

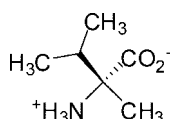
Enantioselective Synthesis and Enantiomeric Amplification of Amino Acids under Prebiotic Conditions

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ABSTRACT



A plausible origin of biomolecular homochirality is advanced, where α -methyl amino acids found on meteorites transfer their chirality in the synthesis of normal amino acids. This asymmetry can be amplified to nearly homochiral levels, thus providing the necessary prerequisite for life to start on this planet and elsewhere in the universe.

One of the most remarkable features of living organisms is their homochirality, the fact that they contain amino acids and sugars of only one enantiomeric form. We have investigated a potential prebiotic source of homochirality: α -methyl amino acids that are delivered to earth by carbonaceous chondritic meteorites (Figure 1). These amino acids, which lack a racemizable

cyanide, and water, all of which have been detected by microwave spectroscopy in interstellar space.^{1,5} The non-methylated amino acids that are also seen in carbonaceous chondritic meteorites are formed from aldehydes, and they are racemic. This must reflect their easy racemization by loss of the α -proton. The α -methyl amino acids, formed from methyl ketones, cannot racemize.

Many theories have been advanced to account for their asymmetry,^{6–8} and one of the most interesting ideas is that synchrotron radiation from supernova remnants contains circularly polarized light.⁹ In a neutron star with circulating electrons, synchrotron radiation would have one chirality above the circulation plane and the opposite below it. Thus, one form of circularly polarized light can be directed into our part of the universe, with the opposite polarized light sent in the other direction.

Circularly polarized light has been shown to preferentially photolyze one enantiomer of a racemic amino acid, leading to some enantioenrichment in the remaining amino acid.¹⁰ Circularly polarized light that struck the parent body of

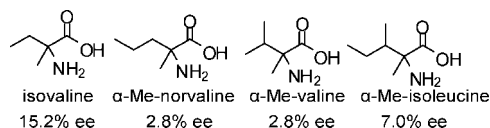


Figure 1. α -Methyl amino acids found on meteorites, and their % ee of the L-enantiomer.

α -methine proton, are found in meteorites with small but measurable L enantiomeric excesses (ee's).¹

Their isotopic distributions confirm that they have extra-terrestrial origins.^{2–4} They are presumably formed by Strecker reactions involving carbonyl compounds, ammonia, hydrogen

- (5) Peltzer, E. T.; Bada, J. L. *Nature* **1978**, 272, 443.
- (6) Bonner, W. A.; Kavasmaneck, P. R.; Martin, F. S.; Flores, J. J. *Science* **1974**, 186, 143.
- (7) Bonner, W. A.; Kavasmaneck, P. R. *J. Org. Chem.* **1976**, 41, 2225.
- (8) Bailey, J.; Chrysostomou, A.; Hough, J. H.; Gledhill, T. M.; McCall, A.; Clark, S.; Ménard, F.; Tamura, M. *Science* **1998**, 281, 672.
- (9) Bonner, W. A. *Origins Life* **1991**, 21, 59.
- (10) Flores, J. J.; Bonner, W. A.; Massey, G. A. *J. Am. Chem. Soc.* **1977**, 99, 3622.

- (1) Pizzarello, S. *Acc. Chem. Res.* **2006**, 39, 231.
- (2) Pizzarello, S.; Feng, X.; Epstein, S.; Cronin, J. R. *Geochim. Cosmochim. Acta* **1994**, 58, 5579.
- (3) Pizzarello, S.; Huang, Y.; Fuller, M. *Geochim. Cosmochim. Acta* **2004**, 68, 4963.
- (4) Pizzarello, S.; Huang, Y. *Geochim. Cosmochim. Acta* **2005**, 69, 599.

carbonaceous chondritic meteorites could thus have resulted in the preferential partial photolysis of one enantiomer of α -methyl amino acids, leading to the observed modest L ee's.

