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Selective prebiotic synthesis of phosphoroaminonitriles and aminothioamides in neutral water

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The central and conserved role of peptides in extant biology suggests that they played an important role during the origins of life. Strecker amino acid synthesis appears to be prebiotic, but the high pK_{aH} of ammonia (pK_{aH} = 9.2) necessitates high pH reaction conditions to realise efficient synthesis, which places difficult environmental constraints on prebiotic amino acid synthesis. Here we demonstrate that diamidophosphate reacts efficiently with simple aldehydes and hydrogen cyanide in water at neutral pH to afford *N*-phosphoro-aminonitriles. *N*-Phosphoro-aminonitrile synthesis is highly selective for aldehydes; ketones give poor conversion. *N*-Phosphoro-aminonitriles react with hydrogen sulfide at neutral pH to furnish aminothioamides. The high yield (73%–Quant.) of *N*-phosphoro-aminonitriles at neutral pH, and their selective transformations, may provide new insights into prebiotic amino acid synthesis and activation.

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Peptides and nucleic acids have universally conserved, interdependent roles in biology that suggest a fundamental link between peptides and nucleotides that may date back to the origins of life. The efficiency and simplicity of Strecker amino acid synthesis from aldehydes (**1**, $R^2 = H$), hydrogen cyanide (HCN) and ammonia[1] suggest that the Strecker reaction is a likely prebiotic amino acid synthesis[2]. The implication of HCN in both amino acid and nucleotide synthesis strongly suggests the importance of cyanide chemistry at the origins of life[2]. However, Strecker synthesis is highly pH dependant and is reversible under sufficiently acidic or alkaline conditions[3]. Moreover, if a successful synthesis of aminonitrile **2** (or amino acid **3**) can be achieved under prebiotic constraints, subsequent activation is required for peptide synthesis. The (prebiotic) electrophilic activation and oligomerisation of amino acids have been achieved with mixed success[4–7], but in extant biology, peptide synthesis begins with phosphorylation of amino acid **3**. Intrigued by the biological relationship between amino acids and nucleotides that is manifest in the genetic code, and the prebiotic reactions of HCN (that can furnish amino acids **3**, as well as nucleobases and simple sugars)[2], we hypothesised that an imine-based reaction might link sugar/nucleotide phosphorylation with amino acid phosphorylation to yield a robust, neutral pH Strecker reaction in water. Specifically, we reasoned that aminonitriles might be accessed at neutral pH by using a low pK_a amine (Fig. 1, EWG-NH_2; EWG = electron-withdrawing group) that is deprotonated, and therefore nucleophilic, at low pH. Moreover, judicious choice of EWG-NH_2 would provide a traceless masked ammonia source that is activated to undergo Strecker reaction at low pH, and subsequent unmasking of the amine moiety would liberate free **2** or an activated derivative of **2** or **3**.

Recently, we reported an α-phosphorylation controlled reaction network that gives access to all the intermediates of triose glycolysis (glyceric acid 2-phosphate, glyceric acid 3-phosphate, PEP (phosphoenol pyruvate) and pyruvate, as well as phosphoserine)[8], which demonstrated that switching the order of oxidation and elimination renders triose glycolysis chemically predisposed. The key to site-selective α-phosphorylation was imine-tethered diamidophosphate (**DAP**)[8–10], which renders intramolecular phosphorylation of the α-hydroxyl moieties of simple sugars highly efficient and remarkably selective, especially at low pH[8].

The importance of **DAP**-imine chemistry in controlling prebiotic glycolysis prompted us to explore the reactivity of **DAP** further, and consider **DAP** as masked ammonia (Fig. 1) in the Strecker reaction. The most-plausible reported prebiotic **DAP** synthesis is achieved by cyclotrimetaphosphate (cTMP) ammonolysis[9–11]. Therefore, it is of note that Rabinowitz reported cTMP-mediated ligation of amino acids (pH 11 at room temperature)[12–15]. The proposed mechanism for cTMP activation involves initial nucleophilic attack of the amine moiety of amino acid **3** at the electrophilic phosphorus atom of cTMP, followed by formation of a five-membered phosphoramide intermediate[15]. This cyclic intermediate is unstable to isolation, but has been observed in situ for glycine and alanine[16,17]. The strongly alkaline conditions required for cTMP-mediated phosphorylation lead to very poor yields and various undesirable side reactions[9,18–21], but we reasoned these problems might be ameliorated by using imine tethering and nucleophilic amine catalysis provide by the ammonolysis of cTMP to form amidotriphosphate and **DAP**[8–10,22].

