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Potentially Prebiotic Synthesis of Pyrimidine β-D-Ribonucleotides by Photoanomerization/Hydrolysis of α-D-Cytidine-2′-Phosphate

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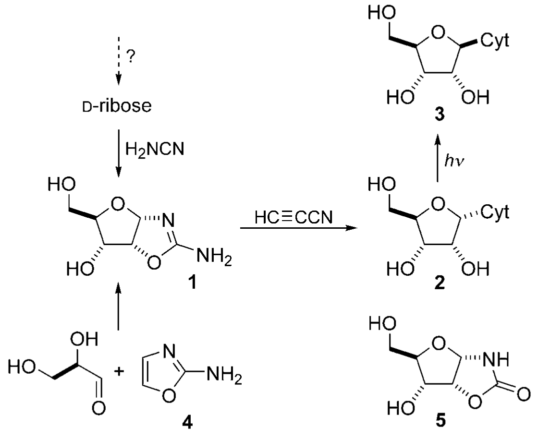
Dedicated to the memory of Leslie Orgel.

The "RNA world" hypothesis is consistent with RNA's being a prebiotic product, or the biotic invention of a precursor genetic system. A prebiotically plausible synthesis of ribonucleotides would help to differentiate between these two possibilities by providing strong support for the first, and, as such, is a major goal.[1]

The efficient conversion of D-ribose aminooxazoline **1** to α-D-cytidine **2**, by treatment with cyanoacetylene was demonstrated over 30 years ago (Scheme 1),[2,3] but has not gained acceptance as part of a route to natural ribonucleotides for two reasons. Firstly, an efficient prebiotic route to D-ribose—from which **1** can be obtained by treatment with cyanamide—has not been demonstrated.[4] Secondly, photoanomerization of **2** to β-D-cytidine **3** was found to occur in only 4% yield.[2]

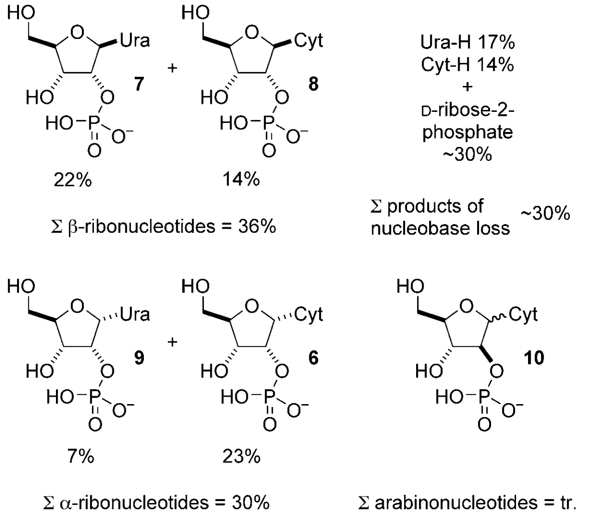


Scheme 1. Potentially prebiotic cytidine synthesis.

We recently showed that the first of these difficulties can be overcome if ribose is bypassed, and **1** is made by the direct reaction of 2-aminooxazole **4** and D-glyceraldehyde.[5] Furthermore, we recently determined that the photoanomerization of **2** to **3** is inefficient partly because of nucleobase loss, but mainly due to the formation of oxazolidinone **5**.[6] In addition to these processes, we found that inversion of stereochemistry at the 2′-position of cytidine derivatives can occur during irradiation,[2,6] as can photohydrolysis to uridine derivatives.[6,7] Since nucleotides are the initial target of our prebiotic RNA assembly chemistry, we are also investigating the effect of phosphorylation of **2** on the photoanomerization. In principle, RNA can derive from polymerization of activated 5′-nucleotides, or nucleoside-2′,3′-cyclic phosphates, and since the latter can easily be produced by cyclization of 2′- or 3′-nucleotides,[8] we initially considered the photoanomerization of all three regioisomeric mononucleotides. The 5′-nucleotide[9] underwent faster photochemistry, and the β-5′-nucleotide was formed in improved yield,[4,6] but nucleobase loss and oxazolidinone formation were again significant competing reactions.[6] Because oxazolidinone formation is also potentially possible with the 3′-phosphate of **2**, we next focused our attention on α-D-cytidine-2′-phosphate **6**. Whatever the mechanism for oxazolidinone formation from **2** or its 5′-phosphate,[6] we reasoned that the blockade of the 2′-hydroxyl group in **6** should prevent such deleterious chemistry from taking place.

