Prebiotic synthesis of cysteine peptides that catalyze peptide ligation in neutral water

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Abstract: Peptide biosynthesis is orchestrated by a complex suite of enzymes, but this must have been predated by a simple chemical synthesis at the origins of life. α-Aminonitriles—prebiotic α-amino acid precursors—are generally produced by Strecker reactions. However, cysteine's aminothiol is incompatible with nitriles. Consequently, cysteine nitrile is not stable and therefore cysteine has long been believed to be a product of evolution, not prebiotic chemistry. We report the first high-yielding, prebiotic synthesis of cysteine peptides. Our biomimetic pathway converts serine to cysteine by nitrile–activated dehydroalanine synthesis. We additionally demonstrate that *N*-acylcysteines catalyze peptide ligation, directly coupling kinetically stable—but energy-rich—α-amidonitriles to proteinogenic amines. This rare example of selective and efficient organocatalysis in water implicates cysteine at the onset of life's evolution.

One Sentence Summary:

Nitrile chemistry unlocks biomimetic synthesis and fuels high-yielding catalytic peptide ligation in water, providing clues to origins of life.

Main Text: Peptides and proteins are essential to all life on Earth, but their biosynthesis is achieved by a highly evolved system of enzyme catalysts (*1, 2*). Although the origin of protein synthesis in biology remains a mystery, life's exploitation of peptides must have predated the evolution of the complex enzymes that are now required to coordinate biosynthesis. At the origins of life, simple chemical processes must have furnished the first peptide catalysts that later evolved to become modern enzymes (*3*). To elucidate these chemical mechanisms, it is essential that we reflect on the biochemical strategies of extant biology to inform the systematic evaluation of prebiotic chemistry. For example, we recently demonstrated α-peptide synthesis in water could be achieved by H_2S-mediated stoichiometric ligation (*4*). Our synthesis exploited a biomimetic N-to-C terminal chain-growth mechanism. This overcame long-standing problems that had prohibited the coupling of α-aminonitriles (*5-7*), and avoided the irrevocable side chain modifications caused by electrophilic agents required to activate amino acids (*8, 9*). Further reflection on the deep-seated role of thiols in non-ribosomal peptide synthesis and core metabolism (*10, 11*) has now led us to hypothesize that cysteine may have originated as a secondary product of sulfide-mediated peptide synthesis, and we suspected that cysteine could be used to deliver (a prebiotically plausible) catalytic peptide ligation (CPL) in water.

Cysteine (**Cys**) is the primary organic source of sulfide in biology and the feedstock for essential cofactors such as glutathione (**GSH**) and co-enzyme A (**CoA**) (*12*). It is also an important residue

within enzyme active sites, with vital functions in catalysis, redox sensing, and electron transfer, as well as being an essential ligand in ancient iron-sulfur proteins (*11, 13, 14*). It therefore seems almost inconceivable that cysteinyl thiols were not present during the development of nascent biological processes on the early Earth, and yet this is not the prevailing view (*15-18*). Numerous, unsuccessful attempts to synthesize and isolate cysteines under prebiotically plausible conditions (*7, 19-21*) have led to a widely-held belief that cysteine is a biological invention (*17, 18*), as well as a late addition to the genetic code (*15-18*). Here, we report a high-yielding prebiotic synthesis of cysteines and demonstrate that these cysteine peptides catalyze non-enzymatic CPL in neutral water. Our results support the hypothesis that **Cys** was available at the origins of life as a secondary product of serine nitrile (**Ser-CN**) synthesis, and that cysteines would have been a cornerstone of early catalytic activity.

The Strecker synthesis of aminonitriles is widely believed to play an important role in the prebiotic origins of amino acids, and we had previously identified glycolaldehyde (**GCA**) and β-mercaptoacetaldehyde (**BMA**) as key nodes in the chemical network required for abiogenesis of RNA and peptides (*3, 22, 23*), and specifically **BMA** as a Strecker precursor of **Cys** (*22*). However, although Strecker reactions are generally highly efficient (*23, 24*), **BMA** forms intractable and insoluble mixtures in Strecker reactions (fig. S1) (*7*). This is in stark contrast to **GCA**, which undergoes the Strecker reaction in excellent yield to afford a stable aminonitrile product, **Ser-CN** (fig. S2) (*23, 24*). The observed disparity between congeners **GCA** and **BMA** is likely due to the rapid reaction of β-aminothiols with nitriles in water (*25*), and because cysteine nitrile (**Cys-CN**) is both an β-aminothiol and an α-aminonitrile it is inherently unstable (*7*). These observations suggested to us that whilst the Strecker reaction of **GCA** could yield serine (**Ser**) under prebiotic conditions, the Strecker reaction of **BMA** would not play a role in prebiotic **Cys** synthesis. To

