Rhenium(V) oxocomplexes with novel pyrazolyl-based N_4- and N_3S-donor chelators

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The novel pyrazolyl-based ligands 3,5-Me_2pz$(CH_2)_2$NH$(CH_2)_2$NH$(CH_2)_2NH_2$ (**1**) and pz*$(CH_2)_2$NH-Gly-CH_2STrit (pz* = pz (**8**), 3,5-Me_2pz (**9**), 4-(EtOOC)CH_2-3,5-Me_2pz (**10**)) were synthesized, and their suitability to stabilize Re(V) oxocomplexes was evaluated using different starting materials, namely $(NBu_4)[ReOCl_4]$, $[ReOCl_3(PPh_3)_2]$ and *trans*-$[ReO_2(py)_4]Cl$. Compound **1** reacts with *trans*-$[ReO_2(py)_4]Cl$ yielding the cationic compound $[ReO(OMe)\{3,5\text{-}Me_2pz(CH_2)_2N(CH_2)_2NH(CH_2)_2NH_2\}](BPh_4)$ (**11**) in a low isolated yield. In contrast, the neutral complexes [ReO{pz*$(CH_2)_2$NH-Gly-CH_2S}] (pz* = pz (**12**), 3,5-Me_2pz (**13**), 4-(EtOOCCH_2)-3,5-Me_2pz (**14**)) were synthesized almost quantitatively by reacting $[ReOCl_3(PPh_3)_2]$ or $(NBu_4)[ReOCl_4]$ with the trityl-protected chelators **8–10**. The X-ray diffraction analysis of **11** and **13** confirmed the tetradentate coordination mode of the respective ancillary ligands. In **11** the monoanionic chelator coordinates to the metal through four nitrogen atoms, while in **13** the chelator is trianionic, coordinating to the metal through three nitrogens and one sulfur atom. Solution NMR studies of **12–14**, including two-dimensional NMR techniques (^{1}H COSY and ^{1}H/^{13}C HSQC), confirmed that the N_3S coordination mode of the chelators is retained in solution. Unlike **11**, complexes **12–14** may be considered relevant in the development of radiopharmaceuticals, as further corroborated by the synthesis of the congener [^{99m}TcO{pz$(CH_2)_2$-NH-Gly-CH_2S}] (**12a**). This radioactive compound was obtained from $^{99m}TcO_4^-$ in aqueous medium, in almost quantitative yield and with high specific activity and radiochemical purity.

Introduction

The development of radiopharmaceuticals is one of the major driving forces for the coordination and organometallic chemistry of technetium and rhenium since ^{99m}Tc is the most used radionuclide in diagnostic nuclear medicine, while Re has two β-emitter radionuclides ($^{186/188}$Re) with excellent physical decay properties for therapeutic applications.[1–4] Re compounds are also usually considered adequate surrogates of the ^{99m}Tc complexes.

We have shown that tridentate pyrazolyl-diamine ligands are efficient bifunctional chelators towards the *fac*-$[M(CO)_3]^+$ (M = Re, ^{99m}Tc) moiety (Chart 1), having relevance in the design of organometallic Tc and Re compounds for radiopharmaceutical applications.[5,6] Within this organometallic approach, these chelators have been already applied for labelling small tumor-seeking peptides, with encouraging results in terms of biological profile and *in vitro*/*in vivo* stability of the *bioactive* organometallic complexes.[7,8] The favourable features of pyrazolyl-diamine ligands led us to evaluate the possibility of preparing pyrazolyl-based tetradentate chelators suitable for the synthesis of Re(V) and Tc(V) oxocomplexes, another class of compounds with a prominent role in the radiopharmaceutical chemistry of these *d*-transition elements.[1,2] The use of Re(V) or Tc(V) oxocomplexes for designing radiopharmaceuticals requires the stabilisation of *trans*-$[MO_2]^+$ or $[MO]^{3+}$ cores (M = Re, Tc) with bifunctional chelators having the right combination of donor atoms. Tetradentate N_4 ligands, of the tetraamine type, are among the best bifunctional chelators for the *trans*-$[MO_2]^+$ core, while a variety of N_3S-donor ligands have been applied with success to stabilise the $[MO]^{3+}$ core.[2,9,10] Therefore the combination of a pyrazolyl group with an aliphatic triamine or with a mercaptoacetylglycine unit would provide potentially tetradentate chelators of the N_4 and N_3S type, which were expected

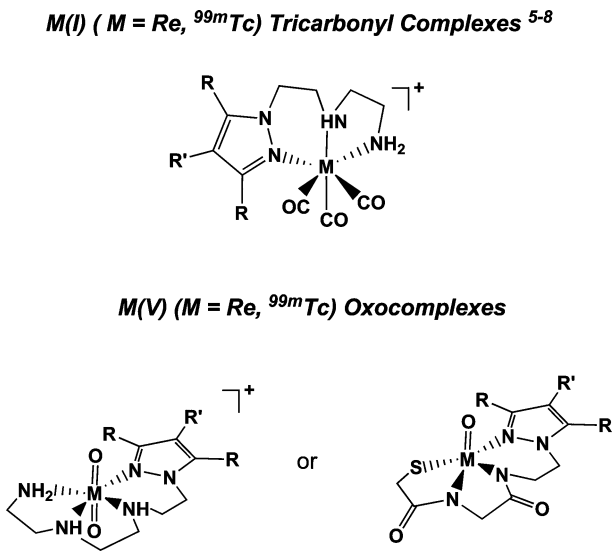

Chart 1 Schematic drawing of reported M(I) tricarbonyl and expected M(V) complexes (M = Re, ^{99m}Tc) anchored by pyrazolyl-based chelators.

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to stabilise the *trans*-$[MO_2]^+$ and the $[MO]^{3+}$ (M = Re, Tc) cores, respectively (Chart 1). The presence of an aromatic pyrazolyl ring in the framework of these chelators would also confer some rigidity to the complexes and allows an easy coupling of biologically relevant molecules to the metal center.[11–14]

Herein, we describe a series of novel tetradentate pyrazolyl-based ligands, 3,5-Me_2pz$(CH_2)_2$NH$(CH_2)_2$NH$(CH_2)_2NH_2$ (**1**) and pz*$(CH_2)_2$NH-Gly-CH_2STrit (pz* = pz (**8**), 3,5-Me_2pz (**9**), 4-(EtOOCCH_2)-3,5-Me_2pz (**10**)), and report on their coordination chemistry towards *trans*-$[ReO_2]^+$and $[ReO]^{3+}$ cores. To gain insight into the suitability of these novel chelators for radiopharmaceutical development, some preliminary studies at the no-carrier added level (^{99m}Tc) have been performed and will be also reported in this work.

Results and discussion

Synthesis and characterization of the pyrazolyl-based chelators

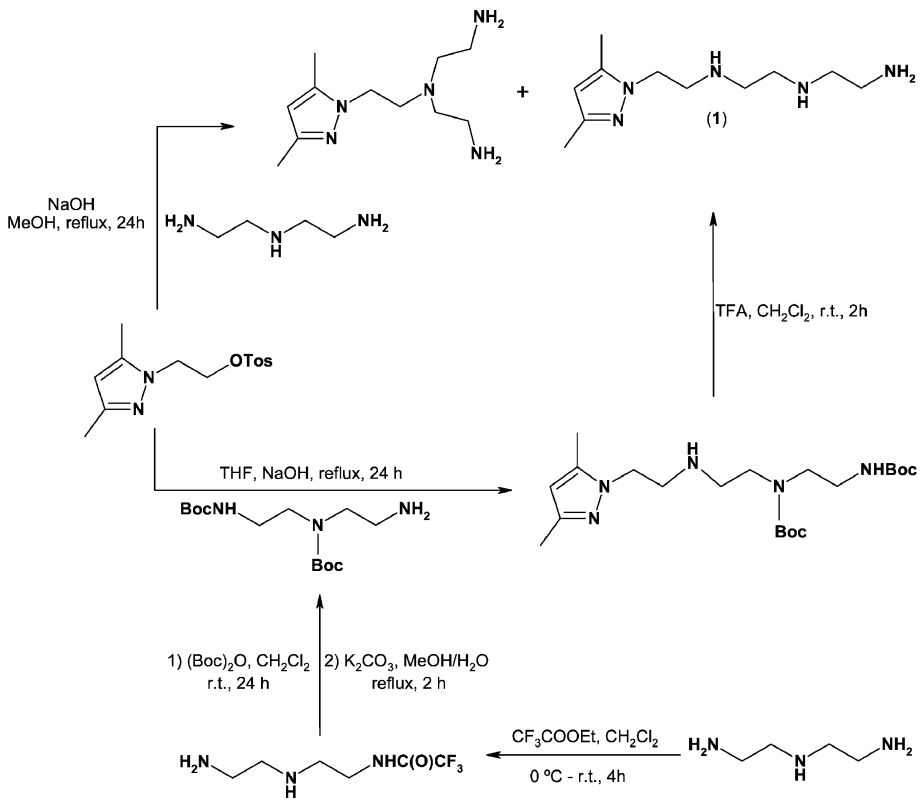
The chelator 3,5-Me_2pz$(CH_2)_2$NH$(CH_2)_2$NH$(CH_2)_2NH_2$ (**1**) has been prepared by two different synthetic approaches (Scheme 1). One of these approaches consisted of reacting *N*-(2-*p*-toluenesulfonylethyl)-3,5-dimethylpyrazole[15] with a large excess of diethylenetriamine (1 : 20) (Scheme 1). This reaction leads to chelator **1**, a pale-yellow oil which has been characterized by ^{1}H and ^{13}C NMR spectroscopy, but also to a side product formed due to alkylation of the secondary amine, as can be seen in Scheme 1. To avoid the formation of the side product, chelator **1** was prepared alternatively by reacting *N*-(2-*p*-toluenesulfonylethyl)-3,5-dimethylpyrazole with the Boc protected diethylenetriamine ($H_2N(CH_2)_2N(Boc)(CH_2)_2NH(Boc)$)[16] (Scheme 1). By exploring this synthetic route we have also taken into account that Boc-protected pyrazolyl-triamine would be useful for the introduction of a pendant arm at the non-protected secondary amine for further coupling to bioactive molecules.