The α -methylated amino acids can serve as the seeds of terrestrial homochirality provided two conditions are met: (i) they must be able to transfer their chirality to biomolecules such as simple amino acids and sugars, and (ii) there must be a mechanism for chiral amplification of meteoritic amino acids or of proteinogenic amino acids up to near homochirality. Here we show that 96% enantiopure L- α -methylvaline can transaminate phenylpyruvate to L-phenylalanine in up to 37% ee and pyruvate to L-alanine in up to 20% ee with copper catalysis. We extend this novel copper-catalyzed decarboxylative transamination to the synthesis of L-valine from L- α -methylisoleucine. Furthermore, we demonstrate that the ee's of various proteinogenic amino acids can be amplified above the thermodynamic limits via preferential kinetic dissolution. Our results demonstrate both conditions that are necessary for meteoritic α -methyl amino acids to be a plausible source of biomolecular homochirality, on this planet and elsewhere in the universe.

Our previously reported decarboxylative transamination (Figure 2) utilized enantiopure D- α -methylvaline to afford

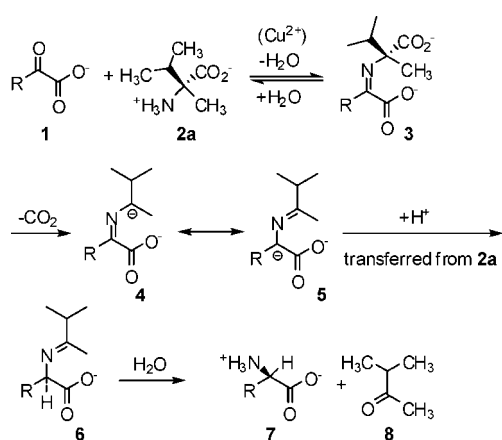
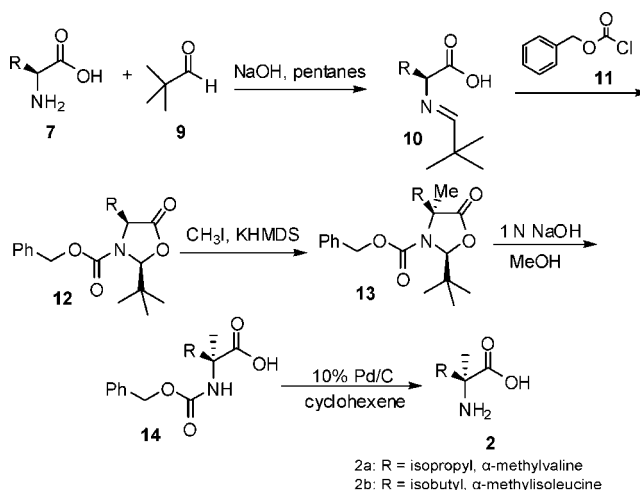


Figure 2. Transamination reaction between α -methylvaline and a ketoacid.

up to 10% L-phenylalanine product under solvent-free conditions, so the meteoritic L- α -methylvaline would have produced D-phenylalanine.¹¹ We have now found that the decarboxylative transaminations that occur when α -methylvaline is heated neat with α -ketoacids can be catalyzed by metal ions.

The α -methylated amino acids were synthesized via stereospecific methylation of the corresponding *cis*-oxazolidinones (Scheme 1).¹² L- α -Methylvaline (**2a**), which has been synthesized previously,¹³ was synthesized in our hands

Scheme 1. Synthesis of L- α -Methyl Amino Acids



in 32% overall yield and 96% enantiopurity (92% ee). While L- α -methylisoleucine has been identified in meteorites,¹ it has not been previously synthesized by chemical means. We synthesized it in 8% overall yield from L-isoleucine. The relative stereochemistry of compound **2b** is assumed by analogy to known compound **2a** (whose stereochemistry is assigned by comparison of its optical rotation to literature values).

The reaction of 4 equiv of L- α -methylvaline with 1 equiv of sodium phenylpyruvate and 1 equiv of cupric sulfate afforded L-phenylalanine. A variety of reaction times (1–120 min) and temperatures (120–160 °C) were screened, and the best results were obtained at 160 °C for 60 min, wherein L-phenylalanine was obtained in 37% ee. Shorter reaction times or lower reaction temperatures led to lower ee's. This is a higher transfer of chirality than we had obtained without the copper salt. Moreover, the desired enantiomer of phenylalanine is now produced: L- α -methylvaline affords L-phenylalanine. Zinc salts (zinc is less abundant than copper in meteorites)^{14,15} were poorer catalysts and did not lead to the reversal of chiral induction that we saw with copper. The reaction conditions (solvent-free, 120–160 °C) mimic plausible conditions on prebiotic earth.

