Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Organometallic Chemistry 689 (2004) 4764–4774
Journal of Organometallic Chemistry
www.elsevier.com/locate/jorganchem

Rhenium(I)- and technetium(I) tricarbonyl complexes anchored by bifunctional pyrazole-diamine and pyrazole-dithioether chelators

Rute F. Vitor, Susana Alves, J.D.G. Correia, António Paulo, Isabel Santos *

Departamento de Química, ITN, Estrada Nacional, 10, 2686-953, Sacavém Codex, Portugal

Received 23 August 2004; accepted 14 September 2004
Available online 14 October 2004

Abstract

The novel pyrazolyl containing ligands 4-(HOOC)pz$(CH_2)_2$NH$(CH_2)_2NH_2$ (***L*^{1}**) and 4-(HOOCCH_2)-3,5-Me_2pz$(CH_2)_2$NH-$(CH_2)_2NH_2$ (***L*^{2}**), and 3,5-Me_2pz$(CH_2)_2$S$(CH_2)_2$SCH_2CH_3 (***L*^{3}**), 3,5-Me_2pz$(CH_2)_2$S$(CH_2)_2$SCH_2COOEt (***L*^{4}**) and 3,5-Me_2pz$(CH_2)_2$S-$(CH_2)_2$SCH_2COOH (***L*^{5}**) were synthesized, and their ability to stabilise complexes with the *fac*-$[M(CO)_3]^+$ (M = Re, ^{99m}Tc) moiety was evaluated. Reactions of ***L*^{1}**–***L*^{5}** with the Re(I) tricarbonyl starting materials $(NEt_4)_2[Re(CO)_3Br_3]$ and/or $[Re(CO)_5Br]$ afforded complexes *fac*-$[Re(CO)_3(\kappa^3$-L)] (L = ***L*^{1}**–***L*^{5}** (***1*–*5***)), which contain the pyrazolyl ancillary ligands coordinated in a tridentate fashion. Complexes **1**–**5** were characterized by the common analytical techniques, which included single crystal X-ray diffraction analysis in the case of **4**. The structural analysis of **4** confirmed the tridentate coordination mode of the pyrazole-dithioether ligand, which is facially coordinated to the Re(I) centre through the nitrogen from the pyrazole ring and the two thioether sulphur atoms, without involvement of the terminal ester functional group. The distorted octahedral coordination environment around the metal is completed by the three facial carbonyl ligands. The radioactive congeners of complexes **1**, **3** and **4**, *fac*-$[^{99m}Tc(CO)_3(\kappa^3$-L)]$^+$ (L = ***L*^{1}** (**1a**), ***L*^{3}** (**3a**), ***L*^{4}** (**4a**)), have been prepared by reacting the precursor *fac*-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ with the corresponding ligands, and their identity confirmed by HPLC comparison with the rhenium surrogates. Complexes **1a** and **3a** have been challenged in the presence of a large excess of histidine or cysteine, in order to evaluate their in vitro stability. Only a negligible displacement was observed, indicating that pyrazole-diamine and pyrazole-dithioether chelators provide a high kinetic inertness and/or stability to organometallic complexes with the *fac*-$[^{99m}Tc(CO)_3]^+$ moiety.

Keywords: Rhenium; Technetium; Carbonyl; Pyrazole; Bifunctional chelators; Radiopharmaceuticals

1. Introduction

The introduction of the low-valent *fac*-$[M(CO)_3(H_2O)_3]^+$ (M = Tc or Re) synthons has introduced a new avenue for the development of radioactive products for diagnostic (^{99m}Tc) and therapeutic ($^{186/188}$Re) medical applications, providing impetus for exploring unusual ligands, bonds and approaches [1–4]. So far, the explored chemistry has shown a high substitution stability for the three CO ligands and a high substitution lability for the three water molecules. The advent of these organometallic synthons motivated a strong research effort on the finding of bifunctional ligands adequate to stabilise the metallic cores and to covalently bind receptor seeking molecules. So far, medium hard bi(tri)dentate ligands containing nitrogen (i.e., pyridines, imidazoles, amines), oxygen (i.e., carboxylates), sulphur (i.e., thioether, thione) or phosphorus donor atoms have been explored [5–15]. The chemical and biological information obtained from all these studies demonstrated that the use of tridentate chelators, as well as the presence of aromatic amines like imidazole, can significantly improve

* Corresponding author. Tel.: +351 21 994 6201; fax: +351 21 994 1455.
E-mail address: isantos@itn.mces.pt (I. Santos).

0022-328X/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jorganchem.2004.09.033

the stability and biological properties of the complexes (i.e., blood clearance or excretion rate). Depending on the physico-chemical properties of the final organometallic building blocks, different bioactive molecules were labelled, like small peptides, sugars, steroids or CNS receptor antagonists [16–18]. To the best of our knowledge, there is only one example of a neurotensin analog labelled with the moiety *fac*-$[^{99m}Tc(CO)_3]^+$ which has been evaluated in patients, in spite of the versatility and unique features of the tricarbonyl approach [18b]. To succeed on using these new labelling tools in the development of clinically relevant radioactive probes, the introduction of novel chelator systems remains of paramount importance. By introducing new chelators, one can tune the physico-chemical properties of the final complexes (e.g. charge, size and lipophilicity) and improve their kinetic and thermodynamic stability, which are determinant for potential medical applications.

Aiming to contribute for the finding of novel bifunctional ligands suitable for the stabilisation of the *fac*-$[M(CO)_3]^+$ (M = ^{99m}Tc or Re) cores, our group has reported recently the chemistry and radiochemistry of symmetric and asymmetric tridentate pyrazolyl containing ligands (Fig. 1) towards these organometallic moieties, as well as the biological behaviour of some of the most promising building blocks [19,20]. This study demonstrated that symmetric ligands of the bis(pyrazolyl) type, with N_3 or N_2S donor atom sets, behave in an ambivalent way and can act as bi- or tridentate, depending on the reaction conditions, namely time and temperature. On contrary, the N_3 and N_2S donor asymmetric ligands, respectively, of the pyrazolyl-diamine or pyrazolyl-thioether-amine types, always coordinate as tridentate ligands to the *fac*-$[M(CO)_3]^+$ moiety (M = Re, ^{99m}Tc).

Taking into account the in vitro and in vivo stability and biological profile of the complexes isolated with asymmetric pyrazolyl tridentate ligands [20], we selected these frameworks for designing novel bifunctional chelators to be used on the labeling of biologically active substrates with the *fac*-$[M(CO)_3]^+$ (M = ^{99m}Tc or $^{186/188}$Re) moieties. A great advantage of these systems is their versatility which is expected to allow, with retained coordination sphere, the covalent coupling of the biomolecules either at the pyrazolyl ring or at the aliphatic side chain. Moreover, an easy introduction of different substituents can also be helpful on adjusting the physico-chemical properties of the complexes with those of the appended targeting biomolecule.

In this paper, we report on the synthesis and characterization of the novel asymmetric pyrazolyl containing ligands 4-(HOOC)pz$(CH_2)_2$NH$(CH_2)_2NH_2$ (***L*^{1}**), 4-$(HOOCCH_2)$-3,5-Me_2pz$(CH_2)_2$NH$(CH_2)_2NH_2$ (***L*^{2}**), 3,5-Me_2pz$(CH_2)_2S(CH_2)_2SCH_2CH_3$ (***L*^{3}**), 3,5-Me_2pz$(CH_2)_2S(CH_2)_2SCH_2$COOEt (***L*^{4}**) and 3,5-Me_2pz$(CH_2)_2S(CH_2)_2SCH_2$COOH (***L*^{5}**), as well as on the corresponding Re(I) tricarbonyl complexes *fac*-$[Re(CO)_3(\kappa^3\text{-L})]$ (L = ***L*^{1}**–***L*^{5}** (**1**–**5**)), which were obtained by reacting ***L*^{1}**–***L*^{5}** with $(NEt_4)_2[Re(CO)_3Br_3]$ and/or $[Re(CO)_5Br]$. Herein, it will be also reported on the synthesis of the radioactive complexes *fac*-$[^{99m}Tc(CO)_3(\kappa^3\text{-L})]^+$ (L = ***L*^{1}** (**1a**), ***L*^{3}** (**3a**), ***L*^{4}** (**4a**)), which were identified by HPLC comparison with the rhenium congeners.

