Technetium(V) Oxo Complexes of Substituted Propylene Diamine Dioxime (PnAO) Ligands: Water-Dependent Interconversion between Syn and Anti Isomers

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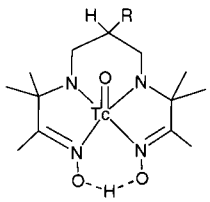
^{99m}Tc and ^{99}Tc complexes of PnAO (propylene diamine dioxime) ligands monosubstituted in the 6-position [PnAO-6-R] were prepared and studied. Ligands substituted with an alkyl group or with no substituent (R = H, CH_3, or $CH_2CH(CH_3)_2$), gave only one Tc complex. However, for several other nonalkyl substituents (R = $COOCH_3$, OH, OCH_3, OCH_2CH_3, F, CN, $NHCOCH_3$, and $NHCOCH_2CH_3$), two Tc complexes **A** and **B** were formed. Products **A** and **B** were assigned to the anti and syn TcO(PnAO-6-R) species, respectively, based on ^{1}H NMR results. X-ray structure analyses supported these assignments. The **A** (anti) isomer of TcO(PnAO-6-OH) had the chemical formula $TcC_{13}H_{25}N_4O_4$ and crystallized in an orthorhombic system with space group $P2_12_12_1$ and $Z = 4$; a = 12.744(2) Å, b = 13.591(2) Å, c = 9.976(2) Å. The **B** (syn) isomer of TcO(PnAO-6-CN) had the chemical formula $TcC_{14}H_{24}N_5O_3$ and was a 1:4 mixture of two monoclinic polymorphs: individual rectangular prisms (space group $P2_1/c$, $Z = 4$) and clusters of intergrown twinned rectangular rods (space group Cc, $Z = 8$). For the prisms, a = 12.457(1) Å, b = 13.932(1) Å, c = 10.336(1) Å, and for the rods, a = 31.344(5) Å, b = 6.993(1) Å, c = 21.657(2) Å. The syn and anti isomers interconverted in the presence of water; nonequilibrium mixtures of epimers remained unchanged under dry conditions. The HPLC behavior under reversed phase conditions was consistent with on-column interconversion (poor resolution), whereas the two isomers were cleanly resolved under drier normal phase conditions. An oxo inversion mechanism involving trans water attack is proposed for the interconversion process. Water also influenced the position of equilibrium of the two isomers. The syn isomer was stabilized in water relative to the anti isomer.

Introduction

PnAO is a propylene diamine dioxime ligand (3,3,9,9-tetramethyl-4,8-diazaundecane-2,10-dione dioxime) which forms a stable, neutral mono-(oxo) complex with technetium.[1−3] Technetium complexes of derivatives of PnAO are important as radiopharmaceuticals. ^{99m}Tc-HMPAO (HMPAO = *d,l*-hexamethyl propylene amine oxime) is a commercially available product for imaging cerebral perfusion (Ceretec).[4] A ^{99m}Tc-PnAO derivative containing a nitroimidazole substituent has been evaluated as a tissue hypoxia imaging agent.[5−8] ^{99m}Tc-PnAO bioconjugates of biotin[9] and octreotide[10] have also been reported for use in detection of tumors.



(1)

TcO(PnAO-6-R)

R = -H, -CH_3, -$CH_2CH(CH_3)_2$, -$COOCH_3$, -OH, -OCH_3, -OCH_2CH_3, -F, -CN, -$NHCOCH_3$, -$NHCOCH_2CH_3$

In this work, several technetium complexes of PnAO ligands **(1)** which are substituted at the central carbon of the propylene

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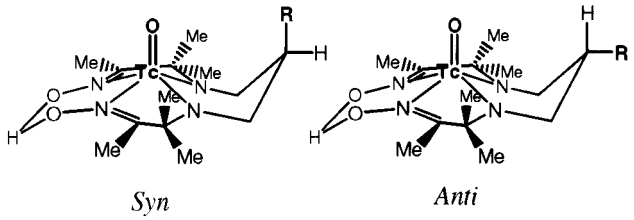

Figure 1. Syn and anti forms of TcO(PnAO-6-R).

bridge (the 6-position) have been investigated. The Tc(V) complex of unsubstituted PnAO, TcO(PnAO), is known to have a distorted square pyramidal structure with a Tc-oxo group perpendicular to the plane of the ligand nitrogen atoms.[3] When the PnAO ligand is monosubstituted at the 6-position, the substituent can be either syn or anti with respect to the Tc-oxo bond (Figure 1). Such isomeric syn/anti pairs have been noted previously with other Tc(V)/Re(V) chelates, such as TcO(DADT),[11−13] TcO(DADS),[14,15] or TcO(MAG3),[16] and individual isomers of these complexes have been isolated and characterized. In addition, there have been several reports of Tc(V)/Re(V) complexes of peptide ligands which comprise syn/anti pairs.[17−19] We have prepared the ^{99}Tc and ^{99m}Tc complexes of several 6-substituted PnAO ligands and found that selected ones yielded two products. We present below details of the preparation, characterization, and chemical behavior of these complexes and show evidence that the two products are a syn/anti pair of isomers which interconvert in the presence of water.

Experimental Section

Reagents. Ammonium pertechnetate (NH_4TcO_4) was obtained from Oak Ridge National Laboratory and recrystallized from dilute aqueous hydrogen peroxide. Caution: ^{99}Tc is a weak β-emitter (0.29 MeV; half-life 2.12×10^5 years) and ^{99m}Tc is a γ-emitter (140 MeV; half-life 6.02 h); all reactions with technetium should be carried out in laboratories approved for the use of radioactivity. $[N(Bu)_4][TcOCl_4]$ was prepared from NH_4TcO_4 using published procedures.[20] The substituted PnAO ligands were prepared by reacting 2-substituted 1,3-propanediamines with 3-bromo-3-methyl-2-butanone, followed by oximation as described elsewhere.[21] The unsubstituted PnAO ligand was prepared by the method of Vassian et al.[22] All solvents were of reagent grade and used as received.

