Electronic Supplementary Information

for manuscript entitled

A Possible Non-Enzymatic Pathway for Metabolite Oxidation in Early Earth Conditions

by

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1. Materials and Methods

Chemicals

Acetophenone, benzyl alcohol, α-ketoglutaric acid, sodium acetate, sodium pyruvate, sodium succinate, L-lactic acid, L-cysteine, malic anhydride, maleic acid, zinc nitrate, and copper nitrate were bought from SRL Pvt. Ltd., India. Copper(I) iodide was purchased from BLD Pharm, India. Deuterium oxide (D_2O; 99.9 % deuterated), sodium sulfide, ethanethiol, ethanedithiol were purchased from TCI (India). Benzaldehyde, acetaldehyde, Amberchrom 50WX8 resin, HPLC-grade acetonitrile and HPLC-grade water were purchased from Sigma Aldrich. Ethanol was purchased from MSB Chemical Ltd., India. All of the chemicals were used without further purification.

High Performance Liquid Chromatography (HPLC)

The Reverse-phase HPLC (RP-HPLC) were performed on Thermo Fisher scientific V/C-P10-A with a diode array detector CG P/N-VC-D11-A. The reference standards and reaction analyte were analysed using LUNA semi-preparative C18-column (100 Å, 10 μm, 250 x 10 mm). During the run, the flow rate was maintained at 1.5 mL/min, with detection at λ = 220 nm using a step gradient flow of water and acetonitrile. All the HPLC data were processed by Chromeleon software (version 7.3.2).

Nuclear Magnetic Resonance Spectroscopy (NMR)

All the NMR spectra were recorded on Bruker AVANCE 400 MHz spectrometer. Samples were prepared using NORELL ST500-7 tubes (5 mm). One-dimensional proton NMR spectra were recorded with relaxation delay, d1 = 10 s for quantitative NMR and d1=2 s for identification of the product. Proton NMR chemical shifts (δ) are reported in parts per million (ppm). Water suppression (at δ =4.71 ppm) was performed using noesygppr1d and zgesgp pulse program. The pH was adjusted to 7 prior to the NMR measurement using 10% D_2O in water. The formation of the 1,3-dihydroxyacetone from the glycerol was confirmed using the Chenomx NMR Suite software (version 12.01). The following abbreviations are used for the multiplicities: s: singlet, d: doublet, t: triplet, m: multiplet. All the data were processed by MestReNova software (version 12.0.0).

Electron Paramagnetic Resonance (EPR)

EPR spectra were recorded on Bruker ELEXSYS 580 (Pulsed X-band EPR Spectrometer). The analysis was performed using microwave frequency of approximately 9.85 GHz,

microwave power of 15 mW, modulation frequency of 100 kHz, and modulation amplitude of 5 G at 25 °C.

General protocol for the oxidation reactions

First, the stock solutions of all the reactants (substrate, metal salt) were prepared at 0.5 M in HPLC-grade water, except for 1-phenylethanol, which was dissolved in HPLC-grade acetonitrile due to its poor aqueous solubility, and cysteine was prepared as a 0.1 M stock solution in water because of its limited solubility. In a 1.5 mL polypropylene vessel, the assay mixture was prepared by the addition of the substrate and the ligand, followed by the addition of metal salt from the respective stock solutions. The total volume of the reaction mixture was made to 1 mL comprising 20 mM of substrate, 20 mM of the ligand and 40 mM of metal salt. Prior to heating, the assay mixture was mixed thoroughly and finally it was placed on a thermostat preheated to 70 °C, under close conditions. No stirring of the assay solution was done during the course of the reaction to follow the restrictions that are conventionally followed in the prebiotic chemistry setup. Following the reaction, the hot mixtures were first allowed to cool to room temperature, and then prepared (or further treated) to ensure their suitability for HPLC-based and ^{1}H-NMR analysis.

To ensure reliability, each assay was performed multiple times. The reported yields represent an average of these replicate assays, and standard deviations were calculated from a minimum of three independent assays. However, the reported yields for the metabolites may be subject to inaccuracy. This is primarily because most of the metabolites used in our assays contain a carboxylic functional group. This functional group is known to form strong adducts with the Cu^{2+} present in the assay solution. While the post-assay resin treatment was performed to mask the free metal ions, it is important to note that the interaction between the metabolites and Cu^{2+} cannot be entirely eliminated by the resin treatment.

Sample preparation for the measurements

Prior to the NMR measurement, the assay solution was subjected to de-salting twice by the treatment of cation exchange resin (NH_4^+-50WX8 resin, 50-100 mesh) to remove metal ions from the reaction mixture. Next, the supernatant was filtered and then adjusted to pH 7 in all cases to prevent pH-induced shifts in the NMR signals. In our assay, multiple product formation was observed, especially with the metabolites. Therefore, maleic acid was added as an internal standard for the quantification of the oxidation-mediated conversion. The products are further confirmed by spiking the assay mixture with authentic standards in all cases.

For HPLC-based analysis, 100 µL of the reaction mixture was pipetted out and subsequently filtered through 0.2 µm syringe filter. From that 20 µL of the filtrate was injected for HPLC

analysis. The conversion to the products in the oxidation assay was determined by the area under the peaks of the HPLC chromatogram recorded at 220 nm.

2. Analytical Data of the Reference Compounds:

NMR Data:

1-phenylethanol: **^{1}H NMR** (400 MHz, 90% H_2O + 10% D_2O) δ = 7.50 – 7.15 (m, 5H), 4.92 (q, *J* = 6.6 Hz, 1H), 1.47 (d, *J* = 6.5, 3H) ppm.

Acetophenone: **^{1}H NMR** (400 MHz, 90% H_2O + 10% D_2O) δ = 8.08 – 7.97 (m, 2H), 7.69 (d, *J* = 7.7 Hz, 1H), 7.63 – 7.52 (m, 2H), 2.67 (s, 3H) ppm.

Benzyl alcohol: **^{1}H NMR** (400 MHz, 90% H_2O + 10% D_2O) δ = 7.40 – 7.23 (m, 5H), 4.56 (s, 2H).

Benzaldehyde: **^{1}H NMR** (400 MHz, 90% H_2O + 10% D_2O) δ = 9.85 (s, 1H), 7.96 – 7.85 (m, 2H), 7.68 (t, *J* = 7.4 Hz, 1H), 7.54 (t, *J* = 7.7 Hz, 2H).

2-phenyl-1,3-dithiolane: **^{1}H NMR** (400 MHz, 10 % D_2O in H_2O) δ (ppm) 7.27-7.03 (m, 2H), 6.99-6.66 (m, 3H), 5.29 (s, 1H), 3.01-2.57 (m, 4H).

2-Methyl-2-phenyl-1,3-dithiolane: **^{1}H NMR** (400 MHz, 10 % D_2O in H_2O) δ (ppm) 7.71 (dd, *J* = 7.9, 1.7 Hz, 2H), 7.37-7.21 (m, 3H), 3.50-3.32 (m, 4H), 2.06 (s, 3H).

For NMR analysis, a mixture of starting material (alcohol) and product (aldehyde or ketone) was prepared in HPLC-grade acetonitrile. Prior to the recording of ^{1}H-NMR, the mixture was diluted with 10% D_2O in water and pH was adjusted to 7.

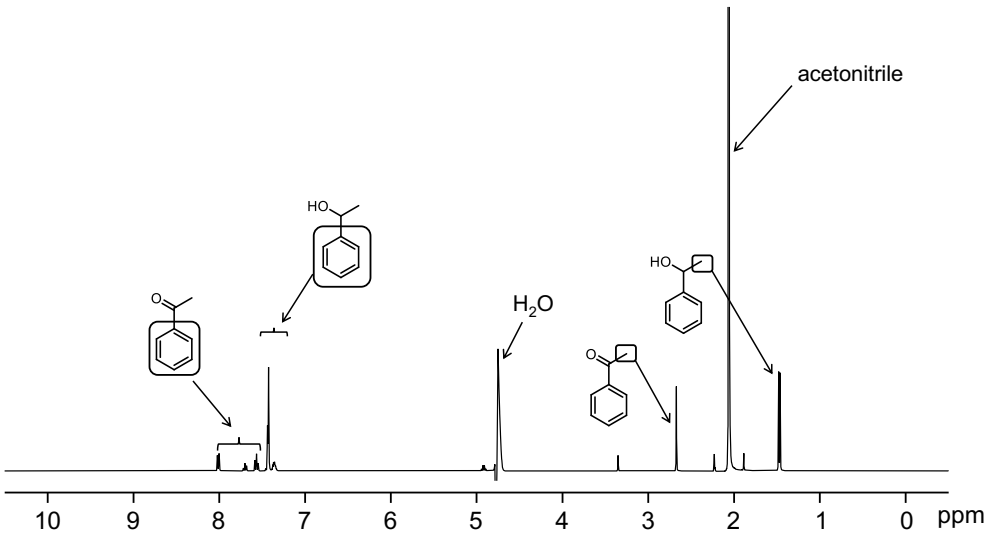


Figure S1. ^{1}H-NMR spectra of the mixture of 1-phenylethanol and acetophenone, recorded at pH 7 in 10% D_2O in water.

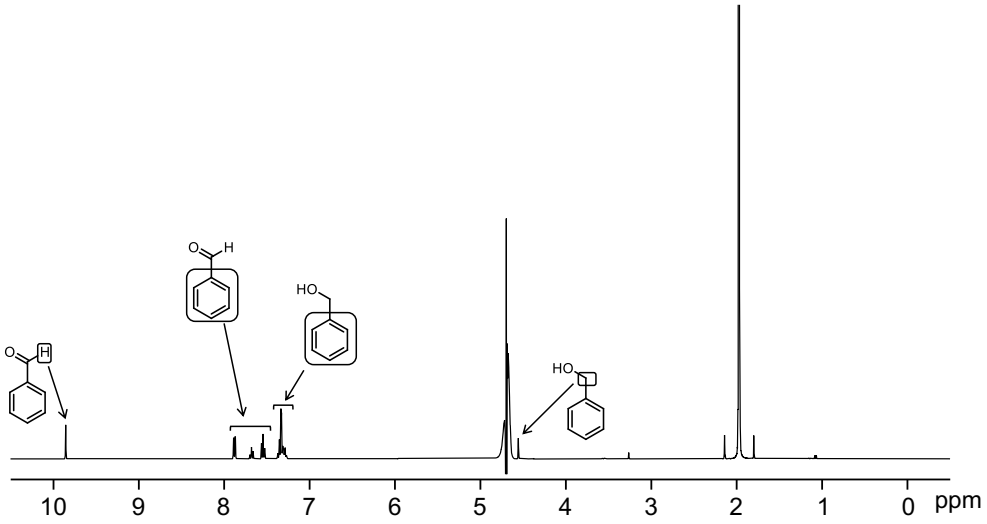


Figure S2. ^{1}H-NMR spectra of the mixture of benzyl alcohol and benzaldehyde, recorded at pH 7 in 10% D_2O in water.

HPLC Data:

For the optimization of the HPLC run profile: At first, stock solutions of 1-phenylethanol, acetophenone, benzyl alcohol and benzaldehyde (5 mM each) were prepared in HPLC-grade water. The samples were prepared by mixing equal volumes (starting material and product) and subsequently injected in RP-HPLC using acetonitrile-water mixture as eluent.

