Deoxypolypeptides bind and cleave RNA

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We have prepared *L*- and *D*-deoxypolypeptides (DOPPs) by selective reduction of appropriately protected polyhistidines with borane, reducing the carbonyl groups to methylenes. The result is a chiral polyamine, not amide, with a mainly protonated backbone and chirally mounted imidazolylmethylene side chains that are mostly unprotonated at neutrality because of the nearby polycationic backbone. We found that, in contrast with the *D*-octahistidine DOPP, the *L*-octahistidine DOPP is able to cooperatively bind to a *D*-polyuridylic acid RNA; this is consistent with results of previous studies showing that, relative to D-histidine, L-histidine is able to more strongly bind to RNA. The L-DOPP was also a better catalyst for cleaving the RNA than the D-DOPP, consistent with evidence that the L-DOPP uses its imidazole groups for catalysis, in addition to the backbone cations, but the D-DOPP does not use the imidazoles. The L-DOPP bifunctional process probably forms a phosphorane intermediate. This is a mechanism we have proposed for models of ribonuclease cleavage and for the ribonuclease A enzyme itself, based on our studies of the cleavage and isomerization of UpU catalyzed by imidazole buffers as well as other relevant studies. This mechanism contrasts with earlier, generally accepted ribonuclease cleavage mechanisms where the proton donor coordinates with the oxygen of the leaving group as the 2-hydroxyl of ribose attacks the unprotonated phosphate.

nucleic acids binding | artificial enzymes | chiral discrimination | deoxygenated peptides

The enzyme ribonuclease A (RNase A) hydrolyzes RNA with bifunctional catalysis, using His-12 as a base and protonated His-119 as an acid to form the first products, a 2,3-cyclic phosphate of one ribose and the free next ribose with a new terminal 5′-hydroxyl group (1, 2). Lys-41 is also involved in stabilizing intermediate anions (3). In a second step the cyclic phosphate is hydrolyzed using the now-protonated His-12 and now-deprotonated His-119 in essentially a reverse of the cyclization step, with water replacing the second ribose unit (4). In the classical mechanism of this process it had been generally accepted, including in textbooks, that the protonated His-119 donates its proton to the leaving group whereas the 2′-hydroxyl group attacks the phosphate (1, 2) [isotope studies show that two protons are "in flight" during the hydrolysis step, essentially the reverse of the cyclization, both moving at the same time (5)]. We also showed the two-proton-in-flight process in a model system (6).

However, our extensive studies on the cleavage of RNA with imidazole–imidazolium catalysts and on catalysis by RNase A itself indicated that a different mechanism is used (Fig. 1) (7, 8). The proton donor BH^+ hydrogen binds to a phosphate oxyanion and donates the proton to the phosphate as the ribose 2′-hydroxyl adds while losing a proton to base :B. This process results in an intermediate phosphorane species. This then expels the 5′-hydroxyl group of the second nucleotide to cleave the P–O bond in a second step. We proposed this two-step phosphorane process to be the preferred path for the enzyme, a proposal further supported by computation (9–11). For a general review of phosphoranes as well-demonstrated intermediates in phosphate reactions, see ref. 12. We believe the phosphorane mechanism likely for the enzyme RNase A is essentially the same as that for some artificial enzymes, in which a simultaneous bifunctional mechanism operates in an enzyme mimic. This was demonstrated in one of our model studies (8).

There are two reasons to create enzyme mimics: (*i*) to create new useful catalysts, and (*ii*) to gain insight into the enzymatic process itself. Ideally one would want to use polyhistidine as an artificial enzyme to cleave RNA, and use sequence recognition to preferentially cleave RNAs that cause disease, but polyhistidine is too insoluble in water at physiological pH to serve this role (13). We previously described a simple polyethylenimine with imidazole groups linked to the nitrogens through methylene chains that indeed catalyzed the cleavage of a polyuridylic acid [poly(U)] RNA (14). The polymer backbone nitrogens were mainly protonated at neutrality whereas the imidazoles were mainly unprotonated because of the cationic charge of the backbone. As with RNase A, we used the imidazole ring of the polymer as the base, but chose the more basic backbone amino groups as the protonated acid catalysts. The enzyme uses a protonated imidazole for this step, from a histidine made more basic by its protein environment, a complexity that is not feasible in a model system. This work was aimed at creating a catalyst that, with sequence recognition, could selectively cleave a viral RNA in a medically useful way, goal 1.

