Inorganic Chemistry

Article

pubs.acs.org/IC

Isostructural Nuclear and Luminescent Probes Derived From Stabilized [2 + 1] Rhenium(I)/Technetium(I) Organometallic Complexes

Tamil Selvi Pitchumony,[†] Laura Banevicius,[‡] Nancy Janzen,[†] Jon Zubieta,[§] and John F. Valliant*[,†,∥]

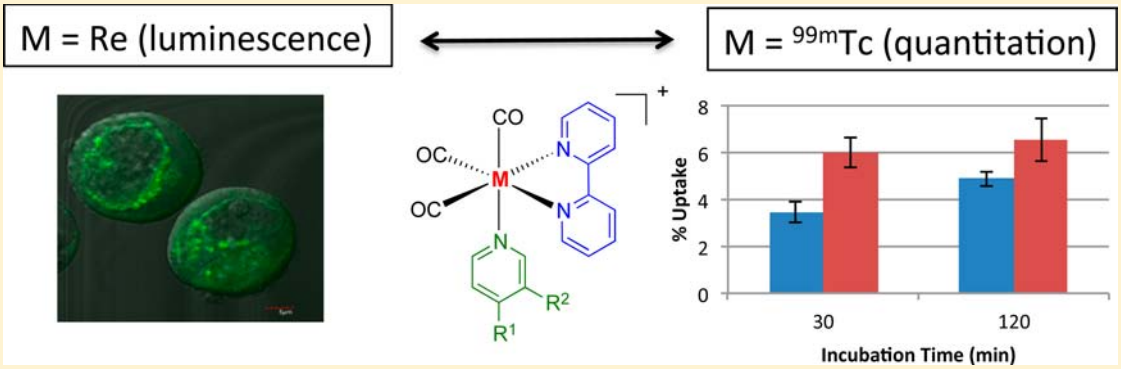
[†]Department of Chemistry and Chemical Biology, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4M1, Canada

[‡]Department of Medical Physics and Applied Radiation Sciences, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4M1, Canada

[§]Department of Chemistry, Syracuse University, Syracuse, New York 13244-4100, United States

[∥]Centre for Probe Development and Commercialization, 1280 Main Street West, Hamilton, Ontario L8S 4M1, Canada

Supporting Information



ABSTRACT: A convenient method to prepare ^{99m}Tc analogues of a class of rhenium(I) luminophores was developed, creating isostructural pairs of nuclear and optical probes. A two-step procedure and a new one-pot procedure were used to produce a series of [2 + 1] complexes of the type $[Tc(CO)_3(bipy)L]^+$ in greater than 80% yield. The plasma stability of the reported compounds was evaluated, where the basicity of the monodentate pyridine type ligand (L) has a significant impact with half-lives ranging from 2 to 20 h. The ability to generate the radioactive complexes makes it possible to quantitate cell uptake of Re luminophores, which was demonstrated in MCF-7 breast cancer cells using ^{99m}Tc analogues of two Re(I)-based mitochondrial targeting dyes.

INTRODUCTION

The interest in molecular imaging probes that can be used with multiple imaging modalities is being spurred on by the increasing availability of hybrid imaging systems and the enhanced information content that can be derived by employing more than one modality.[1−6] The ability to combine and/or contrast data from complementary imaging technologies creates an opportunity to leverage the strengths and overcome limitations of individual methods. Correlating nuclear and fluorescence imaging for instance makes it possible to relate deep-tissue whole-body imaging with histopathology and fluorescence microscopy, providing a convenient way to validate the mechanism of binding, selectivity, and cellular distribution of targeted radiopharmaceuticals.

To this end molecular imaging probes are being developed whereby targeting vectors are being derivatized with both fluorophore- and radionuclide-containing prosthetic groups. This way a single construct can be used to generate fluorescent and nuclear (positron emission tomography or single photon emission computed tomography) images. The challenge in developing probes of this type is that the associated prosthetic groups are unto themselves rarely innocent in that they typically have a significant impact on the ability of the targeting vector to localize in the desired region/organ in vivo and bind to the molecular target of interest. It requires extensive optimization and resources to find the right combination of linker types and sites for derivatization, and in many cases the need to add two prosthetic groups limits the type and size of vectors that can be used.

An alternative strategy is to use radiolabeled dyes or chelates which form a luminescent complex with a nonradioactive metal and an isostructural complex with a radioactive isotope of that same element or its congenor (for second- and third-row

Received: July 30, 2013
Published: November 14, 2013

   dx.doi.org/10.1021/ic401972g | *Inorg. Chem.* 2013, 52, 13521−13528

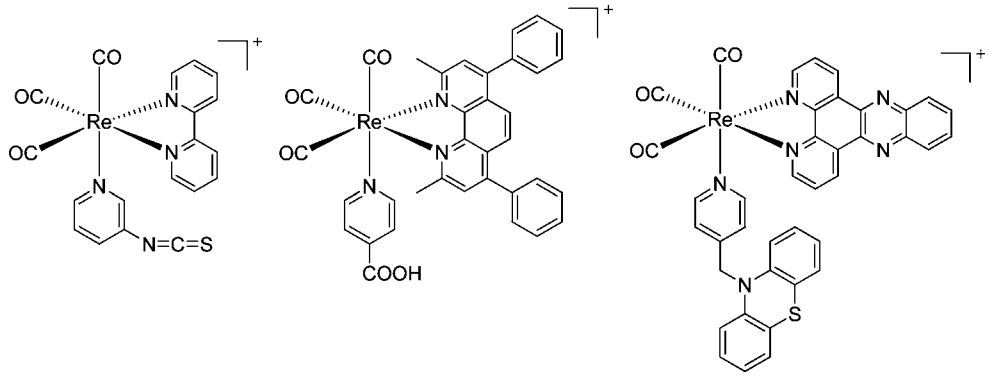
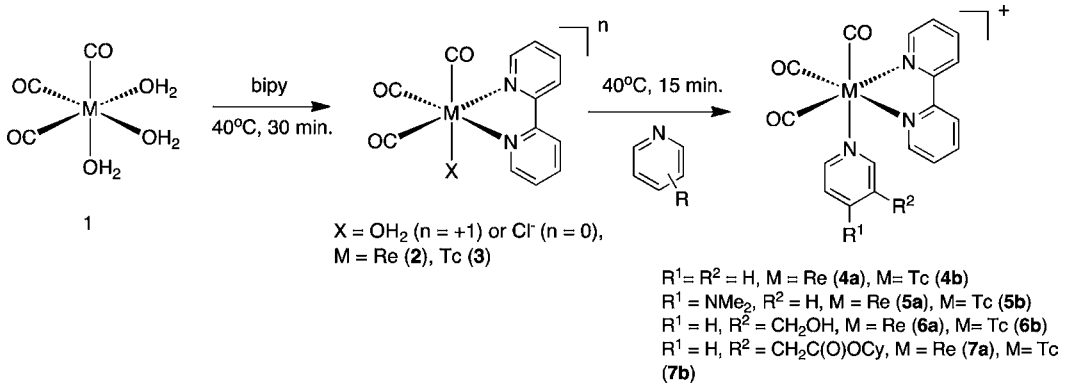


Figure 1. Examples of previously reported [2 + 1] type luminophores based on pyridine complexes of the $[Re(CO)_3]^+$ core.

