An Unusual Determinant of Chirality. Unique Solution Chemistry and Novel NMR Spectroscopic Changes in Fluxional Re(V)═O Complexes with Rearranging *meso* N_2S_2 Ligands

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Abstract: The nature of metal species in aqueous solution is often ambiguous since the two processes, OH^- coordination and ligand deprotonation, have the same pH profile. This problem plagues the assessment of the form of some radiopharmaceuticals present at physiological pH. Representative M(V)═O(N_2S_2) (M = ^{99m}Tc, Re) radiopharmaceuticals include ReO(ECH_3) complexes (ECH_3 = thrice deprotonated ethylene di(cysteine), ECH_6). We found that *syn*-ReO(DL-ECH_3) (**1**) (*meso*-ECH_3, formed from DL-cysteine) and the tetramethyl analog, *syn*-ReO-(DL-$TMECH_3$) (**2**, $TMECH_6$ = ethylene di(penicillamine)), have uniquely informative spectral properties. Two equivalents of OH^- convert **1** and **2** to the dianionic form. This form of **2** has only *one* set of three penicillamine (pen) ^{1}H NMR signals near pH 6. However, these signals underwent major changes from pH 6 to 10 as follows: they broadened and collapsed; *two equal* sets of three signals emerged; these then broadened and collapsed; and the *original* set of three signals reemerged. Combined with the Raman data given below, these results require that one form is present from pH 6 to 10 and that this form be chiral and both conformationally and configurationally fluxional; one N is protonated and the other N bears a lone pair (Lp). The determinant of chirality of this NH/NLp form is the site of the NH group. The magnetic equivalence leading to one set of pen signals observed at the low and high ends of the pH range demonstrates the occurrence of fast exchange of the NH site between the two N's. This exchange leads to rapid inversion of complex chirality through symmetric intermediates, an NH/NH monoanion and an NLp/NLp trianion, in the acid- and base-catalyzed processes, respectively. At neutral pH, the H^+ and OH^- concentrations are low; as a result, the inversion of chirality is slow compared to the NMR time scale, and the two halves of the ligand are magnetically inequivalent. The two Re═O bands found in a fixed ratio in the resonance Raman spectra of **2** between pH 6 and 10 indicate that two major NH/NLp conformers are present throughout the range, including physiological pH. Two conformers of a form axially ligated by OH^- cannot explain the two equal sets of pen NMR signals. However, two NH/NLp conformers differing only in NLp orientation and in rapid interchange via NLp inversion explain all the spectral results. Thus, the use of fast and slow time scale spectroscopies eliminates the ligation/deprotonation ambiguity in this case.

Introduction

Long-lived analogs of ^{99m}Tc radiopharmaceuticals with ^{99}Tc or Re ligated by quadridentate N_2S_2 donor ligands are often difficult to characterize in solution.[1−3] A knowledge of structure, charge, and charge distribution is important in the development of structure−distribution relationships. It is also important to define features of ligands that lead to a single species at physiological pH since it is unlikely that all components of a mixture will have optimal biological properties. Since the definition of the solution form of many types of complicated metal complexes (including metal anticancer drugs) requires superior approaches, we have been seeking methods and strategies for better characterization of metal complexes in aqueous solution.

The ^{99m}Tc(V)═O(N_2S_2) complex formed with the N_2S_2 ligand, LL-ethylene di(cysteine) (LL-ECH_6, Chart 1, formed from L-cysteine; the subscript on H indicates the number of dissociable protons), is a potentially useful radiopharmaceutical for evaluating renal function.[4−7] However, at physiological pH, M(V)═O(LL-ECH_3) complexes (M = ^{99}Tc, Re) have rather complicated spectral properties.[8] We developed the strategy of investigating complexes of the analogous ligand made from penicillamine (pen) instead of cysteine.[3] The ligand, DD-ethylene di(penicillamine) (Chart 1, called DD-$TMECH_6$ for tetramethyl EC and made from D-pen), formed a Re(V)═O

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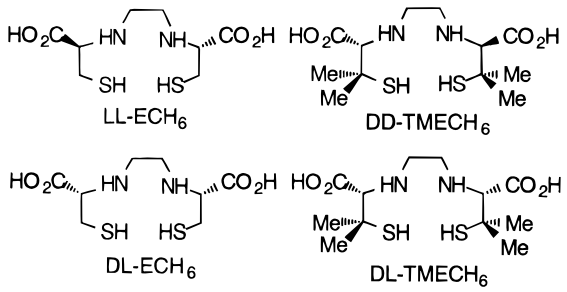
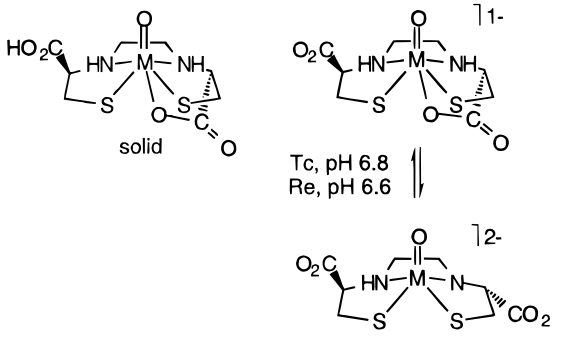
Chart 1



Scheme 1

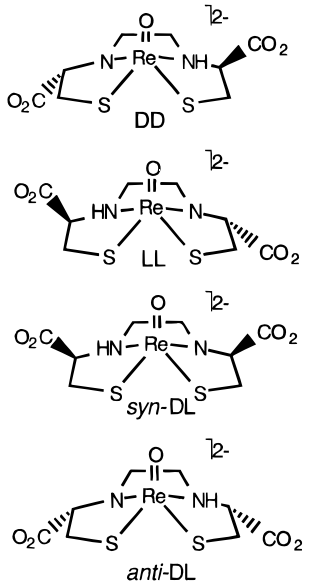


complex that allowed us to model and elucidate many features of the solution chemistry of the M(V)═O(LL-ECH_3) radiopharmaceuticals at physiological pH.[3,9] The TMEC ligand has the advantage that the H_α and Me ^1H NMR signals have no $^1H-^1H$ coupling; therefore, components of mixtures are more readily identified and characterized by NMR spectroscopy.

