# Enrichment of Non-Terrestrial L-Proteinogenic Amino Acids by Aqueous Alteration on the Tagish Lake Meteorite Parent Body

Daniel P. Glavin[1*], Jamie E. Elsila[1], Aaron S. Burton[1], Michael P. Callahan[1], Jason P. Dworkin[1], and Christopher D. K. Herd[2]

[1]NASA Goddard Space Flight Center, Greenbelt, Maryland 20771 USA
[2]Department of Earth and Atmospheric Sciences, University of Alberta, Edmonton, Alberta, T6G 2E3 CANADA

[*]**Corresponding author:** Code 699.0, Tel: (301) 614-6361, Fax: (301) 614-6406, Email: daniel.p.glavin@nasa.gov

**Manuscript Information:** Abstract (245 words), Main Text (39,715 characters with spaces, 6,126 words): 3 Figures and 2 Tables; Supporting Information (SI): 2 Figures and 3 Tables

**Keywords:** Amino acids, aspartic acid, enantioenrichment, origin of homochirality, Tagish Lake meteorite

## ABSTRACT

The distribution and isotopic and enantiomeric compositions of amino acids found in three distinct fragments of the Tagish Lake C2-type carbonaceous chondrite were investigated via liquid chromatography fluorescence detection time-of-flight mass spectrometry and gas chromatography isotope ratio mass spectrometry. Large L-enantiomeric excesses ($L_{ee}$ ~ 43 to 59%) of the $\alpha$-hydrogen aspartic and glutamic amino acids were measured in Tagish Lake, whereas alanine, another $\alpha$-hydrogen protein amino acid, was found to be nearly racemic (D ~ L) using both techniques. Carbon isotope measurements of D- and L-aspartic acid and D- and L-alanine in Tagish Lake fall well outside of the terrestrial range and indicate that the measured aspartic acid enantioenrichment is indigenous to the meteorite. Alternate explanations for the L-excesses of aspartic acid such as interference from other compounds present in the sample, analytical biases, or terrestrial amino acid contamination were investigated and rejected. These results can be explained by differences in the solid-solution phase behavior of aspartic acid, which can form conglomerate enantiopure solids during crystallization, and alanine, which can only form racemic crystals. Amplification of a small initial L-aspartic acid excess during extensive aqueous alteration on the meteorite parent body could have led to the large L-enrichments observed for aspartic acid and other conglomerate amino acids. The detection of non-terrestrial L-proteinogenic amino acid excesses in Tagish Lake and other aqueously altered meteorites provides support for the hypothesis that significant amino acid enantiomeric enrichments could form by abiotic processes prior to the emergence of life.

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## INTRODUCTION

Meteorites provide a record of the chemical processes that occurred in the early solar system before life began on Earth. The delivery of complex organic compounds by carbonaceous chondrites to the early Earth and other planetary bodies could have been an important source of prebiotic compounds required for the emergence of life (1). Of particular interest is the study of meteoritic amino acids and their enantiomeric compositions since these prebiotic molecules are the monomers of proteins common to all life on Earth. The homochirality observed in biological molecules, so called "left-handed" (by analogy with glyceraldehyde) amino acids and "right-handed" sugars, is a property important for molecular recognition processes and is thought to be a prerequisite for life. Most amino acids (including those found in meteorites) are chiral, meaning they possess two non-superimposable mirror image structures, or enantiomers. In contrast to biology, which is dominated by L-amino acids, abiotic processes form racemic amino acids (equal mixtures of L- and D-enantiomers). The questions of why and how the nearly exclusive production of one hand of such molecules arose from what were presumably equal mixtures of L- and D-enantiomers in a prebiotic world continues to be a crucial hurdle in understanding the origin of life.

The first amino acid analysis of the Murchison meteorite shortly after its fall found that the chiral amino acids were racemic (D/L = 1), indicating an abiotic origin with very little, if any, terrestrial amino acid contamination of the meteorite (2). However, slight to significant L-enantiomer excesses for several unusual $\alpha$-dialkyl amino acids in the CM2-type meteorites Murchison and Murray including isovaline, $\alpha$-methylnorleucine, $\alpha$-methylvaline, $\alpha$-methylnorvaline, $\alpha$-methylisoleucine, and the 2-amino-2,3-dimethylpentanoic acid diastereomers have since been discovered (3-5), with L-isovaline excesses as high as 18.5% measured in Murchison (6). An extraterrestrial origin for these $\alpha$-methyl amino acid excesses is supported

by the observation that these non-protein amino acids are extremely rare on Earth (3) and by compound-specific carbon and hydrogen isotopic measurements of D- and L-isovaline enantiomers ($\delta^{13}C$ ~ +12 to +22‰; $\delta D$ ~ 3200‰) in Murchison and Murray (5,7) that are heavily enriched compared to terrestrial sources of isovaline (8). Enantiomeric excesses of ~7 to 12% for the $C_6$ amino acid diastereomers L-isoleucine and D-alloisoleucine in the Antarctic CR2-type meteorites GRA 95529 and LAP 02342 as well as Murchison and Murray have also been reported and are thought to be indigenous based on carbon isotope values that fall outside of the terrestrial range (9). The L-isoleucine and D-alloisoleucine enantiomeric excesses are speculated to have been inherited from initial asymmetry in their chiral $C_5$ aldehyde precursor 2-methylbutyraldehyde (9,10), although enantiomeric excesses for this aldehyde have not been reported in these meteorites. The original source of the hypothesized asymmetry of the aldehyde precursor remains unclear, but may have been produced by asymmetric photolytic decomposition from UV circularly polarized light (CPL) in the presolar cloud (11,12) prior to incorporation inside the meteorite parent body.

The origins of amino acid enantiomeric excesses in meteorites have been difficult to explain since laboratory simulations indicate that the formation of both $\alpha$-hydrogen ($\alpha$-H) and $\alpha$-methyl amino acids from aldehyde and ketone precursors (e.g., by the Strecker-cyanohydrin synthesis) on the parent bodies should have produced racemic amino acid mixtures (13-16). In addition, though modest asymmetric photolytic decomposition or synthesis by UV or other circularly polarized radiation sources have been reported (17), UV CPL alone cannot be used to explain the large L-isovaline excesses observed in CM and CI meteorites for two reasons: 1) the correlation observed between isovaline enantiomeric excess and relative degree of aqueous alteration places the formation of isovaline within the meteorite parent bodies, thus shielded from UV radiation (6), and 2) the most plausible ketone precursor for isovaline (2-butanone) is achiral and thus could not have been chirally biased. Other possible mechanisms for symmetry

breaking and amplification of amino acid excesses in meteorites have also been proposed and are discussed in detail elsewhere (10, 18-23). Nevertheless, the exact mechanism(s) for the formation of L-isovaline and other amino acid enantiomeric excesses found in carbonaceous meteorites still remains unclear.

Interpretation of L-enantiomeric excesses measured for the α-H amino acids present in Murchison and other carbonaceous meteorites has been even more problematic because unlike α-methyl amino acids, the α-H amino acids are common in biochemistry and their measurements are more susceptible to terrestrial contamination. In addition, α-H amino acids racemize (convert from one enantiomer to the other) far more readily than α-methyl amino acids, which are not as prone to geologically rapid racemization under aqueous or radiogenic conditions (24,25). Although it has been argued that racemization in an asteroid would not have completely erased any initial enantiomeric biases present in the α-H amino acids (26,27), some racemization of the α-H amino acids will occur during parent body aqueous alteration and exposure to ionizing radiation. Engel and Nagy (28) reported a wide range of L-enantiomer excesses ($L_{ee}$ ~25 to 67%) for several common α-H protein amino acids including alanine, aspartic acid, and glutamic acid within interior fragments of the Murchison meteorite and were the first to propose that these L-excesses were not likely to be the result of terrestrial contamination after impact since other common protein amino acids (e.g. tyrosine, phenylalanine, lysine, histidine, arginine, etc.) were absent from the meteorite. Furthermore, subsequent nitrogen isotopic measurements of D- and L-glutamic acid and D- and L-alanine detected in Murchison showed that both enantiomers for each amino acid were heavily enriched in $^{15}N$ relative to terrestrial amino acids and had similar $\delta^{15}N$ values ranging from +57 to +60 ‰, suggesting that the large L-glutamic acid and L-alanine excesses were indigenous to the meteorite (29,30). Carbon isotope measurements for D- and L-alanine in Murchison were also shown by Engel et al. (31) to be highly enriched in $^{13}C$ with a $\delta^{13}C$ value of +27 ‰, similar to the

carbon isotope value obtained for isovaline in the same meteorite. However, Pizzarello and Cronin (32) argued that incomplete gas chromatographic resolution of L-alanine and other meteoritic amino acids could have affected both the enantiomeric ratios measured and the $\delta^{15}N$ determinations of Engel and Macko (29).

Given the challenges associated with the analytical measurements and interpretation of L-enantiomeric excesses for $\alpha$-H amino acids in meteorites, it is not surprising that to date there have been so few published claims of non-terrestrial $\alpha$-H amino acid L-excesses in meteorites. Nevertheless, given the importance of $\alpha$-H amino acids to biochemistry, additional scrutiny of the apparent large L-enantiomer excesses of $\alpha$-H amino acids in Murchison and other carbonaceous chondrites using multiple analytical techniques is certainly warranted. Here we report on measurements of the distribution, enantiomeric composition, and carbon isotopic ratios of amino acids in three distinct lithologies of carefully collected (33) fragments of the Tagish Lake meteorite that have undergone a wide range of aqueous alteration on the meteorite parent body (34) by using both ultra-high performance liquid chromatography fluorescence detection and time-of-flight mass spectrometry (LC-FD/TOF-MS) and gas chromatography mass spectrometry and isotope ratio mass spectrometry (GC-MS/IRMS) techniques.

