Re(V)═O(N_2S_2) Complexes with N_2S_2 = Thio-Amido-Secondary Amine-Thio Chelate Ligands: Synthesis, Structure, and Characterization of Solution Forms

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Anti- and *syn*-ReO(L-cysteine-acetyl-cysteamine) (**1** and **2**, respectively), and the dimethyl derivatives *anti*- and *syn*-ReO(L-penicillamine-acetyl-cysteamine) (**3** and **4**, respectively), were synthesized. Anti and syn refer to the relationship of the oxo ligand and carboxyl group of the cysteine/penicillamine residue. In the structures of **1** and **4**, determined by X-ray diffraction, the oxo/carboxyl relationship was anti in **1** and syn in **4**. Such thio-amido-amine-thio (MAMA for monoamido monoamine) type complexes are being investigated as radiopharmaceuticals. The charge and the number of species present under physiological conditions influence biodistribution, and understanding factors influencing the pK_a of the coordinated secondary amine is essential for successful design of such agents. Dissociation of the proton on the secondary amine alters the charge and structure. For both **1** and **2**, the ^{1}H NMR spectra, monitored as a function of pH, exhibited changes in two pH regions: near pH 4 the signals (especially the H_α of the cysteine residue) shifted, and from pH ∼6−8 the signals shifted again and broadened. These shift changes are consistent with dissociation of the carboxyl proton to give a monoanion **I**, and of the amine proton to give a dianion **II**, respectively. The cysteamine chelate ring of **1** and **4**, anchored by the amine donor, is highly puckered in the solid state. Torsion angles, calculated from ^{1}H NMR ^3J values at pH 3, 5 and 8, indicate that in solution the cysteamine chelate ring is also highly puckered in the neutral form and **I**, but relatively planar in **II**. Since five-membered chelate rings are more planar when an anchoring amine is deprotonated, the data are consistent with **II** being an NH-deprotonated form rather than an OH^- axially ligated form. The high acidity of the amine in **1** and **2** compared to analogues with two amine donors indicates that the amido group is a modest donor. The signal broadening observed as **I** and **II** interconvert, an unusual effect for NH/N^- exchange, is due primarily to the low rate of exchange at the OH^- concentration needed to convert **I** to **II**. Making the metal (Re/Tc) more electron rich may decrease the acidity of the NH, but since the NH in **1** and **2** is particularly acidic, this approach is unlikely to work. Our results suggest that introduction of an electron-withdrawing group into the ligand may lower the NH pK_a of the complex below the physiologically relevant range and give a species with a well-defined charge.

Introduction

Quadridentate ligands with N_2S_2 and N_3S donor sets are widely used in radiopharmaceutical chemistry to form ^{99m}Tc and Re chelates. Typical N_2S_2 and N_3S ligands used for chelation of M = Tc and Re are linear and form stable M(V)═O complexes with the N and S donor atoms normally coordinating in the equatorial plane and the oxo ligand in an axial position. The S donors are terminal; two N donors are interior and each anchors two chelate rings upon complexation to the metal. (In N_3S systems the third N is terminal.) The N donors can be amido groups, amines, or a combination of the two donor types. Interior amines can be secondary or tertiary; secondary amines possess one dissociable proton. Thus, secondary amines can have no charge or a mononegative charge, whereas tertiary amine and amido donors are always neutral or mononegative, respectively. Since the charge influences biodistribution, we have been investigating how the nature of the metal ligating groups influences deprotonation.

^{99m}Tc is the preferred radioisotope for nuclear medicine imaging, and since all isotopes of Tc are radioactive, Re derivatives of investigational ^{99m}Tc radiopharmaceuticals are routinely prepared for characterization. Analogous Tc and Re complexes have virtually identical physical properties, permitting correlation of distribution findings obtained using ^{99m}Tc with the structural properties of their Re derivatives. In addition, β-emitting $^{186/188}$Re radiopharmaceuticals are being developed for therapeutic applications.[1,2]

Our interest in the design and development of renal receptor-targeted ^{99m}Tc radiopharmaceuticals led us to study a complex with two secondary amine donors, ReO(LL-ECH_3). The free ligand LL-ECH_6 (ethylene-di-L-cysteine) is shown in Chart 1; the subscripts on H indicate the number of dissociable protons present. The ^{99m}Tc derivative showed renal imaging characteristics superior to those of ^{99m}TcO(N_2S_2)[3] and ^{99m}TcO(N_3S)[4,5] renal agents containing exclusively amido N donors, but the

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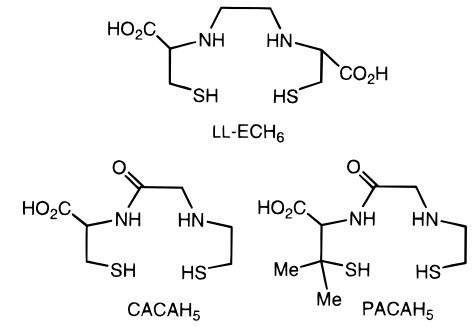
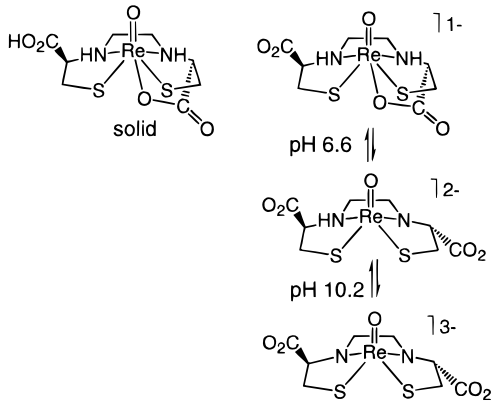
Chart 1



Scheme 1



nature of MO(LL-EC) (M = Tc, Re) complexes in solution was not clear from the structural and spectral data in the literature. Our studies with ReO(LL-ECH_3) and the tetramethyl derivative ReO(DD-$TMECH_3$) revealed that four forms exist for each (Scheme 1).[6] The uncharged form is insoluble in water; the other three species are formed in aqueous solution by successive addition of 3 equiv of hydroxide. In the uncharged form the two amine donors retain the NH group, one carboxyl group is not coordinated but is protonated, and the other carboxyl group is coordinated trans to the oxo ligand and deprotonated. The monoanion shares the same structure but the uncoordinated carboxyl group is deprotonated, giving the complex aqueous solubility. The dianion and trianion are formed by successive deprotonation of the amines. Dissociation of the first NH ("pK_a" 6.6) is coupled to deligation of the CO_2^-. (We place the pK_a value in quotes since it is not a simple pK_a.) The pK_a of the second NH is 10.2.

