# One and two photon fluorescent complexes of rhenium and their technetium analogues $\ensuremath{\dagger}$

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The synthesis of rhenium and 99m-technetium complexes of dipyrazolemethanecarboxylates directly from the tetroxometallates is reported together with a 'one pot' synthesis of mixed ligand M(v) complexes. The rhenium complexes in all three oxidation states show strong fluorescence when the pyrazole groups have aromatic substituents with emission around 330 nm with both one and two photon excitation. The emission lifetimes of the complexes and free ligands differ considerably.

Although metallic complexes of PET and SPECT radioisotopes give valuable diagnostic imaging information at 1-4 mm resolution, little is known on how these complexes behave at the cellular level. This has prompted the search for ligands that would not only bind metallic radionuclides but also have intrinsic fluorescence to open up the possibility of utilizing confocal fluorescence microscopy to track the fate of complexes in cells. Thus far the only complexes of rhenium and technetium described in this context in the literature have involved the tricarbonyl core and only one photon fluorescence has been described.<sup>1</sup> However Re(v) and Tc(v) are the dominant oxidation states in current radiopharmaceuticals and we here describe the simple 'one-pot' synthesis of new oneand two-photon fluorescent complexes of oxidation states (I) and (v) and also for Re(III). The results of 99m-Tc radiolabelling and stability studies are also presented.

We have previously described the synthesis of Re(v) and Re(III) complexes of bis(pyrazole)acetic acid ( $\text{HL}^{1-3}$ ) ligands (Fig. 1) from [ReOCl<sub>3</sub>(PPh<sub>3</sub>)<sub>2</sub>].<sup>2</sup> Significantly for potential radiopharmaceutical applications we now report that these complexes can also be synthesized directly from perrhenate. Reaction of [ReO<sub>4</sub>]<sup>-</sup> with SnCl<sub>2</sub> in ethanol in the presence of HCl and subsequent addition of **HL** at room temperature gave the turquoise blue complexes



Fig. 1 Structures of ligands HL<sup>1-3</sup>.

 $[ReOCl_2(L)] \text{ (complexes 1-3) in good yield. These were identical in all respects to those prepared earlier from [ReOCl_3(PPh_3)_2]. HPLC studies showed that the complexes underwent less than 10% decomposition on solution in aqueous DMF/PBS buffer in the presence of excess cysteine over 4 h.}$ 

[ReOCl<sub>2</sub>L<sup>1</sup>] (1) can also be reacted with dimercaptosuccinic acid (dmsaH<sub>2</sub>) to give the complex [ReO(dmsa)(L<sup>1</sup>)] **4**. The use of the carboxylate groups in [ReO(dmsa)<sub>2</sub>]<sup>-</sup> to conjugate a salmon calcitonin *via* an anhydride intermediate has been reported<sup>3</sup> and the carboxylates in **4** could be used similarly to target the complexes. The 'one-pot' synthesis of complex **4** is achieved directly from perrhenate in acetonitrile by reaction with one equivalent of dmsaH<sub>2</sub> in the presence of acetylhydrazine as reductant and subsequent addition of one equivalent of **HL**<sup>1</sup>.

For the complex [ReO(dmsa)(L<sup>1</sup>)] a strong IR band at 966 cm<sup>-1</sup> was assigned to the Re=O stretching frequency, close to the literature values for Re(v) oxo complexes.<sup>4</sup> ESMS(+) showed the mass ion (100%) at m/z = 574.9911 (calculated for [ReO(dmsa)(L<sup>1</sup>)] 574.9702). The <sup>1</sup>H NMR showed the expected peaks for the protons of L<sup>1</sup> and dmsa with appropriate integrated ratios. The protons of the equivalent CH groups of the pyrazole substituents of L<sup>1</sup> (R in Fig. 1) and the CH groups of the of the backbone dmsa ligand were split into two singlets with a ratio 1:2. This splitting was assigned to the presence of two possible geometric isomers involving the orientation of the carboxyl groups relative to the Re=O group (Fig. 2). Analogous isomers are observed for [ReO(dmsa)<sub>2</sub>]<sup>-</sup>.<sup>5</sup> HPLC in methanol showed a single peak with a retention time of 1.8 min, with a shoulder at *ca.* 1.6 min, these peaks being assigned to the two isomers observed in the <sup>1</sup>H NMR.



Fig. 2 Structures of isomers of  $[ReO(dmsa)L^1]$  4.

Rhenium(I) tricarbonyl complexes of simple bis(pyrazolyl)methane carboxylate ligands have been reported previously.<sup>6</sup> We have now extended the series to include a complex of a ligand bearing aryl groups *i.e.*  $[Re(L^3)(CO)_3]$  **5**. The molecular structure of the complex  $[Re(L^3)(CO)_3]$  **5** (ESI†) with the bulky arylated ligand has not been reported before and shows that planes of the aryl groups lie as expected almost orthogonal to the planes of the pyrazole rings with the 2-methoxy substituents directed away from the metal.