The N_3S-donor ligands, **8–10**, were synthesized by a multistep process which involved a final amide coupling reaction between *N*-(2-aminoethyl)pyrazole precursors, **5–7**, and *N*-hydroxysuccinimidyl 2-[(triphenylmethylthio)methylcarbonyl-amino]ethanoate[11,17] in the presence of DIPEA and in refluxing acetonitrile (Scheme 2). The compounds pz$(CH_2)_2NH_2$ (**5**)[18] and 3,5-Me_2pz$(CH_2)_2NH_2$ (**6**)[19] were prepared by reacting the corresponding bromoethylpyrazoles with potassium phthalimide, followed by hydrazinolysis of the phthalimide intermediates **2** and **3**. Compound 4-(EtOOCCH_2)-3,5-Me_2pz$(CH_2)_2NH_2$ (**7**) has been synthesized *via* a Mitsunobu reaction (Scheme 2)[20] by treatment of 4-(EtOOCCH_2)-3,5-Me_2pz$(CH_2)_2$OH[6] with phthalimide, followed by reaction with hydrazine.

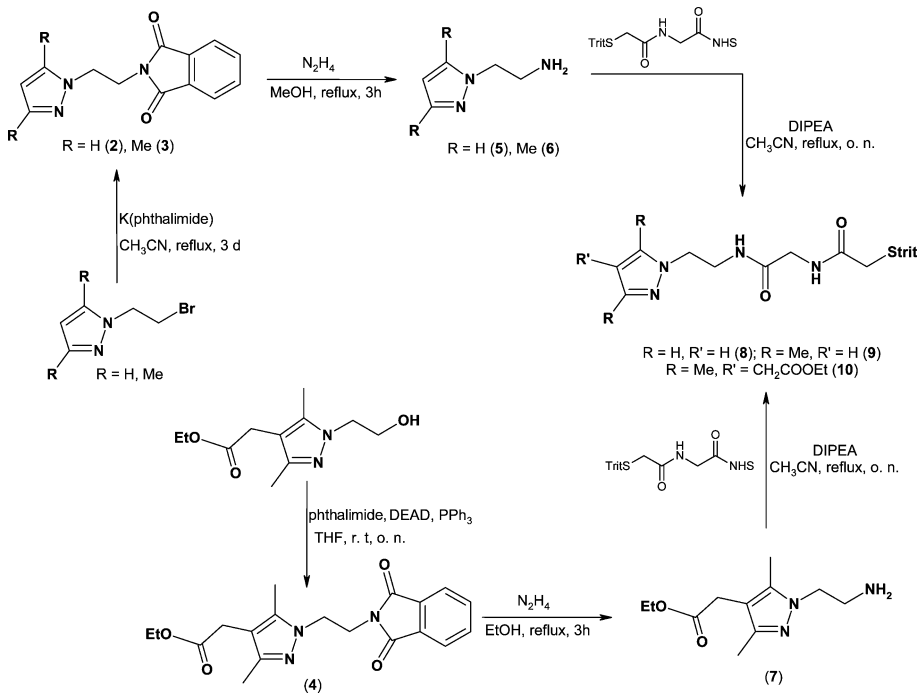
Compounds **8–10** have been purified by column chromatography and were used in the synthesis of the Re(V) oxocomplexes without previous detritylation of the thiol groups, as discussed below. Their formulation was supported by elemental analysis, IR, ^{1}H and ^{13}C NMR spectroscopy.

Synthesis and spectroscopic characterization of the Re(V) oxocomplexes

The reaction of *trans*-$[ReO_2(py)_4]$Cl with **1** (1 : 1 molar ratio) in refluxing methanol yields a dark green solution which, after



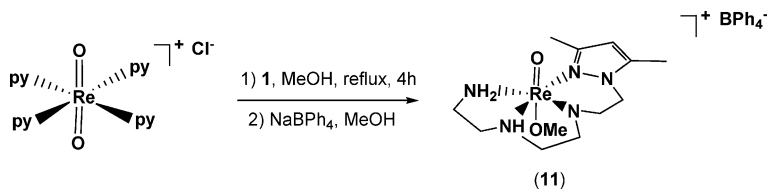
Scheme 1 Synthesis of the ligand 3,5-Me_2pz$(CH_2)_2$NH$(CH_2)_2$NH$(CH_2)_2NH_2$ (**1**).



Scheme 2 Synthesis of pz*$(CH_2)_2$NH-Gly-STrit ligands.

removal of the solvent, was analysed by ^{1}H NMR spectroscopy. The ^{1}H NMR analysis has shown the presence of a major species which clearly had the pyrazolyl-triamine ligand coordinated. This assumption was based on the chemical shift (δ = 6.36 ppm) of the H(4) proton of the pyrazolyl ring, considerably downfield shifted in comparison with the same proton in compound **1** (δ = 5.83 ppm). Moreover, a complex set of multiplets, between 2.73 and 4.57 ppm, could also be easily assigned to the presence of diastereotopic methylenic protons as a consequence of the coordination of the amine groups of **1** to the metal. Expecting to have cationic complexes, the recrystallization of this reaction mixture has been attempted in methanol, using tetraphenylborate as counterion. After standing for several days at room temperature, a few dark green crystals formed which were of a suitable quality for single crystal X-ray diffraction analysis. As discussed below, this structural study confirmed the formation of [ReO(OMe){3,5-Me_2pz$(CH_2)_2$N$(CH_2)_2$NH$(CH_2)_2NH_2$}](BPh$_4$) (**11**) (Scheme 3): a cationic Re(v) oxo-methoxyde complex anchored by a monoanionic pyrazolyl-based N_4-donor ligand. Unfortunately, the crystalline material was only sufficient to perform the X-ray structural study, and **11** has not been studied by other analytical techniques.

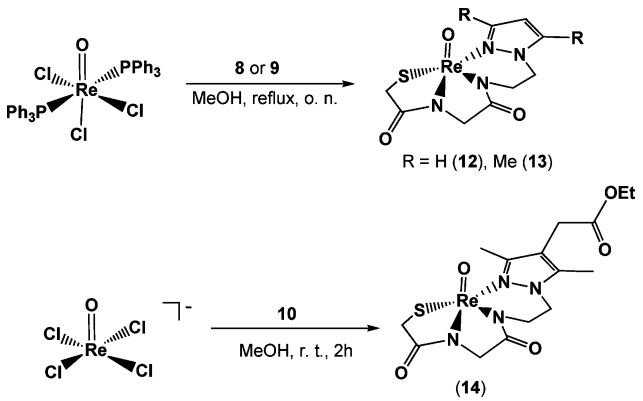
The presence of the pyrazolyl ring, certainly a better $\sigma + \pi$ donor than secondary or primary aliphatic amines, facilitates most probably the formation of the Re(v) monoxocomplex, **11**, and/or the deprotonation of the aliphatic amine of chelator **1**. We have carried out the studies under aqueous conditions, aiming to avoid the formation of the oxo-methoxyde complex and to disfavour the deprotonation of **1**. The precursor *trans*-[$ReO_2(py)_4$]Cl reacts with **1** (1 : 1 molar ratio) in H_2O affording a clear orange solution, after heating at 80 °C for 1 h. The solvent was removed under vacuum and the crude product analysed by ^{1}H NMR spectroscopy. The spectrum obtained in D_2O has shown a pattern similar to the one obtained when the reaction was run in methanol: a highfield shifted resonance at 6.11 ppm due to the H(4) proton of the pyrazolyl ring and a complex set of CH_2 multiplets spanning between 2.66 and 4.06 ppm. We must also mention that the IR spectrum of the crude product did not show any band in the range 900–1000 cm^{-1}, where ν(Re=O) stretching bands usually appear, displaying instead a medium intense band at 800 cm^{-1} which can be assigned to ν_{as}(O=Re=O).[21] These data might indicate the presence of a Re(v) *trans*-dioxocomplex anchored by **1**. However, some unreacted *trans*-[$ReO_2(py)_4$]Cl was still present and, therefore, one could not unequivocally associate the ν_{as}(O=Re=O) band to the novel species formed with chelator **1**. Several attempts have been made to purify this compound, namely precipitation with sodium tetraphenylborate in aqueous solution or in organic medium. All



Scheme 3 Synthesis of a Re(v) oxocomplex with chelator **1**.

the attempts were unsuccessful and the presence of a Re(V) *trans*-dioxocomplex anchored by **1** could not be confirmed.

