Anhydro-2,3-bis-*O*-(*tert*-butyldimethylsilyl)-α-D-ribopyranose**

| | | | | | | | | | | stereoregularity,[e] % | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | amt of ADSR feed | | | | | | | | ABRP | | ADSR | |
| run[a] | amt of ABRP feed, g | g | mol % | temp, °C | time, h | yield, % | $[\alpha]_D^{25}$,[b] deg | $10^{-4}\bar{M}_n$[c] | amt of ADSR unit in polymer,[d] mol % | 1,4-β | 1,5-α | 1,4-β | 1,5-α |
| 1 | 0.30 | 0.20 | 28.6 | 0 | 20 | 53.7 | −45.4 | 0.9 | 26 | 97 | 3 | 100 | 0 |
| 2 | | | | −20 | 22 | 74.1 | −25.9 | 1.1 | 34 | 93 | 7 | 100 | 0 |
| 3 | | | | −40 | 24 | 44.9 | −32.4 | 2.2 | 26 | 88 | 12 | 81 | 19 |
| 4 | 0.20 | 0.30 | 47.8 | 0 | 20 | 56.5 | −30.0 | 0.8 | 47 | 95 | 5 | 100 | 0 |
| 5 | | | | −20 | 22 | 60.1 | −11.4 | 0.9 | 53 | 93 | 7 | 73 | 27 |
| 6 | | | | −40 | 24 | 61.8 | +9.5 | 1.2 | 52 | 89 | 11 | 47 | 53 |
| 7 | 0.10 | 0.40 | 71.0 | 0 | 20 | 43.3 | −8.9 | 0.5 | 76 | 95 | 5 | 100 | 0 |
| 8 | | | | −20 | 22 | 92.4 | +33.5 | 1.2 | 82 | 83 | 17 | 61 | 39 |
| 9 | | | | −40 | 5 | 74.4 | +81.8 | 1.9 | 81 | 74 | 26 | 25 | 75 |
| 10 | 0.05 | 0.45 | 83.6 | 0 | 23 | 83.4 | +25.1 | 1.0 | 85 | 76 | 24 | 72 | 28 |
| 11 | | | | −20 | 24 | 78.3 | +53.7 | 1.7 | 87 | 68 | 32 | 52 | 48 |
| 12 | | | | −40 | 4 | 68.5 | +74.4 | 1.6 | 91 | 52 | 48 | 33 | 67 |

[a] Conditions: solvent, $CH_2Cl_2$; catalyst, $SbCl_5$ (1.0–2.5 mol %). [b] Measured in $CHCl_3$ (*c* 1%). [c] Determined by GPC. [d] Calculated from the $^1H$ NMR spectrum. [e] Calculated from the $^{13}C$ NMR spectrum.

anti diastereomers. Mp: 139.5–141.0 °C (lit.[11] mp 141–146 °C). $[\alpha]_D^{25}$ = −53.0° (*c* 1% in $CHCl_3$) (lit.[11] $[\alpha]_D^{25}$ = −56.6°) (*c* 1% in $CHCl_3$).

1,4-Anhydro-2,3-bis-*O*-(*tert*-butyldimethylsilyl)-α-D-ribopyranose (ADSR) was synthesized by reacting 1,4-anhydro-α-D-ribopyranose with *tert*-butyldimethylsilyl chloride using a modified method of Hakimelahi et al.,[12] $[\alpha]_D^{25}$ = −34.6° (*c* 1% in $CHCl_3$).

**Copolymerization.** The typical procedure for copolymerization is as follows: A mixture of ABRP (0.35 g) and ADSR (0.15 g) was copolymerized with antimony pentachloride (2.0 mol % to the feed) as catalyst in dichloromethane (1 mL) under high vacuum at −40 °C for 24 h. The polymerization was terminated by addition of methanol. The mixture was dissolved in chloroform. The chloroform solution was neutralized with sodium bicarbonate solution, washed with water, dried over sodium sulfate, and concentrated to approximately 10 mL. A copolymer was purified by reprecipitation using the methanol–chloroform system three times and freeze-dried from benzene.

**Removal of *tert*-Butyldimethylsilyl Groups from the Copolymer.** To a tetrahydrofuran (THF) solution of the copolymer was added a 1 M tetra-*n*-butylammonium fluoride solution in THF. The mixture was stirred under reflux for 1 h. After evaporation of THF, the polymer was dissolved in chloroform, purified by dissolution–reprecipitation three times by using the methanol–chloroform system, and then isolated by freeze-drying from benzene.

**Branching of L-Glucose to a Partially Benzylidenated Polymer.** A partially benzylidenated (1→4)-β-D-ribopyranan, 3,4,6-tri-*O*-acetyl-α-L-glucose (1,2-ethyl orthoacetate), and 2,6-lutidinium perchlorate were dissolved in benzene and the mixture was reacted by refluxing for 2 h. The Dean–Stark trap was used during the condensation to remove a small amount of water in the system. After evaporation of benzene, the residue was dissolved in chloroform. The purification of an L-glucose-branched ribopyranan derivative was performed in a way similar to that above.

