Carbon isotope evidence for the substrates and mechanisms of prebiotic synthesis in the early solar system

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Abstract

Meteorites contain prebiotic, bio-relevant organic compounds including amino acids. Their syntheses could result from diverse sources and mechanisms and provide a window on the conditions and materials present in the early solar system. Here we constrain alanine's synthetic history in the Murchison meteorite using site-specific $^{13}C/^{12}C$ measurements, reported relative to the VPDB standard. The $\delta^{13}C_{VPDB}$ values of -29 ± 10 ‰, 142 ± 10 ‰, and -36 ± 10 ‰ for the carboxyl, amine-bound, and methyl carbons, respectively, are consistent with Strecker synthesis of interstellar-medium-derived aldehydes, ammonia, and low-$\delta^{13}C$ nebular or interstellar-medium-derived HCN. We report experimentally measured isotope effects associated with Strecker synthesis, and use them to constrain the $\delta^{13}C$ values of the alanine precursors, which we then use to construct a model that predicts the molecular-average $\delta^{13}C$ values of 19 other organic compounds of prebiotic significance found in Murchison if they were made by our proposed synthetic network. Most of these predictions agree with previous measurements, suggesting that interstellar-medium-derived aldehydes and nebular and/or pre-solar HCN could have served as substrates for synthesis of a wide range of prebiotic compounds in the early solar system.

1. Introduction

Carbonaceous chondrite (CC) meteorites contain amino acids (Cronin and Moore, 1971; Engel et al., 1990; Glavin et al., 2018), the extraterrestrial origins of which are evinced by their chemical and isotopic properties. Known life predominantly synthesizes 20 amino acids that are mostly L enantiomers and ~2 % lower in their $^{13}C/^{12}C$ ratios than the average terrestrial inorganic carbon. On the other hand, the CC meteorites contain over 90 amino acids that are nearly racemic mixtures of D and L enantiomers—likely unchanged from their formation—and are generally

~1-3 % higher in their $^{13}C/^{12}C$ ratios than the average terrestrial inorganic carbon (Sephton, 2002; Burton et al., 2012; Elsila et al., 2016; Glavin et al., 2018).

Proposed mechanisms of meteoritic amino acid synthesis include *i)* ion/radical-molecule reactions in the interstellar medium (ISM) (*e.g.*, with the irradiation of methanol ices (Bernstein et al., 2002)), *ii)* Fischer-Tropsch type (FTT) synthesis in the protosolar nebula (Botta and Bada, 2002), and/or *iii)* aqueous chemistry (*e.g.*, Strecker synthesis or reductive amination) of ISM-derived precursor molecules that were accreted in ices by the meteorite parent bodies and reacted during aqueous alteration (Kerridge, 1999; Pizzarello et al., 2006; Glavin et al., 2018). The molecular-average $\delta^{13}C$ values[1] of individual α-amino acids from the Murchison CM2 CC decrease systematically with increasing carbon number (Pizzarello et al., 1991; Sephton, 2002; Elsila et al., 2012; Glavin et al., 2018), suggesting that they might have been assembled from smaller precursors with each newly added carbon atom being lower in ^{13}C than its source due to kinetic isotope effects (KIEs) (Yuen et al., 1984; Engel et al., 1990; Sephton, 2002). Alternatively, these trends could reflect the dilution of a high-$\delta^{13}C$ carbon atom inherited from ISM-derived CO by carbon from other, lower $\delta^{13}C$ precursors (Elsila et al., 2012). However, in the full set of prior $\delta^{13}C$ measurements of Murchison α-amino acids, $\delta^{13}C$ variations for individual amino acids compared between studies span a range equal to the extent of the

[1] $\delta^{13}C$ quantifies the ratio of $^{13}C/^{12}C$ relative to a standard, Vienna Pee Dee Belemnite (VPDB). Mathematically, $\delta^{13}C_{VPDB} = \frac{{}^{13}C/{}^{12}C_{sa}}{{}^{13}C/{}^{12}C_{st}} - 1$ where *sa* denotes the sample and *st* the VPDB standard. $\delta^{13}C$ is conventionally reported in parts per thousand (‰), *i.e.*, $= \left[\frac{{}^{13}C/{}^{12}C_{sa}}{{}^{13}C/{}^{12}C_{st}} - 1 \right] \times 1000$

proposed correlation between carbon number and $\delta^{13}C$ and so calls these explanations into question.

These and other hypotheses regarding the origins of meteoritic amino acids can be tested through observations of their site-specific carbon isotope distributions (*i.e.*, the $\delta^{13}C$ values of individual carbon positions in each molecule). Here we present site-specific $\delta^{13}C$ measurements of the three carbon sites in alanine extracted from a sample of Murchison and measured using novel techniques conducted with an Orbitrap mass spectrometer.

2. Methods and Materials

2.1 Materials

2.1.1 Meteorite

We analyzed two samples of Murchison meteorite, a Methods Development sample (analyzed winter and spring 2018) and an Analytical sample (analyzed summer 2018). The Methods Development sample was a 5 g piece of Murchison from the Field Museum of Natural History via Clifford N. Matthews's research group that was known to be contaminated; although this contamination means that analytical results are of limited value, it provided a natural sample on which we could assess our novel analytical procedures. The Analytical sample was a 2.6 g sample from a different piece of Murchison and the same source; the sample has been described in Friedrich et al., 2018(Friedrich et al., 2018).

The D/L ratio of alanine from the methods development sample is 0.4, which is far from a pure racemic mixture's value of 1 or past measurements and therefore suggests a high proportion of

terrestrial contamination. The D/L ratio of alanine from the analytical sample is 0.85, which agrees with past measurements of Murchison alanine (Cronin et al., 1995). The overall amino acid content of the Analytical sample is also similar to those measured previously in Murchison (Cronin and Moore, 1971; Martins and Sephton, 2009; Friedrich et al., 2018), which combined with the D/L ratios of amino acids in this sample suggest minimal terrestrial contamination.

2.1.2 Derivatization

Alanine standards used in this study were Alfa Aesar L-alanine (99 % Purity), VWR alanine (Purity >99 %, Lot # 2795C477), and one sample of alanine synthesized by Strecker synthesis (Purity confirmed by NMR, Figure S1) (hereafter, 'Strecker standard'). Calibration of standards is described in Appendix A. Ultrapure water was obtained from a MilliPore ultrahigh-purity water (18.2 MΩ cm; hereafter 'water') system at Caltech. In addition to the standards listed above, alanines with 99 % ^{13}C label at C-1, C-2, or C-3 were purchased from Sigma Aldrich (C-1: Lot # EB2220V, C-2: Lot # SZ0643V, C-3: Lot # EB2211V).

