

Coordination Modes of Multidentate Ligands in *fac*-[Re(CO)₃(polyaminocarboxylate)] Analogues of ^{99m}Tc Radiopharmaceuticals. Dependence on Aqueous Solution Reaction Conditions

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Received September 3, 2009

We study Re analogues of ^{99m}Tc renal agents to interpret previous results at the ^{99m}Tc tracer level. The relative propensities of amine donors versus carboxylate oxygen donors of four L = polyaminocarboxylate ligands to coordinate in *fac*-[Re^I(CO)₃L]ⁿ complexes were assessed by examining the reaction of *fac*-[Re^I(CO)₃(H₂O)₃]⁺ under conditions differing in acidity and temperature. All four L [*N,N*-bis-(2-aminoethyl)glycine (DTGH), *N,N*-ethylenediaminediacetic acid, diethylenetriamine-*N*-malonic acid, and diethylenetriamine-*N*-acetic acid] can coordinate as tridentate ligands while creating a dangling chain terminated in a carboxyl group. Dangling carboxyl groups facilitate renal clearance in *fac*-[^{99m}Tc^I(CO)₃L]ⁿ agents. Under neutral conditions, the four ligands each gave two *fac*-[Re^I(CO)₃L]ⁿ products with HPLC traces correlating well with known traces of the *fac*-[^{99m}Tc^I(CO)₃L]ⁿ mixtures. Such mixtures are common in renal agents because the needed dangling carboxyl group can compete for a coordination site. However, the HPLC separations needed to assess the biodistribution of a single tracer are impractical in a clinical setting. One goal in investigating this Re chemistry is to identify conditions for avoiding this problem of mixtures in preparations of *fac*-[^{99m}Tc^I(CO)₃L]ⁿ renal tracers. After separation and isolation of the *fac*-[Re^I(CO)₃L]ⁿ products, NMR analysis of all products and single crystal X-ray crystallographic analysis of both DTGH products, as well as one product each from the other L, allowed us to establish coordination mode unambiguously. The product favored in acidic conditions has a dangling amine chain and more bound oxygen. The product favored in basic conditions has a dangling carboxyl chain and more bound nitrogen. At the elevated temperatures used for simulating tracer preparation, equilibration was facile (ca. 1 h or less), allowing selective formation of one product by utilizing acidic or basic conditions. The results of this fundamental study offer protocols and guidance useful for the design and preparation of *fac*-[^{99m}Tc^I(CO)₃L]ⁿ agents consisting of a single tracer.

Introduction

Tracers containing the ^{99m}Tc radionuclide are widely used in clinical nuclear medicine.^{1,2} Our goal of developing ^{99m}Tc radiopharmaceuticals with a rapid renal clearance has led us to seek ^{99m}Tc agents having ligands with a dangling

(uncoordinated) carboxyl group,^{3–8} because the interaction of the renal receptor with the carboxyl group is important for clearance of small peptides.^{9–11} Many ligands have been investigated that form robust ^{99m}Tc^{VO} anionic compounds with one or two dangling carboxyl groups.^{2,9,12–14} Such ligands pose a challenge because the carboxyl group can coordinate under some circumstances.¹⁵ The design of these

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$^{99m}\text{Tc}^{\text{VO}}$ tracers has been aided by the evaluation of the inorganic chemistry of the Re^{VO} analogues.^{9,15–24} The use of the Re analogue approach has provided well-defined chemical information that has facilitated the identification of $^{99m}\text{Tc}^{\text{VO}}$ agents with excellent renal clearance in humans; however, $^{99m}\text{Tc}^{\text{VO}}$ tracers do not have ideal properties.^{13,14,25–29}

In recent years, technetium and rhenium complexes in low oxidation states have attracted significant attention.^{30–32} In particular, the $\text{fac}\{-\text{M}^{\text{I}}(\text{CO})_3\}^+$ core (M = Re, ^{99m}Tc) has been assessed because of its small size, its low-spin d^6 electronic configuration, and the high chemical and kinetic stability of its complexes.³⁰ These useful properties of the $\text{fac}\{-\text{M}^{\text{I}}(\text{CO})_3\}^+$ core allow the labeling of low-molecular-weight biomolecules attached through the three remaining facial sites available for substitution to produce agents having high specific activities.^{30,33–38} A convenient kit described by Alberto and colleagues for generating solutions of the $\text{fac}\{-^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3\}^+$ cation is now commercially available.^{39,40} The uncharged aqua ligands in the $\text{fac}\{-^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3\}^+$

precursor can be readily substituted by a wide variety of ligating groups, including amines, imines, thioethers, thiols, phosphines, and charged oxygen donors (carboxylates, phosphates, and phosphonates).^{4,7,41–51} Agents exhibiting the most favorable stability and pharmacokinetics have tridentate chelating ligands.^{42,44,52}

We have been investigating the possibility that this relatively new core will allow us to develop renal agents with properties superior to those of the widely used agents with the $^{99m}\text{Tc}^{\text{VO}}$ core.^{5–8,53} To explore the analogous Re chemistry, we recently developed a reliable and straightforward preparation of an aqueous solution of the $\text{fac}\{-\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3\}^+$ cation.⁴ It is synthetically convenient to utilize polyamino-carboxylic acid ligands with acetate groups. Such ligands, including amino acid analogues, readily form stable complexes with the $\text{fac}\{-\text{M}^{\text{I}}(\text{CO})_3\}^+$ core.^{3,43,54,55} On the other hand, the catholic nature of the binding ability of this core raises concerns about the core's coordination preferences for various donor atoms,^{43,47} especially the relative preference for amine groups versus carboxylate groups.^{56,57}

We initiated our studies with the new $\text{fac}\{-^{99m}\text{Tc}^{\text{I}}(\text{CO})_3\}^+$ core by using polyaminocarboxylate ligands (L).^{3,58,59} Treating $\text{fac}\{-^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3\}^+$ with L formed robust $\text{fac}\{-^{99m}\text{Tc}(\text{CO})_3\text{L}\}^-$ anionic agents having a free carboxyl group useful for renal targeting, but often a mixture of two major anionic agents formed in a ratio dependent on preparative conditions.⁵⁹ Therefore, we decided to utilize the Re analogue approach to investigate how temperature and acidic/neutral/basic conditions affected the formation and properties of $\text{fac}\{-\text{Re}(\text{CO})_3\text{L}\}^-$ analogues, primarily with *N,N*-bis(2-aminoethyl)glycine (DTGH, **1**), but also with *N,N*-ethylenediaminediacetic

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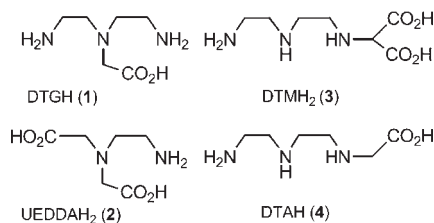
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Chart 1



acid (UEDDAH₂, **2**), diethylenetriamine-*N*-malonic acid (DTMH₂, **3**), and diethylenetriamine-*N*-acetic acid (DTAH, **4**), Chart 1. Note that the L in Chart 1 exist as zwitterions, and the H_x in the names designates the number of dissociable protons. These L contain N and O donor groups appropriately oriented so as to allow facial, tridentate coordination, while at the same time having the potential to bind via different coordination modes (NNN vs NNO; NNO vs ONO). These ligands contain combinations of ethylene amino groups and acetate groups; the groups are attached to N donors in such a way that only five-membered chelate rings can form and such that any dangling group is anchored to Re via an N donor. Note that because all tricarbonyl complexes used or made here have a facial geometry, *fac*- is not used for specific tricarbonyl compounds but is used in general designations such as *fac*-[Re(CO)₃L]ⁿ or *fac*-[^{99m}Tc(CO)₃L]ⁿ.

Experimental Section

DTGH (**1**),⁶⁰ UEDDAH₂ (**2**),⁶¹ DTAH (**4**)⁶⁰ and potassium bromomalonate⁶² were synthesized according to published methods. The trifluoroacetate of diprotonated DTGH (DTGH·2CF₃CO₂H) was obtained during the purification process and was used as a crude product for the next step. An aqueous solution of the precursor, [Re(CO)₃(H₂O)₃]OTf (OTf = triflate anion), was prepared as reported elsewhere.⁴ All solvents were purchased from Aldrich and were used without purification. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA. ¹H NMR spectra were recorded on Varian INOVA 400 or 600 MHz spectrometers and referenced to internal sodium 3-(trimethylsilyl)propionate-*d*₄. HPLC analyses (monitored at 254 nm) were performed on a Waters Breeze system equipped with a Waters 2487 dual wavelength absorbance detector, Waters 1525 binary pump, and XTerra MS C18 column (5 μm; 4.6 × 250 mm). HPLC solvents consisted of the buffer [0.05 M TEAP (aqueous triethylammonium phosphate) at pH 2.5, solvent A] and methanol [solvent B]. The HPLC system started with 100% A from 0 to 3 min. The eluent switched at 3 min to 75% A/25% B, at 6 min to 66% A/34% B, and remained for 3 more min, followed by linear gradients: 66% A/34% B to 34% A/66% B from 9 to 20 min; and 34% A/66% B to 100% A from 20 to 30 min. The flow rate was 1 mL/min. An Orion Research Digital Ion-analyzer/501 equipped with a glass/calomel ultrathin longstem combination pH electrode (Z11,343-3, Aldrich) was used to measure the pH of NMR solutions, including solutions being monitored over time.

