Rhenium and technetium tricarbonyl complexes anchored by pyrazole-based tripods: novel lead structures for the design of myocardial imaging agents†

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This report describes the synthesis and biological evaluation of cationic ^{99m}Tc–tricarbonyl complexes anchored by ether-containing tris(pyrazolyl)methane or bis(pyrazolyl)ethanamine ligands to be applied in the design of radiopharmaceuticals for myocardial imaging: *fac*-[^{99m}Tc(CO)$_3${RC(pz)$_3$}]$^+$ (R = H (**1a**), MeOCH$_2$ (**2a**), EtOCH$_2$ (**3a**), ^{n}PrOCH$_2$ (**4a**)) and *fac*-[^{99m}Tc(CO)$_3${RNHCH$_2$CH(pz)$_2$}]$^+$ (R = H (**5a**), MeO(CH$_2$)$_2$ (**6a**)) (pz = pyrazolyl). At the no carrier added level, complexes **1a–6a** were obtained in high radiochemical yield (> 98%) by reaction of *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ with the corresponding tripod chelator in aqueous medium. All these complexes display a high *in vitro* and *in vivo* stability, except **6a** which metabolizes *in vivo* yielding *fac*-[^{99m}Tc(CO)$_3${HO(CH$_2$)$_2$NHCH$_2$CH(pz)$_2$}]$^+$ (**7a**). Biological studies in mice have shown that among the radiotracers evaluated in this work, **3a**, anchored by a tris(pyrazolyl)methane chelator bearing an ethyl methyl ether substituent, has the highest heart uptake (3.6 ± 0.5%ID g^{-1} at 60 min p.i.). Complex **3a** presents also the best heart : blood, heart : liver and heart : lung ratios, appearing as the most promising as a potential myocardial imaging agent. The chemical identity of **1a–7a** was ascertained by HPLC comparison with the previously reported *fac*-[Re(CO)$_3${HC(pz)$_3$}]Br (**1**) and with the novel *fac*-[Re(CO)$_3${RC(pz)$_3$}]Br (R = MeOCH$_2$ (**2**), EtOCH$_2$ (**3**), ^{n}PrOCH$_2$(**4**)) and *fac*-[Re(CO)$_3${RNHCH$_2$CH(pz)$_2$}]Br (R = H (**5**), MeO(CH$_2$)$_2$ (**6**) HO(CH$_2$)$_2$ (**7**)). The novel Re(I) tricarbonyl complexes, **2–7**, were characterized by the common analytical techniques, including single crystal X-ray diffraction analysis. The solid state structure confirmed the presence of facial and tridentate (κ^3-N$_3$) anchor ligands. Solution NMR studies have also shown that this κ^3-N$_3$ coordination mode is retained in solution for all complexes (**2–7**).

Introduction

The availability of the precursor *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ justifies the growing and outstanding importance of this organometallic aqua ion in radiopharmaceutical chemistry.[1] In addition the attractiveness of this low-valent precursor stems from the high substitution stability of the CO ligands and the easy replacement of the water molecules by chelators of different denticity.[2,3] Tridentate chelators, namely those containing *N*-heterocyclic donors, emerged in recent years as the most suitable to stabilize Re and Tc tricarbonyl complexes for biomedical applications, due to their high *in vitro* and/or *in vivo* stability.[4,5]

Amongst tridentate ligands, pyrazole-based tripods of the tris(azolyl)hydroborate and tris(pyrazolyl)methane type are obvious candidates to stabilize the *fac*-[^{99m}Tc(CO)$_3$]$^+$ unit, due to their topology, denticity and donor-atom set.[6–8] Within our interest on Tc and Re organometallic compounds for biomedical applications, we have evaluated the possibility of synthesizing tris(pyrazolyl)hydroborate ^{99m}Tc tricarbonyl complexes under aqueous conditions required in the preparation of radiopharmaceuticals. In our hands, the synthesis of these type of ^{99m}Tc complexes has not been possible due to the tendency of the boron containing ligands to undergo hydrolysis.[3,9] Considering the hydrolytic stability of the tris(pyrazolyl)methanes, we decided to evaluate the coordination capability of these tripod chelators towards the *fac*-[^{99m}Tc(CO)$_3$]$^+$ core in aqueous medium. Our studies were also extended to bis(pyrazolyl)ethanamine ligands which represent another class of neutral pyrazole-based tripods, recently introduced by Reger *et al.*[10] Tris(pyrazolyl)methanes and bis(pyrazolyl)ethanamines must afford cationic technetium tricarbonyl complexes with a tuneable lipophilicity, due to the possibility of introducing different substituents at the pyrazolyl rings and at the central carbon atom and terminal amine function. These features led us to anticipate that this type of compound might be useful for designing myocardial imaging agents, as it is well known that lipophilic and cationic ^{99m}Tc complexes may have the ability to cross the cardiac cells membrane. The presence of ether functional groups in such complexes has also been considered crucial to achieving efficient ^{99m}Tc radiotracers for heart imaging, considering that they act as modifiers of the lipophilicity and pharmacokinetics. [^{99m}Tc]-sestamibi and [^{99m}Tc]-tetrofosmin are two lipophilic and cationic radiopharmaceuticals in clinical use for heart imaging, containing six and eight ether groups, respectively. However, these two radiopharmaceuticals are far from being the ideal agents for myocardial perfusion studies, suffering from relatively low heart : liver and heart : lung uptake ratios.[11,12]

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The importance of heart imaging in Nuclear Medicine still justifies an interest in finding the best performing ^{99m}Tc radiotracers for myocardial perfusion. Several research groups are very active in this field, trying to explore innovative labelling methodologies, based on Tc(V) nitrido or Tc(I) tricarbonyl metal fragments.[12–18]

In this report, we present a first screening of the usefulness of pyrazole-based tripods for the design of cationic ^{99m}Tc tricarbonyl complexes for myocardial imaging. Two novel families of ^{99m}Tc(I) tricarbonyl complexes (**1a–6a**) anchored by ether-containing tris(pyrazolyl)methane and bis(pyrazolyl)ethanamine ligands (Chart 1) are reported, including studies on their biodistribution and metabolic stability in mice. As depicted in Chart 1, the rhenium congeners, **1–6**, were also prepared and characterized to be used as surrogates of the ^{99m}Tc complexes.

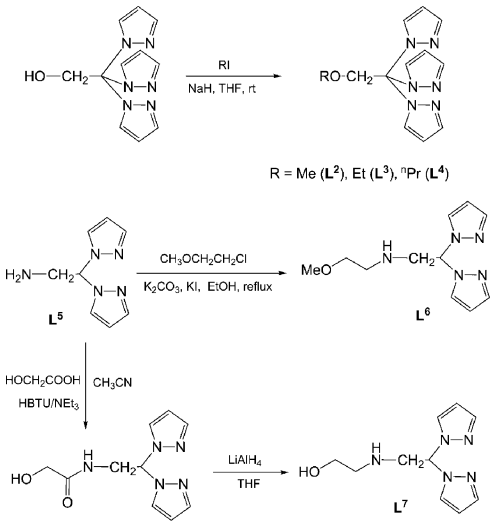
Chart 1 Tris(pyrazolyl)methane and bis(pyrazolyl)ethylamine Re(I) and ^{99m}Tc(I) tricarbonyl complexes discussed in this work.

Results and discussion

Synthesis and characterization of the ligands

Tris(pyrazolyl)methanes and bis(pyrazolyl)ethanamines are neutral tripod chelators, stable in water and easily functionalized through the pyrazolyl rings and/or through the carbon and nitrogen central atoms. With tris(pyrazolyl)methanes some Re(I) tricarbonyl complexes have been reported but, to the best of our knowledge, the possibility of preparing the ^{99m}Tc congeners in aqueous medium has never been explored.[19–22] In order to have a first insight into the interest of these two families of chelators for the design of ^{99m}Tc(I) organometallic cations for heart imaging, we started to evaluate the effect of introducing ether groups in the aliphatic backbone of the chelators. For the tris(pyrazolyl)methane derivatives this has been carried out using 2,2,2-trispyrazolylethanol as the starting material, and following methodologies similar to those reported in the literature for the synthesis of other ether derivatives of this type of chelators.[23] As indicated in Scheme 1, the conversion of $HOCH_2C(pz)_3$ into the ether-containing compounds $ROCH_2C(pz)_3$ (R = Me (**L**^{2}), Et (**L**^{3}), ^{n}Pr (**L**^{4})) comprised its deprotonation with sodium hydride in tetrahydrofuran, followed by reaction with an approximate fivefold excess of the adequate alkyl iodide at room temperature. Compounds **L**^{2}–**L**^{4} were obtained as colourless oils in moderate to high isolated yields (52–97%) after a relatively simple work-up which involved extraction of the crude with diethyl ether and washing with water.

