Contribution from the Departments of Chemistry, University of Cincinnati, Cincinnati, Ohio 45221, and University of Oklahoma, Norman, Oklahoma 73019

Comparison of the Chemical and Biological Properties of *trans*-$[Tc(DMPE)_2Cl_2]^+$ and *trans*-$[Re(DMPE)_2Cl_2]^+$, Where DMPE = 1,2-Bis(dimethylphosphino)ethane. Single-Crystal Structural Analysis of *trans*-$[Re(DMPE)_2Cl_2]PF_6$

JEAN-LUC VANDERHEYDEN,[1] MARY JANE HEEG,[2] and EDWARD DEUTSCH*[1]

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Recent studies on the monocationic ^{99m}Tc complex *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ have shown that this species accumulates in normal myocardium and allows scintigraphic visualization of the hearts of mongrel dogs and humans. The mechanism of action of the myocardial accumulation is still unclear. Because of the periodic relationship between technetium and rhenium, in vivo evaluation of the analogous complex *trans*-$[Re(DMPE)_2Cl_2]^+$ can provide insight on the role of the oxidation–reduction potential of complexes in determining biological behavior. Preparation of *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$ from $^{186}ReO_4^-$ is accomplished analogously to the synthesis of *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ from $^{99m}TcO_4^-$. The rhenium complex prepared with nonradioactive Re is characterized by single-crystal X-ray structural analysis refined to conventional R factors of 0.030 and 0.037. Yellow crystals of the hexafluorophosphate salt crystallize in the monoclinic space group $P2_1/c$ with a = 10.661 (5) Å, b = 10.214 (6) Å, c = 12.835 (6) Å, β = 115.43 (4)°, and Z = 2. This octahedrally coordinated complex has essentially the same structure, bond lengths, and bond angles as does the technetium analogue: Re–P = 2.438 (2) Å and Tc–P = 2.436 (5) Å; Re–Cl = 2.337 (1) Å and Tc–Cl = 2.323 (3) Å. However, cyclic voltammetry shows that the Re complex is 190 mV more difficult to reduce than is the Tc analogue. Tissue distribution studies in rats, and qualitative imaging studies in rats and dogs, show a definite difference in the biodistributions of *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ and *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$, despite the identical size, shape, charge, and lipophilicity of these complexes. This observation strongly implies that in vivo reduction of the cationic Tc(III) complex *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ to the neutral, more lipophilic, Tc(II) analogue *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^0$ determines, at least in part, the biodistribution of *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$. This in vivo reduction detracts from the utility of *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ as a myocardial imaging agent by promoting transfer of radioactivity from the heart to the liver.

Introduction

There has recently been a great amount of interest in the preparation and in vivo evaluation of cationic complexes of ^{99m}Tc as myocardial imaging agents.[3-10] In support of this interest there have also appeared several recent publications on the macroscopic chemistry of monocationic complexes of Tc(V), Tc(III), and also Tc(I) using the long-lived isotope ^{99}Tc.[11-14] Bis(phosphine) ligands, and especially the DMPE ligand (where DMPE represents 1,2-bis(dimethylphosphino)ethane), are the only ligands known to stabilize the technetium center in all three oxidation states.[14] In vivo evaluations of the three Tc(I), Tc(III), and Tc(V) cationic DMPE complexes were systematically performed in order to develop an animal model that would adequately predict the biological behavior of cationic complexes in humans.[15]

Since the first report concerning *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ in 1981,[4] the chemistry and biology of this cation have been extensively studied. Acquisition of rat biodistribution data,[16] basal and kinetic studies in mongrel dogs,[5,6] and evaluation as a myocardial imaging agent in humans[9] using the ^{99m}Tc agent have been accompanied by a full characterization of the ^{99}Tc analogue using classical chemical techniques.[4,14,17] Although there is a definite accumulation of *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ in the normal myocardium of many species, the mechanism of action of this uptake is not known. In vitro studies[18] show that the mechanism of uptake is different from the uptake mechanism of $^{43}K^+$, $^{139}Cs^+$, and $^{201}Tl^+$, the latter being the myocardial imaging agent currently in clinical use. Further in vitro work involving perfused guinea pig hearts[19] has also not defined the biological mechanism of action of *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$.

A totally different approach is presented in this paper. The periodic relationship between technetium and rhenium is used to study very specifically the role of reduction potential of *trans*-$[M(DMPE)_2Cl_2]^+$ (M = Tc, Re) complexes on their in vivo biodistribution. The chemistries of Tc and Re are quite similar due to their relative positions in the periodic table and the lanthanide contraction. The salient difference between the chemistries of analogous Tc and Re complexes is the fact that the Re complexes are about 200 mV more difficult to reduce than the Tc analogues. This difference in redox potentials between Tc and Re analogues is consistent with several observations made on analogous complexes of second- and third-row transition metals[20,21] and is large enough to shift one complex or the other out of the range of potentials readily accessible to biological systems. The

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reduction of *trans*-$[M^{III}(DMPE)_2Cl_2]^+$ to *trans*-$[M^{II}(DMPE)_2Cl_2]^0$ will change the net charge of the complex and therefore alter its biodistribution. Since it is felt that only cationic species accumulate in the myocardium, such reduction is expected to lessen the efficacy of potential myocardial imaging agents.[7]

In this paper are presented the preparation and characterization of the *trans*-$[Re(DMPE)_2Cl_2]^+$ complex, the synthesis and HPLC quality control of its ^{186}Re form, and the structural characterization of *trans*-$[Re(DMPE)_2Cl_2]PF_6$, as well as data comparing the biodistribution of *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$ and *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ in dogs and rats.

Experimental Section

General Considerations. Standard radiation safety procedures must be followed at all times when handling ^{99m}Tc and ^{186}Re isotopes. Technetium-99m emits a 141-KeV γ ray with a half-life of 6.0 h to decay to technetium-99. Rhenium-186 emits a 137-KeV γ ray associated with a 1.07-MeV β particle and decays with a 90-h half-life.

Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Visible–UV spectra were recorded on a Cary 210 spectrophotometer (Varian) and IR spectra were obtained on a Perkin-Elmer 224 grating infrared spectrophotometer.

Octanol/saline partition coefficients were obtained as previously described[3] and represent the average of at least three independent determinations.

Materials. Unless otherwise noted, all chemicals were of reagent grade. The DMPE ligand was obtained from Strem Chemical Co. and was used without further purification. Dilution and stabilization of the ligand for use in the preparations of ^{99m}Tc and ^{186}Re complexes have been previously described.[8] Potassium perrhenate was commercially available from Morton Thiokol, Inc. Sephadex SP C-25 was prepared and stored as recommended by the manufacturer (Pharmacia).

Tetrabutylammonium Perrhenate, $(Bu)_4NReO_4$. $KReO_4$ (600 mg 2.07 mmol) was dissolved in 10 mL of hot water, and 1 mL of 2 M tetrabutylammonium bromide was then added dropwise. The reaction mixture was cooled to room temperature, and the white precipitate was collected and successively washed with water, 10% ethanol/diethyl ether, and diethyl ether; yield 960 mg (94%). IR (KBr pellet) ν, cm^{-1} (intensity): 320 (m), 610 (w), 735 (w), 900 (s), 1390 (m), 1470 (m), 2870 (m), 2960 (s). UV (methanol) λ, nm (ϵ, M^{-1} cm^{-1}): 250 sh, 244 (2780), 240 (3430), 235.5 (3750), 231 (3850), 227 (3850), 223 (3770).

