ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Enhanced Cytotoxicity through Conjugation of a "Clickable" Luminescent Re(I) Complex to a Cell-Penetrating Lipopeptide

Anna Leonidova,[†] Vanessa Pierroz,[†,‡] Luke A. Adams,[§] Nicholas Barlow,[§] Stefano Ferrari,[‡] Bim Graham,*[,§] and Gilles Gasser*[,†]

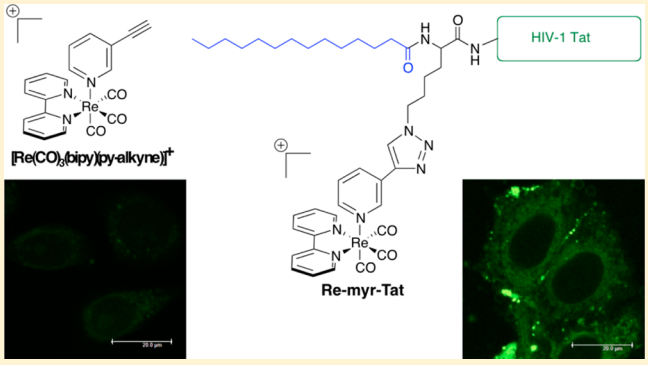
[†]Department of Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

[‡]Institute of Molecular Cancer Research, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

[§]Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia

Supporting Information

ABSTRACT: Re(I) tricarbonyl polypyridine-based complexes are particularly attractive metal complexes in the field of inorganic chemical biology due to their luminescent properties, ease of conjugation to targeting biomolecules, and the possibility to prepare their "hot" ^{99m}Tc analogues for radioimaging. In this study, we prepared and characterized a novel, "clickable" complex, [Re(2,2′-bipyridine)(3-ethynylpyridine)$(CO)_3$](BF_4) ($[\mathbf{Re(CO)_3(bipy)(py\text{-}alkyne)}](\mathbf{BF_4})$), exhibiting the characteristic luminescent properties and moderate cytotoxicity of this general class of compound. Using Cu(I)-catalyzed "click" chemistry, the complex was efficiently attached to a lipidated peptide known to increase cell permeability, namely, the myristoylated HIV-1 Tat peptide (**myr-Tat**), to give **Re-myr-Tat**. Fluorescence microscopy localization in human cervical cancer cells (HeLa) confirmed enhanced cellular uptake of **Re-myr-Tat** compared with $[\mathbf{Re(CO)_3(bipy)(py\text{-}alkyne)}](\mathbf{BF_4})$, and cytotoxicity studies showed that this resulted in an increase in potency to a level comparable with cisplatin (13.0 ± 2.0 μM).



KEYWORDS: *Anticancer, fluorescence microscopy, medicinal organometallic chemistry, peptide, rhenium complexes*

Re(I) tricarbonyl [2 + 1] complexes based on polypyridine-derived ligands, such as $(Re(bipy)(L)(CO)_3)$ (L = monodentate ligand), have attracted considerable attention in the past decade as catalysts,[1] photosensitizers in photocatalytic water reduction,[2,3] and CO-releasing molecules.[4] They remain, however, best known for their outstanding photochemical properties (large Stokes shifts, long emission lifetimes, and resistance to photobleaching),[5] which make them excellent candidates for cellular imaging and ion sensing applications.[6−14] Their use in these areas has been spurred on by their good biocompatibility[15] and the possibility to incorporate targeting vectors via the pyridine or bipyridine-based ligands.[9,16,17] Significantly, the "hot" ^{99m}Tc analogues of these Re compounds could also be prepared, making them promising multimodal agents.[16,18−20]

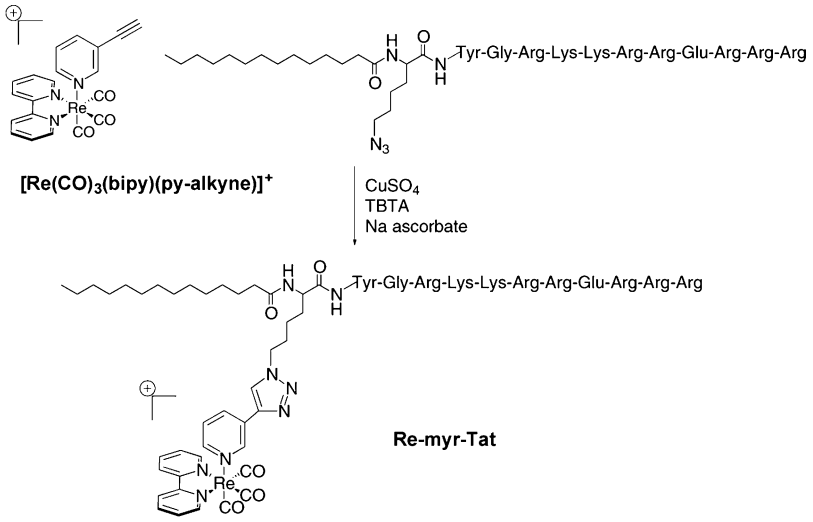
While Re(I) tricarbonyl polypyridine-based complexes are normally only moderately toxic, or essentially nontoxic, several other Re(I) compounds have been reported to be as active or even more potent than cisplatin.[8,21−28] Given how active many organometallic compounds are known to be against cancer cells lines,[29−34] it is surprising that the cytotoxic potential of these Re(I) complexes has not yet been fully explored, especially in light of their aforementioned advantages.