## RESULTS AND DISCUSSION

**Amino Acid Analyses.** Typical liquid chromatography UV fluorescence and TOF-MS mass chromatograms of the 6 M HCl-vapor hydrolyzed, hot-water extracts from the Tagish Lake 5b, 11h, and 11i meteorite specimens and the procedural blank show several peaks that were identified by comparison with amino acid standards, fluorescence, retention time, and mass [Fig. 1 and supporting information (SI) Fig. S1]. Although complete resolution of all $C_2$ to $C_6$ amino acid isomers and enantiomers was not achieved under the chromatographic conditions used, we were able to obtain adequate separation of several $\alpha$-H amino acids including aspartic and glutamic acids, serine, alanine, threonine, and valine and their enantiomers (Fig. 1), which

was the primary focus of this study. The total procedural-blank-corrected amino acid abundances (free + bound) of identified $C_2$ to $C_6$ amino acids in HCl-hydrolyzed hot-water extract of Tagish Lake were ~40 parts-per-billion (ppb) for sample 11i, ~740 ppb for sample 5b, and ~5,400 ppb for sample 11h (SI: Tables S1 and S2). In contrast to samples 5b and 11h, the abundances of many amino acids in 11i were below analytical detection limits of < 0.1 to 1 ppb. These results are consistent with a much higher degree of aqueous alteration experienced by 11i compared to 5b and 11h as also inferred from variations in mineralogy, bulk isotopes, petrology, carboxylic acid abundances, and structure of the insoluble organic matter (34). The low total amino acid abundance in 11i is similar to the levels (< 100 ppb) previously found in another pristine Tagish Lake meteorite stone (35). Overall, the amino acid abundances in the C2 Tagish Lake meteorite (~ 40 to 5,400 ppb) are much lower than the levels measured in other less altered type 2 carbonaceous chondrites, but do fall within the range of amino acid concentrations measured for aqueously altered CI, CM, and CR type 1 carbonaceous chondrites (23).

A large increase in amino acid abundances (> 100% increase) in all three meteorite fragments was observed after acid hydrolysis of the water extracts, which indicates that most of the amino acids in the Tagish Lake specimens are present in an acid-labile or bound form (SI: Tables S1 and S2), consistent with previous analyses of CI, CM, and CR carbonaceous chondrites (23). The most abundant amino acids detected and quantified by LC-FD/TOF-MS in the 5b and 11h extracts were D- and L-aspartic acid, D- and L-glutamic acid, D- and L-alanine, D- and L-serine, L-threonine, β-alanine (BALA), α-amino-*n*-butyric acid (ABA), D,L-β-ABA, γ-ABA, α-aminoisobutyic acid (AIB), D- and L-valine, D,L-norvaline, and D,L-isovaline (Fig. 1 and SI: Fig. S1 and Tables S1 and S2). Only trace levels (< 10 ppb) of L-aspartic and L-glutamic acids, L-serine, L-threonine, glycine, β-alanine, and L-alanine were measured by LC-FD/TOF-MS in the procedural blank, indicating that very little amino acid contamination of the samples

occurred during sample processing (Fig. 1 and SI: Fig. S1). However, the low amino acid abundances in the procedural blank does not rule out the possibility of amino acid contamination of the meteorites during collection, storage, or handling of the samples. The Tagish samples 11i, 5b, and 11h investigated here were collected at the same time from the surface ice within days after the fall and have all been kept at temperatures below 0°C prior to extraction (34); in contrast to these specimens, a non-pristine Tagish Lake fragment (sample 24-24) that was collected from lake meltwater 3 months after the fall contained a significant amount of terrestrial amino acid contamination from the lake itself (36).

The relative distribution of amino acids measured in the Tagish Lake meteorite samples 5b and 11h are clearly distinct from the Tagish Lake ice meltwater, which had higher relative abundances of L-aspartic and L-glutamic acids, L-serine, L-alanine and D,L-α-amino-*n*-butyric acid (SI: Fig. S2). In addition, several non-protein amino acids that are not common in terrestrial samples, including D- and L-β-ABA, D- and L-isovaline, and D- and L-norvaline, were identified above background levels in both 5b and 11h, but have not been reported in the Tagish Lake meltwater or previous analyses of the Tagish lake meteorite (35,36). The differences in relative amino acid abundances in the Tagish Lake meteorite samples and absolute abundances of both protein and non-protein amino acids that increase in order 11i < 5b < 11h (SI: Tables S1 and S2) provide additional support that most of these amino acids are indigenous to the meteorites, since all three fragments were collected, stored, and processed in parallel under identical conditions (34). Herd et al. (34) argued that differences in the absolute abundances of amino and carboxylic acids in these Tagish Lake samples are best explained by differences in the extent of aqueous alteration on the parent body.

***Amino Acid Isotopic Composition and Enantiomeric Measurements.*** Carbon isotope measurements were made for the most abundant amino acids in the Tagish Lake meteorite extracts 5b and 11h (11i had insufficient amino acids for isotope measurements). The

GC-MS/IRMS technique employed provides simultaneous compound-specific structural and carbon isotopic information from a single injection, which permitted three replicate analyses of D- and L-aspartic acid, L-glutamic acid, glycine, D- and L-alanine, β-alanine, and γ-ABA in the meteorite extracts. We previously reported a $\delta^{13}C$ value of +19 ± 4‰ for glycine (34), the most abundant amino acid in Tagish lake 11h, which is similar to carbon isotope values of glycine ($\delta^{13}C$ ~ +22‰, Table 1) that have been measured in the CM Murchison (37) and CI Orgueil (38) meteorites. The GC-MS/IRMS data for the D- and L-aspartic acid and D- and L-alanine peaks in the combined hydrolyzed and non-hydrolyzed Tagish Lake 11h hot-water extracts is shown in Fig. 2. The retention times and mass spectra for both peaks in the 11h extract match those for the TFAA/IPA derivatives of the D- and L-aspartic acid peaks in the standard, with no evidence for other co-eluting compounds (Fig. 2). The corrected $\delta^{13}C$ values for D- and L-aspartic acid peaks in 11h were +24‰ and +29‰ and were similar within a measurement error of ± 4‰ (Table 1). These values fall well outside of the terrestrial range for organic carbon of -6‰ to -40‰ (39) and indicate an extraterrestrial origin for both D- and L-aspartic acid. A corrected D/L aspartic acid ratio of 0.26 ± 0.02 was determined by GC-MS at the same time from the integrated D- and L-aspartic acid peak areas in 11h compared to a racemic aspartic acid standard and corresponds to an L-enantiomeric excess ($L_{ee}$ = L% - D%) of 58.7 ± 6.4% for aspartic acid. A similarly high $L_{ee}$ in the total aspartic acid of 45.5 ± 5.2% was determined independently from the absolute abundances of D- and L-aspartic acid measured by LC-FD/TOF-MS (Table 2). If the observed L-excess for aspartic acid was due to terrestrial contamination, the measured carbon isotope value of L-aspartic acid should have been less enriched in $^{13}C$ compared to D-aspartic acid. For example, Pizzarello *et al.* (37) measured the L- and D-aspartic acid carbon isotope ratios on a sample with an L-enantiomer excess and reported a $\delta^{13}C$ value of -6‰ for L-aspartic acid and +25‰ for D-aspartic acid (Table 1). In this case, the depletion in $\delta^{13}C$ for the L-aspartic acid is a clear indicator that the Murchison sample

contained both extraterrestrial and terrestrial sources of L-aspartic acid. This was not observed in the Tagish lake meteorite sample 11h, where L-aspartic acid actually had a higher $\delta^{13}C$ value compared to D-aspartic acid (Table 1), which cannot be explained by terrestrial L-aspartic acid contamination. No significant carbon isotope fractionation was observed in a racemic aspartic acid standard carried through the same extraction and analytical procedure.

The measured $\delta^{13}C$ values for glycine, D- and L-alanine, L-glutamic acid, β-alanine, and γ-ABA in the Tagish Lake meteorite samples range from -5‰ to +67‰ (Table 1), which all fall outside of the typical terrestrial range for these amino acids. We were unable to determine the $\delta^{13}C$ value for D-glutamic acid in the Tagish Lake samples due to low abundances and chromatographic interference. A D/L ratio of ~ 0.3 was measured by LC-FD/TOF-MS for glutamic acid in Tagish Lake 5b and 11h, which was similar to the values measured for aspartic acid (SI: Table S2). A $\delta^{13}C$ value of -4 ± 9‰ for L-glutamic acid in Tagish 11h could indicate that some terrestrial L-glutamic acid contamination of the meteorite occurred, although in the absence of the D-glutamic acid carbon isotope value, we have no basis for comparison. However, if we assume that the level of L-glutamic acid contamination in 11h is similar to the amount measured in 11i (~ 6 ppb, SI: Table S1), which is reasonable since both samples fell in the same location, were collected at the same time, and were processed under identical conditions, than terrestrial L-glutamic acid contamination represents < 1% of the total L-glutamic acid abundance in 11h (SI: Table S1). A similarly low D/L ratio (D/L ~ 0.3) for glutamic acid has previously been measured in the Murchison meteorite and was argued to be indigenous based on non-terrestrial nitrogen isotopic values for both D- and L-enantiomers that were similar (30). This result seems to contradict the report of large L-enantiomeric excesses (~16 to 47%) for the glutamic acid derivative pyroglutamic acid that have also been reported in Murchison, although the lower $\delta^{13}C$ values for both L-pyroglutamic acid and L-glutamic acid point to a significant terrestrial contribution to these L-excesses (40). The measured D/L ratios for aspartic and

glutamic acids in Tagish Lake are not due to extraction or LC-FD/TOF-MS analytical biases, since these two amino acids were found to be racemic (D/L = 1) in the less altered Antarctic CR meteorites EET 92042 and QUE 99177 using the same analytical technique (23).