Re(CO)₃(DTG) Products (5 and 6). A solution of [Re(CO)₃(H₂O)₃]OTf (2.5 mL, 0.1 M) was added to a solution of DTGH·2CF₃CO₂H (0.097 g, 0.25 mmol) in 2 mL of water,

and the pH of this equimolar reaction mixture was adjusted to 3.5 or (in a single experiment to assess pH effects) to pH 7.5. Normally for reaction mixtures, 1 N NaOH or HCl was used to adjust the pH, which was determined by using the Orion Research pH meter or EDM colorpHast pH test strips. The solution was heated at reflux, and the progress of the reaction was monitored by high-performance liquid chromatography (HPLC). Two products, **5** ([Re(CO)₃(DTGH)-NNO]⁺) and **6** ([Re(CO)₃(DTG)-NNN]), formed, having HPLC retention times (RT) of 3.8 min (**5**) and 7.6 min (**6**). Ratios of **5/6** of ~90:10 at pH 3.5 and ~45:55 at pH 7.5 were observed after 1 h of heating. Reactions in both acidic and basic conditions were repeated several times under similar pH conditions (±0.3 pH units) and the reported ratio of products was based on the average peak ratio, which did not change significantly with time.

5 (NNO Ligand Binding Mode). Solid KPF₆ (5 mg, 0.25 mmol) was added (in pH 3.5 reactions), and the mixture was stirred until the solid dissolved completely. After filtration, the solution was passed through a Sephadex G-15 column, and the products were eluted with deionized water. Eluted fractions containing only the NNO isomer were collected and concentrated to dryness by rotary evaporation, yielding a white solid that was recrystallized from water. Yield, 0.13 g (86%) of pure **5** as the [Re(CO)₃(DTGH)-NNO]PF₆ salt, designated hereafter as the **5** PF₆ salt. Anal. Calcd for C₉H₁₅F₆N₃O₃PRE·H₂O: C, 18.17; H, 2.86; N, 7.06. Found: C, 18.39; H, 2.86; N, 7.12. ¹H NMR [δ (ppm), DMSO-*d*₆]: 2.47 (m, 1H), 2.65 (d, 1H), 2.90 (m, 1H), 3.05 (m, 1H), 3.16 (m, 2H), 3.43 (m, 1H), 3.64 (d, 2H), 3.66 (m, 1H), 4.91 (m, 1H), 5.14 (m, 1H) 7.74 (bs, 3H). [The **5** PF₆ salt was obtained mainly for the X-ray crystal structure determination and elemental analyses. For other low-pH reactions **5** was isolated as a white solid (**5** Cl salt) from low pH reaction mixtures (pH adjusted with 1 N HCl) in the same manner as described above but without the addition of KPF₆.]

6 (NNN Ligand Binding Mode). Pure **6** was isolated when the **5** Cl salt was dissolved in water at pH 12 and the solution was kept at room temperature overnight. White crystals of the neutral NNN product **6** ([Re(CO)₃(DTG)-NNN], neutral solid) were collected, washed with a small amount of water, and vacuum-dried. Anal. Calcd for C₉H₁₄N₃O₃Re·H₂O: C, 24.10; H, 3.60; N, 9.37. Found: C, 24.06; H, 3.57; N, 9.39. ¹H NMR [δ (ppm), D₂O, pH 8.7]: 3.17 (m, 4H), 3.26 (m, 4H), 4.12 (s, 2H). Solids of **5** (PF₆ and Cl forms) and **6** (neutral form) are slightly soluble in water.

Re(CO)₃(UEDDA) Products (7 and 8). **8 (NNO Ligand Binding Mode).** [Re(CO)₃(H₂O)₃]OTf (5 mL, 0.1 M) was added to a solution of UEDDAH₂ (0.081 g, 0.50 mmol) in 5 mL of water, and the pH of the equimolar reaction mixture was adjusted to 7 with 1 N NaOH. The clear pH 7 solution was stirred at 30 to 40 °C. The reaction progress was monitored by HPLC, which showed that the precursor was consumed after 3 h, and one sharp peak was observed (RT = 14.5 min). The solution was concentrated to 2 to 3 mL by rotary evaporation and desalted on a Sephadex G-15 column (eluted with deionized water). The fractions collected were concentrated to dryness by rotary evaporation and then dried under vacuum to give **8**, most probably as a sodium salt (Na[Re(CO)₃(UEDDA)-NNO], NNO ligand binding mode), in 90% yield (0.210 g). A small amount of this white solid was dissolved in 2 mL of water/NaOH; the solution was filtered, the pH was adjusted to ~1 with 1 N HCl, and the solution was allowed to stand at 0 to 5 °C. Crystals of **8**, as the neutral [Re(CO)₃(UEDDAH)-NNO] zwitterion with a protonated carboxylic group, formed overnight and were suitable for X-ray crystallography. Anal. Calcd for C₉H₁₁N₂O₇Re: C, 24.27; H, 2.49; N, 6.29. Found: C, 24.35; H, 2.46; N, 6.27. ¹H NMR [δ (ppm), D₂O, pH 7.0]: 2.72 (m, 1H), 2.99 (m, 1H), 3.14 (m, 1H), 3.40 (m, 1H), 3.96 (d, 1H, *J* = 16.4 Hz), 4.02 (d, 1H, *J* = 17.6 Hz), 4.14 (mb, 1H), 4.18 (d, 1H, *J* = 17.6 Hz), 4.21 (d, 1H, *J* = 16.4 Hz), 4.88 (mb, 1H).

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7 (ONO Ligand Binding Mode). When the above preparative procedure was carried out at pH 3 to 4, instead of at pH 7, HPLC analysis showed that the precursor was consumed after ~4 h, and one sharp major peak (RT = 8.8 min, 96%) was observed. The white solid that precipitated from the reaction mixture overnight was collected by filtration, washed with a small amount of cold water, and dried under vacuum. Yield, 0.15 g (67%). Anal. Calcd for $C_6H_{11}N_2O_7Re \cdot H_2O$: C, 23.33; H, 2.83; N, 6.05. Found: C, 23.25; H, 2.83; N, 5.95. Elemental analysis suggests that **7** is the neutral $[Re(CO)_3(UEDDAH)(ONO)]$ zwitterion, with a protonated amino group, and an isomer of **8**. 1H NMR (δ (ppm), D_2O , pH 4.0) analysis showed that the product has an ONO ligand binding mode (**7**: 3.41 (m, 2H), 3.72 (overlapping d, $J = 16.8$ Hz, 2H and m, 2H), 3.93 (d, 2H, $J = 16$ Hz)). All attempts to obtain crystals of **7** suitable for X-ray crystallography were unsuccessful.

Re(CO)₃(DTM) Products (9 and 10). To prepare diethylenetriamine-*N*-malonic acid (DTMH₂, **3**), an aqueous solution of potassium bromomalonate (4.43 g, 17.1 mmol, 5 mL) was added dropwise to an aqueous solution (5 mL) of diethylenetriamine (1.85 mL, 17.1 mmol) at 25 °C. The pH of the reaction mixture was adjusted to 10 with 2.5 N HCl, and the reaction mixture was maintained at 60 °C and pH 10 for 2.5 h before it was cooled to 25 °C. The solution was acidified with concentrated HCl (pH ~4) and left overnight at 0 to 5 °C. After a white precipitate formed, the mixture was centrifuged, the supernatant liquid removed, and the precipitate was washed with cold water and vacuum-dried; yield, 1.78 g (51%). The 1H NMR spectrum in D_2O (pH 6) indicated that the solid contained ~80% of **3**. Although an elaborate purification method gave product with much cleaner NMR spectra (1H NMR [δ (ppm), D_2O , pH 3.5]: 3.39 (t, $J = 6.6$ Hz, 2H), 3.44 (m, 4H), 3.52 (t, $J = 6.0$ Hz, 2H); ^{13}C NMR [δ (ppm), D_2O , pH 4.3]: 41.3 (NCH₂), 47.0 (NCH₂), 47.9 (NCH₂), 48.1 (NCH₂), 69.4 (CH), 172.2 (CO₂H)), the crude product was sufficiently pure for use in the next step.

