

Spontaneous Prebiotic Formation of a β -Ribofuranoside That Self-Assembles with a Complementary Heterocycle

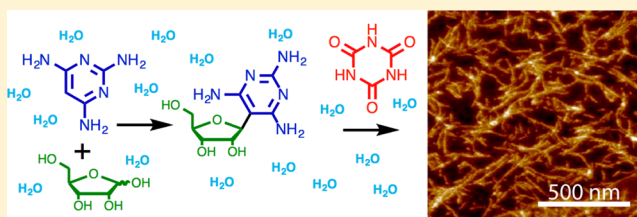
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S Supporting Information

ABSTRACT: The RNA World hypothesis is central to many current theories regarding the origin and early evolution of life. However, the formation of RNA by plausible prebiotic reactions remains problematic. Formidable challenges include glycosidic bond formation between ribose and the canonical nucleobases, as well as the inability of nucleosides to mutually select their pairing partners from a complex mixture of other molecules prior to polymerization. Here we report a one-pot model prebiotic reaction between a pyrimidine nucleobase (2,4,6-triaminopyrimidine, TAP) and ribose, which produces TAP-ribose conjugates in high yield (60–90%). When cyanuric acid (CA), a plausible ancestral nucleobase, is mixed with a crude TAP +ribose reaction mixture, micrometer-length supramolecular, noncovalent assemblies are formed. A major product of the TAP +ribose reaction is a β -ribofuranoside of TAP, which we term TARC. This nucleoside is also shown to efficiently form supramolecular assemblies in water by pairing and stacking with CA. These results provide a proof-of-concept system demonstrating that several challenges associated with the prebiotic emergence of RNA, or pre-RNA polymers, may not be as problematic as widely believed.



INTRODUCTION

Forty years ago, Orgel and co-workers explored abiotic nucleoside formation by drying and heating D-ribose with each of the four canonical nucleobases (adenine, guanine, cytosine, and uracil). Only adenine was found to form nucleosides in detectable amounts (~2% yield).^{1–3} The poor glycosylation of the canonical nucleobases in model prebiotic reactions prompted the Orgel laboratory to explore the possibility that pyrimidine nucleosides were originally synthesized by nucleobase formation on a sugar scaffold,⁴ a hypothesis for which a complete synthetic pathway was more recently demonstrated by Sutherland and co-workers through an elegant, albeit multistep, reaction.^{5,6} Exploring the hypothesis that RNA evolved from a pre-RNA polymer,^{7,8} Miller and co-workers,⁹ and later our laboratory,¹⁰ demonstrated that nucleosides are formed in good yields by reacting alternative heterocycles with ribose, specifically urazole and 2-pyrimidinone. The discovery of alternative heterocycles that readily undergo nucleoside formation suggests that alternative nucleosides could have been more abundant on the prebiotic earth than the canonical nucleosides.

Even if a prebiotic route to all four canonical nucleosides were established, the mechanism by which these nucleosides could have been selected from a complex mixture of molecules and coupled into RNA polymers without the aid of enzymes remains a daunting question. A major challenge arises due to the fact that the canonical mononucleotides and their free bases

do not form Watson–Crick base pairs in water,^{11,12} but rather associate by π -stacking. Thus, it is difficult to imagine how the four nucleobases of contemporary RNA would have been exclusively selected from among similar, but nonpairing, heterocycles for incorporation into the first informational polymers of life. We have proposed that this “paradox of base pairing”¹³ could be overcome if there existed a set of recognition elements that are able to self-sort into noncovalent polymer assemblies prior to linkage by a common backbone.¹⁴ Together, both prebiotic nucleoside formation and nucleobase selection may have not been so difficult if alternative heterocycles amenable to glycosylation and self-assembly formed the first informational polymers.

In search of alternative nucleobases that could form nucleosides robustly and make stable base pairs in water, we systematically considered all possible pyrimidines and purines with $-\text{NH}_2$, $=\text{O}$, or $-\text{H}$ as exocyclic groups. Of more than 80 possible candidates, 2,4,6-triaminopyrimidine (TAP) was one of the molecules chosen for investigation because its structure suggested the potential for enhanced reactivity with ribose,¹⁵ and because TAP is well-known to form supramolecular assemblies with complementary heterocycles.^{16–20} The H-bonding interactions between these heterocycles are similar to those found in Watson–Crick base pairs; however, their ability

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to interact along three “faces” enables the formation of larger structures (see below). Additionally, recent studies from our laboratory demonstrated the capability of modified, monomeric TAP to assemble with cyanuric acid (CA) in aqueous solution to form micrometer-length supramolecular assemblies.²⁰ Here we show that aqueous mixtures of TAP and ribose spontaneously form β -furanosyl nucleosides in high yields. Upon the addition of the complementary CA, the TAP–ribose conjugates assemble (within the unpurified reaction solution) to form micrometer-length noncovalent polymers consisting of thousands of ordered nucleosides.