Now we have designed and prepared new chemically defined polyamines from synthetic chiral polypeptides, histidine octamers, using our previously described process in which polypeptides can be deoxygenated with borane (15). This reaction preserves the chirality of the histidine units, so we compared the octamer derived from *L*-histidine with that from *D*-histidine to get further insight into the details of the catalytic process, goal 2. We now see that the *L*-deoxypolypeptide (*L*-DOPP) **1** binds cooperatively to poly(U), and is also a better catalyst for poly(U) cleavage, than is the *D*-enantiomer (*D*-DOPP **2**). The L-DOPP uses a bifunctional mechanism in which a simple shift of two protons in two hydrogen bonds would lead to the phosphorane (Fig. 1), whereas the DOPP **2** uses principally the backbone nitrogens, probably with one as B: and another as BH^+.

Significance

This paper describes an important new example of our previously described class of compounds, deoxygenated polypeptides (DOPPs), in which polypeptides are converted into polyamines by changing carbonyl groups into methylene groups. Of particular interest, the conversion does not interfere with the chirality or functional groups of the polypeptides, so they become stereospecific species that differentially bind and cleave RNA. This class of polyamines made from polypeptides has great potential as medicinally important binders and selective catalysts, because the polyamine backbone is hydrolytically stable and not cleaved by enzymes, and carries well-defined chirality and potential polyfunctionality in defined places along the chain. They are single species, not polymeric mixtures, just as synthetic polypeptides are.

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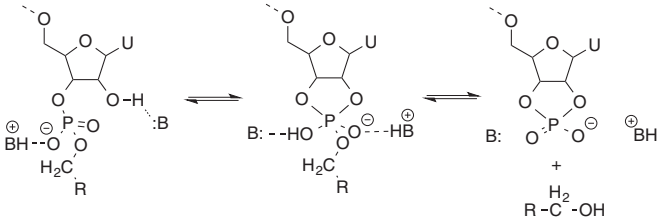


Fig. 1. Combination of a base B: and an acid BH^+ converts a uridine monomer to its phosphorane by a simple shift of two protons. In the second step the formed B: helps transfer a proton to the leaving group oxygen from the new OH group to form the cyclic phosphate and the new R-CH_2-OH.

This work supports our mechanism for RNase A that proceeds through a phosphorane intermediate, not the classical mechanism that involves direct protonation of the leaving group while the nucleophilic 2-OH group is attacking. Protonation of the leaving group is a transfer catalyzed by the backbone amine in our process, or in the enzyme by His-119, and is a second step performed on the phosphorane intermediate. A likely sequence for this second step is protonation of the O^- on the right by the BH^+ with deprotonation of the OH group on the left by B: to form a new monoanion and a new BH^+, then proton transfer of that BH^+ to the leaving oxygen which is expelled by the oxyanion on the left forming a P=O double bond, and then proton reabstraction on the right to form the cyclic anion. Note that the cyclic anion must be formed with B: on the left and BH^+ on the right to explain why the cyclic phosphate is more slowly hydrolyzed as an added substrate by RNase A, with the reverse protonation state, than it is when it is formed by this process (8, 16). The hydrolysis must proceed by a reverse of the cyclization, with H_2O replacing the departed leaving group.

