

Supplementary Information for “Distribution of extraterrestrial nucleobases, other N-heterocycles, and their precursors in a sample from asteroid Bennu”

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Supplementary Methods

Extraction of melamine and its homologues from the Murchison meteorite and the soil at the landing site of the Murchison meteorite

We have performed solvent extraction on the Murchison meteorite and the soil sample from the meteorite's landing site using a method previously described^{S1,S2}. In brief, ~1 g of the Murchison meteorite was exposed to 2% HCl assisted with ultrasonication, followed by cation exchange chromatography to remove inorganic materials from the extract. The obtained fractions were analyzed using an HPLC coupled with an Orbitrap-type mass spectrometer. ~100 mg of the soil sample was analysed in the same manner as mentioned above.

Identification of the synthesized carbonate

The synthesis of CaCO_3 was confirmed by transmission electron microscopy (TEM) as follows: The white powders were dispersed in ethanol by ultrasonic treatment, and 0.5 μL of the dispersion was dropped onto a copper grid equipped with an elastic carbon membrane (ELS-C10, STEM Corp., Tokyo, Japan) and dried. Because the residue contained salts (NaCl, which is a byproduct, and tiny amounts of unreacted Na_2CO_3 and CaCl_2) that interfered with the analysis of calcium carbonates, 1 μL of distilled water was dropped onto the grid and immediately blown off. This procedure was repeated three times to dissolve and remove water-soluble salts.

The sample was observed by a transmission electron microscope (JEM-2100F; JEOL Ltd., Tokyo, Japan) with a field-emission gun operated at an acceleration voltage of 200 kV. We used a One View TEM camera (Gatan, Inc., Pleasanton, CA, USA) to take TEM images and selected area electron diffraction (SAED) patterns and an energy-dispersive X-ray spectroscopy (EDS) system (JEOL JED2300T) for elemental analysis. Electron-diffraction patterns were simulated by Recipro^{S3}.

Recovery of guanine from the synthesized guanosine isomers

Sample solutions containing 20 nmol of guanine, 1 μmol of ribose, and 1 μmol of MgCl_2 in 600 μL were prepared in 1.5 mL Eppendorf microtubes. These were then dried at 85 °C for 20 h in a furnace. One of the samples was dissolved in 600 μL of Milli-Q water. Another sample was added by 2% HCl and sonicated for 30 min at below room temperature. A third sample was dissolved in 1 mL of 20% HCl, then sealed in a glass ampoule with an N_2 atmosphere. This sample was then heated at 110 °C for 2 h.

All samples were diluted and analyzed using liquid chromatography-mass spectrometry (Shimadzu LCMS-8060NX). Separation was conducted at 40 °C using a reversed-phase column (InertSustain PFP HP 3 µm, 2.1 × 250 mm; GL Sciences) with eluents of water and 0.1% formic acid in acetonitrile. The eluent composition was initially 90% water for 10 min, followed by a decrease to 70% over 5 min, after which it was held for 2 min. The flow rate was 0.2 mL/min.

Supplementary Result

TEM analysis of the synthesised calcium carbonate

A typical TEM image of the synthesized calcium carbonate in the residue is shown in Supplementary Fig. 9. An analysis of the SAED pattern (Supplementary Fig. 9b) and EDS (Supplementary Fig. 9d) revealed that the observed calcium carbonate was calcite.

Recovery of guanine from the synthesized guanosine

The water extract, the 2% HCl extract, and the 20% HCl extract contained approximately 20%, 30%, and 100% of the initial guanine, respectively (Supplementary Figure 11). The water extract and the 2% HCl extract both contained abundant guanosine isomers, indicating that approximately 80% of the initial guanine was combined with ribose during the dry-down process, and the 2% HCl extraction slightly hydrolyzed the products to guanine. Conversely, the 20% HCl extract did not contain these isomers and contained 100% of the initial guanine, indicating that the combined guanine was reversibly hydrolyzed by heating in 20% HCl.

Melamine and its homologues in the soil samples at the Murchison landing site

Supplementary Figure 17 shows concentrations of melamine and its homologues detected in the 2% HCl extract from the soil sample collected at the Murchison meteorite strewn field (see Supporting Information of Ref. S1). Melamine, ammeline, ammelide, and cyanuric acid were detected in the extract from the soil. However, the observed distribution was quite different from that for the Murchison meteorite; cyanuric acid was the most abundant, followed by melamine, ammelide, and ammeline. Since the inner portion of the Murchison meteorite was picked up for the analysis of sugars, HMT, and nucleobases^{S1,S2,S4}, these molecules in the soil would not have contributed to the meteorite analysis. Hence, the observed distribution in the Murchison extract should be indigenous to the meteorite.

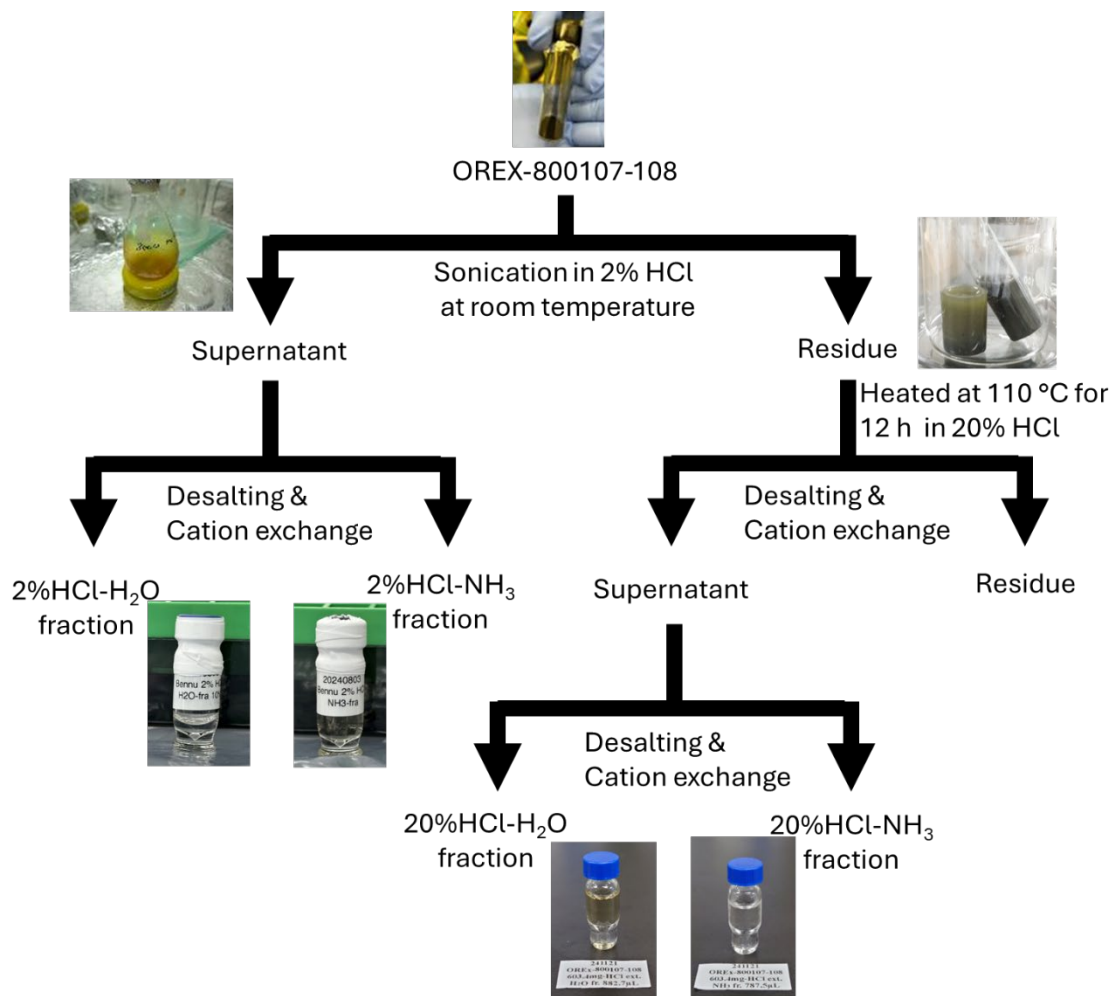
Supplementary References

S1. Oba, Y. *et al.* Extraterrestrial hexamethylenetetramine in meteorites-a precursor of prebiotic chemistry in the inner solar system. *Nat. Commun.* **11**, 6243 (2020).

