Paper A1

SYNTHESIS AND CHARACTERIZATION OF OXOTECHNETIUM COMPLEXES WITH TRIPODAL LIGANDS

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Research in the technetium chemistry has rapidly developed the last years due to the utility of technetium complexes in Nuclear Medicine. A considerable number of investigations have been focused on the development of new backbones to coordinate TcO^{3+} core. In the present work we report the synthesis and characterization of oxotechnetium complexes with tripodal NS_3 and N_2S_2 ligands. The following compounds were synthesized :

N,N-bis(2-mercaptoethyl)-2-ethylthioethylamine (BMTAH_2)

N,N-bis(2-mercaptoehtyl)-N´,N´-diethylethylenediamine (BMDAH_2)

The synthesis was proceed via direct mercaptoethylation of 2-ethylthioethylamine and N,N-diethylethylenediamine respectively using ethylene sulfide. The structure of the ligands was confirmed by IR, ^1H NMR and elemental analysis. The general formula of BMTAH_2 and BMDAH_2 is given in Fig.1.

The Tc(V)-complexes were obtained by the reaction of tetrachlorooxotechnetate with equimolar amount of the ligands. Red crystalls were isolated in 20% yield and characterized by IR, UV-Vis, ^1H NMR, mass spectroscopy and elemental analysis. The two complexes have been formulated as [TcO(BMTA)Cl] (I) and [TcO(BMDA)Cl]*HCl (II) respectively. Analytical data are shown in tables 1-3.

The IR spectra show intense single bands for Tc=O strech at 945cm^{-1} for (I) and 936cm^{-1} for (II). The band in the region from 2650-2480cm^{-1} indicate the formation of an amine salt for complex (II). This is consistant with elemental analysis which showed that (II) is crystallized as a hydrochloric salt. The changes in the ^1H NMR chemical shifts upon complexation, suggest that the two thiolato groups and the tripodal nitrogen are coordinated while the heteroatom S or N (group X) is not participating to chelation.The $\Delta\delta$ values in both complexes caclulated for the methylene protons attached to heteroatom (group X), were lower compared to the values for the methylene protons attached to thiolato groups or tripodal nitrogen.

The proposed structure of the complexes is shown in Fig.1 with two sulfurs and one nitrogen occuping the three equatorial sites while the fourth position is occupied by a chlorine atom. X-ray crystallography studies are in progress in ordrer to confirm the proposed stucture.

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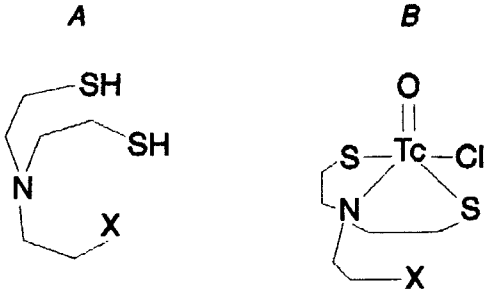
SUPPORTING DATA



X:-SEt, $-NEt_2$

Figure 1. A:General structure of the ligands B:Proposed structure of the complexes.

Table 1. Infrared and UV-Vis Spectral Data

Complex	IR,(cm^{-1}) vN-H,amine salt	vTc=O	UV-Vis,(nm) λ_{max}	Solvent
[TcO(BMTA)Cl]	–	945	268,422	CH_2Cl_2
[TcO(BMDA)Cl]*HCl	2650-2480	936	278.418	MeOH

*Table 2. Elemental Analysis**

	%C	%H	%N	%S
[TcO(BMTA)Cl]	26.97 (25.70)	4.77 (4.58)	3.27 (3.57)	–
[TcO(BMDA)Cl]*HCl	28.94 (28.51)	5.68 (5.50)	6.63 (6.65)	13.88 (15.22)

* calculated values in parentheses.

SUPPORTING DATA

Table 3. ^1H NMR Spectral Data, δ(ppm)

Compound	CH_3	CH_2	XCH_2	$XCH_2C^*H_2N$	$SCH_2C^*H_2N$	$SC^*H_2CH_2N$
$BMTAH_2$[a]	1.110	2.407	2.790	3.140	2.720	2.490
[TcO(BMTA)Cl][a]	1.233	2.548	2.873	3.785	3.590 3.545	3.190 3.001
Δδ	0.123	0.141	0.083	0.645	0.870 0.825	0.700 0.511
$BMDAH_2$[a]	0.975	2.483	2.890	3.180	2.716	2.516
[TcO(BMDA)Cl]*HCl[b]	1.350	3.098	4.225	3.430	3.853 3.720	3.430 3.150
Δδ	0.375	0.610	0.540	1.045	1.137 1.004	0.904 0.634

a: In CD_3Cl
b: In CD_3CN

Tc-PnAO COMPLEXES THAT ARE SUBSTITUTED AT THE CENTRAL CARBON ATOM. J. Cyr, K.E. Linder, P. Nanjappan, N. Raju, K. Ramalingam, D.P. Nowotnik and A.D. Nunn. Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 191, New Brunswick, New Jersey 08903, USA.

As part of a study on the effect of systematic changes in the substituents of neutral lipophilic technetium complexes on physico-chemical parameters such as lipophilicity, solubility and permeability, we have come across some interesting substituent effects with monosubstituted derivatives of the well known ligand, PnAO (propylenediamine dioxime). We synthesized several PnAO ligands, substituted at the central propylene carbon atom [R=H, Me, $(Me)_2$, OH] using the chloronitroso butane reagent [Fig.1] previously described in the literature (1). Compounds prepared from hindered diamines (e.g. R=2-isobutylpropylenediamine) were not readily alkylated. These (and others) required alternate methods of synthesis. (R=F, NH_2, $NHCOCH_3$, NHCOEt, OMe, CN, isobutyl, $(Et)_2$, COOMe).

All PnAO ligands were labelled with ^{99m}Tc by stannous tartrate reduction of pertechnetate at pH > 8. ^{99}Tc complexes [Fig. 2] were also made by this route (2) or via a new synthesis from TcO(ethylene glycol)$_2$ (3). The known crystal structures of Tc-PnAOs (2) suggest that our unsymmetrical PnAO ligands could yield four possible products; one set of boat/chair conformers with R syn to Tc=O, the other with the R group in the anti position [Fig. 3]. To our surprise, only selected PnAO ligands gave us two products. When the substituent on the ligand was H, Me, isobutyl, NHCOMe, or OH, only one Tc complex was detected by reversed phase HPLC. A crystal structure for R=OH showed all OH groups to be anti to the Tc=O core; NMR studies (CD_2Cl_2) on the same crystals showed the OH to be syn in solution. However, when R = -F, -CN, -OMe, or $-COOCH_3$, two apparently interconverting isomers were observed (with both 99m-Tc and 99-Tc). This behavior was seen only when R was a hydrogen bond acceptor [Table 1]. The ratio of the two products depends on the composition of the solvent. Addition of water to the solution shifts the equilibrium. [Fig. 4]. Baseline separation of the 2 peaks could not be attained on PRP-1 [ACN/0.1N NH_4OAc pH 4.6], the chromatograms obtained were characteristic (4) of an on-column reaction (presumed to be isomerization). However, good separation was achieved on a normal phase Spherisorb-NH_2 silica gel column with non-aqueous solvents. This suggests that water promotes inter-conversion. Tc(V) oxo syn/anti isomers have been noted previously (5), but interconversion between two such products has not, to our knowledge, been reported prior to this, except in the presence of base (6).

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Fig. 1. PnAO ligands where R = H, Me, $(Me)_2$, isobutyl, and OH, were prepared via the route below (1). For R= OCH_3, F, $NHCOCH_3$, $NHCOCH_2CH_3$, NH_2, CN, and COOMe, alternate methods of synthesis were used.

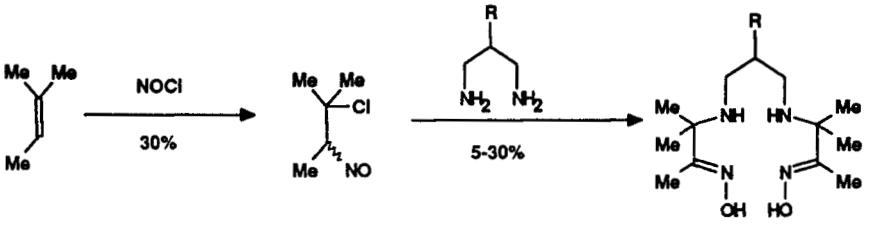


Fig. 2 ^{99}Tc Complexes were synthesized via the routes shown below. All complexes were characterized by elemental analysis, FAB Mass Spectrometry, UV-Vis spectroscopy, IR and ^1H-NMR.

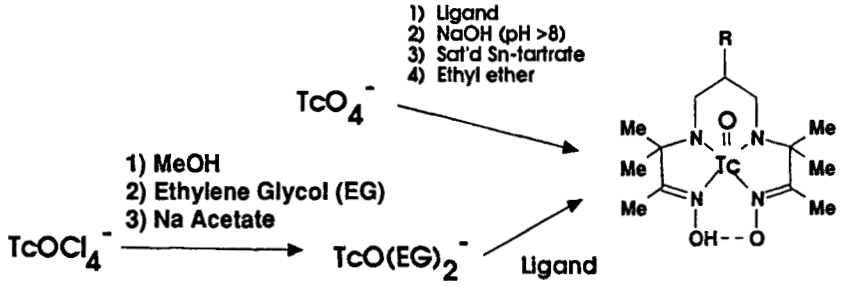


Table 1. Log k' values for TcOPnAO-5-R: *=Two interconverting isomers formed.

R	log k'	R	log k'
H	0.20	OCH_3	-0.17, 0.04*
CH_3	0.28	F	-0.11, 0.15*
$(CH_3)_2$	0.38	$NHCOCH_3$	-0.66
$CH_2CH(CH_3)_2$	0.61	CN	-0.26, 0.02*
OH	-0.47	$COOCH_3$	-0.03, 0.04*

Hamilton PRP-1, 5 micron column. 65/35 Acetonitrile/0.1M NH4OAc, pH 4.6, 1.0 mL/min.

Fig. 3 Possible structures for peaks observed. (based on the known structure of Tc-PnAO).

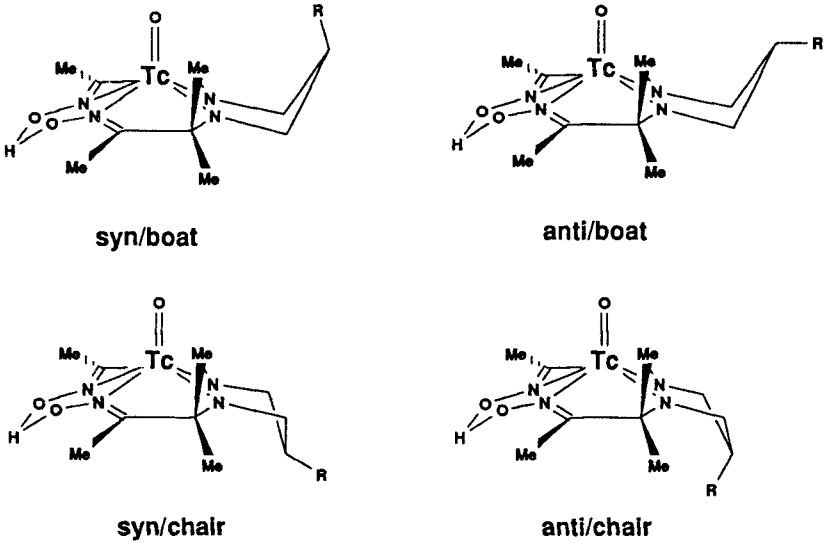
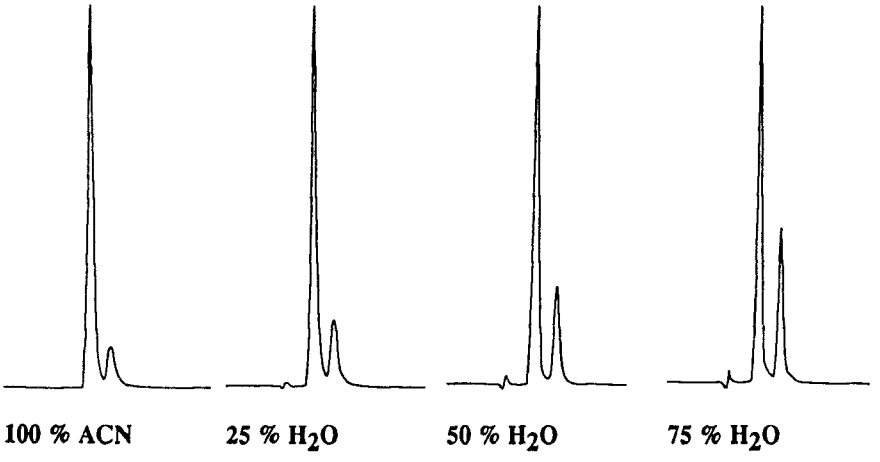


Fig. 4 Normal phase HPLC of the two peaks observed for ^{99}Tc-PnAO-5-OMe in ACN/H_2O (Spherisorb-NH_2 5-micron column [Alltech], 30/70 EtOH/Hexane].



PN-BIDENTATE AND P_2N_2-TETRADENTATE AMINO-PHOSPHINE LIGANDS SUITABLE FOR MO^{3+} (M= Tc, Re) COORDINATION.

