Reactions of Antitumor Active Dirhodium(II) Tetraacetate 
Rh₂(CH₃COO)₄ with Cysteine and Its Derivatives

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S Supporting Information

ABSTRACT: We have combined results from several spectroscopic techniques to investigate the aerobic reactions of Rh₂(AcO)₄ (AcO⁻ = CH₃COO⁻) with l-cysteine (H₂Cys) and its derivatives d-penicillamine (3,3-dimethylcysteine, H₂Pen), with steric hindrance at the thiol group, and N-acetyl-l-cysteine (H₂NAC), with its amino group blocked. Previous investigations have shown that antitumor active dirhodium(II) carboxylates may irreversibly inhibit enzymes containing a thiol group at or near their active sites. Also, cysteine, the only thiol-containing proteinogenic amino acid, interacts in vivo with this class of antitumor compounds, but structural information on the products of such reactions is lacking. In the present study, the reactions of Rh₂(AcO)₄ and H₂L were carried out in aqueous solutions at the pH of mixing (acidic) and at physiological pH, using the different mole ratios 1:2, 1:4, and 1:6, which resulted in the same products in increasing yields. Electrospray ionization mass spectrometry (ESI-MS) indicates formation of dimeric [Rh₃⁺Pen₂]⁺ or oligomeric [Rh₃⁺L₂]⁺ (L = Cys, NAC) complexes with bridging thiolate groups. Analyses of Rh K edge extended X-ray absorption fine structure (EXAFS) data reveal 3–4 Rh–S and 2–3 Rh–(N/O) bonds around six-coordinated Rh(III) ions at mean distances of 2.33 ± 0.02 and 2.09 ± 0.02 Å, respectively. In the N-acetyl-l-cysteine compound, the Rh₃⁺–Rh₃⁺ distance 3.10 ± 0.02 Å obtained from the EXAFS spectrum supports trithiolate bridges between the Rh(III) ions, as was also found when using glutathione as ligand. In the cysteine and penicillamine complexes, double thiolate bridges join the Rh(III) ions, with the nonbridging Cys₂ and Pen₂⁻ ligands in tridentate chelating (S,N,O) mode, which is consistent with the ΔνC=O = 7.3–8.4 ppm shift of the COO⁻ signal in their carbon-13 cross polarization magic angle spinning (CPMAS) NMR spectra. For the penicillamine complex, the 2475.6 eV peak in its S K-edge X-ray absorption near edge structure (XANES) spectrum shows partial oxidation, probably caused by peroxide generated from reduction of dissolved O₂, of thiolato to sulfenato (S⁻O⁻) groups, which were also identified by ESI-MS for all three {Rh₃⁺L₄}, compounds.

INTRODUCTION

The antitumor activity of neutral dirhodium(II) complexes with bridging carboxylate groups in a cage structure, Rh₂(μ-RCOO)₄ (R = CH₃, C₂H₅, and C₃H₇), was initially reported in 1972 by Bear and co-workers.1–3 They observed that the survival time of mice bearing Ehrlich ascites or leukemia L1210 tumors increased when they were treated with dirhodium(II) carboxylates. The treatment was also effective against leukemia P388 and sarcoma 180 tumors. This class of compounds could inhibit in vivo DNA synthesis of Ehrlich ascites tumor cells, and in vitro DNA and RNA polymerase from Escherichia coli.4–6 The metabolism of dirhodium(II) tetraacetate was explored by injecting ¹³C-labeled Rh₂(AcO)₄ in tumor-bearing Swiss mice, which exhaled ¹⁴CO₂ within 2 h after injection, indicating breakdown of the cage structure into carboxylate and rhodium ions. The rhodium was mainly deposited in the liver, with only ~5% excreted through urine within the first 24 h after administrating the drug.7 A similar structural breakdown was observed when reacting dirhodium(II) tetraacetate (1, Rh₂(AcO)₄) with cysteine in the mole ratio 1:4 at pH = 7.5, which resulted in an insoluble Rh(III)–cysteine complex, protons, and free acetate groups.7,8 In vitro studies showed that human serum albumin maintained the cage structure of Rh₂(AcO)₄ by binding to its axial positions via the N-imidazole moieties of its histidine residues. Although enzymes containing thiol (–SH) groups at or near their active site were irreversibly inhibited by dirhodium(II) carboxylates, other enzymes without free –SH groups, or those containing thiol groups away from their active site, were not affected.8,9 Inhibition was proposed to involve reactions of protein–thiol groups with dirhodium(II) carboxylates, leading to breakdown of their cage structure, oxidation of Rh(II), and tight binding of Rh(III) ions to the active site of those enzymes. The results were then correlated to the limited excretion of rhodium from mice treated with

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A calibrated Thermo Scientific Orion Star semi-micro electrode was used for pH measurements. Thermogravimetry (TG), to measure water content, was carried out using a Netzsch STA 409 instrument, and a Johnson Matthey magnetic susceptibility balance was used to analyze the magnetic properties of the solid samples.

**Solid {Rh$_2$(H$_2$Cys)$_2$(Cys)$_2$}$_2$O$_4$** (4). To a completely dissolved solution of dirhodium(II) tetaacetate (0.1123 g, 0.254 mmol) in 40 mL of oxygen-free water, solid cysteine (0.1234 g, 1.018 mmol) was added under a stream of argon to prevent oxidation of the ligand to cystine. The solution color changed from dark red to dark green. The solution was subsequently heated to 100 °C for 3 h until it had cooled down to room temperature. The solid was filtered and dried in vacuo to give 0.0647 g (61% yield) of the products.

**EXPERIMENTAL SECTION**

**Sample Preparation.** L-Cysteine, d-penicillamine, N-acetyll-cysteine, and sodium hydroxide were purchased from Sigma-Aldrich, and dirhodium(II) tetaacetate (pure; 47% Rh) was obtained from Pressure Chemical Company; all were used without further purification. Degassed water and D$_2$O were prepared by bubbling argon through boiled distilled water or D$_2$O for 3 h until they had cooled down to room temperature. Sephadex G-15 was obtained from VWR International.

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gradually changed from emerald green to orange-brown and a precipitate was formed (pH = 3.2). The mixture was stirred for 24 h at room temperature under aerobic conditions. The precipitate was filtered, washed with methanol and ether several times and dried under vacuum in a desiccator. Elemental analysis: calculated for \(\text{[Rh}_{2}(\text{Cys})_{4}][\text{H}2\text{O}]_{4}\) \(\text{C}_{52}\text{H}_{48}\text{O}_{22}\text{S}_{4}\text{Na}_{2}\): %C 33.18, %H 3.30, %N 5.95 (9.5% H2O); found: %C 33.89, %H 3.31, %N 5.89 (9.6% H2O); yield = 91%. Magnetic susceptibility measurements showed the solid bulk, which is insoluble in solvents, to be diamagnetic.

