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Poly(cytidylic acid) and poly(adenylic acid) analogues: synthesis and catalytic activity for hydrolysis of dinucleotides and nucleic acids

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Abstract

Poly(cytidylic acid) and poly(adenylic acid) analogues, resembling closely the structures of the natural polymers, were synthesized by the alternating cyclocopolymerization of a nucleoside derivative and acrylic anhydride, and subsequent hydrolysis. The polymers catalyzed the cleavage of dinuleotides and nucleic acids (DNA and RNA). Poly(cytidylic acid) and poly(adenylic acid) analogues accelerated the hydrolysis of GpG and TpT, respectively, faster than other dinucleotides due to the base pairing.

Keywords: Cyclocopolymerization; Synthetic enzyme; Polynucleotide analogue

1. Introduction

Synthetic enzyme mimics are of great interest since their studies can improve the knowledge of structures and mechanisms of natural enzymes. The enzyme mimics themselves are also important from the practical viewpoint when they overcome the limitations of natural enzymes, namely, denaturation in organic media, difficulties in isolation, and loss of activity on recycling. Additionally, the preservation of their activities in the broad range of pH and temperature can be expected [1,2].

Recently, we reported that the ribose-containing polymers showed catalytic activity for the cleavage of nucleic acids and hydrolysis of a phosphodiester with a rate acceleration of 10^3 compared with that of the uncatalyzed reaction [3]. The catalysis was caused by *vic-cis*-diol groups of the ribose rings attached on the polymer chains as pendent groups [3–6]. Among the natural enzymes, ribozyme, a RNA molecule having enzymatic activity for the transphosphorylation or hydrolysis of RNA as well as the cleavage of DNA [7], has a backbone comprising ribose rings and phosphate groups. The specificity of the ribozyme reactions is much indebted to the nucleic acid bases on the ribose rings, which can bind to their complementary bases through the base pairing. This led us to design a ribose-containing polymer with nucleic acid bases, expecting the catalytic activity for the cleavage of nucleic acids as well as the specificity for the cleavage sites.

In this work, RNA analogues, poly[{2(R)-cytosin-1-yl-3(R),4(S)-dihydroxytetrahydrofuran-5,5-diyl}{2,4-dicarboxypentamethylene}] (**13**) and poly[{2(R)-adenin-9-yl-3(R),4(S)-dihydroxytetrahydrofuran-5,5-diyl}{2,4-dicarboxypentamethylene}] (**17**) were prepared by the alternating cyclocopolymerization of acrylic anhydride with the corresponding nucleoside derivatives and subsequent hydrolysis. Their catalytic activities for the hydrolysis of dinucleotides and the cleavage of nucleic acids were studied.

2. Experimental

2.1. Materials

Chemicals were purchased from Sigma-Aldrich. Supercoiled ds DNA (pBR 322 Vector), BMV RNA, Lambda

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DNA/*Hin*dIII Markers, RNA Markers (0.28–6.58 kb) and Ethidium Bromide Solution were purchased from Promega. Tris-buffer materials (TRIZMA® Base and TRIZMA® HCl) were recrystallized from deionized water and ethanol three times to exclude metal ions. Azobisbutyronitirile (AIBN) was recrystallized from methanol. Solvents were purified in common methods [8]. Other commercially available reagent chemicals were used as received.

2.2. N^4-Acetyl-2′,3′-di-O-acetyl-5′-deoxy-5′-iodocytidine (3)

5′-Iodo-5′-deoxycytidine (**2**) was synthesized according to the literature [9]. To a solution of **2** (2.6 g, 7.4 mmol) in DMF (10 ml), acetic anhydride (15 ml) and pyridine (2 ml) were added. The solution was stirred for 2 h at room temperature. After addition of methanol (10 ml) and methylene chloride (100 ml), the solution was washed with an aqueous 5% sodium bicarbonate solution and with distilled water twice. The organic layer was dried with anhydrous $MgSO_4$. After filtration and evaporation of the solvent, compound **3** was isolated by recrystallization from methanol (mp: 190–192 °C, yield: 85%). ^{1}H NMR (DMSO-d_6): δ 2.00, 2.02 (ss, 6H, 2CH_3CO), 2.22 (s, 3H, N^4–COCH_3), 3.40 (q, 1H, $H^{5'a}$), 3.60 (q, 1H, $H^{5'b}$), 4.15 (q, 1H, $H^{4'}$), 5.25 (t, 1H, $H^{3'}$), 5.50 (t, 1H, $H^{2'}$), 5.98 (d, 1H, $H^{1'}$), 7.45 (d, 1H, H^5), 7.96 (d, 1H, H^6), 10.35 (s, 1H, N*H*). IR (KBr pellet): 3440, 3039, 1749, 1710, 1677, 1572, 1499, 1242, 810, 600 cm^{-1}. Anal. $C_{15}H_{18}IN_3O_7$: calcd C 37.59, H 3.79, N 8.77; found C 37.69, H 3.82, N 8.71.

