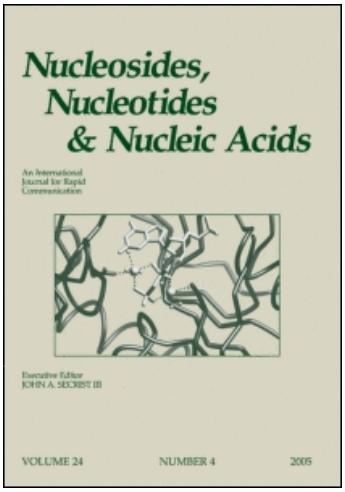

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SYNTHESIS AND CHARACTERIZATION OF POLYAMINE-BASED BIOMIMETIC CATALYSTS AS ARTIFICIAL RIBONUCLEASE

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ABSTRACT

Several polyamine derivatives (**I–V**) conjugated with or without an intercalative moiety were prepared as ribonuclease mimics. Although no DNA-cleaving activity was observed for all compounds tested, mimics **I, III**, and **V** bearing an intercalative moiety along with the primary amine and/or imidazole moieties exhibited potent RNA-cleaving activity at near physiological pH. The RNA-cleaving reactions of the compounds show characteristic bell-shaped pH dependency, and the optimal pH values for **III** and **V** were well correlated to the pK_a values of their active sites, primary amine, and imidazole moieties.

The design and synthesis of a small synthetic molecule that mimics the active center of naturally occurring ribonuclease and is capable of cleaving RNA molecules are an interesting subject. Such artificial nuclease would find several significant applications in the development of new types of therapeutic agents and in the study of molecular biology. For example, the conjugation of an artificial ribonuclease and a nucleic acid binding group, such as an antisense oligonucleotide, will result in a sequence-specific ribonuclease that is capable of cleaving certain mRNA in a sequence-specific manner an-selective manner (2) and will be useful in the study of molecular biology as an artificial restricted RNase that is not known in nature (3).

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Meanwhile, Breslow et al. initiated a series of studies to resolve the mechanism of pancreatic ribonuclease, RNase A, which possesses two histidine groups around the catalytic center (4). Through their studies they proposed that in RNase A, a protonated imidazole moiety of His-119 and a free imidazole moiety of His-12 act as general acid and general base catalysts, respectively, and promote the hydrolysis of the phosphodiester bonds of RNA (4).

Several small organic molecules capable of cleaving RNA in a hydrolytic manner via a possible acid–base catalytic mechanism have also been reported, including simple diamine, guanidine derivatives, imidazole conjugated cyclodextrin (5). More recent reports from several different research groups are quite interesting in terms of RNase mimicking molecule (6–9). These researchers synthesized short peptides containing histidine residues (6) or small molecules containing histamine residues along with an intercalative molecule (7–9). These molecules exhibited potent RNA-cleaving activity and the mechanism of the action of the molecules was assumed to be an acid–base catalytic reaction like that of the natural enzyme mentioned above.

We have also undertaken the study of artificial ribonuclease and reported the preliminary results of the synthesis and the RNA-cleaving activity of polyamine-derived novel artificial ribonuclease (**I–IV**) in a previous paper (10). These compounds were designed to mimic the functions of RNase A. However, one of the imidazole moieties of the natural RNase active center was substituted by a primary amine in the mimics. Thus, the mimics bear a primary amine and/or an imidazole group as catalytic functional groups on certain length of linker arms (Fig. 1). Among the compounds, **I** and **III** exhibited potent RNA-cleaving activity with 1.0 m*M* of concentration at pH 7.4 when 16s/23s ribosomal RNA (16s/23s rRNA) was used as the substrate (6,10). Although the observed cleavage of the RNA molecule was assumed to be hydrolytic in nature, a detailed study to elucidate the mechanism had not been conducted at that time. This situation is also true for most of the analogous RNase mimicking molecules mentioned above.

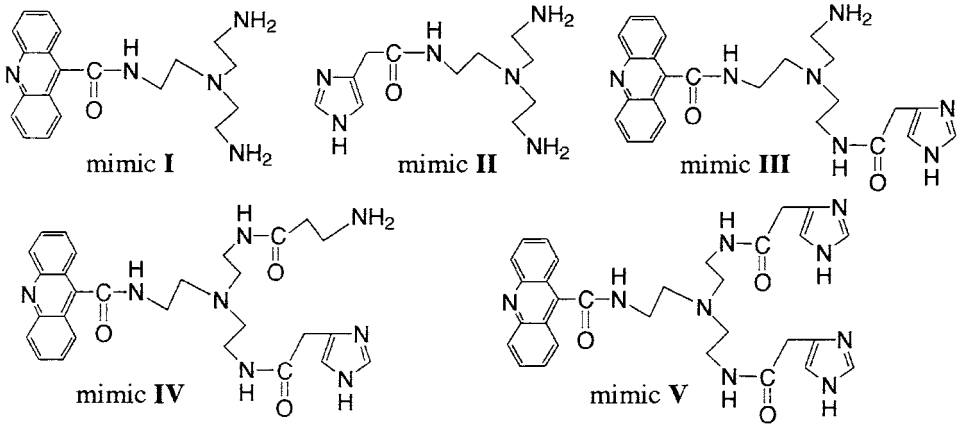

Figure 1. Structure of the RNase mimic compounds.

