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Title: Prebiotically plausible chemoselective pantetheine synthesis in water

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Abstract: Coenzyme A (CoA) is essential to all life on Earth, and its functional subunit, pantetheine, is central to many origins of life scenarios, but how pantetheine emerged on the early Earth remains a mystery. Earlier attempts to selectively synthesize pantetheine failed, leading to suggestions that 'simpler' thiols must have preceded pantetheine at the origin of life. Here we report the first high-yielding prebiotic syntheses of pantetheine by routes that selectively yield its unique structure in water. Chemoselective multicomponent aldol, iminolactone and aminonitrile reactions deliver spontaneous differentiation of pantoic acid and proteinogenic amino acid syntheses, as well as the dihydroxyl, gem-dimethyl, and β-alanine-amide moieties of pantetheine in dilute water. Our results support the role of canonical pantetheine at the outset of life on Earth.

One-Sentence Summary: Multicomponent prebiotic nitrile chemistry is predisposed to yield pantetheine, a universally conserved constituent of cofactor CoA, in water.

Main Text: There are competing views concerning the nature of the chemistry that preceded life on Earth (*1-12*). However, inorganic and organic cofactors play an essential role in both biochemical and prebiotic reactions, so there is a strong consensus across all the conceptual divisions in prebiotic chemistry that cofactors must have played an important role at the origin of life (*2, 3, 5, 9, 13-21*). Coenzyme A (CoA) is unique amongst cofactors: not only is it universally conserved across all living organisms (*19, 22*) like adenosine triphosphate (ATP), RNA and proteins, but it also combines RNA and peptide structural elements within a linchpin of metabolism, making CoA a unique 'molecular fossil' that unites the 'RNA-', 'peptide-' and 'thioester world' hypotheses for the origins of life (*6, 10*).

CoA is the fulcrum about which metabolism turns (*19, 23, 24*). For example, CoA-thioesters drive anabolic pathways, including fatty acid, polyketide, and non-ribosomal peptide syntheses (*13, 24, 25*), and are so integral to ancient autotrophic carbon fixation pathways, including the reverse-Krebs cycle and acetyl-CoA pathway (*5, 9, 10, 23*), that thioester-based protometabolism (a 'thioester world') has been proposed to have paved the way to biochemistry (*2, 3, 13, 16, 21*).

CoA contains two distinct fragments: pantetheine **1** and a nucleotide (Fig. 1A). The nucleotide fragment may have been a later evolutionary modification of **1** (*14, 16*), however its ribozyme-catalyzed incorporation into RNAs (*26-28*) provides a mechanism for **1** to be recruited even prior to genetically coded enzymes and the advent of translation. CoA is also still incorporated into RNAs during the initiation of transcription in modern organisms (*29, 30*). Furthermore, CoA is one of the smallest known ribozymes (a 'coribozyme' (*28*)), that can catalyze prebiotic peptide ligation in water (*31*), and may be an ancient remnant of an RNA-based metabolism (*14, 17, 18*). However, pantetheine **1** is the crucial fragment that, for example, forms high-energy thioesters in enzyme active sites (*3, 13, 25*), whereas the nucleotide is a binding motif or is lost during the attachment of **1** to enzymes. Therefore, particular importance has been placed upon the functional thiol fragment of CoA, pantetheine **1**, acting as an organocatalyst for protometabolic reactions before its recruitment by genetically encoded enzymes (*2, 3, 16*).

Pantetheine biosynthesis is a complex multistep pathway that consumes methylene tetrahydrofolate, nicotinamide dinucleotides, several nucleoside triphosphates, and requires pyruvoyl- and flavin-dependent decarboxylases (*22, 32*). The multistep biosynthesis and structural complexity of pantetheine **1** have led to speculation that 'simpler' thiols may have fulfilled its essential role on the early Earth (*4, 5, 9, 10, 33, 34*). However, **1** is strictly conserved, suggesting it may have persisted from the onset of life. Accordingly, elucidating the prebiotic origin of pantetheine **1**, and why complex thiol **1** is pervasive in enzyme-catalyzed reactions across all domains of life, rather than other simpler thiols, is a key challenge for understanding the origins of life. For pantetheine **1** to be selected as a part of nascent metabolism, it must have been available and in plentiful supply. Therefore, we suspected that a high-yielding selective synthesis of **1** must be chemically predisposed.
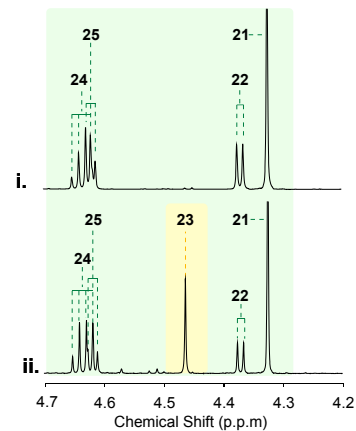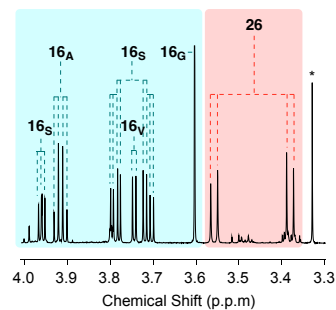
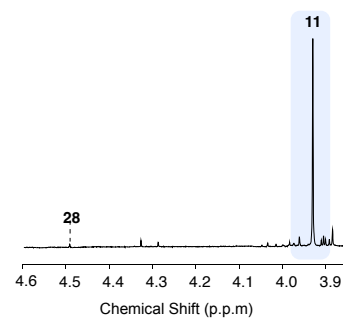## A. Coenzyme A
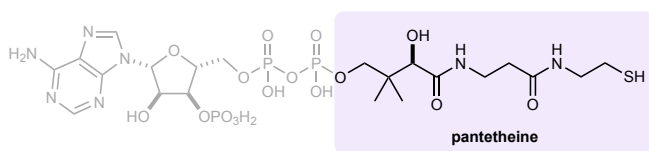
B. Nitrile mediated pantetheine synthesis
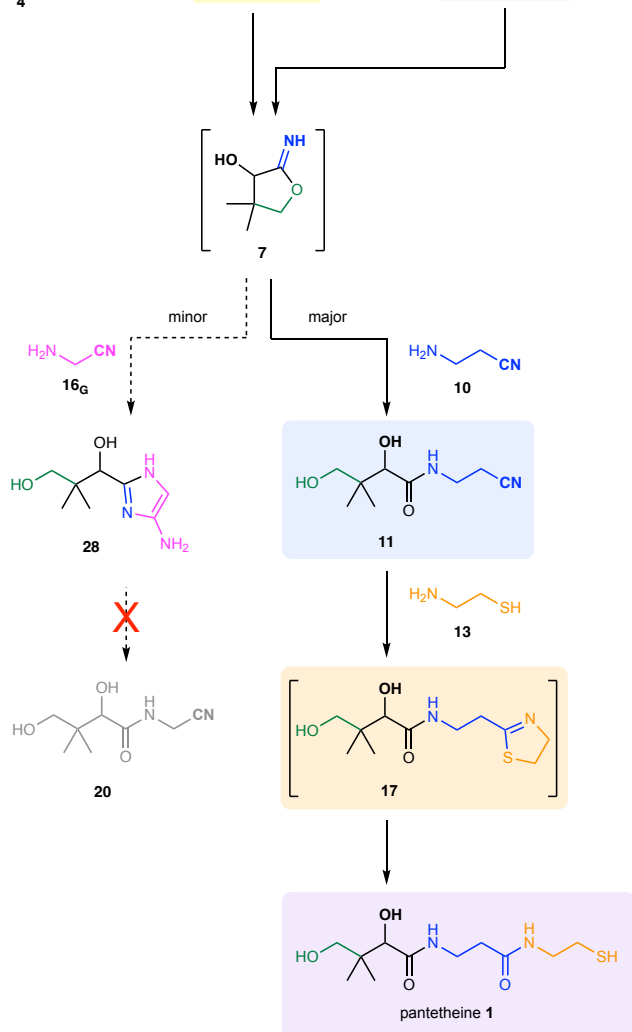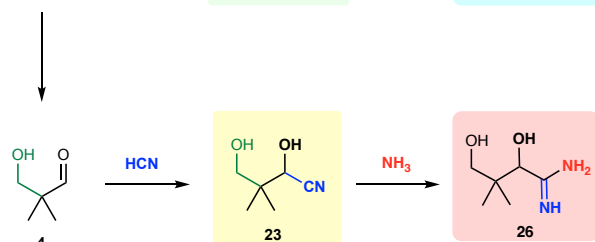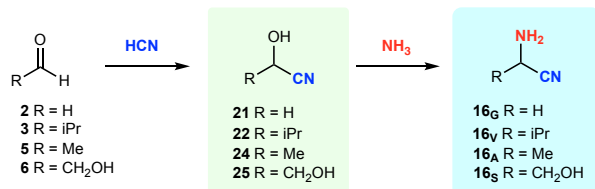
C. Chemoselective aldol