overcome this inherent stability problem, we considered an alternative biomimetic pathway for **Cys** synthesis. We suspected that clues to the prebiotic synthesis of **Cys** might remain embedded within the extant biological pathway and that **GCA**, rather than **BMA**, was the key prebiotic precursor of **Cys**.

The principal mechanism by which reduced inorganic sulfur is incorporated into bioorganic compounds is through **Cys** biosynthesis (*17, 18*). In plants and various archaea and bacterial species, **Cys** biosynthesis begins with the enzymatic conversion of **Ser** to *O*-acetylserine (**Ser^{Ac}**) [or *O*-phosphoserine (**Sep**)] followed by a pyridoxal-5′-phosphate (**PLP**) dependent acetic acid β-elimination, and then sulfide β-addition before disassociation of the cysteine-enzyme complex (Fig. 1A). Throughout this sequence, the α-amine remains covalently bound to **PLP** to promote elimination and to prevent the release and rapid decomposition of highly unstable dehydroalanine (**Dha**) (*26*). The nitrile equivalent of **Dha**, dehydroalanine nitrile (**Dha-CN**), has been synthesized by Eschenmoser and co-workers, but was found to be extremely unstable and failed to react with H_2S to produce **Cys-CN**, instead undergoing rapid degradation, even under anhydrous conditions (*27*). However, we have recently shown that *N*-acylation of α-aminonitriles is a crucial element in initiating peptide synthesis in water, and it prevents hydantoin-, diketopiperazine- and imidazole-induced peptide degradation (*4-6*). Similarly, *N*-acylation would stabilize **Dha-CN**. *N*-Acylation would simultaneously prevent the highly favourable, but unwanted, enamine→imine tautomerization that precludes sulfur addition to **Dha** (*27*), and prevent the degradation of **Cys-CN** that is brought about by its free α-amine (*7*). Consequently, we recognized that serine diacylation presents a simple biomimetic strategy for prebiotic dehydroalanine synthesis, and we identified *N,O*-diacetyl-serine nitrile (**Ac-Ser^{Ac}-CN**) as a key intermediate for prebiotic **Cys** synthesis (Fig. 1B). In addition to the electron-withdrawing effects of the α-nitrile, *N,O*-

diacetylation would further enhance the acidity of the α-proton of **Ac-Ser^{Ac}-CN** as well as activating the serine hydroxyl moiety as a leaving group. We envisaged that these combined effects would promote **Ac-Dha-CN** synthesis at neutral pH, without recourse to the highly alkaline (pH >13) conditions typically required for **Dha** formation in water (*28*) that would also promote peptide degradation (*29*).

We have a long-standing interest in (prebiotic) acylation (*30, 31*), but it was not clear if the hydroxyl moiety of **Ser-CN** could be selectively acetylated in water. Therefore, we were pleased to observe chemoselective *N*,*O*-acetylation of **Ser-CN** with thioacetic acid (**AcSH**) and ferricyanide (*4, 30*) to produce **Ac-Ser^{Ac}-CN** in up to 91% yield within 1 h at room temperature (Supplementary Pages S13–24). Acetylation of **Ser-CN** with *N*-acetylimidazole (**NAI**) (*30, 31*) was equally effective for **Ac-Ser^{Ac}-CN** synthesis in neutral water. We initially observed *O*-acetylserine nitrile **Ser^{Ac}-CN** as the major product (<10 min) with **NAI** (fig. S8), and attribute the nucleophilicity of the β-hydroxyl of **Ser-CN** to the pronounced electron-withdrawing effect of the α-nitrile and the remarkably low pK_{aH} of this amino-alcohol (Supplementary Pages S23–32). More importantly, we also observed the formation of a stable dehydroalanine nitrile, **Ac-Dha-CN**, at near-neutral pH for the first time during these acetylation reactions. Optimal formation of **Ac-Dha-CN** from **Ac-Ser^{Ac}-CN** occurred at pH 8, and after 4 d at room temperature an 85% conversion to **Ac-Dha-CN** was observed (Fig. 1C). This elimination is all the more remarkable because *N,O*-diacetylserinamide (**Ac-Ser^{Ac}-NH$_2$**) underwent near-exclusive hydrolysis at pH 8 (fig. S34–35) (*32*). The switch in reactivity between α-nitrile (acetate elimination) and α-amide (acetate hydrolysis) demonstrates the benefits of α-nitrile activation for acetate elimination, and indicates that **Ac-Dha-CN** is predisposed to form in near-neutral water (*28, 29*).

