Factors Influencing the pK_a of Ligated Amines and the Syn/Anti Isomerization in Cysteine-Based Re(V)═O(N_2S_2) Radiopharmaceutical Analogues As Revealed by a Novel Dominant Tautomer in the Solid State

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Efficient radiopharmaceutical design demands an understanding of factors that lead to one isomeric species in one ionization state at physiological pH. Thus, all pK_a values must be outside the range of 6−9 for the typical M(V)O(N_2S_2) (M = ^{99m}Tc, $^{186/188}$Re) agents. The pendant carboxyl group needed for rapid clearance of renal agents in particular must be either only syn or only anti to the oxo ligand with respect to the N_2S_2 ligand plane. Monoamide-monoamine-dithiol (monoamide-monoamine = MAMA) ligands useful in preparing radiopharmaceuticals typically form M(V)O(N_2S_2) complexes with one core ligand pK_a of ∼6−7 (secondary amine) and with both syn and anti isomers. We designed a new MAMA ligand, mercaptoacetamide-ethylene-cysteine ($MAECH_5$), with the electron-withdrawing carboxyl group separated by only two bonds from the NH group. Only *syn*-ReO($MAECH_2$) was isolated. The structure of the monoanion *syn*-[ReO(MAECH)]$^-$ in the crystal of a $[AsPh_4]^+$ salt reveals lattice H-bonding between the CO_2H of a tautomer (**t$_2$**) with a CO_2H and an amine N^- and the C═O of a neighboring **t$_2$** anion; this interaction results in preferential crystallization of **t$_2$**. However, in aqueous solutions of *syn*-[ReO(MAECH)]$^-$, the predominant monoanionic tautomer (**t$_1$**) has a CO_2^- and an amine NH, as indicated by ^{1}H NMR and resonance Raman spectra. The *endo*-NH configuration favored in M(V)O(N_2S_2) complexes places the NH and CO_2^- groups in **t$_1$** spatially close. The NH is less acidic due to the cancellation of the electron-withdrawing and electrostatic effects of the negative CO_2^-; as a result, *syn*-[ReO(MAECH)]$^-$ has a pK_a value (6.0 ± 0.1) similar to that of the regioisomer *syn*-[ReO(CACAH)]$^-$ in which the carboxyl group and the NH are not close ($CACAH_5$ = cysteine-acetyl-cysteamine). Our results suggest that the carboxyl group position also influences the syn/anti equilibrium. Attachment of the carboxyl group to a puckered ring in *syn*-[ReO(MAECH)]$^-$ appears both to favor the syn isomer and to increase the rate of syn/anti isomerization. ReO($CACAH_2$), with a carboxyl group attached to a less puckered chelate ring anchored by the amido donor, formed as a noninterconverting roughly equal mixture of syn/anti isomers. Thus, for a MAMA ligand to form a syn isomer with a pK_a < 6, it must be designed with a nonionizable electron-withdrawing group near the NH group and a pendant carboxyl on a puckered ring.

Introduction

Tc(V)O and Re(V)O complexes with quadridentate ligands are often used as radiopharmaceuticals. Such ligands usually lack C_2 symmetry, and even if they are achiral, they wrap around the metal in two directions, leading to chiral complexes. In many cases, the ligands contain chiral centers, and thus, even if the starting ligand is resolved or is prepared from resolved material, two isomers are possible. We are interested in developing procedures for preparing radiopharmaceuticals containing only one isomer and existing as only one form under physiological conditions. Such agents must be prepared directly without the need for separation if they are to prove clinically useful. We also are interested in renal radiopharmaceuticals that require a pendant carboxyl group; this group is often attached via a chiral carbon. The potential coordination of this group to the metal complicates the chemistry, and we are exploring approaches to avoid coordination. Such pendant groups also offer a convenient site on which to append other molecules designed to target specific tissues or receptors. Thus, they are of interest beyond the area of renal agents.[1−3] The chemistry of M(V)O(N_2S_2) and M(V)O(N_3S) (M = Tc, Re) complexes is further complicated when one or more donors are secondary amines. Upon coordination, the secondary amines can be neutral (NH) or charged (N^-), and the NH pK_a can be near physiological pH, resulting in the presence of multiple species in vivo.

When the pendant carboxyl group is linked to a chiral carbon in a chelate ring, the isomers arising from the two directions in which the ligand can wrap are named syn and anti, since one has the carboxyl group close to the oxo group and the other has the carboxyl group away from the oxo group. Typically the reaction of a resolved ligand with an appropriate metal precursor leads to a mixture of syn and anti isomers. However,

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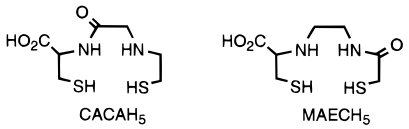
Chart 1



various factors influence the kinetically determined syn/anti ratio during the formation process, the syn/anti ratio at equilibrium, and the kinetics of syn/anti equilibration. At present these factors are not understood. Since the charge, the number of species present, the ligand denticity, and the stereochemistry of M(V)-O(N_2S_2) and M(V)O(N_3S) radiopharmaceuticals influence not only the syn/anti ratio but biodistribution as well, an understanding of the factors that control NH deprotonation, carboxyl coordination, and isomer distribution is essential for designing useful new agents.

Contemporary designs of MO(N_2S_2) complexes have a combination of amido and amine donors and are commonly referred to as MAMA (monoamide-monoamine) type complexes. ReO($CACAH_2$) ($CACAH_5$ is cysteine-acetyl-cysteamine) (Chart 1) is an example of a MAMA complex containing a secondary amine and carboxyl group. In ReO($CACAH_2$) the chelate ring bearing the carboxyl group is anchored by the amido donor, thus precluding carboxyl coordination. Nevertheless, the amine donor is highly acidic (pK_a (syn) 6.0, (anti) 6.6) because the amido group is a relatively weak donor and does not meet the need of the $[Re(V)=O]^{3+}$ center for electron donation.[4] Although at physiological pH *syn*-$[ReO(CACA)]^{2-}$ is the predominant form, the anti isomer exists as an equilibrium mixture of the monoanion, *anti*-$[ReO(CACAH)]^{-}$ (16%), and dianion, *anti*-$[ReO(CACA)]^{2-}$ (84%).

Here we report the synthesis, structure, and characterization of the solution forms of a new MAMA complex, *syn*-ReO(L-$MAECH_2$) (L-$MAECH_5$ is mercaptoacetamide-ethylene-L-cysteine) (Chart 1). Only L-$MAECH_5$ results are discussed here, but we also prepared D-$MAECH_5$ for biological testing. Since the specific chirality of cysteine is of no chemical consequence, the L designation is omitted in this study. We designed and investigated ReO($MAECH_2$) because the carboxyl group is electron withdrawing and separated by only two bonds from the NH group. The acidity of the NH group may be enhanced compared to ReO($CACAH_2$), ensuring a CO_2^- deligated species with a well-defined charge at physiological pH.

