Rhenium(V) and Technetium(V) Oxo Complexes of an $N_2N'S$ Peptidic Chelator: Evidence of Interconversion between the *Syn* and *Anti* Conformations

Ernest Wong,*,† Theresa Fauconnier,‡ Samantha Bennett,‡ John Valliant,‡ Tam Nguyen,† Frank Lau,† Linda F. L. Lu,† Alfred Pollak,† Russell A. Bell,‡ and John R. Thornback†

Resolution Pharmaceuticals Inc., 6850 Goreway Drive, Mississauga, Ontario, Canada L4V 1V7, and Department of Chemistry, McMaster University, 1280 Main Street West, Hamilton, Ontario, Canada L8S 4M1

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Neutral Re(V) and Tc(V) oxo complexes of the peptide dimethylglycyl-L-seryl-L-cysteinylglycinamide (RP294) were prepared and characterized by HPLC, spectroscopic techniques, and X-ray crystallographic analysis. The peptide was prepared as a single peptide chain using solid phase methods and characterized by HPLC and various spectroscopic techniques. The water-soluble Re(V) oxo complex of dimethylglycyl-L-seryl-L-cysteinylglycinamide [ReO(RP294)] was prepared from the reaction of the peptide with either $[ReO_2(en)_2]Cl$ or $ReOCl_3(PPh_3)_2$ in the presence of base. The complex exists as two isomers, the serine CH_2OH group being in the *syn* or *anti* conformation with respect to the Re−oxo bond. The ratio of the isomers at room temperature is 1:1.1. The isomers were separated by reverse-phase HPLC, but the isolation of each isomer was complicated by their rapid interconversion in aqueous solution at room temperature. The molecular structure of the *syn* isomer of the Re complex was determined by X-ray crystallography. Crystals of *syn*-[ReO(RP294)] ($C_{12}H_{20}N_6O_5ReS$) are orthorhombic, of space group $P2_12_12_1$, with $a = 6.954(1)$ Å, $b = 8.0472(1)$ Å, $c = 32.9183(4)$ Å, and $Z = 4$. The structure was solved by direct methods and was refined by full-matrix least-squares procedures to $R = 0.0327$ ($R_w = 0.0838$) for 10 447 reflections with $I > 2\sigma(I)$. The Re metal was coordinated in a distorted square pyramidal geometry with the oxo moiety in the apical position. The peptide coordinated to ReO^{3+} via the N_{amine} atom of dimethylglycine, the $S_{thiolate}$ atom of cysteine, and the two N_{amide} atoms of serine and cysteine (an $N_2N'S$ donor atom set). The Re atom lies ∼0.74 Å above the distorted plane formed by the $N_2N'S$ donor atom set. Variable-pH ^1H NMR spectral data showed the Re complex was stable from pH 5 to 8.5. The reaction of $^{99}TcO_4^-$ with $SnCl_2$, sodium gluconate, and RP294 produced the ^{99}Tc(V) oxo RP294 complex, [^{99}TcO(RP294)]. Like the [ReO(RP294)] complex, [^{99}TcO(RP294)] also exists in the *syn* and *anti* conformations in a ratio of approximately 1:1. The ^{99m}Tc complex of RP294 was prepared at the tracer level from the reaction of $Na[^{99m}TcO_4]$ with excess $SnCl_2$, sodium gluconate, and RP294. The ^{99m}Tc and Re RP294 complexes behaved similarly under identical HPLC conditions.

Introduction

The current interest in the radiolabeling of biologically important molecules (proteins, antibodies, and peptides) with ^{99m}Tc stems from the desire to develop a target-specific diagnostic radiopharmaceutical.[1−10] The advantages of using ^{99m}Tc in diagnostic nuclear medicine are well-known,[11−15] and a number of techniques have been developed for the ^{99m}Tc labeling of biologically important molecules.[16−20] One obvious approach is to coordinate a ^{99m}Tc metal directly with the targeting molecule. This approach is known as the direct labeling method, and it involves the use of a reducing agent to convert disulfide linkages into free thiolates, which then bind to the ^{99m}Tc metal. A major disadvantage of this method is the lack of control over the coordination of the ^{99m}Tc metal and the stability of the resulting metal complex. In addition, the lack of suitable or accessible coordination sites in some proteins and peptides exclude direct labeling as a viable technique. Two common alternatives to direct labeling are the final-step-labeling method and the preformed-chelate approach. Both techniques involve the use of a bifunctional chelator, which provides the site of ^{99m}Tc coordination. The difference between the two approaches lies in the order in which the ^{99m}Tc complex is formed. In the final-step-labeling method, complexation occurs

* To whom correspondence should be addressed. Phone: 905-677-0831. Fax: 905-677-9595. E-mail address: esywong@respharm.com.
† Resolution Pharmaceuticals Inc.
‡ McMaster University.
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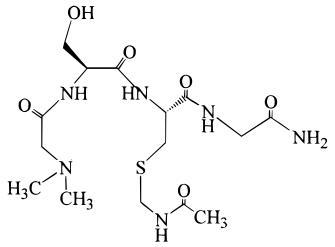
after the chelator has been attached onto the targeting molecule. With the preformed-chelate method, the ^{99m}Tc complex is initially prepared and purified before being attached to the targeting molecule. In both techniques, the bifunctional chelator must coordinate to ^{99m}Tc to form a complex that is stable *in vivo* and the chelator must have an active moiety that can react with a functional group on the targeting molecule. A number of bifunctional chelators have been used in the labeling of proteins, peptides, and monoclonal antibodies. The most successful class of bifunctional chelator is a series of amine/amide thiolate compounds that coordinate to TcO^{3+} via an $(N_{amine/amide})_{4-x}(S_{thiolate})_x$ donor atom set.[2, 9, 10, 17, 21−24]

For several years, we have actively investigated the ^{99m}Tc labeling of small biologically active peptides (5−30 amino acid residues). Instead of attaching a nonpeptidic chelator onto the peptide chain, we have chosen to connect, via the N terminus, a small peptide sequence that has a high binding affinity for ^{99m}Tc. Preliminary molecular modeling has shown that dimethylglycyl-L-seryl-L-cysteinylglycinamide (RP294) would coordinate strongly to TcO^{3+}. In addition to peptides, RP294 would also be an ideal bifunctional chelator for the labeling of proteins, monoclonal antibodies, and small molecules. In order to better understand the coordination of this peptidic chelator to ^{99m}Tc, we have examined the Re and Tc complexes of RP294. Macroscopic studies of Tc coordination chemistry are performed using the low energy β-emitting ^{99}Tc radionuclide, which has a much longer half life ($t_{1/2} = 2.12 \times 10^5$ years) than ^{99m}Tc ($t_{1/2} = 6.01$ h). Investigations of Re coordination chemistry are often performed in conjunction with Tc studies because Re possesses chemical properties similar to those of Tc, particularly in the +5 oxidation state, and Re provides a nonradioactive alternative to working with Tc radioisotopes.[25] In addition, ^{186}Re and ^{188}Re are of great interest to nuclear medicine as they possess nuclear properties favorable for use in therapeutic radiopharmaceuticals.[26−28] In this article, we report the synthesis and characterization of Re, ^{99}Tc, and ^{99m}Tc complexes of dimethylglycyl-L-seryl-L-cysteinylglycinamide:



Experimental Section

Materials. $[ReO_2(en)_2]Cl$ and $ReOCl_3(PPh_3)_2$ were prepared according to literature methods.[29,30] C-18 TLC plates (KC18F reversed phase) were purchased from Whatman. Sodium acetate, $H_2{}^{18}O$, *tert*-butyl methyl ether, and trifluoroacetic acid were purchased from Aldrich Chemicals Inc. Sephadex G10 resin, tin(II) chloride, and sodium gluconate were purchased from Sigma Chemical Co. Sasrin resin (2-methoxy-4-alkoxybenzyl alcohol resin) and ((9-fluorenylmethyl)oxy)-carbonyl (FMOC) protected amino acids were purchased from Bachem Bioscience Inc. 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBT), piperidine, *N*,*N*-dimethylformamide (DMF), *N*-methylpyrrolidone (NMP), and diisopropylethylamine (DIEA) were purchased from Applied Biosystems Inc. All chemicals were used as received.

Caution! ^{99}Tc is a low-energy (0.292 MeV) β^- emitter with a half-life of 2.12×10^5 years. ^{99m}Tc is a γ emitter (140 keV) with a half-life of 6.01 h. All manipulations of solutions and solids were performed in a laboratory approved for the handling of radioisotopes, and normal safety procedures were followed at all times to prevent contamination.