RESULTS AND DISCUSSION

Drying and Mild Heating of TAP with Ribose Produces TAP–Ribose Conjugates. TAP and (D)-ribose were mixed together in water and heated under various conditions to assess the glycosylation potential of TAP. As shown in Figure 1, a considerable fraction of TAP becomes glycosylated to form TAP–ribose conjugates when the mixtures were dried at 35 °C (Figure 1). The reaction is robust, with TAP–ribose conjugates being produced in comparable yields when the ratio of TAP and ribose were varied over a factor of 5, and when the reaction was carried out at temperatures ranging from 35 to 95 °C (Figures S1–S5 Supporting Information [SI]). The yield of single-ribose TAP conjugates was maximized relative to double- and triple-ribose additions to TAP when mixtures were dried at the lower range of temperatures investigated and with TAP:ribose ratios close to unity. As shown in Figure 1B, HPLC analysis with simultaneous MS and UV absorption monitoring reveals that vacuum-assisted drying of a 1:2 TAP:ribose mixture at 35 °C for one day results in over 30% of TAP being modified by conjugation with ribose. Extended reaction times allowed maximum conversion of TAP to TAP–ribose conjugates. For example, the yield of single-ribose TAP conjugates (including nucleosides, see below) increased to around 60% after 10 days of drying at 35 °C, with momentary daily rehydration (which allowed the reaction to be carried out for multiple days under conditions of low water activity while remaining homogeneous) (Figure 1C). Reactions carried out at higher temperatures and with higher ratios of ribose to TAP resulted in the more rapid and nearly complete modification of TAP, with up to 90% of all TAP being modified by the addition of one to three ribose molecules. This reaction was observed to happen even in aqueous solution (i.e., without drying), as TAP–ribose conjugates were formed in the solution state within days at temperatures from 35 to 65 °C, or weeks at 4 °C (Figures S6 and S7 SI).

Drying TAP with ribose results in a thick syrup with free TAP and TAP–ribose conjugates reaching a concentration of ~38% (m/m). The solubility of TAP in the absence of ribose is only 3.6% (m/m), indicating that a substantial fraction of TAP forms associations with ribose, which could include highly labile linkages between open-chain ribose and the exocyclic amines of TAP that may not be observed during HPLC analysis. The increased solubility of TAP in the presence of ribose contrasts with the relatively low solubility of the canonical nucleobases under similar conditions. For example, cytosine (the most soluble of the four RNA nucleobases) has a solubility limit in water of 0.73% (m/m), and this limit does not appear to increase with ribose as a cosolute. In addition to increasing TAP solubility, ribose conjugation with TAP could also prevent ribose degradation by fixing the sugar in its cyclized form, a

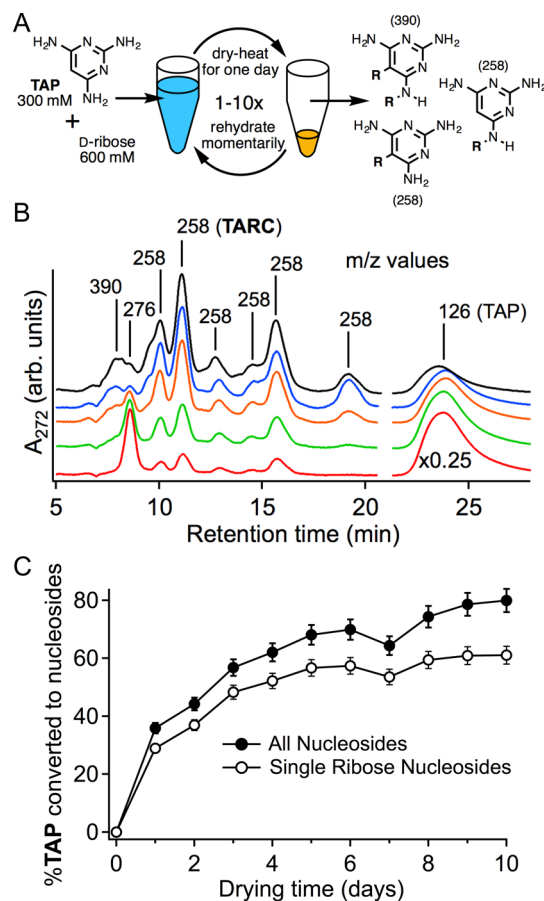


Figure 1. (A) Chemical structure of 2,4,6-triaminopyrimidine (TAP) and the process used to generate TAP-(D)-ribose conjugates (including nucleosides). R groups on TAP represent any possible ribose isomers. (B) HPLC chromatographs of samples of TAP and ribose after 1–5 days of drying, i.e., drying of TAP (300 mM) with ribose (600 mM) (pH 8) at 35 °C (vacuum assisted) with momentary rehydration every 24 h. HPLC traces, from bottom to top, correspond to reaction times of 1, 2, 3, 4, and 5 days. Peaks are labeled with m/z values obtained by simultaneous MS and UV absorption monitoring of LC. The m/z values listed correspond to TAP with one closed-ring ribose conjugate, 258; TAP with two conjugated riboses, 390; and TAP conjugated with an open chain ribose, 276. (C) Plot of percentage of TAP converted to nucleosides as a function of reaction time (same reaction conditions as in B). Error bars represent known sources of experimental uncertainty.

possible rudimentary mechanism to aid the accumulation of ribose that is complementary to previously proposed prebiotic mechanisms, which include ribose complex formation with borate and sequestration by selective crystallization.^{21–23}

The Major Product of the TAP+Ribose Reaction Is a β -Ribofuranosyl C-Nucleoside. To confirm that nucleosides are among the products of the TAP+ribose reaction, the main product (~20%) resulting from the 10-day 35 °C drying reaction of a 1:2 TAP+ribose mixture was isolated by HPLC. NMR spectroscopy revealed that this product is 5- β -ribofuranosyl-2,4,6-triaminopyrimidine (TARC) (Figures 2 and S8–S10 in SI). Two-dimensional (HMBC) analysis confirmed that TARC is a C-nucleoside, with a C–C bond between ribose and the nucleobase (Figure 2B), while 1D ROE experiments established TARC to be of the β -furanose conformation (Figure 2C).

