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Extraterrestrial amino acids and amines identified in asteroid Ryugu samples returned by the Hayabusa2 mission

Eric T. Parker [a,*], Hannah L. McLain [a,b,c], Daniel P. Glavin [a], Jason P. Dworkin [a], Jamie E. Elsila [a], José C. Aponte [a], Hiroshi Naraoka [d], Yoshinori Takano [e], Shogo Tachibana [f], Hikaru Yabuta [g], Hisayoshi Yurimoto [h], Kanako Sakamoto [i], Toru Yada [i], Masahiro Nishimura [i], Aiko Nakato [i], Akiko Miyazaki [i], Kasumi Yogata [i], Masanao Abe [i], Tatsuaki Okada [i], Tomohiro Usui [i], Makoto Yoshikawa [i], Takanao Saiki [i], Satoshi Tanaka [i], Satoru Nakazawa [i], Yuichi Tsuda [i], Fuyuto Terui [j], Takaaki Noguchi [k], Ryuji Okazaki [d], Sei-ichiro Watanabe [l], Tomoki Nakamura [m]

[a] Solar System Exploration Division, NASA Goddard Space Flight Center, Greenbelt, MD 20771, USA
[b] Center for Research and Exploration in Space Science and Technology (CRESST), NASA Goddard Space Flight Center, Greenbelt, MD 20771, USA
[c] Department of Physics, The Catholic University of America, Washington, D.C. 20064, USA
[d] Department of Earth and Planetary Science, Kyushu University, Nishi-ku, Fukuoka 819-0395, Japan
[e] Biogeochemistry Research Center (BGC), Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokosuka, Kanagawa 237-0061, Japan
[f] UTokyo Organization for Planetary and Space Science (UTOPS), The University of Tokyo, 7-3-1 Hongo, Tokyo 113-0033, Japan
[g] Hiroshima University, Higashi-Hiroshima 739-8526, Japan
[h] Hokkaido University, Sapporo 060-0810, Japan
[i] Institute of Space and Astronautical Science (ISAS), Japan Aerospace Exploration Agency (JAXA), Sagamihara 252-5210, Japan
[j] Kanagawa Institute of Technology, Atsugi 243-0292, Japan
[k] Kyoto University, Kyoto 606-8502, Japan
[l] Nagoya University, Nagoya 464-8601, Japan
[m] Tohoku University, Sendai 980-8578, Japan

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ABSTRACT

The hot water and acid extracts of two different Ryugu samples collected by the Hayabusa2 mission were analyzed for the presence of aliphatic amines and amino acids. The abundances and relative distributions of both classes of molecules were determined, as well as the enantiomeric compositions of the chiral amino acids. The Ryugu samples studied here were recovered from sample chambers A and C, which were composed of surface material, and a combination of surface and possible subsurface material, respectively. A total of thirteen amino acids were detected and quantitated in these samples, with an additional five amino acids that were tentatively identified but not quantitated. The abundances of four aliphatic amines identified in the Ryugu samples were also determined in the current work. Amino acids were observed in the acid hydrolyzed and unhydrolyzed hot water extracts of asteroid Ryugu regolith using liquid chromatography with UV fluorescence detection and high-resolution mass spectrometry. Conversely, aliphatic amines were only analyzed in the unhydrolyzed hot water Ryugu extracts. Two- to six-carbon (C_2-C_6) amino acids with individual abundances ranging from 0.02 to 15.8 nmol g^{-1}, and one- to three-carbon (C_1-C_3) aliphatic amines with individual abundances from 0.05 to 34.14 nmol g^{-1}, were found in the hot water extracts. Several non-protein amino acids that are rare in biology, including β-amino-*n*-butyric acid (β-ABA) and β-aminoisobuytric acid (β-AIB), were racemic or very nearly racemic, thus indicating their likely abiotic origins. Trace amounts of select protein amino acids that were enriched in the L-enantiomer may indicate low levels of terrestrial amino acid contamination in the samples. However, the presence of elevated abundances of free and racemic alanine, a common protein amino acid in terrestrial biology, and elevated abundances of the predominately free and racemic non-protein amino acids, β-ABA and β-AIB, indicate that many of the amino acids detected in the Ryugu water extracts were indigenous to the samples. Although the Ryugu samples have been found to be chemically similar to CI type carbonaceous chondrites, the measured concentrations and relative distributions of amino acids and aliphatic amines in Ryugu samples were notably different from those previously observed for the CI1.1 carbonaceous chondrite, Orgueil. This discrepancy could be the result of

* Corresponding author at: NASA Goddard Space Flight Center, 8800 Greenbelt Road, Mail Code 691, Greenbelt, MD 20771, USA.
E-mail address: eric.t.parker@nasa.gov (E.T. Parker).

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differences in the original chemical compositions of the parent bodies and/or alteration conditions, such as space weathering. In addition to α-amino acids that could have been formed by Strecker cyanohydrin synthesis during a low temperature aqueous alteration phase, β-, γ-, and δ-amino acids, including C_3 – C_5 straight-chain *n*-ω-amino acids that are not formed by Strecker synthesis, were also observed in the Ryugu extracts. The suite of amino acids measured in the Ryugu samples indicates that multiple amino acid formation mechanisms were active on the Ryugu parent body. The analytical techniques used here are well-suited to search for similar analytes in asteroid Bennu material collected by the NASA OSIRIS-REx mission scheduled for Earth return in September 2023.

1. Introduction

A variety of extraterrestrial materials, including meteorites (Burton et al., 2012b; Cooper et al., 2001; Glavin et al., 2018; Kvenvolden et al., 1970; Pizzarello, 2006; Simkus et al., 2019), and samples returned from comet Wild 2 (Elsila et al., 2009; Glavin et al., 2008; Sandford et al., 2006) and the JAXA Hayabusa mission to asteroid Itokawa (Parker et al., 2022), have been analyzed for amino acids and other soluble organic compounds in search of prebiotic molecules that could have contributed to the emergence of life on Earth and potentially elsewhere. On December 6, 2020, the JAXA Hayabusa2 mission successfully returned 5.4 g of material from the Cb-type asteroid 162173 Ryugu (Tachibana et al., 2022; Yada et al., 2022). The return of asteroid Ryugu material to Earth presented an opportunity to analyze the organic content of pristine extraterrestrial samples from a known parent body. This contrasts with recovered carbonaceous chondrites, which originated from unknown parent bodies and are often exposed to terrestrial weathering and contamination sources (Kvenvolden et al., 2000).

Among the array of soluble organic compounds that could be explored in Ryugu samples, amino acids and aliphatic amines are particularly intriguing classes of target molecules for a range of reasons. Most notably, these two classes of analytes play key roles in the biochemistry of life on Earth and there are a variety of highly sensitive and selective analytical techniques that can be used to measure the abundances, distributions, and isotopic and enantiomeric compositions of these prebiotic compounds (Simkus et al., 2019). Additionally, amino acid and aliphatic amine abundances and distributions hold implications for synthetic mechanisms and parent body histories. To elaborate, elevated relative abundances of the non-protein amino acid, β-alanine, have been correlated with aqueously altered CI, CM, and CR chondrites (Glavin et al., 2011), while pronounced abundances of straight-chain *n*-ω-amino acids have been correlated with thermally altered CO3 and CV3 chondrites (Burton et al., 2012a). Amino acid structural composition is capable of inferring clues regarding the formation of these biomolecules. For example, a predominance of branched carbon chain amino acid isomers, compared to straight-chain amino acid isomers, suggests that radical and ion chemistry were likely critical components of the amino acid synthesis mechanisms at play (Cronin and Chang, 1993; Herbst, 1995). Furthermore, aliphatic amino acids and amines are structurally related, and thus, the analysis of amines may illuminate our understanding of synthetic pathways that could have led to the formation of these compound classes in the interstellar medium and in Ryugu. The observed molecular distribution in aliphatic amines combined with that of amino acids may help unlock the distinctive processes that the asteroid has experienced (Aponte et al., 2020), such as aqueous alteration and shock heating that may have shaped the abundances we have found in this work. Amino acids were the target of a previous study of microparticles returned by the JAXA Hayabusa asteroid sample-return mission, which collected material from the S-type asteroid Itokawa and predated JAXA's Hayabusa2 mission (Parker et al., 2022). This exploration resulted in the detection of several non-protein amino acids, including some that are rare on Earth; however, the observed distribution was inconsistent with that expected from S-type asteroid fragments (type LL chondrites), so it is possible the detected amino acids in the carbon-rich Hayabusa grains originated from a parent body other than Itokawa (Parker et al., 2022). In contrast, the return of asteroid Ryugu samples by Hayabusa2 is an opportunity to explore the amino acid and aliphatic amine content of carbon-rich material from a known C-type asteroid.

It is worth pointing out the first reporting of amino acids in asteroid Ryugu material was by (Nakamura et al., 2022a), which overviewed a large body of work on the analyses of Ryugu material, a small portion of which included some amino acid analytical results. Amino acids were searched for in unpurified extracts of Ryugu sample C0008 using ultrahigh performance liquid chromatography and high-resolution mass spectrometry. The report concluded that the sample contained 23 amino acids, including such amino acids as glycine, β-alanine, valine, and norvaline (Nakamura et al., 2022a).

Subsequently, Naraoka et al. (Naraoka et al., 2023) published an article on behalf of the Hayabusa2 initial analysis team, which overviewed the soluble organic content (e.g., amino acids, aliphatic amines, carboxylic acids, polycyclic aromatic hydrocarbons, and nitrogen heterocycles) in the hot water extracts of Ryugu sample A0106. In this communication, Naraoka et al. (Naraoka et al., 2023) reported the use of 3-dimensional high performance liquid chromatography with a high-sensitivity fluorescence detector, and liquid chromatography with tandem UV fluorescence detection and high-resolution mass spectrometry to detect and quantify low individual abundances (>6 nmol g^{-1}) of 15 amino acids, while tentatively identifying 5 additional amino acids. The results of the high-resolution mass spectrometry-based technique introduced by Naraoka et al. (Naraoka et al., 2023) are the subject of the current study and will be expanded on in detail here.

To provide quantitative Ryugu amino acid data that can simultaneously be used to compare across different studies and efficaciously evaluate the plausibility of terrestrial contamination influences, we have analyzed asteroid Ryugu samples for their C_2–C_6 amino acid content. An intended goal of this quantitative analytical approach was to help determine possible parent body alteration histories and amino acid formation mechanisms. We have also analyzed these same Ryugu samples for their complementary C_1–C_6 aliphatic amine content. The samples that were analyzed included both the hot water extracts (Extract #7-1) and the subsequent hydrochloric acid (HCl) extracts (Extract #7-2). Free amino acids and aliphatic amines were investigated in the unhydrolyzed (hereafter UNH) fractions of these extracts, while both free and bound amino acids were analyzed in the double distilled (dd) HCl-hydrolyzed (hereafter HYD) fractions of both extracts. The aliphatic amines were not analyzed in the HYD

extracts. Amino acid analyses were performed after derivatization with *o*-phthaldialdehyde/N-acetyl-L-cysteine (OPA/NAC) using ultrahigh performance liquid chromatography with tandem UV fluorescence detection and high-resolution mass spectrometry (LC-FD/HRMS). Aliphatic amines were analyzed after derivatization with AccQ•TAG using ultraperformance liquid chromatography with UV fluorescence detection and time-of-flight mass spectrometry (LC-FD/ToF-MS).

2. Methods and materials

2.1. Sample extraction

Asteroid Ryugu samples A0106 (13.08 mg, composed of asteroid surface material collected at the first touchdown site) and C0107 (10.73 mg, composed of a mixture of asteroid surface and possible subsurface material collected at the second touchdown site) were prepared at Kyushu University for analysis of amino acids and aliphatic amines, along with a single baked serpentine procedural blank (16.21 mg). The purpose of the procedural blank was to control for contamination that samples may have been exposed to during extraction and subsequent sample handing procedures. The subsurface material uncovered by the Hayabusa2 Small Carry-on Impactor (SCI) has been estimated to be from a maximum depth of approximately 1 m (Arakawa et al., 2020; Arakawa et al., 2017). The residues of the blank and samples first underwent hot water extraction at Kyushu University. The water supernatants after hot water extraction were then removed and set aside for later processing. Half of each of the remaining residues then underwent HCl extraction at Kyushu University. The HCl supernatant after extraction was removed and set aside for separate processing. The extraction details for both the hot water extracts and the HCl extracts of Ryugu samples A0106 and C0107 and the controls are provided below and in Figure S1.

Hot water extraction entailed submerging each blank and sample residue in 200 μL of water in its own nitrogen-purged, sealed, glass ampoule. Hot water extraction was then performed at 105 °C for 20 h. The contents (i.e., solid residue + liquid) of each glass ampoule were transferred to separate, intermediary glass vials upon completion of hot water extraction. These intermediary glass vials were then centrifuged at 14,000 rpm for 8 min, and the water supernatants from each intermediary glass vial were transferred to their own, respective sample extract vials. The interiors of each glass ampoule were then rinsed with 200 μL of water. The resultant water solutions were then transferred to their respective intermediary glass vials, which were mixed by shaking. The intermediary glass vials were again centrifuged for 8 min at 14,000 rpm before transferring the supernatants to their respective sample extract vials. This rinsing process was completed one more time, to bring the total volume of each sample extract vial to 600 μL. Each sample extract vial was mixed well by shaking. A total of 250 μL of each blank and sample were shipped at ambient temperature in sealed vials to NASA Goddard Space Flight Center (GSFC) for analysis of amino acids and aliphatic amines.

Half of each hot water extracted residue that remained in its respective intermediary glass vial underwent a subsequent HCl extraction step. Each residue was submerged in a 50:50 H_2O:6 M HCl solution (~3 M HCl) in its own nitrogen-purged, sealed, glass ampoule. Hydrochloric acid extraction was then performed at 105 °C for 20 h. The supernatants of each glass ampoule were transferred to separate, glass sample vials upon completion of HCl extraction. The remaining residues in the glass ampoules were then rinsed by adding 200 μL of water into the glass ampoules. The glass ampoules then underwent ultrasonication for 15 min before being spun down to separate the residues from the rinse solvent. The supernatants were transferred from each glass ampoule to their respective glass sample vials. This rinsing process was completed one more time, to bring the total volume in each glass sample vial to 600 μL. Each glass sample vial was then mixed well by shaking. A total of 200 μL of each blank and sample were shipped at room temperature in sealed vials to NASA GSFC for analysis of amino acids and aliphatic amines.

Prior to the analysis of the Ryugu sample extracts, the sample preparation and analysis workflows were rehearsed to evaluate the efficacies of these procedures before subjecting precious Ryugu samples to them. As part of this rehearsal, 3.24 mg of the CM1.6 Murchison meteorite (source: Chicago Field Museum) were extracted and analyzed using similar protocols planned for Ryugu. These rehearsal findings will be shown in the current work for comparative purposes. As a result, the rehearsal sample preparation and analysis protocols are detailed in §1.4 of the Supplementary Material, for reference.

2.2. Preparation of unhydrolyzed and hydrolyzed samples

Upon arrival at NASA GSFC, extracts were prepared for 6 M ddHCl vapor hydrolysis inside an ISO 5 HEPA-filtered positive pressure laminar flow hood, located in an ISO $\leq$ 8 white room. An analytical blank comprised of ultrapure water was prepared at NASA GSFC simultaneously with Ryugu extracts A0106 and C0107, and the serpentine procedural blank extract. The purpose of the analytical blank was to control for contamination that samples and the baked serpentine procedural blank may have been exposed to during sample handing procedures at NASA GSFC, alone.

A schematic overviewing sample preparation is displayed in Figure S1 but will be discussed in detail here. Hot water extracts and a corresponding analytical blank were prepared for analysis as UNH fractions by drying down 80 μL of each sample and blank in glass vials, under vacuum at room temperature. These sample and blank residues were then set aside until derivatization. It should be noted that the quantification of the non-protein amino acids in the UNH A0106 sample was derived from this 80 μL aliquot of Ryugu sample A0106. The quantification of protein amino acids in Ryugu sample A0106 sample, on the other hand, was derived from a separate 5 μL aliquot of Ryugu sample A0106, which was prepared simultaneously with a 5 μL aliquot of ultrapure water that served as an analytical blank. The 5 μL aliquots of Ryugu sample A0106 and ultrapure water were also dried down under vacuum in glass vials and set aside until derivatization. Portions of the hydrochloric acid extracts and a corresponding analytical blank were prepared for analysis at NASA GSFC without going through 6 M ddHCl vapor hydrolysis (hereafter referred to as UNH) by drying down 60 μL of each sample and blank in glass vials, under vacuum. These sample and blank residues were then set aside until derivatization and analysis.

Portions of the hot water extracts and a corresponding analytical blank were prepared for analysis as HYD fractions via 6 M ddHCl vapor hydrolysis by mixing 80 μL of each sample and blank with 8 μL of 1.5 M ddHCl in test tubes, to minimize evaporative loss of volatile amines, before bringing the solutions to dryness under vacuum. Portions of hydrochloric acid extracts and a corresponding analytical blank were also prepared for 6 M ddHCl vapor hydrolysis at NASA GSFC by mixing 60 μL of each sample and blank with 6 μL of 1.5 M ddHCl in test tubes before drying the solutions under vacuum. Dried residues of each hot water extract and HCl extract were then subjected to acid vapor hydrolysis using 6 M ddHCl for 3 h at 150 °C to determine both free and bound amino acid content. Further details about how samples were prepared for, and exposed to, acid vapor hydrolysis at NASA GSFC are provided in (Glavin et al., 2006). Portions of the HCl extracted samples underwent hydrolysis upon arrival at NASA GSFC to facilitate a

more consistent comparison between the HCl extracted samples and the hot water extracted samples. The higher temperature ddHCl vapor hydrolysis approach was used in the GSFC analyses to enable a direct comparison with previously published amino acid results from carbonaceous meteorite water extracts that were hydrolyzed under the same conditions.

Upon completion of acid vapor hydrolysis, retrieved sample and blank test tubes were dried under vacuum to remove HCl. Sample and blank test tubes were then reconstituted with 100 μL of ultrapure water. These solutions were then transferred from their test tubes into capped glass vials. Each sample and blank test tube was then rinsed using 100 μL of ultrapure water. After mixing well by shaking and spinning down, the supernatants of each test tube were transferred to their respective glass vials. The sample and blank test tubes were subjected to a second rinse using 100 μL ultrapure water followed by solution transfer, resulting in 300 μL of solution in each glass vial. The solutions in the glass vials were then evaporated to dryness under vacuum at room temperature to ensure complete removal of residual HCl from the acid vapor hydrolysis process, and then set aside until derivatization and analysis.

2.3. Additional methodological details

Further information regarding sample handling and analysis are reported in §1 of the Supplementary Material. There, one can find details about the types and purities of reagents used in this work, and contamination control strategies implemented. The Supplementary Material also provides a detailed accounting of sample derivatization procedures utilized and the analytical parameters under which the liquid chromatography- and mass spectrometry-based techniques were operated. Examples of the performance characteristics of the LC-FD/HRMS and LC-FD/ToF-MS techniques used for the analysis of precious Ryugu samples are outlined in Figures S2-S4 and Tables S1-S3 in §2.1. of the Supplementary Material.

3. Results

3.1. Amino acids

Amino acids were not detected above background levels in any of the Ryugu HCl extracts (#7-2 extracts) but were detected in the UNH and HYD hot water extracts (#7-1) above blank levels (Tables S4-S11). These contrasting results suggest that the hot water extraction process, which was performed prior to the HCl extraction process, was efficient in extracting amino acids or their chemical precursors from the Ryugu samples studied here, such that insufficient amino acids and amino acid precursors remained in the residues to be extracted at detectable quantities using a 50:50 H_2O:6 M HCl solution. See §3.1. of the Supplementary Material for more details. Since amino acids were not detected in the HCl extracts, this paper will focus only on the amino acid data from the hot water extracts, and not the amino acid data from the HCl extracts.

A comparison of blank-corrected amino acid data for the HYD and UNH Ryugu samples A0106 and C0107 are presented in Tables 1 and 2, and an example of amino acid detection is shown in Fig. 1. Juxtaposing UNH amino acid data with HYD amino acid data allows for an analysis of the extent to which bound amino acids were liberated by the acid vapor hydrolysis protocol. For each of the HYD and UNH Ryugu samples A0106 and C0107, 11 – 13 different amino acids were detected and quantitated, with an additional set of five C_5 amino acids that were tentatively detected above background, but were not quantitated (Table 2).

The sum of the amino acid abundances identified in the UNH samples were 8.3 ± 0.1 nmol g^{-1} for A0106 and 27.3 ± 1.9 nmol g^{-1} for C0107. The significant difference in UNH amino acid abundances between the hot water extracts of A0106 and C0107 is largely due to the pronounced contrast in glycine (Gly) and alanine (Ala) abundances between these two UNH samples (Figures S5-S7). The UNH C0107 sample contained 15.8 ± 1.8 nmol g^{-1} of glycine, while the UNH A0106 sample contained 1.62 ± 0.04 nmol g^{-1} of glycine. In addition to the UNH C0107 sample containing nearly 10x as much glycine as did UNH A0106, UNH C0107 also contained > 25x as much alanine as did UNH A0106.

3.1.1. Chiral amino acid enantiomeric composition

An important analysis that is complementary to measuring amino acid abundances is determining enantiomeric ratios of chiral amino acids. The benefit of doing so is to help evaluate the likelihood that such chiral species were formed in an extraterrestrial environment, as opposed to being products of terrestrial contamination. In this context, the most intriguing chiral amino acids targeted here include the protein amino acids that are common contaminants, alanine and serine, as well as the non-protein amino acids, β-AIB and β-ABA, which are not used in protein synthesis and are thus less likely to be biological contaminants. Enantiomeric measurements for these chiral species are detailed in Table 3.

