Prebiotic Synthesis of α-Amino Acids and Orotate from α-Ketoacids Potentiates Transition to Extant Metabolic Pathways

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Article

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Abstract

The Strecker reaction of aldehydes is the preeminent pathway to explain the prebiotic origins of a-amino acids. However, biology employs transamination of a-ketoacids to give rise to amino acids which are then transformed to nucleobases, implying subsequent evolution of the biosynthetic pathways – abiotically or biotically. Herein, we show that a-ketoacids react with cyanide and ammonia sources to form the corresponding a-amino acids – via the Bucherer-Bergs pathway. An efficient prebiotic transformation of oxaloacetate to aspartate via N-carbamoyl aspartate enables the simultaneous formation of dihydroorotate, paralleling the biochemical synthesis of orotate as the precursor to pyrimidine nucleobases. Glyoxylate forms both glycine and orotate, and reacts with malonate and urea to form aspartate and dihydroorotate. These results, along with the previously demonstrated protometabolic analogs of the Krebs cycle suggest that there can be a natural emergence of congruent forerunners of biological pathways with the potential for seamless transition from prebiotic chemistry to modern metabolism.

Introduction

Amino acids and nucleobases are among the most elementary prebiotic and metabolic building blocks.[1-3] On the early Earth, amino acids are thought to be available by the Strecker reaction of aldehydes with cyanide and ammonia (Fig. 1a) [1,4-6] and the nucleobases by hydrolysis of cyanide polymers[3], differing significantly from the extant biochemical synthetic pathways.[3,7-9] While the heterotrophic models[10] suggests cyanide and formaldehyde were robust prebiotic source molecules,[7,11] extant biochemistry utilizes a-ketoacids as the building blocks via transamination for the synthesis of a-amino acids (Fig. 1b), which in turn give rise to the nucleobases[9], implying that there must be an evolutionary switch between the prebiotic- and biotic-chemistries. However, the mechanisms of such transformations and such transitions are not clear.[12] An alternative autotrophic approach[10,13] aims to reproduce the biological pathways from the very beginning but has inadequate experimental support.[7,14,15] Discovering chemistries that would be compatible with prebiotic constraints and at the same time allow for the transition to biological pathways can resolve this conundrum.[16] Towards that direction we began exploring the reaction of simple activated carboxylic acids such as a-ketoacids (pyruvate and glyoxylate) and dicarboxylic acids (malonate) and have shown that their inherent reactivity does translate to reactions that gives rise to protometabolic pathways.[17,18] Building on these observations we have recently shown that the reactions of these a-ketoacids and malonate in the presence of cyanide enables a series of transformations that harbors the potential for a reductive glyoxylate cycle of reactions.[19] Herein, we take this system of reactions further and demonstrate that with the inclusion of various ammonia sources, these very same set of reactions begin to naturally produce the next generation products, the corresponding a-amino acids and the precursors to the canonical pyrimidine nucleobases – in a manner that can enable a transition to the types of pathways that are observed in extant metabolism.

Results And Discussion

Recently[17] an a-keto analog of the reductive citric acid pathway (r-TCA) was shown to emerge from reacting two of the simplest a-ketoacids,[18] glyoxylate and pyruvate (Scheme S1). Transamination of the a-ketoacids, pyruvate and a-ketoglutarate with glycine produced the corresponding a-amino acids, alanine and glutamate, respectively, however in low-to-moderate yields.[17] With a view to increase the efficiency of the transamination reaction at neutral pH, we investigated replacing glycine with aminoacetonitrile (pKa 5.3) –based on enhanced nucleophilicity of the amine donors[20]– but, aminoacetonitrile was even less efficient compared to glycine (Supplementary Figs. 1-3). As an alternate to this canonical transamination process which involves the formation of imine of the a-ketoacids and then a hydrogen transfer (Fig. 1b),[9] we proposed the "decarboxylative Strecker equivalent" of a-ketoacids reacting with sources of ammonia (such as diamidophosphate, DAP) and cyanide (Fig. 1c), wherein we envisioned the formation of a carboxylated amino-nitrile intermediate (I) that could decarboxylate to give the a-amino acid. Among the various ammonia sources, we chose to use diamidophosphate (DAP, pKa ≈ 5) for two reasons: (a) DAP is known to release NH_3 and acts as an amine donor in the Strecker reaction with aldehydes;[21,22] and (b) importantly, we anticipated that the amidophosphate of the cyano-adduct (I) formed from the a-ketoacid would assist in an intramolecular hydrolysis of the cyanide to form the a,a-dicarboxylic acid intermediate (II) and, subsequently, enable a decarboxylative reductive amination of intermediate (II) to form the corresponding a-amino acid (Fig. 1c).

