Bis-Arene Complexes: A New Direction in ^{99m}Tc Radiopharmaceuticals

Bis-Arene Complexes: A New Direction in ^{99m}Tc Radiopharmaceuticals

By FIKRICAN DILEK, M.Sc

A Thesis Submitted to the School of Graduate Studies in Partial Fulfillment of the Requirements for the Degree Master of Science

McMaster University MASTER OF SCIENCE (2023) Hamilton, Ontario (Chemistry)

TITLE: Bis-Arene Complexes: A New Direction in ^{99m}Tc Radiopharmaceuticals

AUTHOR: Fikrican Dilek, M.Sc (McMaster University)

SUPERVISOR: Associate Professor, Saman Sadeghi

PAGES: xv, 50

Abstract

Technetium-99m (^{99m}Tc) is one of the most promising radionuclides for SPECT imaging. Multidentate chelators are commonly used to label Tc-99m. The use of a chelator requires derivatization of a targeting molecule with a chelating group followed by the addition of the Tc-99m radionuclide. Tc-99m radiolabeling without using traditional multidentate chelators was shown about 30 years ago with the synthesis of ^{99m}Tc bis-arenes, following Fischer-Hafner reaction conditions. However, the preparation of ^{99m}Tc bis-arenes by this method requires a lengthy and complicated process and was incompatible with functional groups containing oxygen or nitrogen. In recent years, new reaction conditions have been found to successfully prepare ^{99m}Tc bis-arene complexes in a convenient and relatively simple procedure that can be utilized with highly functionalized arenes.

In the first part of the thesis, a new method for Tc-99m radiolabeling of L-Dopa was proposed. This new method was called Sandwich Technetium and involved single-step labeling without the need for any chelators. In addition to radiolabeling experiments, *in vitro* and *in vivo* assays were also performed. Due to the highly hydrophilic character of $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, new L-DOPA derivatives were synthesized to increase the hydrophobicity of ligands and subsequently, the hydrophobicity of the Tc-99m radiolabeled compounds. In the future, hydrophobic L-DOPA derivatives Tc-99m labeling can be prepared in one step.

The second part of the thesis focuses on preparation of ^{99m}Tc bis-arenes using $[^{99m}Tc(\eta^6\text{-naphth})_2]^+$ as a synthon. This two-step reaction procedure is proposed for more complex bis-arene compounds that cannot be achieved in one step. In this part, $[^{99m}Tc(\eta^6$-

naphth)$_2$]$^+$, and [Re(η^6-naphth)$_2$]$^+$ were synthesized with the aim to substitute naphthalene in [^{99m}Tc(η^6-naphth)$_2$]$^+$ with a ligand of interest. This new method may be a new way for Tc-99m compounds, which could not be synthesized in a single step before.

Acknowledgements

First of all, I would like to express my appreciation to Dr. Saman Sadeghi for giving me the great opportunity to carry out my graduate study in his research laboratory and for his encouragement, patience, endless attention, and scientific guidance throughout this study.

Many thanks to Afaf Genady, Mohamed El-Zaria and Daniel Hernández Valdés who always helped me and answered all my questions in the lab.

I would like to thank my labmates, Yusra Marfatia, Rowan Swann, Rojin Falah, Shirley Wang, and for their invaluable help and kind mess in the laboratory.

Besides, I would like to thank all my friends of me in IYTE especially, Yasemin Bilgi, Murat Delman, Meltem Kutluer, Müge Bilgen, and Özge Tuncel for their unfailing encouragement, and never-ending friendship and enjoyable company.

I would like to thank my Turkish friends Dicle Mutlu, Kursat Sozer, Beyza Hatun Kiziltepe and Busra Atar at McMaster University for their support and friendship.

Finally, I am also grateful to my parents, my cousin, and my sister for their endless support, motivation, patience, and limitless love. I know without their support and confidence would never have been able to achieve what I have.

Dedication

I would like to dedicate this thesis to all of my friends and family that have sat and listened to me even when I'm at my saddest over the past 3 years. I would not have made it through without all of your support in those moments and I am forever grateful for all of you.

Table of Contents

List of Figures

List of Table

Chapter 1: Introduction

1.1 Molecular Imaging

Molecular imaging (MI) is a commonly used technique for the non-invasive observation of biochemical markers and processes, for the detection of specific diseases, such as cancer. MI can be used not only for diagnosis but also, to monitor the progression and effectiveness of disease treatment.[1] There are various MI techniques, each with their own strengths and limitations (Table 1.1).[1] Single photon emission computed tomography (SPECT) and positron emission tomography (PET) are nuclear medicine imaging techniques that, unlike CT and MRI, provide metabolic and functional information.Although different biomolecular imaging strategies can be utilized to assess different biological phenomena, it is vital to utilize imaging methods that are appropriate for each reason.[2]

Table 1.1 Summary of the strengths and limitations of various MI techniques.[1,3,4]

Imaging Modality	**Strengths**	**Limitations**
PET	- Unlimited depth penetration - Whole body imaging - Quantitative molecular imaging - High Sensitivity	- Radiation exposure - Expensive - Low spatial resolution - Long acquisition time
SPECT	- Unlimited depth penetration - Whole body imaging - High Sensitivity - Quantitative - Theranostic option	- Radiation exposure - Low spatial resolution - Long acquisition time

Table 1.1 (*cont.*)

Imaging Modality	Strengths	Limitations
Optical imaging	- No ionizing irradiation - Real-time imaging/short acquisition time - Relatively high spatial resolution - **Inexpensive** - High Sensitivity	- Limited depth penetration - Whole body imaging not currently accessible
Ultrasound	- High spatial resolution - No ionizing irradiation - Real-time imaging - Inexpensive	- Limited depth of penetration - Whole body imaging not possible
Photoacoustic imaging	- High spatial resolution - No ionizing irradiation - **Inexpensive** - Quantitation possible - High Sensitivity - Greater depth of penetration vs optical imaging	- Limited depth of penetration vs nuclear methods - Whole body imaging not possible

1.1.1 SPECT

SPECT is a sensitive and widely used molecular imaging technique. It involves injecting a radioactive compound into patients, after which, gamma rays are emitted, generating SPECT images.[2] The medical radioisotopes emit gamma photons that pass through a collimator and are detected by detectors such as thallium doped sodium iodide crystal classic scintillators. The detectors will emit light which will be intensified using photomultipliers, an in turn analyzed and counted as electrical pulses. A scintillation camera

will obtain multiple 3D images from various angles, creating a 3D reconstructed image showing the distribution of the radioactive compound in the body.

Commonly used radioisotopes for SPECT imaging are technetium-99m (^{99m}Tc), iodine-123, xenon-133, thallium-201, and indium-111 (see Table 1.2).^{99m}Tc remains the radionuclide of choice for clinical SPECT imaging because of its favorable physical properties (t1/2 = 6 h, Eγ = 140 keV), low cost, and widespread availability from ^{99}Mo/^{99m}Tc generators.[4]

Table 1.2 Commonly used medical isotopes for SPECT and key nuclear properties.[5]

	$t_{1/2}$	E_{max}
^{99m}Tc	6.02 h	141 keV
^{123}I	13.0 h	529 keV
^{111}In	67.2 h	245 keV

1.1.1.1 Historical Development of Technetium Chemistry

C. Perrier and Nobel laureate E. Segrè discovered and characterized technetium, a man-made element, in 1937.[6] In the early 1950s, isolation of ^{99}Tc from the workup of spent nuclear fuels led to the publication of the first reports on technetium chemistry on a weighable scale.[7-9] Technetium-99 is a pure beta emitter (βmax = 294 keV) and its β^- radiation is easily absorbed in glassware. Technetium was largely ignored as it was viewed as an "exotic" artificial element. This changed when the ^{99}Mo/^{99m}Tc generator and the extremely advantageous properties of the metastable nuclide ^{99m}Tc for use in nuclear medicine were discovered by Brookhaven National Laboratory.[10] A scientific development in technetium chemistry was encouraged by the recognition of the importance and

convenience of ^{99m}Tc for nuclear medicine.[10-11] . From the 1970s to 1990s, technetium coordination compounds and organometallic complexes were synthesized and characterized.[11-12] Technetium chemistry saw the involvement of numerous groups from all over the world, and periodic reviews of progress were published.[13]

Characterizing ^{99m}Tc complexes can be a challenge since only a very small molar fraction of the metal complexes contain radioactive Tc-99m. For instance, the dose of a ^{99m}Tc-labeled radiopharmaceutical used for a clinical scan—typically, 185–925 MBq—corresponds to only 0.95–4.7 nanograms (ng) of metal. This is below the detection limit of methods typically used for the chemical characterization of metal complexes. Furthermore, as there are no stable isotopes of technetium, ^{99}Tc must be used to develop new complexes and study the chemistry of ^{99m}Tc. ^{99}Tc has a half-life of 2.11×10^5 years, and because it undergoes a low-energy beta decay, it can be easily shielded, and milligram quantities can be handled safely. However, the long half-life does create challenges regarding contamination and disposal. Consequently, rhenium, technetium's 5d congener, is often used to prepare reference standards for ^{99m}Tc-containing compounds.[13]

When developing a new ^{99m}Tc-based radiopharmaceutical, convention dictates that a rhenium analogue be prepared on a multi-milligram scale and characterized with techniques such as NMR, mass spectrometry, X-ray crystallography among others. Once the ^{99m}Tc-labeled compound is synthesized, both the ^{99m}Tc and Rhenium complexes can be co-injected into an HPLC. Subsequently, the elution of the Rhenium complex can be monitored via UV or MS, the elution of the ^{99m}Tc complex can be monitored using a radiation detector, and their retention times can be compared to verify co-elution.[13]

1.1.1.2 Opportunities in Technetium Chemistry

Technetium is positioned in the middle of the transition element series on the periodic table. Due to the unique characteristics of both the early and later d- elements, its chemistry is extremely diverse. Rhenium chemistry as a substitute for ^{99}Tc and as a surrogate for ^{99m}Tc as low-valent organometallic chemistry has become increasingly crucial for the labelling of target biomolecules.

Labeling with ^{99m}Tc is based on various techniques, each of which needs extensive knowledge and cross-disciplinary perspectives. ^{99m}Tc is a radionuclide used in many clinical studies. Among them, the highly successful myocardial imaging agent, Cardiolite®, can be given as an example. This molecule is still used in clinical practice.[14,15]

First-generation Tc-99m radiopharmaceuticals (such as ^{99m}Tc-succimer (DMSA), ^{99m}Tc-pentetate (DTPA)) were used in biodistribution applications to determine blood flow and perfusion. Molecules such as peptides, proteins, or small molecules were mainly used as targeting ligands for Tc-99m in a second generation of radiopharmaceuticals. The design and synthesis of tailor-made bifunctional ligands that maintain the affinity to the biological target is required for the preparation of a second-generation Tc-99m based radiopharmaceuticals. This involves the work in a multidisciplinary research field, including medicinal chemistry, inorganic radiochemistry, and biological sciences. MIP-1404, currently in a Phase 3 clinical trial by Lantheus as a prostate imaging agent, is a good example of the successful clinical translation of this interdisciplinary research.[16-18]

1.2 Organometallic Chemistry

In general, organometallic compounds have many structural variations and contain at least one covalent carbon-metal bond. There are four basic categories of organometallic compounds, metallocenes, metalarenes, metalcarbonyls, and metalcarbenes (Figure 1.1).[18] Most complexes have high kinetic stability and are frequently lipophilic, allowing for the metal centre to frequently have no charge and a low oxidation state.[19] Bioorganometallic chemistry is the study of biologically active molecules that contain a carbon atom directly attached to metals or metalloids. Incorporating ferrocene into the structure of an existing drugs is an example of these structures (Figure 1.2). This can allow for improvement in the properties of the drug, such as reducing toxicity. Ferroquine (FQ), a combination of chloroquine (CQ) and ferrocene (Fc), is an example of an organometallic drug candidate.[20]

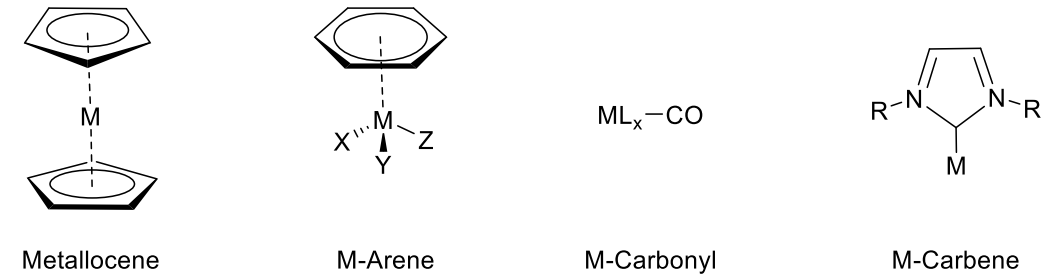

Figure 1.1 An overview of typical classes of organometallic compounds used in medicinal chemistry.[19]

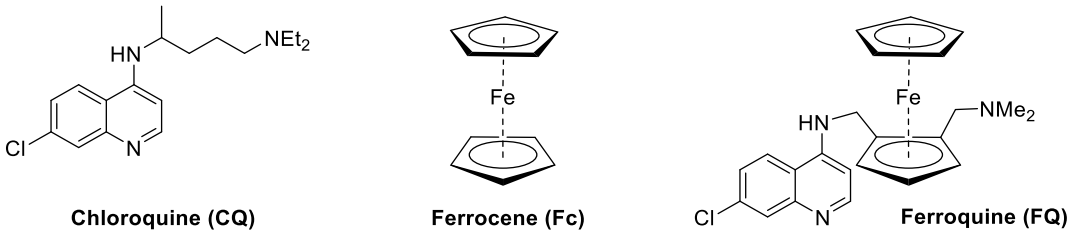

Figure 1.2 Chemical structures of chloroquine (CQ), ferrocene (Fc) and ferroquine (FQ).20

1.2.1 Bis-Arene Complexes in Bioorganometallic Chemistry

The cyclopentadienyl (Cp) ligand is a monoanionic ligand with the formula $[C_5H_5]^-$. The main example of a cyclopentadienyl complex is ferrocene. Bis(cyclopentadienyl) compounds are sometimes referred to as metallocenes or "sandwich compounds". In addition to the negatively charged cyclopentadienyl ligand, isoelectric, uncharged, aromatic, cyclic hydrocarbon moieties have attracted the attention of many research groups. Due to their lower kinetic and thermodynamic stability as well as the difficulties associated with their synthesis, the so-called arenes receive less attention than cyclopentadienyl ligands. When compared to the negatively charged cyclopentadienyl ligand, the neutral arene ligands generally binds less firmly to the metal center, and the coordination properties and hapticity differ. [21-24]

Ernst Otto Fischer and Walter Hafner made the discovery in 1955 of the first metal bis-arene complex, $[Cr(\eta^6\text{-}C_6H_6)_2]$.[25] Using aluminum and the Lewis acid, aluminium trichloride, in benzene as the solvent, $CrCl_3$ can be reduced to Cr^0 and form the bis-benzene complex. Literature has identified bis-arene complexes with the main group elements gallium, tin, lead, and thallium.[24] The main limitation of these reactions is $AlCl_3$'s reactivity, as Lewis acid-Lewis base adducts form during the reaction. This potent Lewis acid is incompatible with oxygen, and nitrogen-containing arenes. In addition, arenes with alkyl substituents like hexamethylbenzene are subject to Friedel-Crafts trans-alkylation reactions.