The neutral ReO(LL-ECH_3) and ReO(DD-$TMECH_3$) complexes dissolve when the dangling CO_2H deprotonates (pK_{a1}); the complexes remain as one monoanionic form at low pH (~4−6) but exist as an equilibrium mixture of monoanionic and dianionic forms at physiological pH.[3,8] Thus, hydroxide has a role in the formation of the dianion. For both forms, the two N and two S donor atoms coordinate in the basal plane with one carboxyl group *syn* and the other *anti* to the oxo ligand (Scheme 1). (The EC-type complex is depicted in schemes and charts for clarity.) However, ligand denticity in the two forms is different.[3] In the monoanion, the *anti*-carboxyl group is ligated *trans* to the oxo ligand. The evidence favors a structure for the dianion with the *anti*-CO_2 deligated as a result of deprotonation of the *anti*-N; the deprotonated *anti*-N bears a lone electron pair (Lp). The pK_a for NH dissociation is enclosed in quotation marks, "pK_{a2}", since the process is more than a simple proton dissociation. Furthermore, alternative forms of the dianion cannot be rigorously eliminated. In particular, the OH^- could ligate axially rather than deprotonate the NH group. Thus, ambiguities exist in defining the nature of the physiologically relevant forms. Similar ambiguities plague the characterization of relevant forms of other types of metal species.

In this report, we definitively elucidate the often elusive features of metal complexes by exploiting the different time domains of NMR and Raman spectroscopy. Our strategy employs ligands with selected stereochemical features, namely *meso* isomers of EC-type ligands (DL-ECH_6, made from D- and L-cysteine, and DL-$TMECH_6$, Chart 1). Ligand stereochemistry also influences renal clearance of radiopharmaceuticals,[10,11] and our studies are thus relevant to the identification of superior

Chart 2



renal agents. When the M(V)═O(N_2S_2) complex has the normal N_2S_2 basal plane coordination, the chiral (LL and DD) EC-type ligands form only one class of complexes with the oxo group *syn* to one and *anti* to the other CO_2 group. However, the analogous *meso* ligands can form two classes of complexes with the oxo group *syn* or *anti* to both CO_2 groups, respectively. In Chart 2, we illustrate gross geometries only for DD, LL, *syn*-DL, and *anti*-DL $[ReO(ECH)]^{2-}$ species with NH deprotonation. Other possible dianions include some with axially ligated OH^- and others with deprotonation at a different N. Also, in Chart 2, we have not indicated the orientation of NH or NLp, both of which can be either *syn* or *anti* to the oxo group. We describe here the unique behavior of *syn*-ReO(DL-ECH_3) and *syn*-ReO(DL-$TMECH_3$) at physiological pH; this behavior has led to unprecedented clarification of the structure and dynamics of such complexes in aqueous solution.

Experimental Section

Isomeric mixtures (DD, LL, and DL) of ECH_6 and $TMECH_6$ were prepared according to literature procedures[12,13] from racemic thiazolidine-4-carboxylic acid, and racemic 5,5-dimethylthiazolidine-4-carboxylic acid, respectively, and isolated as dihydrochloride salts. The isomeric mixtures are referred to subsequently as ECH_6·2HCl and $TMECH_6$·2HCl. $ReIO_2(PPh_3)_2$,[14] ReO(LL-ECH_3), and ReO(DD-$TMECH_3$)[3] were prepared as previously reported.

Treatment of $ReIO_2(PPh_3)_2$ with ECH_6·2HCl or $TMECH_6$·2HCl below pH 7 resulted in the formation of *syn*-DL and *anti*-DL isomers of ReO(DL-ECH_3) or ReO(DL-$TMECH_3$), respectively. Under high pH conditions (with 7 equiv of KOH), only the *syn*-DL isomer was obtained with ECH_6·2HCl; both *syn*- and *anti*-DL isomers were formed with $TMECH_6$·2HCl, but the *anti*-DL isomer was converted to the *syn*-DL isomer by heating the reaction solution at reflux overnight (see below). In all cases, the DD and LL isomers were formed and required separation from the DL products. The separations were achieved by cation-exchange column chromatography with Bio-Rad AG 50WX4 200-400 mesh analytical grade resin equilibrated with 1 N HCl.

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^{1}H NMR spectra were recorded with General Electric QE-300 or 500 or Nicolet NT 360 spectrometers in D_2O; chemical shifts (ppm) were referenced to (trimethylsilyl)propionic acid (TSP). UV−visible spectra were recorded on a Shimadzu 3101 spectrometer in D_2O. The pH (uncorrected) was adjusted with NaOD (2.2 M) and DCl (2.2 M). Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA.

Syntheses. ***syn*-ReO(DL-ECH$_3$) (1)**. $ReIO_2(PPh_3)_2$ (0.43 g, 0.5 mmol) was added to a solution of ECH_6·2HCl (0.17 g, 0.5 mmol) and KOH (0.20 g, 3.5 mmol) in 50% MeOH (40 mL). After the mixture was heated at reflux for 1 h, the resulting orange solution was cooled to 25 °C. Addition of concentrated HCl to pH 2 gave a rose-colored solution, which was reduced in volume to ∼10 mL by rotary evaporation and filtered. From the cation exchange column developed with 1 N HCl, the DD and LL isomers eluted first, followed by **1**. The eluent containing **1** was collected, concentrated to ∼10 mL and desalted by gel filtration (Sephadex G-15 column eluted with H_2O). The pink fraction was collected and evaporated to dryness. The residue was crystallized from H_2O by slow solvent evaporation. Violet crystals were collected and vacuum dried. Yield: 19 mg (8%). ^{1}H NMR in D_2O, pH 12.3: 2.79 (t, J = 12 Hz, 2H), 3.33 (dd, J = 11 Hz, 6 Hz, 2H), 3.50 (dd, J = 12 Hz, 5Hz, 2H), 3.59 (dd, J = 12 Hz, 5 Hz, 2H), 4.10 (dd, J = 11 Hz, 6 Hz, 2H). Anal. Calcd for $C_8H_{13}N_2O_5ReS_2$: C, 20.55; H, 2.80; N, 5.99. Found: C, 20.52; H, 2.81: N, 5.95.

