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Technetium Labeling of Bi, Tri and Tetradentate Ligands Derived from 2-Aminocyclopentene-1-Dithiocarboxylic Acid: Characterization and Biodistribution of Their Oxo and Nitrido ^{99m}Technetium Complexes

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ABSTRACT. We have synthesized and characterized seven ligands derived from 2-aminocyclopentene-1-dithiocarboxylic acid with different donor sets (SN^{2-}, SNO^{2-}, SNN^{2-}, $SNNO^{3-}$ and $SNNN^{3-}$) and different substituents on the sulfur moieties -SR (with R = H, CH_3 or $C_2H_5O(CH_3)CH$). With five of these ligands technetium nitrido complexes have been obtained with high yields (over 95%) using rather harsh conditions (pH ≈ 1, temperature ≥80°C), whereas for technetium oxo complexes similar high yields were only obtained with two ligands but with mild conditions (pH = 7–8, temperature ≈ 50°C). Changing an OH group for an NH_2 has a drastic effect upon labeling yields. The possibility of complexing ligands as either oxo $(TcO)^{3+}$ or nitrido $(TcN)^{2+}$ derivatives increases the number of available labeled agents with different overall change and consequently with different biological behavior. NUCL MED BIOL 23;3:353–357, 1996.

KEY WORDS. 2-Aminocyclopentene-1-dithiocarboxylic acid, Technetium-99m, Nitridotechnetium, Complexes, Aminothiol, Biodistribution

INTRODUCTION

Amino-thiol ligands as diaminodithiol (DADT) are of great interest in the field of nuclear medicine owing to their high affinity with ^{99m}Tc, giving stable neutral lipophilic complexes (4, 12, 15, 20). Amino-thiol ligands have also been functionalized to obtain ^{99m}Tc-label biomolecules (13, 14, 18). In his work devoted to bifunctional DADT derivatives, Shiba (18) reported that complexes were formed with high yields (>95%) at room temperature but at pH = 10, while at physiological pH, high temperatures (70°–80°) were necessary. Till now, most of the complexation methods used far from ideal physiological conditions suitable for labeling biomolecules, and the current challenge is the development of new labeling methods working within these physiological conditions. To this end, the choice of the ligand remains the crucial point. For example, in metallic complexes of Schiff-base-derived ligands, the metal redox potentials are largely dependent on the nature of the donor sites and subtituents (3, 5, 16).

During the last several years, we have prepared and isolated stable oxo and nitrido technetium complexes with symmetrical and unsymmetrical ligands derived from 2-aminocyclopentene-1-dithiocarboxylic acid in which the number and the nature of the donor sites can be systematically changed. Some of these nitrido complexes have shown a good heart uptake in rats (2, 6–8).

In this article we describe our results about the technetium labeling of a new series of bi, tri, and tetradentate ligands (Fig. 1) derived from 2-aminocyclopentene-1-dithiocarboxylic acid with different donor sets (SN^{2-}, SNO^{2-}, SNN^{2-}, $SNNO^{3-}$, and $SNNN^{3-}$) and different substituents on the sulfur moiety SR (R = H, CH_3, $C_2H_5O(CH_3)CH$). Using different pH and temperature conditions, lipophilic oxo and nitrido complexes were isolated and the results of their biodistribution in the rat are reported.

EXPERIMENTAL

Materials and Methods

Technetium complexes were analyzed and purified by high-performance liquid chromatography (HPLC) on a Waters 600E gradient chromatograph with a Waters Lambda Max UV detector, a SAIP radioactivity detector, and an ICS dual integrator for effluent monitoring. Radiochemical purity was assessed by thin-layer chromatography on Nano-sil C18 plates (Macherey-Nagel) with an LB 2832 linear analyzer (Berthold). Elemental analyses were performed on a Fisons EA1108/CHNSO analyzer, and ^{1}H-NMR spectra were recorded on a Brucker 200WH spectrometer in $CDCl_3$ versus TMS (Me_3Si) as internal standard. All chemicals were laboratory grade and used without further purification.

