Supplemental data for: Prebiotic thiol-catalyzed thioamide bond formation

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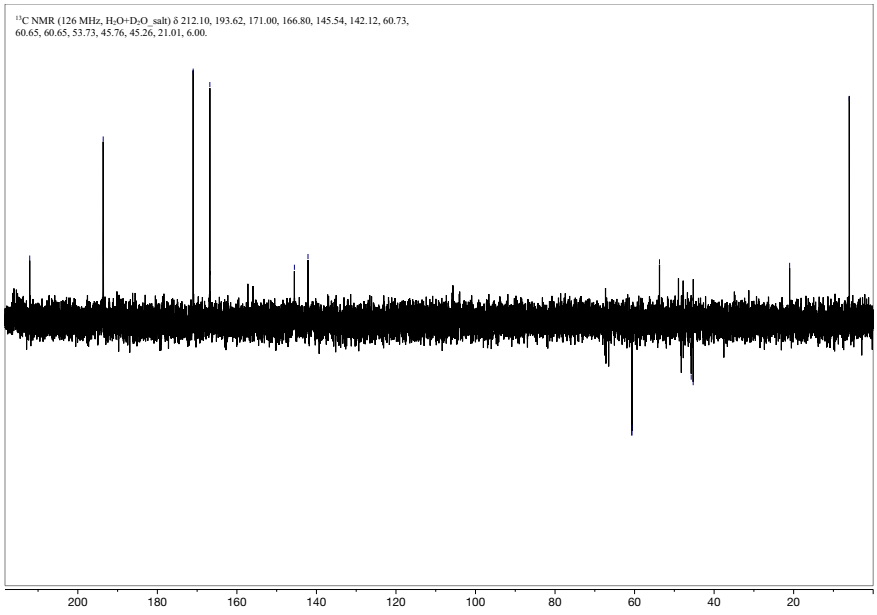
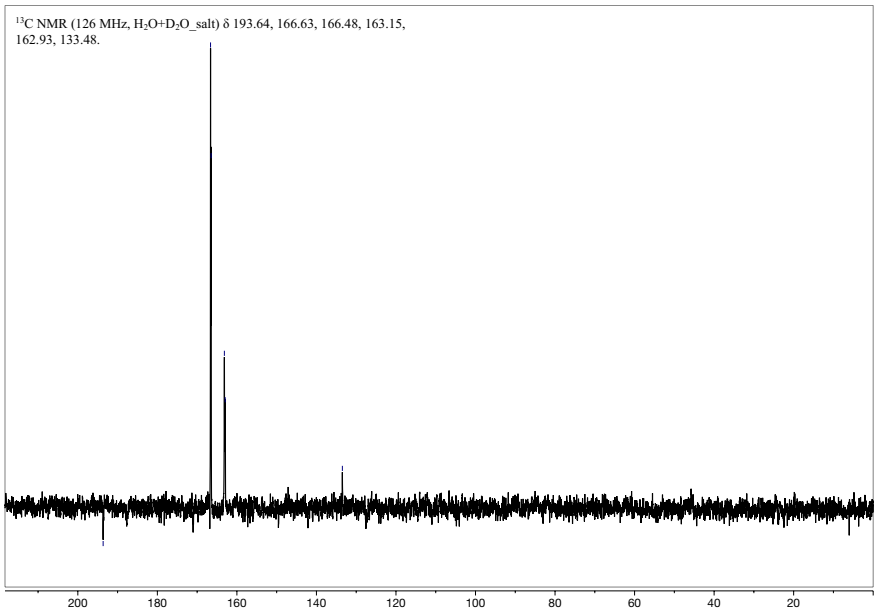
1 Spark-discharge NMR spectra

13C NMR spectra were collected using proton-decoupling (pulse program zgpg30), as described in the main body of the text. Additional 13C spectra were collected for spark discharge experiments to aide in the identification of the compound of interest (thioformamide). Dimensionless Enhancement by Polarization Transfer (DEPT-135) helps elucidate the multiplicity of the carbon atoms (methine and methyl groups have a positive phase, methylene groups have a negative phase, and quaternary carbons do not have peaks). Similarly, the pulse program jmod shows methine and methyl groups with a negative phase and shows methene and quaternary carbons with a positive phase. These spectra are shown below (Figures S1 and S2).



