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Ru(II) Polypyridyl Complexes Derived from Tetradentate Ancillary Ligands for Effective Photocaging

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CONSPECTUS

Metal complexes have many proven applications in the caging and photochemical release of biologically active compounds. Photocaging groups derived from Ru(II) traditionally have been composed of ancillary ligands that are planar and bi- or tridentate, such as 2,2'-bipyridine (bpy), 2,2':6',2''-terpyridine (tpy), and 1,10-phenanthroline (phen). Complexes bearing ancillary ligands with denticities higher than three represent a new class of Ru(II)-based photocaging groups that are grossly underdeveloped. Because high-denticity ancillary ligands provide the ability to increase the structural rigidity and control the stereochemistry, our groups initiated a research program to explore the applications of such ligands in Ru(II)-based photocaging. Ru(TPA), bearing the tetradentate ancillary ligand tris(2-pyridylmethyl)amine (TPA), has been successfully utilized to effectively cage nitriles and aromatic heterocycles. Nitriles and aromatic heterocycles caged by the Ru(TPA) group show excellent stability in aqueous solutions in the dark, and the complexes can selectively release the caged molecules upon irradiation with light. Ru(TPA) is applicable as a photochemical agent to offer precise spatiotemporal control over biological activity without undesired toxicity. In addition, Ru(II) polypyridyl complexes with desired photochemical properties can be synthesized and identified by solid-phase synthesis, and the resulting complexes show properties to similar to those of complexes obtained by solution-phase synthesis. Density functional theory (DFT) calculations reveal that orbital mixing between the π^* orbitals of the ancillary ligand and the Ru–N $d\sigma^*$ orbital is essential for ligand photodissociation in these complexes. Furthermore, the introduction of steric bulk enhances the photolability of the caged molecules, validating that steric effects can largely influence the quantum efficiency of photoinduced ligand exchange in Ru(II) polypyridyl complexes. Recently, two new photocaging groups, Ru(cyTPA) and Ru(1-isocyTPQA), have been designed and synthesized for caging of

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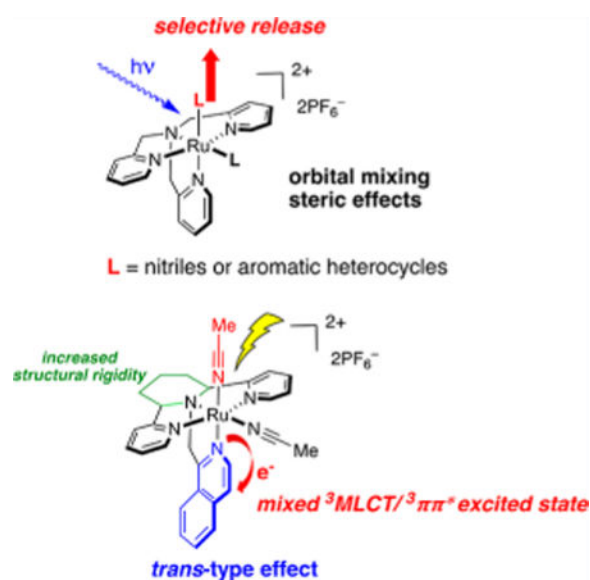
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Notes

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nitriles and aromatic heterocycles, and these complexes exhibit unique photochemical properties distinct from those derived from Ru(TPA). Notably, the unusually greater quantum efficiency for the ligand exchange in $[\text{Ru}(\text{1-isocyTPQA})(\text{MeCN})_2](\text{PF}_6)_2$, $\Phi_{400} = 0.033(3)$, uncovers a *trans*-type effect in the triplet metal-to-ligand charge transfer ($^3\text{MLCT}$) state that enhances photoinduced ligand exchange in a new manner. DFT calculations and ultrafast transient spectroscopy reveal that the lowest-energy triplet state in $[\text{Ru}(\text{1-isocyTPQA})(\text{MeCN})_2](\text{PF}_6)_2$ is a highly mixed $^3\text{MLCT}/^3\pi\pi^*$ excited state rather than a triplet metal-centered ligand-field (^3LF) excited state; the latter is generally accepted for ligand photodissociation. In addition, Mulliken spin density calculations indicate that a majority of the spin density in $[\text{Ru}(\text{1-isocyTPQA})(\text{MeCN})_2](\text{PF}_6)_2$ is localized on the isoquinoline arm, which is opposite to the *cis* MeCN, rather than on the ruthenium center. This significantly weakens the Ru–N6 (*cis* MeCN) bond, which then promotes the ligand photodissociation. This newly discovered effect gives a clearer perception of the interplay between the $^3\text{MLCT}$ and ^3LF excited states of Ru(II) polypyridyl complexes, which may be useful in the design and applications of ruthenium complexes in the areas of photoactivated drug delivery and photosensitizers.