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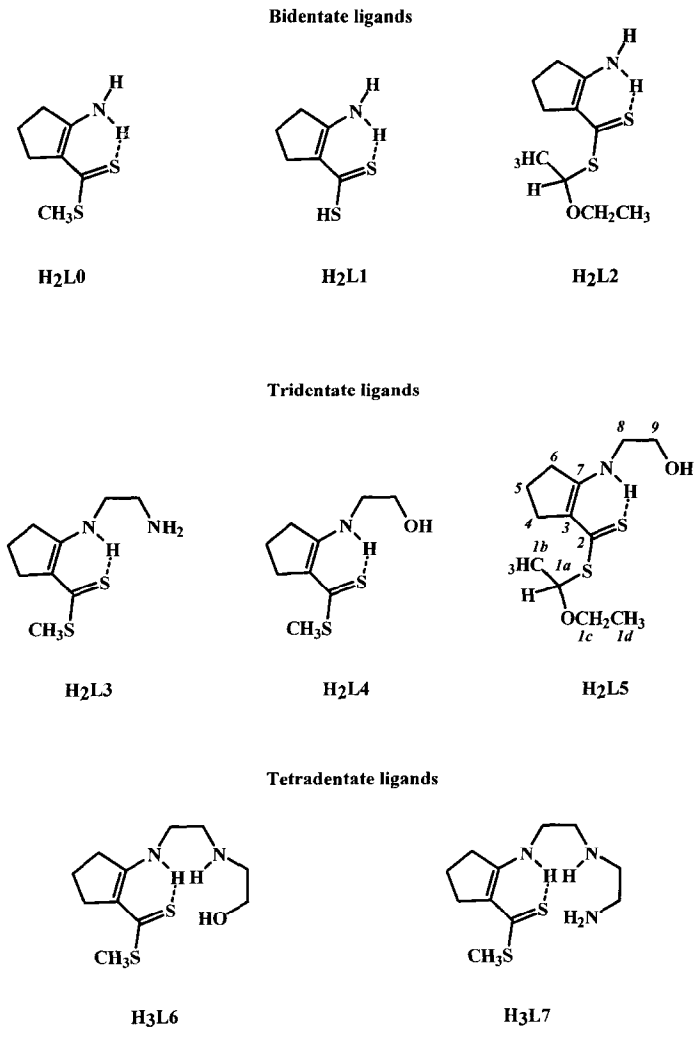

FIG. 1. Ligand structures and labeling schemes.

Synthesis

SYNTHESIS OF H_2L1. Initially the ammonium salt was prepared by stirring a mixture of cyclopentanone (0.3 mol) with carbon disulfide (0.3 mol) in 300 mL of aqueous ammonia (28%) at 0°C for 8 h (19). The acidic hydrolysis (pH 5) of this salt led to the corresponding acid (H_2L1), which was purified by recrystallization in a mixture of methanol and water (1:1).

SYNTHESIS OF H_2L4, H_3L6, H_3L7. The ligands H_2L4, H_3L6, and H_3L7 were synthesized according to the method of Roy *et al.* (17) based on a transamination reaction of the methyl ester of 2-aminocyclopentene-1-dithiocarboxylic acid (H_2L0) with an excess of the appropriate amine in ethyl alcohol:

- 2-amino-1-ethanol for H_2L4,
- *N*(2-hydroxyethyl)ethylene diamine for H_3L6
- diethyltriamine for H_3L7.

Ester H_2L0 was prepared according to a procedure described elsewhere (6).

SYNTHESIS OF H_2L3. H_2L3 is also obtained by Roy's method (17) using an excess of ethylenediamine. The disubstituted derivative *N,N'*-ethylene bis (methyl 2-aminocyclopenten-1-dithiocarboxylate) was first formed and eliminated by filtration. The monosubstituted compound H_2L3 was precipitated in the filtrate by addition of water and purified by recrystallization in a mixture of methanol and water (1:10).

