Synthesis and Characterization of Novel Phosphinimine Ligand Systems for Potential Applications in Radiopharmaceuticals

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by
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Dr. Sheryl Tucker

Dr. Krishna Sharma
Dedicated to,
my parents and teachers.
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“If I have seen further than others, it is by standing upon the shoulders of giants.”

--Isaac Newton—
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ABSTRACT

Multidentate phosphinimine ligands, C_{25}H_{49}N_{2}P_{2}Si_{2}(2) C_{34}H_{38}N_{2}P_{2}Si_{2}(3), and C_{50}H_{66}N_{3}P_{3}Si_{3}(4), were synthesized in near quantitative yields by Staudinger reaction using appropriate phosphines. Coordination chemistry of 2 and 3 with Re was performed and the X-ray crystallographic study of the Re ion-pair complex of ligand 2 is reported. Radiolabeling of ligands 2, 3 and 4 with 99mTc was performed. Simple mixing of ligand solutions with aqueous 99mTcO_{4}^{-} in saline solution produced ion-pairs with more than 95% yields. All three ion-pairs produced are stable for more than 24 h in organic media and in alcohols. The ion-pair produced with ligand 3 ((NH_{2}PPh_{2}CH_{2}PPh_{2}NH or O)^{+} (99mTcO_{4})^{-} ), upon heating has resulted in the neutral complex [(NH_{2}PPh_{2}CH_{2}PPh_{2}NH or O)^{+} (99mTcO_{3})]. Upon heating the ion-pair ([(NH_{2}PPh_{2}CH_{2}PPh_{2}NH)^{+} (ReO_{4})^{-} ] in the presence of Verkade’s superbase led to the rearrangement of the ligand via cleavage of the P-C-P bridge to produce P-N-P bridged compound.
CHAPTER 1

Introduction

1.1 Outlook

The development of specific ligand systems for potential utilization as bifunctional chelating agents (BFCAs) in the field of nuclear medicine has been the focal point of the radiopharmaceutical chemist over the past few decades. Radiopharmaceuticals are drugs containing a radionuclide and are used routinely in nuclear medicine for the diagnosis or therapy of various diseases.\textsuperscript{1,2} Radiopharmaceuticals are administered at extremely low concentrations (10^{-9} to 10^{-12} moles), which generally does not induce any pharmacological response. The Common property of radiopharmaceuticals is radiation such as $\alpha$, $\beta^-$, $\beta^+$ or $\gamma$. The choice of radionuclide depends on the intended application. For example $\beta^+$ or $\gamma$ emitting isotopes are used for diagnostic applications and $\alpha$ or $\beta^-$ emitting isotopes for treating diseases. There has been rapid progress in the production and availability of radionuclides for clinical applications. However, the development of delivery vectors for these radionuclides is still in its infancy.

Among all available radionuclides, radiometals provide a wider range of nuclear properties than non-metals and exhibit diverse chemistry, which is what makes them attractive candidates for the development of new radiopharmaceuticals.\textsuperscript{3-5} In order to utilize a radiometal as a radiopharmaceutical, it must be first chelated to a ligand system
that stabilizes the metal by satisfying its coordination valency. Metal-based radiopharmaceuticals are of two types: 1) Metal essential, where the radiometal ligand chelate is directly administered as is and the biological distribution properties are governed by the physiochemical properties of the coordination complex; 2) Metal tagged, in which case a bifunctional radiometal complex is attached to a suitable biomolecular delivery system (peptide, monoclonal antibody, etc.), which has high affinity for receptors expressed on cells/tissue of interest and controls the biological distribution. In either case, designing a suitable chelate that has optimal properties is of vital importance. The pharmacological advantage of using extremely low concentration has a disadvantage from the chemistry point of view because, it is highly impossible to characterize them using classical spectroscopic (UV/VIS, IR, NMR and Mass) methods. This makes it difficult to understand the chemistry at tracer levels.

Developing novel chelates has great importance in developing a successful radiopharmaceutical. One of the key requirements for a suitable chelate is extreme kinetic stability of the resulting metal complex, which is generally achieved by use of multidentate chelate ligands with appropriate coordinating heteroatoms on the backbone. Phosphorus based multidentate chelates are one of the competent candidates for this task. The phosphinimine family of ligands has a prominent scope as a novel ligand system, as they form kinetically stable compounds with most early transition metals. The chemistry of the phosphinimine ligands with radiometals has been explored before, but has achieved limited success. The primary focus of this thesis project is to synthesize some multifunctional phosphinimine ligands and study their radiometal complexation
chemistry, especially with technetium and rhenium. In this chapter a brief introduction to the topics related to the dissertation project will be discussed in the following sections.

1.2 Radiometals for Pharmaceutical Applications

There are two key components in metal based radiopharmaceuticals. One is the delivery vehicle (a covalent molecule, coordination complex or biomolecule that delivers the drug), which carries the radiometal to the target. And the second, the choice of the radiometal itself. One should consider these two components in designing a radiopharmaceutical. By varying the combination of delivery vector and the radiometal, a radiopharmaceutical can be designed for optimal efficacy. The selection of an appropriate radionuclide is the core determinant in designing any radiopharmaceutical.

The choice of radiometal depends primarily on intended application of the radiopharmaceutical (i.e., either diagnosis or therapy), which indeed depends on the physical characteristics of the radiometal. Other factors such as, availability and the cost of the radiometal can also influence the selection of a radionuclide.

For diagnostic applications, it is necessary that the radionuclide emit gamma photons or positrons (which eventually annihilate with an electron to produce two photons of 511 keV), which can penetrate through the body tissue and be detected externally. SPECT (Single Photon Emission Computed Tomography) imaging is usually used to detect low energy gamma rays (up to 380 keV) and PET (Positron Emission Tomography) is used with positron emitting radionuclides. For therapeutic applications, it is necessary to have particle emitting radionuclides which can deliver lethal doses of radiation to the disease site. The particles emitted by a therapeutic nuclide ($\alpha$, Auger e')
interact with DNA in the cell (to initiate strand breaks by ionization) or the cellular tissues (which can be ionized by the particles (α and β particles) to induce free radicals, which can also induce DNA strand breaks). Either method irreparably damages the DNA inducing cell death.

An ideal radionuclide with the following properties is best suited for radiopharmaceutical applications:

a. Decay mode should have high abundance or high percentage of desired radiation.

b. Emitted γ-rays or particles must have optimum energy for the application intended. For example, for imaging purposes the γ-ray energy should be around within 80-300 keV or for therapy the particle average energy must be around 0.5-3 MeV.

c. Radionuclides must be available in highly purified form and should be free from interfering chemical and radionclidic impurities.

d. Must be easily available and inexpensive.

e. Allow chemical modifications for incorporation onto targeting vectors, etc.

1.2.1 Radiometals for Diagnostic Applications

Widely used imaging modalities in nuclear medicine include X-ray, SPECT and PET. The choice of radiometal here depends on the modality. The energy of gamma photons has significant importance, since most gamma cameras are designed for specific energy windows, generally in the range of 100-300 keV. Radionuclides that decay with gamma energies lower than this range produce too much scatter, while gamma energies >3000 keV are difficult to collimate, and in both cases the image quality is
greatly reduced. In PET (positron emission tomography) imaging, the positron annihilation results in emission of two 511 keV photons 180° apart, which are detected by a circular array of detectors with coincidence circuits designed to specifically detect the 511 keV photons emitted in opposite directions. So, the first and the most important issue that can be addressed in choosing a radiometal for imaging is the energy of gamma photon. Other factors, like the half-life of the radionuclide, the decay mode, cost and availability of the isotope, must also be considered in developing a radiopharmaceutical. A small list of gamma- and positron-emitting radiometals, which have suitable nuclear characteristics is shown in Table 1.1. Of all the metals listed, only $^{68}$Ga, $^{99m}$Tc, $^{111}$In, $^{82}$Rb$^+$ and $^{201}$Tl are currently being employed in FDA approved radiopharmaceuticals for clinical applications.

### Table 1.1. Gamma and Positron Emitting Radiometals

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Half Life(h)</th>
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<th>$E_{\beta^+}$ or $E_{\gamma}$ (keV)</th>
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<tr>
<td>$^{55}$Co</td>
<td>17.5</td>
<td>Cyclotron</td>
<td>$\beta^+$</td>
<td>1513, 1037</td>
</tr>
<tr>
<td>$^{61}$Cu</td>
<td>3.3</td>
<td>Cyclotron</td>
<td>$\beta^+$</td>
<td>1220, 1150</td>
</tr>
<tr>
<td>$^{68}$Ga</td>
<td>1.1</td>
<td>Generator</td>
<td>$\beta^+$</td>
<td>1880, 770</td>
</tr>
<tr>
<td>$^{82}$Rb</td>
<td>0.022</td>
<td>Generator</td>
<td>$\beta^+$</td>
<td>3150</td>
</tr>
<tr>
<td>$^{99m}$Tc</td>
<td>6.0</td>
<td>Generator</td>
<td>IT(γ)</td>
<td>140</td>
</tr>
<tr>
<td>$^{111}$In</td>
<td>67.9</td>
<td>Cyclotron</td>
<td>EC(γ)</td>
<td>172,245</td>
</tr>
<tr>
<td>$^{201}$Tl</td>
<td>72</td>
<td>Cyclotron</td>
<td>EC(X-rays)</td>
<td>80</td>
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The ideal nuclear properties of $^{99m}$Tc and the utility of the $^{99}$Mo/$^{99m}$Tc generator system to produce an essentially carrier-free isotope, makes it an ideal radionuclide for SPECT imaging procedures in diagnostic nuclear medicine. It is most widely used of all the available radiometals because of its favorable nuclear properties.$^{18,19}$ $^{99m}$Tc is
available in the form of $^{99m}$TcO$_4^-$, essentially as a carrier-free isotope, from a $^{99m}$Tc/99Mo generator system. $^{99m}$Tc emits a single 140 keV gamma photon and decays by isomeric transition with a half-life of 6.02 hours, into the very long-lived radioisotope $^{99}$Tc ($t_{1/2} = 2.1 \times 10^5$ yrs). These ideal decay properties, in addition to easy availability via a generator system at no-carrier added (NCA) levels and, most importantly, its very low cost, has made technetium the most popular imaging radionuclide currently being used. It is estimated that technetium-$^{99m}$ is involved in nearly 20 million diagnostic procedures annually worldwide, which is nearly 85 percent of the diagnostic imaging procedures used in nuclear medicine today.\(^{20}\) The current thesis focuses on utilizing technetium based systems for radiopharmaceutical applications.