**Aerobic Reaction of Rh2(AcO)4 with Thiol-Containing Ligands H2L (pH = 7.4).** An emerald green suspension of 0.100 g (0.226 mmol) of dirhodium(II) tetracetate in 30 mL of degassed water was added dropwise to colorless solutions containing one of the following H2L ligands (0.909 mmol of H2Cys, or 0.905 mmol of H2Pen, or 0.906 mmol of H2NAC) dissolved in 90 mL of deoxygenated water. The reactions were carried out under a stream of argon to prevent formation of disulfides by oxidation of the ligand −SH group. When adding 1.0 M NaOH dropwise to adjust the pH of the solutions to 7.4, their color turned to dark red/orange. The reaction mixtures were measured at different time intervals (30 min, 2 h, 24 h, and 48 h after pH adjustment). A rotary evaporator was used (at RT) to reduce the volume before passing the solution through a Sephadex G-15 size exclusion chromatography column with water as eluent. ESI-mass spectra were measured at room temperature (RT) to reduce the volume before passing the solution and 48 h after pH adjustment. A rotary evaporator was used to measure ESI-mass spectra both in positive and negative ion modes. Voltages for capillary, skimmer, and fragmentor were set at 4000, 65, and 80 V, respectively. Solid samples were dissolved in water, and methanol was used as the mobile phase with a continuous injection flow rate of 0.2 mL min⁻¹ and a drying gas flow rate of 7 L min⁻¹ at 200 °C. For voltage variable measurements, the fragmentor voltage varied between 0 and 80 V. The nature of the mass ions and their corresponding m/z values were confirmed using the isotope distribution calculator from Agilent, and a high resolution calculation method chosen from Scientific Instrument Services (SIS).

**Electronic Spectroscopy.** UV−vis absorption spectra were measured at RT using a Cary 300 UV−vis double-beam spectrophotometer. Samples were measured in quartz cells with 1 mm path-length, using water in the reference position.

**X-ray Absorption Spectroscopy Data Collection.** Rh K edge EXAFS spectra of the solid \(\text{[Rh}_{2}(\text{Cys})_{4}][\text{H}2\text{O}]_{4}\) \(\text{C}_{52}\text{H}_{48}\text{O}_{22}\text{S}_{4}\text{Na}_{2}\) (4) precipitated at pH = 3.2, the concentrated solutions (pH = 7.4), and corresponding solid compounds \(\text{[Na}_{2}\text{[Rh}_{2}(\text{Cys})_{4}]\cdot\text{H}2\text{O}\) \(\text{C}_{52}\text{H}_{48}\text{O}_{22}\text{S}_{4}\text{Na}_{2}\) (5), \(\text{Na}_{2}\text{[Rh}_{2}(\text{Pen})_{2}(\text{Pen}++)_{2}]4\text{H}2\text{O}\) \(\text{C}_{52}\text{H}_{48}\text{O}_{22}\text{S}_{4}\text{Na}_{2}\) (6), and \(\text{[Na}_{2}\text{[Rh}_{2}(\text{NAC})_{4}]4\text{H}2\text{O}\) \(\text{C}_{52}\text{H}_{48}\text{O}_{22}\text{S}_{4}\text{Na}_{2}\) (7) were measured at room temperature at BL 7−3 (500 mA) at the Stanford Synchrotron Radiation Lightsource (SSRL) operating under 3 GeV. Higher-order harmonics were rejected by detuning the Si(220) (ϕ = 0°) double-crystal monochromator to 50% of maximum intensity at the end of the Rh K edge scan range. The X-ray energy was internally calibrated by assigning the first inflection point of the absorption edge of a Rh foil placed between the iron chambers I1 and I2 to 23219.80 eV. Three iron chambers (I0, I1, and I2) were filled with nitrogen (N2). Four to ten scans were collected for each sample in transmission mode and compared prior to averaging to ensure that no radiation damage occurred during measurement.

S K edge XANES spectra of the solid compounds 5−7 were measured at BL 4−3 (SSRL), equipped with a Si(111) double-crystal monochromator, a harmonic rejection mirror, and a Vortex fluorescence detector. The solid samples were finely ground, dusted on Mylar tape, and placed in a sample chamber filled with helium. Two scans collected in fluorescence mode were averaged. Energy calibration was achieved by setting the first peak maximum in the S XANES spectrum of Na2S2O3, SH2O to 2472.02 eV.

**XAS Data Analysis.** The WinXAS 3.1 program was used for first order polynomial pre-edge background subtraction and normalization of the Rh K edge XAS spectra, followed by subtraction of a seven segment cubic spline in the post-edge region to extract the EXAFS oscillations. The first inflection point of the absorption edges varied over a narrow range (E0 = 23 226.3−23 226.7 eV). To simulate theoretical EXAFS oscillations, the atomic coordinates of ΔA[Rh{Ir(aet)₃}₂]³⁺ (Haet = 2-aminoethanethiol) with the Cambridge Structural Database (CSD) code AQUVOX,³ were used for the ATOMS and Pegg 7.0 programs, replacing Ir with Rh, as the ionic radii of Rh³⁺ and Ir³⁺ ions are very similar (0.665 Å and 0.68 Å, respectively). Least-squares curve-fitting of the theoretically simulated EXAFS oscillations to the experimental EXAFS spectra was performed over the k-range ∼2.8−18.2 Å⁻¹. The wide k-range EXAFS data of good quality allowed simultaneous refinement of the coordination numbers (CN), bond distances (R), and the Debye–Waller factor parameters (σ²), unless otherwise stated. The ΔE₀ was allowed to float as a common value for all scattering paths involved in the fitting. The amplitude reduction

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The majority of these results are presented in Appendices 1 and 2 in the Supporting Information, where an image of the size exclusion chromatography column is shown for a solution containing Rh$_2$(AcO)$_4$ (m/z = 764.90 and 766.91) and the corresponding Na$^+$ mass ions ($+m/z = 764.90$ and 766.91) as well as intense peaks assigned to unreacted Rh$_2$(AcO)$_4$ ($+m/z = 459.90$, 464.85, and 505.88); see Figures 1 (right) and S-3, and Tables 1 and S-1. No mass peak was related to the mononuclear Rh(III) complex. When the pH of the solution mixture was adjusted to 7.4 after 48 h, the peak intensity at $\lambda_{max} = 367$ nm observed after 48 h (see Figure 1, left). ESI-mass spectra of the same UV−vis samples showed peaks associated with [Rh$_3$(AcO)$_2$($\text{HNAC}$)$_2$]($\pm$ H$^+$) and the corresponding Na$^+$ mass ions ($+m/z = 764.90$ and 766.91) as well as intense peaks assigned to unreacted Rh$_2$(AcO)$_4$ ($+m/z = 459.90$, 464.85, and 505.88); see Figures 1 (right) and S-3, and Tables 1 and S-1. No mass peak was related to the mononuclear Rh(III) complex. When the pH of the solution mixture was adjusted to 7.4 after 48 h, the peak intensity at $\lambda_{max} = 367$ nm substantially decreased within a few minutes. This peak does not appear in the UV−vis spectra when adjusting the reaction pH to 7.4 directly after mixing the reactants (see Figure S-7).