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<sup>&</sup>lt;sup>†</sup> Electronic supplementary information (ESI) available: Syntheses and characterization data for complexes **4**, **5**. X-Ray crystallographic data for complex **5**. Radiolabeling studies for complexes **6-8**. CCDC reference numbers 764250. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b927506a

Compound	Excitation/nm	Emission/nm
L <sup>1</sup> H	355	445 w
L <sup>3</sup> H	270	335 s
$[\text{ReOCl}_2(L^1)]$ 1	357	445 w
$[\operatorname{ReOCl}_2(\mathrm{L}^3)]$ 3	290	335 s
	580	335 s
$[\text{Re}(\text{L}^{3})(\text{CO})_{3}]$ 5	290	335 s
$[Re(NNPh)Cl(L^3)(PPh_3)]$ 9	290	339 m
$[Re(NNC_4H_4OMe-4)Cl(L^3)(PPh_3)] 10$	290	339 s

The <sup>99m</sup>Tc analogues of complexes 1 and 3,  $[TcOCl_2(L^{1,3})]$  6 and 7 respectively were obtained using a standard glucoheptonate kit. The radio HPLC trace showed for 7 showed a predominant peak at 19.60 min (82%) with a smaller peak at 2.9 min due to  $[^{99m}$ TcO<sub>4</sub>]<sup>-</sup>. The corresponding Re complex 3 showed a peak (UV detection) at 19.70 min under the same conditions confining the identity of complex 7. Stability studies in aqueous medium of the <sup>99m</sup>Tc complex 7 by HPLC showed lower stability than 3 and only 40% of the complex remained after 4 h and the parallel increase in the pertechnetate peak suggested hydrolysis to the tetroxometallate. The <sup>99m</sup>Tc analogue of 5 [<sup>99m</sup>Tc(CO)<sub>3</sub>(L<sup>3</sup>)] 8 was obtained in better than 95% radiochemical yield by reaction of  $[^{99m}Tc(CO)_3(H_2O)]^+$  with HL<sup>3</sup>. The radio HPLC showed a single peak at 19.42 min compared with 19.83 min for [Re(CO)<sub>3</sub>L<sup>3</sup>] 5 under the same conditions and 8 showed good stability in serum. To our knowledge these are the first reported examples of technetium complexes of this class of ligand.

#### One- and two-photon fluorescence studies

The emission and absorption spectroscopic data for complexes **1–3** and **5–6** were investigated in DMSO solution using one- or two- photon excitation and representative data is summarized in Table 1. The diazenido complexes [Re(NNPh)Cl(L<sup>3</sup>)(PPh<sub>3</sub>)] **9** and [Re(NNC<sub>4</sub>H<sub>4</sub>OMe-4)Cl(L<sup>3</sup>)(PPh<sub>3</sub>)] **10** were prepared as previously described in a two-step reaction from perrhenate.<sup>7</sup>

The emissive properties of tris(pyrazolyl)borates have been studied in the solid state and solution and show a weak emission at 450 nm assigned to an intraligand  $\pi$ - $\pi$ \* transition. An Ag(I) complex shows similar luminescence.8 However there are no reports on the emission spectra of pyrazolylmethane derivatives. Complex 1 of the unsubstituted pyrazolate carboxylate ligand HL<sup>1</sup> shows only weak fluorescence with emission at 436 nm following excitation at 357 nm. The introduction of the phenyl or 2-methoxy-phenyl substituents at the 3- and 5-positions of the pyrazole in HL<sup>2</sup> and HL<sup>3</sup> causes a large increase in fluorescence emission intensity with emission at 337 nm compared to the weak emission at 445 nm for the unsubstituted ligand. The fluorescence of complex 3 was virtually identical to that of the free ligand HL<sup>3</sup>. However, complex 3 also shows strong fluorescent emission at 340 nm when 2-photon excited at 590 nm. The formally Re(III) diazenido-complexes 9 and 10 are also fluorescent when excited at 290 nm and the introduction of the 4-MeO substituent on the diazenide ligand causes a significant increase in the intensity of fluorescent emission. Finally the Re(I) tricarbonyl complex 5 shows virtually the same fluorescence behavior. This is in contrast to other Re(I) carbonyl complexes where excitation within a charge transfer band causes emission at relatively long wavelengths and two-photon fluorescence has not been studied.1 The fluorescence of these ligand systems is clearly retained on coordination to Re(v) (d<sup>2</sup>), Re(III) (d<sup>4</sup>) and Re(I) (d<sup>6</sup>) diamagnetic cores and emission is ligand rather than metal based. The complex [ $ReOCl_2L^3$ ] has a different relaxation (with lifetimes 350 and 1724 ps) to the free ligand (with lifetimes of 134 and 1372 ps). This suggests that it might be possible to use emission lifetimes to distinguish free ligand from complex and therefore assess the degree of dissociation of a complex *in vitro*. The short observed emission lifetimes also indicate that the emission from these complexes is fluorescence.

### Conclusions

Examples of Re(v) or Re(VII) complexes which are luminescent or fluorescent in solution are rare, being confined to [ReN(MeCN)(Ph<sub>2</sub>PCH<sub>2</sub>CH<sub>2</sub>PPh<sub>2</sub>)<sub>2</sub>]<sup>2+</sup> (emission at 517 nm, d–d transition)<sup>9</sup> and [ReO<sub>3</sub>(8-hydroxyquinolate)] (emission 527 nm).<sup>10</sup> and complexes of the types [ReO<sub>2</sub>(pyridine)<sub>4</sub>]<sup>+</sup> and [ReO<sub>2</sub>(CN)<sub>4</sub>]<sup>3-</sup> which give rise to luminescent emissions at around 650 nm.<sup>11</sup> There are as far as we are aware no reported examples of fluorescent Re(III) complexes or indeed any complexes of rhenium which show 2-photon fluorescence. The use of 2-photon confocal microscopy *in vitro* offers significant potential advantages in terms of greater tissue penetration at longer excitation wavelengths and less photochemical damage. Details of *in vitro* one and two photon fluorescence and emission lifetime studies of these Re complexes and of fluorescent complexes of analogous ligands with Ga and In will be reported elsewhere.

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