The ether functionalization of chelators of the bis(pyrazolyl)-ethanamine type was achieved by *N*-alkylation of the primary amine of $H_2NCH_2CH(pz)_2$ (**L**^{5}) with excess of 1-chloro-2-methoxyethane yielding $CH_3O(CH_2)_2NHCH_2CH(pz)_2$ (**L**^{6}) in poor yield (21%), even after 4 days of reflux (Scheme 1).



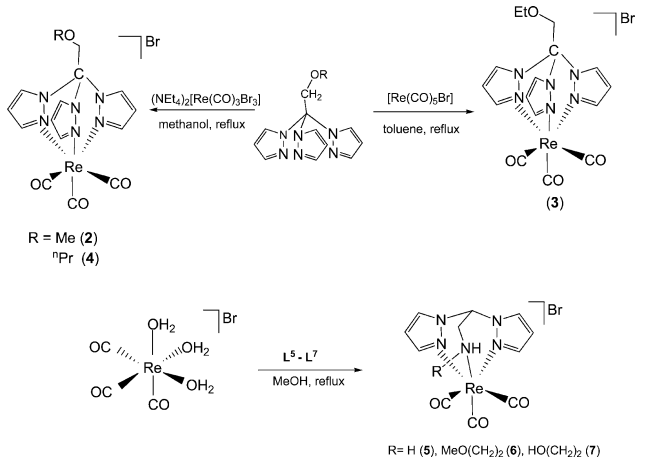
Scheme 1 Synthesis of the pyrazole-based ligands.

Compound **L**^{6} is a brown oil which has been purified by column chromatography. No further efforts have been performed to optimize the yield of this reaction because the amount of isolated ligand was sufficient to proceed with the studies with Re and ^{99m}Tc. **L**^{7} has been obtained by reacting **L**^{5} with glycolic acid followed by the reduction of the resulting amide derivative with $LiAlH_4$ (Scheme 1). **L**^{7} and its Re and ^{99m}Tc complexes have been prepared just to clarify some aspects of the biological behaviour of the ^{99m}Tc complex with **L**^{6}, as discussed below.

All the novel ligands reported in this work, **L**^{2}–**L**^{4}, **L**^{6} and **L**^{7} are air stable compounds which are soluble in the most common organic solvents. Their characterization by ^{1}H and ^{13}C NMR spectroscopy and by mass spectrometry corroborated the respective formulations.

Synthesis and spectroscopic studies of the model rhenium complexes

The use of Re complexes to identify the molecular structure of the ^{99m}Tc congeners, by means of HPLC comparison, is a common and accepted practice in radiopharmaceutical chemistry owing to the similarities between the physico-chemical properties of the compounds of these group 7 elements. Therefore, we have studied the synthesis of Re(I) tricarbonyl complexes with **L**^{1}–**L**^{7} aiming their use as surrogates of the ^{99m}Tc analogues. For **L**^{1}, the Re complex *fac*-$[Re(CO)_3\{HC(pz)_3\}]Br$ (**1**) was already reported and its synthesis has been performed by the literature method.[20] The synthesis of the complexes with the novel ether-containing tris(pyrazolyl)methanes, **L**^{2}–**L**^{4}, has been attempted by reaction with $(NEt_4)_2[ReBr_3(CO)_3]$ in refluxing methanol (Scheme 2). After overnight reflux and removal of the solvent under vacuum, the ^{1}H NMR analysis of the crude confirmed that reactions proceeded almost to completion with formation of the desired compounds *fac*-$[Re(CO)_3\{ROCH_2C(pz)_3\}]Br$ (R = Me (**2**), Et (**3**), ^{n}Pr (**4**)). The synthesis of **2–4** was quite straightforward but the purification of some of these compounds was more demanding owing to the presence of tetraethylammonium bromide. Complex **2** was obtained in high yield (71%) and analytically pure by extraction with tetrahydrofuran followed by washing with toluene. Complex



Scheme 2 Synthesis of the Re(I) tricarbonyl complexes.

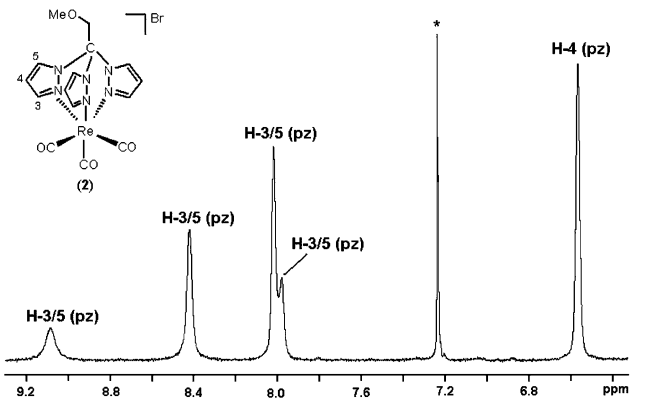


Fig. 1 ^{1}H NMR spectrum in $CDCl_3$ of complex **2** in the region of the pyrazolyl protons (*solvent).

4 was also obtained in a pure form after successive washing with tetrahydrofuran and distilled water, although in a relatively low isolated yield (30%), due to its moderate solubility in these solvents. Complex **3** could only be obtained pure using $[Re(CO)_5Br]$ as starting material (Scheme 2).

By contrast, the synthesis and purification of the bis-(pyrazolyl)ethanamine Re(I) tricarbonyl complexes, *fac*-$[Re(CO)_3\{RNHCH_2CH(pz)_2\}]$ (R = H (**5**), $MeO(CH_2)_2$ (**6**), $HO(CH_2)_2$ (**7**)), have been quite straightforward starting from $[Re(CO)_3(H_2O)_3]Br$ (Scheme 2). Compounds **5–7** have been obtained in high yield (74–90%) in the form of microcrystalline white solids, after a minimal work-up.

The novel organometallic Re(I) tricarbonyl complexes, **2–7**, were characterized by the common spectroscopic techniques (IR, ^{1}H and ^{13}C NMR), and in the case of complex **5** its chemical identity was also ascertained by X-ray diffraction analysis.

The IR spectra of **2–7** show a set of intense ν(C≡O) bands in the region 2042–1888 cm^{-1} with the typical pattern for complexes with the *fac*-$[Re(CO)_3]^+$ moiety. In comparison with the tris(pyrazolyl)methane complexes (**2–4**), those with the bis(pyrazolyl)ethanamine chelators present lower carbonyl stretches. This indicates that the replacement of one pyrazolyl arm by an aliphatic amine arm leads to pyrazole-based tripods with better electron releasing properties, a trend that has been already reported for Re(I) tricarbonyl complexes with related ligands.[21]

As reported in the literature, the three pyrazolyl rings of *fac*-$[Re(CO)_3\{HC(pz)_3\}]Br$ (**1**) are magnetically equivalent at room temperature, in accordance with the C_3 symmetry of this compound.[20] In contrast, the complexes anchored by the ether-containing tris(pyrazolyl)methanes (**2–4**) have ^{1}H NMR spectra presenting at room temperature four resonances for the H-3 and H-5 pyrazolyl protons in a 2 : 1 splitting pattern. Two of these resonances present chemical shifts in quite different ranges, 9.10–9.20 ppm and 8.39–8.41 ppm, while the other two resonances appear in a relatively narrow region (7.98–8.04 ppm). Unlike the H-3/5 protons, the H(4) protons originate a unique resonance centred at 6.58 ppm, as exemplified for complex **2** in Fig. 1. These data are consistent with the structure found in the solid state for compound **2**. In spite of the poor quality of the data (*vide infra*), the determination of the solid state structure has shown that the oxygen atom of the ether linkage straddles two of the pyrazolyl rings, being *anti* to the third one. The set of resonances which span the widest range of chemical shifts must be due to the H(5) protons. These protons are closer to the ether linkage and, therefore, their chemical shifts are more influenced by its orientation. The same type of behaviour has been reported for $[\{1,4\text{-}C_6H_4[CH_2OCH_2C(pz)_3\}_2\{Re(CO)_3\}]_2(Br)_2$, a dimeric Re(I) complex anchored by a bitopic ligand displaying a $-CH_2OR$ linkage.[20] In the case of this complex, it has been claimed that the alignment of the H(5) protons toward the ether linkage upon coordination of the azole rings hinders the rotation around the central C–CH_2, causing the magnetic non-equivalence of the pyrazolyl rings. Most probably, the same process must explain the behaviour exhibited by complexes **2–4**. We must mention that at 70 °C the spectrum of complex **2** in dmso-d_6 shows only three resonances for the pyrazolyl protons, a pattern compatible with free rotation of the ether substituent at high temperature.

