Novel ^{99m}Tc Aminobisthiolato/Monothiolato "3 + 1" Mixed Ligand Complexes: Structure−Activity Relationships and Preliminary in Vivo Validation as Brain Blood Flow Imaging Agents

Ioannis C. Pirmettis, Minas S. Papadopoulos, and Efstratios Chiotellis*

Institute of Radioisotopes-Radiodiagnostic Products, NCSR "Demokritos", POB 60228, 153 10, Aghia Paraskevi, Athens, Greece

Received April 9, 1996[⊗]

A series of neutral, lipophilic ^{99m}Tc mixed-ligand complexes of the general formula $^{99m}TcOL^1L^2$, where L^1H_2 is an N-substituted bis-(2-mercaptoethyl)amine, [X-$CH_2CH_2N(CH_2CH_2SH)_2$], [SNS], and L^2H is a monodentate thiol (RSH), [S], has been synthesized and evaluated in rodents for potential use in brain blood flow imaging. The complexes were prepared by ligand exchange reaction using ^{99m}Tc(V)O-glucoheptonate as precursor and equimolar quantities of the two ligands. In all cases the *syn* isomer was formed in a high yield, whereas the *anti* isomer was not always present. The formation of two isomeric complexes—*syn* and *anti*—was expected, since the N-substituent (X-CH_2CH_2N) can assume *syn* or *anti* configuration with respect to the $^{99m}TcO^{3+}$ core during complexation. One *anti* and all *syn* isomers were isolated by HPLC. Their identity was confirmed by comparative HPLC studies with the analogous ^{99}Tc complexes of established structure. In vivo distribution, in particular brain uptake and retention, greatly depended on the type of either tridentate (L^1H_2) or monodentate (L^2H) ligand. All ^{99m}Tc complexes showed significant brain uptake in mice (0.78−4.35% injected dose per organ at 5 min postinjection). This initial uptake remained nearly constant for at least 30 min for most of the complexes. Structure−activity relationships of novel ^{99m}Tc(V)O SNS/S complexes in mice are reported and discussed. Selected complexes were further studied in rats. High brain uptake, comparable to that of ^{99m}Tc−*d*,*l*-HMPAO, and sufficient retention 60 min postinjection were provided with complex **18** [X = $(C_2H_5)_2N$ and R = *p*-$CH_3OC_6H_4CH_2$].

Introduction

The development of technetium-99m brain perfusion imaging agents suitable for single photon emission computed tomography (SPECT) has been a subject of great interest in radiopharmaceutical research. These agents have to cross the intact blood−brain barrier but also must remain in the brain for sufficient time to allow SPECT imaging.[1]

A variety of tetradentate chelating systems containing the N_2S_2 (DADT, BAT)[2] or the N_4 (PnAO)[3] donor atom set have been extensively studied in the past for their ability to form neutral and lipophilic complexes with ^{99m}Tc. Only one representative of each group, ^{99m}Tc−*d*,*l*-HMPAO (hexamethylpropyleneamine oxime)[4] and ^{99m}Tc−ECD (ethyl cysteinate dimer),[5] has been approved so far for routine clinical application. Despite its wide clinical use, ^{99m}Tc−*d*,*l*-HMPAO is unstable in vitro, thereby complicating routine clinical use.[6] On the other hand, in extreme conditions of low and high brain blood flow, SPECT images obtained with the newly developed and in vitro stable ^{99m}Tc−ECD do not always accurately reflect regional cerebral blood flow (rCBF).[1e] The Tc−HMPAO and Tc−ECD complexes contain the monooxotechnetium core with the technetium center five-coordinated in a square pyramidal geometry.[7,8] The oxo ligand is at the apical position of the pyramid with the basal plane defined by either N_4 or N_2S_2 chelate.

An alternative challenging concept for designing neutral oxotechnetium complexes is based on the simultaneous action of a dianionic tridentate ligand (ONS), (ONO), (SOS), (SSS), (SNN),[9] or (SNS)[10] and a monoanionic monodentate thiolato (R-S) coligand on a suitable TcO^{3+} precursor (mixed ligand approach, "3 + 1" donor combination). The tridentate ligand binds to the TcO^{3+} core, leaving one coordination site *cis* to the oxo group open, which is occupied by the monodentate coligand.

We have recently described the synthesis at technetium carrier level and the characterization of such a series of SNS/S neutral mixed-ligand complexes of the general formula $^{99}TcO\{[X\text{-}CH_2CH_2N(CH_2CH_2S)_2](S\text{-}R)\}$.[11] Two isomers (*syn* and *anti*) are theoretically expected, due to the different orientation of the N-substituent of the tridentate ligand (X-CH_2CH_2N) with respect to the Tc═O core. However, the study[11] shows that the *syn* isomer was formed at a high yield, whereas the *anti* isomer was formed at a very low yield in only four cases. Crystallographic analysis for both isomers revealed that neutral, five-coordinated mixed ligand complexes form containing a TcO^{3+} core and a combination of one tridentate [X-$CH_2CH_2N(CH_2CH_2S)_2$] and one monodentate (R-S) ligand. The *syn* isomers[10b,11] adopt distorted trigonal bipyramidal geometry, a rather rare case for the oxotechnetium(V) core, whereas the *anti* isomer[11] demonstrated the usual distorted square pyramidal geometry (Figure 1).

In this work the synthesis at tracer level (^{99m}Tc) and structure−activity relationships of these complexes are reported. In particular, the effect of X and R substituents of the tri- or monodentate ligand on brain uptake and retention is investigated with the aim of exploring the SNS/S donor atom set for providing potential novel ^{99m}Tc brain blood flow imaging agents.

* Author to whom correspondence should be addressed. Tel. (+301) 6513793, (+301) 6524480. Fax (+301) 6543526.

[⊗] Abstract published in *Advance ACS Abstracts,* June 15, 1997.

S0022-2623(96)00273-7 CCC: $14.00

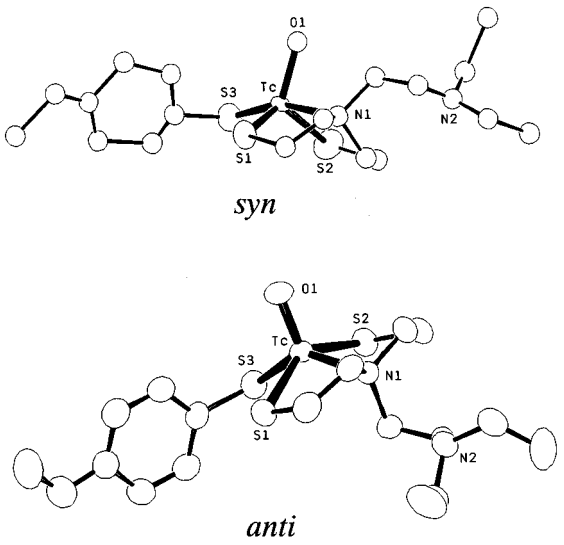
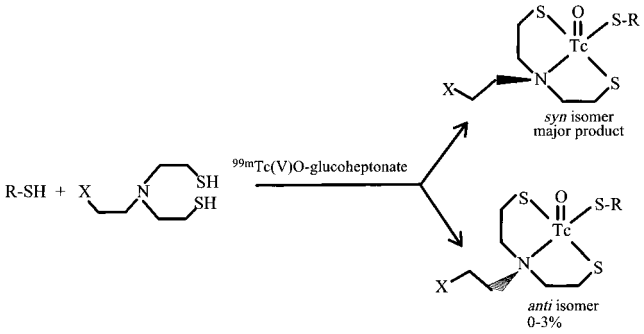


Figure 1. X-ray crystallography determined structure of *syn* (**4**)[10b] and *anti* (**4a**)[11] isomers.

Scheme 1

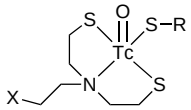


Results and Discussion

The technetium-99m mixed−ligand complexes ^{99m}TcO-{[X-$CH_2CH_2N(CH_2CH_2S)_2$](S-R)} were prepared by ligand exchange reaction with ^{99m}Tc(V)O-glucoheptonate as precursor (Scheme 1, Table 1). Due to the coordinating power of the SNS/S system, the reaction was fast and nearly quantitative, as determined by ITLC-SG and by organic solvent extraction of the aqueous reaction mixture. Aliquots of the organic extracts were analyzed by HPLC. Several systems and two types of columns (normal and reverse phase) were alternatively used in order to separate the complex from excess ligands and small amounts of radiochemical impurities. The best separations for complexes **1**, **2**, and **20** were achieved by elution of a C-18 column with methanol/water (70/30) at a flow rate of 1.5 mL/min. With this system the main radioactive peak (>95%) was observed at 7 min, while the ligands (monitored by UV detector) were eluted 3 to 4 min earlier. For the analysis of complexes **3**−**19** two systems were found suitable: (i) Normal phase column run with dichloromethane/methanol (85/15) and (ii) RP C-18 column run with methanol/water (95/5), both at a 1.0 mL/min flow rate. Ligands were eluted at 4 min with both systems, whereas the major complex appeared at 6−7 min. In some preparations (Figure 2), a small radioactive peak was detected at about 5 min. The structural validation accomplished by chromatographic correlation with the analogous ^{99}Tc complexes of established structure, available from a previous study,[11] demonstrated that the major radioactive species (>95%) in each preparation was the *syn* isomer. In a similar manner, the minor radioactive peak was identified in one preparation as the *anti* isomer (**4a**).