We next investigated **Ac-Dha-CN** synthesis via phosphate elimination. Acetylation of **Sep-CN** (*33*) in water with **AcSH** and ferricyanide at pH 7 yielded **Ac-Sep-CN** (80%) after 1 h. Phosphoserines typically require alkaline pH and Ba^{2+} to promote dehydroalanine formation (*29*), but the formation of **Ac-Dha-CN** (8%) was nonetheless observed after heating **Ac-Sep-CN** at 60 °C for 3 d at pH 7. The sluggish rate of phosphate elimination was enhanced by Mg^{2+}, yielding **Ac-Dha-CN** (24%) after 1 d at 60 ºC (fig. S33). This demonstrates prebiotically plausible dehydroalanine formation can be achieved by **Ser** acetylation or phosphorylation. **Ac-Dha-CN** was found to be highly stable, and we did not observe the addition of acetate, phosphate or hydroxide (even at pH 11) to this dehydroalanine, setting the stage for selective addition of inorganic sulfur to synthesize cysteine.

Pleasingly, **Ac-Dha-CN** underwent near-quantitative conversion to **Ac-Cys-SNH$_2$** upon incubation with H_2S, yielding the first prebiotic synthesis of a stable cysteinyl residue (Fig. 1C). We next investigated the prebiotic acetylating agent, **AcSH**, as a more water-soluble sulfide source at pH 7. Incubation of **Ac-Dha-CN** with **AcSH** led to quantitative thioester formation after 12 h in phosphate buffer (Fig 1C). The cysteine residue was then rapidly liberated by ammonolysis yielding **Ac-Cys-CN** (77%; fig. S44) or thiolysis to yield **Ac-Cys-SNH$_2$** in 95% yield (fig. S39). Prolonged incubation of **Ac-Cys^{Ac}-CN** with H_2S gradually yielded cysteine thioacid **Ac-Cys-SH** and amide **Ac-Cys-NH$_2$** (2:1) (fig. S47–48). High-yielding **Ser-CN→Cys^{Ac}-CN** conversion was also observed for *N*-acetylvalinylserine nitrile (**Ac-Val-Ser-CN**; Supplementary Pages S59–72) demonstrating the efficacy of cysteine synthesis within a sterically encumbered peptide substrate.

Acetyl CoA is biosynthesized from **Cys** and is a universally conserved acetylating agent for protein, carbohydrate and lipid metabolism. The relative simplicity of thioester **Ac-Cys^{Ac}-CN**

suggests it may have been exploited as an acetyl CoA analogue and an activated source of acetate in (proto)metabolism (*10*), so we verified the proficiency of **Ac-Cys^{Ac}-CN** as a source of activated acetate. We suspected that acetyl-transfer from **Ac-Cys^{Ac}-CN** to α-aminonitriles (**AA-CN**) would be promoted by their low pK_{aH} (e.g. **Gly-CN** pK_{aH} = 5.4), which renders **AA-CN** neutral and highly nucleophilic at neutral pH (*4*). To this end, we incubated **Ac-Dha-CN**, **AcSH** and **Gly-CN** in phosphate buffer at pH 7 and room temperature. We observed *in situ* thioester formation and near-quantitative acetyl-transfer from **Ac-Cys^{Ac}-CN** to **Gly-CN**, producing **Ac-Gly-CN** (81%) and **Ac-Gly-SNH$_2$** (13%) after 3 d (fig. S67).

