

Prebiotic Catalytic Peptide Ligation Yields Proteinogenic Peptides by Intramolecular Amide Catalyzed Hydrolysis Facilitating Regioselective Lysine Ligation in Neutral Water

Jyoti Singh,[†] Daniel Whitaker,[†] Benjamin Thoma, Saidul Islam, Callum S. Foden, Abil E. Aliev, Tom D. Sheppard, and Matthew W. Powner^{**}



Cite This: *J. Am. Chem. Soc.* 2022, 144, 10151–10155



Read Online

ACCESS |



Metrics & More



Article Recommendations



Supporting Information

ABSTRACT: The prebiotic origin of catalyst-controlled peptide synthesis is fundamental to understanding the emergence of life. Building on our recent discovery that thiols catalyze the ligation of amino acids, amides, and peptides with amidonitriles in neutral water, we demonstrate the outcome of ligation depends on pH and that high pK_a primary thiols are the ideal catalysts. While the most rapid thiol catalyzed peptide ligation occurs at pH 8.5–9, the most selective peptide ligation, that tolerates all proteinogenic side chains, occurs at pH 7. We have also identified the highly selective mechanism by which the intermediate peptidyl amidines undergo hydrolysis to α -peptides while demonstrating that the hydrolysis of amidines with nonproteinogenic structures, such as β - and γ -peptides, displays poor selectivity. Notably, this discovery enables the highly α -selective protecting-group-free ligation of lysine peptides at neutral pH while leaving the functional ϵ -amine side chain intact.

Peptide synthesis is one of the most important processes in chemistry and biology.¹ Peptide biosynthesis is a highly evolved system,^{2,3} that could not have spontaneously appeared in its current form,⁴ but what nonenzymatic chemistry preceded it and how these reactions influenced the structure of biological peptides remains unknown. We recently reported that α -peptidyl nitriles **1** are activated for biomimetic peptide synthesis⁵ and that the in-built reactivity of **1**⁶ can drive catalytic peptide ligation (CPL; Figure 1). CPL requires no activating agent to ligate **1** with amino acid derivatives (**2**)^{5b} and is a rare example of organocatalysis in water.⁷ The nitrile's kinetic stability means ligation must be thiol catalyzed, and so catalyst-gated reactivity, which is an essential feature of biochemistry, is observed. As a mechanism for prebiotic peptide synthesis CPL has several appealing characteristics: it uses simple prebiotic reactants; is selective for α -amidonitriles, and therefore proteinogenic peptides; generates high peptide yields under conditions where peptides are very stable;⁸ and is orthogonal to (biological) phosphate activation,⁹ which would in principle enable independent catalytic modulation of both peptide and nucleic acid synthesis. Intriguingly, CPL produces amidines **3** when amino acids are the nucleophilic coupling partner (Figure 1; **2**, X = OH), whereas peptides **5'** are formed when α -amino amides or peptides are used (Figure 1; **2'**, X = NHR⁴).^{5b} With this in mind, we set out to explore the conditions under which CPL delivers the highest selectivity for α -peptide formation.¹⁰

Our preliminary study of CPL focused on reactions at neutral pH, but we envisaged that pH would have a profound effect on CPL, as the nucleophile and catalyst could both deprotonate at higher pH. Pleasingly, we observed that ligation of alanine (H-Ala-OH, **2_A**) to nitrile **1** catalyzed by Ac-Cys-OH (**6a**) is more rapid at pH 8.5 than at pH 7 (Table 1). In

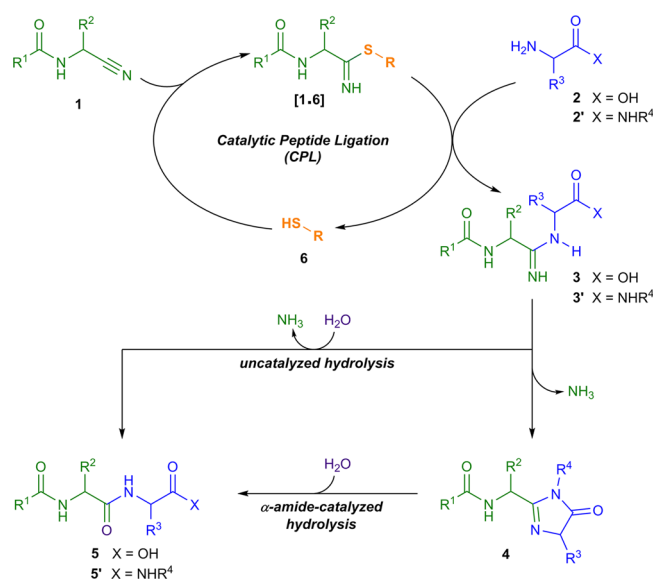
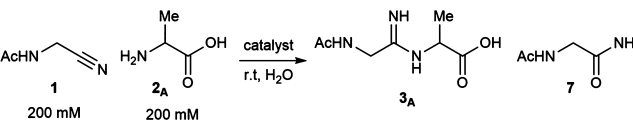






