INVESTIGATING $^{99m}$TECHNETIUM/RHENIUM(V)-CYCLIZED OCTREOTIDE ANALOGUES USING EXPERIMENTAL AND COMPUTATIONAL METHODS

A Dissertation

Presented to

the Faculty of the Graduate School

at the University of Missouri-Columbia

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

By

YAWEN LI

Dr. Silvia S. Jurisson, Dissertation Supervisor

Dr. Carol A. Deakyn, Dissertation Co-Supervisor

Dr. Michael R. Lewis, Dissertation Co-Supervisor

May 2015
The undersigned, appointed by the dean of the Graduate School, have examined the
dissertation entitled

INVESTIGATING $^{99M}$TECHNETIUM/RHENIUM(V)-CYCLIZED OCTREOTIDE
ANALOGUES USING EXPERIMENTAL AND COMPUTATIONAL METHODS

presented by Yawen Li,

a candidate for the degree of doctor of philosophy,

and hereby certify that, in their opinion, it is worthy of acceptance.

______________________________
Professor Silvia S. Jurisson

______________________________
Professor Carol A. Deakyne

______________________________
Professor Michael R. Lewis

______________________________
Professor J. David Robertson
For my parents, grandparents and husband.

献给我的父母,祖父母,外祖父母和爱人
ACKNOWLEDGEMENTS

Many people’s help have been important in the completion of this work and in my education as a graduate student. First I thank Dr. Jurisson, Dr. Deakyne and Dr. Lewis for being my mentors and their guidance and encouragements throughout my graduate career.

I thank the collaborators, postdoctoral fellows and graduate students of my advisors for their support in my research. I thank Dr. Fabio Gallazzi for training in peptide synthesis and LC-ESI-MS, Dr. Lixin Ma for her guidance in 2D NMR spectra interpretation, Dr. Wei Wycoff for her assistance in setting up 2D NMR experiments, Dr. Heather Hennkens and Dr. Dijie Liu for training in competitive binding assays and Dr. John Adams for his help in molecular dynamics simulations.

I thank my committee member, Dr. Robertson for his suggestions and questions that led me to think more deeply on topics related with my research project.

Finally, I thank the faculty and staff of the chemistry department for their help and support throughout my graduate career.
TABLE OF CONTENTS

ACKNOWLEDGEMENTS .................................................................................................................. ii
LIST OF FIGURES .......................................................................................................................... vii
LIST OF TABLES ............................................................................................................................. ix
LIST OF ABBREVIATIONS ................................................................................................ .......... xi
ABSTRACT ....................................................................................................................................... xiv

Chapter 1
General Introduction ......................................................................................................................... 1

1.1 Cancer-targeting Nuclear Medicine ...................................................................................... 2
1.2 Computational Methods ......................................................................................................... 4
1.3 References .............................................................................................................................. 7

Chapter 2
Synthesis, Characterization and In Vitro Evaluation of New $^{99m}$Tc/Re(V)-cyclized Octreotide Analogues: An Experimental and Computational Approach ................................................................. 9

2.1 Introduction ............................................................................................................................ 9

2.2 Experimental and Computational Details ............................................................................ 11

2.2.1 Experimental details ....................................................................................................... 11

2.2.2 Computational details .................................................................................................... 19

2.3 Results and Discussion ......................................................................................................... 21

2.3.1 Choice of the peptide sequence ..................................................................................... 21

2.3.2 Synthesis and characterization of Re(V)-cyclized SDPhe TATE isomers ......................... 23

2.3.3 Calculated equilibrium structures ................................................................................... 28

2.3.4 NMR chemical shift calculations ................................................................................... 32
2.3.5  In vitro receptor binding studies ......................................................... 36
2.3.6  Preparation and characterization of $^{99m}$Tc(V)-cyclized SDPhe-TATE ............................................................. 37
2.3.7  Chemical stability of $^{99m}$Tc(V)-cyclized SDPhe-TATE .................. 39
2.4  Conclusions ......................................................................................... 40
2.5  References and Notes ........................................................................ 41

Chapter 3
Using Potential Energy Surface Scans to Examine the Bond Dissociation Energies of trans-ReOS$_2$N$_2$ and [ReOS$_3$N]$^{1-}$ Model Complexes ........................................ 46
3.1  Introduction ......................................................................................... 46
3.2  Computational Details ......................................................................... 49
3.3  Results and Discussion ......................................................................... 55
  3.3.1  Optimized Geometries .................................................................. 55
  3.3.2  Re–NH$_3$ ...................................................................................... 58
  3.3.3  Re–SH .......................................................................................... 62
  3.3.4  Re–NH$_2$ and Re–N(H)CHO .......................................................... 64
  3.3.5  Effect of solvent ............................................................................ 66
  3.3.6  ReO-222-MAMA ........................................................................... 71
3.4  Conclusions ......................................................................................... 73
3.5  References .......................................................................................... 74

Chapter 4
4.1  Introduction ......................................................................................... 78
4.2  Computational Details ......................................................................... 79
Chapter 4

4.3 Results and Discussion

4.3.1 Calculated equilibrium structures

4.3.2 PES scans

4.4 Summary

4.5 Future Studies

4.6 References

Chapter 5

Modeling Disulfide-cyclized and Re-cyclized Octreotide Derivatives Using Molecular Dynamics (MD) Simulations and Quantum Chemical Methods

5.1 Introduction

5.2 Computational Details

5.2.1 MD simulations

5.2.2 Electronic structure calculations

5.2.3 Computational resources

5.3 Current Results and Discussion

5.3.1 MD simulations

5.3.2 Electronic structure calculations

5.4 Conclusions

5.5 Future Studies

5.5.1 Develop a set of Re(V) parameters for the AMBER force field

5.5.2 Perform MD simulations on Re(V)-cyclized octreotide analogues and Tyr³-TATE

5.5.3 Perform electronic structure calculations on representative conformers selected from MD simulations

5.6 References
Chapter 6

Conclusions and Future Studies ................................................................. 120

References .................................................................................................... 122

Appendix I

The First Re(V)-cyclized Somatostatin Antagonist Peptide Analogue .......... 124

7.1 Introduction ............................................................................................... 124

7.2 Methods and Material .............................................................................. 127

7.2.1 General methods .................................................................................. 127

7.2.2 Synthesis of Lu-DOTA-sst2-antagonist ............................................. 129

7.2.3 Synthesis of Re(V)-cyclized DOTA-sst2-antagonist
(Re-DOTA-sst2-ANT) .................................................................................... 130

7.2.4 ICP-MS analysis .................................................................................. 130

7.2.5 Cell culture .......................................................................................... 131

7.2.6 IC\textsubscript{50} studies ........................................................................ 132

7.3 Results and Discussion ........................................................................... 133

7.3.1 Synthesis and characterization of the metal-peptide complexes ........... 133

7.3.2 \textit{In vitro} sst\textsubscript{2} receptor binding affinity ................................ 136

7.4 Conclusions .............................................................................................. 138

7.5 References ............................................................................................... 138

Appendix II ..................................................................................................... 141

VITA ............................................................................................................... 147
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 2-1. Molecular structures of representative M(V)-cyclized octreotide analogues previously investigated</td>
<td>22</td>
</tr>
<tr>
<td>Figure 2-2. Molecular structure of Re(V)-cyclized SDPhe¹-Tyr³-octreotate</td>
<td>26</td>
</tr>
<tr>
<td>Figure 2-3. The <em>anti</em> isomer of N2 deprotonation (N2-1)</td>
<td>31</td>
</tr>
<tr>
<td>Figure 3-1. PBE0/6-31G(d,p):LANL2TZ minima for trans-ReO(SH)₂NH₂NH₃ and [ReO(SH)₃NH₂]⁻</td>
<td>57</td>
</tr>
<tr>
<td>Figure 3-2. Re–NH₃ relaxed PES scan BDE curves with C₅ symmetry for trans-ReO(SH)₂(NH₂)(NH₃)</td>
<td>59</td>
</tr>
<tr>
<td>Figure 3-3. Re–NH₃ BDE curves for trans-ReO(SH)₂(NH₂)(NH₃) without symmetry constraint</td>
<td>61</td>
</tr>
<tr>
<td>Figure 3-4. Re–SH and Re–NH₂ BDE curves for [ReO(SH)₃(NH₂)]⁻</td>
<td>63</td>
</tr>
<tr>
<td>Figure 3-5. Re–N(H)CHO BDE curves for [ReO(SH)₃(N(H)CHO)]⁻</td>
<td>65</td>
</tr>
<tr>
<td>Figure 3-6. BDE curves for the Re–N(H)CHO, Re–SH and Re–NH₃ bonds in the presence of implicit solvent</td>
<td>67</td>
</tr>
<tr>
<td>Figure 3-7. Re–SH and Re–NH₂ BDE curves for [ReO(SH)₃(NH₂)]⁻ in the presence of implicit solvent</td>
<td>70</td>
</tr>
<tr>
<td>Figure 3-8. The ReO-222-MAMA complex investigated via relaxed PES scans along the Re–N/S bonds</td>
<td>72</td>
</tr>
<tr>
<td>Figure 4-1. Ball-and-stick diagrams for N2-1 to N3-4 of ReO[DPhe-Cys-Tyr][Cys]</td>
<td>84</td>
</tr>
</tbody>
</table>
Figure 4-2. Re‒S/N BDEs obtained at the PBE0/6-31G(d):LANL2DZ level of theory for the 5-coordinate N2-1 isomer of ReO[SDPhe-Cys-Tyr][Cys] .............................................................. 86

Figure 4-3. Re‒S/N BDEs obtained at the PBE0/6-31G(d):LANL2DZ level of theory for the 6-coordinate N3-1 of ReO[SDPhe-Cys-Tyr][Cys] ..... 87

Figure 4-4. Re‒S/N BDEs obtained at the PBE0/6-31G(d,p):LANL2TZ level of theory for the 5-coordinate N2-1 of ReO[SDPhe-Cys-Tyr][Cys] ..... 88

Figure 5-1. Tyr³-TATE conformations observed in simulated annealing results. 99

Figure 5-2. Trajectory of dihedral angle ψ of Cys⁷ .............................................. 100

Figure 5-3. Conformational change caused by replacing the alcohol terminus with a carboxylate group .......................................................... 105

Figure 5-4. Conformational change caused by replacing the terminal NH₂ with a [NH₃]¹⁺ .......................................................... 105

Figure 5-5. Comparison of the effects of charged termini and the other side chains on backbone conformation .............................................. 106

Figure 5-6. The effect of R-groups on the position of the R-groups of neighboring residues ........................................................................ 107

Figure App.1-1. Proposed structure of Re(V)-cyclized DOTA-sst₂-ANT. .... 136

Figure App.1-2. IC₅₀ for Lu-DOTA-sst₂-ANT and Re-DOTA-sst₂-ANT determined in AR42J cells from competitive binding assays with ¹²⁵I-Tyr¹¹-somatostatin-14 ................................................................. 137

Figure App.2-1. Pictures of isomers 1-8. Geometries optimized at the PBE0/6-31G(d):LANL2DZ level of theory ........................................... 144

Figure App.2-2. Selected occupied MOs of the ReO(SH)₃(NH₂) lowest-energy structure ........................................................................ 146
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2-1. ESI-LC-MS results for Re-SDPhe-TATE</td>
<td>24</td>
</tr>
<tr>
<td>Table 2-2. Selected $^1$H and $^{13}$C chemical shifts (ppm) of linear SDPhe-TATE and Re-SDPhe-TATE isomers 1, 2 and 4</td>
<td>25</td>
</tr>
<tr>
<td>Table 2-3. Backbone-backbone NOEs of Re-SDPhe-TATE isomer 1 and Re(V)-cyclized Tyr$^3$-octreotate</td>
<td>28</td>
</tr>
<tr>
<td>Table 2-4. Relative energies of the low energy isomers of ReO[SDPhe-Cys-Tyr][Cys]</td>
<td>30</td>
</tr>
<tr>
<td>Table 2-5. IC$_{50}$ results of the Re-SDPhe-TATE isomers and nat/In-DOTA-octreotide</td>
<td>37</td>
</tr>
<tr>
<td>Table 2-6. HPLC retention times (min) of Re(V)- and $^{99m}$Tc(V)-cyclized SDPhe-TATE</td>
<td>39</td>
</tr>
<tr>
<td>Table 2-7. Chemical stability of $^{99m}$Tc(V)-cyclized SDPhe-TATE in PBS and cysteine solution</td>
<td>40</td>
</tr>
<tr>
<td>Table 3-1. Selected bond lengths and bond angles for trans-ReO(SH)$_2$(NH$_2$)(NH$_3$) obtained with different basis sets</td>
<td>58</td>
</tr>
<tr>
<td>Table 3-2. Comparison of BDEs from infinite separation (Eqn. 3.1 and 3.2) and PES scans</td>
<td>62</td>
</tr>
<tr>
<td>Table 3-3. Comparison of BDEs from infinite separation (Eqn. 3.1 and 3.2) and PES scans in the presence of implicit solvent</td>
<td>67</td>
</tr>
<tr>
<td>Table 4-1. Geometrical arrangements and relative energies of the low energy isomers of ReO[DPhe-Cys-Tyr][Cys]</td>
<td>82</td>
</tr>
<tr>
<td>Table 5-1. Dihedral angles and relative energies of octreotide and derivatives</td>
<td>104</td>
</tr>
<tr>
<td>Table 5-2. Partitioning schemes investigated for ONIOM calculations for an isomer of Re-SDPhe-TATE</td>
<td>110</td>
</tr>
</tbody>
</table>
Table App.1-1. LC-MS characterization of reduced, disulfide-cyclized and metal-complexed peptides. .................................................................................................................. 135

Table App.2-1. $^1$H and $^{13}$C chemical shifts of linear SDPhe-TATE................. 141

Table App.2-2. $^1$H and $^{13}$C chemical shifts of Re(V)-cyclized SDPhe$^1$-Tyr$^3$-octreotate isomer 1................................................................. 142

Table App.2-3. $^1$H and $^{13}$C chemical shifts of Re(V)-cyclized SDPhe$^1$-Tyr$^3$-octreotate isomer 2................................................................. 142

Table App.2-4. $^1$H and $^{13}$C chemical shifts of Re(V)-cyclized SDPhe$^1$-Tyr$^3$-octreotate isomer 4................................................................. 143

Table App.2-5. Calculated NMR chemical shifts of the N2- and N3-deprotonated isomers of ReO[SDPhe-Cys-Tyr][Cys]. ........................................ 145