In this work, we aimed to improve the cytotoxicity of a Re(I) compound, namely, $[\mathbf{Re(CO)_3(bipy)(py\text{-}alkyne)}]^+$, whose moderate cytotoxicity was discovered by serendipity in the course of a separate study. To do this, we envisaged enhancing cellular uptake of the complex via conjugation to a myristoylated HIV-1 Tat peptide (Scheme 1). HIV-1 Tat is a membrane translocation sequence from HIV, while myristic acid is a saturated linear fatty acid that naturally occurs as a post-translational protein modification. Both myristic acid and myristoylated Tat peptide were shown to increase the cellular uptake of compounds when conjugated to them.[35,36] In this study, the Re complex was appended to an azide-modified myristoylated Tat peptide via "click" chemistry. Over the past few years, click chemistry has been successfully employed to couple other organometallic compounds to peptides, either by solid-phase or solution-phase methods.[37−40]

$[\mathbf{Re(CO)_3(bipy)(py\text{-}alkyne)}](\mathbf{BF_4})$ was synthesized following an established literature procedure employed for Re(I) *fac*-tricarbonyl bipyridyl complexes with a substituted pyridine ligand.[41] In brief, the initially formed Re tricarbonyl bipyridyl

Received: April 23, 2014
Accepted: May 15, 2014
Published: May 15, 2014

Scheme 1. Bioconjugation of $[Re(CO)_3(bipy)(py\text{-}alkyne)]^+$ to a Myristoylated Tat Peptide



chloride complex was activated via halide abstraction as the corresponding acetonitrile complex. The acetonitrile ligand was then displaced by 3-ethynylpyridine. The formation and purity of the desired product was confirmed by ^1H and ^{13}C NMR, HR-MS (Figures S1−S3, Supporting Information) and elemental analysis. Of note, loss of the monodentate pyridine ligand (m/z 427.0 $[M\text{-}py]^+$) during ionization was observed in the MS spectrum (Figure S3, Supporting Information). $[Re(CO)_3(bipy)(py\text{-}alkyne)](BF_4)$ exhibits the typical photophysical properties for this type of Re(I) complex.[10,17,25,42−50] The UV−vis absorption spectra showed intense bands at 250−330 nm (absorption coefficients around 15 000 M^{-1} cm^{-1}) and a weaker shoulder at 350−360 nm (about 4500 $M^{-1}cm^{-1}$) (Figure S5, Supporting Information). The former are normally attributed to spin-allowed intraligand transitions ($^1IL = (\pi \rightarrow \pi^*)$ (bipyridine and pyridine), while the latter is assigned to spin-allowed metal-to-ligand charge transfer (^1MLCT) $[d\pi(Re) \rightarrow \pi^*(bipy)]$. Upon irradiation in the MLCT band (355 nm), $[Re(CO)_3(bipy)(py\text{-}alkyne)](BF_4)$ displayed a strong emission centered at 550 nm (Figure S6, Supporting Information). Long, submicrosecond luminescence lifetimes (Table 1) and large Stokes shift indicate the phosphorescent nature of the emission,[51] which can be ascribed to a triplet ^3MLCT $[d\pi(Re) \rightarrow \pi^*(bipy)]$ transition, as observed for similar complexes.[4,12] Typical emission lifetimes and quantum yields of $Re(CO)_3(bipy)(L)$ complexes encompass a relatively broad range (0.05−9.6 μs and 0.002−0.59, respectively).[52] The values measured for $[Re(CO)_3(bipy)(py\text{-}alkyne)](BF_4)$ are, in fact, very close to those of structurally similar $Re(CO)_3(bipy)(L)$ complexes, such as isothiocyanate-substituted pyridine complexes.[17] Of note, shorter lifetimes in water compared to acetonitrile can be explained by the greater polarity of water, which stabilizes the ^3MLCT excited state, thus lowering its energy and facilitating nonradiative processes.[53,54] The complex itself can be solubilized in pure water up to 0.79 ± 0.13 mM (25 °C).

Table 1. Emission Lifetimes and Quantum Yields of $[Re(CO)_3(bipy)(py\text{-}alkyne)](BF_4)$

solvent	aerated	degassed	quantum yields (aerated)
H_2O	117 ± 2 ns	128 ± 3 ns	0.0048 ± 0.0005
acetonitrile	168 ± 6 ns	329 ± 1 ns	0.011 ± 0.001

Since some studies have previously noted the loss of the monodentate pyridine ligand in solution for this type of complex,[16,18−20] the stability of $[Re(CO)_3(bipy)(py\text{-}alkyne)](BF_4)$ in water and human blood plasma was assessed. Following an experimental procedure similar to that previously reported by our group,[28,55−57] the complex and diazepam (used as an internal standard due to its known stability in blood plasma and water) were incubated in human plasma or double distilled water for up to 72 h. The aqueous phase was then extracted with dichloromethane and the organic phase analyzed by UPLC-MS. Two peaks corresponding to $[Re(CO)_3(bipy)(py\text{-}alkyne)]^+$ (1.7 min, m/z 530.1 $[M]^+$ and 427.1 $[M - ligand]^+$) and diazepam (2.1 min, m/z 285.2 $[M + H]^+$) could be clearly identified (Figures S9−S12, Supporting Information). The percentage of decomposed $[Re(CO)_3(bipy)(py\text{-}alkyne)]^+$ was then calculated using diazepam as the internal standard. As shown in Figure 1, decomposition proceeded significantly faster in human blood plasma (half-life of approximately 22 h) than pure water (half-life of approximately 5 days), probably due to the substitution of the pyridine ligand by stonger donor groups present in blood plasma proteins (e.g., histidine or cysteine). These results are consistent with the recent study by the Valliant group, which showed a marked dependence of the

