RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Journal Name

ARTICLE

An Efficient Route to Asymmetrically Diconjugated tris(heteroleptic) Complexes of Ru(II)

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Christopher S. Burke [a] and Tia E. Keyes[a]

A highly efficient and versatile route to preparation of tris(heteroleptic) Ru(II) polypyridyl complexes is described which permits access to two or more independently conjugatable termini in the final structure. The strategy utilizes the well-known $Ru(DMSO)_4Cl_2$ precursor to form the $Ru(N^N)(DMSO)_2Cl_2$ product and then proceeds through an oxalate intermediate which can be cleaved under acidic conditions to control the stoichiometric addition of polypyridyl ligands to the Ru(II) coordination sphere enabling the stepwise assembly of each heteroleptic complex. To exemplify this approach, three complexes were prepared including the novel: $[Ru(dppz)(bpyArCOOH)(bpyArCOOEt)]^{2+}$ (where dppz is dipyridophenazine, bpyArCOOH and bpyArCOOEt are 4-(4-carboxyphenyl)- and 4-(4-ethoxycarbonylphenyl)- 2,2-bipyridine respectively) in which the synthetic yield from the $RuCl_3$ starting material to final product is 82 %. A sequential conjugation-deprotection-conjugation step is then described to yield a Ru(II) complex which is both PEGylated and peptide-conjugated. This synthetic approach offers a useful tool to expand the structural diversity of bis coordinated Ru(II) polypyridyl complexes and provides a simple route to building multifunctionalility into such complexes which should broaden their application in particular in the domain of bioimaging and therapy.

Introduction

The wide utility of luminescent Ru(II) polypyridyl complexes has been demonstrated for decades across diverse photonics applications ranging from dye sensitised solar cells and photocatalysis[1–4] to the more recent burgeoning fields of bio-imaging and metal-based theranostics.[5–7] As the breadth of application of this family of complexes grows so does the demand for greater structural versatility. Although synthetic routes to asymmetric ligand coordination is well established for tridentate ligands related to the 2,2':6',2''-terpyridine ligand.[8,9] A drawback of such complexes is that their luminescence intensities and lifetime are often relatively low, and this limits their application, particularly in bioimaging applications. The *bis*-chelate polypyridyl complexes of Ru(II) offer often excellent photophysical properties but the structural diversity of these complexes is largely limited to constructs of the general form: $[Ru(N^N)_2(N^N)']^{2+}$ (where N^N is an N-donor bidentate polypyridyl ligand). Generally within this model, often it is the ternary ligand (N^N)' that is varied to impart specific functionality, with the '*bis*-ligand' (N^N) most often one of either 2,2'-bipyridine (bpy) or 1,10-phenanthroline (phen). Consequently, the route to the synthesis of such complexes is almost universally through the well-established preparation of the $[Ru(N^N)_2Cl_2]$ intermediate with subsequent conversion to *tris*-chelates.[10] Indeed, synthesis through the dichloride intermediate can itself be problematic due to formation of the intractable side product $[Ru(N^N)_2(CO)Cl]^+$ which until a recent approach reported by Rau *et al.*, blocked chelation of the heteroligand.[11]

The prevalence of the $[Ru(N^N)_2Cl_2]$ synthetic route to *tris bis*-chelated ruthenium complexes has limited somewhat the structural diversity of the synthetic library of Ru(II) polypyridyl complexes. And, as the application areas of such complexes expands, particularly in biological domains there is a growing demand to create structures which have multiple independently modifiable functionalities as shown in Figure 1. This can be achieved through preparation of asymmetric *tris*-heteroleptic metal-ligand systems. Current approaches to forming *tris*-heteroleptic bidentate Ru(II) chelates of the form, $[Ru(N^N)(N^N)'(N^N)'']^{2+}$ (Figure 1); include; (i) decarbonylation using photolysis or otherwise from for example $[Ru(CO)_2Cl_2]_n$ precursor, (ii) cyclometalation from Ru-cymene type starting materials (iii) the use of a $Ru(DMSO)_4Cl_2$ intermediate.[12–17] Beyond this, one-pot syntheses and solid

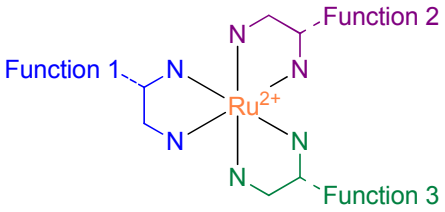


Figure 1 – Schematic illustrating the diversity in functionality that may be incorporated into Ru(II) *tris* heteroleptic chelate complexes.

[a] School of Chemical Sciences, National Centre for Sensor Research, Dublin City University, Glasnevin, Dublin 9, Ireland. E-mail: tia.keyes@dcu.ie

†Electronic Supplementary Information (ESI) available: structural and photophysical characterisation of all Ru(II) complexes including NMR spectra, mass spectra, HPLC traces, additional absorbance and emission spectra and lifetime decay plots . See DOI: 10.1039/x0xx00000x

phase methods have also been reported.[18,19] However, the complexity, synthetic yields and/or generality of these routes are frequently limiting. For example, to the best of our knowledge, the highest yield reported to date was the 5-step procedure reported by Myahkostupov and Castellano who implemented the original protocol described by Mann *et al*. to provide a Ru(II) *tris* heteroleptic complex from $[Ru(Bz)Cl_2]_2$ precursor in 61 % yield.[13,17] Herein, we expand the synthetic diversity of *bis*-chelated Ru(II) complexes through a complete, quite general and high yielding route to Ru(II) *tris*-heteroleptic complexes.

The $Ru(DMSO)_4Cl_2$ intermediate is perhaps the most straight-forward route to *bis*-chelates and was adapted for optimisation as part of the present work. It has been widely demonstrated that stoichiometric addition of a ligand to $Ru(DMSO)_4Cl_2$ yields $Ru(N^N)(DMSO)_2Cl_2$ in high yield.[20–22] Further substitution may then proceed via the classic $Ru(N^N)_2Cl_2$ route with subsequent ternary chelation in aqueous alcohol. Although well established, this approach frequently leads to poor synthetic yields and, as the dichloride intermediate can be difficult to obtain in a pure form due to by-products such as *tris*-chelates and, as described $[Ru(N^N)_2(CO)Cl]^+$, this method frequently requires significant follow on purification.[23] We rationalized that both yield and purity may be improved by avoiding the formation of the dichloride intermediate. Instead we propose the use of an oxalate as an intermediate since their chemistry with Ru(II) has been well studied and they are synthetically easily accessible. Furthermore, oxalate ligand can be hydrolysed in acidic conditions to permit ternary ligand chelation.[24–26] Combining the use of Ru-DMSO and Ru-oxalate intermediates, we describe three examples of the successful implementation of this route to convert $RuCl_3.nH_2O$ to pure $[Ru(N^N)(N^N)'(N^N)'']^{2+}$ with unprecedented yield, in excess of 82 % in each case start-to-finish.

