Thiols in Natural Organic Matter: Molecular Forms, Acidity, and Reactivity with Mercury(II) from First-Principles Calculations and HR-XANES Spectroscopy

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ABSTRACT

Thiol functional groups in natural organic matter form strong complexes with Hg(II) and other soft metal cations, and therefore are an important component of sulfur and metal cycling in the environment. However, characterizing thiol reactivity is difficult because natural organic molecules are complex both in composition and molecular structure. Here, reactivity was assessed by calculating the Gibbs free energies of thiolation and thiol deprotonation reactions for model structures considered to form during abiotic sulfurization of natural organic matter. Gaussian calculations were performed at the CCSD(T) level of theory. Thiol addition is predicted to be faster by as much as 8 orders of magnitude on unsaturated cyclic structures rich in ketone and ether linkages than on open-chain carbonyl structures. The pK_a values of thiols added to carboxyl-rich alicyclic molecules are predicted to be above 10, whereas pK_a values of thiols bonded to lignin-derived polyhydroxyphenols are predicted to be between 2 and 6. Reactivity of thiols with Hg(II) was evaluated by monitoring the kinetics of transformation of Hg(SR)₂ complexes to nanoparticulate metacinnabar in dissolved organic matter with different chemical compositions under oxic conditions using high energy-resolution X-ray absorption near-edge structure spectroscopy (HR-XANES). The most rapid transformation occurred in the material with the greatest quinone carbon (ketone) and polyphenol contents, consistent with the predicted low pK_a values of thiolated ketones near phenolic hydroxyl groups. The slowest transformation occurred in the material with the least amount of cyclic ketone structures, despite having the highest amount of thiols. These results indicate that the sulfur atoms in thiolated ketones are those that are most labile and removed during

the alkyl transfer reaction considered to nucleate metacinnabar, and that the concentration of the low- pK_a thiols controls the transformation rate.

1. INTRODUCTION

Carbon-bonded thiol groups (R-SH) are key reactive functional groups in biological systems and natural organic matter (NOM). The soft thiolate anion (R-S⁻) is a potent nucleophile that complexes with soft metal cations, such as Hg(II), Cu(I), Ag(I), and Au(I). In the cell, protein and non-protein thiols exhibit a large range of reactivity with apparent pK_a values generally between 7.5 and 9.5, occasionally around 5,¹ and exceptionally as low as 3.5 for thiol regulating thioltransferase.² The R-SH group of cysteine has a pK_a of ~8.2.³ Low pK_a 's occur when the thiolate anion is stabilized through hydrogen bond formation with a hydroxyl or an amine group.^{1, 4-8} Some bacterial cell envelopes also contain acidic sulfhydryl protons with pK_a values of 3.6-4.1.^{9, 10}

Characterizing the acid-base properties of thiols in NOM is difficult because NOM is heterogeneous and polyfunctional: carboxyl groups predominate, and pK_a values of thiol, amine, and to some extent carboxyl groups overlap. Insight into the chemical nature and reactivity of the thiol groups is possible by considering molecular structures of the carboxyl groups, which exhibit a large range of pK_a values as low as 0.5 in humic substances.^{11, 12} The strong acid character is explained by the stabilization of the carboxylate anion via intermolecular hydrogen bonding and by the clustering of carboxyl groups on alicyclic ring structures, which favors intramolecular H bond formation. Carboxyl-rich alicyclic molecules (CRAM) are comprised of condensed unsaturated alicyclic structures containing variable amounts of alcohol (R-OH), ether (R-O-R), carbonyl (R-HCO-R), and ester (R-O-HCO-R) linkages.¹³⁻¹⁵

Recently, O-rich cyclic hydrocarbons were also identified as being rich in sulfur atoms in soil humic acid and wetland dissolved organic matter (DOM) using FT-ICR-MS spectroscopy.^{16, 17} In the wetland study, 86% out of 4180 sulfur-containing molecules from the reactive hydrophobic organic acid (HPOA) fraction of DOM had C_cH_hO_oS formulae and were noticeably to considerably H-unsaturated (H/C ~ 0.7-1.5) and oxygenated (O/C ~ 0.1 – 0.7). A large proportion of this sulfur pool should be thiol groups because when DOM is reacted with inorganic sulfide (H₂S and SH⁻), as in a wetland setting, the amount of incorporated sulfur increases proportionally with the sum of carbonyl, carboxyl, and aromatic contents.¹⁸ By virtue of similarity with carboxyl groups in CRAM, one may assume that thiols attached to O-rich ring structures can be strongly acidic, and therefore proportionally more available for binding with soft metals than less acidic thiols. These thiolate anions also may be more reactive with respect to the release of soft metals. In contrast to HPOA, S-bearing formulae from FT-ICR-MS spectra of organic matter rich in open-chain aliphatic carbon have a high H/C atomic ratio (~1.5-2.0), suggesting that the thiol groups are mainly attached to H-

saturated hydrocarbon chains and are therefore probably stronger binding sites, but less available for metal binding.^{19, 20} Here, the hypothesis of a relationship between the molecular structure and relative reactivity of thiol groups in NOM was tested in two ways: theoretically from energetics calculations of thiol addition and deprotonation on model molecules, and experimentally from the rate of change in bonding structures of Hg when complexed to three DOMs having contrasted H/C and O/C quality and differences in predicted reactivity of thiols. The two approaches are described successively below.

Most thiols in NOM are acquired through the addition of bisulfide (HS^-) anion,²¹ in which the sulfur donates its lone pair to a carbon to form a R-SH σ -bond. The nucleophilic addition of HS^- is energetically most likely to occur on an electron-deficient carbon atom, such as a π -bonded carbon (R-HC=CH-R). One sp^2 carbon undergoes a sulfur nucleophile attack upon exposure to HS^- while the second hydrocarbon becomes saturated (R-HCSH-CH₂-R, Figure 1).^{22, 23} However, the two doubly-bonded C atoms need to bear different charges for an anion (HS^-) to be attached to one and a cation (H^+) to the other. A π -bonded C atom may acquire a higher positive charge when an electron-withdrawing group (EWG), such as a carbonyl, is added next to the double bond (R-HC $_{\beta}$ =C $_{\alpha}$ H-CO-R).²⁴ In this example, the carbonyl group increases the partial electronic charge at the β -position and decreases the charge at the α -position. Addition of a nucleophile to α,β -unsaturated carbonyl (enone) is referred to as a Michael reaction.²⁵

Thiol addition to enones, when written as $\text{R-HC}_{\beta}=\text{C}_{\alpha}\text{H-HCO-R} + \text{H}_2\text{S} \rightleftharpoons \text{R-HCSH=CH}_2\text{-HCO-R}$, is exergonic by typically $\Delta G = -3$ to -5 kcal mol^{-1} , which corresponds to an equilibrium constant of about $K = 10^{2-4}$.^{26, 27} However, with a pK_a of 7.0, H_2S is partly dissociated in aqueous solution, and the relevant reaction, $\text{R-HC}_{\beta}=\text{C}_{\alpha}\text{H-HCO-R} + \text{SH}^- + \text{H}^+ \rightleftharpoons \text{R-HCSH=CH}_2\text{-HCO-R}$, has a $\Delta G = -12.5$ to $-14.5 \text{ kcal mol}^{-1}$ and an equilibrium constant of about $K = 10^{9-11}$. Formation of the intermediate $[\text{R-HCSH=C}_{\alpha}\text{H-HCO-R}]^-$ carbanion upon addition of the thiolate conjugate is rate determining with an energy barrier of complexation (transition state structure) on the order of 15-20 kcal mol^{-1} relative to reactants (Figure 1).^{24, 28} Also, nucleophilic addition of HS^- is generally favored at lower pH where this intermediate carbanion is better stabilized.²³

Here, the energetics of thiol addition at circumneutral pH and its subsequent deprotonation were calculated for a range of O-ring Michael acceptors consisting of a bicyclic enone core with ether, carbonyl, carboxyl, and alcohol substituents on the scaffold. The $\text{C}_6\text{H}_8\text{O}_6$ enones are representative of the alicyclic and CO-conjugated ring structures in NOM and coal described in the literature.^{13, 14, 23, 29-31} Other thiolation reactions on electron-deficient carbon are also thermodynamically favorable, such as nucleophilic substitution and bond cleavage on σ -bonded hydrocarbon (Supporting Information, SI).²⁵ Bond cleavage breaks the molecule into two residues when it occurs on an aliphatic straight chain and opens the ring when it occurs on a cyclic molecule. However, these

reactions have high activation barriers and thus are kinetically hindered under ambient conditions without a catalyst. They were not considered in the construction of the $C_cH_hO_oS_s$ models. Notably, the calculations show that hydrogen bonding with alcoholic hydroxyl ($RS\cdots HO$) and between thiols ($RS\cdots HSR$) on enones lowers the thiol pK_a from ≥ 8 to the range of 2 to 6. Therefore, NOM rich in aromatic alcohol, such as lignin-derived polyphenols,³¹ and alicyclic thiols should contain more reactive thiol groups.

The influence of thiol functionality on its reactivity toward Hg(II) was investigated by performing complexation experiments with three different DOM samples and characterizing the evolution of reaction products with time by high energy-resolution XANES (HR-XANES) spectroscopy.³²⁻³¹ Previously we showed that metacinnabar nanoparticles (β -HgS_{NP}) nucleate and grow within days to months directly from linear Hg-dithiolate complexes ($Hg(SR)_2$) in Elliott soil humic acid (ESHA) reacted at pH 6 with 200 ng Hg(II) mg^{-1} ESHA, and that the rate is faster at a lower concentration of Hg(II).³² This result suggests that the ratio of certain types of reactive thiols to Hg(II) is important in controlling the transformation rate of the initially fast-forming $Hg(SR)_2$ complexes. Here, we tested three other IHSS materials: Pony Lake fulvic acid (PLFA), Nordic Aquatic fulvic acid (NAFA), and Leonardite humic acid (LHA) from the International Humic Substances Society (IHSS), each differing in environmental source, sulfur content and functionality, total H/C, O/C and N/C ratios, and quality (Table 1).

Pony Lake fulvic acid has a microbial algal origin,³³⁻³⁶ and thus a high N/C ratio (0.11), a high aliphatic to aromatic carbon ratio (C_a/C_ϕ), and minor carbonyl linkage. It contains as much as 30.3 mg S g^{-1} , of which 50% is exocyclic reduced sulfur (S_{Exo}).³⁷ The majority of the thiol groups is aliphatic and most likely derived from cysteine residues. Nordic Aquatic fulvic acid was isolated from a Norwegian pond in a peaty area. It contains 4.6 mg S g^{-1} , has high aromaticity and the highest proportion of carbonyl functionality of all the IHSS DOMs. A large fraction of the thiol groups should be associated with unsaturated O-rich ring structures, such as plant-derived polyhydroxyphenols.³⁸ Leonardite humic acid contains 7.6 mg S g^{-1} , and was extracted from an oxidized form of lignite, thus is also highly aromatic with abundant aryl-hydroxyl and aryl-carbonyl functionality. LHA is most similar to the ESHA investigated previously.

Although NOM molecules are complex both in composition and molecular structure, we show that our computational results on local thiol structures combined with knowledge of NOM chemical character is sufficient to demonstrate the relative capacities of different NOM samples to form β -HgS_{NP}. Based on our calculations, we predict that β -HgS_{NP} should form faster in NAFA and slower in PLFA solutions of constant Hg concentration given their differences in aromaticity and carbonyl content, and that the rate in LHA will be similar to that measured for ESHA. A faster rate of change of the β -HgS_{NP}/ $Hg(SR)_2$ ratio with time should occur for NOM with higher amounts of thiolated O-

heterocyclic rings, i.e., higher amounts of low- pK_a thiols, a supposition that would underpin the ability of NOM to precipitate β -HgS_{NP} directly from the Hg(SR)₂ complex in oxygenated environments.