$^{111}$In is a cyclotron ($^{112}$Cd (p, 2n) $^{111}$In) produced radionuclide that is currently being used in nuclear medicine for diagnostic imaging procedures. \(^{111}$In emits two gamma photons ($E_{\gamma 1} = 172$ keV (88%), $E_{\gamma 2} = 247$ keV (94%)), has a half life of 68 hours, and decays by electron capture to stable cadmium-$^{111}$.\(^{21}\) This radionuclide is currently being used for the labeling of white blood cells for imaging focal sites of infection and evaluation of cerebral spinal fluid pathways.\(^{22-24}\) $^{111}$In-DTPA-Octreotide is a site specific radiopharmaceutical that has been found to bind to somatostatin receptor-positive tumors.\(^{25,26}\) This complex provides an excellent example of a peptide model for the labeling of biomolecules.

Gallium-$^{68}$ is another interesting radiometal which decays by positron emission (89%) with a half-life of 68 minutes, and it is available from a $^{68}$Ge/$^{68}$Ga generator.\(^{27}\) The long half-life of the parent nuclide $^{68}$Ge (280 days) gives the generator a useful life of 1-2 years, allowing PET imaging at facilities without an onsite cyclotron. The
generator is commercially available, but to date the usage of $^{68}$Ga is limited to a very few number of clinical applications due to problems such as poor target to background ratio, which is due to the fact that, Ga$^{3+}$ mimics Fe$^{3+}$ in vivo and binds to serum proteins (i.e. transferrin) with high affinity thereby increasing the uptake in blood. Another limiting factor for the usage of this radionuclide is its cost.

Thallium–201 another cyclotron produced radionuclide ($^{203}$Tl(p,3n) $^{201}$Pb(p,n) $^{201}$Tl), and has a half-life of 72 hours and decays by electron capture, emitting X-rays of energy 80.3 keV and 167 keV. $^{201}$Tl has been used in myocardial imaging. It is a monocation and mimics potassium and uptakes via K$^+$-Na$^+$ ATPase pump and rapidly leaving the vascular spaces and accumulating in lean muscle tissue including the myocardium, generating the image. $^{201}$Tl has also been used in detecting and differentiating between certain types of cancers.

In addition to these radiometals there are several $\gamma$ or $\beta^+$ emitting radionuclides like $^{82}$Rb, $^{55}$Co, $^{61}$Cu, $^{117m}$Sn etc., that also have potential interest in the field of diagnostic nuclear medicine. $^{29}$

1.2.2 Radiometals for Therapeutic Applications

Radiometals emitting particulate radiation are essential for therapeutic applications. Depending on the nature of the particle emitted, these can be broadly divided into 1) $\alpha$ - particle emitting radionuclides and 2) $\beta$ - emitting radionuclides.

$\alpha$ - particle emitters

$\alpha$ particle emitting radionuclides are attractive to cure very small tumors or micrometastases, where it is more advantageous to use particulate radiation with a range
of only a few cell diameters.\textsuperscript{2,30} $\alpha$ - particles are high energy helium nuclei that produce a high density of ionization along their path and have very high linear energy transfer (LET) over short ranges (usually 40 $\mu$m to 100 $\mu$m). Though there are vast numbers of $\alpha$ - particle emitting radionuclides available, their utility for \textit{in vivo} applications is rare because of very long half-lives of these radionuclides. In addition to this is, the production of these radionuclides with acceptable radionuclidic purity is a hard task. The only $\alpha$-emitters that have received considerable attention for radiotherapeutic applications are astatine-211,\textsuperscript{31} a cyclotron produced isotope ($^{209}$Bi ($\alpha$,2n)$^{211}$At) with a half-life of 7.2 hours and an average $\alpha$ energy of 6.8 MeV, and bismuth-212, which is available through $^{225}$Ac/$^{212}$Bi generator system and has a half life of 1 hour, with average $\alpha$ particle energy of 7.8 MeV. Because of these limitations, the utility of $\alpha$-particle emitting isotopes in radiopharmaceuticals is currently limited.

**$\beta$ - particle emitters**

The most extensively used radionuclides for radiotherapeutic applications in current clinical practice are, radionuclides that emit $\beta$ particles.\textsuperscript{2,32-34} $\beta$ particle emitting radionuclides produce a highly homogeneous dose of radiation, even though their deposition is heterogeneously distributed in target tissues.\textsuperscript{2} The $\beta$ particles are high energy electrons emitted from the nucleus as a spectrum or continuum of energies up to a maximum value. The range of these $\beta$ particles is higher than $\alpha$ particles, which is compensated by low ionization accounting for low LET. The utility of the particles depends on the energy of the $\beta$ particle emitted. They can be used effectively against large tumors (large avg$E_{\beta}$ max) or small tumors (small avg$E_{\beta}$ max), though $\beta$ emitters are
not well suited for microscopic tumors. A list of β emitting radionuclides with potential therapeutic applications is given in Table 1.2.

Table 1.2: Radionuclides with Therapeutic potential

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>$t_{1/2}$ (days)</th>
<th>$E_{\beta_{\text{max}}}$ (MeV)</th>
<th>γ-ray energy (keV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{47}$Sc $^b$</td>
<td>3.4</td>
<td>0.6</td>
<td>159 (68%)</td>
</tr>
<tr>
<td>$^{64}$Cu $^b$</td>
<td>0.5</td>
<td>0.57</td>
<td>511 (38%)</td>
</tr>
<tr>
<td>$^{67}$Cu $^b$</td>
<td>2.6</td>
<td>0.57</td>
<td>184 (48%) 92 (23%)</td>
</tr>
<tr>
<td>$^{89}$Sr $^a$</td>
<td>50.5</td>
<td>1.46</td>
<td>-</td>
</tr>
<tr>
<td>$^{90}$Y $^c$</td>
<td>2.7</td>
<td>2.27</td>
<td>-</td>
</tr>
<tr>
<td>$^{105}$Rh $^a$</td>
<td>1.5</td>
<td>0.57</td>
<td>319 (19%) 306 (5%)</td>
</tr>
<tr>
<td>$^{111}$Ag $^b$</td>
<td>7.5</td>
<td>1.05</td>
<td>342 (6%)</td>
</tr>
<tr>
<td>$^{117}$mSn $^a$</td>
<td>13.6</td>
<td>0.13</td>
<td>158 (87%)</td>
</tr>
<tr>
<td>$^{149}$Pm $^a$</td>
<td>2.2</td>
<td>1.07</td>
<td>286 (3%)</td>
</tr>
<tr>
<td>$^{153}$Sm $^a$</td>
<td>1.9</td>
<td>0.8</td>
<td>103 (29%)</td>
</tr>
<tr>
<td>$^{166}$Ho $^a$</td>
<td>1.1</td>
<td>1.6</td>
<td>81 (6.33)</td>
</tr>
<tr>
<td>$^{177}$Lu $^a$</td>
<td>6.7</td>
<td>0.50</td>
<td>113 (6.4%) 208 (11%)</td>
</tr>
<tr>
<td>$^{186}$Re $^a$</td>
<td>3.8</td>
<td>1.07</td>
<td>137 (9%)</td>
</tr>
<tr>
<td>$^{188}$Re $^d$</td>
<td>0.7</td>
<td>2.11</td>
<td>155 (15%)</td>
</tr>
</tbody>
</table>

$^a$ Radionuclides produced in nuclear reactors. $^b$ Radionuclides produced in charged particle accelerators. $^c$ $^{90}$Y is generated from a long-lived $^{90}$Sr parent, which is reactor produced and supplied only as a NCA $^{90}$Y product. $^d$ $^{188}$Re is produced as a NCA reagent from a $^{188}$W/$^{188}$Re generator system.

There are several β particle emitting radionuclides available but, few like $^{90}$Y, $^{166}$Ho and $^{186,188}$Re became more popular, because they can be obtained via generator systems. Rhenium – 188 is a radiometal with a half-life of 17 hours, emits a single β particle ($E_{\beta_{\text{max}}} = 2.12$ MeV) and can be conveniently obtained via a $^{188}$W/$^{188}$Re generator system. It also emits one γ ray of energy 155 keV (15%), which allows simultaneous in
vivo detection and can be produced at NCA (no carrier added) levels. These ideal nuclear decay properties have made $^{188}$Re one of most widely investigated radiometals.\textsuperscript{35} Another attractive feature, and the driving force for use of rhenium isotopes is having same chemistry as technetium, and can be used as matched pair with $^{99m}$Tc. So, the systems that are effectively used for technetium can often be utilized for rhenium based radiopharmaceuticals. Furthermore, their physical similarities (i.e., shape, size, charge, etc.) suggest the indistinguishability between the matched pair to a biological system. Therefore, information obtained from the diagnostic studies with $^{99m}$Tc can be used to evaluate radiotherapeutic administration using $^{188}$Re.

Rhodium-105 is another attractive radiometal for therapeutic applications in nuclear medicine. $^{105}$Rh can be produced in high specific activity in a nuclear reactor facility by the neutron irradiation of ruthenium-104. It emits $\beta$ particles of energy ($E_{\beta_{\text{max}1}} = 0.56$ MeV and $E_{\beta_{\text{max}2}} = 0.25$ MeV) and two gamma photons of energy 306 keV (5%) and 319 keV (19%), making it an ideal isotope for small tumors and allowing simultaneous in vivo detection.\textsuperscript{36-38}

Yttrium exhibits similar chemistry as Indium. Therefore the matched pair concept can also be effectively utilized in the case of $^{90}$Y. Yttrium-90 has a half life of 2.7 days, emits a high energy $\beta^-$ particle ($E_{\beta_{\text{max}}} = 2.27$ MeV) and is available in carrier free form from a strontium-90 ($t_{1/2} = 28$ years) generator. Although the chemistry of indium and yttrium are presumably alike, differences in the kinetic properties of $^{90}$Y and $^{111}$In have been observed.

Gold-199 is another radionuclide carrying vast potential for the design and development of tumor-specific radiopharmaceuticals for treatment of human
metastases.\textsuperscript{43,32} Gold-199 is prepared by neutron irradiation of a \(^{198}\)Pt target. Gold-199 decays by \(\beta\) emission \((E_{\beta_{\text{max}}} = 0.460 \text{ MeV})\) with a half-life of 3.2 days. A simultaneous gamma emission \((E_\gamma = 158 \text{ keV})\) allows \emph{in vivo} tracking of the \(^{199}\)Au therapeutic dose.

