Aqueous preparation and physiological stability studies of $Re(CO)_3$(tripodal) compounds

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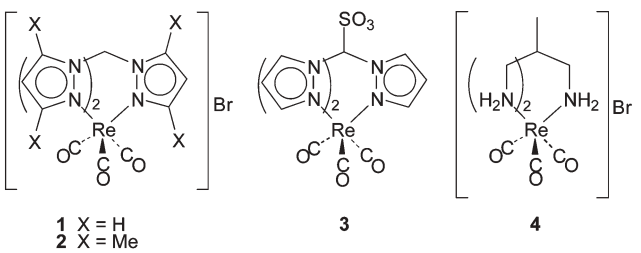
Received (in Berkeley, CA, USA) 20th June 2006, Accepted 4th August 2006
First published as an Advance Article on the web 23rd August 2006
DOI: 10.1039/b608683g

$Re(CO)_3L$ compounds, where L is a methane-derivatized tripodal ligand, can be prepared under aqueous conditions, and one of which displays significant stability under physiological conditions.

Diagnostic imaging using radiopharmaceuticals is an essential tool for managing and treating many common diseases, including heart disease and cancer. ^{99m}Tc is the most important nuclide for diagnostic imaging, with over 7 million scans performed each year.[1] The first generation compounds were ^{99m}Tc-essential, with the archetype being Cardiolite® ($Tc(CNR)_6^+$, R = CH_2CMe_2OMe).[2] The next generation of ^{99m}Tc radiopharmaceuticals will use a bifunctional chelating agent (BFCA) to couple the radionuclide core to a biomolecule that targets a specific receptor or biological process,[1] ensuring that the radiotracer ends up localized at the desired point of imaging to provide superior images. Now that $^{99m}Tc(CO)_3(H_2O)_3^+$ can be readily prepared in aqueous solution from $^{99m}TcO_4^-$,[3] target-specific $^{99m}Tc(CO)_3L$ complexes form a compelling research objective. Cold rhenium congeners are attractive ways to pursue exploratory tests on promising candidates.

We were drawn to examine complexes of tris(pyrazolyl)methane and related neutral tripodal ligands (see Scheme 1) as models of BFCAs, based on several favorable factors. Amine and N-heterocyclic amine donor groups, incorporated into linear tridentate ligands (L), form $M(CO)_3L$ (M = ^{99m}Tc, Re) compounds, which are frequently stable to incubation under physiological conditions and also to *in vivo* injection.[4] Related ligands with C_3 symmetry are attractive because of their ease of synthesis, crystallinity and variety of available derivatives.



Scheme 1 The structures of the compounds discussed in this paper.

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Compounds **1** and **2** have high reported decomposition temperatures of 310 and 250 °C, respectively.[5] Furthermore, the resulting ionic compounds would have solubility advantages in water.

Compounds **1** and **2** were prepared as previously reported.[5] A 4 hour reflux of $Re(CO)_5Br$ in methanol with 1 equivalent of lithium tris(pyrazolyl)sulfonate[6] or TAME (1,1,1-tris(aminomethyl)ethane, purchased from Fluka) gave new compounds **3** and **4** in 55 and 92% yields, respectively.† The compounds can be stored in air indefinitely. The crystal structures of **2–4** (Fig. 1) were determined and compared with the previously determined structure of **1**.‡[7] Compounds **2** and **3** each display approximately C_{3v} symmetry. Compound **2** resides on a crystallographic mirror plane. Compound **3** is a zwitterion. The similar S–O bond distances average 1.44 Å, demonstrating the delocalization of the negative charge. Compound **4** shows a lowered approximate C_3 symmetry for the cation due to a torsional twist of the three methylene groups by an average of 14°. This is likely to be a consequence of the increased flexibility of this ligand. The Re–C and Re–N bond distances, and the various angles around the rhenium metal center are unexceptional. The Re–N bond distances for **2** and **3** average 2.17 Å. This is essentially equal to the average value of 2.18 Å observed for **1**[7] but shorter than the average value of 2.22 Å observed for **4**. The shortening is consistent with the relative p-character of sp^2 *vs.* sp^3 nitrogen atoms.

