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Synthesis and Biodistribution of New Oxo and Nitrido ^{99m}Tc Complexes with Asymmetrical Potentially Dianionic or Trianionic Tetradentate SNNO Ligands Derived from Methyl-2-Aminocyclopentene-1-Dithiocarboxylic Acid

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ABSTRACT. In this work, 10 new asymmetrical tetradentate SNNO ligands were prepared by reaction of the amine function of methyl 2-[(β-aminoethyl)amino]cyclopentene-1-dithiocarboxylate with various bifunctional substituents bearing hydroxyl/ketone and hydroxyl/aldehyde functional groups and with diethyl oxalate. ^{99m}Tc labeling efficiency was optimized by adjusting temperature and pH conditions. Seven nitrido and two oxo ^{99m}Tc complexes were isolated. Six of them proved to be stable near physiological conditions. Biodistribution studies in the rat showed a significant heart uptake for four of them and strong kidney and liver uptake for the other two. NUCL MED BIOL 25;1:65–69, 1998. **© 1998 Elsevier Science Inc.**

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

INTRODUCTION

In recent years, a growing interest has been devoted to the design of new ^{99m}Tc radiopharmaceuticals based on aminothiol ligands (4, 12, 16). These complexes are of interest because their lipophilic properties are closely dependent on their structure (overall charge, nature of substituents on the N_2S_2 backbone, etc.). So far, some of them have been used as imaging agents for the brain, such as TcO(ECD) (15, 25), or kidney, such as $TcO(MAG)_3$ (8, 10, 23). Others, the neutral nitrido complexes, exhibit good heart uptake in animals (5, 7). Furthermore, some of these complexes have proven to exhibit a convenient tissue diffusion and a good stability. For example, *in vitro* the stability of TcO(ECD) (about 6 h) can be advantageously compared to that of TcO(HMPAO): 30 min (11). Owing to their stability in biological media, some of these complexes have been used to label biomolecules (9, 21, 22, 24); unfortunately, synthesis of the precursor complexes involves rather harsh conditions of pH and temperature (2).

In previous papers we have described new series of ^{99}Tc and ^{99m}Tc complexes with unsaturated aminothiol ligands derived from a dithiocarboxylic acid, and comparative biodistribution studies have been presented (2, 6). In the present work, we report the synthesis and the biodistribution behavior of oxo and nitrido ^{99m}Tc complexes of 10 new asymmetrical tetradentate ligands with an SNNO donor set (Fig. 1). They are built with a common bulk part: deprotonated methyl 2-[(β-aminoethyl)amino]cyclopentene-1-dithiocarboxylate (H_2LO) and a different moiety bearing a hydroxyl or a carboxylate group. Our final objective is the design of new ^{99m}Tc complexes labeled in physiological conditions of pH and temperature and stable in biological media.

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MATERIALS AND METHODS

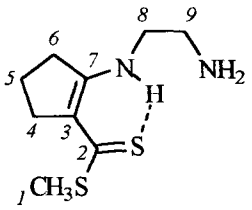
Analysis of ^{99m}Tc-labeled reaction products was performed using high performance liquid chromatography (HPLC) on a Waters 600E gradient chromatograph using a NaI radiodetector (SAIP), a Waters Lambda Max UV detector and an ICS dual integrator for effluent monitoring.

Radiochemical purity was checked by thin-layer chromatography (TLC) on Nanosil C18 plates (Macherey-Nagel) with an LB 2832 linear analyzer (Berthold). Elemental analyses were carried out on a Fisons EA1108/CHNSO analyzer; ^{1}H nuclear magnetic resonance (NMR) spectra were obtained with a Brucker 250 WH spectrometer in $CDCl_3$ versus TMS (Me_4Si) as internal standard. All laboratory chemicals used were of reagent grade.

