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Supplementary information

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A complete set of canonical nucleobases in the carbonaceous asteroid (162173) Ryugu

In the format provided by the authors and unedited

Supplementary Text

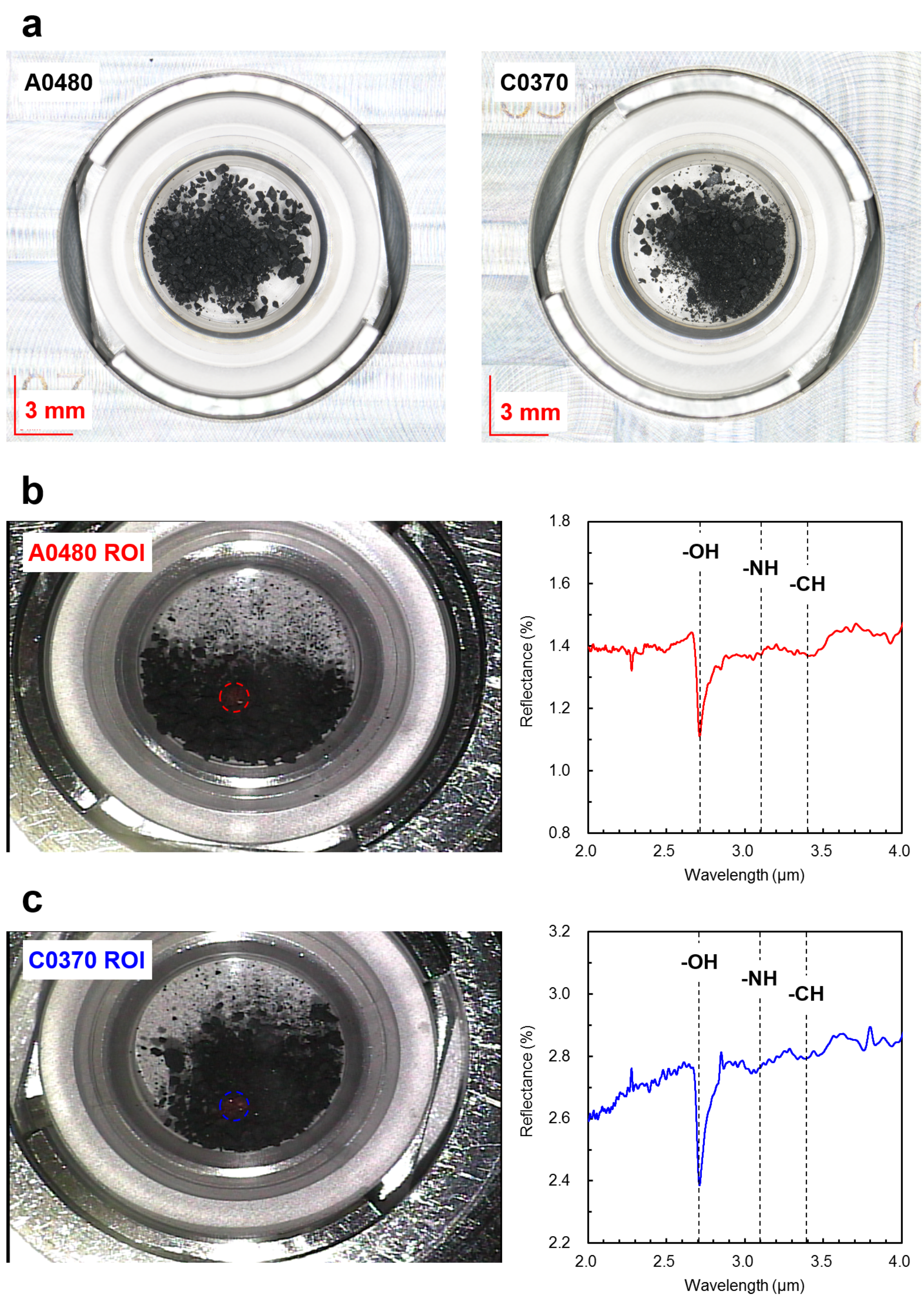
Nitrogen isotopic composition of soluble components in H_2O and HCl extracts. The large ^{15}N enrichment of the soluble components in the H_2O and HCl extracts (+59.8‰ for A0480; +45.1‰ for C0370, Extended Data Table 1) falls well outside the $\delta^{15}N$ terrestrial organics range of –10‰ to +20‰[1]. Although urea was the most abundant individual N-containing molecule detected in the Ryugu samples (Extended Data Table 2), it accounted for only 0.28% and 1.1% of the total nitrogen in the soluble fractions of A0480 and C0370, respectively. It is worth noting that ammonia concentrations in the Ryugu A0106 and C0107 were substantially lower (~0.18 and <0.02 μmol g^{-1}, respectively)[2] compared to asteroid Bennu (~13.6 μmol g^{-1})[3] and Orgueil meteorite (~33.5 μmol g^{-1})[2]; the relative contributions of ammonia to total soluble nitrogen in the Ryugu samples (0.30% and 0.05%, respectively) are comparable to those of urea. Therefore, the bulk of the ^{15}N enrichment in the soluble components is likely attributable to a diverse array of unidentified N-containing molecules, as previously suggested by Fourier transform–ion cyclotron resonance mass spectrometry analyses[4,5].

