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Radiopharmaceuticals: the Application of Technetium-99m and Rhenium Complexes

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Radiopharmaceuticals: the Application of Technetium-99m and Rhenium Complexes

A Capstone Project Submitted in Partial Fulfillment of the Requirements of the Renée Crown University Honors Program at Syracuse University

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Abstract

Nuclear imaging used in diagnostic medicine requires the use of radiopharmaceuticals to make biological areas visible under a gamma camera. Although much success has been found in the use of technetium based imaging agents, their corresponding rhenium complexes can provide insight into the chemical properties of these radiopharmaceuticals without the potentially damaging effects of radiation. Technetium and rhenium complexes utilize a bifunctional chelator to act as a linker between biological vectors and the metal, improving the coordination between the two. Ligands containing thiazole rings have been successfully coordinated to technetium or rhenium tricarbonyl complexes, although it is uncertain whether coordination occurs through the nitrogen or the sulfur of the thiazole ring. Imaging studies of isomers of these compounds have extended understanding of the functioning of these compounds by providing insight into the chemistry of their coordination.

This project involves the study of [$Re(CO)_3$-1,1-bisthiazolate-(1,4)-diaminobutane] as a surrogate for the technetium based complex. The precursor to this complex, N_1,N_1-bis(thiazol-2, 4, or 5-ylmethyl)butane-1,4-diamine has been successfully synthesized using thiazole ring containing isomers, thiazole-2-carboxaldehyde, thiazole-4-carboxaldehyde, and thiazole-5-carboxaldehyde. Reverse-phase high performance liquid chromatography and characterization through ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR) and Electrospray Ionization-Mass Spectrometry (ESI-MS) have been completed to purify and confirm the presence of the desired products.

HPLC chromatograms for N_1,N_1-bis(thiazol-2,4, or 5-ylmethyl)butane-1,4-diamine synthesized with thiazole-2-carboxaldehyde, thiazole-4-carboxaldehyde, or thiazole-5-carboxaldehyde give singular peaks indicating significant ligand purity and relatively poor yield. Following purification, it was determined that the solvent was best removed by lyophilization to minimize the deterioration of the product. ^{1}H-NMR and ESI-MS results confirm the presence of the product, indicating that the desired ligands have been successfully synthesized.

Additional research requires more extensive characterization of the synthesized ligands before the synthesis of the final product using these ligands. Previous research indicates that this product has a high potential for use as fluorescent surrogates to the corresponding technetium complexes. Fluorescence tests on the rhenium complexes should provide insight on the nature of the coordination of rhenium to the chelate and biological vectors. Extensive *in vitro* and *in vivo* studies will require completion before these complexes can be used in a clinical setting.

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Executive Summary

Nuclear imaging techniques, such as single photon emission computed tomography (SPECT) and positron emission tomography (PET), used in diagnostic medicine, require the use of radiopharmaceuticals (radioactive pharmaceuticals) to make biological areas visible. These radiopharmaceuticals are used as tracers that tag the biological areas, making them visible under a camera that detects the gamma rays emitted from the radiopharmaceuticals. Because radioactive substances are damaging to cells and biological tissues, the ideal imaging agent selectively targets the area to be imaged and is effective in that it is removed efficiently from the body to minimize harm. A number of radiopharmaceuticals are based on the radioactive transition metal, technetium. This metal has a half-life of six hours, which gives sufficient time for preparation, cellular absorption, imaging, and elimination of agent.

Although much success has been found in the use of technetium based imaging agents, their corresponding rhenium complexes can provide insight into the chemical properties of these radiopharmaceuticals without the potentially damaging effects of radiation. Rhenium is not radioactive, but emits fluorescent light, which eliminates the cell damage caused by radioactive substances while still allowing the imaging of cells. The chemical and photochemical properties of rhenium are comparable to those of technetium, making it a valid fluorescent surrogate for the radioactive metal.

Technetium and rhenium imaging agents utilize a bifunctional chelator to act as a linker between biological vectors (proteins) and the metal (technetium or rhenium), improving the coordination between the two. Bifunctional refers to the two places for binding, one for the biological vector and the other for the metal, on the chelate, which is simply an ion or molecule that binds metal ions.

Ligands are molecules that bind to a central metal atom to form a coordination complex. Thiazole ring containing ligands include a nitrogen and a sulfur atom in the ring and have been successfully coordinated to technetium or rhenium tricarbonyl ($(CO)_3$) complexes. However, it is uncertain whether the connection to the metal occurs through the nitrogen or the sulfur of the thiazole ring. Imaging studies of isomers, structures with the same chemical formula but different attachments between atoms, of these compounds containing thiazole rings have extended understanding of the functioning of bifunctional chelates by providing insight into the chemistry of their coordination.

This project involves the study of [$Re(CO)_3$-1,1-bisthiazole-(1,4)-diaminobutane] as a fluorescent surrogate for the technetium based complex. This complex contains a tricarbonyl core bound to rhenium, which is also bound to a thiazole ligand. The precursor to this complex, N_1,N_1-bis(thiazol-2, 4, or 5-ylmethyl)butane-1,4-diamine has been successfully synthesized using thiazole ring containing isomers, thiazole-2-carboxaldehyde, thiazole-4-carboxaldehyde, and thiazole-5-carboxaldehyde, which vary in the placement of the nitrogen and

sulfur atoms in the thiazole ring. Reverse-phase high performance liquid chromatography (HPLC) and characterization through ^{1}H-NMR and (ESI-MS) have been completed to purify and confirm the presence of the desired products.

HPLC separates the components of a mixture by passing them through a column filled with absorbent material, in this case, a silica resin. Because of their different chemical properties, specifically polarity and hydrophobicity, each component of the mixture is absorbed at different times, affecting when they flow out of the column. In polar compounds, the electrons on atoms are shared unequally, creating slight positive and negative charges where the electrons are given or taken. Polar molecules tend to be hydrophilic and are attracted to water, while nonpolar molecules are hydrophobic and are repelled by water. Hydrophilic molecules are attracted to the column and are left stationary for longer periods than hydrophobic molecules that are repelled by the column and pass through easily at a quicker rate. The components first appearing in the chromatogram are more hydrophobic and nonpolar than those appearing later.

HPLC produces chromatograms, which display a series of peaks that vary in time and integration. Each peak corresponds to a different component of the mixture with the integration of the peak related to the amount of the component present in the mixture. The larger the integration of the peak, the more pure a component is. HPLC chromatograms for N_1,N_1-bis(thiazol-2,4, or 5-ylmethyl)butane-1,4-diamine synthesized with thiazole-2-carboxaldehyde,

thiazole-4-carboxaldehyde, or thiazole-5-carboxaldehyde give singular peaks with significant integration, indicating significant ligand purity of reasonable yield.