$[Re(CO)_3(H_2O)_3]OTf$ (8.2 mL, 0.1 M) was added to a solution of DTMH₂ (0.168 g, 0.82 mmol) in 15 mL of water, and the pH of the reaction mixture was adjusted to 7 with 1 N NaOH. The solution was stirred at 50 to 60 °C for 2 h. HPLC analysis of the solution showed two peaks with RT = 3.5 min for **9** ($[Re(CO)_3(DTMH)-NNO]$) and RT = 6.8 min for **10** ($[Re(CO)_3(DTM)-NNN]$). 1H NMR (D_2O) analysis of the reaction mixture at pH 7 showed a **9**:**10** ratio of 45:55.

9 (NNO Ligand Binding Mode). Pure **9** as the neutral $[Re(CO)_3(DTMH)-NNO]$ product (**9** neutral) was obtained by adjusting the pH of the reaction mixture to 3.5 with 1 N HCl and stirring the mixture at room temperature for 30 min. The solution was then concentrated to 2–3 mL by rotary evaporation and desalted on a Sephadex G-15 column (eluted with deionized water). The product fractions collected were evaporated to dryness to give **9** neutral as a white solid; yield, 0.162 g (42%). Crystals suitable for X-ray crystallography were obtained by crystallization from water. Anal. Calcd for $C_{10}H_{14}N_3O_7Re$: C, 25.32; H, 2.97; N, 8.86. Found: C, 25.14; H, 3.30; N, 9.06. 1H NMR [δ (ppm), D_2O , pH 6.0]: 2.34 (m, 1H), 3.09–3.26 (m, 6H), 3.43 (m, 1H), 4.01 (s, 1H), 5.75 (m, 1H), 6.15 (s, 1H).

10 (NNN Ligand Binding Mode). Pure **10** was obtained by adjusting the pH of the reaction mixture to 10 with 1 N KOH and stirring at room temperature for 1 h. After the solution was concentrated to 2 to 3 mL and desalted on a Sephadex G-15 column (eluted with deionized water), the fractions containing product were collected and evaporated to dryness. The residue was treated with ethanol (10 mL), and the mixture was kept overnight at 0–5 °C. The white solid that formed was separated by decantation and dried; yield, 0.24 g (55%) as the $K[Re(CO)_3(DTM)-NNN]$ salt. Anal. Calcd for $C_{10}H_{13}KN_3O_7Re \cdot H_2O$: C, 22.63; H, 2.92; N, 7.92. Found: C, 22.93; H, 2.96; N, 7.90. 1H NMR [δ (ppm), D_2O , pH 10.5]: 2.66 (m, 1H), 2.75 (m, 1H), 2.96 (m, 1H), 3.05 (m, 2H), 3.12 (dd, 1H), 3.24 (m, 1H), 3.45 (m, 1H),

4.12 (s, 1H). The spectrum lacked the very characteristic upfield signal of **9** at 2.34 ppm; thus, **9** (the product related to **10** but with the NNO coordination mode) was not formed under these conditions. Compound **10** is freely soluble in water. All attempts to obtain crystals of **10** suitable for X-ray crystallography were unsuccessful.

Re(CO)₃(DTA) Products (11 and 12). During the purification of **10** on a Sephadex G-15 column, several fractions collected contained a small amount of a product with the rhenium tricarbonyl moiety and having an HPLC RT of 7.8 min. Water was removed by rotary evaporation to give a white solid (**12**, $[Re(CO)_3(DTA)-NNN]$). 1H NMR [δ (ppm), D_2O , pH 7.0]: 2.66 (m, 1H), 2.81 (m, 1H), 2.96 (m, 3H), 3.08 (m, 1H), 3.24 (m, 1H), 3.37 (m, 1H), 3.77 (dd, 2H), 4.03 (m, 1H), 4.22 (m, 1H), 5.17 (m, 1H), 6.55 (s, 1H). Crystals suitable for X-ray structural determination were obtained by crystallizing a few milligrams of the crude product from methanol. An aqueous solution of **12** kept at pH ~2 overnight showed no 1H NMR spectral changes. When equimolar amounts (0.5 mmol) of the DTAH (**4**) ligand and $[Re(CO)_3(H_2O)_3]OTf$ in 5 mL of water were heated at pH ~6–7 and 70 °C for 2 h, the HPLC analysis of the solution showed two peaks with RT = 3.6 min and RT = 7.8 min in a 48:52 ratio. Preparative HPLC afforded only a few milligrams of each product. The 1H NMR spectrum of the product with RT = 7.8 min was identical with that of **12** ($[Re(CO)_3(DTA)-NNN]$). The 1H NMR (D_2O , pH 3.0) analysis of the product with RT = 3.6 min (**11**) showed that the ligand in this product has the NNO ligand binding mode; 1H NMR [δ (ppm), D_2O , pH 3.0]: 2.36 (m, 1H), 2.98 (m, 1H), 3.08 (m, 4H), 3.28 (m, 2H), 3.53 (d, 1H, $J = 17.6$ Hz), 3.91 (dd, 1H, $J = 17.6$, 7.6 Hz), 5.75 (bs, 1H), 6.50 (bs, 1H)). We are confident that **11** is ($[Re(CO)_3(DTAH)-NNO]^+$). However, all attempts to isolate **11** in a pure form for the full characterization were unsuccessful.

X-ray Crystal Structural Determination. Suitable crystals of **5**, **6**, **8**, **9**, and **12** were coated with Paratone N oil, suspended in a small fiber loop, and placed in a cooled nitrogen gas stream at 100 K on a Bruker D8 SMART APEX CCD sealed tube diffractometer with graphite monochromated $Cu \text{ K}\alpha$ (1.54178 Å) radiation (for **5**, **6**, **8**, **12**) or $Mo \text{ K}\alpha$ (0.71073 Å) radiation (for **9**). Data were measured by using a series of combinations of phi and omega scans with 10 s frame exposures and 0.5° frame widths. Data collection, indexing, and initial cell refinements were all carried out with APEX 2⁶³ software. SAINT⁶⁴ software was used for frame integration and final cell refinements. The SADABS⁶⁵ program was used to carry out absorption corrections.

The structures were solved by using direct methods and difference Fourier techniques (SHELXTL, V5.10).⁶⁶ All non-hydrogen atoms were located in difference Fourier maps and refined anisotropically except for the N and O atoms in **5**; the C and N atoms in **6**; the C, O4 and O5 atoms in **8**; C9 in **9**; and C1s and O1s in **12**. Hydrogen atoms were placed in their expected chemical positions by using the HFIX command and were included in the final cycles of least-squares with isotropic U_{ij} 's related to the atoms ridden upon. Scattering factors and anomalous dispersion corrections were obtained from the *International Tables for X-ray Crystallography*.⁶⁷ Structure solution, refinement, graphics, and generation of publication materials were performed by using SHELXTL, V5.10 software. Crystal data and refinement parameters for **5**, **6**, **8**, **9**, and **12** are listed in Table 1. Selected bond distances and angles appear in Table 2.

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(64) Bruker SAINT; Bruker AXS, Inc.: Madison WI, 2007.

