Bis(Dithiocarbamato) Nitrido Technetium-99m Radidpharmaceuticals: A Class of Neutral Myocardial Imaging Agents

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The synthesis and biodistribution in various animal models (rat, dog, pig and monkey) of ^{99m}Tc radiopharmaceuticals containing the Tc≡N multiple bond are reported. **Methods:** The complexes are represented by the general formula $^{99m}TcN(L)_2$, where L is the monoanionic form of a dithiocarbamate ligand of the type $[R^1(R^2)\text{-}N\text{-}C(=S)S]^-$, and R^1 and R^2 are variable, lateral groups. The preparations were carried out, both as a liquid and freeze-dried formulation, through a simple procedure involving the initial reaction of $[^{99m}TcO_4]^-$ with S-methyl N-methyl dithiocarbazate $[H_2NN(CH_3)C(=S)SCH_3]$, in the presence of tertiary phosphines or Sn^{2+} ion as reductants, followed by the addition of the sodium salt of the ligand (NaL) to afford the final product. The chemical identity of the resulting complexes was determined by comparing their chromatographic properties with those of the corresponding ^{99}Tc analogs characterized by spectroscopic and x-ray crystallographic methods. The complexes are neutral and possess a distorted, square pyramidal geometry. **Results:** No decomposition of the complexes, in physiological solution, was observed over a period of 6 hr. Imaging and biodistribution studies demonstrated that these radiopharmaceuticals localize selectively in the myocardium of rats, dogs and primates, but that they failed to visualize the pig heart. The kinetics of heart uptake and clearance were studied in rats and dogs, and found to be strongly influenced by variation of the lateral R^1 and R^2 groups. **Conclusion:** The high quality of myocardial images obtained in dogs and monkeys demonstrates that the derivative $^{99m}TcN[Et(EtO)NCS_2]_2$ [^{99m}TcN(NOEt)] exhibits the most favorable distribution properties for further studies in humans.

Key Words: myocardial imaging agents; technetium-99m-bis(dithiocarbamato) nitrido compounds

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In recent years, many successful efforts have been devoted to the synthesis of technetium-99m radiopharmaceuticals containing the monoxo $[Tc=O]^{3+}$ and dioxo $[O=Tc=O]^+$ cores (*1*). These groupings of atoms constitute basic functional arrangements for technetium in the +5 oxidation state and, at present, nearly all mononuclear Tc(V) compounds prepared at tracer level are characterized by the presence of Tc=O or O=Tc=O multiple bonds. It is generally observed that the formation of these multiple bonds from $[TcO_4]^-$, in physiological conditions, is strongly dependent upon various factors such as the nature of the other ligands coordinated to the technetium center, the reducing agent and pH conditions. The careful optimization of these factors is required in order to obtain the final Tc(V) oxo complex. The $[Tc\equiv N]^{2+}$ core constitutes another characteristic functional moiety, in which the Tc^{+5} ion is multiply bonded to a nitride nitrogen atom (N^{3-}). The resulting arrangement of atoms exhibits a very high chemical stability towards both oxidation-reduction reactions involving the technetium ion and pH variations (*2,3*). This suggests that the synthesis of ^{99m}Tc radiopharmaceuticals containing the Tc≡N multiple bond would allow the facile variation of the other ancillary ligands coordinated to the metal center and hence make possible the fine tuning of the biological properties of the resulting compounds. Until now, however, no ^{99m}Tc radiopharmaceuticals containing the Tc≡N multiple bond and exhibiting useful imaging properties have been reported and this was probably due to the lack of a convenient synthesis of this group at tracer level (*4*).

We recently reported on an efficient method for the preparation of ^{99m}Tc radiopharmaceuticals containing the Tc≡N multiple bond under sterile and apyrogenic conditions (*5,6*). This method is based on the reaction of $[^{99m}TcO_4]^-$ with N-methyl S-methyl dithiocarbazate $[H_2N\text{-}N(CH_3)\text{-}C(=S)SCH_3]$ in acidic conditions and in the presence of triphenylphosphine. In this reaction, the species N-methyl S-methyl dithiocarbazate behaves as an efficient donor of nitride nitrogen atoms (N^{3-}) to yield the $[Tc\equiv N]^{2+}$ group. We observed that the formation of the $[Tc\equiv N]^{2+}$ group is independent upon both the choice of the reducing agent and pH conditions, and that it also takes place at neutral pH using $SnCl_2$ as reductant (*7*). These results demonstrate that the basic reaction of pertechnetate

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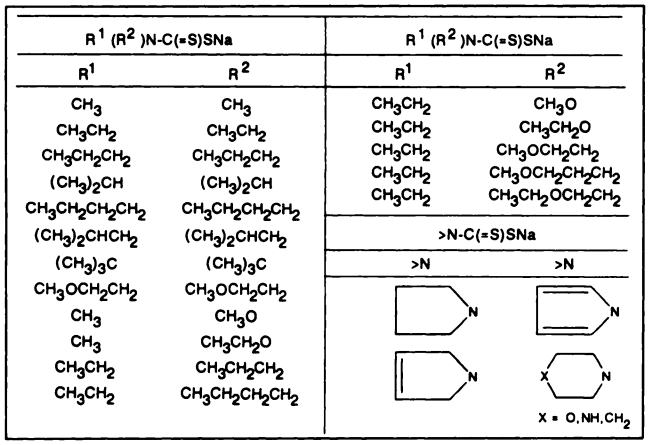