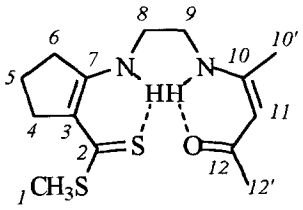
Ligand Synthesis

H_2L1, H_2L2, H_2L3, H_2L4, H_2L5, H_2L6 and H_2L7 were obtained by reacting the amine function of H_2LO with the carbonyl function of the following compounds: 2,4-pentanedione, salicylaldehyde, 2-hydroxyacetophenone, 2-hydroxy-5-nitrobenzaldehyde, 2,3-dihydroxybenzaldehyde, 4,6-dimethoxybenzaldehyde and dehydroacetic acid.

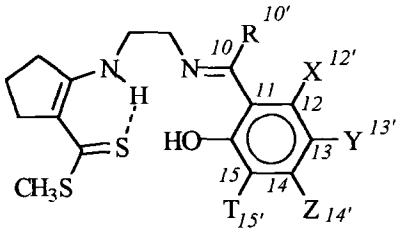
The general procedure was as follows: 1.13 g (9.2 mmol) of salicylaldehyde was added to 2 g (9.2 mmol) of H_2LO dissolved in 50 mL of ethanol. The mixture was stirred for 2 h at room temperature. Then, the yellow precipitate (H_2L2) was filtered off and washed with ethanol and recrystallized from methanol:water (1:3). The

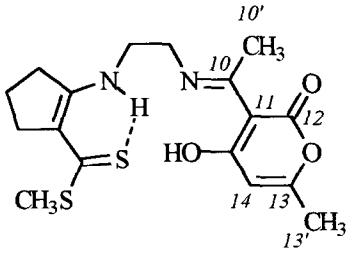


H_2L0



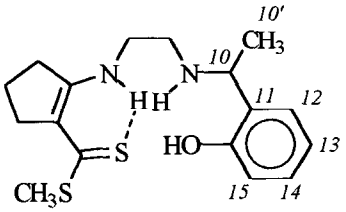
H_2L1



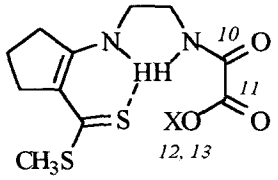


H_2L7

R = H, X = H, Y = H, Z = H, T = H **H_2L2**
R = CH_3, X = H, Y = H, Z = H, T = H **H_2L3**
R = H, X = H, Y = NO_2, Z = H, T = H **H_2L4**
R = H, X = H, Y = H, Z = H, T = OH **H_2L5**
R = H, X = OCH_3, Y = H, Z = OCH_3, T = H **H_2L6**



H_2L8



X= H **H_3L9**
X= C_2H_5 **H_3L10**

FIG. 1. Ligand structure and labeling scheme.

intense yellow color is typical of the presence of an imine bond in those Schiff base ligands.

H_3L9 and H_3L10 were prepared in the same way from the reaction between H_2L0 and diethyloxalate in ethanolic medium and in a KOH solution (5 M), respectively.

H_2L8 was obtained by reducing the imine bond of H_2L3 with $NaBH_4$ according to a procedure from the literature (20).

^{99m}Tc *Labeling*

FORMATION OF NITRIDOTECHNETIUM COMPLEXES. Two methods were used for ^{99m}Tc labeling of ligands.

Direct Complexation Method. Labeling reactions were carried out according to a new protocol developed in our laboratory (2). Typically, to 200 μL (4×10^{-3} M) of ligand dissolved in ethanol were added successively 2 mg of NaN_3, 100 μL of HCl (1 M, 0.1 M and 0.01 M), 100 μL of sodium pertechnetate (74 MBq of $^{99m}TcO_4^-$ in aqueous solution) and 25 μL of stannous chloride in water (5×10^{-4} M). The vial was Teflon capped and the mixture vortex mixed and incubated for 15 min in the temperature range 25°C–80°C.

