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Simple Polyphenyl Zirconium and Hafnium Metallocene Room-Temperature Lumophores for Cell Imaging

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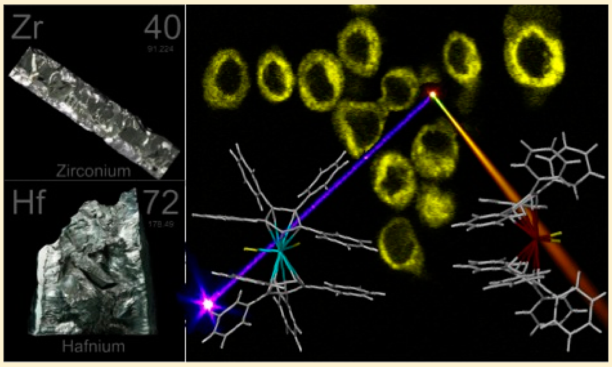
Supporting Information

ABSTRACT: Zirconium and hafnium metallocene dihalides based on simple polyphenyl cyclopentadienes are stable room-temperature lumophores with large Stokes shifts, emitting from states with substantial LMCT character. Their synthesis is described, including by a rapid solvent-free approach compatible with the lifetime of PET-active isotopes (^{89}Zr). Preliminary experiments indicate the viability of these species as lumophores for fluorescence cell-imaging microscopy.



Late transition metals coupled with ligands based around aromatic heterocycles and other conjugated species form an important class of photoactive complexes,[1] which have found a vast range of applications, including photocatalysis,[2] light-emitting devices,[3] photovoltaics,[4] and sensing and imaging.[5] Energy losses through recombination of charge-separated states can be reduced via triplet electronic configurations, which are prevented from relaxing to the ground (singlet) state by spin selection rules[6] and can themselves be accessed by spin−orbit coupling with heavy second- and third-row transition metals (particularly Ru, Ir, Re, and Pt).[7−10] Almost all applications reported to date involve these late transition metals in low oxidation states (0 → 3+) with ligands which can easily be reduced, often in MLCT (or related) processes.[11,12] In contrast, early transition metals in high oxidation states often absorb light through LMCT processes, but these are rarely emissive.[13−17] However, certain metallocene derivatives of the heavier group IV metals emit from ^{3}LMCT states at low temperatures,[18−21] and while these species are underexplored, recently room-temperature emission was reported from a rigid Zr−*ansa*-metallocene dichloride (**1**; Scheme 1).[22] This ground-breaking report from Loukova et al. demonstrated the potential for early-transition-metal lumophores to compete with the better known late-TM species due to its remarkable photophysical properties, with room-temperature quantum yields up to 0.41 and room-temperature lifetimes of up to 17 μs. In addition to their potential for photophysical applications such as luminescent imaging, there are reports of cytotoxicity in group IV metallocene dichlorides,[23−28] and there is a long-lived positron emitting isotope of zirconium, ^{89}Zr ($t_{1/2}$ = 78 h),[29−31] suggesting potential for a bimodal PET/fluorescence imaging agent with integrated therapeutic ability.[32]

The synthesis of the *ansa*-metallocene **1**[22] involves post-coordination modification of ligands, undesirable with radioisotopes. Noting that highly substituted cyclopentadienes may also retard vibrational quenching and thus allow room-temperature emission, following this initial seminal report, we have now explored a series of penta- and tetraphenylcyclopentadienyl Zr and Hf complexes; both are readily available and easily substituted, and their metallocene halides are water-stable, although their syntheses often require long reaction times.[33,34]

Reaction of lithiated tetraphenylcyclopentadiene with $MCl_4(THF)_2$ (M = Zr/Hf) gave the bis(cyclopentadienyl) metallocene dichlorides (Scheme 1), and samples appropriate for X-ray crystallography[35] were grown of both $(Ph_4Cp)_2ZrCl_2$ (**2**) and $(Ph_4Cp)_2HfCl_2$ (**3**) (Figure 1; see the Supporting Information for more details).[36]

The UV−vis spectra of **2** and **3** showed ligand-based (π−π*) transitions between 250 and 350 nm, shifted in comparison to those of the free ligand, and a broad lower-energy band assigned[19,22] as ^{1}LMCT (**2**, 404 nm; **3**, 364 nm; see the Supporting Information) extending into the visible region in each case. Steady-state excitation and emission spectra