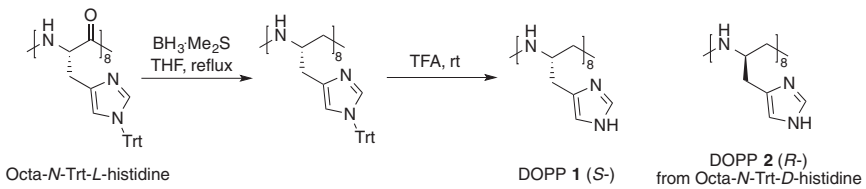
Results and Discussion

Synthesis of Chiral DOPPs 1 and 2. In a previous communication we reported the simple polyethylenimine mixture, with imidazolylmethylene side chains attached to the backbone nitrogens, for the effective cleavage of RNA (14). This mixture was prepared by polymerization, then alkylation by the side chain, and did not have a single length but had a 23-monomer predominant isomer. In the present work the imidazolylmethylene side chains are not on the nitrogen atoms but on the adjacent carbon in a deoxypolyhistidine. Our new catalysts have a single defined length (8-mer), prepared from a synthetic polypeptide. This synthetic process retains a chiral center while maintaining the previously studied functional groups. We prepared two catalysts, DOPPs **1** and **2**, by the borane reduction of commercially supplied octahistidine peptides (Scheme 1).

The protecting group triphenylmethyl was perfect for the protection–deprotection and deoxygenation, whereas other protecting groups for the imidazole were less satisfactory.

Binding Activity Monitoring. Solutions of a commercially available poly(U) RNA (Aldrich) with length distribution ranging from 300 to 1,000 uridine monomers at a concentration of *ca.* 20 nM uracil monomers were mixed with varying numbers of equivalents of *L*-histidine monomers in DOPP **1** in 10 mM phosphate buffer, and the UV absorbance of the poly(U) over a range of 200–350 nm was measured at controlled pH 7.00 (constant before and after the measurements) and 25.0 °C. One equivalent of histidine units in the DOPP is the amount of the 8-mer that would be able to bind to all of the uridine monomers of the poly(U) (17). As we increased the number of such equivalents added we observed an increase in the UV absorbance at 260 nm consistent with increased levels of binding, and at saturation we observed some loss of solubility as the negative charges of RNA became neutralized. The results of these titration experiments are shown in Fig. 2*A*. Binding of **2** to poly(U) was measured using an analogous UV titration, and these data are shown in Fig. 2*B*. Hill plots of the UV titration data of **1** and **2** and fitting of the data to the Hill equation (17) (Fig. 2 *A* and *B*, *Left, Insets*) reveal several interesting features of the poly(U) binding activities of *L*-DOPP and *D*-DOPP. First, as briefly noted above, binding of either DOPP to poly(U) induces an increase in the UV absorbance of poly(U) at 260 nm, with a small red shift of the maximum peak frequency (from 260 to *ca.* 265 nm) (18, 19). Because neither **1** nor **2** shows any significant absorption in the 200–350-nm scanning range, these changes indicate that complexation of the protonated DOPPs to the poly(U) induces a conformational transition of the poly(U) in which any inherent stacking of the uridines has been disrupted [i.e., any inherent helical character of the poly(U) is converted into random coil as the poly(U) assumes a structure that is closer to its denatured form] (19, 20).

However, what differentiates binding of DOPPs **1** or **2** to poly(U) is the observation that **1** exhibits cooperative binding to the poly(U), with fits to the Hill equation yielding a Hill coefficient, n, of 1.7 ± 0.2 and an associated equilibrium binding constant, $K_{1.7}$ of $(2.2 \pm 0.3) \times 10^6\ M^{-1}$ (two runs). Although the cooperative binding of **1** most likely arises from conformational changes of the poly(U) RNA that are induced upon binding of **1** and that facilitate the binding of further molecules of **1**, the details of such a conformational change, and consequently the structural basis for the observed cooperativity, remain unknown. Contrasting with the results of **1**, **2** does not exhibit any significant level of cooperativity, with fits to the Hill equation yielding $n = ca.\ 1$ and $K_1 = 4.6 \times 10^3\ M^{-1}$ (one run). For comparison, the analogous analysis of poly(U) binding data for a simple polyethyleneimine under the same conditions as those reported here yields $n = ca.\ 1$ and $K_1 = 1.9 \times 10^3\ M^{-1}$ (1 run). Thus, whereas **2** binds poly(U) in a manner that is very similar to a simple polyethyleneimine, **1** exhibits more complex, cooperative binding. Although the apparent equilibrium constants all have the units of M^{-1}, they are not directly comparable because one involves cooperative binding with an equilibrium expression that is different from that for the other two. However, if we just compare the midpoints of the titrations for **1** and **2** we see, correcting for the RNA concentrations in the two titrations, that compound **1** is *ca.* a factor of 1.8 more strongly bound than is **2**. The lengths of the DOPPs **1** and **2** are identical, with the only difference being the presence of two enantiomeric carbon centers, *S*- and *R*-, respectively. We note that Ruta et al. used column chromatography of racemates and reported that *L*-histidine binds an RNA aptamer 1,000 times more tightly than does *D*-histidine (21), whereas Majerfeld et al. reported a 130- to