SYNTHESIS OF H_2L2 AND H_2L5. The acid H_2L1 (19 mmol) in dichloromethane was reacted with an excess of ethylvinyl ether at −15°C for 24 h. After evaporation of the solvent the reaction product, *S*-ethoxyethyl-2-aminocyclopentene-1-dithiocarboxylic ester (H_2L2) was purified by recrystallization in a mixture of methanol and water (1:3). H_2L2 (11.5 mmol) was reacted with 2-amino-1-ethanol (23 mmol), giving the *N*-ethanol *S*-ethoxyethyl 2-aminocyclopentene-1-dithiocarboxylic ester (H_2L5). The ethoxyethyl group protecting in the -SR moiety can be easily removed under mild conditions used in some technetium labeling experiences (11).

^{99m}Tc-Labeling

The ligands were labeled either as nitrido or oxo technetium complexes.

PREPARATION OF NITRIDO COMPLEXES. In borosilicate vial containing 2 mg sodium azide, we added ligand solution (200 μL, 4×10^{-3} M, in ethanol), hydrochloric acid (100 μL, concentration from 10^{-3} to 1 M), sodium pertechnetate [$^{99m}TcO_4^-$] (200 μL, 74 MBq) and $SnCl_2,2H_2O$ (25 μL, 5×10^{-4} M, in water). The Teflon-capped vial was heated to various temperatures for 15 min. For H_2L2 and H_2L4 we replaced sodium azide by hydrazine solution (25 μL, 0.1 M), and the vial was heated to various temperatures for 10 min.

PREPARATION OF OXO COMPLEXES. In a borosilicate vial, a solution of ligand (200 μL, 4×10^{-3} M, in ethanol, a buffered solution (500 μL, 5×10^{-2} M), a solution of sodium pertechnetate [$^{99m}TcO_4^-$] (200 μL, 74 MBq) and a solution of $SnCl_2,2H_2O$ (25 μL, 1×10^{-2} M in acetic acid 0.1 M) were added successively. The vial was sealed with a Teflon-lined cap and heated at various temperatures for 15 min. Sodium glucarate buffer was used for H_2L5 and sodium tartarate buffer for H_3L6.

Purification and Characterization

The complexes formed were analyzed by liquid chromatography with a LiChroCART® 125-4 system filled with LiChrospher® 60 RP-select B (5 μm) eluted by methanol/water (80:20) and by a thin-layer chroma-

TABLE 1. Analytical Data for Ligands[a]

Formula	C	H	N	S
$C_6H_9NS_2$ (H_2L1)	45.0 (45.3)	5.4 (5.7)	9.2 (8.8)	39.1 (40.2)
$C_{10}H_{17}NOS_2$ (H_2L2)	52.3 (51.9)	7.5 (7.4)	5.8 (6.1)	28.1 (27.7)
$C_9H_{16}N_2S_2$ (H_2L3)	50.3 (50)	7.3 (7.5)	12.8 (13.0)	29.2 (29.6)
$C_9H_{15}NOS_2$ (H_2L4)	50.2 (49.8)	7.3 (7.0)	6.1 (6.4)	29.1 (29.5)
$C_{12}H_{21}NO_2S_2$ (H_2L5)	52.7 (52.4)	7.9 (7.7)	4.8 (5.1)	23.6 (23.2)
$C_{11}H_{20}N_2OS_2$ (H_2L6)	50.4 (50.8)	7.4 (7.7)	10.4 (10.8)	24.1 (24.6)
$C_{11}H_{21}N_3S_2$ (H_2L7)	50.3 (50.9)	8.4 (8.2)	16.5 (16.2)	24.9 (24.7)

[a] Found (calculated) (%).

TABLE 2. ^{1}H-NMR Shifts (RF = 250 MHz, ppm vs TMS in $CDCl_3$)[a]