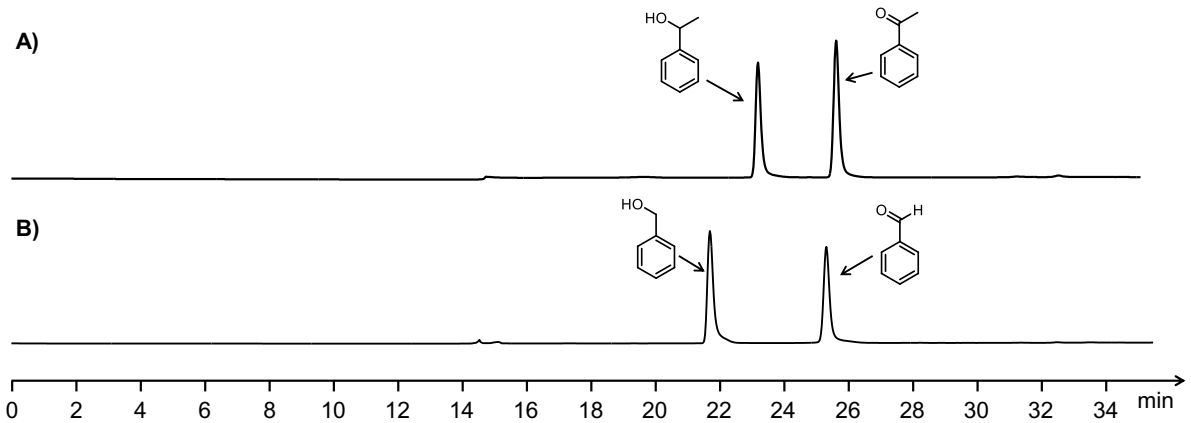


Figure S3. HPLC profile of the references (**A.** 1-phenylethanol and acetophenone; **B.** benzyl alcohol and benzaldehyde) using acetonitrile-water mixture as eluent.

Determination of the Correction Factor for HPLC-based Yield Calculation:

Due to the significant difference in molar extinction coefficients between the starting material and the product, it is important to determine the correction factor to calculate the yield from the HPLC chromatogram. Initially, stock solutions of both compounds (1-phenylethanol and acetophenone) were prepared in water and then mixed at various concentrations (15 mM, 10

mM, 5 mM, and 1 mM) in Eppendorf tubes to investigate the oxidation of 1-phenylethanol to acetophenone. Similarly, stock solutions of benzyl alcohol and benzaldehyde were prepared and mixed at different concentrations (20 mM, 15 mM, 10 mM, 5 mM, and 1 mM) to study the oxidation of benzyl alcohol to benzaldehyde. Then, the equimolar mixtures were injected into the RP-HPLC using a uniform run profile. The area under the peak of the corresponding substance at 220 nm was plotted. From the plot, the slope of the linear fit to the calibration curve was found to be 1.4 for acetophenone/1-phenylethanol and 0.96 for benzaldehyde/ benzyl alcohol (Figure S4), which was used as correction factor for the estimation of conversion of the assay.

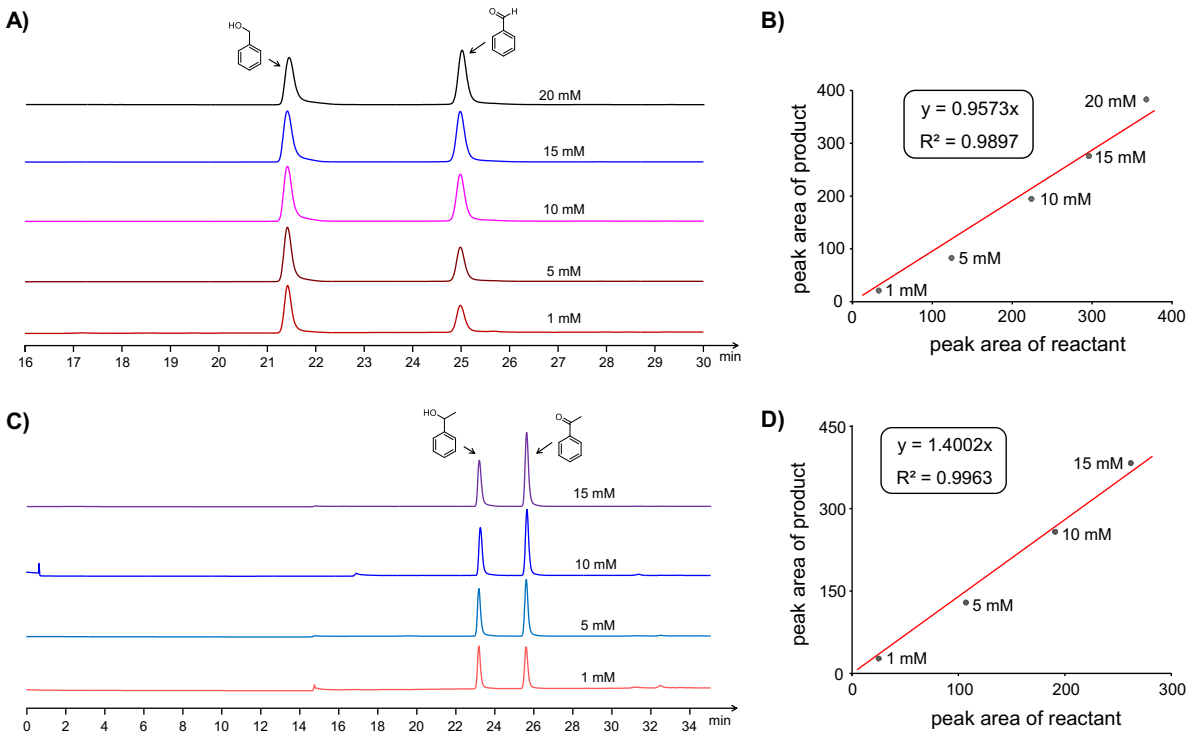


Figure S4. A) HPLC profiles of the equimolar mixture of the starting material (benzyl alcohol) and the product (benzaldehyde) at different concentrations (20 mM, 15 mM, 10 mM, 5 mM, 1 mM); **B)** Plot of the peak area under the curve of the benzyl alcohol (reactant) (x-axis) vs benzaldehyde (product) (y-axis); **C)** HPLC profiles of the equimolar mixture of the starting material (1-phenylethanol) and the product (acetophenone) at different concentrations (15 mM, 10 mM, 5 mM, 1 mM); **D)** Plot of the peak area under the curve of the 1-phenylethanol (reactant) (x-axis) vs acetophenone (product) (y-axis) at different concentrations obtained from the HPLC profile.

3. Analytical Data of the Oxidation Assays:

Yield calculation

NMR-yield. Yield was calculated using the formula[1]:

$$\mathrm{n_{product}} = \frac{\mathrm{I_{product}}}{\mathrm{I_{standard}}} \times \frac{\mathrm{P_{standard}}}{\mathrm{P_{product}}} \times \mathrm{n_{standard}}$$

where, I: signal intensity of obtained from ^{1}H NMR, P: no of protons, n: no of moles.

The NMR yield was calculated using maleic acid (adjusted to pH 7) as an internal standard, which was added after the removal of the metal ion from the reaction mixture by resin treatment.

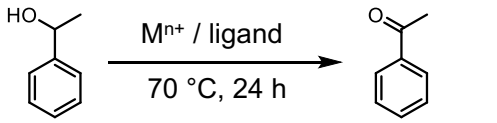
HPLC-yield. Yield was calculated using the formula:

$$\%\ \text{of yield} = \left[\left(\frac{A_{product}}{f}\right) / \left(\frac{A_{product}}{f} + A_{reactant}\right)\right] \times 100$$

Where A is the peak area under the curve obtained from the HPLC profile, and f is the correction factor (*f* =.1.4 for 1-phenylethanol and *f* = 0.96 for benzyl alcohol).

A. Oxidation of secondary alcohol to ketone

Optimization of the Oxidation Assay:



1-phenylethanol acetophenone

Scheme S1. Schematic representation of the oxidation of 1-phenylethanol to acetophenone.

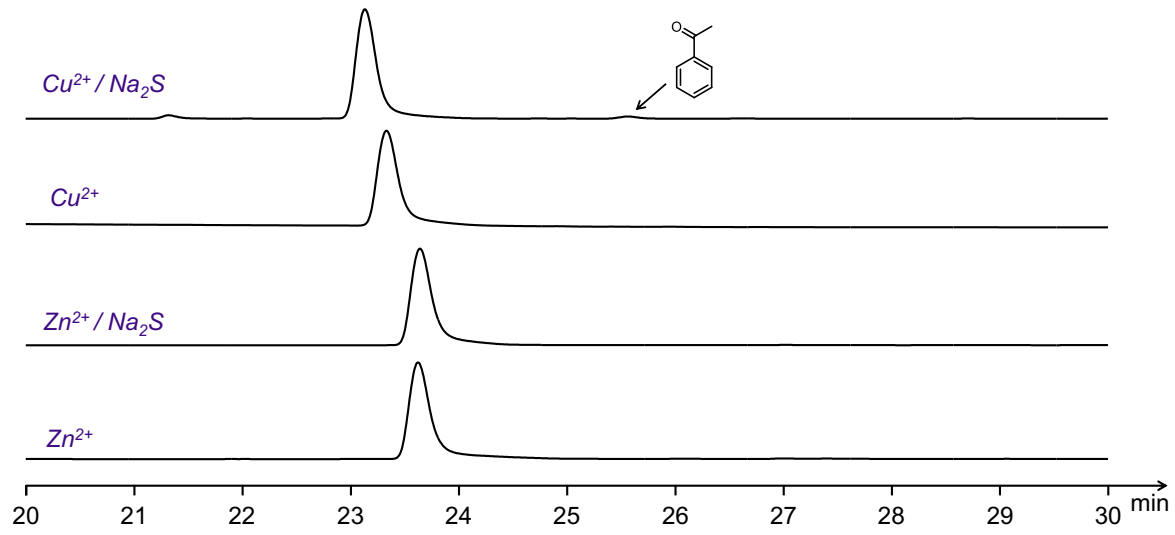


Figure S5. HPLC chromatogram of the assays involving the oxidation of 1-phenylethanol using the combination of different metal ions (Zn^{2+}, Cu^{2+}) and Na_2S after 24 h. Condition: 20 mM of 1-phenylethanol, 40 mM of metal salt and 20 mM of Na_2S in water at 70 °C.

Table S1. Conversion (%) of the oxidation assay of 1-phenylethanol using different combinations of metal ions and sodium sulfide [a,b,c].

Entry	Ligand	M^{n+}	Yield / %
1	-	Zn^{2+}	n.d.
2	-	Cu^{2+}	n.d.
3	Na_2S	-	n.d.
4	Na_2S	Zn^{2+}	n.d.
5	Na_2S	Cu^{2+}	tr.

[a] Analysis of the assays was conducted by HPLC.
[b] Reaction condition: 20 mM of 1-phenylethanol, 40 mM of metal salt and 20 mM Na_2S in water for 24 h at 70 °C
[c] n.d.: not detected; tr.: trace.