FIGURE 1. The dithiocarbamate ligands used in this study.

with N-methyl S-methyl dithiocarbazate is of general applicability and can be easily used to prepare ^{99m}Tc radiopharmaceuticals containing the $[Tc{\equiv}N]^{2+}$ group in a wide range of labeling conditions. Using this reaction, we prepared a series of neutral bis(dithiocarbamato) nitrido technetium(V) complexes of general formula $^{99m}TcN(L)_2$[L = $R^1(R^2)NCS_2$], where the pendant R^1 and R^2 groups on the >NC(=S)S moiety were varied to include different organic functional groups. In this paper, we report the synthesis and characterization of these complexes at tracer level and their biodistributions in various animal models (rats, dogs, pigs and monkeys). It was found that all the compounds localize selectively in myocardium tissue of rats and dogs, and that the washout from the heart is generally slow. High quality gamma camera images of myocardium were obtained in primates using the derivatives $^{99m}TcN[Et(EtO)NCS_2]_2$ and $^{99m}TcN[Et_2NCS_2]_2$. These radiopharmaceuticals, therefore, constitute the first, homogeneous class of neutral technetium-99m imaging agents showing long retention times in myocardium tissue. Preliminary accounts of this work have been communicated (*8–10*).

MATERIALS AND METHODS

Ligands

Tris(m-sulfophenyl)phosphine, $[P(m\text{-}C_6H_4SO_3)_3]Na_3$ (TPPS), was obtained as a gift from the laboratory of Dr. Dartiguenave (CNRS, Toulouse, France). N-methyl S-methyl dithiocarbazate $[H_2N\text{-}N(CH_3)\text{-}C(=S)SCH_3]$ was synthetized as reported previously (*10,11*). The sodium salts of the dithiocarbamate ligands {$[R^1(R^2)NCS_2]Na$ = NaL} used in the preparations are shown in Figure 1. Sodium dimethyldithiocarbamate and sodium diethyldithiocarbamate are commercially available. The other ligands were prepared by reacting the corresponding secondary amines with an equivalent amount of carbon disulfide in NaOH solutions. The following procedure, utilized for the preparation of the sodium salt of piperidine dithiocarbamate, is representative. Piperidine (8.5 g, 0.10 mole) was dissolved in 200 ml of dried diethyl ether and the resulting solution was cooled in an ice-salt bath. Sodium hydroxide (8.0 g, 0.20 mole) was added to this solution under stirring, followed by carbon disulfide (8.4 g, 0.11 mole). The mixture was stirred for 30 min in the ice-salt bath, then allowed to reach room temperature and stirred for an additional hour. A white precipitate formed which was filtered off, washed with diethyl ether and dried over P_2O_5. The crude product was recrystallized from isopropanol/diethyl ether to give white crystals of sodium piperidine dithiocarbamate (yield, 60%).

All the ligands were characterized by elemental analysis (C, H, N, S) and by ^{1}H and ^{13}C NMR, FT IR and mass spectra. Details of the synthesis of the ligands have been reported previously (*10*).

Preparation of ^{99m}Tc Complexes

Both liquid and freeze-dried formulations for the preparation of $^{99m}TcN(L)_2$ complexes have been developed and used for determining tissue distribution in rats, and for gamma camera imaging in pigs, dogs and monkeys, as well as early studies in human volunteers.

All manipulations were carried out using standard procedures to ensure sterile, pyrogen-free preparations. Unless otherwise noted, oxygen was excluded from vials and solutions by nitrogen filling and purging, respectively.

Preparation of $^{99m}TcN(L)_2$[L = $R^1(R^2)NCS_2$] Complexes

The preparations of ^{99m}Tc complexes were carried out using the following two procedures:

Method 1 (Liquid Formulation). One milliliter of saline containing $[^{99m}TcO_4]^-$ (activity ranging from 1.0 MBq to 1.0 GBq) was added to a vial containing 3.0 mg of TPPS and 1.0 mg of $H_2NN(CH_3)C(=S)SCH_3$ dissolved in 1.0 ml of HCl (0.10 mole dm^{-3}). The resulting solution was heated at 100°C for 15 min and then cooled to room temperature. The pH of the solution was raised to 8.0 by adding 1.0 ml of a sodium phosphate buffer (0.20 mole dm^{-3}) and 1.0 ml of a water solution containing 10 mg of the sodium salt of the appropriate ligand. The formation of the final compound occurred almost instantaneously at room temperature.

Method 2 (Lyophilized Formulation). One milliliter of saline containing $[^{99m}TcO_4]^-$ (activity ranging from 1.0 MBq to 1.0 GBq) was added to a vial containing 0.1 mg of $SnCl_2 \cdot 2H_2O$, 10 mg of 1,2-diaminopropane-N,N,N',N'-tetraacetic acid (DPTA) and 1.0 mg of $H_2NN(CH_3)C(=S)SCH_3$ in a freeze-dried form. The resulting solution was heated at 100°C for 15 min and then cooled to room temperature. One milliliter of a water solution containing 10 mg of the sodium salt of the appropriate ligand was then added and the reaction vial stood for 5 min at room temperature.