Instrumentation. NMR spectra were recorded on a Bruker AC-300 (variable-pH ^{1}H NMR) and on a Bruker DRX-500 NMR spectrometer (^{1}H, ^{13}C, COSY, HSQC, HMBC, HSQC-TOCSY)[31] and are reported as δ in ppm from external TMS. Mass spectra (electrospray) were obtained on a Sciex API#3 mass spectrometer in the positive-ion mode. Infrared spectra were recorded from KBr disks on a Bomem Michelson 100 infrared spectrometer in the range 4000−400 cm^{-1} and were referenced to polystyrene film. HPLC analyses and purifications were made on a Beckman gold chromatographic system with a Waters 4 mm radial pak C-18 column. During analytical HPLC analysis (HPLC method A), the mobile phases were changed from 100% water containing 0.1% trifluoroacetic acid to 100% acetonitrile containing 0.1% trifluoroacetic acid over 20 min at a flow rate of 2 mL/min. The HPLC analyses were monitored with a UV detector set at 215 nm. HPLC analyses of ^{99m}Tc samples were made on a Beckman gold chromatographic system with a Vydac 4.6 mm radial pak C-18 column. Two methods were used to analyze the ^{99m}Tc samples. In method B, the mobile phase was changed from 100% water containing 0.1% trifluoroacetic acid to 70% acetonitrile containing 0.1% trifluoroacetic acid over 25 min at a flow rate of 1 mL/min. In method C, the mobile phase was changed from 100% water containing 0.1% trifluoroacetic acid to 30% acetonitrile containing 0.1% trifluoroacetic acid over 45 min at a flow rate of 1 mL/min. The HPLC analyses of the ^{99m}Tc samples were monitored with a UV detector set at 215 nm and a radiometric γ detector. The interconversion of the *syn* and *anti* isomers was monitored using a Waters 625LC HPLC system with a Vydac 4.6 mm radial pak C-18 column. The mobile phase was changed from 100% water containing 0.1% trifluoroacetic acid to 70% acetonitrile containing 0.1% trifluoroacetic acid over 20 min at a flow rate of 1 mL/min. Elemental analyses were performed by Guelph Chemical Laboratories Ltd.

Synthesis of Dimethylglycyl-L-seryl-L-cysteinylglycinamide (RP294). Dimethylglycyl-L-seryl-L-cysteinylglycinamide was prepared via a solid phase peptide synthesis method[32] on an Applied Biosystems Inc. Model 433A peptide synthesizer. Sasrin resin and FMOC-protected amino acids were used. Prior to the addition of each amino acid residue to the peptide chain, the FMOC protection group was removed with 15% piperidine in NMP. Each amino acid residue was activated with 0.45 M HBTU and 0.45 M HOBT in DMF, in the presence of DIEA. The peptide was cleaved from the sasrin resin using 95% aqueous trifluoroacetic acid. The sasrin resin was removed by filtration. The addition of the filtrate to *tert*-butyl methyl ether at 0 °C caused the precipitation of the crude product. The crude RP294 was purified by HPLC. In RP294, the cysteine thiolate was protected with an (acetoamido)methyl (ACM) group and the C terminus was capped as an amide moiety. Mass spectrum (electrospray): $m/z = 421$ ($[M + 1]^+$, $[C_{15}H_{29}N_6O_6S]^+$). HPLC retention time using method A: $R_t = 4.7$ min. ^{1}H and ^{13}C NMR spectral data are given in Tables 1 and 2.

Deprotection of RP294. RP294 (100 mg, 0.238 mmol) was dissolved in 2 mL of 30% acetic acid. Mercury(II) acetate (151 mg,

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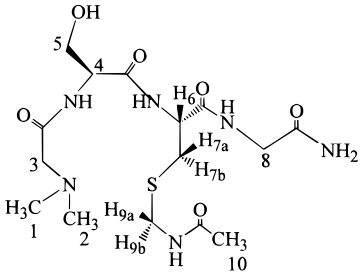
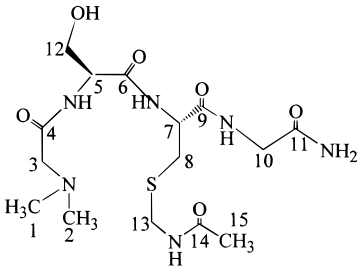
Table 1. ^{1}H NMR Data (500 MHz, D_2O) for RP294



	δ (ppm)	coupling constant (Hz)
H(1) and H(2)	2.85 (s, 6H)	
H(3)	3.99 (s, 2H)	
H(4)	4.45 (t, 1H)	$^3J_{H3-H4} = 5.5$
H(5)	3.77 (d, 2H)	$^3J_{H4-H3} = 5.5$
H(6)	4.53 (t, 1H)	$^3J_{H5-H6} = 5.6$
H(7a)	2.86 (dd, 1H)	$^2J_{H6a-H6b} = 14.2$, $^3J_{H6-H5} = 5.6$
H(7b)	3.01 (dd, 1H)	$^2J_{H6b-H6a} = 14.2$, $^3J_{H6-H5} = 5.6$
H(8)	4.21 (s, 2H)	
H(9a)	3.80 (d, 1H)	$^2J_{H7a-H7b} = 17.1$
H(9b)	3.82 (d, 1H)	$^2J_{H7b-H7a} = 17.1$
H(10)1.90 (s, 3H)		

Table 2. ^{13}C NMR Data (500 MHz, D_2O) for RP294



	δ (ppm)		δ (ppm)		δ (ppm)
C(1) and C(2)	43.8	C(7)	53.4	C(12)	61.1
C(3)	58.1	C(8)	31.5	C(13)	42.2
C(4)	165.6	C(9)	172.4	C(14)	173.8
C(5)	55.6	C(10)	40.9	C(15)	22.1
C(6)	171.6	C(11)	174.3		

0.475 mmol) was added to the solution, and the resulting solution was stirred under Ar at room temperature for 1 h. H_2S gas was then bubbled through the solution for 5 min, causing black HgS to precipitate. The precipitate was removed by vacuum filtration, and the filtrate was frozen and lyophilized overnight. The resulting residue was used immediately in the reaction with the Re starting material.

Synthesis of the Re Complex of RP294, [ReO(RP294)]. Two methods were used to prepare the Re complex of RP294. Different Re starting materials and solvent systems were used in each method.

Method 1. $[ReO_2(en)_2]Cl$ (80.3 mg, 0.215 mmol) was dissolved in 1.5 mL of distilled water. Deprotected RP294 (87.0 mg, 0.250 mmol) and sodium acetate (51.0 mg, 0.375 mmol) were dissolved in 2 mL of distilled water. The two solutions were combined to give a light green solution. The pH of the solution was adjusted to 6 using 1 M NaOH. The solution was refluxed under Ar for 2 h, during which the solution changed from green to red. The solution was frozen and lyophilized overnight, yielding a red solid. Purification of the product was done by HPLC. The fractions collected from the HPLC were lyophilized and characterized by elemental analysis and by various spectrometric techniques. Two fractions corresponding to the two isomers of the Re complex were observed. Slow evaporation of the solvent from an aqueous solution containing both isomeric complexes produced crystals suitable for X-ray crystallographic analysis. A crystal of the *syn* isomer was selected for X-ray crystallographic analysis. Yield: 53 mg (59%). Anal. Calcd (found) for $C_{12}H_{20}N_5O_6ReS{\cdot}0.5H_2O$: C, 25.85 (26.00); H, 3.80 (3.80); N, 12.56 (11.99). Mass spectrum (electrospray): *m/z* = 549 ($[M + 1]^+$, $[C_{12}H_{21}N_5O_6ReS]^+$), *m/z* = 571 ($[M + Na]^+$, $[C_{12}H_{20}N_5NaO_6ReS]^+$). HPLC retention time using method A: fraction 1, R_t = 10.67 min; fraction 2, R_t = 10.83 min. IR (KBr disk): 986 cm^{-1} ($\nu_{Re=O}$). ^{1}H and ^{13}C NMR spectral data are given in Tables 3 and 4, respectively.

A sample of the crystallized [ReO(RP294)] was dissolved in ^{18}O-enriched water. The solution was left at room temperature overnight and lyophilized. The lyophilized product was analyzed by high-resolution mass spectrometry (fast-atom bombardment ionization, FAB). Mass spectrum (electrospray): *m/z* = 550.074 ($[C_{12}H_{20}N_5{}^{16}O_5{}^{18}O^{185}ReS]^+$), *m/z* = 552.077 ($[C_{12}H_{20}N_5{}^{16}O_5{}^{18}O^{187}ReS]^+$).

