Appendix A: Calibration of alanine standards for Site-Specific Isotope Ratio (SSIR) Measurements

The molecular-average $\delta^{13}C$ values of pure alanine standards were measured on a Thermo Fisher Scientific Flash Elemental Analyzer (EA) coupled to a Delta-V isotope ratio mass spectrometer (IRMS) at Caltech. Alanine standards are described above (See Materials: Derivatization). A lab acetanilide standard served as check on accuracy of $\delta^{13}C$ measurements. The $\delta^{13}C$ values and associated uncertainties for the alanine standards are -19.4 ± 0.1 ‰, -20.0 ± 0.2 ‰, and -32.9 ± 0.2 ‰ for Alfa Aesar, VWR, and Strecker alanine respectively (Eiler et al., 2017) (Table 1); acetanilide was measured to have a $\delta^{13}C$ value of -27.6 ± 0.1 ‰ in good agreement with its prior measured value of -27.7 ± 1.7‰.

The Alfa Aesar and Strecker alanine standards were also analyzed at GSFC following protocols from (Elsila et al., 2012) using coupled GC-combustion-IRMS (GC-C-IRMS), which enables isotopic analysis of individual amino acids in mixtures such as those from the Murchison extracts. After accounting for dilution effects from the derivative methyl and isopropyl groups (See Data Processing and (Elsila et al., 2012) for details on dilution effects), the standards' $\delta^{13}C$ values were -19.4 ± 0.2 ‰ and -33.3 ± 0.1 ‰ for Alfa Aesar and Strecker respectively, which is within two standard errors of those measured at Caltech (Table 1).

We also measured the $\delta^{13}C$ values of C-1 in all 3 alanine standards via ninhydrin decarboxylation, following methods from (Van Slyke et al., 1941) and (Abelson and Hoering, 1961). Resulting $\delta^{13}C_{VPDB}$ values for C-1 were -28.5 ± 0.1 ‰, -29.5 ± 0.3 ‰, -43.5 ± 0.1 ‰, for the Alfa Aesar, VWR, and Strecker standards, respectively (Eiler et al., 2017). Combining these

data with the molecular-average $\delta^{13}C$ values from above allowed us to calculate the average $\delta^{13}C$ of their combined C-2 and C-3 sites (See Section 2.3: Data Processing for calculations and Figure 1a in main text for alanine with labelled carbon sites) as -14.8 ± 0.6 ‰, -27.6 ± 0.3 ‰, and -15.6 ± 0.3 ‰. At the time of this publication, we have no independent evidence regarding the individual isotopic compositions of the C-2 and C-3 sites in these standards; however, NMR studies of site-specific carbon isotope ratios of amino acids (R. Robins pers. com.) indicate that all common terrestrial forms of these amino acids, including standards purchased from Sigma Aldrich (BioUltra, >99% Purity, Lot# BCBM6312V), have $\delta^{13}C_{VPDB}$ fractionations between C-2 and C-3 in each molecule that are 10 ‰ or less, which is in the upper range of differences between methyl and adjacent cites for other small organics (Gilbert et al., 2011). The differences we observe in the Murchison sample relative to the Alfa Aesar standard C-2 and C-3 are on the order of 170 ‰, and the error of the C-3 calculation (10 ‰) is within error of the 10 ‰ difference found between C-2 and C-3 in other alanine samples. Consequently, the potential 10 ‰ difference is negligible in our study, and for this study we assume our standards have C-2 and C-3 sites that are identical in $\delta^{13}C$. Future measurements of one or more of the standards used in this study could be used to refine the data presented here in order to account for the likely small differences between C-2 and C-3 in our alanine standards, but we think it implausible that our conclusions could be influenced by the small isotopic differences between these sites likely present in our terrestrial standards.

Site-specific $\delta^{13}C$ values for the Methods Development samples measured in December and March are within error of one another (Table 1). We interpret the differences in site-specific isotope ratios between methods development and analytical samples as being due to terrestrial contamination (though it is also possible that they partially reflect differences in isotopic

composition between the alanine native to these two Murchison samples or fractionations arising from chemical reactions of sample alanine during storage). Regardless, we base our discussion of the Murchison sample only on the analytical sample. We present the data for the methods development sample only in order to document the development of the methods used in this study.

