Energy Conservation via Thioesters in a Non-Enzymatic Metabolism-like Reaction Network

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Life's catabolic processes capture chemical energy from the oxidative breakdown of metabolites. In the catabolic pathways at the core of biochemistry, the oxidation of α -ketoacids or aldehydes is coupled to the synthesis of thioesters, whose energy-releasing hydrolysis is in turn coupled to the production of adenosine 5'-triphosphate (ATP). How these processes became linked before life emerged, and thus how the framework for modern bioenergetics was established, is a major problem for understanding the origins of biochemistry. The structure of biochemical networks suggests that the intermediary role of thioesters in biological energy flows, and their central role in biosynthesis, is a consequence of their entry into metabolism at the earliest stage of biochemical evolution. However, how thioesters could have become embedded within a metabolic network before the advent of enzymes remains unclear. Here we demonstrate non-enzymatic oxidant- or light-driven thioester synthesis from biological α -ketoacids and show it can be integrated within an iron-promoted metabolism-like reaction network. The thioesters obtained are those predicted to be pivotal in computational reconstructions of primitive biochemical networks (acetyl, malonyl, malyl and succinyl thioesters), demonstrating a rare convergence between top-down and bottom-up approaches to the origins of metabolism. The diversity and simplicity of conditions that form thioesters from core metabolites suggests the energetic link between thioester synthesis and catabolism was in place at the earliest stage of prebiotic chemistry, constraining the path for the later evolution of life's phosphorus-based energy currencies.

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Energy conservation via thioesters in a non-enzymatic metabolism-like reaction network

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Life's catabolic processes capture chemical energy from the oxidative breakdown of metabolites. In the catabolic pathways at the core of biochemistry, the oxidation of α -ketoacids or aldehydes is coupled to the synthesis of thioesters, whose energy-releasing hydrolysis is in turn coupled to the production of adenosine 5'-triphosphate (ATP). How these processes became linked before life emerged, and thus how the framework for modern bioenergetics was established, is a major problem for understanding the origins of biochemistry. The structure of biochemical networks suggests that the intermediary role of thioesters in biological energy flows, and their central role in biosynthesis, is a consequence of their entry into metabolism at the earliest stage of biochemical evolution. However, how thioesters could have become embedded within a metabolic network before the advent of enzymes remains unclear. Here we demonstrate non-enzymatic oxidant- or light-driven thioester synthesis from biological α -ketoacids and show it can be integrated within an iron-promoted metabolism-like reaction network. The thioesters obtained are those predicted to be pivotal in computational reconstructions of primitive biochemical networks (acetyl, malonyl, malyl and succinyl thioesters), demonstrating a rare convergence between top-down and bottom-up approaches to the origins of metabolism. The diversity and simplicity of conditions that form thioesters from core metabolites suggests the energetic link between thioester synthesis and catabolism was in place at the earliest stage of prebiotic chemistry, constraining the path for the later evolution of life's phosphorus-based energy currencies.

A major goal of origins of life research is to understand how and why chemistry self-organized into today's biochemistry.^{1,2,3} The recent discovery of chemical reaction networks that break or form bonds in ways closely resembling biochemistry suggests that core metabolic pathways emerged from simpler non-enzymatic precursors, providing a straightforward explanation for why biochemistry came to be the way it is.^{4,5,6,7,8,9,10} However, non-enzymatic

reaction networks that combine metabolite synthesis with life's ability to capture chemical energy from catabolic (breakdown) processes have yet to be demonstrated.

The hydrolysis of thioesters is highly exergonic, which allows them to occupy a central position in biosynthesis and to act as life's sulfur-based energy carriers.¹¹ Accordingly, thioesters are thought to have played a dominant role in early biochemical evolution – a concept that De Duve dubbed the “Thioester World”.^{12,13,14} In biology, two of the main catabolic routes for thioester synthesis are the oxidative decarboxylation of α -ketoacids and the oxidation of aldehydes, both of which employ the thiol cofactor A (CoA), and which constitute key steps in core metabolic pathways such as the Krebs cycle and glycolysis (Figure 1). Recent computational predictions, based on the network structure and thermodynamics of all documented metabolic reactions, also support the existence of an early metabolic network hinging on thioesters.^{15,16} Specifically, acetyl, malonyl, malyl and succinyl thioesters were predicted to emerge within or from a reaction network resembling a primitive (reverse) Krebs cycle.¹⁶ Here we show that the same thioesters can be produced non-enzymatically from biological α -ketoacids and integrated into a Krebs cycle-like chemical reaction network, in good agreement with computational predictions about the structure of primitive metabolism.^{15,16}

Figure 1 Energy conservation via thioesters in biosynthesis. A) Thioester synthesis in the Krebs cycle. Oxidative decarboxylations of α -ketoacids are highlighted using red arrows. Additional biological reactions, such as the aldol reaction of acetyl thioester with glyoxylate to give malyl thioester, are also shown. A similar network was predicted to have been at the core of primitive metabolism, by starting from simple seed molecules and applying a thermodynamically constrained network expansion algorithm to grow the network using known biological reactions.¹⁶ B) Thioester synthesis via aldehyde oxidation in alcohol metabolism and glycolysis. C) Nature's most important thiol, coenzyme A (CoA) and a simpler analogue used in this study, *N*-acetylcysteamine.

Experimental work on prebiotic thioester synthesis is limited, with the most important examples arising from aldehydes^{17,18,19,20} or acetyl thioester synthesis from carbon monoxide and methyl thiol in very low yields.²¹ Inspired by the synthetic organic chemistry literature,^{22,23} we searched for simple aqueous inorganic conditions that might enable thioester synthesis from α -ketoacids and thiols. Our initial investigations focused on the reaction between pyruvate, the prebiotically plausible biological precursor to sugars and several amino acids,^{4,5,6,24} and *N*-

acetylcysteamine, a simpler, potentially ancestral,²⁵ analogue of CoA. Optimization of oxidizing agent, catalyst, and temperature (see Table S-1) converged on sulfate radical-generating conditions identical to those reported to drive Krebs cycle-like breakdown of core metabolites⁵ ($\text{S}_2\text{O}_8^{2-}$ as oxidant, FeS as catalyst, 70 °C), giving the corresponding acetyl thioester in 27% yield (Table 1, entry 1, method A). The remaining material was found mostly to be unreacted pyruvate and a small amount of acetate, according to GC-MS and NMR analysis (Table S-2). Thioester synthesis could equally be carried out with another water-soluble thiol (Scheme S-3), or with a disulfide instead of a thiol (entry 2, method A). Two other key intermediates of the Krebs cycle, α -ketoglutarate and oxaloacetate also underwent decarboxylative thioesterification to give the corresponding succinyl thioester, an analogue of the biosynthetic precursor to many co-factors, and malonyl thioester, an analogue of the precursor to biological fatty acid synthesis, respectively (entries 3-4, method A). Malonyl thioester formation is accompanied by decarboxylation to give acetyl thioester as a secondary product (entry 4). Malyl thioester was also formed under the same conditions starting from 4-hydroxyketoglutarate (entry 5, method A). UV-A light, thought to have constituted a significant portion of the solar spectrum on the early Earth,²⁶ was equally able to generate sulfate radicals from $\text{S}_2\text{O}_8^{2-}$, and therefore to deliver thioesters from α -ketoacids at ambient temperature (entries 1-5, method B). Alternatively, UV-A light alone was found to trigger thioester formation under acidic conditions (entries 1-5, method C). In this case however, the reduced reactivity with disulfides suggests the mechanism is distinct from the light-triggered reaction in the presence of $\text{S}_2\text{O}_8^{2-}$ (entry 2, method C). The direct photochemical reaction was found to be independent of the chemical nature of the acid (Table S-1), ruling out the involvement of photogenerated sulfate radicals from bisulfate.²⁷ Acetaldehyde, a representative aldehyde, was also found to furnish acetyl thioester under thermal and photochemical sulfate radical-forming conditions (entry 6, methods A-B), as well as under direct photochemical conditions similar to those previously reported by Weber (entry 6, method C).¹⁷ Thus, all three sets of conditions are able to generate thioesters from both α -ketoacids and aldehydes.

