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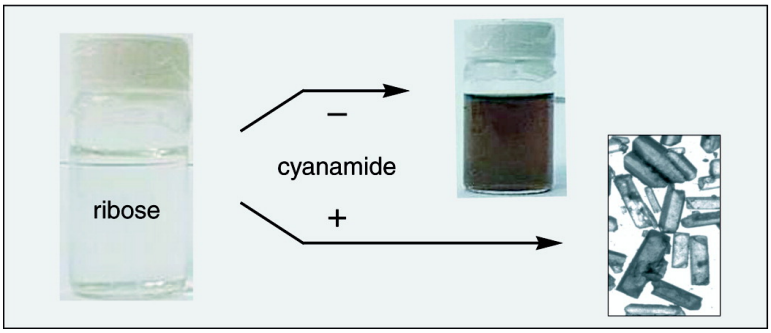

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Selective Derivatization and Sequestration of Ribose from a Prebiotic Mix

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Abstract: Observations regarding the catalytic potential of RNA and the role of RNA in biology have formed the basis for the "RNA world" hypothesis, which suggests that a genetic system based on self-replicating polyribonucleotides preceded modern biology. However, attempts to devise a realistic prebiotic synthesis of nucleic acids from simple starting materials have been plagued by problems of poor chemical selectivity, lack of stereo- and regiospecificity, and similar rates of formation and degradation of some of the key intermediates. For example, ribose would have been only a small component of a highly complex mix of sugars resulting from the condensation of formaldehyde in a prebiotic world. In addition, ribose is more reactive and degrades more rapidly compared with most other monosaccharides. This study demonstrates an approach for the preferential sequestration of ribose relative to other sugars that takes advantage of its greater reactivity. Cyanamide reacts especially rapidly with ribose to form a stable bicyclic adduct. This product crystallizes spontaneously in aqueous solution, whereas the corresponding products derived from threose, galactose, glucose, mannose, and each of the other pentoses do not. Furthermore, when employing a racemic mixture of D- and L-ribose, enantiomerically twinned crystals are formed that contain discrete homochiral domains.

Introduction

Ribose, the sugar component of nucleic acids, is one of many products that result from the condensation of formaldehyde (the formose reaction).[1,2] Under basic conditions, formaldehyde is efficiently converted to a library of higher-carbon compounds, including 4-, 5-, and 6-carbon sugars of all stereochemistries.[3] The proportion of ribose among these products is estimated to be less than 1%.[4] There are, however, methods for increasing the relative yield of ribose. For example, by starting from the sugar precursor glycoaldehyde phosphate in the presence of formaldehyde, ribose-2,4-diphosphate is obtained as the predominant reaction product.[5] Ribose-2,4-diphosphate can be obtained in good yield from dilute solutions of glycolaldehyde phosphate and glyceraldehyde-2-phosphate at near-neutral pH if the reaction is carried out in the presence of layered hydroxide minerals such as hydrotalcite.[6] Alternatively, by adding either lead hydroxide or calcium borate, both of which preferentially complex with furanose *cis*-diols, the yield and stability of the aldopentoses, including ribose, is significantly enhanced.[7,8]

Sugars, and especially ribose, are unstable under the conditions that are required to create them, undergoing facile reactions through enediol intermediates to form various fragmentation and dehydration products. Under alkaline conditions, sugars decompose quickly to form a tarlike mixture of dozens of compounds.[9] The rate of enediol formation from a sugar, measured by tritium uptake from ^3H_2O, demonstrates the especially high lability of ribose,[9] which reacts 4-fold faster than the average of the other pentoses and 16-fold faster than the average of the hexoses. The relatively rapid degradation of ribose is consistent with its higher reactivity in general. For example, ribose reacts with urazole 3.5-fold faster than the average of the other pentoses.[10] This heightened reactivity can be explained in part by the anomeric composition of ribose, which is relatively biased toward the electrophilic open-chain aldehyde.[10] In addition to undergoing rapid decomposition, ribose readily isomerizes under mild conditions, generating the other aldo- and ketopentoses.[11] Therefore, even if a supply of pure ribose were available, it likely would degrade to a complex formose-like distribution of products long before substantial reaction with a nucleobase could occur.[12] Furthermore, nucleosides contain only β-D-ribofuranose, while ribose in a prebiotic mix would exist as the open-chain