In both UNH Ryugu A0106 and UNH Ryugu C0107, alanine was observed to be racemic within error. The D/L ratios of alanine in UNH Ryugu A0106 and C0107 were both 1.09 ± 0.15. Additionally, measurements of the enantiomers of the non-protein amino acid, β-AIB, revealed that this species was racemic in both UNH Ryugu samples, with D/L ratios of 0.95 ± 0.08 for A0106 and 0.98 ± 0.12 for C0107. For both HYD Ryugu samples, β-AIB was slightly enriched in the D-enantiomer for each sample, with L-enantiomeric excess ($\%L_{ee}$) values of −8.42 ± 7.05 for A0106 and −18.2 ± 6.6 for C0107 (Table 3).

3.2. Aliphatic amines

The background-subtracted abundances of aliphatic amines in the water and acid extracts of Ryugu are reported in Table 4. From this data, Extract #7-2 was largely devoid of aliphatic amines with only Ryugu sample A0106 containing detectable quantities of ethylamine, although it should be emphasized that the uncertainty estimate for this measurement is larger than the measurement, itself. Therefore, the detection and quantitation of ethylamine in Ryugu sample A0106 in Extract #7-2 should be interpreted with caution. In contrast, four aliphatic amines were detected in both Ryugu samples of Extract #7-1. Aliphatic amine abundances observed in Ryugu sample A0106 ranged from 0.05 to 23.79 nmol g^{-1}, while those observed in Ryugu sample C0107 ranged from 0.08 to 34.14 nmol g^{-1}, and each individual analyte was present at comparable abundances in each sample. For each sample, the most abundant species was the simplest aliphatic amine, methylamine, and the least abundant species was propylamine. Larger (C_4–C_6) aliphatic amines were not present above the limits of detection.

Demonstration of aliphatic amine detection in samples from Extract #7-1 can be seen in Fig. 2. There, accurate mass chromatograms for methylamine, ethylamine, and propylamine and isopropylamine are displayed for a mixed aliphatic amine standard, the serpentine procedural blank and Ryugu samples A0106 and C0107. Low abundances of methylamine and ethylamine were visible in the serpentine procedural blank, but propylamine and isopropylamine were not detected in the procedural blank. Also observable during the analysis of aliphatic amines were peaks that represented derivatization side products. Despite detecting these untargeted analytes, the derivatization side products did not

Table 1
Averaged, blank-corrected abundances (nmol g^{-1}) of UNH and HYD C_2 – C_6 amino acids in the hot water extracts of the A0106 and C0107 Ryugu samples that were eluted and quantitated using the C_2 – C_6 amino acid method. Reported quantitation of Ryugu amino acids are based on fluorescence data unless otherwise stated below. CI1.1 Orgueil amino acid data are included for comparison purposes.

			A0106 (#7-1)		C0107 (#7-1)		CI1.1 Orgueil[a]	
C#	Amine Position	Amino Acid	UNH	HYD	UNH	HYD	UNH	HYD
2	α	Gly	1.62 ± 0.04	0.46 ± 0.05	15.8 ± 1.8	1.0 ± 0.2	4.0 ± 1.3	11.5 ± 6.0
3	α	D-Ala	0.088 ± 0.005[b]	0.025 ± 0.006[c]	2.2 ± 0.2[b]	0.053 ± 0.005	0.46 ± 0.11	0.90 ± 0.19
3	α	L-Ala	0.08 ± 0.01[b]	< 0.44	2.1 ± 0.2[b]	0.3 ± 0.1	0.67 ± 0.22	1.1 ± 0.25
3	α	D-Ser	0.03 ± 0.02[c,d]	0.06 ± 0.01[c]	0.10 ± 0.03[c]	0.05 ± 0.01[c]	0.03 ± 0.01	<0.01
3	α	L-Ser	0.49 ± 0.01	0.18 ± 0.03	< 0.06	0.6 ± 0.1	0.18 ± 0.06	<0.01
3	β	β-Ala	3.21 ± 0.09	3.3 ± 0.1	3.7 ± 0.4	3.6 ± 0.6	16.4 ± 3.3	30.6 ± 7.6
4	α	D-Asp	0.03 ± 0.01[c]	< 0.06	0.52 ± 0.06	< 0.06	0.22 ± 0.05	0.41 ± 0.23
4	α	L-Asp	0.22 ± 0.01[c]	0.02 ± 0.01	0.11 ± 0.05[d,e]	0.07 ± 0.01	0.67 ± 0.48	0.41 ± 0.21
4	α	D-Thr	< 0.01	< 0.02	< 0.01	< 0.02	n.r.	n.r.
4	α	L-Thr	< 0.01	< 0.04	< 0.01	< 0.04	n.r.	n.r.
4	α	D,L-α-ABA	0.173 ± 0.004	< 0.02	0.23 ± 0.01	< 0.02	0.36 ± 0.30	0.69 ± 0.48
4	α	α-AIB	1.0 ± 0.1	0.38 ± 0.02	0.9 ± 0.1	0.44 ± 0.07	3.2 ± 1.6	3.3 ± 1.4
4	β	D-β-ABA	0.22 ± 0.01[b]	0.32 ± 0.01[b]	0.33 ± 0.03[b]	0.35 ± 0.04	1.7 ± 0.4	2.1 ± 1.1
4	β	L-β-ABA	0.21 ± 0.01[b]	0.32 ± 0.01[b]	0.34 ± 0.03[b]	0.36 ± 0.04	1.8 ± 0.5	1.8 ± 0.6
4	β	D-β-AIB	0.16 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.29 ± 0.02	◇	◇
4	β	L-β-AIB	0.17 ± 0.01	0.17 ± 0.02	0.19 ± 0.02	0.20 ± 0.02	◇	◇
4	γ	γ-ABA	0.46 ± 0.01	3.5 ± 0.2	0.52 ± 0.04	3.9 ± 0.6	1.1 ± 0.5	2.7 ± 1.3
5	α	D-Glu	< 0.01	< 0.03	< 0.01	< 0.03	0.10 ± 0.07	0.32 ± 0.11
5	α	L-Glu	0.033 ± 0.001[c,d]	< 0.03	< 0.02	< 0.03	0.02 ± 0.01	0.56 ± 0.15
5	Multiple	C_5 amino acids (From Table 2)	0.132 ± 0.007	1.3 ± 0.1	0.057 ± 0.002	1.5 ± 0.1	1.66 ± 0.05	13.8 ± 0.4
6	α	D-Leu	< 0.03	< 0.05	< 0.03	< 0.05	n.r.	n.r.
6	α	L-Leu	< 0.2	< 0.06	< 0.2	< 0.06	n.r.	n.r.
6	α	D-Ile	< 0.02	< 0.04	< 0.02	< 0.04	n.r.	n.r.
6	α	L-Ile	< 0.1	< 0.04	< 0.1	< 0.04	n.r.	n.r.
6	ε	ε-ACA	< 0.04	4.5 ± 2.6[b]	< 0.04	< 49.9	0.09 ± 0.01	0.82 ± 0.79
2- 6	Multiple	Sum	8.3 ± 0.1	14.7 ± 2.6	27.3 ± 1.9	12.7 ± 0.9	36 ± 4	71 ± 10

[a]Data taken from (Burton et al., 2014).
[b]Analyte quantitation was performed via HRMS due to an optically fluorescent analyte that interfered with target analyte analysis by FD, but was resolved by HRMS.
[c]Analyte was detected and quantitated by one detector, but not both. Therefore, analyte identification is tentative, and the associated abundance estimate serves as an upper limit estimate.
[d]Averaged value was determined based on two replicate measurements as opposed to three.
[e]L-Asp < D-Asp because trace quantities of L-Asp were present in the baked serpentine procedural blank, but D-Asp was not. Consequently, after blank-correction was executed, the L-Asp blank-corrected abundance was less than the D-Asp blank-corrected abundance.
◇ = Analyte was detected, but not quantitated due to chromatographic interference with other target analytes.
n.r. = not reported.
Note: uncertainties (δ_x) reported for Ryugu samples were calculated as the standard error ($\delta_x = \sigma_x \times (n)^{-1/2}$) based on the standard deviation (σ_x) of the averaged value of triplicate ($n = 3$) measurements, unless the averaged value was otherwise stated above to be based on two replicate measurements.

interfere with the detection and quantitation of the targeted aliphatic amines. Detection metrics for all aliphatic amines detected in the two Ryugu samples are displayed in Tables S13 and S14. It should be noted that the observed mass error for isopropylamine in Ryugu sample A0106 is just outside the 10-ppm mass threshold for the ToF-MS system used to analyze aliphatic amines in this work. As a result, the identification of isopropylamine in Ryugu sample A0106 is tentative, and its associated quantitative data in Table 4 should be interpreted as an upper limit estimate.

4. Discussion

4.1. Amino acids

4.1.1. Glycine and alanine anomalies

Regarding the greater abundances of glycine and alanine in UNH C0107 compared to UNH A0106, as noted in §3.1., above, there are potential explanations that are worthy of further investigation. One possibility is that the samples were exposed to different levels of contamination. However, the similarity in species observed between A0106 and C0107, albeit at somewhat different concentrations, suggests that an amino acid source unique to C0107, such as contamination, is unlikely. Additionally, considering that Ryugu sample C0107 possibly included subsurface material that may have experienced less exposure to space weathering, it is plausible that the elevated abundances of amino acids from UNH C0107 shown in Table 1 (sum = 27.3 nmol g^{-1}) compared to UNH A0106 (sum = 8.3 nmol g^{-1}) reflect a higher degree of amino acid degradation in A0106, possibly due to more surface exposure to space weathering. In this context, examples of space weathering that could have been experienced on Ryugu's parent body include solar wind implantation and sputtering, solar and galactic cosmic radiation, and micrometeorite deposition (Pieters and Noble, 2016). However, such discrepancies in total amino acid abundances between A0106 and C0107 are not commonly observed in the HYD sample extracts (Table 1). To illustrate, the amino acid abundances and distribution are very similar between HYD A0106 and HYD C0107, indicating that space weathering likely was not a dominant process affecting amino acid compositions observed here.

Table 2
Averaged, blank-corrected abundances (nmol g^{-1}) of UNH and HYD C_5 amino acids in the hot water extracts of the A0106 and C0107 Ryugu samples. Reported quantitation of Ryugu amino acids are based on fluorescence data unless otherwise stated below. CI1.1 Orgueil amino acid data are included for comparison purposes.

		A0106 (#7-1)		C0107 (#7-1)		CI1.1 Orgueil[a]	
Amine Position	**Amino Acid**	**UNH**	**HYD**	**UNH**	**HYD**	**UNH**	**HYD**
α	D-Val	< 0.02	< 0.07	< 0.02	< 0.07	<0.01	0.19 ± 0.05
α	L-Val	< 0.2	< 0.06	< 0.2	< 0.06	0.04 ± 0.01	0.48 ± 0.02
α	D-Iva	< 0.01	< 0.05	< 0.01	< 0.05	0.10 ± 0.01	0.31 ± 0.03
α	L-Iva	< 0.04	< 0.05	< 0.04	< 0.05	0.10 ± 0.01	0.42 ± 0.02
α	D-Nva	< 0.01	< 0.04[b]	< 0.01	< 0.04[b]	0.02 ± 0.01	0.11 ± 0.01
α	L-Nva	< 0.01	< 0.04[b]	< 0.01	< 0.04[b]		0.12 ± 0.01
β	R-3-APA	< 0.03	< 0.06[b]	< 0.03	< 0.06[b]	0.18 ± 0.01	1.6 ± 0.1
β	S-3-APA	< 0.04	< 0.08[b]	< 0.04	< 0.08[b]	0.18 ± 0.01	
β	D,L + *allo*-3-A-2-MBA	◇	◇	◇	◇	0.22 ± 0.01	0.55 ± 0.03
β	3-A-3-MBA	◇	◇	◇	◇	<0.35	<0.26
β	3-A-2,2-DMPA	0.057 ± 0.005[b]	0.055 ± 0.002[b]	0.057 ± 0.002[b]	0.051 ± 0.004[b]	0.13 ± 0.01	0.59 ± 0.03
β	R,S-3-A-2-EPA	◇[c]	◇[c]	◇[c]	◇[c]	0.04 ± 0.01	1.5 ± 0.1
γ	D,L-4-APA	◇	◇	◇	◇	0.32 ± 0.03	2.4 ± 0.2
γ	4-A-2-MBA	< 0.1	< 0.17[b]	< 0.1	< 0.17[b]	0.06 ± 0.01	1.5 ± 0.1
γ	4-A-3-MBA	◇	◇	◇	◇	0.17 ± 0.01	2.8 ± 0.1
δ	5-APA	0.075 ± 0.005[b,d]	1.2 ± 0.1[b]	tr.[b]	1.4 ± 0.1[b]	0.10 ± 0.01	1.2 ± 0.2

[a]Data taken from (Burton et al., 2014) and (Glavin and Dworkin, 2009).
[b]Analyte quantitation was performed via HRMS due to an optically fluorescent analyte that interfered with the target analyte, but was resolved by HRMS.
[c]Only the R-stereoisomer was searched for because a stereoisomerically pure standard for the S-stereoisomer was not available.
[d]Averaged value was determined based on two replicate measurements as opposed to three.
◇ = Analyte was tentatively detected, but not quantitated due to chromatographic interference with other target analytes.
tr. = trace abundance.
Note: uncertainties (δ_x) reported for Ryugu samples were calculated as the standard error ($\delta_x = \sigma_x \times (n)^{-1/2}$) based on the standard deviation (σ_x) of the averaged value of triplicate ($n = 3$) measurements, unless the averaged value was otherwise stated above to be based on two replicate measurements.

Glycine and alanine abundances were also observed to be curious for another reason, namely their abundances were notably lower ($\geq$ 3x) in the HYD Ryugu extracts than the UNH Ryugu extracts. The purpose of the acid hydrolysis protocol implemented in the current work is to liberate amino acids that are not detectable in the UNH water extracts as free species. To illustrate, the acid hydrolysis protocol releases amino acids that may be contained within a larger chemical structure and also converts amino acid precursors (e.g., aldehydes and ketones) into their respective amino acids. Such liberated amino acids are referred to as "bound" amino acids. Due to the acid hydrolysis conditions implemented, which were previously determined to be favorable for the recovery of amino acids (Glavin et al., 1999; Simkus et al., 2019), this liberation of bound amino acids typically occurs with minimal concurrent amino acid loss. Therefore, both the free and bound amino acids are detectable in the HYD samples, whereas just the free amino acids are detectable in the UNH samples. Consequently, it is common for amino acid abundances in the HYD water extracts of meteorites to be similar to, or greater than, those measured in the corresponding UNH water extracts.

However, in the case of Ryugu, the two simplest amino acids, glycine and alanine, both experienced a significant reduction in abundances following the acid hydrolysis protocol. Specifically, after acid vapor hydrolysis, the glycine abundances in the hot water extracts dropped by factors of 3.5 and 15.8 in Ryugu samples A0106 and C0107, respectively. Likewise, alanine abundances dropped by factors of 6.7 and 12.2 in Ryugu samples A0106 and C0107, respectively, following acid hydrolysis. While it is unexpected for HYD samples to contain significantly lower abundances of some amino acids than what is observed for the UNH samples, similar observations of lower amino acid abundances in HYD water extracts have been reported before in extraterrestrial samples. For example, glycine depleted by a factor of 3 and alanine depleted by a factor of 7.6 in the primitive achondritic ureilite Elephant Moraine (EET) 83309 (Burton et al., 2012a). Furthermore, an apparent degradation of amino acids following the acid hydrolysis of iron and stony-iron meteorites, possibly due to the presence of abundant metals or cations in the water extracts, has previously been reported (Elsila et al., 2021).

If the presence of metals plays a role in amino acid degradation following acid hydrolysis, it is worth noting that both EET 83309 and Ryugu possess significant iron. For example, EET 83309 contains abundant iron silicides (Ross et al., 2019), and while one analysis of a ~95 mg allotment of Ryugu did not reveal the detection of individual metal grains, it did demonstrate that Ryugu is composed of iron-rich, coarse-grained minerals, such as pentlandite, magnetite, and cubanite (Yokoyama et al., 2022). If the presence of iron contributes to amino acid destruction during acid vapor hydrolysis, the iron in the Ryugu samples analyzed here may be a possible explanation for the amino acid destruction observed in the current research. Regarding the presence of cations interfering with amino acid detection, it is noteworthy that derivatization of amino acids by OPA/NAC can sometimes be complicated by the presence of salts (Glavin and Bada, 1998). However, given the small masses of Ryugu samples analyzed here, it is unlikely that significant cations were present to interfere with amino acid detection in the HYD samples. More details on how this determination was made can be found in §3.1.1. of the Supplementary Material. No additional steps were taken in the Ryugu extraction protocol to remove metals or cations from the water extracts prior to acid hydrolysis, OPA/NAC derivatization, and amino acid analysis. While the temperature implemented during acid hydrolysis is a possible explanation for the observed amino acid destruction, it has not been confirmed why some amino acids might appear to be more susceptible to apparent degradation after acid hydrolysis than other amino acids. As a result of these uncertainties, comparisons of the relative amino acid abundances in the Ryugu HYD extracts with amino acid data from other meteorites should be interpreted with some caution. Although providing a mechanistic understanding of the observed degradation phenomenon lies outside the scope of the current work, the processes responsible for the genesis of the observed amino acid destruction after hydrolysis is worthy of focus in future research.

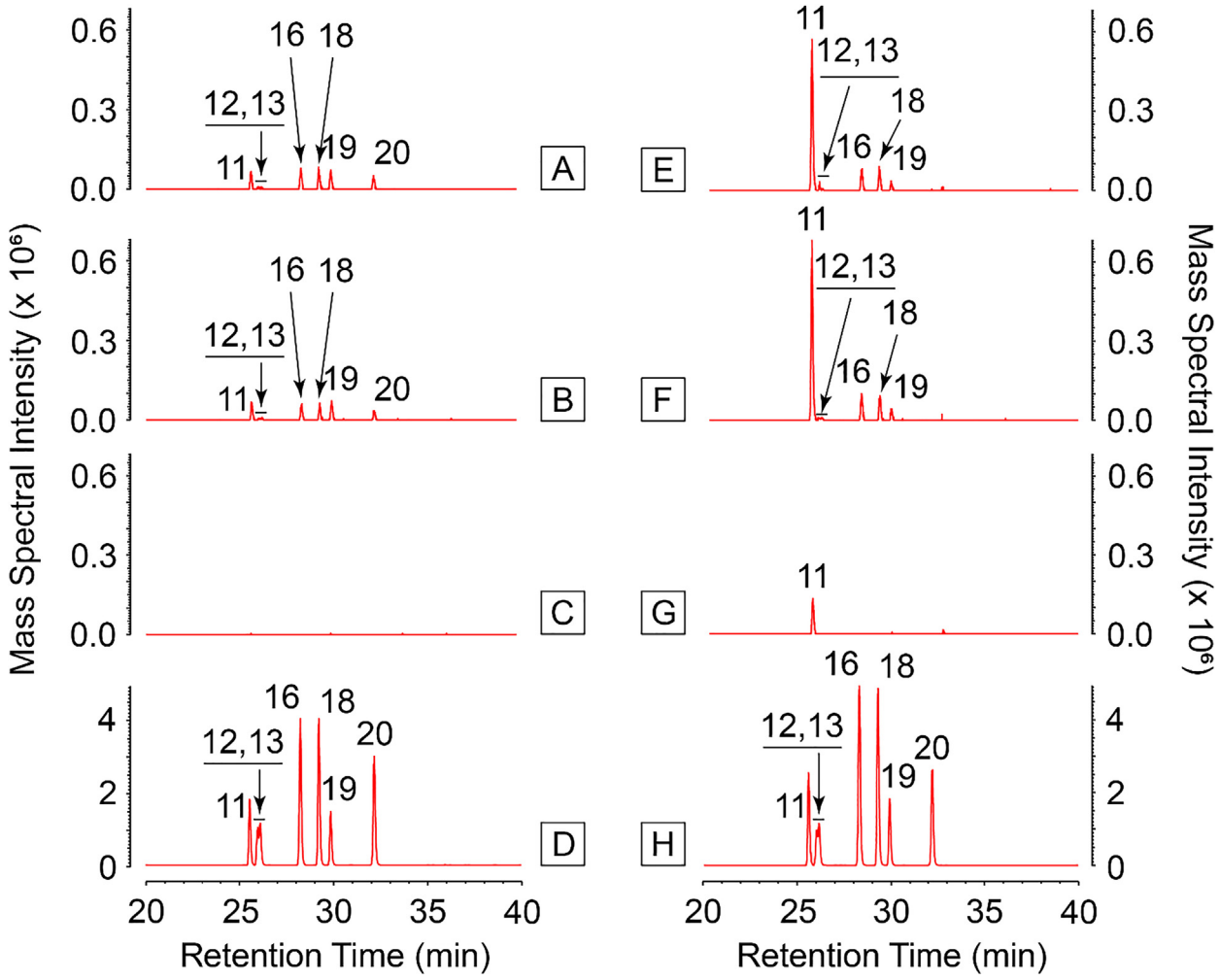

Fig. 1. Several non-protein, C_4 amino acids were detected in the UNH and HYD Ryugu A0106 and C0107 samples. The 20 to 40-minute regions of accurate mass chromatograms for γ-ABA, D,L-β-AIB, D,L-β-ABA, α-AIB, and D,L-α-ABA (*m/z* 365.11657 ± 5 ppm) from A) UNH Ryugu sample C0107, B) UNH Ryugu sample A0106, C) UNH serpentine blank, and D) a mixed amino acid standard. Also shown are the 20 to 40-minute regions of accurate mass chromatograms for γ-ABA, D,L-β-AIB, D,L-β-ABA, α-AIB, and D,L-α-ABA (*m/z* 365.11657 ± 5 ppm) from E) HYD Ryugu sample C0107, F) HYD Ryugu sample A0106, G) HYD serpentine blank, and H) a mixed amino acid standard. The above analyte elution was achieved via the C_2–C_6 amino acid gradient. Analyte identifications shown here are consistent with those listed in Table S1 and are as follows: 11 = γ-ABA, 12 = D-β-AIB, 13 = L-β-AIB, 16 = D-β-ABA, 18 = L-β-ABA, 19 = α-AIB, and 20 = D,L-α-ABA.

