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Structure, Stability, and Biodistribution of Cationic $[M(CO)_3]^+$ (M = Re, ^{99}Tc, ^{99m}Tc) Complexes with Tridentate Amine Ligands

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Bifunctional chelating molecules linking the *fac*-$[^{99m}Tc(CO)_3]^+$ core with targeting biomolecules are required for the development of specific diagnostic radiopharmaceuticals. Diethylenetriamine (1) and N-(pyridin-2-ylmethyl)ethane-1,2-diamine (2) both react readily with $[^{99m}Tc(H_2O)_3(CO)_3]^+$ in 0.9% saline at micromolar concentrations to form the cationic complexes $[^{99m}Tc(1)(CO)_3]^+$ (5) and $[^{99m}Tc(2)(CO)_3]^+$ (6) in quantitative yields. The crystal structures of the corresponding Re or ^{99}Tc complexes were determined and exhibit in particular the small size of 5. Challenging both ^{99m}Tc complexes 5 and 6 with a 10^4 excess of histidine or cysteine showed no decomposition or ligand exchange after 24 hours and both compounds were also stable against reoxidation to $[^{99m}TcO_4]^-$. In normal mice, complex 5 revealed a good and fast clearance from the blood, and most organs. Only limited accumulation in the large intestine was visible after 4 hours. Complex 6 was also excreted relatively quickly from the blood but retention was observed in some tissues after 4 h. In order to illustrate the potential of both ligands to be further functionalized, two derivatives containing potentially DNA binding functionalities, *N*-(2-Amino-ethyl)-*N'*-pyren-1-ylmethyl-ethane-1,2-diamine (3) and *N*-(quinolin-2-ylmethyl)ethane-1,2-diamine (4) were synthesized. The respective Re or ^{99}Tc complexes were fully characterized. Based on these results, it appears that functionalization of biomolecules with acyclic triamine ligands is biologically relevant. Complex 5 in particular could be used to mimic a terminal amino group in, *e.g.*, a peptide due to its small size and positive charge.

Keywords ^{99m}Techentium, rhenium, radiopharmacy, imaging agents, bioorganometallic, biodistribution, labeling

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INTRODUCTION

Due to its convenient decay characteristics and its availability at low cost, ^{99m}Tc is one of the most widely used radioisotopes in diagnostic nuclear medicine. However, most of the routinely used compounds such as ^{99m}Tc-Sestamibi are perfusion but not receptor targeting agents. More specific applications, such as tumor imaging, require bifunctional chelating molecules which link ^{99m}Tc complexes with a targeting biomolecule, e.g., monoclonal antibodies, receptor specific hormones or peptides. The bifunctional systems are crucial and must obey many requirements: an efficient and fast complex formation with the ^{99m}Tc moiety at micromolar concentrations, retention of receptor affinity of the biomolecule and *in vivo* stability, but still show a convenient biodistribution pattern. A wide variety of ^{99m}Tc chelating ligands has been investigated. The established strategies use tetradentate N,S or N,O ligands or the HYNIC system for the labeling of M(V) complexes, M = ^{99m}Tc, $^{186/188}$Re (Alberto and Abram, 2003). A few derivatives based on such systems, TcO-d,l-HM-PAO, TcO-L,L-ECD, and TcO-MAG_3, are now routinely used in diagnostic nuclear medicine (Schwochau, 2000). A more recent strategy is based on the use of the *fac*-Tc(I) $[^{99m}Tc(H_2O)_3(CO)_3]^+$ moiety, whose convenient synthesis in water was first established in 1998 (Alberto et al., 1998). This moiety exhibits very good water solubility and stability over a broad pH range. The three water molecules in $[^{99m}Tc(H_2O)_3(CO)_3]^+$ are labile and can be substituted by a variety of donors, including carboxylic acids, phosphines, thiols, thioethers, amines or amides as monodentate, bidentate or tridentate ligands. Due to the d^6 low spin electronic configuration, the resulting complexes are kinetically very stable. A recently developed CO-free kit for its preparation makes its use now much more attractive (Alberto et al., 2001).

Since the *in vivo* behavior of radiolabeled biomolecules is strongly influenced by the nature of the ^{99m}Tc complex,