***syn*-ReO(DL-TMECH$_3$)·H$_2$O (2)**. The procedure described for **1** was followed using $TMECH_6$·2HCl (0.20 g, 0.5 mmol) except that the reaction solution was heated at reflux overnight. From the cation exchange column, the DD and LL isomers and **2** were eluted with 1 N HCl and with H_2O, respectively. **2** was adsorbed by solid-phase extraction using Whatman SPE ODS-4 cartridges, primed with MeOH. The solid phase was washed with H_2O and eluted with MeOH. The pink eluent was evaporated to near dryness and pale pink microneedles were collected, washed with H_2O, and vacuum dried. Yield: 37 mg (14%). ^{1}H NMR in D_2O, pH 12.4: 1.34 (s, 6H), 1.79 (s, 6H), 3.19 (dd, J = 11Hz, 6Hz, 2H), 3.62 (s, 2H), 4.08 (dd, J = 11Hz, 6Hz, 2H). Anal. Calcd for $C_{12}H_{23}N_2O_6ReS_2$: C, 26.61; H, 4.28; N, 5.17. Found: C, 26.83; H, 4.14: N, 5.12.

Resonance Raman Spectroscopy. Resonance Raman measurements were made with samples (20 mM) in melting point capillaries with excitation at 406.7 nm from a krypton ion laser (Coherent Innova 100). Power at the samples was kept below 20 mW. Raman signals were collected via 90° geometry by a triple monochromator (Spex Model 1877 Triplemate) with a photodiode array detector consisting of a Model IRY-1024 detector and a Model ST-120 controller from Princeton Instruments which was interfaced to a microcomputer. Calibrations were performed for each measurement by using known Raman lines of toluene. Peak positions were reproducible to within ±1 cm^{-1} between runs. Typical resolution was 8 cm^{-1}. No changes (both spectral features and intensities) in the Raman spectra as a function of exposure time to the laser were observed for any sample. Titrations were recorded in D_2O. The pH (uncorrected) was adjusted with NaOD (2.2 M) and DCl (2.2 M).

FTIR Spectroscopy. The solution for FTIR spectroscopy was placed between calcium fluoride windows, and the spectrum was recorded with a Nicolet Magna-IR 560 spectrometer.

Results

All M(V)═O(N_2S_2) type complexes are expected to have the N_2S_2 donor set in the basal coordination plane. However, the neutral solid-state structure of *syn*-ReO(DL-ECH$_3$) (**1**), determined by X-ray diffraction methods, revealed an unusual ligand arrangement with an NOS_2 basal donor set and an axial N donor (Figure 1). This result was reported in a preliminary communication.[2] Below we describe the unique behavior of the solution forms of *syn*-ReO(DL-ECH$_3$) and *syn*-ReO(DL-TMECH$_3$) as observed by NMR, resonance Raman, and UV−visible spectroscopy.

NMR Spectroscopy. The ^{1}H NMR spectra of **1** and **2** were very pH dependent with considerable broadening and shifting of signals. Severe broadening is characteristic of the analogous complexes with chiral LL or DD ligands near neutral pH.[3]

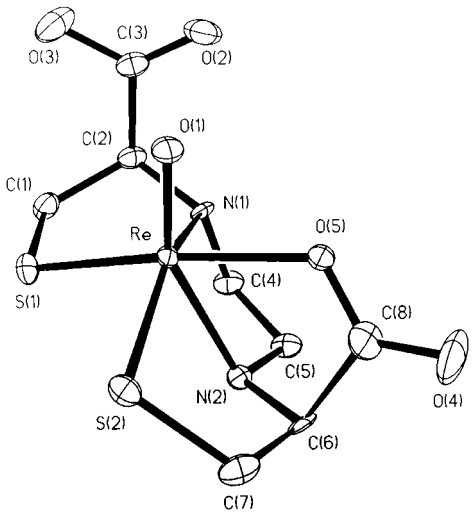


Figure 1. Perspective drawing of *syn*-ReO(DL-ECH$_3$) (**1**) with 50% probability for the thermal ellipsoids.

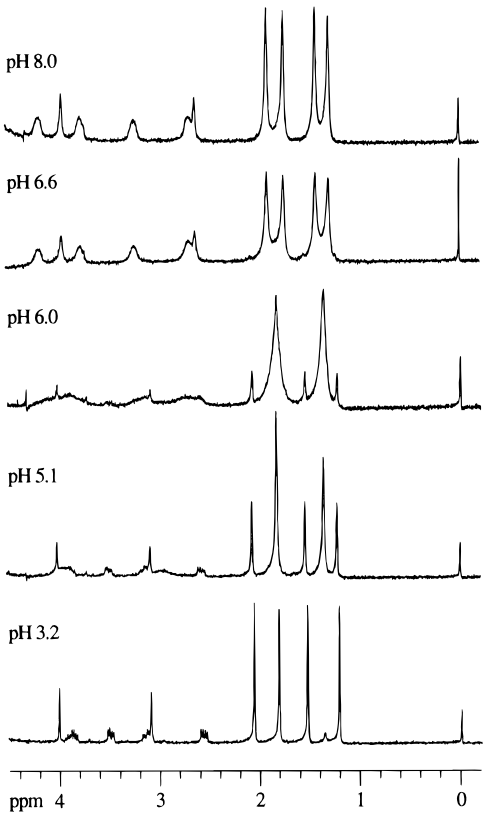


Figure 2. ^{1}H NMR spectra of *syn*-ReO(DL-TMECH$_3$) (**2**) in D_2O from pH (uncorrected) 3.2 to 8.0.

However, in contrast to the spectral behavior of the LL or DD analogs for which sharp spectra were found at low pH, the spectrum of **1** remained uninterpretably broad even at low pH values. The spectrum of **2** was more informative because the Me signals remained distinguishable throughout the experimental pH range (Figures 2 and 3).

The ^{1}H NMR spectrum of **2** at pH 3.2 is shown at the bottom in Figure 2. The signals are sharp and include two sets of equal intensity pen signals, each with one H_α and two Me signals, plus four ethylene bridge (H_{en}) signals. The signals (particularly the H_α signals) did not shift at higher pH values; thus, for **2** the pK_a of the dangling CO_2H is <3, and the species observed at pH 3.2 is the monoanionic form (**I**). In the monoanionic form of the complex, each pen residue is deprotonated at S and CO_2 but remains protonated at N. However, separate signals for the

