# Notes

## Hydrolysis of Double-Stranded and Single-Stranded RNA in Hairpin Structures by the Copper(II) Macrocycle Cu([9]aneN$_3$)Cl$_2$

**Eric L. Hegg, Kim A. Deal, Laura L. Kiessling, and Judith N. Burstyn***

Department of Chemistry,
University of Wisconsin−Madison,
Madison, Wisconsin 53706

*Received August 8, 1996*

RNA is hydrolyzed *in vivo* by ribonucleases via a two-step reaction involving transesterification by the 2′-hydroxyl, with concomitant cleavage of the RNA strand, followed by the hydrolysis of the resulting 2,3′-cyclic phosphodiester. There is much interest in designing synthetic ribonucleases because they could be useful for elucidating the structures and functions of a variety of RNAs and inhibiting gene expression by targeting specific mRNAs. A number of transition metal complexes are known to oxidatively degrade RNA, and many have proven useful as probes of RNA structure.[1−8] For example, the bis-(1,10-phenanthroline-$N^1$,$N^{10}$)copper(I) cation ($(OP)_2Cu^+$) has been used as an RNA-footprinting agent to map the *lacZ* binding site of *Escherichia Coli* mRNA[4] while a Ni(II) macrocycle and $Rh(phen)_2phi^{3+}$ (phen = 1,10-phenanthroline, phi = 9,10-phenanthrenequinone diimine) have been used to probe the 3-dimensional solution structures of the *Tetrahymena* group I intron[2a] and of the 5S rRNA of *Xenopus* oocyte.[7c] $Rh(phen)_2phi^{3+}$ has also been used to probe the effect of bulges on the major groove in the *trans*-activation responsive region (TAR) found in HIV RNA.[8] Finally, $Rh(phen)_2phi^{3+}$ and Ru(tpy)(bpy)-$O^{2+}$ (tpy = 2,2′,:6′,2″-terpyridine, bpy = 2,2′-bipyridine) have been used in concert to gain information about the iron regulatory element (IRE) in ferritin mRNA.[1b]

Metal complexes that cleave RNA via a hydrolytic mechanism, however, have advantages over those that utilize an oxidative mechanism: hydrolytic cleavage agents do not require coreactants and do not produce highly reactive metal oxenes or radical species. Furthermore, because the 2′-hydroxyl on the ribose ring makes RNA nearly 100 000-fold more reactive toward hydrolysis than DNA,[9] complementary DNA could be used to direct site-specific cleavage of mRNA. In contrast, many oxidative cleavage agents cleave DNA more rapidly than RNA,[1a,5] making it impractical to use complementary DNA as a targeting molecule. Finally, because many ribonucleases utilize metal ions, small metal complexes that hydrolyze RNA could also aid in determining the precise role of metal ions in enzymatic reactions.

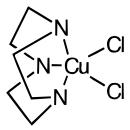
There are numerous examples of metal-promoted hydrolysis of RNA. Several lanthanide[10−14] and transition metal[13a,15−24] ions and complexes promote the transesterification/hydrolysis of RNA dinucleotides, oligonucleotides, and RNA models; in some instances the reactions have been shown to be catalytic.[13b,16c,23a] Similar ions and complexes also promote the