The Bucherer-Bergs (and not the "decarboxylative Strecker") reaction to form a-amino acids. With this expectation, we investigated the reaction of a pyruvate **1** with various equivalents of DAP and cyanide (Fig. 2) over a range of concentrations (0.1-0.5M), pHs (6-9) and temperatures (room temperature – 80°C) in unbuffered and buffered (phosphate & carbonate-bicarbonate) aqueous solutions and monitored the reactions by ^{1}H- and ^{13}C-NMR spectroscopy (Supplementary Figs. 4-25, 29-40). At room temperature, the cyanohydrin adduct **2** and a-aminonitrile **4** of pyruvate were observed, which with time (days) or upon heating (50-80 °C, 24 h) converted (with varying efficiency), first to the 5-methyl hydantoin **6** and finally to alanine **8** as the major product (Fig. 2). Thus, the reaction seems not to proceed as anticipated via the classic Strecker reaction (Fig. 1c) but via a Bucherer-Bergs pathway through the corresponding hydantoin,[23,24] which hydrolyzes to the amino acid (Fig. 1d).

An NMR time-course study of the reaction and spiking with authentic compounds confirmed the formation of 5-methyl hydantoin **6**, which was hydrolyzed to alanine via *N*-carbamoyl alanine **7** (Fig. 2). While the formation of aminonitrile intermediate **4** was expected, the ease of hydantoin formation in water or phosphate buffer was surprising, since it implied efficient *in situ* trapping of the CO_2 generated by decarboxylation. A mechanistic study involving a series of control reactions and with ^{13}C- and ^{15}N-labeled compounds in water and in ^{13}C-bicarbonate buffer revealed that in the absence of added bicarbonate, the C(2)-carbonyl moiety of hydantoin **6** originates from the CO_2H group of the a-ketoacid. The cyanide C becomes the C(4)-carbonyl moiety of hydantoin **6** and ends up as the CO_2H group of the a-amino acid **8**. Reactions in degassed phosphate buffer at room temperature showed little/no formation

of hydantoin **6**, while heating led to production of **6** and acetate indicating that the initial CO_2 originates from the oxidative decarboxylation of the pyruvate (by the traces of oxygen present in water) to acetate. The inefficient reaction in degassed solutions at room temperature could be 'rectified' by adding CO_2, suggesting that it is the availability of CO_2 that is important for initiating the reaction (Supplementary Figs. 13-16). The thus available CO_2 reacts with aminonitrile **4** to form N-carboxy aminonitrile **5**, which is hypothesized (similar to the Bucherer-Bergs pathway) to undergo an intramolecular cyclization to form a putative cyclic a-carboxy imino-oxazolidinone **5a** and **5b** that rearranges to hydantoin **6** with concomitant decarboxylation of the CO_2H group that originated from the a-ketoacid (Fig. 2). The released CO_2 is captured by another a-aminonitrile **4** continuing the cycle of reactions towards hydantoin **6** formation (Fig. 2, green color arrows).

The hydantoin intermediate is stable and usually requires forcing conditions to hydrolyze to the amino acid.[25] However, under these reaction conditions where ammonia is formed by the hydrolysis of DAP, heating at 80 °C was enough to hydrolyze **6** to alanine, as confirmed by the relatively rapid hydrolysis of 5-methylhydantoin **6** in the presence of added DAP versus its absence (Supplementary Fig. 21). ^{31}P- and ^{15}N-NMR spectra confirmed that DAP was hydrolyzed and is acting as a source of NH_3 with the phosphate playing no important role (contrary to what was envisioned in Fig. 1c). With this realization, we investigated the reaction of a pyruvate **1** with various other ammonia sources (NH_4OH, ammonium salts, cyanamide and urea) and observed comparable yields of amino acids (Supplementary Table 1), demonstrating the flexibility of the system with regards to the nitrogen source.

