Inorganic Chemistry

ARTICLE

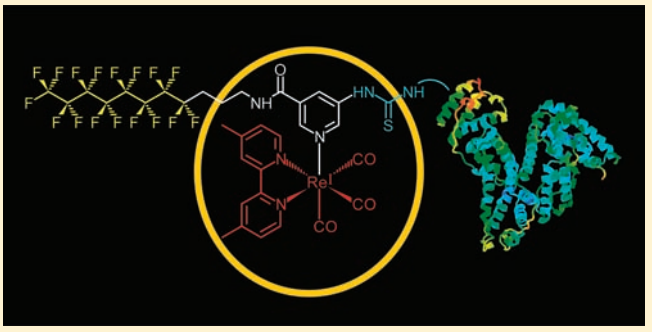
pubs.acs.org/IC

Luminescent Rhenium(I) Polypyridine Fluorous Complexes as Novel Trifunctional Biological Probes

Man-Wai Louie, Tommy Tsz-Him Fong, and Kenneth Kam-Wing Lo*

Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, P. R. China

ABSTRACT: We present the synthesis, characterization, and photophysical properties of three luminescent rhenium(I) polypyridine fluorous complexes $[Re(Me_2bpy)(CO)_3(L)](PF_6)$ (Me_2bpy = 4,4′-dimethyl-2,2′-bipyridine; L = 3-amino-5-(*N*-((3-perfluorooctyl)propyl)aminocarbonyl)pyridine (py-Rf-NH_2) (**1**), 3-isothiocyanato-5-(*N*-((3-perfluorooctyl)propyl)aminocarbonyl) pyridine (py-Rf-NCS) (**2**), 3-ethylthioureidyl-5-(*N*-((3-perfluorooctyl)propyl)aminocarbonyl)pyridine (py-Rf-TU-C_2H_5) (**3**)). The isothiocyanate complex **2** has been used to label bovine serum albumin (BSA) and glutathione (GSH). The photophysical properties of the resultant bioconjugates have been studied. The isolation of the luminescent fluorous rhenium−GSH conjugate from a mixture of 20 amino acids has been demonstrated using fluorous solid-phase extraction (FSPE). Additionally, the cytotoxicity of complexes **1** and **3** toward HeLa cells has been examined by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay. The cellular uptake properties of complex **3** have also been investigated by laser-scanning confocal microscopy.



INTRODUCTION

The fluorous chemistry has contributed to the development of new biphase catalysis that allows the facile separation, recovery, and reuse of catalysts and reagents.[1] Recently, this technology has been implemented to biological applications; for example, the use of fluorous affinity tags provides a platform for the isolation, enrichment, and mass spectrometric characterization of peptides,[2a,b] oligonucleotides,[2c] and glycosphingolipids.[2d] This method is highly selective and has overcome many limitations of traditional bioaffinity-based enrichment strategies. It has also led to the development of simple solid-phase extraction procedures involving inexpensive solvents such as methanol and water. For these reasons, fluorous labeling reagents targeting different functional groups of biomolecules have been designed.[3] Although the development of fluorous chemistry has attracted much attention, the incorporation of perfluorinated alkyl chains into emissive organic or inorganic compounds has only been reported recently.[4] Studies of luminescent transition metal complexes appended with perfluorinated alkyl chains, to the best of our knowledge, are still very limited.[4a,c,f]

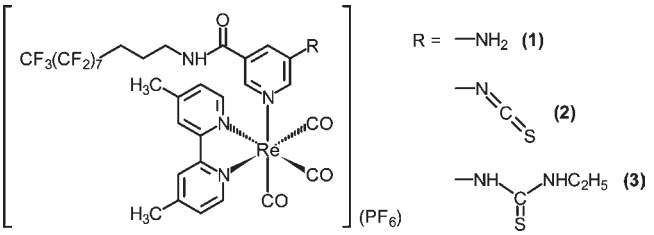
On the basis of the rich photophysical properties of luminescent rhenium(I) polypyridine complexes,[4a,5−15] we have a longstanding interest in the use of these complexes as biological labeling reagents[14] and noncovalent probes for biomolecules and ions.[15] The advantages of using rhenium(I) complexes over other d^6 transition metal complexes are their ease of emission color-tuning and longer-lived excited states, which are useful in the development of multicolor probes for time-resolved applications such as fluorescence lifetime imaging microscopy (FLIM).[16] We anticipate that luminescent fluorous labeling reagents are an important addition to the family of rhenium(I)-based biological probes as they will render the fluorous-labeled biomolecules to possess luminescence properties, which could be applied in the design of new bioassays and imaging experiments. Also, the extent of fluorous labeling of biomacromolecules can be readily determined by spectrofluorometric methods. Additionally, these reagents allow the isolation of the labeled bioconjugates by Fluorous Solid-Phase Extraction (FSPE).

Herein we report three luminescent rhenium(I) polypyridine fluorous complexes $[Re(Me_2bpy)(CO)_3(L)](PF_6)$ (Me_2bpy = 4,4′-dimethyl-2,2′-bipyridine; L = 3-amino-5-(*N*-((3-perfluorooctyl)propyl)aminocarbonyl)pyridine (py-Rf-NH_2) (**1**), 3-isothiocyanato-5-(*N*-((3-perfluorooctyl)propyl)aminocarbonyl)pyridine (py-Rf-NCS) (**2**), 3-ethylthioureidyl-5-(*N*-((3-perfluorooctyl) propyl)aminocarbonyl)pyridine (py-Rf-TU-C_2H_5) (**3**)) (Chart 1). The isothiocyanate complex **2** has been used to label bovine serum albumin (BSA) and glutathione (GSH). The photophysical properties of the resultant bioconjugates have been studied. The isolation of the luminescent fluorous rhenium−GSH conjugate from a mixture of 20 amino acids has been demonstrated using FSPE. Additionally, the cytotoxicity of complexes **1** and **3** toward HeLa cells has been examined by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay. The cellular uptake properties of complex **3** have also been investigated by laser-scanning confocal microscopy.

