ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2018, **54**, 12487

Received 18th July 2018,
Accepted 4th October 2018

DOI: 10.1039/c8cc05809a

rsc.li/chemcomm

Ru(II) complexes with diazine ligands: electronic modulation of the coordinating group is key to the design of "dual action" photoactivated agents†

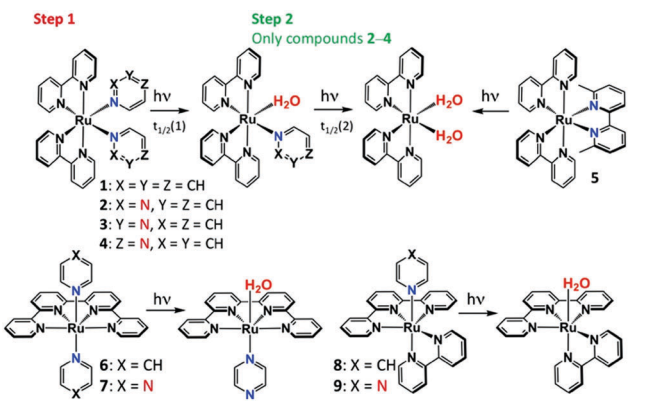
Dmytro Havrylyuk, Megha Deshpande, Sean Parkin and Edith C. Glazer *

Coordination complexes can be used to photocage biologically active ligands, providing control over the location, time, and dose of a delivered drug. Dual action agents can be created if both the ligand released and the ligand-deficient metal center effect biological processes. Ruthenium(II) complexes coordinated to pyridyl ligands generally are only capable of releasing one ligand in H_2O, wasting equivalents of drug molecules, and producing a Ru(II) center that is not cytotoxic. In contrast, Ru(II) polypyridyl complexes containing diazine ligands eject both monodentate ligands, with the quantum yield (ϕ_{PS}) of the second phase varying as a function of ligand pK_a and the pH of the medium. This effect is general, as it is effective with different Ru(II) structures, and demonstrates that diazine-based drugs are the preferred choice for the development of light-activated dual action Ru(II) agents.

Light-triggered Ru(II) molecules that produce active species capable of forming covalent adducts with DNA are being actively explored for photoactivated chemotherapy (PACT).[1] A complementary and potentially compatible approach is "photocaging," where a biologically active, monodentate ligand is masked by coordination to a metal center.[2–7] Photo-release of this ligand allows for temporal and spatial control over its activity. However, a persistent issue that has limited the utility of Ru(II) photocages is the sluggish photochemistry associated with ejecting the monodentate ligand. Strain-inducing bidentate ligands are known to activate dissociative photochemical pathways within cells,[8–11] and have been used to increase the photolability of the monodentate ligand.[12–14]

"Dual action" light-activated Ru(II) compounds, where both the metal center and the liberated ligands induce different, potentially synergistic biological effects, are also of interest.[15,16] The same issue hampers the development of such dual action agents, however, as photocages; the photoreactivity of the second ligand is often orders of magnitude lower than the first.[17] This sub-optimal photochemistry results in the waste of one equivalent of the drug ligand, but more importantly, the opening of the two binding sites on the metal appears important for the creation of the Ru(II) cytotoxic species.[18–20] This is in notable contrast to platinum species such as phenanthriplatin, a highly potent cytotoxin with only one reactive site.[21]

The limitation of photoejection of only one ligand has been demonstrated for Ru(II) photocages containing pyridine, imidazole, aliphatic amine derivatives,[22] and phosphine ligands.[23] These light-induced ligand dissociation phenomena have been explored by ultrafast spectroscopy techniques and computational approaches.[24,25] In contrast, the release of two monodentate ligands has been shown for a complex with 5-cyanouracil,[26] demonstrating that Ru(II) bound nitriles undergo light-activated ligand exchange more efficiently than other monodentate ligands.[7] However, the second ligand ejection is slow, and the majority of nitrile-containing drugs, including anticancer agents, are derivatives of nitrogen-containing heterocycles.[27] Therefore, coordination with a Ru scaffold by a direct synthetic pathway could be complicated by the presence of coordination isomers in the prodrug molecule.