Indirect Complexation Method Using a Ligand Exchange Reaction. Nitrido complexes were formed according to the procedure described by Pasqualini *et al.* (17), mixing in a vial 200 μL of ethanolic solution of *N*-methyl S-methyl dithiocarbazate, 300 μL of tri(phenylsulfonic acid)phosphine $(HO_3S\text{-}Ph\text{-})_3P$ in water (3.5×10^{-3} M), 100 μL of hydrochloric solution (1 M) and 74 MBq of sodium pertechnetate in 500 μL of aqueous solution. The mixture was heated for 30 min at 100°C and cooled to room temperature. Ligand was added after the reaction mixture had been neutralized by NaOH and adjusted to pH 8. The ligand exchange reaction was carried out at various temperatures (40°C–100°C).

TABLE 1. Analytical Data for Ligands

Formula	C	H	N	S
$C_{14}H_{22}N_2OS_2$ H_2L1	56.3 (56.3)[a]	7.4 (7.5)	9.4 (9.3)	21.4 (21.0)
$C_{16}H_{21}N_2OS_2$ H_2L2	59.8 (59.7)	6.5 (6.6)	8.7 (8.7)	19.9 (20.0)
$C_{17}H_{23}N_2OS_2$ H_2L3	60.9 (60.8)	6.9 (6.9)	8.4 (8.4)	19.1 (19.2)
$C_{16}H_{20}N_3O_3S_3$ H_2L4	52.4 (52.2)	5.5 (5.4)	11.5 (11.4)	17.5 (17.4)
$C_{16}H_{21}N_2O_2S_2$ H_2L5	57.0 (56.9)	6.2 (6.3)	8.3 (8.2)	19.0 (18.9)
$C_{13}H_{25}N_2O_3S_2$ H_2L6	56.7 (56.6)	6.6 (6.5)	7.3 (7.3)	16.8 (16.7)
$C_{15}H_{19}N_2O_2S_2$ H_2L7	55.7 (55.6)	5.9 (5.9)	8.7 (8.6)	19.8 (19.6)
$C_{17}H_{24}N_2OS_2$ H_2L8	60.7 (60.6)	7.1 (7.2)	8.3 (8.4)	19.0 (19.0)
$C_{11}H_{15}KN_2O_3S_2$ H_3L9	40.5 (40.3)	4.6 (4.6)	8.6 (8.5)	19.6 (19.4)
$C_{13}H_{20}N_2O_3S_2$ H_3L10	49.3 (49.1)	6.4 (6.3)	8.8 (8.8)	20.3 (20.2)

[a] Calculated (found).

FORMATION OF OXOTECHNETIUM COMPLEXES. To 74 MBq of TcO_4^- in 500 μL of buffer (1×10^{-3} M of citrate or tartrate) over a wide range of pH (2.2–12) were added 200 μL of ethanolic solution of ligand (4×10^{-3} M) and 25 μL of stannous chloride in water (1×10^{-2} M); the mixture was vortex mixed and heated at various temperature (20°C–100°C) in the Teflon-capped vial.

Purification and Characterization

Analysis of complex formation was performed on an HPLC system with a LiChropher 60RP-select B (5 μm) column eluted by methanol:0.01 M trifluoroacetic acid (80:20) at a flow rate of 1 mL/min and by the TLC system Nanosil C18 (Macherey-Nagel) in methanol:water:trifluoroacetic acid (80:20:0.1).

PURIFICATION OF THE ^{99m}TC COMPLEXES. The complexes were extracted in ethyl acetate, concentrated with external heating (about 40°C) to dryness, and reconstituted with Tris–HCl buffer (0.05 M and pH 7.4) for the stability study or with 0.9% physiological saline for the biodistribution study.

STUDY OF COMPLEX STABILITY. The 3.7 MBq of purified ^{99m}Tc complexes in 2 mL of Tris–HCl buffer (0.05 M, pH 7.4) were stored at room temperature under air for 4 h and analyzed by TLC.