Appendix B: Blanks

Multiple procedural blanks were carried through the workup and analyzed alongside the Methods Development and Analytical samples. All blanks start at their listed step (*e.g.*, extraction, transfer, derivatization; see Table S1) and follow all subsequent steps through derivatization as outlined in Figure S2. As an example, blanks designed to test the extraction of amino acids had water added to an empty ampoule after which all subsequent extraction, transfer, and derivatization steps were followed. Thus, all blanks should only contain derivatizing reagents, the products of their reactions with one another, and hexane if sample processing produced no contamination. Procedural Blanks are summarized in Table S1 and consisted of the following: (1a and 1b) blanks that tested reagents used in the derivatization of alanine (our ultimate analytical target), (2) a blank that starts with water leaching at GSFC and continues through chemical derivatization at Caltech, (3) a blank that starts with the water:methanol transfer of the meteorite extract into a GC vial at Caltech, and (4) a blank that starts with analyte derivatization at Caltech (See Figure S2). Procedural Blanks 1a and 1b occurred prior to the day of meteorite extract derivatization while Procedural Blanks 2-4 occurred on the same day as the corresponding meteorite extract derivatization. Additional solvent blanks (injections of hexane

into the Orbitrap) and instrument blanks (temperature ramps with no injection) were run prior to each meteorite analysis to test the instrument background.

Each procedural blank was analyzed in Direct Injection mode on the Orbitrap, and signals were integrated between 6.5 and 8.5 minutes after injection for ^{12}C and ^{13}C counts from *m/z* 140.032 and 141.035 fragment peaks (for conversion from signal intensity to counts see(Eiler et al., 2017). Alanine elutes at ~7.5 minutes and is typically transferred into the reservoir from approximately 7–8 minutes retention time, so counting the background over 2 minutes overestimates possible contamination. As with the sample data (see Site-Specific Isotope Analysis and Data Processing) data used to calculate ^{13}R was culled only to include scans that contained both the monoisotopic and singly ^{13}C-substituted fragment and was computed using a counts-weighted average of all ^{13}R values in the blank. Reported sums of ^{12}C and ^{13}C counts (Dataset S1) use all scans including those which have only the monoisotopic or the singly ^{13}C substituted fragment without the other in order demonstrate the maximum possible error in our measurements. When compared to samples measured with the Reservoir Elution mode, the overestimation is even greater because in Reservoir Elution mode measurements are broadened over many tens of minutes, giving them a lower signal-to-noise ratio (which is inversely proportional to counts reported). The procedural blank for analytical Murchison that had the highest amount of contamination in all metrics was Procedural Blank 2 (Table S1), which started with the meteorite extraction at GSFC. However, compared to the 15 pmol/μL alanine in the analytical sample, Procedural Blank 2 contained 0.15 pmol/μL and could account for only 1.9 % of the integrated ^{12}C counts, 0.7% of the integrated ^{13}C counts, and 0.3 % of the integrated ^{12}C signal intensity relative to the directly injected Murchison sample. The 140.032 and 141.035 *m/z*

fragments are the most abundant ones in the mass spectrum of alanine. Maximum abundances of *m/z* 140.032 and 141.035 ions in blanks were low (see Dataset S1) and did not appear during the 7.41-7.73 window during which alanine elutes, so these background signals likely either represent other compounds derived from column bleed, reagents, etc., and/or part of the instrument background. For chromatograms and spectra of blanks and Murchison, see Figure S3.

Solvent blanks and instrument blanks were run prior to meteorite sample analyses and also processed for integrated ^{12}C and ^{13}C counts from 6.5 to 8.5 minutes elution time (Table S2). These measurements find background ^{12}C and ^{13}C counts arising from the injector, column, transfer lines, etc. to typically account for less than 0.5% of the measured ^{12}C and ^{13}C counts in Murchison samples and a <0.05 ‰ change in ^{13}R values. Of the fragments used to calculate the site-specific isotope ratios of alanine, the highest background signals were observed for the *m/z* 184.021 fragment. In this case, the background counts account for approximately 0.5 % of the measured signal but change the ^{13}R value by only ~0.03 ‰, which is well within the ~10 ‰ standard error of the measurements at the 184.021 fragment. The low procedural blanks and instrument background demonstrate that our ^{13}R values reflect alanine from the meteorite rather than background or contamination.