(1) (a) Neyhart, G. A.; Cheng, C. C.; Thorp, H. H. *J. Am. Chem. Soc.* **1995**, *117*, 1463−1471. (b) Thorp, H. H.; McKenzie, R. A.; Lin, P. N.; Walden, W. E.; Theil, E. C. *Inorg. Chem.* **1996**, *35*, 2773−2779.
(2) (a) Chen, X.; Woodson, S. A.; Burrows, C. J.; Rokita, S. E. *Biochemistry* **1993**, *32*, 7610−7616. (b) Muller, J. G.; Zheng, P.; Rokita, S. E.; Burrows, C. J. *J. Am. Chem. Soc.* **1996**, *118*, 2320−2325.
(3) (a) Pope, L. E.; Sigman, D. S. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 3−7. (b) Murakawa, G. J.; Chen, C. B.; Kuwabara, M. D.; Nierlich, D. P.; Sigman, D. S. *Nucleic Acids Res.* **1989**, *17*, 5361−5375. (c) Mazumder, A.; Chen, C. B.; Gaynor, R.; Sigman, D. S. *Biochem. Biophys. Res. Commun.* **1992**, *187*, 1503−1509.
(4) Murakawa, G. J.; Nierlich, D. P. *Biochemistry* **1989**, *28*, 8067−8072.
(5) Carter, B. J.; de Vroom, E.; Long, E. C.; van der Marel, G. A.; van Boom, J. H.; Hecht, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9373−9377.
(6) Guan, L. L.; Totsuka, R.; Kuwahara, J.; Otsuka, M.; Sugiura, Y. *Biochem. Biophys. Res. Commun.* **1993**, *191*, 1338−1346.
(7) (a) Chow, C. S.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 2839−2841. (b) Chow, C. S.; Behlen, L. S.; Uhlenbeck, O. C.; Barton, J. K. *Biochemistry* **1992**, *31*, 972−982. (c) Chow, C. S.; Hartmann, K. M.; Rawlings, S. L.; Huber, P. W.; Barton, J. K. *Biochemistry* **1992**, *31*, 3534−3542. (d) Chow, C. S.; Barton, J. K. *Biochemistry* **1992**, *31*, 5423−5429.
(8) Neenhold, H. R.; Rana, T. M. *Biochemistry* **1995**, *34*, 6303−6309.
(9) Thompson, J. E.; Kutateladze, T. G.; Schuster, M. C.; Venegas, F. D.; Messmore, J. M.; Raines, R. T. *Bioorg. Chem.* **1995**, *23*, 471−481.
(10) Rordorf, B. F.; Kearns, D. R. *Biopolymers* **1976**, *15*, 1491−1504.
(11) (a) Magda, D.; Miller, R. A.; Sessler, J. L.; Iverson, B. L. *J. Am. Chem. Soc.* **1994**, *116*, 7439−7440. (b) Hall, J.; Hüsken, D.; Pieles, U.; Moser, H. E.; Häner, R. *Chem. Biol.* **1994**, *1*, 185−190.
(12) (a) Komiyama, M.; Matsumura, K.; Matsumoto, Y. *J. Chem. Soc., Chem. Commun.* **1992**, 640−641. (b) Hayashi, N.; Takeda, N.; Shiiba, T.; Yashiro, M.; Watanabe, K.; Komiyama, M. *Inorg. Chem.* **1993**, *32*, 5899−5900. (c) Matsumura, K.; Endo, M.; Komiyama, M. *J. Chem. Soc., Chem. Commun.* **1994**, 2019−2020.
(13) (a) Morrow, J. R.; Buttrey, L. A.; Berback, K. A. *Inorg. Chem.* **1992**, *31*, 16−20. (b) Morrow, J. R.; Buttrey, L. A.; Shelton, V. M.; Berback, K. A. *J. Am. Chem. Soc.* **1992**, *114*, 1903−1905. (c) Kolasa, K. A.; Morrow, J. R.; Sharma, A. P. *Inorg. Chem.* **1993**, *32*, 3983−3984. (d) Amin, S.; Morrow, J. R.; Lake, C. H.; Churchill, M. R. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 773−775. (e) Chin, K. O. A.; Morrow, J. R. *Inorg. Chem.* **1994**, *33*, 5036−5041.
(14) Breslow, R.; Huang, D. L. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4080−4083.
(15) (a) Brown, R. S.; Hingerty, B. E.; Dewan, J. C.; Klug, A. *Nature* **1983**, *303*, 543−546. (b) Brown, R. S.; Dewan, J. C.; Klug, A. *Biochemistry* **1985**, *24*, 4785−4801.
(16) (a) Stern, M. K.; Bashkin, J. K.; Sall, E. D. *J. Am. Chem. Soc.* **1990**, *112*, 5357−5359. (b) Modak, A. S.; Gard, J. K.; Merriman, M. C.; Winkeler, K. A.; Bashkin, J. K.; Stern, M. K. *J. Am. Chem. Soc.* **1991**, *113*, 283−291. (c) Bashkin, J. K.; Jenkins, L. A. *J. Chem. Soc., Dalton Trans.* **1993**, 3631−3632. (d) Bashkin, J. K.; Jenkins, L. A. *Comments Inorg. Chem.* **1994**, *16*, 77−93. (e) Bashkin, J. K.; Frolova, E. I.; Sampath, U. *J. Am. Chem. Soc.* **1994**, *116*, 5981−5982.
(17) (a) Prakash, T. P.; Ganesh, K. N. *J. Chem. Soc., Chem. Commun.* **1994**, 1357−1358. (b) Prakash, T. P.; Kunte, S. S.; Ganesh, K. N. *Tetrahedron* **1994**, *50*, 11699−11708.
(18) Kazakov, S.; Altman, S. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7939−7943.
(19) Chu, F.; Smith, J.; Lynch, V. M.; Anslyn, E. V. *Inorg. Chem.* **1995**, *34*, 5689−5690.
(20) (a) Wall, M.; Hynes, R. C.; Chin, J. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1633−1635. (b) Wahnon, D.; Hynes, R. C.; Chin, J. *J. Chem. Soc., Chem. Commun.* **1994**, 1441−1442. (c) Linkletter, B.; Chin, J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 472−474. (d) Young, M. J.; Chin, J. *J. Am. Chem. Soc.* **1995**, *117*, 10577−10578.
(21) Dempcy, R. O.; Bruice, T. C. *J. Am. Chem. Soc.* **1994**, *116*, 4511−4512.
(22) (a) Matsumoto, Y.; Komiyama, M. *J. Chem. Soc., Chem. Commun.* **1990**, 1050−1051. (b) Irisawa, M.; Takeda, N.; Komiyama, M. *J. Chem. Soc., Chem. Commun.* **1995**, 1221−1222.
(23) (a) Shelton, V. M.; Morrow, J. R. *Inorg. Chem.* **1991**, *30*, 4295−4299. (b) Morrow, J. R.; Shelton, V. M. *New J. Chem.* **1994**, *18*, 371−375.
(24) (a) Breslow, R.; Huang, D. L.; Anslyn, E. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1746−1750. (b) Breslow, R.; Berger, D.; Huang, D. L. *J. Am. Chem. Soc.* **1990**, *112*, 3686−3687. (c) Chapman, W. H., Jr.; Breslow, R. *J. Am. Chem. Soc.* **1995**, *117*, 5462−5469.

