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Methionine Binding to Dirhodium(II) Tetraacetate

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Abstract

The reaction between antitumor active dirhodium(II) tetraacetate and *DL*-methionine (HMet) was followed in aqueous solution and showed initially mixtures of 1:1 and 1:2 adducts $[\text{Rh}_2(\text{AcO})_4(\text{HMet})(\text{H}_2\text{O})]$ ($\text{AcO}^- = \text{CH}_3\text{COO}^-$) and $[\text{Rh}_2(\text{AcO})_4(\text{HMet})_2]$ formed at room temperature (RT), as evidenced by UV-vis spectroscopy and electrospray ionization mass spectrometry (ESI-MS). Rh K-edge extended X-ray absorption fine structure (EXAFS) spectroscopy confirmed methionine thioether binding to the axial positions of the $\text{Rh}_2(\text{AcO})_4$ cage structure. With excess HMet at RT stepwise displacement of the acetate groups was observed after some time using ESI-MS. Heating the solution to 40 °C for 24 h accelerated the substitution reaction leading to stable dirhodium(II) species with two acetate ligands displaced by two methionine groups. The crystal structure of the purple $[\text{Rh}^{\text{II}}_2(\text{AcO})_2(\text{D-Met})(\text{L-Met})] \cdot 6\text{H}_2\text{O}$ compound obtained from the solution revealed tridentate coordination of the methionine ligands to the Rh(II) ions, with the thioether S atoms in equatorial positions. A minor amount of a light orange monomeric $[\text{Rh}^{\text{III}}(\text{Met})_2](\text{AcO})$ complex also formed in the solution was isolated by size exclusion chromatography, and identified by ESI-MS. Crystals of $[\text{Rh}^{\text{III}}(\text{D-Met})(\text{L-Met})]\text{Cl} \cdot 3\text{H}_2\text{O}$ were prepared by reacting RhCl_3 and *DL*-HMet. The crystal structure showed tridentate binding of the methionine ligands to the Rh(III) ion in a *trans-S,N,O* arrangement.

Graphical Abstract

The amino acid methionine (HMet) coordinates reversibly via its sulfur atom to the axial positions of the antitumor active complex, $\text{Rh}_2(\text{CH}_3\text{COO})_4$ (**1**). The current study shows that methionine subsequently forms $\text{Rh}_2(\text{CH}_3\text{COO})_2(\text{S,N,O-Met})_2$ complexes, replacing two of the bridging acetate groups in **1**. This irreversible displacement which occurs relatively fast at 40 °C, would reduce the antitumor activity of **1** *in vivo*.

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: XXXX. Crystal data and selected bond distances for the hydrated complexes of **4DL** and **5**; syntheses and proposed structures of **4L** and **4D**; TD-DFT simulated UV-vis spectra for the aqua complex, the mono- and dithioether adducts; low contribution transitions centered around 270–310 nm for the adducts; separate contributions of different scattering paths in the EXAFS spectra of solution **A** and solid **4**; ESI-MS time study of solution **B** over 37 days and the corresponding peak assignment; ESI-mass spectra for solids **4D** and **4L**, and for different bands on the size-exclusion column, as well as for solution **A** after 24h (and 7 days) stirring at 40 °C and refluxing at 100 °C; assignment of ^{13}C NMR peaks.

Accession Codes

CCDC 1855920–1855921 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

Notes

The authors declare no competing financial interest.

Keywords

Dirhodium(II) tetraacetate; Methionine; Structure; ^{103}Rh -NMR; EXAFS

1. INTRODUCTION

Dirhodium(II) complexes have attracted considerable attention as a possible alternative to cisplatin due to their remarkable cytotoxicity.¹ Since 1972, when Bear *et al.* first described their antitumor activity of this type of compounds, a number of new dirhodium(II) derivatives with improved activities relative to cisplatin have been reported.^{2, 3} Although their mechanism of action still remains ambiguous,⁴⁻⁸ it is well known that dirhodium(II) carboxylates can bind to both single- and double-stranded DNA,^{5, 9} as well as to several nucleotides,¹⁰ and can form intra-strand DNA cross-links,¹¹ inhibiting DNA replication and transcription *in vitro*.^{7, 12, 13} Interactions of amino acids, proteins and enzymes with dirhodium(II) carboxylates have also been investigated.^{8, 14} Among the amino acids with a heteroatom in the side chain, only cysteine, histidine and methionine caused obvious changes in the UV-vis spectrum upon coordination to dirhodium(II) tetraacetate, $\text{Rh}_2(\text{AcO})_4$ (1), with color change from emerald green to yellow, pink and purple, respectively.¹⁵

Methionine (HMet), with a thioether group in its side chain, is one of the two sulfur-containing proteinogenic amino acids. Animal proteins are common dietary sources of methionine, which is primarily metabolized in the liver.¹⁶ Its reaction with ATP generates *S*-adenosyl-*L*-methionine, which serves as the major methyl donor in transmethylation processes, leading to *S*-adenosyl-*L*-homocysteine. The latter can be hydrolyzed to homocysteine, which can be metabolized through transsulfuration to *L*-cysteine and glutathione.¹⁷ However, a significant amount of the homocysteine synthesized in the liver can be remethylated into methionine.^{16, 18} The level of free methionine in the plasma of an average person increases moderately after a high-protein meal,¹⁹ and the normal range for methionine in plasma in adults is 16-30 μM .²⁰ Earlier studies have shown that HMet promotes cisplatin toxicity, probably by forming a toxic complex with this anticancer drug *in vivo*,²¹ and can also reduce the rate of cisplatin reaction with DNA bases in natural DNA.²²

Methionine has three potential coordination sites: the carboxyl group ($-\text{COOH}$), the amino group ($-\text{NH}_2$) and the thioether group ($-\text{SCH}_3$). For *D,L*-methionine, the acid dissociation

constants for the carboxyl and amino groups are $pK_{a1} = 2.27$ and $pK_{a2} = 9.16$, respectively ($I = 1.00\text{ M}$; $25\text{ }^{\circ}\text{C}$).²³ The S atom in methionine is a much weaker σ donor than the thiolate in deprotonated cysteine.²⁴ Depending on the nature of the metal ion, methionine can coordinate in monodentate, bidentate or tridentate mode, as in $[\text{Ti}^{\text{IV}}(\text{O-HMet})_2(\text{Cp})_2]\text{Cl}_2$ ($\text{Cp} = \text{Cyclopentadienyl}$; C_5H_5^-),²⁵ $[\text{Pt}^{\text{II}}\text{Cl}_2(\text{S},\text{N-HMet})]$,²⁶ $[\text{Hg}^{\text{II}}(\text{N},\text{O-Met})_2]$,²⁷ and $[\text{Co}^{\text{III}}(\text{S},\text{N},\text{O-Met})_2](\text{ClO}_4)$, respectively.²⁸

The preparation of solid $[\text{Rh}_2(\text{AcO})_4(\text{S-HMet})_2]$ from an aqueous solution of $\text{Rh}_2(\text{AcO})_4$ and *L*-methionine (1:2 mole ratio) was initially reported by Pneumatikakis and Psaroulis,²⁹ who proposed based on IR spectroscopy that HMet was bound via its thioether group ($-\text{S}-\text{CH}_3$) to the axial positions of the $\text{Rh}_2(\text{AcO})_4$ complex. Later, Jakimowicz *et al.* monitored the same reaction at pH 7.4 via UV-vis spectroscopy over 5 days, and confirmed the formation of $[\text{Rh}_2(\text{AcO})_4(\text{S-HMet})_{1.2}]$ based on “the blue shift of the band I and strong increase of absorption in the range of 430-350 nm”, which was assigned as $\text{S} \rightarrow \text{Rh}$ charge transfer.³⁰ Band I has been attributed to the $\pi^*(\text{Rh}_2) \rightarrow \sigma^*(\text{Rh}_2)$ transition in $[\text{Rh}_2(\text{AcO})_4(\text{H}_2\text{O})_2]$.³¹ More recently, ^1H NMR spectroscopy was used to confirm the *S*-coordination of HMet to $\text{Rh}_2(\text{AcO})_4$ in D_2O by comparing experimental data with calculated ^1H -NMR chemical shifts.³² Axial ligands are generally very labile with rapid exchange,³³ and only one averaged signal was observed for an equilibrium mixture of $[\text{Rh}_2(\text{AcO})_4(\text{S-HMet})(\text{H}_2\text{O})]$ (2) and $[\text{Rh}_2(\text{AcO})_4(\text{S-HMet})_2]$ (3). Evidently, those axial ligands compete with the weakly coordinated water molecules in the diaqua adduct in aqueous solution.^{32, 34}

Sulfur-containing biomolecules play significant roles in the human body and investigations of how they interact with antitumor active complexes are important to assess their *in vivo* activity. We recently reported on structural aspects of the aerobic reaction products of $\text{Rh}_2(\text{AcO})_4$ with glutathione, cysteine and its derivatives.^{35, 36} Here, we describe products formed in the reactions of $\text{Rh}_2(\text{AcO})_4$ and methionine (mole ratios 1:2 and 1:10) in aqueous solution at different temperatures. The products were characterized by combining results from single crystal X-ray diffraction, electrospray ionization mass spectrometry (ESI-MS), UV-vis, ^{13}C NMR, ^{103}Rh NMR, and Rh K-edge X-ray absorption spectroscopy. Our study shows that methionine can irreversibly replace two of the bridging acetate groups in the $\text{Rh}_2(\text{AcO})_4$ cage and bind to the Rh(II) ions in tridentate (*S,N,O*) mode by occupying both axial and equatorial positions in a stable $\text{Rh}_2(\text{AcO})_2(\text{Met})_2$ complex. Such methionine chelation would certainly impair the antitumor efficiency of $\text{Rh}_2(\text{AcO})_4$.