Here, we show that the reaction of aldehydes (**1**, $R^2 = H$), HCN and **DAP** yields *N*-phosphorylated aminonitriles (**4**) at neutral pH. Compounds **4** are stable under the reaction conditions and, unlike **2**, retro–Strecker reaction is not observed under acidic or alkaline conditions. Hydrolysis of *N*-phosphorylated aminonitriles at acidic pH affords the corresponding aminonitriles **2**. Conversely, alkaline hydrolysis selectively yields *N*-phosphoro amino acids (**5**). Finally, we demonstrate that selective thiolysis of the nitrile group allows the synthesis of thioamides **6** at neutral pH. Our synthesis opens a lower pH window for Strecker chemistry, and provides a selective mechanism for amino acid *N*-phosphorylation[23]. We have also demonstrated pH-dependent hydrolysis and selective (prebiotically plausible) thiolysis of *N*-phosphorylated aminonitriles **4**, which may open new avenues for selective peptide ligation controlled by phosphorylation.

Results

Phosphoro–Strecker reaction optimisation. We first studied the reaction of aldehydes **1a–h** (50–200 mM), HCN (1.2 equiv.) and **DAP** (1.2–4 equiv.) in water across a broad pH range (pH 5–10) at room temperature (Fig. 2, Table 1 and Supplementary Table 1). This wide range of (prebiotically plausible) aldehydes underwent phosphoro–Strecker reaction in good yields (73%–Quant.) from their respective phosphoro–aminonitriles **4a–h** (Table 1, entries 1–8; Supplementary Figs. 1–16). The optimal pH for phosphoro–Strecker reaction was pH 7 (Supplementary Fig. 17), which starkly contrasts with typical Strecker reactions that require alkaline (pH 9–10) conditions close to the pK_{aH} of ammonia. In contrast to the phosphoro–Strecker reaction, aldehydes (**1**, $R^2 = H$) react poorly with HCN (1.2 equiv.) and ammonia (4 equiv.) at neutral pH to afford low yields (6–10%) of aminonitriles (**2**) after 4 days (Supplementary Figs. 18–20), with a large amount of aldehyde (**1**) remaining trapped as the corresponding cyanohydrin (**7**). Consequently, the pH dependence of the Strecker reaction allows the pH-controlled preparation of **2** or **4** in a remarkably selective way. The reaction of acetaldehyde **1a**, HCN (1.2 equiv.), **DAP** (4 equiv.) and ammonia (4 equiv.) at pH 7 for 4 days yields **4a** in excellent yield (94%), alongside a low yield of aminonitrile **2a** (6%). However, under alkaline conditions (pH 10) the observed yields for **4a** (3%) and **2a** (90%) are reversed (Supplementary Fig. 18).

Aldehydes **1** ($R^2 = H$) bearing polar and charged functional groups gave good-to-excellent yields of **4** in water (Table 1, entries 10–11, Supplementary Figs. 21–24). For example, 4-oxobutanoic acid (**1j**) furnished *N*-phosphoro-glutamic acid aminonitrile (**4j**) in 88% yield at neutral pH (Table 1, entry 10; Supplementary Figs. 23 and 24). Low aldehyde solubility can lead to poor Strecker reaction yields, but the yield of phosphoro–Strecker reactions for poorly water-soluble aldehydes (e.g. **1g**) was enhanced by the addition of co-solvents. For example, addition of 10% DMSO or formamide improved the solubility of the aldehyde substrates and their respective cyanohydrins. However, the reaction with formaldehyde (**1l**), the precursor of glycine, required slow addition of **1l** (Fig. 3). The general reaction conditions initially yielded cyanohydrin **7l** in excellent yield (Supplementary Figs. 25 and 26), but after 6 day significant amounts of material were sequestered into various oligomers, rather than the desired product **4l**. Excess **1l** yielded aminal **8** (Fig. 3, Method 1 in the Supplementary Methods) as a major product. Aminal **8** has a highly characteristic ^1H NMR phosphorus-coupled diastereotopic spin system [δ_H 5.29 ppm (dt, $J = 14.1$, 7.0 Hz); 4.33 ppm (dt, $J = 21.7$, 14.1 Hz)] (Supplementary Figs. 27 and 28) and was readily precipitated as a calcium salt. Recrystallisation and X-ray analysis (Supplementary Fig. 29, Supplementary Table 2 and Method 2 in the Supplementary Methods) unambiguously proved that **8** was a highly symmetric cage structure furnished by oligomerisation of **DAP**/**1l** (3:6). Slow addition of stoichiometric **1l** to a solution of HCN and **DAP** at pH 7 (Method 3 in the Supplementary Methods) yielded the