2. MATERIALS AND METHODS

2.1. Hg reactivity with DOM. Stock suspensions of each DOM (PLFA, catalog no. 1R109F; NAFA, also referred to as NLFA for Nordic Lake Fulvic Acid,¹⁸ 1R105F; and, LHA, 1S104H) were prepared in 0.01 M NaNO₃ at a concentration of 800 mg DOM L⁻¹. The pH was adjusted to 6.0 ± 0.1, and the solutions were pre-equilibrated for 5 h. Mercury from a 10⁻⁴ M Hg(NO₃)₂ stock solution was added to a specific volume of each DOM stock solution in two different sets of batch experiments. All experiments were conducted at 20.0 ± 0.1 °C in either glass or Teflon-PFA[®] reaction vessels wrapped with aluminum foil and stored in dim light.

In the first set of experiments, mercury was added to 900 mL of the PLFA, NAFA, and LHA suspensions in glass reaction vessels to obtain a concentration of 25 ng Hg mg⁻¹ DOM. The vessels were kept closed with a Teflon-PFA[®]-lined cap for most of the reaction time, but periodically opened to the atmosphere to stir the suspensions and readjust the pH to 6.00 ± 0.03 as needed (pH drift was < 0.2 pH unit). Aliquots of the air-equilibrated suspensions were withdrawn in volumes of 110 mL at reaction times of 0.625, 1.6, 3.6, 9.6, 23.6, 58.6, 146.6, and 365 d. The DOM in each aliquot was immediately separated by filtration at 1000 Da on a stirred ultrafiltration cell (Millipore 8200), and the wet powder was freeze-dried and stored at -20 °C.

In the second set of experiments, mercury was added to 160 mL of the PLFA and NAFA suspensions in Teflon-PFA[®] reaction vessels to obtain a concentration of 25 ng Hg mg⁻¹ DOM. The pH was readjusted after mercury addition. Filtered and humidified air was bubbled continuously into the suspension for 90 d without readjusting the initial pH. To minimize contamination by impurities, the incoming air was subject to a strict cleansing procedure which consisted of eliminating oil mists, condensing water, eliminating fine particles through a 0.01 micron filter, and then circulating through an activated carbon filter (Figure S1, SI). The purified air was then humidified using a wash bottle filled with purified water (Milli-Q reagent grade). Because the reaction vessels were wrapped with aluminum foil, air bubbling was monitored by connecting the top of the vial to a beaker of water. These DOM samples also were collected by ultrafiltration at the end of the experiment, and the wet powders were freeze-dried and stored at -20 °C. All powders from both sets of experiments were pressed into pellets for HR-XANES spectroscopy.

2.2. HR-XANES spectroscopy. Mercury L₃-edge HR-XANES spectra were measured³⁹ with high-reflectivity analyzer crystals⁴⁰ on beamline ID26 at the European Synchrotron Radiation

Facility (ESRF). They were collected at a temperature of 10-15 K and a scan time of 15 s to reduce beam exposure, and repeated at different pristine positions on the sample pellets to increase the signal-to-noise ratio. Scans were monitored carefully for any evidence of radiation damage. The proportions of Hg species were evaluated using least-squares fitting (LSF) of the data with linear combinations of the end-member spectra. The accuracy of the amount of a fitted component was estimated to be equal to the variation of its value when the fit residual (*NSS*) was increased by 20%. *NSS* is the normalized sum-squared difference between two spectra expressed as $\Sigma[(y_{\text{exp}} - y_{\text{fit}})^2] / \Sigma(y_{\text{exp}}^2)$. The fitting program from beamline 10.3.2 at the Advanced Light Source was used.^{41, 42}

2.3. Thermochemical calculations. Calculations were performed with Gaussian 16⁴³ using a computational methodology validated previously on Hg-dithiolate complexes.⁴⁴ All calculations were performed using the polarizable continuum model (PCM⁴⁵) to represent the solvent water. The proton solvation energy was taken to be $-264.0 \text{ kcal mol}^{-1}$,⁴⁶ and the cavity radius⁴⁷ of S to be 1.6 Å such that the calculated solvation energy of HS⁻ agrees with the experimental value of $70.2 \text{ kcal mol}^{-1}$.⁴⁶ The geometry optimizations were performed using the second-order Møller-Plesset perturbation theory (MP2⁴⁸). Electronic energies of the optimized structures are given in Table S1. Atomic charges, in units of elementary charge *e*, were calculated by natural population analysis (NPA⁴⁹) of the molecular orbitals of the optimized structures. Single-point energies were evaluated on the MP2 geometries at the coupled cluster level of theory with single and double substitutions and corrections for triple substitutions (CCSD(T)⁵⁰), and the thermal and entropic contributions were obtained by performing vibrational frequency calculations at the MP2 level. This method is denoted as CCSD(T)//MP2. Transition states obtained by frequency calculations were further verified by IRC calculations.⁵¹ The standard aug-cc-pVDZ basis sets⁵² were employed for the H and C atoms and the aug-cc-pVTZ sets⁵³ for the O and S atoms. All energies are reported at a standard state of 1 mol L^{-1} and 298.15 K. For selected model compounds, relaxed potential energy scans along the C-SH distance were carried out at the MP2 level, and animations of the scans from right to left along the reaction coordinate were made to show the pathway and transition states for HS⁻ addition.

The Gibbs free energy change (ΔG) of the thiolation reactions are calculated reliably close to chemical accuracy ($1\text{-}2 \text{ kcal mol}^{-1}$) using this computational procedure.^{44, 54, 55} Predicting accurately pK_a values is more arduous because an uncertainty of 2 kcal mol^{-1} on ΔG yields an error interval of 1.5 units on pK_a .⁵⁶ A test set of eight measured pK_a values for $C_cH_hO_oS$ compounds showed that predicted pK_a values were systematically overestimated by ~ 1.0 unit over the $2.5 < pK_a < 9.4$ interval with the CCSD(T)/MP2 method and underestimated by ~ 1.0 when single-point energies were calculated at the MP2 level (Table S2 and Figure S2, SI). Since the same implicit solvent model was

used in the CCSD(T)/MP2 and the pure MP2 methods, the systematic deviation from experiment is not a solvation effect. To report accurate acidity constants, pK_a values for the $C_cH_hO_oS_s$ models were calculated at the MP2 level then corrected empirically by +1 unit ($pK_{a(corr)}$). Using this protocol, pK_a values of the test set are predicted well with a root-mean-square deviation (RMSD) of 0.5 pK_a unit.

3. RESULTS

3.1. Identification of electrophilic carbon in O-ether and ketone models. In a symmetric $C^\delta=C^\delta$ double bond, each C atom has a charge $\delta = -0.21$ e in an open-chain hydrocarbon (**1**) and $\delta = -0.23$ e in an aromatic compound (**2**) (Figures 2 and S3). Since the unsaturated C atoms have equal charge, the thiolation reaction can be initiated in principle with H^+ or HS^- on either carbon center. The intermediate products $IP(1, H^+)$ and $IP(1, HS^-)$ are calculated to be 37.2 and 57.8 kcal mol⁻¹ higher in energy than the reactants, respectively. Any transition state (TS) complex would have even higher energy (Figure 1). The activation energies of the reactions of **1** with H^+ and HS^- are too high for the thiolation reaction to be initiated without a catalyst. Breaking the symmetric C=C bond in aromatic compounds costs even more energy because it is accompanied by a loss of the aromatic resonance energy, which amounts to 36 kcal mol⁻¹ for benzene (**2**).²⁵

The C=C bond can be polarized ($C_\beta^{\delta 2}=C_\alpha^{\delta 1}$) with O or N since both are more electronegative than C. When the π -bond asymmetry is created by an O-ether group ($C_\beta H=C_\alpha H-O-R$), it is the proximal C_α atom which becomes positive ($\delta 1 = 0.21$ e) relative to the distal C_β atom ($\delta 2 = -0.34$ e) (**5**) (Figure 2). The delocalization of negative charge by an O-ether is so strong that it is observed also in (cyclo)alkanes. In this case, C_α from $R-C_\alpha H_2-O-R$ has a net charge $\delta = +0.02$ e (**6**), instead of -0.39 e in the absence of O-ether (**3**). Oxygen atoms are so polarizing that two ether oxygens in an acetal functional group $R-O-C_\alpha H_2-O-R$ raise the net charge on the saturated C_α atom to +0.38 e (**7**). ΔG values of HS^- and H^+ addition with O-ether as electron-withdrawing group (EWG) were computed for a large variety of model structures present in NOM (Figures S4 to S7, SI). Many of these reactions are thermodynamically unfavorable, and when they are feasible they have a high energy barrier. The proximal electrophilic carbon is too close to the ether oxygen anion for the HS^- anion to approach C_α . Electrostatic hindrance also prevents the formation of thiocarboxylic esters (e.g., thioacetate group) directly from the reaction of thiols or thiolate anions on carboxylate groups in NOM.

In the standard Michael reaction, the $C_\beta^{\delta 2}=C_\alpha^{\delta 1}$ bond asymmetry is created by a carbonyl group. In contrast to an O-ether EWG, here $\delta 2 > \delta 1$ with $\delta 2 = -0.03$ e and $\delta 1 = -0.41$ e (**8**). The carbonyl group has similar capacity for pulling away the electrons from the C_β atom whether the hydrocarbon chain is open (**8**) or closed (**9**) (Figures 3 and S3). In condensed ring structures the cloud of π -electrons can extend to a longer distance from the carbonyl oxygen than in $C_\beta H=C_\alpha H-CO-R$ ($d(O-$

$C_\beta = 3.60 \text{ \AA}$), therefore the $\delta 2$ charge can be delocalized beyond the C_β atom by the π system. In bicyclic enone **10**, $\delta(C_\beta^2) = -0.24 \text{ e}$ and $\delta(C_\gamma^2) = -0.17 \text{ e}$ compared to $\delta(C_\beta^2) = -0.21 \text{ e}$ and $\delta(C_\gamma^2) = -0.20 \text{ e}$ without the carbonyl group in **4** ($d(O_1-C_\gamma) = 4.16 \text{ \AA}$). Replacing the ketone ring with a quinone brings the partial charge on C_γ to -0.10 e (**11**) ($d(O_2-C_\gamma) = 5.2 \text{ \AA}$). Replacing the first carbonyl carbon with a O-ether while keeping the second quinone carbonyl group yields about the same charge of $\delta(C_\gamma) = -0.08 \text{ e}$ (**12**). The $\delta(C_\gamma)$ charge can be increased to -0.04 e by adding a second carbonyl to **12** (**13**), and to -0.05 e with three carbonyl groups (**14**). In **11**, **12**, **13**, and **14**, C_γ is the only electrophilic distal carbon which can undergo attack by HS^- . Thus, the polar character of a CO group can be extended beyond a C_β carbon in condensed ring enones through delocalization of negative charge by the π -electrons. We conclude from this that thiol groups can be attached to hydrocarbons situated relatively far from carbonyl groups in H-unsaturated and oxygen-rich DOMs, which are universally present in aquatic systems.³¹

3.2. Reaction pathway of thiol addition. The partial charge on carbon atoms in ketone is a good predictor of susceptibility to HS^- addition. When $\delta(C_{\beta/\gamma}) < -0.10 \text{ e}$, no stable intermediate product (IP) or transition state having a high energy barrier ($\Delta G^\ddagger > 50 \text{ kcal mol}^{-1}$) was obtained. The threshold value of -0.10 e is conservative because it was independent of the Michael microenvironment considered. Enones **8** to **15**, all possessing an electrophilic carbon $\delta(C_{\beta/\gamma}) \geq -0.10 \text{ e}$, are predicted to react exergonically with HS^- and H^+ ($-17.9 \leq \Delta G \leq -9.9 \text{ kcal mol}^{-1}$) (Table 2). The relaxed potential energy scans along the C-SH distance from $d(C-S) > 4 \text{ \AA}$ to the minimum bond distance of $1.8\text{-}1.9 \text{ \AA}$ are shown in Figure 4. Animations of molecular motion along the distance trajectory for enones **8**, **9**, **10**, **11**, and **13** can be viewed in Videos S1-S5 in the SI and the formation of the C-SH bond at the transition state for enones **8** and **10** in Videos S6 and S7 in the SI.