Tetrabutylammonium Perrhenate (^{186}Re), $(Bu)_4N^{186}ReO_4$. Enriched (85.84%) ^{185}Re metal (1–2 mg) (Oak Ridge National Laboratory) was weighed into a quartz vial, which was then sealed by flame under vacuum. The vial was then irradiated in a flux of 10^{14} neutrons cm^{-2} s^{-1} at the University of Missouri research reactor (Columbia, MO) for 24 h. The Re metal was then dissolved by the addition of concentrated HNO_3, and the resulting solution was neutralized with ammonia. This solution was then diluted to 4 mL to give a total Re concentration of about 0.004 M and a specific activity of about 30 mCi/mL. To aliquots (0.5–1.0 mL) of this solution were added 0.5 mL of 0.1 M tetrabutylammonium bromide (0.05 mmol). This mixture was passed through a Sep-Pak C_{18} cartridge (Waters) that had been prepared by successive washings with 3 mL of 95% ethanol and 3 mL of 0.01 M tetrabutylammonium bromide. After loading, the cartridge was washed with 20 mL of water and the activity was then eluted in 99% yield with 2 mL of absolute ethanol.

***trans*-Dichlorobis(1,2-bis(dimethylphosphino)ethane)rhenium(III) Hexafluorophosphate, *trans*-$[Re(DMPE)_2Cl_2]PF_6$.** Tetrabutylammonium perrhenate (162 mg, 0.33 mmol) dissolved in 5.0 mL of absolute ethanol was placed in a Teflon-lined bomb reactor. A 1.0-mL portion of 12 M HCl (0.012 mol) was added, and the resulting solution was deaerated for 5 min with a stream of nitrogen. The bomb was capped and transferred into an argon-purged glovebox, where 0.55 mL of neat DMPE (0.494 g, 3.3 mmol) was added. The bomb was then sealed and heated at 155 °C for 5 h. After the mixture was cooled to room temperature, 5.0 mL of H_2O was added to the reaction solution. Subsequent addition of 0.2 mL of a saturated aqueous solution of ammonium hexafluorophosphate yielded a yellow precipitate of the rhenium(III) complex that was collected and washed successively with 10% ethanol/0.01 M KOH, water, cold 95% ethanol, and diethyl ether. Recrystallization of this material from acetonitrile yielded 180 mg (76%) of translucent yellow crystals. The crystals for X-ray structural analysis were obtained by slow evaporation of an acetonitrile solution at room temperature. Anal. Calcd for $ReC_{12}H_{32}P_5Cl_2F_6$: C, 20.52; H, 4.59; Cl, 10.09. Found: C, 19.82; H, 4.66; Cl, 10.21. Visible–UV (CH_3CN) λ, nm (ϵ, M^{-1} cm^{-1}): λ_{max} = 432 (2400); λ_{min} = 421 (2300); λ_{max} = 406 (2620); λ_{max} = 261 (5520); λ_{max} = 236 (3030). IR (KBr pellet) ν, cm^{-1} (intensity): 310 (m), 450 (w), 550 (s), 640 (m), 710 (s), 740 (m), 830 (s), 890 (s), 925 (s), 940 (s), 1240 (w), 1290 (m), 1300 (w), 1390 (w), 1420 (s) 2900 (m), 2990 (w).

Table I. Experimental Crystallographic Data for *trans*-$[Re(DMPE)_2Cl_2]PF_6$

formula	$Re_1P_5Cl_2F_6C_{12}H_{32}$
mol wt	702.35
cryst dimens	0.12 × 0.15 × 0.15 mm
cryst syst	monoclinic
space gp	$P2_1/c$
cell dimens[a]	a = 10.661 (5) Å, b = 10.214 (6) Å, c = 12.835 (6) Å, β = 115.43 (4)°, V = 1262 (1) Å^3, Z = 2, D(calcd) = 1.848 g cm^{-3}
radiation	Mo Kα (λ = 0.71069 Å)
monochromator	graphite crystal
2θ range	2.4–53°
max scan time	45 s
scan angle	0.80 + 0.20 tan θ
monitor reflcns	3 measd for orientn check every 200 reflcns; 3 measd for intensity check every 2 h
variation in monitors	1%
total no. data collcd	2836
no. of unique data	2615
no. of obsd data with $I_o \geq 3\sigma(I)$	1737
μ(Mo Kα)	55.9 cm^{-1}
transmissn coeff	0.37–0.51
F(000)	684
R	0.030
R_w	0.037
w	$(\sigma_F)^{-2}$

[a] Lattice constants calculated from 48 high-angle reflections measured at ±2θ.

***trans*-Dichlorobis(1,2-bis(dimethylphosphino)ethane)rhenium(III) (^{186}Re), *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$.** Purified $(Bu)_4N^{186}ReO_4$ (15–20 mCi) in absolute ethanol was diluted to 1.2 mL with absolute ethanol in a 5-mL borosilicate glass reaction vial. A 0.1-mL portion of 4 M HCl was added, and the mixture was deaerated. A 0.20-mL portion of a 0.54 M DMPE solution in ethanolic HCl[8] was added, and then the vial was sealed with a Teflon-lined cap and placed in a 150 °C dry heat bath for 2 h. (*Caution*! This is a potentially explosive procedure. Reaction vials should be pretested to ensure that they will withstand reaction conditions. Reaction vials should be cooled slowly to prevent their breaking. Only borosilicate reaction vials should be used.) After cooling, 0.075 mL of 4 M NaOH was added and the reaction mixture was diluted to 20 mL with water. This solution was loaded onto a Sephadex SP C-25 cation-exchange column (1.5 × 6 cm), and the column was successively washed with 20–25 mL of 0.005 M NaOH and 30–35 mL of water. The desired radiopharmaceutical was then eluted with 0.15 M NaCl in 5-mL fractions. The aliquot containing the highest specific activity of *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$ was passed through a sterile 0.22-μm filter (Millex SG) and used for in vivo evaluations.

X-ray Characterization and Structure Solution and Refinement of *trans*-$[Re(DMPE)_2Cl_2]PF_6$. Yellow crystals of this material were irregular and multifaceted. A sample was mounted on a Nonius CAD-4 automated diffractometer, and axial photos indicated the monoclinic crystal system. Gaussian integration absorption corrections were applied by the method of indexing crystal faces. The collected intensity measurements showed unusually weak hkl reflections for the condition $k + l$ = odd, which is consistent with the heavy atom being located on the inversion center. The structure was solved by heavy-atom methods and refined as a full matrix in least-squares calculations.[22] Hydrogen atoms were placed in a combination of observed and calculated positions and held invariant with $U(H)$ = 0.09 Å^2; all non-hydrogen atoms were refined anisotropically. In a final cycle of least-squares refinement, the maximum shift was $<0.01\sigma$. No systematic correction for secondary extinction was made; however, one reflection (002) that obviously was affected was removed. Some disorder was evident in the PF_6^- anion; the highest peak on a difference map represented 1.0 e Å^{-3} near the anion. The number of observations was 1737, and the number of variable parameters was 121. Neutral-atom scattering factors and corrections for anomalous dispersion were from ref 23. Other details of the crystallographic experiment are given in Table I.

HPLC Procedure. High-performance liquid chromatography (HPLC) analysis was performed either on analytical C_8 10-μm (Alltech) or C_8 5-μm (Alltech) reverse-phase columns of dimensions 250 × 4.5 mm.

(22) All crystallographic computations were performed by using local modifications of the programs of SHELX-76: Sheldrick, G. M. "SHELX-76"; University Chemical Laboratory: Cambridge, England, 1976.

(23) "International Tables for X-ray Crystallography"; Kynoch Press: Birmingham, England, 1974; Vol. 4.