The principal significance of these findings is that, at physiological pH, MO(LL-ECH_3) species exist as equilibrium mixtures of two forms differing in charge, shape, and ligand denticity. Ideally, receptor-targeted radiopharmaceuticals should exist in a single form since two forms are unlikely to have the same affinity for the receptor site. Hence, we sought a different chelate type in which key structural features of EC could be incorporated but without an amine pK_a near 7.

Subsequently, ^{99m}TcO($CACAH_2$) was reported as a bifunctional chelate system for radiolabeling peptides.[7] The free ligand $CACAH_5$ (cysteine-acetyl-cysteamine) (cysteamine is 2-aminoethanethiol) is shown in Chart 1. This N_2S_2 ^{99m}Tc complex shares features with ^{99m}Tc(LL-ECH_3) but has one amido donor and one amine donor (and only one noncoordinating carboxyl group). The acronym "MAMA" for monoamine monoamide is commonly used in the literature for such N_2S_2 systems. Since the amido donor bears a negative charge and lacks an NH group, ^{99m}TcO($CACAH_2$) has only one dissociable NH, and it was reasonable to expect that dissociation of this NH would occur outside the physiological pH range.[7,8] Thus, we investigated ^{99m}TcO($CACAH_2$) as a dynamic renal imaging agent. Results of biodistribution studies in an animal model were promising.[9] Because characterizing radiopharmaceuticals in the forms that exist near pH 7 helps us to understand their biological behavior, we report here the synthesis, structure, and solution forms of ReO($CACAH_2$) and the new dimethyl derivative ReO(penicillamine-acetyl-cysteamine) [ReO($PACAH_2$)]. The $PACAH_5$ ligand is shown in Chart 1.

Experimental Section

$ReIO_2(PPh_3)_2$ was prepared as described previously.[10] Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA. ^{1}H NMR spectra were recorded on General Electric QE 300, Nicolet NT 360 or Varian 400 spectrometers in D_2O and referenced to internal TSP (3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt) unless specified otherwise.

Syntheses. 2-[[[(*S*-Tritylmercapto)ethyl]amino]acetyl]-*S*-trityl-L-cysteine Ethyl Ester (*S*-Trityl Ethyl Ester of L-Cysteine-acetyl-cysteamine, L-$CACAH_2$). This compound was prepared by a literature procedure[7] with the following modifications: *N*-(bromoacetyl)-*S*-trityl-L-cysteine ethyl ester (1.55 g, 3.0 mmol) and *S*-trityl-2-aminoethanethiol (0.96 g, 3.0 mmol) were dissolved in DMF (25 mL). Sodium carbonate (0.48 g, 4.5 mmol) was added to the solution. The reaction vessel was tightly sealed, and the reaction mixture was stirred for 5 days. Water (100 mL) was added and the precipitate that formed was collected, washed with water, and vacuum-dried. Chromatography over silica (eluent gradient 23−33% hexane/ethyl acetate) gave a pure, foamy product. Yield: 1.17 g (51%).

2-[[[(*S*-Tritylmercapto)ethyl]amino]acetyl]-*S*-trityl-L-Penicillamine Ethyl Ester Dihydrochloride (*S*-Trityl Ethyl Ester of L-Penicillamine-acetyl-cysteamine, L-$PACAH_2$). This new compound was prepared as described for *S*-trityl ethyl ester of L-$CACAH_2$ except that *N*-(bromoacetyl)-*S*-trityl-L-penicillamine ethyl ester (0.70 g, 1.3 mmol) was used in place of the analogous L-cysteine derivative. Yield: 0.69 g (48%). Anal. Calcd for $C_{49}H_{50}N_2O_3S_2 \cdot 2HCl$: C, 69.07; H, 6.15; N, 3.29; Cl, 8.32; S, 7.53. Found: C, 70.25; H, 6.12; N, 3.29; Cl, 8.55; S, 7.56. ^{1}H NMR ($CDCl_3$): 0.94 (s, 3H); 0.97 (s, 3H); 1.22 (t, J = 7 Hz, 3H); 2.37 (t, J = 6 Hz, 2H); 2.52 (t, J = 6 Hz, 2H); 3.10 (s, 2H); 4.07−4.15 (m, 3H); 7.16−7.28 (m 18 H), 7.41 (d, J = 7.2 Hz, 6H); 7.57 (d, J = 7.2 Hz, 6H); 7.91 (d, J = 8.4 Hz, 1H); NH and water signals are overlapping.

ReO(cysteine-acetyl-cysteamine), ReO($CACAH_2$). Et_3SiH (0.16 mL, 1.0 mmol) was added slowly to the *S*-trityl ethyl ester of L-$CACAH_2$ (0.3 g, 0.4 mmol) in CF_3CO_2H (10 mL), and the reaction mixture was stirred for 10 min at room temperature. Hexane (10 mL) and water (10 mL) were added and the resulting mixture was shaken. The aqueous layer was separated from the organic layer and concentrated by rotary evaporation. The residue was dissolved in aqueous NaOH (3 N, 2.5 mL) and the solution stirred for 20 min at room temperature. The pH of the solution was then adjusted to 11 with 1 N HCl. $ReIO_2(PPh_3)_2$ (0.348 g, 0.4 mmol) and MeOH (10 mL) were added to the solution, and the mixture was heated at reflux for 3 h. The pH of the solution was monitored frequently and adjusted to pH 11 with 1 N NaOH. The purple mixture gradually cleared to give an orange-brown solution. The solution was filtered, extracted with $CHCl_3$, and passed through Celite. The filtrate was concentrated by rotary evaporation and acidified to pH 3−4 with 1 N HCl. The two isomers (anti and

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Table 1. Crystallographic Data for *anti*-ReO(CACAH$_2$) (**1**) and *syn*-ReO(PACAH$_2$)·0.67H$_2$O (**4**)

	1	**4**
chemical formula	$C_7H_{11}N_2O_4ReS_2$	$C_9H_{16.33}N_2O_{4.67}ReS_2$
fw	437.50	477.56
T (K)	193 (2)	193 (2)
λ (Å)	0.71073	0.71073
space group	$P2_12_12_1$	$P2_12_12_1$
unit cell dimensions (Å)	$a = 9.172$ (2)	$a = 10.6811$ (2)
	$b = 10.844$ (2)	$b = 10.9709$ (2)
	$c = 11.648$ (2)	$c = 37.5134$ (5)
V (Å^3)	1158.5 (4)	4395.87 (13)
Z	4	12
ρ_{calcd} (mg m^{-3})	2.508	2.165
R indices [$I > 2\sigma(I)$][a]	$R_1 = 0.0147$, $wR_2 = 0.0353$	$R_1 = 0.0248$, $wR_2 = 0.0443$
R indices (all data)[a]	$R_1 = 0.0151$, $wR_2 = 0.0358$	$R_1 = 0.0290$, $wR_2 = 0.0453$

