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Two-Step Potentially Prebiotic Synthesis of α-D-Cytidine-5′-phosphate from D-Glyceraldehyde-3-phosphate

Carole Anastasi, Michael A. Crowe, and John D. Sutherland*

School of Chemistry, The University of Manchester, 12 Oxford Road, Manchester M13 9PL, U.K.

Received September 8, 2006; E-mail: john.sutherland@manchester.ac.uk

A prebiotically plausible synthesis of ribonucleotides is required to support the "RNA world" hypothesis.[1] The conversion of D-ribose-5-phosphate **1** to the aminooxazoline **2**, and thence to α-D-cytidine-5′-phosphate **3**, by sequential treatment with cyanamide and cyanoacetylene was demonstrated over 30 years ago (Scheme 1),[2] but no prebiotic synthesis of **1** has been reported.

Not only is ribose notoriously difficult to synthesize as anything other than a component of a complex mixture,[3] but it is unstable,[4] and prebiotic phosphorylation furnishes the 1-, 2-, and 3-phosphates and not the 5-phosphate.[5]

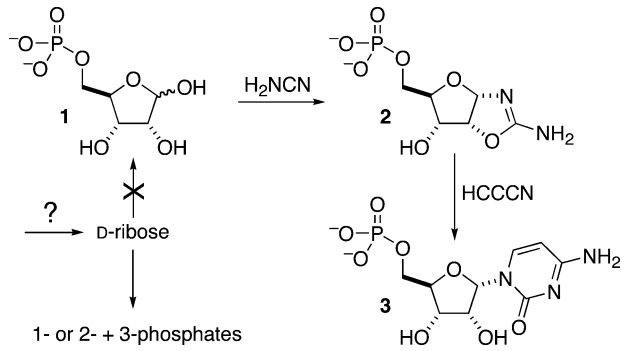
D-Pentose-5-phosphates, including **1**, are theoretically accessible by aldol reaction of glycolaldehyde **4** and D-glyceraldehyde-3-phosphate **5**, but such an approach is likely to be thwarted experimentally by the instability of **5** under the alkaline conditions required to enolize **4** (Scheme 2).

The enolate of **5** is easily formed by intramolecular general base catalysis by the phosphate dianion in mild alkali and eliminates phosphate rapidly.[6] Expectations that this E1cb irreversible elimination behavior of **5** would prevent an aldol synthesis of **1** were realized at the outset of this study. It was found that incubation of a D_2O solution of **4** and **5** for a day at pD = 9.4 resulted in significant elimination of phosphate from **5** even before **4** had undergone significant enolization (as evidenced by the lack of exchange of the CH_2 (and CH) protons of **4** for deuterons). Incubation of a solution of **4** and **5** at neutral pD did not result in elimination of phosphate from **5**, but neither was aldolization evident.

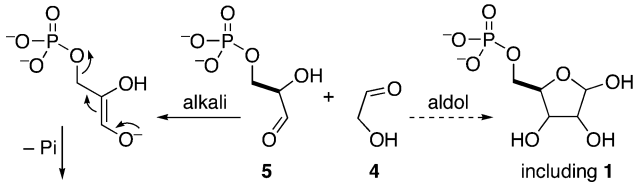
We have recently found that 2-aminooxazole **6**, a condensation product of glycolaldehyde and cyanamide,[7] reacts with glyceraldehyde to give pentose aminooxazolines in a remarkable process that is essentially quantitative and is highly stereoselective for the *ribo*- and *arabino*-products.[8] We thus wondered if it might be possible to produce **2** from **6** and **5** (Scheme 3). However, in addition to the possibility that elimination from **5** might still occur faster than the addition of **6**, there was also the chance that the stereoselectivity found in the earlier reaction of **6** with glyceraldehyde might be altered by the presence of the phosphate group in **5**.[9]