Since DAP was found to be a convenient "in situ" source of ammonia we used it for investigating conditions for optimization of yields. As expected, use of aqueous bicarbonate as the solvent increased the yield of alanine. Thus, reacting pyruvate under optimal conditions (2 equiv. of DAP, 1.2 equiv. of NaCN, pH 8.5, 0.5 M bicarbonate buffer, at room temperature) yielded 88% of 5-methyl hydantoin **6**, which was hydrolyzed at 80 °C to alanine **8** (78%) (Fig. 3a). Applying these optimized conditions to α-ketoglutarate **9** with DAP and cyanide generated the corresponding hydantoin **10** in 96% yield (Fig 3b), which was hydrolyzed to glutamate **12** (19%, with 72% of **10** remaining, Supplementary Figs. 42-44).

Reactions of oxaloacetate and glyoxylate provide simultaneous access to pyrimidines. Unlike pyruvate and a-ketoglutarate, transamination of oxaloacetate **13** to aspartate **16** is challenging since **13** decarboxylates in solution producing pyruvate **1**[26] leading to alanine[17]. Under the optimized conditions at room temperature, oxaloacetate **13** was converted to the corresponding hydantoin **14** (69%), which at 80 °C provided a prebiotically unprecedented access to aspartate **16** (68%, Fig. 3c and 3e) via N-carbamoyl aspartate **15** as an intermediate (Supplementary Figs. 48-52). The formation of N-carbamoyl aspartate provides a natural path to dihydroorotate (DHO) **17** paralleling the biosynthesis of orotate, which is the precursor to the canonical pyrimidines.[9] A simple heating in solution (50 °C, pH 4.5) or wet-dry cycling (pH 4.5, 50°C) of **15** led to the formation of hydantoin **14** (up to 49%) and aspartate **16** (up to 13%) and

dihydroorotate **17** (up to 13%, Supplementary Table 2). Dihydroorotate has been shown to be converted abiotically to orotate **18**.[27]

Similarly, employing the optimized conditions at pH 7, glyoxylate **19** was converted to hydantoin **20**, which at 80 °C produced glycine **22** (14-61%) along with 21-32% of N-carbamoyl glycine **21** (Fig. 3d). This reaction of glyoxylate giving rise to hydantoin becomes significant in the context of the recent prebiotic synthesis of orotate from the reaction of hydantoin with glyoxylate.[28] And thus, not unexpectedly, traces (ca. 1%) of orotate was also observed in the reaction mixture (Supplementary Figs. 54-59). The Bucherer-Bergs chemistry of a-ketoacids documented in Fig. 3a-d above suggests a purely chemical reason as to why the pyrimidines are a natural outcome of this protometabolic set of reactions (that also seems to be reflected in the extant metabolic pathways).

The natural emergence of interconnected pathways between protometabolic pathways. The above reactions provide a natural extension of the previous work[17] where aldol condensation of just the a-ketoacids pyruvate and glyoxylate gives rise to a-ketoglutarate and the corresponding a-ketoacid analogs the constituents of the Krebs cycle (Supplementary Scheme S1). Recently, in a related work, we have shown that reaction of these a-ketoacids (or their condensation products) with cyanide alone leads to selective reductive transformations reminiscent of reductive TCA pathway (Supplementary Scheme S2).[19] Therefore, it was of interest to know what these a-ketoacids and their condensation products would produce in the presence of ammonia sources alone. When the primary condensation products from the reaction of pyruvate and glyoxylate were exposed to various prebiotic sources of ammonia, 12-20% of a-ketoglutarate was formed (Fig. 4a) as opposed to the 2-4% yield in their absence (Supplementary Table S3). Thus, the presence of amines/ammonia enables a more efficient transformation of the pyruvate-glyoxylate condensation products to the stable a-ketoglutarate via a retro-Claisen reaction[17] and/or by enhancing the cross-Cannizzaro reaction[29]. Thus, while the reaction of ammonia alone or cyanide alone with a-ketoacids (or their condensation products) produce differing chemistries related to the Krebs pathway, the combination of all three of them, as shown in this work (Fig. 3), produces the next generation of products: amino acids and the pyrimidine nucleobase precursors, DHO and orotate (Supplementary Scheme S3).

