

DOE-FG02-87ER60526

DOE/ER/60526--002

A NEW APPROACH TO THE ANALYSIS OF RADIOPHARMACEUTICALS

FINAL TECHNICAL REPORT

for the period January 15, 1987 – June 30, 1991

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March 1998

Prepared for  
The U. S. Department of Energy  
Agreement No. DOE-FG02-87ER60526

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## ABSTRACT

The objective of this research was to investigate analytical techniques that could be used in the study of both the basic chemistry and the radiopharmaceutical chemistry of the transition metal technetium. This element, in the form of its shortlived gamma-emitting radionuclide  $^{99m}\text{Tc}$ , has more than any other isotope contributed to the expansion of clinical nuclear medicine in the past twenty years. The work done under this funding was intended to provide a complementary investigation to a broad program of chemistry that has been continuing in Dr. Jones's and Dr. Davison's laboratories since 1975.

First funded in 1981, the work focused initially upon the use of high performance liquid chromatography (HPLC) and various forms of mass spectrometry for the identification of technetium species. In 1978 this group was the first in the world to employ mass spectrometry for the characterization of coordination complexes. Since that time the technique has become standard, not merely in radiopharmaceutical chemistry but, more important, in the field of inorganic chemistry as a whole. This funding allowed us to combine HPLC and mass spectrometry to identify radiopharmaceuticals which, although in clinical use, had not previously been characterized. Much of this is documented in the second renewal submitted in 1986. At that time, however, we could not have predicted the extent to which mass spectrometry was in fact becoming part of the identification of inorganic complexes.

Other techniques that have been examined include resonance Raman spectroscopy and, more significantly,  $^{99}\text{Tc}$  nuclear magnetic resonance spectroscopy (NMR), with the latter not only being used in purely chemical experiments but also in biologic studies. In 1985 a grant to the Department of Chemistry at MIT from DOE allowed the purchase of an X-ray diffractometer and access to this instrument has enabled us to broaden the analytical base with routine structural determinations.

## PROJECT OUTPUT

Complete List of Publications Supported Completely or Partially by  
DE-FG02-87ER60526

### Publications

The following publications were cited as being in press in the 1986 renewal proposal.

#### 1985

- Jones AG, Kronauge JF, Davison A. Biological distribution and structure-function relationships of hexakisisonitrile technetium(+1) complexes. *J Nucl Med Allied Sci* 1985;29:200-1.
- Jones AG, Lister-James J, Costello CE, Davison A.  $\omega$ -Substituted fatty acids derived from neutral  $N_2S_2$ -oxotechnetium chelates. *J Nucl Med Allied Sci* 1985;29:201.

#### 1986

- Davison A, Pearlstein RM, Mabrouk PA, Jones AG, Morelock MM. Resonance Raman studies on technetium complexes. In: Nicolini M, Bandoli G, Mazzi U, eds. *Technetium in chemistry and nuclear medicine 2*. Verona: Cortina International, 1986:25-7.
- Carroll TR, Davison A, Jones AG. Ligand exchange in the synthesis of technetium complexes at C.A. and N.C.A. concentrations. In: Nicolini M, Bandoli G, Mazzi U, eds. *Technetium in chemistry and nuclear medicine 2*. Verona: Cortina International, 1986:169-70.
- English RJ, Jones AG, Davison A, Lister-James J, Campbell S, Holman BL. Imaging considerations for a technetium-99m myocardial perfusion agent. *J Nucl Med Technol* 1986;14:6-10.
- Holman BL, Campbell CA, Lister-James J, Jones AG, Davison A, Kloner RA. Effect of reperfusion and hyperemia on the myocardial distribution of technetium-99m t-butyl-isonitrile. *J Nucl Med* 1986;27:1172-7.
- Patterson GS, Davison A, Jones AG, Costello CE, Maleknia SD. Synthesis and characterization of tris( $\beta$ -diketonato)technetium(III) and -(IV) complexes. *Inorg Chim Acta* 1986;114:141-4.
- Campbell S, Holman BL, Kirshenbaum JM, Antman EM, Lister-James J, Davison A, Kozlowski J, English RJ, Jones AG. The scintigraphic evaluation of myocardial infarction and regional ventricular performance using technetium-99m hexakis(t-butylisonitrile) technetium(I) (TBI): a new myocardial imaging agent. *Eur J Nucl Med* 1986;12:219-25.
- Sia STB, Holman BL, McKusick K, Rigo P, Gillis F, Sporn V, Perez-Balino N, Mitta A, Vosberg H, Szabo Z, Schwartzkopff B, Moretti J-L, Davison A, Lister-James J, Jones A. The utilization of Tc-99m-TBI as a myocardial perfusion agent in exercise studies: comparison with Tl-201 thallous chloride and examination of its distribution in humans. *Eur J Nucl Med* 1986;12:333-6.

#### 1987

- Holman BL, Sporn V, Jones AG, Sia STB, Perez-Balino N, Davison A, Lister-James J, Kronauge JF, Mitta AEA, Camin LL, Campbell S, Williams SJ, Carpenter AT. Myocardial imaging

with technetium-99m CPI: initial experience in the human. *J Nucl Med* 1987;28:13-8.  
Davison A, DePamphilis BV, Jones AG, Franklin KJ, Lock CJL. Synthesis and characterization of complexes containing the bis(1,2-diolato)oxotechnetium(V) core. *Inorg Chim Acta* 1987;128:161-7.

The following publications were supported by this contract through the end of the last no-cost extension in 1991.

#### 1987

Sia STB, Holman BL, Campbell S, Lister-James J, English RJ, Kronauge JF, Davison A, Jones AG. The utilization of technetium-99m CPI as a myocardial perfusion imaging agent in exercise studies. *Clin Nucl Med* 1987;12:681-7.

#### 1988

de Vries N, Dewan JC, Jones AG, Davison A. Complexes of technetium(III) with sterically hindered arenethiolates and their interactions with small  $\pi$ -accepting molecules. *Inorg Chem* 1988;27:1574-80.

Bryson N, Dewan JC, Lister-James J, Jones AG, Davison A. Neutral technetium(V) complexes with amide-thiol-thioether chelating ligands. *Inorg Chem* 1988;27:2154-61.