Prebiotic syntheses, like biosyntheses, are necessarily multistep chemical pathways and therefore, like all multistep processes, are susceptible to diminished overall yields unless the individual reactions proceed with remarkably high efficiency (*3*). In biosynthesis, enzyme catalysis achieves exquisite selectivity and high yields, but modern enzymes are a product of billions of years of evolution. In the absence of enzymes, prebiotic chemistry had to initially exploit unevolved and directly accessible alternatives, such as organocatalysts (*34*). Small molecule catalysts could have functioned as rudimentary 'enzymes' at the origins of life, and although they have been highly sought-after since the watershed rediscovery of proline catalysis, progress has been greatly hampered by the generally poor activity of organocatalysts in water (*35*). However, the importance of nitriles at the origins of life (*3*), as well as their low background reactivity, suggested they may be ideal substrates for a highly selective and high yielding catalytic peptide ligation (CPL) in water, and warranted further investigation.

Peptide fragment ligations, such as native chemical ligation, are important reactions in chemical biology and synthetic chemistry, facilitating rapid synthesis of longer peptides from smaller

subunits (*36-38*). Fragment ligations have been proposed to play an important role in prebiotic chemistry (*39*), but the plausibility of these are diminished by the need for synthetically-prepared C-terminal thioesters (*40*). We have recently demonstrated high-yielding prebiotic fragment ligations (*4*) using stoichiometric H_2S and an activating agent (such as ferricyanide or cyanoacetylene) (Fig 2A.i). Ideally, prebiotic fragment ligations through direct coupling of nitrile and amine fragments would side-step thioamide and thioacid intermediates (*4*). Therefore, we became intrigued by the potential of thiols, such as *N*-acylcysteines, to act as organocatalysts for CPL. Reversible thiol addition to an α-amidonitrile could render peptide synthesis catalytic and redox-neutral via a highly reactive – but transient – thioimidate intermediate (Fig. 2A.ii). We anticipated that these thioimidates would be significantly more reactive than the thioesters conventionally exploited in peptide ligations (*36, 37*), but would also be inherently protected from hydrolysis by reversible thiol-to-nitrile addition, and elimination back to the stable nitrile substrate.

Eschenmoser and co-workers had previously reported a **Cys**–catalyzed ammonolysis of an α-amidonitrile in methanol (*41*) to produce a C-terminal primary amidine. In water, however, the addition of **Cys** to α-amidonitriles near-quantitatively yields thiazolines (Supplementary Pages S75–88), following the mechanism implicated in **Cys-CN** self-degradation (*7*). Thiazoline formation is, however, completely suppressed by cysteine *N*-acylation (*42*), suggesting that our biomimetic **Cys** synthesis—which necessarily yields *N*-acyl-cysteines—is predisposed to furnish catalytically active cysteines. Therefore, we next tested **Ac-Cys-OH** as a catalyst for peptide ligation. We incubated **Ac-Gly-CN** and **Gly** with **Ac-Cys-OH** and, remarkably, observed CPL in water for the first time. This reaction yielded peptidyl amidine **Ac-Gly^{N}-Gly-OH** (60%) after 24 h at 60 °C and pH 7 (fig. S85). Importantly, no activating agents were required to induce ligation.

Moreover, peptide ligation no longer requires a C-terminal thioacid (*4*) or thioester and an N-terminal cysteine residue as the nucleophilic ligation partner (*36, 37, 40*). A broad spectrum of cysteine derivatives were excellent catalysts for peptidyl amidine synthesis in water, including **Ac-Cys-NH$_2$** (69%), *N*-acylcysteine peptides (57–71%), as well as **CoA** (65%) and simple thiols such as co-enzyme M (**CoM**; 79%) (fig. S85). Catalysis is essential to promoting this ligation, and the catalytic potency of simple thiols is highlighted by the lack of background reactivity. In control experiments (with no thiol catalyst) no coupling was observed, and **Ac-Gly^{N}-Gly-OH** (<1%) could only be detected after 7 d (fig. S86). Furthermore, very little cysteine-catalyzed hydrolysis was observed in the absence of an amine nucleophile; incubating **Ac-Gly-CN** with **Ac-Cys-OH** (30 mol%, pH 7, 60 ºC) resulted in only 6% hydrolysis after 24 h (fig. S87). These results underscore the outstanding kinetic stability of α-amidonitriles and the generality of thiol-catalysis, where only a severely hindered tertiary thiol failed to catalyze CPL (fig. S85).