In the animations, one sees that HS^- approaches the enones with its proton directed toward the negative electrostatic charges (shown in red on the electrostatic potential surfaces). Coulomb interaction in this orientation minimizes the electrostatic repulsion between the lone pair orbitals of the sulfur atom and the regions of concentrated electron density in the molecules (π -orbital on the C=C bond and O lone pairs). The energy path of enone **11** is particularly insightful (Video S4, SI): HS^- enters the electrostatic field above the $C_\alpha^1=C_\beta^1$ bond, which has little electrophilic character ($\delta(C_\beta^1) = -0.22 \text{ e}$), then moves toward the $C_\alpha^{1,2}=C_\beta^{1,2}$ bond, and finally reaches the $C_\beta^2=C_\gamma^2$ bond where the nucleophilic attack takes place on C_γ^2 . The formation of the C-SH bond occurs at the maximum (activation) energy at $d(S-C_\gamma^2) = 2.2 \text{ \AA}$ (Video S7, SI). Before reaching the transition state, the HS^- anion forms an edge-on contact with the $C_\alpha^{1,2}=C_\beta^{1,2}$ bonds. This H- π interaction has hydrogen-bond nature with H-C distances as short as $d(H-C_\alpha^{1,2}) = 2.58 \text{ \AA}$ and $d(H-C_\beta^{1,2}) = 2.55 \text{ \AA}$. The Coulomb energy of the H- π interaction is seen in models **10**, **11**, and **12** as a decrease of $\Delta E =$

3 kcal mol⁻¹ on the potential energy scan. Cation- π interactions are common in protein structures and can pull cations out of water into hydrophobic pockets.^{57, 58} Based on these models, the H- π interaction, which is a powerful organizational force in protein structures, may contribute to the enrichment of thiol in O-rich cyclic DOM structures.

3.3. Kinetics of thiol addition. The activation energy is similar for the one-oxygen C $_{\beta}$ enones **8**, **9**, and **10** ($\Delta G^{\ddagger} = 23.6 - 24.9$ kcal mol⁻¹, Table 2), indicating that the thiolation reaction occurs at the same rate regardless of whether or not the R-C $_{\beta}$ H=C $_{\alpha}$ H-CO-R reactant is cyclic or acyclic. The two-oxygen C $_{\gamma}^2$ enones **11** and **12** have about the same activation energy for addition of HS⁻ (24.9 – 28.5 kcal mol⁻¹) as the one-oxygen C $_{\beta}$ enones **9** and **10** (23.8 – 24.9 kcal mol⁻¹). In contrast, the three-oxygen C $_{\gamma}^2$ enones **13** and **14** have lower activation energies ($\Delta G^{\ddagger} = 17.1$ and 12.4 kcal mol⁻¹) and do not need much energy to proceed ($\Delta H^{\ddagger} = 6.8$ and 2.9 kcal mol⁻¹). For the C $_{\gamma}^2$ models, there is a trend of lower activation energy with more O substituents and less negative charge on the C electrophile. This trend is supported by the work of Krenske et al.²⁴, who synthesized a Michael reactor with a NPA partial charge of 0.06 e (Figure S8a, SI) and a $\Delta G^{\ddagger}$ value for thiol addition calculated by DFT of 4.5 kcal mol⁻¹ compared to 5.6 kcal mol⁻¹ calculated here by the more accurate CCSD(T)/MP2 method. The influence of the number of ketone/ether groups on the reactivity of enones is illustrated with Models **10** and **15**. The two structures are C $_{\beta}^1$ electrophiles with a partial charge of -0.06 e, but model **10** has one O substituent whereas model **15** has four. As a result, the energy of activation is decreased from 24.9 kcal mol⁻¹ in **10** to 18.9 kcal mol⁻¹ in **15** (Table 2). The rate constants of the thiolation reactions at 298 K, deduced from the calculated $\Delta G^{\ddagger}$ values and equation $\ln(k/T) = 23.76 - \Delta G^{\ddagger}/RT$,⁵⁹ are on the order of $k = 1.8 \times 10^{-3}$ min⁻¹ for **8**, $k = 1 \times 10^2$ min⁻¹ for **13**, and 3.0×10^5 min⁻¹ for **14**. The addition of HS⁻ can be assumed to be a first-order reaction because electrophilic centers in CRAM are in great excess relative to the bisulfide anion, therefore the rate is independent of their concentration. The first-order thiolation half-lives $t_{1/2} = \ln(2)/k$ are 2.3×10^4 s for **8**, 0.4 s for **13**, and 10^{-4} s for **14**. The kinetics calculated for one- and two-oxygen enones are relatively slow at room temperature. Rates of thiolation can be accelerated by adding a substituent, typically an EWG, to C $_{\alpha}$ to stabilize the anionic charge of the enolate intermediate.^{24, 60} As accurate as these calculations are, care should be exercised when extrapolating theoretical kinetics results to real systems. The modeled reactions are one piece of a much more complex reaction and other factors are at play, such as steric hindrance, the diffusion of the bisulfide into the NOM, the strength of the Coulomb interactions, and the “cavity-effect”⁶¹, as discussed next.

The transition state activation energies were calculated for reaction of the isolated hydrocarbon adduct with free HS⁻ to form a C-S bond. At 298 K and 1 atm, the contribution of the entropy term

$T\Delta S^\ddagger$ to $\Delta G^\ddagger$ ($= \Delta H^\ddagger - T\Delta S^\ddagger$) is relatively large, ranging from 9.5 to 11.3 kcal mol⁻¹ (Table 2). In NOM, the reactants are not isolated, but instead are surrounded by the macromolecular scaffold and have fewer degrees of freedom. Thus, the activation entropy for the reaction that forms the transition state would be much smaller. This “cavity effect” could lower the energy barrier by as much as $T\Delta S^\ddagger = 10$ kcal mol⁻¹. If $\Delta G^\ddagger = \Delta H^\ddagger$, the predicted half-lives are 10⁻⁴ s for **8**, 1.1 10⁻⁸ s for **13**, and 1.8 10⁻¹¹ s for **14**.

When the bisulfide proton is attracted to electron-rich ring structures, such as plant-derived polyphenols and CRAM, the free energy of the thiolate adduct is lower than when both reactants are completely isolated (**10**, **11**, **12**, and **15** in Figures 4 and S3). This electrostatic attraction increases $\Delta G^\ddagger$, but only by about 3 kcal mol⁻¹, approximately equal to the energy of the H-bond. At the same time, in macromolecular DOM the degrees of freedom of HS⁻ would decrease, producing the reverse effect of lowering $\Delta G^\ddagger$. Therefore, we conclude that thiolation in NOM occurs faster on unsaturated cyclic structures rich in ketone and ether linkages than on open-chain carbonyl structures. These findings adequately explain the predominance of alicyclic thiol over aliphatic thiol in soil humic acid and wetland DOM.^{16, 17} They also help understand the linear relationship between the summed carbonyl, carboxyl, and aromatic contents and the propensity of DOM to incorporate HS⁻.¹⁸

3.4. pK_a values of thiols on ketones. After Michael addition of HS⁻ and H⁺ on the C=C double bond, the two C atoms becomes saturated ($\delta \approx -0.4$ e) and less capable of withdrawing electrons. S acquires the negative charge of $\delta \approx -0.1$ e, compared to about $\delta \approx 0.0$ e when bonded to an unsaturated carbon as in thiophenol (Figures 3 and S8b in SI). The thiols on cyclic and acyclic enones are therefore relatively stable with pK_a values above 8 like alkane thiols (Figure 3, Table 2). The proximity of a carboxylate group to the thiol hydrogen, as in CRAM, can raise the pK_a to around 12 through the formation of a RS-H...OOC hydrogen bond (**16**) (Figures 5 and S3). Alternatively, the acidity can be increased through stabilization of the RS⁻ conjugate base by a RS...HR hydrogen bond or by delocalizing the repulsive negative charge of the surrounding carbon atoms on a ring structure.

When a cyclic molecule contains several substituent O groups (**17**), the inductive electrostatic effect can cause pK_a to decrease to around 6-7. Hydrogen bonding can occur with an alcohol group (RS...HO-R), as in polyhydroxyphenols, or between two thiols (RS...HS-R) on polycyclic enones. Since alcoholic hydroxyls are extremely stable, a proximal thiol can acquire a pK_a value of around 2-6, depending on the spatial positioning of the alcohol group (i.e., strength of the H-bond) and number of O groups (i.e., intensity of the electrostatic effect) (models **18-21** in Figure 5). In contrast to the RS...HO-R bonding, RS...HS-R bonding does not lower the pK_a value below about 7-8 (**22**),

unless the two thiols are substituted on O-rich enones, which reinforces the weak H-bonding effect with an electrostatic effect. This case is exemplified with the models for three-carbonyl bicyclic enone **23** ($pK_a = 6.4$) and four-carbonyl bicyclic enone **24** ($pK_a = 6.0$).

Thiols other than cyclic enone-SH can have their acidity constant lowered through H-bonding (Figures 6 and S3). One example is thiophenol, the pK_a of which is decreased from 6.6 to 4.7 when the RS^- conjugate base is H-bonded to an alcohol group (**25**). Much lower and even negative pK_a values are obtained with thioacetate ($pK_a = 3.3$). The high acidity of thioacetate can be attributed to the electrostatic destabilization of the acidic proton by the highly polarized C=O group ($\delta(C) = 0.59$ e; $\delta(O) = -0.75$ e for model **26**). The protonated sulfur atom has a charge of -0.03 e. It can be increased by as much as $+0.09$ e by H-bonding of the carbonyl oxygen to alcohol or carboxylic groups (Models **27** to **29**). A proton attached to a positively charged sulfur atom is extremely acidic with a pK_a around -2 . Occurrence of thioacetate groups in organic matter is, however, hypothetical, whereas aromatic thiols are more likely.⁶² From this analysis, we infer that the acid-base properties of thiol groups are influenced by their microenvironment, such as the nature and spatial positioning of substituent functional groups on the ring structure and intermolecular electrostatic interactions. Any factor which makes the thiolate anion more stable increases the acidity of thiol. A recent FT-ICR-MS analysis of 6166 molecular formulae showed that carboxyl and hydroxyl groups are most dominant in terrestrial and aquatic DOM.³¹ A thiol would retain its proton when close to a carboxylate group and lose it when next to a hydroxyl group. Thus, the fraction of thiol groups bonded to CRAM is predicted to be basic and the fraction bonded to polyhydroxyphenols is predicted to be acidic with pK_a values between 2 and 6, and perhaps even lower.