S0020-1669(96)00955-X CCC: $14.00 

hydrolysis of 2′,3′-cyclic phosphates.[14,16c,23a,25] Using metal ion complexes that facilitate RNA cleavage, site-specific hydrolysis of RNA has been realized using complementary DNA as a targeting molecule. When appropriate metal-binding sites were attached to DNA oligonucleotides, the addition of the lanthanide ions $Lu^{3+}$ and $Eu^{3+}$ and the transition metal $Cu^{2+}$ led to the site-specific hydrolysis of complementary RNA oligomers[11,12c] and HIV mRNA.[16e] These experiments targeting specific RNAs for degradation are an important step toward the goal of developing drugs for gene therapy.

In this paper we report that the transition metal complex copper(II) 1,4,7-triazacyclononane dichloride[26] ($Cu([9]aneN_3)Cl_2$) hydrolyzes RNA hairpin oligonucleotides at physiological




**$Cu([9]aneN_3)Cl_2$**

pH and temperature. We previously established that $Cu([9]aneN_3)Cl_2$ hydrolyzes proteins[27] and cleaves DNA in the absence of an oxidant.[28] This study of RNA hydrolysis completes the survey to define the scope of reactivity of $Cu([9]aneN_3)Cl_2$ toward biopolymers. To our knowledge, this is the first transition metal complex shown to hydrolyze both nucleic acids and proteins. The information obtained from this survey on the reactivity of the synthetic hydrolase $Cu([9]aneN_3)Cl_2$ provides a foundation for the development of new catalysts that selectively hydrolyze specific biological macromolecules.

## Experimental Section

**General Procedures.** All solutions were prepared using water purified by passage through a Millipore purification system and sterilized by treatment with 0.1% diethyl pyrocarbonate (DEPC) followed by autoclaving. All glass and plastic ware were sterilized by soaking overnight in water containing 0.1% DEPC followed by autoclaving.