Scheme 1. Synthesis of chiral DOPPs **1** and **2**.

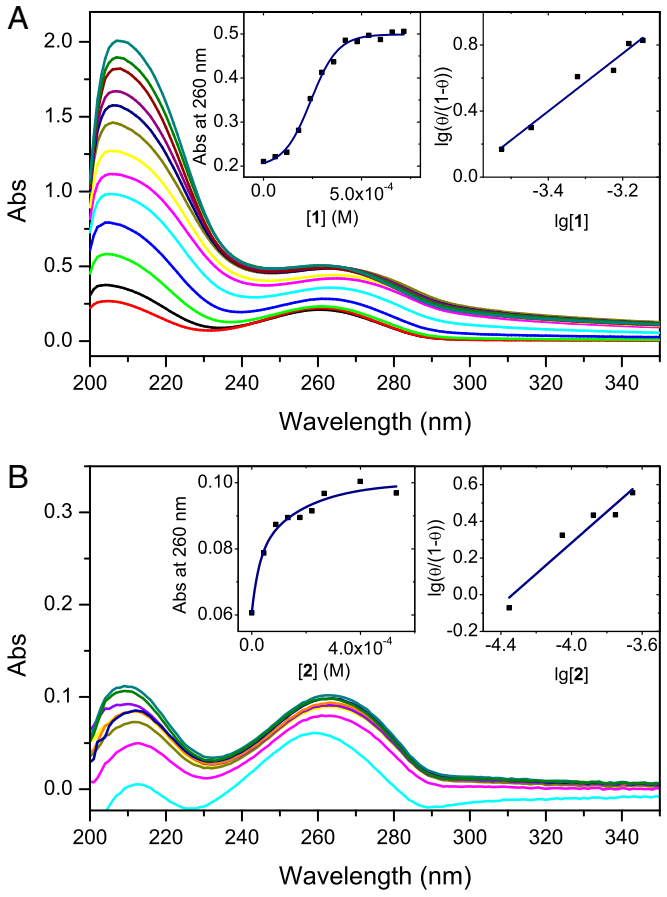


Fig. 2. Binding activity monitoring. (*A*) Change in the UV spectra of poly(U) upon addition of increasing numbers of equivalents of *L*-histidines of **1**. One equivalent of the *L*-histidines of **1** is the amount of **1** that is needed to supply one *L*-histidine for each of the uridines of the poly(U) RNA. Several equivalents are needed to shift the binding equilibrium so as to saturate the RNA. The change in absorbance at 260 nm as a function of [**1**] is plotted in *Left, Inset*. The log(θ/(1-θ)) vs. log[**1**], where $\theta = (A - A_0)/(A_{max} - A_0)$, A is the absorbance at 260 nm for poly(U) at the specified point in the titration, A_o is the initial absorbance at 260 nm for poly(U) before the addition of any **1**, and A_{max} is the maximum absorbance at 260 nm for poly(U) upon its saturation with **1** at the end of the titration (i.e., a Hill plot), is plotted in *Right, Inset*. The blue line in this plot is a fit of the data to the Hill equation (*SI Appendix*) such that the slope of the line provides the Hill coefficient and the *y* intercept provides log*K*, where *K* is the equilibrium association constant. Conditions for UV measurement: Na_2HPO_4 –NaH_2PO_4, c = 10 mM, I = 0.1 M (adjusted by NaCl), pH 7.00 at 25.0 °C. (*B*) Change in the UV spectra of *ca*. 4.5 nM poly(U) upon addition of increasing numbers of equivalents of *D*-histidines of **2**. Data are analyzed and plotted as in *A*.