Graphical abstract



1. INTRODUCTION

Selective release of bioactive molecules can achieve spatiotemporal control over activity, which offers researchers a convenient method to investigate biological systems.^{1–5} Because of the success of transition metal complexes in photoinduced ligand release with low-energy light, ruthenium(II)-based systems have been developed for caging in a manner that is inherently different from traditional organic chromophores that photorelease substrates developed over the past decade.^{6–17}

Ruthenium(II) polypyridyl complexes have emerged and functioned as photocaging groups for many bioactive molecules.^{8,12,18–42} Most Ru(II) polypyridyl complexes exhibit lowest-

lying triplet metal-to-ligand charge transfer ($^3\text{MLCT}$) states with thermally accessible low-lying triplet ligand-field (^3LF) state(s) with $\text{Ru-L}(\sigma^*)$ antibonding character ($\text{L} = \text{ligand}$). Thus, it is generally accepted that ligands coordinated to the metal center can be easily dissociated upon irradiation with light at room temperature through population of the ^3LF state(s).^{27,33,43} In addition, caged molecules are released as a primary photochemical step, thus avoiding undesirable photo-damage that commonly occurs from organic photocages arising from dark reactions.^{1,43–46} The three-dimensional geometry and tunable photophysical properties also make Ru(II) polypyridyl complexes attractive photocaging agents.^{27,46–48}

$[\text{Ru}(\text{bpy})_3]^{2+}$ ($\text{bpy} = 2,2'$ -bipyridine) and its derivatives $[\text{Ru}(\text{bpy})_2(\text{L})_2]^{2+}$ ($\text{L} = \text{leaving ligand}$) exhibit strong singlet MLCT ($^1\text{MLCT}$) absorption in the visible range. Bpy and related planar heteroaromatic ligands (Figure 1) have been largely employed to protect many bioactive molecules.^{12,18,19,36,38,41,49} For example, $\text{Ru}(\text{bpy})_2$ -caged neurotransmitters are able to achieve high levels of spatial and temporal control over receptor activity in live neuronal cells.^{12,33,49} The $\text{Ru}(\text{bpy})_2$ fragment has also been used extensively to cage enzyme inhibitors and small-molecule drugs in live-cell assays.^{21,28,34,36,50,51} Apart from $\text{Ru}(\text{bpy})_2$, Ru(II) -based photocaging groups are frequently composed of bi- or tridentate ancillary ligands, including $2,2':6',2''$ -terpyridine (tpy) and 1,10-phenanthroline (phen).^{8,21,28,35,39,52,53} These imine-based photocages have been effectively applied to release bioactive molecules bearing nitriles, thioethers, and aromatic heterocycles, which generally cannot be protected by traditional organic photocages.

Although Ru(II) polypyridyl complexes bearing these planar bi- or tridentate ancillary ligands have been successfully employed for caging of bioactive molecules, those derived from ancillary ligands with denticities of four or higher have been largely unexplored. Notably, ancillary ligands with higher denticities not only increase the stability of the coordination sphere of Ru(II) polypyridyl complexes but also provide rigidity that reduces nonradiative decay pathways that lead to fast, unproductive excited-state deactivation to the ground state. In this Account, we present the recent development of a new class of Ru(II) photocaging groups based on the structure of the nonplanar tetradentate ligand tris(2-pyridylmethyl)amine (TPA). The TPA ligand, along with its derivatives $2,2'$ -(($2R,6S$)-1-(pyridin-2-ylmethyl)piperidine-2,6-diyl)dipyridine (cyTPA) and 1-((($2R,6S$)-2,6-bis(pyridin-2-yl)piperidin-1-yl)-methyl)isoquinoline (1-isocyTPQA), were shown to be effective ancillary ligands to Ru(II) for caging applications. The corresponding complexes $[\text{Ru}(\text{L}')(\text{L})_2]^+$ ($\text{L}' = \text{TPA}, \text{cyTPA}, 1\text{-isocyTPQA}$; $\text{L} = \text{caged ligand}$) are able to cage groups found in bioactive molecules, including nitriles and aromatic heterocycles. The caged complexes are highly stable in the dark but are able to selectively release the molecules of interest upon irradiation with light. In an effort to obtain Ru(II) polypyridyl complexes that possess the desired photoreactivity, a solid-phase synthetic method was introduced to rapidly synthesize and identify complexes that exhibit strong reactivity with visible light. In addition, we validated that orbital mixing and steric effects strongly influence the quantum efficiency of photoinduced ligand exchange. Finally, we discovered a *trans*-type effect that also plays a critical role in promoting ligand photodissociation from the $^3\text{MLCT}$ state induced by $^3\text{MLCT}/^3\pi\pi^*$ mixing instead of from the expected ^3LF state(s).

2. THE DEVELOPMENT OF Ru(TPA) FOR PHOTOCAGING

Ru(TPA) is distinct from traditional photocages in terms of structure and photoreactivity. As a result of the nonplanar ligand geometry, Ru(TPA) exhibits markedly different photophysical and photochemical properties compared with traditional complexes containing Ru(bpy)₂ and Ru(tpy).^{26,29,42,43,48,54} In addition, the tertiary amine scaffold of TPA is highly amenable to derivatization,⁴⁸ making Ru(TPA) an attractive lead for the design of novel Ru(II)-based photocages.