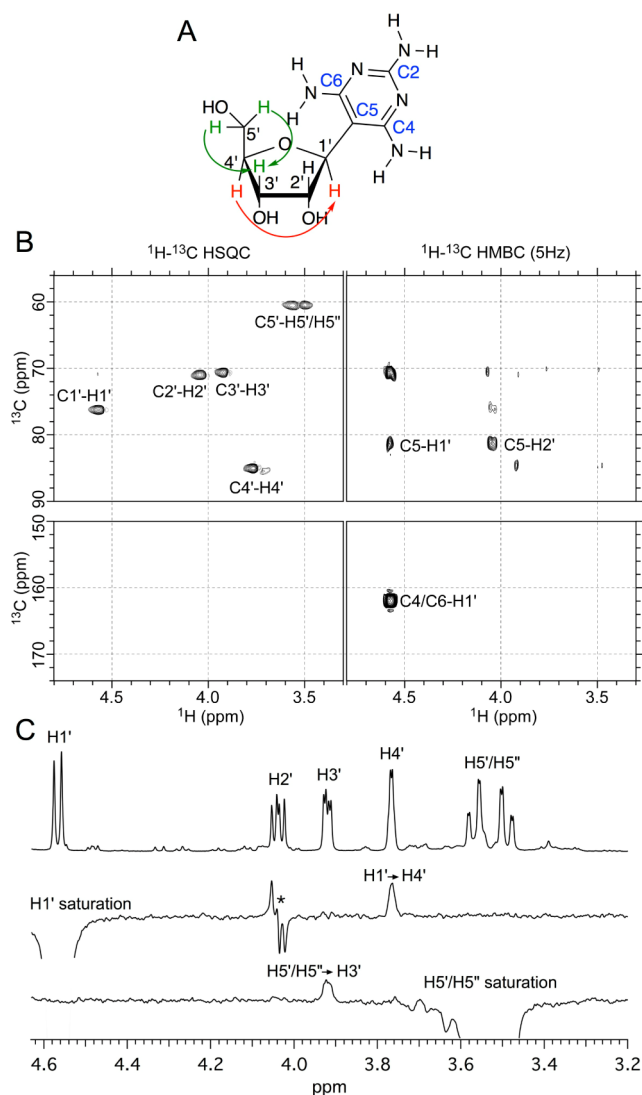


Figure 2. (A) Chemical structure of TARC (*5*- β -ribofuranose-*C*-triaminopyrimidine). Arrows show through space proton–proton magnetization transfer as indicated by ROE analysis in panel (C). (B) HMBC spectrum which was optimized for 5 Hz ^{13}C – ^1H couplings. The ^{13}C chemical shift of 81 ppm is assigned to the C5 of the pyrimidine ring of TARC, and the 162 ppm chemical shift is assigned to the symmetry-related C6 and C4 carbons of the pyrimidine ring. The observed ^1H – ^{13}C correlations between the H1' proton and the C5 chemical shift, and the C4, C6 carbon chemical shifts, support the C-nucleoside structural assignment of TARC. (C) ^1H NMR and 1D ROE spectra of the nonexchangeable (ribose) protons of TARC. (Top) ^1H NMR spectrum with resonance assignments as indicated in (A). No resonances were observed in the aromatic region, consistent with the C-nucleoside assignment. (Middle) Irradiation of the H1' results in through-space magnetization transfer to the H4' proton. * indicates TOCSY transfer from H1' to H2', a through-bond magnetization transfer by these strongly coupled protons (e.g., 9 Hz). (Bottom) Irradiation of the H5'/H5'' protons results in through-space magnetization transfer to the H3' proton. See SI for additional structural characterization of TARC.

Contemporary nucleosides are predominantly *N*-ribosides, but *C*-nucleosides do exist in nature, including pseudouridine, the most common RNA post-transcriptional nucleoside modification.²⁴ The finding that the ribose of TARC is in the β -furanose conformation, like the nucleosides found in extant life, was somewhat unexpected as free ribose exists predom-

inantly in its β -pyranose form.²⁵ This discovery is consistent with previously reported model prebiotic reactions of nucleoside formation, which have also indicated that the ribofuranosyl form of nucleosides could have been available since the earliest abiotic glycosylation reactions.^{9,10}

Like other 2,4,6-substituted pyrimidines, which undergo Mannich reactions,²⁶ TAP is known to be nucleophilic at the C5 position.²⁷ TARC formation is expected to proceed through the reaction of TAP with the free aldehyde form of ribose.²⁸ This proposed pathway, detailed in Figure S11 SI, is supported by the observation of a covalent intermediate in early wet–dry cycles with the mass of an open chain ribose connected to TAP (peak with m/z of 276 in Figure 1B and Figure S2 SI). Consistent with this putative assignment, the 276 m/z product is a transient species, appearing most prominently after the first two days of drying, but vanishing almost completely after the fifth day of drying. In contrast, TARC yield steadily increases up to at least 5 days of drying and does not decrease with additional drying time. It is also a certainty that exocyclic *N*-glycosides are among the TAP–ribose conjugates present in the crude reaction mixture, as multiple products with m/z values corresponding to TAP with one, two, and three covalent ribose additions are observed (which necessitates that some of these products have at least one ribose added to an exocyclic amine of TAP). Furthermore, formation of exocyclic *N*-glycosides involving the natural nucleobases has previously been shown to form in both drying reactions^{1,2} and in solution.²⁹ Hydrolysis studies of the crude TAP–ribose reaction mixture show the rapid conversion of multiple ribose nucleosides to free TAP or a single ribose–TAP conjugate (Figure S12 SI), which is analogous to the facile release of ribose from the exocyclic amine of adenine.^{1,2}