The radiochemical purity (RCP) of the products was evaluated by TLC chromatography and ranged between 93%–98% using both methods of preparation. Radioactivity profiles were quantitated using a procedure described elsewhere (*6*). These complexes each migrated as single radioactive peaks near the solvent front on silica gel plates eluted with toluene or CH_2Cl_2 ($[^{99m}TcO_4]^-$ at the origin), or near the origin on reversed-phase C18 plates eluted with methanol-acetonitrile-tetrahydrofuran-ammonium acetate (0.5 mole dm^{-3}) (3:3:2:2) ($[^{99m}TcO_4]^-$ at the solvent front). R_f values for some selected complexes are reported in Table 1. The chemical identity of all products prepared at tracer level was established by comparing their chromatographic behavior with that of the corresponding compounds prepared at the macroscopic level (see below).

The compound $^{99m}TcN(L)_2$ showed the same R_f values of the corresponding ^{99}Tc analogs. The logarithmic values of $k' = (t_R - t_0)/t_0$ for some representative complexes are given in Table 1. They have been measured as previously demonstrated (*12*) on a HPLC system equipped with a Spectra-Physics SP 8800 ternary pump. Injections were automatically performed using a Spectra-Physics autosampler with a 20-μl Rheodine injector. Elutions were run in the isocratic mode on a RP-18 Lichrosorb column

TABLE 1
Log k' and R_f Values Measured for Various $^{99m}TcN(L)_2$ Complexes

L	log k'	R_f*	R_f†
Me_2NCS_2	−0.030	0.66	0.82
Et_2NCS_2	0.23	0.52	0.88
$[CH_3(CH_2)_3]_2NCS_2$	1.14	0.15	0.94
$Me(MeO)NCS_2$	−0.020	0.65	0.84
$Et(MeO)NCS_2$	0.13	0.57	0.85
$Et(EtO)NCS_2$	0.28	0.48	0.89
$Et(CH_3OCH_2CH_2)NCS_2$	0.20	0.55	0.58
$(CH_3OCH_2CH_2)_2NCS_2$	0.17	0.57	0.22
$Et(C_2H_5OC_2H_4)NCS_2$	0.37	0.45	0.64
$Et(CH_3OC_3H_6)NCS_2$	0.24	0.54	0.32
$C_4H_8NCS_2$	0.21	0.56	0.84

*Reversed phase.
†Silica gel.

(Merck, mean 10 μm, 250 × 4 mm) using He-degassed MeOH/H_2O (75:15) mixtures. The detector system consisted of a ABI Kratos UV-Vis detector (Spectroflow 783) coupled to a 10-μl loop flow-through gamma detector (LB2040 Berthold Spectrometer, Wilbad, Germany). Output signals were analyzed by a Spectra-Physics SP4290 dual-channel integrator. The stability of the products was determined at room temperature by measuring RCP values at different times (1, 3 and 6 hr) after preparation. The stability of both liquid and freeze-dried formulations (stored at 4°C in the dark) were assayed over a period of 1 yr by oxidative titration of the $-C(=S)S^-$ functional group of the ligand [NaL, L = Et_2NCS_2, $Et(EtO)NCS_2$, $Me(MeO)NCS_2$, $C_4H_8NCS_2$] with I_2 in CH_3CN/H_2O (70:30) mixtures. End points were obtained on the corresponding dE/dV plots recorded with a (Ag/AgCl)/Pt combined electrode. The freeze-dried formulation was also monitored for loss of Sn^{2+}, over the same period of time, using procedures reported previously (*7*).

Preparation of ^{99}Tc Complexes

The complexes $^{99}TcN(L)_2$ were prepared by reacting the starting compound $^{99}TcNCl_2(PPh_3)_2$ (PPh_3 = triphenylphosphine) (*13,14*) (0.2 mmole) with an excess of the appropriate ligand (1:3) in chloroform/methanol (1:1) at room temperature for 1 hr. Evaporation of the solvent gave a yellow oily residue which, after dissolution in CH_3CN/ethanol (1:1), afforded yellow crystals of the final compound. The yields, based on ^{99}Tc, ranged between 60% and 85%. The products were characterized by elemental analysis, FT IR and electron impact mass spectra (see Appendix, Table 1A). The diagnostic $\nu(Tc{\equiv}N)$ stretching frequency was found in the usual range 1070–1085 cm^{-1}. The synthesis and characterization of the complex $^{99}TcN[Et(EtO)NCS_2]_2$ has been communicated previously (*9*). The characterization and x-ray crystal structure of the complex $^{99}TcN(Et_2NCS_2)_2$ has been reported elsewhere (*15*).

Biodistribution Studies

Anesthetized (diethyl ether) Wistar male rats (200–250 g) were injected in the pudendal vein with a solution of $^{99m}TcN(L)_2$ (0.10 ml, ca. 74 kBq). The animals were killed under anesthesia by cervical dislocation 5, 30 and 60 min postinjection and the organs of interest and blood were collected. All organs and fluids were assayed for radioactivity in a gamma counter (Packard, Model 500, Meridien, CT). The accumulated radioactivity in each organ and blood was expressed as a percentage of the injected dose.