Table 1. Thioester formation from biological α -ketoacids or aldehydes and *N*-acetylcysteamine.

Entry	Substrate	Thioester Product(s)	Product Yield (%) $\pm$ SD [‡]		
			Method A ^a	Method B ^b	Method C ^c
1	pyruvate	acetyl thioester	27 $\pm$ 1	31.2 $\pm$ 0.4	22.1 $\pm$ 0.4
2 ^d	pyruvate	acetyl thioester	34 $\pm$ 1	32 $\pm$ 2	4.9 $\pm$ 0.2
3	α -ketoglutarate	succinyl thioester	10 $\pm$ 2	25 $\pm$ 2	13 $\pm$ 1
4	oxaloacetate	malonyl thioester	6.7 $\pm$ 0.4	24 $\pm$ 2	8 $\pm$ 2
		acetyl thioester	2.6 $\pm$ 0.3	2.4 $\pm$ 0.3	< 0.5
5	hydroxyketoglutarate ^e	malyl thioester	2.0 $\pm$ 0.1	10 $\pm$ 1	3.5 $\pm$ 0.2
6	acetaldehyde ^f	acetyl thioester	15 $\pm$ 1	13 $\pm$ 2	20 $\pm$ 2

[‡] Reported values were determined by GC-FID after an extraction procedure or by LCMS and represent the average of at least three runs. SD = standard deviation. See the Supplementary Information for additional control experiments. Unless otherwise noted, 0.5 mmol of the substrate and 0.1 mmol of the thio compound were employed. ^aMethod A: K₂S₂O₈ (2.0 equiv), FeS (0.5 equiv) in H₂O, 3 h, 70 °C. ^bMethod B: K₂S₂O₈ (2.0 equiv), FeS (0.5 equiv) in H₂O, 6 h, UV-A. ^cMethod C: KHSO₄ (3 M) in H₂O, 6 h, UV-A. ^d0.05 mmol of *N*-acetyl cysteamine disulfide was used instead of *N*-acetyl cysteamine. ^eHydroxyketoglutarate was freshly prepared from glyoxylate and oxaloacetate without isolation. ^f1.0 mmol of the substrate were used.

We recently reported an iron-promoted non-enzymatic reaction network in which most Krebs cycle metabolites are formed from pyruvate and glyoxylate, including other α -ketoacids.⁸ To mimic the biological integration of α -ketoacid synthesis, α -ketoacid breakdown and thioester synthesis, we simulated non-equilibrium environments where material could cycle between neutral and oxidized regions, or between dark and light regions. In a first experiment, pyruvate, glyoxylate, ferrous iron and *N*-acetylcysteamine were mixed in water at 70 °C for 1 h, after which time K₂S₂O₈ was added and the reaction continued without changing the temperature. At 4 h, acetyl (1.4 mM), succinyl (0.1 mM) and malyl (3.6 mM) thioesters were detected, as well as 8 carboxylic acid metabolites (Table S-3). In a second experiment, pyruvate, glyoxylate and ferrous iron were mixed in water at 70 °C for 1 h, after which time *N*-acetylcysteamine and KHSO₄ were added and the reaction was exposed to UV-A light at 22 °C. At 7 h, acetyl (0.1 mM), succinyl (0.2 mM) and malyl (0.06 mM) thioesters were detected, as well as 5 carboxylic acid metabolites (Table S-3). Thus, different types of non-equilibrium environments (neutral/oxidized or dark/light) can lead to a non-enzymatic reaction network in which α -ketoacid and thioester synthesis are coupled, as in biochemistry (Figure 2).

Figure 2 Thioester formation from an iron-catalysed reaction network generated from pyruvate, glyoxylate and *N*-acetylcysteine, achieved through redox or light cycling. Molecules in black represent those observed in the presence of Fe^{2+} , thiol and oxidant. Molecules in grey represent those observed in the presence of Fe^{2+} only. Additional reactions and observed metabolites have been omitted for clarity.

In summary, inorganic oxidants or light enable non-enzymatic thioester synthesis from metabolites in a way that closely mimics energy conservation within biological catabolism, and that is integrated with metabolite-generating chemical networks. Synthesis and breakdown of metabolites could have been linked in an energy-conserving way from the outset of prebiotic chemistry, in accord with computational models of an “onion-like” growth of metabolism from one initially based on C, H, and O to one incorporating S, N, and then P.^{12,13,15,16} The simplicity of the conditions observed here suggest thioester synthesis would have been difficult to avoid in any near-surface environment where light, α -ketoacids or aldehydes and thiols were present. A primitive form of bioenergetics built around thioesters therefore may have emerged very early on, setting the stage for the complex life-like behaviours characteristic of thioester networks,^{28,29} and imposing constraints on later chemical energy currencies based on phosphorous. Finally,

hydrogen-rich geochemical environments where reductive metabolite synthesis⁶ could occur within proximity of oxidants or light, such as surface hydrothermal vents, warrant consideration for the origin of life.³⁰

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Author contributions: J.M. supervised the research and the other authors performed the experiments. All authors contributed intellectually throughout the study. All authors wrote the paper and E.C.-B. and J.G. assembled the Supplementary information.

Competing financial interests: Authors declare no competing financial interests.

Data and materials availability: All data is available in the main text or the supplementary information file.

Supplementary Information:

Materials and Methods

Figures S-1 to S-9

Tables S-1 to S-3

Scheme S-1 to S-4

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Supporting Information

Energy conservation via thioesters in a non-enzymatic metabolism-like reaction network

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1. General information

All reactions were carried out in oven dried glassware under an atmosphere of argon. All glassware and stir bars were pre-washed with acid, followed by distilled water and acetone, and oven dried to prevent any cross-contamination by metal salts. Unless otherwise noted, all reagents and solvents were purchased from commercial suppliers and used without further purification. Water was obtained from a Milli-Q purification system (18 MΩcm) and was purged with argon before use.

For water suppression ¹H-NMR spectra were recorded on a Bruker Avance300 (300 MHz) spectrometer at ambient temperature in a H₂O:D₂O mixture (6:1) as solvent, with sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) as the internal standard (TMS peak at 0 ppm). Solvent suppression was achieved through excitation sculpting, using the Bruker ZGESGP pulse program adjusted for the water resonance. Integration was performed using *ACD/NMR Processor Academic Version 12.00* software.

Regular ¹H- and ¹³C-NMR spectra were recorded on a Bruker Avance300 (300 MHz), Bruker UltraShield 400 (400 MHz) or a Bruker Avance 500 (500 MHz) spectrometer at ambient temperature and are reported in ppm using the solvent signal as internal reference (CDCl₃ at 7.26 ppm (¹H) or 77.00 ppm (¹³C), DMSO-d₆ at 2.50 ppm (¹H) or 39.51 ppm (¹³C)). Data are reported as: multiplicity (br. s = broad singlet, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (in Hz) and integration. Melting points were obtained on a Büchi Melting Point B-450 apparatus without correction. High resolution mass spectrometry (HRMS) analysis was performed on a Bruker MicroTOF-Q (ESI) instrument.

LC-MS analysis was performed on a ThermoFisher Scientific UltiMate 3000 UHPLC-system equipped with an C₁₈ ThermoFisher Hypersil Gold 10 mm column using an Exactive Plus EMR Orbitrap detector. Acetonitrile (+ 0.1 % formic acid) and water (+ 0.1 % formic acid) were used for the mobile phase. The column was maintained at 25 °C and the solvent gradient was used as following: 2% MeCN for 1 min, linear gradient to 15% MeCN over 3.5 min, linear gradient to 100% MeCN over 1 min, 100% MeCN for 2.5 min, linear gradient to 2% MeCN over 0.5 min, 2% MeCN for 1.5 min. The analytes were quantified using a calibration of authentic samples by integration of the peak area of a specified m/z range. Unless otherwise noted 0.1 µL of the sample solutions were injected.

