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## COMMUNICATION

# Simultaneous synthesis of thioesters and iron-sulfur clusters in water: two universal components of energy metabolism

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**Thioesters are important intermediates in both synthetic organic, and biosynthetic reaction pathways. Here we show that thioesters can be synthesized in an aqueous reaction between thioacetic and thiols. The reaction can be coupled to a second reaction between sulfide and either ferrous or ferric iron, which drives the reaction forward. We furthermore demonstrate that sulfide released during thioester formation can be used in the synthesis of peptide bound [Fe-S] clusters, which like thioesters, are ancient components of metabolism. Together our results reveal a primordial linkage between high-energy ester formation and redox chemistry.**

A key question of the emergence of life is to understand how prebiotically available molecules could have been assembled into self-replicating systems. Biology today replicates by coupling otherwise energetically unfavorable, endergonic reactions to energy yielding reactions, but how such an energy coupling scheme may have worked in the earliest life remains unclear. A large component of the energetic cost of cell replication is derived from polymerization reactions, where monomers must be activated so that dehydration can proceed in water.<sup>1,2</sup> These reactions are typically powered by the hydrolysis of phosphoesters such as adenosine triphosphate (ATP), which today are sustainably regenerated in the cell mainly through electron transfer processes in metabolism.<sup>3</sup> Thioesters are a frequently invoked alternative to ATP in abiotic processes: (i) Both have similar standard free energies of hydrolysis, making them exchangeable from an energetic perspective.<sup>4</sup> (ii) Since thioester formation often precedes phosphoester formation in metabolism, for example in glycolysis<sup>5</sup> and in the Wood–Ljungdahl pathway,<sup>6</sup> it is tempting to consider them as primordial, having operated before phosphoesters in metabolisms.<sup>7</sup> (iii) Recent computational studies employing network

extension algorithms have indicated the possibility of a phosphate-free core metabolic network, where coupling of endergonic reactions to thioesters is sufficient for driving components of the reductive-TCA cycle, amino acid biosynthesis, and the hydroxypropionate bi-cycle.<sup>8</sup> An early thioester enabled metabolism may have been privileged compared to those using phosphoesters, due to solubility issues of phosphates in the ferruginous Archean ocean.<sup>9</sup> Several prebiotically relevant synthetic routes for thioesters have been reported, for example the synthesis of iminothioesters from malononitrile, which can subsequently hydrolyze producing a thioester,<sup>10</sup> thioester synthesis from acetaldehyde and a thiol via UV-irradiation<sup>4</sup>, through the exergonic dehydration of glyceraldehyde<sup>11</sup>, with carbonyldiimidazole as a condensing agent<sup>12</sup>, and also from CO under hydrothermal conditions<sup>13</sup>. Thioacids are possible precursors to thioesters, and can be synthesized by thiolysis of nitriles in water, with subsequent hydrolysis of the produced thioamide,<sup>14</sup> or produced by the reaction of primary amines with carbonyl sulfide.<sup>15</sup> Thioacetic acid (TAA) in particular has been found to be an efficient acetylating agent for amino acids producing peptide bonds in the presence of an oxidant.<sup>14,16</sup> In addition to these synthetic reactions, thioacetate would also have likely had important roles in energy transfer reactions in a prebiotic metabolism: if thioesters could be produced from thioacetate in non-peptide forming reactions, they might be able to form a link between anabolic peptide forming reactions and catabolic energy harvesting reactions. However, information on direct thioester formation from thioacetate in aqueous solution is lacking.

In this work, thioacetate was investigated as a thiol-acylating agent, and the reaction yields and rates of thioacetate with two different thiols were surveyed at different pH. Promotion of the reaction by sequestering sulfide in iron sulfide was investigated, as was the utilization of liberated sulfide in the formation of peptide bound iron sulfur clusters. Thioester formation from thioacetate and a thiol could potentially occur with or without ferrous iron, and in an oxidative process in the presence of ferric iron (Scheme 1).<sup>16,17</sup> Given that the free energy of hydrolysis of thioacetic acid has been calculated to be -15.2 kJ mol<sup>-1</sup> whereas thioester hydrolysis is generally more negative ( $\Delta G^{\circ}_{\text{Hyd}} = -35.3$  kJ mol<sup>-1</sup> for methylthioacetate),<sup>18</sup> we first considered if thiol-acetylation via thioacetic

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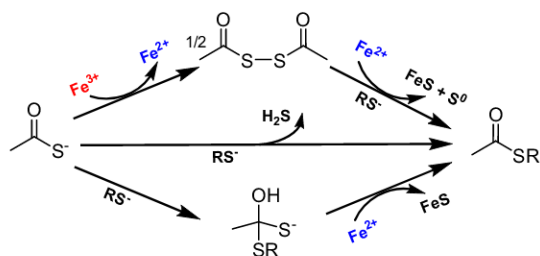
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Electronic supplementary information (ESI) available: Experimental details, and Fig. S1–S12 mentioned in the text.

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acid could be enhanced by coupling to a redox reaction with  $\text{Fe}^{3+}$  or with non-redox formation of iron(II) sulfide ( $\Delta G^0 = -93.6 \text{ kJ mol}^{-1}$ ),<sup>19</sup> with sulfide originating from TAA as shown in Scheme 1. In such a way, low energy TAA could be converted into a high energy thioester, representing a possible route of prebiotic energy conservation between organic chemistry and mineral formation.



Scheme 1 Overview of the proposed reaction mechanisms of thioester synthesis from thioacetate in iron independent and iron dependent reactions. On the top, oxidation of thioacetate to diacetyl disulfide by  $\text{Fe}^{3+}$  precedes thioester formation, and is followed by formation of the thioester along with  $\text{FeS}$  and  $\text{S}^0$  with hydrogen disulfide as the leaving group.<sup>20</sup> In the middle, the non-iron dependent reaction involves degassing of  $\text{H}_2\text{S}$ , and on the bottom, sulfide is sequestered through the formation of iron sulfide ( $\text{FeS}$ ) with  $\text{Fe}^{2+}$ .

The reaction between TAA and a model thiol compound of mercaptoethanesulfonate (also known as Coenzyme M, CoM) at  $70^\circ\text{C}$  follows initially pseudo-zeroth order rate kinetics (Fig. 1). The addition of 5 mM  $\text{Fe}^{2+}$  does not alter the initial reaction rate significantly, but ferric iron (5 mM) increases the reaction rate by a factor of 3 at pH 5.5, and shows a 1.6-times faster reaction at pH 7.5. During separate experiments which ran for several days (Fig. S11, ESI), reactions with the addition of  $\text{Fe}^{3+}$  at pH 5.5 exhibited maximum thioester yields of 39% at 1.7 days, compared to 35% yield at 4.8 days with the addition of  $\text{Fe}^{2+}$  (the percent yields are given with respect to the starting thiol concentration, and are averages from duplicate experiments measured by UPLC, with ranges in % yield between duplicates given in Table S2, ESI). With no metal addition, the maximum yield observed was 29%. At pH 7.5, the highest thioester yields were 19% in the presence of 5 mM  $\text{Fe}^{3+}$  at 0.9 days, 13% with  $\text{Fe}^{2+}$  at 1.7 days, and 9% at 0.9 days with no added metal.

Triethylamine has been employed previously during the acetylation of alcohols and is expected to act as an auxiliary base.<sup>21</sup> With an altered experimental setup using lower concentrations of starting materials (Fig. 1c), we found that the addition of triethylamine accelerated the formation of thioesters with and without the addition of  $\text{Fe}^{2+}/\text{Fe}^{3+}$  (Fig. 1c and d).

Using a tertiary amine containing thiol (2-diethylamino)ethanethiol;  $\text{Et}_3\text{NS}$ ) as the starting compound, a drastic increase in reaction rate was observed (Fig. 2), and the reaction characteristics varied between the two pH conditions investigated. The highest yields were observed at pH 5.5, the highest concentration of acetylated  $\text{Et}_3\text{NS}$  ( $\text{AcEt}_3\text{NS}$ ) was measured after 1h reaction time (within the time frame of the experiment and analysis via UPLC) at both pH values in the case of  $\text{FeCl}_3$  or no metal addition. At pH 5.5, the addition of  $\text{Fe}^{2+}$  changed the production curve markedly, and in the case of ferric iron or no addition, the hydrolysis of the thioester followed pseudo zeroth order kinetics within the first 10h. The hydrolysis of the thioester methyl-thioacetate was previously determined to follow a pseudo first order<sup>18,22</sup> but for  $\text{AcEt}_3\text{NS}$  only the times series obtained

with  $\text{FeCl}_3$  at pH 7.5 showed a similar trend (Fig. 2b).

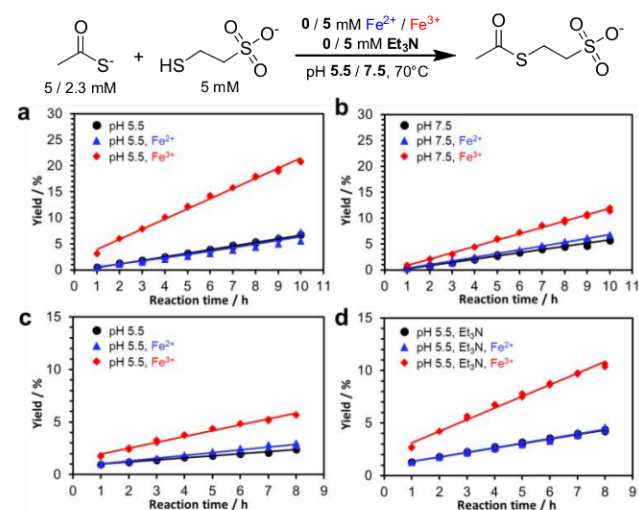


Figure 1 Time course measurement of the synthesized CoM thioester (AcCoM) from 5 mM thioacetate at  $70^\circ\text{C}$  expressed in percent yield in respect to 5 mM CoM as starting material (Panel a and b). Each time point for the respective experiment shows data from experimental duplicates and a regression with the least square fit. The red trace shows the reactions in which  $\text{FeCl}_3$  was added, blue with  $\text{FeCl}_2$  and black with no iron. Panel c and d had an initial concentration of 2.3 mM thioacetic acid and 5 mM of thiol and  $\text{Fe}^{2+}/\text{Fe}^{3+}$ . Panel d contained 5mM triethylamine in addition to the components in c. The y-axis scale is different for panels c and d where lower concentrations were used compared to a and b. Additional reaction rate measurements at lower TAA concentrations are given in Fig. S12, ESI.

In aqueous solution, thioester synthesis competes with the hydrolysis of both the starting compound TAA and the product thioester. An investigation of the hydrolysis rates of methyl-thioacetate at different pH found the highest stability of the thioester at pH 4,<sup>22</sup> which would favor a more acidic pH for a higher yield of acetylated CoM and  $\text{Et}_3\text{NS}$  ( $\text{AcCoM}$  and  $\text{AcEt}_3\text{NS}$ ). In line with this, only traces of the expected thioester could be found in a 100 mM carbonate buffer at pH 10, suggesting rapid hydrolysis of any  $\text{AcCoM}$  produced.

At longer time periods, the concentration of  $\text{AcCoM}$  decreased only marginally at pH 5.5 over the course of five days compared to pH 7.5 where up to half of the thioester produced hydrolyzed (Fig. S11, ESI). At the lower pH, the reaction equilibrium is expected to shift towards thioester formation, due to the continuous removal of  $\text{H}_2\text{S}$  to the vapor phase ( $\text{pK}_a = 7$ ). This – in addition to differences in stability against hydrolysis – could account for the highest yields obtained for  $\text{AcEt}_3\text{NS}$ : in the absence of  $\text{Fe}^{2+}/\text{Fe}^{3+}$ , a threefold higher  $\text{AcEt}_3\text{NS}$  concentration was measured at pH 5.5 (21% yield) than at pH 7.5 (6%) within the first hour of the experiment (Fig. 2a). For  $\text{AcCoM}$ , the highest yields are found at longer time intervals, and the highest average thioester concentration was also associated with the lower pH either with or without the addition of iron (Fig. S11, ESI). Importantly, heating a pH 5.5 acetate buffer with  $\text{Et}_3\text{NS}$  at  $70^\circ\text{C}$  did not yield any detectable thioester, indicating that acetate in solution did not lead to acetylation of the thiol.

Other than sulfide removal by degassing, the formation of  $\text{FeS}$  by reaction with ferrous iron provides another way to sequester sulfide and shift the reaction equilibrium towards the formation of thioesters. Using  $\text{Et}_3\text{NS}$ , a black iron sulfide precipitate is formed

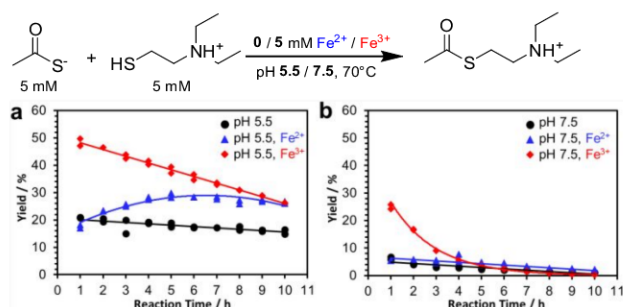
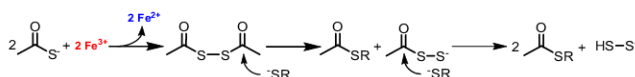


Figure 2 Time course measurement of the production of the AcEt<sub>3</sub>NS thioester at 70°C is displayed as % yield in respect to the initial amount of Et<sub>3</sub>NS at pH 5.5 (a) and pH 7.5 (b) with 5 mM Fe<sup>2+</sup> (blue), Fe<sup>3+</sup> (red) or no metal added (black). Duplicates were performed for each reaction and are shown at the time point.

within a few hours of reaction at 70°C when either Fe<sup>2+</sup> or Fe<sup>3+</sup> were in the reaction. At pH 5.5 and with the addition of FeCl<sub>2</sub>, the highest yields of thioester increased by 9% and 5% for Et<sub>3</sub>NS and AcCoM respectively (Fig. 2b, Fig. S11, ESI). At pH 7.5, a 4% yield increase of AcCoM was observed with the addition of FeCl<sub>2</sub>, which at these lower yields with respect to the starting thiol corresponds to a 1.4-fold concentration increase in respect to the no metal addition (Fig. S11, ESI). However, at pH 7.5 thioester formation was insignificantly increased by FeCl<sub>2</sub> using the Et<sub>3</sub>NS thiol. In the presence of ferric iron, the highest yield of AcEt<sub>3</sub>NS of 48% was obtained after 1h reaction at pH 5.5. Indeed, Fe<sup>3+</sup> was associated with the maximum yields observed in all of the experiments (Fig. 1, Fig. 2, S11, S12). The enhancement of both reaction rate and yield with Fe<sup>3+</sup> suggests that the oxidation of thioacetate is involved as previously reported in the case of amines and peptide bond formation<sup>16,23</sup> and depicted in Scheme 2 in the case of thiols.



Scheme 2 Proposed reactions scheme for oxidation of thioacetate by Fe<sup>3+</sup> to diacetyldithiol and subsequent thioester formation. The leaving hydrogen persulfide likely reacts with Fe<sup>2+</sup> produced in the reaction to form FeS and elemental sulfur as reported earlier.<sup>20</sup>

The energetic efficiency of thioester formation can be assessed by considering the reaction thermodynamics: Acetic anhydride, a commonly used compound for acetylation in organic synthesis,<sup>24</sup> has a standard free energy of hydrolysis of -65.9 kJ mol<sup>-1</sup>,<sup>25</sup> whereas thioacetate amounts to only -15.2 kJ mol<sup>-1</sup>.<sup>18</sup> In the case of thioester formation from iminothioesters<sup>10</sup> and nitriles, the standard free energy of hydrolysis of acetonitrile and hydrogen cyanide to the corresponding amide is calculated to be -27.0 kJ mol<sup>-1</sup> and -69.3 kJ mol<sup>-1</sup> respectively.<sup>26</sup> With fair yields of thioesters obtained at mild conditions in water, thioacetate thus presents an energy efficient route for the acetylation of thiols. As the toxic sulfide produced during the acetylation by thioacetate can be sequestered away as FeS, this reaction scheme might be applicable to green chemistry due to its energy efficiency and the usage of water as the sole solvent. Peptide bound [Fe-S] clusters have prominent and diverse roles in biological metabolism<sup>27,28</sup> and likely emerged early in metabolism bound by short peptides.<sup>29,30</sup> Understanding how [Fe-S] clusters may

form in plausible prebiotic conditions requires research into the availability of thiols, ferric iron, and sulfide.<sup>30</sup> Since thioester formation from thioacetate involves the release of sulfide, we investigated peptide bound [Fe-S] cluster synthesis during thioester forming reactions.