Attached to these was a guard column filled with Pellicular C_8 packing (Anspec). The mobile phase consisted of an 85/15 (v/v) methanol/water solution, the aqueous fraction being 0.01 M in sodium heptanesulfonate and 3 mM in pH 7.0 phosphate buffer. HPLC chromatograms were obtained at ambient temperature, with a flow rate of 1.5 mL/min and a pressure of ca. 1600 or 2500 psi on the 10- and 5-μm columns, respectively. Detection was both radiometric (using a DS-202 Nuclear Chicago Well scintillation detector centered at the 140-keV emission of ^{99m}Tc and ^{186}Re) and spectrophotometric (using a Beckman 153 UV detector with a 254-nm filter insert). In all experiments designed to measure yield, 100 ± 5% of the injected radioactivity was recovered from the column. The relative areas under the peaks were obtained using a Hewlett-Packard 3390 integrating recorder.

Electrochemistry and Electrophoresis. Electrochemical measurements were made with a Bioanalytical Systems Inc. CV-1A apparatus using a platinum-disk working electrode (PDE, Bioanalytical Systems Inc. MF 2013). An aqueous Ag/AgCl/NaCl (3M) electrode and a platinum wire were used as reference and auxiliary electrodes, respectively. Experiments were conducted in *N,N*-dimethylformamide (DMF) with 0.5 M tetraethylammonium perchlorate (TEAP) as supporting electrolyte.

Electrophoresis experiments were conducted with a Gelman electrophoresis chamber and power supply using a pH 6.5–6.8 buffer that is 8 mM in Na_2SO_4 and 2 mM in Na_2HPO_4 and paper electrophoresis strips (Beckman S&S 2043 A). The applied electric field was 200 V for a period of 3 h. The strips were then cut in 1-cm portions and assayed for γ activity in a Isodyne 1186 automatic γ system.

Animal Studies. The tissue distribution studies were conducted with female Sprague-Dawley rats of 200-g average weight. The rats were anesthetized with Metofane (Pitman–Moore), and the radiopharmaceuticals were administered via jugular vein injection. Each injection contained 50 μCi of ^{186}Re activity and/or 150 μCi of ^{99m}Tc activity. At 5, 45, and 90 min after the initial injection, the rats were sacrificed by cervical dislocation for the 5-min biodistribution points and CO_2 asphyxiation for the other time points. Samples of the blood, heart, lung, liver, spleen, and kidney were immediately obtained. Specimens were weighed and counted in a Isodyne Model 1385 automatic γ system (window 90–190 keV) both 14 and 110 h after injection; appropriate standards and blanks for calculation of percent uptake per gram of tissue were counted at the same times. One female Sprague–Dawley rat of ca. 200-g weight was injected with 0.24 mCi of *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$, and scintiphotographs were obtained with a Model 100 Ohio Nuclear γ camera as a function of time. A second, similar, rat was injected with 0.26 mCi of *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$, and an analogous imaging study was performed.

A 16.5-kg male beagle dog was injected with 4.3 mCi of *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$. Blood samples were withdrawn at various times and assayed for radioactivity to monitor the rate of blood clearance. Scintigraphic data were collected in a computer as previously described:[5] time–activity curves were obtained over the heart, lung, and liver.

Results

Synthesis. The preparation of nonradioactive $(Bu)_4NReO_4$ is straightforward. However, the preparation of the ^{186}Re analogue is based on a chromatographic procedure that allows efficient recovery of the purified ^{186}Re complex in yields greater than 99%. This procedure is designed around a reverse-phase C_{18} cartridge upon which $(Bu)_4N^{186}ReO_4$ is loaded and purified. Subsequent elution of the cartridge with absolute ethanol rapidly and cleanly yields the desired product.

Each of the Re and Tc complexes *trans*-$[M(DMPE)_2Cl_2]^+$ (M = Tc, Re) has been prepared from MO_4^- with both macroscopic amounts of material and the very small amount of material encountered in radiochemical syntheses.[24] All four of these preparations are closely related and can be described within a common synthetic route. When a solution of MO_4^- in ethanol/HCl is heated at 140–160 °C with an excess of the DMPE ligand, the desired *trans*-$[M(DMPE)_2Cl_2]^+$ complex is synthesized in yields greater than 95%. Elimination of the excess ligand and the oxidized ligand from the reaction medium is easily performed by cation-exchange chromatography or by purification based upon solubility differences. The overall reaction can be represented as

$$M^{VII}O_4^- + \text{DMPE (excess)} + HCl \rightarrow [M^{III}(DMPE)_2Cl_2]^+ + DMPE(O)_2 + H_2O \quad (1)$$

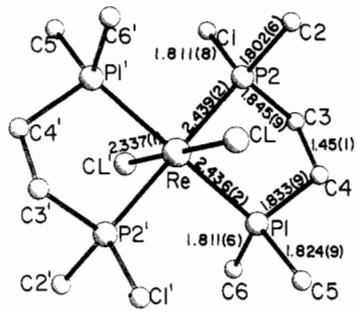

Figure 1. PLUTO drawing of *trans*-$[Re(DMPE)_2Cl_2]^+$ with atom labels and bond lengths (Å). Primed atoms are related by the inversion center at Re.

The detailed syntheses of macroscopic amounts of *trans*-$[^{99}Tc(DMPE)_2Cl_2]^+$ and radiochemical quantities (10^{-8} M) of *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ have been previously documented.[8,25] Syntheses of the nonradioactive and radioactive rhenium analogues differ from these preparations of technetium complexes due to (1) the resistance of ReO_4^- to reduction and (2) the relatively large concentration of nonradioactive Re present in reactor-produced ^{186}Re. Macroscopic amounts of nonradioactive *trans*-$[Re(DMPE)_2Cl_2]PF_6$ are obtained from a bomb reaction utilizing somewhat drastic conditions of temperature, time, and acid concentrations. Under these conditions, the sole detectable Re product is *trans*-$[Re(DMPE)_2Cl_2]^+$, which can be precipitated from solution by the addition of NH_4PF_6. For safety reasons, the radiochemical preparation of *trans*-$[Re(DMPE)_2Cl_2]^+$ cannot be conducted in a bomb reactor. Rather, the synthesis of *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$ is conducted in a borosilicate glass reaction vial under conditions that are carefully controlled to generate acceptable radiochemical yields while maintaining operator safety. These conditions are less drastic than those used in the bomb preparation of the nonradioactive complex, and thus some radiochemical impurities are chromatographically detected in the final product. The cation-exchange chromatography purification is employed to eliminate most of the excess ligand and ligand byproducts, to convert the product into a radiopharmaceutically acceptable form, and also to remove some of the radiolabeled impurities.

Characterization. The *trans*-$[Re(DMPE)_2Cl_2]^+$ complex is identified by single-crystal X-ray structural analysis, elemental analysis, spectroscopy, and chromatography. It has been further characterized by cyclic voltammetry to determine the redox potential, $E^{0\prime}$, of the Re(III)/Re(II) couple. The final product of the radiochemical preparation with either ^{99m}Tc or ^{186}Re is identified by HPLC. Confirmation of the identity of the respective complexes is accomplished by HPLC using a dual-detection system (radiometric and UV). In this procedure, the radioactive complex and the nonradioactive, well-characterized, complex are simultaneously injected. Observation of simultaneously eluting radioactive and UV peaks confirms that the radioactive "unknown" and nonradioactive "known" are indeed the same chemical species. Further characterization of the radioactive complexes is accomplished by electrophoresis, which confirms the cationic charge of $[M(DMPE)_2Cl_2]^+$ (M = Tc, Re) in solution, and by octanol/saline partition coefficients. Table II summarizes the data obtained from these several studies on the rhenium complexes and lists comparable data for the technetium analogues.