GC-MS analysis was performed on a GC System 7820A (G4320) using an Agilent High Resolution Gas Chromatography Column (PN 19091S – 433UI, HP – 5MS UI, 28 m×0.250 mm, 0.25 Micron, SN USD 489634H). The system was connected to an MSD block 5977E (G7036A). Hydrogen (99.999 % purity) was used as carrier gas at a constant flow rate of 1.5 mL/min. The analysis was carried out in a splitless mode with 1 µL injection volume, at the injection port temperature of 250 °C. The column was maintained at 60 °C for 1 min, then ramped at 30 °C/min to 310 °C with 3 min hold, and the total

running time was 12.33 min. The mass spectrometer was turned on after a 2-min solvent delay, and was operated at the electron ionization (EI) mode with quadrupole temperature of 150 °C. Data was acquired in the full-scan mode (50-500 amu).

GC-FID analysis was performed on a GC System GC7890B with an FID-detector using an Agilent High Resolution Gas Chromatography Column (PN 19091S – 433UI, HP – 5MS UI, 28 m×0.250 mm, 0.25 Micron, SN USD 489634H). Hydrogen (99.999 % purity) was used as carrier gas at a constant flow rate of 5.0 mL/min. The analysis was carried out in a splitless mode with 1 µL injection volume, at the injection port temperature of 250 °C. The column was maintained at 60 °C for 1 min, then ramped at 30 °C/min to 300 °C with 3 min hold, and the total running time was 12 min. The FID detector was used with an airflow of 400 mL/min, a H₂ fuel flow of 25 mL/min and a N₂ makeup flow of 10 mL/min. The software used was Agilent OpenLAB CDS ChemStation Edition Rev. C.01.09[144].

2. Analytical methods

2.1. GC-FID analysis

Extraction procedure for GC-FID analysis

A 0.5 mL aliquot of the reaction mixture was added to 0.5 mL of EA, followed by vortexing for 30 s. The EA layer was separated and dried over anhydrous Na_2SO_4 . 40 μL of the dry EA layer was diluted with 960 μL of EA and subjected to GC-FID analysis.

Yield determination and error analysis for GC-FID analysis

1 mL standard aqueous solutions of thioesters at different concentrations (1 mM, 2 mM, 5 mM, 10 mM, 20 mM, 40 mM) were prepared by dilution 100 mM stock solutions of these thioesters. 500 μL of each standard solution were extracted using the above described extraction procedure for GC-FID analysis. For each thioester, a six-point graph was plotted, correlating the characteristic GC peak (as integrated automatically by the Agilent OpenLAB CDS ChemStation Edition Rev. C.01.09[144] software) with substrate concentration (Figure S-1 – S-2). Each data point was obtained from three independent measurements and the correlation line was obtained from the least-squares fitting (intercept = 0). Error bars on graphs are shown as $\pm$ standard deviation for each data point. Overall percentage error of the response factor corresponds to $\pm$ standard deviation for each slope value.

The concentrations of the compounds were calculated by comparing the product peak area with the calibration curve. The yields of the products were calculated by multiplication of the determined concentration with the reaction volume, divided by the amount of limiting starting material (in most cases 0.1 mmol of the thio compound). Each reaction was performed at least three times to ensure reproducibility and reported percentage yields are an average of these three runs, with an error corresponding to $\pm$ standard deviation.

Figure S-1: Correlation between the concentration of an aqueous solution of **3b** and the measured gas chromatography FID peak area.

Figure S-2: Correlation between the concentration of an aqueous solution of **3c** and the measured gas chromatography FID peak area.

2.2. LCMS analysis

Sample preparation of LCMS analysis

After treatment of 1 mL of the reaction mixture with Chelex® 100 resin followed by centrifugation 20 μ L of the supernatant were diluted with 180 μ L of MilliQ-water and subjected to LCMS analysis (injection volume = 0.1 μ L unless otherwise noted).

Yield determination and error analysis for LCMS analysis

1 mL standard aqueous solutions of authentic samples at different concentrations (selection of 0.5 mM, 1 mM, 2 mM, 4 mM, 5 mM, 10 mM, 20 mM, 40 mM, 80 mM) were prepared by dilution of 100 mM stock solutions of these compounds. For each of these solutions 20 μ L were diluted with 180 μ L MilliQ-water and subjected to LCMS-analysis. For each compound, a six-point graph was plotted, correlating the characteristic LCMS-peak (as integrated by the Thermo Xcalibur 4.2.28.14 Qual Browser software) with substrate concentration (Figure S-3 – S-7). Each data point was obtained from three independent measurements and the correlation line was obtained from the least-squares fitting (intercept = 0). Error bars on graphs are shown as $\pm$ standard deviation for each data point. Overall percentage error of the response factor corresponds to $\pm$ standard deviation for each slope value.

The concentrations of the compounds were calculated by comparing the product peak area with the calibration curve. The yields of the products were calculated by multiplication of the determined concentration with the reaction volume, divided by the amount of limiting starting material (in most cases 0.1 mmol of the thio compound). Each reaction was performed at least three times to ensure reproducibility, and reported percentage yields are an average of these three runs, with an error corresponding to $\pm$ standard deviation.

Figure S-3: Correlation between the concentration of an aqueous solution of **5b** and the measured LCMS peak area ($m/z = 219.95\text{--}220.15$).

Figure S-4: Correlation between the concentration of an aqueous solution of **8b** and the measured LCMS peak area ($m/z = 205.54-206.54$).

Figure S-5: Correlation between the concentration of an aqueous solution of **9b** and the measured LCMS peak area ($m/z = 236.04-236.06$).

Figure S-6: Correlation between the concentration of an aqueous solution of **1b** and the measured LCMS peak area (m/z = 119.95–120.15).

Figure S-7: Correlation between the concentration of an aqueous solution of **1d** and the measured LCMS peak area (m/z = 236.97–237.17).

Figure S-8: Correlation between the concentration of an aqueous solution of **3b** and the measured LCMS peak area (m/z = 161.95–161.15).

2.3. GC-MS analysis

Derivatisation procedure for GC-MS analysis

To facilitate GC-MS analysis, a literature ECF-derivatization procedure^{1,2} was applied to the sample to convert carboxy groups to ethyl esters, hydroxy groups to ethyl carbonates, ketones to diethyl ketals, and aldehydes to diethyl acetals, using a mixture of ethanol/ethyl chloroformate (EtOH/ECF). For optimal gas chromatography resolution, heavy metal atoms were first removed using a resin (Chelex[®] 100 sodium form).

A *ca.* 1 mL aliquot of the reaction mixture was added to 50 mg of Chelex[®], briefly shaken and then centrifuged (6000 rpm, 3 min). To 600 μ L of the supernatant was added EtOH (300 μ L) and pyridine (40 μ L), followed by ethyl chloroformate (ECF, 40 μ L). The mixture vortexed for 30 s. A second 40 μ L portion of ECF was added and the mixture was vortexed again for 30 s. Next, CHCl₃ (200 μ L) was added, followed by vortexing (10 s). Finally, NaHCO₃ (600 μ L) was added and the mixture was vortexed again for 10 s. The CHCl₃ layer was separated and dried over anhydrous Na₂SO₄. 50 μ L of the dry CHCl₃ layer were diluted with 150 μ L of ethyl acetate prior to GC-MS analysis.

Product identification

Reaction products derivatized were identified by comparing the mass spectra and retention times with analogously derivatized authentic samples, as described in a previous paper.³

2.4. NMR analysis with water suppression

For ¹H-NMR analysis using water suppression the samples were first treated with Chelex[®] 100 resin followed by centrifugation to remove heavy metal ions. 600 μ L from the supernatant were mixed with 100 μ L of DSS in D₂O (0.05 M) and analyzed by NMR with water suppression. Water suppression was achieved using the Bruker ZGESGP pulse program. Relaxation delay D1 was set to 87 s, with time domain size TD = 32768 and sweep width SWH = 4789.27 Hz (11.963 ppm). 32 scans were acquired for each sample.