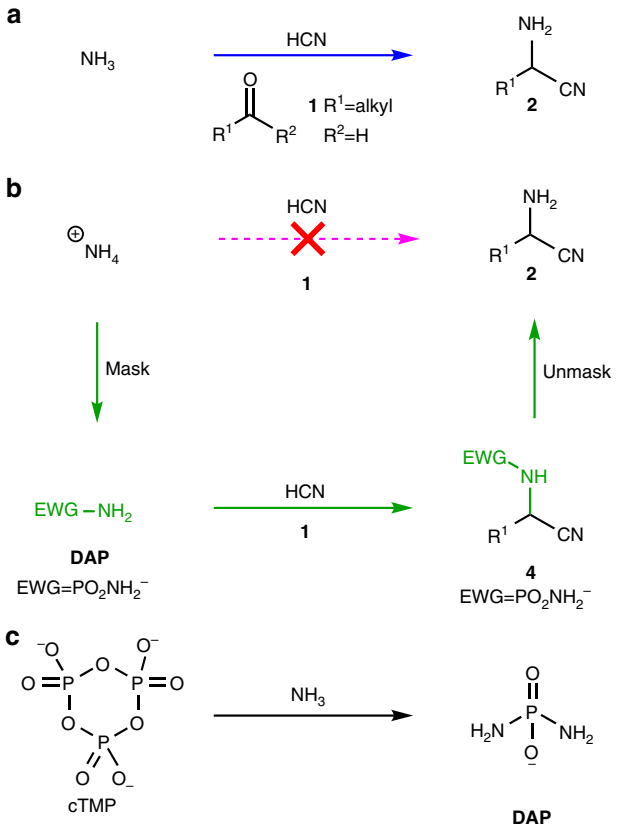

Fig. 1 Masked Strecker reaction. **a** Strecker reactions proceed with ammonia (NH_3) at high pH to furnish aminonitriles (**3**) (blue arrow). **b** Ammonia is protonated (NH_4^+) at neutral pH, which blocks low pH Strecker reaction with ammonia (magenta arrow). If ammonia is masked (green arrows) to yield an amine with a low pK_{aH}, the Strecker reaction can proceed at low pH before the latent primary amine moiety is unmasked to liberate the free NH_2-moiety. **c** Prebiotic synthesis of diamidophosphate (**DAP**) from cyclotrimetaphosphate (cTMP)

desired product **4l** in a satisfactory 48% yield (Table 1, entry 12; Supplementary Figs. 30–32).

Owing to their (retrosynthetic) relationship with proteinogenic amino acids, aldehydes (**1**, $R^2 = H$) are a central focus of prebiotic chemistry. However, ketones (**1**, $R^2 \neq H$) have been shown to emerge alongside aldehydes from prebiotic networks[24]. Their corresponding aminonitriles (**2**) have been reported to form in yields similar to those formed from aldehydes at basic pH, at which conventional Strecker reactions operate[25]. However, cyanohydrin (**7**) formation from ketones and HCN is disfavoured with respect to aldehydes at neutral pH[24,26]. Therefore, we next studied the phosphoro–Strecker reaction of a range of ketones (**1m**–**r**, Table 1, entries 13–18). Simple ketones (**1m**–**p**) gave significantly lower yields (<19%) of α,α-disubstituted *N*-phosphoro-aminonitriles **2m**–**p** (Table 1, entries 13–16; Supplementary Figs. 33–40) than the aldehyde substrates. The phosphoro–Strecker reaction of ketones was not improved by the addition of co-solvents (e.g. 10% DMSO), even when near-quantitative ketone solubility, as a mixture of **4**, ketone (**1m**–**p**) and cyanohydrin (**7**), was observed by NMR spectroscopy. To further probe the importance of carbonyl reactivity in controlling phosphoro–Strecker reaction yields, we investigated ring-strained ketones **1q** and **1r**. Ketones **1q** and **1r** furnished improved yields of *N*-phosphoro-aminonitrile **2q** (38%) and **2r** (41%), respectively (Table 1, entries 17 and 18), which demonstrates that carbonyl stability is a key factor in controlling the phosphoro–Strecker reaction yield. Intrigued by the observed difference between ketone and aldehyde reactivity, we next investigated the competition between aldehydes and ketones in phosphoro–Strecker chemistry. Pleasingly, every combination of aldehyde/ketone (1:1, 200 mM, Method 4 in the Supplementary Methods, Supplementary Figs. 41–46 and Supplementary Tables 3–7) explored, underwent a highly selective reaction to furnish the aldehyde phosphoro-aminonitrile product (**4**, $R^2 =$ H). For example, a 1:1 mixture of acetaldehyde (**1a**) and acetone (**1m**) yielded **4a** (70%) as the major product after 4 days (Supplementary Fig. 41) and only minimal conversion to **4m** (<1%) was observed. There is a clear aldehyde selectivity in the phosphoro–Strecker reaction at neutral pH, therefore it is of note that aldehydes yield proteinogenic aminonitriles, whereas ketones yield α,α-disubstituted products that are not assigned to the universal genetic code.