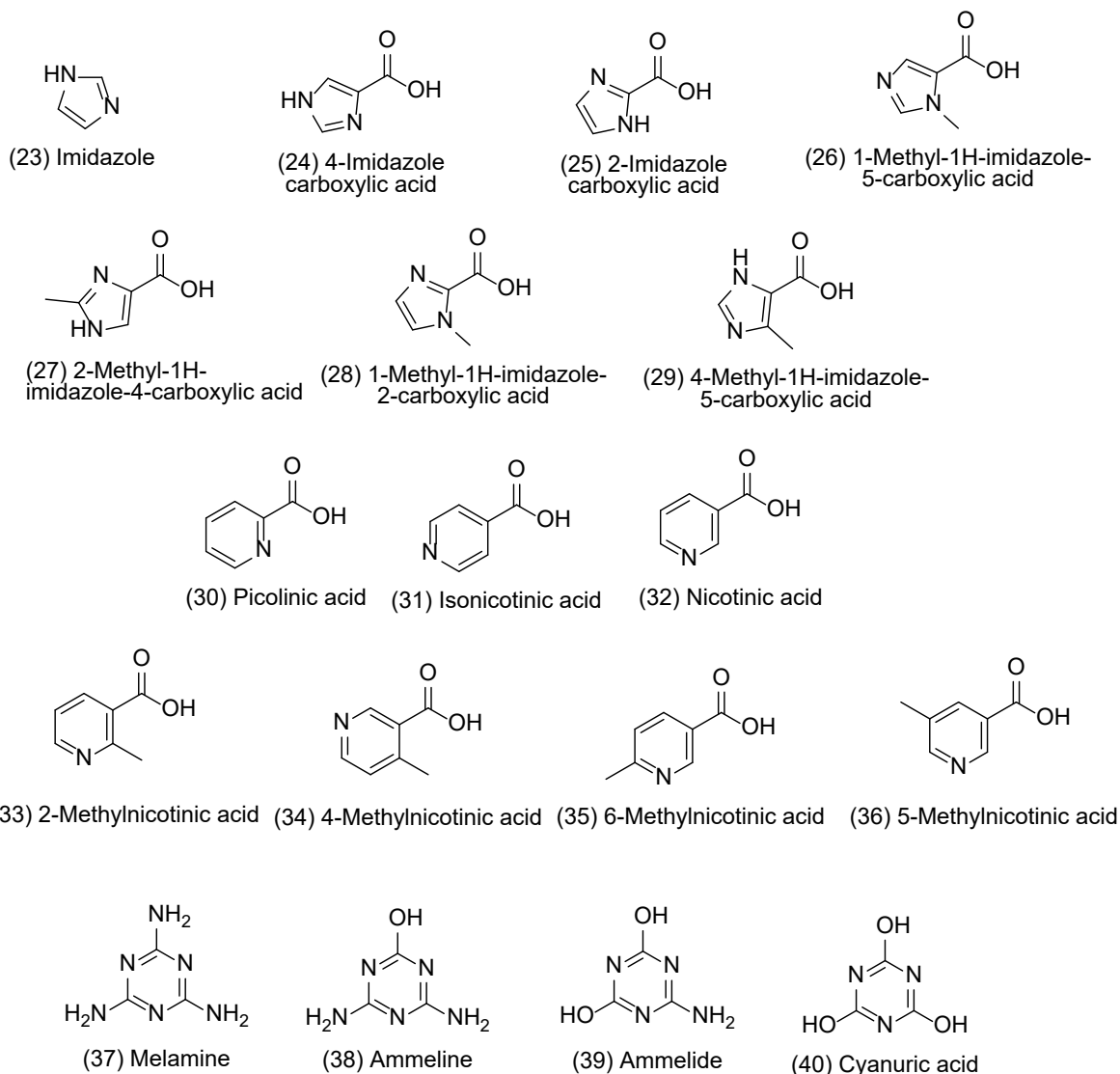
S2. Oba, Y. *et al.* Identifying the wide diversity of extraterrestrial purine and pyrimidine nucleobases in carbonaceous meteorites. *Nat. Commun.* **13**, 2008 (2022).

S3. Seto, Y. & Ohtsuka, M. ReciPro: free and open-source multipurpose crystallographic software integrating a crystal model database and viewer, diffraction and microscopy simulators, and diffraction data analysis tools. *J. Appl. Cryst.* **55**, 397 (2022).

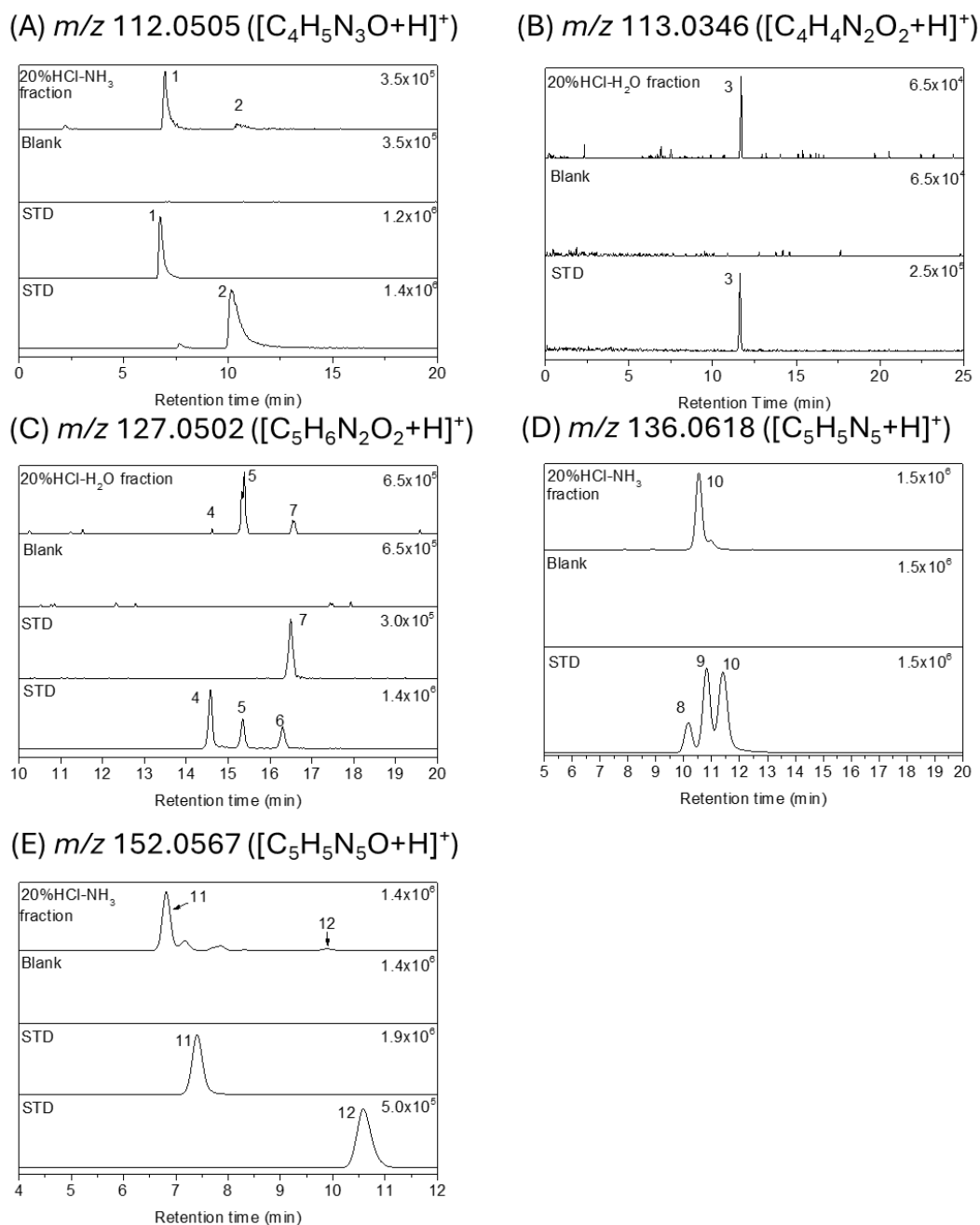
S4. Furukawa, Y. *et al.* Extraterrestrial ribose and other sugars in primitive meteorites. *Proc. Natl. Acad. Sci. USA* **116**, 24440–24445 (2019).