\(^{89}\)Sr (as \(^{89}\)SrCl\(_2\)), with a half-life of 50.5 days and \(E_{\beta_{\text{max}1}}\) of 1.46 MeV, and \(^{153}\)Sm (as \(^{153}\)Sm-EDTMP), with half-life of 1.9 days and \(E_{\beta_{\text{max}1}}\) of 0.8 MeV, which are reactor produced isotopes and have been approved by the FDA for routine use in humans as bone pain palliation agents. Microspheres and particles labeled with \(^{166}\)Ho, \(^{90}\)Y and \(^{153}\)Sm have been used for liver tumors by intra-arterial radionuclide therapy\textsuperscript{39} and radiation synovectomy.\textsuperscript{40,41} Lutetium-177, copper-67\textsuperscript{42} and silver-111 are also being explored for their applications in radiotherapy.

1.3 \textbf{Site Specific Radiopharmaceuticals}

Radiopharmaceuticals are fast emerging as agents of choice for various diseases, especially cancer, in both diagnostic and therapeutic applications because the current systems are failed to detect and/or treat diseases in the early stages. Diagnostic radiopharmaceuticals reveal the physiology and function of an organ or tissue, in contrast to anatomy as registered by usual radiological procedures such as X-rays. Therapeutic radiopharmaceuticals deliver lethal doses of ionizing radiation specifically to the tumor site in sharp contrast to external beam therapy, which most often results in exposure of radiation to non-cancerous and essential tissues/organs, and gives side effects such as depressed immunity and/or increased incidence of secondary cancers.
The mode of incorporating the radionuclide on a target molecule solely depends on the elemental chemistry of the radionuclide. Of all the available radionuclides, radiometals provide a wider range of nuclear properties than nonmetals and exhibit diverse chemistry which makes them attractive candidates. Most of the current radiopharmaceuticals are simple inorganic complexes, which contain a metal chelated by a ligand. Some of the FDA approved radiopharmaceuticals that are currently being used as radiopharmaceuticals are shown in the Figure 1.1. Ceretec™ and Neurolite™ are being used as diagnostic brain imaging agents,⁴⁴⁻⁴⁶ Tc-MAG₃™ is being used for measuring renal function, and Quadramet™ for bone pain palliation therapy.⁴⁷ The biodistribution and other properties of these radiopharmaceuticals depend on the properties of the metal complex on whole and therefore the complex must remain intact to reach the target.

Figure 1.1: Examples of FDA Approved Radiopharmaceuticals
Recent technological advances in the fields of molecular biology, combinatorial chemistry and peptide biochemistry are contributing to the targeting of individual cells on the basis of specific receptors, metabolic pathways, actual genetic sequencing, etc. These advances have led to the development of the concept of site specific delivery of radiopharmaceuticals, in which the radioelement of choice is tagged to a biomolecular vector, that is delivered to the site of the disease (usually cancer), either for diagnostic (imaging) or therapeutic purposes.

The first biomolecular vectors that were used for achieving the site specific delivery of radiometal were monoclonal antibodies. The list now includes small peptides, steroids, small organic molecules, and peptide nucleic acids. With the growing list of the biomolecular vectors available for targeting cancerous tissue, the task at hand is to tag the vector with the radiometals without affecting the biospecificity. To achieve the goal of labeling biomolecular vectors with a radiometal, there are two commonly used methods: 1) the direct labeling method and 2) the indirect labeling method or the bifunctional chelating agent (BFCA) method.

### 1.3.1 Direct labeling method:

In this method of labeling proteins or monoclonal antibodies, naturally occurring functional groups like -SH, -NH₂, -OH, or –COOH present within the protein sequence are used to chelate the metallic radioisotope. This approach offers a simplistic approach to protein or antibody labeling, with procedures often being only one or two steps. Furthermore, this approach allows for the use of short-lived radioisotopes for applications in nuclear medicine. A typical procedure involves heating the solution of biomolecular
vector and radiometal, usually in the presence of reducing agents like Sn^{+2}, 2-mercaptopethanol, dithioerythritol, etc. (for Tc and Re), which reduces the disulfide linkages and hence increases the number of free sulfhydryl functionalities within the protein for efficient metal chelation. A schematic representation of the direct labeling method is shown in Figure 1.2.

$$\text{M-Complex} = ^{99m}\text{Tc} \left( ^{188/186}\text{Re} \right) \text{glucoheptonate or citrate etc.}$$

**Figure 1.2: Direct Labeling Protocol**

This method has been successfully demonstrated for labeling antibodies, peptides like α-CCMSH, etc. and the *in vitro* and *in vivo* studies have indicated that these radiolabeled biomolecules do retain their biospecificity. However, as this method offers a number of advantages for efficient protein labeling, there are also a number of disadvantages that must be considered. First, the method is not universally applicable to all the biomolecules (such as steroids) and is restricted mostly to peptides that contain thiol or disulfide bonds. Second, the reduction of disulfide linkages in peptides and antibodies often results in loss of biospecificity (loss of receptor binding), thereby
making the whole process ineffective. Furthermore, issues like non-specific binding of the metal to the biomolecule, lack of knowledge of the oxidation state of the metal, reduction in binding efficacy to receptors, etc., makes this procedure less attractive. In order to overcome these difficulties the indirect labeling approach or the bifunctional chelating agent (BFCA) approach has been developed.

1.3.2 Indirect labeling or Bifunctional chelating agent method:

This method of labeling peptides or antibodies indirectly is also termed as Bifunctional Chelating Agent (BFCA) method. A bifunctional chelating agent is a chelating ligand that contains an organic functionality such as -NH₂, -COOH, which allows conjugating the ligand to a biomolecule. A schematic representation of the bifunctional chelating method to target receptors is shown in Figure 1.3.

As shown in Figure 1.3, a BFCA consists of three necessary components.

1. Chelate, which binds to the metal.
2. Functionality, which aids in conjugating the biomolecule to the chelate.

3. Linker or spacer prevents chelate’s interference with biomolecule.

Chelate

The most important component of BFCA design is the chelate that contains heteroatoms (W, X, Y, Z), which can donate a lone pair of electrons to metal and form either covalent or coordinate covalent bonds with the radiometal. The primary function of the chelate is to produce kinetically inert radiometal complexes that are stable \textit{in vitro} and \textit{in vivo}. The primary aim of this thesis project is to synthesize new chelating ligands with favorable kinetics.

Functionality

The functionality is usually an amino group or a carboxylate group, which can be utilized in covalently linking the chelate to biomolecule. The functionality must be designed to minimize its affinity towards active sites in the biomolecule and also the radiometal.

Linker

The function of a linker is to direct the chelate away from the biomolecule, so as to minimize interference in the biological functions of the biomolecule by the radiometal chelate. Therefore the linker acts as a spacer between the chelate and biomolecule. The linker can also be effectively used to modify or tune the hydrophobic/hydrophilic properties of the BFCA. By choosing an appropriate chelate and linker, the efficacy of the radiopharmaceutical can be greatly enhanced.
1.4 Radiolabeled peptides for tumor imaging and therapy:

After the discovery of the method for producing monoclonal antibodies (MAbs), these highly specific proteins were radiolabeled and extensive study was done in this area for their utility in tumor imaging and therapy.\(^1\) Though these molecules have high binding affinity and high specificity for tumor tissue, one difficulty with MAbs is their size, which makes them kinetically slow and creates high background. Small peptides became an attractive alternatives to monoclonal antibodies because of their small size and rapid blood clearance. Many naturally-occurring peptides exhibit extremely high affinities for cell-surface receptors. The rapid kinetics also decreases the diagnosis timings. A few radiolabeled peptides include \(^{111}\text{In-DTPA-octreotide,}^{111}\text{In-DOTA-Tyr}^3\text{-octreotide and}^{90}\text{Y-DOTA-Tyr}^3\text{-octreotide.}\(^1\)

1.4.1 Octreotide-based radiopharmaceuticals:

Octreotide was developed as a somatostatin analogue for suppression of hypersecretion to control the symptoms of neuroendocrine disease.\(^1\) Somatostatins are 14 amino acid and 28 amino acid cyclic peptides.\(^68\) The significant appearance of these peptides can be found in several organ systems such as the hypothalamopituitary system, gastrointestinal tract, pancreas, etc. They inhibit a wide spectrum of physiological functions including peptide hormone secretion.
Somatostatin receptors are molecular structures present on the surface of a cell that bind with somatostatin. There are five somatostatin receptor subtypes identified in different organs in the human body: Sst1, Sst2, Sst3, Sst4 and Sst5. A high density of somatostatin receptors are expressed on the majority of neuroendocrine tumors such as growth hormone-secreting pituitary adenomas, gastroenteropancreatic tumors, phaeochromocytomas, etc. Most tumor tissues preferentially express Sst2 receptors, and less frequently express Sst1, Sst3 and Sst5. The Sst4 receptor is only rarely detected. Somatostatins are degraded in vivo by peptidases, causing them to clear from the circulation very rapidly (2-3 min). To overcome this drawback, somatostatin analogues have been prepared. Octreotide, one of these analogues, contains D-amino acids and a C-terminal alcohol group, and a disulfide bond between its two cysteine residues.
Tyr<sup>2</sup>-Octreotide was developed which accommodates labeling this peptide with several iodine isotopes for imaging and therapy. But the success was limited due to the higher uptake in non-targeted tissues and lower physiological life. Among all other radionuclides<sup>111</sup>In has achieved greater success in labeling with Octreotide. <sup>111</sup>In-DTPA-Octreotide (Octreoscan<sup>®</sup>) was the first FDA approved peptide receptor agent that is available in market. But the poor imaging quality necessitates the development of alternates. Several PET based agents have been made and are being studied. As<sup>99m</sup>Tc been the working horse in the field of SPECT imaging, developing novel<sup>99m</sup>Tc metal chelates to label it with Octreotide may solve many problems. But all known chelates for<sup>99m</sup>Tc require Sn<sup>2+</sup> as external reducing agent to facilitate the reduction of generator produced<sup>99m</sup>TcO<sub>4</sub><sup>-</sup> to complex with the chelates. Developing an agent which avoids the usage of external reducing agent with minimum synthetic difficulties is a challenge for the organometallic chemists. Strategies to stabilize Tc(VII) and Re(VII) will provide unprecedented opportunities for the development of Tc(VII) labeled tumor-avid peptides and would open up new routes to Tc(VII) radiopharmaceuticals.