As an example of how naturally productive such “systems-chemistry” can be, we subjected a mixture of malonate and glyoxylate [18] in the presence of urea (as an ammonia surrogate) in phosphate buffer to heating and/or wet-dry cycles (Fig. 4B), which led to the formation of hydantoin **14** (32%), aspartate **16** (10%) and DHO **17** (14%) along with malate (≈2%) (Fig. 4C). The formation of aspartate and DHO independent of oxaloacetate as the source material may be significant since the prebiotic provenance of oxaloacetate (due to its instability) is not resolved.[18] Thus, the reaction of a-ketoacids has the natural potential, at various stages of ‘systems-chemistry’ complexity (Supplementary Scheme S3), to give rise to increasing levels of diverse products that are capable of interacting with the original reaction pathways to

enable the emergence of innate feedback mechanisms that could lead to chemical evolutionary network of reactions that can transition to protobiological systems.[30,31]

Implications for transitioning to extant metabolic pathways. The abiotic conversion of a-ketoacids to amino acids [32] using the same type of cyanide and ammonia sources as for the Strecker reaction, potentiates the prospect of using a-ketoacids instead, which are central to many biological pathways but in a prebiotic scenario. If the prebiotic availability ketoacids on early Earth can be substantiated,[33-35] then this overlap can enable a more seamless transition from the cyanide/ammonia-based chemistry to the next step of using the amino acids themselves as the nitrogen source for transamination.[36,37] The efficient non-enzymatic transamination with an amino acid requires organocatalysts.[38,39] Coincidentally, the demonstration that the DAP, used for decarboxylative reductive aminations reported in this work, is also able to synthesize short peptides starting from the amino acids [40,41] – suggests a model scenario where peptides could naturally emerge to catalyze the switch to using amino acids as the nitrogen and hydrogen source via transamination (Fig. 1b), thus replacing cyanide and ammonia (Fig. 1c). Since the transamination of amino acid would result in regeneration of an a-ketoacid, this sets up a feedback mechanism – potentially making it self-sustaining and weaning the system away from the external cyanide and ammonia sources – a process that seems to have been exploited by biology, as evidenced by how central and universal this reversible transamination process is in extant biochemistry.[9]

In addition, hydantoins **14** and **20** are the only intermediates capable of transiting to DHO and orotate respectively, which is indicative of the natural emergence and the potential connection between such protometabolic pathways and pyrimidine nucleobases – coinciding with the synthesis of orotic acid from aspartate (via DHO) in extant biology.[9] And, such transformations[27] which “appear closer” to biological pathways when compared to the prebiotic generation of orotic acid starting only from cyanide,[42,43] hint at the potential for inherent appearance of forerunners of biological pathways from prebiotic chemistry. Thus, the apparent divide between heterotrophic and autotrophic scenarios connecting prebiotic chemistry and biology need not be incompatible, at least in some cases, so as to invoke a drastic and discontinuous switch from one chemistry to the another – leading to a better understanding of where such transitions are feasible and, more importantly, are implausible.[7,8,44]

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Declarations

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Author contributions

R.K. proposed the project. G.S. and R.K. designed and supervised research; S.P and M.Y. designed and performed the experiments and collected the data. S.P., M.Y., G.S. and R.K. analyzed data. R.K. wrote the manuscript with feedback and edits from S.P., M.Y. and G.S. We thank Dr. Luke Leman for feedback on the manuscript.

Competing interests

Authors declare that they have no competing interests.

Supplementary Information is available for this paper.

This file contains General Experimental, Experimental Procedures, Supplementary Figures 1-71, Supplementary Tables 1-3, Supplementary Schemes 1-3, Supplementary References and NMR spectra – see contents page for details.

Data Availability

All data are available from the corresponding author upon reasonable request. Correspondence and request for materials should be addressed to Ramanarayanan Krishnamurthy, rkrishna@scripps.edu.