Received: May 28, 2011
Published: August 30, 2011

ACS Publications © 2011 American Chemical Society

Chart 1. Structures of the Rhenium(I) Polypyridine Fluorous Complexes



EXPERIMENTAL SECTION

Materials and Synthesis. All solvents were of analytical reagent grade and purified according to standard procedures.[17] 5-Aminonicotinic acid, *N*-hydroxysuccinimide, $AgCF_3SO_3$, KPF_6, $CaCO_3$, thiophosgene, and ethylamine were purchased from Acros. Me_2bpy and $Re(CO)_5Cl$ were obtained from Aldrich. BSA was purchased from Calbiochem. 3-(Perfluorooctyl) propylamine and FSPE columns (2 g, 8 mL tube) were purchased from Fluorous Technologies Inc. The 20 amino acids used in the FSPE purification including alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine were purchased from Aldrich or Acros. All these chemicals were used without further purification. 5-Aminonicotinic acid *N*-hydroxysuccinimidyl ester[14c] and $[Re(Me_2bpy)(CO)_3(CH_3CN)](CF_3SO_3)$[8a] were prepared as described previously. All buffer components were of molecular biology grade. PD-10 size-exclusion columns and YM-30 centricons were purchased from GE Healthcare and Millipore, respectively. GSH and MTT (Sigma) were used without further purification. HeLa cells were obtained from American Type Culture Collection. Dulbecco's modified Eagle's medium (DMEM), MitoTracker Deep Red FM, fetal bovine serum (FBS), phosphate buffered saline (PBS), trypsin−EDTA, and penicillin/streptomycin were purchased from Invitrogen. The growth medium for cell culture contained DMEM with 10% FBS and 1% penicillin/streptomycin.

3-Amino-5-(N-((3-perfluorooctyl)propyl)aminocarbonyl)pyridine, py-Rf-NH$_2$. A mixture of 5-aminonicotinic acid *N*-hydroxysuccinimidyl ester (700 mg, 3 mmol), triethylamine (1.67 mL, 12 mmol), and 3-(perfluorooctyl)propylamine (1.40 g, 3 mmol) in DMF (7 mL) was stirred under an inert atmosphere of nitrogen at room temperature for 12 h. The mixture was then evaporated to dryness under reduced pressure, resulting in a reddish brown solid. The crude product was purified by column chromatography on silica gel with the eluent changing from hexane/ethyl acetate (1:4, *v*/*v*) to CH_2Cl_2/MeOH (10:1, *v*/*v*). The fractions containing the product were collected and the solvent was removed by rotary evaporation to give a white solid. Yield: 1.02 g (57%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, $Si(CH_3)_4$): δ 8.27 (d, J = 1.8 Hz, 1H, H6 of pyridine), 8.13 (d, J = 2.7 Hz, 1H, H2 of pyridine), 7.92 (br, 1H, NH), 7.42 (t, J = 2.4 Hz, 1H, H4 of pyridine), 5.07 (br, 2H, NH_2), 3.49 (t, J = 7.2 Hz, 2H, $NHCH_2CH_2CH_2$), 2.39−2.18 (m, 2H, $NHCH_2CH_2CH_2$), 1.91 (p, J = 6.9 Hz, 2H, $NHCH_2CH_2CH_2$); ^{13}C NMR (75 MHz, acetone-d_6, 298 K, $Si(CH_3)_4$): δ 166.22, 144.41, 139.31, 136.50, 130.84, 118.92, 38.75, 20.81; positive-ion ESI-MS ion clusters at m/z 598 $[M + H]^+$.

*[Re(Me$_2$bpy)(CO)$_3$(py-Rf-NH$_2$)](PF$_6$) (**1**).* A mixture of $[Re(Me_2bpy)(CO)_3(CH_3CN)](CF_3SO_3)$ (0.35 mmol) and py-Rf-NH_2 (209 mg, 0.35 mmol) was refluxed in THF (20 mL) under an inert atmosphere of nitrogen for 12 h. The solution was evaporated to dryness to give a yellow The complex was then converted to the hexafluorophosphate salt by anion exchange with KPF_6 in MeOH and then purified by column chromatography on alumina. The desired product was eluted with CH_2Cl_2/MeOH (20:1, *v*/*v*). Recrystallization of the crude product from CH_2Cl_2/petroleum ether afforded the complex as yellow crystals. Yield: 216 mg (49%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, $Si(CH_3)_4$): δ 9.22 (d, J = 5.7 Hz, 2H, H6 and H6′ of Me_2bpy), 8.61 (s, 2H, H3 and H3′ of Me_2bpy), 8.06 (d, J = 2.4 Hz, 1H, H6 of pyridine), 8.03−7.97 (m, 2H, H2 of pyridine, NH), 7.82 (d, J = 5.7 Hz, 2H, H5 and H5′ of Me_2bpy), 7.53 (t, J = 1.5 Hz, 1H, H4 of pyridine), 5.54 (s, 2H, NH_2), 3.44 (q, J = 6.3 Hz, 2H, $NHCH_2CH_2CH_2$), 2.63 (s, 6H, CH_3 of Me_2bpy), 2.42−2.20 (m, 2H, $NHCH_2CH_2CH_2$), 1.88−1.82 (m, 2H, $NHCH_2CH_2CH_2$); ^{13}C NMR (75 MHz, acetone-d_6, 298 K, $Si(CH_3)_4$): δ 196.09, 192.20, 163.65, 155.84, 154.47, 153.35, 147.15, 139.80, 138.65, 133.64, 129.74, 125.66, 121.56, 38.88, 20.87; IR (KBr) ν/cm^{-1}: 3403 (br, N—H), 2030 (s, C≡O), 1924 (s, C≡O), 1623 (m, C═O), 1241 (m, C—F), 1210 (m, C—F), 1149 (m, C—F), 1030 (m, C—F), 848 (s, PF_6^-); positive-ion ESI-MS ion clusters at m/z 1052 $[M - PF_6]^+$; anal calcd (%) for $C_{32}H_{24}N_5O_4PF_{23}Re$: C, 32.12; H, 2.02; N, 5.85; found: C, 32.35; H, 2.26; N, 5.83.

