Oxorhenium Phosphinophenolato Complexes with Model Peptide Fragments: Synthesis, Characterization, and Stability Considerations

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Received February 14, 2000

The synthesis and characterization of a series of mixed-ligand oxorhenium(V) complexes containing the *o*-diphenylphosphinophenolato ligand (HL) and model peptide fragments acting as the tridentate coligand are reported. Thus, by reacting equimolar amounts of tiopronin, Gly-Gly, Gly-L-Phe, or glutathione (GSH) peptides on the $[(n\text{-}C_4H_9)_4N][ReOCl_3(L)]$ precursor in refluxing MeCN/MeOH or aqueous MeCN/MeOH mixtures, the following complexes were obtained: $ReO\{[SC(CH_3)CONCH_2COO][L]\}[(n\text{-}C_4H_9)_4N]$, **1**, $ReO\{[H_2NCH_2CONCH_2\text{-}COO][L]\}$, **2**, $ReO\{[H_2NCH_2CONCH(CH_2C_6H_5)COO][L]\}$, **3**, and $ReO\{[SCH_2CH(NHCOCH_2CH_2CHNH_2\text{-}COOH)CONCH_2COO][L]\}Na$, **4**. The compounds are closed-shell 18-electron oxorhenium species adopting a distorted octahedral geometry, as demonstrated by classical spectroscopical methods including multinuclear NMR. X-ray diffraction analyses for **1** and **2** are also reported. By comparative stability studies of complexes **1**−**3** against excess GSH it was shown that complex **3** containing the bulky $C_6H_5CH_2$ substituent adjacent to the coordinated carboxylate group of Phe is the most stable complex.

Introduction

The recent proliferation of research on rhenium chemistry has a direct impact on the application of ^{186}Re and ^{188}Re radionuclides in oncology.[1−6] The worldwide search for novel sophisticated $^{186/188}$Re radiopharmaceuticals that act at the molecular level and can thereby efficiently be applied in the therapy of malignant disease is rapidly advancing.[4−6] The fact that cancer cells often overexpress peptide hormone receptors on their surface provides new opportunities for radiotherapy. Thus, radiolabeled tumor-seeking peptide analogues are employed for delivering a sufficient radiation dose with a high specificity to malignant cells by means of high-affinity peptide receptors located on their membrane.[4−6] Tagging of a metal radionuclide onto the biologically active peptide is usually achieved by covalent coupling of a bifunctional chelator at a selected position, according to the so-called "pendant approach". This method has led to the successful paradigm of ^{111}In−DTPA−octreotide used in the diagnosis of somatostatin (SMS) positive tumors and their metastases applying single photon emission computed tomography (SPECT). Recently a lyophilized vial has been commercially available under the trade name OctreoScan-111 which is used routinely for the facile preparation of ^{111}In−DTPA−octreotide.[3,6,7] In the field of radiotherapy, despite the fact that several SMS analogues labeled with therapeutic radionuclides, like ^{64}Cu, $^{186/188}$Re, or ^{90}Y, have been reported, an approved radiotherapeutic compound is not yet available.[3,6,8−10]

The above described route of synthesis of a chelator−peptide conjugate can often be very time-consuming, especially when the independent multistep synthesis of suitably protected bifunctional agents is necessary before coupling to the peptide. Therefore, alternative strategies of the "pendant approach" have been proposed, that involve the elongation of the peptide backbone by a short amino acid sequence that serves as the radiometal binding site. In those examples related to rhenium or technetium at least one cysteine residue is usually included in the attached sequence in order to ensure selective binding of

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10.1021/ic000154f CCC: $19.00
Published on Web 10/21/2000

the radiometal at the desired position but also a sufficient stability of the resulting complex in the biological milieu.[11−13]

As a part of our ongoing work of six-coordinate mixed-ligand oxorhenium(V) complexes containing the bidentate *o*-diphenylphosphinophenolato ligand (HL) and a variety of tridentate coligands of several N and S donor atom combinations, we were able to demonstrate the high stability of the studied octahedral oxorhenium species against the attack of nucleophile competing ligands like glutathione (GSH). It is assumed that the higher stability found in the tested complexes is, to a great extent, a consequence of the formation of closed-shell 18-electron octahedral structures. In the present work we study further the coordination of model peptide fragments to the $[ReO(L)]^{2+}$ unit, in order to explore the effectiveness and limitations of this approach in stabilizing as well peptide-related structures in vivo.[14,15] For this purpose, the following octahedral monooxo Re(V) complexes have been synthesized: ReO{[SC(CH_3)CONCH$_2$COO][L]}[(n-C_4H_9)$_4$N], **1**, ReO{[H_2NCH$_2$CONCH$_2$COO][L]}, **2**, ReO{[H_2NCH$_2$CONCH(CH$_2C_6H_5$)COO][L]}, **3**, and ReO{[SCH$_2$CH(NHCOCH$_2$CH$_2$CHNH$_2$COOH)CONCH$_2$COO][L]}Na, **4**. Complexes **1**−**4** have been characterized by elemental analyses and classical spectroscopical methods. X-ray structure analysis was additionally performed for compounds **1** and **2**. Comparative stability studies of complexes **1**−**3** against GSH are reported as well.

Experimental Section

Materials. All chemicals were reagent grade and used so without further purification. The peptides tiopronin, H_3L^1, Gly-Gly, H_2L^2, Gly-L-Phe, H_2L^3, and glutathione, H_2L,[4] were purchased from Aldrich or from Fluka. The *o*-HOC$_6$H$_4$P(C_6H_5)$_2$ ligand, HL, was synthesized and purified according to published methods.[16,17] Rhenium was purchased from Aldrich as $KReO_4$ and was converted to the [(n-C_4H_9)$_4$N][$ReOCl_4$] precursor as reported previously.[18] Solvents for high-performance liquid chromatography (HPLC) were HPLC grade; they were filtered through membrane filters (0.22 μ, Millipore, Milford/USA) and degassed by helium flux before use. Silica gel packing material from Merck was applied for column chromatography. Thin-layer chromatography (TLC) was performed on 0.25 mm silica gel coated aluminum F_{254} plates from Merck.