2.3. 1-β-(N^4-Acetylcytosin-1-yl)-2′,3′-di-O-acetyl-5′-deoxy-D-erythro-pent-4′-enofuranose (4)

(A) From compound **2**: a solution of **2** (2 g, 5.67 mmol) and DBU (2 ml, 13.3 mmol) in pyridine (20 ml) was stirred at room temperature for 16 h and then acetic anhydride (10 ml) was added. After stirring for 5 h at room temperature, methanol (15 ml) was added. The solution was stirred for 0.5 h at the same temperature and then neutralized by an aqueous 5% sodium bicarbonate solution. Organic compounds were extracted with ethyl acetate. The organic layer was washed with an aqueous HCl solution (1 M) and distilled water twice, and dried over anhydrous $MgSO_4$. After filtration and evaporation of the solvent, the product was isolated by column chromatography on silica gel (eluent: methylene chloride/acetone = 4/1, v/v) to give **4** (yield: 40%).

(B) From compound **3**: **3** (1.2 g, 2.50 mmol) and AgF (1.23 g, 9.69 mmol) were added to pyridine (20 ml) and the suspension was stirred for 4 days at room temperature. The reaction mixture was filtered over celite and the filtrate was evaporated to dryness under the reduced pressure. After dissolving the residue in ethyl acetate, the solution was washed with distilled water three times and dried over anhydrous $MgSO_4$. After filtration and evaporation of the solvent, the product was isolated by column chromatography on silica gel (eluent: methylene chloride/acetone = 4/1, v/v) (yield: 18%). ^{1}H NMR ($CDCl_3$): δ 2.10, 2.13 [ss, 6H, 2CH_3C(=O)], 2.28 [s, 3H, N^4–C(=O)CH_3], 4.46 (d, 1H, $H^{5'a}$), 4.76 (d, 1H, $H^{5'b}$), 5.46 (dd, 1H, $H^{2'}$), 5.83 (d, 1H, $H^{3'}$), 6.25 (d, 1H, $H^{1'}$), 7.51 (d, 1H, H^5), 7.62 (d, 1H, H^6), 10.38 (s, 1H, N*H*). ^{13}C NMR ($CDCl_3$): δ 20.23 [$C^{2'\text{ or }3'}$–C(=O)*C*H$_3$], 20.39 [$C^{3'\text{ or }2'}$–C(=O)*C*H$_3$], 24.70 [N^4–C(=O)*C*H$_3$], 68.92, 72.87, 88.64, 90.10, 97.71, 143.88, 154.76, 156.64, 163.46, 169.35 [$C^{2'\text{ or }3'}$–*C*(=O)CH$_3$], 169.39 [$C^{3'\text{ or }2'}$–*C*(=O)CH$_3$], 172.25 [N^4–*C*(=O)CH$_3$]. IR (KBr pellet): 3447, 3026, 1755, 1677, 1558, 1499, 1321, 1242 cm^{-1}. Anal. $C_{15}H_{17}N_3O_7$: calcd C 51.28, H 4.88, N 11.96; found C 51.4, H 4.77, N 11.89.

2.4. 1′-β-(N^4-Ethoxycarbonylcytosin-1-yl)-5′-deoxy-2′,3′-O-isopropylidene-D-erythro-pent-4′-enofuranose (8)

2′,3′-O-Isopropylidene cytidine **(5)** [10], 5′-deoxy-5′-iodo-2′,3′-O-isopropylidene cytidine (**6**) [11], and 1′-β-(cytosin-1-yl)-5′-deoxy-2′,3′-O-isopropylidene-D-*erythro*-pent-4′-enofuranose **(7)** [12] were synthesized according to the literatures. **7** (0.72 g, 2.7 mmol), ethyl chloroformate (0.52 ml, 5.4 mmol), and pyridine (1.4 ml) were dissolved in methylene chloride (10 ml) and the solution was stirred for 0.5 h at room temperature. The solution was washed with distilled water three times and dried over anhydrous $MgSO_4$. After filtration, the filtrate was concentrated to dryness by evaporation and the solid residue was recrystallized from ethyl acetate to give **8** (mp: 144–146 °C, yield: 85%). ^{1}H NMR ($CDCl_3$): δ 1.32 (t, J = 7.8 Hz, 3H, CH_3CH$_2$O), 1.39 (s, 3H, *exo*-CH_3 of acetonide), 1.53 (s, 3H, *endo*-CH_3 of acetonide), 4.25 (q, J = 7.8 Hz, 2H, CH$_3$CH_2O), 4.42 (d, 1H, $H^{5'a}$), 4.62 (d, 1H, $H^{5'b}$), 5.05 (d, 1H, $H^{3'}$), 5.30 (d, 1H, $H^{2'}$), 5.75 (s, 1H, $H^{1'}$), 7.25 (d, 1H, H^5), 7.60 (d, 1H, H^6), 7.80 (s, 1H, N*H*). ^{13}C NMR ($CDCl_3$): δ 14.13 [N^4–HC(=O)OCH$_2$*C*H$_3$], 25.3, 25.6 (*C*H$_3$ of acetonide), 62.45 [N^4–HC(=O)O*C*H$_2$CH$_3$], 79.57, 83.81, 87.87, 95.18, 97.34 (CH$_3$*C*CH$_3$ of acetonide), 110.63, 140.52, 152.46, 154.58, 163.19, 163.52 [N^4–H*C*(=O)OCH$_2$CH$_3$]. IR (KBr pellet): 3420, 3040, 1795, 1200 cm^{-1}. Anal. $C_{15}H_{19}N_3O_6$: calcd C 53.41, H 5.68, N 12.46; found C 53.59, H 5.77, N 12.31.