*[Re(Me$_2$bpy)(CO)$_3$(py-Rf-NCS)](PF$_6$) (**2**).* A mixture of complex **1** (0.16 mmol) and finely crushed $CaCO_3$ (64 mg, 0.64 mmol) was stirred in acetone (20 mL) at room temperature under an inert atmosphere of nitrogen, and thiophosgene (26 μL, 0.34 mmol) was added slowly to the mixture. The suspension was stirred in the dark at room temperature for 5 min. The suspension was filtered, and the filtrate was evaporated to dryness to give a yellow solid. Recrystallization of the crude product from CH_2Cl_2/petroleum ether afforded the complex as yellow crystals. Yield: 200 mg (89%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, $Si(CH_3)_4$): δ 9.31 (d, J = 5.7 Hz, 2H, H6 and H6′ of Me_2bpy), 8.74 (s, 1H, H6 of pyridine), 8.70 (d, J = 1.2 Hz, 1H, H2 of pyridine), 8.62 (s, 2H, H3 and H3′ of Me_2bpy), 8.42 (s, 1H, H4 of pyridine), 8.29 (br, 1H, NH), 7.84 (d, J = 5.7 Hz, 2H, H5 and H5′ of Me_2bpy), 3.49 (t, J = 6.3 Hz, 2H, $NHCH_2CH_2CH_2$), 2.62 (s, 6H, CH_3 of Me_2bpy), 2.40−2.18 (m, 2H, $NHCH_2CH_2CH_2$), 1.90−1.83 (m, 2H, $NHCH_2CH_2CH_2$); ^{13}C NMR (75 MHz, acetone-d_6, 298 K, $Si(CH_3)_4$): δ 195.56, 191.98, 155.94, 154.61, 153.63, 150.55, 149.33, 137.43, 132.23, 129.89, 127.77, 125.83, 20.95; IR (KBr) ν/cm^{-1}: 3423 (br, N—H), 2111 (m, N═C═S), 2036 (s, C≡O), 1931 (s, C≡O), 1671 (m, C═O), 1241 (m, C—F), 1209 (m, C—F), 1149 (m, C—F), 1032 (m, C—F), 847 (s, PF_6^-); positive-ion ESI-MS ion clusters at m/z 1095 $[M - PF_6]^+$; anal calcd (%) for $C_{33}H_{22}N_5O_4SPF_{23}Re \cdot 1.5CH_2Cl_2$: C, 30.33; H, 1.84; N, 5.47; found: C, 30.31; H, 2.08; N, 5.34.

*[Re(Me$_2$bpy)(CO)$_3$(py-Rf-TU-C$_2$H$_5$)](PF$_6$) (**3**).* A mixture of complex **2** (0.08 mmol) and ethylamine (10 μL, 0.16 mmol) was stirred in acetone (15 mL) at room temperature under an inert atmosphere of nitrogen for 2 h. The solution was evaporated to dryness to give a yellow solid, which was purified by column chromatography on alumina. The desired product was eluted with CH_2Cl_2/MeOH (20:1, *v*/*v*). Recrystallization of the crude product from CH_2Cl_2/petroleum ether afforded the complex as yellow crystals. Yield: 86 mg (83%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, $Si(CH_3)_4$): δ 9.31 (s, 1H, H2 of pyridine), 9.21 (d, J = 5.4 Hz, 2H, H6 and H6′ of Me_2bpy), 8.60−8.53 (m, 4H, H4 and H6 of pyridine, H3 and H3′ of Me_2bpy), 8.36 (s, 1H, NH), 7.78 (d, J = 4.8 Hz, 2H, H5 and H5′ of Me_2bpy), 3.47−3.45 (m, 4H, CH_2 of C_2H_5, $NHCH_2CH_2CH_2$), 2.59 (s, 6H, CH_3 of Me_2bpy), 2.39−2.13 (m, 2H, $NHCH_2CH_2CH_2$), 1.88−1.82 (m, 2H, $NHCH_2CH_2CH_2$), 1.40 (t, J = 7.5 Hz, 3H, CH_3 of Et); ^{13}C NMR (75 MHz, acetone-d_6, 298 K, $Si(CH_3)_4$): δ 195.97, 192.50, 162.97, 155.87, 154.34, 153.50, 152.51, 145.84, 132.77, 129.91, 128.30, 125.81, 124.48, 39.04, 20.93, 20.58, 13.43, 12.28; IR (KBr) ν/cm^{-1}: 3391 (br, N—H), 2034 (s, C≡O), 1925 (s, C≡O), 1654 (m, C═O), 1241 (m, C—F), 1210 (m, C—F), 1149 (m, C—F), 1031 (m, C—F), 848 (PF_6^-); positive-ion ESI-MS ion clusters at m/z 1140 $[M - PF_6]^+$; anal calcd (%) for $C_{35}H_{29}N_6O_4SPF_{23}Re$: C, 38.47, H, 2.01, N, 4.98; found: C, 38.43; H, 2.23, N, 4.73.

Instrumentation and Physical Measurements. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 MHz NMR spectrometer at 298 K. Positive-ion ESI mass spectra were recorded on a Perkin-Elmer Sciex API 365 mass spectrometer. IR spectra were recorded on a Perkin-Elmer 1600 series FT-IR spectrophotometer.

dx.doi.org/10.1021/ic201143f |*Inorg. Chem.* 2011, 50, 9465–9471

Elemental analyses were carried out on a Vario EL III CHN elemental analyzer. Electronic absorption and steady-state emission spectra were recorded on a Hewlett-Packard 8453 diode array spectrophotometer and a SPEX FluoroLog 3-TCSPC spectrophotometer, respectively. Emission lifetimes were measured in the Fast MCS mode with a NanoLED N-375 as the excitation source. Unless specified, all the solutions for photophysical studies were degassed with no fewer than four successive freeze−pump−thaw cycles and stored in a 10 cm^3 round-bottomed flask equipped with a side arm 1 cm fluorescence cuvette and sealed from the atmosphere by a Rotaflo HP6/6 quick-release Teflon stopper. Luminescence quantum yields were measured by the optically dilute method with an aerated aqueous solution of $[Ru(bpy)_3]Cl_2$ (Φ_{em} = 0.028, λ_{ex} = 455 nm) as the standard solution.[18,19]

Labeling of BSA with Complex 2. Complex **2** (1.48 mg, 1.2 μmol) in anhydrous DMSO (50 μL) was added to BSA (10 mg, 151 nmol) in 50 mM carbonate buffer (450 μL) at pH 9.7. The suspension was stirred for 12 h in the dark at room temperature, and the solid residue was removed by centrifugation. The supernatant was then diluted to 1.0 mL with 50 mM potassium phosphate buffer at pH 7.4 and loaded onto a PD-10 column equilibrated with the same buffer. The first elution band with strong green emission was collected. The resultant bioconjugate **Re-BSA** was washed successively with potassium phosphate buffer using a YM-30 centricon, concentrated to 1.5 mL, and stored at 4 °C.

Labeling of GSH with Complex 2. Complex **2** (1.05 mg, 0.85 μmol) in anhydrous DMSO (200 μL) was added to GSH (21.5 mg, 70 μmol) in a mixture of H_2O (1.8 mL) and triethylamine (20 μL). The suspension was incubated for 5 min at room temperature, and the solid residue was removed by centrifugation. The supernatant was loaded onto an FSPE column, which had been activated by DMF (5 mL) and preconditioned with H_2O. The column was operated under gradient elution (H_2O to 40% aqueous MeOH). Finally, the resultant bioconjugate **Re-GSH** was eluted using 60% aqueous MeOH.