Methods. HPLC separations were made using a Spectra Physics SP8700XR LC pump with a Tennelec NaI probe for gamma activity detection or a Kratos SF769Z Spectroflow monitor for UV−visible detection. Reversed phase separations were performed on a 15 cm × 4.1 mm Hamilton PRP-1 HPLC column (5 μm) with acetonitrile/0.1 M ammonium acetate pH 4.6 mobile phase. Normal phase HPLC studies were conducted on a 25 cm × 4.6 mm Alltech Spherisorb NH_2 column with ethanol/hexane or methanol/methylene chloride mobile phases. Normal phase studies of ^{99m}TcO(PnAO-6-OH)) were also conducted on a 15 cm × 4.6 mm Chiracel OD column eluted with isopropyl alcohol/hexane. UV−visible spectra were measured on a Hewlett-Packard 8451A photodiode-array spectrophotometer. ^{1}H NMR studies were conducted on a 400 MHz JEOL-GX-400 spectrometer. Fast atom bombardment (FAB) mass spectra were obtained on a JEOL 102 SX spectrometer from a nitrobenzyl alcohol matrix. Elemental analyses were performed by the Bristol-Myers Squibb microanalytical department.

Preparation of ^{99m}Tc Complexes. The ^{99m}Tc complexes of the PnAO-6-R ligands (R = H, CH_3, $CH_2CH(CH_3)_2$, $COOCH_3$, OH, OCH_3, OCH_2CH_3, F, CN, $NHCOCH_3$, and $NHCOCH_2CH_3$) were readily prepared by reduction of ^{99m}Tc pertechnetate with stannous tartrate in the presence of the PnAO ligand at pH ∼8.5. In a typical preparation, normal saline (1.0−1.5 mL) and 0.1 M $NaHCO_3$ (0.5 mL) were added to ligand (∼3 mg) dissolved in methanol (0.2 mL). $^{99m}TcO_4^-$ as sodium pertechnetate eluent from a Dupont/Pharma generator (0.15−0.75 mL, ∼40 mCi) was then added, followed by 50 μL saturated stannous tartrate in saline. The product complexes were typically formed with >90% radiochemical purity (RCP) within 10 min as measured by reversed phase HPLC.

If necessary, the product complex was purified (separated from hydrophilic impurities, radiocolloid, and nonradioactive kit components) using a solid-phase extraction procedure with PRP-1 C18 resin (Hamilton). The lipophilic ^{99m}TcO(PnAO-6-R) complex was loaded onto reversed phase C18 resin, washed with ethanol/saline, and then eluted with absolute ethanol or acetonitrile. The RCP of the purified product was typically >95%. Purified samples in saline were prepared by evaporating the ethanol/acetonitrile sample under a stream of nitrogen, and redissolving in normal saline.

^{99}TcO(PnAO) from $[NH_4]TcO_4$. The unsubstituted (R = H) ^{99}Tc complex was prepared by following the procedure previously described by Jurisson et al.[2] Yield: 18.0 mg (27% based on Tc). Analysis for $C_{13}H_{25}N_4O_3Tc$ calcd (found): C, 40.62 (40.93); H, 6.56 (6.83); N, 14.58 (14.62).

^{99}TcO(PnAO-6-OH) from $[NH_4]TcO_4$. This complex was prepared following the procedure of Jurisson et al.[2] and recrystallized from ethyl ether. Normal phase HPLC analysis (UV detection at 225 nm) showed a mixture of two TcO(PnAO-6-OH) products. The ratio of the more lipophilic first-eluting species (**A**) to the more hydrophilic second-eluting species (**B**) was **A**/**B** ≈ 98/2 (in other preparations where two products were observed, this convention will be followed). Analysis for $C_{13}H_{25}N_4O_4Tc \cdot 1/4\ C_4H_{10}O$ calcd (found): C, 40.14 (40.25); H, 6.62 (6.65); N, 13.37 (13.93). Slow recrystallization from ethyl ether yielded crystals of product **A** suitable for X-ray analysis.

^{99}TcO(PnAO-6-F) from $[NH_4]TcO_4$. This complex was prepared following the procedure of Jurisson et al.[2] A mixture of two products in a ratio **A**/**B** ≈ 14/86 was obtained. Analysis for $C_{13}H_{24}N_4O_3Tc$ calcd (found): C, 38.81 (39.28); H, 6.01 (6.29); N, 13.92 (13.92).

^{99}TcO(PnAO-6-OMe) from TcO(ethylene glycol)$_2^-$. This complex was prepared by the same method as described below for TcO(PnAO-6-CN). A mixture of two products in an **A**/**B** ratio of 88/12 was obtained. Yield was 21 mg, 38% based on Tc. Analysis for $C_{14}H_{27}N_4O_4Tc$ calcd (found): C, 40.68 (40.25); H, 6.58 (6.65); N, 13.55 (13.93).

^{99}TcO(PnAO-6-CN) from TcO(ethylene glycol)$_2^-$. This complex was prepared from TcO(ethylene glycol)$_2^-$ generated in situ by the method of Brenner et al.,[23] as previously described by Linder et al. for the preparation of TcO(PnAO).[8] After stripping the reaction mixture to a viscous red-orange opaque oil by rotary evaporation, the product was redissolved in CH_2Cl_2, washed with H_2O (× 2), and the solution dried over Na_2SO_4. The solution was filtered and stripped to a small volume by rotary evaporation, causing red-orange crystals to precipitate out of solution. These were redissolved, recrystallized from CH_2Cl_2/hexane, and isolated by suction filtration as Batch 1 (Yield 24.4 mg,

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22.0% based on Tc). Addition of hexane to the mother liquor precipitated out a second batch (Batch 2) of product (Yield 47.2 mg, 42.5% based on Tc).

Normal phase HPLC analysis (UV detection at 225 nm) showed that both Batch 1 and Batch 2 consisted of a mixture of two TcO(PnAO-6-CN) products. The ratios of the first-eluting product/second-eluting product, **A**/**B**, in Batch 1 and Batch 2 were ~5/95 and 65/35, respectively. There was no evidence of impurities in these samples by either reversed phase or normal phase HPLC. Slow recrystallization of Batch 1 product from ether/dichloromethane yielded crystals of product **B** suitable for X-ray crystallography analysis.