4.1.2. Non-protein amino acids

Ryugu samples contained a range of C_3-C_4 non-protein amino acids. The abundances of the C_3 non-protein amino acid, β-alanine, were consistently between 3.2 and 3.7 nmol g^{-1} for both the HYD and UNH hot water extracts of Ryugu A0106 and C0107 samples (Table 1). The C_4 non-protein amino acids were found to be present at similar abundances to one another (Fig. 1). For example, the hot water extracts for both UNH A0106 and UNH C0107 contained γ-amino-*n*-butyric acid (γ-ABA), D,L-β-aminoisobutyric acid (D,L-β-AIB), D,L-β-ABA, α-AIB, and D,L-α-ABA at low abundances that did not exceed 1 nmol g^{-1}. The corresponding HYD samples contained all the same amino acids, except D,L-α-ABA, perhaps suggesting that the acid vapor hydrolysis protocol led to more α-ABA decomposition in the samples. Aside from D,L-α-ABA, the other C_4 non-protein amino acid that was present at notably different abundances between the UNH and HYD samples, was γ-ABA, which was approximately 7.5x more abundant in each HYD sample, compared to the respective UNH samples (Table 1).

Analyses of the C_5 acyclic, aliphatic amino acids revealed that very low abundances of these species were observed in the Ryugu samples (Table 2). The C_5 acyclic, aliphatic amino acids searched for were D,L-valine (D,L-Val), D,L-isovaline (D,L-Iva), D,L-norvaline (D,L-Nva), R,S-3-aminopentanoic acid (R,S-3-APA), D,L + *allo*-3-amino-2-methylbutanoic acid (D,L + *allo*-3-A-2-MBA), 3-amino-3-methylbutanoic acid (3-A-3-MBA), 3-amino-2,2-dimethylpropanoic acid (3-A-2,2-DMPA), R-3-amino-2-ethylpropanoic acid (R-3-A-2-EPA), D,L-4-aminopentanoic acid (D,L-4-APA), D,L-4-amino-2-methylbutanoic acid (D,L-4-A-2-MBA), D,L-4-amino-3-methylbutanoic acid (D,L-4-A-3-MBA), and 5-aminopentanoic acid (5-APA). The most noteworthy examples of C_5 amino acid detections included 3-A-2,2-DMPA and 5-APA, with tentative detections of 3-A-2-MBA, 3-A-3-MBA, 3-A-2-EPA, 4-APA, and 4-A-3-MBA (Figure S8). The C_5 amino acids that were both detected and quantitated in Ryugu had abundances that ranged between 0.033 nmol g^{-1} and 1.4 nmol g^{-1}. The abundances of quantitated C_5 amino acids in Ryugu were primarily consistent between UNH and HYD samples, with the chief contrary example being 5-APA. Upon hydrolysis, the quantities of 5-APA present, rose from < 0.08 nmol g^{-1} to 1.2–1.4 nmol g^{-1}, which constitutes an increase in 5-APA abundance by a factor of more than 15.

4.1.3. Chiral amino acid enantiomeric composition

The racemic enantiomeric data for alanine in the two UNH Ryugu samples (Table 3) indicate that the UNH Ryugu samples contained minimal terrestrial L-protein amino acid contamination

Table 3
Summary of enantiomeric compositions of select amino acids observed in the UNH and HYD hot water extracts of the A0106 and C0107 Ryugu samples. Reported enantiomeric data ($_{D/L}$ ratios and $\%_{Lee}$) for Ryugu are based on data taken from Tables 1 and 2.

			A0106 (#7-1)				C0107 (#7-1)				CI1.1 Orgueil[a]			
			UNH		HYD		UNH		HYD		HYD		UNH	
C#	Amine Position	Amino Acid	D/L Ratio	% L_{ee}	D/L Ratio	% L_{ee}	D/L Ratio	% L_{ee}	D/L Ratio	% L_{ee}	D/L Ratio	% L_{ee}	D/L Ratio	% L_{ee}
3	α	Ala	1.09 ± 0.15	−4.5 ± 7.4	□	□	1.09 ± 0.15	−4.3 ± 7.0	0.16 ± 0.05	72.9 ± 5.8	0.7 ± 0.3	18.6 ± 16.8	0.8 ± 0.3	10.0 ± 14.0
3	α	Ser	0.06 ± 0.04	88.4 ± 4.6	0.31 ± 0.09	53.0 ± 8.2	□	□	0.08 ± 0.03	84.9 ± 3.2	0.17 ± 0.08	71.4 ± 8.3	n.i.	n.i.
4	β	β-AIB	0.95 ± 0.08	2.4 ± 4.3	1.18 ± 0.15	−8.42 ± 7.05	0.98 ± 0.12	1.1 ± 5.9	1.44 ± 0.16	−18.2 ± 6.6	◇	◇	◇	◇
4	β	β-ABA	1.03 ± 0.06	−1.4 ± 3.1	1.01 ± 0.05	−0.4 ± 2.2	0.98 ± 0.14	0.8 ± 7.0	0.98 ± 0.16	0.96 ± 8.08	0.9 ± 0.3	2.9 ± 17.7	1.2 ± 0.7	−7.7 ± 33.5

□Only one enantiomer was detected so enantiomeric data could not be reported.
◇ = Analyte was detected, but not quantitated due to chromatographic interference with other target analytes.
n.i. = enantiomers were not detected, but identities were not confirmed because optically pure standards were unavailable.
Note: uncertainty estimates reported for Ryugu samples are based on the averaged values and associated standard errors reported in Tables 1 and 2, and propagated through the appropriate equations, such that $\%_{Lee} = [(1 - {}_{D/L})/(1 + {}_{D/L})] \times 100$.
[a] Data taken from (Burton et al., 2014).

(see §3.1. of the Supplementary Material for more details), as would be expected for material from a controlled sample return mission (Sakamoto et al., 2022; Sawada et al., 2017). However, this contrasts with the alanine data for HYD Ryugu C0107, which contained a large L-enantiomeric excess. Such an L-enantiomeric excess indicates that either the hydrolysis protocol exposed the Ryugu sample to some L-alanine contamination, or that contamination existed prior to hydrolysis, but was not seen during the amino acid analyses of UNH samples because the contamination consisted primarily of bound L-alanine that was released upon hydrolysis.

When examining enantiomeric data for serine, large L-excesses were also observed in the Ryugu extracts (Table 3). Since serine is an amino acid commonly found in fingerprints (Oró and Skewes, 1965), it is possible that L-serine excesses may be due to contamination. However, it is important to note that serine is only present in proteins and bacteria (Reeck and Fisher, 1973), and the rest of the biosphere (Kozlowski, 2022), at average abundances. This observation underscores that serine is not necessarily a strong indicator of terrestrial contamination and that the presence of an excess of L-serine, without an accompanying excess of the L-enantiomer of another protein amino acid (e.g., L-alanine), is difficult to explain by biological contamination, alone. The UNH hot water extract of Ryugu sample A0106 studied here had an L-serine excess with an accompanying racemic mixture of alanine.

It is worth pointing out that large excesses of L-serine have also been observed in other meteorites, such as the C2.1 Tagish Lake meteorite, lithology 11 h, which was maintained in a frozen state after its fall and prior to amino acid extraction and analysis (Glavin et al., 2012). If the L-serine excesses in Ryugu and other extraterrestrial samples are not of biological origin, one potential explanation for this could be that serine can produce a nearly enantiopure solution from mostly racemic starting conditions in a solid–liquid equilibrium environment (Elsila et al., 2009; Klussmann et al., 2006). Therefore, compound specific stable isotopic measurements (Elsila et al., 2009), which typically require relatively large sample masses, are needed for each serine enantiomer to better evaluate the plausibility of extraterrestrial origins of the L-serine excesses observed here. Due to the small masses of samples analyzed in this work, such isotopic measurements were not possible. Developments in compound specific stable isotopic analyses are needed to perform such measurements on small sample masses.

The slightly larger abundances of D-β-AIB than L-β-AIB found in HYD Ryugu samples were also observed in acid hydrolyzed hot water extracts of grains returned by the Hayabusa mission (Parker et al., 2022) and in CM1.6 Murchison (Koga and Naraoka, 2017), with the latter observation being accompanied by substantial uncertainty estimates. As was the case with the β-AIB measurements made in the Hayabusa grain extracts, the β-AIB abundances measured here were small, and interpretations of D-β-AIB excesses in the HYD Ryugu samples should be done cautiously. The β-ABA enantiomers, on the other hand, were racemic within error for all HYD and UNH Ryugu samples analyzed here (Table 3). The racemic, or very nearly racemic, nature of these non-protein amino acids suggests they are likely to have been indigenous to the samples and are of extraterrestrial origin.

4.1.4. Comparison to previous Ryugu analyses

As stated previously, the samples studied in the current work were also analyzed by a fluorescence detector-based method. A comparison of results obtained from the current work and that of the fluorescence detector-based method is described elsewhere (Naraoka et al., 2023). However, the results of the current study and those of the aforementioned Nakamura et al. (2022a) reporting have not yet been compared. Such a comparison is inherently challenging for reasons that will be outlined here.

Table 4
Summary of blank-corrected abundances (nmol g^{-1}) of aliphatic amines in the UNH Ryugu samples from Extracts #7-1 and #7-2. Reported quantitation of Ryugu aliphatic amines are based on accurate mass data. Hydrolyzed CI1.1 Orgueil aliphatic amine data are included for approximate comparison purposes because unhydrolyzed CI1.1 Orgueil amine data have not been reported.

C#	Aliphatic Amine	A0106 (#7-1)	C0107 (#7-1)	A0106 (#7-2)	C0107 (#7-2)	CI1.1 Orgueil[a]
1	Methylamine	23.79 ± 0.64	34.14 ± 1.74	< 0.1	< 0.1	331.5 ± 0.2
2	Ethylamine	11.37 ± 0.34	10.70 ± 1.98	0.46 ± 0.57	< 0.1	27.3 ± 2.4
3	Isopropylamine	0.59 ± 0.03	0.62 ± 0.06	< 0.1	< 0.1	5.1 ± 0.1
3	Propylamine	0.05 ± 0.01	0.08 ± 0.02	< 0.1	< 0.1	4.8 ± 0.04

[a] Data taken from (Aponte et al., 2015). Note: uncertainty estimates were calculated as the standard errors, as stipulated in Tables 1 and 2.

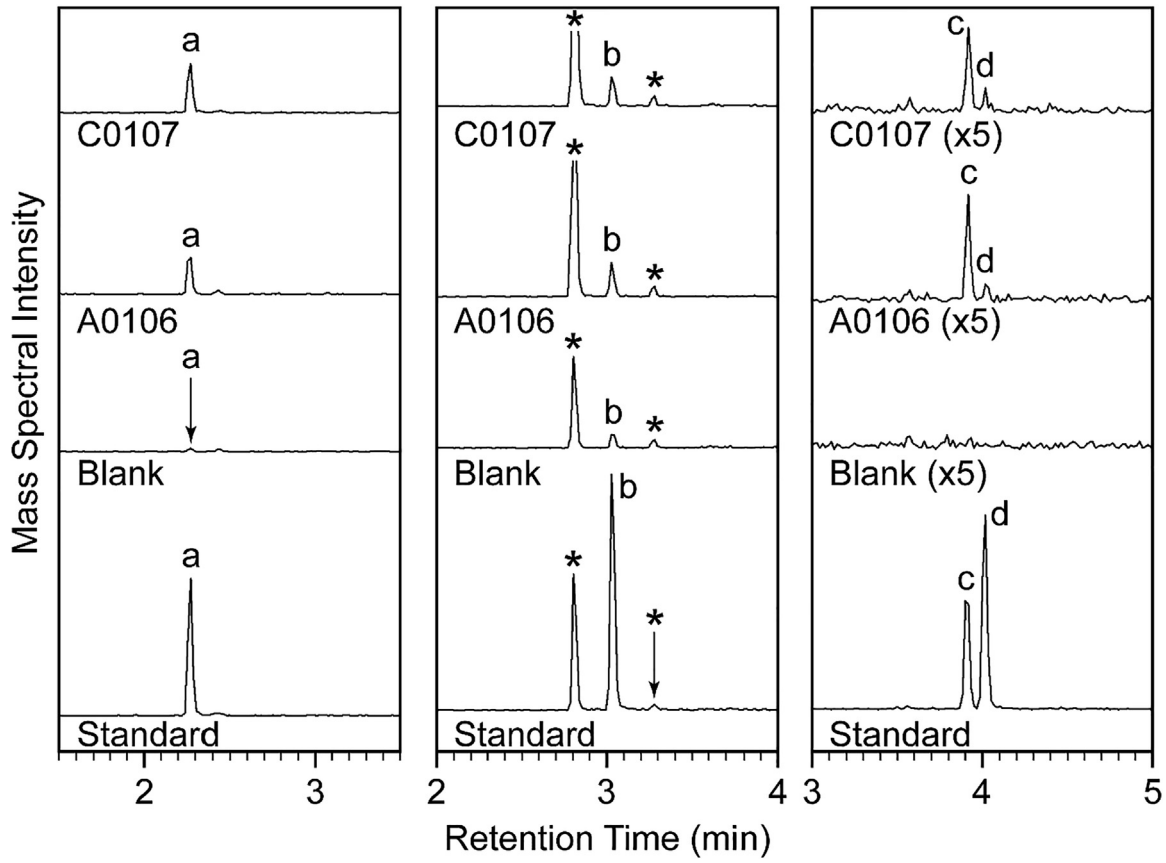

Fig. 2. Aliphatic amines were detected in Ryugu samples A0106 and C0107. Accurate mass chromatograms of the AccQ•Tag derivatized aliphatic amines from the amine standard, serpentine blank, and Ryugu samples A0106, and C0107 are shown here. Compound identifications: left panel, m/z = 202.0908 for methylamine (a), center panel, m/z = 216.1137 for ethylamine (b), and right panel, m/z = 230.1293 for propylamine (c) and isopropylamine (d). Accurate mass chromatograms shown here were plotted using 10-ppm mass windows centered on the theoretical accurate masses of the target analytes. Asterisks represent derivatization side products. Note: all traces are plotted on the same intensity scale except where indicated.

Nakamura et al. (2022a) exposed 1.9 mg of Ryugu sample C0008 to hot water extraction at 110 °C for 20 h followed by derivatization with a 2 M HCl/isopropyl alcohol solution at 110 °C for an unspecified period of time to convert amino acids to their isopropyl ester derivatives. This extraction and derivatization approach is distinctly different than that used in the current work and what has previously been used for amino acid analyses of meteorites and other extraterrestrial samples. Therefore, the choice of extraction and derivatization procedures, alone, is a source of complication for comparative purposes.

Analytical drawbacks also inhibit comparisons. For example, the reporting did not demonstrate that calibration curves, repeat analyses, or chromatographic separations to control ion suppression, were performed (Nakamura et al., 2022a). Additionally, Nakamura et al. (2022a) only represented amino acid detections by raw instrument peak intensities, as opposed to absolute abundances. Lastly, the results of Nakamura et al. (2022a) featured peak identities that were based on a limited set of standards, amino acid chemistry that was not confirmed by a fluorescent label, and amino acid chirality that was not examined, making it impossible to appropriately determine if significant sample contamination occurred.

The combination of Ryugu amino acids being the result of substantially different extraction and workup processes than those used here and the lack of amino acid absolute abundances (Nakamura et al., 2022a), makes it challenging to compare these results to those of any other study. Consequently, the results of Nakamura et al. (Nakamura et al., 2022a) will not be discussed further.

4.1.5. Amino acid synthesis

Comparative abundances of analytes can provide insight into possible formation mechanisms. For example, considering that the quantities of the C_3 non-protein amino acid, β-alanine, in the HYD Ryugu samples are all equivalent to those of the UNH Ryugu samples, within error (Table 1), this finding indicates that β-alanine in Ryugu could not have been in the form of a bound precursor (e.g., lactams) since there were no increases in β-alanine abundances observed after hydrolysis. Instead, β-alanine in the UNH and HYD samples may have been formed by Michael addition of ammonia to cyanoacetylene, or by the addition of ammonia to acrylonitrile followed by hydrolysis (Miller, 1957).

Contrastingly, γ-ABA abundances experienced a sharp increase after acid hydrolysis of the hot water extracts (Table 1), which indicates that both Ryugu samples likely contained abundant γ-ABA precursors, perhaps including γ-lactams (Cooper and Cronin, 1995; Glavin et al., 2020). Alternatively, elevated relative abundances of γ-ABA could be contributed by the amination of hydrothermal formose reactions, which can favor glycine and γ-ABA syntheses (Vinogradoff et al., 2020). However, it should be noted that amino acid abundances after hydrolysis were not reported in the prior study of amination of the formose reaction because the hydrolysis protocol would have inundated the sample with HMT products due to sample workup, which would have complicated downstream amino acid analyses (Vinogradoff et al., 2020). The γ-isomer of ABA can also be produced as a result of α-decarboxylation of glutamic acid (Lie et al., 2018) and could have been formed from glutamic acid decomposition during acid hydrolysis. However, the lack of free glutamic acid in the UNH extracts of both A0106 and C0107 (Table 1) indicates that glutamic acid decarboxylation is not a reasonable explanation for the elevated levels of γ-ABA in the HYD extracts.

Similar to how γ-amino acids may be formed by the hydrolysis of γ-lactam amino acid precursors, δ-amino acid synthesis has also been proposed to occur by the hydrolysis of δ-lactam amino acid precursors (Cooper and Cronin, 1995; Glavin et al., 2020). Further work is needed to confirm the abundance of lactam amino acid precursor species present in Ryugu material, which may elucidate potential formation mechanisms of the corresponding amino acids upon hydrolysis. This additional work is needed because γ- and δ-lactams that may be present in the UNH Ryugu hot water extracts are not detectable by LC-MS using the OPA/NAC derivatization method implemented here because lactams contain a secondary amine, not a primary amine that is detectable using OPA/NAC.

4.1.6. Comparison to amino acids in carbonaceous chondrites

Ryugu has been proposed to be similar to CI chondrites based upon preliminary bulk chemistry and isotopic analyses of Haybausa2 samples (Yokoyama et al., 2022). Direct comparisons of the soluble organic content in Ryugu to that of CI Chondrites, such as CI1.1 Orgueil, can help ascertain if such similarities are concomitantly observed in other facets of the chemistry of Ryugu. As a result, the Ryugu amino acid data reported in Tables 1-3 are accompanied by complementary amino acid data from previous analyses of CI1.1 Orgueil.

In addition to comparing the Ryugu amino acid chemistry to CI1.1 Orgueil, broadening such comparisons to include a larger cohort of previously studied meteorites can be informative to better understand the range of possible chemical environments that likely set the stage for the amino acids observed in Ryugu (Botta et al., 2007; Glavin et al., 2010; Glavin et al., 2006; Martins et al., 2007). One particular subset of amino acids that is particularly useful for this purpose is the C_5 amino acids. This is because the C_5 subset of amino acids is composed of many isomers whose structural compositions can vary widely. Upon comparing the abundances and distribution of these C_5 amino acids with those observed in previously studied carbonaceous chondrites, new insights can be obtained regarding the possible chemical evolution that likely occurred.

An example of such a comparison is shown in Fig. 3, whereby the relative abundances of C_5 amino acids in Ryugu, normalized to the total number of possible structural isomers, are plotted as a function of valeric acid carbon chain backbone. Also included in this comparison are CI1.1 Orgueil, CI1.1 Ivuna, CM1.2 Scott Glacier (SCO) 06043, CR1.3 Grosvenor Mountains (GRO) 95577, C2.1 Tagish Lake 11 h, and CM1.6 Murchison. The 11 h lithology of C2.1 Tagish Lake contained chondrules featuring pyroxene and olivine, likely experienced mild aqueous alteration (Herd et al., 2011), and was comprised of insoluble organic matter with similar characteristics to that of unheated insoluble organic matter from CR2.8 EET 92042, CR1.3 GRO 95577, and CM1.6 Murchison (Alexander et al., 2014). The CM1.6 Murchison data in Fig. 3 includes previously published CM1.6 Murchison data (Glavin et al., 2021) and previously unpublished CM1.6 Murchison data that was collected during the rehearsal analysis phase of the Hayabusa2 mission. The purpose of including previously unpublished CM1.6 Murchison data collected during the Hayabusa2 rehearsal analysis alongside previously published CM1.6 Murchison data is to demonstrate that the analytical protocol applied to investigate Ryugu was similarly applied to investigate a well-studied extraterrestrial sample, and when doing so, yielded similar results to those reported in the literature. Providing such a comparison underscores the efficacy of the analytical protocol implemented here. More details about the analysis of CM1.6 Murchison during the rehearsal analysis phase of the Hayabusa2 mission are included in §1.4. and §3.1.1. of the Supplementary Material.

