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14. ABSTRACT
The project investigated the development of ligand linked Flutamide analogs for complexing M(CO)3 (Re,99mTc) organometallic species to target prostate cancer. The project has been successful in developing and testing new synthetic strategies for this application. General methods were established for preparing the cold Re and 99mTc complexes in excellent yields(>95%) at (10-4,10-5 M) ligand concentration, testing the stability of the complexes (pH, temperature) and in vitro (serum, AR +/- prostate cancer cells). Several 99mTc flutamide analogs were examined. Tridentate ligands (i.e., cysteine, histidine, dipyridylamine, iminodiacetic acid) were successfully prepared and maintained stability in vitro. The "2+1" approach analogs were prepared, however, failed to maintain the complex conformation, when examined under biological conditions. Second generation compounds based on the initial experiments with addition functional group are currently being investigated.

15. SUBJECT TERMS
Imaging and therapy of Prostate cancer

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Introduction

The focus of the research as highlighted in the report is the development of new diagnostic agents for identifying and probing prostate cancer through the expression of the androgen receptor. The proposed work capitalizes on the high affinity of Flutamide, a non steroidal antagonist of the androgen receptor, is one of current medical treatment of prostate cancer.[1] To utilize the targeting potential of Flutamide for diagnostic purposes, organometallic complexes formed with $M(CO)_3^+$ (^{99m}Tc, Re) were incorporated into the molecule's framework. In particular, the ^{99m}Tc complexes provide nuclear imaging capabilities through Single Photon Emission Spectroscopy.

The global goals of the work would 1) non-invasive image the prostate distinguishing between normal and prostate cancer cells based on AR expression 2) provide clinicians with essential tumor information about AR expression as prostate cancer cells becomes hormone refractory with decreased function of the AR. In this report, the initial design and preparation of the covalently linked ^{99m}Tc Flutamide complexes and the preliminary investigations of the complexes will be discussed to reach the global goals.

Body

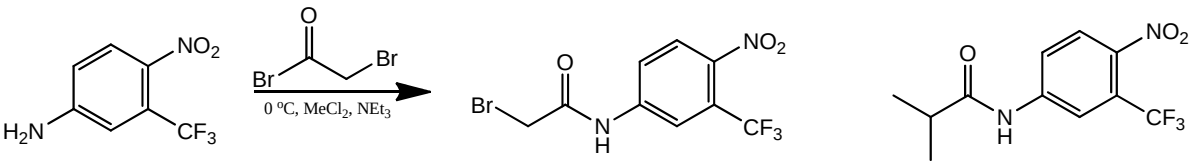
The work conducted during the term of the grant has met the main objectives of the proposal. The primary focuses during course of the project involved the synthesis and characterization of novel flutamide linked chelate compounds, preparation of the corresponding metal complexes formed with natural rhenium and the radioactive technetium-99m, and preliminary investigation of the radiolabeled complexes. The scientific developments of the Flutamide linked complexes achieved during the course of the investigation were not limited to the intrinsic specific aims highlighted in the original proposal. Discoveries within the project led to the exploration of additional methods that directly related to the scope of the project that will be discussed as well.

Project Objectives

Synthesis of Flutamide compounds, Flutamide linked chelates, and metal complexes

As detailed in the year 1 report, one of the key concepts we pursued was the synthetic preparation of a universal Flutamide derivative, compound 1 (Figure 1). This approach developed a modified Flutamide that could be easily covalently bound to a wide variety of molecules. Initially, the preparation of an unreported universal Flutamide molecules was challenging with low yields (10-20%) with moderate to poor purity. We have been able during the course of the project better understand the reaction chemistry and decomposition pathways of the molecules to increase yields significantly (80-95%) with excellent purity (>99%) in multi-gram quantities.

Figure 1. Preparation of a universal Flutamide alkylating agent

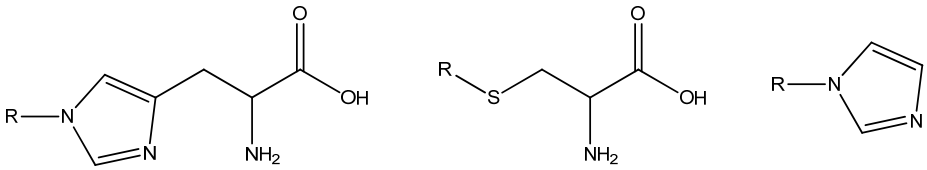


1 **Flutamide**

In this application, the universal Flutamide molecule was utilized to synthesize covalently bonded compounds that could incorporate a number of chelate into the systems. This interchangeable approach permitted the investigation of the impact of the chelate and the complex of the specific binding of the molecules. Initial synthetically strategies to couple the Flutamide with a chelate were hampered by the low yields and purity issues with the initial universal Flutamide alkylating agent. As outlined in further detail in year 1 and 2 reports, this

was also followed by limitations in developing alkylating conditions that could be used to incorporation of the chelate into the flutamide molecule. The first generation studies focuses on investigating three different chelate systems (histidine, cysteine, and "2+1") with the universal Flutamide agent (Figure 2). Particularly, in the cysteine and histidine synthetic approaches, the protecting group chemistry provide significant challenges in determining conditions to the couple the two compounds. Destablilization of the amide bond on the Flutamide portion of the molecule was determined to be the cause of decomposition and readily observed under temperature and pH effects (basic). In the design of the histidine and cystiene chelates, the compounds could be coupled with the universal Flutamide molecule, however, deprotection of the methyl ester in both cases yielded only cleavage products. In circumvent this issue, additional strategies were investigated.

Figure 2. First generation of Flutamide (R) linked chelate compounds



Histidine Cysteine Imidazole

In the cysteine case, new conditions to combine the Flutamide were developed that did not use protecting groups under very mild aqueous conditions (room temperature, pH~8, overnight) in modest yields (20-35%) with only the poorly soluble Flutamide starting material remaining and no decomposition products observed. While this dramatically improved product yields over the protecting group strategy, we further developed novel biphasic conditions that addressed the poor water solubility of **1**, but allowed it to still react with chelate system in the aqueous layer as previously described. This new approach allowed the simple preparation and isolation of cysteine coupled Flutamide analogs in near quantitative yields (85-95%) in ~2-3 hrs. The histidine ligand was prepared by a different approach where the $Re(CO)_3$ was used as a protecting group and could be deprotected under acidic oxidative conditions, where cleavage of the amide bond would not occur as detailed in year 1 and 2 reports.

In conjunction with the cysteine and histidine investigations, a "2+1" approach that utilized two co-ligands for occupying the available three sites on the $M(CO)_3$. The monodentate ligand imidazole was funtionalized with Flutamide to compare against the tridentate ligands.

Although the 2+1 complexes were successfully prepared, the stability of these complexes were determined to be unsatisfactory for future applications.

During year 3 and 4, a second generation compounds utilized the ligand to generate an overall charge of the molecule upon complexation of the metal. Iminodiacetic acid (IDA) and dipyridylamine (DPA) were selected as they have been previously identified to form reasonable complexes with $M(CO)_3$ (Figure 3). The Flutamide linked IDA compounds could be prepared by alkylation of **1** with a t-butyl ester protected IDA followed by acidic deprotection. The Flutamide linked DPA ligands were prepared with similar alkylation conditions to the protected cysteine analogs, however, no protecting groups were needed with this chelate.

Figure 3. Synthesis of the second generation of Flutamide (R) linked chelate compounds

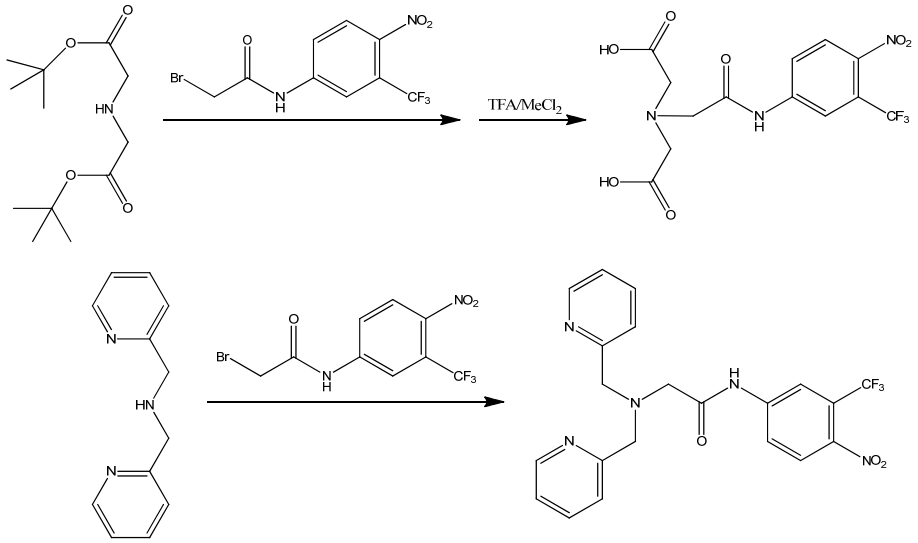
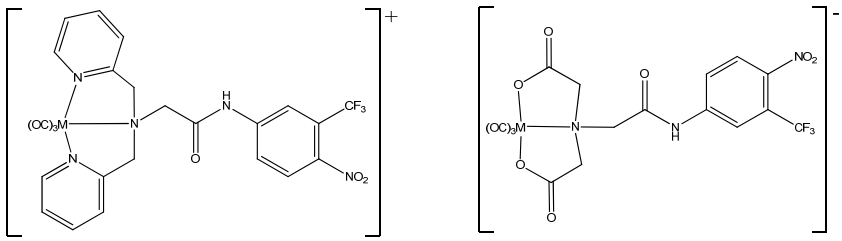


Figure 4. $[M(OH_2)_3(CO)_3]^+$ (M = Re, ^{99m}Tc) complexes formed with and the DPA and IDA Flutamide linked ligands



The hypothesis in the preparation of these compounds was to determine if charge of the metal complex could be utilized to improve affinity of the complexes to the androgen receptor due to charge affinity at the binding site and could be directly compared to the neutral charge first generation compounds (Figure 4). The corresponding Re and ^{99m}Tc complexes were

formed under similar conditions utilized with the previous complexes and had similar macroscopic and radiochemical yields.

Radiochemical stability and in vitro Cell binding assays

The ^{99m}Tc complexes were investigated for stability in a number of conditions (pH, temperature) to ascertain whether they would degrade under biological conditions (7.4, 37 °C). The first generation tridentate complexes (histidine, cysteine) demonstrated stability under biological conditions out to 8 hrs. Whereas, the 2+1 approach showed dissociation of the imidazole ligand within 15 mins proving this approach to be inadequate for further clinical applications. The second generation tridentate complexes (IDA, DPA) showed similar stabilities as the first generation complexes. Both the first and second generation compounds showed stability in mouse serum out to 8 hrs with less than 10 % dissociation of the metal from the complex.

As denoted in year 2 and 3 reports, the ^{99m}Tc compounds were investigated with prostate cancer lines AR positive cells (DU-145) and AR negative (PC-3). The first generation of ^{99m}Tc flutamide complexes was examined with prostate cancer cells. The tridentate analogs (histidine and cysteine) were incubated with AR positive cells (DU-145) and AR negative (PC-3). The compounds illustrated some binding to the AR + cells (~2%). The percent binding was lower than anticipated, but it was not unexpected as the distance between the flutamide binding motif was not optimized yet. These results provided positive feedback for additional studies. Cell studies with the second generation compounds are currently under investigation.

Alternative approaches

During the course of the project, new approaches to ligand design and methodology were also investigated. These methods were investigated on a fundamental level prior to functionalization with the biotargeting agent to prove proof of concept. These included the following

- *Multi-valent targeting*

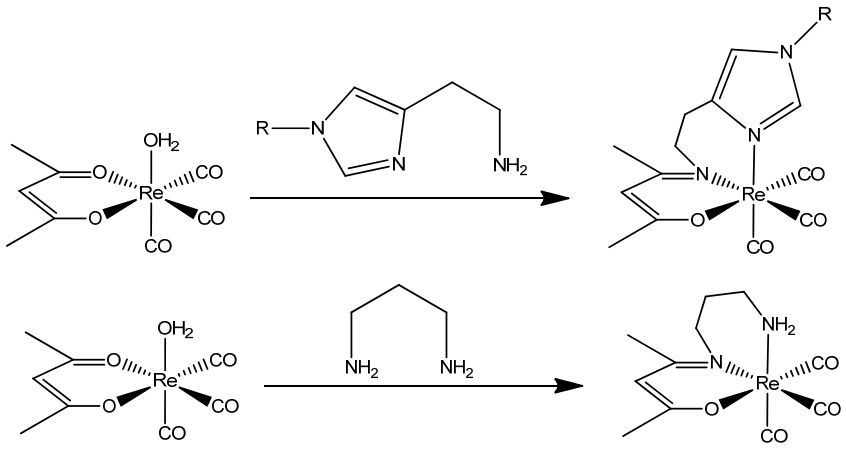
The 2+1 approach was initially investigated for coupling the $Tc(CO)_3$ to a multi-valent flutamide compound for increased binding. The goal was to incorporate two flutamide targeting molecules into the compound structure. The model compounds (benzyl) illustrated the potential for preparing the flutamide compound. Experimental details are noted in the published article to

Inorganic Chimica Acta. Although this bivalent approach is believed to enhance binding, the poor stability of the 2+1 approach based on the serum and stability data limited this potential application. Further study with stable tridentate ligand systems is needed.

- *2+2 = 3 approach*

The development 2+2 = 3 approach was built on the observations of the instability of the 2+1 complex. We hypothesized that two co-ligands could be covalently linked in situ to generate a single tridentate chelate. The conversion of the two ligands into a single tridentate chelate would avoid the dissociation issues of the 2+1 approach. In this approach, an in situ Schiff base or imine was formed by reacting a coordinated acetylacetone with a primary amine. The first of three preliminary studies investigating the design of this approach was reported in Inorganic Chemistry 2008 with a pyridine amine (See attached) with additional manuscripts with histamine and diamine ligands currently in preparation (Figure 5).

Figure 5. Investigation of the 2+2 = 3 with bidentate ligands (histamine, diamine)



- *S-functionalization of cysteine*

Utilizing the reactivity of the sulfur in cysteine, a new class of S-methylpyridyl cysteine ligand was prepared and investigated. As highlighted in the Inorganic Chemistry 2009 paper, we were able to successfully able to understand the synthetic approaches and coordinatation chemistry based on the achievements developed for the cysteine flutamide. The ligand was found to be potent at 10^{-6} M ligand concentrations with $^{99m}Tc(CO)_3$ and more effective than the ethylene version identified in year 1.[4]

The new ligand systems is currently being applied to the flutamide moiety with a longer linker as early attempt to directly couple the ligand and flutamide would have decreased affinity for the AR. The ligand and the synthetic approach developed here can also be applied to a number of types of targeting molecules, such as peptides (linear, cyclized, folded structures).

- *Click chemistry incorporation into the molecule and coupling methodology.*

An additional approach investigated a new method to incorporate the flutamide targeting agent with a ligand through a non-standard covalent approach using "click" chemistry defined as a rapid approach to coupling two molecules together. As described in detail in the submitted communication to Dalton Trans (attached), the concept was to separate the radiolabeling from the click or coupling of the two molecules using Sharpless chemistry of cyclolization of an azide with an alkyne. The premise was to develop a new method to attach radioactive complexes and provide a more universal labeling unit for use in numerous applications. We compared two approaches 1) preparing the molecules then complexing with the metal 2) complexing the metal then coupling. The dipyridylamine ligand was functionalized with an alkyne and readily reacted with an azide functionalized Flutamide or $M(CO)_3$ (^{99m}Tc, Re) in either approach. In short, the second approach is the first example of metal complexes to achieve this chemistry. The real benefit allows the molecules to be coupled in similar concentration as approach 1 (10^{-5}-10^{-6} M), however, the reaction can be successfully carried out in half the time of approach 1 (15 min) and at room temperature (25 °C) compared to 70 °C. This development may have a huge impact on the types of complexes that can be coupled to metal complexes. Temperature sensitive molecules could be efficiently labeled at biological relevant temperatures with the new approach that was not possible with approach 1. Furthermore, the formation of a triazole ring from the click reaction provides two additional points for improving the affinity of the agents to AR. The inclusion of the triazole into the molecule would provide a second aromatic system that generate a crossover agent similar to bicalutimde, which may also serve to improve AR affinity. Second, the click reaction shifts the complex portion of the molecule further away from the binding portion of the molecule.

Key Research Accomplishments

1) Successful preparation and *in vitro* evaluation of the first generation of flutamide compounds linked with chelate and complexed with $Re(CO)_3$ and $^{99m}Tc(CO)_3$

2) Successful development/reporting a new labeling strategy for *in situ* ligand formation for the formation of tridentate ligands from two different ligand systems using the reactivity of acac toward amine donors on the metal center.

3) Successful development and exploration of a new labeling strategy using cysteine and pyridine to generate an *in situ* ligand formation and complexation of $Re(CO)_3$ and $^{99m}Tc(CO)_3$.

4) Discovery and implementation of a new approach of click chemistry in radiopharmaceutical applications to improve specific activity and targeting affinity of androgen targeting agents. Importantly, this room temperature and mild approach allows the use of targeting molecules that would otherwise be inaccessible due to limitations of radiolabeling.

Reportable Outcomes

Published Manuscripts

1) Benny, Paul D.*; Fugate, Glenn A.; Barden, Adam O.; Morley, Jennifer E.; Silva-Lopez, Elsa; Twamley, Brendan. Metal-Assisted In Situ Formation of a Tridentate Acetylacetone Ligand for Complexation of fac-$Re(CO)_3^+$ for Radiopharmaceutical Applications. *Inorganic Chemistry* (2008), 47(7), 2240-2242.

2) He, Haiyang; Morely, Jennifer E.; Silva-Lopez, Elsa; Bottenus, Brienne; Montajano, Maribel; Fugate, Glenn A.; Twamley, Brendan; Benny, Paul D.*. Synthesis and Characterization of Nonsteroidal-Linked $M(CO)_3^+$ (M = ^{99m}Tc, Re) Compounds Based on the Androgen Receptor Targeting Molecule Flutamide. *Bioconjugate Chemistry* (2009) 78–86

3) Benny, Paul D.*; Fugate, Glenn A.; Morley, Jennifer E.; Twamley, Brenden; Trabue, Steven Synthesis and Characterization of 2,5-bis(benzyl thio)-1,3,4-thiadiazole Complexes with *fac*-$ReBr_3(CO)_3^{2-}$ *Inorganica Chimica Acta* (2009) 1289–1294

4) He, Haiyang; Morley, Jennifer E.; Twamley, Brendan; Groeneman, Ryan H.; Bučar, Dejan-Krešimir; MacGillivray; Leonard R. and Benny, Paul D.* Investigation of the Coordination Interactions of S-(Pyridin-2-ylmethyl)-L-Cysteine Ligands with $M(CO)_3^+$ (M = Re, ^{99m}Tc) *Inorganic Chemistry* 2009 (Available on the web as ASAP article) http://pubs.acs.org/doi/abs/10.1021/ic901159r)

5) Benny, P. D.*; He, H.; Morely, J. Functionalized Cysteine Derivatives for Labeling $Tc(CO)_3$ *J. Radiolabeled Compounds* (2009) S442

In press Manuscripts

6) Moore, Adam L.; Bučar*b*, Dejan-Krešimir; MacGillivray, Leonard R.*;* Benny, Paul D.* "Click" Labeling Strategy for $M(CO)_3$ (M= Re, ^{99m}Tc) Prostate Cancer Targeted Flutamide Agents. *Dalton Trans Communication* (Submitted 10-2009)

Manuscripts in preparation

7) Paul D. Benny*, Adam Moore, Elsa Silva-Lopez, Brendan Twamley. Investigation of Charge Dependence on Androgen Receptor binding with ^{99m}Tc Flutamide complexes

8) Paul D. Benny*, Adam Moore, Glenn A. Fugate, Brendan Twamley. Novel 2 + 2 = 3 Approach for Preparing Tridentate Histamine Acac Complexes with *fac*-$M(OH_2)_3(CO)_3^+$ (M= Re, ^{99m}Tc)

9) Paul D. Benny*, Lyndel Raiford, Glenn A. Fugate, Brendan Twamley. Novel 2 + 2 = 3 Approach for Preparing Tridentate Diamine Acac Complexes with *fac*-$M(OH_2)_3(CO)_3^+$ (M= Re, ^{99m}Tc)

Presentations partially or fully supported from the grant

11/4/2009	Chemistry Colloquium, Northeastern University, Boston, MA
10/6/2009	The Palouse Prostate Cancer Support Group, Recent Advances in Prostate Cancer Diagnostic Imaging
8/19/2009	Imaging the androgen receptor in prostate cancer with organometallic Tc-99m compounds, (invited speaker) 238th American Chemical Society meeting, Washington DC, August 15-20, 2009.American Chemical Society, Molecular Imaging Section (invited)
7/14/2009	P. D. Benny*, H. He[†], J. Morley[∞]. Functionalized Cysteine Derivatives for Labeling $Tc(CO)_3$, *Society of Radiopharmaceutical Symposia*, Edmonton, Canada, July 12-17, 2009
4/2/2009	School of Molecular Biosciences Colloquium, Washington State University
3/17/2009	Missouri Baptist University, Student Seminar, Careers in Prostate Cancer Molecular Imaging
3/16/2009	St. Louis University, Chemistry/Biochemistry Seminar,
3/13/2009	University of Missouri-Columbia, Chemistry Colloquium,
12/9/2008	Univ. of Idaho-Moscow, Chemistry Colloquium,
9/9/2008	Stanford University, Molecular Imaging Group
9/10/2008	Probe development Meeting, Univ. of California- San Francisco
3/26/ 2008	Radiochemistry at Washington State University. Paul Benny. The Cure Start Here, Symposium for Medical Isotope Applications, Kennewick, WA.
4/18/2008	Future Directions in Prostate Cancer Imaging, Paul Benny. The Idaho Society of Radiologic Technologists, Lewiston, ID.
9/7/2007	Targeting Prostate Cancer through the Androgen Receptor with Organometallic Tc-99m Flutamide Derivatives. Benny, Paul*; Silva-Lopez, Elsa[‡]; Bottenus, Brienne[∞]; Montejano, Maribel[∞]: Prostate Cancer Research Program Meeting, Atlanta, GA
4/2008, 4/2007 4/2006, 4/2005	Pharmaceutical Applications of Nuclear Medicine. Benny, Paul PharS 534 course, Washington State University, Pullman, WA

3/2007	Paul Benny*, Eileen Morley[∞], Elsa Silva-Lopez[‡], Daniel Vanderbilt[∞]. "Targeting Prostate Cancer through the Androgen Receptor with Organometallic Tc-99m Flutamide Derivatives" Washington State University, Fourth Annual College of Sciences Undergraduate Research Poster Competition.
11/2006	Targeting Prostate Cancer through the Androgen Receptor with Organometallic Tc-99m Flutamide Derivatives. Benny, Paul*; Silva-Lopez, Elsa[‡]; Bottenus, Brienne[∞]; Montejano, Maribel[∞]: 11TH Prouts Neck Meeting on Prostate Cancer NIH/NCI Meeting, Prouts Neck, Maine
9/7/2006	*In situ* formation of tridentate ligands in aqueous media from 2, 4-pentanedione to form imine complexes around $[M(CO)_3(OH_2)_3]^+$ (M=Re, ^{99m}Tc) centers. Fugate, Glenn A.[†]; Bottenus, Brienne N.[∞]; Benny, Paul*. Abstracts of Papers, 232nd ACS National Meeting, San Francisco, CA, United States
6/2006	S-functionalized cysteine ligands for complexation with $M(CO)_3^+$ M=Re, ^{99m}Tc for diagnostic applications. Bottenus, Brienne N.[∞]; Fugate, Glenn A.[†]; Benny, Paul*. Actinides Separations, Conference Pacific Northwest National Lab
6/2006	In situ formation of tridentate ligands in aqueous media from 2, 4-pentanedione to form imine complexes around $[M(CO)_3(OH_2)_3]^+$ (M=Re, ^{99m}Tc) centers. Fugate, Glenn A.[†]; Bottenus, Brienne N.[∞]; Benny, Paul*. Actinides Separations, Conference Pacific Northwest National Lab
3/12/2006	S-functionalized cysteine ligands for complexation with $M(CO)_3^+$ M=Re, ^{99m}Tc for diagnostic applications. Bottenus, Brienne N.[∞]; Fugate, Glenn A.[†]; Benny, Paul*. Abstracts of Papers, 231st ACS National Meeting, Atlanta, GA, United States
3/12/2006	Organometallics in the Design of Radiopharmaceuticals for Molecular Imaging Applications. Department of Pharmaceutical Sciences seminar, Washington State University, Pullman, WA
3/13-17/2005	Radiochemical separations in nuclear medicine: Where are we now and where we can go? Benny, Paul. 229th ACS National Meeting, San Diego, CA, United States,
07/2004, 07/2005 07/2009	Lecturer, Nuclear Medicine Symposium ACS/DOE Nuclear Chemistry Summer School Brookhaven National Lab, Upton, New York

Grants submitted based on concepts developed within the grant or from the experience gained while conducting the work.

Funded projects

1) LSDF 08-01: 12/1/08 – 11/30/10
Life Science Discovery Fund
Role on the Project: (Co-PI) 2.00 calendar
Chemoaffinity Agents for the Detection of Prostate Cancer

2) DE-PS02-08ER08-11 10/1/08 – 9/30/10
DOE
Role on the Project: (PI) 1.00 calendar
Development of Prostate Specific Membrane Antigen (PSMA) Inhibitors Coupled to $^{99m}Tc(CO)3^{+}$ with Enhanced Specific Activity for SPECT Imaging

1) WSU College of Sciences 5/2006-4/2007
Undergraduate Research Mini-grant
Role on the Project: (Mentor)
Development of Androgen Targeting ^{99m}Tc Agents for Prostate Cancer Imaging

Pending Grants

1) PC093686 3 year
DOD- Prostate Cancer Idea Development
Role on the Project: (PI) 1.0 calendar
Designing High Specific Activity Prostate Cancer Imaging Agents

2) BC097294 1 year
DOD-Breast Cancer Concept Award
Role on the Project: (PI)
A Click Chemistry Approach to Multivalent Targeting of the HER-2 receptor with $^{99m}Tc(CO)_3$

Not funded

1 year
1) Wendy Will Case Cancer Foundation
Role on the Project: (PI) 1.0 calendar
Click Chemistry Methodology for Assembling Prostate Cancer Imaging Agents

2) PC081290 3 year
DOD-Prostate Cancer Idea Development
Role on the Project: (PI) 1.0 calendar
Click Chemistry Methodology for Assembling Prostate Cancer Imaging Agents

3) BC085805 1 year
DOD-Breast Cancer Concept Award
Role on the Project: (PI) 1.0 calendar
Novel Labeling Strategies of Affibodies for Imaging of Breast Cancer with $^{99m}Tc(CO)3$.

Student Training and Impact

	Degrees		Current Location (Position)
Brienne Bottenus	BS 5/2006	WSU	Univ. of Missouri-Columbia (Graduate Student)
Eileen Morley[*]	BS 5/2008	WSU	Univ. of California-Santa Barbara (Medical Student)

[*]Honors thesis on prostate cancer research was given "*With Distinction*" by the WSU Honors College

Arianna Wimple[‡]	BS 5/2011		University of Montana (undergraduate)

[‡]Currently conducting her honors thesis research on ^{99m}Tc Flutamide complexes

Conclusions and Future Directions

The results presented here illustrate the potential of $^{99m}Tc(CO)_3$ flutamide molecules for prostate cancer imaging. The successful preparation and radiolabeling of the first generation of compounds illustrates one the key critical objectives being achieved and provided key understanding of the chemistry and the limitations of the synthetic approaches utilized. The initial results have provided a positive foundation to continue to improve new probes for AR targeting. In addition, a number of new fundamental strategies and methodologies are a direct outcome of the work investigated for Flutamide functionalized molecules. The results obtained may not only aide in the goals of $^{99m}Tc(CO)_3$ flutamide molecules, but may also translate to the development of other prostate cancer targeting agents.