TARC Assembles with CA To Form a Hydrogel with Micrometer-Length Polymers. HPLC purified TARC was tested to determine if it is able to assemble with cyanuric acid (CA) in aqueous solution. Mixing TARC with CA in equimolar proportions in a sodium phosphate and boric acid buffer resulted in the formation of a shear-thinning hydrogel (Figure 3A). Hydrogel formation is indicative of self-assembly into supramolecular polymers that further associate to form a continuous matrix.³⁰

Circular dichroism (CD) was used to inspect solutions containing TARC and CA, as dramatic changes in CD-signal intensity are often associated with the formation of supramolecular assemblies by chiral monomers.^{31,32} In the present case, large, positive bands from 210 to 250 and 270–290 nm were observed for TARC and CA mixtures at 4 °C for concentrations greater than 5 mM in each monomer; a TARC and CA mixture at 2.5 mM each exhibited a CD profile essentially identical to TARC alone (Figure 3B and Figures S13 and S14 in SI). Heating of a sample containing 5 mM TARC and CA to 20 °C resulted in a complete loss of the assembly-induced CD signal. The TARC UV absorption spectrum exhibited a red-shift upon the addition of CA, demonstrating the formation of J-type aggregates, a spectral feature that is associated with π – π stacking interactions (Figure 3C).

TARC–CA assemblies were visualized by atomic force microscopy (AFM), which revealed supramolecular polymers with lengths greater than 200 nm (Figure 4A). The diameter of a single fiber, measured perpendicular to the image plane, was 2 nm (inset, Figure 4A). Self-assembled structures of comparable thickness and length were recently observed when a molecule named TAPAS (TAP with succinate conjugated to an exocyclic

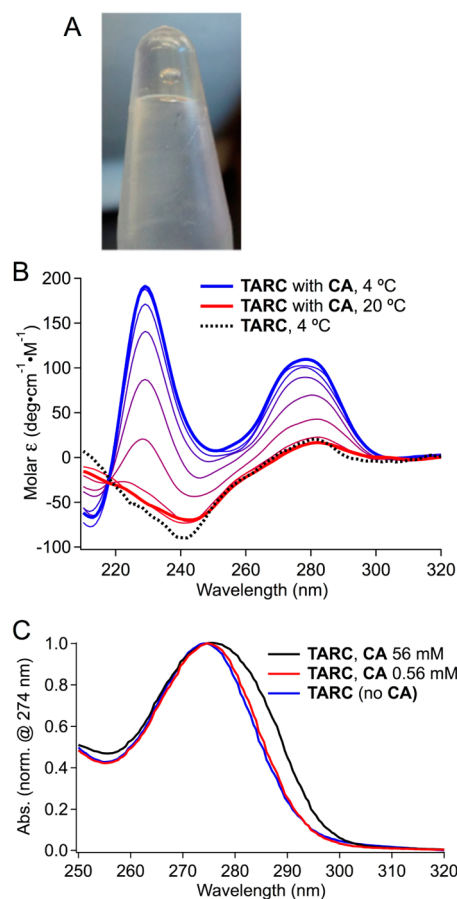


Figure 3. (A) Gel formed by TARC and CA (40 mM in each monomer). High viscosity of the gelled solution is illustrated by a bubble that remained in place indefinitely. (B) CD spectra of 5 mM TARC and CA, in 200 mM sodium phosphate/200 mM sodium borate (pH 7), at temperatures ranging from 4 to 20 °C, in steps of 2 °C. Spectrum of TARC in same buffer without CA is also shown for comparison. (C) The UV spectrum of TARC (56 mM) and CA (56 mM) at 4 °C in sodium phosphate buffer (200 mM, pH 7.0) with sodium borate (200 mM) (black), showing a red-shift characteristic of J-type aggregates when compared to TARC alone (blue). When the same solution was diluted by 100-fold (red), the TARC/CA UV profile matched that of TARC alone. All absorbances were normalized to absorbance maximum within the region shown.

amine) was mixed with CA.²⁰ The similarities of these structures strongly suggest that the fibers shown in Figure 4A are TARC nucleosides assembled with CA in the same manner as TAPAS assembled with CA. That is, CA and TARC arranged within “rosettes” that are π -stacked to form linear supramolecular assemblies (Figure 4B). These assemblies have a predicted width of approximately 1.6 nm for TAP before conjugation with ribose, which is consistent with the measured fiber thickness of 2 nm. Given that the fibers imaged by AFM are up to 500 nm in length, and that the interplane stacking of planar ring systems is 0.34 nm, each of these linear assemblies represents up to 1500 stacked hexads.

The borate anion in the sodium borate buffer was found to be essential for the formation of water-soluble assemblies. Initial mixing experiments with TARC and CA in the absence of borate resulted in immediate precipitation, as observed when unmodified TAP is mixed with CA. The ability for borate to improve the solubility of the TARC–CA complex was not unexpected. Water-soluble rosette assemblies by TAP (or