CPL is remarkably specific and selective for proteinogenic α-peptide synthesis (Fig. 3). For example, the reaction of **Ac-Gly-CN** and **Ac-β-Ala-CN** (1:1; 200 mM) with **Gly** (200 mM) and **Ac-Cys-OH** (30 mol%) results in exclusive α-amidonitrile coupling to furnish **Ac-Gly^{N}-Gly-OH** (65%) with no detectable β-amidonitrile coupling of **Ac-β-Ala-CN**. We also observed only α-ligation upon challenging CPL with *N*-acetylglutamine dinitrile **Ac-Glx-CN**. Finally, we only observed coupling of proteinogenic **Ala** in competition with α,α-disubstituted (non-proteinogenic amino acid) α-aminoisobutyric acid (**Aib**). The observed selectivity may have been an essential element in the emergence of proteinogenic α-peptides in extant biology (*3, 4, 23*).

Thiol-catalyzed coupling of α-amidonitriles with α-amino acids is highly general; all proteinogenic α-amino acids coupled with **Ac-Gly-CN** to give peptidyl amidines **Ac-Gly^{N}-AA-OH** in good yields at pH 7 and 60 °C (Table 1). However, dipeptides derived from **Ser**, **Thr** and **Asn** underwent pronounced amidine hydrolysis to the corresponding peptides (**Ac-Gly^{N}-AA-OH→Ac-Gly-AA-OH**). In the reactions of **Ser** and **Thr** we observed oxazoline intermediates, suggesting that intramolecular catalysis by the amino acid side chain was responsible for rapid amidine hydrolysis (Fig 2B.). Having also observed that the peptidyl amidine derived from **Asn** hydrolyzed, we envisaged that amino amides (and therefore peptides) would behave similarly. We next examined whether amides intramolecularly catalyze amidine hydrolysis by coupling proteinogenic α-amino amides (**AA-NH$_2$**). All **AA-NH$_2$** resulted in selective dipeptide (**Ac-Gly-AA-NH$_2$**) synthesis, irrespective of their side chain (Table 1). This observed hydrolysis is significant because, whilst peptidyl amidines undergo racemization as expected (*41*), N-terminal **Ser**, **Thr**, and **Cys** undergo stereoretentive coupling (Fig 2B.). The origins of biological homochirality remain a formidable challenge, requiring an as-yet unknown symmetry-breaking event, and until that event prebiotic syntheses must produce racemic mixtures (*43*). The observed racemization and epimerization of peptidyl amidines may have limited impact in a racemic (prebiotic) environment, however, the stereoretentive couplings of peptides with nucleophilic side chains and the intramolecular amide-catalyzed hydrolysis of peptidyl amidines offers a route to investigate dynamic kinetic resolution of peptide stereochemistry that may underpin peptide chiral resolution in oligomers, rather than α-amino acid (or α-aminonitrile) monomers (*44*).

Having demonstrated the tolerance of all proteinogenic aminoacyl residues at the ligation junction in thiol-catalyzed dipeptide synthesis, we explored the feasibility of organocatalytic peptide

fragment ligations. We recently demonstrated the prebiotic synthesis of *N*-acylpeptide nitriles (**Ac-AA$_n$-CN**) in water by sulfide-mediated iterative ligation of α-aminonitriles (Fig. 2A.i) (*4*), and therefore we chose to investigate **Ac-Gly$_3$-CN** as a prebiotically plausible peptide nitrile in fragment ligations. Peptide nitrile **Ac-Gly$_3$-CN** (100 mM) readily coupled with various peptides with 3-mercaptopropionic acid (**MPA**) as a catalyst at neutral pH to give excellent yields of peptides, even with stoichiometric (1:1) coupling partners (Table 2). The simplicity and generality of CPL, underscores the privileged nature of thiol-catalyzed peptide ligations in water.

We have combined systems chemistry (*3, 45*) with analysis of the strategy of modern biosynthesis to resolve two long-standing conundrums at the origins of life: the chemical origins of cysteine and catalytic peptide ligation. We note that extant cysteine biosynthesis (*17, 18*) bear a striking resemblance to the prebiotic synthesis we have outlined. However, the non-enzymatic reactions described here are contingent on an α-nitrile, rather than complex enzymes and cofactors. The α-amidonitrile moiety is not only prebiotically plausible, but also ideally poised to activate dehydroalanine formation at near-neutral pH, and provides the in-built energy required to drive peptide synthesis. It is important to note that the α-amidonitrile has a unique balance of kinetic stability and thermodynamic reactivity to deliver aqueous ligation without requiring any electrophilic activation, and the highly selective and reversible reaction that thiols undergo with α-amidonitriles makes thiol-catalyzed nitrile ligations a powerful and selective system for peptide ligation in water. The amidine intermediates observed during CPL suggest that ligation occurs by intermolecular addition of the nucleophilic coupling partner to a catalyst-bound thioimidate (Fig. 2). Importantly, our ligation (in contrast to thioester acylations (*36, 37*)) tolerates all amino acid

side chain residues without protection to deliver high yields in neutral water, and is not limited to ligation of N-terminal cysteine residues via intramolecular amidation.

