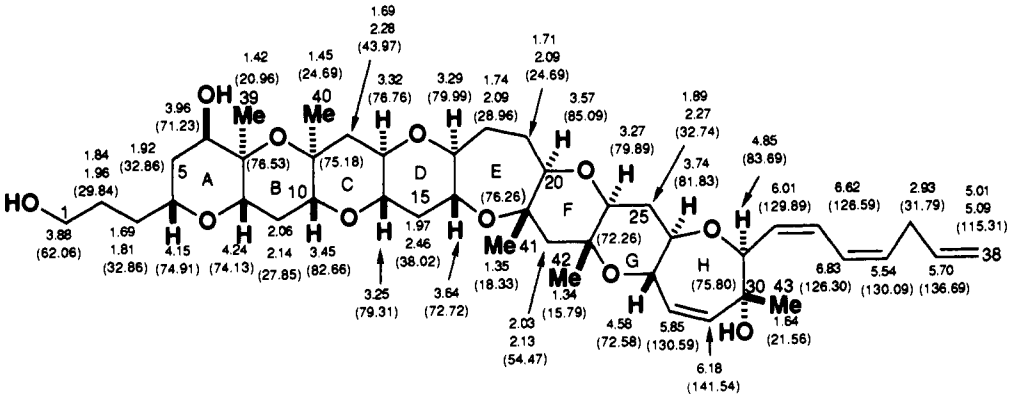

1

Figure 1. Structure and NMR assignments of **1**. ^1H and ^{13}C chemical shifts in parentheses are those in C_5D_5N.

H_2-19 were difficult to connect by COSY because of the closeness of their chemical shifts. However, a 2D HOHAHA spectrum clarified their connectivity by a cross peak due to a multiple relayed coupling between H17 and H20. The number of methylenes present between C17 and C20 was determined to be two by HSQC measurements. Connectivities of protons H17–H20 were thus established. Five oxygen-bearing quaternary carbons observed in the ^{13}C NMR spectra explained the disconnection of spin systems. Clearly, five singlet methyls (1.34–1.64 ppm) observed in the ^1H NMR spectrum resided on these quaternary carbons and were useful for HMBC experiments. Cross peaks due to $^{2,3}J_{CH}$ from methyl groups were shown for Me-39 vs C6/C7/C8, Me-40 vs C10/C11/C12, Me-41 vs C20/C21/C22, Me-42 vs C22/C23/C24, and Me-43 vs C29/C30/C31. Connectivities around the quaternary carbons were thus clarified and allowed us to assemble partial structures into a skeletal structure (Figure 1).

NOEs between angular protons or between an angular proton and a singlet methyl, as observed in NOESY and NOE difference spectra, supported the notion that ether rings A–H were trans-fused. No NOE between H16 and Me-41 was observed at room temperature, probably due to perturbation of ring E, but was clearly detected at −20 °C, as had been the case with ciguatoxin.[2] Coupling constants of angular protons (10 Hz) also supported trans-fusion of rings.[2] Positions of 1-OH and 6-OH were deduced from couplings of the hydroxyl protons with H_2-1 and H6, respectively. HMBC experiments clarified the position of 30-OH, by revealing a cross peak due to $^3J_{CH}$ coupling between the hydroxyl proton and C43. NOE difference spectra showed NOEs between H6 and Me-39, and between H27 and Me-43, thereby indicating that both 6-OH and Me-43 are in β configuration. The geometry of double bonds C32=C33 and C34=C35 was determined to be *Z* from proton coupling constants (11 Hz). The above results led to **1** as the structure of gambierol, including relative stereochemistry. Assignment of all ^1H and ^{13}C signals of **1** was achieved by analysis of HSQC spectra.

Production of **1** by cultured *G. toxicus* and the resemblance between gambierol (**1**) and ciguatoxin in molecular size, chromatographic properties, and symptoms caused in mice strongly support our hypothesis that *G. toxicus* is the true cause of ciguatera. Structural elucidation was accomplished with only 1.5 μmol of the material. The ring system of **1** (6/6/6/6/7/6/6/7) differs from previously known polyethers, e.g., brevetoxins,[8] yessotoxin,[9] and ciguatoxins,[2] thus demonstrating the diversity of ciguatera toxins and the complex biosynthesis of polyether compounds in dinoflagellates.

Acknowledgment. The present study was supported by grants from the Ministry of Education, Science and Culture. We are grateful to Professors T. Ishimaru, Tokyo University of Fisheries; A. Inoue, Kagoshima University; and J. Roux, Louis Malardé, for assistance in collecting dinoflagellates. Critical reading of the manuscript by Professor P. J. Scheuer, University of Hawaii, is also acknowledged.

Supplementary Material Available: 1D NMR, 2D HOHAHA, ^1H–^1H COSY, HSQC, and HMBC spectra (8 pages). Ordering information is given on any current masthead page.