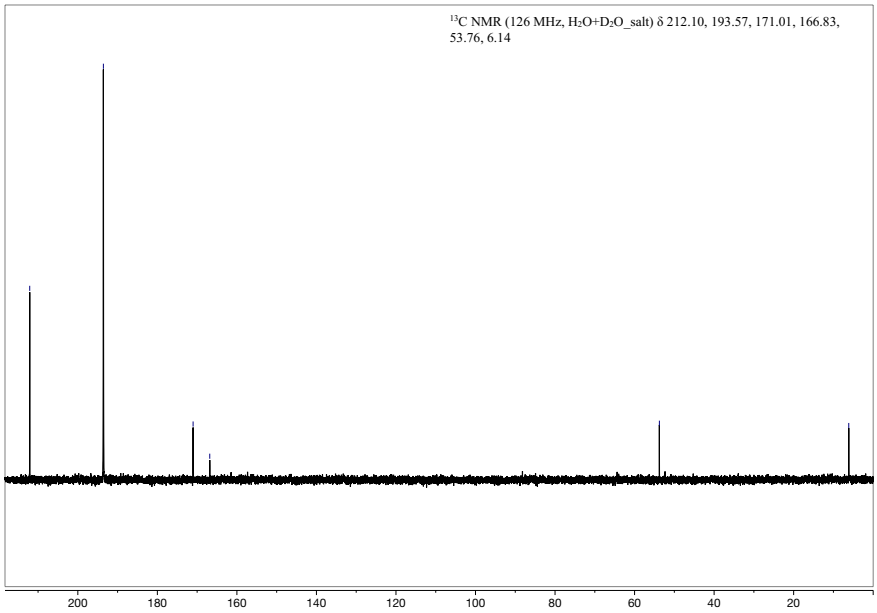
Supplementary Figure 1: 13C NMR spectrum (DEPT-135) for the reducing spark discharge incubation. The peak corresponding to thioformamide (193.6 ppm) is phased up, indicating that the carbon is either a methine or methyl carbon.



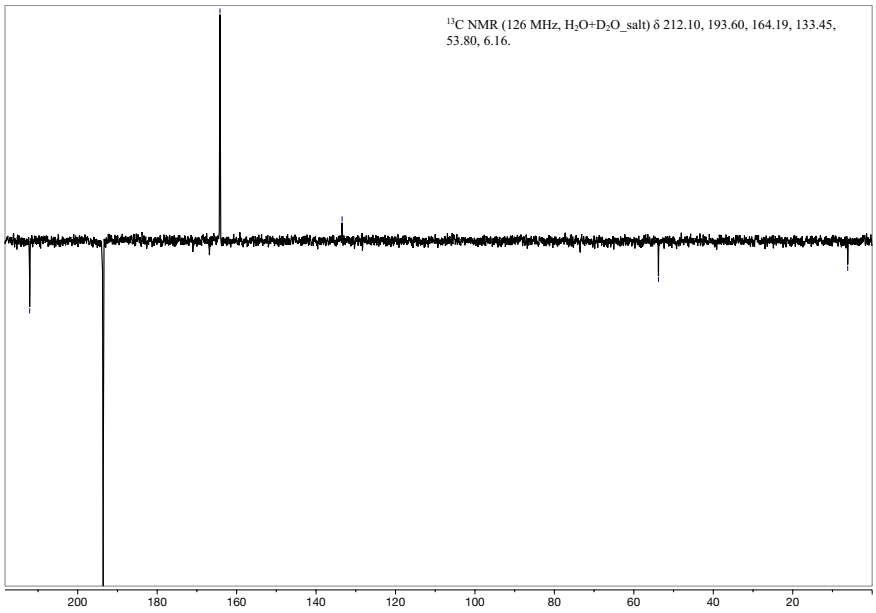
Supplementary Figure 2: 13C NMR spectrum (jmod) for the reducing spark discharge incubation. The peak corresponding to thioformamide (193.6 ppm) is phased down, indicating that the carbon is either a methine or methyl carbon.

2 Additional spectra from HCN reactions

We also used DEPT-135 and jmod pulse programs to characterize the reaction of HCN, CH_3SH, and HS^-. These specta are shown below (Figures S3 and S4).