1.2.2 ^{99m}Tc Bis-Arene Complexes

^{99}Tc and Re bis-arene chemistry started with the work of Fisher *et al*.[24] Although the study opened a new field of research, the Fisher-Hafner reaction procedure was used in all synthesis methods. One drawback of this reaction procedure is that Fischer-Hafner reaction conditions are incompatible with most functional groups.[24]

In 1991, Based on the reactions under Fischer-Hafner conditions, a series of ^{99m}Tc bisarene complexes have been synthesized by Wester et al. Some members of this class of compounds have been shown to have exceptional water stability in *in vivo* studies.[26] The constant variability in the yields of Tc-99m compounds in this study is a clear indication that a new procedure is needed.[26]

Benz *et al*. prepared a variety of ^{99m}Tc bis-arene complexes following a simpler procedure, which still relied on $AlCl_3$. Because $AlCl_3$ reacts with Lewis basic side chains, these conditions prevented access to more decorated arenes. Only a few examples of ^{99m}Tc bis-arenes with a more functionalized arenes have been directly prepared following Fischer-Hafner conditions.[27]Although these studies were a proof of concept of a straightforward radiosynthesis of ^{99m}Tc bis-arene complexes, the limited scope of Fischer-Hafner reaction restricts applications in the radiopharmaceutical field.

Only recently, Nadeem *et al*. showed that $[^{99m}Tc(\eta^6\text{-arene})_2]^+$ type complexes with highly functionalized arenes and even pharmacophores can be prepared directly from $[^{99m}TcO_4]^-$ and water. (Figure 1.3).[28] The concept of conditions arenes directly as ligands in pharmacophores without the use of pendent chelators is the closest thing to the integrated

approach (In the integrated approach, technetium is incorporated into a binding site built into the molecule so that technetium becomes an integral part of the molecule, affecting its conformation and localization *in vivo*). The novel method allows direct access to ^{99m}Tc bis-arene complexes from water and $[^{99m}TcO_4]^-$ as well as arenes incompatible with Fischer-Hafner conditions. Several functionalized ^{99m}Tc bis-arene complexes have been synthesized by utilizing reducing agents such Zn^0, NH_3BH_3 or $NaBH_4$ and modest amounts of sodium dodecylsulfate (SDS) as a surfactant to improve the solubility of the arenes in water. [28]

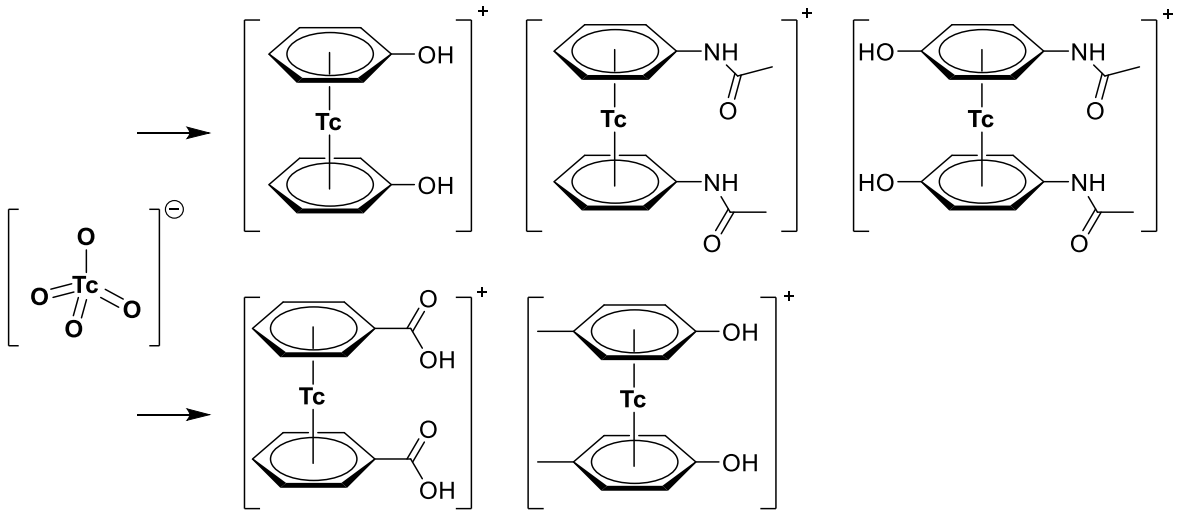

Figure 1.3 Synthesis of bis-arene complexes of ^{99m}Tc directly from $(^{99m}TcO_4)^-$. (Direct synthesis of ^{99m}Tc bis-arene complexes from $[^{99m}TcO_4]^-$ in water. The coordinated arenes are incompatible with Fischer–Hafner conditions. Reducing agents depend on the arene. Typical reducing agents are Zn^0/HCl, $H_3N{\cdot}BH_3$ or $NaBH_4$, and SDS to increase solubility.

In this novel method, the above-reducing agents are used instead of $AlCl_3$ to produce ^{99m}Tc bis-arene complexes providing a way for the "chelator-free" labeling of

several chemical compounds with phenyl groups. The expanded scope of reaction schemes can be used with a wide range of phenyl groups, but the precise conditions must be optimized for each of the derivatives.

1.3 References

(1) Paeng, J. C.; Lee, D. S. Multimodal Molecular Imaging In Vivo. *TONMEDJ* 2010, *2* (1), 145–152. https://doi.org/10.2174/1876388X01002010145.

(2) Deshpande, N.; Needles, A.; Willmann, J. K. Molecular Ultrasound Imaging: Current Status and Future Directions. *Clinical Radiology* 2010, *65* (7), 567–581. https://doi.org/10.1016/j.crad.2010.02.013.

(3) Wu, M.; Shu, J. Multimodal Molecular Imaging: Current Status and Future Directions. *Contrast Media & Molecular Imaging* 2018, *2018*, 1–12. https://doi.org/10.1155/2018/1382183.

(4) Kluba, C.; Mindt, T. Click-to-Chelate: Development of Technetium and Rhenium-Tricarbonyl Labeled Radiopharmaceuticals. *Molecules* 2013, *18* (3), 3206–3226. https://doi.org/10.3390/molecules18033206.

(5) Frangioni, J. V. New Technologies for Human Cancer Imaging. *JCO* 2008, *26* (24), 4012–4021. https://doi.org/10.1200/JCO.2007.14.3065.

(6) PERRIER, C., SEGRÈ, E. Radioactive Isotopes of Element 43. *Nature* **140**, 193–194 (1937). https://doi-org.libaccess.lib.mcmaster.ca/10.1038/140193b0

(7) G. E. Boyd, J. W. Cobble, C. M. Nelson, and W. T. Smith Jr. *Journal of the American Chemical Society* **1952** *74* (2), 556-557 DOI: 10.1021/ja01122a504

(8) C. M. Nelson, G. E. Boyd, and Wm. T. Smith Jr. *Journal of the American Chemical Society* **1954** *76* (2), 348-352 DOI: 10.1021/ja01631a009

(9) J. W. Cobble, Wm. T. Smith Jr., and G. E. Boyd *Journal of the American Chemical Society* **1953** *75* (23), 5777-5782 DOI: 10.1021/ja01119a002

(10) Richards P, Tucker WD, Srivastava SC. Technetium-99m: an historical perspective. Int J Appl Radiat Isot. 1982 Oct;33(10):793-9. doi: 10.1016/0020-708x(82)90120-x. PMID: 6759417.

(11) Knapp, F. F. (Russ); Mirzadeh, S. The Continuing Important Role of Radionuclide Generator Systems for Nuclear Medicine. *Eur J Nucl Med* **1994**, *21* (10), 1151–1165. https://doi.org/10.1007/BF00181073.

(12) Jurisson SS, Lydon JD. Potential technetium small molecule radiopharmaceuticals. Chem Rev. 1999;99(9):2205-2218. doi:10.1021/cr980435t Alberto, R.; Braband, H.; Nadeem, Q. Bioorganometallic Technetium and Rhenium Chemistry: Fundamentals for Applications. *Chimia* 2020, *74* (12), 953. https://doi.org/10.2533/chimia.2020.953.

(13) Holman, B L et al. "Myocardial imaging with technetium-99m CPI: initial experience in the human." Journal of nuclear medicine : official publication, Society of Nuclear Medicine vol. 28,1 (1987): 13-8.

(14) Abrams, M. J.; Davison, A.; Jones, A. G.; Costello, C. E.; Pang, H. Synthesis and Characterization of Hexakis(Alkyl Isocyanide) and Hexakis(Aryl Isocyanide) Complexes of Technetium(I). *Inorg. Chem.* **1983**, *22* (20), 2798–2800. https://doi.org/10.1021/ic00162a007.

(15) Schmidkonz, C, Hollweg, C, Beck, M, et al. 99mTc-MIP-1404-SPECT/CT for the detection of PSMA-positive lesions in 225 patients with biochemical recurrence of prostate cancer. *The Prostate*. 2018; 78: 54–63. https://doi.org/10.1002/pros.23444

(16) Eder, M., Eisenhut, M., Babich, J. *et al.* PSMA as a target for radiolabelled small molecules. *Eur J Nucl Med Mol Imaging* **40**, 819–823 (2013). https://doi.org/10.1007/s00259-013-2374-2

(17) Vallabhajosula S, Nikolopoulou A, Babich JW, et al. 99mTc-labeled small-molecule inhibitors of prostate-specific membrane antigen: pharmacokinetics and biodistribution studies in healthy subjects and patients with metastatic prostate cancer. *J Nucl Med*. 2014;55(11):1791-1798. doi:10.2967/jnumed.114.140426

(18) Gasser, G.; Ott, I.; Metzler-Nolte, N. Organometallic Anticancer Compounds. *J. Med. Chem.* 2011, *54* (1), 3–25. https://doi.org/10.1021/jm100020w.

(19) Biot, C.; Glorian, G.; Maciejewski, L. A.; Brocard, J. S.; Domarle, O.; Blampain, G.; Millet, P.; Georges, A. J.; Abessolo, H.; Dive, D.; Lebibi, J. Synthesis and Antimalarial Activity in Vitro and in Vivo of a New Ferrocene−Chloroquine Analogue. *J. Med. Chem.* 1997, *40* (23), 3715–3718. https://doi.org/10.1021/jm970401y.

(20) Hernández-Valdés, D.; Meola, G.; Braband, H.; Spingler, B.; Alberto, R. Direct Synthesis of Non-Alkyl Functionalized Bis-Arene Complexes of Rhenium and $^{99(m)}$ Technetium. *Organometallics* 2018, *37* (17), 2910–2916. https://doi.org/10.1021/acs.organomet.8b00494.

(21) Muetterties, E. L.; Bleeke, J. R.; Wucherer, E. J.; Albright, T. Structural, Stereochemical, and Electronic Features of Arene-Metal Complexes. *Chem. Rev.* 1982, *82* (5), 499–525. https://doi.org/10.1021/cr00051a002.

(22) Benz, M.; Braband, H.; Schmutz, P.; Halter, J.; Alberto, R. From Tc ^{VII} to Tc ^{I}; Facile Syntheses of Bis-Arene Complexes [$^{99(m)}$ Tc(Arene) $_2$] $^+$ from Pertechnetate. *Chem. Sci.* 2015, *6* (1), 165–169. https://doi.org/10.1039/C4SC02461C.

(23) Meola, G.; Braband, H.; Schmutz, P.; Benz, M.; Spingler, B.; Alberto, R. Bis-Arene Complexes [Re(η ^{6} -Arene) $_2$] $^+$ as Highly Stable Bioorganometallic Scaffolds. *Inorg. Chem.* 2016, *55* (21), 11131–11139. https://doi.org/10.1021/acs.inorgchem.6b01748.

(24) Fischer, E. O.; Hafner, W. Z. Naturforsch. 1955, 10b, 140.

(25) Dennis W. Wester, Joseph R. Coveney, Dennis L. Nosco, Mark S. Robbins, and Richard T. Dean, Journal of Medicinal Chemistry 1991 34 (11), 3284-3290 DOI: 10.1021/jm00115a018

(26) Pampaloni, G. Aromatic Hydrocarbons as Ligands. Recent Advances in the Synthesis, the Reactivity and the Applications of Bis(H6-Arene) Complexes. *Coordination Chemistry Reviews* 2010, *254* (5–6), 402–419. https://doi.org/10.1016/j.ccr.2009.05.014.

(27) Nadeem Q, Meola G, Braband H, et al. To Sandwich Technetium: Highly Functionalized Bis-Arene Complexes [^{99m} Tc(η^6 -arene)$_2$]$^+$ Directly from Water

and [$^{99m}TcO_4$]. *Angew Chem Int Ed Engl.* 2020;59(3):1197-1200. doi:10.1002/anie.201912994

Chapter 2: Synthesis and Biological Evaluation of Sandwich ^{99m}Tc Labeled L-DOPA and L-DOPA Derivative

2.1 Introduction

Research efforts in the last decades to develop ^{18}F-based probes have resulted in several FDA approved imaging agents with recognized application in early detection and diagnosis of various types of cancers and malignant diseases. Some notable examples include [^{18}F]FDOPA and other LAT1substrates, which are used to visualize neuroendocrine tumors (NET). Unfortunately, for many radiopharmaceuticals such as [^{18}F]FDOPA, there are no SPECT analogs, which limits the clinical applications to places with nearby cyclotrons where PET tracers and scanners are available. For example, a developed country like Canada had a total of 512 SPECT and SPECT-CT scanners in 2020, versus only 62 PET-CT or PET-MRI.[1]

This thesis aims at developing new technetium-99 labeled sandwich radiopharmaceuticals. The goal of this initiative is to develop novel ^{99m}Tc -based radiopharmaceuticals that are labeled through direct coordination with biologically active compounds without the need for additional of a chelator. This chapter reviews our efforts

to develop a novel class of technetium imaging agents for L-type amino acid transporter 1 (LAT1) without the need for bulky chelating agents.

2.2 Objectives

The goal of this chapter is to develop a SPECT agent for L-type amino acid transporter 1 (LAT1) imaging. Following the synthesis of $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, , its *in vitro* stability was evaluated by incubating the probe in high-concentration of amino acids and in plasma. Binding assays of $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, were performed with the neuroendocrine tumor cell line pheochromocytomas (PC12), which has a high expression of LAT1. In the last part of this chapter, biodistribution studies with $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, radiotracer was performed in a healthy mouse model and in a PC12 xenograft mouse model.