The ^{1}H NMR spectrum of complex **5** is relatively simple with a unique set of resonances for the protons of the two pyrazolyl rings which are magnetically equivalent. Each type of protons (C*H*, CH_2 or NH_2) from the aliphatic backbone of the bis(pyrazolyl)ethanamine ligand (**L**^{5}) also originate a unique resonance, due to the presence of a symmetry plane in the molecule which contains the metal center, the methylenic carbon and the amine nitrogen atoms. This pattern is consistent with a κ^3-N_3 coordination mode for the chelator, as found in the solid state (*vide infra*). Complexes **6** and **7**, anchored by bis(pyrazolyl)ethanamine ligands bearing ethyl methyl ether or ethanol groups at the terminal amine, present a lower symmetry. As a consequence, the two pyrazolyl rings in **6** and **7** are magnetically different and the methylenic protons are diastereotopic. As discussed above for complexes **2–4**, this behaviour must be related with the barrier to rotation of the ether or ethanol groups around the C–N bond. Due to this barrier, the ether or ethanol containing arms tilts preferentially towards one of the pyrazolyl rings, rendering these rings magnetically non-equivalent. Consistently, the ^{13}C NMR spectra of **6** and **7** show three resonances for the CO ligands. In dmso-d_6, the non-equivalence of the pyrazolyl rings is retained even at high temperature (T = 100 °C), meaning that the barrier to rotation around the C–N bond in **6** and **7** is higher than the barrier to rotation around the C–CH_2 in **4**.

Solid-state structures

Low quality single crystals of **2** were obtained by slow diffusion of *n*-hexane in a saturated dichloromethane solution of **2**. The best crystal measured did not provide a good quality data set to determine a satisfactory structure for **2**.[24] Nevertheless, the connectivity of the atoms was determined unambiguously, confirming the coordination of three pyrazolyl rings with no interaction between the ether group and the metal.

High quality crystals of **5** were grown from a saturated methanolic solution. An ORTEP diagram of the cation of **5** is shown in Fig. 2, together with a selection of bond lengths. The coordination environment around the rhenium atom is defined by the two nitrogen atoms from the pyrazolyl rings, the nitrogen atom from the amine group and the three carbonyl ligands, in a nearly octahedral arrangement. The Re–C and Re–N bond distances can be considered unexceptional, as well as the intraligand bond distances and angles. As expected, the pyrazolyl Re–N bond distances (av. 2.175(4) Å) are shorter than the Re–N bond distance of 2.233(4) Å found for the coordinated amine group. For each type of nitrogen atom (sp^2 *vs.* sp^3), these Re–N distances are almost coincident with those reported for Re(I) tricarbonyl complexes anchored by tripodal ligands of tris(pyrazolyl)methane or tris(aminomethyl)ethane types, respectively.[19–22]

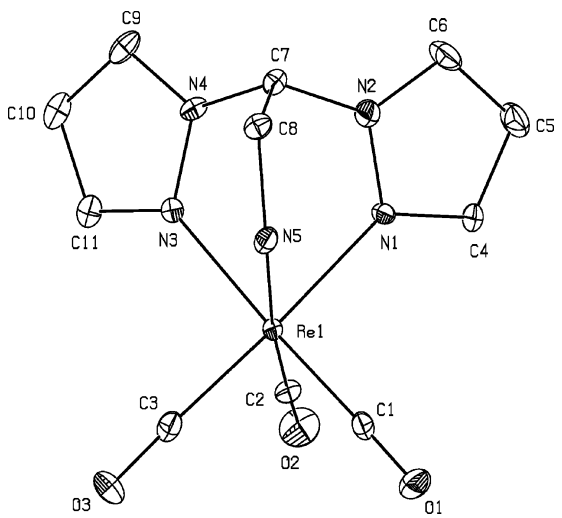


Fig. 2 ORTEP view of complex **5**; thermal ellipsoids are drawn at the 40% probability level. Selected bond lengths (Å): Re1–C1 1.925(5), Re1–C2 1.927(5), Re1–C3 1.927(5), Re1–N1 2.177(4), Re1–N3 2.172(4), Re1–N5 2.233(4).

Table 1 Crystallographic data for compound **5**

Formula	$C_{11}H_{15}BrN_5O_3Re{\cdot}CH_3OH$
M/g mol^{-1}	559.40
Crystal system	Monoclinic
Space group	$P2_1/c$
a/Å	12.5900(2)
b/Å	10.0842(2)
c/Å	14.5569(2)
α/°	90
β/°	110.3190(10)
γ/°	90
V/Å^3	1733.14(5)
Z	4
T/K	130(2)
ρ (calculated)/g cm^{-3}	2.144
μ(Mo-Kα)/mm^{-1}	9.342
Reflections collected	15 143
No. unique reflections	3529 (R_{int} = 0.0702)
R^a	0.0345 (0.0902)^b
$wR_2{}^a$	0.0413 (0.0938)

^{a} The values were calculated for data with $I > 2\sigma(I)$. ^{b} Based on all data.

Synthesis and characterization of the ^{99m}Tc complexes

The ^{99m}Tc congeners **1a–7a** were prepared in high radiochemical yield (>98%) by reaction of *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ with the respective ligand (**L**^{1}–**L**^{7}) in aqueous medium, at temperatures between 75 and 100 °C (Scheme 3). Reactions were almost complete after 30–60 min heating, using final concentrations of the ligands in the range 7.5×10^{-5}–1.5×10^{-3} M (Table 2). The radiochemical purity of complexes **1a–7a** has been determined by HPLC analysis, and their chemical identity ascertained by HPLC comparison with the Re congeners as exemplified for complex **2a** in Fig. 3.

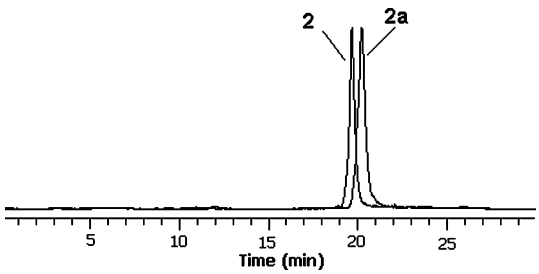
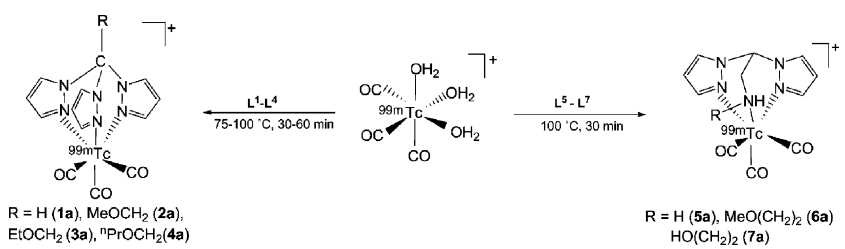


Fig. 3 Comparative HPLC chromatograms of complexes **2** (UV detection) and **2a** (radiometric detection).

At the no carrier added level, the kinetics of the reactions are faster for the bis(pyrazolyl)ethanamine ligands than for tris(pyrazolyl)methanes, most probably due to the replacement



Scheme 3 Preparation of the ^{99m}Tc complexes.