Method 2. Deprotected RP294 (92.9 mg, 0.271 mmol) and sodium acetate (61.3 mg, 0.450 mmol) were dissolved in 2 mL of methanol. $ReOCl_3(PPh_3)_2$ (203.2 mg, 0.244 mmol) was added to the RP294 solution. The $ReOCl_3(PPh_3)_2$ was insoluble in methanol. The mixture was refluxed under Ar for 4 h. As the solution was heated, $ReOCl_3(PPh_3)_2$ gradually dissolved and the solution turned red. The methanol was then removed by a rotary evaporator to yield a red residue. The residue was dissolved in distilled water to give a cloudy red solution. It was filtered and lyophilized. Purification of the crude product was done by HPLC. The collected HPLC fractions were lyophilized and characterized by elemental analysis and by various spectrometric techniques. The purified product was identical to the product obtained from method 1. Yield: 38 mg (26%). Anal. Calcd (found) for $C_{12}H_{20}N_5O_6ReS$: C, 26.27 (26.67); H, 3.67 (3.92); N, 12.77 (12.93). Mass spectrum (electrospray): *m/z* = 549 ($[M + 1]^+$, $[C_{12}H_{21}N_5O_6ReS]^+$), *m/z* = 571 ($[M + Na]^+$, $[C_{12}H_{20}N_5NaO_6ReS]^+$). HPLC retention time using method A: fraction 1, R_t = 10.70 min; fraction 2, R_t = 10.85 min. IR (KBr disk): 986 cm^{-1} ($\nu_{Re=O}$).

X-ray Crystallographic Analysis of *syn*-[ReO(RP294)]. X-ray crystallographic data for *syn*-[ReO(RP294)] were collected from a single-crystal sample, which was mounted on a glass fiber with epoxy glue. Data were collected using a P4 Siemens diffractometer, equipped with a Siemens SMART 1K charge-coupled device (CCD) area detector (using the program SMART[33]) and a rotating anode using graphite-monochromated Mo Kα radiation (λ = 0.710 73 Å). The crystal-to-detector distance was 3.991 cm, and the data collection was carried out in 512 × 512 pixel mode, utilizing 2.2 pixel binning. The initial unit cell determination was carried out by scanning 4.5° in 15 frames over three different parts of reciprocal space (45 frames, reflections total). The strong reflections were then chosen and used in the determination of the unit cell. One complete hemisphere of data was collected, to better than 0.8 Å resolution. Upon completion of the data collection, the first 50 frames were recollected in order to improve the decay correction analysis. Processing was carried out by the use of the program SAINT,[34] which applied Lorentz and polarization corrections to three-dimensionally integrated diffraction spots. The program SADABS[35] was utilized for the scaling of diffraction data, the application of a decay correction, and an empirical absorption procedure in the Siemens SHELXTL program library[36] and refined by full-matrix least-squares methods with anisotropic thermal parameters for all non-hydrogen atoms. Selected crystallographic data are given in Table 5, selected bond lengths and angles are listed in Table 6, and final atomic coordinates and equivalent isotropic thermal parameters are listed in Table 7.

Variable-pH ^{1}H NMR Spectroscopy of [ReO(RP294)]. To study the stability of [ReO(RP294)] at moderate pH, a variable-pH ^{1}H NMR spectroscopic study was conducted. Variable-pH ^{1}H NMR spectra of [ReO(RP294)] were collected in D_2O. The pD values were measured by a Beckman ϕ pH meter equipped with a Beckman 39844 gel-filled combination electrode. The pD values were converted to pH by adding 0.40.[37] The ^{1}H NMR spectra of [ReO(RP294)] were recorded at pD 4.6, 5.8, 6.7, 7.3, 7.9, and 9.4.

Synthesis of the ^{99}Tc Complex of RP294. RP294 (379 mg, 0.917 mmol) was dissolved in 3 mL of distilled water. Tin(II) chloride (205

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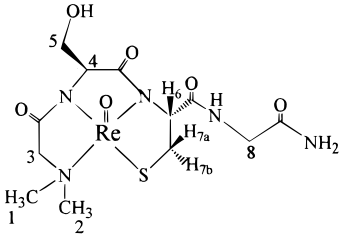
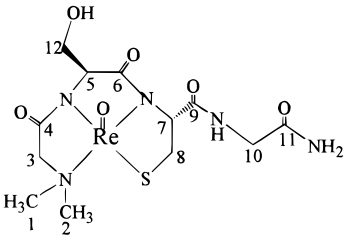
Table 3. ^{1}H NMR Data (500 MHz, D_2O) for the *Syn* and *Anti* Isomers of [ReO(RP294)]



	syn isomer		*anti* isomer	
	δ (ppm)	coupling constant (Hz)	δ (ppm)	coupling constant (Hz)
H(1)	2.51 (s, 3H)		2.59 (s, 3H)	
H(2)	3.63 (s, 3H)		3.64 (s, 3H)	
H(3a)	4.40 (d, 1H)	$^2J_{H3a-H3b} = 15.8$	4.5 (d, 1H)	$^2J_{H3a-H3b} = 16.1$
H(3b)	4.5 (d, 1H)	$^2J_{H3b-H3a} = 15.8$	4.88 (d, 1H)	$^2J_{H3b-H3a} = 16.1$
H(4)	4.76 (dd, 1H)	$^3J_{H4-H5a} = {}^3J_{H4-H5b} = 2.6$	4.875 (dd, 1H)	$^3J_{H4-H5a} = {}^3J_{H4-H5b} \approx 1.8$
H(5a)	4.00 (dd, 1H)	$^2J_{H5a-H5b} = 12.0$, $^3J_{H5a-H4} = 2.3$	3.83 (dd, 1H)	$^2J_{H5a-H5b} = 11.8$, $^3J_{H5a-H4} = 1.8$
H(5b)	4.12 (dd, 1H)	$^2J_{H5b-H5a} = 12.0$, $^3J_{H5b-H4} = 2.9$	4.23 (dd, 1H)	$^2J_{H5b-H5a} = 11.8$, $^3J_{H5b-H4} = 3.1$
H(6)	5.09 (dd, 1H)	$^3J_{H6-H7a} = 8.2$, $^3J_{H6-H7b} = 1.1$	5.31 (dd, 1H)	$^3J_{H6-H7a} = 4.5$, $^3J_{H6-H7b} = 7.6$
H(7a)	3.50 (dd, 1H)	$^2J_{H7a-H7b} = 12.8$, $^3J_{H7a-H6} = 8.2$	3.51 (dd, 1H)	$^2J_{H7a-H7b} = 12.4$, $^3J_{H7a-H6} = 4.6$
H(7b)	3.97 (dd, 1H)	$^2J_{H7b-H7a} = 12.8$, $^3J_{H7b-H6} = 1.1$	3.55 (dd, 1H)	$^2J_{H7b-H7a} = 12.4$, $^3J_{H7b-H6} = 7.7$
H(8a)	3.73 (d, 1H)	$^2J_{H8a-H8b} = 17.2$	3.72 (d, 1H)	$^2J_{H8a-H8b} = 17.2$
H(8b)	3.83 (d, 1H)	$^2J_{H8b-H8a} = 17.2$	3.76 (d, 1H)	$^2J_{H8b-H8a} = 17.2$

Table 4. ^{13}C NMR Data (500 MHz, D_2O) for the Two Isomers of [ReO(RP294)]



	δ (ppm)	
	syn isomer	*anti* isomer
C(1) or C(2)	55.7	56.2
C(1) or C(2)	58.3	58.6
C(3)	71.7	71.8
C(4)	187.2	185.9
C(5)	69.0	69.4 or 69.5[a]
C(6)	193.5 or 198.0[b]	193.5 or 198.0[b]
C(7)	71.5	69.4 or 69.5[a]
C(8)	48.9	46.3
C(9)	174.0−174.5[c]	174.0−174.5[c]
C(10)	42.2	42.4
C(11)	174.0−174.5[c]	174.0−174.5[c]
C(12)	62.0	60.4

[a] Unable to assign the resonances at 69.4 ppm and 69.5 ppm specificially to either C(5) or C(7). [b] Unable to assign the resonances at 193.5 ppm and 198.0 ppm specificially to the C(6) of either the *syn* or the *anti* isomer. [c] Multiple resonances from 174.0 to 174.5 ppm.

mg, 1.08 mmol) and sodium gluconate (200 mg, 0.917 mmol) were added to the peptide solution, followed by Na[$^{99}TcO_4$] (151 mg, 0.812 mmol). The solution was stirred at room temperature for 6 h. The color of the solution changed to orange-red. The solution was frozen and lyophilized overnight, yielding a red solid. The product was purified using a column packed with Sephadex G10 resin, with distilled water as the eluting solvent. During the reaction, the (acetoamido)-methyl protection group was displaced from the cysteine thiolate. Yield: 225 mg (60%). Mass spectrum (electrospray): $m/z = 484$ ([M + Na]$^+$, [$C_{12}H_{20}N_5NaO_6$99TcS]). IR (KBr disk): 977 cm^{-1} ($\nu_{Tc=O}$). ^{1}H NMR spectral data are given in Table 8.