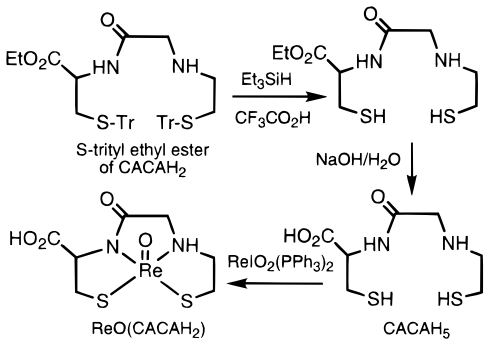
[a] $R_1 = (\sum||F_o| - |F_c||)/\sum|F_o|$; $wR_2 = [\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]]^{1/2}$, where $w = 1/[\sigma^2(F_o^2) + (aP)^2 + bP]$ and $P = [(\text{Max};0,F_o^2) + 2F_c^2]/3$.

syn by order of elution) were separated by reversed-phase HPLC (Beckman Ultrasphere ODS 5 μm 10 × 250 mm, 3% EtOH/0.01 M NaH_2PO_4/pH 6.5). The fractions of each isomer were collected, combined, reduced in volume by rotary evaporation, and then passed down a column of Sephadex G-15 (eluting with deionized water) to remove the phosphate. Later it was observed that the anti and syn isomers had different mobilities on the Sephadex column (syn eluting first) and could be separated without HPLC methodology. Fractions of each isomer obtained by gel filtration were collected, combined, and reduced in volume by rotary evaporation. Solid product, precipitated by slow evaporation of solvent, was collected and vacuum-dried. Yield: anti (**1**), 9% (34 mg), syn (**2**), 9% (31 mg), Anal. Calcd for $C_7H_{11}N_2O_4ReS_2$: C, 19.22; H, 2.53; N, 6.40. Found: anti C, 19.30; H, 2.46; N, 6.30; syn C, 19.31; H, 2.50; N, 6.34.

ReO(penicillamine-acetyl-cysteamine), ReO(PACAH$_2$). This complex was prepared as described for ReO(CACAH$_2$) using the *S*-trityl ethyl ester of L-PACAH$_2$ (0.68 g, 0.88 mmol). The anti and syn isomers were separated by HPLC using a mobile phase of 8% EtOH/0.01 M NaH_2PO_4/pH 6.5. Combined fractions from gel filtration were reduced in volume by rotary evaporation. Slow solvent evaporation yielded crystalline product for both isomers; this was collected and vacuum-dried. Yield: anti (**3**), 12% (54 mg), syn (**4**), 16% (72 mg). Anal. Calcd for *anti*-$C_9H_{15}N_2O_4ReS_2$: C, 23.22; H, 3.23; N, 6.02. Found: C, 23.28; H, 3.21; N, 5.96. Anal. Calcd for *syn*-$C_9H_{15}N_2O_4ReS_2$·0.067H$_2$O: C, 22.89; H, 3.49; N, 5.93. Found: C, 22.95; H 3.24; N, 5.84.

X-ray Crystallography. Brown prisms of *anti*-ReO(CACAH$_2$) (**1**) suitable for X-ray diffraction were obtained by recrystallization from water. Crystals of **1** and **4** with approximate dimensions of 0.18 × 0.16 × 0.12 mm^3 and 0.18 × 0.12 × 0.10 mm^3, respectively, were mounted under Paratone-8277 on a glass fiber and immediately placed in a cold nitrogen stream at −80 °C on the X-ray diffractometer. The X-ray intensity data were collected on a standard Siemens SMART CCD Area Detector System equipped with a normal focus molybdenum-target X-ray tube operated at 2.0 kW (50 kV, 40 mA). For **1**, 1321 frames of data (1.3 hemispheres) were collected using a narrow frame method with scan widths of 0.3° in ω and exposure times of 30 s/frame using a detector-to-crystal distance of 5.09 cm (maximum 2θ angle of 56.6°). The total data collection time was approximately 12 h. Frames were integrated to a maximum 2θ angle of 56.6° with the Siemens SAINT program to yield a total of 7454 reflections, of which 2737 were independent (R_{int} = 2.38%, R_{sig} = 3.55%) and 2689 were above 2σ(I). Laue symmetry revealed an orthorhombic crystal system, and the final unit cell parameters (at −80 °C) were determined from the least-squares refinement of three-dimensional centroids of 7230 reflections. Data were corrected for absorption with the SADABS program based on the method of Blessing.[11] For **4**, 1321 frames of data (1.3 hemispheres) were collected using a narrow frame method with scan widths of 0.3° in ω and exposure times of 60 s/frame using a detector-to-crystal distance of 5.09 cm (maximum 2θ angle of 56.6°). The total data collection time was approximately 25 h. Frames were integrated to a maximum 2θ angle of 56.6° with the Siemens SAINT program to yield a total of 28 049 reflections, of which 10 290 were independent (R_{int} = 3.49%, R_{sig} = 4.80%) and 9617 were above 2σ(I). Laue symmetry revealed an orthorhombic crystal system, and the final unit cell parameters (at −80 °C) were determined from the least-squares refinement of three-dimensional centroids of 8192 reflections. Data were corrected for absorption with the SADABS program.

Scheme 2



The structures were solved by direct methods for **1** and Patterson methods for **4**, and refined by full-matrix least-squares procedures on F^2 using SHELXL 93. All non-hydrogen atoms were refined anisotropically. The amine and carboxyl H-atoms of **1**, and the amine, carboxyl, and water H-atoms of **4**, except for those bound to N5, O7 (carboxyl), and O14B (water), were located from difference maps. All other H atoms were generated at calculated positions (d(C−H) = 0.96 Å, d(N−H) = 0.90 Å, d(O−H) = 0.85 Å). The H-atoms were constrained using a riding model with isotropic thermal parameters that were 20% greater than the U(eq) of the bonded heavy atom. Crystal data and refinement parameters for **1** and **4** are presented in Table 1.