To test their syntheses in aqueous conditions, we prepared the compounds by a 1 hour reflux of $[Re(CO)_3(H_2O)_3]Br$[8] with 1 equivalent of the appropriate ligand in H_2O under nitrogen. Compounds **1–4** were the only organometallic products. Unoptimized yields were greater than 50% for each compound. Successful preparation in water is a crucial result because, ultimately, imaging agents will be prepared in a therapeutic setting in aqueous conditions prior to administration to patients.

Another critical requirement for a therapeutic compound is that, while it is in the body, it withstands physiological challenges without decomposition. Typically ^{99m}Tc radiopharmaceuticals clear the body within 24 hours. This requirement was tested by incubating compounds **1–4** under conditions that mimic the physiological environment. Each compound was exposed to 0.030 M solutions of either histidine methyl ester (histidine-OMe) or cysteine in d_6-DMSO at 37 °C.§ The order of kinetic inertness to these conditions was found to be **4** ≫ **2** > **3** > **1** (Table 1). Of special note, **4** was unchanged after 24 hours in the presence of either histidine methyl ester or cysteine and showed less than 10% decomposition after 1 month in both experiments. **1–3** each showed some resistance to decomposition after 24 hours. In contrast, $TpRe(CO)_3$ (Tp^- = tris(pyrazolyl)borate) and related

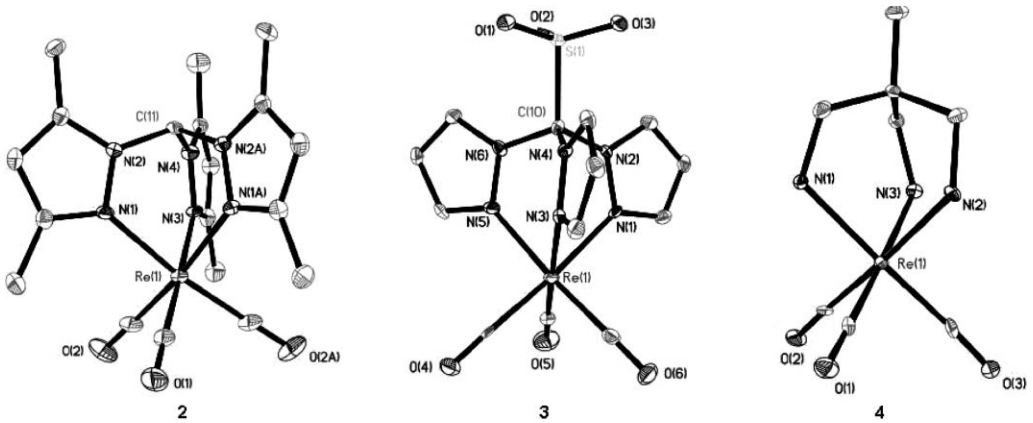

Fig. 1 Molecular structures of **2–4** (cations only) showing 50% thermal ellipsoids. Hydrogen atoms have been omitted for clarity. The A-affixed atoms in **2** were generated by crystallographic mirror symmetry.

Table 1 IR and exchange data summary

Compound	ν(CO)/cm^{-1} [a]	Exchange (*i.e.* % decomposition after 24 h)[b,c]
1	2034, 1912	5(5) / 45(5)
2	2018, 1886(sh), 1858	10(5) / 10(5)
3	2032, 1914	5(5) / 20(5)
4	2018, 1888(sh), 1853	0(5) / 0(5)

[a] Neat solid. [b] Cysteine / histidine-OMe. [c] Values in parentheses are the percentage error.

compounds decompose in water.[9] The extra inertness displayed by **4** presumably results from the increased basicity of amines relative to pyrazole. The observed robust behavior of the four compounds in the exchange experiments suggests that ligands utilizing a tris(amino) chelating scaffold will be more likely to produce promising candidates for use in imaging than tris(pyrazolyl) ligands.

The positions of the ν(CO) bands in the IR spectra of compounds **1–4** are shown in Table 1. Compounds **1** and **3** have virtually identical spectra, showing that the sulfonyl group on the apical carbon in **3** does not significantly alter the CO stretching compared to **1**. As expected, **2**, with the more electron donating ligand, shows lower energy carbonyl stretches than **1** or **3**. Compound **4** has a spectrum similar to **2**. The dramatically different inertness of **4** *vs.* **2** in the exchange experiments suggests that comparisons of the ν(CO) stretching frequencies between different ligand classes do not predict the resistance to decomposition.

