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Supplementary Information for
Stability of Nucleic Acid Bases in Concentrated Sulfuric Acid: Implications for the Habitability of Venus' Clouds

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Supplementary Information Text

1. Molecular Structure Determination of Guanine by NMR in 98% w/w Concentrated Sulfuric Acid. For guanine we find five ^{13}C NMR peaks for carbon that correspond to the five carbons in the purine ring. Each of the five peaks are found in the region of the NMR spectra associated with aromatic compounds.

We assign the carbon peaks by comparison with literature data (Table S1) and by our 2D NMR experiments (Figure S1). We can assign C5 because it is the most magnetically shielded atom in the ring structure with a chemical shift distinctly upfield from the other four carbon peaks (Table S1). The C5 chemical shift also agrees with the literature values (including in solvents DMSO-d_6 and D_2O) (1, 2). To assign C4 and C8 we use our 2D NMR where we correlate the positions of H and C within the ring. Guanine has only one protonated carbon, at C8. Our 2D ^{1}H-^{13}C HMQC shows a signal that corresponds to the C8 carbon peak at 137.53 ppm and H at 8.29 ppm. Further supporting these assignments and the integrity of the imidazole ring, the ^{1}H-^{13}C HMBC correlates the distinct ^{13}C chemical shift of C5 to the nearby H8 which in turn is correlated with C4 (see Figure S1E).

This leaves C2 and C6, which are especially difficult to assign due to their similar magnetically deshielded environment, which results in very similar, almost overlapping chemical shifts. C6 is attached to the carbonyl group that withdraws electrons from the ring resulting in deshielding of the C6 atom. C2 is deshielded by the electron withdrawing amino group. So while the two peaks that have the highest chemical shift values in the 1D ^{13}C NMR spectra belong to carbons that are attached to the most electronegative atoms, C2 and C6, it is difficult to determine which is which. Since the peak at 150.13 ppm has higher intensity than the peak at 150.19 ppm we tentatively assign the 150.13 ppm peak as carbon C2. The higher peak intensity is due to the Nuclear Overhauser Effect (NOE) that is produced by the proton decoupling carried out during the experiment. The NOE is proportional to the distance (r^{-6}) and C2 is closer to the NH_2 protons. The general trend in chemical shifts of C2 and C6 for DMSO-d_6 and D_2O reported in the literature (Table S1), where C2 is more shielded than C6, also supports this assignment. Note that the C2 and C6 can also be distinguished thanks to the very weak signal on 2D ^{1}H-^{13}C HMBC spectra (coordinates: 8.29 ppm, 150.26 ppm; not shown for clarity). The signal corresponds to carbon C6, at 150.19 ppm, at the distance of four bonds from the H8 hydrogen.

To further confirm the structure, we turn to ^{1}H (Figure S1B). For guanine the ^{1}H NMR spectral peak for the H attached to carbon is highly consistent with the peaks in other solvents to about 0.5 ppm (Table S1) and we use this consistency to assign the H attached to C8. The strong peak at 8.29 ppm corresponds to H8 of the purine ring (consistent with the literature data on the ^{1}H NMR of guanine hydrochloride in D_2O (2)).

The other peaks in our ^{1}H NMR spectrum are due to N-H hydrogens. We assign the peaks on the basis of comparison to literature data to other purines in the solvents DMSO-d_6 and D_2O (Tables S1-8). The broad peak at 7.05 ppm corresponds to the hydrogen atoms of the intact amino group of the guanine ring, consistent with literature chemical shifts for other purines. N9 and N7 are chemically similar; we tentatively assign H9 at 12.21 ppm and H7 at 11.73 ppm.

Finally, we use 1D ^{15}N NMR to show that the N atoms in the ring and in the amino group remain intact in concentrated sulfuric acid. The amino group contains the most magnetically shielded N and we therefore can assign the peak at 85.92 ppm to N atom attached to C2. We see four other peaks corresponding to nitrogen atoms of the guanine ring and make assignments (Table S1) based on other purine data available in the literature (see Table S5 and Table S7) and the ^{1}H-^{15}N HMBC experiment (Figure S1F). Together with the results of ^{1}H-^{13}C HMBC discussed above, the ^{1}H-^{15}N HMBC experiment further confirms the integrity of the imidazole ring and allows for tentative assignments of N9 and N7 atoms to signals at 159.34 ppm and 154.76 ppm respectively. We assign N7 and N9 peaks based on the known N7 and N9 chemical shifts of

purine, where N7 is more magnetically shielded (shifted towards lower ppm) in acidic conditions than N9 (Figure 7).

We now turn to the discussion of the protonation state of the guanine ring in concentrated sulfuric acid. The upfield shift (towards lower ppm) in acidic solvent of ^{13}C NMR peaks corresponding to carbon atoms directly adjacent to the nitrogen atoms, as compared to DMSO-d_6 and D_2O (Table S1), could indicate protonation of nitrogen atoms in the guanine ring. As the ring nitrogen atoms get protonated in acidic conditions the neighboring carbon atoms become more shielded which results in the upfield shift of ^{13}C NMR carbon peaks. The upfield shift is particularly pronounced for C2, C4, and C6 ^{13}C NMR peaks, which could indicate the protonation of N1, N3, N9 as well as possibly the carbonyl group oxygen of the carbon C6 in concentrated sulfuric acid. This conclusion is supported by the early work of Wagner and von Philipsborn (3) who claim that nitrogen atoms N1, N3 as well as N7 and N9 of guanine are protonated in fluorosulfuric acid (HSO_3F), a strong acid, analogous to H_2SO_4. The fact that HSO_3F and H_2SO_4 are chemically closely related and that the protonation state of purine is the same in both acids (4) further suggests that protonation of guanine in conc. H_2SO_4 will be analogous to the protonation in HSO_3F reported by Wagner and von Philipsborn (3) (Figure 7 and Figure S15).

2. Molecular Structure Determination of Cytosine by NMR in 98% w/w Concentrated Sulfuric Acid. For cytosine we see four peaks for carbon that correspond to the four carbons in the pyrimidine ring; they are found in the region of the NMR spectra associated with aromatic compounds.

We assign the carbon peaks by comparison with literature data (Table S2) and by our 2D NMR experiments (Figure S2). We can assign C5 because it is the most magnetically shielded atom in the ring structure with a chemical shift distinctly upfield from the other three carbon peaks (Table S8). The C5 chemical shift largely agrees with the literature values (including DMSO and D_2O) (1, 2). To assign C2, C4 and C6 we use our 2D NMR where we correlate the positions of H and C within the ring (Figure S2). Cytosine has only two carbons with directly attached hydrogen, and this is for C5 and C6. Our 2D ^{1}H-^{13}C HMQC shows a signal that corresponds to the C6 carbon peak at 145.28 ppm and H (attached to C6) at 7.17 ppm. The 2D ^{1}H-^{13}C HMQC also further confirms the assignment of C5 at 97.48 ppm. We assign C2 and C4, and confirm the assignments of C5 and C6, on the basis of ^{1}H-^{13}C HMBC data (see Figure S2E).

To further confirm the structure, we turn to ^{1}H NMR (Figure S2B). For cytosine the ^{1}H NMR spectral peak for the H attached to carbons are highly consistent with the peaks in other solvents (Table S2) and we use this consistency to assign the H attached to C5 and C6. The peak at 7.17 ppm corresponds to H6 of the pyrimidine ring, while the second peak at 5.88 ppm corresponds to H5 (the chemical shifts for hydrogen atoms are highly consistent with the literature data on the ^{1}H NMR of cytosine in D_2O (2)). No peaks corresponding to hydrogens attached to nitrogen have been detected. Such non-detection is expected as hydrogen atoms exchange very rapidly between the N atom and the solvent in the acidic solution. Similarly, if the carbonyl oxygens were protonated in concentrated sulfuric acid those hydrogen atoms would not be detected.

Finally, we use 1D ^{15}N NMR to show that the N atoms, especially the amino group remain present in the cytosine structure in concentrated sulfuric acid (Table S2). The amino group contains the most shielded N and we therefore can assign the peak at 102.59 ppm to the N attached to C4. We see an additional two nitrogen atoms of the pyrimidine ring and tentatively assign N1 and N3, to 136.44 ppm and 138.61 ppm based on the ^{1}H-^{15}N HMBC data (Figure S2F). We can further confirm the assignment of the amino group to the 102.59 ppm peak on the basis of the very weak signal on the 2D ^{1}H-^{15}N HMBC spectra (coordinates: 5.86 ppm, 102.29 ppm; not shown for clarity). The signal corresponds to the nitrogen atom of the amino group at the distance of three bonds from the H5 hydrogen.

We now turn to the discussion of the protonation state of the cytosine ring in concentrated sulfuric acid. The upfield shift (towards lower ppm) of C2 and C4 ^{13}C NMR peaks in concentrated sulfuric

acid, as compared to DMSO-d_6 and D_2O (Table S2), suggests protonation of neighboring nitrogen atom N3 and possibly also carbonyl oxygen. The ^{13}C NMR chemical shifts of cytosine in concentrated sulfuric acid reported by Benoit and Frechette are consistent with protonation of cytosine (5) and agree with ours, supporting the protonation of nitrogen N3 and carbonyl oxygen atoms in 98% w/w D_2SO_4 (Table S2). The protonation of cytosine in strong acid is also supported by early studies on protonation of pyrimidines in FSO_3H (6).

We note that the slight shifts of the carbon peaks on the ^{13}C NMR, as the acid concentration changes from 81% to 98% (Figure S7) are likely due to different relative amounts of double protonated (2H) vs single protonated (1H) species in solution as the acidity of the solution changes. Different tautomeric structures of cytosine, including protonation of carbonyl group could also contribute to this effect (6).

3. Molecular Structure Determination of 2,6-Diaminopurine by NMR in 98% w/w Concentrated Sulfuric Acid. For 2,6-diaminopurine, in 1D ^{13}C NMR spectrum (Figure S3A), we find five peaks for carbon that correspond to the five carbons in the diaminopurine ring. Each of the five peaks are found in the region of the NMR spectra associated with aromatic compounds.

There is very scarce NMR data for 2,6-diaminopurine available in the literature (Table S3). We therefore assign the carbon peaks by comparison of the diaminopurine spectra with the spectra of the closely-related molecules, adenine (Figure S5, Table S5) and guanine (Figure 5, Table S7) and by our 2D NMR experiments (Figure S3D, E). As for the other compounds we can assign C5 because it is the most magnetically shielded atom in the ring structure with a chemical shift distinctly upfield from the other four carbon peaks. To assign C4 and C8 we use our 2D NMR spectra where we correlate the positions of H and C within the ring. Diaminopurine has only one protonated carbon, at C8, therefore, the single strong peak at 8.53 ppm in the 1D ^{1}H NMR corresponds to the hydrogen atom H8 of the diaminopurine ring (Figure S3B). Our 2D ^{1}H-^{13}C HMQC correctly shows a signal that corresponds to the C8 carbon peak at 140.87 ppm and H (bonded to C8) at 8.53 ppm (Figure S3C). We assign C4 to the 137.38 ppm peak on the basis of our ^{1}H-^{13}C HMBC data (Figure S3E). Further supporting these assignments and the integrity of the imidazole ring, the HMBC correlates the distinct ^{13}C chemical shift of C5 to the nearby H8 which in turn is correlated with C4.

This leaves C2 and C6 which are especially difficult to assign due to their similarly magnetically deshielded environment which results in very similar chemical shifts. Nevertheless, the presence of a very weak signal in the ^{1}H-^{13}C HMBC spectra (coordinates: 8.52 ppm, 146.82 ppm; not shown for clarity) allows us to distinguish carbons C2 and C6 from each other. The very weak signal in the ^{1}H-^{13}C HMBC spectra corresponds to carbon C6, at 146.80 ppm, at the separation of four bonds from the H8 hydrogen.

Finally, we use 1D ^{15}N NMR to show that the diaminopurine structure contains all six expected N atoms. The 1D ^{15}N spectra (Figure S3C) shows six peaks, as expected, corresponding to six nitrogen atoms, four Ns of the aromatic rings and two Ns belonging to the amino groups. The ^{1}H-^{15}N HMBC experiment (Figure S1F) helps in the identification of N7 and N9. We can tentatively assign N7 and N9 to 156.96 ppm and 160.12 ppm respectively, on the basis of the similarities with purine (Table S7). We assign N7 and N9 peaks based on the known N7 and N9 chemical shifts of purine, where N7 is more magnetically shielded (shifted towards lower ppm) in acidic conditions than N9 (Figure 7). However, based on the data at hand, we cannot unambiguously assign N1, N3 and the two remaining amino groups.

We now turn to the discussion of the protonation state of the diaminopurine ring in concentrated sulfuric acid. There are no studies on protonation of diaminopurine in acidic conditions. The upfield shift (towards lower ppm) of C2, C4, C5, and C6 ^{13}C NMR peaks in concentrated sulfuric acid, as compared to DMSO-d_6 (Table S3), suggests protonation of neighboring nitrogen atoms. As the ring nitrogen atoms get protonated in acidic conditions the neighboring carbon atoms become more shilelded which results in the upfield shift of ^{13}C NMR carbon peaks. Assuming that

the protonation state of diaminopurine is similar to adenine we can hypothesize that N1, N3 as well as N7 and N9 of diaminopurine are protonated in 98% D_2SO_4 (Figure S15).

Taken together the NMR data confirms that the 2,6-diaminopurine ring structure remains intact in 98% w/w D_2SO_4 in D_2O.

4. Molecular Structure Determination of Thymine by NMR in 98% w/w Concentrated Sulfuric Acid. For thymine, in the 1D ^{13}C NMR spectrum (Figure S4A), we see four peaks for carbon that correspond to the four carbons in the pyrimidine ring; they are found in the region of the NMR spectra associated with aromatic compounds. We also see one distinctive peak corresponding to thymine's methyl group.

We assign the carbon peaks by comparison with literature data (Table S4) and by our 2D NMR experiments (Figure S4D, E). We can assign C5 because it is the most magnetically shielded atom in the ring structure with a chemical shift distinctly upfield from the other three carbon peaks (Table S4). We assign the distinct peak at 9.99 ppm to the methyl group carbon (CH_3), following the established literature values (Table S4). To assign C2, C4, and C6 we use our 2D NMR where we correlate the positions of H and C within the ring (Figure S4D, E). Thymine has only one carbon in the pyrimidine ring with a directly attached hydrogen, and this is for C6. Our 2D ^{1}H-^{13}C HMQC shows a signal that corresponds to the C6 carbon peak at 149.59 ppm and H6 (attached to C6) at 7.43 ppm (Figure S4D). We assign C2 and C4, and confirm the assignments of C5 and C6, on the basis of ^{1}H-^{13}C HMBC data (Figure S4E).

To further confirm the structure, we turn to ^{1}H (Figure S4B). For thymine the ^{1}H NMR spectral peak for the H attached to carbons are highly consistent with literature values of peaks in DMSO-d_6 (Table S4) and we use this consistency to assign the Hs attached to the carbon of the methyl group and carbon C6. No peaks corresponding to hydrogens attached to nitrogen have been detected. Such non-detection is not surprising as hydrogen atoms are expected to exchange very rapidly between the N atom and the solvent in the acidic solution. Similarly, if the carbonyl oxygens were protonated in concentrated sulfuric acid those hydrogen atoms would likely also not be detected.

Finally, we use 1D ^{15}N NMR to show the two N atoms of the thymine ring (Figure S4C). We assign N1 and N3 to 146.64 ppm and 154.42 ppm respectively based on the ^{1}H-^{15}N HMBC data (Figure S4F).

We now turn to the discussion of the protonation state of the thymine ring in concentrated sulfuric acid. The ^{13}C NMR chemical shift changes of thymine in different solvents are similar to the chemical shift changes reported for uracil (Table S4). The ^{13}C NMR chemical shifts of thymine in concentrated sulfuric acid reported by Benoit and Frechette (5) have been interpreted as signs of protonation of carbonyl oxygen atoms. The results of Benoit and Frechette (5) generally agree with ours. The change in chemical shifts of C2, C4, C5 and C6 carbon peaks between acidic and neutral media could indicate protonation of the neighboring carbonyl oxygen atoms in 98% w/w D_2SO_4 (Table S4 and Figure S15). The downfield shift of the C2 peak as the concentration of acid increases (Figure S8) could indicate the protonation of the carbonyl oxygen O2. The downfield shift of C6 peak in the acidic medium (Table S4) is consistent with previously reported chemical shifts of thymine in concentrated sulfuric acid (5).
The protonation of thymine carbonyl groups in strong acid is also supported by early studies on protonation of pyrimidines in FSO_3H (6).

Taken together the NMR data confirms that the thymine ring structure remains intact in intact in 98% w/w D_2SO_4 in D_2O.