Functionalities such as nitriles and aromatic heterocycles can be effectively protected and liberated from the Ru(TPA) fragment.^{26,29,42,48} Two complexes containing nitrile ligands for photorelease and three with aromatic heterocycles were prepared, having the general formula [Ru(TPA)(L)₂](PF₆)₂, where L = MeCN (**1**), the cathepsin K inhibitor Cbz-Leu-NHCH₂CN (**2**), pyridine (py) (**3**), nicotinamide (NA) (**4**), and imidazole (Im) (**5**) (Figure 2). These complexes are highly stable in the dark and are capable of selectively releasing the caged molecules upon irradiation with light. Because the ¹MLCT bands in **3–5** are observed at $\lambda > 400$ nm, the uncaging process for these complexes can be initiated with visible light instead of the UV light required for nitrile release in **1** and **2**. By occupying four coordination sites on the ruthenium center, the tetradentate TPA ligand increases structural rigidity, which results in enhanced dark stability of the complexes compared with the Ru(bpy)₂ and Ru(tpy) cages. The quantum yields (Φ_{350}) for the exchange of the nitrile positioned *cis* to the amine N atom (the *cis* nitrile) were measured to be $\Phi_{350} = 0.012(1)$ and $\Phi_{350} = 0.011(1)$ for **1** and **2**, respectively, in H₂O ($\lambda_{\text{irr}} = 350$ nm), which are ~20-fold lower than that of [Ru(bpy)₂(MeCN)₂]²⁺ ($\Phi_{400} = 0.21$ with $\lambda_{\text{irr}} = 400$ nm). The quantum yields for the ligand exchange of the *cis* aromatic heterocycles in **3–5** were lower than those of the related nitrile complexes **1** and **2**, with values of $9.7(8) \times 10^{-3}$ for **3**, $9.1(8) \times 10^{-3}$ for **4**, and $5.3(3) \times 10^{-4}$ for **5** in H₂O ($\lambda_{\text{irr}} = 400$ nm). This decrease in photodissociation efficiency in the latter is attributed to the stronger σ donation from aromatic heterocycles compared with nitriles along with changes in the electronic structures of the complexes.

Importantly, complex **2** functions as a potent inhibitor of human cathepsin K upon irradiation with light, exhibiting similar potency to the free inhibitor Cbz-Leu-NHCH₂CN under similar experimental conditions. Enzyme inhibition by **2** was enhanced by a factor of ~89 upon exposure to light, with IC₅₀ values of 63 nM under irradiation and 5.6 μ M when **2** was kept in the dark (Figure 3). In addition, cell viability measurements for **3–5** against PC-3 cells showed that the caged complexes and the resulting photochemical byproducts are well-tolerated by PC-3 cells, indicating that Ru(TPA) is applicable as a photochemical tool toward the development of Ru(II) polypyridyl complexes for biological research applications.²⁶ Our data also suggest that the complexes do not generate ¹O₂ in addition to the release of the caged nitriles.

Next, in an effort to achieve spectral tuning of Ru(II) polypyridyl complexes into the visible range, Ru(II)-caged nitriles bearing tetra- and pentadentate ancillary ligands were designed and synthesized by a solid-phase method.⁴⁸ Briefly, Ru(II)-caged MeCN complexes were synthesized using a library of TPA derivatives obtained on polystyrene resin, which were then metalated using *fac*-[Ru(DMSO)₃(O₂CCCF₃)₂(H₂O)], converted into caged nitriles

upon treatment with MeCN/H₂O, and cleaved from the resin for photochemical analysis (Figure 4). The data show a wide range of spectral tuning and reactivity with visible light for these complexes. Lead complexes exhibiting strong absorption in the visible range were synthesized by solution-phase methods for comparison, and the results showed that complexes obtained by solid-phase synthesis have similar photochemical behavior as those prepared by traditional solution-phase synthesis. However, we noticed that although some complexes show absorption in the visible range, they do not undergo ligand exchange upon irradiation with visible light. Density functional theory (DFT) calculations revealed that apart from absorbing in the desired wavelength range, orbital mixing is essential for effective ligand photodissociation in Ru(II) polypyridyl complexes. For example, in the lead complex [Ru(TQA)(MeCN)₂](PF₆)₂ (**6**) (TQA = tris(1-isoquinolylmethyl)amine) (Figure 5), the photorelease of the *cis* MeCN can be attributed to the appropriate orbital mixing between the π^* orbitals of the isoquinolyl rings of TQA and the Ru–N (*cis* MeCN) $d\sigma^*$ orbital in the excited state (Figure 5). However, such an orbital overlap is not preferable for the *trans* MeCN, causing the *trans* MeCN to remain bound to the ruthenium center.