Scheme 1. Synthesis of the Isostructural [2 + 1] Re(I) and ^{99m}Tc Complexes[a]



[a]Note that the conditions shown are specifically for the reactions run at the tracer level.

transition metals).[7−12] The advantage here is that only one bioconjugate containing a single prosthetic group needs to be developed and optimized to generate both classes of imaging probes. Complexes derived from Re(I)/^{99m}Tc(I) are particularly attractive because of the widespread use, low cost, and low dose burden of ^{99m}Tc. Quinoline-based ligand systems, for instance, have been used to prepare luminescent and radioactive Re/Tc probes for a number of applications imaging prostate cancer and infection and for monitoring neurosphere transplants.[13−16] Unfortunately, Re complexes of this type possess low quantum yields and their absorbance (peak at 301 nm) and emission (425 and 580 nm) properties are not ideal for cell and tissue imaging. Furthermore, the ligands generally require heating or long reaction times to form the desired ^{99m}Tc complexes.

Re(I)−tricarbonyl complexes derived from one bidentate polypyridine type donor and one monodentate pyridine type ligand (Figure 1) have been used extensively as ion sensing and cell imaging agents.[17−25] These transition-metal luminophores have a number of attractive optical imaging properties but have not been widely investigated as cores for developing radioactive probes because of concerns about premature loss of the monodentate ligand in vivo and potential complexities of the radiochemical synthesis, which could hinder clinical translation.[26−28] Our hypothesis was that, with the appropriate choice of monodentate ligand and the development of a convenient radiochemical method, compounds of the type $[M(CO)_3(bipy)L]^n$ (where M = Re, ^{99m}Tc, bipy = 2,2′-bipyridine, and L= monodentate ligand) could be used to create a new class of isostrucural luminescent and nuclear probes. These complexes should possess desirable optical imaging properties, have low dose consequences when used in vivo, and would possess the synthetic versatility needed to create structurally and functionally diverse high-affinity multimodal molecular imaging probes.[29−31]

RESULTS AND DISCUSSION

Radiochemistry. The initial goal was to prepare a series of technetium analogues of $[Re(CO)_3(bipy)(L)]^+$ in which the basicity of the monodentate ligand was varied to assess the overall impact of the complex on stability. Reference standards **2** and **4a−7a** were prepared following an established methodology in good yields (Scheme 1).[32,33] The rhenium complexes were initially synthesized as the triflate salts but were converted to the corresponding chlorides using an ion-exchange resin so that they would be identical with the ^{99m}Tc analogues which are prepared in the presence of saline.

The corresponding ^{99m}Tc complexes were synthesized from $[^{99m}Tc(CO)_3(H_2O)_3]^+$, which can be produced in a single step from TcO_4^- following the method developed by Alberto et al.[34,35] $[^{99m}Tc(CO)_3(bipy)Cl]$ (**3**) was synthesized in high yield by adding $[^{99m}Tc(CO)_3(H_2O)_3]^+$ to a vial containing bipyridine and heating the reaction mixture to 40 °C. After 15 min the reaction was nearly complete, showing <10% $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and a single new product formed whose HPLC retention time matched that of $[Re(CO)_3(bipy)Cl]$. Following optimization of pH, temperature, and reaction time, quantitative formation of **3** can be achieved in 30 min at 40 °C (Figure 2).

dx.doi.org/10.1021/ic401972g | Inorg. Chem. 2013, 52, 13521−13528

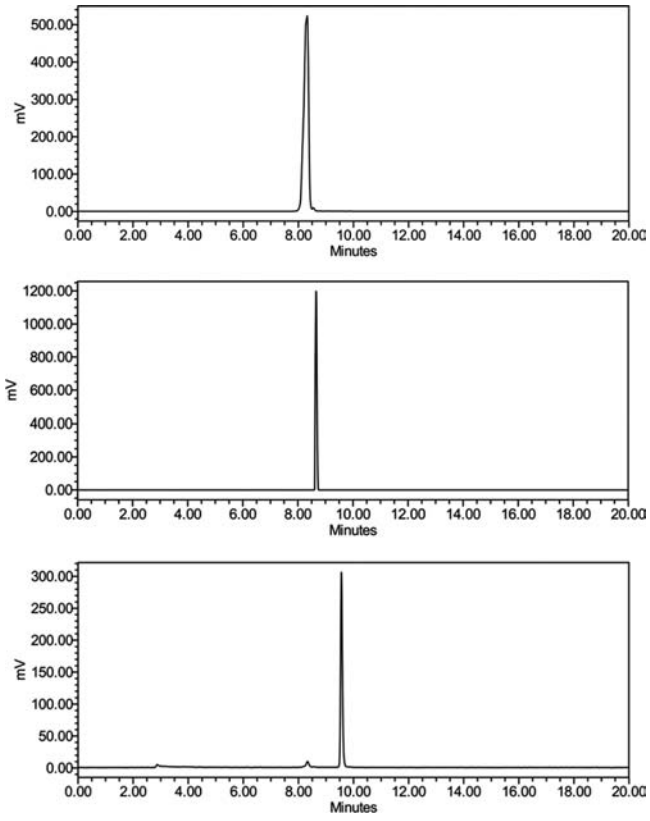


Figure 2. Gamma HPLC traces of (top) $[^{99m}Tc(CO)_3(bipy)Cl]$ (**3**), (middle) $[^{99m}Tc(CO)_3(bipy)(py)]^+$ (**4b**), and (bottom) $[^{99m}Tc(CO)_3(bipy)(DMAP)]^+$ (**5b**).

The ^{99m}Tc analogue of $[Re(CO)_3(bipy)(py)]^+$ (**4a**) was prepared by adding the solution of **3** to pyridine and heating for a further 15 min at 40 °C. The formation of the desired product was achieved in quantitative yield, and there was no evidence of residual starting material under these conditions (Figure 2). The product could be readily isolated by solid-phase extraction (SPE) or semipreparative HPLC free from residual ligand.