Table App.2-6. Effect of changing the position of the R-group and the capped terminal group on the calculated chemical shift ....................... 146
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1D</td>
<td>One-dimensional</td>
</tr>
<tr>
<td>2D</td>
<td>Two-dimensional</td>
</tr>
<tr>
<td>3D</td>
<td>Three-dimensional</td>
</tr>
<tr>
<td>α-MSH</td>
<td>alpha-melanocyte-stimulating hormone</td>
</tr>
<tr>
<td>AIM</td>
<td>Atoms in molecules</td>
</tr>
<tr>
<td>Ala</td>
<td>Alanine</td>
</tr>
<tr>
<td>BDE</td>
<td>Bond dissociation energy</td>
</tr>
<tr>
<td>Cys</td>
<td>Cysteine</td>
</tr>
<tr>
<td>DFT</td>
<td>Density functional theory</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DOTA</td>
<td>1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid</td>
</tr>
<tr>
<td>DSS</td>
<td>2,2-dimethylsilapentane-5-sulfonic acid</td>
</tr>
<tr>
<td>DTPA</td>
<td>Diethylenetriaminepentaacetic acid</td>
</tr>
<tr>
<td>Fmoc</td>
<td>9-fluorenlymethyloxycarbonyl</td>
</tr>
<tr>
<td>HF</td>
<td>Hartree-Fock</td>
</tr>
<tr>
<td>HSQC</td>
<td>Heteronuclear single quantum coherence</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Half maximal inhibitory concentration</td>
</tr>
</tbody>
</table>
in vitro  In an artificial environment outside a living organism

in vivo  Inside a living organism

LC-ESI-MS  Liquid chromatography-electrospray ionization-mass spectroscopy

Lys  Lysine

NMR  Nuclear magnetic resonance

NOE  Nuclear Overhauser effects

NOESY  Nuclear Overhauser effect spectroscopy

ONIOM  Our own N-layered Integrated molecular Orbital and molecular Mechanics

PBS  Phosphate buffered saline

PES  Potential energy surface

PET  Positron-emission tomography

Phe  Phenylalanine

RP-HPLC  Reverse phase high performance liquid chromatography

SDPhe  (R)-2-mercapto-3-phenylpropanoic acid

SPECT  Single photon emission computed tomography

SSTR  Somatostatin receptor

TATE  Tyr$^3$-octreotate

TBA  Tetrabutylammounium

TFA  Trifluoroacetic acid

Thr  Threonine

TLC  Thin layer chromatography

TOCSY  Total correlated spectroscopy

Trp  Tryptophan
<table>
<thead>
<tr>
<th>Tyr</th>
<th>Tyrosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV</td>
<td>Ultra violet</td>
</tr>
</tbody>
</table>
INVESTIGATING $^{99m}$TECHNETIUM/RHENIUM(V)-CYCLIZED OCTREOTIDE ANALOGUES USING EXPERIMENTAL AND COMPUTATIONAL METHODS

Yawen Li

Dr. Silvia S. Jurisson, Dissertation Supervisor
Dr. Carol A. Deakyne, Dissertation Co-Supervisor
Dr. Michael R. Lewis, Dissertation Co-Supervisor

ABSTRACT

Radiolabeled proteolytic degradation-resistant somatostatin analogues have been of long-standing interest as cancer imaging and radiotherapy agents for targeting somatostatin receptor-positive tumors. Our interest in developing $^{186}$Re- and $^{188}$Re-based therapeutic radiopharmaceuticals led to investigation of a new Re(V)-cyclized octreotide analogue, Re(V)-cyclized SDPhe$^1$-Tyr$^3$-octreotate [thiolated-DPhe$^1$-Cys$^2$-Tyr$^3$-DTrp$^4$-Lys$^5$-Thr$^6$-Cys$^7$-Thr(OH)$^8$] (Re-SDPhe-TATE) using both experimental and quantum chemical methods. The metal is directly coordinated to SDPhe$^1$-Tyr$^3$-octreotate through cyclization of the peptide around the [ReO]$^{3+}$ core. Upon complexation, four isomers were observed; the isolated/semi-isolated isomers exhibited different sst$_2$ binding affinities, 0.13 to 1.5 μM, in AR42J cells. Two-dimensional NMR experiments and electronic structure calculations were employed to elucidate the structural differences among the different isomers. According to NMR studies the metal is coordinated to three thiolates and the backbone amide of Cys$^2$ in isomers 1 and 4, whereas the metal is coordinated to three thiolates and the backbone amide of Tyr$^3$ in isomer 2. Quantum chemical methods clarified the stereochemistry of Re-SDPhe-TATE and the possible peptide arrangements around the [ReO]$^{3+}$ core. The Re-cyclization
reaction was translated to the $^{99m}$Tc radiotracer level with four isomers observed on complexation with comparable HPLC retention times as the Re-SDPhe-TATE isomers. About 85% total $^{99m}$Tc labeling yield was achieved by ligand exchange from $^{99m}$Tc-glucoheptonate at 60°C for an hour. About 100% and 51% of $^{99m}$Tc(V)-cyclized SDPhe$^1$-Tyr$^3$-octreotate remained intact in phosphate buffered saline and 1 mM cysteine solution under physiological conditions at 6 h, respectively.

A reliable computational method for modeling Re-peptide complexes may facilitate the design of $^{99m}$Tc/Re(V)-cyclized octreotide analogues for targeting somatostatin receptor-positive tumors. A relaxed potential energy surface scan approach was assessed for trans-ReO(SH)$_2$(NH$_2$)(NH$_3$), [ReO(SH)$_3$(NH$_2$)]$^{1-}$, and [ReO(SH)$_3$(N(H)CHO)]$^{1-}$ model complexes to calculate bond dissociation energies for Re–NH$_3$, Re–NH$_2$, Re–N(H)CHO and Re–SH bonds, common components of Re(V) coordination environments. In addition, the model complexes ReO[SDPhe-Cys-Tyr][Cys] and ReO[DPhe-Cys-Tyr][Cys] which contain chelate rings similar to those in the Re(V)-cyclized octreotide analogues were employed for comparing the stability of N$_2$S$_2$ and NS$_3$ coordination systems. Further, the 3D molecular structures of disulfide-cyclized Tyr$^3$-octreotate (Tyr$^3$-TATE) and octreotide were studied using molecular dynamics (MD) simulations and electronic structure calculations. Tyr$^3$-TATE was observed to have distorted helical and β-turn-like conformations in the MD simulations with explicit water. The amino acid side chains were found to have minor effects on the peptide backbone conformation of octreotide and its derivatives compared with the charged termini,
based on gas-phase electronic structure calculations. However, the presence of some side chains affects the position of the neighboring residues’ side chains. Finally, seven partitioning schemes were explored for modeling Re-SDPhe-TATE using the ONIOM method.
Chapter 1

General Introduction

Nuclear medicine is a division of medicine that involves the application of radionuclides to the diagnosis and treatment of disease. In the 1920s, George de Hevesy studied the distribution of a radiotracer ($^{32}\text{P}$) in rats and established the radiotracer principle, the fundamental premise of nuclear medicine. The radiotracer principle states that a radiotracer is a radiolabeled molecule that can be used to trace biochemical processes in biological systems. Because radioactivity can be detected at very low concentrations (pmol to nmol), only trace quantities of the radiotracer are introduced into the body and the normal activity of the biological systems is not disturbed.

Although an increasing number of radiotherapeutic procedures are being performed in hospitals, the primary application of nuclear medicine is still in nuclear imaging. For example, nuclear imaging procedures are performed to help accurately assess the damage to the heart after a heart attack, or to evaluate the glucose metabolism in the brain and help with the diagnosis of Alzheimer's disease. It was reported in March 2014 that the global nuclear medicine market is expected
to reach 24 billion dollars by 2030.\textsuperscript{1} The growth of nuclear medicine is a multidisciplinary effort, one in which chemistry makes a significant contribution.

### 1.1 Cancer-targeting Nuclear Medicine

Today nuclear medicine, especially the use of site-specific targeting radiopharmaceuticals, is playing an important role in the early diagnosis and treatment of cancers. Ideally, the tumor-targeted radiopharmaceutical should be carried to the cancerous tissues rapidly via the blood stream and be retained only at targeted sites. Any radiopharmaceutical that is not taken up by the tumor should be excreted from the body quickly so that radioactivity does not accumulate in normal tissues. To obtain these ideal properties, factors such as the targeting vector, the choice of radionuclide and the labeling approach need to be considered when designing radiopharmaceuticals, along with many other factors such as clearance and uptake by non-target organs/tissues.

Targeting vectors useful for developing radiopharmaceuticals should have very high affinity (pM to nM binding affinity) and specificity for the cell surface receptors/transporters that are unique for and highly expressed in cancerous tissues. Alternatively the target vector can be a substance actively involved in metabolic processes up-regulated in cancer.

For the selection of radionuclide, $\beta^+$-emitting and low energy (around 100 to 200 keV) $\gamma$-emitting radionuclides are potentially useful for Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT)
imaging, respectively. On the other hand, $\alpha$-emitting and $\beta$-emitting radionuclides are potentially useful for developing tumor-targeted therapeutic radiopharmaceuticals.

The half-life of a radionuclide is an important nuclear property that must be considered when choosing radionuclides. Radionuclides with short half-lives such as $^{11}\text{C}$ (~ 20 min) can be useful if the radionuclide can be produced on site and preparation of the radiopharmaceutical is relatively fast with respect to the half-life of the radionuclide. However, such radionuclides are generally not suitable for labeling large targeting vectors such as antibodies because large biomolecules usually circulate in the blood stream for days before they are taken up by the targeted tissue or excreted from the body. Radionuclides with longer half-lives, such as $^{89}\text{Zr}$ (~ 3.3 days), are a better match for labeling targeting vectors with in vivo circulation half-lives on the order of days. Besides decay mode and half-life, the availability of the radionuclide in relatively high specific activity is also an important factor.

Radionuclides are attached to the targeting vector using a direct or an indirect labeling approach. The indirect labeling approach, or bifunctional chelate approach, incorporates the radionuclide into the targeting vector via prosthetic groups (organic radionuclides) or functionalized chelators (inorganic radionuclides), whereas the direct labeling approach directly incorporates the radionuclides into the targeting vector without the use of prosthetic groups or chelators. The choice of labeling approach depends on the nature of the radionuclide as well as the type of
targeting vector. For example, if tyrosine, an amino acid residue that is readily iodinated, is present in the sequence of a peptide targeting vector, the peptide may be directly labeled with $^{131}$I and $^{124}$I by electrophilic substitution at the tyrosine residue. OctreoScan®, a FDA approved imaging agent for somatostatin receptor-positive tumors, was derived from the indirect labeling approach. In OctreoScan®, the radionuclide $^{111}$In is coordinated to a chelating group, diethylenetriaminepentaacetic acid (DTPA), that is conjugated to the N-terminus of the peptide targeting vector. For both labeling approaches, the ultimate goal is to yield a radiolabeled product with high in vivo stability. A labeling approach that requires mild reaction conditions and short reaction times and results in high labeling yields and few byproducts is favored.

### 1.2 Computational Methods

Molecular Mechanics (MM) methods are computational chemistry methods that treat a molecule as a collection of particles held together by forces such as bond stretching, angle bending, bond torsions, and non-bonded interactions. The overall molecular potential energy functional is the sum of the individual potential energy functions that describe the various types of forces. MM methods are also called force-field methods because the potential energy functions and parameters together constitute a force field. Force fields are also employed in molecular dynamics (MD) methods for the calculation of the potential energy of molecular systems. MD simulations are used for investigating the dynamic behavior of systems, such as the conformational changes of proteins and nucleic acids.
MM methods are well developed for modeling small organic molecules and biomolecules composed of organic elements. Although they have been used to assist with designing organic radionuclide-based radiopharmaceuticals\textsuperscript{3}, MM methods and force fields used in MD simulations generally still need to be improved for modeling drugs that contain metals. Many useful radionuclides are inorganic elements such as technetium, rhenium and copper. Thus developing a reliable MM method for modeling metal-containing molecules is of interest in the radiopharmaceutical sciences.

Quantum mechanical (QM) methods (also called quantum chemical methods) and hybrid methods (QM/MM) provide an alternative for modeling metal-containing molecules, although the computational costs (CPU time) for QM methods are much higher than those for MM methods. The most popular quantum mechanical method for studying relatively large systems (up to 100 heavy atoms) is Density Functional Theory (DFT). DFT methods are computational methods that describe the properties of a many-electron molecular system as a function of the electron density rather than the many-electron wavefunction. DFT methods can generate accurate molecular geometries and energetics at reasonable computational cost, if properly used. As examples, the use of DFT methods provided insight into the chelate design for $^{89}$Zr\textsuperscript{3} and the mechanism of hypoxic selectivity in copper bis(thiosemicarbazone) radiopharmaceuticals\textsuperscript{5}. Hybrid methods can be used for studying larger systems, where only a region of the entire system is treated with a quantum chemical method and the rest of the atoms are accounted for using a MM
method. Hybrid methods are more cost effective but the regions treated with methods of different accuracy must be carefully defined.

Examples of the DFT methods and basis sets of interest in this work are 1) PBE0 and B3LYP and 2) 6-31G(d), 6-31G(d,p), 6-311+G(d), LANL2DZ, LANL2TZ and cc-pVDZ(pp), respectively. In general, for basis sets the longer the name, the more atomic orbitals are assigned to each atom. For example, compared to 6-31G, the basis set 6-31G(d) adds polarization functions to non-hydrogen atoms. Different combinations of DFT methods and basis sets provide calculations of different accuracy. Specifically in this work, the notation “PBE0/6-31G(d,p):LANL2TZ” means the PBE0 DFT method is employed in combination with the basis set 6-31G(d,p) for non-metal atoms and LANL2TZ for metal atoms. Geometries converge sooner than energies do, so single-point calculations are often performed on reliable geometries obtained at a lower calculational level for more accurate energetics. For example, the notation “PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d,p):LANL2TZ” means a single-point energy calculation is performed at the PBE0/6-311+G(d):LANL2TZ level of theory using the PBE0/6-31G(d,p):LANL2TZ geometry. In other words, the double-slashes in computational method notations separate the levels at which single-point energy calculations and geometry optimizations are performed.

In this work, a coordinated experimental and computational effort was made to investigate potential $^{186/188}$Re and $^{99m}$Tc tumor-targeting agents. New $^{99m}$Tc/Re(V)-cyclized somatostatin receptor-targeting peptides were synthesized,
characterized and evaluated in vitro for receptor binding affinity and chemical stability, as described in Chapter 2. Additionally, quantum chemical methods were employed to assist with structural characterization of the metal-peptide complexes. Chapters 3-5 detail the efforts made to develop a computational method for predicting the 3D structure (receptor binding affinity) and chemical stability of Re(V)-cyclized somatostatin receptor-targeting peptides. Conclusions and future directions are summarized in Chapter 6.

