

PLAUSIBLE PREBIOTIC REACTIONS IN HIGHLY COMPLEX ORGANIC MIXTURES LEAD TO THE SYNTHESIS OF PEPTIDE NUCLEIC ACID MONOMERS. L. E. Rodriguez¹, C. H. House¹, and M. P. Callahan², ¹Department of Geosciences and Penn State Astrobiology Research Center, The Pennsylvania State University, 220 Deike Bldg, University Park, PA, 16802, Email: chrishouse@psu.edu. ²Department of Chemistry and Biochemistry, Boise State University, 312 Science Building, Boise, ID 83725. Email: michaelcallahan914@boisestate.edu.

Introduction: Although it is widely believed that RNA preceded DNA as the first genetic material, the prebiotic synthesis of RNA has yet to be demonstrated, which has led to the belief that a simpler genetic molecule (often referred to as pre-RNA) that could spontaneously form from likely available precursors may have come first [1,2]. However, prebiotic chemistry on early Earth likely occurred in complex mixtures (conditions rarely simulated in laboratory experiments), and numerous competitive reactions leading to undesired products were probably common [3]. As a consequence, the synthesis of specific compounds and macromolecules under plausible prebiotic conditions still poses a significant challenge for the origin of life field.

Here, we investigated the reactivity of 53 N-heterocycles in Miller-Urey mixtures formed under both reducing and neutral atmospheres. Miller-Urey reactions produce a highly complex mixture of organics (in the thousands depending on the gas composition sparked [4]), and these mixtures were used to simulate the chemical complexity expected on early Earth and possibly other planetary environments.

Methods: For spark discharge experiments, a 1 L round flask filled with 250 mL of a 0.2 M phosphate buffer solution (pH 8.0) and attached to a Tesla coil and two tungsten electrodes was used to spark either a reducing (0.4 N₂, 0.1 CO₂, 0.25 H₂, and 0.25 CH₄) or neutral (0.5 N₂ and 0.5 CO₂) atmosphere at 1 bar. The flask was placed inside a water bath (~5 °C) and sparked at 40 kV for various time periods (for example, 72 hours). The resulting mixture was then incubated with a single N-heterocycle at different temperatures and times.

We used high-throughput high resolution mass spectrometry to analyze the liquid portion and identify N-heterocycle adducts in our highly complex reactions. For several N-heterocycle adducts, we elucidated their likely reaction pathway by carrying out *isolated* reactions using the specific N-heterocycle and predicted reactant, characterized the product(s) by tandem mass spectrometry (MS/MS), and compared these results to MS/MS data of the N-heterocycle adducts synthesized in the complex reaction. For select cases, we also performed NMR spectroscopy to elucidate where structural modifications took place; we were particularly interested in structures that substituted in favorable positions that maintained the possibility of base pairing.

Results and Discussion: We determined that specific spark organics react readily with a range of N-heterocycles. Robust reactions were identified based on commonly observed adducts, i.e. N-heterocycle adducts with the same $\Delta m/z$ and appended chemical formula (as determined by accurate mass measurements) have the same moiety added (see **Fig. 1** for some examples). Numerous N-heterocycle adducts were likely formed via a Strecker-like synthesis (with glycolonitrile) and Michael additions with 3-carbon acrylic and propiolic derivatives.

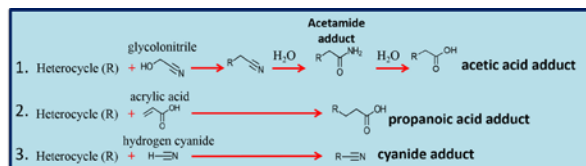


Fig. 1: Types of N-heterocycle adducts that were observed in a large number of N-heterocycle reactions with highly complex Miller-Urey organic mixtures. These results suggest a robust synthesis. Only a small fraction of the types of N-heterocycle adducts we observed are shown here.

One of the more interesting observations is that N-heterocycle adducts with an acetic acid group was very robust since they were the most frequently formed adducts detected in *both* reducing and neutral spark reaction mixtures and N-heterocycles. These adducts could be considered the “nucleoside subunit” of peptide nucleic acid (PNA), which are linked to an N-(2-aminoethyl)glycine (AEG), which acts as a backbone. Furthermore, it was previously demonstrated that AEG forms in Miller-Urey spark-discharges of CH₄, N₂, NH₃, and H₂O [5], and our mass spectrometry data suggests that AEG is also present in our Miller-Urey mixtures.

We explored the reactivity of specific spark adducts, thymine-N-1-acetic acid (1 mM) and uracil-N-1-propanoic acid (1 mM) with AEG. **Fig. 2** shows the DART mass spectrum of reaction containing uracil-1-propanoic acid and AEG and accurate mass measurements assign the peak at m/z 285.1198 as [C₁₁H₁₆N₄O₅ + H]⁺, which corresponds to a molecular formula for the PNA-like monomer shown in the same figure (and represents one possible structure). Additionally, we

determined that AEG does not deter the adduct formation between glycolonitrile and N-heterocycles, which led us to speculate that a one-pot synthesis of these compounds may be possible. Upon closer inspection of reactions involving nitrogen heterocycles and complex mixtures from either reducing or neutral atmospheres, a variety of high molecular weight adducts corresponding to molecular formulae that potentially represent PNA and PNA-like monomers were detected and suggests that (1) a one-pot synthesis of these compounds may be possible despite the great complexity of the reaction mixture, (2) reactions can proceed in aqueous solutions, and (3) PNA may actually be prebiotically relevant!

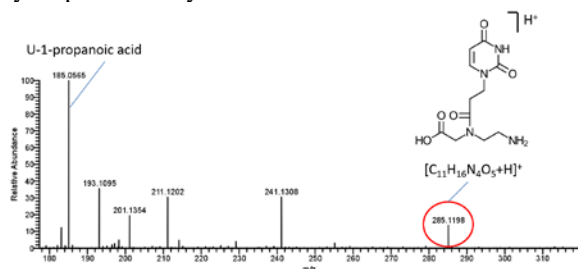


Fig. 2: Mass spectrum of reaction (80 °C, 72 h) containing uracil-1-propanoic acid and N-(2-aminoethyl)glycine.

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