

Prebiotic synthesis of phosphoenol pyruvate by α -phosphorylation-controlled triose glycolysis

Adam J. Coggins and Matthew W. Powner*

Phosphoenol pyruvate is the highest-energy phosphate found in living organisms and is one of the most versatile molecules in metabolism. Consequently, it is an essential intermediate in a wide variety of biochemical pathways, including carbon fixation, the shikimate pathway, substrate-level phosphorylation, gluconeogenesis and glycolysis. Triose glycolysis (generation of ATP from glyceraldehyde 3-phosphate via phosphoenol pyruvate) is among the most central and highly conserved pathways in metabolism. Here, we demonstrate the efficient and robust synthesis of phosphoenol pyruvate from prebiotic nucleotide precursors, glycolaldehyde and glyceraldehyde. Furthermore, phosphoenol pyruvate is derived within an α -phosphorylation controlled reaction network that gives access to glyceric acid 2-phosphate, glyceric acid 3-phosphate, phosphoserine and pyruvate. Our results demonstrate that the key components of a core metabolic pathway central to energy transduction and amino acid, sugar, nucleotide and lipid biosyntheses can be reconstituted in high yield under mild, prebiotically plausible conditions.

The integrated pathways of metabolism are orchestrated by an array of enzymes and chaperones that are ultimately dependent on a highly evolved genetic coding system essential to all life on Earth. However, at the origins of life, before the advent of sophisticated enzymatic catalysis and selection, metabolism was required to provide the fundamental building blocks of life^{1–14}. Although only an intriguingly small constellation of molecules produce the diversity of contemporary life, the function of the individual biological components cannot be appreciated in isolation^{1,2}. Therefore, it must follow that the generation and function of biochemical species at the origins of life requires a concerted and, ideally, unified investigation¹⁴. Seeking to further establish the prebiotic generational relationship between the essential molecular components of biology, we were struck by the constitutional relationship between nucleotide precursors glycolaldehyde (**2**) and glyceraldehyde (**3**) and the carbon framework of phosphoenol pyruvate (**1**), biology's highest-energy phosphate (free energy at pH 7, 25 °C (kJ mol⁻¹): **1** = -61.9; creatine phosphate = -43.0; adenosine triphosphate = -30.5; pyrophosphate = -19.2; glucose-6-phosphate = -13.8)^{15–18}.

Pyruvate (**6**) synthesis has been reported at elevated temperatures (25 °C) and pressures (50–200 MPa) in the presence of iron sulfides (0.07% yield)¹⁹, during the abiotic degradation of sugars and sugar-phosphates^{20,21}, and in good yield by lactic acid oxidation (70%)²². However, access to the high-energy phosphate **1** required by metabolism has not been addressed. Although phosphorylation of glyceric acid (**4**) by cyclotrimetaphosphate (which, notwithstanding contrary opinion³⁰, is considered a promising prebiotic phosphorylating agent^{23–29}) yields a 1:1.2 mixture of glyceric acid 2- and 3-phosphates (**4-2P/4-3P**; 38%)²³ and amide derivatives can be accessed by Passerini reactions³¹, the elimination of **4-2P** (or its amide derivatives) to give **1** has not been demonstrated. Indeed, even under strongly acidic (pH 2), alkaline (pH 10) or general acid–base (1 M phosphate, pH 7) conditions, **4-2P** (60 mM) is remarkably stable for prolonged periods of incubation (>15 h at 60 °C; Supplementary Fig. 1). To overcome the shortcomings of both regioselective phosphorylation and elimination, we reasoned that **1** would be directly accessible with high efficiency and complete regiochemical control by mild, aldehyde-mediated reactivity and

late-stage oxidation. Amidotriphosphate (**7**) and diamidophosphate (**8**)—the ammonolysis products of cyclotrimetaphosphate^{23–32}—effect a remarkably selective phosphorylation of α -hydroxyaldehydes and sugars in aqueous solution^{25,32}, and are commonly regarded to be prebiotically plausible^{24,25,28–32}. It was of particular interest to us that these phosphorylations block entry of the key aldehyde components **2** and **3** into ribonucleotide syntheses^{1,18}, which redirects both **2** and **3** to non-natural phosphorylation patterns and pyranosyl (rather than natural furanosyl) sugar and nucleotide structures³³. In a seminal contribution to prebiotic chemistry, Eschenmoser and co-workers outlined the possibility that this switch in reactivity could have predisposed the prebiotic synthesis to non-canonical nucleotides^{25,32–34}. However, in contrast, we hypothesized that α -phosphorylation would redirect **2** and **3** into another metabolic pathway, and provide a generational link between two distinct pathways within (proto)metabolism. Specifically, under prebiotically plausible conditions¹⁸, rapid dehydration of glyceraldehyde 2-phosphate (**3-2P**) was anticipated to yield phosphoenol pyruvaldehyde (**9**) and, upon oxidation, **1**. Interestingly, given the central role of glycolysis in shaping biochemical amino acid, nucleotide and lipid syntheses and the metabolic relationship between **3**, **4-2P**, **4-3P**, **1** and **6**, the efficient generation of each species is observed after subtle variations of mild aqueous reaction conditions to establish a single chemical network that can assemble each of the components of the triose glycolysis pathway of contemporary metabolism (Fig. 1).

Results

Phosphoenol pyruvaldehyde (9). Eschenmoser and co-workers have reported the phosphorylation of tetrose and pentose sugars with **8** (ref. 32) and magnesium-dependent phosphorylation of **2** and **3** with **7** (ref. 25). We have found that the pH-stabilization provided by phosphate buffer (pH 7), as anticipated, removes the reported³² requirement for periodic addition of both **8** and a proton-exchange resin during diamidophosphate-mediated phosphorylations (to counteract the stoichiometric liberation of ammonia and ensure that the phosphorylation reaction proceeds). Phosphate-buffered phosphorylation of **2** (25 mM) by **8** (100 mM) at ambient temperature gave clean phosphorylation after 22 h.