1.3 References


Chapter 2

Synthesis, Characterization and In Vitro Evaluation of New $^{99m}$Tc/Re(V)-cyclized Octreotide Analogues: An Experimental and Computational Approach

2.1 Introduction

Rhenium radioisotopes are useful for therapeutic radiopharmaceutical development due to their attractive nuclear properties: $^{186}$Re has a half-life of 90 h and emits $\beta^-$ particles with a maximum energy of 1.07 MeV, along with a 137 keV $\gamma$ ray (9%); $^{188}$Re has a half-life of 17 h and emits $\beta^-$ particles of 2.1 MeV maximum energy, along with a 155 keV $\gamma$ ray (15%). The $\beta^-$ particles can be used for killing cancer cells and the $\gamma$ rays are useful for single-photon emission computed tomography (SPECT) imaging for internal dosimetry monitoring. An example of $^{188}$Re-labeled radiotherapeutic agents is $^{188}$Re-1,1-hydroxyethylidene-diphosphonate (HEDP) which is prescribed to treat painful bone metastases in outpatients by taking advantage of the $\gamma$ rays and $\beta^-$ particles emitted by $^{188}$Re and quantitative SPECT/CT imaging in Australia. $^{188}$Re-HEDP has bone marrow toxicity comparable to FDA approved...
bone-targeting radiopharmaceuticals.\textsuperscript{1-3} There are also a number of human studies using \textsuperscript{188}Re-labeled monoclonal antibodies, peptides or carbohydrates (like lipiodol) for the treatment of various cancers.\textsuperscript{4-8}

Rhenium-186/188 has been recognized as the radiotherapeutic matched pair for the diagnostic \textsuperscript{99m}Tc as the 3\textsuperscript{rd} row congener of technetium; however, few \textsuperscript{99m}Tc-chelating systems used for tumor targeted imaging have been successfully translated to use with \textsuperscript{186/188}Re, due to the differences in their redox chemistry and ligand exchange kinetics. Perrhenate \([\text{\textsuperscript{186/188}ReO}_4]^{1-}\) is more difficult to reduce than \([\text{\textsuperscript{99m}TcO}_4]^{1-}\) and once reduced Re(V) has a higher tendency to reoxidize to perrhenate. Endeavors are continuously made in developing \textsuperscript{186/188}Re-chelating systems for specific site targeting therapeutic agents, yet such systems that do not require harsh labeling conditions, have ideal lipophilicity and are stable \textit{in vivo} are still in demand.\textsuperscript{9-12}

Our interest is in developing somatostatin receptor targeting \textsuperscript{186/188}Re therapeutic agents. Somatostatin (SS), also known as somatotropin release-inhibiting factor (SRIF), is a peptide hormone that functions by inhibiting the secretion of several other hormones. SS interacts with five G protein-coupled receptor subtypes (sst\textsubscript{1}-sst\textsubscript{5}). These receptors are present in many organ systems, including the central nervous system and the exocrine and endocrine pancreas. Somatostatin receptors (SSTRs) are expressed in high density on most human endocrine tumors and are thus recognized as molecular targets for localizing to these tumors and their metastases.\textsuperscript{13} Native SS is a cyclic disulfide-containing
peptide containing 14 or 28 residues (SS-14/28) with a short plasma half-life of about 3 min. Therefore, D-amino acids were introduced into the peptide sequence and the natural molecule was shortened to the eight amino acid bioactive core to increase the biological stability. Octreotide [DPhe-cyclo(Cys-Phe-DTrp-Lys-Thr-Cys)-Thr-(ol)] [Sandostatin®] and its derivatives, such as Tyr³-octreotide and Tyr³-octreotate [DPhe-cyclo(Cys-Tyr-DTrp-Lys-Thr-Cys)-Thr-(OH)], are important SS analogues with resistance to enzymatic degradation.¹⁴,¹⁵

The [ReO]³⁺ core can be directly coordinated to octreotide analogues through cyclization of the peptide around the metal, taking advantage of the thiolates and amides in the peptide sequence.¹⁶-¹⁸ The direct labeling approach does not require use of a bifunctional chelator and may lead to increased tumor uptake and retention, as shown by previous studies with Re(V)-cyclized α-melanocyte-stimulating hormone (α-MSH) analogues.¹⁷,¹⁸ In this work, new ⁹⁹ᵐTc/Re(V)-cyclized octreotide analogues, ⁹⁹ᵐTc/Re(V)-cyclized, thiolated DPhe¹-Tyr³-octreotate (⁹⁹ᵐTc/Re-SDPhe-TATE) were synthesized, characterized and evaluated in vitro using experimental and computational methods.

2.2 Experimental and Computational Details

2.2.1 Experimental details

2.2.1.1 General Methods

Most reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO), Thermo Fisher Scientific (Pittsburgh, PA) and Chem-Impex International
(Wood Dale, IL) at the highest quality available and were used without further purification. Trityl-protected (R)-2-mercapto-3-phenylpropanoic acid (TrtSDPhe) and tetrabutylammonium tetrachlorooyxorhenium(V) ([TBA][ReOCl₄]) were synthesized and purified following literature methods.¹⁹,²⁰

Liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) analysis and semi-preparative RP-HPLC purification were carried out on a Beckmann Coulter (Fullerton, CA) System Gold HPLC equipped with the 32 KARAT software package, a 507e auto-injector and a 168 diode array detector coupled to a Thermo Scientific (Waltham, MA) LTQ XL ion trap mass spectrometer. Radiotracer reactions were monitored on a Shimadzu (Columbia, MD) UFLC HPLC equipped with a NaI(Tl) well detector. LC-ESI-MS and radio-RP-HPLC analyses were performed on a Thermo Scientific BetaBasic C18 column (150 Å, 0.46 cm ×15 cm, 5 μm) with linear gradients of solvent B in solvent A (A: 0.1% TFA in water; B: 0.1% TFA in acetonitrile), a 1 mL/min flow rate, and UV detection at 214 and 280 nm. Semi-preparative RP-HPLC purification was performed on a Waters (Milford, MA) Prep Nova-Pak HR C18 column (60 Å, 1.9 cm × 30 cm, 6 μm), also using linear gradients of solvent B in solvent A with flow rates up to 10 mL/min and UV detection at 225/235 and 280 nm.

2.2.1.2 Synthesis and Purification of Linear SDPhe-TATE

[Thiolated-DPhe-Cys-Tyr-DTrp-Lys-Thr-Cys-Thr-(OH)]

The protected peptide precursor of [Cys-Tyr-DTrp-Lys-Thr-Cys-Thr-(OH)] was prepared with an Advanced ChemTech (Louisville, KY) 396 Omega multiple
peptide synthesizer following conventional Fmoc solid-phase peptide synthesis protocols using 2-chlorotrityl resin. The peptide precursor was cleaved from the resin using 25% hexafluoro-2-propanol (HFIP) and 5% triisopropylsilane (TIS) in dichloromethane (DCM) to leave side chains fully protected. The cocktail cleavage was neutralized with 10% pyridine in methanol and the organic solvents were removed by lyophilization. Benzotriazol-1-yl-oxytrityrroldinophosphonium hexafluorophosphate (PyBOP) and N,N-diisopropylethylamine (DIEA) were added to a dimethylformamide (DMF) solution of the TrtSDPhe in a molar ratio of 0.7:1:1.8 to generate the activated ester of the amino acid building block. The crude product of the protected peptide precursor was dissolved in DMF with about 9 molar equiv of DIEA and combined with the activated ester in a 1:5 molar ratio. The reaction mixture was mixed gently at room temperature for 24 h and then treated with a TFA solution containing 2.5% each of thioanisole, phenol, water, ethanedithiol, and TIS to deprotect the side chains. Following lyophilization, the crude product was purified by semi-preparative RP-HPLC. The purity and identity of linear SDPhe-TATE were verified by LC-ESI-MS.

2.2.1.3 Synthesis and Purification of Re-cyclized SDPhe-TATE

Re cyclization was achieved via transchelation reactions of Re(V) from [TBA][ReOCl₄] to linear SDPhe-TATE using previously described procedures. Briefly, the purified linear peptide (5-30 mg) and 3 molar equiv of [TBA][ReOCl₄] were dissolved in anhydrous DMF to give a peptide concentration of 10 mg/mL. The reaction was stirred at room temperature overnight under a positive pressure of
argon. The peach color of the reaction mixture gradually changed to golden yellow. The solvent was removed via a high-pressure vacuum pump, the resulting brown-colored residue was dissolved in a 1:1 acetonitrile (0.1% TFA):water (0.1% TFA) solution, and this solution was centrifuged and filtered to remove the black precipitate, likely ReO₂. The purple magenta filtrate was analyzed by LC-ESI-MS to confirm the formation of the desired complex. Semi-preparative RP-HPLC purification was performed using an optimized multistep gradient.

2.2.1.4 Synthesis of \(^{\text{nalt}}\text{In-DOTA-Tyr}^{3}\text{-octreotide}\)\(^{22}\)

The linear DOTA-Tyr\(^3\)-octreotide, DOTA-[DPhe\(^1\)-Cys\(^2\)-Tyr\(^3\)-DTrp\(^4\)-Lys\(^5\) -Thr\(^6\)-Cys\(^7\)-Thr\(^8\)-(ol)], was prepared with an Advanced ChemTech 396 Omega multiple peptide synthesizer following conventional Fmoc solid-phase peptide synthesis protocols. Disulfide cyclization was achieved by stirring about 20 mg of the linear peptide in 2 mL of 50:25:25 DMSO:acetonitrile:H\(_2\)O solution at room temperature for 72 h. DMSO was diluted to less than 10% by adding ultrapure water (18 MΩ-cm) prior to lyophilization. The recovered solid and 5 molar equiv of \(^{\text{nalt}}\text{InCl}_3\) were dissolved in a 30 mM sodium acetate:25 mM sodium ascorbate solution (pH 5.0) and refluxed for 30 minutes at 99 °C to label the peptide with \([^{\text{nalt}}\text{In}]^{3+}\) via the DOTA chelate. The reaction solution was lyophilized and the crude product was purified using semi-preparative RP-HPLC. The identity of the isolated product was confirmed by LC-ESI-MS.
2.2.1.5 Two-dimensional NMR experiments of Re-cyclized SDPhe-TATE isomers

One-dimensional and two-dimensional (2D) NMR spectra of linear SDPhe-TATE and Re-cyclized SDPhe-TATE isomers 1, 2 and 4 were collected on a Varian Unity Inova 600 MHz spectrometer equipped with a 5-mm [$^{1}$H, $^{15}$N, $^{13}$C] triple-resonance cryoprobe or on a Bruker Avance UltraStabilized 800 MHz spectrometer equipped with a 5-mm automatic match and tuning TCI probe with $^{1}$H and $^{13}$C preamplifiers. NMR experiments, 2D total correlated spectroscopy (TOCSY) (80 ms mixing time), nuclear Overhauser effect spectroscopy (NOESY) (400 ms mixing time) and $^{1}$H–$^{13}$C heteronuclear single quantum coherence (HSQC) spectroscopy, were performed for 1 to 5.3 mM solutions of Re-cyclized SDPhe-TATE isomers in 420 μL of a 40:80:300 D$_{2}$O:CD$_{3}$CN:H$_{2}$O mixture. The pH was in the range of 2.0 to 4.5, as determined using an electronic pH meter equipped with a micro pH probe (IQ Scientific Instruments). The NMR samples were prepared in a SHIGEMI NMR tube (Shigemi Co., Japan), and the experiments were carried out at 25 °C. NMR data were processed with NMRPipe$^{23}$ and analyzed in SPARKY$^{24}$ software. Indirect dimensions were normally extended by linear prediction and zero filled prior to Fourier transformation, and only spectral regions containing signals were retained. The $^{1}$H and $^{13}$C chemical shifts were referenced to 2, 2-dimethylsilapentane-5-sulfonic acid (DSS) as an external standard.

2.2.1.6 NOE assignments and distance calibration

The nuclear Overhauser effect (NOE) cross peaks on NOESY spectra of isomer 1 were assigned based on the chemical shift assignments determined from
TOCSY, $^1$H-$^{13}$C HSQC, and NOESY spectra. The NOE cross peak intensities were translated into upper-distance bounds according to Eqn 2.1, where $I$ is the NOE intensity, $r$ is the proton-proton distance and $k$ is a constant determined using the cross peak intensity and distance of the Trp H$_\beta$ to the H$_\delta$ (2.8 Å), which is considered a medium NOE. The backbone NOEs of isomer 1 were classified as weak (W), medium (M), or strong (S), with corresponding upper bounds of 6.0, 3.5 and 2.7 Å, respectively.\(^{21}\)

$$I = \frac{k}{r^6} \quad \text{Eqn. 2.1}$$

### 2.2.1.7 In vitro receptor binding assays

The somatostatin receptor binding affinities of isomers 1, 2, and 4 and a mixture of isomers 2 and 3 were determined on AR42J rat pancreatic tumor cells from competitive binding assays with $^{125}$I-Tyr$^{11}$-somatostatin-14. The cells were cultured in a modified RPMI 1640 medium (GIBCO-Invitrogen, Carlsbad, CA) with 4.5 g/L glucose supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 50 μg/mL gentamycin. Cells were incubated in a humidified atmosphere of 5% CO$_2$ in air at 37 °C by the Cell and Immunobiology Core Facility at the University of Missouri.

Prior to cell experiments, the cells were harvested by trypsinization with TrypLE Express (GIBCO-Invitrogen, Carlsbad, CA) and the trypsin was quenched with 1 mL of soybean trypsin inhibitor (1 mg/mL; Sigma-Aldrich, St. Louis, MO)
for every 1 mL of TrypLE used. Subsequently, the cells were washed and concentrated to 20 million cells/mL, using a binding buffer, RPMI 1640 medium with 0.25% bovine serum albumin (GIBCO-Invitrogen). AR42J cells (2 million cells/aliquot; in triplicate) were incubated with $^{125}$I-Tyr$^{11}$-somatostatin-14 (~100,000 cpm) and increasing concentrations of the Re(V)-SDPhe-TATE isomers at room temperature for 1 h. Cell-bound radioactivity was recovered by centrifugation and aspiration of the incubation media. The cells were kept at 4 °C and washed three times with ice cold binding buffer to remove any residual unbound radioactivity. The cell pellets were counted on a Wallac 1480 Wizard 3′′ automated gamma counter (PerkinElmer Life Sciences, Gaithersburg, MD) to determine the amount of $^{125}$I-Tyr$^{11}$-somatostatin-14 bound to the cells. The results were analyzed using GraphPad Prism 6.025 to determine the IC$_{50}$ values, the concentrations of the compounds that resulting half-maximal inhibition of $^{125}$I-Tyr$^{11}$-somatostatin-14 binding to the receptors.