860-fold affinity difference between *L*- and *D*-histidine binding to RNA in a similar study (22).

Cleavage of RNA Promoted by DOPPs. We also studied the ability of our DOPPS **1** and **2** to cleave poly(U), using our previously described kinetic assay (23). Table 1 summarizes the kinetic constants k_{obs} obtained from the hydrolysis of poly(U) catalyzed with DOPPs **1** and **2** at 80 ± 1 °C, pH 7.0 (with a buffer compatible with the enzymatic part of the assay). In these studies the RNA was at 2–4 mM, and both **1** and **2** showed saturation binding with five added equivalents. These results show that both DOPPs **1** and **2** can catalyze the cleavage of the phosphate linkage in RNA, but with different effectiveness. The pseudo–first-order cleavage rate in the presence of DOPP **1** increased with increasing amounts of catalyst, until further increasing its concentration led to saturation. The hydrolysis rate reached a plateau, with the highest being *ca*. 13-fold acceleration (Table 1 and Fig. 3). At saturation the rate of cleavage by **1** was 4.9 times that of **2**. Also, catalyst **2** is not a significantly better catalyst than is simple polyethyleneimine hydrochloride (Fig. 3). The geometry of its complex apparently does not allow the imidazole side chain to assist cleavage. As described above, binding by **2** is also similar to that of polyethyleneimine hydrochloride.

We propose that binding to the polycation backbone and the basic imidazole of **1** contribute to the hydrolysis (14). This proposal was further confirmed here by two sets of reference experiments using unsubstituted polyethylenimine salt (PEI·HCl) and natural poly-*L*-histidine·HCl as the catalysts. The linear PEI·HCl afforded only a relatively small hydrolytic acceleration (Fig. 3), showing that the cleavage by **1** involved functional imidazole groups along with the polycation backbone (24, 25). On the other hand, with the natural (not deoxygenated) polypeptide poly-*L*-histidine·HCl, which shares the same chiral configuration (*L*-) with DOPP **1** but lacks positive charged secondary amino groups on the backbone, almost no acceleration was observed (Table 1, entry 14).

Metal Catalyzed Cleavage. Several examples are known of the cleavage of RNA or RNA model substrates by divalent metal cations, such as Zn^{2+}, Cu^{2+}, and Pb^{2+} (26, 27) and by rare earth ions, such as Eu^{3+} and Yb^{3+} (16). Thus, we have also reexamined the system and extended it to a number of metal ions in 1:1 combination with DOPP **1** for the cleavage of poly(U) (Table 2).

Certain metal ions may either participate directly in the cleavage mechanism or are specifically involved in binding with the catalysts and/or polynucleotides complexes. Indeed, the data in Table 2 show the rates as a function of the nature of the specific metal ions added. The reasons for the metal effects are not clear, but we believe that the efficiency is partly correlated with the Lewis acidity of the metals when combining with strong ligands, such as the nitrogen on the polyethylenimine backbone or the imidazoles (14, 28).

Note that a more rapid (1.4-fold) cleavage is observed with $MgCl_2$. Although Mg^{2+} plays an important role in the phosphodiester cleavage mediated by ribozymes, it is not considered as a typical chemical nuclease (29, 30), and the enhancement by Mg^{2+} was not noted in previous research (31). Thus, the unusual acceleration of the hydrolysis observed here in the presence of