Following purification, it was determined that the solvent was best removed by lyophilization to minimize the deterioration of the product. Heating caused by removal of the solvent in a vacuum deteriorated the product. Lyophilization freeze-dries the product, eliminating the loss of product to heat.

^{1}H-NMR studies interactions between the nuclei of hydrogens as described by their magnetic properties. ^{1}H-NMR spectra display peaks of varying integrations and chemical shifts. The integrations of the peaks correspond to the number of identical hydrogens that the peak represents, while chemical shift describes the surrounding environment of the hydrogens giving information about the number and types of neighboring hydrogens. Chemical shift is most affected functional groups nearest to the hydrogens. These groups adjust the electron density of the hydrogen, either donating or withdrawing electrons. Donating groups tend to increase shielding, while withdrawing groups decrease shielding. Shielding refers to when a magnetic field is induced around the hydrogen in opposition to the magnetic field applied to the sample. This effect decreases the chemical shift, moving the peak upfield on the spectra. The opposite is observed for deshielding. The trends in chemical shifts are used to interpret ^{1}H-NMR spectra and confirm the presence of product.

ESI-MS measures the mass of ion fragments from a molecule. Certain fragments are commonly observed, making the determination of the molecular mass straightforward. ESI-MS results gave an m/z peak at 283 for N_1,N_1-bis(thiazol-2-ylmethyl)butane-1,4-diamine. The m/z peak represents the mass of the compound plus one with the one most likely coming from positively charged hydrogen, resulting from the ionization of the sample. The mass of N_1,N_1-bis(thiazol-2-ylmethyl)butane-1,4-diamine is 282, indicating that the desired ligands have been successfully synthesized.

Additional research on these rhenium compounds requires more extensive characterization of the synthesized ligands before the synthesis of the final product using these ligands. These additional characterization methods would confirm the presence of products by focusing on the examination of their various chemical properties. Previous research indicates that the overall product, [$Re(CO)_3$-1,1-bisthiazolate-(1,4)-diaminobutane], has a high potential for use as a fluorescent surrogate to the corresponding technetium complexes. Tests examining the fluorescence of the rhenium complexes synthesized using the different ligands should provide insight on the nature of the coordination of rhenium to the chelate and eventually, to cells. Extensive studies in cells both outside and inside the body will require completion before these complexes can be used in a clinical setting.

Acknowledgements

I would like to thank Professor Jon Zubieta for the opportunity to pursue this project under his supervision. Thank you to Nick Azzarelli and Kelly Henry for their unfaltering guidance and insight throughout the entire Capstone process. Also, thank you to Eric Holzwarth, my Honors advisor.

Chapter 1: Introduction

Background

Within the medical field, the detection and diagnosis of disease often requires the use of imaging and scanning through diagnostic nuclear imaging techniques to provide rapid, noninvasive results. The use of these methods promotes early detection and treatment, improving the prognosis of a disease before it progresses to later stages, and ultimately lowering the cost to the patient. In targeting particular areas, treatments can be specified to the required areas, minimizing side effects and complications.[1]

Medicine utilizes two main imaging techniques, SPECT) and PET.[1] Although both techniques function similarly, utilizing gamma cameras to detect gamma rays emitted from radioactive isotopes injected into the patient, they each have their own advantages and disadvantages.[1] While PET offers higher resolution images, SPECT utilizes more readily available, longer-lived, and cheaper radioactive isotopes, including ^{123}I, ^{111}In, and ^{99m}Tc.[1-5] The ideal imaging agent is effective and selective and ideally improves staging, prognosis, and post-therapy monitoring. Of the commonly used isotopes, ^{99m}Tc is often preferred.[1-5]

Technetium-99m is an isomer of the radioactive transition metal, technetium, and has successfully been used as a radioactive tracer for medical imaging.[6] It emits gamma rays at an ideal energy, 140-keV, for use with the

gamma cameras utilized in SPECT and PET imaging.[1] Additionally, ^{99m}Tc has a half-life of six hours, making it an ideal candidate for use in medical imaging as this length allows sufficient time for on-site preparation and accumulation in the target tissue while reducing the amount of radiation exposure to the patient.[6] The low radiation dose provided by ^{99m}Tc also causes low amounts of tissue damage compared to other isotopes.[7] ^{99m}Tc can be prepared in on-site generators in radiopharmacies in the form of a pertechnetate, $Na^{99m}TcO_4$, with high specific activity, ideal for its use as a molecular imaging agent.[8]

Unfortunately, because ^{99m}Tc is a transition metal, it produces unstable products when substituted in a targeting vector since it cannot be directly substituted for a hydrogen atom.[9] The coordination of technetium to suitable ligands has offered a solution to this problem and has allowed the successful incorporation of technetium into complexes used as molecular imaging probes.[1,6,7,10]

Technetium radiopharmaceuticals are divided into two categories based on the method of technetium incorporation: technetium-essential or technetium-tagged compounds.[7] In technetium-essential compounds, technetium is incorporated into the targeting vector, directly contributing to the structure and overall physicochemical properties of the molecule.[11] Examples include those shown in figures 1 and 2, which are used in heart imaging and brain imaging respectively.[12,13]

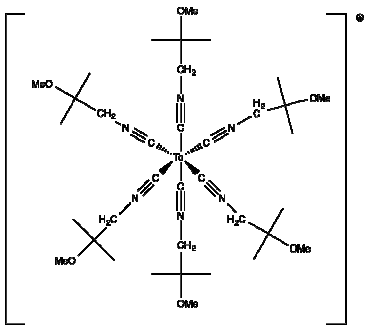


Figure 1. The chemical structure of the technetium-essential heart-imaging agent, Cardiolite.[12]

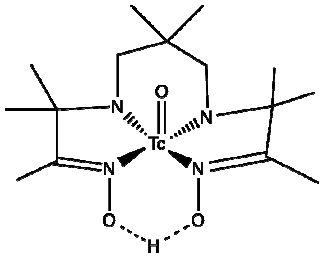


Figure 2. The chemical structure of the technetium-essential brain-imaging agent, [TcO(hexamethylpropylene amine oxide)].[13]

These complexes are useful in the targeting of phagocytosis, hepatocyte clearance, glomerular filtration, bone sorption, and other high capacity systems.[1] Distribution in the body is dictated by blood flow and the complexes tend to have low molecular weights.[7]

