Platinum trimethyl bipyridyl thiolates – new, tunable, red- to near IR emitting luminophores for bioimaging applications†

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Synthetic, spectroscopic, computational and biological imaging studies of platinum trimethyl bipyridyl thiolate complexes of the general formula \([\text{PtMe}_3(bpy)SR]\) reveal these to be easily accessed, tunable bioimaging agents which feature an unusual \(\sigma-\pi^*\) Inter-Ligand Charge Transfer (ILCT) transition, and in some cases emit into the Near infra-red (NIR).

Platinum(IV) trimethyl iodide exists as a cubic tetramer which reacts with a large range of ligands to give stable octahedral complexes of the general formula \([\text{fac-PtMe}_3(L)_2]\) which have been extensively studied. 1 In the case of chelating bis-heterocyclic ligands such as bipyridine, the derived complexes, e.g. \([\text{fac-PtMe}_3(N^N)I]\) 1, are luminescent, showing room temperature phosphorescence from excited states assigned as \(3L \pi-\pi^*\). 1 The photochemistry of these units has been widely explored, with photo-reduction to the square-planar Pt(II) species typically observed, 2 but there are few applications of Pt(IV)Me 3 complexes in luminescence, while there is much data on cyclo-metallated Pt(II) complexes as lumophores. 3–5 The analogous \([\text{fac-Re(CO)}_3(N^N)L]\) complexes are widely applied lumophores 6 in which systematic variations in the \((N^N)\) unit tune the absorption and emission characteristics, while variations in L (usually substituted pyridines) control solubility and other physical characteristics. 7 It is generally the case that substituted complexes, in which L is a nitrogen heterocycle, have attractive photophysical properties and stability while the precursor halido-complexes are unstable and tend to be toxic in biological work due to halide lability. 8 Therefore, an investigation was undertaken of the synthesis and photophysical characteristics of a series of \([\text{fac-PtMe}_3(L)]\) complexes of chelating and monodentate ligands. Reaction of PtMe 3 with substituted bipyridines gave analogues of 1 with electron donating (OMe, complex 2) and withdrawing (CO 2 Me, complex 3) groups in the 4,4 0 positions (Scheme 1).

These complexes showed the respective blue- and red-shifted absorptions and emissions which would be predicted from the electronic structures (Table 1). Complexes of the general formula \([\text{fac-PtMe}_3(bpy)L]\), where L is a substituted pyridine, were easily synthesised by the reaction of 1 with the appropriate pyridine in the presence of KPF 6 to give complexes 4–6 (Scheme 1). However, the yellow colour of 1 had been lost, and while these complexes were luminescent (see Table 1) UV excitation was required. Reaction of 1 with triphenyl phosphine or aniline gave complexes 7 and 8, but these complexes too required u.v. excitation. As it seemed that substituting the iodide led to loss of the low energy band responsible for visible absorption and excitation,
### Table 1 Photophysical data of complexes 1–10

<table>
<thead>
<tr>
<th>Complex</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt; (nm)</th>
<th>Quantum yield (Φ&lt;sup&gt;em&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>372</td>
<td>4.1 × 10&lt;sup&gt;−4&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>280, 289, 299</td>
<td>1.3 × 10&lt;sup&gt;−2&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>300, 314, 297</td>
<td>3.7 × 10&lt;sup&gt;−2&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>300, 314</td>
<td>2.6 × 10&lt;sup&gt;−2&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>300, 310</td>
<td>3.0 × 10&lt;sup&gt;−2&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>290, 300, 310</td>
<td>4.1 × 10&lt;sup&gt;−4&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>303, 313</td>
<td>1.3 × 10&lt;sup&gt;−3&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>290, 298, 310</td>
<td>3.7 × 10&lt;sup&gt;−2&lt;/sup&gt;</td>
</tr>
<tr>
<td>9</td>
<td>301, 308, 358</td>
<td>4.1 × 10&lt;sup&gt;−4&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>310, 350</td>
<td>3.7 × 10&lt;sup&gt;−2&lt;/sup&gt;</td>
</tr>
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* Strongest bands > 275 nm.  † Lowest energy band/shoulder.  + Measurements performed irradiating into ILCT band for each complex in aerated acetonitrile solutions, using [Ru(bpy)]<sub>2</sub>(PF<sub>6</sub>)<sub>2</sub> as a standard (Φ<sub>em</sub> = 0.018)<sup>−2</sup>.  ‡ ILCT band absent.

a A re-examination of the nature of this band was required. It is not clear why substitution of iodide for N- and P-donor ligands leads to complete loss of this transition, which has previously been assigned as a formally disallowed <sup>3</sup>IL π−π* transition.<sup>1</sup>