2.5. 1-β-(N^6-Ethoxycarbonyl-adenin-9-yl)-5-deoxy-2,3-di-O-acetyl-D-erythro-pent-4-enofuranose (10) [13]

1-β-(Adenin-9-yl)-5-deoxy-2,3-di-O-acetyl-D-*erythro*-pent-4-enofuranose (**9**) was synthesized according to the literature [9]. To a solution of **9** (5 g, 15.0 mmol) in methylene chloride (200 ml), pyridine (0.5 ml) and ethyl chloroformate (1.95 ml, 20.4 mmol) were added dropwise and stirred for 1 h at room temperature. The solvent was removed by evaporation and the residue was dissolved in $CHCl_3$ (100 ml). The solution was washed with brine and distilled water, and dried over anhydrous $MgSO_4$. After

filtration and evaporation of the solvent, the product was isolated by recrystallization from chloroform and hexane (yield: 88%, mp: 143–144 °C).

2.6. Copolymerization

Calculated amounts of a monomer and an initiator (AIBN) were charged into a polymerization tube and the least amount of DMF was added to dissolve the reactants. After three freeze-thaw cycles under N_2, the tube was sealed and placed in a water bath at 90 °C for 24 h. The polymer was isolated by precipitation in diethyl ether and further purified by reprecipitation of the polymer solution in DMF in diethyl ether (**12** and **14**) or methyl ethyl ketone (**16**).

2.7. Poly[{1′-β-(N^4-acetylcytosin-1-yl)-2′,3′-di-O-acetyl-5′-deoxy-D-erythro-pent-4′-enofuranose}-alt-{acrylic anhydride}] (12)

^{1}H NMR (DMSO-d_6): δ 1.3–2.7 [15H, $H^{5',7',9'}$, $CH_3C(=O)$], 3.3–3.8 (2H, $H^{6',8'}$), 5.2–6.2 (3H, $H^{1',2',3'}$), 7.1–7.4 (1H, H^5), 7.7– 8.1 (1H, H^6), 10.8–11.2 (1H, NH). IR (KBr pellet): 3467, 1805, 1755, 1637, 1539, 1374, 1236 cm^{-1}.

2.8. Poly[{1′-β-(N^4-ethoxycarbonylcytosin-1-yl)-2′,3′-O-isopropylidene-5′-deoxy-D-erythro-pent-4′-enofuranose}-alt-{acrylic anhydride}] (14)

^{1}H NMR (DMSO-d_6): δ 1.0–1.9 [15H, $H^{5',7',9'}$, CH_3CH_2O, $C(CH_3)_2$ of acetonide], 3.8–4.6 (4H, $H^{6',8'}$, CH_3CH_2O), 5.4–6.0 (2H, $H^{2',3'}$), 6.0–6.4 (1H, $H^{1'}$), 7.0–7.2 (1H, H^5), 7.8–8.2 (1H, H^6), 10.4 –10.8 (1H, N*H*). IR (KBr pellet): 3500, 2990, 1700, 1650, 1500, 1200, 1100 cm^{-1}.

2.9. Poly[{1′-β-(N^6-ethoxycarbonyladenin-9-yl)-2′,3′-di-O-actyl-5′-deoxy-D-erythro-pent-4-enofuranose}-alt-{acrylic anhydride}] (16)

^{1}H NMR (DMSO-d_6): δ 1.2–1.4 (3H, CH_3CH_2O), 2.0–2.3 [6H, $CH_3C(=O)$], 2.3–2.7 (6H, $H^{5',7',9'}$), 4.0–4.2 (2H, $H^{6',8'}$), 4.2–4.6 (2H, CH_3CH_2O), 6.0–6.6 (3H, $H^{1',2',3'}$), 8.0–8.6 (2H, $H^{2,8}$), 10.2–10.7 (1H, N*H*).

2.10. Poly[{2(R)-cytosin-1-yl-3(R),4(S)-dihydroxytetrahydrofuran-5,5-diyl}{2,4-dicarboxypentamethylene}] (13)

Polymer **12** (41.3 mg) was dissolved in 0.1 N NaOH (6 ml) and the solution was stirred for 24 h at room temperature. The polymer solution was dialyzed against distilled water through a cellulose tube with a molecular weight cutoff of 1000. The retent was freeze-dried to give **13** (yield: 82%). ^{1}H NMR (D_2O): δ 1.3–2.7 (6H, $H^{5',7',9'}$), 4.0–4.5 (2H, $H^{6',8'}$), 4.9–5.4 (2H, $H^{2',3'}$), 5.6–6.3 (3H, $H^{1',5'}$), 7.6–7.9 (1H, H^6). IR (KBr pellet): 3400–2500, 2950, 1700, 1565, 1407 cm^{-1}.