Technetium-tagged complexes involve the carrying of the technetium atom by the target vector, indicating that the functioning of the biological target does not depend on the incorporation of the technetium atom.[1,7,11] Biodistribution of this class targets low capacity systems that rely on specific enzymatic or

receptor binding interactions.[7] The technetium atom is incorporated through integration or conjugation.[1,11] Integration allows the retention of binding affinity through the replacement of a receptor ligand with a technetium chelator.[1,11] Conjugation tethers a ^{99m}Tc-moiety to a molecule that exhibits high affinity binding to a receptor.[1,11] In this method, single amino acid chelates are often used to link radioactive metal cations to a biologically active molecule (see figure 3).[1,7,11]

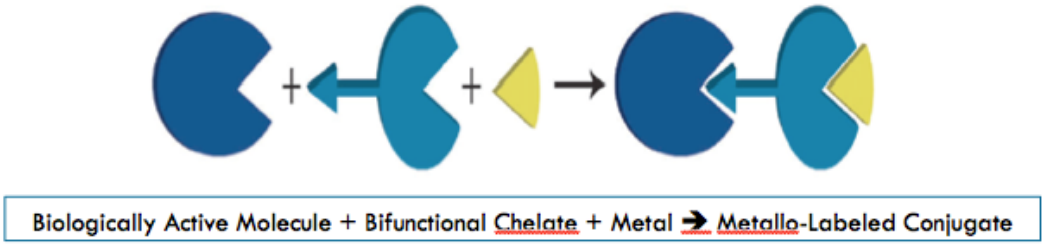


Figure 3. A depiction of how a bifunctional chelate attaches to a radionuclide (metal, technetium-99m) and a biological vector.[1]

Because it is a transition metal, ^{99m}Tc produces an unstable compound upon direct substitution into a target vector, complicating its labeling chemistry, and limiting its usefulness.[1,7,10] Fortunately, the use of single amino acid chelates eliminates this complication. The chelator serves as a connection between the radioactive metal and the biological vector by binding to ^{99m}Tc at one site and binding to the target vector at a separate site.[1,7,10] The chelate also securely binds the metal radionuclide to prevent leakage *in vivo*, while retaining a functional site for connection to the target vector.[1] A $\{Tc(CO)_3\}^+$ core binds to a bifunctional chelate for incorporation into peptide-based targeting vectors.[7,9] The addition of

the bifunctional chelate to the radionuclide is essential as it prevents dissociation of the complex by securely binding to a *fac*-metal core and maintaining the integrity of the biomolecular structure.[1,7,9] The coordination chemistry of this radioactive ^{99m}Tc tricarbonyl core has been further studied using its non-radioactive congener, rhenium.

As an alternative, rhenium makes possible the synthesis of a metallo-labeled conjugate while limiting the presence of radioactive isotopes.[1-15] Rhenium exhibits fluorescence, allowing study of the chelate system to occur at subcellular level before testing in living subjects.[1,6] Additionally, combining this rhenium-based core to a bisthiazole ligand helps produce a stable octahedral complex by facially chelating the Re-*fac* tricarbonyl core through its nitrogen in the amine and thiazole rings, to the biological vectors.[14,15] This method of chelation increases the stability of the compound through σ and π donating from the amine and thiazole rings and allows for the ease of conjugation to the biological vectors.[1] The nitrogen atom donates electrons that promote the formation of the appropriate geometry, while the amine nitrogen provides the electrons that allow the link to the biological vector (a peptide).[1,14]

The thiazole ring allows coordination to isomers through the donation of electrons offered by sulfur or nitrogen. Coordination through thiazole nitrogen is more common as this is a better donor than sulfur. Computational calculations indicate, however, that coordination through the sulfur of one or both thiazole

rings to rhenium does produce a stable structure. Changes in coordination affect the chemical properties of these complexes including their stability and luminescent properties. Further exploration of the nature of the coordination occurring in rhenium based metallo-labeled conjugates can expand understanding on the functioning of these complexes and have the potential to produce a complex with favorable fluorescent properties. As nonradioactive surrogates, the structure and understanding of the properties of these rhenium complexes can then be applied to further understand and study their radioactive technetium counterparts.[14]

Ligand Variation

There has been previous success synthesizing [$Re(CO)_3$-1,1-bisthiazolate-(1,4)-diaminobutane] (P1) using thiazole-4-carboxaldehyde (T4) (figures 4 and 5 respectively).[14] This project successfully utilized isomers of thiazole-4-carboxaldehyde, thiazole-2-carboxaldehyde (T2) and thiazole-5-carboxaldehyde (T5), to synthesize the precursor, N_1,N_1-bis(thiazol-4-ylmethyl)butane-1,4-diamine (L4) (figures 6 though 8 respectively). Synthesized with T2 or T5, this precursor becomes N_1,N_1-bis(thiazol-2-ylmethyl)butane-1,4-diamine (L2) or N_1,N_1-bis(thiazol-5-ylmethyl)butane-1,4-diamine (L5).

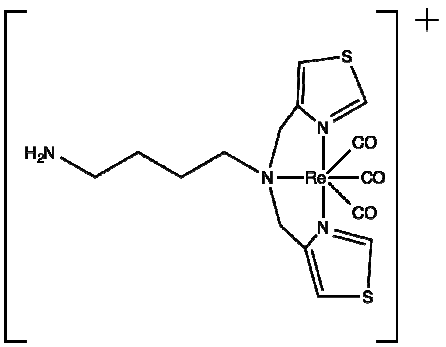


Figure 4. The structure of [$Re(CO)_3$-1,1-bisthiazolate-(1,4)-diaminobutane] (P1).

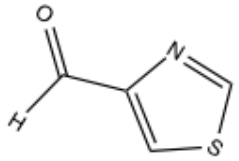


Figure 5. The structure of thiazole-4-carboxaldehyde (T4).

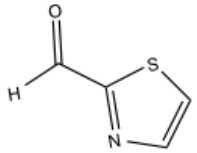


Figure 6. The structure of thiazole-2-carboxaldehyde (T2).

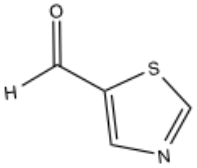


Figure 7. The structure of thiazole-5-carboxaldehyde (T5).