Crystal Structure. Figure 1 shows a perspective view of the *trans*-$[Re(DMPE)_2Cl_2]^+$ cation, defines the atom-numbering scheme, and gives selected bond lengths. Final positional parameters are given in Table III. Both the Re atom and the phosphorus atom of the PF_6^- anion, i.e. P3, occupy crystallographic inversion centers in the unit cell. Listings of bond lengths and bond angles (Table A), observed and calculated structure factors (Table B), thermal parameters (Table C), hydrogen atom positional parameters (Table D), and calculated torsion angles (Table

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Table II. Comparison of Chemical and Physical Properties of *trans*-$[Tc(DMPE)_2Cl_2]^+$ and *trans*-$[Re(DMPE)_2Cl_2]^+$

	trans-$[Tc(DMPE)_2Cl_2]^+$	*trans*-$[Re(DMPE)_2Cl_2]^+$
	Crystal Structure	
M–Cl, Å	2.323 (3) av	2.337 (1)
M–P, Å	2.436 (5) av	2.438 (2) av
Cl–M–P, deg	89.3 (4) av	89.3 (1), 91.4 (1)
P–M–P, deg	81.4 (2) av	81.5 (1)
	UV–Vis Spectroscopy	
λ_{max}, nm (ϵ, M^{-1} cm^{-1})	464 (2140), 278 (3360)	432 (2400), 406 (2620), 261 (5530)
	IR Spectroscopy	
M–Cl stretch,[a] cm^{-1}	345	315
	Cyclic Voltammetry	
$E^{\circ\prime}$[M(III)/M(II)],[b] V	−0.208	−0.398
$E^{\circ\prime}$[M(II)/M(I)],[b] V	−1.370	−1.548
	HPLC Analysis	
k' on 5 μm[c]	2.02–2.27[d]	2.06–2.31[d]
	trans-$[^{99m}Tc(DMPE)_2Cl_2]^+$	*trans*-$[^{186}Re(DMPE)_2Cl_2]^+$
	HPLC Analysis	
k' on 10 μm[c]	3.0	2.7
	Electrophoresis	
dist,[e] cm	6–7	5–6
	Lipophilicity	
octanol/saline partn coeff	3.0 (3)	2.8 (3)

[a] Tentative assignments. [b] Vs. Ag/AgCl in DMF, 0.5 M TEAP. [c] k' values are dependent on the size of the particles used to pack the HPLC column. Columns with both 5- and 10-μm packing have been used. [d] k' values are dependent on the mass of complex injected (see text and Figure 2[26]). Table entries are the observed ranges of k' values. [e] Distance migrated toward the cathode under the conditions outlined in the text.

Table III. Fractional Atomic Positional Parameters[a,b] for *trans*-$[Re(DMPE)_2Cl_2]PF_6$

atom	*x*	*y*	*z*
Re	0.0	0.0	0.0
Cl	0.0469 (2)	−0.0388 (2)	0.1927 (1)
P1	0.2216 (2)	−0.1004 (2)	0.0343 (2)
P2	0.1435 (2)	0.1968 (2)	0.0523 (2)
P3	0.50000	0.0	0.50000
F1	0.4576 (6)	0.1376 (6)	0.4409 (6)
F2	0.520 (1)	−0.050 (1)	0.3983 (7)
F3	0.6504 (7)	0.0378 (9)	0.557 (1)
C1	0.092 (1)	0.3368 (8)	−0.0436 (8)
C2	0.188 (1)	0.2668 (9)	0.1927 (8)
C3	0.311 (1)	0.154 (1)	0.0521 (9)
C4	0.3574 (9)	0.0237 (9)	0.099 (1)
C5	0.288 (1)	−0.236 (1)	0.1359 (7)
C6	0.2418 (9)	−0.157 (1)	−0.0915 (7)

[a] The estimated error in the last digit is given in parentheses. This form is used throughout. [b] The numbering scheme is shown in Figure 1.

E) have been deposited as supplementary material.[26]

HPLC Analyses. The chemical purity of each of the ^{99}Tc and nonradioactive Re complexes is greater than 98% by HPLC analysis using UV detection. The radiochemical purity of the ^{99m}Tc and ^{186}Re radiopharmaceuticals is greater than 98% by the same HPLC analysis using radiometric detection. The observed retention time of *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$ is dependent on the mass of the complex coinjected, just as the observed retention time of *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ is dependent on the mass of *trans*-$[^{99}Tc\text{-}(DMPE)_2Cl_2]^+$ coinjected[8] (Figure 2[26]). For both complexes the retention time (and thus the capacity factor k') decreases as the number of moles injected increases. The largest k' value (3.0) is observed for *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$, which contains the least mass since it is prepared from generator-produced, "no carrier added", $^{99m}TcO_4^-$.[24] The k' value observed for *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$ is smaller (2.7) since this agent is prepared from reactor-produced $^{186}ReO_4^-$ which contains substantial amounts of "carrier" $^{185}ReO_4^-$. When *trans*-$[^{99m}Tc\text{-}(DMPE)_2Cl_2]^+$ and *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$ are mixed for the biological experiment described below, the k' value observed for the resulting, single, HPLC peak is 2.8, reflecting the large mass of rhenium complex coinjected with the technetium complex.

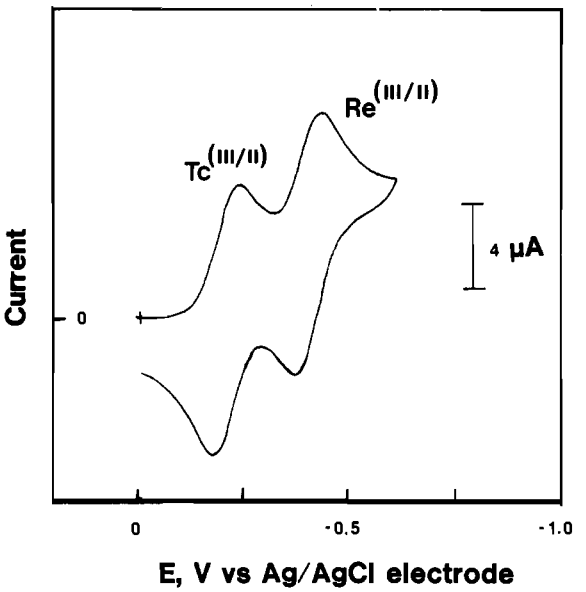

Figure 4. Cyclic voltammogram of a mixture of *trans*-$[Tc(DMPE)_2Cl_2]^+$ and *trans*-$[Re(DMPE)_2Cl_2]^+$. Conditions: 0.5 M TEAP in DMF; platinum electrode; scan range 100 mV/s.

UV–Visible Spectrophotometry. The spectra of the Tc and Re complexes show characteristic, intense absorptions in the visible region. That of the Re complex is split, both components occurring at energies higher than that of the comparable band of the Tc complex. Each complex also exhibits a UV absorption, and again that for the Re complex is at higher energy (Figure 3[26]).

Cyclic Voltammetry. The *trans*-$[Re(DMPE)_2Cl_2]^+$ complex exhibits a Re(III)/Re(II) couple that appears to be electrochemically reversible since (1) the difference between the cathodic and anodic peaks is close to the Nernstian value of 59 mV, (2) the peak currents are both proportional to the square root of the scan rate over the range 25–400 mV/s, and (3) the ratio of anodic to cathodic peak currents is close to unity. A large and significant difference is observed between the Tc(III)/Tc(II) $E^{0\prime}$ value for *trans*-$[Tc(DMPE)_2Cl_2]^+$ and the Re(III)/Re(II) $E^{0\prime}$ value for *trans*-$[Re(DMPE)_2Cl_2]^+$. The Re complex is 190 mV harder to reduce than the Tc complex. Figure 4 shows a cyclic voltammogram of a mixture of the Re and Tc complexes wherein the waves due to the disparate Tc and Re processes can be seen clearly. Also, both the Tc and Re complexes exhiibit a reversible M(II)/M(I) redox couple, the $E^{0\prime}$ of which is ca. 1.15 V more negative than that of the corresponding M(III)/M(II) $E^{0\prime}$ value (Table II). No evidence for a M(IV)/M(III) couople is seen for either the Re or Tc complex.