Synthesis of the ^{99m}Tc Complex of RP294. RP294 (200 mg, 0.476 mmol) was dissolved in 200 mL of saline. Na[$^{99m}TcO_4$] (10 mCi) was added to the solution, followed by tin(II) chloride (7.5×10^3 mg, 39 mmol) and sodium gluconate (1.3×10^3 mg, 5.8 mmol). The solution was left at room temperature for 1 h. As in the synthesis of the ^{99}Tc RP294 complex, the (acetoamido)methyl protecting group was displaced from the cysteine thiolate. The ^{99m}Tc RP294 complex was analyzed by HPLC. A radiochemical yield of 94% was achieved. The Re and ^{99m}Tc complexes of RP294 were co-injected into the HPLC column. HPLC retention time using method B: [ReO(RP294)], $R_t = 13.99$, 14.27 min (UV detector set at 215 nm); [^{99m}Tc(RP294)], $R_t = 14.62$ min (radiometric γ detector). HPLC retention time using method C: [ReO(RP294)], $R_t = 18.26$, 18.85 min (UV detector set at 215 nm); [^{99m}Tc(RP294)], $R_t = 18.33$, 19.09 min (radiometric γ detector).

Table 5. Selected Crystallographic Data for [ReO(RP294)]

empirical formula	$C_{12}H_{20}N_5O_6ReS$
formula weight	548.59
crystal system	orthorhombic
space group	$P2_12_12_1$
a (Å)	6.954(1)
b (Å)	8.0472(1)
c (Å)	32.9183(4)
V (Å^3)	1842.01(3)
Z	4
ρ_{calc} (g/cm^3)	1.978
T (K)	300(2)
radiation (λ, (Å))	Mo Kα (0.710 73)
transm factors	0.40−0.45
R(all data)	0.0335
R_w(all data)	0.0843

Results and Discussion

Dimethylglycyl-L-seryl-L-cysteinylglycinamide (RP294) was prepared in excellent yields using solid phase methods on an automated peptide synthesizer. The cysteine thiolate was protected with an (acetoamido)methyl (ACM) group, and the C terminus was capped as an amide moiety. RP294 was extremely hygroscopic and was soluble in water and aqueous acetonitrile solution. RP294 was purified by HPLC and was characterized using NMR and electrospray mass spectrometry. ^{1}H and ^{13}C NMR spectral data for RP294 are given in Tables 1 and 2, respectively.

The neutral Re(V) oxo complex of RP294 ([ReO(RP294)]) was prepared from the ligand exchange reactions of deprotected RP294 with either [$ReO_2(en)_2$]Cl or $ReOCl_3(PPh_3)_2$ in the presence of sodium acetate (Scheme 1). Prior to the reaction with the Re starting material, the (acetoamido)methyl protection group was removed from the cysteine thiolate using mercury-

Table 6. Bond Lengths (Å) and Bond Angles (deg) for the *Syn* Isomer of [ReO(RP294)]

Re(1)−O(1)	1.682(5)	C(8)−N(7)	1.490(8)
Re(1)−N(7)	1.954(6)	C(8)−C(17)	1.521(10)
Re(1)−N(4)	1.980(5)	C(8)−C(9)	1.530(11)
Re(1)−N(1)	2.165(6)	C(19)−C(20)	1.504(11)
Re(1)−S(1)	2.281(2)	O(17)−C(17)	1.214(10)
S(1)−C(9)	1.827(9)	C(14)−O(15)	1.401(9)
N(1)−C(2)	1.483(9)	C(14)−C(5)	1.525(10)
N(1)−C(11)	1.493(10)	C(6)−N(7)	1.365(9)
N(1)−C(12)	1.514(10)	C(6)−C(5)	1.509(9)
O(13)−C(3)	1.197(10)	C(2)−C(3)	1.516(11)
N(18)−C(17)	1.340(10)	C(20)−O(20)	1.229(10)
N(18)−C(19)	1.449(9)	C(20)−N(20)	1.328(11)
N(4)−C(3)	1.380(9)	O(16)−C(6)	1.236(9)
N(4)−C(5)	1.453(9)		
O(1)−Re(1)−N(7)	113.0(3)	C(6)−N(7)−C(8)	114.8(6)
O(1)−Re(1)−N(4)	111.2(2)	C(6)−N(7)−Re(1)	120.2(5)
N(7)−Re(1)−N(4)	78.1(2)	C(8)−N(7)−Re(1)	124.0(4)
O(1)−Re(1)−N(1)	109.8(3)	N(4)−C(5)−C(6)	106.2(5)
N(7)−Re(1)−N(1)	135.9(2)	N(4)−C(5)−C(14)	114.7(6)
N(4)−Re(1)−N(1)	77.4(2)	C(6)−C(5)−C(14)	109.8(6)
O(1)−Re(1)−S(1)	108.7(2)	O(13)−C(3)−N(4)	125.4(8)
N(7)−Re(1)−S(1)	83.5(2)	O(13)−C(3)−C(2)	123.2(7)
N(4)−Re(1)−S(1)	140.0(2)	N(4)−C(3)−C(2)	111.3(6)
N(1)−Re(1)−S(1)	92.5(2)	C(8)−C(9)−S(1)	110.5(5)
C(9)−S(1)−Re(1)	96.8(3)	O(15)−C(14)−C(5)	112.6(6)
C(2)−N(1)−C(11)	109.6(7)	O(16)−C(6)−N(7)	124.2(7)
C(2)−N(1)−C(12)	110.3(6)	O(16)−C(6)−C(5)	121.9(7)
C(11)−N(1)−C(12)	108.8(7)	N(7)−C(6)−C(5)	113.9(6)
C(2)−N(1)−Re(1)	105.3(4)	N(1)−C(2)−C(3)	110.0(6)
C(11)−N(1)−Re(1)	113.5(5)	O(20)−C(20)−N(20)	123.0(8)
C(12)−N(1)−Re(1)	109.3(5)	O(20)−C(20)−C(19)	123.6(8)
C(17)−N(18)−C(19)	120.0(7)	N(20)−C(20)−C(19)	113.4(7)
C(3)−N(4)−C(5)	121.0(6)	O(17)−C(17)−N(18)	122.5(7)
C(3)−N(4)−Re(1)	119.6(5)	O(17)−C(17)−C(8)	123.0(7)
C(5)−N(4)−Re(1)	119.4(4)	N(18)−C(17)−C(8)	114.5(6)
N(7)−C(8)−C(17)	110.4(6)	N(18)−C(19)−C(20)	114.0(7)
N(7)−C(8)−C(9)	108.6(6)	C(17)−C(8)−C(9)	112.5(6)