Isolation of Re-GSH from a Mixture of Amino Acids with FSPE. A mixture of **Re-GSH** (0.7 mM) and the 20 amino acids (each at 0.1 mg/mL) in water (2 mL) was loaded onto an FSPE column (2 g, 8 mL tube), which had been activated by DMF (5 mL) and preconditioned with H_2O. When the column was operated under gradient elution (H_2O to 40% aqueous MeOH), a luminescent band remained at the top and the eluted solution (flow-through fraction) was collected. When the mobile phase was changed to 60% aqueous MeOH, the luminescent band was eluted and collected (elution fraction). The initial mixture, flow-through fraction, and elution fraction were all analyzed by ESI-MS.

MTT Assays. Cells were seeded in a 96-well flat-bottomed microplate (ca. 10 000 cells per well) in growth medium (100 μL) and incubated at 37 °C under a 5% CO_2 atmosphere for 24 h. The rhenium(I) polypyridine complex was dissolved in the growth medium with 1% DMSO, and the solutions were added to the wells. After the microtiter plate was incubated for 48 h, MTT in PBS (5 mg per mL, 10 μL) was added to each well. The microplate was incubated for another 3 h. The medium was removed carefully and isopropanol (200 μL) was added to each well. The microplate was further incubated for 5 min. All the assays were run in parallel with a positive control, in which cisplatin was used as a cytotoxic agent. The absorbance of the solutions at 570 nm was measured with a SPECTRAmax 340 microplate reader (Molecular Devices Corp., Sunnyvale, CA). The IC_{50} values of the complexes were determined from dose dependence of surviving cells after exposure to the complexes for 48 h.

Live-Cell Confocal Imaging. HeLa cells in growth medium were seeded on a sterilized coverslip in a 60-mm tissue culture dish and grown at 37 °C under a 5% CO_2 atmosphere for 48 h. The growth medium was then removed and replaced with medium/DMSO (99:1, *v*/*v*) containing the rhenium(I) polypyridine complex (5 μM). After incubation for 1 h, the medium was removed and the cell layer was washed with PBS (1 mL × 5). The coverslip was mounted onto a sterilized glass slide and then imaged using a Leica TCS SPE confocal microscope with an oil immersion 63× objective and an excitation wavelength at 405 nm. The emission was measured using a long-pass filter at 532 nm. In the colocalization imaging experiments involving MitoTracker Deep Red FM, the excitation wavelength was 633 nm and the emission was measured at >640 nm.

Table 1. Electronic Absorption Spectral Data of Complexes 1−3 in CH_2Cl_2 at 298 K

complex	λ_{abs}/nm (ε/dm^3 mol^{-1} cm^{-1})
1	254 (25 230), 308 sh (12 050), 318 (12 305), 337 sh (7405), 393 sh (1960)
2	281 (19 815), 303 sh (14 320), 317 (11 220), 355 sh (3765), 379 sh (2130)
3	256 (24 390), 279 sh (20 780), 318 sh (9895), 337 sh (5420) 385 sh (2045)

RESULTS AND DISCUSSION

Complex Design. The design of the rhenium(I) polypyridine fluorous complexes is based on the use of 5-aminonicotinic acid; the carboxyl group of which can be functionalized with a fluorous chain, the amine group can be readily converted to the amine-specific isothiocyanate group, and the pyridine can be coordinated to the rhenium(I) center. Thus, the ligand py-Rf-NH_2 was obtained from the reaction of 3-(perfluorooctyl)propylamine with 5-aminonicotinic acid *N*-hydroxysuccinimidyl ester in DMF at room temperature. The amine complex **1** was synthesized from the reaction of $[Re(Me_2bpy)(CO)_3(CH_3CN)](CF_3SO_3)$ with py-Rf-NH_2 in refluxing THF, followed by anion exchange with KPF_6 and column chromatographic purification. The isothiocyanate complex **2** was prepared from the reaction of complex **1** with thiophosgene in acetone. The amine reactivity of complex **2** was investigated by reacting with a model substrate ethylamine, yielding the thiourea complex **3**. All the complexes were characterized by ^1H and ^{13}C NMR, positive-ion ESI-MS, IR spectroscopy and gave satisfactory elemental analyses. The IR spectra of these complexes showed absorption bands from ca. 1240 to 1100 cm^{-1}, which have been assigned to C—F stretching of the fluorous moiety. The isothiocyanate group of complex **2** was associated with an absorption peak at ca. 2110 cm^{-1}, typical of N═C═S stretching. The moderately intense absorption band at ca. 850 cm^{-1} has been assigned to the P—F stretching of the PF_6^- ion. All three fluorous complexes were yellow in color and very soluble in common organic solvents such as CH_2Cl_2, CH_3CN, and alcohols but sparingly soluble in aqueous solution.

Electronic Absorption and Emission Spectroscopy. The electronic absorption spectral data of complexes **1**−**3** in CH_2Cl_2 at 298 K are summarized in Table 1. The absorption spectra of the complexes are characterized by high energy intraligand ($\pi \rightarrow \pi^*$) (Me_2bpy and pyridine) and low-energy metal-to-ligand charge-transfer (MLCT) ($d\pi(Re) \rightarrow \pi^*(Me_2bpy)$) features at ca. 250−320 and 340−390 nm, respectively. Irradiation of the complexes in fluid solutions at 298 K and *n*-butyronitrile glass at 77 K resulted in intense and long-lived green to yellow emission. The photophysical data are listed in Table 2. The emission of these complexes has been assigned to a ^{3}MLCT ($d\pi(Re) \rightarrow \pi^*(Me_2bpy)$) excited state.[4a,5−15] The

dx.doi.org/10.1021/ic201143f |*Inorg. Chem.* 2011, 50, 9465–9471

Table 2. Photophysical Data of Complexes 1−3

complex	medium (T/K)	λ_{em}/nm	τ_o/μs	Φ
1	CH_2Cl_2 (298)	533	0.70	0.20
	CH_3CN (298)	546	0.35	0.065
	glass (77)[a]	496	4.97	
2	CH_2Cl_2 (298)	526	0.68	0.17
	CH_3CN (298)	539	0.31	0.047
	glass (77)[a]	490	4.55	
3	CH_2Cl_2 (298)	528	0.79	0.098
	CH_3CN (298)	538	0.25	0.014
	glass (77)[a]	490	4.61	

[a] In *n*-butyronitrile glass.