	H1(a)	H1(b)	H1(c)	H1(d)	H4	H5	H6	H8	H9	H10	H11	N-H	S-H	N-H···S
H_2L1					3.1 (t)	2.2 (qt)	3.15 (t)					6.3 (s)	9.8 (s)	11.5 (s)
H_2L2	6.0 (qd)	1.7 (d)	3.7 (qd)	1.2 (t)	2.8 (t)	1.9 (qt)	2.8 (t)					5.9 (s)		11.2 (s)
H_2L3	2.6 (s)				2.7 (t)	1.9 (qt)	2.75 (t)	3.4 (m)	2.9 (m)					12.4 (s)
H_3L4	2.6 (s)				2.7 (t)	1.85 (qt)	2.8 (t)	3.5 (d+t)	3.8 (t)					12.3 (s)
H_3L5	6.1 (qt)	1.6 (d)	3.4 (t)	0.9 (t)	2.7 (t)	1.9 (qt)	2.8 (t)	3.6 (d+t)	3.8 (t)					12.6 (s)
H_3L6	2.6 (s)				2.65 (t)	1.8 (qt)	2.75 (t)	3.5 (m)	3.45 (qd)	2.85 (t)	3.6 (t)			12.4 (s)
H_3L7	2.6 (s)				2.7 (t)	1.9 (qt)	2.8 (t)	3.5 (m)	3.5 (m)	2.8 (m)	2.8 (m)			12.6 (s)

[a] s = singlet; d = double; t = triplet; qd = quadruplet; qt = quintuplet; m = multiplet; the numbering scheme is indicated in Fig. 1.

tography (TLC) system: Nano-sil C18 (Macherey-Nagel) in methanol/H_2O/trifluoro acetic acid (50:50:0.1).

Determination of Octanol/Buffer Partition Coefficients

The lipophilicity of the nitrido and oxo complexes was evaluated by the determination of the partition coefficients between octanol and Tris-HCl buffer (0.05 M, pH = 7.4). In a test tube, 25 μL of an ethanolic solution of the ^{99m}Tc complexes was added to 2 mL octanol and 2 mL buffer. The tubes were vortex-mixed for 1 min and centrifuged for 5 min at 5000 *g*. The supernatant octanol layer was then transferred to another tube and vortex-mixed with 2 mL fresh buffer. The entire procedure was repeated at least three times. After the last partitioning, 20 to 100 μL of each phase were weighed and counted in a γ counter. The partition coefficients were calculated by dividing the cpm/g of octanol from that of the buffer. Results are the means of three determinations.

Biodistribution

All experiments were carried out in compliance with French laws relating to the conduct of animal experimentation.

The biodistribution of technetium-99m complexes was studied in male Wistar rats (Janvier, France), 400 ± 50 g, anesthetized with Nesdonal® (thiopental sodium salt; 0.25 mg intraperitoneously) after injection of 300 μL of a saline solution of the labeled agent into the femoral vein. Three rats were sacrificed 5 and 30 min after injection, and the organs of interest (liver, spleen, heart, lungs, kidney, and brain) were dissected, rapidly rinsed in saline, blotted, and weighed. The organs and a blood sample were counted, and the percentage radioactivity in the blood was calculated assuming that the whole-blood volume was 7% of the body weight.

RESULTS AND DISCUSSION

Ligand Characterization

Microanalysis results given in Table 1 and ^{1}H NMR data in Table 2 are in accordance with the proposed structure of the ligands shown in Fig. 1.

Complex Characterization

The high values of the octanol/buffer partition coefficient (Table 3) indicated a high lipophilicity of the technetium complexes. Nitrido technetium complexes were prepared with high yield (≥95%) for all the ligands except H_3L7 and H_2L3, whereas oxo technetium complexes were formed with a comparable high yield only with H_2L5 and H_2L3. But for nitrido complexes such high yields were only observed when using harsh conditions (pH ≈ 1; 70°–80°C), whereas for oxo complexes good yields were obtained in rather mild conditions (pH ≈ 7–8, 50°C).

TABLE 3. Synthesis of ^{99m}Tc Nitrido and Oxo Complexes: Labeling Conditions, Radiochemical Yields, Partition Coefficients, HPLC Retention Time, and R_f[a]