To evaluate the coordination behaviour of the tritylated ligands pz*$(CH_2)_2$NH-Gly-CH_2STrit (pz* = pz (**8**) 3,5-Me_2pz (**9**)) $[ReOCl_3(PPh_3)_2]$ was used as the Re(V) starting material. In refluxing methanol and using triethylamine as a deprotonating agent, the reactions proceeded smoothly, affording almost quantitatively (97–98%) the neutral complexes [ReO{pz*$(CH_2)_2$-NH-Gly-CH_2S}] (pz* = pz (**12**), 3,5-Me_2pz (**13**)) (Scheme 4). These results showed that the cleavage of the C–S bond of the trityl protecting group is a very efficient process, certainly promoted by the Lewis acidity of the Re(V).[12,22]



Scheme 4 Synthesis of Re(V) oxocomplexes with the pz*-$(CH_2)_2$NH–Gly–CH_2S ligands.

In the case of 4-(EtOOCCH_2)-3,5-Me_2pz$(CH_2)_2$NH-Gly-CH_2STrit (**10**) the reaction with $[ReOCl_3(PPh_3)_2]$ in refluxing methanol was less favourable. The complex [ReO{4-(EtOOCCH_2)-3,5Me_2pz$(CH_2)_2$-NH-Gly-CH_2S}] (**14**) was also formed, but the major part of the protected ligand did not react after 24 h of reflux. As shown in Scheme 4, the use of the more reactive Re(V) starting material $(NBu_4)[ReOCl_4]$ allowed the preparation of **14** in a high isolated yield (80%), even at room temperature.

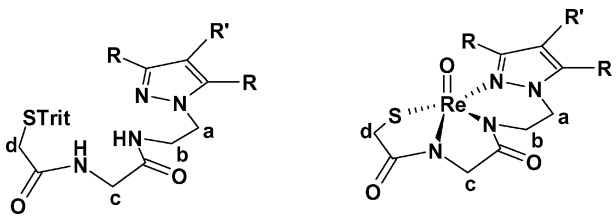
Compounds **12–14** are carmine or orange microcrystalline solids which are soluble in polar organic solvents, like dichloromethane, chloroform, methanol or acetonitrile, but insoluble in water. These Re(V) oxocomplexes are quite stable in the solid state and in solution, even in the presence of air or moisture, as checked by ^{1}H NMR and HPLC analysis. The characterization of **12–14** has been performed by elemental analysis, IR and NMR spectroscopy (^{1}H, ^{13}C, ^{1}H COSY and ^{1}H/^{13}C HSQC) and, in the case of **13**, also by X-ray diffraction analysis.

The IR spectra of **12–14** display intense ν(Re=O) stretching bands in the range 971–984 cm^{-1}.[21] There are also one or two strong bands, spanning between 1628–1655 cm^{-1},[12] which were assigned to the ν(CO) stretching bands of the two amide groups present in the N_3S chelators.

The ^{1}H NMR spectra of **12–14** display a complex array of resonances due to the methylenic protons of the linear backbone of the respective pz*$(CH_2)_2$NH-Gly-CH_2S chelators. These resonances are downfield shifted in comparison with the ones due to the corresponding protons of the free N_3S ligands (Table 1). A characteristic feature of all these spectra is the presence of two sets of doublets between 3.98 and 5.44 ppm belonging to spin systems of the AB type, as exemplified for complex **12** in figure 1. These doublets were assigned to the *endo* (*syn* to the Re=O group) and to the *exo* protons (*anti* to the Re=O group) of the mercaptoacetylglycine unit (Table 1), by comparison with the ^{1}H NMR data reported in the literature for other Re(V) complexes bearing this coordinating motif.[12,22] For each pair of *endo*/*exo* methylenic protons, the resonance attributed to the *endo* proton was the one displaced towards highfield.

For complexes **13** and **14** in $CDCl_3$, the resonances due to the CH_2 protons of the ethylenic bridge linking the mercaptoacetyl unit and the pyrazolyl ring appear as three relatively broad multiplets, spanning between 3.53 and 4.74 ppm (Table 1). These protons should originate four multiplets, due to their

Table 1 ^{1}H and ^{13}C NMR for the methylenic backbone of ligands **8–10** and complexes **12–14**



Compound	^{1}H NMR δ_H (ppm)[c]	^{13}C NMR δ_C (ppm)
pz-Gly-STrit (**8**)[a]	H_a, 4.20; H_b, 3.63; H_c, 3.50; H_d, 3.14	C_c, 43.4; C_d, 35.5; C=O, 168.5, 168.7
3,5-Me_2pz-Gly-STrit (**9**)[a]	H_a, 4.00; H_b, 3.58; H_c, 3.50; H_d, 3.13	C_a, 46.8; C_b, 39.3; C_c, 43.2; C_d, 35.5; C=O, 168.3, 168.5
4-(EtOOC)CH_2-3,5-Me_2-pz-Gly-STrit (**10**)[a]	H_a, 4.07; H_b, 3.54; H_c, 3.54; H_d, 3.08	C_a, 46.8; C_b, 39.3; C_c, 43.2; C_d, 38.0; C=O, 168.4, 168.5
[ReO(pz-Gly-S)] (**12**)[b]	H_a, 5.02/4.49; H_b, 4.60/3.13; H_c, 4.64/4.31; H_d, 4.06/3.83	C_a, 53.7; C_b, 46.8; C_c, 55.9.2; C_d, 35.5; C=O, 188.6, 189.9
[ReO(3,5-Me_2pz-Gly-S)] (**13**)[a]	H_a, 4.50/3.53[d]; H_b, 4.67/3.53[d]; H_c, 5.44/4.35; H_d, 4.05/3.98	C_a, 50.6; C_b, 42.7; C_c, 55.9; C_d, 39.1; C=O, 190.4, 191.2
[ReO{4-(EtOOC)CH_2-3,5-Me_2-pz-Gly-S}] (**14**)[b]	H_a, 4.54/3.47[d]; H_b, 4.74/3.47[d]; H_c, 5.43/4.35; H_d, 4.03/3.91	C_a, 50.8; C_b, 42.5; C_c, 55.9; C_d, 39.1; C=O, 190.4, 191.2

[a] Spectra run in $CDCl_3$. [b] Spectra run in dmso-d_6. [c] Each pair is relative to the H_{exo}/H_{endo} protons attached at the same carbon. [d] There is occasional overlap for these protons.

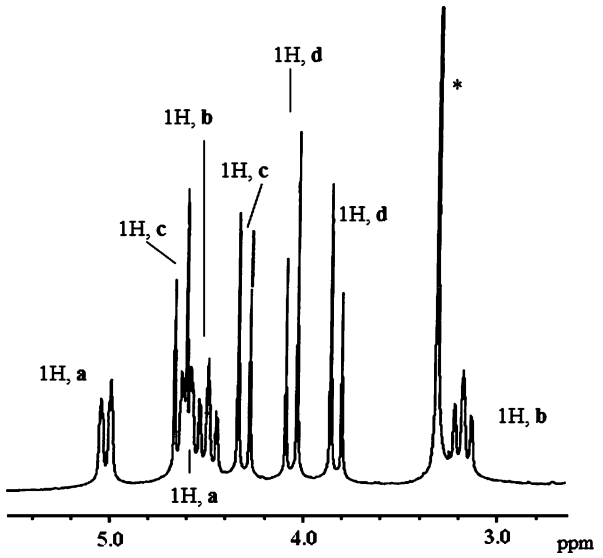


Fig. 1 ^{1}H NMR spectrum of complex **12** (range 5.0–3.0 ppm) in dmso-d_6 (* residual water from the solvent).

diastereotopic character, but there is the occasional overlapping of the two resonances associated with each *exo* proton from the two methylenic groups, as confirmed by two-dimensional NMR techniques (^{1}H COSY and ^{1}H/^{13}C HSQC). The ^{1}H NMR spectrum of **12** in dmso-d_6 display the expected four multiplets for these protons, as shown in Fig. 1.

The CH and CH_3 protons of the pyrazolyl ring are downfield shifted ($\Delta\delta$ = 0.3–1.6 ppm) compared to the corresponding protons in the free ligands, confirming that these rings are coordinated to the $[ReO]^{3+}$ core. In the ^{13}C NMR spectra of **12–14** all the resonances are downfield shifted relative to the corresponding resonances in the free chelators. This trend is more pronounced for the carbons that are close to the coordinating groups, as is the case of the amide carbons ($\Delta\delta$ = 22–23 ppm) or the glycine α carbon ($\Delta\delta$ = 12–13 ppm). In summary, the NMR data obtained for **12–14** are compatible with the formation of diamagnetic Re(v) oxocomplexes anchored by trianionic and tetradentate N_3S-donor ligands, as found in the solid state for one complex of this family (**13**).