Prior to further evaluation, the ^{99m}Tc complexes **1**−**20** were purified by organic extraction and HPLC and used thereafter as a 50% aqueous methanolic solution. The stability and purity of the final solution was tested throughout the time of biological studies by HPLC

Table 1. Molecular Weight and Lipophilicity of the Studied ^{99m}Tc Complexes



compd	X	R	MW	log PC
1	H	*p*-$CH_3OC_6H_4$	417.49	2.77
2	CH_3O	*p*-$CH_3OC_6H_4$	447.52	2.81
3	$(CH_3)_2N$	*p*-$CH_3OC_6H_4$	460.56	2.38
4	$(C_2H_5)_2N$	*p*-$CH_3OC_6H_4$	488.61	2.63
4a	$(C_2H_5)_2N$	*p*-$CH_3OC_6H_4$	488.61	2.54
5	$(C_2H_5)_2N$	*m*-$CH_3OC_6H_4$	488.61	2.58
6	$(C_2H_5)_2N$	*o*-$CH_3OC_6H_4$	488.61	2.60
7	$(C_2H_5)_2N$	C_6H_5	458.59	2.63
8	$(C_2H_5)_2N$	*p*-BrC_6H_4	537.49	2.87
9	$(C_2H_5)_2N$	*p*-IC_6H_4	548.48	2.77
10	$(C_2H_5)_2N$	*p*-$CH_3C_6H_4$	472.61	2.66
11	$(C_2H_5)_2N$	*p*-$C_2H_5C_6H_4$	486.64	2.70
12	$(C_2H_5)_2N$	*p*-*i*-$C_3H_7C_6H_4$	500.67	2.83
13	$(C_2H_5)_2N$	*p*-*n*-$C_4H_9C_6H_4$	514.69	2.99
14	$(C_2H_5)_2N$	*p*-*t*-$C_4H_9C_6H_4$	514.69	2.97
15	$(C_2H_5)_2N$	3,4-$(CH_3)_2C_6H_3$	486.64	2.65
16	$(C_2H_5)_2N$	1-naphthyl	508.65	2.66
17	$(C_2H_5)_2N$	benzyl	472.61	2.38
18	$(C_2H_{5f})_2N$	*p*-$CH_3OC_6H_4CH_2$	502.64	2.61
19	$(C_2H_5)_2N$	*p*-*t*-$C_4H_9C_6H_4CH_2$	528.72	2.53
20	CH_3O	benzyl	431.52	2.66

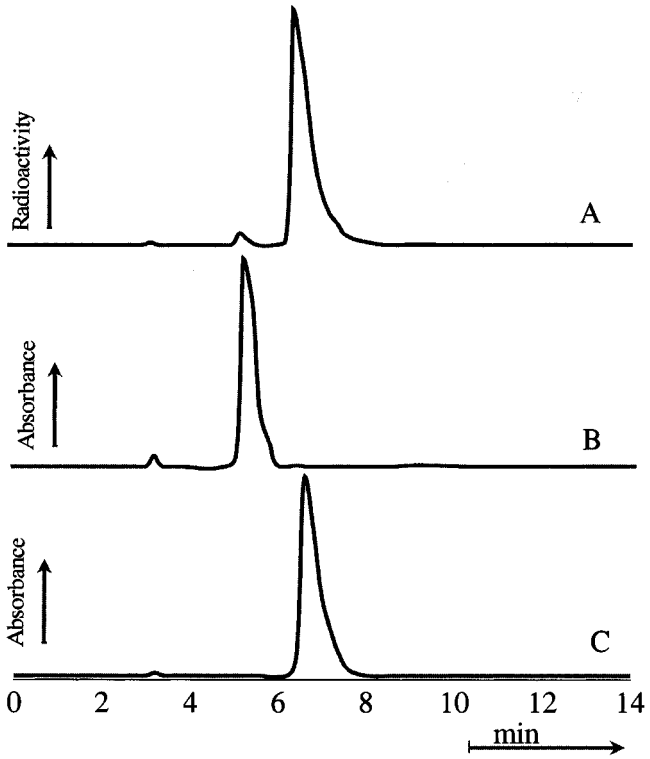


Figure 2. Representative HPLC chromatograms (C-18, methanol/water, 95/5, 1 mL/min): (A) Gamma trace of organic extract (crude) from the preparation of **4** and **4a** (^{99m}Tc); (B) UV trace at 254 nm of pure **^{99}Tc-4a** (*anti* isomer); (C) UV trace at 254 nm of pure **^{99}Tc-4** (*syn* isomer).

Table 2. Biodistribution of ^{99m}Tc Complexes (**1**−**20**) in Mice and Radioactivity in Brain and Blood at 5, 10, 30, and 60 min Postinjection (% ID/Organ ± sd, Average of Five to Seven Animals)

	brain				blood				brain/blood[a]				
compd	5 min	10 min	30 min	60 min	5 min	10 min	30 min	60 min	5 min	10 min	30 min	60 min	retention ratio[b]
1	1.59 ± 0.14	0.81 ± 0.15	0.16 ± 0.01	0.07 ± 0.00	3.20 ± 0.07	2.51 ± 0.08	1.78 ± 0.33	1.82 ± 0.15	2.50	1.42	0.40	0.17	0.10
2	1.84 ± 0.06	0.77 ± 0.02	0.21 ± 0.00	0.13 ± 0.00	3.90 ± 0.46	3.29 ± 0.40	3.52 ± 0.13	2.75 ± 0.28	2.05	1.02	0.27	0.20	0.11
3	2.93 ± 0.36	2.14 ± 0.05	1.55 ± 0.15	0.80 ± 0.14	6.72 ± 0.21	4.95 ± 0.54	3.74 ± 0.14	2.89 ± 0.18	1.96	1.94	1.87	1.30	0.52
4	3.01 ± 0.06	2.47 ± 0.27	1.71 ± 0.09	1.54 ± 0.11	2.02 ± 0.24	1.53 ± 0.20	1.29 ± 0.16	1.21 ± 0.13	4.43	4.71	4.16	4.23	0.57
4a	2.26 ± 0.37		0.56 ± 0.04		6.09 ± 1.34		5.14 ± 0.54		1.70		0.50		0.25
5	2.32 ± 0.21	1.99 ± 0.14	1.23 ± 0.24	0.79 ± 0.06	3.46 ± 0.21	2.70 ± 0.17	1.88 ± 0.12	1.30 ± 0.06	2.67	3.30	2.66	2.56	0.53
6	1.12 ± 0.13	1.10 ± 0.25	0.84 ± 0.07	0.64 ± 0.13	7.99 ± 0.36	6.24 ± 1.43	4.67 ± 1.18	2.65 ± 0.63	0.63	0.85	0.90	1.13	0.75
7	2.60 ± 0.34	2.92 ± 0.19	2.08 ± 0.30	1.10 ± 0.14	3.01 ± 0.38	2.47 ± 0.16	1.82 ± 0.09	1.30 ± 0.13	3.33	4.39	3.94	3.25	0.80
8	2.69 ± 0.28	2.69 ± 0.16	2.28 ± 0.17	1.39 ± 0.14	3.73 ± 0.42	2.70 ± 0.21	2.12 ± 0.26	1.76 ± 0.29	2.35	3.26	3.53	2.62	0.85
9	1.73 ± 0.41	2.25 ± 0.40	1.53 ± 0.37	1.12 ± 0.36	2.98 ± 0.61	2.24 ± 0.10	1.62 ± 0.55	0.89 ± 0.30	1.93	3.26	3.38	3.36	0.88
10	3.32 ± 0.26	3.88 ± 0.33	2.99 ± 0.18	1.77 ± 0.29	2.24 ± 0.24	1.74 ± 0.32	1.27 ± 0.16	1.18 ± 0.18	4.93	6.94	7.06	5.18	0.90
11	2.22 ± 0.07	2.20 ± 0.27	1.72 ± 0.10	1.14 ± 0.15	2.87 ± 0.13	2.01 ± 0.32	1.53 ± 0.24	1.43 ± 0.11	3.09	4.84	5.29	3.56	0.77
12	1.32 ± 0.20	1.38 ± 0.11	1.28 ± 0.11	0.94 ± 0.08	3.91 ± 0.42	2.11 ± 0.17	1.36 ± 0.19	1.23 ± 0.08	1.89	3.13	4.41	3.54	0.96
13	0.78 ± 0.06	0.91 ± 0.11	0.93 ± 0.10	0.61 ± 0.05	2.02 ± 0.61	1.51 ± 0.17	1.11 ± 0.18	0.72 ± 0.09	1.70	2.20	3.08	3.54	1.19
14	1.61 ± 0.28	1.86 ± 0.46	1.69 ± 0.23	1.29 ± 0.12	2.40 ± 0.25	1.74 ± 0.19	1.08 ± 0.08	0.80 ± 0.12	2.43	4.13	5.68	6.15	1.05
15	1.92 ± 0.19	1.85 ± 0.05	1.53 ± 0.18	1.03 ± 0.06	5.61 ± 0.83	3.22 ± 0.79	1.05 ± 0.17	0.81 ± 0.02	1.50	2.77	6.56	5.85	0.80
16	1.72 ± 0.22	1.65 ± 0.28	1.32 ± 0.20	1.00 ± 0.16	4.46 ± 1.70	3.63 ± 1.57	3.58 ± 1.38	2.60 ± 0.55	1.72	2.11	1.75	1.89	0.76
17	3.40 ± 0.39	3.71 ± 0.34	2.95 ± 0.18	1.80 ± 0.16	1.58 ± 0.20	1.31 ± 0.17	1.10 ± 0.03	1.02 ± 0.13	6.93	9.63	9.24	5.80	0.86
18	4.35 ± 0.33	4.22 ± 0.29	3.61 ± 0.47	2.33 ± 0.12	2.43 ± 0.12	1.73 ± 0.11	1.56 ± 0.17	1.35 ± 0.22	5.75	7.75	7.19	5.71	0.82
19	0.94 ± 0.13	0.81 ± 0.06	0.79 ± 0.08	0.65 ± 0.03	3.74 ± 0.29	2.22 ± 0.05	1.43 ± 0.07	1.16 ± 0.03	1.32	1.80	2.86	2.58	0.84
20	1.87 ± 0.29	1.12 ± 0.17	0.42 ± 0.06	0.17 ± 0.02	3.65 ± 0.55	3.60 ± 0.29	2.64 ± 0.53	1.82 ± 0.13	2.39	1.49	0.72	0.47	0.22