Table 2 Experimental conditions for the synthesis of complexes **1a–7a** and their radio-HPLC retention times and log *P* values

Complex	Yield (%)	[L]/M	Time/min	*T*/°C	t_R/min	log $P_{o/w}$
1a	> 98	3.5×10^{-4}	30	75	16.6[a] (16.1)[c]	0.55 ± 0.006
2a	> 98	1.5×10^{-3}	60	75	20.2[b] (19.7)[c]	0.32 ± 0.006
3a	> 98	1.5×10^{-3}	60	100	20.5[b] (20.1)[c]	0.68 ± 0.015
4a	> 98	10^{-3}	60	100	20.2[b] (19.7)[c]	1.18 ± 0.002
5a	> 98	7.5×10^{-5}	30	100	15.5[a] (14.7)[c]	−0.30 ± 0.033
6a	> 98	10^{-4}	30	100	16.4[a] (15.9)[c]	−0.078 ± 0.015
7a	> 98	1.2×10^{-4}	30	100	14.6[a] (14.1)[c]	—

[a] Using a gradient of acetonitrile and aqueous 0.1% CF_3COOH as the solvent. [b] Using a gradient of methanol and aqueous 0.1% CF_3COOH as the solvent. [c] The values in parentheses are for the Re complexes **1–6**.

of one of the azolyl rings by the primary amine coordinating group. All the complexes can be kept in PBS (pH = 7.4, 37 °C) for at least 24 h without any noticeable decomposition, namely oxidation to pertechnetate (HPLC analysis). These findings are not surprising since *N*-heterocyclic ligands usually provide highly stable complexes with the *fac*-[^{99m}Tc(CO)$_3$]$^+$ unit.[2–5]

The lipophilicity of all the complexes has been determined under physiological conditions, *i.e.* using PBS at pH = 7.4. The tris(pyrazolyl)methane ^{99m}Tc(I) tricarbonyl complexes, *fac*-[^{99m}Tc(CO)$_3${RC(pz)$_3$}]$^+$ (R = H (**1a**), MeOCH$_2$ (**2a**), EtOCH$_2$ (**3a**), ^{n}PrOCH$_2$ (**4a**)), are moderately lipophilic (log *P* = 0.32–1.18) following the trend **2a** < **1a** < **3a** < **4a** (Table 2). The bis(pyrazolyl)ethylamine complexes, *fac*-[^{99m}Tc(CO)$_3${RNHCH$_2$CH(pz)$_2$}]$^+$ (R = H (**5a**), MeOCH$_2$CH$_2$ (**6a**)), are more hydrophilic with log *P* values of −0.307± 0.033 and −0.078 ± 0.015, respectively.

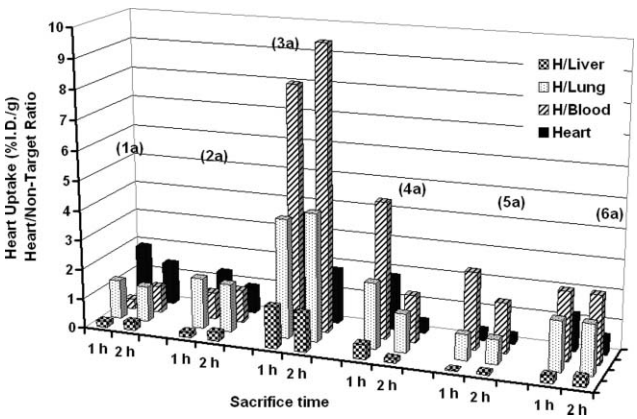


Fig. 4 Comparison of heart uptake (% ID g^{-1}) and heart : non target ratio for complexes **1a–6a**.

Biological evaluation: Biodistribution studies and *in vivo* stability

Biodistribution studies of complexes **1a–6a** were performed in female CD-1 mice at 1 and 2 h after i.v. administration. This animal model was just used for a first screening of the biological profile and to anticipate the potential of **1a–6a** as heart imaging agents (*i.e.* heart uptake, excretory pathway, heart : liver and heart : lung ratios). Data from these studies, expressed as % I.D. g^{-1} organ are presented in Table 3 and are also shown in graphical form in Fig. 4.

In general, the tris(pyrazolyl)methane complexes, **1a–4a**, display a greater tendency to be accumulated by the heart (1.3–3.6% ID g^{-1} at 1 h p.i.) than the bis(pyrazolyl)ethylamine congeners, **5a** and **6a**, which show a poorer radioactivity accumulation (0.33–1.2% ID g^{-1} at 1 h p.i) in this organ. This difference is certainly justified by the highest lipophilicity of the tris(pyrazolyl)methane complexes which present log *P* values in the range (0.5–1.2) well thought-out as necessary to have high heart uptake and a fast liver clearance.[12] All the complexes, **1a–6a**, are excreted mainly through the hepatobiliary pathway with a faster overall excretion (40.8 ± 7.0% ID and 47.9 ± 4.0% ID at 2 h p.i) for the more hydrophilic compounds, **5a** and **6a**, respectively.

Amongst the tris(pyrazolyl)methane complexes (**1a–4a**), the ethoxy derivative (**3a**) is the one which has the highest heart uptake (3.6 ± 0.5% ID g^{-1} at 1 h p.i), a fast blood clearance (0.5 ± 0.2% ID g^{-1} at 1 h p.i) and the highest rate of excretion (33.1 ± 4.3% ID at 2 h p.i) (Table 3 and Fig. 4). Consequently, complex **3a** shows the more favourable heart : non-target organ ratios, *i.e.* a heart : blood ratio of 8.2 ± 2.9, a heart : liver ratio of 1.4 ± 0.9 and a heart : lung ratio of 4.0 ± 0.7 at 1 h p.i (Fig. 7). However, **3a** is not yet an alternative to [^{99m}Tc]-sestamibi which presents in the same animal model the highest heart uptake (7.9 ± 0.6% ID g^{-1}, 1 h p.i.) although showing comparable heart : liver (1.4 ± 0.1, 1 h p.i.) and heart : lung (6.3 ± 0.6, 1 h p.i.) ratios.

The metabolic stability of complexes **1a–6a** has also been evaluated by HPLC analysis of the blood and urine of mice injected with those radiotracers. Complexes **1a–4a** do not undergo any significant metabolic fate, and almost all (> 95%) of the circulating activity corresponds to the intact compounds. The metabolic stability of **1a–4a** compares with that of [^{99m}Tc]-sestamibi which is excreted intact by the kidneys and hepatobiliary tract.[12] We must also mention that recently reported ^{99m}Tc(V)–nitrido and ^{99m}Tc(I)–tricarbonyl complexes, with faster liver clearance and higher heart : liver ratio than [^{99m}Tc]-sestamibi, have also shown a high metabolic stability.[18,25] The influence of the ether groups on the

Table 3 Biodistribution data of complexes **1a–6a** in Charles River mice at 60 and 120 min p.i. (percentage of the injected dose per gram ± standard deviation)