Table 7. Final Atomic Coordinates (Fractional) and B_{eq} Values ($Å^2$) for Non-Hydrogen Atoms in [ReO(RP294]

	x	*y*	*z*	B_{eq}[a]
Re(1)	0.5006(1)	0.8696(1)	0.0870(1)	0.020(1)
S(1)	0.6709(3)	0.9199(3)	0.1449(1)	0.030(1)
N(1)	0.4629(8)	1.1320(8)	0.0748(2)	0.025(1)
O(13)	0.0273(9)	1.0224(8)	0.0246(2)	0.041(2)
N(18)	0.3121(9)	0.5938(8)	0.2268(2)	0.023(1)
O(16)	0.0997(8)	0.5463(7)	0.1306(2)	0.029(1)
N(4)	0.2333(8)	0.8802(8)	0.0664(2)	0.020(1)
O(1)	0.6492(7)	0.7859(7)	0.0522(2)	0.026(1)
C(8)	0.4463(10)	0.6438(10)	0.1605(2)	0.024(2)
C(19)	0.1739(12)	0.6176(10)	0.2592(2)	0.029(2)
O(17)	0.2120(9)	0.8193(7)	0.1930(2)	0.034(1)
C(14)	0.0526(10)	0.6291(10)	0.0424(2)	0.027(2)
C(6)	0.1884(10)	0.6550(9)	1.118(2)	0.020(1)
C(2)	0.3361(12)	1.1402(10)	0.0386(2)	0.027(2)
C(20)	0.2190(12)	0.7618(11)	0.2867(2)	0.028(2)
C(17)	0.3112(11)	0.6954(10)	0.1946(2)	0.025(2)
N(7)	0.3720(8)	0.7030(8)	0.1205(2)	0.018(1)
C(5)	0.0989(9)	0.7489(9)	0.0769(2)	0.018(1)
C(3)	0.1783(12)	1.0108(10)	0.0418(2)	0.026(2)
O(20)	0.3815(8)	0.8190(8)	0.2912(2)	0.039(2)
O(15)	0.2104(10)	0.5304(7)	0.0314(2)	0.037(1)
N(20)	0.0665(11)	0.8192(10)	0.3066(2)	0.039(2)
C(9)	0.6501(12)	0.7113(11)	0.1663(2)	0.030(2)
C(12)	0.3661(14)	1.2134(12)	0.1109(3)	0.042(2)
C(11)	0.6469(14)	1.2210(13)	0.0662(3)	0.046(2)
O(21)	0.7216(9)	0.6507(9)	0.2818(3)	0.050(2)

[a] $B_{eq} = (8/3)\pi^2(U_{11}(aa^*)^2 + U_{22}(bb^*)^2 + U_{33}(cc^*)^2 + 2U_{12}aa^*bb^* \cos\gamma + 2U_{13}aa^*cc^* \cos\beta + 2U_{23}bb^*cc^* \cos\alpha)$.

(II) acetate. [ReO(RP294)] was soluble in aqueous solution and slightly soluble in methanol. Analysis by thin-layer chromatography (C-18 reverse-phase plate and methanol containing 0.05% HCl) showed [ReO(RP294)] to have a R_f value of 0.9, while $[ReO_2(en)_2]Cl$ and $ReOCl_3(PPh_3)_2$ have R_f values of 0.0. [ReO(RP294)] was purified by HPLC and characterized by various spectroscopic techniques, elemental analysis, and X-ray crystallography.

It is well-known that Re(V) oxo complexes exhibit a characteristic $\nu_{Re=O}$ band in the infrared spectra. $ReOCl_3(PPh_3)_2$ has a $\nu_{Re=O}$ band at ~970 cm^{-1}, while $[ReO_2(en)_2]Cl$ has a $\nu_{O=Re=O}$ band at ~820 cm^{-1}.[29−30,38,39] The infrared spectrum of [ReO(RP294)] showed a $\nu_{Re=O}$ stretch at 986 cm^{-1}. Mass spectral data for [ReO(RP294)] were obtained by electrospray mass spectrometry in the positive-ion detection mode. The molecular ion MH^+ and the sodium adduct MNa^+ were detected. Both of these ions exhibited the characteristic Re isotopic pattern. The mass spectral data confirmed the Re RP294 complex as a mononuclear monoligand complex.

There were two fractions of approximately equal magnitude in the HPLC chromatogram of [ReO(RP294)]. Electrospray mass spectroscopic analysis of a sample containing both fractions showed only ions associated with the Re complex. The two HPLC fractions represented the two isomers of [ReO(RP294)], the serine CH_2OH group being in the *syn* or *anti* conformation with respect to the Re−oxo bond (Chart 1). These isomers are often observed in Tc(V) and Re(V) complexes with similar tetradentate amino/amido-thiolato ligands.[40−48] The 1:1 ratio between the *syn* and *anti* isomers was previously observed in Re(V) and Tc(V) oxo complexes with N_2S_2 ligands.[47,48]

^1H and ^{13}C NMR spectra of [ReO(RP294)] were recorded in D_2O at room temperature. The ^1H NMR spectrum showed a complex array of overlapping ^1H NMR resonances from 2.5 to 5.5 ppm. The ^{13}C NMR spectrum showed resonances from 42 to 187 ppm. Assignments of the ^1H and ^{13}C NMR resonances are given in Tables 3 and 4, respectively.

The presence of the *syn* and *anti* isomers of [ReO(RP294)] was evident from the NMR spectral data. In the ^1H NMR spectrum, there were two pairs of singlets associated with the nonequivalent methyl groups in the dimethylglycine residue. Each pair of singlets corresponded to either the *syn* or *anti* [ReO(RP294)] isomer. In addition, there were also two sets of ^1H NMR resonances for the methine and methylene hydrogen atoms of the four amino acid residues. ^{13}C NMR spectrum showed two sets of ^{13}C NMR resonances that corresponded to the two isomers.

In the ^1H NMR spectrum of uncoordinated RP294, the two methyl groups of dimethylglycine were chemically equivalent

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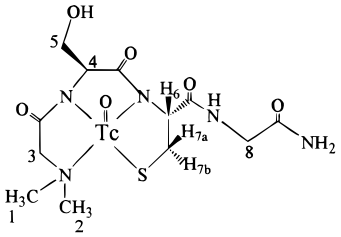
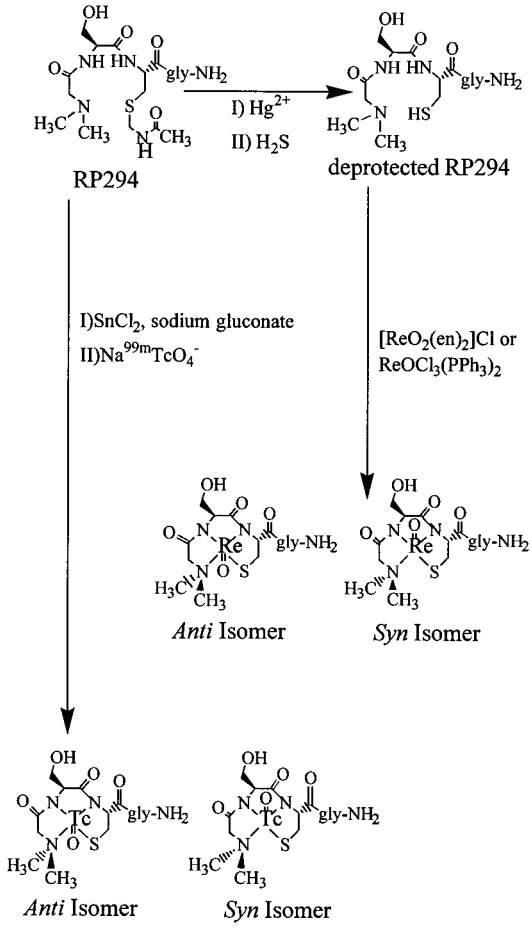
Table 8. ^{1}H NMR Data (500 MHz, D_2O) for the *Syn* and *Anti* Isomers of [^{99}TcO(RP294)]



	syn isomer		*anti* isomer	
	δ (ppm)	coupling constant (Hz)	δ (ppm)	coupling constant (Hz)
H(1)	2.36 (s, 3H)		2.46 (s, 3H)	
H(2)	3.43 (s, 3H)		3.45 (s, 3H)	
H(3a)	4.6 (d, 1H)[a]	$^{2}J_{H3a-H3b} = 15.5$	4.13 (d, 1H)	$^{2}J_{H3a-H3b} = 12.0$
H(3b)	3.74 (d, 1H)	$^{2}J_{H3b-H3a} = 15.5$	3.99 (d, 1H)	$^{2}J_{H3b-H3a} = 12.0$
H(4)	4.79 (dd, 1H)	$^{3}J_{H4-H5a} = 3.5$, $^{3}J_{H4-H5b} = 1.9$	4.52 (dd, 1H)	$^{3}J_{H4-H5a} = {}^{3}J_{H4-H5b} = 2.7$
H(5a)	4.23 (dd, 1H)	$^{2}J_{H5a-H5b} = 11.9$, $^{3}J_{H5a-H4} = 3.5$	4.12 (dd, 1H)	$^{2}J_{H5a-H5b} = 12.0$, $^{3}J_{H5a-H4} = 2.7$
H(5b)	3.82 (dd, 1H)	$^{2}J_{H5b-H5a} = 11.9$, $^{3}J_{H5b-H4} = 1.9$	3.99 (dd, 1H)	$^{2}J_{H5b-H5a} = 12.0$, $^{3}J_{H5b-H4} = 2.7$
H(6)	5.68 (dd, 1H)	$^{3}J_{H6-H7a} = 5.3$, $^{3}J_{H6-H7b} = 6.5$	5.29 (dd, 1H)	$^{3}J_{H6-H7a} = 7.8$, $^{3}J_{H6-H7b} = 1.2$
H(7a)	3.74 (dd, 1H)	$^{3}J_{H7a-H6} = 6.7$, $^{2}J_{H7a-H7b}$[b]	3.77 (dd, 1H)	$^{2}J_{H7a-H7b} = 13.0$, $^{3}J_{H7a-H6} = 7.8$
H(7b)	3.74 (dd, 1H)	$^{2}J_{H7b-H7a} = ...$,[b] $^{3}J_{H7b-H6} \approx 7$[c]	3.87 (dd, 1H)	$^{2}J_{H7b-H7a} = 13.0$, $^{3}J_{H7b-H6} = 1.2$
H(8a)	3.74 (d, 1H)	$^{2}J_{H8a-H8b} = 17.2$	3.74 (d, 1H)	$^{2}J_{H8a-H8b} = 17.2$
H(8b)	3.83 (d, 1H)	$^{2}J_{H8b-H8a} = 17.2$	3.83 (d, 1H)	$^{2}J_{H8b-H8a} = 17.2$