X-Ray crystallography

X-Ray quality crystals of complexes [ReO(OMe){3,5-$Me_2pz(CH_2)_2N(CH_2)_2NH(CH_2)_2NH_2$}]($BPh_4$) (**11**) and [ReO{3,5-$Me_2pz(CH_2)_2$-NH-Gly-$CH_2S$}] (**13**) were obtained by slow evaporation of methanol and acetonitrile solutions, respectively. Crystal data for **11** and **13** are summarized in Table 2.

An ORTEP view of the cation of **11** is shown in Fig. 2, and a selection of bond distances and angles for this complex is presented in Table 3.

Compound **13** crystallized with two independent and chemically different molecules per asymmetric unit cell. The most striking difference between these two molecules is the absence (molecule **A**) or presence (molecule **B**) of a coordinated H_2O ligand in a *trans* position relatively to the $[Re{=}O]^{3+}$ core. Selected bond distances and angles for molecules **A** and **B** of complex **13** are given in Table 4. ORTEP views for each of these molecules are presented in Fig. 3 and 4.

Table 2 Crystal data and structure refinement for compounds **11** and **13**

Compound	**11**	**13**
Formula	$C_{36}H_{45}BN_5O_2Re$	$C_{22}H_{30}N_8O7Re_2S_2$
M	776.78	955.02
Crystal system	Monoclinic	Triclinic
Color	Green	Carmine
Crystal size/mm	0.33 × 0.30 × 0.24	0.20 × 0.10 × 0.08
Space group	$P2_1/n$	$P\bar{1}$
a/Å	12.101(2)	9.4388(2)
b/Å	14.3993(15)	10.3213(3)
c/Å	19.975(2)	14.9377(3)
α/°	90	89.0390(10)
β/°	96.285(13)	80.1750(10)
γ/°	90	89.2970(10)
V/Å^3	3459.6(9)	1433.6(3)
Z	4	2
T/K	293(2)	130(2)
D_c/gcm^{-3}	1.491	2.212
μ (Mo Kα)/mm^{-1}	3.55	8.638
Reflections collected	7102	17158
Independent reflections	6767 [R_{int} = 0.0551]	12499 [R_{int} = 0.0225]
Final R indices [$I > 2\sigma(I)$]		
R_1	0.0663	0.0396
wR_2	0.0910	0.0927
R indices (all data)		
R_1	0.1474	0.0586
wR_2	0.1127	0.1073
GOF	1.047	1.003

Table 3 Selected bond lengths (Å) and angles (°) for the cation of compound **11**

Re–O(1)	1.708(6)	Re–O(2)	1.948(6)
Re–N(1)	2.090(8)	Re–N(3)	1.913(8)
Re–N(4)	2.101(8)	Re–N(5)	2.299(8)
O(1)–Re–O(2)	157.8(3)	O(1)–Re–N(1)	98.7(3)
O(1)–Re–N(4)	92.8(3)	O(1)–Re–N(5)	83.4(3)
O(2)–Re–N(1)	86.4(3)	O(2)–Re–N(3)	94.5(3)
O(2)–Re–N(4)	84.4(3)	O(2)–Re–N(5)	74.5(3)
N(1)–Re–N(3)	89.6(4)	N(1)–Re–N(4)	167.7(3)
N(1)–Re–N(5)	106.4(3)	N(3)–Re–N(4)	83.0(3)
N(3)–Re–N(5)	159.6(3)	N(4)–Re–N(5)	78.9(3)
C(2)–N(3)–Re	130.7(7)	C(3)–N(3)–Re	114.2(7)
C(2)–N(3)–C(3)	115.1(9)		

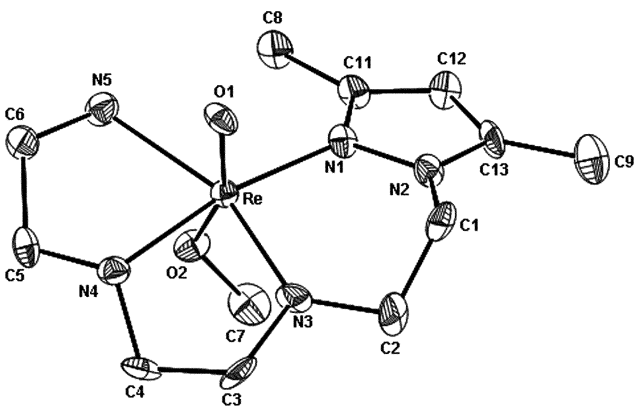


Fig. 2 ORTEP view of the cation of compound **11**. Vibrational ellipsoids are drawn at the 30% probability level.

For complex **11** and for molecule **B** of compound **13** the coordination geometry around the metal is approximately octahedral, whereas the structure of molecule **A** of **13** can be considered as

Table 4 Selected bond lengths (Å) and angles (°) for **13**

Molecule A			
Re(1)–O(1)	1.690(4)	Re(1)–N(1)	2.111(4)
Re(1)–N(3)	1.997(4)	Re(1)–N(4)	1.981(4)
Re(1)–S(1)	2.2847(13)		
O(1)–Re(1)–N(1)	100.74(18)	O(1)–Re(1)–N(3)	113.44(18)
O(1)–Re(1)–N(4)	107.04(18)	O(1)–Re(1)–S(1)	113.18(14)
N(1)–Re(1)–N(3)	88.98(16)	N(1)–Re(1)–N(4)	152.19(17)
N(1)–Re(1)–S(1)	88.27(12)	N(3)–Re(1)–N(4)	78.93(18)
N(3)–Re(1)–S(1)	132.98(12)	N(4)–Re(1)–S(1)	82.15(14)
Molecule B			
Re(2)–O(4)	1.682(3)	Re(2)–O(5)	2.306(3)
Re(2)–N(6)	1.999(4)	Re(2)–N(7)	2.040(3)
Re(2)–N(9)	2.143(8)	Re(2)–S(2)	2.3379(11)
O(4)–Re(2)–O(5)	169.8(15)	O(4)–Re(2)–N(6)	104.11(17)
O(4)–Re(2)–N(7)	101.95(16)	O(4)–Re(2)–N(9)	92.24(16)
O(4)–Re(2)–S(2)	101.63(11)	O(5)–Re(2)–N(6)	86.31(14)
O(5)–Re(2)–N(7)	78.30(14)	O(5)–Re(2)–N(9)	77.25(14)
O(5)–Re(2)–S(2)	80.80(9)	N(6)–Re(2)–N(7)	79.22(15)
N(6)–Re(2)–N(9)	162.16(15)	N(6)–Re(2)–S(2)	83.05(11)
N(7)–Re(2)–N(9)	90.58(15)	N(7)–Re(2)–S(2)	153.31(11)
N(9)–Re(2)–S(2)	100.84(11)		

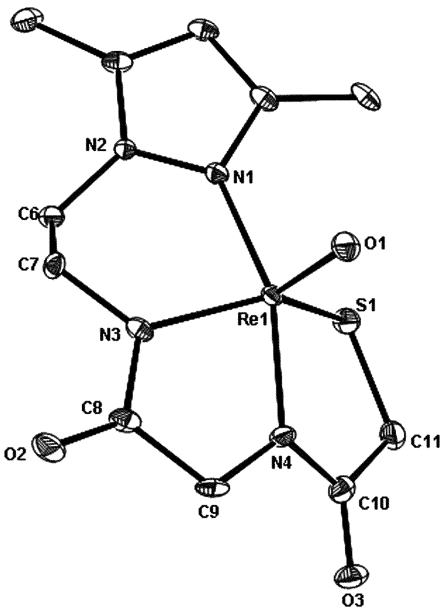


Fig. 3 ORTEP view of molecule **A** of **13**. Vibrational ellipsoids are drawn at the 30% probability level.

distorted square pyramidal. In terms of trigonality index τ, the value found for the latter is 0.32 ($\tau = 0$ for a regular square pyramid, $\tau = 1$ for a regular trigonal bipyramid).[23] In all the structures, the ancillary ligands are coordinated in a tetradentate way, with the respective donor atoms occupying the equatorial plane or the basal square plane. The tetradentate pyrazolyl-triamine ligand in complex **11** is monoanionic, due to deprotonation of the secondary amine adjacent to the pyrazolyl ring. The 3,5-Me_2pz$(CH_2)_2$-NH-Gly-CH_2S ligand is trianionic in both independent molecules of **13**. The oxo ligands occupy the axial (**11** or molecule **B** of **13**) or apical positions (molecule **A** of **13**). In the case of complex **11**, the coordination environment around the metal is completed by

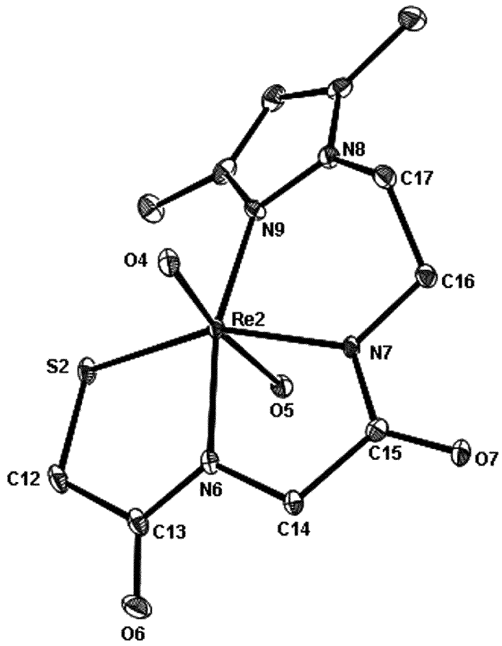


Fig. 4 ORTEP view of molecule **B** of **13**. Vibrational ellipsoids are drawn at the 30% probability level.

a methoxyde ligand coordinated in a *trans*-position relative to the oxo group (O(1)–Re(1)–O(2) angle of 157.8(3)°).