	1a		**2a**		**3a**		**4a**		**5a**		**6a**	
Organ	60 min	120 min	60 min	120 min	60 min	120 min	60 min	120 min	60 min	120 min	60 min	120 min
Blood	5.4 ± 1.2	1.6 ± 0.3	1.5 ± 0.1	0.8 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.36 ± 0.06	0.22 ± 0.06	0.2 ± 0.1	0.3 ± 0.1	0.52 ± 0.05	0.29 ± 0.02
Liver	9.9 ± 2.7	4.9 ± 1.3	6.9 ± 2.3	6.2 ± 2.1	3.8 ± 2.0	1.6 ± 0.6	4.3 ± 1.6	2.1 ± 0.5	6.4 ± 0.4	3.5 ± 0.7	4.9 ± 0.4	2.2 ± 0.5
Heart	1.9 ± 0.3	1.4 ± 0.3	1.3 ± 0.3	0.9 ± 0.2	3.6 ± 0.5	1.8 ± 0.7	1.8 ± 0.4	0.4 ± 0.2	0.33 ± 0.08	0.29 ± 0.06	1.2 ± 0.3	0.65 ± 0.10
Lung	1.4 ± 0.3	1.2 ± 0.2	0.8 ± 0.2	0.8 ± 0.3	0.9 ± 0.1	0.6 ± 0.3	0.9 ± 0.5	0.29 ± 0.04	0.41 ± 0.16	0.40 ± 0.2	0.7 ± 0.1	0.39 ± 0.07
Kidney	7.2 ± 0.5	3.9 ± 0.8	3.3 ± 0.9	2.7 ± 0.4	5.0 ± 1.4	2.8 ± 0.5	8.6 ± 2.1	7.8 ± 3.0	1.9 ± 0.4	1.3 ± 0.3	6.9 ± 0.8	4.0 ± 1.7
Excretion (% I.D)	18.4 ± 4.7	28.1 ± 2.9	20.6 ± 2.4	22.1 ± 3.4	33.1 ± 4.3	34.6 ± 3.9	9.2 ± 1.5	34.7 ± 2.3	39.8 ± 6.8	47.9 ± 4.0	38.4 ± 2.1	40.8 ± 7.0

biological performance of the tris(pyrazolyl)methane complexes discussed herein, **1a–4a**, stems essentially from physicochemical factors which affect their distribution in the tissues and their pharmacokinetics. Apparently, there is no metabolic role for the ether groups, as previously found for other ether-containing lipophilic ^{99m}Tc cations.

By contrast, complex *fac*-[^{99m}Tc(CO)$_3${MeOCH$_2$CH$_2$HNCH$_2$-CH(pz)$_2$}]$^+$ (**6a**) (t_R = 16.4 min) suffers pronounced *in vivo* metabolization. As shown in Fig. 5, HPLC analysis of the urine of mice injected with **6a** revealed the presence of a radioactive metabolite (t_R = 14.6 min) corresponding to 55% of the excreted activity at 2 h p.i. HPLC analysis of blood, kidney and liver homogenates (see Fig. 5) of mice injected with **6a** has also confirmed the presence of this metabolite, albeit in lower percentage in the case of the kidney homogenate.

The biotransformation suffered by complex **6a** must not be caused by a transchelation process because the parent compound *fac*-[^{99m}Tc(CO)$_3${H$_2$NCH$_2$CH(pz)$_2$}]$^+$ (**5a**) (t_R = 15.5 min) displays a high metabolic stability (HPLC analysis of blood and urine). Hydrolytically cleavage of the C–N bond, between the ether group and the central nitrogen atom, could be one possibility to explain the in *vivo* degradation of **6a** which should then be transformed into complex **5a**.[26] However, co-injection of urine samples containing the metabolite of **6a** and authentic samples of complex **5a** unequivocally proved the presence of different compounds. These results led us to consider that the metabolization of **6a** could involve the ether-containing arm. Although we did not observe any metabolization for the other ether-containing complexes evaluated herein, biotransformation of ethers into alcohols or carboxylic acids mediated by cytochrome P450 in human liver microsomes are well known.[27] To clarify this point we have prepared the alcohol derivative *fac*-[^{99m}Tc(CO)$_3${HOCH$_2$CH$_2$HNCH$_2$CH(pz)$_2$}]$^+$ (**7a**) (t_R = 14. 6 min) and co-injected **7a** and urine samples containing the metabolite of **6a.** These studies confirmed unequivocally that the metabolization of **6a** takes place at the ether-containing arm with formation of complex **7a.**

Conclusion

For the first time, we have shown that tris(pyrazolyl)methane or bis(pyrazolyl)ethanamine ligands are suitable to stabilise the *fac*-[^{99m}Tc(CO)$_3$]$^+$ core under the aqueous conditions required for the preparation of radiopharmaceuticals. The cationic ^{99m}Tc tricarbonyl complexes anchored by these tripod ligands, **1a–6a**, have been fully characterized and evaluated as potential radiotracers for myocardium imaging. These studies proved that the introduction of ether functions in the chelator backbone does not affect their coordination capability, and the complexes formed do not transchelate *in vivo.* However, the replacement of an azolyl ring by a primary amine (**1a** *vs* **6a**) or the introduction of ether groups in the central carbon atom (**3a** *vs* **1a**) or in the primary amine (**6a** *vs* **5a**) of the tripod chelators affects significantly the liphophilicity, pharmacokinetics and metabolic stability of the complexes. From all the radiotracers evaluated in this work, *fac*-[^{99m}Tc(CO)$_3${EtOCH$_2$C(pz)$_3$}] (**3a**) is the one which exhibits the most significant heart uptake (3.6 ± 0.5%ID g^{-1} at 1 h p.i.), and the best heart : blood, heart : liver and heart : lung ratios, although it is not yet an alternative to [^{99m}Tc]-sestamibi. Nevertheless, the versatility of the two classes of tripodal ligands evaluated herein

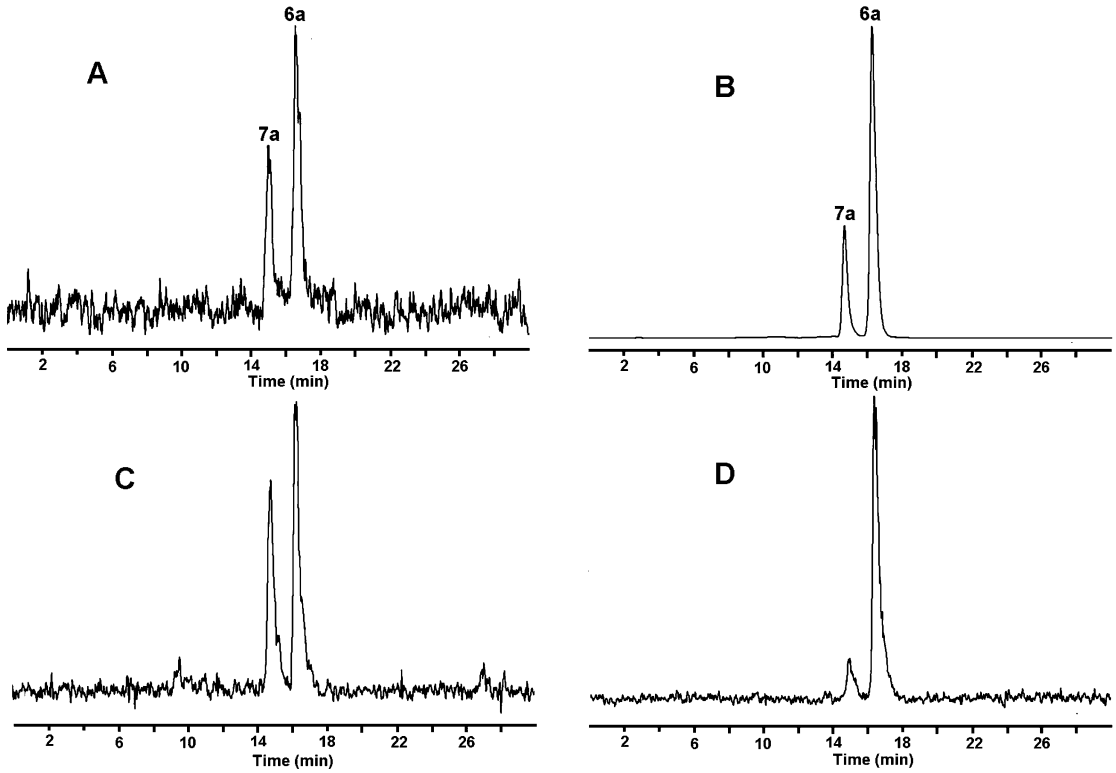


Fig. 5 HPLC analysis (radiometric detection) of serum (A), urine (B), liver homogenate (C) and kidney homogenate (D) from mice injected with **6a**, at 60 min p.i.

make them very promising to obtain ^{99m}Tc tricarbonyl complexes with improved physicochemical properties, biodistribution, and pharmacokinetics in terms of myocardial perfusion imaging. Currently complexes **1a** and **5a** are being used as lead structures for the design of the best performing myocardial imaging agents.