The introduction of steric bulk in Ru(II) polypyridyl complexes is known to enhance the quantum efficiencies of photoinduced ligand exchange.^{27,32,35,39} The steric bulk distorts the pseudo-octahedral geometry around the ruthenium center, lowering the energy of the metal-centered ³LF state(s), thus bringing these state(s) closer to the ³MLCT states.^{32,39} Accordingly, we surmised that steric effects may also influence ligand photodissociation in the Ru(TPA) series. Therefore, in order to investigate steric effects in the Ru(TPA) system, methyl groups were consecutively introduced to the pyridyl arms of TPA, generating three new complexes having the general formula [Ru(L)(MeCN)₂](PF₆)₂, where L = MeTPA (**7**), Me₂TPA (**8**) and Me₃TPA (**9**) (Figure 6).⁵⁴ The data confirmed that **7–9** are less stable in the dark than **1** under similar conditions, where the caged MeCN ligands exchange more readily with solvent without irradiation. For example, **8** and **9** undergo thermal ligand exchange in organic solvents and water in short time periods (<1 h) in the absence of light. In addition, our results indicate that the addition of methyl groups into the structure of TPA facilitates the photodissociation of the *trans* MeCNs in **7–9**. Therefore, steric bulk surrounding the coordination sphere must be balanced to ensure dark stability while enhancing photodissociation, showing the profound effect of steric effects on photoactivated ligand dissociation.

3. THE DEVELOPMENT OF Ru(CYTPA) FOR PHOTOCAGING

Tetradentate ligands based on the structure of TPA, namely, cyTPA (**10**) and 1-isocyTPQA (**11**) (Figure 7), were recently developed in our group.⁵⁵ Each ancillary ligand possesses a piperidine ring that not only increases the structural rigidity of the complexes but also avoids the formation of stereoisomers by locking the two pyridyl donors into the *trans* configuration. In order to examine the photophysical and photochemical properties that arise from Ru(II) complexation of **10** and **11**, we prepared [Ru(cyTPA)(MeCN)₂]⁺ (**12**) and [Ru(1-isocyTPQA)(MeCN)₂]⁺ (**13**), both of which cage MeCN ligands, as well as the pyridine-caged complexes [Ru(cyTPA)-(py)₂]⁺ (**14**) and [Ru(1-isocyTPQA)(py)₂]⁺ (**15**) (Figure 7). Electronic absorption spectra of **12–15** are shown in Figure 8.

All of the complexes derived from TPA preferentially release the MeCN or py ligand positioned *cis* to the tertiary N atom of the piperidine upon irradiation with light, consistent with the data for **1–5**. The introduction of the piperidine ring into the TPA scaffold rigidifies the resulting complexes, and the photoinduced ligand exchange quantum yield of photoinduced ligand exchange in **12** is lower than that of **1**, while those of **14** and **15** are lower than that of **3** (Table 1).^{26,29} Surprisingly, complex **13** has a 5-fold greater quantum yield for ligand exchange relative to **12** and, as expected, a significantly greater quantum yield than the two py-releasing complexes **14** and **15**. DFT calculations indicated that the lowest-energy triplet state in **13** is a highly mixed $^3\text{MLCT}/^3\pi\pi^*$ excited state, rather than a ^3LF state as is the case in **12**, **14**, and **15**.²⁷ This result was confirmed by Mulliken spin density calculations, which show that unlike **12**, **14**, and **15**, in which the spin density in the lowest-energy triplet state is localized on the Ru(II) center, consistent with a ^3LF state, the majority of the spin density in **13** is localized on the isoquinoline ring, which is positioned *trans* to the leaving *cis* MeCN ligand (Figure 9). Such an unusual phenomenon likely weakens the Ru–N6 (*cis* MeCN) bond in **13** in the excited state, leading to the enhancement of the quantum efficiency for the nitrile release. In addition, Mayer bond order (MBO) analyses confirmed this *trans*-type effect, where in the triplet state the bond strength of Ru–N5 (N in the isoquinoline ring) increases and the bond strength of Ru–N6 (*cis* MeCN) decreases (Table 2). Ultrafast transient absorption spectra further indicated that ligand dissociation occurs from the $^3\text{MLCT}/^3\pi\pi$ state in **13** and that higher activation barriers must be overcome in complexes **12**, **14**, and **15** in order to populate the dissociative ^3LF states from their $^3\text{MLCT}$ states, consistent with the lower quantum yields observed for the latter. Taken together, these results demonstrate that this newly discovered *trans*-type effect significantly promotes ligand photodissociation in Ru(II) polypyridyl complexes, which will be useful in the design and applications of these complexes in the areas of photoactivated drug delivery and photosensitizers.

4. CONCLUSION

In this Account, a new class of Ru(II)-based photocages bearing the nonplanar tetradentate ancillary ligands TPA and cyTPA have been discussed. Their photoreactivity was evaluated by making Ru(TPA)- and Ru(cyTPA)-caged nitriles and aromatic heterocycles. We have demonstrated that the caged molecules can be selectively released upon irradiation with light. In addition, solid-phase synthesis was introduced for the preparation of Ru(II) polypyridyl complexes possessing the desired photochemical properties, from which the absorptions of the complexes were able to be tuned into the visible range by altering the structure of TPA. Furthermore, we validated that orbital mixing and steric effects can have a strong influence on photoinduced ligand exchange. Finally, a *trans*-type effect that greatly enhances the quantum efficiency of photodissociation was discovered in the Ru(1-isocyTPQA) system, providing a new mechanism for achieving efficient ligand photodissociation.