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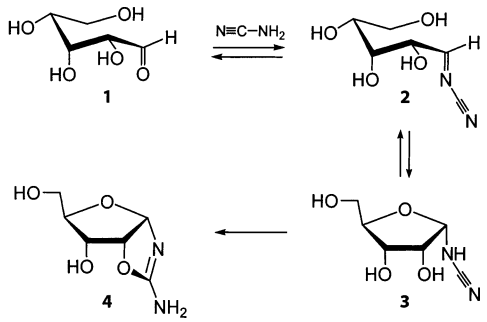
Scheme 1. Reaction of Ribose (**1**) with Cyanamide to Give Intermediates **2** and **3** (Together with the β-Pyranose and β-Furanose Forms, Not Shown), Then Ring Closure of the α-Furanose (**3**) to Generate **4**



aldehyde, the α- and β-pyranoses and furanoses, and the D- and L-stereoisomers.

It is conceivable that the status of ribose as the only sugar within nucleic acids is a reflection of some exceptional aspect of its chemistry compared with other monosaccharides. For example, the greater reactivity of ribose might have a favorable effect on its long-term availability if it resulted in the preferential sequestration of ribose as part of a stable complex under conditions where other sugars are degraded. Pursuing this hypothesis, a series of simple ribose derivatives, including ribosylamines and rearrangement products (e.g., Amadori compounds) were investigated. In basic aqueous solution a variety of ribose products were generated, but they too suffered from poor stability and contained ribose in the pyranosyl rather than the furanosyl form. Ribose has been shown to undergo an efficient reaction with cyanamide (NH_2CN) to form a bicyclic product,[13] with cyanamide joined at both the 2-hydroxyl and the anomeric carbon (Scheme 1). This product, ribose-cyanamide (**4**), was found to be highly stable and contained ribose in the furanosyl form. Furthermore, upon reaction of D,L-ribose with cyanamide, the ribose-cyanamide product crystallized spontaneously out of aqueous solution, forming large, enantiomorphously twinned crystals with discrete homochiral domains. Ribose-cyanamide (as well as arabinose-cyanamide) has been studied previously in the context of the prebiotic chemistry of nucleic acids because it reacts with cyanoacetylene to form pyrimidine nucleosides.[13−15]

Ribose-cyanamide can be regarded as a "depot" form of ribose that may have facilitated its eventual inclusion within nucleic acids. As will be described here, the stability of ribose-cyanamide is much greater than that of free ribose. In addition, ribose reacts preferentially with cyanamide as compared to other sugars, and the ribose-cyanamide product crystallizes readily in aqueous solution while the cyanamide products of other sugars do not. Ribose-cyanamide crystals form just as readily from a racemic mixture of D,L-ribose as from either the D- or L-enantiomer alone. The crystals that are formed from racemic ribose-cyanamide contain a mosaic of pure D and pure L domains, producing an X-ray diffraction pattern identical to that obtained from crystals derived from either pure D- or pure L-ribose-cyanamide.

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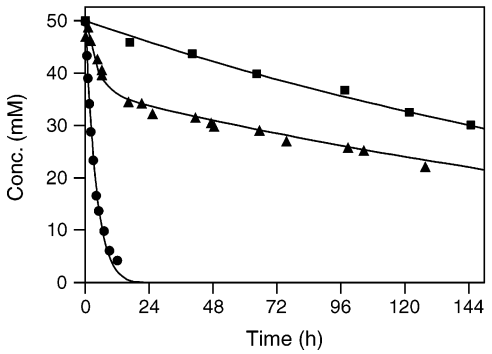

Figure 1. Degradation of ribose and ribose-cyanamide in aqueous solution. (●) Remaining concentration of ribose starting from 0.05 M ribose alone. (▲) Remaining concentration of ribose (both free and as ribose-cyanamide) starting from 0.05 M ribose and 0.1 M cyanamide. (■) Remaining concentration of ribose-cyanamide starting from 0.05 M ribose-cyanamide. All reactions were carried out in 0.2 M $NaHCO_3/Na_2CO_3$ in D_2O at pD 10.2 and 55 °C. DCl or NaOD was added as necessary to maintain the pD.