Planar gamma camera images were taken in mongrel dogs weighing between 15 and 25 kg, large white pigs weighing between 60 and 80 kg and primates (Macaca Fascicularis) weighing between 3 and 6 kg. After 24-hr fasting, dogs were anesthetized with intravenous injection of thiopentane (induction dose 25 mg kg^{-1}, maintenance dose 5 mg kg^{-1} hr^{-1}). Intermittent ventilation (15 b.p.m.) with oxygen-enriched air at positive pressure was supplied via tracheal intubation. After 24-hr fasting, pigs were preanesthetized by ketamine injection (10 mg kg^{-1}) and then maintained under inhalation anesthesia (halothane; N_2O/O_2, 2:1). After 24-hr fasting, primates were anesthetized by intravenous injection of ketamine (10 mg kg^{-1}) and diazepan (1 mg kg^{-1}). The animals were injected intravenously under the gamma camera [injected dose ca. 9 MBq kg^{-1} (0.25 mCi kg^{-1})]. Thoracic images were acquired in a planar or left anterior oblique projection (LAO) using a standard-field gamma camera equipped with a medium-energy parallel-hole collimator. A 20% window, centered at 140 keV, was used. One-minute frames were obtained in a 64 × 64 pixel matrix in experiments with dogs, and in a 128 × 128 pixel matrix in experiments with pigs and primates. The regions of interest (ROIs) were manually selected on the heart, lungs and liver in the experiments with dogs.

RESULTS

Chemistry

The preparation of bis(dithiocarbamato) nitrido ^{99m}Tc radiopharmaceuticals was carried out using two alternative procedures, which differ in the choice of the reducing agent and pH conditions. The first method was based on the reaction of $[^{99m}TcO_4]^-$ with S-methyl N-methyl dithiocarbazate, $H_2NN(CH_3)C(=S)SCH_3$, in the presence of the water-soluble tertiary phosphine, $[P(m\text{-}C_6H_4SO_3)_3]Na_3$ (TPPS) and HCl. The basic chemistry involved in this procedure has been previously described in detail (*5*). In this reaction, the species $H_2NN(CH_3)C(=S)SCH_3$ plays the role of an efficient donor of nitride nitrogen atoms (N^{3-}) and TPPS behaves as acceptor of oxygen atoms from pertechnetate. The preparation of the complexes was carried out in two steps as a liquid formulation. In the first step, the reaction of $[^{99m}TcO_4]^-$ with S-methyl N-methyl dithiocarbazate led to the formation of a mixture of intermediate, reduced complexes all containing the terminal Tc≡N multiple bond. The complete chemical characterization of these intermediate species was not accomplished. However, it was found that all the complexes composing the mixture underwent facile substitution reactions with the ligands $[R^1(R^2)NCS_2]Na$ (NaL) to give the same final disubstituted products $^{99m}TcN(L)_2$ [L = $R^1(R^2)NCS_2$] in high yields (95%–98%).

The compounds $^{99m}TcN(L)_2$ were obtained as a freeze-dried formulation by replacing the reducing agent TPPS with $SnCl_2$. In these conditions, it was possible to perform the initial reaction of $[^{99m}TcO_4]^-$ with S-methyl N-methyl dithiocarbazate to afford the Tc≡N group at neutral pH. The subsequent addition of the appropriate dithiocarbamate ligand led to the formation of the same final

TABLE 2
Radiopharmaceutical Distribution in Rats*

	%ID/Organ							
	$^{99m}TcN(Et_2NCS_2)_2$				$^{99m}TcN[Et(EtO)NCS_2]_2$			
Organ	5 min	30 min	60 min	120 min	5 min	30 min	60 min	120 min
Heart	3.78 ± 0.15	3.05 ± 0.25	2.01 ± 0.32	1.23 ± 0.20	4.28 ± 0.18	3.64 ± 0.17	2.62 ± 0.53	1.53 ± 0.06
Blood	2.10 ± 0.30	2.58 ± 0.61	1.82 ± 0.39	1.88 ± 0.31	2.50 ± 0.06	3.12 ± 0.55	2.82 ± 0.44	2.93 ± 0.08
Muscle	39.2 ± 0.85	25.2 ± 1.2	20.2 ± 1.3	13.0 ± 1.1	23.7 ± 1.8	23.7 ± 0.46	19.1 ± 3.2	16.8 ± 1.4
Lung	5.19 ± 0.85	2.30 ± 0.42	2.08 ± 0.55	1.76 ± 0.36	4.66 ± 0.61	3.09 ± 0.10	1.89 ± 0.72	1.40 ± 0.06
Liver	17.7 ± 1.3	24.8 ± 1.9	27.5 ± 1.6	26.5 ± 1.4	24.9 ± 1.7	25.8 ± 0.15	26.5 ± 2.6	23.6 ± 2.4
Kidney	5.24 ± 0.59	5.02 ± 0.20	4.38 ± 0.61	3.88 ± 0.52	6.28 ± 0.49	6.00 ± 0.50	5.71 ± 0.51	6.42 ± 0.32

*Mean ± s.d. of five animals.