5. Molecular Structure Determination of Adenine by NMR in 98% w/w Concentrated Sulfuric Acid. For Adenine, in the 1D ^{13}C NMR spectrum (Figure S5A), we find five peaks for

carbon that correspond to the five carbons in the adenine ring. Each of the five peaks are found in the region of the NMR spectra associated with aromatic compounds.

We assign the carbon peaks by comparison with literature data (Table S5) and by our 2D NMR experiments (Figure S5D, E). We can assign C5 because it is the most magnetically shielded atom in the ring structure with a chemical shift distinctly upfield from the other four carbon peaks (Table S5). To assign C2, C4, C6, and C8 we use our 2D NMR where we correlate the positions of H and C within the ring. Adenine has two protonated carbons, at C2 and C8. Our 2D ^{1}H-^{13}C HMQC data show two signals that correspond to C2 (peak at 149.55 ppm) and C8 peak (at 143.42 ppm) attached to H2 (at 8.83 ppm) and H8 (at 8.86 ppm) respectively (Figure S5D). We assign C4 and C6 to 137.37 ppm and 146.43 ppm respectively on the basis of ^{1}H-^{13}C HMBC data (Figure S5E). The assignment of C5 also supported by the ^{1}H-^{13}C HMBC NMR. Furthermore, the ^{1}H-^{13}C HMBC data are consistent with carbon atom assignments derived from the ^{1}H-^{13}C HMQC data.

Finally, we use 1D ^{15}N NMR to show that the adenine structure contains all five expected N atoms. The 1D ^{15}N spectra (Figure S5C) shows five peaks corresponding to five nitrogen atoms, four Ns of the aromatic ring and one N belonging to the amino group. The ^{1}H-^{15}N HMBC experiment (Figure S5F) allows for the assignment of the peaks at 161.23 ppm and 160.89 ppm to N7 and N9 respectively. The amino group contains the most magnetically shielded N and we therefore can assign the peak at 113.44 ppm to N atom attached to C6. Based on the data at hand we cannot distinguish N1, N3 from each other.

We now turn to the discussion of the protonation state of the adenine ring in concentrated sulfuric acid. The general upfield shift (towards lower ppm) of ^{13}C NMR carbon peaks in concentrated sulfuric acid, as compared to DMSO-d_6 and D_2O (Table S5), is consistent with protonation of neighboring nitrogen atoms. As the ring nitrogen atoms get protonated in acidic conditions the neighboring carbon atoms become more shielded which results in the upfield shift of ^{13}C NMR carbon peaks.

To the authors' knowledge direct measurements of the protonation state of N atoms in adenine at different concentrations of sulfuric acid have never been published. However, the assessment of the protonation state in a closely related fluorosulfuric acid (HSO_3F) has been attempted for adenine, guanine and purine (3). Since the protonation state of purine is the same in both acids (Figure 7)(3, 4) we can hypothesize that the state of protonation of adenine in conc. H_2SO_4 will be analogous to the state of protonation in HSO_3F. Wagner and von Philipsborn postulate that N1, N3 as well as N7 and N9 of adenine are protonated in HSO_3F (3), we can therefore assume that the same nitrogen atoms get protonated in 98% w/w D_2SO_4 (Figure S15).

Taken together the NMR data confirms that the adenine ring structure remains intact in 98% w/w D_2SO_4 in D_2O.

6. Molecular Structure Determination of Uracil by NMR in 98% w/w Concentrated Sulfuric Acid. For uracil, in the 1D ^{13}C NMR spectrum (Figure S6A), we see four peaks for carbon that correspond to the four carbons in the pyrimidine ring; they are found in the region of the NMR spectra associated with aromatic compounds.

We assign the carbon peaks by comparison with literature data (Table S6) and by our 2D NMR experiments (Figure S6D, E). We can assign C5 because it is the most magnetically shielded atom in the ring structure with a chemical shift distinctly upfield from the other three carbon peaks (Table S6). To assign C2, C4, and C6 we use our 2D NMR data where we correlate the positions of H and C within the ring (Figure S6D, E). Uracil has only two carbons with directly attached hydrogens, and this is for C5 and C6. Our 2D ^{1}H-^{13}C HMQC shows a signal that corresponds to the C6 carbon peak at 153.07 ppm and H (attached to C6) at 7.61 ppm. The 2D ^{1}H-^{13}C HMQC also further confirms the assignment of C5 at 95.93 ppm (Figure S6D). We assign C2 and C4, and confirm the assignments of C5 and C6, on the basis of ^{1}H-^{13}C HMBC data (Figure S6E).

To further confirm the structure, we turn to ^{1}H (Figure S6B). For uracil the ^{1}H NMR spectral peak for the Hs attached to carbons are highly consistent with the peaks of uracil in DMSO-d_6 found in the literature (Table S6) and we use this consistency to assign the Hs attached to C5 and C6. No peaks corresponding to hydrogens attached to nitrogen have been detected. Such non-detection is not surprising as hydrogen atoms are expected to exchange very rapidly between the N atom and the solvent in the acidic solution. Similarly, if the carbonyl oxygens were protonated in concentrated sulfuric acid those hydrogen atoms would likely also not be detected.

Finally, we use 1D ^{15}N NMR to show the two N atoms of the uracil ring (Figure S6C). We assign N1 and N3 to 149.50 ppm and 154.75 ppm respectively based on the ^{1}H-^{15}N HMBC data (Figure S6F).

We now turn to the discussion of the protonation state of the uracil ring in concentrated sulfuric acid. Uracil is protonated in acidic conditions. The ^{13}C NMR chemical shifts of uracil in concentrated sulfuric acid reported by Benoit and Frechette (5) have been interpreted as signs of protonation of carbonyl oxygen atoms. The results of Benoit and Frechette (5) generally agree with ours. The change in chemical shifts of C2, C4, C5 and C6 carbon peaks between acidic and neutral media could indicate protonation of the neighboring carbonyl oxygen atoms in 98% w/w D_2SO_4 (Table S6 and Figure S15). The protonation of uracil carbonyl groups in strong acid is also supported by early studies on protonation of pyrimidines in FSO_3H (6).

Taken together the NMR data confirms that the uracil ring structure remains intact in 98% w/w D_2SO_4 in D_2O.

7. Stability of the Nucleic Acid Bases in 98% w/w Concentrated Sulfuric Acid After Two Week Incubation. To confirm the long-term stability of all eight nucleic acid bases in concentrated sulfuric acid we have incubated 10 to 40 mg of each base in 81-98% w/w D_2SO_4 in D_2O for two weeks, stored in the NMR tubes with room temperature varying from about 18 to 24 °C. After the two-week incubation we acquired 1D ^{13}C NMR spectra of each base, at each of the tested acid concentrations, and compared them to the original 1D ^{13}C NMR spectra collected after ~30-48 h. The two-week spectra and the ~30-48 h spectra look virtually identical for all eight tested bases, at all tested concentrations, confirming long-term stability of the nucleic acid bases in concentrated sulfuric acid solvent (Figure 4 and Figures S12-S14).

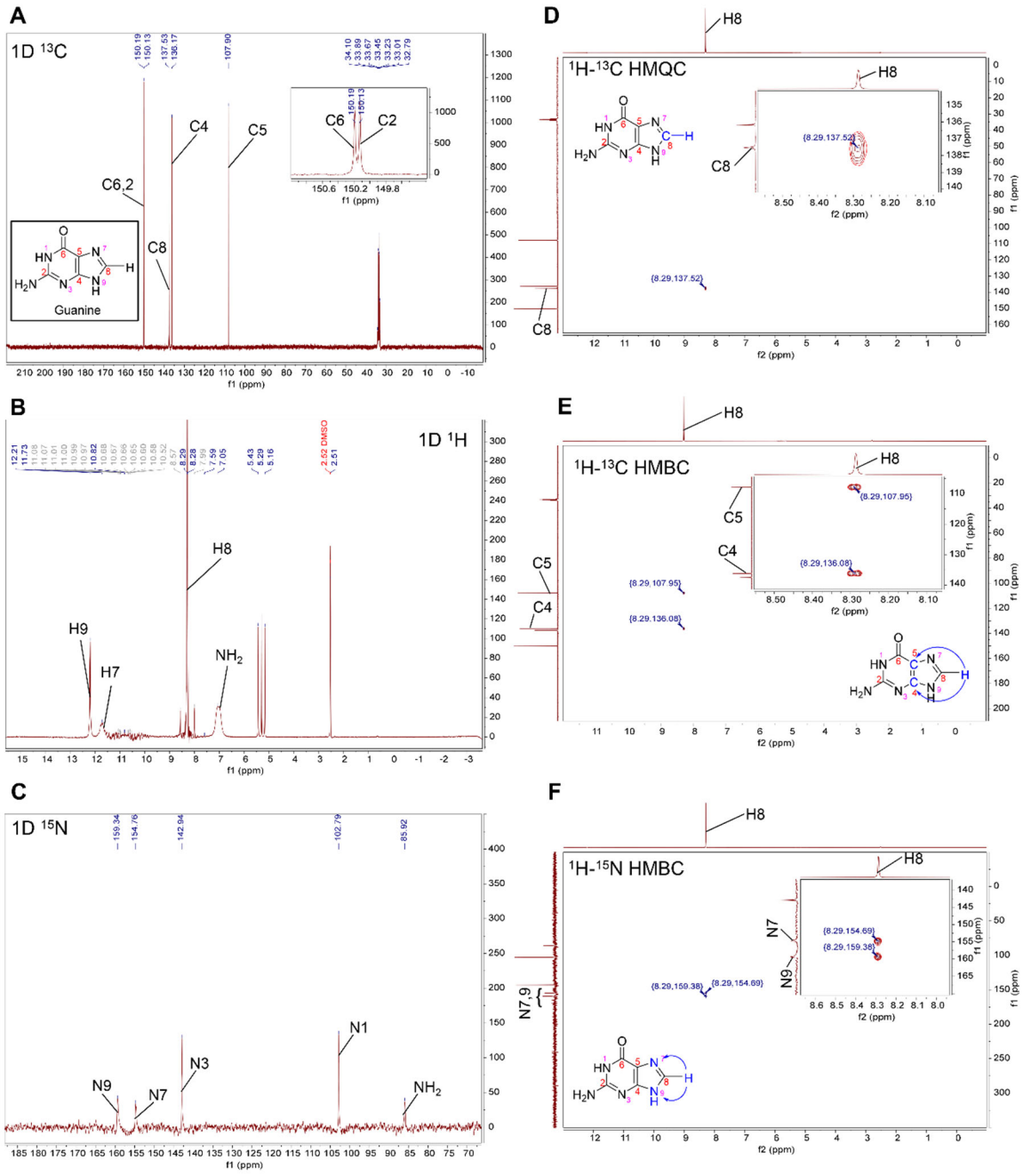

Fig. S1. NMR spectra for guanine in concentrated sulfuric acid (98% D_2SO_4 and 2% D_2O (by weight) with reference DMSO-d_6) at room temperature. The NMR experiments confirm the stability of guanine in concentrated sulfuric acid. **A)** 1D ^{13}C NMR shows five peaks corresponding to five carbons in the guanine ring. DMSO-d_6 reference peak shown at 33.45 ppm. **B)** 1D ^{1}H NMR shows peaks corresponding to hydrogen atoms in the guanine ring, including hydrogens belonging to the C2 amino group. The solvent peak is suppressed for clarity. **C)** 1D ^{15}N NMR further reaffirms the integrity of the guanine ring in the concentrated sulfuric acid by showing four peaks of the nitrogen atoms of the aromatic ring and one nitrogen belonging to the intact amino group attached to carbon C2. **D)** The 2D ^{1}H-^{13}C HMQC NMR shows direct bonding between H and C atoms in the guanine ring structure. As expected it shows only one signal, at the intersection of hydrogen atom H8 (f2: ^{1}H tracer spectra) and carbon atom C8 (f1: ^{13}C tracer spectra). This confirms the identity of 137.53 ppm peak in 1D ^{13}C NMR spectra as carbon C8. **E)** The 2D ^{1}H-^{13}C HMBC NMR shows signals that correspond to hydrogen and carbon atoms

separated from each other by the distance of 3 chemical bonds in the guanine ring structure (blue arrows). As expected for guanine, the spectrum shows only two signals at the expected positions. The two signals correspond to a distance of 3 chemical bonds between H8 and C4 and C5 in the guanine structure. The correct distances between atoms derived from the 2D ^{1}H-^{13}C HMBC NMR confirm the identity of 107.90 ppm peak and the 136.17 ppm peak in 1D ^{13}C NMR spectra as C5 and C4 respectively. **F)** The 2D ^{1}H-^{15}N HMBC NMR shows bond distances between hydrogen atoms attached to carbons and nitrogen atoms (blue arrows). The spectrum shows two signals at the expected positions. The two signals correspond to a distance of 2 chemical bonds between H8 and N7 or N9. The relationships between atoms derived from the 2D NMR, taken together with the 1D NMR data, further confirm peak assignments of the carbon, hydrogen and nitrogen 1D NMR spectra and support the hypothesis that the guanine ring remains unchanged and is stable in 98% w/w concentrated sulfuric acid. The NMR experiments confirm the integrity of guanine in concentrated sulfuric acid.

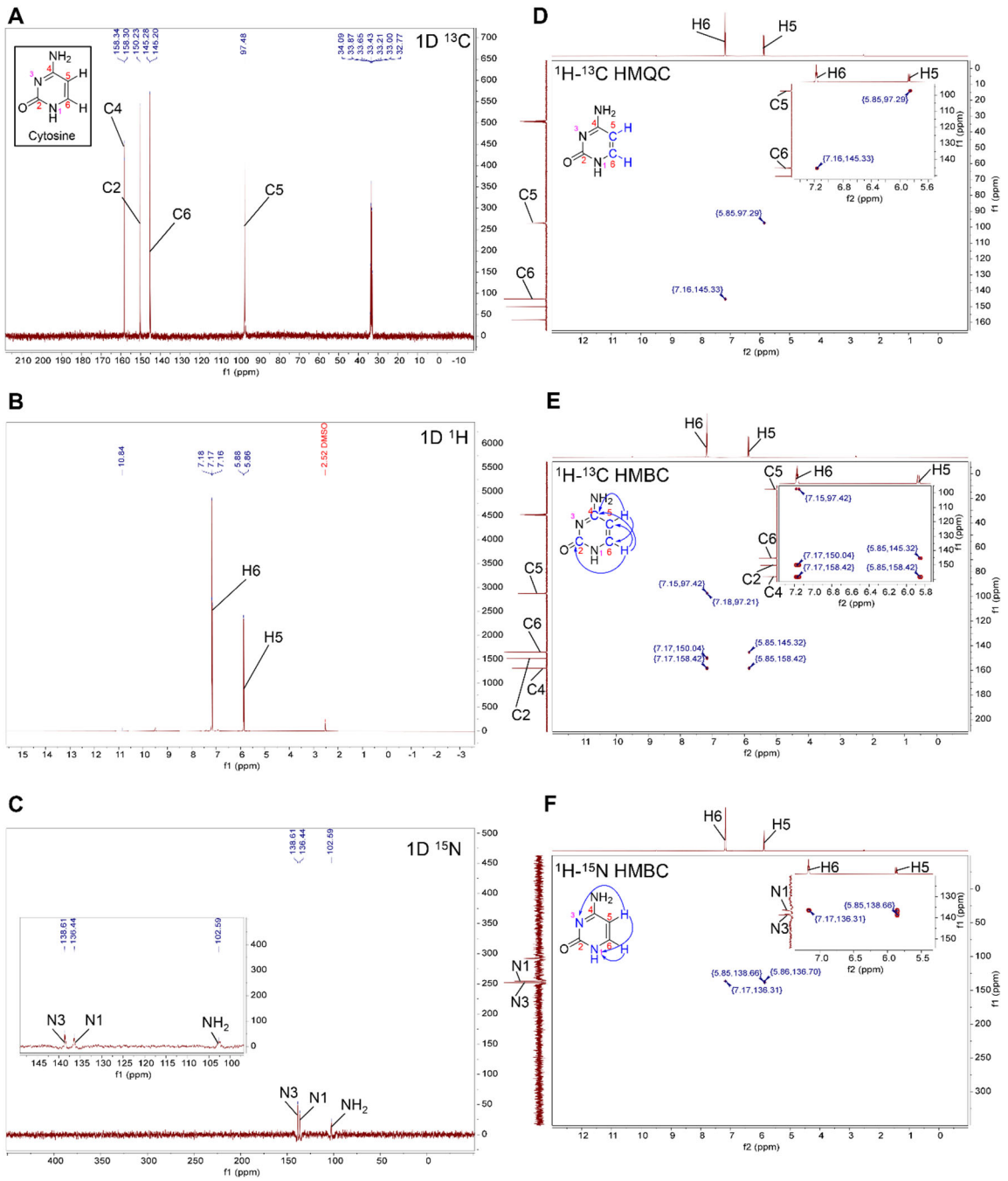

Fig. S2. NMR spectra for cytosine in concentrated sulfuric acid (98% D_2SO_4 and 2% D_2O (by weight) with reference DMSO-d_6) at room temperature. The NMR experiments confirm the stability of cytosine in concentrated sulfuric acid. **A)** 1D ^{13}C NMR shows four peaks corresponding to four carbons in the cytosine ring. The DMSO-d_6 reference peak appears at 33.43 ppm. **B)** 1D ^{1}H NMR shows peaks corresponding to hydrogen atoms attached to carbons in the cytosine ring. The Hs associated with Ns have not been detected. The solvent peak is suppressed for clarity. **C)** 1D ^{15}N NMR further reaffirms the integrity of the cytosine ring in concentrated sulfuric acid by showing two peaks corresponding to the nitrogen atoms of the aromatic ring and one nitrogen belonging to the intact amino group attached to carbon C4. **D)** The 2D ^{1}H-^{13}C HMQC NMR shows direct bonding between H and C atoms in the cytosine ring structure. As expected it shows two signals, at the intersection of hydrogen atoms H5 and H6 (f2: ^{1}H trace spectra) and carbon atoms