Appendix C: Potential additional constraints for alanine SSIR measurement

We attempted to add a fourth constraint to our characterization of the carbon isotope structure of alanine by measuring the ^{13}R of a fragment ion having a monoisotopic mass of 113.0208 ($C_3H_4OF_3$). The straightforward fragment suggested by this mass would be $CF_3CH(O)CH_3$ using C-2 from the parent alanine. However, our studies of labeled alanines suggest that this fragment

only receives sample carbon atoms from C-3 of the parent alanine along with two carbons from the TFAA derivatizing reagents and none from C-2 of the parent alanine. The stoichiometry of this ion suggests it is a recombination product (*i.e.*, because direct fragmentation of the parent molecule cannot create a single piece containing these sites). We infer C-3 of alanine recombines with COH and CF_3 from the TFAA derivatizing reagent either as a two-body reaction or as two stepwise reactions. This complexity calls into question whether such a measurement could yield a consistent constraint on the ^{13}R of C-3 because the yields of recombination reactions generally depend on source pressure and other analytical variables (*i.e.*, we can imagine the same ion might be produced through other pathways when analytical conditions are varied). In any case, when we attempted to apply this method to the derivatized Murchison extract our peak captures of alanine were contaminated by at least one subsequent peak of a different compound. We recognize one such candidate contaminant peak also produces a 113.0208 Da fragment ion. Thus, we consider these measurements to have failed for reasons having to do with our chromatographic separations and peak trapping. We report these results in the for completeness, but we do not use these data as constraints on the Murchison sample carbon isotope structure.

Appendix D: Error Analysis

Errors for the Total Orbitrap and the Combined Orbitrap/GC-C-MS calculations were weighted according to the proportion effect of their value on the final calculation and then added in quadrature (Eqn. A1a-A1c):

Combined Orbitrap(140,184)/GC-C-IRMS Calculation Error

$$^{13}\sigma_{C\text{-}1} = \{(3 \times {}^{13}\sigma_{molec\ avg})^2 + (2 \times {}^{13}\sigma_{C\text{-}2+C\text{-}3})^2\}^{0.5} \qquad (A1a)$$

$$^{13}\sigma_{C\text{-}2} = \{(2 \times {}^{13}\sigma_{C\text{-}1+C\text{-}2})^2 + {}^{13}\sigma_{C\text{-}1}{}^2\}^{0.5} \quad (A1b)$$

$$^{13}\sigma_{C\text{-}3} = \{(2 \times {}^{13}\sigma_{C\text{-}2+C\text{-}3})^2 + {}^{13}\sigma_{C\text{-}2}{}^2\}^{0.5} \quad (A1c)$$

It is important to note that the resulting computed errors for the three alanine sites are highly correlated with one another due to interdependencies among the functions that relate them to the various measured ratios. In particular, the $\delta^{13}C$ of C-2 and C-3 are associated with large errors, yet their average is known to within 1.5 ‰ (1SE). The primary control on the error is the experimental uncertainty in the average C-1 + C-2 $\delta^{13}C$, which is doubled in computing the site-specific uncertainty of the C-2 site (See Eqn. A1b) and then propagated into the calculated $\delta^{13}C$ of the C-3 site. If future studies improve in the precision of the results presented here, it will be productive to focus on these dependencies; in particular, a highly precise molecular-average measurement that includes the derivative carbons, a high precision analysis of the *m/z* = 184.021, and a high precision analysis of the fragment *m/z* = 113.032 fragment with peak capturing that excludes subsequent peaks. These improvements were not possible during this study due to limited sample sizes, but a more ambitious effort to extract and purify alanine from Murchison might achieve errors on the order of ~1 ‰ for all sites (see (Neubauer et al., 2018) for an example of high precision amino acid C isotope structures measured using our techniques).

Appendix E: Alternative Pathways for Alanine Synthesis

In addition to acetaldehyde and cyanide reacting via Strecker synthesis, the alanine carbon isotope structure could be explained by the reductive amination of pyruvic acid(Rustad, 2009; Robins et al., 2015). In this case, the pyruvic acid would form from a ketene (ethenone) which sources its alkyl group (C-2) from the same ^{13}C-deplete CH_x pool and its CO (C-1) from the

same ^{13}C-enriched CO pool described in the main text (See Figure S4). The ethenone would then react with CN and water to form pyruvic acid that could react with NH_3 on later to form alanine. Consequently, assuming a low ^{13}C ISM CN pool, this reaction network could explain our results. Furthermore, as the reaction network (Figure S4) still involves the addition of CN to an sp^2-hybridized carbon and the oxidation of a nitrile to a carboxyl group (Rustad, 2009), the isotope effect and thus predicted initial carbon values should not greatly change between the scenarios (excepting possible changes in isotope effect due to physiochemical conditions).