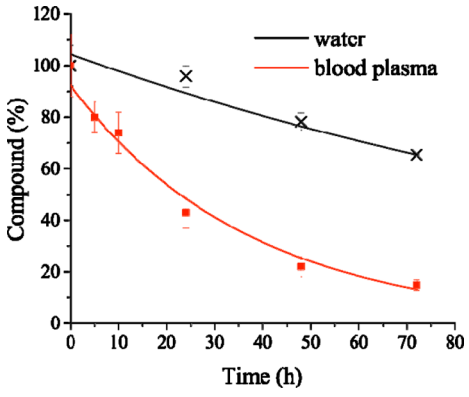


Figure 1. $[Re(CO)_3(bipy)(py\text{-}alkyne)]^+$ stability in human blood plasma and water (double distilled) at 37 °C.

dx.doi.org/10.1021/ml500158w | ACS Med. Chem. Lett. 2014, 5, 809−814

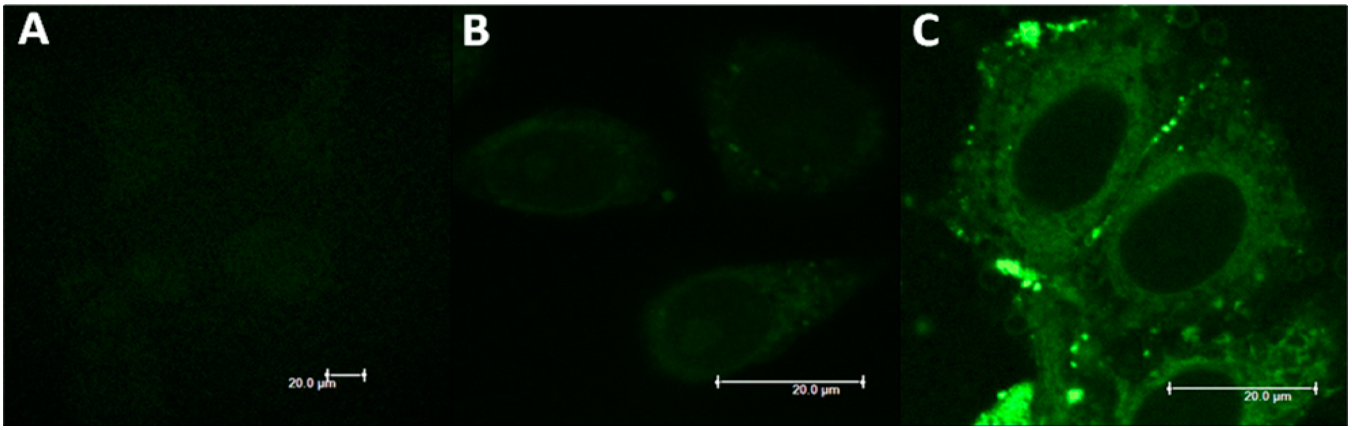


Figure 2. Fluorescence microscopy of HeLa cells fixed after 2 h: (A) untreated cells; (B) treated with $[\mathbf{Re(CO)_3(bipy)(py\text{-}alkyne)}](\mathbf{BF_4})$ at 100 μM; and (C) treated with **Re-myr-Tat** at 20 μM.

pyridine ligand lability on its basicity/leaving group ability.[16] The plasma stability of $[\mathbf{Re(CO)_3(bipy)(py\text{-}alkyne)}](\mathbf{BF_4})$ is on par with the most stable compounds reported by Valliant and co-workers.[16]

The azide-modified myristoylated Tat peptide (**myr-Tat**) was prepared via standard solid-phase peptide synthesis techniques and then purified by preparative HPLC to give a sticky yellow solid, which was unambiguously characterized by HR-MS and HPLC (Figures S13 and S14, Supporting Information). It was then successfully conjugated to $[\mathbf{Re(CO)_3(bipy)(py\text{-}alkyne)}](\mathbf{BF_4})$ via Cu(I)-catalyzed "click" chemistry using similar experimental conditions to those reported by Fokin and co-workers.[58] The crude compound was purified by preparative HPLC to yield **Re-myr-Tat** as a pale yellow solid, which was characterized by UPLC-MS and MALDI-TOF (Figures S15 and S16, Supporting Information). A single peak was observed in the UPLC-MS chromatogram, and consistent with the MS spectrum of the complex, both the desired product and fragments resulting from pyridine ligand dissociation appeared in the MS spectrum (m/z 338.9 $[M - Re(CO)_3(bipy) + 6H]^{6+}$, 406.4 $[M - Re(CO)_3(bipy) + 5H]^{5+}$, 491.6 $[M + 4H]^{5+}$, 507.8 $[M - Re(CO)_3(bipy) + 4H]^{4+}$, 614.2 $[M + 3H]^{4+}$, and 818.7 $[M + 2H]^{3+}$). In the MALDI-TOF spectrum, only the fragmented product (m/z 2026.4 $[M - Re(CO)_3(bipy)+H]^{+}$) was detected due to the harsher ionization conditions compared to ESI-MS. The presence of the *fac*-$Re(CO)_3$ core in the **Re-myr-Tat** bioconjugate was unambiguously confirmed by the presence of the CO stretching bands in the IR spectrum, which appeared in the same range as that of $[\mathbf{Re(CO)_3(bipy)(py\text{-}alkyne)}](\mathbf{BF_4})$ (Figure S17, Supporting Information). The influence of the **myr-Tat** moiety on the lipophilicity of the resulting **Re-myr-Tat** bioconjugate was evaluated by measuring the distribution coefficient between octanol and phosphate buffer, pH 7.01 ($\log D_{7.01}$), using a similar procedure to one used by our group for Ru complexes.[59] Interestingly, although **myr-Tat** contains both a long lipophilic fatty acid chain and a highly positively charged peptide sequence, the net effect is an increase in lipophilicity, namely, from a $\log D_{7.01}$ of -0.36 ± 0.05 for $[\mathbf{Re(CO)_3(bipy)(py\text{-}alkyne)}]^{+}$ to 0.86 ± 0.15 for **Re-myr-Tat**. This change could potentially improve the cellular uptake of **Re-myr-Tat**, as higher lipophilicity can favor the entry of compounds into cells and hence enhance the cytotoxicity.[59−63]

