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New [^{99m}Tc]-Cytectrene Amine Compounds as Specific Brain Imaging Agents

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Summary

Lipophilic tertiary amines attached to cyclopentadienyl technetium-99m tricarbonyl (cytectrene) have been prepared with high radiochemical yield and purity. Biodistribution studies in mice showed that [^{99m}Tc]-cytectrenes, containing in their structure an N-methylpiperidine, were accumulated in the brain up to 2.8 % of injected dose with high brain-to-blood ratios at 15 min p.i.[1]. They therefore indicate some potential as brain imaging agents.

It has to be pointed out that the N-methylpiperidine ester showed similar biological behaviour as the keto derivative. This indicates that the conversion to polar metabolite(s) via hydrolysis of the ester group - as described for [^{99m}Tc]-ECD - is not essential for brain retention.

Key words: ^{99m}Tc, brain, ferrocene

[1]The abbreviations used are: p.i., postinjection; i.v., intravenous; BBB, blood-brain barrier; DADT, diaminodithiol; ECD, ethyl cysteinate dimer; HM-PAO,hexamethylpropyleneamine oxime.

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Introduction

In contrast to commonly used [^{99m}Tc]-chelates with donor atoms such as S, N, O or P, in this paper we report on chemically stable complexes in which ^{99m}Tc is sandwiched between cyclopentadienyl and carbonyl groups. Recently, a new procedure for the synthesis of a [^{99m}Tc]-cytectrene complex bound to very different substituents has been introduced [1, 2]. Because of its high lipophilicity, relatively small molecular size and inert nature [3, 4], this complex is best suited for attachment to biologically active compounds as it does not decisively affect their properties.

Substituted ferrocenes yield cytectrene analogues in a reaction with $Mn(CO)_5Br$ and $^{99m}TcO_4^-$ at 130-150°C wherein the fragment of [Fe-cyclopentadienyl] is exchanged for [^{99m}Tc-tricarbonyl] (Fig.1) [1]. It is remarkable that a cyclopentadienyl ring with a side chain is preferably used to form the new cytectrene derivative. The side chain should have an electron-withdrawing effect to give a high radiochemical yield. If two identical side chains are attached to each ring of ferrocene, the radiochemical yield of cytectrene is often increased.

Investigations on the stability of ferrocenes bound to side chains with electron-withdrawing effects have shown that considerable amounts of ferrocenes (>50%) are decomposed at these temperatures. In contrast to the ferrocenes, the corresponding [^{99m}Tc]-cytectrenes have proven to be stable under these conditions.

The radiochemical reaction conditions to obtain the [^{99m}Tc]-cytectrenes from ferrocenes and the subsequent purification are reported in this study.

It has shown that tertiary piperidine derivatives are retained by brain tissue showing high brain-to-blood ratios. As an example, [^{99m}Tc]-cytectrene attached to N-methylpiperidine via an ester group is reported to have a brain uptake of 2.78 % of injected dose with a considerable brain-to-blood ratio of 16 at 15 min after the i.v. administration to rats [2].

Biodistribution studies with a [^{99m}Tc]-DADT complex bound to a piperidinylethyl side chain showed that about 2.2 % of injected dose was in the brain of mice with a brain-to-blood ratio of 5.3 at 5 min p.i. [5].

To obtain more information about the structure/biodistribution relationship of the piperidine [^{99m}Tc]-cytectrenes with respect to brain uptake, and to achieve better brain retention behaviour, new piperidine derivatives and other tertiary amines bound via the γ-C atom to the functional group of the [^{99m}Tc]-cytectrene backbone were synthesized and investigated in biodistribution studies.