**Synthesis and 5′ $^{32}$P-End-Labeling of RNA Oligonucleotides.** The RNA oligonucleotides were synthesized on an Applied Biosystems 392 DNA/RNA synthesizer and purified by the procedure described in User Bulletin No. 69. 5′ $^{32}$P-end-labeling was accomplished using T4 polynucleotide kinase according to literature techniques.[29]

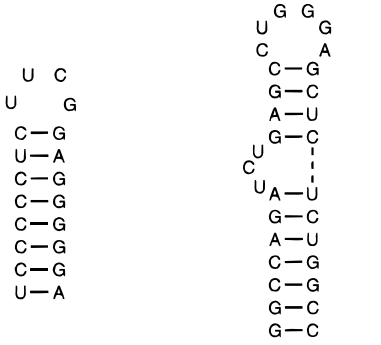
**Hydrolysis of RNA.** A 5′ $^{32}$P-end-labeled RNA hairpin oligonucleotide (20 $\mu$M) was incubated for 6, 12, 24, or 48 h at 37 °C in the presence of varying concentrations of $Cu([9]aneN_3)Cl_2$. The pH was maintained at 7.2 with 20 mM HEPES. At appropriate times, the reactions were quenched via ethanol precipitation. The RNA pellets were redissolved in a loading buffer and stored frozen at −20 °C until analyzed by polyacrylamide gel electrophoresis.

**Reactions in the Presence of SOD and Catalase.** To assess the likelihood that either hydrogen peroxide or superoxide participates in the cleavage reactions, RNA was incubated for 24 h in the presence of 2.0 mM $Cu([9]aneN_3)Cl_2$ and either 50 $\mu$g/mL superoxide dismutase (3.2 units/reaction) or 100 $\mu$g/mL catalase (40 units/reaction). To ascertain if the products obtained by cleavage with the metal complex were consistent with a hydrolytic mechanism, the products were compared with those obtained via alkaline hydrolysis. Alkaline hydrolysis of the RNA hairpins was accomplished using the 76000 RNA sequencing kit from USB. Denatured RNA was incubated with the alkaline buffer for 40 min at 50 °C.

**Product Analysis.** The products were separated on a 20% denaturing polyacrylamide gel containing 7 M urea using an IBI Model STS-45 standard thermoplate sequencer. Electrophoresis was performed at 70 W (2000−2500 V) for approximately 2.0−2.5 h. The cleavage products were visualized by exposure of the gel to Kodak X-Omat AR X-ray film at −80 °C. For quantitative analysis, gels were exposed to a Molecular Dynamics PhosphorImaging Screen overnight at room temperature and imaged using a Molecular Dynamics 425-S PhosphorImager. Quantitation was accomplished using the software ImageQuaNT version 4.2.

## Results and Discussion

The 20-base RNA hairpin oligonucleotide, 5′-UCCCCCUCUUCGGAGGGGGA-3′ (loop region underlined), is cleaved




20-Base Hairpin RNA 31-Base TAR RNA

by $Cu([9]aneN_3)Cl_2$ when incubated at pH 7.2 and 37 °C with varying concentrations of the metal complex. Product analysis was accomplished by separating the resulting polynucleotide fragments via polyacrylamide gel electrophoresis and using autoradiography to image the products (Figure 1). Analogous results were obtained for the 31-base RNA oligonucleotide TAR (5′-GGCCAGAUCUGAGCCUGGGAGCUCUCUGGCC-3′), an RNA oligonucleotide that interacts with the tat protein in HIV and is necessary for the life cycle of the virus (data not shown). Hydrolysis occurs at all phosphodiester bonds, indicating that the cleavage reaction promoted by $Cu([9]aneN_3)Cl_2$ does not depend on interactions with specific nucleotide bases. Furthermore, both single-stranded and double-stranded regions of the hairpins are hydrolyzed, although the loop region is cleaved to a greater extent than the stem. This reactivity of the complex is notable because double-stranded RNA is generally a poorer substrate for both oxidative[2−6,7b,c] and hydrolytic[10,13c,15] cleavage agents.