Using a previously characterized peptide as a model [4Fe-4S] cluster binding sequence,<sup>31</sup> a reaction with Et<sub>3</sub>NS, thioacetate, and FeCl<sub>3</sub> at 70°C produced an absorbance spectrum characteristic of [Fe-S] clusters (Fig. 3, Fig. S10). Absorbance features at ~384nm, and 447nm, which are characteristic of the ligand to metal charge transfer bands in iron-sulfur clusters,<sup>32</sup> bleached with the addition of dithionite, demonstrating redox activity (Fig. S10, ESI). Water soluble thiols such as 2-mercaptoethanol can serve as ligands for [4Fe4S] clusters produced from FeCl<sub>3</sub> and Na<sub>2</sub>S,<sup>33</sup> but while working on a similar timescale as with the peptide, no appreciable amount of [FeS] clusters were self-assembled from reactions that lacked the peptide (Fig. 3). Instead, in the absence of the peptide an increase in absorbance across the spectrum after 6.5h was observed, likely resulting from FeS nanoparticle derived turbidity<sup>34</sup> followed by precipitation by 15h (Fig. S10, ESI).

After 6.5h, the measured yield of AcEt<sub>3</sub>NS was 1.2% compared to 2.3% under the same reaction conditions but without the addition of the peptide. Thioacetate can thus serve as a source of both thioesters and sulfide in [Fe-S] cluster formation. Besides the prebiotic implications of this, molecular release of sulfide from thioacetate may be of use in laboratory chemical reconstitution schemes, where sulfide is usually added slowly in a dropwise manner.<sup>35</sup>

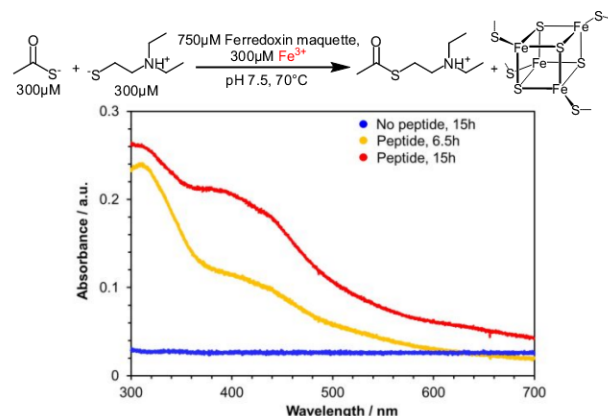


Figure 3 UV-Vis spectrum of the reaction of 300 μM Et<sub>3</sub>NS, thioacetate and FeCl<sub>3</sub> in the presence of 750 μM Ferredoxin peptide maquette at 70°C after 6.5h (yellow), after 15h (red) and without the maquette after 15h (blue).

Iron sulfur derivatives have featured prominently in origins of life research<sup>36,37</sup> and both synthetic [Fe-S] clusters and iron sulfide minerals have been shown experimentally to facilitate CO<sub>2</sub> reduction. Reductive carboxylations and amino acid synthesis have been performed with synthetic [Fe-S] clusters<sup>38</sup> and pyruvate has been synthesized from CO<sub>2</sub> on a greigite (Fe<sub>3</sub>S<sub>4</sub>) working electrode<sup>39</sup> and in reactions involving metallic iron and also iron minerals and hydrogen.<sup>40,41</sup> With the availability of pyruvate for prebiotic chemistry assured, a synergistic process between thioesters and Fe-S clusters can be conceived regarding the work presented here. The enzyme pyruvate ferredoxin-oxidoreductase (PFOR) catalyzes the synthesis of the thioester acetyl-coenzyme A (CoA) from pyruvate via oxidative decarboxylation and uses [4Fe-4S] clusters as electron



mediators.<sup>6</sup> If the acetylation of a thiol by thioacetate and the concomitant formation of [4Fe-4S] clusters were to be connected to the reconstitution of a proto-PFOR, a catalytic feedback could be envisioned, in which continued thioester formation could now be synthesized from both thioacetate and prebiotically available pyruvate. Thus, thioacetate and abiotically produced pyruvate could work synergistically to produce high energy thioesters through two different reactions in an early metabolism.

PFOR, [Fe-S] clusters, and thioesters adopt a central position in the metabolism of bacteria and archaea, connecting the Wood-Ljungdahl (WL) CO<sub>2</sub> fixation pathway with the tricarboxylic acid cycle.<sup>42</sup> The WL pathway itself is thought to be the most ancient autotrophic pathway,<sup>43</sup> and in concert with a complete rTCA cycle as is found in *Thermovibrio Ammonificans*,<sup>44</sup> a hybrid WL-rTCA pathway may have been a robust primordial metabolism due to redundancy in the formation of thioesters as a central metabolite.<sup>45</sup>

The formation of both thioesters and soluble [FeS] clusters from thioacetate thus presents an intriguing prebiotic entry point into both the WL Pathway and the rTCA cycle. Thioester formation with TAA as the acetylating agent and Fe<sup>3+</sup> as an oxidant is reminiscent of oxidative thioesterification reactions in metabolism, such as that in glycolysis and in the decarboxylation of alpha-keto acids. The biological finding of an ancient thioester driven core metabolism<sup>8</sup> enriched in coenzymes that utilize [Fe-S] clusters as catalytic centers, points towards thioacetate being a possible substrate for a metabolic network that also generates both, the necessary catalysts and redox mediators.

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## Conflicts of interest

There are no conflicts to declare.

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**Thioesters are important intermediates in both synthetic organic, and biosynthetic reaction pathways. Here we show that thioesters can be synthesized in an aqueous reaction between thioacetic and thiols. The reaction can be coupled to a second reaction between sulfide and either ferrous or ferric iron, which drives the reaction forward. We furthermore demonstrate that sulfide released during thioester formation can be used in the synthesis of peptide bound [Fe-S] clusters, which like thioesters, are ancient components of metabolism. Together our results reveal a primordial linkage between high-energy ester formation and redox chemistry.**

A key question of the emergence of life is to understand how prebiotically available molecules could have been assembled into self-replicating systems. Biology today replicates by coupling otherwise energetically unfavorable, endergonic reactions to energy yielding reactions, but how such an energy coupling scheme may have worked in the earliest life remains unclear. A large component of the energetic cost of cell replication is derived from polymerization reactions, where monomers must be activated so that dehydration can proceed in water.<sup>1,2</sup> These reactions are typically powered by the hydrolysis of phosphoesters such as adenosine triphosphate (ATP), which today are sustainably regenerated in the cell mainly through electron transfer processes in metabolism.<sup>3</sup> Thioesters are a frequently invoked alternative to ATP in abiotic processes: (i) Both have similar standard free energies of hydrolysis, making them exchangeable from an energetic perspective.<sup>4</sup> (ii) Since thioester formation often precedes phosphoester formation in metabolism, for example in glycolysis<sup>5</sup> and in the Wood–Ljungdahl pathway,<sup>6</sup> it is tempting to consider them as primordial, having operated before phosphoesters in metabolisms.<sup>7</sup> (iii) Recent computational studies employing network

extension algorithms have indicated the possibility of a phosphate-free core metabolic network, where coupling of endergonic reactions to thioesters is sufficient for driving components of the **reductive-TCA cycle**, amino acid biosynthesis, and the hydroxypropionate bi-cycle.<sup>8</sup> An early thioester enabled metabolism may **have been privileged compared** to those using phosphoesters, due to solubility **issues of phosphates in the ferruginous** Archean ocean.<sup>9</sup>

Several prebiotically relevant synthetic routes for thioesters have been reported, for example the synthesis of iminothioesters from malononitrile, which can subsequently hydrolyze producing a thioester,<sup>10</sup> thioester synthesis from acetaldehyde and a thiol via UV-irradiation<sup>4</sup>, through the exergonic dehydration of glyceraldehyde<sup>11</sup> with carbonyldiimidazole as a condensing agent<sup>12</sup>, **and also from CO under hydrothermal conditions**<sup>13</sup>. Thioacids are possible precursors to thioesters, and can be synthesized by thiolysis of nitriles in water, with subsequent hydrolysis of the produced thioamide,<sup>14</sup> or produced by the reaction of primary amines with carbonyl sulfide.<sup>15</sup> Thioacetic acid (TAA) in particular has been found to be an efficient acetylating agent for amino acids producing peptide bonds in the presence of an oxidant.<sup>14,16</sup> In addition to these synthetic reactions, thioacetate would also have likely had important roles in energy transfer reactions in a prebiotic metabolism: if thioesters could be produced from thioacetate in non-peptide forming reactions, they might be able to form a link between anabolic peptide forming reactions and catabolic energy harvesting reactions. However, information on direct thioester formation from thioacetate in aqueous solution is lacking.

In this work, thioacetate was investigated as a thiol-acylating agent, and the reaction yields and rates of thioacetate with two different thiols were surveyed at different pH. Promotion of the reaction by sequestering sulfide in iron sulfide was investigated, as was the utilization of liberated sulfide in the formation of peptide bound iron sulfur clusters. Thioester formation from thioacetate and a thiol could potentially occur with or without ferrous iron, and in an oxidative process in the presence of ferric iron (Scheme 1).<sup>16,17</sup> Given that the free energy of hydrolysis of thioacetic acid has been calculated to be -15.2 kJ mol<sup>-1</sup> whereas thioester hydrolysis is generally more negative ( $\Delta G^{\circ}_{\text{Hyd}} = -35.3$  kJ mol<sup>-1</sup> for methylthioacetate),<sup>18</sup> we first considered if thiol-acetylation via thioacetic

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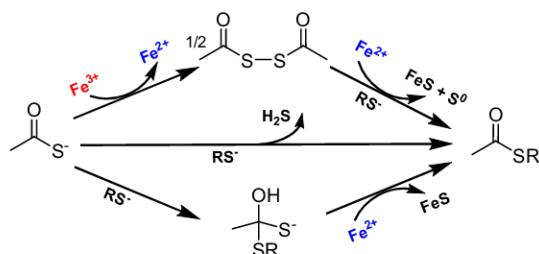
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Electronic supplementary information (ESI) available: Experimental details, and Fig. S1–S12 mentioned in the text.

acid could be enhanced by coupling to a redox reaction with  $\text{Fe}^{3+}$  or with non-redox formation of iron(II) sulfide ( $\Delta G^0 = -93.6 \text{ kJ mol}^{-1}$ ),<sup>19</sup> with sulfide originating from TAA as shown in Scheme 1. In such a way, low energy TAA could be converted into a high energy thioester, representing a possible route of prebiotic energy conservation between organic chemistry and mineral formation.



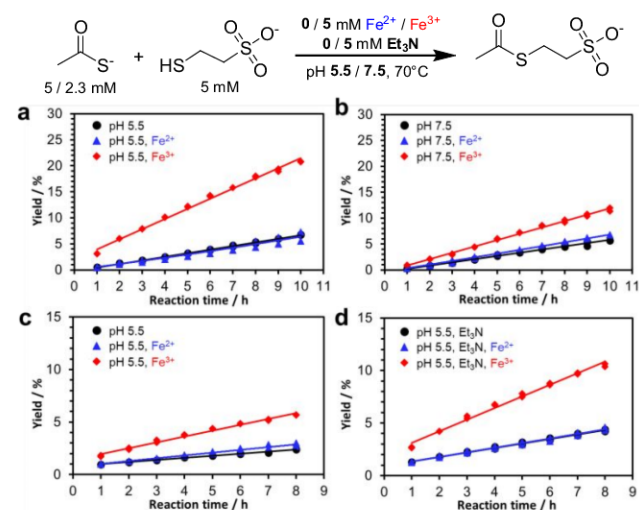
**Scheme 1** Overview of the proposed reaction mechanisms of thioester synthesis from thioacetate in iron independent and iron dependent reactions. On the top, oxidation of thioacetate to diacetyl disulfide by  $\text{Fe}^{3+}$  precedes thioester formation, and is followed by formation of the thioester along with  $\text{FeS}$  and  $\text{S}^0$  with hydrogen disulfide as the leaving group.<sup>20</sup> In the middle, the non-iron dependent reaction involves degassing of  $\text{H}_2\text{S}$ , and on the bottom, sulfide is sequestered through the formation of iron sulfide ( $\text{FeS}$ ) with  $\text{Fe}^{2+}$ .

The reaction between TAA and a model thiol compound of mercaptoethanesulfonate (also known as Coenzyme M, CoM) at  $70^\circ\text{C}$  follows initially pseudo-zeroth order rate kinetics (Fig. 1). The addition of 5 mM  $\text{Fe}^{2+}$  does not alter the initial reaction rate significantly, but ferric iron (5 mM) increases the reaction rate by a factor of 3 at pH 5.5, and shows a 1.6-times faster reaction at pH 7.5. During separate experiments which ran for several days (Fig. S11, ESI), reactions with the addition of  $\text{Fe}^{3+}$  at pH 5.5 exhibited maximum thioester yields of 39% at 1.7 days, compared to 35% yield at 4.8 days with the addition of  $\text{Fe}^{2+}$  (the percent yields are given with respect to the starting thiol concentration, and are averages from duplicate experiments measured by UPLC, with ranges in % yield between duplicates given in Table S2, SI). With no metal addition, the maximum yield observed was 29%. At pH 7.5, the highest thioester yields were 19% in the presence of 5 mM  $\text{Fe}^{3+}$  at 0.9 days, 13% with  $\text{Fe}^{2+}$  at 1.7 days, and 9% at 0.9 days with no added metal.

Triethylamine has been employed previously during the acetylation of alcohols and is expected to act as an auxiliary base.<sup>21</sup> With an altered experimental setup using lower concentrations of starting materials (Fig. 1c), we found that the addition of triethylamine accelerated the formation of thioesters with and without the addition of  $\text{Fe}^{2+}/\text{Fe}^{3+}$  (Fig. 1c and d).

Using a tertiary amine containing thiol (2-diethylamino)ethanethiol;  $\text{Et}_3\text{NS}$ ) as the starting compound, a drastic increase in reaction rate was observed (Fig. 2), and the reaction characteristics varied between the two pH conditions investigated. The highest yields were observed at pH 5.5, the highest concentration of acetylated  $\text{Et}_3\text{NS}$  ( $\text{AcEt}_3\text{NS}$ ) was measured after 1h reaction time (within the time frame of the experiment and analysis via UPLC) at both pH values in the case of  $\text{FeCl}_3$  or no metal addition. At pH 5.5, the addition of  $\text{Fe}^{2+}$  changed the production curve markedly, and in the case of ferric iron or no addition, the hydrolysis of the thioester followed pseudo zeroth order kinetics within the first 10h. The hydrolysis of the thioester methyl-thioacetate was previously determined to follow a pseudo first order<sup>18,22</sup> but for  $\text{AcEt}_3\text{NS}$  only the times series obtained

with  $\text{FeCl}_3$  at pH 7.5 showed a similar trend (Fig. 2b).



**Fig. 1** Time course measurement of the synthesized CoM thioester (AcCoM) from 5 mM thioacetate at  $70^\circ\text{C}$  expressed in percent yield in respect to 5 mM CoM as starting material (Panel a and b). Each time point for the respective experiment shows data from experimental duplicates and a regression with the least square fit. The red trace shows the reactions in which  $\text{FeCl}_3$  was added, blue with  $\text{FeCl}_2$  and black with no iron. Panel c and d had an initial concentration of 2.3 mM thioacetic acid and 5 mM of thiol and  $\text{Fe}^{2+}/\text{Fe}^{3+}$ . Panel d contained 5mM triethylamine in addition to the components in c. The y-axis scale is different for panels c and d where lower concentrations were used compared to a and b. Additional reaction rate measurements at lower TAA concentrations are given in Fig. S12, SI.

In aqueous solution, thioester synthesis competes with the hydrolysis of both the starting compound TAA and the product thioester. An investigation of the hydrolysis rates of methyl-thioacetate at different pH found the highest stability of the thioester at pH 4,<sup>22</sup> which would favor a more acidic pH for a higher yield of acetylated CoM and  $\text{Et}_3\text{NS}$  ( $\text{AcCoM}$  and  $\text{AcEt}_3\text{NS}$ ). In line with this, only traces of the expected thioester could be found in a 100 mM carbonate buffer at pH 10, suggesting rapid hydrolysis of any  $\text{AcCoM}$  produced.