One of the key future goals is to translate the current studies into mouse tumor models to ascertain *in vivo* interactions of the molecules in a prostate cancer model. In conjunction with the in vivo assessment, future investigations will focus to 1) Optimize linker distance to improve affinity by developing a combinatorial approach 2) Improve the flutamide binding moiety with additional functional groups to enhance AR binding following a structure activity relationship study to provide additional information about affinity 3) Investigate multi-valent approaches with small molecules, nanoparticles etc. The work developed under this grant has allowed a number of platforms to be developed and multiple new directions in research investigations to be achieved.

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[3]Schibli, Roger; Schwarzbach, Rolf; Alberto, Roger; Ortner, Kirstin; Schmalle, Helmut; Dumas, Cecile; Egli, Andre; Schubiger, P. August. *Bioconjugate Chemistry* **2002**, 13(4), 750-756.

[4]Fish, Windle; Gafield, Scherer; *Inorg. Chem.*, **1973**, 855-859.

Appendices

List of Personnel supported by this funding

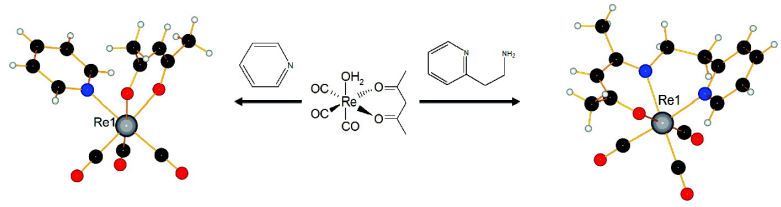
Personnel	Position
Paul Benny	Professor, director of research
Hiayang He	Postdoctoral Research Associate
Elsa Lopez	Graduate student
Eileen Morley	Undergraduate Researcher
Arianna Wimple	Undergraduate Researcher (Univ. of Montana)

Communication

Metal-Assisted In Situ Formation of a Tridentate Acetylacetone Ligand for Complexation of *fac*-Re(CO) for Radiopharmaceutical Applications

Paul D. Benny, Glenn A. Fugate, Adam O. Barden, Jennifer E. Morley, Elsa Silva-Lopez, and Brendan Twamley

Inorg. Chem., **2008**, 47 (7), 2240-2242 • DOI: 10.1021/ic701996e • Publication Date (Web): 26 February 2008



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Metal-Assisted In Situ Formation of a Tridentate Acetylacetone Ligand for Complexation of *fac*-$Re(CO)_3^+$ for Radiopharmaceutical Applications

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Reaction of $[NEt_4]_2[ReBr_3(CO)_3]$ with 2,4-pentanedione (acac) yields a complex of the type *fac*-$Re(acac)(OH_2)(CO)_3$ (**1**) under aqueous conditions. **1** was further reacted with a monodentate ligand (pyridine) to yield a *fac*-$Re(acac)(pyridine)(CO)_3$ complex (**2**). Complex **1** was found to react with primary amines to generate a Schiff base (imine) in aqueous solutions. When a mixed-nitrogen donor bidentate ligand, 2-(2-aminoethyl)pyridine, that has different coordination affinities for *fac*-$Re(acac)(OH_2)(CO)_3$ was utilized, a unique tridentate ligand was formed in situ utilizing a metal-assisted Schiff base formation to yield a complex *fac*-$Re(CO)_3$(3[(2-phenylethyl)imino]-2-pentanone) (**3**). Tridentate ligand formation was found to occur only with the Re-coordinated acac ligand. Reactions of acac with *fac*-$Re(CO)_3Br$(2-(2-aminoethyl)pyridine) (**4**) or a mixture of $[NEt_4]_2[ReBr_3(CO)_3]$, acac, and 2-(2-aminoethyl)pyridine did not yield the formation of complex **3** in water.

Technetium-99m ($t_{1/2}$ = 6.02 h; γ = 140 keV) is the radionuclide of choice in hospitals comprising 90% of all nuclear medicine imaging scans.[1] Development of organometallic technetium complexes, such as *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$, has provided new avenues of complex formation for diagnostic imaging.[2,3] Current labeling strategies include incubation of monodentate, bidentate, tridentate, or combination (2 + 1) ligand systems with the *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ moiety.[4,5] Some of the best ligand systems (histidine, cysteine, and 2,3-diaminopropanoic acid) form tridentate complexes at >90% at 10^{-6} M.[6–9] However, complex formation below 10^{-6} M appears limited by the thermodynamics of ligand substitution of the technetium(I) center.[10]

Interest in developing new modes of complex formation has led us to investigate 2,4-pentanedione or acetylacetone (Acac) as a potential ligand system. acac is a well-established bidentate ligand that coordinates a number of transition metals. Acac can also be synthetically modified to incorporate a linked biotargeting moiety at carbon C1 and/or C3. The Schiff base or imine versions of acac are prepared by reacting a primary amine with the ligand in organic solvents. The stability of the Schiff base ligand in water may be limited by the hydrolytic nature of the imine bond. The mixed-donor (O, N) acac-derived Schiff base ligand provides an excellent ligand for rhenium with improved stability over the acac ligand alone.[11,12]

Acac-based complexes were prepared and characterized with natural rhenium to better understand the chemistry that would be potentially found with radioactive ^{99m}Tc and $^{186/188}Re$ analogues used in nuclear medicine. The reactions reported within were prepared in water to simulate reaction conditions that would be potentially translatable to the analogous radioactive complexes. The rhenium acac complex can be formed by heating $[NEt_4]_2[ReBr_3(CO)_3]$ with acac at 70 °C for 2 h in 10.0 mL of water to yield *fac*-$Re(acac)OH_2(CO)_3$ (**1**; Scheme 1). The product, **1**, remains quite soluble in water but can be isolated as a colorless solid

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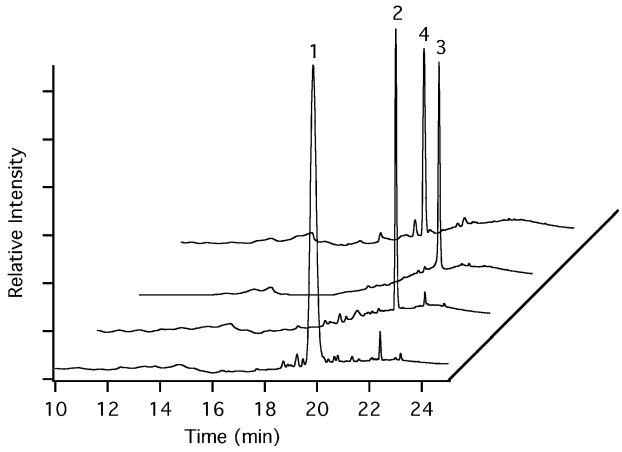
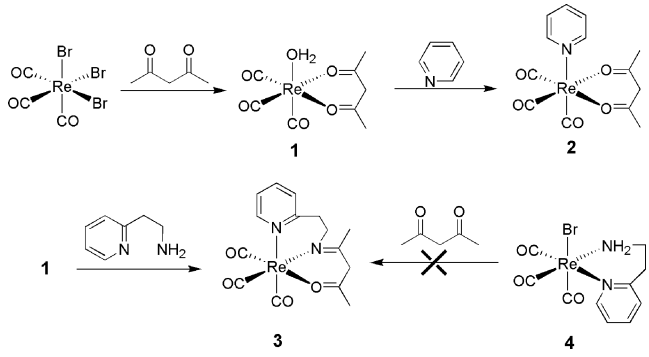


Figure 1. UV−HPLC trace at 220 nm of **1**−**4**.

Scheme 1. Synthetic Route for Preparation of Rhenium acac Complexes and Subsequent Reactions with Mono- and Bidentate Ligands To Yield "2 + 1" and in Situ Formed Tridentate Complexes



in high yield through concentration and cooling of the solution in a refrigerator (∼2 °C) overnight. High-performance liquid chromatography (HPLC) studies of the solution and the isolated solid revealed a single peak of complex **1** at 20.6 min verified by NMR (Figure 1).

Complex **1** is a versatile reagent because it can be isolated as a solid or utilized directly from the reaction mixture. The addition of a second monodentate ligand to **1** generated a "2 + 1" style of complex. When **1** is reacted with pyridine at 70 °C overnight, the complex *fac*-Re(acac)(CO)$_3$py (**2**) can be prepared in high yield. **2** is the only product observed from the solution. Even in the presence of excess pyridine, displacement of the acac ligand was not observed. The formation of **2** can be observed by the appearance of a new peak at 22.3 min in the HPLC (Figure 1). The X-ray structure of **2** was obtained by diffusion of pentane into a dichloromethane solution of **2** (Figure 2).[13] The octahedral complex has comparable Re−CO bonds (1.89–1.92 Å) with an asymmetric axis elongated along the Re−pyr (Re1−N1 2.20 Å) and acac (Re−O1 or Re−O2 2.12 Å) axes. The acac ligands show a minimally constrained bite angle (O1−Re−O2 85.07°). The pyridine N1 is equidistant to the O1/O2 of the acac ligand (O−Re−N1 82.67–83.3°).

(13) **2**, monoclinic space group *P*2(1)/*c* with cell dimensions $a = 14.9940(7)$ Å, $b = 6.8687(3)$ Å, and $c = 14.1746(6)$ Å and $\beta = 104.698(1)°$.

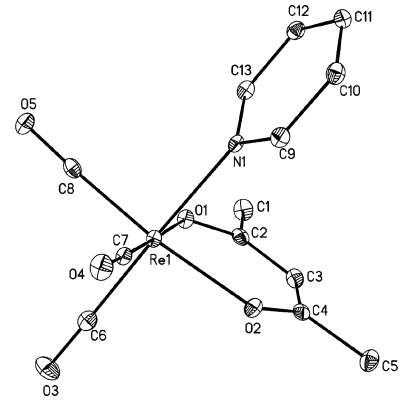


Figure 2. Molecular structure of **2** (thermal displacement 30%). Hydrogen atoms are omitted clarity. Bond distances (Å): Re1−O1 2.1189(19), Re1−O2 2.1226(19), Re1−C6 1.926(3), Re1−C7 1.896(3), Re1−C8 1.903(3), Re1−N1 2.209(2). Bond angles (deg): O1−Re−O2 85.07(8), O1−Re−C6 95.29(10), O1−Re−C7 177.72(10), O1−Re−C8 92.76(10), O2−Re−N1 82.67(8), O2−Re−C6 95.62(11), O2−Re−C7 94.55(10), O2−Re−C8 174.08(10), N1−Re−C6 177.88(10), N1−Re−C7 94.39(10), N1−Re−C8 91.62(11).

Although the "2 + 1" complex **2** is coordinatively saturated, ligand displacement may limit the effectiveness of the complex in vivo. The formation of a tridentate ligand compared to the "2 + 1" complex may have increased stability toward substitution because of the chelate effect. The "2 + 1" complex of **2** can be transformed by an in situ reaction into a tridentate complex utilizing the reactivity of acac in **1** to form an imine from a primary amine. An analogous rhenium pyridine aldehyde complex was previously demonstrated to form a bidentate imine complex system from a primary amine; however, the bidentate complex had limited stability toward ligand substitution.[14,15] *fac*-Re(CO)$_3$(3[(2-phenylethyl)imino]-2-pentanone)] (**3**) was prepared in a two-step process: formation of the acac complex **1** followed by the addition of a second bidentate ligand (Scheme 1). Complex **3** was formed either stepwise or as a single-pot reaction. The formation of the imine bond in **3** was observed by the addition of 2-(2-aminoethyl)pyridine to an aqueous solution of complex **1** followed by heating at 70 °C. The product precipitated as a colorless solid upon cooling to room temperature. The reaction progress was monitored by HPLC, where the disappearance of **1** and the appearance of **3** at 21.4 min were observed in the chromatogram (Figure 1). Crystals of **3** were obtained by slow evaporation of a methanol/water solution at room temperature (Figure 3).

The solid-state structures of the "2 + 1" complex **2** and the tridentate complex **3** have many structural similarities in bond distances (i.e., Re−pyridine ∼2.2 Å, Re−O1 2.13 Å, Re−CO ∼1.9 Å) and angles (O1−Re−N1 82.24(8)° in **3** has a bite angle similar to that of the acac ligand in **2**). However, the methylene carbons (C6 and C7) have larger than typical bond angles (113–115°). C3 of the acac

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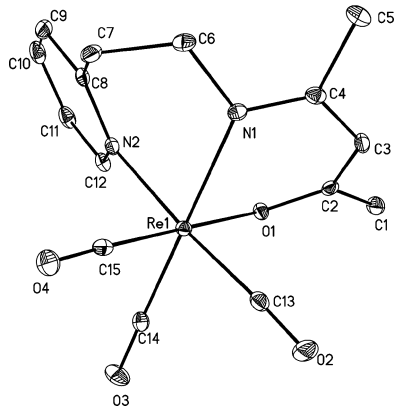

Figure 3. Molecular structure of **3** (30% thermal displacement). Hydrogen atoms are omitted for clarity. Bond distances (Å): Re1−O1 2.1336(18), Re1−N1 2.166(2), Re1−N2 2.197(2) Re1−C13 1.925(3), Re1−C14 1.933(3), Re1−C15 1.901(3). Bond angles (deg): O1−Re−N1 82.24(8), O1−Re−C13 93.27(10), O1−Re−C14 92.46(9), O1−Re−C15 178.39(9), N1−Re−N2 80.36(8), N1−Re−C13 96.05(10), N1−Re−C14 173.77(9), N1−Re−C15 99.16(10), N2−Re1−C13 175.54(10), N2−Re1−C14 95.85(10), N2−Re1−C15 95.76(10).

ligand in **3** is also positioned slightly out of plane because of the steric restraints of the linked pyridine and the imine bond of the tridentate system in **3** compared to **2**.

The Schiff base formation of the tridentate complex utilizes distinct differences in the coordination strength of the bidentate ligand 2-(2-aminoethyl)pyridine, containing a primary amine and an aromatic amine. It is proposed that the pyridine ligand, as opposed to the amine, first coordinates to the rhenium center, replacing the labile aqua ligand. The uncoordinated amine donor is available for nucleophilic attack on the C2 of the coordinated acac ligand. The coordinated oxygen from the acac ligand is converted to water during the Schiff base condensation and probably remains coordinated for a brief moment prior to displacement by the more favorable imine donor from the tridentate ligand. Reactivity of the amine donor with the acac ligand is believed to depend on the effective chelate ring size and steric constraints of the number of methylene carbons between the pyridine and amine.

Displacement of the acac ligand from complex **1** upon introduction of 2-(2-aminoethyl)pyridine was a primary concern. The potential displacement byproduct *fac*-Re(2-(2-aminoethyl)pyridine)$Br(CO)_3$ (**4**) was prepared by refluxing $[NEt_4]_2[ReBr_3(CO)_3]$ with 2-(2-aminoethyl)pyridine in methanol (Scheme 1). HPLC of the reaction yielded a single peak at 20.0 min corresponding to **4**. The complex was characterized and utilized as a reference for HPLC comparison (Figure 1). Single crystals were obtained from a methanol solution of **4** at 0 °C after several days (Figure 4). **4** was further evaluated to determine the dissociation/reactivity of the coordinated 2-(2-aminoethyl)pyridine by introducing excess acac ligand in water (Scheme 1). A second slightly more hydrophilic peak was observed in HPLC over a prolonged period, which may be due to substitution of the coordinated

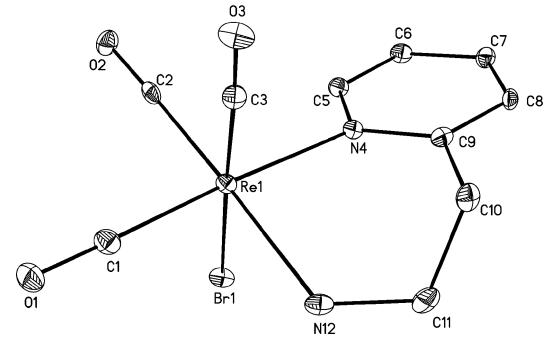

Figure 4. Molecular structure of **4** (thermal displacement 30%). The solvent molecule and hydrogen atoms are omitted for clarity. Bond distances (Å): Re1−Br1 2.1189(19), Re1−O2 2.1226(19), Re1−C6 1.926(3), Re1−C7 1.896(3), Re1−C8 1.903(3), Re1−N1 2.209(2). Bond angles (deg) O1−Re−O2 85.07(8), O1−Re−C6 95.29(10), O1−Re−C7 177.72(10), O1−Re−C8 92.76(10), O2−Re−N1 82.67(8), O2−Re−C6 95.62(11), O2−Re−C7 94.55(10), O2−Re−C8 174.08(10).

bromine in **4** with water. Under the conditions examined, neither the formation of **3** or the displacement of 2-(2-aminoethyl)pyridine by acac yielding **1** was observed, suggesting that 2-(2-aminoethyl)pyridine remains coordinated to the rhenium center in **4** without dissociation or activation of the complex toward Schiff base formation. Although free ligand formation is possible, we examined the possibility that the ligand could be formed in situ by the addition of acac and 2-(2-aminoethyl)pyridine in water followed by the addition of *fac*-$[ReBr_3(CO)_3]^{2-}$. The mixture yielded **4** or the aquo-coordinated complex as observed by HPLC, and no formation of the tridentate ligand complex **3** was observed.

In conclusion, we have demonstrated that acac can be utilized as a bidentate ligand system in a "2 + 1" approach or utilizing coordination differences to generate a tridentate ligand system while coordinated to the rhenium metal center. This new methodology has the potential for linking to targeting molecules, such as small molecules, peptides, and antibodies, for generating in situ tridentate complexes for nuclear medicine applications.

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Supporting Information Available: Full syntheses, characterization of compounds, and X-ray crystallographic bond angles and distances tables (PDF) and X-ray structural information for **2**−**4** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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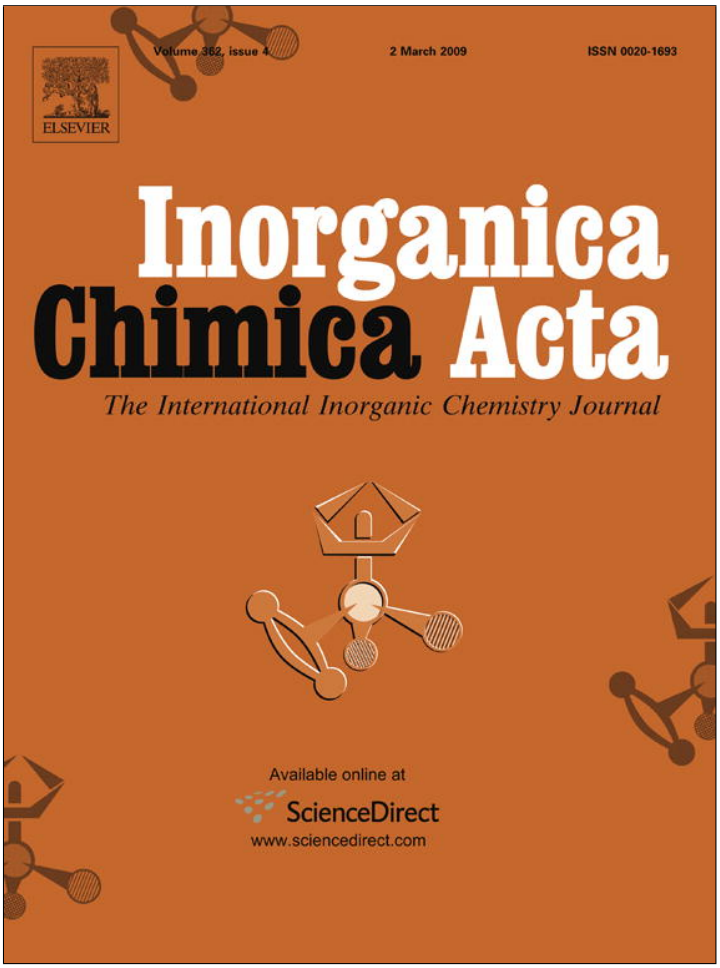

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Synthesis and characterization of 2,5-bis(benzylthio)-1,3,4-thiadiazole complexes with *fac*-$ReBr_3(CO)_3^{2-}$

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ABSTRACT

Reactions of 2,5-bis(benzylthio)-1,3,4-thiadiazole (**1**) with a common organometallic rhenium starting material $[NEt_4]_2[fac\text{-}[Re(I)Br_3(CO)_3]$ yielded two distinct types of complexes. Both complexes coordinate only through the nitrogen of the thiadiazole ring. Reaction of **1** with the rhenium starting material alone yielded a bimetallic complex *fac*-(di-μ-bromo)(μ-2,5-bis(benzylthio)-1,3,4-thiadiazole-κN:κ′N)bis(tricarbonyl rhenium (I)) (**2**). The nitrogens of the thiadiazole ring of **1** each coordinate to a different rhenium center combined with two bridging bromide ligands in **2**. A "2+1" complex was prepared in a two step process by reacting the rhenium starting material with picolinic acid followed by **1** to yield *fac*-Re(I)(2,5-bis(benzylthio)-1,3,4-thiadiazole)$(CO)_3$(picolinate) (**3**). Compounds **2** and **3** were characterized by NMR, IR, UV, elemental analysis, and single crystal X-ray diffraction.

1. Introduction

2,5-Dimercapto-1,3,4-thiadiazole (DTD) and analogs have a broad range of potential applications (i.e., solid state organic crystals, pharmaceuticals, bioinorganic, catalysis) [1–15]. A number of different types of complexes with DTD have been observed with a wide spectrum of transition metals (Scheme 1). DTD can coordinate through several different modes as a mondentate ligand through one of the sulfur donors or a nitrogen in the thiadiazole ring [2–6]. A number of bidentate complexes can also be formed with DTD, where the ligand can interact with the metal center through two sulfur donors similar to a dithiocarbamates or through a combination of thiol and diazole nitrogen analogous to a thioacetimide. The DTD ligand can function as a bidentate ligand with a single metal center [4,7–9] or as a bridging ligand forming a bimetallic complex [8–15]. DTD can also be functionalized by conversion of the thiols into thioethers producing a number of derivatives that provide tunability of the ligand system (Scheme 1). Additional donor ligands (i.e., pyridine, carboxylic acid, acetylacetone) modify the coordination number of the ligand, donors effect, complexes isolated and the chemistry of the complex [7,11,12,16,17].

DTD rhenium complexes have particular applications in photochemical materials, nonlinear optics, radiopharmaceutical applications, and as a surrogate for technetium-99m complexes used in nuclear medicine imaging. Generally, the isolated DTD complexes have been primarily isolated with the rhenium center in lower oxidation states (+1 to +3). Rhenium and technetium(III) complexes formed by reacting a benzothiazole-2-thiol analog revealed a bidentate (S, N) coordination mode with the metal centers [18]. Recently, organometallic rhenium analogs have been reacted with DTD. A common rhenium starting material, $Re(CO)_5(SO_3CF_3)$, was reacted with DTD to yield a mixed monodentate coordination complex [19]. Three octahedral rhenium metal centers were coordinated to a single DTD ligand through the thiols and the nitrogen of the 1,3,4-thiadiazole ring and no displacement of CO ligands was observed. A simplified DTD analog, a diazole ligand was incorporated into tridentate ligand to coordinate to *fac*-$M(CO)_3(OH_2)_3^+$ M = Re, Tc, where a mixture of coordinated diazole species were identified in the reaction mixture [20].

The interesting electronic properties of DTD and the functional capability through alkylation of the thiol into a thioether containing ligand led to the investigation of the thioether analogs with rhenium. Alberto recently reported a benzyl thioether rhenium complex, *fac*-$Re(CO)_3$(*S*-benzyl cysteine), and the corresponding technetium complex, in which the sulfur in the thioether moiety was an acceptable donor for the organometallic core [21]. This paper investigates the interactions of 2,5-bis(benzylthio)-1,3,4-thiadiazole (**1**) with *fac*-$ReBr_3(CO)_3^{2-}$ focusing on the coordination chemistry modes of the ligand and complexes observed. The benzyl analog serves as an important model system for examining the fundamental interactions of the ligand with the *fac*-rhenium center prior to additional electronic tuning of the molecule and

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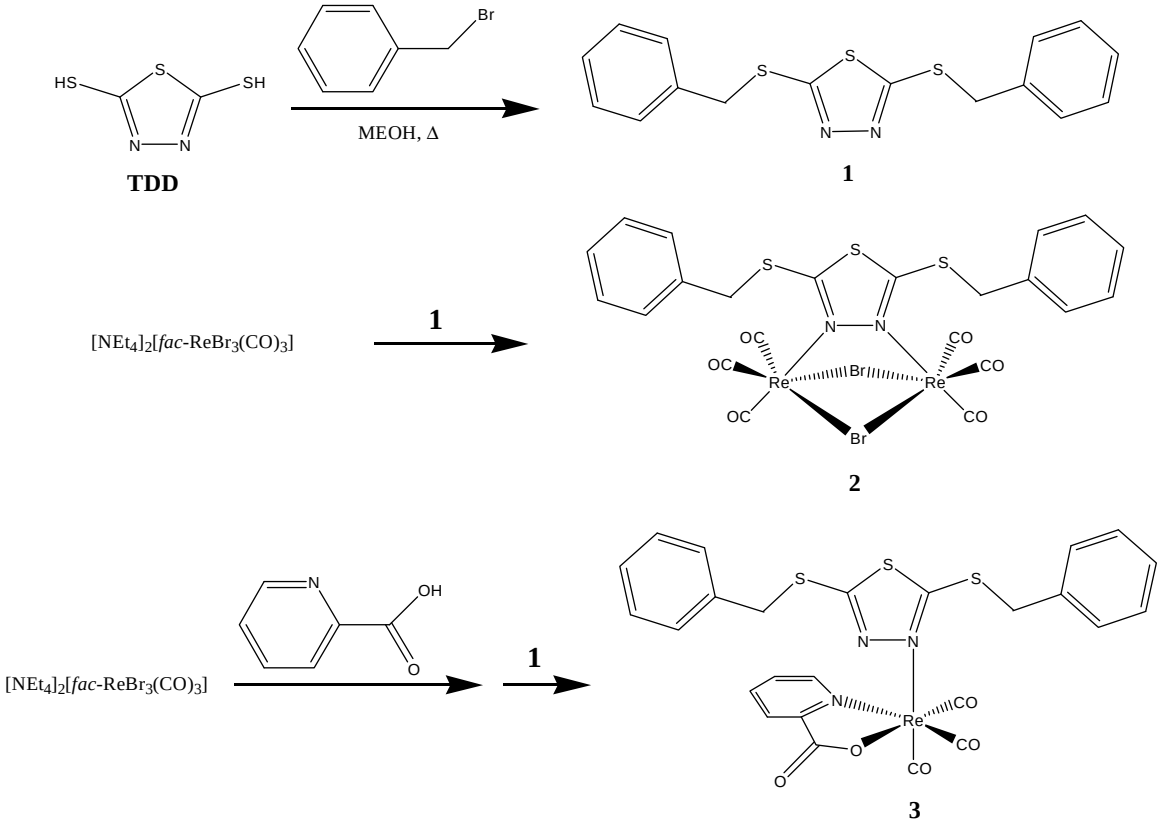

Scheme 1. Synthetic procedures for the formation of **1**, **2** and **3**.

the corresponding absorbance and emission capabilities of the complex.