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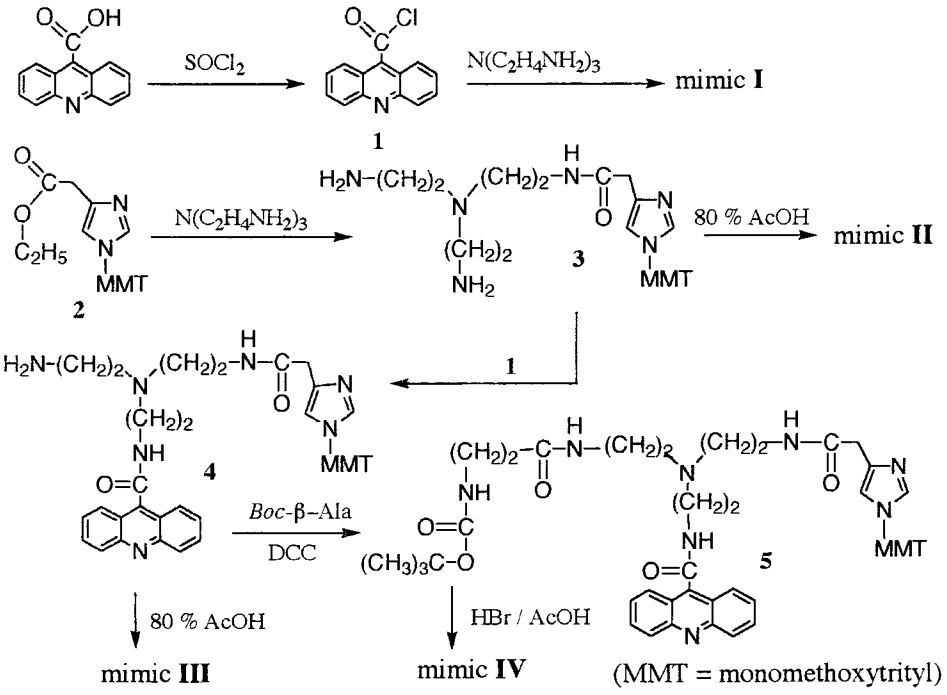
In this paper we wish to describe the results of our further investigation about the mechanism of RNA-cleaving reaction conducted by these polyamine-derived nuclease mimics, as well as the structure–activity relationships. Also, the details of the synthesis of **I–IV** and the new compound **V** having two imidazole moieties as active sites are reported.

Preparation of Artificial Ribonuclease I–IV. The syntheses of artificial ribonuclease **I–IV** mimicking RNase A are outlined in Scheme 1 (10). Reaction of acridine carbonyl chloride (**1**), readily obtained from treatment of acridine carboxylic acid with thionyl chloride, with excess amount of tris(2-aminoethyl)amine gave mimic **I** as a major product (66.5%) along with some di- and a very little of tri-substituted by-products. The desired product was separated from the by-products by silica gel column chromatography. Monomethoxytritylated imidazole carboxylic acid ethyl ester (**2**) (11) was also readily reacted with the same amine to give compound **3**, which was then converted to mimic **II** by brief treatment with 80% acetic acid to remove the trityl group. The treatment of **3** with equimolar of acridine carbonyl chloride (**1**) in CH_2Cl_2 gave compound **4** in moderate yield (52.5%) along with a di-substituted by-product which was easily removed from the desired product by silica gel column chromatography. Compound **4** was detritylated in the same manner as above to give mimic **III**. Condensation of **4** with *Boc*-β-alanine in CH_2Cl_2 in the presence of dicyclohexylcarbodiimide (DCC) (12) gave compound **5**. The treatment of **5** with 30% HBr in glacial acetic acid effected



Scheme 1. Preparation of RNase mimics **I–IV**.

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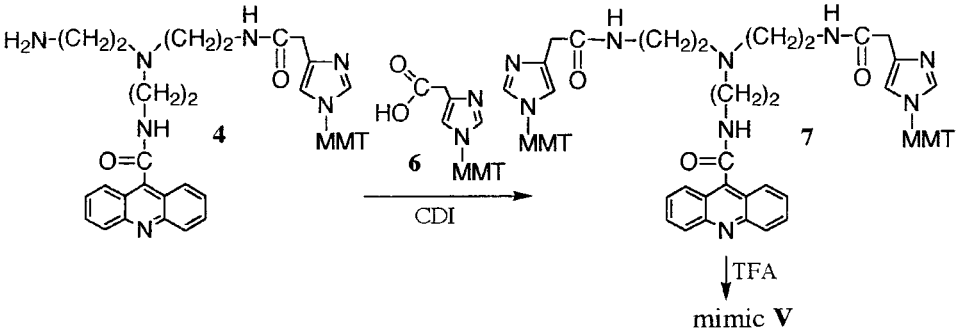

Scheme 2. Preparation of RNase mimic **V**.

complete deprotection (13) to afford mimic **IV**. Mimic **V** bearing two imidazole groups was also prepared by the condensation of **4** and monomethoxytritylated imidazole carboxylic acid (**6**) (14) in the presence of carbonyl diimidazole as a condensation reagent. Subsequent removal of the protecting group as depicted in Scheme 2 gave mimic **V**. All the mimic compounds were purified by either silica gel column chromatography, recrystallization, or a combination of both. These details are described in the Experimental Section.