Conventional synthetic chemistry was used to prepare **6** and standard samples of several potential irradiation products that were not commercially available. We next irradiated **6** for 72 h in aqueous solution at pH 6.5 with a UV lamp having principal emission at 254 nm, then lyophilized the reaction mixture and dissolved the residue in D_2O for analysis by ^1H NMR spectroscopy. By sequentially spiking with standards, we were able to identify the products of the irradiation (Scheme 2).[10]

As we had hoped, the blocking of oxazolidinone formation by the 2′-phosphate group of **6** resulted in an increase in nucleotide products. The long irradiation time also resulted in increased photoanomerization, and significant photohydrolysis



Scheme 2. Products formed through the irradiation of **6**. Some 30% of the nucleotides underwent nucleobase loss, and the sum total of uracil and cytosine was matched by the amount of D-ribose-2-phosphate suggesting a single (hydrolytic) nucleobase loss reaction manifold.

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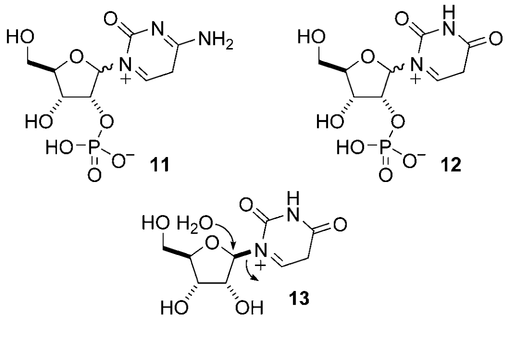
such that both β-D-uridine-2′-phosphate **7** and β-D-cytidine-2′-phosphate **8** were produced in roughly comparable quantity along with residual starting material and α-D-uridine-2′-phosphate **9**. Traces of both α- and β-D-arabinocytidine-2′-phosphate **10** could also be detected amongst the products of the irradiation.[10] Partial nucleobase loss again took place as evidenced by the presence of uracil and cytosine in the sample. In irradiations of cytidine nucleosides, we had previously been unable to identify the sugar products of nucleobase loss, but in the present case, ribose-2-phosphate was clearly evident according to ^{1}H NMR spectroscopic analysis, and was confirmed by sample spiking with an authentic synthetic standard.[6] Thus, in this case, nucleobase loss can be equated with hydrolysis of an excited state or reaction intermediate. We have previously suggested that photoanomerization of pyrimidine nucleosides involves nucleobase iminium ions, and it seems likely that hydrolytic nucleobase loss also proceeds from such species.[6] In the present case the corresponding iminium ions would be the cytidine and uridine derivatives **11** and **12** (Scheme 3). Acid-catalyzed hydrolysis of β-D-uridine in the absence of irradiation proceeds through the related iminium ion **13**[11] by what is presumably a dissociative concerted process.[12] By analogy, we assume that the photochemically-generated species **11** and **12** also undergo hydrolysis by S_N2-type reactions, but at pH values near to neutrality.

These results from the irradiation of α-D-cytidine-2′-phosphate **6** represent a significant improvement relative to those previously reported for the free nucleoside **2**.[2] In the case of the irradiation of **2**, only one β-ribonucleoside was detected in 4% yield, and the β:α ratio was ~0.07. In the irradiation of **6**, two β-ribonucleotides are produced in 36% combined yield, and the β:α ratio is 1.2. This approximately tenfold increase in yield, and >15-fold increase in stereoisomer ratio is still not enough to constitute a plausible prebiotic synthesis of the β-ribonucleotides for RNA, however, and two major problems remain: the issue of the (selective) 2′-phosphorylation of **2**, and the source of purine β-D-ribonucleotides. The phosphorylation of **2** using cyclic trimetaphosphate and sodium hydroxide has been described, and gives **6** and the corresponding 3′-phosphate in high yield,[13] but the alkaline conditions required raise doubts about the prebiotic plausibility of this reaction. We are currently investigating the phosphorylation of **2** under more credible prebiotic conditions.[14] The source of the purine β-D-ribonucleotides remains uncertain, but it might be possible to adapt the reaction manifold of **6** that results in hydrolytic pyrimidine nucleobase loss (Scheme 3) to introduce purine nucleobases.[15] This possibility is also being actively investigated.



Scheme 3. Putative iminium ion intermediates **11** and **12** in the photo-anomerization/hydrolysis of **6**, and a related iminium ion **13** formed in the strong acid-catalyzed hydrolysis of β-D-uridine.

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Keywords: nucleotides · photoanomerization · photohydrolysis · prebiotic · RNA

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[15] Thus, if purine nucleobases (or their precursors) were to outcompete water in the nucleophilic attack of the iminium ions **11** and **12**, purine ribonucleotides would result. For steric reasons it can be anticipated that reaction of the α-anomers of **11** and **12** will be faster than reaction of the β-anomers, and, as a consequence of the S_N2 nature of the displacement reaction, purine β-D-ribonucleotides should predominate.

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