These synthesis conditions were equally effective in producing different pyridine derivatives, including the 4-dimethylaminopyridine (DMAP; **5b**), 3-pyridylmethanol (PM; **6b**), and cyclohexyl nicotinate ester (CHN; **7b**) complexes. These ligands were selected as a preliminary assessment of the impact of the basicity of the pyridine ligand on the stability of the complex and because **6a** and **7a** have been used as cell imaging reagents and were reported to exhibit uptake into specific cellular compartments (vide infra).[33]

An attempt was also made to produce a one-pot labeling method whereby both the bidentate and monodentate ligands are present in the reaction mixture at the same time. Here the kinetics of the reaction and tendency of the two ligands to coordinate at different pH values was exploited. $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was added to a solution containing bipy and DMAP at pH 2 and the mixture heated to 40 °C. After 15 min the pH was adjusted to 9 and the reaction allowed to proceed at 40 °C for a further 30 min, giving the desired product in yield comparable to that for the two-step procedure. HPLC of the reaction mixture at pH 2 and 9 (Figure 3) clearly showed the formation of **3** and **5b**, respectively, which was confirmed through comparison of the retention times to those for the corresponding Re standards.

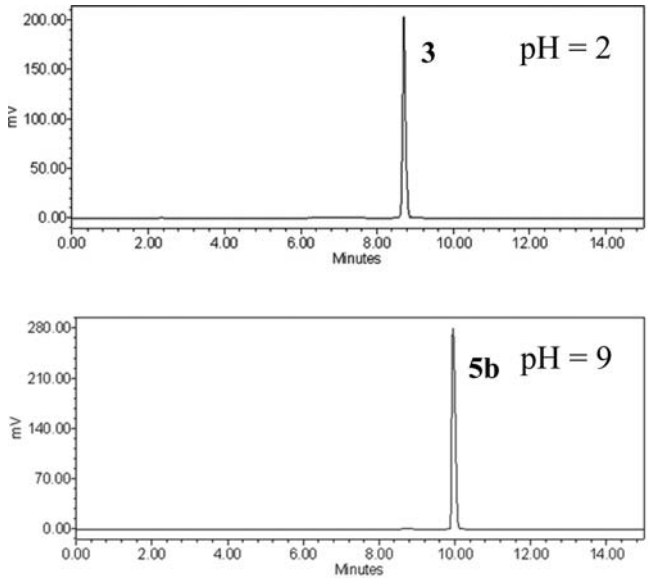


Figure 3. Gamma HPLC traces associated with the one-pot synthesis of $[^{99m}Tc(CO)_3(bipy)(DMAP)]^+$ (**5b**): (top) trace of the reaction mixture after 30 min at pH 2 which shows the formation of $[^{99m}Tc(CO)_3(bipy)Cl]$ (**3**); (bottom) trace of the reaction mixture after an additional 15 min at pH 9 which shows the formation of **5b**.

Stability. The stability of the ^{99m}Tc complexes and the extent of protein binding were evaluated in mouse plasma as a function of time (Figure 4). The loss of the monodentate ligand forms a reactive species that exhibits high binding to proteins (>50% in 1 h) through coordination to good donor groups including cysteine and histidine residues; consequently, the amount of radioactivity in the plasma along with the associated HPLC analysis is a good indicator of stability of the complexes in vitro. For the pyridine derivative **4b**, protein binding at early time points was low; however, the time to achieve 50% protein binding was just over 2 h. HPLC analysis of the supernatant showed predominately the intact complex even after 3 h. The pyridylmethanol derivative **6b** showed comparable stability. The more basic DMAP ligand (pK_a = 9.2), in contrast, showed low protein binding (<20%) after 4 h and only 50% binding at 20 h. When the Re complexes **5a** and **6a** were incubated with plasma over 6 h, which is longer than what is typically used for cell imaging studies, HPLC analysis following protein precipitation showed almost no loss of product due to protein binding, precipitation, or degradation of the complexes.

The results suggest that the basicity/leaving group ability of the monodentate ligand can be exploited to tune the stability of the $[Tc(CO)_3]^+$ complexes. This can be potentially used to address a longstanding concern with tridentate chelate complexes of the tricarbonyl core, which are inert to the extent that they do not bind to proteins or other structures within target cells once they are internalized. This can limit the retention of the complex at the site of interest and ultimately the maximum achievable image contrast (i.e., target to nontarget ratios). In the experiments reported, differences in protein binding between the complexes due to nonspecific binding interactions is unlikely, as the complexes all have the same charge and similar log *P* values (**4b**, −0.32; **5b**, −0.31; **6b**, −0.40; **8b**, −1.07).

Cell Uptake Studies. Extensive work has gone into developing Re luminophores, and several elegant articles on

