VIP Nucleotide Stereochemistry

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# Phosphate-Mediated Interconversion of *Ribo*- and *Arabino*-Configured Prebiotic Nucleotide Intermediates**

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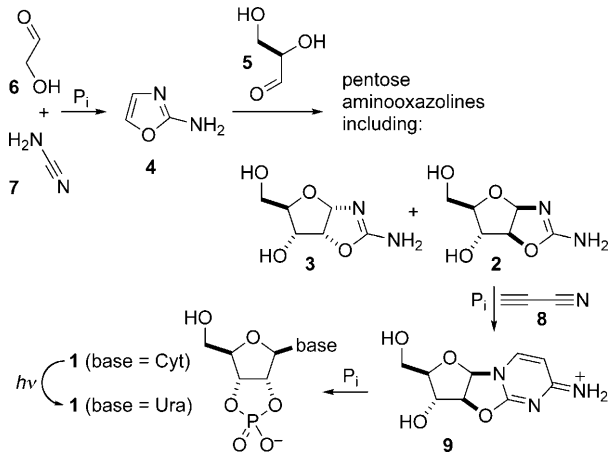
We recently described a prebiotically plausible synthesis of pyrimidine ribonucleoside-2′,3′-cyclic phosphates **1** that proceeds through the intermediacy of arabinose aminooxazoline **2** and involves inorganic phosphate in several of its steps (Scheme 1).[1]



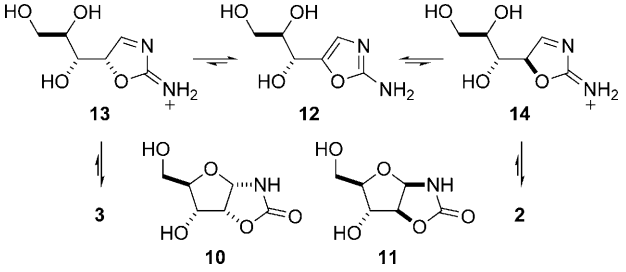

***Scheme 1.*** Potentially prebiotic synthesis of activated pyrimidine nucleotides **1**. $P_i$ = inorganic phosphate.

Compound **2** is produced alongside ribose aminooxazoline **3** and its *lyxo* and *xylo* diastereoisomers when 2-aminooxazole **4** adds to glyceraldehyde **5**. The synthesis of **4** from glycolaldehyde **6** and cyanamide **7** only takes place efficiently at neutral pH when phosphate was included as a general acid–base catalyst. Furthermore, the subsequent reaction of **2** with cyanoacetylene **8**, giving the anhydronucleoside **9**, is also controlled by phosphate at pH 6.5, where it acts as a pH buffer, thereby preventing hydrolysis of **9** to arabinocytidine. Heating **9** with phosphate in formamide or in the dry state then results in the formation of **1** (base = Cyt). In a final step, **1** (base = Cyt) is partly converted into **1** (base = Ura) by UV irradiation in aqueous solution.

Enantiomerically pure monomers are considered essential for the abiogenesis of RNA (racemic or scalemic monomers potentially giving rise to enormously complex diastereomeric mixtures of polymers), but as it stands, the synthesis does not yield enantiomerically pure ribonucleotides **1** unless the starting glyceraldehyde **5** is enantiomerically pure. Whilst enantioselectivity in the formation of glyceraldehyde **5** by aldolization of glycolaldehyde **6** and formaldehyde is easy to imagine if a suitable chiral catalyst could be found, formation of enantiopure **5** seems unlikely. Fractional crystallization of the ribonucleotides **1** or chiral intermediates in the synthesis offers a potential means of further enantioenrichment. The aminooxazolines **2** and **3** are less polar than **1** or the anydronucleosides such as **9**, and so offer the best hope of such a crystallization. However, the *arabino*-configured **2** needed for ribonucleotide synthesis is more water-soluble than the *ribo*-configured **3** along with which it is produced. Indeed, upon cooling or concentration of a solution of all four aminooxazolines, **3** selectively crystallizes.[2] Furthermore, scalemic **3** is enantioenriched by crystallization such that enantiopure **3** can be obtained from **5** with an enantiomeric excess (*ee*) of 60%.[3] We have therefore sought a means whereby **3**, purified and enriched to enantiomeric purity in such a way, might subsequently be partly[4] converted into **2**. Such a conversion would maintain the enantiopurity established by crystallization but transfer it from the *ribo* series to the *arabino* series and from there onwards to the *ribo* nucleotides **1**.