$^{99m}TcN(L)_2$ complexes as obtained using the liquid formulation procedure described above, with comparable yields (93%–97%). These results indicate that the ability of N-methyl S-methyl dithiocarbazate of donating N^{3-} groups does not depend on the specific reaction conditions utilized, and that the formation of the reduced $[Tc(V)\equiv N]^{2+}$ core can be efficiently achieved with other reducing agents and over a wide range of synthetic conditions. The presence of DPTA in the freeze-dried formulation procedure is required in order to prevent the precipitation of the neutral, disubstituted complexes $Sn(L)_2$ after addition of the dithiocarbamate ligand.

RCP measurements showed that no decomposition of the complexes $^{99m}TcN(L)_2$, prepared using both liquid and freeze-dried formulations, occurred over 6 hr, at room temperature. The stability of the two formulations, stored at 4°C in the dark, was assayed by monitoring ligand decomposition and loss of Sn^{2+}. No significant changes in composition were observed over a 1-yr period. As expected, HPLC measurements of k′ showed these neutral tracers to be lipophilic, with log k′ values ranging from −0.030 to 0.37 (Table 1). The variation in the lipophilic character among the complexes reported in Table 1 might be related to the corresponding variation of the lateral functional groups on the ligand side-chain. Based on the previous characterization of the molecular structure of the complexes $^{99}TcN[Et(EtO)NCS_2]_2$ and $^{99}TcN(Et_2NCS_2)_2$ (*9,15*), a square pyramidal geometry was attributed to the complexes $^{99m}TcN(L)_2$, with the Tc≡N multiple bond in an apical position and the four sulfur atoms of the two dithiocarbamate ligands occupying the four sites on the basal plane.

Biodistribution Studies

Biological distribution results in rats for some representative bis(dithiocarbamato) nitrido ^{99m}Tc complexes are shown in Tables 2 and 3. Planar gamma camera images of $^{99m}TcN[Et(EtO)NCS_2]_2$ in dogs, pigs and monkeys are illustrated in Figure 2.

All $^{99m}TcN(L)_2$ complexes accumulate in the rat myocardium, but their biodistributions are strongly dependent on the pendant functional groups bonded to the uncoordinated nitrogen atom of the dithiocarbamate ligand. The highest values of myocardial uptake were found with the derivatives having $R^1 = R^2 = C_2H_5$ and $R^1 = C_2H_5$, $R^2 = C_2H_5O$ as lateral groups. By increasing the length and size of the alkyl substituents, a concomitant decrease of myocardial localization was observed in the order n-propyl > isopropyl > n-butyl > isobutyl > t-butyl, with the liver becoming the principal target organ. The complexes bearing cyclic, symmetric lateral groups showed very low myocardial uptake in rats, and this observation cannot be interpreted in terms of increased steric hindrance for these derivatives, for their sizes and lipophilicities are similar to those of the complex $^{99m}TcN(Et_2NCS_2)_2$ (Table 1). No significant heart uptake was found, in this animal model, for the complexes carrying beta and gamma alkoxy side groups.

Planar gamma camera biodistribution studies carried out in dogs showed that all $^{99m}TcN(L)_2$ complexes exhibited significant heart uptake. Derivatives bearing alkyl groups on the ligand side chains exhibited long retention times in heart tissue as well as in the lungs. Strong interspecies differences were observed for complexes having lateral alkoxy groups. Although these derivatives failed to visualize the rat heart (see above), they gave good quality images of the dog myocardium. In this animal, the introduction of beta and gamma alkoxy groups on the ligand side-chain caused a lowering of the lung uptake and a more rapid myocardial washout. We selected some specific members from the dog group based on their kinetic properties after observing biodistribution of the $^{99m}TcN(L)_2$ radiopharmaceuticals, especially lung uptake and heart clearance which were greatly influenced by the nature of the lateral groups on the ligands. The uptake and clearance of selected complexes in the organs of interest were determined by ROI analysis. The activity in each selected region was expressed in terms of cpm $pixel^{-1}$ mCi^{-1} and the corresponding myocardial time-activity curves are illustrated in Figure 3. ROI ratios are reported in Table 4. We found that the most favorable properties were associated with the derivatives $^{99m}TcN[Et(EtO)NCS_2]_2$[$^{99m}TcN(NOEt)$] and $^{99m}TcN(Et_2NCS_2)_2$, which were chosen for further studies

