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SYNTHESIS AND RNA CLEAVING ACTIVITIES OF POLYAMINE DERIVED NOVEL ARTIFICIAL RIBONUCLEASE

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Abstract: Novel polyamine derivatives (I-IV) bearing imidazole and/or primary amine groups were prepared as artificial ribonuclease. Among the derivatives the compound (III) which has an imidazole and a primary amine groups as catalytic active sites at the ends of certain length of linker arms along with a intercalative RNA binding site exhibited the most potent RNA cleaving activity.

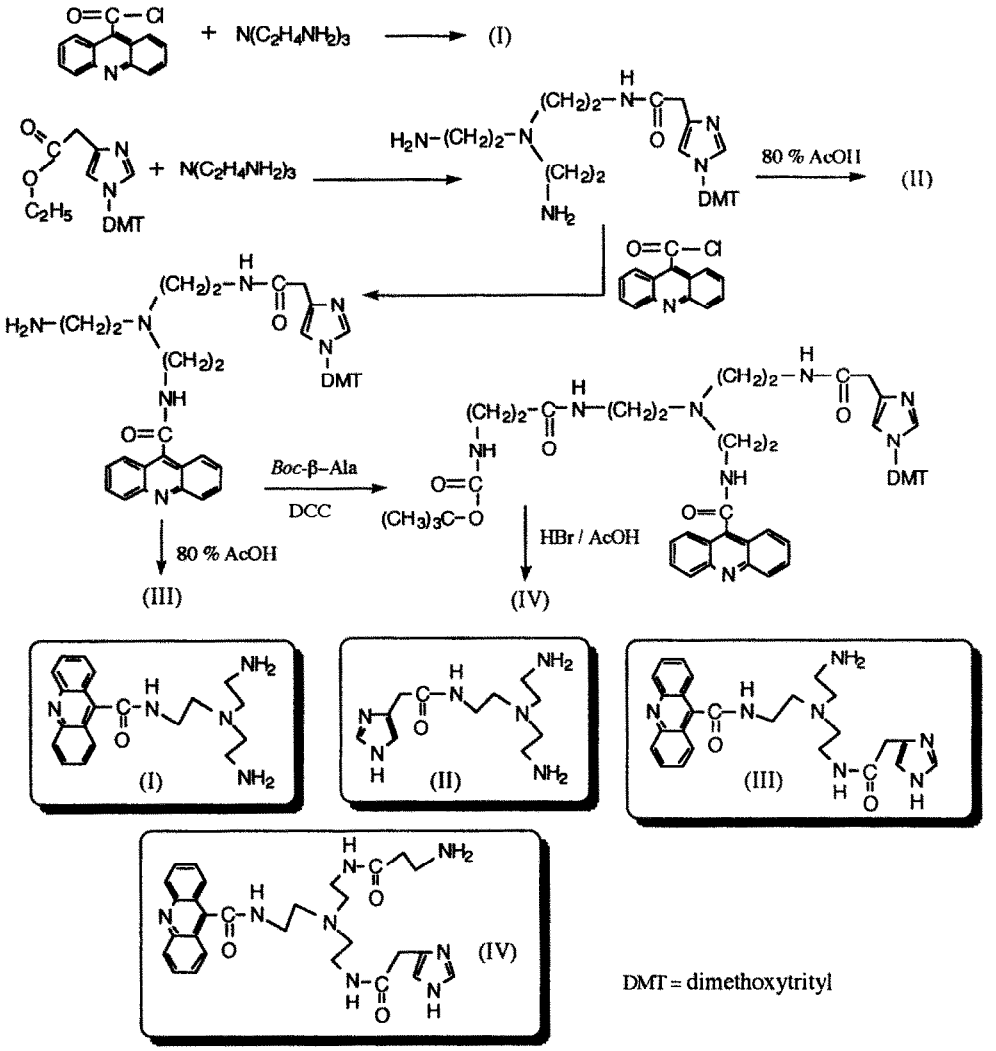
It is a great interest to create artificial nuclease which specifically cleave ribonucleotide in a development of a new therapeutic agent. For example, the artificial nuclease conjugated with antisense oligonucleotide would cleave the target messenger RNA sequence specifically to prevent the gene expression[1]. Such conjugates would also be useful in a study of molecular biology as an artificial restricted RNase[2] which is not known in nature. However, attempts to develop a small synthetic organic molecule which mimics naturally occurring ribonuclease possessing efficient RNA cleaving activity has not been successful yet. Most of the RNA cleaving agents hitherto reported involve metal complexes[3], simple amines[4] and imidazole conjugates of cyclodextrin [5] as cleaving reagent.