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Table 1. Crystal Data and Structure Refinement for [Re(CO)₃(DTGH)-NNO]PF₆·H₂O (**5** PF₆·H₂O), [Re(CO)₃(DTG)-NNN]·H₂O (**6**·H₂O), [Re(CO)₃(UEDDAH)-NNO] (**8**), [Re(CO)₃(DTMH)-NNO]·2H₂O (**9**·2H₂O), and [Re(CO)₃(DTA)-NNN]·CH₃OH (**12**·CH₃OH)

	5 PF ₆ ·H ₂ O	6·H ₂ O	8	9·2H ₂ O	12·CH ₃ OH
empirical formula	C ₉ H ₁₅ F ₆ N ₃ O ₆ Re	C ₉ H ₁₄ N ₃ O ₆ Re	C ₉ H ₁₁ N ₂ O ₇ Re	C ₁₀ H ₁₈ N ₃ O ₉ Re	C ₁₀ H ₁₈ N ₃ O ₆ Re
<i>f</i> w	592.41	446.43	445.40	510.47	462.47
<i>T</i> (K)	173 (2)	100 (2)	173 (2)	100 (2)	173 (2)
<i>λ</i> (Å)	1.54178	1.54178	1.54178	0.71073	1.54178
crystal system	monoclinic	monoclinic	orthorhombic	monoclinic	monoclinic
space group	<i>P</i> 2 ₁ / <i>c</i>	<i>P</i> 2 ₁ / <i>n</i>	<i>P</i> na2 ₁	<i>P</i> 2 ₁ / <i>n</i>	<i>P</i> 2 ₁ / <i>n</i>
unit cell dimensions					
<i>a</i> (Å)	9.7966 (4)	7.8847 (10)	11.8855 (5)	10.241 (9)	8.2324 (9)
<i>b</i> (Å)	12.3907 (5)	12.7776 (2)	7.7212 (3)	13.256 (11)	12.6243 (13)
<i>c</i> (Å)	14.4301 (5)	13.4044 (2)	13.6549 (6)	11.602 (11)	13.6879 (14)
<i>β</i> (deg)	107.363 (2)	94.2740 (10)	90	92.37 (2)	96.321 (14)
<i>V</i> (Å ³)	1671.81 (11)	1346.70 (3)	1253.11 (9)	1574 (2)	1413.9 (3)
<i>Z</i>	4	4	4	4	4
<i>ρ</i> _{calc} (mg/m ³)	2.354	2.202	2.361	2.155	2.173
<i>μ</i> (mm ⁻¹)	16.086	17.929	19.309	7.773	17.103
<i>R</i> indices [<i>I</i> > 4σ(<i>I</i>)] ^a	R1 = 0.0633 wR2 = 0.2006	R1 = 0.0756 wR2 = 0.2629	R1 = 0.0230 wR2 = 0.0583	R1 = 0.0946 wR2 = 0.2390	R1 = 0.0252 wR2 = 0.0661
<i>R</i> indices (all data) ^a	R1 = 0.0690 wR2 = 0.2197	R1 = 0.0890 wR2 = 0.2966	R1 = 0.0230 wR2 = 0.0583	R1 = 0.1051 wR2 = 0.2491	R1 = 0.0257 wR2 = 0.0665

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

Table 2. Selected Bond Distances (Å) and Bond Angles (deg) of [Re(CO)₃(DTGH)-NNO]PF₆·H₂O (**5** PF₆·H₂O), [Re(CO)₃(DTG)-NNN]·H₂O (**6**·H₂O), [Re(CO)₃(UEDDAH)-NNO] (**8**), [Re(CO)₃(DTMH)-NNO]·2H₂O (**9**·2H₂O), and [Re(CO)₃(DTA)-NNN]·CH₃OH (**12**·CH₃OH)

	5 PF ₆ ·H ₂ O	6·H ₂ O	8	9·2H ₂ O	12·CH ₃ OH
Re(1)–N(1)	2.191(8)	2.181(10)	2.242(4)	2.160(7)	2.230(4)
Re(1)–N(2)	2.237(8)	2.252(11)	2.203(5)	2.234(19)	2.235(4)
Re(1)–N(3)		2.200(11)			2.221(4)
Re(1)–O(4)	2.150(6)		2.151(4)	2.119(14)	
O(4)–Re(1)–C(1)	173.9(3)		170.6(3)	174.1(8)	
O(4)–Re(1)–C(2)	96.1(4)		96.79(17)	96.1(8)	
O(4)–Re(1)–C(3)	95.2(3)		98.4(3)	96.7(11)	
O(4)–Re(1)–N(1)	80.9(3)		76.2(3)	76.1(6)	
O(4)–Re(1)–N(2)	78.5(3)		78.61(15)	83.0(8)	
N(1)–Re(1)–C(3)	173.0(3)	175.5(5)	173.9(2)	171.7(10)	89.77(17)
N(1)–Re(1)–C(1)	94.8(4)	92.0(5)	95.1(2)	98.0(9)	98.88(17)
N(1)–Re(1)–C(2)	94.6(4)	95.8(5)	98.3(2)	98.5(9)	174.21(18)
N(2)–Re(1)–C(2)	172.3(4)	167.4(5)	175.05(18)	178.2(8)	97.66(19)
N(2)–Re(1)–C(1)	96.5(4)	98.7(5)	96.5(2)	95.6(10)	172.94(17)
N(2)–Re(1)–C(3)	94.3(3)	98.4(5)	97.4(2)	95.4(9)	96.45(17)
N(3)–Re(1)–C(1)		176.5(5)			96.18(17)
N(3)–Re(1)–C(2)		90.4(5)			93.24(17)
N(3)–Re(1)–C(3)		93.9(5)			173.81(16)
N(1)–Re(1)–N(2)	79.2(3)	77.1(4)	78.86(18)	79.8(7)	76.55(14)
N(1)–Re(1)–N(3)		85.0(4)			85.89(14)
N(2)–Re(1)–N(3)		78.7(4)			78.25(15)

Results and Discussion

Complexes **5** to **12** were prepared by treating each polyaminocarboxylic acid ligand with the *fac*-[Re(CO)₃(H₂O)₃]⁺ precursor in aqueous solution (Scheme 1). For all ligands, two products with different coordination modes were formed, and the ratio of the products depended on whether the reaction mixture was acidic, neutral, or basic. As indicated in Scheme 1, under most conditions, the protonation state of the coordinated ligand is different in the two related complexes. Thus, the complexes in a pair have different charge and are not, strictly speaking, isomers. The *fac*-[Re(CO)₃L]^{*n*} complexes (*n* = +1, 0, or –1) were characterized by analytical and spectroscopic methods; the structures of several complexes (**5**, **6**, **8**, **9**, and **12**) were also confirmed by X-ray crystallographic methods.

Crystallographic Studies. All X-ray structural analyses (Figures 1 to 4, below) confirm that three CO ligands are coordinated to one face of the pseudo-octahedron, while the other face is occupied by tridentate coordinated

ligands; donor sets are ONO in **7**, NNO in **5**, **8**, and **9**, and NNN in **6** and **12**. In all cases, the ligands form two five-membered chelate rings. The structural parameters involving Re are similar and typical for all structures (Table 2), with unexceptional Re–C bond distances (1.900 to 1.924 Å; Table S1 in Supporting Information) consistent with values found in similar complexes.^{3,68–70} The trans angles fall between 170.6° and 178.2°, with the exception of the smaller N(2)–Re(1)–C(2) angle in **6** (167.4(5)°), in which the ligating tertiary nitrogen atom anchors two chelate rings and bears the dangling CH₂CO₂[–] group. The cis angles show large deviations from the idealized octahedral geometry (76.1–98.9°). The

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Scheme 1

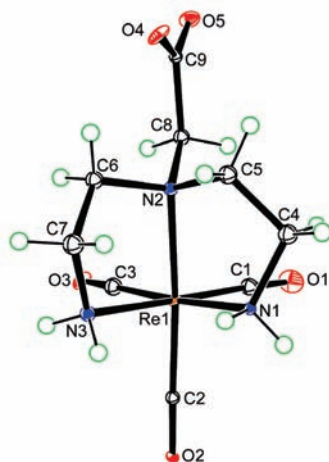
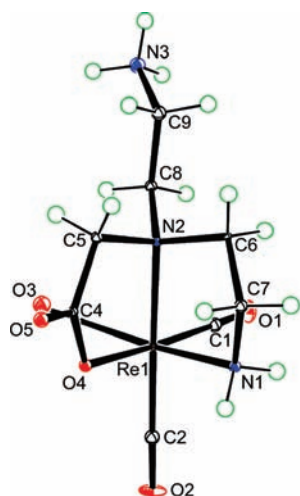
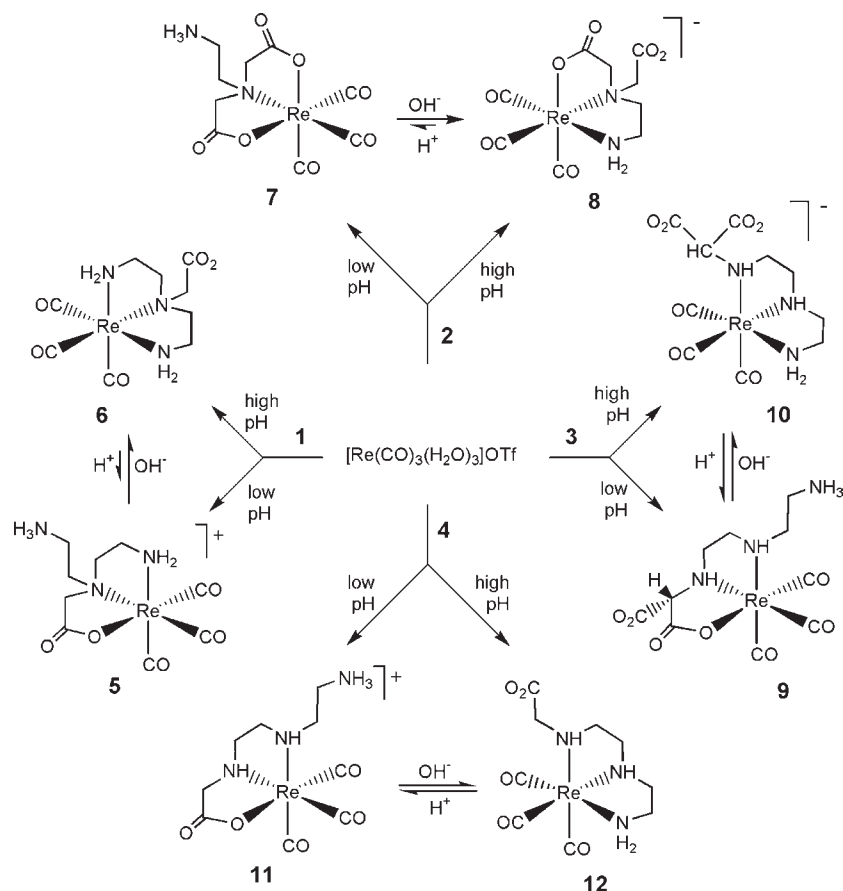