With the desired organometallic complex and bioconjugate in hand, we investigated the intracellular fate of both compounds in human cervical cancer cells (HeLa) by fluorescence microscopy. The cells were first incubated for 2 h with an appropriate concentration of each compound, then fixed with formaldehyde and finally imaged. Figure 2 shows a pronounced difference in emission intensity between the cells treated with $[\mathbf{Re(CO)_3(bipy)(py\text{-}alkyne)}](\mathbf{BF_4})$ (B) or **Re-myr-Tat** (C). Indeed, while the concentration of **Re-myr-Tat** in the cell medium was five times lower than that of the complex, the cells incubated with **Re-myr-Tat** appeared much brighter, indicating significantly higher uptake (it is important to keep in mind that the intensity of the emission signal of a compound in the cells sometimes fails to correlate with uptake, as luminescence can be quenched in the cellular environment).[28,64] In terms of subcellular localization, the complex and the bioconjugate were visualized in the cytoplasm (although weak signals were sometimes detected in the nucleoli of cells incubated with the complex), a localization typically observed for this type of Re complex.[10,12,15,16] Of note, while the HIV-1 Tat peptide sequence has been reported to promote nuclear localization,[65] with a myristoylated Tat derivative dual-labeled with Gd-DOTA and fluorescein previously shown to stain both nucleoli and nuclear membranes,[36] this was not the case for **Re-myr-Tat**. Our microscopy images were recordered at a relatively low **Re-myr-Tat** concentration that left most of the cells alive after 2 h. The Gd-DOTA myr-Tat derivative, however, was imaged at a much higher concentration (260 μM), at which about 60% of the cells were already dying.[36] When HeLa cells were incubated with a higher concentration (2.5-fold increase) of **Re-myr-Tat**, localization in nuclear membrane and nucleoli was also observed (Figure S18, Supporting Information). However, the cells appeared to be already slightly affected by the conjugate at this concentration, so this change in localization could be due to toxin stress.

Next, the cytotoxicity of $[\mathbf{Re(CO)_3(bipy)(py\text{-}alkyne)}](\mathbf{BF_4})$ and **Re-myr-Tat** toward HeLa cells was evaluated by incubating the cells with increasing concentrations of the compounds for 48 h and quantifying cell viability using the resazurin assay.[66] The toxicity of our compounds was compared with that of an established metal-containing anticancer drug, namely, cisplatin. Although several studies report on the cytotoxicity of Re(I) tricarbonyl bipyridine−pyridine complexes, their antiproliferative effect has generally been found to range from nonexistent to moderate; $[\mathbf{Re(CO)_3(bipy)(py\text{-}alkyne)}](\mathbf{BF_4})$ does not deviate from this trend (Table 2). A series of similar complexes, namely, Re(phen)(diaminopy)$(CO)_3$, display IC_{50} values 2−3-

dx.doi.org/10.1021/ml500158w | ACS Med. Chem. Lett. 2014, 5, 809−814

Table 2. Cytotoxicity Data (IC_{50} Values) for $[Re(CO)_3(bipy)(py\text{-}alkyne)](BF_4)$, Re-myr-Tat and Cisplatin Towards HeLa Cells[a]

compd	HeLa IC_{50} (μM)
$[Re(CO)_3(bipy)(py\text{-}alkyne)]^+$	29.9 ± 6.1
Re-myr-Tat	13.0 ± 2.0
cisplatin	9.1 ± 2.8

[a]Experiments were performed in triplicate.

fold higher than cisplatin.[25] Significantly, however, the coupling of $[Re(CO)_3(bipy)(py\text{-}alkyne)]^+$ to the myristoylated Tat peptide was found to increase the cytotoxicity of the resulting bioconjugate, bringing it on par with cisplatin.

In conclusion, in this work, we prepared and characterized a new clickable Re(I) complex, which could be successfully conjugated to an azide-containing myristoylated Tat peptide to give **Re-myr-Tat**. Cytotoxicity and biological studies with HeLa cells revealed that the antiproliferative effect of the complex could be enhanced considerably by the addition of a cell uptake-enhancing biomolecule.