It is noteworthy that complexes derived from Ru(cyTPA) and Ru(1-isocyTPQA) exhibit lower quantum yields than those derived from Ru(TPA) under similar experimental conditions. This may result from the increased structural rigidity introduced by the

piperidine ring, decreasing the role of orbital mixing between the π^* orbitals of the ancillary ligand and the Ru–N6 $d\sigma^*$ orbital. Extending the π conjugation system of the N5 donor or replacing that with fluorophores may reduce the $^3\text{MLCT}$ – ^3LF energy gap, which may in turn promote the quantum efficiency of the complexes in these series.

Our research strongly supports the development of Ru(II) polypyridyl complexes bearing ancillary ligands with high denticities. In the future, we will continue our efforts to design new Ru(II)-based photocages and will also evaluate the properties of these new ligand-release complexes in various biological environments in order to apply them as effective photochemical tools for research applications.

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Biographies

Ao Li graduated from Xiangtan University in China in 2013 with a B.E. in Polymer Materials and Engineering. He received his Ph.D. in Chemistry from Wayne State University in 2018, working under the direction of Prof. Jeremy J. Kodanko. His research focused on the design and synthesis of Ru(II) polypyridyl complexes for effective caging of nitriles and aromatic heterocycles.

Claudia Turro is originally from Argentina and attended Michigan State University, where she earned both her B.S. and Ph.D. in Chemistry. Under the guidance of Profs. Daniel Nocera and George Leroy, her research focused on ultrafast proton-coupled electron transfer and excited states of inorganic complexes. She then investigated the interactions of metal complexes with DNA and other supramolecular systems at Columbia University with Prof. Nicholas Turro as a Jane Coffin Childs Memorial Fellow. She began her independent career as a faculty member at The Ohio State University in 1996.

Jeremy J. Kodanko grew up in northeastern Wisconsin and attended the University of Wisconsin, where he received a B.S. in Chemistry, performing undergraduate research on protease inhibition in the laboratory of Daniel H. Rich. He earned a Ph.D. from the University of California-Irvine under the guidance of Larry E. Overman, where his research focused on natural product synthesis and methods development. He was an NIH postdoctoral fellow at the Massachusetts Institute of Technology in the laboratory of Stephen J. Lippard, focusing on bioinorganic and coordination chemistry. He started his independent career in 2006 at Wayne State University, where his laboratory focuses on the development of metal compounds for biological applications.

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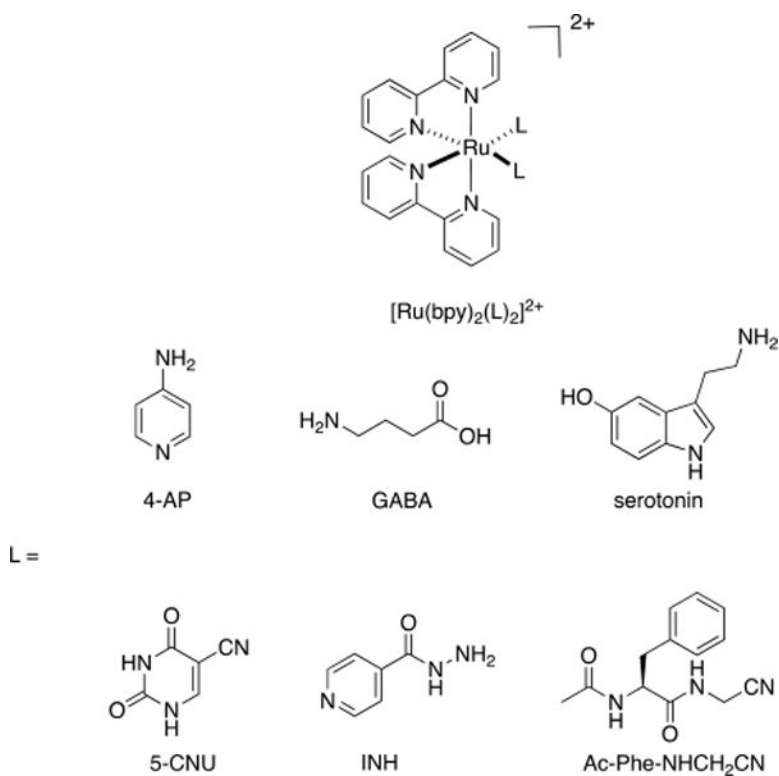


Figure 1. Structures of $[\text{Ru}(\text{bpy})_2(\text{L})_2]^{2+}$, where L = 4-aminopyridine (4-AP), γ -aminobutyric acid (GABA), serotonin, 5-cyanouracil (5-CNU), isoniazid (INH), and the cathepsin inhibitor Ac-Phe-NHCH₂CN.