Figure 1. Perspective drawings of the complex in $[\text{Re}(\text{CO})_3(\text{DTGH})\text{-NNO}]\text{PF}_6 \cdot \text{H}_2\text{O}$ (**5** $\text{PF}_6 \cdot \text{H}_2\text{O}$) (left) and $[\text{Re}(\text{CO})_3(\text{DTG})\text{-NNN}] \cdot \text{H}_2\text{O}$ (**6** $\cdot \text{H}_2\text{O}$) (right) with 50% probability for the thermal ellipsoids. The PF_6^- anion and H_2O molecule are omitted for clarity.

Re–O distances (2.119–2.151 Å) are close to those found in related complexes in which a carboxylate oxygen is coordinated trans to a carbonyl ligand.^{3,42,44,69,70} The Re–N bond distances of 2.160–2.252 Å are consistent with sp^3 -hybridized nitrogen donors. Projecting away from the Re center is the dangling side arm of the ligand, namely, the acetate group in **6**, **12** ($-\text{CH}_2\text{CO}_2^-$; Figures 1 (right) and 4, respectively) and **8** ($-\text{CH}_2\text{CO}_2\text{H}$; Figure 2) and

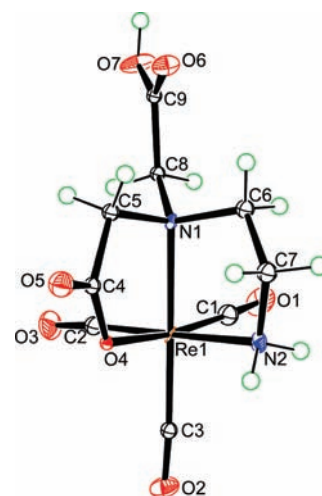


Figure 2. Perspective drawing of $[\text{Re}(\text{CO})_3(\text{UEDDAH})\text{-NNO}]$ (**8**) with 50% probability for the thermal ellipsoids.

the ethylammonium group in **5** and **9** ($-\text{CH}_2\text{CH}_2\text{NH}_3^+$; Figures 1 (left) and 3, respectively). For *fac*- $[\text{Re}(\text{CO})_3\text{L}]^n$ complexes in which the nitrogen anchoring the dangling group is a terminal secondary amine (i.e., coordinated secondary amine not linking two chelate rings), the NH proton could be *endo* (projecting away from the face defined by the tridentate ligand, as in **9**, Figure 3) or *exo* (projecting into this face, as in **12**, Figure 4).

Except for **6**, which in the solid has essentially a mirror plane containing the O2–C2–Re–N2 bonds (cf. Figures 1–4 for

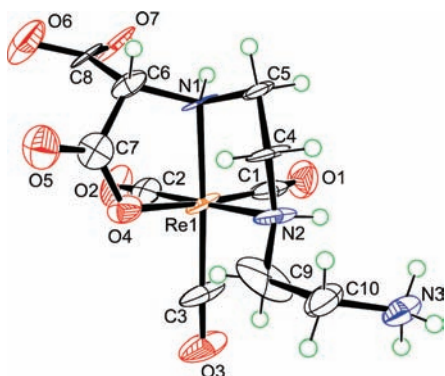


Figure 3. Perspective drawing of the complex in $[\text{Re}(\text{CO})_3(\text{DTMH})\text{-NNO}] \cdot 2\text{H}_2\text{O}$ ($9 \cdot 2\text{H}_2\text{O}$) with 50% probability for the thermal ellipsoids. The H_2O molecules are omitted for clarity.

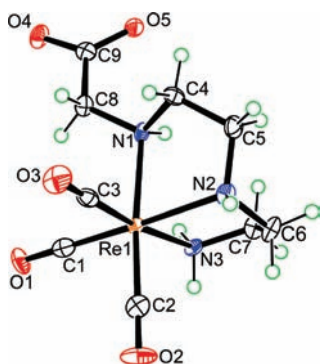


Figure 4. Perspective drawing of the complex in $[\text{Re}(\text{CO})_3(\text{DTA})\text{-NNN}] \cdot \text{CH}_3\text{OH}$ ($12 \cdot \text{CH}_3\text{OH}$) with 50% probability for the thermal ellipsoids. The methanol molecule is omitted for clarity.

numbering), the complexes are asymmetric and the unit cells contain a racemic mixture. The structure of only one enantiomer is illustrated. All complexes exhibit significant intermolecular hydrogen bonding in the solid.

Spectroscopic Properties. The use of ^1H NMR spectra to study Re^{I} analogues of Tc^{I} radiopharmaceuticals is less advanced than in the study of Re^{V} analogues of Tc^{V} radiopharmaceuticals.^{15,21,23,71–74} Here, we employed ^1H NMR spectra to provide needed evidence for assessing the overall geometry of the new *fac*- $[\text{Re}(\text{CO})_3\text{L}]^n$ complexes, especially when crystals could not be obtained. Fundamental studies are ongoing in our program to employ simple ligands lacking dangling groups to interpret the causes of unusual NMR chemical shifts in Re analogues of $^{99\text{m}}\text{Tc}$ agents with dangling groups.⁷⁵ Although we note briefly a few such shifts that appear to be characteristic of some geometric features, the elucidation of the underlying factors influencing these shifts is not the focus of the current study.

Identification of coordination mode relies on comparison of the NMR spectra of the free and coordinated

ligand. For free ligands, the signals for the methylene group adjacent to the carboxyl group are singlets, and the signals of the protons of each ethylene bridge are two triplets. After ligand coordination, the methylene group protons give rise to two strongly coupled doublets having coupling constants ($J = \sim 17$ Hz) consistent with geminal coupling because the protons become non-equivalent in an asymmetric environment. However, when the ligand coordinates in such a way that the resultant Re complex has mirror symmetry (mirror plane through one of the three carbonyl groups, as in **6**), the signal for the methylene group protons of a dangling acetate side chain is a singlet. The methylene signals do not have very different shifts or coupling constants whether the group is in a coordinated or dangling acetate group. The key observations providing information on structure are the presence or absence of coupling and the number of signals. However, from past work,³ the doublets of a coordinated acetate group have a larger coupling constant than those for a dangling group, and we use this criterion for attributing a particular set of doublets to coordinated or dangling acetate groups. In addition, if the coordinated acetate group is attached to a secondary amine, one acetate doublet exhibits weak NH/CH coupling ($J \sim 7$ Hz) because the H–C–N–H torsion angle is about $\sim 20\text{--}30^\circ$ for this CH proton. The signal for the second CH proton (H–C–N–H torsion angle ca. 90°) remains a doublet. On the other hand, both doublets of the dangling acetate group are further split by the coupling to an NH proton if the dangling group is attached to a secondary amine terminating the chelate ring.³

Also after coordination, the two triplets of each ethylene bridge are further split into four multiplets; these sometimes overlap with each other or with other signals. These features are consistent with the facial tridentate coordination mode of these polyaminocarboxylate ligands. In general, because the CH signals can be observed under all pH conditions, the CH signals were more useful than NH signals in assessing the coordination mode of ligands in products that did not form X-ray quality crystals.