Pure **A** and **B** products were isolated from Batch 2 by preparative HPLC using a Spherisorb NH_2 analytical column (Alltech) with 100% CH_2Cl_2 mobile phase at a flow rate of 1.5 mL/min as described below. Samples of each isomer were isolated for MS, UV−visible, and ^1H NMR characterization.

Product A. A 9.70 mg amount of a mixture of the two TcO(PnAO-6-CN) isomers (~65/35 **A**/**B**) was dissolved in 100 μL of CH_2Cl_2 and injected in two separate preparative HPLC runs. Fractions containing peak **A** (retention time approximately 3.3−6.5 min) and peak **B** (retention time approximately 7.7−15.7 min) were collected. The peak **A** product was reduced by rotary evaporation to a gold residue and redissolved in <0.5 mL of CH_2Cl_2. Addition of 2 mL of hexane and slow evaporation precipitated small orange crystals. After an additional 1 mL of hexane, Product **A** was filtered, washed with 1 mL of hexane, and dried in vacuo overnight. Yield was 0.9 mg.

Product B. A 8.10 mg amount of the same product mixture (~65/35 **A**/**B**) was dissolved in 1 mL of acetonitrile (ACN), and 4.0 mL of H_2O was added. After 1 h, the solution was rotary evaporated to an orange residue and redissolved in 1 mL of CH_2Cl_2. This treatment shifted the product equilibrium to favor peak **B** (new **A**/**B** ratio ~26/74). The sample was blown to dryness under nitrogen, redissolved in ~75 mL of CH_2Cl_2, and injected in two separate preparative HPLC runs. Peak B fractions were collected, combined with the peak **B** fractions from the peak **A** isolation, and evaporated to dryness with a rotary evaporator. The residue was redissolved in <0.5 mL of CH_2Cl_2, and upon addition of 1 mL hexane, a fine light orange powder precipitated. The product was filtered, washed with 1 mL of hexane, and dried in vacuo overnight. Yield was 3.04 mg.

HPLC analysis of the isolated TcO(PnAO-6-CN) products **A** and **B** showed >95% purity.

Interconversion of Isolated ^{99}TcO(PnAO-6-OMe) Isomers. Preparative normal phase HPLC was conducted on a small sample of ^{99}TcO(PnAO-6-OMe) to isolate product **A** and product **B** fractions containing 87/13 and 10/90 **A**/**B**, respectively. The HPLC system was the same as that described above for the isolation of ^{99}TcO(PnAO-6-CN) **A** and **B**, except the mobile phase was 10/90 ethanol/hexane. Each fraction was evaporated to dryness under a stream of nitrogen, and the products were redissolved in 75/25 acetonitrile/water. **A**/**B** ratios were then monitored by HPLC over 1 h.

Equilibrium Studies with TcO(PnAO-6-CN). Samples of ^{99}TcO(PnAO-6-CN) (~1.56 mg/mL) were prepared in ACN/H_2O mixtures that contained 15%, 25%, 50%, or 75% H_2O. Two samples of ^{99m}TcO(PnAO-6-CN) were prepared in 85% and 100% H_2O as follows: ^{99m}TcO(PnAO-6-CN) (40 mCi) was prepared and purified on PRP-1 C18 resin as described above, using ACN to extract the complex from the resin. The ACN fraction was blown to dryness under N_2, and the product was redissolved in 0.3 mL of H_2O (this comprised the 100% H_2O sample). ACN was added to a portion of the 100% H_2O sample to make an 85% H_2O sample. The **A**/**B** product ratios for the ^{99}Tc samples were monitored at room temperature (~25 °C) until equilibrium was attained (up to 8 h) using normal phase HPLC (Spherisorb NH_2 column, 50/50 EtOH/hexane, 1.75 mL/min) with UV detection at 300 nm. The ^{99m}Tc samples were allowed to equilibrate for ~75 min at room temperature (~25 °C), and the **A**/**B** ratios were determined by the same normal phase HPLC method with gamma detection.

Crystal Structure Analysis of TcO(PnAO-6-OH) and TcO(PnAO-6-CN).[24] The TcO(PnAO-6-CN) product **B** from ether/dichloromethane was a ~(1:4) mixture of two solvent-free monoclinic polymorphs: individual rectangular prisms ($P2_1/c$, $Z = 4$) and clusters of intergrown twinned rectangular rods (Cc, $Z = 8$). Analysis of the prismatic form was straightforward; however, the crystal structure of the rod form had to be determined from a crystal fragment containing two (**100**) twin members in the approximate ratio (3:2). The molecular structure and conformation of the two independent molecules in the rods were clearly evident and essentially identical to the molecular structure and conformation in the prismatic form. Only one crystal form was found for TcO(PnAO-6-OH) product **A**.

The general comments below apply to all three analyses. Unit cell parameters were obtained through a least-squares analysis of twenty-five high angle reflections. Crystal densities were measured by flotation in carbon tetrachloride/bromoform/hexane mixtures. Intensity data were measured on an Enraf-Nonius CAD4S diffractometer with the $\theta-2\theta$ variable scan technique and were corrected for Lorentz polarization factors and for absorption by the DIFABS method.[25] Background counts were collected at the extremes of the scan for half the time of the scan. Two standard reflections were monitored for decay; no decrease of intensity was observed during the course of the measurements. The structures were solved by heavy atom techniques and refined on the basis of "observed" reflections with $I \geq 3\sigma(I)$. Although most hydrogen positions were evident in difference Fourier maps, only the protons on the hydroxyl groups were introduced in their observed positions. The other hydrogen atoms were introduced in idealized positions, and their scattering was taken into account in the terminal stages of refinement. The final difference maps contained no significant features. All calculations utilized the SDP program package with minor local modifications.[26] For TcO(PnAO-6-OH) and TcO(PnAO-6-CN) prisms, the refined parameters were the coordinates and anisotropic temperature factors of the non-hydrogen atoms. For TcO(PnAO-6-CN) rods, refinement of a fully anisotropic model resulted in nonpositive definite thermal parameters for N1, C7, and C35, which therefore were refined as isotropic atoms.