2. EXPERIMENTAL SECTION

2.1. Sample Preparation.

DL-methionine, *D*- and *L*-methionine were purchased from Fluka Chemicals and Sigma Aldrich, respectively, and used without further purification. Dirhodium(II) tetraacetate was used as supplied by Pressure Chemical Co. Sephadex G-15 was obtained from GE Healthcare Life Sciences. The pH values of the solutions were monitored with a Thermo Scientific Orion Star pH meter calibrated with standard buffers.

Solutions for UV-vis titration.—Stock aqueous solutions of $\text{Rh}_2(\text{AcO})_4$ (2.00 mM), and *DL*-methionine (0.20 M) were prepared with distilled water. Using a micro pipet, 0.400 mL

of the stock $\text{Rh}_2(\text{AcO})_4$ solution (0.80 μmol) was placed in a 1.0 mm cuvette (solution **I**). UV-vis spectra were collected after subsequent additions of 4.0 μL of the HMet stock solution (each time adding 0.80 μmol HMet). During the titration, the solution pH was constant (5.65 ± 0.01).

Another stock solution of $\text{Rh}_2(\text{AcO})_4$ (0.20 mM) was prepared; 4.00 mL of this solution (0.80 μmol) was placed in a 1.0 cm cuvette (solution **II**). The titration with *DL*-methionine was monitored via UV-vis spectroscopy, after stepwise additions of 4.0 μL of the 0.20 M HMet stock solution. The pH variation was insignificant as the mole ratio changed from 1:1 (pH = 5.92) to 1:10 (pH = 5.88)

Solution A ($\text{Rh}_2(\text{AcO})_4$:(*DL*-HMet) mole ratio 1:2).—Addition of a solution of *DL*-methionine (0.121 mmol) in 5.00 mL of water to an emerald green solution of $\text{Rh}_2(\text{AcO})_4$ (**1**, 0.060 mmol) completely dissolved in 25.0 mL of water, immediately changed the solution color to violet (pH = 5.5, C_1 = 2.00 mM, similar to solution **I**), and its ESI-mass spectrum was measured. For EXAFS measurements a more concentrated solution was prepared by adding solid *DL*-methionine (0.456 mmol) to a solution of $\text{Rh}_2(\text{AcO})_4$ (0.226 mmol) in 35.0 mL of water and stirring for 2 hrs (C_1 = 6.45 mM).

Solution B ($\text{Rh}_2(\text{AcO})_4$:(*DL*-HMet) mole ratio 1:10).—Mixing a solution of $\text{Rh}_2(\text{AcO})_4$ (0.060 mmol) completely dissolved in 25.0 mL of water with a solution of *DL*-methionine (0.600 mmol) in 5.00 mL of water, resulted in a burgundy solution (pH = 5.2, C_1 = 2.00 mM, similar to solution **I**) and its ESI-mass spectrum was collected. For ^{103}Rh NMR measurement a more concentrated solution (**B_{conc}**; $C_1 \sim 26$ mM) was prepared by adding solid *DL*-methionine (2.262 mmol) to a solution of $\text{Rh}_2(\text{AcO})_4$ (0.227 mmol) in 35.0 mL of water and stirring for 2 hrs (C_1 = 6.45 mM). Using a rotary evaporator the solution volume was reduced at room temperature to ~ 8.8 mL (pH = 3.8).

$[\text{Rh}_2(\text{AcO})_2(\text{Met})_2] \cdot 5\text{H}_2\text{O}$ (4** + $5\text{H}_2\text{O}$).**—Solid *DL*-methionine (0.452 mmol) was added to an emerald green aqueous solution of $\text{Rh}_2(\text{AcO})_4$ (0.226 mmol). The resulting violet solution was stirred at 40 °C for 24 h, then concentrated to 2 mL and passed through a Sephadex G-15 size exclusion chromatography column using distilled water as eluent. The purple band was isolated and evaporated to dryness resulting in a purple solid (**4** + $5\text{H}_2\text{O}$, yield: 19%). The reaction yield increased to 35% by refluxing the solution at 100 °C for 24 h. Elemental anal. Calcd for $[\text{Rh}_2(\text{AcO})_2(\text{Met})_2] \cdot 5\text{H}_2\text{O}$ (**4** + $5\text{H}_2\text{O}$), ($\text{Rh}_2\text{C}_{14}\text{H}_{36}\text{N}_2\text{O}_{13}\text{S}_2$): %C 23.67, %H 5.11, %N 3.94 (12.7% H_2O); Found %C 23.61, %H 4.97, %N 3.94 (TG: 13.1% H_2O). ^{13}C NMR (400 MHz, D_2O): δ_{C} (ppm) = 19.1, 19.3 (SCH_3), 22.4 (CH_3COO), 27.8, 28.6, 32.5, 33.1 (SCH_2CH_2), 58.4, 59.1 (CHNH_2), 180.9, 181.4 (Met-COO), 189.7 (CH_3COO).

$[\text{Rh}_2(\text{AcO})_2(\text{D-Met})(\text{L-Met})] \cdot 6\text{H}_2\text{O}$ (4DL** + $6\text{H}_2\text{O}$).**—Purple crystals of hexahydrate **4DL** were obtained by vapor diffusion of acetone into an aqueous solution of **4**. ^1H NMR (400 MHz, D_2O): δ_{H} (ppm) = 3.74 (t, $J_{\text{HH}} = 3.9$ Hz, 2H), 3.06 (dt, $J_{\text{HH}} = 4.4$ Hz, $J_{\text{HH}} = 12.8$ Hz, 2H), 2.81 (td, $J_{\text{HH}} = 4.0$ Hz, $J_{\text{HH}} = 12.5$ Hz, 2H), 2.52 (dq, $J_{\text{HH}} = 4.3$ Hz, $J_{\text{HH}} = 16$ Hz, 2H), 2.29 (s, 3H), 2.10-2.20 (m, 2H), 2.14 (s, 6H), 2.11 (s, 3H). ^{13}C NMR (400 MHz, D_2O): δ_{C}

(ppm) = 19.4 (SCH₃), 22.4 (CH₃COO)_{ave}, 27.8, 32.6 (SCH₂CH₂), 58.4 (CHNH₂), 181.4 (Met-COO), 189.7 (CH₃COO)_{ave}.

The compounds [Rh₂(AcO)₂(*L*-Met)₂] $\cdot$ 4.5H₂O (**4L** + 4.5H₂O) and [Rh₂(AcO)₂(*D*-Met)₂] $\cdot$ 4H₂O (**4D** + 4H₂O) were also prepared following the same procedure as for (**4** + 5H₂O), using pure *D*- and *L*-methionine enantiomers (see Supporting Information). However, attempts for preparing single crystals failed.

Synthesis of [Rh(*D*-Met)(*L*-Met)]Cl (5**).—**The compound was prepared following a previously reported procedure with slight modifications.³⁷ To an aqueous solution of *DL*-methionine (1.003 mmol) in 17.0 mL water solid NaOH was added (1.004 mmol), while stirring the solution for 5 minutes. Dark red crystalline RhCl₃ $\cdot$ 3H₂O (0.380 mmol) was then added, refluxing the mixture for 3 hours. After filtering the mixture the filtrate was concentrated. Slow evaporation of this solution led to yellow single crystals of **5**. Before elemental analysis the solid was dried under vacuum. Elemental anal. Calcd for [Rh(*D*-Met)(*L*-Met)]Cl (RhC₁₀H₂₄N₂O₆S₂Cl): %C 27.63, %H 4.64, %N 6.44; Found %C 27.56, %H 4.58, %N 6.41; yield = 29%. The single crystal taken from mother liquor contained [Rh(*D*-Met)(*L*-Met)]Cl $\cdot$ 3H₂O (**5** + **3H₂O**); see Section 3.3.

2.2. Physical Measurements and Methods.

UV-vis absorption spectra were measured using a Cary spectrophotometer. Samples were measured at room temperature in 1.0 mm or 1.0 cm quartz cells using water as reference.