Results

The degradation of sugars under basic conditions can be monitored by ^{1}H NMR using D_2O as the medium. Although complicated by the formation of multiple anomers, signals from the C1-hydrogen (4.9 ppm) and the C2-hydrogen (3.5 ppm) of the β-pyranose form of ribose occur at chemical shifts that are distinct from those of the ribose degradation products. Accurate quantification was achieved by integrating these signals and comparing them to signals resulting from 2-methylpropanol, which was used as an internal standard. The proportion of β-pyranose relative to total ribose was determined at the start of the reaction, and the pD was kept constant so that this ratio would not change. The half-life of ribose at pD 10.2 and 55 °C was found to be less than 3 h (Figure 1), which is more than 6-fold faster than that of arabinose (7.0×10^{-5} and 1.1×10^{-5} s^{-1}, respectively).

Aliphatic amines react with the C1-carbon of ribose to form glycosylamines. The course of this reaction can be monitored by the pH depression resulting from the increased acidity of the glycosylamine relative to the free amine. The increase in acidity is likely a result of the electron-deficient anomeric carbon destabilizing the protonated form of the amine. In a mixture containing 0.75 M piperidine and 2.5 M ribose, the pH decreased from 11.21 to its equilibrium value of 10.75 within 15 min. Similarly, the approach to equilibrium starting from ribosylcyclohexylamine (which had been purified by crystallization) was complete within 15 min, with the pH rising from 10.42 to 10.93.

The products of ribose and various amines were detected on the basis of their characteristic ^{1}H NMR signals. The ribosylamine generated from methylamine gives two methyl signals, from both its α- and β-pyranose forms, at 2.4−2.5 ppm (pH dependent). No furanose form was detected by NMR. The complex of ribose and methylamine has an apparent association constant of 22 M^{-1} at pD 10.3, which was the highest of all the ribosylamines tested, including those of glycine, cyclohexylamine, piperidine, morpholine, and aminopyrimidine. Although ribosylamine complexes formed readily, even the most stable of these did not protect ribose from undergoing rapid degradation. The addition of 2.5 equiv of methylamine to ribose resulted in a ribose degradation profile that was nearly indistinguishable from that of ribose alone.

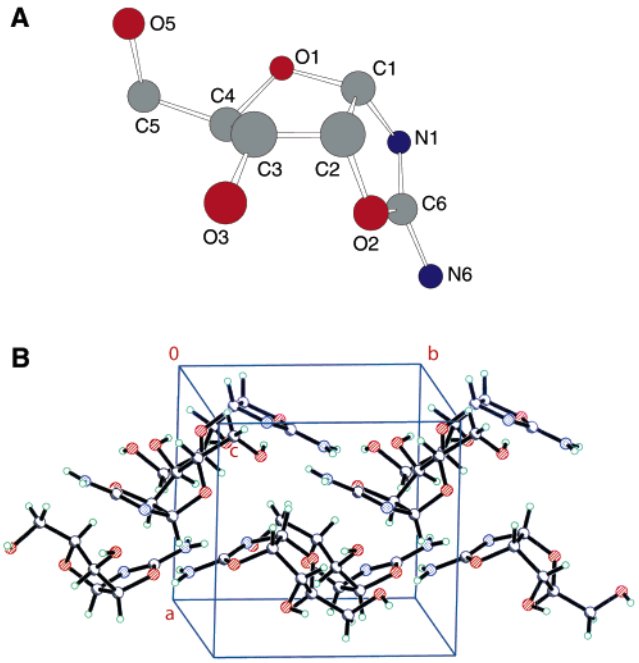

Figure 2. X-ray diffraction structure obtained from crystals of D,L-ribose-cyanamide. (A) Structure of the molecule. (B) Arrangement of molecules within the unit cell.

The addition of cyanamide to ribose gave a dramatically different result, with substantial protection of ribose against degradation (Figure 1). The reaction of ribose and cyanamide could easily be followed by monitoring the ^{1}H NMR signal at a shift of 5.7 ppm, attributed to the C1-hydrogen of the product. This is almost a full ppm downfield from the nearest ribose signal. The spectrum is considerably simplified for ribose-cyanamide compared with ribose because the former exists as a single anomer (**4**, Scheme 1). None of the pyranose or β-furanose forms of ribose-cyanamide were detected by NMR.