2. Experimental

Chemicals and solvents were of reagent grade and were used without further purification, unless stated otherwise. The organometallic precursors $[Re(CO)_5Br]$ and $(NEt_4)_2[Re(CO)_3Br_3]$ were prepared according to published methods [21,22]. The radioactive synthon $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was obtained as described elsewhere [2]. $Na^{99m}TcO_4$ in saline solution was eluted from a ^{99}Mo/^{99m}Tc generator from MDS Nordion S.A., Belgium. The compounds *N*-*tert*-butoxycarbonyl-1,2-ethanediamine, ethyl 2-formyl-3-oxopropionate, ethyl 3-acetyl-4-oxopentantanoate and 1-(3-thia-5-hydroxypentyl)-3,5-dimethylpyrazole (3,5-Me_2pz(CH_2)-S$(CH_2)_2$-OH) were synthesized according to procedures described in the literature [23–26], although introducing slight modifications in some of the syntheses.

^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 300 MHz spectrometer; ^{1}H and ^{13}C chemical shifts were referenced with the residual solvent resonances relative to tetramethylsilane. IR spectra were recorded as KBr pellets or in CsI cells on a Bruker, Tensor 27 spectrometer. C, H and N analysis were performed on an EA110 CE Instruments automatic analyser.

Column chromatography was performed in silica gel 60 (Merck). HPLC analysis were performed on a Shimadzu C-R4A chromatography system equipped with a Berthold-LB 505 γ-detector and with a tunable absorption UV, using a Macherey-Nagel C18 reversed-phase

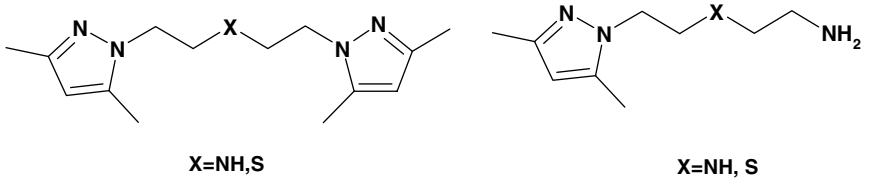


Fig. 1. Symmetric and asymmetric pyrazolyl containing chelators.

column (Nucleosil 10 μm, 250 × 4 mm) and a gradient of methanol/0.1% CF_3COOH or acetonitrile/0.1% CF_3COOH as eluent with a flow rate of 1.0 ml/min. The methanol/TFA gradient has been used to analyze complexes **1/1a**, while an acetonitrile/TFA gradient was applied in the study of complexes **3/3a** and **4/4a**. Method: $t = 0$–3 min: 0% MeOH or acetonitrile; 3–3.1 min: 0–25% MeOH or acetonitrile; 3.1–9 min: 25% MeOH or acetonitrile; 9–9.1 min: 25–34% MeOH or acetonitrile; 9.1–20 min: 34–100% MeOH or acetonitrile; 20–22 min: 100% MeOH or acetonitrile; 22–22.1 min: 100–0% MeOH or acetonitrile; 22.1–30 min: 0% MeOH or acetonitrile.

2.1. Synthesis of the ligands

2.1.1. 4-(HOOC)-pz$(CH_2)_2$NH$(CH_2)_2NH_2$ ($\boldsymbol{L^1}$) and 4-($HOOCCH_2$)-3,5-Me_2pz$(CH_2)_2$NH$(CH_2)_2NH_2$($\boldsymbol{L^2}$)

4-(EtOOC)pz$(CH_2)_2$OH and 4-(EtOOCCH_2)Me_2-pz$(CH_2)_2$OH. A solution of 2-hydroxyethylhydrazine (20 mmol) in EtOH (100 mL) was added dropwise to a solution of ethyl 2-formyl-3-oxopropionate or ethyl 3-acetyl-4-oxopentanoate (20 mmol) in EtOH (20 mL), at 0 °C. After overnight reaction at room temperature, the solvent was vacuum removed and the compounds obtained as yellow oils. Yields: 4-(EtOOC)pz$(CH_2)_2$OH, 3.500 g, 95%; 4-(EtOOCCH_2)Me_2pz$(CH_2)_2$OH, 4.400 g, 97%.

NH(DNS)$(CH_2)_2$NHBoc. *N-tert*-Butoxycarbonyl-1,2-ethanodiamine (345 mg, 2.15 mmol), 2,4-dinitrobenzenesulfonylchloride (DNSCl) (626 mg, 2.35 mmol) and pyridine (0.23 mL, 2.85 mmol) were dissolved in CH_2Cl_2. The reaction was left at room temperature for 4 h. The resulting suspension was filtered off, and the CH_2Cl_2 solution washed three times with H_2O. After removing the CH_2Cl_2, the solid residue obtained was purified by column chromatography (ethylacetate (30–100%)/hexane) yielding a white/yellow solid. Overall yield: 471 mg (1.21 mmol, 56%).

$\boldsymbol{L^1}$. NH(DNS)$(CH_2)_2$NHBoc (200 mg, 0.51 mmol), 4-(EtOOC)pz$(CH_2)_2$OH (189 mg 1.02 mmol) and diethylazodicarboxylate (DEAD) (134 mg, 0.77 mmol) were dissolved in dry THF, and PPh_3 (202 mg, 0.77 mmol) added to the resulting solution. The reaction mixture was allowed to react overnight at room temperature. After this time, the solvent was removed under vacuum and the obtained solid purified by column chromatography (ethylacetate (50–100%)/hexane). The solid (161 mg, 0.29 mmol) was treated with $HSCH_2COOH$ (35 mg, 0.38 mmol) and NEt_3 (0.080 ml, 0.58 mmol) in CH_2Cl_2 at room temperature for 30 min, to remove the protecting DNS group. The mixture was vacuum dried and the residue purified by column chromatography (MeOH/CH_2Cl_2 (20:80)), yielding a yellow solid. The BOC protecting group was removed with TFA, while the saponification of the ethyl ester was achieved using NaOH. The mixture was neutralized with 1 N HCl, the water removed under vacuum and the residue extracted with methanol. The solids were filtered off and the supernatant vacuum dried, yielding a yellow solid formulated as $\boldsymbol{L^1}$. Yield: 37 mg (0.19 mmol, 37%).

$\boldsymbol{L^2}$. This compound was synthesized as above described for $\boldsymbol{L^1}$, starting from NH(DNS)$(CH_2)_2$NHBoc (2.590 g, 6.63 mmol), 4-(EtOOCCH_2)3,5Me_2-pz$(CH_2)_2$-OH (3.0 g, 13.26 mmol), DEAD (1.732 g, 9.94 mmol) and PPh_3 (2.609 g, 9.95 mmol), although its purification required different procedures. Unlike $\boldsymbol{L^1}$, DNS and BOC protected $\boldsymbol{L^2}$ was always contaminated with PPh_3, even after the attempted purification by column chromatography (ethylacetate (30–100%)/hexane). Despite this contamination, confirmed by TLC and ^{1}H NMR spectroscopy analysis, the collected product was used in the deprotection steps without further purification. Thus, for DNS-deprotection, part of the obtained solid (1.410 g) was treated as above described for $\boldsymbol{L^1}$. To the resulting reaction mixture was added a saturated $NaHCO_3$ solution followed by extraction (3×) with CH_2Cl_2. The organic phases were collected, dried over $MgSO_4$, filtered and the solvent evaporated. The residue was first purified by successive precipitation of triethylammonium salts, 2,4-dinitrobenzenesulfonyl impurities, and triphenylphosphine in ethyl acetate/hexane and in diethylether. The final supernatant was evaporated under vacuum and the solid obtained was dissolved in ethyl acetate and a layer of hexane placed over it. After cooling the solution overnight, 400 mg of a yellow crystalline product was isolated. This solid was dried and used in the next step. Hydrolysis of the ester functional group was achieved by refluxing the product in THF with excess NaOH (ca. 10 equiv.) overnight. After evaporation of the solvent, the residue was dissolved in water, washed with diethylether (4×) and dichloromethane (2×), and finally neutralized with 0.01 N HCl solution. The brown solid, obtained after evaporation of the aqueous solution, was extracted with methanol. The solids were filtered off and the supernatant vacuum dried. The residue was purified by column chromatography (MeOH, 100%). The obtained oil (0.104 g) was dissolved in neat TFA for BOC deprotection, and allowed to react for 2 h. After evaporation of the TFA, the residue was dissolved in water and neutralized with 30% NaOH. Evaporation of the water, extraction of the residue with THF (3×), and final evaporation of the solvent, yielded a white-yellowish solid, corresponding to $\boldsymbol{L^2}$ (72 mg, 0.30 mmol).

$\boldsymbol{L^1}$, ^{1}H NMR (D_2O): δ 7.80 (s, H(3,5)pz, 1H); 7.64 (s, H(3,5)pz, 1H); 4.27 (t, CH_2, 2H); 3.24 (t, CH_2, 2H); 3.11–3.00 (m, CH_2, 4H). ^{13}C NMR (D_2O): 168.9 (*C*OOH); 142.0 (C(3,5)pz); 134.5 (C(3,5)pz); 118.1 (C(4)pz); 47.7 (*C*H_2); 47.4 (*C*H_2); 44.5 (*C*H_2); 35.4 (*C*H_2). IR (cm^{-1}): ν(C=O) 1690.