To gain mechanistic insight into this reversal of enantioselectivity in the copper-catalyzed reaction, we performed DFT/B3LYP calculations using the Maestro interface with Jaguar version 7.4 using a 6-31TM** basis set on a dual processor Dell Precision 490 workstation. One potential reaction intermediate is a square planar copper(II) complex containing two molecules of imine **3** as bidentate ligands (Figure 3). Calculations of the van der Waals surface of this complex suggest that only one face of the α -carbon is

Scrimin, P. *J. Org. Chem.* **2004**, 69, 3849. Synthesis of **2a**: (c) Obrecht, D.; Bohdal, U.; Broger, C.; Bur, D.; Lehmann, C.; Ruffieux, R.; Schönholzer, P.; Spiegler, C.; Müller, K. *Helv. Chim. Acta* **1995**, 78, 563.

(14) Strasdeit, H.; Büsching, I.; Behrends, S.; Saak, W.; Barklage, W. *Chem. Eur. J.* **2001**, 7, 1133.

(15) Greenland, L.; Goles, G. G. *Geochim. Cosmochim. Acta* **1965**, 29, 1285.

(11) Breslow, R.; Levine, M. *Tetrahedron Lett.* **2006**, 47, 1809.
(12) Seebach, D.; Fadel, A. *Helv. Chim. Acta* **1985**, 68, 1243.
(13) Synthesis of **12a**: (a) Qi, J.; Roush, W. R. *Org. Lett.* **2006**, 8, 2795. Synthesis of **14a**: (b) Formaggio, F.; Barazza, A.; Bertocco, A.; Toniolo, C.; Broxterman, Q. B.; Kaptein, B.; Brasola, E.; Pengo, P.; Pasquato, L.

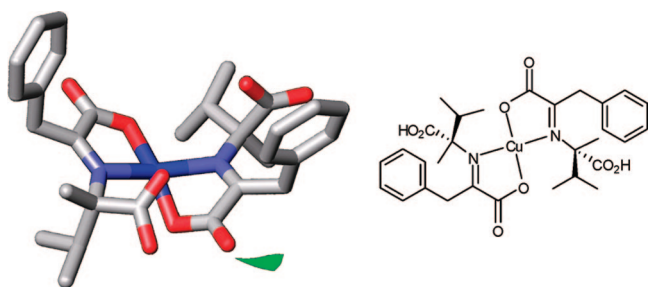


Figure 3. Optimized geometry of Cu(II) complexed with two imine molecules. The green ribbon indicates the face of the α -carbon that is accessible for protonation; H atoms have been omitted for clarity.

accessible to contact with a second molecule, as the other face is blocked by the bulky benzyl groups. Protonation from the accessible face leads to L-phenylalanine.

Further examination of this complex indicates the potential for a competing reaction pathway of a concerted decarboxylative protonation (Figure 4), as the carboxylic acid proton

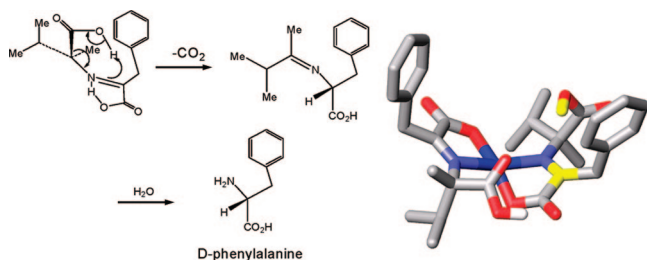


Figure 4. Formation of D-phenylalanine by concerted decarboxylative proton transfer, both without copper and in the copper complex.

in the complex is 2.65 Å above the α carbon. Protonation of the α carbon would lead to D-phenylalanine. A combination of both reaction pathways (enantioselective protonation and concerted decarboxylative protonation) would account for the observed moderate ee's in the phenylalanine product.