Other N-containing molecules detected using CE-HRMS. In addition to N-heterocycles, urea, ethanolamine, and several amino acids were detected in the Ryugu samples by CE-HRMS analyses (Extended Data Fig. 7). Because slight amounts of these compounds were also found in the procedural blank—likely introduced during the desalting process (Methods)—blank-subtracted concentrations are reported in Extended Data Table 2. Urea, the most abundant organic molecule detected (50 nmol g^{-1} in A0480; 132 nmol g^{-1} in C0370), is considered important in prebiotic chemistry as a reactant, catalyst, and solvent[6–8]. Ethanolamine, the head group of a major class of phospholipids in biological membranes, was detected in both Ryugu samples (0.088 nmol g^{-1} in A0480 and 0.33 nmol g^{-1} in C0370) at concentrations comparable to that in the Almahata Sitta meteorite, a fragment of asteroid 2008 TC3 (0.28 nmol g^{-1})[9]. Given the markedly different thermal histories of these bodies—low-temperature aqueous alteration on Ryugu (~40 °C)[10] and intense metamorphism in Almahata Sitta (1200–1300 °C)[11]—the similarity in ethanolamine abundance is noteworthy. C_3 amino acids, including DL-α-alanine, β-alanine, and sarcosine, were also identified in the Ryugu extracts. The HCl extracts showed higher α-alanine concentrations (5.5 and 7.4 nmol g^{-1} for A0480 and C0370, respectively) than those in the blank and in previous analyses of A0106 and C0107 samples (0.17–4.3 nmol g^{-1})[4,12,13]. In contrast, β-alanine concentrations in A0480 and C0370 (0.93 and 0.85 nmol g^{-1}, respectively) were lower than in A0106 and C0107 (3.2 and 3.7 nmol g^{-1})[12]. Sarcosine, not previously reported in Ryugu samples[4,12,13], was identified exclusively in the HCl extracts of both A0480 and C0370. The suite of N-containing molecules identified—including nicotinic acids, urea, ethanolamine, and amino acids—highlights the complex organic chemistry that occurred on Ryugu's parent body.