dx.doi.org/10.1021/ic401972g | *Inorg. Chem.* 2013, 52, 13521−13528

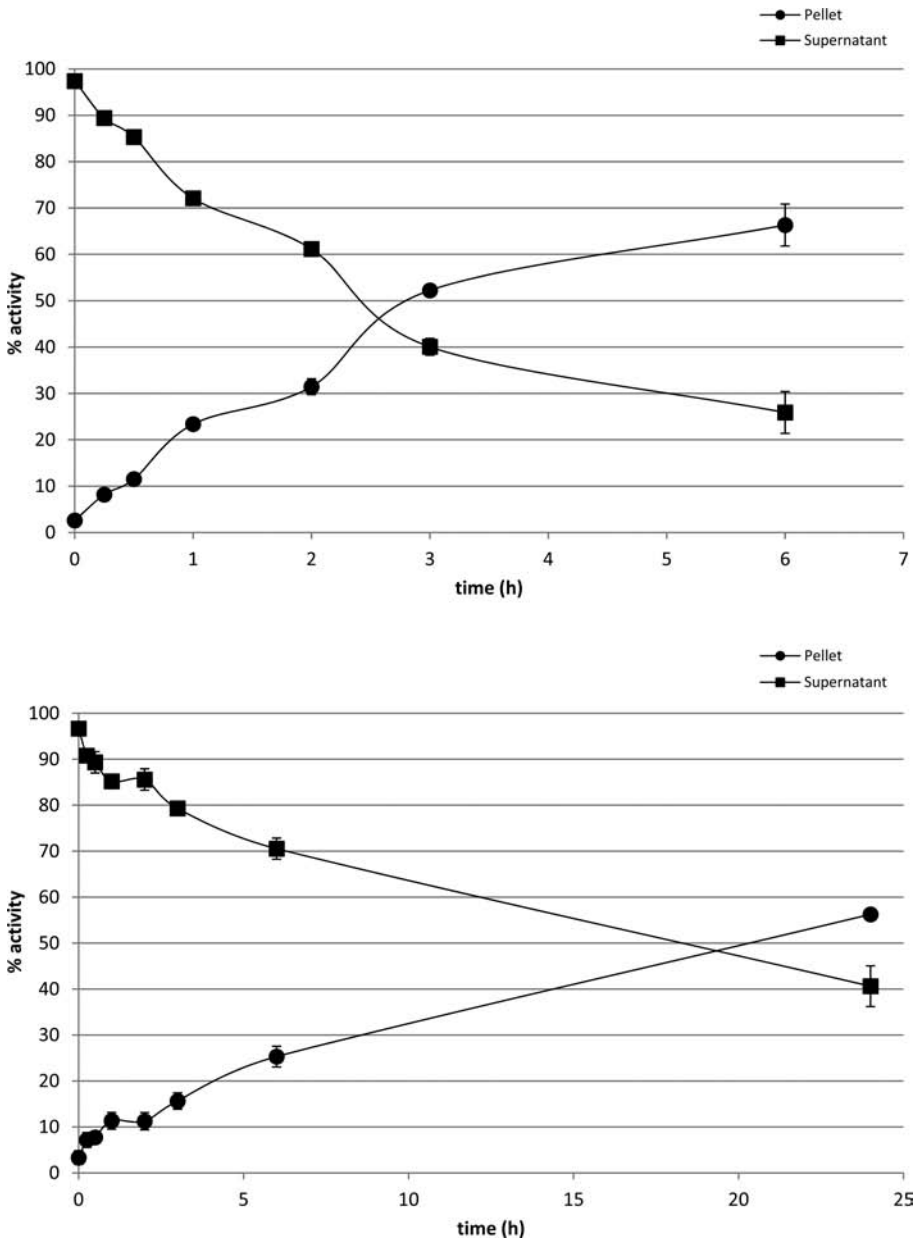


Figure 4. Results of plasma stability studies for (top) **4b** and (bottom) **5b**. Additional stability data can be found in the Supporting Information.

their use as cellular imaging probes have been reported.[17−25] The Re complex **7a**, for example, was effectively imaged in MCF-7 breast cancer cells (Figure 5), and colocalization experiments were performed which match those reported in the literature (see the Supporting Information for images).[33] The advantage of having an isostructural complex derived from ^{99m}Tc is that the radioactive compound can be used to quantitate cell uptake directly without worrying about the impact of the environment on fluorescence intensity.

To demonstrate this, the uptake of the ^{99m}Tc analogue of **6a**, which is reported to stain the mitochondria,[33] was measured in the MCF-7 cells. The uptake of **6b** was approximately 2% of the incubated activity at 20 min and 7% at 120 min (Figure 6). When cells were washed with dilute HCl ,only a small fraction was bound to the cell membrane and the majority of the agent had internalized. For **7b** there was approximately 3% uptake at 30 min and 5% uptake at 120 min (Figure 7). To see if increasing the concentration of the agent would enhance the amount of uptake, cells were incubated with 125 μM of **7a** and the ^{99m}Tc complex simultaneously. At 30 min the added mass had a statistically significant impact on uptake (nearly double) at the early time point; however, there was much less of an effect on the overall uptake at 120 min.

Targeted Agents. One of the advantages of the [2 + 1] concept is that pyridine donors can be incorporated into a wide range of targeting vectors without the need to use complex protecting group chemistry. As an initial example, risedronic acid, a pyridine-containing bisphosphonate derivative that is used to treat osteoporosis, was used as a vector to create a luminescent analogue of Tc-MDP (Scheme 2).

For the Re complex, $[Re(CO)_3(bipy)(H_2O)][CF_3SO_3]$ was combined with risedronic acid at pH 7 and the mixture heated at 75 °C overnight. The product was isolated in high yield (70%) by semipreparative HPLC. MS of the product was consistent with the proposed mass, where N coordination was confirmed by ^{1}H NMR spectroscopy. Coordination to Re(I)

dx.doi.org/10.1021/ic401972g | *Inorg. Chem.* 2013, 52, 13521−13528

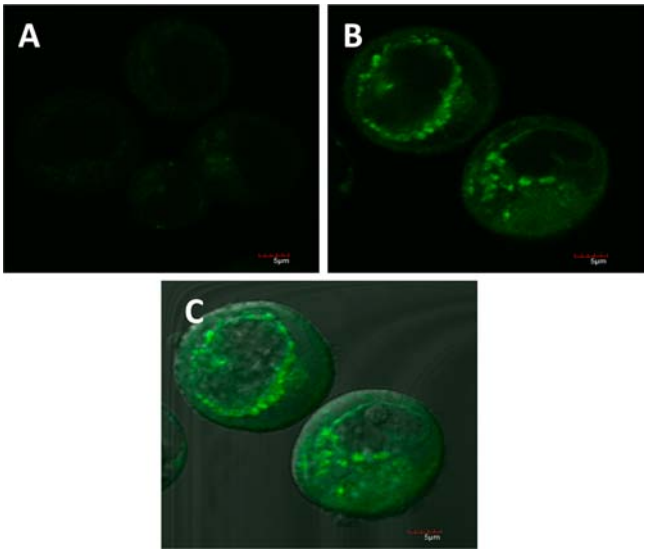


Figure 5. Confocal images of 100 μg/mL **7a** incubated with 5.0×10^5 MCF-7 cells for 1.5 h at room temperature: (A) autofluorescence control; (B) confocal fluorescence image; (C) merged bright-field and fluorescence images.

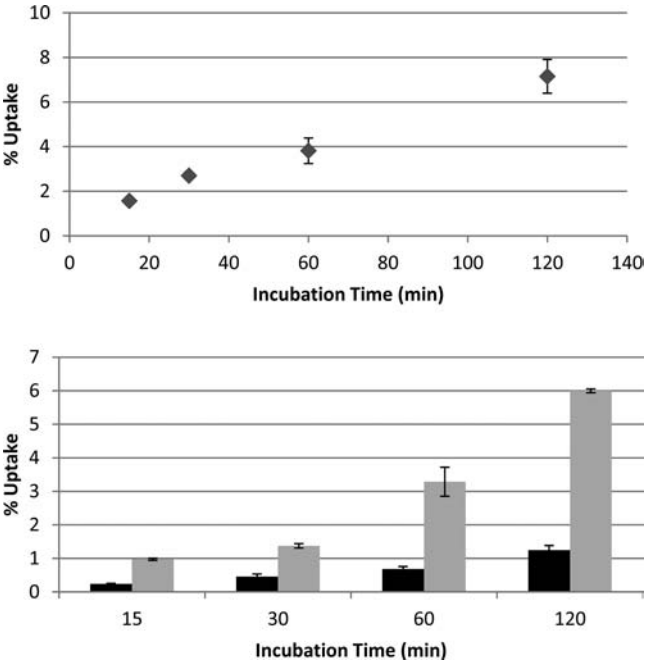


Figure 6. (top) Percent uptake of **6b** in MCF-7 cells as a function of incubation time. (bottom) Results from acid wash experiments indicating the amount of **6b** externally bound (black bars) and internally bound (gray bars) as a function of time.

through the phosphonate groups is possible, as has been shown for other transition metal complexes;[36−38] however, ^{1}H NMR showed that the aromatic protons in close proximity to the pyridine nitrogen exhibit significant chemical shift changes upon complexation with Re(I) in comparison to that of the free ligand. ^{31}P NMR showed the chemical shift of phosphorus to be the same as that in the free ligand. This suggests that the phosphonates are not coordinated to the metal. Unfortunately, attempts to grow single crystals of **8a** were unsuccessful. Nevertheless, compound **8a** when excited at 364 nm showed a