DETERMINATION OF PARTITION COEFFICIENTS. Partition coefficients were measured by vortex-mixing aliquots of each purified ^{99m}Tc complex in a mixture of 2 mL of *n*-octanol and 2 mL of Tris–HCl buffer (0.05 M, pH 7.4) for 1 min and centrifuging for 5 min at 5000 × *g*. The supernatant *n*-octanol layer was transferred to another tube and vortex-mixed with 2 mL of fresh buffer. The whole procedure was repeated at last three times. After the fourth partitioning, 20 and 100 μL of each phase were removed, and the radioactivity in the buffer and *n*-octanol was determined. The partition coefficients were obtained by calculating the ratio of counts per minute per milliliter of *n*-octanol to that of the buffer. The results are the means of three determinations.

TABLE 2. ^{1}H NMR Shifts

	H1	H4	H5	H6	H8	H9	H10	H10′	H11	Phenyls	H12	H12′	H13	H13′	H14	H14′	H15	N-H-S	N-H-O
H_2L1	2.55 (s)	2.75 (t)	1.9 (qt)	2.8 (t)	3.5 (m)	3.5 (m)		1.95 (s)	5.4 (s)			2.0 (s)						12.4	10.9
H_2L2	2.55 (s)	2.6 (t)	1.7 (qt)	2.7 (t)	3.8 (m)	3.8 (m)	8.3 (s)			6.6–7.4 (m)								12.35	
H_2L3	2.5 (s)	2.7 (t)	1.8 (qt)	2.8 (t)	3.8 (m)	3.8 (m)		2.3 (s)		6.8–7.6 (m)								12.45	
H_2L4	2.55 (s)	2.7 (t)	1.8 (qt)	2.75 (t)	3.8 (m)	3.8 (m)	8.45 (s)			7.0–8.3 (m)								13.35	
H_2L5	2.6 (s)	2.7 (t)	1.9 (qt)	2.8 (t)	3.5 (m)	3.5 (t)	8.3 (s)			6.1–7.6 (m)								12.4	
H_2L6	2.55 (s)	2.65 (t)	1.9 (qt)	2.7 (t)	3.65 (m)	3.65 (m)	8.5 (s)					3.8 (s)	5.7 (s)			3.8 (s)	6.0 (s)	12.4	
H_2L7	2.55 (s)	2.65 (t)	1.9 (qt)	2.8 (t)	3.7 (m)	3.7 (m)		2.1 (s)						2.65 (s)	5.8 (s)			12.4	14.5
H_3L8	2.6 (s)	2.7 (t)	1.9 (qt)	2.8 (t)	3.5 (m)	3.5 (m)	4.0 (qt)	1.4 (d)										12.4	
H_2L9	2.6 (s)	2.7 (t)	1.9 (qt)	2.8 (t)	3.6 (m)	3.6 (m)												12.4	
H_3L10	2.6 (s)	2.7 (t)	1.9 (qt)	2.8 (t)	3.5 (m)	3.6 (m)					4.3(qd)		1.35(t)					12.4	

RF = 250 MHz, ppm vs. TMS in $CDCl_3$; abbreviations: s, singlet; d, doublet; m, multiplet; qd, quadruplet; qt, quintuplet; t, triplet.