M^{n+} = Cu^{2+}; ligand = ethanedithiol

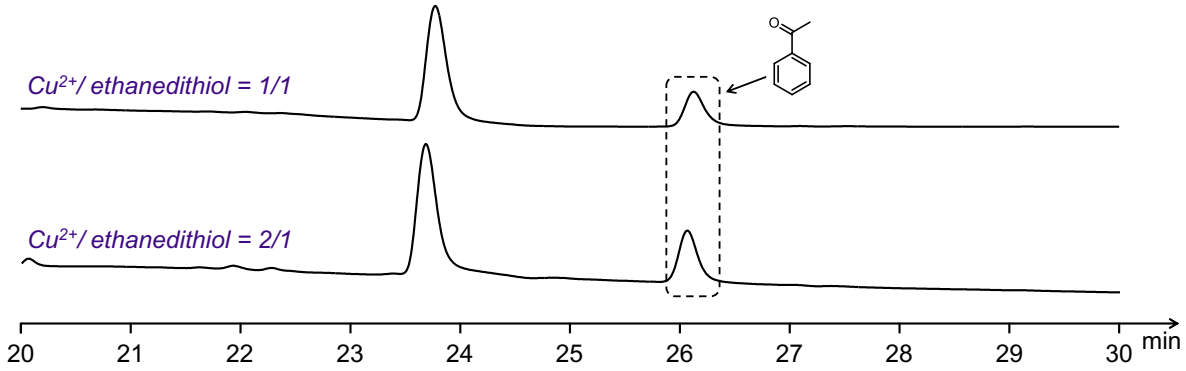


Figure S6. HPLC chromatogram of the assays involving the oxidation of 1-phenylethanol using the different ratio of Cu^{2+} and ethanedithiol (Cu^{2+}(20 mM): ethanedithiol (20 mM) and Cu^{2+}(40 mM): ethanedithiol (20 mM)) in water after 24 h at 70 °C. The substrate 1-phenylethanol was used in 20 mM amount during the optimization assay.

Table S2. Conversion (%) of the control experiment of the oxidation assay of 1-phenylethanol using different combinations of metal ions and ethanedithiol [a,b].

Entry	Cu^{2+} / ethanedithiol	Yield / %
1	1:1	15
2	2:1	21

[a] Analysis of the assays was conducted by HPLC after 24 h reaction time.
[b] Assay condition: In water, 20 mM of 1-phenylethanol and different ratio of Cu^{2+} and ethanedithiol was mixed and heated at 70 °C.

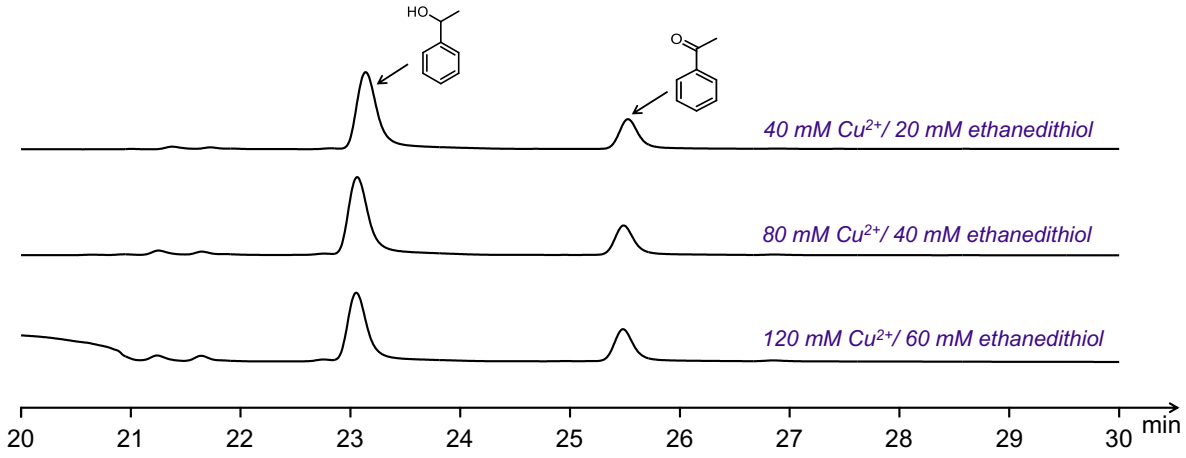


Figure S7. HPLC profile of the oxidation assay of 1-phenylethanol using Cu^{2+} and ethanedithiol (2:1) at different concentrations.

Table S3. Conversion (%) of the oxidation assay of 1-phenylethanol using different concentration of metal ions and ethanedithiol (Cu^{2+} and ethanedithiol ratio = 2:1) [a,b].

Entry	Cu^{2+}/ mM	Ethanedithiol/ mM	Yield / %
1	40	20	21
2	80	40	22
3	120	60	25

[a] Analysis of the assays was conducted by HPLC after 24 h reaction time.
[b] Assay condition: In water, 1-phenylethanol and 2:1 of Cu^{2+} and ethanedithiol at different concentrations were mixed and heated at 70 °C.

M^{n+}= no $metal^{+}$; only different ligand

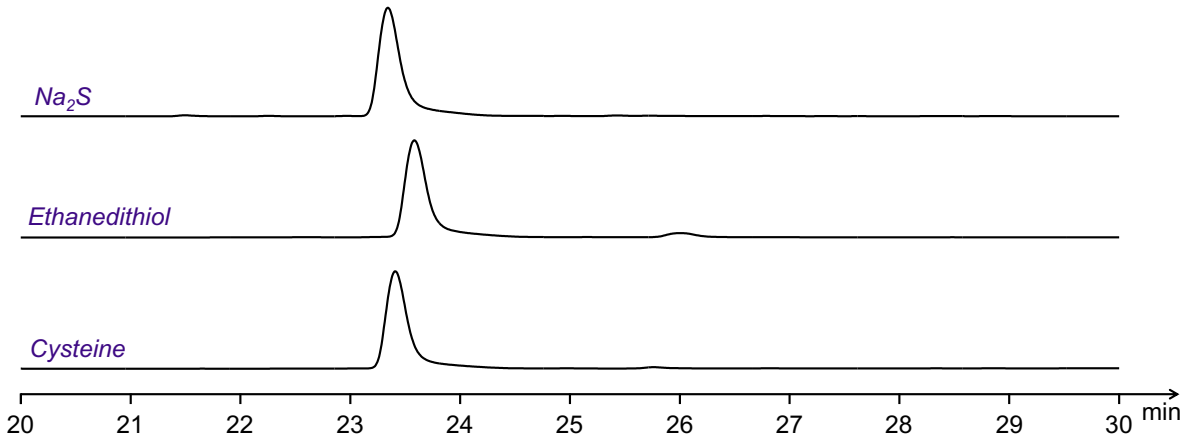


Figure S8. HPLC chromatogram of the assays involving the oxidation of 1-phenylethanol using different ligand only (Na_2S, ethanedithiol, cysteine) after 24 h. Condition: 20 mM of 1-phenylethanol and 20 mM of ligand in water at 70 °C.

M^{n+}= Cu^{2+} and Cu^{+}; ligand = ethanethiol, ethanedithiol, β-marcaptoethanol, cysteine, ethylenediamine, ethylene glycol

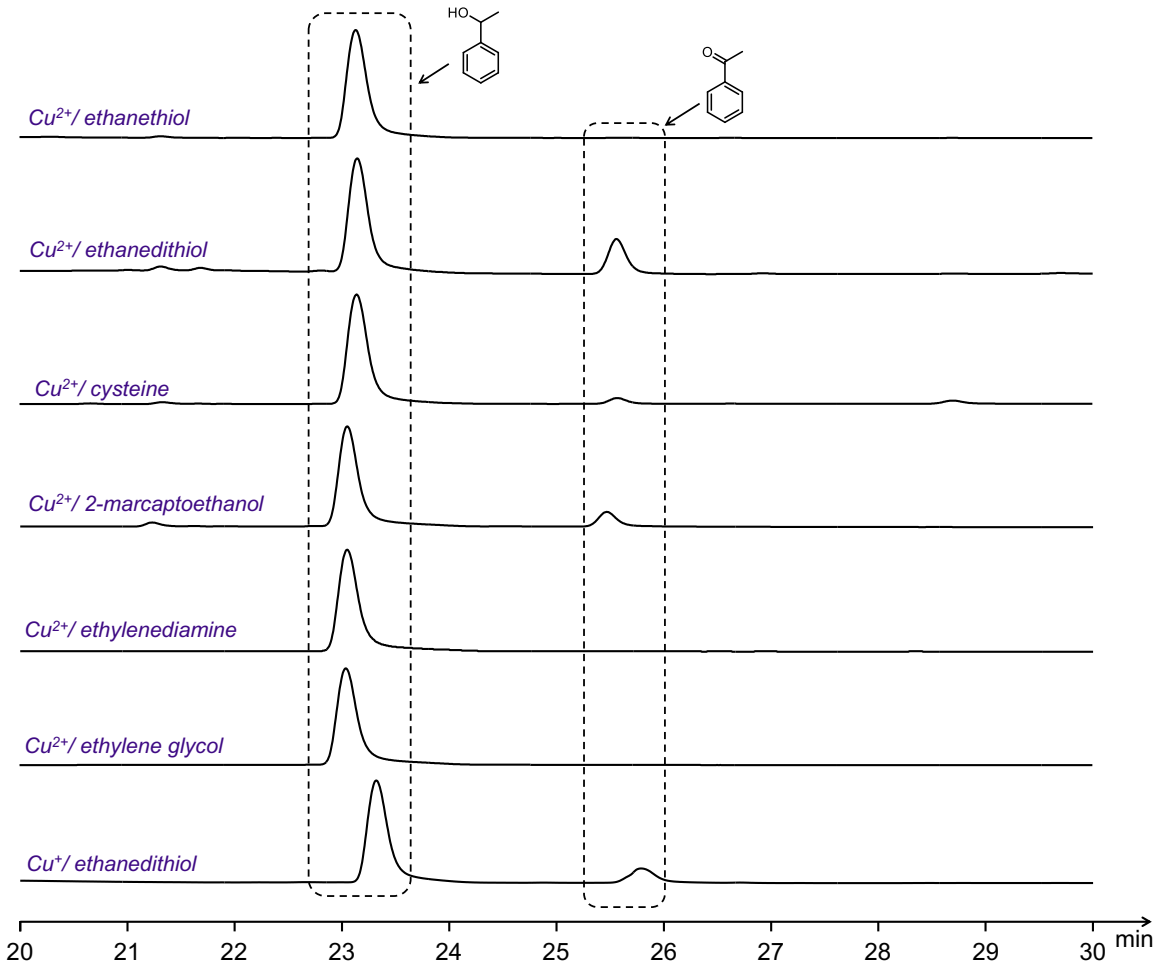


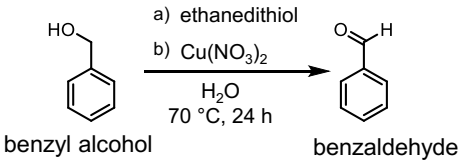
Figure S9. HPLC chromatogram of the assays involving the oxidation of 1-phenylethanol using copper in different oxidation states and ligand system comprising thiol, amine and alcohol functional groups after 24 h reaction time. Condition: 20 mM of 1-phenylethanol, 40 mM of $Cu(NO_3)_2$ and 20 mM of ligand in water at 70 °C.

Table S4. Conversion (%) of 1-phenylethanol to acetophenone in presence of different thiols and Cu-salts [a,b,c,d].

Entry	ligands	M^{n+}	Time / h	Yield (%)
1	Na_2S	Cu^{2+}	24	1
		Cu^{2+}	72	2
2	ethanedithiol	Cu^{2+}	24	21
		Cu^{2+}	72	26
		Cu^{+}	24	10
3	cysteine	Cu^{2+}	24	3
		Cu^{2+}	72	7
4	2-marcaptoethanol	Cu^{2+}	24	8
5	ethylenediamine	Cu^{2+}	24	n.d.
6	ethylene glycol	Cu^{2+}	24	n.d.