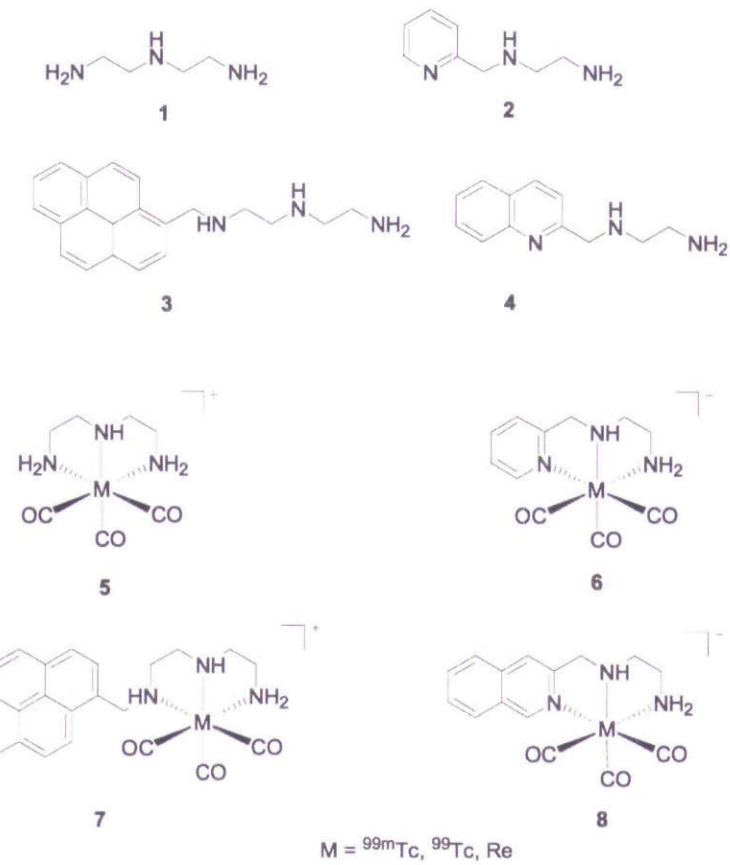
systematic screening of potential ligands is demanded. Schibli et al. performed the first systematic *in vitro* and *in vivo* studies of $^{99m}Tc(CO)_3$ model complexes with bidentate and tridentate N,O ligands resulting in neutral complexes (Schibli, et al., 2000). They found that complexes with bidentate ligands showed high retention in kidneys and liver, presumably due to the substitution of the remaining water molecule in these compounds by functional groups of plasma proteins. On the other hand, tridentate ligands, yielding neutral or anionic complexes, revealed good *in vivo* stability and rapid clearance from organs, tissues and blood. Acyclic tridentate triamine ligands form monopositively charged complexes with the $^{99m}Tc(CO)_3$ core. With one exception, they have not yet been studied in more detail, although highly stable and hydrophilic complexes can be expected (Banerjee et al., 2003a, 2003b; Chen et al., 2001; James et al., 2003).

We report the syntheses, structures and *in vivo* biological properties of the cationic complexes **5** and **6**. These hydrophilic monopositive complexes can be used to mimic, e.g., terminal amino groups or in the context of other functional molecules such as DNA intercalating or groove-binding agents, where a positive charge is fundamental. To illustrate this strategy, we report here the synthesis and structure of complexes **7** and **8**, which contain, in addition to the coordinating units **1** and **2**, the potentially DNA binding moieties pyrene and quinoline (Scheme 1).

EXPERIMENTAL

Chemicals and solvents were purchased from Merck, Dietikon (CH), Aldrich, or Fluka, Buchs (CH) and used without further purification. All reactions were performed under N_2 or Ar. $Na^{99m}TcO_4$ was eluted from a $^{99}Mo/^{99m}Tc$ Generator (Mallinckrodt) in 0.9% saline. The precursors $(NEt_4)_2[Re(Br)_3(CO)_3]$ and $(NEt_4)_2[^{99}Tc(Cl)_3(CO)_3]$ were synthesized according to previously reported methods (Alberto et al., 1996). ^1H and ^{13}C-NMR spectra were recorded on Varian Gemini 300 or Bruker DRX500 spectrometers at 300 and 75 MHz, or 500 and 125 MHz, respectively. Chemical shifts (in ppm) are relative to solvent protons as reference. TLCs were performed on Merck Silica gel 60 R_{254} plates. The compounds were visualized with 254 nm UV light or Schlittler reagent (Schlittler and Hohl, 1952).

HPLC was performed on a Merck L7000 system equipped with a EG&G Berthold LB 508 radiometric detector, using a Macherey-Nagel EC 250/3 Nucleosil 100-5 C18HD column. HPLC solvents were 0.1% trifluoroacetic acid (solvent A) and MeOH HPLC grade (solvent B). Gradient: 0–3 minutes: 100% A; 3.1–9 minutes: 75% A, 25% B; 9.1–20 minutes:



SCH. 1. Structures of the tridentate triamino ligands **1**–**4** and the corresponding complexes **5**–**8** (M = ^{99m}Tc, ^{99}Tc, Re).

linear gradient from 66% A (34% B) to 0% A (100% B); 20–28 minutes: 100% B; 28.1–30: 100% A. Flow rate: 0.5 mL/min, detection at 250 nm. Preparative HPLC: Varian Pro Star system, Macherey-Nagel VP 250/40 Nucleosil 100-7 C18 column, flow rate: 40 mL/min. Electrospray ionization mass spectra (ESI-MS) were recorded on a Merck Hitachi M8000 spectrometer.

Crystal data and experimental details are listed in the supporting information. Suitable crystals were covered with Paratone N oil and mounted on top of a glass fibre and immediately transferred to a STOE IPDS diffractometer. Data was collected at 183(2) K using graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$ Å). Data was corrected for Lorentz and polarization effects as well as for absorption (numerical). Structures were solved with direct methods using SHELXS-97 or SIR97 and were refined by full-matrix least-squares methods on F^2 with SHELXL-97 (Altomare et al., 1999; Sheldrick, 1997a, 1997b).