Experimental Section

Succinimidyl-*S*-benzoylthioglycolate,[5] succinimidyl-*S*-tritylthioglycolate,[6] and $ReIO_2(PPh_3)_2$[7] were prepared according to literature procedures. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA. ^{1}H NMR spectra of the synthetic intermediates were recorded on a Varian 400 or a General Electric QE 300 spectrometer; chemical shifts were referenced to TSP [3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt] in D_2O, TMS [tetramethylsilane] in $CDCl_3$, and the solvent peak in DMSO-d_6.

Syntheses. *N*-Ethylamine-L-cysteine Hydrochloride. Aminoacetaldehyde dimethyl acetal 6.0 g, 57.1 mmol) was dissolved in pyridine (45 mL) and the solution cooled in an ice bath before *p*-toluenesulfonyl chloride (12.2 g, 63.9 mmol) in pyridine (45 mL) was added dropwise. The solution was stirred at room temperature overnight before the solvent was removed by rotary evaporation, and the residue was redissolved in $CHCl_3$. The organic solution was washed with water and dried. Evaporation of $CHCl_3$ afforded an orange oil of *N*-*p*-tosylaminoacetaldehyde dimethyl acetal. Yield: 12.9 g (87%). ^{1}H NMR ($CDCl_3$): δ 2.43 (s, 3H); 3.03 (t, 2H); 3.32 (s, 6H); 4.33 (t, 1 H); 4.67 (t, 1 H); 7.31 (d, 2H); 7.74 (d, 2H).

L-Cysteine (2.86 g, 24 mmol) and *N*-*p*-tosylaminoacetaldehyde dimethyl acetal (6.12 g, 24 mmol) were dissolved in THF (154 mL) by adding concentrated HCl/H_2O (33% v/v, 154 mL). After the solution was stirred at room temperature overnight, the solvent was removed by rotary evaporation, yielding a yellow oil. The excess of EtOH added to dissolve this oil was removed by rotary evaporation (twice). EtOH (100 mL) was added again, and the mixture warmed to dissolve the oil completely. Water was added, and the solution was cooled to 5 °C overnight to induce precipitation of a white solid, 2-(*N*-*p*-tosylamino)methyl-1,3-thiazolidine-4-carboxylic acid, which was collected and washed with cold EtOH. Yield: 4.05 g (53%). ^{1}H NMR (D_2O + NaOD): δ 2.40 (s, 3H); 2.69−2.82 (m 1H); 3.01−3.35 (m, 3H); 3.56−3−69 (m, 1H); 4.41−4.67 (m, 1H); 7.36 (d, 2H); 7.67 (d, 2H).

2-(*N*-*p*-Tosylamino)methyl-1,3-thiazolidine-4-carboxylic acid (2.45 g, 7.8 mmol) was placed in a three-necked flask equipped with a water condenser and a Dewar condenser filled with a dry ice/acetone mixture, and cooled to −60 °C (dry ice/acetone). The system was purged with N_2 before liquid NH_3 (200 mL) was introduced. Na (2.45 g, 0.1 mol), weighed under hexane, was slowly added to the solution through the water condenser. The solution, which developed a blue color over ~30 min, was stirred for 90 min and then quenched by slow addition of saturated NH_4Cl to give a white mixture. The NH_3 was allowed to evaporate overnight. Water (200 mL) was added to the residue, forming a suspension that was filtered through Celite. The filtrate was condensed to a white paste, and concentrated HCl (25 mL) was added to redissolve the product. NaCl was removed by filtration, the filtrate was reduced to dryness by rotary evaporation, and the residue was dissolved in water (20 mL). EtOH (50−60 mL) was added to the solution until a white precipitate formed. The mixture was cooled to 5 °C overnight to complete precipitation. The solid was collected, washed with EtOH, and dried under vacuum. Yield: 1.16 g (63%). ^{1}H NMR (D_2O): δ 3.37−3.47 (m, 6H); 4.07 (t, 1H).

***N*-[(*S*-Benzoylmercaptoacetamide)ethylene]-L-cysteine (*S*-Bz-MAECH$_4$).** *N*-Ethylamine-L-cysteine hydrochloride (0.95 g, 4.0 mmol) was dissolved in water (50 mL). The pH of the solution was adjusted from 2 to 8 by dropwise addition of 1 N NaOH. To the solution was added succinimidyl-*S*-benzoylthioglycolate (1.18 g, 4.0 mmol) dissolved in CH_3CN (45 mL). The resulting suspension was heated to slow reflux for 35 min, as the pH was maintained with addition of 1 N NaOH. Stirring was continued for 1 h at room temperature. The pH of the solution was lowered to 3 with 1 N HCl, and stirring was continued for 15 min. The precipitated product was collected and washed with water. Yield: 1.10 g (80%). ^{1}H NMR (CF_3CO_2D): δ 3.49−3.86 (m, 6H); 3.96 (s, 2H); 4.67 (t, 1 H); 7.48 (t, 2H); 7.67 (t, 1H); 7.92 (d, 2H).

***N*-[(*S*-Benzylmercaptoacetamide)ethylene]-L-cysteine (*S*-Bn-MAECH$_4$).** *S*-Benzylthioglycolic acid (1.82 g, 10.0 mmol) and N-hydroxysuccinimide (1.15 g, 10.0 mmol) in CH_3CN (50 mL) were treated with dicyclohexylcarbodiimide (2.06 g, 10.0 mmol) in CH_3CN (20 mL). The mixture was stirred at ambient temperature for 16 h. Solid dicyclohexylurea was removed by filtration, and the filtrate was evaporated to dryness. The residue was dissolved in hot MeOH; succinimidyl-*S*-benzylthioglycolate precipitated as the solution cooled and was collected, washed with cold MeOH, and vacuum-dried. Yield: 2.20 g (82%). ^{1}H NMR ($CDCl_3$): δ 2.84 (s, 2H); 3.80 (s, 2H); 4.10 (s, 2H); 7.50 (t, 2H), 7.62 (t, 1 H); 7.97 (d, 2H).