Pearlstein RM, Lock CJL, Faggiani R, Costello CE, Zeng C-H, Jones AG, Davison A. Synthesis and characterization of technetium(V) complexes with amine, alcoholate, and chloride ligands. *Inorg Chem* 1988;27:2409-13.

Davison A, Kronauge JF, Jones AG, Pearlstein RM, Thornback JR.  $^{99}\text{Tc}$  NMR spectroscopy of technetium(I) phosphine and phosphite complexes. *Inorg Chem* 1988;27:3245-6.

#### 1989

Piwnicka-Worms D, Kronauge JF, Holman BL, Davison A, Jones AG. Comparative myocardial binding characteristics of hexakis (alkylisonitrile) technetium(I) complexes: effect of lipophilicity. *Invest Radiol* 1989;24:25-9.

Pearlstein RM, Davis WM, Jones AG, Davison A. Preparation and characterization of  $\text{TcCl}_3(\text{PPh}_3)_2(\text{MeCN})$  and its reactions with small  $\pi$ -accepting ligands. *Inorg Chem* 1989;28:3332-4.

de Vries N, Jones AG, Davison A. Technetium thiolate complexes as oxygen atom transfer catalysts. *Inorg Chem* 1989;28:3728-34.

Nicholson T, de Vries N, Davison A, Jones AG. Synthesis, spectroscopy and structural characterization of five-coordinate bis(aryldiazenido) technetium complexes and their protonation reactions. X-ray structure of  $[\text{TcCl}(\text{PPh}_3)_2(\text{NNC}_6\text{H}_4\text{Br})_2]$ . *Inorg Chem* 1989;28:3813-9.

Linder KE, Dewan JC, Davison A. Technetium bis( $\mu$ -oxo) dimers of 1,4,7-triazacyclononane- $N,N',N''$ -triacetate (TCTA). Synthesis and characterization of  $[(\text{TCTA})\text{Tc}(\mu\text{-O})_2(\text{TCTA})]^{n+}$  ( $n = 2,3$ ) and the crystal structure of  $\text{Ba}_2[(\text{TCTA})\text{Tc}(\mu\text{-O})_2\text{Tc}(\text{TCTA})](\text{ClO}_4) \cdot 9\text{H}_2\text{O}$ . *Inorg Chem* 1989;28:3820-5.

Bryson NJ, Brenner D, Lister-James J, Jones AG, Dewan JC, Davison A. Synthesis and molecular structure of a "lantern" dimer,  $(\text{AsPh}_4)_2[\text{Tc}_2\text{O}_2(\text{SCH}_2\text{CONHCH}_2\text{CH}_2\text{NHCO}-\text{CH}_2\text{S})_4]$ . *Inorg Chem* 1989;28:3825-8.

O'Connell LA, Pearlstein RM, Davison A, Thornback JR, Kronauge JF, Jones AG. Technetium-99m NMR spectroscopy: chemical shift trends and long range coupling effects. *Inorg Chim Acta* 1989;161:39-43.

## 1990

Nicholson T, Thornback JR, O'Connell L, Morgan G, Davison A, Jones AG. Synthesis, spectroscopy, and structural characterization of neutral seven-coordinate technetium xanthate complexes: X-ray structure of  $[\text{Tc}(\text{PPh}_3)(\text{S}_2\text{COC}_4\text{H}_9)_3]$ , a capped octahedral technetium(III) complex. *Inorg Chem* 1990;29:89-92.

Breikss AI, Nicholson T, Jones AG, Davison A. Synthesis and characterization of technetium(III) and technetium(II) complexes with mixed phosphine-, chloride-, and nitrogen-donor ligands. X-ray crystal structure of  $\text{TcCl}_3(\text{PPh}_3)(\text{bpy})$ . *Inorg Chem* 1990; 29:640-5.

de Vries N, Cook J, Davison A, Nicholson T, Jones AG. Synthesis and characterization of a technetium(III) nitrosyl compound:  $\text{Tc}(\text{NO})(\text{Cl})(\text{SC}_{10}\text{H}_{13})_3$ . *Inorg Chem* 1990;29:1062-4.

Nicholson T, Davison A, Jones AG. The characterization of technetium organohydrazide chelate complexes. The synthesis of a technetium phenylimido complex. The X-ray crystal structure of  $[\text{TcO}(\text{SC}_6\text{H}_2\text{Pr}^i)_2(\text{PhNNCON}_2\text{HPh})]$ . *Inorg Chim Acta* 1990;168:227-31.

de Vries N, Costello CE, Jones AG, Davison A. Technetium nitrido complexes with amine and thiolate ligands: structural characterization of  $\text{TcN}(\text{SC}_6\text{HMe}_4)_2(\text{NHC}(\text{NMe}_2)_2)_2$ , a complex with coordinatively bound 1,1,2,2-tetramethylguanidine. *Inorg Chem* 1990; 29:1348-52.

Breikss AI, Davison A, Jones AG. Low oxidation state chemistry of technetium with nitrogen donor ligands. In: Nicolini M, Bandoli G, Mazzi U, eds. *Technetium and rhenium in chemistry and nuclear medicine 3*. Verona: Cortina International, 1990:85-93.

Nicholson T, de Vries N, Davison A, Jones AG. Synthesis and characterization of aryldiazenido technetium complexes and their protonation reactions. The X-ray structure of  $[\text{TcCl}(\text{PPh}_3)_2(\text{NNC}_6\text{H}_4\text{Br})_2]$ . In: Nicolini M, Bandoli G, Mazzi U, eds. *Technetium and rhenium in chemistry and nuclear medicine 3*. Verona: Cortina International, 1990:95-108.

Nicholson T, Thornback JR, O'Connell L, Davison A, Morgan GF, Jones AG. Synthesis and characterization of seven coordinate technetium(III) xanthate complexes. The X-ray structure of  $[\text{Tc}(\text{PPh}_3)(\text{S}_2\text{COC}_4\text{H}_9)_3]$ . In: Nicolini M, Bandoli G, Mazzi U, eds. *Technetium and rhenium in chemistry and nuclear medicine 3*. Verona: Cortina International, 1990:207-14.

de Vries N, Kronauge JF, Davison A, Jones AG. Compounds of technetium(III) with the "umbrella" ligand, tris(*o*-thiophenylato)phosphine. In: Nicolini M, Bandoli G, Mazzi U, eds. *Technetium and rhenium in chemistry and nuclear medicine 3*. Verona: Cortina International, 1990:215-9.