Instrumentation. IR spectra were recorded on KBr pellets on a Perkin-Elmer 1600FT-IR spectrophotometer in the region 500−4000 cm^{-1} with polystyrene as a reference. Proton, ^{13}C, and ^{31}P NMR spectra were collected on a Bruker AC-200 instrument, using (CH_3)$_4$Si as an internal reference (for ^{1}H and ^{13}C) and 85% aqueous H_3PO_4 as an external reference (for ^{31}P). Complexes were dissolved in deuterated solvents at a concentration of ca. 1−2%. Chemical shifts are given as δ in ppm. Elemental analyses for C, H, N, and S were conducted on a Perkin-Elmer 2400/II automatic elemental analyzer. HPLC analyses were performed on a Waters chromatograph efficient with a 600 solvent delivery system and coupled to a Waters 996 photodiode array UV detector. The Millennium software by Waters was applied for controlling the HPLC system and processing the data. Chromatographic conditions applied for analyses are given separately for individual compounds below. For purification of complex **4** a preparative HPLC system from Waters was used (Waters Prep LC 4000) coupled to the PDA UV detector used also for analyses. For the separation a Prep Nova-Pak HR C18 cartridge (25 mm × 100 mm, 6 μ) from Waters was used.

Synthesis of ReO(L^n/L) Complexes. ReO{[SC(CH$_3$)CONCH$_2$COO]-[*o*-OC$_6$H$_4$P(C$_6$H$_5$)$_2$]}[(n-C$_4$H$_9$)$_4$N], ReO(L^1/L), 1. By reacting the HL ligand with an equimolar amount of [(n-C_4H_9)$_4$N][$ReOCl_4$] precursor in MeCN the emerald [(n-C_4H_9)$_4$N][$ReOCl_3$(L)] complex was prepared, as reported previously.[14−16] The emerald precursor (200 mg, 0.24 mmol) was dissolved in MeCN (5 mL), and a solution of tiopronin (39.2 mg, 0.24 mmol) in MeOH (5 mL) was added with stirring. This mixture was refluxed with stirring for 60 min while its color was slowly turning from emerald green to orange-red. The solvent was expelled under vacuum and the residue redissolved in a small portion of CH_2Cl_2. Purification followed by means of a silica gel column (20 cm × 1.5 cm), which was eluted with a 100/5 CH_2Cl_2/MeOH solvent mixture. The fraction containing the orange product was collected and concentrated to a small volume, and a small amount of MeOH was added. By slow evaporation of this mixture at ambient temperature orange-red needlelike crystals of complex **1** suitable for X-ray analysis separated.

Yield: 65%. R_f (SiO_2; CH_2Cl_2/MeOH, 10/1): 0.5. t_R (Waters Symmetry Shield RP C18, 5 μ, 3.9 × 150 mm; A, MeOH; B, 20 mM NaH_2PO_4 pH 7.0; from 1 to 20 min 50% to 70% A): 13.4 min, 14.09 min. t_R (Supelco Discovery RP-Amide C16 column, 5 μ, 150 mm × 4.6 mm; A, MeCN; B, 0.2% H_3PO_4 neutralized with NH_3 to pH 7.0; from 0 to 20 min 20% A to 70% A): 16.36 min, 16.68 min. Anal. Calcd (found) for $C_{39}H_{55}N_2O_5PReS$: C, 53.16 (53.08); H, 6.29 (6.35); N, 3.18 (3.42); S, 3.64 (3.75). UV/vis (λ/nm): 285, 310, 380. IR (KBr, ν/cm^{-1}): 3431, 2963, 2873, 1621, 1584, 1455, 1443, 1310, 1270, 1122, 1097, 998, 945 (Re═O str), 905, 856. ^{1}H NMR (200 MHz, Me_4Si, $CDCl_3$): 2.92 (m, 8H), 1.42 (m, 8H), 1.20 (sextet, 8H), 0.87 (t, 12H) [(n-C_4H_9)$_4$N]; 1.63, 1.60 (two doublets, 3H, SCHC*H*$_3$), 3.97 and 4.29 (two quartets, SC*H*CH$_3$,), 4.07 and 4.69 (two quartets, NC*H*$_2$, 2H)), 6.28 (dd, 1H, *o*-C_6H_4ORe), 6.50 (t, 1H, *p*-C_6H_4ORe), 6.98 (t, 1H, *p*-C_6H_4PRe), 7.27 (dd, 1H, *o*-C_6H_4PRe), 7.35−8.25 ((C_6H_5)$_2$P, 10H). ^{13}C NMR (200 MHz, Me_4Si, $CDCl_3$): 13.61, 19.60, 23.80 and 58.55 [(n-C_4H_9)$_4$N]; 21.46 and 22.86 (SCH*C*H$_3$), 53.40 and 53.57 (S*C*HCH$_3$), 53.97 and 54.95 (NCH$_2$), 115.70−137.15 (aromatic carbons), 188.46, 189.30, 189.74, 190.48 (*C*(O)O and *C*(O)N).^{31}P NMR (200 MHz, 85% H_3PO_4, $CDCl_3$): 6.32 (s), 6.38 (s).