[a] Brain/blood: % ID/gram in brain divided by % ID/gram in blood. [b] Retention ratio is the brain uptake (% ID/organ) at 30 min pi divided by the brain uptake at 5 min pi.

analysis. The ^{99m}Tc complexes were found to be stable in dichloromethane and in a 50% methanolic solution for more than 6 h independent of the presence of ligands.

The logarithms of the octanol−buffer (pH 7.4) partition coefficients (log PC) are summarized in Table 1, reflecting the high lipophilicity of the compounds. Values for complexes **1**−**20** ranged between 2.38 and 2.99, and their molecular weight did not exceed 600, thereby satisfying two fundamental prerequisites for blood−brain barrier penetration.[1]

Observed differences in the log PC values of several close structural analogs were not consistent with theoretical log PC differences.[12] For example the log PC of the *p*-*n*-butyl analog **13** was expected to be more than 1 log unit greater than the *p*-methyl analog **10**, and the log PC of the *p*-iodo analog **9** was expected to be greater than the *p*-bromo analog **8**. These discrepancies are difficult to explain. One should take into consideration that the theoretically expected values for log P mainly refer to simple organic compounds in their neutral form, and this cannot always be extrapolated in complexes consisting of these compounds since electronic influences might be increasingly apparent after the binding of critical functional groups to the metal core. In addition, at pH 7.4, the diethylamino analogs are partially ionized, as is evident by the estimation of the log PC of complex **14** at various pH (log PC 1.53, 2.57, 2.95, 2.97, 3.09 at pH 3.0, 6.5, 7.0, 7.4, and 8.0, respectively). Thus, the theoretically expected values for log P cannot be directly applied to these mixed ligand complexes, since the reported values for log PC are the apparent partition coefficients at pH 7.4.

Biodistribution studies of purified ^{99m}TcO{[X-CH_2-CH_2N(CH_2CH_2S)$_2$](S-R)} complexes were carried out in Swiss Albino mice (Tables 2−5). It is generally observed that all ^{99m}Tc mixed-ligand complexes demonstrated a rather fast blood clearance in mice (Table 2).

The only *anti* isomer isolated in this work is **4a**, and pertinent data are included in Tables 2−5. Data for the *syn* isomer **4** of the same derivative have been additionally included herein, although reported previously,[10b] for comparison purposes. Both isomeric forms showed high uptake in the brain at 5 min pi [3.01 ± 0.06% ID/organ for *syn* (**4**) and 2.26 ± 0.37% ID/organ for *anti* (**4a**)]. However, the *syn* isomer showed longer retention, faster blood clearance, and greater brain to blood ratio compared to the *anti* isomer. Since the partition coefficient of the two stereoisomers are almost the same, the difference in brain retention must rely on their different stereochemistry. A similar steric effect for *syn* and *anti* isomers has been reported for substituted ^{99m}Tc−DADT derivatives.[1b,2d,g,j] The better characteristics of the *syn* isomer, as well as the fact that the *anti* isomers were formed in small to negligible amounts, led us to study only the *syn* isomers at this point.

In order to estimate the effect of X substituent on brain uptake and retention, a group of four complexes with *p*-methoxythiophenol as the coligand (**1**−**4**) were prepared and their biodistributions compared (Tables 2−5). This group displayed contrasting brain accumulation curves (Figure 3). Complexes **1** (X = H) and **2** (X = CH_3O) showed significant initial brain uptake (1.59 ± 0.14% and 1.84 ± 0.06% ID/organ respectively at 5 min pi) followed by a rapid clearance phase. A similar pattern was observed for **20** (X = CH_3O and R = C_6H_5-CH_2) as well. On the other hand, the use of an dialkylamino X substituent, dimethylamino **3** or diethylamino **4**, greatly influenced the brain uptake and retention of the forming $^{99m}TcO^{3+}$ complexes. Thus, the practically identical uptake of **3** and **4** (2.93 ± 0.36 and 3.01 ± 0.06% ID/organ at 5 min pi) declined much slower in **4**. Nevertheless, radioactivity from both compounds remained in the brain tissue for longer periods, as compared to the non-alkylamino-substituted derivatives **1**, **2**, and **20**. In addition, complex **4** exhibited a considerably higher brain to blood ratio with respect to **3** at the same time interval. It seems that the presence of amines in the side chain leads to significant changes in biodistribution and brain uptake

Table 3. Biodistribution of ^{99m}Tc Complexes (**1**−**20**) in Mice and Radioactivity in Lungs and Heart at 5, 10, 30, and 60 min Postinjection (% ID/Organ ± sd, Average of Five to Seven Animals)