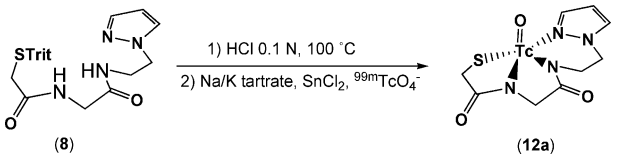
In all the structures the Re atom is out of the distorted basal plane, towards the oxo group. As expected, this is more pronounced for molecule **A** of **13**, with a 0.663(2) Å displacement of the metal from the [N(1), N(3), N(4), S(1)] plane. The Re–O bond distance in **11** (1.708(6) Å) appears at the upper limit usually found for multiple bound oxo ligands in Re(v) oxocomplexes,[21] being longer than the same distance in the two independent molecules of compound **13** (molecule **A**: Re(1)–O(1), 1.690(4) Å; molecule **B**: Re(2)–O(4), 1.682(3) Å). This difference is certainly justified by the competition of the methoxide group with the oxo ligand in π-bonding with the metal, as evidenced by the relatively short Re–O(2) bond distance (1.948(6) Å) found for compound **11**.[24] By contrast, the loosely bound water ligand in molecule **B** of **13** (Re(2)–O(5), 2.306(3) Å)[25] is not expected to compete in an extensive way with the oxo-ligand.

Concerning the coordination of the tetradentate ancillary ligands the Re–N_{pz}, Re–N_{amine}, Re–N_{amide} and Re–S bond distances can be considered normal, taking into account the values reported for the same bond distances in other Re(v) complexes with pyrazolyl containing ligands,[26,27] polyamines[28,29] and MAG3 (mercaptoacetyltriglycine) chelators.[12,21,30,31] In particular, the short Re–N(3) bond distance of 1.913(8) Å in compound **11** is consistent with a certain double character for the coordination of the deprotonated and almost planar sp^2-hybridized nitrogen atom.[28] This bond distance is considerably shorter than the Re–N_{amine} bond distances in **11** (2.090(8)–2.299(8) Å), being more comparable to the Re–N_{amido} bond distances in **13** (1.981(4)–2.040 Å).

Labelling studies: syntheses and *in vitro* evaluation of [^{99m}TcO{pz$(CH_2)_2$-NH-Gly-CH_2S}] (12a)

The stability of the complexes [ReO{pz*$(CH_2)_2$-NH-Gly-CH_2S}] (pz* = pz (**12**), 3,5-Me_2pz (**13**), 4-(EtOOCCH_2)-3,5-Me_2pz (**14**)),

both in the solid state and in solution, together with their excellent isolated yields (80–98%), even using trityl-protected ligands, prompted us to pursue with the studies at the no-carrier added level (^{99m}Tc). In order to gain insight into the possibility of preparing ^{99m}Tc oxocomplexes with this class of chelators, we have focused on pz(CH$_2$)$_2$NH-Gly-CH$_2$STrit (**8**). Immediately before use, an ethanolic solution of **8** was heated (100 °C, 30 min) in the presence of aqueous 0.1 N HCl, to remove the trityl-protecting group. The complex [^{99m}TcO{pz(CH$_2$)$_2$-NH-Gly-CH$_2$S}] (**12a**) was prepared by reacting [^{99m}TcO$_4$]$^-$ with tin(II) chloride, Na/K tartrate, and an aliquot of the trityl-deprotected **8** (Scheme 5).



Scheme 5 Synthesis of a ^{99m}Tc(V) oxocomplex with the pz-(CH$_2$)$_2$NH–Gly–CH$_2$S ligand.

Complex **12a** was obtained with high radiochemical yield (> 90%), at room temperature and using ligand concentrations in the 10^{-5} M range. The TLC analysis of **12a**, using methanol as eluent, has shown the presence of negligible amounts of reduced hydrolyzed technetium (< 5%). In this chromatographic system, complex **12a** shows $R_f = 0.7$, as well as its Re congener [ReO{pz(CH$_2$)$_2$-NH-Gly-CH$_2$S}] (**12**) which was fully characterized, as discussed above. The identity of complex **12a** (t_R = 14.63 min) was further authenticated by HPLC comparison with complex **12** (t_R = 14.00 min) (Fig. 5). Typically, HPLC analysis of the preparations of **12a** has shown that more than 95% of the eluted radioactivity was due to complex **12a**.

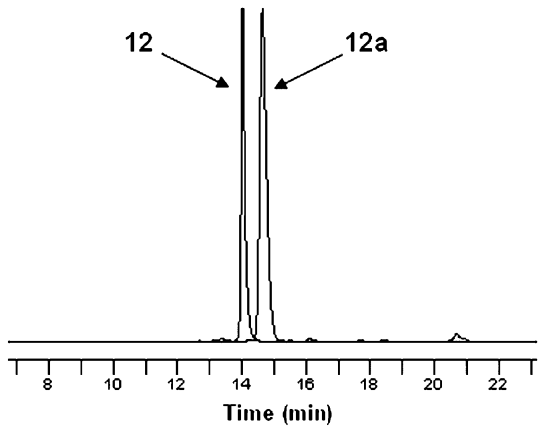


Fig. 5 HPLC chromatograms of co-injected [ReO{pz-(CH$_2$)$_2$NH-Gly-CH$_2$S}] (**12**) (UV detection, t_R = 14.00 min) and [^{99m}TcO{pz-(CH$_2$)$_2$-NH-Gly-CH$_2$S}] (**12a**) (γ detection, t_R = 14.63 min).

Complex **12a** is moderately lipophilic (log $P_{o/w}$ (**12a**) = + 0.73 ± 0.03), and displays a high *in vitro* stability in the presence of a huge excess of glutathione (10–20 mM), since HPLC analysis has shown that no *trans*-chelation occurred, at least for a period of 2 h at 37 °C. Glutathione is a tripeptide, present in relatively high concentrations in the blood stream, with a well known affinity for the [^{99m}TcO]$^{3+}$ core.

Concluding remarks

A novel class of tetradentate chelators containing a pyrazolyl unit have been synthesized and their coordination capability towards the *trans*-[Re(O)$_2$]$^+$ and [M(O)]$^{3+}$ cores (M=Re, ^{99m}Tc) studied. Using the pyrazolyl-triamine ligand (**1**) no Re(V) complex containing a *trans*-[ReO$_2$]$^+$unit could be isolated. The unique compound isolated with **1** was the mono-oxocomplex [ReO(OMe){3,5-Me$_2$pz-(CH$_2$)$_2$N(CH$_2$)$_2$NH(CH$_2$)$_2$NH$_2$}](BPh$_4$) (**11**). This compound has no relevance in radiopharmaceutical research, since the *trans*-[ReO(OMe)]$^{2+}$ unit is not achievable under the aqueous conditions required in the preparation of radiopharmaceuticals.

By contrast, a series of trianionic and tetradentate ligands, combining a pyrazolyl ring with a mercaptoacetylglycine unit, allowed the synthesis and full characterization of well defined complexes, [ReO{pz*-(CH$_2$)$_2$-NH-Gly-CH$_2$S}] (pz* = pz (**12**), 3,5-Me$_2$pz (**13**), 4-(EtOOCCH$_2$)-3,5Me$_2$-pz (**14**)), which may have some relevance in radiopharmaceutical development. These complexes are remarkably stable, either in the solid state or in solution, and can be prepared almost quantitatively using S-protected ligands. The potential relevance of this class of complexes for the design of radiopharmaceuticals was further demonstrated by the synthesis of [^{99m}TcO{pz-(CH$_2$)$_2$-NH-Gly-CH$_2$S}] (**12a**), which was obtained in almost quantitative yield and with high specific activity. The neutral and moderately lipophilic M(V) (M = Re, Tc) complexes anchored on the pz*-(CH$_2$)$_2$-NH-Gly-CH$_2$S ligands must be suitable for labelling small biomolecules, for which free diffusion through the cell membrane can be a crucial issue. Preferentially, these small biomolecules can be attached to the framework of the pz*-(CH$_2$)$_2$-NH-Gly-CH$_2$S chelators through derivatization of the 4-position of the pyrazolyl ring, as exemplified herein with the synthesis of complex **14** which already contains a CH$_2$COOEt ester function useful for coupling biomolecules.