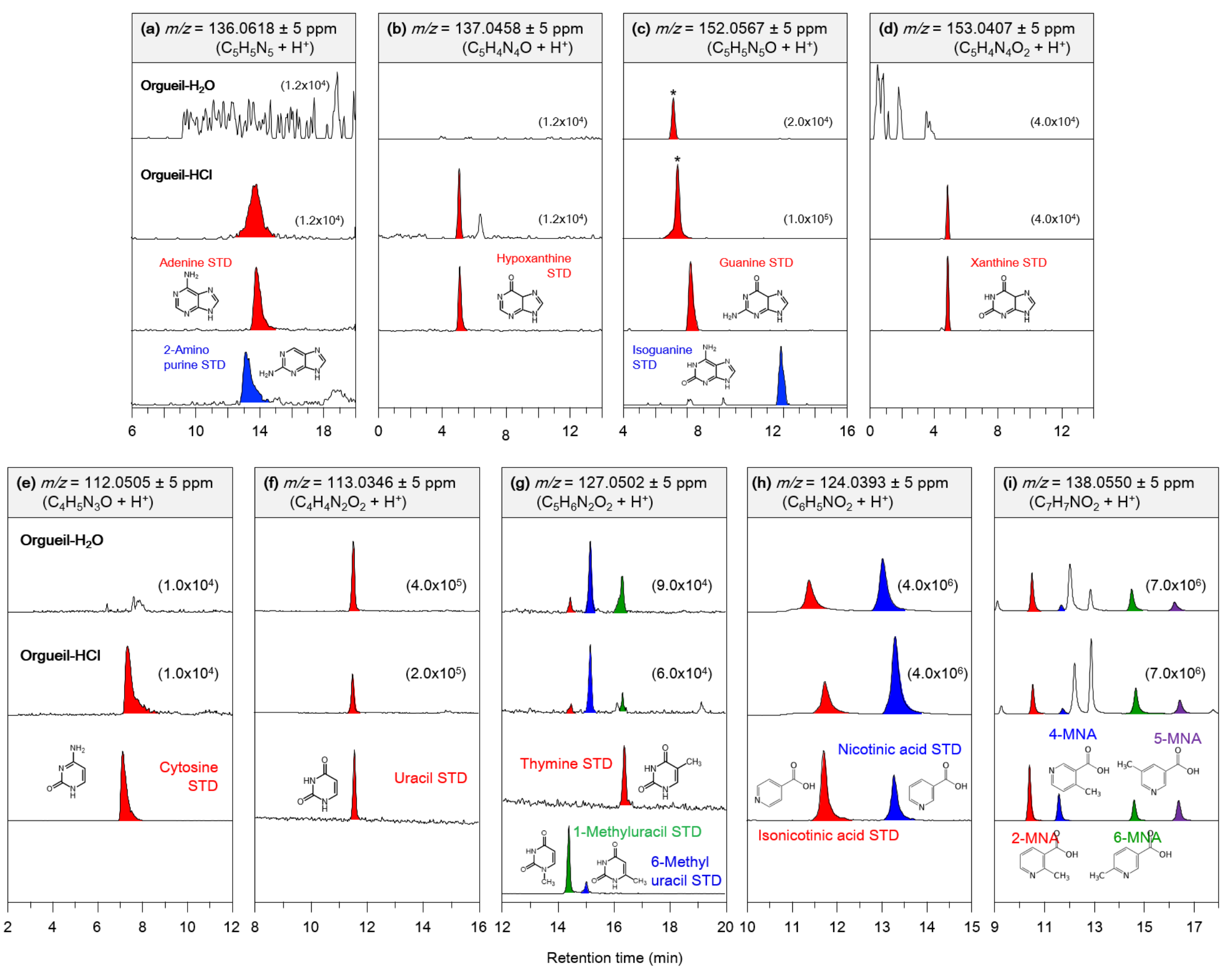
Contrasting H_2O and HCl extractable organics highlight organo–mineral associations. The sequential extraction procedure employed in this study revealed that more than half of the nucleobases in the Ryugu samples are recovered in the second-step HCl extracts, whereas in the Orgueil meteorite, the majority are found in the first-step H_2O extracts (Table 1 and Supplementary Table 2). This contrasting distribution suggests differences in the degree of nucleobase incorporation into, or binding with, mineral matrices and/or the IOM, which in turn affect molecular accessibility and preservation. The higher abundances of ethanolamine and C_3 amino acids in the HCl extracts relative to the H_2O extracts (Extended Data Table 2) further demonstrate the efficacy of hydrochloric acid in liberating a broad spectrum of pristine asteroidal organics. Together, these findings emphasize the critical role of extraction methodology in capturing the full diversity of extraterrestrial organic compounds and indicate that complex organo-mineral associations influence the stability and extractability of SOM. Performing water extraction prior to HCl treatment is expected to minimize the contribution of molecules newly formed under strongly acidic conditions, as water-soluble reactive precursors are likely removed in the initial step; nevertheless, we cannot entirely exclude the possibility that some reactive molecules retained within mineral matrices or insoluble organic matter contributed to the compounds observed in the HCl extracts.