Experimental

General procedures

All chemicals and solvents were of reagent grade and used without purification unless stated otherwise. The syntheses of ligands and respective Re complexes were performed under a nitrogen atmosphere, while the work-up was carried out in air. ^{1}H and ^{11}C NMR spectra were recorded on a Varian Unity 300 MHz spectrometer, ^{1}H and ^{11}C chemical shifts were referenced with the residual solvent resonances relative to tetramethylsilane. IR spectra were recorded as KBr pellets on a Perkin-Elmer 577 spectrometer. C, H and N analyses were performed on an EA 110 CE Instruments automatic analyser. All the new ligands, $\mathbf{L}^2$–$\mathbf{L}^5$ and $\mathbf{L}^6$–$\mathbf{L}^7$, were characterized by Fourier transform ion cyclotron resonance mass spectrometry (FT/ICR-MS). The compounds $HOCH_2C(pz)_3$,[23] $HC(pz)_3$ ($\mathbf{L}^1$)[23] and $H_2NCH_2CH(pz)_2$ ($\mathbf{L}^5$)[10] were prepared according to published methods. The starting materials $[Re(CO)_5Br]$,[28] $(NEt_4)_2[Re(CO)_3Br_3]$[29] and $[Re(H_2O)_3(CO)_3]Br$,[30] and the model rhenium complex *fac*-$[Re(CO)_3\{HC(pz)_3\}]Br$ (**1**)[20] were prepared as described elsewhere. The radioactive precursor *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ was prepared using a IsoLink® kit (Malinckrodt, Inc.). $Na[^{99m}TcO_4]$ was eluted from a $^{99}Mo/^{99m}Tc$ generator with 0.9% saline. HPLC analysis of the Re and ^{99m}Tc complexes was performed on a Perkin-Elmer LC pump 200 coupled to a LC 290 tunable UV/Vis detector and to a Berthold LB-507A radiometric detector. Separations were achieved on a Nucleosil column (10 μm, 250 mm × 4mm), using a flow rate of 1 mL min^{-1}; UV detection, 254 nm, eluents, A = aqueous 0.1% CF_3COOH solution, B = methanol or acetonitrile, method, t = 0–3 min, 0% B, 3–3.1 min, 0–25% B, 3.1–9 min, 25% B, 9–9.1 min, 25–34% B, 9.1–20 min, 34–100% B, 20–22 min, 100% B, 22–22.1 min, 100–0% B, 22.1–30 min, 0% B.

Synthesis of $MeOCH_2C(pz)_3$ ($\mathbf{L}^2$)

To a stirred suspension of NaH (34 mg, 1.42 mmol) in dry THF (10 mL) was added, at room temperature, 2,2,2-tris(pyrazolyl)ethanol (300 mg, 1.23 mmol) dissolved in the same solvent (10 mL), and the mixture was stirred for 1 h. After cooling to 0 °C, a solution of methyl iodide (873 mg, 6.15 mmol) in dry THF (5 mL) was added dropwise. The resulting mixture was warmed to room temperature and stirred for 16 h. After evaporation of THF under vacuum, the residue was extracted twice with 10 mL of diethyl ether. Following washing of the ether extracts with distilled water, compound $\mathbf{L}^2$ was recovered as a colourless oil, after drying the organic phase under vacuum. Yield: 97% (307 mg, 1.19 mmol).

^{1}H NMR ($CDCl_3$): δ_H 7.64 (d, H-5 (pz), 3H), 7.34 (d, H-3 (pz), 3H), 6.32 (dd, H-4 (pz), 3H), 5.03 (s, CH_2, 2H), 3.38 (s, CH_3O, 3H). ^{13}C NMR ($CDCl_3$): δ_C 141.3 (C-3 (pz)), 130.7 (C-5 (pz)), 106.5 (C-4 (pz)), 89.5 (*C*-H), 75.5 (CH_2), 59.9 (CH_3O). FT/ICR-MS (+) (m/z): 258.1 $[M]^+$ (52%).

Synthesis of $EtOCH_2C(pz)_3$ (L^3)

Compound $\mathbf{L}^3$ is a colourless oil with tendency to solidify on standing, which was obtained as above described for $\mathbf{L}^2$, starting from 305 mg (1.25 mmol) of 2,2,2-tris(pyrazolyl)ethanol and from 975 mg (6.25 mmol) of ethyl iodide. Yield: 66% (225 mg, 0.83 mmol).

^{1}H NMR ($CDCl_3$): δ_H 7.63 (d, H-5 (pz), 3H), 7.40 (d, H-3 (pz), 3H), 6.31 (dd, H-4 (pz), 3H), 5.05 (s, CH_2, 2H), 3.50 (q, OCH_2CH$_3$, 2H), 1.09 (tr, OCH$_2$CH_3, 3H). ^{13}C NMR ($CDCl_3$): δ_C 141.2 (C-3 (pz)), 130.9 (C-5 (pz)), 106.4 (C-4 (pz)), 89.7 (*C*H), 73.5 (*C*H$_2$), 67.9 (*C*H$_2$), 15.0 (*C*H$_3$). FT/ICR-MS (+) (*m*/*z*): 272.1 [M]$^+$ (8%).

Synthesis of ^{n}PrOCH$_2$C(pz)$_3$ (L^4)

Compound $\mathbf{L}^4$ is a colourless oil which was obtained as above described for $\mathbf{L}^2$, starting from 350 mg (1.43 mmol) of 2,2,2-tris(pyrazolyl)ethanol and from 1.215 g (7.15 mmol) of *n*-propyl iodide. Yield: 52% (212 mg, 0.74 mmol).

^{1}H NMR ($CDCl_3$,): δ_H 7.63 (d, H-3/5 (pz), 3H), 7.40 (d, H-3/5 (pz), 3H), 6.31 (dd, H-4 (pz), 3H), 5.05 (s, CH_2, 2H), 3.40 (tr, OCH_2, 2H), 1.47 (m, 2H, CH_2), 0.77 (tr, CH_3, 3H). ^{13}C NMR ($CDCl_3$): δ_C 141.1 (C-3 (pz)), 130.8 (C-5 (pz)), 106.2 (C-4 (pz)), 89.6 (*C*H), 73.8 (O*C*H$_2$), 73.7 (O*C*H$_2$), 22.6 (*C*H$_2$), 10.3 (*C*H$_3$). FT/ICR-MS (+) (*m*/*z*): 286.2 [M]$^+$ (12%).

Synthesis of MeO(CH$_2$)$_2$NHCH$_2$CH(pz)$_2$ (L^6)

To a solution of 2,2′-bis(pyrazolyl)ethanamine ($\mathbf{L}^5$) (327 mg, 1.8 mmol) in dry ethanol (15 mL) was added excess of 1-chloro-2-methoxyethane (526 μL; 5.8 mmol), K_2CO_3 (1.281 g, 9.2 mmol) and KI (30 mg, 0.18 mmol), and the mixture refluxed for 4 days. After this time, the solvent was removed under vacuum and the residue was applied on a silica gel column which was eluted with MeOH–$CHCl_3$ (5 : 95). Removal of the solvent from the collected fractions yielded compound $\mathbf{L}^6$ in the form of a brown oil. Yield: 21% (89 mg, 0.38 mmol).

^{1}H NMR ($CDCl_3$): δ_H 7.58 (d, H-3/5 (pz), 2H), 7.53 (d, H-3/5 (pz), 2H), 6.51 (t, C*H*, 1H), 6.25 (t, H-4 (pz), 2H), 3.69 (d, −NHCH_2CH, 2H), 3.41 (t, OCH_2, 2H), 3.28 (s, −OCH_3, 3H), 2.27 (t, NCH_2CH$_2$, 2H). ^{13}C NMR ($CDCl_3$): δ_C 140.3 (C-3 (pz)), 128.9 (C-5 (pz)), 106.6 (C-4 (pz)), 75.0 (*C*H), 71.8 (*C*H$_2$), 58.8 (*C*H$_3$O), 51.6 (*C*H$_2$), 48.7 (*C*H$_2$). FT/ICR-MS (+) (*m*/*z*): 236.2 [M + H]$^+$ (10%).