D. Spontaneous differentiation

E. β-nitrile >> α-nitrile selectivity

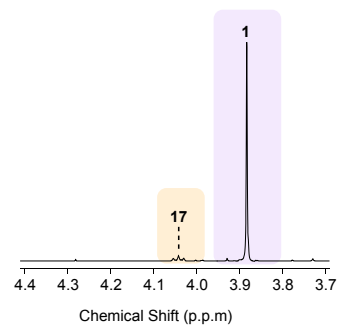## F. Quantitative cysteamine ligation

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Fig. 1. Overview of prebiotic pantetheine synthesis by nitrile-activation. (**A**) Coenzyme A. (**B**) Selective, high-yielding nitrile-mediated pathway to pantetheine **1**. ^{1}H NMR spectra showing selectivity of: (**C**) The aqueous aldol reaction of aldehydes **2** (22 mM), **3** (17 mM), **5** (22 mM) and **6** (22 mM) in PBS (pH 7, 500 mM) after: (i) 20 mins at 20ºC, followed by NaCN (300 mM) at 20ºC; (ii) 1 day at 60°C, followed by NaCN (300 mM) at 20ºC, yielding pantoic acid nitrile **23** (57%). (**D**) Pantoic acid and proteinogenic amino acid differentiation upon reaction of aldehydes **2** (20 mM), **3** (17 mM), **4** (20 mM), **5** (31 mM) and **6** (20 mM) with NH_3 (500 mM) and NaCN (150 mM) after 1 day in PBS (pH 9.5, 500 mM) at 20ºC, yielding proteinogenic aminonitrile **7$_G$**, **7$_A$**, **7$_V$**, **7$_S$** and pantoic acid amidine **26**. * = methanol. (**E**) Pantoylation during the stoichiometric competition of β-alanine-nitrile **10** (6.2 mM) and glycine nitrile **16$_G$** (6.2 mM) with aldehyde **4** (3.1 mM) and HCN (4.7 mM) in PBS (pH 9, 31 mM) at 20ºC after 3 days, yielding pantothenic acid nitrile **11** (44%). The non-canonical α-homolog **20** was not observed. (**F**) Activating-agent-free pantetheine synthesis upon reaction of pantothenic acid nitrile **11** (500 mM) with cysteamine **13** (2 equiv.) in PBS (pH 7, 500 mM) at 20°C after 60 days, yielding pantetheine **1** (93%).

Here we report selective prebiotic syntheses of pantetheine **1** (Fig. 1B) that harness the unique reactivity of the aldehyde and nitrile products of prebiotic hydrogen cyanide (HCN) reduction (*7, 12, 35*). Our multicomponent reaction pathways demonstrate that the neutral pH aldol reaction of glycine (**Gly**) and valine (**Val**) precursors (i.e., formaldehyde **2** and isobutyraldehyde **3**) selectively yield hydroxypivaldehyde **4**, even within mixtures of enolizable aldehydes (e.g., **2** + **3** + **5** + **6**; Fig. 1C). Furthermore, we found that the newly installed hydroxyl moiety of aldehyde **4** excludes it from undergoing a Strecker reaction (*7, 35*) to allow in-situ one-pot spontaneous chemical differentiation of α-hydroxy-pantoic acid and proteinogenic α-amino acids (Fig. 1D). Additionally, we discovered that the hydroxyl moiety of aldehyde **4** also promotes selective incorporation of the β-alanyl-motif of **1** via iminolactone **7** (Fig. 1E). This allows the less reactive lactone **8**, which has previously been proposed as a prebiotic reagent (*20, 36*), to be bypassed. By exploiting the more reactive iminolactone **7**, our synthesis of pantetheine **1** was achieved at extremely low concentration. For example, whereas lactone **8** (50 mM) was only observed to hydrolyze to pantoic acid **9** (Fig. 2A), the formation of iminolactone **7** (from 3 mM aldehyde **4** and HCN) and in-situ reaction with β-alanine-nitrile **10** yields pantothenic acid nitrile **11** as the major product. In addition to observing kinetic and thermodynamic β-alanyl-selectivity in these reactions to favor the canonical structure of pantetheine **1** over its homologs, the nitrile mediated iminolactone pathway also blocks access to non-canonical (undesired) α-analogues of pantetheine **1** that would otherwise arise preferentially from carboxylic acids (*20, 36*). Whilst there remain interesting questions with respect to the (subsequent) evolution of CoA biosynthesis, our results suggest nitrile reactivity could underpin the chemical selection of pantetheine **1** and proteinogenic peptides (*31, 37*), and provide support for the cyanosulfidic origins of life (*7, 8, 11, 12, 31, 35, 37*).