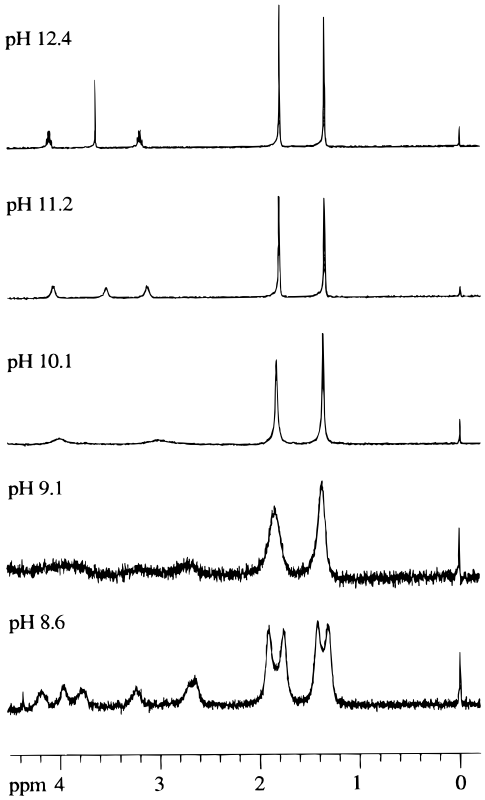


Figure 3. ^{1}H NMR spectra of *syn*-ReO(DL-TMECH$_3$) (**2**) in D_2O from pH (uncorrected) 8.6 to 12.4.

two halves of the TMEC ligand were observed, indicating chemically different environments for the two pen residues. This result suggests that the *trans*-NO,*cis*-NO,*cis*-S_2 structure (observed in the solid state for **1**, Figure 1) persists in solution at low pH.

With increasing pH, the signals of **I** declined in intensity, and signals of a new form (**II**) emerged. The pH dependence indicates that **II** is formed from **I** by reaction with 1 equiv of OH^-. Initially only two Me signals were observed for **II**. With increasing pH, these signals increased in intensity but also broadened. At pH 5.1, two very broad ethylene signals, at ~2.9 and ~3.9 ppm, are also attributable to **II**. Between pH 6.0 and 10.1, the signals of **II** collapsed and reemerged as two sets of equal intensity pen signals (Figure 2); these pen signals were sharpest near pH 7.5−8.0 and then broadened, collapsed, and reemerged as one set of signals (Figure 3). The positions of the pen signals at pH 10.1 were nearly identical to those at pH 5.1 (Figure 4), suggesting that **II** exists as one form on the NMR time scale over this pH range.

Above pH 10.1, some signals shifted, consistent with titration of an additional equivalent of OH^- to give a trianion (**II′**). The presence of only one set of signals indicates a fast **II**/**II′** equilibration on the NMR time scale.

Resonance Raman Spectroscopy. The Re(V)═O stretching frequency of **1** and **2** was monitored by resonance Raman spectroscopy as a function of pH in D_2O. Since the pH dependence of the forms of the analogs with chiral ligands, ReO(LL-ECH$_3$) and ReO(DD-TMECH$_3$), had been interpreted previously with other types of spectroscopic data, similar pH titrations with these species were monitored with resonance Raman spectroscopy. For all four complexes, different frequencies for the Re═O bands were observed in low-, mid-, and high-pH ranges (Table 1). For **1** and **2** these corresponded to the presence of **I**, **II**, and **II′**, respectively, observed by NMR methods.

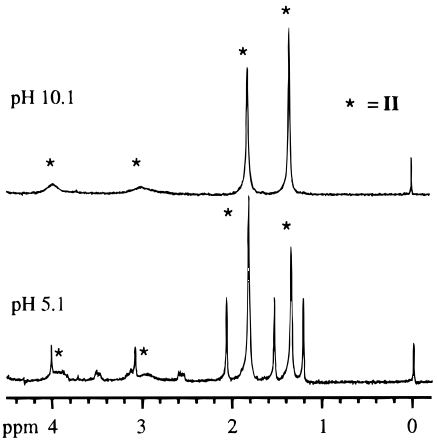


Figure 4. ^{1}H NMR spectra of *syn*-ReO(DL-TMECH$_3$) (**2**) in D_2O at pH (uncorrected) 5.1 and 10.1. The H_{en} signals at pH 10.1 are slightly downfield compared to the H_{en} signals at pH 5.1 because formation of **II′** has just begun to occur at pH 10.1.

Table 1. Resonance Raman Re(V)═O Stretching Frequencies (cm^{-1}) for ReO(ECH$_3$) and ReO(TMECH$_3$) Complexes in D_2O

complex	low pH	mid pH	high pH
syn-ReO(DL-ECH$_3$) (**1**)	952	935	889, 861
syn-ReO(DL-TMECH$_3$) (**2**)	951	942, 929	871
ReO(LL-ECH$_3$)	976	931	901, 882
ReO(DD-TMECH$_3$)	974	930	889, 874

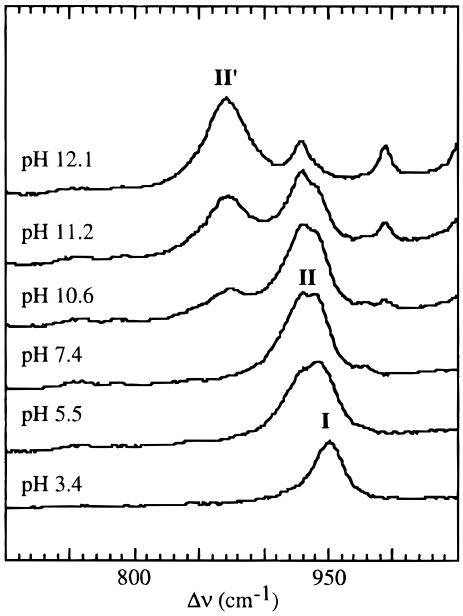


Figure 5. Resonance Raman spectra of *syn*-ReO(DL-TMECH$_3$) (**2**) in D_2O at various pH (uncorrected) values.

The frequencies of the Re═O band clearly differed among the low-pH forms of the four complexes (Table 1). The Re═O frequencies of **1** (952 cm^{-1}) and **2** (951 cm^{-1}) were similar but ≥20 cm^{-1} lower than those of the DD/LL complexes (974, 976 cm^{-1}).