Experimental

General procedures

The synthesis of the ligands and complexes were carried under a nitrogen atmosphere, using standard Schlenk techniques and dry solvents, while the work-up procedures were performed under air. The compounds *N*-(2-*p*-toluenesulfonylethyl-3,5-dimethylpyrazole,[15] *N*-(2-bromoethyl)pyrazole,[32] *N*-(2-bromoethyl)-3,5-dimethylpyrazole,[32] *N*-(2-hydroxyethyl)-3,5-dimethyl-4-(ethylethanoate)pyrazole[6] and *N*-hydroxysuccinimidyl 2-[(triphenylmethylthio)methylcarbonylamino]ethanoate[11,17] were prepared according to published methods. The starting materials *trans*-[ReO$_2$(py)$_4$]Cl,[33] [ReOCl$_3$(PPh$_3$)$_2$][34] and [NBu$_4$][ReOCl$_4$][35] were prepared by the literature methods. Na[^{99m}TcO$_4$] was eluted from a commercial ^{99}Mo/^{99m}Tc generator, using 0.9% saline.

^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 300 MHz spectrometer; ^{1}H and ^{13}C chemical shifts are given in ppm and were referenced with the residual solvent resonances relative to SiMe$_4$. IR spectra were recorded as KBr pellets on a Bruker, Tensor 27 spectrometer. C, H and N analyses were performed on an EA 110 CE Instruments automatic analyser.

Thin layer chromatography (TLC) was done using plates from Merck (silica gel 60 F254). Column chromatography was performed in silica gel 60 (Merck). HPLC analysis 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, using a Macherey-Nagel C18 reversed-phase column (Nucleosil 10 μm, 250 × 4 mm) and a gradient of aqueous 0.1% CF_3COOH (**A**) and acetonitrile (**B**) with a flow rate of 1.0 mL min^{-1}. Method: 0–3 min, 100% **A**; 3–18 min, 100% → 0% **A**; 18–22 min, 0% **A**; 22–25 min 0%→ 100% **A**; 25–30 min, 100% **A**.

Syntheses of the ligands

3,5-Me_2pz$(CH_2)_2$NH$(CH_2)_2$NH$(CH_2)_2NH_2$ (1). Method 1: A solution of *N*-(2-*p*-toluenesulfonylethyl-3,5-dimethylpyrazole (1.75 g, 6 mmol) in THF (5 mL) was added dropwise, at room temperature, to an aqueous solution of diethylenetriamine (13 mL, 0.12 mol) and NaOH (250 mg, 6.25 mmol). After complete addition, the mixture was refluxed for 4 h and extracted with dichloromethane, after cooling to room temperature. After concentration under vacuum, the organic extract was applied on the top of a silica-gel column which was eluted with CH_2Cl_2–MeOH, starting from 0 to 100% MeOH. Compound **1** was obtained as a pale yellow oil upon removal of the solvents from the collected methanol fractions. Yield: 634 mg (2.82 mmol, 48%).

Method 2(i), synthesis of $H_2N(CH_2)_2N(Boc)(CH_2)_2NH(Boc)$: This compound was prepared by a method different from the one described in the literature,[16] starting from diethylenetriamine. A solution of ethyl trifluoroacetate (1.46 g, 10. 3 mmol) in CH_2Cl_2 (12 mL) was added dropwise to a solution of diethylenetriamine (1.06, 10.3 mmol) in the same solvent (12 mL), while keeping the temperature at 0 °C. After complete addition, the mixture was stirred for 2 h at 0 °C and then for 2 h at room temperature. Removal of the solvent under vacuum yielded a yellow oil which was formulated as $H_2N(CH_2)_2NH(CH_2)_2NH(COCF_3)$ and used without further purification. This compound was dissolved in CH_2Cl_2 (20 mL) and the resulting solution was cooled to −10 °C. Di-*tert*-butyl dicarbonate (4.5 g, 20.6 mmol) dissolved in CH_2Cl_2 (20 mL) was added dropwise to the cooled solution, and then the reaction mixture was stirred for 24 h at room temperature. After evaporation of the solvent, $(Boc)NH(CH_2)_2N(Boc)(CH_2)_2NH(COCF_3)$ was obtained in the form of a dense oil, which was dissolved in a mixture of methanol (280 mL) and distilled water (12 mL). Then, K_2CO_3 (55.28 g, 0.4 mmol) was added and the mixture refluxed for 2 h. After filtration, the solvent from the supernatant was removed under vacuum, the residue was redissolved in distilled water and the pH adjusted to pH = 13 with 40% NaOH. Extraction with $CHCl_3$, followed by removal of the solvent from the organic phase, gave a yellow oil corresponding to $H_2N(CH_2)_2N(Boc)(CH_2)_2NH(Boc)$. Yield: 2.05 g (7.6 mmol, 74%).

(ii) Synthesis of **1**: *N*-(2-*p*-toluenesulfonylethyl-3,5-dimethylpyrazole (167 mg, 0.55 mmol) in THF (5 mL) was added dropwise to an aqueous solution (10 mL) of $H_2N(CH_2)_2N(Boc)(CH_2)_2NH(Boc)$ (162 mg, 0.55 mmol) and NaOH (44 mg, 1.1 mmol). The mixture was refluxed for 24 h and, after cooling to room temperature, the solution was extracted with $CHCl_3$. The organic extract was purified by column chromatography (CH_2Cl_2 (0–100%)–MeOH). A pale yellow oil was recovered from the collected methanol fractions and formulated as 3,5-Me_2pz$(CH)_2$NH$(CH_2)_2N(Boc)(CH_2)_2NH(Boc)$. To a solution of this compound in CH_2Cl_2 was added TFA (250 μL). After stirring at room temperature for 2 h, the solvent was removed under vacuum and the residue redissolved in distilled water. After adjustment to pH = 11 with 40% NaOH, the water was removed under vacuum giving a residue which, after extraction with methanol and upon evaporation of the solvent, afforded compound **1**. Yield: 38 mg (0.17 mmol, 31%).

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 2.14 (s, 3H, CH_3-pz), 2.18 (s, 3 H, CH_3-pz), 2.67 (m, 6H, CH_2), 2.77 (t, 2 H, CH_2), 2.97 (t, 2 H, CH_2,); 4.01 (t, 2 H, CH_2), 5.72 (s, 1 H, H(4)-pz). ^{13}C NMR (75.373 MHz, $CDCl_3$): δ_C 11.0 (CH_3-pz), 13.4 (CH_3-pz), 41.3 (CH_2), 48.1 (CH_2), 48.8 (CH_2), 49.1 (CH_2), 51.6 (CH_2), 104.8 (C(4)-pz), 139.1 (C(3,5)-pz), 147.4 (C(3,5)-pz).

***N*-(2-Phthalimideethyl)pyrazole (2).** A mixture of potassium phthalimide (6.446 g, 34.8 mmol) and *N*-(2-bromoethyl)pyrazole (2.06 g, 11.7 mmol) in acetonitrile was refluxed, under nitrogen, for 3 d. After filtration, the solvent was evaporated under vacuum, affording compound **2** as a microcrystalline white solid. Yield: 2.505 g (10.4 mmol, 89%).

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 4.09 (2H, t, CH_2), 4.44 (2H, t, CH_2), 6.19 (1H, t, H(4)-pz), 7.69 (1H, d, H(3/5)-pz), 7.70 (1H, d, H(3/5)-pz), 7.76 (2H, m, Ph), 7.80 (2H, m).

***N*-(2-Phthalimideethyl)-3,5-dimethylpyrazole (3).** Compound **3** was prepared as above described for **2**, starting from 4.694 g (23.0 mmol) of *N*-(2-bromoethyl)-3,5-dimethylpyrazole and 18.821 g (69.0 mmol) of potassium phthalimide, but its purification was done by column chromatography (CH_2Cl_2–MeOH (98 : 2)). Yield: 2.216 g (8.23 mmol, 56%)

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 2.05 (3H, s, CH_3-pz); 2.19 (3H, s, CH_3-pz); 4.01 (2H, t, CH_2); 4.25 (2H, t, CH_2); 5.74 (1H, s, H(4)-pz); 7.72 (2H, m, Ph); 7.80 (2H, m, Ph). R_f (silica-gel, CH_2Cl_2–MeOH (98 : 2)) = 0.30.

***N*-(2-Phthalimideethyl)-4-(methylpropionate)-3,5-dimethylpyrazole (4).** *N*-(2-Hydroxyethyl)-3,5-dimethyl-4-(ethylethanoate)-pyrazole (500 mg, 2.70 mmol) and phthalimide (516 mg, 3.51 mmol) were dissolved in dry THF (12 mL). Next, diethyl azodicarboxylate (582 μL, 3.51 mmol) and PPh_3 (921 mg, 3.51 mmol) were added and the mixture was allowed to stir overnight at room temperature. The resulting red solution was purified by silica gel column chromatography, by elution of a first column with CH_2Cl_2–MeOH (95 : 5) and elution of a second one with ethyl acetate–n-hexane (80 : 20)). Compound **4** was recovered as a white powder, after removal of the solvent from the collected fractions. Yield: 558 mg (1.57 mmol, 58%).