C5 and C6 (f1: ^{13}C trace spectra). The experiment confirms the identity of 97.48 ppm and 145.28 ppm peaks in 1D ^{13}C NMR spectra as carbons C5 and C6 respectively. **E)** The 2D ^{1}H-^{13}C HMBC NMR shows signals that correspond to hydrogen and carbon atoms separated from each other by the distance of 2 or 3 chemical bonds in the cytosine ring structure (blue arrows). The correct distances between atoms derived from the 2D ^{1}H-^{13}C HMBC NMR confirm the identity of carbon peaks in 1D ^{13}C NMR spectra. **F)** The 2D ^{1}H-^{15}N HMBC NMR shows 2 or 3 bond distances between hydrogens attached to carbon and nitrogen atoms (blue arrows). The spectrum shows three signals at the expected positions. The three signals correspond to a distance of 2 or 3 chemical bonds between hydrogens H5 and H6 and nitrogens N1 and N3 in the cytosine structure. The correct distances between atoms derived from the 2D ^{1}H-^{15}N HMBC NMR confirm the identity of nitrogen peaks in the 1D ^{15}N NMR spectra. The relationships between atoms derived from the 2D NMR, taken together with the 1D NMR data, further confirm peak assignments of the carbon, hydrogen and nitrogen 1D NMR spectra and support the hypothesis that the cytosine ring remains unchanged and is stable in 98% w/w concentrated sulfuric acid. The NMR experiments confirm the integrity of cytosine in concentrated sulfuric acid.

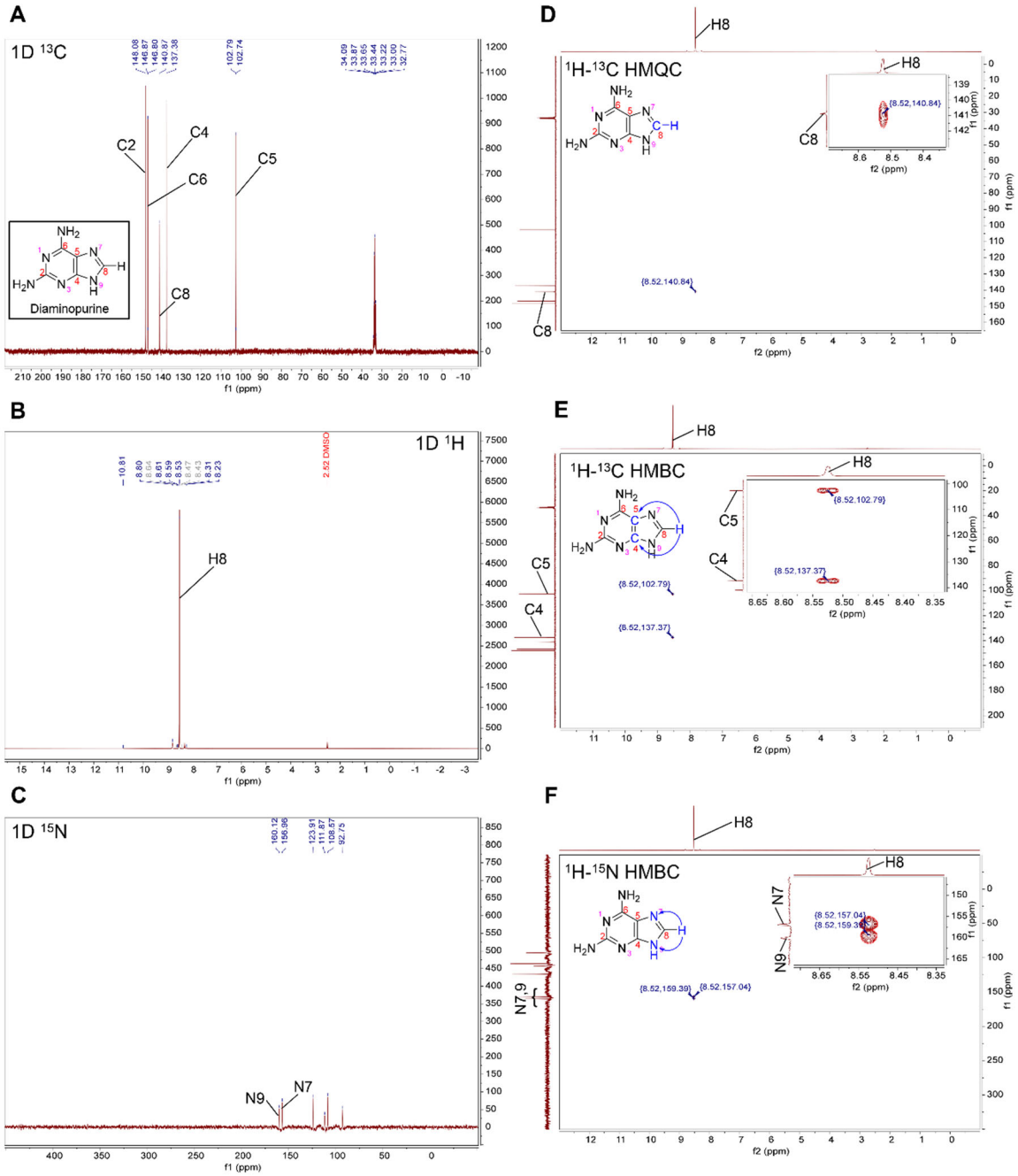

Fig. S3. NMR spectra for 2,6-diaminopurine concentrated sulfuric acid (98% D_2SO_4 and 2% D_2O, by weight, with reference DMSO-d_6) at room temperature. The NMR experiments confirm the stability of 2,6-diaminopurine in concentrated sulfuric acid. **A**) 1D ^{13}C NMR shows five peaks corresponding to five carbons in the 2,6-diaminopurine ring. The DMSO-d_6 reference peak shown at 33.44 ppm. **B**) 1D ^{1}H NMR shows a single peak corresponding to the H8 hydrogen atom. The solvent peak is suppressed for clarity. **C**) 1D ^{15}N NMR further reaffirms the integrity of the 2,6-diaminopurine ring in the concentrated sulfuric acid by showing four peaks of the nitrogen atoms of the aromatic ring and two nitrogen atoms belonging to the intact amino groups attached to carbons C2 and C6. **D**) The 2D ^{1}H-^{13}C HMQC NMR shows direct bonding between H and C atoms in the 2,6-diaminopurine ring structure. As expected it shows only one signal, at the intersection of hydrogen atom H8 (f2: ^{1}H tracer spectra) and carbon atom C8 (f1: ^{13}C tracer spectra). This confirms the identity of 140.87 ppm peak in 1D ^{13}C NMR spectra as carbon C8. **E**)

The 2D ^{1}H-^{13}C HMBC NMR shows signals that correspond to hydrogen and carbon atoms separated from each other by the distance of 3 chemical bonds in the 2,6-diaminopurine ring structure (blue arrows). As expected for 2,6-diaminopurine, the spectrum shows only two signals at the expected positions. The two signals correspond to a distance of 3 chemical bonds between H8 and C4 and C5 in the 2,6-diaminopurine structure. The correct distances between atoms derived from the 2D ^{1}H-^{13}C HMBC NMR confirm the identity of the 102.74 ppm peak and the 137.38 ppm peak in 1D ^{13}C NMR spectra as C5 and C4 respectively. **F**) The 2D ^{1}H-^{15}N HMBC NMR shows bond distances between hydrogen and nitrogen atoms (blue arrows). The spectrum shows two signals at the expected positions. The two signals correspond to a distance of two chemical bonds between H8 and N7 or N9. The relationships between atoms derived from the 2D NMR, taken together with the 1D NMR data, further confirm peak assignments of the carbon, hydrogen and nitrogen 1D NMR spectra and support the hypothesis that the 2,6-diaminopurine ring remains unchanged and is stable in 98% w/w concentrated sulfuric acid.

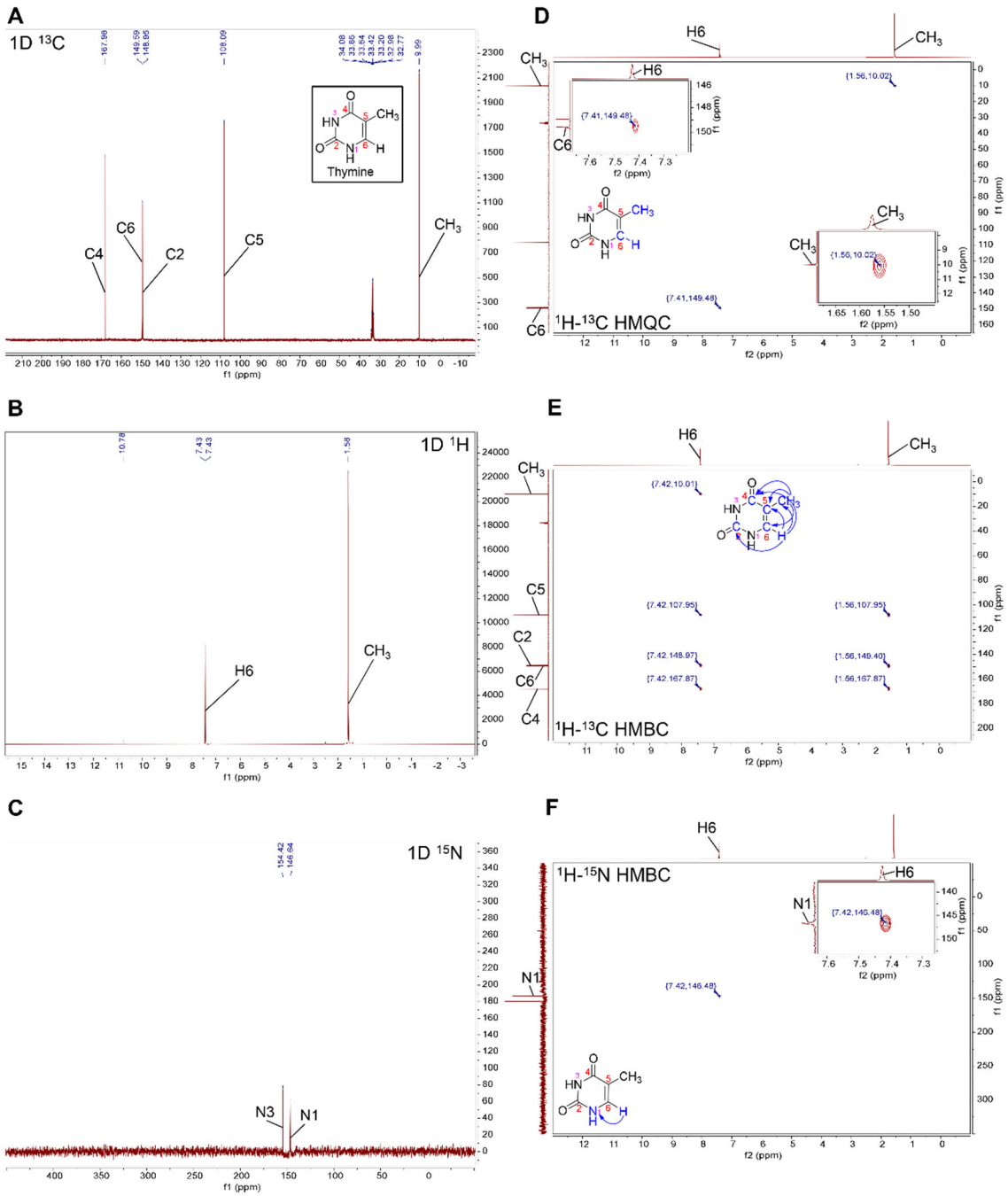

Fig. S4. NMR spectra for thymine in concentrated sulfuric acid (98% D_2SO_4 and 2% D_2O, by weight, with reference DMSO-d_6) at room temperature. The NMR experiments confirm the stability of thymine in concentrated sulfuric acid. **A**) 1D ^{13}C NMR shows four peaks corresponding to four carbons in the thymine ring. The DMSO-d_6 reference peak shown at 33.42 ppm. **B**) 1D ^{1}H NMR shows two peaks corresponding to H6 in the thymine ring and the hydrogens of the methyl group. The Hs associated with Ns have not been detected. The solvent peak is suppressed for clarity. **C**) 1D ^{15}N NMR further reaffirms the integrity of the thymine ring in concentrated sulfuric acid by showing two peaks corresponding to the nitrogen atoms of the aromatic ring. **D**) The 2D ^{1}H-^{13}C HMQC NMR shows direct bonding between H and C atoms in the thymine ring structure. As expected it shows two signals, at the intersection of hydrogen atoms H6 and the hydrogens of the methyl group (f2: ^{1}H trace spectra) and carbon atoms C6 and the carbon atom of the methyl

group (f1: ^{13}C trace spectra). The experiment confirms the identity of 9.99 ppm and 149.59 ppm peaks in 1D ^{13}C NMR spectra as carbons of the methyl group and C6 respectively. **E**) The 2D ^{1}H-^{13}C HMBC NMR shows signals that correspond to hydrogen and carbon atoms separated from each other by the distance of two or three chemical bonds in the thymine ring structure (blue arrows). The correct bond separation between atoms derived from the 2D ^{1}H-^{13}C HMBC NMR confirms the identity of carbon peaks assigned in the 1D ^{13}C NMR spectra. **F**) The 2D ^{1}H-^{15}N HMBC NMR shows two bond separations between hydrogen attached to carbon and a nitrogen atom (blue arrows). The spectrum shows a single signal at the expected position. The signal corresponds to a separation of two chemical bonds between hydrogen H6 and nitrogen N1 in the thymine structure. The correct separations between atoms derived from the 2D ^{1}H-^{15}N HMBC NMR confirm the identity of nitrogen peaks in the 1D ^{15}N NMR spectra. The relationships between atoms derived from the 2D NMR, taken together with the 1D NMR data, further confirm peak assignments of the carbon, hydrogen and nitrogen 1D NMR spectra and support the hypothesis that the thymine ring remains unchanged and is stable in 98% w/w concentrated sulfuric acid.

