

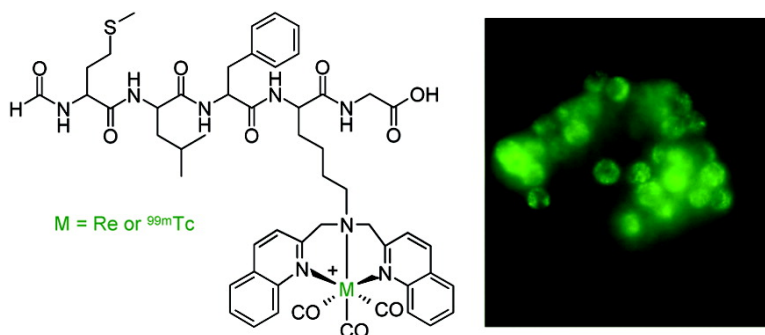
Communication

Bridging the Gap between in Vitro and in Vivo Imaging: Isostructural Re and Tc Complexes for Correlating Fluorescence and Radioimaging Studies

Karin A. Stephenson, Sangeeta Ray Banerjee, Travis Besanger, Oyebola O. Sogbein, Murali K. Levadala, Nicole McFarlane, Jennifer A. Lemon, Douglas R. Boreham, Kevin P. Maresca, John D. Brennan, John W. Babich, Jon Zubieta, and John F. Valliant

J. Am. Chem. Soc., **2004**, 126 (28), 8598-8599 • DOI: 10.1021/ja047751b • Publication Date (Web): 26 June 2004

Downloaded from <http://pubs.acs.org> on March 31, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 4 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

[View the Full Text HTML](#)



ACS Publications
 High quality. High impact.

Bridging the Gap between *In Vitro* and *In Vivo* Imaging: Isostructural Re and ^{99m}Tc Complexes for Correlating Fluorescence and Radioimaging Studies

Karin A. Stephenson,[†] Sangeeta Ray Banerjee,[‡] Travis Besanger,[†] Oyebola O. Sogbein,[†]
Murali K. Levadala,[‡] Nicole McFarlane,[†] Jennifer A. Lemon,[†] Douglas R. Boreham,[†]
Kevin P. Maresca,[§] John D. Brennan,[†] John W. Babich,[§] Jon Zubieta,^{*,‡} and John F. Valliant^{*,†}

Department of Chemistry, McMaster University, Hamilton, Ontario, Canada L8S 4M1, The Medical Physics and Applied Radiation Sciences Unit, McMaster University, Hamilton, Ontario, Canada L8S 4M1,
Department of Chemistry, Syracuse University, Syracuse, New York 13244-4100, and
Molecular Insight Pharmaceuticals Inc., Cambridge, Massachusetts 02142

Received April 19, 2004; E-mail: valliant@mcmaster.ca

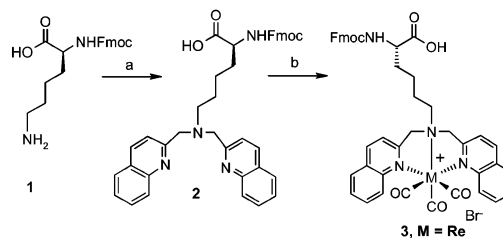
Two of the most widely employed techniques for visualizing specific biological processes are fluorescence microscopy¹ and radioimaging.² Fluorescence microscopy is a powerful tool for looking at the distribution of fluorescent probes *in vitro* and for studying the dynamics of protein motion. The high spatial resolution of this technique allows accurate depiction of processes at the subcellular level and of complex systems such as protein trafficking within cells. Imaging with gamma-emitting radiopharmaceuticals, using nuclear medicine scanners, allows for sensitive detection of biological processes deep within the body at resolutions of a few millimeters.

A constraint in the development of radiopharmaceuticals is the inability to image the behavior and fate of these compounds at the cellular and subcellular level, in real-time, to rapidly elucidate mechanisms responsible for cellular specificity and localization. Substitution of the radiolabel with a fluorescent dye³ may allow for visualization of the vector compound *in vitro*, but will likely alter the physicochemical properties sufficiently to render such substitution of little value. The optimal system would be one in which the fluorescent and radioactive prosthetic groups are isostructural.