3. Synthetic procedures

Compound	Structure	New	Lit	¹ H	¹³ C	GC-FID	HRMS
10		No	⁴	+	-	-	-
1c		No	⁵	+	+	+	-
1d		No	⁶	+	+	+	-
3b		No	⁷	+	+	+	-
3c		No	⁸	+	+	+	-
5b		No	⁹	+	+	-	-
11		Yes	-	+	+	+	+
8b		No	¹⁰	+	+	-	-
12		No	¹¹	+	+	-	-
13		Yes	-	+	+	-	+
9b		Yes	-	+	+	-	+

3.1. Synthesis of starting materials

Scheme S- 1: Synthesis routes toward **1d** and **1c**.

N,N'-(disulfanediyldiis(ethane-2,1-diyl))diacetamide (**1d**)⁶

To a solution of *N*-acetylcysteamine **1b** (1.00 g, 8.39 mmol, 1.0 equiv) in 9 mL of EA was added potassium iodide (7.0 mg, 0.042 mmol, 0.5 mol%) and aqueous hydrogen peroxide (33 wt.%, 857 μL , 9.23 mmol, 1.1 equiv) subsequently and the mixture was stirred at room temperature. After one hour the resulting brown solution was treated with $\text{Na}_2\text{S}_2\text{O}_3$ -solution (5 wt.%, 10 mL) followed by further stirring for 5 minutes. Then the layers were separated, and the aqueous layer was extracted two more times with EA (2 x 20 mL). The combined organic layers were dried (Na_2SO_4) and filtered. The solvent of the filtrate was removed in vacuo which gave access to **1d** (653 mg, 2.76 mmol, 66%) as a colorless white powder.

m.p. 87–88°C **¹H-NMR** (500 MHz, CDCl_3): δ = 2.03 (s, 6 H, 1-H), 2.83 (t, $^3J_{4,3}$ = 6.6 Hz, 4 H, 4-H), 3.56 (dt, $^3J_{3,4} \approx ^3J_{3,\text{NH}}$ = 6.4 Hz, 4 H, 3-H), 6.68 (bs, 2 H, N-H). **¹³C-NMR** (125 MHz, CDCl_3): δ = 23.1, 37.7, 38.6, 170.9.

2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**10**)⁴

For the synthesis of the tosylate to a solution of triethylene glycol monomethyl ether (12.3 g, 75 mmol, 1.0 equiv) in 150 mL DCM was added triethylamine (11.5 mL, 82 mmol, 1.1 equiv), 4-dimethylaminopyridine (0.46 g, 3.75 mmol, 5 mol%) and tosyl chloride (14.3 g, 75 mmol, 1.0 equiv). After stirring the reaction mixture for 5 hours at room temperature DCM (100 mL) was added and the organic layer was washed with 1 M HCl solution (150 mL). The layers were separated, and the organic layer was dried (Na_2SO_4) and filtered. After removal of the solvent in vacuo the crude tosylate **10** (21.9 g) was isolated as a yellow oil and used in the next step without further purification.

¹H-NMR (400 MHz, CDCl_3): δ = 2.44 (s, 3 H, 12-H), 3.36 (s, 3 H, 1-H), 3.53 (m, 2 H, 2-H), 3.56–3.63 (m, 6 H, 3-H, 4-H, 5-H), 3.68 (t, $^3J_{6,7}$ = 4.8 Hz, 2 H, 6-H), 4.15 (t, $^3J_{7,6}$ = 4.8 Hz, 2 H, 7-H), 7.33 (d, $^3J_{10,9}$ = 8.1 Hz, 2 H, 10-H), 7.79 (d, $^3J_{9,10}$ = 8.2 Hz, 2 H, 9-H).

S-(2-(2-(2-methoxyethoxy)ethoxy)ethyl) ethanethioate (**3c**)⁸

To a solution of the tosylate **10** (21.9 g, 68.7 mmol, 1.0 equiv) in 100 mL DMF under N_2 -atmosphere was added potassium iodide (0.57 g, 3.44 mmol, 5 mol%) and potassium thioacetate (9.42 g, 82 mmol, 1.2 equiv). The mixture was stirred at room temperature overnight. After 16 hours 300 mL of EA were added followed by washing with water (3 x 150 mL). The organic layer was dried (Na_2SO_4) and filtered. After removal of the solvent in vacuo the thioester **3c** (10.9 g, 49.2 mmol, 66% over two steps) was isolated as a yellow oil. The compound was used for the synthesis of **1c** and also as an authentic sample for the acetylation reaction.

¹H-NMR (400 MHz, CDCl_3): δ = 2.32 (s, 3 H, 9-H), 3.08 (t, $^3J_{7,6}$ = 6.5 Hz, 2 H, 7-H), 3.37 (s, 3 H, 1-H), 3.54 (m, 2 H, 2-H), 3.56–3.66 (m, 8 H, 3-H, 4-H, 5-H, 6-H). **¹³C-NMR** (100 MHz, CDCl_3): δ = 28.8, 30.5, 59.0, 69.7, 70.3, 70.5, 70.5, 71.9, 195.5.

2-(2-(2-methoxyethoxy)ethoxy)ethane-1-thiol (1c**)**⁵

For the synthesis of the thiol to a slurry of potassium carbonate (9.33 g, 67.5 mmol, 1.5 equiv) in 45 mL of methanol was added a solution of **3c** (10.0 g, 45 mmol, 1.0 equiv) dropwise over 30 seconds in the dark. After stirring at room temperature for one hour the reaction mixture was diluted with DCM (300 mL) and washed with sat. NH_4Cl solution. The aqueous layer was extracted with DCM (100 mL) and the combined organic layers were dried (Na_2SO_4). After filtration the solvent was removed under reduced pressure giving access to **1c** (7.75 g, 43 mmol, 96%) as a pale yellow oil.

^1H -NMR (400 MHz, CDCl_3): δ = 1.57 (t, $^3J_{\text{SH},7}$ = 8.3 Hz, 1 H, S-H), 2.68 (dt, $^3J_{7,\text{SH}}$ = 8.3 Hz, $^3J_{7,6}$ = 6.4 Hz, 2 H, 7-H), 3.37 (s, 3 H, 1-H), 3.50–3.68 (m, 10 H, 2-H, 3-H, 4-H, 5-H, 6-H). **^{13}C -NMR** (100 MHz, CDCl_3): δ = 24.3, 59.0, 70.2, 70.6, 70.6, 71.9, 72.9.

3.2. Synthesis of reference compounds

Scheme S- 2: Synthesis routes toward several reference compounds.

N,S-diacetyl cysteamine (**3b**)⁷

To a solution of *N*-acetyl cysteamine **1b** (2.00 g, 16.8 mmol, 1.0 equiv) in 50 mL of DCM was added DIPEA (4.4 mL, 25.2 mmol, 1.5 equiv) followed by the dropwise addition of acetyl chloride (1.2 mL, 17.6 mmol, 1.05 equiv). The reaction mixture was stirred at room temperature. After 1.5 hours the mixture was diluted with DCM (50 mL) and washed with 1 M HCl solution (30 mL) and sat. NaHCO₃-solution (10 mL). The organic layer was dried (Na₂SO₄) and filtered. Removal of the solvent under reduced pressure afforded **3b** (2.00 g, 12.4 mmol, 74%) as a pale yellow oil.

¹H-NMR (300 MHz, CDCl₃): δ = 1.95 (s, 3 H, 1-H), 2.34 (s, 3 H, 6-H), 3.00 (t, ³J_{4,3} = 6.5 Hz, 2 H, 4-H), 3.40 (dt, ³J_{3,4} ≈ ³J_{3,NH} = 6.3 Hz, 2 H, 3-H), 6.03 (br. s, 1 H, N-H). ¹³C-NMR (75 MHz, CDCl₃): δ = 23.1, 28.7, 30.6, 39.5, 170.4, 196.3.