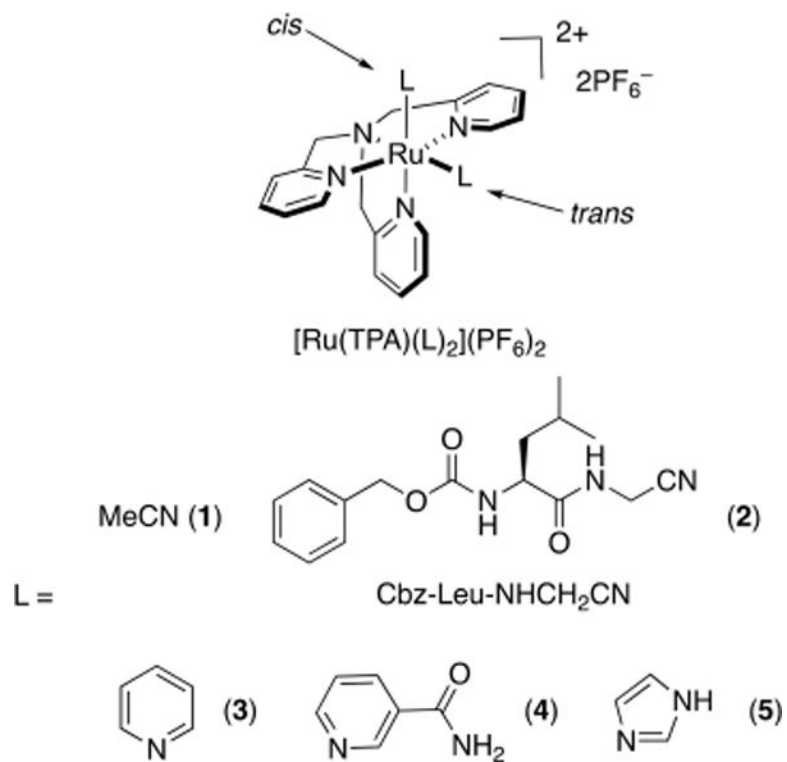


Figure 2. Structures of $[\text{Ru}(\text{TPA})(\text{L})_2](\text{PF}_6)_2$, where L = MeCN (1), Cbz-Leu-NHCH₂CN (2), py (3), NA (4), and Im (5).

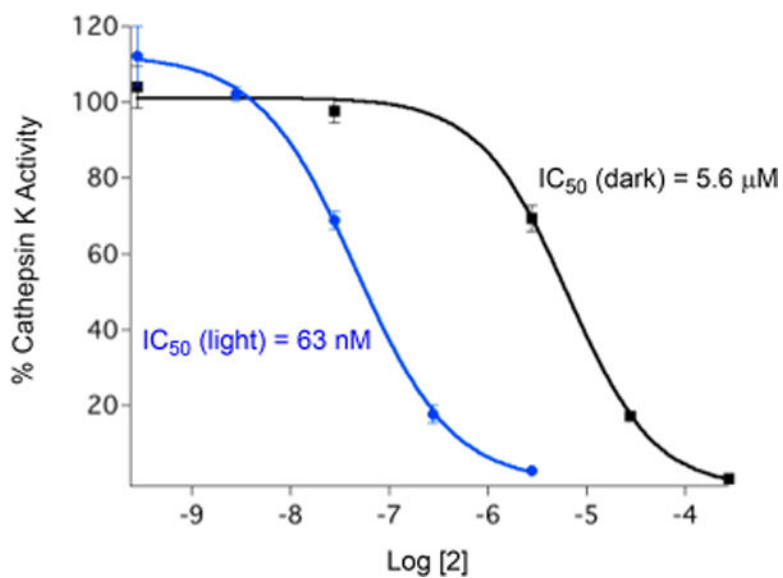


Figure 3. IC₅₀ curves for **2** under light (blue) and dark (black) conditions against human cathepsin K. The reported data are based on three independent experiments. Conditions: 0.4 M acetate buffer (1% DMSO), pH 5.5; [DTT] = 8 mM, [cathepsin K] = 2 nM, and [Z-Gly-Pro-Arg-AMC] = 100 μM; irradiation time ($\lambda_{\text{irr}} = 365 \text{ nm}$) = 15 min; 8 W light source. The enzyme activity was determined with the fluorogenic substrate Z-Gly-Pro-Arg-AMC. Adapted from ref ²⁹. Copyright 2014 American Chemical Society.

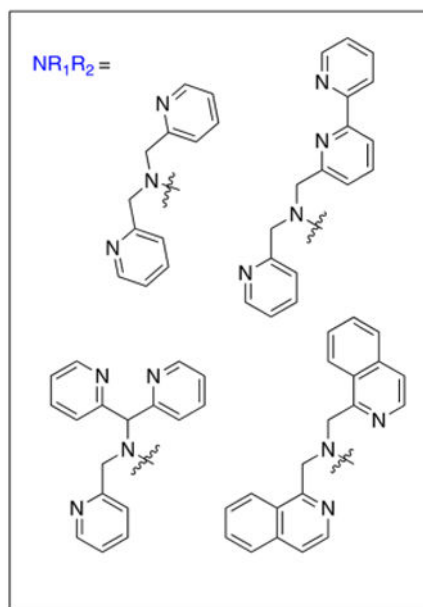
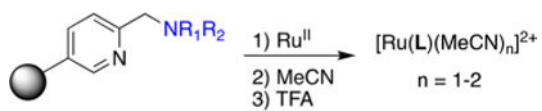


Figure 4.
Solid-phase synthesis of Ru(II)-caged MeCN complexes.