In contrast to aspartic and glutamic acids, the D/L ratio of alanine in 5b and 11 h determined by LC-FD/TOF-MS was found to be racemic (D/L ~ 1) within error (SI: Table 2) and the $\delta^{13}C$ values for D- and L-alanine were measured to be +6 ± 3‰ and +16 ± 4‰, respectively, for sample 11h and +67 ± 7‰ and +55 ± 3‰, respectively, for sample 5b (Table 2). The D/L alanine ratio of sample 11h was also measured independently by GC-MS and also found to be nearly racemic (D/L ~ 0.9). Based on the observation that alanine was racemic in both meteorite samples and is extraterrestrial in origin, we would have expected the carbon isotope values of D- and L-alanine in each sample to be similar. It is possible that the slightly $^{13}C$ depleted value for D-alanine in 11h is due to the isotopically light peak that elutes after D-alanine and cannot be completely resolved from the tail of the D-alanine peak (Fig. 2).

The carbon isotope values measured in Tagish Lake sample 5b for glycine, D- and L-alanine, and β-alanine were all enriched in $^{13}C$ compared to the same amino acids in 11h, with $\delta^{13}C$ values ranging from +30 to +67‰ (Table 1). Carbon isotope values for aspartic and glutamic acids in 5b could not be obtained due to low amino acid abundances; however, low D/L ratios (~ 0.3 to 0.4) were also measured for aspartic and glutamic acids in 5b by LC-FD/TOF-MS, corresponding to large $L_{ee}$ of ~43 to 51% (Table 2). The relatively high $\delta^{13}C$ values in 5b indicate that these amino acids and/or their precursor materials retained a more primitive carbon isotopic signature compared to 11h, consistent with mineralogical differences and isotopic measurements of the insoluble organic matter, demonstrating that 11h has experienced a greater degree of aqueous alteration compared to 5b (34). Since most of the organic carbon in Tagish Lake samples 5b and 11h is depleted with an average bulk $\delta^{13}C$ of -14‰ (34), the lighter carbon isotope values measured for the amino acids in 11h could be explained by their

formation from $^{13}C$-depleted precursor material during a secondary aqueous alteration stage in the parent body. We believe that the elevated abundances of α-amino acids in 11h with depleted carbon isotope ratios compared to 5b (SI: Table S2) is best interpreted as reflecting a secondary pulse of amino acid formation in 11h during parent body alteration from $^{13}C$ depleted precursors by Strecker synthesis (34,41,42), although other formation mechanisms for α– and other amino acids before their incorporation in the parent body have been suggested (43).

Enantiomeric measurements were also obtained by LC-FD/TOF-MS for several other α-hydrogen amino acids in Tagish Lake 5b and 11h including serine, threonine, and valine with $L_{ee}$ values that range from ~19% for valine to >99% for threonine (Table 2). These amino acids were not reported in previous amino acid analyses of the Tagish Lake meteorite (34-36). Since the total abundances of these amino acids increase in the order 11i < 5b < 11h (SI: Tables S1 and S2), it is possible that some fraction of these amino acids and their $L_{ee}$ were formed during parent body aqueous alteration and are indigenous to the Tagish Lake meteorite. However, due to insufficient amino acid concentrations in the samples, we could not measure their carbon isotope ratios. Therefore, at this time we are unable to draw any conclusions about whether the large L-excesses measured for serine, threonine, and valine in the Tagish Lake meteorite are extraterrestrial in origin. In addition, due to a possible interfering mass peak in the procedural blank at the same retention time as L-valine (SI: Fig. S1), the reported $L_{ee}$ for valine could have been overestimated and are given as upper limits in Table 2.

In contrast to most of the α-H protein amino acids in the Tagish Lake 5b and 11h meteorite that displayed large L-excesses ranging from ~19 to 99%, only very small enantiomeric excesses ($L_{ee}$ ~ 0 to 7%) were observed for the chiral non-protein amino acids β-ABA, norvaline, and isovaline and most were racemic within analytical error (Table 2, SI: Table S3). Although the α-methyl amino acid isovaline is highly resistant to racemization (24,25), β-ABA and norvaline will readily racemize under aqueous conditions; therefore, it is not surprising

that no enantiomeric enrichment was observed for these two non-protein amino acids in the Tagish Lake meteorite. A slight L-isovaline excess ($L_{ee}$ = 7.0 ± 1.9%) was measured in the Tagish 5b sample, but no L-isovaline enrichment was observed in the more aqueously altered 11h sample ($L_{ee}$ = 0.0 ± 2.8%). These results are consistent with small L-isovaline enrichments (~ 0 to 3%) that have been reported in pristine Antarctic CR2 carbonaceous meteorites (6,9). It is possible that some radioracemization (≤ 5%) of isovaline from ionizing radiation produced by radioactive decay in the parent body (25) could have reduced the original L-isovaline enrichments in both Tagish Lake meteorite samples, or that a secondary pulse of amino acid formation during aqueous alteration in 11h could have overprinted any original L-isovaline excess with a racemic mixture (34). However, it is surprising that secondary aqueous alteration leading to the higher amino acid abundances observed in 11h did not also reduce the L-enantiomer excesses measured for several α-H amino acids including aspartic and glutamic acids, threonine, and valine. In fact, the L-excesses measured for these amino acids were slightly higher in 11h compared to 5b (Table 2). Given that all of these α-H amino acids racemize under aqueous conditions, another explanation is needed for the presence of these large L-excesses, particularly for L-aspartic acid shown to be indigenous to the Tagish Lake meteorite.

***Enantioenrichment by Racemization During Parent Body Alteration.*** The α-H amino acids alanine and aspartic acid in Tagish Lake 11h both have extraterrestrial isotopic values indicating they are indigenous to the meteorite; however, alanine is racemic while aspartic acid shows a significant L-enantiomeric excess. One possible explanation for this disparity exists in the crystallization behavior of these two amino acids. It has been shown experimentally that aspartic acid and alanine have very different crystallization behaviors in solution – alanine preferentially forms racemic crystals (21), while aspartic acid can form metastable conglomerate crystals in addition to racemic crystals (44, 45). It has been

demonstrated that saturated solutions of aspartic acid with slight enantiomeric excesses can be converted to enantiopurity under solution-phase racemizing conditions such as aldehyde-catalyzed racemization or with the input of UV-circularly polarized light, provided there is a small initial enantiomeric excess (45,46). While we want to make it clear that we do not have the necessary isotope values to establish an extraterrestrial origin for the L-excesses measured for other $\alpha$-H amino acids in Tagish Lake 11h, the tendency to form conglomerate crystals has also been observed for glutamic acid (44,45) and threonine (47), making it possible that these enantiomeric excess could have been formed by the same mechanism proposed for aspartic acid. It has even been suggested that spontaneous resolution of conglomerates is the most likely terrestrial mechanism for the origin of homochirality on the early Earth (48).

The conglomerate crystal amplification process is illustrated in Fig. 3 (top) and is based on the observation that larger conglomerate crystals are more stable than smaller crystals that preferentially dissolve in solution and begin to disappear. Over time, the major enantiomer present in excess will accumulate more material in the solid phase than does the minor enantiomer. This is because the minor enantiomer tends to form smaller crystals that dissolve more rapidly than larger crystals. Racemization of the minor enantiomer will favor production of the major enantiomer in solution, which will eventually precipitate out and help build even larger crystals of the major enantiomer (Fig. 3, top). If we assume that there was a slight initial bias toward L-aspartic and L-glutamic acids on the Tagish Lake meteorite parent body generated via any of the proposed methods of breaking symmetry in amino acids or their precursors, extensive aqueous alteration and racemization of aspartic and glutamic acids could have resulted in a large net enrichment of L- over the D-enantiomers as is observed for these two amino acids in the meteorite. The extent to which the conversion occurs is dependent on the duration of parent body aqueous alteration and the rate of racemization for the individual amino acids, with a theoretical maximum conversion of 100%, based on laboratory experiments (45,46). It is likely that the interaction of amino acids with other organic and inorganic species in

the parent body would also have an effect on amplification. This is a line of research that should be explored in greater detail through laboratory crystallization experiments of free amino acids and their acid-hydrolyzable precursors under relevant parent body conditions. Although the temperature and duration of aqueous alteration on the Tagish Lake parent body was likely highly variable and remains poorly constrained, Mn-Cr isotopic analyses of carbonate in Tagish 11h indicates that this meteorite experienced extensive aqueous alteration in a similar setting and timescale to the CI parent body (49). Temperatures ranging from ~50°C to 150°C have been estimated previously for CIs (50,51), and aqueous alteration periods of ~ $10^2$ to $10^4$ years that have been estimated previously for CM meteorites (52, 53), with recent models suggesting that liquid water could have been present in asteroids for up to millions of years on CI and CM meteorite parent bodies (54,55). Under these conditions, racemization of aspartic acid would have been especially rapid. For example, at a temperature of ~25°C under aqueous conditions at neutral pH, the racemization half life of aspartic acid in natural samples ranges from ~$10^3$ to $10^4$ years, and at a temperature of 100°C the racemization half life of free aspartic acid in aqueous solution is only 30 days (56). However, we note that the racemization rate of glutamic acid is four times slower than aspartic acid under the same conditions, and would be even slower with the formation of pyroglutamic acid (57). It should also be acknowledged that for metastable conglomerates such as aspartic and glutamic acids, changes in conditions on the Tagish Lake parent body such as rapid changes in temperature could trigger a shift from conglomerate crystals to racemic ones, thus stopping enantioenrichment via crystallization. This may explain why the L-aspartic and L-glutamic acid excesses measured in Tagish Lake did not reach 100%.