Scheme 1 Photochemical reactions of complexes included in this study in H_2O.

Department of Chemistry, University of Kentucky, 505 Rose St., Lexington, KY 40506, USA. E-mail: ec.glazer@uky.edu

† Electronic supplementary information (ESI) available. CCDC 1856425 and 1856426. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c8cc05809a

Considering the difference between acid–base properties and photochemical behaviors for Ru(II) complexes with aliphatic amines or pyridine (hard and borderline bases, respectively) and nitriles (soft bases),[28,29] we hypothesized that *cis*-$[Ru(bpy)_2(L_2)]^{2+}$ (bpy = 2,2′-bipyridyl) complexes with monodentate diazines (which are softer bases than pyridine) would efficiently release two ligands upon light irradiation in an aqueous medium. This hypothesis is partially supported by the fact that the incorporation of the bidentate diazine ligand bipyrimidine in Ru(II) complexes results in a photochemically active species without any strain-inducing groups, in marked contrast to the bipyridine complexes.[29,30] Diazines are privileged heterocycles in medicinal chemistry,[9] and are bioisosteres for the pyridine and phenyl rings, so this study was further motivated by the presence of these moieties in several known drugs and the potential for incorporation in many more. The results of this report may be applied as a starting platform to develop improved light-activated photocages and dual-action ruthenium complexes with diazine-based antitumor agents.

Three complexes with isomeric diazine coligands, *cis*-$[Ru(bpy)_2(pyd)_2]^{2+}$ (pyd = pyridazine; compound **2**), *cis*-$[Ru(bpy)_2(pym)_2]^{2+}$ (pym = pyrimidine; **3**), and *cis*-$[Ru(bpy)_2(pyz)_2]^{2+}$ (pyz = pyrazine; **4**) were synthesized and their photochemical properties were compared to *cis*-$[Ru(bpy)_2(py)_2]^{2+}$ (**1**; py = pyridine; Scheme 1). The complexes were prepared under low light conditions by refluxing *cis*-$[Ru(bpy)_2Cl_2]$ with a 10-fold excess of the desired diazine in ethanol : water (1 : 1). Complexes **1**, **2** and **4** have been reported previously,[31,32] including the photophysical properties[33] and some photosubstitution reactions[34] for complexes **1** and **2**. NMR spectroscopy and X-ray crystal structures have been determined for **1**,[32] though noteworthy differences were found in the NMR spectra of compound **1**.[35]

Structural analysis by X-ray crystallography revealed distorted octahedral geometries for complexes **2** and **3**. These complexes exhibited altered [Ru–N] bond lengths in comparison to pyridine-containing **1** (Table S4, ESI†);[32] the Ru–N(pyd) and Ru–N(pym) bonds (**2** and **3**) are equal, in contrast to Ru–N(py) bonds (**1**) where different lengths were found (2.063 and 2.13 Å). The N–Ru–N bond angles between diazine ligands are also distorted from the ideal bond angles of 90° and 180°, with the deviations of N2*–Ru–N2 (angles between the *trans*-nitrogens in the bpy coligands) for complexes **2** (8.04°) and **3** (6.74°) larger than that for **1** (5°) (Fig. S1A and C, ESI†). The pyridazine ligands are bent from N2 (the top bpy nitrogen, Fig. S1B, ESI†) with different bond angles (88.44° and 97.13°), and the bond angle between pyridazines is 92.53° in contrast to 90° between pyridine ligands in complex **1**.[32] This distortion was anticipated to affect the photochemical reactivity of the complexes.

All four Ru(II) complexes (**1–4**) were relatively stable in the dark for 72 h at 37 °C (Fig. S21, ESI†), and exhibited selective photoejection of the first monodentate ligand in water (monitored by absorption spectroscopy; Scheme 1; Fig. S2–S5, ESI†) when irradiated with 470 nm light. The presence of an isosbestic point was interpreted as an indication of direct conversion to a single product. Compound **1** formed *cis*-$[Ru(bpy)_2(py)(H_2O)]^{2+}$ (see absorption profile in Fig. 2B) and no further ligand loss was observed.