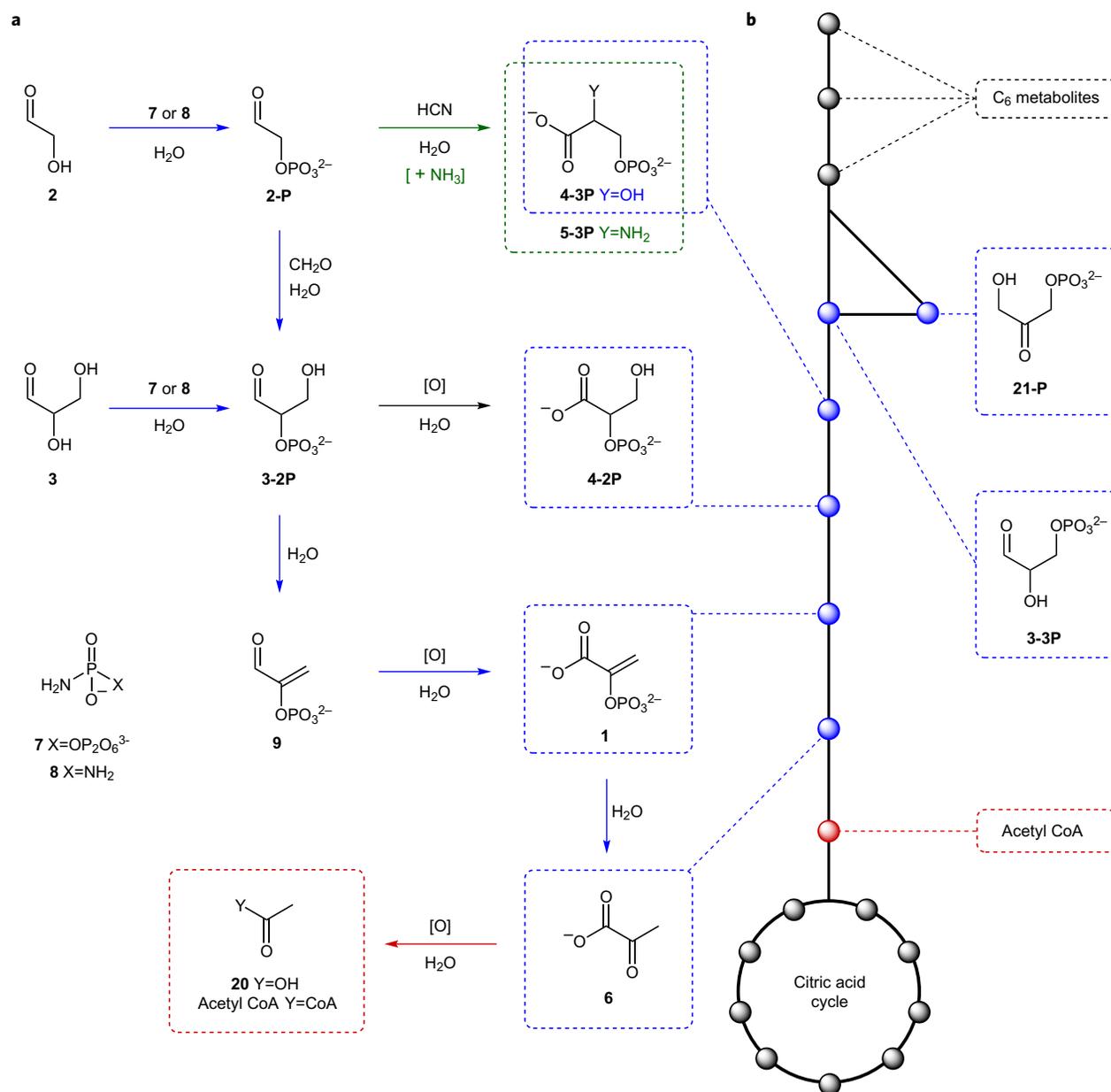


Figure 1 | Reconstitution of the triose glycolysis pathway (blue nodes) in a mild prebiotically plausible aqueous reaction network controlled by α -phosphorylation. **a**, Blue arrows: prebiotic pathway to phosphoenol pyruvate (**1**) and pyruvate (**6**) from glycolaldehyde (**2**) and glyceraldehyde (**3**). Green arrow: divergent prebiotic synthesis of glyceric acid 2-phosphate (**4-3P**; Y = O) and phosphoserine (**5-3P**; Y = NH) from glycolaldehyde-2-phosphate (**2-P**), which provides a generational link between glycolysis (blue) and serine metabolism (green). Red arrow: oxidative decarboxylation of pyruvate (**6**) to yield acetate (**20**). Black arrow: quantitative oxidation of glyceraldehyde 2-phosphate (**3-2P**) to glyceric acid 2-phosphate (**4-2P**). Structures boxed in blue highlight the alignment between the prebiotic network and the specific node on the metabolic triose glycolysis pathway. **b**, Enzymatically controlled pathway from C₆-metabolites to citric acid cycle via glyceraldehyde-3-phosphate (**3-3P**) and dihydroacetone phosphate (**21-P**).

However, as both imine formation and amidophosphate protonation appear essential to the mechanism of phosphorylation, we suspected that the reaction could be promoted at lower pH. Accordingly, we observed an optimal rate of conversion at pH 4 that gave 90–96% phosphorylation of **2** or **3** (25 mM) at ambient temperature in 2 h (Supplementary Fig. 4).

On incubating **3-2P** (80 mM) in phosphate (0.5 M) we observed facile dehydration to **9** (74%, one day, 60 °C; Supplementary Fig. 21). Furthermore, direct synthesis of **9** (50%) was observed during the stoichiometric phosphorylation of **3** in phosphate buffer (pH 4, 0.5 M, 60 °C, five days) to directly establish the high-energy phosphoenol moiety of **1** under mild aqueous conditions.

Having demonstrated the synthesis of **9** from **3**, we next incubated glycolaldehyde phosphate (**2-P**) with formaldehyde. At pH 10.7, **2-P** is converted to **3-2P** (66%) over the course of six days³³; further incubation (60 °C, pH 10, three days) led to elimination to furnish **9** (61%; Supplementary Fig. 22). Alternatively, incubation in phosphate buffer (0.5 M, 60 °C, pH 7, 30 h) led to the direct synthesis of **9** (40%; Supplementary Fig. 23) from formaldehyde and **2-P**. Demonstrating the phosphorylation of both prebiotic aldehyde precursors of RNA can lead, even at neutral pH, to high-energy phosphoenol ether **9**.

Phosphoenol pyruvate (1). Struck by the simplicity and efficiency of this synthesis and the structural relationship between **9** and **1**, we next turned our attention to the oxidation of **9**. The range of

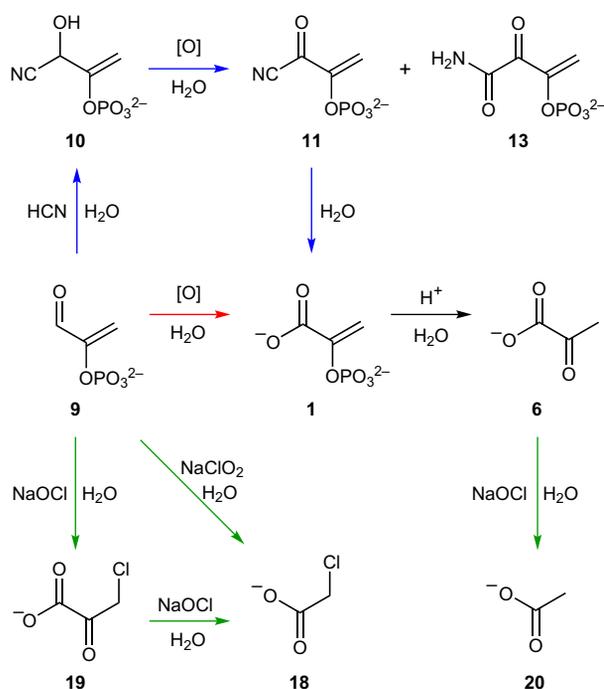


Figure 2 | Oxidation of phosphoenol pyruvaldehyde (9) and oxidative decarboxylation of pyruvates (6 and 19). Oxidant ([O]) and oxidation conditions are provided in Table 1. Blue arrows: aqueous umpolung oxidation of phosphoenol pyruvaldehyde (9) to phosphoenol pyruvate (1) via cyanohydrin (10). Red arrow: direct oxidation of phosphoenol pyruvaldehyde (9) to phosphoenol pyruvate (1). Black arrow: quantitative phosphoenol pyruvate (1) hydrolysis. Green arrows: oxidative decarboxylation of α-ketoacids (6 and 19) yielding acetates (20 and 18), respectively.

potential conditions and oxidants available under prebiotic constraints is unknown and probably highly varied. Therefore, rather than develop a model geochemical scenario for aldehyde oxidation, we sought to demonstrate a range of low-temperature, aqueous model oxidations that operate through significantly varied mechanistic pathways across a broad pH range (pH 3–13).

Activation towards oxidation through cyanohydrin formation³⁵ initially appealed to us because high concentrations of hydrogen cyanide are implicated in nucleotide, sugar and amino acid abiogenesis^{1,5,8,12} and are observed in abiotic high abundance^{36,37}. Incubation of **9** (33–200 mM) with sodium cyanide (167 mM to 1 M) led to rapid (<15 min) and quantitative conversion to cyanohydrin **10** in water (Supplementary Fig. 33). Because iron is likely to be prebiotically abundant²¹ and clearly has biological importance³⁸, we first investigated ferricyanide ([Fe(CN)₆]³⁻) oxidation of **10**. Ferricyanide is a prebiotically plausible model oxidant^{12,39–42} that contains low-spin iron, which is easily reduced to the ferrocyanide ion ([Fe(CN)₆]⁴⁻) in a reversible redox couple and can be photochemically regenerated from ferrocyanide^{12,39,41}. We were pleased to observe oxidation of **10** by ferricyanide between pH 11 and 13 (Fig. 2 and Supplementary Fig. 36). However, expedient oxidation required significantly elevated pH and became limited by alkaline nitrile hydrolysis.