We have devised this approach in the context of our interest in developing receptor-directed Ru(II) polypyridyl complexes for imaging of sub-cellular structures in live cells.[27–31] Indeed, the application of transition metal luminophores to cell imaging is a rapidly growing field of investigation.[32–34] The use of conjugated biomolecules such as peptides or polymeric moieties such as PEG have been demonstrated to improve solubility, reduce cytotoxicity and impart membrane permeability and targeting to metal complex cargo.[35–37] The incorporation of a conjugatable terminus within the metal complex is a prerequisite to progress in this area. In the Ru(II) conjugates reported to date, complexes of the form; $[Ru(N^N)_2(N^N)']^{2+}$ have been applied where the complex is conjugated at an appropriate functional group at the ternary ligand (N^N)' or far less commonly identical groups bound to both of the '*bis*-ligands' (N^N). However, using the approach presented here the incorporation of one, two or three conjugations as indicated in Figure 1 is possible and indeed the implementation of different conjugation termini is easily achieved. In our exemplar here, we describe a synthetic route to a novel dual conjugate which is both PEGylated and peptide conjugated.

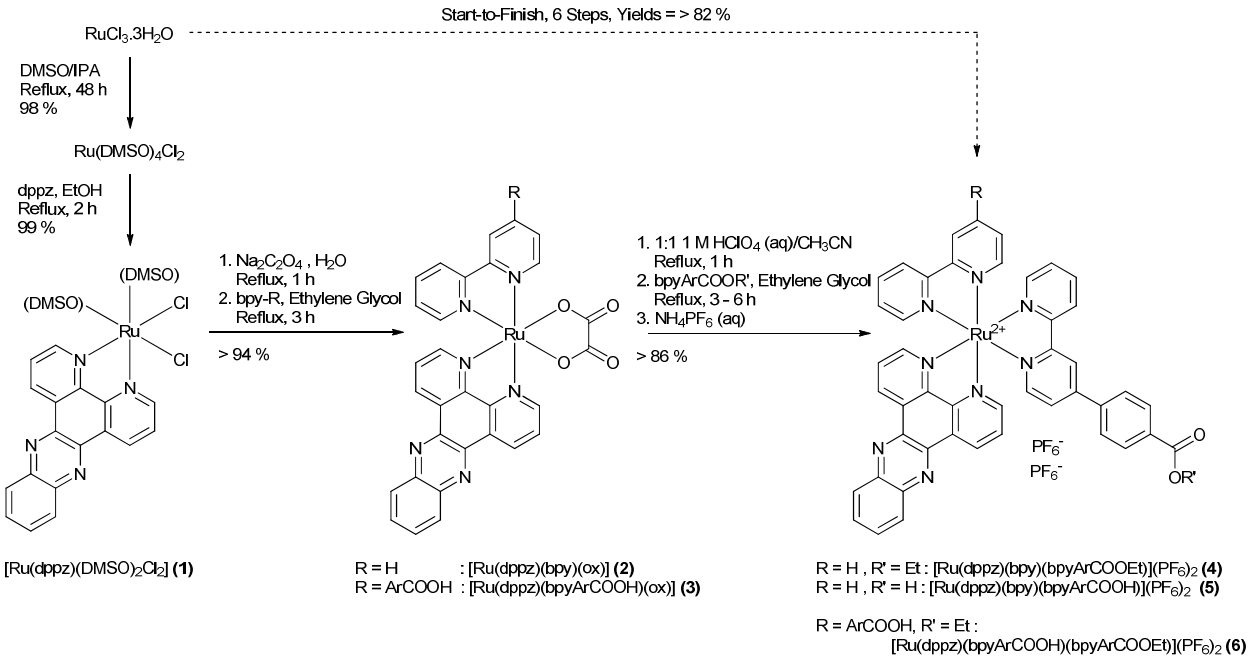
Results and Discussion

Synthesis

The route exploited here to produce Ru(II) polypyridyl *tris*-heteroleptic complexes in high yield is shown in Scheme 1. We adopted $Ru(DMSO)_4Cl_2$ as a Ru(II) precursor for the stepwise addition of successive bidentate polypyridyl ligands to the coordination sphere. In our hands, the original synthesis reported by Evans *et al*.[20] was found to be somewhat inconsistent in terms of the yield and isomeric purity of the products. Consequently, the approach reported by Alston *et al*.[38] was used as this route produced isomerically pure *cis,fac*-$RuCl_2(\kappa S\text{-}DMSO)_3(\kappa O\text{-}DMSO)$ in quantitative yield (98 %). The ability to efficiently displace two DMSO ligands from this precursor and coordinate a single bidentate polypyridyl ligand has been widely reported.[20–22] The ligand of choice in the present case was dipyrido[3,2-a:2',3'-c]phenazine (dppz) – as this ligand is of significant broad interest in ruthenium chemistry due to its capacity to bind DNA and the "solvent switch" effect it induces in complexes of the type; $[Ru(bpy/phen)_2(dppz)]^{2+}$.[39,40] Such complexes exhibit virtually no emission in aqueous media but emit strongly upon protection of the phenazine moiety from hydrogen bonding for example on its intercalation in DNA or a lipid membrane.[28] The Ru(II)-dppz solvate complex, $Ru(dppz)(DMSO)_2Cl_2$ **(1)**; was obtained quantitatively (99 %) under simple ethanolic reflux and structurally characterised by NMR, mass spectrometry and microanalyses (see ESI†). Analysis of the number of aromatic signals in the ^1H NMR spectrum indicates asymmetry in the complex indicating that both of the DMSO ligands and the chlorides conform to a *cis*-configuration relative to one another.

In accordance with the most widely accepted route to preparation of *tris* Ru(II) polypyridyl chelates, i.e. via a $Ru(N^N)_2Cl_2$ intermediate, we first attempted the addition of bpy to $Ru(dppz)(DMSO)_2Cl_2$ **(1)** to yield $Ru(dppz)(bpy)Cl_2$. This route, via reflux in LiCl/DMF[10] or in ethylene glycol[23] was found to be inefficient and the crude isolates in each case typically contained significant quantities of unreacted precursor or under longer reaction times, the *tris*-chelate, $[Ru(dppz)(bpy)_2]^{2+}$; as the majority product. Furthermore, purification of the dichloride was not trivial and impacted on the final yield significantly. A cleaner, more controlled approach was therefore developed which employed dioxane as solvent under reflux with a donor solvent added to catalyse the ligand substitution. The lower reflux temperature and poorer donor ability of dioxane made it easier to limit extent of reaction by controlling water content. Adjustment of the reaction mixture up to 5 % v/v water provided pure $Ru(dppz)(bpy)Cl_2$ in 78 % yield after simple filtration. Unfortunately, the method was found to be quite specific to this ligand system and the analogous $Ru(dppz)(bpyArCOOH)Cl_2$ complex was never isolated by this route.