Phosphate has proved to be a key player in the systems chemistry aspects of the synthesis, so we took the requirement for phosphate to be present in the subsequent conversion of **2** into **9** as a clue that phosphate might also catalyze the conversion of **3** into **2**. Furthermore, we envisaged a potential mechanism for the interconversion of **2** and **3** that appeared to require general acid–base catalysis (Scheme 2), and we had previously found phosphate to be an ideal mediator of such catalysis.[1]

When exploring the effects of pH buffering by phosphate on the reactions of aminooxazolines **2** and **3** with cyanoacetylene **8**, we had not seen any interconversion of **2** and **3**.




***Scheme 2.*** Proposed mechanism for the interconversion of **2** and **3**.

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These experiments involved limited exposure of the aminooxazolines to phosphate before addition of **8** and subsequent rapid cyanovinylation chemistry. It therefore struck us that if phosphate-catalyzed interconversion of **2** and **3** is possible, it must be slow, and we planned our experimental approach accordingly. In a preliminary experiment, **3** was dissolved in deuterated 1M phosphate buffer at pD = 7, and this solution was then incubated for three weeks at 60°C with periodic examination by $^1$H NMR spectroscopy. Over time, a singlet signal appeared at $\delta = 6.54$ ppm, the signal for H1′ of **3** collapsed from a doublet to a singlet, and three major new singlet signals appeared in the chemical shift region for H1′ of aminooxazolines and related species between $\delta = 5.50$ and 6.00 ppm. When this experiment was repeated in $H_2O$ with samples being periodically removed and lyophilized prior to dissolution in $D_2O$ for $^1$H NMR analysis,[5] the signal at $\delta =$ 6.54 ppm was still a singlet, but the other four major downfield signals were doublets. By sample spiking with authentic standards, it was shown that the doublet signals were due to **2** and **3**,[6] and the oxazolidinones **10** and **11**[7] (Scheme 2). The singlet signal at $\delta = 6.54$ ppm was subsequently shown to be due to the pentose aminooxazole **12**, a presumed intermediate in the interconversion of **2** and **3**.

The potential interconversion mechanism (Scheme 2) involves furanose ring-opening of **3** to give the iminium species **13** which can undergo phosphate-mediated deprotonation of C2′ to give **12**. Phosphate-mediated reprotonation of C2′ of **12** can then either regenerate **13**, or give **14** from which **2** can be produced by ring-closure. It is thought that the oxazolidinones **10** and **11** are either products of the direct hydrolysis of **3** and **2**, or the indirect hydrolysis via **13** and **14**. Supporting its validity, the interconversion mechanism accounts for the behavior first observed in $D_2O$ solution, as deuteronation of **12** would give **3** and **2** (and thence the oxazolidinones **10** and **11**) deuterated at C2′ and thus having singlet signals for H1′ in $^1$H NMR spectra.

To confirm the presence of **12** in reaction mixtures, and to provide support for its presumed intermediacy in the interconversion of **3** and **2**, we synthesized **12** using conventional chemistry, and then subjected it to the conditions of the interconversion. The synthesis began with 4,6-*O*-benzylidene-D-glucose **15**, which was elaborated to the vinyl alcohol **16** by diol cleavage with periodate[8] followed by addition of vinyl magnesium bromide (Scheme 3).

Ozonolysis of **16** followed by reductive work-up and condensation with cyanamide **7** then gave **17**, the 3′,5′-*O*-benzylidene derivative of **12**—3′,5′-*O*-benzylidene aminooxazoline derivatives not being formed because of strain in their fused tricyclic framework. As the conversion of **10** into **2** and **3** was believed to require general acid catalysis, we thought that it should be possible to deprotect **17** by specific acid catalysis without the **12** so-produced equilibrating with **2** and **3**. Accordingly, we treated **17** with mineral acid at room temperature and, gratifyingly, it was converted to **12** in quantitative yield.

With an authentic sample of **12** in hand, we were able to compare $^1$H NMR spectral signals and confirm that **12** was indeed present in the mixture of compounds formed by heating **3** in phosphate buffer. We then subjected a synthetic sample of **12** to reaction in 1M phosphate buffer at 40°C and pH 7.0 to confirm the intermediacy of **12** in the equilibration of **3** and **2**. Samples were periodically withdrawn and lyophilized, and the residues redissolved in $D_2O$ for $^1$H NMR analysis. This procedure revealed that **12** was partially converted into **3** and **2**, and, more slowly, the hydrolysis products **10** and **11** (Figure 1).