Results

Synthesis. The CACA/PACA ligands were characterized in their *S*-trityl ethyl ester forms (Scheme 2, only CACA shown for clarity). The ligands were treated with Et_3SiH to remove the trityl groups and then with base to hydrolyze the ester. The deprotected acid (not isolated) was then treated with $ReIO_2(PPh_3)_2$ to give the ReO(CACAH$_2$) and ReO(PACAH$_2$). For each complex, two isomers were obtained since the carboxyl group can be syn or anti to the oxo ligand; both isomers persisted at high pH (>12). In contrast, under such conditions the anti isomers of the closely related complexes, MO(DL-ECH$_3$) and MO(MAECH$_2$) (M = Re, ^{99m}Tc; MAECH$_5$ = mercapto-acetamide-ethylene-cysteine) convert to the syn isomers.[12,13] The

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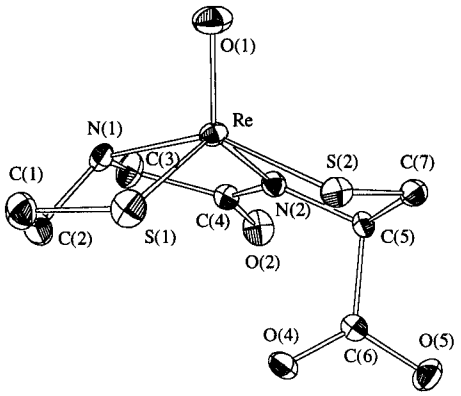

Figure 1. Perspective drawing of *anti*-ReO($CACAH_2$) (**1**) with 50% probability for the thermal ellipsoids.

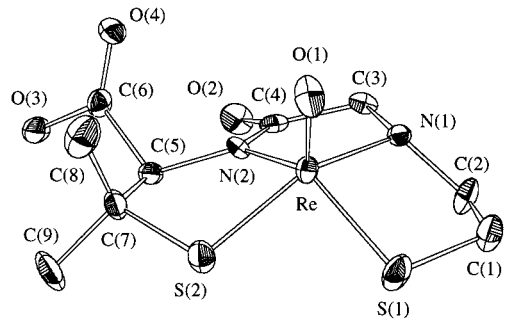

Figure 2. Perspective drawing of *syn*-ReO($PACAH_2$)·0.67H_2O (**4**) with 50% probability for the thermal ellipsoids.

Table 2. Selected Bond Distances (Å) and Angles (deg) for *anti*-ReO(CACA) (**1**) and Molecule 1 in the Asymmetric Unit of *syn*-ReO(PACA)·0.67H_2O (**4**)

	1	**4**
	Bond Distances (Å)	
Re(1)−O(1)	1.691(3)	1.675(4)
Re(1)−N(1)	2.142(3)	2.134(4)
Re(1)−N(2)	2.002(3)	2.001(4)
Re(1)−S(1)	2.2727(11)	2.2704(14)
Re(1)−S(2)	2.2831(10)	2.2687(14)
	Bond Angles (deg)	
O(1)−Re(1)−N(2)	116.9(2)	116.9(2)
O(1)−Re(1)−N(1)	102.51(14)	104.8(2)
N(2)−Re(1)−N(1)	78.77(12)	79.1(2)
O(1)−Re(1)−S(1)	113.77(14)	111.74(14)
N(2)−Re(1)−S(1)	128.73(9)	130.93(13)
N(1)−Re(1)−S(1)	83.52(9)	82.76(11)
O(1)−Re(1)−S(2)	109.19(12)	109.18(13)
N(2)−Re(1)−S(2)	82.82(9)	81.30(12)
N(1)−Re(1)−S(2)	147.95(9)	145.63(11)
S(1)−Re(1)−S(2)	87.92(4)	89.24(5)

syn and anti isomers of ReO($CACAH_2$) and ReO($PACAH_2$) were separated chromatographically.

X-ray Crystallography. The asymmetric unit consisted of one complex molecule for **1** and three independent complex molecules plus two water molecules for **4** ($Z = 12$). No significant differences were found between like molecules of **4**. Perspective drawings of **1** and molecule 1 in the asymmetric unit of **4** appear in Figures 1 and 2, respectively; selected bond distances and angles are listed in Table 2. Both **1** and **4** are typical of Re(V) monoxo complexes; i.e., they have square-pyramidal coordination geometry with the CACA/PACA ligating atoms in the basal plane and the oxo ligand at the apex. All Re−heteroatom bond distances are normal.[14]

The relationship of the oxo and CO_2H groups is anti in **1** and syn in **4**; otherwise, there are no important differences between the two complexes. A fit of the common atoms of **1** and molecule 1 from the asymmetric unit of **4** gave a weighted root-mean-square deviation of 0.12 Å. The largest deviation, 0.26 Å, was found for C(5) (or C_α). In **1** the carboxyl group is not bound to the metal, but the O(4) is tucked under the CACA ligand with a Re−O(4) distance of 3.83 Å. This feature is likely due to attractive electrostatic forces between O(4) and Re. In contrast, the carboxyl group of **4** extends away from the inner coordination sphere, probably due to repulsive electrostatic and steric forces between the carboxyl group and the oxo ligand and the geminal methyl group, respectively. In **1** the cysteamine chelate ring is highly puckered, and the anchoring N(1) (having a *syn*-NH) is positioned 0.58 Å above the basal plane defined by S(1), S(2), and N(2) donor atoms. The Re atom is displaced 0.90 Å above this same plane. A puckered cysteamine chelate ring is also found in **4**, but the displacements of N(1) and Re are less pronounced (0.24−0.44 Å for N(1) and 0.80−0.87 Å for Re).

NMR Spectroscopy. The ^1H NMR spectra of **3** and **4** in aqueous solution at pH ∼12 consisted of signals from three isolated spin systems: the penicillamine (pen) residue (three singlets, 1:3:3 ratio); the acetyl residue (two strongly coupled doublets); the cysteamine residue (four multiplets). For **1** and **2** the spectra are more complicated because each signal integrates to one proton and is spin coupled; however, some signals have chemical shift and coupling patterns virtually identical to those of the acetyl and cysteamine residues of **3** and **4**, respectively (Table 3, pH ∼12). This information allowed us to assign signals in the spectra of **1** and **2** to the acetyl and cysteamine residues. The remaining signals were assigned to the cysteine residue. With each signal assigned to the appropriate residue, specific assignments were made by analysis of coupling constants and simulation of the spectra. Substituents near (endo) and away (exo) from the oxo group are indicated by prime and double prime, respectively. Chart 2 shows the labeling of the hydrogens. For the H_3 and Me protons (in **3** and **4**) we assumed that the endo protons are deshielded,[15] with signals appearing downfield from the signals of their vicinal exo partners. Note that the endo H_α signals in **1** and **3** are downfield of the exo H_α signals of **2** and **4**, respectively (Table 3).

The ^1H NMR spectrum of each ReO($CACAH_2$) isomer (**1** and **2**) was monitored as the pH was lowered (Figures 3 and 4, respectively). No spectral changes were observed from pH 12 to 8. Below pH 8, signals shifted and broadened. Large shifts (Figures 3 and 4) were observed for the $H_{2'/2''}$ signals (Chart 2). The H_α and $H_{\beta''}$ signals also shifted significantly (Table 3). The signals that shifted the most showed the greatest degree of broadening. From pH 6 to 5 the chemical shift changes ceased and the signals sharpened. Below pH 5 the signals remained sharp, but the H_α signals shifted noticeably, and minor shifts were observed for the other signals.

