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Synthesis, Characterization and Biodistribution in Mice of Oxotechnetium(V) (SNS/S) Mixed Ligand Complexes with N,N-bis(2-mercaptoethyl)(2-piperidin-1-ylethyl)amine as Tridentate Ligand.

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SUMMARY

A new SNS tridentate ligand containing piperidine group as pendant amine, N,N-bis(2-mercaptoethyl)(2-piperidin-1-ylethyl)amine, was synthesized by alkylation of bis(2-benzylthioethyl)amine with 1-(2-chloroethyl)piperidine in the presence of NaI, and deprotection of the thiols using Na/NH_3. Three neutral oxotechnetium mixed ligand complexes [SNS/S] with either *p*-toluenethiol, benzylmercaptan, or *p*-methoxybenzylmercaptan, were synthesized at carrier level [^{99}Tc] and characterized by elemental analysis, UV-vis, IR and ^{1}H NMR spectroscopies. The complex with *p*-toluenethiol as coligand was also characterized by x-ray crystallography. The same complexes were also prepared at tracer level [^{99m}Tc], and their in vivo biodistribution was investigated in mice. All ^{99m}Tc complexes have fast blood clearance, high uptake and significant retention into the brain.

Keywords : Brain Imaging, Tc-99, Tc-99m, mixed ligand complexes.

INTRODUCTION

The development of technetium-99m brain imaging agents is an area of radiopharmaceutical research that is still receiving much attention. A brain perfusion agent must be able to penetrate the intact blood-brain barrier and be distributed according to the brain blood flow. Moreover, its distribution into the brain must be unchanged for sufficient time to allow SPECT imaging (1-5).

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Extensive studies generated two technetium-99m radiopharmaceuticals, the ^{99m}Tc-d,l-HMPAO (6-10) and the ^{99m}Tc-l,l-ECD (11-13), which have been approved for clinical use as brain perfusion imaging agents. Both HMPAO (hexamethylpropyleneamine oxime) and ECD (ethyl cysteinate dimer) are linear tetradentate ligands, N_4 and N_2S_2 donor atom set respectively, and their complexation with oxotechnetium results in five-coordinated complexes with a square pyramidal geometry.

Recently mixed ligand oxotechnetium complexes with SNS, SOS SSS tridentate ligands have been used for the development of brain imaging agents (14-18). We have reported the synthesis of technetium ([^{99}Tc] and [^{99m}Tc]) neutral mixed ligand complexes (SNS/S) of the general formula TcO{[X-$CH_2CH_2N(CH_2CH_2S)_2$](S-R)} (19-22). It was found that usually only the *syn* isomer is formed at both carrier and tracer level, although the formation of two isomers (*syn* and *anti*) is possible. The ^{99m}Tc complexes were evaluated in mice and rats as potential brain perfusion imaging agents and they were found capable of penetrating the blood brain barrier and most of them resulted in significant retention into the brain. The brain uptake and retention were grandly influenced by both X and R substituents.

In a continuous effort to develop ^{99m}Tc mixed ligand complexes with improved characteristics new tridentate ligands were prepared. Previous studies have demonstrated that when the piperidinylethyl side chain was attached to the DADT ligand the brain uptake and retention were increased (23-25). In this paper we report the synthesis of a new SNS tridentate ligand, which carries the piperidinylethyl side chain attached to the nitrogen. The preparation of its ^{99}Tc and ^{99m}Tc mixed ligand complexes, with either *p*-toluenethiol, benzylmercaptan, or *p*-methoxybenzylmercaptan as coligand as well as the biodistribution data in mice, are presented.

EXPERIMENTAL

Caution!!! Technetium-99 is a weak β-emitter (0.292 MeV) with a half life of 2.12×10^5 years. All manipulations of solutions and solids were carried out in a laboratory approved for the handling of low level, long-lived radioisotopes. Normal safety procedures were followed at all times to prevent contamination.