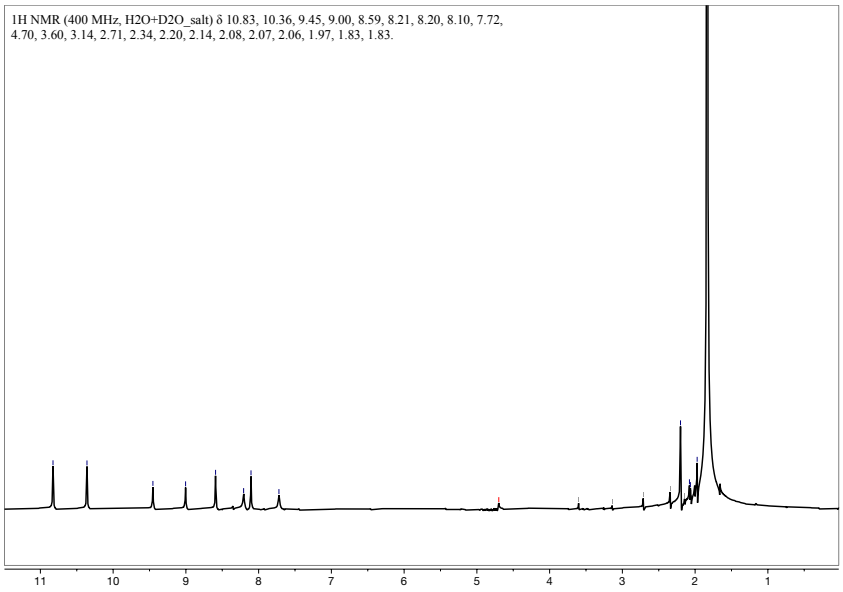


Supplementary Figure 3: 13C NMR spectrum (DEPT-135) for the reaction of HCN, CH_3SH, and HS^-. The peak corresponding to thioformamide (193.6 ppm) is phased up, indicating that the carbon is either a methine or methyl carbon.



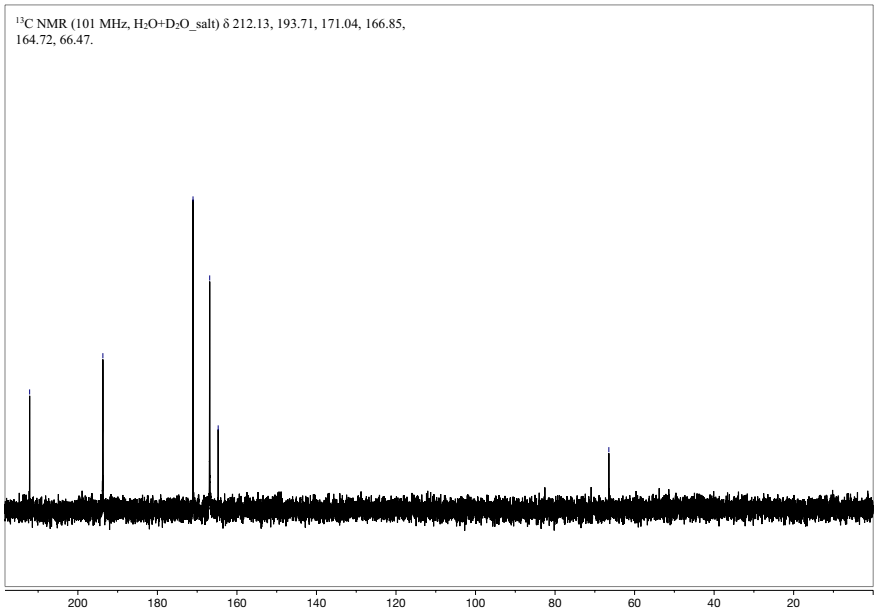
Supplementary Figure 4: 13C NMR spectrum (jmod) for the reaction of HCN, CH_3SH, and HS^-. The peak corresponding to thioformamide (193.6 ppm) is phased down, indicating that the carbon is either a methine or methyl carbon.

Proton NMR (pulse program *zgesgp*) was also used to examine the reaction of cyanide, sulfide, and methanethiol. This spectrum is shown in Figure S5.