Spectra of **1** and **2** were also obtained in DMSO-d_6. Assignments were made by comparison with the respective D_2O spectra (pH ∼3, Table 3) and analysis of the coupling constants. For both complexes, the NH signals appeared as singlets (∼9 ppm). The $H_{2''}$ signals were broad, and couplings to $H_{2'}$, $H_{1'}$, and $H_{1''}$ were unresolved. These results are consistent with strong coupling between the NH signal and the $H_{2''}$ signal. Since the NH−N−C−$H_{2''}$ torsion angles in the solid were 176° (anti, **1**)

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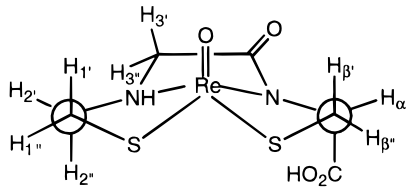
Table 3. ^{1}H NMR Chemical Shifts and Assignments for syn and anti Isomers of ReO(CACA) and ReO(PACA) in Neutral, Monoanionic, and Dianionic Forms

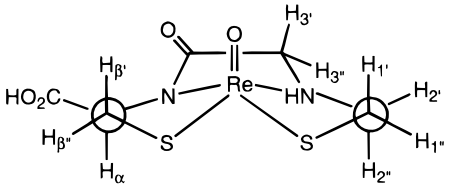
	cys/pen			C_1		C_2		C_3			
solvent, pH	H_α	$H_{\beta'}$/Me′	$H_{\beta''}$/Me″	$H_{1'}$	$H_{1''}$	$H_{2'}$	$H_{2''}$	$H_{3'}$	$H_{3''}$	CO_2H	NH
				anti-ReO(CACA) (**1**)							
D_2O, pH 3.9	5.14	3.59	4.35	3.10	3.36	3.51	1.95	*a*	4.21		
D_2O, pH 4.9	5.11	3.58	4.34	3.11	3.36	3.53	1.96	4.90	4.22		
D_2O, pH 12.4	4.65	3.48	3.97	2.69	3.32	3.98	2.99	5.03	4.45		
DMSO-d_6	5.08	*a*	4.12	2.92	3.14	*a*	1.52	4.69	4.17	12.50	9.14
				anti-ReO(PACA) (**3**)							
D_2O, pH 3.7	4.68	1.87	1.57	3.10	3.34	3.53	*b*	4.90	4.09		
D_2O, pH 5.2	4.60	1.86	1.54	3.07	3.38	3.51	2.01	4.86	4.10		
D_2O, pH 12.0	4.20	1.74	1.55	2.69	3.32	3.95	2.93	5.01	4.34		
				syn-ReO(CACA) (**2**)							
D_2O, pH 3.1	4.35	3.34	4.28	3.05	3.40	3.54	1.94	4.94	3.99		
D_2O, pH 4.5	4.20	3.29	4.20	2.99	3.38	3.55	1.96	4.95	3.95		
D_2O, pH 12.3	4.54	3.26	3.38	2.61	3.29	3.97	2.90	5.14	4.17		
DMSO-d_6	4.09	3.08	3.34	2.83	3.22	3.34	1.82	4.56	3.74	12.50	9.25
				syn-ReO(PACA) (**4**)							
D_2O, pH 3.7	4.04	1.96	1.37	3.04	3.39	3.57	*b*	4.95	3.84		
D_2O, pH 5.1	3.97	1.95	1.37	3.01	3.37	3.59	*b*	4.97	3.82		
D_2O, pH 12.1	3.93	1.81	1.41	2.68	3.26	3.99	2.90	5.19	4.05		

[a] Signal overlaps with the water peak. [b] Signal overlaps with Me′ peak.

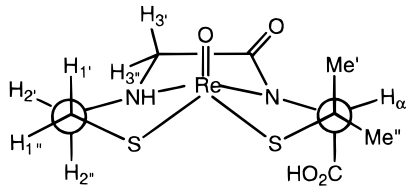
Chart 2



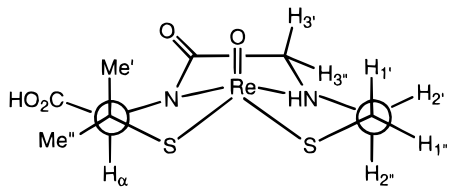
anti-ReO(CACAH$_2$) (**1**)



syn-ReO(CACAH$_2$) (**2**)



anti-ReO(PACAH$_2$) (**3**)



syn-ReO(PACAH$_2$) (**4**)

and 154°(syn, **4**), strong NH/$H_{2''}$ coupling can be expected. Conversely, the NH−N−C−$H_{2'}$ torsion angles were 65° (anti, **1**) and 35° (syn, **4**) in the solid; thus, NH/$H_{2'}$ coupling should be relatively weak and the $H_{2'}$ NMR signals sharp. The $H_{2'}$ signal of **1** overlapped the water peak and was completely obscured; that of **2** overlapped the water peak partially, but a relatively sharp doublet of doublets was discernible.

Discussion

The NMR data for **1** and **2** indicate that for each isomer there are three forms: neutral, monoanionic (**I**), and dianionic (**II**) (Scheme 3). The uncharged form is modestly soluble in water and contains two dissociable protons, CO_2H and NH (as observed in the solid state of **1** and **4**). Conversion of the uncharged form to **I** occurs in a pH range consistent with the pK_a of a carboxylic acid (near 4) and is accompanied by shifts in the ^{1}H NMR signals, especially in the H_α signal. Thus, we expect **I** to have an ionized carboxylate group. The large shifts observed for the $H_{2'}$ and $H_{2''}$ signals of the cysteamine ring as **I** converts to **II** suggest a change in protonation state of the coordinated amine [∼6.0 (**1**); ∼6.6 (**2**); these pK_a values correspond to the pH at which the $H_{2''}$ signals were at the midpoint between the $H_{2''}$ signal of **I** (at pH ∼5) and **II** at (pH ∼12)]. However, the signals of the cysteine residue (far removed from the amine) also shifted significantly as **I** converted to **II**. Thus, it is possible that instead **II** has coordinated hydroxide; consumption of base via coordination or via NH deprotonation can give the same pH profile.