Reagents used in derivatization reactions and cleaning at Caltech included: anhydrous methanol (MeOH; >99.8 % purity, Macron Fine Chemicals, Batch# 0000042997), n-hexane (>98.5 % purity, Millipore Sigma, HPLC grade, multiple lots), acetyl chloride (AcCl; >99 % purity, Sigma Aldrich, Lot# BCBT8141), trifluoroacetic anhydride (TFAA; >99 % purity, Sigma Aldrich, Lot# SHBJ0051), and dichloromethane (DCM, Sigma Aldrich, HPLC Plus, >99.9 % purity). All derivatizing reagents were tested for amino acid contamination prior to use on samples (See Appendix B section for more details).

Prior to derivatization, glassware was cleaned with ultrapure water and combusted twice at 450°C. The second combustion occurred the night before use and with no other glassware present. GC vial PTFE caps were new and handled with forceps that were pre-cleaned with methanol. Any cap whose interior was touched with forceps was discarded. Fumehoods and tubing for nitrogen gas were cleaned prior to derivatization. BioPur pipette tips were used on pipettes to prevent contamination. Chemical lab syringes (Hamilton, 250 μL) were cleaned with methanol prior to and after derivatization reactions, and instrument inlet syringes (Hamilton, 10 μL) were cleaned with 30 μL hexane and 30 μL DCM between and before all analyses.

2.2 Methods

2.2.1 Amino Acid Extraction

Amino acids were extracted from meteorite samples at NASA Goddard Space Flight Center (GSFC) following the protocol from (Elsila *et al.*, (2012). Briefly, each sample was ground to a homogenized powder and sealed in a glass ampoule with 1mL ultrahigh purity water (Millipore Integral 10 UV, 18.2 MΩ cm, <3 ppb total organic carbon) for 24 hours at 100°C. The water extract was separated, dried under vacuum, and hydrolyzed in 6N HCl vapor (Sigma Aldrich, double distilled) for 3 hours at 150°C. This hydrolyzed extract was then desalted on a cation-exchange resin column (AG50W-X8, 100-200 mesh, hydrogen form, Bio-Rad), with the amino acids recovered by elution with 2 M NH_4OH (prepared from ultrahigh purity water and NH_3 (g) *in vacuo*); this eluent was split into two fractions and dried under N_2. The Methods Development sample was processed in this way in November 2017 and the analytical sample in May of 2018.

Upon arrival at Caltech, extracts were triple bagged, boxed, and stored in a freezer. One extract from the Methods Development sample was derivatized and analyzed in December 2017; the other was derivatized and analyzed in March 2018. A portion of each derivatized extract from the Methods Development sample was sent back to GSFC along with derivatized standards for secondary analysis. The extract from the Analytical sample was split between GSFC (85 %) and Caltech (15 %). Analyses were made on the analytical sample in June 2018 at GSFC and between June to July 2018 at Caltech.

2.2.2 Derivatization

A flow chart for handling of samples and blanks are depicted in Figure S2. First, 1.0 mL of water:MeOH (3:1) was added to the centrifuge vials containing meteorite extract that had been shipped from GSFC. Vials with the Methods Development samples were capped, placed in a beaker of water, and sonicated for five minutes. The analytical sample extract sat in the water-methanol mixture for 20 minutes but was not sonicated. Samples were then uncapped and transferred into 2 mL GC vials ('sample vials') via combusted glass pipettes. All original shipped extract vials were rinsed twice more with the 3:1 water-methanol mixture without sonication. The rinse liquid was again transferred into the sample vials via glass pipette. Between the second and third rinse and following the third rinse, GC samples vials were dried under slow N_2 flow.

Standards and Murchison extract samples were then derivatized as N-trifluouroacetyl-O-methyl esters. Samples were brought up in 100 μL of anhydrous MeOH and placed in an ice bath. Using a clean syringe, 25 μL of AcCl was added dropwise to the sample, which was swirled between

drops to limit localized boiling (the reaction with AcCl is strongly exothermic). Forceps were used to lift vials and swirl them in order to minimize potential contamination. Samples were capped and heated to 70°C in a heating block for 1 hour. Samples were then cooled and dried under N_2. To avoid cross-contamination, all samples, blanks, and standards were dried separately. Next, 120 μL hexane and 60 μL TFAA were added and vials were capped and heated to 60°C in a heating block for 30 minutes. Samples were evaporated under N_2 until 50 μL remained. To this, 500 μL hexane was added for the methods development samples and 200 μL hexane was added for the analytical sample.

A split of the analytical sample extract and Caltech alanine standards were also derivatized as N-trifluoroacetate-O-isopropyl esters at GSFC following protocols from Elsila *et al.* (2012) at GSFC. The methods are similar to those listed above but use isopropanol instead of methanol.

2.2.3 Amino Acid Characterization

Amino acid abundance and enantiomeric composition (*e.g.*, abundances of D- and L-alanine) of both the method development and analytical samples were measured at GSFC via liquid chromatography with fluorescence detection and time-of-flight mass spectrometry (LC-FD/ToF-MS) using methods described in Glavin *et al.*, (2010). For the methods development samples, 1 % of the sample was used for amino acid characterization, while for the analytical sample, 0.4 % of the initial 2.6 g sample was used for characterization (details in Friedrich *et al.*, (2018)).

2.2.4 Isotope Ratio Measurements

2.2.4.1 Molecule-average isotope analysis of Murchison samples

Approximately 99 % of the methods development sample was sent as two splits to Caltech where it was derivatized as N-trifluoroacetate-O-methyl ester (See *2.2.2:* Derivatization for further details) on the two analysis dates (winter and spring 2018). One aliquot of each derivatized sample in addition to two derivatized standards (Strecker and Alfa Aesar) were sent back to GSFC where they were analyzed for molecular-average (combining both chiral forms) $\delta^{13}C$ via Gas Chromatography-Combustion-IRMS (GC-C-IRMS) with a TC-5LIMS 30m column. For the analytical sample, the 85 % that remained at GSFC was derivatized as N-trifluoroacetate-O-isopropyl ester and injected into a GC-MS with four 25m Chirasil-L-Val columns (Agilent, CP7495) connected in series. This long chiral column allowed us to separate and measure the $\delta^{13}C$ values of D- and L-alanine.

2.2.4.3 Site-specific isotope analysis

Site-specific carbon isotope ratios of derivatized alanine samples and standards were measured at Caltech The constraints presented in this paper are based on measurements of the bulk carbon isotope ratio of the full molecule by GC-combustion IRMS (yielding the average $\delta^{13}C$ of C-1, C-2, and C-3), along with direct mass spectrometric measurements of $^{13}C/^{12}C$ ('^{13}R') of two fragment ions of the alanine derivative, one of which constrains the average ratio for C-1 and C-2 and the other of which constrains the average ratio for C-2 and C-3. For carbon number identities, see Figure 1. These three independent constraints permit us to calculate the $\delta^{13}C$ of each of C-1, C-2 and C-3 (see *2.3*: Data Processing).