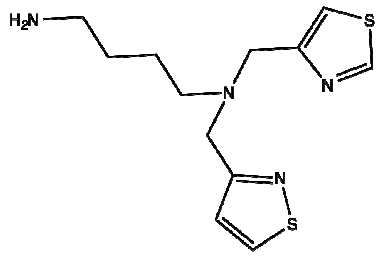
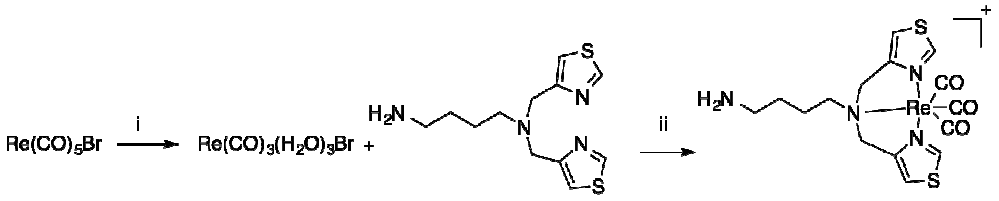


Figure 8. The structure of N_1,N_1-bis(thiazol-4-ylmethyl)butane-1,4-diamine (L4). Synthesis of this compound with T2 or T5 adjusts the placement of the nitrogen and sulfur on the thiazole ring.

The expectation is that rhenium complexes synthesized with these isomers will provide greater insight into the chemistry of the coordination of rhenium complexes and that the synthesized compound will exhibit greater fluorescence. Based on previous research, it is strongly suggested that these rhenium complexes can indeed served as fluorescent, nonradioactive surrogates of technetium based complexes for future use in nuclear imaging.[14]

Chapter 2: Method

Completion of this project rests on the synthesis of P1 using T2, T4, and T5 (see scheme 1).

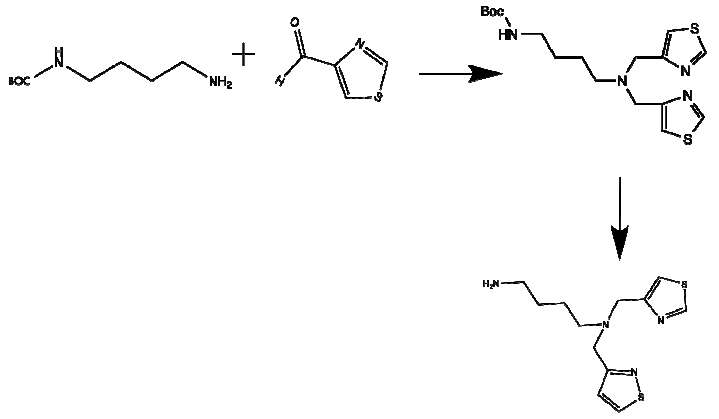


Scheme 1. Synthesis of P1.

This synthesis requires a three-part reaction, the first of which has been successfully synthesized the ligand, L2, L4, and L5, using the respective isomers of thiazole-carboxaldehyde. The ligands were synthesized using T2, T4, and T5 according to the methods described below.

Synthesis of N_1,N_1-bis(thiazol-2, 4, or 5-ylmethyl)butane-1,4-diamine (L2, L4, or L5)

According to the process described by Henry et al., synthesis of the rhenium complex, P1, begins with the initial formation of the ligand, 1,1-bisthiazolate-(1,4)-diaminobutane.[14] The synthetic scheme of this ligand can be viewed in Scheme 2.



Scheme 2. Synthesis of 1,1-bisthiazole-(1,4)-diaminobutane ligand involving the combination of N-BOC-1,4-butanediamine and thiazole-4-carboxaldehyde to give 1,1-bisthiazolate-1,4-diaminobutane after the removal of the BOC protecting group.

This synthesis began by mixing *N*-Boc-1,4-butanediamine (300 mg, 1.5 mmol) and T2, T4, or T5 (360 mg, 3.0 mmol) in dichloroethane (DCE) under nitrogen gas at room temperature. After 30 minutes, sodium triacetoxyborohydride (954 mg, 3.0 mmol) was added to the mixture with additional DCE and was stirred for 16 hours. Then, the solvent was removed in vacuo to give an oily product that was mustard yellow to dark amber in color. The product was dissolved in a mixture of 10% methanol and 10% trifluoracetic acid in water and was stirred for 3 hours. The product was a light yellow liquid. From there, the ligand, 1,1-bisthiazolate-(1,4)-diaminobutane, was purified using reverse-phase HPLC with a gradient of 100% 0.1% TFA in water increased to 20% acetonitrile (MeCN) over 5 minutes, and increased to 40% MeCN over 2.5

minutes, and increased to 100% MeCN over 2.5 minutes. HPLC purification resulted in a clear liquid.

Chapter 3: Characterization Results

The products synthesized at each step were characterized using various methods. Nuclear magnetic resonance (NMR) spectra were obtained using a Bruker Advance DPX 300 MHz spectrometer. High Performance Liquid Chromatography chromatograms were obtained using an Agilent 1200 reverse-phase HPLC instrument with a manual injector and automated fraction collector. Electrospray ionization-mass spectra (ESI-MS) were obtained using a Shimadzu 2000 Electrospray Ionization Mass Spectrometer. Additional characterization methods to be performed in future endeavors are listed in Chapter 4: Anticipated Characterization**.**

^{1}H-Nuclear Magnetic Resonance (NMR) of Crude Ligands

The ^{1}H-Nuclear Magnetic Resonance spectra of L2 and L5 were obtained before the samples were purified by HPLC. The spectra correlate to figures 9 and 10 respectively.

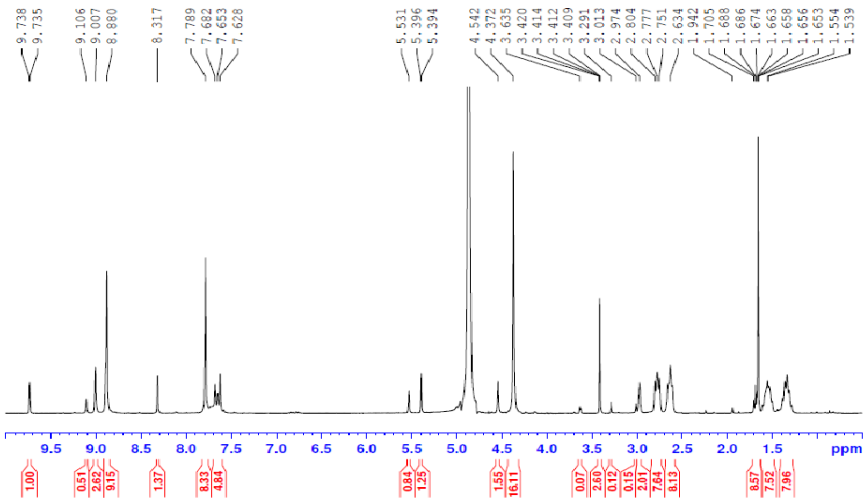


Figure 9. ^{1}H-NMR spectra of crude of L2. The NH_2 peak appears at approximately 4.8 ppm with impurities from methanol and trifluoroacetic acid at approximately 3.5 ppm and 8.8 ppm respectively.

