Published in final edited form as:
ACS Chem Biol. 2010 July 16; 5(7): 655–657. doi:10.1021/cb100093g.

A Stereoelectronic Effect in Prebiotic Nucleotide Synthesis

Amit Choudhary[†], **Kimberli J. Kamer**[⊥], **Matthew W. Powner**[§], **John D. Sutherland**[§,*], and **Ronald T. Raines**[⊥,‖,*]

[†]Graduate Program in Biophysics, University of Wisconsin–Madison, Madisonf, Wisconsin 53706, USA

[⊥]Department of Biochemistry, University of Wisconsin–Madisonf, Madison, Wisconsin 53706, USA

[‖]Department of Chemistry, University of Wisconsin–Madison, Madison, Wisconsin 53706, USA

[§]School of Chemistry, The University of Manchester, Oxford Road, Manchester M13 9PL, UK.

Abstract

A plausible route for the spontaneous synthesis of an activated ribonucleotide that is poised for polymerization has been put forth (Powner et al., *Nature* **2009f**, *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 executing 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**. The last synthetic step requires the phosphorylation of **1** to yield cytidine 2′,3′-cyclic phosphate (**2**), an activated ribonucleotide poised to undergo polymerization (Scheme 1).

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

[*]Corresponding authors, RTR: rtraines@wisc.edu; JDS: john.sutherland@manchester.ac.uk..

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 the lone pair of an oxygen donor and an sp^2 carbon lead 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 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 towards $O_{5'}$ by 0.01 Å from the plane formed by its three pendant atoms.

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 $O_{5'}$. Secondly, 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 1C 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.

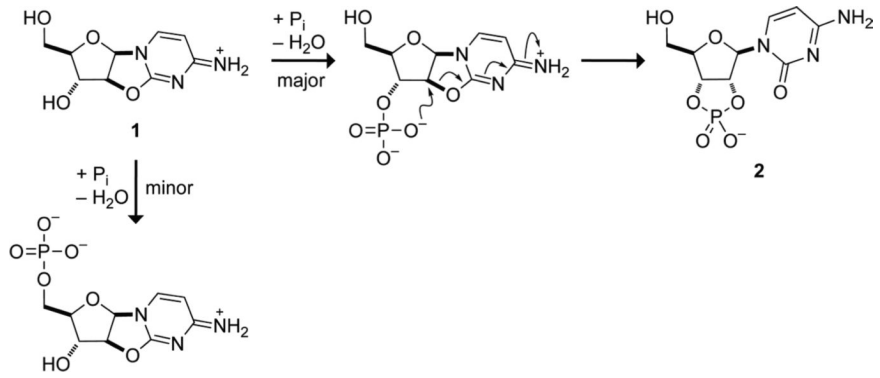
Our analysis supports the hypothesis that an $n\rightarrow\pi^*$ interaction is responsible for the phosphorylation of anhydronucleoside **1** on C_3, as well as its resistance to hydrolysis. We note that the use of an $n\rightarrow\pi^*$ interaction had not been invoked as a means to control the reactivity 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.

Acknowledgments

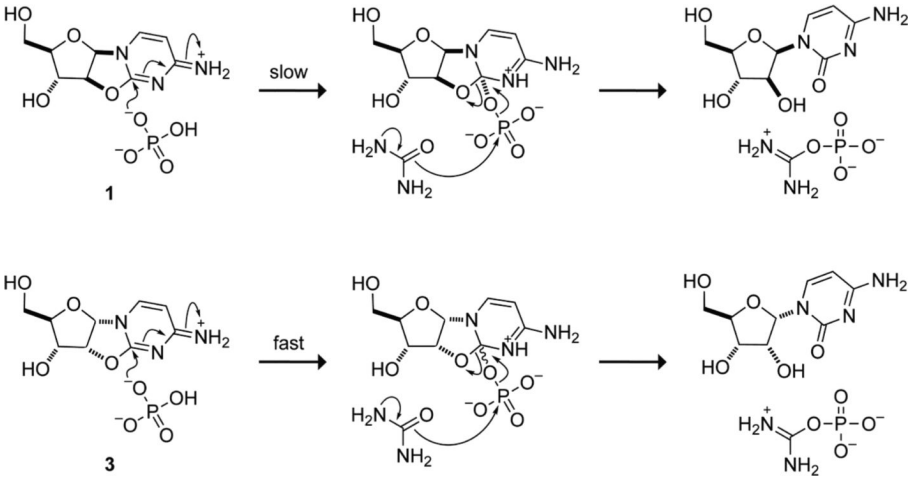
This work was supported by grant R01 AR044276 (NIH) and the UK Engineering and Physical Sciences Research Council.