1.5 Phosphinimine ligand systems for the stabilization of Tc(VII) and Re(VII):

Phosphinimine ligands (e.g., R<sub>3</sub>R<sub>2</sub>R<sub>3</sub>P=NSiMe<sub>3</sub>) are multi electron donors and have the capacity to stabilize transition metal cores in their high oxidation state.<sup>12,13</sup> Earlier work from our laboratory has demonstrated that phosphinimine ligands (R<sub>3</sub>P=NSiMe<sub>3</sub>, R=Ph or alkyl) upon interaction with<sup>99m</sup>TcO<sub>4</sub><sup>-</sup> (or<sup>188</sup>ReO<sub>4</sub><sup>-</sup>) at 25°C produced an ion pair R<sub>3</sub>P=NH<sub>2</sub><sup>+</sup><sup>99m</sup>TcO<sub>4</sub><sup>-</sup> (or R<sub>3</sub>P=NH<sub>2</sub><sup>+</sup><sup>188</sup>ReO<sub>4</sub><sup>-</sup>) almost instantaneously.
in >99% even with low ligand concentrations (~10$^{-4}$ M).\textsuperscript{14,15} Upon heating, the ion pairs can be dehydrated to produce the neutral Tc(VII) or Re(VII) complexes $R_3P=NMO_3$ (M = $^{99m}$Tc or $^{188}$Re).\textsuperscript{14,15} The ion pairs and neutral complexes were fully characterized using spectroscopic and x-ray crystallographic analysis.\textsuperscript{14,15} \textit{In vitro} studies indicated that the neutral Tc(VII) phosphinimine complex $R_3P=NMO_3$ slowly reverts to the ion pair $R_3P=NH_2^+ MO_4^-$ in aqueous solutions of BSA.

\begin{align*}
R_3P=N-SiMe_3 & \xrightarrow{MO_4^-} R_3P=NH_2^+ MO_4^- & \text{Heat} & \rightarrow R_3P=N-MO_3 \\
(M= ^{99m}$Tc, $^{99}$Tc, Re and $^{188}$Re)
\end{align*}

\textbf{Scheme 1: General route for the synthesis of Tc(VII) and Re(VII) metal complexes with phosphinimines}

Provision of additional phosphinimine ligating sites to $^{99m}$Tc (or Re) could increase bonding capacity and therefore, may lead to increased \textit{in vitro/in vivo} stability of the resulting metal complexes. Considering the highly efficient kinetics of phosphinimine ligands towards complex formation with $^{99m}$TcO$_4^-$ (and ReO$_4^-$) and the propensity of such ligands to produce stable and well defined Tc(VII) (or Re(VII)) compounds, the possibility of using bis and tris phosphinimine frameworks to afford kinetic stability to Tc(VII) (and Re(VII)) compounds can be explored.

1.6 \textbf{Research goals of the thesis project:}

Considering the highly efficient kinetics of phosphinimine ligands towards complex formation with $^{99m}$TcO$_4^-$ (and ReO$_4^-$) and the propensity of such ligands to
produce stable and well defined Tc(VII) (or Re(VII)) compounds, possibility of using bis and tris phosphinimine frameworks to afford kinetic stability to Tc(VII) (and Re(VII)) compounds were explored.

The primary objective of this thesis project is to synthesize various multidentate phosphinimine ligands and explore their complexation properties with Re precursors at the macroscopic level and $^{99m}$Tc precursors at the tracer level. Specifically, the goals of this thesis project include:

- the design, synthesis and characterization of multidentate phosphinimine ligand frameworks; and
- investigation of coordination chemistry of these ligand frameworks with Re metal and the radiochemistry of these ligand frameworks with technetium-99m and evaluate in vitro and in vivo properties.
CHAPTER 2
Experimental

2.1 Materials and Methods

All reactions were carried out under purified nitrogen by standard Schlenk techniques. Solvents were purified by standard methods and distilled under nitrogen prior to use. In suitable cases, Sure Seal™ (Aldrich) solvents were used directly without any purification or distillation procedures. Reagents such as 2, 6 Lutidine, Tetramethylethylenediamine (TMEDA), Diisopropyl phosphine chloride, azidotrimethylsilane, 1,1,1-Tris (diphenylphosphinomethyl) ethane and 2-diphenylphosphinopyridine were purchased from Aldrich Chemical Company, and were used without further purification unless noted. Reagents, Ammonium perrhenate, bis (Diphenylphosphino)methane, Verkade super base, were purchased from Strem Chemicals Company and were used without further purification unless noted. For reactions involving n-Butyl Lithium used was a 2.5 M solution in hexanes, purchased as a Sure Seal Bottle from Aldrich Chemical Company, was used as it was received. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker ARX-300 spectrometer using D$_2$O, CDCl$_3$, CD$_3$CN, etc., as solvents. Deuterated solvents were purchased from Aldrich or Cambridge Isotopes and were used directly. The $^1$H (300 MHz) NMR chemical shifts are reported in ppm, downfield from the internal standard tetramethylsilane (TMS). The $^{31}$P NMR (121.5 MHz) spectra were recorded with 85% phosphoric
acid as an external standard with positive chemical shifts downfield from the standard. The signals in NMR are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and so on. X-ray intensity data for 5 and 6 were collected on a Siemens SMART CCD system using the omega scan mode. Data were corrected for absorption using the program SAINT. Crystal decay for the complexes was insignificant and correction deemed unnecessary. The structures were solved using SHELXS 86 and refined by the full matrix least squares method on \( F^2 \) using SHELXL 93. All non-hydrogen atoms were refined anisotropically and hydrogen atoms were placed in calculated positions, with thermal parameters depending on parent atoms.

HPLC analyses of all metal complexes were performed on a Shimadzu LC 10AT VP system equipped with a SPD10AVP UV-VIS detector, a sodium iodide crystal containing radiometric detector and a PC loaded with Class VP data collecting software. HPLC grade acetonitrile was purchased from Fisher Scientific and degassed before use. A Phenomenex Jupiter C-18 (5\( \mu \), 4.1 X 250 mm) column was used with a flow rate of 1.0 mL/min unless specified differently. Technetium-99m was eluted from a \(^{99}\text{Mo}/^{99m}\text{Tc}\) generator provided by Mallinkrodt Medical, Inc.

### 2.2 Synthesis of Phosphinimine Ligand systems

#### 2.2.1 Synthesis of Phosphine C\(_{19}\)H\(_{31}\)P\(_2\) (1):

![Phosphine C\(_{19}\)H\(_{31}\)P\(_2\) (1)](image)
A mixture of 2,6 Lutidine (2.0 mL, 17 mmol), Tetramethylethylenediamine (TMEDA) (5.2 mL, 34.4 mmol) and diethyl ether (40 mL) were taken in an round bottom flask, purged with nitrogen and cooled to 0 °C in an ice bath. To this solution Bu"Li (21.6 mL) was added drop wise at 0 °C. After the addition was complete, the reaction was brought to room temperature and stirred for 10 h. This solution was then added to a cooled (-90 °C) solution of Pr iPr 2 PCl (5.48 mL, 34.43 mmol) in 40 mL of ether. The reaction mixture was stirred for 30 min at -90 °C and then at room temperature for a further 15 h. Filtration and removal of solvent under vacuum gave a yellow oil. Purification at reduced pressure yielded a pale yellow oil. (4.0 g, 70% yield) of the 1. ³H NMR (300 MHz, CDCl3, 25°C): δ (ppm) 0.95-1.17 (m, 24H), 1.70-1.80 (m, 4H), 2.88 (d, $J_{P-H} = 2.14$ Hz, 4H), 7.05 (d, $J = 6.0$ Hz, 2H), 7.38 (t, $J = 6.0$ Hz, 1H). ³¹P NMR (121.5 MHz, CDCl3, 25°C): δ (ppm) 13.67. b.p 110-120 °C at 0.01 Torr.

$$\text{i) n-BuLi, TMEDA, 0}^\circ\text{C-RT} \xrightarrow{\text{ii) iPr}_2\text{PCl, }-90}^\circ\text{C-RT} \text{Ether}$$

\[\text{H}_3\text{C}\text{NCH}_3\]

2.2.2 Synthesis of Phosphinimine ligand C_{25}H_{49}N_2P_2Si_2 (2):

\[\text{iPr}\]_2\text{P} - \text{SiMe}_3 \quad \text{N}

2
A mixture of (Pr₂PCH₂)₂C₅H₃N-2,6 (1) (10.30 g, 30.34 mmol) and Me₃SiN₃ (8.9 mL, 67.05 mmol) in toluene (50 mL) was heated at 110-130 °C for 10 h with stirring. After cooling to room temperature, volatiles were removed under vacuum and the residue was distilled at reduced pressure (0.01 Torr). A pale yellow oil of the title compound (14.49 g, 92.9%) was obtained, b.p = 150-160 °C/0.01 mmHg. ¹H NMR (300 MHz, CDCl₃, 25 °C): δ (ppm) -0.05 (s, 18H,), 0.96-1.16 (m, 24H,), 1.87-1.90 (m, 4H), 3.06 (d, Jₚ-H = 13.0 Hz, 4H,), 7.24 (d, J = 7.7 Hz, 2H,), 7.42 (t, J = 7.51 Hz, 1H). ³¹P{¹H} NMR (121.50 MHz, CDCl₃, 25 °C): δ (ppm) 23.52.