Succinimidyl-*S*-benzylthioglycolate (0.28 g, 1.0 mmol) and *N*-ethylamine-L-cysteine hydrochloride (0.24 g, 1.0 mmol) were coupled according to the procedure given for *S*-Bz-$MAECH_4$. The product was redissolved in water (pH ≈ 8) and the solution filtered. The pH of the solution was lowered to ~5 with HCl. The white solid that precipitated was collected, washed with water, and vacuum-dried. Yield: 0.25 g (75%). Anal. Calcd for $C_{14}H_{20}N_2O_3S_2$·0.25HCl: C, 50.49; H, 6.09; N, 8.41; S, 19.25. Found: C, 50.62; H, 5.80; N, 8.55; S, 19.38. ^{1}H NMR

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(D_2O + NaOD): δ 2.48−2.63 (m, 2H); 2.89−3.15 (m, 6H); 3.35 (t, 1H); 3.80 (s, 2H); 7.35−7.42 (m, 5H).

***N*-[(*S*-Tritylmercaptoacetamide)ethylene]-L-cysteine (*S*-Tr-MAECH$_4$).** Succinimidyl-*S*-tritylthioglycolate (1.81 g, 4.2 mmol) and *N*-ethylamine-L-cysteine hydrochloride (1.0 g, 4.2 mmol) were coupled according to the procedure given for *S*-Bz-MAECH$_4$. The crude product was recrystallized as a white solid from DMF. Yield: 1.85 g (90%). Anal. Calcd for $C_{26}H_{28}N_2O_3S_2$: C, 64.97; H, 5.87; N, 5.83; S, 13.34 Found: C, 64.82; H, 5.91; N, 5.81; S, 13.41. ^{1}H NMR (DMSO-d_6): δ 2.67−2.76 (m, 2H); 2.78 (s, 2H); 2.89−2.99 (m, 1H); 3.12−3.25 (m, 4H); 3.52−3.55 (m 1H), 7.28−7.32 (m, 15 H), 7.99 (s, 1H).

***syn*-ReO[*N*-[(mercaptoacetamido)ethylene]-L-cysteine] [ReO(MAECH$_2$)] (1).** *S*-Bz-MAECH$_4$ (0.21 g, 0.61 mmol) was dissolved in 50% MeOH/H_2O (50 mL) by addition of 1 N NaOH to pH 12. $ReIO_2(PPh_3)_2$ (0.53 g, 0.61 mmol) was added, and the mixture was heated at reflux for 3 h while the pH was maintained at 12 with 1 N NaOH. Stirring was continued overnight at room temperature. The solution was extracted (3×) with CH_3Cl, and reduced to ∼15 mL by rotary evaporation. The solution was acidified to pH 2 by addition of 1 N HCl. The light tan product that precipitated was collected, washed with water, and vacuum-dried. Yield: 0.16 g (59%). An analytically pure sample of **1** was prepared by suspending the complex in hot ethanol for 15 min. After the mixture was cooled to room temperature, the solid was collected and vacuum-dried. Anal. Calcd for $C_7H_{11}N_2O_4ReS_2$ [ReO(MAECH$_2$)·0.25EtOH]: C, 20.06; H, 2.81; N, 6.24. Found: C, 19.82; H, 2.70; N, 6.31.

[AsPh$_4$][ReO(MAECH)] (2). ReO(MAECH$_2$) (**1**) (23 mg, 0.05 mmol) was dissolved in water by addition of 1 N KOH. The final pH of the solution was ∼6. To this solution was added [AsPh$_4$]Cl·H_2O (20 mg, 0.05 mmol). The solution was filtered, and the filtrate concentrated by rotary evaporation and stored at 5 °C for 4 days. Small gold-brown prisms that formed were collected and air-dried. The only solid formed consisted of a few crystals; the yield was insufficient for elemental analysis.

X-ray Crystallography. A crystal of **2** with dimensions of 0.18 × 0.16 × 0.12 mm^3 was used for data collection. The crystal was mounted under Paratone-8277 on a glass fiber and placed in a cold nitrogen stream at −80 °C on a Siemens SMART CCD area detector system equipped with a normal focus Mo target X-ray tube operated at 2.0 kW (50 kV, 40 mA). A total of 1321 frames (1.3 hemispheres) was collected using a narrow frame method with a scan width of 0.3° in ω and an exposure time of 30 s/frame (detector-to-crystal distance 5.09 cm). Collection time was ∼12 h. Frames were integrated to a 2θ maximum of 56.6° by using the Siemens SAINT program to yield 9748 reflections, 6493 of which were independent (R_{int} = 2.61%, R_σ = 5.75%) and 5907 of which were above 2$\sigma(I)$. Laue symmetry revealed a monoclinic crystal system. Final unit cell parameters were determined from least-squares refinement of three-dimensional centroids of 6388 reflections. Data were corrected for absorption using the SADABS program based on the method of Blessing.[8]

The structure was solved by direct methods and refined by full-matrix least-squares procedures on F^2 using SHELXL 93. All non-hydrogen atoms were refined anisotropically. The carboxyl H-atom (see the Results) was located from a late-stage difference map and the position refined. H-atoms bound to carbon were generated at calculated positions (d(C−H) = 0.96 Å) and 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 **2** are presented in Table 1.

NMR Spectroscopy. All spectra of **1** were recorded on a Varian 400 spectrometer. ^{1}H NMR spectra were obtained in DMSO-d_6 (referenced to the solvent peak), and in D_2O at various pH values (uncorrected) ranging from 12.2 to 1.9 (referenced to TSP, 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt). For the D_2O experiments, **1** was dissolved by addition of NaOD (2.2 N); the pH was adjusted with DCl (2.2 N). Signal assignments (cf. Chart 2) were obtained by 2D methods: COSY (D_2O pH 4.8 and 7.4); HETCOR (D_2O pH 4.8). ^{1}H NMR (DMSO-d_6): δ 3.14 (t, $H_{\beta'}$); 3.34 (dd, H_α); 3.59 (td, $H_{3'}$); 3.78 (dd, $H_{4''}$); 3.82 (d, $H_{1'}$); 4.00 (dd, $H_{\beta''}$); 4.31 (d, $H_{1''}$); 4.56 (dd, $H_{3''}$); 9.48 (br, NH); 13.85 (br, CO_2H); $H_{4'}$ overlaps within the solvent peak. ^{1}H NMR (D_2O, pH 4.8): 2.47 (td, $H_{4'}$); 3.03 (dd, H_α); 3.24 (t, $H_{\beta'}$); 3.75 (td, $H_{3'}$); 3.88 (dd, $H_{4''}$); 4.03 (d, $H_{1'}$); 4.10 (dd, $H_{\beta''}$); 4.61 (dd, $H_{3''}$); 4.63 (d, $H_{1''}$).^{13}C NMR (D_2O, pH 4.8): δ 45.0 (C_1); 49.2 (C_β); 55.5 (C_3); 60.5 (C_4); 78.0 (C_α); 175.7 (CO_2); 195.3 (C_2).