[a.] Yields were determined based on the area under the curve of the signals of interest in the HPLC chromatogram at 220 nm.
[b] Correction factor of 1.4 was used to determine the yield.
[c.] n.d. = not detected
[d] Condition: 20 mM of 1-phenylethanol, 40 mM of $Cu(NO_3)_2$ or CuI and 20 mM of ligand in water at 70 °C.

Oxidation of primary alcohol to aldehyde:



Scheme S2. Oxidation of benzyl alcohol to benzaldehyde.

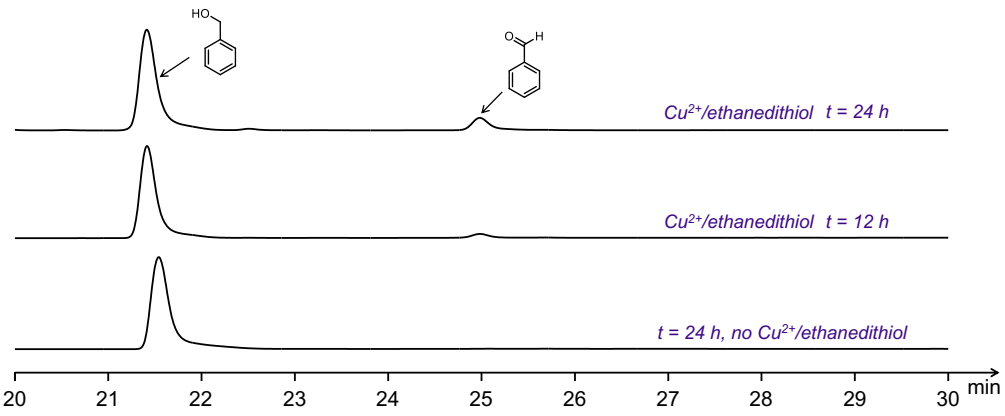


Figure S10. HPLC chromatogram of the assays involving the oxidation of benzyl alcohol to benzaldehyde in presence of 20 mM benzyl alcohol, 40 mM of $Cu(NO_3)_2$ and 20 mM of ethanedithiol in water at 70 °C after 12 h and 24 h, showing the formation of benzaldehyde.

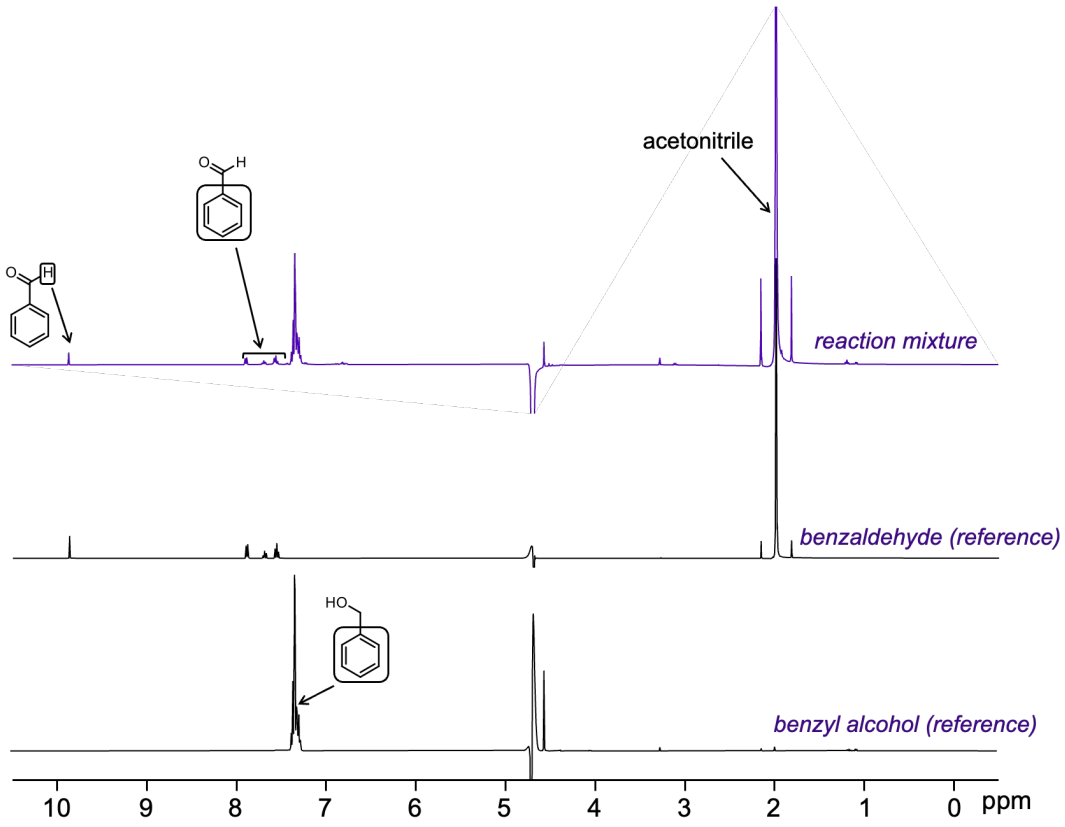
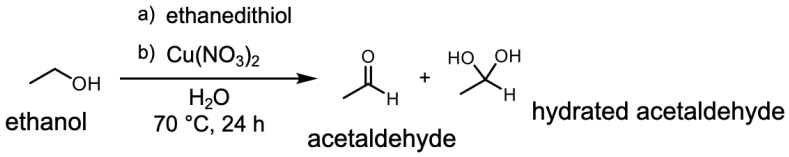


Figure S11. ^{1}H-NMR spectrum of the assays involving the oxidation of benzyl alcohol using Cu^{2+} and ethanedithiol after 24 h reaction time, recorded at pH 7 in 10% D_2O in water. Condition: 20 mM of benzyl alcohol, 40 mM of $Cu(NO_3)_2$ and 20 mM of ethanedithiol in water at 70 °C.



Scheme S3. Schematic representation of the oxidation of ethanol to acetaldehyde.

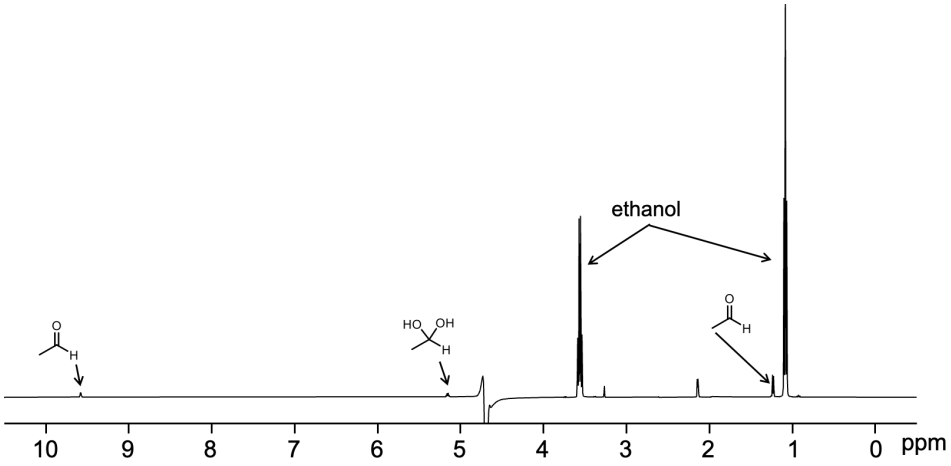


Figure S12. ^{1}H-NMR spectrum of the assay involving the oxidation of ethanol to acetaldehyde using Cu^{2+} and ethanedithiol after 24 h reaction time, recorded at pH 7 in 10% D_2O in water. Condition: 20 mM ethanol, 40 mM of $Cu(NO_3)_2$ and 20 mM of ethanedithiol in water at 70 °C.

$M^{n+} = Cu^{2+}$; ligand = sulfide ion

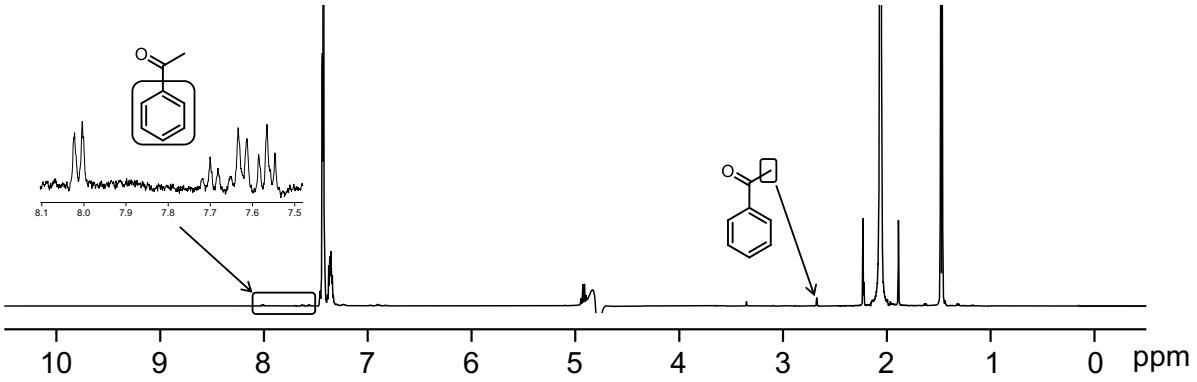


Figure S13. ^{1}H-NMR spectrum of the assays involving the oxidation of 1-phenylethanol using Cu^{2+} and sodium sulfide after 24 h reaction time, recorded at pH 7 in 10% D_2O in water, showing trace amount of product formation. Condition: 20 mM of 1-phenylethanol, 40 mM of $Cu(NO_3)_2$ and 20 mM of Na_2S in water at 70 °C.

$M^{n+} = Cu^{2+}$; ligand = ethanedithiol

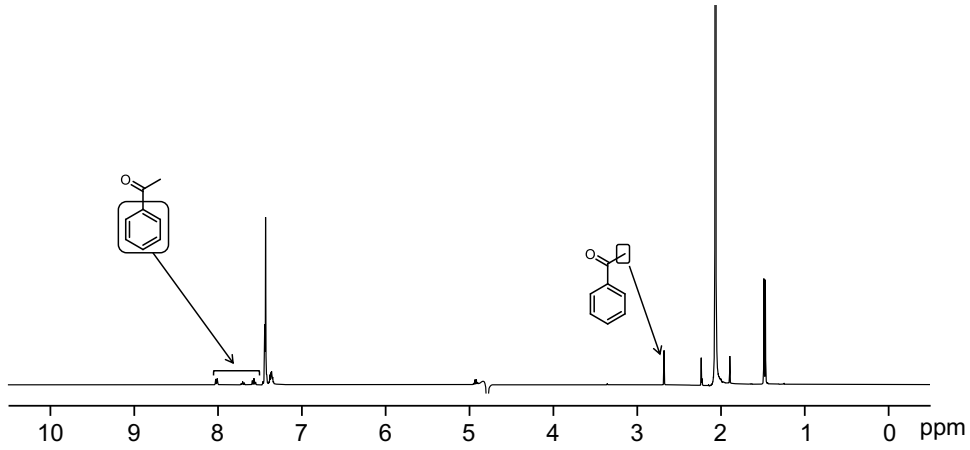


Figure S14. ^{1}H-NMR spectrum of the assays involving the oxidation of 1-phenylethanol using Cu^{2+} and ethanedithiol after 24 h reaction time, recorded at pH 7 in 10% D_2O in water. Condition: 20 mM of 1-phenylethanol, 40 mM of $Cu(NO_3)_2$ and 20 mM of ethanedithiol in water at 70 °C.