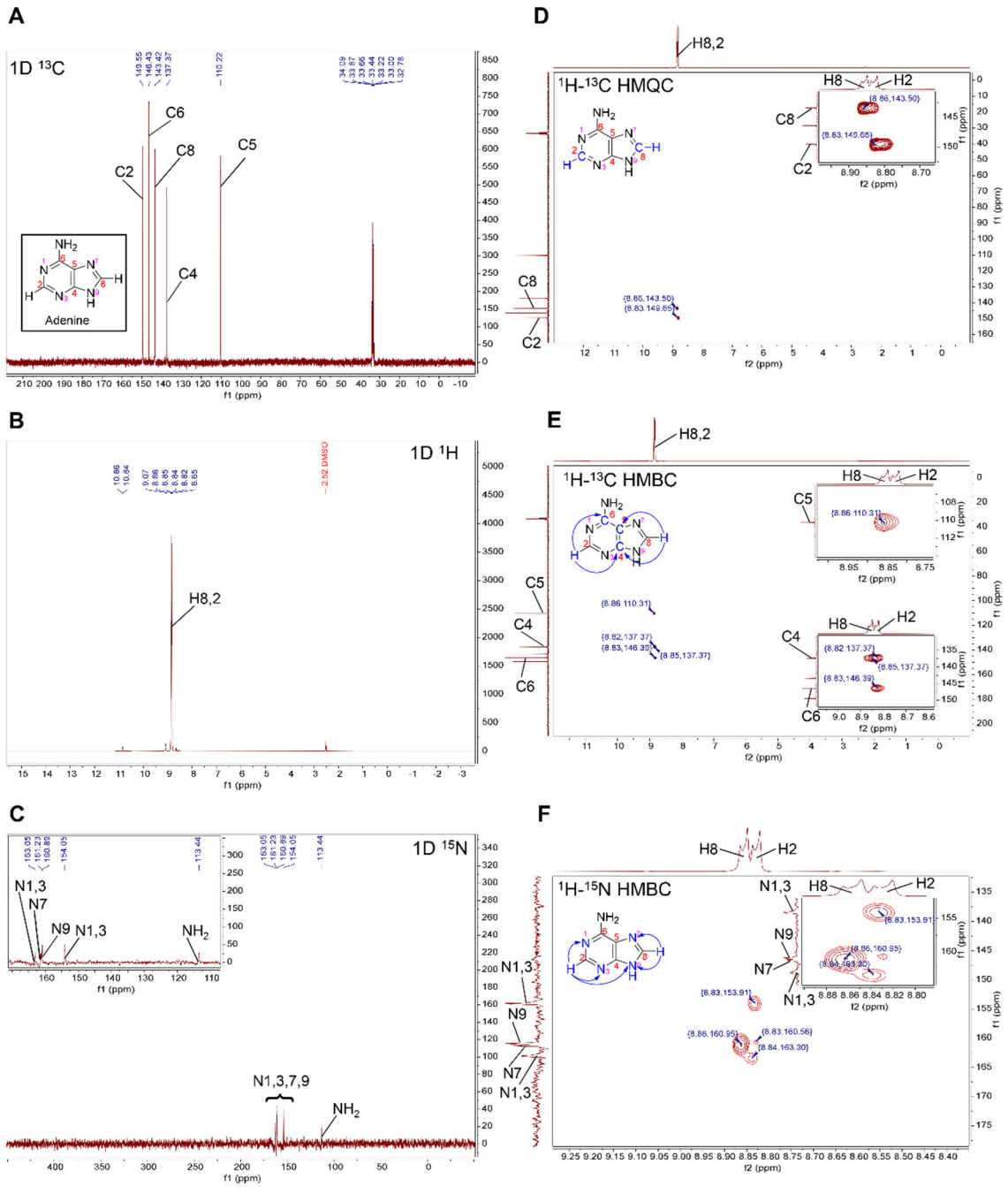

Fig. S5. NMR spectra for adenine concentrated sulfuric acid (98% D_2SO_4 and 2% D_2O, by weight, with reference DMSO-d_6) at room temperature. The NMR experiments confirm the stability of adenine in concentrated sulfuric acid. **A**) 1D ^{13}C NMR shows five peaks corresponding to five carbons in the adenine ring. The DMSO-d_6 reference peak shown at 33.44 ppm. **B**) 1D ^{1}H NMR shows overlapping peaks corresponding to hydrogen atoms H8 and H2 in the adenine ring. The 98% D_2SO_4 and 2% D_2O solvent peak is suppressed for clarity. **C**) 1D ^{15}N NMR further reaffirms the integrity of the adenine ring in concentrated sulfuric acid by showing four peaks of the nitrogen atoms of the aromatic ring and one nitrogen belonging to the intact amino group attached to carbon C6. **D**) The 2D ^{1}H-^{13}C HMQC NMR shows direct bonding between H and C atoms in the adenine ring structure. As expected it shows two signals, at the intersection of hydrogen atoms H8 and H2 (f2: ^{1}H tracer spectra) and carbon atoms C8 and C2 (f1: ^{13}C tracer spectra). This confirms the identity of the 143.42 ppm and 149.55 ppm peaks in the 1D ^{13}C NMR

spectra as carbons C8 and C2 respectively. **E**) The 2D ^{1}H-^{13}C HMBC NMR shows signals that correspond to hydrogen and carbon atoms separated from each other by 3 chemical bonds in the adenine ring structure (blue arrows). As expected for adenine, the spectrum shows three signals at the expected positions. The correct separations between atoms derived from the 2D ^{1}H-^{13}C HMBC NMR confirm the identity of the carbon atom peaks in 1D ^{13}C NMR spectra. **F**) The 2D ^{1}H-^{15}N HMBC NMR shows bond distances between hydrogen atoms attached to carbon and nitrogen atoms (blue arrows). The spectrum shows signals at the expected positions allowing for the assignment of the N7 and N9 nitrogen atoms. The relationships between atoms derived from the 2D NMR, taken together with the 1D NMR data, further confirm peak assignments of the carbon, hydrogen and nitrogen 1D NMR spectra and support the hypothesis that the adenine ring remains unchanged and is stable in 98% w/w concentrated sulfuric acid.

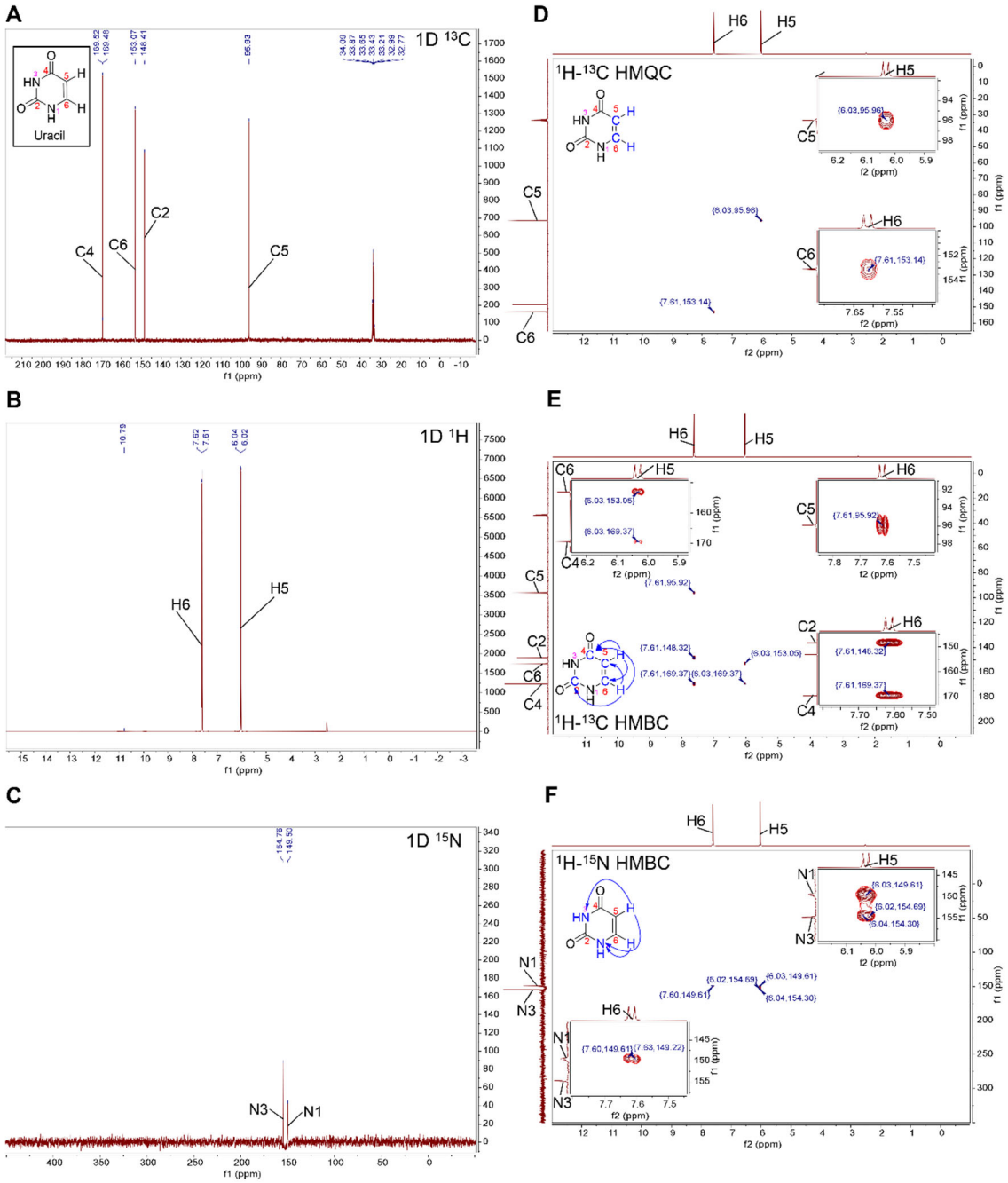

Fig. S6. NMR spectra for uracil in concentrated sulfuric acid (98% D_2SO_4 and 2% D_2O, by weight, with reference DMSO-d_6) at room temperature. The NMR experiments confirm the stability of uracil in concentrated sulfuric acid. **A**) 1D ^{13}C NMR shows four peaks corresponding to four carbons in the uracil ring. DMSO-d_6 reference peak shown at 33.43 ppm. **B**) 1D ^{1}H NMR shows two peaks corresponding to hydrogen atoms attached to carbons in the uracil ring. The Hs associated with Ns have not been detected. The solvent peak is suppressed for clarity. **C**) 1D ^{15}N NMR further reaffirms the integrity of the uracil ring in concentrated sulfuric acid by showing two peaks corresponding to the nitrogen atoms of the aromatic ring. **D**) The 2D ^{1}H-^{13}C HMQC NMR shows direct bonding between H and C atoms in the uracil ring structure. As expected it shows two signals, at the intersection of hydrogen atoms H5 and H6 (f2: ^{1}H trace spectra) and carbon atoms C5 and C6 (f1: ^{13}C trace spectra). The experiment confirms the identity of the 95.93 ppm and 153.07 ppm peaks in 1D ^{13}C NMR spectra as carbons C5 and C6 respectively. **E**) The 2D

^{1}H-^{13}C HMBC NMR shows signals that correspond to hydrogen and carbon atoms separated from each other by two or three chemical bonds in the uracil ring structure (blue arrows). The correct separations between atoms derived from the 2D ^{1}H-^{13}C HMBC NMR confirm the assignment of carbon peaks in 1D ^{13}C NMR spectra. **F**) The 2D ^{1}H-^{15}N HMBC NMR shows 2 or 3 bond distances between hydrogens attached to carbon and nitrogen atoms (blue arrows). The spectrum shows three signals at the expected positions. The three signals correspond to a distance of 2 or 3 chemical bonds between hydrogens H5 and H6 and nitrogens N1 and N3 in the uracil structure. The correct separations between atoms derived from the 2D ^{1}H-^{15}N HMBC NMR confirm the identity of nitrogen peaks in the 1D ^{15}N NMR spectra. The relationships between atoms derived from the 2D NMR, taken together with the 1D NMR data, further confirm peak assignments of the carbon, hydrogen and nitrogen 1D NMR spectra and support the hypothesis that the uracil ring remains unchanged and is stable in 98% w/w concentrated sulfuric acid.

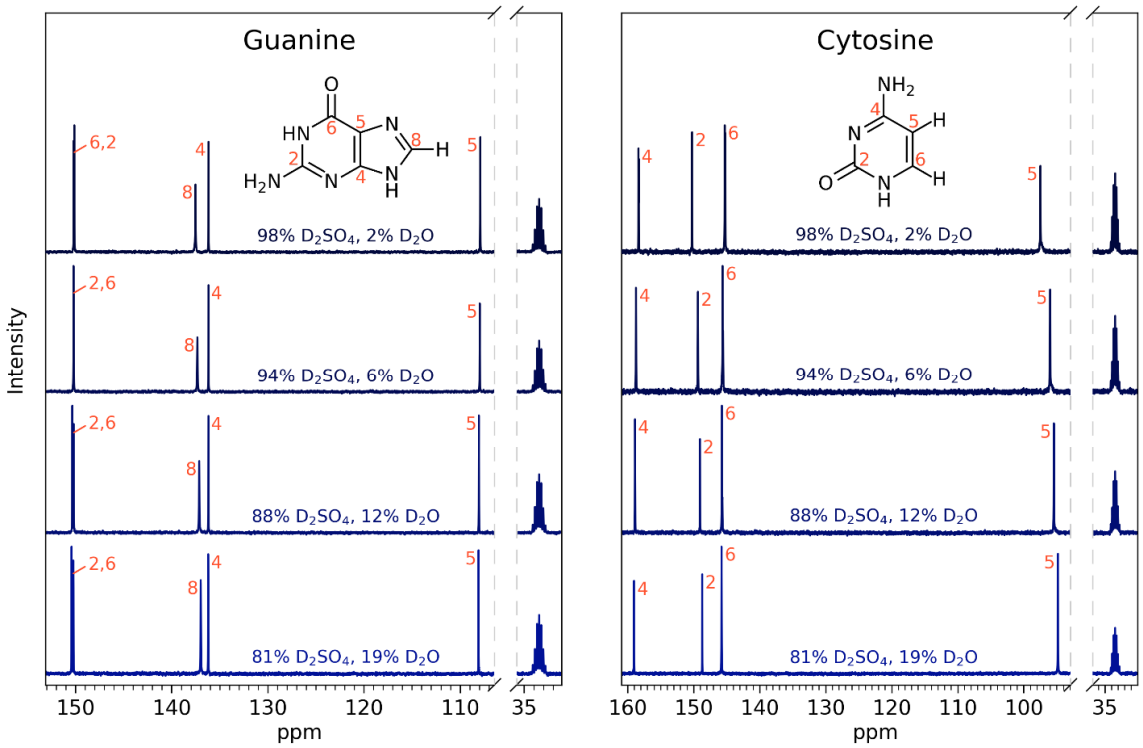

Fig. S7. 1D ^{13}C NMR spectra for guanine (left) and cytosine (right) for a range of sulfuric acid concentrations found in the Venus clouds. From top to bottom are different concentrations (by weight) of sulfuric acid in water: 98% D_2SO_4/2% D_2O; 94% D_2SO_4/6% D_2O; 88% D_2SO_4/12% D_2O; 81% D_2SO_4/19% D_2O with DMSO-d_6 as a reference and at room temperature. The labeled NMR peaks show five peaks corresponding to five carbon atoms in the guanine ring, and 4 peaks corresponding to four carbons of the cytosine ring. All peaks are consistent with the molecules being stable and the structure not being affected by the concentrated sulfuric acid solvent. For a description of peak assignments, see Figure S1 and Figure S2.

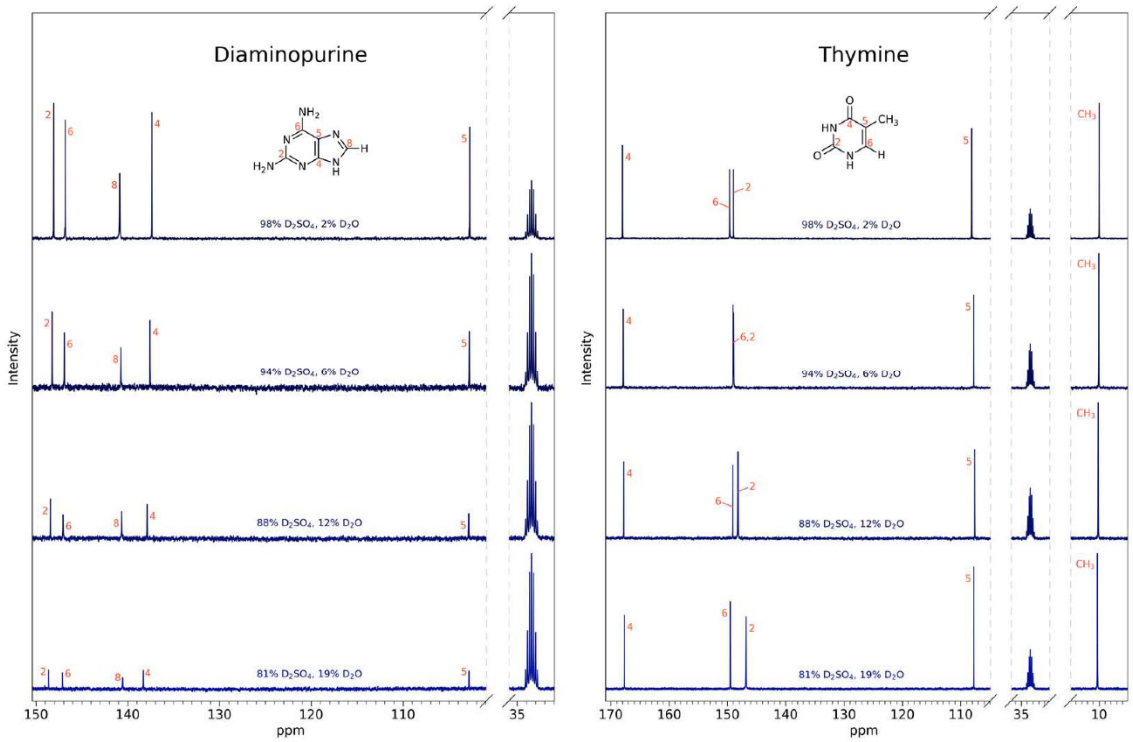

Fig. S8. 1D ^{13}C NMR spectra for 2,6-diaminopurine (left) and thymine (right) for a range of sulfuric acid concentrations found in the Venus clouds. From top to bottom are different concentrations (by weight) of sulfuric acid in water: 98% D_2SO_4/2% D_2O; 94% D_2SO_4/6% D_2O; 88% D_2SO_4/12% D_2O; 81% D_2SO_4/19% D_2O with DMSO-d_6 as a reference and at room temperature. The labeled NMR peaks show five peaks corresponding to five carbon atoms in the 2,6-diaminopurine ring, and 5 peaks corresponding to five carbons of the pyrimidine ring and a methyl group. All peaks are consistent with the molecules being stable and the structure not being affected by the concentrated sulfuric acid solvent. For a description of peak assignments, see Figure S3 and Figure S4.