2. Experimental

2.1. General details

All reagents were of ACS grade or higher were purchased from Aldrich, Fluka, Acros, Strem or Alfa Aesar and were used without further purification. $[NEt_4]_2[fac\text{-}[ReBr_3(CO)_3]$ was prepared in a two step procedure from dirhenium decacarbonyl according to literature methods [22]. ^1H and ^{13}C NMR spectra were recorded at 293 K on a Varian Mercury Vx 300 spectrometer using 5 mm NMR tubes and processed using Varian VNWR 6.1 software using trimethylsilane and/or solvent peaks as an internal reference. IR spectra were recorded on a Thermo Nicolett 6700 FT-IR equipped with an ATR cell and analyzed with OMNIC 7.1a software. UV/Vis spectra were recorded of the compounds in methanol in quartz cuvettes on a Varian Cary 50 Bio and analyzed with Cary WINUV 3.00 software. Elemental analyses were performed by QTI of Whithouse, NJ. Mass spectra of the samples were obtained by electrospray ionization directly infusing the samples into an Agilent 1100 Ion Trap LC/MS/MS and scanning from 50 to 1200 m/z with the drying gas at 12 mL min^{-1} at 350 °C and nebulizer pressure set at 50 psig.

Separation and identification of compounds were conducted on a Perkin Elmer Series 200 High Pressure Liquid Chromatograph (HPLC) with an Agilent Zorbex 30 cm SB-C18 column. The reverse phase gradient system begins with 0.1% trifluoroacetic acid (TFA) aqueous eluent gradually shifting to methanol according to the following method, 0–3.0 min (100% TFA), 3.0–9.0 min (75% TFA, 25% MeOH), 9.0–20.0 min (25–100% MeOH linear gradient), 20.0–25.0 min (100% MeOH) at a flow rate of 1.0 mL/min.

2.2. Preparation of compounds

2.2.1. 2,5-Bis(benzylthio)-1,3,4-thiadiazole (*1*)

2,5-Bis(benzylthio)-1,3,4-thiadiazole (**1**) was prepared by modifying a previously reported procedure to significantly improve yields and purity of the compound [1]. Compound **1** was prepared by combining 2,5-dimercapto-1,3,4-thiadiazole (2.0 g, 0.0133 mol) with benzylbromide (4.76 g, 0.0280 mol) and triethylamine (2.828 g, 0.0280 mol) in 50.0 mL methanol. The solution was stirred and refluxed for 2 h. Upon cooling, the formation of analytically pure white crystalline solid of **1** was observed. The solid was collected by filtration and washed with methanol to yield pure product. Yield 4.038 g, 92%. ^1H NMR (273 K, CD_3COCD_3) 4.54 (4H, s), 7.27–7.38 (3H, m), 7.44–7.48 (2H, m) ^{13}C NMR (273 K, CD_3COCD_3) 38.1, 128.0, 128.8, 129.4, 136.7, 164.8 UV/Vis (nm, (ε)) 294.0 (12, 590) HPLC RT 24.4 min.

2.2.2. [fac-$Re_2(CO)_6(\mu\text{-}Br)_2$($\mu$-2,5-bis(benzylthio)-1,3,4-thiadiazole)] (*2*)

Compound **1** (0.100 g, 0.301 mmol) was combined with *fac*-$[NEt_4]_2[Re(I)Br_3(CO)_3]$ (0.211 g, 0.274 mmol) in 15 mL of methanol. The solution was stirred for 9 h at 60 °C under nitrogen. The methanol was removed by rotary evaporation at 40 °C followed by drying *in vacuo*. The solid was washed three times with methanol to remove residual **1** and $NEt_4^+Br^-$. The solid was redissolved in methylene chloride, filtered, and evaporated to yield the pure complex **2**. Single crystals suitable for X-ray diffraction were produced by slow infusion of pentane into a methylene chloride solution of the complex. Yield: 0.058 g, 41.4%. ^1H NMR (chloroform-d_3): δ = 7.42–7.62 (m, 10H, aromatic H), 4.37 (s, 4, CH_2). ^{13}C NMR (chloroform-d_3): δ = 41.0, 129.0, 129.5, 129.6, 129.7, 131.8, 174.3. IR (solid, cm^{-1}) 2045 m, 2019 m, 1937 s, 1909.1 s, 1889.7 s, 906 s, 729 s, 700 m. UV/Vis (nm, (ε)) 272.0 (45, 900). *Anal.* calc. for

$C_{22}H_{14}Br_2N_2O_6Re_2S_3 \cdot 0.75\ CH_3OH$: C, 25.9; H, 1.62; N, 2.65. Found: C, 28.86; H, 1.64; N, 3.50%.

*2.2.3. [fac-Re(CO)$_3$(picolinate)(2,5-bis(benzylthio)-1,3,4-thiadiazole)] (**3**)*

Picolinic acid (0.035 g, 0.286 mmol) and [NEt$_4$]$_2$[*fac*-Re(CO)$_3$Br$_3$] (0.200 g, 0.260 mmol) were dissolved into 10.0 mL of methanol. Sodium bicarbonate (0.290 mmol) was added to adjust the pH to neutral. The stirred solution was heated at 70 °C under nitrogen for 1 h when HPLC indicated the formation of Re(CO)$_3$(OH$_2$)(picolinate) was complete (RT 18.5 min). Compound **1** (0.094 g, 0.286 mmol) was added to the solution and heated at 70 °C overnight after which time HPLC indicated the formation of *fac*-[Re(CO)$_3$(picolinate) (**1**)] (**3**; RT 23.5 min). Upon evaporation of the solution an off-white precipitate was collected and washed with water multiple times. The product, **3,** was isolated by silica chromatography (RF 0.15; 3:1 ethyl acetate:hexane). Single crystals suitable for X-ray diffraction were produced by slow evaporation of the complex solution in mixed media of acetonitrile and water. Yield: 0.098 g, 48.8%. ^{1}H NMR (chloroform-d_3): δ = 8.76 (d, 1H, HC), 8.13 (d, 1H, aromatic H), 7.92 (t, 1H, aromatic H), 7.40 (m, 6H, aromatic H), 7.29 (m, 3H, aromatic H), 7.14 (m, 2, aromatic H), 4.326 (s, 2, benzyl), 4.18 (d, 1H$_a$, J_{ab} = 12.9, benzyl) 3.98 (d, 1H$_b$, J_{ab} = 12.9, benzyl). ^{13}C NMR (chloroform-d_3): δ = 174.8, 173.0, 163.8, 152.0, 151.9, 139.4, 134.3, 133.1, 129.2, 129.2, 129.1, 128.9, 128.9, 128.8, 128.3, 128.0, 127.2, 126.4, 107.3, 41.2, 38.4. IR (solid, cm^{-1}) 2017 s, 1889 s, 1637 m, 1598 m, 1349 m, 774 m, 691 m. UV/Vis (nm, (ε)) 286.9 (17, 320). MS M$^+$ 745.9. *Anal.* calc. for $C_{25}H_{18}N_3O_5ReS_3$: C, 41.54; H, 2.51; N, 5.81. Found: C, 41.83; H, 2.80; N, 5.89%.

2.3. X-ray experimental

Crystals of **2** and **3** were removed from the flask and covered with a layer of hydrocarbon oil. A suitable crystal was selected, attached to a glass fiber and placed in the low-temperature nitrogen stream [23]. Data for **2** and **3** were collected at ca. 89 K using a Bruker/Siemens SMART APEX instrument (Mo Kα radiation, λ = 0.71073 Å) equipped with a Cryocool NeverIce low temperature device. Data were measured using omega scans of 0.3° per frame for 5 s for both. A full sphere of data was collected in each case with a total of 2400 frames and a final resolution of 0.83 Å. Cell parameters were retrieved using SMART [24] software and refined using SAINTPLUS [25] on all observed reflections. Data reduction and correction for Lp and decay were performed using the SAINTPLUS software. Absorption corrections were applied using SADABS [26]. Each structure was solved by direct methods and refined by least squares method on F^2 using the SHELXTL program package [27]. Compound **2** was solved in the space group $P2(1)/n$ (#14) and **3** in $P\bar{1}$ (#2) by analysis of systematic absences. All non-hydrogen atoms were refined anisotropically. No decomposition was observed during data collection. Details of the data collection and refinement are given in Table 1. Further details are provided in the Supplementary material.

Table 1
Crystallographic data and structure refinement parameters

	2	**3**
Formula	$C_{22}H_{14}Br_2N_2O_6Re_2S_3$	$C_{50}H_{38}N_6O_{11}Re_2S_6$
Molecular weight	1030.75	1463.62
Crystal system, Space group	monoclinic, $P2(1)/n$	triclinic, $P\bar{1}$
a (Å)	13.8647(17)	12.282(4)
b (Å)	12.7260(16)	14.072(4)
c (Å)	15.3535(19)	15.554(4)
α (°)		101.381(4)
β (°)	98.582(2)	104.464(4)
γ (°)		91.371(4)
V (Å^3)	2678.7(6)	2544.2(13)
Z	4	2
T (K)	89(2)	89(2)
λ (Å)	0.71073	0.71073
ρ_{calc} (Mg/m^3)	2.556	1.911
μ (mm^{-1})	12.288	5.068
F(000)	1904	1428
Crystal size (mm^3)	0.27 × 0.16 × 0.08	0.32 × 0.18 × 0.14
θ Range (°)	1.85–25.25	1.38–25.25
Index ranges	$-16 \leqslant h \leqslant 16$, $-15 \leqslant k \leqslant 15$, $-18 \leqslant l \leqslant 18$	$-14 \leqslant h \leqslant 14$, $-16 \leqslant k \leqslant 16$, $-18 \leqslant l \leqslant 18$
Number of reflections collected	39960	37764
Number of independent reflections (R_{int})	4861 (0.0433)	9222 (0.0251)
Data/restraints/parameters	4861/0/334	9222/0/676
Goodness-of-fit	1.100	1.023
R_1[a] [$I > 2\sigma(I)$]	0.0233	0.0207
wR_2[a] [$I > 2\sigma(I)$]	0.0499	0.0495
Largest difference in peak, hole (e Å^{-3})	0.997, −0.934	1.281, −0.629

[a] $R_1 = \sum||F_o| - |F_c||/\sum|F_o|$; $wR_2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]\}^{1/2}$.

3. Results and discussion

*3.1. Synthesis and characterization of compounds **1–3***

Investigation of the interactions of 2,5-bis(benzylthio)-1,3,4-thiadiazole (**1**) with *fac*-ReBr$_3$(CO)$_3^{2-}$ yielded several unexpected results. Ligand **1** functioned in two coordination modes as a monodentate ligand solely through the nitrogen donor or as a bridging ligand through both nitrogen's on the thiadiazole ring. The general synthesis of compounds **1–3** prepared within are illustrated in Scheme 1.

Although the preparation of 2,5-bis(benzylthio)-1,3,4-thiadiazole (**1**) had been reported previously, the synthetic method reported in this work significantly improves both the yield and the purity of the compound [1]. The selection of methanol as the reaction solvent as opposed to previously reported dimethylformamide (DMF) was a better choice for reaction synthesis, isolation and purification. Compound **1** was prepared by reacting two equivalents of benzyl bromide and 2,5-dimercapto-1,3,4-thiadiazole in the presence of triethylamine in refluxing methanol and was isolated in near quantitative yields as an analytically pure colorless crystalline solid from the reaction solution as the reaction mixture slowly cooled from reflux to room temperature. Residual starting materials (benzyl bromide, 2,5-dimercapto-1,3,4-thiadiazole, triethylamine) and the unwanted salt byproducts (triethylamine hydrogen bromide) from the reaction remained quite soluble in the methanol solution, which significantly improved and simplified the isolation and recrystallization of the product.

Compound **1** was reacted in equal molar equivalents directly with the common starting material [NEt$_4$]$_2$[*fac*-ReBr$_3$(CO)$_3$] (**2**, Scheme 1). It was anticipated that several possible species would be identified from the reaction mixture as **1** was expected to function as either a monodentate ligand from one of the three possible N or S donors or as a bidentate ligand through coordinating the sulfur in the thioether and a nitrogen of the thiadiazole ring with the Re(CO)$_3$ core. It was postulated that the isolated compounds could also contain two or three coordinated **1** ligands to the metal center. However, the formation of multiple coordinated **1** ligands with [NEt$_4$]$_2$[*fac*-ReBr$_3$(CO)$_3$] was not observed under the reaction conditions examined. The product *fac*-(di-μ-bromo)(μ-2,5-bis(benzylthio)-1,3,4-thiadiazole-κN:κ′N)bis(tricarbonyl rhenium (I)) (**2**) precipitated from the reaction mixture and was isolated as a colorless solid. In complex **2**, ligand **1** functions as a μ-**1**-κN:κN′ bridging two rhenium centers solely through the nitrogen's of the thiadiazole ring. Complex **2** has two additional coordinated μ bro-

mide ligands bridging the two rhenium centers to generate an overall neutral complex.

Initial experiments varying the concentration of **1** relative to the $[NEt_4]_2[fac\text{-}ReBr_3(CO)_3]$ starting material were conducted to investigate the possibility of inducing other species to form in solution. It was anticipated the reaction mixture may yield monomeric products such as $[fac\text{-}ReBr_2(CO)_3(\mathbf{1})]^-$, $fac\text{-}ReBr(CO)_3(\mathbf{1})_2$, and $[fac\text{-}Re(CO)_3(\mathbf{1})_3]^+$ or dimeric products $[fac\text{-}Re_2\mu\text{-}Br(CO)_3(\mu\text{-}\mathbf{1})_2]^+$ or $[fac\text{-}Re_2\mu\text{-}Br(CO)_3(\mu\text{-}\mathbf{1})_2]^{2+}$ in the course of the reaction. Treatment of $fac\text{-}[ReBr_3(CO)_3]^{2-}$ with excess **1**, which would most likely lead to monomeric species with multiple ligands per rhenium center or multiple bridging ligand between two metal centers did not yield such complexes in isolation. Treatment of $fac\text{-}[ReBr_3(CO)_3]^{2-}$ with limited concentration of **1**, which would most likely lead to bridging complexes with multiple rhenium centers per ligand bridging two, were not isolated as well. In all concentrations (0.5, 1, 2, 3, 5 equiv.) of **1** relative to the rhenium starting material, only compound **2** was isolated from the reaction mixture and no evidence for multiple products was found.

The ^1H and ^{13}C NMR of complex **2** were observed as expected for a symmetric 18 electron bimetallic complex. The coordinated ligand **1** spectral peaks were shifted slightly downfield of the uncoordinated ligand. The NMR spectra for **2** did not show any inequivalent protons or carbons differences based on the coordination geometry or orientation of **1**. The most significant shift in the ^{13}C spectra was the carbon in the thiadiazole ring which notably shifted from 164.8 in **1** to 174.3 ppm in complex **2**. The methylene proton and carbon signals of the benzylthioether were found to quite symmetrical in the ^1H (singlet, 4.37 ppm) and ^{13}C (single peak, 41.0 ppm) spectra. IR spectra of **2** showed the characteristic strong *fac* carbonyl stretches at 1937 and 1909.1 cm^{-1} shifted down from other $fac\text{-}Re(CO)_3$ stretches and equally strong stretches in the fingerprint region at 905 and 728.9 cm^{-1}. Complex **2** exhibited a strong broad UV absorbtion band at 272.0 nm hypsochromically shifted from free **1** at 294 nm. Determination of the complex parent ion with mass spectrometry of complex **2** was unsuccessful. A number of fragmentation peaks were observed, but could not utilized to clearly identify the M^+ parent ion.

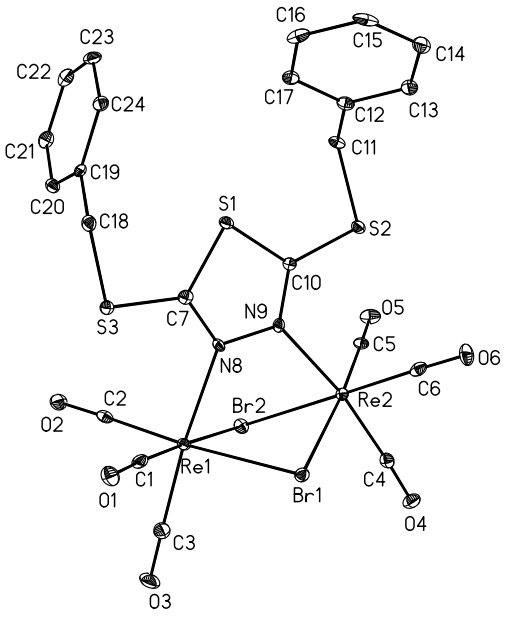

Fig. 1. View of the molecular structure for complex **2**. Thermal ellipsoids are shown with the 30% probability level. Non-functional hydrogen atoms were omitted for clarity.

The bridging nature of compound **1** through the nitrogen's of the thiadiazole ring to generate a bimetallic complex has been previous reported with some copper [3,11,12]. The copper complexes utilized a similar thioether analog compared to the benzyl analogs of DTD reported here. However, the copper complexes utilized a functional version with 2-methyl pyridine that provides two additional coordination donors to the metal centers. The bimetallic copper analogs also have additional μ bridging ligands in coordination complex in conjunction with the pyridyl thioether DTD ligand. Several variations of copper complexes were isolated that contained two μ bridging chloride or bromide ligands or a combination of μ bridging azide and bromide ligands. Unlike the copper complexes, complex **2** is a rare example of a bridging thiadiazole metal complex that does not utilize an additional function group to stabilize the rhenium metal centers. The unique formation of **2** may be due to the lack of available coordination sites as the substitutionally inert nature of the carbonyls in the $Re(CO)_3$ moiety.

In order to eliminate the possibility of bridging bromide ligands and generate a single non bridging rhenium complex, occupation of two of the available coordination sites of the labile bromide ligands in $fac\text{-}[Re(I)Br_3(CO)_3]^{2-}$ was achieved with a second bidentate ligand, picolinate. To compare the reactivity of **1**, a "2+1" style complex was prepared with **1** as a monodentate ligand. The

Table 2
Selected bond distances (Å) and bond angles (°) of **2** and **3**

2		**3**	
Br(1)–Re(1)	2.6379(6)	C(1)–Re(1)	1.916(3)
Br(1)–Re(2)	2.6477(6)	C(2)–Re(1)	1.926(3)
Br(2)–Re(2)	2.6172(6)	C(3)–Re(1)	1.914(3)
Br(2)–Re(1)	2.6294(6)	N(1)–Re(1)	2.175(2)
C(1)–Re(1)	1.900(5)	N(2)–Re(1)	2.203(2)
C(2)–Re(1)	1.916(5)	O(4)–Re(1)	2.137(2)
C(3)–Re(1)	1.929(5)		
C(4)–Re(2)	1.918(5)		
C(5)–Re(2)	1.915(5)		
C(6)–Re(2)	1.903(5)		
N(8)–Re(1)	2.205(4)		
N(9)–Re(2)	2.199(4)		
Re(1)–Br(1)–Re(2)	87.829(15)	C(3)–Re(1)–C(1)	87.05(13)
Re(2)–Br(2)–Re(1)	88.654(16)	C(3)–Re(1)–C(2)	87.39(13)
C(1)–Re(1)–C(2)	89.1(2)	C(1)–Re(1)–C(2)	86.33(13)
C(1)–Re(1)–C(3)	87.0(2)	C(3)–Re(1)–O(4)	94.82(10)
C(2)–Re(1)–C(3)	88.9(2)	C(1)–Re(1)–O(4)	174.93(10)
C(1)–Re(1)–N(8)	98.08(18)	C(2)–Re(1)–O(4)	98.45(11)
C(2)–Re(1)–N(8)	94.54(17)	C(3)–Re(1)–N(1)	91.38(11)
C(3)–Re(1)–N(8)	173.92(18)	C(1)–Re(1)–N(1)	99.74(11)
C(1)–Re(1)–Br(2)	177.82(14)	C(2)–Re(1)–N(1)	173.74(11)
C(2)–Re(1)–Br(2)	92.87(14)	O(4)–Re(1)–N(1)	75.53(8)
C(3)–Re(1)–Br(2)	92.03(16)	C(3)–Re(1)–N(2)	175.15(11)
N(8)–Re(1)–Br(2)	82.79(10)	C(1)–Re(1)–N(2)	95.43(11)
C(1)–Re(1)–Br(1)	94.58(14)	C(2)–Re(1)–N(2)	96.92(11)
C(2)–Re(1)–Br(1)	175.02(14)	O(4)–Re(1)–N(2)	82.37(8)
C(3)–Re(1)–Br(1)	94.68(16)	N(1)–Re(1)–N(2)	84.10(9)
N(8)–Re(1)–Br(1)	81.61(10)		
Br(2)–Re(1)–Br(1)	83.552(16)		
C(6)–Re(2)–C(5)	91.4(2)		
C(6)–Re(2)–C(4)	88.8(2)		
C(5)–Re(2)–C(4)	90.4(2)		
C(6)–Re(2)–N(9)	96.08(17)		
C(5)–Re(2)–N(9)	96.00(18)		
C(4)–Re(2)–N(9)	171.77(18)		
C(6)–Re(2)–Br(2)	177.77(15)		
C(5)–Re(2)–Br(2)	90.25(15)		
C(4)–Re(2)–Br(2)	92.68(15)		
N(9)–Re(2)–Br(2)	82.22(10)		
C(6)–Re(2)–Br(1)	94.79(15)		
C(5)–Re(2)–Br(1)	173.85(15)		
C(4)–Re(2)–Br(1)	89.62(14)		
N(9)–Re(2)–Br(1)	83.42(10)		
Br(2)–Re(2)–Br(1)	83.597(16)		

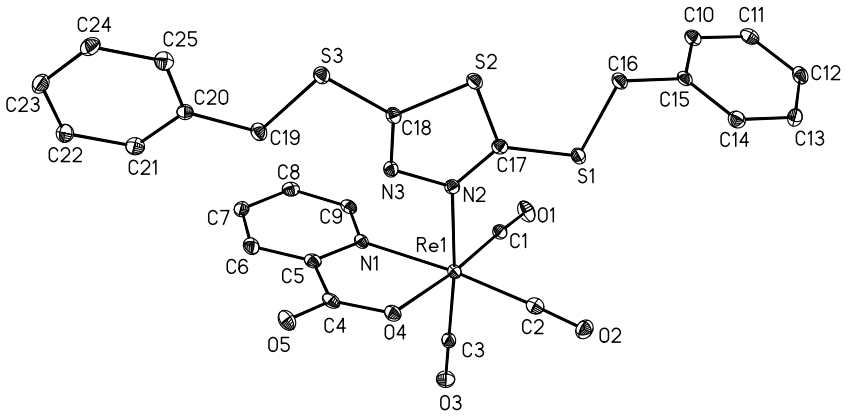

Fig. 2. View of the molecular structure for complex **3**. Thermal ellipsoids are shown with the 30% probability level. Non-functional hydrogen atoms and solvent molecule were omitted for clarity. Only one of the unique molecules in the asymmetric unit is shown.

product **3** was prepared in a two step one pot process. The intermediate *fac*-Re(I)(OH_2)($CO)_3$(picolinate) (**4**) was prepared similarly to previously reported procedures [28]. The formation and completion of the intermediate product **4** (RT18.7 min) was characterized by HPLC after the reaction was heated for 1 h prior to the addition of **1**. Introduction of **1** (RT24.4 min) to the reaction mixture containing **4** resulted in the formation of a new species, [*fac*-Re($CO)_3$(picolinate)(2,5-bis(benzylthio)-1,3,4-thiadiazole)] (**3**; RT23.5 min). The product was isolated and from the reaction mixture and characterized by standard analyses.

The ^{1}H NMR of **3** illustrated unexpected results in the proton interactions of **1** in complex **3**. The most significant observation in the spectra are the methylene carbons of the benzylthioether group in **1**. The methylene carbons had two unique signals depending on which side of the coordinated **1** they were located. As expected, the methylene groups would be inequivalent due to asymmetrical coordination nature of the thiadiazole ring. The first set of methylene protons were identified as a singlet at 4.33 ppm, while the second methylene group exhibited inequivalent H_a and H_b, as a doublet–doublet pattern at 3.98 and 4.18 ppm (J = 12.9). The steric orientation of coordinated **1** in complex **3** is believe to contort the methylene protons of the thiobenzyl analogs to interact with the equatorial plane of the picolinate ligand and CO ligands affecting the magnetic environment of each proton. The ^{13}C NMR further illustrated the asymmetrical coordination of **1** in complex **3**. Two sets of signals were assigned to the methylene carbons at 41.2 and 38.4 ppm. The asymmetry of **1** in **3** was evident in a slight distinction in the aromatic carbons as they also showed a modest shift in the two different benzyl portions of **1**. Complex **3** had a slight hypsochromic shift in the UV/Vis absorbance spectra from 286.9 nm compared to the free ligand at 294 nm. The shift in absorption peak in **3** was not as nearly pronounced as in the bridging complex **2**. Complex **3** illustrated a similar IR spectra to compound **2**, where the *fac*-Re($CO)_3$ exhibited strong stretches at 2017 and 1889. Additional stretches identified were attributed to the picolinate and **1** at 1637, 1598, 1349, 774, and 691. Mass spectrometry of **3** clearly identified the M^+ ion at 745.9 in acidic conditions. A fragmentation peak at 331 was observed and attributed to the free **1**, which probably dissociated upon ionization of the compound.

3.2. X-ray characterization

Single crystals of **2** were isolated by slow diffusion of pentane into a dichloromethane solution. Characterization by single crystal X-ray diffraction yields the complex shown in Fig. 1. This complex crystallizes in the monoclinic $P2(1)/n$ space group and the structure shown is symmetry unique with all atoms lying on general positions (Table 1). The major bond distance and angles in compound **2** are reported in Table 2. The Re–Re distance (3.66 Å) is too long to be considered a bond. The thiadiazole bridges the Re–Re vector with N–Re distances of ca. 2.2 Å. The strain of this chelation affects the bridging bromide atoms which are canted out of the Re–Re plane with Re–Br–Re angles of 87.8° and 88.6° and a Br–Br distance of ca. 3.509 Å. This is seen in other $Re_2(CO)_6$ chalcogenide bridged complexes where the strain of the chelation also distorts the geometry away from the simple orthogonal system [29–33]. The packing structure of **2** also has a close arene–thiadiazole interaction (3.56 Å from S1 to centroid of the C18–C24 ring) which is similar to slipped π–π interactions.

The complex **3** was isolated in crystalline form from a mixed acetonitrile water solution. The complex is less symmetric and was solved in the $P\bar{1}$ space group with two independent complex molecules and a H_2O molecule in the asymmetric unit (Table 1). The structure, shown in Fig. 2, displays monodentate ligation by the thiadiazole and the other octahedral sites are occupied by the picolinate ligand. Similar bond lengths (Table 2) are seen in **2** and **3** as well as in related complexes, e.g. Re($CO)_3$(imidazole)(picolinate COOH) [34]. In **3**, however, the methylene groups are rotated to elongate the thiadiazole parallel to the picolinate–Re–CO plane. This enables closer packing of the phenyl and thiadiazoles with arene–thiadizole π–π interactions of ca. 3.8–3.9 Å (centroid to centroid). This distance is long for a π–π interaction, thereby indicating that this interaction is very weak. The water molecule links two complexes together via strong hydrogen bonding with the picolinate ligand.