TABLE 3. Physical and Chemical Characterization of the ^{99m}Tc Complexes

Complex	% Labeling	Temperature (°C)	pH	Retention time	R.f (CCM)	Stability pH 7.4	Partition coefficient
TcOL1	<15	—	—	—	—	Unstable	—
TcNL1	85	80°	1	2.8	0.33	Stable	420
TcOL2	<15	—	—	—	—	Unstable	—
TcNL2	95	60°	8	2.55	0.43	Stable	10
TcOL3	<15	—	—	—	—	Unstable	—
TcNL3	70	60°	8	5.1	0.19	Unstable	—
TcOL4	<15	—	—	—	—	Unstable	—
TcNL4	30	60°	8	2.5	0.5	Unstable	—
TcOL5	<15	—	—	—	—	Unstable	—
TcNL5	<15	—	—	—	—	Unstable	—
TcOL6	<15	—	—	—	—	Unstable	—
TcNL6	40	60°	8	2	0.25	Unstable	—
TcOL7	<15	—	—	—	—	Unstable	—
TcNL7	<15	—	—	—	—	Unstable	—
TcOL8	<15	—	—	—	—	Unstable	—
TcNL8	50	60°	1	1.9	0.36	Unstable	—
TcOL9	95	40°	6.8	3	0.59	Stable	12
TcNL9	80	70°	1	2	0.87	Stable	0.3
TcOL10	95	50°	2.2	3	0.59	Stable	14
TcNL10	80	70°	1	2	0.87	Stable	0.4

Animal Studies

Purified ^{99m}Tc complexes were diluted to a concentration of 74 MBq/mL in saline, and 300 μL of this solution were injected via the femoral vein into sedated (under Nesdonal intraperitoneal anesthesia) male Wistar rats (weighing 400 ± 50 g). At selected intervals (5 and 30 min) after the injection, the animals were sacrificed while under anesthesia. The organs were excised, weighed and counted. The percent dose per gram of organ was calculated by comparison of organ radioactivity levels with that of all pooled rat tissues.

RESULTS AND DISCUSSION

Ligand Characterization

The reported C, H, N, S microanalysis results (Table 1) fit the proposed formulations (Fig. 1). The ^{1}H NMR data collected in Table 2 verify that the spectra of each ligand is effectively constituted by the six typical signals of H_2LO (2) plus those of the variable moiety.

Comparative Study of the Complex Stability

For the 10 ligands, Table 3 reports the optimum radiolabeling yields and the corresponding pH and temperature conditions. Working in aerobic and high-dilution conditions, seven nitrido Tc complexes (40% > yield >95%) and only two oxo complexes (yield >95%) were isolated. The main objective of our work was to obtain ^{99m}Tc complexes stable in physiological medium; their stability was checked at pH 7.4 and 4 h after preparation. Finally, six complexes satisfied these conditions and were selected for biodistribution studies.

As observed in a previous work (2), nitrido complexes seem to be easier to stabilize than their oxo analogues. An interesting feature of these ligands is the possibility of tuning the partition coefficient of the complexes (and their lipophilicity) over a large range from 0.3 to 420 by performing structural modifications.

In the present case of Schiff base-type ligands H_2L1, H_2L2, H_2L3, H_2L4, H_2L5, H_2L6 and H_2L7, owing to the hydrolysis of the imine bond in acidic medium, the one-step method proposed by Belhadj-Tahar *et al.* (2) to prepare nitrido complexes was not convenient, so we used a two-step method (17) involving first the stabilization of the nitrido core at acid pH and then a ligand transfer reaction at a slightly basic pH. Finally, only the TcNL2 complex appeared stable. The unexpected instability of nitrido complexes of H_2L3, H_2L4, H_2L5, H_2L6 and H_2L7 highlights the importance of the inductive effects of substituents attached to the aromatic ring on the stability of these complexes. Furthermore, the enhancement of the stability of the nitrido complex of H_2L1 may be presumably attributed to the presence of π electron delocalization around the basic Tc-acetylacetone six-membered ring.

H_2L8 ligand was obtained by reduction of the imine bond with $NaBH_4$; however, the corresponding nitrido complex was not stable at pH 7.4. This is in accordance with the results of Pillai *et al.* (20) showing that complexes of reduced amine-phenol Schiff base could only be stabilized near pH 9.

The ligands H_3L9 and H_3L10 gave stable oxo complexes with very good yields (95%); interestingly, the oxo complex of H_3L9 was obtained in almost physiological conditions (pH 6.8, temperature 40°C).

Biodistribution

Biodistribution studies in the rat were performed for the six selected complexes. The distribution of organ activity expressed as a percent of the injected dose per gram of tissue is summarized in Table 4.