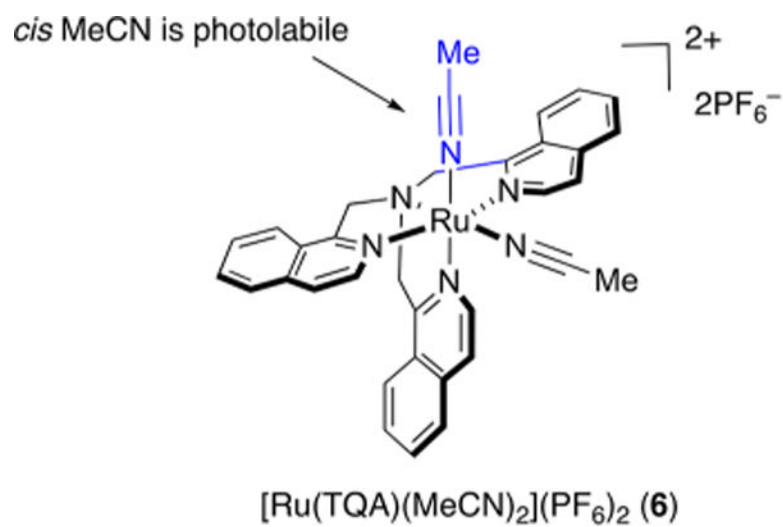


Figure 5.
Structure of $[\text{Ru}(\text{TQA})(\text{MeCN})_2](\text{PF}_6)_2$ (**6**).

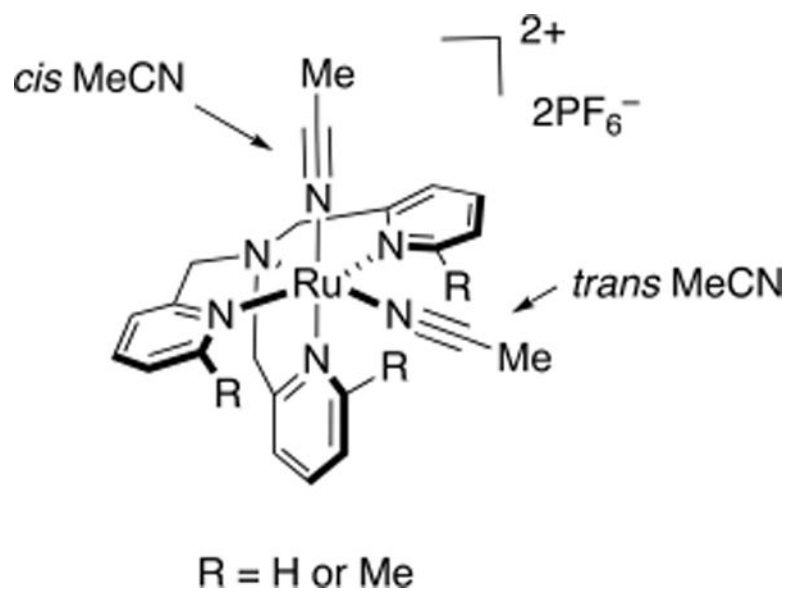


Figure 6. Structures of $[Ru(L)(MeCN)_2](PF_6)_2$, where $L = \text{MeTPA}$ (**7**), Me_2TPA (**8**), and Me_3TPA (**9**).

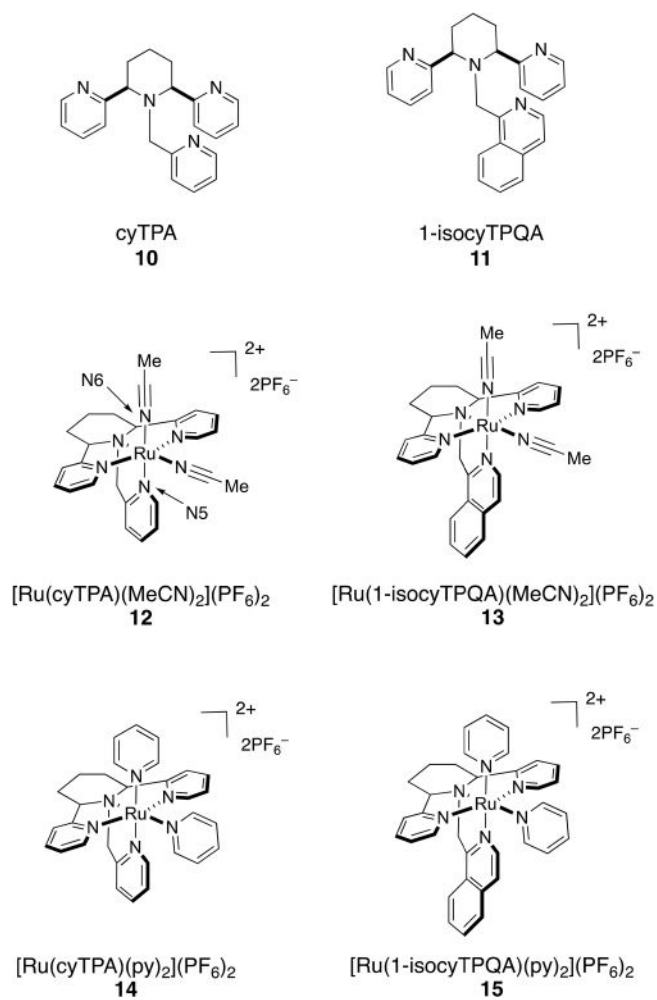


Figure 7. Structures of cyTPA (**10**), 1-isocyTPQA (**11**), Ru–MeCN complexes (**12**) and (**13**), and Ru–py complexes (**14**) and (**15**).