Unlike the Strecker model, the pyruvate model would not provide clear pathways to amines, aldehydes, or monocarboxylic acids. Furthermore, measured values of keto acids are, as of yet, unavailable such that we could not compare predictions of this model to our data. For this reason, we chose to focus on the Strecker synthesis possibility. The agreement between our predictions and measured values across a wide range of compound classes supports the possibility that Strecker synthesis of aldehydes and cyanohydrins produced alanine and other organic compounds.

We also considered whether Murchison alanine could be the product of a reaction network in which alanine carboxyl is derived from high $\delta^{13}C$ HCN, through Strecker chemistry. This hypothesis could be indirectly supported by the observation that monocarboxylic acids in Murchison have high molecular average $\delta^{13}C$ values (Yuen et al., 1984). If these carboxylic acids formed by hydrolysis of nitriles, then those nitriles presumably could have been high in $\delta^{13}C$. And if that ^{13}C enrichment were hosted by the terminal CN group, we should expect co-existing HCN would be ^{13}C enriched. We are not aware of measurements of $\delta^{13}C$ of Murchison

nitriles (and their terminal CN groups are certainly not known). But if their terminal CN groups were enriched enough to account for the 10's of per mil enrichment of carboxylic acids, it would imply a $\delta^{13}C$ value for that group of +100 ‰ or more. This hypothesis is speculative but based on sound chemical principles and so worth considering. Nevertheless, it is strongly contradicted by data (both from previous studies and our study), so we think it must be rejected. Most simply, HCN from Murchison is relatively low in $\delta^{13}C$ (Pizzarello, 2014), and our measurement of alanine carboxyl indicates it is consistent with derivation by Strecker reaction from that measured HCN. We conclude the most parsimonious interpretation is that alanine in fact did form from the HCN present in Murchison, and that this HCN was not derived from a strongly ^{13}C-enriched pre-solar pool.

Finally, we consider the IOM as source of organics. (Huang et al., 2007) argue that monocarboxylic acids and other small organics could be produced by the hydrothermal processing of IOM. Observations that might be taken as evidence of this idea include: 1) Correlations of the $\delta^{13}C$ values of monocarboxylic acids with their carbon numbers are similar to those for moieties from the IOM; and 2) our measurements demonstrate that the IOM has an isotopic composition similar to the ^{13}C pool that was the source of the C-1 and C-3 sites of alanine, perhaps suggesting alanine is also formed by hydrolysis of IOM. This second observation could be understood in the context of the model we present if the IOM and alanine's C-1 and C-3 sites both derive from a primordial low ^{13}C pool (*i.e.*, hydrocarbons and HCN). If, instead, alanine was made from hydrolysis of the IOM, it is not obvious how it would have acquired such an extraordinarily high $\delta^{13}C$ value in its C2 carbon site without evidence of enrichment in the C1 and C3 sites. We are aware of no high ^{13}C chemical moieties of the IOM

that could readily explain this finding, and so we believe this idea could not be developed to provide a satisfactory explanation of this study's results.

Nevertheless, future compound- and site-specific measures may be able to identify IOM processing as a source of soluble organics in Murchison (and perhaps other carbonaceous chondrites). The site-specific $\delta^{13}C$ isotope ratio for compounds produced by IOM processing should mirror those found in the IOM aliphatic side chains (which have compound specific molecular average $\delta^{13}C$ values of 57.9 ‰ to 0.4 ‰). In contrast, the reaction network we propose predicts that the terminal carboxyl (C-1) sites of the carboxylic acids will be highly ^{13}C enriched compared to all other CH_x sites.

Appendix F: Parent-Body Organic Reaction Model

Constraints on the Isotope Effects Associated with Syntheses

To calculate the $\delta^{13}C$ values of alanine precursors and organic synthesis products other than alanine, isotope effects of different synthetic steps were collated from literature review and those for Strecker synthesis were measured via experimental work conducted as part of this study. Isotope effects for Strecker synthesis were further validated by comparison to literature values for isotope effects from similar reaction mechanisms.