**Deprotection of Branched Ribopyranan.** To a liquid ammonia (50-mL) solution containing 0.4 g of sodium was added dropwise the branched ribopyranan (0.5 g) in 1,2-dimethoxymethane (15 mL) at −78 °C under nitrogen. After 1 h of stirring at −78 °C, anhydrous ammonium chloride was added

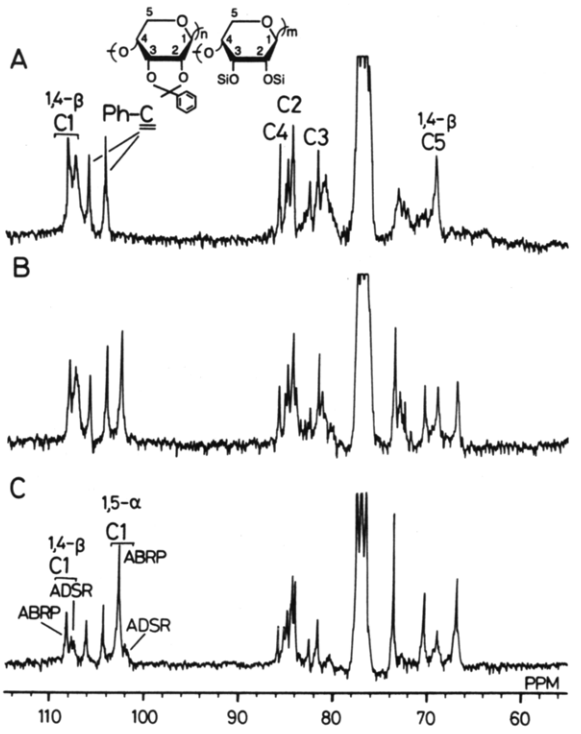


**Figure 1.** 67.8-MHz $^{13}C$ NMR spectra of copoly(ABRP-ADSR) ($CDCl_3$ as solvent). Polymerization temperature: (A) 0 °C, (B) −20 °C, and (C) −40 °C. Monomer feeds: ABRP:ADSR = 29.0:71.0 mol %.

until a dark color disappeared, and then a small amount of methanol was added. After evaporation of ammonia, 50 mL of water was added and then the aqueous layer was washed with dichloromethane and dialyzed with deionized water overnight. The OH-free polysaccharide was freeze-dried from water.

**Sulfation.** Free ribopyranans (0.11–0.55 g) dissolved in DMSO (20–40 mL) were sulfated with piperidine-*N*-sulfonic acid (2.0–3.0 equiv to the hydroxyl group of the glucose residue) at 85 °C for 1 h to give sulfated ribopyranans. The detailed method for the sulfation was described previously.[8]

**Anti-AIDS Virus Activity (=Anti-HIV Activity).** The anti-HIV activity of ribopyranan sulfates in HTLV-$III_B$ (a kind of AIDS virus HIV-1) was assayed by the inhibition of virus-induced cytopathic effects (CPE) in the MT-4 cells, by monitoring spectrophotometrically using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as an indicator.[13] The activity is described as the 50% effective concentration ($EC_{50}$) which is calculated from the dose of the test compound achieving the 50% protection of HIV-induced CPE. The cytotoxicity ($CC_{50}$) was determined by the 50% cytotoxic concentration of the test compound on the MT-4 cell.

**Anticoagulant Activity.** The anticoagulant activity *in vitro* was determined by use of bovine plasma according to the previous papers[14,15] based on the United States Pharmacopoeia.[16]

## Results and Discussion

**Copolymerization of ABRP and ADSR.** The total synthetic scheme of branched ribopyranan sulfates is illustrated in Scheme 1. The results of copolymerization of ABRP and ADSR by $SbCl_5$ catalyst at −40 °C are summarized as well as the those of homopolymerization in Table 1. These monomers were readily homopolymerized into high molecular weight polymers with a large negative specific rotation of −60.5° (ABRP) and a large positive specific rotation of +64.5° (ADSR) (runs 1 and 10). It has been revealed that poly(ABRP) with such a high negative specific rotation had a 1,4-β-linked structure and poly(ADSR) with such a positive specific rotation had mixed structures of 1,5-α- and 1,4-β-linked units.[3] Furthermore, from the results of hydrolysis studies, poly(ABRP) was very slow but the (1→5)-β-ribofuranan derivative was fast, indicating that poly(ABRP) has the 1,4-β-pyranosidic structure.[17] When 13.2 and 20.7 mol % of ADSR in the monomer feeds were used, the copolymers obtained had a completely 1,4-β-pyranosidic structure (runs 2 and 3), the determination of which was carried out by $^{13}C$ NMR spectrometry. However, as the proportion of ADSR in the feed increased, the stereoregularity of copolymers decreased, and finally, a copolymer with a random structure was formed (run 9). The number-average molecular weight ranged from $1.2 \times 10^4$ to $2.2 \times 10^4$. The proportion of 1,4-β- and 1,5-α-units in the polymer backbone was determined by the intensity of respective C1 carbon absorptions in the $^{13}C$ NMR spectrum.