Based on the data in Fig. 3, we can see that Ryugu contained a markedly different C_5 amino acid composition than select carbonaceous chondrites. The C_5 amino acids in Ryugu were composed of a limited diversity and were overwhelmingly dominated by *n*-valeric acid species, followed by smaller amounts of *tert*-valeric acid C_5 amino acids. This is due to the pronounced abundance of 5-APA in the HYD Ryugu samples, followed by smaller amounts of 3-A-2,2-DMPA (Table 2). Aqueously altered CI1.1 chondrites Orgueil and Ivuna also contained more *n*-valeric acids than *iso*-, *sec*-, or *tert*-valeric acids; however, these CI chondrites both possessed markedly lower relative abundances of *n*-valeric acids than Ryugu. In turn, these CI chondrites possessed notably greater relative abundances of *iso*- and *sec*-valeric acids than Ryugu. The C_5 amino acid composition of Ryugu also contrasted with those of the CM, CR, and C_{ung} chondrites shown in Fig. 3, whereby *n*-valeric acids were not the dominant C_5 amino acid species for each specimen. The small abundances of branched chain C_5 amino acids in Ryugu, relative to straight-chain C_5 amino acids, suggests that ion and radical stability may have been of reduced importance during chain formation (Cronin and Chang, 1993; Glavin et al., 2011; Herbst, 1995) of C_5 amino acids.

The depletions of *sec*- and *iso*-valeric acids in Ryugu (Fig. 3), are correlated with depletions of isovaline and valine, respectively (Table 2), both of which are α-C_5 amino acids. This is in contrast to CI1.1 Orgueil and CI1.1 Ivuna, neither of which are depleted in α-C_5 amino acids (Burton et al., 2014; Glavin and Dworkin, 2009). Therefore, the observed depletion of α-C_5 amino acids in Ryugu was unexpected and is worthy of exploration.

One plausible cause of the observed depletion of α-C_5 amino acids in Ryugu that must be examined, is the possibility that the techniques used here may have imposed an analytical bias that contributed to this observation. To evaluate the likelihood that the analytical methods used were responsible for this observed depletion of α-C_5 amino acids, namely isovaline and valine, the C_5 amino acid chemistry of a more well-studied specimen, CM1.6 Murchison, was examined using the analytical techniques

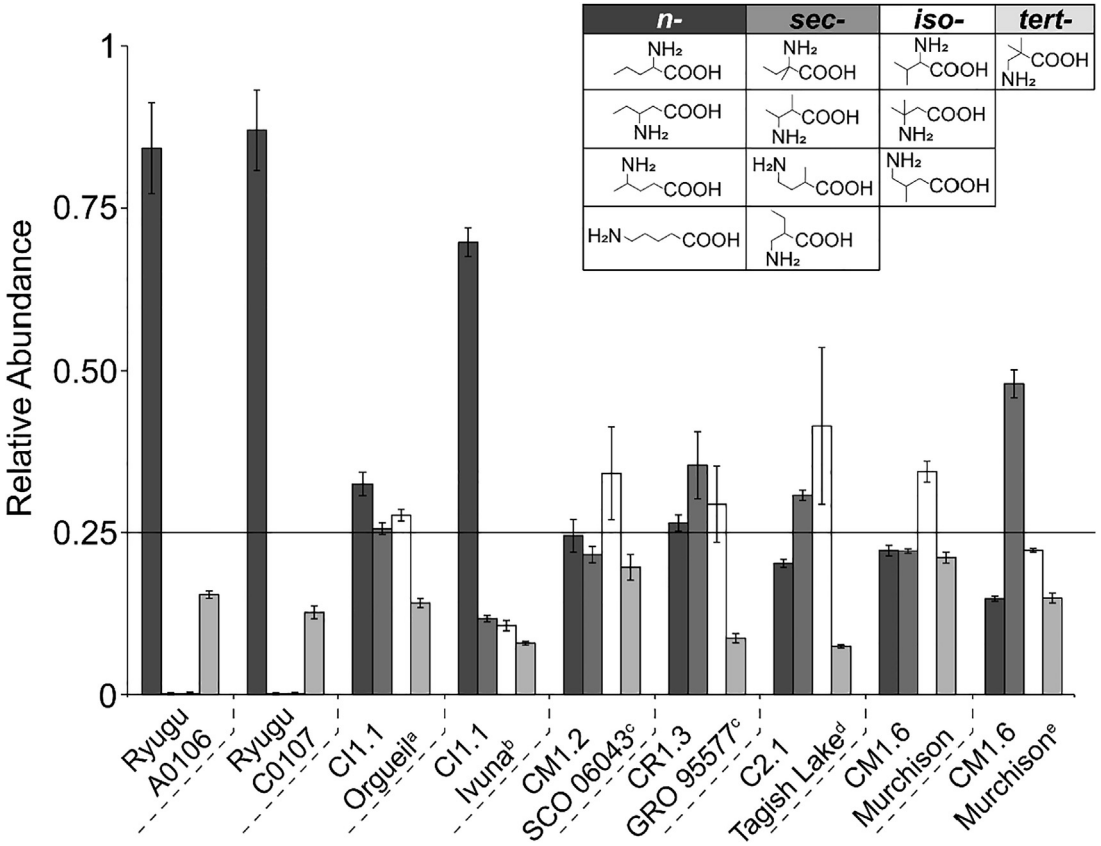

Fig. 3. The relative abundances of C_5 amino acids demonstrate that the Ryugu amino acid composition is clearly distinct from those of meteorites comprising a diversity of petrographic histories. A depiction of C_5 amino acid relative abundances normalized to the number of possible structural isomers, based on valeric acid chain, is displayed here. All data shown here is from acid hydrolyzed hot water extracts. Analytes which were listed as detected but not quantified in Table 2 were taken as 0.001 ± 0.001 nmol/g for the purposes of this figure. Here, two sets of CM1.6 Murchison data are provided. The CM1.6 Murchison (this study) data were collected during the Hayabusa2 rehearsal analysis (§3.1.1. of the Supplementary Material). The other set of CM1.6 Murchison data are from the 80 mg sample from (Glavin et al., 2021). These two sets of CM1.6 Murchison data are included here to illustrate that the sample preparation approach used to process the Ryugu samples was also used to process a well-studied meteorite specimen and resulted in data that are similar to reported literature values, though with depressed α-amino acid abundances and enhanced δ-amino acid abundances. Please see §1.4. of the Supplementary Material for more details of how CM1.6 Murchison was processed and analyzed in this work. Note, in this figure the "sec-" and "*tert-*" structures are both classified as such with respect to their carboxylic acid moieties. [a](Glavin and Dworkin, 2009), [b](Burton et al., 2014), [c](Glavin et al., 2021), [d]Sample 11 h data from (Glavin et al., 2012), [e]80mg sample data from (Glavin et al., 2021).

employed here and compared to previously published results. To execute this comparison, the hot water extract of 3.24 mg of CM1.6 Murchison was analyzed during the Hayabusa2 rehearsal analysis, and the results were compared to a previous reporting of the analysis of a larger (80 mg) mass of the same CM1.6 Murchison sample (Glavin et al., 2021), as seen in Fig. 3.

In this comparison, the distribution and relative abundances of C_5 amino acids from the Hayabusa2 rehearsal analysis of CM1.6 Murchison were generally similar to those of the previously published analysis of an 80 mg CM1.6 Murchison sample, apart from *sec*-valeric acid C_5 amino acids. The difference in *sec*-valeric acid C_5 amino acid relative abundances was chiefly due to the difference in isovaline abundances observed between the two analyses. Approximately 3x more isovaline was observed in the larger CM1.6 Murchison sample than the smaller CM1.6 Murchison sample (Table S12). However, such a difference in observed total abundances of isovaline could reflect heterogeneity effects at play when analyzing two varyingly sized portions of the same sample. Given the stated difference in sizes of the two Ryugu samples studied here, heterogeneity could also play a role in the current Ryugu analyses. Additionally, the Hayabusa2 rehearsal analysis of CM1.6 Murchison revealed slightly larger abundances of other α-C_5 amino acids (i.e., valine and norvaline) compared to the larger CM1.6 Murchison sample analyzed (Table S12). If the analytical techniques used here imposed an analytical bias that resulted in the observation of lower quantities of α-C_5 amino acids, such an outcome would be expected for all α-C_5 amino acids, but this phenomenon was not observed. Therefore, it is unlikely that the methods used here imposed an analytical bias upon α-C_5 amino acids. Instead, it is more likely that the Strecker-cyanohydrin synthesis (Peltzer and Bada, 1978) was minimally involved in the origin of valeric acid species, resulting in very low abundances of α-C_5 amino acids being a feature of the Ryugu samples analyzed here.

4.1.6.1. Ryugu parent body environmental implications based on amino acid observations. As a supplement to learning about the Ryugu parent body chemical environment, it is also possible to make inferences about the Ryugu parent body physical environment by comparing Ryugu amino acid data to those of previously studied carbonaceous chondrites. For example, it has been postulated that the relative abundance of β-alanine can be indicative of a parent body history that features aqueous alteration (Glavin et al., 2011). To this end, Fig. 4 demonstrates the relative abundances of select amino acids, including β-alanine, observed in the hot water extracts of Ryugu and a suite of carbonaceous chondrites. Also included in Fig. 4 are the total amino acid abundances quantitated for each specimen.

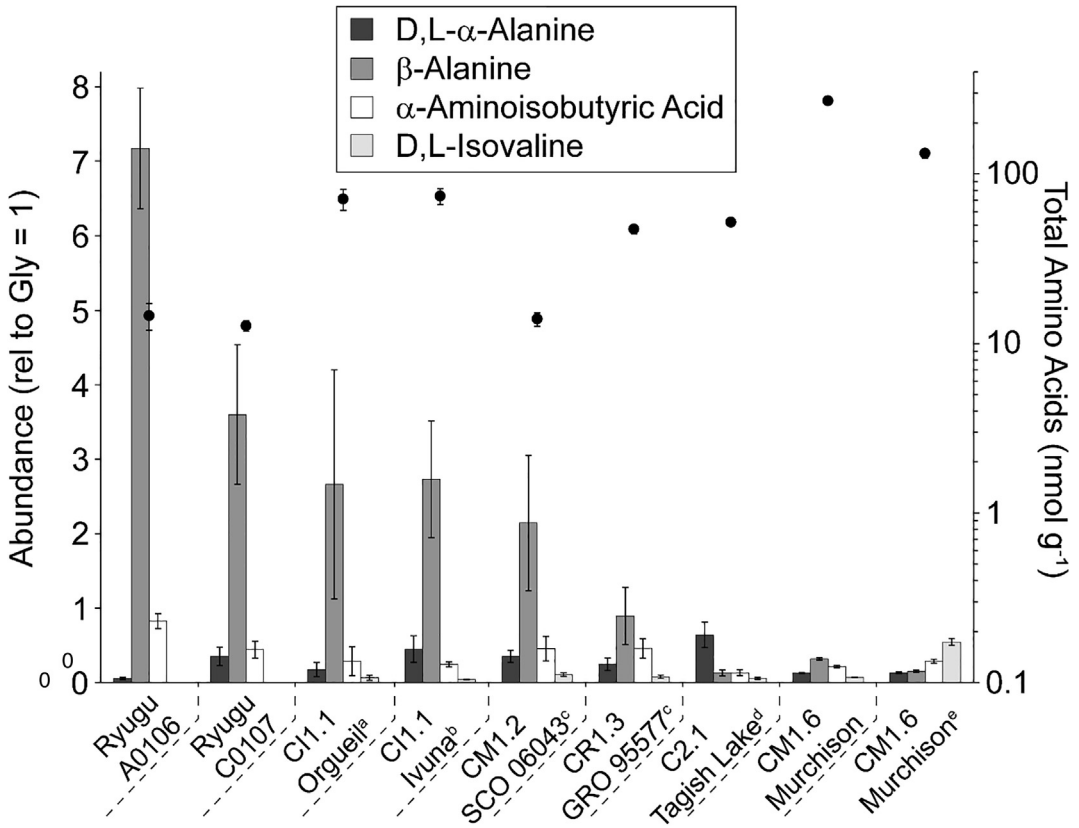

Fig. 4. The relative abundances of non-protein amino acids in Ryugu are not consistent with examples of other meteorites within the terrestrial collection. Featured here is a plot of the abundances (relative to glycine = 1) of non-protein amino acids and the protein amino acid alanine, for comparative purposes, observed for asteroid Ryugu, as well as numerous meteorites. Also shown on the right axis is a plot of the total amino acid abundances for each specimen considered in the figure to compare across samples. All data shown here are from acid hydrolyzed hot water extracts. Note, all meteorite petrographic and sub-petrologic types used throughout this article were taken from (Alexander et al., 2013).[a](Glavin and Dworkin, 2009), [b](Burton et al., 2014), [c](Glavin et al., 2011), [d]Sample 11 h data from (Glavin et al., 2012), [e]80mg sample data from (Glavin et al., 2021).

As can be seen in Fig. 4, the relative abundances of β-alanine in Ryugu samples A0106 and C0107 are more similar to, and even exceed, those of low temperature, aqueously altered CI1.1 chondrites Ivuna and Orgueil, compared to CR1.3 GRO 95577 and CM1.6 Murchison, suggesting the Ryugu parent body may have experienced aqueous alteration (Glavin et al., 2011). However, it should be noted that while the β-alanine relative abundances of Ryugu are similar to CI chondrites, the total amino acid abundances of Ryugu are markedly lower (~5x) than those of CI1.1 Orgueil and CI1.1 Ivuna. Similar amino acid relative abundance profiles paired with contrasting total amino acid abundance characteristics compared to Orgueil and Ivuna indicate that the amino acid chemistry of Ryugu may have shared select parent body history commonalities with aqueously altered CI chondrites, while possibly fostering different starting chemical compositions and formation mechanisms.

In addition to β-alanine, large relative abundances of other straight-chain *n*-ω-amino acids were also observed. The *n*-ω-amino acids of interest in this study were glycine (C_2), β-alanine (C_3), γ-ABA (C_4), 5-APA (C_5), and ε-ACA (C_6). The summed abundances of these species were 5.4 ± 0.1 nmol g^{-1}, 13.0 ± 2.6 nmol g^{-1}, 20.0 ± 1.8 nmol g^{-1}, and 9.9 ± 0.9 nmol g^{-1}, for UNH A0106, HYD A0106, UNH C0107, and HYD C0107, respectively. The summed abundances of these *n*-ω-amino acids represented approximately 65%, 88%, 73%, and 78% of the total amino acid abundances observed for UNH A0106, HYD A0106, UNH C0107, and HYD C0107, respectively. The comparatively large abundances of *n*-ω-amino acids in Ryugu are reminiscent of those observed for thermally altered specimens (Burton et al., 2012a), which would indicate a deviation from the low temperature, aqueous alteration history alluded to by the relative abundances of β-alanine, alone, shown in Fig. 4. Based on individual subsets within the amino acid data of Ryugu that suggest opposing parent body history characteristics, it can be concluded that further amino acid analyses of larger quantities of Ryugu material are needed to evaluate the environmental history of the Ryugu parent body more comprehensively. In particular, compound-specific stable isotopic measurements (Elsila et al., 2009) of Ryugu amino acids are vital to make rigorous determinations of the origins of these biomolecules.

4.2. Aliphatic amines

Aliphatic amines were analyzed for only in the UNH samples in an effort to maximize the amount of HYD sample available for amino acid analysis. Since aliphatic amines and amino acids were searched for using different techniques, analyzing the HYD samples for aliphatic amines, in addition to amino acids, would have required that the HYD sample be split up for two different analyses, limiting the amount of sample available for each analysis, and thus rendering the possibility that neither aliphatic amines nor amino acids may have been concentrated enough in their respective aliquots to exceed analytical detection limits. Therefore, we elected to dedicate the HYD samples solely for amino acid analyses to increase the likelihood of detecting these critical analytes.

4.2.1. Aliphatic amine synthesis

When attempting to evaluate possible formation pathways of the aliphatic amines observed for Ryugu, it is important to consider

the potential ramifications of the molecular relationship between amino acids and aliphatic amines. For example, exposure of amino acids to high temperatures can decarboxylate amino acids, yielding amines (Cox and Seward, 2007; Li and Brill, 2003). To this end, there are molecular similarities observed between the amino acids and aliphatic amines in the Ryugu samples investigated in this work. Ryugu amino acid abundances are largely comprised of straight-chain *n*-ω-amino acids (e.g., glycine, β-alanine, and γ-ABA). Similarly, Ryugu aliphatic amine abundances are dominated by the straight-chain species, methylamine, ethylamine, and propylamine. It is plausible that in these Ryugu samples, methylamine may have derived from glycine, ethylamine from the α- and β- isomers of alanine, and propylamine from the α-, β-, and γ-isomers of ABA (Aponte et al., 2017). However, in order to confirm the genesis of the aliphatic amine species observed in Ryugu, isotopic analyses are required to discern whether the aliphatic amines were indigenous to the Ryugu parent body, or if the aliphatic amines may have been generated by sample work-up procedures inducing amino acid decarboxylation. Therefore, further analyses of Ryugu aliphatic amines, coupled with isotopic analyses, are required to better constrain the origin of the aliphatic amines observed here.

4.2.2. Comparison to aliphatic amines in carbonaceous chondrites

The sums of aliphatic amines for Ryugu samples A0106 and C0107 were 35.8 nmol g^{-1} and 45.5 nmol g^{-1}, respectively (Table 4), which are similar to that (44 nmol g^{-1}) of CR1.3 GRO 95577 (Pizzarello et al., 2012), a highly aqueously altered CR meteorite depleted in aliphatic amines, except for methylamine. When compared to the heavily aqueously altered CI1.1 Orgueil, which possessed a total of 395.9 nmol g^{-1} aliphatic amines (Aponte et al., 2015), the Ryugu samples studied here were comprised of roughly one order of magnitude lower aliphatic amine abundances.

Considering that the Ryugu aliphatic amine data collected in the current work were taken only from UNH samples for reasons stated above in §4.2., it should be noted that aliphatic amine data from UNH CI1.1 Orgueil samples have not previously been reported. Therefore, the aliphatic amine data comparison between Ryugu and CI1.1 Orgueil provided here is intended as an approximate comparison. Even so, the relative ratios of aliphatic amines in UNH and HYD extracts are approximately the same for the two meteorites, CM1.6 Murchison (Aponte et al., 2014) and CR2.5 Graves Nunataks (GRA) 95229 (Aponte et al., 2016), where analyses of HYD and UNH samples were performed for both meteorites. Despite the differences in reported total aliphatic amine abundances between Ryugu and CI1.1 Orgueil, both specimens demonstrate a descending trend of methylamine > ethylamine > isopropylamine > propylamine, suggesting that a comparison between the two data sets remains relevant.

In comparison to the mildly aqueously altered chondrite CM1.6 Murchison (105.8 nmol g^{-1}, (Aponte et al., 2014)), Ryugu samples A0106 and C0107 possessed roughly 2–3x lower aliphatic amine abundances. The minimally altered CR meteorites, CR2.5 GRA 95229 (Aponte et al., 2016), and CR2.8 EET 92042 and CR2.4 Queen Alexandra Range (QUE) 99177 (Pizzarello et al., 2012), each contained aliphatic amine total abundances of 769 nmol g^{-1}, 120 nmol g^{-1}, and 339 nmol g^{-1}, respectively. The abundances of aliphatic amines in these minimally altered CR chondrites represent roughly 3–21-fold greater aliphatic amine abundances compared to the Ryugu samples analyzed in the current work.

The total aliphatic amine abundances observed for the Ryugu samples analyzed in the current work demonstrated consistencies with the mildly aqueously altered CM1.6 Murchison and select heavily and minimally altered carbonaceous chondrites, providing a challenge when identifying a specific correlation with degree of aqueous alteration. However, the distribution of aliphatic amines is far simpler in Ryugu than it is for the aforementioned aqueously altered specimens. To illustrate, each of the named aqueously altered chondrites was comprised of a complex distribution of > 10 different aliphatic amine species, except for CR1.3 GRO 95577 (Aponte et al., 2014; Aponte et al., 2015; Aponte et al., 2016; Pizzarello et al., 2012).

5. Conclusions

In this work, hot water and HCl extracts of samples collected from two different locations on asteroid Ryugu were analyzed by liquid chromatography with fluorescence detection and high-resolution mass spectrometry for amino acids and aliphatic amines. The samples were split into HYD and UNH fractions prior to amino acid analysis to determine both total and free amino acid abundances. The findings of this study revealed that the asteroid Ryugu material analyzed here contained a multiplicity of amino acids and aliphatic amines at low abundances. Total amino acid abundances for an individual sample were < 30 nmol g^{-1}, while total aliphatic amine abundances were < 50 nmol g^{-1} per sample. Despite these low abundances, some very interesting amino acid results were observed, including racemic alanine observed in both UNH Ryugu samples A0106 and C0107, indicating that these samples experienced minimal terrestrial contamination prior to hydrolysis. Also observed were the C_4 non-protein amino acids, β-AIB and β-ABA, present as racemic, or very nearly racemic, mixtures in both the HYD and UNH Ryugu samples A0106 and C0107, indicating that these species were indigenous to the asteroid. Elevated abundances of γ- and δ- non-protein amino acids (e.g., γ-ABA and 5-APA) in the HYD samples compared to the UNH samples suggest that the Ryugu samples may have contained relatively abundant amino acid precursors, such as lactams, that could have been liberated upon hydrolysis (Cooper and Cronin, 1995; Glavin et al., 2020). Further investigations are needed to accurately evaluate the abundance of lactams in Ryugu material. Additional investigations are also warranted to examine the compound-specific stable isotopic composition of amino acids observed here using more sensitive techniques than currently available to properly determine the provenance of Ryugu amino acids.