Experimental

Preparative Chemistry

The organic compounds synthesized were characterized by uncorrected melting point (Büschi, 530), ^{1}H-NMR (WM 250, Bruker), IR spectroscopy (Perkin Elmer, Model 1600, FT-IR Spectrophotometer) and elemental analysis (240C Elemental Analyzer, Perkin Elmer). The following abbreviations for interpretation of ^{1}H-NMR spectra are used: br = broad, s = singlet, d = doublet, t = triplet, q = quadruplet, p = pentet, m = multiplet. Chemical shifts were related to tetramethylsilane as an internal standard. Starting materials were obtained from Aldrich Chemical Co.. Ferrocenes were purified on columns filled with silicagel (Si 60 (0.04-0.063 mm), E.Merck). 1,1'-Ferrocenedicarbonyl chloride was originally prepared by the procedure described by Knobloch et al. [6].

Sodium[^{99m}Tc]pertechnetate was eluted from a commercial ^{99}Mo/^{99}Tc generator (^{99m}Tc Generator 4 GBq, Hoechst). The radioactivity on the thin-layer plates (HPTLC Si 60 F_{252}, E.Merck) was measured on a phosphor imager (Molecular Dynamics 410a, Sunnyvale, CA) to ascertain radiochemical yields. Radiochemical preparations were purified on SEP-PAK cartridges (RP18, NH_2 SEP-PAK, Water Associates, Inc.).

Bis[N-methylpiperidin-4-yloxycarbonyl]ferrocene 1

A solution of 1,1'-ferrocenedicarbonyl chloride (0.4 g, 1.59 mmol), 1-methyl-4-piperidinol (1.26 g, 11 mmol) and triethylamine (0.68 ml, 4.5 mmol) in dry CH_2Cl_2 (50 ml) was heated under reflux for 3 h. The solution was extracted with saturated $NaHCO_3$-solution (2*100 ml). The organic layer was evaporated, and the residue was transferred to a silicagel chromatographic column. On elution with an acetone-ethanol-ammonia mixture (92:6:2), a light-orange fraction was collected, which was dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. 1 was prepared in the form of light-red crystals by recrystallization twice from petroleum ether: yield 0.6 g (81 %); mp 79-81°C; IR (KBr): 1710 cm^{-1} (C=O); NMR ($CDCl_3$) δ(ppm): 4.98 (p, 2H, -O-CH-, J=3.7 Hz), 4.80 (s, 4H, Fc), 4.42 (s, 4H, Fc), 1.87-2.85 (m, 16H, -CH_2-)[15], 2.41 (s, 6H, -CH_3); MS (95°C) m/e: 468(M^+, 100%); Anal. Calcd. for $FeC_{24}H_{32}N_2O_4$: C, 61.54; H, 6.84; N, 5.98; Found: C, 61.42; H, 7.01; N, 5.84.

N-methyl-4-piperidinoylferrocene 2

N-methyl-4-piperidinecarboxylic acid hydrochloride:

To 98% formic acid (18.5 g, 0.394 mol) in a half liter flask cooled in an ice bath, was added piperidine-4-carboxylic acid (8.7 g, 0.067 mol) and 37 % formaline solution (16.5 ml) with stirring. The mixture was stirred and heated at 50°C overnight. 20 ml of 12 M HCl was added to this solution. After concentrating under reduced pressure, the solution was neutralized and then evaporated to dryness in vacuo, leaving a slightly gummy, white residue. The latter was dried over phosphorus pentoxide and recrystallized from

absolute EtOH to give a white powder of N-methylpiperidine-4-carboxylic acid hydrochloride: yield 7.85 g (59.5 %); mp 225-226°C [7].

product:

