Articles

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

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ABSTRACT: Stereoregular (1→5)-α-D-ribofuranan derivatives were synthesized by selective ring-opening copolymerization in the various ratio of 1,4-anhydro-2,3-di-*O*-benzyl- and 1,4-anhydro-2,3-di-*O*-(*tert*-butyldimethylsilyl)-α-D-ribopyranoses with boron trifluoride etherate as catalyst in methylene chloride. Selective removal of the silyl groups gave (1→5)-α-D-ribofuranans which are composed of 1,5-α-D-ribofuranosidic units and benzylated 1,5-α-D-ribofuranosidic units. The partially hydroxyl polymer was reacted with D-mannose, D-glucose, and L-glucose orthoesters to give branched polymers. Those were deprotected by sodium in liquid ammonia to give branched (1→5)-α-D-ribofuranans having branches at 2- and/or 3-positions. Sulfation of the branched ribofuranans was carried out with piperidine-*N*-sulfonic acid to give branched ribofuranan sulfates. The sulfated polysaccharides completely inhibited the infection of AIDS virus to T lymphocytes in the concentration as low as 3.3 μg/mL.

Introduction

The ring-opening polymerization of 1,4-anhydro sugars takes place in a selective and stereoregular manner by choosing hydroxyl protective groups, catalysts, and the polymerization temperature.[1] In the polymerization of 1,4-anhydro sugars, there are two possible ring-opening modes of scission and two anomeric structures, so that four monomeric units, that is, 1,5-α- and 1,5-β-furanosidic units and 1,4-β- and 1,4-α-pyranosidic units, may be included in the polymer backbone. However, it has been revealed in the case of 1,4-anhydroribopyranoses that two monomeric units, that is, the 1,5-α-furanosidic and 1,4-β-pyranosidic units, are almost exclusively formed in the polymer backbone.[2]

It was previously reported that 1,4-anhydro-2,3-di-*O*-benzyl-α-D-ribopyranose (ADBR) was polymerized with boron trifluoride etherate (BF_3OEt_2) as catalyst at low temperature to give completely stereoregular (1→5)-α-D-ribofuranan derivatives with high molecular weights.[2] 1,4-Anhydro-2,3-di-*O*-(*tert*-butyldimethylsilyl)-α-D-ribopyranose (ADSR) was also polymerized in the same conditions as ADBR to form a polymer consisting of 1,5-α-D-ribofuranosidic and 1,4-β-D-ribopyranosidic units.[3] On the other hand, when acryloyl (or methacryloyl) chloride–silver hexafluorophosphate or –silver hexafluoroantimonate complex as catalyst was used, ADSR was polymerized via 1,4-linkage scission to afford a (1→4)-β-D-ribopyranan derivative.[3]

Other 1,4-anhydroribose derivatives with benzylidene, isopropylidene, and cyclohexylidene protective groups were readily polymerized with $SbCl_5$ as catalyst to give (1→4)-β-D-ribopyranan derivatives, which were the first synthetic cellulose-type polysaccharides.[2,4,5]

In the copolymerization of 1,4-anhydro-2,3-di-*O*-benzyl- (ADBX)[6] and 1,4-anhydro-2,3-di-*O*-(*tert*-butyldimethylsilyl)-α-D-xylofuranose (ADSX), polymerization with phosphorus pentafluoride as catalyst afforded a stereoregular copolymer consisting of a (1→5)-α-D-xylofuranosidic structure. The copolymer converted into D-mannosyl-branched xylofuranan sulfate having anticoagulant activity.[7]

Recently, it was found that such sulfated polysaccharides as lentinan sulfate[8] and curdlan sulfate[9] have a potent inhibitory effect against a human immunodeficiency virus (HIV, AIDS virus) infection in vitro.

Of various naturally occurring branched polysaccharides, (1→3)-β-D-glucans with 1,6-α-single glucosyl side chains such as lentinan and schizophyllan, which play an important role for the activation of the human immune system, are used as antitumor drugs.[10] In addition, natural dextrans with a small quantity of branches have been used as a plasma expander.[11] However, natural polysaccharides have too complex structures to be made clear of the relationship between the structure and biological activity. Consequently, it is very important to synthesize artificial branched polysaccharides with a definite structure and to investigate such a relationship.

In this study, we report the synthesis of branched ribofuranans by ring-opening copolymerization of ADBR and ADSR, followed by selective removal of the silyl group and then by branching with D-mannose, D-glucose, and L-glucose orthoesters. Furthermore, we wish to report that the sulfation of the branched ribofuranans with piperidine-*N*-sulfonic acid gives branched ribofuranan sulfates with high anti-AIDS virus activity in vitro.

Results and Discussion

Synthesis of Branched (1→5)-α-D-Ribofuranans. Synthetic routes to branched and linear ribofuranan sulfates were demonstrated in Scheme I, in which various molar ratios of ADBR and ADSR were copolymerized with boron trifluoride etherate as catalyst at low temperature, giving stereoregular 1,5-α-D-furanosidic copolymers. Of all cationic catlaysts examined, boron trifluoride etherate was the best catalyst to form stereoregular 1,5-α-linked