The lack of success by these standard and modified routes to a generalised synthesis of an asymmetric *bis*-polypyridyl



Scheme 1 – Synthesis of the Ru(II) *tris* heteroleptic complexes via Ru-DMSO and Ru-oxalate intermediates.

intermediate prompted us to seek an alternative to the dichloride intermediate that could be obtained in high yield and purity and crucially, which is reactive enough for subsequent ternary chelation. Ru(II)-oxalates have been well studied across the literature though they have rarely been used as synthetic precursors to *tris*-chelated Ru(II) complexes.[24–26] This is despite their advantages of being less labile than the chloride and incapable of deviation from the desired *cis*-type configuration within the Ru(II) coordination sphere. Herein, Ru(dppz)$(DMSO)_2Cl_2$ **(1)** was converted to Ru(dppz)(bpy)(ox) **(2)** and Ru(dppz)(bpyArCOOH)(ox) **(3)** in > 94 % yield in each case following a facile two-stage reflux with isolation of the product in each case by simple filtration. Both oxalate complexes were characterised by ^{1}H NMR, mass spectrometry and microanalyses which confirm their successful preparation. Critically, the oxalate ligand hydrolyses rapidly at low pH to provide Ru(II) solvates.[41] In our hands, the bright orange mixed aquo/acetonitrile solvate was found to be most easily accessible by treatment of a suspension of the Ru-oxalate with aqueous 1 M $HClO_4$ in acetonitrile. Crucially, the solvate complex precipitates in water as the perchlorate salt, permitting its isolation by filtration after which the solid was washed to eliminate residual acid.

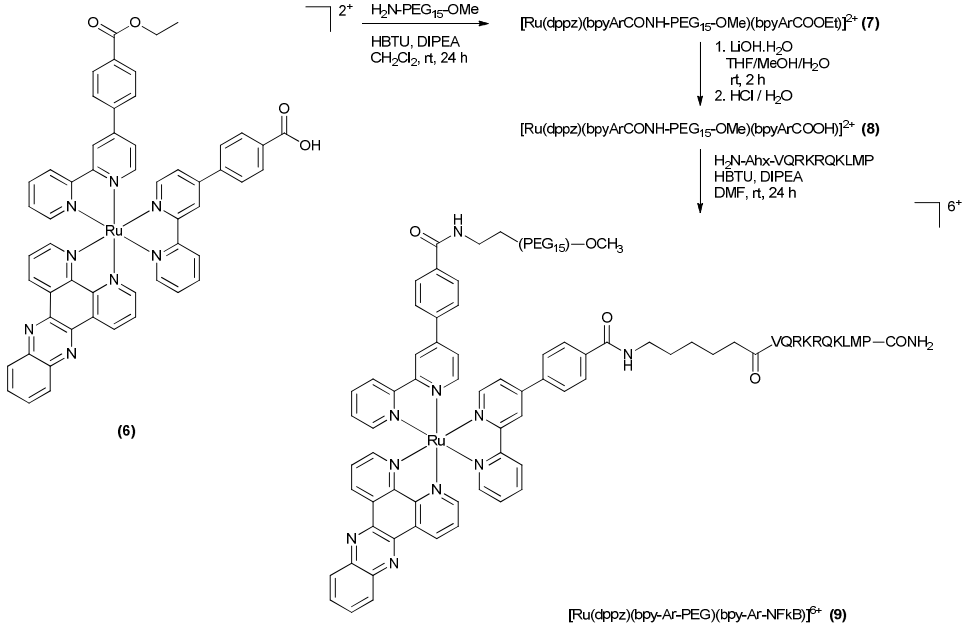
Reaction of the *bis*-chelated Ru(II) solvates in ethylene glycol with stoichiometric quantities of ligand yielded the target *tris*-heteroleptic complexes in yields in excess of 86 % from the oxalate precursor following conventional flash column chromatography on silica. The resulting novel constructs; [Ru(dppz)(bpy)(bpyArCOOEt)]$(PF_6)_2$ **(4)**, [Ru(dppz)(bpy)(bpyArCOOH)]$(PF_6)_2$ **(5)** and [Ru(dppz)(bpyArCOOEt)(bpyArCOOH)]$(PF_6)_2$ **(6)** were obtained as a mixture of isomers as indicated by their respective ^{1}H NMR and COSY spectra (see ESI†). The compounds were fully structurally characterised by NMR, elemental analysis and mass spectroscopy which confirmed their identity and purity. As Scheme 1 indicates the *tris*-heteroleptic complexes were obtained with exceptional yields with overall in each case of more than 82 % from the ruthenium chloride precursor to the final purified product.

Conjugation – Exploiting asymmetry to expand functionality

To demonstrate the practicality of this approach as a route to complexes with mixed conjugation, [Ru(dppz)(bpyArCOOH)(bpyArCOOEt)]$^{2+}$ was subjected to the functionalizations shown in Scheme 2. The complex was prepared so that it contains both a free and protected acid at its periphery in order to enable successive coupling to different vectors.

PEGylation is often performed to improve aqueous solubility and reduce cytotoxicity as well as improve cell uptake of a conjugate.[35,42] Signal peptides can be coordinated to direct cargo to specific organelles within the cell e.g. in the case of imaging to permit study of dynamic cellular processes.[31,43] Therefore, in an imaging application it is useful to be able to combine both PEGylation with a targeting agent at a single probe. Herein, using the *tris*-heteroleptic complex **(6)** we prepared a metal complex luminophore that is both signal peptide conjugated and PEGylated. First, the free acid was exploited for amide conjugation to an amine terminated PEG_{15} chain using the well-known HBTU coupling chemistry. The ester was then de-protected through a facile hydrolysis using LiOH and was followed by acid work up to provide another free acid terminus. Since discrete PEG chains are employed, both the ester and acid Ru-PEG complexes could be unequivocally characterised by ^{1}H NMR and mass spectroscopy where the molecular ion corresponding to the [M – PF_6^-]$^+$ ion

and the ruthenium isotope pattern are clearly evident (see ESI†).



Scheme 2 – Synthesis of the PEGylated and peptide-conjugated Ru(II)-dppz construct.

The nuclear localising signal (NLS) VQRKRQKLMP–NH_2 was then conjugated to the newly de-protected acid site. This peptide is derived from the transcription factor NF-kB, which functions to internalise NF-kB into the nucleus, it has previously been reported as a transmembrane molecular cargo carrier.[29] The NLS was conjugated to the Ru-PEG conjugate again through a HBTU coupling protocol. The resulting diconjugate complex: $[Ru(dppz)(bpy\text{-}Ar\text{-}PEG)(bpy\text{-}Ar\text{-}NFkB)]^{6+}$ was purified on silica plates and obtained as the hexafluorophosphate salt. The ^1H NMR spectrum of the diconjugate exhibits signals that correspond to the Ru(II) core, the PEG chain and also multiple signals in the aliphatic region attributed to the short peptide sequence. The relative integration in ^{1}HNMR was as expected for the ruthenium, PEG and peptide moieties for all but the exchangeable peptide protons. Methanol-d_4 was employed as solvent which simplified the spectrum through deuterium substitution equilibria established with exchangeable protons on the polypeptide backbone at arginine and lysine residues. The mass spectrum of the diconjugate **(9)** was markedly different to that of the monoconjugate **(8)**. Despite the absence of a molecular ion, evidence for the successful amidation coupling was deduced from two peaks at *m/z* values of 1753.58 and 1607.62 which are shifted slightly relative to **(8)** and are assigned to the conversion of the free acid of **(8)** to the amide of **(9)** thus indicating fragmentation at the newly coupled amide. Additionally, an overlay of the mass spectra of **(8)** and **(9)** reveals new Ru isotope clusters in the m/z region of 800 to 1500, which are attributed to ions formed from extensive fragmentation of the polypeptide backbone. Confirmation of peptide conjugation was also confirmed by UV-vis spectroscopy and by HPLC where the elution time of the diconjugate on reverse phase HPLC was extended to 9.93 min compared to the parent (4.84 min) or monoconjugates (8.36 min and 8.65 min for the ester and acid respectively). The purity of all of the conjugates was also confirmed by HPLC to be > 98 % purity in each case.