Supplementary Figure 1. Analytical protocol. Flow charts of the analysis of N-heterocycles in Bennu sample OREX-800107-108.



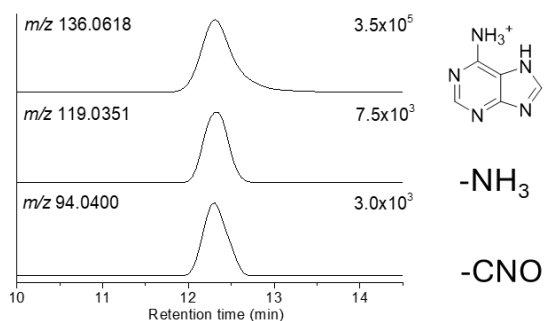
Supplementary Figure 2. Structures of the representative non-nucleobase N-heterocycles. Molecular structures of representative N-heterocycles that we searched for in Bennu sample OREX-800107-108. The numbers in parentheses correspond to those shown in the chromatograms (e.g., Figures 5 and 6).



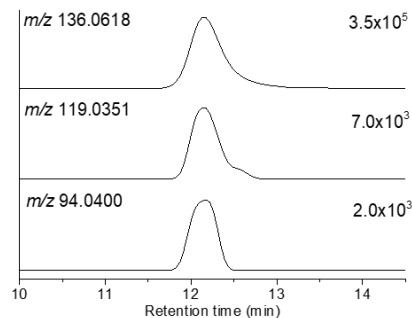
Supplementary Figure 3. Canonical nucleobases in the 2% HCl extract. Extracted ion chromatograms at the mass-to-charge ratio (m/z) of (A) 112.0505, (B) 113.0346, (C) 127.0502, (D) 136.0618, and (E) 152.0567, corresponding to the protonated ions of cytosine, uracil, thymine, adenine, and guanine, respectively, in the 2% HCl extract from OREX-800107-108, and those for the blank samples (SiO_2) and their standard reagents are shown for comparison. Peaks were identified by

comparison of their retention times, exact monoisotopic masses with those in the standard reagents analyzed on the same day, an MS/MS analysis and/or a co-injection measurement, and are designated by peak number as follows: (1) cytosine, (3) uracil, (5) 6-methyluracil, (7) thymine, (10) adenine, and (11) guanine. The numbers in the upper left corners indicate the y-axis scale for each chromatogram.

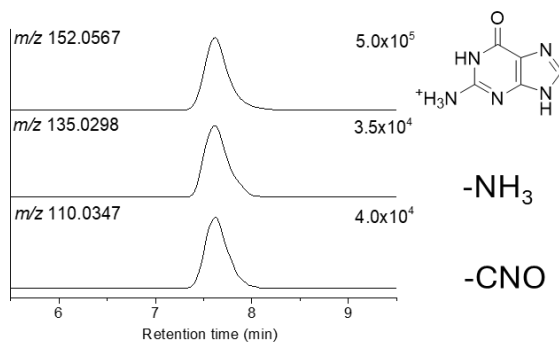
(A) Adenine STD



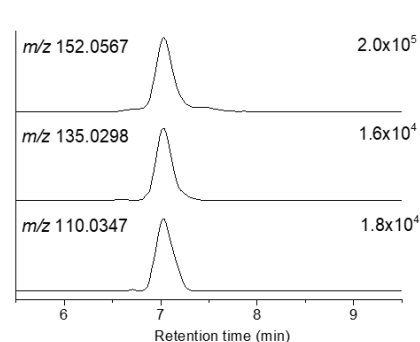
(B) Adenine in 2% HCl-NH_3 fraction



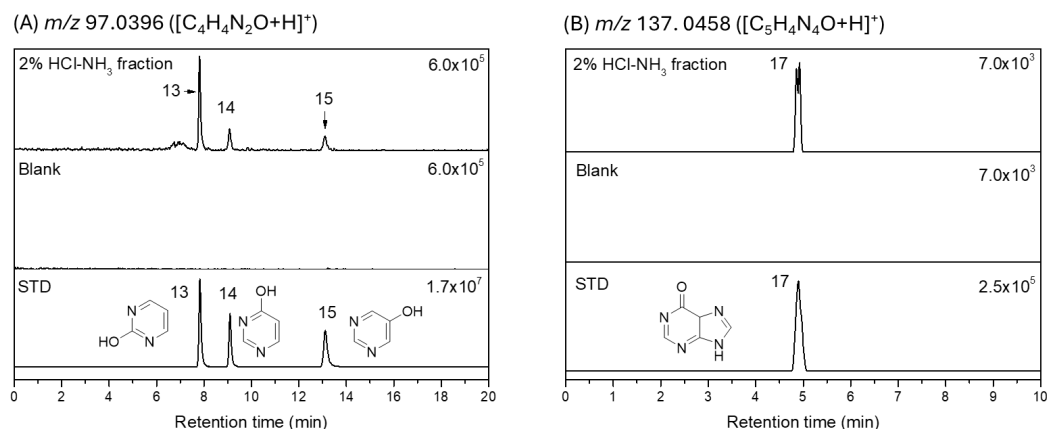
(C) Guanine STD



(D) Guanine in 2% HCl-NH_3 fraction

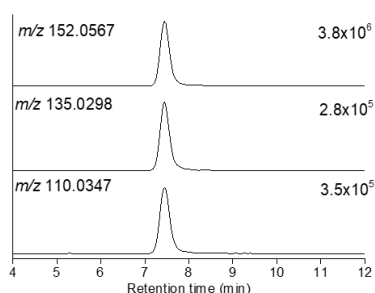


Supplementary Figure 4. Confirmation of adenine and guanine in the 2% HCl extract. Extracted ion chromatograms in MS/MS experiments at the m/z corresponding to the parent ion of adenine and its daughter ions for (A) Bennu sample OREX-800107-108 (2% HCl-NH_3 fraction) and (B) the standard reagent, and that of guanine and its daughter ions for (C) OREX-800107-108 (2% HCl-NH_3 fraction) and (D) the standard reagent (STD). From top to bottom in each panel: chromatograms for the parent ion (m/z 136.0618 for adenine or 152.0567 for guanine), the fragment after the loss of NH_3 (m/z 119.0351 or 135.0298), and that after the loss of CNO (m/z 94.0400 or 110.0347).