TABLE 3
Radiopharmaceutical Distribution in Rats*

Organ	%ID/Organ											
	$^{99m}TcN(Me_2NCS_2)_2$		$^{99m}TcN[(C_3H_7)_2NCS_2]_2$		$^{99m}TcN[Me(MeO)NCS_2]_2$		$^{99m}TcN[Et(MeO)NCS_2]_2$		$^{99m}TcN(C_4H_8NCS_2)_2$		$^{99m}TcN(HNC_4H_8NCS_2)_2$	
	5 min	60 min	5 min	60 min	5 min	60 min	5 min	60 min	5 min	60 min	5 min	60 min
Heart	2.38 ± 0.12	0.67 ± 0.05	0.80 ± 0.07	0.64 ± 0.04	1.92 ± 0.22	0.84 ± 0.09	1.58 ± 0.17	0.54 ± 0.14	0.18 ± 0.12	0.07 ± 0.03	1.26 ± 0.15	0.64 ± 0.08
Blood	3.04 ± 0.18	3.04 ± 0.21	11.4 ± 2.6	4.08 ± 0.16	2.04 ± 0.48	7.80 ± 0.72	7.23 ± 0.31	6.05 ± 0.36	0.96 ± 0.02	2.34 ± 0.09	10.2 ± 3.0	6.06 ± 1.16
Lung	4.46 ± 0.59	8.20 ± 1.3	2.30 ± 0.18	2.02 ± 0.09	4.86 ± 0.80	7.30 ± 1.8	4.48 ± 0.29	2.54 ± 0.15	2.14 ± 0.85	0.65 ± 0.04	4.35 ± 0.28	2.80 ± 0.13
Liver	17.5 ± 1.2	24.5 ± 1.6	47.8 ± 2.4	52.5 ± 3.4	15.9 ± 3.8	22.7 ± 1.9	22.8 ± 1.1	23.0 ± 2.6	45.4 ± 7.6	28.1 ± 4.2	30.3 ± 4.0	26.7 ± 2.4
Kidney	5.24 ± 0.80	1.86 ± 0.28	1.46 ± 0.61	1.20 ± 0.03	5.88 ± 0.97	5.46 ± 0.28	6.06 ± 1.4	8.76 ± 3.7	0.47 ± 0.01	5.60 ± 0.18	7.28 ± 0.35	4.72 ± 1.13

*Mean ± s.d. of five animals.

FIGURE 2. Anterior view of planar gamma camera images of a (A) pig, (B) dog and (C) monkey 1 hr after intravenous injection of $^{99m}TcN[Et(EtO)NCS_2]_2$ (9 MBq kg^{-1}). Prominent liver uptake is observed in all three images.

in pigs and primates. Gamma camera images obtained in a normal dog, pig and monkey 1 hr after injection of ^{99m}TcN-(NOEt) are reported in Figure 2. No heart uptake was observed in pigs, the activity being eliminated mainly through the liver. In contrast, high values of the injected activity were observed in myocardium tissue of monkeys (ca. 4.0% of the total injected activity), along with significant uptake both in the kidneys and the liver. The behavior of the derivative $^{99m}TcN(Et_2NCS_2)_2$ in pigs and primates was quite similar to that of $^{99m}TcN(NOEt)$ and, therefore, the corresponding gamma camera images have not been reported here.

DISCUSSION

The introduction of a simple and efficient method for the synthesis of complexes containing the $[Tc{\equiv}N]^{2+}$ core, at tracer level and in sterile and pyrogen-free conditions, has opened the possibility to explore the biological properties of a wide category of radiopharmaceuticals characterized by the presence of this terminal multiple bond. At first

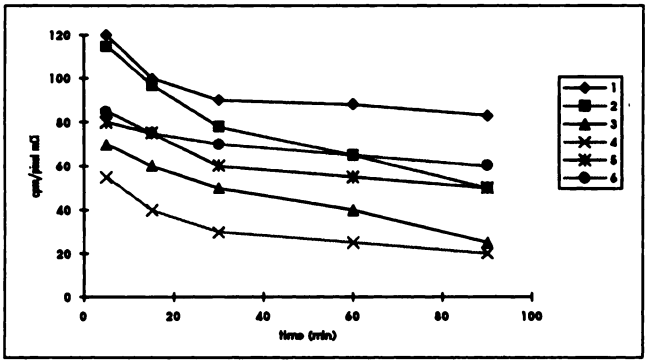

FIGURE 3. Time-activity curves for a selected myocardial region obtained after injection of $^{99m}TcN(L)_2$ complexes in a normal dog: (1) L = $Et(EtO)NCS_2$; (2) Et_2NCS_2; (3) $Et(CH_3OCH_2CH_2)NCS_2$; (4) $(CH_3OCH_2CH_2)_2NCS_2$; (5) $Et(CH_3CH_2OCH_2CH_2)NCS_2$; and (6) $Et(CH_3OCH_2CH_2CH_2)NCS_2$.

sight, it might appear that the synthesis of ^{99m}Tc complexes containing the isoelectronic $[Tc{\equiv}N]^{2+}$ group could not add any significant improvement to the search for new, useful imaging agents due to the availability of ^{99m}Tc radiopharmaceuticals containing the $[Tc{=}O]^{3+}$ group. The finding that $^{99m}TcN(L)_2$ [L = $R^1(R^2)NCS_2$] complexes exhibited high myocardial localization in various animal models prompts the opposite conclusion. In fact, the preparation and biodistribution in rats of some ^{99m}Tc complexes with dithiocarbamate ligands similar to those utilized in this study have been recently reported (*16,17*). These compounds were obtained by the usual procedure involving pertechnetate reduction in the presence of the ligand and demonstrated high in vitro instability and no accumulation into the heart of rats. The observation that the nitrido analogues of these compounds are highly stable and behave as myocardial perfusion tracers suggests that the introduction of the Tc≡N multiple bond into the molecular structure of a radiopharmaceutical may dramatically alter its biological behavior. It is reasonable to speculate that the way by which the formation of the Tc≡N bond affects the biodistribution of a compound should not originate from some biological uniqueness of this group in itself, but from the change in chemical and physical properties (stability, liopophilicity), and on the ligand arrangement around the metal center imparted by the specific electronic requirements of this core.