2.3 Experimental

2.3.1 Materials and Instrumentation

Chemicals and reagents were purchased from Sigma-Aldrich and used without further purification. High-performance liquid chromatography was performed on a Waters 1525 Binary HPLC system connected to a Bioscan ɣ-detector and a 2998 photodiode array detector monitoring at 280 nm. Analysis: HPLC (DOPA buffer isocratic 1mL/min. Column: gemini C18 5u 110A, 250 x 4.60mm. DOPA buffer: 5mM sodium acetate, 1 mM EDTA, O.1% Acetic acid, 0.01% Ascorbic acid. Preparation of the buffer. In 2 L miliQ water add: 1,361 g $NaAc.3H_2O$, 0,744 g EDTA, 0.2 g Ascorbic acid, 2.0 mL Acetic acid. The compound was purified via semi preparative RP-HPLC using isocratic conditions (semi-prep C18 column. Isocratic 4 mL/min)

^{99m}Tc was obtained as [^{99m}TcO$_4$]$^-$ from a ^{99}Mo/^{99m}Tc generator (Lantheus Medical Imaging) in saline (0.9 % NaCl). Caution: ^{99m}Tc is a γ-emitter (Eγ = 140 keV, $t_{1/2}$ = 6h) and should only be used in a licensed and appropriately shielded facility. All other reagents were purchased from Sigma Aldrich. ^{1}H NMR spectra were recorded on a Bruker Avance AV-600 instrument at 300 K.

2.3.2. Synthesis of [^{99m}Tc(η^6-Dopa)$_2$]$^+$ (Tc1)

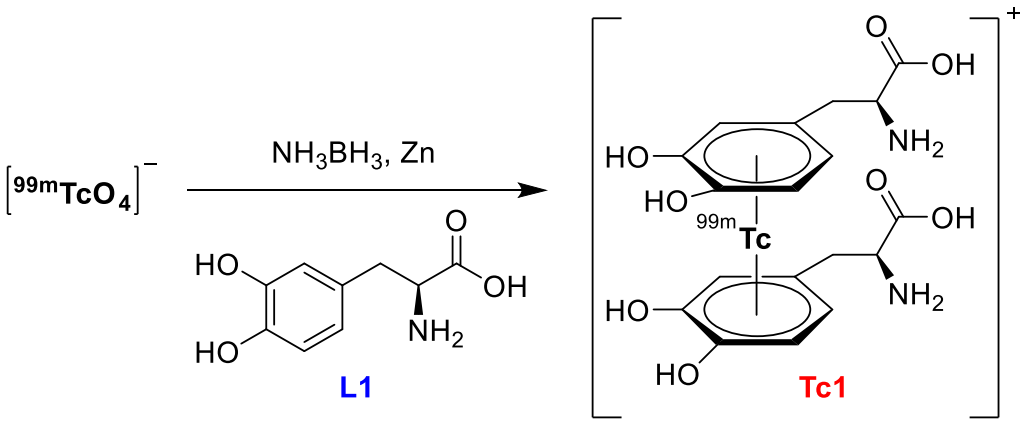

Figure 2.1 Radiolabeling L-DOPA with ^{99m}Tc. One pot labeling reactions are performed in microwave.

Procedure: A U-shape microwave vial is charged with L-DOPA (10 mg, 0.05 mmol), NH_3BH_3 (13 mg, 0.42 mmol), 1-Methyl-3-octylimidazolium chloride (10 mg, 0.04 mmol), and generator eluate (1 mL). The microwave vial is sealed and flushed with argon for 1 min, and then heated at 100^{0}C microwave for 40 min. A clear solution is obtained. Analysis: HPLC (isocratic 1mL/min. Column: Synergi 4U Polar-RP 80A size= 200mm X 4.60mm)

The procedure described above was previously published by Nadeem and co-workers to obtain different bis arene structures, and was used in this study to prepare a

new Tc1 molecule.[2] NH_3BH_3 was used as the reducing agent for the procedure and the reaction was started directly from sodium pertechnetate obtained from the generator. After obtaining Tc1 (Figure 2.1), HPLC analysis was performed with the polar-RP (Synergi 4U Polar-RP 80A size= 200mm X 4.60mm) column. The results showed a peak between 3-4 minutes.

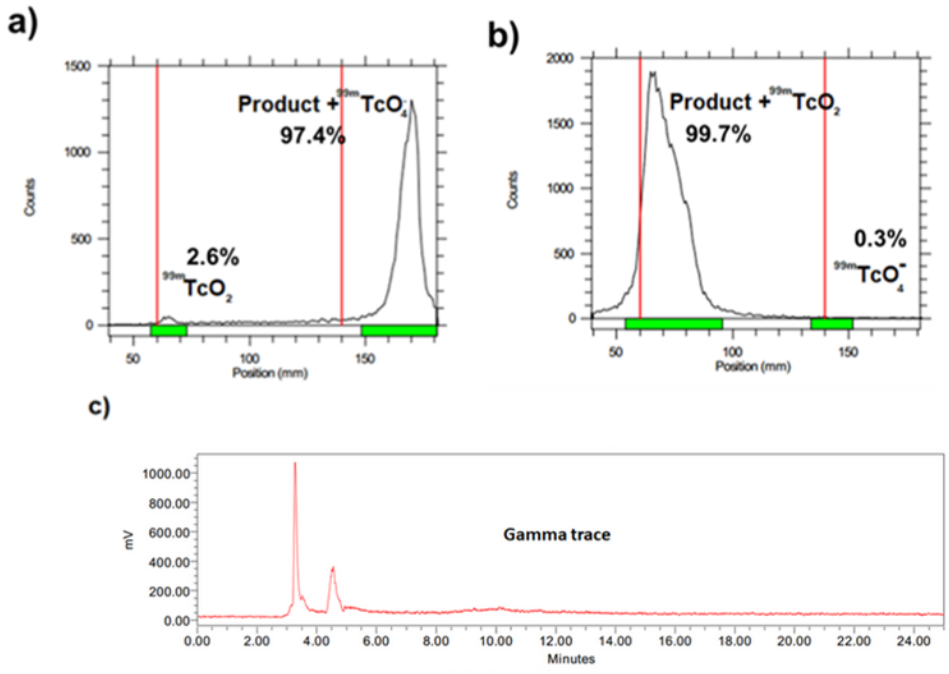

Figure 2.2 Characterization of $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, showing high RCY and RCP. a) i-TLC of crude product in acetone, b) i-TLC of crude product in water, c) Crude radio-HPLC of the product.

Radiochemical yields were evaluated by i-TLC. Two different solvents, water, and acetone were used to determine percentage of TcO_2, $[^{99m}TcO_4]^-$, and product formed during the radiolabeling. The results showed high radiochemical yields (RCY) and high radiochemical purity (RCP) (more than 97% for RCY and RCP based on the radio-TLC results).

A purification C18 column was used in this study. The main reason for purification was to remove L-DOPA. In the first figure, $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, retention time is clearly shown. In the second figure, there is a UV image in which the mobile phase(5 mM NaAc, 1mmM EDTA, 0.1 % HAc, 0.01 % ascorbic acid) and L-DOPA are together.(Figure 2.3)

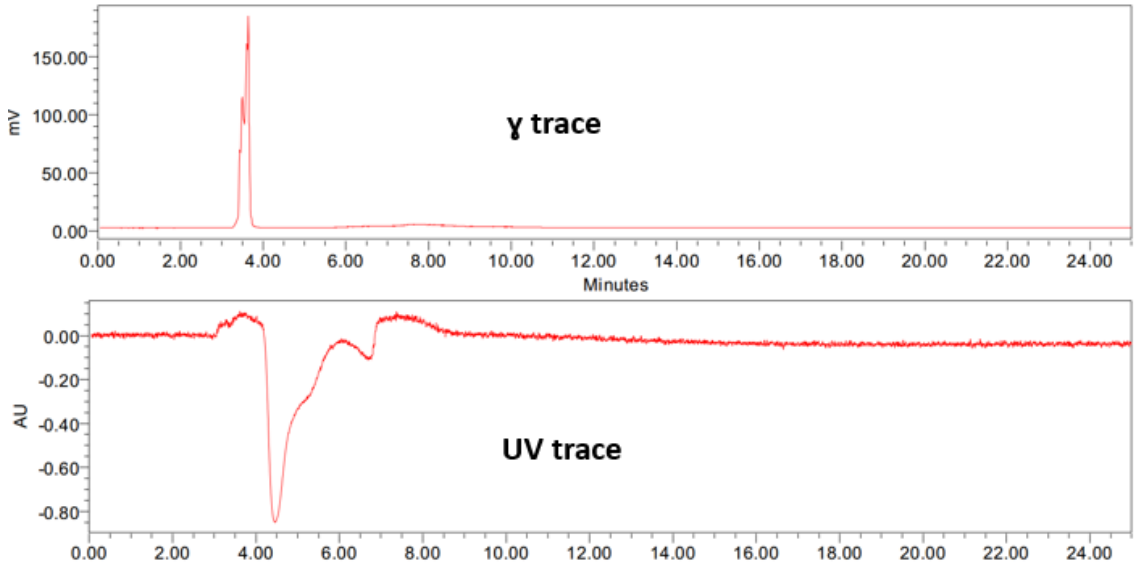

Figure 2.3 Purification test of $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, (Column: Semipreb C18 column)

After purification, an analytical column was used and the HPLC result of the pure compound is seen in figure 2.4. Since the retention time of L-DOPA in Figure 2.5 is 5 minutes, these results clearly show us that L-DOPA has been completely removed by purification. Since the $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, shows a single and clean signal in the gamma trace, the purification was successful.(figure 2.4)

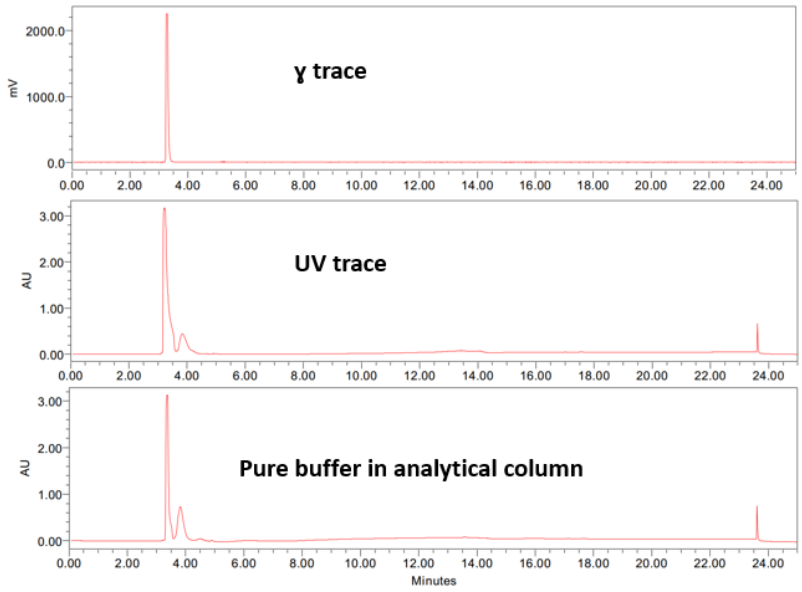

Figure 2.4 HPLC analysis of purified product of $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, with analytical Polar RP column (Column: Synergi 4U Polar-RP 80A size= 200mm X 4.60mm).

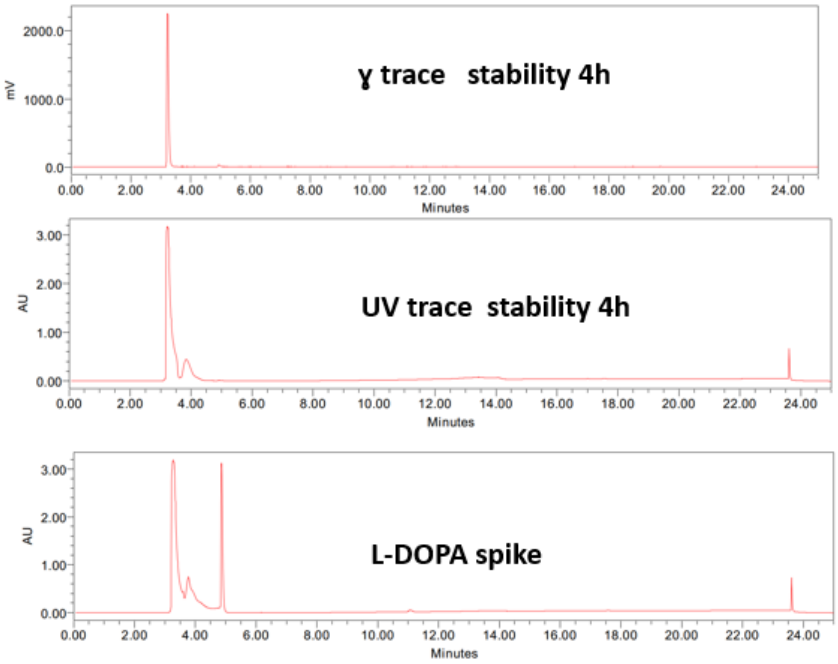

Figure 2.5 Stability test of $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, and cold L-DOPA spike and ascorbic acid with Polar-RP HPLC (Column: Synergi 4U Polar-RP 80A size= 200mm X 4.60mm).

Stability studies were performed for $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, in Figure 2.5. For these studies, HPLC analysis was performed again at hour 4 for stability. An analytical polar RP column was used for this study. As a result of the analysis, the $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, compound was found to be a stable complex and the HPLC retention time did not change. It is clearly seen that the first image contains any dopa in the UV peak (UV trace stability 4h). Finally, both L-DOPA and ascorbic acid were injected together. In the DOPA spike, the retention times of dopa and ascorbic acid are shown separately. These results clearly show that the $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, sandwich compound is stable.

2.3.3 *In vitro* and *In Vivo* study of $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$,

The purpose of this experiment is to measure the binding of $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, to PC12 cells. PC12 cells were transferred to low binding eppendorf tubes in increments of $1x10^3$, $1x10^4$, $1x10^5$, and $1x10^6$ cells and centrifuged at 250g for 10 minutes. The supernatant was then discarded, and blocked with L-DOPA [(huge excess). $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, and non-blocked $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, were mixed with each cell concentration duplicate. All reaction tubes were incubated at room temperature with agitation for 60 minutes. After incubation, cells were spin down(400rpm) for 1 minute, and 200 µL of the supernatant was collected in labeled tubes. The rest of the supernatant was removed, and the cell pellet was rinsed with cold assay buffer. After rinsing, cells were suspended in 250 µL of MilliQ water and incubated for 30 minutes. 200 µL of lysed cells were then transferred to a gamma-counter tube, and the activities were counted using the automated gamma-counter.(figure 2.6)

Titration binding assays with pheochromocytomas PC12 cell line were developed for $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, (Figure 2.6). In this experiment, we used 5 different concentrations and first determined total binding. Then we first used L-DOPA as a blocking agent (**Blocked compound**: 1 mL of 3-5 nM ^{99m}Tc-L-DOPA + 300 µL 50 µM L-DOPA in assay buffer + 1.7 mL assay buffer (in 50 ml tube) to determine non-specific binding$[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$,(**Unblocked compound**: 1 mL of 3-5 nM $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, + 2 mL HBSS + 10 µM HEPES (in 50 ml tube)) for blocked and unblocked experiments. Finally, we determined the specific binding (total binding - non-specific binding). Although the saturation is not reached due to the low molar concentration of the radiotracer, the data show a linear increase in specific binding as is expected before saturation occurs.

