Ribofuranose-Containing Polymers: Synthesis and Catalytic Activity for the Hydrolysis of Phosphodiester and the Cleavage of DNA

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ABSTRACT: Two derivatives of 5-deoxy-D-*erythro*-pent-4-enofuranose (**4** and **6**) and 6-deoxy-α-D-*galacto*-hex-5-enopyranose (**9**) were synthesized. Copolymerization of these monomers with maleic anhydride and subsequent reactions resulted in poly[(5-deoxy-D-*erythro*-pent-4-enofuranose)-*alt*-(maleic acid)] (**11**) and its derivatives, such as dimethyl ester (**12**), 2,3-*O*-isopropylidene (**13**), 1-*O*-methyl (**15**), and poly[(6-deoxy-α-D-*galacto*-hex-5-enopyranose)-*alt*-(maleic acid)] (**17**). Polymers **11**, **12**, and **15** showed catalytic activity for the hydrolysis of phosphodiesters. The rate constant (k_{cat}) for the hydrolysis of ethyl *p*-nitrophenyl phosphate substrate catalyzed by polymer **12** was 9.0×10^{-1} h^{-1} at 50 °C, corresponding to a rate acceleration of about 10^3 as compared with that of the uncatalyzed reaction (9.1×10^{-4} h^{-1}). Competitive and noncompetitive inhibitions for this catalysis were observed on addition of sodium acetate ($K_I = 5.9 \times 10^{-4}$ M) and K_2HPO_4 ($K_I = 2.5 \times 10^{-4}$ M), respectively. The polymers also catalyzed the cleavage of ssDNA of 30 bases and tetradeoxyadenylic acid in Tris buffer (pH 7.4) at ionic strength 0.02 (KCl) at 37 °C. The catalytic activity of the polymers seemed to be attributable to *vic*-*cis*-diol groups of furanose rings on the polymer backbones.

During the past 3 decades there has been great interest in the synthesis of enzyme-like polymers.[1,2] To mimic enzymes, the synthetic polymers should be water-soluble, form active sites, and have catalytic activity. Some synthetic polymers fulfilled such requirements.[3−7]

While investigating polynucleotide analogues,[8−12] we found that the synthetic ribofuranose-containing polymers showed catalytic activity for the hydrolysis of phosphodiesters and the cleavage of oligodeoxyribonucleotides. In order to elucidate the structure of the active center for the synthetic polymer catalyst, five alternating copolymers containing furanose or pyranose rings were synthesized, as shown in Scheme 2. We report here the synthesis and characterization of the relevant monomers and polymers and their catalytic activity. This is the first example that synthetic polymers showed catalysis for the cleavage of DNAs and hydrolysis of phosphodiester. We reported part of the results in another paper.[13]

Results and Discussion

Monomer Synthesis. Vinyl monomers **4** and **6** containing ribofuranose rings were prepared from D-ribose (**1**) as depicted in Scheme 1. The 2,3-dihydroxyl group of D-ribose was blocked by acetonization under acidic conditions[14] and then the 5-hydroxyl group was converted to an iodo group with iodine and triphenylphosphine. After acetylation of the 1-hydroxyl group, hydrogen iodide was eliminated with the aid of AgF to give monomer **4**. In the synthesis of monomer **6**, the 1-hydroxyl group of D-ribose was first methylated and the 2,3-dihydroxyl group was blocked by acetonization. Tosylation of the 5-hydroxyl group[15] and subsequent elimination with potassium *tert*-butoxide resulted in monomer **6**. Vinyl monomer **9** containing a pyranose ring was obtained from compound **7** by tosylation of the 6-hydroxyl group and subsequent elimination with potassium *tert*-butoxide.

Copolymerization. Radical copolymerization of vinyl ethers with maleic anhydride is known to give alternating copolymers by forming charge-transfer complexes of the monomer pairs during copolymerization.[16,17] As the electron-donating character of the vinyl ether groups of monomers **4**, **6**, and **9** is little influenced by either 1-*O*-acetyl, 1-*O*-methyl, or 2,3-*O*-isopropylidene groups on furanose rings or 1,2:3,4-di-*O*-isopropylidene groups on pyranose rings, the copolymers of the monomers and maleic anhydride were expected to have alternating sequences.

The copolymerizations of maleic anhydride with **4**, **6**, and **9** were carried out in bulk in the presence of 2,2′-azobis(isobutyronitrile) (AIBN) to give polymers **10**, **14**, and **16**, respectively (Scheme 2). The copolymerization data are given in Table 1. Neither the monomers **4**, **6**, and **9** nor maleic anhydride was homopolymerized under the same conditions.

^{1}H-NMR spectra of monomer **4** and polymer **10** are given in Figure 1. After polymerization, the proton signals of H_{5a} and H_{5b} of monomer **4** at $\delta = 4.45$ and 4.68 were shifted to $\delta = 1.15-1.75$ and the signals of sugar and succinic anhydride moieties appeared at $\delta = 1.0-2.3$, 2.7, and 3.5. As expected for the alternating structure, the equimolar amounts of comonomers (succinic anhydride moieties: 51 ± 1 mol %) were confirmed by titrations of anhydride groups[18] incorporated into the polymers **10**, **14**, and **16**.

Polymers **11** was obtained by hydrolysis of polymer **10** in dioxane−aqueous HCl solutions, while polymers **14** and **16** were hydrolyzed in aqueous formic acid to give polymers **15** and **17**, respectively. The methoxy groups of **14** were intact under the reaction conditions. Hydrolysis of **10** under basic conditions (0.1 N NaOH) resulted in **13**. The completion of the reactions was monitored by following the disappearance of the isopropylidene, acetyl, or methyl groups with a ^{1}H-NMR spectrometer. After hydrolysis, the carbonyl IR peaks at near 1810 cm^{-1} of anhydride groups of the polymers **10**, **14**, and **16** were shifted to near 1750 cm^{-1}, corresponding to carboxylate groups of polymers **11**, **13**, **15**,