Photophysical characterisation

Preliminary spectroscopic and photophysical characterisation of the complexes and conjugates was carried out for completeness. We were interested in particular to explore the impact, if any, that the conjugated peptide and PEG had on the photophysical properties of the Ru-dppz core in aqueous media.

The unconjugated probes all exhibit spectroscopic and photophysical behaviour typical of Ru(II)-dppz complexes with distinct ligand absorptions at *ca.* 280 and 350 nm and a broad MLCT band centred around 450 nm region (Table 1 and Figure 2). As expected, luminescence is observed in acetonitrile with emission maxima *ca*. 620 nm. The luminescence lifetime for the parent complexes lies in the range 230 – 250 ns increasing to *ca*. 400 ns upon deaeration under N_2 purge. In all cases there was no luminescence observed from the dppz containing complexes when dissolved in water and titration of the luminescent acetonitrile solution of $[Ru(dppz)(bpy)(bpyArCOOH)]^{2+}$ with water indicates that half of the emission is quenched when the water content exceeds 5 % v/v and emission approaches the baseline above 15 %.

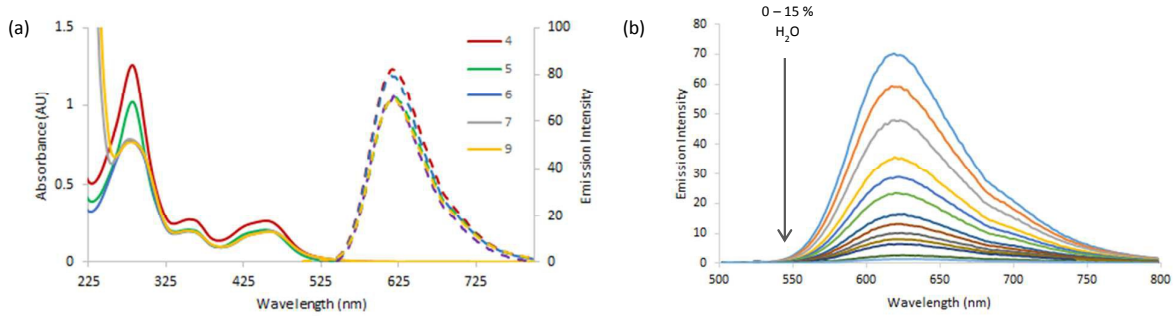


Figure 2 – (a) Absorbance and emission curves for **(4)** – **(9)** in acetonitrile (10 µM, slit widths; 5 nm). (b) Emission quenching of an acetonitrile 10 µM solution of **(5)** upon titration with water up to 15 % v/v.

As shown in Table 1, the luminescent lifetimes for the mono ester compound **(4)** decreases by about 10 ns on de-protection and the lifetime of **(6)** is higher than **(4)** or **(5)** but decreases modestly on conjugation to the PEG moiety, with no significant change on conjugation of the peptide. Luminescent quantum yields in aerated acetonitrile are similar for complexes **(4)** – **(6)** and sit in the range 2.6 – 3.0 % which is typical of Ru(II)-dppz complexes of this type.[5] As expected for bis-chelated Ru(II) polypyridyl complexes, the complexes are stable with no apparent degradation of the complexes **(4)** – **(6)** over the course of two months evident from UV-Vis spectroscopy. In terms of the application of such conjugates to imaging, the photophysical properties of the complex, including the light-switch effect is maintained on *bis*-conjugation.

Experimental

General Information

cis-$Ru(DMSO)_4Cl_2$,[38] dppz,[44] bpyArCOOEt and bpyArCOOH[45] were synthesised according to reported procedures. Discrete methoxy-PEG_{15}-amine, m-dPEG, was purchased from Quanta Biodesign. Peptides (> 98 %) were procured from Celtek Peptides, TN, USA. All other materials were obtained from Sigma Aldrich Chemical Co. and were used without further purification. ^1H and ^{13}C NMR spectra were recorded at either 400 and 100 MHz or 600 and 150 MHz respectively using Bruker spectrometers and deuterated solvent for homo-nuclear lock. The spectra were processed using Bruker Topspin software and were calibrated against solvent peaks according to published values.[46] Elemental analyses were performed at the Microanalytical Laboratory at University College Dublin. High Resolution Mass Spectrometry (HR-MS) was performed at the Mass Spectrometry Facility, University College Dublin or at the Mass Spectrometry Unit, Trinity College Dublin. The syntheses of the Ru(II) complexes described below were performed under nitrogen and in the absence of light. Preparative LC was performed using flash columns or plates as indicated. Analytical HPLC was performed on a Varian 940-LC Liquid Chromatograph using a Hichrom C18 column (4.6 x 250 mm). Gradient elution was employed in the separation using a 0.1% TFA in MeCN/Water mixture starting at 95/5 and changing linearly to 50/50 over 20 minutes at 1.8 mL/min flowrate. PDAD was used for peak detection and the analysis was followed by monitoring 220 nm and 450 nm channels.

Synthesis of the Ru(II) parent complexes

$[Ru(dppz)(DMSO)_2Cl_2]$ (1): *cis*-$Ru(DMSO)_4Cl_2$ (500 mg, 1.03 mmol) and dppz (290 mg, 1.03 mmol) were heated at reflux in ethanol (35 mL) for 2 h. The reaction was then cooled to room temperature and the solvent volume reduced to *ca*. 10 mL *in vacuo*. The precipitate that forms upon cooling was filtered,

Table 1 – Summary of photophysical data for the Ru(II) complexes and conjugates.