2.2.3 Synthesis of Phosphinimine ligand C₃₄H₃₈N₂P₂Si₂ (3):

Bis-(diphenylphosphino)methane (2 g, 5.2 mmol) was taken in a two-necked flask fitted with a condenser and purged with nitrogen. To this, 5 mL of azidotrimethylsilane was added and refluxed for 12 h. The excess azide was evaporated
under vacuum. The resulting white solid was recrystallized using dry acetonitrile to yield a white crystalline powder (2.8 g, 96% yield). $^1$H NMR: δ (ppm) 3.4 (t, 2H), 7.41 (m, 20H), $^{31}$P NMR: δ (ppm) -3.8. b.p 162°C

![Chemical structure](image)

### 2.2.4 Synthesis of Phosphinimine ligand $C_{50}H_{66}N_3P_3Si_3$ (4):

A double-necked round bottom flask purged with nitrogen, loaded with 0.9 g of 1,1,1-tris(diphenylphosphinomethyl)ethane. To this, 3 mL of azidotrimethylsilane was added and refluxed for 12 h. The excess azide was evaporated under vacuum. The resulting white solid was recrystallized using toluene. (0.98 g, 98% yield). $^1$H NMR: δ (ppm) 0.08 (s, 27H) 1.45 (s, 3H) 2.61 (d, 6H) 7.08 (m, 12H), 6.49 (m, 18H), $^{31}$P NMR: δ (ppm) 1.8(s)
2.3 Synthesis of Re (VII) complex ion-pair with Phosphinimine ligand

\( \text{C}_{25}\text{H}_{49}\text{N}_{2}\text{P}_{2}\text{Si}_{2}:: \)

To an CH\(_3\)CN (25 mL) solution of 2 (1 g, 1.94 mmol) was added an aqueous solution (5 mL) of NH\(_4\)ReO\(_4\) (1.04 g, 3.86 mmol) dropwise at room temperature. The mixture was stirred for 1 hr before the volatiles and aqueous solvents were removed under vacuum to produce a cream color solid. Recrystallization of this compound in CH\(_3\)CN produced very pale yellow colored cubic shaped crystals (1.6 g, 94.6\%). \(^{31}\text{P}\) NMR (121 MHz, CDCN, 25\(^\circ\)C) \(\delta\) (ppm) 65.6. m.p. 163 \(^\circ\)C.
2.4 Synthesis of $^{99m}$Tc(VII) complex ion-pair with Phosphinimine ligand 2: $^{+}[\text{(NH}_2\text{P(iPr)}_2\text{CH}_2\text{C}_5\text{H}_3\text{N CH}_2\text{P(iPr)}_2\text{NH}) (^{99m}\text{TcO}_4^-)]$:

To a solution of 2 (2 mg/mL in toluene or CH$_2$Cl$_2$ or CHCl$_3$) 1 mCi (37 MBq) of $^{99m}$TcO$_4^-$ (in a 0.1 mL saline solution) obtained as eluent from $^{99}$Mo/$^{99m}$Tc generator (Mallinckrodt, Inc.) was added. The mixture was vortexed for 1 minute. 5 minutes after vortexing, the organic layer, which has the ion-pair complex, was separated from the aqueous layer. The radiochemical purity and the yield of the complex were determined by paper chromatography and reversed phase HPLC (High Pressure Liquid Chromatography). The mobile phase of the HPLC system consisted 100% CH$_3$CN. Since the ligand is more lipophilic, aqueous buffer solution causes the compound to stick to the stationary phase and makes the elusion difficult. To avoid this issue, 100% CH$_3$CN was used as the mobile phase. The ion-pairing agent TFA also avoided because, the acid cleaves the phosphorous nitrogen bond. The run time was 25 min with a flow rate 1.0 mL/min. The paper chromatography analysis involved spotting the complex near the bottom (origin) of 10x1 cm paper strips (Whatman ET-31 paper). Then the strips were developed with saline (0.9% aqueous NaCl), 100% acetone and 100% ethyl acetate. Quantification of the activity on the paper segments and the R$_f$ values were determined using the AMBIS radio chromatographic scanning system.
2.4.1 Stability studies of $[^99mTcO_4^-](\text{NH}_2\text{P(iPr)}_2\text{CH}_2\text{C}_5\text{H}_3\text{N CH}_2\text{P(iPr)}_2\text{NH})$:

The stability of the complex $\text{NH}_2\text{P(iPr)}_2\text{CH}_2\text{C}_5\text{H}_3\text{N CH}_2\text{P(iPr)}_2\text{NH}^+ (\text{aq. Na }[^99mTcO_4^-])$ was measured as a function of time. The complex obtained was peak purified on HPLC by collecting the fraction corresponding only to the complex. The acetonitrile in the solvent was removed by bubbling nitrogen gas for 1 h. The purified complex was incubated with toluene and EtOH and the radiochemical purity of the complex at different time points was analyzed by paper chromatography.

2.5 Synthesis of $[^99mTc(VII)]$ complex ion-pair with Phosphinimine ligand 3 $[(\text{NH}_2\text{PPh}_2\text{CH}_2\text{PPh}_2\text{NH or O})^+ (\text{aq. Na }[^99mTcO_4^-])$:

To a solution of 3 (2 mg/mL in toluene or CH$_2$Cl$_2$ or CHCl$_3$) 1 mCi (37 MBq) of $[^99mTcO_4^-]$ (in a 0.1 mL saline solution) obtained as eluent from $[^99\text{Mo}]/[^99\text{Tc}]$ generator
(Mallinckrodt, Inc.) was added. The mixture was vortexed for 1 minute and kept idle for 5 min. Then the organic layer, which has the ion-pair complex, was separated from the aqueous layer to determine the radiochemical purity and the yield of the complex using paper chromatography and reversed phase HPLC. The mobile phase of the HPLC system was 100% CH₃CN. The run time was 25 min with a flow rate 1.0 mL/min. The Paper chromatography analysis involved spotting the complex near the bottom (origin) of 10x1 cm paper strips (Whatman ET-31 paper). Then the strips were developed with saline (0.9% aqueous NaCl), 100% acetone and 100% ethyl acetate. Quantification of the activity on the paper segments and the R_f values were determined using the AMBIS radio chromatographic scanning system.
2.5.1 Stability studies of [(NH$_2$PPh$_2$CH$_2$PPh$_2$NH or O)$^+$(99m$^{\text{Tc}}$O$_4^-$)]:

The stability of the complex [(NH$_2$PPh$_2$CH$_2$PPh$_2$NH)$^+$(99m$^{\text{Tc}}$O$_4^-$)] was measured as function of time. The complex obtained was peak purified on HPLC by collecting the fraction corresponding only to the complex. The acetonitrile in the solvent was removed by bubbling nitrogen gas for 1 h. The purified complex was incubated with toluene, EtOH and 50% EtOH solution in saline. The radiochemical purity of the complex at different time points was analyzed by paper chromatography.

2.6 Synthesis of 99mTc(VII) neutral complex with Phosphinimine ligand 3 [(NPPh$_2$CH$_2$PPh$_2$N)$_{99mTcO_3}$]:

To a solution of 3 (5 mg/mL in toluene or CH$_2$Cl$_2$ or CHCl$_3$), 1 mCi (37 MBq) of $^{99mTcO_4}^-$ (in a 0.1 mL saline solution) obtained as eluent from $^{99}$Mo/$^{99mTc}$ generator (Mallinckrodt, Inc.) was added. The mixture was vortexed for 1 minute and kept idle for 5 min. Then the organic layer was separated and heated at 90 °C for 1 h to yield the neutral complex. The formation of the complex was confirmed by the paper chromatographic study and reversed phase HPLC. The mobile phase of the HPLC system was consisted 100% CH$_3$CN. The run time was for 25 min with a flow rate 1.0 mL/min. The paper chromatography analysis involved spotting the complex near the bottom (origin) of 10x1 cm paper strips (Whatman ET-31 paper). Then the strips were developed with saline (0.9% aqueous NaCl), 100% acetone and 100% ethyl acetate. Quantification of the activity on the paper segments and the R$_f$ values were determined using the AMBIS radiochromatographic scanning system.
2.6.1 *In vitro* and stability studies of [(NPPh₂CH₂PPh₂N)⁹⁹ᵐ⁻TcO₃]⁻:

The stability of the complex (NPPh₂CH₂PPh₂N)⁹⁹ᵐ⁻TcO₃ was measured as function of time. The complex obtained was peak purified on HPLC by collecting the fraction corresponding only to the complex. The acetonitrile in the solvent was removed by bubbling nitrogen gas for 1 h. The purified complex was incubated with toluene, EtOH, 50% EtOH solution in saline and 2% HSA solution in saline. The radiochemical purity of the complex at different time points was analyzed by paper chromatography.

2.7 Synthesis of ⁹⁹ᵐ⁻Tc(VII) complex ion-pair with Phosphinimine ligand 4 [(NH₂PPh₂CH₂C(NH or O PPh₂CH₂)₂CH₃)⁺ (⁹⁹ᵐ⁻TcO₄⁻)]:

To a solution of 4 (5 mg/mL in toluene) 1 mCi (37 MBq) of ⁹⁹ᵐ⁻TcO₄⁻ (in a 0.1 mL saline solution) obtained as eluent from ⁹⁹Mo/⁹⁹ᵐ⁻Tc generator (Mallinckrodt, Inc.) was added. The mixture was vortexed for 1 minute and kept idle for 5 min. Then the organic layer, which has the ion-pair complex, was separated from the aqueous layer to
determine the radiochemical purity and the yield of the complex using paper chromatography and reversed phase HPLC. The mobile phase of the HPLC system was consisted 100% CH$_3$CN. The run time was 25 min with a flow rate 1.0 mL/min. Paper chromatography analysis involved spotting the complex near the bottom (origin) of 10x1 cm paper strips (Whatman ET-31 paper). Then the strips were developed with saline (0.9% aqueous NaCl), 100% acetone and 100% ethyl acetate. Quantification of the activity on the paper segments and the $R_f$ values were determined using the AMBIS radio chromatographic scanning system.

2.7.1 Stability studies of [(NH$_2$PPh$_2$CH$_2$C(NH or O PPh$_2$CH$_2$)$_2$CH$_3$)$^+$ (99mTcO$_4^-$)]:

The stability of the complex [(NH$_2$PPh$_2$CH$_2$C(NHPPh$_2$CH$_2$)$_2$CH$_3$)$^+$ (99mTcO$_4^-$)] was measured as function of time in the toluene solution. The complex obtained was taken as it was in toluene solution and the radiochemical purity of the complex at different time points was analyzed by paper chromatography.
2.8 Attempted Synthesis of phosphinimine ligand C₅H₄PPh₂NSiMe₃:

A two necked round bottom flask fitted with a condenser was loaded with 1 g of 2-pyridyl-diphenylphosphine and purged with nitrogen. To this, 25 mL of dry toluene was added. Once the phosphine was dissolved, 2.5 mL of azidotrimethylsilane was added and the resulting solution was refluxed for 10 h. Then the excess azide and solvent were removed under vacuum. The resulting orange colored solid was taken for NMR analysis.