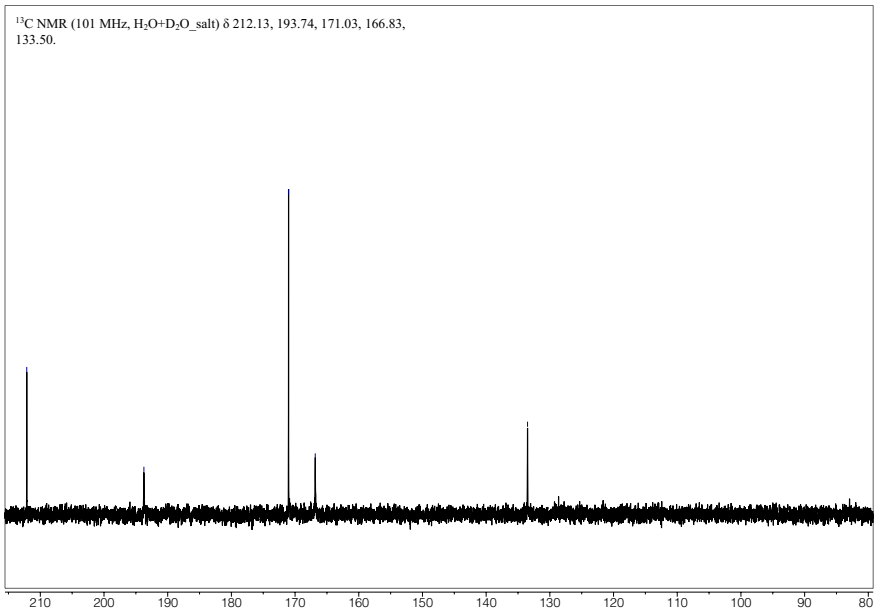


Supplementary Figure 5: 1H NMR spectrum (zgesgp) for the reaction of HCN, CH_3SH, and HS^-. Solvent suppression was used to eliminate the peak for water.

We tested whether or not the phosphate buffer was playing a role in the formation of thioformamide by using both a carbonate buffer (Figure S6) and carrying out an unbuffered reaction (Figure S7). Both of these showed the formation of thioformamide.



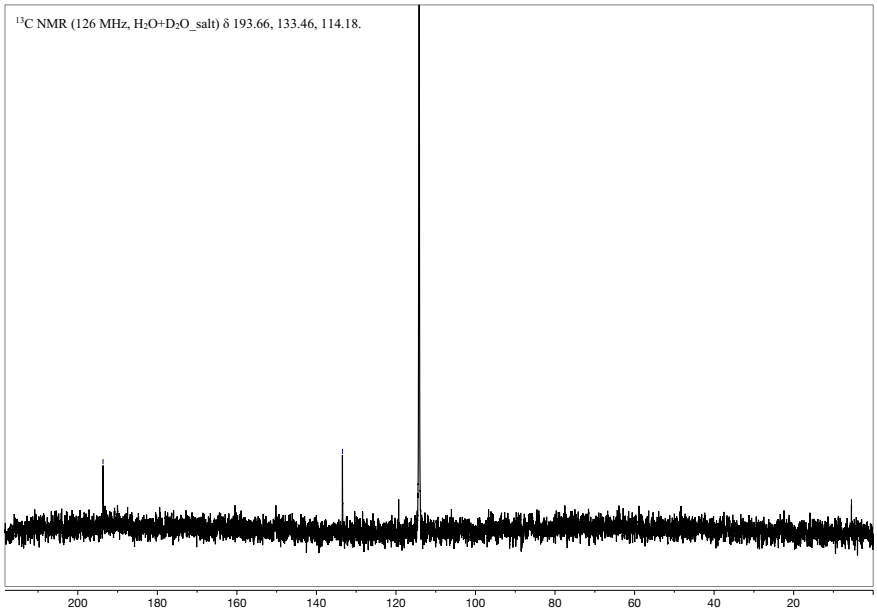
Supplementary Figure 6: 13C NMR spectrum (proton-decoupled) for the reaction of HCN, CH_3SH, and HS^- in carbonate buffer at pH 11.The peak observed at 193.6 ppm corresponds to the carbonyl carbon on thioformamide.



Supplementary Figure 7: 13C NMR spectrum (proton-decoupled) for the reaction of HCN, CH_3SH, and HS^- in water at pH 11.The peak observed at 193.6 ppm corresponds to the carbonyl carbon on thioformamide.