Results

^{99m}TcO(PnAO-6-R) Products. Radiolabeled ^{99m}Tc complexes of the 6-substituted PnAO ligands were readily prepared in >90% yield via stannous tartrate reduction of $^{99m}TcO_4^-$ in the presence of ligand. Normal phase HPLC analysis of the ^{99m}TcO(PnAO-6-R) preparations showed that for ligands where R = H, CH_3, or $CH_2CH(CH_3)_2$, only one product was produced. However, when R was nonalkyl (R = OH, $NHCOCH_3$, $NHCOCH_2CH_3$, OCH_3, OCH_2CH_3, F, CN, and $COOCH_3$), two products were seen. Product (**A**) eluted from normal phase HPLC columns first, followed by the more hydrophilic product (**B**). Product ratios for the pairs of products ranged from 21/79 to 94/6 **A**/**B** (detailed HPLC results including retention times and **A**/**B** product ratios are available as Supporting Information). Complexes where R was OH, $NHCOCH_2CH_3$, OCH_3, and OCH_2CH_3 showed a preference for product **A**, while complexes where R was CN, F, $NHCOCH_3$, and $COOCH_3$ showed a preference for product **B**.

^{99}TcO(PnAO-6-R) Products. ^{99}Tc complexes of the PnAO-6-R ligands (R = CN, OMe, F, OH) were readily prepared either from $^{99}TcO_4^-$ reduced with stannous tartrate, or in higher yield by ligand exchange from TcO(ethylene glycol)$_2^-$. Normal phase HPLC of the ^{99}TcO(PnAO-6-R) samples also showed two products **A** and **B** that coeluted with their ^{99m}Tc analogues (simultaneous UV and gamma detection). The **A**/**B** ratios obtained from the ^{99}Tc synthesis reactions did not correlate to

(24) Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

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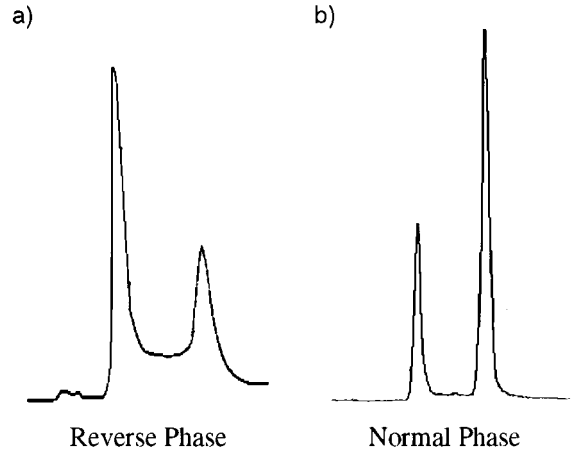


Figure 2. Reversed phase vs normal phase HPLC chromatograms of TcO(PnAO-6-F). Reversed phase conditions: PRP-1 C18 column, 5 μm; isocratic 65/35 ACN/NH_4OAc pH 4.6; flow = 1.0 mL/min. Normal phase conditions: Spherisorb NH_2 column; isocratic 30/70 ethanol/hexane; flow = 1.5 mL/min.

the ratios observed in the ^{99m}Tc radiolabeling studies (for example, the ^{99}Tc **A**/**B** ratio for TcO(PnAO-6-F) was 14/86, while the ^{99m}Tc **A**/**B** ratio was 31/69). FAB MS and elemental analysis of the mixtures of **A**/**B** products gave results consistent with that predicted for a single product formulated as ^{99}TcO-(PnAO-6-R).

HPLC Behavior. Under normal phase conditions, baseline resolution of ^{99m}Tc products **A** and **B** was achieved for all complexes except ^{99m}TcO(PnAO-6-OH) on the Spherisorb NH_2 column. For ^{99m}TcO(PnAO−OH), better resolution of products **A** and **B** was obtained on a Chiracel OD column.

Under reversed phase HPLC conditions the order of elution of products **A** and **B** was inverted from that seen on normal phase. The product pairs did not show baseline separation; a prominent trough above baseline was seen between the two peaks, even when the peaks were widely separated. Figure 2 compares the HPLC behavior of the two products for ^{99m}TcO-(PnAO-6-F) under reversed phase and normal phase conditions. As the flow rate was decreased in the reversed phase system, the two peaks collapsed into one (HPLC chromatograms depicting the flow rate dependence are available as Supporting Information).

X-ray Crystallography.[24] X-ray structure analyses were done on the **A** isomer of TcO(PnAO-6-OH) and the **B** isomer of TcO-(PnAO-6-CN). The **B** isomer of TcO(PnAO-6-CN) crystallized in two distinct forms: rectangular prisms or rectangular rods. The molecular structures of both forms were identical. Structural views of TcO(PnAO-6-OH) isomer **A** and TcO(PnAO-6-CN) isomer **B** (prismatic crystal form) are shown in Figures 3 and 4, respectively. Crystallographic data collection parameters are given in Table 1. Selected bond distances and angles are given in Table 2.

Interconversion Behavior of ^{99}TcO(PnAO-6-OMe) Products. Preparative HPLC separation of ^{99}TcO(PnAO-6-OMe) gave isolated product samples with **A**/**B** ratios of 87/13 and 10/90. These ratios remained unchanged in dry ethanol/hexane (mobile phase). When the samples were dissolved in 75/25 acetonitrile/water, both samples converted to an equilibrium mixture containing 80/20 **A**/**B** within 1 h.