Although asteroid Ryugu is considered to be similar to a low temperature, aqueously altered carbonaceous chondrite, perhaps like the CI1.1 chondrites Orgueil or Ivuna (Yokoyama et al., 2022), the amino acid and aliphatic amine composition of Ryugu is less similar to CI1.1 Orgueil (Tables 1, 2, and 4) and CI1.1 Ivuna (Burton et al., 2014) than one would expect based on inorganic and other chemical analyses of Ryugu (Yokoyama et al., 2022). It should be noted, however, that Ryugu grains are more reduced than CI and CM chondrites based on observed redox chemistry (Nakamura et al., 2022b), which is indicative of less intense hydrothermal activity and may impact relative organic syntheses between Ryugu and carbonaceous chondrites. The Ryugu samples studied here contained substantially elevated relative abundances of β-, γ-, and δ-amino acids compared to aqueously altered meteorites. However, unlike CI1.1 Orgueil or CI1.1 Ivuna, Ryugu amino acids are not necessarily dominated by β-alanine. Instead, the Ryugu samples were comprised of comparatively very large relative abundances of *n*-ω-amino acids, which are more suggestive of a parent body history comprised of thermal alteration (Burton et al., 2012a) or possibly hydrothermal formose reactions (Vinogradoff et al., 2020). Such an observation contrasts with the structural diversity of amino acids produced by the Strecker-cyanohydrin synthesis expected of low temperature aqueously altered meteorites (Burton et al., 2012b). Additional work is needed to better constrain if differences in alteration conditions between Ryugu and

low temperature, aqueously altered carbonaceous chondrites may have contributed to this contrasting observation.

Potential alternative amino acid formation pathways include galactic cosmic radiation (Holtom et al., 2005; Meinert et al., 2011) and ultraviolet photon mechanisms (Bernstein et al., 2002; Munoz Caro et al., 2002). Evaluating the extent to which these mechanisms may have been responsible for the amino acids observed here is beyond the scope of this study; however, these potential pathways warrant future investigation when exploring amino acids in Ryugu. The combination of factors reported here indicates that the amino acid and aliphatic amine chemistry of Cb-type asteroid Ryugu is distinct from that of low temperature aqueously altered meteorites and other solar system small bodies. Lastly, from this work, it can be concluded that the sensitive analytical techniques employed here are well-suited to perform similar analyses of amino acids and aliphatic amines in small quantities of asteroid Bennu material, which are scheduled for Earth return by the NASA OSIRIS-REx mission in September 2023.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

The supplementary material is comprised of a document that provides additional methodological details and results that support the primary results reported here in the main text. Also included are the raw data that were used to generate the fluorescence and accurate mass chromatograms throughout the main text and supplementary material. Supplementary data to this article can be found online at https://doi.org/10.1016/j.gca.2023.02.017.

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Supplementary Material for:

Extraterrestrial Amino Acids and Amines Identified in Asteroid Ryugu Samples Returned by the Hayabusa2 Mission

Eric T. Parker[a,*], Hannah L. McLain[a,b,c], Daniel P. Glavin[a], Jason P. Dworkin[a], Jamie E. Elsila[a], José C. Aponte[a,b,c], Hiroshi Naraoka[d], Yoshinori Takano[e], Shogo Tachibana[f], Hikaru Yabuta[g], Hisayoshi Yurimoto[h], Kanako Sakamoto[i], Toru Yada[i], Masahiro Nishimura[i], Aiko Nakato[i], Akiko Miyazaki[i], Kasumi Yogata[i], Masanao Abe[i], Tatsuaki Okada[i], Tomohiro Usui[i], Makoto Yoshikawa[i], Takanao Saiki[i], Satoshi Tanaka[i], Satoru Nakazawa[i], Yuichi Tsuda[i], Fuyuto Terui[j], Takaaki Noguchi[k], Ryuji Okazaki[d], Sei-ichiro Watanabe[l], Tomoki Nakamura[m]

[a]Solar System Exploration Division, NASA Goddard Space Flight Center, Greenbelt, MD 20771, USA
[b]Center for Research and Exploration in Space Science and Technology (CRESST), NASA Goddard Space Flight Center, Greenbelt, MD 20771, USA
[c]Department of Physics, The Catholic University of America, Washington, D.C. 20064, USA
[d]Department of Earth and Planetary Science, Kyushu University, Nishi-ku, Fukuoka, 819-0395, Japan
[e]Biogeochemistry Research Center (BGC), Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokosuka, Kanagawa, 237-0061, Japan
[f]UTokyo Organization for Planetary and Space Science (UTOPS), The University of Tokyo, 7-3-1 Hongo, Tokyo 113-0033, Japan
[g]Hiroshima University, Higashi-Hiroshima 739-8526, Japan
[h]Hokkaido University, Sapporo 060-0810, Japan
[i]Institute of Space and Astronautical Science (ISAS), Japan Aerospace Exploration Agency (JAXA), Sagamihara 252-5210, Japan
[j]Kanagawa Institute of Technology, Atsugi 243-0292, Japan
[k]Kyoto University, Kyoto 606-8502, Japan
[l]Nagoya University, Nagoya 464-8601, Japan
[m]Tohoku University, Sendai 980-8578, Japan

***Corresponding Author**: Eric T. Parker
Email: eric.t.parker@nasa.gov
Address: NASA Goddard Space Flight Center, 8800 Greenbelt Road, Mail Code 691, Greenbelt, MD 20771, USA
Phone Number: 301-614-5107

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1. MATERIALS AND METHODS

1.1. Chemicals and Reagents used for Ryugu Analyses

Sample handling materials, including glassware, were baked out in air at 500 °C, overnight, to eliminate organic contamination prior to contacting samples. Hot water extraction (Extract #7-1) was performed using ultrapure water (TAMAPURE-AA) purchased from Tama Chemicals Co., Ltd. Hydrochloric acid extraction (Extract #7-2) was performed using ultrapure HCl (TAMAPURE-AA-10) and ultrapure water (TAMAPURE-AA), both purchased from Tama Chemicals Co., Ltd.

After extraction, all water used for sample preparation purposes was Millipore Integral 10 ultrapure water (18.2 MΩ-cm, ≤3 ppb total organic carbon), except for the preparation of liquid chromatography eluents, which utilized LC-MS grade water. A portion of each extract was subjected to acid vapor hydrolysis, which was performed using ultrapure HCl that was doubly distilled (ddHCl, 6 M). For pre-column derivatization of amino acids, 0.1 M sodium borate, *o*-phthaldialdehyde/*N*-acetyl-L-cysteine (OPA/NAC), and 0.1 M hydrazine hydrate were all prepared and used as described elsewhere (Glavin et al., 2011). For pre-column derivatization of aliphatic amines by AccQ•Tag (6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate), the following 4 reagents were used with the first 3 being purchased from Waters Corporation: 1) AccQ•Tag Ultra borate buffer, 2) AccQ•Tag Ultra reagent powder, 3) AccQ•Tag Ultra reagent diluent, and 4) AccQ•Tag derivatization agent. The AccQ•Tag derivatization agent was prepared as described elsewhere (Parker et al., 2020).

For LC-MS analyses of amino acids, different reagents were prepared to enable chromatography of target analytes and to calibrate the mass spectrometer. Liquid chromatography eluents used during the analyses of C_2 – C_6 amino acids were A) 45 mM ammonium formate with 7% methanol, pH adjusted to 9.0 and B) LC-MS grade methanol. Mobile phase A) was prepared by dissolving 2 mL of LC-MS grade formic acid in 1033 mL of LC-MS grade water, before using 1 M aqueous ammonium hydroxide to titrate the solution to pH 9.0, and lastly mixing in 85 mL of LC-MS grade methanol. The 1 M ammonium hydroxide solution was typically made by dilution of a 7.3 M stock solution of aqueous ammonium hydroxide (assay = 28.6%, ammonia in water) with LC-MS grade water until a final concentration of 1 M was reached. The LC eluents used for analyses of C_5 amino acids were C) 45 mM ammonium formate with 7% methanol, pH adjusted to 7.4 and D) LC-MS grade methanol. Mobile phase C) was prepared identically to mobile phase A), except mobile phase C) was titrated to pH 7.4. Mass calibrations were performed on the HRMS system using the Thermo Scientific Pierce LTQ Velos ESI positive ion calibration solution, which was purchased from Fisher Chemical.

For LC-MS analyses of aliphatic amines, two eluents were used: A) AccQ•Tag A buffer and B) AccQ•Tag B buffer. Eluent A was prepared as described elsewhere (Parker et al., 2020), and Eluent B was purchased directly from Waters Corporation. Also used was a 0.5 M aqueous sodium formate solution to perform daily calibrations of the ToF-MS, and a 200 µg L^{-1} leucine enkephalin solution to perform real-time lock mass corrections. These final two solutions were prepared as detailed elsewhere (Glavin et al., 2021).

All reagents (excluding C_5 amino acid standards) used in this study were purchased from Sigma-Aldrich, Mann Research Labs, Acros Organics, Tama Chemicals Co., Ltd., Fisher Chemical, and Waters Corporation. Purchased, individual amino acid crystals (excluding C_5 amino acid standards) used to generate mixed amino acid standards all had purities ≥96.8%, while all other purchased reagents had purities ≥95%, unless stated otherwise. Details about the origins and purities of C_5 amino acid standards used here, are detailed elsewhere (Glavin and Dworkin, 2009). Individual amino acid stock solutions were prepared in ultrapure water at concentrations of 10^{-3} M to 10^{-1} M prior to mixing to generate mixed amino acid standards.

1.2. Amino Acid Derivatization and Analysis of Ryugu Samples

The residues of samples and blanks in the UNH glass vials and the residues of samples and blanks in the HYD derivatization vials, were each resuspended in 20 μL of 0.1 M sodium borate and brought to dryness under vacuum at room temperature. Each derivatization vial was then reconstituted using 20 μL of ultrapure water and derivatized individually with 5 μL of OPA/NAC for 15 minutes at room temperature (≈21 °C). The derivatization reactions were quenched using 75 μL of 0.1 M hydrazine hydrate. Following the completion of the derivatization protocol, each sample and blank was promptly injected for analysis by liquid chromatography with fluorescence detection and high-resolution mass spectrometry (LC-FD/HRMS). After injection was complete, each derivatization vial was immediately stored in a -80 °C freezer to halt the degradation of amino acid derivatives and preserve the integrity of the solution for subsequent injections. Sample and blank derivatization vials were allowed to thaw briefly at room temperature prior to executing replicate injections to collect sufficient data necessary to perform uncertainty estimate calculations for observed analyte abundances.

Amino acid analysis was performed using a Thermo Fisher Scientific Vanquish Horizon LC coupled to a Thermo Fischer Scientific Vanquish fluorescence detector and a Thermo Fisher Scientific Q Exactive hybrid quadrupole-Orbitrap mass spectrometer. Chromatographic retention time, optical fluorescence, and accurate mass measurements were used to confirm sample analyte identifications, upon comparison to a mixed amino acid standard. A 3-ppm mass tolerance was used for accurate mass identification purposes. Once identified, target analytes were quantified by manual integration of peak areas using the Xcalibur software program, followed by background subtraction using data collected from the analysis of the respective serpentine procedural blank, and comparison of background-subtracted sample peak areas to the chromatographic peak area and concentration of the corresponding standard analyte.

Chromatography was performed using a 2.1 x 5 mm, 1.7 μm particle size Waters ACQUITY UPLC BEH C18 VanGuard Pre-column, followed by two stationary phases in series: 1) 2.1 x 150 mm, 1.7 μm particle size Waters ACQUITY UPLC CSH C18 and 2) 2.1 x 150 mm, 1.7 μm particle size Waters ACQUITY UPLC BEH Phenyl. The C_2 - C_6 amino acids were eluted via the following gradient: 0 – 35 min, 0-55% eluent B, 35 – 45 min, 55 – 100% eluent B, 45 – 50 min, isocratic at 100% eluent B, 50 – 50.1 min, 100 – 0% eluent B, 50.1 – 60 min, isocratic at 0% eluent B. The eluent flow rate was 0.15 mL min^{-1} and columns were maintained at 33 °C. The autosampler was held 5 °C and an injection volume of 10 μL was used. The FD was operated at a temperature of 33 °C, while utilizing an emission wavelength of 450 nm and an

excitation wavelength of 340 nm. The LC-FD settings used when analyzing C_5 amino acids were identical to those used when analyzing C_2 – C_6 amino acids, except the chromatography of C_5 amino acids implemented a different aqueous mobile phase as stated in §1.1., above, and an alternative gradient: 0 – 25 min, 15 – 20% eluent D, 25 – 25.06 min, 20 – 35% eluent D, 25.06 – 44.5 min, 35 – 40% eluent D, 44.5 – 45 min, 40 – 100% eluent D, 45 – 50 min, isocratic at 100% eluent D, 50 – 50.1 min, 100 – 15% eluent D, 50.1 – 60 min, isocratic at 15% eluent D.

For all amino acids targeted in this work, the HRMS system used a heated electrospray ionization (HESI) source, with the following operational parameters: sweep gas (N_2) flow rate = 2 a.u., sheath gas (N_2) flow rate = 40 a.u., auxiliary gas (N_2) flow rate = 10 a.u., auxiliary gas heater temperature = 300 °C, spray voltage = 3.50 kV, capillary temperature = 350 °C, and S-lens RF level = 50.0%. For all amino acid analyses, the HRMS system was operated in Full MS – SIM scan mode and implemented the following scan parameters: polarity = positive, mass resolution setting = 70,000 (at full-width-half-maximum for *m/z* 200), scan range = 150 – 2,000 *m/z*, fragmentation = none, automatic gain control target = 1 x 10^6 ions, microscans = 1, and maximum injection time = 50 ms.

The HRMS system was calibrated daily over the 150 – 2,000 *m/z* range using the Thermo Scientific Pierce LTQ Velos ESI positive ion calibration solution, which enabled a typical mass accuracy of < 3 ppm. The calibration solution was comprised of caffeine, MRFA (Met-Arg-Phe-Ala), and Ultramark 1621 in an aqueous solution that contained acetic acid, acetonitrile, and methanol.

1.3. Aliphatic Amine Derivatization and Analysis of Ryugu Samples

Aliphatic amines were targeted for analysis in the UNH extracts of both Extract #7-1 and Extract #7-2. The derivatization agent, AccQ•Tag, was used to provide enhanced specificity for the targeted analysis of aliphatic amines. This derivatization agent is a fluorescent tag that is capable of derivatizing primary amino groups and select secondary amino groups. A detailed description of the derivatization process can be found elsewhere (Boogers et al., 2008), but will be briefly overviewed here. For this work, 10 µL of each UNH extract was separately mixed with 70 µL of AccQ•Tag Ultra borate buffer and 20 µL of the AccQ•Tag derivatization agent before being heated at 55 °C for 10 minutes. Once heating was complete, the derivatization reaction was finished, and standards and samples were ready for analysis. Derivatives were stable at room temperature for 7 days.

Aliphatic amines were analyzed as detailed by (Aponte et al., 2020) but will be summarized here. Aliphatic amine derivatives were analyzed using a Waters ACQUITY H Class ultraperformance liquid chromatograph (UPLC) that was coupled to a Waters ACQUITY UPLC fluorescence detector and a Waters XEVO G2-XS time-of-flight mass spectrometer (LC-FD/ToF-MS). Analyte identification and quantitation were evaluated by comparison of sample and blank analytes to those of known aliphatic amines at known concentrations in a mixed aliphatic amine standard. Calibration curves of the aliphatic amine standard were created by analyzing 5 separate concentrations of the mixed standard. Quantitation was performed by determining analyte peak areas in accurate mass chromatograms for each sample and blank, and

performing a background subtraction from the sample analytes. Sample aliphatic amine abundances are reported as the average values of triplicate injections of the same extract.

1.4. Hayabusa2 Rehearsal Sample Preparation and Analysis Procedures

During the Hayabusa2 rehearsal, 24.37 mg of baked (500 °C for 3 hours) antigorite and a 19.44 mg fragment of pristine CM1.6 Murchison were prepared and analyzed for amino acids. The CM1.6 Murchison sample was provided by the Chicago Field Museum and was stored as detailed elsewhere (Glavin et al., 2021). The rehearsal sample and blank underwent hot water extraction as detailed in Figure S1, except in the rehearsal the initial extraction volume was 300 µL and the extraction conditions were 100 °C for 24 hours. This hot water extraction process resulted in 600 µL of each extract that could be isolated for use in analyses. Of these 600 µL, half of each extract (i.e., 12.19 mg antigorite and 9.72 mg CM1.6 Murchison) was shipped to GSFC as detailed in §2.1. of the main text, for amino acid analysis.

Upon arrival at GSFC, the rehearsal sample and blank extracts were prepared for acid vapor hydrolysis inside an ISO 5 HEPA-filtered positive pressure laminar flow hood, located in an ISO ≤ 8 white room. An analytical blank comprised of 300 µL ultrapure water was prepared at NASA GSFC simultaneously with the rehearsal sample and blank extracts. The rehearsal sample and blank, and the analytical blank, each underwent two successive 300 µL rinses using ultrapure water, bringing the volumes of each to 900 µL. Despite increasing the volumes of the rehearsal blank and sample, it is important to note that the effective sample masses of each were not altered. Of these 900 µL for the rehearsal sample and blank, and the analytical blank, 300 µL of each (i.e., 4.06 mg antigorite and 3.24 mg CM1.6 Murchison) were isolated for amino acid analysis.

Prior to acid vapor hydrolysis, the rehearsal blank and sample, and the analytical blank, were each spiked with 30 µL of 1.5 M HCl to mitigate loss of amines. The sample and blanks were then subjected to acid vapor hydrolysis as described in §2.2. of the main text. The HCl that was used to prepare the rehearsal sample and blanks for hydrolysis and to carry out hydrolysis was ultrapure HCl (TAMAPURE-AA-10), purchased from Tama Chemicals Co., Ltd. Following acid vapor hydrolysis, rehearsal sample and blanks were transferred from their respective test tubes to corresponding glass vials as described in §2.2. of the main text and dried down under vacuum at room temperature to remove residual HCl. Next, the rehearsal sample and blanks were each resuspended in 100 µL of ultrapure water, and 10 µL of each sample and blank was dried down under vacuum at room temperature with 20 L of 0.1 µM sodium borate. At this point, each rehearsal sample and blank were then individually derivatized with OPA/NAC as stated in the second sentence of §1.2, above, and immediately analyzed for amino acids. During the rehearsal period, amino acids were analyzed by LC-FD/ToF-MS and not LC-FD/HRMS. The analytical parameters used for LC-FD/ToF-MS analyses of rehearsal sample and blanks were identical to those detailed in (Glavin et al., 2021), with the following minor differences: 1) the mass resolution at full-width-half-max was 20,000, 2) the detector voltage was 2,450 V, 3) the calibration range was 50 – 1,200 *m/z*, 4) the C_2 – C_4 and C_6 amino acids were eluted using the following gradient 0 min. = 0% organic, 35 min. = 55% organic, 45 min. = 100% organic, 50 min. = 100% organic, 50.1 min. = 0% organic, 60 min. = 0% organic, and 5) the C_5 amino acid

isomers were eluted using the following gradient: 0 min. = 15% organic, 25 min. = 20% organic, 25.06 min. = 35% organic, 44.5 min. = 40% organic, 45 min. = 100% organic, 50 min. = 100% organic, 50.1 min. = 15% organic, 60 min. = 15% organic.

2. RESULTS

2.1. Analytical Performance

In total, 31 analytes were analyzed for using the $C_2 – C_6$ amino acid gradient, including 13 chiral amino acids. The only examples for which chromatographic coelution prevented quantitation, were the D- and L-enantiomers of α-ABA, which could not be individually quantitated due to coelution (Figure S2). While the $C_2 – C_6$ amino acid gradient eluted seven C_5 amino acid analytes, all C_5 amino acid quantitative data reported here were obtained using the C_5 amino acid gradient. The C_5 amino acid gradient targeted 21 C_5 amino acid analytes (Table S2). Of these 21 analytes, six experienced coelution interference: two enantiomers of 3-A-2-MBA and the analytes 4-A-3-MBA, R-3-A-2-EPA, L-γ-AVA, and 3-A-3-MBA (Figure S3). As a result, quantitation for these analytes was not performed, and their identifications were considered tentative. The detection metrics presented in Tables S1 and S2 illustrate the efficacies of the amino acid detections facilitated by the $C_2 – C_6$ and C_5 amino acid gradients implemented in this work, as demonstrated by the consistencies between observed FD and MS retention times, as well as measured mass accuracies of amino acids in mixed amino acid standards.

Fifteen aliphatic amines were targeted for analysis: methylamine, ethylamine, propylamine, isopropylamine, *sec*-butylamine, isobutylamine, *n*-butylamine, *tert*-butylamine, 3-aminopentane, 2-amino-3-methylbutane, *sec*-pentylamine, 2-methylbutylamine, *tert*-pentylamine, isopentylamine, pentylamine, and hexylamine. Despite searching for 15 aliphatic amines, only four were detected (i.e., methylamine, ethylamine, propylamine, and isopropylamine), so only these four $C_1 – C_3$ aliphatic amines will be discussed further. The analytical method employed for aliphatic amines provided confident detection of these species, based on observed optical fluorescence, chromatographic retention time, and accurate mass measurements in a mixed aliphatic amine standard (Figure S4 and Table S3).

3. DISCUSSION

3.1. Amino Acids

One observation that was made when reviewing the amino acid data collected from the sample and blank analyses performed here was that the C_6 amino acid, ε-ACA, was detected at relatively high abundances in the HYD fractions, but not detected in the UNH fractions. This C_6 amino acid was detected in the HYD serpentine blank and both HYD Ryugu samples A0106 and C0107. The HYD analytical blank was also found to contain relatively large abundances of ε-ACA. In contrast, ε-ACA was not observed in the UNH samples and blanks. This combination of factors indicates that ε-ACA was largely a contaminant introduced during the acid hydrolysis protocol. Low levels (4.5 nmol g^{-1}) of ε-ACA were reported for the HYD Ryugu sample A0106 resulting from hot water extraction (Table 1 of the main text); however, this quantity is merely a small abundance that slightly exceeded the abundance of ε-ACA in the procedural blank. Therefore, the presence of low abundances of background-corrected ε-ACA in HYD Ryugu

Sample A0106 from Extract #7-1 is likely to be contamination. The hydrolysis of nylon 6 is known to yield ε-ACA (Glavin et al., 2006). Furthermore, nylon contaminants are commonly present in bags, casters, and wipes, which are often present in laboratories and cleanrooms (Dworkin et al., 2018). As a result, nylon contaminants are difficult to fully remove from the laboratory environment. Consequently, while the specific source responsible for nylon contamination of the samples studied here is not known, it is most likely that the samples became exposed to nylon contamination during acid hydrolysis, which was conducted after extraction. Despite the detection of ε-ACA contamination in the HYD samples and blanks, it is important to underscore that the presence of ε-ACA contamination did not obscure the detection of additional target analytes.