The differences in reactivity toward double-stranded RNA between most cleavage agents and $Cu([9]aneN_3)Cl_2$ may arise from two structural features of RNA duplexes. First, duplex RNA adopts an A-conformation. This form of the double-helix has a much deeper and narrower major grove than the B-conformation typically adopted by DNA, and as a result, many cleavage agents that bind to double-stranded DNA cannot bind to double-stranded RNA.[30] The small size of $Cu([9]aneN_3)Cl_2$ may play a role in its ability to cleave double-stranded RNA; however, the reactivity differences cannot completely be attributed to differences in size since various free metal ions also cleave single-stranded RNA selectively over double-stranded RNA.[10,15] Second, duplex RNA is less flexible than single-

(25) (a) Kuusela, S.; Lönnberg, H. *J. Phys. Org. Chem.* **1992**, *5*, 803−811. (b) Matsumoto, Y.; Komiyama, M.; Takeuchi, K. *Chem. Lett.* **1990**, 427−428. (c) Shiiba, T.; Komiyama, M. *Tetrahedron Lett.* **1992**, *33*, 5571−5574.
(26) Deal, K. A.; Burstyn, J. N. *Inorg. Chem.* **1996**, *35*, 2792−2798.
(27) Hegg, E. L.; Burstyn, J. N. *J. Am. Chem. Soc.* **1995**, *117*, 7015−7016.
(28) Hegg, E. L.; Burstyn, J. N. *Inorg. Chem.* **1996**, *35*, 7474−7481.
(29) Cobianchi, F.; Wilson, S. H. *Methods Enzymol.* **1987**, *152*, 94−110.

(30) $Rh(phen)_2(phi)^{3+}$ has been shown to bind and oxidatively cleave double-stranded RNA in regions where the major groove is widened due to tertiary interactions.[7b] Likewise, $(OP)_2Cu^+$ will bind and oxidatively cleave both strands in the chimeric duplex of polyadenylic acid (poly(rA)) and polydeoxythymidylic acid (poly(T)).[3a]

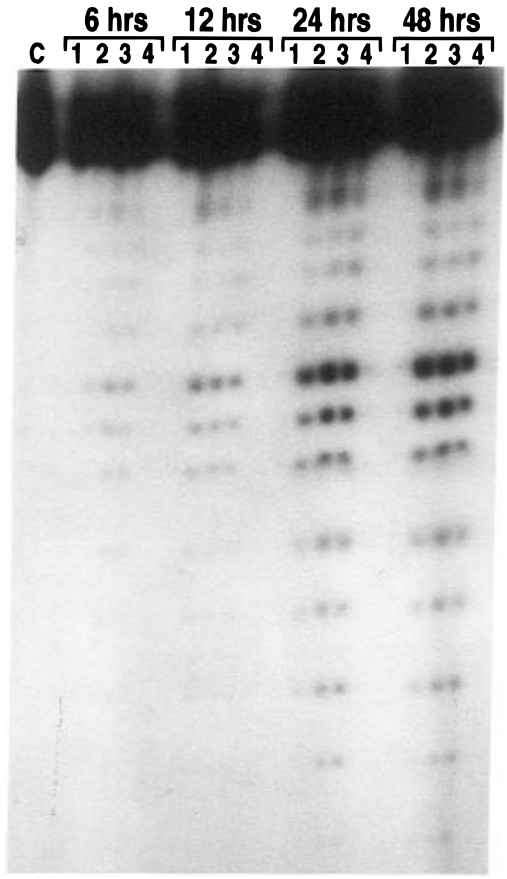



**Figure 1.** The 5′ $^{32}$P-end-labeled RNA hairpin oligonucleotide 5′-UCCCCCUCUUCGGAGGGGGA-3′ (loop region underlined) incubated for up to 48 h with varying concentrations of Cu([9]aneN$_3$)Cl$_2$ at 37 °C and pH 7.2 in 20 mM HEPES buffer. Lane 1: control, no metal complex. Lane 2: 1.0 mM Cu([9]aneN$_3$)Cl$_2$. Lane 3: 2.0 mM Cu([9]aneN$_3$)Cl$_2$. Lane 4: 4.0 mM Cu([9]aneN$_3$)Cl$_2$.