2.2.1.8 Preparation and Characterization of $^{99m}$Tc(V)-cyclized SDPhe-TATE

The purified linear SDPhe-TATE was cyclized with $[^{99m}\text{TcO}_{2}]^{3+}$ by ligand exchange from $^{99m}$Tc-glucoheptonate ($^{99m}$Tc-GH), using a modified published protocol.26 Briefly, 200 μL of degassed 0.2 M sodium glucoheptonate containing 0.13 mg of SnCl$_2$ was added to 100 μL of $[^{99m}\text{TcO}_4]^{-}$ (1-3 mCi, obtained from Mid-America Isotopes, Inc., Ashland, MO). Fifty μL of a 1.5 mg/mL linear SDPhe-TATE solution in 50:50 acetonitrile:water solution was added to the $^{99m}$Tc-GH solution, followed by pH adjustment to 8.0 using about 20 μL of 0.1 M
NaOH. The reaction was heated at 60 °C for 1 h. An aliquot of the reaction mixture was co-injected onto an RP-HPLC column with a solution of natural abundance Re(V)-SDPhe-TATE to confirm the formation of $^{99m}$Tc(V)-cyclized SDPhe-TATE. Sep-Pak C18 Plus Light Cartridges (Waters, Milford, MA) were used for the separation of $^{99m}$Tc(V)-cyclized SDPhe-TATE from unreacted $^{99m}$Tc-GH and salts. A cartridge was preconditioned by washing with 2 mL of ultrapure water, 3 mL of acetonitrile and 5 mL of ultrapure water, consecutively. One to three hundred µL of the reaction mixture was loaded onto the cartridge, followed by 1 mL of ultrapure water. Finally, the cartridge was eluted with fractions of 100 µL of 50:50 acetonitrile:water and an aliquot of the third fraction collected was injected onto an RP-HPLC column for quality control, or diluted in phosphate buffered saline (PBS) or cysteine solution for subsequent in vitro stability studies.

2.2.1.9 In vitro stability studies

Chemical stability of $^{99m}$Tc(V)-cyclized SDPhe-TATE was evaluated in 10 mM PBS and 1 mM cysteine solution under physiological conditions (pH 7.4 and 37 °C). Thirty to fifty µL (about 250 µCi) of $^{99m}$Tc(V)-cyclized SDPhe-TATE, purified by Sep-Pak C18 cartridges, were diluted 10 times with 10 mM PBS, or with 10 mM PBS that containing 1 mM cysteine (from 0.1 M cysteine stock solution freshly made, 6.1 mg of L-cysteine in 0.5 mL of 10 mM PBS). Approximately 0.5 mg of gentisic acid was added to the PBS buffer on the day of the experiment, and the pH was pre-adjusted to 7.4 using 0.1 M NaOH solution as
needed. The diluted solutions were incubated at 37 °C. Aliquots of the incubation mixtures were removed at various time points (2, 4 and 6 h) and analyzed by RP-HPLC to determine the percent of intact radiolabeled peptide remaining.

2.2.2 Computational details

Quantum chemical calculations were carried out using the Gaussian 03/09 software packages\textsuperscript{27,28} to characterize the Re-peptide coordination sphere, which is challenging to determine using $^1$H/$^{13}$C NMR. The model complex $\text{ReO[SDPhe-Cys-Tyr][Cys]}$ was used to model the coordination sphere of Re-SDPhe-TATE, where the terminal amines and carboxylates are capped with acetyl groups and methylamines, respectively. The $[\text{ReO}]^{3+}$ core was coordinated to the deprotonated backbone amide of Cys\textsuperscript{2}, Tyr\textsuperscript{3} or Cys\textsuperscript{7} and the three thiolates to represent the three coordination possibilities, N2, N3 and N7. A variety of trigonal bipyramidal, square pyramidal and octahedral (only for N3 deprotonation) starting geometries were screened at the HF/6-31G(d):LANL2DZ level of theory; the local minima with relative energies smaller than 50 kcal/mol were reoptimized at the PBE0/6-31G(d):LANL2DZ level of theory.\textsuperscript{29} The orientation of the R-groups was varied to identify the lowest energy structures. Vibrational frequencies were computed to verify the optimized structures correspond to minima. Single-point energy calculations were carried out with and without implicit water at the (PCM)-PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d):LANL2DZ level as recommended from our earlier calibration studies.\textsuperscript{30} Correction terms were extracted from the vibrational frequency calculations to obtain enthalpies and
Gibbs free energies. AIMAll (Version 11.12.19) was used to perform quantum theory of atoms in molecules (QTAIM) analyses.

To identify a reliable method for the calculation of NMR chemical shifts, ReO[Phe-Gly-Cys] (designated as [ReO]FGC by Cantorias et al.), a Re(V) complex that has been synthesized and characterized by X-ray crystallography and NMR spectroscopy, was investigated computationally. The crystal structures of ReO[Phe-Gly-Cys] were used as the starting geometries in the PBE0/6-31G(d):LANL2DZ optimizations of the syn and anti diastereoisomers of ReO[Phe-Gly-Cys]. Single-point energy calculations were performed in the presence of implicit water implanted by the integral equation formalism of the polarizable continuum model (IEF-PCM) at the PCM-PBE0/6-31G(d,p):LANL2TZ, PCM-PBE0/6-311+G(d):LANL2TZ, PCM-PBE0/cc-pVTZ-(pp), and PCM-MP2/6-31G(d,p):LANL2TZ levels of theory on the PBE0/6-31G(d):LANL2DZ geometries, to obtain NMR shielding tensors using the Gauge-Independent Atomic Orbital (GIAO) method or the Continuous Set of Gauge Transformations (CSGT) method for the ReO[Phe-Gly-Cys] isomers. Hα and Hβ chemical shifts were calculated referencing to the protons of tetramethylsilane (TMS). The method that reproduced the experimentally observed differences between the syn and anti isomers for ReO[Phe-Gly-Cys] was employed for calculating Hα and Hβ chemical shifts for the lowest energy N2-, N3- and N7-deprotonated structures of ReO[SDPhe-Cys-Tyr][Cys].
2.3 Results and Discussion

2.3.1 Choice of the peptide sequence

The peptide sequence of SDPhe-TATE was designed based on previous structure-activity relationship studies. In earlier work, a series of Re(V)-cyclized octreotide analogues were synthesized and assessed. In these Re-peptide complexes, the $[\text{ReO}]^{3+}$ core was coordinated directly into the peptide taking advantage of the thiolates and amides available from the peptide. Depending on the specific peptide sequence, the $[\text{ReO}]^{3+}$ core formed either an $\text{N}_2\text{S}_2$ or $\text{NS}_3$ five-coordinate coordination sphere with 2 or 3 cysteine thiols and 1 or 2 amides from the peptide backbone. The $\text{N}_2\text{S}_2$ Re-peptide complexes showed sub-micromolar to nanomolar $\text{in vitro}$ $\text{sst}_2$ binding affinities in IC$_{50}$ experiments with Re(V)-cyclized Tyr$^3$-octreotate (Re-TATE) being the most promising candidate with a 29 nM IC$_{50}$ value. However, $^{186/188}$Re-TATE was expected to be unstable $\text{in vivo}$ because $^{99m}$Tc(V)-cyclized Tyr$^3$-octreotate showed poor $\text{in vitro}$ (PBS) and $\text{in vivo}$ stability, which was attributed to the eight-membered ring formed by the $N$-terminal amine coordination to the metal upon cyclization (Figure 2-1a). The $\text{NS}_3$ Re-peptide complexes were investigated subsequently to improve stability. A third cysteine was added to the peptide sequence or substituted for one of the existing residues. Figure 2-1b shows an example of the three-cysteine analogues. Although, in general, the $\text{NS}_3$ $^{99m}$Tc-peptide complexes showed improved $\text{in vitro}$ stabilities, the IC$_{50}$ value of the best binder among 11 $\text{NS}_3$ Re-peptide complexes examined was 3.4 $\mu$M and the IC$_{50}$ values of most of the other $\text{NS}_3$ analogues were greater than 100 $\mu$M. 
Structure-activity relationships for these Re(V)-cyclized octreotide analogues were rigorously evaluated. The 3D molecular structures of the Re-peptide complexes and the disulfide-cyclized Tyr<sup>3</sup>-octreotide were constructed using NOE constraints and analyzed for the effect of metal complexation on the peptide conformation.<sup>21</sup> From previous work on disulfide-cyclized octreotide or octreotide, the β-turn spanning Tyr<sup>3</sup>-DTrp<sup>4</sup>-Lys<sup>5</sup>-Thr<sup>6</sup> is believed to be held in the correct orientation by the disulfide bridge.<sup>36</sup> The DPhe<sup>1</sup> as well as close proximity of the side chains of DTrp<sup>4</sup>-Lys<sup>5</sup> are believed to be important for receptor binding.<sup>37</sup> Thus, the poor receptor binding affinity observed on metal complexation may be rationalized in several ways: 1) metal complexation widens the disulfide bridge and alters the β-turn spanning Tyr<sup>3</sup>-DTrp<sup>4</sup>-Lys<sup>5</sup>-Thr<sup>6</sup>; 2) formation of five- or six-membered chelate rings causes a considerable constraint on the backbone near the site of metal coordination and consequently affects the backbone conformation in the vicinity (Figure 2-1b);<sup>16,21,26</sup> 3) the phenyl group of DPhe<sup>1</sup> is
absent when DPhe$^1$ is substituted with Cys/DCys; $^{21}$ 4) the total charge changes from +2 to neutral or negative upon metal complexation at pH 7.4.

Thiolated-DPhe$^1$-Tyr$^3$-octreotate (SDPhe-TATE) structurally resembles linear Tyr$^3$-octreotate but possesses a thiol in place of the amine terminus. The peptide was designed to examine the influence of substituting a thiolate sulfur for the $N$-terminal amine on the stability and molecular structure of Re-TATE.

2.3.2 Synthesis and characterization of Re(V)-cyclized SDPhe TATE isomers

Purified linear SDPhe-TATE was cyclized via a transchelation reaction with [TBA][ReOCl$_4$] in anhydrous DMF under argon. Greater than 80% yield was achieved for the Re-cyclization reaction, with four isomers of Re-SDPhe-TATE observed by ESI-LC-MS (Table 2-1). Interestingly, only two diastereoisomers were observed for each Re(V)-cyclized octreotide analogue studied previously.$^{16,21}$ Mass spectral data were consistent with the predicted$^{185/187}$Re isotopic pattern and the calculated mass-to-charge ratio of Re-SDPhe-TATE (Table 2-1). The crude product was purified using semi-preparative RP-HPLC and the isomers were isolated from impurities using an optimized multistep gradient. Because stereoisomers of radiopharmaceutical products often have very different receptor binding affinities and biodistribution behavior, isomers 1, 2 and 4 were collected separately with $\geq$ 90% isomeric purity.
Table 2-1. ESI-LC-MS results for Re-SDPhe-TATE.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Isomer</th>
<th>Retention time (min)</th>
<th>Observed m/z (M+H)$^+$</th>
<th>Calculated m/z (M+H)$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.25</td>
<td>1267.80</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>19.57</td>
<td>1268.22</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>19.98</td>
<td>1268.95</td>
<td>1268.31</td>
</tr>
<tr>
<td>4</td>
<td>20.58</td>
<td>1268.05</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}Gradient: 20\% to 35\% B in A over 45 min. A: H$_2$O with 0.1\% TFA; B: acetonitrile with 0.1\% TFA.

Two-dimensional NMR experiments including TOCSY, NOESY and $^{1}$H-$^{13}$C HSQC were carried out to characterize the linear SDPhe-TATE and Re-SDPhe-TATE isomers 1, 2 and 4. The TOCSY and $^{1}$H-$^{13}$C HSQC spectral assignments identified all eight amino acids and the NOESY assignments confirmed the sequential connectivity of the amino acids. $^{1}$H and $^{13}$C Chemical shifts of linear SDPhe-TATE and Re-SDPhe-TATE isomers 1, 2 and 4 can be found in Appendix II (Tables App.2-1 to App.2-4). We were unable to completely separate isomer 3 from isomer 2 and the NMR spectra obtained for the mixture of isomers 2 and 3 could not be assigned due to severe overlap among the chemical shifts of amide protons.

To characterize the metal coordination site, the chemical shifts of each isomer were compared to those of linear SDPhe-TATE (Table 2-2). For isomer 1, significant downfield shifts were observed for the H$_{\alpha}$ (0.70 ppm) and C$_{\alpha}$ (13.9 ppm) of SDPhe$^1$, indicating that the thiolate group of SDPhe$^1$ is coordinated to the metal. Furthermore, the backbone amide proton cross peaks of Cys$^2$ were absent on the TOSCY and NOESY spectra, suggesting the coordination of the amide of Cys$^2$ to
the metal. This suggestion is further supported by the significant downfield shifts of the $H_α$ (0.84 ppm) and $C_α$ (11.7 ppm) of Cys$^2$ of isomer 1. Moreover, the thiolate groups of Cys$^2$ and Cys$^7$ are coordinated to the metal, indicated by the significant downfield shifts of the $H_β$ (0.65, 0.61 ppm) and $C_β$ (18.7 ppm) of Cys$^2$, and the $H_α$ (0.50 ppm), $H_β$ (1.04, 1.20 ppm) and $C_β$ (9.2 ppm) of Cys$^7$ of isomer 1. Thus, the new Re-SDPhe-TATE isomer 1 has an NS$_3$ metal coordination structure: Re is coordinated to the sulfur of SDPhe$^1$, the backbone amide of Cys$^2$, and the sulfurs of Cys$^2$ and Cys$^7$, as shown in Figure 2-2a.

Table 2-2. Selected $^1$H and $^{13}$C chemical shifts (ppm) of linear SDPhe-TATE and Re-SDPhe-TATE isomers 1, 2 and 4.

<table>
<thead>
<tr>
<th></th>
<th>SDPhe$^1$</th>
<th>Cys$^2$</th>
<th>Tyr$^3$</th>
<th>Cys$^7$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$α_{CH}$</td>
<td>$β_{CH}$</td>
<td>NH</td>
<td>$α_{CH}$</td>
</tr>
<tr>
<td>Linear</td>
<td>3.94 (38.4)</td>
<td>3.29 (37.0)</td>
<td>8.15</td>
<td>4.57 (50.8)</td>
</tr>
<tr>
<td>Isomer 1</td>
<td>4.64 (52.3)</td>
<td>3.15, 3.56 (37.2)</td>
<td>5.41 (62.5)</td>
<td>3.35, 3.38 (39.7)</td>
</tr>
<tr>
<td>Isomer 2</td>
<td>4.49$^b$</td>
<td>2.84, 3.82 (37.6)</td>
<td>7.77</td>
<td>5.24 (66.6)</td>
</tr>
<tr>
<td>Isomer 4</td>
<td>4.56$^b$</td>
<td>3.04, 3.64 (37.4)</td>
<td>5.46$^c$</td>
<td>3.38, 3.55$^c$</td>
</tr>
</tbody>
</table>

$^a$Chemical shifts were referenced relative to DSS and were measured at 298 °K. The chemical shifts are generally accurate to 0.01 ppm for $^1$H and 0.1 ppm for $^{13}$C. $^{13}$C chemical shift values are indicated in parentheses. $^b$Chemical shifts of $^{13}$C$_α$ could not be assigned due to resonances overlapping with H$_2$O on the $^1$H-$^{13}$C HSQC spectrum. $^c$Chemical shifts of $^{13}$C$_α$ could not be assigned due to intermediate timescale chemical exchange.
Isomer 4 displayed highly similar chemical shift changes to isomer 1 for SDPhe, Cys and Cys upon Re metallation (Table 2-2). For example, significant downfield shifts were observed for the $H_\alpha$ (0.62 ppm) of SDPhe, the $H_\alpha$ (0.89 ppm) and $H_\beta$ (0.68, 0.78 ppm) of Cys, and the $H_\alpha$ (0.51 ppm), $H_\beta$ (1.17, 1.19 ppm) and $C_\beta$ (8.3 ppm) of Cys in isomer 4 compared to linear SDPhe-TATE. The results indicate a similar metal coordination of isomers 1 and 4, suggesting they may be syn and anti diastereoisomers.