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.13 (3H, t, CH_3), 1.97 (3H, s, CH_3-pz), 2.10 (CH_3-pz), 3.19 (2H, s, CH_2), 3.91 (2H, t, CH_2), 4.00 (2H, q, CH_2), 4.17 (2H, m, CH_2), 7.66 (2H, m, Ph), 7.77 (2H, m, Ph). ^{13}C NMR (75.373 MHz, $CDCl_3$): δ_C 9.4 (CH_3(Et)), 11.6 (CH_3-pz), 14.1 (CH_3-pz), 29.8 (CH_2), 37.5 (CH_2), 46.1 (CH_2), 60.6 (CH_2), 109.5 (C(4)-pz), 123.2 ($C_{phthalimide}$), 133.9 ($C_{phthalimide}$), 137.0 (C(3/5)-pz), 147.0 (C(3/5)-pz), 167.7 ($CO_{phthalimide}$), 171.4 (CO (Et)). R_f (silica gel, ethyl acetate–n-hexane (80 : 20)) = 0.50.

***N*-(2-Aminoethyl)pyrazole (5) and *N*-(2-aminoethyl)-3,5-dimethylpyrazole (6).** To a solution of **2** (1.013 g, 4.20 mmol) or **3** (1.041 g, 3.86 mmol) in methanol were added, respectively, 2.20 mL or 2.00 mL of hydrazine monohydrate. The mixtures were refluxed under nitrogen for 3 h, resulting in the formation of a white precipitate of phthalhydrazide. After cooling to room

temperature, concentrated HCl (**2**, 9.47 mL; **3**, 8.70 mL) was added, leading to the formation of more white precipitate. After filtration and adjustment to pH = 9 by addition of 3M NaOH, the supernatant was extracted with $CHCl_3$ (3 × 20 mL). The organic extracts were dried over $MgSO_4$, filtered and the solvent removed under vacuum to afford yellow oils which were formulated as compounds **5** and **6**. Yield: **5**, 289 mg (2.60 mmol, 62%); **6**, 340 mg (2.44 mmol, 63%).

Compound **5**, ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 3.06 (2H, m, CH_2); 4.11 (2H, t, CH_2); 6.18 (1H, t, H(4)-pz); 7.36 (1H, d, H(3,5)-pz); 7.45 (1H, d, H(3/5)-pz).

Compound **6**, ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 2.17 (3H, s, CH_3-pz); 2.20 (3H, s, CH_3-pz); 3.07 (2H, m, CH_2); 3.98 (2H, t, CH_2); 5.76 (1H, s, H(4)-pz).

***N*-(2-Aminoethyl)-4-(methylpropionate)-3,5-dimethylpyrazole (7).** Compound **7** was prepared and purified in the same way as that described above for **5** and **6**, starting from 194 mg (0.55 mmol) of **4** and running the reaction in ethanol. Yield: 103 mg (0.46 mmol, 84%).

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.21 (3H, t, CH_3); 2.16 (3H, s, CH_3-pz); 2.18 (CH_3-pz); 3.07 (2H, m, CH_2); 3.43 (2H, s, CH_2); 3.97 (2H, t, CH_2); 4.16 (2H, q, CH_2). ^{13}C NMR (75.373 MHz, CD_3OD): δ_C 9.9 (CH_3), 10.0 (CH_3), 14.5 (CH_3), 28.7 (CH_2); 39.3 (CH_2); 46.6 (CH_2); 52.9 (CH_2); 114.7 (C(4)-pz), 126.5 (C(3/5)-pz), 134.2 (C(3/5)-pz); 171.7 (CO).

pz(CH$_2$)$_2$NH-Gly-CH$_2$STrit (8). The precursor *N*-hydroxysuccinimidyl 2-[(triphenylmethylthio)methylcarbonylamino]ethanoate (523 mg, 1.07 mmol) and DIPEA (0.19 mL, 1.07 mmol) were added to a solution of **5** (120 mg, 1.07 mmol) in acetonitrile (25 mL) and the mixture was refluxed overnight. After cooling to room temperature and removal of the solvent, compound **8** was recovered as a white powder by column chromatography on silica gel (CH_2Cl_2–MeOH (95 : 5)). Yield: 251 mg (0.52 mmol, 49%).

Found: C, 65.8; H, 5.2; N, 11.0. $C_{28}H_{28}N_4O_2S{\cdot}0.5CH_2Cl_2$ requires C, 64.9; H, 5.5; N, 10.6%. IR: ν_{max}/cm^{-1} 1559 (CO). ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 3.14 (2H, s, CH_2); 3.50 (2H, d, J = 5.4 Hz, –$COCH_2$N); 3.63 (2H, q, J = 5.4 Hz, CH_2); 4.20 (2H, t, J = 5.1 Hz, CH_2); 5.29 (1H, s, H(4)-pz); 6.46 (1 + 1H, br, NH); 7.18–7.48 (15 + 2H, m, Ph-Trit + H(3/5)-pz). ^{13}C NMR (75.373 MHz, $CDCl_3$): δ_C 35.5 (CH_2); 39.6 (CH_2); 43.4 (CH_2); 50.6 (CH_2); 105.7 (C(4)-pz), 127.04 (CH-Trit), 128.17 (CH-Trit), 129.39 (CH-Trit), 139.465 (C(3/5)-pz), 143.8 (C-Trit, C(3/5)-pz), 168.46 (CO), 168.65 (CO). R_f (sílica gel, CH_2Cl_2–MeOH (95 : 5)) = 0.30.

3,5-Me$_2$pz(CH$_2$)$_2$NH-Gly-CH$_2$STrit (9). Compound **9** is a white solid which has been synthesized and purified as described above for **8**, starting from 121 mg (0.86 mmol) of **6**. Yield: 274 mg (0.53 mmol, 64%).

Found C, 62.5; H, 6.1; N, 9.5. $C_{30}H_{32}N_4O_2S.CH_2Cl_2$ requires C, 62.3; H, 5.7; N, 9.4%. IR: ν_{max}/cm^{-1} 1675 (CO). ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 2.15 (3H, s, CH_3-pz); 2.16 (3H, s, CH_3-pz); 3.13 (2H, s, CH_2); 3.50 (2H, d, J = 5.4 Hz, CH_2); 3.58 (2H, q, J = 5.4 Hz CH_2); 4.00 (2H, t, J = 5.4 Hz, CH_2); 5.75 (1H, s, H(4)-pz); 6.51 (1H, br, NH); 6.55 (1H, br, NH); 7.17–7.21 (10H, m, Ph-Trit), 7.24–7.41 (5H, m, Ph-Trit). ^{13}C NMR (75.373 MHz, $CDCl_3$): 10.7 (pz-CH_3); 13.5 (pz-CH_3); 35.5 (CH_2); 39.3 (CH_2); 43.3 (CH_2); 46.8 (CH_2); 105.2 (H(4)-pz); 127.0 (CH-Trit), 128.14 (CH-Trit), 129.4 (CH-Trit), 139.5 (C(3/5)-pz); 143.8 (C-Trit); 148.0 (C(3/5)-pz); 168.3 (CO), 168.5 (CO). R_f (silica gel, CH_2Cl_2–MeOH (95 : 5)) = 0.30.

4-(EtOOC)CH$_2$-3,5-Me$_2$pz(CH$_2$)$_2$NH-Gly-CH$_2$STrit (10). Compound **10** is a yellow oil which has been obtained as reported above for **8** and **9**, starting from 103 mg (0.45 mmol) of **7**. Yield: 142 mg (0.24 mmol, 41%).

Found C, 63.1; H, 7.7; N, 9.2; S. $C_{34}H_{38}N_4O_4S$ requires C, 64.6; H, 6.1; N, 8.7%. IR: ν_{max}/cm^{-1} 1670 (CO). ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.22 (3H, d, J = 7.2 Hz, CH_3), 2.11 (3H, s, CH_3-pz), 2.12 (3H, s, CH_3-pz), 3.08 (2H, s, SCH_2), 3.28 (2H, s, pzCH_2COO), 3.54 (2H + 2H, m, CH_2), 4.07 (2H + 2H, m, CH_2), 6.74 (1H, br, NH), 6.92 (1H, br, NH), 7.15–7.41 (15H, m, Ph-Trit). ^{13}C NMR (75.373 MHz, $CDCl_3$): δ_C 9.3 (CH_3), 11.6 (pz-CH_3), 14.0 (pz-CH_3), 29.31 (CH_2), 35.4 (CH_2), 39.1 (CH_2), 42.9 (CH_2), 46.9 (CH_2), 60.6 (CH_2), 109.0 (C(4)-pz), 127.3 (CH-Trit), 128.6 (CH-Trit), 129.2 (CH-Trit), 137.5 (C(3/5)-pz); 143.6 (C-Trit), 146.7 (C(3/5)-pz), 168.40 (CO), 168.50 (CO), 171.4 (CO). R_f (silica gel, CH_2Cl_2–MeOH (95 : 5)) = 0.30.