Accurate mass chromatograms confirming amino acids detections in the Ryugu samples analyzed here are shown in Figures S5-S8. More specifically, glycine detections in the HYD and UNH Ryugu samples are shown in Figure S5, including the comparatively large abundance of glycine observed in the UNH Ryugu sample C0107. The detections of β-alanine and racemic α-alanine are shown for the UNH Ryugu samples in Figure S6. The detections of these same C3 amino acid species are shown for the HYD Ryugu samples in Figure S7. Finally, the C5 amino acids detected in the HYD and UNH Ryugu samples A0106 and C0107 are displayed in Figure S8.

Extract #7-2 of the Ryugu samples were similarly depleted in both amino acids and aliphatic amines. This may indicate the target analytes were efficiently captured during hot water extraction, as discussed in the main text. Additionally, the acidic natures of the Extract #7-2 samples could have posed analytical challenges, as progressive deterioration of chromatographic resolution was observed during the analyses of these HCl extracted samples.

3.1.1. Comparison to Amino Acids in Carbonaceous Chondrites

Figure 3 of the main text provided a comparison of the C_5 amino acid abundances relative to the total number of possible structural isomers based on valeric acid chain. In this graph was previously published data from CM1.6 Murchison (Glavin et al., 2021) and previously unpublished CM1.6 Murchison data collected as a part of the current work. The CM1.6 Murchison data collected as part of this work arose from a rehearsal analysis of multiple known extraterrestrial samples that was performed prior to the return of asteroid Ryugu material to Earth in December of 2020. Performing this rehearsal analysis provided the opportunity to test and validate the analytical protocol that was planned to be used on Ryugu material prior to analyzing these very precious asteroid samples. The rehearsal also afforded the possibility of observing potential drawbacks of the analytical protocol intended for application to Ryugu samples, and to modify the approach accordingly prior to analyzing Ryugu material, if indeed drawbacks in the analytical protocol were observed.

One of the analytical protocol parameters that was evaluated during the rehearsal analysis was whether it was necessary to perform a desalting step during sample preparation for amino acid analysis on small (< 10 mg) sample masses similar to those anticipated to be made available for amino acid analysis of Ryugu. The reason why this sample preparation parameter was evaluated was because desalting samples is a potential source of contamination and sample loss. During the rehearsal analysis, 3.24 mg of CM1.6 Murchison were simultaneously prepared for amino acid analysis along with 4.06 mg of a baked antigorite procedural blank. Sample

preparation during the rehearsal analysis involved hot water extraction at 100 °C for 24 hrs. Hot water extracts were then spiked with 30 µL of 1.5 M ddHCl to prevent the loss of amines during subsequent dry down steps. Spiked hot water extracts were brought to dryness under vacuum and underwent acid vapor hydrolysis as described here but were not desalted to mitigate the potential for sample loss and contamination. Following acid vapor hydrolysis, samples were derivatized using OPA/NAC as described in the present work. Following derivatization, samples were analyzed for amino acids by LC-FD/ToF-MS using highly similar chromatography and mass spectrometry instrumental parameters as those used when analyzing Ryugu samples.

The rehearsal analysis of undesalted CM1.6 Murchison yielded an amino acid distribution and abundances that were generally consistent with those published for larger (80 mg) samples of CM1.6 Murchison that were desalted prior to analysis (Glavin et al., 2021). This finding provided compelling evidence that desalting was not required for small (< 10 mg) samples and served as the basis for the decision to not desalt the similarly small Ryugu samples analyzed here. It should be pointed out that the sample preparation and analysis of CM1.6 Murchison during the Hayabusa2 rehearsal analysis was largely similar, but not identical, to that implemented for the analysis of Ryugu; however, minor modifications to amino acid preparation and analysis procedures have yielded similar results to those reported in the literature. Consequently, it is reasonable to conclude that the slight differences in analytical protocols between the rehearsal analysis and the Ryugu analysis introduced inconsequential differences in the analytical results. Therefore, the unique amino acid signatures observed for Ryugu were unlikely to be the result of the lack of a desalting step during sample preparation and are most likely to be genuine organic characteristics of asteroid Ryugu.

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FIGURES

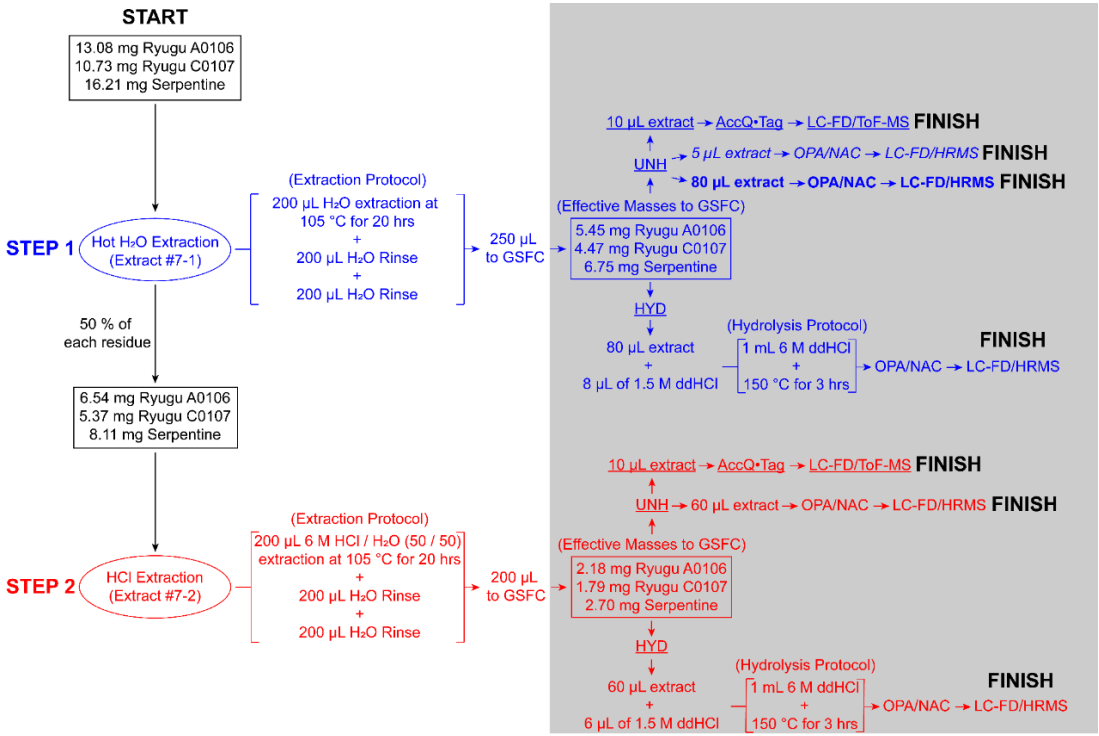

Figure S1. Flow chart depicting the hot water extraction (Extract #7-1) and the HCl extraction (#7-2) protocols implemented to produce the samples prepared for amino acid and aliphatic amine analyses. These extraction steps (non-shaded region) were performed at Kyushu University. After the completion of these extractions, portions of each extract were shipped to NASA GSFC where they underwent further processing for amino acid and aliphatic amine analyses (shaded region). Portions of both the hot water extract and the HCl extract underwent 6 M ddHCl vapor hydrolysis upon arrival at GSFC, to allow for a more direct comparison between samples from these two extraction protocols. The blue text outlines the hot water extraction pathway implemented. The red text outlines the HCl extraction pathway implemented. The bolded blue branch represents the portion of the hot water extraction pathway that was followed for the quantitation of non-protein amino acids of UNH A0106, and both the protein and non-protein amino acids of UNH C0107. The italicized blue branch represents the portion of the hot water extraction pathway that was followed for the quantitation of protein amino acids of UNH A0106. The underlined blue and red branches represent the portions of the respective hot water and HCl extraction pathways that were followed for the quantitation of aliphatic amines in Ryugu samples A0106 and C0107. Aliphatic amines were not analyzed for in the HYD sample.

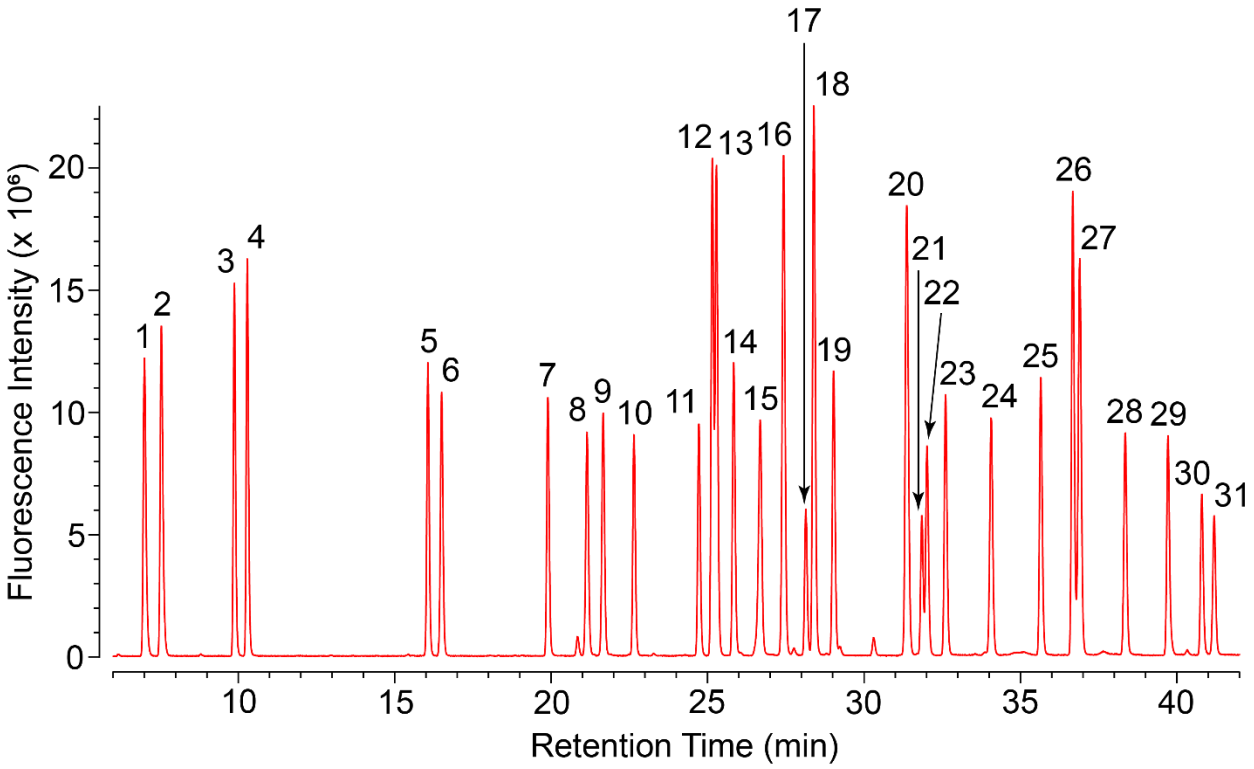

Figure S2. A mixed amino acid standard eluted using the C_2-C_6 amino acid gradient demonstrated good chromatographic resolution of many amino acids and their enantiomers. The 6 to 42-minute region of a fluorescence chromatogram of a mixed amino acid standard is shown here. Chromatographic coelution of target analytes was minimal, which facilitated accurate detection and quantitation of amino acids. Analyte identifications shown here are consistent with those listed in Table S1, and are as follows: 1 = D-Asp, 2 = L-Asp, 3 = L-Glu, 4 = D-Glu, 5 = D-Ser, 6 = L-Ser, 7 = D-Thr, 8 = L-Thr, 9 = Gly, 10 = β-Ala, 11 = γ-ABA, 12 = D-β-AIB, 13 = L-β-AIB, 14 = D-Ala, 15 = L-Ala, 16 = D-β-ABA, 17 = δ-AVA, 18 = L-β-ABA, 19 = α-AIB, 20 = D,L-α-ABA, 21 = ε-ACA, 22 = D-Iva, 23 = L-Iva, 24 = L-Val, 25 = D-Val, 26 = D-Nva, 27 = L-Nva, 28 = L-Ile, 29 = D-Ile, 30 = D-Leu, 31 = L-Leu.

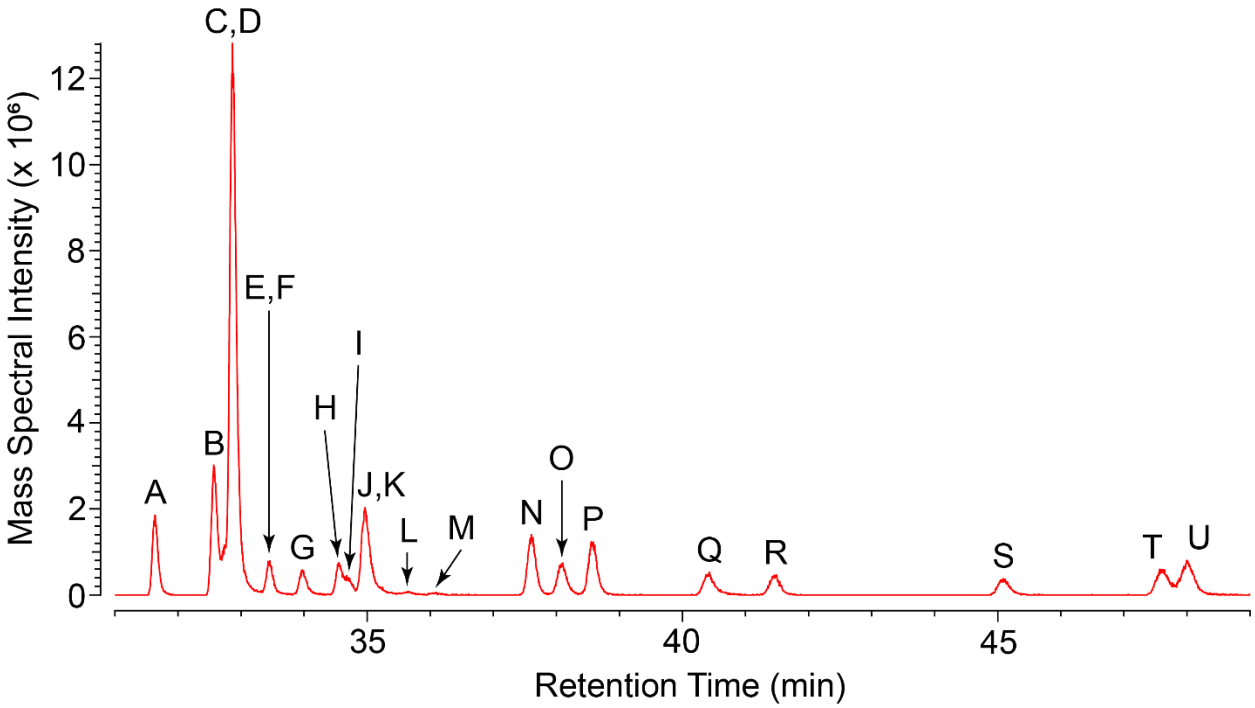

Figure S3. Implementation of a dedicated C_5 amino acid gradient enhanced the chromatographic resolution of a plurality of C_5 amino acid ($C_5H_{11}NO_2$) isomers compared to their elution using the C_2 – C_6 amino acid gradient. The 31 to 49-minute region of an accurate mass chromatogram (*m/z* 379.13222 ± 5 ppm) of a standard mixture of C_5 amino acid isomers, as eluted using the C_5 amino acid gradient. Analyte identifications shown here are consistent with those listed in Table S2, and are as follows: A = 3-A-2,2-DMPA, B = D-4-APA, C = D,L+*allo*-3-A-2-MBA, D = D,L-4-A-3-MBA, E = R-3-A-2-EPA, F = D,L+*allo*-3-A-2-MBA, G = 5-APA, H = L-4-A-2-MBA, I = D-4-A-2-MBA, J = L-4-APA, K = 3-A-3-MBA, L = D,L+*allo*-3-A-2-MBA, M = D,L+*allo*-3-A-2-MBA, N = D-Iva, O = S-3-APA, P = L-Iva, Q = R-3-APA, R = L-Val, S = D-Val, T = D-Nva, U = L-Nva.

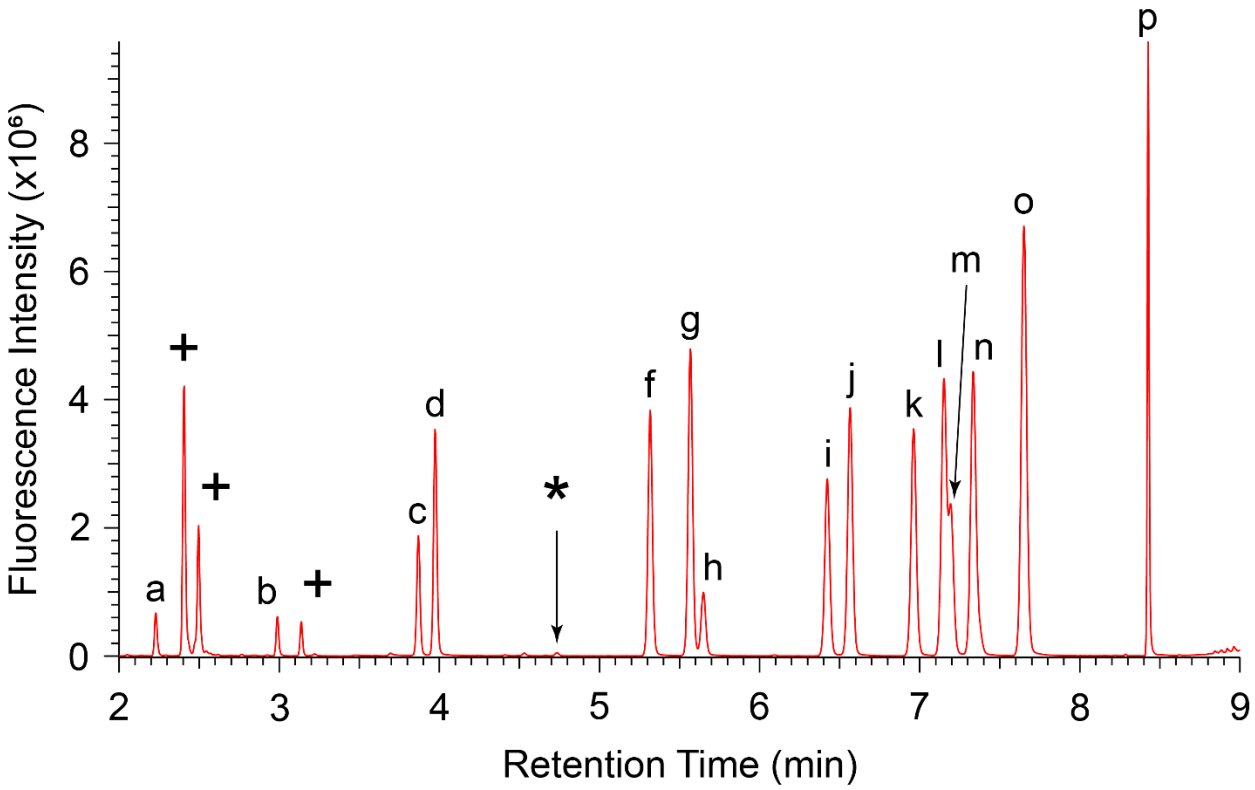

Figure S4. A mixed aliphatic amine standard comprised of 15 aliphatic amines was chromatographically resolved using the AccQ•Tag derivatization agent. The 2 to 9-minute region of a fluorescence chromatogram of a mixed aliphatic amine standard is displayed here. Analyte identifications shown here are consistent with those listed in Table S3, and are as follows: a = methylamine, b = ethylamine, c = propylamine, d = isopropylamine, e = *sec*-butylamine, f = isobutylamine, g = *n*-butylamine, h = *tert*-butylamine, i = 3-aminopentane, j = 2-amino-3-methylbutane, k = *sec*-pentylamine, l = 2-methylbutylamine, m = *tert*-pentylamine, n = isopentylamine, o = pentylamine, and p = hexylamine. Note: *sec*-butylamine is derivatized less efficiently by AccQ•Tag than are the other aliphatic amines targeted here and as a result *sec*-butylamine is not detected by the FD; however, *sec*-butylamine is detected by ToF-MS and the asterisk represents the retention time of *sec*-butylamine as indicated by accurate mass analysis. Also, the three peaks labeled with a cross are derivatization reaction side products that do not interfere with target analyte detection.