The fragment ion measurements were made using the Q-Exactive GC Orbitrap mass spectrometer (hereafter 'Orbitrap'), using techniques described in Eiler *et al.*, (2017). The

Orbitrap can mass resolve a ^{13}C substitution from D, ^{15}N, or ^{17}O substitutions allowing a user to measure the ^{13}R of a fragment directly (*e.g.* without combusting a fragment and then converting carbon into CO) (Figure 1c and 1d insets). The measured fragment ions have monoisotopic peaks (*i.e.*, the isotopologue containing only the most abundant isotope of each element, also known as the 'unsubstituted' isotopologue) of mass/charge (*m/z*) 140.0317 ($C_4H_5ONF_3$) and 184.0214 ($C_5H_5O_3NF_3$) Da (Figure 1). Measurements of their isotope ratios will be referred to by their monoisotopic mass (*i.e.*, 140.032 for the $^{13}C/^{12}C$ ratios derived from ions with masses 141.0350 and 140.0317 Da). The relative contributions of the carbon sites from the parent molecule to each fragment ion were determined by analyzing three mixtures, each with a 10 % ^{13}C enrichment at one carbon site (produced by mixing an appropriate site-specific, 99 % labeled alanine with the unlabeled standard). The *m/z* = 140.032 fragment contains both C-2 and C-3 from the parent alanine along with two carbons from the TFAA reagent (Figure 1c). The *m/z* = 184.021 fragment contains C-1 and C-2 from parent alanine along with all three carbons from the derivatizing reagents (Figure 1d). From labelling studies, both appear to be direct fragmentation products with no obvious evidence for recombination reactions that may add carbon atoms from one sample site into a different molecular ion.

The methods of high-precision isotope ratio analysis by Orbitrap-based mass spectrometry are described in Eiler *et al.* (2017). For the measurements presented in this paper, two configurations were used: direct analysis of analyte peaks eluting from a GC column ('Direct Elution') and analyte capturing from the GC effluent into a reservoir followed by isotopic analysis as it drained from that reservoir ('Reservoir Elution') (Figure 2). The Direct Elution mode was used to characterize the fragmentation pattern and retention time of derivatized alanine (Figure 2c). For

this study, analyte eluting from the GC column was admitted to the ion source continuously following a 5.5-minute delay to avoid the solvent peak. Pre-mass selection using the AQS (quadrupole) system was set to permit all ions between *m/z* 50 and 300 Da to enter the Orbitrap mass analyzer. Reservoir Elution mode was used to measure ion-abundance ratios at a useful precision for study of natural stable isotope variations. Here Reservoir Elution mode measurements were conducted with an initial 5.5-minute solvent delay followed peak monitoring in Direct Elution mode until 30 seconds prior to the elution of derivatized alanine, which could be timed relative to the retention times of earlier-eluting compounds (Figure 2d). At this point, the effluent from the GC column was rerouted into either a 5 or 20 cm^3 glass reservoir, the contents of which were flushed with He into the ion source. The 20 cm^3 reservoir was used for measurements of the relatively high intensity 140.032 fragment and the 5 cm^3 reservoir was used for the weaker intensity 184.021 and 113.021 fragments, in order to increase signal-to-noise ratios (For more information on the 113.021 fragment, see Appendix C). Following the total collection of the derivatized alanine peak, GC column effluent was vented and clean helium was directed into the reservoir to continue purging analyte into to the ion source for the remainder of the measurement. In this fashion the glass reservoir serves as an exponential-dilution flask (Merritt and Hayes, 1994) that broadens the analyte peak from a few seconds to tens of minutes and thereby facilitates the accumulation of more ion counts – and thus greater precision for isotope ratios – by the Orbitrap. Alanine measurements were accumulated for 10- to 60-minutes depending on the reservoir size and the abundance of the fragments of interest (Figure 2d).

Orbitrap measurements produce a series of ‘scans’, each of which reports the apparent ^{13}R of a selected fragment (*i.e.*, for the *m/z* = 140.032 or 184.021 fragments; see values in Dataset S1).

Measurements begin when the alanine peak elutes (*i.e.*, when the NL of the monoisotopic peak is at its minimum immediately prior to alanine's elution). To minimize mass spectrometric artifacts (Eiler et al., 2017), we accept only those analyses in which both the monoisotopic and singly ^{13}C-subsituted fragments are present, in which the monoisotopic ion makes up at least 30 % of the total ion current (TIC) in the observed mass window (Dataset S1), and in which the product of the TIC and injection time (IT) varies over a narrow range (~10's of %, relative) between scans. In some cases, the trace of ion intensities provides evidence that we failed to capture all of the alanine peak in the reservoir and/or a nearly co-eluting peak has been captured with it (*e.g.*, in the case of the 113.021 peak of the Murchison sample discussed in Appendix C); these measurements are also discarded as procedural failures. Standard errors were calculated as the standard deviation of all accepted scans ^{13}R values for a fragment divided by the square root of the number of scans for that fragment.

The accuracy and precision of site-specific measurements was verified via a comparison of differences in δ^{13}C of C-1 measured by the Orbitrap with that measured by ninhydrin decarboxylation for the three standards (See Appendix A for a detailed discussion). The average δ^{13}C values for C-2 and C-3 of Strecker alanine relative to the Alfa Aesar alanine standard measured by the 140.032 Da fragment on the Orbitrap was -17.4 ± 1.6 ‰ (See Appendix D for Error Analysis). This value is just beyond 2 standard errors from the value found from subtraction of δ^{13}C C-1 from the molecular-average δ^{13}C measured by ninhydrin decarboxylation and molecular-average EA-IRMS measurements for Strecker alanine relative to the Alfa Aesar alanine standard (-13.4 ± 0.6 ‰).

Differences in the isotopic composition between the Alfa Aesar and Strecker standards' fragments were constant within the nominal external errors of each measurement over the course of our analysis (Dataset S1) and between analysis sets (Table 1). Each standard had stable measurements of each fragment's ratios of the ^{13}R over the course of our measurements: when source backgrounds are low, the standard deviation for Alfa Aesar's ^{13}R between different injections normalized to the measurements' averages are 4.6 ‰ and 14.0 ‰ for the *m/z* 140.032 and 184.021 fragments, respectively, for quantities of analyte similar to those of Murchison extracts. Furthermore, the variation decreases with increasing quantity of analyte (*i.e.*, the samples that most vary from the mean tend to be of lower intensity fragments and/or measurements) because ^{13}C counts increase with analyte quantity, and the instrument's shot noise limit is inversely proportional to the number of ^{13}C counts.