**Reaction of Rh$_2$(AcO)$_4$ with Thiols at Physiological pH.**

The results presented below refer to the aerobic reactions of Rh$_2$(AcO)$_4$ with thiol-containing ligands (H$_2$L) in the mole ratio of 1:4. Other mole ratios (1:2 and 1:6) led to the same products (S−7) in different yields (44−96% for mole ratios of 1:2 and 1:6, respectively); see Appendix 3 in the Supporting Information, where an image of the size exclusion chromatography purification column is shown for a solution containing Rh$_2$(OAc)$_4$ and H$_2$NAC (mole ratio of 1:4).

**ESI-Mass Spectrometry.** The most intense peaks in the ESI-mass spectra (−ion mode, fragmentor voltage 80 V) of the products obtained from the reactions of 1 with cysteine (5), penicillamine (6), or N-acetylcysteine (7) at pH = 7.4 were

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**Table 1. Assignment of the ESI-MS Peaks Shown in Figure 1 (Right)**

| m/z (M) | isotopic pattern | assignment |
|--------|-----------------|------------|
| 186.02 | M + 1           | $[\text{H}_2\text{NAC} + \text{Na}^+]^+$ |
| 325.05 | M + 1           | $[2\text{H}_2\text{NAC} - 2\text{H}^+ + \text{H}^+]^+$ |
| 347.03 | M + 1           | $[2\text{H}_2\text{NAC} - 2\text{H}^+ + \text{Na}^+]^+$ |
| 459.80 | M + 1           | $[2\text{R}h^{III} + 4\text{AcO}^- + \text{NH}_4^+]^+$ |
| 464.85 | M + 1           | $[2\text{R}h^{III} + 4\text{AcO}^- + \text{Na}^+]^+$ |
| 505.88 | M + 1           | $[2\text{R}h^{III} + 4\text{AcO}^- + \text{CH}_3\text{CN} + \text{Na}^+]^+$ |

"$\text{H}_2\text{NAC} = \text{C}_3\text{H}_7\text{NO}_3\text{S}; 2\text{H}_2\text{NAC} - 2\text{H}^+$ is the oxidized form of N-acetylcysteine with an S–S bond; $\text{AcO}^- = \text{CH}_3\text{COO}^-$.”

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**RESULTS**

Dirhodium(II) tetraacetate, Rh$_2$(AcO)$_4$ (1), has limited solubility in water. When a completely dissolved aqueous solution of 1 (emerald green) is mixed with a fourfold amount of cysteine under aerobic conditions, a red-brown precipitate is formed after 5 min (pH = 3.2), whereas similar reactions with N-acetylcysteine or penicillamine result in clear emerald green solutions. When adjusting the pH to 7.4, the reactions proceed quickly with a color change to orange-red (see Experimental Section). To monitor the early stages of the reaction for the solutions containing N-acetylcysteine or penicillamine, we measured, over a period of time, their ESI-MS, UV−vis, and/or $^1$H NMR spectra at the pH of mixing (acids) at which the reaction is relatively slow. The majority of these results are presented in Appendices 1 and 2 in the Supporting Information (Figures S-1−S-5, Tables S-1, and S-2), including the ESI-mass spectra of pure Rh$_2$(AcO)$_4$ in water (Figure S-6).
associated with \([\text{Rh}^{III}\_4]\)^{2-} ions that had satellite peaks with \((-m/z = 803.84) and \((-m/z = 819.85)\) for \([\text{Rh}^{III}\_4\_2(Pen)]^2-\) and \([\text{Rh}^{III}\_4\_2(Pen)]^2-\) ions, respectively. Because the reaction of \(\text{Rh}_2(\text{AcO})_4\) with cysteine was carried out at the reaction mixtures with \(\text{Rh}_2(\text{AcO})_4/\text{H}_2\text{L}\) in a mole ratio of 1:2, the presence of such a \(\text{Rh}/\text{Cys} 2:5\) complex may indicate fragmentation of higher complexes, for example with a \(\text{Rh}/\text{Cys}\) 4:8 ratio, as observed in the ESI-mass spectrum shown in Figure 2. ESI-mass spectra of the reaction mixtures with \(\text{Rh}_2(\text{AcO})_4/\text{H}_2\text{L}\) in a mole ratio of 1:2 showed the same mass ions, together with large peaks related to unreacted \(\text{Rh}_2(\text{AcO})_4\) (see Appendix 3 in the Supporting Information for \(\text{H}_2\text{Pen}\) reaction).

When setting the fragmentor voltage at 0 V, the main mass peaks observed for the \(\text{Rh}/\text{penicillamine}\) reaction product 6 were those of \([\text{Rh}^{III}\_4\_2(Pen)]^2-\) \((-m/z = 794.96)\), \([\text{Rh}^{III}\_4\_2(Pen)]^2-\) \((-m/z = 801.95)\), and \([\text{Rh}^{III}\_4\_2(Pen)]^2-\) \((-m/z = 826.95)\), indicating that the dinuclear species dominate in 6; see Figure S-8. For the \(\text{Rh}/\text{N-acetylcysteine}\) reaction product 7, the majority of the mass peaks at 0 V were those of the tetranuclear \(\text{Rh}^{III}\_4(\text{NAC})_{8-10}\) species with an added \(\text{O}\) atom or \(\text{H}^-/\text{Na}^+/\text{K}^+\) ions, with only a few peaks detected for the dinuclear \(\text{Rh}^{III}\_4(\text{NAC})_4\) species (see Figure S-9 and Table S-3).

The lowest fragmentor voltage that could generate mass peaks for the \(\text{Rh}/\text{cysteine}\) reaction product 5 was 10 V; however, no isotopic pattern could be observed (Figure S-10 and Table S-4). Similar to \(\text{Rh}/\text{penicillamine}\) 6, peaks were assigned to the dinuclear species \([\text{Rh}^{III}\_2\_2(\text{Cys})_4\_4\_4\_4^2-\) \((-m/z = 682.83)\), \([\text{Rh}^{III}\_2\_2(\text{Cys})_4\_4\_4\_4^2-\) \((-m/z = 698.83)\), and \([\text{Rh}^{III}\_2\_2(\text{Cys})_4\_4\_4\_4^2-\) \((-m/z = 714.81)\) assuming the charge \(-1\). In addition, two less intense peaks were detected at \((-m/z = 803.84) and 819.85\) for \([\text{Rh}^{III}\_2\_2(\text{Cys})_4\_4\_4\_4^2-\) \((-m/z = 803.84) and \([\text{Rh}^{III}\_2\_2(\text{Cys})_4\_4\_4\_4^2-\) \((-m/z = 819.85)\) ions, respectively. The reaction of \(\text{Rh}_2(\text{AcO})_4\) with cysteine was carried out at the mole ratio 1:4; the presence of such a \(\text{Rh}/\text{Cys}\) 2:5 complex may indicate fragmentation of higher complexes, for example with a \(\text{Rh}/\text{Cys} 4:8\) ratio, as observed in the ESI-mass spectrum measured at 80 V (see Table 2).