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In recent years we have devoted most of our research efforts in developing inorganic Tc-chemistry with bidentate functionalized phosphine ligands because of their ability to act both as reductant and coordinating agents. Encouraging results were obtained with phosphino-carboxylate chelates of the type Ph_2P-R-COOH (R= C_6H_4, C_2H_4, CH_2) (shortly P-COOH) (1) and more recently with the ligand (o-aminophenyl)diphenylphosphine (P-NH_2) (2). In both cases Tc(III) complexes were obtained, three mononegative ligands being coordinated around the metal in a meridional configuration. P-COOH ligands are easily and readily labelled with Tc-99m producing neutral and stable $^{99m}Tc(P\text{-}COO)_3$ species, while the isostructural tris-phosphinoamido derivative undergoes protonation reaction and oxidation to oxo-Tc(V) products (3), therefore limiting the labelling procedure. Oxo species of the type $Tc^{V}O(PNH)_2X$ (X= mononegative ligand) can indeed be prepared at macroscopic level directly from pertechnetate by using a 1:3 stoicheiometric metal/ligand ratio. In this case the coordination around the metal is described as distorted octahedral with the X ligand *trans* to the Tc-oxo moiety and the bidentate PNH^- groups lying on the equatorial plane with a mutual *cis*-P configuration. However, solutions of $^{99}Tc(V)$ complexes are also instable and, remaining in contact with air and/or with time, tend to disproportionate to $[TcO_4]^-$ and $Tc^{III}(PNH)_3$ species. The poor stability of $Tc^{V}O(PNH)_2X$ complexes together with the coordinating *cis* position of the phosphorous atoms of the PNH^- chelate prompted us to synthesize the tetradentate $P_2(NH)_2$ chelate by joining with a propylic chain the nitrogen atoms of two PNH_2 ligands. The reduction-substitution reaction onto $[^{99}TcO_4]^-$ with $P_2(NH)_2$ in the correct metal/ligand ratio yield stable Tc(V) species of the type $TcO(P_2N_2)X$. Owing to the high reduction potential of $[ReO_4]^-$, $Re^{V}O(PNH)_2X$ as well as $Re^{V}O(P_2N_2)X$ complexes are easier prepared *via* ligand-exchange by mixing $[Re^{V}OCl_4]^-$ and the appropriate ligand in basic media. All oxo-M(V) complexes have been characterized by elemental analysis, IR, ^1H and ^{31}P NMR, visible-UV, FAB^+ spectroscopies and X-ray crystallography. Some relevant spectroscopic parameters are reported in Table I while Figure I depicts the perspective views of $TcO(P_2N_2)(OMe)$ and $ReO(PNH)_2(OEt)$ complexes.

As expected, $P_2(NH)_2$ ligand can be labelled with Tc-99m producing quite stable $^{99m}Tc(V)$ species and further reduction reactions operated by monotertiary phosphines lead to stable cationic Tc(III) products. Since the tetradentate ligand backbone can be easily derivatized, this new class of compounds may join the already known large series of cationic technetium complexes becoming a canditate to be studied as a potential hearth imaging agent.

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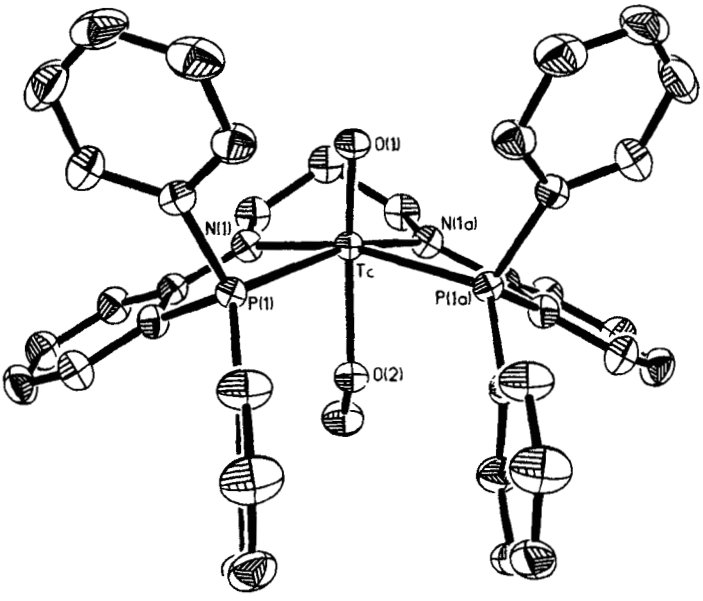
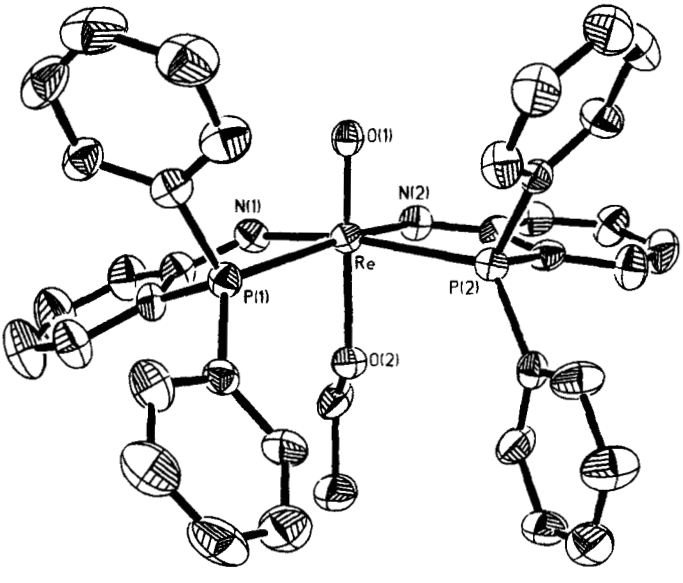
TABLE I. Relevant spectroscopic parameters of the complexes.

Compound	bond lenghts (Å)		infrared (cm^{-1})		nmr (ppm)		
	M=O	M--N_{av}	υ(M=O)	υ(N--H)	^{31}P	^{1}H (N-H)	^{1}H (O--R)
$TcO(PNH)_2(OMe)$	1.700	1.974	879	3303	n.d.	8.76	2.66
$ReO(PNH)_2(OEt)$	1.692	1.995	887	3300	11.97	9.08	2.91(CH_2)
							0.00(CH_3)
$TcO(P_2N_2)(OMe)$	1.691	2.040	891	-	32.67	-	2.40
$ReO(P_2N_2)(OMe)$	1.699	2.049	912	-	8.94	-	2.46



$TcO(P_2N_2)(OMe)$



$ReO(PNH)_2(OEt)$

Paper A4

STUDIES OF FORMATION OF THE Tc≡N BOND FROM THE REACTION OF [99mTcO4]$^-$ WITH DIFFERENT N3$^-$ DONOR LIGANDS.

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Technetium compounds containing the Tc≡N group represent a new class of potentially useful radiopharmaceuticals which are now easily prepared at no-carrier level through a new efficient method based on the following reaction (1):

$$[^{99m}TcO_4]^- + HCl + [P(m\text{-}C_6H_4SO_3)_3]Na_3 + L \longrightarrow {}^{99m}TcN\text{-complexes} \quad (1).$$

In reaction (1),L represents a derivative of dithiocarbazic acid of the type H_2N-N(R)-C(=S)SCH_3 (R=H,CH_3) or $R^1(R^2)C$=N-N(R^3)-C(=S)SCH_3 (R^1=organic moiety;R^2=R^3=H,CH_3).It was found that the ligands L can behave as both coordinating agents and donors of nitride nitrogen atoms (N^{3-}).This remarkable donating property was attributed to the presence of suitable leaving groups on the hydrazine-like arrangement >N-N<.In particular,the -C(=S)SCH_3 functionality appears to strongly enhance the N^{3-} donor capability of the derivates containing the >N-N< group.Mechanistic studies using the class of square pyramidal technetium (V) monoxo complexes with bis(aminoethanethiol) (BAT) ligands [^{99m}TcO(BAT)] {BAT=[HS-CR(R')-CH2-NH]2(CH2)2,R=R'=Me,Et} ,and the technetium (V) dioxo complex [^{99m}TcO2(cyclam)]$^+$ (cyclam=1,4,8,11-tetraazacyclotetradecane) as starting reagents in reaction (1) support the view that the synthesis of the Tc≡N group should result from a balance between its coordinative properties and the ability to undergo successive cleavage to generate the N^{3-} group.

In order to investigate the influence of the substituent groups on the >N-N< arrangement,we carried out reaction (1) using various hydrazine-like derivatives and compared the results with those obtained with H_2N-N(R)-C(=S)SCH_3 (R=H,CH_3).All the reactions were carried out in saline,at room temperature and at 100°C,in the presence of excess HCl (pH=2) and the water-soluble phosphine [P(m-$C_6H_4SO_3$)$_3$]Na3 (TPPS) (ca.2 x 10^{-2} mol dm^{-3}).We used a fixed amount of N^{3-} donor (20 μmol) in all the preparations.Since reaction (1) may lead to a mixture of compounds all containing the Tc≡N bond (1),the yields of the various preparations were obtained by adding to the same reaction solution 0.40 mL of 1.0 x 10^{-2} mol dm^{-3} diethyldithiocarbamate (Et_2NCS_2Na) which is able to convert quantitatively all the ^{99m}TcN-complexes composing the intermediate mixture to the single compound [^{99m}TcN(Et_2NCS_2)$_2$].Measuring the yield of the final product gave the yield of formation of the Tc≡N group through reaction (1).

We studied the reactivity of the following hydrazine-like compounds:

1) H_2N-$N(CH_3)$-C(=S)SCH_3
2) H_2N-N(H)-C(=S)SCH_3
3) H_2N-NH_2
4) H_2N-NH-C(=O)OCH_3
5) H_2N-NH-C(=S)NH_2
6) H_2N-NH-C(=O)NH_2
7) H_2N-NH-C(=S)NH-NH_2
8) H_2N-NH-C(=O)NH-NH_2

Table I reports the obtained yields at room temperature and at 100° C.The results clearly indicate that the leaving group plays an important role in determining the N^{3-}-donor properties of the species **1-8**,and point out that **1** is the species of choice when reaction **(1)** is applied to the preparation of technetium nitrido radiopharmadeuticals in nuclear medicine.

Table I. Yields(%) of reaction **(1)** at room temperature (r.t.) and 100° C

LIGAND	r.t.	100°C
1	97.0	98.8
2	92.0	95.2
3	77.4	90.7
4	45.1	82.4
5	44.3	84.3
6	63.3	63.8
7	79.9	96.4
8	79.7	95.1

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The Characterization and Synthesis of Technetium Complexes Which Contain Organonitrogen Cores from Pertechnetate.

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Abstract:

The organoimido core, (M≡N-R), may prove to be of great synthetic utility in radiopharmacology, since a variety of organic substituents can be incorporated into a stable technetium-nitrogen core. This property may allow the chemical "fine-tuning" of the complexes' biological properties by simply altering the imido group's organic substituent -R. This core can be expected to form a whole new class of technetium complexes based on established technetium-oxo chemistry.

The reaction of $(NH_4)[TcO_4]$ with triphenylphosphine and the organohydrazine N-acetyl, N'-phenylhydrazine, $(PhNHNHCOCH_3)$, in methanol with a minimal amount of $HCl_{(aq)}$ gives the neutral Tc(V) complex $[Tc(NPh)Cl_3(PPh_3)_2]$ in very good yields. The infrared spectrum of this complex displays a strong band in the 1090 cm^{-1} region which has tentatively been assigned to ν(Tc≡N). The (+)FAB mass spectum does <u>not</u> show a peak associated with the neutral species $[TcCl_3(NPh)(PPh_3)_2]$. However, a prominent feature of 784 m/z is associated with the fragment generated from the loss of a chloride ligand, giving the cationic species $[TcCl_2(NPh)(PPh_3)_2]^+$. The diamagnetic Tc(V) complex displays a ^1H NMR spectrum with the proton signals from the imido-phenyl group resolved from those of the phosphine-phenyl groups. The complex displays distorted octahedral molecular coordination geometry, with mutually *trans* triphenylphosphine ligands and equatorial phenylimido and chloride ligands. The technetium-nitrogen bond length is 1.704(4) Å with a technetium-nitrogen-carbon bond angle of 171.8(4)°, which reflects the sp hybridization of the phenylimido nitrogen atom.

In an analogous reaction, $[TcO_4]^-$ reacts with benzoylhydrazine $(PhC{=}ONHNH_2)$ and triphenylphosphine in methanol with HCl to give the Tc(V) nitrido complex $[TcNCl_2(PPh_3)_2]$ in excellent yields.

A mechanism is proposed to account for the formation of these two products from pertechnetate involving a organohydrazine bound intermediate which is protonated at it's β-nitrogen with the addition of HCl. With protonation, the nitrogen-nitrogen bond is cleaved with the loss of acetamide or benzamide, yielding the organoimido containing product.

Paper A6

THE SYNTHESIS OF NITROIMIDAZOLE BATO (BORONIC ACID ADDUCTS OF TECHNETIUM DIOXIME) DERIVATIVES AND THEIR IN-VITRO EVALUATION AS POTENTIAL HYPOXIA IMAGING RADIOPHARMACEUTICALS.

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We are trying to design a technetium-based agent that will image hypoxia in ischemic myocardium, brain and tumors. One class that we have studied are nitroimidazole-BATO complexes. (BATO=Boronic Acid adduct of Technetium diOxime) (1,2). Nitroimidazoles are known (3) to be selectively trapped in hypoxic tissue via enzymatic reduction of the nitro group to reactive species in the absence of oxygen. Other groups have demonstrated hypoxia localization with 18-F and I-labeled nitroimidazoles (4). It is believed that the redox potential of the nitroimidazole (5) is a major factor in how readily it is reduced and trapped. Misonidazole has been suggested as a "gold standard" (4). We selected metronidazole as a negative standard that should be difficult to trap. We wanted to demonstrate that a) our new Tc BATO-nitroimidazoles could be electrochemically reduced at potentials closer to misonidazole (miso) than metronidazole (metro), and b) the complexes could be reduced by xanthine oxidase (XOD), an enzyme that is known (6) to reduce nitro compounds in vivo and in vitro. Compounds synthesized and tested included nitroimidazole boronic acids [$(OH)_2BR$] and BATO nitroimidazoles [$TcX(dioxime)_3BR$] (X=Cl,OH), where R contained a variety of spacer groups linked to an imidazole ring with a nitro group in either the 2- or 4- position. The BATO nitros were prepared from $TcCl(dioxime)_3$ precursors following methods described previously (2). All nitro compounds were characterized by elemental analysis, IR, UV-Vis, NMR and Mass Spectrometry. They were also fully characterized electrochemically.