Oxalyl chloride (13 ml, 0.15 mol) was added dropwise to a solution of N-methylpiperidine-4-carboxylic acid hydrochloride (7.5 g, 42 mmol) in CH_2Cl_2 (100 ml) with stirring and at ice bath temperature at the beginning. After stirring for 12 h, the solution was evaporated to dryness. To the white residue was added ferrocene (5 g, 0.027 mol) in CH_2Cl_2 (250ml) and anhydrous $AlCl_3$ (20 g, 0.15 mol) with stirring and at ice bath temperature. After stirring for 12 h, the solution was poured over ice. 4-5 g $SnCl_2 \cdot 2H_2O$ was added to the solution that was stirred for 30 min. The aqueous liquid was extracted with Et_2O (3*100 ml). The organic layer was concentrated and carefully eluted on a silicagel column with a mixture of acetone, ethanol and ammonia (89:6:5) to remove not reacted ferrocene and a by-product eluting shortly before the main product. The separated main fraction was dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give red crystals of **2** from petroleum ether: yield 170 mg (2 %); mp 106-107°C; IR (KBr): 1651 cm^{-1} (C=O); NMR ($CDCl_3$) δ(ppm): 4.78 (s, 2H, Fc), 4.51 (s, 2H, Fc), 4.21 (s, 5H, Fc), 2.93-2.97, 1.76-2.28 (m, 8H, -C<u>H</u>$_2$-), 2.78 (pent, 1H, -C<u>H</u>-, J=4.3 Hz), 2.32 (s, 3H, -N-C<u>H</u>$_3$); MS(80°C) m/e: 311 (M^+, 100%); Anal. Calcd. for $FeC_{17}H_{21}NO$: C, 65.59; H, 6.75; N, 4.50; Found: C, 66.02; H, 6.98; N, 4.55.

Bis(N-methylpyrrolidin-3-yloxycarbonyl)ferrocene 3

A solution of 1,1'-ferrocenedicarbonyl chloride (1 g, 3.64 mmol), 3-hydroxy-N-methylpyrrolidine (1 g, 9.9 mmol) and triethylamine (1.5 ml, 9.9 mmol) in dry CH_2Cl_2 (100 ml) was heated under reflux for 1 h. The organic liquid was concentrated and purified by silicagel chromatography with the solvent system CH_2Cl_2, MeOH, Et_3N (45:4:1). After drying over anhydrous Na_2SO_4, the organic fraction was evaporated to dryness. The product **3** was recrystallized from Et_2O and isolated as light red crystals: yield 301 mg (18.8 %); mp 119-120°C; IR (KBr): 1694 cm^{-1} (C=O), NMR ($CDCl_3$) δ(ppm): 5.35 (p, 1H, -C<u>H</u>-, J=2.7 Hz), 4.84 (s, 4H, Fc), 4.40 (s, 4H, Fc), 1.94-2.88 (m, 12H, -C<u>H</u>$_2$-), 2.41 (s, 6H, -C<u>H</u>$_3$); MS(120°C) m/e: 440 (M^+, 2%), 83 (100%); Anal. Calcd. for $FeC_{22}H_{28}N_2O_4$: C, 60.00; H, 6.35; N, 6.36; Found: C, 59.98; H, 6.54; N, 6.81.

Bis(2-(piperidin-1-yl)eth-1-yloxycarbonyl)ferrocene 4

A solution of 1,1'-ferrocenedicarbonyl chloride (2.71 g, 8.76 mmol), 2-piperidino-1-ethanol (2.5 g, 19.4 mmol) and triethylamine (2.4 ml, 17.4 mmol) in dry CH_2Cl_2 (200 ml) was heated under reflux for 1 h. The solution was extracted with saturated $NaHCO_3$-solution (2*100 ml). The organic layer was concentrated and transferred to a silicagel column. On elution with a mixture of acetone, ethanol, ammonia (2:6:92), a light-orange fraction was collected, which was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. **4** was isolated in the form of orange crystals by recrystallization twice from petroleum ether: yield 3.45 g (79 %); mp 56-57°C; IR (KBr): 1716 cm^{-1} (C=O); NMR ($CDCl_3$) σ(ppm): 4.84 (s, 4H, Fc), 4.43 (s, 4H, Fc), 4.36

(t, 4H, -O-C<u>H</u>$_2$-, J=5.98 Hz), 2.71 (t, 4H, -C<u>H</u>$_2$-N-, J=5.98 Hz), 2.53 (br, 8H, -N-C<u>H</u>$_2$- (ring)), 1.62 (p, 8H, -C<u>H</u>$_2$-(ring), J=5.4 Hz), 1.46 (br, 4H, -C<u>H</u>$_2$- (ring)); MS (130°C) m/e: 496(M$^+$, 1%), 98(100%); Anal.Calcd. for $FeC_{26}H_{36}N_2O_4$(496): C, 62.90; H, 7.26; N, 5.64; Found: C, 62.62; H, 7.26; N, 5.78.