The data in Table 4 show that the heart activity is significant for the two neutral nitrido complexes of the dianionic ligands H_2L1 and H_2L2 and for the oxo complexes of H_3L9 and H_3L10. Recently, neutral nitrido Tc complexes of ligands derived from either dithiocarbamate or dithiocarboxylic acid proved to exhibit good heart uptake (5, 13, 18, 19). It may be suggested that the four complexes TcNL1, TcNL2, TcOL9 and TcOL10 were neutral and that the ligands H_2L1, H_2L2 on the one hand and H_3L9 and H_3L10 on the other hand thus behave as dianionic and trianionic ligand, respectively. Consequently, the two nitrido complexes TcNL9 and TcNL10 might be considered as monoanionic. This is in accordance (i) with the fact that the diamagnetic oxorhenium complex $Re^{(V)}OL9$ was neutral (1), (ii) with the measured value of the partition coefficients and (iii) with the fact that the nitrido complexes of H_3L9 and H_3L10 show strong uptake by

TABLE 4. Biological Distribution of ^{99m}Tc Complexes in the Rat

Complex	Liver	Spleen	Lung	Kidney	Heart	Brain	Blood
% Injected dose/g organ 5 min post-injection							
TcNL1	1.21 ± 1.12	1.09 ± 0.16	0.79 ± 0.09	1.03 ± 0.20	1.00 ± 0.02	0.25 ± 0.03	0.38 ± 0.07
TcNL2	2.09 ± 0.05	1.25 ± 0.07	1.45 ± 0.12	1.44 ± 0.41	1.34 ± 0.03	0.45 ± 0.04	0.23 ± 0.01
TcOL9	1.87 ± 0.05	1.30 ± 0.01	1.66 ± 0.01	2.41 ± 0.14	0.88 ± 0.01	0.06 ± 0.01	2.23 ± 0.06
TcNL9	5.53 ± 1.02	0.12 ± 0.03	0.32 ± 0.07	6.51 ± 1.01	0.16 ± 0.04	0.02 ± 0.01	0.53 ± 0.11
TcOL10	1.65 ± 0.03	1.40 ± 0.05	1.52 ± 0.10	2.35 ± 0.16	0.91 ± 0.06	0.02 ± 0.01	2.15 ± 0.13
TcNL10	5.70 ± 0.89	0.20 ± 0.01	0.30 ± 0.06	6.60 ± 1.10	0.12 ± 0.04	0.03 ± 0.00	0.47 ± 0.14
% Injected dose/g organ 30 min post-injection							
TcNL1	1.17 ± 0.11	0.55 ± 0.12	0.41 ± 0.05	0.66 ± 0.02	0.40 ± 0.10	0.11 ± 0.01	0.21 ± 0.05
TcNL2	1.55 ± 0.05	0.48 ± 0.09	0.70 ± 0.10	0.90 ± 0.01	0.63 ± 0.04	0.12 ± 0.04	0.07 ± 0.01
TcOL9	1.66 ± 0.08	1.61 ± 0.11	1.59 ± 0.19	2.57 ± 0.10	0.88 ± 0.11	0.05 ± 0.00	2.17 ± 0.03
TcNL9	2.8 ± 0.18	0.08 ± 0.01	0.18 ± 0.02	8.15 ± 0.34	0.09 ± 0.01	0.01 ± 0.00	0.21 ± 0.02
TcOL10	1.68 ± 0.06	1.59 ± 0.10	1.64 ± 0.17	2.49 ± 0.15	0.92 ± 0.09	0.07 ± 0.01	2.20 ± 0.06
TcNL10	2.65 ± 0.16	0.09 ± 0.01	0.16 ± 0.02	8.22 ± 0.28	0.06 ± 0.01	0.01 ± 0.00	0.19 ± 0.02

kidney as generally observed for charged complexes of ligands bearing a carboxylate group (3, 14).

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