We compared the cyclic voltammetry $E_{1/2}$ values of our BATO compounds, and their precursor boronic acids to those of miso and metro. All compounds tested proved to be more difficult to reduce than is miso. The position of the nitro group proved important; all 4- and 5- substituted nitro groups were harder to reduce than the 2-nitroimidazoles. An *in vitro* enzyme assay using xanthine oxidase (XOD) was also developed to determine if the 99-Tc-BATO nitroimidazoles and precursor boronic acids would be recognized by this nitroreductase. The reaction was monitored by UV/Vis, as the nitroimidazole group has a strong absorbance at 300-320 nm, which disappears upon reduction. Under anaerobic conditions, only the nitro absorbance decreased; other peaks were unchanged. As expected, in the presence of O_2, no nitro reduction was noted, nor was any reaction seen in the absence of XOD. However, BATO nitroimidazoles were reduced more slowly by xanthine oxidase than were their precursor boronic acids. These data suggest that Tc-BATO nitros have some of the characteristics required of a hypoxia imaging agent, but that reduction *in vivo* might be slow. Nevertheless, we have demonstrated that the nitroimidazole group on the BATO complexes is selectively recognized by xanthine oxidase.

Comparison of enzyme assay data and redox potentials for nitroimidazole boronic acids and standards.

	$T_{1/2}$ for Nitro Group Loss	Epc (Volts) for NO_2 Reduction *
$(OH)_2BBNO_2$	13.0 ±2.8 min. (n=6)	-1.50
$(OH)_2BPropeneNO_2$	21.6 ±1.7 min (n=5)	-1.54
$(OH)_2BPhEtNO_2$	39.0 ±1.4 min (n=4)	-1.50
$(OH)_2BB4NO_2$	~120 min (n=2)	-1.75
Misonidazole	36.5 ±1.7 min (n=4)	-1.45
Metronidazole	>20 hours (n=2)	-1.59

*In DMF at mercury working electrode (0.1M in TBA BF_4) vs. $Ag/AgNO_3$ (ACN) reference. (Scan rate = 100 mV/sec). In DMF this is a quasi-reversible 1-electron process.

Half lifes for nitro reduction in xanthine oxidase enzyme assay.

COMPLEX	[Tc] or $[(OH)_2BR]$	[DMF]	$T_{1/2}$ For Nitro Loss	$T_{1/2}$ for Corresponding Boronic Acid
Tc-BATO COMPLEXES				
$TcOH(DMG)_3BPropene\text{-}NO_2$	0.10 mM	0.8%	180 min	22 min
$TcOH(DMG)_3BPropene\text{-}NO_2$	0.077 mM	0.8%	175 min.	
$TcOH(DMG)_3BPropene\text{-}NO_2$	0.077 mM	5.0%	185 min.	20 min (n=2)
$TcOH(DMG)_3BPropene\text{-}NO_2$ No Enzyme Control	0.10 mM	0.8%	no reduction seen	
$TcOH(DMG)_3BBNO_2$	0.077 mM	5.0%	240 min. (n=2)	15 min (n=3)
$TcOH(DMG)_3BBNO_2$ No Enzyme Control	0.10 mM	5.0%	no reduction seen	no reduction seen

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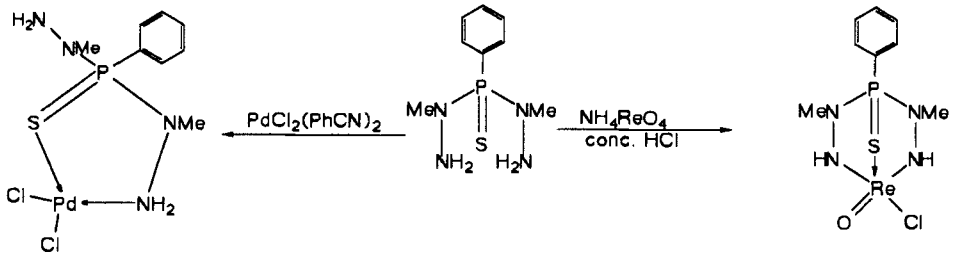
A New and Versatile Multifunctional Phosphorus Hydrazide Ligand for Formulating Neutral-Lipophilic Complexes of ^{99m}Tc, ^{186}Re and ^{109}Pd Radionuclides. *W. A. Volkert, P. R. Singh, K. K. Katti, A. R. Ketring, K. V. Katti., Depts. of Chemistry, Radiology and Research Reactor, University of Missouri and *Research Service, H.S. Truman Memorial Veterans Hospital, Columbia, MO.

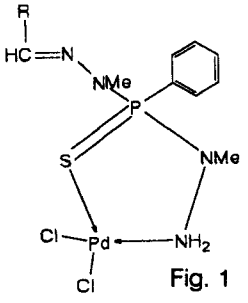
The chemistry of early and late transition metals with hydrazine ligands has attracted considerable attention because the electronic flexibility of such ligands has allowed them to bind to transition metals with a wide variety of bonding modes such as η^1, (2-), 4 electron donor or η^2,(2-), 4 electron donor or η^1, (2-), 2 electron donor etc. Most of the hydrazine type ligands used so far are of the simple straight-chain type and are, generally, monodentate with transition metals [1,2]. The multifunctional chelating hydrazine-based ligands would not only afford increased stability characteristics to the transition metals through the cooperative electronic interaction but may also offer enhanced in vitro and in vivo stabilities of the resulting complexes. Such ligating properties would broaden the scope of the utility of these multifunctional hydrazine ligands to be used in the development of new radiopharmaceuticals. In this paper we report the versatile reactivity shown by the chelating phosphorus hydrazide (PH) ligands towards a variety of early and late transition metal radionuclides to give new, stable and neutral complexes of Tc-99m, Re-188 and Pd-109 radionuclides.

As a model system to examine the reactions of PH ligands, phenyl-bishydrazine phosphine sulfide (PBHP) at "no carrier added" levels with $^{99m}TcO_4^-$, $^{188}ReO_4^-$ and $^{109}PdCl_4^{2-}$, we have studied the reactions of this ligand (1) with the corresponding transition metal precursors at the "carrier added" level as illustrated in Scheme 1.



3 1 2

The chemical constitution of 2 and 3 have been confirmed by spectroscopic (^{1}H, ^{31}P and ^{13}C NMR and IR); C,H,N,Cl elemental analysis and mass spectroscopic data (i.e. parent ion for 2 and 3, are 465.65 and 407.32 respectively). The presence of the uncoordinated substituted or free hydrazine unit seems to be a common feature associated with a number of our Pd(II) complexes derived from closely related ligands. The bonding feature in all of these compounds as inferred from x-ray crystallographic investigations is shown in Figure 1.



R = Ph, C_6H_4F or C_4H_3O.

Fig. 1

The ^{99m}Tc complex of PBHP was formed by simple mixing of TcO_4^- (freshly eluted from a ^{99}Mo/^{99m}Tc generator) with excess (10^{-3}M) ligand 1 in 0.1 N HCl. The reaction mixture was heated for 20 min at 100°C before further analyses. The ^{99m}Tc-PBHP chelate was also obtained by exchange labeling when freshly prepared ^{99m}Tc-glucoheptonate was added to an acidified solution of the PBHP ligand and the mixture was incubated for 60 min at 100°C. The corresponding ^{188}Re complex was prepared under identical conditions to those used for the ^{99m}Tc chelate, wherein $^{188}ReO_4^-$ freshly eluted from ^{188}W/^{188}Re generator was used. The Pd-109 complex of PBHP was formed by mixing of an acidic solution of 1 with $^{109}PdCl_4^{2-}$ which was obtained from the University of Missouri Research Reactor.

The resultant chelates were analyzed by paper chromatography, ITLC-SG, reversed-phase HPLC and paper electrophoreses. None of the chelates measurably moved from the origin during electrophoresis (300 v for 1 hr) in aqueous solutions at pH 7-7.5 (0.05M $NaHCO_3$ buffer). ^{99m}Tc-, ^{188}Re- and ^{109}Pd-PBHP were found to move to the solvent front by paper and TLC chromatographic analysis using acetone as the solvent. In contrast, when normal saline was used as the solvent, no significant migration of the chelates from the origin (R_f=0) occurs. The rf values of the complexes 2 and 3 as evaluated from ITLC/uv-vis (at macroscopic levels) and radio ITLC (at "no carrier added"

levels) were comparable suggesting the formation of identical chemical species at both the macroscopic and "no carrier added" levels.

The results obtained with PBHP(1) show that it forms neutral, lipophilic chelates with ^{99m}Tc, ^{188}Re and ^{109}Pd (Table 1). No significant decomposition of these chelates was measurable for ≥24 hr at pH 6.5-7 in 0.9% aqueous NaCl at room temperature. Reversed phase HPLC analysis using a Hamilton PRP1 column and a mobile phase of 75:25, $CH_3CN:H_2O$ of ^{99m}Tc-PBHP indicate the complex to be of a single chemical species.

The results of these studies indicate that the ^{99m}Tc-, ^{188}Re- and ^{109}Pd-PBHP chelates formed at the tracer level have the same structure as the respective chelate produced at the "carrier added" level. Thus, ^{99m}Tc- and ^{188}Re- complexes are expected to be complexed as the $MOCl^{+2}$ core to PBHP via both terminal hydrazine N-atoms and the S-atom. By comparison, complexation of ^{109}Pd with PBHP involves the S-atom and only one of the two PBHP hydrazine groups (Figure 1). The high stability and neutral lipophilic properties of these chelates indicate the potential utility of PH-derivatives for use in developing new radiopharmaceuticals. Chemical modification of the fundamental PH-ligands is feasible for preparation of a variety of neutral-lipophilic and charged or more polar derivatives that will permit systematic variation of the biolocalization properties of these types of radiolabeled chelates.

Table 1. The radiochemical purity (RCP) of ^{99m}Tc-, ^{188}Re-, ^{109}Pd-PBHP complexes at 1 and 24 hr post formulation and the respective chloroform/water partition coefficients.

Complex	Radiochemical Purity[a]		Chloroform\Saline Ratio
	1 hr	24 hr	
^{99m}Tc-PBHP	95 ± 1.1	93 ± 1.2	5.7 ± 0.3
^{188}Re-PBHP	88 ± 1.3	85 ± 1.1	4.9 ± 0.5
^{109}Pd-PBHP	93 ± 1.2	90 ± 1.3	5.9 ± 0.5

(a) RPC determined by paper and thin layer chromatography. The chelates were incubated for either 1 or 24 hr at 22°C in 0.9% aqueous NaCl at pH 6.5-7. Values are mean ± S.D.

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NEW TECHNETIUM COMPLEXES WITH P_2 AND P_3 LIGANDS.

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Neutral mono- and di-phosphine ligands have been utilized to prepare a variety of cationic ^{99m}Tc complexes.

Technetium(III) complexes have been obtained with monodentate tertiary phosphines as well as with bidentate diphosphine ligands and all these complexes possess an octahedral geometry (1-6). The first cationic ^{99m}Tc complexes to be evaluated in humans were trans-$[TcCl_2(DMPE)]^+$ and $[Tc(DMPE)_3]^+$ (DMPE = 1,2 bis(dimethyl-phosphino)ethane) containing the $[Tc^{+3}Cl_2]^+$ and Tc(I) cores (7-9). With the aim of studing new reducting agents, evaluating their coordination properties and reactivity of the complexes toward substitution reactions we have selected the ligands showed in fig. 1. In this paper we report preliminary data on the synthesis of ^{99}Tc and ^{99m}Tc complexes and biodistribution studies.

Nitruro complexes $[TcNCl_2(PNP)]$ **1a**, $[TcNCl_2(POOP)]$ **1b**, $[TcNCl_2(CP3)]$ **1c** and $[TcCl_4(PNP)]$ **2a** and $[Tc(CP3)_2]^+$ **2b** were obtained by substitution reactions on $[AsPh_4][TcNCl_4]^-$ and $[TcNCl_2(PPh_3)_2]$ and $[AsPh_4][TcOCl_4]$ and $[TcCl_4(PPh_3)_2]$.

The reaction with (POOP) ligand led to an unstable compound.

Up to now, all the complexes were characterized by elemental analysis, i.r. and ^1H n.m.r. spectra, and conductivity measurements. Conductivity data are indicative of neutral

character of the nitruro complexes, i.r. spectra show the TcN stretch to fall in the range 1065 - 1090 cm^{-1}. The ^{1}H n.m.r. signals reveal that they are diamagnetic and consistent with a square-pyramidal geometry for **1a** and **1b** and esa-coordination for **1c** in which a phosphinic group is in <u>trans</u> position to the TcN multiple bond. The formulation of the Tc(IV) and Tc(I) complexes is supported by i.r. spectra in which there is no evidence of the Tc=O stretch, conductivity measurements indicate the complex **2a** to be neutral and **2b** cationic. Finally, ^{1}H n.m.r. spectra show that the complex **2a** is paramagnetic and **2b** diamagnetic.

Syntheses of ^{99m}Tc complexes.

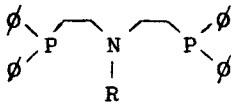
In our preliminary studies the synthesis of ^{99m}Tc complexes was only carried out using PNP ligands. ^{99m}Tc complexes were prepared using solution purged with argon.

In a 5 ml vial containing 2 ml of $Na^{99m}TcO_4$ (about 500 MBq of $^{99m}TcO_4^-$) eluted from a commercial $^{99}Mo/^{99m}Tc$ generator were added 0.2 ml of a solution in ethanol of the PNP ligand (20 mg in 2 ml) and 0.1 ml of aqueous 1N HCl. Finally, a carbonate buffer was added to the mixture to bring the pH to 7.5. The vial was heated at 80°C for 15 minutes.

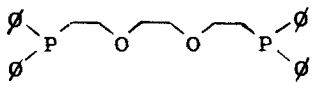
Anesthetized rats (150 - 180 g.) were injected with 0.1 ml of the ^{99m}Tc solution and images showed the whole body biodistributions at 5, 15 min. as well as 1, 2 h. post administration.

The nitrido complexes of ^{99m}Tc were prepared according to our procedure (10). The chromatographic analysis showed the formation of only one product and yield was in the range 92 - 95%.

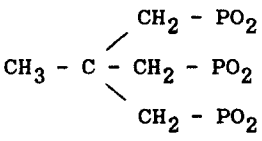
Up to this time, biodistribution studies of these complexes have not yet been performed.