ESI-Mass spectrometry.—Electrospray ionization (ESI) mass spectra were collected both in positive (+) and negative (–) ion modes on an Agilent 6520 Q-ToF instrument. Samples from solutions **A** and **B** (*C*₁ = 2.00 mM) diluted in MeOH were injected and mobilized using an injection flow rate of 0.2 mL/min and a drying gas flow rate of 7 L/min at 200 °C. Capillary, skimmer and fragmentor voltages were set at 4000, 65 and 80V, respectively. Peak assignments were confirmed using the Isotope Distribution Calculator from Agilent and a high resolution Isotope Distribution Calculator from Scientific Instrument Services.³⁸

NMR spectroscopy.—¹H and ¹³C NMR measurements were carried out at room temperature using a Bruker Avance III 400 MHz spectrometer. ¹³C NMR spectra were collected using a 30° pulse, a 26.2 kHz sweep width, a 1-s delay between scans, and 65K data points. A total of 1000 scans were co-added, and the spectra were externally calibrated using CH₃OH in D₂O, resonating at 49.15 ppm.³⁹ ¹H NMR spectra were collected by co-adding 64 scans and internally referenced using the HOD/H₂O peak at 4.80 ppm.⁴⁰

A Bruker Avance III 400 MHz NMR spectrometer was used to collect the ¹⁰³Rh NMR spectra of a saturated aqueous solution of Rh₂(AcO)₄ and the Rh-methionine solution **B_{conc}** (*C*₁ ~ 26 mM) at room temperature. For comparison, the ¹⁰³Rh NMR spectrum of an aqueous solution of RhCl₃ $\cdot$ 3H₂O (0.1 M) was also measured. A 15 mm horizontal selenoidal probe and 5 mm sample tube increased exposure of the solution to the magnetic field and accelerated the measurement time. Spectra were referenced such that zero chemical shift corresponds to an arbitrary ¹⁰³Rh frequency of 3.16 MHz at a field where the protons of

tetramethylsilane would resonate at exactly 100.00 MHz, scaled to the operating field of the spectrometer.⁴¹ Because of the wide spectral chemical shift range the signal collection was run in sections, collecting 10000 to 100000 scans in each case. For the spectra of $\text{RhCl}_3 \cdot 3\text{H}_2\text{O}$ in D_2O , $\text{Rh}_2(\text{AcO})_4$ in $\text{H}_2\text{O}/\text{CH}_3\text{OH}$, and $\text{Rh}_2(\text{AcO})_4$ + methionine mixture (Solution **B_{conc}**), the number of scans were 3779, 46898 and 11211, respectively. The parameters used were: 16K data points, 20000 Hz spectral width, 0.41 s acquisition time, 15.0 s relaxation time with 30 μs pulse.

X-ray absorption spectroscopy (XAS).—Rh K-edge EXAFS spectra of a freshly prepared $\text{Rh}_2(\text{AcO})_4$ – methionine solution **A** (mole ratio 1:2, $C_1 = 6.45 \text{ mM}$) and solid **4** were collected at ambient temperature at beam lines 7-3 and 4-1, respectively, at the Stanford Synchrotron Radiation Lightsource (SSRL), which operates under 3.0 GeV and 500 mA. To remove higher-order harmonics, the Si(220) ($\phi = 90^\circ$) double-crystal monochromator was detuned to 50% of maximum I_0 intensity at the end of the scan. The energy of the incident beam (size $1 \times 1 \text{ mm}$) was internally calibrated by assigning the first inflection point of the absorption spectrum of a Rh foil to 23219.8 eV. All ion chambers were filled with argon gas. Solid **4** was mixed with boron nitride and placed in a 1 mm thick Al frame, using Mylar tape as window material; 15 scans were collected in transmission mode. The Rh-methionine solution **A** was held in a 2 mm Teflon sample holder between 5 μm polypropylene windows; 8 scans were measured in fluorescence mode using a 30-channel Ge detector. All individual scans / channels were compared prior to averaging to ensure that no radiation damage occurred during measurement.

The EXAFS oscillations were extracted using the WinXAS 3.1 program.⁴² The background in the pre-edge region was subtracted using a first-order polynomial, followed by edge step normalization. The energy unit was converted to $k (\text{\AA}^{-1})$, where $k = [(8\pi^2 m_e / h^2)(E - E_0)]^{1/2}$, using the threshold energy of $E_0 = 23225.3 \text{ eV}$ for solution **A** and 23226.1 eV for solid **4**. A seven-segment cubic spline was then subtracted in the post-edge region to obtain the EXAFS oscillation. The crystal structures of two model compounds were used for the ATOMS program,⁴³ which creates the input file for the FEFF 7.0 program:^{44, 45} for solution **A**, the atomic coordinates of $[\text{Rh}_2(\text{AcO})_4((\text{PhCH}_2)_2\text{S})_2]$ (Cambridge Structural Database = CSD code: DEHBIB),⁴⁶ and for solid (**4** + **5H₂O**), the crystal structure of (**4DL** + **6H₂O**). Least-squares curve fitting of theoretically simulated EXAFS oscillations to the extracted EXAFS spectra was performed over k -range $2.8 - 18 \text{ \AA}^{-1}$, allowing the bond distance (R) and the Debye–Waller parameter (σ^2) to be refined for each backscattering path. The E_0 (a common value for all paths) was allowed to float.

Single crystal X-ray diffraction.—A purple single crystal of the dinuclear (**4DL** + **6H₂O**) compound, obtained by vapor diffusion of acetone into an aqueous solution of **4**, and a single crystal of the mononuclear complex (**5** + **3H₂O**) obtained after slow evaporation, were mounted on a glass loop with paratone oil. Data were collected at 173.2 K with a Bruker APEX-II CCD diffractometer (Cu $K\alpha$ radiation, $\lambda = 1.5418 \text{ \AA}$) with a frame width of 0.8° and collection time of 10 s for low angle and 25 s for high angle frames. For (**5** + **3H₂O**), a numerical absorption correction was applied based on mapped crystal faces

using the multi-scan method (SADABS).⁴⁷ Using Olex2,⁴⁸ the structures were solved by ShelXT using Intrinsic Phasing and refined with ShelXL.⁴⁹

Computational calculations.—The Gaussian 16 (G16) package⁵⁰ was used to optimize the molecular structures of $[\text{Rh}_2(\text{AcO})_4(\text{H}_2\text{O})_2]$, the methionine adducts $[\text{Rh}_2(\text{AcO})_4(\text{S-HMet})(\text{H}_2\text{O})]$ and $[\text{Rh}_2(\text{AcO})_4(\text{S-HMet})_2]$. The crystal structure of $[\text{Rh}_2(\text{AcO})_4(\text{H}_2\text{O})_2]$ was used as starting point,⁵¹ replacing the axial water molecules stepwise with methionine molecules. The vibrational frequencies were calculated to ascertain that the optimized structures corresponded to minima on the potential energy surface. The distances in the optimized geometry of the aqua complex were comparable to those in the crystal structure of $[\text{Rh}_2(\text{AcO})_4(\text{H}_2\text{O})_2]$.⁵¹ All calculations employed the BHANDHLYP functional and the 6-31G(d) basis set for non-metal atoms and LANL2DZ for the rhodium ions. The time-dependent density functional theory (TD-DFT) calculations, performed on the optimized geometries in the Continuum Model (PCM) using water as solvent, accounted for the first 100 excited states.

3. RESULTS AND DISCUSSION

3.1 Adducts of $\text{Rh}_2(\text{AcO})_4$ with *DL*-methionine.

ESI-Mass spectrometry.—The ESI-mass spectra in (+) ion mode of solutions **A** and **B** ($C_1 = 2.00$ mM; $\text{Rh}_2(\text{AcO})_4\text{:HMet}$ mole ratios 1:2 and 1:10, respectively) display peaks corresponding to $\text{Rh}_2(\text{AcO})_4(\text{HMet})$ ($+m/z = 591.92$ and 613.90) and $\text{Rh}_2(\text{AcO})_4(\text{HMet})_2$ ($+m/z = 740.97$ and 762.95), while small mass peaks for $\text{Rh}_2(\text{AcO})_4$ ($+m/z = 459.90$, 464.85 and 505.87) could be detected only in the spectrum of solution **A**; see Figure 1 and Table 1.

UV-vis spectroscopy.—The electronic absorption spectra reported for $\text{Rh}_2(\text{AcO})_4$ – methionine mixtures have often been limited to the visible region (400–800 nm).^{29, 30, 32} Figure 2 (*top*) shows the UV-vis spectra of a 2.00 mM aqueous solution of $\text{Rh}_2(\text{AcO})_4$ (**1**), and its reaction mixtures with *DL*-methionine in mole ratios 1:2 (solution **A**) and 1:10 (solution **B**). The inset displays a blue shift for the absorption peak maximum in the visible region, from $\lambda_{\text{max}} = 584$ nm for $[\text{Rh}_2(\text{AcO})_4(\text{H}_2\text{O})_2]$, to 556 and 540 nm as the amount of HMet in solution increases.

This band has been attributed to a $\pi^*(\text{Rh}_2^{4+}) \rightarrow \sigma^*(\text{Rh}_2^{4+})$ transition and is influenced by changes in the type of donor atoms occupying the axial positions of the $\text{Rh}_2(\text{AcO})_4$ structure.^{52, 53} Absorption bands in the reflectance spectra of similar mono- and diethylsulfide adducts of $\text{Rh}_2(\text{AcO})_4$ have been reported at $\lambda_{\text{max}} = 555$ and 541 nm, respectively.⁵⁴

The weak band observed at 440 nm corresponding to the $\pi^*(\text{Rh}_2) \rightarrow \sigma^*(\text{Rh-O}_{\text{eq}})$ transition remains unchanged for both solutions **A** and **B**. However, significant changes occur in the UV region. In Figure 2 (*top*), two strong bands are observed at 283 and 307 nm for solution **A**, while for solution **B** with large excess of methionine, the band at 307 nm is quite intense with a small shoulder at ~ 280 nm.

Two sets of titrations with different $\text{Rh}_2(\text{AcO})_4$ (**1**) concentrations, $C_1 = 2.00$ and 0.20 mM, respectively, were then performed and monitored by UV-vis spectroscopy – volume change was insignificant, as only $4.0\ \mu\text{L}$ of the HMet solution was added stepwise (see Experimental Section). Figure 2 (*bottom*) shows that for the dilute solution ($C_1 = 0.20$ mM) the peak at $283\ \text{nm}$ gradually gains intensity with increasing HMet concentration, together with a shoulder at $307\ \text{nm}$. The UV-vis spectra obtained during titration of the more concentrated solution ($C_1 = 2.00$ mM) in Figure 2 (*middle*) show that the band at $307\ \text{nm}$ (and also $540\ \text{nm}$) becomes dominating with high free ligand concentration, $[\text{HMet}]_{\text{free}}$.