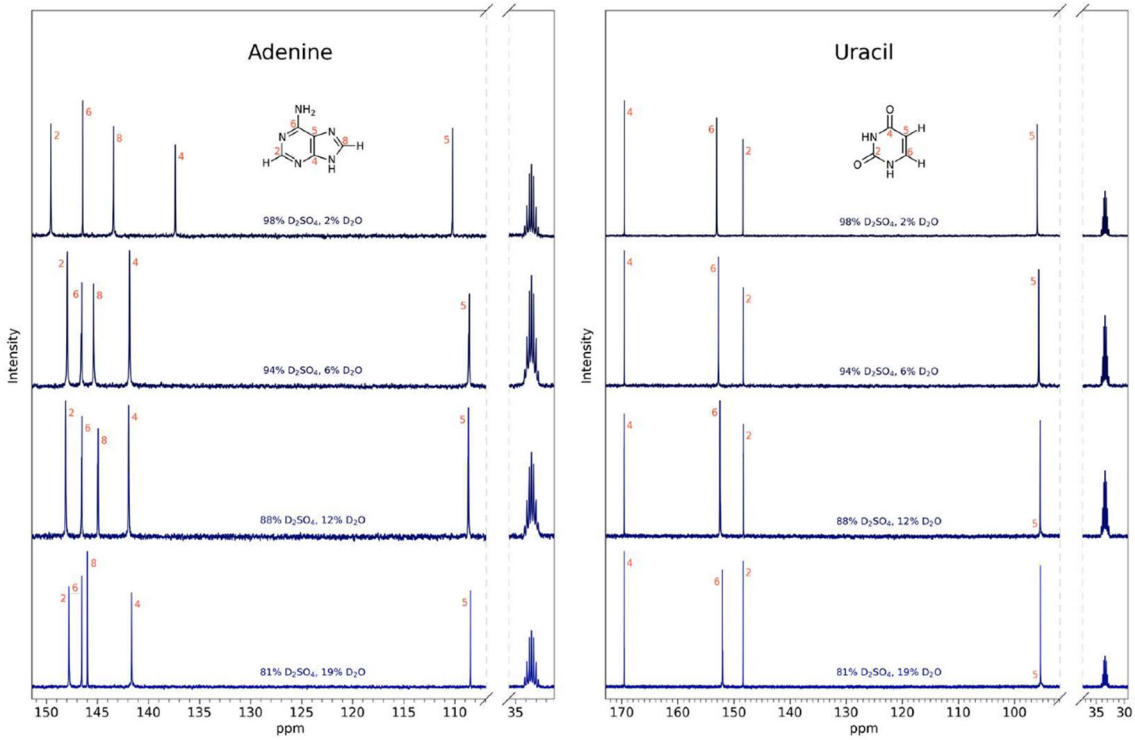

Fig. S9. 1D ^{13}C NMR spectra for adenine (left) and uracil (right) for a range of sulfuric acid concentrations found in the Venus clouds. From top to bottom are different concentrations (by weight) of sulfuric acid in water: 98% D_2SO_4/2% D_2O; 94% D_2SO_4/6% D_2O; 88% D_2SO_4/12% D_2O; 81% D_2SO_4/19% D_2O with DMSO-d_6 as a reference and at room temperature. The labeled NMR peaks show five peaks corresponding to five carbon atoms in the adenine ring, and 4 peaks corresponding to four carbons of the uracil ring. All peaks are consistent with the molecules being stable and the structure not being affected by the concentrated sulfuric acid solvent. For a description of peak assignments, see Figure S5 and Figure S6.

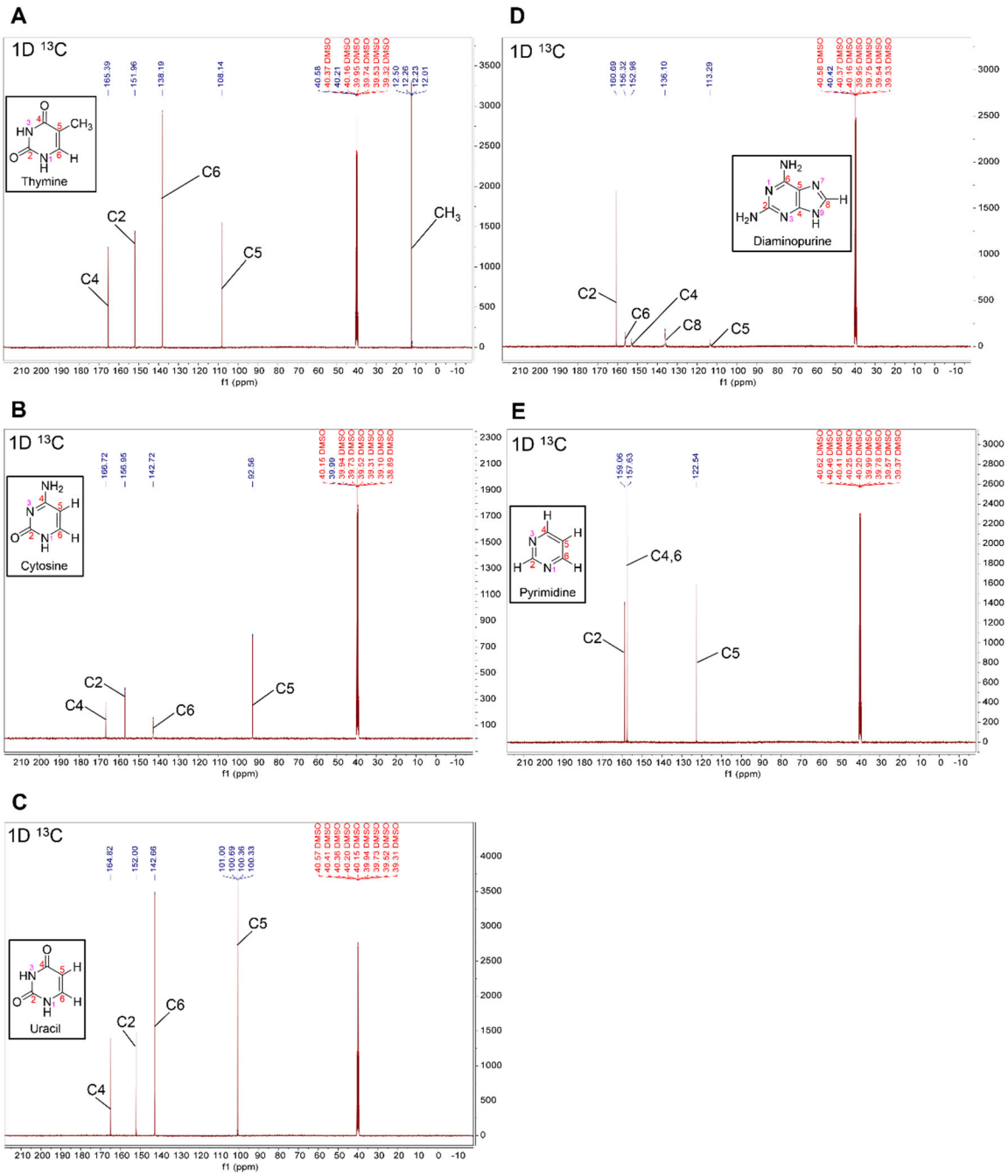

Fig. S10. 1D ^{13}C NMR in DMSO-d_6 solvent for **A**) thymine, **B**) cytosine, **C**) uracil **D**) 2,6-diaminopurine, **E**) pyrimidine. The chemical shifts of the collected spectra are consistent with previously reported values (Table S2, Table S3, Table S4, Table S6 and Table S8). Spectra for adenine, purine and guanine could not be collected due to poor solubility of these compounds in DMSO-d_6 solvent.

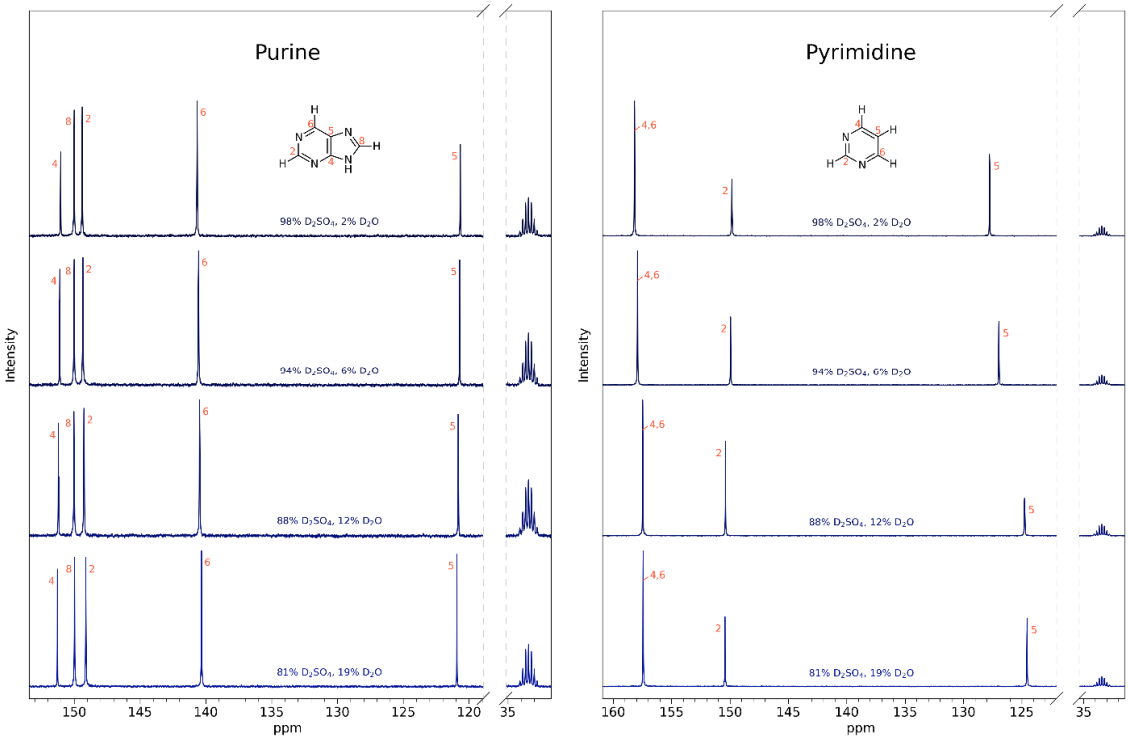

Fig. S11. 1D ^{13}C NMR spectra for purine (left) and pyrimidine (right) for a range of sulfuric acid concentrations found in the Venus clouds. From top to bottom are different concentrations (by weight) of sulfuric acid in water: 98% D_2SO_4/2% D_2O; 94% D_2SO_4/6% D_2O; 88% D_2SO_4/12% D_2O; 81% D_2SO_4/19% D_2O with DMSO-d_6 as a reference and at room temperature. The labeled NMR peaks show five peaks corresponding to five carbon atoms in the purine ring, and 4 peaks corresponding to four carbons of the pyrimidine ring. All peaks are consistent with the molecules being stable and the structure not being affected by the concentrated sulfuric acid solvent. For a description of peak assignments, see Figure 5 and Figure 6 of the main text.

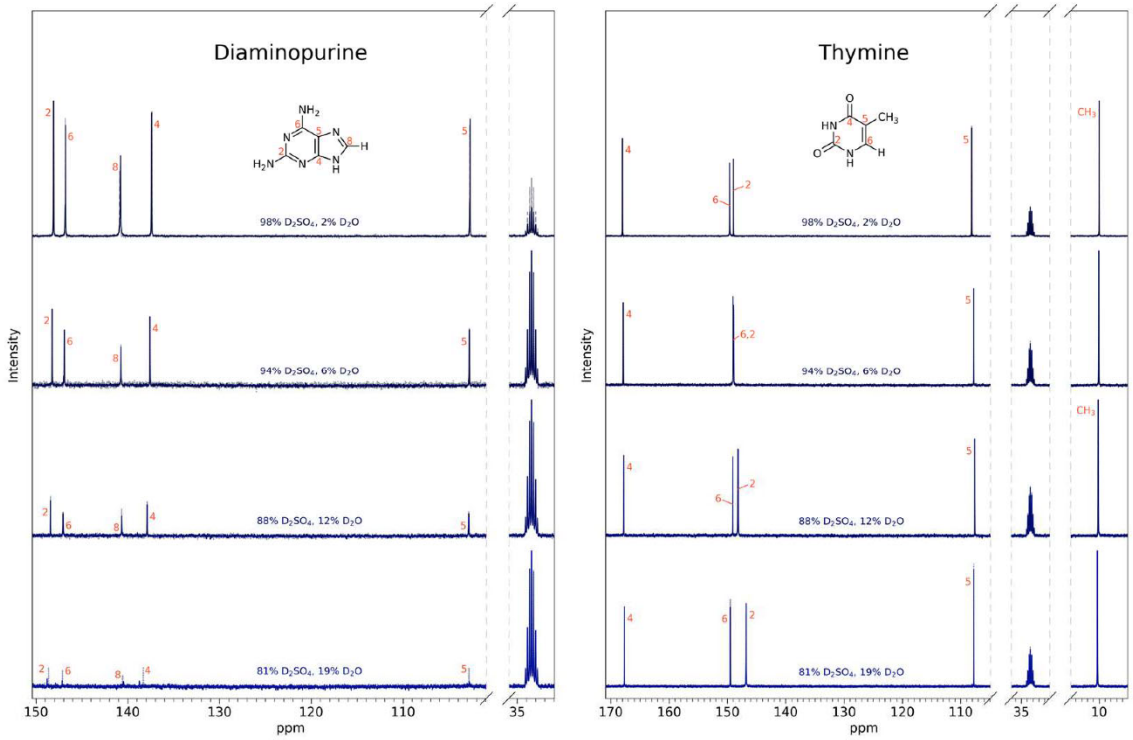

Fig. S12. 2,6-diaminopurine (left) and thymine (right) are stable for two weeks in a range of sulfuric acid concentrations found in the Venus clouds. We have incubated 10-80 mg of each compound in 81-98% w/w D_2SO_4 for two weeks. Due to low solubility of diaminopurine in concentrated sulfuric acid different amounts have been tested. After two-week incubation we have collected the 1D ^{13}C NMR spectra (solid line spectra), at each of the tested acid concentrations, and compared them to the original 1D ^{13}C NMR spectra collected after ~30-48 h (dashed line spectra and Figure S8). The two-week spectra and the ~30-48 h spectra look virtually identical for all tested concentrations, confirming long-term stability of the nucleic acid bases in concentrated sulfuric acid solvent. From top to bottom are different concentrations (by weight) of sulfuric acid in water: 98% D_2SO_4/2% D_2O; 94% D_2SO_4/6% D_2O; 88% D_2SO_4/12% D_2O; 81% D_2SO_4/19% D_2O with DMSO-d_6 as a reference and at room temperature. All peaks are consistent with the molecules being stable and the structure not being affected by the concentrated sulfuric acid solvent.