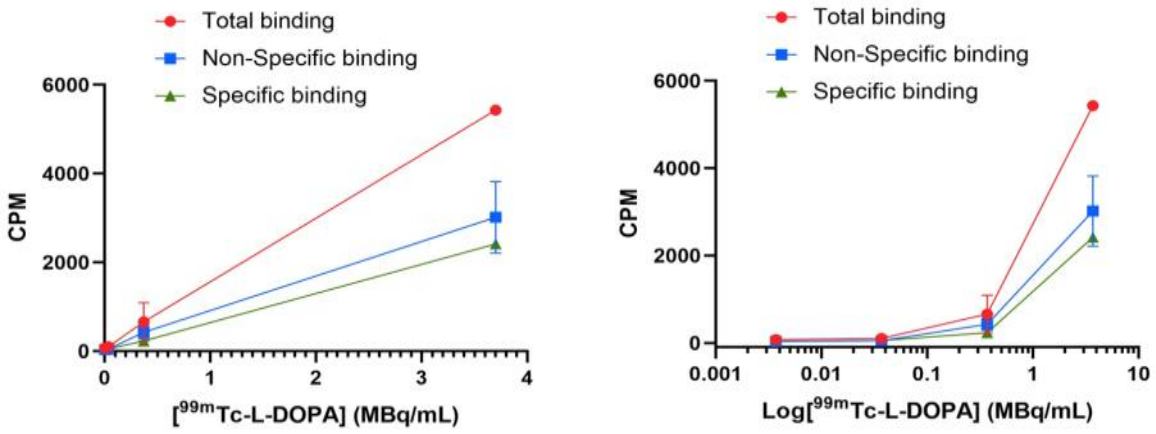

Figure 2.6 *In vitro* and *in vivo* studies with $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, (MBq/mL). a) with $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, and b) Log $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, Titration binding assay

2.3.4 Animal Studies General

All animal studies were approved by the Animal Research Ethics Board at McMaster University. Mice were maintained under clean conditions with 12 h light/dark cycles and given food and water *ad libitum*.

2.3.4.1Biodistribution Study of $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, in Healthy Mice and PC12 Tumor Bearing Mice

A cohort of 6 female Balb/c mice (4-5 weeks) and 6 NCr nude mice (4-5 weeks) were used for the healthy mice and PC12 tumor bearing mice studies respectively. The mice were IP injected with carbidopa (20 μg, 100 μL of 200 mg/mL solution in saline). Carbidopa is a drug given to people with Parkinson's disease in order to inhibit the peripheral metabolism of levodopa(L-DOPA). for non-tumor bearing mice or IP injected with BCH(2-Aminobicyclo-(2,2,1)-heptane-2-carboxylic acid), an inhibitor of system L amino acid transporters for PC12 (average 29747 mg) tumor bearing mice. After 60 min, the mice were administered with $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, formulated in 5 mM sodium acetate, 1 mM EDTA, 0.1 % acetic acid, 0.01 % ascorbic acid and 8.3% (w/v) in PBS via IV injection (20μCi, 100 μL of 200 μCi/mL). At 30 min and 90min post-injection (n=3 per time point), mice were anesthetized with 3% isoflurane and euthanized via cervical dislocation. Blood, stomach, large intestine, bone, spleen, urine and bladder, liver, kidneys, thyroid and trachea, lungs, skeletal muscle, adipose, gall bladder, small intestine, heart, pancreas, and tumour were collected, weighed, and counted in a gamma counter. %ID/g and %ID/organ for each organ was calculated at each time point.

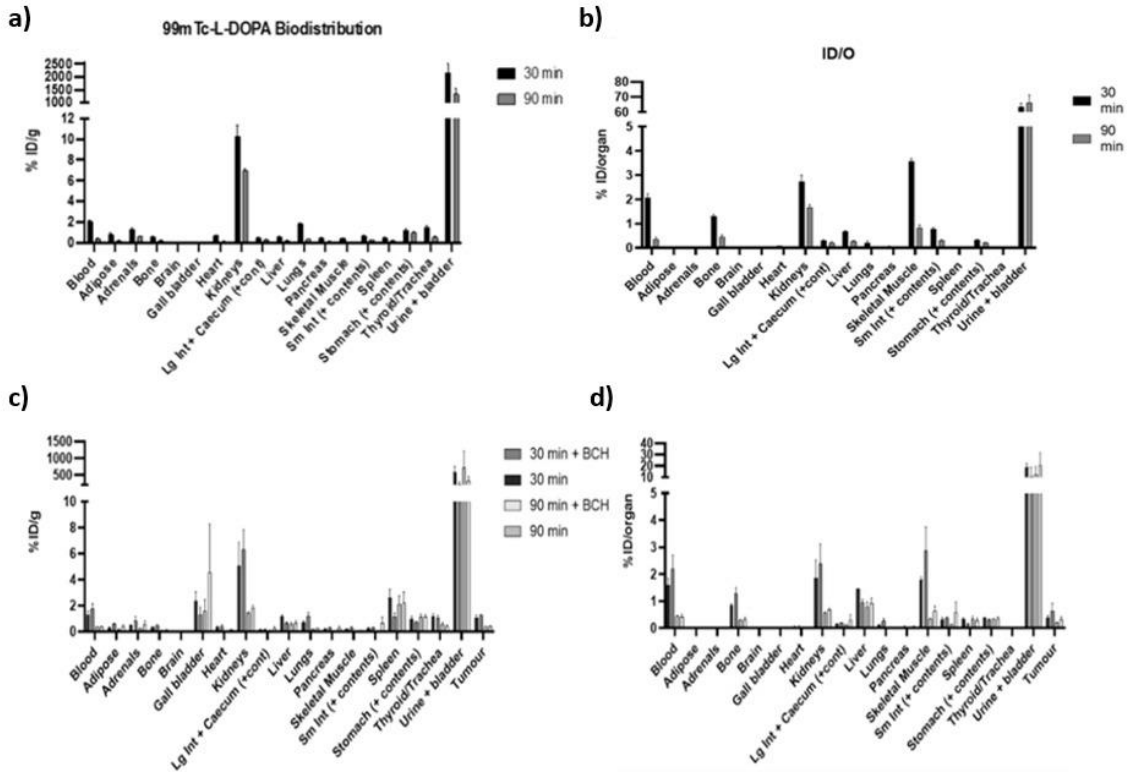

Figure 2.7 Biodistribution studies of [99mTc(η6-Dopa)2]+, with Balb/c healthy mice (a and b) and PC12 tumour-bearing NCr mice (c and d) and. BCH was used for blocking the uptake in PC12 tumours. a) %ID/g at 30 and 90min post-injection in healthy Balb/c mice, b) %ID/O at 30 and 90min post-injection in healthy Balb/c mice.%ID/O at 30 and 90min post-injection in PC12 tumour-bearing NCr mice, c) %ID/g at 30 and 90min post-injection in PC12 tumour-bearing NCr mice, d)

Both a healthy animal model and a xenograft animal model were used for biodistribution studies. Carbidopa (20 μg, 100 μL of 200 mg/mL solution in saline). BCH (100μL of 400μg/mL IP) was used to block uptake into PC12 tumors in the xenograft tumor model. In both mouse models, the results were generally similar. Rapid renal clearance was observed in both the healthy and xenograft mouse models, which is speculated to be due to the hydrophilic nature of compound $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, . There was

also no uptake in the brain, which means that $[^{99m}Tc(\eta^6\text{-Dopa})_2]^+$, has very little or no ability to cross the blood-brain barrier. The results show that Tc1 was rapidly excreted from the urine. Low tumor uptake was clearly demonstrated in both animal models, with no difference between the unblocked and blocked cohorts.

Since Tc1 was found to be remarkably hydrophilic, it was hypothesized that modification of the molecular structure of L-DOPA to make it more hydrophobic could potentially improve pharmacokinetics and biodistribution of the Tc1. Therefore, we aimed to prepare a more hydrophobic L-DOPA derivative to overcome this problem through esterification of the carboxylic acid.

2.3.5 Synthesis of $[Re(L\text{-}DOPA)_2]^+$

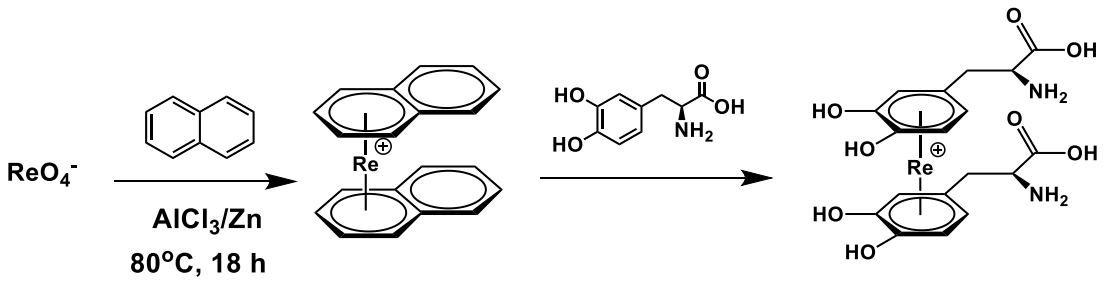

Figure 2.8 Synthesis of $[Re(L\text{-}DOPA)_2]^+$

A two-step synthesis route was determined to obtain $[Re(L\text{-}DOPA)_2]^+$. The first step was to obtain $[Re(napth)_2]^+$ and the second step was to obtain $[Re(L\text{-}DOPA)_2]^+$through a substitution reaction. The first step was successfully (see details 3.3) achieved. Although many different conditions (DMF/100°C microwave, DMF/ 60 - 100°C reflux, H_2O/ 60 – 100°C reflux, H_2O/ 100 - 130°C microwave, DMSO/100°C reflux, H_2O/ [MOIM]Cl 100°C/microwave, H_2O/ NH_3BH_3, [MOIM]Cl 100°C/microwave) were tried in the second

step, the reaction was not successful, and $[Re(L\text{-}DOPA)_2]^+$could not be obtained. The first problem was that L-DOPA decomposes in organic solvents probably because of polymerization.

2.3.6 Synthesis of L-DOPA derivatives and ^{99m}Tc radiolabeling

Synthesis: Thionyl chloride (0.8mL, 5.4 eq) was added dropwise to a solution of L-DOPA (400mg, 1eq) MeOH (5mL) at 0ºC. After 18h at RT, the solvent was evaporated giving compound L2. After the molecule was synthesized, it was purified with a reverse phase combiflash (%95 water, %5 acetonitrile)

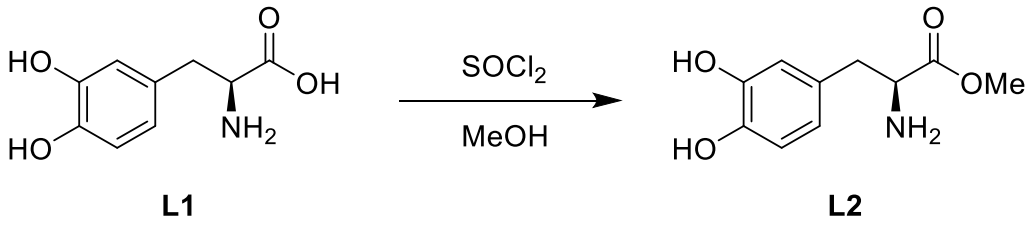

Figure 2.9 Esterification of L-DOPA

After the Tc1 studies, we aimed to synthesize the L2 molecule in a single step. We used L-DOPA for the conversion of a carboxylic acid to an ester using thionyl chloride ($SOCl_2$) and methanol (MeOH). After the molecule was synthesized, HPLC, ESI-MS and NMR was performed to characterize the product (Figures 2.9 and 2.10).

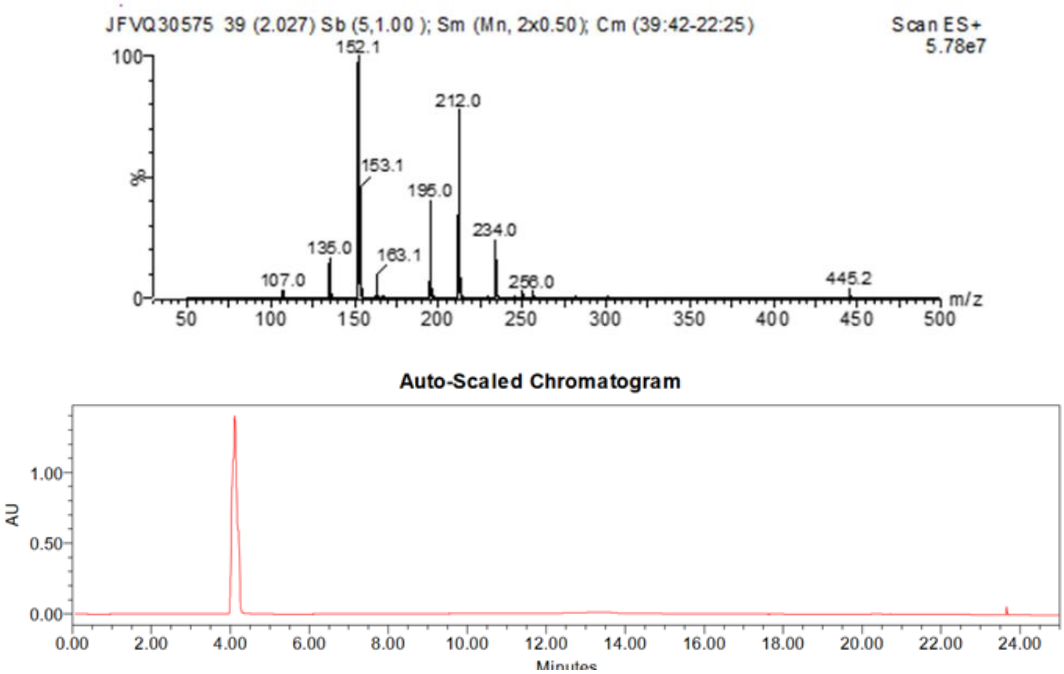

Figure 2.10 Characterization of L2 with ESI-MS and characterization of L2 with Polar-RP HPLC (Column: Synergi 4U Polar-RP 80A size= 200mm X 4.60mm).