**Scheme 2. Proposed Mechanism in the Stereoselective Formation of the 1,4-β-Linkage by a Trialkyloxonium Ion Intermediate**

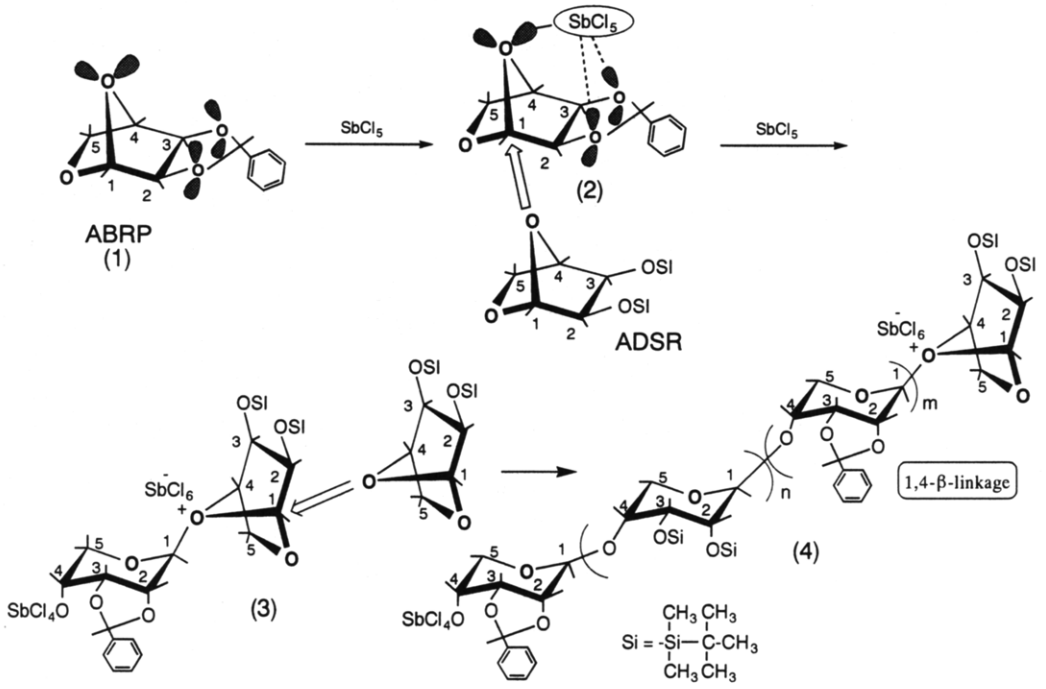

**Table 3. Copolymerization of 1,4-Anhydro-2,3-*O*-benzylidene-α-D-ribopyranose (ABRP) with 1,4-Anhydro-2,3-bis-*O*-(*tert*-butyldimethylsilyl)-α-D-ribopyranose (ADSR) by Lewis Acid Catalysts**

| run[a] | amt of ABRP feed, g | amt of ADSR feed, g | amt of ADSR feed, mol % | catalyst (amt, mol %) | temp, °C | time, h | yield, % | $[\alpha]_D^{25}$,[b] deg | $10^{-4}\bar{M}_n$[c] | amt of ADSR unit in polymer,[d] mol % | stereoregularity,[e] % ABRP 1,4-β | ABRP 1,5-α | ADSR 1,4-β | ADSR 1,5-α |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.25 | 0 | 0 | $SnCl_4$ (3.4) | 0 | 24 | 75.6 | +48.5 | 3.1 | 0 | 38 | 62 | 0 | 0 |
| 2 | 0.40 | 0.10 | 13.2 | $SnCl_4$ (3.7) | 0 | 24 | 70.8 | +48.6 | 1.9 | 49 | 44 | 56 | 32 | 68 |
| 3 | 0.175 | 0.075 | 20.7 | $SnCl_4$ (3.2) | 0 | 22 | 67.4 | +43.7 | 1.9 | 31 | 41 | 59 | 44 | 56 |
| 4 | 0.20 | 0.30 | 47.8 | $SnCl_4$ (5.3) | 0 | 48 | 59.2 | +41.3 | 3.6 | 89 | 39 | 61 | 57 | 43 |
| 5 | 0.10 | 0.40 | 71.0 | $SnCl_4$ (3.7) | 0 | 24 | 66.3 | +42.5 | 1.2 | 93 | 36 | 64 | 54 | 46 |
| 6 | 0 | 0.50 | 100 | $SnCl_4$ (1.6) | 0 | 5 | 91.0 | +93.2 | 7.3 | 100 | 0 | 0 | 16 | 84 |
| 7 | 0.10 | 0.15 | 47.8 | $SnCl_4$ (4.3) | −40 | 23 | 65.4 | +70.6 | 2.2 | 80 | 30 | 70 | 27 | 73 |
| 8 | 0 | 0.50 | 100 | $SnCl_4$ (4.7) | −40 | 2 | 88.1 | +100.3 | 19.7 | 100 | 0 | 0 | 10 | 90 |
| 9 | 0.20 | 0.30 | 47.8 | $BF_3OEt_2$ (10.2) | 0 | 22 | 53.8 | +80.9 | 3.8 | 87 | 21 | 79 | 20 | 80 |
| 10 | 0.10 | 0.15 | 47.8 | $BF_3OEt_2$ (4.0) | −40 | 22 | 65.5 | +71.4 | 5.0 | 83 | 30 | 70 | 33 | 67 |
| 11 | 0.25 | 0.25 | 37.9 | $PF_5$ (2.5) | −60 | 4 | 25.6 | +67.7 | 0.8 | 76 | 60 | 40 | 23 | 77 |
| 12 | 0.25 | 0.25 | 37.9 | $PF_5$ (2.5) | −60 | 24 | 19.6 | +61.8 | 2.2 | 91 | 44 | 56 | 30 | 70 |