Reagent-grade chemicals and HPLC solvents were obtained from commercial sources (Fluka Chemika and Aldrich Chemical). Commercially available *p*-toluenethiol, benzylmercaptan, p-methoxybenzylmercaptan were used. N,N-bis(2-benzylthioethyl) amine was synthesized by literature methods (26). ^{99}Tc was purchased as ammonium pertechnetate from the Oak Ridge National Laboratory. The impure black solid was purified prior to its use by treatment overnight with

hydrogen peroxide and ammonium hydroxide in methanol. Evaporation of the solvent gave ammonium pertechnetate as a white powder. The precursor Tc(V)-gluconate was synthesized by literature methods (27).

[^{99m}Tc]$NaTcO_4$ was obtained in physiological saline either as in house preparation (Techne/Demoscan) or as commercial $^{99}Mo/^{99m}Tc$ generator eluate (Cis International). Commercial glucoheptonate kits containing a lyophilized mixture of 200 mg calcium glucoheptonate and 0.2 mg $SnCl_2$ (Gluco/Demoscan, NCSR"Demokritos") were used.

Melting points were determined using an Electrothermal 9100 capillary melting point apparatus and are uncorrected. IR spectra were recorded as KBr pellets in the range 4000-500 cm^{-1} on a Perkin-Elmer 1600FT-IR spectrophotometer and were referenced to polystyrene. The ^1H NMR spectra were recorded on a Bruker FT-NMR/250 AF spectrometer and were referenced to internal tetramethyl silane (TMS). Elemental analyses were performed on a Perkin-Elmer 2400/II automated analyzer. Flash chromatography was carried out using either Merck 9385 silica gel or aluminum oxide. For thin layer chromatography Merck 5554 silica gel or Fluka 06408 aluminum oxide both on aluminum sheets were used. High performance liquid chromatography (HPLC) analyses were performed on a Waters chromatograph equipped with the 600E solvent delivery system, and a μ-Bondapak C-18 RP (10 μm, 3.9 mm x 300 mm) column using methanol/water, 95/5, as mobile phase at a flow rate of 1.0 mL/min. Detection of complexes was accomplished by a Waters 991 Photodiode Array detector (UV trace for ^{99}Tc and ligands) and a Beckman 171 detector (gamma trace for ^{99m}Tc). The radioactivity content of biological samples was counted in an automatic γ-counter [NaI(Tl) crystal, Canberra Packard Auto-Gamma 5000 series instrument].

Synthesis of the SNS ligand

N,N-bis(2-benzylthioethyl)(2-piperidin-1-ylethyl)amine (1)

1-(2-chloroethyl)-piperidine (3.00 g, 0.02 mol) and NaI (3.00 g, 0.02 mol) were added to a solution of N,N-bis(2-benzylthioethyl)amine (3.4 g, 0.01 mol) in 55 mL dry methylethylketone. The mixture was refluxed for 3h. Subsequently, volatiles were removed, the residue was made alkaline with aqueous 1N NaOH and extracted with dichloromethane (3 x 50 mL). The combined organic extracts were separated, washed with water and brine, dried (K_2CO_3), filtered, and concentrated under reduced pressure. The crude product was submitted to column chromatography on neutral aluminum oxide with ether-petroleum ether (1:10) as the eluent. The amine 1, was isolated as a slightly yellow

colored oil (Rf: 0.6 on TLC, aluminum oxide, ether petroleum ether, 1:1) and analyzed as the corresponding oxalate salt.

^{1}H NMR (δ, $CDCl_3$): 7.22 (m, 10H, C_6H_5CS), 3.67 (s, 4H, C_6CH_2S), 2.77-2.23 (complex, m, 16H, $(CH_2)_2NCH_2CH_2N(CH_2CH_2)_2$), 1.60 (m, 6H, $NpipC_2(CH_2)_3$). Elemental analysis: calculated for $C_{29}H_{40}N_2O_8S_2$ C: 57.22%, H: 6.63%, N: 4.60%, S: 10.51%; found: C: 57.10%, H:6.69 %, N: 4.71%, S:10.73 %. m.p. 176 °C.