Reaction mixtures containing ribose and cyanamide formed millimeter-sized rod-shaped crystals (see Supporting Information). Crystals formed from mixtures of ribose and cyanamide ranging in concentration from 0.065 to 2 M each. At the lowest concentration the crystals appeared after several days, while at the highest concentration they appeared after only a few hours. Crystals were obtained over a pH range of 6.7−12.9, but formed more rapidly at higher pH presumably because of the increased rate of reaction of ribose and cyanamide under these conditions. The crystals typically had masses of 0.1−0.4 mg, measured by dissolving a crystal in 0.3 mL of 0.01 M phosphoric acid (pH 2.8) and comparing its absorption at 197 nm to a standard curve. Employing a mixture of 0.3 M ribose and 0.3 M cyanamide, which was incubated at pH 9.4 and 23 °C, approximately 50% of the total ribose was recovered as ribose-cyanamide crystals after 72 h. Crystals of ribose-cyanamide could be regenerated by first dissolving the crystals in water by sonication and heating (60 mg in 6 mL of H_2O at 45 °C), then allowing slow evaporation of the solution at 34 °C. This demonstrates that crystal formation is not dependent on the reaction conditions that were used to generate ribose-cyanamide.

The X-ray crystal structure confirmed that ribose-cyanamide is in the α-furanose form (Figure 2). The bond lengths within the amino-oxazoline portion of the molecule are 1.45, 1.28, 1.33, and 1.35 Å for the C1−N1, C6−N1, C6−N6, and C6−O2 bonds, respectively. This is in close agreement with the corresponding bond lengths in the reported crystal structure of 2-amino-1,3-oxazole, which are 1.41, 1.29. 1.34, and 1.36 Å, respectively.[16] The C6−N1 and C6−N6 bonds of ribose-cyanamide both have lengths that are consistent with partial double-bond character.

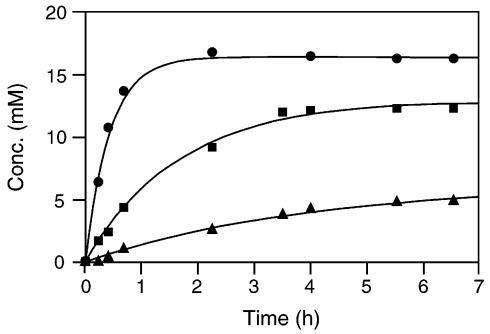

Figure 3. Formation of sugar-cyanamide from ribose (●), arabinose (■), or glucose (▲). Reaction conditions: 0.02 M sugar, 0.04 M cyanamide, and 0.2 M $NaHCO_3/Na_2CO_3$ in D_2O at pD 10.3 and 55 °C.

The rate of reaction of ribose with cyanamide is more than 200-fold faster than the rate of degradation of ribose under the same conditions (1500×10^{-5} and 7.0×10^{-5} s^{-1}, respectively). Thus, the addition of cyanamide quickly stems ribose decomposition (Figure 1). The rate of decomposition of ribose-cyanamide is 70-fold slower than that of free ribose (1×10^{-6} and 70×10^{-6} s^{-1}, respectively, Figure 1), and once ribose-cyanamide enters the crystalline state, it becomes nearly inert. A collection of crystals totaling 184 mg was heated in 5 mL of D_2O at pD 9.6 and 55 °C for 100 h. During this incubation only 9% of the crystals dissolved. The remaining crystalline material exhibited no detectable degradation, as determined by ^{1}H NMR. Ribose-cyanamide is stable in both basic and acidic environments, and its persistence relative to ribose is readily apparent by visual inspection. Solutions containing ribose-cyanamide remain clear under conditions that dehydrate sugars into black chromophoric tars.

Cyanamide reacts more readily with ribose compared with other sugars. The rate of sugar-cyanamide formation was determined for arabinose, a pentose that is structurally similar to ribose, and for glucose, a thermodynamically stable hexose that is a major product of the formose reaction (Figure 3). Under identical conditions at pD 10.3 and 55 °C, ribose reacted about 7-fold faster than arabinose and 30-fold faster than glucose, with rate constants of 150×10^{-4}, 22×10^{-4}, and 4.5×10^{-4} s^{-1} for ribose, arabinose, and glucose, respectively.