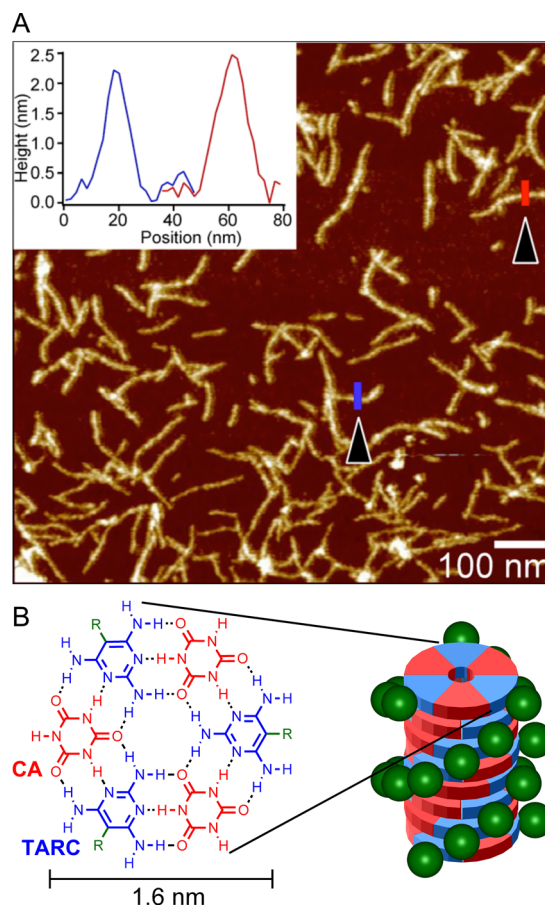


Figure 4. (A) AFM topographic image of self-assembled TARC-CA fibers from a solution containing 50 mM in each molecule. Inset shows the height profile of single fibers delineated by the red and blue lines in the main panel. (B) Structures of TARC and CA, in their H-bonded rosette structure, and proposed higher-order assembly based upon AFM fiber dimensions. Ribose is indicated as R on the TARC chemical structures and green spheres on schematic models of TARC. The hexad stack is depicted with a helical twist to emphasize the presence of a preferred chiral arrangement of monomers as indicated by the CD spectrum shown in Figure 3. The actual handedness and degree of helical twist has not been determined.

melamine, its triazine analogue) and CA have only been obtained when a charged group is incorporated at the periphery of these assemblies,^{20,33} and it was anticipated that the borate anion would add a negative charge to TARC upon complex formation with the vicinal *cis*-diols of ribose.^{22,34} With regard to the emergence of charged nucleosides (e.g., nucleotides) and their associated polymers, it is clear that phosphate is optimal for providing negative charges along the nucleic acid backbones in extant life.³⁵ We do not propose that borate was part of an early pre-RNA. However, other negatively charged moieties, such as gloxylate,³⁶ may have initially been part of a pre-RNA before being replaced by phosphate (just as there may have been different nucleoside bases in pre-RNA polymers).

TAP–Ribose Conjugates in the Crude Reaction Mixture Assemble with CA To Form Hydrogels and Micrometer-Length Fibers. In order to find a more realistic one-pot prebiotic reaction for the formation and selection of nucleosides, we investigated the potential of TAP nucleosides in the reaction mixture to associate with CA while still in solution with unreacted starting materials, nonpairing TAP–

ribose conjugates, and any other reaction products. For this investigation, the crude reaction mixture of 1 part TAP and 2 parts ribose that had been subjected to drying for 10 days was resuspended in sodium borate buffer, pH 8. CA was then added to the solution and heated to 95 °C (to facilitate dissolving CA). Upon cooling to room temperature the solution formed a hydrogel that coexisted with a precipitate (Figure 5A). Control

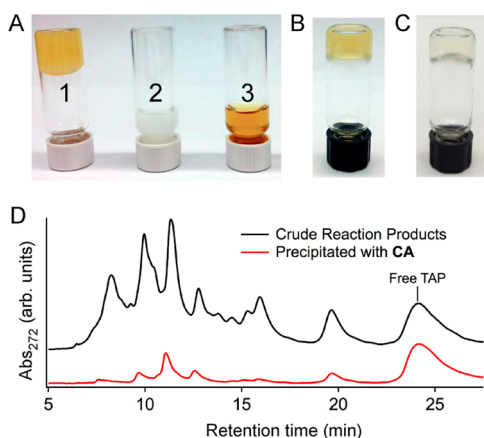


Figure 5. (A) Inverted bottle test showing gel formation of a 10-day reaction of TAP with ribose (1:2::TAP:ribose; vacuum-assisted drying at 35 °C with daily rehydration) after addition of cyanuric acid (CA) to the same concentration as TAP (modified and unmodified) (bottle 1). CA was added with sodium borate (300 mM, pH 8) at room temperature. Gel formation is not observed if procedure is identical to that of bottle 1, but the TAP and ribose solution is not subjected to drying (bottle 2), or if CA is not added to sample (bottle 3). (B) Same as bottle 1 in A, except CA was added before sample was dried to generate TAP–ribose conjugates. (C) Same as bottle 1 in A, except solution was clarified of precipitated material by centrifugation and diluted to 16 mM for both CA- and TAP-containing species. (D) HPLC chromatographs of the crude TAP+ribose reaction product used to make the gel shown in C (upper trace) and of the precipitate that formed when this crude mixture was mixed with CA (lower trace). Chromatographs were normalized to the integrated area of free TAP peaks.

experiments in which TAP and ribose were freshly mixed without drying, or where CA was not added to the postdrying TAP+ribose reaction mixture, did not result in hydrogel formation (Figure 5A), confirming that products of the TAP+ribose reaction and their assembly with CA are the cause of hydrogel formation. Similar hydrogels were also obtained if CA was initially added to an unreacted solution containing TAP and ribose, followed by drying and heating; confirming that the order of CA addition is not critical to the TAP+ribose reaction or hydrogel formation (Figure 5B)

The translucent hydrogel solution formed by the crude TAP+ribose reaction mixture and CA could be clarified of precipitate by centrifugation to yield a transparent hydrogel (Figure 5C). HPLC–UV analyses of the material in the gel and the precipitate revealed that the hydrogel is the preferential phase for TAP–ribose conjugates, whereas the precipitate is the preferential phase for unmodified TAP (Figure 5D). Specifically, the crude TAP+ribose gel contained approximately 87% of TAP as TAP–ribose conjugates and 13% as free TAP. In contrast, the precipitate formed upon the addition of CA was substantially depleted of TAP–ribose conjugates (43%) and enriched in free TAP (57%). We propose that this separation of TAP–ribose conjugates from free TAP by supramolecular

polymer formation/precipitation with CA represents a potential prebiotic mechanism to locally concentrate pairing nucleosides away from nonpairing molecules (e.g., nonpairing TAP–ribose conjugates) and from precipitating molecules that do pair (e.g., free TAP) in a complex chemical mixture.