3 Hydrogen sulfide headspace NMR spectra

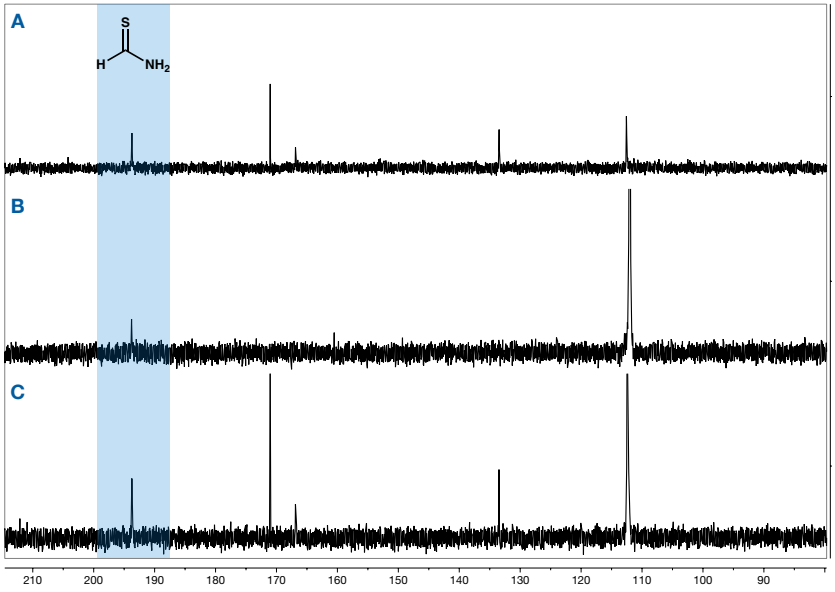
We also mimicked an atmospheric source of sulfide by injecting H_2S into a N_2 headspace in the presence of aqueous HCN and CH_3SH. This also resulted in the formation of thioformamide (Figure S8).



Supplementary Figure 8: 13C NMR spectrum (proton-decoupled) for the reaction of HCN and CH_3SH with an N_2/H_2S headspace at pH 11.The peak observed at 193.6 ppm corresponds to the carbonyl carbon on thioformamide.

4 Thiophosphate reaction

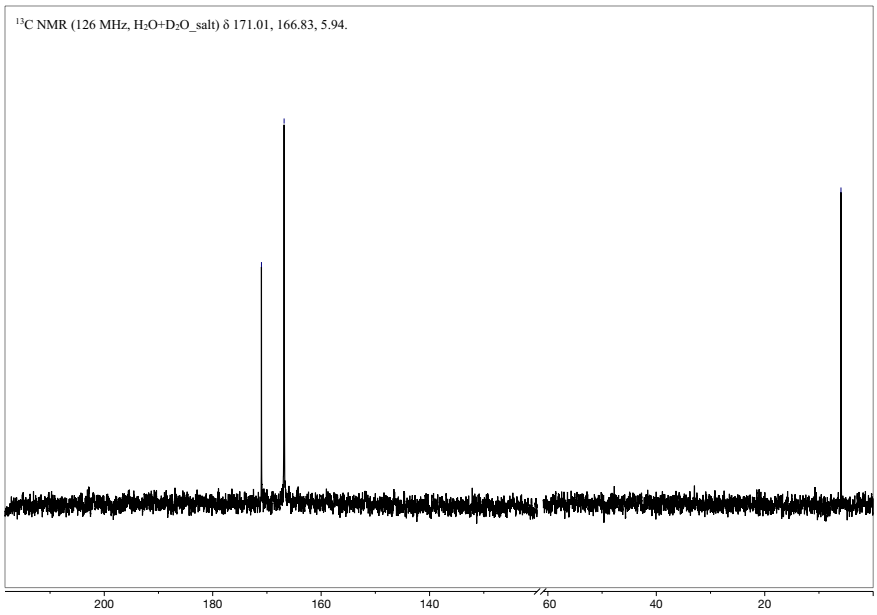
To confirm the identity of thioformamide, we reacted HCN with sodium thiophosphate (as described in the main text). We compared the chemical shifts of this reaction with the products formed from HCN, sulfide, and methanethiol and showed that the main product, thioformamide, is the same in both (Figure S9).