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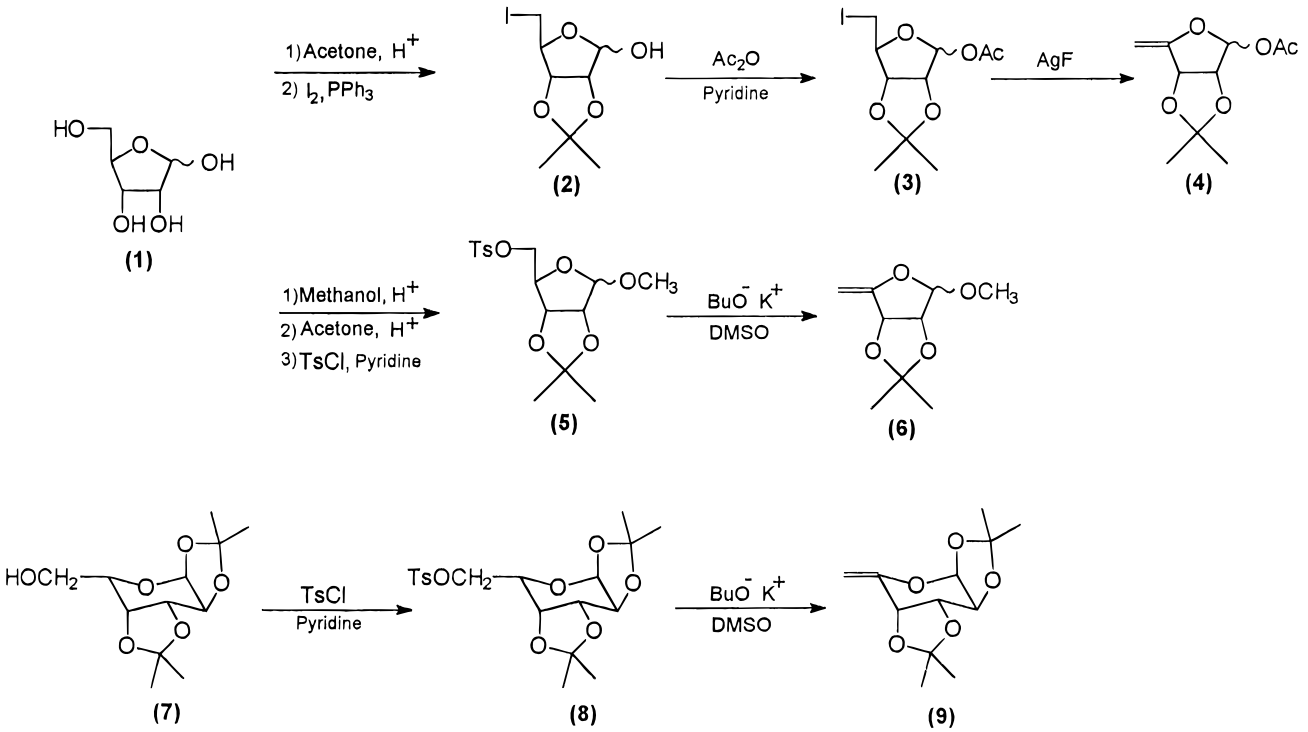
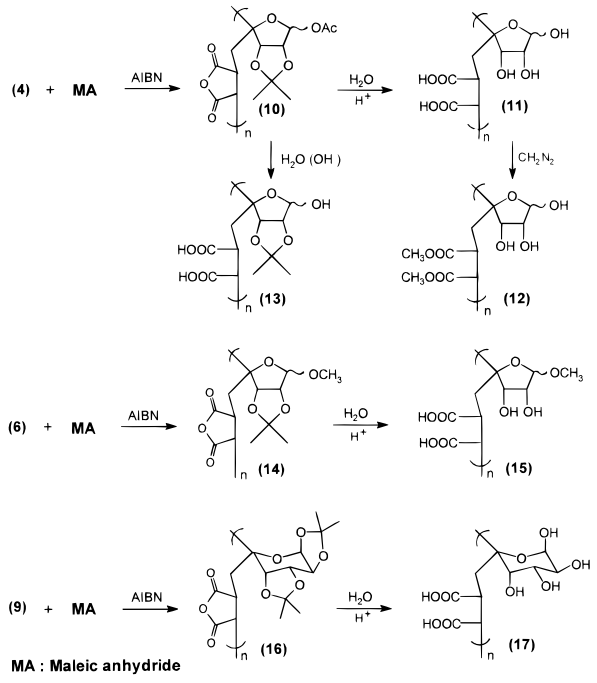
Scheme 1. Monomer Synthesis



Scheme 2. Copolymerization



and **17**. Methylation of **11** with diazomethane gave polymer **12**.

The *vic*-OH pairs at C2 and C3 on the furanose rings of polymers **11**, **12**, and **15** were in the *cis*-configuration, while the hydroxyl groups at C1, C2, C3, and C4 on the pyranose rings of polymer **17** stayed in either *aeea* or *eaae*. Approximately 30% of the hydroxyl groups at C1 of furanose of polymers **11** and **12** stayed in the *cis*-configuration with respect to C2−OH groups on the same furanose rings, which was determined by ^{1}H-NMR spectroscopy analysis of the model reaction products.[19]

Polymers **10**, **14**, and **16** were soluble in polar organic solvents such as ethyl acetate, chloroform, acetone, DMF, and DMSO, whereas they were insoluble in nonpolar solvents, such as diethyl ether and hexane. Polymers **11**−**13**, **15**, and **17** were soluble in water. The molecular weights of the polymers were measured by gel permeation chromatography (Table 1).

Conformation of the Polymer. As polymers **11**, **13**, **15**, and **17** contained carboxylate anions, their conformations were expected to depend strongly on the counterions and pH. CD spectra of polymer **11** measured in tris(hydroxymethyl)aminomethane and its HCl salt (Tris) buffer (pH = 7.4) in the presence of various cations (ionic strength = 0.02) at 50 °C are shown in Figure 2. Polymer **11** showed quite different CD curves depending on the counterions; they showed troughs at 208 nm ($\theta = -6500$) for K^+, at 205 nm ($\theta = -4300$) for Li^+, and at 204 nm ($\theta = -2200$) for Na^+, whereas they showed a peak at 203 nm ($\theta = +1600$) for Mg^{2+}. The polymer conformations seemed to play very important roles for the catalytic activities as discussed below.

Kinetic Study. Ethyl *p*-nitrophenyl phosphate was used as the substrate. Hydrolysis rates of phosphodiesters were determined in Tris buffers (pH = 7.4, ionic strength = 0.02, KCl) at 50 °C in the presence of the polymers. The rates were followed by the measurement of the ultraviolet absorption (λ: 400 nm) of the *p*-nitrophenol evolved.