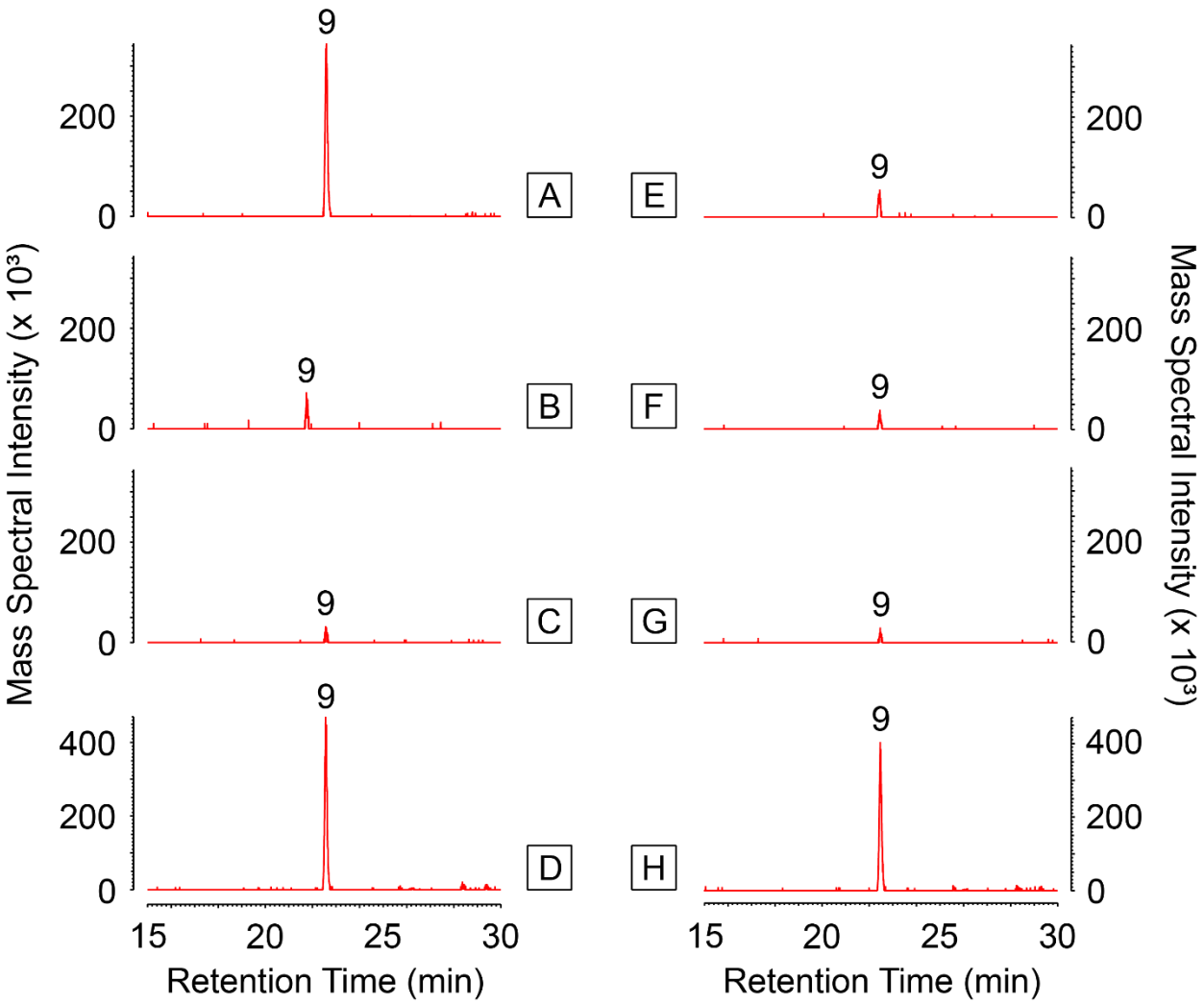

Figure S5. Glycine was detected above blank levels in Ryugu samples A0106 and C0107. The 15 to 30-minute regions of accurate mass chromatograms for glycine (*m/z* 337.08527 ± 5 ppm) in A) UNH Ryugu sample C0107, B) UNH Ryugu sample A0106, C) UNH serpentine blank, and D) a mixed amino acid standard. Also shown are the 15 to 30-minute regions of accurate mass chromatograms for glycine (*m/z* 337.08527 ± 5 ppm) from E) HYD Ryugu sample C0107, F) HYD Ryugu sample A0106, G) HYD serpentine blank, and H) a mixed amino acid standard. The above analyte elution was achieved via the C_2 – C_6 amino acid gradient. Analyte identifications shown here are consistent with those listed in Table S1 and are as follows: 9 = glycine. Note: a slight deviation in retention time exists for B) compared to A), C), and D) because the data for UNH Ryugu sample A0106 was collected on a different day than when the data was collected for A), C), and D).

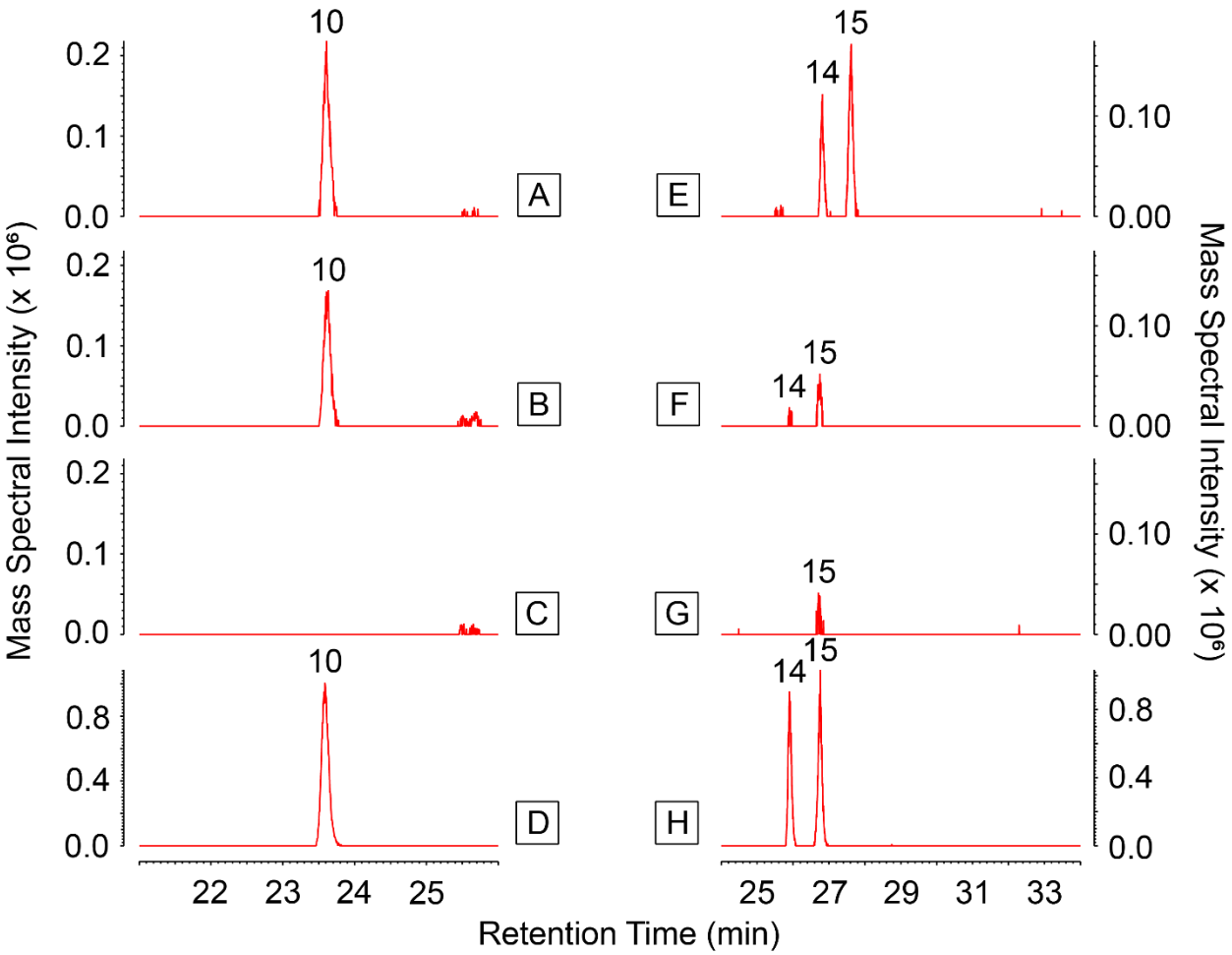

Figure S6. The non-protein amino acid, β-Ala, was detected in both UNH Ryugu samples A0106 and C0107, but not in the blank, and the protein amino acid, Ala, was observed to be racemic in both samples, following blank-correction. The 21 to 26-minute regions of accurate mass chromatograms for β-Ala (*m/z* 351.10092 ± 5 ppm) from A) UNH Ryugu sample C0107, B) UNH Ryugu sample A0106, C) UNH serpentine blank, and D) a mixed amino acid standard. Also shown are the 24 to 34-minute regions of accurate mass chromatograms for D,L-Ala (*m/z* 351.10092 ± 5 ppm) from E) UNH Ryugu sample C0107, F) UNH Ryugu sample A0106, G) UNH serpentine blank, and H) a mixed amino acid standard. The above analyte elution was achieved via the C_2 – C_6 amino acid gradient. Analyte identifications shown here are consistent with those listed in Table S1 and are as follows: 10 = β-Ala, 14 = D-Ala, and 15 = L-Ala. Note: a slight deviation in retention time exists for E) because the D,L-Ala data for UNH Ryugu sample C0107 was collected on a different day than when the D,L-Ala data was collected for F), G), and H).

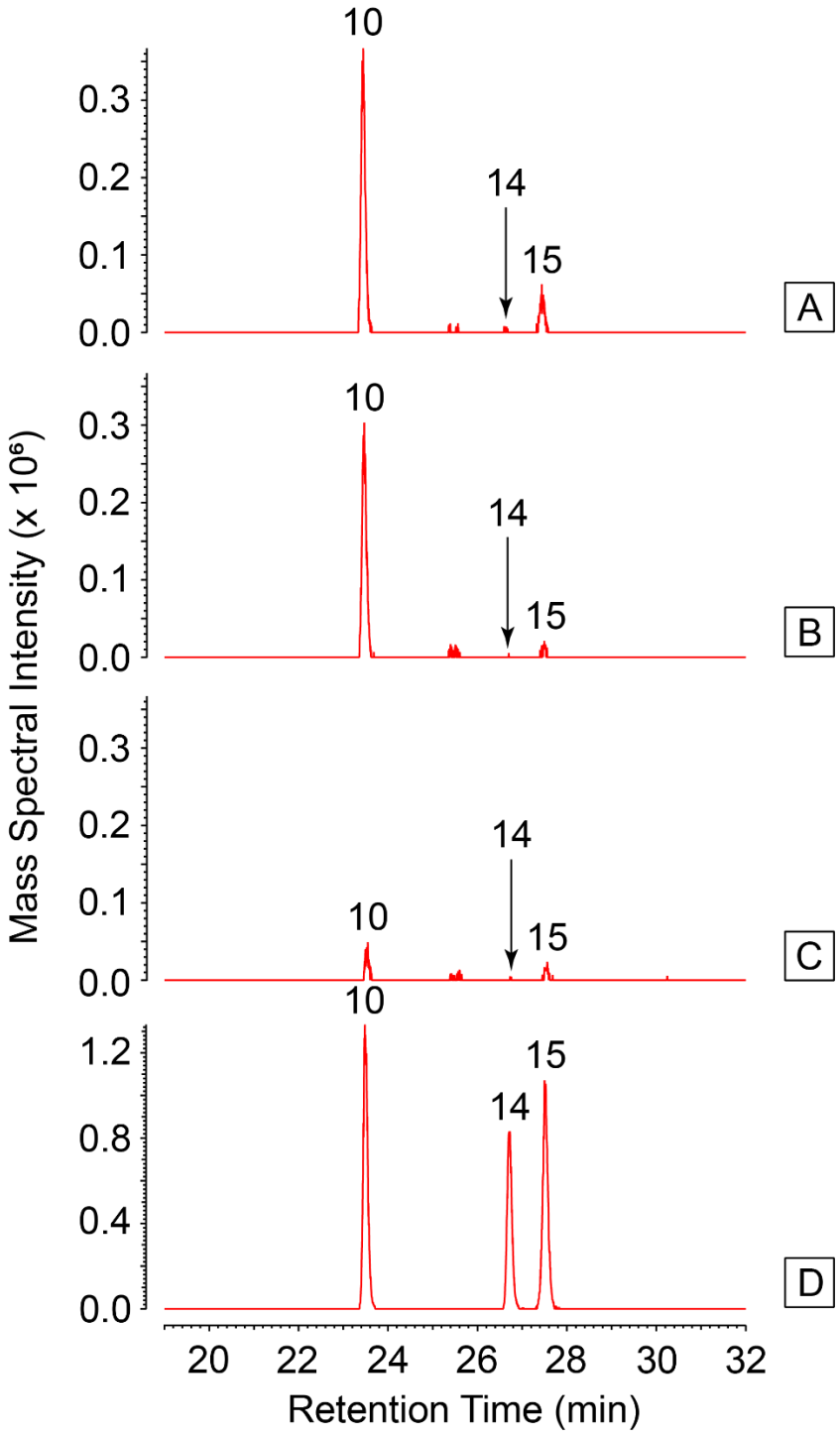

Figure S7. The non-protein amino acid, β-Ala, was detected in both HYD Ryugu samples A0106 and C0107 at abundances significantly greater than what was observed in the blank. The 19 to 32-minute regions of accurate mass chromatograms for β-Ala and D,L-Ala (*m/z* 351.10092 ± 5 ppm) from A) HYD Ryugu sample C0107, B) HYD Ryugu sample A0106, C) HYD serpentine blank, and D) a mixed amino acid standard. The above analyte elution was achieved via the C_2 – C_6 amino acid gradient. Analyte identifications shown here are consistent with those listed in Table S1, and are as follows: 10 = β-Ala, 14 = D-Ala, and 15 = L-Ala.

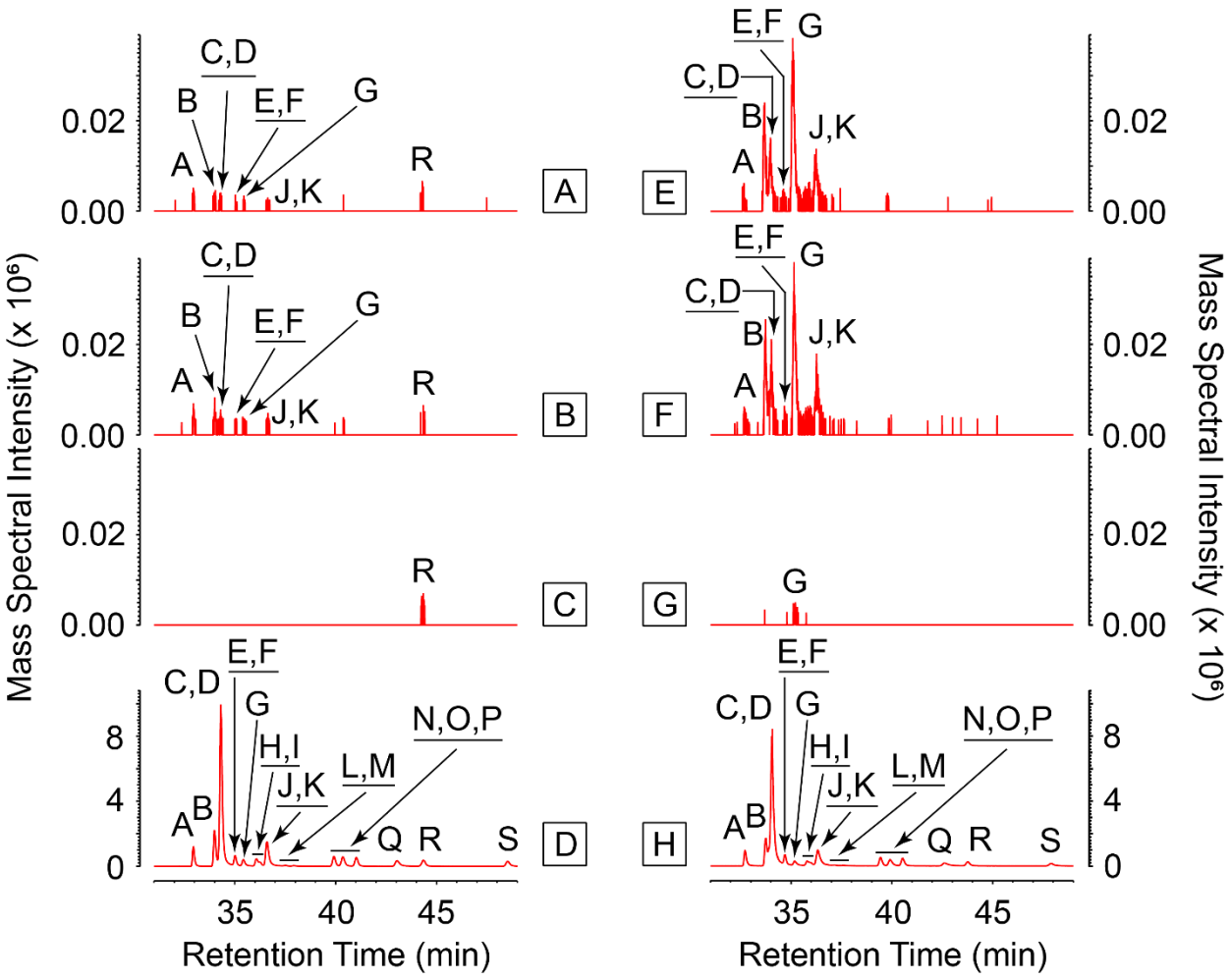

Figure S8. Select C_5 amino acids were detected and quantitated in the UNH and HYD Ryugu A0106 and C0107 samples, while other C_5 amino acids were tentatively identified. The 31 to 49-minute regions of accurate mass chromatograms for 3-A-2,2-DMPA, D,L-γ-AVA, 3-A-2-MBA, 4-A-3-MBA, R-3-A-2-EPA, δ-AVA, D/L-4-A-2-MBA, 3-A-3-MBA, D,L-Iva, S,R-3-APA, and D,L-Val (*m/z* 379.13222 ± 5 ppm) from A) UNH Ryugu sample C0107, B) UNH Ryugu sample A0106, C) UNH serpentine blank, and D) a mixed amino acid standard. Also shown are the 31 to 49-minute regions of accurate mass chromatograms for 3-A-2,2-DMPA, D,L-γ-AVA, 3-A-2-MBA, 4-A-3-MBA, R-3-A-2-EPA, δ-AVA, D/L-4-A-2-MBA, 3-A-3-MBA, D,L-Iva, S,R-3-APA, and D,L-Val (*m/z* 379.13222 ± 5 ppm) from E) HYD Ryugu sample C0107, F) HYD Ryugu sample A0106, G) HYD serpentine blank, and H) a mixed amino acid standard. The above analyte elution was achieved via the C_5 amino acid gradient. Analyte identifications shown here are consistent with those listed in Table S2, and are as follows: A = 3-A-2,2-DMPA, B = D-γ-AVA, C = 3-A-2-MBA, D = 4-A-3-MBA, E = R-3-A-2-EPA, F = 3-A-2-MBA, G = δ-AVA, H = L-4-A-2-MBA, I = D-4-A-2-MBA, J = L-γ-AVA**,** K = 3-A-3-MBA, L = 3-A-2-MBA, M = 3-A-2-MBA, N = D-Iva, O = S-3-APA, P = L-Iva, Q = R-3-APA, R = L-Val, and S = D-Val.

TABLES

Table S1. Example of typical detection metrics observed when performing an analysis of C_2 – C_6 amino acids in a mixed amino acid standard using the gradient specifically designed for C_2 – C_6 amino acids.

Analyte #	Analyte	FD RT (min)	MS RT (min)	$[M+H]^+$ Chemical Formula	Theoretical *m/z*	Experimental *m/z*	Mass Error (ppm)
1	D-Asp	7.00	7.08	$C_{17}H_{19}N_2O_7S$	395.09075	395.09070	-0.12655
2	L-Asp	7.54	7.62	$C_{17}H_{19}N_2O_7S$	395.09075	395.09067	-0.20249
3	L-Glu	9.88	9.94	$C_{18}H_{21}N_2O_7S$	409.10640	409.10645	0.12222
4	D-Glu	10.29	10.36	$C_{18}H_{21}N_2O_7S$	409.10640	409.10648	0.19555
5	D-Ser	16.07	16.13	$C_{16}H_{19}N_2O_6S$	367.09583	367.09570	-0.35413
6	L-Ser	16.50	16.58	$C_{16}H_{19}N_2O_6S$	367.09583	367.09564	-0.51758
7	D-Thr	19.90	19.96	$C_{17}H_{21}N_2O_6S$	381.11148	381.11142	-0.15743
8	L-Thr	21.15	21.20	$C_{17}H_{21}N_2O_6S$	381.11148	381.11142	-0.15743
9	Gly	21.67	21.72	$C_{15}H_{17}N_2O_5S$	337.08527	337.08496	-0.91965
10	β-Ala	22.65	22.72	$C_{16}H_{19}N_2O_5S$	351.10092	351.10074	-0.51267
11	γ-ABA	24.72	24.79	$C_{17}H_{21}N_2O_5S$	365.11657	365.11624	-0.90382
12	D-β-AIB	25.15	25.21	$C_{17}H_{21}N_2O_5S$	365.11657	365.11630	-0.73949
13	L-β-AIB	25.29	25.35	$C_{17}H_{21}N_2O_5S$	365.11657	365.11615	-1.15032
14	D-Ala	25.84	25.90	$C_{16}H_{19}N_2O_5S$	351.10092	351.10077	-0.42723
15	L-Ala	26.68	26.76	$C_{16}H_{19}N_2O_5S$	351.10092	351.10068	-0.68356
16	D-β-ABA	27.43	27.49	$C_{17}H_{21}N_2O_5S$	365.11657	365.11630	-0.73949
17	δ-AVA	28.14	28.21	$C_{18}H_{23}N_2O_5S$	379.13222	379.13196	-0.68578
18	L-β-ABA	28.40	28.46	$C_{17}H_{21}N_2O_5S$	365.11657	365.11621	-0.98599
19	α-AIB	29.03	29.10	$C_{17}H_{21}N_2O_5S$	365.11657	365.11627	-0.82166
20	D,L-α-ABA	31.37	31.44	$C_{17}H_{21}N_2O_5S$	365.11657	365.11624	-0.90382
21	ε-ACA	31.84	31.91	$C_{19}H_{25}N_2O_5S$	393.14787	393.14761	-0.66133
22	D-Iva	32.01	32.07	$C_{18}H_{23}N_2O_5S$	379.13222	379.13205	-0.44839
23	L-Iva	32.61	32.68	$C_{18}H_{23}N_2O_5S$	379.13222	379.13193	-0.76490
24	L-Val	34.06	34.13	$C_{18}H_{23}N_2O_5S$	379.13222	379.13196	-0.68578
25	D-Val	35.65	35.72	$C_{18}H_{23}N_2O_5S$	379.13222	379.13184	-1.00229
26	D-Nva	36.67	36.73	$C_{18}H_{23}N_2O_5S$	379.13222	379.13184	-1.00229
27	L-Nva	36.90	36.96	$C_{18}H_{23}N_2O_5S$	379.13222	379.13187	-0.92319
28	L-Ile	38.35	38.42	$C_{19}H_{25}N_2O_5S$	393.14787	393.14752	-0.89025
29	D-Ile	39.72	39.77	$C_{19}H_{25}N_2O_5S$	393.14787	393.14780	-0.17805
30	D-Leu	40.80	40.87	$C_{19}H_{25}N_2O_5S$	393.14787	393.14749	-0.96656
31	L-Leu	41.19	41.26	$C_{19}H_{25}N_2O_5S$	393.14787	393.14752	-0.89025

Derivatization of amino acids with OPA/NAC induces a shift in mass by 261 Da. Mass errors were calculated using the following equation: [(Experimental *m/z* – Theoretical *m/z*) / Theoretical *m/z*] x 10^6. Analyte numbers correspond to peak numbers specified in chromatograms generated via the C_2 – C_6 amino acid gradient, as shown throughout the manuscript.