stranded RNA. As a result of this decreased flexibility, the hydrolysis of duplex RNA is inhibited since the attacking 2′-OH must properly orient itself for in-line displacement.[13c,31] The binding of Cu([9]aneN$_3$)Cl$_2$ to RNA may alter the orientation of the phosphate backbone, thereby facilitating the nucleophilic attack. Further experiments are necessary, however, to definitively explain the cleavage of both single-stranded and double-stranded RNA by Cu([9]aneN$_3$)Cl$_2$.

The extent of RNA cleavage was determined via densitometric analysis of gels imaged via phosphor screen autoradiography (Figure 2). The extent of hydrolysis is dependent both on the time of incubation and on the metal complex concentration; control reactions where RNA was incubated in the absence of Cu([9]aneN$_3$)Cl$_2$ exhibited no RNA degradation. These results confirm that the metal complex, not the buffer or adventitious metal ions, is responsible for the RNA hydrolysis. The observation that the extent of RNA degradation does not vary linearly with metal complex concentration is to be expected since in solution Cu([9]aneN$_3$)Cl$_2$ exists in a monomer−dimer equilibrium, and it is the monomer that is the active species.[26] Therefore, we expect the extent of cleavage to exhibit a half-order dependence on the metal complex concentration.[26] This predicted relationship was observed within experimental error.

Further experiments demonstrated that the cleavage of RNA oligonucleotides by Cu([9]aneN$_3$)Cl$_2$ proceeds via a hydrolytic mechanism. Reactions performed in the presence of the enzymes catalase and superoxide dismutase exhibit the same

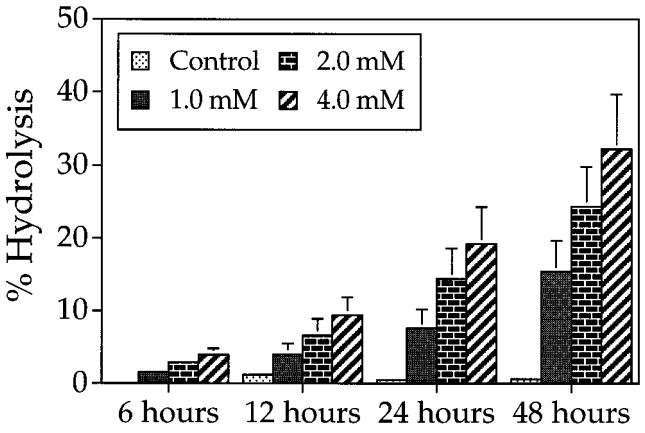



**Figure 2.** Extent of cleavage of the 20-base RNA hairpin oligonucleotide by Cu([9]aneN$_3$)Cl$_2$ determined via phosphor screen autoradiography. The reaction conditions are described in Figure 1. The graph was created with the data from six separate experiments, and the error bars show the standard deviations calculated using the formula for a small number of data points.

**Table 1.** Cleavage of RNA by Cu([9]aneN$_3$)Cl$_2$[a]

| reaction | % RNA cleaved | std dev |
|---|---|---|
| Cu([9]aneN$_3$)Cl$_2$ only | 14 | 6 |
| Cu([9]aneN$_3$)Cl$_2$ + CAT | 13 | 2 |
| Cu([9]aneN$_3$)Cl$_2$ + SOD | 14 | 4 |

[a] The 20-base RNA hairpin oligonucleotide (20 $\mu$M) was incubated at pH 7.2 (20 mM HEPES) and 37 °C with 2.0 mM Cu([9]aneN$_3$)Cl$_2$ for 24 h. Reactions were performed in the presence of either Cu([9]aneN$_3$)Cl$_2$ only, Cu([9]aneN$_3$)Cl$_2$ + catalase, or Cu([9]aneN$_3$)Cl$_2$ + superoxide dismutase. All reactions were performed in quadruplicate.