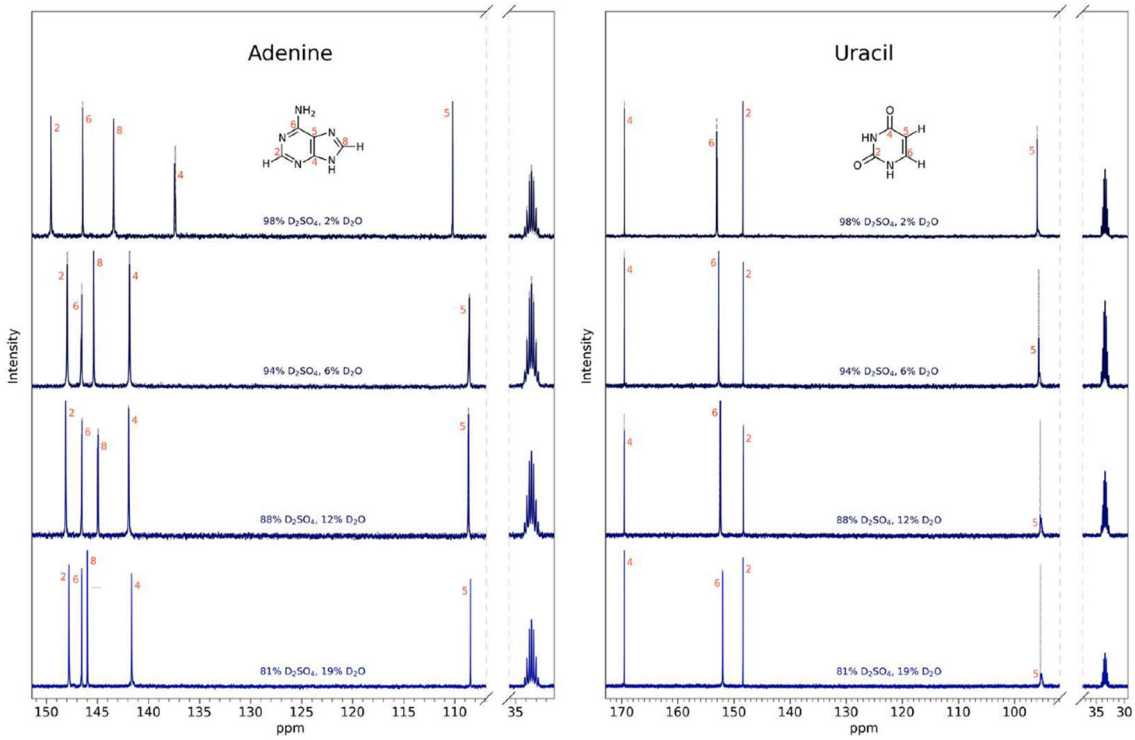

Fig. S13. Adenine (left) and uracil (right) are stable for 2 weeks in a range of sulfuric acid concentrations found in the Venus clouds. We have incubated 30 mg of each base in 81-98% w/w D_2SO_4 for two weeks. After two-week incubation we have collected the 1D ^{13}C NMR spectra (solid line spectra), at each of the tested acid concentrations, and compared them to the original 1D ^{13}C NMR spectra collected after ~30-48 h (dashed line spectra and Figure S9). The two-week spectra and the ~30-48 h spectra look virtually identical for all tested concentrations, confirming long-term stability of the nucleic acid bases in concentrated sulfuric acid solvent. From top to bottom are different concentrations (by weight) of sulfuric acid in water: 98% D_2SO_4/2% D_2O; 94% D_2SO_4/6% D_2O; 88% D_2SO_4/12% D_2O; 81% D_2SO_4/19% D_2O with DMSO-d_6 as a reference and at room temperature. All peaks are consistent with the molecules being stable and the structure not being affected by the concentrated sulfuric acid solvent.

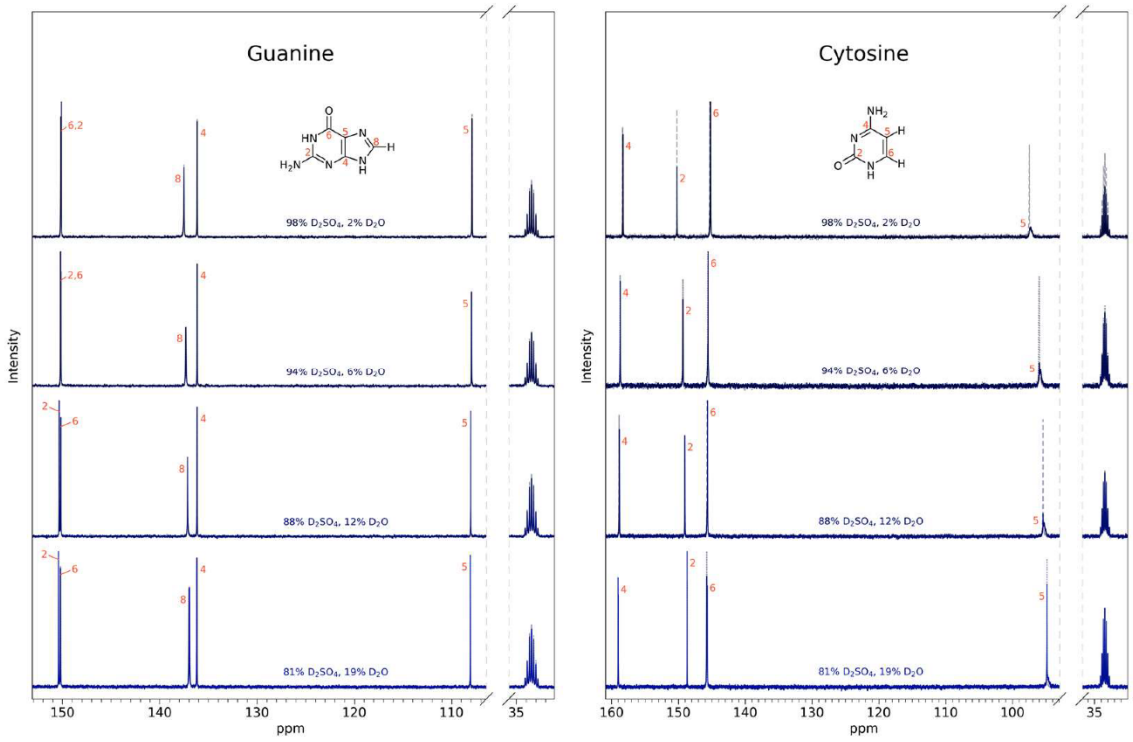

Fig. S14. Guanine (left) and cytosine (right) are stable for 2 weeks in a range of sulfuric acid concentrations found in the Venus clouds. We have incubated 30-40 mg of each base in 81-98% w/w D_2SO_4 for two weeks. After two-week incubation we have collected the 1D ^{13}C NMR spectra (solid line spectra), at each of the tested acid concentrations, and compared them to the original 1D ^{13}C NMR spectra collected after ~30-48 h (dashed line spectra and Figure 4). The two-week spectra and the ~30-48 h spectra look virtually identical for all tested concentrations, confirming long-term stability of the nucleic acid bases in concentrated sulfuric acid solvent. From top to bottom are different concentrations (by weight) of sulfuric acid in water: 98% D_2SO_4/2% D_2O; 94% D_2SO_4/6% D_2O; 88% D_2SO_4/12% D_2O; 81% D_2SO_4/19% D_2O with DMSO-d_6 as a reference and at room temperature. All peaks are consistent with the molecules being stable and the structure not being affected by the concentrated sulfuric acid solvent.

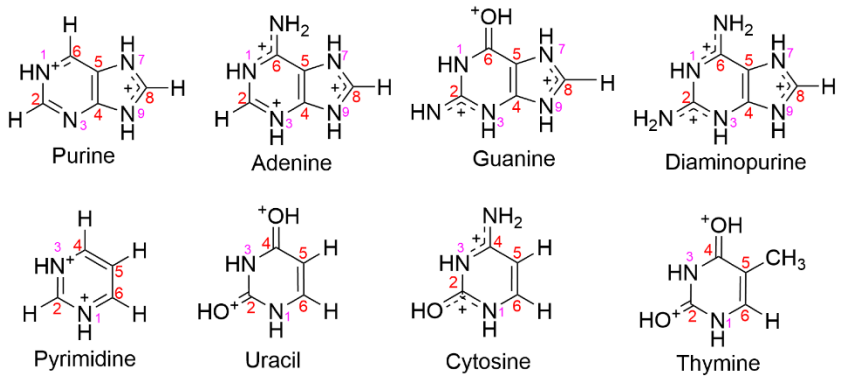

Fig. S15. Inferred protonation states of the nucleic acid bases studied in this paper in concentrated sulfuric acid. See text for details.

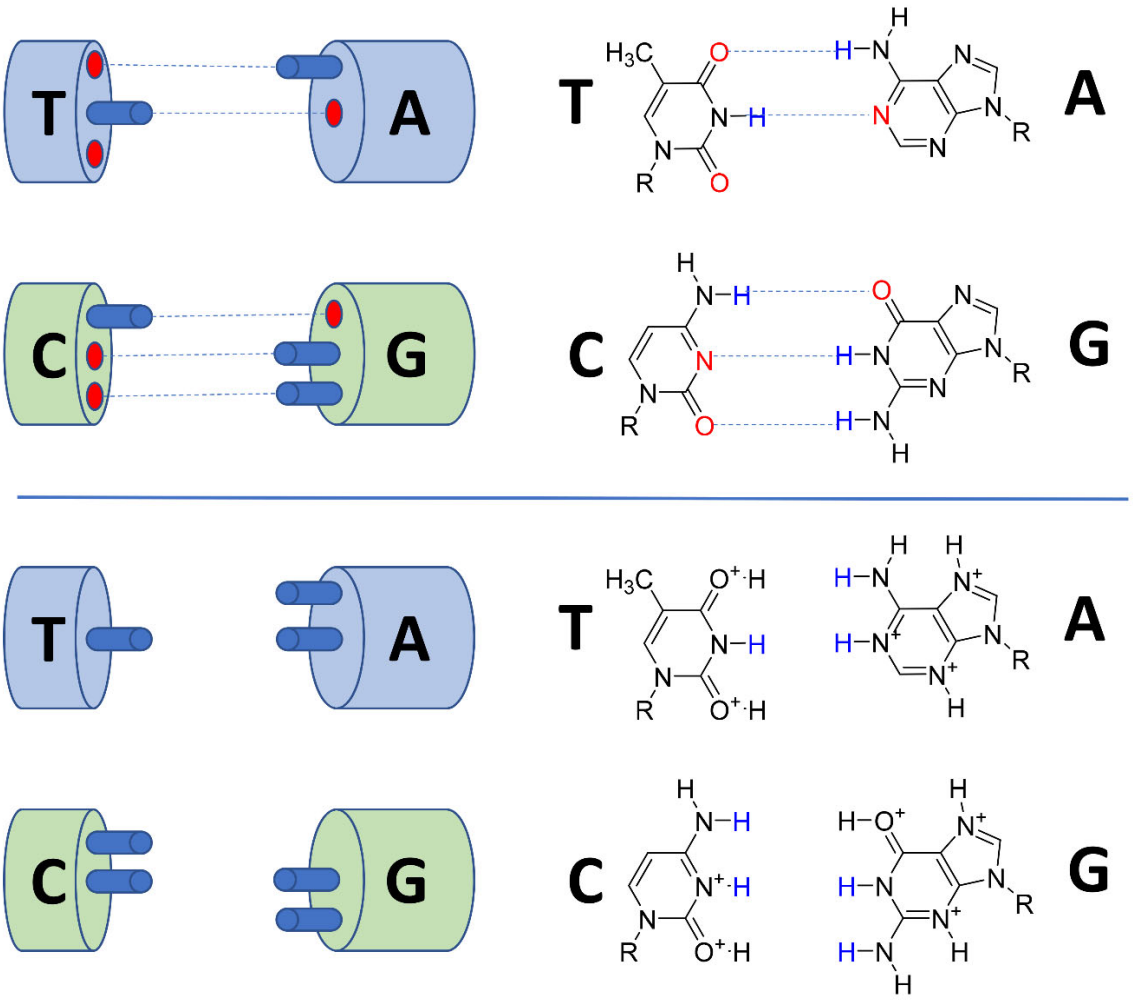

Fig. S16. Protonation and anticipated interference with nucleic acid base pairing. *Right:* Chemical structures of nucleic acid base pairs. *Top*: Formation of base pairs between adenine (A) and thymine (T), cytosine (C) and guanine (G) in water. *Bottom*: The efficient base-pairing of the canonical bases in concentrated sulfuric acid may be perturbed by the protonation of the nitrogen atoms which converts hydrogen bond acceptors (red circles) into hydrogen bond donors (blue rods).

Table S1. An overview and comparison of NMR chemical shifts of guanine from this work, obtained in 98% D_2SO_4, 2% D_2O (by weight) to values in selected solvents reported in the literature. All listed literature NMR data have been obtained at, or close to, room temperature.

Guanine					
^{13}C					
Solvent (reference)	**C2 (ppm)**	**C4 (ppm)**	**C5 (ppm)**	**C6 (ppm)**	**C8 (ppm)**
D_2O pH=13 (1)	160.00	162.20	119.60	168.80	150.10
D_2O/NaOH (7)	160.60	162.80	120.30	169.30	150.50
DMSO-d_6 (2)*	153.80	150.45	108.42	155.74	137.87
DMSO-d_6/HCl (8)	156.00	150.00	108.00	154.00	138.00
98% D_2SO_4, 2% D_2O	150.13	136.17	107.90	150.19	137.53
^{1}H					
Solvent (reference)	**H8 (ppm)**	**H9 (ppm)**	**H7 (ppm)**	**NH_2 (ppm)**	
D_2O (2)*	8.75	–	–	–	
98% D_2SO_4, 2% D_2O	8.29	12.21	11.73	7.05	
^{15}N					
Solvent (reference)	**N1 (ppm)**	**N3 (ppm)**	**N7 (ppm)**	**N9 (ppm)**	**NH_2 (ppm)**
98% D_2SO_4, 2% D_2O	102.79	142.94	154.76	159.34	85.92

(–) Available literature does not provide chemical shift values for the specified atoms or no peaks corresponding to hydrogens attached to nitrogen have been detected.
(*) Data for hydrochloride salt.

Table S2. An overview and comparison of NMR chemical shifts of cytosine from this work, obtained in 98% D_2SO_4, 2% D_2O (by weight) to values in selected solvents reported in the literature. All listed literature NMR data have been obtained at, or close to, room temperature.

Cytosine				
^{13}C				
Solvent (reference)	**C2 (ppm)**	**C4 (ppm)**	**C5 (ppm)**	**C6 (ppm)**
D_2O (2)	159.91	168.10	95.79	144.05
D_2O (2)*	149.74	160.76	94.61	146.92
D_2O (9)	166.40	167.20	94.20	155.30
D_2O (10)	160.20	168.30	95.90	143.90
DMSO-d6 (2)	157.77	167.49	93.35	143.47
DMSO-d6 (11)	156.90	166.60	92.50	142.70
DMSO-d6 (12)	156.80	166.50	92.40	142.70
DMSO-d6**	156.95	166.72	92.56	142.72
conc. H_2SO_4 (5)	150.19	161.03	95.80	147.60
98% D_2SO_4, 2% D_2O	150.23	158.34	97.48	145.28
^{1}H				
Solvent (reference)	**H5 (ppm)**	**H6 (ppm)**	**H1 (ppm)**	**NH_2 (ppm)**
D_2O (2)	5.97	7.50	–	–
D_2O (9)	5.78	7.62	–	–
D_2O (13)	5.94	7.50	–	–
D_2O pH=3.35 (14)	5.89	7.86	–	–
DMSO-d6 (11)	5.58	7.33	–	–
DMSO-d6 (12)	5.56	7.29	10.20	6.95
DMSO-d6 (15, 16)	5.56	7.32	10.39	7.03
conc. H_2SO_4 (5)	6.53	7.90	see (5)	see (5)
98% D_2SO_4, 2% D_2O	5.88	7.17	–	–
^{15}N				
Solvent (reference)	**N1 (ppm)**	**N3 (ppm)**	**NH_2 (ppm)**	
98% D_2SO_4, 2% D_2O	136.44	138.61	102.59	

(–) Available literature does not provide chemical shift values for the specified atoms or no peaks corresponding to hydrogens attached to nitrogen have been detected.
(*) Data for hydrochloride salt.
(**) This study, see Figure S10 in the SI.

Table S3. An overview and comparison of NMR chemical shifts of 2,6-diaminopurine from this work, obtained in 98% D_2SO_4, 2% D_2O (by weight) to values in different solvents reported in the literature.

Diaminopurine						
^{13}C						
Solvent (reference)	**C2 (ppm)**	**C4 (ppm)**	**C5 (ppm)**	**C6 (ppm)**	**C8 (ppm)**	
DMSO-d_6 (17)	160.20	152.77	112.50	155.78	135.91	
DMSO-d_6**	160.69	152.98	113.29	156.32	136.10	
98% D_2SO_4, 2% D_2O	148.08	137.38	102.74	146.80	140.87	
^{1}H						
Solvent (reference)	**H8 (ppm)**	**H9 (ppm)**	**H7 (ppm)**	**NH_2 (ppm)**	**NH_2 (ppm)**	
DMSO-d_6 (18, 19)	7.76	–	–	–	–	
DMSO-d_6 (20)	8.48	–	–	–	–	
98% D_2SO_4, 2% D_2O	8.53	–	–	–	–	
^{15}N						
Solvent (reference)	**N1 (ppm)**	**N3 (ppm)**	**N7 (ppm)**	**N9 (ppm)**	**NH_2 (ppm)**	**NH_2 (ppm)**
98% D_2SO_4, 2% D_2O	see text	see text	156.96	160.12	see text	see text

(–) Available literature does not provide chemical shift values for the specified atoms or no peaks corresponding to hydrogens attached to nitrogen have been detected.
(**) This study, see Figure S10.