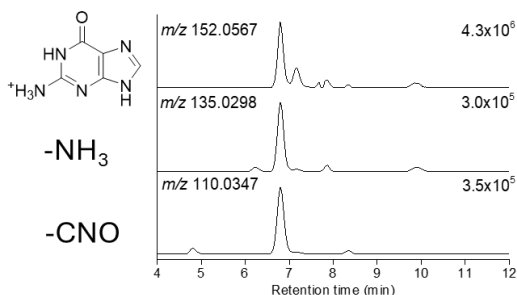


Supplementary Figure 5. Non-canonical nucleobases in the 2% HCl extract. Extracted ion chromatograms at the m/z of (A) 97.0396 and (B) 137.0458, corresponding to the protonated ions of monohydroxypyrimidines and hypoxanthine, respectively, in the 2% HCl extract from Bennu sample OREX-800107-108, and those for the blank sample (SiO₂) and their standard reagent are shown for comparison. Peaks were identified by comparison of their retention times, exact monoisotopic masses with those in the standard reagent analyzed on the same day as follows: (13) 2-hydroxypyrimidine, (14) 4-hydroxypyrimidine, (15) 5-hydroxypyrimidine, and (17) hypoxanthine. The numbers in the right indicate the y-axis scale for each chromatogram.

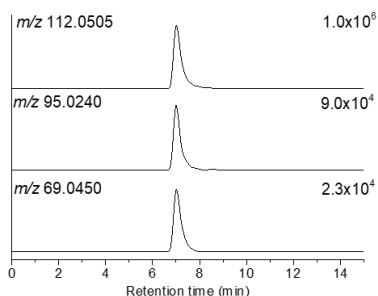
(A) Guanine STD



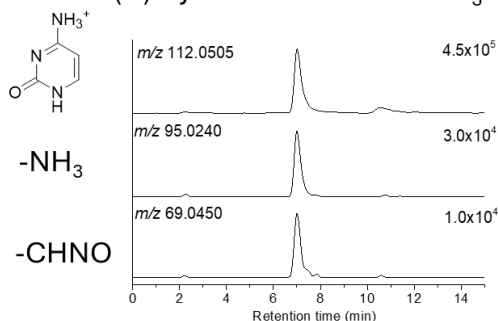
(B) Guanine in 20%HCl-NH₃ fraction



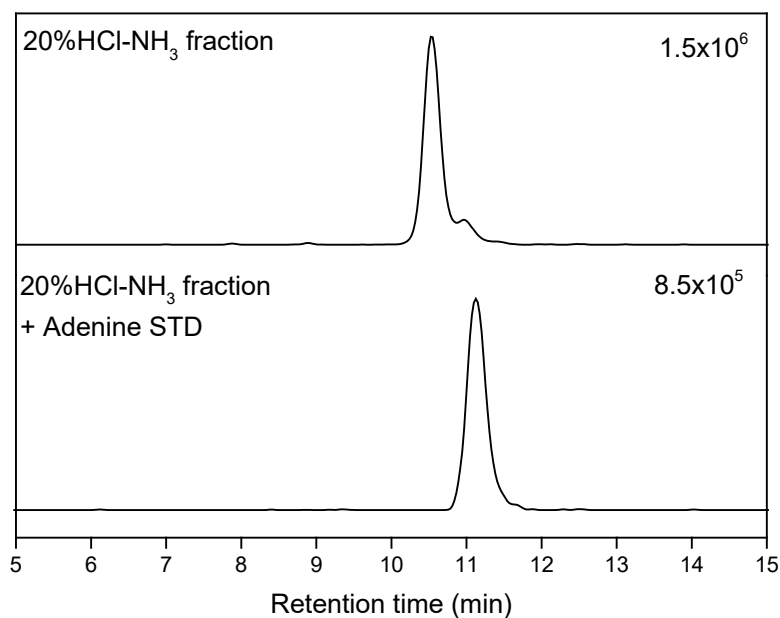
(C) Cytosine STD



(D) Cytosine in 20%HCl-NH₃ fraction

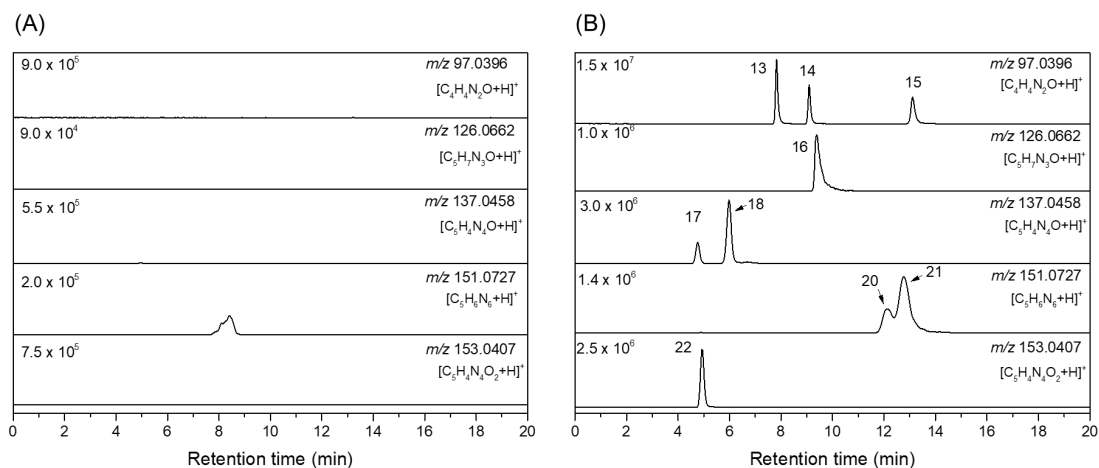


Supplementary Figure 6. Confirmation of guanine and cytosine in the 20% HCl extract. Extracted ion chromatograms in the MS/MS experiments at the m/z corresponding to the parent ion of guanine and its daughter ions for (A) Bennu sample OREX-800107-108 (20%HCl-NH₃ fraction) and (B) the standard reagent, and that of cytosine and its daughter ions for (C) OREX-800107-108 (20%HCl-NH₃ fraction) and (D) the standard reagent. From top to bottom in panels (A) and (B): chromatograms for the guanine parent ion (m/z 152.0567), the fragment after the loss of NH₃ (m/z 135.0298), and that after the loss of CNO (m/z 110.0347). From top to bottom in panels (C) and (D): chromatograms for the cytosine parent ion (m/z 112.0505), the fragment after the loss of NH₃ (m/z 95.0240), and that after the loss of CHNO (m/z 69.0450).