RNA Cleaving Activity. 16s/23s ribosomal RNA (16s/23s rRNA) and a plasmid DNA (pBR322) were used as the substrates in the cleavage reaction since the nucleic acids possess double-stranded regions and are expected to provide the binding sites for the intercalative moiety of some of the mimic compounds (6,7). The cleaving activity of the mimics was monitored by denaturing agarose gel electrophoretic analysis through the experiments. Figure 2 displays typical degradation of the RNA substrate (0.1 OD unit) generated by mimics **I–V** (1 m*M*) under near physiological condition at 37°C. The substrate was simply mixed with the mimics (1 m*M*) and allowed to react for 1 h in PBS buffer (pH 7.4). The reaction mixture was subjected to electrophoresis through denaturing agarose gel. The fainting in the main bands in lanes 3–7 along with the generation of smeary ladder bands was observed by the following ethidium bromide staining of the gel. This is due to the cleavage of intact RNA. As shown in Figure 2, mimics **I, III**,

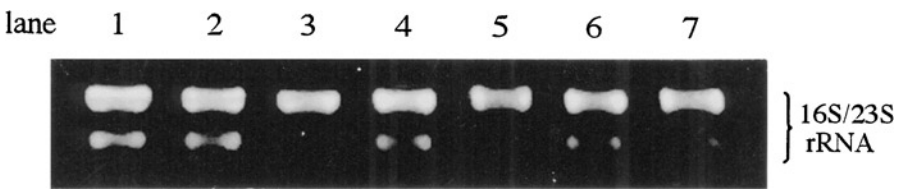

Figure 2. Degradation of 16s/23s rRNA promoted by the RNase mimic compounds. Lane 1: 0-h negative control; lane 2: 1-h negative control (incubation of the RNA without the mimic compound); lane 3: 1-h reaction with mimic **I**; lane 4: 1-h reaction with mimic **II**; lane 5: 1-h reaction with mimic **III**; lane 6: 1-h reaction with mimic **IV**; lane 7: 1-h reaction with mimic **V**. The details of the reaction conditions are described in the Experimental Section.

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and **V** exhibited potent RNA-cleaving activity within 1-h-reaction. The cleavage efficiency of the substrate RNA was estimated by photodensitometric reading of the remaining intact RNA on the gel. The experiment was repeated three times and the average amounts of cleavage resulted in the experiments are none (0-h) – 0; none (1-h) – 6.6; **I** – 41.0; **II** – 11.7; **III** – 56.9; **IV** – 14.3; **V** – 22.5. The results thus obtained for mimics **I–IV** were in good agreement with the previous results (10). Among the compounds, mimic **III** was the most active. Marked difference in activity was observed between mimics **II** and **III** in which the intercalative moiety is absent in mimic **II**, otherwise, both compounds have the same functional moieties. The results indicate that the presence of an intercalative moiety, which presumably acts as the binding group to the RNA, is essential to exhibit the activity (7).

The difference in activity between mimics **III** and **IV** is rather interesting since the compounds have exactly the same active sites, primary amine, and imidazole moieties, along with the intercalative moiety. In mimic **IV**, however, a linker moiety of the primary amine moiety is elongated by the insertion of extra methylene bridges compared to mimic **III**. Therefore, the relative geometry between the primary amine moiety and the imidazole moiety between **III** and **IV** is different. The activity of mimic **V** bearing two imidazole moieties both connected to the same length of linker group in mimic **IV** is also somewhat lower compared to mimic **III**. These results may reflect the fact that the RNA-cleaving activity was brought about by the cooperative action of both active sites. In addition, the active sites should occupy a precise spatial position to accomplish favorable interaction with target site to promote efficient cleavage of the target, as was suggested before (6,7).

None of the mimics exhibited cleaving activity on the double-stranded DNA (pBR 322) substrate in the same experiment even after prolonged incubation (48 h, data not shown), indicating that the presence of 2′-OH in the substrate is essential for the cleavage (15).* Thus, the cleaving activity of mimics **I, III**, and **V** is specific for RNA substrate. It should be noted that the cleavage of the RNA by the mimics was not affected by the presence of a radical trapping agent such as DMSO and DUBCO (data not shown).

Nucleic Acid Binding Activity. Nucleic acid binding activity of mimics **I, III, IV**, and **V** was determined by a spectrophotometrical titration method at pH 7.4. Double-stranded DNA (calf thymus) was used as the nucleic acid component instead of RNA to avoid possible degradation of the nucleic acid during the experiment. The absorption of the mimic compounds at 360 nm, which corresponds to the λ_{max} of the acridine moiety in the mimics, steadily decreased as the amount of the DNA was increased (data not shown). The observed hypochromic effect indicates that the interaction between the mimic compounds and the DNA proceeds upon the mixing

*Takagi et al. reported the cleavage of double-stranded DNA by a Cu(II) complex of polyamine-linked DNA-intercalator[1] which is an analogous compound of mimic **I**. We have also observed a cleavage of pBR322 DNA by mimic **I** under the presence of an equal amount of Cu(II) ion. The magnitude of the cleavage was, however, modest to low in our results (data not shown).