In summary, analyses of the ESI-mass spectra show formation of a dinuclear \([\text{Rh}^{III}\_2\_2(\text{Cys})_4\_4\_4\_4^2-\) complex in 6, dinuclear \([\text{Rh}^{III}\_2\_2(\text{Cys})_4\_4\_4\_4^2-\) or tetranuclear \([\text{Rh}^{III}\_2\_2(\text{Cys})_4\_4\_4\_4^2-\) complexes in 5, and oligomeric \([\text{Rh}^{III}\_2\_2(\text{NAC})_4\_4\_4\_4^2-\) species in 7 (with 1–2 added \(\text{O}\) atoms), and exclude formation of mononuclear \(\text{Rh}\) complexes.

**Sulfur K Edge XANES Spectroscopy.** To examine whether the detected mass ions with 1–2 added \(\text{O}\) atoms correspond to sulfinato \((\text{SO})\) or sulfinato \((\text{SO})\) groups, S K edge XANES spectra of the \(\text{Rh}/\text{cysteinate}\) complex with 3d^8 low-spin configuration and square-planar coordination, \(\text{K}_2[\text{Ni}(\text{S,N-Cys})_4]\), the distinct pre-edge feature observed at 2471.1 eV and the main peak at 2472.8 eV correspond to \(\text{S}\) 1s transitions to an unoccupied frontier orbital with \(\text{S} 3p\) and \(\text{Ni} 3d_{xy}\) character, and to a \(\sigma^* (\text{C}–\text{S})\) orbital, respectively. For the \(\text{Rh}/\text{cysteine}\) reaction product 5 with \(\text{Rh}(\text{III})\) low-spin 3d^6 configuration, it seems likely that the peaks at 2472.0 and 2474.0 eV correspond to the \(\text{S}(1s) \rightarrow \text{lowest unoccupied molecular orbital (LUMO)}\) with \(\text{S}(3p)\) and \(\text{Rh}(4d)\) character and \(\text{S}(1s) \rightarrow \sigma^* (\text{C}–\text{S})\) transitions, respectively. The additional feature appearing in the spectrum of the \(\text{Rh}/\text{penicillamine}\) reaction product 6 at 2475.6 eV (less pronounced or absent for 5 or 7) is probably due to a sulfinato \((\text{SO})\) group bound to...
Table 2. Assignment of the Mass Ions Observed in the ESI-Mass Spectra (− Ion Mode) of Products 5–7 Shown in Figure 2a

| m/z (M) | isotopic pattern | assignment | m/z (M) | isotopic pattern | assignment |
|---------|-----------------|------------|---------|-----------------|------------|
| 340.91  | M + 0.5         | [2RhⅢ + 4H₂Cys − 8H+]^{2−} | 511.87  | M + 0.5         | [3RhⅢ + 6H₂Cys − 11H+]^{2−} |
|         | M + 0.33        | [3RhⅢ + 6H₂Cys − 12H+]^{2−} | 519.87  | M + 0.5         | [3RhⅢ + 6H₂Cys − 11H+ + O]^{2−} |
| 348.91  | M + 0.5         | [2RhⅢ + 4H₂Cys − 8H+ + O]^{2−} | 682.83  | M + 0.5         | [4RhⅢ + 8H₂Cys − 14H+]^{2−} |
| 356.91  | M + 0.5         | [2RhⅢ + 4H₂Cys − 8H+ + 2O]^{2−} |       | M + 1           | [2RhⅢ + 4H₂Cys − 7H+]^{−} |
| 409.42  | M + 0.5         | [2RhⅢ + 5H₂Cys − 8H+ + O]^{2−} | 690.83  | M + 0.5         | [4RhⅢ + 8H₂Cys − 14H+ + O]^{2−} |
| 417.42  | M + 0.5         | [2RhⅢ + 5H₂Cys − 8H+ + 2O]^{2−} | 698.83  | M + 0.5         | [4RhⅢ + 8H₂Cys − 14H+ + 2O]^{2−} |
| 454.89  | M + 0.33        | [4RhⅢ + 8H₂Cys − 15H]^{−} |       | M + 1           | [2RhⅢ + 4H₂Cys − 7H+ + O]^{2−} |
| 339.44  | M + 0.5         | [2RhⅢ + 3H₂Pen + S^{−} − 6H]^{5−} | 794.96  | M + 0.5         | [4RhⅢ + 8H₂Pen − 14H+]^{2−} |
| 347.44  | M + 0.5         | [2RhⅢ + 3H₂Pen + S^{−} − 6H+ + O]^{5−} |       | M + 1           | [2RhⅢ + 4H₂Pen − 7H+]^{−} |
| 355.44  | M + 0.5         | [2RhⅢ + 3H₂Pen + S^{−} − 6H+ + 2O]^{5−} | 810.95  | M + 0.5         | [4RhⅢ + 8H₂Pen − 14H+ + O]^{2−} |
| 396.98  | M + 0.5         | [2RhⅢ + 4H₂Pen − 8H]^{4−} |       | M + 1           | [2RhⅢ + 4H₂Pen − 7H+ + O]^{2−} |
| 404.97  | M + 0.5         | [2RhⅢ + 4H₂Pen − 8H+ + O]^{4−} | 816.94  | M + 1           | [2RhⅢ + 4H₂Pen − 8H+ + Na]^{−} |
| 412.97  | M + 0.5         | [2RhⅢ + 4H₂Pen − 8H+ + 2O]^{4−} | 826.95  | M + 1           | [2RhⅢ + 4H₂Pen − 7H+ + 2O]^{2−} |
| 323.04  | M + 1           | [2H₂NAC − 3H]^{+} | 657.91  | M + 0.5         | [3RhⅢ + 6H₂NAC − 11H]^{−} |
| 344.43  | M + 0.5         | [2RhⅢ + 3H₂NAC − 6H]^{−} |       | M + 0.25        | [6RhⅢ + 12H₂NAC − 22H]^{+} |
| 424.93  | M + 0.5         | [2RhⅢ + 4H₂NAC − 8H]^{+} | 648.90  | M + 0.5         | [3RhⅢ + 6H₂NAC − 12H+ + Na]^{−} |
| 432.93  | M + 0.33        | [3RhⅢ + 6H₂NAC − 12H]^{+} | 850.87  | M + 1           | [6RhⅢ + 12H₂NAC − 24H+ + 2Na]^{+} |
| 506.45  | M + 0.25        | [2RhⅢ + 3H₂NAC − 10H]^{+} |       | M + 0.5         | [4RhⅢ + 8H₂NAC − 14H]^{−} |
| 512.57  | M + 0.33        | [4RhⅢ + 7H₂NAC − 15H]^{+} |       | M + 0.33        | [6RhⅢ + 12H₂NAC − 21H]^{3−} |
| 556.39  | M + 0.5         | [3RhⅢ + 6H₂NAC − 11H]^{+} | 861.87  | M + 0.5         | [4RhⅢ + 8H₂NAC − 15H+ + Na]^{−} |
| 566.92  | M + 0.25        | [6RhⅢ + 10H₂NAC − 22H]^{−} | 872.86  | M + 1           | [2RhⅢ + 4H₂NAC − 8H+ + Na]^{−} |
| 621.26  | M + 0.33        | [4RhⅢ + 8H₂NAC − 15H]^{+} |       | M + 0.33        | [4RhⅢ + 8H₂NAC − 16H+ + 2Na]^{−} |
|         | M + 0.33        | [4RhⅢ + 8H₂NAC − 15H]^{+} |       | M + 0.33        | [6RhⅢ + 12H₂NAC − 24H+ + 3Na]^{−} |