	Labeling conditions						
Complexes	T°C	Reaction time (min)	pH	Yield %	Partition coefficients	HPLC retention time (min)	R_f
$H_2L1[^{99m}TcN]$	80°	15 min	1	95%	4000	2.5	0.22
$H_2L1[^{99m}TcO]$	#	#	#	<15%	#	unstable	#
$H_2L2[^{99m}TcN]$	70°	15 min	1	95%	3990	2.5	0.22
$H_2L2[^{99m}TcO]$	#	#	#	<15%	#	unstable	#
$H_2L3[^{99m}TcN]$	80°	15 min	1	<15%	#	#	#
$H_2L3[^{99m}TcO]$	#	#	#	<15%	#	#	#
$H_2L4[^{99m}TcN]$	70°	10 min	1	>95%	80	2.5	0.52
$H_2L4[^{00m}TcO]$	#	#	#	<15%	#	unstable	#
$H_2L5[^{99m}TcN]$	70°	15 min	1	>95%	495	2.3	0.56
$H_2L5[^{99m}TcO]$	50°	15 min	8	>95%	111	2.1	0.44
$H_3L6[^{99m}TcN]$	70°	15 min	1	90%	396	2.5	0.50
$H_3L6[^{99m}TcO]$	50°	15 min	7.5	95%	423	2.8	0.54
$H_3L7[^{99m}TcN]$	#	#	#	<15%	#	#	#
$H_3L7[^{99m}TcO]$	#	#	#	<15%	#	#	#

[a] For conditions see text.

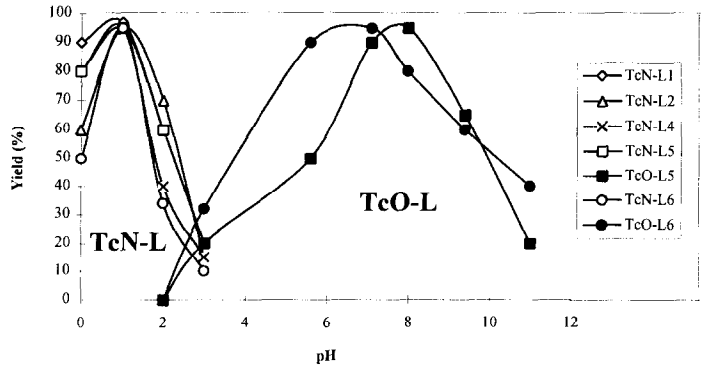

FIG. 2. ^{99m}Tc labeling yields of ligands as a function of pH.

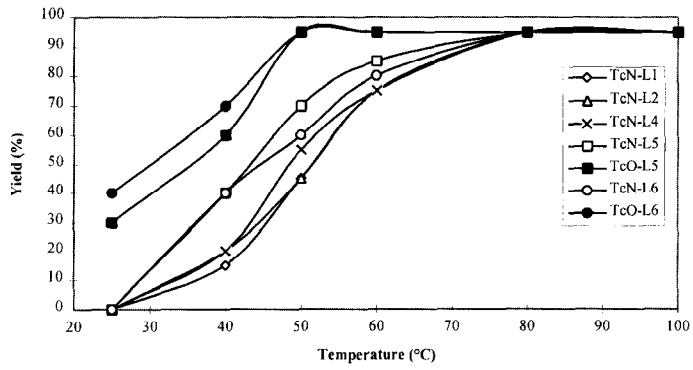

FIG. 3. ^{99m}Tc labeling yields of ligands as a function of temperature.

LIGAND DONOR SET INFLUENCE ON COMPLEX FORMATION. Comparing the technetium labeling yields of the ligands H_2L4 and H_3L6 (Table 3) to those of H_3L7 and H_2L3 shows that the substitution of a hydroxyl group by an amino involves a dramatic decrease of the yields. This could be explained by a higher mobility of the hydroxyl protons in the presence of the metal. That fact was corroborated by the impossibility, until now, of complexing H_2L3 with the long-lived isotope ^{99}Tc.

However, with the other ligands, the studies of ^{99}Tc and Re complexes (in progress) show that:

H_2L1 and H_2L2 behaved as bidentate ligands in $TcN(L2)_2$ and $TcO(L2)_2Cl$ complexes.

H_2L5 behaved as SN^-O^- tridentate ligand as demonstrated by the structure of $^{99}TcNL5,PPh_3$ complex determined by X-ray diffraction (6).

H_2L6 behaved as SN^-NO^- dianionic tetradentate ligand in the complex $[ReOL6]BPh_4$ (8).