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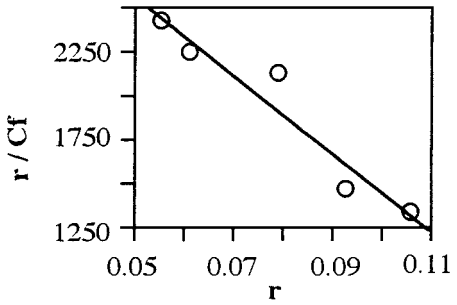

Figure 3. Scatchard plots of mimic **III** by UV-vis spectrophotometric titration.

of these (16). The decrease in the absorption of **III** at 360 nm, where the DNA has no absorption, was converted to Scatchard plots, as shown in Figure 3 as a typical example, and the binding constant (k) was calculated to be $2.2 \times 10^4\ M^{-1}$ by the Scatchard equation (17,18). The binding constants of **I, IV**, and **V** were obtained in a similar manner and are listed in Table 1.

As indicated in Table 1, it is confirmed that the compounds bearing an intercalative moiety have potent binding ability to the double-stranded nucleic acid. It would be safe to assume that the compounds also have nearly the same magnitude of binding ability toward the rRNA (19). Mimics **III–V** showed almost the same binding activity that was slightly smaller than that of mimic **I**. This may be attributed to the presence of two primary amine groups in mimic **I** since the groups are expected to exist in protonated form (see the following section) and can interact with the negatively charged backbone of nucleic acid more strongly than the other mimics (20).

The protonation constants of the active sites of mimics **I, III**, and **V** were determined by a potentiometric titration method. HCl-Salts of the mimics were used for the titration in the presence of 0.1 M NaCl. The titrated data were analyzed by a computer program, SUPERSQUAD (21), to calculate the log values of the protonation constants (pK_a values) and these are listed in Table 2.

The pK_a values for the proposed active site in **III**, the imidazole and the primary amine moieties, thus obtained are 6.48 and 9.57, respectively. Based on this, it is evident that at the pH of the cleaving reaction (pH 7.4), the imidazole exists

Table 1. Nucleic Acid Binding Constant (K) of the Mimics Estimated by the Scatchard Plots

Mimic Compound	$K(M^{-1})$	n
I	1.0×10^5	0.1
III	2.2×10^4	0.2
IV	1.3×10^4	0.1
V	1.1×10^4	0.1

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Table 2. The pK_a Values of Active Sites in Mimics **I, III**, and **V**

Mimic Compound	**I**	**III**	**V**
Active site	$-NH_2$, 8.84	−Im, 6.48	−Im, 6.07
and its pK_a	$-NH_2$, 9.71	$-NH_2$, 9.57	−Im, 7.53

as an unprotonated form whereas the primary amine exists as a protonated form predominantly. The pK_a values of the active sites of mimics **I** and **V** were determined in a similar manner and are also given in Table 2. The pK_a values of the imidazole groups in mimic **V** indicate that these exist in almost equimolar equilibrium between the protonated and unprotonated forms at near-neutral to slightly acidic pH range. Contrary to this, the pK_a values of two primary amine groups in mimic **I** were considerably higher than 7.4. This means both of the amines in mimic **I** exist as predominantly protonated form at the pH of the nucleic acid cleaving reaction described in the earlier section.

It is well known that the rate of cleavage versus pH shows a characteristic bell-shaped relationship in the case of RNase A-mediated cleavage of RNA (Breslow (5); (22)). This is accounted for by the involvement of two essential imidazole moieties of histidine residues (His-12 and His-119) acting as general base and acid catalysts (4). Therefore, we examined the pH dependency of the RNA-cleaving reaction to further elucidate the mechanism of the mimics **I, III**, and **V** (Fig. 4).

As shown in Figure 4B, the total amount of the cleavage as a function of the pH of the reaction exhibited a bell-shaped profile in the case of compound **III** as it was reported previously on the relevant compounds (7). The optimal pH for the reaction of **III** estimated from Figure 4B is about 7. The results strongly suggest that the reaction really proceeds via an acid–base catalytic mechanism. Furthermore, considering the result obtained in the foregoing section, the primary amine and the imidazole in mimic **III** are assumed to predominantly exist as the protonated and unprotonated forms, respectively, at the pH. Thus, in mimic **III** the protonated primary amine and the unprotonated imidazole of **III** act as acid–base catalysts,