To further improve the oxidation reaction and expand the scope of potential model oxidants, we next investigated manganese, which is the most common heavy metal in the Earth's crust (0.1 wt%) after iron⁴³, and readily adopts +2, +3, +4, +6 and +7 oxidation states, although naturally occurring systems are dominated by the +2, +3 and +4 oxidation states. Manganese is abundant in most geological systems, covers 10–30% of the deep Pacific Ocean floor⁴⁴, and is readily found in minerals such as birnessite, pyrolusite, romanechite

and todorokite^{43,44}. Additionally, phylogenetic analysis suggests that manganese may have been the active centre of the earliest enzymes³⁸, and manganese concentrations are thought to have been as high as 50 mM in the oceans of the early Earth⁴⁴. Although the global availability of redox-sensitive Mn(IV) before atmosphere oxygenation has been questioned^{45–47}, the deviation of local environments and near-surface oxygen fugacity from global values⁴⁸ (for example, owing to photo-oxidation, ionization, volcanic outgassing, meteorite impacts and atmospheric water dissociation and hydrogen escape) remains opaque⁴⁹. However, stimulated by the recent elucidation of facile prebiotically plausible sugar, amino acid and lipid syntheses mediated by photo-oxidation of transition metals in aqueous solution^{12,39,41}, and recognizing the prebiotically plausible (photo-)oxidation of Mn(II) to Mn(III) and Mn(IV)^{22,50}, and disproportionation of Mn(III) to Mn(II) and MnO₂ in the absence of complexing ligands, manganese dioxide (MnO₂) was investigated as the most constitutionally simple Mn(IV) oxidant. Interestingly, we observed near-quantitative conversion of **9** (100 mM) to **1** (93%) on incubation with potassium cyanide (500 mM) and manganese dioxide (20 equiv.) after 2 h, which demonstrates the facile synthesis of **1** from both **2** and **3** (Fig. 2 and Supplementary Fig. 37).

We next investigated the direct chemical oxidation of **9** to **1**. The relatively low bond-dissociation energy of an aldehyde hydrogen atom (360–370 kJ mol⁻¹)⁵¹, the probably high prebiotic availability of iron²¹ and prebiotic plausibility of a hydroxyl-radical-mediated oxidation^{24,52} suggested the investigation of Fenton oxidation. Interestingly, incubation of **9** (25 mM) with ferrous chloride (25 mM) and hydrogen peroxide (100 mM) at pH 7–11 furnished **1** (30%, 25 °C, pH 10; Fig. 2 and Table 1). However, these aggressive oxidation conditions also led to the concomitant degradation of **1** to formate and glycolate (**12**).

To make further improvements to the direct oxidation, guided by the conditions of nucleotide synthesis^{14,18}, the prevalence of dry heating and wet-dry cycles in prebiotic synthesis^{12,14,18,29,53}, and the simple photochemical synthesis and high abiotic abundance of the chlorate family of molecules^{54–57}, we were drawn to investigate the role of these abiotic oxidants. Chlorates are ubiquitously formed by stratospheric oxidation of chlorides on Earth and are widely distributed by both wet and dry deposition⁵⁷. Although ozone probably plays a key role on Earth today, chlorates are also widely distributed throughout the solar system (on Mars, the Moon and carbonaceous chondrites including Murchison and Fayetteville). Little is yet known regarding the origin of chlorates on Mars, but the known terrestrial syntheses do not account for their abundance or distribution throughout the solar system^{54,55,57}. Therefore, although the arid environments implicated in chlorate synthesis are debated and the delivery of chlorates to prebiotic chemistry remains unknown, there are clearly abiotic mechanisms for the accumulation of chlorates, which, if followed by wetting, could either mobilize deposits or deliver materials to chlorate-rich locations. Moreover, the disproportionation of atmospheric ClO₂ in aqueous solutions would directly deliver chlorite^{56,58}, which is an excellent mild oxidant for aldehydes in water, with a pH reactivity profile that is an ideal match for **8**-mediated phosphorylation⁵⁹. Therefore, despite the uncertain provenance of chlorates in prebiotic chemistry, the abundance of chlorates in the solar system and their capability to act as mild and highly selective nucleophilic oxidants in water meant that we judged chlorates to be an interesting model oxidant for **9**. Chlorite oxidation of aldehydes can be mediated at low pH (pH 3–6) in the presence of a phosphate buffer, which allows a transition away from high-pH cyanide-catalysed oxidations. On incubation of **9** (70 mM) with chlorite (100 mM) and a hypochlorite scavenger such as methionine (140 mM) or dimethylsulfoxide (DMSO; 140 mM), **9** is quantitatively oxidized to **1** (Supplementary Figs 39 and 40).

Table 1 | Aldehyde oxidation conditions and product yields after 2 h incubation of the substrate and specified oxidant ([O]) in water or phosphate buffer.

Substrate	R ¹	R ²	pH	[O] (equiv.)	Additive (equiv.)	Product	Yield (%)
9	OPO ₃ ²⁻	=CH ₂	13	K ₃ Fe(CN) ₆ (10)	HCN (5)	1 (13)	50 (13)
9	OPO ₃ ²⁻	=CH ₂	9	MnO ₂ (20)	HCN (5)	1	93
9	OPO ₃ ²⁻	=CH ₂	10	FeCl ₂ (1)	H ₂ O ₂ (4)	1 (12)	30 (16)
9	OPO ₃ ²⁻	=CH ₂	4	NaClO ₂ (1.4)	DMSO (2)	1	Quant.
9	OPO ₃ ²⁻	=CH ₂	4	NaClO ₂ (2)	-	18	Quant.
3-2P	OPO ₃ ²⁻	CH ₂ OH	4	NaClO ₂ (1.4)	DMSO (2)	4-2P	Quant.
3-3P	OH	OPO ₃ ²⁻	4	NaClO ₂ (1.4)	DMSO (2)	4-3P	94
3	OH	CH ₂ OH	4	NaClO ₂ (1.4)	DMSO (2)	4	93
2-P	H	OPO ₃ ²⁻	4	NaClO ₂ (1.4)	DMSO (2)	12-P	Quant.
2	H	OH	4	NaClO ₂ (1.4)	DMSO (2)	12	Quant.
6	-	-	4	NaOCl (2)	-	20	Quant.

*Oxidation of phosphoenol pyruvaldehyde (9) to phosphoenol pyruvate (1); †oxidation of glycolaldehyde (2) and glyceraldehyde (3) derivatives to glycolic acid (12) and glyceric acid (4) derivatives, respectively; ‡oxidative decarboxylation of pyruvate (6) to acetate (20). An expanded table of conditions and yields is provided in Supplementary Table 1. Quant., quantitative.

Glyceric acid 2-phosphate (4-2P) and glycolic acid phosphate (12-P). Gratifyingly, the oxidation by chlorite is general and provides quantitative conversion of 2 and 2-P to glycolic acid (12) and glycolic acid phosphate (12-P), respectively (Table 1, Supplementary Figs 41 and 42). Phosphate 12-P is a key intermediate of photorespiration in plants¹⁵. Consequently, chlorite is also an ideal oxidant to convert 3-2P to 4-2P. Following reaction of 3-2P with stoichiometric chlorite, we observed quantitative oxidation to 4-2P. Furthermore, upon phosphorylation of 3 with 8 followed by incubation at 60 °C in phosphate buffer (0.5 M) for 5 h and addition of chlorite/DMSO (1:1.4), we observed quantitative conversion of 3 to 1 and 4-2P (1:1) and thus realized the direct divergent synthesis of two key intermediates of the universally conserved glycolysis pathway (Fig. 3)¹⁷.

Glyceric acid 3-phosphate (4-3P) and phosphoserine (5-3P). Interestingly, the hydrolysis that limited the ferricyanide oxidation of cyanohydrin 10 suggested direct prebiotic access to 4-3P and phosphoserine (5-3P) through cyanohydrin and aminonitrile hydrolysis, which provides access to a fourth component of the glycolysis pathway and a direct link to serine metabolism, respectively. On incubation of 2-P with sodium cyanide, we observed smooth and quantitative conversion to cyanohydrin 14 (Supplementary Fig. 46), which is readily hydrolysed to 15 (27%, 750 mM phosphate, pH 7, three days, 75 °C; Supplementary Fig. 54) and 4-3P (31%, 750 mM phosphate, pH 12, 75 °C, five days; Supplementary Fig. 59) with absolute control over the phosphate regiochemistry (Fig. 4).

Furthermore, incubation of 2-P with ammonium cyanide led to the near-quantitative synthesis of glycolaldehyde phosphate aminonitrile (16; Supplementary Fig. 66). We found that the hydrolysis of aminonitrile 16 to amide 17 was aldehyde-catalysed; the cyanide carbon atom was mildly and efficiently locked into the carbon frame work of serine by a small excess of 2-P (<5%)⁶⁰. This reactivity is only observed for aminonitrile hydrolysis, not cyanohydrin hydrolysis. The aldehyde-catalysed hydrolysis occurs via 5-*exo-dig* hemiaminal cyclization and is quenched by excess cyanide. It is therefore of note that we observed no evidence of intramolecular phosphate-assisted hydrolysis, despite the ostensive potential for 6-*exo-dig* addition to the nitrile, which would stall 5-3P synthesis at glycolic acid 4-3P and, as a result, we observed efficient conversion of 2-P to 5-3P (36%; Supplementary Fig. 83).