For compounds that preferentially form racemic crystals, such as alanine and the majority of the chiral $\alpha$-H proteinogenic amino acids (21), as well as metastable conglomerates such as aspartic and glutamic acids that have switched to racemic crystals, racemization in the solution phase works in the opposite direction, resulting in an increase in the amount of racemic solid

phase and an overall reduction in enantiomeric excess. This is because an enantiomeric excess in solution will drive racemization towards a racemic solution phase, causing more racemic crystals (with lower solubility) to precipitate (Fig. 3, bottom). Since enantiopure crystals are more soluble and will dissolve more rapidly than racemic crystals, preferential dissolution of the major enantiomer in the solid phase will drive racemization to the minor enantiomer, resulting in a net conversion of the major to the minor enantiomer and formation of racemic solid crystals. This hypothesis is consistent with racemic alanine measured in the Tagish Lake meteorite and large $L_{ee}$ values for aspartic acid, provided that there was a slight initial L-bias of aspartic acid. We also note that similarly high L-aspartic acid and L-glutamic acid excesses ($L_{ee}$ ~ 32 to 61%) were measured in the aqueously altered type 1 CI meteorite Orgueil, while alanine was also found to be racemic (38). Although isotopic measurements were not measured for these amino acids in Orgueil, the conglomerate crystallization behavior of aspartic and glutamic acids could explain the observed L-aspartic and L-glutamic acid excesses in Orgueil provided that there was a slight initial L-excess. It has been suggested previously that some amino acids formed by Strecker synthesis in meteorites could have inherited their asymmetry directly from asymmetric photolysis of aldehyde or ketone precursors that were exposed to UV circularly polarized radiation in the solar nebula prior to their incorporation inside the meteorite parent body (9). For example, slight (~ 1%) enantiomeric excesses in alanine have been produced from laboratory interstellar ice analogs exposed to circularly polarized UV light (17). However, aspartic acid would probably not have obtained its initial asymmetry via this mechanism since its most plausible ketone precursor is 3-oxopropanoic acid, which is achiral. Aspartic acid could also have formed from Michael addition of ammonia to fumaric or maleic acid (e.g. http://www.jbc.org/content/89/1/41.full.pdf), although both of these precursor molecules are also achiral. Finally, the enhanced abundance of aspartic acid in the more aqueously altered 11h sample (~600 ppb) compared to 5b (~30 ppb), suggests that most of the aspartic acid was formed inside the parent body during aqueous alteration, thus shielded from any UV CPL.

## CONCLUSION

The large enantiomeric excesses of several α-H protein amino acids reported in the Murchison meteorite (28-30) have now been identified in two different fragments of the C2 Tagish Lake meteorite. We believe that the L-amino acid enrichment found in these meteorites can be adequately explained by their crystallization behaviors, with preferential amplification of meta-conglomerate or conglomerate forming enantiopure crystals during parent body aqueous alteration, although future experiments will be needed to confirm this hypothesis. The finding that the Tagish Lake meteorite also contains a similar enrichment in L-aspartic and L-glutamic acids provides additional support that a wide variety of aqueously altered carbonaceous chondrites could have contributed nonracemic α-hydrogen amino acids leading to the origin of homochirality in life on Earth and possibly elsewhere. As suggested by others previously (44, 48), similar L-enrichments of conglomerate amino acids could have occurred in ancient aqueous sedimentary environments on the Earth. It has been observed that since large enantiomeric excesses of conglomerate-forming α-H amino acids are apparently more easily obtained by crystallization processes compared to racemic or α-methyl amino acids, conglomerate amino acids may have been more common in the first biopolymers on Earth. For example, aspartic and glutamic acids form a key part of the repetitive sequence calculated for Ferredoxine (58), believed to be one of the first proteins formed on Earth (59). Although amino acid homochirality can be an important signature of biological processes in the search for evidence of life elsewhere, the detection of non-terrestrial L-amino acid excesses in carbonaceous meteorites indicates that non-biological processes could also lead to significant enantioenrichment for some amino acids.

## MATERIALS AND METHODS

**Chemicals and Reagents.** Most of the chemicals and reagents used were purchased from Sigma-Aldrich. A stock amino acid solution (~ $10^{-5}$ to $10^{-6}$ M) was prepared by mixing individual amino acid standards (97-99% purity) in Millipore Direct Q3 UV (18.2 MΩ, < 5 ppb total organic carbon) ultrapure water. All chiral α-H amino acid standards were purchased as racemic mixtures (D = L), except for D- and L-threonine (Sigma-Aldrich, >98% purity) and D- and L-isovaline (Acros Organics, >99% purity) which were prepared as racemic mixtures by mixing the appropriate masses of each compound in Millipore water to the standard mixture. The sources of the $C_5$ amino acid standards used are detailed elsewhere (6). The LC-FD/TOF-MS and GC-MS/IRMS reagents used in this study were prepared as previously described (8, 23).

**Meteorite Samples, Controls, and Processing Procedures.** All glassware and sample handling tools were heated in a furnace at 500°C overnight. Three pristine Tagish Lake meteorite specimens (designated 5b, 11h, and 11i) were selected for detailed amino acid and compound specific carbon isotopic analysis. These meteorite samples were all recovered at the same time without direct hand contact from the frozen surface of the Taku arm of Tagish Lake within days of their fall in January 2000 and have been kept at temperatures below 0ºC until extraction for this study (34). Based on several petrologic differences including the relative proportions of matrix and framboidal magnetite and the replacement of chondrule glass by phyllosilicates, these three meteorite fragments represent a large range of parent body aqueous alteration of order 5b < 11h << 11i, where 5b represents the least altered and 11i the most altered material (34). Subsamples (~1-2 cm in size) of each meteorite were removed using a sterile scalpel under cold conditions (in a walk in freezer at -20 °C), weighed (mass 5b = 2.9 g; mass 11h = 2.5 g; mass 11i = 2.0 g), transferred to round-bottom flasks, and then crushed into fine powders using a glass rod in a fume hood. As a control, a procedural blank was carried

through the identical extraction procedure as the meteorite samples. In addition, a crushed serpentine (a hydrated magnesium silicate) sample that had been heated at 500°C in air overnight was processed using the hot water extraction, acid hydrolysis, and desalting protocol.

The powdered meteorite samples were extracted at reflux in 20 mL of Millipore water inside the round-bottom flasks at 100 °C for 6 hours at University of Alberta (UA) in a chemical fume hood. After cooling to room temperature, the water supernatants were filtered (0.2 micron) and the extracts transferred to separate round-bottom flasks and dried by rotoevaporation. At GSFC, the dried extracts of 5b, 11h, and 11i were re-dissolved in 5 mL of Millipore water at room temperature and one half of the water extract transferred to a separate glass tube, dried under vacuum, and the residue subjected to a 6 M HCl acid vapor hydrolysis procedure at 150°C for 3 hours to determine total hydrolyzable amino acid content. The acid-hydrolyzed, hot-water extracts were desalted by using cation-exchange resin (AG50W-X8, 100-200 mesh, hydrogen form, BIO-RAD), and the amino acids recovered by elution with 2 M $NH_4OH$ (prepared from Millipore water and $NH_3$(g) (AirProducts, *in vacuo*). The remaining half of each water extract (non-hydrolyzed fraction) was taken through the identical desalting procedure in parallel with the acid-hydrolyzed extracts to determine the free amino acid abundances in the meteorites. The amino acids in the $NH_4OH$ eluates were dried under vacuum to remove excess ammonia; the residues were then re-dissolved in 100 μ L of Millipore water, transferred to sterile microcentrifuge tubes, and stored at -20°C prior to analysis. Based on our analysis of amino acid standards taken through the entire extraction and acid hydrolysis procedure, we found no evidence of significant decomposition, racemization, or thermal degradation of amino acids during the extraction.

The amino acids in the $NH_4OH$ eluates were derivatized with OPA/NAC followed by separation and analysis by a Waters ACQUITY UPLC and Waters LCT-Premier mass spectrometer. The amino acids and their enantiomeric ratios were then quantified from the peak areas generated from both fluorescence detection and from the mass chromatogram of

their OPA/NAC derivatives from a minimum of 6 separate analyses. A more detailed description of the analytical technique and quantification methods used is described elsewhere (23). For the carbon isotopic measurements, total amino acids in the Tagish Lake 5b and 11h extracts were esterified with isopropanol and the isopropyl esters reacted with trifluoroacetic anhydride (TFAA) prior to analysis of the TFAA-isopropyl derivatives by GC-MS/IRMS and determination of the average $\delta^{13}C$ values for individual amino acids from three separate analyses as previously described (8). Based on the low amino acid abundances measured in 11i, we determined that there was insufficient meteorite sample available for this study to make carbon isotope measurements of amino acids in this sample. In addition, nitrogen and/or hydrogen isotope measurements of the amino acids in Tagish Lake samples 11h and 5b were not possible in this study and would require at least three times the mass of meteorite sample used for the carbon isotope measurements.

## ACKNOWLEDGMENTS

Funding support was received from the National Aeronautics and Space Administration (NASA) Astrobiology Institute and the Goddard Center for Astrobiology, the NASA Cosmochemistry Program, and the Natural Sciences and Engineering Research Council of Canada. A. S. Burton is supported by a NASA Postdoctoral Program fellowship administered by Oak Ridge Associated Universities. We thank D. N. Simkus and R. W. Hilts for assistance with the meteorite sample preparation and solvent extractions; and J. L. Bada and D. G. Blackmond for helpful discussions and comments on the manuscript.