2.9 Reaction studies of Phosphinimine ligand 3 with NH₄ReO₄:

To a CH₃CN (25 mL) solution of 3 (1 g, 1.94 mmol) was added an aqueous solution (5 mL) of NH₄ReO₄ (0.96 g, 3.86 mmol) dropwise at room temperature. The mixture was stirred for 1 hr before the solvents were removed under vacuum to produce a cream colored solid. (1.6 g) $^{31}$P NMR (121 MHz, CDCl₃, 25°C) δ (ppm) 39.72 m.p. 163°C.
2.10 Investigation of reaction of 3 with NH$_4$ReO$_4$ in presence of Verkade super base:

To a CH$_3$CN (25 mL) solution of 3 (1 g, 1.94 mmol) was added an aqueous solution (5 mL) of NH$_4$ReO$_4$ (0.96g, 3.86 mmol) dropwise at room temperature. The mixture was stirred for 1 hr before the volatiles and aqueous solvents were removed under vacuum to produce a cream colored solid. This solid was totally dried under vacuum overnight and purged with nitrogen and redissolved in dry acetonitrile. To this solution, 0.37 g of Verkade super base was added quickly and refluxed for 1 h. Removal of solvents gave a cream colored solid. Recrystallization of this solid gave pure cubic shaped crystals. These crystals were collected for the X-ray crystal analysis.
CHAPTER 3

Results and Discussion

This chapter contains a detailed discussion of the results obtained in the present study on synthesis, characterization, structural aspects of a variety of phosphinimine based ligand systems and their Re metal complexes, the results of labeling strategies with Tc-99m complexes of these phosphinimine based ligands and the stability of resulting complexes are discussed.

3.1 Synthesis and characterization of phosphinimine ligand 2:

![Scheme 2: Synthesis of phosphinimine ligand 2](image)

The air sensitive phosphinimine ligand 2 was synthesized according to the reported procedure\(^7\) shown in Scheme-2. Initially 2,6-Lutidine was lithiated using n-BuLi and then reacted with phosphine chloride to yield the corresponding phosphine. The impurities formed and tiny amounts of phosphine oxide were separated using a vacuum distillation process. Vacuum distillation was done at 0.01 torr and the phosphine distilled at 150-160 °C. The pure phosphine is a pale yellow oil. Before proceeding to the second
step the phosphine was characterized by NMR spectroscopy ($^1$H, $^{31}$P). A clean singlet at 13.67 in $^{31}$P NMR corresponds to the pure phosphine. The Staudinger reaction of this phosphine with azidotrimethylsilane gives phosphinimine 2 in reasonably high yields. Purification of the final ligand was done by vacuum distillation at 0.01 torr. NMR spectroscopy ($^1$H, $^{31}$P) confirms the structure of the resulting phosphinimine. A clean singlet at 23.52 in $^{31}$P NMR corresponds to the phosphinimine 2.

![Figure 3.1: $^{31}$P NMR of phosphinimine 2](image)

### 3.2 Synthesis and characterization of phosphinimine ligand 3:

![Scheme 3: Synthesis of phosphinimine ligand 3](image)
The synthesis of this phosphinimine was carried out with a reported synthetic procedure\textsuperscript{71} that involves the Staudinger reaction of DPPM bis(diphenylphosphino)methane with azidotrimethylsilane. The reaction route is given in Scheme-3. The reaction was carried under inert atmosphere. The resulting phosphinimine is reasonably stable in air. The NMR spectroscopy ($^1$H, $^{31}$P) confirms the structure of the compound. This phosphinimine hydrolyzes very quickly when it is reacted with water.

![Figure 3.2: $^{31}$P NMR of the phosphinimine ligand 3](image)
3.3 Synthesis and characterization of phosphinimine ligand 4:

Scheme 4: Synthesis of phosphinimine ligand 4

Synthesis route for this ligand is given in Scheme-4. This ligand is extremely insoluble in many polar solvents and also in organic solvents. It is soluble in toluene. The extreme solubility problems with this ligand make it an inconvenient choice for radiolabeling studies.

Figure 3.3: $^{31}$p NMR of phosphinimine ligand 4
3.4 Synthesis and characterization of Re complex of ligand 2:

Scheme 5: Synthesis of Re complex ion-pair of ligand 2

The synthetic route to produce the ion-pair is shown in Scheme 5. The synthesis of the phosphinimine starting material was described in Section 2.2.2. The ligand was dissolved in dry acetonitrile and to this an aqueous solution of ammonium perrhenate was added slowly using an addition funnel. The reaction mixture was stirred for 1 h and the solvents were removed under vacuum. The resulting solid was recrystallized using acetonitrile.

The crystals were characterized using X-ray crystallographic analysis and NMR spectroscopy ($^1$H, $^{31}$P, $^{13}$C). The $^{31}$P NMR spectrum recorded in CD$_3$CN shows a single peak at 65.68 ppm. The melting point of the crystal was recorded and found to be 163 °C. Crystals suitable for single crystal X-ray diffraction studies of 5 were obtained from an acetonitrile solution of compound 5. The detailed results of the crystal structure are discussed in the subsection 3.4.1. The attempt to form a neutral complex by heating 5 was unsuccessful. Heating the ligand overnight at 100 °C did not bring any change in the phosphorous chemical shift, indicating this ion-pair remains intact. The reaction kinetics may not be favorable for the removal of water from this specific complex.
3.4.1 X-ray crystallographic analysis of Re complex ion-pair 5:

The Re complex ion pair 5 crystallizes in the Pbca space group, belonging to orthorhombic crystal system. Table 3.1 gives the crystal data for compound 5. The ORTEP plot of 5, shown in Figure 3.5 confirms the molecular constitution of the new Re complex ion pair of the bis-phosphinimine 2. Selected bond lengths and bond angles are given in Table 3.2 and Table 3.3. As shown in the diagrams, the Re-Re distance is about 7.2. There is a hydrogen bonding observed between N(2) to the O(3) on Re(1) and N(3) to O(5) on Re(2).
Figure 3.5: ORTEP diagram of crystal structure of 5

Table 3.1: Crystal data for compound 5

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<tr>
<th>Compound</th>
<th>5</th>
</tr>
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<tbody>
<tr>
<td>Emp. formula</td>
<td>C_{19}H_{39}N_{3}O_{8}P_{2}Re_{2}</td>
</tr>
<tr>
<td>Formula weight</td>
<td>871.87</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Orthorhombic</td>
</tr>
<tr>
<td>Space group</td>
<td>Pbca</td>
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<tr>
<td>$a$ /Å</td>
<td>15.0971(8)</td>
</tr>
</tbody>
</table>
\[
\begin{align*}
  b / \text{Å} & \quad 13.9229(7) \\
  c / \text{Å} & \quad 27.1491(14) \\
  \alpha / \text{deg} & \quad 90.00 \\
  \beta / \text{deg} & \quad 90.00 \\
  \gamma / \text{deg} & \quad 90.00 \\
  V / \text{Å}^3 & \quad 5706.6(5) \\
  Z & \quad 8 \\
  D_{\text{calc}} / \text{g cm}^{-3} & \quad 2.030 \\
  F(000) & \quad 3344 \\
  \text{Crystal size [mm]} & \quad 0.4 \times 0.32 \times 0.3 \\
  2\theta \text{ max.} & \quad 54.26 \\
  \text{Observed reflections} \quad (I>2\sigma(I)) & \quad 5251 \\
  \text{Data/ restraints/ parameters} & \quad 6299/ 6/ 327 \\
  S & \quad 1.019 \\
  R1 [I>2\sigma(I)] & \quad 0.0472 \\
  wR2 [\text{all data}] & \quad 0.0257 \\
  \text{Max./min. residual electron dens. [eÅ}^{-3}] & \quad 2.216 / -1.494 
\end{align*}
\]
Table 3.2  Selected bond lengths [Å] and for 5 with esd’s in parentheses

<table>
<thead>
<tr>
<th>Bond</th>
<th>Length</th>
<th>Bond</th>
<th>Length</th>
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<tbody>
<tr>
<td>Re(1)-O(3)</td>
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<td>Re(1)-O(4)</td>
<td>1.703(4)</td>
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<td>Re(1)-O(1)</td>
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<td>Re(1)-O(2)</td>
<td>1.720(3)</td>
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<tr>
<td>P(1)-N(3)</td>
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<td>P(1)-C(8)</td>
<td>1.807(5)</td>
</tr>
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<td>1.807(4)</td>
<td>P(1)-C(9)</td>
<td>1.815(4)</td>
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<td>N(1)-C(1)</td>
<td>1.335(5)</td>
<td>N(1)-C(5)</td>
<td>1.344(5)</td>
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<td>Re(2)-O(7)</td>
<td>1.711(3)</td>
<td>Re(2)-O(6)</td>
<td>1.721(3)</td>
</tr>
<tr>
<td>Re(2)-O(8)</td>
<td>1.723(3)</td>
<td>Re(2)-O(5)</td>
<td>1.739(3)</td>
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<tr>
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<td>P(2)-C(7)</td>
<td>1.803(5)</td>
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<td>1.803(4)</td>
<td>P(2)-C(14)</td>
<td>1.812(5)</td>
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<td>P(2)-C(15)</td>
<td>1.818(5)</td>
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Table 3.3  Selected bond angles [°] for 5 with esd’s in parentheses

<table>
<thead>
<tr>
<th>Bond</th>
<th>Angle</th>
<th>Bond</th>
<th>Angle</th>
</tr>
</thead>
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<tr>
<td>O(3)-Re(1)-O(4)</td>
<td>109.2(2)</td>
<td>O(3)-Re(1)-O(4)</td>
<td>110.8(3)</td>
</tr>
<tr>
<td>O(4)-Re(1)-O(1)</td>
<td>107.9(3)</td>
<td>O(3)-Re(1)-O(2)</td>
<td>111.1(2)</td>
</tr>
<tr>
<td>O(4)-Re(1)-O(2)</td>
<td>108.7(2)</td>
<td>O(1)-Re(1)-O(2)</td>
<td>109.1(19)</td>
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<tr>
<td>N(2)-P(1)-C(8)</td>
<td>110.2(2)</td>
<td>N(2)-P(1)-C(6)</td>
<td>107.0(2)</td>
</tr>
<tr>
<td>C(8)-P(1)-C(6)</td>
<td>112.2(2)</td>
<td>N(2)-P(1)-C(9)</td>
<td>111.5(2)</td>
</tr>
<tr>
<td>C(8)-P(1)-C(9)</td>
<td>107.7(2)</td>
<td>C(6)-P(1)-C(9)</td>
<td>108.2(2)</td>
</tr>
<tr>
<td>C(1)-N(1)-C(5)</td>
<td>118.1(4)</td>
<td>N(1)-C(1)-C(2)</td>
<td>123.1(4)</td>
</tr>
<tr>
<td>N(1)-C(1)-C(6)</td>
<td>115.9(4)</td>
<td>O(7)-Re(2)-O(6)</td>
<td>109.69(18)</td>
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<td>O(7)-Re(2)-O(8)</td>
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<td>O(6)-Re(2)-O(8)</td>
<td>108.66(16)</td>
</tr>
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<td>O(7)-Re(2)-O(5)</td>
<td>111.41(17)</td>
<td>O(6)-Re(2)-O(5)</td>
<td>110.15(17)</td>
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<td>O(8)-Re(2)-O(5)</td>
<td>108.05(15)</td>
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<td>110.0(2)</td>
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<td>N(3)-P(2)-C(14)</td>
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<td>113.1(2)</td>
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<td>N(1)-C(5)-C(7)</td>
<td>116.5(4)</td>
<td>C(7)-C(6)-P(1)</td>
<td>116.6(3)</td>
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<td>C(5)-C(7)-P(2)</td>
<td>117.4(3)</td>
<td>C(11)-C(8)-P(1)</td>
<td>112.8(4)</td>
</tr>
</tbody>
</table>

3.5  Synthesis and chromatographic studies of $^{99m}$Tc complex ion-pair of ligand 2:

The coordination chemistry of the complexation studies of ligand 2 with Re metal is further extended to the radiochemical counter part $^{99m}$Tc to check the similar
complex formation. The schematic diagram for the formation of ion-pair complex of 2 with $^{99m}\text{Tc}$ is given in Scheme-6.