2.11. Poly[{2(R)-cytosin-1-yl-3(R),4(S)-O-isopropylidenetetrahydrofuran- 5,5-diyl}{2,4-dicarboxypentamethylene}] (15)

Polymer **14** (37 mg) was dissolved in 0.1 N NaOH (6 ml) and the solution was stirred for 24 h at room temperature. The polymer was purified as described for **13** to give **15** (yield: 76%). ^{1}H NMR (D_2O): δ 1.0–1.6 [6H, $C(CH_3)_2$ of acetonide], 1.8–2.7 (6H, $H^{5',7',9'}$), 3.9–4.3 (2H, $H^{6',8'}$), 4.9–5.4 (2H, $H^{2',3'}$), 5.5–6.2 (2H, $H^{1',5}$), 7.6–8.0 (1H, H^6). IR (KBr pellet): 3500–2600, 1700, 1600, 1350, 1200, 1100 cm^{-1}.

2.12. Poly{[2(R)-adenin-9-yl-3(R),4(S)-dihydroxytetrahydrofuran-5,5-diyl][2,4-dicarboxypentamethylene]} (17)

Polymer **16** (200 mg) was hydrolyzed in 0.1 N NaOH (11 ml) for 24 h at 60 °C. The solution was dialyzed against distilled water through a cellulose tube with a molecular weight cutoff of 3500 for 72 h and then the retent was freeze-dried to give **17** (yield: 65%). ^{1}H NMR (D_2O): δ 1.5–2.8 (6H, $H^{5',7',9'}$), 4.0–4.5 (2H, $H^{6',8'}$), 5.0–5.5 (2H, $H^{2',3'}$), 6.0–6.3 (1H, $H^{1'}$), 8.0–8.3 (2H, $H^{2,8}$).

2.13. Measurements

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

2.14. Catalysis for the cleavage of nucleic acids

Supercoiled ds DNA (pBR322 DNA Plasmid, 800 ng) and polymer **13** or **15** (10.7 pmol) were dissolved in 20 μl of Tris-buffer (pH 7.4 and ionic strength of 0.02, KCl). RNA (BMV RNA, 250 ng) and polymer **13** or **15** (10.7 pmol) were dissolved in 20 μl of Tris-buffer (pH 7.4 and ionic strength of 0.02, KCl). The both solutions were incubated at 37 °C for 6 h. The cleaved products were analyzed by electrophoresis on EtBr-agarose gel and visualized with UV light. Lambda DNA/*Hin*dIII Markers and RNA Markers were used as markers for DNA and RNA, respectively.

2.15. Catalysis for the hydrolysis of dinucleotides

A dinucleotide (5×10^{-4} M) and polymer **13** or **15** (2.5×10^{-4} M) were dissolved in Tris-buffer (pH 7.4 and ionic strength of 0.02, KCl). The solution was incubated at 50 °C. The reaction mixture was investigated by HPLC

(conditions: Kromacil C18 reverse phase column, UV detector $\lambda = 265$ nm, eluent; pH 8 phosphate buffer 0.025 M, flow rate; 0.7 ml/min).

3. Results and discussion

3.1. Monomer synthesis

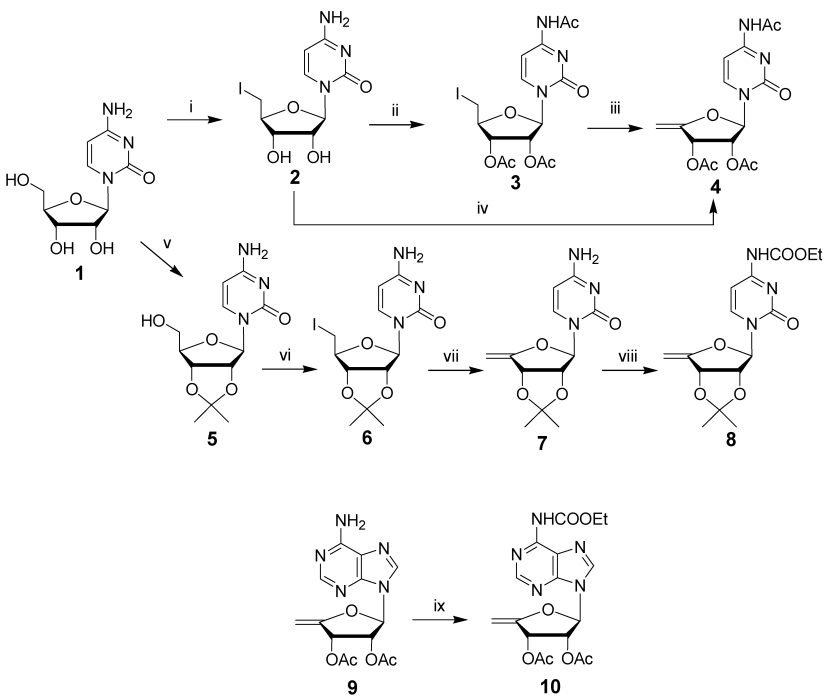
Iodination of cytidine (**1**) with triphenyl phosphine and iodine gave **2**, which was acetylated by acetic anhydride to result in **3**. Monomer **4** was obtained by elimination of HI with the aid of silver fluoride. Monomer **4** could be also obtained from **2** by elimination with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and following acetylation with acetic anhydride. Acetonization of **1** gave **5**, which was iodinated with the aid of methyl triphenoxy phosphonium iodide to give **6**. Elimination of HI with sodium methoxide gave **7**, which was reacted with ethyl chloroformate to give **8**. Monomer **10** was synthesized by the reaction of **9** with ethyl chloroformate (Scheme 1). The ^{1}H NMR spectra of **4** and **10** are given in Fig. 1.