4-[[2-(Acetylamino)ethyl]thio]-4-oxobutanoic acid (**5b**)⁹

To a solution of *N*-acetyl cysteamine **1b** (99.2 mg, 0.83 mmol, 1.0 equiv) in 3 mL of acetonitrile was added DMAP (10.2 mg, 0.083 mmol, 0.1 equiv), pyridine (197.4 mg, 2.50 mmol, 3.0 equiv) and succinic anhydride (83.26 mg, 0.83 mmol, 1.0 equiv). The reaction mixture was stirred at room temperature. After 2 hours the mixture was diluted with sat. NaHCO₃-solution (3 mL) and washed with Et₂O (5 mL). The organic layer was discarded. The aqueous layer was acidified with 1 M HCl solution to pH 1 and was extracted with EA (4 × 10 mL). The combined organic layers were dried (Na₂SO₄). After filtration the solvent was removed under reduced pressure giving access to **5b** (88 mg, 0.40 mmol, 48 %) as a colorless powder.

m.p. 78–80 °C ¹H-NMR (500 MHz, CDCl₃): δ = 1.97 (s, 3 H, 8-H), 2.73 (t, ³J_{3,2} = 6.6 Hz, 2 H, 3-H), 2.90 (t, ³J_{2,3} = 6.6 Hz, 2 H, 2-H), 3.06 (t, ³J_{5,6} = 6.2 Hz, 2 H, 5-H), 3.45 (dt, ³J_{6,5} = ³J_{6,N-H} = 6.2 Hz, 2 H, 6-H), 5.99 (s, 1 H, N-H). ¹³C-NMR (125 MHz, CDCl₃): δ = 23.1, 28.5, 28.9, 38.3, 39.7, 171.0, 175.5, 198.5.

3-((2-acetamidoethyl)thio)-3-oxopropanoic acid (**8b**)¹⁰

To a solution of malonyl monothiophenylester¹² (125 mg, 0.64 mmol, 1.0 equiv) and NaHCO₃ (107 mg, 1.27 mmol, 2.0 equiv) in 1 mL of MilliQ water was added *N*-acetylcysteamine **1b** (85 mg, 0.71 mmol, 1.1 equiv) dissolved in 1 mL of MilliQ water over 30 minutes. After stirring at room temperature for one hour, the mixture was acidified to pH = 1 with 1 M HCl. The aqueous layer was washed with chloroform (3 x 10 mL) and DCM (2 x 10 mL). The aqueous layer was lyophilized. The resulting solid was suspended in acetonitrile (20 mL) and filtered to remove NaCl. The filtrate was dried in vacuo giving access to the malonyl thioester **8b** (80 mg, 0.39 mmol, 61 %) as a pale yellow oil.

¹H-NMR (500 MHz, CDCl₃, DMSO-d₆): δ = 1.81 (s, 3 H, 7-H), 2.95 (t, ³J_{4,5} = 6.6 Hz, 2 H, 4-H), 3.27 (dt, ³J_{5,4} ≈ ³J_{5,NH} = 6.4 Hz, 2 H, 5-H), 3.43 (s, 2 H, 2-H), 6.76 (br. s, 1 H, N-H). ¹³C-NMR (125 MHz, CDCl₃, DMSO-d₆): δ = 22.7, 28.7, 38.6, 49.3, 167.6, 170.3, 191.5.

2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-yl)acetic acid (**12**)¹¹

A solution of racemic malic acid (3.00 g, 22.4 mmol, 1.0 equiv) and *p*-toluene sulfonic acid monohydrate (85 mg, 0.45 mmol, 2 mol%) in a mixture of 25 mL of 2,2-dimethoxypropane and 25 mL acetone was stirred at room temperature for 5 hours. Then sodium bicarbonate (38 mg, 0.45 mmol, 2 mol%) and 50 mL of water were added. The mixture was extracted with DCM (3 x 100 mL) and the combined organic layers were dried (Na₂SO₄). After filtration and removal of the solvent in vacuo the crude product was recrystallized from PE/Et₂O (97:3). The protected malic acid **12** (1.74 g, 10.0 mmol, 45%) was isolated as a white solid.

m.p. 74–77°C ¹H-NMR (400 MHz, CDCl₃): δ = 1.56 (s, 3 H, 6-H), 1.61 (s, 3 H, 6-H'), 2.85 (dd, ²J_{2a,2b} = 17.2 Hz, ³J_{2a,3} = 6.6 Hz, 1 H, 2-H_a), 2.99 (dd, ²J_{2b,2a} = 17.2 Hz, ³J_{2b,3} = 3.8 Hz, 1 H, 2-H_b), 4.71 (dd, ³J_{3,2a} = 6.6 Hz, ³J_{3,2b} = 3.8 Hz, 1 H, 3-H), 10.23 (br. s, 1 H, COO-H). ¹³C-NMR (100 MHz, CDCl₃): δ = 25.8, 26.7, 36.0, 70.4, 111.4, 171.8, 175.1.

S-(2-acetamidoethyl) 2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-yl)ethanethioate (13)

To a mixture of the protected malic acid **12** (300 mg, 1.72 mmol, 1.05 equiv) and DIPEA (344 μ L, 1.97 mmol, 1.2 equiv) in 17 mL of DCM was added ethyl chloroformate (158 μ L, 1.64 mmol, 1.0 equiv) dropwise at 0 °C. After 5 minutes, *N*-acetylcysteamine **1b** (196 mg, 1.64 mmol, 1.0 equiv) was added and the mixture was allowed to warm to room temperature. After 3 hours the reaction mixture was diluted with DCM (30 mL) and washed once with 1 M HCl-solution (10 mL) and sat. NaHCO₃-solution (10 mL). The organic layer was dried (Na₂SO₄) and filtered. After removal of the solvent in vacuo the thioester **13** (369 mg, 1.34 mmol, 82%) was isolated as a colorless liquid.

¹H-NMR (500 MHz, CDCl₃): δ = 1.57 (s, 3 H, 10-H), 1.62 (s, 3 H, 10-H'), 2.00 (s, 3 H, 8-H), 3.06 (dd, ²*J*_{3a,3b} = 16.5 Hz, ³*J*_{3a,2} = 6.7 Hz, 1 H, 3-H_a), 3.11 (td, ³*J*_{5,6} = 6.4 Hz, ⁴*J*_{5,NH} = 2.1 Hz, 2 H, 5-H), 3.19 (dd, ²*J*_{3b,3a} = 16.5 Hz, ³*J*_{3b,2} = 4.0 Hz, 1 H, 3-H_b), 3.46 (m, 2 H, 6-H), 4.76 (dd, ³*J*_{2,3a} = 6.7 Hz, ³*J*_{2,3b} = 4.3 Hz, 1 H, 2-H), 6.29 (br. s, 1 H, N-H). **¹³C-NMR** (100 MHz, CDCl₃): δ = 23.0, 25.8, 26.8, 28.8, 39.3, 44.8, 70.5, 111.4, 170.7, 171.8, 195.0. **HRMS**: [C₁₁H₁₇NO₅S+K]⁺ calculated 314.0450 found: 314.0459, *R*_f(silica, EA) = 0.40.

4-((2-acetamidoethyl)thio)-2-hydroxy-4-oxobutanoic acid (9b)

A solution of the thioester **13** (87 mg, 0.317 mmol, 1.0 equiv) in a mixture of 2.5 mL THF and 1 mL of 1 M HCl solution was heated to 50 °C. After one hour, TLC-analysis showed complete conversion of the starting material. The reaction was cooled to room temperature and extracted with EA (5 mL). The organic layer was again extracted with 2 mL of 1 M HCl-solution and the combined aqueous layers were dried in vacuo giving access to the malyl thioester **9b** (51.4 mg, 0.218 mmol, 69%) as a colorless resin.

¹H-NMR (500 MHz, D₂O): δ = 1.89 (s, 3 H, 8-H), 3.01 (m, 2 H, 5-H), 3.07 (dd, ²J_{3a,3b} = 16.2 Hz, ³J_{3a,2} = 6.7 Hz, 1 H, 3-H_a), 3.13 (dd, ²J_{3b,3a} = 15.9 Hz, ³J_{3b,2} = 4.6 Hz, 1 H, 3-H_b), 3.32 (t, ³J_{6,5} = 6.3 Hz, 2 H, 6-H), 4.57 (dd, ³J_{2,3a} = 6.9 Hz, ³J_{2,3b} = 4.1 Hz, 1 H, 2-H). **¹³C-NMR** (125 MHz, DMSO-d₆): δ = 25.2, 30.8, 40.8, 50.6, 69.6, 172.0, 176.8, 198.4. **HRMS**: [C₈H₁₃NO₅S+H]⁺ calculated 236.0587 found: 236.0583.