Figures

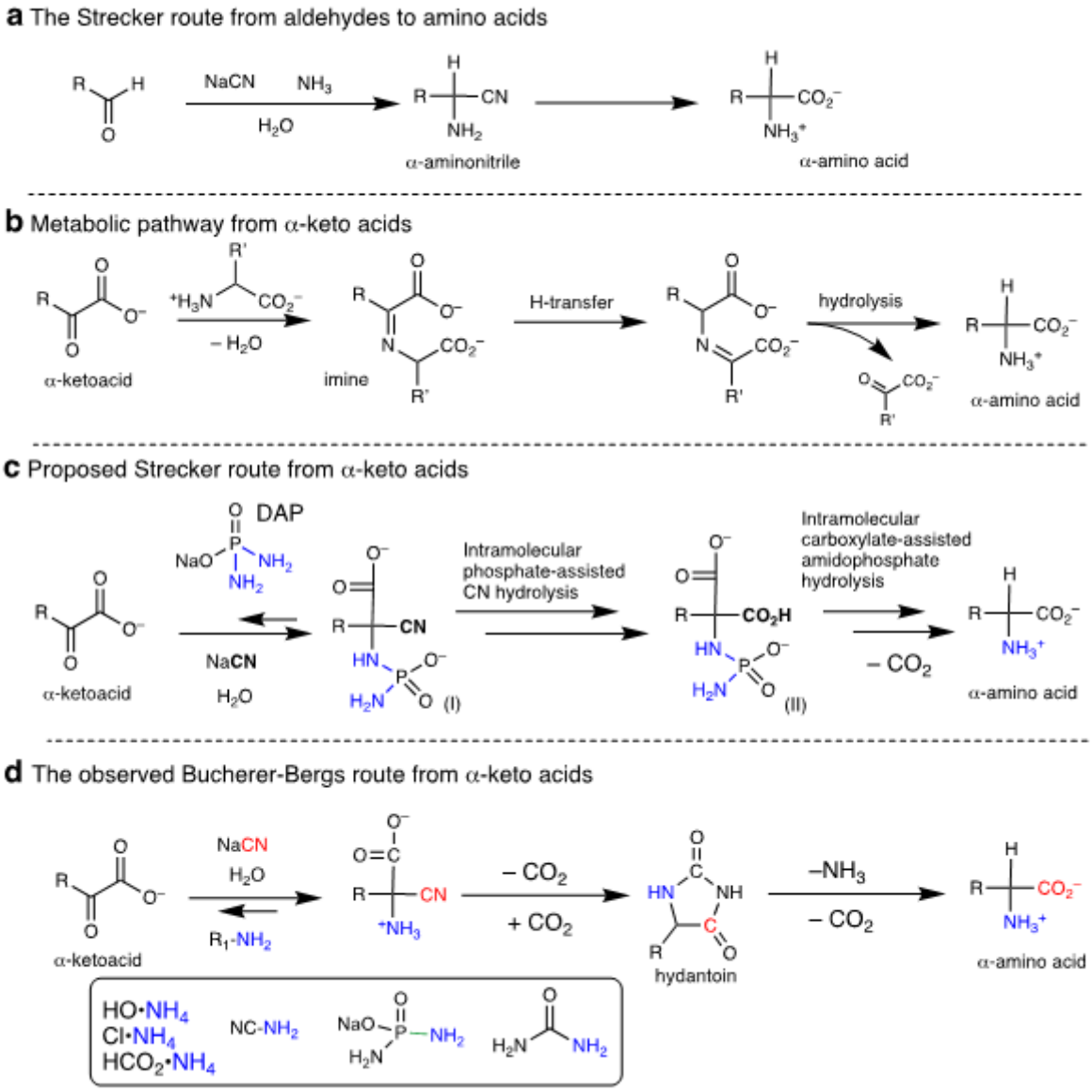

Figure 1

Comparison of the prebiotic and biotic routes to α-amino acids. (a) Strecker reaction starting from aldehydes. (b) The biochemical transamination pathway in extant metabolism. (c) The proposed ‘decarboxylative’ Strecker-transformation of α-ketoacid to α-amino acid in the presence of an ammonia source (DAP) and cyanide. (d) The actually observed (Bucherer-Bergs reaction) pathway for conversion of an α-ketoacid with various ammonia sources to the corresponding α-amino acid (via hydantoin as the intermediate).