Table S2. Example of typical detection metrics observed when performing an analysis of C_5 amino acids in a mixed amino acid standard using the gradient specifically designed for C_5 amino acids. Mass errors were calculated as done in Table S1.

Analyte Letter	Analyte	FD RT (min)	MS RT (min)	$[M+H]^+$ Chemical Formula	Theoretical *m/z*	Experimental *m/z*	Mass Error (ppm)
A	3-A-2,2-DMPA	31.57	31.64	$C_{18}H_{23}N_2O_5S$	379.13222	379.13144	-2.05733
B	D-4-APA	32.51	32.57	$C_{18}H_{23}N_2O_5S$	379.13222	379.13144	-2.05733
C	D,L+*allo*-3-A-2-MBA	32.82	32.86	$C_{18}H_{23}N_2O_5S$	379.13222	379.13129	-2.45297
D	D,L-4-A-3-MBA	32.82	32.86	$C_{18}H_{23}N_2O_5S$	379.13222	379.13129	-2.45297
E	R-3-A-2-EPA	33.38	33.46	$C_{18}H_{23}N_2O_5S$	379.13222	379.13132	-2.37384
F	D,L+*allo*-3-A-2-MBA	33.38	33.46	$C_{18}H_{23}N_2O_5S$	379.13222	379.13132	-2.37384
G	5-APA	33.91	33.97	$C_{18}H_{23}N_2O_5S$	379.13222	379.13165	-1.50343
H	L-4-A-2-MBA	34.50	34.55	$C_{18}H_{23}N_2O_5S$	379.13222	379.13141	-2.13646
I	D-4-A-2-MBA	34.60	34.71	$C_{18}H_{23}N_2O_5S$	379.13222	379.13126	-2.53210
J	L-4-APA	34.90	34.97	$C_{18}H_{23}N_2O_5S$	379.13222	379.13150	-1.89907
K	3-A-3-MBA	34.90	34.97	$C_{18}H_{23}N_2O_5S$	379.13222	379.13150	-1.89907
L	D,L+*allo*-3-A-2-MBA	35.58	35.64	$C_{18}H_{23}N_2O_5S$	379.13222	379.13123	-2.61123
M	D,L+*allo*-3-A-2-MBA	35.99	36.09	$C_{18}H_{23}N_2O_5S$	379.13222	379.13156	-1.74082
N	D-Iva	37.54	37.60	$C_{18}H_{23}N_2O_5S$	379.13222	379.13132	-2.37384
O	S-3-APA	38.02	38.10	$C_{18}H_{23}N_2O_5S$	379.13222	379.13144	-2.05733
P	L-Iva	38.51	38.56	$C_{18}H_{23}N_2O_5S$	379.13222	379.13153	-1.81995
Q	R-3-APA	40.33	40.43	$C_{18}H_{23}N_2O_5S$	379.13222	379.13156	-1.74082
R	L-Val	41.40	41.49	$C_{18}H_{23}N_2O_5S$	379.13222	379.13159	-1.66169
S	D-Val	45.02	45.09	$C_{18}H_{23}N_2O_5S$	379.13222	379.13156	-1.74082
T	D-Nva	47.52	47.57	$C_{18}H_{23}N_2O_5S$	379.13222	379.13141	-2.13646
U	L-Nva	47.94	48.01	$C_{18}H_{23}N_2O_5S$	379.13222	379.13110	-2.95411

Derivatization of amino acids with OPA/NAC induces a shift in mass by 261 Da. Analyte numbers correspond to peak numbers specified in chromatograms generated via the C_5 amino acid gradient, as shown throughout the manuscript.

Table S3. Example of typical detection metrics observed when performing an analysis of aliphatic amines in a mixed aliphatic amine standard by LC-FD/ToF-MS. Mass errors were calculated as done in Table S1.

Analyte Letter	Analyte	FD RT (min)	MS RT (min)	$[M+H]^+$ Chemical Formula	Theoretical *m/z*	Experimental *m/z*	Mass Error (ppm)
a	Methylamine	2.23	2.27	$C_{11}H_{11}N_3O$	202.0980	202.0976	-1.9792
b	Ethylamine	2.99	3.03	$C_{12}H_{14}N_3O$	216.1137	216.1126	-5.0899
c	Propylamine	3.87	3.92	$C_{13}H_{16}N_3O$	230.1293	230.1281	-5.2145
d	Isopropylamine	3.97	4.02	$C_{13}H_{16}N_3O$	230.1293	230.1284	-3.9108
e	*sec*-Butylamine[a]	-	4.78	$C_{14}H_{18}N_3O$	244.1450	244.1439	-4.5055
f	Isobutylamine	5.32	5.37	$C_{14}H_{18}N_3O$	244.1450	244.1441	-3.6863
g	*n*-Butylamine	5.57	5.62	$C_{14}H_{18}N_3O$	244.1450	244.1442	-3.2767
h	*tert*-Butylamine	5.65	5.71	$C_{14}H_{18}N_3O$	244.1450	244.1439	-4.5055
i	3-Aminopentane	6.42	6.48	$C_{15}H_{20}N_3O$	258.1606	258.1595	-4.2609
j	2-Amino-3-Methylbutane	6.57	6.63	$C_{15}H_{20}N_3O$	258.1606	258.1597	-3.4862
k	*sec*-Pentylamine	6.96	7.01	$C_{15}H_{20}N_3O$	258.1606	258.1595	-4.2609
l	2-Methylbutylamine	7.15	7.19	$C_{15}H_{20}N_3O$	258.1606	258.1595	-4.2609
m	*tert*-Pentylamine	7.21	7.25	$C_{15}H_{20}N_3O$	258.1606	258.1595	-4.2609
n	Isopentylamine	7.33	7.38	$C_{15}H_{20}N_3O$	258.1606	258.1593	-5.0356
o	Pentylamine	7.65	7.71	$C_{15}H_{20}N_3O$	258.1606	258.1593	-5.0356
p	Hexylamine	8.43	8.48	$C_{16}H_{22}N_3O$	272.1763	272.1750	-4.7763

[a]The AccQ•Tag derivative of *sec*-Butylamine does not fluoresce well, so it was not detected by the FD. However, the AccQ•Tag derivative of *sec*-Butylamine was detected by accurate mass analysis.

Table S4. Detection metrics of select amino acids in the hot water extracted UNH A0106 Ryugu sample, as analyzed using the $C_2 – C_6$ amino acid gradient. Mass errors were calculated as described in Table S1.

		STANDARD	#7-1 UNH A0106		
Amino Acid	**Theoretical *m/z***	**MS RT (min)**	**MS RT (min)**	**Experimental *m/z***	**Mass Error (ppm)**
Gly	337.08527	21.72	21.70	337.08505	-0.65265
β-Ala	351.10092	23.59	23.63	351.10074	-0.51267
D-Ala	351.10092	25.90	25.88	351.10046	-1.31016
L-Ala	351.10092	26.76	26.73	351.10062	-0.85446
γ-ABA	365.11657	25.68	25.70	365.11621	-0.98599
D-β-AIB	365.11657	26.11	26.12	365.11581	-2.08153
L-β-AIB	365.11657	26.22	26.25	365.11575	-2.24586
D-β-ABA	365.11657	28.38	28.40	365.11636	-0.57516
L-β-ABA	365.11657	29.36	29.33	365.11649	-0.21911
α-AIB	365.11657	29.97	29.99	365.11627	-0.82166
D,L-α-ABA	365.11657	32.31	32.34	365.11627	-0.82166

Table S5. Detection metrics of select C_5 amino acids in the hot water extracted UNH A0106 Ryugu sample, as analyzed using the C_5 amino acid gradient. Mass errors were calculated as described in Table S1.

		STANDARD	#7-1 UNH A0106		
Amino Acid	**Theoretical *m/z***	**MS RT (min)**	**MS RT (min)**	**Experimental *m/z***	**Mass Error (ppm)**
3-A-2,2-DMPA	379.13222	32.95	32.97	379.13211	-0.29014
D-γ-AVA	379.13222	33.98	34.02	379.13229	0.18463
3-A-2-MBA	379.13222	34.31	34.33	379.13229	0.18463
4-A-3-MBA	379.13222	34.31	34.33	379.13229	0.18463
3-A-2-MBA	379.13222	35.01	35.06	379.13260	1.00229
R-3-A-2-EPA	379.13222	35.01	35.06	379.13260	1.00229
δ-AVA	379.13222	35.43	35.44	379.13220	-0.05275
L-γ-AVA	379.13222	36.58	36.64	379.13205	-0.44839
3-A-3-MBA	379.13222	36.58	36.64	379.13205	-0.44839

Table S6. Detection metrics of select amino acids in the hot water extracted HYD A0106 Ryugu sample, as analyzed using the $C_2 – C_6$ amino acid gradient. Mass errors were calculated as described in Table S1.

		STANDARD	#7-1 HYD A0106		
Amino Acid	**Theoretical *m/z***	**MS RT (min)**	**MS RT (min)**	**Experimental *m/z***	**Mass Error (ppm)**
Gly	337.08527	22.46	22.44	337.08527	0.00000
β-Ala	351.10092	23.48	23.43	351.10101	0.25634
D-Ala	351.10092	26.69	n.d.	n.d.	n.d.
L-Ala	351.10092	n.d.	n.d.	n.d.	n.d.
γ-ABA	365.11657	25.57	25.54	365.11676	0.52038
D-β-AIB	365.11657	26.00	25.95	365.11694	1.01337
L-β-AIB	365.11657	26.14	26.08	365.11710	1.45159
D-β-ABA	365.11657	28.28	28.23	365.11658	0.02739
L-β-ABA	365.11657	29.25	29.23	365.11667	0.27389
α-AIB	365.11657	29.88	29.82	365.11661	0.10955
D,L-α-ABA	365.11657	32.20	n.d.	n.d.	n.d.

n.d. = Analyte was not detected. See Table 1 of the main text for details.

Table S7. Detection metrics of select C_5 amino acids in the hot water extracted HYD A0106 Ryugu sample, as analyzed using the C_5 amino acid gradient. Mass errors were calculated as described in Table S1.

		STANDARD	#7-1 HYD A0106		
Amino Acid	**Theoretical *m/z***	**MS RT (min)**	**MS RT (min)**	**Experimental *m/z***	**Mass Error (ppm)**
3-A-2,2-DMPA	379.13222	32.71	32.70	379.13226	0.10550
D-γ-AVA	379.13222	33.76	33.74	379.13242	0.52752
3-A-2-MBA	379.13222	34.06	34.03	379.13223	0.02638
4-A-3-MBA	379.13222	34.06	34.03	379.13223	0.02638
3-A-2-MBA	379.13222	34.71	34.69	379.13229	0.18463
R-3-A-2-EPA	379.13222	34.71	34.69	379.13229	0.18463
δ-AVA	379.13222	35.16	35.15	379.13223	0.02638
L-γ-AVA	379.13222	36.23	36.26	379.13223	0.02638
3-A-3-MBA	379.13222	36.23	36.26	379.13223	0.02638

Table S8. Detection metrics of select amino acids in the hot water extracted UNH C0107 Ryugu sample, as analyzed using the C_2 – C_6 amino acid gradient. Mass errors were calculated as described in Table S1.

		STANDARD	#7-1 UNH C0107		
Amino Acid	**Theoretical *m/z***	**MS RT (min)**	**MS RT (min)**	**Experimental *m/z***	**Mass Error (ppm)**
Gly	337.08527	22.56	22.57	337.08444	-2.46228
β-Ala	351.10092	23.59	23.59	351.10034	-1.65195
D-Ala	351.10092	26.81	26.82	351.10010	-2.33551
L-Ala	351.10092	27.60	27.60	351.10034	-1.65195
γ-ABA	365.11657	25.68	25.70	365.11615	-1.15032
D-β-AIB	365.11657	26.11	26.09	365.11661	0.10955
L-β-AIB	365.11657	26.22	26.21	365.11588	-1.88981
D-β-ABA	365.11657	28.38	28.38	365.11633	-0.65732
L-β-ABA	365.11657	29.36	29.34	365.11627	-0.82166
α-AIB	365.11657	29.97	29.97	365.11621	-0.98599
D,L-α-ABA	365.11657	32.31	32.30	365.11609	-1.31465

Table S9. Detection metrics of select C_5 amino acids in the hot water extracted UNH C0107 Ryugu sample, as analyzed using the C_5 amino acid gradient. Mass errors were calculated as described in Table S1.

		STANDARD	#7-1 UNH C0107		
Amino Acid	**Theoretical *m/z***	**MS RT (min)**	**MS RT (min)**	**Experimental *m/z***	**Mass Error (ppm)**
3-A-2,2-DMPA	379.13222	32.95	32.94	379.13214	-0.21101
D-γ-AVA	379.13222	33.98	33.98	379.13214	-0.21101
3-A-2-MBA	379.13222	34.31	34.30	379.13269	1.23967
4-A-3-MBA	379.13222	34.31	34.30	379.13269	1.23967
3-A-2-MBA	379.13222	35.01	35.01	379.13275	1.39793
R-3-A-2-EPA	379.13222	35.01	35.01	379.13275	1.39793
δ-AVA	379.13222	35.43	35.45	379.13263	1.08142
L-γ-AVA	379.13222	36.58	36.58	379.13235	0.34289
3-A-3-MBA	379.13222	36.58	36.58	379.13235	0.34289

Table S10. Detection metrics of select amino acids in the hot water extracted HYD C0107 Ryugu sample, as analyzed using the $C_2 – C_6$ amino acid gradient. Mass errors were calculated as described in Table S1.

		STANDARD	#7-1 HYD C0107		
Amino Acid	**Theoretical *m/z***	**MS RT (min)**	**MS RT (min)**	**Experimental *m/z***	**Mass Error (ppm)**
Gly	337.08527	22.46	22.46	337.08496	-0.91965
β-Ala	351.10092	23.48	23.47	351.10083	-0.25634
D-Ala	351.10092	26.69	26.67	351.10071	-0.59812
L-Ala	351.10092	27.50	27.46	351.10049	-1.22472
γ-ABA	365.11657	25.57	25.55	365.11642	-0.41083
D-β-AIB	365.11657	26.00	25.96	365.11664	0.19172
L-β-AIB	365.11657	26.14	26.11	365.11676	0.52038
D-β-ABA	365.11657	28.28	28.27	365.11612	-1.23248
L-β-ABA	365.11657	29.25	29.19	365.11655	-0.05478
α-AIB	365.11657	29.88	29.83	365.11609	-1.31465
D,L-α-ABA	365.11657	32.20	n.d.	n.d.	n.d.

n.d. = Analyte was not detected. See Table 1 of the main text for details.

Table S11. Detection metrics of select C_5 amino acids in the hot water extracted HYD C0107 Ryugu sample, as analyzed using the C_5 amino acid gradient. Mass errors were calculated as described in Table S1.

		STANDARD	#7-1 HYD C0107		
Amino Acid	**Theoretical *m/z***	**MS RT (min)**	**MS RT (min)**	**Experimental *m/z***	**Mass Error (ppm)**
3-A-2,2-DMPA	379.13222	32.71	32.66	379.13196	-0.68578
D-γ-AVA	379.13222	33.76	33.70	379.13242	0.52752
3-A-2-MBA	379.13222	34.06	33.98	379.13235	0.34289
4-A-3-MBA	379.13222	34.06	33.98	379.13235	0.34289
3-A-2-MBA	379.13222	34.71	34.66	379.13248	0.68578
R-3-A-2-EPA	379.13222	34.71	34.66	379.13248	0.68578
δ-AVA	379.13222	35.16	35.08	379.13226	0.10550
L-γ-AVA	379.13222	36.23	36.22	379.13239	0.44839
3-A-3-MBA	379.13222	36.23	36.22	379.13239	0.44839

Table S12. Averaged, blank-corrected abundances (nmol g^{-1}) of HYD C_5 amino acids in the hot water extracts of Antigorite and CM1.6 Murchison observed during the Hayabusa2 rehearsal analysis. Also included for comparative purposes is complementary data from a previously published HYD hot water extract of the same CM1.6 Murchison sample.

Amine Position	Amino Acids	Antigorite	CM1.6 Murchison (3.24 mg Extract, Current Work)	CM1.6 Murchison (80 mg Extract, Glavin et al., 2021)
α	D-Val	< 0.01	1.3 ± 0.1	0.55 ± 0.02
α	L-Val	0.12 ± 0.02	2.6 ± 0.2	2.8 ± 0.1
α	D-Iva	< 0.01	3.80 ± 0.02	10 ± 0.5
α	L-Iva	< 0.01	3.82 ± 0.05	11.8 ± 0.7
α	D-Nva	< 0.01	0.28 ± 0.03	0.1 ± 0.1
α	L-Nva	< 0.01	0.28 ± 0.01	0.05 ± 0.03
β	R,S-3-APA[a]	< 0.01	1.9 ± 0.1	2.7 ± 0.1
β	D,L+*allo*-3-A-2-MBA[a]	< 0.01	1.5 ± 0.1	0.29 ± 0.04
β	3-A-3-MBA	< 0.01	11.6 ± 0.7	4.76 ± 0.04
β	3-A-2,2,-DMPA	< 0.01	3.3 ± 0.1	1.9 ± 0.1
β	R,S-3-A-2-EPA[b]	< 0.01	1.06 ± 0.01	< 0.3
γ	D,L-4-APA[a]	< 0.01	2.0 ± 0.2	2.9 ± 0.1
γ	4-A-2-MBA[b]	< 0.01	3.4 ± 0.1	2.1 ± 0.6
γ	4-A-3-MBA[b]	< 0.01	0.45 ± 0.01	0.42 ± 0.02
δ	5-APA	< 0.01	8.9 ± 0.5	1.8 ± 0.1
Multiple	Total	0.12 ± 0.02	46.2 ± 0.9	42.2 ± 1.1

Uncertainties (δ_x) are based on the standard deviation (σ_x) of the average value of separate measurements (n) and are calculated as the standard error of the mean: $\delta_x = \sigma_x \cdot (n)^{-1/2}$.

[a]Enantiomers or stereoisomers were chromatographically resolved, but elution order could not be determined due to a lack of enantiopure or stereoisomerically pure standards.

[b]Enantiomers were not separated by the chromatographic conditions employed.

Table S13. Detection metrics of aliphatic amines in the hot water extracted UNH Ryugu A0106 sample, as analyzed by LC-FD/ToF-MS. Mass errors were calculated as described in Table S1.

		STANDARD	#7-1 UNH A0106		
Amino Acid	**Theoretical *m/z***	**MS RT (min)**	**MS RT (min)**	**Experimental *m/z***	**Mass Error (ppm)**
Methylamine	202.0980	2.27	2.27	202.0966	6.9273
Ethylamine	216.1137	3.04	3.03	216.1137	0.0000
Propylamine	230.1293	3.92	3.92	230.1288	2.1727
Isopropylamine	230.1293	4.03	4.02	230.1262	13.4707[a]

[a]Since the observed mass error of isopropylamine is outside the 10-ppm threshold for the ToF-MS system used to analyze aliphatic amines, the detection of isopropylamine in A0106 is considered tentative and the quantitative estimate provided in Table 4 of the main text for this analyte is an upper limit estimate.

Table S14. Detection metrics of aliphatic amines in the hot water extracted UNH Ryugu C0107 sample, as analyzed by LC-FD/ToF-MS. Mass errors were calculated as described in Table S1.

		STANDARD	#7-1 UNH A0106		
Amino Acid	**Theoretical *m/z***	**MS RT (min)**	**MS RT (min)**	**Experimental *m/z***	**Mass Error (ppm)**
Methylamine	202.0980	2.27	2.27	202.0977	1.4844
Ethylamine	216.1137	3.03	3.03	216.1120	7.8662
Propylamine	230.1293	3.92	3.92	230.1277	6.9526
Isopropylamine	230.1293	4.02	4.02	230.1286	3.0418