2,5,8,15,18,21-hexaoxa-11,12-dithiadocosane (11)

A solution of the thiol **1c** (300 mg, 1.66 mmol, 1.0 equiv) in 2 mL of EA was treated with sodium iodide (1 mg, 8 μmol, 0.5 mol%) and aqueous hydrogen peroxide solution (33 wt.%, 160 μL, 1.83 mmol, 1.1 equiv) and stirred at room temperature. After one hour the resulting brown solution was treated with Na₂S₂O₃ solution (5 wt.%, 2 mL), followed by further stirring for 5 minutes. Then the layers were separated, and the aqueous layer was extracted two more times with EA (2 x 5 mL). The combined organic layers were dried (Na₂SO₄) and filtered. The solvent of the filtrate was removed in vacuo which gave access to **11** (287 mg, 0.80 mmol, 96%) as a pale yellow oil.

¹H-NMR (400 MHz, CDCl₃): δ = 2.88 (t, ³J_{7,6} = 6.7 Hz, 4 H, 7-H), 3.37 (s, 6 H, 1-H), 3.55 (m, 4 H, 2-H), 3.62–3.72 (m, 12 H, 3-H, 4-H, 5-H), 3.72 (t, ³J_{6,7} = 6.7 Hz, 4 H, 6-H). **¹³C-NMR** (125 MHz, CDCl₃): δ = 38.3, 59.0, 69.6, 70.3, 70.51, 71.52, 71.9. **HRMS**: [C₁₄H₃₀O₆S₂+Na]⁺ calculated: 381.1376 found: 381.1384.

3.3. Formation of thioesters under prebiotic conditions

General procedures for thioester formation:

Method A ($K_2S_2O_8$, FeS, 70 °C):

To a solution of the thiol (0.1 mmol) in 3 mL of MilliQ water in a Pyrex pressure tube with a stirring bar was added the corresponding α -keto acid (0.50 mmol; 0.42 mmol for hydroxyketoglutarate) or acetaldehyde (1.0 mmol) under a constant argon flow. Iron(II) sulfide (0.05 mmol) and potassium persulfate (0.2 mmol) were added. The reaction vessel was closed and heated to 70 °C for 3 hours. After cooling to room temperature, the amount of thioester formed was determined by GC-FID analysis or LCMS analysis (see section 2.1 and 2.2). Additionally selected samples were analyzed by NMR using water suppression (see section 2.4).

Method B ($K_2S_2O_8$, FeS, UV-A):

To a solution of the thiol (0.1 mmol) in 3 mL of MilliQ water in a quartz tube with a stirring bar was added the corresponding α -keto acid (0.50 mmol; 0.42 mmol for hydroxyketoglutarate) or acetaldehyde (1.0 mmol) under a constant argon flow. Iron(II) sulfide (0.05 mmol) and potassium persulfate (0.2 mmol) were added. The tube was closed with a septum and irradiated in a Luzchem LZO-ORG photoreactor equipped with 10 LZO-UVA lamps for 6 hours. The amount of thioester formed was determined by GC-FID analysis or LCMS analysis (see section 2.1 and 2.2). Additionally selected samples were analyzed by NMR using water suppression (see section 2.4).

Method C ($KHSO_4$, UV-A):

To a solution of the thiol (0.1 mmol) in 3 mL of a $KHSO_4$ -solution (3 M in MilliQ water) in a quartz tube with a stirring bar was added the corresponding α -keto acid (0.50 mmol; 0.42 mmol for hydroxyketoglutarate) or acetaldehyde (1.0 mmol) under a constant argon flow. The tube was closed with a septum and irradiated in a Luzchem LZO-ORG photoreactor equipped with 10 LZO-UVA lamps for 6 hours. The amount of thioester formed was determined by GC-FID analysis or LCMS analysis (see section 2.1 and 2.2). Additionally selected samples were analyzed by NMR using water suppression (see section 2.4).

Hydroxyketoglutarate was synthesized by adapting a literature procedure without isolation of the product.³ To a solution of glyoxylic acid hydrate (506 mg, 5.50 mmol, 1.1 equiv) in 30 mL of MilliQ water was added oxaloacetic acid (660 mg, 5.00 mmol, 1.0 equiv) and NaHCO₃ (840 mg, 10.0 mmol, 2.0 equiv) under argon. The resulting mixture was stirred at room temperature for two hours. A concentration of 139 mM for the resulting hydroxyketoglutarate was determined by NMR analysis with water suppression using DSS as an internal standard.

Table S-1: Optimization of the oxidative decarboxylation of sodium pyruvate with 1b.

Entry	Additive (equiv/conc)	Light source/T	t [h]	Yield(3b) [%]
1	none	UVA/-B/-C/CW	3	0.6 ± 0.2
2	none	70 °C	3	0.0 ± 0.0
3	K ₂ S ₂ O ₈ (2 equiv)	rt	3	1.5 ± 0.7
4	K ₂ S ₂ O ₈ (2 equiv)	50 °C	3	9.0 ± 0.9
5	K ₂ S ₂ O ₈ (2 equiv)	70 °C	3	14.8 ± 0.7
6	K ₂ S ₂ O ₈ (2 equiv)	100 °C	3	8.6 ± 0.8
7	K ₂ S ₂ O ₈ (2 equiv)	UVA/-B/-C/CW	3	6.4 ± 1.8
8	K ₂ S ₂ O ₈ (2 equiv) + FeS (0.5 equiv)	UVA/-B/-C/CW	3	23.1 ± 2.7
9	K ₂ S ₂ O ₈ (2 equiv) + FeS (0.5 equiv)	70 °C	3	21.2 ± 0.5
10	KHSO ₄ (3 M)	UVA	6	22.1 ± 0.4
11	Fe(ClO ₄) ₃ (1.0 equiv)	UVA	6	4.8 ± 0.4
12	K ₂ S ₂ O ₈ (2 equiv) + FeS (0.5 equiv)	UVA	6	31.2 ± 0.4
13	K ₂ S ₂ O ₈ (2 equiv) + FeS (0.5 equiv)	70 °C	3	26.6 ± 0.7
14	KHSO ₄ (1 M)	UVA/-B/-C/CW	3	6.2 ± 0.2
15	KHSO ₄ (3 M)	UVA/-B/-C/CW	3	8.4 ± 1.9
16	NaHSO ₄ (1 M)	UVA/-B/-C/CW	3	4.4 ± 0.9
17	H ₂ SO ₄ (1 M)	UVA/-B/-C/CW	3	6.8 ± 0.3
18	H ₃ PO ₄ (3 M)	UVA/-B/-C/CW	3	6.1 ± 0.3
19	HCl (0.5 M)	UVA/-B/-C/CW	3	2.5 ± 0.2
20	H ₂ SO ₄ (1 M) or H ₃ PO ₄ (3 M) or HCl (0.5 M) or KHSO ₄ (3 M)	70 °C	3	0 ± 0
21	none ^a	UVA/-B/-C/CW	3	1.8 ± 0.0
22	KHSO ₄ (1 M)	none	3	0.5 ± 0.1
23	KHSO ₄ (1 M)	UVA	3	14.2 ± 0.7
24	KHSO ₄ (1 M)	UVB	3	9.5 ± 1.0
25	KHSO ₄ (1 M)	UVC	3	0.8 ± 0.0
26	KHSO ₄ (1 M)	CW	3	0.6 ± 0.1
27	KHSO ₄ (3 M) ^b	UVA	6	4.9 ± 0.2
28	Fe(ClO ₄) ₃ (1.0 equiv) ^b	UVA	6	0.5 ± 0.1
29	K ₂ S ₂ O ₈ (2 equiv) + FeS (0.5 equiv) ^b	UVA	6	31.9 ± 0.7