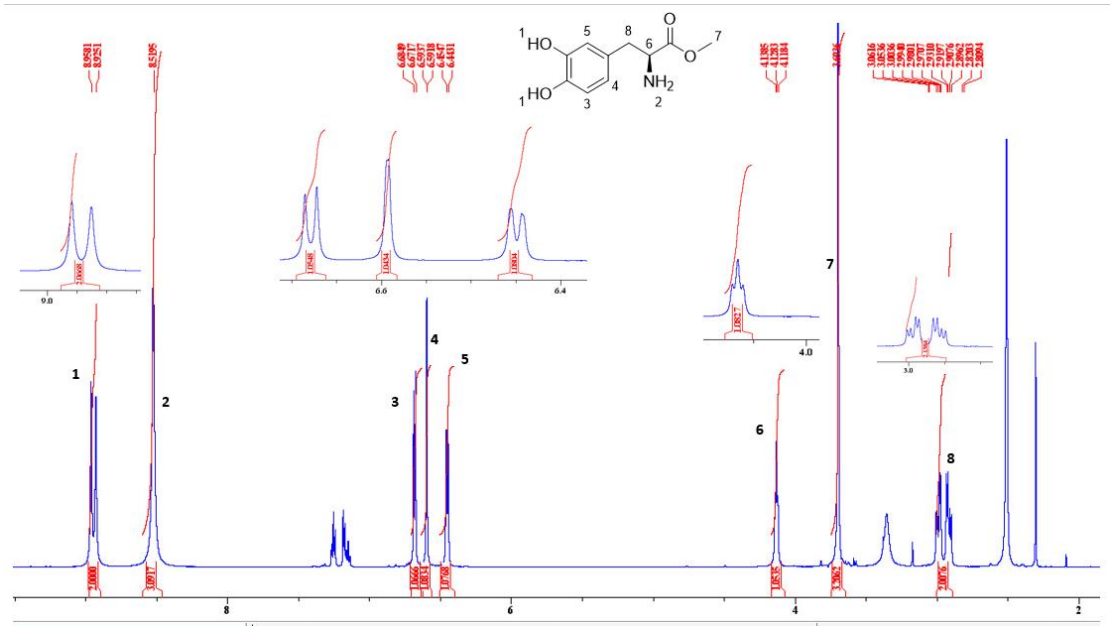

Figure 2.11 Characterization of L2 with ^{1}H-NMR. (600MHz)

NMR analysis confirmed the purity and identity of L2. The calculated exact mass of the molecule was 211. A peak at m/z = 212 corresponding to the $[M+H+]^+$ was observed in the positive mode after mass spectrometry analysis. The peak corresponding to m/z = 152 was hypothesized to be an in-source fragment of L2, as it almost completely vanishes at low voltages. HPLC results show a single and a clean peak.

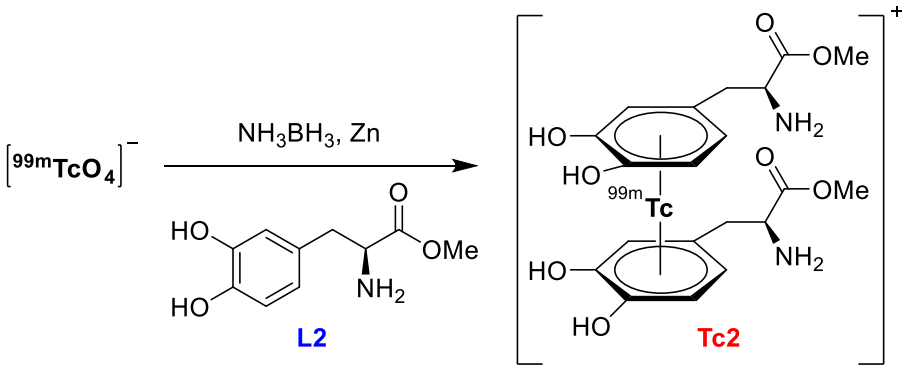

Figure 2.12 Radiolabeling hydrophobic L-DOPA with 99mTc. One pot labeling reaction is performed in the microwave.

Procedure: A U-shape microwave vial is charged with L2 (11 mg, 0.05 mmol), NH_3BH_3 (13 mg, 0.42 mmol), 1-Methyl-3-octylimidazolium chloride (10 mg, 0.04 mmol), and generator eluate (1 mL). The microwave vial is sealed and flushed with argon for 1 min, and then heated at 100^0C microwave for 40 min. A clear solution was obtained.

After successfully synthesizing L2, same reaction conditions previously used to prepare Tc1 were attempted to prepare Tc2. Unfortunately, the radiolabeling (Injection activity: 0.37 MBq) experiments were unsuccessful due to most likely decomposition of the ester moiety under acid and base conditions (Figure 2.12).

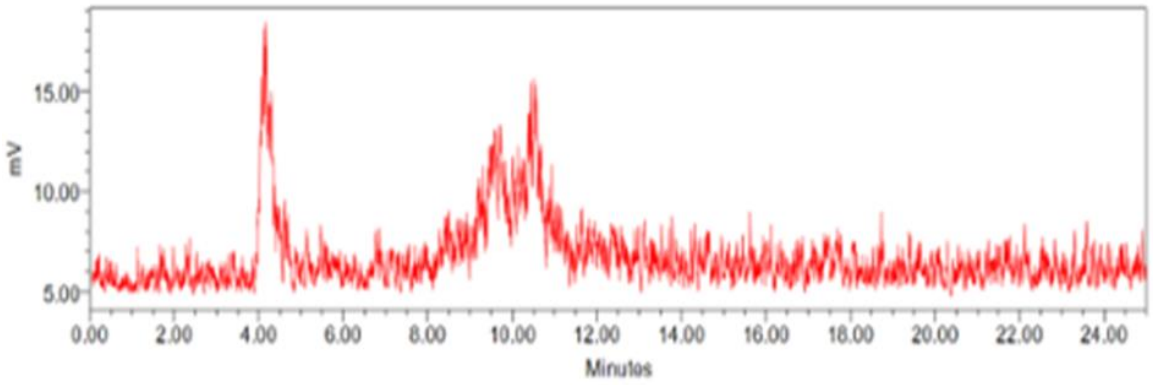

Figure 2.13 Characterization of a crude sample of Tc2 with radio-HPLC. (Column: Synergi 4U Polar-RP 80A size= 200mm X 4.60mm)

2.3.7 Conclusions

Despite several attempts, $[Re(L\text{-}DOPA)_2]^+$could not be obtained. Predicting the lipophilicity of novel imaging agent is for L-dopa. The log P values calculated for $[^{99m}Tc(\eta^6\text{-}Dopa)_2]^+$, in water is -3.84.LogP calculations showed that the $[^{99m}Tc(\eta^6\text{-}Dopa)_2]^+$, was hydrophilic, and to solve this problem, hydrophobic derivatives of $[^{99m}Tc(\eta^6\text{-}Dopa)_2]^+$, were synthesized. After obtaining the L2 molecule by chemical synthesis, ^{99m}Tc radiolabeling experiments showed that hydrolysis of L2 would lead to the formation of $[^{99m}Tc(\eta^6\text{-}Dopa)_2]^+$, instead of the desired product. We propose that other hydrophobic $[^{99m}Tc(\eta^6\text{-}Dopa)_2]^+$, derivatives could potentially alter the pharmacokinetic profile of the radiotracer and thus improve tumor uptake in PC12 tumor-bearing NCr mice in future studies.

2.4 References

(1) Chao YS, Sinclair A, Morrison A, Hafizi D, Pyke L. The Canadian Medical Imaging Inventory 2019–2020 [Internet]. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health; 2021 Jan. PMID: 34990091.

(2) Nadeem Q, Meola G, Braband H, Bolliger R, Blacque O, Hernández-Valdés D, Alberto R. To Sandwich Technetium: Highly Functionalized Bis-Arene Complexes [99m Tc(η6 -arene)2]+ Directly from Water and [99m TcO4]. Angew Chem Int Ed Engl. 2020 Jan 13;59(3):1197-1200. doi: 10.1002/anie.201912994. Epub 2019 Dec 12. PMID: 31680387.

Chapter 3: Naphthalene Substitution in $[M(\eta^6\text{-napht})_2]^+$ with Pharmaceuticals and Arenes (M=Re/^{99m}Tc)

3.1 Introduction

The discovery of a highly versatile $[^{99m}Tc(CO)_3]^+$ core 25 years ago allowed for the preparation of organometallic complexes in aqueous solution, leading to an avalanche of fascinating technetium chemistry in the last decades.[1] By treating $[^{99m}TcO_4]^-$ with borohydride in the presence of CO gas, Alberto and colleagues demonstrated a method to produce the reactive Tc(I) species $[^{99m}Tc(CO)_3(OH_2)_3]^+$. However, because CO is a hazardous gas, it should not be used in medical facilities or in commercial radiopharmaceutical kits. This problem has been solved by using boron carbonate or $K_2[H_3BCO_2]$. The three face-directed water molecules in this complex are sufficiently labile to be rapidly replaced by a variety of monodentate, bidentate, and tridentate ligands. The reaction consists of two steps (Figure 3.1). In the first step, $[^{99m}Tc(CO)_3(OH_2)_3]^{+\cdot}$ is formed, and the labile water molecules are subsequently replaced by ligands in the reaction of the precursor.[1]

Inspired by the tricarbonyl strategy, we propose a new method for the preparation of ^{99m}Tc bis-arene complexes (Figure 3.1). In this method, $[^{99m}Tc(\eta^6\text{-naphth})_2]^+$ is obtained

and then the desired compound is obtained by substituting the labile naphthalene for incoming ligands. The purpose of this chapter is to determine which method is better by performing Tc-99m labeling in two steps, such as lidocaine or lenalidomide, which could previously be done in one step. The second purpose, some challenging ^{99m}Tc bis-arene complexes that are performed in low yields or that cannot be performed at all in a single-step reaction, can be tried via a substitution reaction from the $[^{99m}Tc(\eta^6\text{-naphth})_2]^+$ in a two-step reaction.

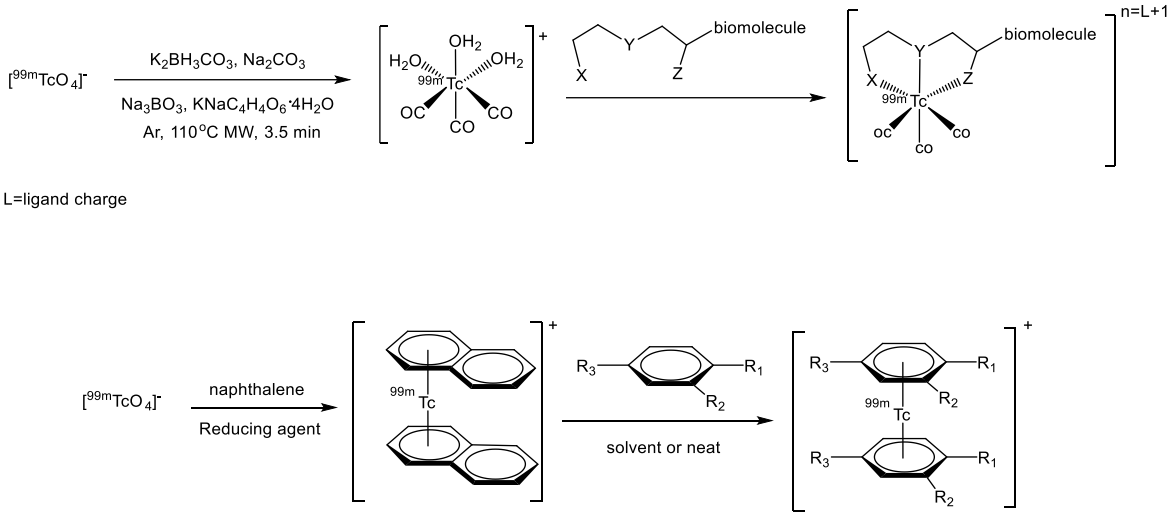

Figure 3.1 Two steps synthetic strategy for the formation of ^{99m}Tc-tricarbonyl and ^{99m}Tc bis-arene complexes, Formation of $[^{99m}Tc(H_2O)_3(CO)_3]^+$ and subsequent substitution of the aqua ligands by an incoming ligand (top)[2], Formation of $[^{99m}Tc(\eta^6\text{-naphth})_2]^+$ and subsequent substitution of the naphthalene by an incoming ligand (bottom).

Previous reports have demonstrated a method of synthesizing sandwich complexes of formula $[Re(\eta^6\text{-arene})_2]^+$ from $[Re(\eta^6\text{-napht})_2]^+$. In this study, firstly $[Re(\eta^6\text{-napht})_2]^+$ was first obtained and then desired $[Re(\eta^6\text{-compound})_2]^+$ was obtained by substitution reaction.[3]

The success of the above-mentioned $[Re(\eta^6\text{-napht})_2]^+$ compound in substitution reactions was the inspiration to attempt the same approach with $[^{99m}Tc(\eta^6\text{-napht})_2]^+$. In the first part of our study, $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ and $[Re(\eta^6\text{-napht})_2]^+$ were obtained. It was then investigated whether $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ could be used in substitution reactions.

3.2 Materials and Instrumentation

All reagents were purchased from Sigma Aldrich, Life Technologies, ThermoFisher. The formation of product $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ was confirmed by RP-HPLC UV/vis-detector paired with gamma-detector and through comparison of retention times of the co-injected Re analogue. ^{99m}Tc was obtained as $[^{99m}TcO_4]^-$ from a $^{99}Mo/^{99}mTc$ generator (Lantheus Medical Imaging) in saline (0.9 % NaCl).

3.3 Synthesis of $[Re(\eta^6\text{-naphth})_2]^+$

Procedure: $KReO_4$ (500 mg, 1.73 mmol), zinc dust (339 mg, 5.18 mmol) and anhydrous $AlCl_3$ (2.3 g, 17.3 mmol) were suspended in a mixture of naphthalene (4.43 g, 34.6 mmol). The reaction mixture was stirred at 100°C for 18h. The mixture was then cooled to room temperature and washed under vigorously stirring with Et_2O (2 x 50 mL). The solid residue was suspended in 20 ml of H_2O . All aqueous solutions were reduced in volume to less than 8 mL. The aqueous phase was filtered and f LiOTf (3-4eq)was added. The resulting mixture was extracted 3 times with 80 mL DCM and dried under vacuum.

Results: We synthesized $[Re(\eta^6\text{-naphth})_2]^+$ following the above procedure and it was characterized by HPLC and mass spectrometry analyses. ESI-MS m/z calculated for

$[Re(\eta 6\text{-naphth})_2]^+$: 443 found 443.. HPLC results show a single peak.(ESI-MS: m/z =443.0 $[M]^+$)

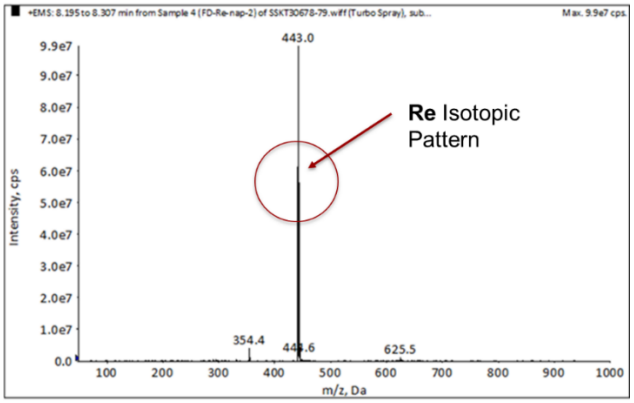

Figure 3.2 Characterization of $[Re(\eta^6\text{-naphth})_2]^+$ with ESI. m/z = 443.0 corresponds with calculated exact mass of the product $[M]^+$.