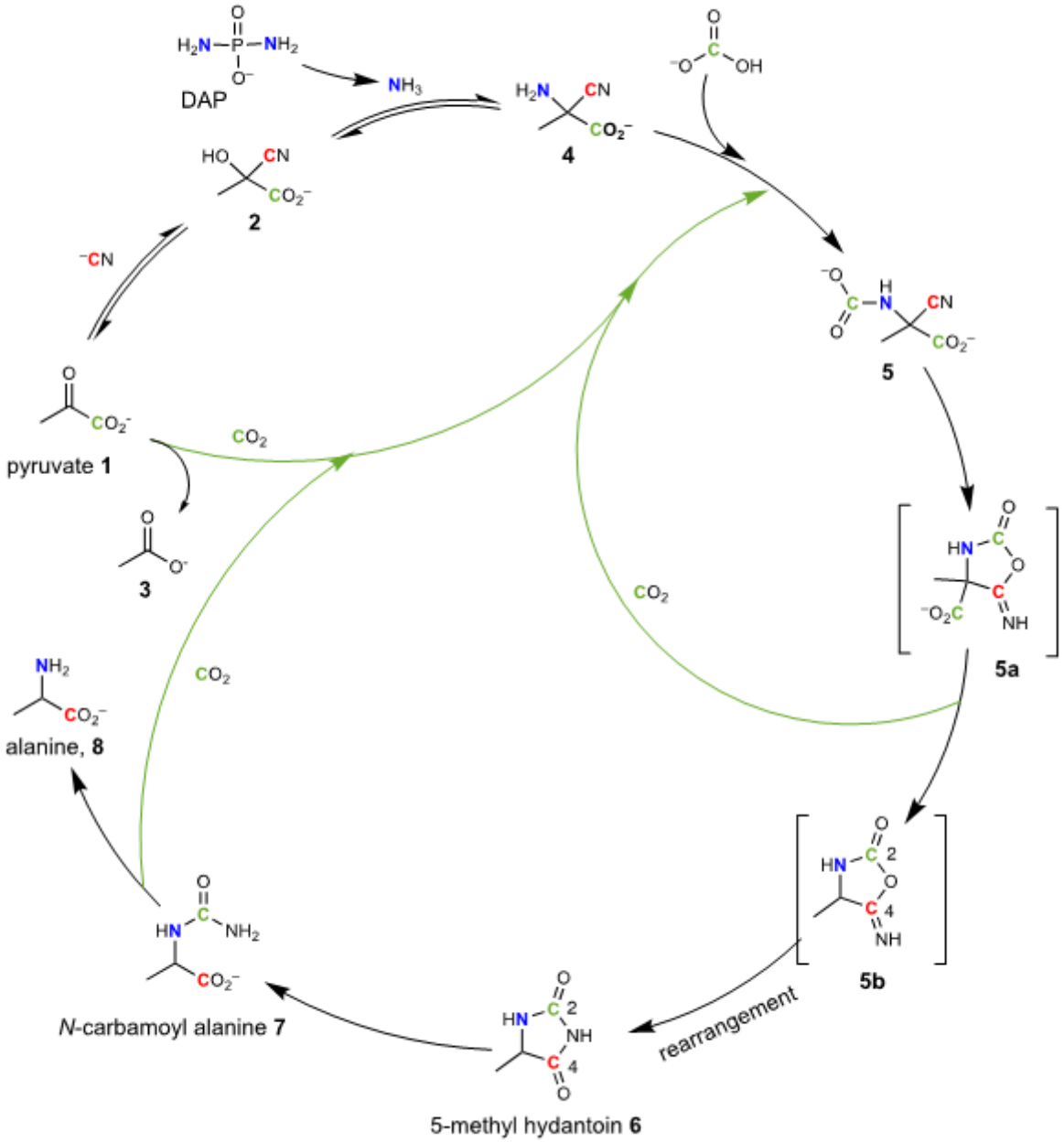

Figure 2

Mechanistic pathway for the conversion of α-ketoacids to α-amino acids via the Bucherer-Bergs reaction. The reaction of pyruvate 1 with DAP and cyanide proceeds via the Bucherer-Bergs reaction pathway through 5-methylhydantoin 6 as the stable intermediate, as elucidated by 13C- and 15N labeling studies. The green color arrows highlight the reincorporation of CO2 generated by the in situ decarboxylation processes.