Among the mid-pH forms of the four complexes, the Re═O band frequencies were more similar, differing by ≤13 cm^{-1} (929−942 cm^{-1}). For **2**, the mid-pH band (corresponding to **II**) was resolved into two peaks (929 and 942 cm^{-1}) (Figure 5). Between pH 6 and 10, the intensities of these two bands were approximately equal and did not change with pH. Small differences in the relative peak height of the two bands were observed below pH 6 and above pH 10 due to overlap with the bands of **I** and **II′**, respectively. Both bands of **II** were replaced

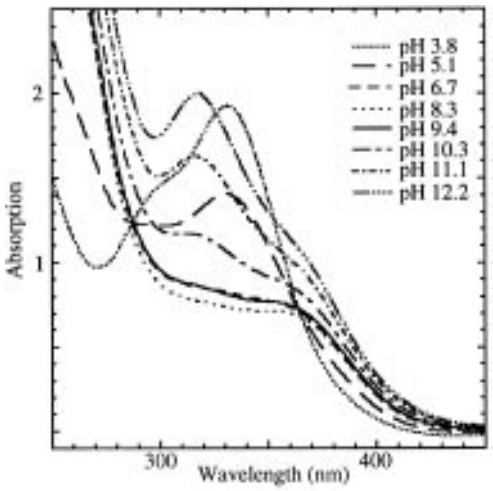


Figure 6. UV spectra of *syn*-ReO(DL-TMECH$_3$) (**2**) in D_2O at various pH (uncorrected) values (4.62×10^{-4} M: pH 3.8, $\lambda_{max} = 331$ nm, $\epsilon = 4.16 \times 10^3$ M^{-1}; pH 12.2, $\lambda_{max} = 319$ nm, $\epsilon = 4.23 \times 10^3$ M^{-1}).

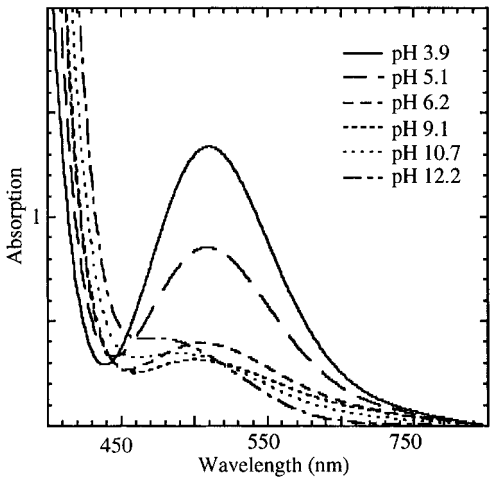


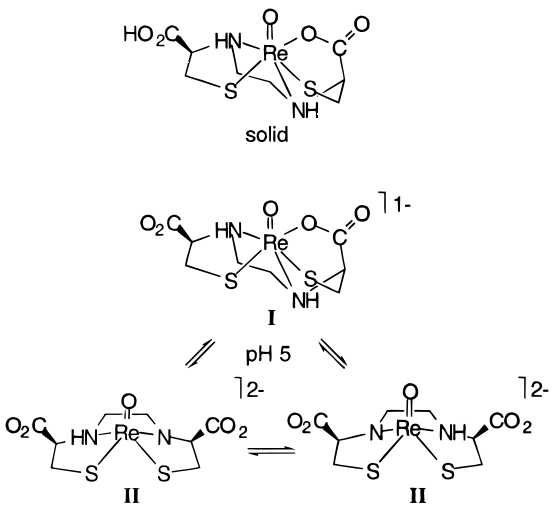
Figure 7. Visible spectra of *syn*-ReO(DL-TMECH$_3$) (**2**) in D_2O at various pH (uncorrected) values (9.23×10^{-3}M: pH 3.9, $\lambda_{max} = 511$ nm, $\epsilon = 145$ M^{-1}; pH 12.2, $\lambda_{max} = 477$ nm, $\epsilon = 45$ M^{-1}).

by overlapped bands (887 and 890 cm^{-1}, pH 7.5) following ^{18}O exchange. (^{18}O-Labeled **2** was prepared by converting *anti*-ReO(DL-TMECH$_3$) to **2** in $H_2{}^{18}O$ at high pH.) For **1**, the mid-pH band had a single peak; however, the line shape of the band was non-Lorentzian, indicating (as with **2**) a mixture of mid-pH forms (**II**).

UV−Visible Spectroscopy. UV and visible spectra of *syn*-ReO(DL-TMECH$_3$) (**2**) at various pH values are presented in Figures 6 and 7, respectively. In both the UV and visible regions, the absorption bands present below pH 4 (331 and 511 nm) decreased in intensity above pH 6. Between pH 6 and 10, the absorption bands were relatively weak and broad, and little change in shape and intensity of the bands occurred. Above pH 10, new absorption bands emerged (319 and 477 nm), which increased in intensity with increasing pH. Little change occurred near and above pH 12. The spectral changes were consistent with the presence of **I**, **II**, and **II′** observed by NMR and resonance Raman spectroscopy.

Main Features of the Solution Chemistry. It is well established that ReO(LL-ECH$_3$) and ReO(DD-TMECH$_3$) dissolve in aqueous solution as monoanions when the pH of the solution is just above their first pK_a (pH ∼4, deprotonation of the *syn*-CO_2H) (Scheme 1).[3] The monoanions have an N_2S_2 basal donor set and the *anti*-CO_2 group bound *trans* to the oxo ligand. In this low pH range, **1** and **2** are also monoanions (**I**). However,

Scheme 2



the relatively low frequencies of the Re═O bands of **1** and **2** (Table 1) at low pH indicate that form **I** has either a different donor atom set, or a different arrangement of donor atoms compared to the complexes with the chiral ligands. The FTIR spectrum of **1** in aqueous solution at pH 3.6 showed two C═O bands at 1640 and 1613 cm^{-1}. The presence of two well-separated C═O bands is consistent with the *trans*-NO,*cis*-NO,*cis*-S_2 (Scheme 2) ligand arrangement, as observed in the solid. Such a unique arrangement of donor atoms probably accounts for the relatively low Re═O band frequencies for form **I** of **1** and **2**.

Near pH 5, a mid-pH species (**II**) was formed by reaction of **I** of **1** and **2** with OH^-. **II** is a dianion. The mid-pH forms (**II**) of ReO(LL-ECH$_3$) and ReO(DD-TMECH$_3$) are also dianions (Scheme 1). The similarity of the Raman Re═O band frequencies of the mid-pH forms of **1**, **2**, ReO(LL-ECH$_3$), and ReO(DD-TMECH$_3$) indicates that the mid-pH form (**II**) of **1** and **2** has the same N_2S_2 basal coordination as form **II** of ReO(LL-ECH$_3$) and ReO(DD-TMECH$_3$) (Scheme 1). Thus, for the two *syn*-DL species, hydroxide promotes *syn*-CO_2 deligation and NOS_2 to N_2S_2 ligand rearrangement (Scheme 2). Such a ligand rearrangement process should be slower than a simple proton exchange process and should lead to a more symmetrical species in solution. The NMR spectra observed for **2** show that the formation of **II** from **I** is slow relative to the NMR time scale and that **II** appears to be a more symmetrical species than **I**. For **1**, the very broad spectra observed between pH 3 and 6 are consistent with an intermediate rate of NOS_2 to N_2S_2 ligand rearrangement on the NMR time scale.