N,N-bis(2-mercaptoethyl)(2-piperidin-1-ylethyl)amine (2)

Sodium (0.47 g, 20 mmol) cut in small pieces was placed in a round-bottom flask immersed in a liquid N_2 bath. With stirring under a nitrogen atmosphere, dry (CaO lumps) liquid NH_3 (100 mL) was collected in the flask. Then 2.5 g (5.8 mmol) of 1 in 5 mL dry ether were syringed into the resulting deep blue solution. Stirring was continued for 1h. In case of decoloration a small piece of sodium was added to restore the color of the solution. The NH_3 was subsequently swept away under a stream of N_2 and a solution of ethanol/2-propanol (2:1, 100 mL) saturated with HCl was added to the residue. The warm mixture was then immediately filtered to remove salt. The filtrate was condensed under reduced pressure and water (100 mL) and ether (100 mL) were added to the residue. The pH of the medium was adjusted to 7.5 with aqueous NaOH (2.5 N). The organic phase was separated, washed with water and brine, dried ($MgSO_4$), and filtered. The filtrate was saturated with dry HCl and converted to the hydrochloric salt. Recrystallization from ethanol/ether afforded pure product. m.p. 190 °C. ^{1}H NMR (δ, $CDCl_3$): 2.80 - 2.32 (complex, m, 16H, $(CH_2)_2NCH_2CH_2N(CH_2CH_2)_2$), 1.85 (bs, 2H, SH), 1.60 (m, 6H, $NpipC_2(CH_2)_3$). Elemental analysis: calculated for $C_{11}H_{26}N_2S_2Cl_2$ C: 41.24%, H: 8.19%, N: 8.75%, S: 19.98%; found: C: 41.30 %, H: 8.24 %, N: 8.19 %, S: 20.80 %.

Synthesis of ^{99}Tc-complexes

All three ^{99}Tc complexes were prepared and isolated by the same method.

[p-Methylbenzenethiolato][N,N-bis(2-mercaptoethyl)(2-piperidin-1-ylethyl)amine]oxotechnetium (V) (3)

A solution of stannous chloride (45 mg, 0.24 mmol) in HCl (1.0 mL, 1N) was added dropwise to an aqueous solution of NH_4TcO_4 (36.2 mg, 0.2 mmol) containing $^{99m}TcO_4^-$ (0.1 mL, 0.5 mCi) and sodium gluconate (200 mg) to obtain the ^{99}Tc-gluconate. The pH of the solution was adjusted to 7.5 with NaOH (1 N). This solution was added, with stirring, to a mixture of N,N-bis(2-mercaptoethyl)(2-piperidin-1-ylethyl)amine (49.6 mg, 0.2 mmol) and *p*-toluenethiol (24.8 mg, 0.2 mmol). The solution was stirred for 30 min. and then extracted with dichloromethane (3 x 10 mL). The organic phase was separated, dried over $MgSO_4$ and filtered. Analysis of the solution by HPLC

(C18, RP column using methanol/water, 95:5, as mobile phase) showed the presence of one complex. The volume of the solution was reduced to 5 ml and then 5 ml of methanol were added. Slow evaporation of the solvents at room temperature afforded the product as dark red crystals. Yield 55 mg (57%). Elemental analysis calculated for $C_{18}H_{29}N_2S_3OTc$: C: 44.70%, H: 6.04%, N: 5.79%, S: 19.89%; found: C: 44.36%, H: 5.87%, N: 5.61%, S: 19.51%. ^{1}H NMR (δ, $CDCl_3$): 7.52 (d, 2H, o-ArH), 7.22 (d, 2H, m-ArH), 3.92 (t, 2H, NCH_2CNpip), 3.59 (m, 4H, $NCCH_2S$ endo, NCH_2CS endo), 3.04 (m, 2H, $NCCH_2S$ exo), 2.68 (t, 2H, $NpipCH_2N$), 2.64 (m, 2H, NCH_2CS exo), 2.41 (m, 4H, $Npip(CH_2)_2$), 2.38 (s, 3H, Ar-CH_3), 1.60 (m, 6H, $NpipC_2(CH_2)_3$). IR (KBr, cm^{-1}) 926 (Tc=O str).