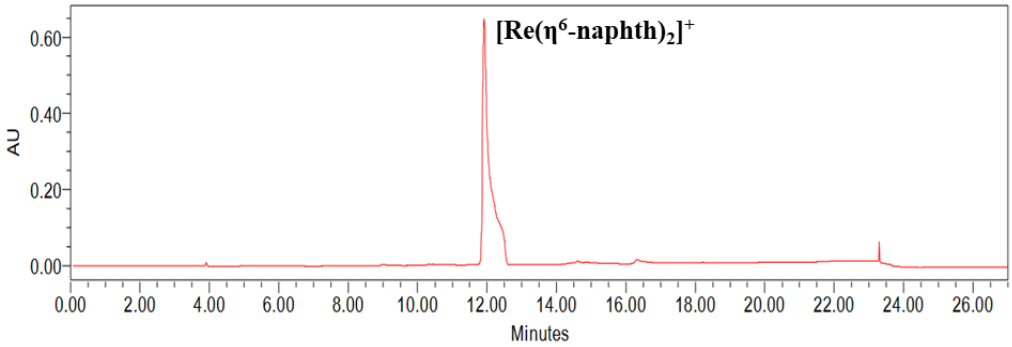

Figure 3.3 Characterization of $[Re(\eta^6\text{-naphth})_2]^+$ with Polar-RP HPLC (Column: Synergi 4U Polar-RP 80A size= 200mm X 4.60mm).

3.4 Synthesis of $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ complex

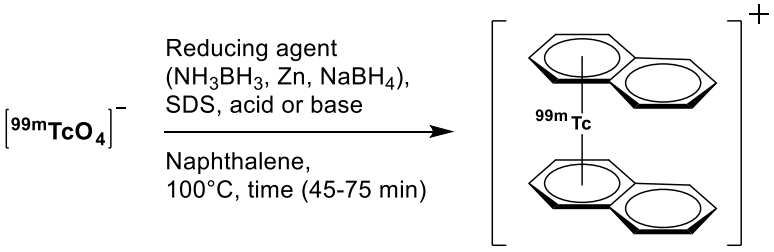

Figure 3.4 Synthesis of bis-arene $[^{99m}Tc(\eta^6\text{-napth})_2]^+$

The HPLC retention time of pertechnetate, which we used as the starting material in each experiment, is shown in the Figure 3.5 for both analytical columns used.

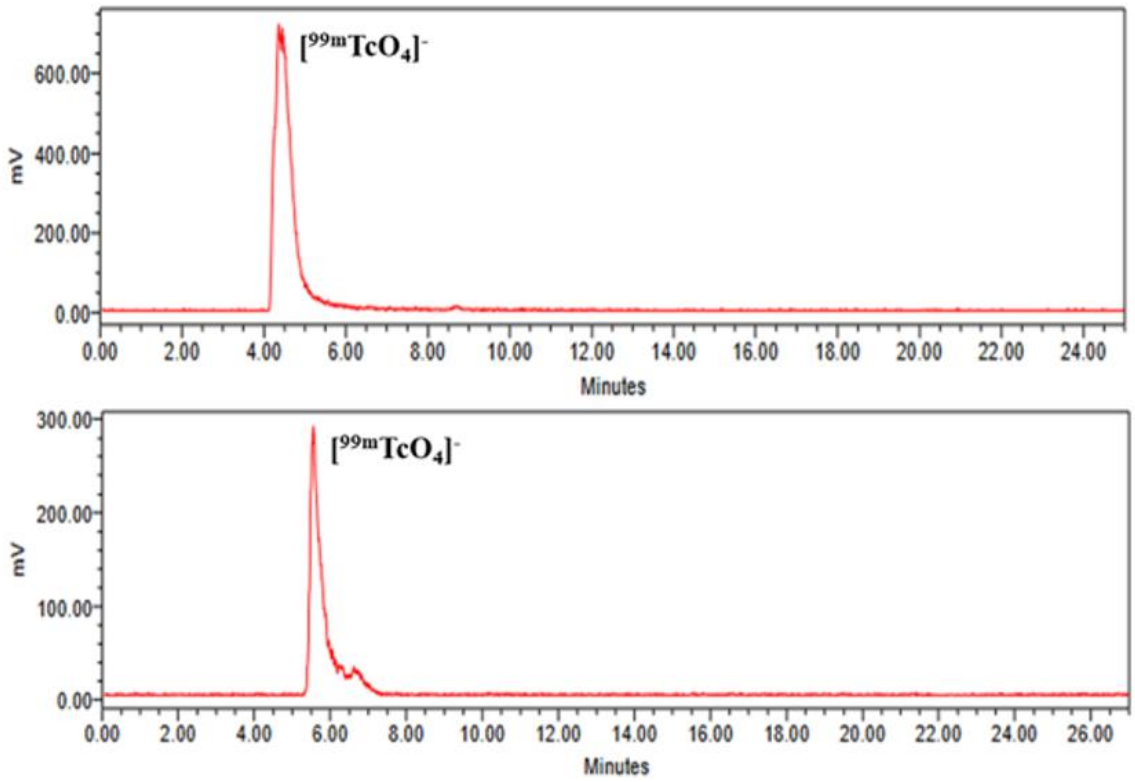

Figure 3.5 Radio-HPLC analysis of pertechnetate in two analytical columns used in this project: Top: Synergi 4U Polar-RP 80A size= 200mm X 4.60mm, Bottom: Gemini C18 5u 110A, 250 x 4.60mm).

Note: All HPLC analysis were conducted by injecting 100 µL of sample with an activity of 0.74-1.48 MBq

First procedure: A vial was charged with sodium dodecyl sulfate (SDS, 4mg, 0.013mmol), NH_3BH_3 (13mg, 0.42mmol), and naphthalene (32 mg, 0.25mmol). After $[^{99m}TcO_4]^-$ from the generator (1mL) was injected into the vial, added 1M NaOH (50µL). The vial was sealed and flushed with argon for 1 minute and then heated at 100°C for 40 min.

Results: NH_3BH_3 was used as the reducing agent in the first procedure. After obtaining $[^{99m}Tc(\eta^6\text{-naphth})_2]^+$, the retention time was compared with $[Re(\eta^6\text{-naphth})_2]^+$ after HPLC analysis. Although the results were promising in terms of retention time, since a clear peak could not be obtained, it was aimed to use other procedures. (Figure 3.6).

Another problem is that although the reaction started with 266.4 MBq of pertechnetate, and only obtained 8.5 MBq after the reaction, was to lose too much activity. Researching a different reducing agent was the most logical solution to these problems. (Activity yield=3.2%)

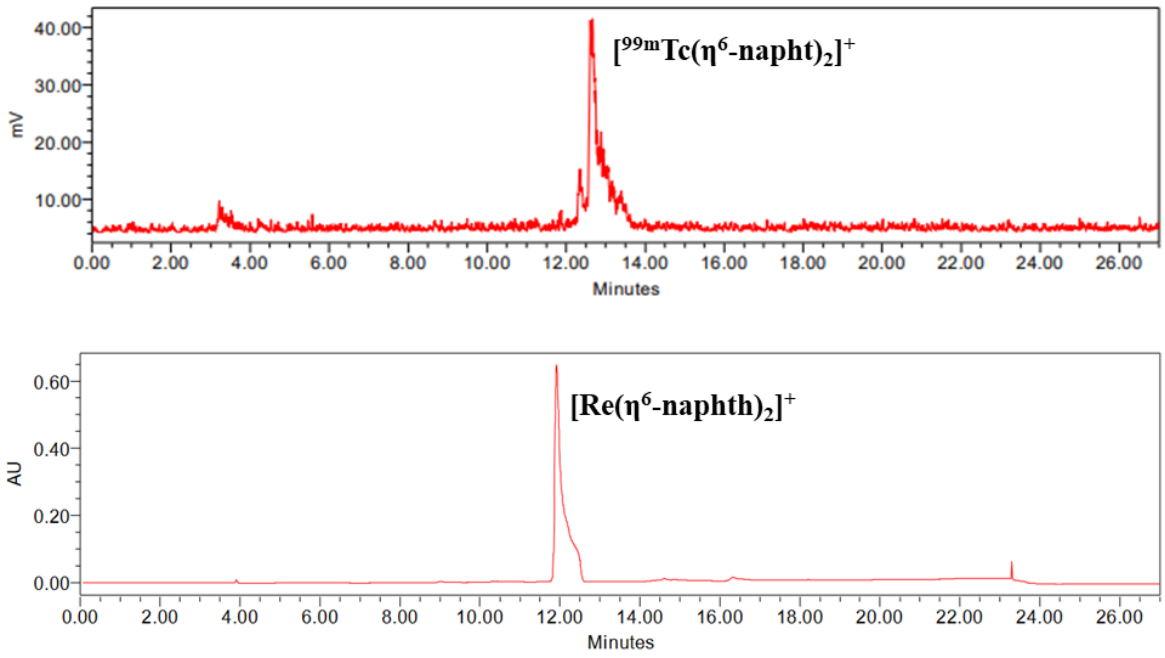

Figure 3.6 Characterization of $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ (first procedure) with Radio-HPLC (Top) and Rhenium-naphthalene(bottom) with 280 nm HPLC (Column: Synergi 4U Polar-RP 80A size= 200mm X 4.60mm).

Second Procedure: A vial was charged with naphthalene (32 mg, 0.25mmol), SDS (4mg, 0.013mmol), and $NaBH_4$ (15mg, 0.40mmol). $[^{99m}TcO_4]^-$ (1mL) was added and the solution then added tetrabutylammonium hydroxide ([TBA]OH, 40% in H_2O) (100µL) and closed and flushed with argon for 1 minute and then heated at 100°C for 30min.

In the second procedure, $NaBH_4$ was used as the reducing agent and the retention time was compared with $[Re(\eta^6\text{-naphth})_2]^+$ after HPLC analysis. Although a clear result was achieved in retention time as with the previous procedure, Due to a split peak, another procedure was applied (Figure 3.6).

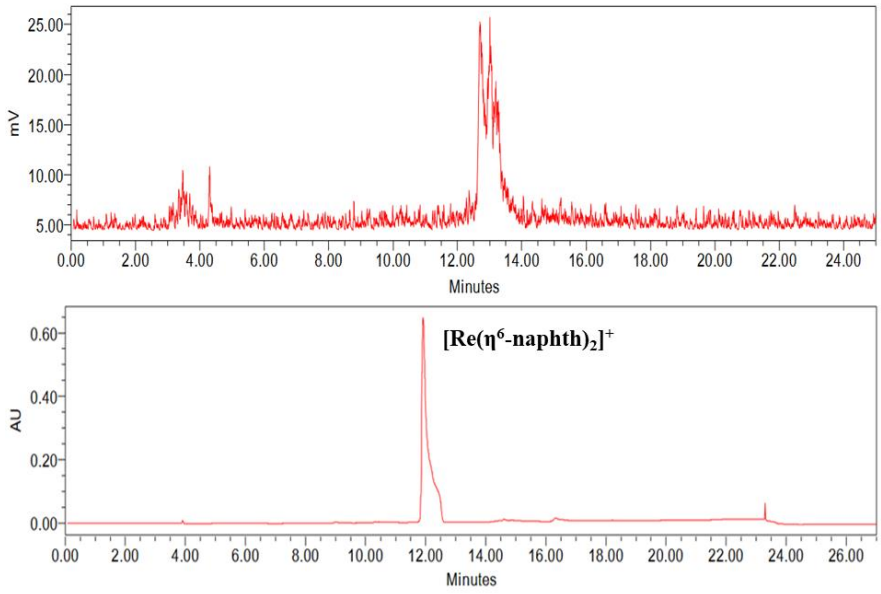

Figure 3.7 Characterization of $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ (second procedure) with radio-HPLC (top) and Rhenium-naphthalene(bottom) with 280 nm HPLC (Column: Synergi 4U Polar-RP 80A size= 200mm X 4.60mm).

Third Procedure: A vial was charged with naphthalene (35mg, 0.25mmol), SDS (4mg, 0.013mmol), and Zn (9-11mg). $[^{99m}TcO_4]^-$ (1mL) was added and the solution then

added 3N HCl (50µL) and closed and flushed with argon for 1 min and then heated at 100°C different minutes (15-30-60-75)

In the last procedure, Zn was used as the reducing agent and more than one trial was made. As a result of all trials, it was seen that the experiment time gave the best result 75 minutes.

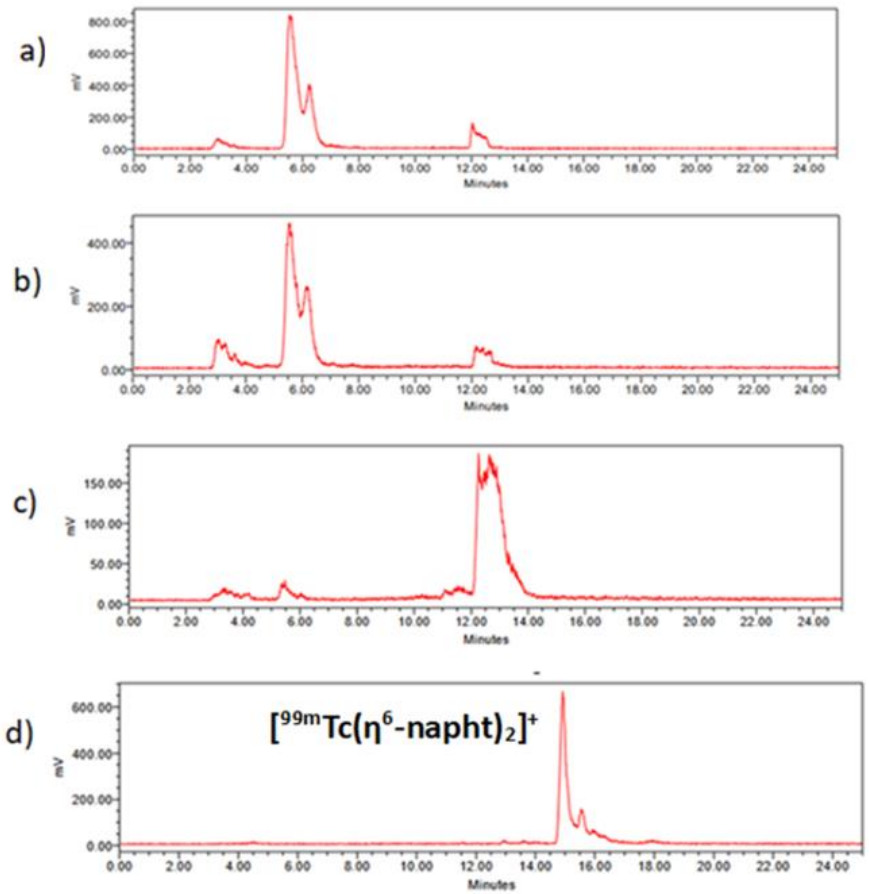

Figure 3.8 Characterization of $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ a) Reaction time: 15 minutes b) Reaction time: 30 minutes c) Reaction time: 60 minutes d) Reaction time: 75 Minutes General: (left) and Rhenium-naphthalene(right) with HPLC (Column: Synergi 4U Polar-RP 80A size= 200mm X 4.60mm).