Entry	Additive (equiv/conc)	Light source/T	t [h]	Yield(3b) [%]
30	K ₂ S ₂ O ₈ (2 equiv) + FeS (0.5 equiv) ^b	70 °C	3	32.9 ± 0.8
31	KHSO ₄ (0.5 M)	UVA	3	7.2 ± 0.5
32	KHSO ₄ (0.41 M) + K ₂ SO ₄ (0.09 M)	UVA	3	6.6 ± 0.3
33	KHSO ₄ (0.33 M) + K ₂ SO ₄ (0.17 M)	UVA	3	5.5 ± 0.3
34	KHSO ₄ (0.25 M) + K ₂ SO ₄ (0.25 M)	UVA	3	4.4 ± 0.7
35	KHSO ₄ (0.17 M) + K ₂ SO ₄ (0.33 M)	UVA	3	3.0 ± 0.1
36	KHSO ₄ (0.09 M) + K ₂ SO ₄ (0.41 M)	UVA	3	1.4 ± 0.1
37	KHSO ₄ (0.46 M) + K ₂ SO ₄ (0.04 M)	UVA	3	0.8 ± 0.1
38	K ₂ SO ₄ (0.5 M)	UVA	3	0.5 ± 0.0
39	Fe(ClO ₄) ₃ (2 equiv)	UVA/-B/-C/CW	3	4.4 ± 0.3
40	FeCl ₂ (2 equiv)	UVA/-B/-C/CW	3	2.8 ± 0.1
41	FeCl ₃ (2 equiv)	UVA/-B/-C/CW	3	3.1 ± 0.1
42	FeS (2 equiv)	UVA/-B/-C/CW	3	1.1 ± 0.2
43	KHSO ₄ (3 M) ^c	UVA	6	0.0 ± 0.0
44	K ₂ S ₂ O ₈ (2 equiv) + FeS (0.5 equiv) ^c	UVA	6	0.0 ± 0.0
45	K ₂ S ₂ O ₈ (2 equiv) + FeS (0.5 equiv) ^c	70 °C	3	0.0 ± 0.0
46	KHSO ₄ (3 M) ^d	UVA	6	0.0 ± 0.0
47	K ₂ S ₂ O ₈ (2 equiv) + FeS (0.5 equiv) ^d	UVA	6	0.0 ± 0.0
48	K ₂ S ₂ O ₈ (2 equiv) + FeS (0.5 equiv) ^d	70 °C	3	0.0 ± 0.0

^a pyruvic acid was used instead of sodium pyruvate ^b 0.5 equiv disulfide **1d** instead of thiol **1b**.

^ccontrol experiment without sodium pyruvate ^dcontrol experiment without thiol **1b**

To show that the described method can also be applied to other thiols, the formation of the acetyl thioester **3c** starting from pyruvate and the thiol **1c** has been investigated.

Scheme S-3: Thioester formation with an alternative thiol 1c analyzed by GC-FID.

Additionally, the use of α -hydroxy acids instead of α -keto acids has been investigated.

Scheme S-4: Thioester formation using lactic acid analyzed by GC-FID.

Figure S-9: Time dependence of the thioester formation under optimized conditions

Table S-2: Stability and decomposition products of different keto acids using methods A–C

Ketoacid	Thio-cmpd.	Method	Remaining Ketoacid ^a	Decomposition ^a
pyruvate	1b	A	48% ^b	acetate (7%)
pyruvate	1b	B	48% ^b	acetate (10%)
pyruvate	1b	C	64% ^b	acetate (2%)
pyruvate	1c	A	63% ^b	acetate (4%)
pyruvate	1c	B	48% ^b	acetate (9%)
pyruvate	1c	C	63% ^b	acetate (4%)
pyruvate	1d	A	54% ^b	acetate (4%)
pyruvate	1d	B	56% ^b	acetate (13%)
pyruvate	1d	C	80% ^b	acetate (1%)
α -ketoglutarate	1b	A	66% ^b	succinate (5%)
α -ketoglutarate	1b	B	82% ^b	succinate (7%)
α -ketoglutarate	1b	C	57% ^b	succinate (1%)
oxaloacetate	1b	A	0%	pyruvate (52% ^b)
oxaloacetate	1b	B	0%	pyruvate (32% ^b)
oxaloacetate	1b	C	44% ^b	pyruvate (4% ^b)

^a determined by NMR with DSS as internal standard using water suppression ^b the shown value is the sum of the ketoacid and its hydrate form

3.4 Thioester formation from a non-enzymatic reaction network

A solution of sodium pyruvate **2** (110 mg, 1.0 mmol, 2.0 equiv), glyoxylic acid monohydrate (46 mg, 0.5 mmol, 1.0 equiv) and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (200 mg, 1.0 mmol, 2.0 equiv) in 3 mL of MilliQ water was prepared in a Pyrex pressure tube equipped with a stirring bar under a constant argon flow. For oxidation procedure A *N*-acetylcysteamine **1b** (59 mg, 0.5 mmol, 1.0 equiv) was added. The reaction vessel was closed and heated to 70 °C for 1 h or 3 h, followed by an oxidation procedure.

Oxidation procedure A:

To the solution prepared as described above, potassium persulfate (270 mg, 1.0 mmol, 1.0 equiv) was added under a constant argon flow. The reaction vessel was closed and heated to 70 °C for 3 hours. After cooling to room temperature, the amount of thioesters formed was determined by GC-FID analysis or LCMS analysis (see section 2.1 and 2.2), the different intermediates of the network were determined by GC-MS analysis (see section 2.3).

Oxidation procedure B: (in this case, *N*-acetylcysteamine was added at a later stage)

To the solution prepared as described above, KHSO_4 (1.2 g, 9.0 mmol, to form a ~3 M solution) and *N*-acetylcysteamine (59 mg, 0.50 mmol 1.0 equiv) were added, the mixture was transferred in a quartz tube under a constant argon flow. The tube was closed with a septum and irradiated in a Luzchem LZC-ORG photoreactor equipped with 10 LZC-UVA lamps for 3 hours. The amount of thioesters formed was determined by GC-FID analysis or LCMS analysis (see section 2.1 and 2.2), the different intermediates of the network were determined by GC-MS analysis (see section 2.3).

Table S-3: Thioester formation from a non-enzymatic reaction network

Entry	equiv pyruvate	addition 1 (equiv)	conditions 1	addition 2 (equiv)	conditions 2	c(3b) ^a [mM]	c(5b) ^b [mM]	c(9b) ^b [mM]
1	2		70 °C, 1 h	Thiol (1) KHSO ₄ (3 M)	UVA, 3 h	0.11 ± 0.01	0.19 ± 0.04	0.06 ± 0.00
2	2	Thiol (1)	70 °C, 1 h	K ₂ S ₂ O ₈ (2)	70 °C, 3 h	1.41 ± 0.11	0.11 ± 0.01	3.60 ± 0.30
3	2	Thiol (1)	70 °C, 3 h	K ₂ S ₂ O ₈ (2)	70 °C, 3 h	1.11 ± 0.14	0.11 ± 0.02	3.63 ± 0.28
4	1	Thiol (1) KHSO ₄ (3 M)	UVA, 3 h	-	-	0.06 ± 0.05	-	-
5	2	Thiol (1) KHSO ₄ (3 M)	UVA, 6 h	-	-	0.16 ± 0.02	-	-
6	1	Thiol (1) KHSO ₄ (3 M)	UVA, 3 h	-	-	0.11 ± 0.05	-	-
7	2	Thiol (1) KHSO ₄ (3 M)	UVA, 6 h	-	-	0.31 ± 0.05	-	-
8	2	Thiol (1)	70 °C, 1 h	KHSO ₄ (3M)	UVA, 3 h	0.12 ± 0.01	-	-
9	2	Thiol (1)	70 °C, 1 h	KHSO ₄ (3M)	UVA, 6 h	0.15 ± 0.01	-	-
10	2	Thiol (1)	UVA, 1 h	KHSO ₄ (3M)	UVA, 3 h	0.22 ± 0.02		

^a Determined by GC-FID analysis after extraction with ethyl acetate ^b Determined by LCMS-analysis. For the network experiments 0.5 μ L instead of 0.1 μ L were injected, the resulting value calculated using the calibration curves was divided by 5 afterwards. See section 4.10 for GCMS-chromatograms of the network products.

