A new \( \alpha \)-cytidine derivative was synthesised from the prebiotic reaction of ribose aminooxazoline and dicyanoacetylene. The tetra-cyclic structure of the product was confirmed by X-ray diffraction and then an alternative 6-step synthetic pathway to the product was found which was suitable for large-scale synthesis.

In seminal prebiotic chemistry studies which prompted our follow-on work, Sanchez and Orgel reported that reaction of ribose aminooxazoline 1 with cyanoacetylene 2 gives a \( \text{ribo-configured anhydronucleoside} \) 3 which subsequently hydrolyses to give \( \alpha \)-cytidine 4 (Scheme 1).\(^1\) Their efforts to extend this sequence of reactions into a plausible synthesis of the canonical pyrimidine nucleotides were thwarted, however, by their inability to photo-anomerize \( \alpha \)-cytidine 4 in anything more than 4% yield, and by difficulties in synthesizing ribose from which 1 was generated by condensation with cyanamide. We subsequently uncovered two prebiotically plausible syntheses of the canonical pyrimidine nucleotides involving pentose aminooxazolines intermediates made by the addition of 2-aminoxazole to glyceraldehyde.\(^2,3\)

Our first synthesis proceeded via arabinose aminooxazoline and involved stereoinversion of C-2,\(^2\) the second proceeded via ribose aminooxazoline 1 and involved high-yielding photo-anomerisation of \( \alpha \)-2-thiocytidine.\(^4\)

Although both ribose and arabinose aminooxazolines are formed in comparable yields in our synthetic routes, the \( \text{ribo-configured material} \) 1 has the advantage over the *arabino*-isomer in that it crystallises out of the mixture of reaction products and residual starting materials. Not only does this crystallisation allow for a spontaneous chemical purification of ribose aminooxazoline 1, it also amplifies any enantionic excess initially present in solution because this highly crystalline compound is a conglomerate.\(^4\)

This behaviour could have contributed to the formation of enantiopure RNA at the origin of life. Given the foregoing and the need for a prebiotically plausible synthesis of purine nucleotides – previous efforts being low yielding or of questionable prebiotic plausibility – we wondered if these latter canonical nucleoside derivatives might also derive from ribose aminooxazoline 1. Since ribose aminooxazoline 1 functions as a nucleophile, we began to consider other electrophiles with which it might react to give adenosine and guanosine precursors. As it happens, this line of thinking has not (yet) resulted in a synthesis of the purine nucleotides, but it led serendipitously to the results described herein and so we relate it here. In particular, we were drawn to electrophiles which were synthetically related to cyanoacetylene 2 which we showed could be produced as its copper(II) complex, CuC\(\text{CN} \), by copper(ii) oxidative coupling of hydrogen cyanide and acetylene.\(^6\) Therefore, it did not seem unreasonable to consider the potential product of a further copper(ii) promoted oxidative coupling between CuC\(\text{CN} \) and hydrogen cyanide, which would be dicyanoacetylene 5. Although this latter compound could not form a stable \( \sigma \)-complex with Cu(c), it could potentially form a \( \pi \)-complex. In addition, 5 has been detected by IR spectroscopy in Titan’s atmosphere.\(^7\) Although we have not demonstrated a synthesis of dicyanoacetylene 5 by oxidative coupling, we now report that the reaction of ribose aminooxazoline 1 with 5 furnishes a new \( \alpha \)-cytidine derivative, which was analysed by NMR spectroscopy and X-ray diffraction. This compound was then made by another, more conventional synthetic route in 6 steps starting from \( \alpha \)-cytidine 4.

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\( ^{†} \) Electronic supplementary information (ESI) available. CCDC 1528414. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c7cc00693d
The potentially prebiotic reaction of ribose aminooxazoline 1 with dicyanoacetylene 5 was performed at room temperature in aqueous solutions at pH = 6.5–7.0 and was monitored by 1H NMR spectroscopy. The only product we could isolate from the reaction mixture was a white crystalline compound, to which we assigned the structure of the amide acetal 11 on the basis of NMR data. The new structure was then verified by X-ray crystallography (Fig. 1). At first glance, the structure of 11 may appear surprising, but by drawing an analogy to the reaction of ribose aminooxazoline 1 and cyanoacetylene 2 (Scheme 1), a cascade of reactions leading to 11 from 1 and dicyanoacetylene 5 can easily be envisaged (Scheme 2). Thus, we propose that conjugate addition of 1 to 5 gives an intermediate 6, which undergoes spontaneous 6-exo-dig addition to form the anhydronucleoside 7 – the 6-cyano-analogue of anhydro-β-cytidine 3. Again by comparison to the reaction of ribose aminooxazoline 1 and cyanoacetylene 2, the next step in the cascade leading from 1 and dicyanoacetylene 5 is expected to be the hydrolysis of the anhydronucleoside 7 induced by an increase in the pH of the system due to protonation of the free base form of the anhydronucleoside. Indeed, the pH of the reaction mixture after the addition of dicyanoacetylene 5 was monitored by 1H NMR spectroscopy. The only product we could isolate from the reaction mixture was a white crystalline compound, to which we assigned the structure of the amide acetal 11 on the basis of NMR data. The new structure was then verified by X-ray crystallography (Fig. 1).

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found to afford the amide acetal 11, which was also synthesised by more conventional means. Coincidentally, both the procedures had the same total yield (32%), but the second one can be used more efficiently and safely at larger scales. Even though nucleosides having the α-anomeric configuration are not found in natural nucleic acids, they constitute important intermediates in the prebiotic synthesis of the nucleotides and also some of them have been found to exhibit pronounced antimetabolic activities – whether this is true of 11 has not been established.

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Notes and references

‡ Crystal data. C_{10}H_{12}N_{4}O_{5}/C_{1}H_{2}O, M = 286.25, orthorhombic, a = 7.4674(2), b = 10.9703(3), c = 13.9309(4) Å, U = 1141.21(5) Å³, T = 180 K, space group P2₁2₁2₁ (no. 19), Z = 4, 13 491 reflections measured, 1996 unique (R(int) = 0.030), which were used in all calculations. The final wR(F²) was 0.069 (all data).

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