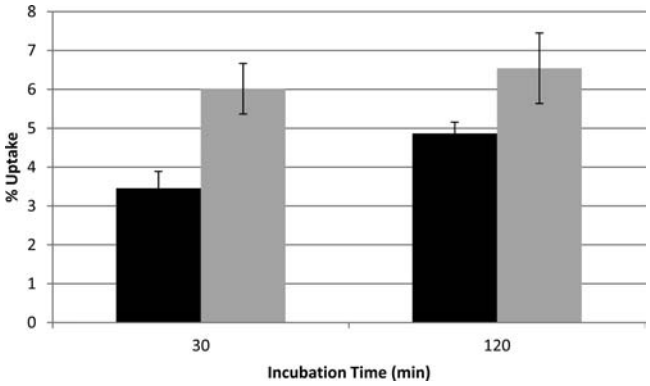
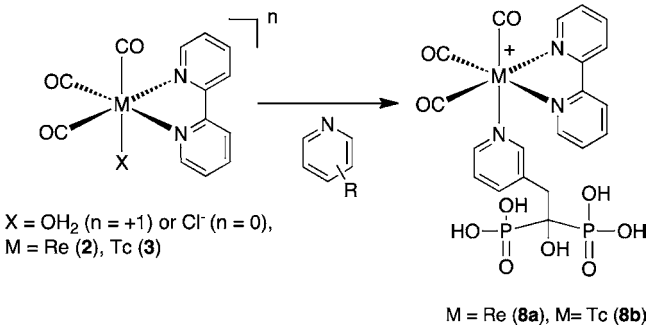


Figure 7. Percent uptake of **7b** in MCF-7 cells at 30 and 120 min (black bars). The experiment was repeated in the presence of **7a** (gray bars).

Scheme 2. Synthesis of Isostructural Re(I) and ^{99m}Tc Complexes with Risedronic Acid



peak emission at 574 nm which is similar to that for compounds **6a** and **7a**.

For the Tc complex **8b**, $[^{99m}Tc(CO)_3(bipy)Cl]$ was combined with risedronic acid in water at pH 7 at 45 °C for 30 min. The product could be isolated by HPLC and was obtained in 85% RCY. In vitro binding studies to different calcium salts compared to Tc-MDP, the gold-standard radiopharmaceutical for imaging bone metastases, was evaluated. As MDP is prepared using an instant kit in the presence of excess ligand, to allow for direct comparison of the in vitro screening studies **8b** was prepared similarly. Compound **8b** showed comparable binding to hydroxyapatite and dibasic calcium phosphate and superior binding to calcium carbonate. Interestingly, MDP more effectively bound calcium pyrophosphate and calcium oxalate (Figure 8). Differences of this nature for MDP and other novel bone-seeking radiopharmaceuticals have been reported previously.[39] The utility of **8a,b** for doing correlated in vitro and in vivo imaging of bone metastases in the appropriate cell and animal models will be reported separately in due course.

CONCLUSIONS

A convenient method to prepare ^{99m}Tc analogues of a class of established rhenium luminophores was developed. The plasma stability of the complexes is impacted by the leaving group ability of the monodentate ligand, making it possible to tune the robustness of the complexes and to prepare products using a pH-controlled one-pot reaction. The ability to generate the ^{99m}Tc complexes makes it possible to quantitate uptake of the corresponding Re luminophores and evaluate their distribution

dx.doi.org/10.1021/ic401972g | Inorg. Chem. 2013, 52, 13521−13528

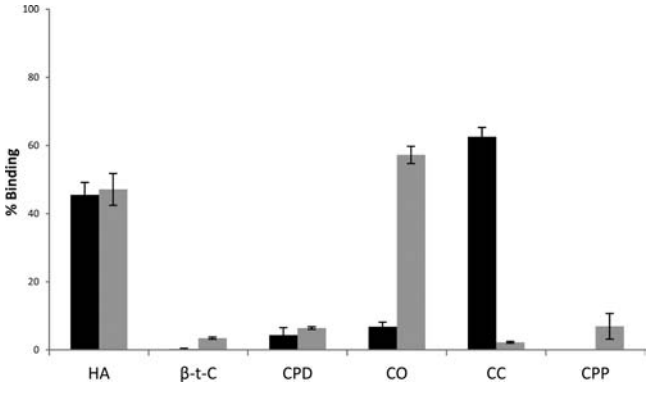


Figure 8. In vitro calcium salt binding comparison between [^{99m}Tc(CO)$_3$(bipy)(RA)] (**8b**) (black bars) and ^{99m}Tc-MDP (gray bars). Abbreviations: HA, hydroxyapatite; β-t-C, β-tricalcium phosphate; CPD, calcium phosphate dibasic; CO, calcium oxalate; CC, calcium carbonate; CPP,calcium pyrophosphate.

in vivo. As a corollary, the ability to access the fluorescent form of complexes will be a useful tool in validating the molecular mechanisms of targeted Tc probes derived from the [2 + 1] construct. There are an increasing number of Re luminophores being developed which have different bifunctional ligands beyond bipy and enhanced chemical and optical properties.[1,32] Furthermore, Alberto and co-workers have already demonstrated the potential utility of a [2 + 1] strategy by combining a targeting vector with a nuclear targeting interchelator linked via a M(I) core.[40] With the methods reported here it will be possible to use one-pot or two-step labeling reactions to create new families of targeted [2 + 1] type isostructural fluorescent and nuclear probes.

EXPERIMENTAL SECTION

General Considerations. Unless otherwise noted, all reagents and solvents were ACS grade and used without further purification from commercial suppliers. 2,2′-Bipyridine, pyridine, 4-dimethylaminopyridine, 3-pyridinemethanol (PM), nicotinic acid, cyclohexanol, silver trifluoromethanosulfonate, and Re(CO)$_5$Br were purchased from Sigma-Aldrich. Tetrahydrofuran, acetone, and methanol were purchased from Caledon. Risedronic (RA) acid was purchased from Santa Cruz Biotechnology, Inc. Distilled water was used for all experiments. Compounds **4a**−**7a** were prepared according to literature methods.[32,33] Triflate salts were converted to the corresponding chlorides using Bio-Rad Prefilled Poly Prep columns containing AG 1-X8 (100−200) chloride ion-exchange resin. ^{99m}Tc was obtained as TcO_4^- from a ^{99} Mo/^{99m}Tc generator (Lantheus Medical Imaging) in saline (0.9% NaCl). *Caution! ^{99m}Tc is a γ-emitter (E_γ = 140 keV, $t_{1/2}$ = 6 h) and should only be used in a licensed and appropriately shielded facility.*