Assuming stepwise replacement of the axial aqua ligands, the bands at 283 and $307\ \text{nm}$ can then be assigned to the $[\text{Rh}_2(\text{AcO})_4(\text{HMet})(\text{H}_2\text{O})]$ (**2**) and $[\text{Rh}_2(\text{AcO})_4(\text{HMet})_2]$ (**3**) complexes, respectively.

Chen *et al.* also reported two peaks at 308 and $537\ \text{nm}$ (with a shoulder at $445\ \text{nm}$) for $[\text{Rh}_2(\text{AcO})_4(\text{HMet})_2]$, indicating that the two axially coordinated methionine ligands are bound to the Rh(II) ions through their thioether groups.¹⁵ Similar bands have been observed in the electronic spectra of the dibenzylsulfide adduct $[\text{Rh}_2(\text{AcO})_4(\text{S}(\text{CH}_2\text{Ph})_2)_2]$ ($308, 432\ \text{sh}, 546\ \text{nm}$ in CH_2Cl_2).⁴⁶

Computational calculations.—Time-dependent density functional theory (TD-DFT) was employed to identify the electronic transitions in $[\text{Rh}_2(\text{AcO})_4(\text{HMet})(\text{H}_2\text{O})]$ (**2**) and $[\text{Rh}_2(\text{AcO})_4(\text{HMet})_2]$ (**3**) that correspond to the UV absorption bands at 283 and $307\ \text{nm}$, respectively; see Experimental Section. The simulated UV-vis spectra for the aqua complex $[\text{Rh}_2(\text{AcO})_4(\text{H}_2\text{O})_2]$, and the methionine complexes **2** and **3** (Figure S1) are in good agreement with the experimental results shown in Figure 2. The energy levels of the molecular orbitals (MOs) that contribute to the bands observed in their experimental UV-vis spectra are displayed in Figure 3.

The simulated spectrum in Figure S1 shows for the mono-adduct (**2**) an intense UV band for the transition with the highest oscillator strength ($f > 0.5$) at $272\ \text{nm}$, and for the di-adduct (**3**) at $301\ \text{nm}$ ($f > 0.8$). In both cases the excitation with highest contribution is $\sigma(\text{Rh}_2^{4+}) \rightarrow \sigma^*(\text{Rh}_2^{4+})$, i.e. from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO); see Figure 3. For the aqua complex the simulated $\sigma(\text{Rh}_2^{4+}) \rightarrow \sigma^*(\text{Rh}_2^{4+})$ band at $239\ \text{nm}$ is close to the experimental value $247\ \text{nm}$ (energy difference $7.825\ \text{eV}$).⁵⁵ Figure 3 shows that the energy of the $\sigma(\text{Rh}_2^{4+})$ orbital increases for the thioether axial adducts. The destabilization is due to a better overlap of the $\sigma(\text{Rh}_2^{4+})$ orbital with the thioether ligand orbitals than with the aqua ligands, and the contribution of

$\sigma^*(\text{Rh-S})$ to the $\sigma(\text{Rh}_2^{4+})$ orbital,⁵⁵ resulting in red shift and increasing intensity of the above UV transition.

Other transitions also contribute to these UV bands, including $\pi(\text{Rh}_2^{4+}) \rightarrow \sigma^*(\text{Rh}_2^{4+})$, as well as a transition from the $\sigma(\text{Rh}_2^{4+})$ to an orbital centered on the water molecule for (**2**), and $\sigma(\text{Rh}_2^{4+}) \rightarrow \sigma^*(\text{Rh-O}_{\text{eq}})$ and $\pi(\text{Rh}_2^{4+}) \rightarrow \sigma^*(\text{Rh}_2^{4+})$ transitions for (**3**). For the graphic representation of the orbitals that are involved in these transitions, see Table S1.

EXAFS spectroscopy.—The k^3 -weighted Rh K-edge EXAFS spectra and the corresponding Fourier transforms of a freshly prepared $\text{Rh}_2(\text{AcO})_4$ – methionine solution **A** ($C_1 = 6.45$ mM; $C_{\text{HMet}} = 13.0$ mM) and the solid $[\text{Rh}_2(\text{AcO})_2(\text{Met})_2] \cdot 5\text{H}_2\text{O}$ (**4** + **5H₂O**) are shown in Figure 4. Least-squares curve fitting results are shown in Table 2 with separate contributions of scattering paths displayed in Figures S2 and S3. The EXAFS fitting model for the solid pentahydrate **4** was constructed based on the crystal structure of (**4DL** + **6H₂O**); see Section 3.2. The distances obtained from EXAFS curve fitting of the solid pentahydrate **4** are similar to the average bond lengths/ distances in the crystal structure of the hexahydrate **4DL** (Table S4).

Based on the ESI-MS data (*see above*), solution **A** ($C_1 = 2.00$ mM) contains a mixture of the $[\text{Rh}_2(\text{AcO})_4(\text{HMet})(\text{H}_2\text{O})]$ (**2**) and $[\text{Rh}_2(\text{AcO})_4(\text{HMet})_2]$ (**3**) species, with 0.5 Rh- S_{axial} and 1.0 Rh- S_{axial} bonds per Rh(II) ion, respectively. The intensities of the peaks at 283 and 307 nm in the UV-vis spectrum of solution **A** ($C_1 = 2.00$ mM) in Figure 2 (*top*) indicate that (**2**) and (**3**) are in equilibrium with (**2**) dominating. Therefore, the Rh- S_{axial} frequency/ coordination number was fixed to 0.6 (Table 2). The refined mean Rh- S_{axial} distance 2.58 ± 0.02 Å is comparable to the Rh- S_{axial} distances ($2.517 - 2.561$ Å) in crystalline thioether adducts of dirhodium(II) carboxylates.^{46, 56-58}

The refined Rh-Rh distance, 2.41 ± 0.02 Å, is slightly longer than the corresponding distance in $\text{Rh}_2(\text{AcO})_4$ (2.38 ± 0.02 Å) obtained from EXAFS,³⁶ but is within the limits of dimeric Rh(II) complexes.³³ This elongation corresponds to the *trans* influence that the axially *S*-coordinated methionine ligands exert on the Rh-Rh bond, possibly through a π -back donation from the metal to the $\sigma^*(\text{C-S})$ molecular orbital. This π donation would originate from the high energy MOs with π symmetry (rather than π^*) in the metal complex, weakening the Rh-Rh bond.⁵⁹

3.2 Displacement of acetate with methionine in $\text{Rh}_2(\text{AcO})_4$.

ESI-Mass spectrometry.—Figure 1 (*right*) displays the ESI-mass spectrum of a freshly prepared $\text{Rh}_2(\text{AcO})_4$ – methionine solution **B** ($C_1 = 2.00$ mM; $C_{\text{HMet}} = 20.0$ mM). When re-measured after 5 weeks (37 days) being at room temperature (see Figure 5 and Table 3), new peaks appeared at $+m/z = 620.93$ and 709.96 , which correspond to $[\text{Rh}_2(\text{AcO})_2(\text{Met})_2 + \text{H}^+]^+$ and $[\text{Rh}_2(\text{AcO})(\text{Met})_3 + \text{H}^+]^+$ mass ions, respectively, revealing that the methionine ligands had replaced the acetate groups in $\text{Rh}_2(\text{AcO})_4$. This observation prompted a time study to follow changes in solution **B** at room temperature (after 1, 8, 15, 25, 30 and 37 days) using ESI-MS (see Figure S4 and Table S2). After 1 day the predominant complex was $[\text{Rh}^{\text{II}}_2(\text{AcO})_4(\text{HMet})]$ ($+m/z = 591.92$ for the protonated species). Other peaks associated with $[\text{Rh}^{\text{II}}_2(\text{AcO})_4(\text{HMet})_2]$ ($+m/z = 740.97$) and

$[\text{Rh}^{\text{II}}_2(\text{AcO})_3(\text{HMet})_2]^+$ (680.95) were also detected. After 8 days, new peaks appeared corresponding to $[\text{Rh}^{\text{II}}_2(\text{AcO})_2(\text{Met})_2 + \text{H}^+]^+$ ($+m/z = 620.93$), $[\text{Rh}^{\text{II}}_2(\text{AcO})_2(\text{Met})_3 + 2\text{H}^+]^+$ (769.98), and $[\text{Rh}^{\text{II}}_2(\text{AcO})(\text{Met})_3 + \text{H}^+]^+$ (709.96), in which two or three acetate groups had left the Rh(II) coordination sphere. Moreover, additional peaks related to monomeric Rh(III) species emerged: $[\text{Rh}^{\text{III}}(\text{Met})_2]^+$ ($+m/z = 398.99$), $[\text{Rh}^{\text{III}}(\text{Met})_2(\text{AcO}) + \text{H}^+]^+$ (459.01) and $[\text{Rh}^{\text{III}}(\text{Met})_3 + \text{H}^+]^+$ (548.04), which could indicate decomposition of some Rh(II) species in the solution. After 25 days dirhodium(III) species with four methionine and one acetate groups, $[\text{Rh}^{\text{III}}_2(\text{Met})_4(\text{AcO})]^+$ ($+m/z = 856.99$), were detected for the first time. At that time the peak with the second highest relative intensity (after $+m/z = 150.06$) was related to $[\text{Rh}^{\text{II}}_2(\text{AcO})_2(\text{Met})_2]^+$ ($+m/z = 620.93$), while beyond 30 days the most intense peak was for $[\text{Rh}^{\text{III}}(\text{Met})_2]^+$ species at $+m/z = 398.99$.