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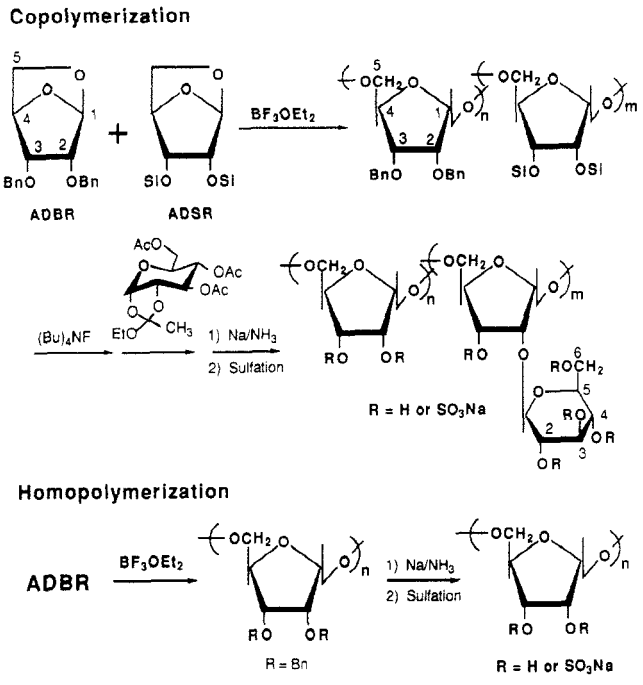
Scheme I
Synthesis of Sulfated Branched and Linear Ribofuranans



copolysaccharides. Desilylation, and subsequent branching with the orthoacetates, and then deprotection afforded OH-free branched ribofuranans. The polysaccharides were sulfated with piperidine-*N*-sulfonic acid in DMSO to give branched ribofuranan sulfates. A Linear ribofuranan sulfate was also synthesized by homopolymerization of ADBR as described in the previous paper.[2] The reason why ADSR monomer was used for the copolymerization is that the *tert*-butyldimethylsilyl group is a good protective group that can be removed selectively and readily from the copolymer backbone without causing debenzylation.

The result of copolymerization with boron trifluoride etherate as catalyst is summarized in Table I. Homopolymerization of ADBR (no. 1) gave a complete stereoregular 2,3-di-*O*-benzyl-(1→5)-α-D-ribofuranan in 79.6% yield with a high molecular weight (40.9×10^4) and a high positive specific rotation of +143.2°. Homopolymerization of ADSR (no. 10) under the same conditions afforded a polymer with a mixed structure of 1,5-α-D-furanosidic and 1,4-β-D-pyranosidic units in the ratio of 94 and 6 mol %, respectively. The stereoregularity of the polymers was determined from C1 absorptions in the ^{13}C NMR spectrum.

Copolymerization of ADBR and ADSR was examined in the various monomer feeds at −40 °C to give the corresponding copolymers in high yields. Up to 67 mol % of ADSR in the feed (no. 7), the copolymer had a completely stereoregular 1,5-α-furanosidic structure. The proportion of ADSR units in the copolymer was slightly larger than the molar ratio of monomer in the feed. Higher positive specific rotations were observed for the copolymer containing high molar ratios of the ADBR unit. The specific rotation decreased with increasing molar ratio of the ADSR unit in the copolymer to reach +103.7° of ADSR homopolymer, as shown in no. 10. The number average molecular weight of the copolymers was high ranging from 11×10^4 to 36.4×10^4.

In Figure 1, the 67.8-MHz ^{13}C NMR spectra of (A) poly(ADBR), (B) poly(ADSR), (C) copoly(ADBR–ADSR) (66:34 mol % in feeds), and (D) copoly(ADBR–ADSR) (19:81 mol %) are exhibited. The C1 carbon absorption of poly(ADBR) in Figure 1A appeared as a single peak at 103.0 ppm due to the 1,5-α-furanosidic unit. On the other hand, in Figure 1B, the C1 carbon absorption appeared as two peaks at 104.0 and 108.4 ppm, which are ascribed to the C1 absorptions of the 1,5-α-furanosidic and 1,4-β-pyranosidic units, respectively. In Figures 1C,D, there is no peak around 108 ppm due to the 1,4-β-pyranosidic unit, indicating that the copolymers have a completely 1,5-α-furanosidic structure. The stereoregular structure consisting exclusively of 1,5-α-D-ribofuranosidic units can be attained by an oxonium ion mechanism, which was proposed in the synthesis of linear (1→5)-α-D-ribofuranan[2] and (1→5)-α-D-xylofuranan.[6]

Effects of temperature on ring-opening copolymerization of ADBR and ADSR was examined using boron trifluoride etherate as catalyst. The result is summarized in Table II. The copolymerization at −40 °C (nos. 3, 6, and 8) afforded copolymers with complete 1,5-α-stereoregularity ($[\alpha]^{25}$ +138.8, +122.5, and +120.5°, respectively). As polymerization temperature increased, both α-stereoregularity and ADSR units decreased in the copolymer, indicating that a low polymerization temperature around −40 °C was suitable for obtaining a stereoregular copolymer. Previously, we reported that in the copolymerization of 1,4-anhydro-2,3-*O*-benzylidene-α-D-ribopyranose with other 1,4-anhydro-α-D-ribopyranose monomers, higher polymerization temperatures work effectively to afford copolymers with 1,4-β-stereoregularity.[12,13]

The *tert*-butyldimethylsilyl group was removed from the three copolymers containing 45, 54, and 66 mol % ADBR units by use of tetrabutylammonium fluoride to give partially benzylated ribofuranans having hydroxyl groups in good yields. The carbon peaks due to the *tert*-butyldimethylsilyl group in the ^{13}C NMR spectrum disappeared completely after the desilylation, indicating that the silyl group was thoroughly removed from the polymer.