(PNP)



(POOP)



(CP3)

(PNP) = bis(diphenylphosphinoethyl)propylamine

(POOP) = bis(diphenylphosphinoethyl)ethylenglycol

(CP3) = 1,1,1 bis(diphenylphosphinomethylethane

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Quantitative Study of the Structure-Stability of TcO(III)-Complexes Based on CNDO/2 Method

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In developing new Tc-99m labeled radiopharmaceuticals, one of the important criteria is the *in vitro* and *in vivo* stability of the Tc-99 complex. Due to technical difficulties associated with characterization of carrier-added Tc-99 complexes, the stability constants are not easily obtainable. Recently, we reported the evaluation of the stability of Tc-99m complexes based on the cone-packing model (1). This model uses solid angle sum (SAS) value as a parameter to indicate *in vitro* stability. The order of stability of a series of Tc-99m complexes is consistent with the experimental result from ligand exchange reaction (2). In order to expand our systematic evaluation of this important factor in Tc-99m radiopharmaceutical development, we have calculated the formation energy of nine $Tc^{V}ON2S2$ complexes (in which the x-ray crystallography data are known) using the completed neglect differential overlap (CNDO/2) method.

The structure-stability relationship of $Tc^{V}ON2S2$ complexes using a stability indicator formation energy value provides an alternative method for evaluating structural features associated with complex stability. The results may be useful in designing new Tc-99m radiopharmaceuticals, especially considering the second order effect which was produced by atoms other than the coordinating atoms or groups immediately involving TcO complex formation. The following equations are used to calculate the formation energy of $Tc^{V}ON2S2$ and the net charge for various coordinating atoms and Tc core.

$$E_i^M = \sum_{n=1}^{i} \frac{e^+_{Tc} \cdot e^-_i}{\Gamma_i} \qquad \text{Eq. 1}$$

$$\text{Net Charge } \Delta e = Z_A - P_{AA} \qquad \text{Eq. 2}$$

E_i^M : **Formation Energy of Tc Complexes**

e^+_{Tc} : **net charge of Tc**

e^-_i : **net charge of ligand atom i**

Γ_i : **distance between Tc & ligand atom i**

Z_A : **Atomic Core Number**

$P_{AA} : \sum_{\mu}^{A} (P^{\alpha}_{\mu\mu} + P^{\beta}_{\mu\mu})$

$P^{\alpha}_{\mu\mu}$: **Population no. of φ_μ of A atom of α electron**

$P^{\beta}_{\mu\mu}$: **Population no. of φ_μ of A atom of β electron**

Net charge and formation energy of the nine $Tc^{V}ON2S2$ complexes based on bond distances obtained by x-ray crystallography data are presented in Table 1 (2). The computer programs for quantum mechanical calculations are validated by comparing calculation results of this program with the published data of known compounds.

The results in Table 2 suggest that stability of $Tc^{V}ON2S2$ complex, the SAS value as calculated by the cone-packing model (1), is parallel to that obtained by quantum mechanical calculation based on the CNDO/2 method. The formation energy for the whole molecule, Emi, showed the same order as that of SAS values. Using the CNDO/2 method, the formation energy based on coordinate atoms (CA), backbone of complexes (BB), or whole molecules (W), can be calculated separately. The substitution groups may either stabilize or destabilize the complexes; the differences in formation energy clearly indicate the importance of this effect. The CNDO/2 method can be used to estimate the substitution effects more precisely than that of cone packing model (SAS values). By comparing data from both methods, it may be feasible to fine tune the substitution groups outside the center Tc core to achieve a different stability and to change in vivo biodistribution of Tc-99m complexes. Additional investigation is also needed to correlate the quantitative stability information with the in vivo biodistribution data.

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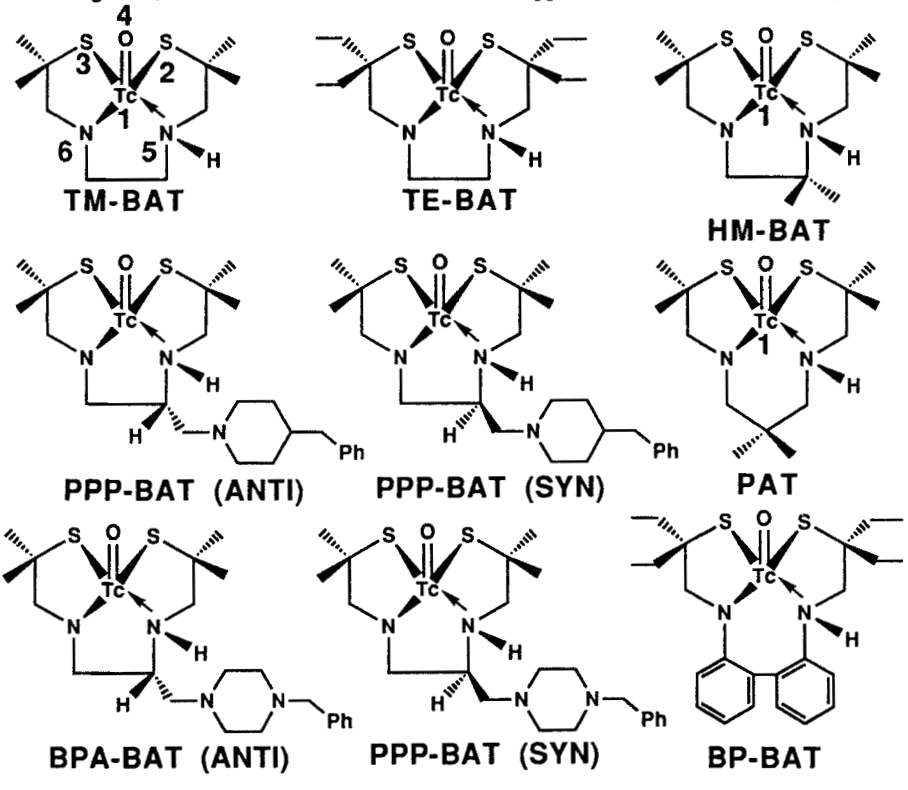

Table 1. Net Charge and Formation Energy (Ei^m) of Nine $Tc^VON_2S_2$ Complexes

	Tc	S_2	S_3	O_4	N_5	N_6	H	V	Ei^m
TM(CA)	1.537	-0.189	-0.341	-0.403	-0.583	-0.437	0.209	0.97201	1.49398
TM(BB)	1.740	-0.160	-0.557	-0.436	-0.754	-0.554	0.312		2.12669
TM(W)	1.88	-0.315	-0.191	-0.511	-0.805	-0.501	0.314	1.16251	2.18550
BPA-SYN(CA)	1.258	-0.003	-0.073	-0.529	-0.515	-0.462	0.220	0.83245	1.04723
BPA-SYN(BB)	1.984	-0.073	-0.134	-0.577	-0.492	-0.582	0.149	1.05949	2.10203
BPA-SYN(W)	1.945	-0.049	-0.151	-0.644	-0.681	-0.527	0.175	1.06733	2.07595
BPA-ANTI(CA)	1.09	-0.188	0.052	-0.407	-0.662	-0.556	0.221	0.87942	0.95856
BPA-ANTI(BB)	1.815	-0.094	-0.041	-0.525	-0.600	-0.908	0.226	1.12251	2.03736
BPA-ANTI(W)	1.177	-0.076	-0.012	-0.520	-0.743	-0.922	0.268	1.1725	1.3800
HM(CA)	0.969	-0.197	-0.174	-0.190	-0.399	-0.483	0.203	0.70866	0.68669
HM(BB)	1.78	-0.230	-0.190	-0.460	-0.510	-0.628	0.208	1.01620	1.8088
HM(W)	1.133	-0.346	-0.205	-0.419	-0.475	-0.500	0.163	1.03310	1.17053
PAT(CA)	1.171	-0.433	0.030	-0.176	-0.569	-0.397	0.199	0.74177	0.86862
PAT(BB)	1.695	-0.198	-0.255	-0.470	-0.500	-0.645	0.273	1.0375	1.75857
PAT(W)	0.387	0.120	0.070	-0.358	-0.503	-0.622	0.243	0.2800	0.72320
TE(CA)	1.771	-0.108	-0.315	-0.181	-0.066	-0.275	0.245		0.82577
TE(BB)	1.904	-0.178	-0.215	-0.581	-0.566	-0.475	0.198		1.95340
TE(W)	1.196	-0.196	-0.331	-0.475	-0.499	-0.629	0.233		1.28130
BP(CA)	1.583	-0.021	-0.023	-0.228	-0.018	-0.381	0.231		0.56353
BP(BB)	1.581	-0.121	-0.123	-0.528	-0.588	-0.281	0.340		1.31840
BP(W)	0.912	-0.269	-0.195	-0.381	-0.321	-0.487	0.390		0.75490
PPP-ANTI(CA)	1.199	-0.030	-0.113	-0.386	-0.459	-0.078	0.244		0.67490
PPP-ANTI(BB)	1.711	-0.230	-0.123	-0.586	-0.359	-0.578	0.325		1.69180
PPP-ANTI(W)	1.090	-0.222	-0.008	-0.377	-0.314	-0.477	0.398		0.77030
PPP-SYN(CA)	1.750	-0.063	-0.207	-0.249	-0.192	-0.324	0.186		0.85787
PPP-SYN(BB)	1.484	-0.363	-0.217	-0.622	-0.640	-0.774	0.234		1.95610
PPP-SYN(W)	1.747	-0.373	-0.138	-0.244	-0.340	-0.474	0.275		1.34000

Table 2. Comparison of SAS Values and Formation Energies (E^m_i) of Nine $Tc^VON_2S_2$ Complexes

	SAS	E^m_i
TM-BAT	0.9436	2.18550
BPA-SYN	0.9424	2.07595
BPA-ANTI	0.9384	1.38000
PPP-SYN	0.9368	1.34000
TE	0.9320	1.28130
HM-BAT	0.9292	1.17053
PAT	0.9287	1.10961
PPP-ANTI	0.9229	0.77030
BP	0.9221	0.75490

Paper A10

KINETIC AND THERMODYNAMIC STABILITIES OF TcO_2-OXO AND DIOXO CYCLAMS

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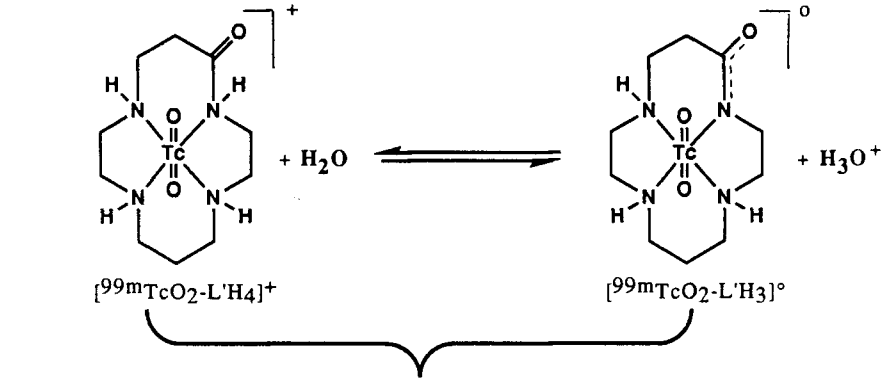
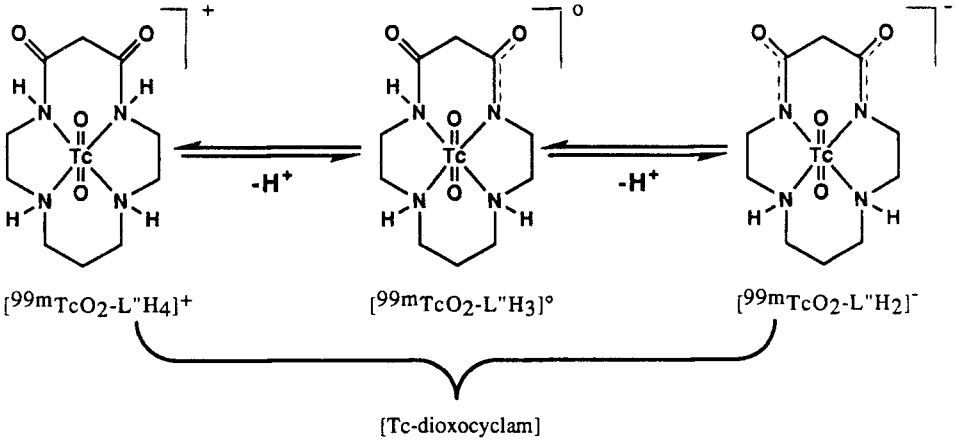
We previously studied (1) the complexation of ^{99m}Tc with 2-oxo-1,5,8,12-tetraazacyclotetradecane (monoxocyclam, L'H4) and 2,4-dioxo-1,5,8,12-tetraazacyclotetradecane (dioxocyclam, L"H4), both of them being derivatives of 1,4,8,11-tetraazacyclotetradecane (cyclam, LH4); these ligands complex the $^{99m}TcO_2^+$ core leading to the following equilibria :





At pH 7,4, neutral species $[^{99m}TcO_2\text{-}L'H_3]^\circ$ and $[^{99m}TcO_2\text{-}L"H_3]^\circ$ are preponderant,

while at pH 11,5, significant amounts of $[^{99m}TcO_2\text{-}L'H_2]^-$ are observed.

The stabilities of these derivatives, compared to the stability of $[^{99m}TcO_2\text{-}LH_4]^+$ were studied by exchange of ligands; because of the short half-life of ^{99m}Tc (6 hrs), only kinetic stability of complexes could be reached. The stability order of these complexes is as follows : [Tc-cyclam] ≈ [Tc-oxocyclam] > [Tc-dioxocyclam]

To enable the study of their thermodynamic stability, ^{95m}Tc having a relatively long half-life (61 d) was used thus allowing to reach thermodynamic equilibrium.

^{95m}Tc decays mainly by E.C. (96%) and has abundant γ-rays such as 204,1 keV (66,5%). For the preparation of ^{95m}Tc, a thick sample of natural metallic Mo (9 foils of 0,1mm) was irradiated in a deuteron beam (28 MeV, 10 μA) for 8 hrs. During this irradiation, ^{95m}Tc is formed by $^{93+x}Mo(d,xn)\ ^{95m}Tc$ reactions; contaminant radionuclides are eliminated by radioactive decay (cooling time : 1 month).