Figure 1. Catalytic peptide ligation (CPL) in water. Thiol-catalyzed coupling of peptide nitriles (**1**) with amines (**2**, X = OH or **2'**, X = NHR⁴). R = alkyl or aryl; R¹ = peptide or alkyl; R² and R³ = aminoacyl side chain; R⁴ = H or peptide; X = OH, NH₂ or peptide. Compounds **2**–**5** and **2'**–**5'** are labeled with subscripts corresponding to the single letter amino acid code.

Received: April 1, 2022

Published: May 31, 2022



Table 1. Effect of pH and Catalyst on Amidine 3 Formation¹⁰


Entry	Catalyst (30 mol%)	Thiol pK _a	pH	3 _A / % 36 h	3 _A / % 120 h
1			5	0	0
2	 6a	9.7	7	42	85
3			8.5	69	85
4			10	27	28
5	 6b	10.8	8.5	90	85
6			10.2	85	85
7	 6d	6.6	8.5	4	13
8			8.4	8.5	41
9	 6f	10.3	8.5	60	78
10			9.2	8.5	64

line with our prediction, we observed negligible reactivity at pH 5 but, surprisingly, slow and low yielding CPL at pH 10. This is likely due to suppressed thioimidate [1•6] protonation at pH 10. Accordingly, the optimal rate for 6a-catalyzed CPL was observed at pH 8.5–9.0.

3-Mercaptopropionic acid (6b) and thioglycolic acid (6c) promoted CPL faster than 6a, giving 85–90% amidine (3_A) after 36 h, at pH 8.5 and rt (Table 1). Low pK_a thiols (e.g., 6d and 6e) are sluggish, with 6d only furnishing 4% amidine 3_A after 36 h. Sterically hindered 6f also retarded the rate of CPL. Limited hydration of 1 to Ac-Gly-NH₂ (7) (~5%) was observed with most catalysts (Figure S12), but 6g yielded significant amide 7 (25%). We suspect 6g undergoes S-to-O acyl transfer, followed by thiirane formation, leading to 7 (Figures S13 and S14).¹¹ These results demonstrate high-pK_a primary thiols are best suited as CPL-catalysts. This stands in stark contrast to thioester ligations (e.g., native chemical ligation), which are accelerated by low pK_a thiols such as 6d.¹²

High amidine 3 yields (82–95%) were observed in H-Gly-OH (2_G), H-Ala-OH (2_A), H-Leu-OH (2_L), H-Ile-OH (2_I), H-Phe-OH (2_F), H-Met-OH (2_M), H-Val-OH (2_V), H-Arg-OH (2_R), H-Glu-OH (2_E), H-Asp-OH (2_D), H-Gln-OH (2_Q), H-Trp-OH (2_W), H-Pro-OH (2_P), and H-His-OH (2_H) couplings (Figures S16–S51). H-Cys-OH (2_C) coupling does not require catalysis, due to the thiol side chain.^{5b} H-Lys-OH (2_K, 86%) exhibited moderate α-selectivity (2:1, α/ε) with 60% α-amidine formation (Figure S37), while β-hydroxy amino acids (i.e., H-Ser-OH, 2_S and H-Thr-OH, 2_T) yielded peptides rather than amidines. We have postulated that this is due to the formation and hydrolysis of oxazoline 8 (Figure 2A). Here, at room temperature, we observed 8 as the major product (8_S (55%) and 8_T (67%); Figures S45 and S49). Oxazoline 8 formed rapidly from nitrile 1 and 2_S or 2_T, but its