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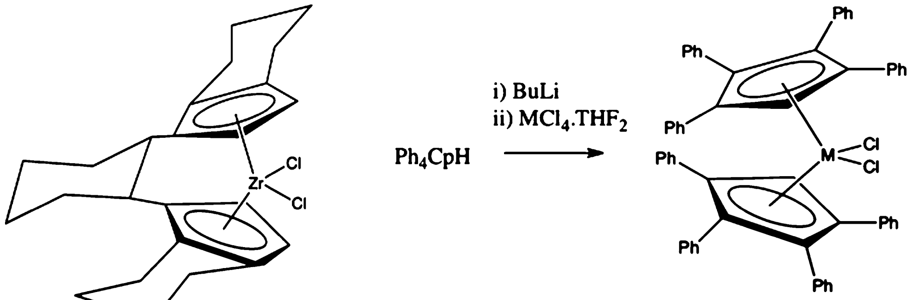
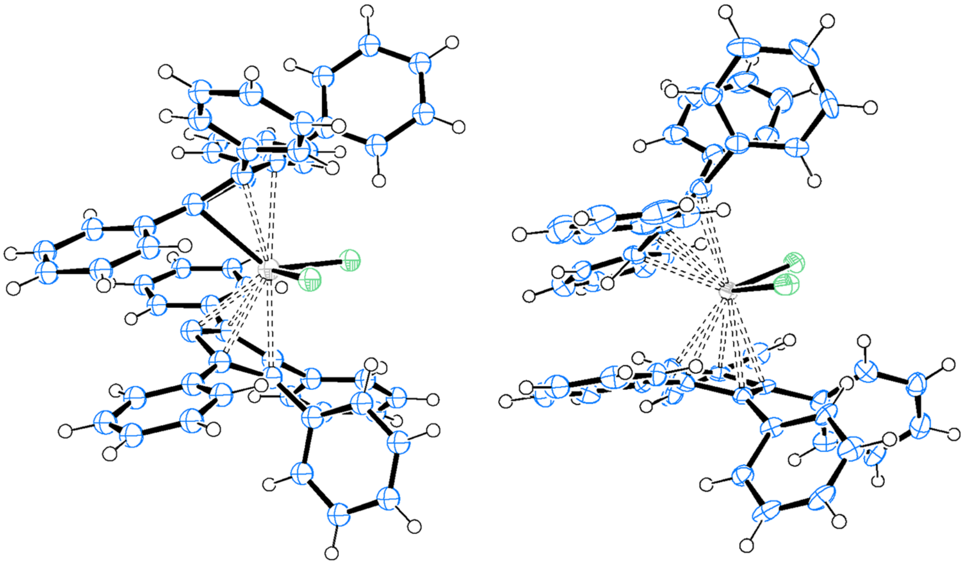
Scheme 1. Structure of 1 and Synthesis of 2 and 3



1

2 M = Zr
3 M = Hf



2 3

Figure 1. Crystal structures of **2** and **3**.

confirmed the desired room-temperature luminescence, with excitation spectra matching the UV−vis spectra, demonstrating that the Zr species **2** is better excited at long wavelengths than the Hf analogue **3** (λ_{max}(excitation) 334 and 269 nm, respectively) (Figure 2). The emission spectra were very similar, with a single broad peak centered at 462 and 445 nm (a Stokes shift of over 100 nm). Time-resolved studies showed that both **2** and **3** had lifetimes too low to be accurately measured, although the lifetimes extended to 5−10 ns in degassed solutions, the extension of lifetime being typical of triplet lumophores due to their sensitivity to quenching by $^3O_2$. $^3O_2$ is the archetypal quencher for triplet charge transfer states of heavy-metal complexes which have been widely used as $^3O_2$ sensors as a result of this behavior.[37] A full description of the procedures for the time-resolved spectroscopy is given in the Supporting Information. Given that the explanation for the long luminescence lifetime of **1** is related to its rigidity, while less rigid simple species such as Cp_2ZrCl_2 are nonemissive unless cooled to reduce vibration, it seems likely that complexes **2** and **3** have long intrinsic lifetimes but suffer from vibrational quenching, which suggests that ligand substitution may offer other simple species with long lifetimes. The quantum yields of these complexes, while low in comparison to organic fluorophores, are moderate to good by comparison with many metallo or organometallic lumophores. The zirconium species **2** has a quantum yield (room-temperature aerated solution) of 0.5% and the hafnium analogue **3** of 1.2%, even higher than for the archetypal $[Ru(bpy)_3]$ dication.