The Orgueil meteorite analyzed in this study exhibits a distinct nucleobase distribution between the H_2O and HCl extracts: the H_2O extract is dominated by pyrimidines (~83%), whereas the HCl extract is dominated by purines (~95%) (Supplementary Table 2). A similar distribution pattern has been reported for the Murchison meteorite[14], suggesting differential preservation of purine and pyrimidine nucleobases within mineral matrices and/or insoluble organic matter (IOM) in carbonaceous meteorites. Although Ryugu A0480 shows nearly equal abundances of purine and pyrimidine nucleobases in the H_2O and HCl extracts (Supplementary Table 2), Ryugu C0370 exhibits a broadly similar distribution, with ~76% of purines recovered in the HCl extract (Supplementary Table 2). Although the differences between A0480 and C0370 are difficult to explain, considering that C0370 includes sub-surface rock fragments, the nucleobase distribution in A0480 may originally have resembled that of C0370 but was subsequently altered by exposure to ultraviolet and cosmic rays on the surface of Ryugu's parent body.

Standards and reagents. Authentic standards for the nucleobases and other N-heterocyclic compounds were purchased from Tokyo Chemical Industry, Sigma-Aldrich, FUJIFILM Wako Pure Chemical, Combi-Blocks, Toronto Research Chemicals, and BLD Pharmatech Ltd. Stock mixed N-heterocyclic compound solutions were prepared by dissolving individual analyte crystals (purities ranging from 96 to 100%) in Milli-Q water (Millipore Milli-Q grade, 18.2 MΩ) or a 20 mM NaOH solution prepared by mixing a 3 M NaOH solution (Kanto Chemical Industry Co., Ltd., Japan; ultrapur^{TM} grade) with Milli-Q water. Ultrapure water and 6 M hydrochloric acid (HCl) (Tama Chemicals Co., Ltd., Japan; Tama pure AA-10 grade), 3 M NaOH solution, and ammonia solution (Kanto Chemical Industry Co., Ltd., Japan; ultrapur^{TM} grade, 28.0–30.0% in water) were obtained for the extraction and purification procedures. Solutions of 1.0 M and 0.1 M HCl, 1 M NaOH, and 10% ammonia in water (NH_4OH) were prepared and diluted from the abovementioned solvents using ultrapure water (Tama pure AA-10 grade). Ultrapure water (QTof-MS grade), acetonitrile (QTof-MS grade), and formic acid (LC–MS grade; >99.5% purity) were sourced from FUJIFILM Wako Pure Chemical for HPLC/ESI-HRMS analyses. All glassware and sea sand (FUJIFILM Wako Pure Chemical Corporation, Japan; 30–50 mesh) used for procedural blanks were rinsed with Milli-Q water, wrapped in aluminium foil, and subsequently heated at 450 °C for 5 h in air prior to use.



