LETTER

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A Stereoelectronic Effect in Prebiotic Nucleotide Synthesis

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ABSTRACT A plausible route for the spontaneous synthesis of an activated ribonucleotide that is poised for polymerization has been put forth (Powner *et al.* (2009) *Nature*, *459*, 239–242). A key step in this route necessitates the regioselective phosphorylation of the secondary alcohol on $C_{3'}$ of an anhydroarabinonucleoside in the presence of the primary alcohol on $C_{5'}$. Here, we propose that this regioselectivity relies on electron delocalization between a lone pair (*n*) of $O_{5'}$ and an antibonding orbital (π^*) of $C_2{=}N_3$. This $n \rightarrow \pi^*$ interaction modulates reactivity without the use of a protecting group. Thus, a stereoelectronic effect could have opened a gateway to the "RNA world", the chemical milieu from which the first forms of life are thought to have emerged on Earth some 4 billion years ago.

According to the "RNA world" hypothesis (*1*), the extant DNA- and protein-based life on Earth was preceded by self-replicating RNA (*2–4*). This hypothesis is supported by the observation that RNA is capable of self-replication (*5, 6*), as well as execution of the functions of both DNA and proteins. RNA, like DNA, can store genetic information (*2–4*) and, like protein-based enzymes, can catalyze reactions (*7, 8*). The validation of this hypothesis mandates the synthesis of RNA building blocks—activated ribonucleotides—under prebiotic conditions. Recently, some of us proposed a concise synthetic route to an activated ribonucleotide using plausible prebiotic feedstocks (*9*). The key intermediate in this route is anhydroarabinonucleoside **1** (Scheme 1). The last synthetic step requires the phosphorylation of **1** to yield cytidine 2′,3′-cyclic phosphate (**2**), an activated ribonucleotide poised to undergo polymerization.

Examination of anhydroarabinonucleoside **1** reveals two phosphorylation sites: the primary alcohol on $C_{5'}$ and the secondary alcohol on $C_{3'}$. On simple steric grounds alone, a primary alcohol should be phosphorylated faster than an otherwise similar secondary alcohol, yet under multiple reaction conditions, 3′-phosphorylation was found to proceed selectively over 5′-phosphorylation (*9*). This surprising regioselectivity is critical because 5′-phosphorylation would not yield an activated ribonucleotide. We sought to determine its origin.

The crystal structure of anhydronucleoside **1** revealed that $O_{5'}$, the oxygen of the primary alcohol, is in a short contact with C_2 (Figure 1, panels a and b) (*9, 10*). Indeed, the van der Waals surfaces of $O_{5'}$ (r_O = 1.52 Å) and C_2 (r_C = 1.70 Å) interpenetrate to an extraordinary extent: 0.52 Å. To ascertain whether this intimacy was an artifact of crystal lattice forces, we optimized the geometry of **1** in the gas phase by using hybrid density functional theory at the B3LYP/6-311+G(2d,p) level of theory with Gaussian 03 (*11*). The short contact observed in the crystal structure ($r_{O\cdots C}$ = 2.70 Å) was preserved in the calculated structure ($r_{O\cdots C}$ = 2.88 Å).

Some of us have shown that a short contact between an oxygen donor and an sp^2 carbon leads to electron delocalization (*12*). To reveal contributions from electron delocalization in anhydronucleoside **1**, we resorted to natural bond orbital (NBO) analysis (*13–15*). Geometry optimization and NBO analyses were performed at the B3LYP/6-311+G(2d,p) level of theory. The stabilization afforded by the various donor–acceptor orbital interactions, such as $E_{n\rightarrow\pi^*}$, was calculated using second-order perturbation theory, as implemented in NBO 5.0.