The hydrogel phase formed by the crude TAP+ribose reaction mixture and CA was imaged by AFM, again revealing the formation of micrometer-length fibers (Figure 6). The

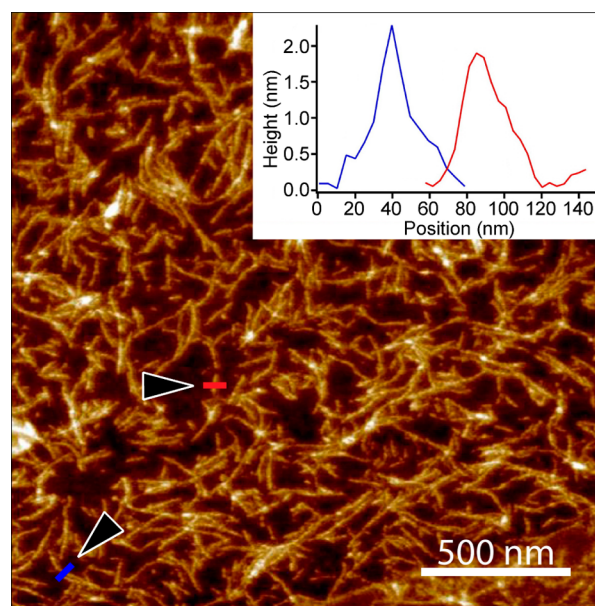


Figure 6. AFM topographical image of assemblies formed by the crude TAP+ribose reaction mixed with CA (gel shown in Figure 5C). (Inset) Height measurements across individual fibers indicated by red and blue lines and arrowheads in main image.

thickness of single fibers was determined to be 2 nm (inset, Figure 6), as found with purified TARC assembled with CA. While the TARC–CA combination is likely to be a large constituent of these noncovalent assemblies, other TAP–ribose conjugates may also be part of these assemblies. We are currently investigating how differences in the TAP–ribose conjugates, such as sugar structure and conformation, may affect their selection and inclusion into the supramolecular assemblies. Regardless, the ability of the TAP–ribose conjugates and CA to assemble into ordered supramolecular structures without the need for starting from a pure source of monomer demonstrates a robust and potentially prebiotic pathway for monomer isolation which may allow for a pathway for ‘self-selection’ by a stepwise constitutional preferential exclusion/inclusion mechanism.

CONCLUSION

In summary, we have shown that TAP readily forms nucleosides with ribose in a one-pot model prebiotic reaction. The high yield of the β -ribofuranoside, TARC, from the TAP+ribose reaction contrasts with the low or nonexistent nucleoside formation found in ribose reactions with the canonical nucleobases. These observations lend support to the hypothesis that there could have been alternative prebiotic nucleobases that were more amenable to spontaneous nucleoside formation.^{14,37,38} Furthermore, the addition of a complementary H-bonding heterocycle with TAP nucleosides, even in the crude TAP+ribose reaction mixture, results in the

self-assembly of micrometer-length noncovalent polymers. Spatial ordering and increased local concentration of monomers is critical for efficient polymerization, and among the principal mechanisms by which enzymes (including polymerases) catalyze bimolecular reactions. Because highly evolved enzymes would not have existed at the origin of biopolymers (or at the origin of ancestral biopolymers), it has long been speculated that mineral surfaces^{39,40} or small, organic molecules⁴¹ may have played an important role in locally concentrating and organizing monomers prior to polymerization. Likewise, self-assembling nucleosides, like TARC and CA, would facilitate monomer coupling (with appropriate ligation chemistry) and thereby the *de novo* formation of covalent, proto-biopolymers. In this context, the results presented begin to address how alternative nucleobases that readily self-assemble could have set the stage for the emergence of the early informational polymers of life by both facilitating nucleoside formation and the selection of pairing bases from complex mixtures, before the emergence of enzymes.

METHODS

Drying–Heating Reactions of TAP with Ribose. Stock solutions of 2,4,6-triaminopyrimidine (Acros Organic) and D-ribose (Amresco and Sigma-Aldrich) were prepared in nanopure H₂O. When dried at 45–95 °C, an Eppendorf tube with 1 mL of the stock solution was placed in a dry block heater at the specified temperature. For drying at 35 °C, tubes were placed in a vacuum centrifuge. Samples that were reacted for multiple days were dried for 24 h, the resultant highly viscous solution was momentarily resuspended in the original volume of nanopure H₂O, and the process was repeated for the indicated number of days. Momentary hydration was performed to ensure that the solution was homogeneous during the course of the reaction. Additionally, daily hydration of the reaction mixtures models environmental day/night cycles, a process which would have been operative on the prebiotic Earth. Analytical HPLC of samples was performed by running 95% 0.010 M NH₄HCO₃ (pH 7.2) and 5% CH₃OH at 0.5 mL·min⁻¹ through a Phenomenex Synergi Polar-RP 80 Å column (250 mm × 4.60 mm × 4 μm).