N-(pyridine-2-ylmethyl)ethane-1,2-diamine (2)

A portion of 500 mg (4.67 mmol) of pyridine-2-carbaldehyde and 524 mg (5.14 mmol) of *N*-(2-aminoethyl)acetamide were stirred in 50 mL EtOH for 14 hours at room temperature. The solution was cooled to 0 °C and 355 mg (9.34 mmol) of $NaBH_4$ was added over 1 hour. The reaction was then allowed to proceed for 4 hours. Excess of $NaBH_4$ was quenched by addition of 1 mL of H_2O and subsequent stirring for 10 minutes at 50 °C. After removal of the solvent under vacuum, the resulting light brown solid was refluxed in a 2N HCl solution for 6 hours. The solvent was removed under vacuum and the product was purified by flash chromatography (MeOH/25% NH_4OH, 20 : 1) to yield 473 mg (67%) of a pale yellow oil. ^{1}H NMR (D_2O): δ 8.83 (d, 1H, H^1), 8.58 (m, 1H, H^3), 8.14 (d, 1H^4), 8.04 (m, 1H, H^2), 4.74 (s, 2H, H^6), 3.61 (t, 2H, H^8), 3.45 (m, 2H, H^7). ^{13}C NMR (D_2O): δ 145.0, 142.9, 142.0, 126.0, 125.9, 45.8, 42.6, 33.3. MS (ESI): m/z 151.84 [$(M + H)^+$], calc. for $C_8H_{14}N_3^+$ 152.12. HPLC $R_t = 2.34$ min. TLC $R_f = 0.37$ (MeOH/25% NH_4OH, 10 : 1).

N-(2-amino-ethyl)-N′-pyrene-1-ylmethyl-ethane-1,2-diamine (3)

A portion of 1.08 g of pyrene-1-carbaldehyde (4.69 mmol) and 1.452 g (1.528 mL) (14.07 mmol) was dissolved in 50 mL EtOH. A few mL of dichloromethane were added until the solution became clear. After addition of 5 g of molecular sieve, the solution was stirred for 36 hours at room temperature. The solution was cooled to 0 °C and 356 mg (9.38 mmol) of $NaBH_4$ were added in small portions to the solution. After 12 hours, the excess of $NaBH_4$ was quenched with 1 mL of water. The solution was filtered and dried over vacuum. The resulting oil was purified by flash chromatography. (CH_2Cl_2/MeOH/25% NH_4OH, 10 : 3 : 0.3), yielding 649 mg (44%) of a light yellow oil. ^{1}H NMR ($CDCl_3$) δ 8.22 (d, 1H), 8.07 (d, 2H), 8.01 (m, 2H), 7.90 (m, 4H), 4.34 (s, 2H), 2.99 (s, 4H), 2.74 (t, 2H), 2.66 (t, 2H), 2.60 (t, 2H), 2.51 (t, 2H). ^{13}C NMR ($CDCl_3$) δ 133.4, 131.1, 130.6, 130.5, 128.9, 127.5, 127.3, 127.0, 125.8, 125.0, 124.9, 124.7, 124.6, 124.5, 123.0, 51.1, 50.6, 48.5, 40.6. MS (ESI) m/z 318.27 [$(M + H)^+$], calc. for $C_{21}H_{24}N_3^+$ 318.19. HPLC $R_t = 17.8$ min. TLC $R_f = 0.15$ (CH_2Cl_2/MeOH/25% NH_4OH, 10 : 3 : 0.3).

N-(quinoline-2-ylmethyl)ethane-1,2-diamine (4)

To a solution containing 500 mg (3.18 mmol) of quinoline-2-carbaldehyde in 30 mL of methanol was added 325 mg (3.18 mmol) of *N*-(2-aminoethyl)acetamide. The solution was stirred for 3 hours at room temperature and subsequently cooled to 0 °C. A portion of 241 mg (6.36 mmol) of $NaBH_4$ was then slowly added within 30 minutes. The reaction was allowed to proceed for 4 hours. Excess of $NaBH_4$ was quenched by addition of 1mL of water and subsequent stirring for one hour. After removal of the solvent under vacuum, the resulting light brown solid was refluxed in a 2N HCl solution for 6 hours. Water was then removed under vacuum and the product was purified by flash chromatography (CH_2Cl_2/MeOH/25% NH_4OH, 10:2:0.2) to yield 390 mg (61%) of **4**. ^{1}H NMR (D_2O): δ 8.60 (d, 1H), 8.10 (t, 2H), 7.92 (m, 1H), 7.75 (m, 1H), 7.67 (d, 1H), 4.71 (s, 1H), 3.60 (t, 2H), 3.48 (t, 2H). ^{13}C NMR (D_2O): δ 145.4, 144.8, 137.8, 133.3, 128.2, 127.0, 126.6, 119.8, 119.5, 46.6, 42.8, 33.4. MS (ESI) m/z 202.00 (100) [$(M + H)^+$], calc. for $C_{12}H_{16}N_3^+$ 202.13, HPLC: $R_t = 12.10$ min.