We tested our ability to trap and analyze derivatized alanine in amino acid mixtures by measuring alanine in a standard mixture comprising the 20 most abundant amino acids in Murchison in relative abundances that match those in Martins and Sephton (2009), as well by measuring alanine in the methods development sample in two analytical periods. We used the Alfa Aesar alanine standard in the standard mixture and compared it to measurements of pure Alfa Aesar alanine (*i.e.* not in a mixture) to ensure that the methodology used to measure mixtures would not fractionate alanine. The standard mixture was handled in a manner similar to that of the meteorite samples including being transferred in a water methanol mixture and dried down prior to derivatization. Relative to the Alfa Aesar standard, the standard mixture had a $\delta^{13}C$ of 2 ‰, which was within error of its measurement. Furthermore, excepting one methods development sample that was contaminated during derivatization (November 2017), the

averaged C-2 + C-3 $\delta^{13}C$ (*i.e.*, that of the 140.032 Da fragment) and the averaged C-1 + C-2 $\delta^{13}C$ (184.021 Da fragment) values for two aliquots of methods development measured via GC-C-IRMS at GSFC and on the Orbitrap at Caltech in January and March of 2019 were within error of one another (Table 1, Appendix A). The summer 2018 analysis of the Strecker alanine is also within one standard error of the spring and winter 2018 C-2 + C-3 averaged $\delta^{13}C$ value and C-1 + C-2 averaged $\delta^{13}C$ value.

2.3 Data processing

2.3.1 Calculations of Site-Specific Isotope Ratios

Several arithmetic operations were required to calculate the site-specific $\delta^{13}C$ values for C-1, C-2, and C-3 in alanine. First, all accepted analyses for each fragment were combined (see Section 2.2.4.1 for criteria of accepted scans and Table S3 for analyses used) and the ^{13}R of each fragment was calculated as a weighted average of all counts (monoisotopic and singly ^{13}C-substituted) for the fragment (Eqn. 1)

$$^{13}R_{fragment} = \Sigma[^{13}R_{scan} \times \frac{^{12}C_{cts,scan} + {}^{13}C_{cts,scan}}{\Sigma^{12}C_{cts,scan} + \Sigma^{13}C_{cts,scan}}] \quad (1)$$

where $^{13}R_{fragment}$ is the ^{13}R value used for a fragment measurement, $^{13}R_{scan}$ is the ^{13}C counts/^{12}C counts for a single scan as defined in Eiler *et al* (2017) with a C_N (the charge conversion constant) of 3.6, $^{i}C_{cts,scan}$ is the and number of counts of isotope, *i,* for a single scan.

This ^{13}R value was then converted into $\delta^{13}C_{VPDB}$. To this end, the measured ^{13}R of each fragment was standardized to Alfa Aesar by dividing it by the same fragment ion's ^{13}R of an Alfa Aesar

alanine measured under the same analytical conditions (*i.e.*, same elution times into reservoir, same AGC conditions, similar TICxIT ranges) and temporally close ($^{13}R_{sa}/^{13}R_{Alfa\ Aesar}$). The standardized ^{13}R for the alanine carbon site(s) in a fragment were then corrected for the dilution by carbon(s) from derivatizing reagents present in the fragment of interest (as these carbons have the same source in the sample and standard; see Table 1). This correction occurred by multiplying the $\delta^{13}C$ of the sample relative to the Alfa Aesar standard by a dilution correction factor defined in Equation S1 as nC_{frag}/nC_{ala}. The corrected $\delta^{13}C$ of the sample relative to the Alfa Aesar standard was then converted back into a ratio of ^{13}R values and multiplied by the ^{13}R value for Alfa Aesar for those sites on the VPDB scale. These steps are defined in the following equation:

$$^{13}R_{corr} = (((^{13}R_{sa,meas}/^{13}R_{AA,meas} - 1) \times nC_{frag}/nC_{ala}) + 1) * {}^{13}R_{AA,fVPDB} \qquad (2)$$

where $^{13}R_{corr}$ is the standardized ^{13}R value for a given fragment, $^{13}R_{sa,meas}$ is the ^{13}R value for a sample directly measured for a fragment on the Orbitrap, $^{13}R_{AA,meas}$ is the ^{13}R value for the Alfa Aesar standard directly measured for the same fragment on the Orbitrap, nC_{frag} is the total numbers of carbons in a fragment (*e.g.* 4 carbons for the 140.035 fragment), nC_{ala} is the numbers of carbons from alanine in that fragment (*e.g.* 2 carbon for the 140.035 fragment), and $^{13}R_{AA,fVPDB}$ Alfa Aesar's ^{13}R value for the alanine carbons in the fragment on the VPDB scale (for more information on these values see Eiler *et al.*, (2017). Finally, the standardized and corrected ^{13}R values were transcribed into $\delta^{13}C_{VPDB}$ values (Table 1). The corrected values assume that the derivative carbons between samples and standards have the identical ^{13}R values at each site between sample and standard (*i.e.*, such that ratios may be treated as conservatively

mixed properties) and that they have the same $\delta^{13}C$ values as the Alfa Aesar standards. We examined this assumption and found that variations less than ~50‰ would result in errors below the analytical uncertainty

Once each fragment was assigned a $\delta^{13}C_{VPDB}$ value, we calculated the site-specific $\delta^{13}C_{VPDB}$ of each of the three alanine sites. Our measurements of the analytical sample extracts provided three independent constraints on the site-specific $\delta^{13}C$ values of alanine: the molecule-average isotope ratio measured by compound-specific GC-C-IRMS and the two ratios measured by the Orbitrap (for the 140.032 and 184.032 Da fragment ions). The assumption that derivative carbons are the same in the sample as the alanine standard provided a fourth constraint. Each constraint is associated with its own uncertainty and weighted effect on the $\delta^{13}C$ of each alanine carbon site.

For the site-specific isotope calculation, the GC-C-MS measurement of molecular-average $\delta^{13}C$, and the Orbitrap measurements of the averaged C-1 + C-2 and the averaged C-2 + C-3 $\delta^{13}C$ were converted to fractional abundances ($^{13}F_{avg}$, $^{13}F_{C1+C2}$, $^{13}F_{C2+C3}$ respectively), which were then used to solve the following set of mass balance expressions (Eqn. 3a-3c):

$$^{13}F_{C\text{-}1} = (^{13}F_{molec\ avg} - \tfrac{2}{3}\,^{13}F_{C\text{-}2+C\text{-}3}) \times 3 \qquad (3a)$$

$$^{13}F_{C\text{-}2} = (^{13}F_{C\text{-}1+C\text{-}2} - \tfrac{1}{2}\,^{13}F_{C\text{-}1}) \times 2 \qquad (3b)$$

$$^{13}F_{C\text{-}3} = (^{13}F_{C\text{-}2+C\text{-}3} - \tfrac{1}{2}\,^{13}F_{C\text{-}3}) \times 2 \qquad (3c)$$

Once fractional abundances of ^{13}C in each site were calculated, they were converted $\delta^{13}C$ values. Error analysis is discussed in Appendix C.