To investigate this potential reaction, we incubated a solution of **5** and **6** (both 69 mM) in H_2O at pH = 7 and room temperature. After 2 days, an aliquot was removed and lyophilized, and the residue was dissolved in D_2O for ^1H NMR analysis. A series of doublets ($J = 5-6$ Hz) in the range of $\delta = 5.4-6.0$ ppm suggested that pentose aminooxazoline-5′-phosphates had been formed in good yield (Figure S1, Supporting Information).[10] One stereoisomer was dominant to the extent that it exceeded the sum of the other stereoisomers. By sample spiking with an authentic standard of **2** prepared from **1**, we were able to determine that this predominant stereoisomer was **2**. To characterize the other products, the mixture was treated with alkaline phosphatase, divided into aliquots, and then separately spiked with standards of the pentose aminooxazolines.[2,11] In this way, the ratio of the various stereoisomers was

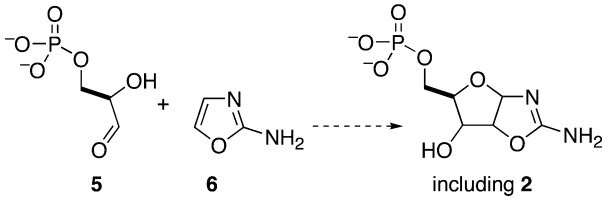
Scheme 1



Scheme 2. Potential Aldolization and the Instability of **5**



Scheme 3



determined to be *ribo* (**2**):*arabino*:*lyxo*:*xylo*, 6.5:3.5:1.4:1. Although **6** can be easily sublimed,[12] some was still present after lyophilization. In addition, there was some evidence that partial elimination of phosphate from **5** had taken place.[6] We therefore decided to increase the ratio of **5**:**6** in the reaction from 1:1 to 2:1 by increasing the concentration of **5** to 138 mM. To enable the products, byproducts, and any residual **6** to be quantified, we carried out this and subsequent reactions in D_2O (pD = 7.4) so that lyophilization was not necessary prior to ^1H NMR analysis (Table 1).

In the reaction of **6** with glyceraldehyde, we had found that the *ribo*-product was formed in 44% yield. To now find that the *ribo*-product **2** is also formed in a similar yield (38% after 4 days) in the reaction of **5** and **6** is noteworthy. However, the selectivity for the *ribo*-product in the present case (*ribo*:*arabino*, 2.7:1) is greater than it is in the nonphosphorylated series (*ribo*:*arabino*, 1.5:1). We next investigated the effect of temperature and carried out a reaction at 4 °C again with the 2:1 ratio of **5**:**6**. At this lower temperature, the reaction was slower, but after 4 days, **2** had still been formed in 37% yield. We are currently investigating whether the stereoselectivity in the reaction of **5** and **6** is due to kinetic or thermodynamic factors, or both.

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Table 1. Formation of Aminooxazoline-5′-phosphates

conditions	Products and Residual **6** (%)[a]					
	6	**2**	*arabino*	*xylo*	*lyxo*	byproducts[b]
2 days, rt	15	34	16	5	5	25
4 days, rt	8	38	14	4	5	28
2 days, 4 °C	34	22	14	6	2	17
4 days, 4 °C	13	37	18	4	3	23

[a] Determined from (H1′) signal integration relative to the total integration of all signals in the range of $\delta = 5.4-6.0$ ppm + the upfield signal for **6** at 6.7 ppm (corroborated by H2′ signal integration where possible). [b] Total integral of unassigned signals in the range of $\delta = 5.4-6.0$ ppm.

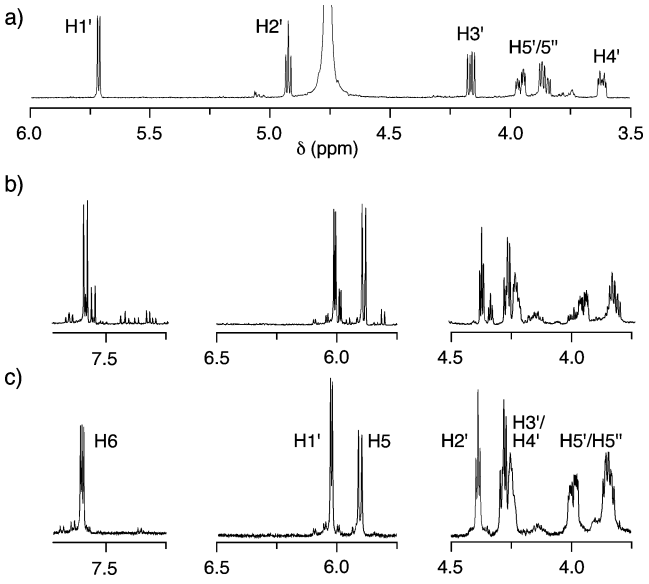

Figure 1. ^{1}H NMR analysis (500 MHz, D_2O) of the conversion of **2** to **3**. (a) Spectrum of **2** as prepared from **1**; (b) spectrum of the crude reaction products of **2** and cyanoacetylene (4 equiv, 60 °C, 24 h) in H_2O after lyophilization and dissolution in D_2O; (c) spectrum of a purified sample of **3**.