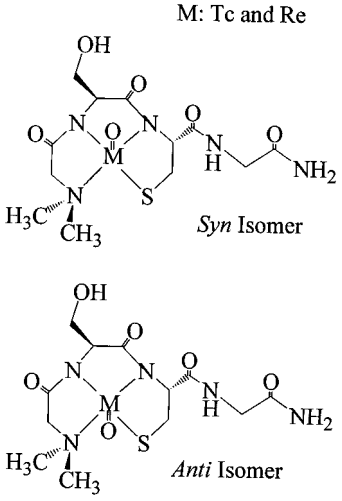
[a] Overlapping with the ^{1}H NMR resonance of water. [b] Unable to accurately determine $^{2}J_{H7a-H7b}$. [c] Unable to accurately determine $^{3}J_{H7b-H6}$ from the TOSCY experiment.

Scheme 1



and a singlet was observed for these six methyl hydrogen atoms. Upon the coordination of RP294 to ReO^{3+}, the two methyl groups became nonequivalent and two singlets were observed for the hydrogen atoms of each methyl group. In addition, the singlet of the two methylene hydrogen atoms in dimethylglycine also became nonequivalent and shifted downfield from 3.99 ppm

Chart 1



to 4.5−4.8 ppm. These changes in the ^{1}H NMR resonances from the free RP294 to [ReO(RP294)] indicates the coordination of the dimethylglycine N_{amine} atom to the Re center.

Changes to the serine and cysteine ^{1}H NMR resonances from the uncoordinated RP294 to the Re complex were also observed. The serine and cysteine methine hydrogen atoms shifted downfield approximately 0.4 and 0.8 ppm, respectively. The resonances of the nonequivalent methylene hydrogen atoms on the cysteine side chain (H_{7a} and H_{7b}) underwent a downfield shift of 0.5−0.65 ppm. The downfield shift of the ^{1}H NMR resonances clearly points to the coordination of the serine and cysteine N_{amide} atoms and of the cysteine $S_{thiolate}$ atom to the ReO^{3+} core.

The coordination of RP294 to ReO^{3+} and the subsequent formation of the five-membered chelate rings imposed a rigidity onto the peptidic backbone, which limited the rotation of the groups within the peptide chain. The two hydrogen atoms of the methylene groups α to the coordinated dimethylglycine N_{amine} atom became nonequivalent as the CH_2 groups could no longer freely rotate about the C(3)−N_{amine} bond (refer to diagram in

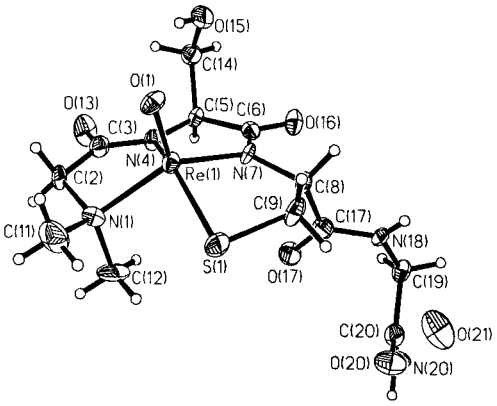


Figure 1. ORTEP drawing of *syn*-[ReO(RP294)] showing the crystallographic numbering. 50% probability thermal ellipsoids are shown for the non-hydrogen atoms.

Table 3). In a similar manner, the two methyl groups in dimethylglycine became nonequivalent upon the coordination of the N_{amine} atom as the $N(CH_3)_2$ group could not rotate about the $C-N_{amine}$ bond. The two methylene hydrogen atoms on the cysteine side chain were also nonequivalent as the CH_2 groups could not freely rotate about the $C(8)-S_{thiolate}$ bond.

For [ReO(RP294)], it is interesting to note that the coordination of the peptide to the ReO^{3+} core resulted in the serine $CH-CH_2OH$ side chain adopting a strong preference for a single conformation. The two coupling constants, J_{AX} and J_{BX}, of the ABX spin system ($CH_X-CH_AH_BOH$) were remarkably small in both the *syn* and *anti* isomers (*syn* $J_{AX} = 3.1$ Hz, $J_{BX} = 1.8$ Hz; *anti* $J_{AX} = 2.3$ Hz, $J_{BX} = 2.9$ Hz) and such small vicinal couplings show the CH_X proton to be preferentially gauche to both H_A and H_B. That is the OH group of the serine has a very strong preference for the conformation where it is pointing in toward the Re atom in both isomers. This unusual situation in solution is exactly the same as observed for the *syn* isomer in the solid state, as noted below. An H-bond between the serine hydroxyl group and the oxygen atom of the ReO^{3+} core is possible; however, the observed distance between the hydroxyl group and the oxo oxygen atom does not support this suspicion. In addition, the proposed H-bond would not explain the nonequivalent nature of the serine methylene hydrogen atoms in the *anti*-[ReO(RP294)] complex, as the serine CH_2OH group and the Re−oxo bond are on the opposite side of the plane formed by the peptidic backbone.

Variable-pH ^{1}H NMR experiments were performed on [ReO(RP294)] to study its stability in aqueous solution in the moderate pH range. ^{1}H NMR spectra of [ReO(RP294)] were recorded at pH 5.0, 6.2, 7.1, 7.7, 8.3, and 9.8. No differences in the ^{1}H NMR spectra were observed from pH 5.0 to 8.3. Decomplexation of the Re atom was observed at pH 9.8. This was evident from the appearance of the ^{1}H NMR resonances associated with the uncoordinated RP294 ligand.

Slow evaporation of the solvent from an aqueous solution of [ReO(RP294)] produced crystals suitable for X-ray crystallographic analysis. An ^{1}H NMR spectrum of the crystals showed both isomers present in solution. A crystal of the *syn* isomer was selected for crystallographic analysis. Attempts to find a crystal of the *anti* isomer were unsuccessful. Selected crystallographic data for the *syn*-[ReO(RP294)] complex are listed in Table 5. An ORTEP drawing of the *syn*-[ReO(RP294)] complex is shown in Figure 1; bond lengths and bond angles are listed in Table 6. Crystallographic analysis confirmed the tetracoordination of the ligand to ReO^{3+} via the tertiary amine nitrogen atom of dimethylglycine, the sulfur atom of cysteine, and the two amide nitrogen atoms of serine and cysteine. This is consistent with the NMR spectral data. The Re is in a distorted square pyramidal coordination environment with an oxygen atom in the apical position. The square plane defined by the $N_2N'S$ donor set is distorted, with the cysteine N_{amide} and the N_{amine} atoms being ∼0.14 Å above the plane and the serine N_{amide} and the $S_{thiolate}$ atoms lying ∼0.14 Å below the plane.[49] The Re atom sits above the distorted plane by approximately 0.74 Å. The distortion of the plane is most likely caused by the steric constraint of the three five-membered chelate rings and the coordination of the Re metal to two types of nitrogen atoms (N_{amide} and N_{amine}).