The absence of the cross peaks from the backbone amide proton of Tyr on the TOSCY and NOESY spectra of isomer 2 indicate that the metal is coordinated to the amide of Tyr. Significant downfield shifts were observed for the $H_\alpha$ (0.55 ppm) of SDPhe, the $H_\alpha$ (0.67 ppm), $C_\alpha$ (15.8 ppm), $H_\beta$ (0.94, 1.13 ppm) and $C_\beta$ (19.4 ppm) of Cys, and the $H_\alpha$ (0.53 ppm) and $H_\beta$ (1.10, 1.25 ppm) of Cys in isomer 2, compared to linear SDPhe-TATE, confirming the coordination of the sulfurs to the metal (Table 2-2). Thus, in isomer 2 the metal is coordinated to the backbone amide...
of Tyr$^3$ and the sulfurs of SDPhe$^1$, Cys$^2$ and Cys$^7$, as shown in Figure 2-2b. Isomer 2 has a unique structure compared to isomers 1 and 4, a conclusion supported by the significant differences in the $H_\beta$ chemical shifts of SDPhe$^1$ and Cys$^2$ of isomer 2 from isomers 1 and 4 (Table 2-2).

NOE assignments and bond distance calibrations were carried out to elucidate the secondary structure of Re-SDPhe-TATE isomer 1. The intensities of the NOE cross peaks were converted into upper-distance bounds and the NOEs were classified as weak, medium and strong with upper bounds of 6.0, 3.5 and 2.7 Å, respectively. The backbone NOEs observed for isomer 1 are compared to those of Re(V)-cyclized Tyr$^3$-octreotate$^{17}$ in Table 2-3. The sequential alpha to amide ($H_\alpha^i-H_\alpha^{i+1}$) NOE for SDPhe$^1$-Cys$^2$ is missing for isomer 1 due to the absence of the amine group in SDPhe$^1$. For the same reason, the $H_\alpha^i-H_\alpha^{i+1}$ NOE for Cys$^2$-Tyr$^3$ and Tyr$^3$-DTrp$^4$ were not observed for Re(V)-cyclized Tyr$^3$-octreotate. In addition, the $H_N^i-H_N^{i+1}$ NOE for DTrp$^4$-Lys$^5$ was observed for isomer 1, rather than the $H_N^i-H_N^{i+2}$ and $H_\alpha^i-H_\alpha^{i+2}$ NOEs observed for DTrp$^4$-Thr$^6$ for Re(V)-cyclized Tyr$^3$-octreotate, indicating a backbone conformational change in the Tyr$^3$-DTrp$^4$-Lys$^5$-Thr$^6$ region. Also the backbone conformation at the C-terminus is different, as evidenced by the presence of a strong $H_N^i-H_N^{i+1}$ NOE for Cys$^7$-Thr$^8$ for isomer 1.
Table 2-3. Backbone-backbone NOEs of Re-SDPhe-TATE isomer 1 and Re(V)-cyclized Tyr³-octreotate.

<table>
<thead>
<tr>
<th>NOE</th>
<th>Intensity</th>
<th>Distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cys² aH–Tyr³ NH</td>
<td>M</td>
<td>1.8-3.5</td>
</tr>
<tr>
<td>Tyr³ aH–DTrp⁴ NH</td>
<td>S</td>
<td>1.8-2.7</td>
</tr>
<tr>
<td>DTrp⁴ NH–Lys⁵ NH</td>
<td>M</td>
<td>1.8-3.5</td>
</tr>
<tr>
<td>Lys⁵ aH–Thr⁶ NH</td>
<td>M</td>
<td>1.8-3.5</td>
</tr>
<tr>
<td>Thr⁶ aH–Cys⁷ NH</td>
<td>S</td>
<td>1.8-2.7</td>
</tr>
<tr>
<td>Cys⁷ NH–Thr⁸ NH</td>
<td>S</td>
<td>1.8-2.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NOE</th>
<th>Intensity</th>
<th>Distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPh³ aH–Cys² NH</td>
<td>M</td>
<td>1.8-3.5</td>
</tr>
<tr>
<td>DTrp⁴ aH–Thr⁶ NH</td>
<td>S</td>
<td>1.8-2.7</td>
</tr>
<tr>
<td>DTrp⁴ NH–Thr⁶ NH</td>
<td>S</td>
<td>1.8-2.7</td>
</tr>
<tr>
<td>DTrp⁴ aH–Lys⁵ NH</td>
<td>M</td>
<td>1.8-3.5</td>
</tr>
<tr>
<td>Lys⁵ aH–Thr⁶ NH</td>
<td>S</td>
<td>1.8-2.7</td>
</tr>
<tr>
<td>Thr⁶ aH–Cys² NH</td>
<td>M</td>
<td>1.8-3.5</td>
</tr>
<tr>
<td>Cys⁷ αH–Thr⁸ NH</td>
<td>W</td>
<td>1.8-6.0</td>
</tr>
<tr>
<td>Cys⁷ NH–Thr⁸ αH</td>
<td>W</td>
<td>1.8-6.0</td>
</tr>
</tbody>
</table>

2.3.3 Calculated equilibrium structures

Quantum chemical studies were performed to elucidate detailed ligand arrangements around the [ReO]³⁺ core in the Re-SDPhe-TATE isomers. A total of eight low energy isomers were found, representing different arrangement of ligands following N2, N3 and N7 deprotonation (Table 2-4). The parameter τ given in Table 2-4 is a measurement used to distinguish distorted square pyramidal and trigonal bipyramidal coordination geometries. The τ value is calculated using Eqn. 2.2, where α and β are the largest and second largest angles around the central metal atom in a 5-coordinate complex. If τ is equal to 0 or 1, the geometry is a perfect square pyramid or a perfect trigonal bipyramid, respectively.

$$\tau = \frac{\alpha - \beta}{60^\circ}$$

Eqn. 2.2

The N2-deprotonated structures are found to be the thermodynamically most stable. The syn and anti N2-deprotonated diastereoisomers adopt 5-coordinate
square pyramidal geometries with the apical oxo group aligned in the same or the opposite direction as the R-groups of SDPhe$^1$ and Tyr$^3$. The basal SDPhe$^1$ thiolate is \textit{trans} to that of Cys$^2$, and the basal Cys$^7$ thiolate is \textit{trans} to the backbone nitrogen of Cys$^2$. The \textit{syn} and \textit{anti} isomers are essentially identical in energy in the presence or absence of implicit solvent. A ball-and-stick diagram of the lowest energy isomer is shown in Figure 2-3 and diagrams of the other 7 isomers are available in Appendix II (Figure App.2-1).
Table 2-4. Relative energies of the low energy isomers of ReO[SDPhe-Cys-Tyr][Cys].

<table>
<thead>
<tr>
<th>Molecular geometry (τ)</th>
<th>cis/trans isomerism</th>
<th>Τ</th>
<th>Implicit water</th>
<th>∆E&lt;sup&gt;298&lt;/sup&gt; (kcal/mol)</th>
<th>∆H&lt;sup&gt;298&lt;/sup&gt; (kcal/mol)</th>
<th>∆G&lt;sup&gt;298&lt;/sup&gt; (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N2-1</strong>&lt;sup&gt;(anti)&lt;sup&gt; b&lt;/sup&gt;</td>
<td>Square pyramid</td>
<td>S1,S2&lt;sup&gt;trans&lt;/sup&gt; N2,S7&lt;sup&gt;trans&lt;/sup&gt;</td>
<td>0.08</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>N2-2</strong>&lt;sup&gt;(syn)&lt;/sup&gt;</td>
<td>Square pyramid</td>
<td>S1,S2&lt;sup&gt;trans&lt;/sup&gt; N2,S7&lt;sup&gt;trans&lt;/sup&gt;</td>
<td>0.19</td>
<td>-1.3</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>N3-1</strong></td>
<td>Distorted octahedron</td>
<td>S1,S2&lt;sup&gt;cis&lt;/sup&gt; N3,S7&lt;sup&gt;cis&lt;/sup&gt; oxo, N2&lt;sup&gt;trans&lt;/sup&gt;</td>
<td>N/A</td>
<td>36.9</td>
<td>38.8</td>
<td>38.3</td>
</tr>
<tr>
<td><strong>N3-2</strong></td>
<td>Distorted octahedron</td>
<td>S1,S2&lt;sup&gt;trans&lt;/sup&gt; N3,S7&lt;sup&gt;cis&lt;/sup&gt; oxo,N2&lt;sup&gt;cis&lt;/sup&gt;</td>
<td>N/A</td>
<td>33.1</td>
<td>38.9</td>
<td>38.2</td>
</tr>
<tr>
<td><strong>N3-3</strong></td>
<td>Distorted octahedron</td>
<td>S1,S2&lt;sup&gt;trans&lt;/sup&gt; N3,S7&lt;sup&gt;trans&lt;/sup&gt; oxo, N2&lt;sup&gt;cis&lt;/sup&gt;</td>
<td>N/A</td>
<td>38.3</td>
<td>42.3</td>
<td>41.9</td>
</tr>
<tr>
<td><strong>N3-4</strong></td>
<td>Distorted octahedron</td>
<td>S1,S2&lt;sup&gt;cis&lt;/sup&gt; N3,S7&lt;sup&gt;cis&lt;/sup&gt; oxo, N2&lt;sup&gt;trans&lt;/sup&gt;</td>
<td>N/A</td>
<td>40.2</td>
<td>44.7</td>
<td>44.0</td>
</tr>
<tr>
<td><strong>N7-1</strong>&lt;sup&gt;(anti)&lt;/sup&gt;</td>
<td>Distorted trigonal bipyramid</td>
<td>S1,N7&lt;sup&gt;trans&lt;/sup&gt;/axial S2,S7,O equatorial</td>
<td>0.63</td>
<td>40.9</td>
<td>42.0</td>
<td>42.1</td>
</tr>
<tr>
<td><strong>N7-2</strong>&lt;sup&gt;(syn)&lt;/sup&gt;</td>
<td>Distorted square pyramid</td>
<td>S1,N7&lt;sup&gt;trans&lt;/sup&gt; S2,S7&lt;sup&gt;trans&lt;/sup&gt;</td>
<td>0.39</td>
<td>41.4</td>
<td>42.1</td>
<td>42.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>The integral equation formalism of the polarizable continuum model (IEF-PCM) was used. <sup>b</sup>The gas-phase total energy and the correction terms for enthalpy and Gibbs free energy are -3160.35025, 0.67533 and 0.54263 hartrees, respectively. In the presence of implicit solvent the total energy is -3160.43035 hartrees.
Figure 2.3. The *anti* isomer of N2 deprotonation (N2-1). Oxygen-red; nitrogen-blue; sulfur-yellow; carbon-grey and hydrogen is omitted for clarity.

The N3-deprotonated isomers adopt distorted octahedral geometries with three adjacent 5-membered chelate rings; two adjacent 5-membered rings are observed for the N2-deprotonated isomers. Additional ring strain might decrease the stability of the 6-coordinate isomers. For N3-1, N3-2, N3-3, and N3-4, the backbone nitrogen of Cys² shows increased sp³ character from donating electrons to the [ReO]³⁺ core. The AIM analysis locates a bond critical point between the Re atom and the backbone nitrogen of Cys². The Re–N2 bond length is 2.3±0.1 Å, less than 10% longer than that of the Re–N3 bond (2.10±0.06 Å); concurrently the N2–C(O) bond (1.46±0.03 Å) is about 8% longer than the average amide bond (1.351±0.004 Å). The sixth ligand in the N3-deprotonated isomers may add to the decreased stability. In the presence of implicit solvent, the enthalpy of N3-2, where the oxo group is *trans* to N3, is lower by about 4, 5 and 7 kcal/mol compared to those of N3-1, N3-3 and N3-4, respectively, where the oxo group is *trans* to N2 (N3-1 and N3-3) or S2 (N3-4). A similar trend is observed in the gas phase: the
difference in energetics among isomers N3-1, N3-2 and N3-3 is on the order of the uncertainty of the method (about 5 kcal/mol). The largest difference is observed for N3-4, which is consistent with the fact that a sulfur atom being trans to the oxo group is less common for oxo Re(V) complexes. Overall, the N3-deprotonated isomers are more than 37 kcal/mol less stable than the N2-deprotonated isomers. However, as long as there is a pathway available for their formation, the N2-deprotonated and N3-deprotonated isomers may co-exist in solution because the barrier between them is likely to be high in energy.

Two of the N7-deprotonated isomers located are similar in energy to the most stable N3-deprotonated isomers. The first is a distorted trigonal bipyramid with the oxo group, S2 and S7 in the equatorial positions and S1 and N7 in the axial positions. The second is a distorted square pyramid with an apical oxo group and the other donor atoms in the equatorial plane. Because the sulfurs are thought to bind to the metal first on reaction, the preferred deprotonation site changes with respect to the order of sulfur attachment. Although similar in energy, N7-deprotonated isomers may be less likely to be observed experimentally because the two sulfurs on the N-terminus side likely bring the metal in the proximity of the amide of Cys² or Tyr³.

2.3.4 NMR chemical shift calculations

NMR chemical shift calculations were carried out in an attempt to connect the experimental NMR data and the possible coordination systems found using the ReO[SDPhe-Cys-Tyr][Cys] model complex. For purposes of calibration, NMR
shielding tensors were first calculated for ReO[Phe-Gly-Cys]\textsuperscript{32} using the GIAO\textsuperscript{34} and CSGT methods\textsuperscript{35} by performing single-point energy calculations in the presence of IEF-PCM\textsuperscript{33} water. Among the various levels of theory considered, the PCM-PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d):LANL2DZ differences in the CSGT H\textsubscript{a} and H\textsubscript{b} resonances between the syn and anti isomers most reliably reproduced those from experiment. Indeed, the data obtained at this level of theory allows us to distinguish between the syn and anti isomers (Chart 2-1a). Although the differences in calculated chemical shifts are generally more pronounced than the time-averaged experimental values, only the trend for the H\textsubscript{a} resonances of the Phe residues is predicted incorrectly. In water, the experimental H\textsubscript{a} resonances of the Phe residues for the two isomers are identical, whereas the calculated values differ by 0.28 ppm.
Chart 2.1. Experimentally observed versus calculated differences in proton chemical shifts between isomers.

<table>
<thead>
<tr>
<th>Isomer 1 - Isomer 4</th>
<th>(N2-2) - (N2-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe Hα</td>
<td>Phe Hβ#</td>
</tr>
<tr>
<td>Gly Hα#</td>
<td>Cys Hα</td>
</tr>
<tr>
<td>Cys Hβ#</td>
<td>Tyr Hα</td>
</tr>
<tr>
<td>Tyr Hβ#</td>
<td>Cys Hα</td>
</tr>
<tr>
<td>Cys Hβ#</td>
<td>-2.7</td>
</tr>
<tr>
<td>-1.8</td>
<td></td>
</tr>
<tr>
<td>-0.9</td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>2.7</td>
<td></td>
</tr>
</tbody>
</table>

- The Hβ chemical shifts are averages of the two β protons.