Phosphoro–aminonitrile reactivity. Selective precipitation provided a facile route to isolate **4** without the use of chromatography. For example, the addition of methanol or ethanol to enhance solvent miscibility, followed by diethyl ether as an anti-solvent resulted in effective precipitation of **4** (Supplementary Figs. 47–54). With pure **4a**–**i** and **4m** in hand, we turned our attention to their reactivity. Aminonitriles (**2a**–**k**) undergo hydrolysis in water to give their respective cyanohydrins **7a**–**k** (for example, α-aminopropionitrile **2b** $t_{1/2} = 0.5$ h at 50 °C and pH 7.0)[27]. Under these conditions **4b** underwent minimal decomposition to **7b** (<5%). α,α-Disubstituted aminonitriles (**2m**–**p**) are stable at pH 7–13[27–29], where as α,α-disubstituted phosphoro–aminonitriles **4m**–**p** readily undergo retro–phosphoro–Strecker reaction at near-neutral pH. For example, when **4m** (25–50 mM) was dissolved in D_2O (pD 7.2), **1m** (>90%) was liberated after 24 h at room temperature (Supplementary Fig. 55). Conversely, **4a**–**i** were remarkably stable at pH 6–9 and room temperature; no decomposition was observed over a 24 h period, and aminonitrile **2** was only observed after long incubation times (>10 day).

Although **4a**–**i** are highly stable at near-neutral pH, they hydrolyse under strongly acidic or alkaline conditions. Compounds **4a**–**f** (100 mM) were incubated in acidic solution (initial pH 1.5, 50 °C, 24 h) and afforded the corresponding aminonitriles **2a**–**f** in near-quantitative yield (Table 1, entries 1–6; Supplementary Figs. 56–59). A switch in reactivity was observed in alkaline solution.

Incubation of **4a**–**f** (100 mM) under alkaline conditions (initial pH 13, 50 °C) led to very clean hydrolysis to afford **5** after 24 h (Table 1, entries 1–6; Supplementary Figs. 60–67) and, unexpectedly, small amounts of amino acid **3** (<10%). Whether these extreme pH conditions are relevant to prebiotic chemistry is not clear, but they clearly demonstrate a straightforward chemical methodology for the divergent synthesis of **2** and **5**, and highlight the pronounced pH dependence on the reactivity of **4**.

Intrigued by the stability of **4** in solution in neutral water, we next investigated (prebiotically plausible) thiolysis of **4**. Thioamides **6** have been proposed as a reactive species for polymerisation to yield peptides[3], but thiolysis of aminonitriles **2** is reported to yield a variety of products, including dithiopiperazines **9**[30], rather than the corresponding thioamides **6**. We strongly suspected that thiolysis of *N*-phosphoramide-masked nitriles **4** would prevent the formation of **9** (Fig. 2). Accordingly, we incubated **4a**–**i** (100 mM) with hydrogen sulfide (H_2S; 10 equiv., pH 9.0) and observed conversion to *N*-phosphoro-thioamides **10a**-**i**, respectively (Supplementary Figs. 68–86). During thiolysis the solution pH increased to pH 11. Compound **10** was observed as the major product but, interestingly, thioamide **6** (<20%) was also observed, and *N*-phosphoro-amino acids **5** were detected in