$\boldsymbol{L^2}$, ^{1}H NMR (CD_3OD): δ 4.07 (t, CH_2, 2H); 3.23 (s, CH_2, 2H); 2.99 (t, CH_2, 2H); 2.88 (m, CH_2, 2H); 2.79

(m, CH_2, 2H), 2.20 (s, CH_3, 3H); 2.13 (s, CH_3, 3H). ^{13}C NMR (CD_3OD): δ 179.5 (*C*OOH); 147.9 (C(3,5)pz); 139.4 (C(3,5)pz); 113.6 (C(4)pz); 46.7 (*C*H_2); 39.6 (*C*H_2); 32.5 (*C*H_2); 11.7 (pz-*C*H_3); 9.7 (pz-*C*H_3). ^{13}C NMR (D_2O): δ 183.2 (*C*OOH); 150.6 (C(3,5)pz); 141.9 (C(3,5)pz); 115.4 (C(4)pz); 50.5 (*C*H_2); 49.1 (*C*H_2); 47.8 (*C*H_2); 40.3 (*C*H_2); 34.3 (*C*H_2); 13.3 (pz-*C*H_3); 11.4 (pz-*C*H_3); IR (cm^{-1}): ν(C=O)1683.

2.1.2. 3,5-$Me_2pz(CH_2)_2S(CH_2)_2SCH_2CH_3$ (***L*^{3}**)

To a solution of 3,5-$Me_2pz(CH_2)_2S(CH_2)_2OH$ (400 mg, 2.02 mmol) in chloroform was added PBr_3 (0.19 mL; 2.00 mmol) and the resulting solution refluxed for 24 h under N_2. After cooling to room temperature, the reaction mixture was washed with 20 mL of 10% $NaHCO_3$ and the collected organic phase was dried over magnesium sulphate. Removal of the solvent under vacuum gave 3,5-$Me_2pz(CH_2)_2S(CH_2)_2Br$ as a brown/yellow oil. Yield: 329 mg (1.25 mmol, 63%).

3,5-$Me_2pz(CH_2)_2S(CH_2)_2Br$. ^{1}H NMR ($CDCl_3$): δ 5.82 (s, H(4)-pz, 1H), 4.15 (t, CH_2, 2H), 3.36 (t, CH_2, 2H), 3.00 (t, CH_2, 2H), 2.70 (t, CH_2, 2H), 2.26 (s, CH_3, 3H), 2.23 (s, CH_3, 3H).

Under N_2, dry ethanol was added to metallic sodium (105 mg, 4.56 mmol), and the mixture was stirred at room temperature until complete conversion to sodium ethoxide. To this mixture was added dropwise an ethanolic solution of ethanethiol (0.50 ml, 4.56 mmol), followed by addition of 3,5-$Me_2pz(CH_2)_2S(CH_2)_2Br$ (1.20 g, 4.56 mmol) in ethanol. The reaction mixture was stirred overnight at room temperature and, after this time, the solvent was removed under vacuum. The resulting oil was dissolved in chloroform and washed with water. After drying over magnesium sulphate, chloroform was removed under vacuum yielding 3,5-$Me_2pz(CH_2)_2S(CH_2)_2SCH_2CH_3$ (***L*^{3}**) as a yellow oil, which was further purified by chromatography on silica gel (eluent: gradient from 100% ethyl acetate to 100% MeOH). Yield: 737 mg (3.02 mmol, 66%).

*3,5-$Me_2pz(CH_2)_2S(CH_2)_2SCH_2CH_3$ (***L*^{3}**)*. ^{1}H NMR ($CDCl_3$): δ 5.82 (1H, s, H(4)-pz), 4.14 (m, CH_2, 2+2H), 3.25 (s, CH_2, 2H), 2.92 (t, CH_2, 2H), 2.75 (t, CH_2, 2H), 2.57 (t, CH_2, 2H), 2.2 (s, CH_3, 3H), 2.16 (s, CH_3, 3H), 1.25 (t, –CH_2CH_3, 3H); ^{13}C NMR ($CDCl_3$): δ (ppm) 147.4 (C(3,5)pz); 138.9 (C(3,5)pz); 104.5 (C(4)pz); 48.3 (*C*H_2); 31.73 (*C*H_2); 31.71 (*C*H_2); 31.2 (*C*H_2); 25.5 (*C*H_2); 14.4 (*C*H_3); 13.1 (pz-*C*H_3); 10.8 (pz-*C*H_3). Anal. Calc. for $C_{11}H_{20}N_2S_2$: C, 53.69; H, 8.20; N, 11.48. Found: C, 54.14; H, 8.70; N, 12.04%.

2.1.3. 3,5-$Me_2pz(CH_2)_2S(CH_2)_2S(CH_2CO_2Et)$ (***L*^{4}**)

***L*^{4}** was synthesized as above described for ***L*^{3}**, starting from ethyl 2-mercaptoacetate (0.50 mL, 4.56 mmol) and 3,5-$Me_2pzCH_{22}S(CH_2)_2Br$ (1.20 g, 4.56 mmol). ***L*^{4}** was obtained as a yellow oil after purification by chromatography on silica gel (eluent: gradient from 100% CH_2Cl_2 to 100% ethylacetate and to 100% methanol). Yield: 72% (1.00 g, 3.30 mmol).

^{1}H NMR ($CDCl_3$): δ 5.82 (s, H(4)-pz, 1H), 4.14 (m, CH_2, 4H), 3.25 (s, CH_2, 2H), 2.92 (t, CH_2, 2H), 2.75 (t, CH_2, 2H), 2.57 (t, CH_2, 2H), 2.20 (s, CH_3, 3H), 2.16 (s, CH_3, 3H), 1.25 (t, CH_3, 3H);^{13}C RMN ($CDCl_3$): δ (ppm) 170.2 (C=O); 147.8 (C(3,5)pz); 139.2 (C(3,5)pz); 105.0 (C(4)pz); 61.37 (O*C*H_2CH$_3$); 48.6 (*C*H_2C=O); 33.4 (*C*H_2); 32.4 (*C*H_2); 32.0 (*C*H_2); 31.4 (*C*H_2); 14.1 (*C*H_3); 13.4 (pz-*C*H_3); 11.1 (pz-*C*H_3). IR (cm^{-1}): ν(C=O) 1731; Anal. Calc. for $C_{13}H_{22}N_2O_2S_2$: C, 51.46; H, 7.26; N, 9.24. Found: C, 51.42; H, 7.34; N, 9.42%.

2.1.4. 3,5-$Me_2pz(CH_2)_2S(CH_2)_2S(CH_2CO_2H)$ (***L*^{5}**)

To a solution of ***L*^{4}** (117 mg, 0.39 mmol) in THF was added NaOH (77 mg, 1.93 mmol), dissolved in the minimum volume of water, and the resulting mixture refluxed overnight. After cooling to room temperature, the reaction mixture was neutralized with 1N HCl and the solvents were removed under vacuum. After washing the resulting residue with water, compound ***L*^{5}** was recovered as a white oil. Yield: 66 mg (0.24 mmol, 62%).

^{1}H NMR ($CDCl_3$): δ 5.75 (s, H(4)-pz, 1H), 4.14 (t, CH_2, 2H,), 3.21 (s, CH_2, 2H), 2.86 (t, CH_2, 2H), 2.77 (t, CH_2, 2H), 2.65 (t, CH_3, 3H), 2.20 (s, CH_3, 3H), 2.16 (s, CH_3, 3H). ^{13}C RMN ($CDCl_3$): δ (ppm) 172.4 (C=O); 147.6 (C(3,5)pz); 139.6 (C(3,5)pz); 105.4 (C(4)pz); 47.8 (*C*H_2C=O); 34.3 (*C*H_2); 32.4 (*C*H_2); 30.6 (*C*H_2); 30.0 (*C*H_2); 12.8 (*C*H_3); 10.9(pz-*C*H_3); IR (cm^{-1}): ν(C=O) 1703.

2.2. Synthesis of the rhenium complexes

2.2.1. [$Re(CO)_3(\kappa^3$-(4-HOOC)$pz(CH_2)_2NH(CH_2)_2NH_2$)] Br (***1***) and [$Re(CO)_3(\kappa^3$-(4-HOOCCH_2)3,5-$Me_2pz(CH_2)_2NH(CH_2)_2NH_2$)]Br (***2***)

[$ReBr(CO)_5$] (100 mg, 0.25 mmol) was reacted with equimolar amounts of the compounds ***L*^{1}** and ***L*^{2}** in refluxing H_2O for 2 h. The complexes precipitate, as white solids from the aqueous solutions, upon concentration and cooling in an ice bath.