At longer time periods, the concentration of  $\text{AcCoM}$  decreased only marginally at pH 5.5 over the course of five days compared to pH 7.5 where up to half of the thioester produced hydrolyzed (Fig. S11, ESI). At the lower pH, the reaction equilibrium is expected to shift towards thioester formation, due to the continuous removal of  $\text{H}_2\text{S}$  to the vapor phase ( $\text{pK}_a = 7$ ). This – in addition to differences in stability against hydrolysis – could account for the highest yields obtained for  $\text{AcEt}_3\text{NS}$ : in the absence of  $\text{Fe}^{2+}/\text{Fe}^{3+}$ , a threefold higher  $\text{AcEt}_3\text{NS}$  concentration was measured at pH 5.5 (21% yield) than at pH 7.5 (6%) within the first hour of the experiment (Fig. 2a). For  $\text{AcCoM}$ , the highest yields are found at longer time intervals, and the highest average thioester concentration was also associated with the lower pH either with or without the addition of iron (Fig. S11, ESI). Importantly, heating a pH 5.5 acetate buffer with  $\text{Et}_3\text{NS}$  at  $70^\circ\text{C}$  did not yield any detectable thioester, indicating that acetate in solution did not lead to acetylation of the thiol.

Other than sulfide removal by degassing, the formation of  $\text{FeS}$  by reaction with ferrous iron provides another way to sequester sulfide and shift the reaction equilibrium towards the formation of thioesters. Using  $\text{Et}_3\text{NS}$ , a black iron sulfide precipitate is formed

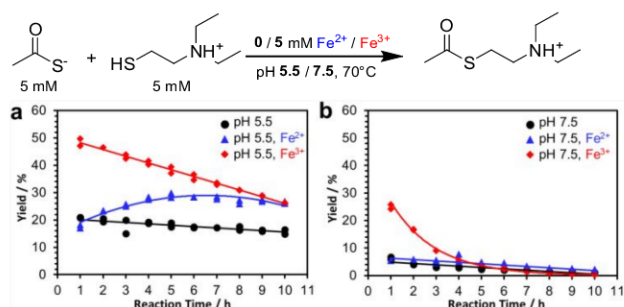
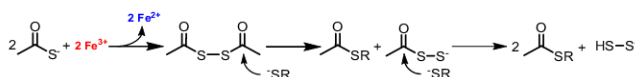


Figure 2 Time course measurement of the production of the AcEt<sub>3</sub>NS thioester at 70°C is displayed as % yield in respect to the initial amount of Et<sub>3</sub>NS at pH 5.5 (a) and pH 7.5 (b) with 5 mM Fe<sup>2+</sup> (blue), Fe<sup>3+</sup> (red) or no metal added (black). Duplicates were performed for each reaction and are shown at the time point.

within a few hours of reaction at 70°C when either Fe<sup>2+</sup> or Fe<sup>3+</sup> were in the reaction. At pH 5.5 and with the addition of FeCl<sub>2</sub>, the highest yields of thioester increased by 9% and 5% for Et<sub>3</sub>NS and AcCoM respectively (Fig. 2b, Fig. S11, ESI). At pH 7.5, a 4% yield increase of AcCoM was observed with the addition of FeCl<sub>2</sub>, which at these lower yields with respect to the starting thiol corresponds to a 1.4-fold concentration increase in respect to the no metal addition (Fig. S11, ESI). However, at pH 7.5 thioester formation was insignificantly increased by FeCl<sub>2</sub> using the Et<sub>3</sub>NS thiol. In the presence of ferric iron, the highest yield of AcEt<sub>3</sub>NS of 48% was obtained after 1h reaction at pH 5.5. Indeed, Fe<sup>3+</sup> was associated with the maximum yields observed in all of the experiments (Fig. 1, Fig. 2, S11, S12). The enhancement of both reaction rate and yield with Fe<sup>3+</sup> suggests that the oxidation of thioacetate is involved as previously reported in the case of amines and peptide bond formation<sup>16,23</sup> and depicted in Scheme 2 in the case of thiols.



Scheme 2 Proposed reactions scheme for oxidation of thioacetate by Fe<sup>3+</sup> to diacetyldithiol and subsequent thioester formation. The leaving hydrogen persulfide likely reacts with Fe<sup>2+</sup> produced in the reaction to form FeS and elemental sulfur as reported earlier.<sup>20</sup>

The energetic efficiency of thioester formation can be assessed by considering the reaction thermodynamics: Acetic anhydride, a commonly used compound for acetylation in organic synthesis,<sup>24</sup> has a standard free energy of hydrolysis of -65.9 kJ mol<sup>-1</sup>,<sup>25</sup> whereas thioacetate amounts to only -15.2 kJ mol<sup>-1</sup>.<sup>18</sup> In the case of thioester formation from iminothioesters<sup>10</sup> and nitriles, the standard free energy of hydrolysis of acetonitrile and hydrogen cyanide to the corresponding amide is calculated to be -27.0 kJ mol<sup>-1</sup> and -69.3 kJ mol<sup>-1</sup> respectively.<sup>26</sup> With fair yields of thioesters obtained at mild conditions in water, thioacetate thus presents an energy efficient route for the acetylation of thiols. As the toxic sulfide produced during the acetylation by thioacetate can be sequestered away as FeS, this reaction scheme might be applicable to green chemistry due to its energy efficiency and the usage of water as the sole solvent. Peptide bound [Fe-S] clusters have prominent and diverse roles in biological metabolism<sup>27,28</sup> and likely emerged early in metabolism bound by short peptides.<sup>29,30</sup> Understanding how [Fe-S] clusters may

form in plausible prebiotic conditions requires research into the availability of thiols, ferric iron, and sulfide.<sup>30</sup> Since thioester formation from thioacetate involves the release of sulfide, we investigated peptide bound [Fe-S] cluster synthesis during thioester forming reactions.

Using a previously characterized peptide as a model [4Fe-4S] cluster binding sequence,<sup>31</sup> a reaction with Et<sub>3</sub>NS, thioacetate, and FeCl<sub>3</sub> at 70°C produced an absorbance spectrum characteristic of [Fe-S] clusters (Fig. 3, Fig. S10). Absorbance features at ~384nm, and 447nm, which are characteristic of the ligand to metal charge transfer bands in iron-sulfur clusters,<sup>32</sup> bleached with the addition of dithionite, demonstrating redox activity (Fig. S10, ESI). Water soluble thiols such as 2-mercaptoethanol can serve as ligands for [4Fe4S] clusters produced from FeCl<sub>3</sub> and Na<sub>2</sub>S,<sup>33</sup> but while working on a similar timescale as with the peptide, no appreciable amount of [FeS] clusters were self-assembled from reactions that lacked the peptide (Fig. 3). Instead, in the absence of the peptide an increase in absorbance across the spectrum after 6.5h was observed, likely resulting from FeS nanoparticle derived turbidity<sup>34</sup> followed by precipitation by 15h (Fig. S10, ESI).

After 6.5h, the measured yield of AcEt<sub>3</sub>NS was 1.2% compared to 2.3% under the same reaction conditions but without the addition of the peptide. Thioacetate can thus serve as a source of both thioesters and sulfide in [Fe-S] cluster formation. Besides the prebiotic implications of this, molecular release of sulfide from thioacetate may be of use in laboratory chemical reconstitution schemes, where sulfide is usually added slowly in a dropwise manner.<sup>35</sup>

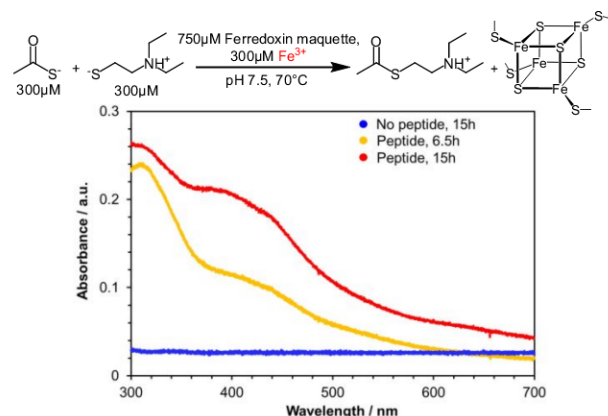


Figure 3 UV-Vis spectrum of the reaction of 300 μM Et<sub>3</sub>NS, thioacetate and FeCl<sub>3</sub> in the presence of 750 μM Ferredoxin peptide maquette at 70°C after 6.5h (yellow), after 15h (red) and without the maquette after 15h (blue).

Iron sulfur derivatives have featured prominently in origins of life research<sup>36,37</sup> and both synthetic [Fe-S] clusters and iron sulfide minerals have been shown experimentally to facilitate CO<sub>2</sub> reduction. Reductive carboxylations and amino acid synthesis have been performed with synthetic [Fe-S] clusters<sup>38</sup> and pyruvate has been synthesized from CO<sub>2</sub> on a greigite (Fe<sub>3</sub>S<sub>4</sub>) working electrode<sup>39</sup> and in reactions involving metallic iron and also iron minerals and hydrogen.<sup>40,41</sup> With the availability of pyruvate for prebiotic chemistry assured, a synergistic process between thioesters and Fe-S clusters can be conceived regarding the work presented here. The enzyme pyruvate ferredoxin-oxidoreductase (PFOR) catalyzes the synthesis of the thioester acetyl-coenzyme A (CoA) from pyruvate via oxidative decarboxylation and uses [4Fe-4S] clusters as electron



mediators.<sup>6</sup> If the acetylation of a thiol by thioacetate and the concomitant formation of [4Fe-4S] clusters were to be connected to the reconstitution of a proto-PFOR, a catalytic feedback could be envisioned, in which continued thioester formation could now be synthesized from both thioacetate and prebiotically available pyruvate. Thus, thioacetate and abiotically produced pyruvate could work synergistically to produce high energy thioesters through two different reactions in an early metabolism.

PFOR, [Fe-S] clusters, and thioesters adopt a central position in the metabolism of bacteria and archaea, connecting the Wood-Ljungdahl (WL) CO<sub>2</sub> fixation pathway with the tricarboxylic acid cycle.<sup>42</sup> The WL pathway itself is thought to be the most ancient autotrophic pathway,<sup>43</sup> and in concert with a complete rTCA cycle as is found in *Thermovibrio Ammonificans*,<sup>44</sup> a hybrid WL-rTCA pathway may have been a robust primordial metabolism due to redundancy in the formation of thioesters as a central metabolite.<sup>45</sup>

The formation of both thioesters and soluble [FeS] clusters from thioacetate thus presents an intriguing prebiotic entry point into both the WL Pathway and the rTCA cycle. Thioester formation with TAA as the acetylating agent and Fe<sup>3+</sup> as an oxidant is reminiscent of oxidative thioesterification reactions in metabolism, such as that in glycolysis and in the decarboxylation of alpha-keto acids. The biological finding of an ancient thioester driven core metabolism<sup>8</sup> enriched in coenzymes that utilize [Fe-S] clusters as catalytic centers, points towards thioacetate being a possible substrate for a metabolic network that also generates both, the necessary catalysts and redox mediators.

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## Conflicts of interest

There are no conflicts to declare.

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**Simultaneous synthesis of thioesters and iron-sulfur clusters in water:  
two universal components of energy metabolism**

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- 4. Ultra-Performance-Liquid-Chromatography**
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- 6. UV-VIS Spectrophotometry**
- 7. Additional Reaction Rate measurements**
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**1. General Methods**

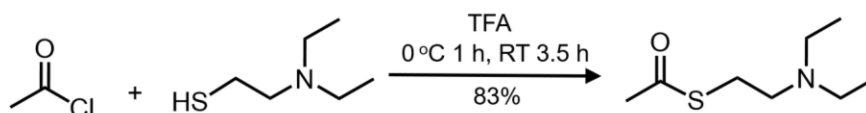
**1.1 General considerations.** All experiments were carried out in 18.2 MΩ water using a MilliQ (Merck Millipore, Billerica, MA, USA) purification system. All reagents were purchased from Sigma Aldrich (Tokyo, Japan) or TCI (Tokyo Chemical Industry, Japan) and used without further purification.

**1.2 Reaction conditions in water.** All solutions were degassed with 99.999% N<sub>2</sub> for 2h and transferred into a Coy glovebox, charged with 5% H<sub>2</sub> and 95% N<sub>2</sub> gas mix, at least one day prior to the reaction, resulting in an equilibration with the atmospheric O<sub>2</sub> concentration of <25 ppm. After preparation of fresh 0.1 M FeCl<sub>2</sub>, FeCl<sub>3</sub>, 0.2 M Et<sub>3</sub>NS or CoM, and 0.5 M potassium thioacetate (98%, Sigma-Aldrich) or thioacetic acid (>95%, TCI) stock solutions in DI water, all reactants were diluted to 5 mM in 100 mM acetic acid buffer (pH 5.5) or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, pH 7.5) buffer (final buffer concentration 92 mM or 97 mM respectively in case of a metal free reaction). 10ml vials were heated inside a dry water bath to 70 ± 1°C and to avoid water evaporation, sealed with Teflon coated butyl rubber stoppers (AS ONE, Japan). Initial experiments were conducted with 2-mercaptoethane-1-sulfonate at room temperature, but the yields did not exceed 4% in the absence of FeCl<sub>3</sub>, and with FeCl<sub>3</sub> the yield was 7% after 5 days.

Prior to analysis, the samples were passed through 13mm, 0.22μm Millex-GV PVDF syringe filters (Merck) and diluted 1:5 with MQ water. Details on experimental sampling and quantification are given below.

## 2. Synthesis of thioester standards.

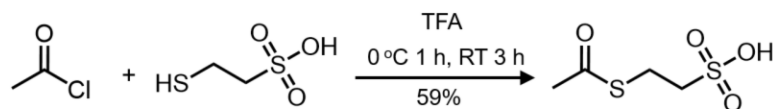
### 2.1. Standard synthesis of S-(2-(diethylamino)ethyl) ethanethioate (AcEt<sub>3</sub>NS)



The chloride salt of 2-(diethylamino)ethane-1-thiol (2.5 mmol, >96%, Tokyo Chemical Industry, TCI) was dissolved in 10 ml TFA (99%, Aldrich) at 0°C and 1.1 equivalents of acetyl-chloride (2.75 mmol, 98%, Sigma-Aldrich) were added dropwise to the solution under stirring. After 1h, the reaction temperature was raised to room temperature (25°C) and after stirring for 3.5 h, the reaction mixture was quenched with 0.1 ml methanol, and concentrated under reduced pressure to obtain AcEt<sub>3</sub>NS as colorless oil. The crude product was used as a standard without further purification. The yield of AcEt<sub>3</sub>NS was calculated from <sup>1</sup>H-NMR spectra (**Figure S1**) using 3-(Trimethylsilyl)-1-propanesulfonic acid-d<sub>6</sub> sodium salt (98 atom % D, Isotec) as an internal standard. From this calibration, a 83% yield of the AcEt<sub>3</sub>NS was determined. <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): δ 3.19–3.33 (m, 8H, CH<sub>2</sub>), 2.41 (s, 3H, CH<sub>3</sub>), 1.31 (t, 6H, *J* = 7.4 Hz, CH<sub>3</sub>). <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O): δ 200.08 (C=O), 50.75 (CH<sub>2</sub>), 48.11 (CH<sub>2</sub>), 30.21 (SCH<sub>2</sub>), 23.12 (CH<sub>3</sub>, Ac), 8.52 (CH<sub>3</sub>). ESI-HRMS: *m/z* [M + H]<sup>+</sup> calculated for C<sub>8</sub>H<sub>17</sub>NOS: 176.110 *m/z*; found: 176.114 *m/z*.