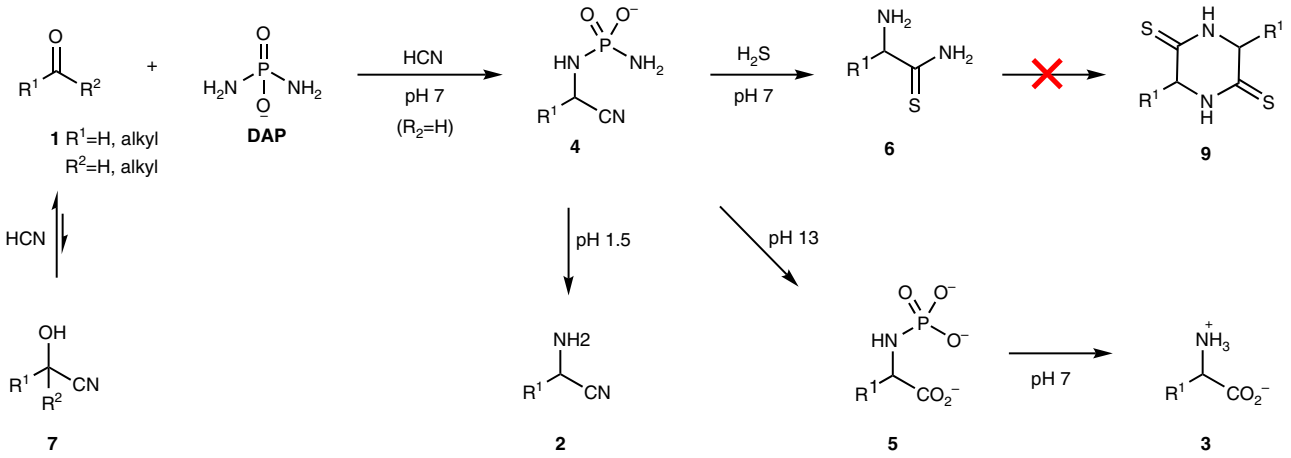

Fig. 2 Phosphoro-Strecker reaction. *N*-Phosphoro-aminonitriles (**4**) are synthesised from aldehydes **(1)** (R^1 = alkyl or H, R^2 = H), HCN and diamidophosphate (**DAP**) in excellent yields at pH 7. Ketones **(1)** ($R^1 \neq H$, $R^2 \neq H$) give α,α-disubstituted *N*-phosphoro-aminonitriles **(4)** ($R^2 \neq H$) in low yields alongside their respective cyanohydrins (**7**). *N*-Phosphoro-aminonitriles **(4)** afford their respective aminonitriles (**2**) upon acid hydrolysis, *N*-phosphoro amino acids (**5**) upon alkaline hydrolysis, and thioamides (**6**) upon thiolysis at neutral pH. See Table 1 for reaction conditions and yields

Table 1 Phosphoro-Strecker reaction products and yields

Entry	1	R^1	R^2	Yield[a] (%)			
				4[b] pH 7	2[e] pH 1.5	5[g] pH 13	6[h] pH 7
1	a	$-CH_3$	H	62 (Quant.[c])	Quant.[f]	93	69
2	b	$-CH_2CH_3$	H	63 (89[c])	98	91	82
3	c	$-CH(CH_3)_2$	H	44 (75[c])	93	83	72
4	d	$-CH_2CH(CH_3)_2$	H	63 (74[c])	Quant.	80	80
5	e	$-CH(CH_3)CH_2CH_3$	H	76 (76[c])	Quant.	57	72
6	f	$-CH_2CH_2CH_3$	H	60 (79[c])	97	86	80
7	g	$-CH(CH_2CH_3)CH_2CH_3$	H	39 (74[c])	93	70	94
8	h	$-CH_2CH_2CH_2CH_3$	H	53 (73[c])	85	85	96
9	i	$-CH_2CH_2SCH_3$	H	34 (79[c])	90	75	84
10	j	$-CH_2CH_2CO_2H$	H	88	—	—	—
11	k	$-CH_2OPO_3H_2$	H	60	—	—	—
12	l	H	H	48[d]	—	—	—
13	m	$-CH_3$	$-CH_3$	19	—	—	—
14	n	$-CH_2CH_3$	$-CH_3$	14	—	—	—
15	o	$-CH(CH_3)_2$	$-CH_3$	5	—	—	—
16	p	$-CH_2CH_2CH_3$	$-CH_3$	9	—	—	—
17	q	$-CH_2CH_2CH_2$-		38	—	—	—
18	r	$-CH_2CH_2CH_2CH_2$-		41	—	—	—

[a]Yields were measured by integration of signals in the ^{1}H NMR spectrum relative to pentaerythritol as an internal standard
[b]Yield of **4** observed after incubation of aldehyde/ketone **1** (200 mM), HCN (1.2 equiv.) and **DAP** (4 equiv.) at pH 7 for 4 day at room temperature in water
[c]Yield of **4** observed with co-solvent DMSO (10%)
[d]Slow addition of stoichiometric **1l** (200 mM) to a solution of **HCN** (1.2 equiv.) and **DAP** (4 equiv.) at pH 7
[e]Yield of **2** observed upon incubation of **4** at pH 1.5 after 1 day (Supplementary Figs. 58–60) at 50 °C in water
[f]Yield of **2a** observed upon incubation of **4a** at pH 1.5 after 4 h (Supplementary Fig. 57) at 50 °C in water
[g]Yield of **5** observed upon incubation of **4** at pH 13 after 1 day (Supplementary Figs. 61–69) at 50 °C in water
[h]Yield of **6** observed upon incubation of **4** and H_2S at pH 7 after 4–35 day (Supplementary Figs. 70–88) at room temperature

small amounts (<5%) after significantly longer (>30 day) incubation times. However, highly selective initial diamidophosphate hydrolysis was observed to liberate ammonia, which suggests that intramolecular nucleophilic catalysis promotes the release of ammonia (Fig. 4, black arrows). Encouraged by this observation, we decided to incubate **4a**–**i** (50–100 mM) with H_2S (10 equiv., pH 7.0), which afforded mixtures of the respective thioamides **6** (>60%) and thioamides **10**. Again, the pH of these reactions increases to pH 9.0–9.2, but upon adjusting (or buffering) the solution to pH 7, thioamides **6a**–**i** were obtained in excellent yields (Table 1, entries 1–9; and Supplementary Figs. 70–85). Importantly, this protocol represents a novel way to directly access to α-aminothioamides (**6**) in aqueous solution at neutral pH. Masking the amine of the Strecker product as a phosphoramide also avoids uncontrolled oligomerisation and, importantly, the formation of cyclic species (e.g. **9**), which leads to a remarkably well-controlled synthesis of thioamides **6**.