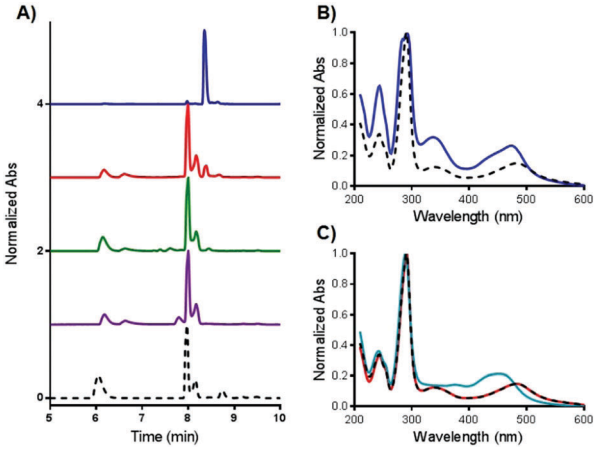


Fig. 1 Determination of photoejection products by HPLC. (A) Chromatogram of **1** (blue line, after 60 min irradiation), **2** (red line), **3** (green line) and **4** (violet line) after 120 min irradiation in comparison to **5** (black dash line, after 15 min irradiation). (B) Absorption profile of Ru(II) photoproducts of **1** (blue line, RT = 8.35 min, *cis*-$[Ru(bpy)_2(py)(H_2O)]^{2+}$) and **5** (black dash line, RT = 7.87 min, *cis*-$[Ru(bpy)_2(H_2O)_2]^{2+}$). (C) Absorption profile of Ru(II) photoproducts of **2** after ejection of the first (cyan line, 5 min irradiation, RT = 8.36 min) and second pyridazine ligand (red line, 120 min irradiation, RT = 7.99 min) overlaid with *cis*-$[Ru(bpy)_2(H_2O)_2]^{2+}$ (black dash line, RT = 7.87 min).

In marked contrast, a second photoreaction was observed for compounds **2–4** (Fig. 1A, C and Fig. S3–S5, ESI†). The quantum yields of photosubstitution by water (ϕ_{PS}) are shown in Table 1 and half-lives ($t_{1/2}$) are provided in Table S13 (ESI†). Only the diazine complexes ejected two ligands. The first ligand photoejection is facile, with $t_{1/2}(1)$ of less than 1 min for complexes **1–4** and ϕ_{PS} of 0.031–0.11. In contrast, the ϕ_{PS} (2) for **2–4** in water were inverted relative to ϕ_{PS} (1), indicating both the sensitivity of the photochemistry to the identity of the diazine ligand, and the presence of a specific chemical feature that drives the disparity in the yields of these sequential processes. The ϕ_{PS} values for the second ligand ejection ranged from 0.0005–0.0033, with **4** exhibiting the highest quantum yield. The product was identified as *cis*-$[Ru(bpy)_2(H_2O)_2]^{2+}$ by both HPLC and absorption spectroscopy (Fig. 1A and Fig. S3–S5, ESI†). The retention time (RT) and absorption profile were compared

Table 1 Photophysical and photochemical[a] properties of compounds **1–9** in H_2O

	λ_{max}^{abs} [b] (nm)		ϕ_{PS} [c]	
Compound	*A*	*B*	(1)	(2)
1	455	—	0.031	—
2	420	450	0.11	0.0005
3	415	460	0.070	0.0011
			0.059[d]	0.0013[d]
4	405	445	0.11	0.0033
5			0.022	—
6			nd	nd
7			nd	nd
8			nd	—
9			0.007	—

[a] Measured using a 13 mW cm^{-2} 470 nm LED. [b] For the MLCT. [c] See ESI for a detailed description. [d] Determined by HPLC.