The results of the calculations in this and the previous sections indicate that within NOM thiolate anions have a range of acid/base character that is a function of local microstructure, implying that they also have a range of reactivity with respect to metal binding. It has been demonstrated for low-molecular-mass thiols that pK_a values and binding constant strengths for Hg are positively correlated: thiols with low pK_a values bind Hg less strongly than thiols with high pK_a values.⁷ The variability in thiol pK_a in our NOM structures can be used to explain how the alkyl transfer reaction initiates the nucleation of nanoparticulate metacinnabar.^{32, 63} When two linear $RS-Hg-SR$ complexes react, bonds to two thiolate anions must break according to the reaction: $RS-Hg-SR + RS-Hg-SR \Rightarrow RS-Hg-S-Hg-SR + R-S-R$. Which bonds break will be those that are weaker, implying that they are bonds to thiolate anions from lower- pK_a thiols. In the next section, we link the computational results to results from a set of experiments designed to test whether variability in predicted relative amounts of reactive thiols in NOM can explain the formation rate of metacinnabar from the linear complexes.

3.5. Kinetics of formation of β -HgS_{NP} from Hg(SR)₂ complexes in NOM. We tested the hypothesis that unsaturated O-rich cyclic structures rich in OH groups have more reactive thiols than open-chain aliphatic structures by aging PLFA, NAFA, and LHA that initially contained Hg(SR)₂ complexes for periods of up to one year and examining the products using HR-XANES spectroscopy. We had shown previously with Elliott Soil humic acid (ESHA) that the Hg(SR)₂ complex transforms into β -HgS_{NP} with a half-time of 7.4 ± 0.5 days at a mercury concentration of 200 ng Hg mg⁻¹ ESHA.³² The reaction proceeded to a greater extent at 28 ng Hg mg⁻¹ ESHA. Here, the test was performed at 25 ng Hg mg⁻¹ DOM. PLFA has the lowest aromaticity and quantity of cyclic ketone structures, while being the richest in sulfur (Table 1). NAFA has intermediate aromaticity and S content, but is extremely rich in quinone carbon because it derives from vascular plant debris.^{64, 65} Leonardite humic acid has the highest proportion of aromatic carbon, has less quinone carbon than NAFA but more phenolic hydroxyls, and its S content is between those of PLFA and NAFA. NAFA and LHA also differ significantly from one another in the size of their constitutive molecules. Fulvic acids are much smaller, and therefore more likely to participate in intermolecular H-bond interactions than humic acids.

Near the beginning of the kinetic experiments ($t = 0.625$ d), the HR-XANES spectrum for LHA has an intense near-edge peak at 12279.2 eV and a bell-shaped top edge, two features that are characteristic of Hg(II) linearly-coordinated to two thiol sulfur ligands (Hg(SR)₂ complex)⁶⁶ (Figure 7a). The near-edge peak is lower in intensity in PLFA but the top edge still has a rounded shape. The reduction in amplitude is explained by secondary bonding to N ligands from amine groups,³⁹ which are abundant in this algal-derived DOM (N/C=0.11). The Hg[(SR)₂+N] coordination is observed at 25 ng Hg mg⁻¹ DOM, but not at 200 ng Hg mg⁻¹ DOM.³² The NAFA $t = 0.625$ d spectrum is distinct from those of LHA and PLFA. It has features like those of β -HgS_{NP} including a weak near-edge peak and a bumpy profile in the above-edge region indicative of Hg-Hg pairs.³² With time, the three DOM spectra progressively look more similar to β -HgS_{NP}, although with different time-dependence and degree of similarity after one year. The $t = 365$ d spectra of NAFA and LHA are closer to the β -HgS_{NP} spectrum than PLFA.

The fractional amount of each Hg species at a given time was calculated by fitting linear combinations of the $t = 0.625$ d (LHA and PLFA) and β -HgS_{NP} spectra to the data sets. Since the NAFA $t = 0.6$ d spectrum is a mixture of Hg(SR)₂ and β -HgS_{NP}, we used the spectrum of Hg-NAFA at 200 ng Hg mg⁻¹¹⁶⁶ as its Hg(SR)₂ end-member. The transformation of the Hg(II)-dithiolate complex into β -HgS_{NP} vs. log time is sigmoidal and approaches a plateau at a mole fraction, f , of Hg as β -HgS_{NP} equal to about 0.7 in the three DOMs (Figure 7b). The conversion reaction of Hg-DOM complexes to β -HgS_{NP} approached completion without consuming all the Hg in the DOM. The transformation curves were fit with the Avrami-Erofeev equation

$$f = a(1 - \exp[-kt^n]) = a(1 - \exp[-0.693(\frac{t}{t_{1/2}})^n]) ,$$

for nucleation-growth reactions in the solid state,⁶⁷ where a is a constant, t is time, n is the reaction order, and k is the Avrami rate coefficient described in terms of the crystallization half-time $t_{1/2}$. The reaction order is close to 1 ($n \approx 0.93 \pm 0.15$), whereas it was 2.0 ± 0.3 for ESHA at $200 \text{ ng Hg mg}^{-1}$ DOM.³² At 25 ng Hg mg^{-1} , the transformation rate is probably limited by the Hg concentration. The reactive thiol groups would be in great excess relative to Hg, and so have a zero order effect on rate. The crystallization half-time is $t_{1/2} = 0.3 \text{ d}$ for NAFA, 8 d for LHA, and 61 d for PLFA. The most reactive DOM is NAFA, which is the richest in quinone carbon, and the least reactive is PLFA, which has the least quinone carbon. In the experiments continuously bubbled with filtered air, the amount of $\beta\text{-HgS}_{\text{NP}}$ formed after 90 d was the same as that formed over a similar time period in the experiments that were periodically opened and equilibrated with air for one year (Figure S9, SI). Thus, $\beta\text{-HgS}_{\text{NP}}$ is a transformation product of Hg-dithiolate complexes in DOM under oxic conditions, and not a reaction product of Hg(II) with free sulfide, which dissimilatory microorganisms might have been able to generate. The $\beta\text{-HgS}_{\text{NP}}$ is formed from Hg(II)-dithiolate complexes by dealkylation.⁶³

We conclude that thiolate groups attached to unsaturated (O,OH)-rich ring structures are most reactive toward the formation of $\beta\text{-HgS}_{\text{NP}}$. Such structures may also explain the faster dissolution rates observed for cinnabar in the presence of DOM with high aromaticity.^{68, 69} Nucleation of mercury sulfide is probably facilitated by the clustering of these reactive thiolate groups in hydrophobic domains of DOM. Also, the smaller molecular size of fulvic acid molecules may facilitate intermolecular H-bonding, in particular via alcohol groups that are more abundant on ring structures. It remains to be determined if the $\beta\text{-HgS}_{\text{NP}}$ nucleated by the highly labile thiols in DOM can be easily methylated by microbial activity. Recent work indicated that the mercury in nanoparticulate metacinnabar formed under oxic conditions in DOM may be dissolved by microbially-exuded thiols and converted to $\text{Hg}(\text{SR})_2$ forms that are bioaccessible to fish.⁷⁰ However, the relative susceptibility to microbial methylation of nanoparticulate metacinnabar vs. $\text{Hg}(\text{SR})_2$ forms of mercury in even highly sulfurized DOM is still unknown.¹⁸

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Description of the thiolation reaction videos, supplementary table and figures, and Cartesian coordinates of some of the enones (PDF)

Thiolation reactions (AVI)

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Notes

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LEGENDS TO FIGURES

Figure 1. Gibbs free energy profile and structure of the complexes for addition of HS[−] and H⁺ to a C₇ hydrocarbon Michael acceptor (enone **8**) computed at the CCSD(T)/MP2 level of theory in PCM water. TS is the transition state and IP is the intermediate product (enolate). Free energies are in kcal mol^{−1} at 1 mol L^{−1} and 298.15 K.

Figure 2. Atomic charges in hydrocarbon molecules calculated by natural population analysis (NPA) of the geometry optimized structures. Oxygen, carbon, and proton are represented as red, grey, and light grey, respectively. Atomic charges, in units of elementary electron charge, are in blue.

Figure 3. Optimized structures of the enone models **8** to **15** before and after thiolation. Atomic charges are in blue. Sulfur, oxygen, carbon, and proton are represented as yellow, red, grey, and light grey, respectively. The thiolation reactions can be viewed in Videos S1 to S7 (SI) and are described in the SI. For enone **8**, the electrophilic addition of HS[−] can be viewed in Video S1 (SI) and the formation of the C_β-SH bond at the maximum energy of the reaction pathway (TS) is shown in Video S6 (SI), which represents the frequency of the transition state. Videos S2, S3, S4, and S5 (SI) show the addition of HS[−] to enones **9**, **10**, **11**, and **13**, and, Video S7 (SI) shows the transition state for enone **10**.

Figure 4. Potential energy scans of the thiolation reactions for enones **8** to **15** along the C-S distance. The geometry was re-optimized at the MP2 theory level at each distance. Electrostatic potential energy maps are inset. Negatively and positively charged surfaces are represented as red and blue shades, respectively.

Figure 5. Optimized structures of the thiolated enones **16** to **25** with atomic charges in blue and predicted pK_a values.

Figure 6. Optimized structures of thioacetates **26** to **29** with atomic charges in blue and predicted pK_a values.

Figure 7. (a) Time series Hg L₃-edge HR-XANES spectra of Nordic Aquatic fulvic acid (NAFA), Leonardite humic acid (LHA), and Pony Lake fulvic acid (PLFA) reacted with 25 ng Hg(II) mg⁻¹ DOM in air-equilibrated water for up to one year. The three sets of spectra are binary, each consisting of variable mixtures of a dithiolate complex (Hg(SR)₂) and nanoparticulate β-HgS. (b) Proportion of β-HgS_{NP} as a function of log time. Amounts of β-HgS_{NP} were obtained by fitting each aged Hg-DOM spectrum with Hg(SR)₂ and β-HgS_{NP} spectra. The sigmoidal curve indicates a nucleation–growth mechanism, here described by the Avrami–Erofeev equation. The rate of the reaction follows the order NAFA > LHA > PLFA.

Table 1. Sulfur concentration, elemental ratios, and C species composition estimated on the basis of ^{13}C -NMR of the three DOM samples

Sample ^a	[S] % g/g	H/C	O/C	N/C	C species % (δ ppm)				Aromaticity ^b
					alkyl-C O-alkyl-C (0-110)	aryl-C O-aryl-C (110-165)	COO-C (165-190)	CO-C (190-220)	
PLFA ^c	3.00	1.23	0.45	0.11	69.6	12	17	1.2	0.15
NAFA ^d	0.46	0.91	0.65	0.01	37	31	24	10	0.46
LHA ^e	0.76	0.69	0.37	0.02	19	58	15	8	0.75

^aSource: IHSS, <https://humic-substances.org/>. ^bAromaticity = (% of 110-165 ppm) / (% of 0-165 ppm). ^cPLFA = Pony Lake Fulvic Acid.

^dNAFA = Nordic Aquatic Fulvic Acid. ^eLHA is a humic acid extracted from leonardite, an oxidation product of lignite.

Table 2. Computed atomic charge of the electrophilic C atom, thermodynamics of thiol addition to enone acceptors, and acidity constant of the thiol groups. Enones before and after thiolation are shown in Figure 3.