In order to check whether there is any side reaction other than the hydrolysis (Scheme 3), the catalysis reaction mixtures of ethyl *p*-nitrophenyl phosphate and polymer **11** or **12** in Tris buffer under the conditions of [S] = 4.67×10^{-4} M, [polymer] = 1.62×10^{-5} M, pH = 7.4 at 50 °C, ionic strength = 0.02, and a time of 24 h were examined by ^{31}P NMR. Two peaks at 2.69 ppm for ethyl phosphate and −5.97 ppm for ethyl *p*-nitrophenyl phosphate (substrate) relative to phosphoric acid at 0 ppm were observed. The same result was obtained by liquid chromatography, indicating that the hydrolysis reaction (Scheme 3) took place exclusively.

In Figure 3 are plotted the concentrations of *p*-nitrophenol produced during the hydrolysis in the buffer solution alone and in the presence of the monomer pair

Table 1. Copolymerization of Monomers 4, 6, and 9 with Maleic Anhydride with the Aid of AIBN Initiator[a]

copolymer	mole ratios of monomers	polymerizat. temp (°C)	polymerizat. time (h)	yield (%)	M_n[b]	$[\eta]$[c] (g/dL)
10	**4**:MA = 1:2	80	13	59	8 400	0.021
		90	13	57		
14	**6**:MA = 1:2	90	8	35	12 900	0.057
		90	13	34		
16	**9**:MA = 1:2	90	13	81	16 900	0.081
		90	20	80		

[a] Initiator concentration: 2 mol % of the total monomers. [b] M_n of the hydrolyzed polymers (**11**, **15**, and **17**) were measured in 0.1 N $NaNO_3$ aqueous solutions by GPC with poly(ethylene oxide) standard. [c] Intrinsic viscosities of the hydrolyzed polymers **11**, **15**, and **17** were measured in 0.1 N $NaNO_3$ aqueous solutions.

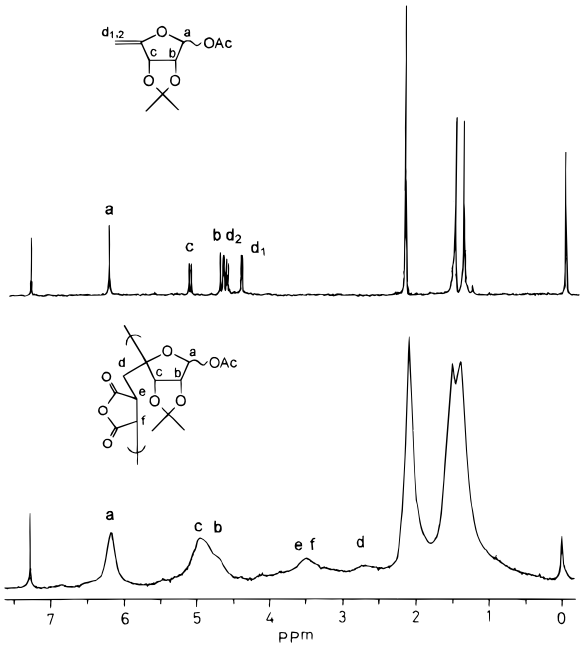


Figure 1. ^{1}H-NMR spectra of monomer **4** and polymer **10** in $CDCl_3$.

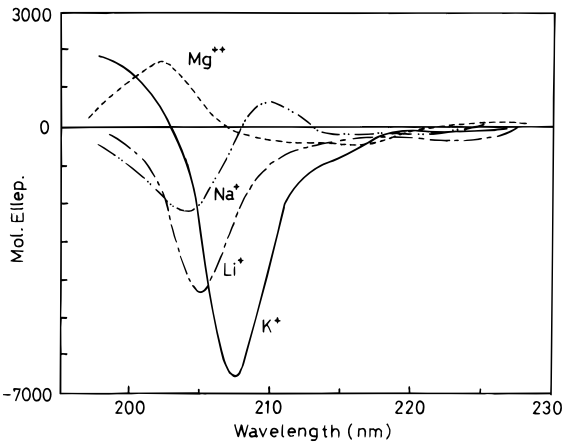
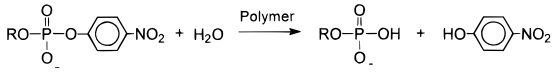


Figure 2. CD spectra of polymer **11** in Tris buffer (pH = 7.4) in the presence of different cations (ionic strength = 0.02) at 50 °C.

Scheme 3. Hydrolysis



(succinic acid and ribofuranose) and polymers **11**−**13**, **15**, and **17** at 50 °C and ionic strength of 0.02 (KCl) as a function of time. Polymers **11**, **12**, and **15** with *vic*-*cis*-diols of furanose rings accelerated the hydrolysis, while no catalytic activities were observed in polymer **13**, whose diol groups were blocked by isopropylidene groups, and polymer **17** containing pyranose rings.

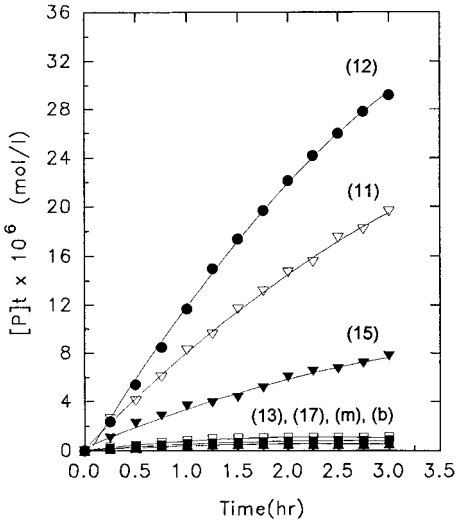


Figure 3. Concentrations of *p*-nitrophenol evolved $[P]_t$ during hydrolysis of ethyl *p*-nitrophenyl phosphate as a function of time in the presence of polymers **11**−**13**, **15**, and **17**, monomer pairs (succinic acid and ribofuranose) (m), and Tris buffer (b) at pH = 7.4, 50 °C, and ionic strength = 0.02 (KCl). [substrate] = [monomer pair] = 4.67×10^{-4} M; [polymer] = 3.24×10^{-5} M.

These results imply that *vic*-*cis*-diols of furanose rings were responsible for the catalytic activity in the hydrolysis of the phosphodiesters. As the monomer pairs showed no activity, the polymeric effect was obvious in this catalysis. The *vic*-dicarboxyl groups of the polymers were of little importance for the catalytic activity because **13** and **17** contained them, while they were blocked by methyl groups in **12**.