Equilibrium Studies. Samples of ^{99}TcO(PnAO-6-CN) were allowed to equilibrate in water/ACN mixtures containing 15%, 25%, 50%, and 75% H_2O and analyzed by normal phase HPLC with UV detection to determine the effect of water on the **A**/**B** ratio. The low solubility of the complex in water precluded ^{99}Tc studies in solutions with % H_2O higher than 75%, so samples of the ^{99m}Tc complex (made in approximately nanomolar amounts) were equilibrated in 85% and 100% H_2O, and product ratios were measured with gamma detection. A plot of the **B**/**A** isomer ratio vs concentration of water (available as Supporting Information) for both the ^{99}Tc and the ^{99m}Tc complexes showed a linear relationship with a slope = 0.036 M^{-1}.

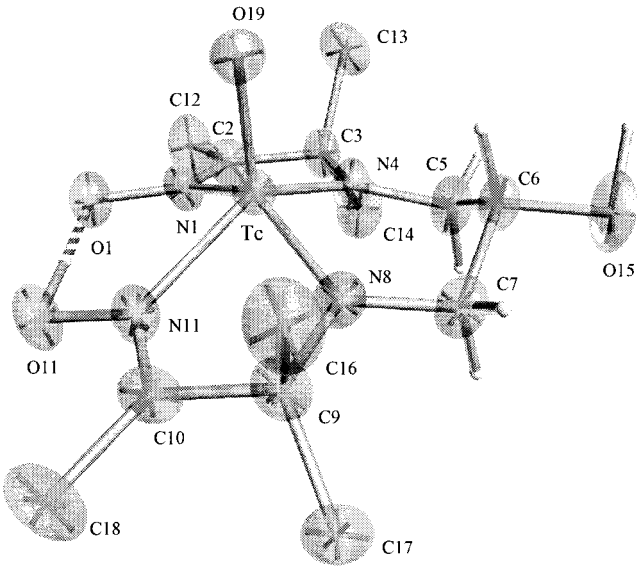


Figure 3. Structure of the **A** isomer of TcO(PnAO-6-OH).

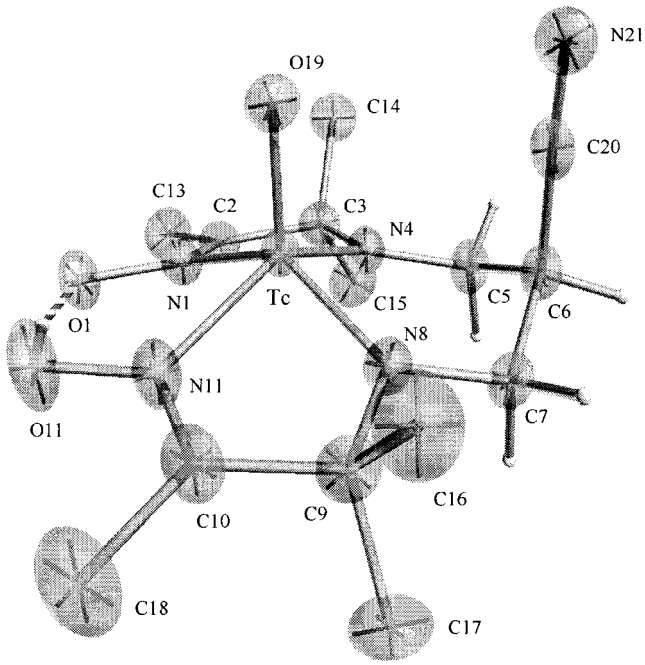


Figure 4. Structure of the **B** isomer of TcO(PnAO-6-CN) (prisms).

Isolation and Characterization of the ^{99}TcO(PnAO-6-CN) Products. The original sample of ^{99}TcO(PnAO-6-CN) consisted mostly of the more lipophilic product **A** (**A**/**B** ≈ 65/35). A sample which favored the **B** product (**A**/**B** ≈ 26/74) was obtained by evaporating a solution of the 65/35 mixture in partially aqueous solution to dryness and redissolving in a dry solvent (CH_2Cl_2). Pure (>99%) samples of **A** and **B** were isolated by preparative normal phase HPLC. FAB mass spectral analyses of the two isolated products and the original mixture showed that all possessed the same molecular ion, $(M + H)^+ = 410$, $(M - H)^- = 408$, corresponding to the formulation TcO-

Table 1. Crystallographic Data for TcO(PnAO-6-OH) Isomer **A** and TcO(PnAO-6-CN) Isomer **B**[a]

	TcO(PnAO-6-OH)	TcO(PnAO-6-CN)prisms	TcO(PnAO-6-CN) rods
chemical formula	$TcC_{13}H_{25}N_4O_4$	$TcC_{14}H_{24}N_5O_3$	$TcC_{14}H_{24}N_5O_3$
formula weight	400.4	409.4	409.4
a, Å	12.744(2)	12.457(1)	31.344(5)
b, Å	13.591(2)	13.932(1)	6.993(1)
c, Å	9.976(2)	10.336(1)	21.657(2)
β, deg	−	104.68(1)	133.12(1)
V, Å^3	1727.7(9)	1735.3(5)	3465(2)
Z	4	4	8
space group	$P2_12_12_1$	$P2_1/c$	*Cc*
T, °C	22	22	22
λ, Å	0.71069	1.5418	1.5418
D_{obsd}, g cm^{-3}	1.54	- -	- -
D_{calcd}, g cm^{-3}	1.539	1.567	1.569
μ, cm^{-1}	8.4	70.2	70.4
R	0.034	0.026	0.052
R_w	0.039	0.039	0.059

[a] Least squares weights, $w = \sigma^{-2}(F_o)$ were calculated with the assumption that $\sigma^2 = \epsilon^2 + (\rho I)^2$ where ϵ is the statistical counting error and $\rho = 0.04$. The function minimized in the least squares refinements were $\sum_w(|F_o| - |F_c|)^2$. *R* is defined as $\sum||F_o| - |F_c||/\sum|F_o|$ while $R_w = [\sum_w(|F_o| - |F_c|)^2/\sum_w|F_o|^2]^{1/2}$.