This copper-catalyzed decarboxylative transamination can be extended to the synthesis of amino acids that are more prebiotically relevant. In particular, both alanine and its corresponding α -hydroxy acid, lactic acid, have been found on meteorites.¹⁶ A simple oxidation of either the alcohol or the amine would yield pyruvic acid as a substrate for our enantioselective transamination. This oxidation could have been accomplished in at least two ways: (i) iron oxide found on meteorites can oxidize lactic acid to pyruvic acid,¹⁷ and

(ii) serine, also found on meteorites, can be dehydrated and hydrolyzed under prebiotic conditions to yield pyruvic acid.¹⁸

Heating 96% enantiopure L- α -methylvaline with sodium pyruvate and cupric sulfate at 160 °C for 60 min under solvent-free conditions yields L-alanine in 20% ee. In the absence of copper, only low ee's of the undesired D-alanine were obtained.

Furthermore, both valine and its corresponding 2-hydroxy-3-methyl butanoic acid have been found on meteorites.^{16,5} A simple oxidation of the alcohol or amine would yield the desired valine ketoacid precursor. The oxidation of the alcohol can occur via the iron oxide found on meteorites.¹⁷ Moreover, pyruvic acid, formed from the dehydration and hydrolysis of serine, can further react with normal amino acids, such as valine, to produce 3-methyl-2-oxobutanoic acid and the byproduct alanine.¹⁸

Valine can be synthesized via our enantioselective transamination. L- α -Methylisoleucine, found on meteorites in 7.0% L ee,¹⁹ reacted with 3-methyl-2-oxobutanoate in the presence of cupric sulfate to produce L-valine in up to 23% ee. In the absence of copper, no reaction occurred.

This modest ee was then amplified via the preferential kinetic dissolution of L-valine crystals more quickly than the racemate crystal. A 500 mg dry mixture of DL-valine and L-valine was placed in a pipet plugged with glass wool, and 1 mL of room temperature water was passed through. The solution that passed through was enantioenriched in L-valine. When we started with 5% ee L-valine and subjected the mixture to three rounds of preferential dissolution, we found that the ee of the solution was enriched up to 46% L ee (a 73:27 ratio) (Figure 5). This is identical with the 45% ee

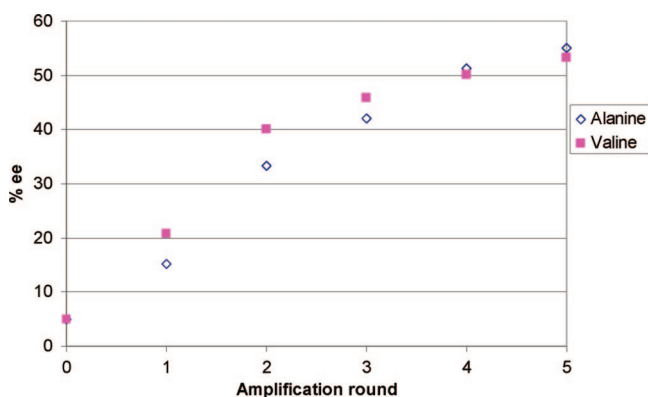


Figure 5. Amplification of valine and alanine via preferential kinetic dissolution.

room temperature thermodynamic limit reported by Blackmond.²⁰ By switching to 1 mL of ice-cold water and subjecting the 46% ee L-valine mixture to two more rounds

(16) Cronin, J. R.; Pizzarello, S. *Meteorites and the Early Solar System*; Kerridge, J., Matthews, M. S., Eds.; University of Arizona Press: Tucson, AZ, 1988; pp 819–857..

(17) Kerridge, J. F.; MacKay, A. L.; Boynton, W. V. *Science* **1979**, 205, 395.

(18) Bada, J. L.; Glavin, D. P.; McDonald, G. D.; Becker, L. *Science* **1998**, 279, 362.

(19) Cronin, J. R.; Pizzarello, S. *Science* **1997**, 275, 951.

(20) Klusmann, M.; Iwamura, H.; Mathew, S. P.; Wells, D. H.; Pandya, U.; Armstrong, A.; Blackmond, D. G. *Nature* **2006**, 441, 621.

of preferential kinetic dissolution, the enantiomeric enrichment increased up to 53% ee L-valine.