Results

By-passing β-alanine, wet-dry cycles, and electrophilic activation in water

Earlier attempts to uncover a prebiotic synthesis of pantetheine **1** (*20*) assumed the condensation of pantolactone **8** (*36*), β-alanine **12** (*1, 7, 36, 38, 39*), and cysteamine **13** (*39-44*) would generate pantetheine **1**. However, the reaction of lactone **8** with β-alanine **12** in water produced only very low yields of pantothenic acid **14** at high (>500 mM) concentration, and completely failed to yield **14** at low (<100 mM) concentration, due to preferential hydrolysis (Fig. 2A & table S2). Furthermore, electrophilic activation of pantothenic acid **14** led to fragmentation of **14** back to β-alanine **12**, lactone **8** and pantoic acid **9** (Fig. 2C). Importantly, at high concentration, where amino

acids can be acylated by lactone **8**, α-amino acid **Gly** reacts preferentially with lactone **8** over β-alanine **12** (Fig. 2B). Therefore, the reaction of lactone **8** with amino acids selectively yields non-canonical homolog **15**, and these carboxylic acid studies provide no rationale for the observed canonical structure of pantetheine **1** (*36*).

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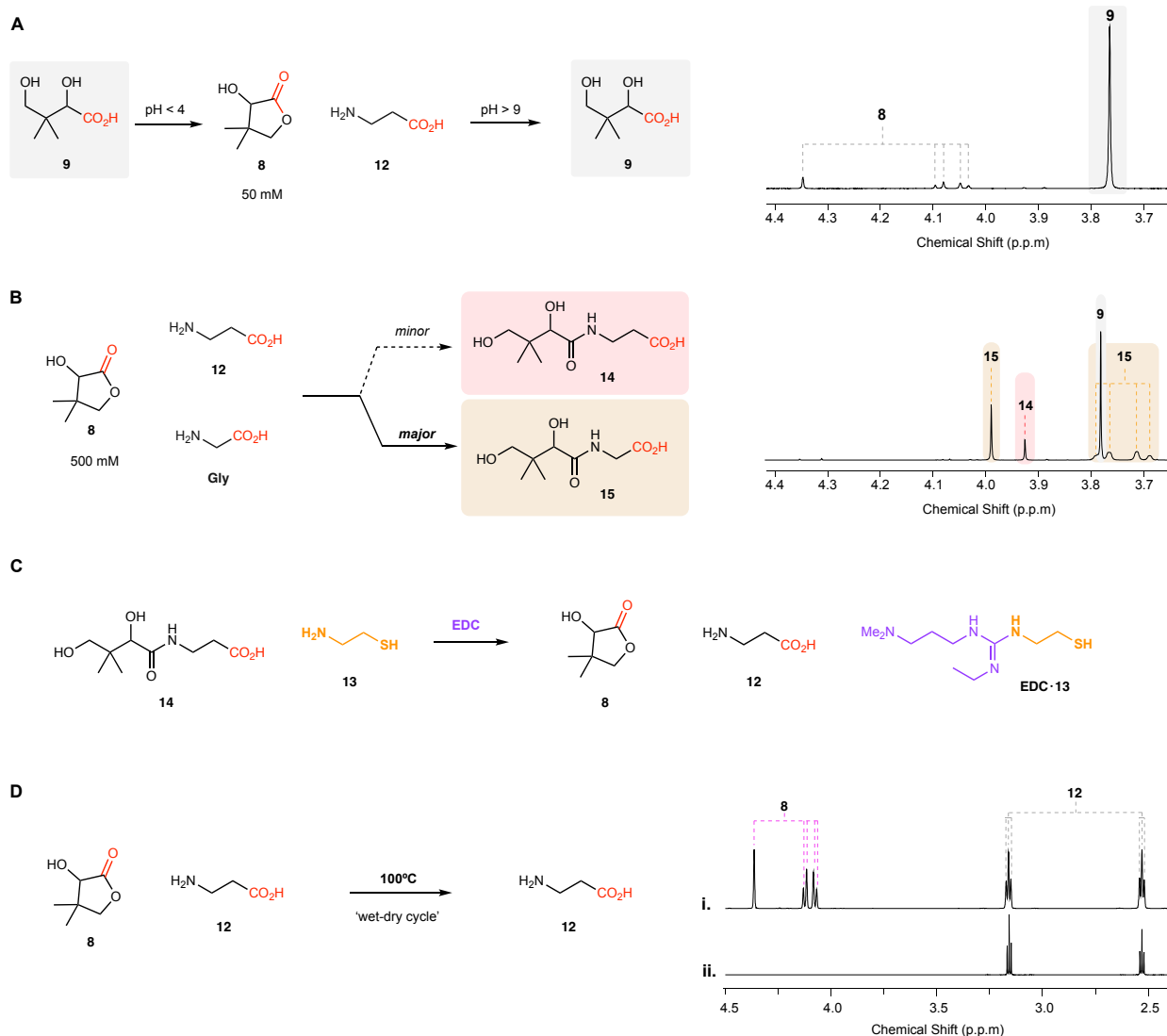
Fig. 2 Previous work: Failed carboxylic acid-mediated pathways to pantetheine. ^{1}H NMR spectra to show: (**A**) Incubating lactone **8** (50 mM) with β-alanine **12** (2 equiv.) in PBS (pH 9; 500 mM) at 20ºC gave hydrolysis product, pantoic acid **9** (94%) after 8 days. (**B**) Incubating lactone **8** (500 mM) with glycine **Gly** (2 equiv.) and β-alanine **12** (2 equiv.) in PBS (pH 9; 500 mM) at 20ºC gave hydrolysis product pantoic acid **9** (59%) and the non-canonical pantoyl-α-glycine **15** (29%) as the major products after 7 days, alongside only 11% of canonical pantothenic acid **14** as the minor product. (**C**) Failed coupling of cysteamine **13** and pantothenic acid **14** with model electrophilic activating agent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (**EDC**). Electrophilic activation of **14** with **EDC** resulted in fragmentation of pantoyl-amide **14**, whilst the rapid reaction of **13** with **EDC** blocks onward reaction and synthesis of pantetheine **1**. See Supplementary Text 3 for further details. (**D**) Failed synthesis of pantothenic acid **14** by wet-dry cycling. ^{1}H NMR spectra to show: (i) a solution of pantolactone **8** (500 mM) and β-alanine **12** (500 mM; pH 7.0), and (ii) products of this solution after a slow stream of air was passed over

the solution for 2.5 days at 20ºC, followed by heating the residue at 100°C for 24 hours and then dissolution in H_2O (1 mL), which revealed the sublimation of pantolactone **8** (>99%). See Supplementary Text 2 for further details.