We therefore carried out time-dependent density functional theory (TD-DFT)<sup>9–16</sup> calculations on 1 and 4 to compare the absorption bands in these species in detail (see ESI† for details). This predicts absorption bands for 1 at 358 and 294 nm, in reasonable agreement with the experimental values of 344, 308 and 297 nm. In contrast, 4 is predicted to absorb at 294 and 284 nm (experimental values 311 and 300 nm). The band at 358 nm in 1 corresponds to excitation from an orbital made up largely of a Pt–I σ-bond, along with a lesser amount of trans-Pt–Me, to the π* MO on bpy, as illustrated in Fig. 1. Hence this low energy band is best described as an Inter-Ligand Charge Transfer rather than either <sup>3</sup>IL π−π* or MLCT. ILCT between halogen and bipyridine antibonding orbitals is also seen admixed with MLCT in the analogous Re complexes,<sup>6</sup> which occasionally may cause problems in biological studies due to halide lability leading to interaction of the heavy metal centre with biomolecules (e.g. DNA).<sup>18</sup> These results therefore call into question the literature assignment, but do explain the change in absorption patterns between 1 and 4, since the latter does not contain a low energy orbital of the correct symmetry to reproduce such absorption. They also suggest a strategy for combining the desirable properties of visible absorption and kinetic stability in [Pt(Me<sub>2</sub>N<sup>+</sup>N L)]<sub>2</sub> complexes, i.e. to find L that combines a strong Pt–L bond with suitable electronic structure for interaction with bpy π*.

Thiolates satisfy both requirements, so further TD-DFT calculations were performed for [Pt(Me<sub>2</sub>N<sup>+</sup>N L)]<sub>2</sub> (Scheme 2: full data in ESI†), from which we predict an absorption band at 393 nm corresponding to S lone pair/Pt–S σ-bond to bpy π* orbitals (Fig. 1).

Having obtained computational support for the hypothesis that thio-substituted complexes should show low energy absorption, methyl-4-mercaptopbenzoate was selected as a model ligand which would (through variations of the ester) allow the incorporation of a variety of substituents at the sulphur ligand, giving the tuneable lipophilicity etc. which has proven important in the development of metallo-imaging agents. Treatment of 1 with a small excess of methyl-4-mercaptopbenzoate in acetonitrile in the presence of sodium tert-butoxide gave the thiobenzoate-substituted complex 9 (Scheme 2: full data in ESI†), and reaction with 3 gave the ester-substituted analogue 10. Electronic spectroscopy confirmed that 9 exhibits low energy absorption, observed as a shoulder centred at 440 nm, along with higher energy bands around 300/350 nm (TD-DFT predicts absorption at 440 nm due to S LP → π*/S–Pt → π*, Fig. 1). Luminescence spectroscopy indicated that the maximum excitation band was centred at ca. 450 nm, correlating with the likely true maximum of the band observed as a shoulder at 440 nm in the UV-vis spectrum. Exciting at 450 nm gave rise to intense emission as a broad band centred around 660 nm (Fig. 2).

Complex 9 is air- and water stable, resistant to ligand substitution of the coordinated thiolate under physiological...
conditions, and with visible excitation, a Stokes shift of over 200 nm and red emission is an ideal candidate for fluorescence imaging experiments.

Complex 3 bearing electron withdrawing ester substituents was likewise converted to the methyl 4-mercaptobenzoate complex 10 and showed a low energy absorption band centred at ca. 500 nm (DFT prediction 532 nm), and an excitation maximum at 490 nm, (Fig. 2) confirming that the photophysics of these complexes is susceptible to significant variations accessible by ligand variations. However, unexpectedly the emission maximum of 10 was blue-shifted in comparison to that of 9 indicating that simple assumptions regarding substituent effects can be misleading, especially in the case of emission from triplet states where the Stokes shift is a function of energy losses through relaxation into triplet geometries, the magnitude of which cannot be estimated intuitively from electron donating/withdrawing arguments.

As a preliminary assessment of the potential for applications of complexes such as 9, a study of cellular uptake was undertaken by flow cytometry and fluorescence microscopy. This used the well characterised HeLa human cervical carcinoma cell line, and was performed at 0–4 °C to inhibit endocytosis. Flow cytometry detecting between 655–735 nm (Fig. 3, PerCP-Cy5.5), and an excitation maximum at 490 nm, (Fig. 2) confirmed that the photophysics of these complexes is susceptible to significant variations accessible by ligand variations. However, unexpectedly the emission maximum of 10 was blue-shifted in comparison to that of 9 indicating that simple assumptions regarding substituent effects can be misleading, especially in the case of emission from triplet states where the Stokes shift is a function of energy losses through relaxation into triplet geometries, the magnitude of which cannot be estimated intuitively from electron donating/withdrawing arguments.

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Preliminary experiments show that biological applications as fluorescent agents are possible with these complexes. The NIR emission can be used to differentiate agent-based emission from autofluorescence even at low levels of uptake and emission intensity.

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Notes and references

9 M. J. Frisch, et al., Gaussian 09, Revision C. 01, Gaussian, Inc., Wallingford, CT, 2010.