Complex **1**, Yield: 95 mg (0.17 mmol, 68%). ^{1}H NMR (D_2O): δ 8.22 (s, H(3)pz, 1H); 8.20 (s, H(5)pz, 1H); 6.62 (s, br, N*H*, 1H); 4.94 (s, br, NH_2, 1H); 4.43 (m, CH_2, 1H); 4.25 (m, CH_2, 1H); 4.05 (s, br, NH_2, 1H); 3.52 (m, CH_2, 1H); 2.92 (m, CH_2, 1H); 2.76 (m, CH_2, 2H); 2.53 (m, CH_2, 1H); 2.14 (m, CH_2, 1H). ^{13}C-RMN (D_2O): 196.0 (Re*C*O); 195.8 (Re*C*O); 195.6 (Re*C*O); 168.0 (COOH); 149.3 (C(3)pz); 139.4 (C(5)pz); 119.2 (C(4)pz); 57.0 (*C*H_2); 55.2 (*C*H_2); 54.3 (*C*H_2); 42.8 (*C*H_2). IV (cm^{-1}): ν(C≡O), 2010, 1885 (v.br); ν(C=O) 1690. HPLC (gradient 0.1% TFA/MeOH): R_t = 18.2 min.

Complex **2**, Yield: 90 mg (0.15 mmol, 60%). ^{1}H NMR (CD_3OD): δ 6.78 (br. tr, N*H*, 1H); 5.38 (br. t, NH_2, 1H); 4.48 (dt, CH_2, 1H); 4.11 (m, CH_2, 1H); 3.91 (br.t, NH_2,

1H), 3.45 (m, CH_2, 1H); 3.27 (s, CH_2, 2H), 2.89 (m, CH_2, 1H); 2.79–2.62 (m, CH_2, 3H), 2.44 (m, CH_2, 1H), 2.36 (s, CH_3, 3H), 2.27 (s, CH_3, 3H). ^{13}C NMR (CD_3OD): 194.7 (Re*C*O); 194.5 (Re*C*O); 178.7 (*C*OOH); 153.1 (C(3,5)pz); 143.0 (C(3,5)pz); 116.7 (C(4)pz); 55.8 (*C*H_2); 47.9 (*C*H_2) 43.5 (*C*H_2); 43.4 (*C*H_2); 33.2 (*C*H_2), 14.4 (*C*H_3); 10.2 (*C*H_3). IV (cm^{-1}): ν(C≡O), 2027, 1992, 1900, 1874; ν(C=O) 1678. Anal. Calc. for $C_{14}H_{20}N_4O_5BrRe$: C, 28.48; H, 3.41; N, 9.49. Found: C, 28.58; H, 3.57; N, 9.58%.

2.2.2. [Re(CO)$_3$(κ^3-(3,5-Me$_2$pz(CH$_2$)$_2$S(CH$_2$)$_2$S(CH$_2$-CH$_3$)))]Br (**3**)

A solution of [Re(CO)$_5$Br] (100 mg, 0,25 mmol) and ligand ***L*^{3}** (65 mg, 0.27 mmol) in dry methanol was refluxed overnight under N_2. After this time, methanol was removed under vacuum, and the resulting residue was washed with THF. The remaining solid was extracted into water and, after centrifugation, the resulting solution was vacuum dried yielding complex **3** as a white microcrystalline solid. Yield: 69 mg (0.12 mmol, 48%).

^{1}H NMR (CD_3OD): δ (ppm) 6.23 (s, H(4)pz, 1H); 4.73 (m, CH_2, 1H); 3.96 (m, CH_2, 1H); 3.78 (m, CH_2, 1H); 3.38 (m, CH_2CH$_3$, 2H); 3.23 (m, CH_2, 1H); 3.05 (m, CH_2, 2H); 2.61 (m, CH_2, 1H); 2.57 (s, CH_3, 3H); 2.35 (s, CH_3, 3H); 2.01 (m, CH_3, 1H); 1.43 (t, CH$_2$CH_3, 3H); ^{13}C NMR (CD_3OD): δ (ppm) 160.0 (C=O); 156.17 (C(3,5)pz); 146.57 (C(3,5)pz); 110.3 (C(4)pz); 37.3 (*C*H_2); 34.4 (*C*H_2); 34.0 (*C*H_2); 32.4 (*C*H_2); 17.1 (CH$_2$*C*H$_3$); 13.6 (pz-*C*H$_3$); 12.11 (pz-*C*H$_3$). IR (cm^{-1}): ν(C≡O) 2039, 1919; Anal. Calc. for $C_{14}H_{20}N_2O_3S_2$-ReBr: C, 28.26; H, 3.37; N, 4.71. Found: C, 28.01; H, 3.31; N, 4.65%. HPLC (gradient 0.1% TFA/CH_3CN): R_t = 18.4 min.

2.2.3. [Re(CO)$_3$(κ^3-(3,5-Me$_2$pz(CH$_2$)$_2$S(CH$_2$)$_2$S(CH$_2$-CO$_2$Et)))]Br (**4**)

A solution of (NEt$_4$)$_2$[Re(CO)$_3$Br$_3$] (100 mg, 0.13 mmol) and ***L*^{4}** (40 mg, 0.13 mmol) in dry methanol was refluxed overnight under N_2. After removal of methanol, the residue was dissolved in water from which complex **4** precipitated as a white microcrystalline solid. Yield: 45 mg (0.07 mmol, 53%).

^{1}H NMR (CD_3OD): δ (ppm) 6.25 (s, H(4)pz, 1H); 4.73 (m, CH_2, 1H); 4.33 (m, CH_2, 2H); 3.94 (m, CH_2, 1H); 3.77 (m, CH_2, 1H); 3.47 (m, CH_2, 2H); 3.00 (m, CH_2, 2H); 2.62 (s, CH_3, 3H); 2.52 (m, CH_2, 2H); 2.35 (s, CH_3, 3H); 2.02 (m, CH_2, 1H,); 1.32 (t, CH_3, 3H).^{13}C NMR (CD_3OD): δ (ppm) 192.6 (Re-*C*O); 168.4 (*C*=O); 156.36 (C(3,5)pz); 146.7 (C(3,5)pz); 110.4 (C(4)pz); 63.8 (OCH$_2$CH$_3$); 41.0 (*C*H_2); 37.5 (*C*H_2); 35.0 (*C*H_2); 32.4 (*C*H_2); 17.0 (OCH$_2$CH$_3$); 14.4 (pz-*C*H$_3$); 12.2 (pz-*C*H$_3$). IR (cm^{-1}): ν(C≡O), 2036, 1947; ν(C=O) 1721; Anal. Calc. for $C_{16}H_{22}N_2O_5S_2$-ReBr: C, 29.42; H, 3.37; N, 4.29. Found: C, 29.57; H, 3.91; N, 4.27%. HPLC (gradient 0.1% TFA/CH_3CN): R_t = 17.1 min.

2.2.4. [Re(CO)$_3$(κ^3-(3,5-Me$_2$pz(CH$_2$)$_2$S(CH$_2$)$_2$S(CH$_2$-CO$_2$H)))]Br (**5**)

Complex **5** was synthesized as above described for **3**, starting from [Re(CO)$_5$Br] (93 mg, 0.23 mmol) and ***L*^{5}** (66 mg, 0.24 mmol). **5** was purified by recrystallization from THF/*n*-hexane. Yield: 65 mg (0.10 mmol, 43%).

^{1}H NMR (CD_3OD): δ (ppm) 6.24 (s, H(4)pz, 1H); 4.72 (m, CH_2, 1H); 4.23–3.97 (m, CH_2, 2H); 3.94–3.89 (m, CH_2, 1H); 3.80–3.70 (m, CH_2, 1H); 3.53–3.41 (m, CH_2, 2H); 3.01 (m, CH_2, 1H); 2.62 (m, CH_2 + CH_3, 1 + 3H); 2.36 (m, CH_2, 3H); 2.01 (m, CH_2, 1H); ^{13}C NMR (CD_3OD): δ (ppm) 190.3 (Re*C*O); 169.7 (*C*=O); 156.4 (C(3,5)pz); 146.6 (C(3,5)pz); 110.3 (C(4)pz); 49.2 (O-*C*H$_2$); 41.3 (*C*H_2); 37.6 (*C*H_2); 34.9 (*C*H_2); 32.4 (*C*H_2); 17.0 (pz-*C*H$_3$); 12.2 (pz-*C*H$_3$). IR (KBr, ν/cm^{-1}): ν(C≡O) 2036, 1944, ν(C=O) 1702; Anal. Calc. for $C_{14}H_{18}N_2O_5S_2ReBr$: C, 26.90; H, 2.88; N, 4.49. Found: C, 27.2; H, 3.02; N, 4.52%. HPLC (gradient 0.1% TFA/CH_3CN): R_t = 16.4 min.