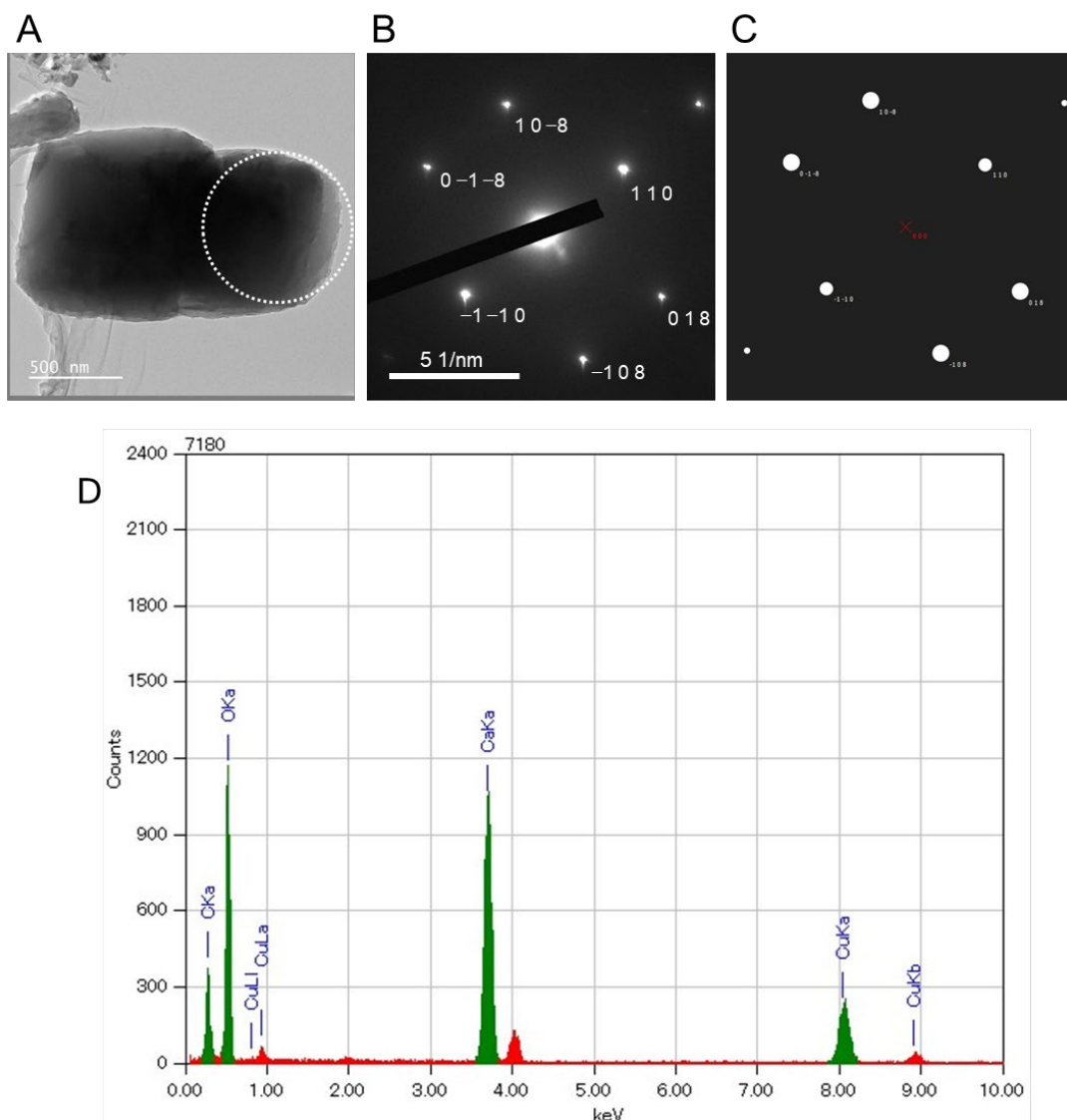


Supplementary Figure 7. Confirmation of adenine in the 20% HCl extract.

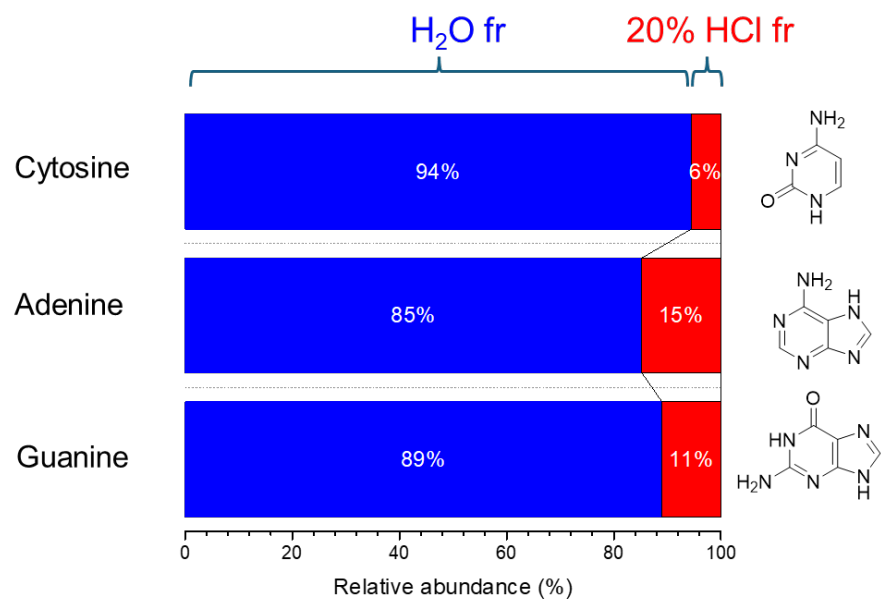
Extracted ion chromatograms at the m/z of 136.0618 for (top) the 20% HCl-NH₃ fraction and (bottom) co-injected samples of the 20% HCl-NH₃ fraction and the standard reagent of adenine. In the chromatogram for the co-injection, where the volume of the Bennu extract was 40% of the single injection and an approximately equal amount of adenine was coinjected, no additional peak was observed, indicating that the predominant peak in the sample chromatogram was derived from adenine. The observed difference in the peak retention time could be due to the different degree of matrix effect from the sample in each measurement.



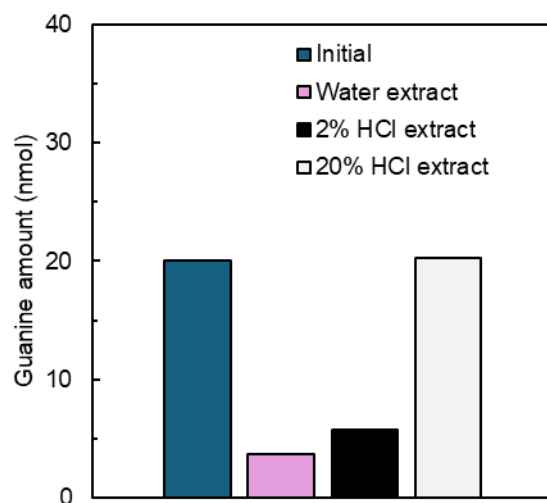
Supplementary Figure 8. Procedural blanks and standard reagents for non-canonical nucleobases. Extracted ion chromatograms at the m/z of 97.0396, 126.0662, 137.0458, 151.0727, and 153.0407 for (A) the 20% HCl-NH₃ fraction from the blank sample (SiO₂) and (B) the standard reagents: (13) 2-hydroxypyrimidine, (14) 4-hydroxypyrimidine, (15) 5-hydroxypyrimidine, (16) 5-methylcytosine, (17) hypoxanthine, (18) 7H-purin-8-ol, (20) 6,8-diaminopurine, (21) 2,6-diaminopurine, and (22) xanthine. The corresponding sample chromatograms are shown in Figure 4. The numbers in the left indicate the y-axis scale for each chromatogram.