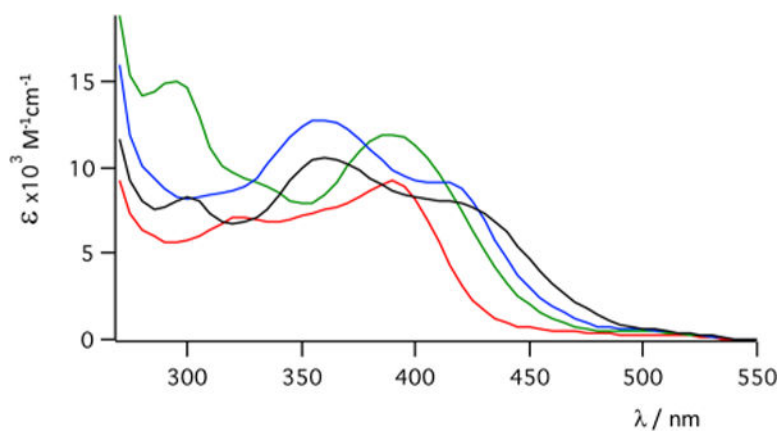


Figure 8.
Electronic absorption spectra of **12** (red), **13** (green), **14** (blue), and **15** (black).

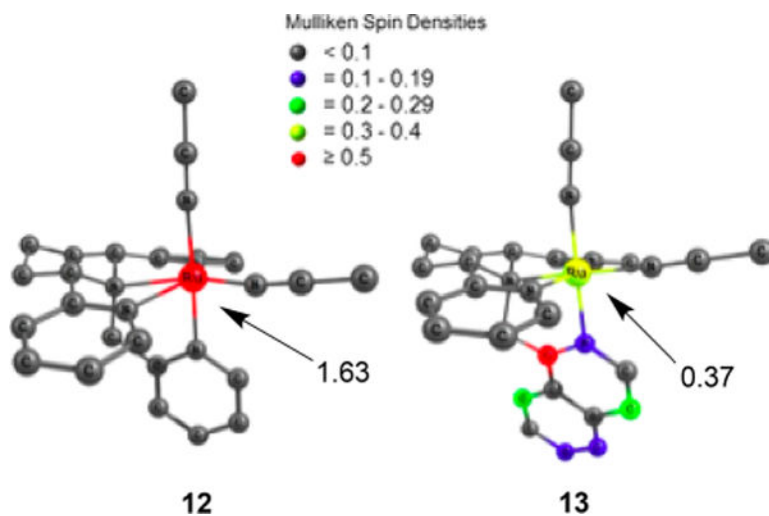


Figure 9. Mulliken spin densities of the lowest-energy triplet state calculated for **12** and **13**. Adapted from ref ⁵⁵. Copyright 2017 American Chemical Society.

Table 1

Photophysical Characterization Data for Complexes 1, 3, and 12–15

	complex	Φ	λ_{max} (nm)	$\epsilon \times 10^3$ ($\text{M}^{-1} \text{cm}^{-1}$) ^c
L = MeCN	1	0.012(1) ^a	380	11200
	12	0.0066(3) ^b	390	9200
	13	0.033(3) ^b	390	11900
L = py	3	0.0097(8) ^b	355	10800
	14	0.0012(1) ^b	360	12800
	15	0.0013(1) ^b	360	10600

^aQuantum yields (Φ) for the first ligand exchange process of **1** in H₂O (<5% acetone) at 298 K ($\lambda_{\text{irr}} = 350$ nm).

^bQuantum yields (Φ) for the first ligand exchange process of **3** and **12–15** in H₂O (<5% acetone) at 298 K ($\lambda_{\text{irr}} = 400$ nm).

^cExtinction coefficients of **1**, **3** and **12–15** in DMSO.

Table 2

MBO Analyses of 12–15 in the Singlet Ground State and Lowest-Energy Triplet State

complex	Ru–N6 ^a		Ru–N5 ^b	
	singlet	triplet	singlet	triplet
12	0.611	0.612	0.434	0.446
13	0.599	0.518	0.415	0.652
14	0.164	0.168	0.397	0.421
15	0.148	0.144	0.369	0.380

^aN6 is the N atom of the *cis* MeCN in **12** and **13** and *cis* py in **14** and **15**.

^bN5 is the N atom of the py arm of cyTPA in **12** and **14** and the isoquinoline arm of 1-isocyTPQA in **13** and **15**.