Table S4. An overview and comparison of NMR chemical shifts of thymine obtained in 98% D_2SO_4, 2% D_2O (by weight) to values in selected solvents reported in the literature. All listed literature NMR data have been obtained at, or close to, room temperature.

Thymine					
^{13}C					
Solvent (reference)	**C2 (ppm)**	**C4 (ppm)**	**C5 (ppm)**	**C6 (ppm)**	**CH_3 (ppm)**
D_2O (2)	153.90	168.29	110.88	139.79	12.06
DMSO-d_6 (2)	151.46	164.87	107.66	137.63	11.72
DMSO-d_6 (21)	151.49	164.93	107.68	137.72	11.79
DMSO-d_6 (22)	151.50	164.80	107.40	137.80	11.70
DMSO-d_6 (23)	151.50	164.90	107.60	137.80	11.80
DMSO-d_6 (24)	151.51	165.48	108.12	138.19	12.24
DMSO-d6 (11)	151.40	164.90	107.60	137.70	–
DMSO-d_6**	151.96	165.39	108.14	138.19	12.26
conc. H_2SO_4 (5)	150.86	169.60	109.72	150.57	11.96
98% D_2SO_4, 2% D_2O	148.95	167.98	108.09	149.59	9.99
^{1}H					
Solvent (reference)	**H6 (ppm)**	**H1 (ppm)**	**H3 (ppm)**		**CH_3 (ppm)**
D_2O/PBS (25)	7.39	–	–		1.87
D_2O (26)	7.38	–	–		1.89
D_2O (27)	7.65	–	–		1.90
D_2O (28, 29)	7.41	–	–		1.91
D_2O (30)	7.38	10.60	10.96		–
D_2O (31)	7.38	–	–		1.89
DMSO-d_6 (2)	7.28	10.60	11.00		1.75
DMSO-d_6 (21)	7.24	10.98	10.57		1.72
DMSO-d_6 (22)	6.80	–	–		1.26
DMSO-d_6 (23, 24)	7.25	–	–		1.72
DMSO-d6 (11)	7.24	–	–		–
conc. H_2SO_4 (5)	7.82	see (5)	see (5)		2.09
98% D_2SO_4, 2% D_2O	7.43	–	–		1.58
^{15}N					
Solvent (reference)	**N1 (ppm)**	**N3 (ppm)**			
98% D_2SO_4, 2% D_2O	146.64	154.42			

(–) Available literature does not provide chemical shift values for the specified atoms or no peaks corresponding to hydrogens attached to nitrogen have been detected.
(**) This study, see Figure S10.

Table S5. An overview and comparison of NMR chemical shifts of adenine from this work, obtained in 98% D_2SO_4, 2% D_2O (by weight) to values in selected solvents reported in the literature. All listed literature NMR data have been obtained at, or close to, room temperature.

Adenine					
^{13}C					
Solvent (reference)	**C2 (ppm)**	**C4 (ppm)**	**C5 (ppm)**	**C6 (ppm)**	**C8 (ppm)**
D_2O pH=13 (1)	150.5	160.4	121.0	155.0	153.7
DMSO-d_6 (2)	153.41	151.71	119.07	156.36	140.30
DMSO-d_6 (32)	152.40	151.30	117.50	155.30	139.30
DMSO-d_6 (33)	152.20	151.60	121.90	155.60	139.50
DMSO-d_6 (34)	152.35	151.15	117.54	155.41	139.63
DMSO-d_6 (17, 35)	152.37	151.30	117.61	155.30	139.29
DMSO-d_6 (36)	152.46	151.44	117.41	155.37	139.38
DMSO-d_6/HCl (8)	144.00	150.00	114.00	152.00	146.00
98% D_2SO_4, 2% D_2O	149.55	137.37	110.22	146.43	143.42
^{1}H					
Solvent (reference)	**H2 (ppm)**	**H8 (ppm)**	**H9 (ppm)**	**H7 (ppm)**	**NH_2 (ppm)**
D_2O (3)	8.62	8.57	–	–	–
D_2O (37)	8.27	8.22	–	–	–
D_2O (38)	8.05	8.09	–	–	–
D_2O/NaOD (39)	8.23	8.12	–	–	–
$CDCl_3$ (2)	8.14	8.11	12.80	–	7.09
DMSO-d_6 (33)	8.11	8.10	12.78	–	7.10
DMSO-d_6 (36)	8.12	8.11	12.75	–	7.10
DMSO-d_6 (18)	–	8.14	–	–	–
98% D_2SO_4, 2% D_2O	8.83	8.86	–	–	–
^{15}N					
Solvent (reference)	**N1 (ppm)**	**N3 (ppm)**	**N7 (ppm)**	**N9 (ppm)**	**NH_2 (ppm)**
DMSO-d_6 (40)	236.00	230.20	241.40	159.10	80.50
98% D_2SO_4, 2% D_2O	see text	see text	161.23	160.89	113.44

(–) Available literature does not provide chemical shift values for the specified atoms or no peaks corresponding to hydrogens attached to nitrogen have been detected.

Table S6. An overview and comparison of NMR chemical shifts of uracil from this work, obtained in 98% D_2SO_4, 2% D_2O (by weight) to values in selected solvents reported in the literature. All listed literature NMR data have been obtained at, or close to, room temperature.

Uracil				
^{13}C				
Solvent (reference)	**C2 (ppm)**	**C4 (ppm)**	**C5 (ppm)**	**C6 (ppm)**
DMSO-d_6 (2)	152.27	165.09	101.01	142.89
DMSO-d_6 (21)	151.39	164.20	100.11	142.07
DMSO-d_6 (33)	151.40	164.30	100.10	142.10
DMSO-d_6 (22)	151.40	164.20	100.10	142.10
DMSO-d_6 (24)	151.98	164.80	100.67	142.67
DMSO-d_6 (41, 42)	151.39	164.20	100.10	142.07
DMSO-d_6 (42)	151.45	164.26	100.28	142.13
DMSO-d_6**	152.00	164.82	100.69	142.66
conc. H_2SO_4 (5)	150.30	171.60	97.30	153.90
98% D_2SO_4, 2% D_2O	148.41	169.48	95.93	153.07
^{1}H				
Solvent (reference)	**H5 (ppm)**	**H6 (ppm)**	**H1 (ppm)**	**H3 (ppm)**
D_2O (37)	5.85	7.57	–	–
D_2O (31)	5.85	7.51	–	–
D_2O/PBS (25)	5.78	7.49	–	–
DMSO-d_6 (2)	5.47	7.41	10.82	11.02
DMSO-d_6 (21)	5.45	7.39	11.00	10.60
DMSO-d_6 (33)	5.45	7.39	10.93	10.93
DMSO-d_6 (43)	5.37	7.29	–	–
DMSO-d_6 (22)	5.44	7.38	–	–
DMSO-d_6 (24)	5.45	7.39	10.85	10.85
DMSO-d_6 (41, 42)	5.45	7.39	10.80	11.00
DMSO-d_6 (42)	5.82	7.74	11.31	11.31
conc. H_2SO_4 (5)	6.70	8.30	see (5)	see (5)
98% D_2SO_4, 2% D_2O	6.02	7.61	–	–
^{15}N				
Solvent (reference)	**N1 (ppm)**	**N3 (ppm)**		
DMSO-d_6 (43)	135.00	162.5		
98% D_2SO_4, 2% D_2O	149.50	154.76		

(–) Available literature does not provide chemical shift values for the specified atoms or no peaks corresponding to hydrogens attached to nitrogen have been detected.
(**) This study, see Figure S10.

Table S7. An overview and comparison of NMR chemical shifts of purine from this work, obtained in 98% D_2SO_4, 2% D_2O (by weight) to values in selected solvents reported in the literature. All listed literature NMR data have been obtained at, or close to, room temperature.

Purine					
^{13}C					
Solvent (reference)	**C2 (ppm)**	**C4 (ppm)**	**C5 (ppm)**	**C6 (ppm)**	**C8 (ppm)**
D_2O (2)	152.39	155.74	129.04	145.36	148.04
DMSO-d_6 (2)	152.03	154.54	130.31	145.53	146.07
DMSO-d_6 (32)	152.10	154.70	130.40	145.50	146.10
DMSO-d_6 (17)	152.10	154.77	130.46	145.50	146.09
DMSO-d_6 (44)	151.80	154.50	130.20	145.30	145.90
DMSO-d_6 (45)	152.21	152.50	133.05	145.80	147.12
98% D_2SO_4, 2% D_2O	149.39	151.04	120.66	140.66	150.00
^{1}H					
Solvent (reference)	**H2 (ppm)**	**H6 (ppm)**	**H8 (ppm)**	**H9 (ppm)**	**H7 (ppm)**
D_2O (2)	8.72	8.83	8.50	–	–
D_2O (37)	9.00	9.19	8.64	–	–
DMSO-d_6 (2)	8.99	9.21	8.70	13.50	–
DMSO-d_6 (32)	8.85	9.05	8.54	–	–
DMSO-d_6 (46)	8.90	9.10	8.60	–	–
DMSO-d_6 (18, 19)	–	–	8.68	–	–
DMSO-d_6 (44)	8.88	9.09	–	12.80	–
DMSO-d_6 (45)	8.91	9.12	8.61	13.45	–
98% D_2SO_4, 2% D_2O	9.01	9.20	9.20	–	–
^{15}N					
Solvent (reference)	**N1 (ppm)**	**N3 (ppm)**	**N7 (ppm)**	**N9 (ppm)**	
H_2O (40)	267.60	252.50	195.70	191.60	
DMSO-d_6 (40)	278.90	261.30	210.50	190.00	
90% D_2SO_4, 10% D_2O (4)*	186.03	260.03	158.33	163.43	
98% D_2SO_4, 2% D_2O	185.99	262.08	158.11	163.02	

(–) Available literature does not provide chemical shift values for the specified atoms or no peaks corresponding to hydrogens attached to nitrogen have been detected.
(*) chemical shift values converted from σ in the original source (4) to δ for consistency (47).

Table S8. An overview and comparison of NMR chemical shifts of pyrimidine from this work, obtained in 98% D_2SO_4, 2% D_2O (by weight) to values in selected solvents reported in the literature. All listed literature NMR data have been obtained at, or close to, room temperature.

Pyrimidine			
^{13}C			
Solvent (reference)	**C2 (ppm)**	**C4,6 (ppm)**	**C5 (ppm)**
D_2O (48)	157.1	157.0	122.3
D_2O/H_2SO_4 (49, 50)	152.2	158.8	125.1
$CDCl_3$ (2)	159.08	156.92	121.61
DMSO-d_6 (51)	158.39	156.90	121.86
DMSO-d_6 (52)	158.60	157.12	122.04
DMSO-d_6**	159.06	157.63	122.54
98% D_2SO_4, 2% D_2O	149.85	158.18	127.77
^{1}H			
Solvent	**H2 (ppm)**	**H4,6 (ppm)**	**H5 (ppm)**
D_2O (48)	8.98	8.67	7.47
D_2O (37)	9.19	8.86	7.65
D_2O/DCl (49)	9.74	9.45	8.34
$CDCl_3$ (2)	9.27	8.78	7.38
DMSO-d_6 (52)	9.24	8.86	7.58
CH_2Cl_2 (53)	9.15	8.69	7.29
98% D_2SO_4, 2% D_2O	9.68	9.20	8.35
^{15}N			
Solvent (reference)	**N1,3 (ppm)**		
CH_2Cl_2 (53)	294.40		
DMSO-d_6 (54)	295.3		
98% D_2SO_4, 2% D_2O	201.21		

(**) This study, see Figure S10.

Table S9. Concentrations and peak maxima for UV spectroscopy in this study.

Compound	Concentration (μM)	UV Maxima (nm)
Adenine	7	197.0, 258.0
Cytosine	100	214.0, 266.0
2,6-Diaminopurine	50	194.0, 217.0, 276.0
Guanine	60	233.0, 255.0
Purine	40	258.0
Pyrimidine	0.6	246.0
Thymine	80	194.0, 284.0
Uracil	70	193.0, 276.0

Dataset S1. Dataset S1 contains original UV-Vis data.

The folder "UV-VIS_plots-data-code-tables DATASET S1.zip" containing the original UV-Vis data can be downloaded from Zenodo at https://zenodo.org/

Dataset S2. Dataset S2 contains original NMR data.

The folder "ORIGINAL NMR DATA_DATASET S2.zip" containing the original NMR data can be downloaded from Zenodo at https://zenodo.org/ and contains the following data:

a) all 2 week stability data – that contains all the 1D-^{13}C NMR data collected after 2 week incubation in concentrated sulfuric acid.

b) DMSO solvent bases data – 1D-^{13}C NMR data for the selected bases measured directly in DMSO-d_6 solvent.

The 1D-^{13}C NMR measurements in sulfuric acid at different concentrations follows the following naming convention. Different concentrations (by weight) of sulfuric acid in water are denoted by different letters from H-K: 98% D_2SO_4/2% D_2O; 94% D_2SO_4/6% D_2O; 88% D_2SO_4/12% D_2O; 81% D_2SO_4/19% D_2O, all with DMSO-d_6 as a reference and at room temperature.

H - 81% D_2SO_4/19% D_2O with DMSO-d_6 as a reference and at room temperature
I - 88% D_2SO_4/12% D_2O with DMSO-d_6 as a reference and at room temperature
J - 94% D_2SO_4/6% D_2O with DMSO-d_6 as a reference and at room temperature
K - 98% D_2SO_4/2% D_2O with DMSO-d_6 as a reference and at room temperature

c) Cytosine NMR folder with the following files:

1D-^{13}C H-K - SZssea-Cytosine-H-DMSO-101322_1.zip, SZssea-Cytosine-I-DMSO-101322_1.zip, SZssea-Cytosine-J-DMSO-101322_1.zip, SZssea-Cytosine-K-DMSO-101322_1.zip (the same as SZssea-Cytosine-K-13C-DMSO-101322_1.zip)

1D-^{13}C - SZssea-Cytosine-K-13C-DMSO-101322_1.zip

1D-^{1}H - SZssea-1H-Cytosine-K-DMSO-101322_1.zip

1D-^{15}N - SZssea-Cytosine-K-15N-102022_1.zip

2D-^{1}H-^{13}C-HMQC - SZssea-Cytosine-K-HC-HMQC-101822_1.zip

2D-^{1}H-^{13}C-HMBC - SZssea-Cytosine-K-HC-HMBC-101822_1.zip

2D-1H-15N-HMBC - SZssea-Cytosine-K-HN-HMBC-101822_1.zip

d) Guanine NMR folder with the following files:

1D-^{13}C H-K - SZssea-Guanine-H-DMSO-101122_1.zip, SZssea-Guanine-I-DMSO-101122_1.zip, SZssea-Guanine-J-DMSO-101122_1.zip, SZssea-Guanine-K-DMSO-101122_1.zip (the same as SZssea-Guanine-K-13C DMSO-101122_1.zip)

1D-^{13}C - SZssea-Guanine-K-13C DMSO-101122_1.zip

1D-^{1}H - SZssea-Guanine-K-1H-DMSO-101122_1.zip

1D-^{15}N - SZssea-Guanine-K-15N-DMSO-101722_1.zip

2D-^{1}H-^{13}C-HMQC - SZssea-Guanine-K-HC-HMQC-DMSO-101722_1 (2).zip

2D-^{1}H-^{13}C-HMBC - SZssea-Guanine-K-HC-HMBC-DMSO-101722_1 (2).zip

2D-^{1}H-^{15}N-HMBC - SZssea-Guanine-K-HN-HMBC-DMSO-101722_1.zip

e) Adenine NMR folder with the following files:

1D-^{13}C H-K - SZssea-Adenine-H-DMSO-101322_1.zip, SZssea-Adenine-I-DMSO-101322_1.zip, SZssea-Adenine-J-DMSO-101322_1.zip, SZssea-Adenine-K-DMSO-101322_1.zip

1D-^{13}C - SZssea-Adenine-K-DMSO-101322_1.zip

1D-^{1}H - SZssea-1H-Adenine-K-DMSO-101322_1.zip

1D-^{15}N - SZssea-Adenine-K-15N-DMSO-110422_3.zip

2D-^{1}H-^{13}C-HMQC - SZssea-Adenine-K-HC-HMQC-102122_2.zip

2D-^{1}H-^{13}C-HMBC - SZssea-Adenine-K-HC-HMBC-102122_2.zip

2D-1H-^{15}N-HMBC - SZssea-Adenine-K-HN-HMBC-102122_2.zip

f) Diaminopurine NMR folder with the following files:

1D-^{13}C H-K - SZssea-Diap-H-DMSO-101422_1.zip, SZssea-Diap-H-DMSO-101422_2.zip, Szssea-Diap-H-DMSO-101422_4.zip, SZssea-Diap-I-DMSO-101422_1.zip, SZssea-Diap-I-DMSO-101422_2.zip, SZssea-Diap-J-101422_1.zip, SZssea-Diap-K-101422_1.zip