2.3. Synthesis of the ^{99m}Tc(I) complexes (**1a**, **3a** and **4a**)

General method. In a glass vial under nitrogen, 100 μL of a 10^{-3} M (***L*^{1}**) aqueous solution or 100 μL of a 10^{-2} M (***L*^{2}** and ***L*^{3}**) ethanolic solution of the ligands were added to 900 μl of [^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ (1–2 mCi) in PBS. The reaction was incubated at 100 °C for 60 min and then analyzed by HPLC.

Cysteine and histidine challenge. Aliquots of 100 μl of the ^{99m}Tc complexes were added to 900 μl of 10^{-3} M or 10^{-2} M cysteine or histidine solutions in PBS (pH 7.4), with final ligand concentrations of 10^{-5} M (***L*^{1}**) and 10^{-4} M (***L*^{3}**), respectively. The solutions were incubated at 37 °C and aliquots were removed at 1, 2, 4 and 6h, at which time HPLC analysis was run.

2.4. X- ray diffraction

White crystals of complex **4** were obtained by recrystallization from a saturated water solution, and mounted in thin-walled glass capillaries. Data were collected at room temperature on an Enraf-Nonius CAD-4 diffractometer with graphite-monochromated Mo Kα radiation, using an ω-2θ scan mode. The crystal data are summarized in Table 1.

The data were corrected for Lorentz and polarization effects, and empirically for absorption by Ψ scans [27]. The heavy atom positions were located by Patterson methods using SHELXS-97 [28]. The remaining atoms were located in successive Fourier-difference maps and refined by least-squares refinements on F^2 using SHELXL-97 [29]. Two remaining residual peaks

Table 1
Crystallographic data for complex **4**

Empirical formula	$C_{16}H_{26}BrN_2O_7S_2Re$
M	688.62
Crystal system	Triclinic
Space group	$P\bar{1}$
a (Å)	9.8592(10)
b (Å)	11.0784(17)
c (Å)	11.8537(18)
α (°)	66.412(12)
β (°)	76.308(11)
γ (°)	85.189 (11)
V (Å)	1152.7(3) Å^3
Z	2
D_c (g cm^{-3})	1.984
μ(Mo Kα) (mm^{-1})	7.233
No. reflections measured	4616
No. unique reflections	4383 (0.0182)[a]
R_1[b]	0.0368
wR_2	0.0841

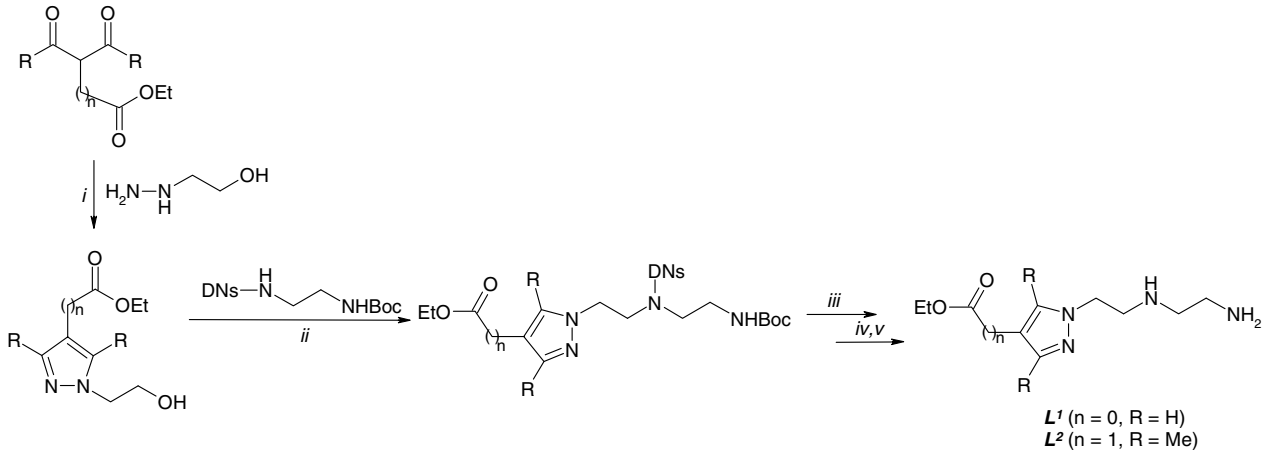
[a] Value of R(int).
[b] The values were calculated for data with $I > 2\sigma(I)$.

were assigned as oxygen atoms of two water molecules. The solvent oxygen atoms were refined anisotropically and the corresponding hydrogen atoms were ignored. With exception of the carbonyl oxygen O5, all the non-hydrogen atoms were refined anisotropically; The O5 atom is disordered and split positions have been considered in the structure calculation with a site occupation of 0.52 and 0.48, respectively. Because of this disorder, their refinement was performed isotropically with an imposed C9–O5 distance. The contributions of the hydrogen atoms were included in calculated positions, constrained to ride on their carbon atoms with group U_{iso} values assigned. Atomic scattering factors and anomalous dispersion terms were as in SHELXL-97 [29]. The drawings were made with ORTEP-3 [30].

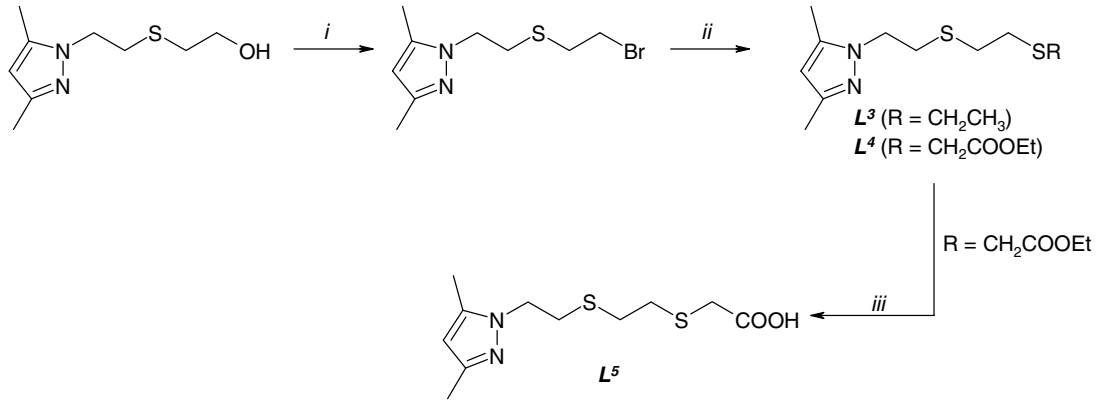
3. Results and discussion

Following our previous work [19,20], and focused on the labelling of biologically relevant molecules, we synthesized novel asymmetric pyrazolyl containing ligands with N_3 and NS_2 donor atom sets and with a carboxylate functional group for direct coupling of biomolecules. The asymmetric and bifunctional pyrazolyl ligands with N_3 donor atom sets, ***L*^{1}** and ***L*^{2}**, were prepared by the multi-step synthetic procedure depicted in Scheme 1. A key step on the preparation of ***L*^{1}** and ***L*^{2}** was the synthesis of the functionalised 1-ethylamine-pyrazole precursors, 4-(EtOOC)pz$(CH_2)_2$OH and 4-(EtOOCCH_2)Me$_2$pz$(CH_2)_2$OH, which were obtained by cyclization reactions between the corresponding dialdehyde or diketone compounds with 2-hydroxyethylhidrazine, following well established procedures for the synthesis of pyrazole derivatives [24]. The ligands ***L*^{1}** and ***L*^{2}** were finally obtained by a Mitsunobu reaction [31], i.e., by reacting the 2-hydroxyethylpyrazole precursors with NH(DNS)$(CH_2)_2$NHBoc, followed by removal of the protecting DNS and BOC groups with mercaptoacetic acid and TFA, respectively.

The related asymmetric ligands of the pyrazole-dithioether type, i.e., with NS_2 donor atom sets, 3,5-Me$_2$pz$(CH_2)_2$S$(CH_2)_2$S(CH_2CO_2Et) (***L*^{4}**) and 3,5-Me$_2$pz$(CH_2)_2$S$(CH_2)_2$S(CH_2CO_2H) (***L*^{5}**), were synthesized as indicated in Scheme 2. Being aware of possible competition of the carboxylate group in the coordination to the *fac*-$[M(CO)_3]^+$ moieties, the ligand 3,5-Me$_2$pz$(CH_2)_2$S$(CH_2)_2$SCH$_2$CH$_3$ (***L*^{3}**) was also prepared in order to compare its coordination behaviour with the one of the functionalised congeners ***L*^{4}** and ***L*^{5}**. As depicted in Scheme 2, the preparation of ***L*^{3}** and ***L*^{4}** involved 3,5-Me$_2$pz$(CH_2)_2$S$(CH_2)_2$OH as a common starting material [26]. Reaction of this compound with PBr_3 afforded the brominated analogue, which by treatment



Scheme 1. Synthesis of (4-HOOC)pz$(CH_2)_2$NH$(CH_2)_2NH_2$ (***L*^{1}**) and (4-HOOCCH_2)-3,5-Me$_2$pz$(CH_2)_2$NH$(CH_2)_2NH_2$ (***L*^{2}**). (i) Ethanol, 0 °C. (ii) DEAD, PPh_3, THF, room temperature. (iii) $HSCH_2CO_2H$, NEt_3, CH_2Cl_2, room temperature. (iv) TFA, room temperature. (v) NaOH, THF, H_2O, reflux, overnight.