The reduction of aldehydes into imines via reductive amination has a maximum measured isotope effect of 0.6 ‰ (Billault et al., 2007), which is lower than our measurement errors so was treated as a 0 ‰ fractionation in the model. Studies for carbon isotope effects during the oxidation of aldehydes have observed a range of effects from negligible (aldehyde to

thiohemiacetal conversion) (Canellas and Cleland, 1991) to large deuterium isotope effects that suggest possible concurrent carbon isotope effects (Wiberg, 1954); although these have not been measured. To consider both possibilities, we consider two endmember cases of 1) no isotope effect and 2) a 30‰ normal kinetic isotope effect, similar to intrinsic KIE's associated with other carbon oxidation reactions (Cleland, 2005). Mechanisms and associated isotope effects are portrayed in Figure 3 in the main text. Differences in our solution between the 0 ‰ carbonyl oxidation KIE and the 30 ‰ normal KIE case are depicted in Figure 4 in the main text.

Experimental work was conducted to constrain the isotope effects in Strecker synthesized alanine from ammonium chloride, acetaldehyde, sodium cyanide, and water at temperatures ranging from 20°C to 25°C for the creation of the aminonitrile and 80°C to 120°C for its acid hydrolysis. We measured the average isotopic composition of solid reagents and products via EA-IRMS, of acetaldehyde via combustion over CuO into CO_2 which was measured on a dual-inlet IRMS, and the site-specific isotopic composition of alanine produced by the synthesis was measured for $\delta^{13}C$ of the C-2 + C-3 (140.032 fragment) on the Orbitrap as described above. Our measurements indicated that the average $\delta^{13}C$ of C-2 and C-3 of alanine produced by Strecker synthesis (-30.6 ± 0.9 ‰) is approximately 12‰ depleted in ^{13}C relative to the reactant acetaldehyde ($\delta^{13}C$ = -19.1 ‰) regardless of yield. Because C-3 does not participate in the Strecker reaction, we assumed the difference in the average $\delta^{13}C$ for C-2 and C-3 is due to a -24 ‰ isotope effect on C-2, which is consistent with other CN addition reactions (Lynn and Yankwich, 1961). C-1 (found by a subtraction of C-2 and C-3 from the molecular average) exhibited a normal KIE that had an average value of 22 ‰ for alanine produced between a 10 % and 55 % yield

(-54.1 ± 3.2 ‰ relative to a starting CN $\delta^{13}C$ of -31.8 ± 0.2 ‰). This KIE also agrees with literature values for amide oxidation (Robins et al., 2015).

Our reaction network model assumes a low yield of products and unlimited supply of reactants relative to the products such that isotope effects would be apparent in products and but would not significantly alter the $\delta^{13}C$ of the reactants (and, consequently, other compounds produced from them). The agreement between our predicted isotope ratios and measurements in literature, particularly for acetaldehyde and HCN, is consistent with this assumption. However, below we analyze the possibility that variations in certain factors would impact our results:

Temperature: The isotope effects associated with reactions in our hypothesized reaction network range up to 30 ‰. Given that the temperatures of aqueous alteration of the CM chondrites have been demonstrated to have varied between 20 and 71 ˚C (293.15 – 344.15 K (Guo and Eiler, 2007)) through clumped isotope thermometry, and given that chemical isotope effects commonly exhibit approximately linear variations in amplitude with $1/T^2$, we estimate that these model estimates could have varied by several per mil. For moderate variations in reaction progress (below), these should lead to variations of just a few per mil in predicted $\delta^{13}C$ values of products. This is comparable to full procedural analytical precision and less than otherwise unexplained variability in the data, and so we consider it insignificant (in the context of the constraints and goals of our model).

Reaction progress: Our model presumes that essential reactants (water, aldehydes, ammonia and HCN) are more abundant than products that are created in our reaction network. If the

proportions of these compounds in the Murchison parent body initially resembled those in comets (*e.g.*, Biver *et al.*, (2019)), this assumption would be well justified. However, if organic synthesis reactions such as the Strecker chemistry locally went to near completion (consuming most of reactants), isotope effects associated with synthesis reactions would be mitigated, as isotopic proportions in products would approach those of reactants. The largest kinetic isotope effects associated with our reaction network model (30 ‰) could be diminished in this way — in the extreme limit of quantitative yield, reduced to nothing.