■ ASSOCIATED CONTENT

Supporting Information

Characterization of the compounds (NMR, MS, UPLC-MS, UV, emission, and IR spectra), emission quantum yields measurements, additional information on stability measurements (UPLC-MS) and fluorescence microscopy images. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*(G.G.) E-mail: gilles.gasser@chem.uzh.ch. Fax: +41 44 635 68 03. Tel: +41 44 635 46 30. Website: www.gassergroup.com.

*(B.G.) E-mail: bim.graham@monash.edu. Fax: +61 3 99039582. Tel +61 3 9903 9706. Website: www.bimgrahamgroup.com.

Funding

This work was supported by the Swiss National Science Foundation (Professorship No. PP00P2_133568 and Research Grants No. 200021_129910 and No. 200020_146776 to G.G.), the University of Zurich (G.G and S.F.), the Stiftung für wissenschaftliche Forschung of the University of Zurich (G.G and S.F.), the Stiftung zur Krebsbekämpfung (S.F.), the Huggenberger-Bischoff Stiftung (S.F.), the University of Zurich Priority Program (S.F.), and the Australian Research Council through the award of a Future Fellowship to B.G.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank the Center for Microscopy and Image Analysis (University of Zurich) and PD Dr. Laurent Bigler and Urs Stalder for their help to record MALDI-TOF and HR-ESI spectra.

■ ABBREVIATIONS USED

Bipy, bipyridine; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; ESI, electrospray ionization; HeLa, cervical cancer cell line; HIV, human immunodeficiency virus; HPLC, high-performance liquid chromatography; L, ligand; NMR, nuclear magnetic resonance; HR-MS, high-resolution mass spectroscopy; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; MLCT, metal-to-ligand charge transfer; Py, pyridine; Tat, trans-activator of transcription (here, a cell-permeating peptide sequence derived from HIV-1 Tat protein); UPLC, ultraperformance liquid chromatography; UV−vis, ultraviolet−visible light

■ REFERENCES

(1) Kutal, C.; Weber, M. A.; Ferraudi, G.; Geiger, D. A mechanistic investigation of the photoinduced reduction of carbon dioxide mediated by tricarbonylbromo(2,2′-bipyridine)rhenium(I). *Organometallics* **1985**, *4*, 2161−2166.

(2) Probst, B.; Rodenberg, A.; Guttentag, M.; Hamm, P.; Alberto, R. A highly stable rhenium-cobalt system for photocatalytic H_2 production: unraveling the performance-limiting steps. *Inorg. Chem.* **2010**, *49*, 6453−6460.

(3) Probst, B.; Guttentag, M.; Rodenberg, A.; Hamm, P.; Alberto, R. Photocatalytic H_2 production from water with rhenium and cobalt complexes. *Inorg. Chem.* **2011**, *50*, 3404−3412.

(4) Pierri, A. E.; Pallaoro, A.; Wu, G.; Ford, P. C. A luminescent and biocompatible photocorm. *J. Am. Chem. Soc.* **2012**, *134*, 18197−18200.

(5) Worl, L. A.; Duesing, R.; Chen, P.; Ciana, L. D.; Meyer, T. J. Photophysical properties of polypyridyl carbonyl complexes of rhenium(I). *J. Chem. Soc., Dalton Trans.* **1991**, 849−858.

(6) Lo, K. K.-W.; Choi, A. W.-T.; Law, W. H.-T. Applications of luminescent inorganic and organometallic transition metal complexes as biomolecular and cellular probes. *Dalton Trans.* **2012**, *41*, 6021−6047.

(7) Lo, K. K.-W.; Zhang, K. Y.; Li, S. P.-Y. Recent exploitation of luminescent rhenium(I) tricarbonyl polypyridine complexes as biomolecular and cellular probes. *Eur. J. Inorg. Chem.* **2011**, 3551−3568.

(8) Louie, M.-W.; Choi, A. W.-T.; Liu, H.-W.; Chan, B. T.-N.; Lo, K. K.-W. Synthesis, emission characteristics, cellular studies, and bioconjugation properties of luminescent rhenium(I) polypyridine complexes with a fluorous pendant. *Organometallics* **2012**, *31*, 5844−5855.

(9) Louie, M.-W.; Liu, H.-W.; Lam, M. H.-C.; Lam, Y.-W.; Lo, K. K.-W. Luminescent rhenium(I) polypyridine complexes appended with an α-D-glucose moiety as novel biomolecular and cellular probes. *Chem.—Eur. J.* **2011**, *17*, 8304−8308.

(10) Fernàndez-Moreira, V.; Ortego, M. L.; Williams, C. F.; Coogan, M. P.; Villacampa, M. D.; Gimeno, M. C. Bioconjugated rhenium(I) complexes with amino acid derivatives: synthesis, photophysical properties, and cell imaging studies. *Organometallics* **2012**, *31*, 5950−5957.

(11) Thorp-Greenwood, F. L.; Balasingham, R. G.; Coogan, M. P. Organometallic complexes of transition metals in luminescent cell imaging applications. *J. Organomet. Chem.* **2012**, *714*, 12−21.

(12) Choi, A. W.-T.; Poon, C.-S.; Liu, H.-W.; Cheng, H.-K.; Lo, K. K.-W. Rhenium(I) polypyridine complexes functionalized with a diaminoaromatic moiety as phosphorescent sensors for nitric oxide. *New J. Chem.* **2013**, *37*, 1711−1719.