Encouraged by the results for ReO[Phe-Cys-Gly], we calculated NMR shielding tensors for N2-1,2, N3-1,2,3,4, N7-1,2 and selected higher energy N2-deprotonated systems using the above protocol to assist with characterizing the
coordination environments of the experimentally observed isomers 1-4. The latter N2-deprotonated systems were included to determine the effect of changing the position of the R-group and the capped terminal group on the chemical shift. The chemical shifts for the α and β protons of ReO[SDPhe-Cys-Tyr][Cys] in the vicinity of the [ReO]$_{3}^{3+}$ core were compared to those of the corresponding protons in isomers 1, 2 and 4. Although isomers 1 and 4 were shown to be N2-deprotonated from experimental NMR, the syn and anti isomers could not be assigned. The chemical shift differences between isomers 1 and 4 were compared to those between N2-1 and N2-2, but the calculated trends fail to match those from the experimental NMR data and do not allow assignment of the syn and anti isomers, as shown in Chart 2-1b. The chemical shift differences between the N2-deprotonated isomer 1 (or 2) and N3-deprotonated isomer 2 were also compared to those between N2-1 (or N2-2) and N3-1 (or N3-2,3,4), respectively. Charts 2-1c and 2-1d show two examples of comparisons, but again the calculated results do not completely match the experimental results. Part of the discrepancy is attributed to the absence of Thr$^{8}$ and the loop formed by residues DTrp$^{4}$, Lys$^{5}$, and Thr$^{6}$. In addition, some of the differences in the experimental chemical shifts among isomers 1, 2 and 4 are within the uncertainty of the calculation method.$^{39}$ NMR shielding tensor calculations are known to be less reliable for systems with more flexibility in solution.$^{40,41}$ Although five- and/or six-membered chelate rings form around the [ReO]$_{3}^{3+}$ core of Re-SDPhe-TATE, as in ReO[Phe-Gly-Cys], the number of pendant groups is larger for the former complex than the latter. As suggested by the ReO[SDPhe-Cys-Tyr][Cys] N2-deprotonated systems, the orientations of the
R-groups have a considerable effect (≈0.3 ppm) on the chemical shifts of the α and β protons, so molecular dynamics simulations are warranted to find averaged conformations, which is currently impeded by the lack of terms for the electrostatic interaction energy in the available force fields for Re(V) complexes.\textsuperscript{42,43}

2.3.5 \textit{In vitro} receptor binding studies

As shown in Table 2-5, the binding affinities of Re-SDPhe-TATE isomers 1, 2, 4, a mixture of the isomers 2 and 3, as well as \textsuperscript{nat}In-DOTA-octreotide were evaluated in AR42J rat pancreatic tumor cells, which are known to express sst\textsubscript{2} receptors in high density.\textsuperscript{44} As a reference, the IC\textsubscript{50} value of \textsuperscript{nat}In-DOTA-octreotide, an analogue known for its high affinity to sst\textsubscript{2} receptors,\textsuperscript{45} was determined to be 8 ± 3 nM in this study. The binding affinities of Re-SDPhe-TATE isomers 2 and 4 are both in the sub-micromolar range with the more hydrophilic isomer 2 showing slightly higher affinity. This is perhaps because the overall conformation of isomer 2 best resembles that of Re-TATE with [ReO]\textsuperscript{3+} binding to the three thiolates and the amide of Tyr\textsuperscript{3} in isomer 2. The binding affinity of the most hydrophilic isomer, isomer 1, is in the low-micromolar range. Isomer 3 is probably not as good a binder as isomer 2 because the IC\textsubscript{50} (0.5±0.2 μM) of a mixture of the two has a value similar to that of pure isomer 4 (0.4±0.1 μM) but larger than that of pure isomer 2 (0.13±0.08 μM). Compared to Re(V)-cyclized NAc-Cys\textsuperscript{1}-TATE (Ac-Cys-Cys-Tyr-DTrp-Lys-Thr-Cys-Thr(OH)), the best binder among the NS\textsubscript{3} Re(V)-cyclized octreotide analogues previously investigated,\textsuperscript{21} the Re-SDPhe-TATE isomers exhibit improved \textit{in vitro} receptor binding affinities to
sst2 receptors. However, for useful tumor-targeting agents, the IC50 values ideally should be in the low nanomolar range, similar to that of \(^{nat}\)In-DOTA-octreotide. Further modification of the peptide sequence is warranted for improving the receptor binding affinity of the Re(V)-cyclized analogues.

Table 2-5. IC50 results of the Re-SDPhe-TATE isomers and \(^{nat}\)In-DOTA-octreotide.

<table>
<thead>
<tr>
<th>Isomer</th>
<th>IC50</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>2+3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{nat})In-DOTA-octreotide</td>
<td>8±3 nM</td>
<td>1.5±0.7 μM</td>
<td>0.13±0.08 μM</td>
<td>0.4±0.1 μM</td>
<td>0.5±0.2 μM</td>
</tr>
</tbody>
</table>

\(^{a}\)Isomer 3: Isomer 2 is about 60:40.

2.3.6  Preparation and characterization of \(^{99m}\)Tc(V)-cyclized SDPhe-TATE

Reacting linear SDPhe-TATE with \(^{99m}\)Tc-glucoheptanate yields four products that have comparable HPLC retention times to the four isomers of Re-SDPhe-TATE, as shown in Table 2-6. The metal-peptide complexes are slightly more hydrophilic than the uncomplexed peptide, indicated by their shorter retentions on the C18 column compared to linear SDPhe-TATE (22.0 min) under the same conditions. The total yield of the \(^{99m}\)Tc cyclization reaction is about 85\%, approximately three to five times higher than the labeling yields previously reported for the cyclization of other analogues.\(^2\)\(^6\) The increased linear peptide concentration (from about 0.012 mM to about 0.21 mM) is likely to have improved the labeling yield. The third fraction recovered from C18 Sep-Pak cartridge purification contains only the \(^{99m}\)Tc-labeled peptides based on HPLC analysis, which is approximately 50\% of the total activity loaded onto the cartridge.
Characterization of $^{99m}$Tc complexes is often accomplished by chromatographic correlation with the corresponding Re complexes.\textsuperscript{26,46-50} Although in some cases co-elution could be observed upon HPLC analysis,\textsuperscript{26,46,47} in other cases the retention times of the $^{99m}$Tc complexes were found to differ by approximately half a minute to two minutes, which has not always been noted in the literature.\textsuperscript{48-51} In addition, it has been reported that $^{99g}$Tc complexes and their Re surrogates with almost identical crystal structures can exhibit different elution behavior.\textsuperscript{52,53} Although the mechanism behind this observation remains unclear, the substitution inertness of Re complexes compared to $^{99g/99m}$Tc complexes is believed to play an important role.\textsuperscript{52} Because a 0.4 to 1.1 minutes difference in retention times between the corresponding $^{99m}$Tc- and Re-SDPhe-TATE isomers was observed, we are unable to conclude whether $^{99m}$Tc is coordinated to SDPhe-TATE identically to Re, based on the current experimental data. It is likely the $[^{99m}\text{TcO}]^{3+}$ reacted first with the three thiolates in SDPhe-TATE and the difference in reaction conditions (ie, slightly basic pH; $^{99m}$Tc$^{VII}$O$_4^-$ vs. Re$^{V}$OCl$_4^-$ starting materials) may have resulted in coordination to different backbone amides. It is also possible that the retention time difference between the corresponding $^{99m}$Tc- and Re-SDPhe-TATE isomers is just due to different elution behavior of structurally identical $^{99m}$Tc- and Re-complexes.
Table 2-6. HPLC retention times (min) of Re(V)- and $^{99m}$Tc(V)-cyclized SDPhe-TATE.\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Re-SDPhe-TATE</th>
<th>$^{99m}$Tc-SDPhe-TATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.6</td>
<td>16.2</td>
</tr>
<tr>
<td>2</td>
<td>18.5</td>
<td>17.9</td>
</tr>
<tr>
<td>3</td>
<td>18.9</td>
<td>19.8</td>
</tr>
<tr>
<td>4</td>
<td>19.5</td>
<td>20.6</td>
</tr>
</tbody>
</table>

\(^a\)Gradient: 20% to 35% B in A over 45 min. A: H\(_2\)O with 0.1% TFA; B: acetonitrile with 0.1% TFA.

2.3.7 Chemical stability of $^{99m}$Tc(V)-cyclized SDPhe-TATE

The stability of the $^{99m}$Tc(V)-cyclized SDPhe-TATE was investigated in phosphate buffered saline (PBS) solution with a mixture of the four isomers under physiological conditions (Table 2-7). Very little (<1%) reoxidation of the metal center to $[^{99m}\text{TcO}_4]^{-}$ was observed in PBS solution at 6 h. Isomers 1 and 3 are thermodynamically more stable as the percentage of isomers 2 and 4 (eluted at 17.9 and 20.6 minutes, respectively) decreased over time and the percentage of isomer 3 (eluted at 19.8 minutes) increased accordingly. The percentage of isomer 1 (eluted at 20.6 minutes) remained unchanged.

Substitution of a thiolate for the N-terminal amine effectively improved the stability of $^{99m}$Tc(V)-Tyr\(^3\)-TATE in PBS; however, the resultant isomers were shown to be vulnerable toward ligand exchange with thiolates. In the presence of 1 mM cysteine, a soft nucleophile, about 51% of the product was observed to be intact at 6 h. The percentages of all the isomers declined, but isomer 1 decreased at a slower rate compared to isomers 2, 3 and 4. In our previous work, the NS\(_3\) $^{99m}$Tc(V)-cyclized octreotide analogues with thiolates all from cysteine were observed to be stable in 10 mM cysteine for 24 h.\(^{26}\) The cysteine thiolate is a better
nucleophile than the thiolate from SDPhe ((R)-2-mercapto-3-phenylpropanoic acid) due to the presence of a methylene between the electron withdrawing carbonyl and the thiolate. Moreover, the chelate effect is likely to have an influence on stability. Based on the NMR data of the Re-SDPhe-TATE isomers, the \([^{99m}\text{TcO}]^{3+}\) core is likely to be coordinated to the three thiolates and the backbone amide of Cys\(^2\) or Tyr\(^3\), forming either fused five-membered chelate rings or fused eight- and six-membered chelate rings, respectively (Figure 2-2). The current data suggests neither of these chelate ring systems is more stable than the fused six- and five-membered rings formed for the NS\(_3\) analogues\(^{16,21,26}\) where two neighboring cysteines are present in the peptide sequence.

Table 2-7. Chemical stability of \(^{99m}\text{Tc}(\text{V})\)-cyclized SDPhe-TATE in PBS and cysteine solution. (n=3)

<table>
<thead>
<tr>
<th>Time</th>
<th>10 mM PBS</th>
<th>1 mM Cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>1</td>
</tr>
<tr>
<td>2 h</td>
<td>100±0%</td>
<td>10±2%</td>
</tr>
<tr>
<td>4 h</td>
<td>100±0%</td>
<td>10±3%</td>
</tr>
<tr>
<td>6 h</td>
<td>100±1%</td>
<td>10±3%</td>
</tr>
</tbody>
</table>

### 2.4 Conclusions

The effect of substituting a thiolate for the N-terminal amine on the sst\(_2\) receptor binding affinity and stability of \(^{99m}\text{Tc}/\text{Re}-\text{TATE}\) was investigated by evaluating new \(\text{NS}_3\) \(^{99m}\text{Tc}/\text{Re}(\text{V})\)-cyclized octreotide analogues, \(^{99m}\text{Tc}/\text{Re}-\text{SDPhe}-\text{TATE}\). Four isomers were observed for \(^{99m}\text{Tc}/\text{Re}-\text{SDPhe}-\text{TATE}\), whereas only two isomers were observed for \(\text{Re}-\text{TATE}\) and the other \(\text{N}_2\text{S}_2/\text{NS}_3\) \(^{99m}\text{Tc}/\text{Re}(\text{V})\)-cyclized octreotide analogues.\(^{16,21,26}\) The IC\(_{50}\) studies showed
Re-SDPhe-TATE isomers are less potent than Re-TATE but possess greater sst$_2$ receptor binding affinities in rat pancreatic tumor cells compared to any of the previously reported NS$_3$ Re(V)-cyclized octreotide analogues.$^{16,21}$ Two-dimensional NMR experiments and quantum chemical calculations were employed to characterize the Re-SDPhe-TATE isomers and understand the influence of replacing the $N$-terminal amine with a thiol on the molecular structure. Technetium-99m-SDPhe-TATE isomers have improved chemical stability compared to $^{99m}$Tc-TATE but are more susceptible to ligand exchange than the NS$_3$$^{99m}$Tc(V)-cyclized octreotide analogues with three cysteines.$^{26}$

2.5 References and Notes


22. The abbreviation DOTA stands for 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid.


25. Nonlinear regression was performed using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA, [www.graphpad.com](http://www.graphpad.com)


29. Due to the size of the model complex, systematic variation of all the dihedral angles can be prohibitively expensive.


Chapter 3

Using Potential Energy Surface Scans to Examine the Bond Dissociation Energies of trans-ReOS$_2$N$_2$ and [ReOS$_3$N]$^{1-}$ Model Complexes


Introduction

Relaxed potential energy surface (PES) scans have been widely used for understanding reaction mechanisms, locating transition structures, studying bond stability and evaluating conformational flexibility.$^{1-8}$ This method does not require thorough exploration of the PES of both the reactant and product, so it is advantageous in terms of computational cost for treating large systems. It is especially advantageous for metal-multidentate ligand systems, which have extensive applications in the radiopharmaceutical sciences.$^{9-13}$ By evaluating each bond individually, the relative strength of each coordination bond in the context of the ligand system can be compared. This information provides a rationale for improving the ligand systems. In vivo chemical stability is critical for radiopharmaceuticals; if the radiometal dissociates from its targeting vehicle in
vivo, it will not reach its target site and may result in radiation to normal tissues. For example, $^{99m}$Tc(V)-cyclized DPhe-c(Cys-Tyr-DTrp-Lys-Thr-Cys)-Thr-(OH) ($^{99m}$Tc-Tyr$^3$-TATE) has been shown to be unstable in mice. The radiometal dissociates from the ligand, accumulates in the stomach and is excreted through the kidneys.

Computational chemistry may aid in the design of chelate systems for rhenium(V), which is of interest due to the attractive nuclear properties of $^{186}$Re and $^{188}$Re. Rhenium-186 has a 90-hour half-life and emits beta particles of 1.07 MeV maximum energy, along with 137 keV (9%) gamma rays; rhenium-188 has a 17-hour half-life and emits beta particles of 2.1 MeV maximum energy, along with 155 keV (15%) gamma rays. The emitted beta particles would be useful for the treatment of tumors and the emitted gamma rays would allow for internal dosimetry monitoring. Since the late 1980s, $^{186}$Re/$^{188}$Re-1,1-hydroxyethylidene-diphosphonate (HEDP) has been recognized as an effective agent for the treatment of bone metastases in patients with primary breast, prostate or lung cancer.