3. Results

The 267 ng sample of alanine recovered from an acid-hydrolyzed hot water extract of the Murchison sample studied here comprises 0.665 ppm by weight of the bulk meteorite, is a nearly racemic mixture of D and L enantiomers, and has molecular-average $\delta^{13}C_{VPDB}$ values of 25 ± 3 ‰ and 26 ± 3 ‰ for the D and L enantiomers, respectively, which is consistent with prior measurements of alanine from samples of Murchison (Engel et al., 1990; Sephton, 2002; Elsila et al., 2012). Acid hydrolysis increases yield in our samples from 2.37 ± 0.23 and 2.30 ± 0.16 nmol/g (water-extractable, or 'free' alanine) to 5.30 ± 0.88 and 5.98 ± 1.03 nmol/g (total alanine) for D- and L-alanine respectively (Friedrich et al., 2018). Past studies have demonstrated that 'free' and total alanine are indistinguishable in $\delta^{13}C$ ((Burton et al., 2013); similar results are found for other water-soluble organics as with (Aponte et al., 2014)). Procedural blanks typically yielded alanine abundances that were less than 1 % of the recovered meteoritic material (see Appendix B, Figure S3, and Tables S1 and S2). Although the enantiomeric proportions of amino acids cannot conclusively establish their biogenicity, the weight of the preceding observations lead us to conclude our sample contains no detectable terrestrial contamination and closely approaches the properties of indigenous alanine found in Murchison. The site-specific $\delta^{13}C$ values for alanine are -29 ± 10 ‰, 142 ± 10 ‰, and -36 ± 10 ‰ for the C-1, C-2, and C-3 sites, respectively (Table 2, see Figure 1a for carbon site identities). Errors in each site-specific value are highly correlated due to the more precisely known molecular-average value (25.5 ± 3 ‰) and even more precisely known average of the C-2

and C-3 sites (64.6 ± 1.5 ‰); see the Appendix A for details. The carbon isotope structure we observe for Murchison alanine, particularly the marked ^{13}C enrichment of the C-2 site, provides new constraints on the mechanism, precursors, and setting of its synthesis.

4. Discussion

In the local ISM (*i.e.*, 8 ± 0.2 parsecs from the galactic center in the same general direction as the solar system), CO and HCO^+ are between 1.08 and 1.51 times (80 - 510 ‰) more ^{13}C-enriched than H_2CO, which is considered to reflect the composition of the reduced carbon ('CH_x') pool (Milam et al., 2005). One possible exception is CN, which has been measured and modeled to have a $\delta^{13}C$ value that is either similar to the ^{13}C-enriched CO or to the ^{13}C-depleted C^+-derived pools of carbon-bearing molecules (Langer et al., 1984; Langer and Penzias, 1990; Milam et al., 2005). Our finding of a high $\delta^{13}C$ value for the C-2 site in alanine provides a strong indication that this site is derived from a precursor that was itself synthesized in the ISM from CO, HCO^+, and/or CN.

However, our finding of a relatively low $\delta^{13}C$ value of the C-1 site is inconsistent with experimental constraints on amino acid synthesis via the irradiation of methanol ices and ammonia in the ISM. An experimental irradiation of isotopically labelled methanol ices at 40 K (Elsila et al., 2007) produced adequate amounts of serine for site-specific analyses and found that both the C-1 and C-2 sites are inherited from CN, implying that this mechanism should not lead to marked differences between the carbon isotopic compositions of the C-1 and C-2 sites. Assuming that alanine follows a similar formation pathway, we conclude that alanine from Murchison inherited the C-2 carbon from a precursor that was itself formed from the CO, HCO^+,

and/or CN pools in the ISM and that its ^{13}C depletion in the C-1 carbon was contributed from another, lower $\delta^{13}C$ precursor through reactions that likely occurred either in the early solar nebula or in Murchison's parent body. However, we note that further experiments should explore the potential of to form alanine through alternate pathways in the ISM such as by gas phase and other gas-grain reactions.

Our findings are also inconsistent with the hypothesis that this nebular or parent body chemistry followed a predominantly FTT mechanism. FTT-synthesized alanine inherits all its carbons from the source CO, with each added carbon being only subtly lower in $\delta^{13}C$ than the CO pool due to a KIE of approximately 0-10 ‰ (Mccollom and Seewald, 2006; Taran et al., 2007). Although this reaction mechanism is incapable of directly generating the ~170 ‰ contrast we observe between the $\delta^{13}C$ values of the C-2 site compared to the C-1 and C-3 sites, it is possible that alanine could form by an FTT-like process if the carbon in the C-2 site were derived from a secondary product of small molecules other than CO. In some conditions, FTT chemistry can create CO_2 and CH_4 that differ from one another by up to ~50 ‰ (Taran et al., 2007)— a contrast approaching that required by our data. In this case, alanine synthesis by FTT would require that the C-2 carbon is a secondary product of the ^{13}C-enriched CO_2 produced by FTT synthesis, whereas the C-3 and – most problematically – C-1 carbon are secondary products of low ^{13}C FTT-derived CH_4. We can think of no plausible chemical reaction sequences in which this would happen.

For these reasons, we conclude that alanine in Murchison likely formed via Strecker synthesis or reductive amination, that it was synthesized in the solar nebula, possibly in the meteorite's parent

418 body, and that it had at least one reactant that itself was derived from CO or CN in the ISM.
419 Drawing on past models and measurements, Elsila *et al.* (2012) and Aponte *et al.* (2017) argued
420 that meteoritic alanine formed by Strecker synthesis from ISM-derived acetaldehyde with a ^{13}C-
421 enriched carbonyl carbon inherited from CO and ^{13}C-depleted methyl carbon inherited from the
422 CH_x pool, in addition to NH_3, and ^{13}C-depleted HCN. These reactants would lead to alanine with
423 a high $\delta^{13}C$ value at the C-2 site and lower $\delta^{13}C$ at the C-1 and C-3 sites (Elsila et al., 2012)
424 (Figure 3). The results presented here are consistent with this argument. If instead alanine formed
425 by reductive amination, one of its precursors would have been pyruvic acid. If the precursor were
426 pyruvic acid formed solely by CO grain chemistry (Elsila et al., 2012), then all of its carbon sites
427 and those on the subsequently produced alanine will be equally ^{13}C-enriched, in disagreement
428 with our findings. If, however, pyruvic acid formed via a ketene or aldehyde reacting with HCN
429 and water in the ISM(Cooper et al., 2011) or cyanohydrin in the parent body, it could result in a
430 carbon isotope structure broadly resembling that produced by Strecker synthesis (See Appendix
431 E). We consider these two mechanisms equally plausible based on the constraints of our
432 alanine's C isotope structure. Non-α-amino acids (*e.g.*, β-, γ-) cannot be produced via the
433 Strecker pathway and require other mechanisms of production.
434
435 To help us predict the isotopic contents and structures for the precursors to alanine in Murchison,
436 we synthesized alanine via Strecker synthesis and measured its site-specific carbon isotope
437 effects relative to the starting acetaldehyde and NaCN (see Appendix F). Experiments indicate
438 that production of the α-aminopropanonitrile intermediate has a $\delta^{13}C$ that is 12 ‰ below its
439 acetaldehyde precursor at moderate (~60-70 %) yields. Because the C-3 carbon does not gain or
440 lose covalent bonds in the Strecker reaction, and thus will not experience large isotope effects