## 2.2. Standard synthesis of 2-(acetylthio)ethane-1-sulfonic acid (AcCoM)



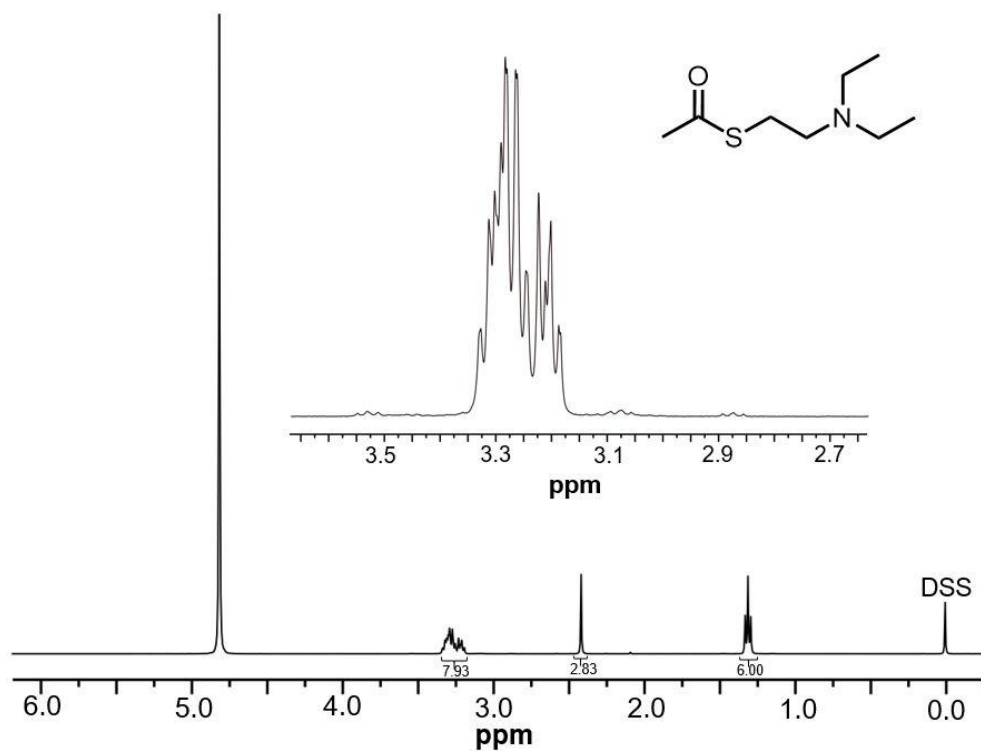
The sodium salt of 2-mercaptoethane-1-sulfonic acid (2.5 mmol, >96%, TCI) was dissolved in 10 ml 99% TFA at 0 °C. Acetyl-chloride (2.75 mmol) was added dropwise to the solution and stirred for 1 h. The reaction was subsequently warmed to room temperature and after stirring for 3h, the reaction mixture was quenched with 0.1 ml methanol, and concentrated under reduced pressure to obtain AcCoM as colourless salt. The concentrated product was used as standard without further purification. The yield was calculated via integration from <sup>1</sup>H-NMR spectra (**Figure S3**) using 3-(Trimethylsilyl)-1-propanesulfonic acid-d<sub>6</sub> sodium salt (98 atom % D, Isotec) as an internal standard. With this calibration, a 59% yield of AcCoM was calculated. <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): δ 3.11-3.15 (m, 2H, *J* = 2.0, 6.0, 11.0 Hz, CH<sub>2</sub>), 3.19-3.23 (m, 2H, *J* = 2.0, 6.0, 11.0 Hz, CH<sub>2</sub>), 2.38 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O): δ 200.88 (C=O), 50.63 (CH<sub>2</sub>), 30.29 (SCH<sub>2</sub>), 23.96 (CH<sub>3</sub>, Ac). ESI-HRMS: *m/z* [M + H]<sup>+</sup> calculated for C<sub>4</sub>H<sub>8</sub>O<sub>4</sub>S<sub>2</sub>: 182.988 *m/z*; found: 182.984 *m/z*.

## 3. Nuclear Magnetic Resonance Spectroscopy

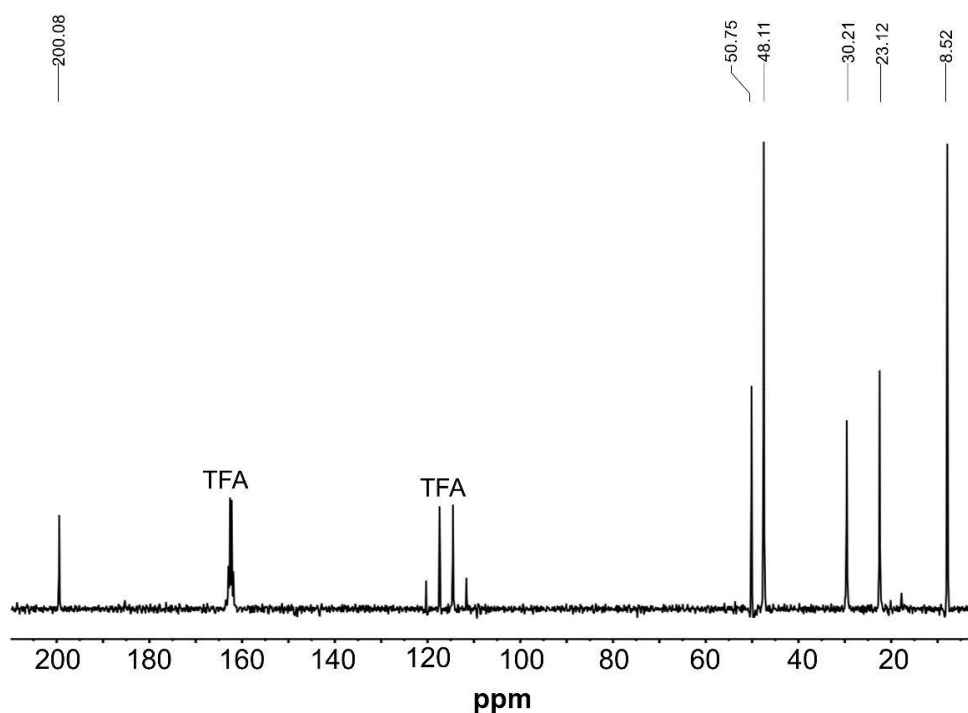
**3.1. Identification and Quantification via Nuclear Magnetic Resonance (NMR).** <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded with a Bruker AVANCE III 400 MHz spectrometer and a 5mm <sup>1</sup>H/<sup>13</sup>C dual probe (400 MHz for <sup>1</sup>H, 100 MHz for <sup>13</sup>C). Carbon chemical shifts are reported in parts per million (ppm) values on the δ scale, and were typically referenced to trifluoroacetic acid and <sup>1</sup>H NMR spectra were referenced to the d6-DSS signal (δ = 0.00 ppm). All NMR spectra were recorded at room temperature and were analyzed using Topspin 3.6.1 (Bruker) and MestReNova (MestreLab Research, Santiago de Compostela, Spain).

To determine the concentration of the thioesters synthesized via acetyl chloride, four independent samples were prepared for AcCoM and three samples of AcEt<sub>3</sub>NS and dissolved in D<sub>2</sub>O with the addition of a known amount of 3-(Trimethylsilyl)-1-propanesulfonic acid-d<sub>6</sub> sodium salt (98 atom % D, Isotec) as an internal standard. A 90° pulse sequence with a calibrated T<sub>1</sub> was used for the integration of the singlet originating from the methyl-residue of the acetyl-group. The total amount of thioester in the synthetic samples were 58.83% (standard deviation 2.65%, n=4) for the AcCoM salt and 83.25% (standard deviation 0.54%, n=3) for the AcEt<sub>3</sub>NS oil.

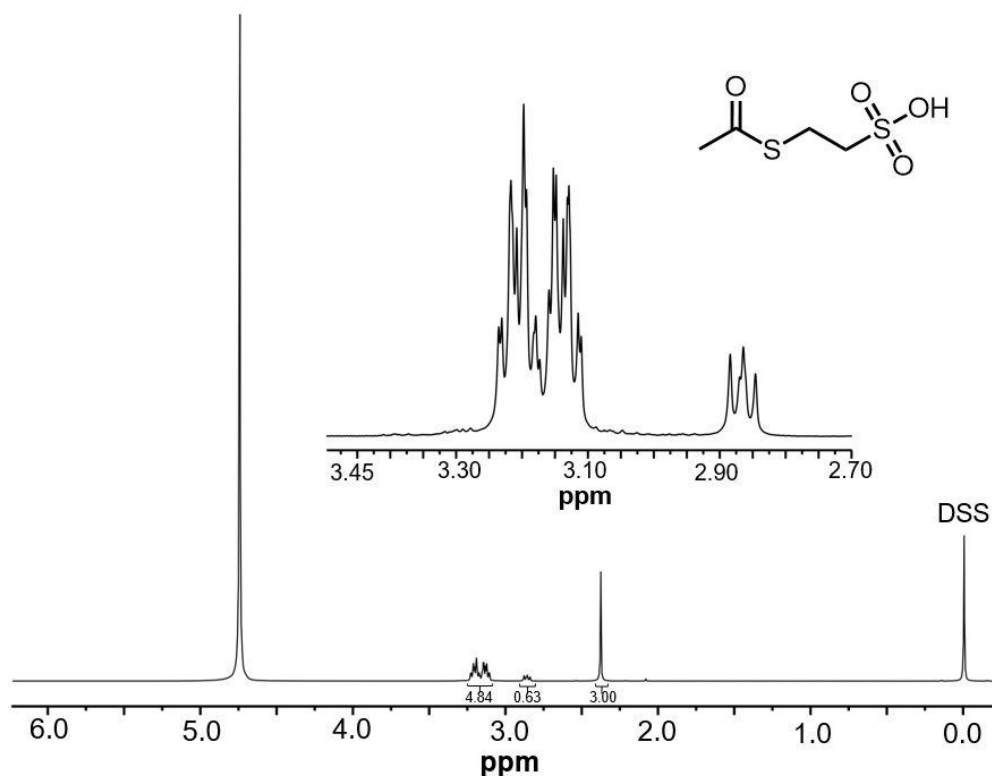
**Figure S1** depicts a <sup>1</sup>H-NMR spectrum of the crude oil of AcEt<sub>3</sub>NS and **Figure S2** the corresponding <sup>13</sup>C-NMR spectrum. The <sup>1</sup>H-NMR spectrum of the crude salt of AcCoM is shown in **Figure S3** and the <sup>13</sup>C-NMR in **Figure S4**.



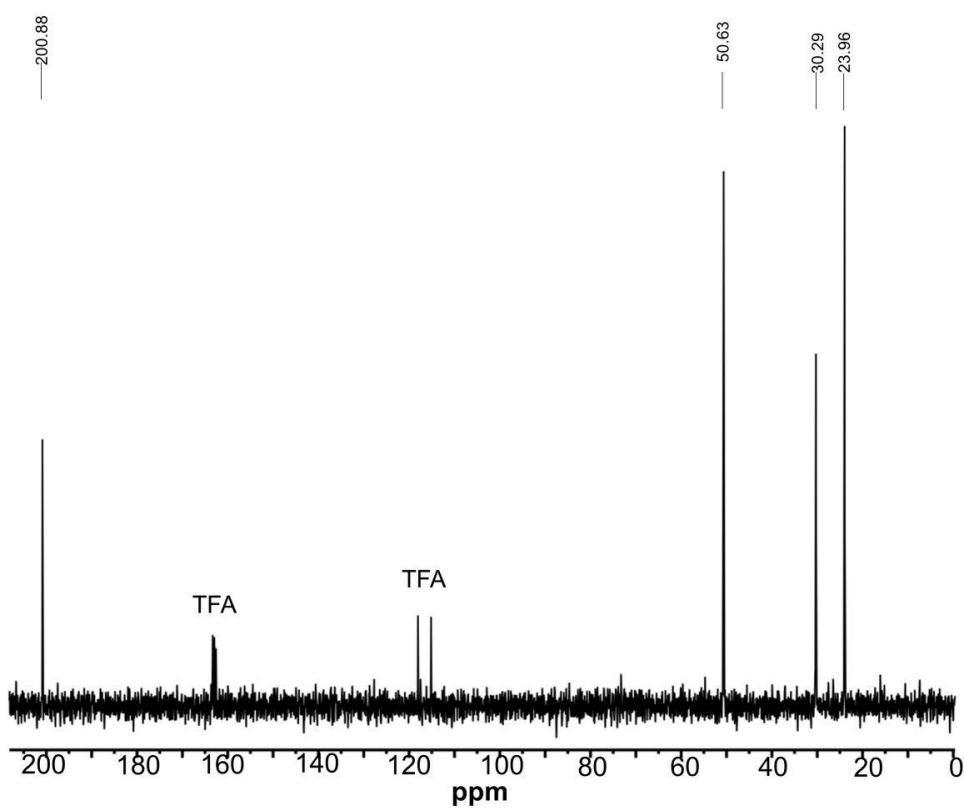
**Figure S1** <sup>1</sup>H-NMR of AcEt<sub>3</sub>NS in D<sub>2</sub>O and 3-(Trimethylsilyl)-1-propanesulfonic acid-d<sub>6</sub> as an internal reference. The integrated areas of the peaks are indicated with brackets and are given in reference to the ethyl amine methyl groups.



**Figure S2** <sup>13</sup>C-NMR of AcEt<sub>3</sub>NS in D<sub>2</sub>O and in the presence of trifluoroacetic acid (TFA).



**Figure S3**  $^1\text{H}$ -NMR of AcCoM in  $\text{D}_2\text{O}$  with 3-(Trimethylsilyl)-1-propanesulfonic acid- $\text{d}_6$  as an internal reference. The integrated areas of the peaks are indicated with brackets and are given in reference to the acetyl methyl group.



**Figure S4**  $^{13}\text{C}$ -NMR of the AcCoM reference compound in  $\text{D}_2\text{O}$  and in the presence of trifluoroacetic acid.

#### 4. Ultra-Performance-Liquid-Chromatography

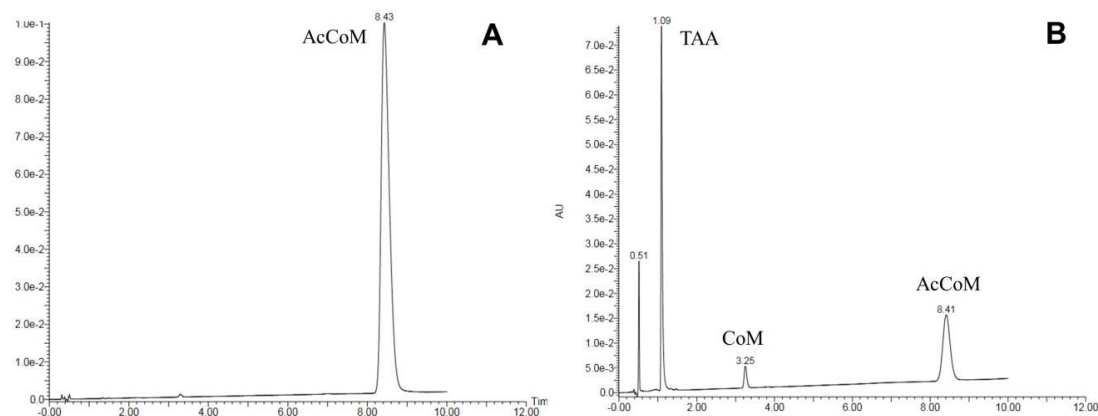
**Quantification via Ultra-Performance-Liquid-Chromatography (UPLC).** The thioester yields were determined through chromatographic separation by an Acquity UPLC H-Class BIO (Waters) equipped with an autosampler, quaternary solvent manager and a photodiode array detector (PDA  $e\lambda$ ). For the AcCoM thioester, a similar chromatography condition as previously reported was chosen<sup>[1]</sup>, employing an isocratic gradient of 5% methanol and 95% 5 mM tetrabutylammonium hydrogen sulfate (>98%, TCI) in water at a flow rate of 0.35 ml min<sup>-1</sup>. The AcEt<sub>3</sub>NS thioester concentration was determined using the gradient given in **Table S1**, using 0.1% formic acid in water as solvent A and 100% acetonitrile as solvent B. Both chromatography conditions employed an Acquity UPLC BEH C18 column with 50 mm length. Representative chromatograms for both thioester standards and thioesters produced in aqueous solution are given in **Figure S5** for AcCoM and **Figure S6** for AcEt<sub>3</sub>NS.