[Benzylthiolato][[N,N-bis(2-mercaptoethyl)(2-piperidin-1-ylethyl)amine]oxotechnetium(V) (4)

Brown crystals. Yield 62 mg (64%). Elemental analysis: calculated for $C_{18}H_{29}N_2S_3OTc$: C: 44.70%, H: 6.04%, N: 5.79%, S: 19.89%; found: C: 44.63%, H: 5.85%, N: 5.62%, S: 19.74%. ^{1}H NMR (δ, $CDCl_3$): 7.43 (m, 2H, o-ArH), 7.28(m, 2H, m-ArH), 7.18 (m, 1H, p-ArH), 4.85 (s, 2H, $ArCH_2S$), 3.94 (t, 2H, NCH_2CNpip), 3.59 (m, 4H, $NCCH_2S$ endo, NCH_2CS endo), 3.04 (m, 2H, $NCCH_2S$ exo), 2.68 (t, 2H, $NpipCH_2CN$), 2.64 (m, 2H, NCH_2CS exo), 2.40 (m, 4H, $Npip(CH_2)_2$), 1.60 (m, 6H, $NpipC_2(CH_2)_3$). IR (KBr, cm^{-1}) 919 (Tc=O str).

[p-Methoxybenzylthiolato][N,N-bis(2-mercaptoethyl)(2-piperidin-1-ylethyl)amine]oxotechnetium(V) (5)

Brown crystals. Yield 70 mg (68%). Elemental analysis: calculated for $C_{19}H_{31}N_2S_3O_2Tc$: C: 44.43%, H: 6.08%, N: 5.45%, S: 18.72%; found: C: 44.05%, H: 5.82%, N: 5.57%, S: 18.26%. ^{1}H NMR (δ, $CDCl_3$): 7.28 (d, 2H, o-ArH), 7.13 (d, 2H, m-ArH), 4.83 (s, 2H, $ArCH_2S$), 3.92 (t, 2H, NCH_2CNpip), 3.71 (s, 3H, CH_3O-Ar), 3.59 (m, 4H, $NCCH_2S$ endo, NCH_2CS endo), 3.04 (m, 2H, $NCCH_2S$ exo), 2.68 (t, 2H, $NpipCH_2N$), 2.64 (m, 2H, NCH_2CS exo), 2.39 (m, 4H, $Npip(CH_2)_2$), 1.60 (m, 6H, $NpipC_2(CH_2)_3$). IR (KBr, cm^{-1}) 918 (Tc=O str).

X-ray crystal structural determination of 3.

Diffraction measurements were made on a $P2_1$ Nicolet diffractometer upgraded by Crystal Logic using Zr-filtered Mo radiation. Unit cell dimensions were determined and refined by using the angular settings of 25 automatically centered reflections in the range $11°<2\theta<23°$ and they appear in Table 1. Intensity data were recorded using a θ-2θ scan to 2θ(max)=50 deg with scan speed 1.5 deg/min, scan range $2.5+\alpha_1\alpha_2$ separation.

Three standard reflections monitored every 97 reflections showed less than 3% variation and no decay. Lorentz, polarization and ψ-scan absorption correction were applied using Crystal Logic

Table 1. Summary of Crystal, Intensity Collection and Refinement Data for complex 3.