[a] Conditions: solvent, $CH_2Cl_2$. [b] Measured in $CHCl_3$ (*c* 1%). [c] Determined by GPC. [d] Calculated from the $^1H$ NMR spectrum. [e] Calculated from the $^{13}C$ NMR spectrum.

**Table 4. Glycosylation of Partially Benzylidenated Ribopyranans with Glucose Orthoacetates**

| run[a] | amt of ribopyranan, g | orthoacetate (amt, g) | amt of catalyst, mg | amt of solvent, mL | time, h | yield, g | $[\alpha]_D^{25}$,[e] deg | $10^{-4}\bar{M}_n$[f] | degree of branching,[g] % |
|---|---|---|---|---|---|---|---|---|---|
| 1[b] | 0.25 | L-glucose (1.0) | 5 | 10 | 3 | 0.36 | −80.7 | 2.2 | 13 |
| 2[b] | 0.28 | L-glucose (1.5) | 6 | 10 | 3 | 0.24 | −40.8 | 1.8 | 24 |
| 3[b] | 0.50 | L-glucose (2.0) | 15 | 15 | 5 | 0.78 | −32.3 | 1.7 | 23 |
| 4[c] | 0.31 | L-glucose (1.5) | 15 | 15 | 5 | 0.58 | −27.8 | 2.1 | 35 |
| 5[d] | 0.51 | D-glucose (2.0) | 15 | 12 | 5 | 1.09 | +20.0 | 0.9 | 38 |

[a] Conditions solvent: benzene; temperature, reflux; catalyst, 2,6-lutidinium perchlorate. [b] 50 mol % of ABRP unit in polymer. [c] 40 mol % of ABRP unit in polymer. [d] 30 mol % of ABRP unit in polymer. [e] Measured in $CHCl_3$ (*c* 1%). [f] Determined by GPC. [g] Determined by the $^{13}C$ NMR spectrum.

In order to synthesize copolymers with a complete 1,4-β-pyranosidic structure, the effects of polymerization temperature were examined (Table 2). The 1,4-β-stereoregularity of copolymers increased with increasing polymerization temperature. The polymerization at 0 °C in various monomer feeds (runs 1, 4, and 7) afforded copolymers with complete 1,4-β-stereoregularity ($[\alpha]_D^{25}$ = −45.4, −30.0, and −8.9°, respectively). In run 7, 29.0 mol % of ABRP in the feed led to a 1,4-β-pyranosidic copolymer. However, in run 10, the copolymer with a mixed structure was obtained when a low molar ratio (16.4 mol %) of ABRP was fed. At 0 °C, the molar ratio of the ADSR unit in the copolymer was approximately the same as that in the feed, suggesting that the two monomers had almost equivalent monomer reactivity ratios.

Figure 1 shows the 67.8-MHz $^{13}C$ NMR spectra of copolymers which were prepared by the copolymerization of ABRP and ADSR in the same feed ratio at different temperatures. C1 absorptions due to the 1,5-α-furanosidic and 1,4-β-pyranosidic units appeared around 102 and 108 ppm, respectively, in the spectrum in Figure 1C. On the other hand, in Figure 1A, the C1 absorption at 102 ppm due to the 1,5-α-furanosidic unit disappeared, suggesting that a higher polymerization temperature worked effectively to give a copolymer with the 1,4-β-pyranosidic structure. It was revealed that when a large proportion of ADSR (71.0 mol %) in the feed was used, the copolymer had a 1,4-β-pyranosidic structure consisting of 76 mol % ADSR unit in the polymer main chain. These results suggest that the initiating species at 0 °C was derived from ABRP but not from ADSR, because in the homopolymerization the initiating species of ADSR formed by $SbCl_5$ catalyst did not afford the 1,4-β-ribopyranosidic structure. Previously, it was reported that the polymerization of ABRP with $SbCl_5$ as catalyst proceeds by a trialkyloxonium ion mechanism to lead to the formation of 1,4-β-ribopyranan.[3] By application of this assumption to the present copolymerization, the following mechanism can be considered, as shown in Scheme 2. In the initiation step at higher copolymerization temperatures, almost exclusive complexation of bulky $SbCl_5$ among 1,4-linked oxygen and the C2 and C3 oxygens of the ABRP monomer (**1**) occurred, affording a trialkyloxonium ion intermediate (**2**). The 1,4-linked oxygen of an approaching monomer (ABRP or ADSR) attacked the propagating end from the backside direction of the trialkyloxonium ion (**2**) to generate an active initiating species with the 1,4-β-pyranosidic structure (**3**). Both ABRP and ADSR monomers might approach exclusively from their 1,4-linked oxygen in the active species to form the 1,4-β-linked copolymer backbone (**4**).