441 from the synthesis, the 12 ‰ shift in the average C-2 and C-3 $\delta^{13}C$ value suggests a -24 ‰
442 isotope effect on the C-2 carbon (see Figure 3), which is consistent with the KIE on a carbonyl
443 carbon from the addition of CN (Lynn and Yankwich, 1961). If we assume a large initial
444 acetaldehyde reservoir such that its isotopic value is effectively constant during alanine
445 production, and account for the reactant aldehyde's fractionation by adding 12 ‰ to the C-2 and
446 C-3 site's average $\delta^{13}C$, we predict that the initial acetaldehyde reservoir parental to Murchison
447 alanine had a molecular-average $\delta^{13}C$ of 64.6 ± 1.5 ‰. This value is within error of 64 ± 1 ‰, a
448 molecular-average value for acetaldehyde recently measured in Murchison (Figure 4, Dataset 2,
449 and (Aponte, Whitaker, et al., 2019)). This agreement is consistent with our suggestion that
450 alanine had an acetaldehyde precursor and thus reinforces the possibility that alanine was
451 synthesized by Strecker reaction rather than reductive amination; it also suggests that the initial
452 aldehyde pool was not fractionated during the synthesis of alanine and was therefore either large
453 in amount relative to the alanine produced or that aldehyde was continuously produced (*e.g.*,
454 from the hydrolysis of other acetaldehyde-derived compounds) as it was consumed in alanine
455 syntheses. We note that other measurements of the molecular-average $\delta^{13}C$ of acetaldehyde have
456 found values of 25-27 ‰ (Simkus et al., 2019) possibly due to sample heterogeneity or
457 fractionation of volatile molecules during laboratory extraction (Aponte, Whitaker, et al., 2019).
458 Future site-specific isotope ratio studies of Strecker synthesis reactants (*e.g.*, aldehyde, CN) and
459 products from the same sample could resolve the reason for this discrepancy and further test our
460 hypothesis.
461
462 The Strecker experiments also indicate that the acid hydrolysis of α-aminonitrile to an amino
463 acid has a KIE on the C-1 site of up to -50 ‰ for a 13 % conversion of cyanide to alanine and a

mean value of -22 ‰ for a 20 % to 55 % conversion of α-aminonitrile to alanine (Figure 4). Therefore, if alanine in Murchison formed by Strecker synthesis with moderate yield in its second step (20 – 50 %), it should have inherited its C-1 carbon from reactant CN that had $\delta^{13}C$ of -7 ± 10 ‰ (For error analysis, see Appendix D). This value is within error of the previously reported 5 ± 3 ‰ $\delta^{13}C$ of HCN in Murchison (Pizzarello, 2014), which suggests a reactant reservoir that was large relative to its products . Other combinations of substrate $\delta^{13}C$ values and reaction yields are also possible, but the agreement of this scenario with independent constraints for acetaldehyde and HCN support its plausibility.

The preceding findings enable us to create a testable hypothesis in the form of a chemical network connecting the synthesis of alanine in Murchison and the formation of other organic compounds, including C_1 to C_6 aldehydes, amines, carboxylic acids, and other α-amino acids in the Murchison parent body **(**See Appendix F, Dataset 2, and references Aponte *et al.* (2017); Simkus *et al.* (2019)). Our model above predicts an acetaldehyde precursor of alanine having carbonyl and methyl groups with $\delta^{13}C$ values of 166 ± 10 ‰ and -36 ± 10 ‰, respectively (noting that the average of these two sites is predicted with a much narrower error of ±1.5 ‰). The model we present presumes that alanine and the other soluble organics we consider were synthesized from a pool of precursors (H_2O, aldehydes, HCN, NH_3) that was not significantly depleted by their growth (excepting HCN, which we assume underwent 10's of % consumption by the Strecker chemistry, as in our experiments), that all reactions occurred at the same temperature, and that none of the studied compounds are residual to losses by a fractionating side-reaction. These assumptions are clearly simplifications, but generally similar models that relax these constraints (*i.e.*, allowing for variable temperature, reaction progress or side

reactions) do not strongly impact our predictions (Appendix F). If formaldehyde and acetaldehyde have the same carbonyl source as expected for ISM-derived aldehyde, then the $\delta^{13}C$ of formaldehyde should be 166 ± 10 ‰. Likewise, larger aldehyde precursors would be predicted to have molecular $\delta^{13}C$ values equal to the weighted average of their one ^{13}C-rich carbonyl carbon and some additional number of ^{13}C-poor R-group carbons similar in ^{13}C isotopic composition to acetaldehyde's methyl group. These predictions agree with some molecular-average measurements of individual linear aldehydes having 2-5 carbon atoms from Murchison (Figure 4a), but they over-predict the $\delta^{13}C$ measured for formaldehyde (Simkus et al., 2019) and under-predict the measured differences between branched and linear compounds (Figure 4b, *refs 23* and *24*). Data from Simkus *et al.*, (2019) disagree with our predicted acetaldehyde value but agree with our predictions for C_4 and C_5 linear aldehydes and still display modest ^{13}C-enrichments for C_2 and C_3 linear aldehydes.

We hypothesize that other molecules with amine functional groups in Murchison were formed by reductive amination of the same aldehyde precursors that formed alanine through Strecker synthesis. Past measurements of reductive amination have demonstrated negligible KIEs of less than 1 ‰ (Billault et al., 2007), so the carbon backbones of other organic amines should resemble the parent aldehyde in our proposed mechanism. This hypothesis leads to $\delta^{13}C$ predictions of 166 ± 10 ‰, 64.6 ± 1.5 ‰, 31 ± 4 ‰, and 14 ± 5 ‰ for methyl-, ethyl-, propyl-, and butylamine, respectively, which resemble previous measurements from Murchison (Figure 4 and (Aponte et al., 2016)). However, the predictions cannot account for the lack of measured difference in $\delta^{13}C$ between the C_3 and C_4 amines.