Pyruvate (6). Compound (1) is highly stable at high pH (6% hydrolysis to 6 observed over 2 days at pH 10 and 60 °C; Supplementary Fig. 93). However, 1 is readily hydrolysed to 6 at lower pH in quantitative yield (pH 4–7, 2 days, 60 °C; Supplementary Fig. 94). Hydrolysis thus provides access to a second linchpin of contemporary metabolism with excellent control and efficiency in water. Accessing 6 from 1 delivers improved yields (up to 69% from 3, over four steps) relative to previously reported multiple-step syntheses (984-fold and 7-fold)^{19,20} and a comparable yield to the single-step oxidation of lactic acid (70%)²². More importantly, the pathway mimics a metabolic trajectory with absolute specificity under mild aqueous conditions from simple prebiotic aldehydes.

Acetate (20). It is of significant interest that during our investigation of phosphoenol pyruvaldehyde (9) oxidation with chlorite that in the absence of a hypochlorite scavenger we observed over-oxidation to give chloroacetate (18; >98% yield with 2 equiv. chlorite; Supplementary Fig. 95). It is likely that 18 was accessed by electrophilic chlorination and oxidative decarboxylation of α -ketoacid 19 (Fig. 2), which is supported by the observed quantitative oxidative decarboxylation of 6 to yield 20 (ref. 61). Pyruvates 1 and 6 are both key nodes of central metabolism, but one of the most important metabolic connections is achieved by oxidative decarboxylation of 6 to access acetyl coenzyme A (acetyl CoA)⁶², ketogenesis and the citric acid cycle (Fig. 1)^{6–9}. The direct liberation of 20 by oxidation of 6 suggests an essential prebiotic link between C₃ and C₂ metabolism within our system; an investigation of (prebiotic) pathways to acetyl transfer reagents^{39,41} and C₂ metabolism is currently under way in our laboratory.

Discussion

Glycolysis is one of the most highly conserved metabolic pathways of life and it is instrumental to the biosynthesis of amino acids, sugars, nucleotides and lipids, phosphorylation and the entry to the Krebs (citric acid) cycle^{2,16,17}. The interrelated syntheses of 1, 4, 4-2P, 4-3P, 5-3P, 6, 12, 12-P and 20 demonstrated herein suggest that prebiotic availability, as well as metabolic versatility, may have positioned glycolysis at the heart of modern cellular metabolism. The generational simplicity of this network also suggests that α -phosphorylation and oxidation warrant further investigation with respect to the essential roles they may have played in coordinating the first steps of life on Earth. Specifically, the remarkable chemical control that α -phosphorylation achieves

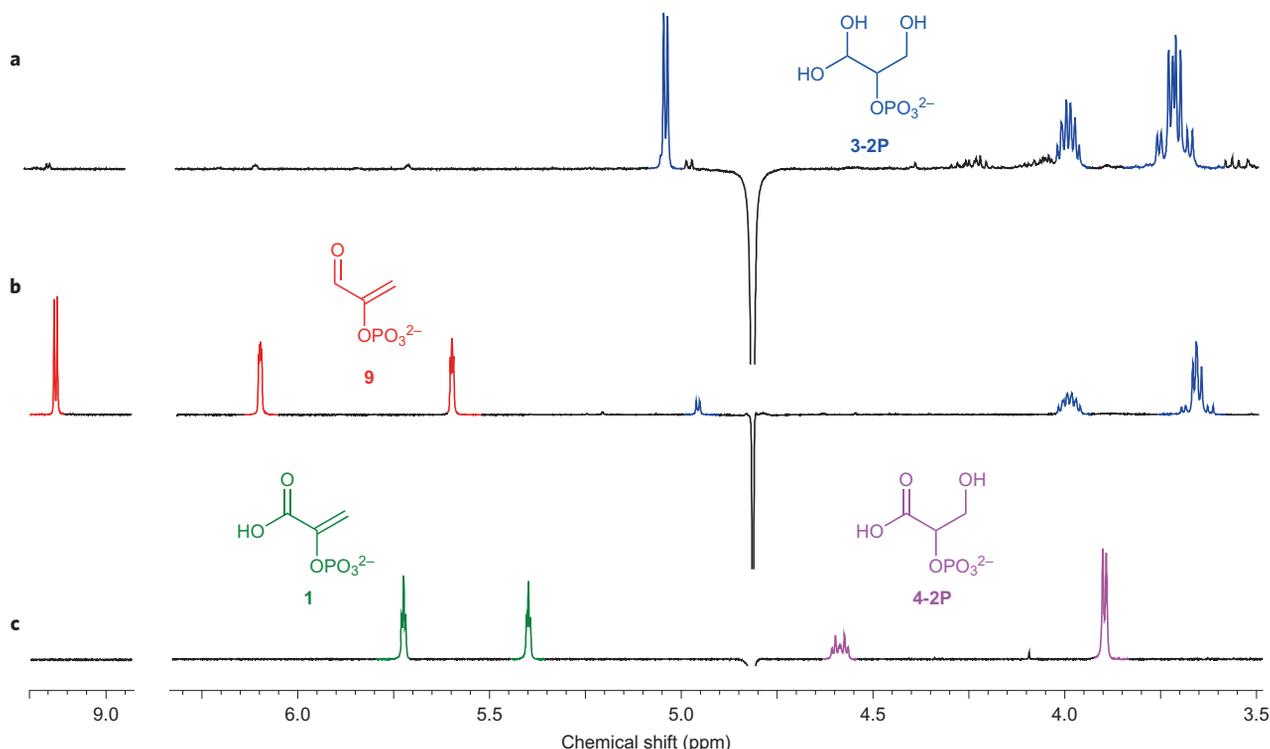


Figure 3 | ^1H NMR spectra (600 MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}$ 9:1, 25 °C) showing quantitative divergent synthesis of phosphoenol pyruvate (1)/glyceric acid 2-phosphate (4-2P) in 0.5 M phosphate buffer. **a, Spectrum showing the incubation of glycerinaldehyde (3; 25 mM) and diamidophosphate (8; 50 mM) at initial pH 4 and 25 °C for 1 h in phosphate buffer, which yields glycerinaldehyde-2-phosphate (3-2P; blue). **b**, Spectrum to show incubation of glycerinaldehyde-2-phosphate (3-2P) in pH 7 phosphate buffer at 60 °C for 5 h, yielding a 1:1 mixture of glycerinaldehyde-2-phosphate (3-2P; blue) and phosphoenol pyruvaldehyde (9; red). **c**, Spectrum showing the quantitative concomitant oxidation of glycerinaldehyde-2-phosphate (3-2P) and phosphoenol pyruvaldehyde (1) to glyceric acid 2-phosphate (4-2P) and phosphoenol pyruvate (9) by chlorite/DMSO (1:1.4) in pH 4 phosphate buffer at 25 °C after 1 h.**

in reconstituting the pathway of glycolysis indicates that a transition from α -phosphorylation to terminal phosphorylation may greatly simplify the transition from abiotic reactions to contemporary metabolism. The essential and ubiquitous roles of 4-2P and 1 in modern biochemistry may be vestiges of an earlier reactivity upon which modern glycolysis/gluconeogenesis are built from the 'bottom up'¹⁷. Although modern metabolism enters triose glycolysis through glycerinaldehyde 3-phosphate (3-3P), 3-3P is unstable with respect to its ketose isomer (21-P) and is predisposed to undergo elimination to methyl glyoxal with concomitant loss of phosphate activation. Biochemically, the isomerization of triose sugar phosphates is mediated to the limit of diffusion by triose-phosphate isomerase, but at equilibrium, the formation of 21-P is still favoured 20:1 (ref. 63). It is of note, however, that we have demonstrated that glycerinaldehyde 2-phosphate (3-2P) blocks these deleterious reaction pathways without the requirement for enzymatic control. Furthermore, by retaining aldehyde activation to E1cb-type elimination, 3-2P readily undergoes a predisposed elimination to access the high-energy phosphoenol moiety, which is kinetically prohibited for 4-2P and its amide derivatives.

Aldehyde oxidations are commonplace in chemistry, and a variety of oxidants can be used in the facile oxidation of 9 to 1. The conditions on the early Earth are far from certain, and we have not sought to specify geochemical constraints for aldehyde oxidation. We have, however, demonstrated *umpolung* and conventional polarity oxidation, nucleophilic, electrophilic and radical oxidation, heterogeneous and homogenous oxidation, and both outer- and inner-sphere oxidation of 9 to give 1, and consequently suspect that the facile oxidation of 9 in water is extremely general and thus prebiotically plausible. Nonetheless, further investigation of redox-sensitive elements (including Mn, Fe, Co, Ni and Cu) on

the early Earth is warranted before unification of prebiotic and geochemical redox strategies⁴⁹.