*H₂Cys = C₃H₇NO₃S; H₂Pen = C₅H₁₀NO₃S; H₂NAC = C₆H₁₄NO₃S; 2H₂NAC − 2H+ is the oxidized form of N-acetylcysteine with a S=S bond.

Figure 3. Sulfur K edge XANES spectra (left) and the corresponding smoothed second derivatives (right) of the solid products obtained from the reactions of Rh(III)(AcO)₄ with cysteine (5), penicillamine (6), or N-acetylcysteine (7) at a mole ratio of 1:4 at pH = 7.4.

Rh K Edge EXAFS Spectroscopy. Because no single crystals could be prepared, we used Rh K edge X-ray absorption spectroscopy to determine the local structure, that is, nature/number of the nearest neighbors and bond distances, around the Rh(III) ions in the solid reaction products 5–7. The k²-weighted Rh K edge EXAFS spectra and the corresponding Fourier transforms (FTs) for the solids 5–7 are shown in Figure 4 with the least-squares curve-fitting results presented in Table 3. For comparison, EXAFS data for a concentrated solution of the related Rh–glutathione compound 8, [Na₂[RhⅢ(3′(HA)₄)₇H₂O]₆ (HA = glutathione), have also been included. For all three H₃L ligands, the EXAFS spectra collected from the solid Rh products (5–7) overlapped with...
those of the corresponding solution (Figure S-11), whereas the EXAFS spectra of 5–8 did not completely match (Figure S-12).

The curve-fitting results in k-space revealed that the mean Rh−(N/O) and Rh−S distances vary over a narrow range of 2.08–2.10 and 2.315–2.34 Å, respectively, with reasonable σ2 values (0.003–0.005 Å2). The Rh−Rh scattering path included in the fitting models for the Rh−N-acetylcysteine reaction product 7 resulted in the Rh−Rh distance 3.10 ± 0.02 Å (σ2 = 0.0047 ± 0.001 Å2), which is similar to that of the Rh−glutathione reaction product 8.23 For the Rh−cysteine (5) and Rh−penicillamine (6) reaction products, the introduction of a Rh−Rh scattering path to explain the small FT features at ~3.0 and ~2.8 Å, respectively, did not yield conclusive results for such a long and diffuse interaction (see Table S-5).

13C NMR Spectroscopy. To find out whether the carboxyl group in the thiol-containing ligands is bound to the Rh(III) ions, carbon-13 cross-polarization magic angle spinning (CPMAS) NMR spectra of the solids 4–7 were measured, and are compared with those of the pure ligands and Rh2(AcO)4 in Figure 5. We previously assigned the peaks for the CH3OH signal at 49.15 ppm).27

Table 3. Least-Squares Curve-Fitting Results for EXAFS Spectra of the Solid Reaction Products 5–7 and a Concentrated Solution of the Rh−Glutathione Reaction Product 5

| sample | Rh−(N/O) | Rh−S | Rh−Rh |
|--------|----------|------|--------|
| 5      | CN | R (Å) | σ2 (Å2) | CN | R (Å) | σ2 (Å2) | CN | R (Å) | σ2 (Å2) | ΔEi | R2 |
| 6      | 2.0 | 2.10 | 0.0037 | 4.1 | 2.34 | 0.0044 | 2.0 | 3.44 | 0.0142 | 0.7 | 14.7 |
| 7      | 3.2 | 2.10 | 0.0039 | 3.2 | 2.315 | 0.0042 | 0.77 | 3.03 J | 0.0078 | −0.4 | 18.5 |
| 8      | 2 J | 2.09 | 0.0032 | 4.6 | 2.32 | 0.0050 | 0.80 | 3.10 | 0.0047 | −0.2 | 17.5 |
| 9      | 2.5 | 2.08 | 0.0034 | 4.1 | 2.33 | 0.0050 | 0.85 | 3.11 | 0.0046 | 1.2 | 17.6 |

See Figure 4. 8Ampplitude reduction factor (S02) = 0.92 fixed;23 J = fixed value; estimated errors: R ± 0.02 Å; σ2 ± 0.001 Å2; CN ± 10–15%. 9Uncertain values for 5 and 6 (see text). 10R = fitting residual (%).
Acidic pH

When crystallized from a HCl(aq) solution, the chemical shift of Rh₂(AcO)₄ at 181.0 ppm has been reported, with the deshielding probably attributed to the tridentate coordination of a penicillaminate ligand with a COO⁻ group. This peak has lower intensity relative to the corresponding resonance in the spectrum of the Rh(III) compounds. For the carboxylate group in a Pb(II) complex, 1.0 ppm deshielded relative to that of the pure ligand (average 174.1 ppm). Also, for the Δ₀-fac-[Rh(N₃S-Cys)₃] complex crystallized from a HCl(aq) solution, the chemical shift δ_C = 181.0 ppm has been reported, with the deshielding probably due to weak forces between −COO⁻ and the counter H⁺ ions.

Previously, we observed a similar shift of Δ_f([₃¹]C) = 6.0 ppm for the carboxylate group in a Pb(II)–penicillamine solution (C_Pb(II) = 10 mM, C_H₄Pen = 20 mM, pH = 9.6), which was then attributed to the tridentate coordination of a (S,N,O)-Pen−ligand to the Pb(II) ion. Therefore, we assign the resonance at 181.4 ppm to a (S,N,O)-Pen ligand bound to a Rh(III) ion in 6. The other peak at 176.1 ppm is shifted only ~2.0 ppm downfield relative to penicillamine, which indicates a penicillamine ligand with a COO− group that is not bound to Rh(III). The small shift could be due to ionic interactions of the COO− group.