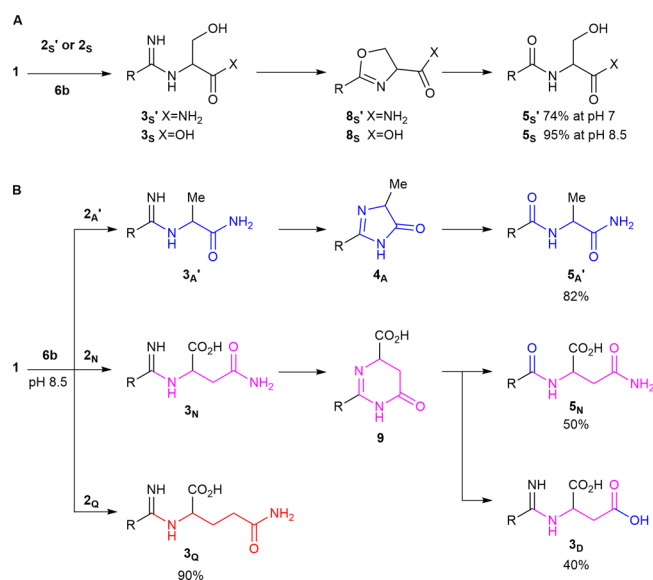


Figure 2. Intramolecular catalysis of amidine hydrolysis. (A) Coupling of nitrile 1 (200 mM) with serinamide (2_S', 2 equiv) yields peptide 5_S' at pH 7, via oxazoline 8, whereas serine (2_S, 1 equiv) yields peptide 5_S at pH 7 (ref 5b) or pH 8.5. (B) Coupling of 1 (200 mM) with alaninamide (2_A', 2 equiv), asparagine (2_N, 1 equiv), glutamine (2_Q, 1 equiv) demonstrates the effect of α-, β-, and γ-amides amidine hydrolysis during CPL. R = CH₂NHCOCH₃.

hydrolysis is slow at 25 °C. However, heating 8_S and 8_T at 90 °C for 12 h resulted in high yielding conversion to 5_S and 5_T.

Like 2_S and 2_T, α-amino amide (2') nucleophiles directly form peptides 5' in CPL. This selective peptide formation, promoted by the α-peptide backbone, warranted further investigation. Amidine 3' can in principle hydrolyze through substitution of ammonia or amino amide (2'), and their similar pK_{aH} values suggested that direct hydrolysis should yield peptide 5' and amide 7 in comparable yields. However, peptide 5' forms selectively, implicating intramolecular catalysis. Upon coupling H-Ala-NH₂ (2_A') and nitrile 1, we observed slow hydrolysis of amidine 3_A' to 5_A'. At room temperature, we also observed an imidazolone intermediate (4_A) (Figure 2B, Figures S55 and S118–S120). This cyclization explains the selective formation of peptide 5', with intramolecular substitution promoting loss of ammonia.

We speculated this selectivity would be uniquely effective for (biogenic) α-peptides. To test this, we used alaninamide (H-Ala-NH₂, 2_A'), asparagine (H-Asn-OH, 2_N), and glutamine (H-Gln-OH, 2_Q) as homologous nucleophiles (with α-, β-, and γ-amides) to investigate amide-catalyzed amidine hydrolysis (Figure 2B). α-Amides cyclize to 5-membered imidazolone 4 and hydrolyze selectively to α-peptides 5'. Although β-amides (e.g., 2_N) also cyclize, they yield 6-membered dihydropyrimidone 9 that hydrolyze with poor selectivity yielding a mixture of peptide 5_N (50%) and amidine 3_D (40%). Thus, unlike α-amides, β-amides undergo significant β-peptide hydrolysis (Figure S22). Extending the series further inhibited cyclization completely, and γ-amide 2_Q only formed amidine 3_Q (Figure S28). These results demonstrate the disposition of α-amino amides (i.e., proteinogenic peptides) to catalyze selective amidine-to-peptide hydrolysis, while nonproteinogenic β- or γ-amino amides are either poor catalysts or catalytically inactive.