Similarly, we hypothesize that aldehyde precursors in Murchison can be oxidized into monocarboxylic acids via hydration and hydrogen abstraction at the carbonyl carbon. In the presence of water and metal oxides, aldehydes can be oxidized (Rajesh and Ozkan, 1993) to form carboxylic acids. Metal oxide catalysts are present in Murchison and other CM2 meteorites (Bunch and Olsen, 1975; Hanowski and Brearley, 2000), supporting the plausibility of this scenario. Accounting for previously measured KIEs associated with addition reactions to aldehydes (a 0 to -19 ‰ KIE for carbonyl carbons; (Yamataka et al., 1997; Yamataka et al., 2001)) and the likely upper limit of a ~-30 ‰ KIE for the oxidation of a carbonyl carbon, the $\delta^{13}C$ values of the C_1-C_5 monocarboxylic acids can be calculated as a mixture of a ^{13}C-enriched carbonyl carbon and ^{13}C-depleted methyl carbons. The final predicted monocarboxylic acid molecular-average $\delta^{13}C$ values vary little between the 0 ‰ and -30 ‰ isotope effects on the carbonyl carbon, so we will consider the -30 ‰ predictions that closely agree with previous measurements for the C_3-C_5 species from Yuen *et al* (1984) and with the trends presented in more recent studies by Huang *et al.* (2005) and (Aponte *et al*. (2019) (Figure 4a). The overprediction of acetic acid's $\delta^{13}C$ relative to data from all studies (Yuen et al., 1984; Huang et al., 2005; Aponte, Woodward, et al., 2019), in conjunction with the larger range of measured $\delta^{13}C$ values for acetic acid (~75 ‰) versus those for other monocarboxylic acids (0-20 ‰) (Figure 4a) supports the argument that the acetic acid measured on Murchison is a mixture of two or more sources (Huang et al., 2005). Furthermore, the differences in past monocarboxylic acid $\delta^{13}C$ measurements from both our predictions and from each other (Yuen et al., 1984; Huang et al., 2005; Aponte, Woodward, et al., 2019) could reflect spatial $\delta^{13}C$ heterogeneity of these components that our model does not capture as it bases its predictions on $\delta^{13}C$ values from one compound from one meteorite sample (see Appendix F). Studies of site-specific isotope

ratios of monocarboxylic acids and aldehydes could provide a means of further testing and refining our understanding of the relationships amongst these compounds in Murchison as they play a critical role in the network of reactions in which amino acid synthesis occurs. Despite these complexities in the prior carbon isotope data, the relatively straightforward, unified chemical reaction network we propose provides a coherent and accurate explanation for the measured $\delta^{13}C$ values of alanine, reactant HCN, and most aldehydes, amines and monocarboxylic acids in Murchison, based only on two assumed $\delta^{13}C$ values (that for CO and CH_x precursors in the ISM; see Figure 3 and Appendix F). The most noteworthy disagreements between our model and prior data for Murchison extracts are for formaldehyde and glycine. These are among the most volatile and easily contaminated compounds that we considered, and we suggest their high variability among prior studies and lower-than-predicted average values may reflect particularly poor preservation.

Four complicating factors prevent us from extending our model to all amino acids in Murchison that have $\delta^{13}C$ measurements: 1) prior studies yield ranges of up to 30 ‰ in $\delta^{13}C$ for individual amino acids (Engel et al., 1990; Pizzarello et al., 2004; Elsila et al., 2012), possibly reflecting spatial variation in precursors, reaction progress, and/or terrestrial contamination between sub-samples; 2) amino acids as a whole are structurally diverse and draw on a variety of precursors that may not have been uniform in their ^{13}C contents; 3) amino acids can be subject to side reactions not considered in the simple reaction network outlined above; and 4) Strecker synthesis can only produce α-amino acids, so all others (*e.g.*, β, γ, δ) require other synthetic routes. Nevertheless, it is straightforward to extend our hypothesis to an approximate prediction of the molecular-average $\delta^{13}C$ values of the α-amino acids. If we assume all the C-1 and C-2 sites in α-

amino acids have $\delta^{13}C$ values that are identical to those observed in alanine and that all other carbon atoms have $\delta^{13}C$ values equal to that of the C-3 site in alanine (as would occur if all form by Strecker synthesis from a closely related pool of aldehyde precursors and HCN as outlined above and in Figure 3), then we can calculate the molecular average $\delta^{13}C$ values of the other individual α-amino acids. The results are similar to most prior measurements of the C_2-C_5 α-H-amino acids, except a subset of glycine measurements; there are several possible explanations for this discrepancy, but we note that glycine is unusual in being achiral and is suspected to have been synthesized by multiple mechanisms (Figure 4) (Engel et al., 1990; Pizzarello et al., 2004; Elsila et al., 2012).

The model presented here consistently under-predicts $\delta^{13}C$ values of both branched aldehydes and α-CH_3-amino acids (Engel et al., 1990; Pizzarello et al., 2004; Elsila et al., 2012) (Figure 4b). One possible cause of higher measured $\delta^{13}C$ values in the amino acids, particularly the α-CH_3 amino acids, in Murchison samples is isotopic exchange between carboxyl sites and dissolved inorganic carbon (DIC) present during parent-body aqueous alteration. Theoretical calculations demonstrate that this exchange can occur for amino acids (Rustad, 2009; Pietrucci et al., 2018), has lower energy barriers for α-CH_3 species than for α-H species (Pietrucci et al., 2018), and high-$\delta^{13}C$ carbonate minerals in Murchison attest to the presence of a ^{13}C-rich DIC pool (est. with the highest measured value of +80 ‰ (Sephton, 2002) to present the full possible range of $\delta^{13}C$ values, see SI) during aqueous alteration. The measured molecular-average $\delta^{13}C$ values of the α-CH_3 amino acids are similar to those predicted by our model of Strecker synthesis if it is followed by equilibration of carboxyl sites with the DIC pool (purple arrows in Figures 3 and 4). Our predictions represent a maximum $\delta^{13}C_{VPDB}$ change in the amino acids (top

of the purple arrows in Figure 4). The isotopic composition of DIC varies on Murchison samples; therefore, a lower amount of exchange and/or exchange with a less enriched $\delta^{13}C_{VPDB}$ DIC pool would result in $\delta^{13}C_{VPDB}$ values that span the length of the purple arrows in Figure 4. It may be that partial exchange and/or lower $\delta^{13}C$ DIC pools explain why some of the α-H amino acids have $\delta^{13}C_{VPDB}$ values lower than predicted by our model. This mechanism would not function on the monocarboxylic acids without moieties on the C-2 site with a lone pair (*e.g.*, NH_2 or OH) as the C-2 site could not switch between sp^3 and sp^2 as easily. This explanation for the $\delta^{13}C$ values of the amino acids is not unique; however, it captures the full range of observations with a single plausible addition to an already parsimonious model. Branched-aldehydes and branched-sidechain amino acids require different explanations as both would require a less favorable exchange of C in saturated hydrocarbon chains. The differences in isotopic content between linear and branched compounds is another attractive target for further studies of site-specific isotopic contents of meteoritic organics.