	lungs				heart			
compd	5 min	10 min	30 min	60 min	5 min	10 min	30 min	60 min
1	1.87 ± 0.10	1.52 ± 0.53	0.83 ± 0.15	0.51 ± 0.02	0.39 ± 0.03	0.27 ± 0.06	0.09 ± 0.00	0.06 ± 0.01
2	1.37 ± 0.02	0.80 ± 0.11	0.49 ± 0.03	0.37 ± 0.04	0.40 ± 0.05	0.25 ± 0.02	0.11 ± 0.01	0.08 ± 0.00
3	10.82 ± 0.92	5.55 ± 1.19	1.48 ± 0.15	0.98 ± 0.02	0.89 ± 0.03	0.52 ± 0.03	0.26 ± 0.02	0.13 ± 0.01
4	15.37 ± 1.63	9.78 ± 1.33	3.09 ± 0.36	1.79 ± 0.14	0.74 ± 0.05	0.46 ± 0.07	0.19 ± 0.01	0.12 ± 0.01
4a	11.03 ± 1.37		3.49 ± 0.55		0.81 ± 0.04		0.28 ± 0.03	
5	13.79 ± 2.67	6.59 ± 1.32	2.58 ± 0.49	1.30 ± 0.35	0.98 ± 0.15	0.48 ± 0.07	0.23 ± 0.02	0.14 ± 0.04
6	6.46 ± 0.97	3.96 ± 0.20	2.05 ± 0.17	1.52 ± 0.07	0.67 ± 0.08	0.51 ± 0.06	0.26 ± 0.02	0.17 ± 0.03
7	14.06 ± 1.67	10.70 ± 1.73	3.80 ± 1.46	1.16 ± 0.14	0.69 ± 0.13	0.50 ± 0.07	0.21 ± 0.05	0.10 ± 0.01
8	14.56 ± 0.59	12.91 ± 0.93	7.99 ± 0.48	4.12 ± 0.65	1.19 ± 0.14	0.69 ± 0.09	0.26 ± 0.05	0.18 ± 0.02
9	7.39 ± 0.48	11.44 ± 4.19	6.56 ± 2.46	2.33 ± 0.46	1.18 ± 0.09	0.81 ± 0.28	0.18 ± 0.05	0.08 ± 0.02
10	18.76 ± 2.65	14.54 ± 1.49	6.45 ± 0.47	2.42 ± 0.24	1.24 ± 0.29	0.73 ± 0.08	0.29 ± 0.04	0.14 ± 0.02
11	10.26 ± 0.65	9.08 ± 1.54	4.70 ± 0.28	2.04 ± 0.39	1.05 ± 0.09	0.69 ± 0.10	0.29 ± 0.01	0.16 ± 0.02
12	8.49 ± 1.37	6.76 ± 1.07	4.84 ± 0.51	2.51 ± 0.36	1.23 ± 0.15	0.77 ± 0.08	0.34 ± 0.03	0.14 ± 0.03
13	6.02 ± 0.43	8.21 ± 2.26	7.20 ± 1.83	2.99 ± 0.64	1.31 ± 0.06	1.06 ± 0.23	0.36 ± 0.09	0.11 ± 0.02
14	14.56 ± 1.57	14.29 ± 3.54	9.48 ± 1.69	6.53 ± 1.07	1.98 ± 0.34	1.34 ± 0.16	0.49 ± 0.04	0.23 ± 0.04
15	11.72 ± 1.76	10.52 ± 2.09	3.87 ± 0.66	1.80 ± 0.17	1.25 ± 0.16	1.07 ± 0.11	0.46 ± 0.04	0.19 ± 0.01
16	9.43 ± 1.28	8.29 ± 0.70	4.31 ± 1.40	2.63 ± 0.38	1.37 ± 0.18	0.90 ± 0.06	0.28 ± 0.04	0.17 ± 0.02
17	13.01 ± 1.05	11.91 ± 1.06	5.98 ± 1.67	2.39 ± 0.15	0.65 ± 0.02	0.53 ± 0.07	0.25 ± 0.05	0.13 ± 0.01
18	16.91 ± 2.05	14.20 ± 0.52	6.26 ± 0.38	3.36 ± 0.34	0.92 ± 0.08	0.60 ± 0.04	0.34 ± 0.04	0.19 ± 0.02
19	5.38 ± 0.28	4.30 ± 0.35	4.11 ± 0.25	2.96 ± 0.49	1.60 ± 0.15	0.85 ± 0.08	0.31 ± 0.01	0.19 ± 0.02
20	1.33 ± 0.09	1.14 ± 0.10	0.85 ± 0.08	0.57 ± 0.06	0.46 ± 0.03	0.30 ± 0.14	0.18 ± 0.03	0.09 ± 0.01

Table 4. Biodistribution of ^{99m}Tc Complexes (**1**−**20**) in Mice and Radioctivity in Liver and Intestines at 5, 10, 30, and 60 min Postinjection (% ID/Organ ± sd, Average of Five to Seven Animals)

	liver				intestines			
compd	5 min	10 min	30 min	60 min	5 min	10 min	30 min	60 min
1	25.92 ± 1.24	36.80 ± 5.50	43.89 ± 3.59	40.08 ± 2.07	12.69 ± 1.34	17.88 ± 0.13	25.59 ± 1.37	30.07 ± 1.31
2	28.56 ± 1.42	35.57 ± 2.05	42.33 ± 2.82	40.80 ± 2.69	8.61 ± 0.89	13.99 ± 2.01	24.77 ± 1.49	35.23 ± 2.82
3	16.28 ± 0.30	26.39 ± 2.27	31.97 ± 3.92	28.92 ± 1.31	8.38 ± 0.40	9.74 ± 0.43	15.89 ± 0.55	20.13 ± 1.10
4	10.73 ± 1.85	14.14 ± 0.84	25.58 ± 2.87	35.12 ± 1.56	8.31 ± 0.55	9.61 ± 0.72	18.99 ± 0.96	33.70 ± 0.80
4a	17.06 ± 2.33		27.33 ± 1.84		9.22 ± 0.63		21.19 ± 1.11	
5	12.81 ± 2.21	23.05 ± 3.22	30.17 ± 4.88	34.55 ± 1.47	8.46 ± 1.43	11.70 ± 1.72	18.39 ± 1.40	35.73 ± 4.90
6	14.22 ± 0.92	18.88 ± 4.77	23.25 ± 5.03	23.62 ± 2.94	9.12 ± 0.91	13.44 ± 0.34	18.61 ± 1.84	25.74 ± 3.86
7	8.97 ± 0.82	15.74 ± 1.64	28.60 ± 3.83	25.73 ± 3.23	6.37 ± 0.65	9.93 ± 1.18	19.56 ± 2.34	26.01 ± 3.19
8	16.18 ± 1.63	25.91 ± 3.33	35.30 ± 5.78	36.34 ± 5.38	7.19 ± 0.75	8.85 ± 1.05	20.56 ± 2.39	35.23 ± 3.10
9	23.25 ± 2.71	29.04 ± 4.82	35.89 ± 3.50	32.34 ± 5.79	6.87 ± 1.37	7.81 ± 0.32	18.75 + 2.14	31.46 ± 5.27
10	11.26 ± 2.76	18.09 ± 1.46	29.03 ± 2.10	29.85 ± 2.07	7.91 ± 1.24	9.94 ± 0.80	18.13 ± 1.33	35.67 ± 3.63
11	15.46 ± 2.71	20.57 ± 0.91	32.00 ± 1.99	35.78 ± 2.36	7.38 ± 0.77	8.10 ± 0.90	13.84 ± 0.84	20.65 ± 4.13
12	15.15 ± 0.98	20.40 ± 1.16	31.04 ± 3.57	32.41 ± 5.41	5.42 ± 0.51	7.47 ± 0.99	10.95 ± 0.78	18.25 ± 2.43
13	28.93 ± 2.37	30.41 ± 5.28	35.95 ± 2.47	45.62 ± 3.00	5.33 ± 0.38	6.49 ± 0.49	12.68 ± 1.18	21.32 ± 4.72
14	14.62 ± 2.88	22.31 ± 3.13	30.42 ± 2.54	33.04 ± 1.63	5.81 ± 0.94	7.73 ± 0.92	13.04 ± 1.33	22.92 ± 2.87
15	15.94 ± 1.40	19.23 ± 3.04	26.32 ± 0.93	29.96 ± 1.24	6.94 ± 0.56	7.78 ± 0.61	14.16 ± 0.91	22.63 ± 1.66
16	18.30 ± 2.11	22.65 ± 1.86	31.02 ± 3.09	31.87 ± 4.88	6.68 ± 0.52	7.45 ± 1.10	12.52 ± 1.21	23.09 ± 3.27
17	10.78 ± 1.01	14.35 ± 2.31	22.55 ± 3.00	25.50 ± 2.77	8.07 ± 0.24	10.64 ± 1.76	18.42 ± 1.58	29.30 ± 3.54
18	12.34 ± 1.64	17.91 ± 3.09	30.02 ± 1.62	30.61 ± 1.79	8.22 ± 0.84	11.18 ± 0.69	19.55 ± 1.48	29.37 ± 2.13
19	19.53 ± 1.95	25.65 ± 0.91	30.79 ± 0.85	29.90 ± 1.28	3.38 ± 0.82	6.05 ± 0.65	11.25 ± 1.53	16.13 ± 0.82
20	29.87 ± 2.44	36.11 ± 3.60	43.12 ± 3.63	37.18 ± 3.54	7.95 ± 1.18	9.85 ± 1.86	22.66 ± 2.15	32.68 ± 1.49

profiles with respect to simple alkyl-substituted analogues. The pH shift mechanism[1a] for trapping weak bases within the brain and/or subsequent binding to cellular components may be responsible for this result. Similar results were also reported by other workers for dialkylamino derivatives of ^{99m}Tc diaminodithiol complexes.[1c,2d,g] Therefore, *N*,*N*-bis(2-mercaptoethyl)-*N*′,*N*′-diethylethylenediamine has clearly proved to be the tridentate ligand of choice in terms of brain retention. Furthermore, by keeping this tridentate ligand, brain retention may be further prolonged by modification of the monodentate thiolato coligand.

Thus, in a second group of ^{99m}Tc(V)O SNS/S complexes (**4**−**6**) the position of the methoxy substituent on the coligand was varied for comparison [X = $(C_2H_5)_2N$]. It was found that meta (**5**) or ortho (**6**) substitution caused a drop in brain uptake (2.32 ± 0.21% ID/organ for **5** and 1.12 ± 0.13% ID/organ for **6** at 5 min pi). Moreover, the blood clearance was slower. Note that the activity in blood at 5 min pi for **6** was the highest of all studied complexes. It is apparent that para substitution (**4**) resulted in more desirable biological characteristics.