Kronauge JF, Kawamura M, Lepisto E, Holman BL, Davison A, Jones AG, Costello CE, Zeng C-H. Metabolic studies of the myocardial perfusion agent Tc-MIBI. In: Nicolini M, Bandoli G, Mazzi U, eds. *Technetium and rhenium in chemistry and nuclear medicine 3*. Verona: Cortina International, 1990:677-81.

- Breikss AI, Davison A, Jones AG. Ligand substitution reactions of tetrachlorobis(triphenylphosphine)technetium(IV). *Inorg Chim Acta* 1990;170:75-9.
- Bryson N, Lister-James J, Jones AG, Davis WM, Davison A. Protecting groups in the preparation of thiolate complexes of technetium. *Inorg Chem* 1990;29:2948-51.
- O'Connell LA, Dewan J, Jones AG, Davison A. Technetium(I) isocyanide complexes with bidentate aromatic amine ligands: structural characterization of  $[\text{Tc}(\text{CNtBu})_4(\text{bpy})]\text{PF}_6$ , a complex with "Tc(III) character". *Inorg Chem* 1990;29:3539-47.

## 1991

- Nicholson T, Mahmood A, Morgan G, Jones AG, Davison A. The synthesis and characterization of  $[\text{M}(\text{C}_8\text{H}_5\text{N}_2\text{N}=\text{NH})_3](\text{BPh}_4)$ , where M = Tc or Re. tris-Diazeno chelate complexes of technetium(I) and rhenium(I). *Inorg Chim Acta* 1991;179:53-7.
- de Vries N, Cook J, Jones AG, Davison A. Technetium(III) complexes with the tetradentate "umbrella" ligand tris(*o*-mercaptophenyl)phosphinate: X-ray structural characterization of  $\text{Tc}(\text{P}(\text{o}-\text{C}_6\text{H}_4\text{S})_3)(\text{CNC}_3\text{H}_7)$  and  $\text{Tc}(\text{P}(\text{o}-\text{C}_6\text{H}_4\text{S})_3)(\text{CNC}_3\text{H}_7)_2$ . *Inorg Chem* 1991;30:2662-5.
- Nicholson T, Davison A, Mahmood A, Morgan G, Jones AG. The synthesis and characterization of  $[\text{M}(\text{C}_8\text{H}_5\text{N}_2\text{N}=\text{NH})_3](\text{BPh}_4)$ , {where M = Tc or Re}: tris-diazeno chelate complexes of technetium(I) and rhenium(I). *J Labelled Compd Radiopharm* 1991;30:10-2.
- Kronauge JF, Davison A, Roseberry AM, Costello CE, Maleknia S, Jones AG. Synthesis and identification of the monocation  $\text{Tc}(\text{CPI})_6^+$  in  $\text{Tc}(\text{CNC}(\text{CH}_3)_2\text{COOCH}_3)_6\text{Cl}$  and its hydrolysis products. *Inorg Chem* 1991;30:4265-71.

## Patents

The following patents were filed on the basis of information partially gleaned using the techniques employed in the first phase of this funding. Although most were filed in the USA before this current renewal period, related foreign patents have continued to appear. All US filings have been previously reported to the agency.

Jones AG, Abrams MJ, Davison A. Isonitrile radionuclide complexes for labeling and imaging agents.

US Patent No. 4,452,774 Filed: 4/30/82 Issued: 6/5/84

Greek Patent No. 77,476 Issued: 5/27/83

Canadian Patent No. 1,218,666 Issued: 3/3/87

Italian Patent No. 1,168,943 Issued: 5/20/87

European Patent No. 0 107 734 Issued: 7/29/87

Irish Patent No. 55024 Filed 5/27/83 Issued: 4/25/90

Patents have issued in all European countries designated in the EPO application, including Austria, Belgium, France, W Germany, Luxembourg, The Netherlands, Sweden, 6/26/92.

Patents applied for: Australia



Assignees: The President and Fellows of Harvard College and the Massachusetts Institute of Technology.

Davison A, Brenner D, Lister-James J, Jones AG. Bisamide bisthiol compounds useful for making technetium radiodiagnostic renal agents.

US Patent No. 4,673,562 Filed: 4/19/83 Issued: 6/16/87  
European Patent No. 0 135 160 Filed: 8/17/84 Issued: 4/26/89  
Canadian Patent No. 1,268,596 Filed: 8/2/84 Issued 5/1/90  
Greek Patent No. 80069 Issued: 8/9/84

Patents applied for in the countries designated in first citation above.

Assignees: The President and Fellows of Harvard College, the Massachusetts Institute of Technology, and Children's Hospital Corporation.

Jones AG, Davison A, Abrams MJ. Metal-isonitrile adducts for preparing radionuclide complexes for labeling and imaging agents.

US Patent No. 4,707,544 Filed: 11/28/84 Issued: 11/17/87  
Divisional application filed 2/22/87  
Canadian Patent No. 1,249,998 Filed: 10/18/85 Issued: 2/14/89  
European Patent No. 0 183 555 Filed: 11/28/85 Issued: 1/22/92

Patents applied for in the countries designated in first citation above.

Assignees: The President and Fellows of Harvard College and the Massachusetts Institute of Technology.

Jones AG, Lister-James J, Davison A. Technetium radiodiagnostic fatty acids derived from bisamide bisthiol ligands.

US Patent No. 4,746,505 Filed: 4/26/85 Issued: 5/24/88  
Divisional application filed 2/24/88  
European Patent No. 0 200 492 Filed: 4/25/86 Issued: 2/8/89  
Canadian Patent No. 1,273,950 Filed: 4/26/85 Issued: 9/11/90

Patents applied for in the countries designated in first citation above.

Assignees: The President and Fellows of Harvard College, the Massachusetts Institute of Technology, and Children's Hospital Corporation.