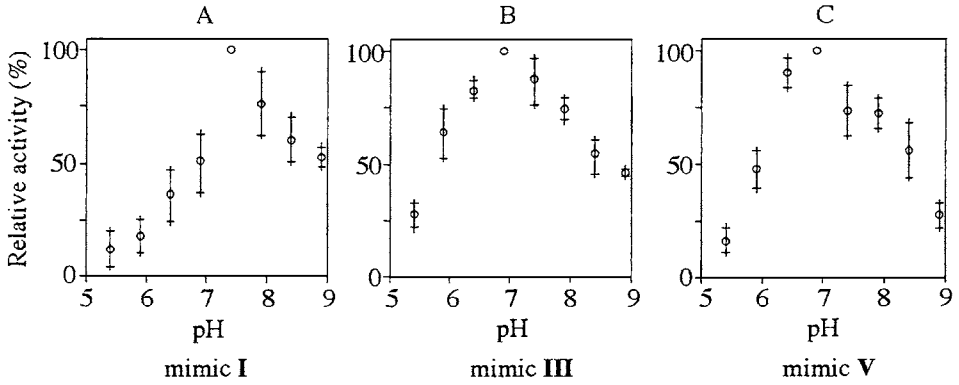

Figure 4. The pH dependent activity of the RNase mimic compounds.

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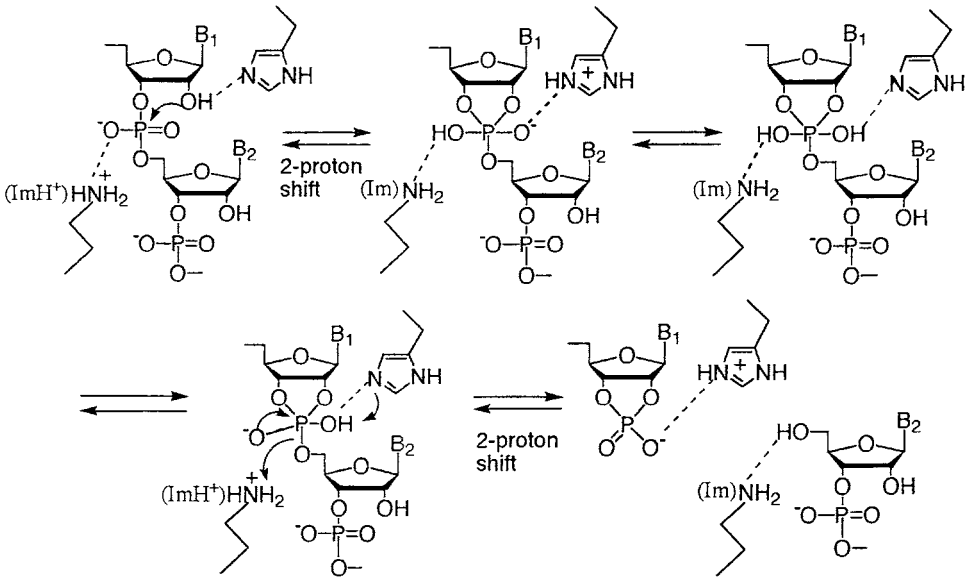
respectively, in the RNA-cleaving reaction. Essentially the same result was obtained for compound **V** in which the optimal pH value was also about 7 (Fig. 4C) and two imidazole groups of **V** were supposed to exist in an almost equimolar equilibrium between the protonated and unprotonated forms at that pH (see Tab. 2).

Thus, the reaction mechanism of mimics **III** and **V** could be accounted for by a general base and general acid hydrolysis mechanism like a natural RNase A-type enzyme. In this mechanism, a free amine or imidazole group acts as the general base, which deprotonates 2′-OH of the target. On the other hand, a protonated imidazole group acts as the general acid that would transfer its proton to negatively charged oxygen on the phosphodiester linkage being cleaved. This is summarized in Scheme 3. It may be worth noting that the RNA-cleaving activity of mimic **III** at near optimal pH (pH 6.9) for the two mimics was higher than that of mimic **V** (data not shown).

In the case of compound **I**, some discrepancies exist between the optimal pH value for the RNA cleaving reaction and the pK_a values (8.84 and 9.71, see Tab. 1) of the primary amine moieties. At the optimal pH of **I** (Fig. 4A, pH 7.4), both of the primary amine moieties of compound **I** are expected to exist as protonated form. Therefore, these could act as a general acid catalyst, but not as a general base catalyst. At this stage we do not understand the exact reason behind this observation. It may be possible that 1,3-bis[tris(hydroxymethyl)methylamino]propane, the buffer component used for the study of pH dependence of RNA cleaving reaction, is involved in the reaction since the compound is a polyimine derivative and, at least, one of its imino functions would be able to act as the base component in



Scheme 3. Proposed mechanism of RNA cleavage by RNase mimic **III** (and **V**) (Breslow et al. J. Am. Chem. Soc. (4)).

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the reaction (23) whereas the primary amine moieties of compound **1** may act as the general acid. In any case, the pH dependence of the reaction also supports the general base and acid type of reaction mechanism in the RNA-cleaving reaction mediated by compound **I** and, therefore, the reaction is hydrolytic in nature.