Formula	$C_{18}H_{29}N_2OS_3Tc$
FW	483.61
Temp, K	298
Wavelength	Mo Kα 0.71073
Space group	$P2_1/n$
a (Å)	11.735 (1)
b (Å)	10.980 (1)
c (Å)	17.118 (2)
β, deg	107.994 (3)
V (Å^3)	2097.7 (4)
Z	4
D_{calcd} /D_{measd} (Mg m^{-3})	1.531/1.51
Abs coeff, (μ), mm^{-1}	0.994
Max. abs. Correction mode	1.11
Scan mode/speed (deg/min)	θ-2θ/1.5
Scan range (deg)	2.5+$\alpha_1\alpha_2$ separation
θ range (deg)	1.87 to 24.99
Reflections collected	3875
Independent reflections	3683 [R(int)=0.0256
Range of h, k, l	0-13, 0-13, -20-19
F (000)	1000
$[\Delta/\sigma]_{max}$	0.001
W[(1)]	a=0.0263 b=0.000
$[\Delta\rho]_{max}[\Delta\rho]_{min}$ (e/Å^3)	0.583 and -0.308
Refinement method	Full matrix least-squares on F^2
Data/restraints/parameters	3683/0/342
Goodness of fit on F^2	1.103
R indices [2446 refs I >2σ(I)][(2)]	R1=0.0371 wR2=0.0803
R indices (all data)	R1=0.759 wR2=0.0974

(1) $W=1/[\sigma^2(Fo^2)+(a*P)^2+b*P]$ and $P=(Max\ (Fo^2, 0)+2*Fc^2)/3$
(2) R1 based on F's, wR2 based on F^2

software. The structure was solved by direct methods using SHELXS-86 (28) and refined by full-matrix least-squares techniques on F^2 with SHELXL-93 (29) using 3683 reflections and refining 342 parameters. All hydrogen atoms were located by difference maps and their positions were refined isotropically. All non-hydrogen atoms were refined anisotropically.

Biodistribution Studies in Mice

Complexes prepared at the tracer level (^{99m}Tc) were studied in mice (Swiss Albino, 29±5g). Three groups of male mice (at least 5 animals per group) were injected in the tail vein with HPLC purified and 30% methanol reconstituted ^{99m}Tc-complex (0.1 mL, 2-3 μCi). The animals were sacrificed by cardiectomy under slight ether anesthesia at a predetermined time interval (1, 10 and 45 min). The organs of interest were excised, weighed and counted in an automatic gamma counter. Bladder and

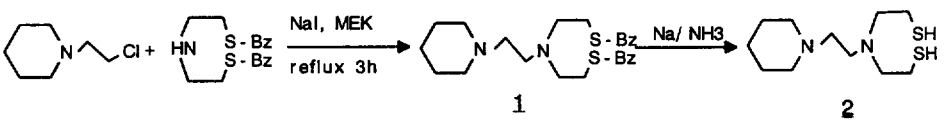
excreted urine were not weighed. The stomachs and intestines were not emptied of food contents prior to radioactivity measurements. The percentage of injected dose per organ (%ID/organ) was calculated by comparison of sample radioactivity to standard solutions containing 1% of the injected dose. The calculation for blood was based upon measured activity, sample weight and body composition data (considering that blood comprises 7% of body weight). The percentage of injected dose per gram (%ID/g) was calculated by dividing the %ID/organ by the weight of the organ or tissue. The brain to blood and the lungs to liver ratio were calculated by dividing the respective %ID/g values.