The chemical separation of ^{95m}Tc from Mo matrix includes the following basic steps :
- dissolution of irradiated sample (HNO_3 40%) and evaporation to dryness; in these conditions Tc and Mo are in their highest oxidation states +VII and +VI,
- dissolution of the residue in NaOH 7N, leading to a TcO_4^- and MoO_4^{2-} solution and solvent extraction of $^{95m}TcO_4^-$ using methylethylcetone,
- purification on alumina column using NaCl 0,9% as eluant.

With this method, 37 MBq of $^{95m}TcO_4^-$ solution (E.O.B.) were obtained. The reduction of $^{95m}TcO_4^-$ in presence of LH_4, $L'H_4$ and $L''H_4$ leads to the same complexes as those obtained with ^{99m}Tc. The thermodynamic study leads to the following results :

(^{95m}Tc)	11 d, 20°C	24hrs or *72hrs, 60°C	64hrs, 105°C
Tc-cyclam + oxocyclam + cyclam	Tc-cyclam 100%	Tc-cyclam * 100%	Tc-oxocyclam 100%
Tc-oxocyclam + cyclam + oxocyclam	Tc-oxocyclam 100%	Tc-oxocyclam 100%	Tc-oxocyclam 100%
Tc-cyclam + dioxocyclam + cyclam	Tc-cyclam 100%	Tc-cyclam * 100%	Tc-cyclam 50% Tc-dioxocyclam 50%
Tc-dioxocyclam + cyclam + dioxocyclam	Tc-dioxocyclam 65% Tc-cyclam 35%	Tc-dioxocyclam 57% Tc-cyclam 43%	Tc-dioxocyclam 50% Tc-cyclam 50%

These data confirm kinetic stabilities of [Tc-cyclam] and [Tc-oxocyclam] and show that thermodynamic equilibria can be reached at 105°C after 64hrs.

(Eq. 3) [Tc-cyclam] + oxocyclam ⇌ [Tc-oxocyclam] + cyclam

(Eq. 4) [Tc-cyclam] + dioxocyclam ⇌ [Tc-dioxocyclam] + cyclam

Equilibrium (Eq. 3) has a very high constant whereas (Eq. 4) has a constant close to unity. The complex [Tc-oxocyclam] is therefore a very good biological tracer; thus this basic structure could be of importance for the further elaboration of radiopharmaceuticals, and antibodies labelling.

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Int. J. Appl. Radiat. Isot. 1991 (in press).

Tc-99m U-BAT: A Case of BAT (N_2S_2) Ligand with Uneven Amines.

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Many neutral Tc-99m BAT (bis-aminoethanethiol) (N_2S_2) complexes have been reported, including Tc-99m-ECD (ethylene cysteine dimers, Fig. 1), a new brain perfusion imaging agent (1-5). Based on the differences in acidity of N-H groups of arylamines and alkylamines, BAT ligands with an unsymmetrical N-H group (U-BAT) was developed. With which neutral complexes with a $Tc^{v}O^{+3}$ center core can be prepared(Fig. 1). Ligand synthesis is described in scheme 1. By reducing 2-aminobenzamide with diborane/THF to the corresponding 2-aminobenzylamine and reacting the diamine with 2-[(p-methoxy)-o-benzylthio]-2-methylpropionyl chloride, the desired diamide was achieved. After another diborane/THF reduction and deprotection of the p-methoxy-benzyl group, the final "U-BAT" ligand was obtained. Radiolabeling with Tc-99m was achieved by reacting the ligand with Tc-99m sodium pertechnetate in the presence of stannous tartrate. The neutral and lipid-soluble product was stable at room temperature. However, in the presence of sodium bicarbonate and heating (85° C for 30 min) in air, the same reaction mixture produced an oxidized form, Tc-99m OU-BAT, which is more lipid-soluble. It is possible to reduce the Tc-99m OU-BAT back to Tc-99m U-BAT by sodium borohydride. The x-ray crystallography data (Fig. 2) for Tc-99 U-BAT shows the Tc-N amine and Tc-N amide distances are different from those of the oxidized form, Tc-99 OU-BAT. The bond distances of the oxidized form indicate extended conjugation of the N-Tc-N chelating bonds. HPLC profiles of the Tc-99m and Tc-99 U-BAT displayed identical retention time (Fig. 3). As expected, both Tc-99m U-BAT and Tc-99m OU-BAT are neutral and penetrate the intact blood-brain barrier. Initial brain uptake after an iv injection was 1.54 and 1.07 %dose/organ, respectively. Since there is no built-in trapping mechanism, both compounds washed out from the rat brain at later intervals. The advantages of the U-BAT ligand system compared to other BAT ligands are: a) the ligand system has a set of uneven amines--the position of ionizable NH proton can be controlled; b) the Tc-99m U-BAT and OU-BAT complexes display a unique reversible redox reaction; c) the ligand can be derivatized through substitutions on the phenyl ring; d) the substitution on the phenyl ring will not induce the formation of additional stereoisomers; e) the compact Tc-99m complex is incorporated into a phenyl system, which may be useful for designing biologically selective Tc-99m BAT complexes.

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Scheme 1. Synthesis of ligand U-BAT

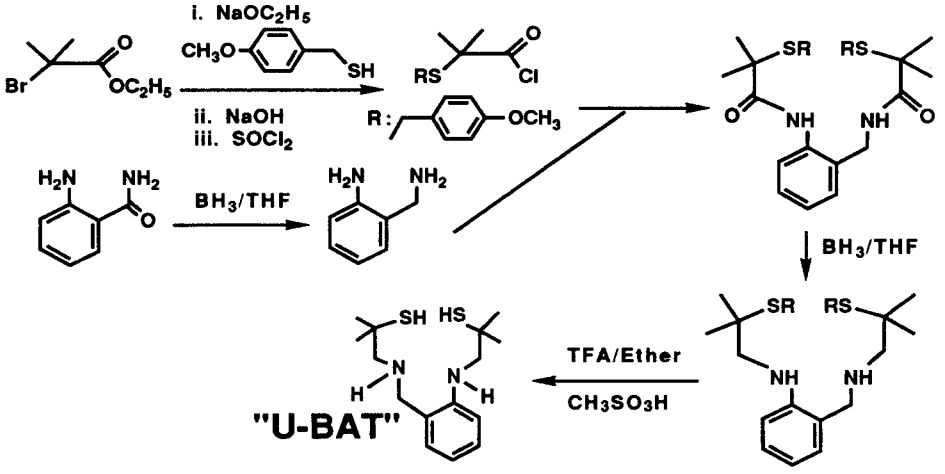


Figure 1. Chemical structures of Tc-99m ECD and the formation of Tc-99m U-BAT and OU-BAT.

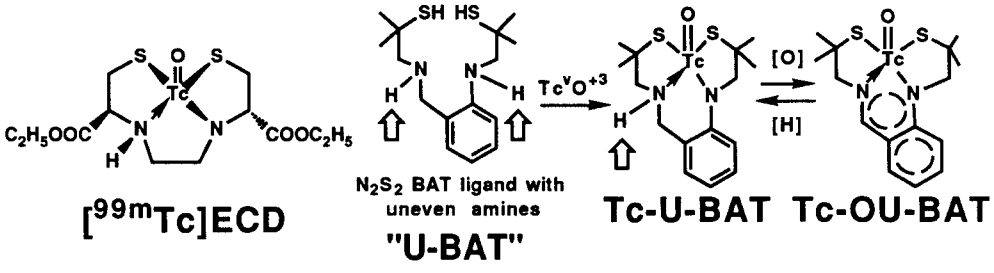


Figure 2. X-ray crytallography data of Tc-99 U-BAT and OU-BAT.

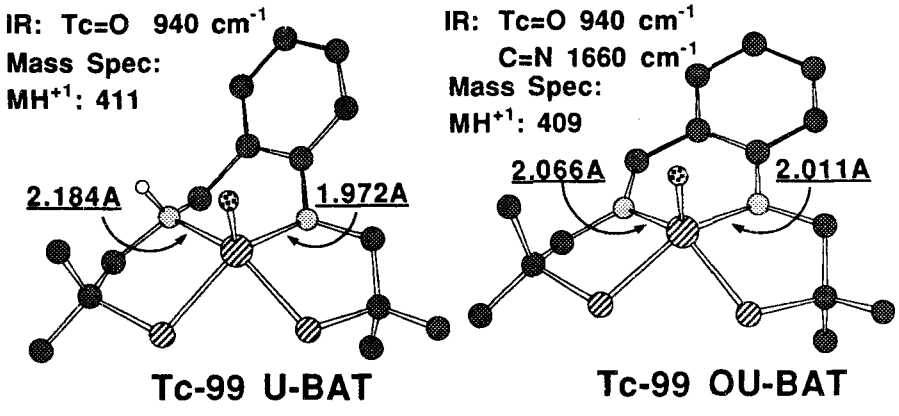
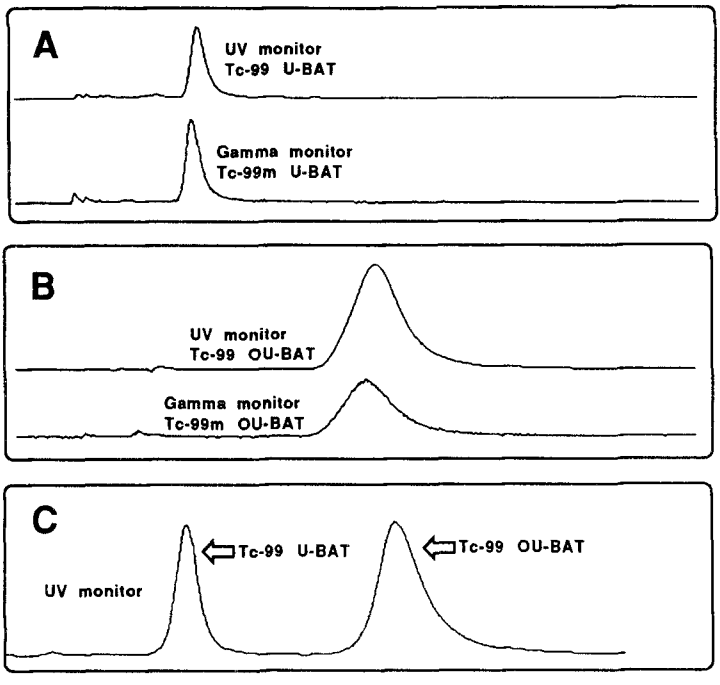


Figure 3. HPLC profiles of Tc U-BAT and OU-BAT: A) Simultaneous injection of Tc-99 and Tc-99m U-BAT. B) Simultaneous injection of Tc-99 and Tc-99m OU-BAT. C)Simultaneous injection of Tc-99 U-BAT and the oxidized form Tc-99 OU-BAT. HPLC conditions: reverse-phase column (PRP-1 column), acetonitrile/buffer pH 7.0 (90/10, v/v) at 1cc/min.



Acknowledgements: This work is supported by a grant awarded by NIH (NS-18509).

Paper A12

EVALUATION OF INSOLUBLE MACROMOLECULAR Sn(II) COMPLEXES FOR PREPARATION OF ^{99m}Tc RADIOPHARMACEUTICALS

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The reduction of $^{99m}TcO_4^-$ from +7 to a lower level of oxidation is essential for the preparation of ^{99m}Tc radiopharmaceuticals. Despite its inherent disadvantages of being easily hydrolyzable and oxidizable, it is generally recognized that stannous chloride($SnCl_2$) is an appropriate reducing agent for the preparation of ^{99m}Tc complexes. Insoluble macromolecular Sn(II)(R-Sn) complexes(1) were proposed as one way to resolve these problems in the preparation of ^{99m}Tc radiopharmaceuticals.

Various kinds of polymer matrix were examined for the preparation of R-Sn complexes (Table 1). Among them, a chelating resin containing aminophosphonic acid groups showed a high capacity for Sn(II), which was bound strongly to the resin by chelation (Fig. 1). These R-Sn complexes had a high reducing ability as that of $SnCl_2$ and preserved the ability in the aqueous solution for a long term without hydrolytic polymerization of Sn(II).

Since the R-Sn complexes can be prevent Sn(II) contamination of ^{99m}Tc labelled compound, the R-Sn complexes were applied to the ^{99m}Tc labeling of protein such as human serum albumin(HSA). HSA is heterogeneous with respect to sulfhydryl(SH) content. We prepared mercaptalbumin(HMA) and nonmercaptalbumin(HNA) by treatment of HSA with dithiothreitol and cystine, respectively. The HMA(SH content: 0.94 mol/mol HSA) and HNA(SH content: 0.04 mol/mol HSA) were directly labeled with ^{99m}Tc at pH 2-3 using R-Sn complexes or $SnCl_2$ for the reduction. Each HSA component labeled with ^{99m}Tc was characterized *in vitro* and *in vivo* experiment. The ^{99m}Tc labelled HMA and HNA with R-Sn complexes demonstrated marked differences in the activity remaining in mice serum. In the case of the HSA components labeled with $SnCl_2$, this difference was not observed (Table 2). These results suggest that R-Sn is effectively available as reducing agent for the ^{99m}Tc labeling of protein containing SH group that is responsible for the binding of ^{99m}Tc. The application of R-Sn complexes to the direct labeling of antibody is now under way.

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Table 1. Characteristics of the Polymer Matrix Tested for the Preparation of R-Sn

Polymer Matrix			Sn(II) Adsorbed* (mmol/g-resin)	
Functional Group	N Content (mmol/g)	P Content (mmol/g)	KCl Concentration 0.1 M	1.0 M
-$NHCH_2PO_3H_2$	2.5	3.3	1.8	1.7
-$N(CH_2COOH)_2$	3.5	-	0.6	0.6
-$C(NH_2)NOH$	6.1	-	0.4	0.4
-SO_3H	-	-	1.1	0.1

* Batch operation: 0.01 M $SnCl_2$ in 0.1 M HCl, 30°C, 1 hr.