Supplementary Figure 9. Synthesized CaCO_3 . (A) Typical TEM image of a calcium carbonate washed with water. The area enclosed by the white dotted circle is where selected area electron diffraction (SAED) and elemental analysis were performed. (B) The SAED pattern of the crystal in (A). The SAED pattern corresponds to the $[-88-1]$ SAED pattern of calcite. (C) The simulated $[-88-1]$ SAED pattern of calcite. The SAED pattern was simulated by *Recipro* and crystallographic data from the American Mineralogist Crystal Structure Database (ID: 0009873). (D) The EDS spectrum of the crystal in (A). The EDS spectrum was obtained from the area enclosed by the white dotted circle in panel (A). No elements other than calcium carbonate and TEM grid (Cu) materials were observed.

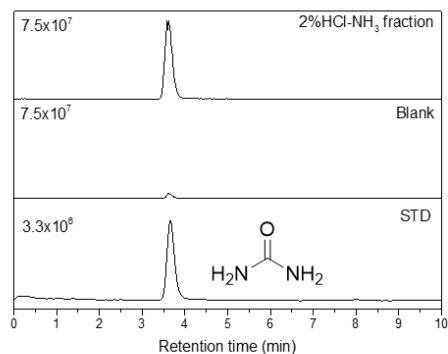


Supplementary Figure 10. Nucleobases trapped in carbonates. Recovery of nucleobases (cytosine, adenine, and guanine) from the synthesized CaCO_3 by successive extractions with pure water (blue) and 20% HCl (red).

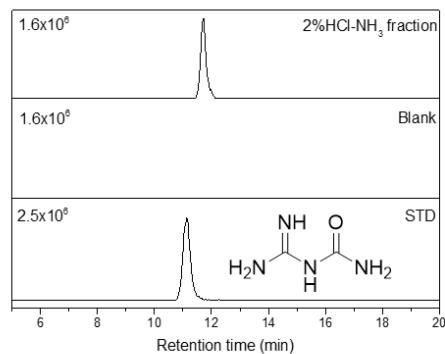


Supplementary Figure 11. Guanine recovery experiment. The amount of guanine (in nmol) used for the synthesis of guanosine isomers, extracted from the synthesized guanosine isomers with water, 2% HCl, and 20% HCl.

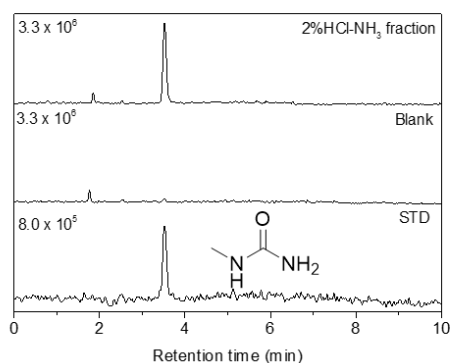
(A) Urea



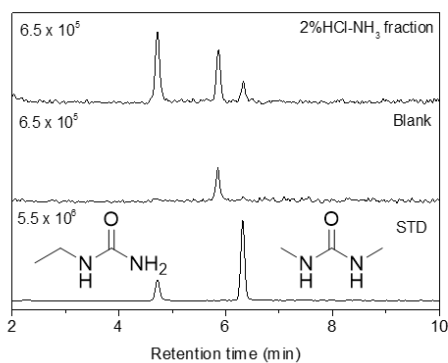
(B) Guanylurea



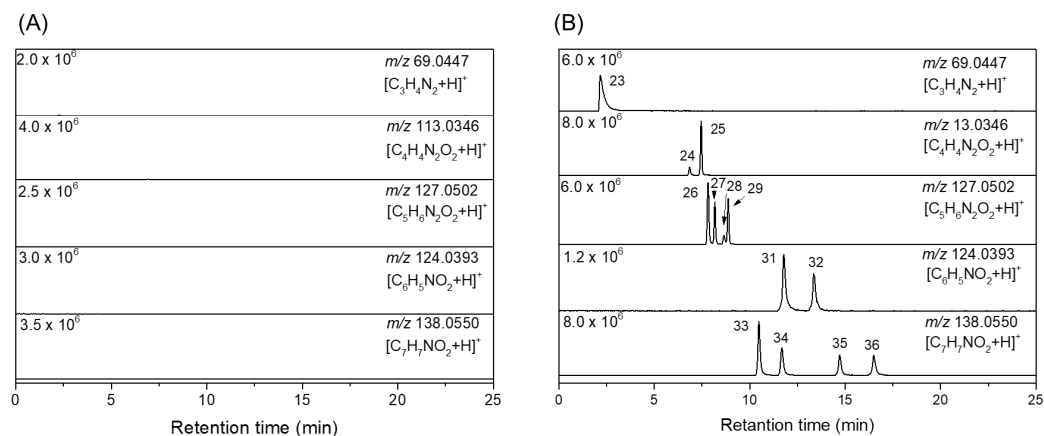
(C) 1-Methylurea



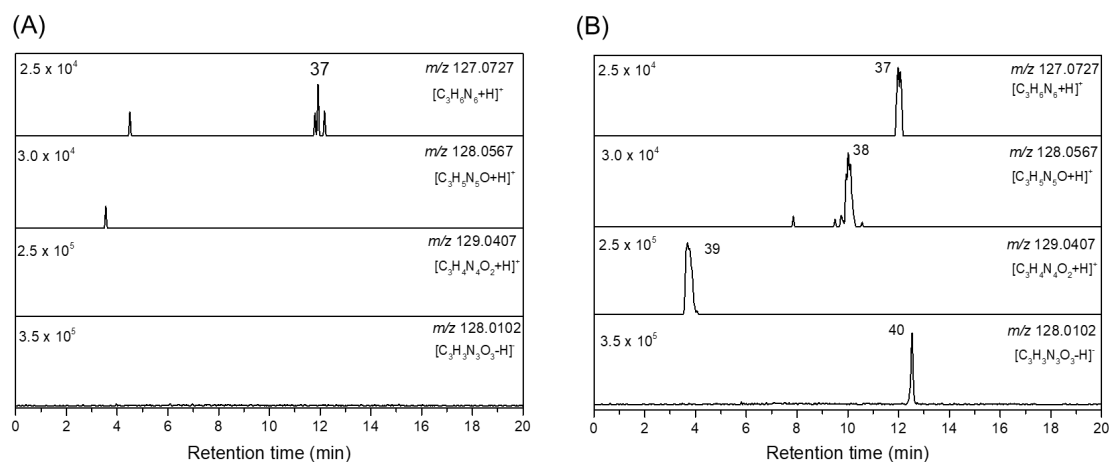
(D) C2-urea



Supplementary Figure 12. Urea and its analogues in the 2% HCl extract. Extracted ion chromatograms at the *m/z* corresponding to (A) urea, (B) guanylurea, (C) 1-methylurea, and (D) C2-urea (1-ethylurea at ~4.8 min and 1,3-dimethylurea at 6.3 min) in the 2% HCl extract from the sample (top), the blank sample (SiO₂; middle), and their standard reagents (bottom). The numbers in the left indicate the y-axis scale for each chromatogram.