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Enhanced Imidazole-Catalyzed RNA Cleavage Induced by a Bis-Alkylguanidinium Receptor

Joseph Smith, Katsuhiko Ariga, and Eric V. Anslyn*

The Department of Chemistry and Biochemistry
The University of Texas at Austin
Austin, Texas 78712

Received October 6, 1992

DNA and RNA cleavage agents have received considerable attention for multiple purposes,[1,2] including such health-related goals as the coupling of an RNA hydrolytic catalyst to antisense DNA[3] for use in mRNA gene therapy.[4] RNA hydrolytic catalysts have typically been metal complexes and amines. Metal complexes are quite efficient,[5,6] but they can be complicated by lability and toxicity.[6] Simple amine catalysts yield appreciable cleavage,[7] but they often require elevated concentrations and/or

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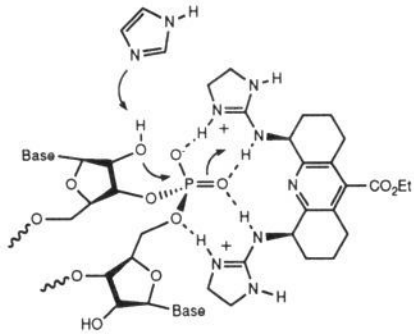
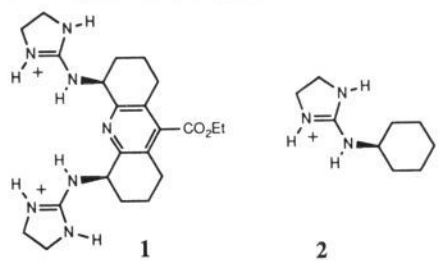
Scheme I



temperatures.[8,9] Likewise, polyamines such as polylysine and polyarginine peptides also yield significant cleavage,[10] but they are susceptible to cellular proteases.

In a different approach to catalyst development, enzyme mimics formed by the incorporation of active site functional groups of nucleases can potentially access nature's enormous hydrolytic rates.[11] For example, staphylococcal nuclease[12] (SNase) imparts a 10^{16}-fold rate enhancement for DNA hydrolysis.[13] Our strategy is to develop a water-soluble enzyme mimic (such as **1**) that initially focuses on only one of the catalytic features in SNase: the two essential arginines.[12b,c] The design for **1** uses amino-imidazoline groups, preorganized by a relatively rigid spacer,[14] as two-point hydrogen-bonding recognition moieties designed to orient a phosphate[15] and to enhance nucleophilic attack.[16] Similar SNase mimics have been studied by Hamilton with a *p*-nitrophenyl-activated RNA analog in acetonitrile.[17] Herein, we report the first utilization of this strategy for aqueous mRNA transesterification and cleavage under physiological conditions with micromolar receptor concentrations.



1 **2**

Figure 1 shows a denaturing polyacrylamide gel electrophoresis of mRNA (0.6 kb) made by run-off transcription[18] from the vector pBM-PRP2,[19] incubated with and without imidazole and with and without receptor **1**. Lanes 1–4 show insignificant RNA hydrolysis by imidazole or **1** alone, but the combination of imidazole and **1** shows substantial cleavage, thus supporting cooperativity between receptor **1** and general base.[20] Comparable concentrations of **2** showed no increased catalysis with imidazole.

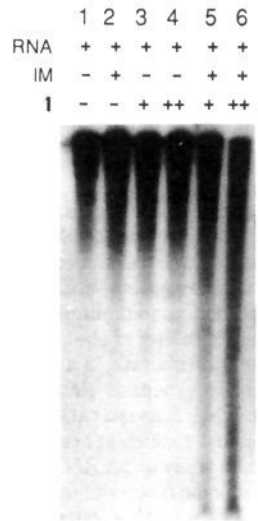


Figure 1. Representative cleavage patterns of ^{32}P-labeled mRNA as shown by autoradiography on a 5% polyacrylamide denaturing gel. IM = 250 mM imidazole (pH 7.05). + = 25 μM **1**. ++ = 200 μM **1**. Incubation for each lane was for 24 h at 37 °C.

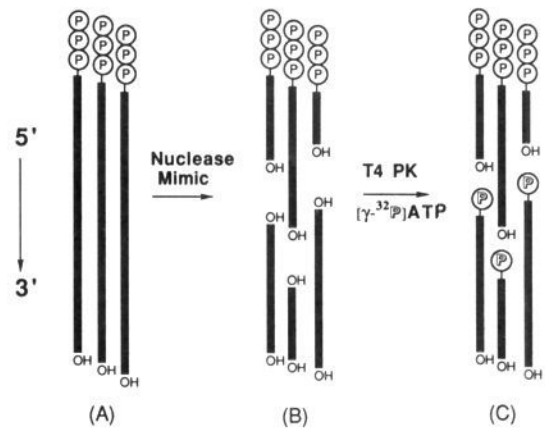


Figure 2. (A) Run-off transcription yielding mRNA with [α,β,γ]-phosphates on the 5′ end.[18] (B) Nuclease mimic catalyzing cleavage of mRNA by transesterification leaving intact 5′-hydroxyl groups. (C) T4 polynucleotide kinase (PK) catalyzing the transfer of the γ-phosphate of [γ-^{32}P]ATP to the 5′-hydroxyl,[27] thereby radiolabeling the mRNA strands that have been cleaved.