Table 1. Crystallographic Data for [AsPh$_4$][ReO(MAECH)] (**2**)

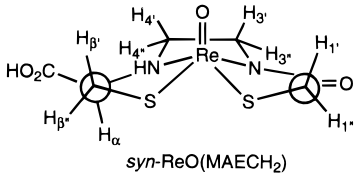
empirical formula	$C_{31}H_{30}N_2O_4ReS_2$	V (Å^3)	1539.2(5)
		Z	2
fw	819.81	ρ_{calcd} (mg m^{-3})	1.769
T (K)	193(2)	abs coeff (mm^{-1})	5.188
λ (Å)	0.710 73	R indices	R1 = 0.0338,
space group	$P2_1$	[$I > 2\sigma(I)$][a]	wR2 = 0.0628
unit cell dimens		R indices	R1 = 0.0399,
a (Å)	12.130(2)	(all data)[a]	wR2 = 0.0653
b (Å)	10.308(2)		
c (Å)	13.374(3)		
β (deg)	113.01(3)		

[a] $R1 = (\sum||F_o| - |F_c||)/\sum|F_o|$. $wR2 = [\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]]^{1/2}$, where $w = 1/[\sigma^2(F_o^2) + (aP)^2 + bP]$ ($P = [(\max;0,F_o^2) + 2F_c^2]/3$).

Table 2. Selected Bond Distances (Å) and Angles (deg) for [AsPh$_4$][ReO(MAECH)] (**2**)

Bond Distances (Å)			
Re−O(1)	1.692(3)	Re−S(1)	2.305(2)
Re−N(1)	2.021(6)	Re−S(2)	2.290(2)
Re−N(2)	1.947(6)		
Bond Angles (deg)			
O(1)−Re−N(1)	109.8(3)	S(2)−Re−S(1)	89.19(7)
O(1)−Re−N(2)	111.8(3)	C(1)−S(1)−Re	100.2(2)
O(1)−Re−S(1)	108.8(2)	C(7)−S(2)−Re	98.5(3)
O(1)−Re−S(2)	110.2(2)	C(2)−N(1)−C(3)	120.8(6)
N(1)−Re−S(1)	81.5(2)	C(2)−N(1)−Re	125.6(5)
N(1)−Re−S(2)	139.9(2)	C(3)−N(1)−Re	113.4(4)
N(2)−Re−N(1)	78.8(2)	C(4)−N(2)−C(5)	110.9(6)
N(2)−Re−S(1)	138.8(2)	C(4)−N(2)−Re	120.3(5)
N(2)−Re−S(2)	83.3(2)	C(5)−N(2)−Re	123.3(4)

Chart 2



syn-ReO(MAECH$_2$)

Resonance Raman Spectroscopy. Resonance Raman (RR) scattering was excited at 406.7 nm with a Kr^+ ion laser (Spectra Physics, 2016) and detected with a CCD (Astromed CCD, 3200) attached to a single polychromator (Ritsu Oyo Kogaku, DG-1000). The slit width and slit height were set to 200 μm and 10 mm, respectively. The excitation laser beam power (at the sample point) was adjusted to 12 mW. Measurements were carried out at room temperature with a spinning cell (3000 rpm). The data accumulation time was 500 s for each spectrum. Raman shifts were calibrated with acetone, and the accuracy of the peak positions of the Raman bands was ±1 cm^{-1}. **1** was dissolved in D_2O by addition of concentrated NaOD (15 mM, pH 12.7). A Raman spectrum was obtained, and the pH of the solution was then lowered successively by addition of concentrated DCl for subsequent measurements.

Results

Synthesis. Three new mercaptoacetamide-ethylene-cysteine ligand (MAECH$_5$) precursors were prepared with different thiol protecting groups: benzoyl (Bz), benzyl (Bn), and trityl (Tr). The Bn and Tr derivatives gave satisfactory analytical and spectral results. The Bz derivative also gave good spectral results but could not be obtained analytically pure. Nevertheless, *S*-Bz-MAECH$_4$ was used to prepare the Re complex because the benzoyl group was easily cleaved (in situ) under the basic

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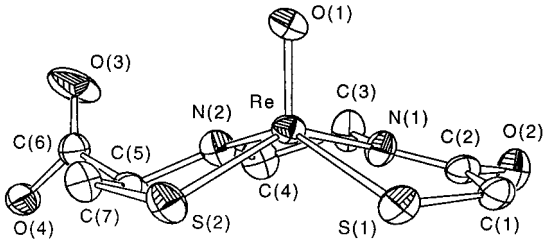

Figure 1. Perspective drawing of *syn*-[ReO(MAECH)]$^-$ (anion of **2**), with 50% probablity for the thermal ellipsoids.

conditions normally used to promote ligand exchange with $ReIO_2(PPh_3)_2$. Under these conditions we isolated only the syn isomer, although the MAEC ligand, since it contains a resolved L-chiral center within a chelate ring and it lacks C_2 symmetry, can form both syn and anti stereoisomers of ReO(MAEC).

In an effort to isolate the anti isomer, the Bz group of *S*-Bz-$MAECH_4$ was first removed by refluxing at pH 12 in 50% THF/H_2O. Next the pH of the cooled ligand solution was lowered to 8 before $ReIO_2(PPh_3)_2$ or $ReOCl_3(Me_2S)(OPPh_3)$[9,10] (0.5 equiv) was added. We suspected that the anti isomer forms, but converts rapidly to the syn isomer at high pH; the lower pH was employed to slow the anti to syn conversion rate. This procedure resulted in the detection of both the anti and syn isomers by HPLC (Beckman Ultrasphere ODS, 4.6 × 250 mm^2, 5 μm; 3% EtOH, 0.01 M NaH_2PO_4, pH 6.0; 1 mL/min; retention volume (anti) 4.6 mL, (syn) 7.6 mL). However, since mild conditions were used (pH 8, room temperature), complex formation was sluggish, and the overall yield of the complex very low. Furthermore, although the rate of anti to syn conversion was slowed, conversion was not halted. Hence all attempts to separate the isomers and isolate pure *anti*-ReO(MAEC) were unsuccessful.

The question remained, however, whether the anti to syn isomerization proceeded to completion, or whether it was reversible but with the equilibrium strongly favoring the syn isomer. Therefore, a solution of *syn*-$ReO(MAECH_2)$ (**1**) (5 mg, 0.1 mmol) in H_2O (10 mL) and NaOH (1 N) was added dropwise to dissolve the complex and raise the pH to 13, and the resulting solution was heated at reflux for 3 h. The solution composition was monitored periodically by HPLC. In addition to the peak corresponding to *syn*-$ReO(MAECH_2)$, a second peak with the retention volume of the anti isomer (see above) was detected. The ratio between the two peaks, anti (6%):syn (94%), became constant after 1 h. This result indicates that although *syn*-$ReO(MAECH_2)$ is thermodynamically preferred over *anti*-$ReO(MAECH_2)$, the isomerization is reversible.