Jones AG, Davison A, Kronauge JF, Abrams MJ. Carboxy, carboalkoxy, and carbamile substituted isonitrile radionuclide complexes.

US Patent No. 4,735,793 Filed: 8/30/85 Issued: 4/5/88

Canadian Patent No. 1,254,901 Filed: 8/29/86 Issued: 5/30/89

Greek Patent No. 86,2179 Issued: 8/22/86

Australian Patent No. 587637 Filed: 8/27/86 Issued: 8/24/89

European Patent No. 0 213 945 Filed: 8/29/86 Issued: 9/26/90

Patents applied for in the countries designated in first citation above.

Assignees: The President and Fellows of Harvard College and the Massachusetts Institute of Technology.

Jones AG, Davison A, Abrams MJ. Method for preparing radiopharmaceutical complexes.

US Patent No. 4,826,961. Filed: 1/22/87 Issued: 5/2/89

Divisional of US Patent No. 4,707,544.

Patents applied for in the countries designated in first citation above.

Assignees: The President and Fellows of Harvard College and the Massachusetts Institute of Technology.

Jones AG, Davison A, Kronauge JF, Abrams MJ. Carboxy, carboalkoxy, and carbamile substituted isonitrile radionuclide complexes.

US Patent No. 4,872,561. Filed: 12/29/87 Issued: 10/10/89

Divisional of US Patent No. 4,735,793.

Patents applied for in the countries designated in first citation above.

Assignees: The President and Fellows of Harvard College and the Massachusetts Institute of Technology.

## TRAINING ACTIVITIES

The analytical techniques supported by this funding were used in part in the following doctoral theses.

1. Kronauge JF. Functionalized isonitrile complexes of technetium as radiopharmaceuticals (Ph.D. diss, Massachusetts Institute of Technology, 1987).
2. Pearlstein RM. Applications of ligand exchange reactions of technetium complexes (Ph.D. diss, Massachusetts Institute of Technology, 1988).
3. De Vries NHC. Synthesis and study of complexes of technetium with sterically hindered arene thiolates (Ph.D. diss, Massachusetts Institute of Technology, 1988).
4. O'Connell LA. Investigations of the chemistry of technetium isonitrile compounds (Ph.D. diss, Massachusetts Institute of Technology, 1989).
5. Breikss AI. Low oxidation state technetium complexes with nitrogen-donor ligands (Ph.D. diss, Massachusetts Institute of Technology, 1989).

The following master's thesis was supported by this grant:

1. Roseberry AM. Investigation of the synthesis and reactivity of technetium nitrosyl complexes (Master's thesis, Massachusetts Institute of Technology, 1990).

The following postdoctoral personnel, junior faculty and visiting faculty used mass spectrometry,  $^{99}\text{Tc}$  NMR and X-ray diffraction facilities.

James F. Kronauge, Ph.D.	1983-1987	Graduate student, MIT
	1987-1992	Instructor in Radiology, HMS
	1992-	Assistant Professor in Radiology, HMS
Nadine de Vries, Ph.D.	1984-1988	Graduate Student, MIT
	1988-1989	Postdoctoral Associate, MIT
		Currently Group Leader, Central Research and Development, E.I. Du Pont de Nemours and Company
Lynne A. O'Connell, Ph.D.	1985-1989	Graduate Student, MIT
	1989-1990	Postdoctoral Associate, MIT
		Currently Undergraduate Laboratory Director, Department of Chemistry, Boston College
Gillian Morgan, Ph.D.	1987-1988	Postdoctoral Associate Currently Clinical Trial Specialist, Gilford Pharmaceuticals, Baltimore

John R. Thornback, Ph.D.	1987-1988	Postdoctoral Associate Currently General Manager, Resolution Pharmaceuticals, Toronto, Canada
Mieko Kawamura, Ph.D.	1987-1989	Sabbatical Visitor, Assistant Professor, Department of Nutrition, Tohoku University, Japan
Terrence L. Nicholson, Ph.D.	1987-1989 1989-	Research Fellow in Radiology, HMS Instructor in Radiology, HMS
Yasushi Arano, Ph.D.	1989-1990	Sabbatical Visitor, Associate Professor of Pharmaceutical Sciences, Kyoto University, Japan
Ashfaq Mahmood, Ph.D.	1989-1991 1991-	Postdoctoral Associate, HMS Instructor in Radiology, HMS
Eva Barbarics, Ph.D.	1990-	Research Fellow in Radiology, HMS also Research Staff, National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary

## FINAL REPORT

The objective of this research was to investigate analytical techniques that could be used in the study of both the basic chemistry and the radiopharmaceutical chemistry of the transition metal technetium. This element, in the form of its shortlived gamma-emitting radionuclide  $^{99m}\text{Tc}$ , has more than any other isotope contributed to the expansion of clinical nuclear medicine in the past twenty years. The work done under this funding was intended to provide a complementary investigation to a broad program of chemistry that has been continuing in Dr. Jones's and Dr. Davison's laboratories since 1975.

First funded in 1981, the work focused initially upon the use of high performance liquid chromatography (HPLC) and various forms of mass spectrometry for the identification of technetium species. In 1978 this group was the first in the world to employ mass spectrometry for the characterization of coordination complexes. Since that time the technique has become standard, not merely in radiopharmaceutical chemistry but, more important, in the field of inorganic chemistry as a whole. This funding allowed us to combine HPLC and mass spectrometry to identify radiopharmaceuticals which, although in clinical use, had not previously been characterized. Other techniques that have been examined include resonance Raman spectroscopy and, more significantly,  $^{99}\text{Tc}$  nuclear magnetic resonance spectroscopy (NMR), with the latter not only being used in purely chemical experiments but also in biologic studies. In 1985 a grant to the Department of Chemistry at MIT from DOE allowed the purchase of an X-ray diffractometer and access to this instrument has enabled us to broaden the analytical base with routine structural determinations.

The following results indicate the usefulness of this multifaceted approach in extending the understanding of the basic chemistry of technetium and its complex formation and in applying this knowledge to the production of technetium-labeled radiopharmaceuticals.