ReO{[H$_2$NCH$_2$CONCH$_2$COO][*o*-OC$_6$H$_4$P(C$_6$H$_5$)$_2$]}, ReO(L^2/L), 2. To an emerald solution of [(n-C_4H_9)$_4$N][$ReOCl_3$(L)] (200 mg, 0.24 mmol) in CH_3CN (10 mL) a solution of Gly-Gly (31.71 mg, 0.24 mmol) in MeOH (10 mL) and H_2O (3 mL) was added with stirring. The mixture was refluxed for 60 min, while the color changed from emerald to orange-red. The solvent was expelled under vacuum and the dark red residue redissolved in a small quantity of CH_2Cl_2. The aubergine-red suspension was loaded on a silica gel column (20 cm × 1.5 cm) eluted with a 10/1 CH_2Cl_2/MeOH solvent mixture. The fraction containing the red compound was collected and concentrated to a small volume under vacuum, and MeOH was added. By slow evaporation from this dark red mixture separated aubergine-red needlelike crystals suitable for X-ray analysis.

Yield: 85%. R_f (SiO_2; CH_2Cl_2/MeOH, 10/1): 0.3. t_R (Merck Lichrospher 100 RP C18 column, 10 μ, 4.6 × 250 mm; A, MeOH; B, 20 mM NaH_2PO_4 pH 7.0; from 1 to 20 min 50% to 80% A): 14.13 min. t_R (Supelco Discovery RP-Amide C16 column, 5 μ, 150 mm × 4.6 mm; A, MeCN; B, 0.2% H_3PO_4 neutralized with NH_3 to pH 7.0; from 0 to 20 min 20% A to 70% A): 14.64 min. Anal. Calcd (found) for $C_{22}H_{20}N_2O_5PRe$: C, 43.35 (43.20); H, 3.31 (3.03); N, 4.60 (4.50). UV/vis (λ/nm): 285, 370. IR (KBr, ν/cm^{-1}): 3448, 3159, 3055, 1677, 1629, 1584, 1454, 1444, 1382, 1297, 1255, 1240, 1177, 1101, 1024, 1012, 968 (Re═O str), 900, 860. ^{1}H NMR (200 MHz, Me_4Si, d_6-DMSO): 3.41−3.86 (H_2NCH_2C(O), 2H), 4.16 (dd, NCH_2C(O)O), 6.49 (dd, 1H, *o*-C_6H_4ORe), 6.75 (t, 1H, *p*-C_6H_4ORe), 7.20 (t, 1H, *p*-C_6H_4PRe), 7.41 (dd, 1H, *o*-C_6H_4PRe), 7.35−8.25 ((C_6H_5)$_2$P, 10H). ^{13}C NMR (200 MHz, Me_4Si, d_6-DMSO): 50.12, 50.85 (H_2NCH_2C(O) and NCH_2C(O)O), 115.70−137.15 (aromatic carbons), 188.46, 189.30,

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189.74, 190.48 (*C*(O)O and *C*(O)N), 115.04−134.42 (aromatic carbons), 183.85, 188.16 (*C*(O)O and *C*(O)N). ^{31}P NMR (200 MHz, 85% H_3PO_4, d_6-DMSO): 4.65 (s).

ReO{[$H_2NCH_2CONCH(CH_2C_6H_5)COO$][*o*-$OC_6H_4P(C_6H_5)_2$]}, ReO($L^3$/L), 3. The emerald [($n$-$C_4H_9$)$_4$N][$ReOCl_3$(L)] precursor (200 mg, 0.24 mmol) was diluted in CH_3CN (10 mL), and a solution of Gly-L-Phe dipeptide (53.34 mg, 0.24 mL) in MeOH (10 mL) and H_2O (5 mL) was added with stirring. The pH of the mixture was set to 8 by addition of 1 N NaOH (≈0.5 mL), whereupon the color turned from emerald to orange. The mixture was refluxed with stirring for 60 min with the color changing to orange-red. The solvent was expelled under vacuum and the oily residue redissolved in a small amount of CH_2Cl_2 and loaded on a silica gel column (20 cm × 1.5 cm). The column was eluted with a 10/1 CH_2Cl_2/MeOH solvent mixture, the fraction containing the aubergine-red complex was collected and concentrated to a small volume under vacuum, and MeOH was added. By slow evaporation at ambient temperature the CH_2Cl_2/MeOH mixture afforded aubergine-red needlelike crystals of **3**.

Yield: 80%. R_f (SiO_2; CH_2Cl_2/MeOH, 10/1): 0.5. t_R (Waters Symmetry Shield RP C18, 5 μ, 3.9 × 150 mm; A, MeOH; B, 0.2% H_3PO_4 adjusted to pH 7.0 with concentrated NH_3; from 1 to 20 min 50% to 80% A): 15.72 min, 11.7 min. t_R (Supelco Discovery RP-Amide C16 column, 5 μ, 150 mm × 4.6 mm; A, MeCN; B, 0.2% H_3PO_4 neutralized with NH_3 to pH 7.0; from 0 to 20 min 20% A to 70% A): 18.36 min. Anal. Calcd (found) for $C_{29}H_{26}N_2O_5PRe$: C, 49.78 (49.69); H, 3.75 (3.91); N, 4.00 (4.12). UV/vis (λ/nm): 277, 360. IR (KBr, ν/cm^{-1}): 3449, 3058, 2926, 1624, 1585, 1456, 1444, 1302, 1268, 1241, 1101, 1028, 979 (Re═O str), 858. ^{1}H NMR (200 MHz, Me_4Si, CD_3OD): 3.53 (m, CH_2−Ph, 2H), 3.73 (dd, H_2NCH_2C(O), 2H), 4.71 (m, C*H*CH_2Ph, 1H) 6.49 (dd, 1H, *o*-C_6H_4ORe), 6.72 (t, 1H, *p*-C_6H_4-ORe), 7.18 (t, 1H, *p*-C_6H_4PRe), 7.25 (dd, 1H, *o*-C_6H_4PRe), 7.20−7.85 ((C_6H_5)$_2$P, 10H + $CH_2C_6H_5$, 5H). ^{13}C NMR (200 MHz, Me_4Si, CD_3-OD): 38.95 (*C*H_2−Ph), 52.75 (NH_2CH_2C(O)), 62.70 (*C*HCH_2Ph), 110.66−137.86 (aromatic carbons), 185.04, 186.68 (*C*(O)O and *C*(O)N). ^{31}P NMR (200 MHz, 85% H_3PO_4, CD_3OD): 3.0 (s).