3,7-Dimethyl-3,7-diazabicyclo[3.3.1.]nonan-9-yloxycarbonylferrocene 5

N,N'-dimethylbispidone:

It was prepared via double Mannich condensation of commercially available 1-methyl-4-piperidone with formaldehyde and methylamine as described by Douglass et al. [8]. The diamine was purified by distillation at 65°C (0.04 mbar) to give 27.8 g (29 %) of a colourless liquid.

N,N'-dimethylbispidinol:

To a solution of N,N'-dimethylbispidone (3 g, 17.8 mmol) in Et_2O (100 ml) held under N_2, was added $LiAlH_4$ (0.4 g, 10.5 mmol) with stirring and at ice bath temperature at the beginning. After stirring for 30 min, H_2O and THF (1:10) was added until the hydrogen development had finished. The organic layer, dried over anhydrous Na_2SO_4, was filtered and evaporated under reduced pressure to give N,N'-dimethylbispidinol: yield 2.6 g (86 %); mp 123-128°C [9].

product:

A solution of ferrocene carboxylic acid (1.5 g, 6 mmol) and oxalyl chloride (1.6 ml, 14 mmol) in CH_2Cl_2 (100 ml) was stirred at 25°C in a 250 ml round-bottom flask equipped with a reflux condenser for 30 min. The solution was evaporated to dryness, and the residue was taken up in CH_2Cl_2 (100 ml). Following the addition of N,N'-dimethylbispidinol (1.08 g, 6.35 mmol) the solution was heated under reflux for 1 h, concentrated and transferred to a short silicagel column. On elution with CH_2Cl_2, an apolar red band was rapidly collected. On elution with a mixture of acetone, ethanol and ammonia (2:6:92), a dark red band was separated from the black material remaining on the column. After repeated chromatography carried out with the mixture of CH_2Cl_2, methanol and triethylamine (18:6:1), a dark red-coloured fraction was eluted to give <u>5</u> as red crystals after drying over anhydrous Na_2SO_4, evaporating to dryness and recrystallization twice from petroleum ether. <u>5</u>: yield 240 mg (10.5 %); mp 129-130°C; IR (KBr): 1706 cm^{-1} (CO); NMR ($CDCl_3$) δ(ppm): 4.89 (p, 1H, -O-C<u>H</u>-, J=3.5Hz), 4.85 (s, 2H, Fc), 4.43 (s, 2H, Fc), 4.22 (s, 5H, Fc), 2.38-3.17 (m, 8H, -C<u>H</u>$_2$-N-)[18], 2.34 (s, 3H, -N-C<u>H</u>$_3$), 2.26 (s, 3H, -N-C<u>H</u>$_3$), 2.07 (br, 2H, -C<u>H</u>-); MS(100°C) m/e: 382 (M$^+$, 88%), 58 (100%); Anal. Calcd. for $FeC_{20}H_{26}N_2O_2$: C, 62.83; H, 6.80; N, 7.33; Found: C, 62.56; H, 6.94; N, 7.41.

1,1'-Bis(N-trifluoracetylpiperidin-4-yloxycarbonyl)ferrocene 6

To a mixture of <u>1</u> (2 g, 4.27 mmol) and $AlCl_3$ (4.54 g, 34 mmol) in CH_2Cl_2 (50ml) placed in a 100-ml round-bottom flask equipped with a dropping funnel and cooled in an ice bath, was added trifluoracetic acid anhydride (2.82 ml, 17 mmol). The closed flask was maintained at -25°C for 24 h and then the contents were poured over ice. After the organic phase had been separated, the aqueous layer was extracted with