The partially benzylated ribofuranans were glycosylated by such sugar orthoesters as D-mannose, D-glucose, and L-glucose orthoesters at the free hydroxyl groups to form branched ribofuranans. The result of glycosylation is shown in Table III. The glycosylation of partially benzylated ribofuranan (66 mol % ADBR unit in polymer backbone) with 3,4,6-tri-*O*-acetyl-β-D-mannose-1,2-(methylorthoacetate) was carried out with 2,6-lutidinium perchloride as catalyst in refluxing benzene to give a ribofuranan derivative bearing 13 mol % D-mannose branching (no. 1 in Table III). The degree of branching, which was calculated from the C1 absorption in the ^{13}C NMR spectrum, increased with increasing molar ratio of catalyst and with decreasing amount of solvent. As shown in nos. 2 and 3 of Table III, the branching reaction readily occurred to form 30 and 37 mol % L- and D-glucose branches, respectively. The number average molecular weight of the branched ribofuranans ranged from 2.3×10^4 to 5.8×10^4. In comparison with the 67.8-MHz ^{13}C NMR spectra of the copolymer (Figure 2C) (54 mol % ADBR unit in the main chain), partially benzylated ribofuranan (Figure 2B), and L-glucose-branched (30 %) ribofuranan derivative (Figure 2A), the C1 and C6 peaks of the L-glucose branches appeared as single and broad absorptions at 97.8 and 62–64 ppm, respectively (Figure 2A). Other ^{13}C absorptions due to the branches were overlapped with those due to the ribofuranan residue. There are two possible branching points on the ribofuranosidic residue, i.e., the 2- and 3-hydroxyl groups. However, it was impossible to determine the individual ratio of branches at the 2- and 3-positions from the NMR measurement. The debenzylation of linear

Table I
Ring-Opening Copolymerization of 1,4-Anhydro-2,3-di-*O*-benzyl- (ADBR) and 1,4-Anhydro-2,3-di-*O*-(*tert*-butyldimethylsilyl)-α-D-ribopyranoses (ADSR)[a]

no.	amt of ADBR feed, g	amt of ADSR feed, g	amt of ADSR feed, mol fraction	amt of catalyst, mol %	temp, °C	time, min	yield, %	$[\alpha]^{25}_D$,[b] deg	$\bar{M}_n$[c]	ADSR unit in polymer,[d] mol fraction	1,5-α-furanose unit,[e] %
1	0.50	0	0	2.0	−40	30	79.6	+143.2	40.9	0	100
2	0.45	0.05	0.09	3.0	−40	60	72.0	+142.2	12.0	0.17	100
3	0.40	0.10	0.18	3.0	−40	60	72.0	+141.5	20.0	0.29	100
4	0.35	0.15	0.27	3.0	−40	30	76.7	+138.8	11.0	0.34	100
5	0.30	0.20	0.38	3.0	−40	15	73.4	+138.6	36.4	0.46	100
6	0.25	0.25	0.47	3.0	−40	30	86.9	+122.5	20.5	0.61	100
7	0.15	0.35	0.67	3.0	−40	60	78.5	+120.5	24.0	0.81	100
8	0.10	0.40	0.77	3.0	−40	60	85.1	+113.3	15.8	0.78	95
9	0.05	0.45	0.89	3.0	−40	60	86.9	+113.4	14.1	0.85	94
10	0	0.50	1.00	4.0	−60	60	80.4	+103.7	24.7	1.00	94

[a] Conditions: monomer concentration, 40–50% w/v; solvent, CH_2Cl_2; catalyst, BF_3OEt_2. [b] Measured in $CHCl_3$ (c, 1%). [c] Determined by GPC. [d] Determined by ^1H NMR spectrum. [e] Calculated from ^{13}C NMR spectrum.

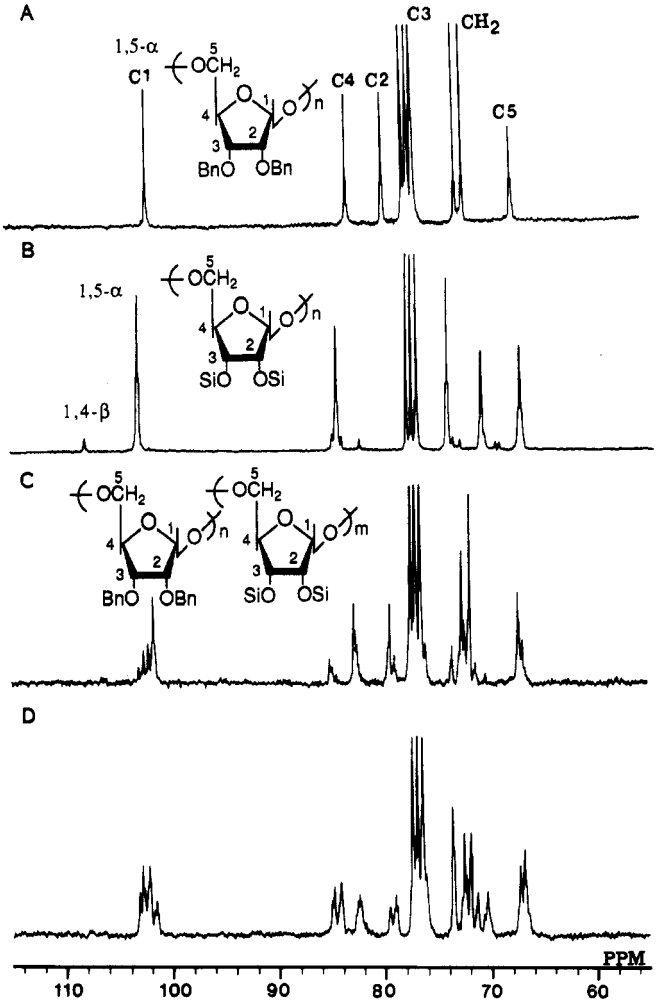


Figure 1. 67.8-MHz ^{13}C NMR spectra of (A) benzylated ribofuranan (poly(ADBR)), (B) silylated ribofuranan (poly(ADSR)), (C) copoly(ADBR–ADSR) (66 mol % ADBR unit in polymer), and (D) copoly(ADBR–ADSR) (19 mol % ADBR unit in polymer) ($CDCl_3$ as solvent).

and branched ribofuranans was carried out with sodium in liquid ammonia at −78 °C to give free linear and branched ribofuranans in high yields, respectively, as shown in Table IV. The OH-free polymers were soluble in water and dimethyl sulfoxide. The specific rotation of free linear and branched ribofuranans was a high positive value except for L-glucose-branched ribofuranan (+35.1°).