For the Rh–cysteine reaction product 5 that precipitated at pH = 3.2, there are three peaks in the carboxylate region at 174.0, 182.0, and 194.3 ppm. The latter peak was attributed to a sulfenato group (S=O) bound to Rh(III); see Results section.

Reactions occur much faster at pH = 7.4, leading to compound 6, Na₂[Rh₂(Pen)₂(Pen(SO))₂].4H₂O, with a structure similar to (d).

**DISCUSSION**

**Aerobic Reaction of 1 with Penicillamine.** Within the first few hours after mixing the reagents Rh₂(AcO)₄ (1) and penicillamine at a mole ratio of 1:4 (initial pH 4.1), the species [Rh₃⁺(AcO)₄(H₂Pen)₁₋₂] (Scheme 2 a) and [Rh₂⁺(AcO)₃−(HPen)⁺] (+ or −H⁺) with a Rh(II,II) core could be detected with ESI-MS. Oxidation of the rhodium ions was observed when two deprotonated penicillamine ligands were present, as in the [Rh₃⁺(AcO)₄(HPen)₂] complex (Scheme 2 b). [Rh₃⁺(Pen)₂]₅⁻ was detected within the first 10 min of reaction (see Appendix 2 in the Supporting Information). The reaction occurs much faster at pH = 7.4.

When two deprotonated penicillamine ligands were present, as in the [Rh₂⁺(AcO)₄(HPen)₂] complex (Scheme 2 b), ESI-mass spectrum in the − ion mode of the Rh–penicillamine compound Na₂[Rh₂(Pen)₂(Pen(SO))₂].4H₂O (6) dissolved in water was dominated by mass peaks for [Rh₃⁺Pen₄− + H⁺]⁻ (m/z = 794.96) at fragmentor voltage = 0 V, and [Rh₃⁺Pen₄]⁻ (−m/z = 396.98) at 80 V, and their m/z +8 and +16 satellites. An absorption peak at 2475.6 eV in the S K edge XANES spectrum of 6 further revealed the presence of a sulfenato group (S=O) bound to Rh(III); see Results section.

Oxidation of metal-bound thiolates by peroxides to sulfenato groups has been previously reported for thiolato Co(III) and thiolato Ru(II) polypyridyl complexes, as well as for glutathione-bound Ru(II)–arene and organo-Ir(III) anticancer compounds. Liu and Sadler proposed that hydrogen peroxide (detected by a H₂O₂ test stick), formed from O₂(g), provides the sulfenate (S=O) oxygen atom, as previously suggested by the Dunbar group. Here, we also propose that oxidation of the Rh₂⁺ core in 1 to Rh₂⁶⁺ in 6 occurs by...
reduction of oxygen (O₂) to peroxide (equation 1), which in turn may oxidize a coordinated penicillamine thiolato to a sulfenato (S=O) group (equation 2). Formation of peroxide in the reaction mixture was confirmed by an iodine test (see Appendix 1 in the Supporting Information).  

\[
[Rh^{III}_2(AcO)_4] + 4H_2Pen + O_2(g) + 2OH^- \rightarrow [Rh^{II}_2(Pen)_4]^{2-} + H_2O_2(aq) + 4AcOH + 2H_2O \tag{1}
\]

\[H_2O_2(aq) + R - S - Rh^{III} \rightarrow R - S(O) - Rh^{III} + H_2O \tag{2}
\]

The structure of the Rh–penicillamine compound 6 was probed by Rh K edge EXAFS, complemented by 13C CPMAS NMR spectroscopy, which revealed 3.1 Rh–(N/O) and 3.2 Rh–S distances at 2.09 ± 0.02 and 2.32 ± 0.02 Å, respectively (Figure 4 and Table 3). The observation of two 13C NMR resonances at 176.3 ppm and a shoulder at 182.3 ppm (Figure 5) assigned to bidentate (S,N)-donor ligand as that in the RhIII(HCys)2(Cys)2 complex, similar to those found for the RhIII(Cys)4 complex. The EXAFS Fourier transform in Figure 4 provides evidence for two types of carboxylate groups, and thus supports two coordination modes for the Rh(III)-bound carboxylate groups (see Results section). Compound 6 is expected to have a similar structure as that shown in Scheme 2d.

Scheme 2 displays our proposed pathway for the aerobic reaction between Rh3(ACO)4 and penicillamine (mole ratio of 1:4). In the proposed structure for Na3[Rh2(Pen)2(Pen(SO))2].4H2O (6), the O atom is bound to the S atom of a tridentate (S,N,O-Pen) ligand because in Rh–S–C, the S atom, with bonds to two other atoms, is sterically less hindered for nucleophilic attack by peroxide than the bridging S atom in Rh–S(O)–Rh with three bonds (Scheme 2).

In our CSD search, only three crystalline dithiolato-bridged binuclear Rh(III) complexes were found, for which the average Rh–Rh distance is 3.54 ± 0.03 Å (see Table S-6a–c in the Supporting Information). In the binuclear [RhIIIIL(µ-S,N-C6H6NS)(η²-S-C6H4NS)(bpy)]2+ complex with a similar (S,N)-donor ligand as that in the Rh–penicillamine compound 6, the Rh–Rh distance is 3.51(3) Å.12 The contribution of Rh···Rh scattering to the overall Rh K edge EXAFS spectrum of 6 would be insignificant for a long nonbonded distance (with high σ²), and does not appear as a well-resolved peak in the EXAFS Fourier transform in Figure 4.

**Aerobic Reaction of 1 with Cysteine.** The aerobic reaction of Rh3(ACO)4 with cysteine at a mole ratio of 1:4 differs from that with penicillamine. The diamagnetic precipitate gradually formed at pH = 3.2 was separated and analyzed with the empirical formula [RhIII(Cys)2(HCys)2(Cys)2.4H2O]n (4). When raising the pH of the reaction mixture, the precipitate dissolved completely at pH = 3.9. The solid product obtained after initially adjusting the pH of the reaction mixture to 7.4 (see Experimental Section) empirically corresponds to the formula [Na5[RhIII(Cys)4]3·SH2O]n (5).

The Rh K edge EXAFS spectrum of this solid is identical to that of 4 (Figure S-13), but had a different phase and amplitude when compared with that of the Rh–penicillamine compound 6 (see Figure S-12, top). Least-squares curve-fitting of the EXAFS oscillation for the solid 5 revealed contributions from 2.0 Rh–(N/O) and 4.1 Rh–S bond distances at 2.10 ± 0.02 and 2.34 ± 0.02 Å, respectively (see Figure 4 and Table 3).

The 13C CPMAS NMR spectrum of the solid 5 displays a main peak at 176.3 ppm and a shoulder at 182.3 ppm (Figure 5) assigned to bidentate (S,N-Cys) and tridentate (S,N,O-Cys), respectively (see Results section). The different relative intensity of these two peaks, as compared with the 13C NMR spectrum of the Rh–penicillamine compound Na2[Rh2(Pen)2(Pen(SO))2]·4H2O (6), implies that the bidentate (S,N)-mode of the cysteinate ligands in 5 dominates.