INFLUENCE OF TEMPERATURE AND PH ON COMPLEX FORMATION. The influence of pH on complex formation at optimum temperature conditions (80°C for TcN and 50°C for TcO derivatives) is illustrated in Fig. 2. It appears that nitrido complexes were obtained with high yield only in a narrow range at low pH (≈1), whereas oxo complexes are formed over a wider pH range (pH 6.5–8.5) and closer to physiological conditions. The rather rough conditions necessary to reach the TcN core might be related, as suggested by Davison (9), with the difficulty of removing the last oxygen atom from the technetium internal coordination sphere, particularly in acidic conditions. Furthermore, Alagui et al. (1) have shown that the nitrido anion $[^{99m}TcNCl_4]^-$ was only stable for pH < 4. Fig. 3 illustrates the influence of temperature upon nitrido and oxo complex formation yields at optimum pH conditions (pH = 1 for TcN and pH = 7.5 for TcO derivatives). It can be seen that higher temperatures were needed to form nitrido than to form oxo complexes. This could be due to the fact that the thermal decomposition of sodium azide (10) or hydrazine is necessary to achieve the TcN core formation.

BIODISTRIBUTION IN THE RATS. The biodistribution results of oxo and nitrido ^{99m}Tc-complexes expressed as a % of the injected dose/g of tissue are summarized in Tables 4 and 5. Biodistribution patterns varied for each labeled compound and showed significant liver and spleen uptake especially for TcO(L5). This can be related to the cleavage of the $(C_2H_5O)(CH_3)CH$-S group in alkaline conditions. Although all complexes exhibited high lipophilicity, as demonstrated by high-partition coefficients, brain uptake was low, but heart uptake was interesting for TcN(L1), TcN(L2), and TcN(L4).

CONCLUSION

In this study oxo and nitrido technetium complexes of seven ligands derived from 2-aminocyclopentene-1-dithiocarboxylic acid have been prepared. Complexation yields have been optimized with respect with temperature and pH conditions. The influence of the nature of the donor sets and lability of substituents on labeling yield and biodistribution was highlighted. Others works are in progress to tentatively rationalize these effects.

TABLE 4. Biological Distributions of [^{99m}TcN] and [^{99m}TcO] Complexes at 5 min Postinjection in Rats[a]

	% Injected dose/g organ						
Complexes	**Liver**	**Spleen**	**Lungs**	**Kidneys**	**Heart**	**Brain**	**Blood**
$H_2L1[^{99m}TcN]$	1.51 ± 0.37	1.13 ± 0.30	0.68 ± 0.15	0.83 ± 0.28	0.84 ± 0.10	0.40 ± 0.01	0.21 ± 0.05
$H_2L2[^{99m}TcN]$	1.70 ± 0.40	1.20 ± 0.30	0.70 ± 0.10	0.80 ± 0.31	0.90 ± 0.15	0.30 ± 0.01	0.30 ± 0.01
$H_2L4[^{99m}TcN]$	2.86 ± 0.36	2.36 ± 0.22	1.26 ± 0.43	1.10 ± 0.40	1.32 ± 0.10	0.20 ± 0.03	0.23 ± 0.05
$H_2L5[^{99m}TcN]$	3.83 ± 0.53	1.15 ± 0.26	1.01 ± 0.49	1.87 ± 0.10	0.32 ± 0.04	0.01 ± 0.00	0.33 ± 0.01
$H_2L5[^{99m}TcO]$	5.38 ± 0.76	8.00 ± 0.05	0.45 ± 0.01	0.23 ± 0.01	0.10 ± 0.00	0.01 ± 0.00	0.56 ± 0.05
$H_3L6[^{99m}TcN]$	2.72 ± 0.47	0.86 ± 0.24	0.68 ± 0.10	6.24 ± 0.66	0.44 ± 0.06	0.04 ± 0.01	0.80 ± 0.01
$H_3L6[^{99m}TcO]$	1.62 ± 0.18	0.49 ± 0.06	1.33 ± 0.51	1.94 ± 0.29	0.70 ± 0.25	0.17 ± 0.03	1.47 ± 0.40

[a] Mean of determination ± SD.