ReO{[$SCH_2CH(NHCOCH_2CH_2CHNH_2COOH)CONCH_2COO$]-[*o*-$C_6H_5P(C_6H_5)_2$]}Na, ReO($L^4$/L), 4. The emerald [($n$-$C_4H_9$)$_4$N]-[$ReOCl_3$(L)] precursor (200 mg, 0.24 mmol) was dissolved in CH_3CN (10 mL), and a solution of GSH (73.8 mg, 0.24 mmol) in MeOH (6.5 mL) and H_2O (3.5 mL) was added with stirring. The reaction mixture was refluxed with stirring for 60 min while the color turned orange-red. The solvent was expelled by rotary evaporation and the brown residue redissolved in a small quantity of CH_2Cl_2 and purified further on a silica gel column (17 cm × 3 cm) by using a 150/300/3 CH_2Cl_2/MeOH/50% CH_3COOH solvent mixture as the eluent. The fraction containing the orange-red product was collected and concentrated to a small volume. The product was further purified by preparative HPLC. The column was eluted isocratically with 45/55 Et_3N/H_3PO_4 buffer pH 7.0/MeOH at a flow rate of 10 mL/min. The fraction eluting at 12 min was collected and concentrated to a small volume under vacuum. The solution was loaded on an activated OASIS solid-phase extraction cartridge (Waters) and rinsed with physiological saline and H_2O and the product eventually collected in MeOH. Slow evaporation at ambient temperature afforded complex **4** as an orange-red solid.

Yield: 60%. R_f (SiO_2; CH_2Cl_2/MeOH/CH_3COOH, 5/10/0.1): 0.3. R_f (SiO_2; $CHCl_3$/MeOH, 1/1): 0.3. t_R (Merck Lichrospher 100, RP C18, 10 μ, 4.6 × 250 mm; A, MeOH; B, 2% Et_3N/H_3PO_4 pH 7.1; 1 to 20 min 0% A to 10% A): 21.5 min. t_R (Supelco Discovery RP-Amide C16 column, 5 μ, 150 mm × 4.6 mm; A, MeCN; B, 0.2% H_3PO_4 neutralized with NH_3 to pH 7.0; from 0 to 20 min 20% A to 70% A): 12.54 min. Anal. Calcd (found) for $C_{28}H_{28}N_3NaO_8PReS$: C, 40.00 (39.81); H, 3.36 (3.42); N, 5.00 (5.21); S, 3.81 (3.99). UV/vis (λ/nm): 291, 370, 410. IR (KBr, ν/cm^{-1}): 3392, 1613, 1584, 1442, 1384, 1304, 1268, 1098, 952 (Re═O str), 854, 746, 692. ^{1}H NMR (200 MHz, Me_4-Si, d_6-DMSO): 1.91 (m, −CH_2C*H*$_2$CHC(O)OH, 2H), 2.34 (q, −CH_2-CH_2CHC(O)OH, 2H), 2.81 (t, HNCHCH_2S-*exo*, 1H), 3.34 (t, −CH_2-CH_2C*H*C(O)OH, 1H), 3.57 (m, HNCHCH_2S-*endo*, 1H), 3.61 (d, C(O)OCH_2N-*exo*, 1H), 4.80 (m, HNC*H*CH_2S, 1H), 4.96 (d, C(O)-OCH_2N-*endo*, 1H), 6.26 (dd, 1H, *o*-C_6H_4ORe), 6.63 (t, 1H, *p*-C_6H_4-ORe), 7.11 (t, 1H, *p*-C_6H_4PRe), 7.34 (dd, 1H, *o*-C_6H_4PRe), 7.35−8.15 ((C_6H_5)$_2$P, 10H), 8.18 (t, *H*NCHCH_2S, 1H). ^{13}C NMR (200 MHz, Me_4-Si, d_6-DMSO): 27.17, 32.05, 42.92, 53.48, 54.2, 56.78 (aliphatic carbons), 114.78−135.54 (aromatic carbons), 170.75, 171.10, 176.87, 183.59 (C(O)-like carbons). ^{31}P NMR (200 MHz, 85% H_3PO_4, d_6-DMSO): 10.9 (s, 5%), 12.2 (s, 95%).