The type of substituent at the para position in the coligand also influenced brain uptake and retention, as evident in complexes **7**−**14** [X = $(C_2H_5)_2N$]. Complex **7**, containing the unsubstituted thiophenol as the coligand, showed maximum brain accumulation at 10 min pi (2.92 ± 0.13% ID/organ), which thereafter decreased with time (2.08 ± 0.30% ID/organ at 30 min and 1.10 ± 0.14% ID/organ at 60 min pi). Replacement of the *p*-methoxy group by a bromine (**8**) or an iodine atom (**9**) resulted in a longer brain retention although initial brain uptake was lower. Thus, the initial brain uptake of *p*-halide-substituted derivatives remained practically unchanged for 30 min pi (2.69 ± 0.28% ID/organ at 5 min and 2.28 ± 0.17% ID/organ at 30 min pi for **8** and 1.73 ± 0.41% and 1.53 ± 0.37% ID/organ for **9**).

Diethylamino complexes [X = $(C_2H_5)_2N$] containing *p*-alkylthiophenol as the coligand (**10**−**14**) showed brain

Table 5. Biodistribution of ^{99m}Tc Complexes (**1**−**20**) in Mice and Radioactivity in Bladder plus Urine, Stomach, and Spleen at 60 min Postinjection (% ID/Organ ± sd, Average of Five to Seven Animals)

compd	bladder[a]	stomach	spleen
1	8.04 ± 0.94	0.75 ± 0.32	0.04 ± 0.01
2	10.48 ± 1.13	0.37 ± 0.14	0.06 ± 0.01
3	18.36 ± 2.13	1.59 ± 0.12	0.33 ± 0.13
4	6.87 ± 1.37	2.34 ± 0.74	0.21 ± 0.02
4a	2.24 ± 0.03[b]	2.43 ± 0.28[b]	0.61 ± 0.30[b]
5	13.12 ± 2.15	0.99 ± 0.53	0.20 ± 0.10
6	27.81 ± 8.48	1.70 ± 0.34	0.12 ± 0.02
7	10.78 ± 2.97	1.66 ± 0.81	0.11 ± 0.02
8	7.32 ± 3.60	1.71 ± 0.32	0.27 ± 0.03
9	18.42 ± 7.38	1.01 ± 0.24	0.07 ± 0.04
10	7.08 ± 0.56	2.56 ± 0.66	0.26 ± 0.02
11	5.78 ± 0.69	2.94 ± 0.28	0.41 ± 0.11
12	6.28 ± 0.41	1.86 ± 0.35	0.42 ± 0.15
13	4.69 ± 0.95	1.69 ± 0.68	0.19 ± 0.04
14	3.44 ± 1.45	1.76 ± 0.42	0.45 ± 0.03
15	5.57 ± 0.85	1.50 ± 0.14	0.67 ± 0.15
16	13.25 ± 3.65	1.41 ± 0.39	0.38 ± 0.09
17	4.35 ± 0.74	1.43 ± 0.48	0.30 ± 0.06
18	6.49 ± 1.00	2.13 ± 0.87	0.37 ± 0.08
19	4.80 ± 1.23	1.40 ± 0.29	0.63 ± 0.24
20	6.14 ± 2.72	1.53 ± 0.75	0.06 ± 0.00

[a] Bladder plus excreted urine. [b] At 30 min.

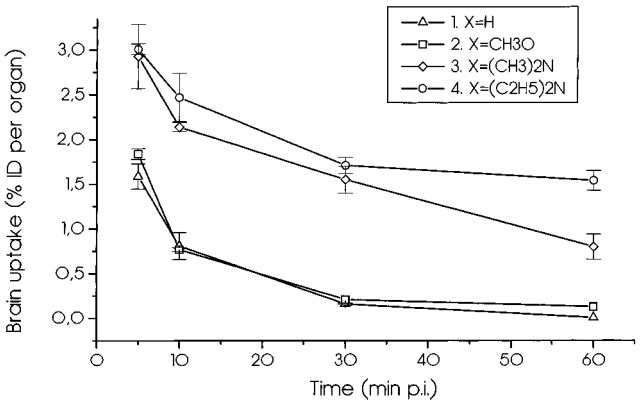


Figure 3. Time course of radioactivity in mouse brain for complexes **1**−**4** that have 4-methoxythiophenol as the coligand. Results are shown as the mean ± sd (SEM) for groups containing five to seven mice at various times after intravenous injection. Data are expressed as percent dose per organ.

uptake and retention depending on alkyl chain length. Initial brain uptake dropped, but retention was prolonged, as the chain length increased. Thus, complex **10** (R = *p*-$CH_3C_6H_4$) showed one of the highest brain uptakes (3.88 ± 0.33% ID/organ at 10 min pi), while the *p*-*tert*-butylthiophenol derivative **14** exhibited the most persistent brain retention in the series (the high initial brain uptake of 1.61 ± 0.28% ID/organ at 5 min pi did not drop below 1.29 ± 0.12% ID/organ at 60 min pi). The retention of **14** was more evident by the increase of brain to blood ratio with time (Table 2). The above values qualify complex **14** for further evaluation as a potential brain blood imaging agent.

Neither 3,4-dimethylthiophenol (**15**) nor α-thionaphthol (**16**) coligands improved brain uptake or retention of resulting ^{99m}Tc complexes. On the other hand, the use of benzyl mercaptan (**17**) or 4-methoxybenzyl mercaptan (**18**) as the coligand caused an increase in brain uptake in comparison to respective thiophenol derivatives (**7** and **4**). Complex **18**, which exhibited the highest brain uptake in the series (4.35 ± 0.33% ID/organ at 5 min pi), was of particular interest. The uptake remained nearly constant for up to 30 min pi

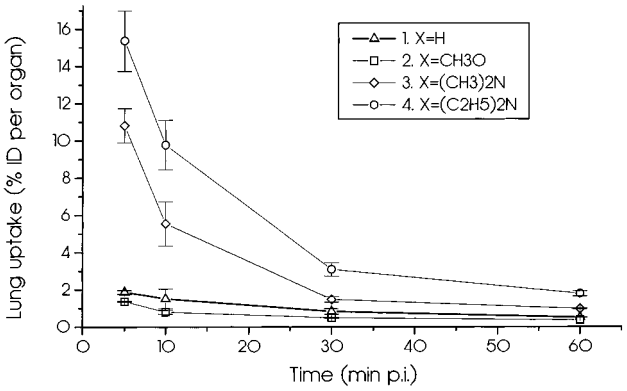


Figure 4. Time course of radioactivity in mouse lungs for complexes **1**−**4** that have 4-methoxythiophenol as the coligand. Results are shown as the mean ± sd (SEM) for groups containing five to seven mice at various times after intravenous injection. Data are expressed as percent dose per organ.

(3.61 ± 0.47% ID/organ) and thereafter started to drop (2.33 ± 0.12% ID/organ at 60 min pi). Thus, complex **18** can also be characterized as a candidate for further evaluation as a potential brain blood imaging agent.

Significant accumulation of radioactivity was observed in the lungs ranging between 1.33% and 18.76% ID/organ at 5 min pi (Table 3). However, no correlation between lipophilicity—as determined by the octanol buffer partition coefficient at pH 7.4—and lung uptake was evident. Besides coligand type, the presence of a dialkylamino substituent on SNS ligand was found to be crucial in affecting lung uptake. This is better shown in the representative lung clearance profiles for complexes containing the *p*-methoxythiophenol coligand (**1**−**4**) summarized in Figure 4. In fact, derivatives with a pendant free dialkylamino group (**3** and **4**) exhibited a much higher lung uptake in respect to their non-alkylamino-substituted analogues (**1** and **2**). These results indicate that lung uptake of ^{99m}Tc SNS/S complexes may involve the amine uptake system of the lungs, as reported by other workers.[2,13]

In a similar manner, heart uptake was greatly influenced by the presence of a free dialkylamino group on the tridentate ligand and/or the type of the coligand (Table 3). Thus, a lower heart uptake (0.39% to 0.46% at 5 min pi) was displayed by complexes **1**, **2**, and **20** lacking the free dialkylamino group, with respect to the dialkylamino derivatives (**4**−**19**). This increase in heart uptake observed in dialkylamino analogues was further enhanced by the introduction of alkyl or halide substituents on the aromatic ring of the coligand. In particular, the introduction of a *tert*-butyl substituent on the coligand (**14** and **19**) resulted in the highest myocardial uptake observed in this series (1.98% and 1.60% ID/organ at 5 min pi).