Re Complexes: General Procedure

A solution containing 0.2 mmol of the appropriate ligand, 0.8 mmol of triethylamine, and 0.2 mmol of $(NEt_4)_2[ReBr_3(CO)_3]$ in 10 mL of MeOH was refluxed for 8 hours. After removal of the solvent under vacuum, the crude product was purified by preparative HPLC.

[Re(1)(CO)$_3$](CF$_3$COO$^-$) (5)

Yield: 74%. ^{1}H NMR (D_2O) δ 3.08 (m, 2H), 2.98 (m, 4H), 2.85 (m, 2H). ^{13}C NMR (D_2O): 194.2, 192.9, 50.1, 40.7. MS (ESI) m/z (%) 373. 87 [$(M)^+$], calc. 374.05. HPLC $R_t = 6.3$ min.

[Re(2)(CO)$_3$](CF$_3$COO$^-$) (6)

Yield: 73%. ^{1}H NMR (MeOD) δ 8.83 (d, 1H), 8.07 (t, 1H), 7.69 (d, 1H), 7.51 (t, 1H), 7.22 (br. S, 1H), 5.29 (br.s, 1H), 4.63 (d, 2H), 4.53 (br. s, 1H), 3.05 (m, 1H), 2.78 (m, 2H), 2.16 (m, 1H).

^{13}C NMR (MeOD): δ 197.4, 196.7, 195.6, 163.3, 154.6, 141.8, 126.8, 124.8, 124.1, 62.0, 56.1, 43.6. MS (ESI) m/z (%) 422.24 [$(M)^+$], calc. 422.05. HPLC: $R_t = 14.4$ min.

[Re(3)(CO)$_3$](CF$_3$COO$^-$) (7)

Yield: 62%. ^{1}H NMR (most lipophilic diastereomer) (CD_3CN): δ 8.40 (d, 1H), 8.26 (m, 5H), 8.2-8.05 (m, 3H),

5.84 (br. S, 1H), 5.67 (br. s, 1H), 5.25 (br. s, 1H), 5.14 (q, $^1J = 24$ Hz, $^2J = 8$ Hz, 1H), 4.90 (q, $^1J = 24$ Hz, $^2J = 15$ Hz, 1H), 4.26 (br. s, 1H), 3.23 (m, 1H), 2.94 (m, 3H), 2.79 (m, 1H), 2.63 (m, 1H), 2.4 (br. S, 2H). ^{13}C NMR (second diastereomer) (CD_3CN): δ 196.9, 196.5, 195, 132.5, 132.3, 131.7, 131.6, 130.6, 129.7, 129.3, 128.9, 128.4, 127.5, 126.7, 126.5, 126, 125.6, 125.4, 123.8, 59.6, 54.5, 53.7, 49.9, 40.6. MS (ESI): m/z 587.81 [$(M)^+$], calc. for $C_{24}H_{23}N_3O_3Re$ 588.13. HPLC $R_t = 22.2$ and 23.2 min.

[Re(4)(CO)$_3$](CF$_3$COO$^-$) (8)

Yield: 68%. ^1H NMR (CD_3CN) δ 8.58 (d, 1H), 8.56 (d, 1H), 8.09 (d, 1H), 7.99 (t, 1H), 7.76 (t, 1H), 7.70 (d, 1H), 6.99 (br., 1H), 4.91 (br. 2H), 4.66 (br. 1H), 3.45 (br., 1H), 3.31 (m, 1H), 2.75 (m, 2H), 2.19 (m, 1H). ^{13}C NMR (CD_3CN): δ 197.4, 196.0, 195.1, 165.9, 148.3, 142.4, 133.7, 130.6, 130.5, 129.3, 121.5, 63.6, 52.7, 47.3. MS (ESI) m/z (%) 472.07 [$(M)^+$], calc. 472.07. HPLC: $R_t = 18.5$ min.

Synthesis of ^{99}Tc Complexes 6 and 8

Complexes **6** and **8** were synthesized from $(NEt_4)_2[^{99}Tc(Cl)_3(CO)_3]$ according to the same procedure as the Re complexes. Crystals were obtained by slow diffusion of Et_2O into a methanol solution of the complex.

^{99m}Tc Complexes: General Procedure

A 10 mL vial containing 100 µl of a 10^{-4} or 10^{-5} M aqueous solution of the ligand of interest was sealed and flushed with nitrogen. After addition of 900 µL of a solution containing *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ the vial was heated to 90°C for 30 minutes. The reaction solution was then cooled in an ice bath, and analyzed by HPLC with radiodetection.

Cysteine and Histidine Challenge

To 500 µL of a $2 \cdot 10^{-3}$ M cysteine solution and of a $2 \cdot 10^{-3}$ M histidine solution in PBS (pH 7) were added 500 µL of the ^{99m}Tc complex solution (final triamine ligand concentration 10^{-5} M). The resulting solutions were incubated at 37°C under atmospheric conditions and analyzed by HPLC after 4 and 24 hours.