CH_2Cl_2 (2*100 ml). The combined organic layers were concentrated and purified by elution with CH_2Cl_2 from a silicagel column to separate **6** from **1** and from the mono-N-trifluoracetyl derivative. The fraction with the purified **6** was dried over anhydrous Na_2SO_4, evaporated to dryness and then taken up in petroleum ether to give **6** as yellow crystals: yield 50 mg (1.9 %); mp 117-119°C; IR (KBr): 1719 (C=O), 1664 (C=O); NMR ($CDCl_3$) δ(ppm): 5.22 (p, 1H, -CH-, J=3.2 Hz), 4.81 (s, 4H, Fc), 4.45 (s, 4H, Fc), 3.59-3.98 (m, 8H, $-CH_2-N-$), 1.87-2.10 (m, 8H, $-CH_2-CH-$); MS(180°C) m/e: 632 (M^+, 100%); Anal. Calcd. for $FeC_{26}O_6N_2F_6H_{26}$: C, 49.36; H, 4.11; N, 4.43; Found: C; 49.60; H, 4.15; N, 4.36.

Bis(N-dimethyl-3-aminoprop-1-yloxycarbonyl)ferrocene 7

A solution of ferrocenedicarbonyl chloride (1g, 3.2 mmol), N-dimethyl-3-aminopropan-1-ol (2 ml, 16.9 mmol) and triethylamine (0.89 ml, 6.44 mmol) in dry CH_2Cl_2 (100 ml) was heated under reflux for 2 h. The solution was extracted with saturated $NaHCO_3$-solution (2*100 ml), dried over anhydrous Na_2SO_4, and evaporated to dryness.

7 was isolated as a red oil from petroleum ether: yield 1.05 g (73.4 %); IR (film): 1715cm^{-1} (CO); NMR ($CDCl_3$) δ(ppm): 4.83 (s, 4H, Fc), 4.41 (s, 4H, Fc), 4.27 (t, 4H, $-CH_2-O-$, J=6.5Hz), 2.42 (t, 4H, $-CH_2-N-$, J=7.3 Hz), 2.27 (s, 12H, $-CH_3$), 1.91 (p, 4H, $-CH_2-$, J=6.9 Hz); MS(70°C) m/e: 444 (M^+, 78%), 360 (100 %); Anal. Calcd. for $FeC_{22}H_{32}N_2O_2$: C, 59.46; H, 7.21; N, 6.30; Found: C, 59.27; H, 7.48; N, 6.06.

Table 1. Radiochemical yields and R_f-values of **1a-7a** as determinated from TLC (solvent systems for elution of **1a-4a**, **6a-7a**: acetone-ethanol-ammonia (92:6:2), for elution of **5a**: CH_2Cl_2-methanol-triethylamine (18:6:1)

substance	radiochemical yield [%]	R_f-value
1a	89	0.45
2a	90	0.4
3a	91	0.6
4a	38	0.75
5a	15	0.3
6a	43	0.9
7a	77	0.6

Radiolabelling and purification

To the ferrocene compound (2 mg) and $Mn(CO)_5Br$ (2 mg) placed in a glass tube, was added THF (80-90 μl) and $^{99m}TcO_4^-$-eluate (5-20 μl, 2-11 MBq) to give a total volume of 100 μl. The glass tube was sealed by melting and heated at 150°C for 1 h. After opening the tube a small portion of solution was transferred to an HPTLC-plate that was developed to determine radiochemical yields of [^{99m}Tc]-cytectrene formed in the reaction.

[^{99m}Tc]-cytectrenes used for biodistribution studies were purified on cartridges and determined to be >90% pure by radiochromatography. **1a, 2a, 3a, 7a** were each applied to RP-18 cartridges and eluted with 0.5 ml

H_2O and then with 0.5 ml EtOH to obtain [^{99m}Tc]-cytectrenes. **4a, 5a, 6a** were purified by eluting from NH_2-cartridges with 0.5 ml EtOH. The purification was necessary to remove insoluble black particles and $^{99m}TcO_2$ formed in the labelling reaction.