extent of RNA degradation as reactions performed in the absence of these enzymes (Table 1), indicating that neither hydrogen peroxide nor superoxide is involved in the cleavage mechanism. Furthermore, all of the observable products from cleavage of the RNA hairpin comigrate with the products of alkaline hydrolysis when separated via polyacrylamide gel electrophoresis and viewed by autoradiography.[32] (Alkaline hydrolysis produces 2′- and 3′-phosphates and 5′-hydroxyls as the only products. Although cleavage by oxidative mechanisms may afford these products, they also yield other fragments that can be observed as bands migrating between those generated via alkaline hydrolysis.) Thus, only the products expected for hydrolysis are present. If an oxidative process exists for the cleavage of RNA by Cu([9]aneN$_3$)Cl$_2$, it occurs at a level below the experimental detection limit; catalase and superoxide dismutase have no observable effect on the extent of degradation, and the only bands observed from the polyacrylamide gel are those consistent with hydrolytic cleavage. Therefore, although under aerobic conditions approximately 30% of the cleavage of DNA by Cu([9]aneN$_3$)Cl$_2$ was shown to be $O_2$-dependent (presumably occurring via an oxidative mechanism),[28] we do not see any evidence of an oxidative mechanism in the cleavage of RNA. Finally, it is interesting to note that an analogous 20-base hairpin oligodeoxynucleotide was not cleaved to any appreciable extent when incubated with Cu([9]aneN$_3$)Cl$_2$ under similar conditions (data not shown). All of these results are consistent with a hydrolytic mechanism of RNA cleavage where the 2′-OH facilitates RNA strand scission.

The results we have obtained for the cleavage of the RNA hairpin oligonucleotide by Cu([9]aneN$_3$)Cl$_2$ are consistent with those obtained by Young and Chin[20d] for the cleavage of the

(31) Usher, D. A. *Nature (London) New Biol.* **1972**, *235*, 207−208.

(32) Because product analysis utilizes autoradiography, only products containing $^{32}$P (i.e., the original 5′-labeled-phosphate) will be observed.

simple RNA dinucleotide adenylyl(3′→5′)adenosine (ApA). The only products observed from the cleavage of ApA by Cu([9]aneN$_3$)Cl$_2$ were adenosine, adenosine 2′-monophosphate (2′-AMP), and adenosine 3′-monophosphate (3′-AMP). The presence of both 2′-AMP and 3′-AMP imply a cleavage mechanism involving attack of the 2′-OH on the phosphate resulting in a 2′,3′-cyclic adenosine monophosphate (2′,3′-cAMP) as an intermediate. 2′,3′-Cyclic AMP is rapidly hydrolyzed to 3′-AMP and 2′-AMP in the presence of the metal complex; therefore the cyclic phosphodiester does not accumulate to a detectable extent. This detailed product analysis provides direct evidence for the hydrolytic cleavage of oligoribonucleotides by Cu([9]aneN$_3$)Cl$_2$.

In conclusion, Cu([9]aneN$_3$)Cl$_2$ cleaves RNA hairpin oligonucleotides at physiological pH and temperature. The reaction, which appears to occur by a hydrolytic mechanism, is not dependent on base sequence. Significantly, Cu([9]aneN$_3$)Cl$_2$ cleaves both single-stranded and double-stranded RNA, a notable feature, given that most of the RNA cleavage agents that have been tested do not cleave duplex RNA.[2−7,10,13c,15] Cu([9]aneN$_3$)Cl$_2$ could therefore be useful in RNA footprinting. The synthetic hydrolase Cu([9]aneN$_3$)Cl$_2$ is only a prototype, however, and future experiments will aim at utilizing the information gained in this and other reactivity studies to both increase the rate of hydrolysis and tune the selectivity of the catalyst.

**Acknowledgment.** We thank Ross Weatherman and Amy Dean at the University of Wisconsin—Madison for their assistance and expertise in the synthesizing, handling, and electrophoresis of RNA oligonucleotides. Funding was provided by NIH Grant 5T32GMO8349 (Biotechnology Training Grant for E.L.H.) and the University of Wisconsin Graduate School Research Committee. J.N.B. acknowledges the Alfred P. Sloan Foundation for a research fellowship. L.L.K. acknowledges the NSF NYI Program for support.

IC960955B