Cyclization of $3_{A'}$ to 4_A and hydrolysis of 4_A to peptide $5_{A'}$ exhibits a strong pH dependence, and both are rapid at pH 10 and sluggish at pH 7 (Figures 3, S124, and S126). However, at

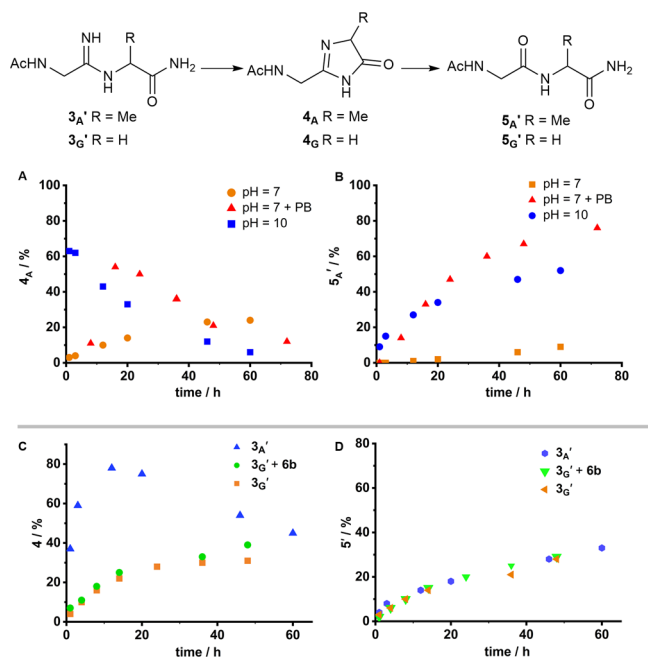


Figure 3. Effect of pH, buffer and catalyst on imidazolone formation and hydrolysis. Time courses to show the (A) formation of imidazolone 4_A from amidine $3_{A'}$ (25 mM) at rt and pH 10 or pH 7, with and without phosphate buffer (PB, 100 mM); (B) formation of peptide $5_{A'}$ from $3_{A'}$ (25 mM) at rt and pH 10 or pH 7, with and without PB (100 mM); (C) formation of 4_A from $3_{A'}$ (25 mM) and 4_G from $3_{G'}$ (25 mM) at rt and pH 9, with and without **6b** (100 mM); (D) formation of peptides $5_{A'}$ from $3_{A'}$ (25 mM) and $5_{G'}$ from $3_{G'}$ (25 mM) at rt and pH 9, with and without **6b** (100 mM).

pH 7, both are accelerated by phosphate buffer (100 mM, Figure S123) but not by **6b** (Figure 3C). This suggests that both hydrolysis and cyclization are general acid–base catalyzed. Moreover, hydrolysis can be catalyzed by a combination of intra- and intermolecular catalysis, and orthogonal catalysts (i.e., thiols and phosphates) can independently catalyze amidine ligation and hydrolysis.

To investigate the effect of side chains on amidine hydrolysis, proteinogenic amino amides ($2'$) were studied in CPL with **1**. Peptides were formed in 70–90% yield with H-Ala-NH₂ ($2_{A'}$), H-Val-NH₂ ($2_{V'}$), H-Leu-NH₂ ($2_{L'}$), H-Phe-NH₂ ($2_{F'}$), H-Arg-NH₂ ($2_{R'}$), H-His-NH₂ ($2_{H'}$), H-Pro-NH₂ ($2_{P'}$), H-Tyr-NH₂ ($2_{Y'}$), H-Trp-NH₂ ($2_{W'}$), H-Glu-NH₂ ($2_{E'}$) and H-Asp-NH₂ ($2_{D'}$) (Figure S54–S101). H-Ile-NH₂ ($2_{I'}$) forms a mixture of diastereomers (11:9 ratio) in high yield (86%). Since H-Ile-OH (2_I) forms only one amidine diastereomer, this implies racemization occurs during the cyclization-hydrolysis process. Slightly lower yields were observed with H-Gly-NH₂ ($2_{G'}$, 50%, Figure S70), H-Gln-NH₂ ($2_{Q'}$, 66%, Figure S68) and H-Met-NH₂ ($2_{M'}$, 63%, Figure S81). All NMR data were consistent with formation of intermediates **4**. The course of the reaction was different for the amino amides (H-Ser-NH₂ $2_{S'}$, H-Thr-NH₂ $2_{T'}$ and H-Asn-NH₂ $2_{N'}$) with side chains that promote amidine hydrolysis. Asparaginamide ($2_{N'}$) formed a mixture of Asn and Asp peptides in 50% yield after 10 days, alongside **7** (45%; Figure S62), but Asn peptides undergo facile hydrolysis,¹³ so

this low yield is likely intrinsic to this side chain and no attempt was made to optimize H-Asn-NH₂ coupling.