**Table S1.** Chromatography profile for AcEt<sub>3</sub>NS

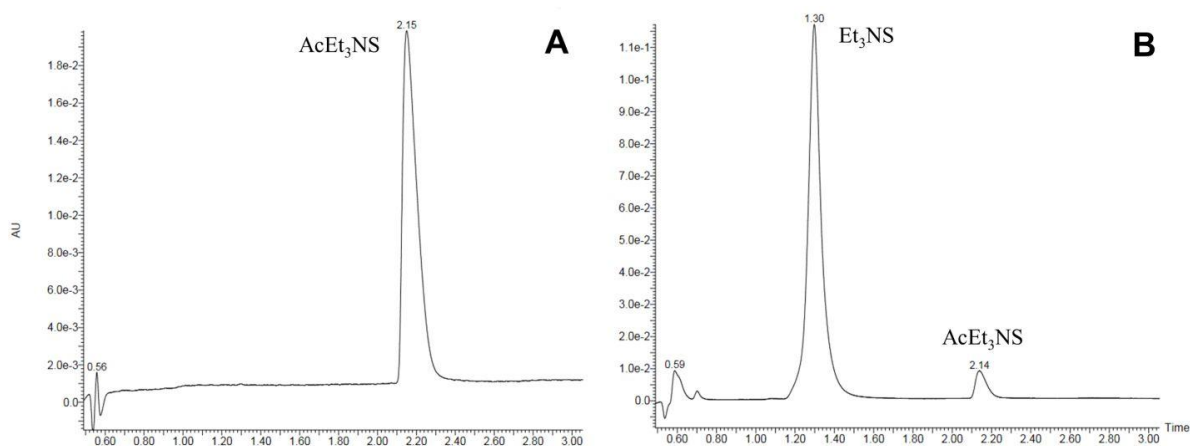
| Time     | Solvent A | Solvent B |
|----------|-----------|-----------|
| 0.00 min | 100%      | 0%        |
| 4.00 min | 100%      | 0%        |
| 8.00 min | 60%       | 40%       |
| 9.00 min | 15%       | 85%       |
| 9.30 min | 0%        | 100%      |
| 9.48 min | 0%        | 100%      |
| 10.00min | 100%      | 0%        |

For the time course measurements conducted within a time interval of 10h, a maximum of 6 samples were taken from the reaction vials at one time and injected into the UPLC to minimize the production of thioesters at room temperature and the influence of oxygen. Therefore, measurement intervals of 1h were chosen to analyze 3 different reaction conditions, prepared in duplicates, within the given time frame to allow consistent experimental conditions throughout the measurement series.



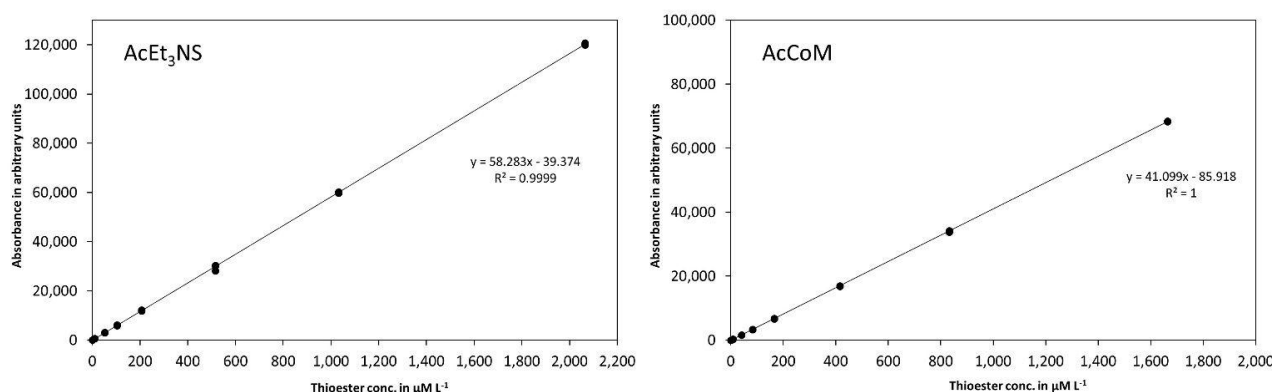


**Figure S5** UPLC chromatograms of the synthetic AcCoM standard (8.43min) at 230nm (A) and a reaction of thioacetic acid with CoM at 70°C (B).



**Figure S6** UPLC chromatograms at a 229nm wavelength of the AcEt<sub>3</sub>NS reference compound at 2.15min (A) and a reaction at 70°C between Et<sub>3</sub>NS and thioacetic acid (B).

The thioesters were quantified using their characteristic absorbance at 228nm for AcEt<sub>3</sub>NS and 229nm for AcCoM and the thioester yields in aqueous solution were calculated from standard curves, in which each concentration of the dilution series was prepared in triplicates (**Figure S7**).

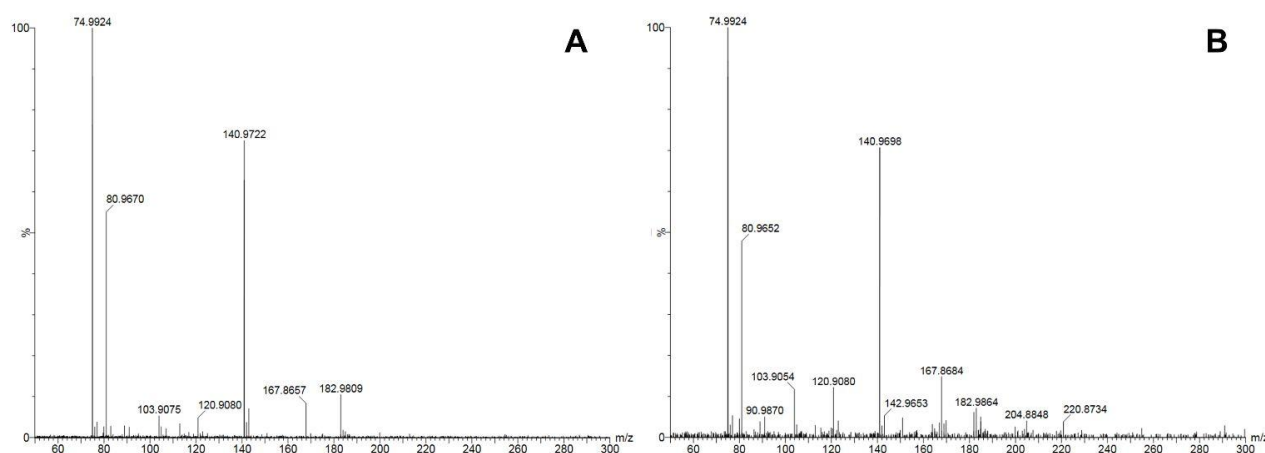


**Figure S7** Calibration curves of AcEt<sub>3</sub>NS and AcCoM generated from a dilution series of their synthetic standards. Linear regressions were calculated using the LINEST function of Microsoft Excel and the linear functions and the corresponding coefficients of determination of the least square fit are displayed.

## 5. Liquid chromatography mass spectrometry.

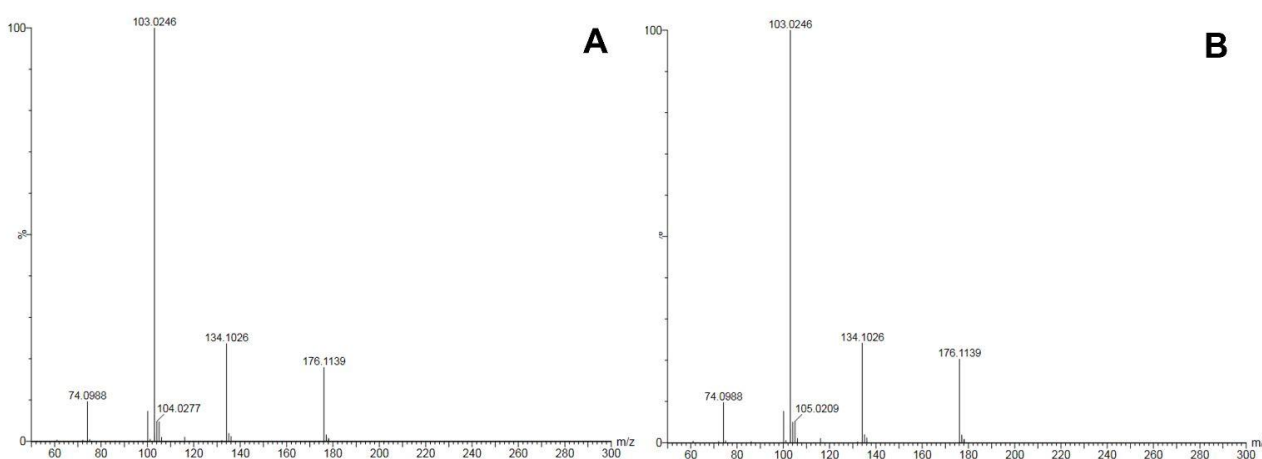
**5. Time of flight mass spectrometry (TOF-MS).** High-resolution mass data analyses were performed on a Xevo G2-XS Q-TOF-MS platform (Waters Corporation, Manchester, UK) operated in electrospray ionization mode, combined with an Ultra-Performance Liquid Chromatography (UPLC) system (Acquity H-class; Waters Corporation, MA, USA). Liquid chromatography was carried out on an UPLC BEH C18 column (1.7  $\mu\text{m}$ , 2.1 mm x 100 mm, Waters) using chromatography conditions as described for AcEt<sub>3</sub>NS (See Section 4., Ultra-Performance-Liquid-Chromatography). The measured mass was calibrated during the sample measurement against Leu-enkephalin as a lock mass.

Mass spectra of the reference compound AcCoM and a reaction of CoM and thioacetic acid are shown in **Figure S8**. These spectra were measured in negative ion mode with the following settings: Capillary voltage 2.5kV, Sampling cone 90.0, source temperature 100°C, desolvation temperature 300°C, cone gas flow 100 L h<sup>-1</sup>. Selecting the parent ion of 182.9 m/z for a MS-MS measurement at a collision energy of 21 eV, the following major mass fragments were obtained: 74.992 m/z, 80.967 m/z and 140.972 m/z (**Figure S8**). The 95% confidence band for the calibration of the mass range from 50 to 1200 m/z with sodium formate was found to be 1.0 ppm and the residual 0.2 ppm (0.1mDa). Expected mass: 182.988 m/z, mass found in the total ion chromatogram: 182.984 m/z.



**Figure S8** MS-MS spectrum of the AcCoM reference compound (A) and CoM and thioacetic acid reacted at 70°C for 4 days (B).

The high-resolution mass spectra for AcEt<sub>3</sub>NS were measured in positive ion mode with the following settings: Capillary voltage 3.0kV, Sampling cone 10.0, source temperature 100°C, desolvation temperature 250°C, cone gas flow 100 L h<sup>-1</sup>. With a collision energy of 15eV, the following major fragments were observed selecting the expected mass of AcEt<sub>3</sub>NS for MS-MS analysis: 74.099 m/z, 103.025 m/z and 134.103 m/z (**Figure S9**). The 95% confidence band for this measurement was determined to be 0.4 ppm and the residual mass 0.3 ppm (0.2 mDa). The expected mass was 176.110 m/z, and 176.114 m/z was measured.

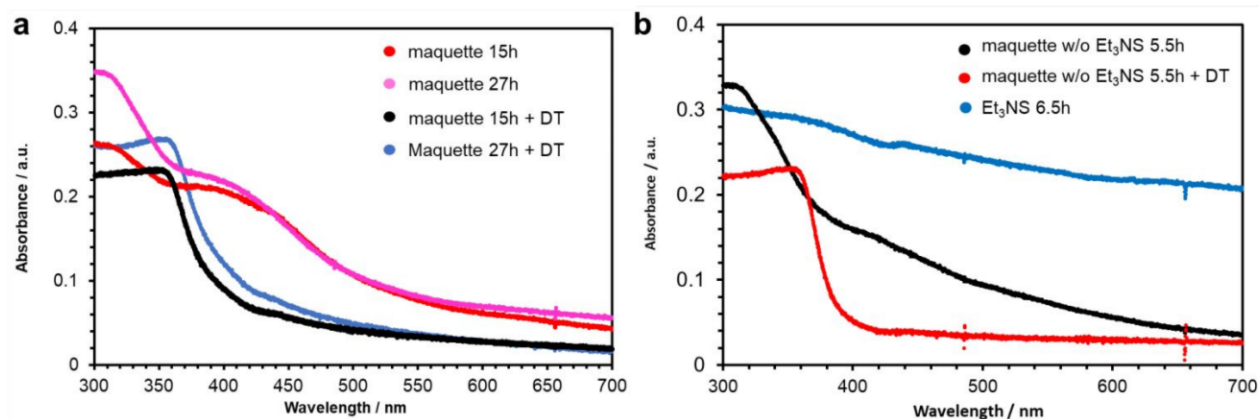


**Figure S9** MS-MS spectrum of the synthetic standard of AcEt<sub>3</sub>NS (A) and Et<sub>3</sub>NS and thioacetic acid reacted at 70°C for 3h (B).

## 6. UV-VIS-spectrophotometry

**6. UV-VIS-spectrophotometry of [FeS] clusters.** Based on an earlier study of minimum binding sequences of [4Fe4S] clusters<sup>[2]</sup>, the C-terminal amidated peptide (91%, GenScript, Japan) with the sequence DLCEGGCIACGACGGD was used as a model iron-sulfur cluster binding peptide. For the FeS cluster reconstitution, Et<sub>3</sub>NS, FeCl<sub>3</sub> and potassium thioacetate with final concentrations of 300 μM were added to 2.2 mg (~750 μM) of the above peptide and heated to 70°C in a 100 mM HEPES buffer at pH 7.5. UV-Vis spectra were acquired in a quartz cuvette under anaerobic conditions with an AvaSpec-ULS4096CL-EVO (Avantes) spectrophotometer and measurements were acquired at different time points, with and without the addition of Et<sub>3</sub>NS or the maquette (**Figure S10**). For the reduction of the FeS cluster, sodium hydrosulfite (>82%, Sigma-Aldrich) was added to achieve a final concentration of 5 mM hydrosulfite (DT).

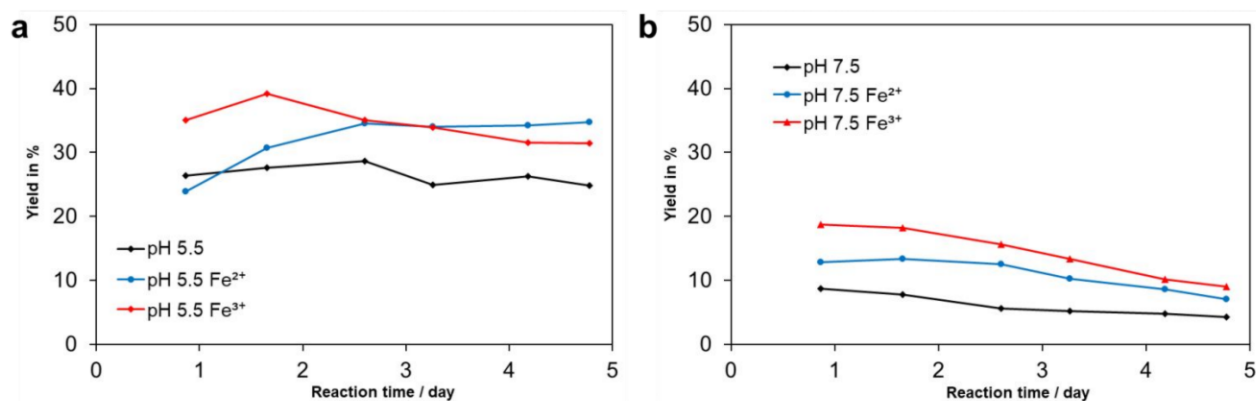
Whereas previous work with this peptide established the incorporation of a [4Fe4S] species after reconstitution with excess iron and sulfide,<sup>[2]</sup> our experiments contained the peptide in excess, perhaps leading to the formation of a mixture of cluster species, although future work is needed to investigate this.



**Figure S10** UV-Vis spectra of 300  $\mu\text{M}$   $\text{FeCl}_3$  and TAA reacted in the presence of a Ferredoxin maquette and 300  $\mu\text{M}$   $\text{Et}_3\text{NS}$  (a) and in the absence of  $\text{Et}_3\text{NS}$  or the maquette (b). The spectra in (a) were taken after 15h with (blue) and without (red) the addition of hydrosulfite (DT) and similarly after 27h in a separate reaction with (blue) and without DT (magenta). UV-Vis spectra without the addition of 300  $\mu\text{M}$   $\text{Et}_3\text{NS}$  are displayed in (b) after 5.5h (black) and after adding 5mM DT (red). A measurement of the reaction of 300  $\mu\text{M}$   $\text{FeCl}_3$ , TAA and  $\text{Et}_3\text{NS}$  is displayed in blue.

## 7. Additional reaction rate measurements

**Reaction yields of AcCoM at an extended timescale.** The buffer pH and concentration, reactant concentrations and temperature match those give in **Section 1.2**. The yields of AcCoM are depicted in percent yield relative to the initial amount of CoM (5 mM) in **Figure S11**. The experiments were conducted in duplicates and the average yields of two experiments are connected to a trend line.