**The utilization of technetium-99m CPI as a myocardial perfusion imaging agent in exercise studies.** In 1982 Jones and Davison developed a new family of hexakisisonitrile technetium analogs. Carbomethoxyisopropylisonitrile (CPI) was synthesized as a technetium-labeled tracer of myocardial perfusion in an attempt to overcome some of the shortcomings of *t*-butylisonitrile, the first of this family to be successfully applied in humans.

**Synthesis and characterization of neutral technetium(V) complexes with amide-thiol-thioether chelating ligands.** The coordination chemistry of amide and thiolate ligands has potential in the preparation of new classes of chelate complexes of technetium that can direct the biodistribution of the radiotracer. We have modified the chelate *N,N'*-ethylenebis(2-mercaptoacetamide) ( $\text{H}_4\text{ema}$ ) to give a series of trianionic mono-S-alkylated derivatives,  $\text{H}_3\text{emaR}$ . These ligands react to form the five-coordinate neutral oxotechnetium(V) complexes  $[\text{TcO}(\text{emaR})]$ ; when  $\text{R} = \text{CH}_2\text{CH}_2\text{NR}_2$ , these complexes react further generating the thio-ether-dealkylated product  $\text{TcO}(\text{ema})$ . The compounds have been characterized by IR,  $^1\text{H}$  and  $^{13}\text{C}$  NMR, UV-vis and mass spectra, and X-ray diffraction. The use of FAB+ mass spectrometry indicates strong molecular ions and ions at double mass  $\text{M}_2\text{H}^+$ , interpreted as due to association of a cation-neutral pair and not to dimeric impurities. Similar observations had been made by others for neutral complexes of other metals. Fragmentation of the molecular ion is seen for  $[\text{TcO}(\text{ema}(\text{undec}))]$  and  $\text{TcO}(\text{ema}(\text{Bzl}))$  and peaks corresponding to loss of side chain, R, have been identified.

**Synthesis and characterization of technetium(V) complexes with amine, alcoholate, and chloride ligands.** Technetium complexes with aromatic amine, chloride, and alkoxide ligands have been prepared. Ligand substitution reactions of  $[\text{TcOCl}_4]^-$  with pyridine (py) and  $[\text{TcO}_2(\text{py})_4]^+$  with chloride ion yield the same products,  $\text{TcOCl}_2(\text{py})_2(\text{OR})$ . The alkoxide ligand can be formed by deprotonation of either MeOH or EtOH under acidic conditions. In an extension of this reaction  $\text{TcOCl}(\text{C}_2\text{H}_4\text{O}_2)(\text{C}_{12}\text{H}_8\text{N}_2)$  is prepared from  $(n\text{-Bu}_4\text{N})[\text{TcOCl}_4]$  with 1,2-ethanediol (eg) and 1,10-phenanthroline (phen) in methanol. This complex has been characterized by IR, UV-vis, and  $^1\text{H}$  and  $^{99}\text{Tc}$  NMR spectroscopies and by X-ray structure determination. The FAB+ mass spectrometry of the diolate species dissolved in nitrobenzyl alcohol indicates some hydrolysis with the low-abundance peak at  $m/z$  726 ( $\text{Tc}_2\text{O}_3(\text{eg})_2(\text{phen})_2^+$ ) corresponding to hydrolysis of both chloride ligands (product could not be isolated), and the other ion in this mass region with  $m/z$  745 ( $\text{Tc}_2\text{ClO}_2(\text{eg})_2(\text{phen})_2^+$ ) possibly corresponding to a cluster of two molecules with one of the chlorine atoms dissociated. Fragmentation patterns in the molecular ion region of the mass spectrum show that the mass of the most abundant ion in this region corresponds to that for loss of the chloride ligand from the parent which is expected considering the exceptionally long Tc-Cl bond length. A fragment of mass 327 ( $\text{TcO}_3(\text{phen})^+$ ) corresponds to a loss of chloride and ethylene from the molecule and is reminiscent of the known Tc(VII) species  $\text{TcO}_3\text{Cl}(\text{phen})$ .

**$^{99}\text{Tc}$  NMR spectroscopy of technetium(I) phosphine and phosphite complexes.** Studies using  $^{99}\text{Tc}$  NMR spectroscopy are reported which correct the data published by others for resonance of  $[\text{Tc}(1,2\text{-bis}(\text{dimethylphosphino})\text{ethane})_3]^+$  and  $[\text{Tc}(\text{P}(\text{OMe})_3)_6]^+$ .

**Preparation and characterization of  $\text{TcCl}_3(\text{PPh}_3)_2(\text{MeCN})$  and its reactions with small  $\pi$ -accepting ligands.** An improved route has been developed for making  $\text{TcCl}_3(\text{PPh}_3)_2(\text{MeCN})$  to be used as a synthetic agent in  $^{99\text{m}}\text{Tc}$ -phosphine complex production. The compound reacts with NO and CO to give  $\text{TcCl}_3(\text{PPh}_3)_2(\text{NO})$  and  $\text{TcCl}_3(\text{PPh}_3)_2(\text{CO})$ , respectively. The intermediate and the products have been characterized by IR,  $^1\text{H}$  NMR, UV-vis, and FAB+ mass spectroscopies and the X-ray structure determination of  $\text{TcCl}_3(\text{PPh}_3)_2(\text{CO})$  has been obtained.

**Technetium thiolate complexes as oxygen atom transfer catalysts.** The chemistry of oxygen atom transfer reactions of technetium, largely unknown, is explored, since oxotechnetium complexes are important in diagnostic radiopharmaceuticals. The Tc(III) compounds  $\text{Tc}(\text{tmbt})_3(\text{MeCN})_2$  and  $\text{Tc}(\text{tmbt})_3(\text{py})_2$ , incorporating the sterically hindered ligand 2,3,5,6-tetramethylbenzenethiolate (tmbt), can be oxidized to Tc(V) oxo species by oxygen atom transfer. The initial product of the reaction of  $\text{Tc}(\text{tmbt})_3(\text{MeCN})_2$  with DMSO, unstable in solution, has been characterized by FAB+ mass spectrometry and X-ray crystallography and determined to be the result of axial ligand substitution rather than an oxo atom transfer reaction. However,  $\text{Tc}(\text{tmbt})_3(\text{MeCN})_2$ , treated with pyridine *N*-oxide, forms  $\text{Tc}(\text{tmbt})_3(\text{py})$ , a stable Tc(V) oxo compound.  $\text{Tc}(\text{tmbt})_3(\text{py})_2$  reacts with several oxygen atom transfer reagents to produce  $\text{Tc}(\text{tmbt})_3(\text{py})$ . The Tc(V) oxo complex can be reduced by phosphines via oxygen abstraction reactions to produce the Tc(III) product. The oxidative and reductive oxo-transfer reactions can be coupled to provide a catalytic cycle. All stable intermediates have been characterized by IR,  $^1\text{H}$  and  $^{31}\text{P}$  NMR, UV-vis, and FAB+ mass spectroscopies.