(13) Clede, S.; Lambert, F.; Sandt, C.; Kascakova, S.; Unger, M.; Harte, E.; Plamont, M.-A.; Saint-Fort, R.; Deniset-Besseau, A.; Gueroui, Z.; Hirschmugl, C.; Lecomte, S.; Dazzi, A.; Vessieres, A.; Policar, C. Detection of an estrogen derivative in two breast cancer cell lines using a single core multimodal probe for imaging (SCoMPI) imaged by a panel of luminescent and vibrational techniques. *Analyst* **2013**, *138*, 5627−5638.

(14) Clede, S.; Lambert, F.; Sandt, C.; Gueroui, Z.; Refregiers, M.; Plamont, M.-A.; Dumas, P.; Vessieres, A.; Policar, C. A rhenium tris-carbonyl derivative as a single core multimodal probe for imaging (SCoMPI) combining infrared and luminescent properties. *Chem. Commun.* **2012**, *48*, 7729−7731.

(15) Fernandez-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. Uptake and localisation of rhenium fac-tricarbonyl polypyridyls in fluorescent cell imaging experiments. *Org. Biomol. Chem.* **2010**, *8*, 3888−3901.
(16) Pitchumony, T. S.; Banevicius, L.; Janzen, N.; Zubieta, J.; Valliant, J. F. Isostructural nuclear and luminescent probes derived from stabilized [2 + 1] rhenium(I)/technetium(I) organometallic complexes. *Inorg. Chem.* **2013**, *52*, 13521−13528.
(17) Lo, K. K.-W.; Ng, D. C.-M.; Hui, W.-K.; Cheung, K.-K. Luminescent rhenium(I) polypyridine complexes with an isothiocyanate moiety-versatile labelling reagents for biomolecules. *Dalton Trans.* **2001**, 2634−2640.
(18) Gottschaldt, M.; Koth, D.; Müller, D.; Klette, I.; Rau, S.; Görls, H.; Schäfer, B.; Baum, R. P.; Yano, S. Synthesis and structure of novel sugar-substituted bipyridine complexes of rhenium and 99m-technetium. *Chem.—Eur. J.* **2007**, *13*, 10273−10280.
(19) Zhang, X.; Chen, X. Preparation and characterization of $^{99m}Tc(CO)_3$-BPy-RGD complex as alphav beta-3 integrin receptor-targeted imaging agent. *Appl. Radiat. Isot.* **2007**, *65*, 70−78.
(20) Alberto, R. The chemistry of technetium−water complexes within the manganese triad: challenges and perspectives. *Eur. J. Inorg. Chem.* **2009**, 21−31.
(21) Zhang, J.; Vittal, J. J.; Henderson, W.; Wheaton, J. R.; Hall, I. H.; Hor, T. S. A.; Yan, Y. K. Tricarbonylrhenium(I) complexes of phosphine-derivatized amines, amino acids and a model peptide: structures, solution behavior and cytotoxicity. *J. Organomet. Chem.* **2002**, *650*, 123−132.
(22) Wang, W.; Yan, Y. K.; Hor, T. S. A.; Vittal, J. J.; Wheaton, J. R.; Hall, I. H. Synthesis, X-ray structures, and cytotoxicity of rhenium(I) carbonyl 2-(dimethylamino)ethoxide complexes. *Polyhedron* **2002**, *21*, 1991−1999.
(23) Bartholoma, M. D.; Vortherms, A. R.; Hillier, S.; Ploier, B.; Joyal, J.; Babich, J.; Doyle, R. P.; Zubieta, J. Synthesis, cytotoxicity, and insight into the mode of action of $Re(CO)_3$ thymidine complexes. *ChemMedChem* **2010**, *5*, 1513−1529.
(24) Can, D.; Peindy N'Dongo, H. W.; Spingler, B.; Schmutz, P.; Raposinho, P.; Santos, I.; Alberto, R. The $[(Cp)M(CO)_3]$ (M = Re, ^{99m}Tc) building block for imaging agents and bioinorganic probes: perspectives and limitations. *Chem. Biodiversity* **2012**, *9*, 1849−1866.
(25) Choi, A. W.-T.; Louie, M.-W.; Li, S. P.-Y.; Liu, H.-W.; Chan, B. T.-N.; Lam, T. C.-Y.; Lin, A. C.-C.; Cheng, S.-H.; Lo, K. K.-W. Emissive behavior, cytotoxic activity, cellular uptake, and pegylation properties of new luminescent rhenium(I) polypyridine poly(ethylene glycol) complexes. *Inorg. Chem.* **2012**, *51*, 13289−13302.
(26) Ho, J.; Lee, W. Y.; Koh, K. J. T.; Lee, P. P. F.; Yan, Y.-K. Rhenium(I) tricarbonyl complexes of salicylaldehyde semicarbazones: synthesis, crystal structures and cytotoxicity. *J. Inorg. Biochem.* **2013**, *119*, 10−20.
(27) Viola-Villegas, N.; Rabideau, A. E.; Cesnavicious, J.; Zubieta, J.; Doyle, R. P. Targeting the folate receptor (FR): imaging and cytotoxicity of Re(I) conjugates in FR-overexpressing cancer cells. *ChemMedChem* **2008**, *3*, 1387−1394.
(28) Kitanovic, I.; Can, S.; Alborzinia, H.; Kitanovic, A.; Pierroz, V.; Leonidova, A.; Pinto, A.; Spingler, B.; Ferrari, S.; Molteni, R.; Steffen, A.; Metzler-Nolte, N.; Wolfl, S.; Gasser, G. Photophysical, theoretical and biological evaluation and cell death mechanisms of a Re(I) organometallic complex. *Chem.—Eur. J.* **2013**, *20*, 2496−2507.
(29) Gasser, G.; Ott, I.; Metzler-Nolte, N. Organometallic anticancer compounds. *J. Med. Chem.* **2010**, *54*, 3−25 and references therein..
(30) Gasser, G.; Metzler-Nolte, N. The potential of organometallic complexes in medicinal chemistry. *Curr. Opin. Chem. Biol.* **2012**, *16*, 84−91 and references therein..