Enone ^a	Atomic charge (δ) ^c	Transition state			IP ^b ΔG^{IP}	Product		Predicted $\text{p}K_{\text{a}}$
		$\Delta H^{\ddagger d}$	$\Delta G^{\ddagger}$	$T\Delta S^{\ddagger}$		ΔH	ΔG	
8	-0.03	12.3	23.6	-11.3	19.0	-26.1	-15.7	10.1
9	-0.04	13.5	23.8	-10.3	21.6	-25.1	-14.9	9.5
10	-0.06	14.8	24.9	-10.1	23.7	-23.2	-13.3	9.4
11	-0.10	15.4	24.9	-9.5	9.7	-27.2	-17.3	9.5
12	-0.08	18.5	28.5	-10.0	22.1	-27.5	-17.4	9.1
13	-0.04	6.8	17.1	-10.3	11.1	-25.5	-15.2	8.1
14	-0.05	2.9	12.4	-9.5	-3.1	-25.2	-15.0	8.1
15	-0.06	9.1	18.9	-9.8	17.0	-19.8	-9.9	5.2

^aEnones shown in Figure 3 (before thiolation) and Figure 4 (after thiolation). ^bIP = intermediate product.

^c δ in electron unit. ^dAll thermodynamic values for transition state, intermediate product and final product in kcal mol⁻¹ and PCM water at 298.15 K and the standard molar concentration of 1 mol L⁻¹.

Figure 1

Figure 2

Optimized structures and atomic charges before thiolation

Optimized structures and atomic charges after thiolation

Figure 3

Figure 4

Figure 5

(26) - Protonated - $pK_a = 4.9$

(27) - Protonated - $pK_a = 1.9$

(28) - Protonated - $pK_a = -0.9$

(29) - Protonated - $pK_a = -2.2$

Figure 6

Figure 7

Supporting Information for:

Thiols in Natural Organic Matter: Molecular Forms, Acidity, and Reactivity with Mercury(II) from First-Principles Calculations and HR-XANES Spectroscopy

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Content

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5. Cartesian coordinates of enones 8-29

1. Description of the videos

Video Number	Enone Model Structure (Figure 3)
S1	8
S2	9
S3	10
S4	11
S5	13
S6	Transition state 8
S7	Transition state 10

The animated Michael reaction on enone **8** (Video S1) shows that the HS⁻ nucleophile attack takes place through two Coulomb interactions. The first is an edge-on attraction of the proton on the π -electrons of the C $_{\alpha}$ =C $_{\beta}$ bond. The proton initially approaches the proximal C $_{\alpha}$ carbon at 2.51 Å, then the distal C $_{\beta}$ carbon at 2.48 Å. At this point, the orientation of the HS⁻ anion flips in the direction of the carbonyl oxygen to form a strong H $\cdots$ O bond at 2.48 Å that stabilizes the S atom 3.09 Å above the electrophile C $_{\beta}$ carbon ($\delta = -0.07e$). The nucleophile attack starts at this position with a progressive diminution of the C $_{\beta}$ -S distance while maintaining the H $\cdots$ O bond distance at 2.5-2.6 Å (Figure 1). The C $_{\beta}$ -SH bond is formed at 2.33 Å and is accompanied by a sudden rotation by 60° of the thiol proton away from the carbonyl oxygen, thus

breaking the H $\cdots$ O bond. The formation of the C $_{\beta}$ -SH bond is shown in Video S6 as an animation of the imaginary frequency of the transition state.

In bicyclic enones having a negative surface charge (enones **9**, **10**, and **11**), HS $^{-}$ moves in the π -electron cloud until reaching an electrophile carbon. The nucleophilic attack takes place without formation of an H $\cdots$ O bond. In enone **9**, the bisulfide proton cannot cross the H-saturated C $_{\alpha}^{1,2}$ -C $_{\beta}^{1,2}$ bond. When the enone has three electron-withdrawing O groups, such as enone **13**, the ring surface becomes positively charged and the bisulfide proton is attracted by van der Waals forces at a distance of 2.8-2.9 Å from the ring (Video S5). In bicyclic enones **13** and **14**, the C $_{\gamma}$ -S bond formation is followed by a reorganization of the enolate structure with an out-of-plane to in-plane rotation of the thiol group (Video S7). This reorganization has an activation energy of several kcal mol $^{-1}$ (Figure 4).

2. Supplementary tables

Table S1. Total electronic energy (EUMP2) in Hartree atomic units (E $_h$)

1	-235.0882	9-SH	-861.154696	14-SH	-1007.82581	19-S	-1009.77189	24-S	-1404.27235
2	-231.543223	10	-461.021885	15	-644.855857	20-SH	-1084.16523	25-SH	-897.963731
3	-236.300721	10-SH	-859.953551	15-SH	-1043.77925	20-S	-1083.71961	25-S	-897.516824
4	-388.272621	11	-534.957991	16-SH	-1083.73731	21-SH	-1159.30954	26-SH	-778.244089
5	-349.393662	11-SH	-933.89513	16-S	-1083.27364	21-S	-1158.8672	27-SH	-743.923047
6	-311.419807	12	-496.967488	17-SH	-931.818693	22-SH	-1332.82583	28-SH	-778.70135
7	-425.724381	12-SH	-895.905721	17-S	-931.366931	22-S	-1332.37287	29-SH	-968.987977
8	-348.221847	13	-570.904751	18-SH	-971.032876	23-SH	-1368.77663		
8-SH	-747.157762	13-SH	-969.839854	18-S	-970.583121	23-S	-1368.32545		
9	-462.219405	14	-608.891889	19-SH	-1010.22041	24-SH	-1404.72287		

Table S2. Experimental and calculated pK $_a$ values^a

Chemical	pK $_a$ (exp)	<pK $_a$ > ^a	pK $_a$ (mp2)	pK $_a$ (mp2) + 1
Thiobenzoic acid ^b	2.5	2.4	1.4	2.4
Thioacetic acid	3.3	2.8	1.8	2.8
PhSH+NO $_2$ +CO $_2$ ^c	4.4	5.0	4.1	5.1
PhS+COOH para ^d	6.0	6.8	5.8	6.8
Thiophenol	6.6	6.2	5.1	6.1
4-Hydroxythiophenol ^e	7.0	6.6	5.6	6.6
Hydrogen sulfide	7.0	7.1	6.2	7.2
Benzylthiol	9.4	9.6	8.7	9.7
Ethanethiol	9.7	11.2	10.3	11.3
Methanethiol	10.3	11.2	10.4	11.4
3-Mercaptopropionic acid	10.8	12.3	11.4	12.4

^aAverage of the MP2 and CCSD(T) values. ^bRef.¹. ^cRef.². ^dRef.³. ^eRef.⁴

3. Supplementary figures

Figure S1. Schematic diagram of the air-saturated experiment 2.

Figure S2. Benchmarking of the quantum-chemical method used to obtain accurate pK_a values. (a) $pK_a(\text{MP2})$: Gibbs free energy of deprotonation calculated with the MP2 method. $\langle pK_a \rangle$: average value of the $pK_a(\text{MP2})$ and $pK_a(\text{CCSD(T)/MP2})$ values. $pK_a(\text{exp})$: experimentally determined values from the literature (Table S1). (b) In the CCSD(T)/MP2 method, the single point total electronic energy of the optimized structure (E) is calculated at the CCSD(T) level and the sum of the thermal and entropic corrections are calculated at the MP2 theory level. The prediction of the $\langle pK_a \rangle$ values is excellent in the [2.5,9.5] pH interval, whereas the $pK_a(\text{MP2})$ values are underestimated by 1 pK_a unit. (b) Linear regression of calculated $pK_a(\text{MP2})+1$ values against experimental pK_a values. The root-mean-square deviation (RMSD) is 0.5 pK_a unit in the [2.5,9.5] pH interval.

1

2

3

4

5

6

7

8

8-SH

9

9-SH

10

10-SH

11

11-SH

12

12-SH

13

13-SH

14

14-SH

15

15-SH

16-SH

16-S

17-SH

17-S

18-SH

18-S

19-SH

Figure S3. Line diagrams of models 1-29

Figure S4. Gibbs free energy (kcal mol⁻¹) of thiolation of ether model compounds. These reactions are kinetically hindered.

Figure S5. Gibbs free energy (kcal mol⁻¹) of thiolation of alkane ether and ketone model compounds. These reactions are kinetically hindered.

Figure S6. Gibbs free energy (kcal mol⁻¹) of thiolation of furan (a), tetrahydrofuran (b), and thiophene (c). Thiophene cannot be thiolated with HS⁻ and H₂S at ambient condition without a catalyst.

Figure S7. Examples of model structures and coordination environments unsuitable for chemical thiolation at room temperature without a catalyst.

Figure S8. (a) Optimized structure and NPA atomic charges of adduct **3** of Krenske et al.⁵ (b) Optimized structure and NPA atomic charges of thiophenol.

Figure S9. (a, b) Hg L₃-edge HR-XANES spectra of NAFA and PLFA reacted with Hg(II) for 0.625 d and 90 d in air-saturated solutions compared to the β -HgSNP spectrum (experiment 2). Mercury sulfide

is formed even when air is dispersed constantly in solution. (c, d) Comparison of the air-saturated spectra after 90 d (experiment 2) to those from the nearest time periods in the one-year experiments in air-equilibrated solution (experiment 1). The near-perfect matching of the spectra shows that β -HgS_{NP} formed at the same rate in the two types of experiments. (e) NAFA and PLFA spectra after 90 d of reaction in the air-saturated solution compared to the β -HgS_{NP} spectrum. Nanoparticulate metacinnabar also formed faster in NAFA than PLFA in the air-saturated solution (experiment 2) like in the air-equilibrated solution (experiment 1, Figure 6).

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5 Cartesian coordinates of enones 8-29

Enone 8 protonated

16	2.916173000	2.665559000	1.991297000
6	4.546966000	-0.119324000	1.097966000
6	4.163695000	0.987224000	0.108397000
6	-1.261488000	-0.103649000	-0.945396000
6	2.838685000	1.701732000	0.425536000
6	-0.899725000	0.418460000	0.460894000
6	1.664435000	0.727711000	0.553220000
8	0.146611000	2.536633000	0.048225000
6	0.291616000	1.352260000	0.353755000
1	4.063394000	0.559897000	-0.906607000
1	-2.131803000	-0.774149000	-0.889928000
1	1.758640000	-0.069620000	-0.209753000
1	1.667796000	0.204540000	1.526261000
1	3.810356000	-0.938142000	1.090564000
1	4.965769000	1.743360000	0.057845000
1	-0.424175000	-0.666454000	-1.387276000
1	-1.744248000	0.985712000	0.881057000
1	-1.509624000	0.735273000	-1.613125000
1	-0.665149000	-0.424638000	1.129010000
1	5.524850000	-0.548944000	0.830560000
1	4.615942000	0.274024000	2.123938000
1	3.854490000	3.542897000	1.591667000
1	2.608017000	2.421219000	-0.375410000

Enone 9 protonated

16	-0.616051000	0.097362000	1.184644000
8	2.787332000	1.297620000	0.053100000
6	0.627587000	-0.826502000	-1.627429000
6	0.646436000	-1.877549000	-2.743223000
6	1.810486000	0.150306000	-1.849655000
6	1.918105000	-2.735932000	-2.649720000
6	3.109683000	-0.590262000	-2.037544000
6	3.150805000	-1.898022000	-2.392138000
6	-0.688943000	-0.046673000	-1.547308000
6	1.828862000	1.153689000	-0.706383000
6	-0.667501000	1.001157000	-0.419973000
6	0.542822000	1.950430000	-0.561787000
1	-1.535225000	-0.739538000	-1.399746000
1	-1.599204000	1.586762000	-0.432401000
1	0.611837000	-1.357413000	-3.718296000
1	2.057790000	-3.315741000	-3.578234000
1	0.404073000	2.547283000	-1.482849000
1	4.041877000	-0.038281000	-1.877064000
1	4.127225000	-2.386060000	-2.499086000
1	-0.855405000	0.482238000	-2.504464000
1	0.800675000	-1.349026000	-0.666109000
1	1.571093000	0.741185000	-2.759622000
1	-0.250651000	-2.516388000	-2.675208000
1	1.810727000	-3.480035000	-1.838267000
1	-0.723211000	1.181661000	1.973995000
1	0.623970000	2.633742000	0.296486000