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## MAIN TEXT FIGURES

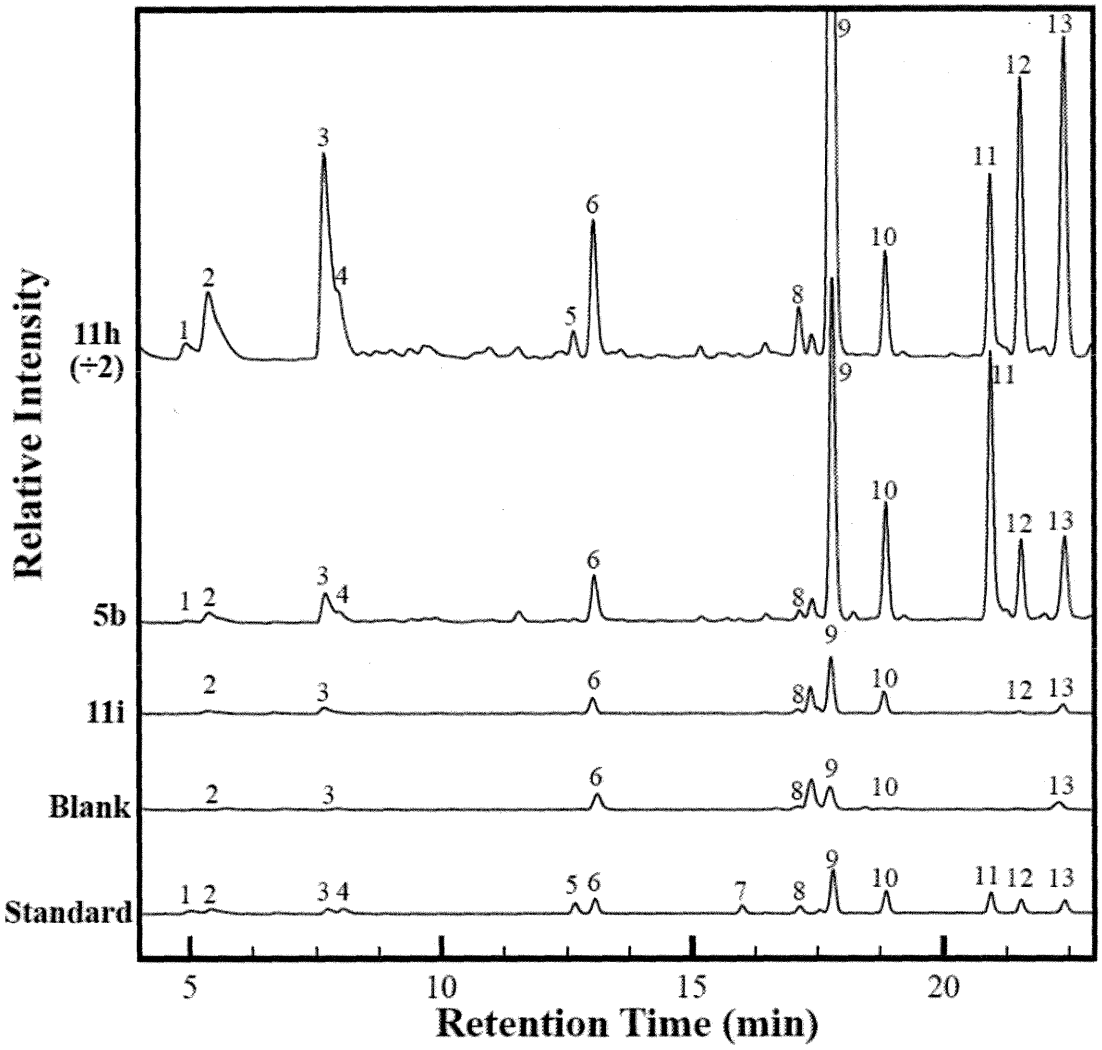


**Fig. 1.** The 4- to 22-min region of the LC-FD chromatograms. OPA/NAC derivatization (15 min) of amino acids in the 6 M HCl-hydrolyzed, hot-water extracts from the standard, procedural blank, and Tagish Lake meteorite samples 11i, 5b, and 11h are shown. Separation was achieved using a Waters BEH C18 column (2.1 x 50 mm, 1.7-μm bead) followed by a second Waters BEH phenyl column (2.1 x 150 mm, 1.7-μm bead). The conditions for amino acid separations for the mobile phase at 30.0°C were as follows: flow rate, 150 μL/min; solvent A (50 mM ammonium formate, 8% methanol, pH 8.0); solvent B (methanol); gradient, time in minutes (%B): 0 (0), 35 (55), 45 (100). The peaks were identified by comparison to the fluorescence retention time and exact molecular mass to those in the amino acid standard run on the same day. Fluorescent peaks that did not have corresponding peaks in the single ion TOF-MS chromatograms (shown in Fig. S1) with m/z values of the OPA/NAC amino acid derivative in the standard were not identified and quantified. Peak identifications: 1, D-aspartic acid; 2, L-aspartic acid; 3, L-glutamic acid; 4, D-glutamic acid; 5, D-serine; 6, L-serine; 7, D-threonine; 8, L-threonine; 9, glycine; 10, β-alanine; 11, D-alanine; 12, L-alanine; and 13, γ-amino-*n*-butyric acid.

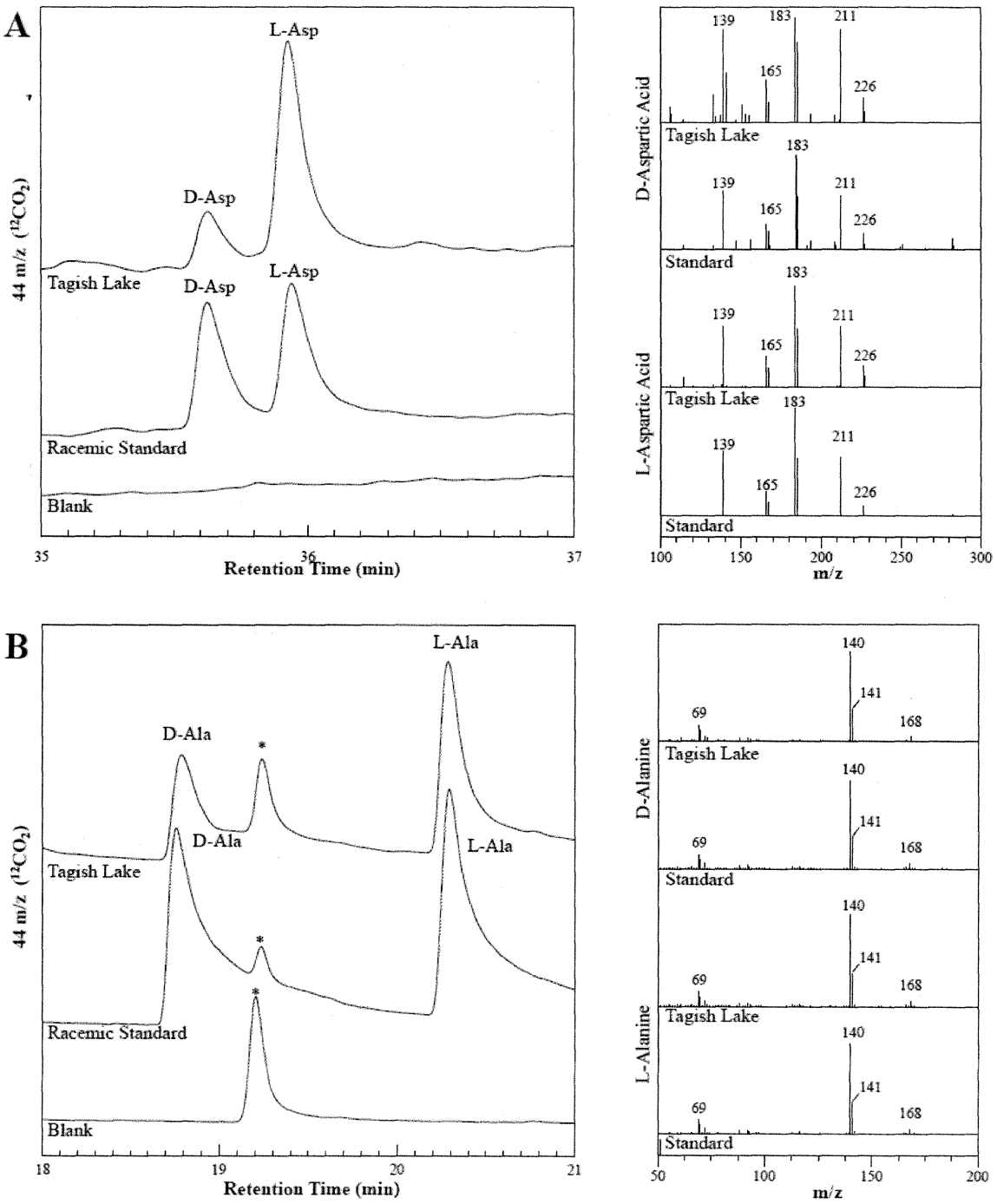


**Fig. 2.** Gas chromatography separation and mass spectrometry analysis of D- and L-aspartic acid (A) and D- and L-alanine (B) of derivatized extracts of the Tagish Lake 11h meteorite, a racemic standard, and the procedural blank. The traces on the left show the m/z 44 ($^{12}CO_2$) peak produced and measured from GC-IRMS for the peaks assigned to D- and L-aspartic acid. The inset shows the simultaneously collected mass spectral fragmentation pattern for these peaks in the Tagish Lake meteorite and standard and the structure of aspartic acid derivatized with trifluoroacetic acid/isopropanol (TFAA/IPA).