Crystallization of the sugar-cyanamide is highly selective for ribose. In solutions containing an equimolar mixture of the four pentoses (ribose, arabinose, lyxose, and xylose), only ribose-cyanamide was detected among the crystals that formed. The same result was obtained with solutions containing the four pentoses as well as galactose, glucose, and mannose (Figure 4). To determine whether this behavior simply is a reflection of ribose's enhanced reactivity with cyanamide, crystallization experiments were conducted with cyanamide and various sugars tested individually. D-Ribose, L-ribose, D-arabinose, D-lyxose, D-xylose, D-threose, D-galactose, D-glucose, and D-mannose all were examined under the same reaction conditions, employing

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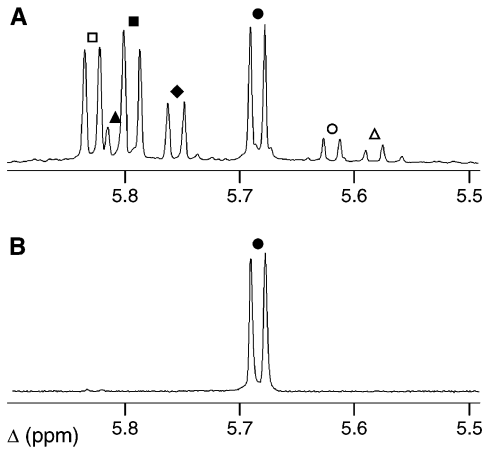

Figure 4. ^{1}H NMR spectra of sugar-cyanamides displaying signals from the C1 proton. (A) Reaction mixture containing 0.7 M cyanamide and 0.1 M each of ribose (●), arabinose (■), lyxose (○), and xylose (□), galactose (◆), glucose (▲), and mannose (Δ). Reaction conditions: 0.2 M $NaHCO_3$/Na_2CO_3 in D_2O at pD 9.1 and 23 °C for 5 d. (B) Solution obtained from crystals that were collected after 8 d and dissolved in D_2O at pD 9.1.

0.3 M sugar and 0.3 M cyanamide, but only solutions containing either D- or L-ribose gave rise to crystals, even after several months. Attempts to obtain ribose-cyanamide starting from either formaldehyde or a mixture of glycoaldehyde and glyceraldehyde were thwarted by the very rapid reaction of cyanamide with these simple aldehydes.

Crystals obtained from a racemic mixture of D,L-ribose had the same general morphology as those obtained from either D- or L-ribose alone. Crystals from all three sources gave identical X-ray diffraction patterns, with the same homochiral $P2_12_12_1$ space group. However, crystals formed from D,L-ribose had different chiroptical properties compared with homochiral crystals (Figure 5). Crystals formed from either D- or L-ribose exhibited chirally specific signals of the appropriate sign in both circular dichroism and polarimetry studies, whereas crystals formed from *rac*-ribose showed no ellipticity or specific rotation. Twenty-seven crystals of various sizes from five different crystallization experiments employing *rac*-ribose all displayed no ellipticity when analyzed by CD spectroscopy.

In an otherwise achiral environment (and in the absence of anomalous X-ray dispersion), crystals of D- and L-ribose-cyanamide necessarily give the same diffraction pattern.[17] It may at first seem surprising that crystals of D,L-ribose-cyanamide give this pattern as well. This can be understood by recognizing that, in this case, the unit cell contains molecules all of the same handedness (Figure 2), and that a very large number of unit cells exist within each crystal domain. The crystal as a whole contains many such domains of either pure D or pure L stereochemistry, occurring in roughly equal proportion, which causes the dissolved crystal to be optically inactive. Such crystals are said to be enantiomorphously twinned, a phenomenon that is unusual, but well recognized in chemical crystallography.[18,19]

Discussion

The reaction of ribose and cyanamide likely proceeds through the open-chain aldehyde form of ribose to give the glycosylamine. Ring closure and a shift to an internal C−N double bond forms the amino-oxazoline ring of **4**. Although the pyranose form predominates for both free ribose and ribosylamines,[20] the α-furanose-cyanamide intermediate adopts a conformation with nearly eclipsed C1-amine and C2-hydroxyl bonds, which likely gives the best orientation for hydroxyl attack on the nitrile carbon (**3**, Scheme 1).

Chirality is an important feature of biopolymers, and the chirality of nucleosides derives from their ribose component. Crystals of ribose-cyanamide obtained from solutions of racemic ribose have the same morphology and space group as those obtained from either D- or L-ribose alone, but the racemate-derived crystals are enantiomorphously twinned, containing numerous domains of either pure D- or pure L-chirality. The most prevalent associations within the crystal lattice occur along the x $^1/_4$ and x $^3/_4$ axes of the unit cell (Figure 2B). The lack of association between the two planes likely creates an opportunity for the switch between enantiomeric domains. The presence of discrete homochiral domains within the crystals of ribose-cyanamide does not lead to symmetry breaking on the macroscopic scale. However, it may be possible to increase the extent of chiral differentiation within the crystals by employing processes such as repeated crystallization and dissolution or contact with chiral materials. Recent advances in microscopic circular dichroism imaging may be useful in developing a more complete understanding of the twinning phenomenon.[21]