TABLE 5. Biological Distributions of [^{99m}TcN] and [^{99m}TcO] Complexes at 30 min Postinjection in Rats[a]

Complexes	% Injected dose/g organ						
	Liver	Spleen	Lungs	Kidneys	Heart	Brain	Blood
$H_2L1[^{99m}TcN]$	1.86 ± 0.56	2.30 ± 0.48	0.35 ± 0.15	0.64 ± 0.12	0.35 ± 0.10	0.12 ± 0.01	0.05 ± 0.01
$H_2L2[^{99m}TcN]$	1.90 ± 0.52	2.50 ± 0.41	0.40 ± 0.15	0.60 ± 0.13	0.40 ± 0.10	0.10 ± 0.02	0.09 ± 0.01
$H_2L4[^{99m}TcN]$	2.02 ± 0.29	1.76 ± 0.05	0.75 ± 0.07	0.91 ± 0.30	0.76 ± 0.15	0.17 ± 0.05	0.09 ± 0.02
$H_2L5[^{99m}TcN]$	4.09 ± 0.16	0.45 ± 0.04	0.57 ± 0.13	0.98 ± 0.15	0.36 ± 0.03	0.02 ± 0.00	0.13 ± 0.01
$H_2L5[^{99m}TcO]$	5.42 ± 0.79	4.00 ± 0.04	0.33 ± 0.01	0.23 ± 0.01	0.06 ± 0.00	0.00 ± 0.00	0.28 ± 0.06
$H_3L6[^{99m}TcN]$	1.62 ± 0.29	0.46 ± 0.05	0.35 ± 0.07	2.83 ± 1.18	0.28 ± 0.06	0.02 ± 0.00	0.32 ± 0.06
$H_3L6[^{99m}TcO]$	1.49 ± 0.14	0.46 ± 0.01	0.64 ± 0.03	2.35 ± 0.24	0.35 ± 0.03	0.12 ± 0.00	0.75 ± 0.08

[a] Mean of determination ± SD.