**Synthesis and molecular structure of a 'lantern' dimer,  $(\text{AsPh}_4)_2[\text{Tc}_2\text{O}_2(\text{SCH}_2\text{CONHCH}_2\text{CH}_2\text{NHCOCH}_2\text{S})_4]$ .**  $(\text{AsPh}_4)_2[\text{Tc}_2\text{O}_2(\text{SCH}_2\text{CONHCH}_2\text{CH}_2\text{NHCOCH}_2\text{S})_4]$  has been synthesized and characterized as part of an ongoing exploration of the coordination complexes of Tc(V) with amide-thiol chelating ligands.  $(\text{Bu}_4\text{N})[\text{TcOCl}_4]$  has been reacted with a fivefold excess

of *N,N'*-ethylenebis(2-mercaptoacetarnide) ( $H_4ema$ ) in methanolic sodium methoxide to give a blue precipitate which has been metathesized to give the title compound which has been characterized by IR,  $^1H$  NMR, UV-vis, and FAB- mass spectroscopies and the structure determined by single-crystal X-ray crystallography. The compound can be converted to  $(AsPh_4)[TcO(ema)]$  when heated in solution or reacted with aqueous base.  $(AsPh_4)_2[Tc_2O_2(SCH_2CONHCH_2CH_2NHCOCH_2S)_4]$  and its conversion to  $(AsPh_4)[TcO(ema)]$  may have some consequences for the design and coordination chemistry of thiol-containing ligands with technetium.

**$^{99}Tc$  NMR spectroscopy: chemical shift trends and long range coupling effects.** To extend the basic knowledge of technetium coordination chemistry and provide information useful in designing radiopharmaceuticals,  $^{99}Tc$  NMR spectroscopy has been performed on a number of technetium complexes. Values for the chemical shifts and line widths of a variety of compounds in oxidation states V, III and I have been obtained and correlations between the shift and oxidation state, ligand field strength and shielding effects for these, and other previously measured compounds, have been made. The relatively large chemical shift differences for the various hexakis(alkylisonitrile) cations suggest that  $^{99}Tc$  NMR spectroscopy is a useful tool in studying the metabolic activity of this class of radiopharmaceuticals. The long range technetium-proton coupling observed in the methylisonitrile compound suggests the potential of this analytic technique in illuminating the mode of technetium coordination to biologically relevant molecules.

**Synthesis, spectroscopy, and structural characterization of neutral seven-coordinate technetium xanthate complexes: X-ray structure of  $[Tc(PPh_3)(S_2COC_4H_9)_3]$ , a capped octahedral technetium(III) complex.** A series of neutral seven-coordinate technetium xanthate complexes have been prepared by the reaction of  $(Bu_4N)[TcOCl_4]$  with triphenylphosphine and an excess of potassium alkyl xanthate to yield  $[Tc(PPh_3)(S_2COR)_3]$  with  $R$  = ethyl, isopropyl, *n*-butyl, neopentyl. The complexes have been characterized by IR,  $^1H$  and  $^{99}Tc$  NMR, UV-vis and FAB+ mass spectroscopies, with single-crystal X-ray structural determination of the *n*-butyl xanthate complex. These compounds possess the capacity for variation at both the axial ligand site and the xanthate terminus, thus allowing manipulation of their lipophilicity, an essential determinant in modifying the biodistribution of radiopharmaceuticals.

**The characterization of technetium organohydrazide chelate complexes. The synthesis of a technetium phenylimido complex.** The X-ray crystal structure of  $[TcO(SC_6H_2Pr^i)_2(PhNNCON_2HPh)]$ . Complexes that incorporate the organimido core ( $Tc=N-R$ ), a species isoelectronic to the oxo species, have been synthesized and are reported for the first time. It is hoped that the organic moiety on the imido unit might provide a method of altering the properties of the complex, thus permitting manipulation of the compound's biodistribution. The  $Tc(V)$  oxo complex  $(Bu_4N)[TcOCl_4]$  is reacted with (phenylazo)formic acid 2-phenylhydrazide and 1,5-diphenylcarbazine to yield the phenylimido complex  $[TcCl(NPh)(PhN_2CON_2HPh)_2]$  and the  $Tc(V)$  oxo *tris*(thiolate) complex  $[TcO(SC_6H_2Pr^i)_3(C_5H_5N)]$  is reacted with (phenylazo)formic acid 2-phenylhydrazide in methanol to give  $[TcO(SC_6H_2Pr^i)_2(PhNNCON_2HPh)]$ . The compounds have been characterized by IR and UV-vis spectroscopies. FAB+ mass spectroscopy demonstrates protonated parent ions for both complexes, and decomposition profiles indicate the generation of a phenylimido-containing species for each chelated organohydrazide ligand present in the parent species.