To decide between these two possible roles for hydroxide, we first consider the data in more detail to determine if NH deprotonation is reasonable. The X-ray structures of **1** and **4** show that the cysteamine chelate ring is highly puckered. Also, the anchoring amine N(1) is significantly displaced from the basal plane defined by S(1), S(2), and N(2). These two distinct features are probably due to the combination of one flexible chelate ring with two relatively rigid rings which are anchored by the planar amido donor. A Karplus type equation[16] was used to calculate torsion angles of **1** and **2** in solution, from the

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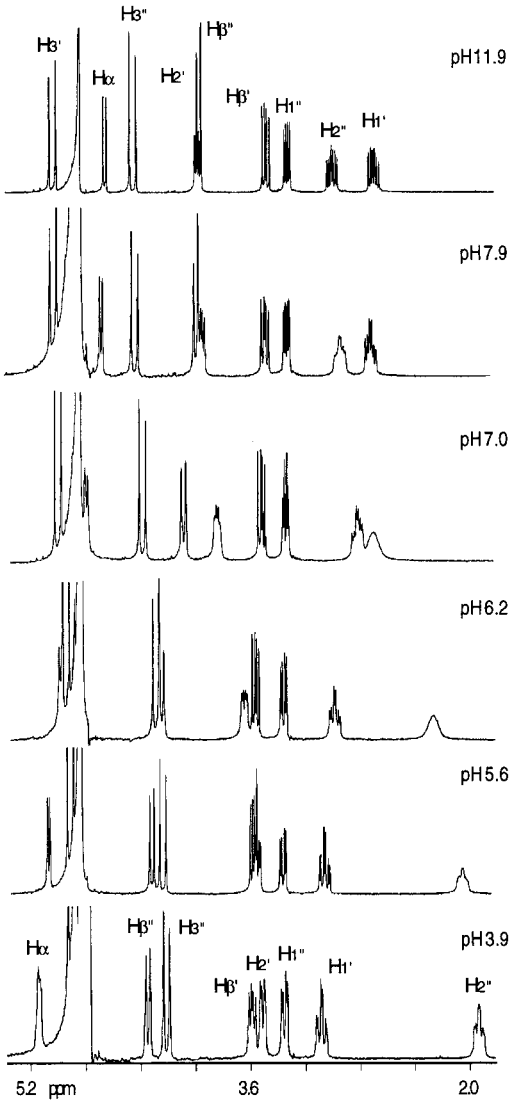


Figure 3. ^{1}H NMR spectra of *anti*-ReO(CACA) (**1**) in D_2O at various pH values.

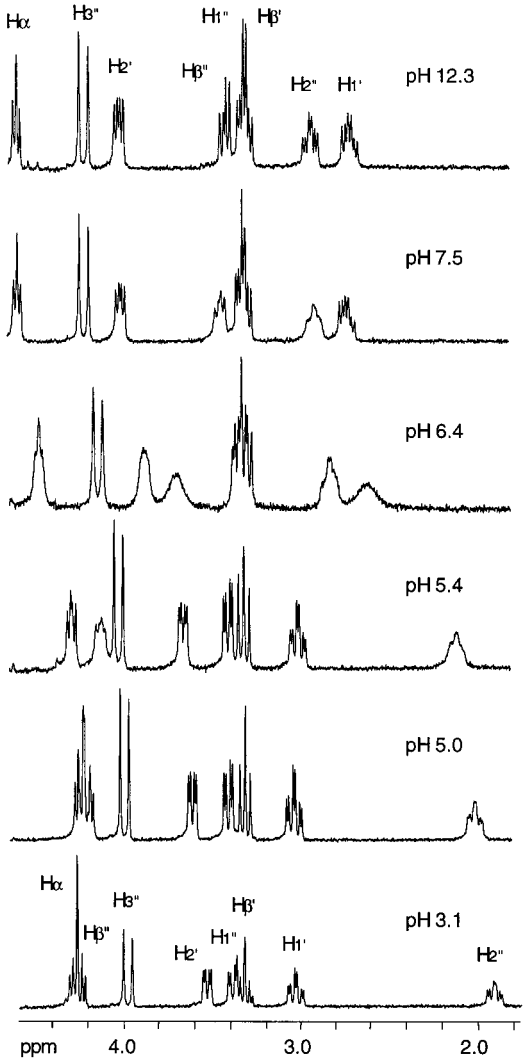
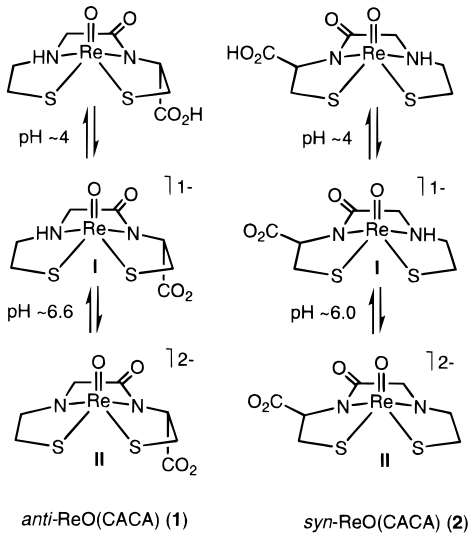


Figure 4. ^{1}H NMR spectra of *syn*-ReO(CACA) (**2**) in D_2O at various pH values.

coupling constants of their respective ^{1}H NMR signals. The calculated torsion angles were compared with those found in the solid forms (Table 4). In solution at pH 5 and below (where the uncharged form and **I** predominate), the cysteamine signals of **1** and **2** have coupling constants that give calculated torsion angles correlating well with those found in the solid of **1** and **4**, respectively. The only large changes observed on deprotonation of the carboxyl group (pH 3−5) were the couplings for the cysteine signals of **1** (Table 5). With increasing pH (5−8) the coupling constants of the cysteamine signals of **1** and **2** increased, indicating a change in the conformation of the chelate ring as **I** converts to **II**. The larger coupling constants give smaller calculated torsion angles. Models show that smaller torsion angles are consistent with a more planar, less puckered ring. Since anchoring amines are more planar when deprotonated than when protonated (and pseudotetrahedral), and five-membered chelate rings anchored by a deprotonated amine are usually nearly planar,[6,17,18] the data are consistent with **II** having an ionized amine donor.