Biodistribution

Planar scintigraphic imaging measurements were performed on female Wistar rats (1 animal per compound) with a SPECT camera for a total of 24 minutes after injection via the tail vein of 250 MBq/0.3 mL of the ^{99m}Tc complexes. Regions of interest (ROIs) analysis was performed to obtain cps over the main organs. Standard bioassay examinations were performed as follows: Male NMRI (Swiss) mice weighing 20–26 grams (3 animals per group) were injected 18 MBq/30 µl of ^{99m}Tc labeled compounds via the tail vein. After 30 minutes and 4 hours, respectively, the animals were sacrificed, the organs removed, weighted and assayed for radioactivity. Results are given as injected dose/gram organ.

RESULTS AND DISCUSSION

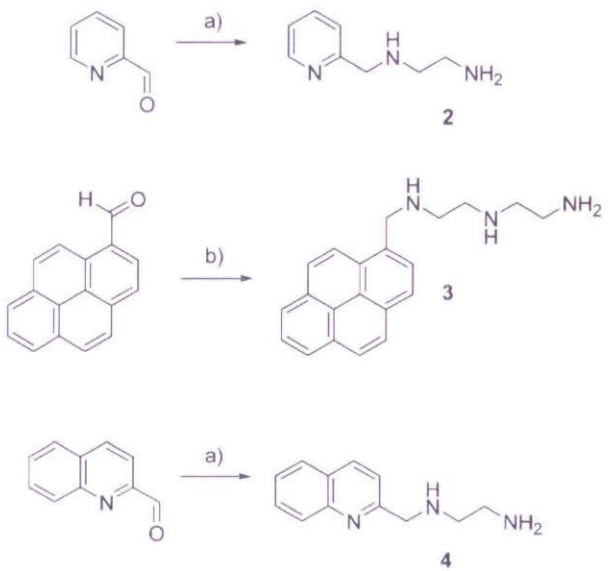
Ligand Synthesis

N'-pyridine-2-ylmethyl-ethane-1,2-diamine (**2**) and *N*-(2-quinolinylmethyl)-ethane-1,2-diamine (**4**) were prepared by reacting pyridine-2-carbaldehyde and 2-quinolinecarboxaldehyde, respectively, with *N*-(2-aminoethyl)acetamide. Subsequent reduction of the imine formed with sodium borohydride and deprotection of the acetamide protecting group with 2N HCl afforded **2** and **4** with an overall yield of 67% and 61%, respectively (Scheme 2). *N*-(2-amino-ethyl)-*N'*-pyrene-1-ylmethyl-ethane-1,2-diamine (**3**) was synthesized by reacting pyrene-1-carbaldehyde with an excess of diethylenetriamine. Subsequent reduction of the imine formed afforded **3** with an overall yield of 44% (Scheme 2).

Re and ^{99}Tc Complexes: Properties and Structures

All four ligands **1**–**4** react with stochiometric amounts of $(NEt_4)_2[ReBr_3(CO)_3]$ or $(NEt_4)_2[^{99}TcCl_3(CO)_3]$ in water or methanol at 75°C to form the cationic complexes **5**–**8** in quantitative yields in solution (Scheme 1).

Complex **5** is achiral. It has a HPLC retention time of about 7 minutes consistent with a hydrophilic character. Crystals suitable for x-ray analysis could be obtained by slow diffusion of Et_2O in a methanol solution of the complex. For a better understanding, an ORTEP representation is given in Figure 1 (Mundwiler et al., 2004). The small size of the complex is remarkable since the structure fits in a sphere of 7 Å diameter. Therefore, **1** could be the ligand of choice for applications with small biologically active molecules in which small size and hydrophilicity of the labeled compound plays a crucial role.



SCH. 2. Synthesis of **2**, **3**, and **4**. a) 1. N-(2-aminoethyl)acetamide, EtOH, r.t. 2. $NaBH_4$, EtOH, r.t. 3. 2N HCl, r.t. b) 1. 3 eq. of diethylenetriamine, EtOH/CH_2Cl_2, r.t. 2. $NaBH_4$, EtOH, r.t.

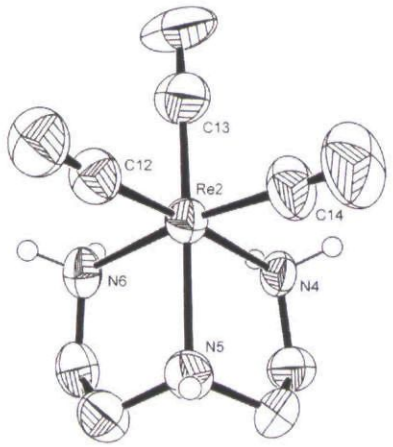


FIG. 1. ORTEP drawing of one of the two complexes **5** in the asymmetric unit, ellipsoids shown with 50% probability. Counter ion and selected hydrogens are omitted for clarity. Re(2)-C(12): 1.924 Å; Re(2)-C(13): 1.934 Å; Re(2)-C(14): 1.956 Å; Re(2)-N(4): 2.241 Å; Re(2)-N(5): 2.215 Å; Re(2)-N(6): 2.226 Å.

In order to enhance lipophilicity, complex **6** was prepared in the same way. In contrast to **5**, **6** possesses now two chiral centers that are dependent on each other. Thus, two enantiomeric forms must exist. Compared with **5**, **6** is significantly more lipophilic and has a HPLC retention time of about 17 minutes. An ORTEP representation of one enantiomer of the ^{99}Tc complex is shown in Figure 2. Obviously, complex **6** is substantially larger than **5** and it fits in an ellipsoid of about 7 (minimum) and 10 Å (maximum) diameter.