The $Re-N_{amide}$ bond lengths are 1.980(5) Å (Re−N(4)) and 1.954(6) Å (Re−N(7)), while the $Re-N_{amine}$ bond length is 2.165(6) Å (Re−N(1)). These bond lengths are consistent with the bond lengths observed in other Re oxo amino/amido−thiolato complexes.[48,50−52] The $Re-N_{amide}$ bond lengths are approximately 0.2 Å shorter than the typical of M−N single bond (ca. 2.15 Å).[53−57] The angles involving the N_{amide} atom are ∼120°, while the corresponding angles around the N_{amine} atom are ∼110°. This data suggest that the Re−amide nitrogen bond has some double-bond character and the N_{amide} atom is considered to be sp^2 hybridized. On the other hand, the $Re-N_{amine}$ bond length is within the range of the typical metal−N_{amine} bond and the N_{amine} is considered to be sp^3 hybridized.

The Re−S bond length is 2.281(2) Å and is consistent with the bond lengths observed in other Re complexes with coordinated thiolato groups.[48,50−52,58,59] The Re−oxo bond length is 1.682(5) Å and is also consistent with the metal−oxo bond observed in other Tc(V) and Re(V) oxo complexes. The distance from the oxygen atom of the serine hydroxyl group to the oxygen atom of the Re oxo moiety was ∼3.7 Å. The distance is longer than that expected for a hydrogen bond; however the presence of an H-bond in solution cannot be ruled out.

The *syn* and *anti* isomers of [ReO(RP294)] were observed as two HPLC fractions with a difference in retention time of about 0.2 min. The separation of the isomers was accomplished using HPLC; however, the isolation of each species was not possible, as each isomer undergoes relatively rapid conversion to the other isomer at room temperature. To confirm the presence of this conversion, an HPLC experiment was performed in which each of the two HPLC fractions were collected and then monitored over a period of 18 h. Within 15 min after the collection of each HPLC fraction, the emergence of the other

(49) The best plane formed by the donor set was calculated from the following equation: $-4.275(0.009)x + 3.273(0.016)y + 22.242(0.039)z = 3.377(0.016)$.
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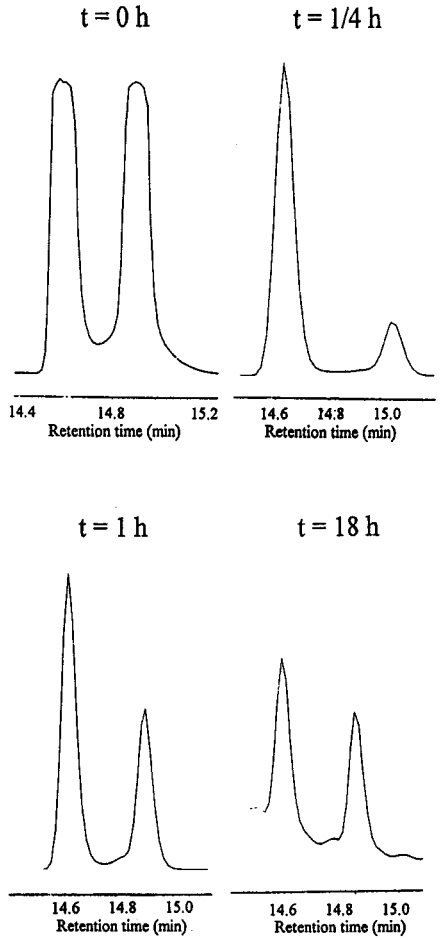

Figure 2. HPLC chromatograms of [ReO(RP294)] at $t = 0$ $^1/_4$ h, 1 h, and 18 h after the collection of fraction A.

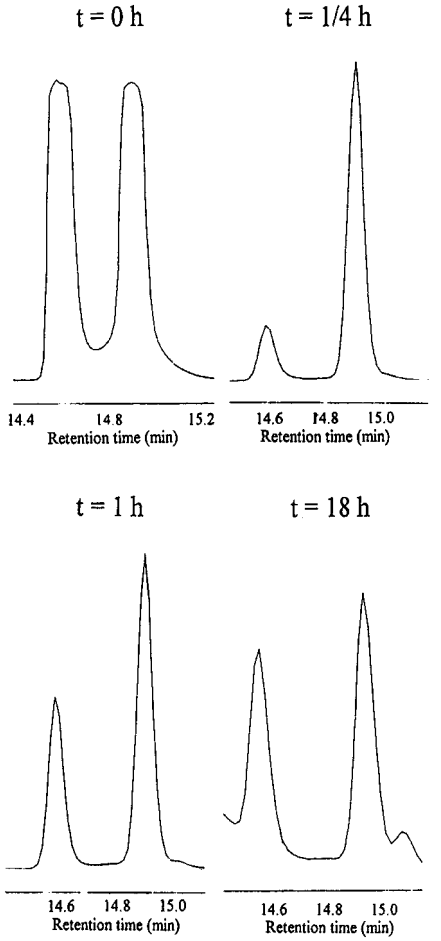

Figure 3. HPLC chromatograms of [ReO(RP294)] at $t = 0$, $^1/_4$ h, 1 h, and 18 h after the collection of fraction B.

fraction was observed (Figures 2 and 3). After 1 h, the ratio of the two isomer had reached 2:3. After 18 h, the ratio of the two isomer was almost 1:1.

The conversion of the *anti* to the *syn* isomeric complex in the presence of excess ligand and with 3 h of heating was reported by Kung's group for some ^{99m}Tc complexes with bis-(amino thiolato) ligands; the conversion was only 5−10%.[48,60] No conversion of the *syn* to the *anti* isomer was reported. The corresponding ^{99}Tc and Re complexes showed no signs of interconversion after being in solution for several weeks at ambient temperature. In contrast, the *syn* isomer of [ReO-(RP294)] converted to the *anti* isomer, and vice versa, in less than 18 h at room temperature and in the absence of free RP294.

It is suspected that the conversion between the *syn* and *anti* isomers of [ReO(RP294)] is possible because of the coordination of a water molecule in the sixth position, trans to the Re−oxo bond. Scheme 2 illustrates a proposed mechanism for the conversion between the [ReO(RP294)] isomers. After the coordination of the water molecule, it is proposed that the coordinated water molecule loses a H^+ ion and becomes a coordinated hydroxide. This is then followed by the formation of a ReO_2^+ group, accompanied by the protonation of the tertiary N_{amine} atom and the breaking of the $M-N_{amine}$ bond. Protonation of the alternate oxygen atom and the regeneration of the the $M-N_{amine}$ bond produce an intermediate that now has the Re oxo group on the opposite side of the $N_2N'S$ plane. The protonation of the coordinated hydroxide, followed by the release of the water molecule, results in the formation of the other isomer.

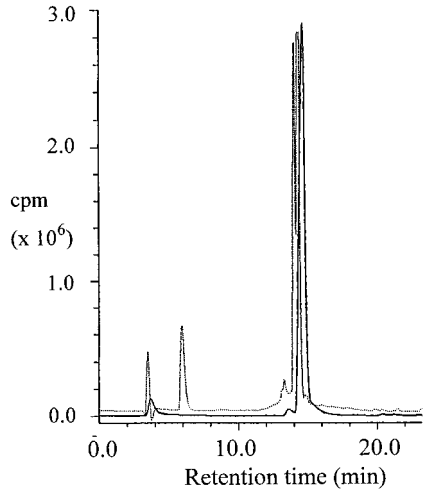

Figure 4. HPLC chromatograms of co-injected [ReO(RP294)] (···, UV detector) and [^{99m}TcO(RP294)] (—, radiometric γ detector) complexes using HPLC method B.