4. Conclusions

2,5-Bis(benzylthio)-1,3,4-thiadiazole (**1**) was found to generate two types of complexes when reacted with *fac*-[$NEt_4]_2$[Re($CO)_3Br_3$]. A single ligand of **1** was found to bridge two rhenium centers to yield **2** even in the presence of excess **1**. A monodentate complex **3** with **1** was prepared using a "2+1" approach with the bidentate picolinate as a coligand. In both complexes **2** and **3**, ligand **1** coordinated through the nitrogen in the 1,3,4-thiadiazole ring only. No evidence was suggested that the ligand would coordinate through either of the thioether donors in **1**. Compound **1** and analogs with rhenium may provide interesting chemistry in potential radiopharmaceutical, bioinorganic and material applications.

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Appendix A. Supplementary material

CCDC 682999 and 683000 contain the supplementary crystallographic data for **2** and **3**. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ica.2008.06.018.

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Article

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Synthesis and Characterization of Nonsteroidal-Linked $M(CO)_3^+$ (M = ^{99m}Tc, Re) Compounds Based on the Androgen Receptor Targeting Molecule Flutamide

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Androgen receptors are overexpressed in most primary and metastatic prostate cancers. A series of single photon emission computed tomography imaging agents (SPECT) utilizing the organometallic radioactive imaging species, *fac*-^{99m}Tc$(OH_2)_3(CO)_3^+$, were prepared on the basis of the structure of Flutamide, a potent nonsteroidal antiandrogen prostate cancer drug. Novel bifunctional chelate-linked Flutamide analogues were prepared using a newly developed universal alkylating reagent, 2-bromo-*N*-[4-nitro-3-(trifluoromethyl)phenyl]-acetamide, **1**. From compound **1**, several ligands (i.e., cysteine **2**, histidine **5**, imidazole **3**) were conjugated to the flutamide derivative to yield targeting ligands capable of either tridentate or monodentate coordination in a "2 + 1" complex. *fac*-$Re(CO)_3^+$ complexes were prepared and characterized with the functionalized conjugates to yield *fac*-$Re(CO)_3$(2-amino-3-(1-(2-(4-nitro-3-(trifluoromethyl)phenylamino)-2-oxoethyl)-1*H*-imidazol-4-yl) propanoate), **4**, *fac*-Re $(CO)_3$(2-(*S*-cysteinyl)-*N*-[4-nitro-3-(trifluoromethyl) phenyl]-acetamide), **6**, and *fac*-$Re(CO)_3$(picolinate)(2-(1*H*-imidazol-1-yl)-*N*-[4-nitro-3-(trifluoromethyl)phenyl]-acetamide), **7**. The corresponding radioactive ^{99m}Tc analogues were prepared and stability studies of the radioactive compounds were also conducted.

INTRODUCTION

Prostate cancer is the second leading cancer affecting men in the United States (*1*). Current screening methods utilized in medicine for prostate cancer involve a digital rectal exam, prostate specific antigen (PSA), and/or prostate core biopsies (*2, 3*). Each of these methods has their own limitations in specificity and accuracy in diagnosing prostate cancer. Major limitations of current diagnostic methods are caused by the inability to noninvasively image and spatially resolve cancerous tissues amidst normal prostate tissue without perturbation of the organ. Prostate cancer cells have been found to overexpress the androgen receptor (AR) during the cancer development (*4*). The AR provides an excellent biological target for discriminating from normal prostate cells due to the increased up-regulation of the AR. The overexpression of AR is most likely due to the increased transportation of testosterone into the cancerous cells, which has been attributed to a role in DNA transcription and cell proliferation (*5*).

Current treatment of prostate cancer involves single or combination treatment strategies from hormone treatment, radiation implants, surgical removal of the prostate, and/or chemical castration for eliminating testosterone production. Major side effects of the treatment methods involve decrease or elimination of normal sexual function and loss of urinary function. Hormone therapy, targeting the androgen receptor, is typically regarded as the first round of treatment for prostate cancer to control the prostate size and tumor growth. Several nonsteroidal antiandrogens, such as nilutamide, bicalutamide, flutamide, and the corresponding metabolite hydroxyflutamide, have been utilized as hormonal therapeutic agents in a clinic setting for a number of years (Figure 1) (*6*−*11*). Although hormone therapy may have some initial success, recurrent or hormone refractory prostate cancers may occur 1−3 years after hormone treatment as an advanced form of metastatic cancer that is resistant to chemotherapy and is believed to be androgen-independent due to a mutation of the AR (*12*).

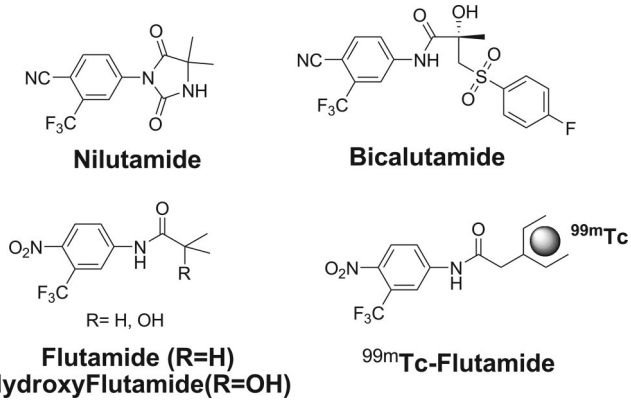

Figure 1. Examples of nonsteriodal antiandrogens clinically utilized in hormone therapy for prostate cancer and the ^{99m}Tc complex-linked flutamide reported within.

The presence or absence of AR in prostate cancer could be identified by a noninvasive imaging probe and would provide a key indicator in early screening as well as disease progression. Several positron emission tomography (PET) AR targeting analogues (i.e., flutamide, testosterone, dihydrotestosterone) have been previously reported for imaging. However, limitations in the half-life of positron (β^+) emitting isotopes, production, and PET imaging instrumentation required may have restricted clinical applications (*13*−*17*). Albeit with decreased resolution compared to PET, the development of a clinically routine single photon emission computed tomography (SPECT) imaging agent

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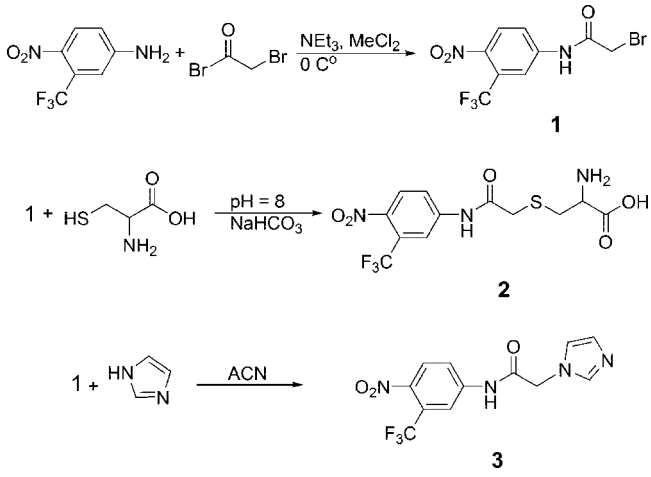
Scheme 1. Preparation of 2-Bromo-*N*-[4-nitro-3-(trifluoromethyl)phenyl]acetamide, 1, and the Corresponding Ligand-Linked Flutamide Compounds by Conjugation of 1 with Cysteine and Imidazole to Generate the Respective Compounds 2 and 3



utilizing ^{99m}Tc (E_γ = 140 keV, I_γ = 89%, $t_{1/2}$ = 6.02 h) would provide an alternative for hospitals already equipped with a SPECT camera. A number of small-molecule-linked ^{99m}Tc(V) oxo complexes with steroids, such as testosterone, have been reported in the literature for prostate imaging (*18, 19*). The recent development of the organometallic precursor, *fac*-^{99m}Tc-$(OH_2)_3(CO)_3^+$, has several important advantages over Tc(V) oxo complexes for biological targeting of prostate cancer (*20*−*24*). The *fac*-^{99m}Tc$(OH_2)_3(CO)_3^+$ species can be conveniently prepared in aqueous conditions from pertechnetate isolated from a ^{99}Mo/^{99m}Tc generator with an Isolink kit produced by Tyco (*25*). The ^{99m}Tc organometallic complexes have a reduced molecular weight, molecular volume, polarity, and ligand size compared the ^{99m}Tc(V) oxo complexes. The stable CO ligands also help increase the lipophilicity of the compounds, which may assist in hepatic clearance rather than renal clearance. The chemical and structural differences of the two types of Tc compounds can have important implications in biological interactions of complexes. This is of particular importance, as bladder collection of more polar ^{99m}Tc agents would significantly impact the clarity of the images by increasing the background or completely obstructing the prostate.

Our investigation reported herein was designed to develop a model system to explore the feasibility of ^{99m}Tc-linked flutamide derivatives that utilized the *fac*-^{99m}Tc$(OH_2)_3(CO)_3^+$ precursor. We developed a central strategy that would generate a library of flutamide analogues capable of complexing the technetium species. The universal flutamide derivative was prepared and conditions determined to use general alkylation methods to covalently link a number of ligand systems to the flutamide derivative (Scheme 1). The flutamide-linked ligands were examined with both the nonradioactive rhenium for chemical and structural characterization followed by the radioactive ^{99m}Tc analogues to determine labeling effectiveness and radiochemical stability.

EXPERIMENTAL SECTION

All reagents and organic solvents were purchased from Aldrich, Acros, or Fluka in reagent grade or better and were used without further purification. Rhenium starting materials $Re(CO)_5OTf$, and *fac*-$[Re(CO)_3(H_2O)_3]OTf$, were prepared by literature methods from $Re_2(CO)_{10}$ purchased from Strem (*24, 26*). Rhenium complexes *fac*-$Re(CO)_3$(Histidine) and *fac*-$Re(OH_2)(CO)_3$(picolinate) were prepared by reacting the rhenium starting materials with the appropriate ligand according to reported methods (*27, 28*). Normal mouse serum (L10410) with 0.1% sodium azide was purchased from Caltag Laboratories and was centrifuged to remove particulate matter prior to use. Elemental analyses were performed by Quantitative Technologies, Inc., New Jersey. Separation and identification of compounds were conducted on a Perkin-Elmer Series 200 high pressure liquid chromatograph (HPLC) equipped with a UV/vis series 200 detector and a Perkin-Elmer Radiomatic 610TR detector utilizing a 30 cm 5 μm Agilent Zorbex SB-C18 column. The compounds were separated with a reverse-phase gradient system beginning with 0.1% trifluoroacetic acid (TFA) aqueous eluent gradually shifting to methanol according to the following method, 0−3.0 min (100% TFA), 3.0−9.0 min (75% TFA, 25% MeOH), 9.0−20.0 min (25% to 100% MeOH linear gradient), 20.0−25.0 min (100% MeOH) at a flow rate of 1.0 mL/min or 5.0 mL/min for separation. ^{1}H NMR spectra were recorded on a Varian 300 MHz spectrometer, and chemical shifts were referenced to the internal standard sodium 3-(trimethylsilyl) propionate-d_4 (TSP, 0.00 ppm) in D_2O or the residual solvent signal and/or tetramethylsilane (TMS) in organic solvents. Spectra were processed using Varian VNWR 6.1 software. IR spectra were collected with a Thermo Nicolet 6700 FT-IR with an ATR cell and analyzed with OMNIC 7.1a software. Mass measurements were performed as Q3 scans on an API4000 triple quadrupole (Applied Biosystems). Sample concentrations of ~0.1 μg/μL in methanol were infused at 10 μL/min, with orifice heating on, declustering potential 20 V, and entrance potential 10 V.

2-Bromo-*N*-[4-nitro-3-(trifluoromethyl)phenyl]acetamide, 1. Et_3N (5.9 mL, 42 mmol) was added slowly to a solution of bromoacetyl bromide (8.1 g, 40 mmol) and 5-amino-2-nitrobenzotrifluroride (7.0 g, 34 mmol) in CH_2Cl_2 (200 mL) at 0 °C. The solution was stirred for 2 h at 0 °C, which resulted in a clear solution. The solution was continuously stirred and allowed to reach rt overnight. The solution was washed with 0.6 M of HCl three times (200 mL × 3), followed by water. The organic phase was dried over Na_2SO_4, filtered, and evaporated to dryness to give a pure slight yellow product (9.9 g, 89%). Anal. Calcd for $C_9H_6BrF_3N_2O_3$: C, 33.05; H, 1.85; N, 8.57. Found: C, 33.00; H, 1.62; N, 8.33. ^{1}H NMR [δ (ppm), $CDCl_3$] 8.53 (s, 1H), 8.02 (m, 3H), 4.08 (s, 2H). ^{13}C NMR [δ (ppm), $CDCl_3$] 164.5, 141.3, 127.4, 125.6 (q), 123.6, 122.7, 120.0, 118.7 (q), 29.1. MS [$M^+ - Na^+$] 349.0, 351.1.

2-(*S*-Cysteinyl)-*N*-[4-nitro-3-(trifluoromethyl)phenyl]acetamide, 2. To a solution of excess cysteine (1.21 g, 10 mmol) in water (50 mL) was added $NaHCO_3$ (10 mL, 1 M), followed by **1** (1.0 g, 3.0 mmol) in CH_2Cl_2 (50 mL). The biphasic mixture was vigorously stirred overnight at rt. A small amount off-white solid product was observed in the reaction mixture and additional material was formed after selective evaporation of CH_2Cl_2 from the mixture. The off-white solid was filtered and dried under vacuum (0.75 g, 67%). Anal. Calcd for $C_{12}H_{12}F_3N_2O_5S \cdot 0.6H_2O$: C, 38.12; H, 3.52; N, 11.11. Found: C, 37.89; H, 3.28; N, 11.38. ^{1}H NMR [δ (ppm), DCl/D_2O, 0.25 M] 7.98 (d, J = 2.1 hz, 1H), 7.95 (d, J = 9.0 hz, 1H), 7.82 (dd, J = 2.1 and 9.0 hz, 1H), 4.48 (dd, J = 4 and 8 hz, 1H), 3.70 (s, 2H), 3.46 (dd, J = 4 and 15 hz, 1H), 3.29 (dd, J = 8 and 15 hz, 1H). ^{13}C NMR [δ (ppm), DCl/D_2O, 0.25 M] 173.4, 173.1, 145.2, 145.0, 130.3, 126.6, 125.9, 121.6, 55.4, 39.2, 35.0, 29.1. MS [M^+] 367.9, [$M^+ - Na^+$] 389.9.

2-(1*H*-Imidazol-1-yl)-*N*-[4-nitro-3-(trifluoromethyl)phenyl]acetamide, 3. To an acetonitrile solution (10 mL) containing imidazole (1.57 g, 5 mmol) was added **1** (0.33 g, 1 mmol). The solution was stirred overnight at rt, then evaporated to dryness under vacuum to yield a faint yellow solid. Water (10 mL) was added to the solid and the solution filtered. HCl (0.5 M, 10

mL) was added to the collected solid, and the corresponding mixture was centrifugated, and the solution collected, and the solid discarded. The slow addition of $NaHCO_3$ (1 M, 4 mL) to the centrifuged solution caused the formation of a yellow precipitate, which was filtered and dried to yield the desired faint yellow product **3** (0.26 g, 82%). Anal. Calcd for $C_{12}H_9F_3N_4O_3$: C, 45.87; H, 2.89; F, 18.14; N, 17.83; O, 15.28. Found: C, 44.60; H, 2.84; N, 17.07. ^{1}HNMR [δ (ppm), methanol-d_4] 9.04 (t, 1H), 8.23 (d, 1H), 8.03 (m, 2H), 7.69 (t, 1H), 7.27 (t, 1H), 5.36 (s, 2H). ^{13}CNMR [δ (ppm), methanol-d_4] 166.8, 142.9, 138.3, 127.0, 126.9, 124.5, 124.0, 122.5, 121.1, 117.9, 117.8, 49.5. MS [M^+] 315.0, [$M^+ - Na^+$] 337.0.

***fac*-Re(CO)$_3$(2-amino-3-(1-(2-(4-nitro-3-(trifluoromethyl)phenylamino)-2-oxoethyl)-1*H*-imidazol-4-yl)propanoate), 4.** To a room temperature acetonitrile solution (80 mL) of *fac*-Re(CO)$_3$(Histidine) (0.84 g, 2 mmol) and **1** (0.72 g, 2.2 mmol) was added Cs_2CO_3 (0.60 g, 2.2 mmol) and stirring continued overnight. The solution was filtered to remove the residual Cs_2CO_3 and salt byproducts, and the filtrate was collected and evaporated to dryness. The solid residue obtained from the filtrate was washed with a minimal amount of ethanol and water and dried under vacuum to yield the desired faint yellow product **4** (1.2 g, 90%). Anal. Calcd for $C_{18}H_{13}F_3N_5O_8Re$: C, 32.24; H, 1.95; N, 10.44. Found: C, 31.81; H, 2.17; N, 9.96. ^{1}H NMR [δ (ppm), acetone-d_6] 11.90 (s, 1H), 8.26 (d, 1H, J = 1.8 Hz), 8.12 (m, 3H), 7.18 (s, 1H), 5.75 (dd, J = 11.4 and 6.0 Hz, 1H, 1H), 5.4 (d, J = 17.1 Hz, 1H), 4.35 (overlapped, 1H), 4.34(overlapped, 1H), 3.51 (dd, J = 13.5 and 3 Hz, 1H), 3.37 (dd, J = 13 and 3 Hz, 1H). ^{13}C NMR [δ (ppm), acetone-d_6] 197.9, 196.5, 183.9, 165.8, 143.9, 142.9, 142.5, 134.9, 127.6, 124.4, 124.2, 123.9, 122.3,120.8,117.5, 52.4, 49.1. IR [cm^{-1}] 2018, 1879, 1604, 1525, 1342, 1147. MS [M^+] 672.3, [$M^+ - Na^+$] 694.2.

2-Amino-3-(1-(2-(4-nitro-3-(trifluoromethyl)phenylamino)-2-oxoethyl)-1*H*-imidazol-4-yl)propanoic Acid, 5. To an acetone solution (15 mL) containing compound **4** (0.34 g, 0.5 mmol) was added HCl solution (15 mL, 1 M), followed by H_2O_2 (0.6 mL) at rt. The mixture was continuously stirred and monitored by HPLC to determine reaction progress. After 3 d, the starting material **4** was no longer observed in the chromatogram, indicating the complete conversion of the Re starting material to the ligand **5**. The reaction mixture was neutralized by Na_2CO_3 (1 M), and the solution was directly purified by preparative HPLC to yield the yellow product **5** as a semisolid. (0.169 g, 83%). Anal. Calcd for $C_{19}H_{18}F_9N_5O_{10}$: C, 35.25; H, 2.80; F, 26.41; N, 10.82; O, 24.71. 35.29, H, 2.70 N, 9.35. ^{1}H NMR [δ (ppm), methanol-d_4] 9.14 (s, 1H), 8.30 (s, 1H), 8.06 (m, 2H), 7.74 (s, 1H), 5.43 (s, 2H), 4.47 (dd, J = 6 and 7 Hz, 1H), 3.56 (dd, J = 6 and 16 Hz, 1H), 3.46 (dd, J = 7 and 16.0 Hz, 1H). ^{13}C NMR [δ (ppm), methanol-d_4] 168.9, 165.3, 143.1, 142.6, 137.5, 127.6, 127.1, 124.5, 124.0, 122.8, 120.4, 118.0, 51.7, 51.5, 25.5. MS [M^+] 402.2.

***fac*-Re(CO)$_3$(2-(S-cysteinyl)-*N*-[4-nitro-3-(trifluoromethyl)phenyl]acetamide), 6.** To a solution of **2** (0.18 g, 0.5 mmol) and [Re(CO)$_5$OTf] (0.25 g, 0.52 mmol) in methanol (30 mL) was added Et_3N (75 μL, 0.54 mmol). The mixture was refluxed for 2 h, concentrated to 5 mL by rotary evaporation, and purified by preparative HPLC to yield the product **6** as a pale yellow solid (0.22 g, 69%). Anal. Calcd for $C_{15}H_{11}F_3N_3O_8ReS$: C, 28.30; H, 1.74; N, 6.60. Found: C, 27.99; H, 1.81; N, 6.47. ^{1}H NMR [δ (ppm), methanol-d_4, −14 °C] 8.25 (d, J = 2.1 hz, 1H), 7.98−8.09 (m, 2H), 6.24 (d, J = 10 hz, 1H), 5.02 (d, J = 10 hz, 1H), 4.36 (s, 1H), 4.24 (m, 1H), 3.92 (d, J = 15.3 Hz, 1H), 3.80 (d, J = 15.3 Hz, 1H), 3.04 (m, 2H). ^{13}C NMR [δ (ppm), methanol-d_4] 195.4, 193.3, 181.4, 167.4, 144.3, 143.9, 128.2, 125.5 (q),123.9, 121.6, 119.2 (q), 59.4, 41.7, 33.1. IR [cm^{-1}] 3000, 2029, 1903, 1530, 1267, 1146, 755. MS [M^+] 638.1, [$M^+ - Na^+$] 660.2.

Table 1. X-ray crystal data and structure refinement for compound 7

empirical formula	$C_{24}H_{19}F_3N_5O_9Re$	
formula weight	764.64	
temperature	90(2) K	
wavelength	0.71073 Å	
crystal system	monoclinic	
space group	*C*2/*c*	
unit cell dimensions	a = 19.0902(11) Å	α = 90°.
	b = 14.6852(8) Å	β = 100.5910(10)°.
	c = 20.6888(12) Å	γ = 90°.
volume	5701.2(6) Å^3	
Z	8	
density (calculated)	1.782 mg/m^3	
absorption coefficient	4.341 mm^{-1}	
F(000)	2976	
crystal size	0.34 × 0.25 × 0.23 mm^3	
crystal color and habit	yellow block	
diffractometer	Bruker/Siemens SMART APEX	
θ range for data collection	1.76° to 27.50°.	
index ranges	$-24 \le h \le 24$, $-19 \le k \le 19$, $-26 \le l \le 26$	
reflections collected	42 304	
independent reflections	6552 [R(int) = 0.0260]	
completeness to θ = 27.50°	100.0%	
absorption correction	semiempirical from equivalents	
max. and min. transmission	0.367 and 0.290	
solution method	XS, SHELXTL v. 6.14 (Bruker, 2003)	
refinement method	full-matrix least-squares on F^2	
data/restraints/parameters	6552/0/384	
goodness-of-fit on F^2	1.040	
final R indices [$I > 2\sigma(I)$]	$R1$ = 0.0201, w$R2$ = 0.0478	
R indices (all data)	$R1$ = 0.0233, w$R2$ = 0.0495	
largest diff. peak and hole	1.817 and −0.533 e.Å^{-3}	

***fac*-Re(CO)$_3$(picolinate)(2-(1*H*-imidazol-1-yl)-*N*-[4-nitro-3-(trifluoromethyl)phenyl]acetamide), 7.** To a methanol solution (5 mL) of *fac*-Re(OH$_2$)(CO)$_3$(picolinate) (0.033 g, 0.081 mmol) was added **3** (0.028 g, 0.089 mmol), and the solution was stirred at 50 °C for 3 h. The reaction solution was evaporated to dryness, then purified by preparative HPLC to yield the product **7** as a yellow solid. (0.040 g, 70%). X-ray quality single crystals of compound **7** were isolated as faint yellow rectangular blocks by slow evaporation of the compound in a 1:1 mixture of hexane/acetone. Anal. Calcd for $C_{21}H_{13}F_3N_5O_8Re$: C, 35.70; H, 1.85; N, 9.91. Found: C, 36.15; H, 1.97; N, 9.56. ^{1}H NMR [δ (ppm), methanol-d_4] 8.97 (dt, 1H), 8.05 (m, 6H), 7.76 (m, 1H), 7.14 (t, 1H), 6.96 (t, 1H), 4.90 (s, 2H). ^{13}C NMR [δ (ppm), methanol-d_4] 196.6, 174.2, 165.9, 152.2, 149.5, 141.2, 140.5, 129.2, 128.5, 124.2, 127.1, 124.5, 124.1, 122.5, 117.8, 117.7, 49.9. IR [cm^{-1}] 2021, 1912, 1653, 1537, 1345, 1167. MS [M^+] 708.4, [$M^+ - Na^+$] 730.5.

X-ray Crystallography. Crystals of compound **7** were removed from the flask; a suitable crystal was selected, attached to a glass fiber, and data were collected at 90(2) K using a Bruker/Siemens SMART APEX instrument (Mo Kα radiation, λ = 0.71073 Å) equipped with a Cryocool NeverIce low-temperature device. Data were measured using omega scans 0.3° per frame for 5 s, and a full sphere of data were collected. A total of 2400 frames were collected with a final resolution of 0.77 Å. Cell parameters were retrieved using SMART software (*29*) and refined using SAINTPlus (*30*) on all observed reflections. Data reduction and correction for Lp and decay were performed using the SAINTPlus software. Absorption corrections were applied using SADABS (*31*). The structure was solved by direct methods and refined by least-squares method on F^2 using the SHELXTL program package (*32*). The structure was solved in the space group *C*2/*c* by analysis of systematic absences. No decomposition was observed during data collection. Details of the data collection and refinement are given in Table 1. Further details are provided in the Supporting Information.

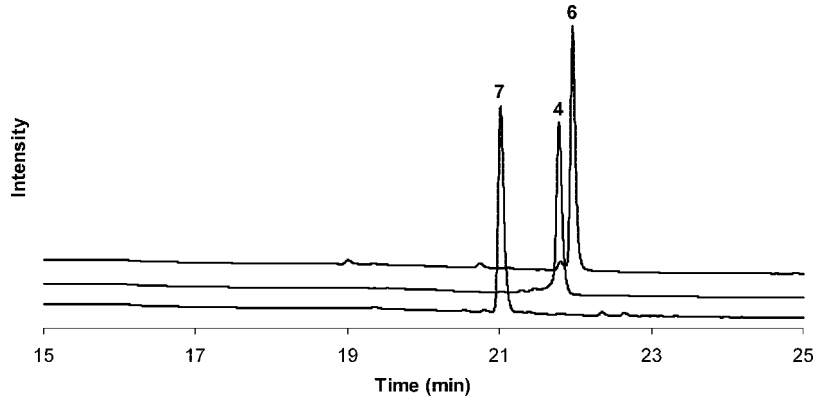


Figure 2. UV/vis HPLC (220 nm) chromatograph of Flutamide-linked rhenium tricarbonyl complexes of **4**, **6**, and **7**.

Radiochemistry. ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$) was purchased from Cardinal Health in Spokane, WA. IsoLink kits to prepare the $^{99m}Tc(H_2O)_3(CO)_3^+$ precursor were obtained as a gift from Tyco, Inc. The radiolabeled products were characterized by Perkin-Elmer Series 200 HPLC under the same mobile-phase conditions previously mentioned, except the γ emissions were detected with a Perkin-Elmer Radiomatic 610TR detector equipped with a Gamma B cell (80 μL loop). The *fac*-$^{99m}Tc(H_2O)_3(CO)_3^+$ precursor was prepared according to the manufacture's procedure, the addition via syringe of 10−20 mCi of $Na^{99m}TcO_4$ in 1 mL saline to the Isolink kit followed by heating at 95 °C for 20 min. The resulting solution was cooled in an ice bath, neutralized with HCl (∼100 μL, 1 M) and checked on the γ-HPLC system to ensure complete formation of the precursor.

General $^{99m}Tc(H_2O)_3(CO)_3^+$ Radiolabeling Procedure. The ligand **2** or **5** (100 μL, 10^{-4} or 10^{-5} M) and phosphate buffer (800 μL, 0.1 M) at pH 7.4 was added to a sealable labeling vial (5.0 mL). The vial was sealed and degassed with nitrogen for ∼10 min. The $^{99m}Tc(H_2O)_3(CO)_3^+$ precursor solution (100 μL) was added to the degassed vial and the vial heated for 30 min at 70 °C. The reaction mixture was then allowed to cool on an ice bath prior to injection and analysis by radio-HPLC.