X-ray Crystallography. The tautomer of *syn*-[ReO(MAECH)]$^-$ present in the crystalline $[AsPh_4]^+$ salt (**2**) is shown in Figure 1. In the pseudo-square-pyramidal coordination geometry, the oxo ligand is apical and the MAEC ligand is coordinated in a typical fashion with the two N and two S donor atoms in the basal plane. The carboxyl group is syn to the oxo ligand and uncoordinated. The Re−S and Re−N(1) (amido donor) bond distances are normal for these donor groups when charged (deprotonated). The complex contains one ionizable H atom, and on the basis of relative acidity, we expected retention of the H atom by the amine donor [N(2)] and ionization of the carboxyl group [O(3)−C(6)−O(4)]. However, the short Re−N(2) bond distance (1.947(6) Å) indicated a deprotonated amine donor. Although the C(4)−N(2)−C(5) bond angle is 110.9 (6)° (approaching the ideal value for a tetrahedral NH center), the Re−N(2)−C(4) and Re−N(2)−C(5) bond angles of 120.3(5)° and 123.3(4)°, respectively, are typical of a hybridized N$^-$ center. In comparison, the analogous angles found for the N(1) (amido) center are similar (120.8(6)°, 125.6(5)°, and 113.4(4)°), with one angle significantly smaller than the other two. We thus attribute the small C−N−C angle in **2** to chelate strain, not to the geometry of an NH donor. Moreover, a significant difference was found between the O(3)−C(6) and O(4)−C(6) bond distances (1.193(8) and 1.304(7) Å, respectively), and there was evidence of intermolecular H-bonding between O(4) and O(2) (2.561 Å; symmetry position $-x$, $1.5 + y$, $-z$). In the late stages of least-squares refinement of the structure, a difference peak consistent with an H atom in intensity and distance from O(4) was observed. The peak was assigned accordingly and included in the final refinement cycles.

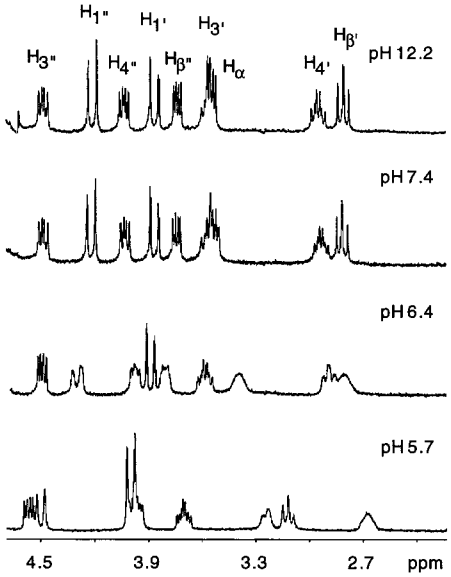

Figure 2. ^{1}H NMR spectra of *syn*-$ReO(MAECH_2)$ (**1**) in D_2O from pH 12.2 to pH 5.7.

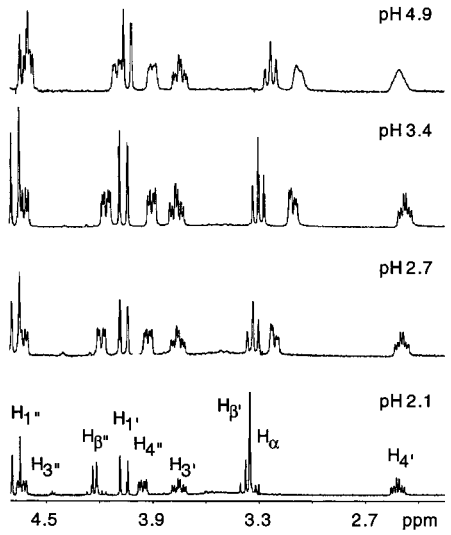

Figure 3. ^{1}H NMR spectra of *syn*-$ReO(MAECH_2)$ (**1**) in D_2O from pH 4.9 to pH 2.1

NMR Spectroscopy. ^{1}H NMR spectra of **1** in D_2O at various pH values (uncorrected) are presented in Figures 2 and 3. At pH 12.2, separate signals were observed for each proton, with the H_α and $H_{3'}$ signals partially overlapping. No changes were observed between pH 12.2 and pH 7.4. As the pH was lowered

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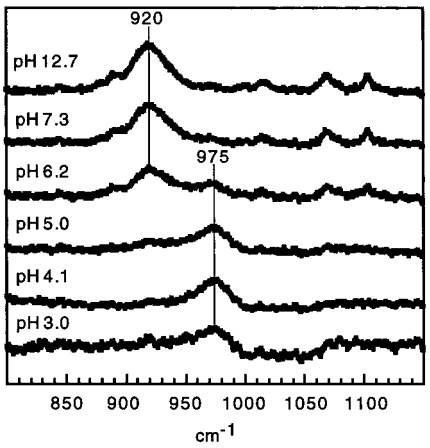


Figure 4. Resonance Raman spectra of *syn*-ReO(MAECH$_2$) (**1**) in D_2O from pH 12.7 to pH 3.0.

below 7.4, the signals shifted, broadened, and resharpened. Those signals that shifted the most showed the greatest degree of broadening. At lower pH values (pH 3.4−2.1, Figure 3), the signals shifted again but remained sharp.

A clear demarcation was observed between the two pH regions where the signals are shifting (pH 7−5 and pH 4−2) because some signals (most notably H_α) shift in the opposite direction within each pH region. In the pH 7−5 region, large changes in chemical shift (0.6 ppm) and in line width were observed for the H_α and $H_{4'}$ signals. In the pH 4−2 region the H_α, $H_{\beta'}$ and $H_{\beta''}$ signals shifted the most, but the overall changes were small.

Resonance Raman Spectroscopy. The Re═O stretching frequency of **1** was monitored by Raman spectroscopy as a function of pH in D_2O (Figure 4). At pH 12.7, the Re═O band was observed at 920 cm^{-1}. This band persisted at this frequency as the pH was lowered to neutrality. From pH 7 to pH 5, the band diminished in intensity and was replaced by a new band at 975 cm^{-1}. No detectable changes were observed below pH 5. Near pH 3, the complex partially precipitated, decreasing the intensity of the spectrum.