$M^{n+} = Cu^{2+}$; ligand = cysteine

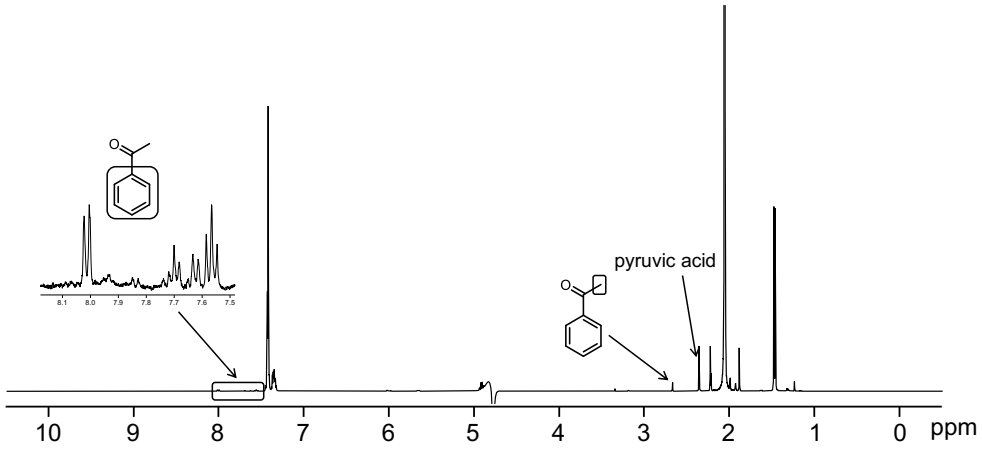
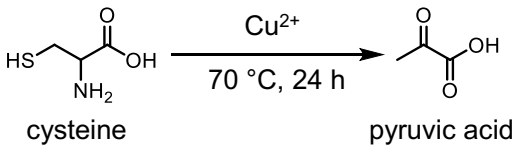


Figure S15. ^{1}H-NMR spectrum of the assays involving the oxidation of 1-phenylethanol using Cu^{2+} and cysteine after 24 h reaction time, recorded at pH 7 in 10% D_2O in water. Condition: 20 mM of 1-phenylethanol, 40 mM of $Cu(NO_3)_2$ and 20 mM of cysteine in water at 70 °C.



cysteine pyruvic acid

Scheme S4. Schematic representation of the formation of pyruvic acid from cysteine.

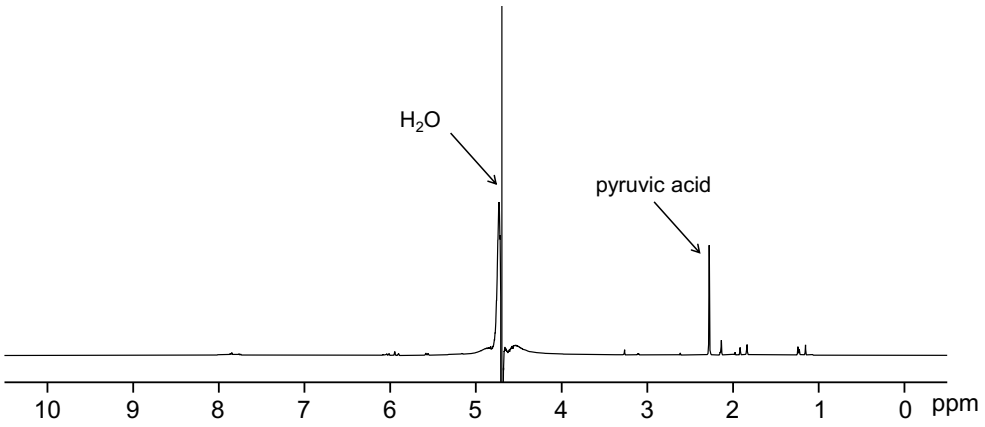


Figure S16. ^{1}H-NMR spectrum of the assays involving the heating of Cu^{2+} and cysteine after 24 h reaction time, recorded at pH 7.0 in 10% D_2O in water. The assay resulted in the formation of pyruvic acid (δ = 2.28 ppm). Condition: 40 mM of $Cu(NO_3)_2$ and 20 mM of cysteine in water at 70 °C.

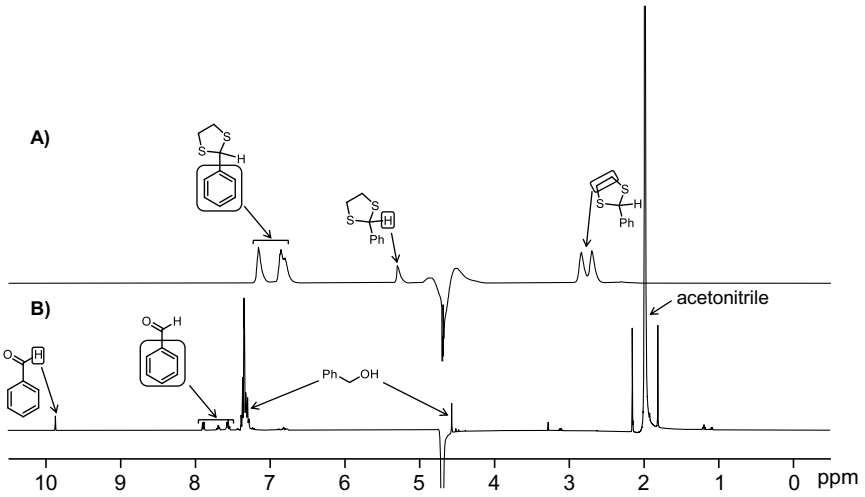


Figure S17. Stacked representation of the ^{1}H NMR spectra of A) reference (2-phenyl-1,3-dithiolane): thioacetal from benzaldehyde; and B) reaction mixture of the oxidation of benzyl alcohol after 24 h, recorded at pH 7 in 10% D_2O in water, showing no formation of thioacetal. Condition: 20 mM of benzyl alcohol, 40 mM of $Cu(NO_3)_2$ and 20 mM of ethanedithiol in water at 70 °C.

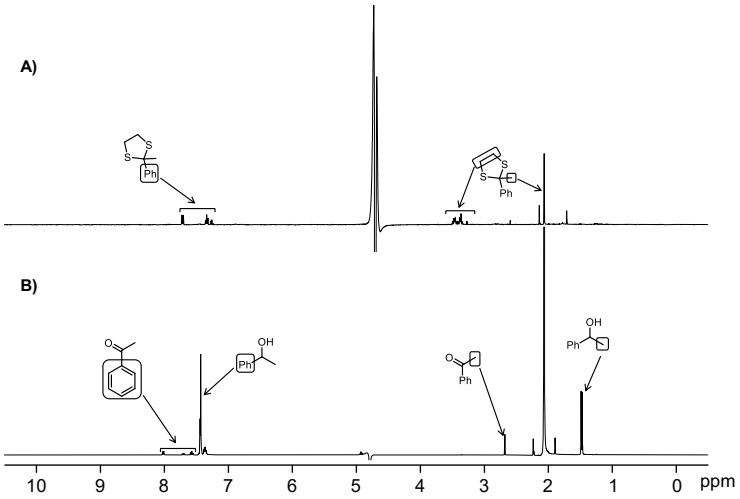


Figure S18. Stacked representation of the ^{1}H NMR spectra of A) reference (2-methyl-2-phenyl-1,3-dithiolane): thioketal formed from acetophenone; and B) reaction mixture of the oxidation of 1-phenyl ethanol after 24 h, recorded at pH 7 in 10% D_2O in water, showing no formation of thioketal. Condition: 20 mM of 1-phenyl ethanol, 40 mM of $Cu(NO_3)_2$ and 20 mM of ethanedithiol in water at 70 °C.

Oxidation of Lactic acid:

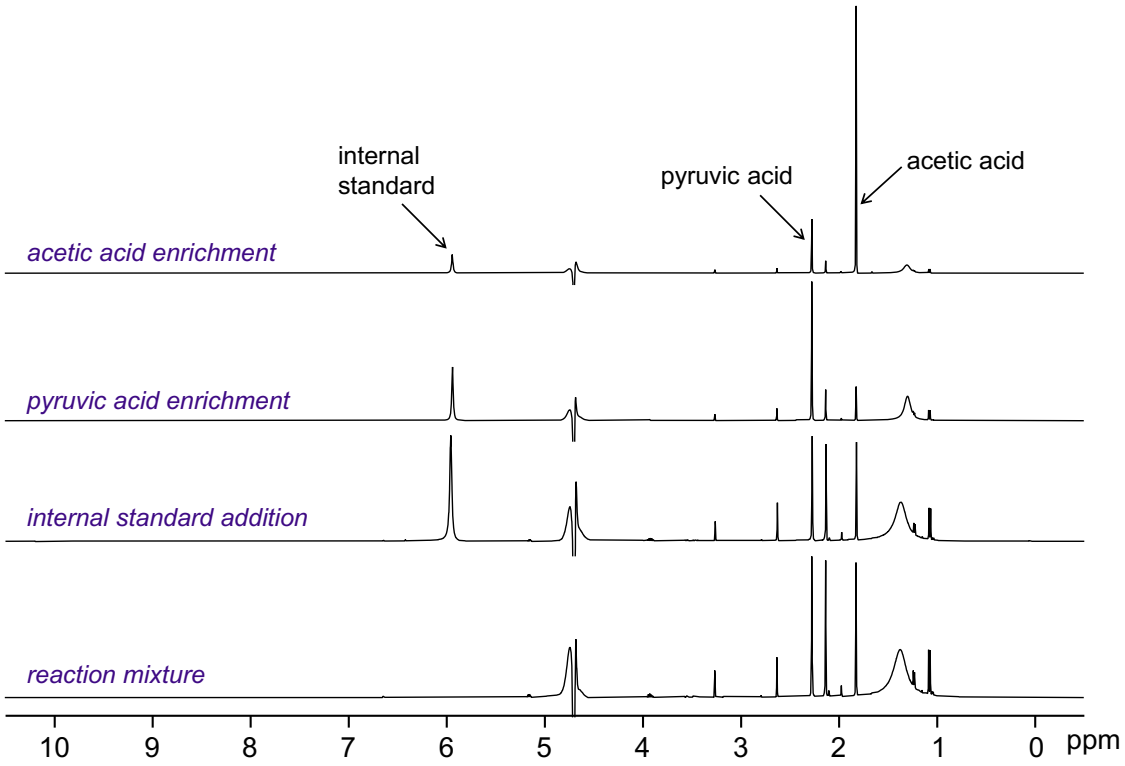


Figure S19. Stacked representation of ^{1}H NMR spectra of the assay for the oxidation of lactic acid in presence of Cu^{2+} and ethanedithiol after 24 h reaction time, recorded at pH 7 in 10% D_2O in water. For yield determination, maleic acid was introduced into the assay mixture, followed by enrichment with pyruvic acid and acetic acid to confirm the identity of the product formed in the assay. Condition: 20 mM of lactic acid, 40 mM of $Cu(NO_3)_2$ and 20 mM of ethanedithiol in water at 70 °C.