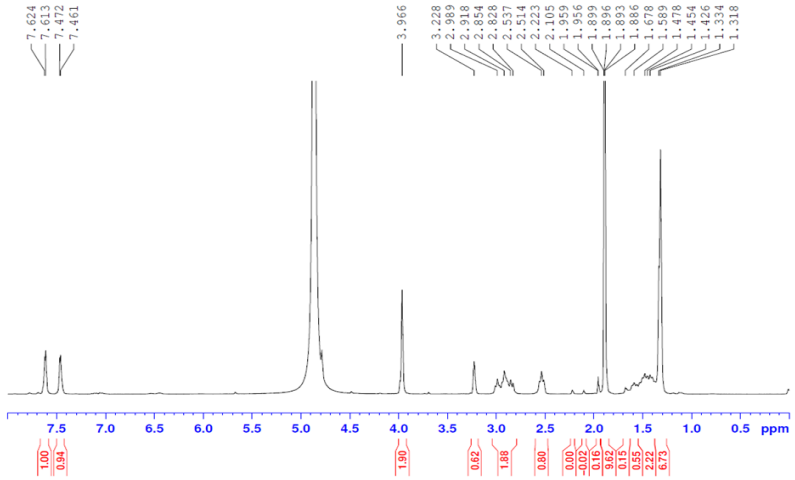


Figure 10. ^{1}H-NMR spectra of crude L5. The NH_2 peak appears at approximately 4.8 ppm, while impurities from methanol appear at approximately 3.3 ppm.

Solubility Tests

Solubility tests were performed on the crude L2 and L5 to determine an ideal method for purification. Water, methanol, acetonitrile, toluene, acetone, chloroform, pyridine, pentane, dichloromethane (DCM), hexane, ethyl acetate, butanol, tetrahydrofuran (THF), 2-propanol, and diethyl ether were tested. Both ligands exhibited solubility in only water, methanol, acetone, pyridine, and ethyl acetate, and neither were soluble in acetonitrile. There does not appear to be a correlation between the solubility of the ligands and solvent polarity. The results may be viewed in chart 1.

Solvent	Crude L2	Crude L5
H_2O	Soluble	Soluble
Methanol	Soluble	Soluble
Acetonitrile	Insoluble	Insoluble
Toluene	Insoluble	Insoluble
Acetone	Soluble	Soluble
Chloroform	Insoluble	Insoluble
Pyridine	Soluble	Soluble
Pentane	Insoluble	Insoluble
Dichloromethane	Insoluble	Insoluble
Hexane	Insoluble	Insoluble
Ethyl Acetate	Soluble	Soluble
Butanol	Insoluble	Insoluble
THF	Insoluble	Insoluble
2-propanol	Insoluble	Insoluble
Diethyl Ether	Insoluble	Insoluble

Chart 1. The results of solubility tests performed on crude L2 and L5 using a variety of solvents.

Thin Layer Chromatography (TLC)

Thin Layer Chromatography was performed on the crude L2 and L5 ligands, N-Boc-butane-1,4-diamine, T2, and T5 to explore the possibility of performing normal phase-HPLC. All samples were dissolved in methanol and eluted with acetone to promote clear separation on the TLC plates. Two R_f values were calculated for the ligand sample at 0 and approximately 0.778, with the value at 0 corresponding to the ligand. The spots at 0.778 indicate a synthetic impurity, as would be expected for the crude ligands. Based on the R_f values for T2 and T5, it can be concluded that the spots at 0.778 are not due to remaining starting material. Because the lack of mobility displayed by the ligand, normal-phase HPLC was eliminated as a possible purification method. The R_f values calculated for each sample can be viewed in chart 2.

Compound	R_f values
L2	0 and 0.786
L5	0 and 0.773
N-Boc-butane-1,4-diamine	0.702
T2	0.869
T5	0.940

Chart 2. R_f values calculated for samples dissolved in methanol and eluted with acetone.

Reverse Phase High-Performance Liquid Chromatography (HPLC)

Reverse Phase High-Performance Liquid Chromatography was performed to purify L2, L4, and L5 according to the procedures outlined above in the Synthesis of N_1,N_1-bis(thiazol-2, 4, or 5-ylmethyl)butane-1,4-diamine (L2, L4,

L5) section of Chapter 2: Method. The HPLC chromatograms for the experimentally synthesized and purified ligands can be viewed in figures 11, 12, and 13 respectively. L2 gives a singular peak at 8.779 minutes, indicating significant ligand purity. L4 shows peaks at 4.534 and 5.738 minutes, indicating the significant presence of two components. Based on the intensity of the peaks, the peak at 5.738 minutes corresponds to pure L4. L5 shows the presence of several components, with the peak at 5.675 minutes having the greatest intensity and corresponding to pure L5.

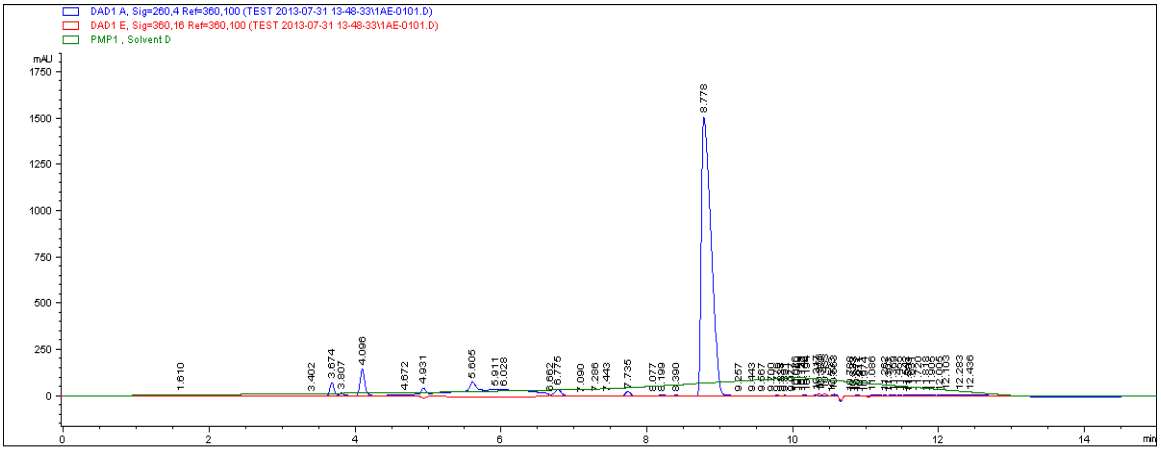


Figure 11. HPLC chromatogram obtained for the purification of L2. HPLC was performed with the parameters of 100% 0.10% trifluoroacetic acid in water and 0% methanol to 75% methanol over ten minutes and then increased to 100% methanol over two minutes.