Biological Results. Table IV compares the biological distribution data of a mixture of *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$ and *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ in rats with the distribution of *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ alone. The data of Table V show how the $[^{99m}Tc]/[^{186}Re]$ ratio varies in several tissues as a function of time. Variations of tissue uptake ratios over the range 0.14–6.40

(26) Supplementary material. This includes all tables designated by alphabetic characters.

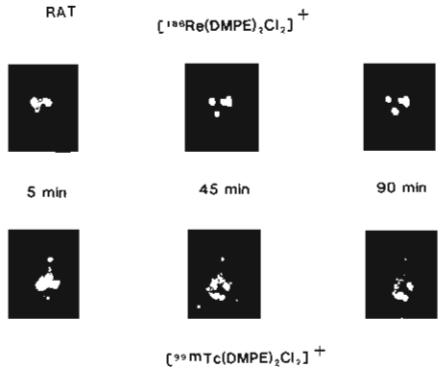

Figure 5. Comparative scintiphotographs of rats imaged with *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$ and with *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ at 5, 45, and 90 min post injection.

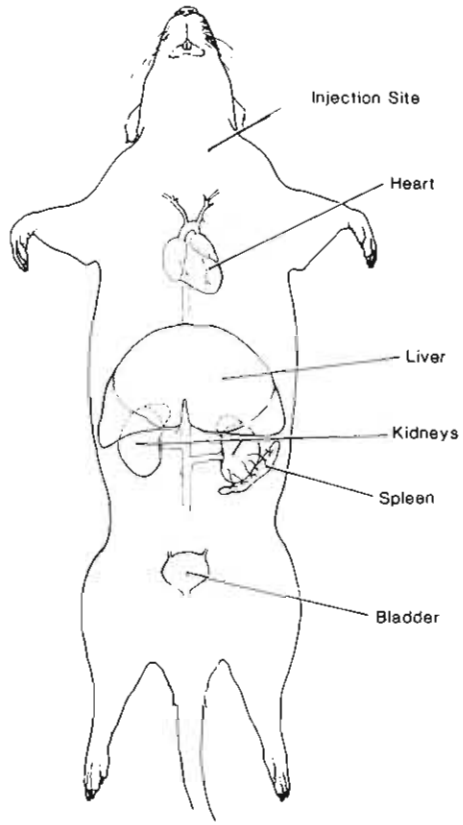

Figure 6. Schematic diagram illustrating the major sites and organs visualized in the scintiphotographs of Figure 5.

are statistically significant and reproducible. The manifestations of these biodistribution data in scintigraphic images are illustrated in Figure 5, which displays comparative scintiphotographs of rats imaged with *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ or *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$. The schematic diagram given in Figure 6 identifies the major sites and organs visualized in the scintiphotographs of Figure 5.

Figure 7 illustrates the blood clearance of *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ and *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$ in dogs, while Figure 8 illustrates the computer-derived biodistribution of these two complexes in dogs. In both of these figures the data for *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ are taken from a previous study.[5] Figure 9 displays comparative scintiphotographs of mongrel dogs imaged with *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ and with *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$.

Discussion

The following abbreviations are used in this section: [M] = *trans*-$[M(DMPE)_2Cl_2]^+$ (M = Tc, Re); [Re] = *trans*-$[Re(DMPE)_2Cl_2]^+$; $[^{186}Re]$ = *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$; $[^{99}Tc]$ = *trans*-$[^{99}Tc(DMPE)_2Cl_2]^+$; $[^{99m}Tc]$ = *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$.

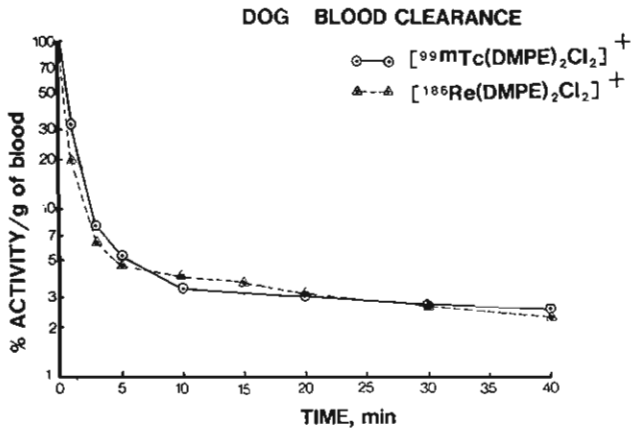

Figure 7. Blood clearance of *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$ and *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ in the dog. Data for the ^{99m}Tc complex taken from ref 5.

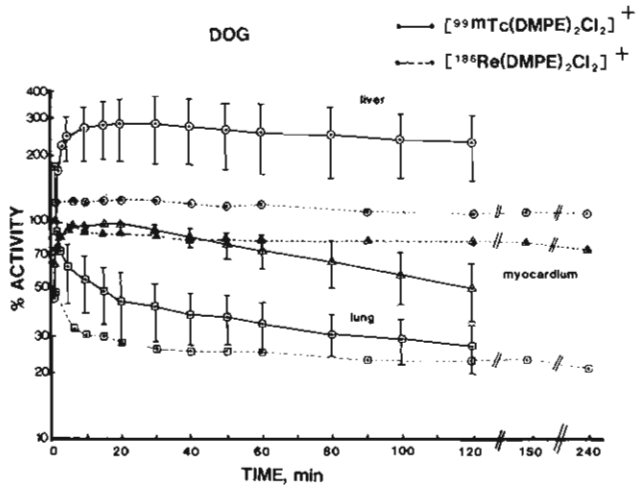

Figure 8. Computer-derived, time-dependent biodistribution data for *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$ and *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ in the dog. Data for the ^{99m}Tc complex taken from ref 5.

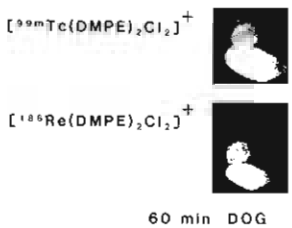

Figure 9. Comparative scintiphotographs (30° LAO) of dogs imaged with *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$ and with *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ 1 h after injection. The heart is visualized as the characteristic doughnut-shaped image, while the liver appears as the large crescent-shaped image below the heart.

Preparation of $(Bu)_4N^{186}ReO_4$. Preparation of $(Bu)_4N^{186}ReO_4$ is effected both to convert $^{186}ReO_4^-$ to a form soluble in alcohol and also to purify $^{186}ReO_4^-$ from NH_4^+ and NO_3^-. When unpurified $^{186}ReO_4^-$ is used as starting material, some labeling does occur, but it is inconsistent and is related to the amount of excess ligand and the volume of $^{186}ReO_4^-$ solution used in the preparation. The C_{18} reverse-phase purification functions because the $(Bu)_4N^+,^{186}ReO_4^-$ ion pair is retained on the cartridge while the $(Bu)_4N^+,NO_3^-$ ion pair is eluted with H_2O (even though the initial NO_3^- concentration is calculated to be ca. 1.5 mmol/mL). The cartridge eluate is tested with diphenylamine/H_2SO_4 until this qualitative reaction is negative (ca. 10^{-7} mol of nitrate/mL).[27]

(27) "Reagent Chemicals: ACS Specifications"; American Chemical Society: Washington, DC, 1981; p 27.