It is remarkable that a single amino acid residue, cysteine, provides robust catalysis for peptide ligation in water. This is strong evidence for the catalytic role of simple cysteines (and thiols) foreshadowing enzymes at the origin of life, and it is now easier to envisage the (reciprocal) evolution of (coded) catalytic peptides that catalyze peptide ligation (*39*). The inherent catalytic activity of simple cysteinyl peptides makes them an excellent starting point from which to evolve more complex (folded) enzymes (*14, 39*) and catalysts for protometabolic reactions in an abiotic environment. Finally, our data support a scenario in which nitriles served as an early energy currency on the primordial Earth, perhaps acting as a forerunner to ATP and thioesters that drive reactions in extant biology (*10, 11*).

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S.I. assembled and wrote the Supplementary Materials with contributions from C.S.F. and C.F-G. C.S.F. and S.I. contributed equally to the experiments and are listed alphabetically. S.I. and M.W.P. wrote the manuscript with assistance from T.D.S.

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Data and materials availability: All data is available in the main text and the supplementary materials.

Supplementary Materials:

Materials and Methods

Figures S1-S209

Tables S1-S14

References (*46-51*)

Figures

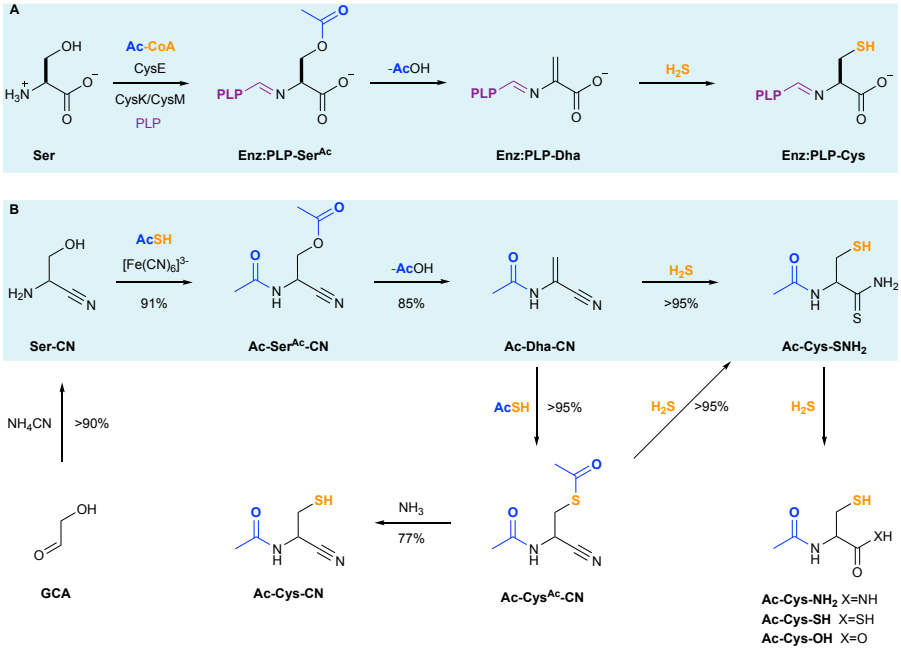

Fig. 1. Prebiotic cysteine synthesis. Biomimetic conversion of serinyl nitriles to cysteinyl nitriles. **A**. **Biosynthesis:** Pyridoxal-5′-phosphate (**PLP**)-dependent enzymatic cysteine synthesis pathway. **B. Prebiotic synthesis:** Nitrile activated synthesis of cysteines in water starting from **Ser-CN**, the stable Strecker product of **GCA**.