Table 1. Hydrolysis of poly(U) with catalysis by DOPPs 1 and 2

Entry	Catalyst	$k_{obs} \times 10^3$ h^{-1}	k_{acc}
1	None	8.76 ± 0.18	1.00
2	**1**, 1 eq.	33.47 ± 4.12	3.82
3	**1**, 2 eq.	56.06 ± 1.98	6.40
4	**1**, 3 eq.	77.80 ± 4.55	8.88
5	**1**, 4 eq.	96.11 ± 6.14	10.97
6*	**1**, 5 eq.	109.08 ± 4.00	12.45
7*	**1**, 6 eq.	98.23 ± 5.40	11.21
8	**2**, 1 eq.	12.33 ± 1.94	1.41
9	**2**, 2 eq.	15.67 ± 1.63	1.79
10	**2**, 3 eq.	17.48 ± 1.32	2.00
11	**2**, 4 eq.	20.54 ± 1.88	2.34
12*	**2**, 5 eq.	22.33 ± 3.27	2.55
13*	**2**, 6 eq.	23.22 ± 2.31	2.65
14*	Poly-*L*-His·HCl, 5 eq.	9.10 ± 2.93	1.04

Hydrolysis in Trizma-HCl buffer, c = 0.1 M, I = 0.1 M (adjusted by NaCl), pH = 7.0 (80 °C). The ratios refer to the histidine units in DOPPs vs. uridine units in poly(U). Data are represented as mean ± SEM.
*Turbidity was observed during the reaction.

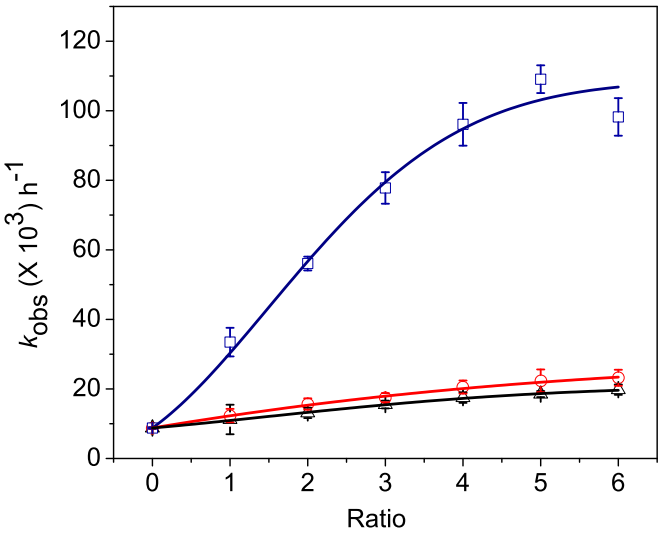


Fig. 3. Rate of poly(U) cleavage promoted by DOPPs **1**, **2**, and PEI·HCl as a function of the ratio of catalyst/poly(U) [□, DOPP **1**; ○, DOPP **2**; △, PEI·HCl; Ratios refer to the ethylenimine units in the catalysts vs. uridine units in poly(U)]. Conditions: Trizma-HCl buffer, c = 0.1 M, I = 0.1 M (adjusted by NaCl), pH = 7.0 (80 °C). RNA at 2–4 mM. See *SI Appendix* for full data.

$MgCl_2$ is presumably due to the complexation of Mg^{2+} with a phosphodiesterase group and it may also stabilize the secondary structure of RNA (29, 32). This is consistent with the importance of Mg^{2+} in ribozymes, and such metal selectivity in enzyme mimics could be of considerable interest with respect to imitating biological selectivities (33).

Conclusion

DOPPs can be used not only as new catalysts in the case of ribonuclease mimics, but also to afford some chemical insight into how the enzymes themselves could work. This is especially true with the DOPPs we have worked with in this study, in which the chiralities of *D*-histidine or *L*-histidine are retained during deoxygenation. Their chiralities lead to differences in binding poly(U), with **1** showing cooperative binding while **2** does not.

Compound **1** is also a more effective catalyst for the hydrolysis of poly(U) than is compound **2**.