Table 1. Summary of Crystal Data for Compounds **1** and **2**

	1	**2**
formula	$C_{39}H_{55}N_2O_5PReS$	$C_{22}H_{20}N_2O_5PRe$
fw	881.08	609.57
a, Å	8.910(3)	10.135(6)
b, Å	23.466(8)	13.266(7)
c, Å	19.114(8)	15.679(8)
β, deg	89.99(1)	99.33(2)
V, Å^3	3996(1)	2080(2)
Z	4	4
D_{calcd}, Mg/m^3	1.464	1.947
space group	$P2_1/n$	$P2_1/c$
temp, K	298	298
radiation; λ, Å	Mo Kα; 0.71073	Mo Kα; 0.71073
abs coeff (μ), cm^{-1}	3.176	5.957
octants collected	$h,k,\pm l$	$\pm h,-k,-l$
GOF on F^2	1.186	1.098
R1	0.0410[a]	0.0296[b]
wR2	0.0918[a]	0.0763[b]

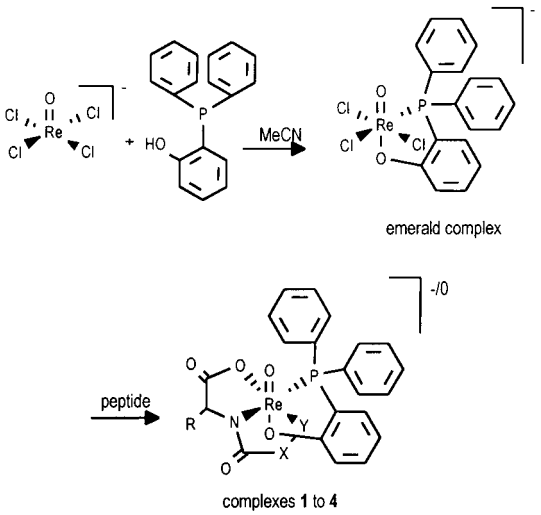
[a] For 5045 reflections with $I > 2\sigma(I)$. [b] For 3073 reflections with $I > 2\sigma(I)$.

X-ray Crystal Structure Determination for Compounds 1 and **2.** Diffraction measurements for **1** and **2** were performed on a Crystal Logic dual goniometer diffractometer using graphite-monochromated Mo Kα radiation. Unit cell dimensions were determined and refined by using the angular settings of 25 automatically centered reflections, and they appear in Table 1. Intensity data were recorded using a $\theta-2\theta$ scan. Three standard reflections monitored every 97 reflections showing less than 3% variation and no decay. Lorentz, polarization, and ψ-scan absorption corrections were applied using Crystal Logic software. The structures were solved by direct methods using SHELXS-86[19] and refined by full-matrix least-squares techniques on F^2 with SHELXL-93.[20] Further crystallographic details on **1**: $2\theta_{max} = 48°$, scan speed 2.7 deg/min, scan range 2.3 + $\alpha_1\alpha_2$ separation, reflections collected/unique/used = 6710/6268 [R_{int} = 0.0186]/6268, 504 parameters refined, R1/wR2 (for all data) = 0.0579/0.1002, $[\Delta\rho]_{min}/[\Delta\rho]_{max}$ = 0.706/−0.575 e/Å^3, $[\Delta/\sigma]$ = 0.105. All hydrogen atoms (except those of C2 and those of the tetrabutyl ammonium counterion which were introduced at calculated positions as riding on bonded atoms) were located by difference maps and were refined isotropically. All non-H atoms were refined anisotropically except C46, which was refined isotropically. The positions of the methyl group (C5) and the hydrogen (on C4) are disordered, so both syn and anti isomers are present. The methyl group was refined anisotropically in two positions with occupancy factor 0.5. Further crystallographic details on **2**: $2\theta_{max} = 49°$, scan speed 4.2 deg/min, scan range 2.4 + $\alpha_1\alpha_2$ separation, reflections collected/unique/used = 3584/3446 [R_{int} = 0.0341]/3446, 360 parameters refined, R1/wR2 (for all data) = 0.0352/0.0802, $[\Delta\rho]_{min}/[\Delta\rho]_{max}$ = 0.782/−1.932 e/Å^3, $[\Delta/\sigma]$ = 0.026. All hydrogen atoms were located by difference maps and were refined isotropically while all non-H atoms were refined anisotropically.

Interaction of ReO(L^n/L) Complexes with GSH (n = 1−3). In an Eppendorf tube a 1 mM solution (50 μL) of the respective complex in EtOH was placed and EtOH (200 μL) and 0.1 M phosphate buffer (pH 7.4) were added. An 0.1 M solution of GSH (50 μL) was added to this mixture and incubated under a nitrogen atmosphere at 37 °C. All above solutions were purged with nitrogen. Incubation of the respective mixtures containing H_2O instead of 0.1 M GSH (50 μL) was also performed and used for reference. Incubates of 24 h were analyzed by HPLC. Additional challenge experiments of complex **1** versus excess

(19) Sheldrick, G. M. *SHELXS-86: Structure Solving Program*; University of Göttingen: Göttingen, Germany, 1986.
(20) Sheldrick, G. M. *SHELXL-93: Crystal Structure Refinement Program*; University of Göttingen: Göttingen, Germany, 1993.

Scheme 1. Two-Step Synthesis of ReO(L^n/L) Complexes



Complex	*Peptide*	*R*	*X*	*Y*
1	tiopronin	H	$CHCH_3$	S
2	Gly-Gly	H	CH_2	NH_2
3	Gly-L-Phe	$C_6H_5CH_2$	CH_2	NH_2
4	GSH	H	*	S

*$CH[NHCO(CH_2)_2CHNH_2COOH]CH_2$

Gly-Gly and complex **2** versus tiopronin dipeptide were conducted according to the above described protocol.

Results and Discussion

Synthesis. The ReO(L^n/L) complexes **1**−**4** were prepared by a two-step synthesis, as shown in Scheme 1. In the first step, [(*n*-C_4H_9)$_4$N][$ReOCl_4$] was reacted with the phosphinophenol ligand HL in a 1/1 molar ratio in refluxing MeCN, affording almost quantitatively the emerald precursor [(*n*-C_4H_9)$_4$N][$ReOCl_3$(L)].[14−16] In the second step, the emerald precursor was reacted in equimolar quantities with the respective peptide in refluxing MeCN/MeOH (for complex **1**) or aqueous MeCN/MeOH (for complexes **2**−**4**) mixtures, leading to the formation of complexes **1**−**4** in good yields. The reaction products were purified over a silica gel column eluted with CH_2Cl_2/MeOH mixtures of the appropriate composition. Complex **4** was additionally purified by preparative HPLC. Analysis by TLC and reverse phase HPLC in connection to a PDA detector revealed the purity of the compounds. Crystals for X-ray analyses were raised for complexes **1** and **2** by slow evaporation from CH_2Cl_2/MeOH mixtures.