In some cases, NH signals were also studied (see below). In our previous studies of *fac*- $[\text{Re}(\text{CO})_3\text{L}]^n$ analogues of $^{99\text{m}}\text{Tc}$ renal agents with dangling carboxyl groups, we found unusual shift dispersion of NH ^1H NMR signals in several solvents, including D_2O and $\text{DMSO-}d_6$ (where $\text{L} = S$ -methyl-L-cysteine,⁴ methionine,⁴ lanthionine,⁶ or ethylenediamine- N,N' -diacetic acid³). Although much more study is needed, some guidelines are developing. NH protons projecting into the face defined by the tridentate ligand (designated as *exo*-NH protons) sometimes have unusually upfield shifts compared to NH protons projecting away from this face (designated as *endo*-NH protons), even for the same primary amine. An *endo*-NH proton in a terminal secondary amine group has a shift that is typically less downfield than that of the NH of a secondary amine anchoring the two chelate rings. The latter protons also project away from the tridentate face of the pseudo octahedron. However, complexes of ligands with dangling groups and asymmetric centers have complicated spectra with signals having shifts not readily understood, as mentioned above.⁶ In studies with prototypical simpler ligands directed at investigating the

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possible cause of the NH dispersion,⁷⁵ we concluded that the more downfield shift of the *endo*-NH signals versus the *exo*-NH signals is related to greater solvent exposure of the *endo*-NH proton. Hydrogen bonding in solution causes downfield shifts.⁷⁵

N versus O Binding. To evaluate factors affecting the relative binding ability of N versus O donors, we selected the DTGH ligand because it is symmetrical and has both one carboxyl group and one tertiary amine, but it also has two primary amines.

Compounds Derived from the DTGH Ligand. Treatment of *fac*-[Re(CO)₃(H₂O)₃]⁺ with DTGH (**1**, Chart 1) at reflux gave two products, **5** (RT = 3.8 min) and **6** (RT = 7.6 min) (Scheme 1). Both **5** and **6** were isolated and fully characterized, including by X-ray crystallography (see above). The product favored at low pH (**5**) is [Re(CO)₃(DTGH)-NNO]⁺; the NNO coordination mode consists of two amine (one primary and one tertiary) and one carboxyl group donors, leaving the -CH₂CH₂NH₃⁺ group dangling (Figure 1, *left*). The product favored at high pH (**6**) is [Re(CO)₃(DTG)-NNN]; the NNN coordination mode consists of three amine groups of the ligand, and the CH₂CO₂⁻ moiety is the pendant arm (Figure 1, *right*). As expected for complexes with different coordination modes, the ¹H NMR spectra of **5** and **6** differ greatly. For **5** (cf. Figure S1 in Supporting Information, see Figure 1 (*left*) for atom labeling), two signals (4.94 and 4.32 ppm) are attributable to the coordinated primary amine N1H₂ group. The N1H signal at 4.94 ppm had cross-peaks to two of the signals of the four resolved ethylene bridge multiplets (3.19 and 2.75 ppm); these CH signals, which had cross-peaks to each other, were assigned to the C7H₂ group (next to N1H₂). Two almost overlapping signals at 3.02 and 3.05 ppm (these signals were better resolved in DMSO-*d*₆) had cross-peaks with both H7 signals and were assigned to the C6H₂ group. The signals (3.86 and 3.69 ppm) of the C9H₂ group next to the protonated primary amine group (N3H₃⁺) of the dangling ethylammonium arm are about 0.3 ppm downfield from the signal for the C8H₂ protons (multiplet at 3.48 ppm integrating for two protons) next to the coordinated tertiary amine (N2). The signals of the methylene group of the coordinated acetate group of **5** appeared as a set of doublets with a coupling constant of 16.8 Hz at 3.99 and 3.75 ppm. In contrast to this complicated spectrum, **6** has a very simple spectrum (in D₂O at pH 8.7 there are two multiplets at 3.26 and 3.17 ppm for eight ethylene protons and one singlet at 4.12 ppm for two methylene protons of the dangling acetate group); these values are consistent with the symmetry of **6**.

The ratio of products **5** and **6** obtained from reaction mixtures at reflux temperatures depended on the pH of the reaction mixture (Scheme 1). While **5** and **6** were formed in nearly equal abundance (45:55 ratio of **5/6**) at pH ~7, **5** (in a protonated form) was the major product (~90%) at pH ~3–5, whereas **6** was the only product (100%) at pH ~9–12 (Table 3). To determine whether products from the synthetic reactions were thermodynamically or kinetically controlled, we assessed both whether the products interconvert and whether interconversion occurs on a relevant time scale. Experiments conducted at both elevated and room temperatures are described in the next paragraph.

Table 3. Relative Abundance of Members of Product Pairs Under Acidic, Neutral, and Basic Conditions (from Preparative Reaction Mixtures, Except As Noted)

form of complex at pH 7	composition (%)		
	pH 3–5	pH ~ 7	pH 9–12
[Re(CO) ₃ (DTGH)-NNO] ⁺ (5)	90	45	0
[Re(CO) ₃ (DTG)-NNN] (6)	10	55	100
[Re(CO) ₃ (UEDDAH)-ONO] (7)	96	2	0
[Re(CO) ₃ (UEDDA)-NNO] ⁻ (8)	4	98	100
[Re(CO) ₃ (DTMH)-NNO] (9)	100	45	0
[Re(CO) ₃ (DTM)-NNN] ⁻ (10)	0	55	100
[Re(CO) ₃ (DTAH)-NNO] ⁺ (11)	98 ^a	42	20 ^b
[Re(CO) ₃ (DTA)-NNN] (12)	2 ^a	58	80 ^b

^a Equilibrated mixture (pH ~ 2) at 100 °C, starting from isolated **12**.

^b Equilibrated mixtures (pH ~ 8) at both 25 and 100 °C, starting from isolated **12**.

HPLC and ¹H NMR techniques were used to measure the relative abundance of the [Re(CO)₃(DTGH)-NNO]⁺ (**5**) and [Re(CO)₃(DTG)-NNN] (**6**) products present in solutions prepared with each pure isomer and maintained at various pH values at room temperature for 3 or more days. No changes were observed from pH 3 to pH 5 for **5** and from pH 9 to pH 12 for **6**. Mixtures of **5** and **6** were observed between pH 5 and pH 9. The [Re(CO)₃(DTGH)-NNO]⁺ (**5**) product undergoes complete conversion to the [Re(CO)₃(DTG)-NNN] (**6**) product at high pH (>9) almost immediately. However, interconversion of **5** and **6** was much slower at neutral pH and room temperature. At pH 7.0, the same final mixture (30:70) of isomers **5/6** was observed after about 1 week whether we started with [Re(CO)₃(DTGH)-NNO]⁺ (**5**) or [Re(CO)₃(DTG)-NNN] (**6**). Note that for the preparative reaction solution, which was heated at reflux, a 45:55 ratio of **5/6** was achieved at pH 7.5 in a short time (~1 h). Because these results suggest that the formation reactions at reflux starting with **1** and the *fac*-[Re(CO)₃(H₂O)₃]⁺ cation were under thermodynamic, not kinetic control, we also heated a solution of **6** at pH ~3 and found that it converted to **5** in 1–1.5 h, a time comparable to that of the typical preparative reaction. These results show clearly that the typical labeling conditions will give the thermodynamic distribution at elevated temperature, regardless of pH, but that at room temperature the change in coordination mode occurs readily only at high pH, where the dangling amine exists to a high extent in the unprotonated state (pK_a of the CH₂CH₂NH₃⁺ group = ~9–11). This observation, in turn, implies that the change in coordination mode may involve either an S_N2 mechanism with the free amine attacking the Re^I center or an S_N1CB reaction with a deprotonated coordinated amine labilizing the Re–O bond, possibly generating an intermediate with reduced coordination number. Extensive studies would need to be performed to establish the likely mechanism, but some results described below suggest that the S_N1CB mechanism is unlikely.

Compounds Derived from the Other Ligands. Because other polyaminocarboxylate ligands (**2**, **3**, and **4**) afforded isomers showing a pH dependence of the coordination mode reflective of that observed for isomers formed by **1** (Table 3), the products formed by each polyaminocarboxylate ligand were studied in less depth than those from **1**. We shall discuss mainly the noteworthy results in

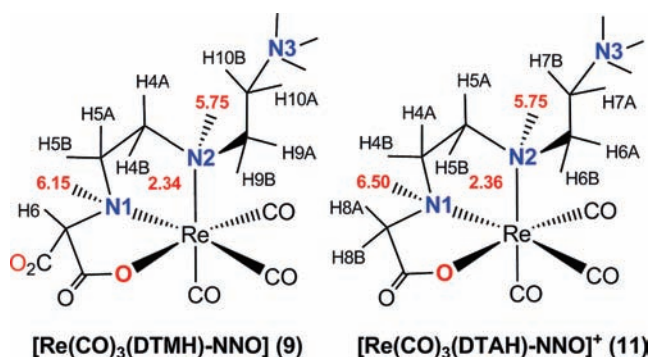
solution for each set of compounds derived from these ligands.