Table 2. Hydrolysis of poly(U) with catalysis by DOPP 1 and metal ions

Entry	Catalyst	$k_{obs} \times 10^3$ h^{-1}	k_{acc}
1	None	8.763 ± 0.182	1.00
2	DOPP **1**	109.079 ± 3.996	12.45
3	DOPP **1** + $MgCl_2$	155.762 ± 7.728	17.77
4*	DOPP **1** + $ZnCl_2$	99.848 ± 7.472	11.39
5	DOPP **1** + $TbCl_3$	107.612 ± 10.920	12.28
6	DOPP **1** + $YbCl_3$	111.312 ± 8.467	12.70
7*	DOPP **1** + $CuCl_2$	43.116 ± 4.625	4.92
8*	DOPP **1** + $MnCl_2$	135.855 ± 10.760	15.50

Hydrolysis in Trizma-HCl buffer, c = 0.1 M, I = 0.1 M (adjusted by NaCl), pH = 7.0 (80 °C). The ratios refer to the imidazole units in DOPP vs. the uridine units in poly(U). Data are represented as mean ± SEM. Ratio: DOPP **1**/metal salt/poly(U) = 5/5/1.
*Turbidity was observed during the reaction.

Our selectivity in catalysis reveals that the L-DOPP uses a bifunctional mechanism in which both the backbone cationic groups and the imidazole side chains play a role, whereas the D-DOPP uses monofunctional catalysis by the backbone cationic groups alone.

Materials and Methods

Experimental details for the synthesis and characterization of DOPPs **1**, **2**, and intermediates, supplementary figures, tables, detailed methods, calculation results, binding and kinetic cleavage procedures can be found in *SI Appendix*. As described previously, the reduction of polypeptides to polyamines was performed with borane dimethylsulfide (15). The method to determine rates of RNA cleavage utilized the phosphodiesterase from rattlesnake venom (23).

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1. Richards FM, Wyckoff HW (1971) *The Enzymes*, ed Boyer PD (Academic, New York), Vol 4, pp 647–806.
2. Blackburn P, Moore S (1982) *The Enzymes*, ed Boyer PD (Academic, New York), Vol 15, pp 317–433.
3. Raines MT (2004) *Artificial Nucleases*, ed Zenkova MA (Springer, Berlin), pp 19–32.
4. Findlay D, Herries DG, Mathias AP, Rabin BR, Ross CA (1961) The active site and mechanism of action of bovine pancreatic ribonuclease. *Nature* 190:781–784.
5. Matta MS, Vo DT (1986) Proton inventory of the second step of ribonuclease catalysis. *J Am Chem Soc* 108(17):5316–5318.
6. Anslyn E, Breslow R (1989) Proton inventory of a bifunctional ribonuclease model. *J Am Chem Soc* 111(24):8931–8932.
7. Breslow R, Huang DL, Anslyn E (1989) On the mechanism of action of ribonucleases: Dinucleotide cleavage catalyzed by imidazole and Zn^{2+}. *Proc Natl Acad Sci USA* 86(6): 1746–1750.
8. Breslow R, Dong SD, Webb Y, Xu R (1996) Further studies on the buffer-catalyzed cleavage and isomerization of uridyluridine. Medium and ionic strength effects on catalysis by morpholine, imidazole, and acetate buffers help clarify the mechanisms involved and their relationship to the mechanism used by the enzyme ribonuclease and by a ribonuclease mimic. *J Am Chem Soc* 118(28):6588–6600.
9. Haydock K, Lim C, Brünger A, Karplus M (1990) Simulation analysis of structures on the reaction pathway of RNase A. *J Am Chem Soc* 112(10):3826–3831.
10. Wladkowski BD, Krauss M, Stevens WJ (1995) Transphosphorylation catalyzed by ribonuclease A: Computational study using *ab initio* effective fragment potentials. *J Am Chem Soc* 117(42):10537–10545.
11. Elsässer B, Fels G, Weare JH (2014) QM/MM simulation (B3LYP) of the RNase A cleavage-transesterification reaction supports a triester $A(_N)$ + $D(_N)$ associative mechanism with an O2′ H internal proton transfer. *J Am Chem Soc* 136(3):927–936.
12. Holmes RR, et al. (1992) *Phosphorus Chemistry*, eds Walsh EN, Griffith EJ, Parry RW, Quin LD (American Chemical Society, Washington, DC), pp 18–40.
13. Shen WC (1990) Acid-sensitive dissociation between poly(lysine) and histamine-modified poly(glutamate) as a model for drug-releasing from carriers in endosomes. *Biochim Biophys Acta* 1034(1):122–124.
14. Cheng L, Abhilash KG, Breslow R (2012) Binding and biomimetic cleavage of the RNA poly(U) by synthetic polyimidazoles. *Proc Natl Acad Sci USA* 109(32):12884–12887.
15. Levine M, Kenesky CS, Zheng S, Quinn J, Breslow R (2008) Synthesis and catalytic properties of diverse chiral polyamines. *Tetrahedron Lett* 49(40):5746–5750.
16. Breslow R, Huang DL (1991) Effects of metal ions, including Mg^{2+} and lanthanides, on the cleavage of ribonucleotides and RNA model compounds. *Proc Natl Acad Sci USA* 88(10):4080–4083.
17. Freifelder D (1982) *Physical Biochemistry: Applications to Biochemistry and Molecular Biology* (Freeman, San Francisco).
18. Richards EG, Flessel CP, Fresco JR (1963) Polynucleotides. VI. Molecular properties and conformation of polyribouridylic acid. *Biopolymers* 1(5):431–446.
19. Helene C, Maurizot JC (1981) Interactions of oligopeptides with nucleic acids. *CRC Crit Rev Biochem* 10(3):213–258.
20. Steely HT, Jr., Gray DM, Ratliff RL (1986) CD of homopolymer DNA-RNA hybrid duplexes and triplexes containing A-T or A-U base pairs. *Nucleic Acids Res* 14(24):10071–10090.
21. Ruta J, et al. (2007) Chiral resolution of histidine using an anti-D-histidine L-RNA aptamer microbore column. *J Chromatogr B Analyt Technol Biomed Life Sci* 845(2):186–190.
22. Majerfeld I, Puthenvedu D, Yarus M (2005) RNA affinity for molecular L-histidine; genetic code origins. *J Mol Evol* 61(2):226–235.
23. Corcoran R, Labelle M, Czarnik AW, Breslow R (1985) An assay to determine the kinetics of RNA cleavage. *Anal Biochem* 144(2):563–568.
24. Breslow R (1991) How do imidazole groups catalyze the cleavage of RNA in enzyme models and in enzymes? Evidence from "negative catalysis." *Acc Chem Res* 24(11):317–324.