Characterization. Elemental analyses and spectroscopic data are given for individual compounds in the Experimental Section and confirm the formulas assigned to ReO(L^n/L) complexes **1**−**4**. Given that the atoms of the deprotonated SNO^{3-} donor atom set of tiopronin or GSH peptide are bound to the [ReO(L)]$^{2+}$ moiety, a net charge of −1 results for complexes **1** and **4**. On the other hand, complexes **2** and **3** are neutral oxorhenium species as a result of coordination of the doubly deprotonated ONN^{2-} set of Gly-Gly or Gly-L-Phe peptide, respectively, to the [ReO(L)]$^{2+}$ fragment. The Re═O stretching vibration of ReO(L^n/L) complexes is evident by the characteristic bands at 945, 968, 979, and 952 cm^{-1} in the IR spectra of complexes **1**, **2**, **3**, and **4**, respectively, as reported for similar oxorhenium complexes.[14,15] Additional bands in the region 750−690 cm^{-1} correspond to the coordinating phosphinophenolato ligand. The UV/vis spectra show maxima in the region 360−380 nm, that fall within the range of values reported for similar complexes.[14,15]

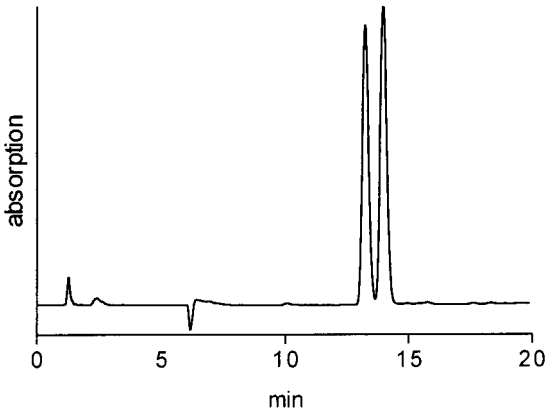


Figure 1. Representative HPLC chromatogram of complex **1** revealing the presence of two isomers in a ∼1/1 ratio in solution; t_R (Waters Symmetry Shield RP C18, 5 μ, 3.9 × 150 mm; A, MeOH; B, 20 mM NaH_2PO_4 pH 7.0; from 1 to 20 min 50% to 70% A): 13.4 min, 14.09 min.

The NMR data reported in the Experimental Section are in agreement with octahedral diamagnetic compounds having a low-spin d^2 configuration. The ^{31}P spectra are diagnostic of phosphinophenolate coordination, the pertinent signal moving downfield from −31.2 ppm in the uncoordinated ligand to the 4.5−12.2 ppm region. Neutral complex **2** exhibits as expected a pure singlet signal. Surprisingly, complex **3**, despite the presence of a chiral center in the Gly-L-Phe ligand, also gives a unique signal. According to the behavior previously observed for related "3 + 2" oxorhenium compounds,[14] the benzyl group of the L-Phe fragment is assumed to be exclusively syn oriented with respect to the oxo oxygen. However, less encumbered substituents at the tridentate framework, such as the methyl group incorporated in tiopronin, allows the isolation of both syn and anti isomers of complex **1**. The ^{31}P spectrum displays two close singlets of equal intensity, and the aliphatic portion of the proton and carbon spectra exhibit two sets of signals for coordinated tiopronin. Analogously, HPLC chromatography of complex **1** shows two well-separated equal peaks according to the presence of both isomers (Figure 1)**.** In the GS-containing complex **4**, also the γ-Glu residue assumes the two orientations. However, one isomer is strongly preferred (likely the syn one), as indicated by the integration of the peaks in the proton not decoupled ^{31}P spectrum. The two-dimensional contour map (Figure 2) shows the diastereotopic nature of the methylene protons of the Gly residue, the cysteine network including the uncoordinated amidic proton, and the γ-Glu system.

Description of the Structures. ORTEP diagrams of compounds **1** and **2** are given in Figures 3 and 4, respectively, while selected bond distances and angles are listed in Table 2. In complex **1** tiopronin is triply deprotonated and coordinated to rhenium through the carboxylate oxygen, the peptide nitrogen, and the sulfur atoms, which along with the PO donor atoms of the HL and the oxo group fulfill the coordination requirements of the metal. In this case, the rhenium complex is anionic and the charge is neutralized by one tetrabutyl ammonium cation. In the case of **2**, the coordination environment about the rhenium atom is fulfilled by the tridentate Gly-Gly ligand through the deprotonated carboxylate oxygen, the deprotonated peptide nitrogen, and the amine nitrogen and it is completed by the PO

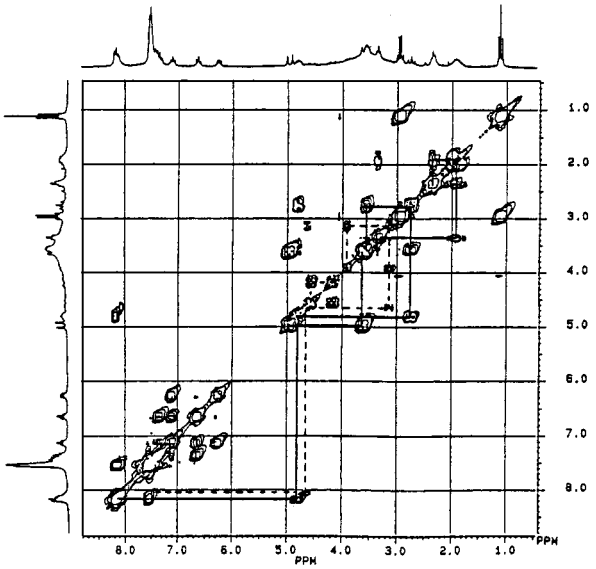


Figure 2. 2D contour map over the 0.5−9.0 ppm region of complex **4**. Solid lines indicate the three proton networks of coordinated GS in the syn isomer (Cys $-HN-C(R)H-CH_2-$, Gly $-CH_2-$, and γ-Glu $-CH_2-CH_2-CH_2-$ in order of increasing shielding). Dotted lines evidence the Cys- and Gly networks in the anti isomer.