It is of note that chlorite oxidation is not only quantitative but that its reactivity profile is also pH matched with the maximal reactivity of the prebiotic phosphorylating agent 8 (optimal α -phosphorylation at low pH). At the concentrations investigated (25–500 mM), background hydrolysis does not compete with Schiff base-mediated phosphoryl transfer; however, the increased efficiency of Schiff base formation and increased proportion of protonated amidophosphate leads to a significantly improved rate of phosphorylation. This pH profile also matches the pH requirements (pH < 7) for controlled ribonucleotide assembly by a leading model for prebiotic synthesis¹⁸. Therefore, it is also interesting to note that an acidic drying phase is required during ribonucleotide synthesis to induce phosphorylation and stereocontrolled rearrangement^{14,18}, and these arid, low-pH conditions have remarkable parity with the proposed environmental pathways to chlorine oxides^{54–57}. However, the relevance of oxidized chlorine reagents in prebiotic chemistry can certainly be questioned, and further investigation of the potential to accumulate and transport chlorates^{55,57} and chlorine dioxide^{56,58} would be needed to verify facile prebiotic access to 4-2P, which we only found to be efficient by chlorite oxidation. It is also of note that although 4-2P is a biological intermediate of glycolysis, it is not an intermediate of our prebiotic network; rather, it is a terminal node (Fig. 1; blue arrows), and without the aldehyde activation exploited to yield 9, it is difficult to envisage elimination to deliver 1 (Supplementary Fig. 1).

It is striking that the prebiotically plausible nucleotide synthesis reported by Powner *et al.*¹⁸ is achieved, like prebiotic entry to glycolysis, through a regiochemical phosphorylation isomer of the canonical form. Ribonucleotide synthesis¹⁸ occurs via 2',3'-cyclic phosphates rather than terminal 5'-phosphates, and triose glycolysis is accessed

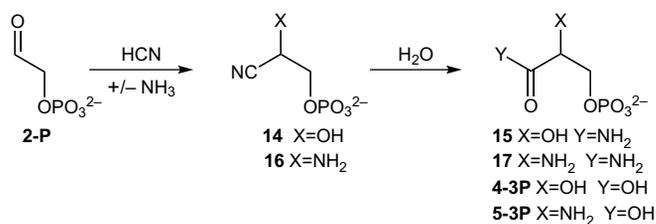


Figure 4 | Divergent synthesis of glyceric acid 3-phosphate (4-3P) and phosphoserine (5-3P) from glyceraldehyde-2-phosphate (2-P). By incubating 2-P with hydrogen cyanide, cyanohydrin (14) or aminonitrile (16) (in the presence of ammonia) can be formed quantitatively. Subsequent nitrile hydrolysis furnishes 15 and 4-3P with absolute control over the phosphate regiochemistry.

via 3-2P rather than terminal 3'-phosphate 3-3P. The parity of these model reaction conditions and the ostensive regiochemical switch from α -phosphorylation both suggest that an integrated and unifying system to concurrently establish nucleotide synthesis and triose metabolism may have been fundamentally important in constructing the first steps towards contemporary metabolism.

Methods

Phosphorylation. Aldehyde (25–500 mM) and sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS, 2.5 mM) were dissolved in phosphate buffer (500 mM, H₂O/D₂O 9:1, pH 4–7). Diamidophosphate (8; 1–4 equiv.) was added, and the solution was incubated at ambient temperature. ¹H and ³¹P NMR spectra were periodically acquired, and phosphorylation was quantified with respect to the internal DSS standard. Aldehyde phosphates were isolated on a preparative scale by ion-exchange chromatography (Dowex-1 \times 8, HCO₃⁻ form, eluent 0 \rightarrow 0.5 M Et₃NH₂HCO₃) and barium (Ba(OAc)₂, 1 equiv.) or calcium (Ca(OAc)₂, 1 equiv.) salt precipitation from aqueous ethanol (EtOH/H₂O 4:1) or aqueous acetone (Me₂CO/H₂O 1.2:1). Sodium salts were acquired by ion-exchange chromatography (Dowex-50 W \times 8, Na⁺ form) followed by lyophilization.

Glycolaldehyde 2-phosphate (2-P). Yield: 90% (4 equiv. 8, pH 4, 25 mM, 4 h, room temperature (RT)). M.p. 135–145 °C (dec). ¹H NMR (600 MHz, D₂O) δ _H 5.11 (1H, t, J = 4.5 Hz, (C1)-H), 3.75 (2H, dd, J = 7.4, 4.5 Hz, (C2)-H₂). ¹³C NMR (151 MHz, D₂O) δ _C 89.9 (d, J = 7.2 Hz, C1), 67.4 (d, J = 4.9 Hz, C2). ³¹P NMR (162 MHz, D₂O) δ _P 4.47 (t, J = 7.4 Hz). m/z (ESI⁻): 139 (100%, [M-H]⁺).

Glyceraldehyde 2-phosphate (3-2P). Yield: 96% (4 equiv. 8, pH 4, 25 mM, 4 h, RT). M.p. 110–120 °C (dec). ¹H NMR (600 MHz, D₂O) δ _H 5.05 (1H, d, J = 3.8 Hz, (C1)-H), 4.09 (2H, dddd, J = 8.8, 5.7, 4.5, 3.8 Hz, (C2)-H), 3.78 (1H, ABX, J = 12.1, 4.5 Hz, (C3)-H_a), 3.75 (1H, ABX, J = 12.1, 5.7 Hz, (C3)-H_b). ¹³C NMR (151 MHz, D₂O) δ _C 90.1 (d, J = 3.6 Hz, C1), 77.5 (d, J = 5.5 Hz, C2), 62.3 (d, J = 4.4 Hz, C3). ³¹P NMR (161 MHz, D₂O) δ _P 3.6 (d, J = 8.8 Hz). m/z (ESI⁺): 215 (100%, [M-H]⁺). High-resolution mass spectrometry (HRMS) ([C₃H₅O₆PNa₂+H]⁺) calcd 214.9692, found 214.9690.

Phosphoenolpyruvaldehyde (9). Method A: Glyceraldehyde 2-phosphate (3-2P; 70 mM) and pentaerythritol (10 mM) were dissolved in phosphate buffer (500 mM, H₂O/D₂O 9:1, pH 7) and incubated at 60 °C. NMR spectra were periodically acquired and dehydration was quantified with respect to the internal pentaerythritol standard. A maximum yield of 9 (74%) with residual 3 (12%) was observed at 23 h.

Method B: Glycolaldehyde 2-phosphate (2-P; 80 mM), formaldehyde (800 mM) and DSS (15 mM) were dissolved in phosphate buffer (500 mM, H₂O/D₂O 9:1, pH 7) and incubated at 60 °C. NMR spectra were periodically acquired and the yield of 9 was quantified with respect to the internal DSS standard. A maximum yield of 9 (40%) was observed at 30 h.

Compound 9 was isolated on preparative scale by barium (Ba(OAc)₂, 1 equiv.) salt precipitation from aqueous ethanol (EtOH/H₂O 4:1). Sodium salts were acquired by ion-exchange chromatography (Dowex-50W \times 8, Na⁺ form) followed by lyophilization. An analytical sample was prepared by reversed-phase HPLC (isocratic gradient of 0.1% NEt₃H₂CO₃ in water, retention time = 2.68 min). M.p. 100–115 °C (decomposed). Infrared (IR, cm⁻¹, powder) 1,686, 1,618, 1,562. ¹H NMR (600 MHz, D₂O) δ _H 9.25 (1H, d, J = 3.0 Hz, (C1)-H), 6.17 (1H, dd, J = 2.8, 1.8 Hz, (C3)-H_a), 5.84 (1H, dd, J = 2.8, 2.0 Hz, (C3)-H_b), 5.29 (0.04H, s, (C1)-H^{hydrate}), 4.87 (0.04H, ap^t, J = 2.1 Hz, (C3)-H^{hydrate}), 4.85 (1H, ap^t, J = 2.1 Hz, (C3)-H^{hydrate}), 3.18 (7H, q, J = 7.3 Hz, NCH₂CH₃), 1.26 (10.5H, t, J = 7.4 Hz, NCH₂CH₃). ¹³C NMR (151 MHz, D₂O) δ _C 191.8 (d, J = 6.1 Hz, C1), 152.9 (d, J = 7.2 Hz, C2), 152.7 (C^{hydrate}), 119.5 (d, J = 3.9 Hz, C3), 93.6 (C^{hydrate}), 87.3 (C^{hydrate}), 47.3 (NCH₂CH₃), 8.9 (NCH₂CH₃). ³¹P NMR (161 MHz, D₂O) δ _P -4.1 (ap^s, P^{hydrate}), -4.2 (ap^s, P). m/z (ESI⁺): 197 (100%, [M + H]⁺). HRMS ([C₃H₅O₅PNa₂+H]⁺) calcd 196.9586, found 196.9583.