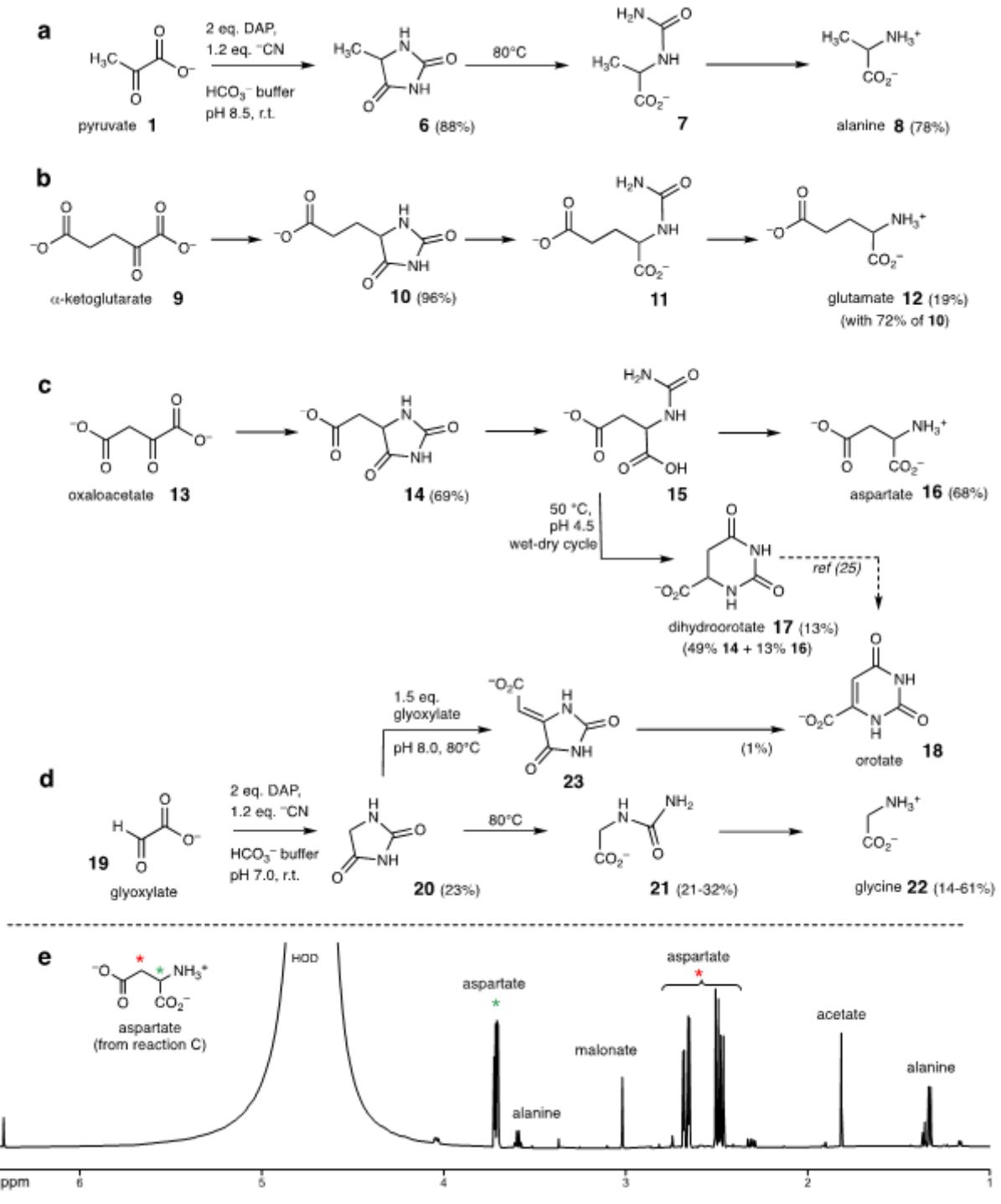

Figure 3

Synthesis of α-amino acids and orotate from α-ketoacids using DAP and cyanide. (a-d) Formation of alanine, glutamate, aspartate and glycine by the Bucherer-Bergs reaction of α-ketoacids under the optimized conditions. (c-d) Hydantoins 14 and 20 are converted to dihydroorotate 17 and orotate 18 respectively. (e) 1H NMR (D2O) spectrum of the reaction of oxaloacetate to form aspartate under optimized conditions (after 14 days).