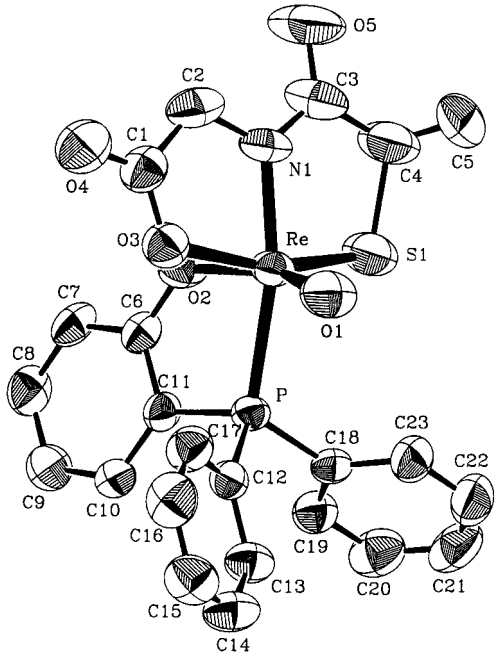


Figure 3. ORTEP diagram of the anion of complex **1** with 50% thermal probability ellipsoids.

donor atoms of the HL ligand and the oxo group. Thus, compound **2** is neutral. The equatorial plane of the distorted octahedron in **1** and **2** is defined by the phosphorus atom of the HL and the donor atoms of the peptide ligand (SNO/NNO in **1** and **2**, respectively), while the doubly bonded oxygen and the phenolate oxygen of HL occupy the apical positions. Rhenium lies ∼0.30 Å out of the equatorial plane toward the oxo group in both structures. The $Re-O_{oxo}$ axis is inclined at 79.2° and 81.4°, in **1** and **2**, respectively, with respect to the equatorial plane.

The dihedral angles of the tridentate chelating backbone, S1−C3−C4−N1 and N1−C2−C1−O3, in **1** are 20.8° and 2.2°, respectively, while the corresponding angles in **2** (N1−C1−C2−N2 and N2−C3−C4−O5) are 23.1° and 8.8°, respectively.

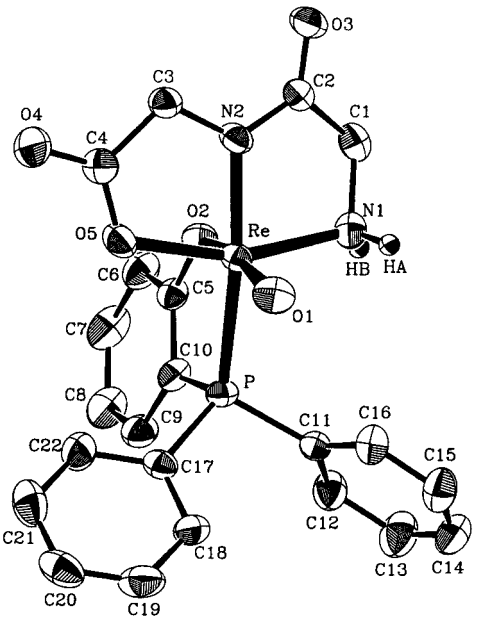


Figure 4. ORTEP diagram of complex **2** with 50% thermal probability ellipsoids.

Table 2. Selected Bond Distances (Å) and Angles (deg) for **1** and **2**

	1	**2**
Re−O(1)	1.692(5)	1.681(4)
$Re-N_{amide}$	2.007(5)	1.991(5)
$Re-O_{phenolate}$	2.076(4)	2.028(3)
$Re-O_{carboxylate}$	2.126(5)	2.046(4)
Re−X	2.294(2)[a]	2.148(5)[b]
Re−P	2.435(2)	2.456(2)
$O(1)-Re-N_{amide}$	109.2(2)	108.2(2)
$O(1)-Re-O_{phenolate}$	161.4(2)	165.6(2)
$N_{amide}-Re-O_{phenolate}$	85.6(2)	85.3(2)
$O(1)-Re-O_{carboxylate}$	93.2(2)	101.3(2)
$N_{amide}-Re-O_{carboxylate}$	78.9(2)	80.6(2)
$O_{phenolate}-Re-O_{carboxylate}$	78.3(2)	85.6(2)
O(1)−Re−X	103.3(3)	95.5(2)
$N_{amide}-Re-X$	84.3(2)	80.4(2)
$O_{phenolate}-Re-X$	89.0(2)	81.5(2)
$O_{carboxylate}-Re-X$	159.6(2)	157.7(2)
O(1)−Re−P	89.0(2)	89.4(1)
$N_{amide}-Re-P$	161.7(2)	162.4(1)
$O_{phenolate}-Re-P$	76.1(1)	77.1(1)
$O_{carboxylate}-Re-P$	98.5(1)	97.2(1)
X−Re−P	93.7(1)	97.4(1)

[a] X = S(1). [b] X = N(1).