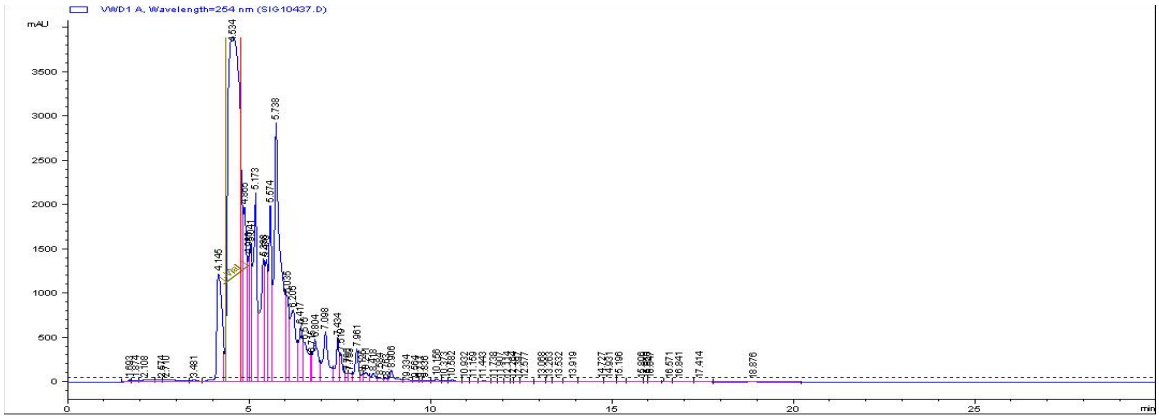


Figure 12. HPLC chromatogram obtained for the purification of L4. HPLC was performed with the parameters of 100% 0.10% trifluoroacetic acid in water and 0% acetonitrile to 20% acetonitrile over 7.5 minutes and then increased to 40% acetonitrile over 2.5 minutes, and then to 100% acetonitrile over 2.5 minutes with a two minutes hold.

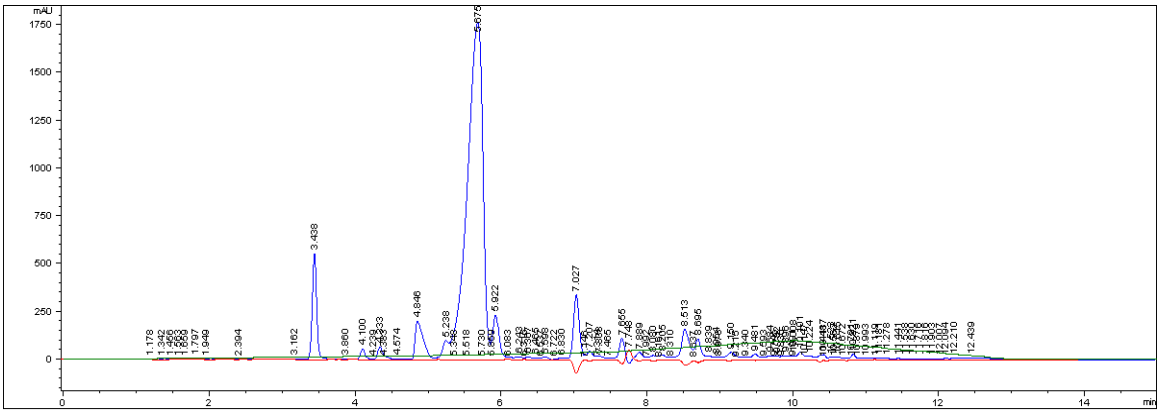


Figure 13. HPLC chromatogram obtained for the purification of L5. HPLC was performed with the parameters of 100% 0.10% trifluoroacetic acid in water to 20% methanol over five minutes with a two minute hold, and then increased to 40% methanol over 2.5 minutes, and finally to 100% methanol over 2.5 minutes.

^{1}H-NMR of Pure Ligands

^{1}H-NMR results showed that the desired produce was being deteriorated from the heat produced in the process of solvent removal in vacuo (see figure 14). To prevent deterioration, lyophilization was used instead. The freezing methods utilized in this process eliminate the heat causing the deterioration of product and is a less damaging way of removing solvent. This method also eliminates the bumping observed in in vacuo solvent removal processes, maximizing the amount of product obtained. The spectra for L2 and L4 following lyophilization can be seen in figures 15 and 16 respectively. L5 appears to be less stable and showed a lack of product in its ^{1}H-NMR spectra.

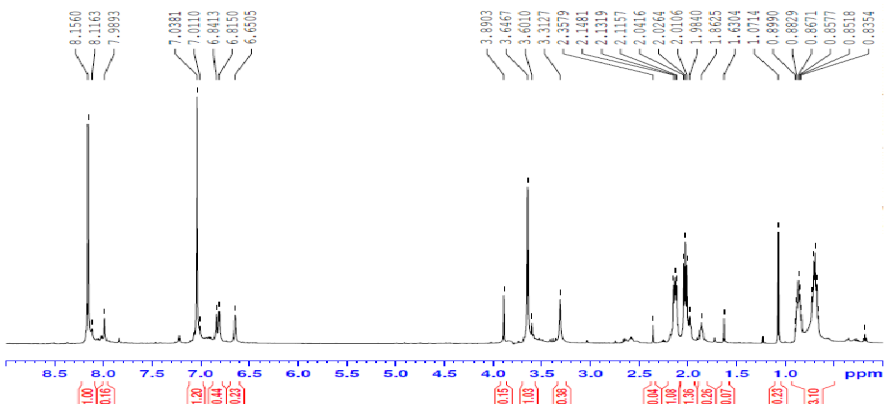


Figure 14. ^{1}H-NMR spectra of L2 following the removal of solvent in vacuo. The spectrum indicates product deterioration due to the heat produced by this solvent removal method. There is a lack of NH_2 peak in the 4 to 5 ppm range, indicating the lack of product. The peak at 3.6 ppm corresponds to methanol.