Compound	Solvent	λ Absorbance (ε) nm (x10^{-3} M^{-1} cm^{-1})	λ Emission nm	τ ns	ϕ_{lum}
$[Ru(dppz)(bpy)(bpyArCOOEt)]^{2+}$ (4)	MeCN	282 (112.6), 355 (29.0), 454 28.1).	617	239 ± 1	0.027
	H_2O	281 (86.1), 359 (20.3), 452 (18.5).	None		
$[Ru(dppz)(bpy)(bpyArCOOH)]^{2+}$ (5)	MeCN	282 (94.8), 354 (24.0), 454 (23.2).	620	228 ± 1	0.026
	H_2O	281 (68.8), 358 (18.3), 455 (17.1).	None		
$[Ru(dppz)(bpyArCOOH)(bpyArCOOEt)]^{2+}$ (6)	MeCN	277 (72.9), 355 (19.4), 459 (19.6).	616	253 ± 2	0.030
	H_2O	279 (73.3), 357 (20.2), 457 (20.0).	None		
$[Ru(dppz)(bpyAr-PEG)(bpyArCOOEt)]^{2+}$ (7)	MeCN	279 (78.7), 354 (20.1), 459 (19.9).	619	246 ± 2	
	H_2O	279 (73.0), 357 (20.7), 458 (19.1).	None		
$[Ru(dppz)(bpyAr-PEG)(bpyAr-NFkB)]^{6+}$ (9)	MeCN	280 (76.8), 354 (20.1), 459 (19.6).	621	249 ± 1	
	H_2O	280 (66.5), 356 (18.8), 457 (17.1).	None		

All solutions were measured at concentrations of 10 µM. Slit widths for absorbance and emission were set to 5 nm. Lifetime data was recorded in triplicate and curve fitting conformed to tailfit criteria of $0.9 < \chi^2 < 1.10$. Quantum yields were measured in aerated acetonitrile using $[Ru(bpy)_3]^{2+}$ ($\phi = 0.018$)[47] as standard.

washed with minimal cold ethanol and copious amounts of hexane/diethyl ether and dried under nitrogen. In general, the product is isolated pure in this manner, otherwise extraction into acetone and re-precipitation using ether/hexane yields the pure Ru(II) DMSO-solvate. Yield: light-brown solid, 625 mg (1.02 mmol, 99 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 10.22 (d, 1 H); 10.05 (d, 1 H); 9.79 (d, 1 H); 9.69 (d, 1 H); 8.43 (m, 2 H); 8.11 (t, 1 H); 8.05 (m, 2 H); 7.93 (t, 1 H); 3.65 (s, 3 H); 3.60 (s, 3 H); 3.26 (s, 3 H); 2.70 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 157.66, 154.26, 152.02, 150.162, 142.90, 139.58, 139.49, 134.89, 133.91, 132.04, 130.36, 129.88, 129.68, 126.10, 126.03, 47.20, 46.47, 45.49, 44.39. Anal. Calculated (Found) for $C_{22}H_{22}Cl_2N_4O_2S_2Ru$: C 43.28 (43.78); H 3.63 (3.35); N 9.18 (9.26); Cl 11.61 (11.42). HR-MS (ESI-TOF) m/z: Calculated for $C_{22}H_{22}Cl_2N_4O_2S_2Ru$ $[M]^+$: 609.9605; Found: 609.9604.

General Procedure for synthesis of [Ru(dppz)(N^N)(ox)]

$Ru(dppz)(DMSO)_2Cl_2$ (312 mg, 0.511 mmol) and sodium oxalate (100 mg, 0.746 mmol) were heated at reflux in water (15 mL) for 1 h. The reaction was then cooled to room temperature and added to a hot solution of the polypyridyl ligand (0.511 mmol) in 15 mL ethylene glycol. The resulting mixture was heated at reflux for 3 h, cooled to room temperature and then added dropwise to 50 mL of stirring water. After 30 minutes, the precipitates were filtered through a 0.4 µm membrane. The solids were washed with copious amounts of water and minimal acetone before drying thoroughly under a nitrogen stream.

[Ru(dppz)(bpy)(ox)] (2): Yield: purple-black fine powder, 313 mg (0.499 mmol, 98 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 9.62 (d, 1 H); 9.32 (d, 1 H); 9.26 (d, 1 H); 9.00 (d, 1 H); 8.83 (d, 1 H); 8.67 (d, 1 H); 8.53 (d, 1 H); 8.47 (d, 1 H); 8.36 (dd, 1 H); 8.22 (q, 1 H); 8.15 (m, 3 H); 7.92 (t, 1 H); 7.80 (t, 1 H); 7.70 (m, 2 H); 7.12 (t, 1 H). Anal. Calculated (Found) for $C_{30}H_{18}N_6O_4Ru.2H_2O$: C 54.70 (54.30); H 2.89 (3.34); N 12.72 (12.66). HR-MS (ESI-TOF) m/z: Calculated for $C_{30}H_{18}N_6O_4RuNa$ $[M + Na]^+$: 651.0325; Found: 651.0358.

[Ru(dppz)(bpyArCOOH)(ox)] (3): Yield (from 200 mg Ru(II) starting material): black solid, 231 mg (0.308 mmol, 94 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.16 (br s, 1 H, COOH); 9.61 (d, 1 H); 9.33 (d, 1 H); 9.26 (d, 1 H); 9.11 (d, 1 H); 9.01 (2s, 2 H); 8.51 (d, 1 H); 8.46 (d, 1 H); 8.37 (dd, 1 H); 8.26 (m, 1 H); 8.15 (m, 3 H); 8.03 (s, 4 H); 7.94 (t, 1 H); 7.76 (d, 1 H); 7.73 (dd, 1 H); 7.50 (dd, 1 H). Anal. Calculated (Found) for $C_{37}H_{22}N_6O_6Ru.H_2O$: C 58.04 (57.50); H 3.16 (2.98); N 10.98 (11.17).

General Procedure for [Ru(dppz)(bpy)(bpyArCOOR)]$(PF_6)_2$

Ru(dppz)(bpy)(ox) (100 mg, 0.159 mmol) was suspended in 2 mL acetonitrile and 2 mL of 1 M perchloric acid was added. After refluxing for 1 h, a red-brown solution of the Ru-solvate was obtained and after cooling it was poured on 10 mL stirring water. The solids that precipitated were filtered and dried yielding the crude burnt-orange *bis*-solvated Ru(II) complex. The intermediate was dissolved in ethylene glycol (10 mL) with the bpyArCOOR ligand (0.16 mmol) and heated at reflux for 4 – 6 h. The deep red mixture was cooled to room temperature and poured on stirring aqueous ammonium hexafluorophosphate to precipitate the crude complex as the hexafluorophosphate salt. The solids were filtered, washed with water and dried under a nitrogen stream to afford the target complexes as a mixture of geometric isomers. Purification was performed on short silica flash columns using 90/10/1 MeCN/H_2O/20% w/v KNO_3 (aq). The product fraction was concentrated *in vacuo*, precipitated using ammonium hexfluorophosphate and filtered. The solids were taken up in minimum acetone, filtered, concentrated and re-precipitated by slow addition to stirring diethyl ether. Filtration yielded the bright orange pure complexes as a mixture of geometric isomers.

[Ru(dppz)(bpy)(bpyArCOOEt)]$(PF_6)_2$ (4): Yield: orange solid, 166 mg (0.146 mmol, 92 %). ^{1}H NMR (600 MHz, CD_3CN) δ (ppm): 9.68 (m, 2 H); 8.80 (2x dd, 1 H); 8.73 (2x dd, 1 H); 8.56 (2x t, 2 H); 8.49 (m, 2 H); 8.12 – 8.25 (m, 8 H); 8.00 – 8.07 (m, 2 H); 7.91 (m, 5 H); 7.49 – 7.79 (2x m, 3 H); 7.29 – 7.48 (2x m, 3 H); 4.37 (2x q, 2 H); 1.38 (2x t, 3 H). ^{13}C NMR (150 MHz, CD_3CN) δ (ppm): 166.56, 158.78, 158.57, 158.20, 158.17, 157.97, 157.92, 154.71, 154.63, 153.33, 153.16, 153.06, 153.01, 152.96, 151.47, 151.42, 149.41, 143.77, 141.01, 140.88, 140.78, 139.03, 138.94, 138.86, 134.56, 133.54, 133.20, 133.12, 131.89, 131.16, 131.09, 128.76, 128.69, 128.61, 128.50, 128.44, 128.39, 126.10, 125.91, 125.59, 125.53, 125.34, 125.28, 123.21, 123.15, 62.20, 14.48. Anal. Calculated (Found) for $C_{47}H_{34}N_8O_2P_2F_{12}Ru$: C 49.79 (49.18); H 3.02 (2.68); N 9.88 (9.59). HR-MS (ESI-TOF) m/z: Calculated for $C_{45}H_{30}N_8O_2PF_6Ru$ $[M - PF_6]^+$: 989.1490; Found: 989.1477.