Oxidation of 2-hydroxypentanedioic acid:

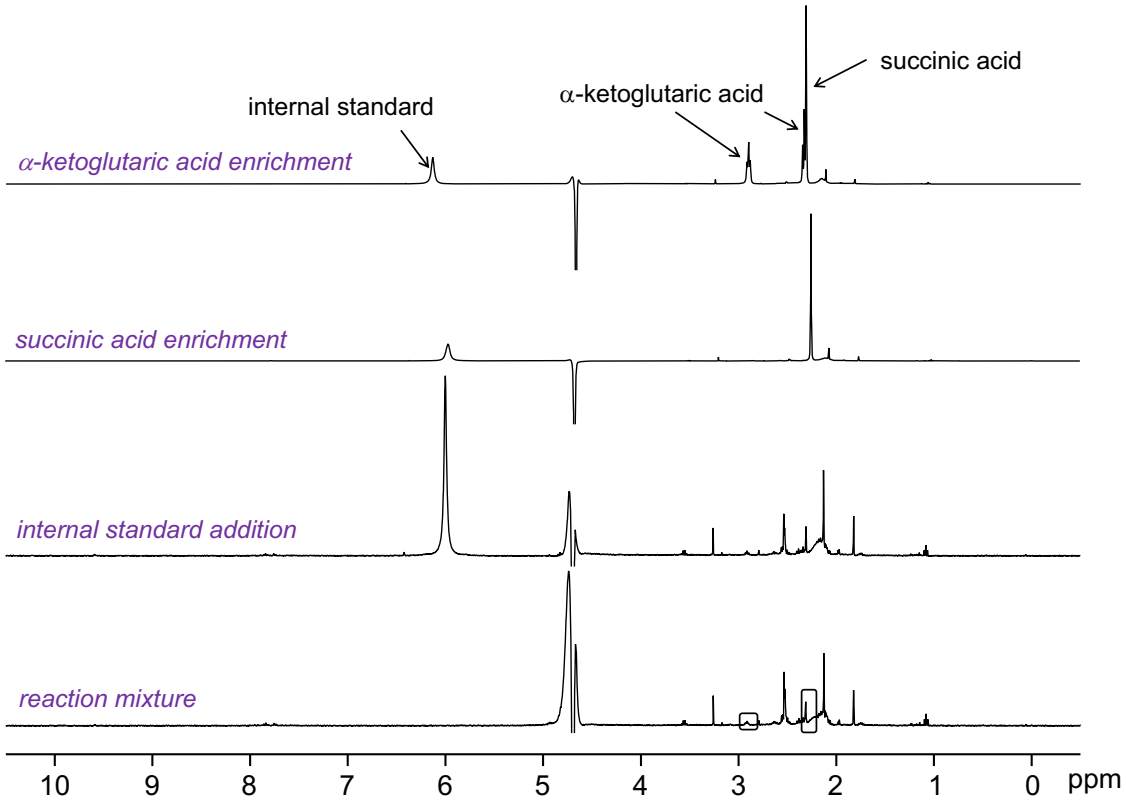
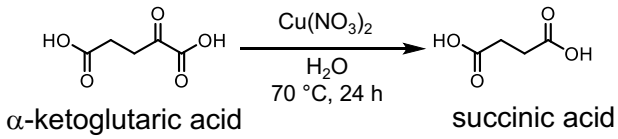


Figure S20. Stacked representation of ^{1}H-NMR spectra of the assay for the oxidation of β-hydroxypentanedioic acid in presence of Cu^{2+} and ethanedithiol after 24 h reaction time, recorded at pH 7 in 10% D_2O in water. For yield determination, maleic acid was introduced into the assay mixture, followed by enrichment with pyruvic acid and acetic acid to confirm the identity of the product formed in the assay. Condition: 20 mM of 2-hydroxypentanedioic acid, 40 mM of $Cu(NO_3)_2$ and 20 mM of ethanedithiol in water at 70 °C.

Decarboxylation of α-ketoglutaric acid



Scheme S5. Cu^{2+}-mediated oxidative decarboxylation of α-ketoglutaric acid.

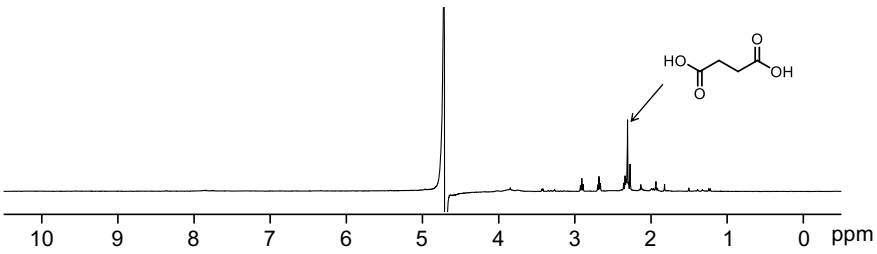


Figure S21. ^{1}H-NMR spectrum of the assays involving the heating of Cu^{2+} and α-ketoglutaric acid after 24 h reaction time, recorded at pH 7.0 in 10% D_2O in water. The assay resulted in the formation of succinic acid via oxidative decarboxylation. Condition: 40 mM of $Cu(NO_3)_2$ and 20 mM of α-ketoglutaric acid in water at 70 °C.

Oxidation of malic acid:

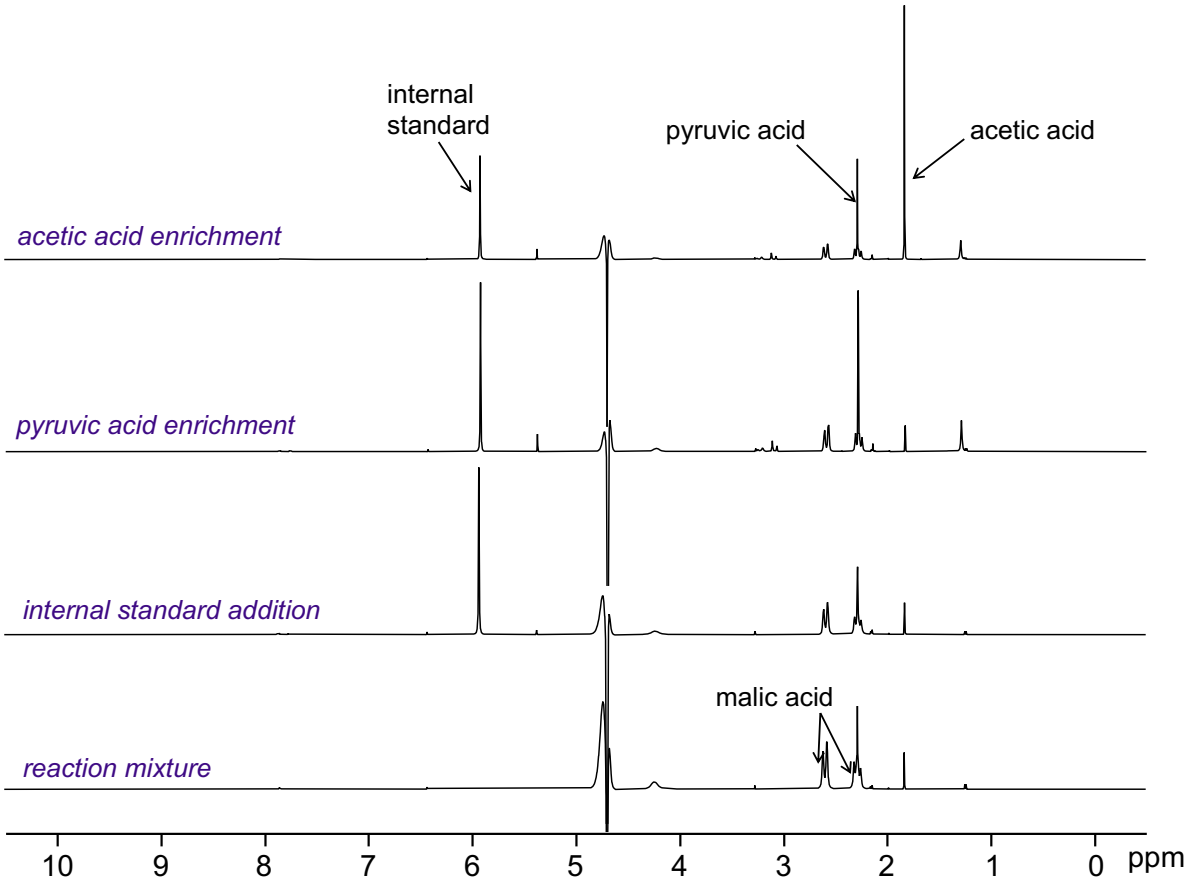
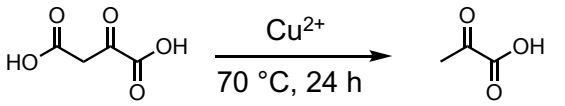


Figure S22. Stacked representation of ^{1}H-NMR spectra of the assay for the oxidation of malic acid in presence of Cu^{2+} and ethanedithiol after 24 h reaction time, recorded at pH 7 in 10% D_2O in water. For yield determination, maleic acid was introduced into the assay mixture, followed by enrichment with pyruvic acid and acetic acid to confirm the identity of the product formed in the assay. Condition: 20 mM of malic acid, 40 mM of $Cu(NO_3)_2$ and 20 mM of ethanedithiol in water at 70 °C.

Decarboxylation of oxaloacetic acid



oxaloacetic acid pyruvic acid

Scheme S6. Schematic representation of decarboxylation of oxaloacetic acid.

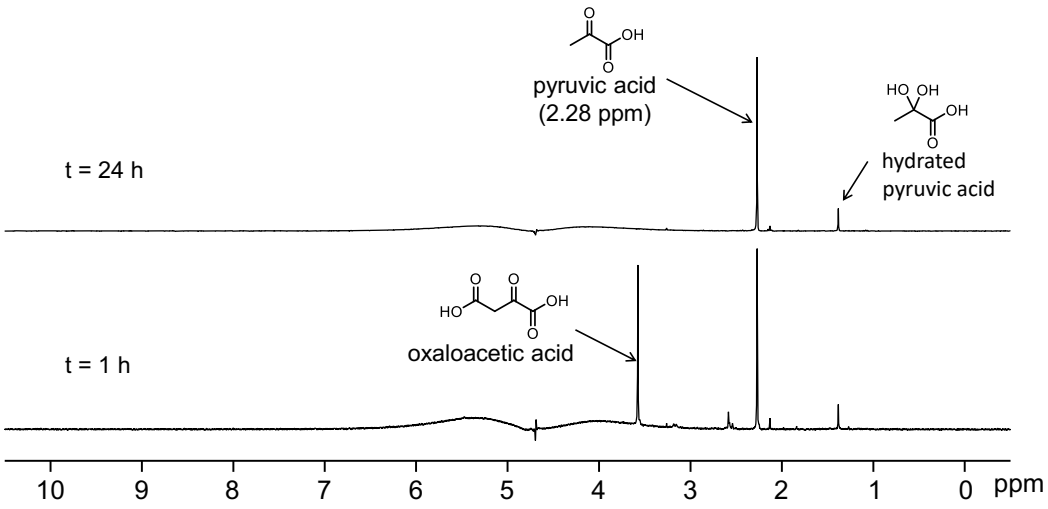
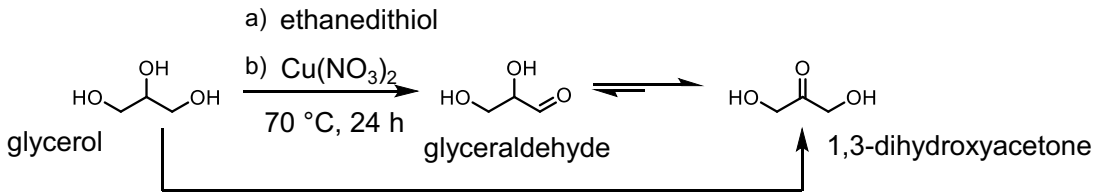


Figure S23. Stacked representation of ^{1}H-NMR spectra of the assay showing the formation of pyruvic acid from oxaloacetic acid via decarboxylation in presence of Cu^{2+} after 1 h and 24 h reaction time, recorded at pH 7 in 10% D_2O in water.