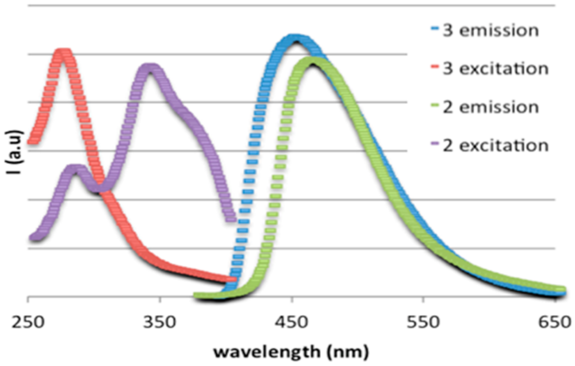


Figure 2. Photophysical behavior of **2** and **3** (room temperature, MeCN).

Tuning photophysics is vital to many applications, and so mixed-ligand systems were examined to see whether only bis-polyphenyl species were emissive and, if not, what the effect of substituting for a simpler ligand would be. Reaction of $CpZrCl_3$ with 1 equiv of lithiated tetraphenylcyclopentadiene gave **4** (Figure 3) in good yield, although this species shows ligand

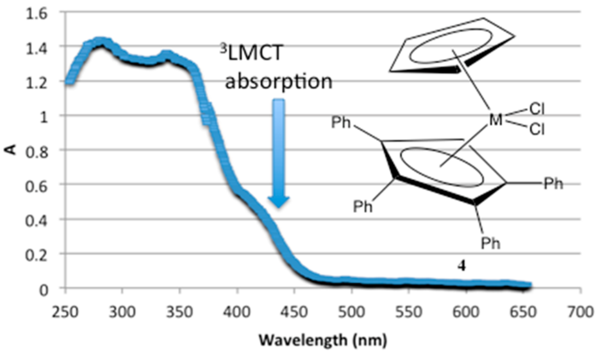


Figure 3. Structure of **4** and UV−vis spectrum.

redistribution at high temperatures to give **2** and Cp_2ZrCl_2 and must be handled with care (Scheme S1, Supporting Information). Interestingly, not only did **4** retain useful luminescence (Figure S2, Supporting Information) but also it showed the most intense absorption at low energy, with the LMCT shoulder extending well into the visible region with reasonable absorption up to 450 nm (Figure 3). Initially it was assumed that the emission would emanate from the substituted Cp ring, so that the effect of the other ligands would be low, and therefore in order to understand why substitution of one of the tetraphenyl-Cp rings with an unsubstituted Cp should have such a marked effect on the photophysics, computational studies were undertaken. As charge transfer species present problems for some DFT methods and Zr and Hf are not represented in standard basis sets, the computational methodology was chosen to reflect these systems (Hybrid DFT with effective core potential (ECP)/basis set) and full details are included in the Supporting Information.

For the simplest model complex Cp_2ZrCl_2, the lowest excited triplet state in simulated CH_3CN was calculated by TD-DFT to be above the ground singlet state at the triplet geometry by 451 nm, which is in excellent agreement with the low-temperature emission reported in the solid state (22120 cm^{-1}, 452 nm)[19] and frozen toluene solution (21800 cm^{-1}, 459 nm), a pure HOMO−LUMO transition.[19] Interestingly, however, the nature of the ground state was less clear than formal oxidation states may suggest, with the HOMO mainly located on Cp π orbitals, but with significant contribution from chlorides and metal d orbitals (Figure S3, Supporting Information).

Assignment of the transition as having predominantly LMCT nature is supported by calculated natural bond orbital (NBO) charges: on Zr these are +0.81 and +0.50 e in the singlet and triplet states, respectively (both at triplet optimum geometry); each Cp has a charge of −0.11 (singlet) and +0.13 (triplet), and chloride charges are −0.30 (singlet) and −0.38 (triplet). Thus, Zr accepts electron density when the complex is excited to the emissive state, meaning that emission involves transfer of density back from the metal to the ligand, which as an emissive process is the direction of charge transfer associated with LMCT (Figure 4). However, as suggested from orbital plots, chloride is also involved in this transition, increasing its negative charge in the triplet state over the singlet state: i.e., charge transfer to the ligand. The NBO electron configuration suggests a more complex picture: the description of Zr in the singlet ground state [core] $4d^{2.26}5p^{0.59}6s^{0.25}$ does not indicate formal zero d orbital population in the ground state. In the triplet state, the NBO configuration of [core] $4d^{2.62}5p^{0.54}6s^{0.12}$ indicates not only charge transfer from Cp into Zr d orbitals but also reduced population of valence s and p orbitals. (Analogous data for Cp_2HfCl_2 (**3**) are given in Table S2 (Supporting Information)).