Enone 10 protonated

16	-0.755549000	0.380868000	0.719294000
8	2.845444000	1.825322000	-0.440015000
6	0.623767000	-0.469151000	-2.119890000
6	0.686925000	-1.766186000	-2.895395000
6	1.792562000	0.039179000	-1.603468000
6	1.716146000	-2.733712000	-2.293921000
6	3.053820000	-0.711149000	-1.731536000
6	3.035614000	-2.030283000	-2.064531000
6	-0.695847000	0.251565000	-2.012916000
6	1.811629000	1.369259000	-0.947087000
6	-0.712288000	1.288719000	-0.882906000
6	0.528065000	2.181708000	-0.979615000
1	-1.506823000	-0.483789000	-1.878503000
1	-1.628737000	1.894355000	-0.943350000
1	0.979101000	-1.521164000	-3.937609000
1	1.847478000	-3.601126000	-2.958961000
1	0.564400000	2.932958000	-0.176291000
1	3.990279000	-0.189564000	-1.516964000
1	3.969267000	-2.600720000	-2.108782000
1	-0.901104000	0.771553000	-2.970189000
1	-0.308932000	-2.234845000	-2.939156000
1	1.343164000	-3.123069000	-1.325325000
1	-0.901900000	1.467805000	1.498744000
1	0.503430000	2.717185000	-1.949534000

Enone 11 protonated

16	0.332228000	4.020096000	-0.515623000
8	3.815284000	0.729359000	-3.163137000
8	0.051157000	-2.704644000	-1.527896000
6	0.767360000	-0.442066000	-1.638341000
6	0.914389000	-1.900829000	-1.913041000

6	1.751066000	0.431990000	-2.025470000
6	2.109221000	-2.384618000	-2.651479000
6	2.940974000	-0.061777000	-2.776704000
6	3.067006000	-1.513909000	-3.062960000
6	-0.481390000	0.000828000	-0.916417000
6	1.685846000	1.913916000	-1.738858000
6	-0.675712000	1.522263000	-0.980796000
6	0.649135000	2.222426000	-0.653980000
1	-0.418494000	-0.335501000	0.134366000
1	-1.464458000	1.826991000	-0.274170000
1	1.015760000	1.867180000	0.324143000
1	3.957171000	-1.834865000	-3.611404000
1	-1.346424000	-0.522875000	-1.353881000
1	2.171924000	-3.459962000	-2.841006000
1	2.686816000	2.264843000	-1.439578000
1	-0.996125000	1.820702000	-1.995063000
1	1.605036000	4.393305000	-0.287157000
1	1.426767000	2.454610000	-2.667765000

Enone 12 protonated

16	1.095028000	4.091477000	-0.752571000
8	-0.175656000	-2.627631000	-1.554480000
6	0.793282000	-0.453152000	-1.648662000
6	0.772908000	-1.892378000	-1.918613000
6	1.872322000	0.302256000	-2.040270000
6	1.935615000	-2.392635000	-2.642148000
8	2.941464000	-0.224487000	-2.704640000
6	2.947866000	-1.548516000	-2.995871000
6	-0.375132000	0.186077000	-0.935938000
6	2.034279000	1.777151000	-1.803443000
6	-0.368935000	1.717006000	-1.066975000
6	1.027496000	2.261583000	-0.749498000
1	-0.348842000	-0.108215000	0.128548000
1	-1.114668000	2.154357000	-0.383954000
1	1.348164000	1.912245000	0.246468000
1	3.852418000	-1.837276000	-3.532810000
1	-1.304630000	-0.236035000	-1.350818000
1	2.007563000	-3.450365000	-2.903635000
1	3.070547000	1.974474000	-1.483855000
1	-0.638803000	2.013953000	-2.096167000
1	1.874490000	2.313596000	-2.757441000
1	0.261423000	4.275281000	0.287124000

Enone 13 protonated

16	0.660759000	3.646848000	0.481668000
8	2.917075000	-0.004301000	-2.323882000
8	-0.225469000	-2.638852000	-2.458604000
8	-1.564043000	-0.736932000	-0.838724000
6	0.639051000	-0.500536000	-1.741316000
6	0.719462000	-1.832592000	-2.385578000
6	1.737783000	0.338231000	-1.758648000
6	2.029547000	-2.127787000	-2.968406000
6	3.045094000	-1.225697000	-2.917373000
6	-0.607760000	-0.000297000	-1.081187000
6	1.798722000	1.724191000	-1.180649000
6	-0.646297000	1.478905000	-0.715696000
6	0.698942000	1.931026000	-0.136150000
1	-1.470623000	1.633285000	-0.005061000
1	0.931498000	1.336519000	0.764734000
1	4.045447000	-1.366123000	-3.327632000
1	2.192627000	-3.089138000	-3.459428000

1	2.799637000	1.884179000	-0.751358000
1	-0.858444000	2.055842000	-1.635753000
1	0.355251000	4.217010000	-0.699744000
1	1.670225000	2.447854000	-2.007111000

Enone 14 protonated

16	0.533846000	3.650934000	0.543477000
8	-0.306483000	-2.392375000	-2.696315000
8	-1.388073000	-0.850105000	-0.592254000
8	4.013001000	0.740095000	-2.437418000
6	0.677395000	-0.434670000	-1.731218000
6	0.715241000	-1.730231000	-2.484697000
6	1.770576000	0.397548000	-1.725861000
6	2.017945000	-2.179885000	-3.040922000
6	3.040733000	-0.026711000	-2.404181000
6	3.110019000	-1.371804000	-3.019159000
6	-0.567256000	-0.025551000	-0.990666000
6	1.783637000	1.758342000	-1.075842000
6	-0.688985000	1.459184000	-0.701690000
6	0.633855000	1.939092000	-0.079834000
1	-1.540662000	1.620613000	-0.026134000
1	0.841883000	1.348017000	0.829951000
1	4.065819000	-1.665822000	-3.461687000
1	2.029197000	-3.165427000	-3.515112000
1	1.704586000	2.514592000	-1.880425000
1	-0.863268000	2.001332000	-1.649579000
1	0.266898000	4.220299000	-0.647744000
1	2.757114000	1.921686000	-0.589080000

Enone 15 protonated

16	1.456049000	-0.735915000	-5.371454000
8	4.434136000	-2.116841000	-3.137972000
8	-1.321893000	0.359418000	-3.221231000
8	3.220539000	1.771219000	-0.715721000
8	3.303483000	-0.382123000	-2.321900000
6	0.958828000	-0.118617000	-2.817770000
6	0.982471000	-1.333576000	-3.696020000
6	2.106282000	0.263056000	-2.180739000
6	2.004684000	-2.320830000	-3.119359000
6	3.342560000	-1.653592000	-2.905057000
6	-0.273879000	0.683927000	-2.645890000
6	2.172573000	1.462866000	-1.289956000
6	-0.221302000	1.887710000	-1.772672000
6	0.925000000	2.251383000	-1.138179000
1	-0.017762000	-1.784967000	-3.748863000
1	0.981205000	3.131523000	-0.492199000
1	-1.148341000	2.458063000	-1.668922000
1	1.648812000	-2.672179000	-2.132940000
1	1.345909000	-1.929466000	-5.986850000
1	2.159943000	-3.190405000	-3.771493000

Enone 16 protonated

16	0.301655000	-1.934496000	-4.247797000
8	2.908732000	-0.063777000	-2.623136000
8	4.126503000	-1.769684000	-3.355644000
8	-1.090799000	0.765068000	-3.902423000
8	-2.498079000	1.645169000	-2.364631000
6	-1.493194000	0.962588000	-2.705323000
6	0.583565000	-0.375432000	-1.988156000
6	0.662287000	-1.803683000	-2.430932000
6	1.702519000	0.397976000	-2.076081000

6	2.079882000	-2.324684000	-2.164118000
6	3.114855000	-1.410290000	-2.779981000
6	-0.693286000	0.293395000	-1.533214000
6	1.728799000	1.802423000	-1.686499000
6	-0.382003000	1.345198000	-0.451357000
6	0.714523000	2.280905000	-0.911291000
1	-1.367024000	-0.467238000	-1.099336000
1	-0.047843000	0.836958000	0.476387000
1	-0.069388000	-2.423137000	-1.890480000
1	2.256879000	-2.336726000	-1.072534000
1	0.727789000	3.321003000	-0.570796000
1	2.240925000	-3.335748000	-2.563104000
1	-0.355702000	-0.711194000	-4.263120000
1	2.568950000	2.425976000	-2.005217000
1	-1.302151000	1.899468000	-0.218270000

Enone 17 protonated

16	-1.342847000	1.930525000	-0.750802000
8	-0.492400000	-1.622494000	-0.437223000
8	1.681451000	1.114547000	0.662809000
8	-2.699666000	-1.848790000	-0.326473000
6	0.553608000	0.396236000	0.372908000
6	-0.766237000	0.995493000	0.727035000
6	0.643518000	-0.881562000	-0.103770000
6	-1.713688000	-0.150431000	1.108463000
6	-1.717374000	-1.249938000	0.066350000
6	1.934004000	-1.539598000	-0.198569000
6	2.790581000	0.755483000	-0.205789000
6	3.033059000	-0.734100000	-0.173732000
1	1.988211000	-2.631352000	-0.208995000
1	3.644366000	1.333168000	0.168007000
1	-0.643413000	1.697128000	1.565647000
1	-1.356494000	-0.600492000	2.053402000
1	4.050405000	-1.132456000	-0.154497000
1	2.527507000	1.090243000	-1.229859000
1	-2.748694000	0.189320000	1.245788000
1	-2.436256000	2.470446000	-0.176939000

Enone 18 protonated

16	0.172670000	-1.734328000	-4.217426000
8	4.149224000	-1.568070000	-3.578076000
8	-1.397316000	0.777371000	-2.594250000
8	2.932124000	0.051623000	-2.667940000
6	0.652974000	-0.364900000	-1.950427000
6	0.740186000	-1.771822000	-2.458033000
6	1.736115000	0.453295000	-2.069132000
6	2.185993000	-2.262972000	-2.324039000
6	3.162846000	-1.281287000	-2.928774000
6	-0.650376000	0.238886000	-1.475689000
6	1.741451000	1.848577000	-1.644232000
6	-0.426367000	1.394024000	-0.500320000
6	0.697104000	2.314725000	-0.908047000
1	-1.255488000	-0.543009000	-0.982931000
1	-0.191155000	0.972779000	0.496761000
1	0.060249000	-2.433734000	-1.901150000
1	2.429890000	-2.341062000	-1.247956000
1	2.346512000	-3.240569000	-2.797778000
1	0.001597000	-3.062144000	-4.372253000
1	-1.373503000	1.947482000	-0.398837000
1	-1.465915000	0.074067000	-3.264264000
1	2.592836000	2.476394000	-1.919039000

1	0.695168000	3.349710000	-0.553276000
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Enone 18 deprotonated