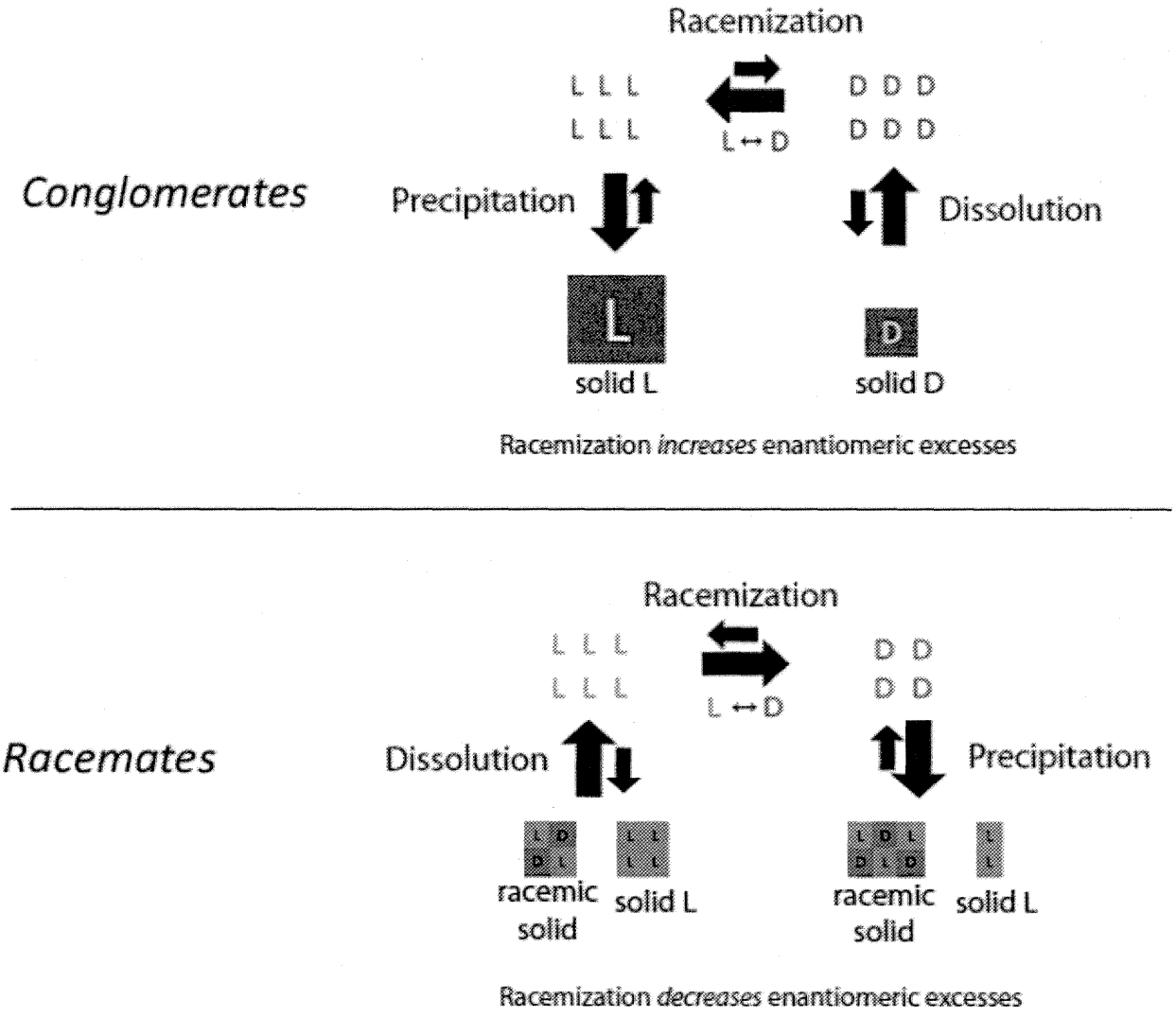


**Fig. 3.** Schematic illustrating the solid liquid phase behavior of amino acids that form conglomerate (top) and racemic (bottom) solid crystals. For the conglomerates, amplification of the L-enantiomer will occur through racemization and crystallization assuming that there is a slight initial L-excess. For racemates, any initial L- excess will decrease over time through racemization.

## MAIN TEXT TABLES

**Table 1**. Summary of the $\delta^{13}C$ values (‰, PDB) of amino acids in the 6M HCl-acid hydrolyzed extracts of Tagish Lake samples 5b and 11h compared to the Murchison meteorite[a].

| Amino acids | *Tagish Lake 5b* | *Tagish Lake 11h* | *Murchison* |
|---|---|---|---|
| | *This study* | *This study* | *Previous work* |
| *D-aspartic acid* | nd | +24 ± 4 | +25[b] |
| *L-aspartic acid* | nd | +29 ± 4 | -6[b] |
| *D-glutamic acid* | nd | nd | +29[b], +32[d] |
| *L-glutamic acid* | nd | -4 ± 9 | +7[b], +6[c], +15[d] |
| *glycine* | +39 ± 6 | +19 ± 4 | +41[b], +22[c] |
| *D-alanine* | +67 ± 7 | +6 ± 3 | +52[b], +30[c] |
| *L-alanine* | +55 ± 3 | +16 ± 4 | +38[b], +27[c] |
| β-alanine | +30 ± 6 | -5 ± 4 | +5[b] |
| γ-ABA | nd | +4 ± 3 | +18[b] |

[a]The $\delta^{13}C$ values were calculated from the $^{13}C/^{12}C$ values using the following equation: $\delta^{13}C$ (‰) = $[(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{PDB}-1] \times 10^3$. For Tagish Lake sample 11h, isotope values were determined from measurements of the combined non-hydrolyzed and acid-hydrolyzed water extracts. Protein amino acids are shown in italics.
[b]Data obtained from (37).
[c]Data obtained from (31).
[d]Data obtained from (40).
nd = value not determined due to low amino acid abundances or chromatographic interferences.

**Table 2**. Summary of the L-enantiomeric excesses measured for several amino acids in the 6M-HCl hydrolyzed extracts of Tagish lake samples 11h and 5b and racemic standards[a].

| Amino acids | *Tagish Lake 5b* | | *Tagish Lake 11h* | | *Racemic Standards* | |
|---|---|---|---|---|---|---|
| | $L_{ee}$ (%) | δx (n) | $L_{ee}$ (%) | δx (n) | $L_{ee}$ (%) | δx (n) |
| *aspartic acid* | 43.1 | ± 8.6 (8) | 45.5<br>58.7[b] | ± 5.2 (8)<br>± 1.8 (3)[b] | 6.6<br>4.7[b] | ± 2.9 (9)<br>± 2.3 (3)[b] |
| *glutamic acid* | 51.0 | ± 1.5 (6) | 55.1 | ± 3.6 (6) | -2.6 | ± 2.1 (9) |
| *alanine* | -4.8 | ± 5.5 (9) | -3.2<br>7.5[b] | ± 6.7 (9)<br>± 1.1 (3)[b] | 1.0<br>1.0[b] | ± 1.6 (9)<br>± 1.4 (3)[b] |
| *serine* | 80.5 | ± 3.9 (6) | 55.5 | ± 3.6 (6) | 3.3 | ± 1.5 (9) |
| *threonine* | 89.2 | ± 4.9 (6) | 99.4 | ± 0.3 (6) | 0.3 | ± 2.1 (9) |
| *valine* | < 19.2 | ± 7.1 (6) | < 68.2 | ± 2.2 (8) | 0.0 | ± 0.4 (14) |
| β-ABA | 5.3 | ± 7.2 (6) | 3.7 | ± 6.4 (6) | 0.8 | ± 1.1 (9) |
| norvaline | 1.9 | ± 4.2 (6) | 4.9 | ± 3.8 (6) | 1.0 | ± 0.8 (8) |
| isovaline | 7.0 | ± 1.9 (8) | 0.0 | ± 2.8 (8) | -2.3 | ± 1.3 (14) |

[a]The standard errors (δx) for the meteorites are based on the errors given for n separate measurements propagated through the equation $L_{ee}$ (%) = [(L-D)/(L+D)]*100. Negative % values indicate D excesses. For the standards, the errors are based on the standard deviation of the average $L_{ee}$ value of n separate analyses using LC-FD/TOF-MS or GC-MS. Protein amino acids are shown in italics.
[b]Values determined from the average D- and L-peak areas measured by GC-MS.