Figure 3 shows ^{13}C NMR spectra of (A) D-glucose-branched, (B) L-glucose-branched, (C) D-mannose-branched, and (D) linear ribofuranans. In Figure 3D, five sharp peaks including a single C1 absorption at 103 ppm suggest that the polymer has a completely stereoregular 1,5-α-furanosidic structure. The assignment of peaks was determined by measuring on H–H and C–H COSY spectra. The C1 peak due to the 1,4-β-D-ribopyranosidic unit appeared around 108 ppm. On the other hand, the clear appearance of the C6 peak due to the branches at 63.0 ppm in A, 63.0 ppm in B, and 63.8 ppm in C confirmed that the scission of branches did not take place during the debenzylation.

Branched polysaccharides have unique biological activities. It was reported that synthetic branched dextrans, i.e., 1,6-α-glucans with 1,3-α-mannosidic branches, precipitated antibodies raised in rabbits by injecting N4 dextran–concanavalin A conjugate.[14] Therefore the establishment of a synthetic route on branched polysaccharides is important to contribute a supply of artificial materials for clinical purposes.

Sulfation of Branched Ribofuranans into Anti-HIV Compounds. The linear and branched ribofuranans were sulfated with piperidine-*N*-sulfonic acid in dimethyl sulfoxide to give ribofuranan sodium sulfates. The results are summarized in Table V. Sulfur content (%) of the sulfated ribofuranans was 17.6% for linear, 16.1% for D-mannose-branched, 16.3% for L-glucose-branched, and 16.8% for D-glucose-branched ribofuranans. The number average molecular weight of the sulfates ranged from 0.7×10^4 to 1.7×10^4. The specific rotation showed high positive values except for L-glucose-branched ribofuranan sulfate which had a small positive one.

Figure 4 shows the 67.8-MHz ^{13}C NMR spectra of branched and linear ribofuranan sulfates. It was revealed that the spectra suggested high degrees of sulfation, because each absorption appeared as a sharp absorption and shifted to lower or upper field compared with that of the corresponding ribofuranans before sulfation. The number of sulfate groups per glucose unit was determined to be 1.7–1.9 by the elemental analysis (shown in Table V).

In 1987, it was revealed that sea alga and other natural sulfated polysaccharides inhibited the infection of retrovirus in vitro.[15] Synthetic linear dextran, xylofuranan, and ribofuranan also showed considerably high anti-AIDS virus activity in vitro.[16] Recently, it was reported that curdlan was successfully sulfated to give highly active curdlan sulfate which inhibited the infection of AIDS virus in the concentration as low as 3.3 μg/ml.[8,17] Thus, the effects of branched and linear ribofuranans against AIDS virus infection were evaluated, and the result is summarized, as well as the anticoagulant activity, in Table VI and Figure 5.

Table II
Effect of Temperature on Ring-Opening Copolymerization of 1,4-Anhydro-2,3-di-*O*-benzyl- (ADBR) and 1,4-Anhydro-2,3-di-*O*-(*tert*-butyldimethylsilyl)-α-D-ribopyranoses (ADSR)[a]

no.	amt of ADBR feed, g	amt of ADSR feed, g	amt of ADSR feed, mol fraction	temp, °C	time, min	yield, %	$[\alpha]^{25}_D$,[b] deg	$\bar{M}_n$[c]	ADSR unit in polymer,[d] mol fraction	1,5-α-furanose unit,[e] %
1	0.35	0.15	0.27	0	60	77.2	+135.1	6.9	0.25	96
2				−20	60	77.5	+134.8	6.8	0.25	98
3				−40	30	76.7	+138.8	11.0	0.34	100
4	0.25	0.25	0.47	0	60	83.3	+124.0	5.9	0.61	97
5				−20	60	81.8	+124.7	8.1	0.40	97
6				−40	30	86.9	+122.5	20.5	0.61	100
7	0.15	0.35	0.67	0	60	70.2	+112.5	4.3	0.62	92
8				−40	60	78.5	+120.5	24.0	0.81	100

[a] Conditions: monomer concentration, 40–50% w/v; solvent, CH_2Cl_2; catalyst, BF_3OEt_2, 2.0–2.5 mol %. [b] Measured in $CHCl_3$ (c, 1%). [c] Determined by GPC. [d] Determined by ^1H NMR spectrum. [e] Calculated from ^{13}C NMR spectrum.

Table III
Glycosylation of Partially Benzylated Ribofuran[a]

amt of ribofuranan, g	amt of orthoacetate, g	amt of catalyst, mg	amt of solvent, mL	time, h	yield, g	$[\alpha]^{25}_D$,[f] deg	$\bar{M}_n$[g]	degree of branching,[h] %
0.38[b]	D-mannose (2.0)	3	80	0.5	0.43	+117.7	5.8	13
0.50[c]	L-glucose (2.0)	15	10	5	0.95	+35.1	5.3	29
0.16[d]	D-glucose (1.5)	15	10	5	0.26	+137.7	2.3	37
0.25[d]	D-glucose (3.0)	20	10	5	0.51	+145.9	2.5	30
0.41[e]	D-glucose (2.5)	20	10	5	0.81	+138.2	3.5	23

[a] Reaction condition: solvent, benzene; temperature, reflux; catalyst, 2,6-lutidinium perchlorate. [b] 66 mol % of ADBR unit in copolymer, $[\alpha]^{25}_D = +138.1°$, $\bar{M}_n = 5.4 \times 10^4$. [c] 54 mol % of ADBR unit in polymer, $[\alpha]^{25}_D = +142.8°$, $\bar{M}_n = 5.6 \times 10^4$. [d] 45 mol % of ADBR unit in polymer, $[\alpha]^{25}_D = +135.7°$. [e] 50 mol % of ADBR unit in polymer, $[\alpha]^{25}_D = +135.1°$, $\bar{M}_n = 3.8 \times 10^4$. [f] Measured in $CHCl_3$ (c, 1%). [g] Determined by GPC. [h] Determined by ^{13}C NMR spectrum.