The limits one should place on this argument are difficult to evaluate because all of the reactants are more volatile than the products (*e.g.*, alanine is essentially involatile whereas its proposed substrates, acetaldehyde and HCN have boiling points of 20 and 26˚C, respectively). Thus, the abundance ratios of aldehydes to amino acids in the Murchison meteorite are likely a poor guide to their proportions early in the history of the Murchison parent body. If the synthesis chemistry had yields comparable to laboratory Strecker synthesis (10's of %), then the effective KIE's would be approximately halved, or reduced by approximately 10 ‰. That would be degrade the level of agreement between our model prediction and the measured $\delta^{13}C$ of some compounds in our model (and improve the level of agreement for others), but by amounts that are a small fraction of the isotopic variations (*i.e.*, site-specific and intermolecular differences) that motivate our model. We therefore consider it implausible that this factor significantly impacts the overall reasonableness of our model.

Alanine destruction: Free and total alanine in Murchison are about one-third as abundant as in the most alanine-rich CM chondrite (~0.20 and ~0.65 ppm, respectively), implying that it could

be residual to 10's of % destruction. If this destruction was accompanied by a ^{13}C kinetic isotope effect in the range typical of irreversible organic reactions (~10-30 ‰) and operated on one or two atomic sites, then the residual alanine could have been enriched in $\delta^{13}C$ by several per mil up to perhaps 10 ‰. The most likely mechanisms for alanine destruction (NH_2 replacement with OH, or decarboxylation) should either enrich the C-2 site or enrich both the C-1 and C-2 sites equally in the residue. These effects are less than or just at the margin of the level of significance addressed by our model and are a small fraction of the 150 ‰ site-specific effect our model was tailored to describe. Moreover, the $\delta^{13}C$ values of alanine from CM chondrites do not exhibit an inverse concentration with their concentration in the samples, so there is no empirical evidence to suggest such a fractionating loss mechanism. We conclude loss of alanine through these side reactions is unlikely to significantly impact our conclusions.

Calculation of reactant $\delta^{13}C$ values

To estimate the site-specific $\delta^{13}C$ values of reactants in our network model, we subtracted site-specific isotope effects constrained by our Strecker synthesis experiments from the measured $\delta^{13}C$ values for alanine in the analytical Murchison sample. Based on these results, the reactant CN is estimated to have a $\delta^{13}C_{VPDB}$ value of -7 ‰ and the initial acetaldehyde is estimated to have $\delta^{13}C_{VPDB}$ values of 166 ± 10‰ and -36 ± 10 ‰ for the carbonyl ($C\text{-}1_{acetaldehyde}$) and methyl ($C\text{-}2_{acetaldehyde}$) carbons, respectively. Combining our results with the ISM chemical networks described in (Elsila et al., 2012) and references therein, we predict that the carbonyl carbon in all aldehyde functional groups are from the ^{13}C-enriched CO pool in the ISM and that all alkyl carbons are from another, ^{13}C-depleted pool (that include C_xH_y compounds). Thus, in our model we assigned $\delta^{13}C$ values of 166 ± 10 ‰ to all carbonyl carbons and -36 ± 10 ‰ to all alkyl

carbons. Equivalently, we calculated the molecular-average $\delta^{13}C$ values of aliphatic aldehydes with two or more carbons by calculating the carbon-weighted average values of acetaldehyde (64.6 ± 1.5 ‰) and additional aliphatic carbons (-36 ± 10 ‰) (Eqn. A2; See Appendix C).

$$^{13}F_{Cx\text{-}aldehyde} = \left(\frac{2}{x}\right)^{13}F_{molec\ avg,\ acetaldehyde} + \left(\frac{x-2}{x}\right)^{13}F_{C\text{-}2,\ acetaldehyde} \qquad \textit{(Eqn. A2)}$$

where x is the carbon chain length and C_x-aldehyde is a molecule with one aldehyde carbon and x methylene carbons. All such calculations are made using ^{13}C mole fraction ("fractional abundance") rather than $\delta^{13}C$ values to avoid systematic errors arising from non-linearities of the δ scale.