Surprisingly, coupling with $2_{S'}$ led to decomposition at pH 8.5, forming no detectable peptide (Figure S88). Oxazoline $8_{S'}$ (Figure 2) was, however, observed at pH 7, and heating $8_{S'}$ at 60 °C led to peptide $5_{S'}$ (74%) after 36 h (Figure S89). Similarly, $2_{T'}$ was converted to $5_{T'}$ (85%) after 36 h at 60 °C and pH 7 (Figure S97) as a single diastereomer. On the other hand, nonproteinogenic *O*-methyl serinamide $2_{MeS'}$ decomposed rather than forming peptide even at pH 7 (Figure S142). These results demonstrate that, at pH 7, oxazoline formation overcomes the incompatibility of β -hydroxyl residues with CPL at elevated pH. Likewise, CPL with peptide nucleophiles (e.g., H-Ala-Gly-Ala-OH 2_{AGA} ; Figures S102–S106) at pH 8.5 and 60 °C only furnished tetrapeptide Ac-Gly-Ala-Gly-Ala-OH (5_{AGA}) in 50% yield, alongside substantial **7** (30%, Figure S104), whereas, at pH 7 and 60 °C, CPL was much more selective and ligation was observed to yield 5_{AGA} (81%) (Figure S102). Thus, though faster at pH 8.5, CPL is only universally compatible and high yielding with proteinogenic peptides at neutral pH.

We next turned our attention to (uncatalyzed) hydrolysis of amidine **3** (X = OH). Whereas high selectivity of amidine-to-peptide hydrolysis was observed for α -amide $3_{A'}$, α -acid 3_A furnished a mixture of **7** and 5_A . At 80 °C, moderate selectivity for hydrolysis of 3_A to **7** (2:1 $7/5_A$) was observed at pH 7–9 (Table 2, entries 2–4). We postulated that this selectivity arose

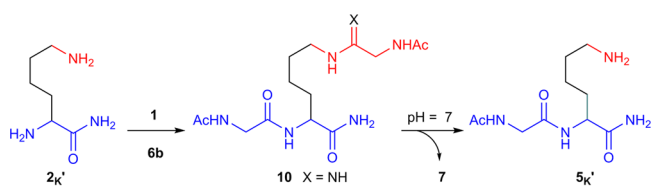
Table 2. Hydrolysis of Amidine 3_A ^a

entry	pH	temp, °C	buffer (500 mM)	time	3_A , %	5_A , %	2_A , %
1	7	20		30 days	100	0	0
2	7	80		18 h	34	15	36
3	7	80	PB	18 h	11	31	52
4	9	80	BB	18 h	0	38	61

^aPB = phosphate buffer, BB = borate buffer.

due to the difference in amine pK_{aH} ($2_A = 9.7$; ammonia = 9.2), with the higher pK_{aH} amine selectively substituted. This suggested a new mechanism to effect selective α -ligation of lysine peptides in water.^{5a,14} The high pK_{aH} of the ϵ -amine (10.8) compared to ammonia (9.2) suggested that hydrolysis of ϵ -lysyl amidines would selectively yield the free ϵ -amine. Furthermore, because α -lysyl peptide amidines (e.g., $3_{K'}$) undergo effective (intramolecular amide-catalyzed) hydrolysis to α -peptides, we envisaged α -peptide ligation and ϵ -hydrolysis would operate together and reinforce selectivity for proteinogenic peptide ligation.

Upon coupling of H-Lys-NH₂ ($2_{K'}$) and **1**, at pH 9, we observed that α -peptide $5_{K'}$ (20%) was a minor product, formed alongside ϵ -amide **11** (15%) and substantial amounts of *N*-acetylglycinamide **7** (59%) after 24 h at 80 °C (Table 3, entry 2 and Figure S135). This demonstrates that α -ligation is disfavored at pH 9 and the predominant product is hydration (i.e., **7**). However, at neutral pH, the selectivity for α -ligation was dramatically increased. At pH 7 peptide $5_{K'}$ was the major product after 6 days, yielding $5_{K'}$ (58–65%) and only 7% ϵ -amide **11** (α : ϵ 9:1; Figure S137). Neutral pH ligation of lysyl peptides was similarly effective; H-Lys-Gly-OH (2_{KG}) and H-