RESULTS AND DISCUSSION

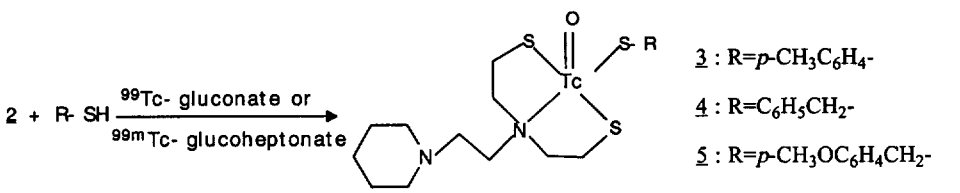
The synthesis of the tridentate ligand is shown in Scheme 1. The N,N-bis(2-benzylthioethyl)amine was synthesized according to the literature (26). The protected piperidine derivative, 1, was synthesized by alkylation of N,N-bis(2-benzylthioethyl)amine with 1-(2-chloroethyl)piperidine in the presence of NaI, in a molar ratio 1:2:2, in refluxing methylethylketone (3h). The tridentate ligand, N,N-bis(2-mercaptoethyl)(2-piperidin-1-ylethyl)amine, 2, resulted by thiol deprotection using Na/NH_3. Purification in both steps was effected through column chromatography (silica or alumina) and the products 1 and 2 were isolated as oxalate or hydrochloric salt respectively.

The ^{99}Tc complexes (carrier level) were prepared (Scheme 2) in good yield (57-68 %), by ligand exchange reaction using ^{99}Tc-gluconate as precursor and equimolar quantities of the tridentate ligand, 2, and the appropriate monodentate thiol (p-$CH_3C_6H_4SH$, $C_6H_5CH_2SH$, p-$CH_3OC_6H_4CH_2SH$ for 3, 4 and 5 respectively). The complexes were extracted into CH_2Cl_2 and isolated as crystalline products

Scheme 1



Scheme 2



(3: dark red, 4 and 5: brown). Elemental analysis performed for C, H, N, S was consistent with the proposed structure. The complexes were soluble in dichloromethane and acetone, slightly soluble in alcohols and insoluble in pentane and water.

Although two isomers are theoretically possible, only the formation of the *syn* isomer was confirmed. The formation of the *anti* isomer, was not confirmed by HPLC (is expected to have shorter retention and different UV spectrum). The electronic absorptions were also determined from the HPLC eluent using the PDA detector. The UV-vis spectra of complex 3 is characterized by an intensive band at 511 nm while for complexes 4 and 5, this band was shifted to lower wavelengths (480 and 484 nm, for 4 and 5 respectively).

The infrared spectra of the complexes showed the expected Tc=O stretch for the monooxo species in the region of 918-926 cm^{-1}. These are at the low energy end of the range thus far observed for TcO-complexes (890-1020 cm^{-1}) (30-34).

The ^1H NMR data of the complexes confirmed the presence of both ligands in the complex in a ratio 1:1. The coligands, L_2H, showed the expected signals which can be unambiguously assigned. The signals of the tridentate ligands, L_1H_2, fell in the region of 1.6-4.2 ppm. In all three complexes, the methylene protons (H-5) of the N-substitution near the coordinated nitrogen atom were found in a deshielded environment, at 3.92-3.94 ppm, indicating that all have the *syn* configuration.

The X-ray crystallography for complex 3 established the *syn* configuration of the ethyl piperinidyl side chain. An ORTEP diagram is shown in Figure 1, and selected bond distances and angles are given in Table 2.

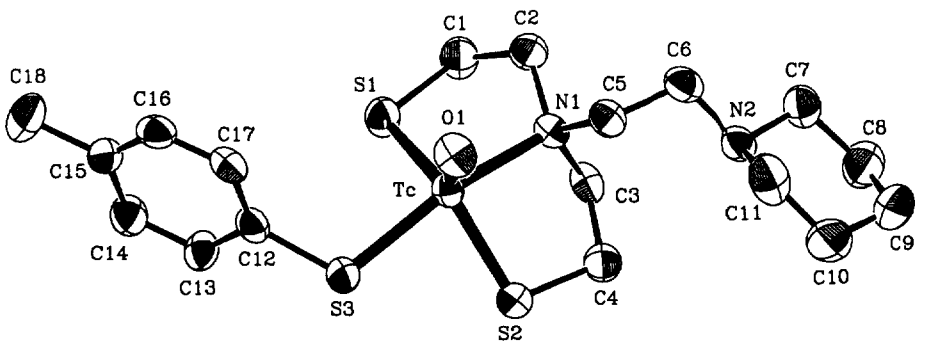


Figure 1. ORTEP diagram of complex 1, with 50% thermal probability ellipsoids showing atomic labelling scheme.