It is conceivable that complimentary pairs of fluorescent and radioactive probes can be prepared using technetium and rhenium. ^{99m}Tc is the most widely used radionuclide in diagnostic medicine, owing to its ideal nuclear properties, low cost, and widespread availability.⁴ Re(I) complexes on the other hand, have been used to prepare luminescent probes.⁵ Fluorescent Re complexes are particularly useful for studying biological processes *in vitro* because of their long-lifetime, polarized emission, and large Stoke's shift. On the basis of the fact that the coordination chemistry of the two congeners is similar, it should be possible to design a ligand that forms a fluorescent Re complex and a stable ^{99m}Tc complex. Such a system, which should also possess the ability to be linked to a targeting agent, would allow images obtained on a fluorescent microscope to be directly correlated with *in vivo* radioimaging studies because the structures of the two probes would be effectively identical.

Recently, we reported the synthesis of a Tc(I)/Re(I) binding ligand based a lysine-derived bis(pyridyl) amine which was referred to as a single amino acid chelate (SAAC).⁶ The SAAC forms an inert complex with the M(CO)₃⁺ core (M = Re, Tc), and it can be incorporated into peptides as if it were a natural amino acid. To prepare a SAAC-type ligand whose Re complex is fluorescent, while retaining the ability to bind ^{99m}Tc, *N*-α-Fmoc-L-lysine was

Scheme 1^a



^a Conditions: a) NaBH(OAc)₃, Q2A. b) [NEt₄]₂[Re(CO)₃Br₃].

reacted with quinoline-2-aldehyde (Q2A) in the presence of Na(OAc)₃BH to give the bifunctional ligand **2** (Scheme 1). The desired product, which can be produced in multigram quantities, was isolated in excellent yield (86%) following column chromatography. The Re complex **3** was synthesized by reacting **2** with [NEt₄]₂[Re(CO)₃Br₃] in methanol. The complex was isolated in 80% yield as the bromide salt.

Compound **3** displays an appreciable absorbance in the UV and blue regions of the UV–visible spectrum with a peak absorbance at 301 nm. The extinction coefficients for **3** were 13200 M⁻¹ cm⁻¹ at 301 nm and 2250 M⁻¹ cm⁻¹ at 366 nm. The emission spectrum shows that the rhenium probe has two distinct transitions giving rise to peak fluorescence intensities at 425 and 580 nm. Compound **3** has a lifetime of 4.31–9.76 μs, depending upon the solvent and amount of oxygen present, which is sufficiently prolonged to enable time-gating techniques to be used during *in vitro* imaging studies to overcome interferences from endogenous fluorescence. Compound **3** shows fluorescence anisotropy with a limiting anisotropy of 0.35 at 424 nm (at -20 °C), which indicates that the probe can also be used to study small-molecule–receptor or protein–protein interactions in which the rotational mobility of the probe is monitored as a function of binding. The quantum yield for **3**, which ranges from 0.003 in chloroform in the presence of air to 0.015 in ethylene glycol under nitrogen, is low but not significantly different from the quantum yields reported for other transition-metal based fluorescence probes.^{5a,7}

Having established that **3** has the necessary properties to be used as a fluorescent probe, the subsequent objective was to develop a method for preparing bioconjugates that can deliver the ligand to specific receptors. Not coincidentally, the SAACQ ligand and the SAACQ–Re complex are amino acid analogues which enables them to be linked to, or incorporated within, peptide-based targeting agents using a conventional automated synthesizer. To demonstrate this feature, the SAACQ and SAACQ–Re complexes were integrated within fMLF, a targeting sequence which has been used to guide radionuclides to the formyl peptide receptor (FPR), which

[†] McMaster University.

[‡] Syracuse University.

[§] Molecular Insight Pharmaceuticals Inc.

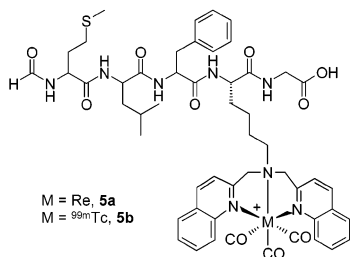


Figure 1. fMLF[(SAACQ-M(CO)₃)⁺]G containing all L-isomers.