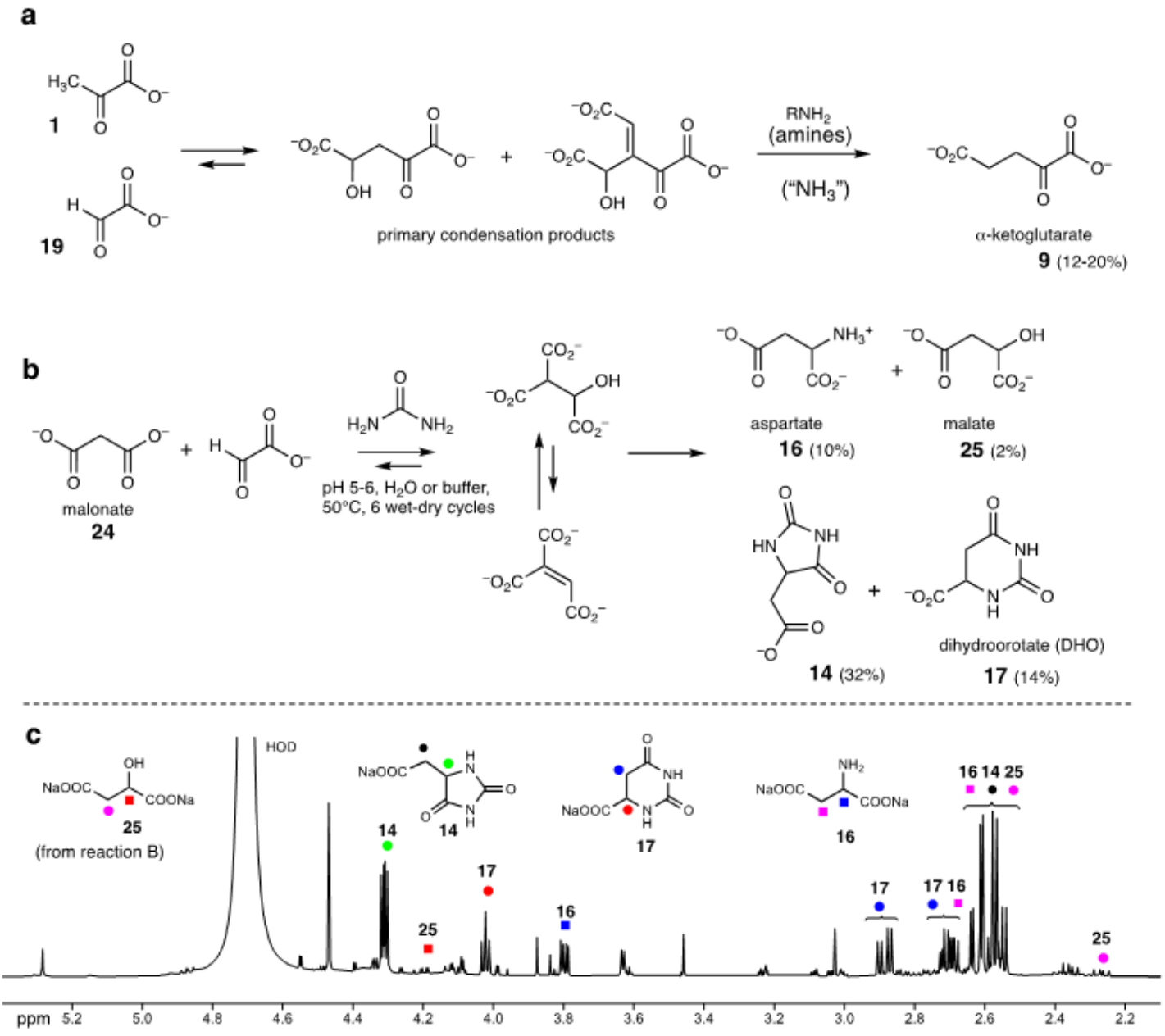

Figure 4

Reaction of α-ketoacid condensation products with ammonia sources produces compounds found in the Krebs cycle and its secondary metabolites. (a) Ammonia/amines promote and enhance the conversion of the condensation products of pyruvate and glyoxylate to α-ketoglutarate. (b) Reaction of malonate and glyoxylate in the presence of urea forms aspartate and DHO, thus bypassing the need for oxaloacetate. (c) 1H NMR (D2O) of the reaction of malonate, glyoxylate in the presence of urea under the wet-dry cycle protocol.

Supplementary Files

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