The activity from the new ^{99m}Tc compounds was excreted mainly through the hepatobiliary system. Thus, 23.6−45.6% and 16.1−35.7% of the injected activity was found in the liver and intestines at 60 min pi (Table 4). Elimination of the activity through the urinary tract is also evident as indicated by bladder values ranging from 3.4% to 27.8% of the injected dose at 60 min pi (Table 5). Stomach and spleen values at 60 min pi (Table 5) were within acceptable levels, indicating no significant decomposition of the complex in vivo at times as long as 60 min pi.

Table 6. Biodistribution of ^{99m}Tc Complexes (**14**, **17**, and **18**) in Rats and Uptake of Radioactivity in Brain and Blood at 5, 10, 30, and 60 min Postinjection (% ID/Organ ± sd, Average of Three to Five Animals)

compd	5 min	10 min	30 min	60 min
		Brain		
14	0.75 ± 0.12	0.92 ± 0.05	0.74 ± 0.14	0.61 ± 0.05
17	1.78 ± 0.21	1.93 ± 0.13	1.28 ± 0.07	0.70 ± 0.12
18	2.27 ± 0.30	2.29 ± 0.25	2.28 ± 0.36	1.40 ± 0.21
HMPAO[b]	1.92 ± 0.17		2.24 ± 0.21[c]	1.88 ± 0.19
		Blood		
14	6.67 ± 0.46	7.81 ± 1.52	5.73 ± 2.73	3.16 ± 1.52
17	19.63 ± 1.13	13.69 ± 1.75	8.81 ± 0.35	4.07 ± 0.74
18	9.92 ± 1.01	10.20 ± 0.73	5.76 ± 0.54	4.50 ± 0.34
HMPAO[b]	11.25 ± 0.57		9.97 ± 0.79[c]	8.74 ± 0.95
		Brain/ Blood[a]		
14	0.86	0.95	1.36	1.46
17	0.69	1.10	1.25	0.93
18	0.95	1.00	1.49	1.27

[a] Brain/Blood: % ID/gram in brain divided by % ID/gram in blood. [b] Data from ref 4a. [c] At 20 min pi.

Complexes **14**, **17**, and **18** were further evaluated in rats. In Table 6, the accumulation of radioactivity in brain and blood is presented in comparison to literature data[4a] for ^{99m}Tc−*d*,*l*-HMPAO. Blood clearance for complexes **14** and **18** was faster, compared to ^{99m}Tc−*d*,*l*-HMPAO, while complex **17** showed higher blood activity at 5−10 min pi. Complex **18** displayed high brain uptake, similar to that of ^{99m}Tc−*d*,*l*-HMPAO. Prolonged retention was shown by complex **14** although it resulted in lower brain values compared to complexes **17** and **18**. In general the washout rates of the three novel complexes from brain tissue were faster than that of ^{99m}Tc−*d*,*l*-HMPAO. However brain activity of compound **18** remained relatively high at 60 min pi. Thus, compound **18** can be further evaluated as a possible brain blood flow imaging agent.

In conclusion, neutral and lipid-soluble $^{99m}TcO^{3+}$ mixed ligand complexes can be easily prepared in high yield and radiochemical purity by reacting one tridentate [X-$CH_2CH_2N(CH_2CH_2SH)_2$] and one monodentate thiolato (RSH) ligand on ^{99m}Tc(V)O-glucoheptonate precursor. Although two isomers (*syn* and *anti*) are theoretically possible, the *syn* isomer formed almost quantitatively, whereas the *anti* isomer formed in negligible amounts. The studied complexes were found to be stable in aqueous media and also in organic solvents.

The biodistribution study in mice demonstrated that this new family of ^{99m}Tc(V)O SNS/S complexes, after intravenous administration, are rapidly cleared from circulation and are well transported in the brain and other tissues of interest. Complex **18** showed the most promising biological properties, in rats, as compared to ^{99m}Tc−*d*,*l*-HMPAO. Therefore it has been selected for further evaluation as a potential brain blood flow imaging agent.

The investigation of structure−activity relationships in mice derived the following conclusions:

(i) The *syn* isomer—in which the N-substituent is positioned *syn* to the metal oxo core—displays better characteristics in terms of brain uptake and retention.

(ii) The type of substituent on the tridentate and/or the monodentate ligand is crucial in determining brain uptake and retention but also the overall biodistribution.

(iii) Dialkylamino substitution on the tridentate ligand prolonged brain retention.

(iv) Substitution of coligands at the para position of the aromatic ring resulted in a higher brain uptake and higher brain/blood ratios. In addition, brain retention was enhanced by introduction of bulky para substituents on the coligand.

This initial work on $^{99m}TcO^{3+}$ mixed-ligand complexes containing the SNS/S donor atom set also provides a basis for the design and development of new ^{99m}Tc complexes which could be oriented to other organs or tissues by modifying the X substituent of the tridentate or the monodentate ligand.

Experimental Section

All chemicals were reagent grade. Solvents used in chromatographic analysis were HPLC grade. The tridentate ligands and the monothiols, which were not commercially available, were synthesized by the previously described methodology.[11,14]

All ^{99}Tc complexes (20 *syn* and one *anti* isomers) were available from a previous study.[11]

[^{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 0.2 mg of $SnCl_2$ and 200 mg of calcium glucoheptonate (Gluco/Demoscan, NCSR "Demokritos") were used.

High-performance liquid chromatography (HPLC) was conducted on a Waters chromatograph equipped with a 600E solvent delivery system. A μ-Bondapak C-18, RP, 10 μm, 3.9 mm × 300 mm column eluted with methanol/water, 95/5, at a flow rate of 1.0 or 70/30 at 1.5 mL/min and a Porasil, 10 μm, 3.9 mm × 300 mm column eluted with dichloromethane/methanol (85/15) at a flow rate of 1.0 mL/min were used. Detection of complexes was accomplished by a Waters 991 photodiode array detector (UV trace for ^{99}Tc and ligands) and a Beckman 171 detector (γ trace for ^{99m}Tc). The radioactivity content of biological samples, and solutions used for partition coefficient measurements, was counted in an automatic γ-counter [NaI(Tl) crystal, Canberra Packard Auto-Gamma 5000 series instrument].

Radiochemistry. General Method for the Preparation of $^{99m}TcOL^1L^2$ Complexes: A Gluco/Demoscan kit was reconstituted with 10 mL of water, and then, a 1.0 mL aliquot was mixed with 0.5−1.0 mL of [^{99m}Tc]pertechnetate solution (5−10 mCi). The ^{99m}Tc(V)O-glucoheptonate solution was added to a centrifuge tube containing equimolar quantities (0.02 mmol) of a tridentate (L^1H_2) and a monodentate ligand (L^2H). The mixture was agitated in a vortex mixer and left to react at room temperature for 10 min. This time was sufficient for a quantitative exchange between ^{99m}Tc(V)O-glucoheptonate and the ligands (ITLC-SG; physiological saline $R_f = 1$ for ^{99m}Tc(V)O-glucoheptonate and $R_f = 0$ for ^{99m}Tc mixed-ligand complex). The complex was extracted with CH_2Cl_2 (3 × 1.5 mL), and the combined organic extracts were dried over $MgSO_4$, and filtered. The extraction was nearly quantitative for all preparations (85−99%).

In all preparations the HPLC analysis of the organic extracts (50 μL, 50−100 μCi) showed one major radioactive peak (>95%), which was proven to be the *syn* isomer. In some preparations a minor peak (~3%) was observed and in case of **4a** was identified as the anti isomer.

The characterization of ^{99m}Tc complexes was accomplished by chromatographic correlation (HPLC) with the analogous ^{99}Tc complexes, available from a previous study.[11]

^{99m}Tc complexes of interest, one *anti* (**4a**, minor peak) and all of the *syn* isomers (major peak), were isolated by manual collection of HPLC eluents. Collected fractions were diluted to a 50% methanolic solution. In the case of the normal phase eluents, the sample was evaporated to dryness, under a mild stream of nitrogen followed by the addition of 50% methanol.

Determination of Partition Coefficient. The apparent partition coefficient for complexes **1**−**20** was determined by mixing aliquots of each purified ^{99m}Tc complex with 1-octanol and phosphate buffer (0.125 M, pH 7.4). In a centrifuge tube, containing 2 mL of each phase, 0.1 mL of the ^{99m}Tc complex solution was added, and the mixture was agitated on a Vortex mixer and finally centrifuged at 5000 rpm for 5 min. Three samples (0.2 mL each) from each layer were counted in a γ-counter. The partition coefficient was calculated as the mean value of each cpm/mL of octanol layer divided by that of the buffer. A sample (1.0 mL) from the octanol layer was subsequently repartitioned in octanol/buffer until constant values were obtained. This was usually achieved with the third repartition. In a similar manner, the apparent partition coefficient at pH 3.0, 6.5, 7.0, and 8.0 of complex **14** was carried out.