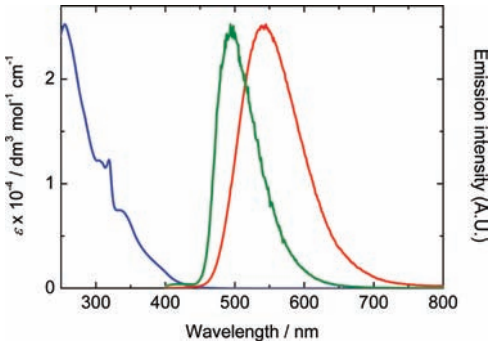


Figure 1. Electronic absorption spectrum in CH_2Cl_2 (blue) and emission spectra of complex **1** in CH_3CN at 298 K (red) and in *n*-butyronitrile (green) at 77 K.

emission energies of the complexes followed the order: **1** < **2** ≈ **3**. The occurrence of lower emission energy for complex **1** has been attributed to its electron-donating amine group, which enriched the electron density of the metal center and hence destabilized the dπ(Re) levels. The absorption and emission spectra of complex **1** are shown in Figure 1 as an example.

Labeling of BSA and GSH with Complex 2. As the isothiocyanate complex **2** has been demonstrated to be reactive toward ethylamine, it was used to label a model protein BSA. The resultant bioconjugate **Re-BSA** was isolated and purified by size-exclusion chromatography and ultrafiltration. Upon irradiation, the bioconjugate exhibited intense and long-lived green emission in degassed 50 mM potassium phosphate buffer at pH 7.4. The electronic absorption and emission spectra of the bioconjugate in buffer are shown in Figure 2. We have assigned the emission band at 523 nm to a ^{3}MLCT excited state.[4a,5−15] The observed biexponential decay (τ_1 = 0.17 μs (60%), τ_2 = 0.54 μs (40%)) of the emission is not uncommon for biomolecules labeled with luminescent transition metal complexes.[14] On the basis of the spectroscopic data, the rhenium-to-BSA ratio was determined to be ca. 4.1, which is comparable to those of other rhenium−protein conjugates (ca. 1.8−4.8).[14] Thus, despite the rigid and bulky fluorous chain, the rhenium(I) polypyridine isothiocyanate complex **2** is capable of functioning as a luminescent fluorous labeling reagent. We have also tagged the small peptide GSH with the same complex. The resultant bioconjugate **Re-GSH** was purified by FSPE and characterized by ESI-MS (m/z = 1401). Upon irradiation, the bioconjugate showed intense and long-lived yellow emission (λ_{em} = 565 nm, τ_o = 0.43 μs) in degassed 60% aqueous MeOH (Figure 3),

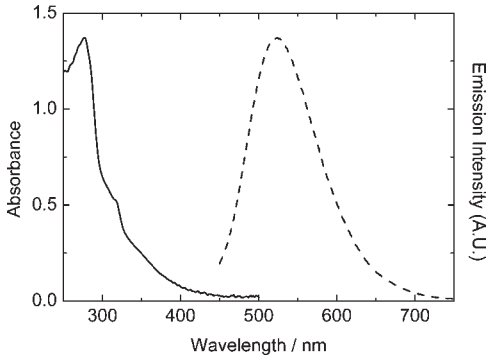


Figure 2. Electronic absorption (solid line) and emission (dashed line) spectra of **Re-BSA** in 50 mM phosphate buffer at pH 7.4 at 298 K.

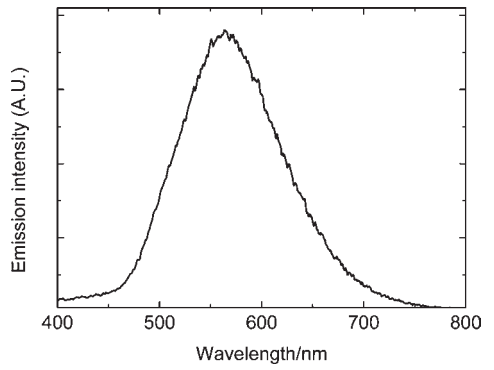


Figure 3. Emission spectrum of **Re-GSH** in degassed 60% aqueous MeOH at 298 K.

which has been assigned to a ^{3}MLCT excited state.[4a,5−15] Interestingly, unlike **Re-BSA**, the modified glutathione exhibited single exponential decay. Also, the emission energy of **Re-GSH** (565 nm) was lower than that of its BSA counterpart (523 nm). It is conceivable that the double exponential decay and higher emission energy of **Re-BSA** are a consequence of the hydrophobic local environment of the luminescent complex.[14]

Isolation of Re-GSH from a Mixture of Amino Acids with FSPE. To study the potential use of the fluorous labeling reagent complex **2** in biomolecular purification and enrichment,[2a] the isolation of **Re-GSH** from a mixture of amino acids has been performed. In this experiment, a mixture of **Re-GSH** and 20 amino acids was loaded onto an FSPE column, which was then washed with 40% aqueous MeOH. As expected, the flow-through fraction was not emissive upon irradiation. Importantly, changing the mobile phase to 60% aqueous MeOH led to the elution of a luminescent species. The ESI mass spectra of (a) the initial mixture, (b) flow-through fraction with 40% aqueous MeOH, and (c) elution fraction with 60% aqueous MeOH are shown in Figure 4. The ESI mass spectrum of the elution fraction (Figure 4c) revealed a peak at m/z = 1401, corresponding to **Re-GSH**. This signal was absent in the mass spectrum of the flow-through fraction (Figure 4b), meaning that the 20 unlabeled amino acids were washed out in the flow-through fraction whereas the fluorous conjugate **Re-GSH** was retained on the FSPE column due to highly selective fluorous−fluorous interactions.

Cytotoxic Activity and Cellular Uptake. The cytotoxic activity and cellular uptake behavior of luminescent rhenium(I) polypyridine complexes have received much attention recently.[12a,13,14c,15f−15h] In