Preparative Synthesis of 5-β-Ribofuranosyl-2,4,6-triaminopyrimidine. 2,4,6-Triaminopyrimidine (1.5 mmol) and D-ribose (3 mmol/2 equiv) were dissolved in 5 mL of nanopure H₂O. The sample was subjected to drying in a vacuum centrifuge for one week. The resulting light-brown, highly viscous crude mixture was resuspended in 2.5 mL of 50 mM CH₃COONa buffer at pH 4.6. The solution was then loaded onto a gravity column containing Sephadex C50 sulfopropyl cation-exchange media, and was eluted with a gradient of CH₃COONH₄ in 50 mM CH₃COONa buffer at pH 4.6. The resultant product-containing fractions were concentrated under vacuum to remove water. TARC was purified using an Agilent 1200 Infinity system by ion-pairing chromatography on a semipreparative Phenomenex Prodigy ODS(3) 100 Å column (250 mm × 10 mm × 5 μm), with a mobile phase of 100% 0.1 M triethylammonium acetate. The resultant product-containing fractions were concentrated under vacuum, removing the majority of volatile salts from the various purification steps. Additional desalting was accomplished by reverse phase chromatography on the previously mentioned semipreparative column. Maximum yield of TARC obtained was 20%. UV/vis (H₂O, pH 7): λ_{max} = 276 nm ε₂₇₆ = 13,600 mol·L⁻¹·cm⁻¹. ¹H NMR (500 MHz, D₂O) δ 4.57 (d, 9.1 Hz, H1'); 4.04 (dd, 9.1, 6.2 Hz, H2'); 3.92 (dd, 2.6, 6.2 Hz, H3'); 3.77 (m, 2.5 Hz, H4'); 3.57 (m, ABX, 2.0, 12.5 Hz, H5'a); 3.49 (m, ABX, 2.4, 12.6 Hz, H5'b). ¹³C NMR (126 MHz, D₂O) δ 161.93 (C4/C6); 160.6 (C2); 84.9 (C4'); 76.2 (C1'); 81.3 (C5); 70.9 (C2'); 70.6 (C3'); 60.4 (C5'). HRMS (pos. m/z): C₉H₁₆O₄N₅ theoretical mass: 258.1197, actual mass: 258.1199.

Crude and TARC Gel Preparation. The crude gel was formed by combining CA (1.5 mmol/1 equiv) with the 10-day dried TAP + ribose reaction product (see Preparative Synthesis above) and then

dissolved in 300 mM sodium borate (pH 8) to reconstitute the sample at 5 mL. Reactions containing CA were carried out the same as with the TAP + ribose reactions except one equivalent of CA was included with the starting material. In all cases the samples were then heated to 95 °C for 5 min to dissolve the CA and cooled at room temperature. A cloudy, yellow hydrogel was observed with an off-white precipitate collected at the bottom. Samples from the original crude reaction, precipitate, and hydrogel were collected and analyzed by HPLC-DAD. Co-injection and LC–MS analysis were used to identify TAP from TAP–ribose conjugates. Clarified hydrogels were obtained by centrifugation of the crude gel through a 0.2 μm Millipore spin column. Gels prepared with purified TARC were made by the same procedure with the addition of 200 mM sodium phosphate to buffer at pH 7.

Analytical Techniques. CD spectra were obtained on a Jasco J-720 CD spectrometer, and acquired by scanning 200–350 nm at a rate of 500 nm/min, with averaging of 20 measurements. UV absorption analysis was performed on an Agilent 8453 UV–vis spectrophotometer equipped with an 89090A temperature controller. All samples that were inspected by CD or UV contained 200 mM sodium phosphate and 200 mM boric acid (pH 7) and were analyzed in a torsion-free sandwich cell with either a 0.1 or 0.01 mm path length. The structure of TARC was then confirmed by NMR using ¹H NMR, ¹³C NMR, COSY, HSQC, HMBC, and ROE, all collected on a Bruker DRX-500. High-resolution mass spectrometry was performed on a Waters Synapt G2; purity was confirmed by analytical HPLC as described in the analytical synthesis of TARC.

AFM Imaging. Samples were imaged by AFM over a silicon surface. Prior to deposition, silicon wafers were rinsed with nanopure water followed by ethanol and dried under a N₂ stream. After rinsing, silicon surfaces were cleaned with UV-ozone for 30 min (JE- LIGHT UVO Cleaner model #42) and stored in a Petri dish until use. A 1.5-μL sample that was stored on ice was spin coated (Laurell Technologies) at 4 °C for 30 s (2000 rpm, Figure 2C; 4000 rpm, Figure 3D). After spin coating, the sample was dried at room temperature in a Petri dish overnight. The AFM imaging was performed on a Multimode AFM with Nanoscope IIIa controller and a “J” scanner (Veeco Instruments) in tapping mode in air, using Si tips (Vistaprobes, 40 N/m).