The complexes $^{99m}TcN(L)_2$ are neutral and exhibit high myocardial uptake in rats and dogs. High quality myocardial images were obtained in monkeys using the complexes $^{99m}TcN(NOEt)$ (Fig. 2) and $^{99m}Tc(Et_2NCS_2)_2$. However, these two derivatives failed to localize in the pig heart suggesting a strong interspecies dependence of the biological behavior of this class of compounds. These results show that $^{99m}TcN(L)_2$ complexes constitute the first examples of neutral ^{99m}Tc radiopharmaceuticals exhibiting a prolonged retention in myocardium tissue. In contrast, neutral ^{99m}Tc-BATO complexes are rapidly eliminated from the heart region within a few minutes after injection (*18*). Transient retention of neutral, lipophilic complexes would be expected on the basis of a nonspecific partitioning of these agents into the hydrophobic environment of the cell (*19*). However, stable retention of cationic imaging agents in heart tissue is generally observed. This localization appears to originate by the action of large negative membrane potentials which act to trap these positively charged compounds (*20*). Presently, the true localization mechanism of $^{99m}TcN(L)_2$ complexes remains undetermined. However, the observation of the prolonged myocardial retention of $^{99m}TcN(L)_2$ complexes seems to indicate that other localization mechanisms different from those involved in the uptake of cationic complexes which are not simply describable in terms of hydrophobic partitioning of a neutral lipophilic compound should exist.

CONCLUSIONS

Technetium-99m nitrido radiopharmaceuticals with dithiocarbamate ligands have been successfully prepared at tracer level and in sterile and apyrogen conditions through this efficient method which is simple enough to be

TABLE 4
ROI Ratios for $^{99m}TcN(L)_2$ Complexes in Dogs*

L		5 min	15 min	30 min	60 min	90 min
$Et(EtO)NCS_2$	H/Li	2.0	1.4	1.2	1.1	0.9
	H/Lu	1.1	1.3	1.6	1.7	2.0
Et_2NCS_2	H/Li	2.0	1.4	1.2	1.2	1.0
	H/Lu	0.7	1.1	1.5	1.8	2.1
$Et[CH_3O(CH_2)_2]NCS_2$	H/Li	1.4	1.0	0.9	0.8	0.7
	H/Lu	2.8	2.6	2.4	2.0	1.7
$[CH_3O(CH_2)_2]_2NCS_2$	H/Li	1.3	0.6	0.4	0.4	0.3
	H/Lu	2.0	1.7	1.4	1.2	1.1
$Et[C_2H_5O(CH_2)_2]NCS_2$	H/Li	1.4	1.0	1.0	0.6	0.6
	H/Lu	1.4	1.7	1.7	2.8	3.0
$Et[CH_3O(CH_2)_3]NCS_2$	H/Li	1.0	0.7	0.6	0.3	0.3
	H/Lu	1.4	2.2	2.4	2.0	1.9

*Values are expressed as cpm $pixel^{-1}$ mCi^{-1}; H = Heart, Lu = Lung, Li = Liver.

utilized in nuclear medicine for routine clinical use. Biodistribution studies show that the resulting neutral complexes, $^{99m}TcN(L)_2$, are retained in the myocardium of rats, dogs and primates and exhibit peculiar characteristics not previously shown by either neutral ^{99m}Tc-BATO and cationic myocardial imaging agents. The heart uptake and washout were found to be influenced by the nature of the variable, lateral groups on the dithiocarbamate ligands. Based on these results, we found that the derivative $^{99m}Tc-N[Et(EtO)NCS_2]_2$ [^{99m}TcN(NOEt)] exhibits the most promising properties for further studies in human subjects.