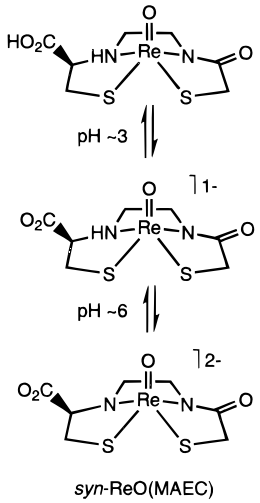
Discussion

Neutral *syn*-ReO(MAECH$_2$) (**1**) has two dissociable protons, NH and CO_2H. ^1H NMR signals for these protons were observed in DMSO-d_6: NH, 9.5 ppm; CO_2H, 13.9 ppm. For **1** in aqueous solution, signals for these protons cannot be observed. pH-dependent spectral changes occurred in two nonoverlapping pH ranges (∼2−4 and ∼5−7) in D_2O; these changes must be from formation of a monanion and then a dianion. (Although aqueous spectra were recorded in D_2O, the interpretations apply to H_2O, and we use protic species in the discussion for simplicity.) The solid-state structure of **2** (*syn*-[ReO(MAECH]$^-$) has the proton associated with the carboxyl group rather than the amine. This result might appear to suggest that the Re(V) is so electron deficient that the low pH changes for **1** are from a very acidic NH rather than from the CO_2H.

The pH range (∼2−4) of the first process is typical of CO_2H pK_a values. The ^1H NMR signals shift but remain sharp over this range. Those signals that shift the most (H_α, H_β, and $H_{\beta''}$) are for the protons in the chelate ring carrying the carboxyl group. All these features are consistent with CO_2H dissociation being responsible for the changes in the pH range 2−4.

Although coordination of hydroxide could produce spectral changes in the ∼5−7 pH range, we discount this possibility because the solid-state structure shows that the NH is acidic.

Scheme 1



syn-ReO(MAEC)

As mono- and dianions interconvert, the H_α and $H_{4'}$ signals (Chart 2) undergo large changes in chemical shift (0.6 ppm); the $H_{4''}$ signal also shifts significantly. Since the H_α and $H_{4'/4''}$ signals are vicinal to the NH group, the shift patterns are consistent with NH deprotonation with a pK_a of 6.0 (this value corresponds to the pH at which the signals were at the midpoint between chemical shifts observed at high pH and pH 4.2).

For **1** the second pK_a value and the NMR line-broadening due to NH/N$^-$ exchange are similar to the NH pK_a (∼6.0) and accompanying changes in the NMR line width of *syn*-ReO(CACAH$_2$).[4] The low pK_a values indicate that the [Re(V)═O]$^{3+}$ center is highly electron deficient and that the amido group is a modest donor. Previously we ranked the abilities of coordinated N centers to donate electron density to a [Re(V)═O]$^{3+}$ center in the order deprotonated amine ≫ amido ≥ protonated amine. This order was constructed by comparing the influence of these donors on the NH acidity of [ReO(LL-ECH$_2$)]$^-$ and [ReO(CACAH)]$^-$.[4] This work supports this ranking.

The RR data support our interpretation of the NMR data. At pH ≈ 3 the solubility of **1** is low and the Re═O band (975 cm^{-1}) is weak. Since this pH value is near the pK_a of the neutral complex, we can attribute the poor solubility to the neutral form, and the observable band to the monoanion. From pH 5 to pH 7, the band at 975 cm^{-1} was replaced by a new band at 920 cm^{-1}. This result is consistent with an increase in the electron density of the [Re═O]$^{3+}$ center, indicating ionization of the donor NH group. Ionization of the carboxyl group (which does not interact with the metal) would not be expected to change the Re═O frequency. In ReO(ECH$_3$) (ECH$_6$ is ethylenedicysteine) type complexes, the simple ionization of an NH group (pK_{a3}) lowered the frequency of the Re═O band by ≥30 cm^{-1}; however, ionization of the CO_2H (pK_{a1}) produced no changes in the frequency of the Re═O band.[11]

The spectroscopic data support a solution equilibrium for **1** consisting of a five-coordinate monoanion and five-coordinate dianion that interconvert via NH deprotonation/protonation (Scheme 1). Because the X-ray structure of **2** contains an unusual tautomeric form, we now discuss the structure and other experimental results in depth.

In MO(N$_2$S$_2$) complexes, neutral amine donors that anchor two chelate rings form a bond to a carbon in each of the two chelate rings, and a third bond to the metal. The fourth

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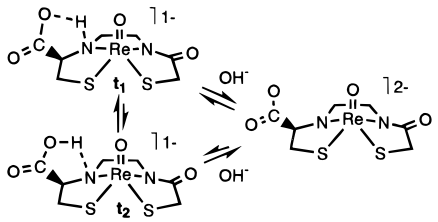
Scheme 2



substituent (NH or NR) can be endo (near the oxo ligand) or exo (away from the oxo ligand), but is usually endo,[4,12−15] suggesting that the endo configuration is thermodynamically preferred (probably for steric reasons). In **2**, where the monoanion has no NH group (tautomer 2, $\mathbf{t_2}$), the N(2)−O(3) distance, 2.99 Å, is typical of distances between atoms that are weakly linked by a hydrogen bond.[16] In solution, the predominant monoanion has a tetrahedral NH group (tautomer 1, $\mathbf{t_1}$); thus, the chelate rings anchored by the amine center are puckered. Models show that the combination of an *endo*-NH and tetrahedral geometry shortens the N(2)−O(3) distance. Thus, in $\mathbf{t_1}$, a moderate to strong intramolecular H-bond between the NH and the CO_2^- is likely. Transfer of the proton from the amine to the carboxyl group gives a rotamer of tautomer $\mathbf{t_2}$ (Scheme 2) which differs from the rotamer in **2** (Figure 1) simply by rotation about the $C_\alpha-CO_2H$ bond.