The reason for the preferential 1,4-β scission of the monomers at higher temperatures was assumed to be as follows: At such a high temperature as 0 °C, the initiating species originating from ABRP is much more reactive than that from ADSR. The former species intrinsically leads to the formation of the 1,4-β-structure. In Table 2, the proportion of ADSR unit in the copolymer was 81 mol % (71.0 mol % in the feed) at −40 °C (run 9) and 76 mol % at 0 °C (run 7). It showed a 5 mol % decrease at 0 °C. This result suggested that the reactivity of ABRP increased with increasing polymerization temperature and the initiating species originating from ABRP became dominant in the 1,4-β scission polymerization.

Table 3 summarizes the result of copolymerizations by other Lewis acid catalysts such as tin tetrachloride, boron trifluoride, and phosphorus pentafluoride. These catalysts gave copolymers with positive specific rotations. The yield and molecular weight of the copolymers, except for runs 11 and 12, were relatively higher than those of the copolymers prepared by the $SbCl_5$ catalyst. However, no 1,4-β-linked stereoregular copolymer was obtained. The copolymers were composed of higher proportions of 1,5-α-units. Therefore, these catalyst were unsuitable for synthesizing 1,4-β-stereoregular copolymers, as was the case for the homopolymers.

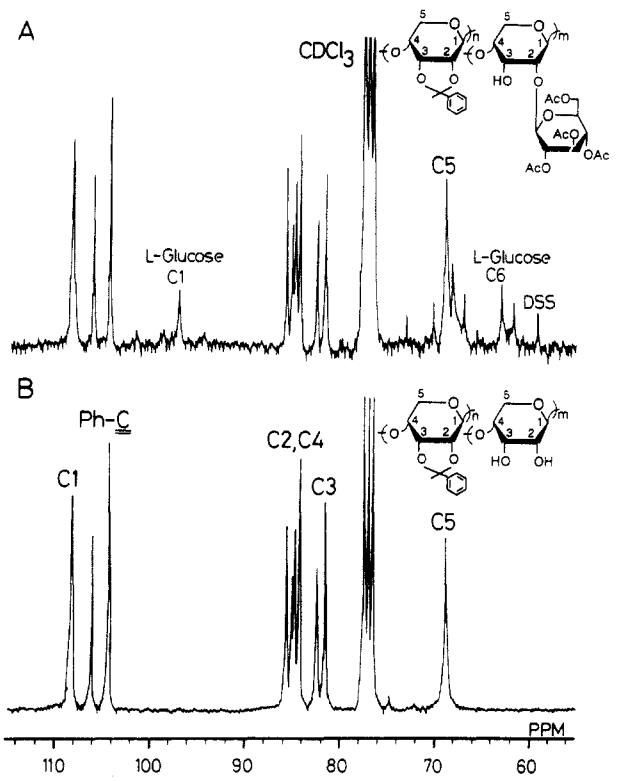


**Figure 2.** 67.8-MHz $^{13}C$ NMR spectra of (A) the L-glucose-branched (23 mol %) ribopyranan derivative and (B) partially protected ribopyranan ($CDCl_3$ as solvent).

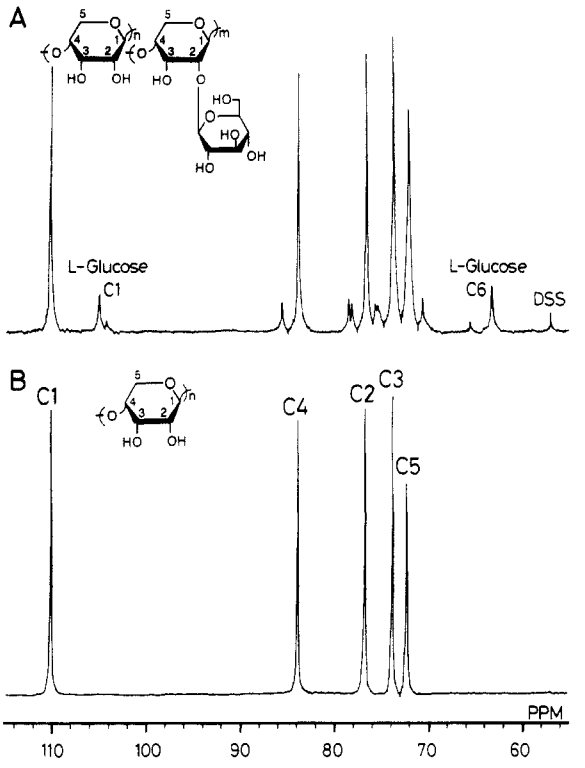


**Figure 3.** 67.8-MHz $^{13}C$ NMR spectra of (A) L-glucose-branched (23 mol %) and (B) linear (1→4)-β-D-ribopyranans ($D_2O$ as solvent, at 37 °C).