To eluted radioactive solutions was added saline to give ratios of $EtOH/H_2O$ <0.05.

Biodistribution studies

Female mice weighing 20-30 g and female rats weighing 160-180 g were each injected intravenously with purified [^{99m}Tc]-complex (0.1 ml, 18-37 kBq for mice and 0.1 ml, 37-70 kBq for rats) through a tail vein. The mice and rats were sacrificed at different time points p.i. and blood was collected immediately. The organs of interest were excised, weighed, and radioactivity was counted in an auto gamma counter (1282 CompuGamma CS, Pharmacia-Wallac).

RESULTS AND DISCUSSION

Reaction schemes for the preparation of ferrocenes and [^{99m}Tc]-cytectrenes are shown in Fig.1 and 2. Results of labelling experiments are given in table 1, those of biodistribution studies are given in table 2.

Among the investigated [^{99m}Tc]-cytectrenes, the N-methylamines **1a**, **2a**, **3a** and **7a** showed the highest brain uptake at 15 min p.i., which decreased to about the half at 30 min p.i..

The dissociation constants (pK_a-values) of the tertiary amines of the investigated [^{99m}Tc]-cytectrenes do not correlate with their brain uptake. Hence, basic strengths of amines do not seem

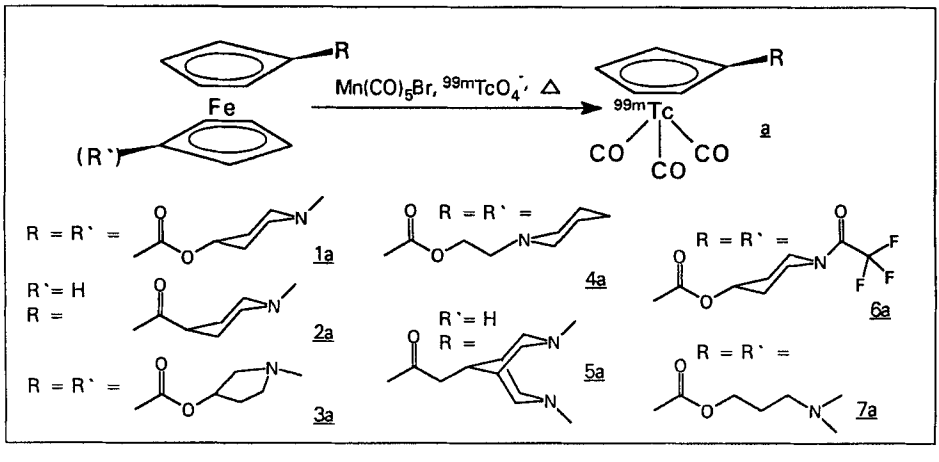

Fig.1 Structures of investigated [^{99m}Tc]-cytectrenes

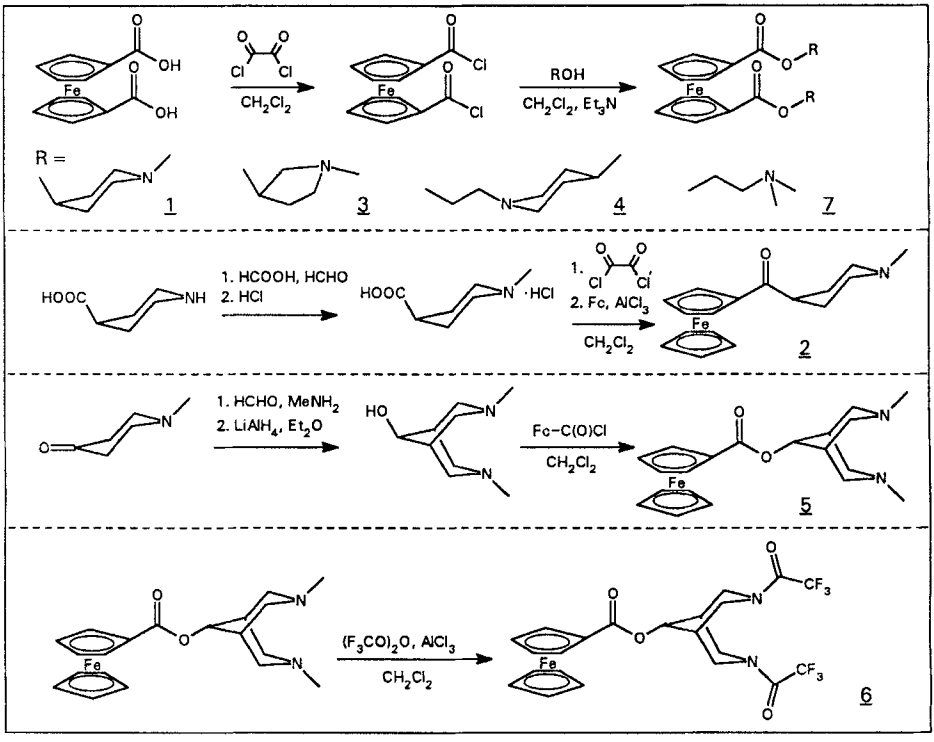

Fig.2 Synthetic route for ferrocene derivatives which form [^{99m}Tc]-cytectrens with the same side chains

Table 2. Biodistribution of [^{99m}Tc]-cytectrenes [% of injected dose / organ] and brain-to-blood ratios [% of injected dose/g / % of injected dose/g] in female mice at 15 min p.i. (mean ± s.d. of 3 animals).

	1a	2a	3a	4a	5a	6a	7a
Brain	2.78	2.75	1.27	0.65	0.12	0.40	1.66