Crystals of **2** formed from an aqueous solution at pH ≈ 6; at this pH, the concentrations of mono- and dianions were approximately equal. Crystallization from aqueous solution of dianions similar to $[ReO(MAEC)]^{2-}$ and rich in oxygen groups has been accomplished by the use of a small cation such as Na^+ or K^+.[17,18] The small cations are highly coordinated to oxygen groups from the dianion and cocrystallized water molecules, resulting in extensively bridged networks of cation, dianion, and water. Thus, since the bulky $AsPh_4^+$ lacks coordination sites, it crystallized the monoanion but as the $\mathbf{t_2}$ tautomer. The K_a of the CO_2H of the $\mathbf{t_2}$ monoanion, *syn*-$[ReO(MAECH)]^-$, is likely to be ∼10× higher than that of the CO_2H of the neutral parent form, *syn*-$[ReO(MAECH_2)]$, since $\mathbf{t_2}$ is a charged monoanion and the deprotonated N will have some inductive effect. The percentage of $\mathbf{t_2}$ must be very low since it was not observed by RR spectroscopy; we would expect the frequency of a Re═O band for $\mathbf{t_2}$ to be similar to that of the dianion because both species have a N^- donor. Thus, at pH < 6 a band at ∼920 cm^{-1} would be observed if substantial amounts of $\mathbf{t_2}$ were present. If we assume that the CO_2H K_a of $\mathbf{t_2}$ is $\sim10^{-4}$ and the NH K_a of $\mathbf{t_1}$ is $\sim10^{-6}$, then only 1% of the monoanion is in the $\mathbf{t_2}$ form. The pK_a measured is essentially that of $\mathbf{t_1}$. Because the concentration of $\mathbf{t_2}$ is low, the preferential crystallization of $\mathbf{t_2}$ must arise from the lower solubility of $\mathbf{t_2}$ compared to $\mathbf{t_1}$. A factor that probably contributes to the lower solubility and crystallization of $\mathbf{t_2}$ is the observed intermolecular H-bonding between the CO_2H and C═O of a neighboring anion in the crystals of **2**. A different counterion or a racemic mixture may favor crystallization of $\mathbf{t_1}$.

Since our goal is to develop procedures for preparing radiopharmaceuticals in a single form at physiological pH (7.4), complexes containing secondary amine donors must all have NH pK_a values that are <6 or >9. Also when syn and anti isomers are possible, one isomer must be thermodynamically preferred, and equilibration between the two isomers must be fast. *syn*-MO(MAEC) comes close to meeting these requirements. The syn isomer is favored at equilibrium (Re 94%, this work; ^{99m}Tc 94%, unpublished results), and the rate of equilibration between syn and anti isomers is fast. The NH pK_a of 6.0 for *syn*-$ReO(MAECH_2)$ results in predominance of the dianionic form (96%) at physiological pH; Furthermore, preliminary investigations of *syn*-^{99m}TcO(MAEC) in animals have yielded excellent results with respect to renal imaging.[19,20] Analysis of these properties produces some understanding of the attributes of the MAEC ligand that have led to these favored features. The analysis which follows offers guidelines by which we can tune the ligand design. We begin with the factors influencing the NH pK_a, since in complexes with quadridentate N_2S_2 ligands NH deprotonation and syn/anti isomerization are linked.

From this and past work, we found that it was futile to attempt to shift the NH pK_a to above 9. The $[M(V)═O]^{3+}$ center is highly electron deficient, and although thiolates are strong donors, complexes with linear quadridentate ligands must have at least two nonthiolate coordinating groups. When one of these groups is a secondary amine, the nature of the other determines the NH pK_a because an amine is a modest donor when neutral and a strong donor when charged. The NH pK_a can be >9 when there are three strong sister donors present.[15] When there is a modest sister donor present, the amine compensates by deprotonating below pH 9.[4] The pK_a values of secondary amines in MAMA type complexes are <7 because the amido group is a modest donor. With MO(MAEC) we attempted to engineer an NH pK_a value of <6 by positioning an electron-withdrawing CO_2^- near the NH. We now understand that in this arrangement the electrostatic and inductive effects cancel. In the future we need to introduce a neutral electron-withdrawing group near the amine.

Next we consider syn/anti isomerization (Scheme 3). The simplest cases involve complexes with two NS ligands. In these complexes, the ligands have a cis orientation in the basal plane and hydroxide adds axially in competition with, or in preference to, NH deprotonation.[21,22] We have demonstrated that, in $[ReO(OH)(penH)_2]^{2-}$ ($penH_3$ is penicillamine) complexes, proton transfer rapidly exchanges the oxo and hydroxo sites. In simple terms syn/anti interconversion in square-pyramidal $[M(V)═O]^{3+}$ complexes consists of breaking the M═O bond and re-forming the bond on the other side of the basal plane (oxo ligand inversion). However, for quadridentate ligands, the amine center(s) must also invert to retain the *endo*-NH config-

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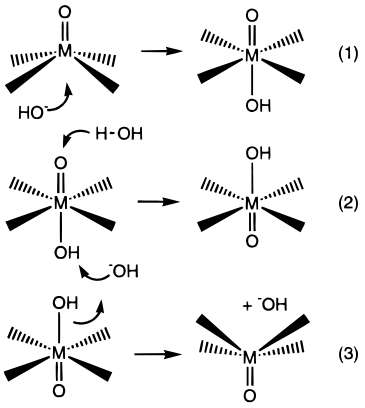
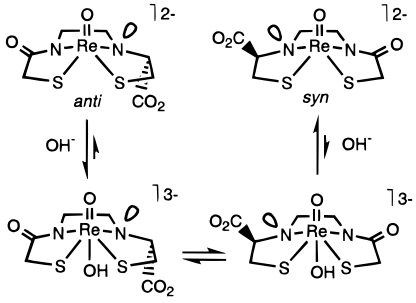
Scheme 3



Scheme 4[a]



[a] For clarity, the syn species (right) are shown flipped by 180° with respect to the anti species (left). The chirality at NLp differs for the syn and anti species.

uration. The amine cannot invert unless it is deprotonated and carries a lone electron pair (NLp). M(V)O(N_2S_2) complexes with even a single tertiary amine evidently do not isomerize.[14,23−25] The rate of syn/anti interconversion for complexes with secondary amines depends on the concentration of the N^- form. For example, for the meso forms of MO(ECH_3) and MO($TMECH_3$) ($TMECH_6$ is ethylene-dipenicillamine or tetramethyl-EC), the syn isomers are highly favored. However, interconversion of *anti*-MO(DL-EC)/*syn*-MO(DL-EC) and *anti*-MO(DL-TMEC)/*syn*-MO(DL-TMEC) does not occur at an observable rate until the pH is raised to a value similar to the pK_{a3} value (corresponding to ionization of the second NH). This NH is more acidic in the EC complex than in the TMEC complex, and the EC complex isomerizes more rapidly.[26] Syn/anti isomerization of ReO(MAEC) (Scheme 4) is particularly facile because a form with deprotonated amine exists in aqueous solution at essentially all pH > 4, since the **t₂** tautomer has a deprotonated amine.