In the hydrolysis, the measured rate (v_m) was the sum of the rate of catalyzed reaction (v_c) and uncatalyzed reaction (v_b). The value of v_c was therefore obtained from eq 2.

$$v_m = v_c + v_b \quad (1)$$

$$v_c = v_m - v_b \quad (2)$$

The initial v_c's were measured at constant concentrations of **11**, **12**, and **15** by changing the substrate concentrations in Tris buffer (pH = 7.4) at 50 °C and ionic strength of 0.02 (KCl). Michaelis−Menten kinetics for the hydrolysis catalyzed by the polymers were confirmed by performing the double reciprocal plot of Lineweaver and Burk ($[v]^{-1}$ *vs* $[\text{substrate}]^{-1}$) (Figure 4), which gave K_m and V_{max}. The k_{cat} was obtained from eq 3. We assumed that each polymer chain formed one

$$k_{cat} = V_{max}/[E]_0 \quad (3)$$

active site, since no significant catalytic activity for the polymers with number-average molecular weights lower

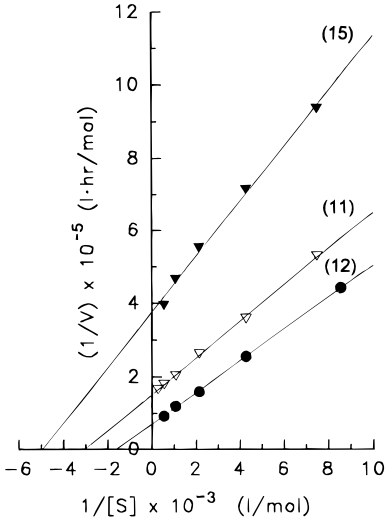


Figure 4. Reciprocals of the initial rates as a function of the reciprocals of the substrate concentrations at constant concentrations of polymers **11**, **12** and **15**: [polymer] = 1.62×10^{-5} M, in Tris buffer (pH = 7.4) at an ionic strength of 0.02 (KCl), at 50 °C.

Table 2. Kinetic Parameters of the Hydrolysis of Ethyl *p*-Nitrophenyl Phosphate Catalyzed by the Polymers in Tris Buffer (pH = 7.4) at 50 °C, [polymer] = 1.62×10^{-5} M, and Ionic Strength = 0.02 (KCl)

catalysts	k_{blank} (h^{-1})	K_m (M)	V_{max} (M h^{-1})	k_{cat} (h^{-1})
blank	9.1×10^{-4}			
polymer **11**		3.8×10^{-4}	6.8×10^{-6}	4.2×10^{-1}
polymer **15**		3.0×10^{-4}	2.7×10^{-6}	1.7×10^{-1}
polymer **12**		6.1×10^{-4}	1.4×10^{-5}	9.0×10^{-1}

than 4000 (polydispersity = 4) was observed. The kinetic parameters are summarized in Table 2.

The hydrolysis of ethyl *p*-nitrophenyl phosphate in the buffer solution at pH 7.4 in the absence of polymers was found to be pseudo-first-order with respect to the substrate and k_{blank} to be 9.1×10^{-4} h^{-1} under the same conditions as mentioned above (Table 2). The value of k_{cat} for **12** was about 10^3 larger than k_{blank}(uncatalyzed reaction). The values of k_{cat} for the polymers were found to be in the order of **12** > **11** > **15**, coinciding with the results shown in Figure 3. Higher catalytic activities of polymers **12** and **11** than that of **15** were attributable to a 30% increase of *vic*-*cis*-diol groups in the former. The highest increase of rate constant by polymer **12** may be caused by a conformational change resulting from methylation of carboxyl groups that favors binding of the substrate.

Competitive and Noncompetitive Inhibition. Competitive inhibition was observed in the catalysis on addition of acetate ions (Figure 5). The dissociation constant of the inhibitor−polymer complex (K_I = [P][I]/[PI]) was found to be 5.9×10^{-4} M by plotting the slopes against inhibitor concentrations. By addition of K_2HPO_4, the noncompetitive inhibition was observed (Figure 6) and the dissociation constant (K_I) was measured to be 2.5×10^{-4} M by plotting the intercepts against inhibitor concentrations. The acetate ion seemed to compete with the substrate for forming hydrogen bonds with the polymeric catalyst. The phosphate ions, as dianions, was a stronger hydrogen bond acceptor with the *vic*-*cis*-diol groups of the polymer catalyst than the substrate, leading to a noncompetitive inhibition.

Temperature and Cation Effect on the Catalytic Activity. The k_{cat} values were measured at different temperatures in Tris buffer (pH 7.4) at ionic strength

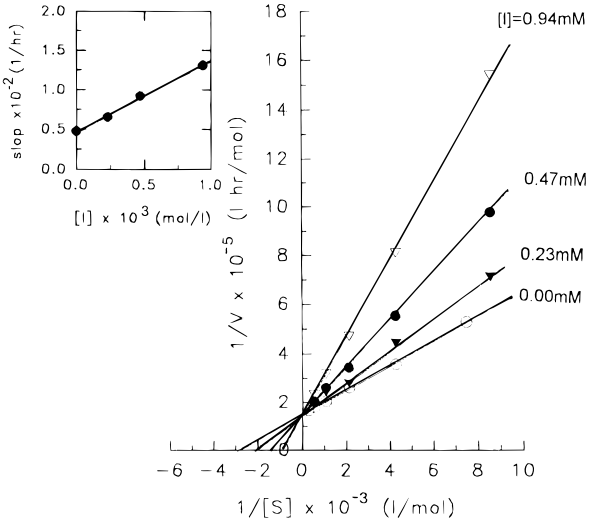


Figure 5. Double-reciprocal plots (1/*v* versus 1/[S]) measured at different concentrations of the inhibitor, sodium acetate, in the presence of polymer **11** in Tris buffer (pH = 7.4) at an ionic strength of 0.02 (KCl), at 50 °C. [polymer] = 1.62×10^{-5} M; [I] = [CH_3COONa]. K_I determination is shown in the upper left plot.