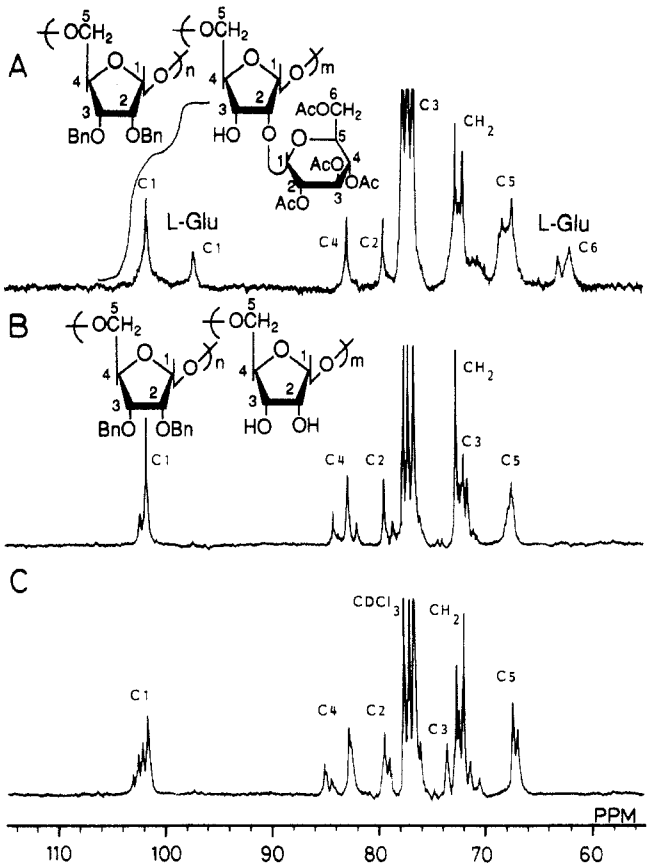


Figure 2. 67.8-MHz ^{13}C NMR spectra of (A) L-glucose-branched (29%) ribofuranan derivative, (B) partially benzylated ribofuranan, and (C) copoly(ADBR–ADSR) (54 mol % ADBR unit in polymer) ($CDCl_3$ as solvent).

The anti-AIDS virus activity of linear (RFS0) and D-mannose-branched ribofuranan sulfates (RMFS1) was examined by the indirect immunofluorescence method[16] and of D-glucose- (RDFS1) and L-glucose-branched ribofuranan sulfates (RLFS1) by the MTT method,[18] respectively. Figure 5 exhibits the effects of linear (A) and D-mannose-branched ribofuranan sulfates (B) on cell growth and HIV-induced cytophagic effects and on the percentage of immunofluorescence (IF) positive cells. When less than 1 μg/mL of the sulfates was used, MT-4 cells were infected and then killed by HIV after a 6-day culture. However, these ribofuranan sulfates completely protected from the infection of AIDS virus in the concentration of 3.3 μg/mL, because there were no IF-positive cells, i.e., no AIDS virus-induced cells. The cytotoxicity of the sulfates was relatively low, because the number of viable MT-4 cells in the coculture was large enough in the cell culture experiment in the presence of a high concentration of the sulfates (1000–5000 μg/mL). According to the another bioassay method (MTT method), i.e., the calculation of EC_{50}, L- and D-glucose-branched ribofuranan sulfates protected 50% MT-4 cells from the infection of AIDS virus in very low concentrations of 0.27 and 2.37 μg/mL, respectively. Under the same conditions, curdlan (a natural 1,3-β-D-glucan) sulfate as reference showed an EC_{50} of 0.43 μg/mL, which is comparable to 3.3 μg/mL of complete-inhibition concentration as described above. Consequently, it was revealed that these ribofuranan sulfates have a very strong anti-HIV activity in vitro.

It is known that sulfated polysaccharides also have anticoagulant activity. A natural anticoagulant polysaccharide, heparin, is a polysaccharide containing sulfate and sulfamide groups, and the active site for blood anticoagulation is a pentasaccharide portion in the polymer backbone. However, for an anti-AIDS virus active compound low anticoagulant activity must be necessary. The blood anticoagulant activity in vitro of the synthetic sulfated ribofuranans was assayed by use of bovine plasma according to a modification of the United States Pharmacopoeia.[19] In Table V, these sulfated ribofuranans showed higher anticoagulant activity (24–56 unit/mg) than that of a commercial dextran sulfate (Meito Sangyo Co., NC-1020, 19.3 unit/mg). The anticoagulant activity of the sulfated ribofuranans was almost the same value as

Table IV
Deprotection of Benzylated Polymer by Sodium in Liquid NH_3

polymer			free polysaccharide				
	$[\alpha]^{25}_D$, deg	$\bar{M}_n$	yield, %	$[\alpha]^{25}_D$,[a] %	$\bar{M}_n$[b]		stereoregularity, %
ribofuranan	+143.2	40.9	88	+142.2	15.7	1,5-α-F	>98
D-mannose-branched (13%) ribofuranan	+117.7	5.8	90	+125.8	1.8	1,5-α-F	>98
L-glucose-branched (29%) ribofuranan	+35.1	5.1	95	+46.8	1.3	1,5-α-F	>95
D-glucose-branched (23%) ribofuranan	+138.2	3.5	95	+141.3	1.3	1,5-α-F	>95

[a] Measured in H_2O or DMSO (c, 1%). [b] Determined by GPC.