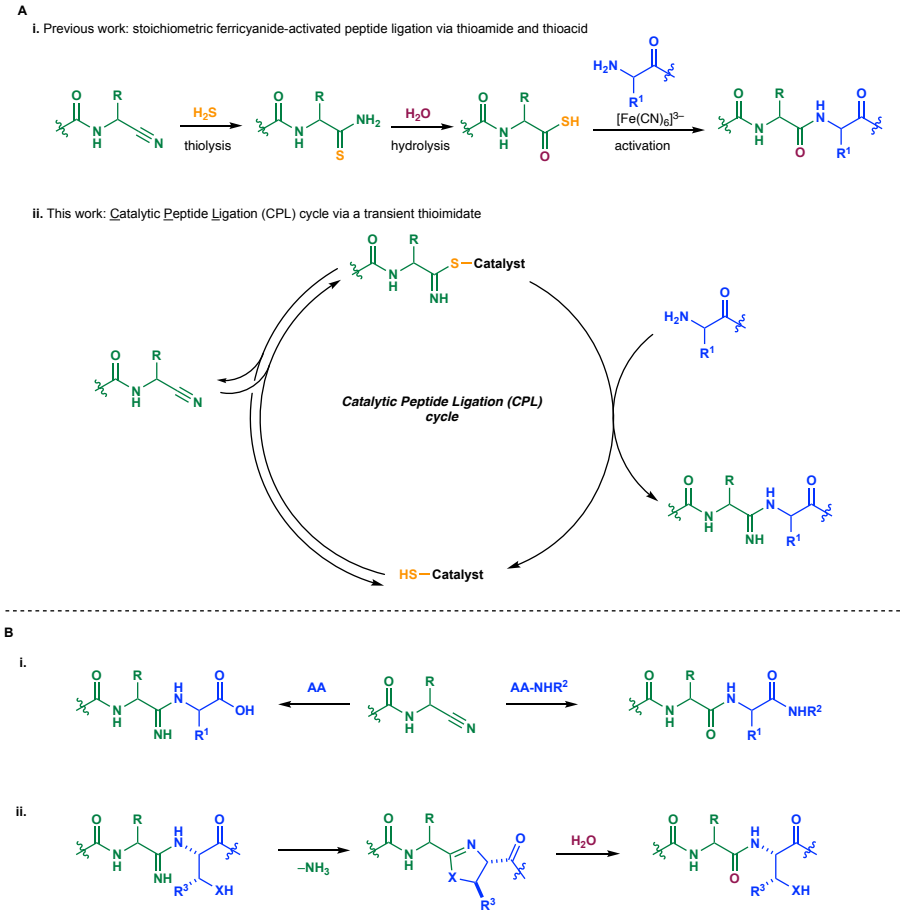

Fig. 2. Thiol-catalyzed peptide synthesis in neutral water. (A) i. Previous work: Stoichiometric ferricyanide-activated peptide thioacid ligation, which requires temporally separated thiolysis and activation steps (*4*). **ii. This work:** Thiol-catalyzed peptide ligation via a transient thioimidate intermediate that is chemoselectively intercepted by an amine nucleophile in water to form a peptidyl amidine directly from a stable nitrile without any activating agents. **(B) i.** Peptidyl amidines persist if α-amidonitriles are coupled with an α-amino acid (**AA**), except **Ser**, **Thr** and **Cys** (see Fig. 2Bii). Intramolecular amide-assisted hydroysis of the peptidyl amidine yields the native peptide bond (e.g. **Asn** or R^2=H or peptide). **ii.** The intramolecular cyclization of serinyl (R^3=H; X=O), threoninyl (R^3=CH_3; X=O), or cysteinyl (R^3=H; X=S) residues promote stereoretentive hydrolysis of peptidyl amidines to native peptides.