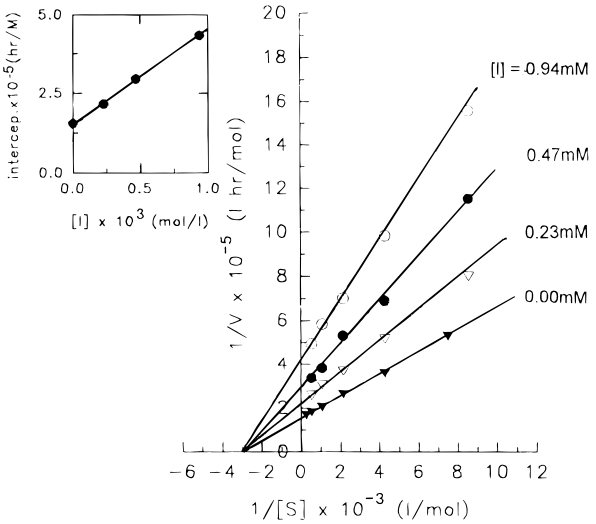


Figure 6. Double-reciprocal plots (1/*v* versus 1/[S]) measured at different concentrations of the inhibitor, K_2HPO_4, in the presence of polymer **11** in Tris buffer (pH = 7.4) at an ionic strength of 0.02 (KCl), at 50 °C. [polymer] = 1.62×10^{-5} M; [I] = [K_2HPO_4]. K_I determination is shown in the upper left plot.

0.02 (KCl) and plotted in Figure 7. In contrast to natural enzymes whose activities generally drop above 60 °C due to denaturation, the catalytic activities of polymer **11** increase up to 80 °C. From an Arrhenius plot, the activation energy of the reaction catalyzed by polymer **11** was found to be 6.9 kcal/mol, which was 6.6 kcal/mol lower than that (13.5 kcal/mol) of the uncatalyzed reaction.

As observed by the CD spectroscopy (Figure 2), the counterions changed the conformation of polymer **11**, which would influence the catalytic activity of the polymer. The catalytic rate constants for the hydrolysis of ethyl *p*-nitrophenyl phosphate catalyzed by polymer **11** in Tris buffer (pH 7.4) at 50 °C in the presence of different cations (ionic strength = 0.02) decreased in the order of K^+ (k_{cat} = 0.42 h^{-1}) > Li^+ (0.33) > Na^+ (0.14) > Mg^{2+} (0.08). The decrease in catalytic activities of the polymer was accompanied by a blue shift and intensity

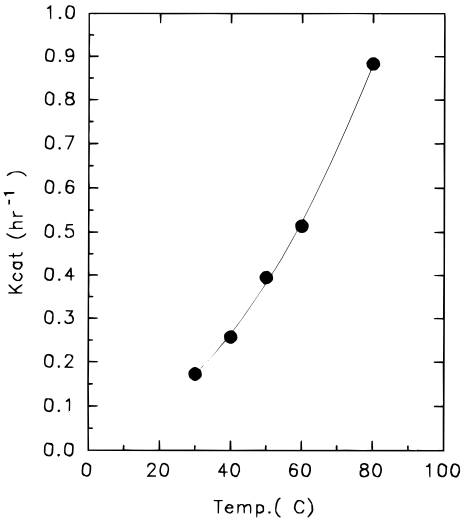


Figure 7. Catalytic rate constants as a function of temperature in Tris buffer (pH 7.4) at ionic strength 0.02 (KCl).

decreases of the troughs in the CD spectra of the polymer (Figure 2), indicating that a specific conformation was required for high catalytic activity. Although Mg^{2+} ion is known to be essential for efficient catalysis of ribozymes and most nucleases, it showed a low k_{cat} in this system and its chelating effect seemed to be negligible.

Catalysis for the Hydrolysis of Oligonucleotides. Since the polymers catalyzed the hydrolysis of phosphodiesters, they were expected to have catalytic activity for the DNA cleavage. ^{32}P-labeled on 5′ of ssDNA of 30 bases, d(CATGGCAAAGCCAGTATACAAATTGTAATA), corresponding to the human foamy virus proviral DNA in position of nucleotides 3634−3611,[20] was incubated in Tris buffer (pH 7.4) at 37 °C and ionic strength 0.02 (KCl) in the presence of polymers **11**−**13** and **15**. Autoradiograms of acrylamide gel electrophoretic analysis of the reaction mixtures are shown in Figure 8A. No DNA cleavage was observed in buffer as well as in the presence of polymer **13**, while polymers **11**, **12**, and **15** catalyzed hydrolysis of DNA.

Tetradeoxyadenylic acid [d(A)$_4$] was also incubated in Tris buffer (pH 7.4) at 37 °C and ionic strength 0.02 (KCl) in the presence of polymers **11**−**13**, **15**, and **17**. TLC chromatograms of the reaction mixtures and authentic samples are shown in Figure 8B. The hydrolysis of d(A)$_4$ [d(A)$_4$ → d(A)$_3$ + d(A)$_2$ + d(A)$_1$] was catalyzed by polymers **11**, **12**, and **15**, while no cleavage was observed in buffer or in the presence of polymers **13** and **17**. The extents of the hydrolysis in both reactions were observed in the order of polymers **12** > **11** > **15**, which coincided with the order of catalytic rate constants of the polymers for the hydrolysis of phosphodiester (Table 2). Both experimental results indicate that only the polymers containing *vic*-*cis*-diol groups of the furanose ring showed DNase activity.

Mechanism. Since no activity was found in the monomer pair, the polymers were likely to form active sites with a specific conformation. It seems possible that the furanose rings having *vic*-*cis*-diol groups were located inside of the active sites, where the phosphodiester substrates were also accommodated. The *vic*-*cis*-diol groups seemed to form hydrogen bonds with the two oxygen atoms of the phosphate so as to activate the phosphorus atoms to be attacked by nucleophiles (H_2O) leading to the expulsion of the alkoxy groups. Further investigation on the mechanism is in progress.