Conclusion. We have successfully synthesized several polyamine derivatives mimicking the active center of pancreatic ribonuclease (RNase A). Among them, mimics **I, III**, and **V** bearing an intercalative moiety along with the active sites exhibited potent RNA-cleaving activity at near-physiological condition. In particular, mimic **III** cleaved almost 60% of the substrate. Meanwhile, these mimics did not show any detectable DNA-cleaving activity under the same conditions. The result indicates that the existence of a nucleic acid binding function in the mimic compound as well as the 2′-OH group in the substrate molecule are the indispensable factors for the derivatives to show nucleic acid-cleaving activity. The RNA-cleaving reaction mediated by the above compounds showed characteristic bell-shaped pH dependency. Thus, the reaction of these compounds proceeds through a general base–acid type of hydrolytic mechanism as that of natural RNase. Considering this with the results of the pK_a analysis of the mimic's active sites, one of the two active sites existing as a protonated form may act as the acid catalyst. Meanwhile, the other active site existing as an unprotonated form may act as the base catalyst at that pH.

The study also indicates that the RNA-cleaving activity was brought about by the cooperative action of the two active sites, therefore, consideration should be taken to design RNase mimic compounds in order to achieve precise interaction of the active sites to their target sites.

The current results will help to design potent RNA-cleaving agents. In our group, a study to incorporate such an agent into a molecule that is able to recognize RNA in sequence-specific manner (24),* is now in progress and the results will be reported elsewhere.

EXPERIMENTAL

Ribosomal RNA(16s/23s) and calf thymus DNA were purchased from Boehringer Mannhaim. ^{1}H NMR spectra were measured on a Varian Gemini-200 spectrometer. All chemical shifts (δ) are reported in ppm down-field from internal tetramethylsilane (TMS) or 3-(trimethylsilyl)propionic acid sodium salt (TMSP). J values are given in Hz. Melting points were determined on a Yanagimoto MP-500D electrothermal apparatus and uncorrected.

*A recent report of Ushijima et al. describes a site-specific cleavage tRNA mediated by β-oligoDNA functionalized at the 5′-terminus with polyamine derivatives[26] which are essentially same as mimic **I** and **III** described in this article.

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2-[(Acridin-9-yl)carbonyl]amino-2′,2″-diaminotriethylamine (I). 9-Acridine carboxylic acid (400 mg, 1.8 mmol) was treated with excess thionyl chloride in benzene, then the mixture was evaporated to dryness. The residue was dissolved in dichloromethane and the solution was added by dropwise to a stirred solution of tris(2-aminoethyl)amine (1.3 g, 3.7 mmol) in dichloromethane under cooling in an ice-bath. After 30 min of stirring, the mixture was evaporated in vacuo. The residue was purified by silica gel chromatography, using the mixture of dichloromethane:methanol:conc. ammonia (4:1:0.1) to afford 421 mg (1.19 mmol, 66.5%) of mimic **I**, m.p. 55.6–55.8°C (Found: C, 65.04; H, 7.28; N, 18.74. $C_{20}H_{25}N_5O \cdot 1H_2O$ requires C, 65.05; H, 7.37; N, 18.96%); δ_H(200 MHz; D_2O) 2.63–2.77 (8H, m, $(-(CH_2)_2-(NH_2)_2)$, 2.83 (2H, t, $J = 5.5$, $-CH_2-CH_2-NH-CO-$), 3.77 (2H, t, $J = 5.5$, $-CH_2-CH_2-NH-CO-$), 7.60–8.02 (8H, m, Acr).

Purified mimic **I** (100 mg, 0.27 mmol) was dissolved in dichloromethane (20 ml). HCl gas was bubbled through the solution for 20 min under cooling in an ice-bath. The deposited, very hygroscopic crystals were collected by filtration and washed with dichloromethane, then quickly dried under reduced pressure in a desiccator overnight. The yield of mimic **I** · 5HCl salt thus obtained was almost quantitative (93.3%) (Found: C, 43.59; H, 5.76; N, 12.78. $C_{20}H_{25}N_5O \cdot 5HCl \cdot 1H_2O$ requires C, 43.71; H, 5.86; N, 12.75%).

2-[1-*H*-(Imidazol-4-yl)acetyl]amino-2′,2″-diaminotriethylamine (II). 1-Dimethoxytrityl-1-*H*-imidazole-4-acetic acid ethyl ester (**11**) (355 mg, 0.78 mmol) was treated with excess of tris(2-aminoethyl)amine at room temperature for 2 days. The whole mixture was applied for silica gel column chromatography and the product was eluted by a mixed solvent of dichloromethane:methanol:conc. ammonia (4:1:0.25). The isolated imidazole acetic acid conjugate of tris(2-aminoethyl)amine (**1**) (332 mg, 76.5%) was treated with 80% acetic acid for 30 min, then the mixture was evaporated to dryness. The residue was applied on silica gel chromatography, and the product was eluted with the mixture of dichloromethane:methanol:conc. ammonia (1:1:0.3) to afford 140 mg (91.7%) of mimic **II**. The compound was further purified as a hydrochloride salt, m.p. 163.0–164.0°C (Found: C, 32.71; H, 6.62; N, 20.51. $C_{11}H_{22}N_6O \cdot 4HCl \cdot 0.5H_2O$ requires C, 32.42; H, 6.68; N, 20.64%); δ_H(200 MHz; D_2O) 2.82 (2H, t, $J = 7$, $-CH_2-CH_2-NH-CO-$), 2.91–3.21 (8H, m, $(-(CH_2)_2-NH_2)_2)$, 3.40 (2H, t, $J = 7-CH_2-CH_2-NH-CO-$), 7.37 (1H, s, H-5 of Im), 8.68 (1H, s, H-2 of Im).