**Technetium nitrido complexes with amine and thiolate ligands: structural characterization of  $TcN(SC_6HMe_4)_2(NHC(NMe_2)_2)_2$ , a complex with coordinatively bound**

**1,1,2,2-tetramethylguanidine.** The nitrido and oxo groups are isoelectronic. Extending the chemistry of oxoTc(V) complexes with sterically hindered arenethiolate ligands, a methanol solution of the Tc(VI) complex  $(\text{Ph}_4\text{As}[\text{TcNCl}_4])$  is reduced in the presence of pyridine to give the Tc(V) complex  $[\text{TcN}(\text{OH})(\text{py})_4](\text{Ph}_4\text{B})$ . As with the oxo analog  $[\text{TcO}_2(\text{py})_4](\text{Ph}_4\text{B})$ , the pyridine ligands are labile and undergo exchange with free pyridine in solution. The  $\text{TcO}_2^+$  and  $\text{TcN}(\text{OH})^+$  cores appear to be electronically similar since the  $^{99}\text{Tc}$  NMR chemical shifts for these two compounds are not significantly different.  $[\text{TcN}(\text{OH})(\text{py})_4](\text{Ph}_4\text{B})$  reacts with 2,3,5,6-tetramethylbenzenethiolate (tmbt) to give *trans*- $\text{TcN}(\text{tmbt})_2(\text{py})_2$ . In an attempt to prepare  $[\text{TcN}(\text{tmbt})_4]^-$ ,  $[\text{TcNCl}_4]^+$  has been reacted with Htmibt in the presence of 1,1,3,3-tetramethylguanidine (TMG). Instead of the desired tetrathiolate complex,  $\text{TcN}(\text{tmbt})_2(\text{TMG})_2$ , a rare example of TMG coordination, is obtained. The compound shows dimer formation in FAB spectra, and the decomposition of the species has been studied by tandem mass spectrometry. The structure of  $\text{TcN}(\text{tmbt})_2(\text{TMG})_2$  has been confirmed by single-crystal X-ray structure determination.

**Synthesis and characterization of aryldiazenido technetium complexes and their protonation reactions.** The X-ray structure of  $[\text{TcCl}(\text{PPh}_3)_2(\text{NNC}_6\text{H}_4\text{Br})_2]$ . Technetium complexes incorporating the aryldiazenido ligands ( $\text{M}-\text{N}=\text{NR}$ ), which are isoelectronic to the nitrosyl ligand and, in addition, can be easily functionalized, have been prepared. These ligands with  $\text{sp}^2$  hybridization on the  $\beta$ -nitrogen may display a number of structural conformations. Both the  $\alpha$ - and  $\beta$ -nitrogen in the aryldiazenido moiety are basic and subject to protic attack. Five aryldiazenido technetium complexes have been synthesized and characterized by IR,  $^1\text{H}$  NMR and FAB+ mass spectroscopies and electronic measurements. The single-crystal X-ray structure of  $[\text{TcCl}(\text{PPh}_3)_2(\text{NNC}_6\text{H}_4\text{Br})_2]$ , prepared by reaction of  $[\text{TcCl}_4(\text{PPh}_3)_2]$  and an excess of *p*-bromophenylhydrazine and diisopropylethylamine in methanol, has been determined.

**Metabolic studies of the myocardial perfusion agent Tc-MIBI.** To provide successful myocardial imaging, the perfusion agent must not only have good uptake and retention in normal heart muscle, but also rapid clearance from nontarget tissue. To achieve rapid blood clearance an agent must be rapidly extracted by either the kidneys or liver. One approach is to select functional groups for the agent that promote hepatobiliary clearance. Toward this end, the technetium-containing species found in the gall bladder and liver contents of guinea pigs, rats, and mice injected with  $^{99\text{m}}\text{Tc}(2\text{-methoxy-isobutylisocyanide})\text{Cl}$  and  $^{99}\text{Tc}(2\text{-methoxy-isobutylisocyanide})_6\text{Cl}$  have been analyzed using an RP-HPLC system. Radiometric detection of the RP-HPLC analysis of guinea pig bile from samples taken 15 min after injection of  $^{99\text{m}}\text{Tc}$ -MIBI indicates the presence of seven new technetium-containing species of increasing hydrophilicity in addition to the parent radiopharmaceutical. The consistent decrease in abundance of the more hydrophilic peaks is in line with sequential altering of the six identical isonitrile ligand isomers. The integral ratios for sequential peaks approach the theoretical values predicted for random sequential alteration of an octahedral complex. After injection of a mixture of  $^{99\text{m}}\text{Tc}$ -MIBI and  $^{99}\text{Tc}$ -MIBI, 78% of the parent radiopharmaceutical cation is found in the bile compared with 34% in that of the  $^{99\text{m}}\text{Tc}$ -MIBI injected animals. It appears that two processes of excretion are present and that one is saturated at higher concentrations of the substrate. FAB+ mass spectroscopy shows the  $m/z = 777$  peak characteristic of the parent radiopharmaceutical. The percentage of catabolites is too low to allow their assessment. Species differences have been observed in the mouse and rat samples.

**Protecting groups in the preparation of thiolate complexes of technetium.** We have found that the thiol-protecting groups S-benzamidomethyl, S-acetamidomethyl and S-benzyl, which have been used to prepare polyfunctional ligands containing amide and thiolate functionalities, may



not need to be removed prior to metal complexation. If intermediate or product complexes are reduced by thiols, the use of the S-protected ligand may be an advantage. The nitrogen-sulfur ligand (N-[2-((2-(((acetylamino)methyl)thio)acetyl)amino)ethyl]2-pyridinecarboxamide),  $H_2PIC(Acm)$ , reacts with  $(Bu_4N)[TcOCl_4]$  in ethanolic solution to yield the neutral complex [oxo(N-{2-(2-thioacetylido)ethyl}-2-pyridinecarboximido)technetium(V)],  $[TcO(PIC)]$ . The thiol-protected derivatives  $H_2PIC(Bzm)$  and  $H_2PIC(Bzl)$  similarly give  $[TcO(PIC)]$  in almost quantitative yield. In contrast, the unprotected  $H_3PIC$  with  $(Bu_4N)[TcOCl_4]$  in methanol produces a mixture of reduced technetium species and only traces of the neutral product. The compounds have been characterized by IR,  $^1H$  and  $^{13}C$  NMR, and UV-vis spectroscopies. In addition,  $[TcO(PIC)]$  has been characterized by FAB+ mass spectroscopy and its structure determined from X-ray diffraction data.