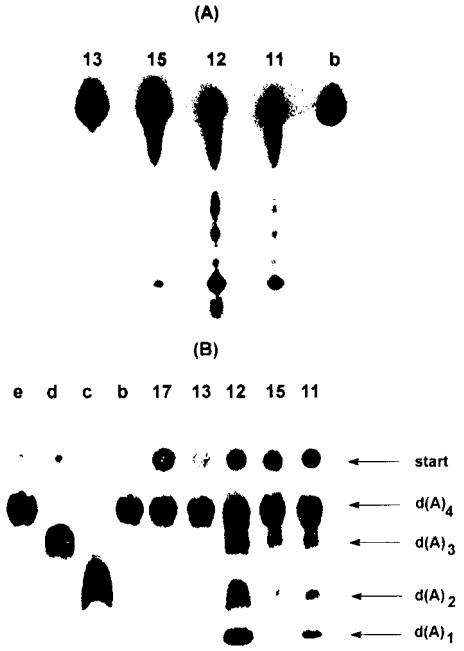


Figure 8. (A) Autoradiogram of 7 M urea−8% acrylamide gel electrophoretic analysis of the reaction mixtures: ^{32}P-labeled ssDNA of 30 bases was incubated at 37 °C, ionic strength = 0.02 (KCl), and pH = 7.4 (Tris buffer) for 12 h (lane b), in the presence of polymers **11** (lane 11), **12** (lane 12), **15** (lane 15), and **13** (lane 13). [DNA] = 7.5×10^{-4} M, [polymer] = 7.3×10^{-5} M. (B) TLC (silica gel) chromatogram of the authentic samples and the reaction mixtures developed by CH_3OH:H_2O = 4:1 and subsequently ethanol:methanol = 4:1 (v/v); lane e for the authentic sample of d(A)$_4$, lane d for d(A)$_3$, and lane c for d(A)$_2$. d(A)$_4$ was incubated at pH = 7.4 (Tris buffer), ionic strength = 0.02 (KCl), and 37 °C for 24 h (lane b), in the presence of polymer **17** (lane 17), polymer **13** (lane 13), polymer **12** (lane 12), polymer **15** (lane 15), and polymer **11** (lane 11). [d(A)$_4$] = 8.4×10^{-4}; [polymer] = 7.3×10^{-5} M.

Conclusion

Five alternating copolymers of maleic acid with ribofuranose or pyranose derivatives were synthesized to investigate the catalysis for phosphodiester hydrolysis. The polymers containing ribofuranose exhibited catalytic activity for the hydrolysis of phosphodiesters. The polymers followed the standard enzyme kinetics, such as the saturation curves, the Michaelis−Menten relationship, and competitive and noncompetitive inhibition, which was not previously found in synthetic polymer catalysis. The rate constants (k_{cat}) for the hydrolysis of phosphodiesters catalyzed by the polymers were found to be about 10^3 higher than those for the uncatalyzed reactions. The polymers showed also DNase activity for oligodeoxyribonucleotides. The catalytic activity of the polymers appeared to be attributable to *vic*-*cis*-diol groups of ribofuranose rings on the polymer backbones.

Experimental Section

Materials. D-Ribose, 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose, and *p*-nitrophenyl phosphorodichloridate, purchased from Aldrich Chemical Co., were used as received. Maleic anhydride was sublimed under vacuum. AIBN was crystallized from methanol. All other commercially available reagent chemicals were used without further purification.

Synthesis of Monomers. 5-Iodo-5-deoxy-2,3-*O*-isopropylidene-D-ribofuranose (2). 2,3-*O*-Isopropylidene-D-ribofuranose (3.13 g, 16.5 mmol),[14] pyridine (2.5

mL), iodine (5.02 g, 19.8 mmol), and triphenylphosphine (5.19 g, 19.8 mmol) were dissolved in 100 mL of dioxane and stirred for 12 h at room temperature. After addition of 5 mL of methanol, the solution was stirred for 30 min at room temperature. After evaporating the solvent, the residue was taken up with 250 mL of ethyl acetate and washed with 5% sodium thiosulfate and water each twice. After drying the organic phase with anhydrous $MgSO_4$ and evaporating the solvent, the residue was purified by column chromatography on silica gel (eluent, CCl_4:acetone = 2:1, v/v) and then recrystallized from CCl_4−acetone (2:1, v/v) to give 2.0 g of **2** (mp, 75−78 °C yield, 40%).

^{1}H NMR: δ 1.33, 1.48 (ss, 6H, acetonide), 3.31 (q, 2H, H_5, J = 8 and 4 Hz), 4.45 (q, 1H, H_4, J = 6 and 6 Hz), 4.67 (d, 1H, H_3, J = 4 Hz), 4.81 (d, 1H, H_2, J = 4 Hz), 5.56 (d, 1H, H_1, J = 2.2 Hz).

Anal. Calcd for $C_8H_{13}O_4$: C, 32.02; H, 4.36; O, 21.32. Found: C, 32.17; H, 4.35; O, 21.19.

1-*O*-Acetyl-5-iodo-5-deoxy-2,3-*O*-isopropylidene-D-ribofuranose (3). Compound **2** (2 g, 6.67 mmol) and acetic anhydride (1.36 g, 13.3 mmol) were dissolved in 10 mL of pyridine and stirred for 12 h at room temperature. After addition of 5 mL of methanol, the solution was stirred for 30 min. The reaction mixture was taken up with 200 mL of ethyl acetate and washed with H_2O twice. After drying with anhydrous $MgSO_4$ and evaporating the solvent, the residue was recrystallized from ethyl acetate−hexane to give 1.71 g of **3** (mp, 53−54 °C; yield, 75%).

^{1}H NMR: δ 1.35, 1.50 (ss, 6H, acetonide), 2.08 (s, 3H, acetyl-CH_3), 3.10 (t, 1H, H_{5a}, J = 10 Hz), 3.27 (q, 1H, H_{5b}, J = 6 and 6 Hz), 4.53 (q, 1H, H_4, J = 6 and 6 Hz), 4.74 (d, 1H, H_3, J = 6 Hz), 4.84 (d, 1H, H_2, J = 6 Hz), 6.30 (s, 1H, H_1).

Anal. Calcd for $C_{10}H_{15}O_5$: C, 35.11; H, 4.42; O, 23.38. Found: C, 35.17; H, 4.40; O, 23.32.

1-*O*-Acetyl-5-deoxy-2,3-*O*-isopropylidene-D-*erythro*-pent-4-enofuranose (4). Compound **3** (1.7 g, 5.15 mmol) was dissolved in the suspension of AgF (1.5 g, 11.8 mmol) in 10 mL of pyridine and stirred for 5 days at room temperature. After filtering through Celite, the reaction mixture was diluted with 100 mL of ethyl acetate and washed with H_2O twice. After drying with anhydrous $MgSO_4$ and evaporating the solvent, the residue was purified by column chromatography on silica gel (eluent, CCl_4:acetone = 2:1, v/v) to give 0.29 g of syrupy **4** (yield, 27%).