These results showed that methionine ligands initially coordinate to the axial positions of the $\text{Rh}_2(\text{AcO})_4$ complex, then under aerobic conditions gradually replace the bridging acetate groups, forming e.g. $[\text{Rh}_2(\text{AcO})_2(\text{Met})_2]$ and $[\text{Rh}_2(\text{AcO})(\text{Met})_3]$ complexes, and eventually split the Rh^{II}-Rh^{II} bond generating mononuclear species. In this process Rh(II) partially oxidizes to Rh(III) and some thioether (-S-) to sulfoxide (-S(O)-), as shown by the peaks at $+m/z = 564.04$ and 166.05 for $[\text{Rh}^{\text{III}}(\text{Met})_3 + \text{O} + \text{H}^+]^+$ and $[\text{HMet} + \text{O} + \text{H}^+]^+$, respectively (see Figure 5). Similar oxidation was observed in the aerobic reaction of $\text{Rh}_2(\text{AcO})_4$ with glutathione or *N*-acetylcysteine, and O_2 was reduced to peroxide. The peroxide could then oxidize Rh^{III}-bound terminal thiolates to sulfenato (S=O) groups.^{35, 36}

The next question was whether higher temperature, e.g. body temperature, would facilitate the methionine substitution of the acetate groups in $\text{Rh}_2(\text{AcO})_4$. Therefore, two solutions with the same composition as solution A ($C_1 = 2.00$ mM, $C_{\text{HMet}} = 4.00$ mM) were prepared: one was kept at 40 °C for 24 h, and the other refluxed for 24 h at 100 °C. The ESI-mass spectra of the reaction mixtures measured after 24 h showed that for the solution heated to 40 °C the peak at $+m/z = 620.93$ associated with $[\text{Rh}^{\text{II}}_2(\text{AcO})_2(\text{Met})_2]^+$ had the highest relative intensity, while for the refluxed solution $[\text{Rh}^{\text{III}}(\text{Met})_2]^+$ had the most intense mass peak ($+m/z = 389.99$; see Figure S5).

The refluxed solution was concentrated and revealed three bands (see Figure S6) when passed through a Sephadex G-15 size-exclusion chromatography column: a major purple band (Band 1), followed by a small orange-yellow layer (Band 2), and a blue-green band (Band 3). In the ESI-mass spectra the $[\text{Rh}^{\text{II}}_2(\text{AcO})_2(\text{Met})_2]$ and $[\text{Rh}^{\text{III}}(\text{Met})_2]^+$ complexes dominated in Bands 1 and 2, respectively, while the blue-green B and 3 mainly contained $\text{Rh}_2(\text{AcO})_4(\text{aq})$. This complex probably is regenerated from $[\text{Rh}_2(\text{AcO})_4(\text{HMet})_{1-2}]$ by exchanging the axially coordinated methionine groups with the eluent water in the column (see Figure S6). Although the ESI-mass spectrum (Figure S5 *right*) indicated $[\text{Rh}^{\text{III}}(\text{Met})_2]^+$ ($+m/z = 389.99$) to dominate after refluxing the reaction mixture for 24 h at 100 °C, the separation with column chromatography revealed that $[\text{Rh}^{\text{II}}_2(\text{AcO})_2(\text{Met})_2]$ (**4**) was the main product (yield: 35% for (**4** + 5H₂O); see Experimental Section). The purple Band 1 was separated, and single crystals were prepared by means of vapour diffusion of acetone into the aqueous solution of the complex.

Crystallography.—The crystal structure of *cis*-[Rh₂(AcO)₂(*D*-Met)(*L*-Met)]·6H₂O (**4DL** + 6H₂O) displayed in Figure 6 shows tridentate methionine coordination to the Rh(II) ions, which are bridged by two adjacent acetate groups; see crystal data and selected bond distances and angles in Tables S3 and S4. Two other amino acids, valine and *L*-phenylalanine, form *cis*-[Rh₂(AcO)₂(*N,O*-amino acid)₂] complexes,^{60, 61} where each amino acid occupies the equatorial positions, binding via its –NH₂ and –COO[–] groups to the same Rh(II) ion. In the crystal structure of (**4DL** + 6H₂O) the (*S,N,O*)-methionine ligands bind to both equatorial and axial positions around the Rh₂(AcO)₂ core. The Rh–Rh distances (Rh1–Rh2 2.5389(9), Rh3–Rh4 2.5747(8) Å) in (**4DL** + 6H₂O) are considerably longer than in Rh₂(AcO)₄(H₂O)₂ (2.3855(5) Å),⁵¹ but within the range (2.356 – 2.653 Å) in bis(μ-carboxylato) dirhodium(II) complexes.³³ The mean Rh–Rh distance, 2.55 ± 0.02 Å, obtained from EXAFS for the solid [Rh(AcO)₂(Met)₂]·5H₂O (**4** + 5H₂O) compound (Table 2) is in good agreement with the crystallographic distances in (**4DL** + 6H₂O). Based on molecular mechanics calculations Boeyens *et al.* indicated that for tetra(carboxylato) dirhodium(II) complexes such as [Rh₂(AcO)₄(H₂O)₂], changing one of the O atoms in the CH₃COO[–] ligands with an S atom would increase the Rh–Rh distance from 2.385 to 2.540 Å.⁶² This distance is a bit longer in (**4DL** + 6H₂O), as the Rh(II) ions are bridged by only two carboxylate groups.

The Rh–S bond distances in (**4DL** + 6H₂O) (2.255(3) – 2.266(3) Å) are similar to those reported for other dirhodium(II) complexes with *S*-donor ligands in equatorial positions (2.260 – 2.279 Å),^{56, 63–65} but considerably shorter than the average Rh–S axial distances (2.58 ± 0.02 Å) obtained from EXAFS for solution **A** containing [Rh₂(AcO)₄(HMet)_{1–2}] (see Table 2).

An interesting feature about the structure of (**4DL** + 6H₂O) is that both methionine groups are enantiomers; therefore, each dirhodium(II) complex contains a *D*-methionine (with *R* configuration) as well as an *L*-methionine (with *S* configuration). This geometry forces the two methionine ligands to adapt a *cis*-configuration relative to each other, with the two N atoms being on the same side of the Rh–Rh bond, i.e. the complex is in *meso*-form with *C_s* symmetry. We examined formation of such complexes using only pure enantiomers, and prepared purple [Rh₂(AcO)₂(*L*-Met)₂]·4.5H₂O (**4L** + 4.5H₂O) and [Rh₂(AcO)₂(*D*-Met)₂]·4H₂O (**4D** + 4H₂O) solids (see *Supporting Information*), which were characterized by ESI-MS, ¹H and ¹³C NMR spectroscopy. Their ESI-mass spectra (Figure S7) show pure solids, which resemble that of **4DL** (Figure S6, Band 1). Attempts to obtain single crystals of the **4L** and **4D** were not successful.

¹³C NMR spectroscopy.—Figure 7 displays the ¹³C NMR spectrum of a D₂O solution of [Rh₂(AcO)₂(Met)₂] (**4**) obtained from the reaction of Rh₂(AcO)₄ (**1**) with *DL*-methionine. This spectrum had twelve signals, with those corresponding to the methionine ligands doubled; see peak assignments in Table S5. Surprisingly, the ¹³C NMR spectrum of the **4DL** hexahydrate crystals dissolved in D₂O only showed one set of signals for the carbon atoms of the Rh^{II}-bound methionine ligands (Met). Moreover, the D₂O solutions of the hydrated solids **4L** and **4D**, prepared from pure *L*-methionine or *D*-methionine, showed a single set of Met peaks with identical chemical shifts (δ_c) for the enantiomer pair, which were slightly

different from those of their diastereomer **4DL** (Figure 7). A comparison reveals that the ^{13}C NMR signals in **4** are a combination of those in **4DL** and **4L/4D**. The integral ratios ($\sim 2:1$) of the doubled signals in **4** indicate that a mixture of **4D**, **4DL** and **4L**, approximately in 1:1:1 ratio, is formed when *DL*-methionine reacts with $\text{Rh}_2(\text{AcO})_4$. This distribution differs from the expected random distribution 1:2:1 of DD:(DL + LD):LL, indicating that the *meso*-form (DL/LD), where the S atoms are in eclipsed position (Figure 6), is less favoured. However, when the same solution is set for crystallization under vapor diffusion of acetone-water, only **4DL** crystallizes while **4D** and **4L** remain in solution. Proposed structures for **4L** and **4D** are shown in Figure S8.

In all $[\text{Rh}_2(\text{AcO})_2(\text{Met})_2]$ complexes (**4DL**, **4D/4L**), the methionine carboxylate peak becomes deshielded relative to pure methionine ($\delta_{\text{C}} = 174.5$ ppm) upon binding to the Rh(II) ions, $\delta(^{13}\text{C}) > +6.4$ ppm, due to the loss of electron density around the carbon atom. Similar downfield shift of the $-\text{COO}^-$ signal was previously observed in the ^{13}C NMR spectra of the reaction products of $\text{Rh}_2(\text{AcO})_4$ with cysteine or penicillamine (solid state),³⁵ and of the Pb(II)-cysteinate complex formed in solution.⁶⁶ However, the ^{13}C NMR signals from the $\mu\text{-COO}^-$ acetate groups in **4DL** and **4D/4L** show an upfield shift ($\delta(^{13}\text{C}) = -3.9$ ppm) with respect to those in $\text{Rh}_2(\text{AcO})_4$ (**1**) ($\delta_{\text{C}} = 193.6$ ppm). This is probably due to an increased electron density from the soft S ligands *trans* to the bridging CH_3COO^- groups (O5, O6, O15, O16), making these Rh-O_{eq} bond lengths somewhat longer (2.056 – 2.075 Å) than those in $\text{Rh}_2(\text{AcO})_4(\text{H}_2\text{O})_2$ (2.029 – 2.047 Å);⁵¹ see Table S4.