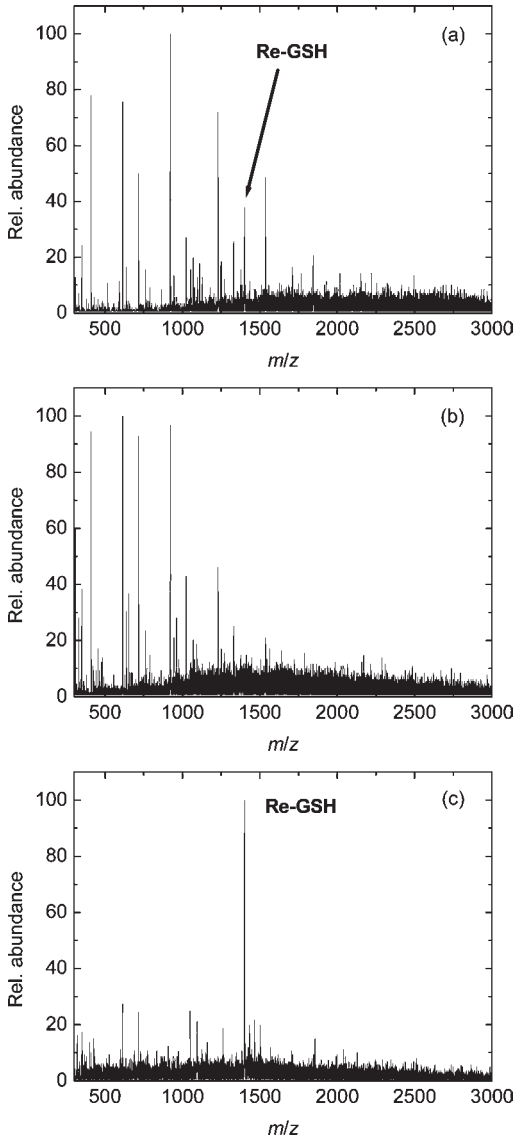


Figure 4. ESI mass spectra of (a) **Re-GSH** and a mixture of 20 amino acids in water, (b) flow-through fraction with 40% aqueous MeOH, and (c) elution fraction with 60% aqueous MeOH from an FSPE column.

this work, the cytotoxicity of the amine complex **1** and the thiourea complex **3** has been examined by the MTT assay using HeLa cells as a model cell line.[20] Complex **2** was omitted in this study because the isothiocyanate group is unstable in aqueous solution. The 48-h IC_{50} value of complex **1** (ca. 8.70 μM) was significantly lower than that of cisplatin (ca. 17.80 μM), indicative of high cytotoxicity. Additionally, the IC_{50} value of complex **3** (ca. 17.02 μM) was similar to those of related rhenium(I) thiourea complexes (IC_{50} = 17.5−28.5 μM)[14c] but slightly higher that of complex **1**. Thus, the conversion of the amine group of complex **1** to the thiourea group has rendered this complex more biocompatible. It is important to mention that the cytotoxicity of these complexes can be readily modified with a choice of ligands with different lipophilicity, and the use of poly(ethylene glycol) is known to substantially reduce the cytotoxic activity of related transition metal complexes.[21]

The cellular uptake and intracellular localization of complex **3**, which can be considered as a model of fluorous-labeled biomolecules, have been investigated. Incubation of HeLa cells with the complex

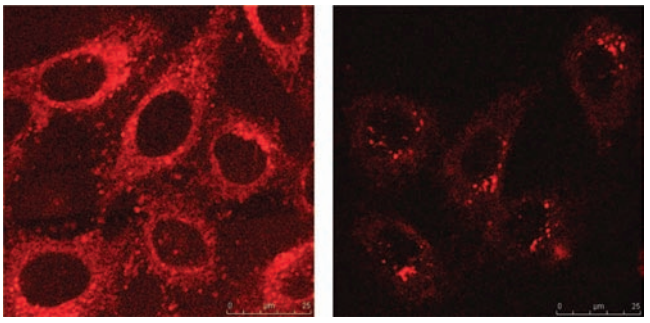


Figure 5. Laser-scanning confocal microscopy images of HeLa cells (pseudocolored) incubated with complex **3** (5 μM) at 37 °C (left) and 4 °C (right) for 1 h.

(5 μM) at 37 °C under a 5% CO_2 atmosphere for 1 h resulted in cellular uptake, as revealed by laser-scanning confocal microscopy (Figure 5, left). The complex was not homogeneously distributed within the cytoplasm but localized in the juxtanuclear region with bright punctate staining. In contrast, the nuclei showed much weaker emission, indicative of negligible nuclear uptake. Incubation at 4 °C led to much reduced uptake efficiency (Figure 5, right), suggesting that the internalization occurred through an energy-requiring pathway such as endocytosis.[22] Most of the punctate emissive dots are presumably due to staining of mitochondria, which are prevalent around the nucleus. To confirm this, HeLa cells pretreated with complex **3** (5 μM, 1 h, λ_{ex} = 405 nm) were coincubated with MitoTracker Deep Red FM (100 nM, 20 min, λ_{ex} = 633 nm), whose spectral properties do not interfere. The significant overlap in the image shown in Figure 6 indicates that the complex was predominantly enriched in mitochondria. The mitochondrial-targeting properties of the complex can be attributed to its cationic charge and highly hydrophobic nature.[13,14c,15f−h] In view of the fact that the MitoTracker only stains mitochondria in living cells, the cells incubated with the complex remained viable under our experimental conditions. These findings highlight that small molecules conjugated with the fluorous isothiocyanate complex **2** can be readily followed and examined by confocal microscopy. This strategy is particularly useful for those molecules that cannot penetrate living cells on their own.

■ CONCLUSION

The main theme of this work is the development of rhenium(I) complexes as novel trifunctional biological probes that have luminescence properties for detection, a fluorous moiety for selective isolation and interactions, and a reactive functional group for bioconjugation. Since the development of fluorous labeling reagents was reported several years ago,[2a] there is an increasing interest in using these reagents in proteomics, metabolomics, and microarray studies.[2,3] Not only can the fluorous-tagged molecules be separated and enriched on FSPE, but also they possess excellent mass spectrometry characteristics, enabling efficient characterization. In addition to these properties, the rhenium(I) complexes in this work offer interesting luminescence behavior to the labeled molecules, which would allow new assay design, easy quantitation of degree of fluorous modification of the target molecules, and the possibility of studying the cellular uptake of the labeled molecules by confocal microscopy. Indeed, we have demonstrated that (1) biomolecules can be readily labeled with the isothiocyanate complex **2** to afford luminescent

dx.doi.org/10.1021/ic201143f |*Inorg. Chem.* 2011, 50, 9465–9471

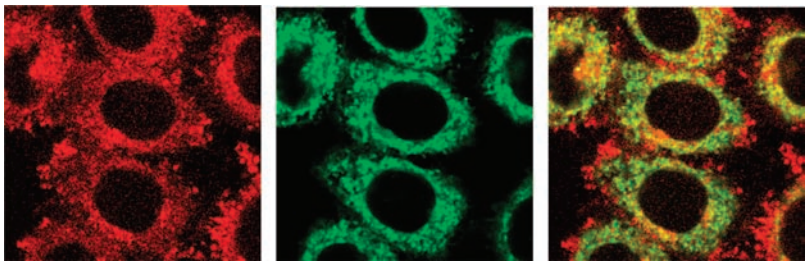


Figure 6. Laser-scanning confocal microscopy images of HeLa cells (pseudocolored) upon incubation successively with complex **3** (5 μM, 1 h, λ_{ex} = 405 nm, left) at 37 °C and MitoTracker Deep Red FM (100 nM, 20 min, λ_{ex} = 633 nm, middle). The overlaid confocal images are shown on the right.

bioconjugates and (2) the separation and purification of the GSH bioconjugate can be performed readily on an FSPE column. Although the emission behavior and cellular uptake properties of many luminescent rhenium(I) complexes have been reported in the literature, the fluorous moiety has offered new and unique properties to the new complexes in this work, rendering them novel trifunctional biological probes. The design of related luminescent transition metal fluorous complexes as a handle to isolate and identify intracellular biological receptors is underway.