	(±0.22)	(±0.15)	(±0.21)	(±0.08)	(±0.04)	(±0.18)	(±0.27)
Lung	2.35	3.86	2.41	1.37	4.57	3.66	2.37
	(±0.48)	(±0.58)	(±1.14)	(±0.08)	(±0.97)	(±0.36)	(±0.83)
Heart	0.30	0.30	0.23	0.18	0.22	0.20	0.35
	(±0.04)	(±0.03)	(±0.10)	(±0.02)	(±0.01)	(±0.05)	(±0.03)
Liver	22.87	33.3	16.88	22.23	31.83	38.04	30.93
	(±1.16)	(±4.45)	(±1.06)	(±1.63)	(±3.02)	(±4.82)	(±3.00)
Kidneys	8.75	3.73	20.86	15.44	15.60	3.17	9.38
	(±0.20)	(±0.20)	(±2.54)	(±1.36)	(±1.79)	(±0.11)	(±2.17)
Brain							
———	3.51	3.49	1.78	1.18	0.13	1.23	1.11
Blood	(±0.29)	(±0.68)	(±0.42)	(±0.16)	(±0.05)	(±0.63)	(±0.33)

to have an influence on it, except for the strong basic N,N'-dimethylbispidine in **5a** that is assumed to be rapidly protonated at physiological pH and thus unable to cross the BBB (Tab.2) ($pK_a = 11.88$ [8], other amines $pK_a < 10.48$ [10]).

In comparison with published data to [^{99m}Tc]-d,l-HMPAO [11, 12], which was the first of the technetium cerebral perfusion tracers to become commercially available, the N-methylpiperidine compounds **1a** and **2a** showed a 30 % higher accumulation at 15 min p.i..

Therefore, biodistribution studies were performed in rats, too (Fig.3). Values of radioactivity concentration in the brain of rats are comparable to those of mice. The considerably high brain-to-blood ratios (>10) in rats within the first 30 min p.i. exceed the corresponding ratios given for mice (Fig.4, Tab.2). Just as in mice, **1a** and **2a** were retained by brain of rats to a higher degree during the initial phase than [^{99m}Tc]-d,l-HMPAO [13].

Biodistribution studies of **1a** performed in rats at 3 min p.i. showed that 3.5 % of injected dose was in the brain indicating a high uptake of activity in the first pass extraction [14].