**Figure S11** Time course measurement of the AcCoM thioester from thioacetate (5mM) expressed in percent yield relative to the amount of CoM (5 mM) at pH 5.5 (a) and pH 7.5 (b). The red trace corresponds to a reaction in which FeCl<sub>3</sub> was added, blue FeCl<sub>2</sub> and black contained only CoM and TAA.

Reactions presented in the main text (Figure 1 and Figure 2) and in this section were conducted as duplicates. Table S2 depicts the ranges of thioester yields that were measured via UPLC in regards to the maximum yield obtained by using either CoM or Et<sub>3</sub>NS and with and without the addition of FeCl<sub>2</sub> or FeCl<sub>3</sub> at 70°C.

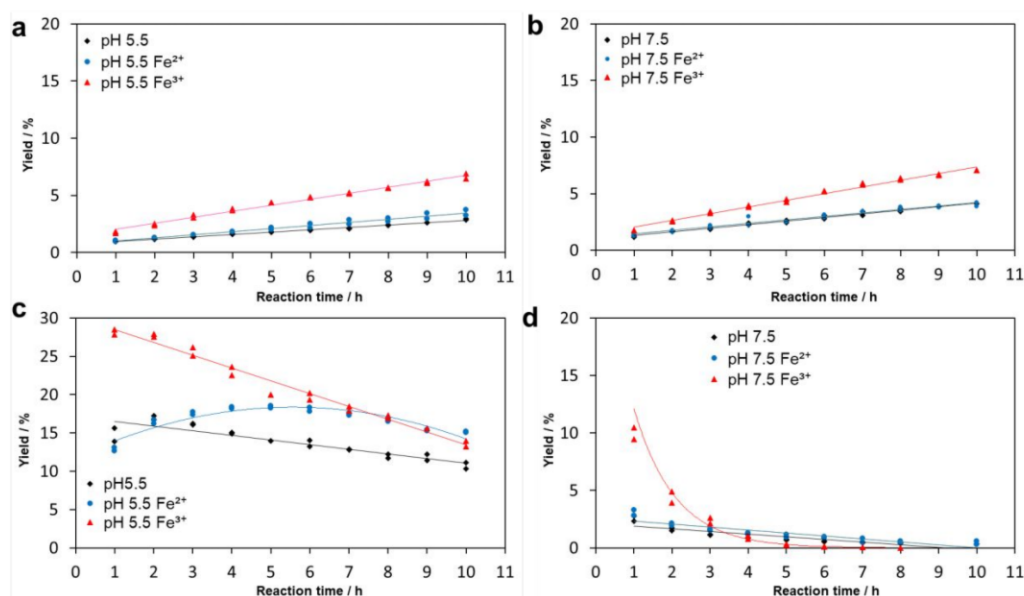
**Table S2.** Highest obtained thioester yields in percent relative to the initial thiol concentration and the reaction time after which these yields were measured in hours.

| Experiment                  | No metal addition    | Fe <sup>2+</sup>      | Fe <sup>3+</sup>     |
|-----------------------------|----------------------|-----------------------|----------------------|
| AcCoM pH 5.5                | 28.64 ± 2.22 % (62h) | 34.71 ± 0.25 % (115h) | 39.23 ± 2.61 % (40h) |
| AcCoM pH 7.5                | 8.69 ± 0.69 % (21h)  | 13.36 ± 0.15 % (21h)  | 18.74 ± 1.08 % (40h) |
| AcEt <sub>3</sub> NS pH 5.5 | 20.85 ± 0.05 % (1h)  | 29.02 ± 0.79 % (5h)   | 48.37 ± 1.35 % (1h)  |
| AcEt <sub>3</sub> NS pH 7.5 | 6.29 ± 0.44% (1h)    | 5.49 ± 0.04 % (1h)    | 24.93 ± 0.81 % (1h)  |

### Reaction rates at lower thioacetic acid concentrations

Following the sample preparation for the thioester synthesis at 70°C, additional reactions were run with a thioacetic acid (96% Sigma-Aldrich) concentration of 2.3 mM and 5 mM of Et<sub>3</sub>NS, CoM and with and without 5 mM FeCl<sub>2</sub> or FeCl<sub>3</sub>. The results for the reaction rate measurements are given in **Figure S12**.





**Figure S12** Time course measurement of the yields of AcCoM (**a** and **b**) and AcEt<sub>3</sub>NS (**c** and **d**) with an initial thiol concentration of either 2.3 mM (pH 5.5) or 5 mM (pH 7.5). The reactions without the addition of an iron chloride are depicted as black traces. Addition of FeCl<sub>2</sub> as blue, and addition of FeCl<sub>3</sub> as red. Reactions in the main text (Figure 1 a, b and Figure 2 a, b) contained 5mM thiol, and for comparison for the data series containing Et<sub>3</sub>N, reactions at a 2.3 mM thiol concentration are given here. Data points from duplicate experiments are shown.

## 8. References

- [1] Z. Apostolides, N. M. J. Vermeulen, D. J. J. Potgieter, High-performance liquid chromatography of some coenzyme M (2-mercaptoethanesulphonic acid) derivatives by ion pairing on reversed-phase columns. *Journal of Chromatography A*, **1982**, 246, 304–307.
- [2] B. R. Gibney, S. E. Mulholland, F. Rabanal, P. L. Dutton, *PNAS* **1996**, 93, 15041–15046.

**Simultaneous synthesis of thioesters and iron-sulfur clusters in water:  
two universal components of energy metabolism**

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\* Corresponding authors: sanden@elsi.jp, mcglynn@elsi.jp

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- 2. Synthesis of Thioester Standards**
- 3. Nuclear Magnetic Resonance Spectroscopy**
- 4. Ultra-Performance-Liquid-Chromatography**
- 5. Liquid Chromatography Mass Spectrometry**
- 6. UV-VIS-Spectrophotometry**
- 7. Additional Reaction Rate measurements**
- 8. References**

**1. General Methods**

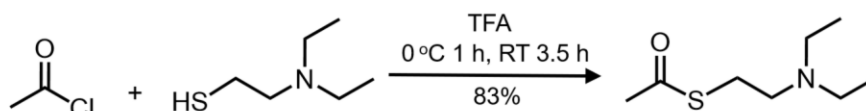
**1.1 General considerations.** All experiments were carried out in 18.2 MΩ water using a MilliQ (Merck Millipore, Billerica, MA, USA) purification system. All reagents were purchased from Sigma Aldrich (Tokyo, Japan) or TCI (Tokyo Chemical Industry, Japan) and used without further purification.

**1.2 Reaction conditions in water.** All solutions were degassed with 99.999% N<sub>2</sub> for 2h and transferred into a Coy glovebox, charged with 5% H<sub>2</sub> and 95% N<sub>2</sub> gas mix, at least one day prior to the reaction, resulting in an equilibration with the atmospheric O<sub>2</sub> concentration of <25 ppm. After preparation of fresh 0.1 M FeCl<sub>2</sub>, FeCl<sub>3</sub>, 0.2 M Et<sub>3</sub>NS or CoM, and 0.5 M potassium thioacetate (98%, Sigma-Aldrich) or thioacetic acid (>95%, TCI) stock solutions in DI water, all reactants were diluted to 5 mM in 100 mM acetic acid buffer (pH 5.5) or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, pH 7.5) buffer (final buffer concentration 92 mM or 97 mM respectively in case of a metal free reaction). 10ml vials were heated inside a dry water bath to 70 ± 1°C and to avoid water evaporation, sealed with Teflon coated butyl rubber stoppers (AS ONE, Japan). **Initial experiments were conducted with 2-mercaptoethane-1-sulfonate at room temperature, but the yields did not exceed 4% in the absence of FeCl<sub>3</sub>, and with FeCl<sub>3</sub> the yield was 7% after 5 days.**

Prior to analysis, the samples were passed through 13mm, 0.22μm Millex-GV PVDF syringe filters (Merck) and diluted 1:5 with MQ water. Details on experimental sampling and quantification are given below.

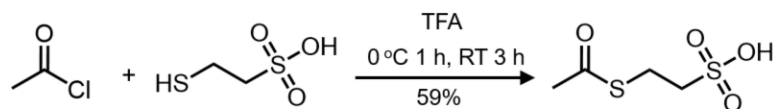
## 2. Synthesis of thioester standards.

### 2.1. Standard synthesis of S-(2-(diethylamino)ethyl) ethanethioate (AcEt<sub>3</sub>NS)



The chloride salt of 2-(diethylamino)ethane-1-thiol (2.5 mmol, >96%, Tokyo Chemical Industry, TCI) was dissolved in 10 ml TFA (99%, Aldrich) at 0°C and 1.1 equivalents of acetyl-chloride (2.75 mmol, 98%, Sigma-Aldrich) were added dropwise to the solution under stirring. After 1h, the reaction temperature was raised to room temperature (25°C) and after stirring for 3.5 h, the reaction mixture was quenched with 0.1 ml methanol, and concentrated under reduced pressure to obtain AcEt<sub>3</sub>NS as colorless oil. The crude product was used as a standard without further purification. The yield of AcEt<sub>3</sub>NS was calculated from <sup>1</sup>H-NMR spectra (**Figure S1**) using 3-(Trimethylsilyl)-1-propanesulfonic acid-d<sub>6</sub> sodium salt (98 atom % D, Isotec) as an internal standard. From this calibration, a 83% yield of the AcEt<sub>3</sub>NS was determined. <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): δ 3.19–3.33 (m, 8H, CH<sub>2</sub>), 2.41 (s, 3H, CH<sub>3</sub>), 1.31 (t, 6H, J = 7.4 Hz, CH<sub>3</sub>). <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O): δ 200.08 (C=O), 50.75 (CH<sub>2</sub>), 48.11 (CH<sub>2</sub>), 30.21 (SCH<sub>2</sub>), 23.12 (CH<sub>3</sub>, Ac), 8.52 (CH<sub>3</sub>). ESI-HRMS: m/z [M + H]<sup>+</sup> calculated for C<sub>8</sub>H<sub>17</sub>NOS: 176.110 m/z; found: 176.114 m/z.

## 2.2. Standard synthesis of 2-(acetylthio)ethane-1-sulfonic acid (AcCoM)



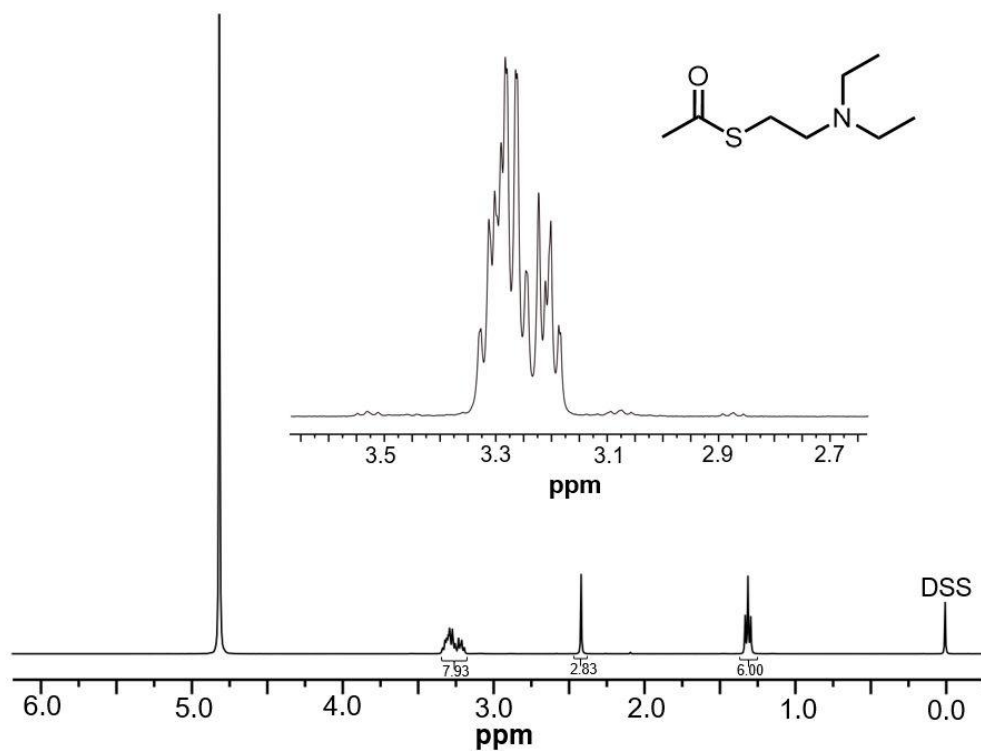
The sodium salt of 2-mercaptoethane-1-sulfonic acid (2.5 mmol, >96%, TCI) was dissolved in 10 ml 99% TFA at 0 °C. Acetyl-chloride (2.75 mmol) was added dropwise to the solution and stirred for 1 h. The reaction was subsequently warmed to room temperature and after stirring for 3h, the reaction mixture was quenched with 0.1 ml methanol, and concentrated under reduced pressure to obtain AcCoM as colourless salt. The concentrated product was used as standard without further purification. The yield was calculated via integration from  $^1\text{H}$ -NMR spectra (**Figure S3**) using 3-(Trimethylsilyl)-1-propanesulfonic acid- $\text{d}_6$  sodium salt (98 atom % D, Isotec) as an internal standard. With this calibration, a 59% yield of AcCoM was calculated.  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  3.11-3.15 (m, 2H,  $J$  = 2.0, 6.0, 11.0 Hz,  $\text{CH}_2$ ), 3.19-3.23 (m, 2H,  $J$  = 2.0, 6.0, 11.0 Hz,  $\text{CH}_2$ ), 2.38 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  200.88 ( $\text{C}=\text{O}$ ), 50.63 ( $\text{CH}_2$ ), 30.29 ( $\text{SCH}_2$ ), 23.96 ( $\text{CH}_3$ , Ac). ESI-HRMS:  $m/z$   $[\text{M} + \text{H}]^+$  calculated for  $\text{C}_4\text{H}_8\text{O}_4\text{S}_2$ : 182.988  $m/z$ ; found: 182.984  $m/z$ .

## 3. Nuclear Magnetic Resonance Spectroscopy

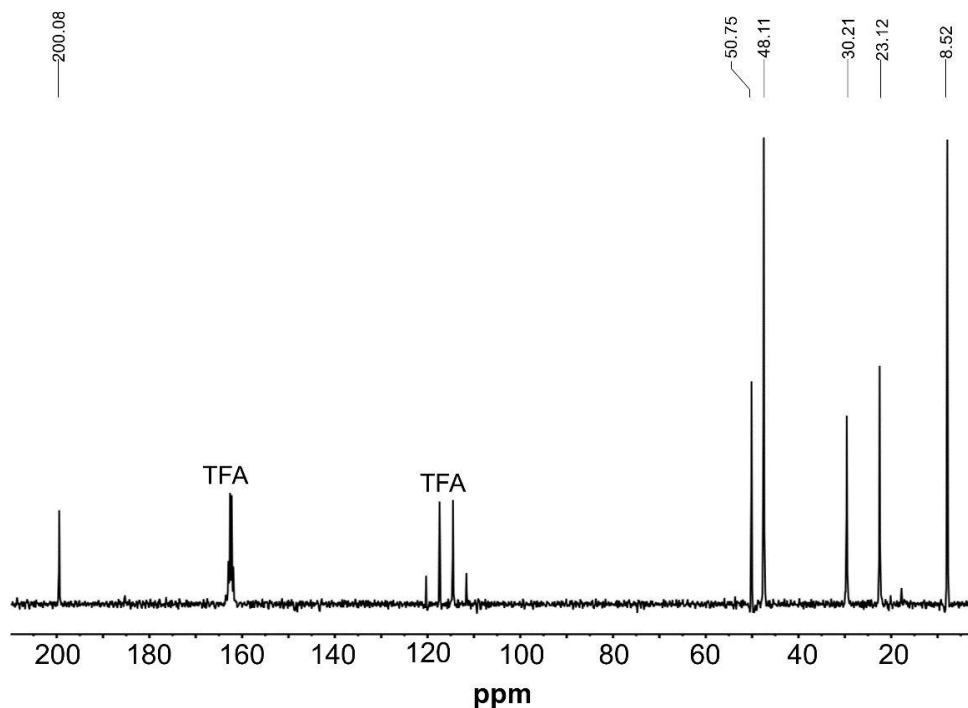
**3.1. Identification and Quantification via Nuclear Magnetic Resonance (NMR).**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded with a Bruker AVANCE III 400 MHz spectrometer and a 5mm  $^1\text{H}/^{13}\text{C}$  dual probe (400 MHz for  $^1\text{H}$ , 100 MHz for  $^{13}\text{C}$ ). Carbon chemical shifts are reported in parts per million (ppm) values on the  $\delta$  scale, and were typically referenced to trifluoroacetic acid and  $^1\text{H}$  NMR spectra were referenced to the  $\text{d}_6$ -DSS signal ( $\delta$  = 0.00 ppm). All NMR spectra were recorded at room temperature and were analyzed using Topspin 3.6.1 (Bruker) and MestReNova (MestreLab Research, Santiago de Compostela, Spain).