In an attempt to overcome these problems, Miller and co-workers proposed a dry-state synthesis of pantetheine **1** (*20*). However, we found pantetheine **1** could only be detected in trace yield (<1%) in an artificially sealed reaction vessel (fig. S11). The reaction failed completely if dried lactone **8**, β-alanine **12**, and cysteamine **13** were not sealed in an airtight reaction vessel before heating (fig. S10) due to the sublimation of lactone **8** (Fig. 2D). To compound the problems of dry-state heating, we discovered pantothenic acid **14** decomposes to β-alanine **12** and pantoic acid **9** under hot-dry conditions (fig. S7). These results demonstrate that the dry-state synthesis of pantetheine **1** cannot be prebiotically plausible since it demands an artificially sealed reaction vessel (*20*). This led us to suspect that alternative prebiotic substrates were needed for the effective synthesis of pantetheine **1**.
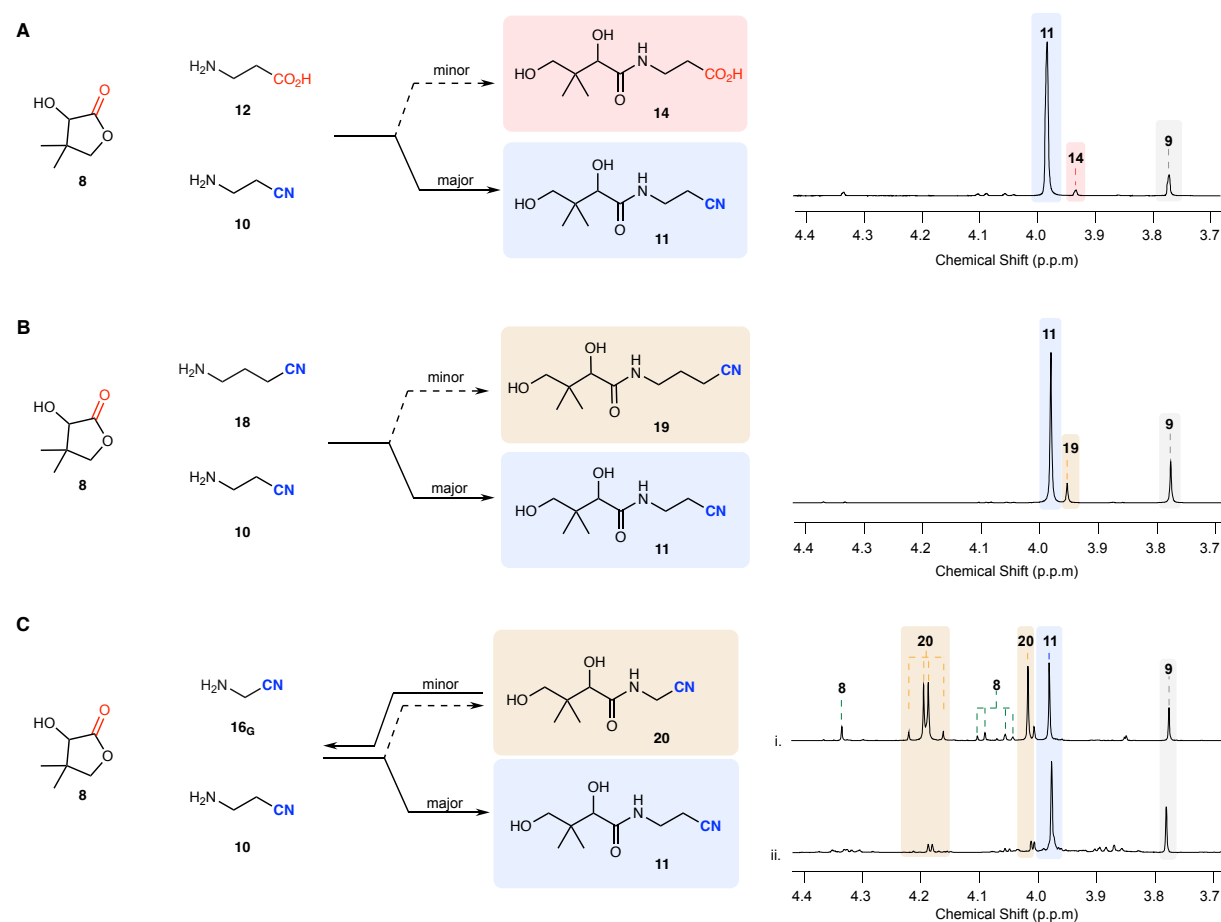
Fig. 3. Chemoselective pantoylation of aminonitriles. ^{1}H NMR spectra to show: (**A**) Incubating lactone **8** (500 mM) with β-alanine-nitrile **10** (2 equiv.) and β-alanine **12** (2 equiv.) in PBS (pH 9; 500 mM) at 20ºC yields pantothenic acid nitrile **11** (84%) as the major product after 2 days, alongside **9** (11%) and **14** (3%). (**B**) Incubating lactone **8** (500 mM) with β-alanine-nitrile **10** (2 equiv.) and γ-aminobutyric-acid-nitrile **18** (2 equiv.) in PBS (pH 9; 500 mM) at 20ºC yields pantothenic acid nitrile **11** (71%) as the major product after 2 days, alongside **9** (19%) and **19** (10%). (**C**) Incubating lactone **8** (500 mM) with β-alanine-nitrile **10** (2 equiv.) and glycine nitrile **16**G (2 equiv.) in PBS (pH 9; 500 mM) at 20ºC yields: (i) after 6 hours,

11/20(~1:1), and then (ii) after 6 days, canonical pantothenic acid nitrile **11** as the major product (**11/20**; >5:1).

Aminonitriles are prebiotic precursors of amino acids (*7, 35*), but their hydrolysis to amino acids dissipates the energy stored within the nitrile moiety. However, taking advantage of the latent nitrile activation of α-aminonitriles **16**, we recently reported a chemoselective synthesis of proteinogenic α-peptides in water (*31, 37*). These mechanisms by-passed α-amino acids to generate α-peptides without the electrophilic carboxylate-activation that would be necessary with amino acids. Electrophilic activation is not only incompatible with various proteinogenic amino acid side chains (*31, 37*), but is also incompatible with pantetheine **1** synthesis. To demonstrate this, our attempts to synthesize pantetheine **1** from cysteamine **13** and carboxylic acids **9**, β-alanine **12** and pantothenic acid **14** were thwarted by a myriad of detrimental reactions (fig. 2C & Supplementary Text S3). Chief amongst these problems were the incompatibility of cysteamine **13** with electrophilic carboxylate-activation (fig. S24) (*45*), and the fragmentation of pantothenic acid **14** (fig. S20). Reflection upon our recent α-peptide coupling strategies (*31, 37*) led us to suspect that latent nitrile-activation could be exploited to achieve pantetheine **1** synthesis and overcome these problems. We recognized that different chemistries would be required for selective pantetheine **1** synthesis, which contains an α-hydroxy-acid and a β-amino acid, rather than proteinogenic α-amino acids (*31, 37*). Specifically, we hypothesized that β-alanine-nitrile **10** (pK_{aH} = 7.8) would possess a key nucleophilic advantage over β-alanine **12** (pK_{aH} = 10.5) to allow selective coupling of β-alanine-nitrile **10** with lactone **8** to generate pantothenic acid nitrile **11** in water. Importantly, **11** would retain latent activation, within its nitrile moiety, which would allow its onward activating-agent-free reaction with cysteamine **13** to furnish pantetheine **1**.