2-[1-*H*-(Imidazol-4-yl)acetyl]amino-2′-[(acridin-9-yl)carbonyl]amino-2″-aminotriethy lamine (III). 9-Acridine carboxylic acid (170 mg, 0.76 mmol) was treated with excess thionyl chloride in benzene (10 mL), then the mixture was evaporated to dryness. The residue was dissolved in dichloromethane and the solution was added dropwise to a stirred mixture of imidazole acetic acid conjugate of tris(2-aminoethyl)amine (**1**) (423 mg, 0.76 mmol) and triethylamine (3.8 mmol) in dichloromethane under cooling in ice-bath. After 30 min of stirring, the insoluble matter was removed by filtration and the filtrate was evaporated in vacuo. The residue was purified by silica gel chromatography, using the mixture of

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dichloromethane:methanol:conc. ammonia (95:5:1) to afford compound (**2**) (304 mg, 52.5%). Compound **2** was treated with 80% acetic acid for 30 min, then evaporated to dryness. Silica gel chromatography of the residue, using the same mixture as above and the recrystallization from ethanol afforded mimic **III** (131 mg, 71.5%), m.p. 75.0–75.5°C (Found: C, 62.59; H, 6.52; N, 20.41. $C_{25}H_{29}N_7O_2 \cdot 1H_2O$ requires C, 62.88; H, 6.54: N, 20.53%); δ_H(200 MHz; D_2O) 2.58–2.79 (6H, m, $-(CH_2)_2-NH_2$ and $-CH_2-CH_2-NH-CO-$Im), 2.81 (2H, t, $J=5$, $-CH_2-CH_2-NH-CO-$Acr), 2.88 (2H, s, $-CH_2$Im), 3.28 (2H, t, $J=6$, $-CH_2-CH_2-NH-CO-CH_2-$Im), 3.77 (2H, t, $J=5$, $-CH_2-CH_2-NH-CO-$Acr), 6.53 (1H, s, H-5 of Im), 7.27 (1H, s, H-2 of Im), 7.63–8.25 (8H, m, Acr).

2-(β-Alanyl)amino-2′-[1-*H*-(imidazol-4-yl)acetyl]amino-2″-[(acridin-9-yl)carbonyl] aminotriethy lamine (IV). Compound **2** (145 mg, 0.19 mmol) was reacted with *N*-(*tert*-butoxycarbonyl) $-\beta$-alanine (*Boc*-β-alanine) (49 mg, 0.26 mmol) in dichloromethane in the presence of dicyclohexylcarbodiimide (DCC) (160 mg) for 24 h. The reaction was quenched by the addition of methanol and the mixture was allowed to stand at 4°C overnight. The deposited urea was removed by filtration and the filtrate was evaporated in vacuo. The obtained crude mixture of compound **3** was treated with 30% HBr in acetic acid. Addition of ether to this mixture caused precipitation and the supernatant was removed by decantation. After repetition of the same treatment, the precipitates were dissolved in water (10 mL) and extracted with ethylacetate (20 mL × 3). The separated aqueous layer was adjusted the pH at around 11 with triethylamine and allowed to stand at 4°C overnight. The solution was concentrated in vacuo and the residue was applied for silica gel column chromatography, using the mixture of dichloromethane:methanol:conc. ammonia (1:1:0.1) to afford mimic (**IV**) (82 mg, 83.2%), m.p. 67.5–68.0°C (Found: C, 61.47; H, 6.58; N, 20.27. $C_{28}H_{34}N_8O_3 \cdot 1H_2O$ requires C, 61.28; H, 6.62; N, 20.43%); δ_H(200 MHz; CD_3OD) 2.49 (2H, t, $J=6.5$, $H_2N-(CH_2)_2-CO-NH-CH_2-CH_2-$), 2.64–2.82 (4H, m, H_2N-CH_2- and $-CH_2-CH_2-NH-CO-CH_2-$Im), 2.85 (2H, t, $J=6$, $-CH_2-CH_2-NH-CO-$Acr), 3.02 (2H, t, $J=6.5$, $H_2N-(CH_2)_2-CO-NH-CH_2-$), 3.21–3.39 (4H, m, $HN-CH_2-CH_2-$ and $-CH_2-NH-CO-CH_2-$Im), 3.29 (2H, s, $-CH_2-$Im), 3.72 (2H, t, $J=6$, $-CH_2-NH-CO-$Acr), 6.86 (1H, s, H-5 of Im), 7.51 (1H, s, H-2 of Im), 7.65–8.20 (8H, m, Acr).