25. Breslow R, Labelle M (1986) Sequential general base-acid catalysis in the hydrolysis of RNA by imidazole. *J Am Chem Soc* 108(10):2655–2659.
26. Butzow JJ, Eichhorn GL (1965) Interactions of metal ions with polynucleotides and related compounds. IV. Degradation of polyribonucleotides by zinc and other divalent metal ions. *Biopolymers* 3(1):95–107.
27. Dahm SC, Uhlenbeck OC (1991) Role of divalent metal ions in the hammerhead RNA cleavage reaction. *Biochemistry* 30(39):9464–9469.
28. Kimura E, Shiota T, Koike T, Shiro M, Kodama M (1990) A zinc(II) complex of 1,5,9-triazacyclododecane ([12]aneN_3) as a model for carbonic anhydrase. *J Am Chem Soc* 112(15):5805–5811.
29. Uchimaru T, Uebayasi M, Tanabe K, Taira K (1993) Theoretical analyses on the role of Mg^{2+} ions in ribozyme reactions. *FASEB J* 7(1):137–142.
30. Morrow JR (1994) Artificial ribonucleases. *Adv Inorg Biochem* 9:41–74.
31. AbouHaidar MG, Ivanov IG (1999) Non-enzymatic RNA hydrolysis promoted by the combined catalytic activity of buffers and magnesium ions. *Z Naturforsch C* 54(7-8): 542–548.
32. Serra MJ, et al. (2002) Effects of magnesium ions on the stabilization of RNA oligomers of defined structures. *RNA* 8(3):307–323.
33. Morrow JR, Iranzo O (2004) Synthetic metallonucleases for RNA cleavage. *Curr Opin Chem Biol* 8(2):192–200.