For ReO(LL-ECH$_3$) and ReO(DD-TMECH$_3$),[3] the pH of the transition between the low- and mid-pH Raman bands, coincided with the pH for *anti*-CO_2 deligation (Scheme 1) ("pK_{a2}" ∼7). For **1** and **2**, the first transition occurred at a lower pH, near 5. Increased acidity in the *syn*-DL complexes may be caused by the net lower electron donation of the *meso* ligands due to coordination of the poorer CO_2 donor in the basal plane. As a consequence, the Re would be more electron deficient in **1** and **2** than in the complexes with the chiral ligands**.** The different pH dependence supports the conclusion that the basal coordination differs for the low pH form of **1** and **2** compared to ReO(LL-ECH$_3$) and ReO(DD-TMECH$_3$).

The ^1H NMR spectrum of **2** (Figures 2 and 3) changed considerably between pH 6 and 10. Despite the changes in the NMR spectrum of **2**, there was little change in the Raman spectra of **1** and **2** (Figure 5) and the UV−visible spectrum of **2** (Figures 6 and 7); these results demonstrate that no new

species was formed in significant concentration between pH 6 and 10. These results are discussed in depth below. At this point, it is sufficient to say that the NMR spectral changes indicate that **II** is fluxional.

At very high pH, our past studies show that ReO(LL-ECH$_3$) and ReO(DD-TMECH$_3$) form trianions (form **II′**).[3] The **II/II′** transition is fast on the NMR time scale for both. The NMR results for **2** and the Raman results show that the behavior of **1** and **2**, including even the pH of the **II/II′** transition, is exactly analogous to that of ReO(LL-ECH$_3$) and ReO(DD-TMECH$_3$). The nature of **II′** will be analyzed in a future report.[15] For the present study, we note that the similarities in the **II/II′** transition are further evidence that form **II** has N_2S_2 basal coordination for all four complexes.

Discussion

As described above, the effect of OH^-, which can either add as a ligand or deprotonate a coordinated ligand, is usually ambiguous. In this work, we have made observations that provide clear evidence that allows us to distinguish between these roles of hydroxide. Although our studies involved D_2O, the results apply to H_2O, and we will use protic species (e.g., NH, OH^-, etc.) in the discussion.

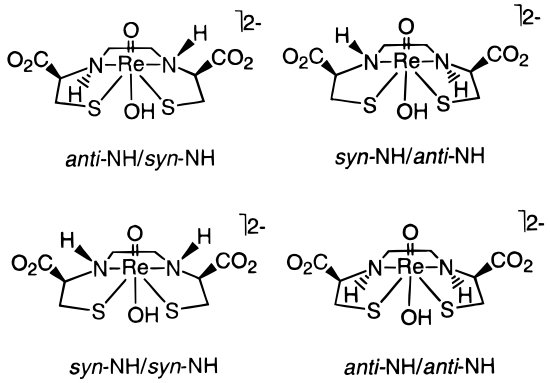
When a nitrogen donor anchors two adjacent chelate rings such that the three ligating atoms form a trigonal face of a coordination compound, the N stereochemistry is fixed. However, when the ligating atoms form a meridional edge, the N normally can have two stereochemistries. For the complexes studied here, the H on N can be either *syn* or *anti* to the oxo group. These stereochemistries can interconvert readily through inversion of the resulting N lone pair (NLp) formed by NH deprotonation. This interconversion may be too rapid for each species to be identified by such a relatively slow time scale method as NMR spectroscopy. The position of an NH site vs an NLp site is also normally difficult to define. Our studies provide evidence which allows us to suggest the probable N stereochemistry.

For **1** and **2**, if form **II** is a hydroxo complex, it can be symmetrical; however, if form **II** is an NH-deprotonated complex, it cannot be symmetrical since one N is protonated and the other is not. For **2**, form **II** at pH 5.1 (Figure 2) has one set of pen ^{1}H NMR signals for the two halves of the TMEC ligand. This result has two alternative interpretations: either form **II** is a hydroxo complex or it is a NH/NLp complex with one NH position in fast exchange between the two N's, resulting in time-averaged signals. Below, we show that for complexes of the *meso* EC-type ligands it is possible to determine which interpretation is correct. For the complexes with the chiral DD/LL EC-type ligands, the two halves of the coordinated ligand are not equivalent (Chart 2), and two sets of signals (one for each half of the EC ligand) would be observed, even with a rapid NH exchange rate. Previous studies have suggested that hydroxide does not coordinate and that the *anti*-N is deprotonated (Scheme 1).[3] However, this interpretation cannot be proved when the EC-type ligand is chiral.

Both the pH dependence of the NMR signals of form **II** of **2** and the presence of two Re═O bands can be explained by NH deprotonation. The simplest process consistent with the pH dependence of the ^{1}H NMR signals of **II** is shown in Scheme 2. The position of the NH equilibrates between the two N's. The rate of interconversion is catalyzed by both H^+ and OH^-; it is fast at pH ~5 and ~10, when the H^+ or OH^- concentration is high, but it is slow near neutral pH (~7.5−8.0).

(15) Hansen, L.; Yue, K. T.; Xu, X.; Taylor, A., Jr.; Marzilli, L. G. Manuscript in preparation.