2 + 1 Radiolabeling Procedure. *Method 1: One-Pot Preparation.* Ligand **3** (100 μL, 10^{-2} M), picolinic acid (100 μL, 10^{-4} M), and phosphate buffer (700 μL, 0.1 M) at pH 7.4 were added in order to a sealable labeling vial (5.0 mL). The vial was sealed and degassed with N_2 for ∼10 min. The $^{99m}Tc(H_2O)_3(CO)_3^+$ precursor solution (100 μL) was added to the degassed vial and heated for 30 min at 80 °C. The reaction mixture was allowed to cool on an ice bath prior to analysis by radio-HPLC.

Method 2: Stepwise Formation. Picolinic acid (100 μL, 10^{-4} M) and phosphate buffer (700 μL, 0.1 M) at pH 7.4 were added to a sealable vial (5.0 mL). The vial was purged with nitrogen for ∼10 min. The $^{99m}Tc(H_2O)_3(CO)_3^+$ precursor solution (100 μL) was added to the degassed vial and heated for 30 min at 80 °C. After the formation of *fac*-$^{99m}Tc(H_2O)(CO)_3$(picolinate) was confirmed by radio-HPLC, ligand **3** (100 μL, 10^{-2} M) was added to the vial and the sample heated for an additional 30 min at 80 °C.

Biological pH Stability Studies. The respective ^{99m}Tc labeled compound (100 μL) prepared with ligands **2**, **3**, or **5** as directed above was added to phosphate buffer (900 μL, 0.1 M) at pH 7.4. The vial was sealed and incubated at 37.0 °C for the duration of the study. The solution was examined by radio-HPLC at 1, 2, and 4 h during the incubation to determine the effective stability of the compounds.

Mouse Serum Stability Studies. The respective ^{99m}Tc complexes (100 μL) prepared from ligands **2**, **3**, or **5** as directed above was added to an Eppendorf 1.5 mL flextube containing 400 μL of normal mouse serum with 0.1% sodium azide that had been previously clarified by centrifugation. The tube was sealed and constantly heated at 37.0 °C for the duration of the study. The serum was examined by radio-HPLC at 1, 2, and 4 h of incubation to observe decomposition or loss of the radiolabeled complex due to a shift in the retention time or decrease in the product peak intensity.

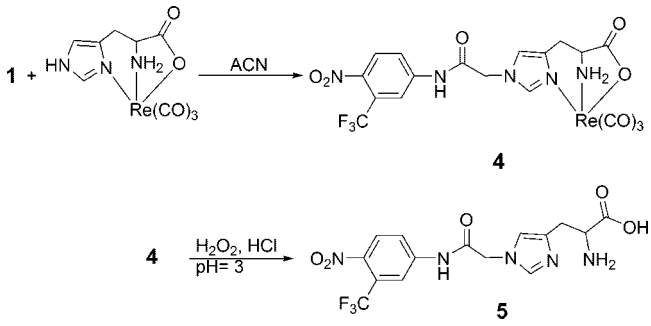
Cell Studies. *In vitro* analysis of the ^{99m}Tc complexes were conducted with DU-145 according to established methods prepared from ligand **2** or **5** as directed above was added. Aliquots of compounds (4, 6, 8 μL of a 0.1 mCi/100 μL) were incubated in triplicate with androgen receptor positive DU-145 prostate cancer cell line for 2 h in a CO_2 incubator at 37 °C. The cells were washed twice with cold PBS to remove nonspecific bound ^{99m}Tc complex; the cells were collected and counted with a Cobra II γ counter for 1 min.

RESULTS AND DISCUSSION

Nonsteroidal antiandrogen drugs, i.e., flutamide, bicalutamide, nilutamide, have been found to effectively mitigate early-stage prostate cancer growth (Figure 1) (*10*). We report within this paper the first analogues of flutamide that incorporate technetium-99m complexes into the structure. Flutamide was modified to incorporate a number of ligand systems capable of coordinating the ^{99m}Tc species, *fac*-$^{99m}Tc(OH_2)_3(CO)_3^+$ (Figure 2). We report within the synthetic methods used to prepare the bifunctional flutamide compounds, characterization of rhenium complexes formed, and radiochemical preparation and stability studies of the technetium-99m complexes formed.

The focus of this work was to develop a flutamide analogue that could be conveniently functionalized with a variety of ligand systems quite readily. The designs of such analogues were restricted to modifications along the carbonyl axis of flutamide's amide bond as the dominent binding motif for the compound to the androgen receptor is understood to be the functionalized aromatic ring (*6, 7, 10, 34, 35*). Transforming the alkyl backbone into an alkyl halide provided an accessible group that could function as a synthetic handle for nucleophilic substitution by a number of reactive species (i.e., amines, thiols, alcohols) (*13,15,36,37*). The brominated flutamide compound, 2-bromo-*N*-[4-nitro-3-(trifluoromethyl)phenyl]-acetamide, **1**, was chosen as the congener of fluatamide in order for later incorporation of chelators. Compound **1** was prepared in high yield by the reaction of bromoacetyl bromide and 5-amino-2-nitrobenzotrifluroride in CH_2Cl_2 in the presence of triethylamine (Scheme 1). The crude product **1** isolated from the reaction mixture after acid washing the reaction mixture was clean enough to use for the subsequent experiments without further purification based on ^{1}H and ^{13}C NMR spectroscopy. Compound **1** could also be further purified by silica gel chromatography (ethyl acetate/hexane 1:5) or

Scheme 2. Preparation of Histidine-Linked Flutamide Ligand (5) by Alkylation with 1 of the Rhenium Histidine to Yield the Rhenium Histidine-Linked Flutamide Complex (4) and Followed by Demetallation to Generate the Free Ligand (5)



recrystallized from ethyl acetate. Compound **1** was found to be a strong irritant as a dry powder, and care was utilized when handling the material.

The reactivity of the acetyl bromide in compound **1** provided an excellent synthetic handle to functionalize with corresponding ligand systems. Introduction of compound **1** afforded a series of molecules **2**, **3**, and **5** that incorporate flutamide and ligands (cysteine, imidazole, histidine, respectively). These ligands had been previously identified for coordination with a $M(CO)_3$ ($M = Re$, ^{99m}Tc) species due to high-efficiency labeling methods (*27, 28, 38*). The synthetic routes to **2**, **3**, and **5** are divided into two categories, direct organic synthesis and metal-coordinated synthesis. Direct synthesis involved a standard alkylation synthesis route to couple **1** with the ligand. The ligands cysteine and imidizole were attached to **1** via the direct synthesis route in a one-step substitution reaction to yield the flutamide-coupled ligands, compounds **2** and **3**, respectively (Scheme 1). The metal-coordinated synthesis of compound **5** involves the use of a rhenium as a protecting group on the histidine (Scheme 2).

Direct synthetic methods allowed the preparation of the compounds directly from compound **1** (Scheme 1). The cysteine-linked flutamide analogue, **2**, was successfully prepared in high yield after a number of synthetic attempts. The reported method has an important synthetic advantage by utilizing the reactive differences between thiol and amine groups to yield the desired product without the use of protecting groups. Early attempts utilizing protecting groups on cysteine on the amine and the carboxylic acid with Boc-Cys-OMe did yield the corresponding alkylation product with **1**. However, deprotection with base of the methyl ester was found to also cleave the amide bond as indicated by the presence of 5-amino-2-nitrobenzotrifluroride in the reaction mixture. Potential amide cleavage was anticipated at high pH due to the strong electron-withdrawing groups (NO_2, CF_3) on the aromatic ring weakening the amide bond. By treatment of **1** with excess cysteine in aqueous conditions at slightly basic pH, alkylation selectivity of the thiol over the amine in cysteine and the avoidance of protecting groups that require basic deprotection methods yielded the desired product, **2**. Initially, the reaction was conducted in aqueous solution only, but it proceeded slowly with low yields (<25%) due to the limited solubility of compound **1**. Attempts to improve reactivity and solubility with increased base and/or heat yielded undesired cleavage products. It is noteworthy to mention that, when the reaction was carried out in biphasic reaction conditions (aqueous/methylene chloride), compound **1** readily dissolved in the methylene chloride and the reaction proceed smoothly without the need of a phase transfer catalyst at room temperature with a significant improvement in yield. Careful control of the solution pH in the reaction was critical to yield the desired product, **2**. The cystine dimer was observed as the major product instead of **2** when the reaction was carried out in acidic conditions (pH < 5). Other water-miscible solvents (i.e., DMF, methanol) did dissolve **1**; however, the reaction did not afford compound **2** in any appreciable yield. The conditions reported here are mild for alkylation of a thiol compared to refluxing 1 M NaOH that can lead to racemic mixtures or utilizing carcinogenic solvents. Ligand **2** was characterized by elemental analysis and ^1H and ^{13}C NMR spectroscopy.

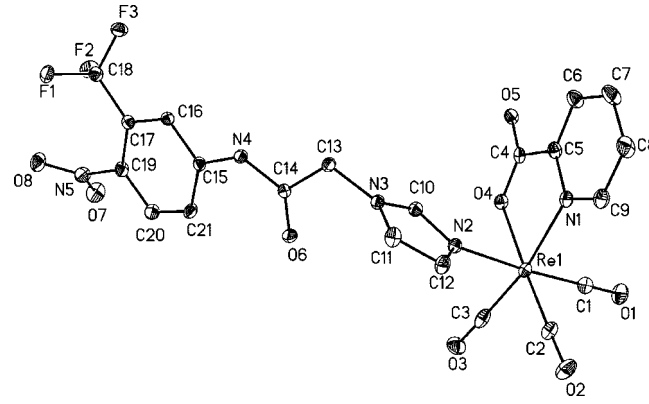
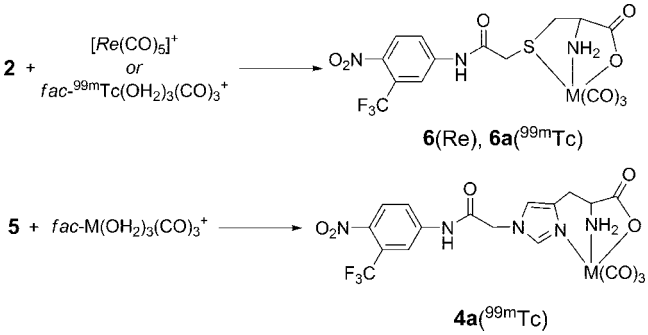


Figure 3. A modeled X-ray structure of *fac*-$Re(CO)_3$(Picolinate)(**3**), **7**, with 30% thermal ellipsoids. Hydrogen atoms are excluded for clarity.

An imidazole-linked flutamide analogue was prepared by treatment of **1** with excess imidazole in acetontrile gave **3** in high yield (Scheme 1). Under the reported reaction conditions, only the monosubstituted product was isolated, where the imidazole ligand was functioning as both the reactant and the proton scavenger. However, when the imidazole was added in stoichiometric concentrations and in the presence of an additional base (i.e., Cs_2CO_3) in acetonitrile, a mixture of the mono- and disubstituted products were observed. The mixed species were also observed when the reaction was conducted in a biphasic solvent system ($H_2O/MeCl_2$) similar to compound **2**. ^1H and ^{13}C NMR reported for **3** were consistent with the proposed compound.

Preparation of a N^{ϵ} histidine-linked flutamide compound ligand with **1** was originally attempted utilizing protecting groups similarly to previous reports (*28*, *39*, *40*). However, deprotection methods yielded cleavage products at the amide bond as noted previously in the synthesis of **2**. Therefore, utilizing a metal-coordinated synthetic route for the preparation of the ligand was a more appropriate method. In this procedure, $Re(CO)_3$ was coordinated in a tridentate fashion to the histidine ligand in place of more traditional organic protecting group (*28*). Alkylation of $Re(CO)_3$(histidine) with compound **1** in the presence of solid Cs_2CO_3 in an acetonitrile solution yielded compound **4** in near quantitative yields (Scheme 2). The reaction proceeds smoothly with no unwanted side products as indicated by HPLC, as the metal coordination bonds sufficiently protect the bound amine, carboxylate, and imidizole functional groups from substitution reactions (Figure 3). The coordinated imidazolyl ligand was of particular importance to maintain the coordination mode and strength for the subsequent ^{99m}Tc labeling. Dissociation of the nitrogen ligands from the rhenium center could lead to substitution at the NH_2 terminus or N^{τ} or disubstitution of the ring, similar to what was observed as a side product in the formation of compound **3**. The complexes were characterized by NMR, and comparison of **4** to the $Re(CO)_3$(Histidine) starting material illustrated similar splitting and slight downfield shifts for the protons associated with the coordinated histidine, indicating no change in the coordination mode of the ligand on the metal center. ^1H NMR of **4** in acetone showed two peaks associated with the imidazolyl group at 8.26

Scheme 3. Complexation of Flutamide-Linked Ligand Analogues with *fac*-M(CO)$_3$ (M = Re, ^{99m}Tc (a)) with Tridentate Ligands 2 and 5 to Generate *fac*-M(CO)$_3$(2) (6, 6a) and *fac*-M(CO)$_3$(5) (4, 4a) Complexes



and 7.18 ppm, slightly shifted downfield from the Re(CO)$_3$(Histidine) starting material at 7.06 and 8.08 ppm. The aromatic protons of the molecule were overlapped in the spectrum to yield a multiplet at 8.12 ppm of 3H. The metal coordinated amine protons are observed as diastereotopic protons of equal integration of one proton as a doublet at 5.4 and mulitplet at 5.75 ppm. The chiral Hα was also observed as a multiplet at 4.35 ppm, and the beta protons Hβ were also diastereotopic, exhibiting a dd pattern at 3.51 and 3.37 ppm.

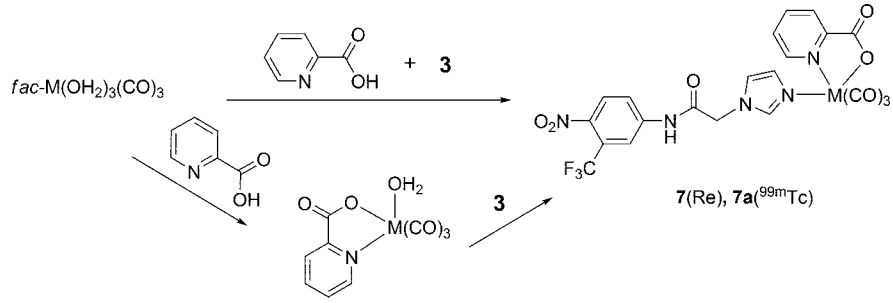
Removal of the coordinated rhenium tricarbonyl complex in **4** to generate the free ligand compound **5** was achieved under acidic oxidative conditions (Scheme 2). The selective oxidation and decomplexation of the rhenium center of **4** occurs by the addition of a 10-fold quantity of H_2O_2 under acidic conditions at room temperature. The reaction was monitored by HPLC and was completed after 3 days. Although a lower pH and increased H_2O_2 concentration would potentially decrease the reaction time, minimal amide cleavage products of the ligand were observed under the conditions reported. The free ligand **5** was isolated by preparatory HPLC from the reaction mixture in reasonable yields. Characterization of **5** by ^{1}H and ^{13}C NMR was consistent with the proposed structure and simplified in comparison to the corresponding rhenium complex, **4**.

Complexes were prepared from the ligands prepared above with rhenium and technetium-99m carbonyl precursors to yield M(CO)$_3$(Ligand/s) complexes. The preparation and characterization of the rhenium complexes provides a stable compound for characterization by standard methods and a reference for comparison to the radioactive ^{99m}Tc complexes in HPLC (Figure 2). The rhenium complexes of the flutamide-linked ligands were prepared by reacting the ligand with a rhenium carbonyl starting material as indicated in Scheme 3, except for complex **4a**, which was prepared directly from the Re(CO)$_3$(Histidine) complex as mentioned above. Ligands **2** and **3** utilized slightly different Re starting materials according to the solubility of the ligand system. Ligand **2** was reacted with the organic soluble [Re(CO)$_5$OTf] in methanol, due to the limited solubility of macroscopic amounts of **2** in water. The reaction involves the displacement of two CO molecules from the rhenium center upon complexation of **2** to generate the neutral complex Re(CO)$_3$(**2**), **6**, in high yield and isolated as a pale yellow solid. HPLC analysis of the product showed a single sharp peak with retention time of 22.1 min (Figure 2). Elemental analysis of **6** confirmed the existence of 1:1 ratio of rhenium to ligand. ^{13}C NMR profile of **6** is in consistent with the proposed structure. Relatively broad signals were observed in the ^{1}H NMR spectrum of **6** in CD_3OD at room temperature. At low temperature (−14 °C), sharp signals were observed, showing two sets of signals indicative of two coordination isomers in solution. The coordinated ligand **2** has a chiral carbon and prochiral sulfur resulting in the formation of diastereoisomers upon metal coordination. Two isomers can equilibrate fast by inversion of the sulfur configuration at room temperature. However, the minor species was observed at approximately 10% in the solution, indicating a preference for one coordination species with the Re(CO)$_3$ most likely due to interactions of functionalized thioether moiety driven by steric factors. Similar observations were reported for other thioether derivatives of cysteine Re(CO)$_3$ complexes (*26*, *38*).

Mundwiler et al. (*41*) reported a "2 + 1" mixed-ligand complex approach consisting of a bidentate and a monodentate ligand coordinated to the M(CO)$_3$ (M = Re, ^{99m}Tc) core provides an alternative method to single ligand coordination. This methodology was adopted to prepare a "2 + 1" flutamide derivative with the M(CO)$_3$ M = Re, ^{99m}Tc core. The rhenium complex, Re(CO)$_3$(pic)(**3**), **7**, prepared within this paper consisted of a bidentate picolinate and a monodentate flutamide fuctionalized imidazole, **3**. Two synthetic approaches, one-pot and stepwise synthesis, were attempted to prepare complex **7** (Scheme 4). The one-pot reaction yielded some of the product **7**; however, several other species were observed in the reaction mixture attributing multiple coordinating ligands of **3**. The stepwise synthesis provided a more clearly defined product in high yields. The compound *fac*-Re(OH$_2$)(CO)$_3$(picolinate) was prepared and isolated according to previous methods (*27*) and reacted with **3** in methanol to yield compound **7** as the only product in the HPLC chromatogram (Figure 2). Elemental analysis, ^{1}H and ^{13}C NMR as well as X-ray crystallography confirm the formation of complex **7**. ^{1}H NMR spectrum of **7** had increased overlapping signals at ∼8 ppm of the aromatic protons from the four protons of the coordinated picolinate and the three protons **3**. The methylene protons of **3** displayed a singlet signal, in contrast to doublet signals observed in **4**, suggesting that the monodentate ligand **3** is more flexible than the analogous tridentate ligand **5** in rhenium complexes. Crystals suitable for X-ray crystallography were obtained by diffusion of hexane into acetone solution of **7** (Figure 3). The structural parameters are listed in Table 1 and selected bond angles (°) and distances (Å) in Table 2. The rhenium center in complex **7** is a distorted octahedron with three CO ligands occupying the facial geometric sites in near-equivalent Re−C distances (1.89−1.92 Å) and C−Re−C angles (88.4−90.2°) with respect to each other. The other coordination sites are occupied by the coordinated "2 + 1" ligands, picolinate (pic) and **3**. Pic functions as a planar bidentate ligand (N, O) with distances and angles comparable to other reported structures (*27*, *41*, *42*). The monodentate ligand **3** is coordinated to the rhenium center through an imidazole nitrogen at N(2)−Re(1) 2.186 Å. The imidazole ligand itself is slightly tilted toward the plane of the pic ligand (∼83°) yielding an acutely distorted octahedron along the pic and **3** coordination sites. Compound **7** also exhibits interesting intermolecular NH−O (2.79 Å) hydrogen bonding between two molecules through the amide proton donor and the noncoordinated oxygen of the picolinate as a proton acceptor, yielding two hydrogen bond interactions per set of molecules in the unit cell.

Investigation of the radioactive ^{99m}Tc complexes were conducted by reacting ligands (**2**, **3**, and **5**) with the *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ generated from a Tyco Isolink kit in aqueous conditions. The general labeling procedure for tridentate ligands **2** and **5** involved mixing the ligand with the *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ at pH 7.4 and heating at 70 °C for 30 min (Scheme 3). Concentrations (10^{-4}, 10^{-5}, 10^{-6} M) were investigated at these conditions to determine effective labeling of the ligand. Both ligands **2** and **5** demonstrated good labeling of >90% at 10^{-4} and 10^{-5} M to yield *fac*-[^{99m}Tc(CO)$_3$(**2**)] **4a** and *fac*-[^{99m}Tc(CO)$_3$(**5**)] **6a** complexes (Figure 4). Labeling yields for both ligands **2** and **5** at 10^{-6} M were slightly diminished, 28% and 20% respectively, compared to the

Scheme 4. 2 + 1 Labeling Strategy for Reacting Ligand 3 with *fac*-M(OH$_2$)$_3$(CO)$_3$ (M = Re, ^{99m}Tc (a)) to Yield Complexes *fac*-M(CO)$_3$(pic)(3), (7, 7a)[a]



[a] Two strategies are reported: (1) single pot reaction; (2) stepwise addition of ligands.

Table 2. Selective Bond Angles (°) and Distance (Å) of *fac*-Re(CO)$_3$(Picolinate)(3), 7

bond lengths [Å]		angles [°]	
C(1)−Re(1)	1.920(3)	C(2)−Re(1)−C(3)	89.79(11)
C(2)−Re(1)	1.898(3)	C(2)−Re(1)−C(1)	88.41(11)
C(3)−Re(1)	1.902(3)	C(3)−Re(1)−C(1)	90.24(10)
N(1)−Re(1)	2.185(2)	C(2)−Re(1)−O(4)	175.05(9)
N(2)−Re(1)	2.186(2)	C(3)−Re(1)−O(4)	94.71(9)
O(4)−Re(1)	2.1424(17)	C(1)−Re(1)−O(4)	93.60(9)
		C(2)−Re(1)−N(1)	100.09(10)
		C(3)−Re(1)−N(1)	169.25(9)
		C(1)−Re(1)−N(1)	94.20(9)
		O(4)−Re(1)−N(1)	75.26(7)
		C(2)−Re(1)−N(2)	93.93(9)
		C(3)−Re(1)−N(2)	92.73(9)
		C(1)−Re(1)−N(2)	176.23(9)
		O(4)−Re(1)−N(2)	83.84(7)
		N(1)−Re(1)−N(2)	82.48(7)

Table 3. *fac*-^{99m}Tc(OH$_2$)$_3$(CO)$_3^+$ Labeling Yields Prepared at 30 min at 70 °C with pH 7.4 at Various Concentrations with Ligands (2, 3, and 5)

ligand (M)	10^{-4}	10^{-5}	10^{-6}
^{99m}Tc(CO)$_3$(**5**), (**4a**)	>99%	95%	20%
^{99m}Tc(CO)$_3$(**2**), (**6a**)	>99%	90%	28%
	10^{-2}		
Single Pot[a]			
^{99m}Tc(CO)$_3$(pic)(**3**), (**7a**)	71		
Stepwise Addition[b]			
^{99m}Tc(CO)$_3$(pic)(**3**), (**7a**)	70		

[a] The picolinate (pic) at 10^{-5} M and **3** were added to directly to the same vial. [b] ^{99m}Tc(CO)$_3$ was added to a picolinate (10^{-5} M) solution and heated for 30 min, then followed by the addition of **3** and 30 min additional heating.

Table 4. Stability Studies of ^{99m}Tc Complexes (4a, 6a, and 7a) Formed with Ligands (2, 3, and 5) at Biological pH (7.4) at 37 °C and in Normal Mouse Serum at 37 °C

	1 h	2 h	4 h
pH = 7.4 at 37 °C			
^{99m}Tc(CO)$_3$(**5**), (**4a**)	>95	>95	>95
^{99m}Tc(CO)$_3$(**2**), (**6a**)	88	88	88
Normal Mouse Serum at 37 °C			
^{99m}Tc(CO)$_3$(**5**), (**4a**)	85	82	80
^{99m}Tc(CO)$_3$(**2**), (**6a**)	95	95	95
^{99m}Tc(CO)$_3$(pic)(**3**), (**7a**)	81	77	70

previously reported ^{99m}Tc labeling with similar ligands at higher temperatures (95 °C) (*28*, *38*, *43*). When the reaction of *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ with **2** or **5** was carried out at 95 °C, decreased labeling yields of **4a** and **6a** and the corresponding amide cleavage product as a ^{99m}Tc(CO)$_3$ free carboxylate ligand complex were observed in the radiochromatograms. The pH of

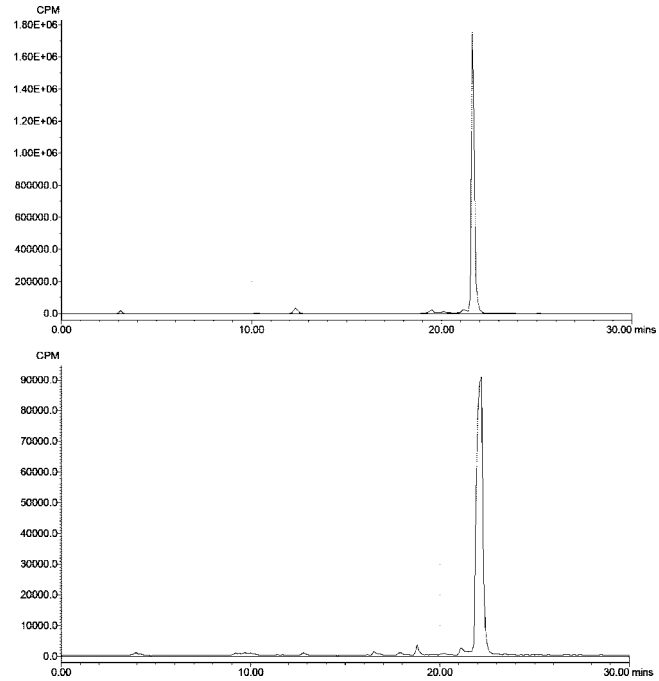


Figure 4. Radio HPLC of the ^{99m}Tc(CO)$_3$ complexes **4a** (top) and **6a** (bottom) formed with ligands **5** and **3**, respectively.

the labeling solution was also found to significantly impact the labeling yields observed. Reactions conducted in slightly acidic conditions between pH 6.5 and 7.4 would yield the desired product, **4a** and **6a**, without the appearance of cleavage products. Attempts to prepare **4a** and **6a** by direct addition of Isolink kit to the reaction mixture in 0.1 M phosphate buffer partially yielded the products and cleavage products. However, neutralization of the Isolink kit with hydrochloric acid to pH ~7.0 prior to addition to the ligand solution was found to be the imperative step in the labeling procedure as the kit solution is quite basic (pH ~11) during the production of *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$. Despite the combination of heating and pH effects impacting the labeling yields, carefully control of the reaction conditions afforded the desired compounds in good yield. The complexes **4a** and **6a** were further studied to determine the stability of the compound in solution at 37 °C in phosphate buffer (pH 7.4) and in mouse serum at 1, 2, and 4 h (Table 4). Both compounds, **4a** and **6a**, were found to be stable for up to four hours in the pH stability and the serum studies with minimal decomposition of complex or cleavage products observed in the radio-HPLC.