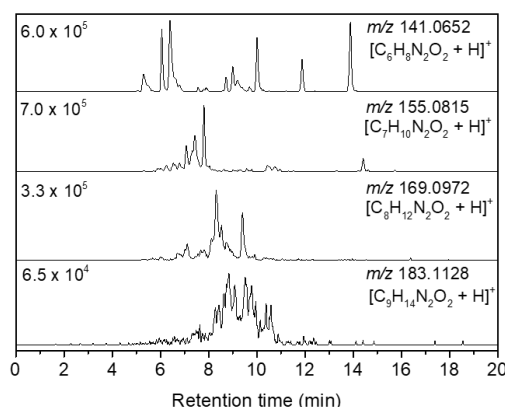


Supplementary Figure 13. Blanks and standard reagents of non-nucleobase N-heterocycles. Extracted ion chromatograms at the m/z of (from top to bottom) 69.0447, 113.0346, 127.0502, 124.0396, and 138.0550, (A) in the 20% HCl extract from the blank sample (SiO_2) and standard reagents: (23) imidazole, (244) 4-imidazole carboxylic acid, (25) 2-imidazole carboxylic acid, (26) 1-methyl-1H-imidazole-5-carboxylic acid, (27) 2-methyl-1H-imidazole-4-carboxylic acid, (28) 1-methyl-1H-imidazole-2-carboxylic acid, (29) 4-methyl-1H-imidazole-5-carboxylic acid, (31) isonicotinic acid, (32) nicotinic acid, (33) 2-methylnicotinic acid, (34) 4-methylnicotinic acid, (35) 6-methylnicotinic acid, and (36) 5-methylnicotinic acid. The numbers in the left indicate the y-axis scale for each chromatogram.

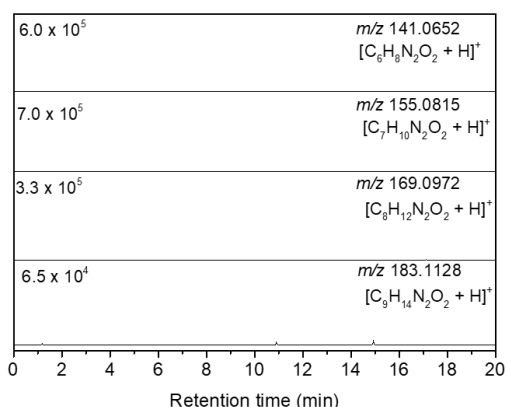


Supplementary Figure 14. Blanks and standard reagents of melamine and its homologues. Extracted ion chromatograms at the *m/z* of (from top to bottom) 127.0727, 128.0567, 129.0407, and 128.0102, (A) in the 2% HCl extract from the blank sample (SiO₂) and (B) standard reagents: (37) melamine, (38) ammeline, (39) ammelide, and (40) cyanuric acid. The bottom spectra were obtained in the negative ion mode. The numbers in the left indicate the y-axis scale for each chromatogram.

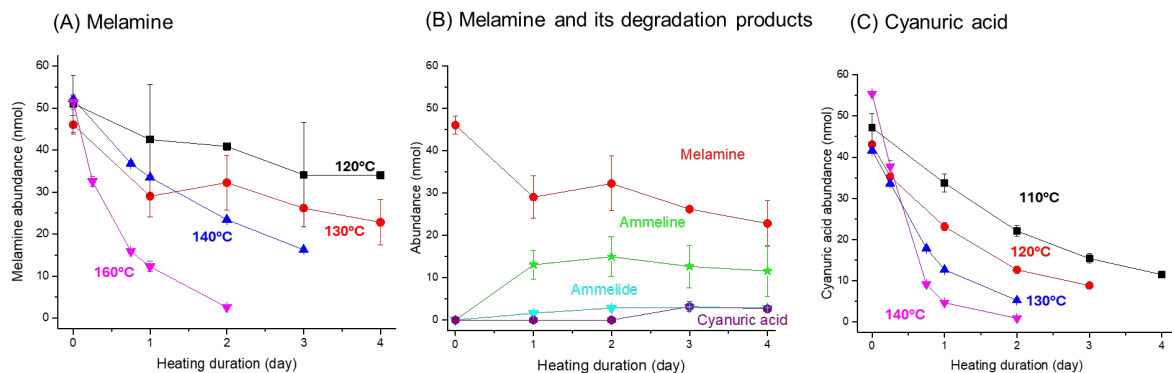
(A) OREX-800107-108 20% HCl-NH_3 fr.



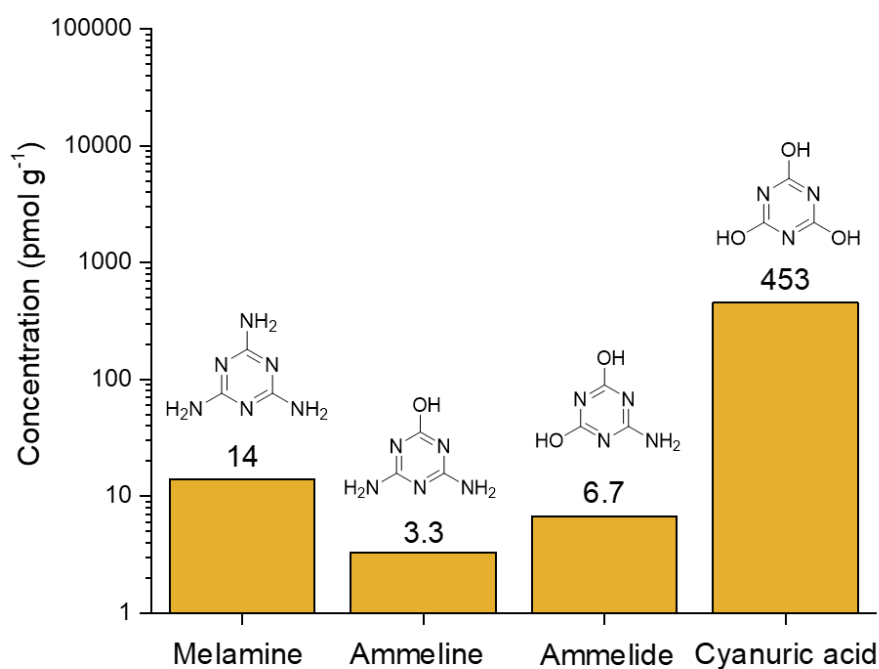
(B) Blank 20% HCl-NH_3 fr.



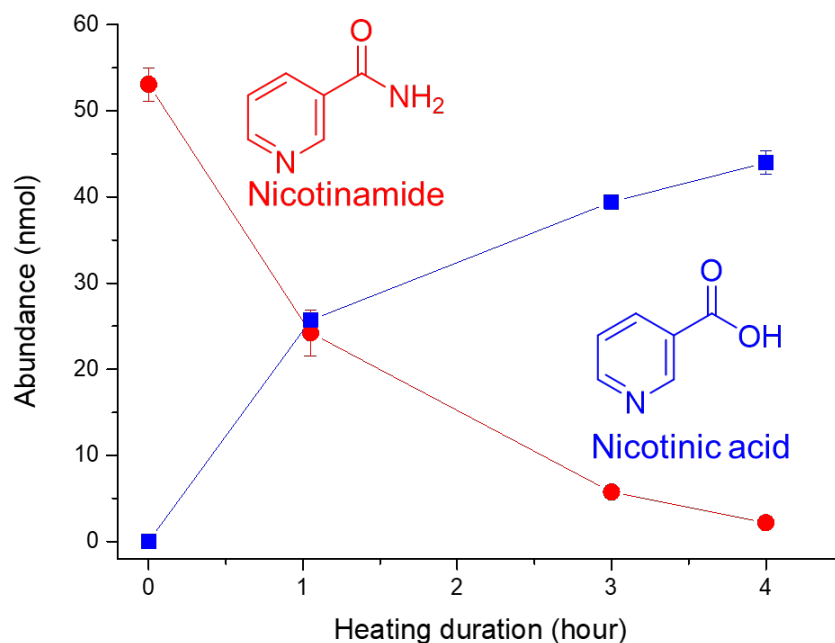
Supplementary Figure 15. Diverse suite of N-heterocycles. Extracted ion chromatograms at the m/z of (from top to bottom) 141.0652, 155.0815, 169.0972, and 183.1128, which correspond to those of the alkylated homologues of imidazole carboxylic acids, (A) in the 20% HCl extract from OREX-800107-108 and (B) blank samples (SiO_2). The numbers on the left indicate the range of peak intensity. Although a number of peaks were detected in the sample chromatograms, none of them can be assigned to any specific molecules due to the lack of their authentic chemical reagents. Based on the current analytical procedure and the mass accuracy, we expect that these peaks are derived from alkylated imidazole carboxylic acids. The numbers in the left indicate the y-axis scale for each chromatogram.