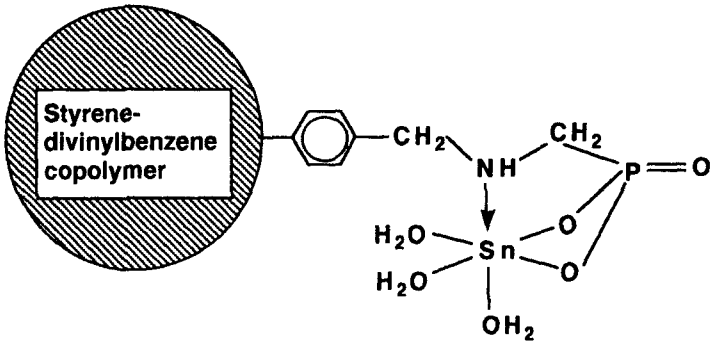

Fig. 1. Schematic structure of R-Sn complexes.

Table 2. Blood Clearance for ^{99m}Tc Labeled HMA and HNA in Mice.

HSA Component	% Injected Dose/g of Blood* R-Sn 0.5 hr	R-Sn 3.0 hr	$SnCl_2$ 0.5 hr	$SnCl_2$ 3.0 hr
HMA	41.53 ± 3.67	24.24 ± 3.33	14.91 ± 1.87	7.72 ± 1.25
HNA	17.45 ± 2.59	8.93 ± 0.92	20.54 ± 2.02	8.76 ± 0.94

* Data given as mean ± S.D.(n=5)

Paper A13

THE DETERMINATION OF CHARGE OF TECHNETIUM COMPEXES BY ION EXCHANGE HPLC. D.P. Nowotnik# and A.L.M. Riley, Amersham International plc, Amersham, UK

As the overall charge of a technetium complex is an important determinant of its biodistribution, knowledge of the absolute charge is essential to the development of meaningful structure-distribution relationships (SDRs). However, because of the extremely low chemical concentration of most Tc-99m preparations ($<10^{-6}$M), the charge of a Tc-99m complex is usually inferred from knowledge of the structure of its Tc-99 counterpart. Ideally, the charge on Tc-99m complexes should be determined directly from measurements of these complexes.

Anion exchange methods have been reported for the determination of the charge of Tc-99m anions (1,2). Wilson and Pinkerton (3) used an anion-exchange HPLC method which was based upon the relationship $\log (R_i) = \text{constant} - \frac{m}{a} \log (A)$, where R_i is the retention of a test substrate (of charge m) on the HPLC column attributable to the ion-exchange mechanism, and A is the activity of a counter ion of charge a. Using this relationship, m was determined by measuring R_i over a range of counter ion concentrations. Wilson and Pinkerton obtained R_i by correcting the observed retention time for column dead-time, but did not account for the contribution of other mechanisms to the observed retention. This ommision could lead to errors. We now report an ion exchange HPLC method for charge determination of anions which includes the terms R_O, the column dead-time and R_C, the retention time of a solute which is independent of counter ion stregth. The retention/counter ion strength relationships of several anionic Tc-99m complexes were obtained on a SAX column (250 x 4.6 mm) using aqueous sodium sulphate as eluent, and the charges of the complexes determined by a non-linear regression method using a programmed spreadsheet.

We also report an adaptation of this method for the determination of charge of cations. Lipophilic Tc-99m cations have been extensively studied over the past decade as potential myocardial perfusion tracers. The high lipophilicity of many of these compounds makes the term R_C of even greater significance in the determination of charge of such complexes. The charges of several cationic Tc-99m complexes were determined using either a TSK or Partisil SCX column with K^+ or NH_4^+ as counterions, and, to minimize the R_C term, aqueous acetonitrile as eluent.

Both the anion and cation HPLC methods appear to provide reliable estimates of the charges of Tc-99m complexes, and these techniques should prove useful in the development of SDRs.

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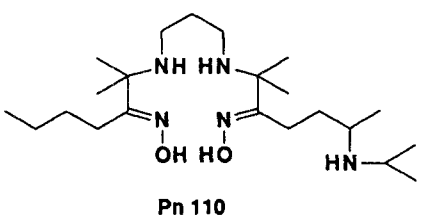
#. Present address: Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 191, New Brunswick, NJ 08903, U.S.A.

Results using the anion exchange method:

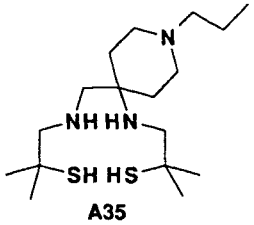
Sulphate Concentration mM	Observed retention times (R_t) in minutes						
	TcO_4^-	Tc-DTPA	Tc-EHIDA		TDG	Tc-TDG	
			#1	#2		#1	#2
100	4.32	9.53		5.70	5.6	4.59	5.19
50		13.33		6.16	7.0	5.28	6.34
35		16.62			8.0	5.80	7.16
25		20.25	5.51	7.51	9.75	6.59	8.38
15	6.21	31.60	5.72	9.55	13.4	8.52	11.71
10	7.52		6.05	11.47	17.3	10.89	15.74
7.5			6.61	13.47	21.75	13.17	19.69
5.0	9.37	80.68	6.85	18.21			
3.5							
2.0	13.26						
1.0	18.59						
0.5	24.97						
Determined charge	-1.13	-2.00	-1.00	-2.01	-1.93	-2.08	-2.14

(TDG = thiodiglycolic acid)

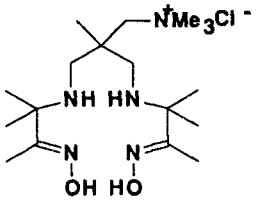
Structures of the tetradentate ligands:



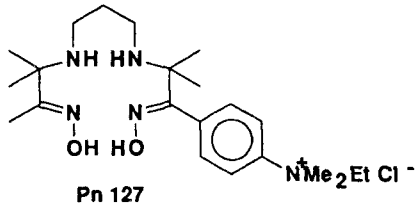
Pn 110



A35



Pn 116



Pn 127

Results using the cation exchange methods:

Compound	SCX Column	Counter ion	% ACN	Determinmed Charge
Tl-201	Partisil	NH_4^+	0	0.95
Tl-201	Partisil	NH_4^+	30	1.07
Tl-201	Partisil	NH_4^+	70	1.02
Mn(II)-54	Partisil	K^+	0	2.00
Tc-99m $(TBI)_6$	Partisil	NH_4^+	30	0.93
Tc(I)-99 $(dmpe)_3$	Partisil	NH_4^+	30	1.06
Tc-99m $Cl_2(dmpe)_2$	Partisil	NH_4^+	30	0.98
Tc-99m $O_2(dmpe)_2$	Partisil	NH_4^+	30	0.97
Tc-99m A35	Partisil	NH_4^+	30	0.93
Tc-99m Pn 110	Partisil	NH_4^+	30	0.96
Tc-99m Pn 110	Partisil	K^+	30	0.94
Tc-99m Pn 116	TSK	K^+	30	0.94
Tc-99m Pn 127	TSK	K^+	30	0.92

ACKNOWLEDGEMENTS

The authors wish to thank Drs. Jon Cummins and Andy Cummings (Amersham International) for the syntheses of the Pn ligands, and Dr. Vaughn Griffiths (University of Keele) for ligand A35.

Paper A14

Electronic Structure of d,l- and meso-Isomeric Forms of TcO(HM-PAO) Complexes and Radioanalytical Composition of Corresponding Radiopharmaceutical Preparation

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3,6,6,9-tetramethyl- 4,8-diazaundecane- 2,10-dione dioxime (HM-PAO) is well known in nuclear medicine tetradentate amine oxime ligand for Tc^{99m}. On the base of this compound the original radiopharmaceutial preparation with Tc^{99m} was created for diagnostic of regional cerebral bloodflow. In radiopharmaceutical Tc^{99m} (HM-PAO) the complex of metal ion with d,l-isomer is the main one and the complex with meso-form is radiochemical impurity.

In the present work the quantum-chemical analysis of electronic structure peculiarities was performed for complexes of Tc^{99m} with d,l- and meso-isomeric forms of HM-PAO. The extended Huckel method with self-consistent atomic charges was used for calculations. The report contains the result data about effective charges of central ion and atoms of its ligand encirclement as well as the energy and composition of molecular orbits being near the occupation border. The results received in these investigations were used to find out the peculiarities of complexes and also to interpretate the data about radioanalytical composition data.

Paper A15

APPLICATION OF NMR AND MOESSBAUER SPECTROSCOPY FOR THE DEVELOPMENT OF ^{99m}Tc - RADIOPHARMACEUTICALS

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Institute of Biophysics, Ministry of Health, USS

By the development of the ^{99m}Tc- radiopharmaceuticals, on the bases of tin complexes, it is desirable to know which of the donor atoms of the ligand take place in the formation of the complex with metal ion, the range of pH complex stability in aqueous solution, the Tc valency, the formation ability of the triple complexes Tc-Sn-ligand and others. The joint use of NMR and Moessbauer spectroscopy makes possible solving these questions.

PH dependence of NMR chemical shifts (^{1}H,^{31}P,^{13}C) of the aqueous solution as ligand as of its Sn(II) complex studied for this purpose. The range of Sn- complexes, their structure and the influence of the pH changing on the formatiuon of different complex forms have been determined in comparison of these date with pH dependence of isomer shifts and quadrupole splittings of ^{119}Sn Moessbauer spectra of the shock-frosen aqueous solutions of ^{119}Sn(II) complexes.

^{119}Sn Moessbauer spectroscopy shows that oxidation of Sn(II) complexes results to the formation of Sn(IY) complexes. Sometimes the tryple complexes can be formed.

Sn(II)+ligand+$KTcO_4$ solutions with varying component ratio have been studied by Sn Moessbauer spectroscopy for the determination of Tc valence. The spectra corresponding to Sn(IY) have been observed at the low Sn content; by increasing relative Sn content the appearance of the Sn(II) lines in spectrum points out the completion of Tc reduction. Tc valence has been determined from the component ratio.

The described method was used for the investigation of the radiopharmaceuticals on the basis of oxabis(ethylennitrilo)tetramethylphosphonic acid (oxabiphore) [1,2], diethylentriaminepentaacetic acid (DTPA), calcium-sodium salt of DTPA (pentacine) [3], hlodiphone [4]. It was found that Tc is reduced as to Tc(IY) in the complexes Sn-Tc-oxabiphore and Sn-Tc-hlodiphone as to Tc(III) in the complexes Sn-Tc-DTPA and Sn-Tc-pentacine.

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CHARACTERIZATION OF TECHNETIUM-99 COMPLEXES WITH S-UNPROTECTED MERCAPTOACETYLPEPTIDES (MAG_3, MAG_2, MAG_1 and Derivatives)

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The use of S-benzoyl protected ligand in the preparation of the renal function agent ^{99m}Tc-MAG_3 implies some inconvenience and restrictions in the labelling procedure. Therefore, we studied the suitability of purified unprotected MAG_3 and further developed a corresponding kit formulation (1,2). With the unprotected ligand, having a free thiol group of much higher reactivity toward the Tc(V)oxo core than sulfur in a thiol ester group, different complexation reactions and products in the Tc/ligand system have to be anticipated. To gain a better understanding of the underlying chemistry, complexation reactions have been studied and $^{99/99m}$Tc complexes characterized. Such studies also have been motivated by the topical great interest in the structural features of the ^{99m}Tc N_3S complex necessary for efficient renal tubular secretion (3-6) as well as the use of MAG_3-type chelator in S-unprotected form for bifunctional chelating agent application (7).

Both in ligand exchange reactions starting from Tc(V) precursors and by reduction of pertechnetate with stannous chloride in the presence of mercaptoacetyltriglycine (MAG_3), mercaptoacetyldiglycine (MAG_2), or mercaptoacetylglycine (MAG_1) up to three different Tc(V)oxo complexes of the corresponding ligand occur.

With MAG_3 as the ligand, the well-known anionic 1:1 complex possessing a free carboxyl group is formed if the pH is >11 in the stannous chloride procedure (1,2,8) or the ligand/Tc ratio in ligand exchange reactions is kept low. Any excess of ligand over Tc favours the 1:2 Tc/ligand complex and, at higher excess, the 1:4 complex (Fig.1). In the latter case, MAG_3 only reacts as a monodentate thiol.

MAG_2 also gives a stable 1:1 complex. The X-ray crystal structure (Fig.2) of Ph_4As[TcO(MAG_2-S,N,N,O)] shows the carboxyl group coordinated to the Tc=O core in equatorial position. Besides this complex, 1:2 and 1:4 species exist (Table 1). MAG_2 methylester did not provide a 1:1 complex. The smallest product obtained was a 1:2 complex . Likewise, with the tridentate MAG_1 only 1:2 and 1:4 compounds occured at adequate ligand/ Tc ratios.

The studies performed show the formation of various Tc(V)oxo complexes of MAG_3-type ligands in case the S-unprotected form of the ligand is used. The versatility results from the good donor quality of the mercapto group which outbalances the other donor atoms if one fails to meet the strict conditions for formation of 1:1 complexes.

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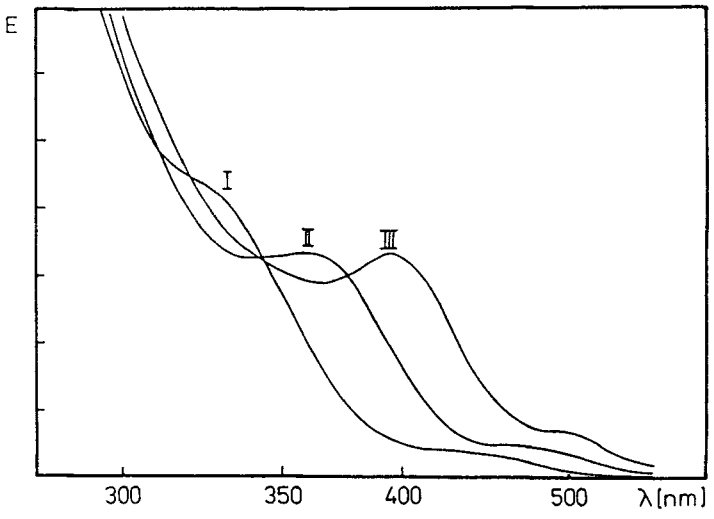

Fig.1: UV-visible spectra of 1:1(I), 1:2(II) and 1:4(III)complexes formed at increasing ligand to Tc ratios in ligand exchange reaction of ^{99}Tc(V)gluconate with MAG_3 at pH 6 after 30 min.