Aldehyde oxidation. Method A: Aldehyde (30–100 mM), sodium cyanide (5 equiv. or none) and an internal standard (DSS, pentaerythritol, or acetate (20)) were dissolved in H₂O/D₂O (9:1) or D₂O and adjusted to the specified pH/pD with 1 M HCl/NaOH. Cyanohydrin formation was monitored by ¹H NMR spectroscopy and, after equilibration (0.1–1 h), the oxidant (K₃Fe(CN)₆, or MnO₂) was added. The solution was incubated at ambient temperature and the specified pH for 1–2 h. The solution was filtered and NMR spectra were acquired. The carboxylic acid product was confirmed by sample spiking and the yield (Table 1 and Supplementary Table 1) was quantified with respect to the internal standard.

Method B: Phosphoenolpyruvaldehyde (9; 25 mM) and ferrous chloride (0–2 equiv.) were dissolved in phosphate buffer (0.75 M, H₂O, pH 10) and stirred rapidly at 0 °C. Hydrogen peroxide (30% wt/wt, 1–4 equiv.) was added and the reaction was stirred at 0–15 °C for 30 min. The reaction was warmed to ambient temperature over 30 min and the solids removed by centrifugation. The supernatant was either combined with D₂O (10% vol/vol) or lyophilized and re-dissolved in D₂O (1 ml). NMR spectra were acquired, and the formation of 1 was confirmed by sample spiking. The yield (Table 1 and Supplementary Table 1) was quantified with respect to an internal standard (20) and titration of an external standard (DSS).

Method C: Aldehyde (70–350 mM), an internal standard (DSS or 20) and the specified additive (none, NH₄Cl, H₂O₂, DMS, DMSO, sulfamic acid or L-methionine) were dissolved in phosphate buffer (60 mM, D₂O, pH 4–7). Sodium chlorite (5 M, 1.4 equiv.) was added in five portions over 1 h at 0 °C, then the solution was warmed to ambient temperature and NMR spectra were acquired. The carboxylic acid product was confirmed by sample spiking, and the yield (Table 1 and Supplementary Table 1) was quantified with respect to the internal standard.

Phosphoenolpyruvaldehyde cyanohydrin (10). ¹H NMR (400 MHz, D₂O, pD 9.5) δ _H 5.10 (1H, s, CH), 4.95 (1H, t, J = 1.8 Hz, CH₂), 4.83 (1H, t, J = 1.8 Hz, CH₂). ³¹P NMR (161 MHz, D₂O, pH 9.5) δ _P 0.2 (ap^s).

Phosphoenolpyruvate (1). ¹H NMR (400 MHz, D₂O, pD 4) δ _H 5.78 (1H, t, J = 2.2 Hz, (C3)-H_a), 5.44 (1H, t, J = 2.2 Hz, (C3)-H_b). ¹³C NMR (151 MHz, D₂O) δ _C 168.1 (d, J = 7.2 Hz, C1), 146.2 (d, J = 7.2 Hz, C2), 108.6 (d, J = 3.9 Hz, C3). ³¹P NMR (161 MHz, D₂O) δ _P -4.0 (ap^s).

Glyceric acid (4). ¹H NMR (400 MHz, D₂O, pD 4) δ _H 4.28 (1H, dd, J = 4.5, 3.8 Hz, CH), 3.81 (1H, ABX, J = 12.1, 3.8 Hz, CH₂), 3.78 (1H, ABX, J = 12.1, 4.5 Hz, CH₂). ¹³C NMR (151 MHz, D₂O) δ _C 176.8 (C1), 72.3 (C2), 64.0 (C3).

Glyceric acid 2-phosphate (4-2P). ¹H NMR (400 MHz, D₂O, pD 4) δ _H 4.61 (1H, dt, J = 9.5, 3.6 Hz, CH), 3.89 (2H, d, J = 3.6 Hz, CH₂). ¹³C NMR (151 MHz, D₂O) δ _C 175.0 (d, J = 3.9 Hz, C1), 75.9 (d, J = 5.0 Hz, C2), 63.7 (d, J = 5.0 Hz, C3). ³¹P NMR (161 MHz, D₂O) δ _P 0.1 (d, J = 9.5 Hz).

Glycolic acid (12). ¹H NMR (400 MHz, D₂O, pD 4) δ _H 4.13 (2H, s, CH₂). ¹³C NMR (151 MHz, D₂O) δ _C 177.4 (C1), 60.2 (C2).

Glycolic acid phosphate (12-P). ¹H NMR (400 MHz, D₂O, pD 4) δ _H 4.37 (2H, d, J = 8.0 Hz, CH₂). ¹³C NMR (151 MHz, D₂O) δ _C 175.1 (C1), 63.2 (d, J = 5.0 Hz, C2). ³¹P NMR (161 MHz, D₂O) δ _P 0.0 (ap^s).

Chloroacetic acid (18). ¹H NMR (400 MHz, D₂O) δ _H 4.02 (2H, s, CH₂).

Acetic acid (20). ¹H NMR (400 MHz, D₂O) δ _H 1.96 (3H, s, CH₃).

Pyruvate (6). Phosphoenolpyruvate (1; 63 mM) and pentaerythritol (16 mM) were dissolved in H₂O/D₂O (9:1, pH 4–10), or phosphate buffer (0.5 M, H₂O/D₂O, 9:1, pH 7) and incubated at 60 °C for 50 h. ¹H NMR spectra were acquired periodically and the yield was quantified with respect to the internal pentaerythritol standard. Yield 95–97% (pH 4 or phosphate buffer, 50 h, 60 °C). ¹H NMR (600 MHz, H₂O/D₂O 9:1, pH 4) δ _H 2.27 (3H, s, CH₃^{ket}), 1.44 (0.7H, s, CH₃^{hydrate}). ¹³C NMR (151 MHz, H₂O/D₂O 9:1, pH 4) δ _C 205.2 (C1^{ket}), 177.2 (C1^{hydrate}), 170.5 (C2^{ket}), 93.9 (C2^{hydrate}), 27.1 (C3^{ket}), 25.9 (C3^{hydrate}).

Cyanohydrin/aminonitrile hydrolysis. Aldehyde (200 mM), sodium cyanide (0.95–5 equiv.), ammonium chloride (0–5 equiv.) and DSS (20–25 mM) were dissolved in H₂O/D₂O (9:1; adjusted to pH/pD 2, 7 or 9.5 with 4 M HCl or 4 M NaOH) or in phosphate buffer (750 mM, H₂O/D₂O 9:1, pH 7). Cyanohydrin and aminonitrile formation were monitored by NMR spectroscopy. Upon equilibration (5–100 h), the solution was incubated at 25, 60 or 75 °C (3–6 days) and NMR spectra were periodically acquired to monitor nitrile/amide hydrolysis. The solution was adjusted to pH/pD 12 with 4 M NaOH/NaOD and incubation was continued (5–30 h). NMR spectra were periodically acquired to monitor amide hydrolysis.

Glycolaldehyde phosphate cyanohydrin (14). Yield 94–98% (5 h, pH 2–9.5, RT). ¹H NMR (400 MHz, H₂O/D₂O 9:1, pH 7) δ _H 4.75 (obs., CH), 3.95 (2H, m, CH₂). ¹³C NMR (151 MHz, H₂O/D₂O 9:1, pH 7) δ _C 120.1 (C1), 65.7 (d, J = 4.4 Hz, C3), 62.3 (d, J = 6.6 Hz, C2). ³¹P NMR (161 MHz, H₂O/D₂O 9:1, pH 7) δ _P 3.8 (ap^s).