Biodistribution. Complexes prepared at the tracer level (^{99m}Tc) were studied in mice (Swiss Albino, 19−34g) and rats (Wistar rats, 95−180g). Four groups of male mice (at least five animals per group) were injected in the tail vein with HPLC-purified and 50% 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 (5, 10, 30, and 60 min). The organs of interest were excised, weighed, and counted in an automatic γ-counter. Bladder and excreted urine were not weighted. 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/blood ratio was calculated by dividing the respective % ID/g values. The retention ratio was also calculated and is defined as the ratio of the mean % ID/organ of brain at 30 min pi to that at 5 min pi. In rat experiments, the animals were injected through the femoral vein with 0.1 mL of 3−6 μCi of HPLC purified and 50% methanol reconstituted ^{99m}Tc complex under ether anesthesia. Data were collected for 5, 10, 30, and 60 min time intervals.

Acknowledgment. This work was partially supported by Mallinckrodt Medical B.V., Petten, Holland.

References

(1) (a) Nowotnik, D. P. Technetium-based Brain Perfusion Agents. In *Radiopharmaceutical Chemistry and Pharmacology*; Nunn, A. D., Ed.; M. Dekker: New York, 1992; pp 37−95. (b) Kung, H. F. New Technetium 99m-labeled brain perfusion imaging agents. *Semin. Nucl. Med.* **1990**, *20*, 150−158. (c) Kung, H. F.; Ohmomo, Y.; Kung, M. Current and Future Radiopharmaceuticals for Brain Imaging With Single Photon Emission Computed Tomography. *Semin. Nucl. Med.* **1990**, *20*, 290−302. (d) Holman, B. L.; Devous, M. D. Functional Brain SPECT: The Emergence of A Powerful Clinical Method. *J. Nucl. Med.* **1992**, *33*, 1888−1904. (e) Moretti, J. L.; Caglar, M.; Weinmann, P. Cerebral Perfusion Imaging Tracers for SPECT: Which One to Choose? *J. Nucl. Med.* **1995**, *36*, 359−363.

(2) (a) Kung, H. F.; Molnar, M.; Billings, J.; Wicks, R.; Blau, M. Synthesis and biodistribution of neutral lipid soluble Tc-99m complexes that cross the blood brain barrier. *J. Nucl. Med.* **1984**, *25*, 326−332. (b) Lever, S. Z.; Burns, H. D.; Kervitsky, T. M.; Goldfarb, H. W.; Woo, D. V.; Wong, D. F.; Epps, L. A.; Kramer, A. V.; Wagner, H. N. Design preparation and biodistribution of a technetium-99m triaminedithiol complex to assess regional cerebral blood flow. *J. Nucl. Med.* **1985**, *28*, 1287−1294. (c) Epps, L.; Burns, H. D.; Lever, S. Z.; Goldfarb, H. W.; Wagner, H. N. Brain imaging agents: Synthesis and characterization of (N-piperidinyl hexamethyl diamino dithiolate) oxo technetium (V) complexes. Technetium aminothiolates as brain agents. *Appl. Radiat. Isot.* **1987**, 661−664. (d) Efange, S. M. N.; Kung, H. F.; Billings, J.; Guo, V. Z.; Blau, M. Technetium-99m bis (Aminoethanethiol) Complexes with Amine Sidechains-Potential Brain Perfusion Imaging Agents for SPECT. *J. Nucl. Med.* **1987**, *28*, 1012−1019. (e) Efange, S. M. N.; Kung, H. F.; Billings, J. J.; Blau, M. The synthesis and biodistribution of ^{99m}Tc-labeled piperidinyl bis(aminoethanethiol) complexes: Potential brain perfusion imaging agents for single photon emission computed tomography. *J. Med. Chem.* **1988**, *31*, 1043−1047. (f) Chiotellis, E.; Varvarigou, A. D.; Maina, Th.; Stassinopoulou, C. I. Comparative evaluation of ^{99m}Tc-labeled aminothiols as possible brain perfusion imaging agents. *Nucl. Med. Biol.* **1988**, *15*, 215−233. (g) Scheffel, U.; Goldfarb, H. W.; Lever, S. Z.; Gungon, R. L.; Burns, D.; Wagner, H. N. Comparison of technetium-99m aminoalkyl diaminodithiol analogs as potential brain blood flow imaging agents. *J. Nucl. Med.* **1988**, *29*, 73−82. (h) Papadopoulos, M.; Stathaki, S.; Mastrostamatis, S.; Varvarigou, A.; Chiotellis, E. ^{99m}Tc-DADT complexes substituted with heterocycles amines: Effect of substitution on in vivo reactivity. *Nucl. Med. Biol.* **1993**, *20*, 105−115. (i) Papadopoulos, M.; Chiotellis, E.; Varvarigou, A.; Mastrostamatis, S.; Cotsyfakis, C.; Vavouraki, H.; Stathaki, S. Correlation of lipophilicity to biodistribution of Tc-99m labeled aminothiols. *Nucl. Med. Biol.* **1993**, *20*, 101−104. (j) Kung, H. G.; Guo, Y. Z.; Yu, C. C.; Billings, J.; Subramanyam, V.; Calabrese, J. New Brain Perfusion Imaging Agents Based on ^{99m}Tc bis-Aminoethanethiol (BAT) Complexes: Stereoisomers and Biodistribution. *J. Med. Chem.* **1989**, *32*, 433−437.

(3) (a) Cumming, S. A.; Nechvatal, G.; Canning, L. R.; Nowotnik, D. P.; Pickett, R. D.; Neirinckx, R. D.; Volkert, W. A.; Trounter, D. E.; Holmes, R. A. Development of technetium-99m regional cerebral blood flow agents based upon the propylene amine oxime ligand (PnAO). *Eur. J. Nucl. Med.* **1985**, *11*, A107. (b) Nowotnik, D. P.; Canning, L. R.; Cumming, S. A.; Harrison, R. C.; Higley, B.; Nechvatal, G.; Pickett, R. D.; Piper, I. M.; Bayne, V. J.; Forster, A. M.; Weisner, P. S.; Neirinckx, R. D.; Volkert, W. A.; Trounter, D. E.; Holmes, R. A. Development of ^{99m}Tc-labelled radiopharmaceutical for SPECT imaging. *Nucl. Med. Commun.* **1985**, *6*, 499−506.

(4) (a) Neirinckx, R. D.; Canning, L. R.; Piper, I. M.; Nowotnik, D. P.; Pickett, R. D.; Holmes, R. A.; Volkert, W. A.; Forster, A. M.; Weisner, P. S.; Marriot, J. A.; Chaplin, S. B. Technetium-99m d,l,HMPAO: A new radiopharmaceutical for SPECT imaging of regional cerebral blood perfusion. *J. Nucl. Med.* **1987**, *28*, 191−202. (b) Podreka, I.; Suess, E.; Goldenberg, G.; Steiner, M.; Brucke, T.; Muller, Ch.; Lang, W.; Neirinckx, R. D.; Deecke, L. Initial experience with technetium-99m HM-PAO brain SPECT. *J. Nucl. Med.* **1987**, *28*, 1657−1666. (c) Sharp, P. F.; Smith, F. W.; Gemmell, H. G.; Lyall, D.; Evans, N. T. S.; Gvozdanovic, D.; Davidson, J.; Tyrrell, D. A.; Pickett, R. D.; Neirinckx, R. D. Technetium-99m HMPAO stereoisomers as potential agents for imaging regional cerebral blood flow: Human volunteer studies. *J. Nucl. Med.* **1986**, *27*, 171−177. (d) Leonard, J. P.; Nowotnik, D. P.; Neirinckx, R. D. Tc-99m d,l-HMPAO: A new radiopharmaceutical for imaging regional brain perfusion using SPECT-A comparison with iodine-123 HIPDM. *J. Nucl. Med.* **1986**, *27*, 1819−1823.

(5) (a) Holman, B. L.; Hellman, R. S.; Goldsmith, S. J.; Mena, I. G.; Leveille, J.; Gherardi, P. G.; Moretti, J.-L.; Bischof-Delaloye, A.; Hill, T. C.; Rigo, P. M.; Van Heertum, R. L.; Ell, P. J.; Buell, U.; De Roo, M. C.; Morgan, R. A. Biodistribution, dosimetry, and clinical evaluation of technetium-99m ethyl cysteinate dimer in normal subjects and in patients with chronic cerebral infraction. *J. Nucl. Med.* **1989**, *30*, 1018−1024. (b) Walovitch, R. C.; Hill, T. C.; Garrity, S. T.; Cheesman, E. H.; Burgess, B. A.; O'Leary, D. H.; Watson, A. D.; Ganey, M. V.; Morgan, R. A.; Wiliams, S. J. Characterization of technetium-99m-L,L-ECD for brain perfusion imaging, part 1: Pharmacology of technetium-99m ECD in nonhuman primates. *J. Nucl. Med.* **1989**, *30*, 1892−1901. (c) Leveille, J.; Demonceau, G.; De Roo, M.; Rigo, P.; Taillefer, R.; Morgan, R. A.; Kupranick, D.; Walovitch, R. C. Characterization of technetium-99m-L,L-ECD for brain perfusion imaging, Part 2: Biodistribution and brain imaging in humans. *J. Nucl. Med.* **1989**, *30*, 1902−1910.