We found that the lone pair (*n*) of $O_{5'}$ is delocalized over the antibonding orbital (π^*) of the $C_2{=}N_3$ bond (Figure 1, panels c

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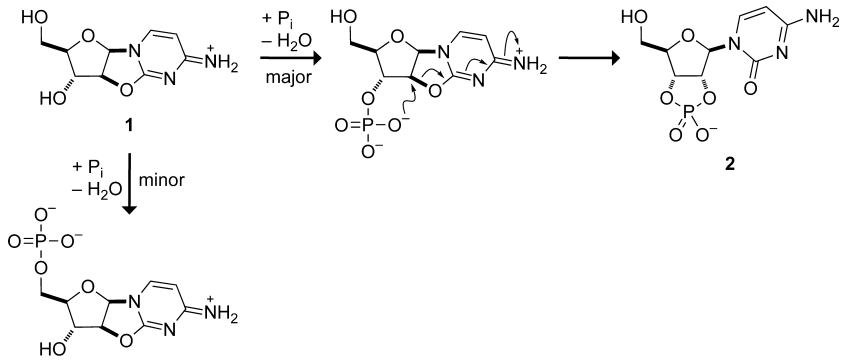
Scheme 1. Phosphorylation of anhydroarabinonucleoside 1 to yield cytidine 2′,3′-cyclic phosphate (2)



and d) with $E_{n\rightarrow\pi^*}$ = 1.09 kcal/mol. Analogous electron delocalization between two carbonyl groups has been reported previously (*12*). Such $n\rightarrow\pi^*$ electronic delocalization is reminiscent of the nucleophilic attack on carbonyl groups along the Bürgi–Dunitz trajectory (*16*), and is accompanied by pyramidalization of the acceptor carbon (*12*). Both of these signatures are apparent in the crystal structure of anhydronucleoside **1**. The $O_{5'}\cdots C_2{=}N_3$ angle is 99.2°, which is close to the Bürgi–Dunitz trajectory; C_2 is displaced toward $O_{5'}$ by 0.01 Å from the plane formed by its three pendant atoms.