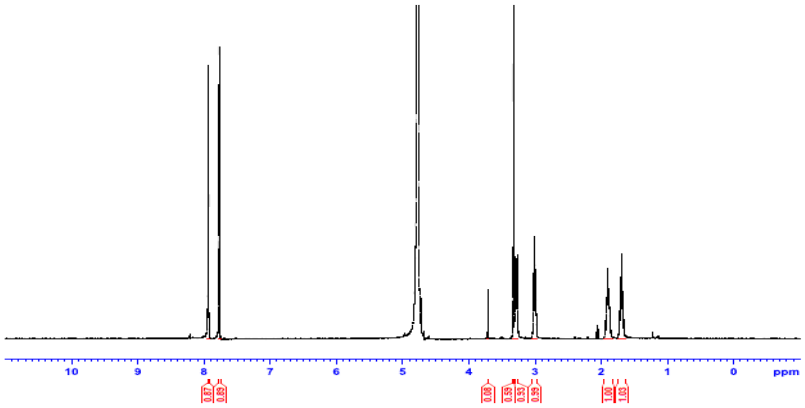


Figure 15. ^{1}H-NMR spectrum of L2 following the removal of solvent through lyophilization. The spectrum shows minimal product deterioration and a slight impurity attributed to methanol. The peak at 5.8 ppm is attributed to NH_2 and confirms the presence of the L2. The methanol impurity is observed at 3.3 ppm.

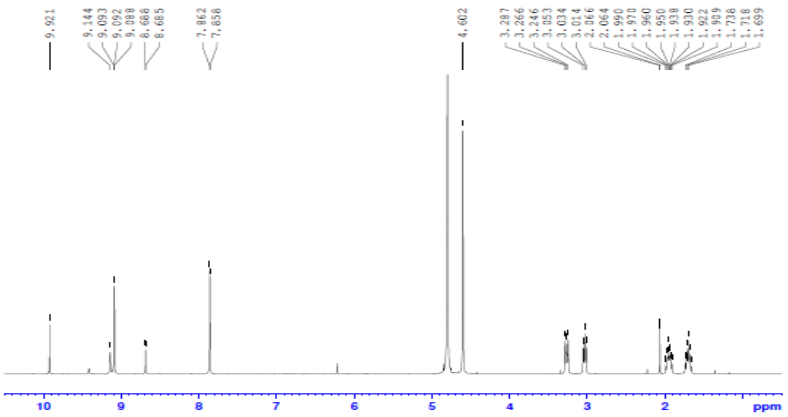


Figure 16. ^{1}H-NMR spectrum L5 following the removal of solvent through lyophilization. The spectrum indicates minimal product deterioration. The NH_2 peaks of L5 appear at 4.8 ppm and the peak at 3.4 ppm can be attributed to a slight impurity from methanol.

Electrospray Ionization-Mass Spectrometry (ESI-MS)

Electrospray Ionization-Mass Spectroscopy (ESI-MS) of L2 indicates an m/z peak at 283 and may be seen in figure 16. The m/z peak appears where expected as L2 has a molecular weight of 282 grams, confirming the presence of the product.

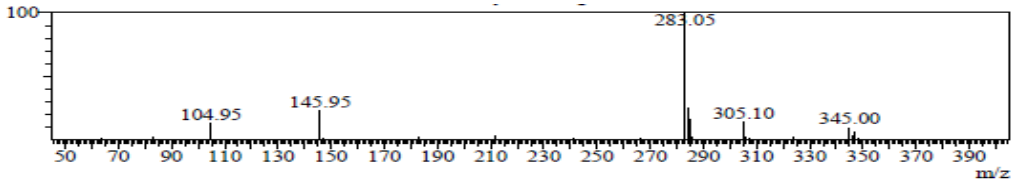


Figure 17. ESI-MS of L2 indicating an m/z peak at 283.

Crystallization Studies

Crude samples of the ligands synthesized with each isomer and $[Re(H_2O)_3(CO)_3]^+Br^-$ and were refluxed at temperatures greater than 65°C for 3 hours in methanol. A variety of slow evaporation and solvent diffusion techniques were tested. Samples were dissolved in methanol or dichloromethane and allowed to evaporate slowly. Diffusion techniques included the use of methanol/ether and acetonitrile/isopropyl alcohol. Unfortunately, the lack of crystal products suggests that the ligands need additional purification due to their oily nature.

Chapter 4: Future Research

Based on previous research results, P1 synthesized using L2, L4, and L5 show high potential for their use as fluorescent surrogates for the corresponding technetium complexes.[14] Before the final product can be synthesized however, the purified ligands require additional characterization. The final product will also require purification and extensive characterization, including fluorescence tests, to provide insight on the nature of the coordination to the chelate and biological vectors. Beyond this, extensive clinical studies need to be completed before the information gained can be applied to radioactive technetium complexes for practical use as nuclear imaging agents.

Further Synthesis

Continuation of this project first requires the synthesis of the final product, [$Re(CO)_3$-1,1-bisthiazolate-(1,4)-diaminobutane] (P1), using the ligands synthesized using T2, T4, and T5 by the methods described by Henry et al.[14] This procedure entails the synthesis of $Re(H_2O)_3(CO)_3$, which is then coupled with the ligand, L2, L4, or L5, to create the final product. Additional research needs to be done on the purification method to find a better way of purifying the ligands by minimizing the deterioration and maximizing product yield.

Additional Characterization

Characterization of the products created at each step of the synthesis (thiazole ligands (L2, L4, and L5), rhenium compound, and P1 synthesized with L2, L4, and L5) requires further attention. ^{1}H-NMR and mass spectrometry in the form of ESI-MS and/or Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI ToF MS) would be useful in confirming the presence of synthesized compounds. Electronic Absorption Spectroscopy (EAS) would also be useful in exploring the nature of the synthesized compounds.