(31) Hartinger, C. G.; Metzler-Nolte, N.; Dyson, P. J. Challenges and opportunities in the development of organometallic anticancer drugs. *Organometallics* **2012**, *31*, 5677−5685 and references therein..
(32) Bruijincx, P. C. A.; Sadler, P. J. New trends for metal complexes with anticancer activity. *Curr. Opin. Chem. Biol.* **2008**, *12*, 197−206 and references therein..
(33) Hartinger, C. G.; Dyson, P. J. Bioorganometallic chemistry: from teaching paradigms to medicinal applications. *Chem. Soc. Rev.* **2009**, *38*, 391−401 and references therein..
(34) Jaouen, G.; Metzler-Nolte, N. *Medicinal Organometallic Chemistry*; Springer-Verlag: Berlin, Germany, 2010.
(35) Nelson, A. R.; Borland, L.; Allbritton, N. L.; Sims, C. E. Myristoyl-based transport of peptides into living cells. *Biochemistry* **2007**, *46*, 14771−14781.
(36) Sturzu, A.; Klose, U.; Echner, H.; Beck, A.; Gharabaghi, A.; Kalbacher, H.; Heckl, S. Cellular uptake of cationic gadolinium-DOTA peptide conjugates with and without N-terminal myristoylation. *Amino Acids* **2009**, *37*, 249−255.
(37) Gasser, G.; Hüsken, N.; Köster, S. D.; Metzler-Nolte, N. Synthesis of organometallic PNA oligomers by click chemistry. *Chem. Commun.* **2008**, 3675−4677.
(38) Gasser, G.; Jäger, K. Z.; Bergmann, M.; Steinbach, R.; Stephan, J.; Metzler-Nolte, H. N. Preparation, ^{99m}Tc-labeling and biodistribution studies of a PNA oligomer containing a new ligand derivative of 2,2′-dipicolylamine. *J. Inorg. Biochem.* **2010**, *104*, 1133−1140.
(39) Patra, M.; Gasser, G.; Bobukhov, D.; Merz, K.; Shtemenko, A. V.; Metzler-Nolte, N. Sequencial insertion of three different organometallics into a versatile building block containing a PNA backbone. *Dalton Trans.* **2010**, *39*, 5617−5619.
(40) Pfeiffer, H.; Rojas, A.; Niesel, J.; Schatzschneider, U. Sonogashira and ″Click″ reactions in the N-terminal and side chain functionalization of peptides with $[Mn(CO)_3(tpm)]^+$-based CO releasing molecules (tpm = tris(pyrazolyl)methane). *Dalton Trans.* **2009**, 4292−4298.
(41) Amoroso, A. J.; Coogan, M. P.; Dunne, J. E.; Fernandez-Moreira, V.; Hess, J. B.; Hayes, A. J.; Lloyd, D.; Millet, C.; Pope, S. J. A.; Williams, C. Rhenium fac tricarbonyl bisimine complexes: biologically useful fluorochromes for cell imaging applications. *Chem. Commun.* **2007**, 3066−3068.
(42) Wrighton, M.; Morse, D. L. Nature of the lowest excited state in tricarbonylchloro-1,10-phenanthrolinerhenium(I) and related complexes. *J. Am. Chem. Soc.* **1974**, *96*, 998−1003.
(43) Caspar, J. V.; Meyer, T. J. Application of the energy gap law to nonradiative, excited-state decay. *J. Phys. Chem. A* **1983**, *87*, 952−957.
(44) Sullivan, B. P. Large solvatochromism of metal-to-ligand charge-transfer transitions in organometallic complexes of rhenium(I). *J. Phys. Chem. A* **1989**, *93*, 24−26.
(45) Kalyanasundaram, K. Luminescence and redox reactions of the metal-to-ligand charge-transfer excited state of tricarbonylchloro-(polypyridyl)rhenium(I) complexes. *J. Chem. Soc., Faraday Trans.* **1986**, *82*, 2401−2415.
(46) Tapolsky, G.; Duesing, R.; Meyer, T. J. Synthetic control of excited-state properties in ligand-bridged complexes of rhenium(I). Intramolecular energy transfer by an electron-transfer/energy-transfer cascade. *Inorg. Chem.* **1990**, *29*, 2285−2297.
(47) Zipp, A. P.; Sacksteder, L.; Streich, J.; Cook, A.; Demas, J. N.; DeGraff, B. A. Luminescence of rhenium(I) complexes with highly sterically hindered alpha-diimine ligands. *Inorg. Chem.* **1993**, *32*, 5629−5632.
(48) Stufkens, D. J.; Vlçek, A., Jr. Ligand-dependent excited state behaviour of Re(I) and Ru(II) carbonyl-diimine complexes. *Coord. Chem. Rev.* **1998**, *177*, 127−179.
(49) Thornton, N. B.; Schanze, K. S. A chromophore-quencher-based luminescence probe for DNA. *Inorg. Chem.* **1993**, *32*, 4994−4995.
(50) Lo, K. K.-W.; Hui, W.-K.; Ng, D. C.-M. Novel rhenium(I) polypyridine biotin complexes that show luminescence enhancement and lifetime elongation upon binding to avidin. *J. Am. Chem. Soc.* **2002**, *124*, 9344−9345.
(51) Uppal, B. S.; Booth, R. K.; Ali, N.; Lockwood, C.; Rice, C. R.; Elliott, P. I. P. Synthesis and characterisation of luminescent rhenium tricarbonyl complexes with axially coordinated 1,2,3-triazole ligands. *Dalton Trans.* **2011**, *40*, 7610−7616.
(52) Lo, K. K.-W.; Choi, A. W.-T.; Law, W. H.-T. Applications of luminescent inorganic and organometallic transition metal complexes