Compounds Derived from the UEDDAH₂ Ligand. UEDDAH₂ (**2**, Chart 1) has two amine nitrogen and two carboxyl oxygen donors available for coordination. As observed for other ligands studied here, the UEDDAH₂ ligand also formed a pair of complexes differing in coordination mode. The coordination modes were ONO or NNO, depending on the pH of the reaction mixture. Syntheses performed at low pH and moderate temperature (30 to 40 °C) led to the formation of one major complex, in the neutral zwitterionic form, [Re(CO)₃(UEDDAH)-ONO] (**7**, RT = 8.8 min; ~96% yield by HPLC). The ¹H NMR spectrum of **7** in D₂O (pH 4.0) is very simple and confirmed the formation of only one isomer, having a dangling –CH₂CH₂NH₃⁺ moiety. The two acetates give only one pair of methylene doublets (at 3.72 and 3.93 ppm, *J* = 16.8 Hz, each integrating for two protons) indicating a symmetrical coordination of the UEDDAH[–] ligand via the two carboxylates and the tertiary amine group. A very similar spectrum with *J* = 16.8 Hz for the acetate methylene doublets was obtained for the Re analogue of the promising [^{99m}Tc(CO)₃(nitilotriacetate)]^{2–} renal imaging agent.^{8,53} As found for **5** (the other product made here with the –CH₂CH₂NH₃⁺ moiety dangling from a tertiary N), the multiplet (3.72 ppm) for the protons of the CH₂ group of **7** next to the protonated amine group is 0.31 ppm downfield from the multiplet (3.41 ppm) for the protons of the CH₂ group next to the coordinated tertiary amine.

However, syntheses performed at pH ~7 and moderate temperature (30 to 40 °C) produced a different new complex, [Re(CO)₃(UEDDA)-NNO][–], which formed almost exclusively (**8**, RT = 14.5 min, ~98%). Depending on the protonation state of the dangling uncoordinated carboxyl group, the complex has a negative charge (deprotonated carboxyl at and above physiological pH) or neutral (protonated carboxylate group at low pH). The latter form of **8** ([Re(CO)₃(UEDDAH)-NNO]) was obtained as crystals (Figure 2).

The ¹H NMR spectrum of **8** revealed the presence of four multiplets (each integrating for one proton) and two distinct sets of two sharp doublets, confirming the unsymmetrical NNO coordination of the UEDDA^{2–} ligand and the asymmetry of **8**. On the basis of previous studies in which the complexes contained ligands allowing unambiguous assignments of coordinated and dangling acetate, for the CH₂ signals,^{3,8} the doublets with the lower *J* (16.4 Hz, 4.21 and 3.96 ppm), are attributed to the C₈H₂ protons of the uncoordinated acetate group, and the doublets with higher *J* (17.6 Hz, 4.18 and 4.02 ppm) are attributed to the C₅H₂ protons of the coordinated acetate group. Complex **8** persisted under all pH conditions investigated at room temperature, including at low pH, where X-ray quality crystals were grown, allowing a structural determination establishing the NNO coordination mode (Figure 2). We then investigated whether **8** would convert to **7** (with the ONO coordination mode) at low pH and elevated temperature. Heating at reflux a pH 2 aqueous solution of **8** converted ~25% of **8** to **7**, giving the equilibrium ratio of 25:75 (**7/8**). As in other cases, immediately after the pH of this solution was adjusted to 9 at room temperature, the only product detected by

Chart 2



HPLC was **8**. Thus, **7** (with the ONO coordination mode) is a kinetic product formed at low pH and moderate temperature (25–40 °C). In contrast to the behavior of other pairs of complexes, in which both exist in comparable amounts under neutral conditions, **7** rearranges almost completely to **8** (NNO coordination mode), the thermodynamic product, under neutral conditions. Indeed, even at low pH, **8** is much more favored than **7** when samples of **8** are heated under conditions allowing a change in coordination mode.

Compounds Derived from the DTMH₂ Ligand. Treatment of [Re(CO)₃(H₂O)₃]⁺ with DTMH₂ (**3**, Chart 1) at pH ~7 gave a mixture of two products, [Re(CO)₃(DTMH)-NNO] (**9**; RT = 3.5 min) and [Re(CO)₃(DTM)-NNN][–] (**10**; RT = 6.8 min), in an almost 45:55 ratio of **9/10**. The molecular structure of the pseudo-octahedral neutral complex **9** reveals that the DTMH[–] ligand is coordinated via an NNO donor set (the two neutral secondary amines and one terminal carboxylate group) (Figure 3); two groups, –CO₂[–] and –CH₂CH₂NH₃⁺, are dangling. This neutral zwitterion form of **9**, [Re(CO)₃(DTMH)-NNO], crystallized from low pH (~3.5) aqueous solution. However, at pH ~3 only the cationic form of **9** ([Re(CO)₃(DTMH₂)-NNO]⁺) with a protonated carboxylic group) was observed. Likewise, only [Re(CO)₃(DTM)-NNN][–] (**10**) was observed for preparative reactions at pH ~10. Although it could be separated and characterized, all attempts to obtain crystals suitable for X-ray crystallography of this NNN product, **10**, have been unsuccessful.

The ¹H NMR spectrum of **9** in aqueous solution at pH 6 (Figure S2 in Supporting Information), in accord with its X-ray structure, consists of the two NH signals, (6.15 (s, 1H, N1H) and 5.75 ppm (m, 1H, N2H)), a singlet for C₆H–CO₂[–] (4.01 ppm; the H₆ signal is a singlet because it has about a 90° torsion angle relationship to the N1H amine proton), and several multiplets with very similar chemical shifts, indicating that the signals of one ethylene bridge belong to a dangling arm. The NMR spectrum of **9** revealed a very characteristic upfield signal at 2.34 ppm assigned by COSY to H₄B (Chart 2) on the basis of the H₄B–C₄–N₂–H₂ torsion angle value of –161.36° from the X-ray crystal structure (Figure 3). Such a ~2.3 ppm signal for a proton of the methylene group attached to the coordinated secondary amine (N₂H) terminating the chelate ring has been observed in related Re(CO)₃ complexes.³

On the other hand, the eight clearly separated multiplets (each integrating for one proton) for the two ethylene

bridges of **10** in H₂O at pH 9 (cf. Figure S3 in Supporting Information) are consistent with facial coordination of all three amine groups. While no NH signal was observed for the dangling $-\text{CH}_2\text{CH}_2\text{NH}_3^+$ group in **9**, as expected because the protons of an uncoordinated protonated primary amine group are undoubtedly in fast exchange with water, two signals (5.21 and 3.98 ppm) were observed in H₂O for the coordinated primary amine protons in **10**, identified by the presence of a COSY cross-peak between these signals.

No changes were observed by NMR and HPLC for up to 10 days for solutions at room temperature of **9** at pH 3 to 5 or **10** at pH 9 to 12, results similar to those observed for solutions of the $\text{Re}(\text{CO})_3(\text{DTG})$ complexes, **5** and **6**. In addition, the $\text{Re}(\text{CO})_3(\text{DTM})$ complexes slowly converted into each other at room temperature when the pH was changed from acidic to basic (**9** converted to **10**) and from basic to acidic (**10** converted to **9**), indicating that both **9** and **10** are the thermodynamically favored products at the pH conditions under which they were isolated (**9** at pH 3.5 and **10** at pH 10).

Compounds Derived from the DTAH Ligand. After the high pH synthesis of **10**, it was purified on a Sephadex G-15 column. The purification revealed a small amount of another new *fac*- $[\text{Re}(\text{CO})_3\text{L}]^n$ complex having an HPLC RT of 7.8 min (**12**). Analytical, spectroscopic, and X-ray crystallographic methods revealed that **12**, neutral $[\text{Re}(\text{CO})_3(\text{DTA})\text{-NNN}]$, contains the fully deprotonated DTA ligand, facially coordinated through three amine donor groups (Figure 4). Under alkaline conditions, heating is well known to lead to decarboxylation of carboxylic acids with a β -carbonyl group, such as the malonic acid moiety of DTMH_2 . Because **10** was stable at high pH and no decarboxylation was observed, some decarboxylation of the DTMH_2 ligand must have occurred during the synthesis of **10**. The newly formed acetate group ($-\text{CH}_2\text{CO}_2^-$) of the resulting DTAH ligand is dangling in **12** (Figure 4).