Table IV. Tissue Distribution of a Mixture of *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$ and *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ in Rats Compared to the Tissue Distribution of *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ Alone

I. Mixture of *trans*-$[^{186}Re(DMPE)_2Cl_2]^+$ and *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$

	5 min		45 min		90 min	
	Tc	Re	Tc	Re	Tc	Re
blood	0.095 ± 0.014	0.685 ± 0.056	0.095 ± 0.016	0.350 ± 0.015	0.078 ± 0.013	0.247 ± 0.023
heart	3.67 ± 0.45	3.50 ± 0.37	1.89 ± 0.46	3.43 ± 0.82	1.33 ± 0.23	2.82 ± 0.27
lung	1.70 ± 0.16	0.91 ± 0.08	0.790 ± 0.067	0.45 ± 0.11	0.709 ± 0.076	0.317 ± 0.026
liver	1.95 ± 0.26	0.79 ± 0.13	1.62 ± 0.23	0.285 ± 0.037	1.39 ± 0.13	0.218 ± 0.005
spleen	2.62 ± 0.34	1.56 ± 0.17	3.07 ± 0.28	0.89 ± 0.12	3.28 ± 0.33	0.79 ± 0.12
kidney	6.18 ± 0.88	8.4 ± 1.2	3.34 ± 0.22	4.17 ± 0.44	2.61 ± 0.28	2.87 ± 0.49

II. *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$

	5 min	45 min	90 min
blood	0.13 ± 0.04	0.093 ■ 0.016	0.069 ± 0.009
heart	4.11 ± 0.42	2.03 ± 0.17	1.27 ± 0.11
lung	2.20 ± 0.17	0.93 ± 0.12	0.622 ± 0.090
liver	2.27 ± 0.27	1.96 ± 0.23	1.96 ± 0.18
spleen	2.35 ± 0.11	2.79 ± 0.31	3.08 ± 0.29
kidney	6.81 ± 0.17	4.00 ± 0.41	2.60 ± 0.23

[a] Table entries are average percent injected dose per gram of organ at the listed time of assay. The quoted error is ±1 standard deviation. Five rats were sacrificed at each time. The radiopharmaceuticals were injected in 0.3 mL of normal saline. Data are corrected for the physical decay of the isotopes.

Table V. *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^+$/*trans*-$[^{186}Re(DMPE)_2Cl_2]^+$ Tissue Uptake Ratios as a Function of Time[a]

	5 min	45 min	90 min
blood	0.14 ± 0.02	0.27 ± 0.04	0.31 ± 0.04
heart	1.05 ± 0.06	0.55 ± 0.02	0.47 ± 0.06
lung	1.87 ± 0.13	1.81 ± 0.33	2.24 ± 0.16
liver	2.49 ± 0.10	5.70 ± 0.36	6.40 ± 0.62
spleen	1.85 ± 0.20	3.48 ± 0.25	4.18 ± 0.38
kidney	0.73 ± 0.02	0.81 ± 0.04	0.93 ± 0.14

[a] Quoted error is ±1 standard deviation. Five rats were sacrificed at each time. Data are corrected for the physical decay of the isotopes.

The $(Bu)_4N,^{186}ReO_4$ ion pair is then eluted from the cartridge with absolute ethanol in a form suitable for subsequent syntheses.

Preparation of [Re] and [^{186}Re]. The fundamental chemistry differentiating the preparations of the [M] complexes involves the fact that TcO_4^- is a better oxidant than is ReO_4^-. The $E^{0\prime}$ values governing the MO_4^-/MO_2 complex are 0.782 and 0.510 V vs. NHE, respectively.[28] Thus, TcO_4^- reacts with a reducing agent such as DMPE under milder conditions than does ReO_4^-. Efficient reduction of ReO_4^- by DMPE requires either a bomb reaction (for nonradioactive ReO_4^-) or high temperature and prolonged reaction time (for $^{186}ReO_4^-$). The different procedures used for the preparation and purification of [Re] and [^{186}Re] are dictated primarily by safety considerations, the disparate amounts of rhenium present in the two preparations, and constraints determined by the ultimate use of [^{186}Re] as a radiopharmaceutical. The [Re] complex is prepared under conditions that do not yield any other detectable complexes. Using milder reaction conditions with rhodium(III), Butter and Chatt[29] prepared both *cis*- and *trans*-$[Rh(DMPE)_2Cl_2]^+$; however, no evidence for the cis isomer is obtained in the synthesis of [Re] presumably because the more stringent reaction conditions used in the rhenium preparation favor formation of the thermodynamically stable trans isomer. Similarly, Hughes et al.[30] report the five-coordinate complex $[ReCl(DPPE)_2]$, where DPPE represents 1,2-bis(diphenylphosphino)ethane, but no evidence for an analogous complex with DMPE is seen in the preparation of [Re].

The preparation of [^{186}Re] in acceptable yield and purity requires careful control of reaction conditions, especially temperature, time, concentration of DMPE and of HCl, and absence of oxygen or other oxidizing agents. HPLC analysis of preparations conducted at 130 °C for 1–2 h shows ca. 80% yield of [^{186}Re], the presence of unreacted $^{186}ReO_4^-$, and two unidentified impurities. Better yields are obtained at higher temperatures (145–150 °C) and longer reaction times (2–5 h). The excess DMPE and HCl used in the preparation are removed by a cation-exchange chromatographic purification, which also generates [^{186}Re] in a medium (0.15 M NaCl) suitable for injection. Typical concentrations of HCl and DMPE used in the synthesis are 0.3 and 0.075 M, respectively, while the total concentration of ReO_4^- is usually ca. 0.002 M. Lowering the concentration of DMPE to 0.009 M reduces the yield of [^{186}Re] to zero. The presence of oxygen or other oxidants (e.g., nitrate) interferes with the preparation of [^{186}Re] by preferentially reacting with DMPE and thus decreasing the amount of DMPE available to reduce and complex rhenium.

Crystal Structure. Single structural analyses of [^{99}Tc][14] and [Re] show that they are very similar with respect to bond lengths and angles, as well as overall dimensions and thus volume (Table II). This is to be expected since the oxidation state and ligand environment of the two metal centers are identical. In addition, the weakly screening 4f electrons of Re cause rhenium and technetium to have about the same atomic radius. Thus, the crystal radii of four-coordinate Tc(VII) and Re(VII) are 0.51 and 0.52 Å, respectively,[31] the M–Cl bond length in both $[Tc^{IV}Cl_6]^{2-}$ and $[Re^{IV}Cl_6]^{2-}$ is 2.35 (1) Å,[32,33] and the radii of Re(0) and Tc(0) in the free metals are identical.[34]

Table F[26] lists 23 mononuclear rhenium complexes, containing tertiary phosphine and/or chloride ligands, that have been structurally characterized. Observed Re–P and Re–Cl bond lengths are dependent on (1) steric crowding, with respect to coordination number and especially with respect to multiply bonded ligands, (2) structural trans effects (STE) of strongly bonded ligands, (3) oxidation state of the metal center, and (4) π-acceptor ability of the ligand, which is clearly more important in the lower oxidation states.[35] These interacting, and sometimes conflicting, factors contribute to a varying degree from one table entry to another, but the overall effects are surprisingly small.