Syntheses of the rhenium complexes

[ReO(OMe){3,5-Mepz(CH$_2$)$_2$N(CH$_2$)$_2$NH(CH$_2$)$_2$NH$_2$}](BPh$_4$) (11). To a solution of *trans*-[$ReO_2(py)_4$]Cl (98 mg, 0.17 mmol) in methanol (15 mL) was added **1** (43 mg, 0.19 mmol) dissolved in the minimum volume of the same solvent and the mixture was refluxed for 4 h, affording a dark green solution. Recrystallization of the crude product in methanol and in the presence of $NaBPh_4$ gave a few crystals of **11**, which has been characterized only by X-ray diffraction analysis.

[ReO{pz-(CH$_2$)$_2$NH-Gly-CH$_2$S}] (12). To a solution of compound **8** (116 mg, 0.24 mmol) and $ReOCl_3(PPh_3)_2$ (200 mg, 0.24 mmol) in dry methanol (20 mL) was added NEt_3 (84 μL, 0.60 mmol), and the mixture was refluxed overnight. After cooling to room temperature, the solvent was evaporated and the crude product was purified by silica gel column chromatography (CH_2Cl_2 : MeOH (95 : 5)), giving **12** as an orange solid. Yield: 159 mg (0.24 mmol, 98%).

Found C, 25.2; H, 3.0; N, 12.3. $C_9H_{11}N_4O_3SRe$ requires C, 24.5; H, 2.5; N, 12.7. IR: ν_{max}/cm^{-1} 984 (Re=O), 1628 (CO). ^{1}H NMR (300 MHz, dmso-d_6): δ_H 3.17 (1H, t, J = 12.3 Hz, CH_2); 3.83 (1H, d, J = 17.1 Hz, CH_2); 4.06 (1H, d, J = 17.1 Hz, CH_2), 4.30 (1H, d, J = 18 Hz, CH_2); 4.60 (2H + 1H, m, CH_2); 5.02 (1H, d, J = 18 Hz, CH_2); 6.86 (1H, t, H(4)-pz); 8.57 (1H + 1H, m, H(3/5)). ^{13}C NMR (75.373 MHz, dmso-d_6): 38.0 (CH_2); 46.8 (CH_2); 53.7 (CH_2); 55.90 (CH_2); 109.65 (C(4)-pz); 138.40 (C(3/5)-pz), 148.32 (C(3/5)-pz); 188.65 (CO), 189.93 (CO). R_f (sílica gel, CH_2Cl_2–MeOH (95 : 5)) = 0.45. R_f (silica gel/MeOH) = 0.70. HPLC: t_R = 14.00 min.

[ReO{3,5-Me$_2$pz-(CH$_2$)$_2$NH-Gly-CH$_2$S}] (13). Compound **13** is a carmine solid which was synthesized and purified in the same way as that described above for **12**, starting from 200 mg (0.24 mmol) of $ReOCl_3(PPh_3)_2$ and 123 mg (0.24 mmol) of ligand **11**. Yield: 111 mg (0.24 mmol, 97%).

Found C, 27.8; H, 3.8; N, 11.7. $C_{11}H_{15}N_4O_3SRe$ requires C, 28.1; H, 3.2; N, 11.9. IR: ν_{max}/cm^{-1} 971 (Re=O); 1636 (CO). ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 2.49 (3H, s, CH_3-pz); 2.92 (3H, s, CH_3-pz); 3.55 (1H + 1H, m, CH_2); 3.91 (1H, d, J = 18 Hz, CH_2); 4.04 (1H, d, J = 18 Hz, CH_2) 4.42 (1H, d, J = 18 Hz CH_2); 4.49 (1H, m,

CH); 4.66 (1H, m, CH_2); 5.44 (1H, d, $J = 18$ Hz CH_2); 6.40 (1H, s, H(4)-pz). ^{13}C NMR (75.373 MHz, $CDCl_3$): δ_C 11.9 (CH_3-pz), 16.9 (CH_3-pz), 39.2 (CH_2); 42.7 (CH_2); 50.6 (CH_2), 55.9 (CH_2), 110.5 (C(4)-pz), 146.6 (C(3/5)-pz), 158.6 (C(3/5)-pz), 190.3 (CO), 191.2 (CO). R_f (sílica gel, CH_2Cl_2–MeOH (95 : 5)) = 0.50.

[ReO{4-(EtOOC)CH$_2$-3,5-Me$_2$pz-(CH$_2$)$_2$NH-Gly-CH$_2$S}] (14). To a solution of $[NBu_4][ReOCl_4]$ (27 mg, 0.045 mmol) in dry methanol (5 mL) was added compound **10** (27 mg, 0.045 mmol) dissolved in the minimum volume of methanol, followed by addition of neat NEt_3 (26 μL, 0,18 mmol) and the resulting mixture was stirred for 2 h at room temperature. After this time, the solvent was evaporated and the crude product purified by silica gel column chromatography (ethyl acetate : methanol (90 : 10)), giving **14** as a carmine solid. Yield: 20 mg (0.036 mmol, 80%).

Found C, 32.1; H, 4.2; N, 7.2. $C_{15}H_{21}N_4O_5SRe$ requires C, 32.4; H, 3.8; N, 7.2. IR: ν_{max}/cm^{-1} 980 (Re=O); 1624 (C=O); 1734 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.28 (3H, t, $J = 6.9$ Hz, CH_3), 2.47 (3H, s, CH_3-pz), 2.89 (3H, s, CH_3-pz), 3.54 (2H + H + H, m, CH_2), 4.14 (2H + 2H, m, CH_2S, pzCH_2COO), 4.34 (1H, d, $J = 18.3$ Hz, CH_2), 4.50 (1H, m, CH_2); 4.73 (1H, m, CH_2); 5.43 (1H, d, $J = 18.3$ Hz, CH_2). ^{13}C NMR (75.373 MHz, $CDCl_3$): 10.6 (CH_3-pz), 14.2 (CH_3-pz), 15.2 (CH_2), 29.7 (CH_2), 39.1 (CH_2), 42.5 (CH_2), 50.8 (CH_2), 55.9 (CH_2), 61.6 (CH_2), 114.6 (C(4)-pz), 145.6 (C(3/5)-pz), 156.9 (C(3/5)-pz), 169.8 (CO), 190.4 (CO), 191.2 (CO). R_f (sílica gel, CH_2Cl_2–MeOH (95 : 5)) = 0.40.

X-Ray diffraction analysis

The X-ray diffraction analysis of compound **11** has been performed on an Enraf-Nonius CAD4 diffractometer and the diffraction data for **13** have been collected on a Bruker AXS APEX CCD area detector diffractometer, using graphite monochromated Mo Kα radiation (0.71073 Å). Empirical absorption correction based on ψ-scans.[36] was used for **11**, while for **13** an empirical absorption correction was carried out using SADABS.[37] Data collection and data reduction for **11** were done with CAD4 and XCAD programs[38] whereas for **13** the SMART and SAINT programs[39] were used. The structures were solved by direct methods with SIR97[40] and refined by full-matrix least-squares analysis with SHELXL97[41] using the WINGX[42] 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.[43] A summary of the crystal data, structure solution and refinement parameters are given in Table 2 and selected bond lengths and angles are shown in Tables 3 and 4.

CCDC reference numbers 616646 and 616647.

For crystallographic data in CIF or other electronic format see DOI: 10.1039/b611034g

Synthesis and *in vitro* evaluation of [^{99m}TcO{pz-(CH$_2$)$_2$NH-Gly-CH$_2$S}] (12a)

Synthesis of 12a. 500 μL of an ethanolic solution of the protected ligand **8** (0.1 mg mL^{-1}) were added to a nitrogen purged vial, followed by the addition of 500 μL of 0.1N HCl, and the resulting solution was heated for 30 min at 100 °C. After cooling to room temperature, 1 mL of 0.1 M phosphate buffer (pH = 6.2), 100 μL of an aqueous solution of Na/K tartrate (300 mg mL^{-1}), 15 μL of $SnCl_2$ in 0.1 N HCl (4 μg μL^{-1}) and 800 μL of $Na^{99m}TcO_4$ (2–6 mCi) were successively added to the ligand solution. The final preparation was incubated at room temperature for 30 min and analysed by TLC and HPLC.

TLC (silica gel/MeOH): $R_f = 0.7$; HPLC: $t_R = 14.63$ min.

Octanol–water partition coefficient. The log $P_{o/w}$ value of **12a** was determined by the multiple back extraction method under physiological conditions (*n*-octanol/0.1 M PBS, pH 7.4).[44] log $P_{o/w}$ (**12a**) = + 0.73 ± 0.03.

Glutathione challenge. In nitrogen purged vials, 200 μL of freshly prepared solutions of reduced glutathione, 10 mM and 20 mM in PBS (pH = 7.4), were added to 200 μL aliquots of complex **12a**. The samples were incubated at 37 °C and aliquots were analyzed by HPLC at 15 min, 1 h and 2 h time intervals.

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