Table V
Sulfation of Ribofurananns

	amt of polymer, g	amt of DMSO, mL	amt of PSA,[a] g	temp, °C	time, min	yield, g	$[\alpha]^{25}_D$,[b] deg	$\bar{M}_n$[c]	elemental analysis, % C	H	S	DS[d]
linear ribofuranan	0.16	20	1.86	75	60	0.17	+83.0	1.7	17.15	2.90	17.6	1.9
D-mannose-branched (13%) ribofuranan	0.20	25	2.20	75	60	0.30	+78.4	1.2	17.44	2.82	16.1	1.7
L-glucose-branched (29%) ribofuranan	0.15	15	0.53	85	60	0.10	+11.2	0.7	20.02	3.05	16.3	1.5
D-glucose-branched (23%) ribofuranan	0.20	25	2.20	85	60	0.25	+71.6	1.0	19.56	3.01	16.8	1.6

[a] Piperidine-*N*-sulfonic acid. [b] Measured in H_2O (c, 1%). [c] Determined by GPC. [d] The number of sulfate groups per sugar unit in ribofuranan sulfate.

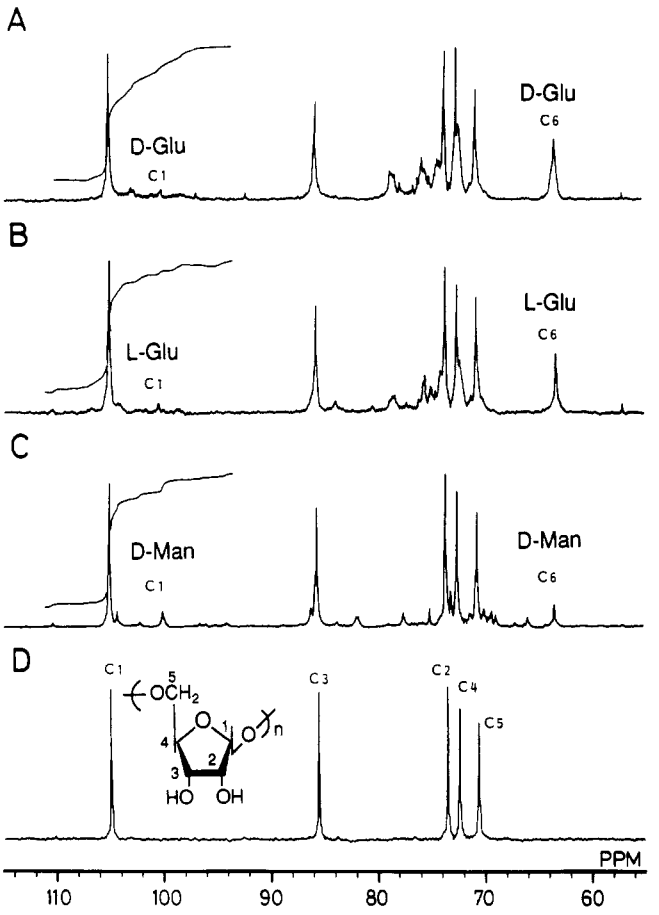


Figure 3. 67.8-MHz ^{13}C NMR spectra of (A) D-glucose-, (B) L-glucose-, and (C) D-mannose-branched and (D) linear ribofuranans (D_2O as solvent, 37 °C).

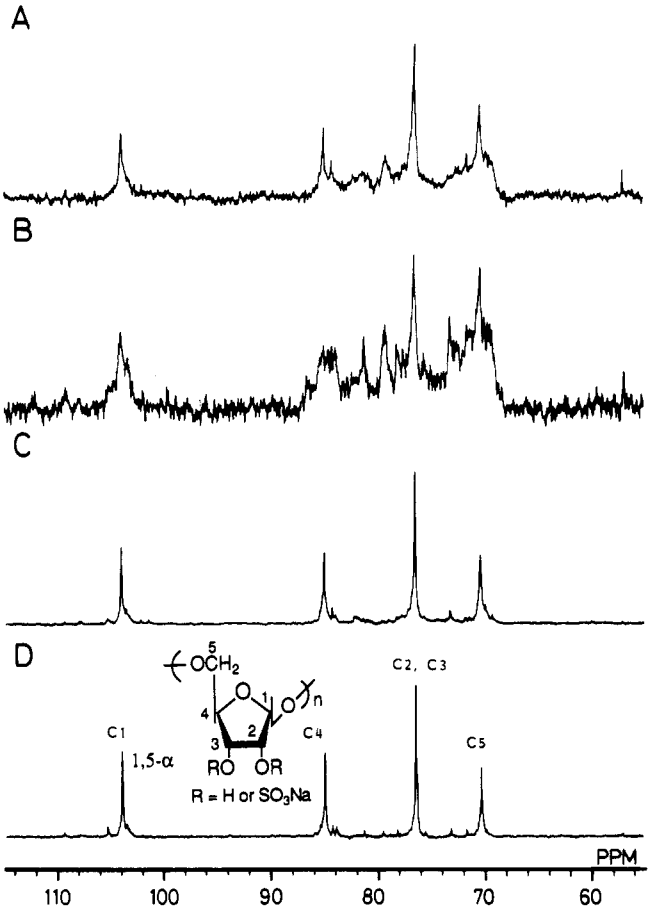


Figure 4. 67.8-MHz ^{13}C NMR spectra of sulfated (A) D-glucose-, (B) L-glucose-, and (C) D-mannose-branched and (D) linear ribofuranans (D_2O as solvent, 37 °C).

previous reported.[20] However, it was reported that curdlan sulfate had low anticoagulant activity in spite of very high anti-AIDS virus activity.[8] It is assumed that although such furanan-type polysaccharides as ribofuranan and xylofuranan sulfates have anti-AIDS virus activity, they intrinsically have as well high anticoagulant activity because of their flexible polymer backbones.