Synthesis of HO(CH$_2$)$_2$NHCH$_2$CH(pz)$_2$ (L^7)

2,2′-Bis(pyrazolyl)ethanamine (215 mg, 1.21 mmol), glycolic acid (109 mg, 1.43 mmol), *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) (543 mg, 1.43 mmol) were dissolved in DMF (15 mL). This solution was treated with triethylamine (263 mg, 2.60 mmol) and the reaction mixture was stirred overnight at room temperature. After this time, the solvent was removed under vacuum and ^{1}H NMR analysis of the mixture has shown that the amide HOCH$_2$C(O)NHCH$_2$CH(pz)$_2$ was formed almost quantitatively. This compound was recovered by silica gel column chromatography with MeOH–EtOAc (40 : 60) as eluent (^{1}H NMR (CD_3OD): δ_H 7.83 (d, H-3 (pz), 2H), 7.54 (d, H-5 (pz), 2H), 6.76 (t, C*H*, 1H), 6.32 (t, H-4 (pz), 2H), 4.32 (d, −NHCH_2CH, 2H), 3.89 (s, HOCH_2, 2H)). Although not strictly pure, the collected amide derivative was dissolved in dry THF (20 mL) and treated with 6.90 mL of 1M $LiAlH_4$ in diethyl ether, and the mixture was stirred at room temperature over 4 days. After this time, the reaction was quenched with water (1 mL) and 10% NaOH (0.360 mL). After filtration to remove a white insoluble material, the filtrate was dried under vacuum and the residue applied to a silica gel column which has been eluted with a gradient from 100% EtOAc to 100% MeOH. Removal of the solvent from the colleted fractions yielded compound $\mathbf{L}^7$ in the form of a yellow–brown solid. Yield: 29% (78 mg, 0.35 mmol).

^{1}H NMR (CD_3OD): δ_H 7.82 (d, H-3 (pz), 2H), 7.53 (d, H-5 (pz), 2H), 6.65 (t, C*H*, 1H), 6.32 (t, H-4 (pz), 2H), 3.71 (d, −NHCH_2CH, 2H), 3.57 (t, HOCH_2, 2H), 2.72 (t, NCH_2CH$_2$, 2H). ^{13}C NMR ($CDCl_3$): δ_C 141.4 (C-3 (pz)), 130.8 (C-5 (pz)), 107.5 (C-5 (pz)), 75.4 (*C*H), 71.8 (*C*H$_2$) 61.7 (*C*H$_2$), 51.9 (*C*H$_2$). FT/ICR-MS (+) (*m*/*z*): 222.1 [M + H]$^+$ (54%).

Synthesis of *fac*-[Re(CO)$_3${MeOCH$_2$C(pz)$_3$}]Br (2)

A solution of $(NEt_4)_2[Re(CO)_3Br_3]$ (80 mg, 0.104 mmol) and compound $\mathbf{L}^2$ (27 mg, 0.104 mmol) in methanol (15 mL) was refluxed overnight. The solvent was removed under vacuum and the residue was extracted with THF. Compound **2** was recovered as a beige solid, after removal of THF, washing with toluene and drying under vacuum. Yield: 71% (45 mg, 0.074 mmol).

Anal. Calcd. for $C_{15}H_{14}N_6O_4BrRe$: C, 29.61; H, 2.32; N, 13.81%. Found: C, 29.27; H, 2.48; N, 13.23%. ^{1}H NMR ($CDCl_3$): δ_H 9.10 (br, H-3/5 (pz), 1H), 8.41 (br, H-3/5 (pz), 2H), 8.04 (br, H-3/5 (pz), 2H), 7.99 (br, H-3/5 (pz), 1H), 6.58 (br, H-4 (pz), 3H), 6.17 (s, CH_2, 2H), 4.07 (s, CH_3, 3H). ^{13}C NMR ($CDCl_3$): δ_C 193.8 (br *C*O,), 147.2 (C-3/5 (pz)), 135.8 (C-3/5 (pz)), 134.6 (C-3/5 (pz)), 109.9 (C-4 (pz)), 109.0 (C-4 (pz)), 85.2 (*C*pz$_3$), 70.4 (*C*H$_2$), 60.7 (*C*H$_3$). IR (KBr, ν_{max}/cm^{-1}): 2042s, 1914vs (C≡O).

Synthesis of *fac*-[Re(CO)$_3${EtOCH$_2$C(pz)$_3$}]Br (3)

A solution of [Re(CO)$_5$Br] (50 mg, 0.123 mmol) and $EtOCH_2C(pz)_3$ ($\mathbf{L}^3$) (34 mg, 0.125 mmol) in toluene was refluxed for 24 h. After refluxing, we obtained a white suspension from which a white insoluble solid was separated by filtration, after cooling to room temperature. This precipitate was washed several times with warm toluene and dried under vacuum to afford compound **3** as a white microcrystalline solid. Yield: 69% (53 mg, 0.085 mmol).

Anal. Calcd. for $C_{16}H_{16}N_6O_4BrRe$: C, 30.87; H, 2.59; N, 13.50%. Found: C, 30.22; H, 2.38; N, 13.07%. ^{1}H NMR ($CDCl_3$): δ_H 9.22 (br, H-3/5 (pz), 1H), 8.49 (d, H-3/5 (pz), 2H), 8.02 (br, H-3/5 (pz), 2H), 7.98 (1H, br, H-3/5 (pz)), 6.58 (tr, H-4 (pz), 3H,), 6.27 (s, CH_2, 2H), 4.38 (q, CH_2, 2H), 1.37 (tr, CH_3, 3H,). δ_C 193.4 (br, *C*O), 147.2 (C-3/5 (pz)), 135.2 (C-3/5 (pz)), 134.3 (C-3/5 (pz)), 109.8 (C-4 (pz)), 108.9 (C-4 (pz)), 85.4 (*C*pz$_3$), 68.5 (*C*H$_2$), 68.3 (*C*H$_2$), 15.2 (*C*H$_3$). IR (KBr, ν_{max}/cm^{-1}): 2042s, 1946s, 1926s (C≡O).

Synthesis of *fac*-[Re(CO)$_3${^{n}PrOCH$_2$C(pz)$_3$}]Br (4)

A solution of $(NEt_4)_2[Re(CO)_3Br_3]$ (100 mg, 0.13 mmol) and compound $\mathbf{L}^4$ (40 mg, 0.14 mmol) in methanol (15 mL) was refluxed for 16 h. The solvent was evaporated under vacuum and

the residue was washed with THF and water. The insoluble solid was dried under vacuum and formulated as compound **4**. Yield: 30% (25 mg, 0.039 mmol).

Anal. Calcd. for $C_{17}H_{18}N_6O_4BrRe$: C, 32.08; H, 2.85; N, 13.20%. Found: C, 31.39; H, 2.41; N, 12.72%. ^{1}H NMR ($CDCl_3$): δ_H 9.20 (br, H-3/5 (pz), 1H), 8.39 (br, H-3/5 (pz), 2H), 8.04 (br, H-3/5 (pz), 2H), 7.99 (br, H-3/5 (pz), 1H), 6.58 (br, H-4 (pz), 3H), 6.25 (br, CH_2, 2H), 4.27 (br, CH_2, 2H), 1.75 (br, CH_2, 2H), 0.98 (br, CH_3, 3H). ^{13}C NMR ($CDCl_3$): ^{13}C NMR ($CDCl_3$): δ_C 193.4 (br, *C*O), 147.3 (C-3/5 (pz)), 134.9 (C-3/5 (pz)), 134.3 (C-3/5 (pz)), 109.8 (C-4 (pz)), 108.9 (C-4 (pz)), 85.4 (*C*pz$_3$), 74.4 (*C*H$_2$), 68.4 (*C*H$_2$), 22.9 (*C*H$_2$), 10.7 (*C*H$_3$). IR (KBr, ν_{max}/cm^{-1}): 2042s, 1940vs (C≡O).

Synthesis of *fac*-[Re(CO)$_3${H$_2$NCH$_2$CH(pz)$_2$}]Br (5)

To a solution of 2,2′-bis(pyrazolyl)ethanamine (**L**[5]) (32 mg, 0.18 mmol) in methanol (15 mL) was added [Re(CO)$_3$(H$_2$O)$_3$]Br (80 mg, 0.20 mmol), and the resulting mixture was refluxed overnight. After cooling to room temperature, the solvent was removed under vacuum and the residue was washed with $CHCl_3$. The insoluble fraction was recovered by centrifugation and dried under vacuum, affording a white microcrystalline solid which was formulated as **5**. Yield: 74% (70 mg, 0.13 mmol).