The "2 + 1" ^{99m}Tc complex, **7a**, with ligand **3** was prepared by similar methods to the rhenium analogues: one-pot and stepwise (Scheme 4). The preparation of **7a** by either method at 70 °C and pH 7.4 yielded a peak with a similar retention

time to the analogous rhenium complex (Table 3). Each of these methods had comparable labeling results of ~70%. The formation of the "2 + 1" complex **7a** required much higher concentrations (10^{-2} M) of **3** compared to the tridentate ligands **2** and **5**. HPLC purified complex **7a** was incubated in phosphate buffer (0.1 M, pH 7.4) and 37 °C to determine stability of the complex at 1, 2, and 4 h (Table 4). During this study, ligand **3** was observed to partially dissociate during incubation from complex **7a** to yield the intermediate species *fac*-^{99m}Tc(CO)$_3$(H$_2$O)(pic) as identified by retention time. Mouse serum studies further confirmed the instability of the "2 + 1" complex as multiple species in addition to the dissociation of **3** were observed in the radio-HPLC. Despite the previously reported and successful synthesis with the rhenium analogue (*41*), the "2 + 1" complex **7a** containing functionalized imidazole **3** may have limited application due to stability of the complex.

In vitro analysis were conducted with the ^{99m}Tc complexes (**4a**, **6a**) to determine cellular uptake with an androgen-positive DU-145 prostate cancer cell line. Incubation of the ^{99m}Tc complexes with the prostate cancer cells displayed a modest ~2% binding relative to control experiments. Although the uptake was much lower than expected, the decrease binding of these particular model compounds was anticipated. The changes in the chemical nature (i.e., polarity, steric bulk) of the native flutamide compound by introduction of the ^{99m}Tc complexes may have impacted the internalization of the modified compounds. Additionally, the functionalized flutamide complexes may also have decreased AR affinity due to poor metabolic conversion. The native flutamide compound is metabolized to the more potent α-hydroxyl antagonist, hydroxyflutamide, by the AR. Although it is uncertain the potential affect of the ^{99m}Tc complexes will have on the AR with limited internalization of the compounds, additional studies will be required.

CONCLUSION

The study presented here demonstrates the first $M(CO)_3$-linked flutamide compounds (M = Re, ^{99m}Tc) prepared for prostate cancer imaging. Although the amide bond of the flutamide was considered to be unstable due to hydrolytic cleavage, conditions were found to prepare the compounds in high yield. Reactions involving *fac*-^{99m}Tc(CO)$_3$(H$_2$O)$_3$ with tridentate ligands, cysteine and histidine, offered higher labeling yields and greater stability than the "2 + 1" approach utilizing a functionalized imidazole analogue. The tridentate ligands exhibited excellent radiochemical stability at 37 °C at physiological pH and in mouse serum. *In vitro* assays did show limited uptake in DU-145 prostate cancer cells. However, further investigations are required to conclusively determine internalization mechanism and androgen inhibition. Further studies involving metabolic activation of ^{99m}Tc(CO)$_3$ linked flutamide complexes to the hydroxyflutamide analogue and *in vivo* tumor model experiments are currently underway.

ACKNOWLEDGMENT

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Supporting Information Available: X-ray structural information for **7** (cif). This material is available free of charge via the Internet at http://pubs.acs.org.

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Investigation of the Coordination Interactions of S-(Pyridin-2-ylmethyl)-L-Cysteine Ligands with $M(CO)_3^+$ (M = Re, ^{99m}Tc)

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Development of new ligands for *fac*-$M(OH_2)_3(CO)_3^+$ (M = Re, ^{99m}Tc) led the investigation with S-(pyridin-2-ylmethyl)-L-cysteine, **1**. The ligand **1** has potential to coordinate with the metal through three different tridentate modes: tripodal through cysteine (O,N,S) and two linear involving the S-pyridyl and cysteine (O,S,N_{Py}, N,S,N_{Py}). From the reaction with **1**, two species were observed in the ^{1}H NMR, where the primary product was the linear *fac*-Re(N,S,N_{Py}-**1**)$(CO)_3^+$, **2a**, complex. To identify the coordination mode of the minor product, functionalized analogues of **1** were prepared from S-(pyridin-2-ylmethyl)-Boc-L-cysteine-methyl ester, **3**, with orthogonal protecting groups on the C terminus (methyl ester) in S-(pyridin-2-ylmethyl)-L-cysteine methyl ester, **4**, or N terminus (Boc) in S-(pyridin-2-ylmethyl)-Boc-L-cysteine, **6**, that specifically directed the coordination mode of *fac*-$M(H_2O)_3(CO)_3^+$ to either N,S, N_{Py} or O,S,N_{Py}, respectively. Two diastereomers [*fac*-$Re(CO)_3$(N,S,N_{Py}-**4**)]$^+$, **5a** and **5b**, were observed and independently characterized by X-ray structure analysis and NMR in high yield with **4**. Surprisingly, the O,S,N_{Py} Re complex with ligand **6** was not observed and simplified versions, 3-(pyridin-2-ylmethylthio) propanoic acid, **7**, and 2-(pyridin-2-ylmethylthio)acetic acid, **8**, were investigated. Ligand **7** did not yield the desired linear tridentate O,S,N_{Py} product. However, the shorter ligand **8** formed *fac*-$Re(CO)_3$(O,S,N_{Py}-**8**), **9**, in high yield. ^{99m}Tc labeling studies were conducted and yielded similar results to the rhenium complex and effective (>99%) at 10^{-5} M ligand concentration.

Introduction

In recent years, imaging and radio-therapeutic applications of the second and third row congeners, ^{99m}Tc (γ, 140 keV, $t_{1/2}$ = 6.0 h) and $^{186/188}$Re (β^-_{max} (1.1 MeV), $t_{1/2}$ = 3.7 d; β^-_{max} (2.1 MeV), $t_{1/2}$ = 17 h) has significantly increased in nuclear medicine.[1−3] The water-soluble organometallic *fac*-$M(OH_2)_3(CO)_3^+$ (M = Re, ^{99m}Tc) complex has yielded considerable interest because of the compact stable nature of the tricarbonyl core.[4−11] Several general coordination strategies (i.e., monodentate, "2 + 1", tridentate ligands) have been investigated with *fac*-$M(OH_2)_3(CO)_3^+$.[12−17] Tridentate ligands have typically offered higher chemical and biological stability that are further subdivided into two general classes: linear[18−22] and

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tripodal.[20−29] Both classes utilize similar design strategies in donors atoms (i.e., N, O, P, S) and coordination ring size (5 or 6) to generate comparable complexes, albeit different ligand conformations.

Investigation of new bifunctional chelates (BFC) to effectively coordinate $M(CO)_3^+$ while linking a biological targeting molecule (i.e., receptor, transporter recognition) continues to be an active area of research. New methods are needed for radiolabeling for large peptides prepared by recombinant techniques rather than protecting group based solid phase synthesis. His-tag labeled peptides have provided initial success with $M(CO)_3^+$ by coordination through multiple imidazole donors with reasonable stability. However, the exact orientation of the metal center within His-tag remains undefined.[30] Alternative labeling strategies are needed to improve the specific $M(CO)_3^+$ coordination, while maintaining mild synthetic and minimal purification requirements found with His-tag.

The reactive nature of thiol in cysteine provides an excellent synthetic handle for conversion of the terminal amino acid into a tridentate ligand capable of coordinating the $M(CO)_3^+$ in a specific mode, while maintaining its association to a peptide. Several analogous linear tridentate and tripodal ligands utilizing thiols and thioethers for complexing $M(CO)_3^+$ provide a basis for determining a S-pendent donor.[26,31−34] Pyridine was selected for functionalizing of the cysteine moiety as it provides an excellent soft donor for the $M(CO)_3$ core and has been demonstrated as an effective ligand as reported with other metals.[35−38] The model ligand S-(pyridin-2-ylmethyl)-L-cysteine, **1**, was prepared and investigated to probe the ligand interactions with $M(CO)_3^+$. Because of the number of available donors (four) and coordination sites (three), ligand **1** has the potential to form three different tridentate coordination complexes with $M(CO)_3^+$, one tripodal (N,S,O of cysteine) and two linear

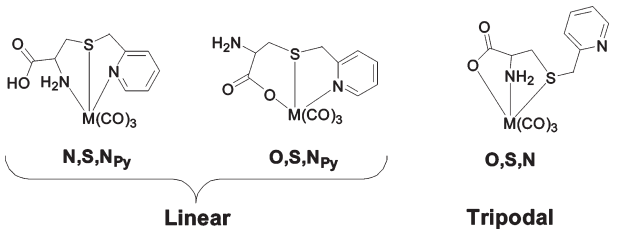

Figure 1. Three possible tridentate coordination compounds illustrating two types of coordination styles (Linear, Tripodal) that could be formed in the reaction of **1** with $M(OH_2)_3(CO)_3^+$ M = Re, ^{99m}Tc.

(N,S,N_{Py} or O,S,N_{Py}) (Figure 1). Tripodal thioether S-functionalized cysteine complexes have been observed with non coordinating extensions (i.e., methyl, propyl, benzyl).[26,27] However, the addition of a S-pendent donor to cysteine has led to discussion of the types of complexes (tripodal vs linear) reported in the literature with $M(CO)_3^+$ because of the prochiral nature of the thioether. Two S functionalized aromatic thioether amino acid variations (S-(2-2′-pyridyl)ethyl-D,L-homocystiene (tripodal N,S,O) and 1,2,3 triazole cysteine (linear N,S,N_{Tri} and tripodal N, S,O) reported in the literature have differing results with *fac*-$Re(CO)_3^+$.[29,32] Although these compounds are similar in nature, subtle difference in the donor strength of the S pendent ligand (pyridine vs triazole) and coordination ring size may have attributed to the observed differences in the isolated complexes.

In light of the ambiguity in the literature, we investigated ligand **1** for potential complex formation with *fac*-$M(CO)_3^+$ (M = Re, ^{99m}Tc). Different than previously investigated ligand systems, **1** is a composite ligand that features the strengths of both ligands, a smaller chelate ring size (cysteine vs homocysteine), and improved donor strength (pyridine vs triazole). The studies presented here provide a convenient aqueous synthetic route for preparing pyridine functionalized cysteine ligands and a fundamental evaluation of the coordination chemistry to determine the most effective coordination mode. **1** also provides important insights into evaluating the coordination of *fac*-$M(CO)_3^+$ in the presence of multiple donors. Protected ligand variations at the C and N terminus of **1** were also prepared to specifically control the coordination mode of the ligand (N,S,N_{Py} or O,S,N_{Py}) with *fac*-$M(CO)_3^+$ (M = Re, ^{99m}Tc). These ligands provide essential controls to determine the effectiveness of the linear systems with $M(CO)_3$ and to simulate conditions found with peptide analogues prior to implementation in a biological targeting molecule.

Experimental Section

All reagents and organic solvents of reagent grade or better were used as purchased from Aldrich, Acros, or Fluka without further purification. Rhenium starting materials $Re(CO)_5SO_3CF_3$, and *fac*-$[Re(CO)_3(H_2O)_3][SO_3CF_3]$, were prepared by literature methods from $Re_2(CO)_{10}$ purchased from Strem.[12,26] Elemental analyses were performed by Quantitative Technologies, Inc., NJ. Analytical identification of compounds were conducted on a Perkin-Elmer Series 200 High Pressure Liquid Chromatograph (HPLC) equipped with a UV/vis Series 200 detector, a Radiomatic 610TR detector, and a 30 cm Agilent ZORBAX SB-C18 5 μm particle column using a reverse phase gradient system beginning with 0.1% trifluoroacetic acid (TFA) aqueous eluent gradually shifting to methanol was utilized: 0−3.0 min (100%

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TFA), 3.0−9.0 min (75% TFA, 25% MeOH), 9.0−20.0 min (25% to 100% MeOH linear gradient), 20.0−25.0 min (100% MeOH) at a flow rate of 1.0 mL/min. Semipreparatory separations were carried out with a Hitachi D-7000 series HPLC system equipped with a UV L-7400 detector on a 21.2 × 250 mm SB-C18 7 μm particle Agilent ZORBAX column at 10.0 mL/min under a similar gradient conditions as the analytic system. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 300 MHz spectrometer, and chemical shifts were referenced to internal sodium 3-(trimethylsilyl)propionate-d_4 (TSP, 0.00 ppm) in D_2O or the residual solvent signal in organic solvents. Mass measurements were performed as Q3 scans on an Applied Biosystems AP14000 triple quadrupole.

S-(pyridin-2-ylmethyl)-L-cysteine, 1. 2-(Bromomethyl)pyridine hydrobromide (0.51 g, 2.0 mmol) and L-cysteine (0.32 g, 2.6 mmol) were added as a solid to 20 mL of water and adjusted to pH 8 by $NaHCO_3$ (1 M) followed by the addition of methanol (10 mL). The solution was stirred overnight at room temperature. The product was purified and isolated by preparatory HPLC as a colorless semisolid (0.62 g, 69%). ^{1}H NMR [δ(ppm), D_2O], 8.72 (d, 1H), 8.54 (dd, 1H), 8.06 (d, 1H), 7.94 (m, 1H), 4.20 (s, 2H), 4.12 (dd, 1H), 3.10 (dd, 1H), 3.02 (dd, 1H). ^{13}C NMR [δ(ppm), D_2O], 171.4, 152.4, 147.5, 141.9, 127.9, 126.1, 52.8, 32.4, 31.5. MS [$(M+H)^+$], 213.

[*fac*-Re(CO)$_3$(N,S,N$_{Py}$-1)][CF$_3$SO$_3$], 2a and 2b. To a solution of **1** (0.17 g, 0.5 mmol) in 5 mL of deionized H_2O was added *fac*-[$Re(CO)_3(H_2O)_3$][SO_3CF_3] (5 mL, 0.1 M). The solution (pH = 2) was heated at 70 °C for 2 h. $NaHCO_3$ was added periodically to adjust the solution to pH = 2. After heating, HPLC analysis showed a single peak (Rt = 17.54 min). After cooling, the mixture was concentrated and desalted on a Sephadex G-15 column (eluted with Deionized water) to give a white solid (0.27 g, 90%). ^{1}H NMR of the crude product showed the presence of two isomers indicated as **2a** (major isomer) and **2b** (minor isomer). Anal. Calcd for $C_{14}H_{12}F_3N_2O_7ReS$: C, 28.24; H, 2.03; N, 4.70. Found: C, 27.90; H, 1.94; N, 4.57.

2a. Pure crystals of major isomer **2a** were obtained by fractional recrystallization of the purified products (**2a** and **2b**) from deionized water. ^{1}H NMR of the isolated crystals indicated they were a single isomer corresponding to major species. X-ray quality crystals of **2a** were obtained by slow evaporation of the MeOH/H_2O solution. Anal. Calcd for $C_{14}H_{12}F_3N_2O_7ReS$: C, 28.24; H, 2.03; N, 4.70. Found: C, 27.76; H, 1.72; N, 4.55. ^{1}H NMR [δ(ppm), D_2O], 9.05 (d, 1H), 8.04 (dd, 1H), 7.75 (d, 1H), 7.46 (dd, 1H), 6.00 (d, 1H), 4.98 (d, J = 17.4 Hz, 1H), 4.58 (d, J = 17.4 Hz, 1H), 4.06 (dd, 1H), 3.82 (m, 2H), 3.59 (dd, 1H), 2.53 (dd, 1H). [δ(ppm),CD_3COCD_3], 9.19 (d, 1H), 8.14 (dd, 1H), 7.94 (d, 1H), 7.59 (dd, 1H), 6.21 (d, 1H), 5.30 (d, J = 17.4 Hz, 1H), 4.77 (d, J = 17.4 Hz, 1H), 4.24 (dd, 1H), 3.83 (m, 2H), 2.66 (dd, 1H). ^{13}C NMR [δ(ppm), D_2O], 177.9, 161.8, 158.2, 143.5, 128.6, 128.2, 65.4, 48.2, 40.0. MS [M^+], 483, 481.

S-(pyridin-2-ylmethyl)-Boc-L-cysteine Methyl Ester, 3. To a solution of 2-(bromomethyl) pyridine hydrobromide (0.34 g, 1.3 mmol) in acetonitrile (10 mL) was added triethylamine (0.36 mL, 2.6 mmol), whereupon the solution turned pink. Then a solution of *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester (0.29 g, 1.2 mmol) in acetonitrile (5 mL) was added. The solution was stirred overnight at room temperature under N_2. The solution was concentrated to dryness and was purified by silica gel chromatography. The crude material was loaded as a dry solid onto a column conditioned with a combination of ethyl acetate: hexanes (1:6) that was gradually shifted to ethyl acetate: hexanes (1:3) to elute the product. Upon evaporation, the product was collected as colorless oil. (0.28 g, 71%) ^{1}H NMR [δ(ppm), CD_3COCD_3], 8.65 (d, 1H), 8.01 (dd, 1H), 7.65 (d, 1H), 7.49 (dd, 1H), 6.62 (b, 1H), 3.98 (dd, 2H), 3.68 (s, 3H), 4.42 (m, 1H), 3.00 (dd, 1H), 2.89 (dd, 1H), 1.48 (s, 9H). ^{13}C NMR [δ(ppm), CD_3COCD_3], 172.2, 158.5, 156.3, 147.9, 140.3, 125.2, 123.9, 79.5, 54.5, 52.4, 36.8, 34.0, 28.5. MS [M^+], 326.

S-(pyridin-2-ylmethyl)-L-cysteine Methyl Ester, 4. To a solution of **3** (0.15 g, 0.38 mmol) in methylene chloride (2.7 mL) was added trifluoroacetic acid (0.3 mL) [1:4 TFA/CH_2Cl_2]. The solution was stirred overnight at room temperature, then evaporated to dryness. The product was isolated as a colorless oil (0.14 g, 95%). ^{1}H NMR [δ(ppm), D_2O], 8.73 (m, 1H), 8.54 (td, 1H), 8.02 (d, 1H), 7.96 (m, 1H), 4.40 (dd, 1H), 4.21 (s, 2H), 3.81 (s, 3H), 3.19 (dd, 1H), 3.06 (dd, 1H). ^{13}C NMR [δ(ppm), D_2O], 168.8, 152.2, 147.5, 142.0, 127.7, 126.1, 54.0, 52.1, 32.4, 30.9. MS [$(M+H)^+$], 227.

[*fac*-Re(CO)$_3$(N,S,N$_{Py}$-4)][CF$_3$CO$_2$], 5a and 5b. To a solution of **4** (0.17 g, 0.5 mmol) in deionized H_2O (5 mL) was added *fac*-[$Re(CO)_3(H_2O)_3$][SO_3CF_3] (5 mL, 0.1 M). The solution (pH 2) was heated at 70 °C for 2 h. $NaHCO_3$ was added periodically to adjust pH back to 2. Analytical HPLC analysis of the reaction mixture showed two peaks with retention times (Rt) of 17.50 and 18.26 min. After cooling, the mixture was desalted by preparative HPLC, and the collected product was evaporated to dryness (0.29 g, 89%). ^{1}H NMR of the solid showed the presence of two isomers. Anal. Calcd for $C_{13}H_{14}N_2O_5ReS\cdot 2/3CF_3CO_2\cdot 1/3CF_3SO_3\cdot 1/3CF_3CO_2H$: C, 27.88; H, 2.17; N, 4.24. Found: C, 28.06; H, 1.75; N, 4.14. Two diastereomers (**5a** and **5b**) were separated and isolated by successive preparative HPLC purification.

5a. (Rt = 17.50 min). ^{1}H NMR [δ(ppm),CD_3COCD_3], 9.21 (d, 1H), 8.20 (td, 1H), 8.00 (d, 1H), 7.64 (m, 1H), 6.24 (d, 1H), 5.38 (d, J = 17.7 Hz, 1H), 4.80 (d, J = 17.7 Hz, 1H), 4.36 (dd, 1H), 4.07 (m, 2H), 3.63 (s, 3H), 2.74 (dd, 1H). ^{13}C NMR [δ(ppm), CD_3COCD_3], 169.8, 159.7, 156.2, 140.9, 126.3, 125.9, 61.4, 52.6, 45.9, 35.9. MS [M^+], 497, 495. Crystals suitable for X-ray crystallography were obtained by slow evaporation of aqueous solution of **5a**.

5b. (Rt = 18.26 min). ^{1}H NMR [δ(ppm),CD_3COCD_3], 9.15 (d, 1H), 8.18 (t, 1H), 7.98 (d, 1H), 7.60 (t, 1H), 6.05 (d, 1H), 5.95 (dd, 1H), 5.38 (d, 1H), 4.92 (dd, 1H), 3.54 (s, 3H), 3.50 (m, 3H). ^{13}C NMR [δ(ppm), CD_3COCD_3], 169.9, 160.6, 156.2, 140.7, 125.9, 125.0, 57.9, 52.6, 45.4, 37.5. MS [M^+], 497, 495. Crystals suitable for X-ray crystallography were obtained by diffusion of methyl *tert*-butyl ether into methanolic solution of **5b**.

S-(pyridin-2-ylmethyl)-Boc-L-cysteine, 6. To a solution of **3** (0.72 g, 2.2 mmol) in 15 mL of methanol was added 2.5 mL of 1 M NaOH and stirred at room temperature for 3 h. The solution was then neutralized with 2.5 mL of 1 M HCl and concentrated to ~5 mL by rotary evaporation then placed in the refrigerator overnight. The product was isolated as a colorless solid. (0.45 g, 65%). ^{1}H NMR [δ(ppm), CD_3COCD_3], 8.51 (d, 1H), 7.77 (dd, 1H), 7.46 (d, 1H), 7.26 (dd, 1H), 4.44 (m, 1H), 3.93 (dd, 2H), 3.05 (dd, 1H), 2.93 (dd, 1H), 1.42, (s, 9H). ^{13}C NMR [δ(ppm), CD_3COCD_3], 171.9, 159.0, 155.6, 149.1, 137.2, 123.4, 122.3, 78.7, 53.6, 37.7, 33.5, 27.9. MS [M^+], 312.

3-(Pyridin-2-ylmethylthio)propanoic acid, 7. To a solution of 2-picolyl chloride hydrochloride (1.64 g, 10 mmol) and methyl 3-mercapto propanoicate (1.2 mL, 10.8 mmol) in acetonitrile (40 mL) was added cesium carbonate (7.15 g, 22 mmol). The mixture was stirred overnight at room temperature and filtered. The filtrate was evaporated to dryness to yield the crude methyl ester product. The methyl ester intermediate was redissolved in 10 mL of MeOH, followed by 10 mL of 1 M NaOH solution. The solution was stirred for 6 h then neutralized with 12 mL of 1.0 M HCl solution. The product was purified by preparative HPLC yielding a white solid (1.63 g, 83%). ^{1}H NMR [δ(ppm), D_2O], 8.52 (d, 1H), 8.36 (dd, 1H), 7.88 (d, 1H), 7.78 (dd, 1H), 4.01 (s, 2H), 2.60 (t, 2H), 2.47 (t, 2H). ^{13}C NMR [δ(ppm), CD_3COCD_3], 172.8, 160.2, 150.0, 145.3, 132.1.2, 129.8, 55.5, 38.7, 35.6. MS [$(M-H)^-$], 196.

2-(Pyridin-2-ylmethylthio)acetic acid, 8. 2-Picolyl chloride hydrochloride (1.64 g, 10 mmol) and mercaptoacetic acid (0.77 mL, 11 mmol) in H_2O (40 mL) was added dropwise to NaOH (16 mL, 1 M) in 1 h. The mixture was stirred overnight,

Table 1. Crystallographic Data and Structure Refinement Parameters for compound **2a**, **5a**, **5b**, and **9**

	2a	5a	5b	9
formula	$C_{14}H_{12}F_3N_2O_7ReS$	$C_{14}H_{14}F_3N_2O_8ReS_2$	$C_{15}H_{14}F_3N_2O_7ReS$	$C_{11}H_8NO_5ReS$
formula weight	595.52	645.59	609.54	452.44
space group	P2(1)2(1)2(1)	P2(1)/n	P2(1)2(1)2(1)	P-1
a (Å)	8.2755(3)	8.6579(5)	10.7633(12)	7.2461(8)
b (Å)	10.7053(4)	17.2829(10)	16.2152(17)	9.3299(10)
c (Å)	20.7099(7)	13.1663(8)	33.494(4)	10.3539(11)
α (deg)				101.925(5)
β (deg)		96.3680(10)		106.904(5)
γ (deg)				101.832(5)
V (Å^3)	1834.73(11)	1958.02(2)	5845.68(11)	628.45(12)
Z	4	4	12	4
T (K)	90(2)	90(2)	190(1)	190(1)
D_{calcd} (Mg/m^3)	2.156	2.190	2.078	2.391
μ (mm^{-1})	6.807	6.494	6.412	9.848
GOF	1.034	1.038	1.06	1.113
R_1 [$I > 2\sigma(I)$][a]	0.0162	0.0192	0.0246	0.0213
wR_2 [$I > 2\sigma(I)$][a]	0.0336	0.0436	0.0309	0.0549

[a] $R_1 = \sum||F_o| - |F_c||/\sum|F_o|$; $wR_2 = \{\sum w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2\}^{1/2}$.

neutralized to pH 2 with HCl, and concentrated to 20 mL. A dark solid was formed, collected, and decolored by charcoal in EtOH-H_2O solution. Concentration of the colorless filtrate to ~10 mL yielded the product as a solid, which was washed sparingly with water and collected. (0.65 g, 36%). ^{1}H NMR [δ(ppm), D_2O/NaOD], 8.27 (d, 1H), 7.64 (dd, 1H), 7.18 (d, 1H), 7.15 (dd, 1H), 3.66 (s, 2H), 2.93 (s, 2H). ^{13}C NMR [δ(ppm), D_2O/NaOD], 180.2, 159.6, 151.2, 141.5, 127.1, 125.8, 39.6, 38.9. MS [M^+], 183.

***fac*-Re(CO)$_3$(O,S,N$_{Py}$-8), 9**. To a solution of **8** (0.10 g, 0.55 mmol) in MeOH (5 mL) was added aqueous solution of *fac*-[$Re(CO)_3(H_2O)_3$][SO_3CF_3] (5 mL, 0.1 M). The solution was refluxed for 2 h and HPLC analysis showed only a peak with Rt of 19.14 min. The mixture was concentrated to 5 mL producing a precipitate (0.20 g, 90%). Suitable X-ray quality single crystals were prepared by recrystallizing **9** in CH_2Cl_2/acetone to yield colorless plates. Anal. Calcd for $C_{11}H_8N_2O_5ReS \cdot 0.2CH_2Cl_2$: C, 28.59; H, 1.78; N, 2.98. Found: C, 28.17; H, 1.42; N, 2.98. ^{1}H NMR [δ(ppm), CD_3COCD_3], 9.05 (d, 1H), 8.17 (dd, 1H), 7.91 (d, 1H), 7.59 (dd, 1H), 5.12 (d, J = 17.4 Hz, 1H), 4.74 (d, J = 17.4, Hz 1H), 3.58 (d, J = 17.7 Hz, 1H), 3.20 (d, J = 17.7 Hz, 1H). ^{13}C NMR [δ(ppm), d$_6$-DMSO], 179.3, 160.2, 154.9, 141.2, 126.3, 125.9, 46.0, 33.4. MS [$(M - H)^-$], 452.