AUTHOR INFORMATION

Corresponding Author

*E-mail: bhkenlo@cityu.edu.hk. Fax: (+852) 3442 0522. Tel: (+852) 3442 7231.

ACKNOWLEDGMENT

We thank The Hong Kong Research Grants Council (Project No. CityU 102109) and City University of Hong Kong (Project No. 7002679) for financial support. M.-W.L. acknowledges the receipt of a Postgraduate Studentship, a Research Tuition Scholarship, and an Outstanding Academic Performance Award all administered by City University of Hong Kong.

REFERENCES

(1) (a) Horváth, I. T.; Rábai, J. *Science* **1994**, *266*, 72–75. (b) *Handbook of Fluorous Chemistry*; Gladysz, J. A., Curran, D. P., Horváth, I. T., Eds.; Wiley-VCH: Weinheim, Germany, 2004. (c) Curran, D. P.; Zhang, W. *Tetrahedron* **2006**, *62*, 11837–11865.

(2) (a) Brittain, S. M.; Ficarro, S. B.; Brock, A.; Peters, E. C. *Nat. Biotechnol.* **2006**, *23*, 463–468. (b) Go, E. P.; Uritboonthai, W.; Apon, J. A.; Trauger, S. A.; Nordstrom, A.; O'Maille, G.; Brittain, S. M.; Peters, E. C.; Siuzdak, G. *J. Proteome Res.* **2007**, *6*, 1492–1499. (c) Beller, C.; Bannwarth, W. *Helv. Chim. Acta* **2005**, *88*, 171–179. (d) Li, Y.; Arigi, E.; Eichert, H.; Levery, S. B. *J. Mass Spectrom.* **2010**, *45*, 504–519.

(3) (a) Filippov, D. V.; van Zoelen, D. J.; Oldfield, S. P.; van der Marel, G. A.; Overkleeft, H. S.; Drijfhout, J. W.; van Boom, J. H. *Tetrahedron Lett.* **2002**, *43*, 7809–7812. (b) Matsugi, M.; Hasegawa, M.; Sadachika, D.; Okamoto, S.; Tomioka, M.; Ikeya, Y.; Masuyama, A.; Mori, Y. *Tetrahedron Lett.* **2007**, *48*, 4147–4150.

(4) (a) Dunn, A. R.; Belliston-Bittner, W.; Winkler, J. R.; Getzoff, E. D.; Stuehr, D. J.; Gray, H. B. *J. Am. Chem. Soc.* **2005**, *127*, 5169–5173. (b) Mancino, G.; Ferguson, A. J.; Beeby, A.; Long, N. J.; Jones, T. S. *J. Am. Chem. Soc.* **2005**, *127*, 524–525. (c) Jelliss, P. A.; Minteer, S. D.; Patel, M.; Siemiarczuk, A.; Watt, M.; Winter, R. E. K. *J. Mater. Chem.* **2009**, *18*, 2104–2111. (d) Li, Y.-T.; Chen, H.-H.; Babu, B. H.; Hsieh, Y.-Y.; Chen, S.-H. *Appl. Surf. Sci.* **2010**, *256*, 6908–6913. (e) Lim, J.; Swager, T. M. *Angew. Chem., Int. Ed.* **2010**, *49*, 7486–7488. (f) Leung, S.-K; Liu, H.-W.; Lo, K. K.-W. *Chem. Commun.* **2011**, DOI: 10.1039/C1CC11423A.

(5) (a) Busby, M.; Gabrielsson, A.; Matousek, P.; Towrie, M.; Di Bilio, A. J.; Gray, H. B.; Vlček, A., Jr. *Inorg. Chem.* **2004**, *43*, 4994–5002. (b) Gabrielsson, A.; Matousek, P.; Towrie, M.; Hartl, F.; Záliš, S.; Vlček, A., Jr. *J. Phys. Chem. A* **2005**, *109*, 6147–6153. (c) Vlček, A., Jr. *Top. Organomet. Chem.* **2010**, *29*, 73–114. (d) Blanco-Rodríguez, A. M.; Towrie, M.; Sýkora, J.; Záliš, S.; Vlček, A., Jr. *Inorg. Chem.* **2011**, *50*, 6122–6134.

(6) (a) Sun, S.-S.; Lees, A. J. *Organometallics* **2002**, *21*, 39–49. (b) Sun, S.-S.; Lees, A. J. *Coord. Chem. Rev.* **2002**, *230*, 171–192. (c) Sun, S.-S.; Lees, A. J.; Zavalij, P. Y. *Inorg. Chem.* **2003**, *42*, 3445–3453. (d) Kumar, A.; Sun, S.-S.; Lees, A. J. *Top. Organomet. Chem.* **2010**, *29*, 1–35.

(7) (a) Metcalfe, C.; Webb, M.; Thomas, J. A. *Chem. Commun.* **2002**, 2026–2027. (b) Foxon, S. P.; Phillips, T.; Gill, M. R.; Towrie, M.; Parker, A. W.; Webb, M.; Thomas, J. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 3686–3688.

(8) (a) Caspar, J. V.; Meyer, T. J. *J. Phys. Chem.* **1983**, *87*, 952–957. (b) Walters, K. A.; Dattelbaum, D. M.; Ley, K. D.; Schoonover, J. R.; Meyer, T. J.; Schanze, K. S. *Chem. Commun.* **2001**, 1834–1835. (c) Dattelbaum, D. M.; Martin, R. L.; Schoonover, J. R.; Meyer, T. J. *J. Phys. Chem. A* **2004**, *108*, 3518–3526. (d) Patrocinio, A. O. T.; Brennaman, M. K.; Meyer, T. J.; Murakami Iha, N. Y. *J. Phys. Chem. A* **2010**, *114*, 12129–12137.