1D-^{13}C - SZssea-Diap-K-101422_1.zip

1D-^{1}H - SZssea-1H-Diap-K-101422_1.zip

1D-^{15}N - SZssea-Diapurine-K-15N-102122_1.zip

2D-^{1}H-^{13}C-HMQC - SZssea-Diaminopurine-K-HC-HMQC-102122_2.zip

2D-1H-^{13}C-HMBC - SZssea-Diaminopurine-K-HC-HMBC-102122_2.zip

2D-1H-^{15}N-HMBC - SZssea-Diaminopurine-K-HN-HMBC-102122_2.zip

g) Purine NMR folder with the following files:

1D-^{13}C H-K - SZssea-Purine-H-DMSO-101122_1.zip, SZssea-Purine-I-DMSO-101122_1.zip, SZssea-Purine-J-DMSO-101122_1.zip, SZssea-Purine-K-DMSO-101122_1.zip

1D-^{13}C - SZssea-Purine-K-DMSO-101122_1.zip

1D-^{1}H - SZssea-Purine-1H-K-DMSO-101722_2.zip

1D-^{15}N - SZssea-Purine-K-15N-102122_1.zip

2D-^{1}H-^{13}C-HMQC - SZssea-Purine-K-HC-HMQC-101722_1.zip

2D-^{1}H-^{13}C-HMBC - SZssea-Purine-K-HC-HMBC-101722_1.zip

2D-^{1}H-^{15}N-HMBC - SZssea-Purine-K-HN-HMBC-101722_1.zip

h) Pyrimidine NMR folder with the following files:

1D-^{13}C H-K - SZssea-Pyr-H-DMSO-101322_1.zip, SZssea-Pyr-I-DMSO-101322_1.zip, SZssea-Pyr-J-DMSO-101322_1.zip, SZssea-Pyr-K-DMSO-101322_1.zip

1D-^{13}C - SZssea-Pyr-K-DMSO-101322_1.zip

1D-^{1}H - SZssea-1H-Pyr-K-DMSO-101322_1.zip

1D-^{15}N - SZssea-Pyrimidine-K-15N_101922_1.zip

2D-^{1}H-^{13}C-HMQC - SZssea-Pyrimidine-K-HC-HMQC-102022_2.zip

2D-^{1}H-^{13}C-HMBC - SZssea-Pyrimidine-K-HC-HMBC_101922_1.zip

2D-^{1}H-^{15}N-HMBC - SZssea-Pyrimidine-K-HN-HMBC_101922_1.zip

i) Thymine NMR folder with the following files:

1D-^{13}C H-K - SZssea-Thymine-H-DMSO-101322_1.zip, SZssea-Thymine-I-DMSO-101322_1.zip, SZssea-Thymine-J-DMSO-101322_1.zip, SZssea-Thymine-K-DMSO-101322_1.zip

1D-^{13}C - SZssea-Thymine-K-DMSO-101322_1.zip

1D-^{1}H - SZssea-1H-Thymine-K-DMSO-101322_1.zip

1D-^{15}N - SZssea-Thymine-K-15N-102122_1.zip

2D-^{1}H-^{13}C-HMQC - SZssea-Thymine-K-HC-HMQC-102022_1.zip

2D-^{1}H-^{13}C-HMBC - SZssea-Thymine-K-HC-HMBC-102022_1.zip

2D-^{1}H-^{15}N-HMBC - SZssea-Thymine-K-HN-HMBC-102022_1.zip

j) Uracil NMR folder with the following files:

1D-^{13}C H-K - SZssea-Uracil-H-DMSO-101322_1.zip, SZssea-Uracil-I-DMSO-101322_1.zip, SZssea-Uracil-J-DMSO-101322_1.zip, SZssea-Uracil-K-DMSO-101322_1.zip

1D-^{13}C - SZssea-Uracil-K-DMSO-101322_1.zip

1D-^{1}H - SZssea-1H-Uracil-K-DMSO-101322_1.zip

1D-^{15}N - SZssea-Uracil-K-15N-102122_1.zip

2D-^{1}H-^{13}C-HMQC - SZssea-Uracil-K-HC-HMQC-101822_1.zip

2D-^{1}H-^{13}C-HMBC - SZssea-Uracil-K-HC-HMBC-101822_1.zip

2D-^{1}H-^{15}N-HMBC - SZssea-Uracil-K-HN-HMBC-101822_1.zip

SI References

1. L. G. Purnell, D. J. Hodgson, Carbon-13 nmr studies of purines and 8-azapurines in basic aqueous medium. *Org. Magn. Reson.* **10**, 1–4 (1977).
2. T. Saito, *et al.*, Spectral database for organic compounds (sdbs). *Natl. Inst. Adv. Ind. Sci. Technol.* (2006).
3. R. Wagner, W. von Philipsborn, Protonierung von Purin, Adenin und Guanin NMR.-Spektren und Strukturen der Mono-, Di-und Tri-Kationen. *Helv. Chim. Acta* **54**, 1543–1558 (1971).
4. M. Schumacher, H. Günther, Beiträge zur 15N-NMR-Spektroskopie Protonierung und Tautomerie in Purinen: Purin und 7-und 9-Methylpurin. *Chem. Ber.* **116**, 2001–2014 (1983).
5. R. L. Benoit, M. Frechette, 1H and 13C nuclear magnetic resonance and ultraviolet studies of the protonation of cytosine, uracil, thymine, and related compounds. *Can. J. Chem.* **64**, 2348–2352 (1986).
6. R. Wagner, W. von Philipsborn, Protonierung von Amino-und Hydroxypyrimidinen NMR-Spektren und Strukturen der Mono-und Dikationen. *Helv. Chim. Acta* **53**, 299–320 (1970).
7. M. D. Oza, R. Meena, K. Prasad, P. Paul, A. K. Siddhanta, Functional modification of agarose: A facile synthesis of a fluorescent agarose–guanine derivative. *Carbohydr. Polym.* **81**, 878–884 (2010).
8. T. Kozluk, I. D. Spenser, Carbon-13 NMR spectroscopy as a biosynthetic probe. The biosynthesis of purines in yeast. *J. Am. Chem. Soc.* **109**, 4698–4702 (1987).
9. S. Kopf, *et al.*, Base-Mediated Remote Deuteration of N-Heteroarenes–Broad Scope and Mechanism. *European J. Org. Chem.* **2022**, e202200204 (2022).
10. A. A. Shaw, M. D. Shetlar, 3-Ureidoacrylonitriles: novel products from the photoisomerization of cytosine, 5-methylcytosine, and related compounds. *J. Am. Chem. Soc.* **112**, 7736–7742 (1990).
11. Y. Shalom, J. Blum, R. G. Harvey, Adducts of phenanthrene 9, 10-imine and of benz [a] anthracene 5, 6-imine to some nitrogen heterocycles. *J. Heterocycl. Chem.* **33**, 681–686 (1996).
12. J. H. Clark, E. M. Goodman, The cytosine—fluoride interaction. *Spectrochim. Acta Part A Mol. Spectrosc.* **42**, 457–460 (1986).
13. S. Shirotake, Complexes between Nucleic Acid Bases and Bivalent Metal Ions. III. Syntheses and Spectral Analyses of Cytosine-Calcium Chloride Complexes. *Chem. Pharm. Bull.* **28**, 956–963 (1980).
14. A. Bourafai-Aziez, *et al.*, Development, Validation, and Use of 1H-NMR Spectroscopy for Evaluating the Quality of Acerola-Based Food Supplements and Quantifying Ascorbic Acid. *Molecules* **27**, 5614 (2022).
15. S. P. Samijlenko, *et al.*, Structural peculiarities of 6-azacytosine and its derivatives imply intramolecular H-bonds. *J. Mol. Struct.* **484**, 31–38 (1999).
16. S. P. Samijlenko, *et al.*, 1H NMR investigation on 6-azacytidine and its derivatives. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **55**, 1133–1141 (1999).
17. M. C. Thorpe, W. C. Coburn Jr, J. A. Montgomery, The 13C nuclear magnetic resonance spectra of some 2-, 6-, and 2, 6-substituted purines. *J. Magn. Reson.* **15**, 98–112 (1974).
18. W. C. J. Coburn, M. C. Thorpe, J. A. Montgomery, K. Hewson, Correlation of the Proton Magnetic Resonance Chemical Shifts of Substituted Purines with Reactivity Parameters. II. 6-Substituted Purines. *J. Org. Chem.* **30**, 1114–1117 (1965).
19. W. C. J. Coburn, M. C. Thorpe, J. A. Montgomery, K. Hewson, Correlation of the Proton Magnetic Resonance Chemical Shifts of Substituted Purines with Reactivity Parameters. I. 2,6-Disubstituted Purines. *J. Org. Chem.* **30**, 1110–1113 (1965).
20. L.-L. Xu, *et al.*, Chiroptical Activity from an Achiral Biological Metal–Organic Framework. *J. Am. Chem. Soc.* **140**, 11569–11572 (2018).
21. D. Kubica, S. Molchanov, A. Gryff-Keller, Solvation of uracil and its derivatives by DMSO:

A DFT-supported 1H NMR and 13C NMR study. *J. Phys. Chem. A* **121**, 1841–1848 (2017).
22. S. Kan, *et al.*, Chemical constituents from the roots of Xanthium sibiricum. *Nat. Prod. Res.* **25**, 1243–1249 (2011).
23. A. W. Newaz, K. Yong, W. Yi, B. Wu, Z. Zhang, Antimicrobial metabolites from the Indonesian mangrove sediment-derived fungus Penicillium chrysogenum sp. ZZ1151. *Nat. Prod. Res.*, 1–7 (2022).
24. J. E. Okokon, *et al.*, In vivo antihyperglycaemic and antihyperlipidemic activities and chemical constituents of Solanum anomalum. *Biomed. Pharmacother.* **151**, 113153 (2022).
25. S. Lamichhane, *et al.*, Strategy for nuclear-magnetic-resonance-based metabolomics of human feces. *Anal. Chem.* **87**, 5930–5937 (2015).
26. A. Sułkowska, Effect of temperature on the stability of association of pyrimidine bases with serum albumin: Proton NMR study. *Appl. Spectrosc.* **51**, 428–432 (1997).
27. M. Martini, J. Termini, Peroxy radical oxidation of thymidine. *Chem. Res. Toxicol.* **10**, 234–241 (1997).
28. P. J. W. Pouwels, R. Kaptein, R. F. Hartman, S. D. Rose, Photo-CIDNP study of pyrimidine dimer splitting I: reactions involving pyrimidine radical cation intermediates. *Photochem. Photobiol.* **61**, 563–574 (1995).
29. P. J. W. Pouwels, R. Kaptein, R. F. Hartman, S. D. Rose, Photo-CIDNP study of pyrimidine dimer splitting II: reactions involving pyrimidine radical anion intermediates. *Photochem. Photobiol.* **61**, 575–583 (1995).
30. H. Asanuma, T. Hishiya, M. Komiyama, Direct evidences for the hydrogen bonding in water by polymeric receptors carrying diaminotriazine. *Chem. Lett.* **27**, 1087–1088 (1998).
31. A. Sulkowska, A. Michnik, Proton NMR studies on the interaction of alkyl derivatives of pyrimidine bases, their nucleosides and nucleotides with bovine serum albumin. *J. Mol. Struct.* **348**, 73–76 (1995).
32. M. T. Chenon, R. J. Pugmire, D. M. Grant, R. P. Panzica, L. B. Townsend, Carbon-13 magnetic resonance. XXVI. Quantitative determination of the tautomeric populations of certain purines. *J. Am. Chem. Soc.* **97**, 4636–4642 (1975).
33. L. RunHui, *et al.*, N-containing compounds from the traditional Chinese medicine ChanSu. *Chem. Nat. Compd.* **45**, 599–600 (2009).
34. K. Dybiec, S. Molchanov, A. Gryff-Keller, Adenine and some of its analogues in DMSO-d6 solution: an NMR and GIAO-DFT study. *Pol. J. Chem.* **83**, 857–868 (2009).
35. P. Chittepu, V. R. Sirivolu, F. Seela, Nucleosides and oligonucleotides containing 1, 2, 3-triazole residues with nucleobase tethers: Synthesis via the azide-alkyne ‘click’reaction. *Bioorg. Med. Chem.* **16**, 8427–8439 (2008).
36. H. Moriyama, T. Iizuka, M. Nagai, K. Hoshi, Adenine, an inhibitor of platelet aggregation, from the leaves of Cassia alata. *Biol. Pharm. Bull.* **26**, 1361–1364 (2003).
37. H. J. Schneider, *et al.*, Complexation of nucleosides, nucleotides, and analogs in an azoniacyclophane. Van der Waals and electrostatic binding increments and NMR shielding effects. *J. Am. Chem. Soc.* **114**, 7704–7708 (1992).
38. A. Sułkowska, Temperature effect on the stability of the complexes between purine derivatives and serum albumin: proton NMR study. *J. Mol. Struct.* **410**, 23–25 (1997).
39. M. Mano, T. Seo, K. Imai, Synthesis of 6-methylaminopurine by thermal cyclization of 4, 6-bis (methylamino)-5-phenylazopyrimidine. *Chem. Pharm. Bull.* **31**, 3454–3459 (1983).
40. R. Marek, V. Sklenar, NMR Studies of Purines. *Annu. reports NMR Spectrosc.* **54**, 201–242 (2005).
41. E. Bednarek, *et al.*, Theoretical and experimental 1H, 13C, 15N, and 17O NMR spectra of 5-nitro, 5-amino, and 5-carboxy uracils. *J. Mol. Struct.* **482**, 333–337 (1999).
42. E. Bednarek, *et al.*, Theoretical and experimental 1H, 13C, 15N, and 17O NMR chemical shifts for 5-halogenouracils. *J. Mol. Struct.* **554**, 233–243 (2000).
43. J. H. Clark, J. S. Taylor, A. J. Goodwin, Multinuclear NMR studies of the fluoride-uracil complex. *Spectrochim. Acta Part A Mol. Spectrosc.* **38**, 1101–1104 (1982).
44. A. Unciti-Broceta, M. J. Pineda de las Infantas, M. A. Gallo, A. Espinosa, Reduction of Different Electron-Poor N-Heteroarylhydrazines in Strong Basic Conditions. *Chem. Eur. J.*

13, 1754–1762 (2007).
45. M. Česnek, *et al.*, Synthesis and properties of 2-guanidinopurines. *Collect. Czechoslov. Chem. Commun.* **71**, 1303–1319 (2006).
46. T. H. Graham, W. Liu, D.-M. Shen, A Method for the Reductive Scission of Heterocyclic Thioethers. *Org. Lett.* **13**, 6232–6235 (2011).
47. R. K. Harris, E. D. Becker, S. M. Cabral de Menezes, R. Goodfellow, P. Granger, NMR Nomenclature: Nuclear Spin Properties and Conventions for Chemical Shifts. IUPAC Recommendations 2001. *Solid State Nucl. Magn. Reson.* **22**, 458–483 (2002).
48. K. Goel, S. Bera, M. Singh, D. Mondal, Synthesis of dual functional pyrimidinium ionic liquids as reaction media and antimicrobial agents. *RSC Adv.* **6**, 106806–106820 (2016).
49. J. Clark, G. Hitiris, Covalent hydration of 5-substituted pyrimidines. *Spectrochim. Acta Part A Mol. Spectrosc.* **40**, 75–79 (1984).
50. R. J. Pugmire, D. M. Grant, Carbon-13 magnetic resonance. X. Six-membered nitrogen heterocycles and their cations. *J. Am. Chem. Soc.* **90**, 697–706 (1968).
51. J. Riand, M. T. Chenon, N. Lumbroso-Bader, Etude par rmn du carbone-13 des effets de substituants dans le noyau de la pyrimidine. *Tetrahedron Lett.* **15**, 3123–3126 (1974).
52. A. Y. Denisov, V. I. Mamatyuk, O. P. Shkurko, Additivity of 13C-1H and 1H-1H spin-spin coupling constants in six-membered aromatic nitrogen-containing heterocycles. *Chem. Heterocycl. Compd.* **21**, 821–825 (1985).
53. K. J. Sheehy, L. M. Bateman, N. T. Flosbach, M. Breugst, P. A. Byrne, Identification of N- or O-Alkylation of Aromatic Nitrogen Heterocycles and N-Oxides Using 1H–15N HMBC NMR Spectroscopy. *European J. Org. Chem.* **2020**, 3270–3281 (2020).
54. A. Dokalik, H. Kalchhauser, W. Mikenda, G. Schweng, NMR spectra of nitrogen-containing compounds. Correlations between experimental and GIAO calculated data. *Magn. Reson. Chem.* **37**, 895–902 (1999).