X-ray Experimental. Crystals of compounds **2a**, **5a**, **5b**, and **9** were removed from the flask and covered with a layer of hydrocarbon oil. A suitable crystal was selected, attached to a glass fiber, and placed in the low-temperature nitrogen stream.[39] Data (**2a** and **5a**) were collected at low temperatures using a Bruker/Siemens SMART APEX instrument (Mo Kα radiation, λ = 0.71073 Å) equipped with a Cryocool NeverIce low temperature device. Data for **2a** and **5a** was measured using omega scans of 0.3° per frame for various exposures, and a full sphere of data was collected in each case. A total of 2400 frames were collected with final resolutions of 0.77 Å. Cell parameters were retrieved using SMART[40] software and refined using SAINTPlus[41] on all observed reflections. Data reduction and correction for Lp and decay were performed using the SAINTPlus software. Absorption corrections were applied using SADABS.[42] Data for **5b** and **9** were collected at low temperature on a Nonius Kappa CCD (Mo Kα radiation, λ = 0.71073 Å) using phi and omega scans. A total of 893 and 522 frames were collected for **5b** and **9** respectively. The unit cells were refined with 25460 reflections for **5b** and 2994 reflections for **9**. The diffraction intensities for **5b** and **9** were collected to a 0.76 Å resolution. Absorption corrections were performed using HKL Scalepack. Structures **2a** and **5a** were solved by direct methods and refined by the least squares method on F^2 using the SHELXTL program[43] package. Structures **5b** and **9** were solved by either the heavy-atom (**5b**) or direct (**9**) method using SHELXS-97.[44] The least squares refinements performed were conducted with SHELXL-97.[44] All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were in all cases refined in geometrically constrained riding positions. No decomposition of crystals was observed during the data collection process. Details of the data collection and refinement are given in Table 1. Further experimental details are provided in the Supporting Information.

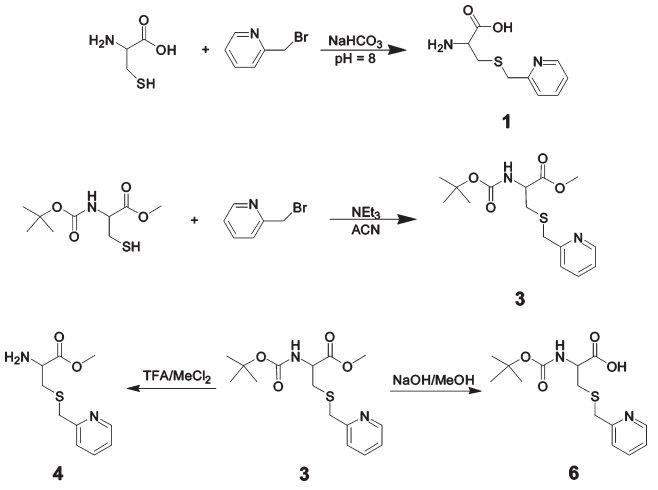
General ^{99m}Tc(H$_2$O)$_3$(CO)$_3^+$ Radiolabeling Procedure. The ligand (100 μL, 10^{-4} or 10^{-5} M) and phosphate buffer (800 μL, 0.1 M) at pH 5.0 or 7.4 was added to a sealable labeling vial (5.0 mL). The vial was sealed and degassed with nitrogen for ~10 min. The $^{99m}Tc(H_2O)_3(CO)_3^+$ precursor solution (100 μL) prepared according to the Isolink kit from Tyco specifications was added to the degassed vial, and the vial heated for 1 h at 90 °C. The reaction mixture was carefully allowed to cool on an ice bath prior to injection and analysis by radio-HPLC.

Results and Discussion

Several variations of S-(pyridin-2-ylmethyl)-L-cysteine ligands were synthesized in a straightforward manner (Scheme 1). The general strategy involved a similar approach to other cysteine compounds reported in the literature.[35,36,45] A protected cysteine at the C and N terminus (Boc-Cys-OMe) was reacted with 2-(bromomethyl) pyridine in acetonitrile in the presence of a base to yield the product S-(pyridin-2-ylmethyl)-Boc-L-cysteine methyl ester, **3**. The orthogonal protecting groups could be selectively removed by either acid (trifluoroacetic acid/methylenechloride) or basic (NaOH/methanol) conditions to yield S-(pyridin-2-ylmethyl)-L-cysteine methyl ester, **4**, and S-(pyridin-2-ylmethyl)-Boc-L-cysteine, **6**, respectively. Both groups could be subsequently deprotected to generate the unprotected version S-(pyridin-2-ylmethyl)-L-cysteine, **1**, in three steps.

(39) Hope, H. *Prog. Inorg. Chem.* **1994**, *41*, 1–19.
(40) *SMART*; Bruker AXS: Madison, WI, 2001.
(41) *SAINTPlus*; Bruker AXS: Madison, WI, 2003/2004.
(42) *SADABS*; Bruker AXS: Madison, WI, 2004.
(43) *SHELXTL*; Bruker AXS: Madison, WI, 2004.
(44) Sheldrick, G. M. *Acta Crystallogr., Sect. A* **2008**, *64*, 112–122.
(45) Ijuin, R.; Umezawa, N.; Higuchi, T. *Bioorg. Med. Chem.* **2006**, *14*, 3563–3570.

Scheme 1. Synthesis of S-Functionalized S-(pyridin-2-ylmethyl)-L-cysteine Ligands



To minimize multistep synthesis for application with peptides, an alternative strategy to direct alkylation of the thiol of cysteine without the use of protecting groups was investigated. Literature methods for aqueous alkylation of the thiol in cysteine utilize strongly basic conditions (i.e., 1 M NaOH) and high temperature (100 °C), which may present problems for pH sensitive peptides because of racemization of the amino acids and structural deformations. To address this issue, cysteine was utilized as a model for peptides to investigate mild aqueous alkylation conditions of thiols. It was found that S-(pyridin-2-ylmethyl)-L-cysteine, **1**, could be prepared by directly reacting cysteine without the use of protecting groups with 2-(bromomethyl) pyridine at pH ~8 in a bicarbonate buffer at room temperature overnight. Only the S alkylated product **1** was observed in the reaction mixture identified by ^{1}H NMR and purified by preparatory HPLC in good yield (69%). The new thiol alkylation conditions provide a facile strategy for generating S functionalized cysteine ligands for peptide applications, while minimizing possible side reactions caused by high pH and heating.

The S-(pyridin-2-ylmethyl)-L-cysteine ligands were reacted with *fac*-$M(OH_2)_3(CO)_3^+$ (M = Re, ^{99m}Tc) to elucidate the possible coordination modes, tripodal (N,S,O of cysteine) or linear (N,S,N_{Py} or O,S,N_{Py}), that would be observed by the ligand.

Each of the three ligands (**1**, **4**, and **6**) representing the different types of $M(CO)_3$ complexes was examined to provide essential information on how to best incorporate them into targeting molecules. Ligand **1** has the potential to form all three types of complexes. Whereas, ligands **4** and **6** reduce the ambiguity to a single coordination mode through selective removal of only one of the protecting groups yielding linear tridentate ligands and $M(CO)_3$ complexes (N,S,N_{Py} or O,S,N_{Py}), respectively. Together these ligands provided important insights in the specific interactions of $M(CO)_3$ with S-(pyridin-2-ylmethyl)-L-cysteine ligands.

In the reaction of **1** with *fac*-$[Re(OH_2)_3(CO)_3]^+$, several factors such as overall complex charge, neutral (tripodal and linear O,S,N) versus cationic (linear N,S,N), chelate ring size, donor ligands, and conformation (linear vs tripodal) were anticipated to affect the coordination modes observed with $M(CO)_3$ and potentially yield several species in the reaction mixture. HPLC analysis throughout the course of the reaction yielded a single peak at 17.5 min (Figure 2). This observation did not preclude the possibility of multiple coordination modes present in the sample, yet insufficient separation of the species under the column conditions. ^{1}H NMR was conducted on the isolated peak and revealed two distinct sets of signals that indicated two products in a 6:1 ratio. Both products exhibited similar H_a-H_b splitting of the methylene protons (S-CH_2-Pyr). The doublets were slightly shifted (4.58, 4.98 ppm) and (4.8, 5.3 ppm), but had identical coupling constants (J = 17.4) for each complex. Whereas, H_a-H_b splitting of the peak of the similar methylene protons (S-CH_2-Ph) in the tripodal *fac*-$Re(CO)_3$(S-benzyl-cystiene) complex was not previously observed.[27] This suggested pyridine was involved in metal coordination in both species and eliminated tripodal as a possible coordination mode present in the sample. However, noticeable shifts in ^{1}H NMR of the cysteine protons (CH−CH_2−S) between the major and the minor species were noted and suggested distinctly different compounds.

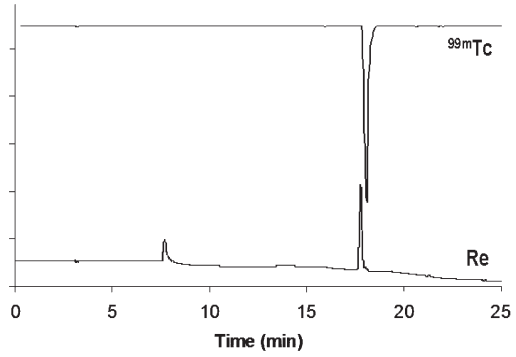
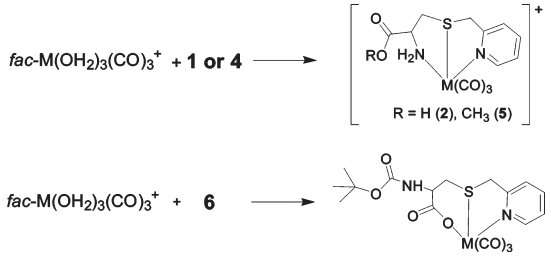


Figure 2. HPLC chromatograms of the reaction of **1** with $M(OH_2)_3(CO)_3^+$, Re (UV 220 nm, bottom), and ^{99m}Tc (NaI, top) to yield *fac*-$[M(CO)_3(N,S,N_{Py}$-**1**$)]^+$, **2a** and **2b**.

Scheme 2. Reaction of S-(pyridin-2-ylmethyl)-L-cysteine Ligands with *fac*-$M(OH_2)_3(CO)_3^+$ (M = Re, ^{99m}Tc)



Attempts to separate the two products by chromatographic methods were unsuccessful. However, fractional recrystallization of the crude material led to the isolation of the major species of the reaction [*fac*-$Re(CO)_3(N,S,N_{Py}$-**1**$)$]-$[CF_3CO_2]$, **2a**, (Scheme 2). Single crystal X-ray diffraction analysis of the isolated crystals definitively illustrated the N,S,N_{Py} orientation of **1** on the metal center (Figure 3). Crystallographic parameters and selected bond angles and distances for **2a** are found in Tables 1 and 2 and are discussed in more detail later. ^{1}H NMR of the isolated crystals **2a** was correlated with the crude sample, where **2a** was observed as the major product (Figure 4). However, isolation of a pure sample of the second minor product from the mother liquor proved elusive as trace amounts of the major product **2a** present in the sample cocrystallized with the minor product **2b**.

In an effort to better understand the ligand interactions of **1**, identify the minor species of **2b**, and evaluate the feasibility of S-(pyridin-2-ylmethyl)-L-cysteine ligands for C or N terminal ligands in peptides, ligands **4** and **6** were investigated with *fac*-$Re(OH_2)_3(CO)_3^+$. The protecting group (methyl ester or boc) on **4** and **6** would specifically limit the formation of a single coordination mode of $M(CO)_3$ to either N,S,N_{Py}, or O,S,N_{Py}, respectively (Scheme 2).

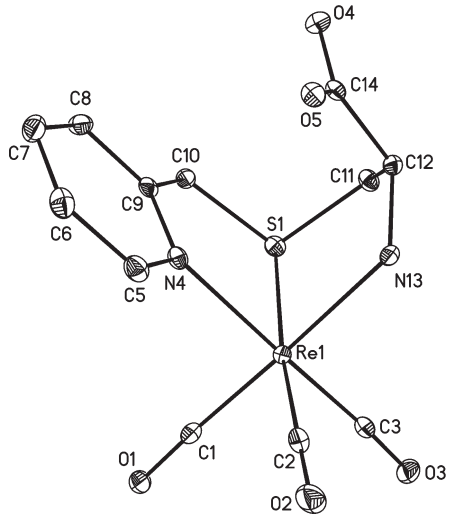


Figure 3. Molecular structure of *fac*-$[Re(CO)_3(N,S,N_{Py}\text{-}\mathbf{1})]^+$, **2a**. Thermal ellipsoids are shown at the 30% probability level. Hydrogen atoms omitted and only the Re cation is shown for clarity.

Table 2. Selected Bond Distances (Å) and Angles (deg) for Compounds **2a**, **5a**, **5b**, and **9**

	2a	5a	5b	9
$Re-NH_2$	2.227(3)	2.219(2)	2.220(5)	
$Re-N_{Py}$	2.210(2)	2.205(2)	2.218(5)	2.203(3)
Re−S	2.4516(8)	2.4530(7)	2.4468(2)	2.4529(10)
Re−CO	1.924(3)	1.932(3)	1.928(7)	1.915(5)
	1.922(3)	1.922(3)	1.942(8)	1.942(4)
	1.918(3)	1.908(3)	1.943(7)	1.903(5)
Re−O				2.138(3)
$S-CH_2$	1.809(3)	1.830(3)	1.834(6)	1.818(4)
	1.822(3)	1.810(3)	1.811(6)	1.808(4)
$N_{Py}-Re-NH_2$	85.26(10)	84.85(9)	83.69(18)	
NH_2-Re-S	81.29(7)	81.27(6)	80.77(12)	
$N_{Py}-Re-S$	80.39(7)	80.52(6)	79.23(13)	79.50(9)
$N_{Py}-Re-O$				78.73(10)
O−Re−S				80.65(8)
CH_2-S-CH_2	102.67(14)	101.78(15)	101.2(3)	101.9(2)

The reaction of **4** with *fac*-$Re(OH_2)_3(CO)_3^+$ yielded similarities and differences to those observed with ligand **1**. Two peaks of near equal intensity at 17.5 and 18.3 min were observed in the reaction mixture by HPLC chromatogram (Figure 5). ^1H NMR spectra of the crude reaction mixture were nearly identical of those of the crude product of **2a** and **2b** except that the former exhibits two additional singlet signals for methyl esters. Each of these peaks was separated by preparatory HPLC and fully characterized. The purified product (17.5 min) was found to be the anticipated complex *fac*-$[Re(CO)_3(N,S,N_{Py}\text{-}\mathbf{4})][CF_3CO_2]$, **5a**. X-ray analysis of the single crystals of **5a** had the same N,S,N_{Py} coordination and structural configuration as **2a** (Figure 6). ^1H NMR of sample **5a** yielded nearly identical splitting patterns and peaks as identified for **2a** with the addition of the singlet for the methyl ester at 3.63 ppm. The second product (18.3 min) isolated from prep HPLC was found to be the diastereomer *fac*-$[Re(CO)_3(N,S,N_{Py}\text{-}\mathbf{4})][CF_3CO_2]$, **5b**. Single crystals suitable for X-ray analysis of **5b** confirmed the N,S, N_{Py} orientation of **4** and the intact methyl ester (Figure 7). ^1H NMR spectra of **5b** available in the Supporting Information except for a methyl ester singlet was found to correlate with the minor species **2b** identified in the reaction mixture with

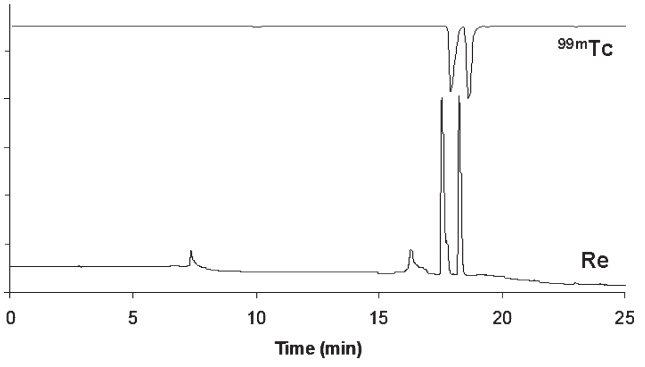


Figure 5. HPLC chromatograms of the reaction of **4** with $M(OH_2)_3(CO)_3^+$, Re (UV 220 nm, bottom), and ^{99m}Tc (NaI, top) to yield *fac*-$[M(CO)_3(N,S,N_{Py}\text{-}\mathbf{4})]^+$, **5a** (1st peak) and **5b** (2nd peak).

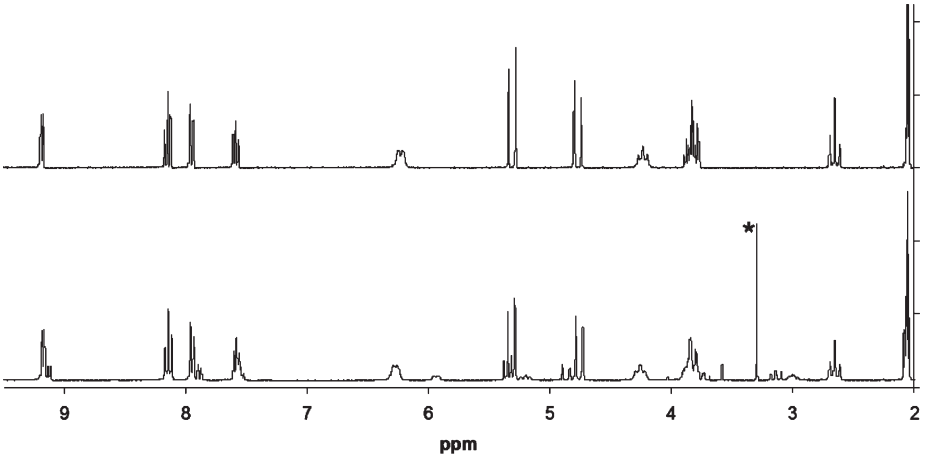


Figure 4. ^1H NMR spectra observed for the major species, **2a**, isolated as crystals (top) and unpurified reaction mixture (bottom) of *fac*-$[Re(CO)_3(N,S,N_{Py}\text{-}\mathbf{1})]^+$, **2a** and **2b**.. *Residual methanol (3.3 ppm)

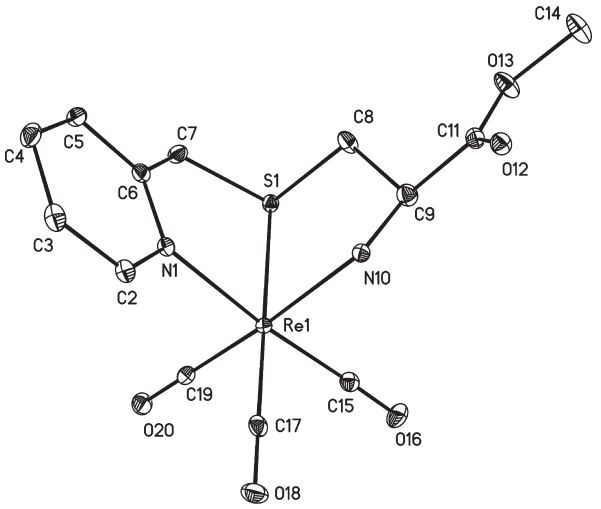


Figure 6. Molecular structure of *fac*-$[Re(CO)_3(N,S,N_{Py}\text{-}\mathbf{4})]^+$, **5a**. Thermal ellipsoids are shown at the 30% probability level. Hydrogen atoms omitted and only the Re cation is shown for clarity.

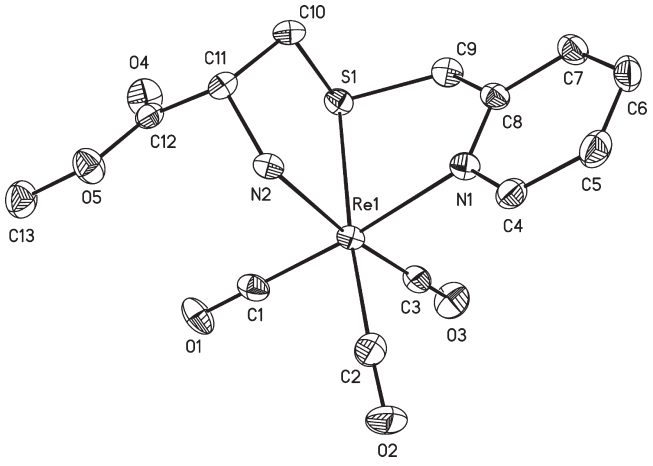


Figure 7. Molecular structure of *fac*-$[Re(CO)_3(NSN_{Py}\text{-}\mathbf{4})]^+$, **5b**. Thermal ellipsoids are shown at the 30% probability level. Hydrogen atoms omitted and only the Re cation is shown for clarity.

ligand **1**. A structural overlay of the two diastereomers (**5a**, **5b**) illustrated the differences in the absolute configurations of **4**, which is believed to have impacted the ^{1}H NMR spectra (Figure 8). In particular, the orientation of the methyl ester within the complex was found to be either pointing away from the molecule (**5a**) or parallel to Re-CO (**5b**), which shifted the H_α and H_β of cysteine into different magnetic environments. Interestingly to note, the reaction pH was critical to the purity and isolation of **5a** and **5b**. Complexation at neutral or slightly basic conditions yielded an additional peak in the chromatogram at 17.6 min. ^{1}H NMR of this sample indicated hydrolysis of the methyl ester and the presence of the N,S,N_{Py} complexes (**2a**, **2b**) as well as (**5a**, **5b**). Conducting the complexation under acidic conditions avoided the issue of methyl ester hydrolysis and permitted the formation of only **5a** and **5b**.

To ensure the correct assessment of the N,S,N_{Py} linear diastereomeric coordination mode of **2b**, the linear S-(pyridin-2-ylmethyl)-Boc-L-cysteine, **6**, ligand that permitted only O,S,N_{Py} coordination of the $M(CO)_3^+$ complex was also investigated. The reaction of *fac*-$Re(OH_2)_3(CO)_3^+$ with **6** did not proceed as expected. A number of low yield

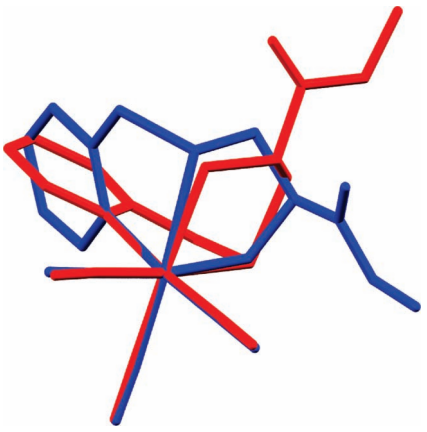
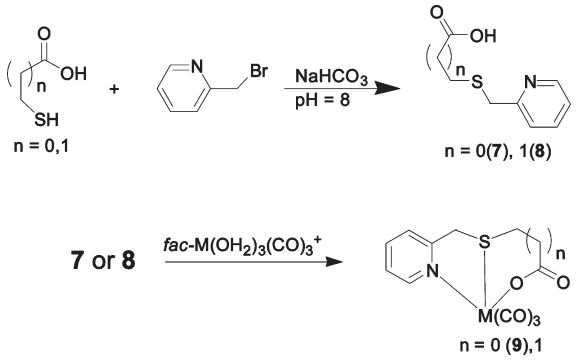


Figure 8. Structural overlay of the L-diasteromers **5a** (red) and **5b** (blue) illustrating ligand orientation of **4** in the complexes. Hydrogen atoms and atom labels omitted for clarity.

Scheme 3. Synthesis of O,S,N_{Py} Coordinating S-(pyridin-2-ylmethyl) Derived Ligands (**7**, **8**) and Subsequent Reactions with $M(OH_2)_3(CO)_3^+$ (M = Re, ^{99m}Tc)



species were observed by HPLC chromatogram with no single predominant species as in reactions with **1** or **4**. This result was unanticipated as the O,S,N_{Py} coordination of **6** would have generated five and six member coordination rings with the rhenium center, well within the expected realm of linear tridentate ligands for $Re(CO)_3^+$. Multiple attempts to isolate any compound from the reaction mixture proved unsuccessful. Additional synthetic approaches, such as adjusting pH (2−9), organic solvents (MeOH, $MeCl_2$), and other rhenium starting materials ($ReBr(CO)_5$, $[Re(CO)_5]^+$), did not yield the desired O,S,N_{Py} coordinated species either.

It was postulated that the steric bulk of the boc group and the proximity of the amine may be affecting the O,S,N_{Py} coordination of the ligand with the $Re(CO)_3^+$ core. Simplified variations eliminating steric bulk and chirality of cystiene, 3-(pyridin-2-ylmethylthio)propanoic acid, **7** and 2-(pyridin-2-ylmethylthio)acetic acid, **8**, were prepared by reacting the respective mercapto carboxylic acid with 2-picolyl chloride to generate the thioether pyridine methyl analogues (Scheme 3). Ligands **7** and **8** were reacted independently with *fac*-$Re(OH_2)_3(CO)_3^+$ to investigate O,S,N_{Py} complex formation (Scheme 3). The reaction of **7** with *fac*-$Re(OH_2)_3(CO)_3^+$ did not yield the expected O,S,N_{Py} complex under all the conditions examined (pH, solvent, and rhenium starting material). The results observed with **7** were similar to the cysteine analogue **6** indicating the steric effect of the boc amine group may not have impacted O,S,

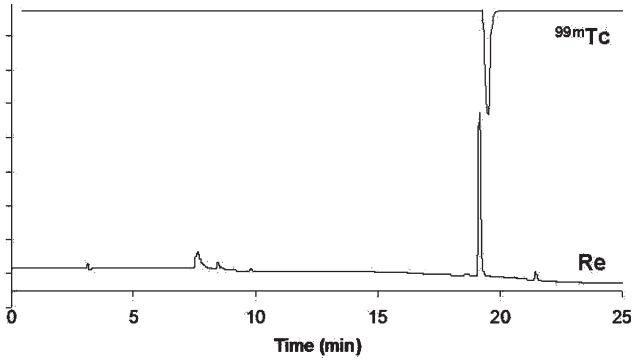


Figure 9. HPLC chromatograms of the reaction of **8** with $M(OH_2)_3(CO)_3^+$, Re (UV 220 nm, bottom) and ^{99m}Tc (NaI, top) to yield *fac*-[$M(CO)_3$(N,S,N_{Py}-**1**)]$^+$, **9**.

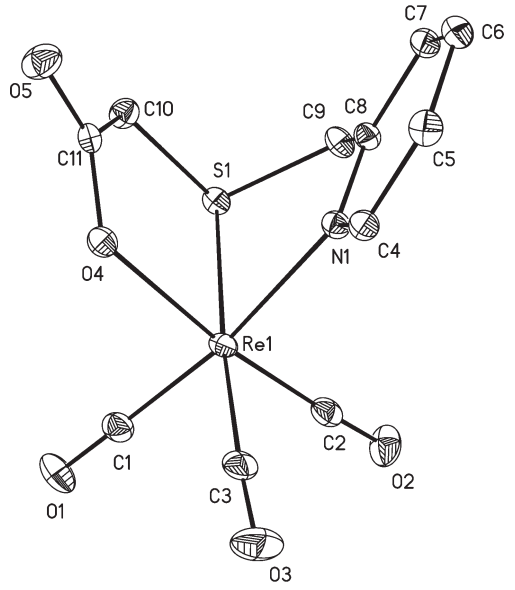


Figure 10. Molecular structure of *fac*-[$Re(CO)_3$(O,S,N_{Py}-**8**)], **9**. Thermal ellipsoids are shown at the 30% probability level and the hydrogen atoms were omitted for clarity.

N_{Py} complex formation and indirectly supporting the formation N,S,N_{Py} diastereomer **2b** from **1**.

Ligand **8** reduced the coordination ring size from six to five by removing one methylene group between the carboxylic acid and the sulfur. Unlike ligands **6** and **7**, the reaction of *fac*-$Re(OH_2)_3(CO)_3^+$ with **8** generated the expected product *fac*-$Re(CO)_3$(O,S,N_{Py}-**8**), **9**, in near quantitative yield as a single product by HPLC at 19.1 min (Figure 9). Single crystals of **9** for X-ray diffraction analysis were obtained by slow evaporation of an acetone solution that is discussed later (Figure 10). The ^1H NMR spectrum of **9** shows the typical chemical shifts of the O,S,N_{Py} ligand coordinated by $Re(CO)_3$. H_a-H_b splitting of the methylene protons, SCH_2CO_2 and SCH_2Py, were observed as doublets (3.60, 3.20 ppm with $J = 17.7$ and 5.12, 4.75 ppm with $J = 17.4$), respectively. In particular, the chemical shifts and coupling constants of SCH_2Py and aromatic pyridine protons in **9** corresponded to those observed with the pyridine coordinated complexes, **2a**, **2b**, **5a**, and **5b**. The change in ring size from six in **7** to five in **8** directly impacted the successful isolation of the O,S,N_{Py} coordinated $Re(CO)_3^+$ complex.