**Synthesis of Branched (1→4)-β-D-Ribopyranans.** Selective desilylation was carried out from the three copolymers containing approximately 50, 60, and 70 mol % of ADBR units to give in good yields partially benzylidenated ribopyranans, which were soluble in such organic solvents as chloroform and benzene. Then, the condensation of partially benzylidenated ribopyranans with L- and D-glucose orthoacetates was performed by use of 2,6-lutidinium perchlorate as the catalyst in benzene. The obtained polymes were L- and D-glucose branched ribopyranan derivatives, as shown in Table 4. The degree of branching determined from the C1 carbon NMR absorptions was in the range 13–38 mol % in relation to the ribose unit of the polymer main chain. The D-glucose branched ribopyranan derivative showed a positive specific rotation (+20.0°) and the L-glucose branched ribopyranans had negative specific rotations (−20.8 to −80.7°). The molecular weight of the branched polymers ranged from $0.9 \times 10^4$ to $2.2 \times 10^4$. The $^{13}C$ NMR spectra of the partially benzylidenated and L-glucose branched ribopyranans are shown in Figure 2. The C1 carbon absorption due to the L-glucose branches appeared around 98 ppm, and that due to the main chain at 108.5 ppm as a single peak which corresponds to the 1,4-β-pyranosidic structure (Figure 2A). The complexity and peak splitting of the NMR spectra are assumed to originate from branchings and conformational differences due to the syn and anti diastereomers of the ABRP unit.

The removal of benzylidene and acetyl groups from the branched polymers was carried out with sodium in liquid ammonia to afford OH-free branched ribopyranans in high yields. The results of debenzylidenation are given in Table 5. The polymers in runs 1 to 3 had a complete 1,4-β-stereoregularity, while those in runs 4 and 5 were composed of mixed structures of different proportions of 1,5-α- and 1,4-β-units. The OH-free ribopyranans had number-average molecular weights from $0.9 \times 10^4$ to $2.4 \times 10^4$ and specific rotations from −66.9 to +67.1°. The 67.8-MHz $^{13}C$ NMR spectra of L-glucose branched and linear ribopyranans are shown in Figure 3. All absorptions were assigned by use of 2-dimensional NMR spectroscopy. A single absorption at 110.5 ppm was explicitly assigned to the C1 carbon of (1→4)-β-D-ribopyranan (Figure 3B). In Figure 3A, the C1 and C6 absorptions of the L-glucose branches appeared clearly at 105 and 63 ppm, respectively.

The OH-free ribopyranans were sulfated with piperidine-*N*-sulfonic acid to give sulfated ribopyranans. The results are summarized in Table 6. All specific rotations

**Table 5. Deprotection of Benzylidenated Polymers**

| | polymer | | | | | OH-free polysaccharide | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | stereoregularity,[c] % | |
| run | ratio of α and β (ratio of branching) | | amt, g | $[\alpha]_D^{25}$, deg | $10^{-4}\bar{M}_n$ | yield, g | $[\alpha]_D^{25}$,[a] deg | $10^{-4}\bar{M}_n$[b] | 1,4-β | 1,5-α |
| 1 | RP1 | β 100% | 1.80 | −60.1 | 4.9 | 0.36 | −66.9 | 2.4 | 100 | 0 |
| 2 | RP2 | β 100% | 0.80 | −60.2 | 6.0 | 0.43 | −66.9 | 1.9 | 100 | 0 |
| 3 | RP3 | β 100% | 0.80 | −55.0 | 5.0 | 0.38 | −60.1 | 2.0 | 100 | 0 |
| 4 | RP4 | α 74%, β 26% | 0.32 | +30.1 | 1.5 | 0.17 | −3.3 | 1.4 | 75 | 25 |
| 5 | RP5 | α 25%, β 75% | 0.30 | +61.8 | 2.0 | 0.14 | +67.1 | 1.1 | 26 | 74 |
| 6 | RPD1 | (D-glucose, 38%) | 1.00 | +20.0 | 0.9 | 0.63 | +25.6 | nd | 100 | 0 |
| 7 | RPL2 | (L-glucose, 23%) | 0.70 | −32.3 | 1.7 | 0.29 | nd | 0.9 | 100 | 0 |
| 8 | RPL2 | (L-glucose, 35%) | 0.50 | −27.8 | 2.1 | 0.35 | −27.4 | nd | 100 | 0 |

[a] Measured in $H_2O$ or DMSO (*c* 1%). [b] Determined by GPC. [c] Calculated from the $^{13}C$ NMR spectrum.