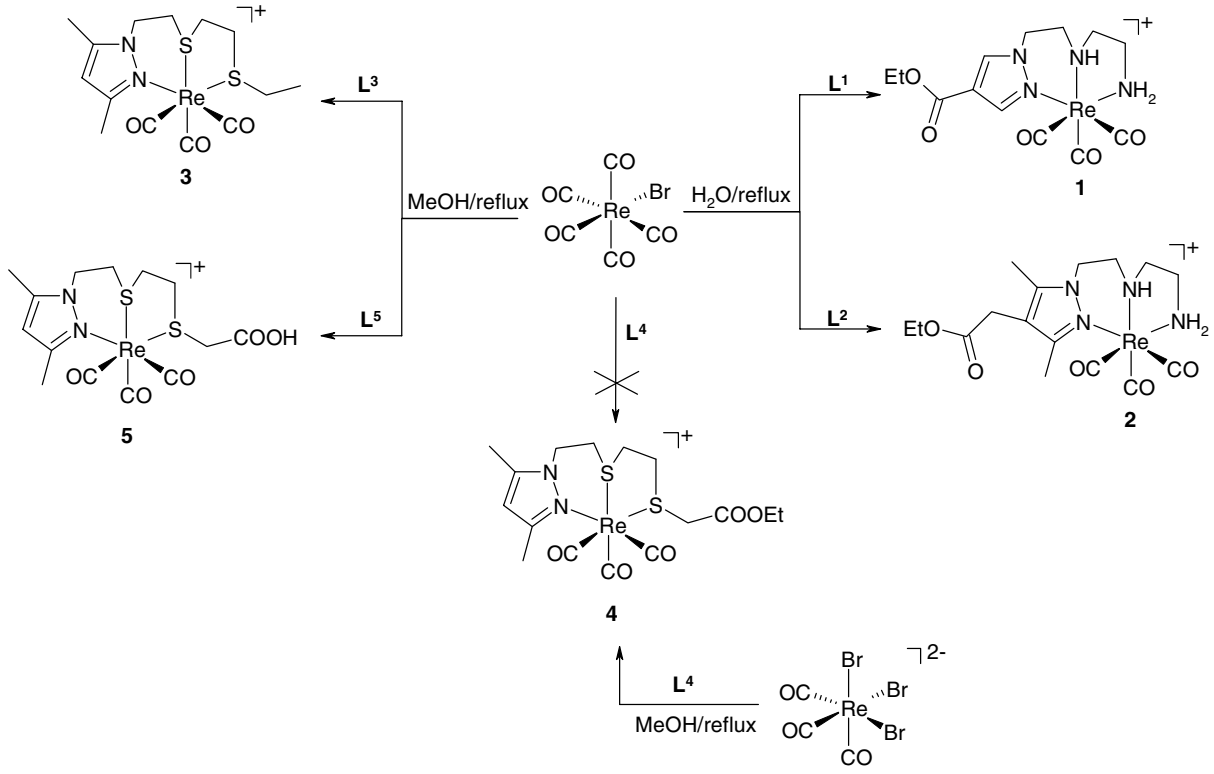
Scheme 2. Synthesis of 3,5-$Me_2pz(CH_2)_2S(CH_2)_2SR$ (R = CH_2CH_3 (***L*** ^{3}), CH_2COOEt (***L*** ^{4}), CH_2COOH (***L*** ^{5})): (i) PBr_3, $CHCl_3$, reflux, 24 h. (ii) RSH (R = CH_2CH_3, CH_2COOEt), NaOEt, EtOH, room temperature, overnight. (iii) NaOH, THF/H_2O, reflux, overnight.

with the adequate thiol yielded the final ligands ***L*** ^{3} and ***L*** ^{4} in a straightforward way. ***L*** ^{5} was prepared by the hydrolysis of ***L*** ^{4}.

L ^{1}–***L*** ^{5} are air and water stable compounds that are soluble in most common polar organic solvents, such as alcohols or acetonitrile. While the pyrazole-diamine ligands (***L*** ^{1} and ***L*** ^{2}) are quite soluble in water, the pyrazole-dithioethers (***L*** ^{3}–***L*** ^{5}) have a poor solubility in this solvent. Compounds ***L*** ^{1}–***L*** ^{5} were characterized by the common analytical techniques.

Reactions of the starting materials $[Re(CO)_5Br]$ and/or $(NEt_4)_2[Re(CO)_3Br_3]$, with ***L*** ^{1}–***L*** ^{5} led to the formation of cationic tricarbonyl complexes, **1**–**5**, where the pyrazolyl based anchors act as tridentate ligands, as we previously reported for related non-functionalised parent complexes (Scheme 3) [19].

Complexes **1**–**3** and **5** were preferentially synthesized using $[Re(CO)_5Br]$ as the starting material. Although the complexes could also be obtained starting from $(NEt_4)_2[Re(CO)_3Br_3]$, as checked by following the reactions by ^{1}H NMR spectroscopy, separation of the tetraethylammonium salts from the complexes is not an easy task, due their similar solubility in most common solvents. By contrast, the successful synthesis of complex **4** bearing a terminal ester functional group was only achieved when ***L*** ^{4} reacted with $(NEt_4)_2[Re(CO)_3Br_3]$. The reaction of



Scheme 3. Synthesis of the rhenium complexes **1**–**5**.

the Re(I) pentacarbonyl precursor with ***L*⁴** was always accompanied by hydrolysis of the ester functional group, which certainly reflects slower rate of reaction for $[Re(CO)_5Br]$ compared to $(NEt_4)_2[Re(CO)_3Br_3]$.

Compounds **1**–**5** are white microcrystalline solids, stable towards air oxidation or hydrolysis, and are soluble in most common polar organic solvents. Reflecting their cationic character, **1**–**5** display a moderate to high solubility in water. As expected, the complexes with the pyrazole-diamine ligands (**1**–**2**) show an enhanced water-solubility. The characterization of **1**–**5** involved IR, ^1H and ^{13}C NMR spectroscopies, and in the case of **4** also X-ray diffraction analysis.

The most significant feature of the IR spectra of compounds **1**–**5** is the presence of strong bands due to the ν(CO) stretching mode, in the range 1874–2039 cm^{-1} and with the typical pattern for complexes with the "*fac*-$Re(CO)_3$" moiety. In comparison with **1** and **2**, there is a slight increase of the ν(CO) frequencies for complexes **3**–**5**. This is probably due to the poorer σ-donor character and better acceptor properties of thioethers comparatively to amines. With the exception of **3**, the IR spectra of the complexes show the presence of medium to strong bands, spanning from 1678 to 1702 cm^{-1}, which were attributed to the ν(CO) stretching mode associated with the corresponding carboxylic (**1**, **2** and **5**) or ester functions (**4**). These frequencies are quite close to the values found for the corresponding free ligands. For complexes **4** and **5**, this clearly shows that the terminal ester or carboxylate groups of the ligands are not involved in the coordination to the metal. This behaviour was further corroborated by the ^1H NMR data collected for these complexes and by the X-ray structural analysis of **4**, as discussed below.

The ^1H NMR data obtained for complexes **1** and **2** are compatible with the tridentate coordination mode of the pyrazole-diamine ligands, ***L*¹** and ***L*²**, respectively. The chemical shifts and splitting of the diastereotopic N*H* and methylenic protons are comparable to those found for the previously reported *fac*-$[Re(CO)_3(\kappa^3\text{-}(3,5\text{-}Me_2pz(CH_2)_2NH(CH_2)_2NH_2))]$, characterized in solution and by X-ray diffraction analysis [19]. The resonances due to the H(3,5) and methyl protons from the pyrazolyl moiety of ***L*¹** and ***L*²**, respectively, are downfield shifted relatively to the same resonances in the corresponding free ligands, which is consistent with the involvement of the azole ring in the coordination to the metal.