3.2. Copolymerization

It was reported that the alternating copolymer was obtained when a cyclic vinyl ether was copolymerized with acrylic anhydride by the radical initiation under proper conditions [14]. During copolymerization an acrylic anhydride radical was converted to a glutaric anhydride radical by cyclization at the growing chain end, which propagated on the vinyl ether monomer. By repetition of the cross-reactions between them, the alternating copolymer was produced (Scheme 2). In the case that the coploymerization proceeded otherwise, the difunctional acrylic anhydride would give rise to the polymer structure having either cross-linkings or acrylic groups as pendants by incomplete cyclizations. Acrylic anhydride could be homopolymerized by the radical initiation, but monomers **4**, **8**, and **10** were not. The excess feeding mole ratios of the latter monomers at the onset of copolymerization was necessary for obtaining the alternating copolymer. The polymerization results are summarized in Table 1.

The alternating structures of the polymers were verified by ^{1}H NMR spectroscopy and a titration method. The anhydride copolymers **12**, **14**, and **16** were soluble in polar solvents, such as DMSO and DMF, showing that the cross-linking did not occur. The double bond protons of the acrylic groups of acrylic anhydride gave three signals at δ 6.57 (dd), 6.19 (q), and 6.07 (dd) in $CDCl_3$. These signals were not found in the ^{1}H NMR spectra of polymers **12**, **14**, and **16**, indicating that no acryl groups remained on the polymer chains as pendants (Fig. 2). Titration of anhydride groups of polymers **12** and **16** also showed that the polymers were composed of an equimolar amount of the comonomers (51 and 50 mol% of glutaric anhydride moieties in polymers **12** and **16**, respectively).

Polymers **12**, **14**, and **16** were hydrolyzed in aqueous 0.1 N NaOH to give polymers **13**, **15**, and **17**, respectively. Polymer **13** is an alternating copolymer of cytidine and a 2,4-dicarboxypentamethylene group, which is analogous to poly(cytidylic acid), an alternating copolymer of cytidine



Scheme 1. Synthesis of monomers: (i) PPh_3, I_2, Py.; (ii) Ac_2O, Py.; (iii) AgF; (iv) DBU, Ac_2O; (v) acetone, *p*-TsOH; (vi) methyltriphenoxyphosphonium iodide; (vii) $NaOCH_3$; (viii) ethyl chloroformate; (ix) ethyl chloroformate.