Glyceramide 3-phosphate (15). Yield 27% (phosphate buffer, 3 d, 75 °C). ¹H NMR (600 MHz, H₂O/D₂O 9:1, pH 7) δ_H 4.25 (1H, dd, *J* = 5.2, 3.2 Hz, CH), 4.00 (1H, ABXY, *J* = 11.6, 7.6, 3.2 Hz, CH₂), 3.92 (1H, ABXY, *J* = 11.6, 7.6, 5.2 Hz, CH₂). ¹³C NMR (151 MHz, H₂O/D₂O 9:1, pH 7) δ_C 179.4 (C1), 73.5 (d, *J* = 7.2 Hz, C2), 67.5 (d, *J* = 4.4 Hz, C3). ³¹P NMR (161 MHz, H₂O/D₂O 9:1, pH 7) δ_P 4.4 (t, *J* = 7.6 Hz).

Glyceric acid 3-phosphate (4-3P). Yield 31% (phosphate buffer, 3 days, 75 °C, then pH 12, 5 h, 75 °C). ¹H NMR (600 MHz, H₂O/D₂O 9:1, pH 7) δ_H 4.11 (1H, dd, *J* = 6.1, 2.8 Hz, CH), 3.93 (1H, ABXY, *J* = 11.2, 5.8, 2.8 Hz, CH₂), 3.80 (1H, ABXY, *J* = 11.2, 6.1, 5.8 Hz, CH₂). ¹³C NMR (151 MHz, H₂O/D₂O 9:1, pH 7) δ_C 179.6 (C1), 73.5 (C2), 67.5 (d, *J* = 4.4 Hz, C3). ³¹P NMR (161 MHz, H₂O/D₂O 9:1, pH 7) δ_P 4.0 (t, *J* = 5.8 Hz).

Glycolaldehyde phosphate aminonitrile (16). Yield 90% (pH 9.5, 100 h, RT). ¹H NMR (600 MHz, H₂O/D₂O 9:1, pH 9.5) δ_H 4.09 (1H, t, *J* = 5.3 Hz, CH), 3.91 (1H, ABXY, *J* = 10.3, 5.9, 5.3 Hz, CH₂), 3.85 (1H, ABXY, *J* = 10.3, 5.9, 5.3 Hz, CH₂). ¹³C NMR (151 MHz, H₂O/D₂O 9:1, pH 9.5) δ_C 122.0 (C1), 65.4 (d, *J* = 4.0 Hz, C3), 44.5 (d, *J* = 8.4 Hz, C2). ³¹P NMR (161 MHz, H₂O/D₂O 9:1, pH 9.5) δ_P 3.8 (t, *J* = 5.9 Hz).

Phosphoserinamide (17). Yield 65% (pH 9.5, 3 d, RT). ¹H NMR (400 MHz, H₂O/D₂O 9:1, pH 9.5) δ_H 3.86 (1H, ABXY, *J* = 11.6, 5.4, 5.2 Hz, CH₂), 3.84 (1H, ABXY, *J* = 11.6, 5.4, 5.2 Hz, CH₂), 3.65 (1H, t, *J* = 5.2 Hz, CH). ¹³C NMR (151 MHz, H₂O/D₂O 9:1, pH 9.5) δ_C 178.5 (C1), 66.5 (d, *J* = 4.4 Hz, C3), 55.4 (d, *J* = 7.7 Hz, C2). ³¹P NMR (161 MHz, H₂O/D₂O 9:1, pH 7) δ_P 4.1 (t, *J* = 5.4 Hz).

Phosphoserine (5-3P). Yield 36% (pH 9.5, 3 days, RT, then pH 12, 3 days, 75 °C). ¹H NMR (400 MHz, H₂O/D₂O 9:1, pH 12) δ_H 3.93 (1H, ABXY, *J* = 10.1, 4.5, 4.5 Hz, CH₂), 3.81 (1H, ABXY, *J* = 10.1, 6.2, 4.5 Hz, CH₂), 3.46 (1H, dd, *J* = 6.2, 4.5 Hz, CH). ¹³C NMR (151 MHz, H₂O/D₂O 9:1, pH 12) δ_C 181.3 (C1), 67.9 (d, *J* = 5.0 Hz, C3), 57.6 (d, *J* = 7.7 Hz, C2). ³¹P NMR (161 MHz, H₂O/D₂O 9:1, pH 12) δ_P 4.4 (t, *J* = 4.5 Hz).