16	0.077373000	-1.795419000	-4.134591000
8	4.087006000	-1.527054000	-3.677373000
8	-1.342533000	0.681639000	-2.611254000
8	2.942751000	0.050099000	-2.614205000
6	0.680708000	-0.393371000	-1.858413000
6	0.740179000	-1.785411000	-2.404114000
6	1.753424000	0.437659000	-1.983535000
6	2.197085000	-2.266930000	-2.334301000
6	3.139411000	-1.266923000	-2.956459000
6	-0.634633000	0.218730000	-1.434275000
6	1.739713000	1.841969000	-1.591315000
6	-0.454486000	1.410579000	-0.493928000
6	0.673322000	2.328002000	-0.899123000
1	-1.248897000	-0.550336000	-0.929524000
1	-0.247492000	1.025015000	0.524509000
1	0.119099000	-2.457661000	-1.788654000
1	2.497181000	-2.364124000	-1.272492000
1	2.339636000	-3.231475000	-2.840019000
1	-1.410034000	1.957464000	-0.440157000
1	-1.147982000	0.011261000	-3.307464000
1	2.593113000	2.469453000	-1.863425000
1	0.656721000	3.374473000	-0.579467000

Enone 19 protonated

16	-1.098970000	2.017323000	-0.568519000
8	-3.556273000	-1.015823000	-0.297231000
8	2.100108000	2.393714000	-0.362835000
8	-1.396661000	-1.434322000	-0.604196000
6	0.319571000	-0.063997000	0.431799000
6	-0.756499000	0.892757000	0.862177000
6	-0.064294000	-1.173395000	-0.262355000
6	-2.009295000	0.085342000	1.223140000
6	-2.416194000	-0.800081000	0.066356000
6	1.805056000	0.157442000	0.673529000
6	0.873190000	-2.173167000	-0.764652000
6	2.526912000	-1.198907000	0.816825000
6	2.140330000	-2.178659000	-0.269599000
6	2.448279000	1.005882000	-0.442431000
1	1.929832000	0.718131000	1.616639000
1	2.265565000	-1.648093000	1.795542000
1	-0.428224000	1.510291000	1.710625000
1	-1.776931000	-0.569788000	2.083206000
1	2.858085000	-2.934762000	-0.601863000
1	-2.865753000	0.723531000	1.479098000
1	1.142922000	2.478088000	-0.542855000
1	0.526754000	-2.895599000	-1.508388000
1	3.616903000	-1.036033000	0.823422000
1	3.543771000	0.973842000	-0.329232000
1	2.185662000	0.589542000	-1.432968000
1	-1.798781000	2.949061000	0.108485000

Enone 19 deprotonated

16	0.604681000	-1.626386000	-4.526041000
8	-1.811728000	0.232101000	-3.827303000
8	3.049756000	-0.127235000	-2.423173000
8	4.235307000	-1.844227000	-3.181527000
6	-1.265761000	1.109302000	-2.840003000
6	0.657834000	-0.336196000	-2.068561000

6	0.709070000	-1.708991000	-2.678250000
6	1.822544000	0.365070000	-1.954290000
6	2.040182000	-2.348637000	-2.252309000
6	3.190102000	-1.468682000	-2.677932000
6	-0.628547000	0.360439000	-1.652107000
6	1.903176000	1.720076000	-1.419879000
6	-0.363020000	1.326686000	-0.479394000
6	0.840603000	2.211743000	-0.725067000
1	-1.355352000	-0.402505000	-1.320125000
1	-0.178867000	0.738083000	0.442132000
1	-0.125572000	-2.321647000	-2.300005000
1	2.079266000	-2.432709000	-1.148688000
1	0.882205000	3.213539000	-0.286611000
1	2.188327000	-3.340623000	-2.699857000
1	-1.068097000	-0.324184000	-4.179290000
1	2.817958000	2.298339000	-1.579077000
1	-1.262737000	1.936265000	-0.292575000
1	-2.111044000	1.714298000	-2.470914000
1	-0.518224000	1.792318000	-3.287999000

Enone 20 protonated

16	0.824188000	-1.326029000	-4.579413000
8	4.327908000	-1.913705000	-2.955068000
8	-1.364910000	2.166209000	-0.247436000
8	-2.002285000	-0.245825000	-3.580220000
8	3.148139000	-0.148594000	-2.299080000
6	-1.412578000	0.859179000	-2.905014000
6	0.732872000	-0.246435000	-2.102326000
6	0.768573000	-1.603202000	-2.750961000
6	1.925247000	0.384201000	-1.896242000
6	2.029262000	-2.333325000	-2.272023000
6	3.259034000	-1.500811000	-2.555143000
6	-0.545018000	0.423718000	-1.678258000
6	2.043901000	1.683422000	-1.259863000
6	-0.379484000	1.681881000	-0.823503000
6	0.942954000	2.321161000	-0.756987000
1	-1.169124000	-0.283166000	-1.102036000
1	-0.134802000	-2.182802000	-2.513919000
1	1.964755000	-2.481986000	-1.178313000
1	1.023120000	3.283248000	-0.244464000
1	2.159676000	-3.309086000	-2.758812000
1	-1.321377000	-0.635185000	-4.164176000
1	3.041527000	2.125501000	-1.181834000
1	-2.241448000	1.471465000	-2.519305000
1	-0.797168000	1.468460000	-3.591435000
1	0.657239000	-2.614570000	-4.938530000

Enone 20 deprotonated

16	0.751289000	-1.456649000	-4.582921000
8	-1.393805000	2.091080000	-0.177544000
8	-1.888322000	-0.138089000	-3.703068000
8	4.295263000	-1.850795000	-3.088749000
8	3.128184000	-0.120399000	-2.330207000
6	-1.352482000	0.918454000	-2.926065000
6	0.719737000	-0.268030000	-2.089080000
6	0.756696000	-1.622064000	-2.739612000
6	1.902566000	0.388439000	-1.896145000
6	2.045885000	-2.315631000	-2.271285000
6	3.240910000	-1.462794000	-2.620826000
6	-0.556746000	0.408530000	-1.672980000
6	2.003410000	1.685282000	-1.263135000

6	-0.406949000	1.640784000	-0.783864000
6	0.899301000	2.307184000	-0.739625000
1	-1.224979000	-0.303695000	-1.157483000
1	-0.114725000	-2.218554000	-2.425779000
1	2.032807000	-2.436667000	-1.170733000
1	0.975589000	3.264513000	-0.217476000
1	2.179743000	-3.297163000	-2.745183000
1	-1.123181000	-0.574356000	-4.167011000
1	2.994342000	2.145013000	-1.192827000
1	-2.203487000	1.512295000	-2.557165000
1	-0.687275000	1.566595000	-3.527669000

Enone 21 protonated

16	0.840861000	-1.317656000	-4.568330000
8	3.151399000	-0.160964000	-2.290569000
8	4.331873000	-1.940765000	-2.904082000
8	3.184891000	2.207799000	-1.027148000
8	-1.120934000	1.696746000	0.251503000
8	-2.002537000	-0.139245000	-3.641789000
6	-1.416677000	0.915082000	-2.886439000
6	0.738250000	-0.230202000	-2.109359000
6	0.775928000	-1.597955000	-2.742802000
6	1.939777000	0.387776000	-1.911221000
6	2.021957000	-2.338856000	-2.244760000
6	3.260463000	-1.521048000	-2.523689000
6	-0.574729000	0.382910000	-1.686605000
6	2.083905000	1.725483000	-1.283615000
6	-0.368267000	1.526795000	-0.701673000
6	0.785968000	2.464826000	-0.998224000
1	-1.189507000	-0.381682000	-1.181309000
1	-0.137429000	-2.163093000	-2.509818000
1	1.945631000	-2.479960000	-1.150881000
1	0.934996000	3.181245000	-0.179466000
1	2.143484000	-3.319438000	-2.723648000
1	-1.301575000	-0.530525000	-4.200476000
1	-2.253680000	1.506428000	-2.484141000
1	-0.791175000	1.564620000	-3.524466000
1	0.607135000	-2.597891000	-4.918166000
1	0.529838000	3.024071000	-1.919625000

Enone 21 deprotonated

16	0.772438000	-1.365588000	-4.565411000
8	3.156496000	-0.174657000	-2.283633000
8	4.293746000	-1.921973000	-3.046692000
8	3.200586000	2.189057000	-1.000699000
8	-1.088832000	1.594626000	0.331355000
8	-1.878877000	0.009700000	-3.745527000
6	-1.328125000	1.003448000	-2.896431000
6	0.745358000	-0.248807000	-2.085739000
6	0.762428000	-1.604044000	-2.734170000
6	1.947619000	0.370465000	-1.875863000
6	2.023182000	-2.343312000	-2.266257000
6	3.242598000	-1.517812000	-2.588958000
6	-0.563501000	0.386661000	-1.683606000
6	2.097990000	1.691847000	-1.241867000
6	-0.351064000	1.474434000	-0.643059000
6	0.803897000	2.421412000	-0.902973000
1	-1.225117000	-0.377739000	-1.241467000
1	-0.132401000	-2.175104000	-2.441202000
1	1.995217000	-2.486300000	-1.168871000
1	0.967686000	3.088715000	-0.045819000

1	2.130104000	-3.319215000	-2.758069000
1	-1.112473000	-0.441960000	-4.191589000
1	-2.174342000	1.594036000	-2.509707000
1	-0.647008000	1.673161000	-3.453634000
1	0.541980000	3.036393000	-1.785805000

Enone 22 protonated

16	-1.480242000	1.059140000	-2.955976000
16	0.336017000	-1.908750000	-4.242746000
8	0.312028000	3.629035000	-0.794018000
8	3.977608000	-2.011960000	-3.625114000
6	0.670560000	-0.280431000	-1.994249000
6	0.687305000	-1.724904000	-2.433280000
6	1.796772000	0.483951000	-2.032390000
6	2.067966000	-2.364738000	-2.193983000
6	3.115658000	-0.045322000	-2.545457000
6	3.133209000	-1.529594000	-2.872155000
6	-0.633093000	0.304061000	-1.496644000
6	1.803878000	1.935438000	-1.612607000
6	-0.392894000	1.356327000	-0.401415000
6	0.537592000	2.427635000	-0.926608000
1	-1.282390000	-0.497918000	-1.113454000
1	0.103596000	0.857424000	0.452692000
1	-0.080126000	-2.293998000	-1.886363000
1	2.261509000	-2.354989000	-1.105799000
1	2.111552000	-3.399711000	-2.564789000
1	-0.766844000	-1.135473000	-4.255746000
1	-1.332612000	1.814545000	-0.059832000
1	-2.642738000	1.344494000	-2.335949000
1	2.636804000	2.106759000	-0.902666000
1	2.005323000	2.591941000	-2.478065000
1	3.898567000	0.122664000	-1.779331000
1	3.440053000	0.513921000	-3.440813000

Enone 22 deprotonated

16	-1.657579000	0.899717000	-2.857187000
16	0.319551000	-1.796066000	-4.239393000
8	0.223588000	3.635699000	-1.049310000
8	3.995811000	-2.014564000	-3.613948000
6	0.681267000	-0.288900000	-1.942059000
6	0.696437000	-1.718811000	-2.424500000
6	1.813052000	0.467474000	-1.986102000
6	2.068302000	-2.381683000	-2.212248000
6	3.123525000	-0.057500000	-2.526879000
6	3.141023000	-1.538753000	-2.866849000
6	-0.631360000	0.272473000	-1.447224000
6	1.825401000	1.923055000	-1.577108000
6	-0.383376000	1.386358000	-0.406929000
6	0.509883000	2.437729000	-1.015805000
1	-1.195526000	-0.537246000	-0.952753000
1	0.150919000	0.949468000	0.460003000
1	-0.078452000	-2.300495000	-1.900791000
1	2.269549000	-2.412609000	-1.125273000
1	2.100472000	-3.404243000	-2.618487000
1	-0.614968000	-0.805374000	-4.166787000
1	-1.328444000	1.839406000	-0.074027000
1	2.592357000	2.080323000	-0.791523000
1	2.119686000	2.566552000	-2.425401000
1	3.924364000	0.105284000	-1.777613000
1	3.432993000	0.504734000	-3.426591000