^{1}H NMR: δ 1.38, 1.49 (ss, 6H, acetonide), 2.07, 2.09 (ss, 3H, acetyl), 4.45 (d, 1H, H_{5a}, J = 2 Hz), 4.63 (d, 1H, H_3, J = 6 Hz), 4.68 (d, 1H, H_{5b}, J = 2 Hz), 5.10 (d, 1H, H_2, J = 6 Hz), 6.23, 6.29 (ss, 1H, H_1).

Anal. Calcd for $C_{10}H_{14}O_5$: C, 56.07; H, 6.54; O, 37.39. Found: C, 56.17; H, 6.55; O, 37.28.

1-*O*-Methyl-5-deoxy-2,3-*O*-isopropylidene-D-*erythro*-pent-4-enofuranose (6). Methyl 2,3-isopropylidene-5-*O*-*p*-toluenesulfonyl-D-ribofuranoside (**5**) was synthesized from D-ribose (**1**) through methylation, acetonization, and tosylation.[15] The dimethyl sulfoxide solution (5 mL) containing **5** (2.6 g, 7.25 mmol) was dropped in the DMSO solution (10 mL) containing potassium *tert*-butoxide (2.44 g, 21.74 mmol), and the reaction mixture was stirred for 1 h at room temperature. After neutralization with 0.87 mL of acetic acid, the reaction mixture was diluted with 200 mL of ethyl acetate and washed with water three times. After drying with $MgSO_4$ and evaporating the solvent, the residue was purified by column chromathography on silica gel (eluent, diethyl ether) to give 0.55 g of syrupy **6** (yield, 41%).

^{1}H NMR: δ 1.34, 1.46 (ss, 6H, acetonide), 3.39 (s, 3H, $-CH_3$), 4.37 (d, 1H, H_{5a}, J = 2.2 Hz), 4.58 (d, 1H, H_{5b}, J = 2.2 Hz), 4.47 (d, 1H, H_2, J = 6 Hz), 4.99 (d, 1H, H_3, J = 6 Hz), 5.09 (s, 1H, H_1).

Anal. Calcd for $C_9H_{14}O_4$: C, 58.05; H, 7.58; O, 34.37. Found: C, 58.07; H, 7.59; O, 34.34.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-*p*-toluenesulfonyl-α-D-galactopyranose (8). This compound was synthesized by tosylation of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose according to the literature.[21]

6-Deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-hex-5-enopyranose (9). The dimethyl sulfoxide solution (12 mL) containing **8** (5 g, 16.16 mmol) was added dropwise to the DMSO solution (25 mL) of potassiun *tert*-butoxide (4.063 g, 36.2 mmol). The solution was stirred for 1 h at room temperature and neutralized by addition of 1.5 mL of acetic acid. After addition of 250 mL of ethyl acetate, the solution was washed with H_2O three times and dried with anhydrous $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography on silica gel (eluent, ethyl acetate:hexane = 2:5, v/v) to give 1.08 g of **9** (mp, 86−87 °C; yield, 37%).

^{1}H NMR: δ 1.34, 1.39, 1.47, 1.48 (ssss, 12H, acetonide), 4.26 (q, 1H, H_4, J = 3 Hz), 4.56 (ss, 2H, H_2, H_3), 4.67, 4.78 (ss, 2H, H_{6b}, H_{6a}), 5.60 (d, 1H, H_1, J = 3.8 Hz).

Anal. Calcd for $C_{12}H_{18}O_5$: C, 59.50; H, 7.44; O, 33.06. Found: C, 59.36; H, 7.35; O, 33.29.

Ethyl *p*-Nitrophenyl Phosphate (Substrate). *p*-Nitrophenyl phosphorodichloridate (10.4 g, 58.6 mmol) was added to a stirred solution of dry diethyl ether (100 mL) containing absolute ethanol (3.5 mL, 58.6 mmol) and 2,6-lutidine (6.8 mL, 58.6 mmol) over 30 min at 0 °C, and the solution was stirred overnight at room temperature. The resulting white precipitates were filtered off and washed with fresh ether. The solvent was evaporated to give ethyl *p*-nitrophenyl chlorophosphate. It was dissolved in THF (100 mL), and the solution was added to cold water (200 mL) containing several drops of diluted HCl. The solution was extracted with CH_2Cl_2 (2 × 200 mL) and dried with Na_2SO_4. After evaporation of the solvent under reduced pressure, residues were recrystallized from ethyl acetate−petroleum ether to give 4.66 g of the product (mp, 93-94 °C; yield, 32.3%).

^{1}H NMR: δ 1.34 (t, 3H, methyl, J = 7 Hz), 4.21 (q, 2H, methylene, J = 7.8 Hz), 7.32, 8.21 (dd, 4H, Bz, J = 8.8 Hz).

Anal. Calcd for $C_8H_{10}O_6NP$: C, 38.88; H, 3.86; N, 5.67. Found: C, 38.85; H, 3.88; N, 5.62.

Copolymerization. Calculated amounts of monomers and initiators (AIBN) were charged into polymerization tubes without solvents. These tubes were then immersed into a Dewar flask containing dry ice and acetone. After a number of freeze−thaw cycles under N_2, the tubes were then sealed and placed in an water bath at a fixed temperature for various periods of time as listed in Table 1. After dissolving the polymerization products in acetone, poly[(6-deoxy-α-D-*galacto*-hex-5-enopyranose)-*alt*-(maleic anhydride)] (**16**) was precipitated in diethyl ether−hexane (4:1, v/v) and poly[(1-*O*-acetyl-2,3-*O*-isopropylidene-5-deoxy-D-*erythro*-pent-4-enofuranose)-*alt*-(maleic anhydride)] (**10**) and poly[(1-*O*-methyl-2,3-isopropylidene-5-deoxy-D-*erythro*-pent-4-enofuranose)-*alt*-(maleic anhydride)] (**14**) were pre-

cipitated in diethyl ether several times. The products were dried in vacuo over P_2O_5 at room temperature.

Hydrolysis of Copolymers. Poly[(5-deoxy-D-*erythro*-pent-4-enofuranose)-*alt*-(maleic acid)] (11). Polymer **10** (150 mg) was dissolved in the mixture of dioxane (20 mL) and 1 N HCl (5 mL). This solution was refluxed for 24 h. The reaction mixture was dialyzed through a cellulose membrane (Spectrum Medical Ind. Inc. MWCO-3500) using a constant flow of distilled water for 2 days at room temperature. The retentate was freeze-dried to give 103.3 mg of **11** (yield; 86%).