To test the first element of our hypotheses we incubated lactone **8** with β-aminonitrile **10** in water. Pleasingly, we observed pantothenic acid nitrile **11** in up to 94% yield, but near-quantitative coupling required high (>100 mM) lactone **8** concentration (table S5). Nevertheless, incubating lactone **8** with equimolar β-alanine-nitrile **10** and β-alanine **12** returned 84% pantothenic acid nitrile **11**, alongside only 3% acid **14** (fig. 3A) in a clear demonstration of the superior reactivity of β-alanine-nitrile **10**.

Latent electrophilic nitrile activation

We next investigated the latent activation of pantothenic acid nitrile **11**. Despite their latent activation, we had previously observed β-alanyl-nitriles resisted reaction with thiol nucleophiles which blocked their unwanted incorporation into peptides by thiol-catalyzed peptide ligation (*31*). However, the ambident nucleophilicity of cysteamine **13**, and irreversible thiazoline formation, were found to switch on β-alanine-nitrile reactivity. Therefore, incubating pantothenic acid nitrile **11** with cysteamine **13** led to the formation of thiazoline **17** in good-to-excellent yield across a broad pH range (table S7). Furthermore, incubating pantothenic acid nitrile **11** with cysteamine **13** at neutral pH directly yielded pantetheine **1** (93%) (fig. S33). Therefore, in three high-yielding activating-agent-free steps, pantetheine **1** was produced through the remarkable nucleophilicity of β-alanine-nitrile **10** and the latent electrophilicity of pantothenic acid nitrile **11** in water.

Nitrile-controlled β-alanyl-selective pantoylation

Pantetheine **1** possesses a unique β-alanyl-motif, so we next questioned whether lactone **8** would discriminate β-alanine-nitrile **10** from its shorter and longer homologs, α-aminonitrile **16_G** and γ-aminonitrile **18** (Fig. 3). γ-Aminonitrile **18** (pK_{aH} = 10.2) is substantially more basic than β-alanine-nitrile **10**, and so **18** did not effectively couple with lactone **8**. Indeed, the reaction of lactone **8** with equimolar **10** and **18** selectively produced pantothenic acid nitrile **11** (71%), alongside only 10% of γ-homolog **19** (Fig. 3B). Under the same conditions, we observed the reaction of lactone **8** with equimolar β-alanine-nitrile **10** and glycine-nitrile **16_G** produced nearly equal amounts of pantothenic acid nitrile **11** and pantoyl-α-glycyl-nitrile **20** after 6 hours. However, upon further incubation an unanticipated equilibration yielded nitrile **11** as the major product (**11/20**; >5:1) after 6 days (Fig. 3C). This dynamic reactivity was confirmed by incubating isolated nitrile **20** with β-alanine-nitrile **10**, which yielded pantothenic acid nitrile **11** in up to 93% yield (table S10). These results demonstrate the reactivity of β-alanine-nitrile **10** markedly favors the synthesis of the canonical structure of pantetheine **1** over both shorter and longer homologs in water.
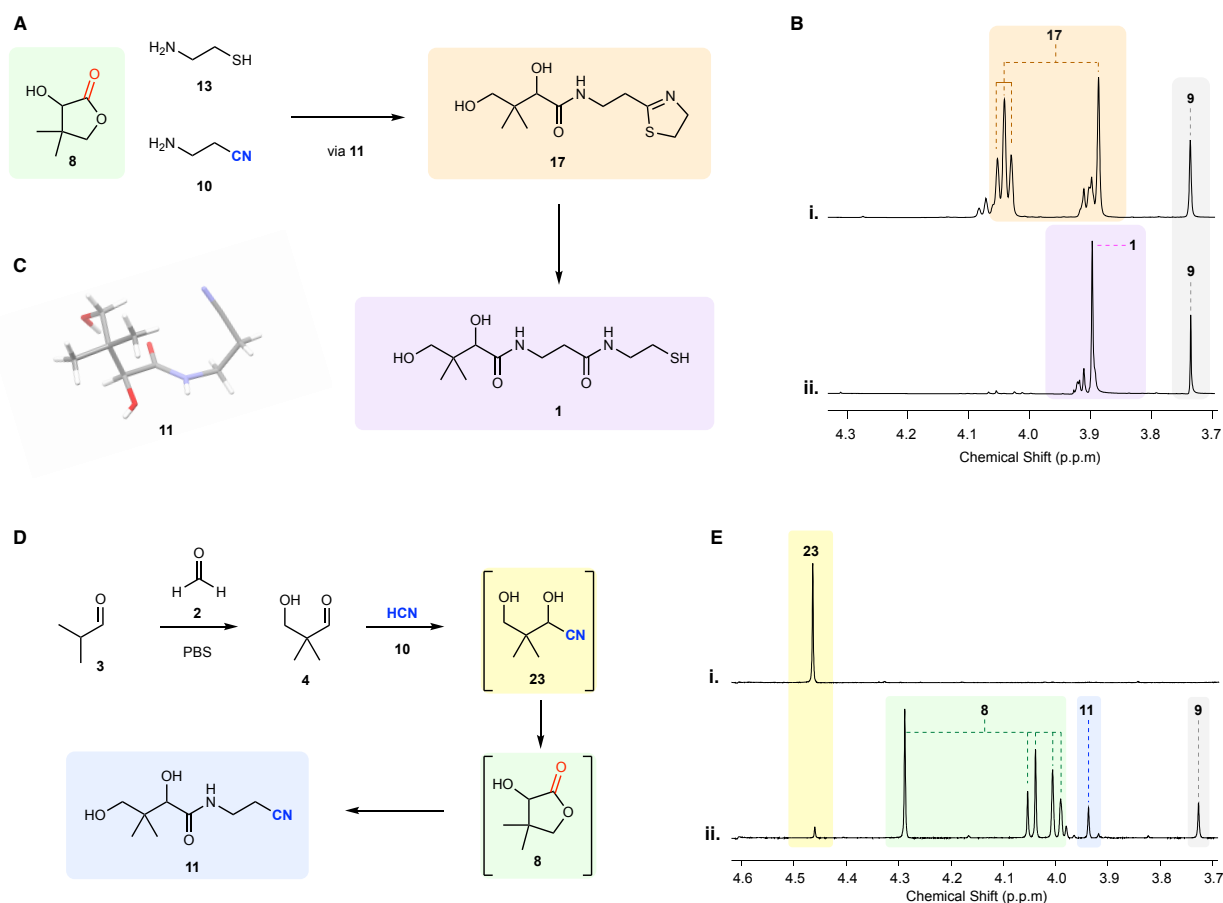
Fig. 4. Multicomponent syntheses of pantetheine via lactone 8. (A) One-pot multicomponent reaction of lactone **8**, β-alanine-nitrile **10**, and cysteamine **13** yields pantetheine **1** in water. (**B**) ^{1}H NMR spectra of lactone **8** (500 mM), **10** (2 equiv.) and **13** (2 equiv.) in PBS (pH 9, 500 mM) at 20ºC which, (i) after 4 days yields **17** (57% from **8**); and then (ii) after in-situ (rapid) hydrolysis of **17** at pH 4, yields pantetheine **1** (57% from **8**). For experimental details see Supplementary Pages S64–S66. (**C**) Single crystal x-ray structure of pantothenic acid nitrile **11**. (**D**) Aldol-product hydroxypivaldehyde **4** reacts in-situ with HCN and β-alanine-nitrile **10** to yield pantoic acid nitrile **23** which undergoes intramolecular γ-hydroxyl-catalyzed interrupted nitrile hydrolysis to yield pantolactone **8**. This locks the final carbon atom of pantoic