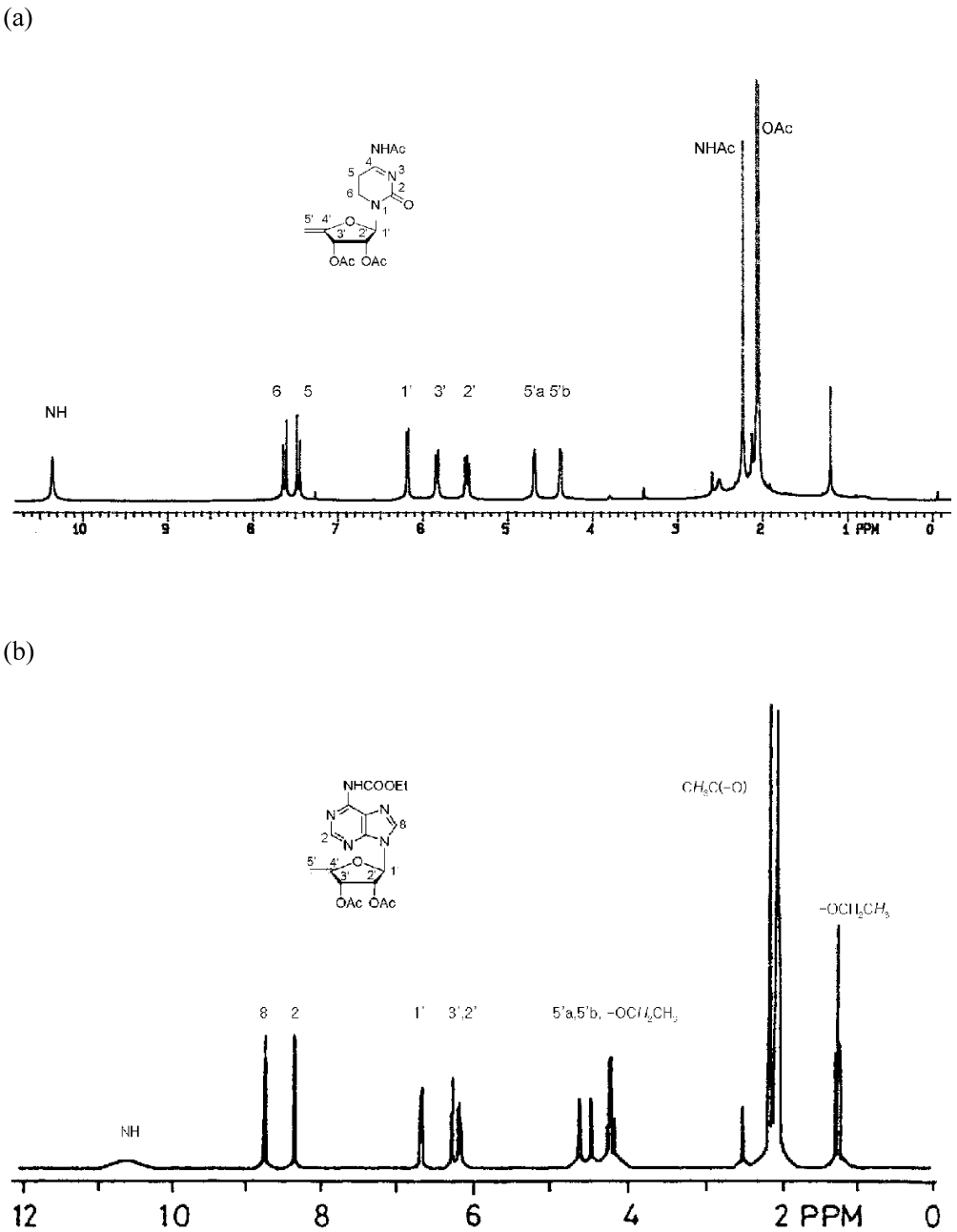


Fig. 1. ^{1}H NMR spectra of monomers **4** and **10**.

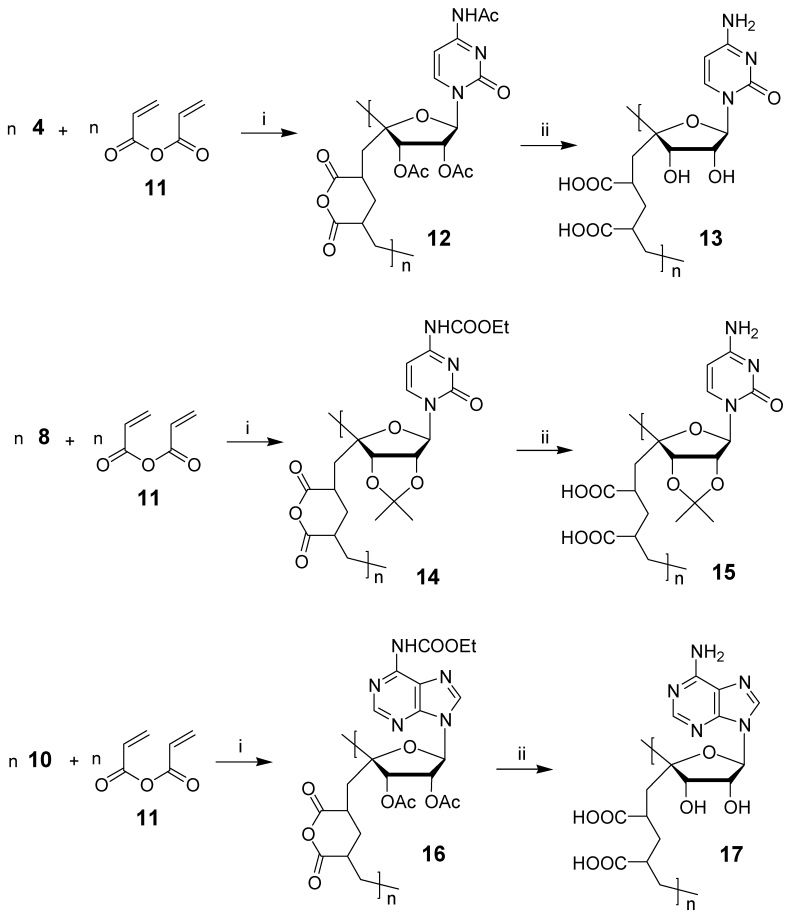
and a methylene phosphate group. Polymer **17** is also a poly(adenylic acid) analogue. As the polymers contained two carboxyl groups per a repeating unit, they were soluble in water as the nucleic acids.

3.3. Cleavage of nucleic acids

Supercoiled double-stranded DNA (pBR 322) and RNA (BMV) were incubated in a Tris-buffer solution and in the presence of polymers **13** and **15** at pH 7.4, 37 °C for 6 h and developed on the agarose gel by electrophoresis. The electrophoresis diagrams clearly showed that polymer **13** catalyzed the cleavage of DNA and RNA. The circular supercoiled DNA (form I) was converted to circular relaxed DNA (form II) via single-strand cleavage. BMV RNA was cleaved in the presence of polymer **13** to yield lower base pair segments. Only partial cleavage was observed in a buffer solution alone and in the presence of polymer **15** (Fig. 3).

3.4. Hydrolysis of dinucleotides

In order to find any differences in the cleavage rates due to the base interaction, the dinucleotides, adenylyl ($3' \rightarrow 5'$)adenosine (ApA), cytidylyl ($3' \rightarrow 5'$)cytidine (CpC),



Scheme 2. Synthesis of polymers: (i) AIBN, DMF; (ii) NaOH.

guanylyl (3′ → 5′)guanosine (GpG), thymidylyl (3′ → 5′)thymidine (TpT), were incubated in the presence of polymers **13** and **17** in Tris-buffer (pH 7.4), at 50 °C and ionic strength of 0.02 (KCl), and the reaction mixture was investigated by HPLC.