Scheme 3



We next consider whether a form of **II** with coordinated hydroxide agrees with the data. This form would be six-

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Table 4. Correlation of Coupling Constants and Torsion Angles (Solid State) of *anti*-ReO(CACA) (**1**) and *syn*-ReO(PACA) (**4**)

	^3J (Hz)		torsion angle (deg)[a]	
	NMR	X-ray[b]	NMR[c]	X-ray
	anti-ReO(CACA) (**1**) (D_2O, pH 3.9)			
$H_\alpha/H_{\beta'}$	0	1.7	90	78
$H_\alpha/H_{\beta''}$	6.0	5.9	40	41
$H_{1'}/H_{2'}$	4.0	4.0	54	55
$H_{1'}/H_{2''}$	12.4	12.3	180[d]	174
$H_{1''}/H_{2'}$	0	2.7	90	65
$H_{1''}/H_{2''}$	4.0	4.0	54	54
	syn-ReO(PACA) (**4**) (D_2O, pH 3.7)			
$H_{1'}/H_{2'}$	4.0	4.0	54	54
$H_{1'}/H_{2''}$	12.8	12.3	180[d]	174
$H_{1''}/H_{2'}$	0	2.7	90	65
$H_{1''}/H_{2''}$	4.0	4.0	54	54

[a] Absolute value. [b] Coupling constants calculated from the equation $^3J = 11.0 \cos^2 \Phi - 1.4 \cos \Phi + 1.6 \sin^2 \Phi$, using a torsion angle Φ obtained from the X-ray structure. [c] NMR torsion angles were calculated from the above quadratic equation by using the observed coupling constants. [d] $^3J > 12.4$ Hz (the ^3J maxima of the equation at $\Phi = \pm 180°$).

Table 5. Comparison of Vicinal Coupling Constants (Hz) and Calculated Torsion Angles[a] (deg) for ReO(CACA) Complexes as a Function of pH

	$\alpha\beta'$		H1′H2′		H1″H2″	
	^3J	angle	^3J	angle	^3J	angle
			1			
pH 3.9	6.0	40	4.0	54	4.0	54
pH 5.6	7.2	33	4.4	51	4.0	54
pH 8.6	8.4	23	6.8	35	5.4	45
pH 11.9	8.4	23	6.6	37	5.6	44
			2			
pH 3.1	9.8	142	4.0	54	4.6	50
pH 5.0	10.4	153	4.3	52	4.9	48
pH 7.5	7.3	135	6.1	40	5.5	44
pH 12.3	7.3	135	6.1	40	5.5	44

[a] Torsion angles were calculated from the equation $^3J = 11.0 \cos^2 \Phi - 1.4 \cos \Phi + 1.6 \sin^2 \Phi$, using observed ^3J values.

coordinate and octahedral (or pseudooctahedral). In six-coordinate Re and Tc monoxo complexes, the metal atom is modestly displaced from the equatorial plane toward the oxo ligand (~0.4 Å) and the $O_{oxo}-Re-X$ (X = equatorial donor atom) bond angles fall in the 89−109° range.[6,19,20] In contrast, in five-coordinate Re and Tc monoxo complexes (including the solid forms of **1** and **4**), the displacement of the metal from the basal plane is greater (~0.7−0.9 Å) and the $O_{oxo}-Re-X$ bond angles are obtuse (109−117°),[6,14,21] creating steric crowding at the vacant axial coordination site. Thus, if **I** converted to a six-coordinate form of **II**, we expect that in order to accommodate an axial OH^-, the Re atom would drop toward the equatorial plane and the O(1)−Re−X bond angles would decrease. However, this structural rearrangement is unlikely to be favorable since the coordinated amine, remaining protonated and tetrahedral, would impede the rearrangement of atoms necessary for the Re−OH bond to form. Furthermore, if **II** had a hydroxide coordinated, we would expect proton exchange from the hydroxo ligand to oxo ligand and equilibration of the oxo and hydroxo sites. Such exchange is facile in $ReO(OH)(penH)_2$ complexes.[22,23] However, oxo/hydroxo exchange would interconvert **1** and **2**, and form **II** of **1** and **2** would have identical ^1H NMR spectra. Also, **II** would have two dissociable protons (NH and OH) and we would expect at least one of these protons to dissociate as the pH was raised further.[22,23] No changes were observed in the NMR spectra of **1** and **2** from pH 8 to 12. Thus, we conclude that **II** is the NH-deprotonated form and not the hydroxide-coordinated form.

Thio-amido-amine-thio ligands were originally designed to obtain neutral M(V)=O complexes;[14,24,25] the amine donors were tertiary, having no dissociable proton, and thus the coordinated ligands were trianionic; i.e., one amido and two thio groups were formed upon complexation. In recent investigations of such ligands with secondary amine donors, characterization of their Tc/Re complexes has been limited.[7,8] By analogy to the original ligands, the ligands containing secondary amines are described as trianionic, with the assumption that the amine donor remains protonated upon metal complexation (at least around neutral pH).[7,8] Our results show that in this type of complex a secondary amine can be predominantly ionized near neutral pH and the MAMA-type ligands can be tetraanionic.

As **I** and **II** interconverted, only one set of ^1H NMR signals was observed; all the signals shifted, but the most shifted signals also broadened. These spectral features indicate that **I/II** exchange occurs at a rate below the fast-exchange NMR limit (where exchange affects the band shape, which is a function of $(\delta\nu)^2$).[26] Signal broadening is an unusual effect for NH/N^- exchange. (Although the NMR spectra were recorded in D_2O, the interpretations apply to H_2O and we use protic species in the discussion for simplicity.) Coordinated amines are normally weakly acidic, and thus the concentration of OH^- is high when detectable concentrations of the dissociated form ordinarily exist. In contrast, free amines exist at lower pHs, and the pK_a's of protonated amines are ordinarily lower than for coordinated amines.[27] Since the rate law for NH/N^- dissociation is usually second order, depending on the concentrations of both the amine and hydroxide, the observed rate is generally very fast for free amines[28] and slow for coordinated amines.[27] Thus, NH/N^- exchange rates and the NMR time domain do not generally coincide. For **1** and **2**, two factors contribute to the observed line broadening. The principal factor is that NH dissociation occurs in a pH range of low OH^- concentration; hence the NH/N^- exchange rate is below the NMR limit for fast exchange. A secondary factor is that some signals undergo large changes in chemical shift (up to 1 ppm for $H_{2''}$) as **I** converts to **II** (or vice versa). At the pH corresponding to the NH pK_a of **1** and **2**, broadening of 2 Hz (minimum for loss of coupling resolution) for the signal with the largest $\delta\nu$ ($H_{2''}$) places an upper limit of 7×10^{-6} s on the half-life of **I** and **II**. (Since the line width of

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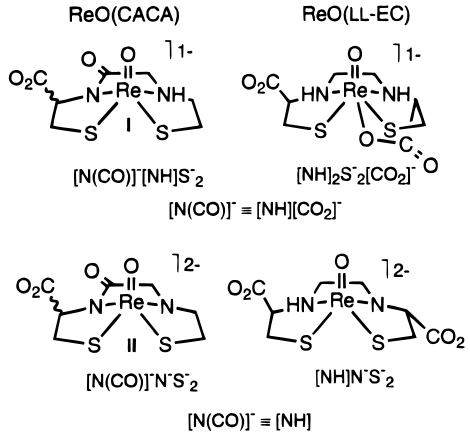
(28) Eigen, M.; DeMaeyer, L. In *Techniques of Organic Chemistry, Part II*; Friess, S. L., Lewis, E. S., Weissburger, A., Eds.; Interscience: New York, 1963; Vol. VIII.