There are many obstacles to the synthesis of RNA molecules under prebiotic conditions. Prominent among these are the selection of ribose from the mixture of products of the formose reaction, protection of ribose from degradation, and activation of ribose for nucleoside formation. Finding solutions to all of these problems has proven difficult. Alternatively, RNA may never have occurred prebiotically and instead may have been produced biosynthetically in the context of a genetic system based on a chemistry that excluded ribose.[22] The results presented here support the possibility that a substantial supply of ribose may have been available under prebiotic conditions, not as free ribose, but sequestered as ribose-cyanamide, which is 70-fold more stable than ribose in solution and essentially inert once it forms crystals. Ribose is selected from the other sugars both by its faster rate of reaction with cyanamide and by its unique propensity to form sugar-cyanamide crystals in aqueous solution. It should be noted, however, that cyanamide reacts directly with formaldehyde, glycoaldehyde, and glyceraldehyde to form compounds that do not participate in the formose reaction. Thus, exposure to cyanamide must take place after a mixture of sugars has been generated, but before those sugars have been degraded.

The nucleosidation of free ribose is extremely difficult because of the lack of nucleophilicity of nucleobases and the ease of hydrolysis of the activated ribose intermediates. Once ribose has been converted to ribose-cyanamide, it reacts readily with cyanoacetylene to form cytosine nucleosides in good yield.[13] However, these nucleosides contain the α- rather than β-furanose form of ribose, and the isomerization of α-cytidine to β-cytidine is an inefficient reaction. Alternatively, arabinose-3′-phosphate reacts with cyanamide to form an amino-oxazoline

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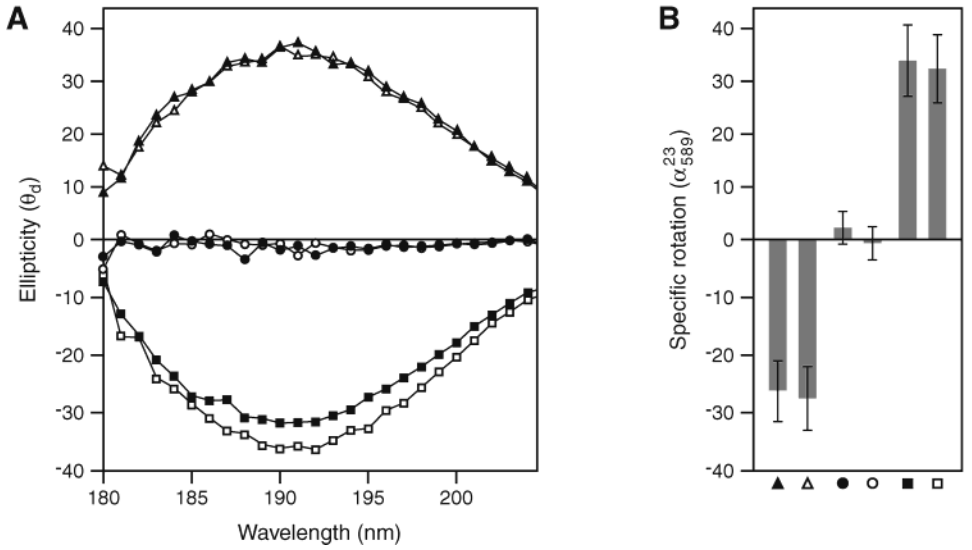

Figure 5. (A) Circular dichroism spectra of single crystals of ribose-cyanamide obtained from a solution of 0.33 M cyanamide and 0.33 M D-, L-, or D,L-ribose. (B) α_{589}^{23} values from polarimetry measurements of the same crystals. Masses of the D-ribose-cyanamide crystals were 0.19 and 0.17 mg (▲,Δ, respectively), the L-ribose-cyanamide crystals were 0.16 and 0.18 mg (■,□, respectively), and the D,L-ribose-cyanamide crystals were 0.19 and 0.18 mg (●,○, respectively).

derivative, which in turn reacts with cyanoacetylene to form a cyclonucleotide that hydrolyzes to give a mixture of β-cytidine-3′-phosphate and β-cytidine-2′,3′-cyclophosphate.[14,15] This sequence of reactions does lead to the β-furanose, but is less efficient than the reaction of ribose, cyanamide, and cyanoacetylene to give the α-furanose. Furthermore, the use of arabinose-3′-phosphate rather than ribose does not take advantage of the special propensity of ribose-cyanamide to form crystals in aqueous solution.