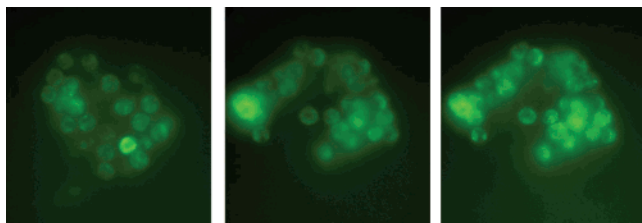


Figure 2. Fluorescence microscopy images of human leukocytes incubated with 1 nM **5a** (left), 50 nM fluorescein-labeled fNLFNTK (middle), and 50 nM **5a** (right).

is expressed on neutrophils, as a means of imaging the trafficking of white blood cells.⁸ The peptides fMLF(SAACQ)G (**4**) and fMLF-[(SAACQ-Re(CO)₃)⁺]G (**5a**, Figure 1) were prepared, using compounds **2** and **3**, respectively, following a conventional automated Fmoc synthesis method using glycine-loaded SASRIN resin and HBTU as the coupling agent. The peptides were isolated using a standard cleavage cocktail and the products purified by HPLC. The HPLC purification was needed as a result of epimerization of the formyl-methionine residue which is known to occur under normal solid-phase synthesis conditions.⁹ The affinities of **4** and **5a** for human FPRs were determined by flow cytometry using fluorescein-labeled fNLFNTK as the reference ligand.¹⁰ Compounds **4** and **5a** show *K_d* values of 11 ± 3 and 27 ± 13 nM, respectively, which is comparable to that for the parent targeting agent.

To demonstrate that the Re-peptide complex can be used as a probe for fluorescence microscopy studies, a 1 nM solution of compound **5a** was incubated with human leukocytes and its uptake observed using a fluorescence microscope (Figure 2). Samples were excited by a mercury lamp using a commercially available filter set consisting of a dichroic 360 nm excitation filter, a 400 nm dichroic long-pass filter, and a 550 nm long-pass emission filter. At low temperatures, compound **5a** could readily be seen bound to the periphery of the leukocytes but internalized into the cytoplasm of the cells when samples were allowed to warm to room temperature. These observations match the results reported for microscopy studies of other fluorescently labeled chemotactic peptides.¹¹ Along those lines, a 1:1 mixture of the peptide and a well-established fluorescent FPR probe, fluorescein-labeled fNLFNTK, were incubated with the same cells and microscopy images taken at different excitation and emission wavelengths so that only one probe was visualized at a time.¹² The images (Figure 2), in qualitative terms, are effectively identical, which strongly suggests that the two probes are targeting the same cell populations. A more quantitative head-to-head comparison of the two probes is currently underway.

The ^{99m}Tc-labeled analogue of **5a** was prepared by reacting compound **4** (500 μg) with 703 MBq (19 mCi) of [^{99m}Tc(CO)₃-

(OH₂)₃]⁺; a precursor that can be prepared in any radiopharmacy from ^{99m}TcO₄⁻.¹³ The desired product, whose HPLC retention time matched that of **5a**, was isolated in high yield (90%) via a simple solid-phase extraction procedure. The stability of the ^{99m}Tc-labeled peptide was subsequently tested by incubating two separate samples of **5b** with an excess of cysteine and histidine in PBS buffer heated to 37 °C. After 24 h there was no dissociation of the metal which indicates that the SAACQ ligand forms a Tc-complex that is suitably robust for use in vivo.

The work reported here demonstrates that: (1) The Re SAACQ complex has the appropriate fluorescence properties to be used for in vitro microscopy studies, (2) the SAACQ ligand and its Re complex can be easily incorporated into peptides using a conventional automated synthesizer, and (3) the SAACQ ligand can be labeled with ^{99m}Tc in high yield and the resulting complex is robust.

In light of these findings it is now possible to use the SAACQ synthon to prepare complementary fluorescent and radioactive peptide probes. This offers the unique opportunity to directly correlate in vitro and in vivo imaging studies, which goes a long way toward bridging the gap between work in isolated cells and studies carried out in living subjects.