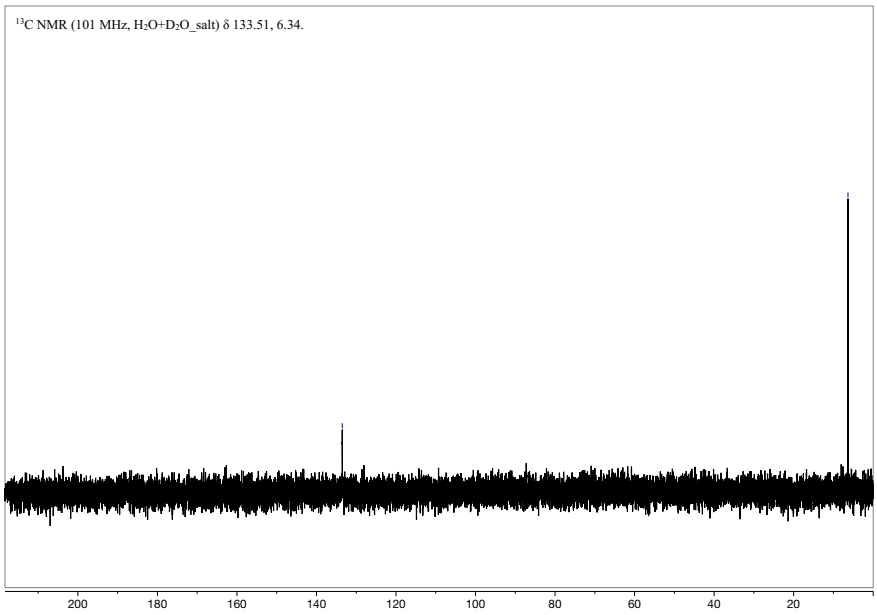
Supplementary Figure 9: 13C NMR spectrum (proton-decoupled) confirming the identity of thioformamide. Spectrum A shows the formation of thioformamide from HCN, sulfide, and methanethiol. Spectrum B shows the formation of thioformamide from HCN and thiophosphate, following the methods of Ritson et al, 2017. Spectrum C shows these two reaction mixtures combined, illustrating that the chemical shift of the carbonyl carbon (highlighted in blue) is identical.

5 Alternate thioformamide precursors

We also investigated whether other compounds (namely formamide and thiocyanate) could serve as precursors to thioformamide. We reacted these compounds with HS^- in the presence of CH_3SH and did not observe the production of a detectable quantity of thioforamide (Figures S10 and S11).



Supplementary Figure 10: 13C NMR spectrum (proton-decoupled) for the reaction of formamide and CH_3SH at pH 11. No thioformamide was observed in this reaction.



Supplementary Figure 11: 13C NMR spectrum (proton-decoupled) for the reaction of SCN^- and CH_3SH at pH 11. No thioformamide was observed in this reaction.

6 Output from Gaussian

	$\varepsilon_0 + \varepsilon_{ZPE}$	$\varepsilon_0 + E_{tot}$	$\varepsilon_0 + H_{corr}$	$\varepsilon_0 + G_{corr}$
Thioformamide	-492.89105	-492.887449	-492.886504	-492.91644
Formamide	-169.933165	-169.929564	-169.92862	-169.957523
Thioformic acid	-512.772139	-512.768486	-512.767542	-512.797600
H_2O	-76.450024	-76.447189	-76.446244	-76.467669
H_2S	-399.41745	-399.414601	-399.413656	-399.437003
NH_3	-56.558457	-56.555589	-56.554645	-56.577523

Table 1: Thermochemical output from Gaussian 16 for several compounds used in standard state Gibbs energy of formation calculations. All values are given in Hartrees/particle. ε_0 is the electronic energy, $\varepsilon_{\mathrm{ZPE}}$ is the zero-point energy; E_{tot} is the thermal correction to energy (where $E_{\mathrm{tot}} = E_t + E_r + E_v + E_e$ for energies of translation, rotation, vibration, and electronic energy); H_{corr} is the thermal enthalpy (where $H_{\mathrm{corr}} = E_{\mathrm{tot}} + k_B T$); and G_{corr} is the thermal correction to Gibbs energy (where $G_{\mathrm{corr}} = H_{\mathrm{corr}} - TS_{\mathrm{corr}}$).