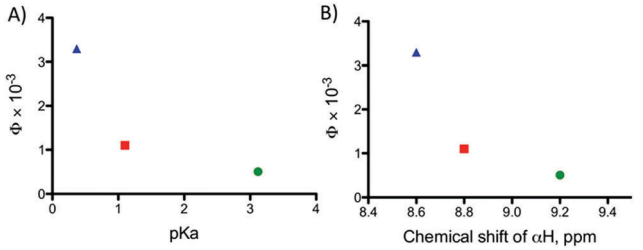


Fig. 2 Correlation between quantum yields for the second ligand ejection for **2** (green ○), **3** (red □), and **4** (blue Δ) and pK_a of the protonated free diazines (A) or chemical shifts of the α protons of diazine ligands (B).

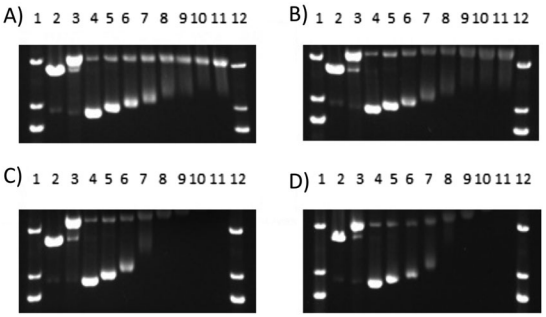


Fig. 3 Agarose gel electrophoresis showing the dose response of compounds (A) **1**, (B) **2**, (C) **3** and (D) **4** incubated with 40 μg mL^{-1} pUC19 DNA with irradiation (470 nm light). Lanes 1 and 12, DNA ladder; lane 2, EcoRI; lane 3, $Cu(OP)_2$; and lane 4–11, 0–500 μM. EcoRI and $Cu(OP)_2$ are controls for linear and relaxed circle DNA. See ESI,† for full gels.

with $[Ru(bpy)_2]$-based products after irradiation of the strained compound **5** $[Ru(bpy)_2(dmbpy)]^{2+}$ (dmbpy – 6,6′-dimethyl-2,2′-bipyridyl).[8] Further exposure of *cis*-$[Ru(bpy)_2(H_2O)_2]^{2+}$ to light after photoejection resulted in a decrease in the intensity of the MLCT (metal-to-ligand charge-transfer) absorption band at 490 nm following irradiation for 90 (**4**), 120 (**3**) and 150 (**2**) minutes, likely due to the oxidation of the Ru(II) center.[13]

An inverse relationship was found between ϕ_{PS} (**2**) for complexes **2–4** and the pK_a values for the protonated parent ligands, as shown in Fig. 2A. The weaker basic diazine ligands are more photolabile, and there was no ejection of the pyridine, which is the strongest base in this series. The quantum yields also correlate with the chemical shifts of the α protons of the diazine ligands (Fig. 2B). In addition, ϕ_{PS} and $t_{1/2}$ (**2**) were found to be sensitive to the environment (pH), as compounds **2–4** demonstrated 1.7–2-fold faster ligand ejection in HCl–KCl buffer, pH = 2 than in sodium phosphate buffer, pH = 7.4 (Table S4, ESI†). A similar effect was observed with 1.8–3.5-fold faster $t_{1/2}$ values in D_2O *vs.* H_2O. A possible explanation is that engagement of the non-bonding electrons of the uncoordinated aza nitrogen, either through protonation or hydrogen bonding, accelerates the photochemistry either by forming a pre-encounter complex with the incoming ligand or by polarizing the electrons on the diazine ligand.

DNA damage was assessed by gel electrophoresis (Fig. 3). Incubation of each Ru(II) complex with plasmid DNA in the dark showed no interactions (Fig. S8, ESI†), in contrast to the two types of DNA damage observed upon irradiation with 470 nm light. The diazine complexes **2–4** undergo ligand loss and covalent attachment to DNA, as observed by the reduction in DNA mobility and loss of ethidium bromide (EtBr) staining. Complex **1** created a combination of covalent damage and single strand breaks to form relaxed circular DNA, possibly through sensitization of singlet oxygen ($^1O_2$). There is some indication of single strand breaks for compound **2** as well, which has the slowest $t_{1/2}$ (**2**) among the diazine-containing complexes. Systems that photoeject and create reactive oxygen species have previously been identified as useful dual mechanism agents;[8,36] if a diazine-containing drug were ligated, these would become triple action agents.