References

1. Alagui A., Apparu M., Du Moulinet d'Hardemare A., Riche F. and Vidal M. (1989) Etude de la stabilité de la liaison ^{99m}Tc≡N en fonction du pH: Mise en évidence de la transformation de $[^{99m}TcNCl_4]^-$ en $^{99m}TcO_4^-$. *Int. J. Radiat. Appl. Instrum.* **40,** 813–817.
2. Belhadj-Tahar H., Cros G., Darbieu M. H., Tafani J. A. M., Coulais Y. and Guiraud R. (1994) Etude des potentialités de complexation de nouvelles bases de Schiff non-symétriques dérivées de l'acide dithiocarboxylique avec le technétium. *Med. Nucl.* **18,** 243.
3. Chen L. S. and Cummings S. C. (1978) Synthesis and characterisation of Cobalt(II) and some Nickel(II) complexes with *N,N'*-ethylene bis(*p*-X-benzoylacetone iminato) and *N,N'* ethylene bis(*p*-benzoylmonothioacetone iminato) ligands. *Inorg. Chem.* **17,** 2358–2361.
4. Chiotellis E., Varvarigou A. D., Maina Th. and Stassinopoulou C. I. (1988) Comparative evaluation of ^{99m}Tc-labeled aminothiols as possible brain imaging agents. *Nucl. Med. Biol.* **15,** 215–223.
5. Costes J. P., Cros G., Darbieu M. H. and Laurent J. P. (1982) Electrochemical study of some cobalt Schiff base complexes and their methyl derivatives: Relation to the behaviour of the cobalt-carbon bond. *Transition Met. Chem.* **7,** 219–223.
6. Coulais Y., Cros G., Darbieu M. H., Tafani J. A. M., Belhadj-Tahar H., Bellande E., Pasqualini R. and Guiraud R. (1994) Synthesis, characterization and biodistribution of new ^{99m}Tc oxo and nitrido complexes with bi- and tetradentate unsaturated NS and N_2S_2 Schiff bases derived from 2-aminocyclopentene-1-dithio-carboxylic acid as potential heart imagining agents. *Nucl. Med. Biol.* **21,** 263–268.
7. Cros G., Coulais Y., Belhadj-Tahar H., Gleizes A. and Guiraud R. (1994) Synthesis and characterization of oxo and nitrido complexes of technetium-99(V) and rhenium(V) with new SNO and SN_2O ligands: Biodistribution study of the related ^{99m}Tc-complexes. In *Technetium and Rhenium in Chemistry and Nuclear Medicine* (Edited by Nicolini M., Bandoli G. and Mazzi U.), pp. 85–87. Servizi Grafici Editoriali, Padova.
8. Cros G., Belhadj-Tahar H., de Montauzon D., Gleizes A., Coulais Y., Guiraud R., Bellande E. and Pasqualini R. (1994) Synthesis and characterization of neutral oxorhenium(V) and nitrido-technetium(V) complexes with a tetradendate N_2S_2 unsaturated ligand derived from dithiocarboxylic acid. *Inorg. Chim. Acta.* **227,** 25–31.
9. Davison A. (1983) The coordination chemistry of technetium. In *Technetium and Rhenium in Chemistry and Nuclear Medicine* (Edited by Nicolini M., Bandoli G. and Mazzi U.), pp. 3–14. Raven Press, New York.
10. Griffith W. P. (1972) Transition metal nitrido complexes. *Coord. Chem. Rev.* **8,** 369–396.
11. Kasina S., Rao T. N., Srinivasan A., Sanderson J. A., Fitzner J., Reno J. N., Beaumier P. L. and Fritzberg A. R. (1991) Development and biologic evaluation of kit for performed chelate technetium-99 m. radiolabeling of an antibody Fab fragment using a diamide dimercaptide chelating agent. *J. Nucl. Med.* **32,** 1445–1451.
12. Kung H. F., Guo Y. Z., Yu C. C., Billings J. B., Subramanyam V. and Calabrese J. C. (1989) New brain perfusion imaging agents based on ^{99m}Tc-bis (amino-ethanethiol) complexes: Stereoisomers and biodistribution. *J. Med. Chem.* **32,** 433–437.
13. Lever S. Z., Kwamena E., Baidoo K. E., Mahmood A., Matsumura K., Schefefel U. and Wagner H. N. Jr. (1994) Novel technetium ligands with affinity for muscarinic cholinergic receptor. *Nucl. Med. Biol.* **21,** 157–164.
14. Liu S. and Edwards D. S. (1994) New N_2S_2 diamidedithiols and N_3S triamidithiols as bifunctional chelating agents for labeling small peptides with ^{99m}Tc. In *Technetium and Rhenium in Chemistry and Nuclear Medicine* (Edited by Nicolini M., Bandoli G. and Mazzi U.), pp. 383–393. Servizi Grafici Editoriali, Padova.
15. Oldendorf W. H. (1978) Need for new radiopharmaceuticals. *J. Nucl. Med.* **19,** 1182.
16. Patterson G. S. and Holm R. H. (1975) Structural and electronic effects on the polarographic half-wave potentials of copper(II) chelate complexes. *Bioinorg. Chem.* **4,** 257–275.
17. Roy R., Parimal P. and Nag K. (1984) Metal complexes of sulfur-nitrogen chelating agents: Part 12—The chemistry of nickel(II), palladium(II), cobalt(II) and copper(II) complexes of N_2S_2 and N_3S_2 donor systems. *Transition Met. Chem.* **9,** 152–155.
18. Shiba K., Mori H., Matsuda H., Tsuji S., Kinuya K. and Hisada K. (1991) Synthesis of technetium-99m labeled diaminodithiol for bifunctional chelating agents. *Appl. Radiat. Isot.* **42,** 1159–1164.
19. Takeshima T., Yokoyama M., Imamoto T. and Asaba H. (1969) The reaction of active methylene compounds with carbon disulfide in the presence of ammonia. III. The reactions of cyclopentanone and cycloheptanone. *J. Org. Chem.* **34,** 730–732.
20. Walowitch R. C., Makuch J., Watson A. D. and Williams S. J. (1988) Brain retention of ^{99m}Tc-ECD is related to in vivo metabolism. *J. Nucl. Med.* **29,** 747.