**Table 6. Sulfation of Ribofuranans[a]**

| run | polymer | amt, g | amt of DMSO, mL | amt of PSA,[b] g | yield, g | $[\alpha]_D^{25}$,[c] deg | $10^{-4}\bar{M}_n$[d] | elemental anal. C | H | S | DS[e] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | RPS1, β 100% | 0.21 | 16 | 0.7 | 0.27 | +2.8 | 0.9 | 21.2 | 3.4 | 16.5 | 1.5 |
| 2 | RPS2, β 100% | 0.25 | 20 | 1.2 | 0.33 | +7.8 | 1.2 | 18.9 | 3.3 | 16.1 | 1.6 |
| 3 | RPS3, β 100% | 0.13 | 15 | 1.5 | 0.10 | +8.7 | 1.2 | 17.9 | 2.8 | 17.9 | 1.6 |
| 4 | RPS4, β 100% | 0.10 | 12 | 1.0 | 0.09 | +16.3 | 1.1 | 17.5 | 2.6 | 15.5 | 1.7 |
| 5 | RPS5, β 26%, α 74% | 0.16 | 20 | 1.3 | 0.11 | +17.5 | 1.4 | 16.2 | 2.6 | 14.7 | 1.7 |
| 6 | RPS6, β 75%, α 25% | 0.11 | 15 | 1.0 | 0.10 | +46.2 | 0.9 | 16.7 | 2.6 | 14.2 | 1.6 |
| 7 | RPDS1 (D-glucose 38%) | 0.30 | 20 | 1.8 | 0.45 | +30.6 | 1.5 | 20.2 | 3.2 | 16.7 | 1.5 |
| 8 | RPLS1 (L-glucose 23%) | 0.20 | 16 | 0.7 | 0.22 | +8.0 | 1.2 | 18.9 | 3.0 | 16.8 | 1.7 |
| 9 | RPLS2 (L-glucose 35%) | 0.55 | 30 | 2.2 | 0.84 | +9.5 | 1.1 | 19.2 | 3.1 | 16.8 | 1.6 |

[a] Conditions: temperature, 85 °C; time, 60 min. [b] Piperidine-*N*-sulfonic acid. [c] Measured in $H_2O$ (*c* 1%). [d] Determined by GPC. [e] The number of sulfate groups per sugar unit in ribopyranan sulfate.

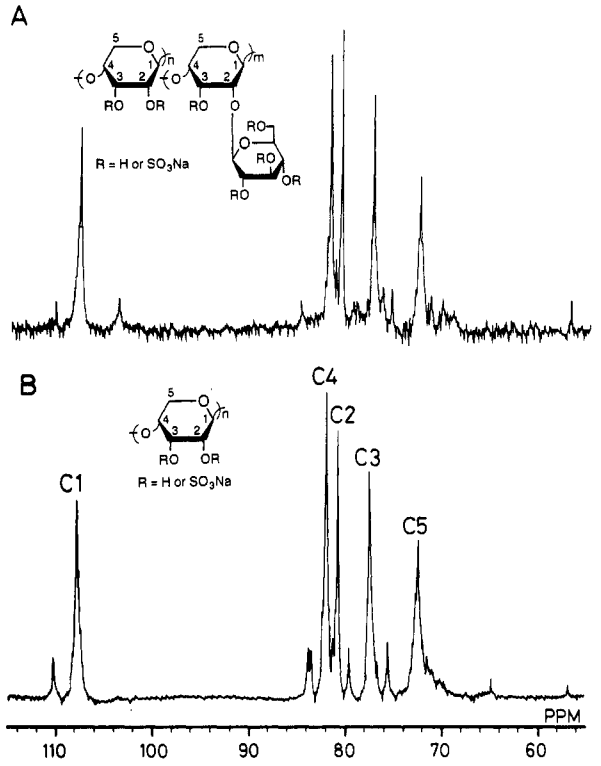


**Figure 4.** 67.8-MHz $^{13}C$ NMR spectra of (A) L-glucose-branched ribopyranan sulfate (DS = 1.7) and (B) (1→4)-β-D-ribopyranan sulfates (DS = 1.6) ($D_2O$ as solvent, at 37 °C).

changed to positive, and the number-average molecular weight was almost the same as before sulfation. The structure of the sulfated ribopyranans was examined by the $^{13}C$ NMR spectrum, as shown in Figure 4, in which each absorption was assigned by the C–H COSY NMR spectra. The C2 and C3 absorptions were shifted downfield, suggesting that the hydroxyl groups were substituted to give sulfate groups. The degree of sulfation was 1.7 for the L-glucose branched and 1.6 for the linear ribopyranans by calculating from the elemental analysis data. The C1 peak of the L-glucose branches appeared at 103.5 ppm, though other absorptions due to branches were unclear because of overlapping with the absorptions of main chain ribopyranoside units.

**Anti-AIDS Virus Activity *in vitro*.** The anti-AIDS virus activity of sulfated ribopyranans *in vitro* was determined by measuring the concentration of the compound necessary to prevent HIV-induced cytopathic effects (CPE) in MT-4 cells. The results are summarized in Table 7. The activity of such reference compounds as curdlan sulfate[7,18] and 2′,3′-dideoxyazidothymidine (AZT)[18] are also listed. Previously, it was reported that curdlan sulfate, which completely inhibitied the AIDS virus infection at a concentration of 3.3 μg/mL,[7] showed a 50% effective concentration ($EC_{50}$) of 0.43 μg/mL; that is to say, the compound inhibitied 50% of cytophatogenicity in AIDS-infected MT-4 cells with a concentration as low as 0.43 μg/mL.[18]