The design of suitable Re(V)-cyclized octreotide analogues for targeting neuroendocrine tumors would be facilitated by a combined computational/experimental approach. Previous experimental studies showed Re(V)-cyclized Tyr$^3$-octreotate had the best somatostatin receptor binding affinity among many analogues evaluated, but its $^{99m}$Tc counterpart showed poor chemical stability in phosphate buffered saline. The metal is bound to the
thiolates of Cys\(^2\) and Cys\(^7\), the amide of Tyr\(^3\) and the amine of Phe\(^1\) in Re(V)-cyclized Tyr\(^3\)-octreotate. It was proposed that the Re/Tc-amine bond is the weak link (due to the 8-membered chelate ring formed) that leads to the breakdown of the metal-peptide complex \textit{in vivo}. Several analogues in which the metal binds to three cysteine thiolates and one backbone nitrogen were synthesized to overcome the instability, and they showed good in vitro chemical stability but poor receptor binding affinity.\(^{17}\) The ultimate goal is to use computational methods to evaluate relative ligand bond strengths for a variety of coordination systems and to use the computational results to guide further modifications to the peptide sequence. Identifying the weakest metal-ligand bond is of particular interest with respect to preventing the \textit{in vitro}/\textit{in vivo} breakdown of a monooxorhenium(V) complex via attack by a sixth ligand \textit{trans} to the Re=O bond.

In earlier work, we assessed a range of computational methods and basis sets with respect to the equilibrium structures and bond dissociation energies (BDEs) of the Re(V) model complexes, ReOX\(_3\) and [ReOX\(_4\)]\(^{1-}\), where X = Cl\(^-\), NH\(_2\)\(^-\), PH\(_2\)\(^-\), OH\(^-\), SH\(^-\) and SeH\(^-\).\(^{18}\) On the basis of this assessment, we recommended the PBE0/6-31G(d,p):LANL2TZ computational level for predicting geometries, whereas the PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d,p):LANL2TZ level was recommended for predicting bond dissociation energies. In the current study, Re-ammine, Re-amide and Re-thiolate bonds are studied using the 5-coordinate \textit{trans}-ReO(SH)\(_2\)(NH\(_2\))(NH\(_3\)), [ReO(SH)\(_3\)(NH\(_2\))]\(^{1-}\) and [ReO(SH)\(_3\)(N(H)CHO)]\(^{1-}\) model complexes because they better represent the mixed ligand coordination environment of the Re(V)-cyclized octreotide analogues investigated.
experimentally. The approach is also applied to an experimentally observed monoamine-monoamide dithiol complex [ReO(S(CH$_2$)$_2$NC(O)CH$_2$NH(CH$_2$)$_2$S)] (ReO-222-MAMA$^{19}$). N$_2$S$_2$ and NS$_3$ mixed ligand environments are common for Re(V) complexes because the thiols help to prevent oxidation of Re(V) to Re(VII), thus improving the stability of the complex, whereas the amides/amines help to counteract the lipophilicity of the thiol moieties.

The three main questions addressed in this work with respect to obtaining BDEs for Re(V) complexes via relaxed PES scans are the following: 1) Are the methods recommended in our earlier work$^{18}$ also reliable for the non-equilibrium structures encountered along a scan pathway? 2) Do the BDE curves plateau at a distance that is accessible for complexes with multidentate ligands? 3) Do the PES scans predict the same relative bond energies as the dissociation reactions do?

**Computational Details**

For metal complexes with multidentate ligands, it is challenging to study the bond dissociation energy of a specific bond or the overall binding energy of all the metal-donor bonds using dissociation reactions, such as Eqn. 3.1-3.3, without disturbing the integrity of the systems. Selecting the “best” product for a reaction analogous to Eqn. 3.3 for a metal-multidentate ligand complex reactant is not always straightforward, and more than one reaction may need to be evaluated. Thus, one advantage of PES scans is that they allow evaluation of an individual bond as opposed to the multidentate ligand as a unit. By leaving the ligand in situ, the
effects of changing the coordinating atom, ring size and ring substituents on individual BDEs, all important aspects of ligand design, can be assessed.

\[
\text{ReOXYZ}_2 \rightarrow \text{ReOYZ}_2 + X \quad \text{Eqn.3.1}
\]

\[
[\text{ReOYZ}_3]^{1-} \rightarrow \text{ReOZ}_3 + Y \quad \text{Eqn.3.2}
\]

\[
[\text{ReOX}_nY_mZ_k]^{3-m-k} \rightarrow \text{ReO}^{3+} + nX + mY + kZ \quad \text{Eqn.3.3}
\]

Here, \(X = \text{NH}_3; Y = \text{NH}_2^-\) or \([\text{N(HCHO)}]^{1-}\); \(Z = \text{SH}^{1-}\); \(n + m + k = 4\).

In the past, PES scans have been used mainly for studying homolytic bond dissociations with DFT and/or multi-reference ab initio methods.\(^3\),\(^4\),\(^6\) Gas-phase PES scan calculations alone may not be sufficient to reveal reaction mechanisms or locate transition structures for heterolytic bond dissociations, and solvent effects, zero-point energy corrections, basis set superposition errors and entropy effects may need to be taken into account to develop realistic computational models.\(^20\),\(^21\) However, carefully calibrated calculations in which implicit solvent effects are taken into account are reliable and cost effective for obtaining qualitative results. In this work, BDEs for Re–N and Re–S bonds obtained with Eqn. 3.1 or 3.2 and with the PES scan method are compared for a variety of method and basis set combinations. The protocol developed from this investigation will be applied to larger, more representative models of Re(V)-cyclized octreotide analogues.

All geometry optimization, vibrational frequency and single-point energy (SPE) calculations were carried out with the Gaussian 03/09 software packages.\(^22\)–\(^24\) For trans-ReO(SH)\(_2\)(NH\(_2\))(NH\(_3\)), fully optimized structures for all
possible arrangements of the hydrogen atoms were obtained using the B3LYP method and the cc-pVDZ-(pp) basis set. The geometries of the global minimum and selected local minima located at this level of theory were reoptimized at the CCSD/cc-pVDZ-(pp), PBE0/cc-pVDZ-(pp), PBE0/6-31G(d,p):LANL2TZ and PBE0/6-31G(d):LANL2DZ levels of theory. The selected local minima included only those used as starting equilibrium structures in the scans. On the basis of the scan results for \textit{trans}-ReO(SH)$_2$(NH$_2$)(NH$_3$), the conformers of [ReO(SH)$_3$(NH$_2$)]$^1$ were screened at the PBE0/cc-pVDZ-(pp) level of theory. The structures of the relevant minima were reoptimized at the three remaining levels. Vibrational frequencies were computed at each optimization level to verify that the stationary points found correspond to minima.

Re-ammime, Re-amide and Re-thiolate bond dissociation energies were calculated by Eqn. 3.1 and 3.2 at the PBE0/6-311+G(d):LANL2TZ level of theory on geometries optimized at the PBE0/6-31G(d,p):LANL2TZ level, the protocol recommended from our earlier calibration study.$^{18}$ PESs of the 4-coordinate subunits of \textit{trans}-ReO(SH)$_2$(NH$_2$)(NH$_3$) and [ReO(SH)$_3$(NH$_2$)]$^1$, specifically ReO(SH)$_2$(NH$_2$), ReO(SH)$_3$, and NH$_3$, NH$_2^-$ and SH$^-$, were explored to locate the global minima. All possible tetrahedral and square planar arrangements of the ligands around the metal center were examined, as well as all possible orientations of the hydrogen atoms.

For the PES scans, the chemical bond being evaluated was elongated by either 3.5 or 6.5 Å starting from the equilibrium bond length, in increments of 0.2, 0.5 or
1.0 Å. At each step, the relevant bond length was fixed, while the remaining geometric parameters were optimized. Initially, the Re–NH$_3$ bond in trans-ReO(SH)$_2$(NH$_2$)(NH$_3$) was scanned with a C$_S$ symmetry restriction (the symmetry of the global minimum), which allowed CCSD(T)/aug-cc-pVTZ-(pp) SPEs to be evaluated. B3LYP/cc-pVDZ-(pp), PBE0/cc-pVDZ-(pp), CCSD/cc-pVDZ-(pp), PBE0/6-31G(d):LANL2DZ, and PBE0/6-31G(d,p):LANL2TZ PES scans were performed and CCSD(T)/aug-cc-pVTZ-(pp)//PBE0/cc-pVDZ-(pp), CCSD/cc-pVDZ-(pp)//PBE0/6-31G(d):LANL2DZ, PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d,p):LANL2TZ, PBE0/6-311+G(d,p):LANL2TZ//PBE0/6-31G(d,p):LANL2TZ and PBE0/aug-cc-pVTZ-(pp)//PBE0/cc-pVDZ-(pp) SPEs were calculated at selected points along the energy curve.

Dissociations of Re–NH$_3$, Re–SH and Re–NH$_2$ bonds without symmetry restrictions (C$_1$ point group) were studied with trans-ReO(SH)$_2$(NH$_2$)(NH$_3$) and [ReO(SH)$_3$(NH$_2$)]$^{1-}$. The protocol followed for the Re–NH$_3$ and Re–SH bond dissociations was similar to that described above; however, scans were repeated only for those methods that reproduced the CCSD(T)/aug-cc-pVTZ-(pp)//PBE0/cc-pVDZ-(pp) Re–NH$_3$ bond dissociation energy within 3 kcal/mol. That is, no B3LYP/cc-pVDZ-(pp) scans were performed and SPEs were obtained only at the CCSD/cc-pVDZ-(pp)//PBE0/6-31G(d):LANL2DZ and PBE0/6-311+G(d)/LANL2TZ//PBE0/6-31G(d,p):LANL2TZ levels of calculation.
In addition, the effect on the dissociation energy curve of inclusion of tight d-functions on the sulfur was assessed by carrying out a PBE0/cc-pV(D+d)Z-(pp) scan. The dissociation of NH$_2^\text{1-}$ from [ReO(SH)$_3$(NH$_2$)$_2$]$^{1-}$ was scanned at only the PBE0/6-31G(d,p):LANL2TZ level of theory as a result of the large discrepancy between the dissociation energies obtained from Eqn. 3.2 and the scan. PBE0/6-311+G(d):LANL2TZ/PBE0/6-31G(d,p):LANL2TZ SPEs were also computed. Local minima were used as the initial structures for both the Re-NH$_2$ and Re-SH bond dissociations to prevent non-physical hydrogen abstractions.

To further understand the discrepancy observed in the Re-NH$_2$ results, the formamide complex [ReO(SH)$_3$(N(H)CHO)]$^{1-}$ was studied. The complex and deprotonated formamide were fully optimized at the PBE0/6-31G(d,p):LANL2TZ level and PBE0/6-311+G(d):LANL2TZ/PBE0/6-31G(d,p):LANL2TZ SPEs were computed. Various trigonal bipyramidal and pentagonal planar starting geometries were examined to identify the lowest-energy structure of the complex. The PES scan was carried out following the protocol used for Re–NH$_2$ in [ReO(SH)$_3$(NH$_2$)$_2$]$^{1-}$.

For the experimentally observed ReO-222-MAMA complex$^{19}$, PES scans were also performed at the PBE0/6-31G(d,p):LANL2TZ and PBE0/6-311+G(d):LANL2TZ/PBE0/6-31G(d,p):LANL2TZ levels of theory. The Re-S/N bonds were elongated up to 2 Å (1.4 Å and 1.5 Å for the Re–NHR$_2$ bond and Re–S$_2$ bond, respectively) beyond the equilibrium bond length, in increments of 0.1 Å.
T1 diagnostic tests were performed for all coupled-cluster calculations to ascertain whether a single reference method is appropriate for these systems. In all cases the diagnostic met the criterion ($\leq 0.05$) suggested by Wilson and coworkers\textsuperscript{25}. The T1 values average $0.0229 \pm 0.0004$ for all of the coupled-cluster calculations performed to scan the Re–NH$_3$ bond, suggesting an acceptable recovery of the dynamic electron correlation energy using a single reference method for this system. For the dissociation of SH\textsuperscript{1−} from ReO(SH)$_3$(NH$_2$), the value rises from 0.02152 to 0.03597 as the equilibrium bond length is increased from 0 to 3 Å, resulting in an average of $0.029 \pm 0.007$. The keyword “int=ultrafine” was used for all DFT calculations. Natural charges were calculated with Natural Bond Orbital (NBO) theory,\textsuperscript{26,27} as implemented in the Gaussian programs. AIMAll (Version 11.12.19)\textsuperscript{28} was used to perform AIM analyses.

Because radiopharmaceuticals are often injected into the bloodstream, the interaction of the drug with solvent molecules is an important factor in its pharmacokinetics and pharmacodynamics. In the current work, the effect of water as a polar medium on the BDEs of the Re–N/S bonds was studied using implicit solvent calculations. In particular, the integral equation formalism of the polarizable continuum model (IEF-PCM)\textsuperscript{29} was used, with the default dielectric constant of 78.3553 for the water solvent.\textsuperscript{30} SPEs were obtained at the PCM-PBE0/6-311+G(d):LANL2TZ level on the gas-phase PBE0/6-31G(d,p):LANL2TZ geometries of the reactants and products in Eqn. 3.1 and 3.2 for the Re–NH$_3$, Re–SH, and Re–N(H)CHO bond dissociations, as well as on selected geometries along the gas-phase PES scan energy curves for these
dissociations. Additionally, the reactants and products yielding the Re–NH₂ and Re–SH implicit water BDE (BDE_{water}) values via Eqn. 3.2 were reoptimized at the PCM-PBE0/6-31G(d,p):LANL2TZ level before obtaining the PCM-PBE0/6-311+G(d)/LANL2TZ//PCM-PBE0/6-31G(d,p):LANL2TZ SPEs. Relaxed PES scans were performed for the Re–NH₂ and Re–SH bonds of [ReO(SH)₃(NH₂)]¹⁻ at the PCM-PBE0/6-31G(d):LANL2DZ and PCM-PBE0/6-31G(d,p):LANL2TZ levels of theory and SPEs were obtained at the PCM-CCSD/cc-pVDZ-(pp)//PCM-PBE0/6-31G(d):LANL2DZ, PCM-PBE0/6-311+G(d)/LANL2TZ//PCM-PBE0/6-31G(d,p):LANL2TZ, and PCM-CCSD/aug-cc-pVDZ-(pp)//PCM-PBE0/6-31G(d):LANL2DZ (Re–NH₂ only) levels of theory. For ReO-222-MAMA, PES scans were performed at the PCM-PBE0/6-31G(d,p):LANL2TZ level and SPEs were evaluated at the PCM-PBE0/6-311+G(d)/LANL2TZ//PCM-PBE0/6-31G(d,p):LANL2TZ level for all the Re–N/S bonds.