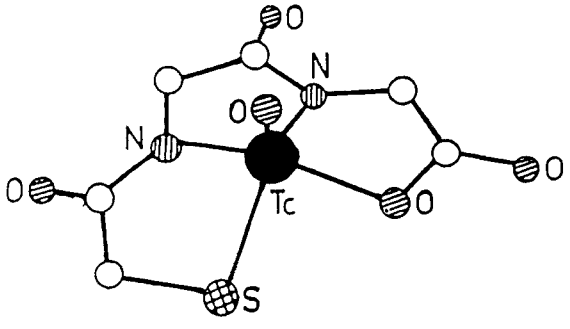

Fig.2: PLUTO drawing of $[Tc(MAG_2)]^-$. The structure of the tetraphenylarsonium counterion has been omitted for clarity.

Table1: Percent portion of different complexes formed by ligand exchange of 99Tc(V) gluconate with MAG_3, MAG_2, or MAG_1 at various TC/ligand ratios (t=30 min), as determined by TLC on silica gel with 95% ethanol

Molar Ratio	Content [%]			
	1:1 complex	1:2 complex	1:4 complex	Tc gluconate
TC/MAG_3				
1:1	70.5	12.0	4.0	13.5
1:2	20.0	63.0	12.0	-
1:4	3.0	67.5	29.5	-
1:5	2.5	60.5	37.0	-
TC/MAG_2				
1:1	83.0	-	4.5	12,5
1:2	84.0	-	16.0	-
1:7	51.0	31.0	18.0	-
1:100	-	39.0	61.0	-
TC/MAG_1				
1:1	-	49.2	-	50.8
1:2	-	91.0	9.0	-
1:7	-	90.0	10.0	-
1:100	-	85.0	15.0	

Paper A17

STRUCTURE AND ACTIVITY OF TECHNETIUM(V) DIPHOSPHINE COMPLEXES

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The uptake of lipophilic Technetium-99m cations in heart tissue in animal models is a widely recorded phenomenon. In general, however, these promising results have not translated to comparable performance in man because of poor clearance from non-target tissues, especially blood and liver and sometimes lung. To overcome these difficulties, the strategy for molecular design which has thus far proved most successful is the incorporation in the ligand of ether functions (1,2,3). This approach has led to the design of a $[TcO_2L_2]^+$ cation, where L = 1,2-bis[bis(2-ethoxyethyl)phosphino]ethane, which has been demonstrated to have significant potential as a myocardial perfusion imaging agent (4).

In the course of development of this agent, we have synthesised a substantial range of ether-functionalised diphosphines. Their Tc(V) dioxo complexes were screened after labelling by the simple addition of the phosphino-ether ligand to pertechnetate at room temperature. HPLC retention times were used as a measure of relative lipophilicity using a Hamilton PRP-1 column (4.1 x 150 mm) with a tetrahydrofuran/pH 7 phosphate buffer gradient (0 - 100% THF over 15 minutes; flow rate of 2.0 ml/min; 5mM phosphate, pH 7.0).

Some representative series of results for dioxo complexes are shown in the accompanying tables.

Within certain narrowly defined series, relationships between structure and biological distribution can be identified, but paradoxical effects are apparent in other cases. Using conventional quantitative structure-activity approaches (5), it is not possible to describe the overall behaviour of these technetium dioxo diphosphine complexes. Qualitative explanations may lie in the packing of ligand around the metal, in particular the extent of exposure of the oxygen atoms of the ether substituents or of the dioxo core.

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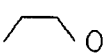
Table 1:
HPLC retention time and biodistribution for $[TcO_2L_2]^+$: L= $R_2PCH_2CH_2PR_2$

		Biodistribution at 1 hour p.i.				
R	Ret	%H	%K+U	%Li+Gl	%Li	%Bl
	4.5′	0.1	76	10	1	0.5
	6.2′	0.5	39	27	2	0.7
	7.2′	0.6	27	43	8	1.6
	8.3′	1.0	16	42	7	0.7

Table 2:
HPLC retention time and biodistribution for $[TcO_2L_2]^+$: L = $R_2P(CH_2)_nPR_2$

		Biodistribution at 1 hour p.i.				
R	Ret	%H	%K+U	%Li+Gl	%Li	%Bl
n = 2						
	8.3′	0.3	9	73	9	1.6
	7.2′	1.7	9	42	1	1.0
	8.3′	1.0	16	42	7	0.7
n = 3						
	7.5′	0.3	12	72	11	3.2
	5.7′	0.2	26	59	11	4.5

POTENTIOMETRIC AND SPECTROPHOTOMETRIC STUDY OF ^{99}Tc-DPD COMPLEXES

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In our previous spectrophotometric study[1], it was found out that DPD (2,3-dicarboxypropane-1,1-diphosphonic acid) and Tc, previously reduced by tin/II/, form coloured complex compounds with absorption maxima at 410 nm (pH 3-7) and at 515 nm (pH 5-9.6). Molar ratios Sn:Tc providing reduction were found to be 2.5:1 and 4.5:1. Our further research was focused on acidic medium (pH 3).

By use of Job's method (Fig.1),formation of complexes with ratio DPD:Tc of 1:1 and 2:1 was found with both mentioned Sn:Tc. Reduction to the same valence state of Tc took place independently of Sn/II/ quantity.

In order to determine valence state of Tc in the complex, taking into account that formation of ^{99}Tc-DPD complex does not occur in absence of stannous ions, two kinds of redox potentiometric titrations have been performed: 1) solutions TcO_4^- $1x10^{-3}$M were titrated with Sn/II/ $1x10^{-2}$M, both pH 3, with DPD $1x10^{-2}$M; 2) solutions TcO_4^- $1x10^{-3}$M, pH 3 were titrated with Sn/II/ $1x10^{-2}$M, pH 1.5, both without the ligand. The difference in pH of Sn/II/ solutions was due to hydrolysis of tin in absence of DPD at pH higher than 1.5, meaning that Sn/II/ also forms complex with DPD.

Pt indicator electrode and saturated calomel electrode as reference were used, while potentials were measured on pH-meter Model 3550, Beckman. Solutions were prepared in nitrogen atmosphere, pH od Sn/II/ solutions was adjusted in nitrogen atmosphere, also titrations were performed in a double-jacketed vassel in nitrogen atmosphere, while temperature was maintained at 78°C by means of warm water circulating from a thermostat. Magnetic stirring was used, and increments were added in measured time intervals of 6 min (by a chronometer).

From potentiometric curves, shown in Fig.2, it can be seen that in the presence of DPD, reduction is going to Tc-IV, whereas in absence of DPD to Tc-III. This difference is due to complexation of Tc, as well as the complexation of Sn with DPD. However, since Sn does not form coloured complexes, it is not possible to determine Sn-DPD composition by spectrophotometric method.

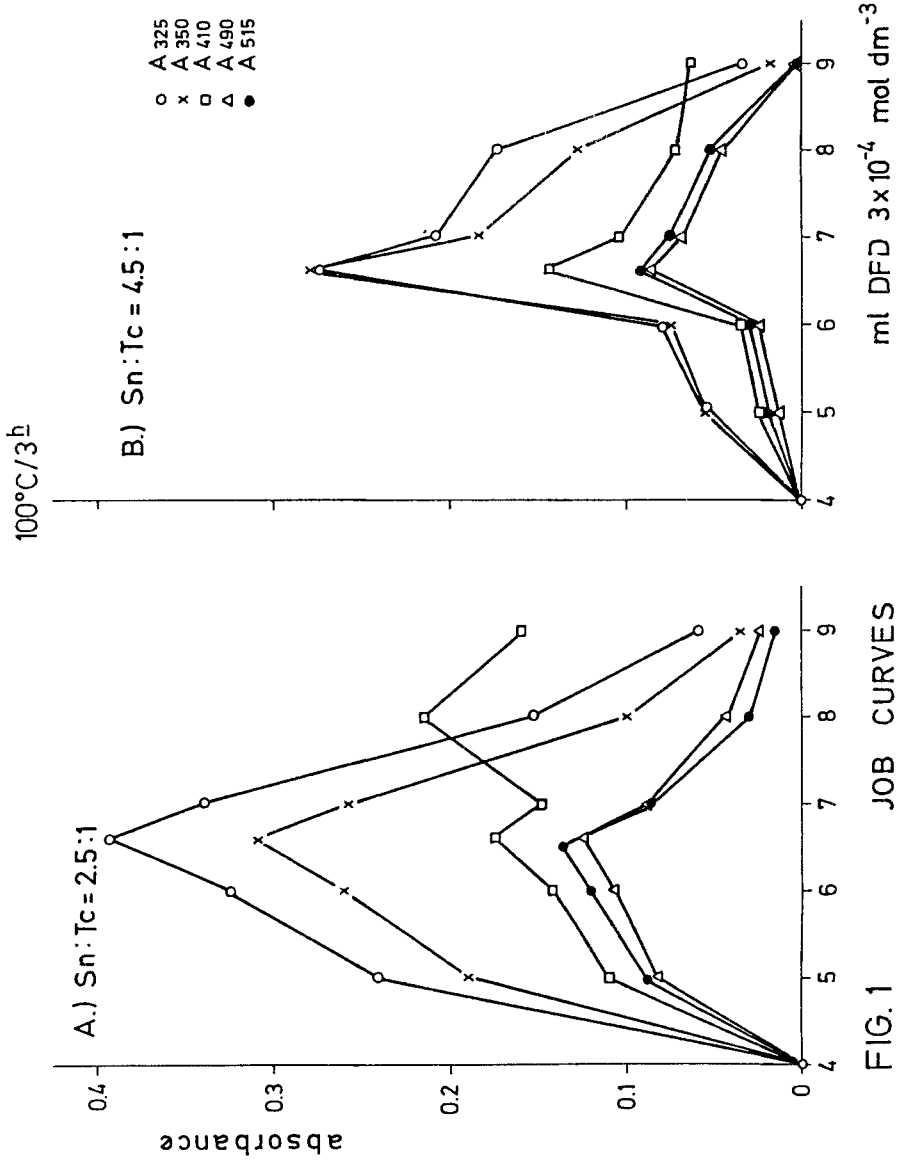

FIG. 1 JOB CURVES

Reference:
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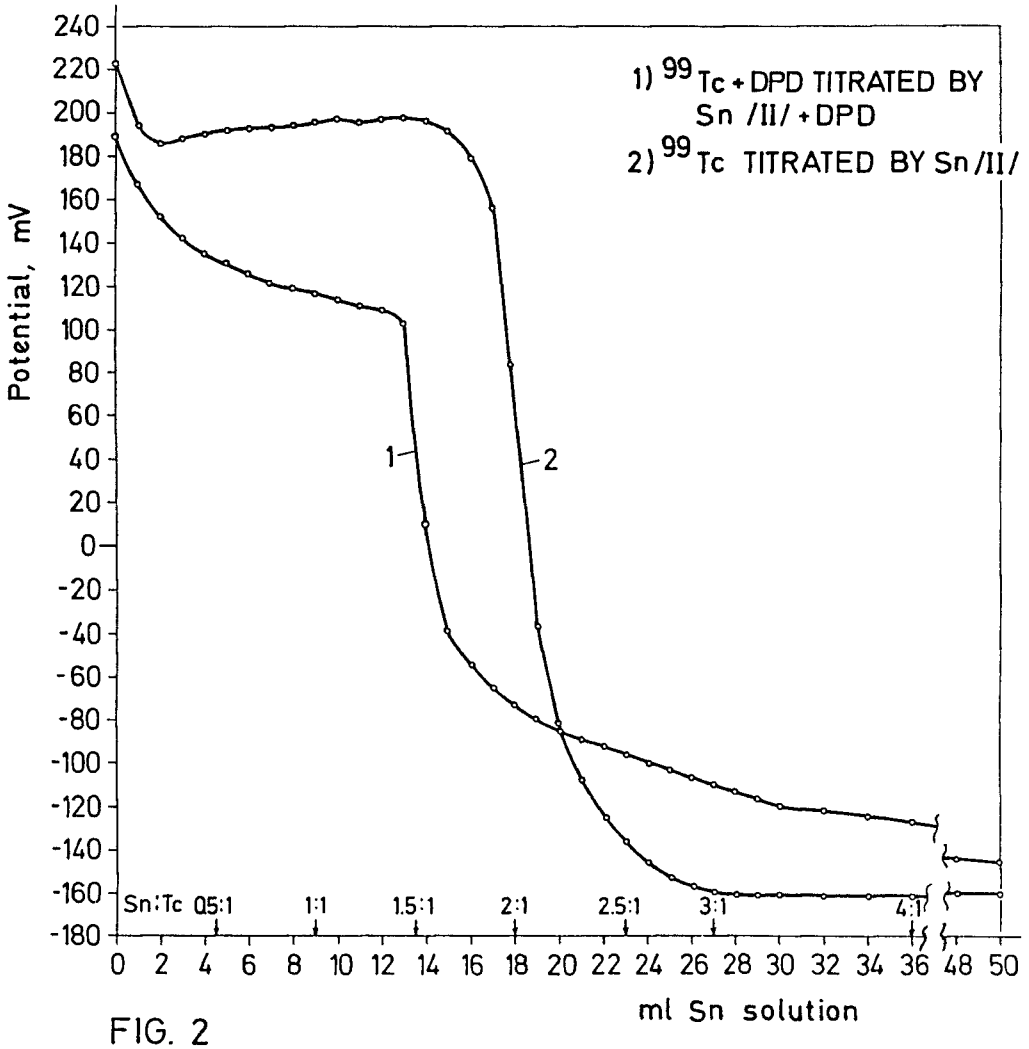

FIG. 2

Paper A19

TECHNETIUM (V) COMPLEXES WITH SCHIFF BASE LIGANDS CONTAINING THE ONSSNO DONOR ATOM SET.

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*Department of Pharmacy, University of Athens, Athens, Greece.

Hexadentate Schiff bases (ONOONO) have been reported previously to form stable, monomeric tetracoordinated complexes with pentavalent rhenium[1]. In a continuation of our investigation into the ability of the polydentate ligands to bind technetium, we investigated the formation of complexes between reaction hexadentate ONSSNO ligands and tetrachlorooxotechnetate.