The weak absorption at 2475.6 eV in the S K edge XANES spectrum of the Rh–cysteine compound 5 shows a significantly smaller amount of sulfenato (S=O) groups than that in 6 (Figure 3). However, in the ESI-mass spectrum of 5 (dissolved in water), the m/z = 682.83 correspond to one or two additional oxygen atoms to the [Rh3(Cys)4]2+ and [Rh3(Cys)4 + H+]2+ mass ions (Figure 2 and Table 2), respectively, indicating that ESI-MS is a more sensitive probe for detecting small amounts of additional O atoms. Even at the low fragmentor voltage of 10 V, intense m/z = 682.83 and/or 16 peaks were identified for the mass ions [Rh3(Cys)4 + H+]2+ (m/z = 682.83) and [Rh3(Cys)4 + 3H+]4+ (m/z = 803.84); see Figure S-10 and Table S-4. It is unlikely that the added O atoms are generated from oxidation in the ESI-MS instrument because, for example, no m/z = 682.83 and/or 16 satellites were observed for [RhIII(2AcO)(HNAC)]2 + Na+ or Na-acetylcysteine [H2NAC–H+] (m/z = 162.02) in Figures S-2 and S-6, respectively.

At the fragmentor voltage of 80 V, additional mass peaks corresponding to tri- and tetranuclear species appeared, such as: [Rh3(Cys)6]5− (m/z = 340.91), [Rh3(Cys)6 + H+]2+ (m/z = 511.87), [Rh3(Cys)6 + H + O]3+ (m/z = 519.87), [Rh4(Cys)6 + H+]3+ (m/z = 454.89), and [Rh4(Cys)6 + 2H + O]2+ (m/z = 690.83). The ESI-MS analysis of the Rh–penicillamine reaction product 6 also shows minor amounts of similar tetrarners [Na2[Rh5(Pen)6]n (n = 2), based on the isotopic patterns of the peaks (m/z) = 794.96 and 810.95 for the [Rh5(Pen)6 + 3H+]7+ and [Rh5(Pen)6 + 2H + 3O]2+ ions, respectively (see Table 2). Although some cluster formation could also be observed in the ESI-mass spectra of pure Rh3(ACO)4 in water (Figure S-6), the difference between the EXAFS spectra of 5 and 6 (Figure S-12) can only be explained by the presence of oligomeric species in 5.

On the basis of the combined results, we conclude that in the reaction product 5 of Rh3(ACO)4 and cysteine (pH = 7.4), the Rh(III) ions are connected via double thiolate bridges probably in at least up to tetrameric species, although some dimeric Na2[Rh3(Cys)4] complexes, similar to those found for the Rh–penicillamine compound 6, cannot be ruled out. Scheme 3 displays our proposed structure for a tetraron [Na2[Rh5(Cys)6]n (n = 2) and its oxidized form, as identified by ESI-MS.

In a large oligomeric structure for 5, there are four Rh–S and two Rh–(N/O) bonds around the Rh(III) ions (except for the terminal units), as indicated by the EXAFS data analysis (Table 3). Only the S atoms with two bonds in the terminal tridentate (S,N,O-Cys) ligands are expected to be oxidized by peroxide to sulfenato (S=O) groups because the bridging thiolate groups with three bonds are less susceptible to nucleophilic attack by peroxide. The number of unbound COO− groups is considerably higher than that of the bound terminal COO−, which gives rise to a small shoulder at δ = 182.3 ppm in the 13C CPMAS NMR spectrum of 5 (Figure 5). The close overlap of the EXAFS spectra (Figure S-13) indicates that the structures of the Rh–cysteine precipitate 4, [Rh3(Cys)4(HCys)2·4H2O]n from acidic (pH = 3.2) media, and 5 are similar, apart from the protonated −COOH groups in 4, shown by the 13C NMR resonance at δ = 194.3 ppm (Figure S-5).
The chiral $[\text{Rh(S-N-Cys)}_3]^{3-}$ complex readily functions as a S-donating ligand to form linear S-bridged trinuclear $[\text{M(Rh-S-N-Cys)}_3]^{2-}$ complexes with various metal ions ($\text{M} = \text{Cr}^{3+}, \text{Co}^{II}$ or $\text{Co}^{III}$, and $\text{Ni}^{II}$) with $\text{MS}_6$ coordination, as well as in the T-cage S-bridged polynuclear complex $[\{\text{Rh(S-N-Cys)}_4\}_4\text{Zn}_4\text{O}]^{6-}$, which has been studied for decades.

Polynuclear S-bridged structures such as the tetranuclear one proposed in Scheme 3 for the $[\text{Rh}^{III}_2(\text{S-N-Cys})_4]^{2-}$ ($n = 2$) complex are other examples of this type of multinuclear compound, formed in absence of a different metal ion ($\text{M}$).

Such oligomers could be formed with morphologically similar but not identical $\text{Rh}^{III}$ centers, for example, with different orientations/conformations of Cys$^{3-}$ or Pen$^{2-}$ rings. The variations in the local environment of the carbon atoms in these ligands would result in broad signals in the $^{13}\text{C}$ NMR spectra in Figure 5. Recent $^{1}H$ NMR spectra were reported for a D$_2$O solution of S-methylcysteine coordinated to the axial position of $\text{Rh}_{2}(\text{AcO})_4$ (mole ratio of 1:1); the broad signals observed in this spectrum were attributed to $J(\text{H,H})$ coupling.

**Aerobic Reaction of 1 with $N$-Acetylcysteine.** Monitoring the slow reaction of $\text{Rh}_2(\text{AcO})_4$ (1) with $N$-acetylcysteine (mole ratio of 1:4) at the pH of mixing (pH = 1.9) with ESI-MS revealed that species with one $N$-acetylcysteine ligand (in protonated or even fully deprotonated form) still keep the $\text{Rh}^{II}-\text{Rh}^{III}$ core, as in $[\text{Rh}^{II}_2(\text{AcO})_4(\text{H}_2\text{NAC})]$ and $[\text{Rh}^{II}_2(\text{AcO})_2(\text{HNAC})]$ (with $\pm \text{H}^+$ or $\pm \text{Na}^+$) as well as $[\text{Rh}^{III}_2(\text{AcO})_2(\text{NAC})]$. Similar to the penicillamine reaction, oxidation to $\text{Rh}^{III}$ is observed in species with two deprotonated $N$-acetylcysteine ligands: $[\text{Rh}^{III}_2(\text{AcO})_4(\text{HNAC})_2]$ and $[\text{Rh}^{III}_2(\text{AcO})_2(\text{HNAC})_2]^+$ (see Appendix 1 in the Supporting Information).