In our model, amines form from a reactant aldehyde's reductive amination (Figure 3, main text), which is proposed to have an insignificant KIE, so we estimated that the $\delta^{13}C$ value of the amine molecule is equal to that of an aldehyde molecule with the same carbon backbone (See Dataset S2). Monocarboxylic acids formed from the oxidation of aldehyde precursors were assigned to have isotope effects that range from 0 ‰ to -30 ‰. In the first case, the product carboxylic acids have $\delta^{13}C$ values equal to their aldehyde precursors (See Dataset S2). For the alternate case of a fully expressed -30 ‰ KIE during oxidation of the aldehyde's carbonyl site, the isotope effect is assumed to only occur on the C-1 carbon, so the molecular-average $\delta^{13}C$ for acetic acid was calculated accounting for the isotope effect only occurring on this site (Eqn. A3). Higher carbon chain carboxylic acids (C_2 and above) were calculated as the carbon-weighted average values of acetaldehyde (64.6 ± 1.5 ‰) and additional CH_x groups (-36 ± 10 ‰) to decrease error (Eqn. A4).

$$^{13}R_{\text{molec avg, acetic acid}} = (1\text{-}0.050/2)\ ^{13}R_{\text{molec avg, acetaldehyde}} \quad (A3)$$

$$^{13}F_{\text{C-x-carboxylic acid}} = \left(\frac{2}{x}\right)^{13}F_{\text{molec avg, acetic acid}} + \left(\frac{x-2}{x}\right)\ ^{13}F_{\text{C-2, acetaldehyde}} \quad (A4)$$

All α-amino acids (*i.e.* not only alanine) were assumed to undergo fractionation in Strecker synthesis as described above. Because our analytical Murchison alanine measurements include $\delta^{13}C$ for sites that have undergone the same fractionations associated with their synthesis (*e.g.*, the C-1 and C-2 carbons of all alpha amino acids formed by Strecker synthesis are predicted to be fractionated in the same way we predict for our model of alanine formation), we used alanine's site-specific isotopic composition as our building blocks for other amino acids. Glycine's $\delta^{13}C$ was predicted based on the 184.021 *m/z* fragment measurement (corrected for dilution with carbons from derivatizing agents) and alanine was assigned to have the $\delta^{13}C$ value directly measured in this study, 25.5 ‰ (*e.g.*, it is not predicted but serves as the basis for predicting other species, particularly acetaldehyde and HCN). All amino acids with longer alkyl chains than alanine were assumed to have additional alkyl carbons (*i.e.:* with a $\delta^{13}C$ equal to that of C-3 in alanine) comprising the balance of the molecular carbon inventory (Eqn. A5).

$$^{13}F_{\text{Cx-amino acid}} = \left(\frac{3}{x}\right)^{13}F_{\text{molec avg, alanine}} + \left(\frac{x-3}{x}\right)\ ^{13}F_{\text{C3, alanine}} \quad (A5)$$

In addition to Strecker synthesis, we also considered the possibility that C-1 in amino acids could equilibrate with the dissolved inorganic carbon (DIC) pool on meteorites (*e.g.*, the carbonate pool). The DIC pool is 3000 times more abundant than all amino acids on Murchison combined (Sephton, 2002). Consequently, in the case of equilibration between the two reservoirs, the $\delta^{13}C$

value of DIC would control that of C-1 in amino acids. We assumed a DIC reservoir with a $\delta^{13}C$ of 80 ‰, equal to the highest measured literature value for CM chondrites (Sephton, 2002) (and thus the maximum effect on amino acids with which it equilibrates). Using ε values for CO_3^{2-}-CO_2 and CO_2-amino acid carboxyl group equilibration from (Rustad et al., 2008) and (Rustad, 2009) respectively, we predicted the $\delta^{13}C$ of different amino acids on Murchison that had equilibrated with its carbonate pool (Dataset S2). Of amino acids with molecular-average $\delta^{13}C$ values measured on Murchison, only glycine and alanine also have ε values for CO_2 and amino acid carboxyl group in (Rustad, 2009). These values are 4.4 ‰ and 4.9 ‰, so we adopted an average value of 4.65 ‰ for $\varepsilon_{CO2\text{-amino acid C-1 site}}$ in our calculations for all amino acids.

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