Supplementary Fig. 1. Microscope images and infrared reflectance spectra of the Ryugu aggregate samples analyzed in this study. a, Samples A0480 (11.9 mg) and C0370 (8.3 mg), collected from the first and second touchdown sites, respectively[14]. The aggregate samples were placed on a sapphire glass dish (~9 mm in diameter) set in a stainless-steel container. **b, c,** Non-destructive near-infrared spectra (2.0–4.0 µm) of the initial bulk samples acquired using MicrOmega, showing absorption features attributed to –OH, –NH, and –CH functional groups[15,16]. The source data are available in ref 17.



Supplementary Fig. 2. Purine and pyrimidine nucleobases detected in the H_2O and HCl extracts of the Orgueil meteorite sample using HPLC/HRMS analysis. Mass chromatograms at *m/z* values of (**a**) 136.0618, (**b**) 137.0458, (**c**) 152.0567, (**d**) 153.0407, (**e**) 112.0505, (**f**) 113.0346, (**g**) 127.0502, (**h**) 124.0393 and (**i**) 138.0550, corresponding to the protonated molecular ions of adenine ($C_5H_5N_5$), hypoxanthine ($C_5H_4N_4O$), guanine ($C_5H_5N_5O$), xanthine ($C_5H_4N_4O_2$), cytosine ($C_4H_5N_3O$), uracil ($C_4H_4N_2O_2$), thymine ($C_5H_6N_2O_2$), nicotinic acid ($C_6H_5NO_2$), and methylnicotinic acids ($C_7H_7NO_2$), respectively. Chromatograms of authentic standards are shown for comparison. Numbers in parentheses indicate the vertical axis range of each sample chromatogram.

Supplementary Table 1. Retention times of nucleobases in Ryugu samples and authentic standards analyzed by HPLC/HRMS with (A) PFP and (B) Hypercarb columns.

(A) PFP column

A0480-H_2O extract	Adenine*	1σ	Hypoxanthine	1σ	Guanine*	1σ	Xanthine	1σ
A0480-H2O_#1	14.99		5.14		8.83		n.d.	
A0480-H2O_#2	13.93		5.13		8.20		n.d.	
Average (n = 2)	**14.46**	**±0.75**	**5.14**	**±0.01**	**8.52**	**±0.45**		

A0480-HCl extract	Adenine	1σ	Hypoxanthine	1σ	Guanine	1σ	Xanthine	1σ
A0480-HCl_#1	13.63		5.11		8.12		4.90	
A0480-HCl_#2	13.73		5.15		8.13		4.91	
Average (n = 2)	**13.68**	**±0.07**	**5.13**	**±0.03**	**8.13**	**±0.01**	**4.91**	**±0.01**

C0470-H_2O extract	Adenine	1σ	Hypoxanthine	1σ	Guanine	1σ	Xanthine	1σ
A0480-H2O_#1	n.d.		5.11		8.24		4.87	
A0480-H2O_#2	n.d.		5.13		8.20		4.89	
Average (n = 2)			**5.12**	**±0.01**	**8.22**	**±0.03**	**4.88**	**±0.01**

C0370-HCl extract	Adenine	1σ	Hypoxanthine	1σ	Guanine	1σ	Xanthine	1σ
A0480-HCl_#1	13.99		5.11		8.22		4.84	
A0480-HCl_#2	13.95		5.08		8.20		4.85	
Average (n = 2)	**13.97**	**±0.03**	**5.10**	**±0.02**	**8.21**	**±0.01**	**4.85**	**±0.01**

Standard	Adenine*	1σ	Hypoxanthine	1σ	Guanine*	1σ	Xanthine	1σ
Standard_#1	13.92		5.15		8.35		4.88	
Standard_#2	14.87		5.39		8.45		4.96	
Standard_#3	14.13		5.29		8.51		4.96	
Standard_#4	13.86		5.11		8.26		4.85	
Standard_#5	13.86		5.14		8.29		4.90	
Standard_#6	14.43		5.20		8.66		4.93	
Standard_#7	13.83		5.11		8.26		4.87	
Average (n = 7)	**14.13**	**±0.39**	**5.20**	**±0.11**	**8.40**	**±0.15**	**4.91**	**±0.04**