The results show that pertechnetate, which is the starting material, did not produce a significant amount of product after 15 and 30 minutes. After 75 minutes, it produced a clean signal. These results show that the ideal reaction time is 75 minutes. Initially, 259 MBq (1mL) pertechnetate was used, and obtained 62.9 MBq(Activity yield=12%) activity at the end of the experiment. This reaction procedure was used to obtain $[^{99}mTc(\eta^6\text{-napht})_2]^+$ before all substitution reactions, as a clear peak and better recovery activity were obtained on HPLC at the end of the experiment. (Radiochemical yield (RCY) = 86%)

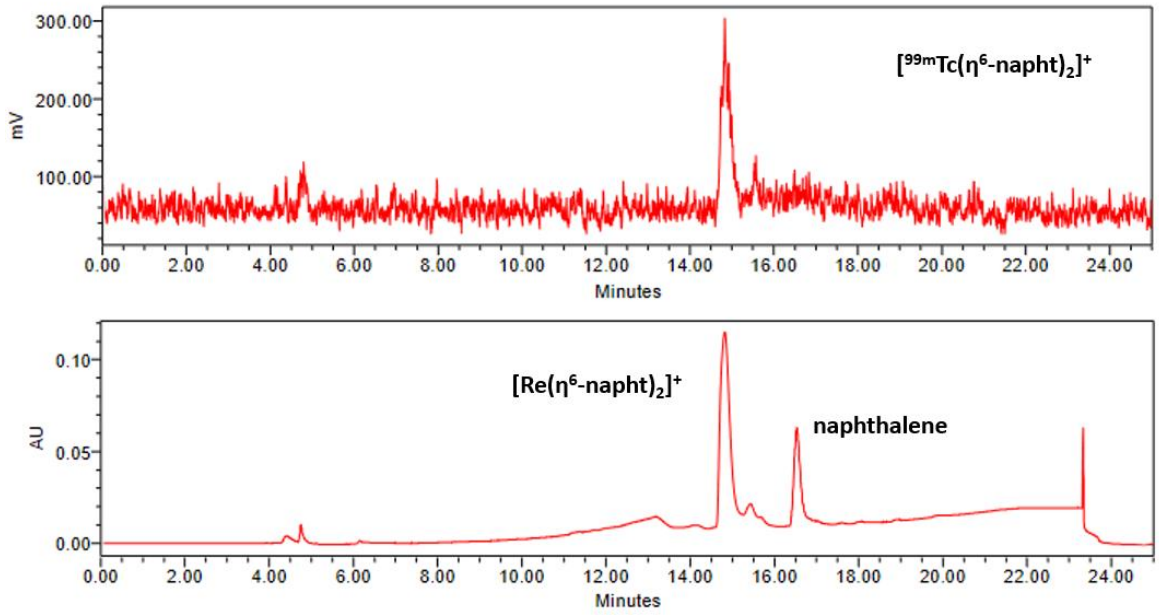

Figure 3.9 Co-injection HPLC ($[^{99m}Tc(\eta^6\text{-napht})_2]^+$ and $[Re(\eta^6\text{-napht})_2]^+$) (Column= Gemini C18 5u 110A, 250 x 4.60mm).

3.5 Substitution reaction with $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ complexes

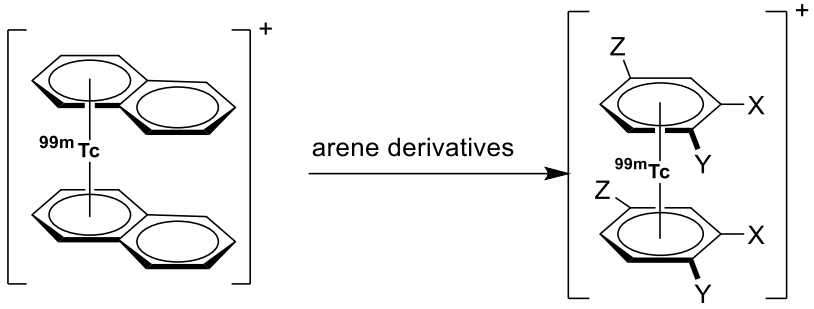

Figure 3.10 General procedure for substitution reactions

A vial was charged with $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ (after obtaining ($[^{99m}Tc(\eta^6\text{-napht})_2]^+$), then Sep-Pak C18 was used to reformulate the pure $[^{99m}Tc(\eta^6\text{-napht})_2]^+$.The solvent was removed under argon gas. Pharmaceuticals including L-DOPA, lidocaine, lenalidomide or benzene were added to $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ in the presence of solvent (water, acetonitrile or 1,4 dioxane). The vials were closed and flushed with argon for 1 min and then heated at 100°C different minutes (20-40). To prove two-step Tc-99m labeling, experiments were performed with lidocaine previously labeled with rhenium and Tc-99m (one-step labeling).[3] This was followed by lenalidomide, which was successfully synthesized in our laboratory and labeled with rhenium and Tc-99m (one-step labeling). In another substitution reaction, L-DOPA was tried. Finally, pure benzene was used instead of a solid compound.

3.5.1. Substitution reaction with $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ and Lidocaine

Procedure: A vial was charged with $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ (after ($[^{99m}Tc(\eta^6\text{-napht})_2]^+$), purification was performed with a Sep-Pak® C18 Cartridge (to remove remaining pertechnetate) was used to reformulate pure $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ Then, The

solvent was removed under argon gas. Lidocaine (20 mg) was added together with 0.6 mL (water or 1,4 dioxane). The vial was closed and flushed with argon for 1 minute and then heated at 100°C for 20 minutes.

Results: After $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ was successfully synthesized, the substitution reaction was started. For this, $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ was used as the starting material and its effect on the substitution reaction with various chemicals and pharmaceuticals was investigated.

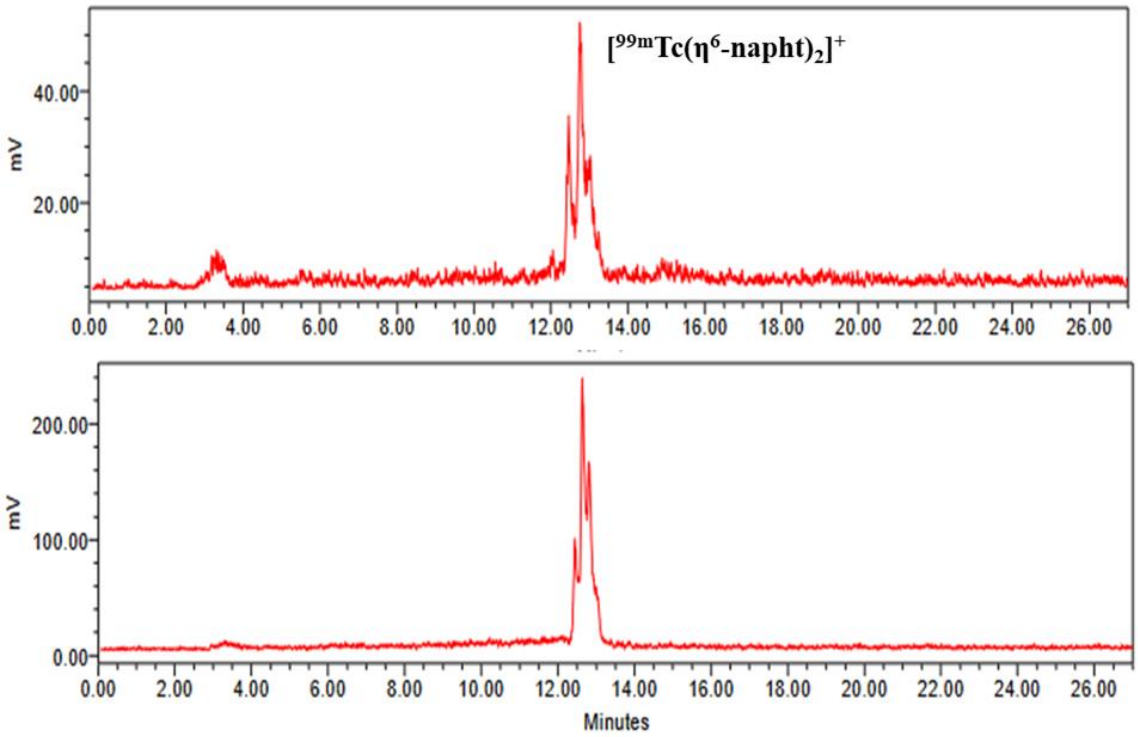

Figure 3.11 Substitution reaction with lidocaine (top=before substitution reaction Radio- HPLC trace ($[^{99m}Tc(\eta^6\text{-napht})_2]^+$), bottom=after substitution reaction Radio-HPLC trace)((Column=Synergi 4U Polar-RP 80A size= 200mm X 4.60mm)

No change was observed when water was used as solvent. These results showed that water does not allow any reaction.

Therefore, the same procedure as for $[Re(\eta^6\text{-lidocaine})_2]^+$ was used. 1,4-Dioxane and NMP were used as solvents for this procedure. The HPLC results show that instead of obtaining ^{99m}Tc-lidocaine at the end of the substitution reaction, pertechnetate, which is the starting material, is obtained again.

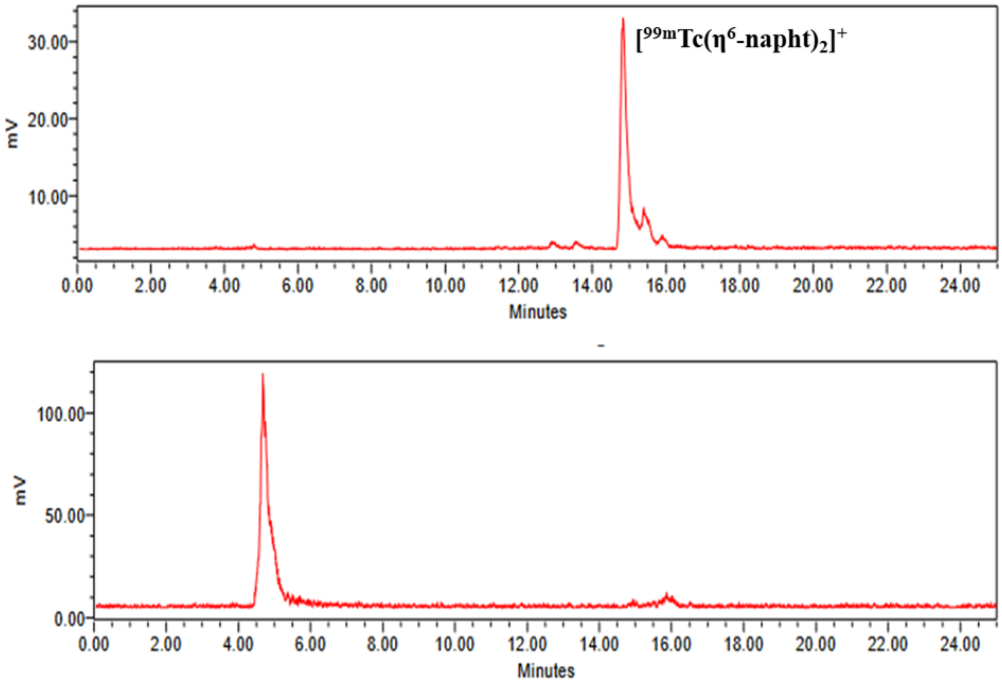

Figure 3.12 Substitution reaction with lidocaine (top=before reaction Radio-HPLC trace ($[^{99m}Tc(\eta^6\text{-napht})_2]^+$), bottom=after reaction Radio-HPLC trace) (Column: Synergi 4U Polar-RP 80A size= 200mm X 4.60mm).

3.5.2 Substitution reaction with $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ and L-DOPA

Procedure: A vial was charged with $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ (after obtaining ($[^{99m}Tc(\eta^6\text{-napht})_2]^+$) purification was performed with a Sep-Pak® C18 Cartridge(to remove remaining

pertechnetate) was used to reformulate pure ($[^{99m}Tc(\eta^6\text{-napht})_2]^+$ Then, The solvent was removed under argon gas. L-DOPA (20 mg) was added together with solvent 0.6 mL (water). The vial was closed and flushed with argon for 1 minute and then heated at 100°C for 40 minutes.

Results: In another substitution reaction, L-DOPA was used as incoming ligand and water as the solvent. The results were compared with lidocaine. The results of both experiments showed that water was not an appropriate solvent to allow the substitution reaction with any other pharmaceutical.

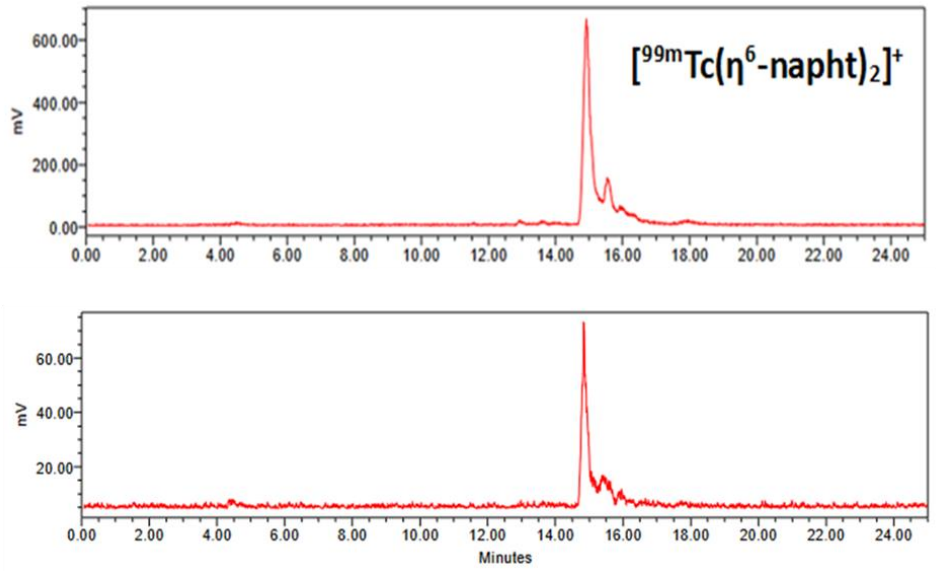

Figure 3.13 Substitution reaction with L-DOPA (top=before reaction Radio-HPLC trace ($[^{99m}Tc(\eta^6\text{-napht})_2]^+$), bottom=after reaction Radio-HPLC trace) HPLC (Column: Synergi 4U Polar-RP 80A size= 200mm X 4.60mm).