Two distinct scenarios can be envisaged for this chemistry. In the first, **5** and **6** are formed in different locations, and then one is somehow delivered to the location of the other. In the second, **6** is formed from glycolaldehyde and cyanamide in the presence of **5** and then undergoes reaction with it. To investigate this latter scenario, we first established the mildest conditions under which glycolaldehyde and cyanamide react to give significant quantities of **6**. In the presence of 1 M phosphate buffer at pH 7, a solution 55 mM in both cyanamide and glycolaldehyde gave **6** in ∼50% yield after 5 days at rt by ^{1}H NMR analysis. When this reaction was repeated with inclusion of 55 mM **5**, no pentose aminooxazoline-5′-phosphates were detected however. Furthermore, we have found that **5** undergoes elimination of phosphate on treatment with cyanamide alone. This appears to rule out the second scenario but leaves open the first, and the ease of sublimation of **6** suggests a means whereby it could be removed from its place of synthesis and delivered to the location of **5** by "rain-in". In support of this, we found that, when a sample of **6** was left on a 50 °C surface overnight, about half of it sublimed. Thus, synthesis of **6** in solution followed by evaporation, sublimation, and subsequent rain-in could result in the synthesis of pentose aminooxazoline-5′-phosphates at a separate location. Whatever the case, the reaction is remarkably stereoselective for the *ribo*-product **2**, and so we decided to study the further elaboration of **2** to ribonucleotides.

It had previously been shown that the one-pot conversion of **1** to **3** proceeded in 28% isolated yield.[2] Since it was unclear whether this relatively low yield was the result of a low yield in either of the chemical steps or due to losses during purification, we investigated the conversion of **2** to **3** by ^{1}H NMR spectroscopy (Figure 1). This analysis revealed that the conversion of **2** to **3** is clean and high yielding (>80% by ^{1}H NMR spectroscopy, 62% isolated yield).[13] It is thus apparent that α-D-cytidine-5′-phosphate **3** can be prepared from D-glyceraldehyde-3-phosphate **5** in two steps in water at neutral pH and at, or near, ambient temperature in >30% overall yield. In the first step, the sugar and half the nucleobase are assembled by stereoselective addition of 2-aminooxazole **6** to **5**, and in the second step, the remaining half of the nucleobase is appended by reaction of **2** with cyanoacetylene. If a prebiotically plausible route to **5** and an efficient anomerization of **3** can be found,[14] then a predisposed route to pyrimidine nucleotide precursors of RNA will be realized.

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Supporting Information Available: Experimental procedures for the reactions described herein and characterization data for the products. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) The preponderance of the *ribo*- and *arabino*-products in the reaction of **6** with glyceraldehyde had been attributed to attack on the least hindered face of the carbonyl group in a six-membered H-bonded chelate involving the 3-OH group (ref 8).
(10) The chemical shifts of the doublet signals ($J = 5-6$ Hz) for H1′ of the corresponding, nonphosphorylated pentose aminooxazolines are somewhat pH dependent but fall in this range (ref 8).
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(13) We found (again by ^{1}H NMR analysis) that the conversion of **1** to **2** is essentially quantitative (>95%, Figure 1a and Supporting Information). By sample spiking with a known quantity of pentaerythritol, we showed that the overall conversion of **1** to **3** proceeds in 82% yield. The conversion of **2** to **3** must therefore proceed in >80% yield. It would therefore appear that the relatively low yield of **3** reported in ref 2 is due to losses incurred during purification.
(14) Photoanomerization of **3** to β-D-cytidine-5′-phosphate has been reported (ref 2) but is low yielding.

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