(28) Weast, R. C. "CRC Handbook of Chemistry and Physics"; CRC Press: Boca Raton, FL, 1983; p D-159.
(29) Butter, S. A.; Chatt, J. *J. Chem. Soc. A* **1970**, 1411.
(30) Hughes, D. L.; Pombeiro, A.; Pickett, C. J.; Richards, R. L. *J. Organomet. Chem.* **1983**, *248*, C26.
(31) Shannon, R. D. *Acta Crystallogr., Sect. A: Cryst. Phys., Differ., Theor. Gen. Crystallogr.* **1976**, *A32*, 751.
(32) Elder, M.; Fergusson, J. E.; Gainsford, G. J.; Hickford, J. H.; Penfold, B. R. *J. Chem. Soc. A* **1967**, 1423.
(33) Adman, E.; Margulis, T. N. *Inorg. Chem.* **1967**, *6*, 210.
(34) (a) Gmelin, L. "Handbuch der Anorganischem Chemie"; Verlag Chemie: Berlin, Germany, 1941; Rhenium. (b) Gmelin, L. "Handbuch, der Anorganischen Chemie"; Springer-Verlag: Berlin, Germany, 1983; Technetium, Suppl. Vol. 2.
(35) Cotton, F. A.; Dunbar, K. R.; Falvello, L. R.; Tomas, M.; Walton, R. A. *J. Am. Chem. Soc.* **1983**, *105*, 4950.

For 16 Re–P lengths the average is 2.44 (6) Å, while for 33 Re–Cl lengths the average is 2.41 (7) Å. Re–P distances are generally longer than Re–Cl distances, but the latter can be lengthened by the presence of a trans ligand that induces a large STE. There are three clear instances of the STE of tertiary phosphines lengthening the Re–Cl bond (relative to a Re–Cl bond trans to chloride). Within the context of this summary, the Re–P and Re–Cl bond lengths of [Re] (Table II) are quite normal.

Characterization. In addition to the single-crystal structural analyses described above, [Re] and [Tc] have been characterized by a variety of chemical and physical techniques.

(a) Visible–UV spectroscopy. The visible spectrum of [Tc] is dominated by an intense band that, by comparison to the visible spectra of related Tc(III) complexes, has been unequivocally assigned as arising from a halogen-to-metal charge-transfer (HTMCT) transition.[25] The visible spectrum of [Re] exhibits an analogous band at higher energy since the Re(III) center is a poorer oxidant than the Tc(III) center. The [Re] HTMCT is split into two components, possibly due to transitions from the π and σ chlorine orbitals. It is unlikely that the splitting represents transitions to the e_g and t_{2g} orbitals of Re(III) since the difference in energy between the split components is only 0.21 eV, much less than the anticipated $10Dq$ value for a low-spin Re(III) complex. Analogous splitting of the visible band has been reported for the related Re(III) complexes *trans*-$[Re(DIARS)_2Cl_2]^+$ and *trans*-$[Re(DPPE)_2Cl_2]^+$,[20] where DIARS represents *o*-phenylenebis(dimethylarsine) and DPPE represents 1,2-bis(diphenylphosphino)ethane. The UV spectra of both [Tc] and [Re] exhibit an intense absorption, with again the [Re] band occurring at higher energy. In fact, the difference in energy between these two UV bands (0.29 eV) is approximately the same as the difference in energy between the two visible HTMCT bands (0.30 eV average). On this basis, the UV bands are assigned as arising from phosphine-to-metal charge-transfer (PTMCT) transitions. Thus, for a given [M] complex the difference between the energy of the PTMCT and the HTMCT bands (ca. 1.9 eV) reflects the relative abilities of tertiary phosphorus and chloride to function as 1-equiv electron donors to M(III).

(b) Cyclic Voltammetry. The above analysis of spectroscopic data suggests that the Tc(III) center in [Tc] is a better oxidant than the Re(III) center in [Re] by about 0.3 eV. A more direct determination by cyclic voltammetry shows that [Tc] is a better oxidant than [Re] by 0.19 V. This difference is consistent with a previously reported summary of data obtained for four pairs of analogous Tc and Re complexes, the range of the observed effect for the four pairs being from 0.19 to 0.32 V.[21] The general agreement between the 0.3-eV difference determined by spectroscopy and the 0.19-V difference determined by cyclic voltammetry is expected on the basis of an extensive study of related Tc(III) complexes.[25] This study showed that, for analogous complexes, the relationships between the energy of the HTTCT band and the $E^{0\prime}$ value governing the Tc(III)/Tc(II) couple can be described as linear with slopes in the range of ca. 0.7 to ca. 2.5.

(c) IR Spectroscopy. As expected, the IR spectra of [Re] and [Tc] are virtually identical. The only anticipated differences in the two spectra are slight shifts in the M–Cl and M–P stretching frequencies, with the Re stretch expected to be lower in energy than the Tc stretch.[36] The tentative M–Cl band assignments listed in Table II are consistent with this expectation.

(d) HPLC Analysis and Lipophilicity. The capacity factors, k', exhibited by [Tc] and [Re] upon reverse-phase chromatography are essentially identical (Table II). For both complexes the value of k' is dependent on the amount of complex injected;[8] the form and magnitude of this effect are the same in both cases (Figure 2[26]). Since k' values in reverse-phase chromatography are determined primarily by lipophilicity, these HPLC studies establish that, as expected, [Tc] and [Re] have identical liophilicities. This conclusion is supported by the independent determination of octanol/saline partition coefficients for both [^{99m}Tc] and [^{186}Re]. Within experimental error, the partition coefficients of these two complexes are identical (Table II). Both have about three times more affinity for the lipophilic phase than for the hydrophilic phase.

Rationale for the Biological Experiments. The [^{99m}Tc] and [^{186}Re] complexes are essentially identical in size, shape, charge, and lipophilicity. However, the properties of these complexes differ—some in ways that are not expected to affect the biological behavior of the complexes (such as UV–visible and IR absorptions) and some in ways that might reasonably be expected to influence biological distribution. The latter category consists of (a) the anticipated difference in rate of ligand substitution onto Tc(III) and Re(III) centers, (b) the fact that the number of moles of rhenium injected as [^{186}Re] is much greater than the number of moles of technetium injected as [^{99m}Tc], and (c) the established difference in redox potential between the Tc(III) and Re(III) complexes.

On the basis of established periodic trends, ligand substitution onto Tc(III) is anticipated to be faster than ligand substitution onto Re(III), and this factor might influence the biodistribution of [^{99m}Tc] relative to that of [^{186}Re]. However, both complexes are very inert to substitution under conditions similar to those encountered in vivo, and after injection into test animals the more labile [Tc] complex can be recovered from tissues (heart, liver, bile) and shown by HPLC analysis not to have undergone any ligand substitution.[37] Thus, it is safe to conclude that the anticipated rate of ligand substitution onto Tc(III) and Re(III) is not likely to affect the relative biodistributions of [^{99m}Tc] and [^{186}Re].

Since ^{186}Re is produced by neutron bombardment of ^{185}Re, it is always associated with large amounts of the ^{185}Re "carrier". On the other hand, ^{99m}Tc is produced by β decay of ^{99}Mo in a generator system that yields ^{99m}Tc associated with only very small amounts of "carrier" ^{99}Tc.[24] Because of this difference the [^{186}Re] radiopharmaceutical contains about 10^3 more molecules than does the [^{99m}Tc] radiopharmaceutical, and it is conceivable that the larger amount of [^{186}Re] injected might saturate biological sites and thereby affect the biodistribution of [^{186}Re] relative to that of [^{99m}Tc]. This possibility is eliminated by the results of the two biological distribution experiments summarized in Table IV. In the first experiment a mixture a [^{186}Re] and [^{99m}Tc] is injected, while in the second experiment [^{99m}Tc] is injected alone. If the mass of the [^{186}Re] radiopharmaceutical were to affect biodistribution by saturating biological sites, then the biodistribution of the coinjected [^{99m}Tc] should also be affected. However, the data of Table IV show that the biodistribution of [^{99m}Tc] is the same, within experimental error, whether it is injected alone or it is coinjected with [^{186}Re]. Thus, it can be concluded that the larger number of molecules associated with [^{186}Re] does not affect its biodistribution. This conclusion is consistent with our observation that addition of "carrier" [^{99}Tc] to [^{99m}Tc] preparations does not affect the biodistribution of this radiopharmaceutical[38] and with the fact that even with [^{186}Re] typically only 10^{-8} mol of complex are being injected.