Alanine can also be amplified using preferential kinetic dissolution. When 1 mL of ice-cold water was passed through a 500 mg mixture of 5% ee L-alanine over 3 min, the solution that passed through contained 15% ee L-alanine (Figure 5). Repeating this procedure four more times led to a 55% ee L-alanine solution, which is close to the 60% ee room temperature eutectic point of alanine reported by Blackmond.²⁰

Previously, we had amplified aqueous solutions with 1%, 5%, or 10% ee of phenylalanine up to 90% ee, by slow evaporation that enriched the ee of the solution as the racemate precipitated.²¹ With most amino acids, including phenylalanine, the racemic crystals are less soluble than the homochiral crystals.²² Blackmond independently used a related process to achieve enantioenrichment, allowing small amounts of a partially racemic amino acid to dissolve in water at equilibrium.^{20,23} With our evaporation process, involving selective partial crystallization, we see that tryptophan can be amplified up to 89% ee (Table 1). These limits

Table 1. Enantiomeric Concentration of Tryptophan after Two Partial Crystallizations from Water^a

component	initial ee, %	final ee, %
D	10	89.0 ± 0.6
	5	86.7 ± 1.6
	1	86.0 ± 0.2
L	10	84.6 ± 4.6
	5	86.9 ± 4.2
	1	81.1 ± 4.3

^a All values represent an average of two runs.

reflect equilibrium values, dictated by thermodynamics.

Preferential kinetic dissolution is able to overcome these thermodynamic limits, because the on and off rates for homochiral crystallizations can involve a smaller kinetic barrier than those rates for the racemate. Via kinetic dissolution, a 91% ee of L-phenylalanine was produced from an initial mixture of 85% L ee, which is similar to the 90% ee obtained at equilibrium. As in the case of valine and alanine, using ice-cold water for the kinetic dissolution of phenylalanine increases the selectivity, allowing us to obtain

an average phenylalanine ee of 95%, although the results in this case are only slightly higher than the margin of error (Table 2). Preferential kinetic dissolution of tryptophan can

Table 2. Enantiomeric Amplification of Amino Acids after Preferential Kinetic Dissolution^a

component	initial ee, %	final ee, %, rt	final ee, %, 0 °C
L-phenylalanine	80	90.3 ± 1.0	94.3 ± 1.4
	85	91.4 ± 3.2	95.2 ± 0.03
L-tryptophan	80	97.5 ± 1.1	
	85	97.8 ± 1.3	

^a All values are L ee and represent an average of three runs.

proceed even with room temperature water, to yield a final average ee of 98%, significantly higher than the 89% ee thermodynamic maximum (Table 2).

We have thus demonstrated both conditions necessary for the α -methyl amino acids found on meteorites to be a plausible source of biological homochirality. First, these enantioenriched amino acids can transfer their chirality to several proteinogenic amino acids, including some that were present on prebiotic earth. This transfer takes place under credible prebiotic conditions. Second, the moderate ee's produced in the proteinogenic amino acids can be amplified via a straightforward, prebiotically relevant procedure, via either evaporation of water or preferential dissolution by Blackmond's equilibration, or by our kinetic process. Amplification via evaporation of water could have occurred on prebiotic earth in a drying lake bed near a site of meteorite landing. Preferential dissolution may have occurred when river or rainwater passed over an amino acid mixture, dissolving the single enantiomer with enriched enantiopurity and carrying it downstream. After such enrichment, biology could start and produce dominant organisms that use the dominant enantiomers, on this planet and elsewhere in the universe.

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Supporting Information Available: The syntheses of α -methyl amino acids, HPLC parameters, and optimization of the transamination reaction. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(21) Breslow, R.; Levine, M. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 12979.

(22) Huang, J.; Yu, L. *J. Am. Chem. Soc.* **2006**, *128*, 1873.

(23) Klusmann, M.; Izumi, T.; White, A. J. P.; Armstrong, A.; Blackmond, D. G. *J. Am. Chem. Soc.* **2007**, *129*, 7657.