Enone 23 protonated

16	1.823454000	1.890618000	-0.529094000
16	-1.911685000	1.874173000	-0.642480000
8	3.505100000	-1.543638000	-0.608630000
8	-3.466478000	-1.678206000	-0.424449000
8	-1.244887000	-1.632701000	-0.479811000
6	-0.007417000	0.186954000	0.508901000
6	-1.296824000	0.899871000	0.804987000
6	-0.051888000	-1.031341000	-0.085874000
6	-2.370309000	-0.150341000	1.120239000
6	-2.450229000	-1.183949000	0.020069000
6	1.326877000	0.789208000	0.869340000
6	1.130233000	-1.866899000	-0.474358000
6	2.387944000	-0.296061000	1.131321000
6	2.456018000	-1.251381000	-0.041518000
1	1.214062000	1.421307000	1.763456000
1	2.074050000	-0.871581000	2.022882000
1	-1.179266000	1.582584000	1.659359000
1	-2.086740000	-0.677375000	2.048758000
1	-3.369246000	0.288640000	1.250020000
1	-0.754710000	2.523717000	-0.871909000
1	1.049142000	-2.860654000	0.005296000
1	3.379559000	0.143742000	1.309583000
1	2.839863000	2.507072000	0.105998000
1	1.148678000	-2.034113000	-1.563783000

Enone 23 deprotonated

16	1.868885000	1.916493000	-0.476035000
16	-1.777042000	1.936662000	-0.683129000
8	3.466717000	-1.505040000	-0.674110000
8	-3.485206000	-1.598547000	-0.491573000
8	-1.264847000	-1.642343000	-0.440852000
6	-0.021677000	0.175312000	0.547810000
6	-1.306796000	0.921116000	0.788747000
6	-0.067449000	-1.051779000	-0.026502000
6	-2.391531000	-0.119572000	1.107530000
6	-2.470637000	-1.142487000	0.003169000
6	1.306969000	0.789208000	0.888471000
6	1.110342000	-1.893689000	-0.417236000
6	2.401520000	-0.272083000	1.107741000
6	2.437025000	-1.237184000	-0.058978000
1	1.207790000	1.399934000	1.799629000
1	2.141829000	-0.845854000	2.016741000
1	-1.188124000	1.585490000	1.660849000
1	-2.127115000	-0.662641000	2.035900000
1	-3.383091000	0.338745000	1.220945000
1	1.066648000	-2.868903000	0.104514000
1	3.393239000	0.186700000	1.237569000
1	0.602353000	2.280973000	-0.824664000
1	1.096865000	-2.104353000	-1.499470000

Enone 24 protonated

16	1.820722000	1.898508000	-0.611451000
16	-1.977335000	1.893754000	-0.551486000
8	3.330024000	-1.805610000	-0.506325000
8	-3.317964000	-1.810375000	-0.501324000
8	-1.098728000	-1.651201000	-0.504378000
8	1.110473000	-1.648953000	-0.508601000
6	0.002906000	0.245554000	0.527568000
6	-1.323322000	0.868644000	0.844232000
6	0.005373000	-0.955683000	-0.093924000

6	-2.344374000	-0.249976000	1.098177000
6	-2.350409000	-1.265934000	-0.021759000
6	1.335405000	0.864135000	0.838290000
6	2.351116000	-0.255954000	1.101305000
6	2.362258000	-1.266515000	-0.021636000
1	1.260370000	1.515955000	1.720889000
1	2.055291000	-0.794419000	2.020927000
1	-1.247155000	1.505185000	1.737800000
1	-2.057018000	-0.786156000	2.020317000
1	-3.368841000	0.130007000	1.212391000
1	-0.843453000	2.591432000	-0.752876000
1	3.374246000	0.123693000	1.222418000
1	2.908845000	2.460275000	-0.048417000

Enone 24 deprotonated

16	1.829736000	1.917965000	-0.601323000
16	-1.857549000	1.959596000	-0.561077000
8	3.333098000	-1.763071000	-0.517790000
8	-3.317462000	-1.759060000	-0.582907000
8	-1.098518000	-1.667842000	-0.470906000
8	1.114060000	-1.651173000	-0.466923000
6	-0.015585000	0.232696000	0.579342000
6	-1.347967000	0.875054000	0.844283000
6	-0.000871000	-0.968973000	-0.036206000
6	-2.367991000	-0.251253000	1.086004000
6	-2.358558000	-1.247111000	-0.046159000
6	1.304541000	0.884621000	0.847394000
6	2.366663000	-0.197213000	1.077930000
6	2.361073000	-1.232008000	-0.025089000
1	1.243646000	1.535428000	1.732353000
1	2.133471000	-0.728002000	2.019119000
1	-1.282152000	1.482052000	1.762382000
1	-2.094073000	-0.809805000	2.002321000
1	-3.390591000	0.135925000	1.185591000
1	3.383373000	0.214517000	1.145427000
1	0.554077000	2.283196000	-0.905590000

Enone 25 protonated

16	-0.566468000	-2.570746000	-2.967598000
8	-1.127999000	0.481520000	-4.238265000
6	0.760600000	-0.282378000	-2.155020000
6	0.891535000	-1.565040000	-2.742363000
6	1.931903000	0.502738000	-1.950284000
6	2.162367000	-2.045944000	-3.140601000
6	3.194094000	0.019049000	-2.359073000
6	3.305992000	-1.250067000	-2.953592000
6	-0.583348000	0.338152000	-1.822962000
6	1.815177000	1.796312000	-1.257075000
6	-0.502995000	1.195948000	-0.546982000
6	0.683548000	2.132706000	-0.582283000
6	-1.036787000	1.213909000	-3.009078000
1	0.102597000	-3.714665000	-3.193929000
1	-1.334943000	-0.456359000	-1.675734000
1	-0.426122000	0.539674000	0.341411000
1	0.640872000	3.070805000	-0.018810000
1	4.084469000	0.635145000	-2.196472000
1	4.282931000	-1.627660000	-3.269876000
1	2.254677000	-3.036066000	-3.597163000
1	-0.239453000	0.144441000	-4.441441000
1	2.686910000	2.459981000	-1.253906000
1	-1.439952000	1.767768000	-0.433576000

1	-2.050182000	1.598395000	-2.815198000
1	-0.348556000	2.070898000	-3.120878000

Enone 25 deprotonated

16	-0.511037000	-2.454166000	-3.345664000
8	-1.055920000	0.541906000	-4.296274000
6	0.807365000	-0.246838000	-2.205693000
6	0.914025000	-1.504148000	-2.872138000
6	1.981638000	0.503695000	-1.911115000
6	2.213842000	-1.986544000	-3.192469000
6	3.257216000	0.012116000	-2.270277000
6	3.369632000	-1.238958000	-2.902407000
6	-0.538670000	0.368925000	-1.870754000
6	1.859095000	1.794211000	-1.210975000
6	-0.479500000	1.215191000	-0.585337000
6	0.707178000	2.151452000	-0.583116000
6	-1.036519000	1.237114000	-3.041624000
1	-1.265160000	-0.448172000	-1.723980000
1	-0.414502000	0.544474000	0.293957000
1	0.651619000	3.094792000	-0.028918000
1	4.149279000	0.605734000	-2.042141000
1	4.354115000	-1.633533000	-3.175271000
1	2.304104000	-2.957191000	-3.691741000
1	-1.082011000	-0.424163000	-4.100258000
1	2.738863000	2.447678000	-1.177237000
1	-1.420455000	1.781837000	-0.475629000
1	-2.052635000	1.604728000	-2.799651000
1	-0.375089000	2.112180000	-3.166822000

Enone 26 protonated

16	3.875742000	2.841461000	-3.233095000
8	7.591577000	-0.920638000	-1.441053000
8	5.187396000	2.589752000	-0.937787000
8	6.622204000	0.466080000	-2.957753000
6	4.183840000	0.529412000	-1.734913000
6	5.300037000	-0.300111000	-1.101023000
6	4.551138000	1.999886000	-1.801032000
6	6.618138000	-0.246490000	-1.905469000
1	3.260051000	0.473495000	-1.128861000
1	3.940909000	0.168832000	-2.745309000
1	4.289636000	4.062371000	-2.846872000
1	4.982969000	-1.352838000	-1.018596000
1	5.506130000	0.051057000	-0.077294000

Enone 27 protonated

16	2.965067000	3.051395000	-2.134674000
8	5.567443000	2.593354000	-2.119189000
8	7.448102000	0.589634000	-2.090530000
6	7.334758000	-1.684409000	-1.425734000
6	4.192752000	0.587658000	-1.973173000
6	5.275434000	-0.231527000	-1.243850000
6	4.448827000	2.081165000	-2.069281000
6	6.551312000	-0.532395000	-2.044864000
1	7.567125000	-1.459083000	-0.372156000
1	8.279554000	-1.831719000	-1.970702000
1	3.213913000	0.441987000	-1.488445000
1	4.078035000	0.228644000	-3.013448000
1	3.613778000	4.216620000	-2.315512000
1	4.812416000	-1.198925000	-0.985641000
1	6.753084000	-2.618108000	-1.471072000
1	6.897185000	1.395667000	-2.173604000

1	6.259496000	-0.806162000	-3.079274000
1	5.558068000	0.255764000	-0.294072000

Enone 28 protonated

16	3.102953000	3.004411000	-2.328727000
8	7.110182000	-1.647639000	-1.743741000
8	5.680210000	2.559017000	-1.973466000
8	7.356320000	0.516277000	-2.210387000
6	4.316873000	0.553836000	-1.965840000
6	5.320292000	-0.176162000	-1.045713000
6	4.555841000	2.051409000	-2.063524000
6	6.657796000	-0.510975000	-1.692116000
1	3.293632000	0.383679000	-1.598489000
1	4.352405000	0.153994000	-2.995138000
1	3.768545000	4.168897000	-2.443797000
1	4.881079000	-1.128384000	-0.721265000
1	6.838516000	1.359701000	-2.089452000
1	5.513640000	0.433913000	-0.146852000

Enone 29 protonated

16	1.369140000	1.260969000	-2.471989000
8	2.727842000	-0.622488000	-1.322153000
8	3.402328000	-0.812721000	3.031289000
8	3.895681000	-1.305046000	0.916479000
6	-0.521650000	-0.677749000	0.497071000
6	0.743398000	-1.347796000	0.977172000
6	-0.606024000	0.656504000	0.280768000
6	1.729030000	-0.241084000	1.401167000
8	0.464675000	1.535681000	0.362483000
6	1.727194000	0.868781000	0.320097000
6	3.080077000	-0.791614000	1.849614000
6	2.006668000	0.361349000	-1.116794000
1	0.519965000	-1.988766000	1.845874000
1	1.322175000	0.255559000	2.296313000
1	1.177806000	-1.997363000	0.198521000
1	2.481563000	1.643852000	0.536392000
1	3.492753000	-1.161580000	0.014427000
1	-1.528671000	1.178541000	0.019683000
1	-1.434480000	-1.264439000	0.365851000
1	0.700238000	2.153414000	-1.718005000