In Fig. 4 are plotted the concentrations of dinucleotides as a function of time in the presence of polymers **13** and **17**. The polymer **13** containing cytidines as pendants showed the highest catalytic activity for the cleavage of GpG. This result can be ascribed to the base pairing of cytosine in the polymer **13** with the guanine of the dinucleotide, which will allow the catalytic polymer to access the substrate easily. For the same reason, polymer **17** showed the highest catalytic activity for the cleavage TpT.

Table 1
Polymerization data with AIBN initiator at 90 °C for 24 h

Copolymer	Mole ratio of monomer	AIBN (mol%)	Yield (%)	Mn[a]	PD[b]
12	**4**:AA = 2:1	1.0	85		
13	–		82	18,600	1.5
14	**8**:AA = 2:1	1.0	62		
15	–		76	14,200	1.8
16	**10**:AA = 2:1	1.0	58		
17	–		65	7800	1.6

Minimum amount of DMF was added to dissolve monomers.

[a] Mn of the hydrolyzed polymers (**13**, **15**, and **17**) were measured by GPC (Waters Co.) with poly(ethylene oxide) standards (eluent: an aqueous 0.1 N $NaNO_3$ solution).

[c] Polydispersity.

3.5. Mechanism

In our previous work [4,5], we found that the *vic-cis*-diol groups of a ribose-containing polymer were responsible for the catalysis in the hydrolysis of a phosphate. The *vic-cis*-diol group formed hydrogen bonds with the two oxygen atoms of the phosphate and thereby activated the phosphorous atoms to be attacked by a nucleophile (H_2O). The formation of strong hydrogen bonds was born out by a theoretical study of the interaction between 3,4-dihydroxytetrahydrofuran and $H_2PO_4^{-1}$ [5].

Nucleic acids have phosphate linkages, connecting nucleotide units. The RNA analogue likely held a nucleic acid through non-covalent interactions in a way to facilitate the formation of hydrogen bonds between the *vic-cis*-diol and the phosphate. The results for the hydrolysis of dinucleotides implied that the analogues could interact more effectively with the substrate part having complementary bases through the base pairing.

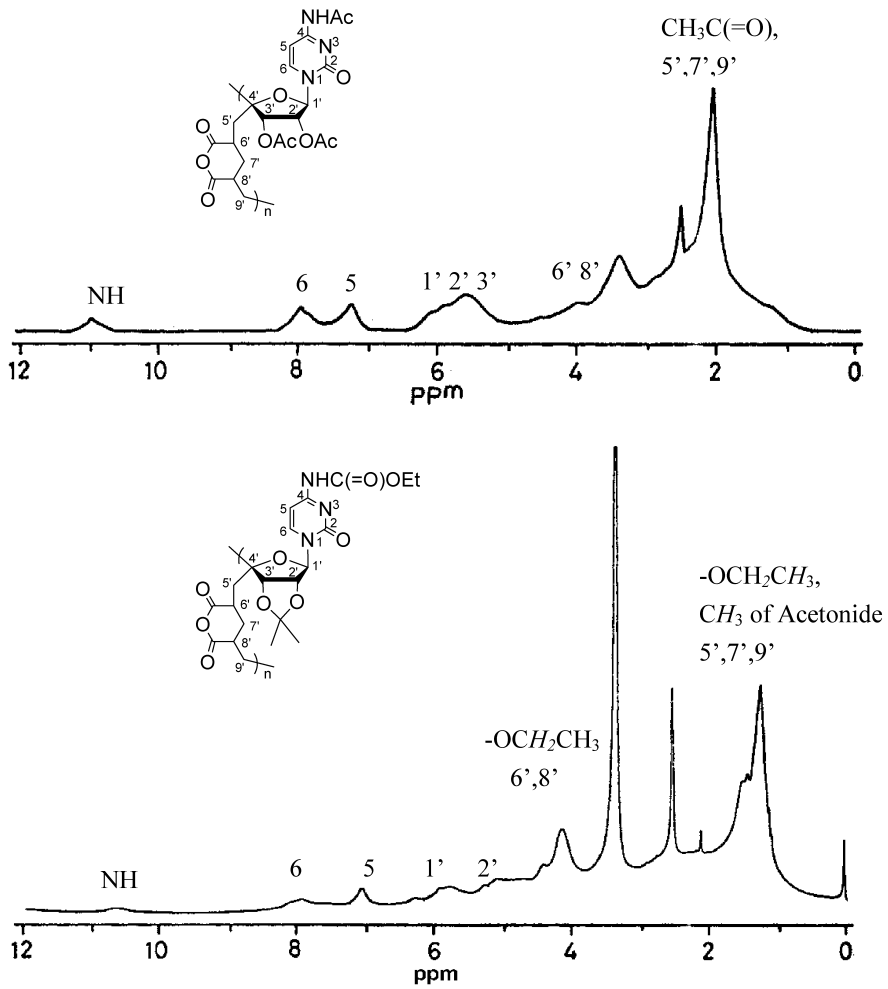


Fig. 2. ^{1}H NMR spectra of polymers **12** and **14**.