In order to determine if the incorporation of diazines for the improvement of Ru(II) complexes photo-lability is generalizable to other Ru(II) structures, the photochemistry of two *trans*-Ru(II) complexes containing pyridine (**6**) and pyrazine (**7**) ligands was investigated. Previously, a *trans*-Ru(II) complex containing thermally exchangeable ligands exhibited higher potency than the analogous *cis* compound,[37] making this an appealing scaffold for the creation of dual action agents. Accordingly, *trans*-$[Ru(qpy)(pyz)_2]^{2+}$ (**7**; qpy = 2,2′:6′,2″:6″,2‴-quaterpyridine) was synthesized and compared to the pyridine analogue **6**, which is photo-stable in aqueous media. In contrast, the *trans*-Ru(II) complex with pyrazine ligands ejected the pyrazine ligand upon irradiation for nine hours (Fig. S9, ESI†).

Finally, to demonstrate the useful applications of a pure "photocaging" approach,[38,39] two Ru(II) complexes (**8** and **9**) were synthesized using a $[Ru(tpy)(bpy)]^{2+}$ scaffold (tpy = 2,2′;6′-2″-terpyridine). The photochemical reaction of the complex containing pyridine, $[Ru(tpy)(bpy)(py)]^{2+}$ (**8**), did not reach completion after nine hours irradiation (Fig. 4A and B), which is consistent with previous reports of $\phi_{PS} < 10^{-5}$ in MeCN.[40,41] In contrast $[Ru(tpy)(bpy)(pyz)]^{2+}$ (**9**) exhibited significantly enhanced photolability of pyrazine upon irradiation in aqueous media,

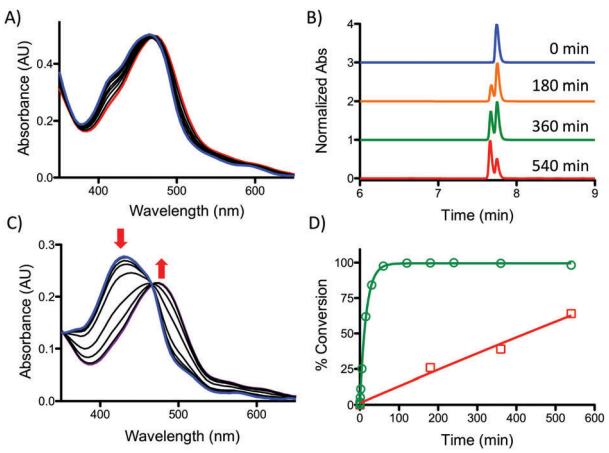


Fig. 4 Photochemistry of $[Ru(tpy)(bpy)(L)]^{2+}$ complexes. (A) Absorption spectra of **8** with irradiation (blue line, t = 0, red line, t = 540 min). (B) HPLC of **8** as a function of irradiation time. (C) Absorption spectra of **9** with irradiation (blue line, t = 0, red line, t = 100 min). (D) Comparison of % conversion for **8** (red open □) and **9** (green open ○). The integrated area under the peaks from (B) was used for % conversion of **8**.

with $t_{1/2}$ = 10 min and complete ligand exchange in two hours (Fig. 4C and D, ϕ_{PS} = 0.007 in water). This significantly improved photochemistry makes $[Ru(tpy)(bpy)]^{2+}$ a useful photocage in water if a diazine ligand is used.

In conclusion, rather than incorporating strain-inducing bidentate ligands in Ru(II) scaffolds[8–10,42] or substituting monodentate ligands with electron withdrawing groups,[43] these results demonstrate that simply using diazine ligands radically improves photochemical features. The approach works for a variety of Ru(II) scaffolds, and facilitates the ejection of two ligands from the *cis*-$[Ru(bpy)_2]^{2+}$ cage. We posit that simple electronic tuning by switching from pyridine to diazine systems is a far more efficient and flexible approach for the creation of functional light-activated metal complexes. These results suggest that photochemistry can be tuned by judicious use of ligands based on pK_a values and HSAB theory.