REFERENCES

1. Gilbert W. The RNA World. Nature. 1986; 319:618.
2. Woese, CR. The Genetic Code. Harper & Row; New York: 1967.
3. Crick FHC. The origin of the genetic code. J. Mol. Biol. 1968; 38:367–379. [PubMed: 4887876]
4. Orgel LE. Evolution of the genetic apparatus. J. Mol. Biol. 1968; 38:381–393. [PubMed: 5718557]
5. Johnston WK, Unrau PJ, Lawrence MS, Glasner ME, Bartel DP. RNA-catalyzed RNA polymerization: Accurate and general RNA-template primer extension. Science. 2001; 292:1319–1325. [PubMed: 11358999]
6. Lincoln TA, Joyce GF. Self-sustained replication of an RNA enzyme. Science. 2009; 323:1229–1232. [PubMed: 19131595]
7. Altman S. Enzymatic cleavage of RNA by RNA (Nobel lecture). Angew. Chem. Int. Ed. 1990; 29:749–758.
8. Cech TR. Self-splicing and enzymatic activity of an intervening sequence RNA from *Tetrahymena* (Nobel lecture). Angew. Chem. Int. Ed. 1990; 29:759–768.
9. Powner MW, Gerland B, Sutherland JD. Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions. Nature. 2009; 459:239–242. [PubMed: 19444213]
10. Brennan T, Sundaralingam M. Molecular structure of 2,2′-anhydro-1-β-d-arabinofuranosyl cytosine hydrochloride (cyclo ara-C): A highly rigid nucleoside. Biochem. Biophys. Res. Commun. 1973; 52:1348–1353. [PubMed: 4717753]
11. Frisch, MJ., et al. Gaussian 03, Revision C.02. Gaussian, Inc.; Wallingford, CT: 2004.
12. Choudhary A, Gandla D, Krow GR, Raines RT. Nature of amide carbonyl–carbonyl interactions in proteins. J. Am. Chem. Soc. 2009; 131:7244–7246. and references therein. [PubMed: 19469574]
13. Weinhold, F. Natural Bond Orbital Methods. In: Schleyer, P. v. R.; Allinger, NL.; Clark, T.; Gasteiger, J.; Kollman, PA.; Shaefer, HF., III; Schreiner, PR., editors. Encyclopedia of Computational Chemistry. John Wiley & Sons; Chichester, UK: 1998. p. 1792-1811.
14. Glendening ED, Badenhoop JK, Reed AE, Carpenter JE, Bohmann JA, Morales CM, Weinhold F. NBO 5.0, NBO 5.0. 2001
15. Weinhold, F.; Landis, CR. Valency and Bonding: A Natural Bond Orbital Donor–Acceptor Perspective. Cambridge University Press; Cambridge, UK: 2005.
16. Bürgi HB, Dunitz JD, Shefter E. Geometrical reaction coordinates. II. Nucleophilic addition to a carbonyl group. J. Am. Chem. Soc. 1973; 95:5065–5067.



Scheme 1.
Phosphorylation of anhydroarabinonucleoside **1** to yield cytidine 2′,3′-cyclic phosphate (**2**)
(Do not reduce. Print at 100%.)



Scheme 2.

Phosphate-mediated hydrolysis of anhydroarabinonucleoside **1** and anhydroribonucleoside **3**

(Do not reduce. Print at 100%.)

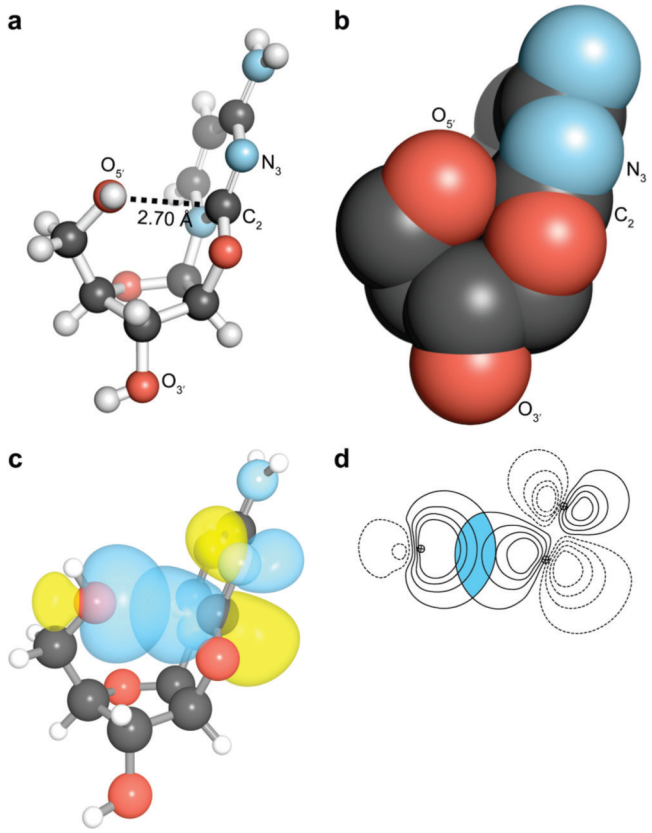


Figure 1.

$n \rightarrow \pi^*$ 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.

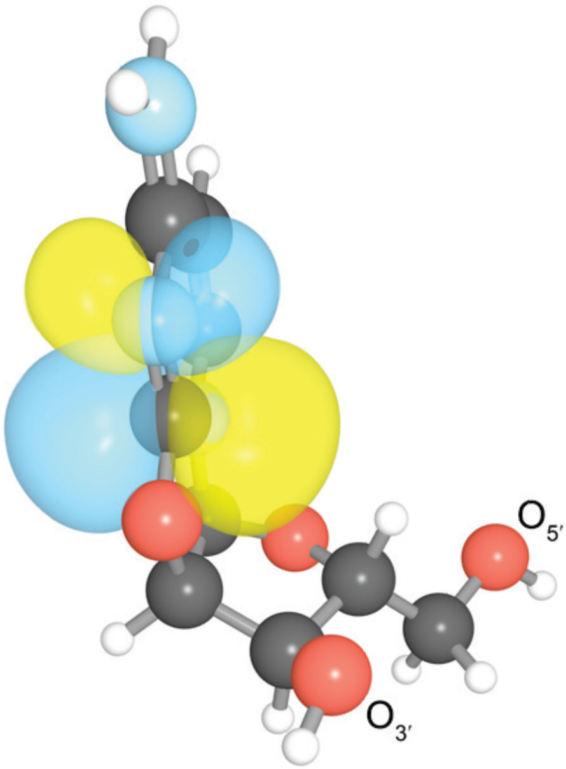


Figure 2.
π^* orbital of C_2=N_3 in the preferred conformation of anhydroribonucleoside **2**.