Chart 3



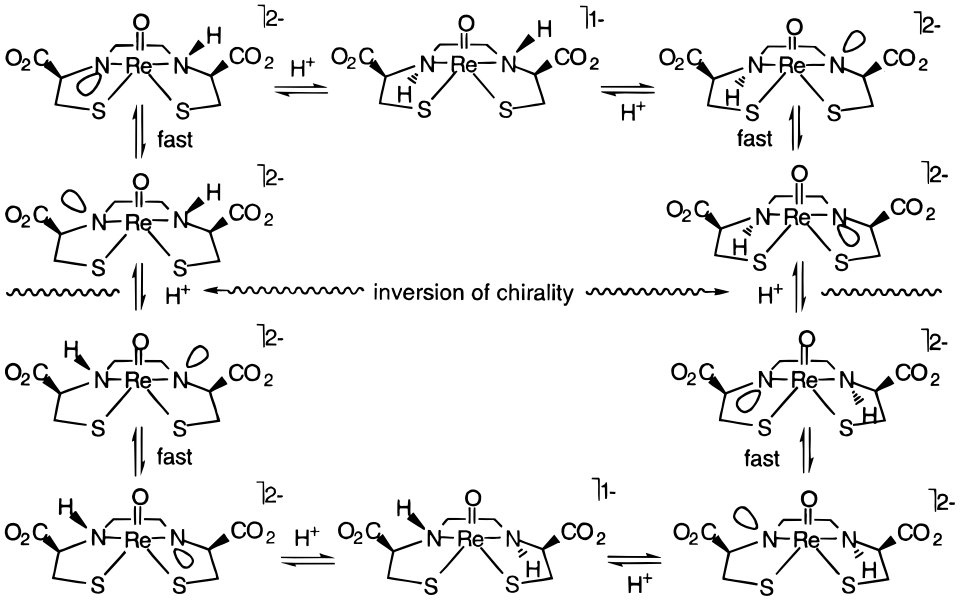
If form **II** is a hydroxo complex with N_2S_2 basal coordination, there are three conceivable geometric isomers for **1** and **2**; they differ in NH stereochemistry (Chart 3). Two of the isomers (with *anti*-NH/*anti*-NH and *syn*-NH/*syn*-NH stereochemistry, respectively) are symmetrical. For **2** these symmetrical isomers would each have only one set of pen signals. The other geometric isomer is dissymmetrical and exists as an enantiomeric pair with *syn*-NH/*anti*-NH and *anti*-NH/*syn*-NH stereochemistry; these enantiomers are indistinguishable by NMR spectroscopy and would have two equal intensity sets of pen NMR signals. Since in the mid-pH range there are two equal intensity sets of pen signals, the enantiomeric pair is a reasonable explanation for the observed NMR spectra. There are thus two explanations consistent with the NMR data: an NH-deprotonated species or the dissymmetrical OH^--ligated species.

On the basis of the NMR data, we cannot resolve the ambiguity about the role of hydroxide. However, the dissymmetrical OH^--ligated species can have only *one* Re═O band, but *two* were found for **2**. The non-Lorentzian line shape of the mid-pH Re═O band for **1** also indicates a mixture of forms. To use OH^- axial ligation for explaining both the two sets of pen signals and the two Re═O bands, one must invoke a coincidental 50:50 mixture of the symmetrical *anti*-NH/*anti*-NH and *syn*-NH/*syn*-NH geometric isomers. A 50:50 mixture of isomers is highly improbable because the *anti*-NH/*anti*-NH isomer would be less stable (see below). Furthermore, at lower pH, form **II** has only one set of pen signals, indicating a dynamic process averaging the signals. The two symmetrical geometric isomers would need to interconvert. For this interconversion to occur in a hydroxo-ligated species, both NH's would have to deprotonate sequentially and the NLp would need to invert during the deprotonated state of each N. Since NH deprotonation is favored at higher (not lower) pH, the averaging of signals at low pH, but not at intermediate pH, cannot be explained with a hydroxo-ligated complex.

Thus, for these *meso* EC-type complexes, we can rule out the hydroxo ligated forms. Form **II** must be an NH-deprotonated form (Scheme 2). The stereochemistry of the previously studied complexes with the chiral ligands, ReO(LL-ECH$_3$) and ReO(DD-TMECH$_3$) (Scheme 1), requires that there are always two sets of cys or pen signals, regardless of the role of OH^- or the rate of dynamic processes; however, the similar Re═O band frequencies of the dianions (compared to the *meso* EC-type complexes) strongly indicate that the ligands of the mid-pH form of ReO(LL-ECH$_3$) and ReO(DD-TMECH$_3$) are also NH deprotonated.

Insight into Additional Features of 1 and 2. Now we turn our attention to features of **1** and **2** that are more difficult to determine experimentally, namely the stereochemistry of the coordinated amines in **II**. Since one N is protonated and the

Scheme 3



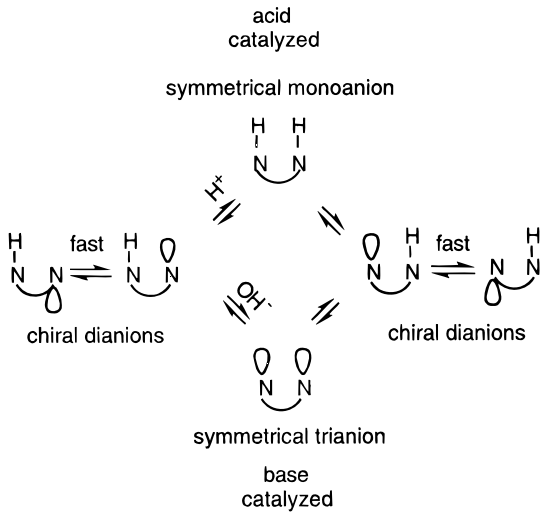
second N bears a Lp, four enantiomeric pairs of **II**, differing in the orientation of NH and NLp are possible, e.g. *syn*-NH/*syn*-NLp, *syn*-NH/*anti*-NLp, *anti*-NH/*syn*-NLp, and *anti*-NH/*anti*-NLp (Scheme 3).

At the corners of Scheme 3, we show the dianionic conformers derived from the structure of the neutral complex by removal of one NH proton and the CO_2H proton. Exchange of the single NH proton between the two N's through a protonated (NH/NH) monoanionic intermediate should be very fast on the NMR time scale, thus making the isomers indistinguishable by NMR methods. These species will be interconverted rapidly also by base catalysis (not shown in Scheme 3 for simplicity). If only the processes along the horizontal were occurring, we would see magnetic inequivalence of the two halves of the ligand. Under most conditions, we observe magnetic equivalence of the two ligand halves. Therefore, at least one of the two vertical processes (interconversion of the two species at the left corners or the two at the right corners) takes place.

Scheme 3 is divided into two parts: the top half has one set of enantiomers, and the bottom half the second set of enantiomers. Interconversion between the members of each pair (one pair at left side and the other at the right side) passes through the respective dianionic conformers with the same stereochemistry at both N's (*syn*-NH/*syn*-NLp on the left and *anti*-NH/*anti*-NLp on the right). Inversion of chirality occurs at this juncture by acid or base catalysis and involves intermediates not shown in Scheme 3. These additional intermediates are symmetrical, formed either by deprotonation of the dianions to form a trianion or by protonation to form a monoanion in base- and acid-catalyzed processes, respectively (Scheme 4). Therefore, inversion of chirality of the dianions with the same chirality at both N's (shown as acid catalyzed in Scheme 3 or stepwise in Scheme 4) involves simple proton exchange alone. Proton exchange is ordinarily very fast and unlikely to be observable on the NMR time scale. However, since the dianionic species are favored over a broad pH range, the concentrations of the symmetrical mono and trianionic intermediates are low. When the concentration of the catalyst (H^+ or OH^-) is low, interconversion between the NH and NLp sites is slow. Therefore, inversion of chirality is remarkably slow, leading to the observation of two sets of pen signals for **2**.