[Ru(dppz)(bpy)(bpyArCOOH)]$(PF_6)_2$ (5): Yield: orange solid, 148 mg (0.134 mmol, 86 %). ^{1}H NMR (600 MHz, CD_3CN) δ (ppm): 9.68 (m, 2 H); 8.81 (2x dd, 1 H); 8.73 (2x d, 1 H); 8.56 (2x t, 2 H); 8.48 (m, 2 H); 8.22 (m, 3 H); 8.14 (m, 5 H); 8.04 (m, 2 H); 7.84 – 7.97 (m, 5 H); 7.54 – 7.76 (m, 3 H); 7.28 – 7.49 (m, 3 H). ^{13}C NMR (150 MHz, CD_3CN) δ (ppm): 168.65, 158.68, 158.47, 158.26, 158.21, 158.04, 157.99, 157.96, 154.71, 154.66, 153.21, 153.15, 153.07, 152.98, 151.49, 143.77, 141.02, 139.00, 138.93, 138.84, 134.53, 133.53, 131.88, 131.35, 131.28, 130.62, 128.71, 128.61, 128.57, 128.49, 128.44, 128.25, 128.17, 126.03, 125.85, 125.60, 125.54, 125.34, 125.27, 123.13, 123.08. Anal. Calculated (Found) for $C_{45}H_{30}N_8O_2P_2F_{12}Ru$: C 48.88 (49.41); H 2.73 (2.58); N 10.13 (10.10). HR-MS (ESI-TOF) m/z: Calculated for $C_{45}H_{30}N_8O_2PF_6Ru$ $[M - PF_6]^+$: 961.1183; Found: 961.1190.

[Ru(dppz)(bpyArCOOH)(bpyArCOOEt)]$(PF_6)_2$ (6): The crude product was obtained using an identical procedure to that described for **(4)** and **(5)**. Purification was performed on silica using 70/26/4/2 $CHCl_3$/MeOH/H_2O/AcOH. The concentrated product fraction was treated with aqueous hexafluorophosphate to precipitate the product salt which was filtered. Dissolution in minimum acetone and re-precipitation from diethyl ether yielded the final complex as a mixture of isomers. Yield: orange solid, 152 mg (0.121 mmol, 91 %). ^{1}H NMR (600 MHz, CD_3CN) δ (ppm): 9.67 (m, 2 H); 8.80 (m, 4 H);

8.46 (m, 2 H); 8.28 (t, 1 H); 8.24 (m, 1 H); 8.10 – 8.22 (m, 7 H); 8.07 (m, 1 H); 8.01 (d, 1 H); 7.98 (t, 1 H); 7.89 – 7.96 (m, 5 H); 7.86 (d, 1 H); 7.81 (m, 2 H); 7.75 (qd, 1 H); 7.55 (m, 1 H); 7.52 (m, 1 H); 7.32 (t, 1 H); 4.37 (2x q, 2 H); 1.38 (2x t, 3 H). ^{13}C NMR (150 MHz, CD_3CN) δ (ppm): 166.55, 166.51, 158.26, 154.75, 154.67, 153.30, 151.48, 149.90, 143.76, 140.99, 140.89, 138,99, 138.90, 134.58, 133.52, 131.90, 131.33, 131.27, 131.14, 131.09, 130.62, 128.71, 128.62, 128.49, 128.24, 128.17, 125.95, 125.89, 125.63, 123.23, 123.17, 123.07. Anal. Calculated (Found) for $C_{54}H_{38}N_8O_4P_2F_{12}Ru$: C 51.72 (51.11); H 3.05 (3.05); N 8.94 (8.59). HR-MS (ESI-TOF) m/z: Calculated for $C_{54}H_{38}N_8O_4PF_6Ru$ $[M - PF_6]^+$: 1109.1701; Found: 1109.1757.

Synthesis of the Ru(II) conjugates

$[Ru(dppz)(bpy\text{-}Ar\text{-}PEG)(bpyArCOOEt)](PF_6)_2$ (7): A suspension of DIPEA (15 µL, 0.086 mmol), HBTU (3.5 mg, 0.009 mmol) and **(6)** (10 mg, 0.008 mmol) in 2 mL dichloromethane was allowed to stir for 15 minutes at room temperature. To this was added a solution of m-$dPEG_{15}$-amine (7 mg, 0.010 mmol) in dichloromethane (2 mL). The mixture was left to stir for 16 hours and was then concentrated to dryness under a nitrogen stream. The residue was purified by column chromatography on silica using 9/1 CH_2Cl_2/MeOH as eluent. The product fraction was evaporated to dryness under nitrogen to provide the product after acetone/diethyl ether reprecipitation as a sticky red solid. Yield: isomer mixture, red tacky solid, 11 mg (0.006 mmol, 71 %). HPLC (PDAD, 450 nm): Indicative Ru(II) purity vs parent: 99.2 %. ^{1}H NMR (600 MHz, $(CD_3)_2CO$) δ (ppm): 9.80 (m, 2 H); 9.25 (m, 2 H); 9.15 (m, 2 H); 8.69 (m, 1 H); 8.59 (m, 1 H); 8.52 (m, 2 H); 8.32 (m, 2 H); 8.09 – 8.26 (m, 13 H); 7.98 – 8.08 (m, 4 H); 7.78 (m, 1 H); 7.69 (m, 1 H); 7.46 (m, 1 H); 4.37 (2x q, 2 H, OEt -CH_2-); 3.34 – 3.72 (m, 60 H, PEG -OCH_2-); 3.25 (s, 3 H, PEG –OCH_3); 1.37 (2x t, 3 H, OEt –CH_3). HR-MS (MALDI-QTOF) m/z: Calculated for $C_{85}H_{101}N_9O_{18}PF_6Ru$ $[M - PF_6]^+$: 1782.5950; Found: 1782.6034.