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References

- Eschenmoser, A. & Loewenthal, E. Chemistry of potentially prebiological natural products. *Chem. Soc. Rev.* **21**, 1–16 (1992).
- Jeong, H., Tombor, B., Albert, R., Oltvai, Z. N. & Barabási, A. L. The large-scale organization of metabolic networks. *Nature* **407**, 651–654 (2000).
- Oparin, A. I. *The Origin of Life* (MacMillan, 1938).
- Huber, C. & Wächterhäuser, G. Activated acetic acid by carbon fixation on (Fe, Ni)S under primordial conditions. *Science* **276**, 245–247 (1997).
- Miller, S. L., Schopf, J. W. & Lazcano, A. Oparin's 'origin of life': sixty years later. *J. Mol. Evol.* **44**, 351–353 (1997).
- Morowitz, H. J., Kostelnik, J. D., Yang, J. & Cody, G. D. The origin of intermediary metabolism. *Proc. Natl Acad. Sci. USA* **97**, 7704–7708 (2000).
- Zhang, X. V. & Martin, S. T. Driving parts of Krebs cycle in reverse through mineral photochemistry. *J. Am. Chem. Soc.* **128**, 16032–16033 (2006).
- Eschenmoser, A. On a hypothetical generational relationship between HCN and constituents of the reductive citric acid cycle. *Chem. Biodiv.* **4**, 554–573 (2007).
- Orgel, L. E. The implausibility of metabolic cycles on the prebiotic Earth. *PLoS Biol.* **6**, 5–13 (2008).
- Cooper, G., Reed, C., Nguyen, D., Carter, M. & Wang, Y. Detection and formation scenario of citric acid, pyruvic acid, and other possible metabolism precursors in carbonaceous meteorites. *Proc. Natl Acad. Sci. USA* **108**, 14015–14020 (2011).
- Sagi, V. N., Punna, V., Hu, F., Meher, G. & Krishnamurthy, R. Exploratory experiments on the chemistry of the 'glyoxylate scenario': formation of ketosugars from dihydroxyfumarate. *J. Am. Chem. Soc.* **134**, 3577–3589 (2012).
- Patel, B. H., Percivalle, C., Ritson, D. J., Duffy, C. D. & Sutherland, J. D. Common origins of RNA, protein and lipid precursors in a cyanosulfidic protometabolism. *Nat. Chem.* **7**, 301–307 (2015).
- De Duve, C. *Singularities: Landmarks on the Pathway of Life* (Cambridge Univ. Press, 2005).
- Powner, M. W. & Sutherland, J. D. Prebiotic chemistry: a new modus operandi. *Phil. Trans. R. Soc. B* **366**, 2870–2877 (2011).
- Nelson, D. L. & Cox, M. M. in *Lehninger Principles of Biochemistry* 4th edn, Ch. 13, 493–497 (W. H. Freeman 2004).
- Walsh, C. T., Benson, T. E., Kim, D. H. & Lees, W. J. The versatility of phosphoenolpyruvate and its vinyl ether products in biosynthesis. *Chem. Biol.* **3**, 83–91 (1996).
- Potter, S. & Fothergill-Gilmore, L. A. Molecular evolution: the origin of glycolysis. *Biochem. Mol. Biol. Educ.* **21**, 45–48 (1993).
- Powner, M. W., Gerland, B. & Sutherland, J. D. Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions. *Nature* **459**, 239–242 (2009).
- Cody, G. D. *et al.* Primordial carbonylated iron–sulfur compounds and the synthesis of pyruvate. *Science* **289**, 1337–1340 (2000).
- Weber, A. The sugar model: catalysis by amines and amino acid products. *Orig. Life Evol. Biosph.* **31**, 71–86 (2001).
- Keller, M. A., Turchyn, A. V. & Ralser, M. Non-enzymatic glycolysis and pentose phosphate pathway-like reactions in a plausible Archean ocean. *Mol. Syst. Biol.* **10**, 725–737 (2014).
- Guzman, M. I. & Martin, S. T. Prebiotic metabolism: production by mineral photoelectrochemistry of α-ketocarboxylic acids in the reductive tricarboxylic acid cycle. *Astrobiology* **9**, 833–842 (2009).
- Kolb, V. & Orgel, L. E. Phosphorylation of glyceric acid in aqueous solution using trimetaphosphate. *Orig. Life Evol. Biosph.* **26**, 7–13 (1996).
- Pasek, M. A., Kee, T. P., Bryant, D. E., Pavlov, A. A. & Lunine, J. I. Production of potentially prebiotic condensed phosphates by phosphorus redox chemistry. *Angew. Chem. Int. Ed.* **47**, 7918–7920 (2008).
- Krishnamurthy, R., Arrhenius, G. & Eschenmoser, A. Formation of glycolaldehyde phosphate from glycolaldehyde in aqueous solution. *Orig. Life Evol. Biosph.* **29**, 333–354 (1999).
- Rabinowitz, J., Lores, J., Krebsbach, R. & Rogers, G. Peptide formation in the presence of linear or cyclic polyphosphates. *Nature* **224**, 795–796 (1969).
- Saffhill, R. Selective phosphorylation of the *cis*-2',3'-diol of unprotected ribonucleosides with trimetaphosphate in aqueous solution. *J. Org. Chem.* **36**, 2881–2883 (1970).
- Mullen, L. B. & Sutherland, J. D. Formation of potentially prebiotic amphiphiles by reaction of β-hydroxy-*n*-alkylamines with cyclotriphosphate. *Angew. Chem. Int. Ed.* **46**, 4166–4168 (2007).
- Orgel, L. E. Prebiotic chemistry and the origin of the RNA world. *Crit. Rev. Biochem. Mol. Biol.* **39**, 99–123 (2004).
- Keefe, A. D. & Miller, S. L. Are polyphosphates or phosphate esters prebiotic reagents? *J. Mol. Evol.* **41**, 693–702 (1995).
- Sutherland, J. D., Mullen, L. B. & Buchet, F. F. Potentially prebiotic Passerini-type reactions of phosphates. *Synlett* **14**, 2161–2163 (2008).
- Krishnamurthy, R., Guntha, S. & Eschenmoser, A. Regioselective α-phosphorylation of aldoses in aqueous solution. *Angew. Chem. Int. Ed.* **39**, 2281–2285 (2000).
- Muller, D. *et al.* Chemie von α-Aminonitrilen. Aldomerisierung von Glycolaldehyd-phosphat zu racemischen Hexose-2,4,6-triphosphaten und (in Gegenwart von Formaldehyd) racemischen Pentose-2,4-diphosphaten: *rac*-Allose-2,4,6-triphosphat und *rac*-Ribose-2,4-diphosphat sind die Reaktionshauptprodukte. *Helv. Chim. Acta* **73**, 1410–1468 (1990).
- Eschenmoser, A. Chemical etiology of nucleic acid structure. *Science* **284**, 2118–2124 (1999).
- Corey, E. J., Gilman, N. W. & Ganem, B. E. New methods for the oxidation of aldehydes to carboxylic acids and esters. *J. Am. Chem. Soc.* **90**, 5616–5617 (1968).
- Goldman, N., Reed, E. J., Fried, L. E., William Kuo, I.-F. & Maiti, A. Synthesis of glycine-containing complexes in impacts of comets on early Earth. *Nat. Chem.* **2**, 949–954 (2010).
- Öberg, K. I. *et al.* The comet-like composition of a protoplanetary disk as revealed by complex cyanides. *Nature* **520**, 198–201 (2015).
- Osterberg, R. Origins of metal ions in biology. *Nature* **249**, 382–383 (1974).
- Braterman, P. S., Cairns-Smith, A. G. & Sloper, R. W. Photo-oxidation of hydrated Fe²⁺-significance for banded iron formations. *Nature* **303**, 163–164 (1983).
- Liu, R. & Orgel, L. E. Oxidative acylation using thioacids. *Nature* **389**, 52–54 (1997).
- Keefe, A. D. & Miller, S. L. Was ferrocyanide a prebiotic reagent? *Orig. Life Evol. Biosph.* **26**, 111–129 (1996).
- Bowler, F. R. *et al.* Prebiotically plausible oligoribonucleotide ligation facilitated by chemoselective acetylation. *Nat. Chem.* **5**, 383–389 (2013).
- Rudnick, R. L. & Fountain, D. M. Nature and composition of the continental crust: a lower crustal perspective. *Rev. Geophys.* **33**, 267–309 (1995).
- Post, J. E. Manganese oxide minerals: crystal structures and economic and environmental significance. *Proc. Natl Acad. Sci. USA* **96**, 3447–3454 (1997).
- Tebo, B. M. *et al.* Biogenic manganese oxides: properties and mechanisms of formation. *Annu. Rev. Earth Planet. Sci.* **32**, 287–328 (2004).
- Hazen, R. M. *et al.* Mineral evolution. *Am. Mineral.* **93**, 1693–1720 (2008).
- Maynard, J. B. The chemistry of manganese ores through time: a signal of increasing diversity of Earth-surface environments. *Econ. Geol.* **105**, 535–552 (2010).
- Trail, D., Watson, E. B. & Tailby, N. D. The oxidation state of Hadean magmas and implications for early Earth's atmosphere. *Nature* **480**, 79–82 (2011).
- Hazen, R. Paleomineralogy of the Hadean eon: a preliminary species list. *Am. J. Sci.* **13**, 807–843 (2013).
- Anbar, A. D. & Holland, H. D. The photochemistry of manganese and the origin of banded iron formations. *Geochim. Cosmochim. Acta* **56**, 2595–2603 (1992).
- Chatgililoglu, C., Crich, D., Komatsu, M. & Ryu, I. Chemistry of acyl radicals. *Chem. Rev.* **99**, 1991–2069 (1999).

52. Kasting, J. F., Holland, H. D. & Pinto, J. R. Oxidant abundances in rainwater and the evolution of atmospheric oxygen. *J. Geophys. Res.* **90**, 10497–10510 (1987).
53. Forsythe, J. G. *et al.* Ester-mediated amide bond formation driven by wet–dry cycles: a possible path to polypeptides on the prebiotic Earth. *Angew Chem. Int. Ed.* **54**, 9871–9875 (2015).
54. Kim, Y. S., Wo, K. P., Maity, S., Atreya, S. K. & Kaiser, R. I. Radiation-induced formation of chlorine oxides and their potential role in the origin of Martian perchlorates. *J. Am. Chem. Soc.* **135**, 4910–4913 (2013).
55. Leshin, L. A. *et al.* Volatile, isotope, and organic analysis of Martian fines with the Mars Curiosity rover. *Science* **341**, 6153–6162 (2013).
56. Solomon, S., Sanders, R. W., Garcia, R. R. & Keys, J. G. Increased chlorine dioxide over Antarctica caused by volcanic aerosols from Mount Pinatubo. *Nature* **363**, 245–248 (1993).
57. Jackson, W. A. *et al.* Widespread occurrence of (per)chlorate in the Solar System. *Earth Planet Sci. Lett.* **430**, 470–476 (2015).
58. Halperin, J. & Taube, H. The transfer of oxygen atoms in oxidation–reduction reactions. III. The reaction of halogenates with sulfite in aqueous solution. *J. Am. Chem. Soc.* **74**, 375–380 (1952).
59. Bal, B. S., Childers, W. E. & Pinnick, H. W. Oxidation of α,β -unsaturated aldehydes. *Tetrahedron* **37**, 2091–2096 (1981).
60. Pascal, R., Taillades, J. & Commeyras, A. Strecker's and related systems. IX. Acetone as catalyst for the hydration of tertiary α -aminonitriles in aqueous basic solution. *Bull. Soc. Chim. Fr.* **2**, 177–184 (1978).
61. Nwaukwa, S. O. & Keehn, P. M. Oxidative cleavage of α -diols, α -diones, α -hydroxyketones and α -hydroxy- and α -ketone-acids with calcium hypochlorite. *Tetrahedron Lett.* **23**, 3135–3138 (1982).
62. Keefe, A. D., Newton, G. L. & Miller, S. L. A possible prebiotic synthesis of pantetheine, a precursor to coenzyme A. *Nature* **373**, 683–685 (1995).
63. Nargoski, R. W. & Richard, J. P. Mechanistic imperatives for aldose–ketose isomerization in water: specific, general base- and metal ion-catalyzed isomerization of glyceraldehyde with proton and hydride transfer. *J. Am. Chem. Soc.* **123**, 794–802 (2001).

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Author contributions

The research was conceived by M.W.P. Experiments were conducted by A.J.C. Both authors contributed to the design and analysis of experiments, and to writing the paper.

Additional information

Supplementary information and chemical compound information are available in the [online version of the paper](#). Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to M.W.P.

Competing financial interests

The authors declare no competing financial interests.