Table 2. Selected Bond Distances (Å) and Bond Angles (deg) for TcO(PnAO-6-OH) Isomer **A** and TcO(PnAO-6-CN) Isomer **B**[a]

TcO(PnAO-6-OH)		TcO(PnAO-6-CN) prisms	
	Distances		
Tc−O19	1.672(4)	Tc−O19	1.674(2)
Tc−N1	2.066(5)	Tc−N1	2.075(3)
Tc−N4	1.905(4)	Tc−N4	1.918(2)
Tc−N8	1.917(5)	Tc−N8	1.913(3)
Tc−N11	2.089(4)	Tc−N11	2.080(3)
O1−N1	1.372(6)	N1−O1	1.368(3)
O11−N11	1.382(6)	N1−C2	1.273(4)
O15−C6	1.427(7)	C2−C3	1.515(5)
N1−C2	1.289(8)	C2−C13	1.497(5)
N4−C3	1.475(8)	C3−N4	1.484(4)
N4−C5	1.467(7)	C3−C14	1.540(5)
N8−C7	1.455(8)	C3−C15	1.537(4)
		N4−C5	1.457(4)
	Angles		
O19−Tc−N1	107.9(2)	N1−Tc−N4	77.5(1)
O19−Tc−N4	110.3(2)	N1−Tc−N8	138.9(1)
O19−Tc−N8	109.6(2)	N1−Tc−N11	87.4(1)
O19−Tc−N11	110.3(2)	N1−Tc−O19	109.3(1)
N1−Tc−N4	77.1(2)	N4−Tc−N8	92.7(1)
N1−Tc−N8	142.3(2)	N4−Tc−N11	143.7(1)
N1−Tc−N11	87.5(2)	N4−Tc−O19	109.8(1)
N4−Tc−N8	93.1(2)	N8−Tc−N11	77.2(1)
N4−Tc−N11	139.2(2)	N8−Tc−O19	111.6(1)
N8−Tc−N11	76.5(2)	N11−Tc−O19	106.3(1)
O1−N1−C2	119.0(5)	O1−N1−C2	119.1(3)
C3−N4−C5	116.1(4)	C3−N4−C5	114.2(2)
C7−N8−C9	115.0(5)	C7−N8−C9	114.1(3)
O11−N11−C10	118.5(5)	C10−N11−O11	119.2(3)
N1−C2−C3	115.0(5)	N1−C2−C3	115.1(3)
N1−C2−C12	122.3(6)	N1−C2−C13	122.6(3)
N4−C3−C2	107.0(5)	N4−C3−C2	106.6(2)

[a] Numbers in parentheses are estimated standard deviations in the least significant digits.

(PnAO-6-CN). The isolated products exhibited identical UV−visible absorption spectra (available as Supporting Information), which were comparable to known Tc(V) oxo PnAO complexes.[2]

NMR Characterization. ^{1}H NMR studies were conducted on the isolated **A** and **B** products of ^{99}TcO(PnAO-6-CN) and on samples containing **A**/**B** mixtures of ^{99}TcO(PnAO-6-OMe) (**A**/**B** ratio = 88/12), ^{99}TcO(PnAO-6-F) (**A**/**B** ratio = 14/86), and ^{99}TcO(PnAO-6-OH) (**A**/**B** ratio = 98/2). NMR resonances could be assigned to the **A** and **B** species of the CN, OMe, and F products based on relative ratios of **A** and **B** (from HPLC analysis) and on the prior results for the isolated TcO(PnAO-6-CN) products. Because the **A**/**B** ratio for the OH-substituted product greatly favored species **A**, only results for the **A** isomer of ^{99}TcO(PnAO-6-OH) were obtained. NMR results (along with syn/anti assignments) are summarized in Table 3.

In general, the chemical shift values for equivalent protons on the two products **A** and **B** were similar. The exceptions were the multiplets ascribed to H(6), which showed dramatically different chemical shift values between the syn and anti products. Coupling constants for the H(4), H(5), and H(6) protons in the isolated TcO(PnAO-6-CN) products are given in Table 4.

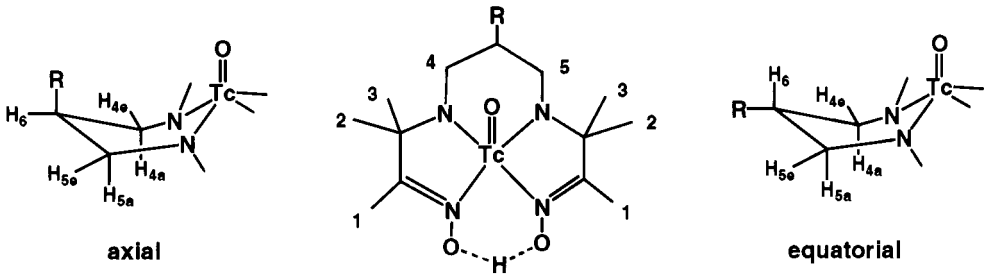
Discussion

The PnAO-6-R ligands radiolabeled well with either ^{99m}Tc or ^{99}Tc to yield one or two product complexes. The ratios of the two ^{99m}TcO(PnAO-6-R) product complexes **A**/**B** did not in general agree with the ratios obtained in the ^{99}TcO(PnAO-6-R) syntheses. Since the **A**/**B** ratio was found to be directly influenced by the amount of water present in sample solution (vide infra), the differences in ratios between the ^{99m}Tc and ^{99}Tc products are ascribed to differences in the amount of water present in the preparations.

No significant differences were noted between the UV−visible or FAB-MS results of the isolated TcO(PnAO-6-CN) products. Elemental analysis and FAB-MS results for **A**/**B** mixtures of the TcO(PnAO-6-OMe) and TcO(PnAO-6-F) products were consistent with the formulation: TcO(PnAO-6-R). These results are consistent with the **A** and **B** products of TcO(PnAO-6-R) being an isomeric syn/anti pair.