$[Ru(dppz)(bpy\text{-}Ar\text{-}PEG)(bpyArCOOH)](PF_6)_2$ (8): The Ru-PEG precursor **(7)** (10 mg, 0.005 mmol) was dissolved in 1.25 mL of a 4/1 THF/methanol mixture under stirring at room temperature. To this was added an aqueous solution of $LiOH.H_2O$ (1 mg in 0.25 mL). After 2 h, the mixture was concentrated under nitrogen to *ca*. 0.5 mL and treated with 0.5 mL of 0.1 M HCl and 0.5 mL of saturated aqueous ammonium hexafluorophosphate. After diluting with 1 mL water, the product was extracted into 4 x 2 mL dichloromethane and the combined organic phase was washed with 5 mL water. The separated organic layer was dried over anhydrous magnesium sulphate, filtered and evaporated to dryness under nitrogen. Yield = red solid, 9 mg (0.005 mmol, 91 %). HPLC (PDAD, 450 nm): Indicative Ru(II) purity vs precursors: 99.7 %. ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 9.80 (d, 2 H); 9.25 (tm, 2 H); 9.16 (m, 2 H); 8.70 (m, 1 H); 8.62 (m, 1 H); 8.53 (m, 2 H); 8.26 – 8.41 (m, 3 H); 8.08 – 8.26 (m, 11 H); 7.96 – 8.08 (m, 4 H); 7.87 – 7.96 (m, 1 H); 7.78 (m, 1 H); 7.70 (m, 1 H); 7.46 (m, 1 H); 3.38 – 3.80 (m, 60 H, PEG –OCH_2-); 3.28 (s, 3 H, PEG –OCH_3). HR-MS (MALDI-QTOF) m/z: Calculated for $C_{83}H_{97}N_9O_{18}PF_6Ru$ $[M - PF_6]^+$: 1754.5637; Found: 1754.5691.

$[Ru(dppz)(bpy\text{-}Ar\text{-}PEG)(bpy\text{-}Ar\text{-}NFkB)](PF_6)_6$ (9): A DMF (1 mL) solution of **(8)** (5 mg, 0.006 mmol) and HBTU (12 mg, 0.031 mmol) was prepared and to this stirring solution at room temperature was added DIPEA (10 uL, 0.06 mmol). After 15 minutes, a solution of the short peptide in DMF (5 mg in 0.2 mL, 0.007 mmol) was added to the reaction mixture. The solution was allowed to stir for 24 h and was then treated with diethyl ether to precipitate a crude mass. The suspension was poured on a short flash column (RP-C18) and the reaction solvent rinsed free using acetonitrile (0.1 % TFA) as eluent. Residual starting material is first eluted using 0.1 % TFA in 95/5 MeCN/H_2O. The product band was then eluted using 0.1 % TFA in 50/50 MeCN/H_2O. The product fraction was concentrated under a nitrogen stream and the conjugate precipitated by the addition of solid ammonium hexafluorophosphate. The precipitated solids were filtered and dried to afford the purified Ru-peptide conjugate. Yield = orange sticky solid. HPLC (PDAD, 450 nm): Indicative Ru(II) purity vs precursors: 100 %. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 9.74 (d, 2H); 9.06 (d, 2 H); 8.97 (m, 2 H); 8.49 (m, 2 H); 8.42 (m, 1 H); 8.33 (d, 1 H); 8.22 (td, 1 H); 7.95 – 8.16 (m, 15 H); 7.91 (m, 3 H); 7.70 (m, 1 H); 7.60 (m, 1 H); 7.41 (m, 1 H); 5.34 (t, 1 H); 3.85 – 4.65 (m, 5 H); 3.62 – 3.76 (m, 16 H); 3.42 – 3.62 (m, 67 H; PEG-*H*); 3.37 (s, 1 H); 3.13 – 3.26 (m, 5 H); 2.98 (m, 15 H); 2.88 (s, 1 H); 2.67 (s, 1 H); 2.63 (s, 1 H); 2.24 – 2.54 (m, 3 H); 2.17 - 2.21 (m, 5 H); 1.85 – 2.23 (m, 9 H); 1.50 - 1.83 (m, 8 H); 1.44 (m, 2 H); 1.27 – 1.39 (m, 28 H); 1.25 (s, 6 H); 1.03 (dd, 2 H); 0.87 - 0.95 (m, 6 H). HR-MS (MALDI-QTOF) m/z: Calculated for $C_{83}H_{98}N_{10}O_{17}PF_6Ru$ $[Ru(dppz)(bpy\text{-}PEG)(bpyArCONH_2) + PF_6^-]^+$ (loss of Ahx-peptide): 1753.5791; Found: 1753.5808. Calculated for $C_{83}H_{97}N_{10}O_{17}Ru$ $[Ru(dppz)(bpy\text{-}PEG)(bpyArCONH_2) - H^+]^+$ (loss of Ahx-peptide): 1607.6066; Found: 1607.6180.

Photophysical characterisation

UV-Vis spectra were recorded on a Jasco spectrophotometer. All analyses were performed using quartz cuvettes and background correction was applied prior to measurement. Emission spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer with excitation and emission slit widths of 10 nm. Luminescent lifetimes were obtained using a time correlated single photon counting (TCSPC) PicoQuant system and an exciting 450 nm laser. Lifetime decay plots were analysed using PicoQuant NanoHarp software applying tailfit criteria; $0.9 < \chi^2 < 1.10$. All photophysical measurements were performed in triplicate at room temperature (293 K).

Conclusions

An efficient, high yielding and versatile 6 step route to *tris*-heteroleptic polypyridyl Ru(II) complexes was described. Synthesis proceeds from commercially available $RuCl_3$, through the $[Ru(DMSO_4)Cl_2]$ precursor through stepwise coordination

of ligands via an oxalate intermediate which is readily cleaved for coordination of the final ligand through hydrolysis. The reaction was demonstrated here for three derivatives of the complex; $[Ru(dppz)(bpyArCOOH)(bpyArCOOEt)]^{2+}$ (where dppz is dipyridophenazine, bpyArCOOH and bpyArCOOEt are 4-(4-carboxyphenyl)- and 4-(4-ethoxycarbonylphenyl)- 2,2-bipyridine respectively). In the three examples provided overall synthetic yields from the $RuCl_3$ to *tris* heteroleptic complex exceeded an unprecedented 80%.

A key objective of this work was to create a route to preparation of luminescent ruthenium containing bidentate ligands in which multiple dissimilar conjugations could be achieved. This was demonstrated by inclusion of ligands within the *tris*-heteroleptic complex that contained both acid and ester termini at which stepwise PEGylation and then following deprotection of the ester, peptide conjugation of each ligand could be achieved through HBTU coupling. The *tris* chelates presented herein all contain a dppz ligand which, as expected, rendered the MLCT emission sensitive to quenching in aqueous environment. We confirmed that the switch effect persists after dual functionalisation of the complex with PEG and peptide moieties. This feature suggests the diconjugated probes are potential candidates for future studies in cellular imaging. Although focussed on conjugation in this example, the facile and high yielding route to *tris*-heteroleptic Ru(II) complexes presented here should be useful across the many domains to which Ru(II) luminophores are applied, from photocatalysis to biophotonics.

Acknowledgements

The authors gratefully acknowledge the Irish Research Council for postgraduate funding and Science Foundation Ireland for funding under grant number 14/IA/248.