^{103}Rh NMR spectroscopy.—The only stable rhodium isotope, ^{103}Rh , has 100% abundance and nuclear spin quantum number $I = 1/2$, but quite small gyromagnetic ratio, $\gamma(^{103}\text{Rh}) = -0.846 \times 10^7$ rad/T·s, leading to a receptivity as low as 3.12×10^{-5} relative to ^1H . Another difficulty is that ^{103}Rh often has a long spin-lattice relaxation time (T_1).⁴¹ Moreover, the ^{103}Rh NMR chemical shifts span over a wide range, from -2000 to $+10,000$ ppm, often making ^{103}Rh NMR signals for new compounds difficult to find.^{41, 67} The ^{103}Rh NMR chemical shift is sensitive to different factors, such as Rh chemical environment, coordination number and geometry, type of donor atom(s) in the ligand (e.g. polarizability, “weak” vs. “strong” ligand field, good σ -donor / π -acceptor), solvent and temperature. Weak-field ligands with non-polarizable or hard donor atoms like H_2O and Cl lead to high-frequency/ high chemical shifts, while strong-field ligands with polarizable or soft donors like PR_3 generate low-frequency/low chemical shifts.

As standard we measured the ^{103}Rh NMR spectrum of a 0.1 M solution of $\text{RhCl}_3 \cdot 3\text{H}_2\text{O}$ in D_2O , showing two signals at 8549.0 and 8793.2 ppm for the *cis*- $[\text{RhCl}_4(\text{OH}_2)_2]^-$ and *fac*- $[\text{Rh}(\text{H}_2\text{O})_3\text{Cl}_3]$ with $\text{Rh}^{\text{III}}\text{O}_2\text{Cl}_4$ and $\text{Rh}^{\text{III}}\text{O}_3\text{Cl}_3$ coordination, respectively (Figure 8; $\delta_{\text{Rh}} = 244.2$ ppm). Previously reported chemical shifts for these species were $\delta_{\text{Rh}} = 8486$ and 8753 ppm at 3 °C (0.5 M Rh^{3+} , $[\text{Cl}^-]_{\text{total}} = 2$ M, $[\text{H}^+] = 1$ M, $[\text{ClO}_4^-] = 0.5$ M).⁶⁸ This difference could be due to the extreme sensitivity of ^{103}Rh NMR chemical shift to temperature (1.7 – 2.3 ppm shift/ degree), and different composition of the solution.⁶⁸

We obtained a signal at $\delta_{\text{Rh}} = 7476.2$ ppm for $[\text{Rh}_2(\text{AcO})_4(\text{H}_2\text{O}/\text{CH}_3\text{OH})_2]$ from a saturated solution of $\text{Rh}_2(\text{AcO})_4$ (**1**) using a 1:1 $\text{H}_2\text{O} + \text{CH}_3\text{OH}$ solvent mixture; see Figure 8. This

chemical shift is comparable to $\delta_{\text{Rh}} = 7455$ ppm observed for $\text{RhO}_4(\text{Rh})$ in $[\text{Rh}_2(\text{AcO})_4(\text{PPh}_3)]$; see Table 4.

Eight days after preparing solution **B_{conc}** containing $\text{Rh}_2(\text{AcO})_4$ (**1**) and *DL*-methionine (1:10 mole ratio; $C_1 \sim 26$ mM; see Experimental section), two signals were detected at 4685.1 and 4616.3 ppm with intensity ratio 1:1 (Figure 8). However, the lack of a ^{103}Rh NMR chemical shift database for related dirhodium(II) compounds makes it necessary to correlate these chemical shifts with the UV-vis and ESI-mass spectra of the similar solution **B** for a plausible assignment.

The UV-vis spectrum of a freshly prepared solution **B** ($C_1 = 2.0$ mM, $\lambda_{\text{max}} = 307$ nm; see Figure 2) showed $[\text{Rh}_2(\text{AcO})_4(\text{S-HMet})_2]$ (**3**) as the predominant species, although in its ESI-mass spectrum (Figure 1, *right*), as well as in the spectrum measured one day later (Figure S4), the most intense peak was at $+m/z = 591.92$ associated with $[\text{Rh}_2(\text{AcO})_4(\text{HMet})(\text{H}_2\text{O})]$ (**2**). This could, however, be an effect of the extensive dilution of the sample before injection (see Section 2.2). After 8 days the ESI-mass spectrum of the same solution **B** showed peaks related to **2**, **3** and $[\text{Rh}_2(\text{AcO})_2(\text{Met})_2]$; see Figure S4. Therefore, after 8 days the ^{103}Rh NMR solution **B_{conc}** ($C_1 \sim 26$ mM) may contain $[\text{Rh}_2(\text{AcO})_4(\text{HMet})_2]$ (**3**) and/or $[\text{Rh}_2(\text{AcO})_2(\text{Met})_2]$ (**4**). Both Rh(II) ions have the same coordination environment, $\text{S}_{\text{ax}}\text{RhO}_4(\text{Rh})$, in complex **3** and only a single ^{103}Rh NMR signal would be expected. Since the coordination environment around the Rh(II) ions in **4**, $\text{O}_{\text{ax}}\text{RhO}_2\text{NS}_{\text{eq}}(\text{Rh})$, differs considerably from that of **3**, it is unlikely that the two ^{103}Rh NMR signals with only $\delta_{\text{Rh}} \sim 69$ ppm and 1:1 intensity would originate from an equi-molar mixture of **3** and **4**.

Results from ^{13}C NMR spectroscopy showed that when using *DL*-methionine, a mixture of **4DL**, **4L** and **4D** formed, approximately in 1:1:1 ratio. The **4L** and **4D** enantiomer pairs had the same ^{13}C NMR chemical shifts, while those of the *meso*-complex **4DL** were slightly different with an integral ratio of 2:1 for **(4L + 4D):4DL** (Figure 7). Since the coordination environment of the two Rh(II) ions in the **4DL** and **4D/4L** complexes is the same, splitting of the signals due to $^1J(^{103}\text{Rh}, ^{103}\text{Rh})$ coupling can be ruled out. A more plausible explanation would be that one of the two observed ^{103}Rh NMR signals originates from the Rh nuclei with exactly the same chemical shift (δ_{Rh}) in the two enantiomers **4D/4L**, in a similar way as in their ^{13}C NMR spectrum (Figure 7). The other ^{103}Rh NMR signal with $\delta_{\text{Rh}} \sim 69$ ppm could then correspond to the *meso*-form **4DL**. One reason why the integral ratio of the ^{103}Rh NMR signals (1:1) differs from the 2:1 integral ratio in ^{13}C NMR spectrum of **4** could be different relaxation times for the Rh nuclei in **4DL** and **4D/4L**.

3.3 Crystal structure of $[\text{Rh}^{\text{III}}(\text{L-Met})(\text{D-Met})]\text{Cl} \cdot 3\text{H}_2\text{O}$ (**5** + $3\text{H}_2\text{O}$)

As shown in the ESI-MS time study of the reaction of $\text{Rh}_2(\text{AcO})_4$ with a large excess of methionine (Figure S4) the peak at $+m/z = 620.93$, associated with $[\text{Rh}^{\text{II}}_2(\text{AcO})_2(\text{Met})_2]$, was generally accompanied by a peak at $+m/z = 398.99$ attributed to the decomposition product $[\text{Rh}^{\text{III}}(\text{Met})_2]^+$. This peak was also predominant in the ESI-mass spectrum of the orange Band 2 separated on the chromatography column (see Figure S6). In order to obtain a sufficient amount of this product for characterization the reaction was performed at 100°C , refluxing solution **A** ($C_1 = 2.00$ mM, $C_{\text{HMet}} = 4.00$ mM) for more than a week. Although the

ESI-mass spectrum showed the highest relative intensity for the peak at $+m/z = 398.99$ (Figure S9), subsequent purification revealed that the major product was still $[\text{Rh}^{\text{II}}_2(\text{AcO})_2(\text{Met})_2]$. We then attempted another approach using $\text{RhCl}_3 \cdot 3\text{H}_2\text{O}$ as starting material, leading to crystalline $[\text{Rh}^{\text{III}}(\text{D-Met})(\text{L-Met})]\text{Cl} \cdot 3\text{H}_2\text{O}$ (**5** + $3\text{H}_2\text{O}$); see Experimental Section.