During our attempt to develop simple synthetic molecules which mimic naturally occurring RNase, two intriguing reports by C-H. Tung *et al.* and by M. A. Podyminogin *et al.* have appeared recently[6,7]. In the latter report, particularly, intercalator conjugated short peptides bearing two histamine residues showed high RNA cleaving activity through a hydrolytic manner. These prompted us to report the design and synthesis of novel polyamine derivatives as artificial ribonuclease system. These derivatives bear a primary amine and/or an imidazole groups on certain length of linker arms as acid-base catalytic sites along with or without intercalating agent. The cleaving activities of the mimic compounds towards ribosomal RNA is also reported.

The design of the RNase mimic is based on the proposed mechanism of RNase A. In RNase A, a protonated imidazole moiety of His^{119} and a free imidazole moiety of His^{12} are supposed to act as acid-base catalysts, respectively and promote the hydrolysis of phosphodiester bonds of RNA[8]. In our mimic compounds, however, a primary amine is substituted for one of the imidazole moieties of natural RNase. We chose ribosomal RNA(16S/23S rRNA) as the substrate and a intercalating agent, acridine, as the simplified binding moiety to the substrate. Ribosomal RNA is known to possess several double stranded regions which are expected to provide the binding sites for the intercalator.

The general synthetic scheme and the structures of the mimic compounds (I-IV) are illustrated in Scheme 1. The DMT bearing imidazole acetic acid ethyl ester was prepared according to the literature method[9]. Obtained

mimic compounds were purified either by silica gel column chromatography using the mixture of CH_2Cl_2 : MeOH : conc. ammonia (4 : 1 : 0.1 for mimic I; 1 : 1 : 0.25 for mimic II; 1 : 1 : 0.1 for mimic IV) or recrystllization (ethanol for mimic III). In the case of mimic (II), the column purified oily material was converted to the HCl salt and recrystallized from ethanol[10].



Scheme 1 Preparation and the structures of synthetic RNase mimic compounds

Degradation of the rRNA by the mimic compounds was analyzed by denaturing agarose gel (1 %, containing 2.2 M of formaldehyde) electrophoresis. As shown in Fig. 1, degradation of the rRNA by the mimic compounds (1 mM) was observed within 1-h with different extent [11]. When double stranded DNA (pBR 322) was used as the substrate, no degradation of nucleic acid was detected even after 48-h (data not shown).

Although it is hard to elucidate the exact mechanism of the degradation, these data suggest that the observed degradation of rRNA is hydrolytic in nature, in which 2'-OH is participated, and not oxidative[12]. It should also be noted that the addition of divalent metals, such as Cu^{2+}, Mg^{2+}, Zn^{2+}, to the reaction mixture in the presence or absence of mimic compounds showed only a small effect toward the degradation of the rRNA.

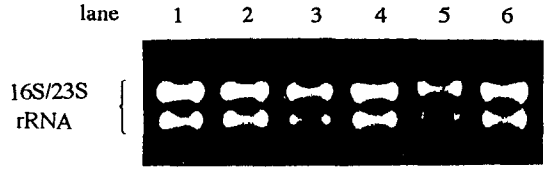


Fig. 1 RNA cleaving activity of mimic compounds. The cleavage reactions were carried out at 37°C for 1-h in 50 mM PBS buffer (pH 7.4) containing 0.1 unit (A_{260}) of 16S/23S rRNA in the presence (1 mM) or absence of mimic compounds. lane 1; 0-h negative control. lane 2; 1-h negative control. lane 3; reaction in the presence of mimic (I). lane 4; reaction in the presence of mimic (II). lane 5; reaction in the presence of mimic (III). lane 6; reaction in the presence of mimic (IV).