acid's carbon-framework into lactone **8**, whilst retaining chemical activation towards amide-bond formation. (**E**) ^{1}H NMR spectra of **4** (3.1 mM), **10** (6.3 mM) and NaCN (3.4 mM) in PBS (pH 7; 31 mM) after: (i) 10 mins, and (ii) 11 days at 20°C, yielding lactone **8** (54% from **4**) and pantothenic acid nitrile **11** (13% from **4**). For experimental details see Supplementary Pages S113–S115.

One-pot multicomponent synthesis of pantetheine from pantolactone in water

We next investigated the one-pot multicomponent synthesis of pantetheine **1** (Fig. 4A). Incubating lactone **8** (500 mM) with β-alanine-nitrile **10** (2 equiv.) and cysteamine **13** (2 equiv.) yielded thiazoline **17** (57%) after 3 days at pH 9 (Fig. 4B). Moreover, incubating lactone **8** with β-alanine-nitrile **10**, cysteamine **13** and glycine-nitrile **16$_G$** under the same conditions furnished thiazoline **17** (33%) as the major pantoyl-amide product (fig. S52–S53). During this four-component reaction, the rapid reaction of glycine-nitrile **16$_G$** with cysteamine **13** suppressed the reaction of the α-aminonitrile with lactone **8**. This favored the addition of β-alanine-nitrile **10** to lactone **8**. Remarkably, we also observed cysteamine **13** was released back into solution to drive thiazoline **17** synthesis. Thiazoline **17** synthesized in these multicomponent reactions was observed to hydrolyze in near-quantitative yield at neutral or acidic pH (pH 7 – 4) to yield pantetheine **1** (up to 57% yield from lactone **8**). Our results demonstrate a nitrile-directed multicomponent synthesis of pantetheine **1** from lactone **8** in water – albeit at high concentration. However, we suspected that further investigation of the role of nitriles in pantoic acid synthesis would resolve the apparent need for high reagent concentrations. Therefore, we turned our attention to the origins of pantoic acid precursors from the aldehydes generated by prebiotic reduction of HCN (*7, 12*).

Chemoselective aldol synthesis of hydroxypivaldehyde and pantolactone at neutral pH

We found that the conversion of formaldehyde **2** and isobutyraldehyde **3** to hydroxypivaldehyde **4** was highly effective at neutral pH and catalyzed by phosphate (*46, 47*). For example, incubation of **2** (22 mM) and **3** (17 mM) in phosphate buffer solution (PBS) at pH 7 gave hydroxypivaldehyde **4** (94%) after 2 days at 60ºC (fig. S61). To test the selectivity of this aldol reaction, we next incubated **2**, **3**, and another enolizable aldehyde, acetaldehyde **5**, at pH 7. We again observed the formation of **4** (94%) after 2 days, but now alongside quantitative recovery of acetaldehyde **5** (fig. S64). Finally, incubation of **2**, **3**, acetaldehyde **5** and glycolaldehyde **6** also yielded hydroxypivaldehyde **4** as the major aldol product (fig. S67–70, table S25), with excellent recovery of acetaldehyde **5**. Interestingly, partial conversion of glycolaldehyde **6** to dihydroxyacetone – which is a C3-sugar precursor of nucleic acids, amino acids, and lipids (*7, 12, 35*) – was also observed (table S25). Subsequent addition of HCN led to in-situ quantitative conversion of aldehydes **2-6** to their respective cyanohydrins **21-25** (fig. S67–70), however, continued incubation under the same conditions provided a mild one-pot conversion of cyanohydrin **23** to lactone **8** (54% from hydroxypivaldehyde **4**) (Fig. 4E, fig. S80). Encouraged by the facile synthesis of lactone **8**, we next investigated the chemoselectivity required to integrate pantoate and proteinogenic α-aminonitrile syntheses (*7, 35*).

Differentiation of pantoate from proteinogenic α-aminonitriles and in-situ formation of pantoyl-amides

The selective concurrent synthesis of pantoate and proteinogenic α-aminonitriles **16** represents an intrinsic challenge because the pantoate precursor, hydroxypivaldehyde **4**, must not form an α-aminonitrile. The aminonitrile of hydroxypivaldehyde **4** would have an α-amine, not the canonical pantoate α-hydroxyl moiety. Conversely, at the same time and under the same conditions, amino

acid precursors aldehydes **2**, **3**, **5** and **6** must form α-aminonitriles **16** that possess an α-amine moiety necessary for proteinogenic α-peptide synthesis (*31, 37*). Remarkably, we found incubating aldehydes **2-6** under Strecker conditions (*7, 35*) with cyanide and ammonia led to the complete differentiation of peptide and pantoate precursors (fig. S73). Chemoselective proteinogenic α-aminonitrile **16$_G$**, **16$_V$**, **16$_A$**, and **16$_S$** formation was observed, but hydroxypivaldehyde **4** was crucially excluded from α-aminonitrile synthesis by rapid formation of α-hydroxy-amidine **26**. This spontaneous differentiation of proteinogenic α-amino acid and pantoate syntheses demonstrates the required reactivity to selectively deliver the α-hydroxyl moiety of pantetheine **1**.