Anal. Calcd. for $C_{11}H_{11}N_5O_3ReBr$: C, 25.05; H, 2.10; N, 13.28%. Found: C, 24.09; H, 1.90; N, 12.63%. ^{1}H NMR (CD_3OD): δ_H 8.28 (d, H-3/5 (pz), 2H), 8.22 (d, H-3,5 (pz), 2H), 7.52 (t, C*H*, 1H), 6.65 (t, H-4 (pz), 2H), 5.48 (t, NH_2, 2H), 3.34–3.11 (m, CH_2, 2H). ^{13}C NMR (CD_3OD): δ_C 150.8 (C-3/5 (pz)), 137.2 (C-3/5 (pz)), 112.0 (C-4 (pz)), 75.5 (*C*H), 45.8 (*C*H$_2$). IR (KBr, ν_{max}/cm^{-1}): 2032s, 1931s, 1909sh, 1888s (C≡O).

Synthesis of *fac*-[Re(CO)$_3${CH$_3$O(CH$_2$)$_2$NHCH$_2$CH(pz)$_2$}]Br (6)

Complex **6** is a white solid which was synthesized as above described for **5**, by reacting **L**[6] (20 mg, 0.082 mmol) with [Re(CO)$_3$(H$_2$O)$_3$]Br (38 mg, 0.094 mmol). The reaction mixture was dried under vacuum and the residue obtained was dissolved in chloroform. After centrifugation, to remove any insoluble material, the solvent was evaporated yielding a white oil. Successive washings of this oil with *n*-hexane, followed by removal of the solvent and drying under vacuum, yielded compound **6** as a white microcrystalline solid. Yield: 90% (43 mg, 0.073 mmol).

Anal. Calcd. for $C_{14}H_{17}N_5O_4ReBr.0.5CHCl_3$: C, 26.95; H, 2.86; N, 10.84. Found: C, 27.42; H, 2.24; N, 10.61%. ^{1}H NMR (CD_3CN): δ_H 8.45 (br, H-3/5 (pz), 1H), 8.43 (br, H-3/5 (pz), 1H), 8.14 (d, H-3/5 (pz), 1H), 8.08 (d, H-3/5 (pz), 2H), 8.03 (br, C*H*, 1H), 6.65 (m, H-4 (pz), 1 + 1H), 5.35 (m, N*H*, 1H), 3.90–3.72 (m, CH_2, 1H), 3.68–3.61 (m, CH_2, 1H), 3.53–3.46 (m, CH_2, 1H), 3.38–3.28 (m, CH_2 + OCH_3, 1 + 3H), 3.12-2.99 (m, CH_2, 1 + 1H). ^{13}C NMR (CD_3CN): δ_C 196.6 (*C*O), 195.1 (*C*O), 193.2 (*C*O), 148.0 (C-3/5 (pz)), 147.8 (C-3/5 (pz)), 135.3 (C-3/5 (pz)), 134.8 (C-3/5 (pz)), 109.3 (C-4 (pz)), 109.2 (C-4 (pz)), 71.0 (*C*H), 70.2 (*C*H$_2$), 59.3 (*C*H$_3$O), 58.8 (*C*H$_2$), 51.8 (*C*H$_2$). IR (KBr, ν_{max}/cm^{-1}): 2028s, 1921vs (C≡O).

Synthesis of *fac*-[Re(CO)$_3${HO(CH$_2$)$_2$NHCH$_2$CH(pz)$_2$}]Br (7)

Complex **7** is a white–brown solid which was synthesized as above described for **5**, by reaction of **L**[7] (20 mg, 0.090 mmol) with [Re(CO)$_3$(H$_2$O)$_3$]Br (40 mg, 0.099 mmol). The reaction mixture was dried under vacuum and the complex was extracted with chloroform. After precipitation with *n*-hexane compound **7** was obtained in the form of a white–brown microcrystalline solid. Yield: 70% (0.036 g, 0.063 mmol).

Anal. Calcd. for $C_{13}H_{15}N_5O_4ReBr.CHCl_3$: C, 24.34; H, 2.33; N, 10.14%. Found: C, 24.24; H, 1.71; N, 9.61%. ^{1}H NMR (CD_3CN): δ_H 8.20 (d, H-3/5 (pz), 1H), 8.17 (d, H-3/5 (pz), 1 + 1H), 8.11 (d, H-3/5 (pz), 1H), 7.36 (br, C*H*, 1H), 6.55 (m, H-4 (pz), 1 + 1H), 5.43 (m, N*H*, 1H), 3.90–3.80 (m, CH_2, 1H), 3.68–3.62 (m, CH_2, 1 + 1H), 3.50 (t, CH_2OH, 1H), 3.35–3.27 (m, CH_2, 1 + 3H), 3.06-2.94 (m, CH_2, 1 + 1H). ^{13}C NMR (CD_3CN): δ 197.9 (*C*O), 196.8 (*C*O), 193.9 (*C*O), 148.2 (C-3/5 (pz)), 148.0 (C-3/5 (pz)), 135.5 (C-3/5 (pz)), 135.0 (C-3/5 (pz)), 109.4 (C-4 (pz)), 109.4 (C-4 (pz)), 71.2 (*C*H), 63.2 (*C*H$_2$), 59.6 (*C*H$_2$), 51.9 (*C*H$_2$). IR (KBr, ν_{max}/cm^{-1}): 2034, 1908 (C≡O).

X-Ray diffraction analysis

The X-ray diffraction analysis of compound **5** has been performed on a Bruker AXS APEX CCD area detector diffractometer, using graphite monochromated Mo-Kα radiation (0.71073 Å). Empirical absorption correction was carried out using SADABS.[31] Data collection and data reduction were performed with the SMART and SAINT programs.[32] The structure of **5** was solved by direct methods with SIR97[33] and refined by full-matrix least-squares analysis with SHELXL97[34] using the WINGX42[35] suite of programmes. Non hydrogen atoms were refined with anisotropic thermal parameters whereas H-atoms were placed in idealised positions and allowed to refine riding on the parent C atom. Molecular graphics were prepared using ORTEP3.[36] A summary of the crystal data, structure solution and refinement parameters are given in Table 1.

Synthesis of the ^{99m}Tc complexes (1a–7a)

General method. In a nitrogen-purged glass vial, 100 μL of a 7.5×10^{-4}–1.5×10^{-3} M ethanolic solution of compounds **L**^{1}—**L**^{7} were added to 900 μL (5–15 mCi) of the organometallic precursor *fac*-[^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$, and the mixture was heated at 70–100 °C for 30–60 min. Complexes **1a–7a** have been obtained typically with a radiochemical yield ≥ 98%, as checked by gradient HPLC analysis, and used in the biodistribution studies without further purification. The chemical identity of **1a–7a** was confirmed by comparing their HPLC chromatograms with the HPLC profile of the analogue Re complexes. Table 2 summarizes the radiochemical yield, labelling conditions and retention time for complexes **1a–7a**.

Octanol–water partition coefficient

The log $P_{o/w}$ values of complexes **1a–6a** (Table 2) were determined by the multiple back extraction method[37] under physiological conditions (*n*-octanol/0.1 M PBS, pH 7.4).

Biodistribution studies

The biodistribution of the complexes was evaluated in groups of 5 female CD-1 mice (randomly bred, Charles River) weighing approximately 20–25 g each, at 1 h and 2 h after intravenous administration with 100 μL (1.5–8.0 MBq) of each preparation

via the tail vein as previously described.[9] [^{99m}Tc]-sestamibi was also evaluated in the same animal model just for comparative purposes. Studies were carried out according to the EU guidelines for Animal Care and Ethics for Animal Experiments.

Biodistribution results were expressed as percentages of the injected dose per gram of tissue (% ID g^{-1}) and are shown in Table 3. Blood and urine samples, collected at the sacrifice time, were analysed by HPLC to check the *in vivo* stability of complexes **1a–6a**. Prior to HPLC analysis urine samples were centrifuged and the serum from the blood samples was separated and treated with ethanol to precipitate proteins. The supernatant from these biological samples was analyzed using the conditions referred above for the HPLC analysis of the Re and ^{99m}Tc complexes.

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