In conclusion, the aqueous synthesis of four rhenium tricarbonyl tripodal compounds, including two new compounds, are reported. Exchange experiments, through incubation under physiological conditions, are promising for **4** and suggest further testing of congeners of this ligand as a BFCA in $^{99m}Tc(CO)_3L$ compounds. This work, as well as studies to identify other tripodal ligands as BFCAs, is in progress.

C. J. Z. and R. S. H. acknowledge the Petroleum Research Fund (PRF# 39625-G5M). R. S. H. acknowledges the Simeon J. Fortin Charitable Trust and the Research Corporation. C. J. Z. acknowledges the University of Akron for a faculty research grant (FRG-1565). We also wish to acknowledge NSF grant CHE-0116041 for funds to purchase the Bruker Nonius diffractometer.

Notes and references

† *Characterization data for new compounds*. **3**: m.p. = 254.7–257.7 °C. Clear crystals of **3** were obtained by vapor diffusion of ether into acetonitrile. Anal. found: C, 27.77; H, 1.64; N, 14.51. $C_{13}H_9N_6O_6S$ requires C, 27.71; H, 1.61; N, 14.90%. ^{1}H NMR (d_6-DMSO): δ 8.87, 8.63 (m, m, 3 H, 3 H, 3,5-*H* pz) and 6.71 (m, 3 H, 4-*H*, pz). **4**: m.p. = 277 °C (decomposes). Clear crystals of **4** were obtained by vapor diffusion of $CHCl_3$ into CH_3OH. Anal. found: C, 20.39; H, 3.51; N, 8.79. $C_8H_{15}N_3O_3BrRe$ requires C, 20.56; H, 3.24; N 8.99%. ^{1}H NMR (D_2O): δ 4.43 (m, 2 H, NH_2), 2.53 (t, ^3J = 6.2 Hz, 6 H, CH_2) and 0.56 (s, 3 H, CH_3).

‡ *Crystallographic data*. Data was collected at 100 K (Bruker KRYO-FLEX) on a Bruker SMART APEX CCD-based X-ray diffractometer. Crystallographic summary for **2**: $ReC_{19}H_{22}N_6O_3Br$, M = 648.54, colorless block 0.20 × 0.20 × 0.10 mm, orthorhombic, space group *Pnma*, Z = 4, a = 11.9161(18), b = 11.8438(18), c = 15.996(2) Å, α = 90, β = 90, γ = 90°, V = 2257.5(6) Å^3, D_c = 1.908 Mg m^{-3}, μ(Mo-Kα) = 7.184 mm^{-1}, F(000) = 1248, final R-indices on 2886 independent reflections [$I > 2\sigma(I)$]: R_1 = 0.0277, wR_2 = 0.0536, CCDC 610271. Crystallographic summary for **3**: $ReC_{13}H_9N_6O_6S$, M = 563.52, colorless needle 0.50 × 0.10 × 0.10 mm, monoclinic, space group *P*2(1)/*n*, Z = 4, a = 8.8316(17), b = 8.9876(17), c = 20.941(4) Å, α = 90, β = 96.909(3), γ = 90°, V = 1650.1(5) Å^3, D_c = 2.268 Mg m^{-3}, μ(Mo-Kα) = 7.539 mm^{-1}, F(000) = 1072, final R-indices on 3962 independent reflections [$I > 2\sigma(I)$]: R_1 = 0.0399, wR_2 = 0.0748, CCDC 610272. Crystallographic summary for **4**·CH_3OH: $ReC_9H_{19}N_3O_4Br$, M = 499.38, colorless block 0.50 × 0.50 × 0.20 mm, monoclinic, space group *P*2(1)/*c*, Z = 4, a = 6.7916(9), b = 13.0578(16), c = 17.449(2) Å, α = 90, β = 98.273(2), γ = 90°, V = 1531.4(3) Å^3, D_c = 2.166 Mg m^{-3}, μ(Mo-Kα) = 10.554 mm^{-1}, F(000) = 944, final R-indices on 3596 independent reflections [$I > 2\sigma(I)$]: R_1 = 0.0580, wR_2 = 0.1528, CCDC 610273. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b608683g

§ *Exchange experiments* were performed with a known concentration of cysteine or histidine-OMe and the appropriate metal compound in d_6-DMSO (ferrocene internal reference). ^{1}H NMR spectroscopy was used to monitor the metal complexes at 37 °C.

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