Table 3. Selectivity for Ligation at α - or ϵ -Amine of Lysyl Peptides^a

entry	pH	ϵ -amidine 10, %	total amide ($\alpha + \epsilon$), %	ratio $\alpha:\epsilon$ acylation
1	9 ^b	0	35	1.3
2	7 ^b	19	67	3.5
3	7 ^c	0	72	9.3

^aSelective α -lysyl peptide (blue) over ϵ -lysyl amine (red) coupling of 200 mM **2k'** with 200 mM **1**, 30 mol % **6b** at 80 °C. ^bYields after 1 day. ^cYields after 6 days.

Lys-Lys-OH (**2_{KK}**) were ligated selectively to afford peptide Ac-Gly-Lys-Gly-OH **5_{KG}** ($\alpha:\epsilon$ 7:1; Figures S138 and S139) and Ac-Gly-Lys-Lys-OH **5_{KK}** ($\alpha:\epsilon$ 5:1; Figures S140–141).

In conclusion, we have demonstrated that proteinogenic substrates undergo selective CPL to furnish racemic α -peptides catalyzed by the adjacent α -amide/peptide at neutral pH. β -Hydroxyl α -amides retain chirality via hydroxyl catalysis, but O-methylation inhibits peptide formation in these substrates even at neutral pH. The impact and value of peptide racemization and stereoretention, within a (likely racemic) prebiotic environment, during self-catalyzed peptidyl-amidine hydrolysis remains an open question.^{5b} By studying the (uncatalyzed) hydrolysis of amidines we have discovered a preference for the substitution of the higher pK_{aH} amine. This uncatalyzed reaction operates in tandem with α -amide-catalyzed hydrolysis to enhance the selectivity for lysine α -peptide synthesis at neutral pH in water while retaining the lysyl (functional) ϵ -amine group.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacs.2c03486>.

Experimental procedures and spectroscopic data (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Matthew W. Powner – Department of Chemistry, University College London, London WC1H 0AJ, United Kingdom; orcid.org/0000-0002-6368-3190; Email: matthew.powner@ucl.ac.uk

Authors

Jyoti Singh – Department of Chemistry, University College London, London WC1H 0AJ, United Kingdom

Daniel Whitaker – Department of Chemistry, University College London, London WC1H 0AJ, United Kingdom

Benjamin Thoma – Department of Chemistry, University College London, London WC1H 0AJ, United Kingdom

Saidul Islam – Department of Chemistry, University College London, London WC1H 0AJ, United Kingdom; Department of Chemistry, King's College London, London SE1 1DB, United Kingdom; orcid.org/0000-0001-8686-2565

Callum S. Foden – Department of Chemistry, University College London, London WC1H 0AJ, United Kingdom

Abil E. Aliev – Department of Chemistry, University College London, London WC1H 0AJ, United Kingdom

Tom D. Sheppard – Department of Chemistry, University College London, London WC1H 0AJ, United Kingdom; orcid.org/0000-0002-3455-1164

Complete contact information is available at: <https://pubs.acs.org/10.1021/jacs.2c03486>

Author Contributions

[†]J.S. and D.W. contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank K. Karu (UCL Mass Spectrometry Facility). We thank the EPSRC (EP/K004980/1, EP/M507970/1, EP/P020410/1), Leverhulme Trust (RPG-2019-214), Simons Foundation (318881FY19), and Volkswagen Foundation (94743) for financial support.