The X-ray crystallography results for TcO(PnAO-6-CN) product **B** and TcO(PnAO-6-OH) product **A** are similar to results obtained for other TcO(PnAO) complexes.[2,3,8] Both complexes are five-coordinate and have square-pyramidal geometry with the oxo group occupying the apical position. The ligands lose three protons (one oxime and two amine protons) on coordination to technetium, forming a neutral Tc(V) complex with strong intramolecular hydrogen bonding between oxime oxygen atoms. The average Tc−O bond distance of 1.67 Å is consistent with that seen in other TcO(PnAO) structures.[2,3,8] Tc−N (amide and amine) distances and the angles in the chelate rings are also comparable to those seen previously (Table 2). In both of the TcO(PnAO-6-R) compounds structurally characterized here, the six-member chelate ring that includes the propylene bridge adopted a boat conformation. Of five other

Table 3. Proton NMR[a] Chemical Shifts (ppm) for TcO(PnAO-6-R)



R[b]	product	6H(1)	6H(2,3)	6H(2,3)	2H(4a,5a)	2H(4e,5e)	H(6)	3H(7)[c]
anti CN	A	2.28 (s)	1.42 (s)	1.48 (s)	3.55 (dd)	3.77 (dd)	2.99 (tt)	-
syn CN	B	2.30 (s)[d]	1.41 (s)	1.51 (s)	3.58 (dd)	3.69 (dd)	3.63 (m)	-
anti OMe	A	2.27 (s)	1.41 (s)	1.49 (s)	3.21 (dd)	3.75 (dd)	~3.35 (m*)	3.48 (s)
syn OMe	B	2.27 (s)	1.41 (s)	1.49 (s)	~3.35 (m*)	~3.35 (m*)	3.93 (m)	3.26 (s)
anti F	A	2.33 (s)	1.42 (s)	1.51 (s)	~3.70 (m*)	~3.70 (m*)	4.68 (dm)	-
syn F	B	2.33 (s)	1.42 (s)	1.51 (s)	~3.70 (m*)	~3.70 (m*)	5.24 (dm)	-
anti OH	A	2.31 (s)	1.42 (s)	1.50 (s)	3.42 (dd)	3.64 (dd)	3.97 (m)	-

[a] R = CN and OMe samples run in CD_2Cl_2; R = F and OH samples run in $CDCl_3$. [b]Syn or anti assignments based on NMR and X-ray structural results as described in text. [c]H(7) corresponds to the methyl protons of the methoxy substituent. [d]Letters in parentheses signify type of resonance: s = singlet, dd = doublet of doublets, tt = triplet of triplets, m = multiplet, dm = doublet of multiplets, m* = an unresolved multiplet comprised of more than one resonance.

Table 4. Proton NMR Coupling Constants (Hz) for H(4,5,6) Protons of the Two Isolated TcO(PnAO-6-CN) Products

product	$J_{H45a-H45e}$	$J_{H45a-H6}$	$J_{H45e-H6}$
A (anti)	12.46	10.99	2.93
B (syn)	12.83	2.57	3.30

PnAO complexes crystallographically characterized,[2,3,8] all but one were found to adopt a boat conformation. In the latter case,[2] both chair and boat configurations were found in the unit cell.

The assignments of anti and syn to products **A** and **B**, respectively, are supported by the X-ray crystallography results. The crystallography data revealed that isomer **A** of TcO(PnAO-6-OH) had its OH substituent anti to the Tc═O group, and that isomer **B** of TcO(PnAO-6-CN) had its CN substituent syn to the Tc═O group.

^{1}H NMR results (Table 4) also support the assignments of anti and syn to products **A** and **B**, respectively. The boat conformation of TcO(PnAO-6-R) has the H(6) proton in an axial position on the anti isomer and in an equatorial position on the syn isomer (Figure 1). The H(6) proton of Product **A** displays vicinal coupling to H(4)/H(5) that is expected for an axial proton (J_{aa} = 10.99 Hz; J_{ae} = 2.93 Hz), while the H(6) proton of product **B** displays vicinal coupling characteristic of an equatorial proton (J_{ea} = 2.57 Hz; J_{ee} = 3.30 Hz).[15] These results are consistent with an equatorial (anti) CN substituent on product **A** and an axial (syn) CN substituent on product **B**.

The ^{1}H NMR chemical shift values for TcO(PnAO-6-CN) product **A** and product **B** are similar for equivalent protons, with the exception of H(6) (Table 3). The H(6) proton chemical shift for TcO(PnAO-6-CN) product **A** is found upfield of the H(6) proton chemical shift for product **B** by ~0.6 ppm. This large upfield shift likely results from a diamagnetic anisotropic shielding interaction with the Tc═O bond. Anisotropic interactions of this type have been reported for Tc═O[13,27] and Re═O[18] complexes. The axial proton of isomer **A** is better oriented to interact with the π-electrons of the Tc═O bond than the equatorial proton.

In ^{1}H NMR studies of ^{99}TcO(PnAO-6-OMe) or ^{99}TcO(PnAO-6-F) **A**/**B** product mixtures, two widely separated H(6) signals were also identified. By correlating the integration ratio of the two H(6) signals to the known ratio of products **A** and **B** (from HPLC analyses) in the mixtures, it was evident that the upfield H(6) signal corresponded to product **A** in all cases. These results were consistent with TcO(PnAO-6-R) product **A** possessing an H(6) proton which is shielded by the Tc═O group, and therefore axial. The product with an axial H(6) proton is expected to have an equatorial (anti) substituent. Hence, the ^{1}H NMR analyses of the OMe and F ^{99}Tc products also support the assignments of anti and syn epimers to products **A** and **B**, respectively.

The interconversion studies of isolated ^{99}TcO(PnAO-6-OMe) products showed that the syn and anti species are in equilibrium and that they can each convert one to the other to reestablish the equilibrium ratio when it is disrupted. The rate of interconversion was observed to be strongly dependent on water. In the absence of water, samples containing nonequilibrium mixtures remained unchanged for days. This phenomenon allowed for the preparation of "nonequilibrium" samples of the TcO(PnAO-6-R) products, and for isolation of the individual epimers under water-free conditions.