Poly[(5-deoxy-D-*erythro*-pent-4-enofuranose)-*alt*-(dimethyl maleate)] (12). After dissolving **11** (89 mg) in the mixture of H_2O (0.5 mL) and methanol (10 mL), the solution was dropped in 15 mL of an etheric diazomethane solution[22] at room temperature. After evaporation of the solvent, the residue was dissolved in water and dialyzed under the same conditions as those for the purification of polymer **11** to give 59.8 mg of **12** (yield, 61.3%).

Poly[(2,3-*O*-isopropylidene-5-deoxy-D-*erythro*-pent-4-enofuranose)-*alt*-(maleic acid)] (13). Polymer **10** (100 mg) was dissolved in 13 mL of 0.1 N NaOH. This solution was stirred for 24 h at room temperature, acidified with 0.1 N HCl, and dialyzed under the same conditions as those for the purification of polymer **11** to give 72.6 mg of the sodium salt of polymer **13** (yield, 63%).

Poly[(1-*O*-methyl-5-deoxy-D-*erythro*-pent-4-enofuranose)-*alt*-(maleic acid)] (15). Polymer **14** (167.4 mg) was dissolved in the mixture of 20 mL of formic acid and 5 mL of water and stirred for 13 h at 107 °C. The reaction mixture was dialyzed under the same conditions as those for the purification of **11** to give 0.124 g of **15** (yield, 81%).

Poly[(1,2:3,4-di-*O*-isopropylidene-6-deoxy-α-D-*galacto*-hex-5-enopyranose)-*alt*-(maleic acid)] (17). Polymer **16** (0.3 g) was dissolved in 15 mL of formic acid and stirred for 13 h at 100 °C. After evaporation of formic acid under reduced pressure, the residue was dialyzed under the same conditions as those for the purification of polymer **11** to give 0.189 g of the sodium salt of polymer **17** (yield, 77%).

Measurements. ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 spectrometer. IR spectra were obtained with a Nicolet Magna IR-550 spectrophotometer. Measurement of molecular weights was carried out by gel permeation chromatography (Waters 150-CV with RI detector) under the following conditions: Waters ultrahydrogel 250 column with a 0.1 N $NaNO_3$ aqueous solution eluent at a flow rate of 0.8 mL/min. Poly(ethylene oxide) was used as the molecular weight standards. Elemental analysis was performed at KRICT.

Kinetic Measurements. The solutions of the polymeric catalyst (7.3×10^{-5} M) and the substrate, ethyl *p*-nitrophenyl phosphate (7.0×10^{-3} M), were prepared in water buffered with 0.02 M Tris (pH 7.4), and the ionic strength was adjusted to 0.02 with KCl (or LiCl, NaCl, or $MgCl_2$).

The definite portions of the catalyst and the substrate solutions were mixed in a measuring cell. A solution of the same substrate concentration buffered with Tris (pH 7.4) at the ionic strength of 0.02 (KCl) was filled in the reference cell. The reaction rates were determined by measuring the absorption (A_t) of *p*-nitrophenol (400 nm) as a function of time (t) with a Hitachi (Model 200-20) spectrophotometer thermostated (±0.1 °C).

Catalysis for the Cleavage of ssDNA. The 5′ end labeling of single-stranded oligonucleotide of 30 bases was carried out with [γ-^{32}P]ATP (5000 Ci/mmol) and T4 polynucleotide kinase as described by Maniatis et al.[23] Radiolabeled ssDNAs were fractionated with denaturing polyacrylamide gel electrophoresis (7 M urea−15% acrylamide) and eluated from gel slice to remove unincorporated ^{32}P-ATP. The 5′ end-labeled ssDNAs were dissolved in water (2 pmol) buffered with 0.02 M Tris (pH 7.4), and the ionic strength was adjusted to 0.02 with KCl. The polymer catalyst (2 pmol) previously dissolved in the same buffer was added to perform catalysis reaction for 12 h at 37 °C in a total volume of 10 μL. After completion of the reaction, the mixture was 10-fold diluted in a gel-loading buffer (80% formamide−0.1% bromophenol blue−0.1% xylene cyanol FF). After heat-denaturing of the reaction mixture, an appropriate volume of mixture was applied to 7 M urea−8% polyacrylamide gel. To reveal the catalytic pattern, dried gel was exposed to X-ray film at 70 °C overnight without intensifying screen.

Catalysis for Hydrolysis of Tetradeoxyadenylic Acid. The solutions of the polymer catalyst (1.5×10^{-4} M) and tetradeoxyadenylic acid (1.7×10^{-3} M) were prepared in water buffered with 0.02 M Tris (pH 7.4), and the ionic strength was adjusted to 0.02 with KCl. The definite portions of catalyst and $d(A)_4$ solutions were mixed in a microtube and incubated at 37 °C for 24 h. The reaction mixture was spotted on a TLC plate (silica gel) and developed with methanol−water (4:1, v/v) until the oligonucleotide and hydrolyzed products were just separated from the polymer catalysts, which remained at the starting line. After drying the TLC plate with a heat gun, it was developed with ethanol−methanol (4:1, v/v).

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(19) In order to determine the configuration of the C1 atom of the furanose ring in polymers **11** and **12**, 1,2,3-tri-*O*-acetyl-5-*O*-trityl-D-ribofuranose was prepared by tritylation and subsequent acetylation from D-(−)-ribose, from which the monomer **4** was also prepared. The configuration of the C1 atom will remain intact during polymerization and during protection and deprotection reactions of the C1 hydroxyl group. In the α form, the C1 proton is *cis* with respect to the C2 proton, while in the *β* form, the C1 proton is *trans* with respect to the C2 proton. In the ^{1}H NMR spectrum of 1,2,3-tri-*O*-acetyl-5-*O*-trityl-D-ribofuranose in $CDCl_3$, two peaks appeared at 6.54 (d, $J = 4$ Hz) and 6.22 (s) ppm from the C1 protons of α and *β* form. The singlet peak at 6.22 ppm and doublet peak at 6.54 ppm were assigned to the proton of *β* form and the proton of α form, respectively, since the dihedral angle between the protons of C1 and C2 of α form was nearly zero and thus a larger coupling constant was expected. Approximately 30% of hydroxyl groups was found to stay in α form by calculation of the peak area ratio.
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