Experimental Section

Measurement. ^1H NMR (270 MHz) and ^{13}C NMR (67.8 MHz) spectra were recorded on a JEOL JMN GX-270 spectrometer. Specific rotations were measured for a solution in $CHCl_3$ or in H_2O by means of a Perkin-Elmer 241 polarimeter using a 1-mL cell. Molecular weights were determined by organic phase GPC (column, Toyo Soda TSK-gel, G2000H, G3000H, G4000, G5000H,

Table VI
Anti-AIDS Virus Activity of Sulfated Ribofuranans

sulfated ribofuranan[a] (branch)	S content, %	$10^{-4}\bar{M}_n$[b]	EC_{50},[c] μg/mL	CC_{50},[d] μg/mL	SI[e]	AA,[f] unit/mg
RFS0	17.6	1.7	3.3[g]			56
RMFS1 (D-mannose, 13%)	16.1	1.2	3.3[g]			55
RDFS1 (D-glucose, 23%)	16.8	1.1	0.27	783	2900	
RLFS1 (L-glucose, 29%)	16.3	0.7	2.37	>1000	>422	24
CS[h]	14.1	7.9	0.43	>1000	>2330	
AZT (μM)			0.0019	6.43	3400	

[a] Key: RFS0, sulfated linear ribofuranan; RMFS1, sulfated ribofuranan with D-mannose branches; RDFS1, sulfated ribofuranan with D-glucose branches; RLFS1, sulfated ribofuranan with L-glucose branches. [b] Determined by GPC. [c] 50% effective concentration. [d] 50% cytotoxic concentration. [e] Selectivity index, CC_{50}/EC_{50}. [f] Anticoagulant activity, dextran sulfate NC-1020, 19.3 unit/mg. [g] Minimum effective concentration. [h] Standard curdlan sulfate.