Its crystal structure revealed a distorted octahedral coordination geometry around the Rh(III) ion (Figure 9; see Tables S3 and S4 for crystal data and selected bond distances). As in the **4DL** compound, one *D*-methionine and one *L*-methionine is coordinated *trans* to each other in complex **5**, *trans-S,N,O*- $[\text{Rh}(\text{D-Met})(\text{L-Met})]^+$, leading to a highly symmetric *meso*-structure with a centre of inversion. The conformation around the sulfur atom is *R* for the coordinated *L*-methionine and *S* for the *D*-methionine. In comparison, structures of three geometrical isomers of *bis*(*L*-methionato)cobalt(III) complexes have been reported: (*trans*-*S*, *trans*-*N* and *trans*-*O*) $[\text{Co}(\text{L-Met})_2]^+$.²⁸ As expected for the larger Rh(III) ion (ionic radii 0.805 Å Rh^{3+} vs. Co^{3+} 0.685 Å; CN = 6),⁷⁶ the Rh-O, Rh-N and Rh-S bond distances in **5**, 2.0338(14), 2.0674(17) and 2.350(5) Å, respectively, are slightly longer than those reported for the $[\text{Co}(\text{L-Met})_2]^+$ isomers, with corresponding distances in the ranges 1.886-1.898, 1.947-1.959 and 2.245-2.301 Å.²⁸ The Rh-S distances in **5** are close to those reported for other thioether-bound Rh(III) complexes such as *fac*(*S*)- $[\text{Rh}(\text{mtea})_3]$ (mtea = $\text{NH}_2\text{CH}_2\text{CH}_2\text{SCH}_3$) with RhS_3N_3 coordination (Rh-N 2.112 – 2.116 Å; Rh-S 2.317 – 2.338 Å).⁷⁷

Attempts to synthesize and isolate a complex with 2Rh and 4 methionines, similar to the species corresponding to the ESI-MS peak in Figure S4 at $+m/z$ 856.99, $[\text{Rh}_2^{\text{III}}(\text{Met})_4(\text{AcO})]^+$, also resulted in formation of the complex **5**.

4. CONCLUSIONS

We have shown using ESI-MS and UV-vis spectroscopy that the amino acid methionine initially binds through its thioether group to the axial positions of hydrated $\text{Rh}_2(\text{AcO})_4$ (**1**) in aqueous solution, replacing the water molecules in $[\text{Rh}_2(\text{AcO})_4(\text{H}_2\text{O})_2]$ to form complexes $[\text{Rh}_2(\text{AcO})_4(\text{S-HMet})(\text{H}_2\text{O})]$ (**2**, $\lambda_{\text{max}} = 283$ nm) and $[\text{Rh}_2(\text{AcO})_4(\text{S-HMet})_2]$ (**3**, $\lambda_{\text{max}} = 307$ and 540 nm). Based on TD-DFT calculations, $\sigma(\text{Rh}_2^{4+}) \rightarrow \sigma^*(\text{Rh}_2^{4+})$ transitions are mainly responsible for their characteristic UV-absorptions bands. The Rh-Rh and average Rh-S(axial) distances determined by EXAFS are 2.41 ± 0.02 Å and 2.58 ± 0.02 Å, respectively. After some time, or accelerated when the reaction mixture is kept at 40 °C or refluxed at 100 °C, a stable complex $[\text{Rh}_2(\text{AcO})_2(\text{Met})_2]$ (**4**) predominates. Carbon-13 NMR spectroscopy revealed that when using *DL*-methionine a mixture of $[\text{Rh}_2(\text{AcO})_2(\text{L-Met})_2]$ (**4L**), $[\text{Rh}_2(\text{AcO})_2(\text{D-Met})_2]$ (**4D**) and $[\text{Rh}_2(\text{AcO})_2(\text{D-Met})(\text{L-Met})]$ (**4DL**) complexes formed, approximately in mole ratio 1:1:1. Only the latter (**4DL**) crystallized in hexahydrated form, with its methionine ligands binding to the Rh(II) ions in a tridentate (*S,N,O*)-mode, with average Rh-Rh and Rh-S (equatorial) bond lengths of 2.557 Å and 2.261 Å, respectively. The ^{103}Rh NMR spectrum of an aqueous solution of $[\text{Rh}_2(\text{AcO})_4(\text{H}_2\text{O})_2]$ ($\delta_{\text{Rh}} = 7476.2$ ppm) mixed with large excess of *DL*-methionine (mole ratio 1:10) showed (after 8 days) two signals at 4685.1 and 4616.3 ppm with 1:1 intensity, one assigned to the enantiomer pair **4D/4L**, the other to the *meso*-form **4DL**.

A monomeric Rh(III) species, $[\text{Rh}(\text{Met})_2]^+$, was identified by ESI-MS as a stable decomposition product. The crystal structure of the trihydrated complex $[\text{Rh}(\text{L-Met})(\text{D-Met})]\text{Cl}$ (**5**) prepared from $\text{RhCl}_3 \cdot 3\text{H}_2\text{O}$ revealed that the tridentate methionine ligands form a *trans*-S, *trans*-O, *trans*-N chelate with the Rh(III) ion. Scheme 1 displays a summary of the reactions that occur when mixing $\text{Rh}_2(\text{AcO})_4$ with excess methionine; ions detected by ESI-MS could be present in solution, or formed inside the instrument in gas phase.

This is the first report which shows that free methionine can not only bind reversibly to the axial positions of the antitumor active dirhodium(II) tetraacetate, but also gradually replace two of the CH_3COO^- groups at body temperature and occupy both the axial and the equatorial positions, forming tridentate (*S,N,O*)-chelates. Since the availability of the axial positions in $\text{Rh}_2(\text{RCOO})_4$ complexes seems crucial in the antitumor activity of this family of compounds,⁷⁸ displacement of the carboxylate groups by methionine and formation of $[\text{Rh}_2(\text{AcO})_2(\text{Met})_2]$ species would reduce their efficiency and cytotoxicity.

We conclude that under aerobic conditions both the amino acids methionine (as a free molecule, not as a protein residue) and cysteine (even bound in proteins and peptides such as glutathione, GSH) can decrease the efficiency of $\text{Rh}_2(\text{AcO})_4$, but through different mechanisms. Free methionine can irreversibly block the axial positions while retaining the $\text{Rh}^{\text{II}}\text{-Rh}^{\text{II}}$ bond in a dirhodium(II) unit $[\text{Rh}_2(\text{AcO})_2(\text{Met})_2]$, which eventually may decompose forming mononuclear Rh(III) species, *e.g.* $[\text{Rh}(\text{Met})_2](\text{AcO})$. Cysteine and glutathione can form oligomeric species $\{\text{Rh}^{\text{III}}_2(\text{Cys/GSH})_4\}_n$ with tri-thiolate bridges between dimeric units of Rh(III) ions.^{35, 36}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

EXAFS	extended X-ray absorption fine structure spectroscopy
ESI-MS	electrospray ionization mass spectrometry

TD-DFT

time-dependent density functional theory

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Figure 1. ESI-mass spectra (+ ion mode) of freshly prepared solutions containing $\text{Rh}_2(\text{AcO})_4$ ($C_1 = 2.00 \text{ mM}$) and methionine: *left*) solution **A** ($C_{\text{HMet}} = 4.00 \text{ mM}$), and *right*) solution **B** ($C_{\text{HMet}} = 20.0 \text{ mM}$).

Figure 2.

Top) Comparison between the UV-vis spectra of an aqueous solution of $\text{Rh}_2(\text{AcO})_4$ (**1**) and solutions with $\text{Rh}_2(\text{AcO})_4$ + methionine in mole ratios 1:2 (solution **A**) and 1:10 (solution **B**) ($C_1 = 2.00$ mM). UV-vis spectra from two series of aqueous solutions of $\text{Rh}_2(\text{AcO})_4$ with methionine at different mole ratios using *middle*), $C_1 = 2.00$ mM, and *bottom*) $C_1 = 0.20$ mM, respectively. At the highest mole ratio (1:10) the minimum free ligand concentration $[\text{HMet}]_{\text{free}}$ is 16.0 mM and 1.60 mM, respectively.

Figure 3.

TD-DFT calculated molecular orbital (MO) energies for the axial mono- and di-methionine $\text{Rh}_2(\text{AcO})_4$ complexes, with graphic representations of the MOs involved in transitions observed experimentally in the 270-310 nm region. The smaller energy difference between the $\sigma(\text{Rh}_2^{4+})$ and $\sigma^*(\text{Rh}_2^{4+})$ orbitals for the di-adduct explains its observed red shift in the UV region (λ_{max} 307 nm vs. 283 nm in Figure 2). For larger images of the $\sigma(\text{Rh}_2^{4+})$ and $\sigma^*(\text{Rh}_2^{4+})$ MOs, see Table S1.

Figure 4.

Left) Least-squares curve fitting to the k^3 -weighted Rh K-edge EXAFS spectra and *right*) the corresponding Fourier transforms for: *a*) $\text{Rh}_2(\text{AcO})_4$ – methionine solution **A** ($C_1 = 6.45$ mM; mole ratio 1:2) and *b*) the pentahydrate solid **4**; see Table 2.

Figure 5.

ESI-mass spectrum (+ ion mode) of the $\text{Rh}_2(\text{AcO})_4$ – methionine solution **B** ($C_1 = 2.00$ mM; 1:10 mole ratio) after 37 days; see Table 3.

Figure 6.

The crystal structure of $[\text{Rh}_2(\text{AcO})_2(\text{D-Met})(\text{L-Met})] \cdot 6\text{H}_2\text{O}$ (**4DL** + $6\text{H}_2\text{O}$) contains two crystallographically non-equivalent complexes in *meso*-form with the methionine ligands eclipsed; H atoms and water molecules are omitted for clarity.

Figure 7.