ASSOCIATED CONTENT

Supporting Information

Additional characterization of the formation of TARC is given in Figures S1–S7 and S12, detailed NMR characterization of TARC is given in Figures S8–S10, a proposed reaction mechanism for the formation of TARC from TAP and ribose is presented in Figure S11. Additional CD characterization of TARC assemblies is given in Figures S13–S14. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Fuller, W. D.; Sanchez, R. A.; Orgel, L. E. *J. Mol. Biol.* **1972**, *67*, 25–33.
- (2) Fuller, W. D.; Sanchez, R. A.; Orgel, L. E. *J. Mol. Evol.* **1972**, *1*, 249–257.
- (3) Maurel, M.-C.; Convert, O. *Origins Life Evol. B* **1990**, *20*, 43–48.
- (4) Sanchez, R. A.; Orgel, L. E. *J. Mol. Biol.* **1970**, *47*, 531–543.
- (5) Powner, M. W.; Gerland, B.; Sutherland, J. D. *Nature* **2009**, *459*, 239–242.
- (6) Powner, M. W.; Sutherland, J. D.; Szostak, J. W. *Synlett* **2011**, 1956–1964.
- (7) Joyce, G. F.; Schwartz, A. W.; Miller, S. L.; Orgel, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 4398–402.
- (8) Orgel, L. E. *Crit. Rev. Biochem. Mol. Biol.* **2004**, *39*, 99–123.
- (9) Kolb, V. M.; Dworkin, J. P.; Miller, S. L. *J. Mol. Evol.* **1994**, *38*, 549–557.
- (10) Bean, H. D.; Sheng, Y. H.; Collins, J. P.; Anet, F. A. L.; Leszczynski, J.; Hud, N. V. *J. Am. Chem. Soc.* **2007**, *129*, 9556–9557.
- (11) Solie, T. N.; Schellman, J. A. *J. Mol. Biol.* **1968**, *33*, 61–77.
- (12) Ts'o, P.; Melvin, I.; Olson, A. *J. Am. Chem. Soc.* **1963**, *85*, 1289–1296.
- (13) Engelhart, A. E.; Hud, N. V. *Cold Spring Harbor Perspect. Biol.* **2010**, *2*, 10.1101/cshperspect.a002196.
- (14) Hud, N. V.; Cafferty, B. J.; Krishnamurthy, R.; Williams, L. D. *Chem. Biol.* **2013**, *20*, 466–474.
- (15) Rembold, H.; Schramm, H. J. *Chem. Ber.* **1963**, *96*, 2786–2797.
- (16) Lehn, J. M.; Mascal, M.; Decian, A.; Fischer, J. *J. Chem. Soc., Perkin Trans. 2* **1992**, 461–467.
- (17) Bohanon, T. M.; Denzinger, S.; Fink, R.; Paulus, W.; Ringsdorf, H.; Weck, M. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 58–60.
- (18) Marchi-Artzner, V.; Artzner, F.; Karthaus, O.; Shimomura, M.; Ariga, K.; Kunitake, T.; Lehn, J. M. *Langmuir* **1998**, *14*, 5164–5171.
- (19) Rakotondradany, F.; Palmer, A.; Toader, V.; Chen, B. Z.; Whitehead, M. A.; Sleiman, H. F. *Chem. Commun.* **2005**, 5441–5443.
- (20) Cafferty, B. J.; Gállego, I.; Chen, M. C.; Farley, K. I.; Eritja, R.; Hud, N. V. *J. Am. Chem. Soc.* **2013**, *135*, 2447–2450.
- (21) Prieur, B. E. *C.R. Acad. Sci. Paris, Chim./Chem.* **2001**, *4*, 667–670.
- (22) Ricardo, A.; Carrigan, M. A.; Olcott, A. N.; Benner, S. A. *Science* **2004**, *303*, 196–196.
- (23) Springsteen, G.; Joyce, G. F. *J. Am. Chem. Soc.* **2004**, *126*, 9578–9583.
- (24) Charette, M.; Gray, M. W. *IUBMB Life* **2000**, *49*, 341–351.
- (25) Angyal, S.; Pickles, V. *Aust. J. Chem.* **1972**, *25*, 1695–1710.
- (26) Han, B.; Rajwanshi, V.; Nandy, J.; Krishnamurthy, R.; Eschenmoser, A. *Synlett* **2005**, *16*, 0744–0750.
- (27) Delia, T. J.; Kirt, D. D.; Sami, S. M. *J. Heterocycl. Chem.* **1983**, *20*, 145–147.
- (28) Dworkin, J. P.; Miller, S. L. *Carbohydr. Res.* **2000**, *39*, 359–365.
- (29) Johnson, K. M.; Price, N. E.; Wang, J.; Fekry, M. L.; Dutta, S.; Seiner, D. R.; Wang, Y.; Gates, K. S. *J. Am. Chem. Soc.* **2012**, *135*, 1015–1025.
- (30) Estoff, L. A. H.; A, D. *Chem. Rev.* **2004**, *104*, 1201–1217.
- (31) Fenniri, H.; Packiarajan, M.; Vidale, K. L.; Sherman, D. M.; Hallenga, K.; Wood, K. V.; Stowell, J. G. *J. Am. Chem. Soc.* **2001**, *123*, 3854–3855.
- (32) Hirschberg, J. H. K. K.; Brunsveld, L.; Ramzi, A.; Vekemans, J. A. J. M.; Sijbesma, R. P.; Meijer, E. W. *Nature* **2000**, *407*, 167–170.
- (33) Ma, M. M.; Bong, D. *Langmuir* **2011**, *27*, 8841–8853.
- (34) Springsteen, G.; Wang, B. *Tetrahedron* **2002**, *58*, 5291–5300.
- (35) Westheimer, F. H. *Science* **1987**, *235*, 1173–1178.
- (36) Bean, H. D.; Anet, F. A. L.; Gould, I. R.; Hud, N. V. *Orig. Life Evol. B* **2006**, *36*, 39–63.
- (37) Rios, A. C.; Tor, Y. *Astrobiology* **2012**, *12*, 884–891.
- (38) Rios, A. C.; Tor, Y. *Isr. J. Chem.* **2013**, *53*, 469–483.
- (39) Bernal, J. D. *The Physical Basis of Life*; Routledge & Kegan Paul: London, 1951.
- (40) Ferris, J. P.; Aubrey R. Hill, J.; Liu, R.; Orgel, L. E. *Nature* **1996**, *381*, 59–61.
- (41) Jain, S. S.; Anet, F. A. L.; Stahle, C. J.; Hud, N. V. *Angew. Chem., Int. Ed.* **2004**, *43*, 2004–2008.