## SUPPORTING FIGURES

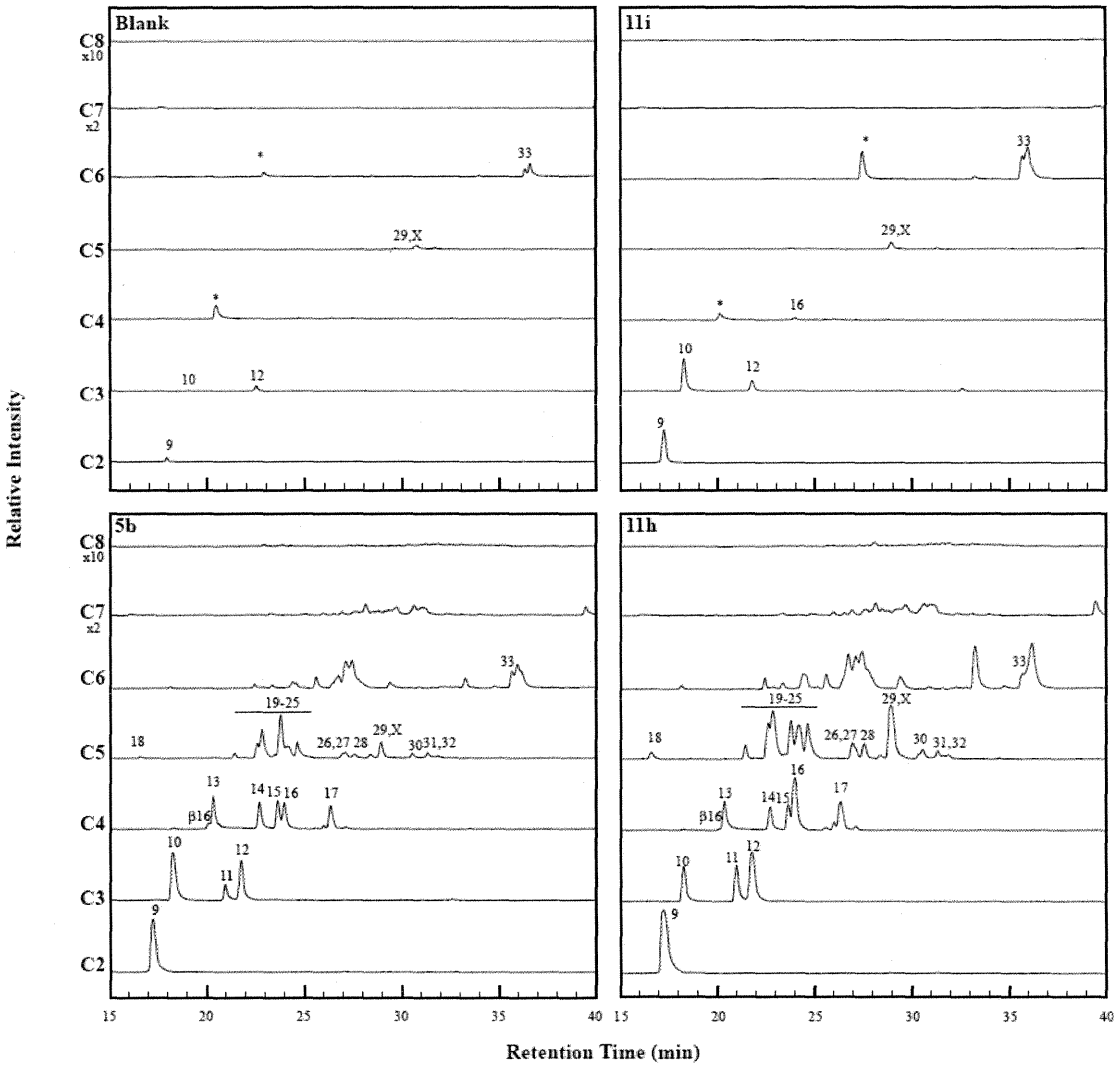


**Fig. S1.** The 15- to 40-min. region of the LC-TOF-MS single ion chromatograms ($C_2$: *m/z* = 337.09; $C_3$: *m/z* = 351.10; $C_4$: *m/z* = 365.12; $C_5$: *m/z* = 379.13; $C_6$: *m/z* = 393.15; $C_7$: *m/z* = 407.16; $C_8$: *m/z* = 421.18) in positive electrospray ionization mode. OPA/NAC derivatization (15 min) of amino acids in the the 6M HCl-hydrolyzed, hot-water extracts of Tagish Lake samples 11h, 5b, 11i, and a procedural serpentine blank are shown. Similar single ion chromatograms were obtained for the non-hydrolyzed extracts. Peaks were identified by comparison of the retention time and molecular mass to those in amino acid standards run on the same day. Non-fluorescent mass artifacts are labeled X. Peak identifications: 9, glycine; 10, β-alanine (BALA); 11, D-alanine; 12, L-alanine; 13, γ-amino-*n*-butyric acid; 14, D-β-amino-*n*-butyric acid; 15, L-β-amino-*n*-butyric acid; 16, α-aminoisobutyric acid; 17, D,L-α-amino-*n*-butyric acid (ABA); 18, 3-amino-2,2-dimethylpropanoic acid; 19, D,L-4-aminopentanoic acid; 20, D,L-4-amino-3-methylbutanoic acid; 21, D,L-3-amino-2-methylbutanoic acid; 22, D,L-3-amino-2-ethylpropanoic acid; 23, 5-aminopentanoic acid; 24, D,L-4-amino-2-methylbutanoic acid; 25, 3-amino-3-methylbutanoic acid; 26, D-isovaline; 27, D,L-3-aminopentanoic acid; 28, L-isovaline; 29, L-valine; 30, D-valine; 31, D-norvaline; 32, L-norvaline; and 33, D,L-norleucine (internal standard). Asterisks designate non-fluorescent mass artifacts that were not identified.

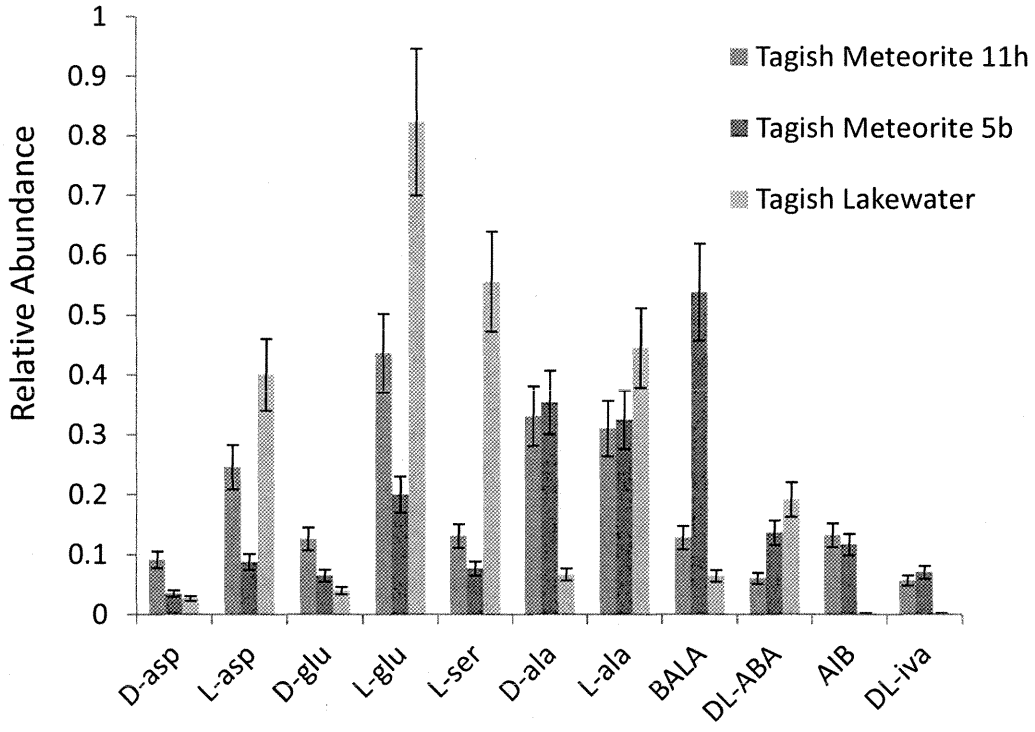


**Fig. S2.** A comparison of the relative molar abundances of several amino acids (glycine = 1.0) in the 6M-HCl hydrolyzed hot water extracts of the Tagish Lake meteorite samples 11h and 5b and a 250 ml sample of Tagish Lake meltwater. The relative abundance data for the Tagish Lake meteorite samples were determined from the absolute abundances measured in this study. Relative amino acid abundances for the Tagish Lake meltwater were calculated from the concentrations reported in (36).

**Table S1**. Summary of the average abundances (in ppb) of identified two- to six-carbon amino acids in the non-hydrolyzed (free) and 6 M HCl-hydrolyzed (total) hot-water extracts of the Tagish Lake meteorite measured by LC-FD/TOF-MS[a].

| Amino acid | ***Tagish Lake 11i*** | | ***Tagish Lake 5b*** | | ***Tagish Lake 11h*** | | ***Tagish Lake 24-24[b]*** |
|---|---|---|---|---|---|---|---|
| | *This study* | | | | | | *Previous work* |
| | *free* | *total* | *free* | *total* | *free* | *total* | *total* |
| *D-aspartic acid* | < 0.3 | < 1 | 1.7 ± 0.4 | 8.0 ± 1.8 | 13.6 ± 2.5 | 161 ± 14 | 11 ± 1 |
| *L-aspartic acid* | 0.7 ± 0.2 | 2.6 ± 0.5 | 8.7 ± 0.6 | 20.1 ± 3.3 | 55.0 ± 5.6 | 430 ± 65 | 83 ± 8 |
| *D-glutamic acid* | < 0.2 | < 0.2 | 1.6 ± 0.2 | 16.4 ± 0.5 | 41.2 ± 1.3 | 244 ± 23 | 16 ± 2 |
| *L-glutamic acid* | 0.2 ± 0.1 | 5.8 ± 1.6 | 2.7 ± 0.6 | 50.6 ± 2.3 | 53.5 ± 13.8 | 844 ± 89 | 306 ± 48 |
| *D-serine* | < 0.2 | < 0.2 | 1.3 ± 0.1 | 1.5 ± 0.1 | 23.6 ± 2.3 | 51.8 ± 7.3 | nd |
| *L-serine* | 1.8 ± 0.1 | 2.1 ± 0.9 | 17.3 ± 1.5 | 13.9 ± 4.2 | 124 ± 23 | 181 ± 29 | nd |
| *D-threonine* | < 0.1 | < 0.1 | < 0.1 | < 0.2 | < 0.2 | < 0.3 | nd |
| *L-threonine* | 0.9 ± 0.3 | 1.3 ± 0.4 | 7.4 ± 1.8 | 3.5 ± 1.6 | 55.2 ± 5.3 | 97.3 ± 17.8 | nd |
| *Glycine* | 2.4 ± 0.4 | 9.7 ± 4.2 | 90.0 ± 6.4 | 129 ± 21 | 619 ± 184 | 987 ± 257 | 147 ± 17 |
| β-alanine | 0.1 ± 0.1 | 13.5 ± 0.7 | 70.4 ± 14.1 | 82.3 ± 9.5 | 107 ± 19 | 150 ± 30 | 64 ± 10 |
| γ-amino-*n*-butyric acid | 0.1 ± 0.1 | < 1 | 7.2 ± 1.0 | 216 ± 24 | 41.1 ± 2.8 | 374 ± 50 | 77 ± 10 |
| *D-alanine* | < 0.1 | < 0.5 | 25.7 ± 0.7 | 54.1 ± 3.3 | 252 ± 32 | 387 ± 25 | 20 ± 5 |
| *L-alanine* | 1.2 ± 1.0 | 1.6 ± 0.5 | 25.1 ± 0.7 | 49.7 ± 4.3 | 240 ± 33 | 363 ± 41 | 75 ± 18 |
| D-β-amino-*n*-butyric acid | < 0.1 | < 0.1 | 13.3 ± 2.0 | 11.5 ± 1.2 | 19.0 ± 3.8 | 36.0 ± 3.9 | < 26[d] |
| L-β-amino-*n*-butyric acid | < 0.1 | < 0.1 | 12.5 ± 2.0 | 12.8 ± 1.4 | 17.4 ± 4.8 | 38.8 ± 3.0 | |
| α-aminoisobutyric acid (α-AIB) | 0.3 ± 0.1 | 1.3 ± 0.2 | 9.2 ± 0.8 | 20.7 ± 2.1 | 161 ± 29 | 179 ± 23 | < 27 |
| D,L-α-amino-*n*-butyric acid[c] | < 0.1 | < 0.2 | 10.2 ± 0.1 | 24.2 ± 3.9 | 62.1 ± 16.5 | 82.2 ± 15.2 | 84 ± 40 |
| ε-amino-*n*-caproic acid (EACA) | < 0.2 | < 0.2 | < 0.2 | < 0.2 | < 0.3 | < 0.3 | nd |
| $C_5$ amino acids (from Table S2) | < 0.7 | < 1 | ~40 | ~210 | ~190 | ~790 | nd |
| **Total (ppb)** | **~8** | **~40** | **~340** | **~740** | **~2,100** | **~5,400** | **~880** |