To determine the concentration of the thioesters synthesized via acetyl chloride, four independent samples were prepared for AcCoM and three samples of  $\text{AcEt}_3\text{NS}$  and dissolved in  $\text{D}_2\text{O}$  with the addition of a known amount of 3-(Trimethylsilyl)-1-propanesulfonic acid- $\text{d}_6$  sodium salt (98 atom % D, Isotec) as an internal standard. A  $90^\circ$  pulse sequence with a calibrated  $T_1$  was used for the integration of the singlet originating from the methyl-residue of the acetyl-group. The total amount of thioester in the synthetic samples were 58.83% (standard deviation 2.65%,  $n=4$ ) for the AcCoM salt and 83.25% (standard deviation 0.54%,  $n=3$ ) for the  $\text{AcEt}_3\text{NS}$  oil.

**Figure S1** depicts a  $^1\text{H}$ -NMR spectrum of the crude oil of  $\text{AcEt}_3\text{NS}$  and **Figure S2** the corresponding  $^{13}\text{C}$ -NMR spectrum. The  $^1\text{H}$ -NMR spectrum of the crude salt of AcCoM is shown in **Figure S3** and the  $^{13}\text{C}$ -NMR in **Figure S4**.

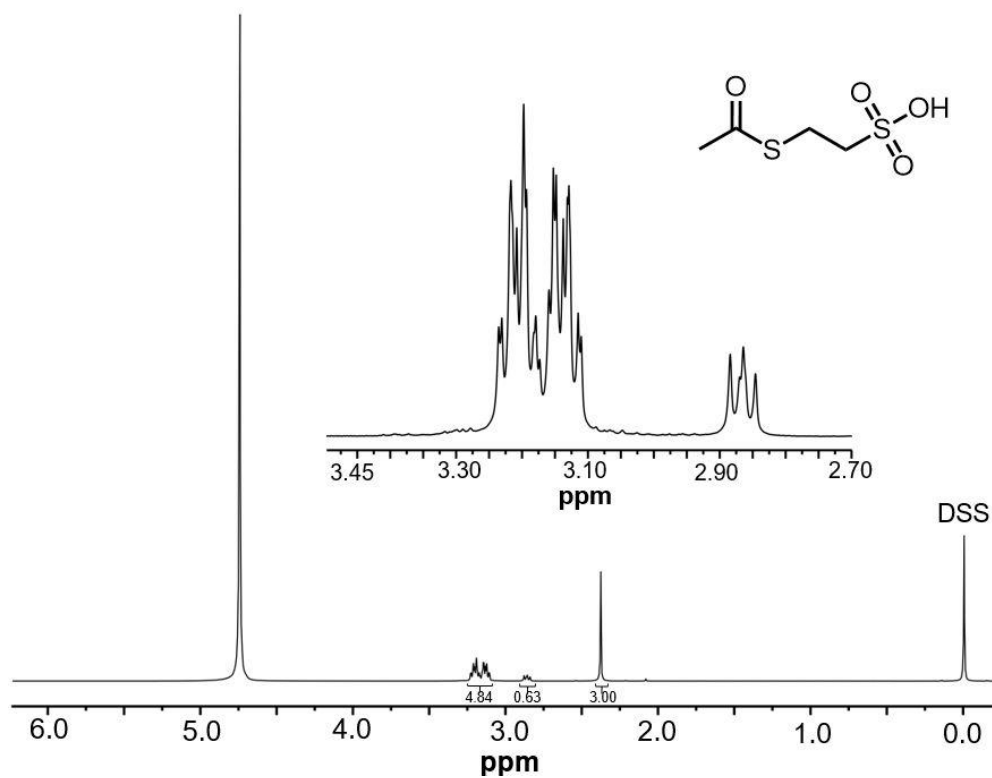


**Figure S1** <sup>1</sup>H-NMR of AcEt<sub>3</sub>NS in D<sub>2</sub>O and 3-(Trimethylsilyl)-1-propanesulfonic acid-d<sub>6</sub> as an internal reference. The integrated areas of the peaks are indicated with brackets and are given in reference to the ethyl amine methyl groups.

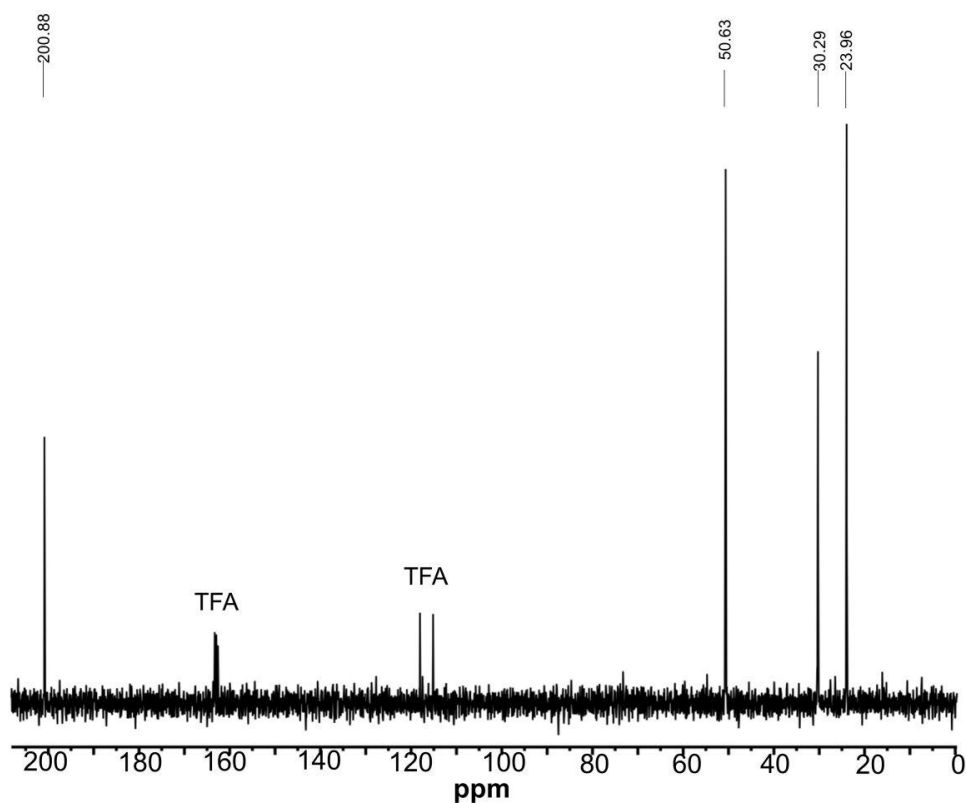


**Figure S2** <sup>13</sup>C-NMR of AcEt<sub>3</sub>NS in D<sub>2</sub>O and in the presence of trifluoroacetic acid (TFA).





**Figure S3**  $^1\text{H}$ -NMR of AcCoM in  $\text{D}_2\text{O}$  with 3-(Trimethylsilyl)-1-propanesulfonic acid- $\text{d}_6$  as an internal reference. The integrated areas of the peaks are indicated with brackets and are given in reference to the acetyl methyl group.



**Figure S4**  $^{13}\text{C}$ -NMR of the AcCoM reference compound in  $\text{D}_2\text{O}$  and in the presence of trifluoroacetic acid.

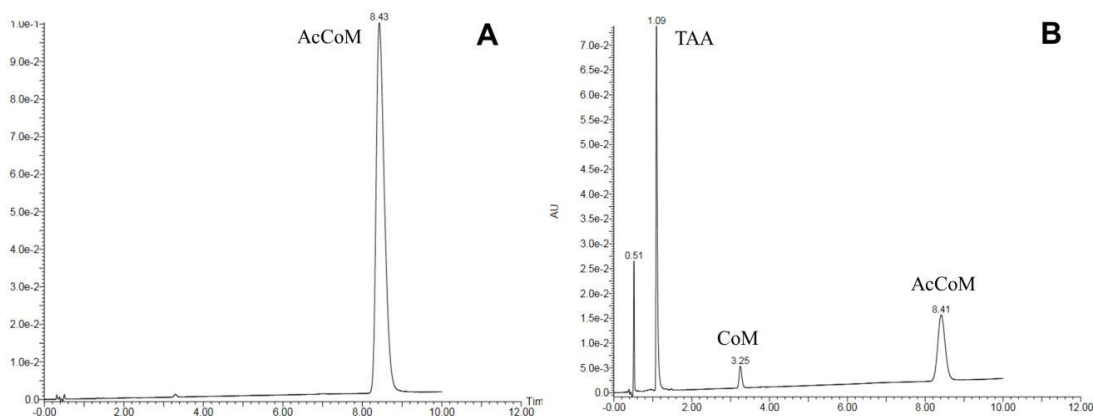
#### 4. Ultra-Performance-Liquid-Chromatography

**Quantification via Ultra-Performance-Liquid-Chromatography (UPLC).** The thioester yields were determined through chromatographic separation by an Acquity UPLC H-Class BIO (Waters) equipped with an autosampler, quaternary solvent manager and a photodiode array detector (PDA  $e\lambda$ ). For the AcCoM thioester, a similar chromatography condition as previously reported was chosen<sup>[1]</sup>, employing an isocratic gradient of 5% methanol and 95% 5 mM tetrabutylammonium hydrogen sulfate (>98%, TCI) in water at a flow rate of 0.35 ml min<sup>-1</sup>. The AcEt<sub>3</sub>NS thioester concentration was determined using the gradient given in **Table 1**, using 0.1% formic acid in water as solvent A and 100% acetonitrile as solvent B. Both chromatography conditions employed an Acquity UPLC BEH C18 column with 50 mm length. Representative chromatograms for both thioester standards and thioesters produced in aqueous solution are given in **Figure S5** for AcCoM and **Figure S6** for AcEt<sub>3</sub>NS.

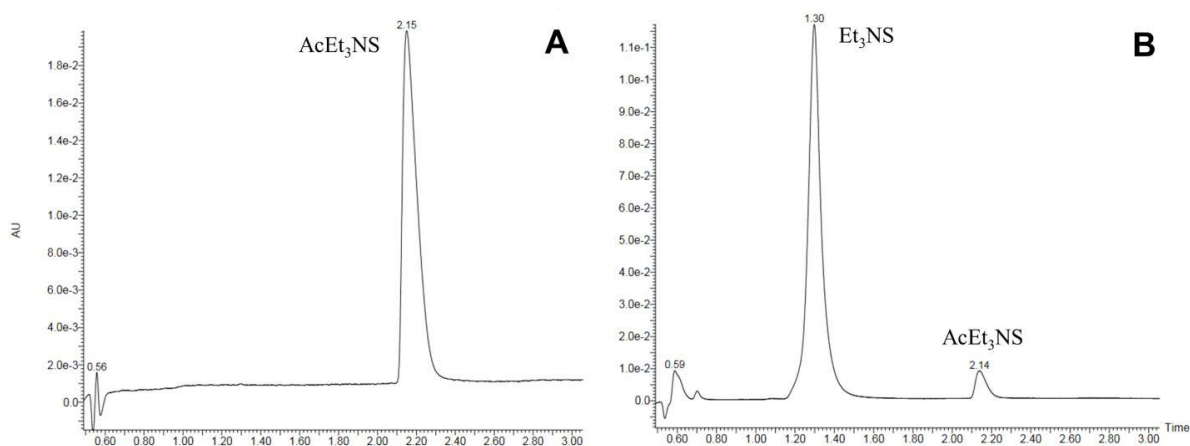
**Table 1.** Chromatography profile for AcEt<sub>3</sub>NS

| Time     | Solvent A | Solvent B |
|----------|-----------|-----------|
| 0.00 min | 100%      | 0%        |
| 4.00 min | 100%      | 0%        |
| 8.00 min | 60%       | 40%       |
| 9.00 min | 15%       | 85%       |
| 9.30 min | 0%        | 100%      |
| 9.48 min | 0%        | 100%      |
| 10.00min | 100%      | 0%        |

For the time course measurements conducted within a time interval of 10h, a maximum of 6 samples were taken from the reaction vials at one time and injected into the UPLC to minimize the production of thioesters at room temperature and the influence of oxygen. Therefore, measurement intervals of 1h were chosen to analyze 3 different reaction conditions, prepared in duplicates, within the given time frame to allow consistent experimental conditions throughout the measurement series.

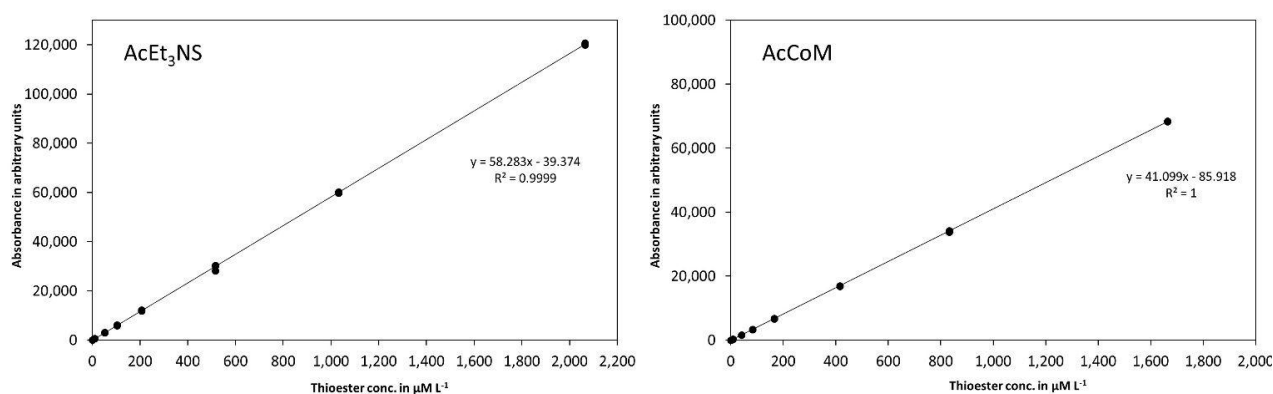


**Figure S5** UPLC chromatograms of the synthetic AcCoM standard (8.43min) at 230nm (A) and a reaction of thioacetic acid with CoM at 70°C (B).



**Figure S6** UPLC chromatograms at a 229nm wavelength of the AcEt<sub>3</sub>NS reference compound at 2.15min (A) and a reaction at 70°C between Et<sub>3</sub>NS and thioacetic acid (B).

The thioesters were quantified using their characteristic absorbance at 228nm for AcEt<sub>3</sub>NS and 229nm for AcCoM and the thioester yields in aqueous solution were calculated from standard curves, in which each concentration of the dilution series was prepared in triplicates (**Figure S7**).

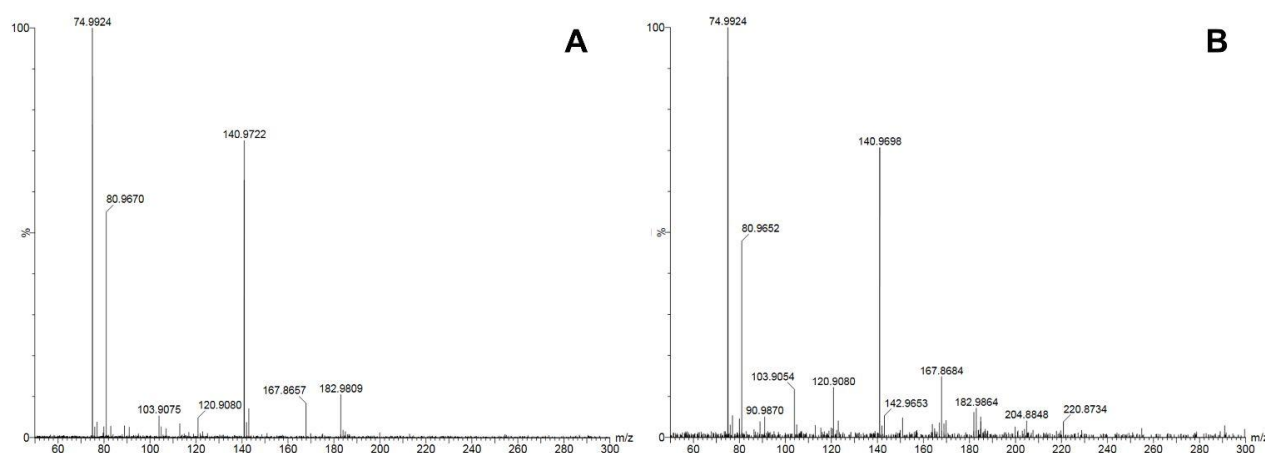


**Figure S7** Calibration curves of AcEt<sub>3</sub>NS and AcCoM generated from a dilution series of their synthetic standards. Linear regressions were calculated using the LINEST function of Microsoft Excel and the linear functions and the corresponding coefficients of determination of the least square fit are displayed.