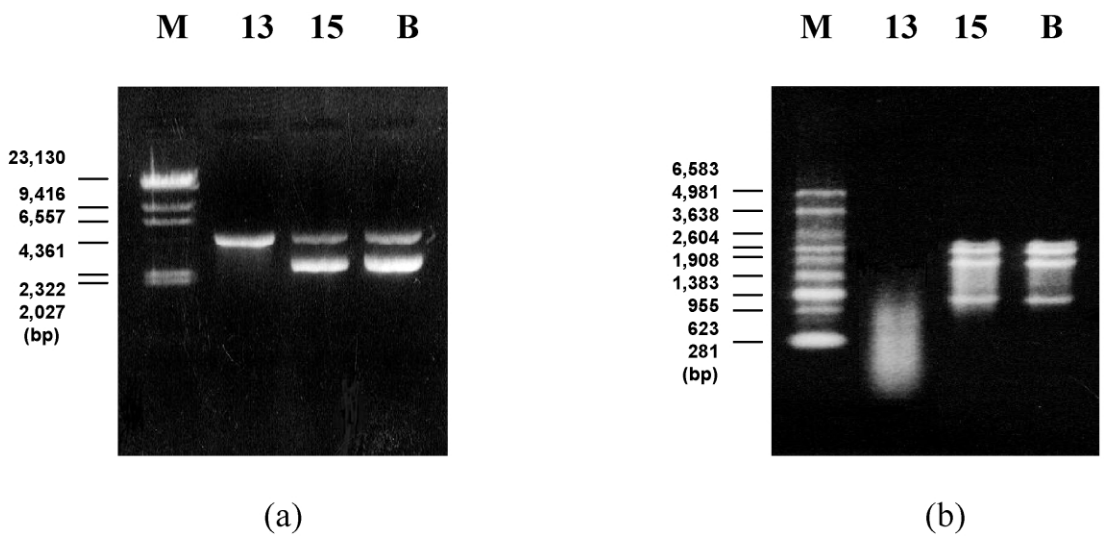
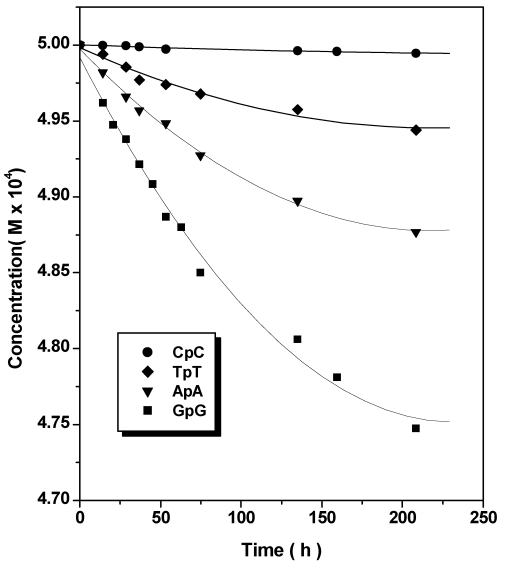
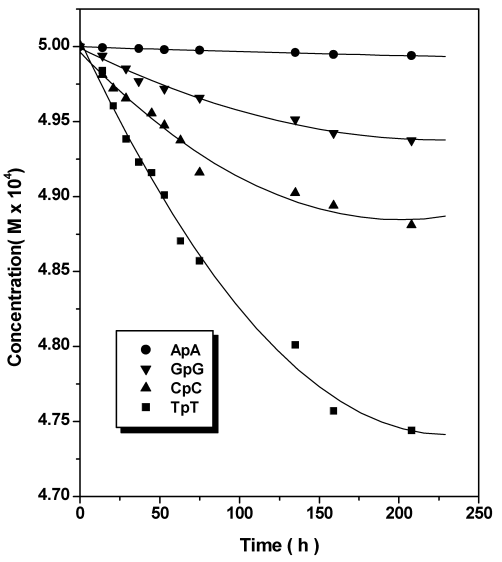


Fig. 3. Electrophoresis diagrams of the reaction mixtures on the agarose gel for (a) supercoiled double-stranded DNA (pBR 322) and (b) RNA (BMV) in the presence of polymer **13**, in the presence of polymer **15**, and in a buffer solution.



(a)



(b)

Fig. 4. Concentration of dinuclotides as a function of time. Conditions: (a) in the presence of polymer **13** and (b) in the presence of polymer **17** at pH 7.4 (Tris-buffer), 50 °C, and $\mu = 0.02$ (KCl).

4. Conclusion

We prepared RNA analogues having ribose rings with *vic-cis*-diol groups as pendants. The analogues showed catalytic activities for the hydrolysis of dinucleotides and the cleavage of nucleic acids. They exhibited the highest catalytic activities for the hydrolysis of dinucleotides with complementary bases. In the biological system, the ribozyme (RNA) contains *vic-cis*-diols of ribose at the 3′-OH termini or at apurinic sites, which might play an important role in its catalytic activity for the cleavage of nucleic acids. We hope that this report will contribute to better understanding of the ribozyme functions.

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