(B) Hypercarb column

A0480-H_2O extract	Cytosine	1σ	Uracil	1σ	Thymine	1σ	6-Methyluracil	1σ
A0480-H2O_#1	7.51		11.50		16.32		15.18	
A0480-H2O_#2	7.45		11.49		16.35		15.15	
A0480-H2O_#3	7.26		11.53		16.36		15.18	
Average (n = 3)	**7.41**	**±0.13**	**11.51**	**±0.02**	**16.34**	**±0.02**	**15.17**	**±0.02**

A0480-HCl extract	Cytosine	1σ	Uracil	1σ	Thymine	1σ	6-Methyluracil	1σ
A0480-HCl_#1	7.47		11.52		16.35		15.17	
A0480-HCl_#2	7.49		11.56		16.33		15.17	
A0480-HCl_#3	7.50		11.55		n.d.		n.d.	
Average (n = 2 or 3)	**7.49**	**±0.02**	**11.54**	**±0.02**	**16.34**	**±0.01**	**15.17**	**±0.00**

C0470-H_2O extract	Cytosine	1σ	Uracil	1σ	Thymine	1σ	6-Methyluracil	1σ
C0370-H2O_#1	7.45		11.52		16.34		15.15	
C0370-H2O_#2	7.41		11.51		16.32		15.15	
C0370-H2O_#3	7.29		11.53		16.34		15.18	
C0370-H2O_#4	7.29							
Average (n = 3 or 4)	**7.36**	**±0.08**	**11.52**	**±0.01**	**16.33**	**±0.01**	**15.16**	**±0.02**

C0370-HCl extract	Cytosine	1σ	Uracil	1σ	Thymine	1σ	6-Methyluracil	1σ
C0370-HCl_#1	7.06		11.52		16.34		15.15	
C0370-HCl_#2	7.12		11.51		16.32		15.15	
C0370-HCl_#3			11.57		16.36		15.17	
C0370-HCl_#4			11.56		16.37		15.21	
Average (n = 2 or 4)	**7.09**	**±0.04**	**11.54**	**±0.03**	**16.35**	**±0.02**	**15.17**	**±0.03**

Standard	Cytosine	1σ	Uracil	1σ	Thymine	1σ	6-Methyluracil	1σ
Standard_#1	7.35		11.54		16.33		15.34	
Standard_#2	7.26		11.53		16.35		15.14	
Standard_#3	7.32		11.52		16.34		15.26	
Average (n = 3)	**7.31**	**±0.05**	**11.53**	**±0.01**	**16.34**	**±0.01**	**15.25**	**±0.10**

*Analyses using the PFP column occasionally exhibited slight shifts in retention times for adenine and guanine; importantly, these shifts occurred in a coordinated manner, such that when the retention time of adenine increased or decreased, guanine's retention time shifted in parallel. This coordinated behavior allows us to robustly estimate the retention time of adenine based on the MS/MS-validated retention time of guanine. Despite these minor coordinated variations, the retention time ranges for the samples were consistent with those of the standards, ensuring reliable identification of each nucleobase.

Supplementary Table 2. Relative abundances of nucleobases in the HCl extracts expressed as a fraction of the total (H_2O + HCl extracts)[a].

Ratio (HCl / H_2O + HCl)	A0480	C0370	Orgueil	Murchison
	this study	this study	this study	Koga et al.[14]
Sum all Purines	**54 ± 3%**	**76 ± 2%**	**95 ± 4%**	**84 ± 10%**
Sum all Pyrimidines	**67 ± 8%**	**44 ± 3%**	**17 ± 1%**	**15 ± 1%**
Sum all Nucleobases	**60 ± 4%**	**61 ± 2%**	**24 ± 1%**	**68 ± 7%**

[a]Calculated from the values listed in Table 1.

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