Chart 3



the uncoupled signals can be determined simply and accurately, work is in progress to replace $H_{2'}$ and $H_{2''}$ with methyl groups.) This exceptional result for a metal complex is due primarily to the high acidity of the NH. The exchange occurs at a rate between the very fast rate of free amines and the ordinarily slow rate of coordinated amines.

We used the NH acidities of ReO(CACAH$_2$) (**1** and **2**) and ReO(LL-ECH$_3$)[6] to evaluate the ability of the different N-donor types to meet the electronic requirements of the metal in Re(V)═O(N_2S_2) complexes. We compared the donor sets of the NH forms (**I**) of **1** and **2**, and [ReO(LL-ECH$_2$)]$^-$ (Chart 3) and the respective pK_a values. The donor sets are [N(CO)]$^-$[NH]S$^-_2$ for [ReO(CACAH)]$^-$ and [NH]$_2$S$^-_2$[CO$_2$]$^-$ for [ReO(LL-ECH$_2$)]$^-$. The pK_a values are ∼6.6 for *anti*-[ReO(CACAH)]$^-$ (**1**), ∼6.0 for *syn*-[ReO(CACAH)]$^-$ (**2**), and 6.6 for [ReO(LL-ECH$_2$)]$^-$. The similar pK_a values indicate roughly equivalent donor abilities between donor sets. Focusing on the differences between the donor groups in the CACA and EC system, we see that the electron density provided to the Re by the amido group of [ReO(CACAH)]$^-$ approximates that provided by the neutral amine plus the carboxylate group in [ReO(LL-ECH$_2$)]$^-$. The ligating CO_2^- probably increases the NH pK_a value of [ReO(LL-ECH$_2$)]$^-$, since the CO_2^- provides some electron density to the metal. However, considering that the CO_2^- is weakly bound to Re (the Re−O bond is long (2.252 (9) Å) in the solid),[6] the amido group in [ReO(CACA)]$^-$ is at best only a slightly better donor than a neutral amine in [ReO(LL-ECH$_2$)]$^-$. Clearly, the amido group is a modest donor since in **1** and **2** it is unable to prevent dissociation of the amine NH except under acidic conditions.

The N$^-$ forms (**II**) of **1** and **2**, and [ReO(LL-ECH)]$^{2-}$ (Chart 3) exist over a wide pH range. [ReO(CACA)]$^{2-}$, lacking a dissociable proton, persists at pH values up to 12. The NH pK_a of [ReO(LL-ECH)]$^{2-}$ (10.2) is only slightly lower than the NH pK_a of cysteine ($^+$NH$_3$−CH(CH$_2$S$^-$)−CO$_2^-$, 10.8), suggesting that the amine nitrogen atom of EC donates roughly the same amount of electron density to the metal as the N of cysteine donates to a proton. Thus, the electronic requirements of the [Re(V)═O]$^{3+}$ center are largely met by one secondary amine, one deprotonated amine, and two thiolates in the N_2S_2 donor set. However, the NH pK_a (10.2) of [ReO(LL-ECH)]$^{2-}$ is much lower than for Co(III) amine complexes (pK_a > 14), and thus the [Re(V)═O]$^{3+}$ center is still more electron deficient than Co(III). In summary, we rank N donor abilities to a [Re(V)═O]$^{3+}$ center as follows: deprotonated amine ≫ amido ≥ protonated amine.

Conclusion

One of our goals is to design a small ^{99m}Tc chelate that exists as a single form at physiological pH and is rapidly excreted into the urine by active renal transport. Cysteine is an excellent ligand precursor because it provides both a CO_2^- group (considered important for recognition by the renal transport receptor)[29] and N and S donor groups for bonding to the metal. ^{99m}TcO(LL-EC) is an example of a N_2S_2 complex derived from cysteine that has characteristics approaching our design goal. However, this compound exists as more than one form near pH 7 because of an acidic secondary amine (pK_a 6.6). A complicating factor is that the complex has a coordinating CO_2^-, and deprotonation of the NH results in CO_2^- deligation. In our model ReO(CACA) complexes, the cysteine N is an amido donor, precluding CO_2^- coordination. The absence of CO_2^- coordination should modestly lower the NH pK_a of [ReO(CACAH)]$^-$ compared to [ReO(LL-ECH$_2$)]$^-$. Compared to the latter species, the NH pK_a of *syn*-[ReO(CACAH)]$^-$ (**2**) is lower (∼6.0) but that of *anti*-[ReO(CACAH)]$^-$ is essentially the same (∼6.6). Thus the lack of CO_2^- coordination does not guarantee an NH pK_a outside the physiologically relevant range. Replacing the NH with an N-alkyl group would eliminate the NH pK_a of [ReO(CACAH)]$^-$; however, alkyl groups generally have an undesirable effect on biodistribution, and additional isomers are possible since coordinated tertiary amines are chiral.[30] Another approach toward ensuring a single form at physiological pH is to make the metal (Re/Tc) more electron rich, and thus decrease the acidity of the NH. However, our work shows that the amine is particularly acidic, and such an approach is unlikely to prove successful since [Re(V)═O]$^{3+}$, and undoubtedly [Tc(V)═O]$^{3+}$, are still more electron deficient than a typical metal even when good donors are present. We rank N donor abilities to a [Re(V)═O]$^{3+}$ center as follows: deprotonated amine ≫ amido ≥ protonated amine. Thus, a thio-amido-secondary amine-thio ligand is probably a poor choice to provide a trianionic ligand if a neutral Re/Tc(V)═O coordination sphere is desired. Alternatively, the opposing approach is likely to work: introduction of an electron-withdrawing group into the ligand to increase the acidity of the NH and lower the pK_a of the complex below the physiologically relevant range. This approach would lead to complexes that exist as a single species with an anionic coordination sphere in vivo. The complex would not have a chiral alkylamine influencing biodistribution.

Acknowledgment. This work was supported by the National Institutes of Health (Grant No. DK38842). We thank Dr. Rene Lachicotte of the University of Rochester for collection of the X-ray data, and the NIH and NSF for supporting the purchase of instruments.

Supporting Information Available: Crystallographic data for **1** and **4** including tables of positional parameters, bond distances and angles, anisotropic displacement coefficients, and H-atom coordinates (12 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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