For complexes **3**–**5** anchored by the pyrazole-dithioether ligands ***L*³**–***L*⁵**, the resonances due to the H(4) and methyl protons from the pyrazolyl rings are also downfield shifted comparatively to the corresponding resonances in the spectra of the free ligands. This is also a clear indication of the coordination of the azole ring to the metal, showing in the case of complexes **4** and **5** that the pyrazole group from ***L*⁴** and ***L*⁵** has a greater affinity for Re(I) than the ester or carboxylic functional groups, respectively. In spite of some occasional overlap of resonances, the presence in the ^1H NMR spectra of **3**–**5** of a series of multiplet signals due to the methylenic protons, integrating each for one proton, are clearly consistent with the (NS_2) tridentate coordination mode for ***L*³**–***L*⁵**.

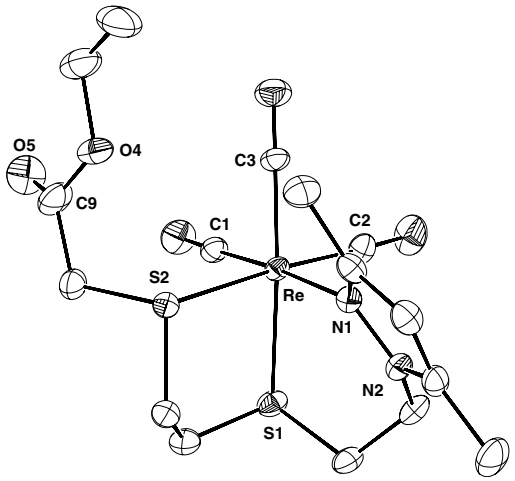


Fig. 2. ORTEP view of complex **4**.

The determination of the molecular structure of **4** by X-ray diffraction analysis confirmed that ***L*⁴** is acting as a tridentate ligand through one nitrogen atom of the pyrazolyl ring and the two sulphur atoms of the thioethers, as can be seen in the ORTEP diagram presented in Fig. 2. No intra- or intermolecular interaction involving the dangling ester function was found. The rhenium atom is six-coordinated and displays an approximately octahedral coordination geometry. The ***L*⁴** ligand is facially coordinated, with the remaining coordination positions occupied by the three CO ligands. As can be verified by the values given in Table 2, the Re–C distances are almost identical, spanning from 1.915(7) to 1.918(7) Å, being comparable to the values found for other Re(I) tricarbonyl complexes anchored by tridentate pyrazolyl-based ligands that we have previously reported [19]. The Re–S bond distances, averaging 2.484 Å, are also normal and similar to the values reported

Table 2
Selected bond lengths (Å) and angles (°) for complex **4**

Re–C(1)	1.915 (7)	Re–C(2)	1.918(7)
Re–C(3)	1.916(7)	Re–S(1)	2.479(2)
Re–S(2)	2.488(2)	Re–N(1)	2.206(5)
C(1)–O(1)	1.144(8)	C(2)–O(2)	1.147(9)
C(3)–O(3)	1.143(2)		
C(1)–Re–C(2)	89.8(3)	C(1)–Re–C(3)	88.0(3)
C(2)–Re–C(3)	86.7(3)	C(1)–Re–S(1)	87.2(2)
C(1)–Re–S(2)	94.9(2)	C(1)–Re–N(1)	175.1(3)
C(2)–Re–S(1)	93.4(2)	C(2)–Re–S(2)	174.6(2)
C(2)–Re–N(1)	94.6(2)	C(3)–Re–S(1)	175.3(2)
C(3)–Re–S(2)	96.0(2)	C(3)–Re–N(1)	94.3(2)
S(1)–Re–S(2)	84.33(6)	S(1)–Re–N(1)	90.38(14)
S(2)–Re–N(1)	80.59(13)		

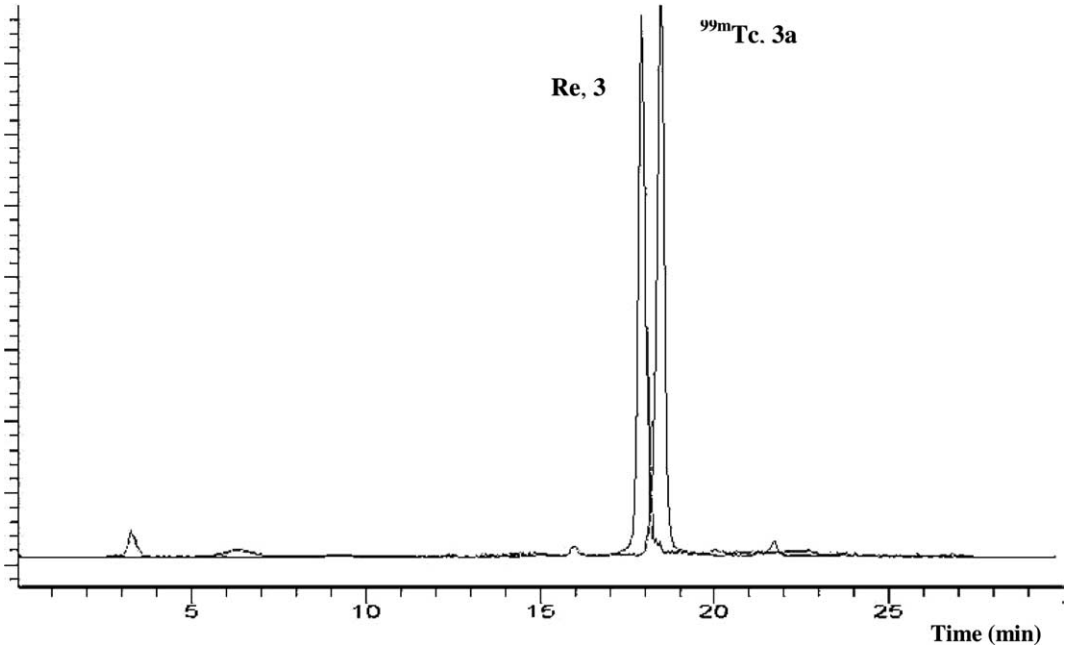


Fig. 3. HPLC trace of complex **3** (254 nm) and γ-trace of the radioactive congener **3a**.

for other thioether containing rhenium(I) complexes [19,32]. All the other metrical parameters of the structure of compound **4** are normal and do not deserve a more exhaustive discussion.

The possibility of preparing at non-carrier added level (^{99m}Tc) representative examples of the complexes with the pyrazole-diamine (***L*^{1}**) and pyrazole dithioether ligands (***L*^{3}** and ***L*^{4}**) has been evaluated. In these studies the identity of the ^{99m}Tc complexes has always been checked by HPLC comparison with the corresponding rhenium complexes.

For L^1, the corresponding radioactive complex *fac*-$[^{99m}Tc(CO)_3(\kappa^3\text{-}L^1)]^+$ (**1a**) (R_t = 18.8 min) was obtained almost quantitatively (>90%) by heating the *fac*-$[^{99m}Tc(H_2O)_3(CO_3)]^+$ precursor at 100 °C in physiological buffer (PBS; pH 7.4) and in the presence of a 10^{-4} M concentration of ***L*^{1}**. Under the same experimental conditions the kinetic of the reaction with the pyrazole-dithioether ligand ***L*^{3}** is less favourable. After boiling for 1 h, the complex *fac*-$[^{99m}Tc(CO)_3(\kappa^3\text{-}L^3)]^+$ (**3a**) (R_t = 18.7 min) was present, but mixed with a significant amount of the unreacted precursor and also with a more hydrophilic impurity (R_t = 15.2 min), which was not identified. However, the radiochemical yield improved considerably when the ***L*^{3}** concentration was increased to 10^{-3} M. Under these conditions, complex **3a** was obtained in almost quantitative yield (>90%) (Fig. 3). The effect of the concentration of ***L*^{3}** in the radiochemical purity of complex **3a** is summarized in Fig. 4.

The labelling of ***L*^{4}** has been also attempted using the reaction conditions optimized for ***L*^{3}** (i.e. $[L^4] = 10^{-3}$ M, 100 °C, 1 h). Using these conditions, the reaction proceeds with formation of *fac*-$[^{99m}Tc(CO)_3(\kappa^3\text{-}L^4)]^+$ (**4a**) (R_t = 17.3 min) but this compound is partially hydrolysed yielding the complex *fac*-$[^{99m}Tc(CO)_3(\kappa^3\text{-}L^5)]^+$ (**5a**) (R_t = 16.8 min).