The following ligands were synthesized:

L_1H_2: $(2\text{-}HOC_6H_4CH{=}NCH_2CH_2S)_2$ (Hsal)2thiatrien

L_2H_2: $(HOC(CH_3){=}CHC(CH_3){=}NCH_2CH_2S)_2$ (Hacac)2thiatrien

Synthesis[2,3] procceded via condensation of 1,8-diamine-3,6-dithiaoctane with salicylaldehyde (L_1H_2) or acetylacetone (L_2H_2). The reaction of one equivalent of $nBu_4NTcOCl_4$ with one equivalent of L_1H_2 in methanol at room temperature immediately produced a deep red precipitate in high yield. Reaction of $TcOCl_4^-$ and L_2H_2 in methanol refluxed under argon, yielded brown solid in 50% yield. The solids were washed several times with hot methanol to remove the reactants and used further. The Tc(V)-complexes were characterized by IR, UV-Vis, spectroscopy and elemental analysis. The data are presented in Table 1.

The IR spectra showed that the ν(C=N) and ν(C-O) bands of the ligands L_1H_2 are shifted upon complexation suggesting coordination of nitrogen and oxygen. The elemental analysis of technetium compound corresponded to binuclear complexes where the L_1H_2 and L_2H_2 being coordinated with two oxotechnetium centers bridged by one oxygen atom. The empirical formula of the complexes was thus estimated as $Tc_2O_3L_1Cl_2$ and $Tc_2O_3L_2Cl_2$ respectively. Because of solubility problems, proton and carbon-13 NMR spectra were initially studied in DMSO-d_6. This solvent is not suitable, due to competition with the ligands in complexation at the first coordination sphere of the metal. As a consequence the spectra corresponded to mixtures the composition of which changed upon standing of the solutions. Studies by NMR and mass spectroscopy (FAB^+) for further characterization of $Tc_2O_3L_1Cl_2$ and $Tc_2O_3L_2Cl_2$ are in progress.

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SUPPORTING DATA

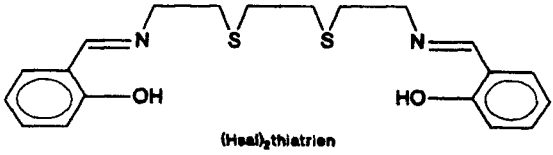
Table 1. *Analytical data of hexadentate ONSSNO technetium (V) complexes.*

Complex	IR (KBr disc) ν(C=N)cm^{-1}	ν(C-O)cm^{-1}	ν(Tc=O)cm^{-1}	UV-Vis(in DMF) λ_{max} (nm)
$Tc_2O_3L_1Cl_2$	1615	1278	945	320(sh), 455
$Tc_2O_3L_2Cl_2$	1575	---	945(w)	307, 375

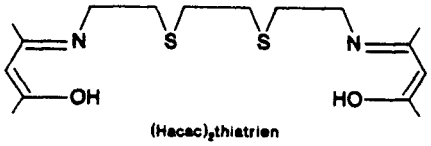
*Elemental Analysis**

	C	H	N	S
$Tc_2O_3L_1Cl_2$	34.03 (34.15)	3.43 (3.15)	3.89 (3.98)	10.10 (9.12)
$Tc_2O_3L_1Cl_2$	28.73 (29.14)	4.34 (3.97)	4.32 (4.25)	11.01 (9.72)

* *caclulated values in parentheses*



$(Hsal)_2$thiatrien



$(Hacac)_2$thiatrien

Paper A20

REACTION AND STORAGE TEMPERATURE EFFECTS ON THE PROPORTION OF LIPOPHILIC AND HYDROPHILIC COMPLEXES OF [^{99m}Tc]HMPAO

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The utility of the lipophilic chelate ^{99m}Tc-HMPAO for cerebral blood perfusion studies and granulocyte labelling for inflammation imaging is hampered by its instability in aqueous solutions (1). The addition of gentisic acid (2) and tartrate (3) to the formulation or preparation in ethanol (4) has been shown to stabilize the product. Other factors related to the quality of the pertechnetate, radiolysis and concentration of ^{99}Tc have been studied (5,6). Since the storage of[^{99}Tc]-HMPAO at 4 °C has been shown to improve stability (7), we undertook to study the effect of reaction and storage temperature on [^{99m}Tc]-HMPAO stability.

The *d,l*-HMPAO was prepared and purified by a modification of the method as described by Neirinckx (1). The [^{99m}Tc]-HMPAO was prepared by the addition of 1 GBq [^{99m}Tc]pertechnetate to 0.5 mg HMPAO in a total of 5.0 mL saline in a nitrogen purged vial. Stannous ion was generated electrolytically (0.004 mg) in the vial at the appropriate temperature (waterbath) with ultrasonic agitation during electrolysis. All reagents and system components were pre-equilibrated to the appropriate temperature (4, 20, 37, 50 and 70 °C). In one series of experiments, each reaction product was stored at the reaction temperature. In a second series, storage temperatures differed from reaction temperatures. In a third series of experiments sodium tartrate was added to stabilize the [^{99m}Tc]-HMPAO. The radiochemical purity of each preparation was determined at 5, 60, 120, and 180 minutes after preparation using the three chromatogram method of Neirinckx(1). Analysis of the reaction products indicated that the concentration of hydrophilic complexes increased with reaction and storage temperature (Figures 1 and 2). Since previous work (8) describing the biodistribution and metabolism of the secondary complex used aged Ceretec (160 min post preparation) which contained approximately 23 % secondary and 53 % primary complex, we took the opportunity to compare the biodistribution, in mice, of a "more well defined" preparation enriched in hydrophilic and depleted in lipophilic complex concentration with a normal [^{99m}Tc]-HMPAO preparation. The lipophilic [^{99m}Tc]-HMPAO was extracted from the reaction mixture in ethyl acetate. The aqueous phase, consisting of 70 - 80 % hydrophilic complex, 15 - 20 % free [^{99m}Tc]-pertechnetate and the remainder as hydrolysed, reduced ^{99m}Tc, was used without further purification in the animal studies. The most notable result was the very high stomach uptake which continued to rise throughout the study while the thyroid gland did not show marked uptake suggesting free pertechnetate was not responsible (Figures 5 and 6). This lends support to suggestions that abdominal activity observed following injection of [^{99m}Tc]-HMPAO labelled granulocytes may be due to hydrophilic complexes back diffusing across the granulocyte membrane into the blood.

The decomposition half-time ($T_{1/2(decomp)}$) was calculated for each set of conditions. The $T_{1/2(decomp)}$ decreased by a factor of two for every 15 °C increase in temperature for both the stabilized and unstabilized formulations (Figures 3 and 4). Changing the storage temperature after preparation had a profound effect on product stability. Samples prepared and stored at ambient temperature (clinical situation) had a mean $T_{1/2(decomp)}$ of 300 min. Preparation at 70 °C and storage at ambient temperature increased the $T_{1/2(decomp)}$ from 36 min (storage at 70°C) to 258 min while preparation at 0°C and storage at 50 °C decreased the $T_{1/2(decomp)}$ from 420 min (storage at 0 °C) to 220 min. Interestingly, the major decomposition product observed at higher temperatures was not free [^{99m}Tc]-pertechnetate but rather a product behaving chromatographically identical to the hydrophilic complex observed routinely, in lower yields, at ambient temperature (Figure 1). The $T_{1/2(decomp)}$ of the secondary complex prepared at 50 C and stored at ambient temperature was 14.75 hours compared to 3.67 hours for the primary complex under similar conditions. These results suggest that the shelf-life of [^{99m}Tc]-HMPAO could be improved by preparing and storing the product at 0 - 4 °C. Alternatively, the reaction could be conducted at ambient temperature and the product stored at 0 - 4 °C, however the preparation would need to be cooled very quickly to take advantage of the temperature change.

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Fig 1. Time Dependent Composition
Tc-99m HMPAO (Ambient Temperature)

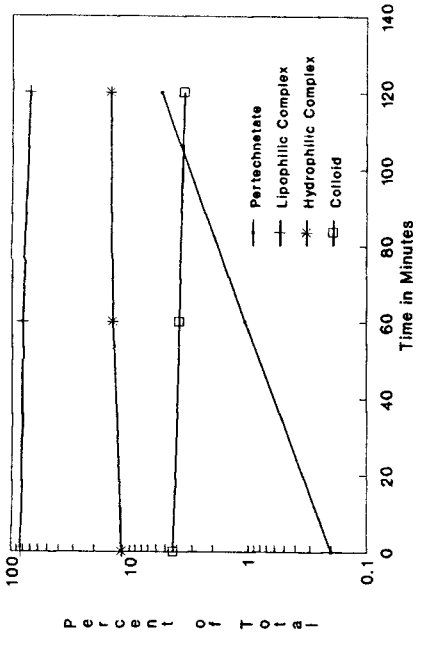


Fig 2. Time Dependent Composition
Tc-99m HMPAO (50 C)

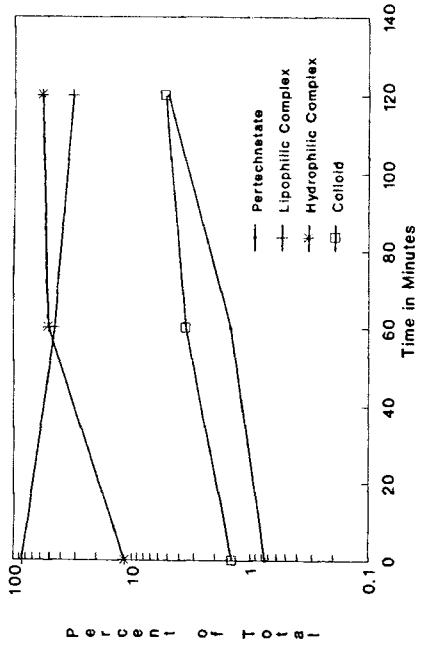


Fig 3. Temperature Effect on Stability
Stored at Reaction Temperature

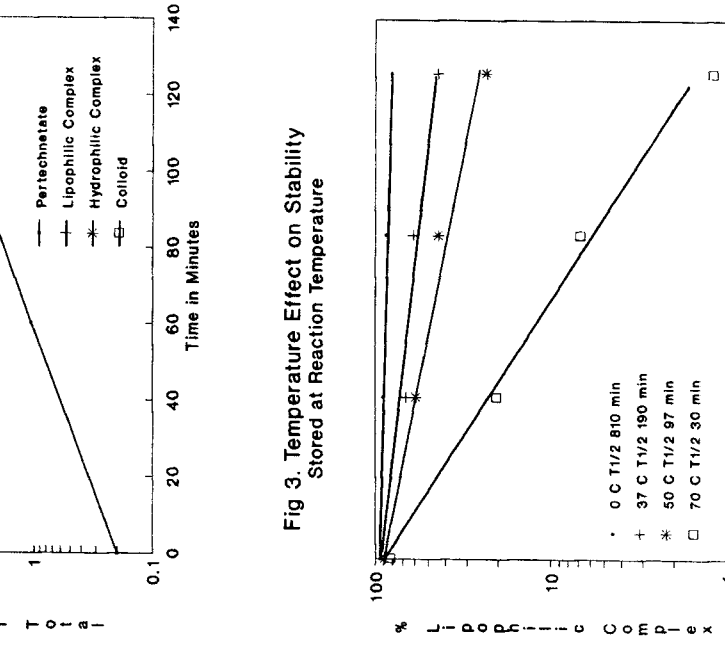
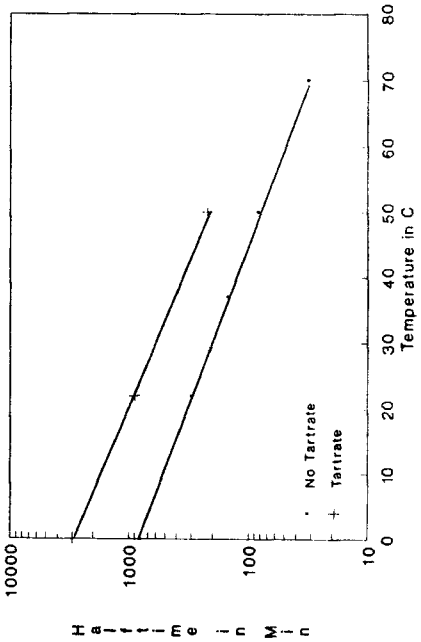


Fig 4. Temperature Effect on Stability
Decomposition Halftime



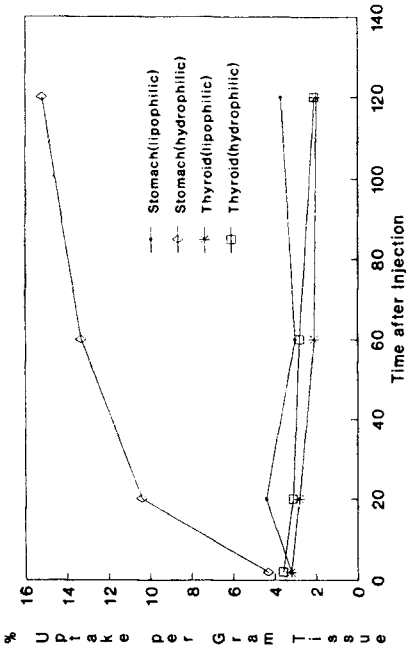


Fig 6. Tc-99m HMPAO Hydrophilic Complex
Biodistribution
Stomach and Thyroid

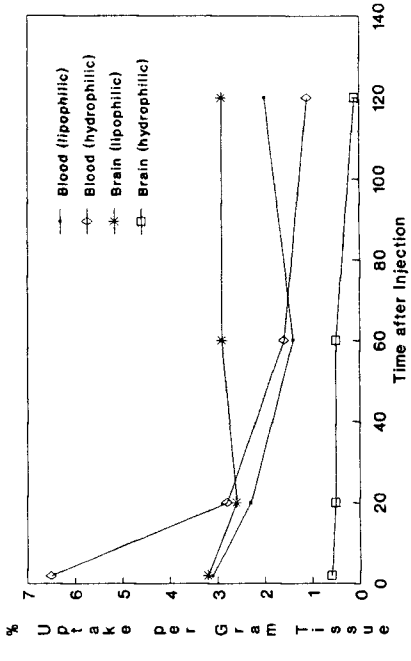


Fig 5. Tc-99m HMPAO Hydrophilic Complex
Biodistribution
Blood and Brain