■ REFERENCES

- (1) (a) Constable, D. J. C.; Dunn, P. J.; Hayler, J. D.; Humphrey, G. R.; Leazer, J. L., Jr.; Linderman, R. J.; Lorenz, K.; Manley, J.; Pearlman, B. A.; Wells, A.; Zaks, A.; Zhang, T. Y. Key green chemistry research areas—a perspective from pharmaceutical manufacturers. *Green Chem.* **2007**, *9*, 411–420. (b) Valeur, E.; Bradley, M. Amide bond formation: beyond the myth of coupling reagents. *Chem. Soc. Rev.* **2009**, *38*, 606–631. (c) Pattabiraman, V. R.; Bode, J. W. Rethinking amide bond synthesis. *Nature* **2011**, *480*, 471. (d) de Figueiredo, R. M.; Suppo, J.-S.; Campagne, J.-M. Nonclassical Routes for Amide Bond Formation. *Chem. Rev.* **2016**, *116*, 12029–12122. (e) Sabatini, M. T.; Boulton, L. T.; Sneddon, H. F.; Sheppard, T. D. A green chemistry perspective on catalytic amide bond formation. *Nat. Catal.* **2019**, *2*, 10–17. (f) Wang, X. Challenges and outlook for catalytic direct amidation reactions. *Nat. Catal.* **2019**, *2*, 98–102.
- (2) (a) Nissen, P.; Hansen, J.; Ban, N.; Moore, P. B.; Steitz, T. A. The Structural Basis of Ribosome Activity in Peptide Bond Synthesis. *Science* **2000**, *289*, 920–930. (b) Fischbach, M. A.; Walsh, C. T. Assembly-line enzymology for polyketide and nonribosomal peptide antibiotics: Logic, machinery, and mechanisms. *Chem. Rev.* **2006**, *106*, 3468–3496. (c) Schmeing, T. M.; Ramakrishnan, V. What recent ribosome structures have revealed about the mechanism of translation. *Nature* **2009**, *461*, 1234–1242.
- (3) Knowles, J. R. Enzyme catalysis: not different, just better. *Nature* **1991**, *350*, 121–124.
- (4) (a) Ruiz-Mirazo, K.; Briones, C.; de la Escosura, A. Prebiotic Systems Chemistry: New Perspectives for the Origins of Life. *Chem. Rev.* **2014**, *114*, 285–366. (b) Frenkel-Pinter, M.; Samanta, M.; Ashkenasy, G.; Leman, L. J. Prebiotic Peptides: Molecular Hubs in the Origin of Life. *Chem. Rev.* **2020**, *120*, 4707–4765.
- (5) (a) Canavelli, P.; Islam, S.; Powner, M. W. Peptide ligation by chemoselective aminonitrile coupling in water. *Nature* **2019**, *571*, 546–549. (b) Foden, C. S.; Islam, S.; Fernandez-Garcia, C.; Maugeri, L.; Sheppard, T. D.; Powner, M. W. Prebiotic synthesis of cysteine peptides that catalyze peptide ligation in neutral water. *Science* **2020**, *370*, 865–869.
- (6) (a) Sutherland, J. D. The Origin of Life—Out of the Blue. *Angew. Chem., Int. Ed.* **2016**, *55*, 104–121. (b) Islam, S.; Powner, M. W. Prebiotic Systems Chemistry: Complexity Overcoming Clutter. *Chem.* **2017**, *2*, 470–501. (c) Islam, S.; Bučar, D.-K.; Powner, M. W. Prebiotic selection and assembly of proteinogenic amino acids and natural nucleotides from complex mixtures. *Nat. Chem.* **2017**, *9*, 584–589.
- (7) (a) Barbas, C. F., III Organocatalysis Lost: Modern Chemistry, Ancient Chemistry, and an Unseen Biosynthetic Apparatus. *Angew. Chem., Int. Ed.* **2008**, *47*, 42–47. (b) van der Helm, M. P.; Klemm, B.

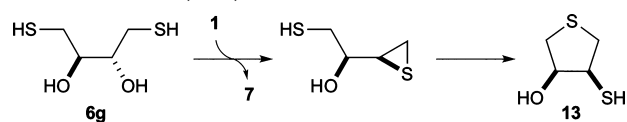
Eelkema, R. Organocatalysis in aqueous media. *Nat. Rev. Chem.* **2019**, *3*, 491–508.

(8) (a) Steinberg, S. M.; Bada, J. L. Peptide decomposition in the neutral pH region via the formation of diketopiperazines. *J. Org. Chem.* **1983**, *48*, 2295–2298. (b) Radzicka, A.; Wolfenden, R. Rates of Uncatalyzed Peptide Bond Hydrolysis in Neutral Solution and the Transition State Affinities of Proteases. *J. Am. Chem. Soc.* **1996**, *118*, 6105–6109. (c) Sun, Y.; Frenkel-Pinter, M.; Liotta, C. L.; Grover, M. A. The pH dependent mechanisms of non-enzymatic peptide bond cleavage reactions. *Phys. Chem. Chem. Phys.* **2020**, *22*, 107–113.