^{13}C NMR spectra of the hydrated solids **4**, **4DL**, **4D/4L** and *DL*-methionine and $\text{Rh}_2(\text{AcO})_4$ dissolved in D_2O ; assignments for chemical shifts are listed in Table S5. $[\text{Rh}_2(\text{AcO})_2(\text{Met})_2] \cdot 5\text{H}_2\text{O}$ (**4** + $5\text{H}_2\text{O}$); $[\text{Rh}_2(\text{AcO})_2(D\text{-Met})(L\text{-Met})] \cdot 6\text{H}_2\text{O}$ (**4DL** + $6\text{H}_2\text{O}$); $[\text{Rh}_2(\text{AcO})_2(D\text{-Met})_2] \cdot 4\text{H}_2\text{O}$ (**4D** + $4\text{H}_2\text{O}$); $[\text{Rh}_2(\text{AcO})_2(L\text{-Met})_2] \cdot 4.5\text{H}_2\text{O}$ (**4L** + $4.5\text{H}_2\text{O}$).

Figure 8.

^{103}Rh NMR spectra of *top*) $\text{RhCl}_3 \cdot 3\text{H}_2\text{O}$ in D_2O ($C_{\text{Rh}} = 0.1 \text{ M}$), *middle*) $\text{Rh}_2(\text{AcO})_4$ (**1**) in a 1:1 $\text{H}_2\text{O} + \text{CH}_3\text{OH}$ solution, and *below*) $\text{Rh}_2(\text{AcO})_4$ and *DL*-methionine in aqueous solution **B_{conc}** (mole ratio 1:10; $C_1 \sim 26 \text{ mM}$), measured eight days after the initial synthesis.

Figure 9. Crystal structure of $[\text{Rh}^{\text{III}}(\text{D-Met})(\text{L-Met})]\text{Cl}\cdot 3\text{H}_2\text{O}$ (**5** + $3\text{H}_2\text{O}$); chloride, H atoms and water molecules are omitted for clarity.

Scheme 1.

Summary of the reactions that occur between $\text{Rh}_2(\text{AcO})_4$ and excess methionine.

Table 1.Assignment of Mass Ions in the ESI-Mass Spectra Shown in Figure 1. ^a

<i>m/z</i>	Assignment	<i>m/z</i>	Assignment
150.06	[HMet + H ⁺] ⁺	470.13	[3HMet + Na ⁺] ⁺
161.04	[2HMet + H ⁺ + Na ⁺] ²⁺	486.11	[3HMet + K ⁺] ⁺
172.04	[HMet + Na ⁺] ⁺	505.87	[2Rh ^{II} + 4 AcO ⁻ + CH ₃ CN + Na ⁺] ⁺
299.11	[2HMet + H ⁺] ⁺	591.92	[2Rh ^{II} + 4 AcO ⁻ + HMet + H ⁺] ⁺
321.09	[2HMet + Na ⁺] ⁺	613.90	[2Rh ^{II} + 4 AcO ⁻ + HMet + Na ⁺] ⁺
459.90	[2Rh ^{II} + 4 AcO ⁻ + NH ₄ ⁺] ⁺	740.97	[2Rh ^{II} + 4 AcO ⁻ + 2HMet + H ⁺] ⁺
464.85	[2Rh ^{II} + 4 AcO ⁻ + Na ⁺] ⁺	762.95	[2Rh ^{II} + 4 AcO ⁻ + 2HMet + Na ⁺] ⁺

^a HMet = C₅H₁₁NO₂S; AcO⁻ = C₂H₃O₂

Table 2.

Parameters Obtained from Least-Squares Curve Fitting to the k^3 -Weighted Rh K-Edge EXAFS Spectra of the $\text{Rh}_2(\text{AcO})_4$ – methionine solution **A** and Solid (**4** + $5\text{H}_2\text{O}$); see Figure 4.^a

Sample	Scattering Path	CN	R (Å)	σ^2 (Å ²)
Solid (4 + $5\text{H}_2\text{O}$)	Rh-(N/O)	3	2.04	0.0017
	Rh-O _{axial}	1	2.11	0.0017 c
	Rh-S	1	2.26	0.0017
	Rh-Rh	1	2.55	0.0024
	Rh $\cdots$ C _{AcO} ⁻ + Met	4	2.94	0.0085
Solution A	Rh-O	4	2.04	0.0024
	Rh-Rh	1	2.41	0.0018
	Rh-S _{axial}	0.6	2.58	0.0014
	Rh $\cdots$ C _{AcO} ⁻	4	2.89	0.0023
	Rh $\cdots$ O'	4	3.08	0.0061

^aCoordination numbers were fixed; amplitude reduction factor (S_0^2) was refined to 0.87 for (**4** + $5\text{H}_2\text{O}$) and fixed at this value for solution **A**; estimated errors: $R \pm 0.02$ Å and $\sigma^2 \pm 0.001$ Å²; c = correlated to σ^2 (Rh-N/O).

Table 3.Assignment of Mass Peaks Detected in the ESI-Mass Spectrum Shown in Figure 5.^a

<i>m/z</i>	Assignment	<i>m/z</i>	Assignment
150.06	[HMet + H ⁺] ⁺	459.01	[Rh ^{III} + 2HMet - H ⁺ + AcO ⁻] ⁺
166.05	[HMet + H ⁺ + O] ⁺	480.99	[Rh ^{III} + 2HMet - 2H ⁺ + AcO ⁻ + Na ⁺] ⁺
172.04	[HMet + Na ⁺] ⁺	548.04	[Rh ^{III} + 3HMet - 2H ⁺] ⁺
188.03	[HMet + Na ⁺ + O] ⁺	564.04	[Rh ^{III} + 3HMet - 2H ⁺ + O] ⁺
299.11	[2HMet + H ⁺] ⁺	620.93	[2Rh ^{II} + 2AcO ⁻ + 2HMet - H ⁺] ⁺
321.09	[2HMet + Na ⁺] ⁺	709.96	[2Rh ^{II} + AcO ⁻ + 3HMet - 2H ⁺] ⁺
398.99	[Rh ^{III} + 2HMet - 2H ⁺] ⁺	856.99	[2Rh ^{III} + AcO ⁻ + 4HMet - 4H ⁺] ⁺
417.00	[Rh ^{III} + 2HMet - 2H ⁺ + H ₂ O] ⁺		

^aHMet = C₅H₁₁NO₂S; AcO⁻ = C₂H₃O₂

Table 4.¹⁰³Rh NMR chemical shifts for selected dirhodium(II) complexes. ^a

	Coordination	δ_{Rh} (ppm)	Experimental conditions	Reference
$\text{Rh}_2(\text{AcO})_4(\text{H}_2\text{O}/\text{CH}_3\text{OH})_2$	$\text{O}_{\text{ax}}\text{RhO4}(\text{Rh})$	7476.2	$\text{H}_2\text{O}/\text{CH}_3\text{OH}$	this work
$\text{Rh}_2(\text{AcO})_4(\text{PPh}_3)$	$\text{RhO4}(\text{Rh})$ $(\text{Rh})\text{RhO4P}_{\text{ax}}$	7455 6963	CDCl_3	69
$\text{Rh}_2(\text{AcO})_4\{\text{P}(\text{OCH}_3)_3\}_2$	$(\text{Rh})\text{RhO4P}_{\text{ax}}$	6694	CD_2Cl_2 , 213 K	70, 71
$\{\text{Rh}_2(\text{mhp})_4\}_2$	$\text{RhO3N}(\text{Rh})$ $(\text{Rh})\text{RhON3}$	7644 4322	$\text{CD}_2\text{Cl}_2/\text{CH}_2\text{Cl}_2$	72
$\text{Rh}_2(\text{mhp})_4$	$\text{RhO2N2}(\text{Rh})$	5745	$\text{CD}_2\text{Cl}_2/\text{CH}_2\text{Cl}_2$	72
$[\text{Rh}_2(\text{AcO})_2(\text{Met})_2]^c$ (<i>D</i> -, <i>L</i> -Met isomers)	$\text{O}_{\text{ax}}\text{RhO2NS}(\text{Rh})$	4685.1 4616.3	H_2O	this work
$\text{Rh}_2(\text{AcO})_2(\text{COOCH}_3)_2(\text{CO})_2\{\text{P}(\text{OPh})_3\}_2$	$\text{P}_{\text{ax}}\text{RhO2C2}(\text{Rh})$	1372	CDCl_3	73
$[\text{Rh}_2(\text{DPhF})_2(9\text{-EtGH})_2(\text{CF}_3\text{COO})_2]$	$\text{O}_{\text{ax}}\text{RhON3}(\text{Rh})^b$	5551.98	CD_3CN	74
$[\text{Rh}_2(\text{DTolF})_3(\text{NO}_3)(\text{PPh}_3)]$	$\text{O}_{\text{ax}}\text{RhON3}(\text{Rh})$ $(\text{Rh})\text{RhN3P}$	6472 3007	CDCl_3	75
$[\text{Rh}_2(\text{DTolF})_3(\text{NO}_3)(\text{C}_5\text{H}_5\text{N})]$	$\text{O}_{\text{ax}}\text{RhON3}(\text{Rh})$ $(\text{Rh})\text{RhN4}$	6060 4332	CDCl_3	75
$[\text{Rh}_2(\text{DTolF})_2(\text{CH}_3\text{CN})_6](\text{BF}_4)_2$	$\text{N}_{\text{ax}}\text{RhN4}(\text{Rh})$	4648	CD_3CN	74

^aDTolF = *N,N'*-*p*-ditolylformamidinate; DPhF = *N,N'*-diphenylformamidinate; 9-EtGH = 9-ethylguanine; Hmhp = 6-methyl-2-hydroxypyridine;^bAccounting for the head-to-tail isomer and axially coordinated CF_3COO^- group;^cHMet = methionine; mixture of **4DL**, **4L**, **4D** (1:1:1 ratio)