There is still the question of why the syn isomer of ReO(MAEC) is strongly favored thermodynamically over the anti isomer. As mentioned above, the *endo*-NH configuration is preferred in M(V)O(N_2S_2) complexes. In our analysis of the isomerization process, we assumed that an amine carrying a lone electron pair (NLp) would also exhibit this preference for the endo configuration. The *endo*-NLp configuration is indeed found in **2** (Figure 1). This configuration determines the conformation of the chelate rings anchored by the amine, and thus the orientation of the substituent on the ring with respect to the metal coordination sphere. For compounds which have an *endo*-NLp configuration and a substituent on the carbon bond to the amine, a syn substituent is extended away from the metal coordination sphere, while an anti substituent is drawn in toward the metal coordination sphere. For complexes that bear a CO_2^- substituent, it is likely that the short-range interaction between the CO_2^- and the metal coordination sphere in the anti isomer is repulsive. The amine can adopt the *exo*-NLp configuration, but empirical evidence suggests that the *exo*-NLp configuration should be unfavorable because the metal is usually displaced out of the basal plane toward an endo substituent. Thus, for the anti isomer, complexes with either of the two possible NLp configurations have destabilizing features. On the other hand, the syn complex with the *endo*-NLp configuration lacks destabilizing features and is favored over both anti forms and over the syn form with an *exo*-NLp configuration.

ReO(CACA) and ReO(MAEC) are closely related, and both form dianions with increasing pH with a midpoint of about pH 6.[4] However, in ReO(CACA) the chelate ring carrying the CO_2^- is anchored by the amido group, and the position of the CO_2^- is fixed with respect to the metal coordination sphere. This apparently minor structural change leads to two significant differences between ReO(CACA) and ReO(MAEC). First, both *syn*- and *anti*-ReO(CACA) isomers were separated and isolated. Second, no syn/anti interconversion was detected for ReO(CACA). Since the isomers do not interconvert, their relative thermodynamic stability cannot be determined. Therefore, we do not know how (or if) the fixed position of the CO_2^- influences the relative stability of these isomers. However, as described below, we believe that the difference in the CO_2^- position between ReO(CACA) and ReO(MAEC) complexes can be used to explain why the syn and anti isomers of ReO(MAEC) isomerize, while those of ReO(CACA) do not.

As mentioned earlier, in $[M(V)=O]^{3+}$ complexes the metal is normally displaced ~0.7 Å from the basal plane toward the oxo ligand. Thus, for syn/anti isomerization to occur, the metal must move ~1.4 Å to the opposite side of the basal plane. Such a rearrangement certainly requires some degree of flexibility within the chelate. In complexes with N_2S_2 and N_3S ligands containing only amido N donors (diamido-dithiol, triamido-monothiol), all the chelate rings are rigidly anchored and isomerization is unknown.[27−29] However, in complexes with chelate rings anchored by (secondary) amines, isomerization is known.[26,30,31] We believe isomerization takes place because chelate rings anchored by amines are flexible.

Both ReO(CACA) and ReO(MAEC) have chelate rings anchored by an amine. The critical difference between the complexes is the position of the amido carbonyl group. In ReO(MAEC) the carbonyl group forms part of the terminal mercaptoacetamide chelate ring, leaving the central and cysteine chelate rings flexible. (We associate ring puckering with flexibility; note the puckering of the central and cysteine rings

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of $[ReO(MAECH)]^-$ in Figure 1). Hence, for ReO(MAEC) isomerization occurs with ease.

In ReO(CACA) the carbonyl group is within the central chelate ring. The X-ray crystal structures of *anti*-$ReO(CACAH_2)$ and *syn*-$ReO(PACAH_2)$ ($PACAH_5$ is penicillamine-acetylcysteamine) show that the interior portions of the basal ligand including the central and cysteine/penicillamine rings) are very rigid, while the terminal cysteamine ring, anchored by the amine, is highly puckered and does not lie along a typical square-pyramidal edge.[4] The cysteamine ring orientation is characterized by two unusual features. First, the amine N is positioned out of the basal plane (as much as 0.6 Å) defined by the three other basal donors. The structure is nonetheless square-pyramidal ($\tau = 0.32$); ideal τ values are zero for square-pyramidal and unity for trigonal-bypyramidal.[32] Second, the metal is displaced 0.8−0.9 Å from this same plane toward the oxo ligand; the normal displacement is ∼0.7 Å.[15,27,33] These two extreme features are probably due to the presence of only one very flexible chelate ring combined with the two relatively rigid rings. These features are expected to be somewhat less pronounced in the N^- form, but a major structural rearrangement would nevertheless be necessary for isomerization to take place. The net rigidity of the complex most likely prevents rearrangement and, hence, isomerization.

Conclusions

Hydroxide can deprotonate secondary amines in $M(V)=O(N_2S_2)$ complexes or add axially to the complex (since they are typically five-coordinate or have a labile axial ligand). Although in some cases it has been possible to establish that secondary amines are deprotonated,[15] or that hydroxide coordinates,[21,22] most experimental results do not distinguish between these possibilities. The X-ray structure of **2** provides very compelling evidence that hydroxide does not add to this MAMA type complex but deprotonates the ligand. The similar pK_a of ReO(MAMA) type complexes suggests that this is a general property of these adducts.

We found clear evidence for one tautomer ($\mathbf{t_2}$) for *syn*-$[ReO(MAECH)]^-$ in the solid state and for a different tautomer ($\mathbf{t_1}$) in aqueous solution. Thus, the pK_a of about 6 reflects a tautomeric $\mathbf{t_1}/\mathbf{t_2}$ mixture. However, since the $\mathbf{t_1}$ greatly dominates, the pK_a measured is essentially that of $\mathbf{t_1}$. The results strongly indicate that the inductive effect of the carboxyl group is offset by the proximity of the negative group to the dissociable proton. For either $\mathbf{t_1}$ or $\mathbf{t_2}$, a negative group is close to the proton. In $\mathbf{t_2}$, the deprotonated N will donate electron density to the CO_2^-, making it more basic. In $\mathbf{t_1}$, the NH is less acidic due to the nearby negative charge on the carboxyl group.

For $ReO(MAECH_2)$, the syn isomer is highly favored at equilibrium and the rate of equilibration is fast. This isomer is favored because it contains the *endo*-NH/NLp configuration, which minimizes steric strain, and the repulsive interaction between the CO_2^- and metal coordination sphere. During isomerization (oxo ligand inversion) the metal must move ∼1.4 Å from one side of the basal plane to the other, and the amine must invert to retain the *endo*-NH configuration; the amine inversion is possible only when the amine is deprotonated. In $ReO(MAECH_2)$ two of the three chelate rings are relatively flexible and the NH in highly acidic. Thus, syn/anti isomerization is particularly facile and the rate of equilibration is fast when the chelate ligand bearing the pendant group is puckered and anchored by an acidic NH group. This new understanding of the factors that control syn/anti isomerization will be most valuable in the future design of radiopharmaceuticals which can be prepared directly as only one isomer.

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Supporting Information Available: Crystallographic data for **2**, including tables of positional parameters, bond distances and angles, anisotropic displacement coefficients, and H-atom coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.

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