Supplementary Figure 16. Degradation of melamine and cyanuric acid by heating. Variations in the abundance of (A) melamine after heating in aqueous ammonia solution (0.3%) at 120-160 °C for up to 4 day, (B) melamine, ammeline, ammelide, and cyanuric acid after the heating of melamine in the ammonia solution at 130 °C for 4 days, and (C) cyanuric acid after heating in aqueous ammonia solution (0.3%) at 100-140°C for up to 3 days. Lines are guide for eyes. The error bar represents a standard error of two measurements ($n = 2$), $\delta_x = \sigma_x \cdot (n)^{0.5}$.

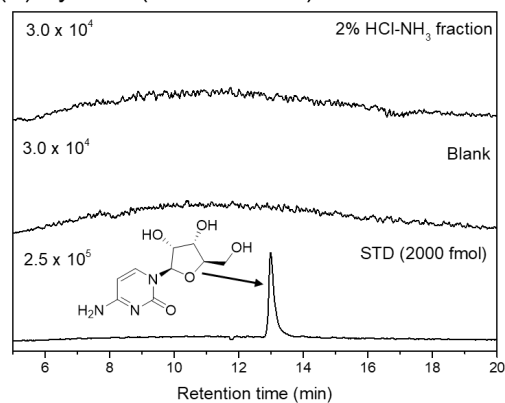


Supplementary Figure 17. Melamine and its homologues in soil samples from the Murchison meteorite landing site. Concentrations of melamine, ammeline, ammelide, and cyanuric acid detected in the 2% HCl extract from the soil at the landing site of the Murchison meteorite^{S1,S2}. The number above each bar corresponds to their concentrations in picomoles per gram.

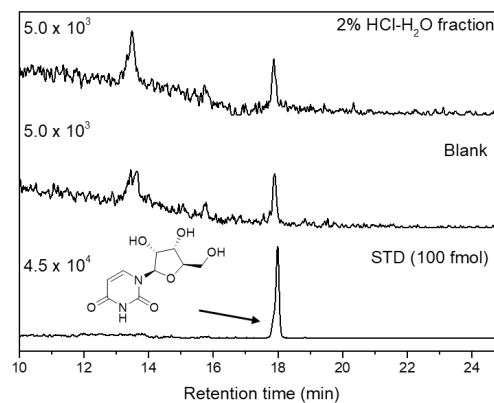


Supplementary Figure 18. Conversion from nicotinamide to nicotinic acid in NH_3 -rich fluid. Variations in the abundance (in nanomoles) of nicotinamide (red circle) heated in an aqueous 0.3% NH_3 solution at 110 °C for up to 4 days, as well as those of nicotinic acid (blue square) formed in the same solution for comparison. Lines are guide for eyes. The error bar represents a standard error of two measurements ($n = 2$), $\delta_x = \sigma_x \cdot (n)^{0.5}$.

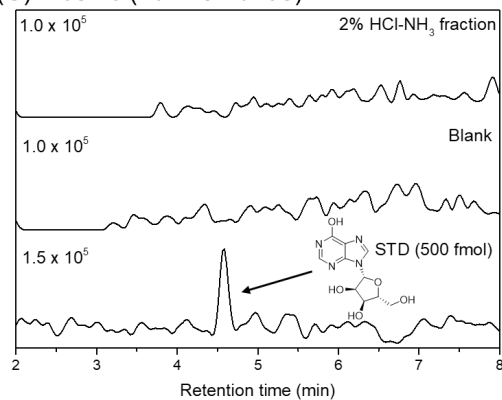
(A) Cytidine (m/z 242.0782)



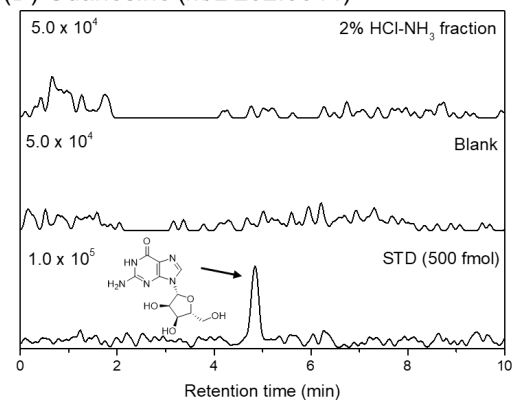
(B) Uridine (m/z 243.0623)



(C) Inosine (m/z 267.0735)



(D) Guanosine (m/z 282.0844)



Supplementary Figure 19. Non-detection of nucleosides in the Bennu sample. Extracted ion chromatograms (negative ion mode) at the m/z corresponding to (A) cytidine, (B) uridine, (C) Inosine, and (D) guanosine in the 2% HCl extract from the Bennu sample (top), the SiO₂ blank (middle), and their standard reagents (bottom). The numbers in the left indicate the y-axis scale for each chromatogram.

Supplementary Table 1. DOIs of the measurement data products from the Bennu sample analysed in this study that will be archived at <https://astromat.org>.

LCMS Data		
DOI	Product Name	Product Type
LCMS	OREX-800107-108	
10.60707/35y7-jb12	20241127_LC-MS_KU_OREX-800107-108_1_LCMSCollection_1.zip	LCMSCollection
10.60707/a55a-q487	20241127_LC-MS_KU_OREX-800107-108_1_LCMSCollection_2.zip	LCMSCollection
10.60707/b15a-z961	20241127_LC-MS_KU_OREX-800107-108_1_LCMSCollection_3.zip	LCMSCollection
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10.60707/q6ey-7d66	20241127_LC-MS_KU_OREX-800107-108_1_LCMSCollection_5.zip	LCMSCollection
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10.60707/2tbh-1g54	20241127_LC-MS_KU_OREX-800107-108_1_LCMSCollection_7.zip	LCMSCollection
10.60707/5k8s-jq94	20241127_LC-MS_KU_OREX-800107-108_1_LCMSCollection_8.zip	LCMSCollection
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10.60707/m34s-m036	20241127_LC-MS_KU_OREX-800107-108_1_LCMSCollection_11.zip	LCMSCollection
10.60707/jbdq-nt31	20241127_LC-MS_KU_OREX-800107-108_1_LCMSCollection_12.zip	LCMSCollection
10.60707/cafp-v173	20241127_LC-MS_KU_OREX-800107-108_1_LCMSCollection_13.zip	LCMSCollection
10.60707/7wxc-wj08	20241127_LC-MS_KU_OREX-800107-108_1_LCMSCollection_14.zip	LCMSCollection
10.60707/cjz6-wm31	20241127_LC-MS_KU_OREX-800107-108_1_LCMSCollection_15.zip	LCMSCollection
10.60707/a8vh-k192	20241127_LC-MS_KU_OREX-800107-108_1_LCMSCollection_16.zip	LCMSCollection
10.60707/ry7k-5014	20241127_LC-MS_KU_OREX-800107-108_1_LCMSCollection_17.zip	LCMSCollection