2,2′-Bis-[1-*H*-(imidazol-4-yl)acetyl]amino-2″-[(acridin-9-yl)-carbonyl]-aminotriethy lamine (V). Mono-methoxytritylated imidazole carboxylic acid sodium salt (**6**) (14) (159 mg, 0.40 mmol) and carbonyl diimidazole (326 mg, 3.0 mmol) were dissolved in dry THF (20 mL) and the solution was stirred at 0°C for 2 h under argon atmosphere. To this solution the THF solution (20 mL) of compound **4** (200 mg, 0.267 mmol) was added and the whole mixture was stirred at 0°C for 3 h, then at room temperature for 12 h under argon atmosphere. After quenching the reaction by the addition of dry ethanol (1 mL), the mixture was evaporated to dryness. The residue was dissolved in CH_2Cl_2 (50 mL) and the organic portion was washed with water (30 mL × 2). The obtained organic layer was dried over $MgCl_2$, then evaporated to a thick oil, which was supposed to contain

compound **7**. The oil was treated with trifluoroacetic acid (2 mL) for 20 min, then evaporated to dryness. The residue was purified by silica gel column chromatography, using the mixture of dichloromethane:methanol:conc. ammonia (4:1:0.2) to afford mimic **V** (39 mg, 17.1%), m.p. 75.8–76.2°C (Found: C, 53.98; H, 6.26; N, 18.73. $C_{30}H_{33}N_9O_3 \cdot 5.5H_2O$ requires C, 54.03; H, 6.66; N, 18.91%); δ_H(200 MHz; D_2O) 2.69–2.79 (4H, m, 2 × $-CH_2-CH_2-$NH−CO−Im), 2.88 (2H, t, $J = 6$, $-CH_2-CH_2-$NH−CO−Acr), 3.11–3.28 (4H, m, 2 × $-CH_2-CH_2-$NH−CO−Im), 3.18 (4H, s, 2 × $-CH_2-$Im), 3.64 (2H, t, $J = 6$, $-CH_2-CH_2-$NH−CO−Acr), 6.75 (2H, s, 2 × H-5 of Im), 7.44–7.98 (10H, m, 2 × H-2 of Im and Acr).

The Assay of RNA Cleaving Activity. 16s/23s rRNA (0.1 unit A_{260}) was allowed to react with or without mimic compounds (1 m*M*) in 10.4 μL of PBS buffer (50 m*M*, pH 7.4) containing 120 m*M* of NaCl and 2.7 m*M* of KCl at 37°C for an appropriate period. After incubation, the whole reaction mixture was subjected to electrophoresis through denaturing (18% formaldehyde) agarose gel (1.0%), followed by ethidium bromide staining. The amount of remaining intact RNA was estimated by photodensitometric reading of the gel by ATTO AE-6900 M Densitograph connected with Apple computer system. The experiment was repeated three times and the average values of the intact RNA were calculated from the results obtained by the above procedure.

Nucleic Acid Binding Activity. Nucleic acid-binding activity of the mimics was determined by UV- vis spectrophotometrical titration method using calf thymus DNA in a Tris–acetate buffer (pH 7.4). The binding constants (K) of the mimics with DNA were estimated by the Scatchard plot method (17) based on the result of the titration. A fixed amount (0.1 m*M*) of the mimics was titrated with DNA over the range of DNA concentration of 0–2.5 m*M*. The UV spectra were measured after equilibration. The differences in the absorbance at 360 nm in the acridine moiety of the mimics were applied to the following Scatchard equation $r/Cf = K(n - r)$ (19). Here, K is the association constant of the mimic with DNA, r is the number of mimics bound per DNA nucleotide residue, where $r = Cb/Cp$, Cf is the molar concentration of the free mimic, and n is the number of binding sites per DNA. Cb is the concentration of the bound mimic and Cp is the input concentration of the DNA (18).

The Measurement of pK_a Values. The acid–base equilibria were determined by potentiometric titration of 1.0 m*M* of mimic–HCl salts and I = 0.10 (NaCl) at 25.0°C, using a Fisher Accumet pH meter. The titration points were obtained from separately prepared solutions to which were added desired quantity of 0.10 *M* NaOH solutions. The titrated data were analyzed by a computer program, SUPERSQUAD (21), to calculate the log values of the protonation constants (pK_a values).

pH Dependence of the RNA Cleavage Reaction. 16s/23s rRNA (0.1 unit A_{260}) was allowed to react in the presence of each mimic compound (1 m*M*) at 37°C for 1 h. MES buffer (50 m*M*) containing 120 m*M* of NaCl and 2.7 m*M* of KCl was used for the reactions at pH of 5.4, 5.9, and 6.4. 1,3-Bis[tris(hydroxymethyl) methylamino]-propane–HCl buffer (50 m*M*)) containing 120 m*M* of NaCl and 2.7 m*M* of KCl was used for the reactions at pH of 6.9, 7.4, 7.9, 8.4, and 8.9.

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The total volume of each reaction mixture was 10.4 μL. After the reaction, the whole reaction mixtures were subjected to electrophoresis through denaturing (18% formaldehyde) agarose gel (1.0%), followed by ethidium bromide staining quantitation of RNA cleavage was done by photodensitometrically using ATTO AE-6900 M Densitograph connected with Apple computer system as described above. The experiment was repeated three times.

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