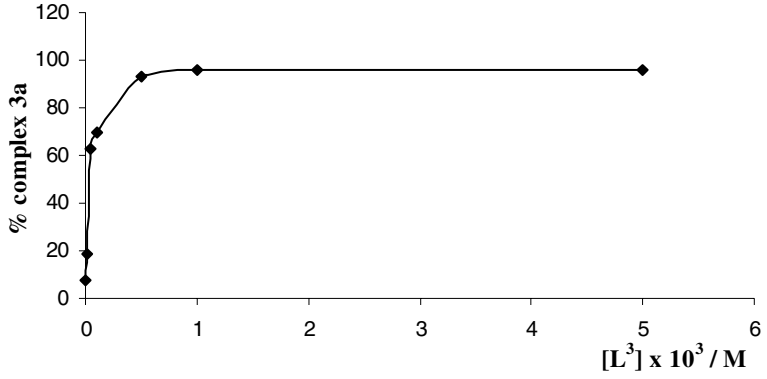


Fig. 4. Radiochemical purity of **3a** versus concentration of ***L*^{3}**.

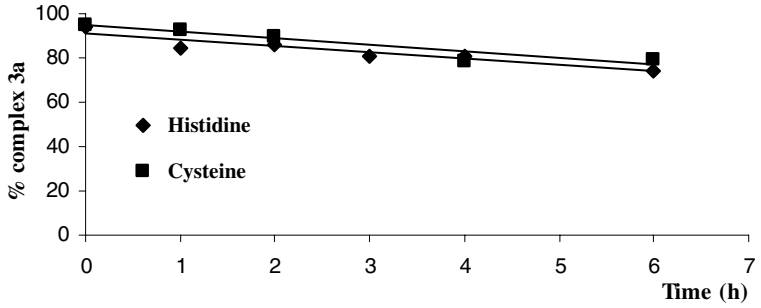


Fig. 5. Stability of **3a** in the presence of histidine and cysteine ([aminoacid]/[L^3] = 100).

The organometallic ^{99m}Tc-complexes **1a** and **3a** were challenged in vitro in PBS buffer and in the presence of a large excess of histidine or cysteine, which display great affinity for the *fac*-[^{99m}Tc(CO)$_3$]$^+$ moiety. In both cases, only a minor decomposition of the complexes could be observed, even for the less stable pyrazole-dithioether complex, **3a**, as can be observed in Fig. 5.

These findings indicate that pyrazole-diamine and pyrazole-dithioether chelators provide a high kinetic inertness and/or stability to organometallic complexes with the *fac*-[^{99m}Tc(CO)$_3$]$^+$ moiety.

4. Concluding remarks

We have synthesized and characterized novel asymmetric bifunctional pyrazolyl containing ligands with N_3 and NS_2 donor atom sets. These compounds react with $(NEt_4)_2[Re(CO)_3Br_3]$ and/or $[Re(CO)_5Br]$ affording cationic complexes of the type *fac*-$[Re(CO)_3(\kappa^3$-L)]. In these complexes the pyrazolyl anchors act as tridentate ligands without any interference of the carboxylate and/or ester functional groups, which stay available for coupling to biological relevant molecules. Preliminary results at non-carrier added level have shown that is possible to synthesize radioactive complexes anchored by these ligands with high yield and high radiochemical purity. However, the pyrazole-diamine ligands allow the synthesis of complexes with higher specific activity than the pyrazole-dithioether ligands.

5. Supplementary material

Crystallographic data (excluding structure factors) for the structure of compound **4** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC-247611. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223/336 033; E-mail: deposit@ccdc.cam.ac.uk].

Acknowledgements

Rute Vitor and Susana Alves thank the Fundação para a Ciência e Tecnologia (National Foundation for Science and Technology) for Ph.D. research grants. This work was partially supported by Mallinckrodt-Tyco Inc.

References

[1] R. Alberto, R. Schibli, A. Egli, P.A. Schubiger, U. Abram, T.A. Kaden, J. Am. Chem. Soc. 120 (1998) 7987.
[2] R. Alberto, K. Ortner, N. Wheatley, R. Schibli, P.A. Schubiger, J. Am. Chem. Soc. 123 (2001) 3135.
[3] R. Alberto, R. Schibli, R. Waibel, U. Abram, P.A. Schubiger, Coord. Chem. Rev. 190–192 (1999) 901.
[4] R. Schibli, R. Schwarzbach, R. Alberto, K. Ortner, H. Schmalle, C. Dumas, A. Egli, P.A. Schubiger, Bioconjug. Chem. 13 (2002) 750.
[5] R. Schibli, R. La Bella, R. Alberto, E.G. Garayoa, K. Ortner, U. Abram, P.A. Schubiger, Bioconjug. Chem. 11 (2000) 345.
[6] J.K. Pak, P. Benny, B. Spingler, K. Ortner, R. Alberto, Chem. Eur. J. 9 (2003) 2053.
[7] S.R. Banerjee, M.K. Levadala, N. Lazarova, L. Wei, J.F. Valliant, K.A. Stephenson, J.W. Babich, K.P. Maresca, J. Zubieta, Inorg. Chem. 41 (2002) 6417.
[8] S. Seifert, J.-U. Künstler, A. Gupta, H. Funke, T. Reich, H.-J. Pietzsch, R. Alberto, B. Johanssen, Inorg. Chim. Acta 322 (2001) 79.
[9] R. Garcia, A. Paulo, A. Domingos, I. Santos, K. Ortner, R. Alberto, J. Am. Chem. Soc. 122 (2000) 11240.
[10] R. Garcia, A. Paulo, A. Domingos, I. Santos, J. Organomet. Chem. 632 (2001) 41.
[11] R. Garcia, A. Paulo, A. Domingos, I. Santos, R. Alberto, Inorg. Chem. 41 (2002) 2422.
[12] R. Schibli, K.V. Katti, W.A. Volkert, C.L. Barnes, Inorg. Chem. 40 (2001) 2358.
[13] K.V. Katti, K. Raghuraman, N. Pillarsetty, W.A. Volkert, S. Jurisson, T.J. Hoffman, in: M. Nicolini, U. Mazzi (Eds.), Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine, 6, SGE Editoriali, Padova, Italy, 2002, pp. 69–72.
[14] J.D.G. Correia, A. Domingos, I. Santos, R. Alberto, K. Ortner, Inorg. Chem. 40 (2001) 5147.
[15] T. Kniess, J.D.G. Correia, A. Domingos, E. Palma, I. Santos, Inorg. Chem. 42 (2003) 6130.
[16] R. Schibli, P.A. Schubiger, Eur. J. Nucl. Med. 29 (2002) 1529, and references therein.

[17] S.M. Okarvi, Med. Res. Rev. 24 (2004) 357, and references therein.
[18] (a) J. Petrig, R. Schibli, C. Dumas, R. Alberto, P.A. Schubiger, Chem. Eur. J. 7 (2001) 1868;
(b) F. Buchegger, F. Bonvin, M. Kosinki, A.O. Schaffland, J. Prior, J.C. Reubi, P. Blauenstein, D. Tourwé, E.G. Garayoa, A.B. Delaloye, J. Nucl. Med. 44 (2003) 1649.
[19] (a) S. Alves, A. Paulo, J.D.G. Correia, A. Domingos, I. Santos, J. Chem. Soc., Dalton Trans. 24 (2002) 4714;
(b) S. Alves, A. Paulo, J.D.G. Correia, L. Gano, I. Santos, in: M. Nicolini, U. Mazzi (Eds.), Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine, vol. 6, SGE Editoriali, Padova, Italy, 2002, pp. 139–141.
[20] S. Alves, A. Paulo, J.D.G. Correia, L. Gano, I. Santos (submitted).
[21] S.P. Schmidt, W.C. Trogler, F. Basolo, Inorg. Synth. 28 (1990) 160.
[22] R. Alberto, A. Egli, U. Abram, K. Hegetschweiler, V. Gramlich, P.A. Schubiger, J. Chem. Soc., Dalton Trans. (1994) 2815.
[23] A.P. Krapcho, C.S. Kuell, Synth. Commun. 20 (1990) 2559.
[24] W. Holzer, G. Seiringer, J. Heterocyclic Chem. 30 (1993) 865.
[25] D.P. Shrout, D.A. Lightner, Synth. Commun. 20 (1990) 2075.
[26] J. Garcia-Antón, J. Pons, X. Solans, Mercè Font-Bardia, Josep Ros, Eur. J. Inorg. Chem. (2003) 2992.
[27] C.K. Fair, MOLEN, Enraf-Nonius, Delft, The Netherlands, 1990.
[28] G.M. Sheldrick, SHELXS-97, University of Göttingen, Germany, 1997.
[29] G.M. Sheldrick, SHELXL-97, University of Göttingen, Germany, 1997.
[30] L.J. Farrugia, Appl. Cryst. 30 (1997) 565.
[31] O. Mitsunobu, Synthesis (1981) 1.
[32] H.-J. Pietzsch, A. Gupta, M. Reisgys, A. Drews, S. Seifert, R. Syhre, H. Spies, R. Alberto, U. Abram, P.A. Schubiger, B. Johannssen, Bioconjug. Chem. 11 (2000) 414.