as biomolecular and cellular probes. *Dalton Trans.* **2012**, *41*, 6021−6047.
(53) Kuimova, M. K.; Alsindi, W. Z.; Blake, A. J.; Davies, E. S.; Lampus, D. J.; Matousek, P.; McMaster, J.; Parker, A. W.; Towrie, M.; Sun, X.-Z.; Wilson, C.; George, M. W. Probing the solvent dependent photophysics of fac-[$Re(CO)_3$(dppz-X_2)Cl] (dppz-X_2 = 11,12-X_2-dipyrido[3,2-a:2′,3′-c]phenazine); X = CH_3, H, F, Cl, CF_3). *Inorg. Chem.* **2008**, *47*, 9857−9869.
(54) Sacksteder, L.; Lee, M.; Demas, J. N.; DeGraff, B. A. Long-lived, highly luminescent rhenium(I) complexes as molecular probes: intra- and intermolecular excited-state interactions. *J. Am. Chem. Soc.* **1993**, *115*, 8230−8238.
(55) Patra, M.; Ingram, K.; Pierroz, V.; Ferrari, S.; Spingler, B.; Keiser, J.; Gasser, G. Ferrocenyl derivatives of the anthelminc praziquantel: design, synthesis and biological evaluation. *J. Med. Chem.* **2012**, *55*, 8790−8798.
(56) Patra, M.; Ingram, K.; Pierroz, V.; Ferrari, S.; Spingler, B.; Gasser, R. B.; Keiser, J.; Gasser, G. (η-Praziquantel)$Cr(CO)_3$ derivatives with remarkable in vitro anti-schistosomal activity. *Chem.—Eur. J.* **2013**, *19*, 2232−2235.
(57) Patra, M.; Ingram, K.; Leonidova, A.; Pierroz, V.; Ferrari, S.; Robertson, M. N.; Todd, M. H.; Keiser, J.; Gasser, G. In vitro metabolic profile and in vivo antischistosomal activity studies of (η-Praziquantel)$Cr(CO)_3$ derivatives. *J. Med. Chem.* **2013**, *56*, 9192−9198.
(58) Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. Polytriazoles as copper(I)-stabilizing ligands in catalysis. *Org. Lett.* **2004**, *6*, 2853−2855.
(59) Pierroz, V.; Joshi, T.; Leonidova, A.; Mari, C.; Schur, J.; Ott, I.; Spiccia, L.; Ferrari, S.; Gasser, G. Molecular and cellular characterization of the biological effects of ruthenium(II) complexes incorporating 2-pyridyl-2-pyrimidine-4-carboxylic acid. *J. Am. Chem. Soc.* **2012**, *134*, 20376−20387.
(60) Ghezzi, A.; Aceto, M.; Cassino, C.; Gabano, E.; Osella, D. Uptake of antitumor platinum(II)-complexes by cancer cells, assayed by inductively coupled plasma mass spectrometry (ICP-MS). *J. Inorg. Biochem.* **2004**, *98*, 73−78.
(61) Puckett, C. A.; Ernst, R. J.; Barton, J. K. Exploring the cellular accumulation of metal complexes. *Dalton Trans.* **2010**, *39*, 1159−1170.
(62) Puckett, C. A.; Barton, J. K. Methods to explore cellular uptake of ruthenium complexes. *J. Am. Chem. Soc.* **2006**, *129*, 46−47.
(63) Mari, C.; Pierroz, V.; Rubbiani, R.; Patra, M.; Hess, J.; Spingler, B.; Oehninger, L.; Schur, J.; Ott, I.; Salassa, L.; Ferrari, S.; Gasser, G. DNA intercalating Ru^{II} polypyridyl complexes as effective photosensitizers in photodynamic therapy, 2014. Submitted for publication.
(64) Gasser, G.; Pinto, A.; Neumann, S.; Sosniak, A. M.; Seitz, M.; Merz, K.; Heumann, R.; Metzler-Nolte, N. Synthesis, characterisation and bioimaging of a fluorescent rhenium-containing PNA bioconjugate. *Dalton Trans.* **2012**, *41*, 2304−2313.
(65) Vivès, E.; Brodin, P.; Lebleu, B. A truncated HIV-1Tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus. *J. Biol. Chem.* **1997**, *272*, 16010−16017.
(66) Ahmed, S. A.; Gogal, R. M. J.; Walsh, J. E. A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [3H]thymidine incorporation assay. *J. Immunol. Methods* **1994**, *170*, 211−224.