^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker Avance AV-600 instrument at 300 K. High-resolution mass spectra (HRMS) were collected on a Waters/Micromass Q-Tof Global Ultima spectrometer. Microwave reactions were performed on a Biotage Initiator 60 instrument. High-performance liquid chromatography (HPLC) was performed using a Waters XBridge analytical column (5 μm, 4.6 × 100 mm, phenyl) or XBridge prep column (5 μm, 10 × 100 mm, C18) with a Waters 1525 system connected to a Bioscan γ detector and a 2998 photodiode array detector monitoring at 254 nm operated using the Empower software package. Flow rates were 1 mL/min (analytical) or 4 mL/min (prep) using the following methods. Method A: solvent A, water + 0.1% TFA; solvent B, acetonitrile + 0.1% TFA; gradient, 10% B (0−2 min), 100% B (2−12 min), 100% B (12−14 min), 10% B (14−16 min), 10%B (16−20 min). Method B: solvent A, water + 0.1% TFA; solvent B, acetonitrile + 0.1% TFA; gradient, 10% B (0−2 min), 100% B (2−12 min), 100% B (12−14 min), 10% B (14−15 min.). Method C: solvent A, 0.4% ammonium formate (w/v) in water; solvent B, acetonitrile; gradient, 10% B (0−2 min), 100% B (2−12 min), 100% B (12−14 min), 10% B (14−15 min).

Cell Culture and Processing. MCF-7 breast adenocarcinoma cells (ATCC no. HTB-22) were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin streptomycin. The cell line was maintained at 37 °C and 5% CO_2. The buffer used for the MCF-7 cell uptake assay consisted of DMEM growth media and 0.5% bovine serum albumin (BSA). RIPA buffer was prepared with 100 mM Tris pH 8, 50 mM NaCl, 1% NP40, 0.5% sodium deoxycholate, and 0.1% SDS (Bioshop). Two solutions were required for the hydrochloric acid wash on the MCF-7 cells. Solution 1 was comprised of 50 mM glycine HCl and 100 nM NaCl. Solution 2 was made up of 0.5 M HEPES and 100 nM NaCl. Confocal images were generated using an Olympus FluoView 1000 Laser Scanning Confocal Microscope at the wavelengths indicated.

*[Re(CO)$_3$(bipy)(CHN)][CF$_3$SO$_3$] (**7a**).* Compound **7a** was prepared according to the literature method with minor modifications.[33] Cyclohexyl nicotinate (30 mg, 0.15 mmol) was dissolved in DCM (5 mL) and added to [Re(CO)$_3$(bipy)(CH$_3$CN)][CF$_3$SO$_3$] (62 mg, 0.10 mmol) in DCM (10 mL) and heated to 60 °C overnight. The formation of the desired product was confirmed by TLC (DCM/MeOH 10/1). The crude reaction mixture was then purified by column chromatography using DCM/MeOH 10/1 as eluent to obtain the product as a yellow solid. Characterization data matched those found in the literature. Yield: 40 mg (52%).

*[Re(CO)$_3$(bipy)(RA)][CF$_3$SO$_3$] (**8a**).* The pH of a solution of freshly prepared [Re(CO)$_3$(bipy)(H$_2$O)][CF$_3$SO$_3$] (50 mg, 0.08 mmol) in water (10 mL) was adjusted to 7 with 0.1 M NaOH and added with constant stirring to a solution of risedronic acid (25.0 mg, 0.09 mmol) in water (5 mL) which had been adjusted to pH 7 with 0.1 M NaOH. The reaction mixture was then heated to 75 °C overnight, whereupon the solution was cooled to room temperature and concentrated to dryness by rotary evaporation, leaving a yellow solid. Unreacted starting material was removed by the addition of CH_2Cl_2 (5 mL) followed by filtration. The residue was dissolved in MeOH and the product isolated (40 mg, 70%) as a yellow solid by semipreparative HPLC (method C). Mp: 200 °C dec. ^{1}H NMR (D$_2$O, 600 MHz): δ (ppm) 9.25 (d, 2H, Ar−H); 8.38 (s, 1H, Ar−H); 8.32 (d, 2H, Ar−H); 8.23 (s, 1H, Ar−H); 8.17 (t, 2H, Ar−H); 8.07 (d, 1H, Ar−H); 7.79 (d, 1H, Ar−H); 7.71 (q, 2H, Ar−H); 7.07 (q, 1H, Ar−H); 3.01 (t, 2H, −CH$_2$−). ^{31}P NMR (D$_2$O, 242 MHz): δ (ppm) 16.83. ^{19}F NMR (D$_2$O, 470 MHz): δ (ppm) −75.5 (s, CF$_3$SO$_3$). HRMS (ESI+): *m/z* calcd for C$_{20}$H$_{19}$N$_3$O$_{10}$P$_2$Re [M − CF$_3$SO$_3$] 710.01 [M + H]$^+$, found 710.01. HRMS (ESI−): *m/z* calcd for C$_{20}$H$_{19}$N$_3$O$_{10}$P$_2$Re [M − CF$_3$SO$_3$] 708.01 [M − H], found 707.99. HPLC (UV 254 nm, method C): R_t = 6.5 min.

General Procedure for the Preparation of [^{99m}Tc(CO)$_3$(bipy)(L)]$^+$. Sodium boranocarbonate (10.0 mg, 0.10 mmol), sodium carbonate (15.0 mg, 0.14 mmol), sodium borate (20 mg, 0.05 mmol), and sodium potassium tartrate (22 mg, 0.08 mmol) were placed in a microwave vial and purged with argon for 10 min. To this mixture was added Na[^{99m}TcO$_4$] (1 mL), and the vial was heated in a microwave at 110 °C for 3.5 min to form [^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$. The solution was cooled, the pH wa adjusted to 6 with 0.1 M HCl (0.2 mL), and the solution was added to bipyridine (1 mg, 6 mmol) in a 5 mL microwave vial, which had been purged with argon. The reaction mixture was stirred at 40 °C for 30 min and the solution added to one of the monodentate ligands (300−500 mmol) under argon and the mixture stirred at 40 °C for 20 min to give [^{99m}Tc(CO)$_3$(bipy)(L)]$^+$. The formation of products was confirmed by analytical HPLC using the HPLC methods A (**4b** and **5b**) and C (**6b** and **7b**): **4b**, R_t = 8.6 min, RCY = 90 ± 2% (n = 3); **5b**, R_t = 9.7 min, RCY = 87 ± 3% (n = 3); **6b**, R_t = 8.3 min, RCY = 83 ± 2% (n = 3); **7b**, R_t = 11.1 min, RCY = 17 ± 5% (n = 3).