Importantly, the differentiation of hydroxypivaldehyde **4** implicated iminolactone **7** as an intermediate and suggested a mechanism to overcome the high-concentration requirements for pantetheine **1** synthesis from lactone **8**. We anticipated that HCN addition to a mixture of hydroxypivaldehyde **4** and β-alanine-nitrile **10** would initiate a reaction cascade that would generate **7**, which would then be intercepted by β-alanine-nitrile **10** to yield pantothenic acid nitrile **11** (Fig. 5). We envisaged that this multicomponent reaction would streamline pantetheine **1** synthesis by creating new carbon-carbon and amide bonds, as well as bypassing the less electrophilic lactone **8**, in a single step and at low concentration.

To investigate this hypothesis, we next monitored the reaction of aldehyde **4** and HCN across a broad pH range. We observed transient formation of iminolactone **7** between pH 7.5–9.8 (tables S15–S21). Furthermore, the multicomponent reaction of aldehyde **4**, β-alanine-nitrile **10** and HCN furnished pantothenic acid nitrile **11**. The optimal yield of **11** was observed between pH 9–9.5 (tables S27–S28), where the optimal formation of iminolactone **7** was also observed. At lower pH the synthesis of **11** was slower and more pantolactone **8** was observed. However, pleasingly, nitrile **11** synthesis was observed across a broad concentration range (1.6–100 mM); for example, incubating hydroxypivaldehyde **4** (3.1 mM), β-alanine-nitrile **10** (2 equiv.) and HCN (1.5 equiv.) in PBS (pH 9, 31 mM) returned pantothenic acid nitrile **11** in 44% yield (fig. S77). This demonstrated the enhanced electrophilicity of iminolactone **7** and provides a new mechanism for pantoyl-amide bond formation that is effective even at high dilution.

Next, it was essential to establish that the conditions for α-aminonitrile **16** syntheses were compatible with pantothenic acid nitrile **11** formation, and that amidine **26** formation did not block synthesis of pantothenic acid nitrile **11** from iminolactone **7**. Surprisingly, the in-situ addition of β-alanine-nitrile **10** to crude amidine **26** yielded pantothenic acid nitrile **11** (64% from hydroxypivaldehyde **4**) through intramolecular γ-hydroxyl-catalyzed transamidation, even in the presence of a large (25 equiv.) excess of ammonia (Fig. 5D & table S28). The subsequent addition of cysteamine **13** and hydrolysis of the resulting thiazoline **17** – still in the presence of ammonia – resulted in the in-situ conversion of pantothenic acid nitrile **11** to pantetheine **1** (51% from hydroxypivaldehyde **4**; fig. S90).

Blocking non-biological pantoyl-α-amino acid analogs

The synthesis of pantothenic acid nitrile **11** via iminolactone **7** suggested an inherent mechanism to block the synthesis of (non-canonical) α-homologs by intramolecular (5-exo-dig) cyclization (i.e., **27** to **28**; Fig. 5). Therefore, we carried out competition reactions with β-alanine-nitrile **10** and α-aminonitrile **16$_G$** in anticipation that pantothenic acid nitrile **11** would emerge as the only pantoyl-amide capable of onward reaction with cysteamine **13**, and thus would selectively yield pantetheine **1**. Pleasingly, the reaction of aldehyde **4**, β-alanine-nitrile **10**, glycine-nitrile **16$_G$**, and cyanide resulted in a highly chemoselective formation of pantothenic acid nitrile **11** (44% from

aldehyde **4**). Only a trace yield of aminoimidazole **28** (<4%) was observed (Fig. 5C) and, importantly, pantoyl-α-glycine-nitrile **20** was not detected. Under comparable conditions, the competition of amino acids (i.e., **Gly** and **12**) not only resulted in poor coupling yields, but also favored the synthesis of non-canonical pantoyl-α-glycine **15** (16%) over the canonical pantothenic acid **14** (9%) (Fig. 5B). These results demonstrate that the reaction of iminolactone **7** with amino acids disfavors pantothenic acid **14** synthesis, whereas the reaction of **7** with aminonitriles overwhelmingly favors the synthesis of the canonical pantothenic acid nitrile **11**. The reaction of **7** with aminonitriles also irrevocably blocks the synthesis of non-biological pantetheine analogues by a mechanism unique to aminonitriles. Therefore, nitrile reactivity provides the selectivity essential for pantetheine **1** synthesis by routes that unequivocally account for the chemical basis of the β-alanyl fragment of pantetheine **1**.
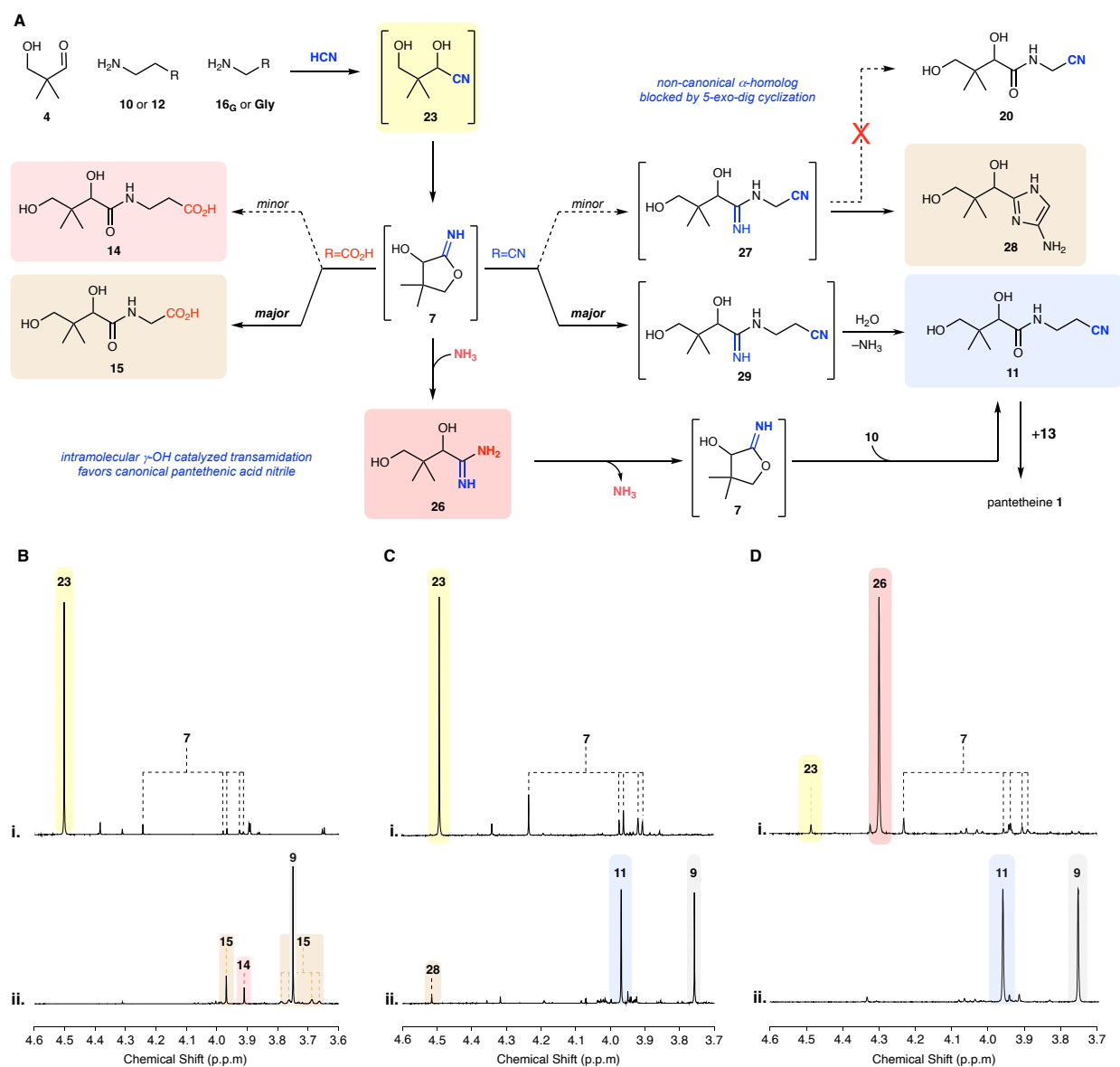