Complex **12** was kept in solution at pH \sim 2 at room temperature. Analysis by HPLC of the low pH solution revealed the presence of a second peak (RT = 3.6 min, $[\text{Re}(\text{CO})_3(\text{DTAH})\text{-NNO}]^+$ (**11**)) in addition to the peak of **12** (7.8 min); the peak for **11** grew very slowly and after 4 days the peaks were present in a 30:70 ratio (**11/12**). However, when a solution of **12** at pH \sim 2 was heated for 30 min, **12** converted almost completely (\sim 98%) to **11**. At this low pH, the dangling group of **12** is a carboxylic acid, and apparently the $[\text{Re}(\text{CO})_3(\text{DTAH})\text{-NNN}]^+$ cation does not easily convert to the NNO product with a dangling protonated ethylamine group at room temperature; however, the conversion is achieved at elevated temperature. About 1 h after adjusting the pH of the low pH solution to \sim 8 and at room temperature, the ratio changed to 85:15 (**11/12**), but after additional heating at \sim 100 °C for 30 min the ratio changed to 20:80 (**11/12**), and it did not change with time, even at elevated temperature; thus, 20:80 is the thermodynamic distribution of products at pH \sim 8. To verify these results, we synthesized the DTAH ligand to investigate whether this ligand could in fact form $[\text{Re}(\text{CO})_3(\text{DTAH})\text{-NNO}]^+$ (**11**) by reaction with *fac*- $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$. Indeed, HPLC analysis of the reaction mixture of DTAH with the precursor at pH 6 to 7 and 70 °C for 2 h showed two peaks (RT = 3.6 min for **11** and RT = 7.8 min for **12**) in a 42:58 ratio.

The ¹H NMR spectrum of the more abundant product is identical with that of $[\text{Re}(\text{CO})_3(\text{DTA})\text{-NNN}]$ (**12**). The ¹H NMR spectrum (at pH 3) of the less abundant product ($[\text{Re}(\text{CO})_3(\text{DTAH})\text{-NNO}]^+$, **11**) has relatively downfield acetate signals (3.53 ppm, d, $J = 17.6$ Hz; 3.91 ppm, dd, $J = 17.6, 7.6$ Hz; see Experimental Section). This pattern is characteristic of the NNO coordination mode of facially coordinated five-membered chelate rings such as we previously observed in $\text{Re}(\text{CO})_3(\text{ENDAC})$ and $\text{Re}(\text{CO})_3(\text{ENAC})$.³ We can assign the doublet of doublets at 3.91 ppm of **11** H8B (Chart 2) because the H1–N1–C8–H8B torsion angle is expected to be \sim 20–30° (a weak H1/H8B coupling).³ We assign the doublet at 3.53 ppm to H8A (Chart 2) for which an about 90° torsion angle relationship to the N1H proton is expected.

The pH 3 spectrum of **11** also exhibits an NH signal with a shift of 5.75 ppm (as we observed for **9**) and the unusually upfield multiplet at 2.36 ppm, also as we observed for **9**. The multiplet is assigned to H5B (Chart 2) on the chelate ring carbon attached to the coordinated secondary amine terminating the ring on the basis of the assignment for **9**. This similarity indicates that the both **11** and **9** have the same configuration at the coordinated secondary amine group. Note that the X-ray structure of **9** (Figure 3) establishes that this is the configuration at N2 having an *endo*-NH proton. The somewhat downfield 5.75 ppm shift is consistent with our past studies of related complexes with an *endo*-NH proton in the coordinated secondary amine terminating a chelate ring.^{3,75} The other NH signal of **11** has a more downfield chemical shift, 6.50 ppm, consistent with an NH signal for a proton on the central N1.

In addition to the other similarities, the signals for the dangling $\text{CH}_2\text{CH}_2\text{NH}_3^+$ group have similar shifts in **11** and **9**. Thus the proposed similarity in the structures for **11** and **9** (as shown in Scheme 1) with an NNO coordination mode and a dangling $-\text{CH}_2\text{CH}_2\text{NH}_3^+$ attached to a secondary amine N terminating one chelate ring is well supported by the NMR data. It is interesting that the *exo* dangling group attached to a secondary N of **11** and **9** lacks the somewhat downfield multiplet characteristic of this dangling group in **5** and **7**, complexes for which the dangling group is attached to a tertiary N and is therefore necessarily *endo*. Further research on other compounds is needed before one can determine if the differences in shift are related to the type of nitrogen to which the dangling group is attached or to the *endo* versus *exo* disposition of the dangling group.

The more downfield NH signal for **12** at 6.55 ppm can be assigned to the N2H (the central coordinated amine) and the more upfield NH shift at 5.17 ppm can be attributed to N1H (an *exo*-NH proton, Figure 4); this assignment is consistent with our past studies of related complexes with amines terminating a chelate ring.^{3,75} The C8H₂ signals for the dangling acetate have similar shifts (3.79 and 3.74 ppm) giving an AB spin system. This has an octuplet pattern arising from geminal coupling (³ $J = 16.8$ Hz) combined with the vicinal coupling (\sim 7 Hz) to the NH. Similar vicinal coupling of \sim 7 Hz has been observed previously and is attributable to the expected rapid free rotation of the uncoordinated acetate group around the N–C bond.³

Effect of Base on the Ease of Interconversion of Coordination Mode. When D₂O solutions of the various

products were made basic (above pH 8), the CH signals sharpened and exhibited simpler coupling patterns. The NH signals diminished in size and eventually disappeared. However, the shifts of the CH signals did not change with this increase in basicity, even at very high pH > 10. The relatively rapid NH/ND exchange indicates some NH deprotonation, because this process requires coordinated amine deprotonation.^{21,23,76} However, the absence of the shifting of the CH signals indicates deprotonation is not favorable enough to produce detectable amounts of NH-deprotonated complexes. This behavior is in contrast to our findings for Re^VO analogues of ^{99m}Tc^VO radiopharmaceuticals, for which detectable amounts of NH-deprotonated forms are present even under physiological conditions.^{15,21,23,71–74}

As mentioned, the rapid NH/ND exchange under basic conditions means that a minor amount of a species with a deprotonated coordinated N must exist. However, we can rule out this species as an intermediate during a change in coordination mode. **7** does not have a coordinated amine bearing an NH group. However, both the **7** to **8** and the **5** to **6** change in coordination mode occur readily in basic solution; the likely reason for the facile process is that the dangling amine becomes deprotonated and undergoes intramolecular exchange with a coordinated carboxyl.

Conclusions

On the basis of the observations in this study, where chelate ligands forming five-membered chelate rings were compared, we are able to provide some guiding principles for radiopharmaceutical design. At elevated temperature, the rate of formation and equilibration of the Re^I complexes is sufficiently rapid that equilibrium is reached in an hour or less. Because Tc^I complexes are expected to equilibrate more

rapidly than Re^I complexes, most high-temperature preparations of Tc^I tracers should be at equilibrium. Experience suggests that the Tc^I products persist at room temperature, and thus it is desirable to form the tracer under conditions in which one or primarily one product is present. In low-pH solutions, the product with one carboxylate oxygen bound to the *fac*-{Re^I(CO)₃}⁺ core, leaving one potential amine donor as a dangling protonated group, is often both kinetically and thermodynamically favored. A greater affinity of the *fac*-{Re^I(CO)₃}⁺ core for the amine group relative to the carboxylate donor in high pH aqueous solution is established by the isolation of Re tricarbonyl complexes in which the polyaminocarboxylate ligand binds through the amine nitrogen donors in preference to carboxylate oxygen donors. Thus, for renal tracers, labeling can be done at elevated *T* at low pH, and the sample can then be converted to the N coordination mode at room temperature by raising the pH. Basic conditions facilitate the change in coordination mode to the species with the dangling carboxyl group whether or not the coordinated amines in the tracer formed at low pH have a dissociable proton. Just before injection, the pH of all ^{99m}Tc tracer doses is always adjusted to 7.4.

Acknowledgment. This work was supported by the National Institutes of Health (R37 DK038842). The authors thank Dr. Kenneth Hardcastle of Emory University for useful discussions and for determining the structures by using instruments supported by NIH Grant S10-RR13673 and NSF Grant CHE 9974864. We also thank Theshini Perera (Louisiana State University) for help in preparing the figures.

Supporting Information Available: X-ray crystallographic data in CIF format for **5** PF₆·H₂O, **6**·H₂O, **8**, **9**·2H₂O, and **12**·CH₃OH (CCDC 667840–667844). An additional table of Re–C bond distances and C–Re–C bond angles and ¹H NMR spectra for **5**, **9**, and **10**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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