Fluorescence studies are essential to the progress of this project. These studies would determine if the isomers show the same unusual photophysical properties exhibited by P1 as explored by Henry et al. Emission and excitation spectra should be examined.[14]

In vitro Studies

Before *in vivo* testing, these complexes require testing in *in vitro* samples. These studies would provide insight into protein motion and other subcellular processes. The use of $\{Re(CO)_3\}^+$ as a surrogate for $\{^{99m}Tc(CO)_3\}^+$ minimizes the variation in physicochemical properties between fluorescence and radioactive imaging tests.[14] Valliant et al. have found success applying rhenium tricarbonyl chelates to neural stem cells, indicating that this would be an ideal subject for *in vitro* testing.[1]

Human Subjects

As this goal of this project is to develop an imaging agent for imaging in humans, the agents would need to be tested in human subjects before being used on a wider scale in a clinical setting. β-amyloid plaques, leukocytes, carbohydrates, biotin, folate, and vitamin B12 have been successfully imaged using ^{99m}Tc chelates, suggesting the promise of ^{99m}Tc tricarbonyl chelates synthesized with a thiazole ring.[1]

Chapter 5: In Conclusion

Diagnostic medicine requires the use of radioactive imaging agents to view the biological vectors, with ^{99m}Tc based imaging agents commonly preferred. $\{^{99m}Tc(CO)_3\}^+$ is coordinated to single amino acid chelates to promote the stability of the complex. These chelates serve as a link between a biological vector and the radioactive metal. Rhenium is a congener of ^{99m}Tc and allows creation of fluorescent complexes that can be used to study the properties of the Tc-based complexes without the harmful effects of radiation. The study of rhenium complexes can also provide additional insight into the coordination of metal tricarbonyls to bifunctional chelators. Rhenium complexes were coordinated to thiazole ring containing compounds to explore coordination and fluorescence properties. The two thiazole ring system allows coordination through nitrogen, sulfur, or both. The use of isomers of thiazole ring compounds provides insight into the various coordination methods and, while fluorescence can provide information regarding the photophysical properties of the rhenium based complexes, and ultimately, of their technetium counterparts.

N_1,N_1-bis(thiazol-2-ylmethyl)butane-1,4-diamine, N_1,N_1-bis(thiazol-4,-ylmethyl)butane-1,4-diamine, and N_1,N_1-bis(thiazol-5-ylmethyl)butane-1,4-diamine were synthesized and characterized as precursors to [$Re(CO)_3$-1,1-bisthiazolate-(1,4)-diaminobutane]. Characterization through HPLC, ^{1}H-NMR, and ESI-MS indicate successful synthesis of desired products of reasonable

purity. Additional characterization by EAS, MALDI-ToF MS, and fluorescence studies are required to gain further information about the properties of the synthesized ligands and explore their value in imaging.

Completion of this project requires the synthesis of the final product, [$Re(CO)_3$-1,1-bisthiazolate-(1,4)-diaminobutane], using each of the varied isomers. Following characterization of the product, *in vitro* and *in vivo* studies can be performed to explore the photophysical properties of the complexes. Ideally, an effective, selective imaging agent will be discovered and be applied to radioactive technetium complexes for clinical use in nuclear imaging.

Works Cited

1. Valliant, John, Zubieta, Jon, et al. “Single Amino Acid Chelates (SAAC): A Strategy for the Design of Technetium and Rhenium Radiopharmaceuticals.” *Chemical Communications* 5 (2009): 493-512.
2. Alberto, R. and Abram, U. “Technetium and rhenium – coordination chemistry and nuclear medical applications.” *Journal of the Brazilian Chemical Society* 17 (2006): 1486-1500.
3. Liu, S. and Edwards, D. “ ^{99m}Tc Labeling of Highly Potent Small Peptides.” *Bioconjugate Chemistry* 8 (1997): 621-636.
4. Hom, R. K. and Katzenellenbogen, J. A. “Technetium-99m-labeled receptor-specific small-molecule radiopharmaceuticals: recent developments and encouraging results.” *Nuclear Medicine and Biology* 24 (1997): 485-498.
5. Eckelman, W. C. “Radiolabeling with technetium-99m to study high-capacity and low-capacity biochemical systems.” *European Journal of Nuclear Medicine* 22 (1995): 249-263.
6. Zeglis, B. M., et al. “Underscoring the Influence of Inorganic Chemistry on Nuclear Imaging with Radiometals.” *Inorganic Chemistry* 53 (2014): 1880-1899.
7. Maresca, Kevin P., et al. “Comprehensive Radiolabeling, Stability, and Tissue Distribution Studies of Technetium-99m Single Amino Acid Chelates (SAAC).” *Bioconjugate Chemistry* 20 (2009): 1625-1633.

8. Alberto, R., et al. “A Novel Organometallic Aqua Complex of Technetium for the Labeling of Biomolecules: Synthesis of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ from $[^{99m}TcO_4]^-$ in Aqueous Solutions and Its Reaction with a Bifunctional Ligand.” *Journal of the American Chemical Society* 120 (1998): 7987-7988.
9. Papagiannopoulou, D., et al. “Rhenium(I) and technetium(I) *fac*-$M(NSO)(CO)_3$ (M = Re, ^{99m}Tc) tricarbonyl complexes, with a tridentate NSO bifunctional agent: Synthesis, structural characterization, and radiochemistry.” *Polyhedron* 29 (2010): 876-880.
10. Banerjee, Sangeeta R., et al. “Direct Reductive Alkylation of Amino Acids: Synthesis of Bifunctional Chelates for Nuclear Imaging.” *Synthesis* 11 (2004): 1759-1766.
11. Bartholoma, Mark D., et al. “Technetium and Gallium Derived Radiopharmaceuticals: Comparing and Contrasting the Chemistry of Two Important Radiometals for the Molecular Imaging Era.” *Chemical Reviews* 110 (2010): 2903-2920.
12. Pasqualini, R., et al. “Synthesis and characterization of the new neutral myocardial imaging agent $[^{99m}TcN(Hnoet)_2]$ (Hnoet = N-ethyl-N-ethoxydithiocarbamato).” *Chemical Communications* 18 (1992): 1354-1355.
13. Leonard, J.P., Nowotnik, D.P., and Neirinckx, R.D. “Technetium-99m-d, 1-HM-PAO: a new radiopharmaceutical for imaging regional brain

perfusion using SPECT – a comparison with iodine-123 HIPDM." *Journal of Nuclear Medicine* 27 (1986): 1819-1823.

14. Henry, Kelly E., et al. "Emission Wavelength Variation with Changes in Excitation in a Re(I)bisthiazole Ligand Complex that Breaks the Kasha-Vavilov Rule." *Chemical Science* 4 (2013): 2490-2495.
15. Machura, B., et al. "Tricarbonyl rhenium(I) complex of benzothiazole – Synthesis, spectroscopic characterization, X-ray crystal structure and DFT calculations." *Journal of Organometallic Chemistry* 724 (2013): 82-87.