Purification of ^{99m}Tc Complexes. Purification of [^{99m}Tc(CO)$_3$(bipy)Cl] and [^{99m}Tc(CO)$_3$(bipy)(L)]$^+$ (**4b**−**6b**) was achieved by solid phase extraction (SPE) or semipreparative HPLC. For SPE, the reaction mixture was diluted with water (2 mL) and then loaded on a Waters C18 Sep-Pak Plus cartridge, which had been activated

dx.doi.org/10.1021/ic401972g | Inorg. Chem. 2013, 52, 13521−13528

with EtOH (1 × 6 mL) followed by H_2O (1 × 6 mL). After the reaction mixture was loaded, the column was washed with 15% ACN in H_2O (2 × 6 mL), followed by 25% ACN in 0.4% (w/v) aqueous ammonium formate (1 × 6 mL). The desired products were eluted with a 1/1 v/v mixture of 0.4% aqueous ammonium formate and ACN.

*One-Pot Synthesis of [^{99m}Tc(CO)$_3$(bipy)(DMAP)]$^+$ (**5b**).* [^{99m}Tc(CO)$_3$(H$_2$O)$_3$] was added to an argon-purged solution of bipyridine (1 mg, 6 mmol) and DMAP (40 mg, 330 mmol) in water (0.5 mL). The pH of the solution was adjusted to 2 with 0.1 M HCl and the mixture stirred at 40 °C for 15 min, whereupon the pH of the solution was adjusted to 9 with 1 M NaOH and the reaction mixture stirred at 40 °C for 30 min. A single product (R_t = 10.1 min) was observed in the gamma trace, and the product was isolated in 80% yield by HPLC (method B).

*[^{99m}Tc(CO)$_3$(bipy)(RA)] (**8b**).* The pH of a solution of [^{99m}Tc(CO)$_3$(bipy)Cl] (370 MBq) was adjusted to 7 with 1 M NaOH and added to an argon-purged solution of risedronic acid in water (88 μM) whose pH was adjusted to 7 with 1 M NaOH. The reaction mixture was stirred at 45 °C for 30 min and the product isolated by semipreparative HPLC (method C): R_t = 6.5 min, RCY = 85 ± 3% (n = 3).

Plasma Stability. A 100 μL portion (111 MBq) of the ^{99m}Tc complex was added to 900 μL of prewarmed (37 °C) mouse plasma (Innovative Research, IMS-CD1-N), and the mixture was vortexed and incubated at 37 °C. At each time point (t = 0, 0.25, 0.5, 1, 2, 3, 6 h for **4b** and t = 0, 0.25, 0.5, 1, 2, 3, 6, 24 h for **5b**), a sample (100 μL) was removed and added to ice-cold acetonitrile (200 μL). The resulting mixture was vortexed and then centrifuged at maximum speed (15000 rpm) for 10 min. The amount of activity in the sample was measured using a dose calibrator (Capintec Inc., CRC-25R). The supernatant was separated from the pellet, and the activities from the supernatant and the pellet were measured separately. For all time points except t = 0, pellets were washed with ice-cold PBS (50 μL) prior to measuring the activity. The experiment was performed in duplicate and the percent bound to blood proteins calculated as follows: [(amount of activity in washed pellet)/(amount of activity in unwashed pellet) + (amount of activity in supernatant)] × 100%. The values reported are an average of the two experiments ± the standard deviation.

MCF-7 Uptake Assay. MCF-7 cells were detached from the flask using trypsin-EDTA and were resuspended in buffer consisting of DMEM growth media and 0.5% BSA. Six 1 mL aliquots containing 5.0 × 10^5 cells in suspension and 33.3 kBq of either **6b** or **7b** were prepared for each incubation time point. These aliquots were incubated at 37 °C for the specified time. Following the incubation period, the supernatant was removed, and the cells were washed three times with PBS. On the remaining three aliquots, a hydrochloric acid wash was performed. The cells were resuspended in the HCl wash solution 1 and gently vortexed for 3 min. The cells were then treated with HCl wash solution 2. The supernatant was removed, and cells were washed an additional three times with PBS before being resuspended in RIPA buffer and incubated at 37 °C for 30 min. All cells and supernatants were transferred to gamma tubes and counted for 1 min using an automated gamma counter. This experiment was repeated whereby 125 μM of **7a** was incubated along with **7b**.

Confocal Microscopy. MCF-7 cells were removed from the flask using trypsin-EDTA. The cells were distributed into aliquots containing 5.0 × 10^5 cells and 100 μg of the either **6a** or **7a** (or equivalent volume of DMSO for the control) in 1 mL of DMEM media. The aliquots were incubated at room temperature for 1.5 h, after which they were washed three times with PBS. The cells were resuspended in 100 μL of PBS and mounted on the slide for imaging. Cell images were obtained by excitation at 405 nm and emission collection from 562−601 nm; PlanApo 60x oil, NA 1.4 lens, and a zoom factor of 4 were applied.

Hydroxyapatite Binding. The method for in vitro calcium salt binding followed a literature procedure.[39] Briefly, 10 μL (0.8 MBq) of either **8b** or ^{99m}Tc-MDP (as a positive control) was added to 1.5 mL of 1 mg/mL solutions of hydroxyapatite, β-tricalcium phosphate, calcium phosphate dibasic, calcium oxalate, calcium carbonate, and calcium pyrophosphate in a 50 mM Tris buffer (pH 6.9). A no salt control was also included. Samples were incubated with gentle shaking for 1 h at room temperature and then centrifuged for 5 min at 10000 rpm. A 60 μL aliquot of the supernatant was counted using a gamma counter. The percent binding was calculated using the following formula, where CPMs is the counts per minute of each sample and CPMc is the counts per minute for the no salt control:

$$\%\ \text{binding} = \left(1 - \frac{\text{CPMS}}{\text{CPMc}}\right) \times 100$$

The reported percent binding values are an average of two experiments done in triplicate.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figures giving ^{1}H and ^{13}C NMR and HRMS spectra, log P values (^{99m}Tc complexes), absorbance and emission data, and HPLC chromatograms for reported compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*J.F.V.: tel, (905) 525-9140 ext. 20182; fax, (905) 522-7776; e-mail, valliant@mcmaster.ca.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge the Natural Sciences and Engineering Research Council (NSERC) of Canada for financial support of this work and Mr. Feng Xu and Mr. James Jonkman at The Advanced Optical Microscopy Facility (AOMF), University Health Network (UHN), for their technical assistance.

■ REFERENCES

(1) Spagnul, C.; Alberto, R.; Gasser, G.; Ferrari, S.; Pierroz, V.; Bergamo, A.; Gianferra, T.; Alessio, E. *J. Inorg. Biochem.* **2013**, *122*, 57−65.

(2) Thorp-Greenwood, F. L.; Coogan, M. P. *Dalton Trans.* **2011**, *40*, 6129−6143.

(3) Jennings, L. E.; Long, N. J. *Chem. Commun.* **2009**, 3511−3524.

(4) Kobayashi, H.; Longmire, M. R.; Ogawa, M.; Choyke, P. L. *Chem. Soc. Rev.* **2011**, *40*, 4626−4648.

(5) Jennings, L. E.; Long, N. J. *Chem. Commun.* **2009**, 3511−3524.