The metal-oxygen bond distance is 1.662 (4) Å well within the range of several characterized monooxo Tc-complexes (30-34). The metal sulfur bond distances are in the range of 2.267(1)-2.317(1) Å, while the Tc-N1 bond distance is 2.207 (1) Å. All of these parameters are in general agreement with the structural parameters for a large number of complexes containing the same donor atoms (14, 15). The bond angles between the diametrically opposite coordinated atoms N1-Tc-S3 and S1-Tc-S2 are 156.3 (1) and 122.54 (6) Å respectively. Analysis of the shape-determining angles using the approach of Addison et al (35), yields a trigonality index, τ, of 0.56 (τ=0 for square pyramidal geometry and τ=1 for trigonal bipyramidal geometry). Thus, the coordination geometry can be described as distorted trigonal bipyramid. The basal plane is formed by the S1, S2, and the oxo group with the N1 and S3 atoms occupying the two apical positions. Technetium lies 0.07Å out of the basal plane toward the monodentate thiol. The crystallographic data also demonstrated that all sulfur atoms undergo ionization during complexation so that the complex is neutral.

Table 2. Selected bond distances (Å) and angles (degrees) for complex 3.

Bond		Length (Å)	Angle			Degrees (°)	Angle			Degrees (°)
Tc	-O1	1.662 (4)	O1	-Tc	-S1	117.9 (1)	N1	-Tc	-S3	156.3 (1)
Tc	-S1	2.267 (1)	O1	-Tc	-S2	119.2 (1)	S1	-Tc	-N1	84.1 (1)
Tc	-S2	2.289 (1)	O1	-Tc	-S3	106.2 (1)	N1	-Tc	-S2	83.2 (1)
Tc	-S3	2.317 (1)	O1	-Tc	-N1	97.2 (2)	S2	-Tc	-S3	82.04 (5)
Tc	-N1	2.207 (3)	S1	-Tc	-S2	122.54 (6)	S3	-Tc	-S1	88.25 (5)

Labelling with ^{99m}Tc was carried out by reacting equimolar quantities of the tridentate and monodentate ligands with the ^{99m}Tc-glucoheptonate precursor with yields of about 85-95%.

The radiochemical purity of the extracts was checked by HPLC. A major peak was monitored in the radiochromatogram for all complexes (C-18 μ-Bondapak, MeOH:H_2O, 95:5, 1.0 ml/min). The radioactivity of the peak in each case was more than 95% of the radiochromatogram. When ^{99}Tc and ^{99m}Tc complexes were coinjected both radioactivity (for tracer) and UV (for carrier) detectors exhibited identical chromatographic profiles, demonstrating that the same chemical species (*syn* isomer) were formed under both chelating conditions. The ^{99m}Tc mixed ligand complexes of interest were isolated by HPLC and reconstituted to a 30% methanolic solution for the biological studies.

The in vivo distribution of the new ^{99m}Tc mixed ligand complexes (3-5) was examined in Swiss Albino mice (Table 3). After an intravenous injection all complexes showed high uptake and significant retention into the brain. Among them, the highest brain uptake values, at 1, 10 and 45 min p.i., were measured for complex 4 (7.25%, 6.17% and 4.59% dose/gram respectively). This complex also showed the fastest blood clearance. Thus, an increasing high brain to blood ratio was calculated, indicating its selectivity for the brain. The longest brain retention was measured for complex 3 which, compared to 4, has slightly lower initial brain uptake (6.16% dose/gram). Thus, the radioactivity into

Table 3. Tissue distribution (% dose/g) in mice (4-5 animals per group) following i.v. administration of the ^{99m}Tc complexes.