Scheme 6: Synthesis of $^{99m}\text{Tc}$ complex ion-pair with ligand 2

To a solution of 2 (2 mg/mL in an organic solvent) was mixed with $\approx$ 1 mCi (37 MBq) $^{99m}\text{TcO}_4^-$ in a 0.1 mL saline solution obtained as eluent from $^{99}\text{Mo}/^{99m}\text{Tc}$ Generator (Mallinckrodt, Inc.). The synthesis was performed in 3 different organic solvents. The procedure is same for all three solvents. After vortexing the mixture for 1 minute, the solution was kept idle for 5 minutes and the organic layer was separated, which has the ion pair. The % of $^{99m}\text{Tc}$ in the organic layer was determined using a dose calibrator. The yields were calculated in comparison to the initial activity added. The plausible structures
for the product were showed in Scheme-6. P=NH have a tendency to hydrolyze to P=O in presence of water. The radiochemical purity (RCP) of the complex was determined using paper chromatography. The rapid transfer of $^{99m}$Tc from the aqueous layer to organic layer is an indication of the interaction of the ligand with pertechnetate. To conform it further experiment was conducted using an organic solvent with no ligand present in it. $\approx$1 mCi of $^{99m}$TcO$_4^-$ was added to 1 mL of organic solvent and vortexed for 1 minute. Then the solution was kept idle for 5 minutes and the organic layer was separated to check the % of $^{99m}$Tc in the organic layer. It was found that only about 1% of the total activity moved in to organic layer. Further, the paper chromatography (PC) of the $^{99m}$Tc-phosphinimine complex formed in organic solvents at room temperature indicated that a single $^{99m}$Tc species was formed by simply mixing the organic solutions containing bis phosphinimine ligand with a normal saline solution containing tracer levels of $^{99m}$TcO$_4^-$ (Scheme-6). Since no reducing agent is present, it can be assumed that the Tc-phosphinimine compounds are produced with Tc remaining in the +7 oxidation state. The reaction of $^{99m}$TcO$_4^-$ with this ligand occurs rapidly and must involve interaction of the two reactants at the interface between the two solvents. Since this $^{99m}$Tc species is highly soluble in variety of non-polar solvents (e.g., CH$_2$Cl$_2$, CHCl$_3$ and toluene) (Table 3.4), it can be assumed to have an overall neutral charge. Further evidence that this $^{99m}$Tc species is an ion pair is provided by paper chromatography. The R$_f$ of this $^{99m}$Tc-ion-pair, using EtOAc as the eluent, is 0.85 – 1.0 on PC. Whereas the R$_f$ of the free $^{99m}$TcO$_4^-$ is 0.0, which is the diagnostic test for the formation of an ion-pair. The % yield values of the complex in different solvent system are given in Table 3.4.
The results show that the yield is greater than 95% in all solvents used and the maximum yield of the ion-pair was obtained in toluene.

**Table 3.4: % yield values of $^{99m}$Tc complex ion-pair of ligand 2:**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>% of $^{99m}$Tc in Organic Layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene</td>
<td>98.6 ± 0.5</td>
</tr>
<tr>
<td>CH$_2$Cl$_2$</td>
<td>97.5 ± 0.5</td>
</tr>
<tr>
<td>CHCl$_3$</td>
<td>95.7 ± 0.5</td>
</tr>
</tbody>
</table>

The reversed phase HPLC analysis of $^{99m}$Tc complex was performed to check the radiochemical purity of the ion-pair. Mobile phase of the HPLC system was consisted 100% CH$_3$CN. The run time was for 25 min with a flow rate 1.0 mL/min. The HPLC chromatogram is given in **Figure 3.6**. The retention time for the ion-pair is found to be 1.667 min.

![HPLC chromatogram](image)

**Figure 3.6: HPLC chromatogram of $^{99m}$Tc complex ion-pair of ligand 2**
3.5.1: Stability of $^{99m}$Tc complex ion-pair of ligand 2 in various solvents:

The ion-pair formed by the reaction of $^{99m}$TcO$_4^-$ with ligand 2 in toluene solution was taken in a test tube and the solvent was evaporated by passing a stream of nitrogen into it. The resulting solid was incubated in 100% toluene and 100% EtOH solution. The stability of the ion-pair at different time intervals is given in Table: 3.5. From the results it is evident that the ion-pair formed is stable over a period of 24 h. The % RCP of the ion-pair in toluene after 24 h is 97.1% and in EtOH it is 96.7%.

Table 3.5: Stability of $^{99m}$Tc complex ion-pair of ligand 2 in various solvents:

<table>
<thead>
<tr>
<th>Incubation Solvent</th>
<th>Radio Chemical Purity in %RCP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Toluene</td>
<td>98.5 ± 0.3</td>
</tr>
<tr>
<td>100% EtOH</td>
<td>98.2 ± 0.3</td>
</tr>
</tbody>
</table>

3.6 Synthesis and chromatographic studies of $^{99m}$Tc complex ion-pair of ligand 3:

The synthetic route for the ion-pair complex is shown in Scheme-7. To a solution of 3 (2mg/mL in Toluene or CH$_2$Cl$_2$ or CHCl$_3$) 1 mCi (37MBq) of $^{99m}$TcO$_4^-$ (in a 0.1mL saline solution) obtained as eluent from $^{99}$Mo/$^{99m}$Tc Generator (Mallinckrodt, Inc.) was added. The mixture was vortexed for 1 minute and kept idle for 5 min. Then the organic layer, which has the ion-pair complex, was separated from the aqueous layer. Upon mixing the entire activity moved from the aqueous layer to organic layer, which is an indication of the reaction of the metal and the ligand at the interface of the two layers. The paper chromatographic pattern of ion-pair, in ethyl acetate as eluent, indicates that
the ion-pair moves to the top of the strip, whereas the free pertechnetiate stays at the bottom. The reversed phase HPLC also shows same retention time as free pertechnetate. As the mobile phase is a polar solvent, the $^{99m}$TcO$_4^-$ ion of the ion-pair behaves as a free ion in this polar solvent. The analogous monodentate ligand Ph$_3$PNSiMe$_3$ forms a stable ion-pair [Ph$_3$P=NH$_2^+$][TcO$_4^-$] with Tc, $^{99m}$Tc, Re and $^{188}$Re.$^{13,14,15}$ Because of the structural similarity, it is assumed that, the ligand 3 also forms an ion-pair. Further evidence is that, in paper chromatography (PC), the ion-pair have moved to the top of the strip in all three developing solvents (Acetone, Ethyl Acetate and Saline) used. This pattern matches with the paper chromatographic behavior of the ion-pair of the monodentate ligand Ph$_3$PNSiMe$_3$. Thus, the paper chromatographic pattern did not behave identically to $^{99m}$TcO$_4^-$.

Scheme 7: Synthesis of $^{99m}$Tc complex ion-pair with ligand 3.
The Paper Chromatography (PC) and HPLC of the $^{99m}$Tc-phosphinimine complex formed in organic solvents at room temperature indicated that a single $^{99m}$Tc species is formed by simply mixing the organic solutions containing bis phosphinimine ligand with a normal saline solution containing tracer levels of $^{99m}$TcO$_4^-$. The % of $^{99m}$Tc moved in to organic layer to form the ion-pair complex in various solvents is showed in Table 3.6.

### Table 3.6: % yield values of $^{99m}$Tc complex ion-pair of ligand 3:

<table>
<thead>
<tr>
<th>Solvent</th>
<th>% of $^{99m}$Tc in Organic Layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene</td>
<td>98.9 ± 0.2</td>
</tr>
<tr>
<td>CH$_2$Cl$_2$</td>
<td>97.4 ± 0.5</td>
</tr>
<tr>
<td>CHCl$_3$</td>
<td>96.1 ± 0.3</td>
</tr>
</tbody>
</table>

The reversed phase HPLC analysis of $^{99m}$Tc complex was performed to check the radiochemical purity of the ion-pair. Mobile phase of the HPLC system was consisted 100% CH$_3$CN. The run time was for 25 min with a flow rate 1.0 mL/min. The HPLC chromatogram is shown in Figure 3.7. The retention time for the ion-pair was found to be 1.8 min.
3.6.1 Stability studies of $^{99m}$Tc complex ion-pair of ligand 3:

The ion-pair formed by the reaction of $^{99m}$TcO$_4^-$ with ligand 3 in toluene solution was taken in a test tube and the solvent was evaporated by passing a stream of nitrogen in to it. The resulting solid was incubated in 100% Toluene, 100% EtOH and 50% EtOH and Saline solution. The stability of the ion-pair at different time intervals were determined using paper chromatography and are given in **Table: 3.7**.

**Table 3.7: Stability of $^{99m}$Tc complex ion-pair of ligand 3 in various solvents**

<table>
<thead>
<tr>
<th>Incubation Solvent</th>
<th>Radio Chemical Purity in %RCP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Toluene</td>
<td>98.7 ± 0.2</td>
</tr>
<tr>
<td>100% EtOH</td>
<td>97.7 ± 0.3</td>
</tr>
<tr>
<td>50% EtOH/Saline</td>
<td>97.4 ± 0.5</td>
</tr>
</tbody>
</table>

**Figure 3.7: HPLC Chromatogram of $^{99m}$Tc complex ion-pair of ligand 3.**
The stability studies reveal that the ion-pair formed is a very stable and the standard deviation values indicate that it is highly reproducible.