(9) (a) Wenger, O. S.; Henling, L. M.; Day, M. W.; Winkler, J. R.; Gray, H. B. *Inorg. Chem.* **2004**, *43*, 2043–2048. (b) Belliston-Bittner, W.; Dunn, A. R.; Nguyen, Y. H. L.; Stuehr, D. J.; Winkler, J. R.; Gray, H. B. *J. Am. Chem. Soc.* **2005**, *127*, 15907–15915.

(10) (a) Sacksteder, L.; Lee, M.; Demas, J. N.; DeGraff, B. A. *J. Am. Chem. Soc.* **1993**, *115*, 8230–8238. (b) Hueholt, B. B.; Xu, W.-Y.; Sabat, M.; DeGraff, B. A.; Dema, J. N. *J. Fluoresc.* **2007**, *17*, 522–527.

(11) (a) Villegas, J. M.; Stoyanov, S. R.; Huang, W.; Rillema, D. P. *Dalton Trans.* **2005**, 1042–1051. (b) Villegas, J. M.; Stoyanov, S. R.; Huang, W.; Rillema, D. P. *Inorg. Chem.* **2005**, *44*, 2297–2309. (c) Kirgan, R. A.; Sullivan, B. P.; Rillema, D. P. *Top. Curr. Chem.* **2007**, *281*, 45–100.

(12) (a) Stephenson, K. A.; Banerjee, S. R.; Besanger, T.; Sogbein, O. O.; Levadala, M. K.; McFarlane, N.; Lemon, J. A.; Boreham, D. R.; Maresca, K. P.; Brennan, J. D.; Babich, J. W.; Zubieta, J.; Valliant, J. F. *J. Am. Chem. Soc.* **2004**, *126*, 8598–8599. (b) Banerjee, S. R.; Schaffer, P.; Babich, J. W.; Valliant, J. F.; Zubieta, J. *Dalton Trans.* **2005**, 3886–3897. (c) Wei, L.; Babich, J.; Eckelman, W. C.; Zubieta, J. *Inorg. Chem.* **2005**, *44*, 2198–2209. (d) Wei, L.; Babich, J.; Zubieta, J. *Inorg. Chim. Acta* **2005**, *358*, 3691–3700. (e) James, S.; Maresca, K. P.; Babich, J. W.; Valliant, J. F.; Doering, L.; Zubieta, J. *Bioconjugate Chem.* **2006**, *17*, 590–596.

(13) (a) Amoroso, A. J.; Coogan, M. P.; Dunne, J. E.; Fernández-Moreira, V.; Hess, J. B.; Hayes, A. J.; Lloyd, D.; Millet, C.; Pope, S. J. A.; Williams, C. *Chem. Commun.* **2007**, 3066–3068. (b) Amoroso, A. J.; Arthur, R. J.; Coogan, M. P.; Court, J. B.; Fernández-Moreira, V.; Hayes, A. J.; Lloyd, D.; Millet, C.; Pope, S. J. A. *New J. Chem.* **2008**, *32*, 1097–1102. (c) Fernández-Moreira, V.; Thorp-Greenwood, F. L.; Coogan, M. P. *Chem. Commun.* **2010**, *46*, 186–202. (d) Fernández-Moreira, V.; Thorp-Greenwood, F. L.; Amoroso, A. J.; Cable, J.; Court, J. B.; Gray, V.; Hayes, A. J.;

Jenkins, R. L.; Kariuki, B. M.; Lloyd, D.; Millet, C. O.; Williams, C. F.; Coogan, M. P. *Org. Biomol. Chem.* **2010**, *8*, 3888–3901.

(14) (a) Lo, K. K.-W.; Ng, D. C.-M.; Hui, W.-K.; Cheung, K.-K. *J. Chem. Soc., Dalton Trans.* **2001**, 2634–2640. (b) Lo, K. K.-W.; Hui, W.-K.; Ng, D. C.-M.; Cheung, K.-K. *Inorg. Chem.* **2002**, *41*, 40–46. (c) Lo, K. K.-W.; Louie, M.-W.; Sze, K.-S.; Lau, J. S.-Y. *Inorg. Chem.* **2008**, *47*, 602–611.

(15) (a) Lo, K. K.-W.; Lau, J. S.-Y.; Fong, V. W.-Y. *Organometallics* **2004**, *23*, 1098–1106. (b) Lo, K. K.-W.; Tsang, K. H.-K. *Organometallics* **2004**, *23*, 3062–3070. (c) Lo, K. K.-W.; Tsang, K. H.-K.; Hui, W.-K.; Zhu, N. *Inorg. Chem.* **2005**, *44*, 6100–6110. (d) Lo, K. K.-W.; Tsang, K. H.-K.; Zhu, N. *Organometallics* **2006**, *25*, 3220–3227. (e) Lo, K. K.-W.; Tsang, K. H.-K.; Sze, K.-S.; Chung, C.-K.; Lee, T. K.-M.; Zhang, K. Y.; Hui, W.-K.; Li, C.-K.; Lau, J. S.-Y.; Ng, D. C.-M.; Zhu, N. *Coord. Chem. Rev* **2007**, *251*, 2292–2310. (f) Louie, M.-W.; Liu, H.-W.; Lam, M. H.-C.; Lau, T.-C.; Lo, K. K.-W. *Organometallics* **2009**, *28*, 4297–4307. (g) Louie, M.-W.; Lam, M. H.-C.; Lo, K. K.-W. *Eur. J. Inorg. Chem.* **2009**, 4265–4273. (h) Louie, M.-W.; Liu, H.-W.; Lam, M. H.-C.; Lam, Y.-W.; Lo, K. K.-W. *Chem.—Eur. J.* **2011**, *17*, 8304–8308. (i) Lo, K. K.-W.; Zhang, K. Y.; Li, S. P.-Y. *Eur. J. Inorg. Chem.* **2011**, 3551–3568.

(16) (a) Wallrabe, H; Periasamy, A. *Curr. Opin. Biotech.* **2005**, *16*, 19–27. (b) Suhling, K.; French, P. M. W.; Phillips, D. *Photochem. Photobiol. Sci.* **2005**, *4*, 13–22.

(17) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: New York, 1988.

(18) Crosby, G. A.; Demas, J. N. *J. Phys. Chem.* **1971**, *75*, 991–1024.

(19) Nakamura, K. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2697–2705.

(20) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.

(21) Li, S. P.-Y.; Liu, H.-W.; Zhang, K. Y.; Lo, K. K.-W. *Chem.—Eur. J.* **2010**, *16*, 8329–8339.

(22) Reaven, E.; Tsai, L.; Azhar, S. *J. Biol. Chem.* **1996**, *271*, 16208–16217.