	1 min	10 min	45 min
		^{99m}Tc-3	
Blood	6.89±1.36	2.01±0.33	1.27±0.13
Brain	6.16±0.67	4.86±0.41	4.01±0.49
Lungs	82.93±5.65	43.54±4.60	16.05±1.01
Heart	25.41±3.49	6.74±0.87	4.06±0.47
Kidneys	10.68±1.81	13.51±1.57	9.97±0.66
Liver	7.88±1.45	19.47±0.69	17.35±2.65
Intestines	1.51±0.31	3.74±0.07	6.51±0.67
Br/Bl[1]	0.89	2.42	3.16
L/L[2]	10.52	2.24	0.92
		^{99m}Tc-4	
Blood	3.59±0.70	1.16±0.20	0.72±0.04
Brain	7.25±0.72	6.17±0.56	4.59±0.65
Lungs	86.88±12.44	45.22±12.25	10.74±0.72
Heart	23.64±2.67	7.40±0.95	2.58±0.44
Kidneys	10.07±5.01	14.02±0.89	9.97±1.02
Liver	3.76±1.30	7.63±1.01	12.03±1.27
Intestines	1.58±0.34	3.01±0.11	5.65±0.10
Br/Bl[1]	2.02	5.32	6.38
L/L[2]	23.11	5.93	0.89
		^{99m}Tc-5	
Blood	3.75±0.6	1.23±0.38	0.97±0.23
Brain	6.04±0.80	4.72±0.20	3.19±0.46
Lungs	64.47±15.81	41.21±9.79	11.08±3.56
Heart	17.80±3.18	5.49±2.34	1.94±0.14
Kidneys	11.45±3.34	13.56±2.56	7.87±1.77
Liver	5.76±2.35	16.56±2.59	18.63±1.70
Intestines	1.99±0.20	3.36±0.27	7.12±1.41
Br/Bl[1]	1.61	3.84	3.29
L/L[2]	11.19	2.49	0.59

[1] Brain to Blood ratio. [2] Lungs to Liver ratio

the brain at 10 min p.i. did not change significantly at 45 min p.i. (4.86±0.41 vs 4.01±0.49 respectively). Due to the higher blood values the brain to blood ratio was lower than that of 4, but it was still increasing by the time (0.89, 2.42, 3.16 at 1, 10 and 45 min respectivly).

The radioactivity from the studied ^{99m}Tc complexes was excreted mainly through the hepatobiliary system. At 45 min p.i., the radioactivity concentration observed in liver and intestines, ranged from 12.03-18.63 % dose/g and 5.65-7.12 % dose/g respectively. Elimination of the activity through the urinary tract is also evident. At the same time interval, 4.42-6.45 % of the injected radioactivity was detected in urine.

Significant concentration of radioactivity was measured in the lungs, especially at early times (64.47-86.88% and 41.21-45.22% dose/gram at 1 and 10 min p.i respectively). Although the complexes are excreted via the hepatobiliary system, the high lungs values resulted in high lungs to liver ratios.

CONCLUSIONS

In conclusion, a new tridentate ligand containing the SNS donor atom set, 2, has been synthesized by a new synthetic procedure, which may be used for the synthesis of SNS derivatives carrying pharmacologically interesting groups. Using the new tridentate ligand, three neutral oxotechnetium mixed ligand complexes [SNS/S] 3, 4, 5, were synthesized at carrier [^{99}Tc] and tracer level [^{99m}Tc]. All ^{99m}Tc complexes showed fast blood clearance, high uptake and significant retention in the mouse brain.

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Supplementary Material Available : Tables of fractional atomic coordinates and anisotropic thermal parameters for all non-hydrogen atoms, fractional atomic coordinates of H-atoms, and full bond lengths and angles. Ordering information is given on any current masthead page.

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