Therefore, the salient difference of biological importance between [^{99m}Tc] and [^{186}Re] is the fact that the technetium complex is more easily reduced than is the rhenium complex. If [^{99m}Tc] is reduced in vivo, the large 0.19 V difference in $E^{0\prime}$ values between complexes makes it very unlikely that [^{186}Re] will also be susceptible to in vivo reduction. Reduction of the cationic [^{99m}Tc] complex would convert it to the neutral Tc(II) analogue *trans*-$[^{99m}Tc(DMPE)_2Cl_2]^0$, which is expected to exhibit considerably different biological behavior. Thus, it can be determined whether or not the redox behavior of [^{99m}Tc] is influencing its biological behavior by coinjecting [^{99m}Tc] with [^{186}Re] into test animals and monitoring the relative biodistributions of these agents in various animal tissues. If neither complex is reduced in vivo, then the

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(38) Libson, K.; Deutsch, E., unpublished data.

relative biodistributions will be the same. But if [^{99m}Tc] undergoes in vivo reduction, then the relative tissue distributions of [^{99m}Tc] and [^{186}Re] should differ considerably.

Biological Experiments. The data of Table IV clearly show that when [^{99m}Tc] and [^{186}Re] are coinjected into rats, the resulting [^{99m}Tc]/[^{186}Re] tissue uptake ratio is dependent upon both the type of tissue sampled and the time after injection. Within the above rationale, this establishes that the difference in redox potential between [^{99m}Tc] and [^{186}Re] affects the relative biodistributions of these agents. Since the Tc(III)/Tc(II) couple of [^{99m}Tc] is biologically accessible, it is reasonable to conclude that [^{99m}Tc] is reduced in vivo whereas [^{186}Re] is not. The following aspects of the biodistribution data support this conclusion: (1) The [^{99m}Tc] complex is taken up in the liver and spleen much more avidly than is the [^{186}Re] complex, and this difference increases with time. This is consistent with gradual in vivo reduction of Tc(III) to Tc(II) since the neutral Tc(II) complex is insoluble in aqueous media and may precipitate in colloidal form. This observation and interpretation are consistent with a previous report of [^{99m}Tc] uptake by the reticuloendothelial system (RES).[5] This RES uptake implies that [^{99m}Tc] is present in vivo in both colloidal and noncolloidal forms,[5] even though laser-scattering experiments show that before injection [^{99m}Tc] is completely in solution. The scintigraphic images of Figure 9 distinctly show the difference in RES uptake of the two agents in dogs. In the [^{99m}Tc] image,[5] the ribs and backbone are visualized due to uptake by the marrow (part of the RES), whereas these bones are not visualized in the [^{186}Re] image. Tissue distribution experiments show that in dogs the [^{99m}Tc] activity is in, or on, the ribs and is not associated with the soft tissue around the ribs.[38] (2) Initially [^{99m}Tc] and [^{186}Re] are taken up by the heart to the same extent. The data of Table III show that both agents then wash out of the heart, but the data of Table IV and Figure 8 show that [^{99m}Tc] definitely washes out more rapidly. Again, this is consistent with Tc(III) undergoing preferential reduction in the heart and then being washed out as Tc(II). The more prolonged heart uptake of [^{186}Re] is consistent with it *not* being reduced to Re(II); the technetium(I) complex [^{99m}Tc(DMPE)$_3$]$^+$ also does not wash out of the heart,[10,15] and it too is incapable of being reduced to a neutral form.[39]

The quantitative biodistribution data of Table IV are more readily visualized in the time-dependent scintiphotographs shown in Figure 5. These comparative photographs show one rat imaged with [^{99m}Tc] and another imaged with [^{186}Re]. The schematic diagram in Figure 6 illustrates the location of the organs of interest to this study, as well as the location of the injection site (jugular vein), which is irrelevant to this discussion. The scintiphotographs of Figure 5 confirm that the [^{186}Re] activity persists in the heart longer than does the [^{99m}Tc] activity and also show that the [^{186}Re] activity clears primarily through the kidneys and then concentrates in the bladder. This clearance pattern is that which is expected for a small, water-soluble molecule. Contrariwise, [^{99m}Tc] is cleared primarily through the liver with activity subsequently being delivered to the intestines. This is the clearance pattern expected for a lipophilic molecule and is consistent with in vivo reduction of [^{99m}Tc] to the more liophilic Tc(II) analogue.

Further evidence for the different biological behavior of [^{186}Re] and [^{99m}Tc] results from injecting [^{186}Re] into a dog and monitoring the resultant activity distribution by serial collection of blood samples and a computer-assisted γ camera. In Figures 7 and 8 the data generated from this experiment are compared to the analogous, but more extensive, data previously obtained[5] upon injecting [^{99m}Tc] into dogs. Figure 7 shows that [^{99m}Tc] and [^{186}Re] clear from the blood at the same rate. Figure 8 shows that, similar to the results of the experiments in rats, [^{186}Re] washes out of the heart much more slowly than does [^{99m}Tc], and the liver uptake of [^{99m}Tc] is much higher than is the liver uptake of [^{186}Re]. The low liver activity, and relatively high heart activity, of the [^{186}Re] agent lead to very good-quality heart images even 4 h after injection. This contrasts to heart images obtained with [^{99m}Tc] (in dogs) that markedly degrade in quality after ca. 1 h.[5]

Conclusions

The salient conclusion to be reached from this work is that *trans*-[^{99m}Tc(DMPE)$_2$Cl$_2$]$^+$ and *trans*-[^{186}Re(DMPE)$_2$Cl$_2$]$^+$ behave differently in vivo even though these complexes have identical charge, lipophilicity, size, and overall molecular configuration. This different in vivo behavior is most reasonably ascribed to the 0.19 V difference in redox potential between the two complexes. Reduction of Tc(III) to Tc(II) is biological accessible, but reduction of Re(III) is not. In vivo reduction of *trans*-[^{99m}Tc^{III}(DMPE)$_2$Cl$_2$]$^+$ to the more lipophilic *trans*-[^{99m}Tc^{II}(DMPE)$_2$Cl$_2$]^0 detracts from the utility of *trans*-[^{99m}Tc^{III}(DMPE)$_2$Cl$_2$]$^+$ as a myocardial imaging agent by promoting transfer of radioactivity from the heart to the liver.

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Supplementary Material Available: Listings of bond lengths and bond angles (Table A), observed and calculated structure factors (Table B), thermal parameters (Table C), hydrogen atom positional parameters (Table D), calculated torsion angles (Table E), and bond distance data for other mononuclear Re complexes (Table F) and figures depicting the dependence of HPLC retention time on number of moles injected (Figure 2) and the visible–UV spectra of *trans*-[Re(DMPE)$_2$Cl$_2$]$^+$ and *trans*-[Tc(DMPE)$_2$Cl$_2$]$^+$ (Figure 3) (16 pages). Ordering information is given on any current masthead page.

(39) Ichimura, A.; Heineman, W. R.; Vanderheyden, J.-L.; Deutsch, E. *Inorg. Chem.* **1984**, *23*, 1272.