In Scheme 3, we show two pairs of dianionic enantiomers for each vertical. These could lead to as many as eight sets of

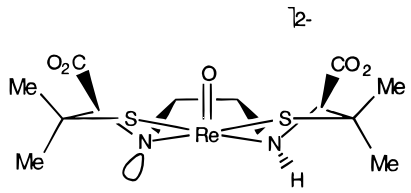
Scheme 4



pen signals. To understand why at most only two sets are observed, let us consider in more detail the interconversion processes along the vertical. In Scheme 4 we show generic species: if the oxo group on Re is up, Scheme 4 represents the left vertical of Scheme 3; and if the oxo group is down, it represents the right vertical. In both pathways, the NLp inverts, a process that should be fast and pH independent. As shown in Scheme 4, the NH position can then be transferred from one N to the other, either by protonation of the Lp followed by dissociation of the proton from the original NH site (acid-catalyzed pathway, top) or by deprotonation by OH^- followed by protonation at the original deprotonated site (base-catalyzed pathway, bottom). These processes should be facile when the concentration of either catalyst is high, and our evidence suggests that they both take place. However, equilibration between enantiomers slows when the concentration of catalyst is low, and since the dianions are nonsymmetrical, the two halves of the ligand become distinguishable by NMR. The ^1H NMR signals of the equilibrating diastereomers (the two on the left and the two on the right in Scheme 4) remain time averaged because interconversion between these conformers involves only NLp inversion, which is fast on the NMR time scale.

In Scheme 3, the pathways along the horizontal interconverting the two corner species at the top and the two at the

Chart 4



bottom are also acid and base catalyzed. Thus, we would also expect to see a pH dependence for these processes by NMR. However, if the horizontal processes do take place, they remain fast on the NMR time scale throughout the mid-pH range. Otherwise, we would see separate signals for the set of dianions at the right and those at the left of Scheme 3. Since this is not the case, there is no NMR evidence that the horizontal processes take place.

The Raman data also do not support the occurrence of the processes shown along the horizontal in Scheme 3. Only two resolved Re═O bands were observed for **2** between pH 5.5 and 10.6 (Figure 5). The relative intensities of the two bands were approximately equal and remained constant throughout the mid-pH range. The data demonstrate that there are two conformers of **II** and indicate that these conformers have nearly equal stability. This result is consistent with the occurrence of only one or the other of the processes along the vertical in Scheme 3: one Re═O band for the two enantiomeric dianions in the corners and the second band for the dianions in the middle of the Scheme. The relative intensities of the bands for these species remained constant as the pH was varied because the equilibrium involves only pH-independent inversion of the NLp.

The Raman data also help to decide which species are present (the set of dianions on the right or those on the left of Scheme 3). In the typical stable Re(V)═O compounds, the metal−oxo group is displaced to one side of the ligand(s) in the basal plane. Such a displacement is more readily accommodated by *syn*-N stereochemistry. The stereochemistry of the N's in the *anti*-NH/*anti*-NLp species (middle-right in Scheme 3) positions both ethylene C's (C_{en}'s) and both C_α's above the N_2S_2 plane (Chart 4). Displacement of the Re−oxo group away from the bulk of the TMEC ligand is not feasible. These enantiomers are likely to be highly disfavored. In the *anti*-NH/*syn*-NLp species, the positioning of one C_{en} and one C_α below the N_2S_2 plane allows the metal−oxo group to move away from the TMEC ligand. Therefore, we believe that the *syn*-NH/*anti*-NLp and the *syn*-NH/*syn*-NLp conformers (on the left in Scheme 3) account for the two Re═O bands of similar intensity.

Conclusions

In this study, we have illustrated the power of combining the rapid time scale method, resonance Raman spectroscopy, with the slower time scale method, NMR spectroscopy. Using these methods, we found that ReO(DL-$TMECH_3$) (**2**) has one predominant form between pH 6 and 10 with the usual N_2S_2 basal donors. However, this form has an unusual determinant of chirality, the position of a proton on one of two chemically equivalent N's. This chiral form undergoes a fluxional inversion of chirality that is both acid and base catalyzed. Under acidic or basic conditions, the fluxional process is fast on the NMR time scale. Under neutral conditions, the inversion rate is slow enough to demonstrate the chiral nature of the dianions. Raman spectroscopy detects the presence in the chiral form of two components; these maintain a constant ratio over a wide pH range and are thus conformers. The NMR results show that these conformers interconvert very rapidly. The only reasonable explanation for these combined spectroscopic results is that the conformers differ only in the stereochemistry of the deprotonated N. The rate of inversion at this nitrogen is slow on the Raman time scale but too rapid for detection by the NMR method. Similar fluxional processes probably occur for other related $M(V)ON_2S_2$ radiopharmaceuticals. However, the stereochemistry of these other complexes, e.g., the analogous complexes with chiral ligands (ReO(LL-ECH_3) and ReO(DD-$TMECH_3$)),[3] or the rate of the fluxional processes generally preclude the type of analysis possible in the current study. Our combined stereochemical and multispectroscopic strategy allows us to eliminate the ambiguity between hydroxo ligation and NH deprotonation, an ambiguity that plagues the study of metal compounds in solution.

Our work was motivated by an interest in radiopharmaceuticals, but other classes of metal compounds could be examined by the dual spectroscopic approach described here. The combined methodology is quite powerful, and recently the prediction from NMR methods that coenzyme B_{12}[16] in solution was a mixture of two rapidly interconverting conformers has been strongly supported by specialized resonance Raman spectroscopic methods.[17]

With NMR and Raman methods, we also found that ReO(DL-ECH_3) (**1**) and ReO(DL-$TMECH_3$) (**2**) have an NOS_2 basal donor set in aqueous solution below pH 5; such an unusual arrangement was established previously only for **1** in the solid state.[2] Near pH 5, NH deprotonation, CO_2 deligation, and ligand rearrangement yield dianionic species with the usual N_2S_2 basal donors.

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