To further shed light on the mechanism of diamidophosphate hydrolysis, **4a**–**f** (100 mM) were incubated under alkaline conditions (initial pH 13) at room temperature. *N*-Phosphoro-amino acids **5** were obtained in good yields, but at room temperature these hydrolyses were sluggish (>6 day to

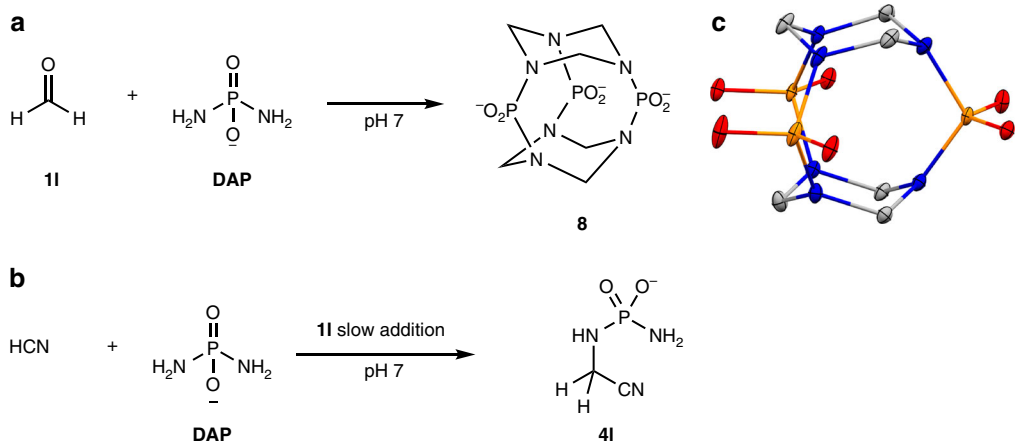

Fig. 3 Formaldehyde-DAP phosphoro–Strecker reaction. **a** Aminal (**8**) is formed upon oligomerisation of formaldehyde (**1l**) and **DAP** at pH 7 and room temperature. **b** Slow addition of formaldehyde (**1l**) to a solution of **DAP** and HCN at pH 7 and room temperature yields phosphoro-aminonitrile **4l** (48%). **c** Crystal structure of (**8**); thermal ellipsoids are shown at the 50% probability level. All hydrogen atoms, water molecules, calcium cations and atom labels are omitted for clarity (crystal packing is shown in Supplementary Fig. 28). Colour scheme: carbon = grey, nitrogen = blue, oxygen = red, phosphorus = orange

completion), and numerous intermediates were observed. Interestingly, nitrile hydrolysis always preceded phosphoramide (P-NH_2 moiety) hydrolysis to afford *N*-phosphoroamide **11** (Supplementary Fig. 87). Direct diamidophosphate hydrolysis/thiolysis to afford **12** was not observed. It is likely that after nitrile hydrolysis (or thiolysis) transient intramolecular amide/thioamide addition to phosphorous (to form cyclic phosphoramidate **13**) promotes the release of ammonia (Fig. 4, black arrows). Cyclic intermediate **13**, which is highly reminiscent of the activated five-membered phosphoramide intermediate reported by Rabinowitz (and others)[12–16], rapidly opens to afford *N*-phosphoro-thioamide **10** or *N*-phosphoro-amide **14** (Fig. 4, blue arrows). This mechanism also explains the formation of small amounts of monoamidophosphate (**MAP**) and **3** or thioamide **6** under basic conditions (Fig. 4, red arrows). To test our hypothesised mechanism, we investigated the hydrolysis/thiolysis of **4** in stoichiometric competition with **DAP**. Rapid formation of *N*-phosphoro-amino acid **5** (or *N*-phosphoro thioamide **10**) was observed, whereas **DAP** did not undergo hydrolysis and remained unchanged throughout the transformation of **4** to **5** or **10**.

Finally, to probe the chemical continuity of the synthesis *N*-phosphoro aminonitriles and their subsequent reactions we investigated a one-pot synthesis and hydrolysis/thiolysis of *N*-phosphoro-aminonitriles **4**. Compounds **4a–f** (200 mM) were obtained in water at neutral pH, and the crude products were diluted (to 100 mM concentration) with acidic (pH 1.5) or alkaline solution (pH 13) and then incubated at 50 °C for 24 h to achieve complete hydrolysis. Alternatively, H_2S (10 equiv.) was added to the crude compounds **4a–f** (100 mM, pH 9), which where then incubated at room temperature until complete thiolysis was observed by NMR spectroscopy. The corresponding aminonitriles **2a–f**, *N*-phosphoro-amino acids **5a–f** and *N*-phosphoro-thioamides **10a–f** were all obtained in comparable yields to the reactions performed with purified **4** (Supplementary Figs. 88–117, Supplementary Table 8).