Following the reaction in acidic solution with UV−vis spectroscopy showed after 48 h, an intense absorption peak at $\lambda_{\text{max}} = 367$ nm (Figure 1, left), which is attributed to a $S \rightarrow \text{Rh}^{III}$ ligand-to-metal charge-transfer band of the $[\text{Rh}^{III}_2(\text{AcO})_4(\text{HNAC})]$, which is the main $\text{Rh}^{III}$ complex according to the ESI-mass spectrum ($^{+}m/z = 766.91$, 788.90, and 810.88 for $^{+}\text{H}^+/\text{Na}^+$ ions, Table 1 and Figure 1 right). The EXAFS spectrum of this complex could not be obtained due to the presence of unreacted $\text{Rh}_2(\text{AcO})_4$ (see Appendix 1).

The solid reaction product at pH = 7.4, purified by size exclusion chromatography, has the empirical formula $\{\text{Na}_2[\text{Rh}_2(\text{NAC})_4] \cdot 4.5\text{H}_2\text{O}\}_n$ ($7$), based on elemental analysis. The $\text{Rh}^{III}$ edge EXAFS spectra of 7 and of the corresponding $\text{Rh}^{III}$−cysteine reaction product 5 show main FT peaks of similar magnitude, but with an additional feature at $\sim 2.7$ Å for 7, as also found for the $\text{Rh}^{III}$−glutathione reaction product 8 (Figure S-12). Its EXAFS oscillation was best modeled with 2 $\text{Rh}^{III}−\text{O}$, 4.6 $\text{Rh}^{III}−\text{S}$, and 0.8 $\text{Rh}^{III}−\text{Rh}$ interactions at mean distances of 2.09 ± 0.02, 2.32 ± 0.02, and 3.10 ± 0.02 Å, respectively, which are similar to those obtained for 8 (see Figure 4 and Table 3). The $\text{Rh}−\text{O}$ scattering contribution is better resolved for 8, appearing as a shoulder in the main FT peak, probably due to less noise in its EXAFS oscillation. Simultaneous refinement of the $\text{Rh}−\text{O}$ coordination number for 7 resulted in an unusually high value (5.7) for the $\text{Rh}−\text{S}$ coordination.

**Scheme 3. Proposed Structures for a Tetrameric $\{\text{Na}_2[\text{Rh}_2(\text{Cys})_4]\}$ Complex in Compound 5**

The lower image is its oxidized form, detected by ESI-MS, with sulenato ($\text{S}=\text{O}$) groups.

**Scheme 4. Proposed Oligomeric Structures for the $\{\text{Na}_2[\text{Rh}_2(\text{NAC})_4]\cdot 4.5\text{H}_2\text{O}\}_n$ (Compound 7)**
number, and very small $\sigma^2$ for Rh–O (see Table S-5). In the trimeric $\Delta\Lambda_{[\text{Rh}(\text{aet})_2]}$ complex (CSD code: AQU-VOX), the central Rh(III) ion is surrounded by six bridging thiolate groups with the average Rh–S distance 2.381 Å, which is longer than the refined mean Rh–S distance in 7. Therefore, a model including two Rh–O and four Rh–S scattering paths seems reasonable. The Rh(III)–Rh(III) distance in 7 is comparable with the Rh(III)–Ir(III) distance of 3.0199(3) Å in the $[\text{Rh}_{2}(\text{Ir}(\text{aet})_3)_2]$ complex with three bridging thiolate groups between the Rh(III) and Ir(III) ions (see Figure S-9 and Table S-3).

The 13C CPMAS NMR spectrum of 7 only shows one set of broad peaks for the coordinated N-acetylcysteine, with the COO– group resonating at $\delta = 177.1$ ppm, shifted downfield only by $\Delta\delta (1^5\text{C}) = 1.7$ ppm relative to that of the pure ligand (Figure 5), indicating that the carboxylate group is not directly bonded to the Rh(III) atoms in 7. The ESI-mass spectrum of this product in water at fragmentor voltage 0 V was dominated by mass peaks for tetranuclear Rh(III) complexes with three bridging thiolate groups, sterically hindered by bonds to three atoms, are not oxidized by peroxide, leaving only the terminal thiolates (S–Rh(III)) exposed to oxidation to sulenates groups.

The results of this study reveal the structure of products that could form when antitumor active rhodium(II) tetraacetate, Rh$_2$(AcO)$_4$, interacts with thiol-containing proteins and peptides, whether acting like N-acetylcysteine or glutathione as a monothiolate entity, or as the ($S,N$-) chelate cysteine in an N-terminal site of a protein.

### CONCLUSIONS

Aerobic reactions of Rh$_2$(AcO)$_4$ with the thiol-containing amino acid cysteine (H$_2$Cys) and its derivatives penicillamine (H$_2$Pen) and N-acetylcysteine (H$_2$NAC) in different mole ratios (1:2, 1:4, and 1:6) at pH = 7.4 in reaction mixture containing Rh$_2$(AcO)$_4$ and N-acetylcysteine (mole ratio 1:4, C$_1$ = 4.5 mM, pD = 2.3); ESI-mass spectra of pure Rh$_2$(AcO)$_4$ and pure N-acetylcysteine in water, of the reaction mixture containing Rh$_2$(AcO)$_4$ and N-acetylcysteine (mole ratio 1:4, C$_1$ = 1 and 4.5 mM, acidic pH), and of the reaction mixture of Rh$_2$(AcO)$_4$ and penicillamine (mole ratio 1:4, C$_1$ = 1.9 mM, acidic pH), and assignment of the corresponding mass peaks; UV–vis spectra of the latter solution and the reaction mixture containing Rh$_2$(AcO)$_4$ and N-acetylcysteine (mole ratio 1:4, C$_1$ = 1.0 mM, pH = 7.4) at different time intervals; fragmentor voltage varied ESI-mass spectra of Na$_2$[Rh$_2$(Pen)$_2$(Pen(SO)$_2$)$_2$·4.5H$_2$O] (6), Na$_2$[Rh$_{11+2}$(Cys)$_4$·5H$_2$O] (5), and Na$_2$[Rh$_2$(NAC)$_4$]·4.5H$_2$O (7); UV–vis spectra of aqueous solutions of 6 and 7; comparison between the $k$-weighted EXAFS spectra of the concentrated aqueous solutions and their corresponding solid products 5–7 and those of solids {Rh$_{11+2}$(HCys)$_4$·4H$_2$O} (4) and 5; comparison between the $k$-weighted EXAFS spectra of the solid products 5–7 and the Rh–glutathione complex 8; comparison between the first derivative EPR spectra of Cu(ClO$_4$)$_2$·6H$_2$O and solid 7; comparison between the yields of reaction products 5–7 when using different Rh$_2$(AcO)$_4$/H$_2$O mole ratios at pH = 7.4, and ESI-mass spectra for the Rh$_2$(AcO)$_4$/H$_2$Pen reaction mixture (mole ratio 1:2, pH = 7.4) (PDF)

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