Table 1. Amounts of the remaining intact 16S/23S rRNA estimated by the photodensitometric reading of the gel electrophoresis results.

mimic compound	none (0-h)	none (1-h)	(I)	(II)	(III)	(IV)
intact rRNA (%)	100	92.8	57.4	93.0	42.3	84.7

We estimated the activity of the mimics by photodensitometric reading of the intact rRNA remaining on the gel. The order of the activity was found to be as follows; mimic (III) > (I) >> (IV) > (II) (Table 1). Observed low activity of the mimic (II) is understandable since this mimic lacks the binding site to the substrate. The most efficient degradation of the rRNA brought by the mimic (III) is presumably due to the existence of imidazole moiety in (III). Under the condition for the assay (pH 7.4), about 75 % of imidazole moiety is expected to exist as free form[13]. On the other hand, the primary amine group may exist as a protonated form. Thus, in the mimic (III) a primary amine and an imidazole will nicely act as acid-base catalysts, respectively.

Considerably high activity observed for the mimic (I) can be explained, at least in part, by the strong interaction of the mimic (I) to the substrate. The binding constants (K) for the mimics (I) and (III) towards nucleic acid, calf thymus DNA[14], estimated by the Scatchard Plots[15] are 1.5×10^5 and 2.2×10^4 M^{-1} respectively[16]. Thus, the mimic (I) may possess even higher binding ability towards the substrate compared to the mimic (III). Also in the mimic (I), two primary amine residues could act as co-operative acid-base catalysts as like certain diamines reported previously[4-a,b,17]. These may cause relatively high activity on the mimic (I).

Deference in activities observed between the mimics (III) and (IV) is rather interesting since the both compounds have exactly same functional groups. The mimic (IV) also retains the binding ability toward double stranded nucleic acid ($K = 1.6 \times 10^4$ M^{-1}). In the mimic (IV), however, primary amine moiety is positioned further away from imidazole moiety. These suggest that the both active sites, imidazole and primary amine, should occupy the precise spatial position to achieve efficient cleavage of RNA as like natural enzyme does. A recent reports of Podyminogin *et al*[7] also indicates that some spatial arrangement is required for catalytic active sites of RNase mimics to exhibit efficient cleavage of RNA. The results of current study would help to develop

new class of simple RNA cleaver. Further investigation to incorporate such mimic compounds to DNA strand to develop sequence specific chemical ribonuclease is currently going on.

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10. Physical properties of the mimics are as follows; mimic (I), mp. 55.6-55.8 °C. Calcd. for $C_{20}H_{25}N_5O$ + 1 H_2O: C, 65.05; H, 7.37; N, 18.96. Found: C, 65.04; H, 7.28; N, 18.74. mimic (II), mp. 163.0-164.0 °C. Calcd. for $C_{11}H_{22}N_6O$ + 4 HCl + 0.5 H_2O: C, 32.42; H, 6.68; N, 20.51. Found: C, 32.71; H, 6.62; N, 20.51. mimic (III), mp. 75.0-75.5 °C. Clacd. for $C_{25}H_{29}N_7O_2$ + 1 H_2O + 0.5 EtOH: C, 62.38; H, 6.85; N, 19.59. Found: C, 62.38; H, 6.62; N, 19.44. mimic (IV), mp. 67.5-68 °C. Calcd. for $C_{28}H_{34}N_8O_3$ + 1 H_2O: C, 61.28; H, 6.62; N, 20. 43. Found: C, 61.47; H, 6.58; N, 20.27.
11. After the gel electrophoresis the bands were stained with ethydium bromide.
12. It should be noted that the reactions were carried out in the absence of redox-active metal species.
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