The $EC_{50}$ values of such linear ribopyranans as RPS1, RPS2, RPS3, and RPS4 were 1.5, 1.4, 0.1, and 0.2 μg/mL, respectively, suggesting that the linear ribopyranan sulfates having lower molecular weights and lower degrees of sulfation (DS) showed relatively lower anti-AIDS virus activity. In addition, polyribose composed of 1,4-β-pyranosidic and 1,5-α-furanosidic units (runs 5 and 6) exhibited high anti-AIDS virus activities. In the previous report, another type of stereoregular synthetic poly(ribose sulfate), i.e., sulfated ribofuranan, which had a molecular weight higher than about $1 \times 10^4$ and a high degree of sulfation, exhibited a potent anti-AIDS virus activity.[4] These results suggest that the anti-AIDS virus activity of sulfated polysaccharides depends to a large extent on both the degree of sulfation and the molecular weight, but it does not depend so much on the stereoregularity of the polymer main chain. The molecular weight of sulfated polysaccharides is a very important factor for high anti-

**Table 7. Anti-AIDS Virus Activity of Sulfated Ribopyranans**

| run | sulfated ribopyranan (branch) | S content, % | $10^{-4}\bar{M}_n$[a] | $EC_{50}$,[b] μg/mL | $CC_{50}$,[c] μg/mL | SI[d] | AA[e] unit/mg |
|---|---|---|---|---|---|---|---|
| 1 | RPS1, β 100% | 16.5 | 0.9 | 1.5 | 510 | 340 | 26 |
| 2 | RPS2, β 100% | 16.2 | 1.2 | 1.4 | >1000 | >670 | 36 |
| 3 | PRS3, β 100% | 17.9 | 1.2 | 0.1 | 420 | 4200 | nd |
| 4 | RPS4, β 100% | 15.5 | 1.1 | 0.2 | 627 | 3130 | nd |
| 5 | RPS5, β 26%, α 74% | 14.7 | 1.4 | 0.5 | >1000 | >2000 | 29 |
| 6 | RPS6, β 75%, α 25% | 14.2 | 0.9 | 0.5 | >1000 | >2000 | 25 |
| 7 | RPDS1 (D-glucose 38%) | 16.7 | 1.5 | 0.4 | 706 | 1660 | 34 |
| 8 | RPLS1 (L-glucose 23%) | 16.8 | 1.2 | 0.9 | 524 | 530 | 47 |
| 9 | RPLS2 (L-glucose 35%) | 16.8 | 1.1 | 0.3 | >1000 | >2770 | 34 |
| 10 | curdlan sulfate | 14.1 | 7.9 | 0.43 | >1000 | >2330 | |
| 11 | AZT (mM) | | | 0.0019 | 6.43 | 3400 | |

[a] Determined by GPC. [b] 50% effective concentration. [c] 50% cytotoxic concentration. [d] Selectivity index, $CC_{50}/EC_{50}$. [e] Anticoagulant activity, dextran sulfate NC-1032 20.6 unit/mg.

AIDS virus activity. Sulfated polysaccharides with lower molecular weights need higher degrees of sulfation to provide compounds with high activities.[7,8] Recently, it was revealed that such sulfated alkyl oligosaccharides with medium molecular weights as sulfated octadecyl maltohexaoside (DS = 1.9) and sulfated dodecyl laminalioligosaccharide with the average degree of polymerization of 11.3 showed high anti-AIDS virus activity with $EC_{50}$'s of 0.4 and 0.6 μg/mL, respectively.[18] On the other hand, sulfated oligosaccharides without alkyl groups had low anti-AIDS virus activities. Accordingly, it is concluded that for sulfated polysaccharides with effective anti-AIDS virus activities a molecular weight ($\bar{M}_n$) of more than $0.9 \times 10^4$ to $1.0 \times 10^4$ is required.

The branched ribopyranan sulfates RPDS1, RPLS1, and RPLS2 (runs 7–9) exhibited high anti-AIDS virus activities, i.e., $EC_{50}$'s of 0.4, 0.9, and 0.3 μg/mL, which were high activities almost equivalent to that of curdlan sulfate. The activity increased with the increasing proportion of branches to the pentapyranan main chain, probably because the introduction of hexose branches can lead to an increase in the porportion of sulfate groups in the polymer. The branched ribopyranan sulfates had a little higher sulfur content than linear ones, suggesting that the glucose branches were subjected to higher degrees of sulfation than the backbone ribose unit.

Concerning the cytotoxicity of ribopyranan sulfates, the $CC_{50}$ was in the range of 510 to more than 1000 μg/mL, suggesting that the ribopyranan sulfates had low cytotoxicities. One of the important biological activities of sulfated polysaccharides is anticoagulant activity. However, this activity is regarded as a serious side effect for anti-AIDS virus drugs. Dextran sulfate, which has both high anti-AIDS virus activity and high anticoagulant activity, was tested as an AIDS drug for humans, causing severe side effects related to the anticoagulation.[19] As shown in Table 7, the anticoagulant activity of ribopyranan sulfates was moderate to high in the range 25–47 unit/mg, being higher than that of a standard dextran sulfate (20.6 unit/mg). The high anticoagulant activity may possibly be attributed to high degrees of sulfation.

Since there is no enzyme to degrade L-glucose in the human blood, the sulfated polysaccharides having L-glucose branches are expected to retain the anti-AIDS virus activity for a long period. For linear and branched ribopyranan sulfates, the measurement of retention time in blood *in vivo* is under investigation and the results will be published elsewhere.

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