Both N-methylpiperidine compounds, in which the side chain is bound to the [^{99m}Tc]-cytectrene backbone via an ester (**1a**) and a keto group (**2a**), respectively, showed similar brain retention behaviour (mice→Tab.2, rats→Fig.3). Therefore, it can be supposed that hydrolysis of **1a** to a [^{99m}Tc]-cytectrenecarboxylic acid within the brain interstice is not responsible for brain retention as has been suggested for [^{99m}Tc]-ECD [15], since **2a** cannot be metabolized by hydrolysis.

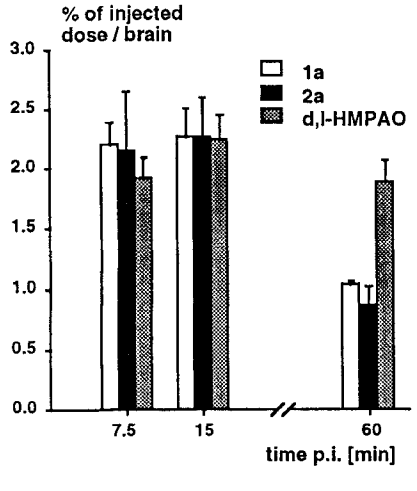

Fig.3. Brain uptake of **1a** and **2a** (n=3) and [^{99m}Tc]-d,l-HMPAO (n=6) [13] in rats at the indicated time. Bars are mean ± s.d..

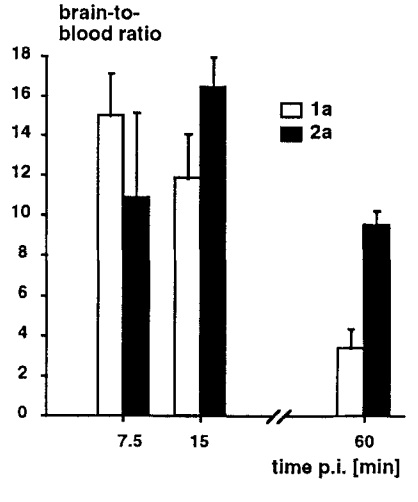

Fig.4. Brain-to-blood ratios of **1a** and **2a** in rats (n=3) sacrificed at the indicated time points.

Elimination studies showed that in general [^{99m}Tc]-cytectrene esters were rapidly washed out by kidneys in contrast to the keto [^{99m}Tc]-cytectrene **2a**, that tended to be cleared via the hepatobiliary system. The elimination half-life of radioactivity after i.v. injection of **2a** in mice was about 8 h (Fig.5).

In the case of specific binding of methylamines to brain receptors, the uptake should depend on the specific radioactivity of [^{99m}Tc]-cytectrenes **1a-3a**, **7a**. Further biodistribution studies in mice injected with different specific activities did not show any dependence of brain uptake on specific radioactivity.

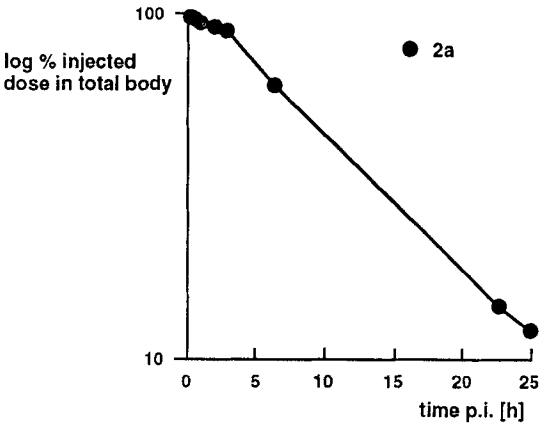

Fig.5. Elimination of radioactivity after injection of **2a** in four female mice.

Brain uptake of **4a** in mice (Tab.2) is in the same order as that of an analogous N-ethylpiperidinyl [^{99m}Tc]-DADT complex (about 1 % of injected dose at 15 min p.i.) as reported by Lever and coworkers [5] indicating the similar biological behaviour of both [^{99m}Tc]-complexes.

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