Autoradiography qualitatively displayed the rate enhancement imparted by **1**, but another method was needed to quantify pseudo-first-order initial rate kinetics. Breslow has described an efficient HPLC assay for measuring hydrolysis kinetics at 80 °C and 6.2×10^{-2} M polyU,[21] and Barbier and Brack[10b] have used an HPLC assay for 4.4×10^{-5} M $(Ap)_9A$ cleavage at 37 °C. For physiological conditions of 37 °C and 2.9×10^{-7} M mRNA,[22] a more sensitive assay was developed using radiolabeling (Figure 2). It involves labeling the 5′ end of the RNA with [^{32}P]ATP after incubation with the nuclease mimic **1** and imidazole. With RNA, each new cleavage site produces a new 5′-OH which can proportionally be ^{32}P labeled. Acid precipitation followed by gel

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filtration[23] removes the unincorporated ^{32}P, and a plot of scintillation counts converted to concentration versus time yields rate constants. Because of the sensitivity of the assay, kinetic studies can be completed in 1 day even when the hydrolytic rate is quite slow. Studies done at approximately equal equivalents of **1** and RNA phosphates (37 °C and pH 7.05) at concentrations of 7-500 μM yield rate enhancements of 8-20-fold over 250 mM imidazole alone.[24]

Scheme I shows a mechanistic proposal in which imidazole acts as a general base to deliver the 2'-OH to the phosphodiester linkage.[25] The phosphorane transition state is stabilized by ion pairing and/or guanidinium general-acid proton transfer from **1**.[16] It is often postulated that guanidiniums play the role of electrophilic catalysts at the active sites of phosphoryl transfer enzymes.[26] These mRNA results corroborate, in a totally synthetic system, the role of guanidinium groups as electrophilic activators.

In conclusion, the RNA cleavage catalyzed by **1** at micromolar concentrations in aqueous solution at neutral pH and 37 °C, along with the parallel recent study on an RNA analog,[17] demonstrates that mimicking the arginine functionalities of SNase is a successful approach for catalyzing phosphodiester cleavage. Further rate enhancements can be expected by expanding the structure of **1** to include additional SNase functionalities such as an intramolecular general base and a metal binding site. In addition, the design of **1** includes an ester group for potential coupling of an RNA recognition moiety.

Acknowledgment. We gratefully acknowledge financial support from the Searle Foundation (Chicago Community Trust), a PYI award to E.V.A., and a fellowship to J.S. from The University of Texas.

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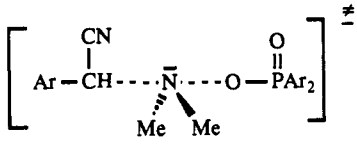
Theoretical Characterization of the Transition Structure for an S_N2 Reaction at Neutral Nitrogen

Michael Bühl and Henry F. Schaefer, III*

Center for Computational Quantum Chemistry
University of Georgia, Athens, Georgia 30602

Received September 8, 1992

Nucleophilic displacement reactions at nitrogen, intriguing analogies to the classical organic S_N type reactions, are attracting an increasing amount of attention.[1-7] The preparation of amines via reaction of organometallic compounds with *N*-haloamines is a useful example of reaction umpolung.[1] The reaction of ultimate carcinogens of aromatic amines with bionucleophiles has been studied in detail; while nitrenium intermediates have been proposed,[2] model reactions have been shown to follow an S_N2 mechanism.[3,4] Recently, experimental evidence for a classical S_N2 transition state involving nitrogen (**1**) has been given by means of double labeling experiments.[6]



1

While the S_N2 reaction at carbon has been studied theoretically in detail,[8,9] little is known about the corresponding nitrogen species. We now report ab initio quantum mechanical evidence[10] for the degenerate model reaction

$$F^- + H_2NF \rightarrow F^-\cdot H_2N\text{-}F \xrightarrow{[F\text{-}NH_2\text{-}F]^-*} F\text{-}NH_2\cdot F^- \rightarrow NH_2F + F^- \quad (1)$$

supporting the existence of transition structures like **1**. We chose this particular system as a model since it allows treatment at relatively high levels of theory.

As in gas-phase S_N2 reactions at a carbon center,[11] the first step in our model reaction is the formation of an ion-dipole complex, $F^-\cdot NH_2F$. This complex possesses C_1 symmetry and is characterized by a single, essentially linear $F^-\cdots H\text{-}N$ hydrogen bond (**2b**, see Figure 1). The C_s symmetry complex **2a** with two $F^-\cdots H$ contacts is the transition state for migration of the F^- from one H to the other. This situation is somewhat different from that in the analogous $F^-\cdot CH_3F$ complex (with C_{3v} symmetry and three $F^-\cdots H\text{-}C$ contacts), but is in agreement with theoretical findings for the related $F^-\cdot H_2O$ complex.[12] The actual dis-

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