The three five-membered rings in the coordination sphere adopt the stable envelope configuration. In particular in **1**, S1 and N1 are displaced by 0.45 and 0.06 Å, respectively, out of the mean plane defined by the four remaining atoms. In compound **2**, C1 is 0.30 Å out of the mean plane defined by Re, N1, C1, and C2, while N2 is 0.20 Å out of the Re, C3, C4, and O5 best mean plane. In both compounds, the metal is displaced by 0.35 and 0.30 Å (for **1** and **2**, respectively) out of the mean plane defined by the O−C−C−P chelating atoms of the HL. The dihedral angle between the equatorial plane of the octahedron (SNOP and NNOP in **1** and **2**, respectively) and the OCCP chelating plane of the HL is 75.7° and 84.8° for **1** and **2**, respectively.

The $Re=O_{oxo}$, $Re-O_{phenolate}$, and Re−S bond distances are in the ranges observed in analogous compounds,[14,15] while the Re−P distances (2.435(2) and 2.456(2) Å in **1** and **2**, respectively) are slightly longer than those found in the precursor

compound [(*n*-$(C_4H_9)_4N$][$ReOCl_3(L)$] (2.422(2) Å)[16] and in [$ReO\{(CH_3CH_2)N(CH_2CH_2S)_2\}(L)$] and [$ReO\{(CH_3CH_2SCH_2CH_2)N(CH_2CH_2S)_2\}(L)$] (~2.40 Å).[14] This lengthening of the Re−P bond distances in **1** and **2** with respect to the previously mentioned complexes is reasonable due to the shortening of the bond trans to the phosphorus atom (Re−Cl = ~2.37 Å and $Re-N_{sp3}$ = ~2.18 Å vs $Re-N_{sp2}$ = ~2.0 Å in our case). The angles around the peptide nitrogens are close to the ideal 120°, as expected for the sp^2 hybridization of these atoms, and the Re, the $N_{peptide}$, and the carbon atoms adjacent to the nitrogen are nearly coplanar. The $Re-O_{carboxylate}$ bond distances (2.126(5) and 2.046(4) Å for **1** and **2**, respectively) are in the ranges observed for analogous complexes. The $Re-NH_2$ distance in **2** is 2.148(5) Å as in the case of the analogous [$ReO(H_2NCH_2CH_2NCH_2CH_2NH_2)(L)$].[15]

The coordinated amine group in **2** is hydrogen bonded to the carbonyl [O(3)] and carboxylate [O(4)] oxygen atoms of neighboring molecules [HN(1A)···O(3′) (−*x*, −*y*, −*z*) = 2.144 Å, N1···O(3′) = 2.976 Å, N1−HN(1A)···O(3′) = 156.5°; HN(1B)···O(4′) (−*x*, 0.5 − *y*, 0.5 − *z*) = 2.029 Å, N1···O(4′) = 2.801 Å, N1−HN(1B)···O(4′) = 179.6°].

Interaction of $ReO(L^n/L)$ Complexes with GSH (n = 1−3). The three $ReO(L^n/L)$ complexes **1**, **2**, and **3** were incubated for a period of 24 h with a 100 molar excess of GSH in a 4/1 EtOH/H_2O medium due to solubility limitations. Analysis of the incubates by HPLC revealed their decomposition mainly to perrhenate and, to a lesser extent, to several short-lived metal species of unknown structure. Thus, in this case GSH attack leads to complex breakdown and not to substitution of the L ligand by GS, as was observed for other ReO(SNS/S) mixed-ligand complexes.[21] It is interesting to note that complex **3** exhibited an exceptionally higher stability, given that around 40% of the original compound remained intact during incubation. In contrast, only traces of complex **1** and none of complex **2** were found present in the respective 24 h incubates. A tentative explanation is related to the $C_6H_5CH_2$ group of Phe adjacent to the coordinated carboxylate group in complex **3**. If GSH attack is initiated at this position, the steric constraints imposed by the $C_6H_5CH_2$ group may lead to a higher stability of complex **3** against GSH in comparison to complexes **1** and **2**, which lack a bulky substituent at this position.

Additional stability experiments were conducted whereby complex **2** was challenged with excess tiopronin and complex **1** with excess Gly-Gly dipeptide. In the first case HPLC analysis of 24 h incubates revealed that complex **2** was partially converted to complex **1**, evident by the characteristic double diastereoisomer peak in the chromatographic profile, as well as several unidentified rhenium species, and partially decomposed to perrhenate. In contrast, complex **1** remained intact within the 24 h time scale of this study.

In conclusion, the preliminary findings of this work suggest that thiolate challenge ligands provoke complex decomposition (with or without parallel ligand substitution) that is initiated at the coordinated carboxylate in the series of complexes tested. This attack can be hampered to some degree by steric effects, when a bulky substituent is present in the immediate vicinity of the carboxylate group. On the other hand, the fact that complex **1** survived a 24 h exposure to excess Gly-Gly hints at the role that thiolate groups in the peptide chain may play in the thermodynamic stability of the resulting complexes. Further studies are presently under way with sulfur-containing peptides having bulky substituents at the carboxylic function with the aim of developing in vivo robust oxorhenium phosphinophenolato complexes with peptides.

Acknowledgment. The authors wish to thank the General Secretariat of Research & Development of Greece and the Italian Ministry of Foreign Affairs for financial support through the program “Sapfo”. Additional financial support by Mr. John Boutaris and the Agricultural Bank of Greece (ATE) to A.T. is also appreciated.

Supporting Information Available: Tables of fractional atomic coordinates and thermal parameters for all atoms, full bond lengths, and angles. Crystallographic data, in CIF format, for compounds **1** and **2**.This material is available free of charge via the Internet at http://pubs.acs.org.

IC000154F

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