3.7 Synthesis and chromatographic studies of $^{99m}$Tc neutral complex with ligand 3:

The reaction scheme for the synthesis is given in Scheme-8. The ion-pair was synthesized according to the procedure given in section 2.5, was heated in toluene for 1 h at 90 °C to yield the neutral complex. The formation of the complex is conformed by paper chromatographic studies and also using reversed phased HPLC. The solvent system used was 100% CH3CN. The chromatography pattern is completely different when it is developed in aqueous saline solution. The $R_f$ of the neutral complex in saline is 0.00 where as for the ion-pair is 0.85-1.0. Further evidence for the formation of the neutral
complex is the reversed phase HPLC. The retention time of the complex changed to 5.0 min. This is due to the increased lipophilicity of the complex. **Figure 3.8** shows the HPLC chromatograms of the ion-pair, neutral and the mixture of the two complexes.

**Figure 3.8: HPLC Chromatograms of ion-pair, neutral and ion-pair + neutral mixture.**

Note: The void volume of the column is approximately 2.8 mL. The pertechnetate elutes with the void volume.

**Figure 3.9** shows the mobility of the ion-pair and neutral complex in acetone, ethyl acetate and Saline.
3.7.1 Stability and *in vitro* studies of $^{99m}$Tc neutral complex of ligand 3:

The neutral Tc(VII) complex in toluene was evaporated to dryness and redissolved in 100% EtOH. The PC and HPLC analysis of the neutral complex dissolved in EtOH showed that the complex remained stable (>95%) over 24h. By sharp contrast, previous studies had demonstrated that the monodentate-phosphinimine Tc(VII) complex, $\text{Ph}_3\text{PNTcO}_3$ was not stable in EtOH and readily converted to the ion pair $\text{Ph}_3\text{P}^+\text{NH}_2^+\text{TcO}_4^-$. Paper chromatography Bioscan analysis of the 1:1 mixtures of Human serum albumin (HAS) (10 mg/mL in 0.9% aqueous NaCl) and EtOH containing neutral complex indicated that this Tc(VII)-phosphinimine complex was stable up to 3 hours (>90%) with 5-7% conversion to the ion pair. In marked contrast to the Tc-VII compound stabilized by the monodentate phosphinimine ligand (i.e., $\text{Ph}_3\text{PNTcO}_3$),

\[ ^{99m}\text{TcO}_4^- \quad \text{Ion-Pair} \quad \text{Neutral} \]

\[ \text{Acetone} \]

\[ \text{Ethyl Acetate} \]

\[ \text{Saline} \]

Figure 3.9: Mobility on ion-pair and neutral complex in different solvents.
the bidentate phosphinimine 3 has demonstrated a substantial increase in *in vitro* stability. Over the 24 hour period the complex was completely decomposed to phosphine oxide.

**Table 3.8** shows the % RCP values of the neutral complex in HSA solution and 100% EtOH.

**Table 3.8: *in-vitro* Stability of $^{99m}$Tc- neutral complex of ligand 3.**

<table>
<thead>
<tr>
<th>Incubation Solvent</th>
<th>Radio Chemical Purity in %RCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% EtOH</td>
<td>97.8 ± 0.3, 97 ± 0.5, 96.6 ± 0.5</td>
</tr>
<tr>
<td>HSA</td>
<td>96.8 ± 0.3, 96.0 ± 0.2, 0.0</td>
</tr>
</tbody>
</table>

3.8 Synthesis, chromatographic and stability studies of $^{99m}$Tc complex ion-pair of ligand 4:

Scheme 9: Synthesis of $^{99m}$Tc complex ion-pair with ligand 4.
Scheme-10 describes the synthetic route for the preparation of the $^{99m}$Tc ion-pair complex of the ligand 4. This ligand has extreme solubility problems. The synthesis was attempted in dichloromethane, chloroform and toluene. Except for toluene ligand showed turbidity in the rest of the solvents. The % RCP of this complex was determined at different time intervals to check the stability of this ion-pair in toluene solution. The results are shown in Table 3.9.

Table 3.9: Stability of $^{99m}$Tc complex ion-pair of ligand 4:

<table>
<thead>
<tr>
<th>Incubation Solvent</th>
<th>Radio Chemical Purity in %RCP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Toluene</td>
<td>99.8</td>
</tr>
</tbody>
</table>

3.9 Attempted synthesis of phosphinimine ligand $C_5H_4PPh_2NSiMe_3$:

The synthetic route for the attempted synthesis is given in Scheme-10. This procedure involved a regular Staudinger reaction of phosphine with azidotrimethylsilane. The reaction was refluxed for 48 h with continuous monitoring of $^{31}$P at regular time intervals. Even after 48 h the starting material remained intact and the reaction did not occur. Further attempt was made with excess azide and with no other solvent present. Still the starting material remained as it is and some minor side products were formed in both attempts. The mass spectrum (Figure 3.10) of the product indicates the formation of
multiple products including the starting material. The Mass spectrum of the reaction product is shown in Figure 3.10.

\[
\text{Ph}_2\text{P} \xrightarrow{\text{Toluene, Reflux}} \text{SiMe}_3 \quad \text{N}_3\text{SiMe}_3
\]

Scheme 10: synthesis of phosphinimine ligand C$_5$H$_4$PPh$_2$NSiMe$_3$

Figure 3.10: Mass Spectrum of the product obtained for the attempted synthesis of ligand C$_5$H$_4$PPh$_2$NSiMe$_3$. 

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The possible reason for the reaction not to take place might be the lone pair electrons on the pyridine nitrogen. According to the mechanism, the driving force for the reaction is the donation of electrons from phosphorous to the azide nitrogen.

\[
PR_3 + N≡N−N−R \rightarrow R_3PN=N=N−R
\]

Aza-Ylide

Scheme 11: Staudinger reaction mechanism.

Whereas in case of 2 pyridyl diphenylphosphine, the lone pair of electrons on pyridine are more freely available than those on phosphorous and are not allowing phosphorus to donate electrons to the azide nitrogen.

In the case of 2,6-diisopropylphosphino Lutidine, (Scheme-2), the inductive effect due to the isopropyl groups on the phosphorous makes phosphorous more nucleophilic than the pyridine nitrogen and it undergoes the Staudinger reaction to give the phosphinimine 2.

3.10 Synthesis of Re complex ion-pair of ligand 3:

Scheme 12: Synthesis of Re complex ion-pair with ligand 3
Synthesis route for this reaction is shown in **Scheme-12**. This reaction has resulted in two products. Due to extreme sensitivity of the starting phosphinimiine ligand to water, it forms the phosphine oxide in almost 50%. Careful washing with 40:60 mixtures of dichloromethane and chloroform for 20 times reduced the amount of hydrolyzed product to 30%. But in this process there is a significant loss of the ion-pair product too. Proton NMR of the product after washings is shown in **Figure 3.10**.

![Figure 3.11: $^{31}$P NMR of Re complex ion-pair of ligand 3.](image)
3.11 Investigation of reaction of Re- complex ion-pair with ligand 3 with Verkade Super-base:

![Reaction Scheme]

Scheme 13: Reaction of Re complex of ligand 3 with super base.

As earlier methods available to remove the methylene proton to form a stable neutral metal complex with ligand 3,\textsuperscript{72} we have used the same strategy to produce a neutral complex with Re metal. But the result was completely different than our hypothesis. A rearrangement of phosphorous-carbon phosphorous bridge to phosphorous-nitrogen-phosphorous was observed. This kind of rearrangement was known in the literature for the same ligand,\textsuperscript{73} where the cleavage of phosphorous bond was assisted by organogermanium or organotin. The current result shows that, even a strong base can also trigger this rearrangement. The proposed mechanism for the rearrangement is shown in Scheme-14.
Scheme 14: Mechanism of rearrangement of P-C-P bridge to P-N-P bridge.

Further evidence for the rearrangement came from the molecular structure of the product obtained from the x-ray crystal structure analysis. The ORTEP diagram of the crystal structure is given in Figure 3.12.

Figure 3.12: ORTEP diagram of rearranged ion-pair of ligand 3.
3.12 Summary and Future work:

The current investigation demonstrates that the bidentate phosphinimines, 2 and 3 impart kinetic stability via chelate interactions with $^{99m}$Tc(VII) and Re(VII) cores. Attempts to make a neutral complex using ligand 2 with $^{99m}$Tc were not successful. However ligand 3 forms a neutral complex with $^{99m}$Tc upon heating of the corresponding ion-pair. This neutral complex is stable in HSA for 3 hours. Similar studies were carried out with the analogous nonradioactive metal Re. The X-ray crystallographic studies of the ion-pair complex of ligand 2 with Re metal indicates that it is an ion-pair.

The four phenyl groups in 3 may be unsuitable for further use of this backbone in labeling tumor-avid peptides. However, the aliphatic functionalized 2 may possess ideal lipophilicity for subsequent use in the chemical architecture of phosphinimine-based BFCA’s for use in labeling biomolecules. The tris ligand 4 has extreme solubility problems to continue the studies. Decreasing the lipophilicity by replacing the phenyl groups with polar substituents may result in increased solubility. Though there is increased in vitro stability with bis phosphinimines compared to the monophosphinimines, it is not sufficient to utilize this system further. In case of the neutral complex of the bisphosphinimine 3, the dramatic electronic effects excreted by the electronegative substituents on phosphinimine ligand were observed. It seems that the highly electronegative substituents on phosphorous decrease the basicity of the $–\text{N}=\text{PPh}_2$ nitrogen which in turn reduces the strength of the coordination interaction of this nitrogen with the metal center.\textsuperscript{74-77} Having electron donating substituent on the organic part of the ligand may stabilize the Re-N bond in the neutral complex.
Investigations of modifying the reaction conditions to make the neutral complex of ligand 3 leads to a rearrangement to occur. Changing the base choices to take the proton off from the methylene carbon and optimizing the reaction conditions might result in the desired product.

Future work includes changing the organic backbone on the phosphinimine in order to increase kinetic stability of the formed metal complexes. Investigating the Staudinger reaction with different phosphines and make a library of phosphinimines to check the coordination chemistry of these ligands with Tc and Re.
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VITA

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