Oxidation of glycerol:



Scheme S7. Schematic representation of the oxidation of glycerol.

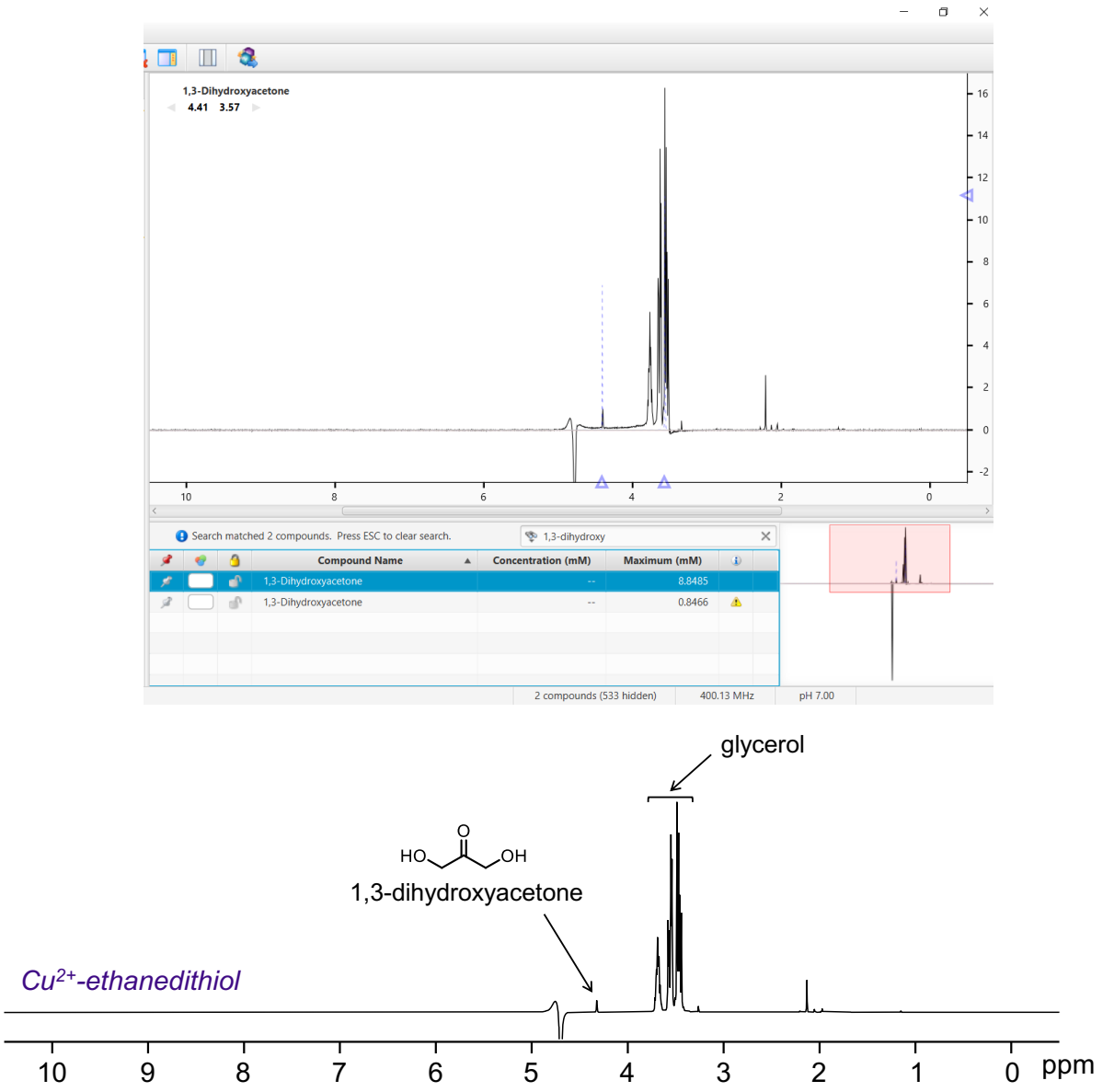


Figure S24. ^{1}H-NMR spectrum of the assay involving the oxidation of glycerol using Cu^{2+} and ethanedithiol after 24 h reaction time, recorded at pH 7 in 10% D_2O in water. Condition: 20 mM glycerol, 40 mM of $Cu(NO_3)_2$ and 20 mM of ethanedithiol in water at 70 °C. Above spectrum was matched with the profiled spectrum (at pH 7 ± 0.5) of 1,3-dihydroxyacetone with the Chenomx NMR Suite software 12.01.

Control experiment:

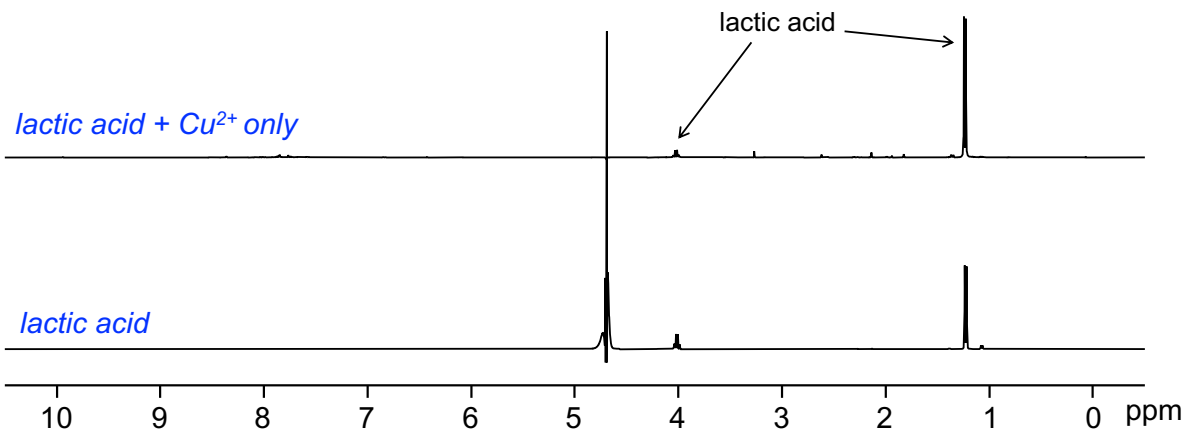


Figure S25. Stacked representation of ^{1}H-NMR spectra of the control assay, in which lactic acid was heated with only Cu^{2+} for 24 h, alongside the reference lactic acid. Both of the NMR were recorded at pH 7 in 10% D_2O in water. Condition of the control assay: 20 mM lactic acid and 40 mM $Cu(NO_3)_2$ in water 70 °C.

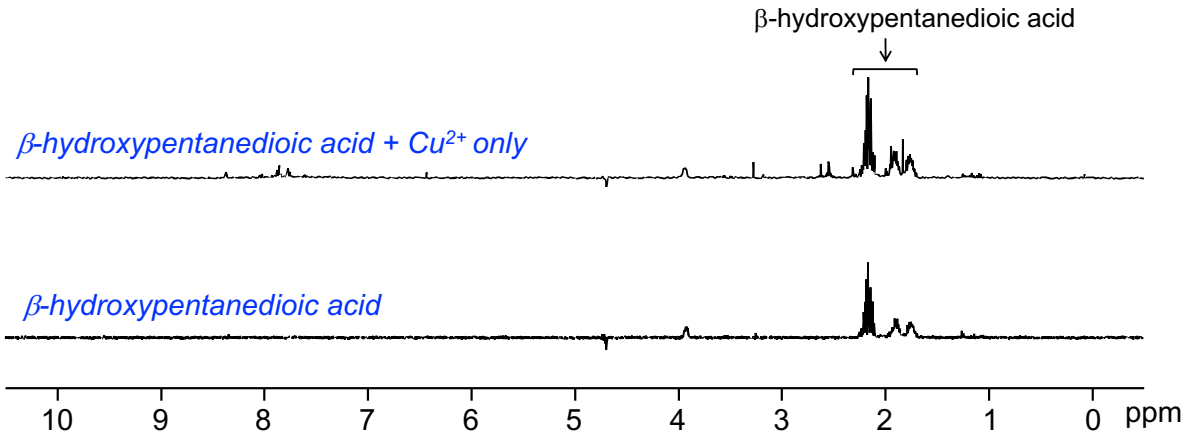


Figure S26. Stacked representation of ^{1}H-NMR spectra of the control assay, in which 2-hydroxypentanedioic acid was heated with Cu^{2+} only for 24 h, alongside the reference 2-hydroxypentanedioic acid. Both of the NMR were recorded at pH 7 in 10% D_2O in water. Condition of the control assay: 20 mM 2-hydroxypentanedioic acid and 40 mM $Cu(NO_3)_2$ in water 70 °C.

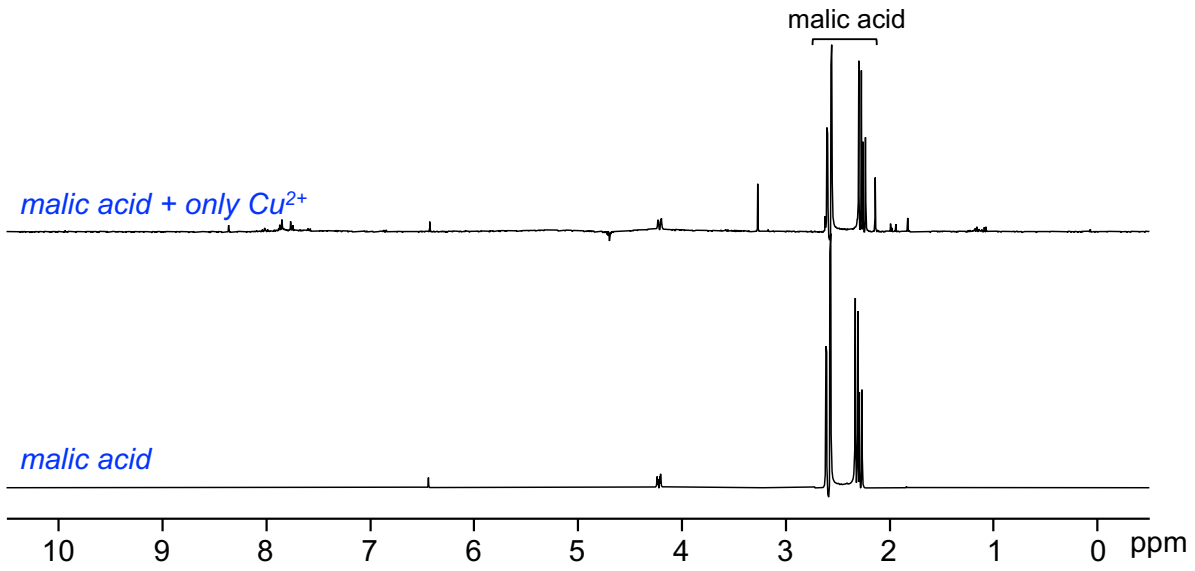


Figure S27. Stacked representation of ^{1}H-NMR spectra of the control assay, in which malic acid was heated with Cu^{2+} only for 24 h, alongside the reference malic acid. Both of the NMR were recorded at pH 7 in 10% D_2O in water. Condition of the control assay: 20 mM malic acid and 40 mM $Cu(NO_3)_2$ in water 70 °C.