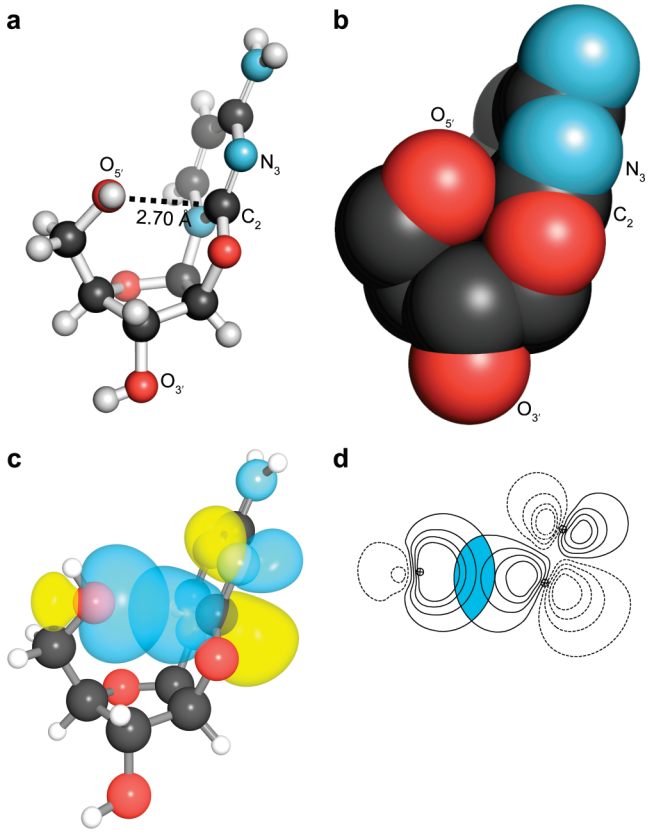


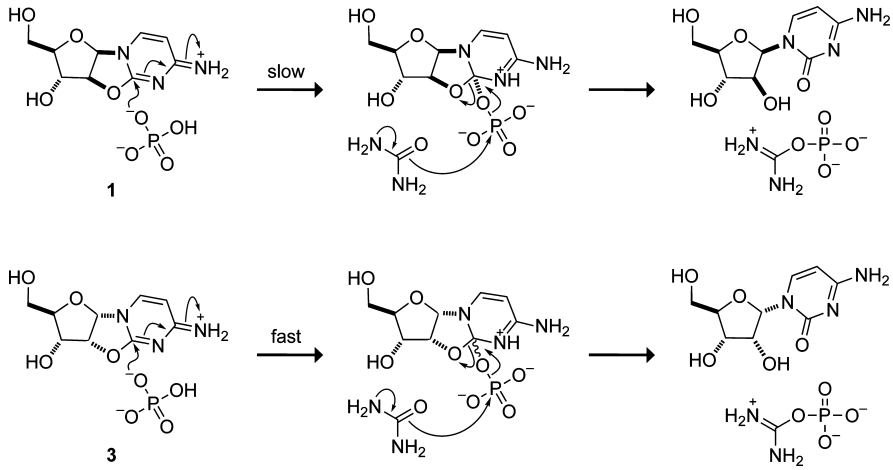
Figure 1. $n\rightarrow\pi^*$ interaction in anhydroarabinonucleoside 1. a) Ball-and-stick and b) space-filling (without hydrogens) representation of crystalline 1 (*9*). c) Overlap between *n* of $O_{5'}$ and π^* orbital of $C_2{=}N_3$ in the preferred conformation of 1. d) Overlap integral (0.1295) from panel c.

Engaging $O_{5'}$ in an $n\rightarrow\pi^*$ interaction is likely to diminish its reactivity in two distinct ways. First, the enforced proximity of $O_{5'}$ and C_2 increases steric crowding near $O_{5'}$. Second, the delocalization of electron density from *n* into π^* decreases the intrinsic nucleophilicity of $O_{5'}$. Neither of these factors affects the reactivity of $O_{3'}$, which hence undergoes selective phosphorylation (*9*).

Complementary support for the existence of an $n\rightarrow\pi^*$ interaction in arabinose anhydronucleoside **1** comes from its *ribo*-diastereomer, **3**, which undergoes deleterious phosphate-mediated hydrolysis much more rapidly (Scheme 2) (*9*). An inspection of the crystal structure and gas-phase optimized geometry of **3** indicates that its $O_{5'}$ cannot participate in an $n\rightarrow\pi^*$ interaction; the donor and acceptor groups are too distal. Thus, whereas both faces of the $C_2{=}N_3$ bond of **3** are accessible to inorganic phosphate, only one face is accessible in **1** (*cf.* Figure 1, panel C and Figure 2). In addition to this steric effect, an electronic effect is also operative. An $n\rightarrow\pi^*$ interaction in **1** increases the energy of the π^* orbital of its $C_2{=}N_3$ bond, thereby reducing the electrophilicity of C_2. As in the regioselectivity of the phosphorylation reaction (Scheme 1), the differing rates of the hydrolysis reaction (Scheme 2) are a manifestation of the steric and electronic effects that arise from an $n\rightarrow\pi^*$ interaction. Notably, ribose anhydronucleoside **3**, which lacks the $n\rightarrow\pi^*$ interaction of arabinose anhydronucleoside **1**, is phosphorylated primarily on $O_{5'}$ (*9*), as expected on simple steric grounds.

Our analysis supports the hypothesis that an $n\rightarrow\pi^*$ interaction is responsible for

Scheme 2. Phosphate-mediated hydrolysis of anhydroarabinonucleoside 1 and anhydroribonucleoside 3



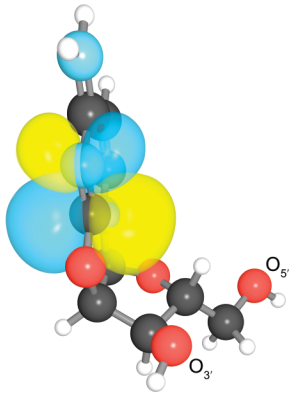


Figure 2. π* orbital of $C_2{=}N_3$ in the preferred conformation of anhydroribonucleoside 2.

the phosphorylation of anhydronucleoside **1** on C_3, as well as its resistance to hydrolysis. We note that the use of an $n{\rightarrow}\pi^*$ interaction had not been invoked as a means to control the reactivity of a nucleic acid. In effect, the ensuing electron delocalization obviates the need for a protecting group. We propose that a stereoelectronic effect played a key role in the prebiotic synthesis of activated ribonucleotides.

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