(6) (a) Hung, J. C.; Corjila, M.; Volkert, W. A.; Holmes, R. A. Kinetic analysis of technetium-99m d,l-HMPAO decomposition in aqueous media. *J. Nucl. Med.* **1988**, *29*, 1568−1576. (b) Verbrugen, A. M. Radiopharmaceuticals: State of the Art. *J. Nucl. Med.* **1990**, *31*, 346−363.

(7) Jurisson, S.; Schlemper, E. O.; Trounter, D. E.; Canning, L. R.; Nowotnick, D. P.; Neirinckx, R. D. Synthesis and characterization and X-ray structural determinations of technetium (V)-oxo-tetradentate amine oxime complexes. *Inorg. Chem.* **1986**, *25*, 543−549.

(8) Edwards, D. S.; Cheesman, E. H.; Watson, N. M.; Maheu, L. J.; Nguyen, S. A.; Dimitre, L.; Nason, T.; Watson, A. D.; Walovitch, R. Synthesis and characterization of technetium and rhenium complexes of N,N′-1,2-ethylenediyl-bis-L-cysteine. Neurolite and its metabolites. In *Technetium and Rhenium in Chemistry and Nuclear Medicine 2*; Nicolini, M., Bandoli, G., Mazzi, U., Eds.; Raven Press: New York, 1984; pp 433−444.

(9) (a) Pietzsch, H.-J.; Spies, H.; Hoffmann, S.; Stach, J. Lipophilic technetium complexes-V. Synthesis and characterization of (3-Thiapentane-1,5-dithiolato)(thiophenolato) oxotechnetium (V). *Inorg. Chem. Acta* **1989**, *161*, 15−16. (b) Spies, H.; Pietzch, H.-J.; Syhre, R.; Hoffmann, S. Lipophilic technetium complexes-VIII. Preparation and animal studies of oxotechnetium (V)

complexes with tridentate/monodentate ligand coordination. *Isotopenpraxis* **1990**, *26*, 159−162. (c) Pietzch, H.-J.; Spies, H.; Hoffmann, S.; Scheller, D. Lipophilic technetium complexes-VII. Neutral oxotechnetium(V) complexes of tridentates shiff-bases containing monothiols as co-ligands. *Appl. Radiat. Isot.* **1990**, *41*, 185−188. (d) Spies, H.; Fietz, Th.; Glaser, M.; Pietzsch, H. J.; Johannsen, B. The "n+1" Concept in the synthesis Strategy of Novel technetium and Rhenium Tracers. In *Technetium and Rhenium in Chemistry and Nuclear Medicine 4*; Nicolini, M., Bandoli, G., Mazzi, U., Eds.; SGE: Padova, 1995; pp 243−246. (e) Papadopoulos, M. S.; Pelecanou, M.; Pirmettis, I. C.; Spyriounis, D.M.; Raptopoulou, C. P.; Terzis, A.; Stassinopoulou, C. I.; Chiotellis, E. A New Donor Atom System [(SNN)(S)] for the Synthesis of Neutral Oxotechnetium(V) Mixed-Ligand Complexes. *Inorg. Chem.* **1996**, *35*, 4478−4483.

(10) (a) Mastrostamatis, S.; Papadopoulos, M.; Varvarigou, A. D.; Pirmettis, I.; Chiotellis, E.; Mixed Ligands Tc(V)-complexes with Tripodal and Monodentate Thiol as Coligand: Preparation and Biological Studies. *Eur. J. Nucl. Med.* **1992**, *19*, 591. (b) Mastrostamatis, S. G.: Papadopoulos, M. S.; Pirmettis, I. C.; Paschali, E.; Varvarigou, A. D.; Stassinopoulou, C. I.; Raptopoulou, C. P.; Terzis, A.; Chiotellis, E. Tridentate Ligands Containing the SNS Donor Atom Set as a Novel Backbone for the Development of Technetium Brain-Imaging Agents. *J. Med. Chem.* **1994**, *37*, 3212−3218. (c) Spyriounis, D.; Pelecanou, M.; Stassinopoulou, C. I.; Raptopoulou, C. P.; Terzis, A.; Chiotellis, E. Synthesis and Characterization of Oxotechnetium(V) Complexes with Aza-Substituted 2,6,Dimethyl-4-azaheptane-2,6-dithiol Ligands and Benzyl Mercaptan as Coligand. *Inorg. Chem.* **1995**, *34*, 1077−1082.

(11) Pirmettis, I. C.; Papadopoulos, M. S.; Mastrostamatis, S. G.; Raptopoulou, C. P.; Terzis, A.; Chiotellis, E. Synthesis and Characterization of Oxotechnetium(V) Mixed Ligand Complexes Containing a Tridentate N-substituted Bis-(2-mercaptoethyl)-amine and a Monodentate Thiol. *Inorg. Chem.* **1996**, *35*, 1685−1691.

(12) (a) Fujita, T.; Iwasa, J.; Hansch, C. A New Substituent Constant, *∂*, Derived from Partition Coefficients. *J. Am. Chem. Soc.* **1964**, *80*, 5175−5180. (b) Hansch, C.; Quinlan, J. E.; Lawrence G. L. The Linear Free-Energy Relationship between Partition Coefficients and the Aqueous Solubility of Organic Liquids. *J. Org. Chem.* **1968**, *33*, 347−350. (c) Hancsh, C.; Anderson, S. M. The effect of Hydrophobic Bonding on Partition Coefficients. *J. Org. Chem.* **1967**, *32*, 2583−2586.

(13) (a) Touya, J. J.; Rahimian, J.; Grubbs, D. E.; Corbus, H. F.; Bennett, L. A Noninvasive Procedure for In vivo Assay of Lung Amine Endothelial Receptor. *J. Nucl. Med.* **1985**, *26*, 1302−1307. (b) Lever, S. Z.; Wagner, H. N., Jr. The status and future of technetium-99m radiopharmaceuticals. In *Technetium and Rhenium in Chemistry and Nuclear Medicine 3*; Nicolini, M., Bandoli, G., Mazzi, U., Eds.; Raven Press: New York, 1989; pp 649−659.

(14) (a) Huntress, E. H.; Carten, F. H. Identification of Organic Compounds. I. Chlorosulfonic Acid as a Reagent for the Identification of Aryl Halides. *J. Am. Chem. Soc.* **1940**, *62*, 511−514. (b) Huntress, E. H.; Autenrieth, J. S. Identification of Organic Compounds. IV. Chlorosulfonic Acid as a Reagent for the Identification of Alkylbenzenes. *J. Am. Chem. Soc.* **1941**, *63*, 3446−3446. (c) Adams, R.; Marvel, C. S. Thiophenol. *Organic Synthesis*; Wiley: New York, 1932; Collect. Vol. I, pp 504−506. (d) Reid, E. E. *Organic Chemistry of Bivalent Sulfur*; Chemical Publishing: New York, 1958; Vol. I, pp 15−106 and references therein. (e) Lewis, T. R.; Archer, S. Alkyl Phenyl-(2-pyridyl)-methyl Sulfones. Sulfonium Salts as Alkylating Agents. *J. Am. Chem. Soc.* **1951**, *73*, 2109−2113. (f) Kuliev, A. M.; Aliev, A. B.; Mamedov, R. M. *Ser. Khim. Nauk.* **1972**, *4*, 39. (g) Snyder, H. R.; Steward, J. M.; Ziegler, J. B. The Synthesis of Amino Mercaptans from Olefin Sulfides. *J. Am. Chem. Soc.* **1947**, *69*, 2672−2674. (h) Harley-Mason, J. Some Aliphatic Thiols and their Derivatives. Part I Aliphatic Mercaptans. *J. Chem. Soc.* **1947**, 320−322. (i) Mann, F. Hofman's Ethylene Bases. Synthesis of *ββ'*-Diaminodiethylamine and *ββ'*-Diaminodiethylmethylamine. *J. Chem. Soc.* **1934**, 461−466. (j) Corbin, J. L.; Miller, K. F.; Pariyadath, N.; Wherland, S.; Bruce, L. A.; Stiefel, E. I. Preparation and Properties of Tripodal and Linear Tetradentate N,S-Donor Ligands and their Complexes Containing the MoO_2^{2+} Core. *Inorg. Chim. Acta* **1984**, *90*, 41−51. (k) Wineman, R. J.; Gollis, M. H.; James, J. C.; Pomponi, A. M. Thioethylation of Amines with Ethylene Sulfide. *J. Org. Chem.* **1962**, *27*, 4222−4226.

JM960273Y