[a]Tagish Lake meteorite 11i, 5b, and 11h extracts were analyzed by OPA/NAC derivatization (15 min.) and UPLC separation with UV fluorescence and time of flight mass spectrometry (ToF-MS) detection. For the LC-MS data, the fluorescence peaks and the mono-isotopic masses of each protonated OPA/NAC amino acid derivative ($M + H^+$) was used for quantification and final peak integrations included background level correction using a serpentine blank and a comparison of the peak areas with those of an amino acid standard run on the same day. The final values were normalized using the desalting and derivatization recoveries of an internal D,L-norleucine standard (recoveries ranged from 60-100% for the meteorite extracts). The uncertainties (δx) are based on the standard deviation of the average value of three to ten separate measurements (n) with a standard error, $\delta x = \sigma_x \cdot (n-1)^{-1/2}$. Protein amino acids in italics.

[b]For comparison, HPLC-FD amino acid measurements of a non-pristine Tagish Lake meteorite sample 24-24 reported by (36)

[c]Enantiomers could not be separated under the chromatographic conditions.

[d]Upper limits for D+L enantiomers.

nd = amino acid abundances not determined.

**Table S2**. Summary of the average $C_5$ amino acid abundances (in ppb) in the non-hydrolyzed (free) and 6 M HCl-hydrolyzed (total) hot-water extracts of the Tagish Lake meteorite measured by LC-FD/TOF-MS[a].

| $C_5$ Amino acid detected | | *Tagish Lake 11i* | | *Tagish Lake 5b* | | *Tagish Lake 11h* | |
|---|---|---|---|---|---|---|---|
| | | *free* | *total* | *free* | *total* | *free* | *total* |
| α | D-norvaline (D-2-apa) | < 0.2 | < 0.4 | 2.2 ± 0.2 | 2.6 ± 0.2 | 5.4 ± 0.2 | 6.8 ± 0.4 |
| | L-norvaline (L-2-apa) | < 0.2 | < 0.3 | 1.9 ± 0.1 | 2.7 ± 0.1 | 5.6 ± 0.2 | 7.5 ± 0.4 |
| | D-isovaline (D-2-a-2-mba) | < 0.2 | < 0.5 | 2.5 ± 0.2 | 6.6 ± 0.2 | 42.7 ± 1.6 | 43.7 ± 1.8 |
| | L-isovaline (L-2-a-2-mba) | < 0.2 | < 0.5 | 3.1 ± 0.2 | 7.6 ± 0.2 | 41.9 ± 1.5 | 43.7 ± 1.7 |
| | *D-valine (D-2-a-3-mba)* | < 0.2 | < 0.4 | 1.9 ± 0.1 | 5.9 ± 0.1 | 7.1 ± 0.2 | 16.4 ± 0.5 |
| | *L-valine (L-2-a-3-mba)* | < 0.7 | < 1 | < 4[b] | < 9[b] | < 37[b] | < 86[b] |
| β | D,L-3-apa[c] | < 0.2 | < 0.4 | 12.7 ± 0.5 | 10.6 ± 0.4 | 13.4 ± 0.4 | 12.7 ± 0.7 |
| | D,L- and allo-3-a-2-mba[c] | < 0.1 | < 0.2 | 2.1 ± 0.2 | 2.6 ± 0.2 | 4.3 ± 0.2 | 5.4 ± 0.7 |
| | 3-a-3-mba[d] | < 0.1 | < 0.1 | < 0.5 | < 1.5 | < 2.6 | < 12 |
| | 3-a-2,2-dmpa | < 0.1 | < 0.1 | 1.5 ± 0.1 | 3.8 ± 0.1 | 9.2 ± 0.3 | 17.7 ± 0.6 |
| | D,L-3-a-2-epa[e] | < 0.5 | < 1 | 2.7 ± 0.2 | 10.7 ± 1.6 | 6.1 ± 0.2 | 10.2 ± 0.4 |
| γ | D,L-4-apa[c] | < 0.1 | < 0.1 | 1.3 ± 0.2 | 24.5 ± 0.8 | 5.3 ± 0.7 | 112 ± 6 |
| | D,L-4-a-2-mba[e] | < 0.3 | < 0.5 | 1.7 ± 0.2 | 34.2 ± 0.7 | 4.5 ± 0.3 | 191 ± 7 |
| | D,L-4-a-3-mba[e] | < 0.2 | < 0.3 | 1.0 ± 0.1 | 42.9 ± 1.3 | 4.3 ± 0.2 | 183 ± 5 |
| δ | 5-apa | < 0.1 | < 0.2 | 1.2 ± 0.1 | 46.3 ± 1.2 | 2.7 ± 0.1 | 53.6 ± 1.1 |
| | **Total (ppb)** | **< 0.7** | **< 1** | **~40** | **~210** | **~190** | **~790** |

[a] All values are reported in parts-per-billion (ppb) on a bulk sample basis. Extracts were analyzed by OPA/NAC derivatization (15 min.) and HPLC separation with UV fluorescence and time of flight mass spectrometry (ToF-MS) detection. For the LC-ToF-MS data, the mono-isotopic masses (m/z 379.13 ± 0.02) of each protonated OPA/NAC amino acid derivative ($M + H^+$) was used for quantification and final peak integrations included background level correction using a procedural blank and a comparison of the peak areas with those of an amino acid standard run on the same day. The final values were normalized using the desalting and derivatization recoveries of an internal D,L-norleucine standard (recoveries ranged from 60-100% for the meteorite extracts). The uncertainties (δx) are based on the standard deviation of the average value of six to eight separate measurements (n) with a standard error, $\delta x = \sigma_x \cdot (n-1)^{-1/2}$. Protein amino acids in italics.

[b] Peak detected above blank levels, however only upper limit reported due to unidentified coeluting peak X in LC-TOF-MS analysis of the procedural blank.

[c] Enantiomers were separated but could not be identified due to the lack of optically pure standards.

[d] 3-a-3-mba co-elutes with one of the enantiomers of D,L-4-apa, therefore upper limits for 3-a-3-mba were estimated by taking the difference in peak areas of the two D,L-4-apa enantiomers.

[e] Enantiomers could not be separated under the chromatographic conditions.

**Table S3**. Amino acid enantiomeric ratios (D/L) measured in the non-hydrolyzed (free) and 6M HCl-hydrolyzed (total) hot-water extracts of the Tagish Lake meteorite[a].

| Amino Acids | ***Tagish Lake 5b*** | | ***Tagish Lake 11h*** | | ***Tagish Lake 24-24*** |
|---|---|---|---|---|---|
| | *This study* | | | | *Previous work*[b] |
| | *free* | *total* | *free* | *total* | *total* |
| *Aspartic acid* | 0.20 ± 0.05 | 0.40 ± 0.11 | 0.25 ± 0.05 | 0.37 ± 0.07<br>0.26 ± 0.02[c] | 0.13 ± 0.02 |
| *Glutamic acid* | 0.59 ± 0.15 | 0.32 ± 0.02 | 0.77 ± 0.20 | 0.29 ± 0.06 | 0.05 ± 0.01 |
| *Serine* | 0.08 ± 0.01 | 0.11 ± 0.03 | 0.19 ± 0.04 | 0.29 ± 0.06 | nd |
| *Threonine* | < 0.01 | < 0.06 | < 0.01 | < 0.01 | nd |
| *Alanine* | 1.02 ± 0.04 | 1.09 ± 0.12 | 1.05 ± 0.20 | 1.06 ± 0.14<br>0.86 ± 0.02[c] | 0.27 ± 0.09 |
| *Valine* | > 0.48 | > 0.65 | > 0.19 | > 0.19 | nd |
| β-ABA | 1.06 ± 0.23 | 0.90 ± 0.35 | 1.09 ± 0.37 | 0.80 ± 0.31 | nd |
| Norvaline | 1.16 ± 0.12 | 0.96 ± 0.08 | 0.96 ± 0.05 | 0.91 ± 0.07 | nd |
| Isovaline | 0.81 ± 0.08 | 0.87 ± 0.03 | 1.02 ± 0.05 | 1.00 ± 0.06 | nd |

[a]The uncertainties are based on the absolute errors shown in Tables S1 and S2. Protein amino acids in italics.
[b]Ratios calculated from data in (36).
[c]Values determined by GC-MS. Error based on the standard deviation of three separate measurements.
nd = ratio not determined due to low amino acid abundances or values that were not reported.