The arguments and data presented here suggest that Strecker synthesis is likely the origin of alanine in the Murchison meteorite and that aldehydes formed from CO and CH_x in the ISM are essential precursors to a wide range of the prebiotic organic compounds observed in Murchison. These organic compounds include amino acids, amines, and carboxylic acids that formed when the ISM-sourced aldehydes reacted with HCN, NH_3, and water. Following the production of amino acids, isotopic exchange between the carboxyl group and ^{13}C-rich DIC pool might have occurred in at least some α-amino acids, approaching equilibrium for the relatively exchangeable α-CH_3 amino acids. The success of a simple reaction model (Figure 3) in explaining most of the $\delta^{13}C$ values previously measured for these diverse compounds supports the idea that the various

chemical reactions called on occurred concurrently, in a single environment, and drawing on a common pool of precursors, some of which likely originated in the ISM(de Marcellus et al., 2015). Aqueous alteration in the Murchison parent body is a plausible setting where this could have transpired.

Acknowledgments: We would like to acknowledge Dr. Max Klatte and Dr. Carl Blumenfeld for aiding with the Strecker synthesis of alanine. **Funding:** This project was supported by NASA through LARS grant number NNX17AE52G and through part of the Planetary Science Division Internal Scientist Funding Program through the Fundamental Laboratory Research (FLaRe) work package of the Planetary Science Division Internal Scientist Funding Program, by DOE grant DE-SC0016561, by Thermo Fisher, and by the Simons Foundations; **Author contributions:** LC, BD, ALS, and JME designed methods for site-specific carbon isotope measurements. JEE, JPD, and JA provided Murchison sample. JEE extracted amino acids and measured molecular-average isotope ratios of alanine. LC and JME created the Monte Carlo simulation to calculate isotope ratios. LC measured alanine on Murchison meteorite, processed data, and calculated site-specific isotope ratios. LC, JEE, JPD, JA, ALS, and JME contributed ideas to form the parent-body organic synthesis model.; **Competing interests:** Authors declare no competing interests.; and **Data and materials availability:** All data is available in the main text or the Supplementary Information.

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Figure Legends:

Figure 1: Mass spectra and fragment images for alanine from Murchison meteorite sample measured in this study. (a) Alanine with carbon sites are labelled. Mass spectra and labelled fragment images are presented for (b) *m/z* 140 and (c) *m/z* 184. In panels (b) and (c), fragments are in black with the rest of the derivative in gray, and carbon sites from alanine being measured are bolded. Insets for each mass spectra displays ^{13}C-substituted peak to demonstrate resolution from potential isobars.

Figure 2: Schematic of custom inlet system for Orbitrap for (a) direct injection and (b) reservoir elution. (a) Chromatogram of 50-300 Da for direct injection that was used to find elution of alanine in Murchison sample. (d) Chromatogram of the 140.032 Da peak for reservoir elution during a typical measurement for Murchison sample.

Figure 3: Proposed mechanisms for syntheses of organic compounds related to alanine (with R of CH_3) on the Murchison parent body, with associated carbon isotope effects. In this scheme, CO and CH_x are derived from the ISM. Reaction steps between the aldehyde and imine and between the imine and aminonitrile are reversible (Van Trump, 1975). Isotopic values for the initial CO and $n$$CH_x$ are back-calculated using our measured alanine value and the isotopic effects shown.

Figure 4: (A) Comparison of $\delta^{13}C$ measurements from this study, model predictions, and literature values for each carbon site in alanine and for molecular-averages of precursors, product aldehydes, amines, monocarboxylic acids, and α-H-amino acids with linear carbon sidechains. (B) Comparison of $\delta^{13}C$ measurements from this study, model predictions, and literature values for α-CH_3-amino acids with linear carbon sidechains (denoted with no subscript) and for aldehydes and α-CH_3-amino acids with branched carbon sidechains (denoted with *br* subscript). We only include compounds with isotopic values recorded in the literature and with alpha chiral sites as well as glycine (and possible compounds made from its proposed precursor, formaldehyde) due to its biological importance. The pink

arrows display the range of values predicted based on the range of KIEs for aldehyde oxidation on the reactant CO site (the terminal COOH site in monocarboxylic acids). The purple arrows highlight the expected range of values for Strecker synthesis followed by carbon isotope exchange between DIC and the C-1 sites of α-amino acids. The subscripts denote the total number of carbons in the molecule. The error bars for carboxylic acids are smaller than symbols and are not included in the data for the Engel *et al.* (1990) measurements as they are not provided in the 1990 paper.

Table 1: Fragment ^{13}R values and $\delta^{13}C$ values (AA and VPDB scales) for samples, standards, blanks. All delta values are dilution corrected. Standard error values are listed in parentheses. The first two columns of data (Molecular-average $\delta^{13}C$ and Fragment ^{13}R) were directly measured while Fragment $\delta^{13}C$ values relative to Alfa Aesar and VPDB were calculate using equation S1. The $\delta^{13}C$ values used in the Monte Carlo simulation are in the last columns (Fragment $\delta^{13}C_{VPDB}$).

Table 2: Fragment and site-specific $\delta^{13}C_{VPDB}$ values for hydrolyzed alanine from a Murchison meteorite hot water extract. The full molecular-average direct measurement $\delta^{13}C$ value was measured via GC-C-IRMS, and the fragments' $\delta^{13}C$ values were measured on the Q-Exactive GC Orbitrap mass analyzer. The site-specific $\delta^{13}C$ values were calculated using the average of the D- and L-alanine molecular averages and the fragment $\delta^{13}C$ values.

Supplementary Information:

Appendices A-F

Figures S1-S3

Tables S1-S3

Electronic Annex 1:

Values for ^{13}R, ^{12}C counts, and ^{13}C counts of each analysis. ^{13}R values are a weighted average for the entire run and only include scans for which the monoisotopic and singly ^{13}C-subsituted peaks are present. The ^{12}C counts and ^{13}C counts are summed from all scans including those that only contained monoisotopic or singly ^{13}C-subsituted peaks. Further discussion of culling procedures for the ^{13}R can be found in the Site-Specific Isotope Analysis and Blanks sections.

Electronic Annex 2:

Predictions of molecular-average $\delta^{13}C$ values for prebiotic compounds. Literature values are from (*a*) Pizzarello (2014), (*b*) Simkus *et al.* (2019), (*c*) Aponte, Whitaker, *et al.* (2019), (*d*) (Aponte *et al.*, (2016), (*e*) (Yuen *et al.*, (1984), (f) Huang *et al.*, (2005), (*g*) Aponte, Woodward, *et al.*, (2019), *(h*) Engel *et al.*, 1990), (*i*) Pizzarello *et al.*, (2004), (*j*) Elsila *et al.*, (2012). Alternate predictions refer to a fully expressed 19 ‰ normal KIE for carboxylic acids and DIC

equilibration with the carboxyl site for amino acids. Data in which cis-trans isomerism was measured separately, data from cis-isomers are denoted with (*Z*) and trans are denoted with (*E*).