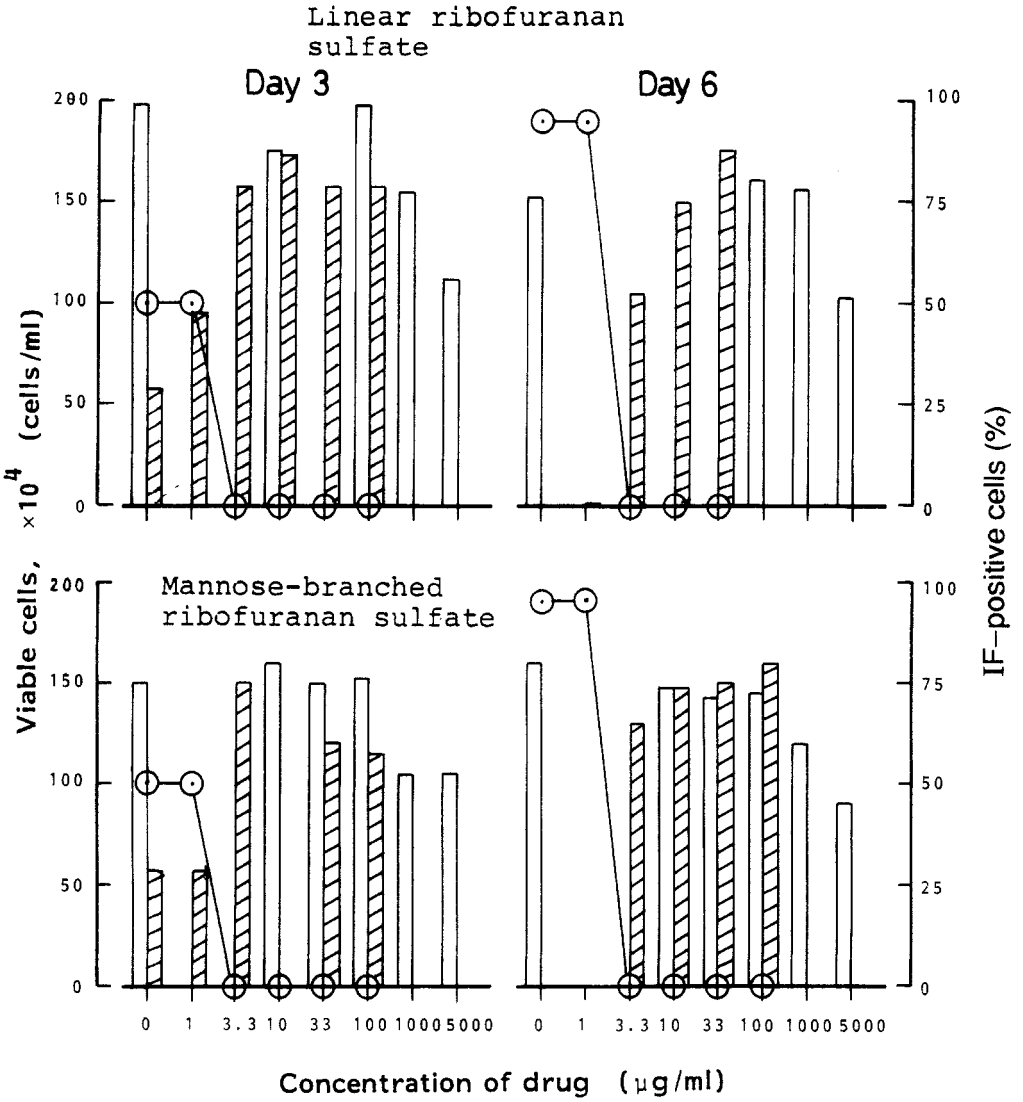


Figure 5. Inhibitory effects of sulfated ribofuranans on cell growth and HIV-induced cytophagic effects (CPE): (A, top) sulfated linear and (B, bottom) sulfated D-mannose-branched ribofuranans. Key: open bars, MT-4 cells; slash bars; MT-4 cells and HIV-infected cells (0.2%); open circles, percentage of IF-positive cells determined by counting the IF-positive cells per approximately 500 cells after 3 and 6 days of infection.

7.6 mm × 600 mm × 4 mm; eluent, THF) using polystyrene standards and by aqueous phase GPC (column, Toyo Soda 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-2,3-di-*O*-benzyl-α-D-ribopyranose (ADBR) and 1,4-anhydro-2,3-di-*O*-(*tert*-butyldimethylsilyl)-α-D-ribopyranose (ADSR) were prepared by the protection of 1,4-anhydro-α-D-ribopyranose which was synthesized by pyrolysis of D-ribose with benzyl chloride and *tert*-butyldimethylsilyl chloride, respectively.[2,3]

Copolymerization. The typical procedure for copolymerization is as follows: A high-vacuum technique was used. A mixture of ADBR (0.30 g) and ADSR (0.20 g) was dired in a polymerization ampule under vacuum pressure (~10^{-5} mmHg) overnight and then methylene chloride (1 mL) was transferred into the polymerization ampule through a glass breakable seal under the same vacuum pressure. After the monomer mixture was frozen with liquid nitrogen, boron trifluoride etherate (2 mol % to the feed) as a catalyst was transferred into the mixture and then the copolymerization was started at −40 °C. After 1 h, the copolymerization was terminated by addition of methanol (~20 mL) to give a copolymer as a white precipitate. The copolymer was dissoved in chloroform, purified by reprecipitation using chloroform–methanol three times, and isolated by freeze-drying from benzene to give a white powder. The results were shown in Tables I and II.

Desilylation. To a tetrahydrofuran (THF) solution of the copolymer was added a 1 M tetra-*n*-butylammonium fluoride solution in THF followed by refluxing for 1 h with stirring. After evaporation of THF, a polymer was dissolved in chloroform, purified by reprecipitation using chloroform–methanol three times, and then freeze-dried from benzene.

Glycosylation. A partially benzylated (1→5)-α-D-ribofuranan and 3,4,6-tri-*O*-acetyl-α-D-glucose-(1,2-ethyl orthoacetate) were dissolved in benzene. A small amount of water in the solution was distilled as the benzene azeotrope at atmospheric pressure, and then a catalytic amount of 2,6-lutidinium perchlorate was added. The mixture was refluxed for 1 h. After evaporation of benzene, a product was dissolved in chloroform, purified by reprecipitation using chloroform–methanol three times and then freeze-dried from benzene.

Deprotection of Branched Ribofuranan. The branched ribofuranan (0.5 g) was dissolved in 15 mL of 1,2-dimethoxyethane, and the solution was added to a sodium (0.5 g)–liquid ammonia solution (50 mL) at −78 °C. After 60 min, ammonium chloride was added 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 solution was washed with methylene chloride and dialyzed against deionized water for 1 day. It was freeze-dried from water.

Sulfation. Piperidine-*N*-sulfonic acid (2.20 g, 0.012 mol) was added to a dimethyl sulfoxide solution (40 mL) of ribofuranan (0.20 g). The temperature of the mixture was gradually elevated to 85 °C for 5 min, and then the mixture was stirred for additional 1 h. After cooling by use of an ice bath, the reaction mixture was neutralized by saturated $NaHCO_3$ solution and then acetone was added until a precipitate appeared. The precipitate was collected by centrifugation, washed with acetone three times, and redissolved into water, and the solution was dialyzed against deionized water for a day. The dialyzate was freeze-dried to give ribofuranan sodium sulfate (abbreviated as ribofuranan sulfate).

Anti-HIV Assay. HIV-infected and uninfected MT-4 cells (3×10^5 cells/mL), a human T4-positive cell line carrying HTLV-1 (human T-lymphotropic virus type I), were cultured with the various concentrations of ribofuranan sulfates for 3 and 6 days. Anti-HIV activity of the various concentrations of test compounds was assayed after 6 days of HIV infection by measuring the decrease in the number of viable cells and by the percentage of immunofluorescence (IF) positive cells as described in a previous paper.[16] The HIV-1 strain was prepared by the supernatant of MOLT-4/$HIV_{HTLV-IIIB}$ cells. The MTT method[18] was also used for the anti-HIV assay, in which the viability of both HIV- and mock-infected cells was assayed spectrophotometrically via the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial dehydrogenases of metabolically active cells to a blue formazan product. MT-4 cells were infected with HIV at the multiplicity (MOI) of 0.1, and HIV-infected and mock-infected MT-4 cells were incubated in the presence of various concentrations of ribofuranan sulfates for 5 days at 37 °C in a CO_2 incubator. The dose achieving 50% protection of MT-4 cells from HIV was defined as the 50% effective dose (EC_{50}), and CC_{50} means the 50% cytotoxic dose of drugs.

Anticoagulant Activity. Anticoagulant activity in vitro was performed by use of bovine plasma according to a modification of the United States Pharmacopoeia.[19]

References and Notes

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