(6) Morais, G. R.; Paulo, A.; Santos, I. *Organometallics* **2012**, *31*, 5693−5714.

(7) Ghosh, S. C.; Ghosh, P.; Wilganowski, N.; Robinson, H.; Hall, M. A.; Dickinson, G.; Pinkston, K. L.; Harvey, B. R.; Sevick-Muraca, E. M.; Azhdarinia, A. *J. Med. Chem.* **2013**, *56*, 406−416.

(8) Priem, T.; Bouteiller, C.; Camporese, D.; Brune, X.; Hardouin, J.; Romieu, A.; Renard, P. Y. *Org. Biomol. Chem.* **2013**, *11*, 469−479.

(9) Zibo, L.; Liu, T. P.; Liu, S.; Huang, C.-W.; Hudnall, T. W.; Gabbaï, F. P.; Conti, P. S. *Chem. Commun.* **2011**, *47*, 9324−9326.

(10) Botchway, S.; Dilworth, J. R.; Salichou, M. *Dalton Trans.* **2010**, *39*, 5219−5220.

(11) Boulay, A.; Artigau, M.; Coulais, Y.; Picard, C.; Mestre-Voegtlé, B.; Benoist, E. *Dalton Trans.* **2011**, *40*, 6206−6209.

(12) Clède, S.; Lambert, F.; Sandt, C.; Gueroui, Z.; Réfrégiers, M.; Plamont, M.-A.; Dumas, P.; Vessières, A.; Policar, C. *Chem. Commun.* **2012**, *48*, 7729−7731.

(13) Schaffer, P.; Gleave, J. A.; Lemon, J. A.; Reid, L. C.; Pacey, L. K. K.; Farncombe, T. H.; Boreham, D. R.; Zubieta, J.; Babich, J. W.; Doering, L. C.; Valliant, J. F. *Nucl. Med. Biol.* **2008**, *35*, 159−169.

(14) Banerjee, S. R.; Babich, J. W.; Zubieta, J. *Chem. Commun.* **2005**, 1784−1786.

(15) 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.
(16) Gasser, G.; Pinto, A.; Neumann, S.; Sosniak, A. M.; Seitz, M.; Merz, K.; Heumann, R.; Metzler-Nolte, N. *Dalton Trans.* **2012**, *41*, 2304−2313.
(17) Lo, K. K.-W.; Choi, A. W.-T.; Law, W. H.-T. *Dalton Trans.* **2012**, *41*, 6021−6047.
(18) Lo, K. K.-W.; Zhang, K. Y.; Li, S. P.-Y. *Eur. J. Inorg. Chem.* **2011**, 3551−3568.
(19) Louie, M.-W.; Liu, H.-W.; Lam, M. H.-C.; Lam, Y.-W.; Lo, K. K.-W. *Chem. Eur. J.* **2011**, *17*, 8304−8308.
(20) Louie, M.-W.; Choi, A. W.-T.; Liu, H.-W.; Chan, B. T.-N.; Lo, K. K.-W. *Organometallics* **2012**, *31*, 5844−5855.
(21) Lo, K. K.-W.; Ng, D. C.-M.; Hui, W.-K.; Cheung, K.-K. *Dalton Trans.* **2001**, 2634−2640.
(22) Choi, A. W.-T.; Louie, M.-W.; Li, S. P.-Y.; Lin, H. W.; Chen, B. T.-N.; Lam, T. C.-Y.; Lin, A. C.-C.; Cheng, S.-H.; Lo, K. K.-W. *Inorg. Chem.* **2012**, *51*, 13289−13302.
(23) Fernández-Moreira, V.; Ortego, M. L.; Williams, C. F.; Coogan, M. P.; Villacampa, M. D.; Gimeno, M. C. *Organometallics* **2012**, *31*, 5950−5957.
(24) Thorp-Greenwood, F. L. *Organometallics* **2012**, *31*, 5686−5692.
(25) Choi, A. W.-T.; Poon, C.-S.; Liu, H.-W.; Cheng, H.-K.; Lo, K. K.-W. *New J. Chem.* **2013**, *37*, 1711−1719.
(26) Alberto, R. *Eur. J. Inorg. Chem.* **2009**, *1*, 21−31.
(27) Gottschaldt, M.; Koth, D.; Müller, D.; Klette, I.; Rau, S.; Görls, H.; Schäfer, B.; Baum, R. P.; Yano, S. *Chem. Eur. J.* **2007**, *13*, 10273−10280.
(28) Zhang, X.; Chen, X. *Appl. Radiat. Isot.* **2007**, *65*, 70−78.
(29) Zelenka, K.; Borsig, L.; Alberto, R. *Bioconjugate Chem.* **2011**, *22*, 958−967.
(30) Schibli, R.; La Bella, R.; Alberto, R.; Garcia-Garayoa, E.; Ortner, K.; Abram, U.; Schubiger, P. A. *Bioconjugate Chem.* **2000**, *11*, 345−351.
(31) Mundwiler, S.; Kündig, M.; Ortner, K.; Alberto, R. *Dalton Trans.* **2004**, 1320−1328.
(32) Probst, B.; Guttentag, M.; Rodenberg, A.; Hamm, P.; Alberto, R. *Inorg. Chem.* **2011**, *50*, 3404−3412.
(33) 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. Ff.; Coogan, M. P. *Org. Biomol. Chem.* **2010**, *8*, 3888−3901.
(34) Alberto, R.; Schibli, R.; Egli, A.; Schubiger, P. A.; Abram, U.; Kaden, T. A. *J. Am. Chem. Soc.* **1998**, *120*, 7987−7988.
(35) Alberto, R.; Ortner, K.; Wheatley, N.; Schibli, R.; Schubiger, P. A. *J. Am. Chem. Soc.* **2001**, *123*, 3135−3136.
(36) Zhao, J.; Wu, J.; Hu, J.; Hou, H.; Fan, Y. *Inorg. Chim. Acta* **2010**, *363*, 662−668.
(37) Demoro, B.; Caruso, F.; Rossi, M.; Benítez, D.; Gonzalez, M.; Cerecetto, H.; Parajón-Costa, B.; Castiglioni, J.; Galizzi, M.; Docampo, R.; Otero, L.; Gambino, D. *J. Inorg. Biochem.* **2010**, *104*, 1252−1258.
(38) Li, C.; Jiao, C. Q.; Sun, Z. G.; Chen, K.; Wang, C. L.; Zhu, Y. Y.; Zhu, J.; Zhao, Y.; Zheng, M. J.; Sun, S. H.; Chu, W.; Tian, H. *CrystEngComm* **2012**, *14*, 5479−5486.
(39) de Rosales, R. T. M.; Finucane, C.; Mather, S. J.; Blower, P. J. *Chem. Commun.* **2009**, 4847−4849.
(40) Zelenka, K.; Borsig, L.; Alberto, R. *Org. Biomol. Chem.* **2011**, *9*, 1071−1078.