Complex **7** possesses the same set of donors as **5**. However, due to the presence of the pyrenyl, group **7** exhibits now three chiral centers, two of them being independent. Thus, this complex exists as two diastereomeric pairs of enantiomers. Indeed, HPLC with achiral column showed two peaks with retention times of 22.2 and 23.2 minutes in a ratio of about 1 : 2. An ORTEP plot of one isomer is shown in Figure 3 (Häfliger et al., *in press*). HPLC analysis showed that the obtained crystal corresponds to the peak with the longer retention time. The structure shows that the two functions, the potential intercalating polyaromatic unit and the triamine complex with *fac*-$[Re(CO)_3]^+$ point away from each other so that the latter should not sterically affect the DNA binding of the aromatic system.

In a similar way than **6**, complex **8** also exists in two enantiomeric forms. Its HPLC retention time is about 19 minutes and therefore the lipophilicity is enhanced again. A crystal of one isomer of ^{99}Tc complex **8** could be obtained. An ORTEP representation is given in Figure 4.

^{99m}Tc Complexes—Syntheses

^{99m}Tc complexes **5**–**8** were prepared from $[^{99m}TcO_4]^-$ in aqueous solution as previously described (Alberto et al., 1998). They were characterized by comparing their HPLC radioactive traces with the HPLC UV traces (absorption at 250 nm) of their Re analogues. If the ^{99m}Tc complexes exhibit the same retention times than the cold Re complex, the two compounds are considered as identical. All four ligands **1**–**4** formed the desired complexes **5**–**8** with no side products (purity > 98%) at micromolar concentrations. Ligands **1** and **3**, which contain three aliphatic amines, reacted quantitatively in water within 30 min at 90°C at 10^{-5} M concentration. Under the same conditions, the reaction of ligands **2** and **4**, containing two aliphatic and one aromatic amine, was complete at a concentration of 10^{-6} M. We assume that the faster rate of complex formation with the ligands **2**/**4** compared to **1**/**3** is due to availability of higher amounts of unprotonated nitrogen donors. For the aliphatic ligands, at least two amines are protonated, whereas for the aromatic amines the pyridine nitrogen is available and not protonated at pH = 7.4.

Stability Against Ligand Exchange and Reoxidation

In order to determine the stability of complexes **5** and **6** against ligand exchange and reoxidation, complexes were

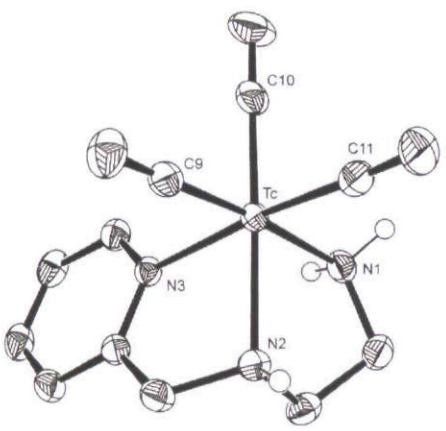


FIG. 2. ORTEP drawing of one enantiomer of complex **6**, ellipsoids shown with 50% probability. Counter ion and selected hydrogens are omitted for clarity. Tc-C(9): 1.913 Å; Tc-C(10): 1.926 Å; Tc-C(11): 1.927 Å; Tc-N(1): 2.205 Å; Tc-N(2): 2.200 Å; Tc-N(3): 2.202 Å.

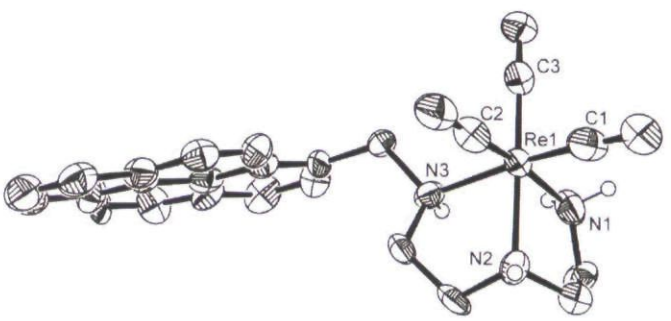


FIG. 3. ORTEP drawing of one isomer of complex **7**, ellipsoids shown with 50% probability. Counter ion and selected hydrogens are omitted for clarity. Re(1)-C(22): 1.974 Å; Re(1)-C(23): 1.862 Å; Re(1)-C(24): 1.887 Å; Re(1)-N(1): 2.228 Å; Re(1)-N(2): 2.221 Å; Re(1)-N(3): 2.264 Å.