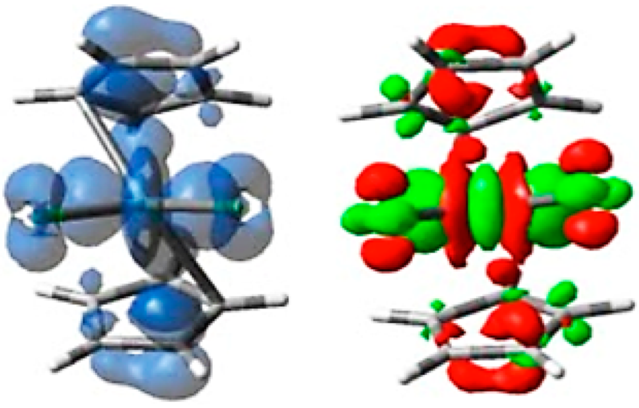


Figure 4. Spin density in the triplet state (left) and difference in density between singlet and triplet states (red denotes more density in the triplet state).

Similar calculations on **2** and **4** were then performed using the same methodology (summarized in Table S1, Supporting Information) and indicate that the HOMO ($CpPh_4$ based) is noticeably higher in energy than in Cp_2ZrCl_2, whereas LUMO (metal-based) energies are barely affected. In free $[CpPh_4]^-$, conjugation lowers the HOMO, whereas in the metal complexes steric crowding apparently prevents efficient overlap between Cp π and metal d orbitals, increasing the HOMO energy (Figure S4, Supporting Information). Calculations on **4** indicate that emission maxima with λ 430−460 nm are pure HOMO−LUMO transitions, and excitation singlet states were found at 359 and 339 nm but not the low-energy shoulder. However, an excited triplet state was calculated to lie at 435 nm relative to the ground state, representing a rare direct ^{3}LMCT absorption, which is formally forbidden but occasionally observed in heavy-metal systems which show strong spin−orbit coupling.[38−41] A spin−orbit coupled TD-DFT calculation using the zeroth-order regular approximation (ZORA) approach found a triplet absorption with low but nonzero intensity ($f = 1.84 \times 10^{-5}$ au) at 439 nm, as well as a more intense singlet band (0.120 au) at 386 nm, both purely HOMO−LUMO transitions, that match the experimental spectra well. Triplet excitations are not observed or calculated for **2** or **3**, demonstrating a remarkable richness in the electronic transitions available in these complexes.

As a very preliminary example of applications of these lumophores, cell imaging studies in MCF-7 human adenocarcinoma cells were undertaken with **4**, as this complex has the best absorption at 405 nm, exploiting the ^{3}LMCT excitation, and imaging studies with metal complexes are now gaining in importance.[42,43] The cell sample showed good uptake (Figure 5A), even though **4** is neutral (many metal complexes show good uptake only as cations),[11] across most of the sample (Figure 5B) and a healthy morphology, indicating low toxicity. **4** shows localization in a range of perinuclear organelles (Figure 5C) (but has not been shown to be specific by colocalization), with little in the nucleus or plasma membranes.

In summary, Zr and Hf complexes of the general formula Cp_2MCl_2, in which at least one of the Cp rings bears aromatic substituents, offer a family of easily accessible room-temperature lumophores which emit from triplet excited states, are air- and water-stable, and offer a new concept in metal-based cell

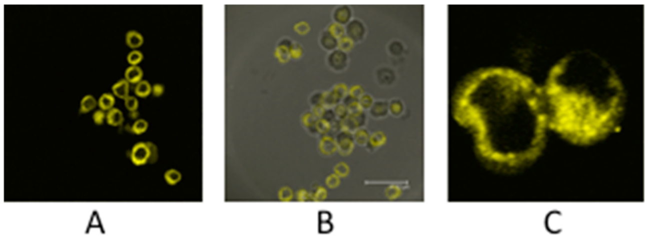


Figure 5. Imaging of MCF-7 human adenocarcenoma cells with **4**.

imaging agents. As they can be accessed rapidly in one step from simple metal salts, they may also find application in bimodal PET/luminescence studies. Beyond the field of biomedical imaging, such stable, charge-separating triplet state lumophores have potential in areas as diverse as OLEDs and photovoltaics.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figures, tables, text, and CIF files giving structural parameters relating to the crystallography, additional photophysical data, and computational details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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