We gratefully acknowledge the National Institutes of Health (Grant GM107586) for the support of this research. Crystallographic work was made possible by the National Science Foundation (NSF) MRI program, grants CHE-0319176 and CHE-1625732.

Conflicts of interest

There are no conflicts to declare.

Notes and references

1 N. J. Farrer, L. Salassa and P. J. Sadler, *Dalton Trans.*, 2009, 10690–10701.
2 L. Zayat, C. Calero, P. Albores, L. Baraldo and R. Etchenique, *J. Am. Chem. Soc.*, 2003, **125**(4), 882–883.
3 L. Zayat, M. Salierno and R. Etchenique, *Inorg. Chem.*, 2006, **45**(4), 1728–1731.
4 T. Respondek, R. N. Garner, M. K. Herroon, I. Podgorski, C. Turro and J. J. Kodanko, *J. Am. Chem. Soc.*, 2011, **133**(43), 17164–17167.
5 A. Li, C. Turro and J. J. Kodanko, *Chem. Commun.*, 2018, **54**(11), 1280–1290.
6 A. K. Renfrew, *Metallomics*, 2014, **6**(8), 1324–1335.
7 J. Mosquera, M. I. Sanchez, J. L. Mascarenas and M. Eugenio Vazquez, *Chem. Commun.*, 2015, **51**(25), 5501–5504.
8 B. S. Howerton, D. K. Heidary and E. C. Glazer, *J. Am. Chem. Soc.*, 2012, **134**(20), 8324–8327.
9 E. Wachter, D. K. Heidary, B. S. Howerton, S. Parkin and E. C. Glazer, *Chem. Commun.*, 2012, **48**(77), 9649–9651.
10 A. N. Hidayatullah, E. Wachter, D. K. Heidary, S. Parkin and E. C. Glazer, *Inorg. Chem.*, 2014, **53**(19), 10030–10032.
11 D. Havrylyuk, D. K. Heidary, L. Nease, S. Parkin and E. C. Glazer, *Eur. J. Inorg. Chem.*, 2017, 1687–1694.
12 K. Arora, J. K. White, R. Sharma, S. Mazumder, P. D. Martin, H. B. Schlegel, C. Turro and J. J. Kodanko, *Inorg. Chem.*, 2016, **55**(14), 6968–6979.
13 M. Huisman, J. K. White, V. G. Lewalski, I. Podgorski, C. Turro and J. J. Kodanko, *Chem. Commun.*, 2016, **52**(85), 12590–12593.
14 J. Wei and A. K. Renfrew, *J. Inorg. Biochem.*, 2018, **179**, 146–153.
15 A. Zamora, C. A. Denning, D. K. Heidary, E. Wachter, L. A. Nease, J. Ruiz and E. C. Glazer, *Dalton Trans.*, 2017, **46**, 2165–2173.
16 H. Chan, J. B. Ghrayche, J. Wei and A. K. Renfrew, *Eur. J. Inorg. Chem.*, 2017, 1679–1686.
17 N. A. Smith, P. Zhang, S. E. Greenough, M. D. Horbury, G. J. Clarkson, D. McFeely, A. Habtemariam, L. Salassa, V. G. Stavros, C. G. Dowson and P. J. Sadler, *Chem. Sci.*, 2017, **8**(1), 395–404.
18 The cytotoxicity appears to depend on the coligands used.
19 J. A. Cuello-Garibo, M. S. Meijer and S. Bonnet, *Chem. Commun.*, 2017, **53**(50), 6768–6771.
20 M. A. Sgambellone, A. David, R. N. Garner, K. R. Dunbar and C. Turro, *J. Am. Chem. Soc.*, 2013, **135**(30), 11274–11282.
21 G. Y. Park, J. J. Wilson, Y. Song and S. J. Lippard, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**(30), 11987–11992.