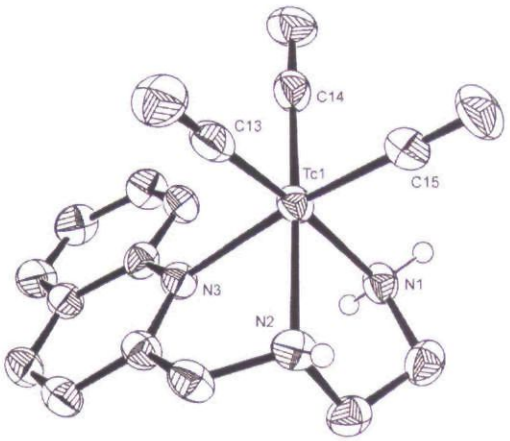


FIG. 4. ORTEP drawing of one of the two complexes **8** in the asymmetric unit, ellipsoids shown with 50% probability. Counter ion and selected hydrogens are omitted for clarity. Tc(1)-C(13): 1.933 Å; Tc(1)-C(14): 1.933 Å; Tc(1)-C(15): 1.924 Å; Tc(1)-N(1): 2.219 Å; Tc(1)-N(2): 2.210 Å; Tc(1)-N(3): 2.244 Å.

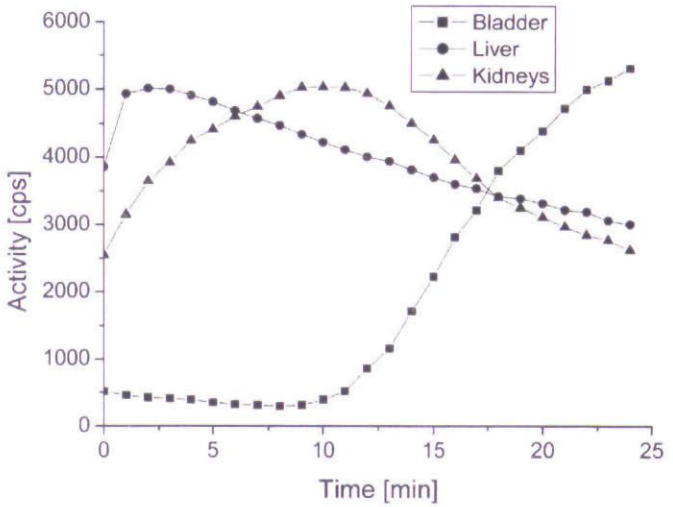


FIG. 5. Planar scintigraphic imaging using regions of interest (ROIs) analysis in a rat for complex **5**.

challenged with 1 mM (10^4 excess) of cysteine and histidine in PBS buffer (pH 7) for 24 hours under atmospheric conditions at 37 °C. The samples were analyzed by HPLC after 4 and 24 hours. Both complexes were found to be very stable against ligand exchange since no significant formation of cysteine or histidine Tc tricarbonyl complexes was observed. Only little reoxidation to $^{99m}TcO_4^-$ (<5%) occured within 24 h. Although complexes with tridentate amine ligands are not expected to have a sufficiently high thermodynamic stability to resist biological conditions at high dilution, the d^6 nature of the M(I) center renders the complexes very robust and mechanistically prevents substitution and decomposition.

Biodistribution of ^{99m}Tc Complexes 5 and 6

The biodistribution of cationic complexes comprising the *fac*-$[Tc(CO)_3]^+$ moiety has not yet been studied. We investigated the biodistribution of complexes **5** and **6** in mice. It was of particular interest to verify if these cationic species would show heart accumulation, confirming the old (and wrong) hypothesis that cations follow the K^+ pathway in Na^+/K^+ ATP pumps. For this purpose, body images of rats were taken every minute by planar scintigraphic imaging using regions of interest (ROIs) analysis to obtain cps over the main organs during 24 minutes post injection. Additionally, the radiolabelled compounds were injected into mice to perform standard bioassays. The mice were sacrificed after 30 minutes and 4 hours post injection, and the organs were removed, weighted and assayed for radioactivity.

Figure 5 and Table 1 show the results of the planar scintigraphic imaging experiment and standard bioassay performed for compound **5**. The planar scintigraphic imaging data show that this complex exhibits a fast uptake into the liver, from where the activity is slowly released. Kidney uptake is slightly delayed with a maximum level obtained after about 10 minutes, which could be a hint that metabolism takes place in the liver. Complex **5** or its metabolites are then quickly excreted via the urinary bladder, where most of the radioactivity can be found after 25 minutes. Standard bioassay experiments show that after 30 minutes unexcreted **5** is mostly to be found in the liver, the kidneys and the gastrointestinal tract. 80.6% ± 4.5% of the total activity is however removed after 4 hours. Significant accumulation can only be observed in the gastrointestinal tract (3.7% ± 1.2% ID/g tissue). Interestingly, despite its positive charge, **5** does not display a noticeable affinity to the heart.