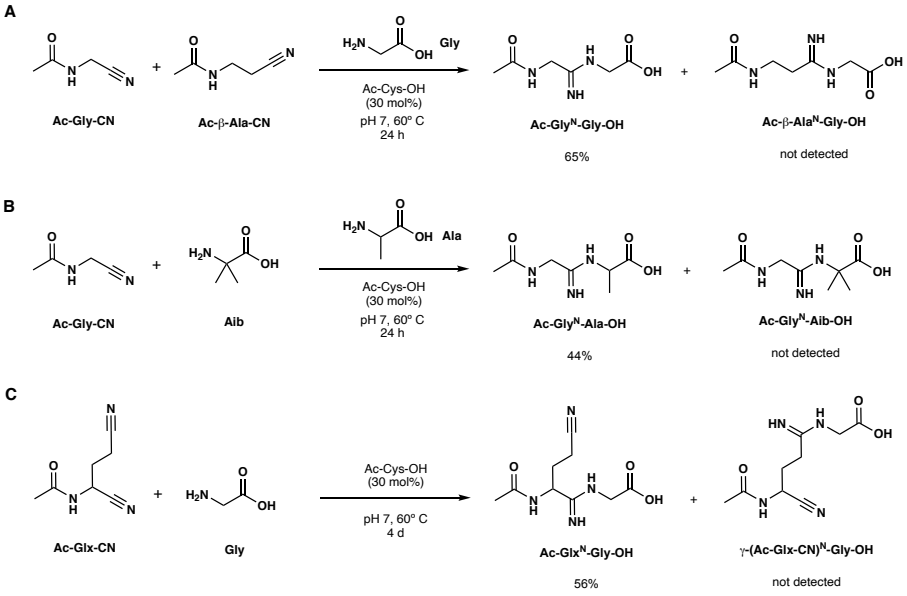

Fig. 3. Chemo- and regioselective organocatalytic proteinogenic α-peptide ligation. CPL selectively yields native α-peptidyl bonds. Selective ligation of: (**A**) **Ac-Gly-CN** (200 mM) with **Gly** (200 mM) affords **Ac-Gly^{N}-Gly-OH** in a stoichiometric competition with **Ac-β-Ala-CN** (200 mM). (**B**) **Ac-Gly-CN** (200 mM) with **Ala** (200 mM) to afford **Ac-Gly^{N}-Ala-OH** in a stoichiometric competition with **Aib** (200 mM). (**C**) **Ac-Glx-CN** (200 mM) with **Gly** (200 mM) to afford **Ac-Gly^{N}-Gly-OH**.

AA^1-OH	Ac-Gly^N-AA^1-OH (%)	Ac-Gly-AA^1-OH (%)	AA^1-NH_2	Ac-Gly^N-AA^1-NH_2 (%)	Ac-Gly-AA^1-NH_2 (%)
Gly	60	-	Gly	21	52
DL-Ala	43	-	D-Ala	3	63
Arg	37	-	Arg	14	56
Asn	9	45	Asn	-	72
Asp	58	-	Asp	6	58
Gln	56	-	Gln	-	43
Glu	58	-	Glu	-	64
His	73	-	His	-	67
Ile	55	-	Ile	12	47
Leu	53	-	D-Leu	5	54
Lys	70	-	Lys	25	52
DL-Met	72	-	Met	5	62
Phe	21	-	Phe	8	52
Pro	58	-	Pro	-	21
Ser	-	61	Ser	-	68
Thr	-	51	Thr	-	69
Trp	32	5	Trp	4	45
Tyr	20	-	Tyr	3	62
Val	42	6	Val	7	50

Table 1. Organocatalytic ligation of *N*-acetylglycine nitrile with α-amino acids and α-amino amides. Yields for **Ac-Cys-OH** (30 mol%) catalyzed formation of peptidyl amidines (**Ac-Gly^N-AA^1-X**) and peptides (**Ac-Gly-AA^1-X**) by coupling of **Ac-Gly-CN** (200 mM) with **AA^1-X** (1 equiv., pH 7, 60 °C, 24 h). See Supplementary Table S6 and S7 for improved yields by varying reaction conditions, and further examples with other α-amidonitriles **Ac-AA-CN**.

AA_n-OH	Ac-Gly_3-AA_n-OH (%)
Met-Gly	80
Ala-Ala-Ala	90
Ala-Gly-Ala	84
Gly-Ala-Gly	87
Gly-Gly-Gly	89
Gly-Gly-His	89
Leu-Leu-Leu	76[A]
Met-Ala-Ser	77
Phe-Gly-Gly	77[B]

Table 2. Organocatalytic peptidyl-nitrile to peptide fragment ligations. Yields for 3-mercaptopropionic acid (**MPA**; 160 mM) catalyzed ligation of **Ac-Gly_3-CN** (100 mM) and peptides **AA_n-OH** (100 mM, 24 h, 60 °C, pH 7.0). [A]48 h. [B]**Ac-Gly_2-Gly^N-Phe-Gly_2-OH** (12%) also observed. Total ligation yield 89%.