Acknowledgment. We acknowledge NSERC of Canada, The Canadian Foundation for Innovation, The Ontario Innovation Trust, The Ontario Research Challenge Fund for funding, and Dr. Karen Gulenchyn and Mr. Ferid Rashid from the Department of Nuclear Medicine, Hamilton Health Sciences, for providing access to ^{99m}TcO₄⁻.

Supporting Information Available: Synthetic procedures for **2**, **3**, **5a**, **5b** and fluorescence data and procedures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Wang, X. F.; Herman, B. *Fluorescence Imaging Spectroscopy and Microscopy*; John Wiley and Sons: New York, 1996.
- (2) Mettler, F. A.; Guiberteau, M. J. *Essentials of Nuclear Medicine Imaging*, 4th ed; W. B. Saunders Company: Toronto, 1998.
- (3) (a) Gallazzi, F.; Wang, Y.; Jia, F.; Shenoy, N.; Landon, L. A.; Hannink, M.; Lever, S. Z.; Lewis, M. R. *Bioconjugate Chem.* **2003**, *14*, 1083. (b) Bullok, K. E.; Dyszlewski, M.; Prior, J. L.; Pica, C. M.; Sharma, V.; Piwnicka-Worms, D. *Bioconjugate Chem.* **2002**, *12*, 1226.
- (4) Jurisson, S. S.; Lydon, J. D. *Chem. Rev.* **1999**, *99*, 2205.
- (5) (a) Guo, X.; Castellano, F. N.; Li, L.; Lakowicz, J. R. *Anal. Chem.* **1998**, *70*, 632. (b) Guo, X.; Castellano, F. N.; Li, L.; Szmajcinski, H.; Lakowicz, J. R.; Sipior, J. *Anal. Biochem.* **1997**, *254*, 179. (c) Shen, Y.; Maliwal, B. P.; Lakowicz, J. R. *J. Fluoresc.* **2001**, *11*, 315.
- (6) Stephenson, K. A.; Zubieta, J.; Banerjee, S. R.; Levadala, M. K.; Taggart, L.; Ryan, L.; McFarlane, N.; Boreham, D. R.; Maresca, K. P.; Babich, J. W.; Valliant, J. F. *Bioconjugate Chem.* **2004**, *15*, 128.
- (7) Lo, K. K.-W.; Hui, W.-K.; Ng, D. C.-M.; Cheung, K.-K. *Inorg. Chem.* **2002**, *41*, 40.
- (8) Liu, S.; Edwards, D. S. *Chem. Rev.* **1999**, *99*, 2235.
- (9) Greathouse, D. V.; Goforth, R. L.; Crawford, T.; van der Wel, P. C. A.; Killian, J. A. *J. Peptide Res.* **2001**, *57*, 519.
- (10) (a) Waller, A.; Pipkorn, D.; Sutton, J. L.; Linderman, J. L.; Omann, G. M. *Cytometry* **2001**, *45*, 102. (b) Fay, S. P.; Posner, R. G.; Swann, W. N.; Sklar, L. A. *Biochemistry* **1991**, *30*, 5066. (c) Hoffman, J. F.; Keil, M. L.; Riccobene, T. A.; Omann, G. M.; Linderman, J. L. *Biochemistry* **1996**, *35*, 13047.
- (11) (a) Johansson, B.; Wymann, M. P.; Holmgren-Peterson, K.; Magnusson, K. E. *J. Cell Biol.* **1993**, *121*, 1281. (b) Niedel, J. E.; Kahane, I.; Cuatrecasas, P. *Science* **1979**, *205*, 1412.
- (12) Loitto, V. M.; Rasmusson, B.; Magnusson, K. E. *J. Leukocyte Biol.* **2001**, *69*, 762.
- (13) (a) Alberto, R.; Ortner, K.; Wheatley, N.; Schibli, R.; Schubiger, A. P. *J. Am. Chem. Soc.* **2001**, *123*, 3135. (b) Alberto, R.; Schibli, R.; Egli, A.; Schubiger, A. P. *J. Am. Chem. Soc.* **1998**, *120*, 7987.

JA047751B