(9) (a) Reese, C. B.; Simons, C.; Pei-Zhuo, Z. The synthesis of 2'-thiouridylyl-(3'→5')-uridine. *J. Chem. Soc., Chem. Commun.* **1994**, *1*, 1809–1810. (b) Dantzman, C. L.; Kiessling, L. L. Reactivity of a 2'-thio nucleotide analog. *J. Am. Chem. Soc.* **1996**, *118*, 11715–11719. (c) Hamm, M. L.; Nikolic, D.; Van Breemen, R. B.; Piccirilli, J. A. Unconventional origin of metal ion rescue in the hammerhead ribozyme reaction: Mn²⁺-assisted redox conversion of 2'-mercapto-cytidine to cytidine. *J. Am. Chem. Soc.* **2000**, *122*, 12069–12078.

(10) (a) Portillo-Ledesma, S.; Sardi, F.; Manta, B.; Tourn, M. V.; Clippe, A.; Knoops, B.; Alvarez, B.; Coitiño, E. L.; Ferrer-Sueta, G. Deconstructing the Catalytic Efficiency of Peroxiredoxin-5 Peroxidatic Cysteine. *Biochemistry* **2014**, *53*, 6113–6125. (b) Keire, D. A.; Strauss, E.; Guo, W.; Noszál, B.; Rabenstein, D. L. Kinetics and Equilibria of Thiol/Disulfide Interchange Reactions of Selected Biological Thiols and Related Molecules with Oxidized Glutathione. *J. Org. Chem.* **1992**, *57*, 123–127. (c) Lindley, H. A. Study of the Kinetics of the Reaction between Thiol Compounds and Chloroacetamide. *Biochem. J.* **1960**, *74*, 577–584. (d) Johnson, E. C. B.; Kent, S. B. H. Insights into the Mechanism and Catalysis of the Native Chemical Ligation Reaction. *J. Am. Chem. Soc.* **2006**, *128*, 6640–6646. (e) Patel, V. M.; Joshi, J. D. Equilibrium Study on the Complex Formation of Europium-, Terbium-, Dysprosium- and Thulium(III) with Some Oxyacids, Thioacids and Phenols. *J. Indian Chem. Soc.* **1998**, *75*, 100–101. (f) Singh, R.; Lamoureux, G. V.; Lees, W. J.; Whitesides, G. M. Reagents for Rapid Reduction of Disulfide Bonds. In *Methods in Enzymology*; 1995; pp 167–173.

(11) **6g** has recently been used as the catalyst for CPL on an RNA template. Based on our data, and the observed concomitant degradation of **6g** and hydration of **1**, we suspect higher yields could have been achieved using **6b**. See: Müller, F.; Escobar, L.; Xu, F.; Węgrzyn, E.; Naintyé, M.; Amatov, T.; Chan, C.; Pichler, A.; Carell, T. A Prebiotically Plausible Scenario of an RNA–Peptide World. *Nature* **2022**, *605*, 279–284.



(12) (a) Dawson, P.; Muir, T.; Clark-Lewis, I.; Kent, S. Synthesis of proteins by native chemical ligation. *Science* **1994**, *266*, 776–779. (b) Kulkarni, S. S.; Sayers, J.; Premdjee, B.; Payne, R. J. Rapid and efficient protein synthesis through expansion of the native chemical ligation concept. *Nat. Rev. Chem.* **2018**, *2*, 0122.

(13) (a) Clarke, S. Propensity for spontaneous succinimide formation from aspartyl and asparaginyl residues in cellular proteins. *Int. J. Pept. Protein Res.* **1987**, *30*, 808–821. (b) Friedrich, M. G.; Wang, Z.; Schey, K. L.; Truscott, R. Mechanism of protein cleavage at asparagine leading to protein-protein cross-links. *Biochem. J.* **2019**, *476*, 3817–3834.

(14) Frenkel-Pinter, M.; Haynes, J. W.; Martin, C.; Petrov, A. S.; Burcar, B. T.; Krishnamurthy, R.; Hud, N. V.; Leman, L. J.; Williams, L. D. Selective incorporation of proteinaceous over nonproteinaceous cationic amino acids in model prebiotic oligomerization reactions. *Proc. Natl. Acad. Sci. U.S.A.* **2019**, *116*, 16338–16346.