Experimental Section

General Methods. Samples for ^{1}H NMR were dissolved in D_2O, with 2-methyl-2-propanol employed as an internal standard. NMR spectra were recorded on a Bruker DRX 500 or 600 MHz spectrometer (16 scans, 5-s delay (d1)). UV−visible spectra were obtained using an HP 8452 spectrophotometer. Specific optical rotation was measured using an Autopol III polarimeter (10-cm path length, sodium lamp, 23 °C), and ellipticity was determined using an Aviv 202 circular dichrometer (0.4-mL solution, 0.1-cm path length, 1-nm bandwidth, 25 °C, two scans, 10-s acquisition time).

Materials. All reagents employed in this study were obtained from commercial sources and used without further purification. Cyanamide was purchased from Aldrich as a 50% (wt) solution in water. D_2O was obtained from Cambridge Isotope Laboratories. Solutions of D_2O were adjusted to the desired pD using NaOD (5 N in D_2O) or DCl (35% (wt) solution in D_2O).

Kinetic Analysis. The rate of degradation of various sugars and sugar-cyanamides was determined by fitting the experimental data to a single-exponential equation:

$$C_t = C_0[\exp(-kt)]$$

where C_t is the concentration at time t, C_0 is the initial concentration, and k is the rate of degradation.

In reaction mixtures containing both ribose and cyanamide, the total remaining concentration of ribose (both free and as ribose-cyanamide) was determined by fitting the experimental data to a double-exponential equation:

$$C_t = F_1[\exp(-k_r t)] + F_2[\exp(-k_c t)]$$

where C_t is the concentration at time t, F_1 and F_2 are the amplitudes of the two phases of the reaction, and k_r and k_c are the rates of degradation of ribose and ribose-cyanamide, respectively.

The rate of formation of various sugar-cyanamides was determined by fitting the experimental data, collected over the initial linear portion of the reaction, to a single-exponential equation:

$$C_t = C_{max}[1 - \exp(-kt)]$$

where C_t is the concentration at time t, C_{max} is the maximum extent determined at long reaction times, and k is the rate of formation.

Chiroptic Analysis. Crystals were obtained from a mixture of 0.3 M ribose and 0.3 M cyanamide at pH 9.4 that was allowed to stand at 23 °C for 1−3 d. The crystals were washed three times with deionized water, then dried under vacuum at 23 °C. Single crystals without obvious satellites were chosen under a microscope, transferred to a 0.5-mL microcentifuge tube, and dissolved in 0.3 mL of 0.01 M phosphoric acid (pH 2.8). After 2 h of sonication at 40 °C, the tubes were removed and checked for homogeneity of the solution. The concentration of ribose-cyanamide was determined by comparing the UV absorbance of the solution at 197 nm to that of a standard curve. An 0.1-mL volume of 0.01 M sodium phosphate (pH adjusted to 12.1 with 5 N NaOH) was added prior to polarimetry and circular dichroism studies.

Conclusions

Discoveries concerning the catalytic power of RNA and the role of RNA in biology have given substantial support to the theory of an earlier form of life based on RNA. This theory has fostered efforts to describe a possible abiotic origin of RNA through the synthesis of nucleosides from plausible prebiotic compounds. But the many difficulties posed by ribose, including its nonspecific synthesis, chemical instability, structural similarity to other sugars, and lack of reactivity with nucleobases have led many to abandon ribose in their pursuit of the origins of the first genetic molecule. Cyanamide conveys unique properties upon ribose compared with other sugars, both through the preferential formation of ribose-cyanamide and the stabilization of ribose as a cyanamide adduct. Furthermore, ribose-cyanamide has a special propensity to crystallize in aqueous solution,

forming chirally pure crystal domains even from racemic starting materials. Together, these properties may have allowed the establishment of a solid-phase reservoir of ribose-cyanamide under prebiotic conditions. From that reservoir it would have been straightforward to derive substantial amounts of the pyrimidine nucleosides, albeit in the α- rather than β-furanose form.

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Supporting Information Available: NMR spectra, image of crystals, and X-ray crystallographic coordinates (CIF, PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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