Discussion

We demonstrate that **4** is obtained in good-to-excellent yields from the reaction of aldehydes (**1a–l**), HCN and **DAP** at neutral pH. The phosphoro–Strecker reaction provides a selective strategy for prebiotic amino acid synthesis: it is selective towards aldehydes, with poor conversion for ketone substrates, which thereby avoids the synthesis of non-biological α,α-disubstituted amino acids and leaves prebiotic ketones available to undergo other reaction pathways[24,25]. Furthermore, **4a–l** are remarkably stable at near-neutral pH, whereas α,α-disubstituted products **4m–r** degrade quickly to their corresponding ketones, HCN and **DAP**.

Thioamides **6** are easily obtained in near-quantitative yield at neutral pH from the reaction of **4** with H_2S. The formation of **4**, and the consequent desymmetrisation of diamidophosphate (**DAP**), promotes highly selective amidophosphate hydrolysis that cleaves the NH_2 moiety and retains the amino acid moiety to selectively to furnish *N*-phosphoro amino acids derivatives (and not **MAP**). This methodology can be readily applied to the controlled synthesis of thioamides (**6**) to provide a mild protocol for their synthesis in neutral water, which warrants further investigation of **6** and its phosphorylated precursors in prebiotic peptide ligations. In the context of the origins of life, it is interesting to note that **4** is not only readily synthesised but also highly stable at neutral pH; this stands in stark contrast to the instability of aminonitriles **2** synthesised from ammonia. Prebiotic scenarios that result in pH oscillations and gradients can be envisaged[31], and it is reasonable to consider that these oscillations/gradients may have played an important role during the origins of life. However, it is not clear that the extreme pH conditions required for the conversion of **4** into **2** or **3** would be available en route to life. More likely the efficient reactivity of H_2S with the nitrile moiety of **4**, which then leads to the synthesis of thioamide **10** and rapid (intramolecular) phosphoramide hydrolysis, would be crucial to the forward reactivity of **4** under geochemically plausible conditions. We are currently exploring the polymerisation, ligation and onward reactivity of **4**, **6** and **10** following their efficient synthesis at near-neutral pH.

Methods

Phosphoro–Strecker reaction protocol A. Aldehyde (**1a–c**, 2 mmol), sodium cyanide (1.2 equiv.) and **DAP** (4 equiv.) were dissolved in water (10 mL) at pH 7.0. This solution was then stirred at room temperature and monitored periodically by ^{1}H and ^{31}P NMR spectroscopy. When the NMR spectra showed no further change in composition, ethanol (40–60 mL) was added to precipitate **DAP**. **DAP** was removed by filtration and the residual solution was concentrated in vacuo. The residue was dissolved in water/ethanol (1:2, 20 mL) and **4a–c** were precipitated by addition of diethyl ether (20–40 mL) and isolated by filtration.