Notes and references

1 J. G. Vos and J. M. Kelly, *Dalton Trans.*, 2006, 4869–4883.
2 S. Bonnet and J.-P. Collin, *Chem. Soc. Rev.*, 2008, **37**, 1207–1217.
3 V. B. A. Juris, *Coord. Chem. Rev.*, 1988, **84**, 85–277.
4 V. Balzani and A. Juris, *Coord. Chem. Rev.*, 2001, **211**, 97–115.
5 M. R. Gill and J. A. Thomas, *Chem. Soc. Rev.*, 2012, **41**, 3179–3192.
6 J. D. Knoll and C. Turro, *Coord. Chem. Rev.*, 2015, **282–283**, 110–126.
7 F. Li, J. G. Collins and F. R. Keene, *Chem. Soc. Rev.*, 2015, **44**, 2529–2542.
8 A. K. Pal, S. Serroni, N. Zaccheroni, S. Campagna and G. S. Hanan, *Chem. Sci.*, 2014, **5**, 4800–4811.
9 A. C. Benniston, A. Harriman, P. Li, C. A. Sams and M. D. Ward, *J. Am. Chem. Soc.*, 2004, **126**, 13630–13631.
10 B. P. Sullivan, D. J. Salmon and T. J. Meyer, *Inorg. Chem.*, 1978, **17**, 3334–3341.
11 N. Zabarska, J. G. Vos and S. Rau, *Polyhedron*, 2015, **102**, 173–175.
12 L. Spiccia, G. B. Deacon and C. M. Kepert, *Coord. Chem. Rev.*, 2004, **248**, 1329–1341.
13 D. A. Freedman, J. K. Evju, M. K. Pomije and K. R. Mann, *Inorg. Chem.*, 2001, **40**, 5711–5715.
14 P. A. Anderson, G. B. Deacon, K. H. Haarmann, F. R. Keene, T. J. Meyer, D. A. Reitsma, B. W. Skelton, G. F. Strouse and N. C. Thomas, *Inorg. Chem.*, 1995, **34**, 6145–6157.
15 J. A. Treadway and T. J. Meyer, *Inorg. Chem.*, 1999, **38**, 2267–2278.
16 D. Mulhern, S. Brooker, H. Görls, S. Rau and J. G. Vos, *Dalton Trans.*, 2006, 51–57.
17 M. Myahkostupov and F. N. Castellano, *Inorg. Chem.*, 2011, **50**, 9714–9727.
18 D. Hesek, Y. Inoue, S. R. L. Everitt, H. Ishida, M. Kunieda and M. G. B. Drew, *Inorg. Chem.*, 2000, **39**, 308–316.
19 S. P. Mulcahy, S. Li, R. Korn, X. Xie and E. Meggers, *Inorg. Chem.*, 2008, **47**, 5030–5032.
20 I. P. Evans, A. Spencer and G. Wilkinson, *J. Chem. Soc. Dalton Trans.*, 1973, 204–209.
21 S. Dutta, S. Pal and P. K. Bhattacharya, *Polyhedron*, 1999, **18**, 2157–2162.
22 S. Le Gac, M. Foucart, P. Gerbaux, E. Defrancq, C. Moucheron and A. Kirsch - De Mesmaeker, *Dalton Trans.*, 2010, **39**, 9672.
23 C. Viala and C. Coudret, *Inorganica Chim. Acta*, 2006, **359**, 984–989.
24 I. Bratsos, G. Birarda, S. Jedner, E. Zangrando and E. Alessio, *Dalton Trans.*, 2007, 4048–4058.
25 S. A. Adeyemi, A. Dovletoglou, A. R. Guadalupe and T. J. Meyer, *Inorg. Chem.*, 1992, **31**, 1375–1383.
26 A. Dovletoglou, S. A. Adeyemi and T. J. Meyer, *Inorg. Chem.*, 1996, **35**, 4120–4127.
27 U. Neugebauer, Y. Pellegrin, M. Devocelle, R. J. Forster, W. Signac, N. Moran and T. E. Keyes, *Chem. Commun.*, 2008, 5307–5309.
28 L. Cosgrave, M. Devocelle, R. J. Forster and T. E. Keyes, *Chem. Commun.*, 2010, **46**, 103–105.
29 L. Blackmore, R. Moriarty, C. Dolan, K. Adamson, R. J. Forster, M. Devocelle and T. E. Keyes, *Chem. Commun.*, 2013, **49**, 2658–2660.
30 K. Adamson, C. Dolan, N. Moran, R. J. Forster and T. E. Keyes, *Bioconjug. Chem.*, 2014, **25**, 928–944.
31 A. Martin, A. Byrne, C. S. Burke, R. J. Forster and T. E. Keyes, *J. Am. Chem. Soc.*, 2014, **136**, 15300–15309.
32 M. P. Coogan and V. Fernández-Moreira, *Chem. Commun.*, 2013, **50**, 384–399.
33 K. K.-W. Lo, A. W.-T. Choi and W. H.-T. Law, *Dalton Trans.*, 2012, **41**, 6021–6047.
34 C. A. Puckett, R. J. Ernst and J. K. Barton, *Dalton Trans.*, 2010, **39**, 1159–1170.
35 S. P.-Y. Li, H.-W. Liu, K. Y. Zhang and K. K.-W. Lo, *Chem. – Eur. J.*, 2010, **16**, 8329–8339.
36 C. A. Puckett and J. K. Barton, *J. Am. Chem. Soc.*, 2009, **131**, 8738–8739.
37 C. A. Puckett and J. K. Barton, *Bioorg. Med. Chem.*, 2010, **18**, 3564–3569.
38 J. R. Alston, S. Kobayashi, T. J. Younts and J. C. Poler, *Polyhedron*, 2010, **29**, 2696–2702.
39 A. E. Friedman, J. C. Chambron, J. P. Sauvage, N. J. Turro and J. K. Barton, *J. Am. Chem. Soc.*, 1990, **112**, 4960–4962.
40 R. M. Hartshorn and J. K. Barton, *J. Am. Chem. Soc.*, 1992, **114**, 5919–5925.
41 J. J. Concepcion, J. W. Jurss, J. L. Templeton and T. J. Meyer, *J. Am. Chem. Soc.*, 2008, **130**, 16462–16463.

42 S. P.-Y. Li, C. T.-S. Lau, M.-W. Louie, Y.-W. Lam, S. H. Cheng and K. K.-W. Lo, *Biomaterials*, 2013, **34**, 7519–7532.
43 A. Byrne, C. Dolan, R. D. Moriarty, A. Martin, U. Neugebauer, R. J. Forster, A. Davies, Y. Volkov and T. E. Keyes, *Dalton Trans.*, 2015, **44**, 14323–14332.
44 J. Dickeson and L. Summers, *Aust. J. Chem.*, 1970, **23**, 1023–1027.
45 A. Martin, A. Byrne, C. Dolan, R. J. Forster and T. E. Keyes, *Chem. Commun.*, 2015.
46 G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw and K. I. Goldberg, *Organometallics*, 2010, **29**, 2176–2179.
47 K. Suzuki, A. Kobayashi, S. Kaneko, K. Takehira, T. Yoshihara, H. Ishida, Y. Shiina, S. Oishi and S. Tobita, *Phys. Chem. Chem. Phys.*, 2009, **11**, 9850–9860.