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APPENDIX

TABLE A1
Elemental Analyses and Mass Spectral Data of ^{99}Tc Complexes

Formula*	%C†	%H†	%N†	%S†	%Tc†	m/e
$TcN(Me_2NCS_2)_2$	20.58	3.46	11.92	35.98	27.95	353
($C_6H_{12}N_3S_4Tc$)	(20.39)	(3.42)	(11.89)	(36.29)	(28.01)	
$TcN(Et_2NCS_2)_2$	29.60	4.95	10.34	30.96	24.01	409
($C_{10}H_{20}N_3S_4Tc$)	(29.33)	(4.92)	(10.26)	(31.31)	(24.17)	
$TcN[(n\text{-}Pr)_2NCS_2]_2$	36.44	6.13	9.10	27.01	20.98	465
($C_{14}H_{28}N_3S_4Tc$)	(36.11)	(6.06)	(9.02)	(27.54)	(21.26)	
$TcN[(iso\text{-}Pr)_2NCS_2]_2$	36.60	6.18	9.24	26.97	20.95	465
($C_{14}H_{28}N_3S_4Tc$)	(36.11)	(6.06)	(9.02)	(27.54)	(21.26)	
$TcN[(n\text{-}Bu)_2NCS_2]_2$	42.03	7.01	8.13	24.00	18.21	521
($C_{18}H_{36}N_3S_4Tc$)	(41.44)	(6.95)	(8.05)	(24.58)	(18.97)	
$TcN[(iso\text{-}Bu)_2NCS_2]_2$	41.83	7.16	8.22	23.94	18.18	521
($C_{18}H_{36}N_3S_4Tc$)	(41.44)	(6.95)	(8.05)	(24.58)	(18.97)	
$TcN[(ter\text{-}Bu)_2NCS_2]_2$	40.98	6.87	8.00	23.98	18.25	521
($C_{18}H_{36}N_3S_4Tc$)	(41.44)	(6.95)	(8.05)	(24.58)	(18.97)	
$TcN\{[MeO(CH_2)_2]_2NCS_2\}_2$	32.06	5.47	8.06	23.89	18.12	529
($C_{14}H_{28}N_3O_4S_4Tc$)	(31.75)	(5.33)	(7.93)	(24.21)	(18.69)	
$TcN[Me(MeO)NCS_2]_2$	18.82	3.19	11.02	33.00	25.07	385
($C_6H_{12}N_3O_2S_4Tc$)	(18.70)	(3.14)	(10.90)	(33.27)	(25.69)	
$TcN[Me(EtO)NCS_2]_2$	23.84	3.91	10.28	30.80	23.03	413
($C_8H_{16}N_3O_2S_4Tc$)	(23.24)	(3.90)	(10.16)	(31.01)	(23.94)	
$TcN[Et(n\text{-}Pr)NCS_2]_2$	33.15	5.79	9.88	28.99	22.05	437
($C_{12}H_{24}N_3S_4Tc$)	(32.94)	(5.53)	(9.60)	(29.31)	(22.62)	
$TcN[Et(n\text{-}Bu)NCS_2]_2$	36.26	6.43	9.31	26.86	21.08	465
($C_{14}H_{28}N_3S_4Tc$)	(36.11)	(6.06)	(9.02)	(27.54)	(21.26)	
$TcN[Et(MeO)NCS_2]_2$	23.72	4.01	10.39	30.84	23.22	413
($C_8H_{16}N_3O_2S_4Tc$)	(23.24)	(3.90)	(10.16)	(31.01)	(23.94)	
$TcN[Et(EtO)NCS_2]_2$	27.32	4.56	9.54	28.90	22.14	441
($C_{10}H_{20}N_3O_2S_4Tc$)	(27.20)	(4.57)	(9.52)	(29.04)	(22.42)	
$TcN\{Et[MeO(CH_2)_2]NCS_2\}_2$	31.10	5.65	9.00	26.96	20.75	469
($C_{12}H_{24}N_3O_2S_4Tc$)	(30.69)	(5.15)	(8.95)	(27.31)	(21.08)	
$TcN\{Et[MeO(CH_2)_3]NCS_2\}_2$	34.18	5.98	8.86	25.17	19.00	497
($C_{14}H_{28}N_3O_2S_4Tc$)	(33.79)	(5.67)	(8.44)	(25.77)	(18.89)	
$TcN\{Et[EtO(CH_2)_2]NCS_2\}_2$	33.56	5.15	8.24	25.20	19.12	497
($C_{14}H_{28}N_3O_2S_4Tc$)	(33.79)	(5.67)	(8.44)	(25.77)	(19.89)	
$TcN(C_4H_8NCS_2)_2$	29.83	3.91	10.29	31.03	23.99	405
($C_{10}H_{16}N_3S_4Tc$)	(29.62)	(3.98)	(10.36)	(31.63)	(24.41)	
$TcN(C_4H_4NCS_2)_2$	30.41	2.30	10.82	31.84	23.78	397
($C_{10}H_8N_3S_4Tc$)	(30.22)	(2.03)	(10.57)	(32.27)	(24.91)	
$TcN(C_4H_6NCS_2)_2$	30.17	3.25	10.57	31.66	24.07	401
($C_{10}H_{12}N_3S_4Tc$)	(29.92)	(3.01)	(10.47)	(31.94)	(24.66)	
$TcN(C_5H_{10}NCS_2)_2$	33.43	4.84	9.78	29.00	22.25	433
($C_{12}H_{20}N_3S_4Tc$)	(33.25)	(4.65)	(9.69)	(29.58)	(22.83)	
$TcN(OC_4H_8NCS_2)_2$	27.81	3.60	10.00	28.83	21.83	437
($C_{10}H_{16}N_3O_2S_4Tc$)	(27.45)	(3.69)	(9.61)	(29.31)	(22.63)	
$TcN(HNC_4H_8NCS_2)_2$	27.80	4.27	16.66	28.74	22.30	435
($C_{10}H_{18}N_5S_4Tc$)	(27.58)	(4.17)	(16.08)	(29.45)	(22.73)	

*Me = methyl; Et = ethyl; Pr = propyl; and Bu = butyl. Empirical formulas are in parentheses.
†Calculated values are in parentheses.

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