4. Experimental data

4.1. NMR spectra of the synthesized compounds

N,N'-(disulfanediyldis(ethane-2,1-diyl))diacetamide (**1d**)

2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (10)

S-(2-(2-(2-methoxyethoxy)ethoxy)ethyl) ethanethioate (3c)

2-(2-(2-methoxyethoxy)ethoxy)ethane-1-thiol (1c)

***N,S*-diacetyl cysteamine (3b)**

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4-[[2-(Acetylamino)ethyl]thio]-4-oxobutanoic acid (5b)

3-((2-acetamidoethyl)thio)-3-oxopropanoic acid (8b)

2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-yl)acetic acid (12)

S-(2-acetamidoethyl) 2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-yl)ethanethioate (13)

4-((2-acetamidoethyl)thio)-2-hydroxy-4-oxobutanoic acid (9b)

4.2. NMR spectra of reference compds. in H₂O/D₂O with DSS as IS with water suppression

N-acetyl cysteamine (1b) in H₂O/D₂O+DSS

2-(2-(2-methoxyethoxy)ethoxy)ethane-1-thiol (1c) in H₂O/D₂O+DSS

***N,N'*-(disulfanediy)bis(ethane-2,1-diyl)diacetamide (1d) in H₂O/D₂O+DSS**

***N,S*-diacetyl cysteamine (3b) in H₂O/D₂O+DSS**

S-(2-(2-(2-methoxyethoxy)ethoxy)ethyl) ethanethioate (3c) in H₂O/D₂O+DSS

4-[[2-(Acetylamino)ethyl]thio]-4-oxobutanoic acid (5b) in H₂O/D₂O+DSS

2,5,8,15,18,21-hexaoxa-11,12-dithiadocosane (11) in H₂O/D₂O+DSS

3-((2-acetamidoethyl)thio)-3-oxopropanoic acid (8b) in H₂O/D₂O+DSS

sodium pyruvate (1)

oxaloacetic acid (6)

α -ketoglutaric acid (4) in H₂O/D₂O+DSS

succinic acid in H₂O/D₂O+DSS

malonic acid in H₂O/D₂O+DSS

4.3. NMR spectra of selected reaction mixtures showing prebiotic thioester formations

Table 1, Entry 1, Method A

Table 1, Entry 1, Method B

Table 1, Entry 1, Method C

Table 1, Entry 2, Method A

Table 1, Entry 2, Method B

Table 1, Entry 2, Method C

Table 1, Entry 3, Method A

Table 1, Entry 3, Method B

Table 1, Entry 3, Method C

Table 1, Entry 4, Method A

Table 1, Entry 4, Method B

Table 1, Entry 4, Method C

Table 1, Entry 6, Method A

Table 1, Entry 6, Method B

Table 1, Entry 6, Method C

Scheme S-3 Method A

Scheme S-3 Method B

Scheme S-3 Method C

4.4. GC-FID chromatograms of authentic samples

N-acetylcysteine (1b)

2-(2-(2-methoxyethoxy)ethoxy)ethane-1-thiol (1c)

***N,N'*-(disulfanediy)bis(ethane-2,1-diyl)diacetamide (1d)**

***N,S*-diacetyl cysteamine (3b)**

S-(2-(2-(2-methoxyethoxy)ethoxy)ethyl) ethanethioate (3c)

2,5,8,15,18,21-hexaoxa-11,12-dithiadocosane (11)

4.5. GC-FID chromatograms of the prebiotic thioester formation

Table 1, Entry 1, Method A

Table 1, Entry 1, Method B

Table 1, Entry 1, Method C

Table 1, Entry 2, Method A

Table 1, Entry 2, Method B

Table 1, Entry 2, Method C

Table 1, Entry 6, Method A

Table 1, Entry 6, Method B

Table 1, Entry 6, Method C

Scheme S-4, Method A

Scheme S-4, Method B

Scheme S-4, Method C

Scheme S-3, Method A

Scheme S-3, Method B

Scheme S-3, Method C

4.6. GC-FID chromatograms of the network combined with thioester formation

Table S-3, Entry 1

Table S-3, Entry 2

Table S-3, Entry 3

4.7. LC-MS chromatograms of authentic samples

N-acetyl cysteamine (1b) $m/z = 119.95\text{--}120.15$

***N,N'*-(disulfanediy)bis(ethane-2,1-diyl)diacetamide (1d) $m/z = 236.97\text{--}237.17$**

***N,S*-diacetyl cysteamine (3b) $m/z = 161.95\text{--}162.15$**

JG220-4 #179 RT: 1.69 AV: 1 SB: 1003 0.00-1.50, 2.02-10.02 NL: 5.44E7
T: FTMS + p ESI Full ms [70.0000-700.0000]

4-[[2-(Acetylamino)ethyl]thio]-4-oxobutanoic acid (5b) $m/z = 219.95\text{--}220.15$

3-((2-acetamidoethyl)thio)-3-oxopropanoic acid (8b) $m/z = 205.54\text{--}206.54$

CL030-Calib-20mM-1 #89 RT: 0.84 AV: 1 SB: 1037 0.99-10.01, 0.00-0.80 NL: 1.01E8
T: FTMS +p ESI Full ms [70.0000-700.0000]

4-((2-acetamidoethyl)thio)-2-hydroxy-4-oxobutanoic acid (9b) $m/z = 236.04\text{--}236.06$

JG233-18 #96 RT: 0.91 AV: 1 SB: 504 1.24-5.25, 0.00-0.80 NL: 8.56E7
T: FTMS +p ESI Full ms [70.0000-700.0000]

4.8. LC-MS chromatograms of thioester formation under prebiotic conditions

Table 1, Entry 3, Method A

Table 1, Entry 3, Method B

Table 1, Entry 3, Method C

RT: 0.00 - 10.01

NL:
2.67E7
m/z=
219.95-
220.15
MS
ECB494-2-
B1

Table 1, Entry 4, Method A

Table 1, Entry 4, Method B

RT: 0.00 - 10.02

NL:
4.59E7
m/z=
205.54-
206.54
MS
JG218-5

RT: 0.00 - 10.02

NL:
6.79E6
m/z=
161.95-
162.15
MS
JG218-5

Table 1, Entry 4, Method C

Table 1, Entry 5, Method A

Table 1, Entry 5, Method B

Table 1, Entry 5, Method C

4.9. LC-MS chromatograms of the network combined with thioester formation

Table S-3, Entry 1

RT: 0.00 - 10.01

NL:
5.06E6
m/z=
219.95-
220.15
MS
ECB-508-1

RT: 0.00 - 10.01

NL:
5.24E5
m/z=
236.04-
236.06
MS
ECB-508-1

Table S-3, Entry 2

RT: 0.00 - 10.02

NL:
1.48E7
m/z=
219.95-
220.15
MS
ECB-509-
A1

sample	retention time 5b (min)	peak area
1	2.31	2.40E+07
2	2.32	2.19E+07
3	2.31	2.13E+07

RT: 0.00 - 10.02

NL:
1.66E7
m/z=
236.04-
236.06
MS
ECB-509-
A1

sample	retention time 9b (min)	peak area
1	1.07	2.06E+08
2	1.05	1.78E+08
3	1.07	2.03E+08

Table S-3, Entry 3

RT: 0.00 - 10.02

NL:
8.40E6
m/z=
219.95-
220.15
MS
ECB-509-
B1

sample	retention time 5b (min)	peak area
1	2.32	1.86E+07
2	2.31	2.58E+07
3	2.32	2.32E+07

RT: 0.00 - 10.02

NL:
1.74E7
m/z=
236.04-
236.06
MS
ECB-509-
B1

sample	retention time 9b (min)	peak area
1	1.04	1.74E+08
2	1.06	2.09E+08
3	1.08	1.87E+08

4.10. GC-MS chromatograms of authentic samples

N-acetylcysteine (1b)

N-acetylcysteamine (1b) after derivatization

4.11. GC-MS chromatograms of the network after thioester formation

Table S-3, Entry 1

Table S-3, Entry 2

Table S-3, Entry 3

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