X-ray Structure Analysis. Complexes **2a**, **5a**, **5b**, and **9** were characterized by single crystal X-ray diffraction analysis. The crystallographic experimental data, space group, and structure refinement parameters for the crystals are reported in Tables 1. Selected bond angles and distances for the complexes are found in Table 2. Complexes **2a**, **5a**, **5b**, and **9** were distorted octahedral complexes with the facial orientation of the carbonyl ligands nearly equidistant bond lengths (Re−C 1.90−1.94 Å) and bond angles (86.6−88.6°) indicative of $Re(CO)_3$(L) complexes. In each of the complexes, the ligands (N,S,N_{Py} or O,S, $N_{Py,}$) were coordinated in a linear tridentate fashion around the metal center. Both types of complexes are composed of two slightly distorted five member coordination rings formed along the axis of ligand, which yielded several similarities in the structures. The elongated Re−S (∼2.45 Å) and C−S (1.81−1.83 Å) bonds significantly attributed to this perturbation compared to related Re−N (∼2.2 Å) and C−N or C−C (∼1.5 Å) bonds. The bond angles in the N,S,N_{Py} complexes (**2a**, **5a**, **5b**) between the N_{Py}−Re-S (79.2−80.5°) and N−Re−S (80.8−81.3°) and the bond distances (Re−N (∼2.22 Å), Re−S (∼2.45 Å), Re−N_{Py} (∼2.21 Å)) were generally consistent between each of the structures in terms of bonding of the ligand with the metal center.

Although the structures (**2a**, **5a**, **5b**) had similar bond angles and distances, the absolute configuration of the cysteine ligand yielded two distinct configurations on the metal center. The α-carbon on cysteine provided the impetus for diastereomer formation as it dictated the orientation of the uncoordinated carboxylic acid or methyl ester group. In the first diastereomer, the uncoordinated group (carboxylic acid, **2a** or methyl ester, **5a**) is oriented away from the $Re(CO)_3$ moiety and bent backward toward the coordinated ligand. This feature is more pronounced in **2a** than **5a** because of a hydrogen bond (3.114 Å) of the carbonyl (O5) with the pyridine ring (N4). Interestingly, **5a** crystallized in an achiral space group with the entantomeric pair equally observed in the structure, which mostly likely occurred because of racemization of ligand or complex.[38,46−48] In the second diastereomer **5b**, the methyl ester group of the ligand was orientated along the carbonyl bond of the $Re(CO)_3$ moiety and away from the coordinated ligand. Structural comparison of the ligand orientation of two diastereomers (**5a**, **5b**) is represented in the structural overlay of complexes (Figure 8).

Although ligand **8** has a carboxylate donor instead of an amine, the structure of the O,S,N_{Py} rhenium complex **9** was similar to the N,S,N_{Py} rhenium complexes in bond distances (Re−N_{Py} (2.20 Å), Re−S (2.45 Å)) and ligand conformation. The ligand in **9** appears to be more constrained with smaller coordination ring bond angles (O−Re−S (80.65 Å) and N_{Py}−Re-S (79.51 Å) and O−Re−N_{Py} (78.73 Å)) than the N,S,N_{Py} complexes. The observed differences are most likely due to a shorter Re−O bond (2.14 Å) and the sp^2 hybridization of the carboxylate, but are well within ranges for other thio/thioether complexes with $Re(CO)_3$.[19,26,32,34]

(46) Kovacs, J.; Mayers, G.; Johnson, R.; Cover, T.; Ghatak, U. *J. Org. Chem.* **1970**, *35*, 1810–1815.
(47) Yoshikawa, S.; Saburi, M.; Yamaguchi, M. *Pure Appl. Chem.* **1978**, *50*, 915–921.
(48) Siedler, F.; Weyher, E.; Moroder, L. *J. Pept. Sci.* **1996**, *2*, 271–275.

The metal chelate ring size appears to be the critical factor for the formation of the Re complex because of the longer C−S bonds (~1.8 Å) compared to typical C−C or C−N bond (~1.5 Å) and Re−S (2.45 Å) distances to Re-NH_2 or ReN_{Py} (~2.2 Å). On the basis of the reported data, it is not as surprising that the ligand S-(2-(2′-pyridyl)ethyl)-D,L-homocystiene prefers a tripodal (N,S,O) coordination mode of homocysteine residue with Re-$(CO)_3$, even though pyridine is a potent donor.[32] The impact of the ethyl linker and longer covalent/coordination bonds of the thioether may have limited the participation of pyridine donor favoring the tripodal conformation. Also, the preferential formation of N,S,N_{Py} Re$(CO)_3$ diastereomers reported here over neutral complexes tripodal or linear (O,S,N_{Py}) modes highlights the importance of the donor strength of the pendent ligand. The previous report of S functionalized triazole cysteine Re$(CO)_3$ complexes suggested two different species, tripodal (N,S,O) and a linear (N,S,N_{Tri}), were formed in the absence of X-ray data.[29] Whereas, the S-(pyridin-2-ylmethyl)-L-cysteine Re$(CO)_3^+$ complexes clearly yielded N,S,N_{Py} diastereomers.

^{99m}Tc$(CO)_3^+$ Labeling Studies. To compare to the rhenium complexes, the ligands were also reacted with ^{99m}Tc$(OH_2)_3(CO)_3^+$ to determine radiolabeling yields at different ligand concentrations (10^{-5} and 10^{-6} M) and pHs (5.0 and 7.4) (Table 3). The reaction of **1** with ^{99m}Tc$(OH_2)_3(CO)_3^+$ yielded a single peak at 17.6 min of the product ^{99m}Tc(N,S,N_{Py}-**1**)$(CO)_3^+$, **2a** + **2b**, which correlated with the observed HPLC peak for the rhenium analogue (Figure 2). The labeling results were comparable at >99% at 10^{-5} M for both pH values. At 10^{-6} M, the formation of **2a** was significantly higher at pH 7.4 (86%) over pH 5.0 (44%). The lower yield at pH 5.0 is most likely due to protonation of the amine donor and electrostatic repulsion of the Tc core and the ligand. The reaction of **4** with ^{99m}Tc$(OH_2)_3(CO)_3^+$ yielded the expected products ^{99m}Tc(N,S,N_{Py}-**4**)$(CO)_3^+$, **5a** + **5b**, as two separate peaks of equal intensity (17.7 and 18.4 min) in the HPLC chromatogram in good yields at 10^{-5} and 10^{-6} M (Figure 5). The relative ratio of the two peaks observed at pH 5.0 correlated well with the rhenium complexes. Interestingly, at pH 7.4 the ratio of the two peaks shifted to favor the ~17.7 min peak (~60:40) at both concentrations. The ratio shift is most likely due to hydrolysis of the methyl ester in the ligand **4** as a faint shoulder of the peak on the front end of the 17.7 min peak indicative of the complex observed with ligand **1**. Prolonged heating of the sample at pH 7.4 confirmed hydrolysis as only a single peak at 17.6 min that corresponded to the results observed with ligand **1**.

The O,S,N_{Py} ligands (**6**, **7**, **8**) based on the cysteine backbone were investigated to determine reactivity with ^{99m}Tc$(OH_2)_3(CO)_3^+$. Even though we were unable to isolate the corresponding rhenium complexes, ligands (**6**, **7**) were examined and yielded the proposed compounds ^{99m}Tc(O,S,N_{Py}-**6**)$(CO)_3$ and ^{99m}Tc(O,S,N_{Py}-**7**)$(CO)_3$ in reasonable labeling yields at 10^{-5} M, but declined at 10^{-6} M. As anticipated by the observations of the N,S,N ligand, **4**, the complex of the boc protected cysteine ligand **6** yielded two peaks (17.6, 18.6 min) of unequal intensity (75:25) in the radiochromatogram, whereas ligand **7** had a single peak at 19.4 min with the ^{99m}Tc precursor. It was surprising based on the similar nature of the ligands, the labeling yields with ^{99m}Tc$(OH_2)_3(CO)_3^+$ for the cysteine analogue **6** were markedly superior than the linear analogue **7**. Even though labeling of these ligands was achieved, the exact speciation of the complexes is uncertain without structural conformation of the ^{99}Tc complexes. However, it is reasonable to propose a tridentate or bidentate (S,N_{Py}) complex with ^{99m}Tc-$(CO)_3^+$. The truncated O,S,N_{Py} ligand **8** was also reacted with ^{99m}Tc$(OH_2)_3(CO)_3^+$ to yield a single product ^{99m}Tc-(O,S,N_{Py}-**8**)$(CO)_3$, **9**, at 19.2 min that correlated with the rhenium analogue (Figure 8). The labeling yields of **8** at 10^{-5} M (>95%) and 10^{-6} (69, 52% at pH 5.0, 7.4) were significantly better than **7** as expected because of the smaller coordination ring.

Table 3. Labeling Yields (%) Observed for *fac*-^{99m}Tc$(OH_2)_3(CO)_3^+$ with the Respective Ligands at pH = 5.0 and 7.4

	pH = 5.0		pH = 7.4	
complex	10^{-5} M	10^{-6}	10^{-5}	10^{-6}
^{99m}Tc(N,S,N_{Py}-1)$(CO)_3^+$	>99	44	>99	86
^{99m}Tc(N,S,N_{Py}-4)$(CO)_3^+$	>99[1]	87[1]	>99[1]	87[1]
^{99m}Tc(O,S,N_{Py}-6)$(CO)_3$	92[1]	76[1]	90[1]	68[1]
^{99m}Tc(O,S,N_{Py}-7)$(CO)_3$	72	26	31	0
^{99m}Tc(O,S,N_{Py}-8)$(CO)_3$	>99	69	95	52

[1] Combined yield for the two species observed in the chromatogram.

Conclusions

A series of S-(pyridin-2-ylmethyl)-L-cysteine ligands were prepared and investigated with *fac*-M$(CO)_3^+$ (M = Re, ^{99m}Tc) to determine the preferred coordination mode, either tripodal (O,N,S) or linear (O,S,N_{Py}, N,S,N_{Py}). S-(pyridin-2-ylmethyl)-L-cysteine, **1**, was found to preferentially favor only the linear N,S,N_{Py} coordination mode among the three possibilities. The methyl ester protected version, **4**, helped elucidate the presence of N,S,N_{Py} diastereomers observed with **1** by limiting the coordination to a single mode. Independent structural characterization of the diastereomers (**5a**, **5b**) formed with **4** provided key insight into understanding the effect of ligand orientation on the metal center to the ^{1}H NMR spectra observed. The boc protected version, **6**, also provided indirect supporting evidence as the O,S,N_{Py} ligands were ineffective at complexing Re$(CO)_3^+$ under conditions examined. Only upon reducing the coordination ring size could a Re(O,S,N_{Py}-**8**)$(CO)_3$ product be observed in good yield.

^{99m}Tc$(CO)_3^+$ labeling studies with the S-(pyridin-2-ylmethyl)-L-cysteine ligands were effective at reasonable concentrations (10^{-5}−10^{-6} M) and correlated with the N,S,N_{Py} rhenium complexes, especially with the two diastereomer peaks observed with **4**. Surprisingly, ^{99m}Tc$(CO)_3^+$ complexed the O,S,N_{Py} ligands at 10^{-5} M illustrating a noteworthy difference between Re and ^{99m}Tc results, where further investigation with ^{99}Tc is needed. Overall, S-(pyridin-2-ylmethyl)-L-cysteine ligands have excellent potential with the N,S,N_{Py} coordination mode for M$(CO)_3^+$ (M = Re, ^{99m}Tc) applications with small molecules, peptides, and other targeting biomolecules.

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Supporting Information Available: Complete X-ray structural information for **2a**, **5a**, **5b**, and **9** is available as a CIF file, and ^{1}H NMR spectra of selected complexes as a PDF. This material is available free of charge via the Internet at http://pubs.acs.org.

"Click" Labeling Strategy for $M(CO)_3$ (M= Re, ^{99m}Tc) Prostate Cancer Targeted Flutamide Agents

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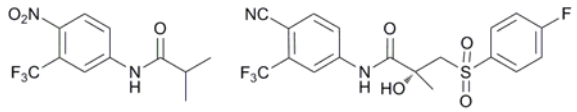
Two distinct "click" chemistry labeling approaches were investigated with dipyridylamine alkyne derivatives and $M(CO)_3^+$ (M= Re, ^{99m}Tc). The triazole ring was found uncoordinated and was incorporated into the preparation of a crossover androgen receptor targeting inhibitor for prostate cancer.



Used in over 90% of clinical diagnostic imaging scans, technetium-99m (γ= 140 KeV, $t_{1/2}$= 6.0 hr) has a long history as a Single Photon Emission Computed Tomography (SPECT) radionuclide. A number of labeling strategies (ligands and complexes) have been proposed over the years that have primarly consisted of mid-valent coordination complexes.[1] A unique organometallic alternative, $[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$ has provided a smaller molecular volume and weight species to improve biological activity.[2-5] Several $[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$ labeling strategies (tridentate, 2+1, monodentate) have been identified.[6-10] However, thermodynamic limitations of complexation still require high temperatures (~90 °C) and large ligand concentrations ($\geq 10^{-6}$ M) for effective yields, even with the most promising tridentate ligand systems (i.e., cysteine, dipyridylamine, histidine, iminodiacetic acid).[6, 11, 12]

To improve radiolabeling efficiency, new labeling strategies for $[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$ utilizing fast and efficient chemical reactions are being examined to develop an alternative to current labeling conditions. The catalytic, Huisgen [3+2] cyclolization, or "click" reaction of an azide and an alkyne, has provided an excellent platform for coupling two molecules for a variety of applications (nanoparticles, polymers, biomolecules).[13] Sutcliff *et al* pioneered the use of "click" chemistry in radiopharaceutical applications with ^{18}F that has led to an explosion of applications with this isotope in the literature.[14, 15] Whereas, investigations of "click" chemistry with radiometals have been limited; mostly likely due to competition of the copper catalyst for complexation.

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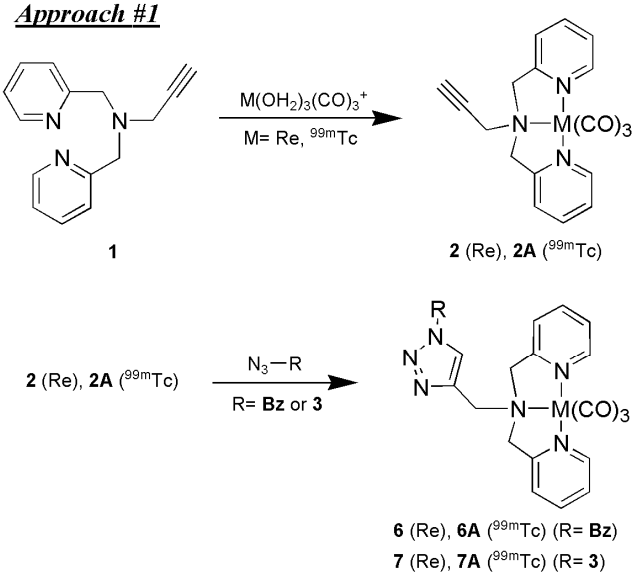
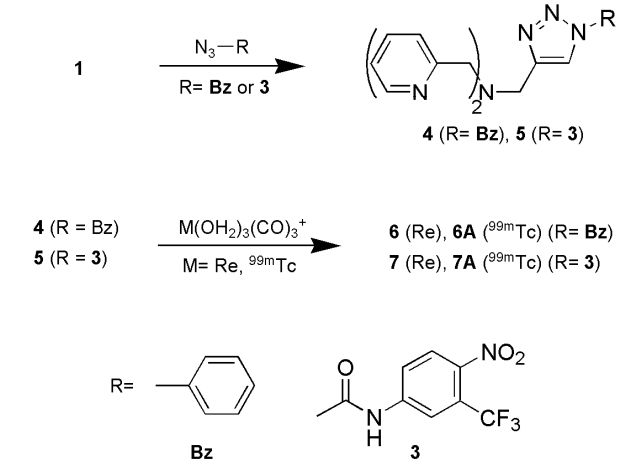
Approach #1



Approach #2



Recently, "click to chealate" used the "click" reaction to generate a combitorial series of ligands, where the triazole ring coordinates to $^{99m}Tc^{I}(CO)_3$.[16, 17] However, this approach still utilizes strong radiolabeling conditions and does not take advantage of the "click" chemistry for coupling of radiometals to biotargeting agents, particularly temperature sensitive molecules.

Interest in adapting "click" chemistry for prostate cancer diagnostic imaging, has led us to investigate functionalized versions of non-steroidal antagonists (i.e., Flutamide,

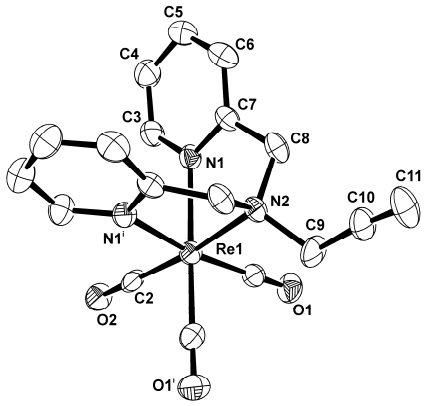


Fig. 1 Molecular structure of *fac*-$[Re(CO)_3(\mathbf{1})]^+$, **2**. Thermal ellipsoids are shown at the 30% probability level. Hydrogen atoms are omitted for clarity.

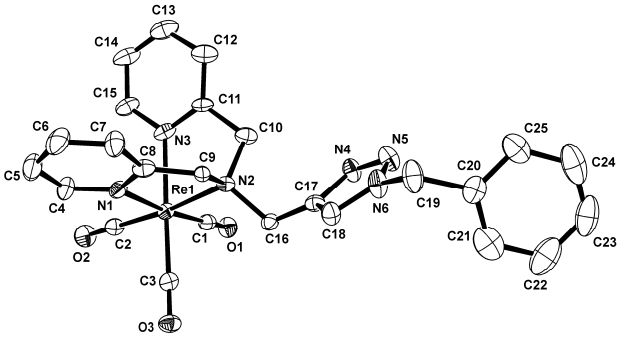


Fig. 2 Molecular structure of *fac*-$[Re(CO)_3(\mathbf{4})]^+$, **6**. Thermal ellipsoids are shown at the 30% probability level. Hydrogen atoms are omitted for clarity.

Bicalutamide) for targeting the androgen receptor (AR) overexpressed in early stage prostate cancer.[18] Previously, we reported $^{99m}Tc^{I}(CO)_3$ Flutamide derivatives that showed AR affinity.[19] To improve AR activity, we proposed to utilize "click" chemistry to generate a crossover molecule by incorporating a second aromatic group into the targeting molecule mimicing Bicalutamide and increasing the distance between the inhibitor core and the $^{99m}Tc^{I}(CO)_3$ complex, while developing a facile method to couple the two molecules under mild conditions.

Two general "click" chemistry stratagies were employed to determine the most effective route of complexation (Scheme 1). Approach #1 involves the complexation of the ligand with $M(CO)_3^+$ followed by a second "click" reaction. Approach #2 involves "clicking" the two molecules followed by complexation with $M(CO)_3^+$. Several distinct advantages can be predicted with approach #1 over #2: separate optimization of radiolabeling without degradation of the biomolecule, limit $M(CO)_3^+$ to a single coordination mode, minimize deactiviation of the copper catalyst through complexation. In both approaches, dipyridylamine (dpa) was utilized to achieve high labeling efficiency and specificity for $M(CO)_3^+$. The alkyne functionalized dpa ligand, **1**, was prepared by alkylation of the secondary amine with propargylbromide.[20]

In approach #1, the dpa alkyne, **1,** was reacted with $[Re^{I}(CO)_3(OH_2)_3]^+$ in equal concentrations and heated at 70 °C for 2 hours. The product, *fac*-$[Re(CO)_3(\mathbf{1})]^+$, **2**, precipitated out of solution as a colorless solid (37%) and was characterized by normal analytical methods and HPLC (Rt 19.0 min). In the ^{1}H NMR of **2**, the pyridyl moieties shift down field to 8.86, 7.95, 7.60, 7.38 ppm and the magnetically unequivalent methylene protons were shifted down field as an AB quartet centered at 4.75 ppm (J_{AB} = 12 Hz) corresponding to literature values.[20]. The X-ray structure confirmed the dpa coordination of $Re(CO)_3$ core in **2** and had similar bond angles and distances to other dpa complexes (Fig. 1). The "click" reaction of **2** was carried out with standard Sharpless conditions first using the model, benzyl azide to probe the conditions and complex speciation. The reaction of **2** with benzylazide proceeded smoothly and in a reasonable time frame (90 min. r.t.) to produce *fac*-$[Re(CO)_3(\mathbf{4})]^+$, **6**, in good yield (84%). As expected in approach #1, the triazole ligand was found uncoordinated to the $Re(CO)_3$ core in both the X-ray structure and the ^{1}H NMR (Fig. 2). Upon triazole formation, the pyridyl resonances shifted slightly upfield to 8.85, 7.89, 7.49 and 7.34 ppm and included a singlet at 8.32 ppm corresponding to the 1,2,3-triazole backbone proton as well as benzyl (5.68 ppm) and triazole (4.98 ppm) methylene singlets. No rearrangement of the $Re(CO)_3$ coordination environment from the dpa to triazole was observed during the course of the reaction. Several biologically relevent temperatures (25, 37, 50, 70 °C) and time points (15, 45, 90 min.) were also examined to probe efficiency of the triazole formation in **6**. At 70 °C, triazole formation was completed in 15 min., while at room temperature it required 90 min. to reach completion.

In approach #2, the assembled ligand was prepared by "clicking" ligand **1** with an azide under standard Sharpless conditions prior to complexation. The "clicked" benzyl azide ligand, **4**, was reacted with $[Re^{I}(CO)_3(OH_2)_3]^+$. Interestingly, the reaction of **4** with $[Re^{I}(CO)_3(OH_2)_3]^+$ at 70 °C for 2 hours produced a colorless solid (74%) that corresponded to the dpa coordinated product *fac*-$[Re^{I}(CO)_3(\mathbf{4})]^+$, **6**, prepared in #1.

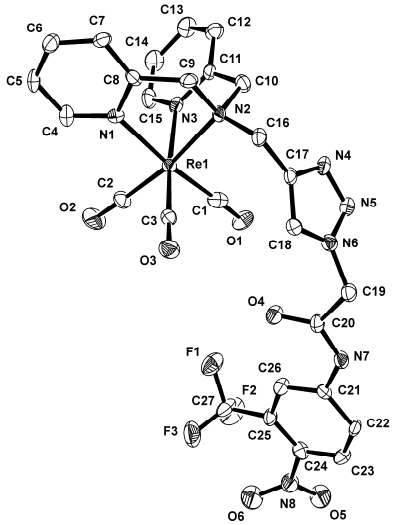


Fig. 3. Molecular structure of *fac*-$[Re(CO)_3(\mathbf{5})]^+$, **7**. Thermal ellipsoids are shown at the 30% probability level. Hydrogen atoms are omitted for clarity.

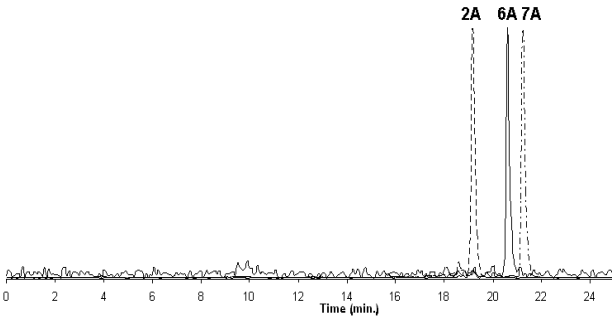


Fig. 4. Normalized Radio (γ) HPLC traces of $^{99m}Tc(CO)_3$(**1**), **2A**, $^{99m}Tc(CO)_3$(**4**), **6A**, and $^{99m}Tc(CO)_3$(**5**), **7A**.

HPLC analysis of the reaction mixture indicated a single peak (Rt 20.2 min.). Reaction progression followed by ^{1}H NMR showed the presence of the free ligand and the dpa coordinated complex. Attempts to generate the triazole coordinated complex or shift the coordination geometry of the system by extended heating or initial cooling of the sample did not pertabate the $Re^I(CO)_3$ dpa coordination in **6**.

Approaches #1 & #2 were also investigated with the flutamide analog, either the azide, **3**, or the dpa "clicked" ligand, **5**. In both approaches, the reactions yielded the same product, *fac*-$[Re^I(CO)_3(\mathbf{5})]^+$, **7**. ^{1}H NMR and X-ray structure analysis confirmed $Re^I(CO)_3$ dpa coordination and the uncoordinated triazole ligand (Fig. 3).

Radiolabeling studies with $[^{99m}Tc^I(CO)_3(OH_2)_3]^+$ were conducted to compare the results obseverd with $Re^I(CO)_3$ and to evaluate the efficiency of approaches #1 and #2 in the benzyl and flutamide systems. Direct labeling of the dpa ligands, both the alkyne (**1**) for approach #1 and "clicked" ligand (**4**, **5**) in approach #2 with $[^{99m}Tc^I(CO)_3(OH_2)_3]^+$ was carried out at 70 °C for 60 min. The direct formation of $^{99m}Tc(CO)_3$(**1**), **2A**, $^{99m}Tc(CO)_3$(**4**), **6A**, and $^{99m}Tc(CO)_3$(**5**), **7A**, showed excellent labeling yields at 10^{-5}-10^{-6} M as expected for dpa systems. In particular, "pre-clicked" ligands **4** and **5** yielded single peaks in the radio (γ) HPLC corresponding to the rhenium analogs. Investigation the "click" reaction in approach #1 using purified $^{99m}Tc(CO)_3$(**1**), **2A**, was carried at several biologically relevant temperatures (25, 37, 50, 70 °C) and azide concentrations (Table 1). The "click" reaction proceeds to completion at all temperatures examined at 10^{-4}-10^{-5} M benzylazide in 15 min. However, at 10^{-6} M benzylazide, incomplete reaction yields were observed at 70 °C and yields declined as the reaction temperature decreased. The reaction of **2A** with the Flutamide azide yielded similar radiolabling yields.

Table 1. "Clicked" triazole formation yields of $^{99m}Tc(CO)_3$(**4**), **6A**, from $^{99m}Tc(CO)_3$(**1**), **2A**, with benzyl azide (10^{-4} to 10^{-6} M) and temperatures (°C) in a 15 min. reaction time.

	10^{-4}	10^{-5}	10^{-6} (M)
70 °C	100 100		75
50 °C	100 100		61
37 °C	100 100		23
25 °C	100 93		23

In conclusion, we have successfully compared two approaches using "click" chemistry with a dpa alkyne ligand, azides, and $[M(CO)_3]^+$. In both cases, the dpa ligand exclusively favored coordination to the metal over the triazole demonstrating the power of click chemistry to provide a specific coordination mode and permit the incorporation of the triazole into the structural design of targeting molecules. Furthermore, the "chelate then click" approach demonstrates the incredible promise of fast efficient room temperature labeling of $M(CO)_3$ (M= Re. ^{99m}Tc) that is particularly relevant to temperature sensitive biomolecules.

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Notes and references

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