**Technetium(I) isocyanide complexes with bidentate aromatic amine ligands: structural characterization of  $[Tc(CNtBu)_4(bpy)]PF_6$ , a complex with "Tc(III) character".** The hexakis(isocyanide)technetium(I) monocations are potentially useful starting materials for the preparation of low-oxidation-state technetium compounds. A series of compounds of the type  $[Tc(CNR)_4(NN)]PF_6$  ( $NN$  = bidentate aromatic amine) is synthesized either by photolysis of  $[Tc(CNR)_6]PF_6$  in the presence of excess bidentate aromatic amine or by reduction of  $NH_4[TcO_4]$  in the presence of excess bidentate aromatic amine and isocyanide. A variety of bidentate aromatic amine ligands have been used while keeping the *t*-butyl isocyanide ligand constant to give compounds of the type  $Tc(CNtBu)_4(NN)$  ( $NN$  = *bpy*,  $Me_2bpy$ , *phen*,  $Me_4phen$ ,  $NO_2phen$ ). Similarly the isocyanide ligand has also been varied while the amine ligand remains constant as 2,2'-bipyridine to synthesize compounds of the type  $Tc(CNR)_4(bpy)^+$  ( $R$  = *t*Bu, Me, *m*Xyl).  $^1H$  and  $^{99}Tc$  NMR, UV-vis, and FAB+ mass spectroscopies and electrochemical measurements have been used for characterization of the compounds. A single-crystal X-ray structure determination has been made on  $[Tc(CNtBu)_4(bpy)]PF_6$ . The coordination sphere is an octahedron, and a deviation from linearity in one of the isonitrile ligands is present at the nitrogen atom. The structural features together with the infrared and  $^{99}Tc$  NMR spectral data suggest that the bent isocyanide is the result of a "pseudo" internal oxidation from Tc(I) to Tc(III). Protonation of the bent isocyanide ligand produces an unstable aminocarbyne complex which deprotonates to reform the starting material.

**Technetium(III) complexes with the tetradentate "umbrella" ligand tris(*o*-mercaptophenyl)phosphinate: X-ray structural characterization of  $Tc(P(o-C_6H_4S)_3)(CNC_3H_7)$  and  $Tc(P(o-C_6H_4S)_3)(CNC_3H_7)_2$ .** The ligand 2,3,5,6-tetramethylbenzenethiolate (*tmbt*) has been shown to stabilize technetium in the +3 oxidation state. Compounds such as  $Tc(tmbt)_3(MeCN)_2$  exhibit trigonal-bipyramidal geometry with three thiols in the equatorial plane and two  $\pi$ -accepting ligands in the axial positions. The acetonitrile ligands are labile and undergo substitution. We have now designed a chelating "umbrella" ligand, tris(*o*-mercaptophenyl)phosphinate (*PS3*) that provides technetium with three thiolate ligands and one of the axial  $\pi$ -accepting ligands while leaving the fifth coordination site open for ligand exchange. With this system the six-coordinate complex can be isolated, indicating that this is the intermediate involved in the ligand-exchange reactions of the trithiolate compounds. *PS3* binds to Tc(III) as a tetradentate ligand to form the formally 14-electron complex  $Tc(PS3)(CNMe)$ . A single-crystal X-ray structure determination of the isopropyl isocyanide derivative  $Tc(PS3)(CN-i-Pr)$  shows that the complex has a trigonal-bipyramidal geometry with phosphorus and isonitrile carbon in the axial positions and the sulfurs bound in the equatorial plane. In the presence of a large excess of isonitrile, this electron-deficient complex binds a sixth ligand. The six-coordinate complex  $Tc(PS3)(CN-i-Pr)_2$  has also been structurally characterized. IR,  $^1H$  and  $^{31}P$  NMR, UV-vis and FAB+ mass spectroscopies have been obtained where appropriate.

**Synthesis and identification of the monocation  $\text{Tc}(\text{CPI})_6^+$  in  $\text{Tc}(\text{CNC}(\text{CH}_3)_2\text{COOCH}_3)_6\text{Cl}$  and its hydrolysis products.** These data are part of an attempt using functionalized isocyanide ligands and their technetium complexes to produce a  $^{99\text{m}}\text{Tc}$ -labeled myocardial perfusion agent with good uptake and retention in normal myocardium and fast clearance from nontarget tissues. The strategy is to incorporate reactive organic functional groups on the ligands that might be recognized as substrates and metabolized in the nontarget organs. We have synthesized a technetium(I) hexakis(isonitrile) complex containing functionalized alkyl isocyanide ligands possessing a terminal methyl ester group.  $\text{Tc}(\text{CNC}(\text{CH}_3)_2\text{COOCH}_3)_6\text{Cl}$  (cation =  $\text{Tc}(\text{CPI})^+$ ) has been prepared from  $\text{TcO}_4^-$  by aqueous  $\text{Na}_2\text{S}_2\text{O}_4$  reduction in the presence of the functionalized isocyanide ligand. The compound has been hydrolyzed to  $\text{Tc}(\text{CNC}(\text{CH}_3)_2\text{COOH})_6$  and intermediate products in the sequential hydrolysis process have also been obtained. The nine predicted carboxylic-acid-containing species have been separated by RP-HPLC and characterized by IR,  $^{99}\text{Tc}$  MNR and FAB+ mass spectroscopies. In addition,  $\text{Tc}(\text{CPI})_6^+$  has been enzymatically hydrolyzed in the serum of humans, mice, and rats and the  $k'$  values from the RP-HPLC of the hydrolysis products used to identify the technetium-containing metabolites. Varying rates of in-vitro enzymatic hydrolysis at the terminal ester moieties of the coordinated ligands have been shown to occur in serum from the different animal species.

#### FINAL SUMMARY

This funding from the Department of Energy proved invaluable in the early development of mass spectrometry for applications in technetium radiopharmaceutical chemistry and in combining this technique with high performance liquid chromatography (HPLC). In the last phases of the contract, the usefulness of technetium-99 nuclear magnetic resonance spectroscopy ( $^{99}\text{Tc}$  NMR) for the characterization of particular classes of technetium and rhenium complexes was also explored. Towards the end of this cycle, attempts were made to design an appropriate renewal application incorporating mass spectrometry. However, the use of this technique for characterizing inorganic complexes had grown to such an extent that it proved difficult to generate a proposal sufficiently unique that the agency would be prepared to fund it. This widespread adoption of the technique could not have been predicted at the outset of the project.