The results of the biodistribution experiments performed for complex **6** are shown in Figure 6 and Table 2. The planar scintigraphic imaging experiment shows that complex **6** is quickly removed from the blood and taken up by the liver and the kidneys. Excretion in the urinary bladder starts already after

TABLE 1
Biodistribution of complex **5** in NMRI mice, % injected dose per gram tissue (average of 3 ± SD), as a function of time after intravenous administration

Organ	30 min	4 hours
Blood	0.57 ± 0.12	0.20 ± 0.02
Muscle	0.16 ± 0.03	0.10 ± 0.01
Bone	0.26 ± 0.06	0.13 ± 0.01
Heart	0.59 ± 0.21	0.29 ± 0.05
Liver	11.36 ± 3.34	3.84 ± 0.46
Spleen	1.57 ± 0.70	0.78 ± 0.09
Kidneys	6.35 ± 1.76	0.99 ± 0.12
Gastrointestinal tract	6.98 ± 1.56	3.75 ± 1.18
Total activity (in % ID)	44.15 ± 1.65	19.42 ± 4.48

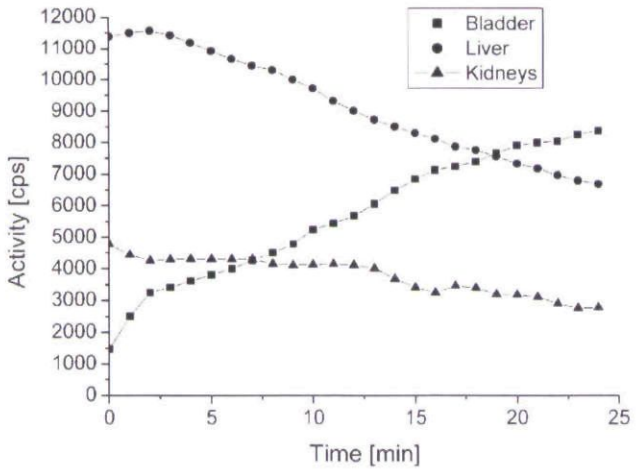


FIG. 6. Planar scintigraphic imaging using regions of interest (ROIs) analysis in a rat for complex **6**.

a few minutes. Standard bioassay examinations showed that after 30 min the complex can be found in the liver and the kidneys, but also in the gastrointestinal tract. After 4 hours, the values for the spleen and the liver decreased, but accumulation in the gastrointestinal tract (7.62% ± 0.91%) is obvious. 69.5% ± 6.5% of the injected dose has been excreted by then. Despite its overall positive charge, complex **6** also does not display any noteworthy affinity for the heart.

When comparing the biodistribution of complexes **5** and **6**, it is obvious that **5** shows a relatively fast excretion with only little undesired accumulation in the gastrointestinal tract, while **6** exhibits a somewhat slower excretion with more accumulation in these tissues. Thus, among these two complexes **5** displays the more favorable biodistribution for future application.

Due to the different experimental conditions used in both studies, a direct comparison with the results obtained by Schibli and coworkers is limited. In general and as expected, **5** and **6** show much better pharmacokinetic behavior with less accumulation in the kidneys and the liver than complexes with the bidentate ligands histamine, 2-picolinic acid, and 2,4-dipicolinic acid. Complex **5** displays, 30 minutes post injection, a smaller accumulation in the blood than the neutral tridentate histidine complex studied by Schibli and coworkers. On the other hand, **5** has an increased tendency to accumulate in the liver and the kidneys, but complex **6** displays less uptake in the kidneys, but more retention in the liver and the spleen than the histidine complex.

TABLE 2
Biodistribution of complex **6** in NMRI mice, % injected dose per gram tissue (average of 3 ± SD), as a function of time after intravenous administration

Organ	30 min	4 hours
Blood	0.20 ± 0.15	0.03 ± 0.01
Muscle	0.12 ± 0.03	0.06 ± 0.03
Bone	0.57 ± 0.69	0.99 ± 1.19
Heart	0.92 ± 0.87	0.07 ± 0.03
Liver	7.63 ± 0.41	1.02 ± 0.44
Spleen	4.88 ± 4.76	0.15 ± 0.26
Kidneys	2.55 ± 0.80	0.56 ± 0.21
Gastrointestinal tract	4.91 ± 3.73	7.62 ± 0.91
Total activity (in % ID)	45.87 ± 4.82	30.05 ± 6.51

CONCLUSION

The first *in vitro* and *in vivo* experiments of $[^{99m}Tc(CO)_3]^+$ model complexes with tridentate triamine ligands, yielding positively charged complexes, are presented. The two ligands reacted quickly at micromolar concentrations with *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ to form the desired complexes **5** and **6** in quantitative yields. Both complexes are stable against reoxidation and ligand exchange. According to biodistribution studies, **5** revealed a good and fast clearance from the blood and organs with only limited accumulation in the large intestine. On the other hand, **6** is removed relatively quickly from the blood but later shows some accumulation in certain organs. Although both complexes are monopositively charged, none of them displays a noticeable accumulation in the heart. Both ligands were further derivatized with DNA binding functionalities and successfully labeled with $[^{99m}Tc(OH_2)_3(CO)_3]^+$. The *in vitro* and *in vivo* results presented indicate that acyclic tridentate amine chelators and in particular **1**, are a biologically relevant alternative to other ligands such as histidine or iminodiacetic acid.

Supplementary Material Available

Crystallographic data for the structural analysis of complexes **6** and **8** have been deposited in the Cambridge Crystallographic Data Centre, CCDC 234733 and CCDC 234732. Copies of this information may be obtained from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336-033; email: deposit@ccdc.cam.ac.uk or www:http://www.ccdc.cam.ac.uk).

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