## 5. Liquid chromatography mass spectrometry.

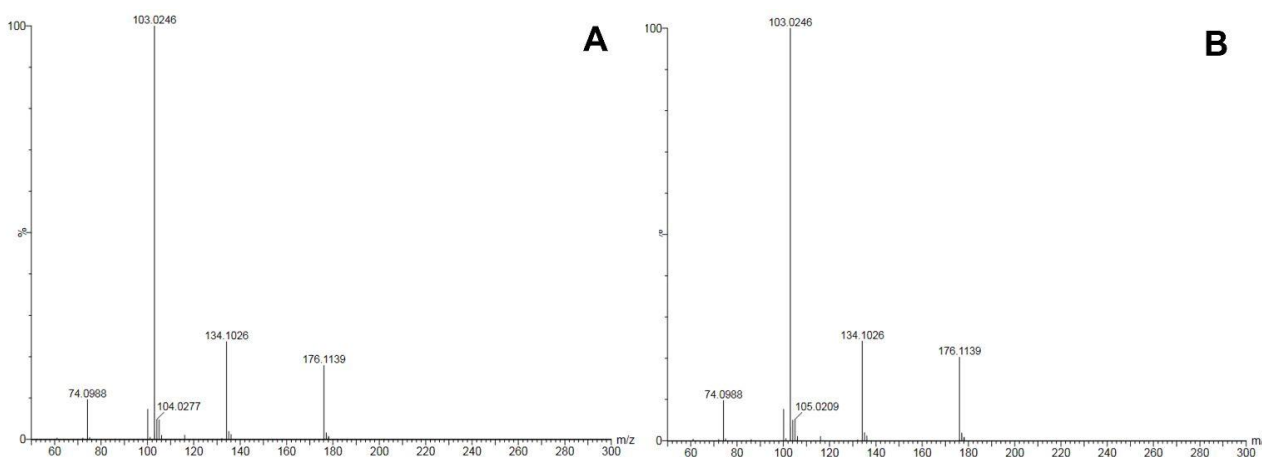
**5. Time of flight mass spectrometry (TOF-MS).** High-resolution mass data analyses were performed on a Xevo G2-XS Q-TOF-MS platform (Waters Corporation, Manchester, UK) operated in electrospray ionization mode, combined with an Ultra-Performance Liquid Chromatography (UPLC) system (Acquity H-class; Waters Corporation, MA, USA). Liquid chromatography was carried out on an UPLC BEH C18 column (1.7  $\mu\text{m}$ , 2.1 mm x 100 mm, Waters) using chromatography conditions as described for AcEt<sub>3</sub>NS (See Section 4., Ultra-Performance-Liquid-Chromatography). The measured mass was calibrated during the sample measurement against Leu-enkephalin as a lock mass.

Mass spectra of the reference compound AcCoM and a reaction of CoM and thioacetic acid are shown in **Figure S8**. These spectra were measured in negative ion mode with the following settings: Capillary voltage 2.5kV, Sampling cone 90.0, source temperature 100°C, desolvation temperature 300°C, cone gas flow 100 L h<sup>-1</sup>. Selecting the parent ion of 182.9 m/z for a MS-MS measurement at a collision energy of 21 eV, the following major mass fragments were obtained: 74.992 m/z, 80.967 m/z and 140.972 m/z (**Figure S8**). The 95% confidence band for the calibration of the mass range from 50 to 1200 m/z with sodium formate was found to be 1.0 ppm and the residual 0.2 ppm (0.1mDa). Expected mass: 182.988 m/z, mass found in the total ion chromatogram: 182.984 m/z.



**Figure S8** MS-MS spectrum of the AcCoM reference compound (A) and CoM and thioacetic acid reacted at 70°C for 4 days (B).

The high-resolution mass spectra for AcEt<sub>3</sub>NS were measured in positive ion mode with the following settings: Capillary voltage 3.0kV, Sampling cone 10.0, source temperature 100°C, desolvation temperature 250°C, cone gas flow 100 L h<sup>-1</sup>. With a collision energy of 15eV, the following major fragments were observed selecting the expected mass of AcEt<sub>3</sub>NS for MS-MS analysis: 74.099 m/z, 103.025 m/z and 134.103 m/z (**Figure S9**). The 95% confidence band for this measurement was determined to be 0.4 ppm and the residual mass 0.3 ppm (0.2 mDa). The expected mass was 176.110 m/z, and 176.114 m/z was measured.

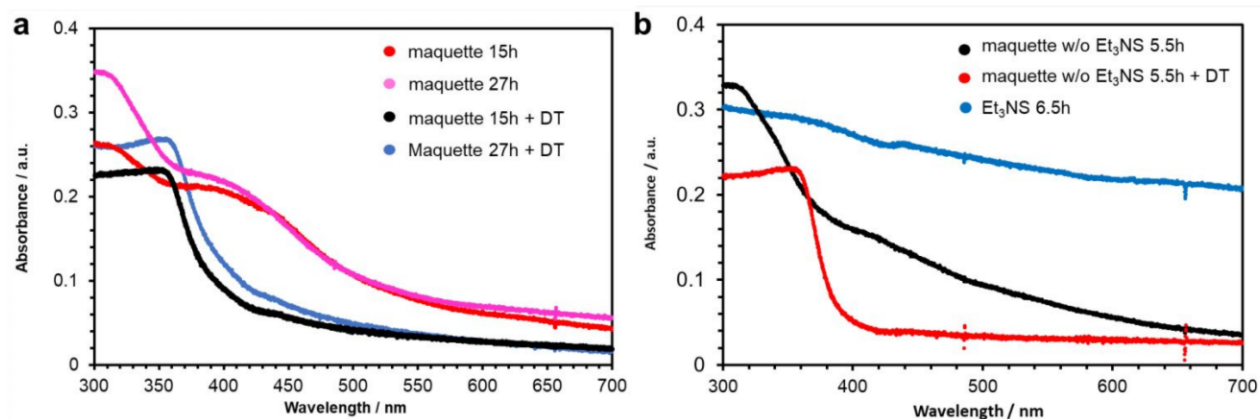


**Figure S9** MS-MS spectrum of the synthetic standard of AcEt<sub>3</sub>NS (A) and Et<sub>3</sub>NS and thioacetic acid reacted at 70°C for 3h (B).

## 6. UV-VIS-spectrophotometry

**6. UV-VIS-spectrophotometry of [FeS] clusters.** Based on an earlier study of minimum binding sequences of [4Fe4S] clusters<sup>[2]</sup>, the C-terminal amidated peptide (91%, GenScript, Japan) with the sequence DLCEGGCIACGACGGD was used as a model iron-sulfur cluster binding peptide. For the FeS cluster reconstitution, Et<sub>3</sub>NS, FeCl<sub>3</sub> and potassium thioacetate with final concentrations of 300 μM were added to 2.2 mg (~750 μM) of the above peptide and heated to 70°C in a 100 mM HEPES buffer at pH 7.5. UV-Vis spectra were acquired in a quartz cuvette under anaerobic conditions with an AvaSpec-ULS4096CL-EVO (Avantes) spectrophotometer and measurements were acquired at different time points, with and without the addition of Et<sub>3</sub>NS or the maquette (**Figure S10**). For the reduction of the FeS cluster, sodium hydrosulfite (>82%, Sigma-Aldrich) was added to achieve a final concentration of 5 mM hydrosulfite (DT).

Whereas previous work with this peptide established the incorporation of a [4Fe4S] species after reconstitution with excess iron and sulfide,<sup>[2]</sup> our experiments contained the peptide in excess, perhaps leading to the formation of a mixture of cluster species, although future work is needed to investigate this.

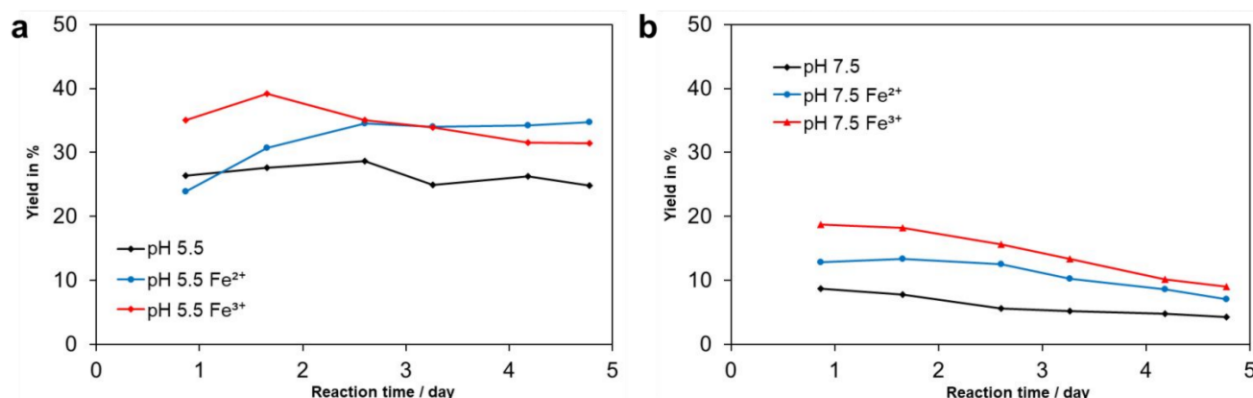


**Figure S10** UV-Vis spectra of 300  $\mu\text{M}$   $\text{FeCl}_3$  and TAA reacted in the presence of a Ferredoxin maquette and 300  $\mu\text{M}$   $\text{Et}_3\text{NS}$  (a) and in the absence of  $\text{Et}_3\text{NS}$  or the maquette (b). The spectra in (a) were taken after 15h with (blue) and without (red) the addition of hydrosulfite (DT) and similarly after 27h in a separate reaction with (blue) and without DT (magenta). UV-Vis spectra without the addition of 300  $\mu\text{M}$   $\text{Et}_3\text{NS}$  are displayed in (b) after 5.5h (black) and after adding 5mM DT (red). A measurement of the reaction of 300  $\mu\text{M}$   $\text{FeCl}_3$ , TAA and  $\text{Et}_3\text{NS}$  is displayed in blue.



## 7. Additional reaction rate measurements

**Reaction yields of AcCoM at an extended timescale.** The buffer pH and concentration, reactant concentrations and temperature match those give in **Section 1.2**. The yields of AcCoM are depicted in percent yield relative to the initial amount of CoM (5 mM) in **Figure S11**. The experiments were conducted in duplicates and the average yields of two experiments are connected to a trend line.



**Figure S11** Time course measurement of the AcCoM thioester from thioacetate (5mM) expressed in percent yield relative to the amount of CoM (5 mM) at pH 5.5 (a) and pH 7.5 (b). The red trace corresponds to a reaction in which FeCl<sub>3</sub> was added, blue FeCl<sub>2</sub> and black contained only CoM and TAA.

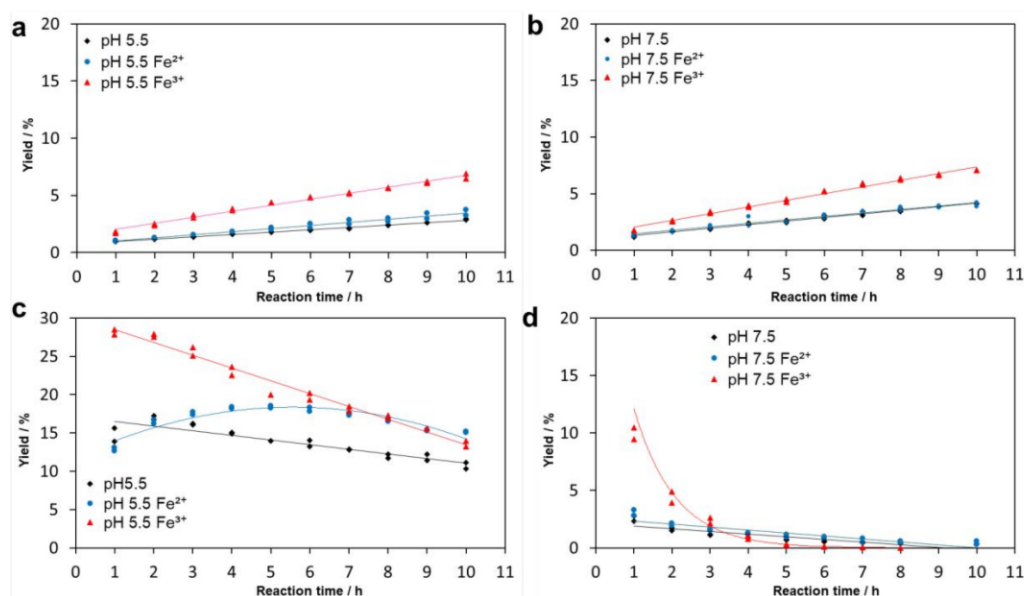
Reactions presented in the main text (Figure 1 and Figure 2) and in this section were conducted as duplicates. Table S2 depicts the ranges of thioester yields that were measured via UPLC in regards to the maximum yield obtained by using either CoM or Et<sub>3</sub>NS and with and without the addition of FeCl<sub>2</sub> or FeCl<sub>3</sub> at 70°C.

**Table S2.** Highest obtained thioester yields in percent relative to the initial thiol concentration and the reaction time after which these yields were measured in hours.

| Experiment                  | No metal addition    | Fe <sup>2+</sup>      | Fe <sup>3+</sup>     |
|-----------------------------|----------------------|-----------------------|----------------------|
| AcCoM pH 5.5                | 28.64 ± 2.22 % (62h) | 34.71 ± 0.25 % (115h) | 39.23 ± 2.61 % (40h) |
| AcCoM pH 7.5                | 8.69 ± 0.69 % (21h)  | 13.36 ± 0.15 % (21h)  | 18.74 ± 1.08 % (40h) |
| AcEt <sub>3</sub> NS pH 5.5 | 20.85 ± 0.05 % (1h)  | 29.02 ± 0.79 % (5h)   | 48.37 ± 1.35 % (1h)  |
| AcEt <sub>3</sub> NS pH 7.5 | 6.29 ± 0.44% (1h)    | 5.49 ± 0.04 % (1h)    | 24.93 ± 0.81 % (1h)  |

### Reaction rates at lower thioacetic acid concentrations

Following the sample preparation for the thioester synthesis at 70°C, additional reactions were run with a thioacetic acid (96% Sigma-Aldrich) concentration of 2.3 mM and 5 mM of Et<sub>3</sub>NS, CoM and with and without 5 mM FeCl<sub>2</sub> or FeCl<sub>3</sub>. The results for the reaction rate measurements are given in **Figure S12**.



**Figure S12** Time course measurement of the yields of AcCoM (**a** and **b**) and AcEt<sub>3</sub>NS (**c** and **d**) with an initial thiol concentration of either 2.3 mM (pH 5.5) or 5 mM (pH 7.5). The reactions without the addition of an iron chloride are depicted as black traces. Addition of FeCl<sub>2</sub> as blue, and addition of FeCl<sub>3</sub> as red. Reactions in the main text (Figure 1 a, b and Figure 2 a, b) contained 5mM thiol, and for comparison for the data series containing Et<sub>3</sub>N, reactions at a 2.3 mM thiol concentration are given here. Data points from duplicate experiments are shown.

## 8. References

- [1] Z. Apostolides, N. M. J. Vermeulen, D. J. J. Potgieter, High-performance liquid chromatography of some coenzyme M (2-mercaptoethanesulphonic acid) derivatives by ion pairing on reversed-phase columns. *Journal of Chromatography A*, **1982**, 246, 304–307.
- [2] B. R. Gibney, S. E. Mulholland, F. Rabanal, P. L. Dutton, *PNAS* **1996**, 93, 15041–15046.