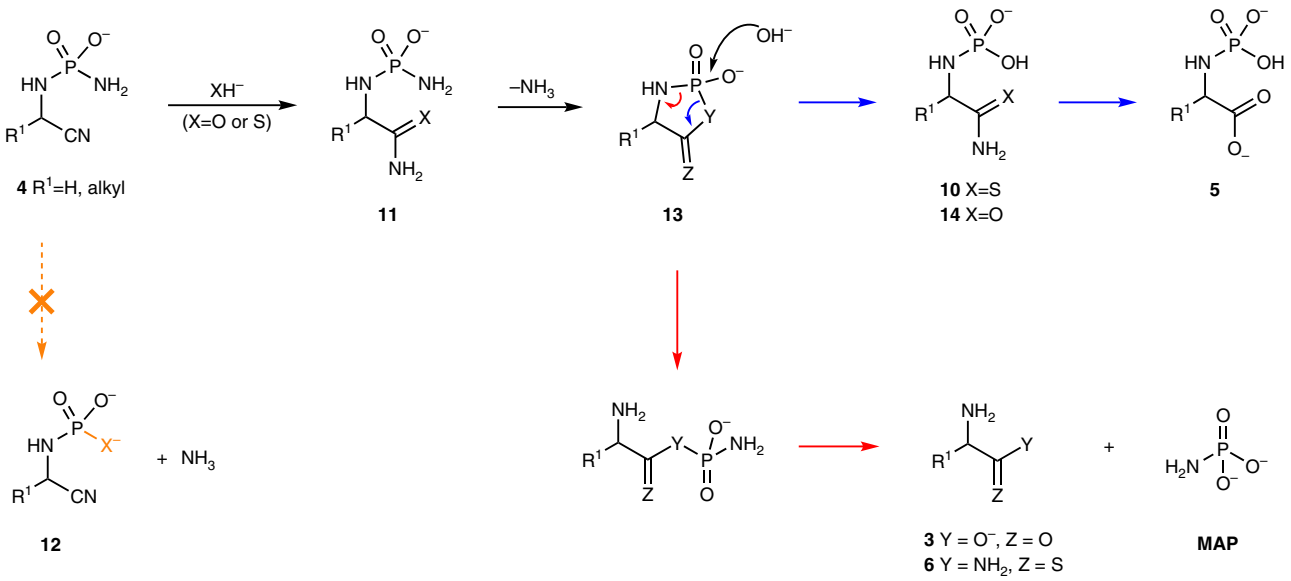

Fig. 4 Proposed mechanism for the selective cleavage of the NH_2 moiety of *N*-phosphoro-amino acid derivatives. Black arrows: Proposed intramolecular catalysis. Blue arrows: Major pathway; yields *N*-phosphoro-amino acid derivatives (**10**) (X = S) or (**14**) (X = O). Red arrows: Minor pathway; furnishes amino acids (**3**) or thioamides (**6**) and monoamidophosphate (**MAP**). Dashed arrow: Direct diamidophosphate hydrolysis (X = O) or thiolysis (X = S) is not observed

Phosphoro–Strecker reaction protocol B. Aldehyde **1d–i** (2 mmol), sodium cyanide (1.2 equiv.) and **DAP** (4 equiv.) were dissolved in water (10 mL) at pH 7.0. This solution was then stirred at room temperature and monitored periodically by ^{1}H and ^{31}P NMR spectroscopy. When the NMR spectra showed no further change in composition, methanol/ethanol (1:4–1:6, 50–70 mL) were added to precipitate **DAP**. **DAP** was removed by filtration and the residual solution was concentrated in vacuo. The residue was dissolved in water/methanol/ethanol (1:1:2, 20 mL), and **4d–i** were precipitated by addition of diethyl ether (20–40 mL) and isolated by filtration (See Supplementary Methods for compound data).

Phosphoro–Strecker reaction protocol C. Aldehyde **1a–i** (2 mmol), sodium cyanide (1.2 equiv.) and **DAP** (4 equiv.) were dissolved in water/formamide or water/DMSO (10 mL, 9:1) at pH 7.0. This solution was then stirred at room temperature and monitored periodically by ^{1}H and ^{31}P NMR spectroscopy. When the NMR spectra showed no further change in composition the solution was lyophilised. The residue was dissolved in water (10 mL). **DAP**, and subsequently **4a–c** or **4d–i**, were then precipitated as described in Protocol A or Protocol B above, respectively (See Supplementary Methods for compound data).

Acid hydrolysis. Phosphoro-amino nitrile **4** (100 mM) was dissolved in H_2O/D_2O (9:1). The pH of the solution was adjusted to pH 1.5 by addition of 4 M HCl. The reaction mixture was heated at 50 °C. The reaction pH was monitored and periodically adjusted to pH 1.5. Once the pH of the solution had stabilised at pH 1.5, the solution was analysed by NMR spectroscopy.

Alkaline hydrolysis. Phosphoro-amino nitrile **4** (100 mM) was dissolved in H_2O/D_2O (9:1). The pH of the solution was adjusted to pH 13.5 by addition of 4 M NaOH. The reaction mixture was stirred at 50 °C and monitored by ^{1}H and ^{31}P NMR spectroscopy (See Supplementary Methods for compound data).

Thiolysis. Phosphoro–amino nitrile **4** (50–100 mM) and NaSH (10 equiv.) were dissolved in H_2O/D_2O (9:1) or 500 mM phosphate buffer at pH 7 or 9 and stirred at room temperature. The reaction was monitored by ^{1}H and ^{31}P NMR spectroscopy until complete consumption of the diamidophosphate was observed, then the excess H_2S was purged with Ar at pH 7 (See Supplementary Methods for compound data).

Data availability

The authors declare that data supporting the findings of this study are available within the paper and its Supplementary Information files and figures or by reasonable request from the corresponding author. X-Ray crystallographic data (Supplementary Data 1—crystallographic information file for compound **8**) was deposited at the Cambridge Crystallographic Data Centre (CCDC) under the following CCDC deposition number: 1878815. These can be obtained free of charge from the CCDC via [https://www.ccdc.cam.ac.uk/structures/]. Compound characterisation data are available in the Supplementary Methods.

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Author contributions

M.W.P. conceived the research. M.W.P., C.F.G. and K.A. designed and analysed the experiments. C.F.G. and K.A. conducted the experiments. A.J.C. isolated and crystallised the aminal cage structure. M.K.C. and D.K.B. performed the crystallographic analyses. M. W.P., C.F.G. and K.A. wrote the paper. C.F.G. and K.A. contributed equally.

Additional information

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Competing interests: Kathryn Ashe is now an Associate Editor at *Nature Chemistry*. *Communications Chemistry* and *Nature Chemistry* are editorially independent of each other. The remaining authors declare no competing interests.

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