22 J. K. White, R. H. Schmehl and C. Turro, *Inorg. Chim. Acta*, 2017, **454**, 7–20.
23 S. Franco, S. R. Manuel, N. G. Samira and R. Antonio, *Eur. J. Inorg. Chem.*, 2016, 1528–1540.
24 L. Salassa, C. Garino, G. Salassa, R. Gobetto and C. Nervi, *J. Am. Chem. Soc.*, 2008, **130**(29), 9590–9597.
25 S. E. Greenough, G. M. Roberts, N. A. Smith, M. D. Horbury, R. G. McKinlay, J. M. Zurek, M. J. Paterson, P. J. Sadler and V. G. Stavros, *Phys. Chem. Chem. Phys.*, 2014, **16**(36), 19141–19155.
26 R. N. Garner, J. C. Gallucci, K. R. Dunbar and C. Turro, *Inorg. Chem.*, 2011, **50**(19), 9213–9215.
27 F. F. Fleming, L. Yao, P. C. Ravikumar, L. Funk and B. C. Shook, *J. Med. Chem.*, 2010, **53**(22), 7902–7917.
28 J. E. House, *Inorganic Chemistry*, Academic Press, 2nd edn, 2012, pp. 297–298.
29 R. J. Crutchley and A. B. P. Lever, *Inorg. Chem.*, 1982, **21**(6), 2276–2282.
30 P. Vicendo, S. Mouysset and N. Paillous, *Photochem. Photobiol.*, 1997, **65**(4), 647–655.
31 S. A. Adeyemi, E. C. Johnson, F. J. Miller and T. J. Meyer, *Inorg. Chem.*, 1973, **12**(10), 2371–2374.
32 P. B. Hitchcock, K. R. Seddon, J. E. Turp, Y. Z. Yousif, J. A. Zora, E. C. Constable and O. Wernberg, *J. Chem. Soc., Dalton Trans.*, 1988, **7**, 1837–1842.
33 J. V. Caspar and T. J. Meyer, *Inorg. Chem.*, 1983, **22**(17), 2444–2453.
34 D. V. Pinnick and B. Durham, *Inorg. Chem.*, 1984, **23**(10), 1440–1445.
35 In contrast to the described ^{1}H NMR spectrum (360 MHz, $(CD_3)_2SO$) for **1**, some resonances for H5 and H5′ of bpy coligands (**1–4**) are present as a doublet of doublets of doublets (ddd) (Fig. S10, ESI†). Detailed NMR spectra and interpretations are presented in the ESI†.
36 L. M. Loftus, J. K. White, B. A. Albani, L. Kohler, J. J. Kodanko, R. P. Thummel, K. R. Dunbar and C. Turro, *Chem. – Eur. J.*, 2016, **22**(11), 3704–3708.
37 E. Wachter, A. Zamora, D. K. Heidary, J. Ruiz and E. C. Glazer, *Chem. Commun.*, 2016, **52**(66), 10121–10124.
38 L. N. Lameijer, D. Ernst, S. L. Hopkins, M. S. Meijer, S. H. C. Askes, S. E. Le Devedec and S. Bonnet, *Angew. Chem., Int. Ed.*, 2017, **56**(38), 11549–11553.
39 A. Li, R. Yadav, J. K. White, M. K. Herroon, B. P. Callahan, I. Podgorski, C. Turro, E. E. Scott and J. J. Kodanko, *Chem. Commun.*, 2017, **53**(26), 3673–3676.
40 Ru(II) complexes have higher quantum yields in MeCN than in water.
41 J. D. Knoll, B. A. Albani, C. B. Durr and C. Turro, *J. Phys. Chem. A*, 2014, **118**(45), 10603–10610.
42 J. D. Knoll, B. A. Albani and C. Turro, *Acc. Chem. Res.*, 2015, **48**(8), 2280–2287.
43 A. T. Vu, D. A. Santos, J. G. Hale and R. N. Garner, *Inorg. Chim. Acta*, 2016, **450**, 23–29.