Fig. 5. Chemoselective multicomponent reaction cascades. **(A) Nitriles (R=CN):** Incubating hydroxypivaldehyde **4**, β-alanine-nitrile **10**, glycine-nitrile **16_G** and HCN selectively yields canonical

pantothenic acid nitrile **11**. α-Homolog **20** synthesis is blocked by cyclization of amidine **27**. Incubating aldehyde **4**, HCN and NH_3 yields α,γ-dihydroxyamidine **26**, which undergoes selective transamidation with β-alanine-nitrile **10** to yield canonical pantothenic acid nitrile **11**. **Amino acids (R=CO₂H):** Incubation of aldehyde **4**, β-alanine **12**, glycine **Gly** and HCN selectively yields non-natural **15**, not canonical pantothenic acid **14**. ^{1}H NMR spectra of: (**B**) **4** (3.1 mM), **12** (6.3 mM), **Gly** (6.3 mM) and NaCN (4.7 mM) in PBS (pH 9, 31 mM) at 20°C after (i) 10 mins and (ii) 8 days, yielding **9** (65%), **15** (16%) and **14** (9%). (**C**) **4** (3.1 mM), **10** (6.2 mM), **16G** (6.2 mM) and HCN (4.7 mM) in PBS (pH 9, 31 mM) at 20°C after (i) 10 mins and (ii) 3 days, yielding **11** (44%). See fig. S85 for spectra that demonstrate β-alanine-nitrile **10** outcompetes β-alanine **12** (2:1) in a direct stoichiometric competition. (**D**) **4** (20 mM), NaCN (30 mM) and NH_3 (500 mM) in PBS (pH 9.5, 500 mM) at 20ºC, after (i) 4.5 hours, yielding **26** (82%), followed by (ii) the addition of **8** (40 mM) yielding **11** (46% from **4**) after 6 days. See fig. S90 for one-pot synthesis of pantetheine **1** via **26**.

Discussion

We have discovered a series of reactions that are exclusive to prebiotic nitrile chemistry that contribute to the synthesis of pantetheine **1**, the key functional component of CoA, with unprecedented selectivity over non-biological homologs. By querying the chemical relationship of pantetheine **1** to Strecker aldehyde precursors of α-peptides, we have discovered the phosphate-catalyzed reaction of formaldehyde **2** and isobutyraldehyde **3** (the Strecker precursors of **Gly** and **Val** (*7, 12, 35*)) yields hydroxypivaldehyde **4** in the first step towards **1**. This aldol condensation is highly effective at low concentration and neutral pH, even within mixtures that include other enolizable aldehydes. The reaction of aldol-product **4** with HCN and β-alanine-nitrile **10** selectively generates pantothenic acid nitrile **11**, even in direct competition with α-aminonitriles **16**. No activating agents are required for the synthesis of pantetheine **1** by nitrile chemistry; latent nitrile-activation is preinstalled within pantoic acid nitrile **23** and pantothenic acid nitrile **11**. Moreover, pantoyl-amide formation requires no external catalysis because the ideally poised γ-hydroxyl moiety of pantoic acid nitrile **23** is an intramolecular nucleophilic catalyst for amide bond formation. The γ-hydroxyl of pantoic acid nitrile **23** also blocks α-aminonitrile synthesis and provides a highly selective mechanism to differentiate proteinogenic α-aminonitriles from α-hydroxy-pantoate derivatives. Although pantoic acid nitrile **23** was transformed into pantoic acid amidine **26** under Strecker conditions (necessary for α-aminonitrile **16** synthesis), the γ-hydroxyl-catalyzed transamidation of ammonia with β-alanine-nitrile **10** yielded pantothenic acid nitrile **11**, even in the presence of a large excess of ammonia. Collectively, our results suggest that the chemical origins of pantetheine **1** are best rationalized through prebiotic nitrile, not carboxylic acid, chemistry.

The selective syntheses of pantetheine **1** in water challenges the persistent dogma that, despite it being the 'solvent of life' (*48*), water is problematic (or even a 'poison') for prebiotic chemistry (*49*). We observed highly effective nitrile-activated amide bond formations in water, even below physiological CoA concentrations within modern cells (*50*). This chemistry not only favors the canonical structure of **1** but also closely aligns with previously reported prebiotic pathways to α-peptides, RNA, and lipids (*7, 12, 31, 35, 37*). Therefore, our results suggest that **1** would have been a product of cyanosulfidic reaction pathways prior to the emergence of life on Earth (*7, 12*). Once available, it is simple to envisage how pantetheine **1** could have been deployed at the origins of life, for example, as a (nucleotide-coded) catalyst or cofactor to enhance the functional limitations of early ribozymes (*14, 26, 28*) or peptide catalysts (*31*). This would mirror its essential role in

augmenting the functional repertoire of enzymes in extant biochemistry (*14, 17, 22*), and provide a mechanism to couple **1** to the evolutionary development of life.

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Supplementary Materials

Materials and Methods

Supplementary Text

Figs. S1 to S118

Tables S1 to S29

References (*51–71*)