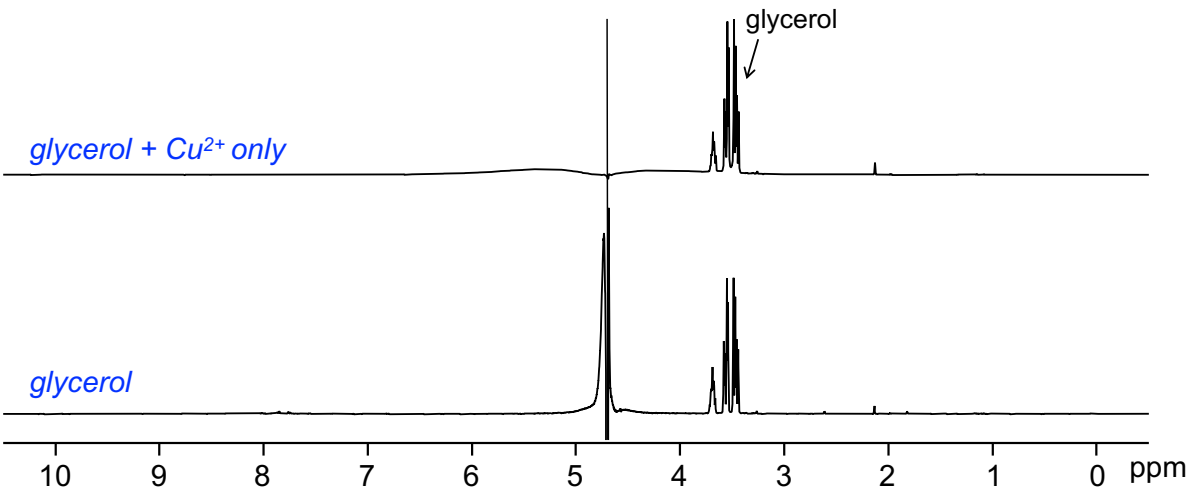


Figure S28. Overlay Stacked representation of ^{1}H-NMR spectra of the control assay, in which glycerol was heated with Cu^{2+} only for 24 h, alongside the reference glycerol. Both of the NMR were recorded at pH 7 in 10% D_2O in water. Condition of the control assay: 20 mM glycerol and 40 mM $Cu(NO_3)_2$ in water 70 °C.

Characterization of copper oxidation state (post-reaction)

Prior to EPR analysis, the recovered pale-green solid was thoroughly washed and dried. Elemental analysis (C-H-S) yielded the following composition: C, 18.08%; H, 2.98%; S, 48.96%, corresponding to an approximate molecular formula of $C_{10}H_{20}Cu_3S_{10}$. Based on this formula, 5.1 mg of the solid was weighed alongside a stoichiometric equivalent of copper(II) nitrate tetrahydrate. Both EPR spectra were recorded under identical instrumental parameters (microwave frequency: 9.85 GHz, microwave power: 15 mW, modulation frequency: 100 kHz, and modulation amplitude: 5 G at 25 °C), and the resulting data were processed using Origin software.

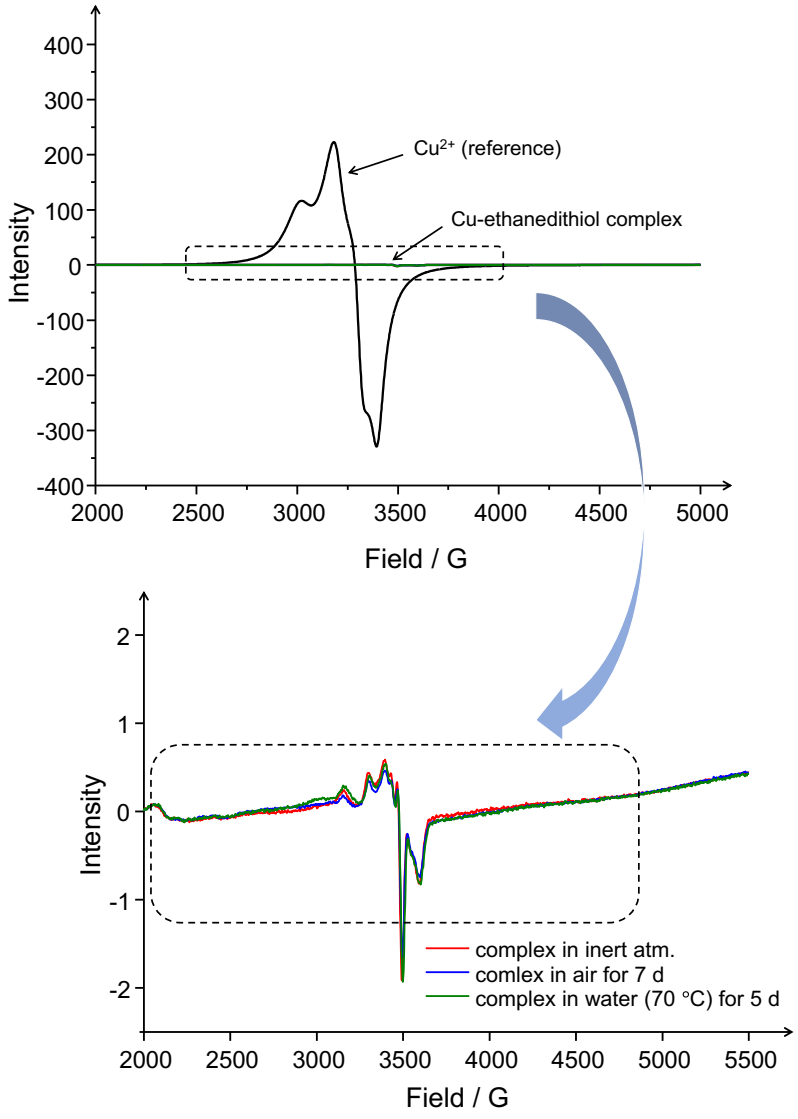
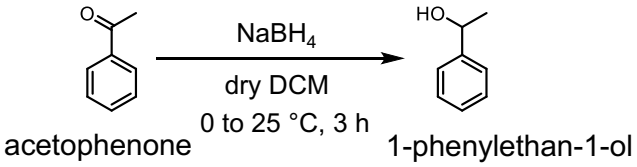


Figure S29. EPR spectra of the Cu-ethanedithiol complex under varying conditions: EPR analysis of the Cu-ethanedithiol complex at different conditions. The spectra were recorded after exposing the Cu^+-ethanedithiol complex at three different atmospheres (inert (—), in air (—), and in water (—) at 70 °C for 5 days). No significant change in the EPR signal was observed, indicating the stability of the Cu^+ in the complex form at different conditions.

The disproportionation of Cu^{+} to Cu^{2+} and Cu^{0} occurs in water. To check the stability of the Cu^{+}-ethanedithiol complex, we performed the following experiment: An assay was first carried out on a relatively large scale with 1-phenylethanol using the Cu^{2+}/ethanedithiol system, and after 24 h a pale green precipitate formed, which was thoroughly washed with water and dried. One portion of the resulting pale green solid was stored under inert atmosphere for 7 days, a second portion was kept in air for 7 days, and the remaining portion was dispersed in water at 70 °C for 5 days. Three of these samples were examined by EPR spectroscopy, and the EPR signals remained largely unchanged (no conversion of Cu^{+} to Cu^{2+}), demonstrating that the Cu^{+} center is well-stabilized by the ethanedithiol ligands even under thermal and oxidative stress.

4. Synthesis of Starting Material:

Synthesis of 1-phenylethanol:

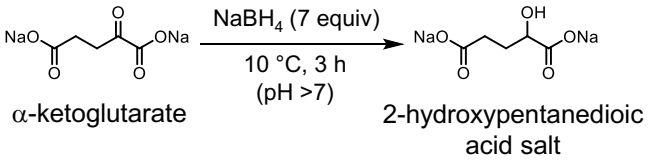


Scheme S8. Synthesis of 1-phenylethanol.

1-phenylethanol was synthesized by the reduction of acetophenone following the literature procedure[2].

^{1}H-NMR (400 MHz, 10% D_2O in water) δ = 7.50 – 7.15 (m, 5H), 4.92 (q, *J* = 6.6 Hz, 1H), 1.47 (d, *J* = 6.5, 3H) ppm.

Synthesis of 2-hydroxypentanedioic acid[3]



Scheme S9. Synthesis of 2-hydroxypentanedioic acid.

To a clean 100 mL round bottom flask, $NaBH_4$ (35 mmol) was dissolved in 35 mL water and the solution was allowed to cool at 0 °C. Then, sodium α-ketoglutarate (5 mmol, pH > 7) was also dissolved separately in 20 mL of water and allowed to cool to 0 °C. Next, the cold solution of sodium α-ketoglutarate was slowly added over a period of 30 min to the aqueous $NaBH_4$ solution at 0 °C. During the addition, the solution temperature was controlled and kept below 10 °C. The reaction mixture was then stirred at 10 °C for 3 hours. The product formation was confirmed by ^{1}H NMR.

^{1}H NMR (400 MHz, 10% D_2O in water) δ = 2.21 – 2.01 (m, 2H), 1.90 – 1.77 (m, 1H), 1.74 – 1.60 (m, 1H) ppm.

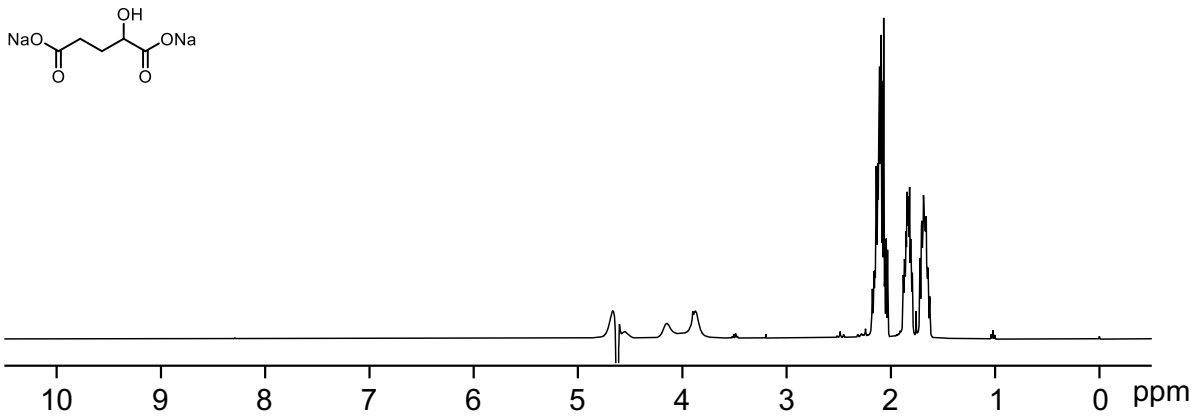


Figure S30. ^{1}H-NMR spectra of 2-hydroxypentanedioic acid recorded in 10% D_2O in water at pH 7.

5. References:

1. S. K. Bharti and R. Roy, *TrAC*, 2012, **35**, 5-26.
2. Z. Wu, T. Li, Y. Ding and A. Hu, *ACS Appl. Polym. Mater.*, 2020, **2**, 5414-5422.
3. E. B. Reid and J. R. Siegel, *J Chem Soc (Resumed)*, 1954, DOI: 10.1039/JR9540000520, 520-524.