In an attempt to confirm the role of water in the conversion process, crystals of the [ReO(RP294)] complex were dissolved in ^{18}O-enriched water. The solution was allowed to stand at room temperature overnight. The solution was then lyophilized, and the solid residue was analyzed by high-resolution mass spectrometry. The mass spectrum showed ions at $m/z = 550.07$ and at $m/z = 552.08$. The ion at $m/z = 550.07$ is consistent with the formulation of a ^{185}Re(V) oxo RP294 complex having one ^{16}O atom replaced by one ^{18}O atom ($C_{12}H_{21}N_5{}^{16}O_5{}^{18}O^{185}$-ReS). The ion at $m/z = 552.08$ is consistent with the

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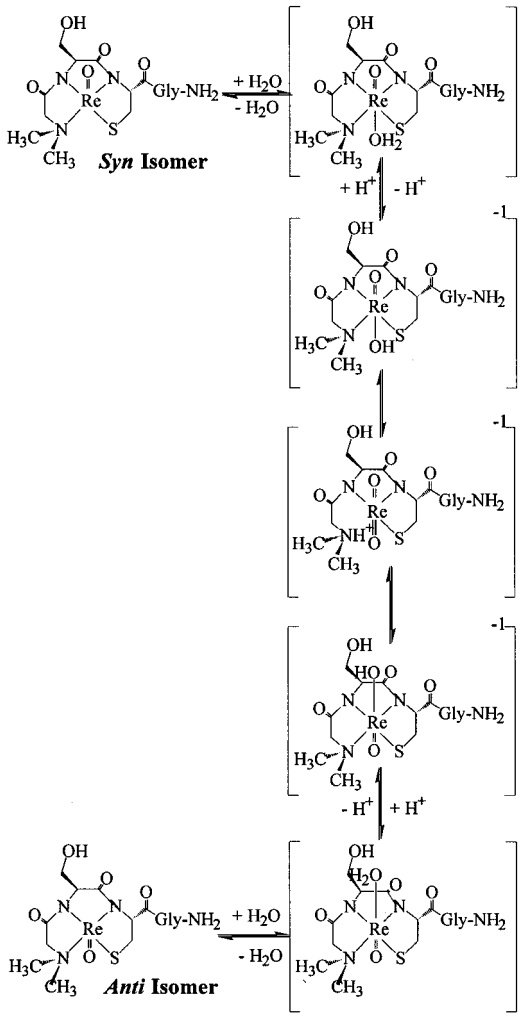
Scheme 2



formulation of a ^{187}Re(V) oxo RP294 complex having five ^{16}O atoms and one ^{18}O atom ($C_{12}H_{21}N_5{}^{16}O_5{}^{18}O^{187}ReS$). Since $H_2{}^{18}O$ was the only source of the ^{18}O atoms in this experiment, the incorporation of the ^{18}O atom into the Re complex confirms the role of water in the conversion process; however more data will be needed to confirm the proposed mechanism.

The [^{99}TcO(RP294)] complex was prepared from the reaction of $^{99}TcO_4^-$ with tin(II) chloride, sodium gluconate, and RP294. The complex exhibited the characteristic $\nu_{Tc=O}$ stretch at 977 cm^{-1}. The difference in the metal−oxo stretching frequencies is often observed between analogous Tc and Re complexes.[48,61−63] The mass spectrum of [^{99}TcO(RP294)] showed the sodium adduct MNa^+. The mass spectral data confirmed the ^{99}Tc RP294 complex as a mononuclear monoligand complex.

Aside from the minor differences in the chemical shifts of the NMR resonances, the ^{1}H NMR spectral data for the [^{99}TcO(RP294)] complex (Table 8) are very similar to those for the analogous Re complex. The data indicated that the coordination of RP294 to $^{99}TcO^{3+}$ was via the amine nitrogen atom of dimethylglycine, the sulfur atom of cysteine, and the two amide nitrogen atoms of serine and cysteine. The presence of the *syn* and *anti* conformations was also evident from the ^{1}H NMR spectral data for the [^{99}TcO(RP294)] complex. As in the case of the [ReO(RP294)] complex, two sets of NMR resonances were observed for the various ^{1}H atoms in [^{99}TcO(RP294)]. Integration of the ^{1}H NMR resonances of the methyl hydrogen atoms in dimethylglycine showed the ratio of the two isomers was approximately 1:1. The interconversion between the *syn* and *anti* isomers is suspected to be occurring for the [^{99}TcO(RP294)] complex.

As in the case of the Re complex, the coordination of the peptide to $^{99}TcO^{3+}$ caused the serine $CH-CH_2OH$ side chain to adopt a strong preference for a single conformation. The two coupling constants, J_{AX} and J_{BX}, of the ABX spin system ($CH_X-CH_AH_BOH$) were again remarkably small in both *syn* and *anti* isomers (*syn* $J_{AX} = 3.5$ Hz, $J_{BX} = 1.9$ Hz; *anti* $J_{AX} = 2.7$ Hz, $J_{BX} = 2.7$ Hz). These small vicinal couplings indicate the CH_X hydrogen to be preferentially gauche to both H_A and H_B. The hydroxyl group of the serine has a strong preference for the conformation where it is pointing in toward the ^{99}Tc atom in both isomers. This is consistent with the Re complex.

The ^{99m}Tc complex of RP294 was prepared at the tracer level in the same manner as the ^{99}Tc complex, with the exception that the tin(II) chloride, sodium gluconate, and RP294 were present in large excess. A radiochemical yield of greater than 94% was obtained in consecutive labeling experiments. The ^{99m}Tc and Re complexes of RP294 were co-injected into the HPLC column. The Re complex was observed using a UV detector, while the ^{99m}Tc complex was monitored with the radiometric γ detector (Figure 3). The UV detector observed two fractions at 13.99 and 14.27 min, which corresponded to the two isomeric complexes of [ReO(RP294)]. For the [^{99m}TcO(RP294)] complex, one fraction was observed at 14.62 min. The Re and ^{99m}Tc complexes eluted out of the column within 0.5 min of each other. There was a difference between the retention times of the Re and ^{99m}Tc complexes because the UV and the γ detectors were arranged in series. Though the HPLC chromatogram of [^{99m}TcO(RP294)] showed only one fraction, it is suspected that the ^{99m}Tc complex exists as two isomers in the same manner as the analogous ^{99}Tc and Re complexes. The Re and ^{99m}Tc complexes were re-examined by HPLC using a slower HPLC gradient (method C). Using this HPLC method, the *syn* and *anti* isomers of the ^{99m}Tc complex were observed, and they had retention times similar to that of the Re complex under identical HPLC conditions. The similar retention times of the Re and the ^{99m}Tc complexes support the hypothesis that the ^{99m}Tc complex has a structure similar to that of the Re complex. The stability of the ^{99m}Tc complex is presently being investigated by performing *in vivo* and *in vitro* rat experiments. Preliminary data showed the ^{99m}Tc complex remaining intact in rat urine and blood plasma.

Concluding Remarks

The peptide dimethylglycyl-L-seryl-L-cysteinylglycinamide (RP294) was prepared in excellent yield via the solid state peptide synthesis method. The neutral Re(V) oxo complex of RP294 was prepared via ligand exchange reactions and was stable in aqueous solution at intermediate pH. The ^{99}Tc complex of RP294 was prepared via the reduction of TcO_4^- in the presence of RP294, $SnCl_2$, and sodium gluconate. The ^{99m}Tc RP294 complex was prepared in the same manner as the ^{99}Tc complex. The Re complex eluted with the ^{99m}Tc complex when co-injected, indicating the chemical species are similar under the HPLC conditions.

The coordinations of RP294 to $^{99}TcO^{3+}$ and ReO^{3+} were observed to be very similar. NMR spectral data for [ReO(RP294)] and [^{99}TcO(RP294)] indicated the peptide was coordinated to the MO^{3+} moiety via the dimethylglycine, serine,

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and cysteine residues. HPLC and NMR spectral data showed the complex existing as two isomers, the serine CH_2OH group being in the *syn* and *anti* conformations with respect to the metal−oxo bond. X-ray crystallographic analysis of the *syn*-[ReO(RP294)] complex showed ReO^{3+} being coordinated by an N_{amine}, an $S_{thiolate}$, and two N_{amide} atoms in a distorted square pyramidal geometry with the oxo group in the apical position. The crystallographic data were consistent with the NMR spectral data.

A conversion between the *syn* and *anti* isomers of the [ReO-(RP294)] complex was observed in aqueous solution at room temperature. This conversion prevented the isolation of each individual isomer. Rapid conversion between the two isomers at room temperature has not been previously reported for Re and Tc oxo complexes with amino/amido−thiolato ligands. Preliminary data support a conversion mechanism involving the coordination of water in the position trans to the metal−oxo bond. Investigations into the rate and mechanism of the *syn*−*anti* conversion are presently underway. The presence of the conversion in other Re and Tc complexes with similar peptidic chelators is also being investigated.

The dimethylglycine, serine, and cysteine residues of RP294 coordinate to a MO^{3+} core (M: Tc and Re) to form a stable neutral, water-soluble complex. The last glycine residue acts a linker for attaching the peptidic chelator to molecules of interest. RP294 is a candidate for use as a bifunctional chelator for the labeling of peptides, proteins, and small biologically important molecules.[64]

Supporting Information Available: Complete tables of crystallographic data, hydrogen atom parameters, anisotropic thermal parameters, bond lengths, and bond angles for [ReO(RP294)] (4 pages). Ordering information is given on any current masthead page.

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