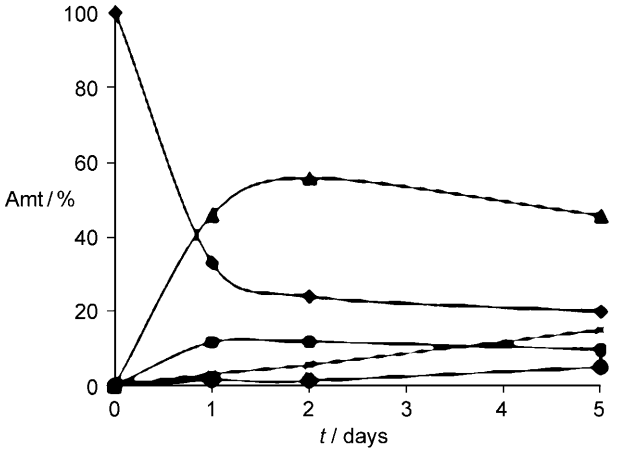
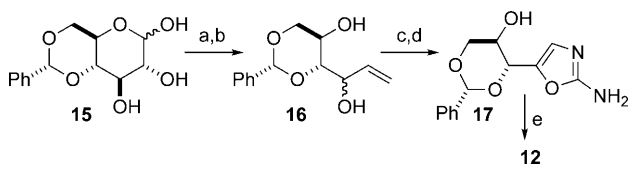



***Figure 1.*** Time course of the reaction of **12** in phosphate buffer. Amt = amount of each species as determined by $^1$H NMR integration. ◆ **12**, ▲ **3**, ■ **2**, × **10**, ● **11**.

With the major products of this reaction now identified,[9] and the intermediacy of **12** confirmed, we next sought to assess the effect of temperature and pH on the product distribution (Table 1).

The reaction of **3** is slow at room temperature, and needs several days at higher temperatures for equilibration with **2** and **12** to occur. Hydrolysis to **10** and **11** always accompanies this equilibration. At pH 6 and above, the apparent thermo-




***Scheme 3.*** a) $NaIO_4$, $CH_2Cl_2/H_2O$, RT; b) $CH_2$=CHMgBr, THF, 0°C, then $NH_4Cl$, $H_2O$, 59% from **15**; c) $O_3$, MeOH, −78°C, then $Me_2S$, MeOH, −78°C→RT; d) $H_2NCN$, *N*,*N*-dimethylacetamide, 60°C, 75% from **16**; e) HCl, dioxane/$H_2O$, RT, quantatitive.

***Table 1:*** Products of incubation of **3** in 1M phosphate buffer.[a]

| pH | *T* [°C] | Product distribution ratio[b] | | | | |
|---|---|---|---|---|---|---|
| | | **12** | **2** | **3** | **11** | **10** |
| 5.0 | 60 | 0.1 | 0.5 | 1.0 | 1.4 | 4.1 |
| 7.0 | 60 | 0.5 | 0.4 | 1.0 | 3.0 | 9.1 |
| 8.0 | 60 | 0.4 | 0.3 | 1.0 | 1.0 | 2.9 |
| 6.0 | 40 | 0.3 | 0.3 | 1.0 | 0.2 | 0.5 |
| 7.0 | 40 | 0.3 | 0.3 | 1.0 | 0.2 | 0.5 |
| 7.0 | 20 | 0 | 0 | 1.0 | 0 | 0 |

[a] After 6 days incubation. [b] Normalized relative to **3**.

dynamic stabilities decrease in the order **3** > **12** ≈ **2**, whereas at pH 5, **12** is disfavored relative to both **3** and **2**. Furthermore, at this lower pH, the **2**:**3** ratio is at its highest.

As regards the overall synthesis of **9**, the formation of oxazolidinones **10** and **11** during the equilibration reaction is not critical, as they do not react in the subsequent cyanovinylation reaction. More important is the **2**:**3** ratio, as **3**, like **2**, reacts with cyanoacetylene to give an anhydronucleoside intermediate.[1] The indication is that the highest yield of **9** relative to its *ribo*-configured counterpart (but see [4]) will be obtained if **3** is first allowed to equilibrate in phosphate buffer at a pH between 5 and 6, and the resultant mixture then allowed to react with cyanoacetylene **8** at pH 6.5. Whether or not enantiomerically pure ribonucleotide monomers **1** can be produced under prebiotically realistic conditions now depends on a synthesis of enantioenriched glyceraldehyde **5** under similar conditions. If the synthesis of **5** with an *ee* of more than 60 % can be demonstrated, the prebiotic formation of enantiomerically pure pyrimidine ribonucleotides **1** (base = Pyr) must then be considered plausible.

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[4] A partial conversion of **3** to **2** was sought because it is possible that activated purine ribonucleotides **1** (base = Ade, Gua) might result from nucleophilic displacement at C1′ of cyanovinylated and phosphorylated derivatives of **3**.
[5] Full experimental details are given in the Supporting Information.
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[9] We also identified low levels of diastereoisomeric hydrates of **13** and **14** (**18** and **19**, respectively; see Supporting Information) formed by the reversible addition of water to C1′.

  