The reversed phase HPLC behavior of the TcO(PnAO-6-R) products also indicated an interconversion process with a water dependence. The syn/anti products displayed poor resolution, which got poorer as the flow rate was decreased. This behavior is characteristic of two species which interconvert on the column during HPLC analysis.[28] In this case, the interconversion reaction is fast relative to the HPLC separation, resulting in a chromatogram that is distorted as the two species elute over a range of time. In contrast, the two TcO(PnAO-6-R) products were baseline resolved under normal phase HPLC conditions. It is likely that on-column interconversion was observed only in the reversed phase HPLC system because the mobile phase was partially aqueous. In the relatively water-free environment of the normal phase HPLC system, interconversion was suppressed, and clean chromatographic behavior was observed.

Most of the syn/anti Tc(V)/Re(V) oxo epimers that have been characterized previously have not been reported to interconvert.[11−16,18] We have found only two other reported examples

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(28) Melander, W. R.; Lin, H.-J.; Jacobson, J.; Horváth, C. *J. Phys. Chem.* **1984**, *88*, 4527−4536.

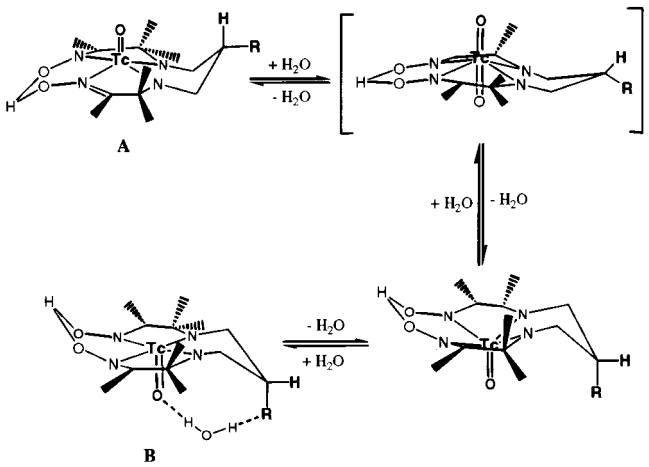
of interconversion behavior between syn/anti Tc═O complexes, both involving tetrapeptide ligands.[17,19] No influence of water was noted in either of these systems. We have reported previously that the two enantiomers of TcO(PnAO-1-2-nitroimidazole) could be resolved by chiral HPLC, but isolated enantiomers racemized with time.[8] Racemization was promoted by water in the same fashion that interconversion between **A** and **B** is in the TcO(PnAO-6-R) complexes. Since both of these processes involve a PnAO ligand core, it is probable that the same mechanism is in effect.

A mechanism for Re(V) oxo inversion leading to interconversion of syn and anti products has been proposed[17] in which a water molecule initially coordinates trans to the oxo group. The coordinated water deprotonates to form a Re−OH species, and subsequently a trans-dioxorhenium intermediate is formed. Support for this mechanism was provided by studies with ^{18}O-enriched water, which showed incorporation of the ^{18}O into the Re complexes. Because interconversion of the TcO(PnAO-6-R) syn/anti epimers strongly depends on water, we also favor the coordinated water mechanism for Tc oxo inversion. Tc(V) trans-dioxo complexes are well-known,[29] and several have been prepared from monooxo starting materials.[30,31] In addition, a Tc(V) trans-aqua-oxo species, $TcO(H_2O)(CN)_4^-$ has been isolated and characterized.[32]

The anti/syn equilibrium ratio was strongly influenced by the amount of water present in the sample solution. The equilibrium studies of TcO(PnAO-6-CN) in ACN/H_2O mixtures ranging from 15 to 100% water indicated that species **B** (the syn isomer) is stabilized in the presence of water relative to species **A** (the anti isomer).

The **A**/**B** equilibrium ratio (anti/syn) in ^{99m}TcO(PnAO-6-R) preparations varied over a wide range (21−94% **A**) depending on the nature of the substituent at the 6-position. Only one TcO(PnAO-6-R) product was observed when the substituent was H or an alkyl group, while two products were observed for R = OH, $NHCOCH_3$, $NHCOCH_2CH_3$, OCH_3, OCH_2CH_3, F, CN, and $COOCH_3$. All of the substituents that yielded two (syn and anti) TcO(PnAO-6-R) product complexes were hydrogen-bond acceptors. This suggests that the stabilizing interaction in product **B** that mediates the **A**/**B** equilibrium ratio may involve hydrogen-bonding between water and the substituent R. A possible scheme which describes such an interaction in TcO(PnAO-6-R) is shown in Scheme 1. The anti species **A** interconverts in the presence of water to give syn **B** which is stabilized by a hydrogen-bonded water bridging the Tc═O oxygen and the substituent R (note intervening protonation steps following water addition to the complex or deprotonation steps preceding water loss from the complex have been omitted in Scheme 1 for the sake of brevity).

Scheme 1



In conclusion, ^{1}H NMR and X-ray structure results confirm that for technetium complexes of PnAO ligands substituted at the 6-position, two products comprising a syn/anti pair of isomers can be formed. The more lipophilic isomer **A** has an anti substituent (with respect to the Tc═O group), and the less lipophilic isomer **B** has a syn substituent. The syn and anti isomers interconvert, and the rate of interconversion as well as the equilibrium ratio of the two was dependent upon the amount of water present. The interconversion process probably involves Tc oxo inversion initiated by water attack trans to the oxo group. The interaction that mediates the equilibrium ratio of the syn/anti products may involve water hydrogen-bonded to the substituent at the 6-position.

Supporting Information Available: Tables giving HPLC data for ^{99m}TcO(PnAO-6-R) complexes, atomic fractional coordinates of the non-hydrogen atoms of TcO(PnAO-6-OH), TcO(PnAO-6-CN) prisms, and TcO(PnAO-6-CN) rods, and additional details of data collection and refinement, parameters for the hydrogen atoms, thermal parameters, and listings of bond distances and angles. Figures depicting the effect of flow rate on reversed phase HPLC behavior of TcO(PnAO-6-OMe), the UV−visible absorption spectra of the two isolated isomers of TcO(PnAO-6-CN) in dichloromethane, and the dependence of TcO(PnAO-6-CN) **A**/**B** equilibrium ratio on water concentration. This material is available free of charge via the Internet at http://pubs.acs.org.

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