3.5.3 Substitution reaction with $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ and Lenalidomide

Procedure: A vial was charged with $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ (after obtaining ($[^{99m}Tc(\eta^6\text{-napht})_2]^+$) purification was performed with a Sep-Pak® C18 Cartridge (to remove

remaining pertechnetate) was used to reformulate pure ($[^{99m}Tc(\eta^6\text{-napht})_2]^+$ Then, The solvent was removed under argon gas.Lenalidomide (20 mg) was added, and the solution then added solvent (1,4 dioxane-NMP) was closed and flushed with argon for 1 min and then heated at 100°C for 40 minutes.

Results: Lenalidomide was used in another substitution reaction. The procedure previously used by the Alberto group for the substitution of naphthalenes in $[Re(\eta^6\text{-naphth})_2]^+$ was used as the reaction procedure here. The results showed that after the reaction was completed, the $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ decomposed when any organic solvent was used (1,4 dioxane, NMP) and pertechnetate was obtained.

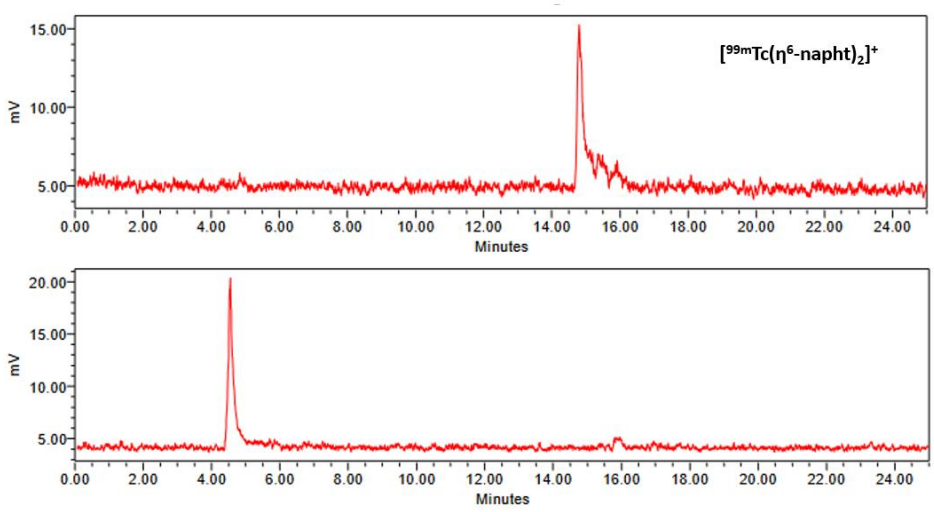

Figure 3.14 Substitution reaction with lenalidomide (top=before reaction Radio-HPLC trace ($[^{99m}Tc(\eta^6\text{-napht})_2]^+$), bottom=after reaction Radio-HPLC trace) (Column: Synergi 4U Polar-RP 80A size= 200mm X 4.60mm).

3.5.6 Substitution reaction with $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ and Benzene

Procedure: A vial was charged with $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ (after obtaining ($[^{99m}Tc(\eta^6\text{-napht})_2]^+$), purification was performed with a Sep-Pak® C18 cartridge (to remove

remaining pertechnetate) was used to reformulate pure ($[^{99m}Tc(\eta^6\text{-napht})_2]^+$ Then, The solvent was removed under argon gas. Benzene (0.5mL) was added, flushed with argon for 1 minute, and then heated at 100°C for 40 minutes.

Results: Since all the substances used above were solid and in all cases, no formation of desired product was seen, the strategy was changed and a liquid arene was used as incoming ligand instead of a solid arene to avoid the use of organic solvents. The substitution reaction was then attempted in neat benzene without using solvents.

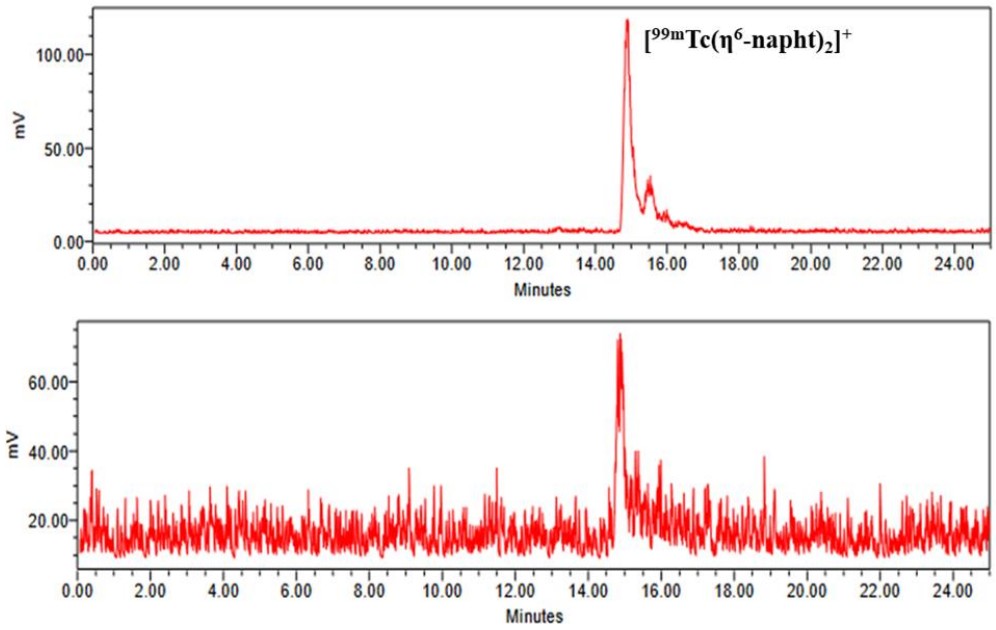

Figure 3.15 Substitution reaction with benzene (top=before reaction Radio-HPLC trace($[^{99m}Tc(\eta^6\text{-napht})_2]^+$), bottom =after reaction Radio-HPLC trace) (Column: Synergi 4U Polar-RP 80A size= 200mm X 4.60mm).

Since there was no change when benzene was used (Figure 3.15), in a following up reaction, acetonitrile was added. According to the HPLC results, decomposition of $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ was seen and pertechnetate was once again obtained (Figure 3.16).

Unfortunately, there was no change in all the substitution reactions attempted. It is hypothesized that $[^{99m}Tc(\eta^6\text{-napht})_2]^+$is not a good candidate compound for substitution reactions, as unsuccessful substitution of the ligands was seen in all previous attempts.

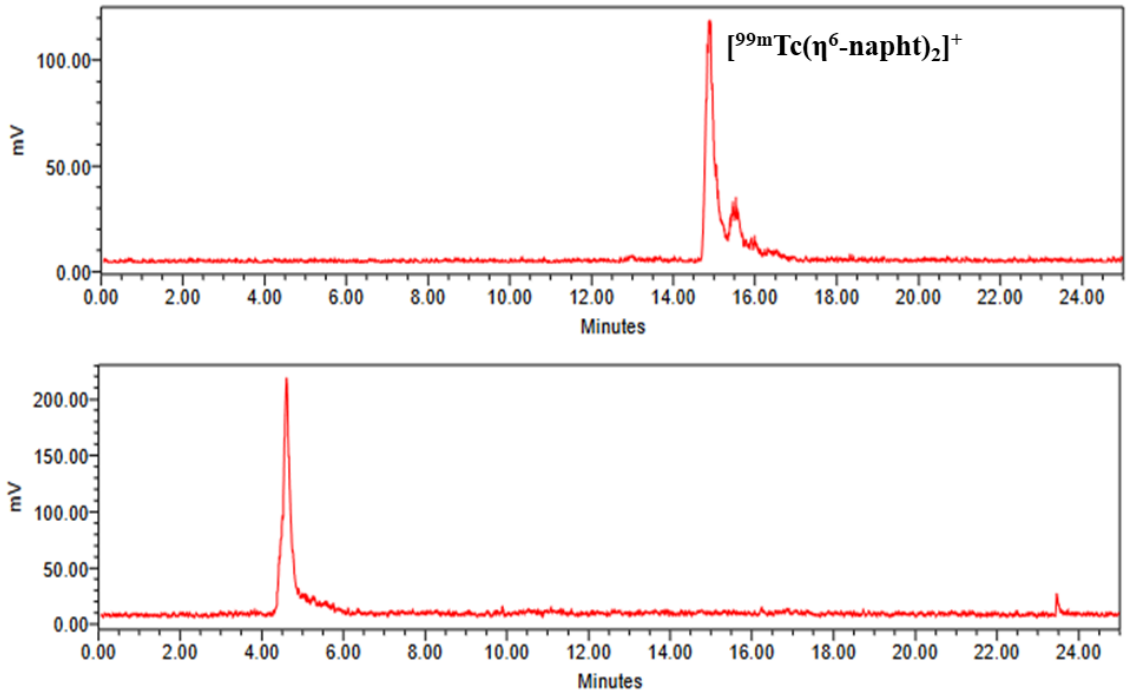

Figure 3.16 Substitution reaction with benzene (top=before reaction Radio-HPLC trace , ($[^{99m}Tc(\eta^6\text{-napht})_2]^+$), bottom=after reaction Radio-HPLC trace) (Column: Synergi 4U Polar-RP 80A size= 200mm X 4.60mm).

3.6 Conclusion

In the first part of the study, 3 different synthesis methods were used to obtain $[^{99m}Tc(\eta^6\text{-napht})_2]^+$. The method using Zn as a reducing agent was successful. This procedure was tried with different reaction times. For the experimental period, 75 minutes gave the best HPLC results. Then substitution reactions were attempted with various pharmaceuticals or simple arenes. First of all, 3 different solid compounds were tried:

Lidocaine, Lenalidomide, and L-DOPA. The final product could not be obtained in all results, so it was finally decided to use neat benzene. Unfortunately, all the substitution reactions attempted were unsuccessful. The fact that a procedure that previously worked for rhenium did not work for Tc-99m clearly shows the difference between these two elements, even though they are in the same group of elements. Further investigation is required to evaluate whether $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ is a good synthon for a two-step bis-arene formation.

3.7 References

(1) Roger Alberto, Roger Schibli, André Egli, August P. Schubiger, Ulrich Abram, and Thomas A. Kaden Journal of the American Chemical Society 1998 120 (31), 7987-7988 DOI: 10.1021/ja980745t

(2) Meola, G.; Braband, H.; Jordi, S.; Fox, T.; Blacque, O.; Spingler, B.; Alberto, R. Structure and Reactivities of Rhenium and Technetium Bis-Arene Sandwich Complexes [M(η6--Arene)2]+. Dalton Trans. 2017, 46, 14631– 14637, DOI: 10.1039/C7DT02072D

(3) Nadeem, Q.; Battistin, F.; Blacque, O.; Alberto, R. Naphthalene Exchange in [Re(η6-Napht)2]+ with Pharmaceuticals Leads to Highly Functionalized Sandwich Complexes [M(η6-Pharm)2]+ (M=Re/99mTc). Chem. - Eur. J. 2022, 28, 1213– 1216, DOI: 10.1002/chem.202103566

Chapter 4. Conclusion and Future Work

4.1 Conclusion

The first objective of this work was to label L-DOPA with Tc-99m in a one-step reaction without the use of a chelator

In chapter 2, the preparation of $[^{99m}Tc(\eta^6\text{-L-DOPA})^2]^+$ was successfully demonstrated. Since a clear and single peak was obtained in the HPLC results, the preparation of $[Re(\eta^6\text{-L-DOPA})^2]^+$ was attempted but all experiments were unsuccessful, and $[Re(\eta^6\text{-L-DOPA})^2]^+$ could not be obtained. The log P values obtained for $[^{99m}Tc(\eta^6\text{-L-DOPA})^2]^+$ showed a very hydrophilic character, which was confirmed by the *in vivo* biodistribution results. To overcome this problem, a hydrophobic L-DOPA (L2) derivative was synthesized and Tc-99m radiolabeling experiments were attempted. Unfortunately, the Tc-99m labeling experiments showed hydrolysis of the ligand forming $[^{99m}Tc(\eta^6\text{-L-DOPA})_2]^+$ instead of the desired product.

The second goal was to evaluate a twostep synthetic strategy to form ^{99m}Tc bis-arene complexes. In order to achieve that, $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ would be prepared as a synthon and the naphthalene ligand would be substitute by incoming highly functionalized arenes. The first step was to obtain $[^{99m}Tc(\eta^6\text{-napht})_2]^+$, and then prepare the substitution reaction with the corresponding phenyl groups.

In Chapter 3, $[Re(\eta^6\text{-napht})_2]^+$ was obtained and characterized according to literature procedures. Various reaction procedures for the formation $[^{99m}Tc(\eta^6\text{-napht})_2$ were tested and the procedure using Zn as a reducing agent was found to be the most suitable. The formation of $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ was confirmed by co-injection with it rhenium analogue $[Re(\eta^6\text{-napht})_2]^+$ and $[^{99m}Tc(\eta^6\text{-napht})_2]^+$. After successful formation of $[^{99m}Tc(\eta^6\text{-napht})_2]^+$, substitution reactions were tried with various pharmaceutical compounds and simple arenes. Various solvents were used including water, acetonitrile, NMP and 1,4-dioxane but either no reactivity of $[^{99m}Tc(\eta^6\text{-napht})_2]^+$ or total decomposition and subsequent formation of pertechnetate was observed.

4.2 Outlook

Although it seems exciting to label Tc-99m in a single step without the use of a chelator, this approach needs further development and more literature studies. An important challenge that needs to be addressed is that the formation of rhenium analogs for proper characterization of ^{99m}Tc-bis arenes by co-injection experiments is not straightforward. This difficulty was observed with in the unsuccessful formation of $[Re(\eta^6\text{-L-DOPA})_2]^+$. An alternative solution to this could be the stepwise synthesis of $[Re(\eta^6\text{-L-DOPA})_2]^+$ starting from $[Re(\eta^6\text{-benzene})_2]^+$. $[Re(\eta^6\text{-benzene})_2]^+$ has been successfully shown to be an adequate building block for the synthesis of highly functionalized Re-bis arenes. Although this strategy is straightforward, it can easily become a multistep synthesis depending on the structure of the desired complex.

The hydrophobic character of $[^{99m}Tc(\eta^6\text{-L-DOPA})_2]^+$ was a limitation in the *in vivo* studies, this problem can be overcome by using hydrophobic linker derivatives of L-DOPA without losing the carboxylic acid structure.

Unfortunately, all the conditions tested for the substitution of $[^{99m}Tc(\eta^6\text{-napth})_2]^+$ were unsuccessful. However, similar conditions have previously been used for the analogue $[Re(\eta^6\text{-napht})_2]^+$. The difference in reactivity between $[^{99m}Tc(\eta^6\text{-napth})_2]^+$ and $[Re(\eta^6\text{-napth})_2]^+$ could be explained by differences in rhenium and Tc-99m chemistry.