**Results and Discussion**

**Optimized Geometries**

All minima identified for trans-ReO(SH)₂(NH₂)(NH₃), [ReO(SH)₃(NH₂)]¹⁻ and [ReO(SH)₃(N(H)CHO)]¹⁻ have a square pyramidal arrangement of the ligands around the ReO³⁺ core, and the four atoms bonded to Re lie in essentially the same plane. (The dihedral angles are: < NH₂–S–NH₃–S = 21° and < NHR–S–S–S = 4°.) The ReO³⁺ core lies above that plane. Despite starting with both tetrahedral and
square planar coordination environments around the ReO$^{3+}$ core in the product complexes of Eqn. 3.1 and 3.2, only tetrahedral coordination environments were found for the equilibrium structures. The NH$_2^2$ adopts a trigonal planar rather than pyramidal geometry in all of the minima, which agrees with crystal structure data for ReO[N$_2$S$_2$]/ReO[NS$_3$] complexes$^{31-33}$. Examination of the molecular orbitals (MOs) for [ReO(SH)$_3$(NH$_2$)]$^{1-}$ shows that the N lone pair MO has sp-character and the Re-NH$_2$ bonding MOs have primarily p-character on the N atom (Figure App.2-2). The PBE0/6-31G(d,p):LANL2TZ lowest-energy structures for trans-ReO(SH)$_2$(NH$_2$)(NH$_3$) and [ReO(SH)$_3$(NH$_2$)]$^{1-}$ and two local minima for [ReO(SH)$_3$(NH$_2$)]$^{1-}$ are shown in Figure 3-1. The two local minima depicted (Figures 3-1c and 3-1d) were used as starting geometries for the Re–SH (Figure 3-1c) and Re–NH$_2$ (Figure 3-1d) PES scans to have the hydrogen atoms oriented away from the dissociating ligand, making proton abstraction less likely.
In our earlier work, we focused on the basis sets of Pople and coworkers, but in this work the correlation consistent basis sets of Dunning and coworkers were also examined. As shown in Table 3-1, there is little difference between the Re–X bond lengths and O=Re–X bond angles of trans-ReO(SH)$_2$(NH$_2$)(NH$_3$) optimized at the PBE0/6-31G(d,p):LANL2TZ and PBE0/cc-pVDZ-(pp) levels of calculation. Also, the Re–X bond lengths and O=Re–X bond angles in the calculated equilibrium structures generally lie within the ranges observed from crystallographic studies of typical ReO-N$_2$S$_2$ and -NS$_3$ complexes. Similar
results were observed for \([\text{ReO(SH)}_3(\text{NH}_2)]^-\). Thus, the benchmark CCSD(T)/aug-cc-pVTZ-(pp) SPEs for trans-ReO(SH)_2(NH_2)(NH_3) were computed for only the PBE0/cc-pVDZ-(pp) geometries.

Table 3-1. Selected bond lengths and bond angles for trans-ReO(SH)_2(NH_2)(NH_3) obtained with different basis sets.\(^a\)

<table>
<thead>
<tr>
<th>Method</th>
<th>Re=O</th>
<th>Re–NH\textsubscript{3}</th>
<th>Re–NH\textsubscript{2}</th>
<th>Re–SH</th>
<th>O=Re–NH\textsubscript{3}</th>
<th>O=Re–NH\textsubscript{2}</th>
<th>O=Re–SH</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBE0/6-31G(d,p):LANL2TZ</td>
<td>1.682</td>
<td>2.244</td>
<td>1.911</td>
<td>2.313</td>
<td>95.3</td>
<td>106.5</td>
<td>113.4</td>
</tr>
<tr>
<td>PBE0/cc-pVDZ-(pp)</td>
<td>1.677</td>
<td>2.239</td>
<td>1.904</td>
<td>2.312</td>
<td>94.8</td>
<td>107.0</td>
<td>113.7</td>
</tr>
<tr>
<td>Experimental data(^b)</td>
<td>1.7±0.1</td>
<td>2.2±0.03</td>
<td>2.0±0.06</td>
<td>2.3±0.04</td>
<td>100±5</td>
<td>115±4</td>
<td>107±5</td>
</tr>
</tbody>
</table>

\(^a\)Bond lengths are in Å and bond angles are in degrees. \(^b\)Average values from multiple crystal structures.\(^{31-33}\)

**Re–NH\textsubscript{3}**

The lowest-energy structure of trans-ReO(SH)_2(NH_2)(NH_3) was used to perform relaxed PES scans along the Re–NH\textsubscript{3} bond that were restricted to C\textsubscript{S} symmetry, and the BDE curves obtained at the various levels of theory are given in Figure 3-2. The benchmark calculation, CCSD(T)/aug-cc-pVTZ-(pp)//PBE0/cc-pVDZ-(pp), predicts the BDE to be 39.7 kcal/mol. All but three of the calculational levels reproduce the benchmark BDE within 3 kcal/mol. Among the three levels that have the larger errors, PBE0/6-311+G(d,p):LANL2TZ, with polarization functions included for the hydrogen atoms, does not perform better than PBE0/6-311+G(d):LANL2TZ. Also, increasing the size of the basis set from valence double-zeta to valence triple-zeta and by adding diffuse functions fails to improve the accuracy of the calculated BDE, as shown for example by the PBE0/aug-cc-pVTZ-(pp)//PBE0/cc-pVDZ-(pp) vs. PBE0/cc-pVDZ-(pp) energy
curves. Finally, B3LYP/cc-pVDZ-(pp) underestimates the BDE by about 5 kcal/mol. That these three levels of calculation do not perform as well as the remaining levels agrees with our previous findings.\textsuperscript{18}

Figure 3-2. Re–NH\textsubscript{3} relaxed PES scan BDE curves with C\textsubscript{5} symmetry for trans-ReO(SH)\textsubscript{2}(NH\textsubscript{2})(NH\textsubscript{3}).

Our earlier work, in which combinations of the two well-known DFT functionals B3LYP and PBE0 and a series of increasingly more complete basis sets were evaluated against the benchmark CCSD(T)/aug-cc-pVTZ-(pp)//MP2/aug-cc-pVTZ-(pp), focused on identifying equilibrium structures for ReOX\textsubscript{3} and [ReOX\textsubscript{4}]\textsuperscript{1–} systems, where X = Cl\textsuperscript{1–}, NH\textsubscript{2}\textsuperscript{1–}, PH\textsubscript{2}\textsuperscript{1–}, OH\textsuperscript{1–}, SH\textsuperscript{1–} and SeH\textsuperscript{1–}. Overall, PBE0/6-31G(d,p):LANL2TZ and PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d,p):LANL2TZ outperformed the other levels of calculation for predicting geometries and BDEs, respectively.\textsuperscript{18} In the current work, the recommended levels are also shown to be reliable for treating non-equilibrium structures, such as those along the scan pathways (Figure 3-2). Even the level of
theory recommended for predicting geometries reproduces the CCSD(T)/aug-cc-pVTZ-(pp)//PBE0/cc-pVDZ-(pp) energetic results for the nitrogen and sulfur mixed ligand systems investigated here. As shown in Figure 3-2, the PBE0/6-31G(d,p):LANL2TZ energy curve levels off at 39.7 kcal/mol and the PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d,p):LANL2TZ curve levels off at 38.0 kcal/mol. Both are within 1 kcal/mol of the CCSD(T)/aug-cc-pVTZ-(pp)//PBE0/cc-pVDZ-(pp) BDE. The results also suggest that reliable geometries, at least, are obtained with the more cost-effective PBE0/6-31G(d):LANL2DZ combination of method and basis set, which will be important for investigating larger Re(V)-containing radiopharmaceuticals.

Since our ultimate goal is to apply the relaxed PES scan method to multidentate systems, it is important that essentially all of the BDE is recovered at a dissociation distance that can be reached without significantly distorting the multidentate system. As shown in Figure 3-2, all of the BDE curves begin to level off after the bond has been elongated 2 Å beyond the equilibrium bond length, and approximately 90% of the BDE obtained at 6.5 Å beyond the equilibrium bond length is recovered. The energy curves each plateau from 3 to 3.5 Å beyond the equilibrium bond length, recovering about 98% of the BDE. Although lengthening the bond by 3 to 3.5 Å is more desirable, in multidentate systems bond elongation may be limited to 2 Å, a distance at which the relaxed PES scans still provide a good estimate of the bond strength. All of the subsequent gas-phase PES scans reported herein begin to level off and plateau at the same distances; consequently, the BDE curves for these scans have been truncated at 3.5 Å beyond the
equilibrium bond length. The distance found in this study for the BDE curves to plateau agrees with results from analogous studies on biochemical systems.\(^3\),\(^4\),\(^6\)

Relaxed PES scans without symmetry restrictions were also performed along the Re-NH\(_3\) bond of \textit{trans}-ReO(SH)\(_2\)(NH\(_2\))(NH\(_3\)). The energy curves are shown in Figure 3-3. Again, the BDEs calculated at the selected calculational levels differ by less than 3 kcal/mol. As shown in Table 3-2, the BDEs obtained from the PES scans are essentially identical to those obtained from Eqn. 3.1. As expected, when the BDEs evaluated with and without the symmetry restriction are compared, the former is about 4 kcal/mol too high. In the C\(_1\) scan the ReO(SH)\(_2\)(NH\(_2\)) rearranges to an asymmetric local minimum as the Re–NH\(_3\) bond is elongated, whereas in the C\(_S\) scan, the ReO(SH)\(_2\)(NH\(_2\)) rearranges to a transition structure. To ensure a meaningful comparison, the corresponding transition structure and local minimum of ReO(SH)\(_2\)NH\(_2\) were used for the calculation of the Re–NH\(_3\) BDEs using Eqn. 3.1.

![Figure 3-3. Re–NH\(_3\) BDE curves for trans-ReO(SH)\(_2\)(NH\(_2\))(NH\(_3\)) without symmetry constraint.](image-url)
Table 3-2. Comparison of BDEs from infinite separation (Eqn. 3.1 and 3.2) and PES scans.\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>( \Delta E ) (kcal/mol)</th>
<th></th>
<th>( \Delta E ) (kcal/mol)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eqn. 3.1</td>
<td>PES scan</td>
<td>Eqn. 3.2</td>
<td>PES scan</td>
</tr>
<tr>
<td>( \text{trans-ReO(SH)}_2(\text{NH}_2)(\text{NH}_3) )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Re–NH(_2) (C(_1))</td>
<td>34.6</td>
<td>34.4\textsuperscript{b}</td>
<td>Re–NH(_2) (C(_1))</td>
<td>128.3</td>
</tr>
<tr>
<td>Re–NH(_3) (C(_3))</td>
<td>38.0</td>
<td>38.0\textsuperscript{c}</td>
<td>Re–SH (C(_1))</td>
<td>73.9</td>
</tr>
</tbody>
</table>

\textsuperscript{a}PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d,p):LANL2TZ data. The point group is given in parentheses after the bond. \textsuperscript{b}BDE at 3.5 Å from the equilibrium bond length. \textsuperscript{c}BDE at 6.0 Å from the equilibrium bond length, the corresponding value at 3.5 Å is 37.5 kcal/mol.

\textbf{Re–SH}

The BDE curves obtained at the various levels of theory for the dissociation of the \( \text{SH}^{1–} \) ligand from the \([\text{ReO(SH)}_3(\text{NH}_2)]^{1–}\) complex are shown in Figure 3-4. The BDE is predicted to be about 69 kcal/mol at the PBE0/6-31G(d,p):LANL2TZ, PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d,p):LANL2TZ and PBE0/6-31G(d):LANL2DZ levels of theory, with good consistency among the different levels. However, the agreement among all the calculational levels is not as good as was found for the dissociation of \( \text{NH}_3 \) from \textit{trans-ReO(SH)}\(_2(\text{NH}_2)(\text{NH}_3)\).

The BDE predicted at the PBE0/cc-pVDZ-(pp) level (73.0 kcal/mol) is about 4 kcal/mol higher than that of the above levels, and the BDE predicted at the CCSD/cc-pVDZ-(pp) level (83.9 kcal/mol) is another 11 kcal/mol higher. The tight-d correlation consistent basis set cc-pV(D+d)Z-(pp) behaves essentially identically to the cc-pVDZ-(pp) basis set for this bond dissociation, although use of the tight-d basis sets has been shown previously to improve the energetic results for third-row elements.\textsuperscript{39} The larger BDE obtained with the CCSD method primarily arises from a proton abstraction, allowed by the reorientation of the hydrogen
atoms, which is complete at 3.5 Å and is not observed at the other levels of calculation.

Figure 3-4. Re–SH and Re–NH$_2$ BDE curves for [ReO(SH)$_3$(NH$_2$)$_2$]$^{1-}$.

NBO and AIM analyses were performed at the PBE0/6-31G(d,p):LANL2TZ calculational level to verify that the PES scan for the dissociation of the Re–SH bond is producing ReO(SH)$_2$(NH$_2$) and SH$^{1-}$ (the products of Eqn. 3.2). The AIM analysis shows that a bond critical point remains between the Re and S at 3.5 Å, but the electron density at the bond critical point decreases by more than two orders of magnitude compared to the value at the equilibrium distance (i.e., from 9.2 x 10$^{-2}$ to 5.6 x 10$^{-4}$). The net NBO charges on SH at these two Re–S distances change from -0.15 to -0.71 as the bond is elongated. A similar result is observed for the Mülliken charges; the net charge on SH changes from -0.18 to -0.69 as the Re-S bond is elongated by 3.5 Å. These results demonstrate that the interaction between the Re and S is extremely weak at 3.5 Å, consistent with the BDE recovery at that distance, and that the desired products (ReO(SH)$_2$(NH$_2$) and SH$^{1-}$) are observed. In fact, the Re–SH BDEs obtained from the PES scans at the
PBE0/6-31G(d,p):LANL2TZ, PBE0/6-311+G(d):LANL2TZ/PBE0/6-31G(d,p):LANL2TZ and PBE0/6-31G(d):LANL2DZ levels of theory (Figure 3-4) show reasonable agreement with the BDE obtained from Eqn. 3.2. The BDE values differ by 5.5 kcal/mol (Table 3-2), on the order of the error of the method.\textsuperscript{18}

**Re–NH\(_2\) and Re–N(H)CHO**

As shown in Table 3-2, the Re–NH\(_2\) BDE from the PES scan for \([\text{ReO(SH)}\(_3\)(\text{NH}_2)]\)\(^{1-}\) is 28.5 kcal/mol lower than that obtained from Eqn. 3.2; that is, the dissociation pathway followed by the PES scan does not yield the desired products (ReO(SH)_3 and NH\(_2^1\)\(^-\)). In Figure 3-4, even the PBE0/6-31G(d,p):LANL2TZ BDE of the Re–NH\(_2\) bond (105.9 kcal/mol) differs much more (6 kcal/mol) from the PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d,p):LANL2TZ BDE (99.8 kcal/mol) than is observed in the PES scans for the other bonds (\(\leq 1\) kcal/mol). [ReO(SH)_3(NH_2)]\(^{1-}\) was initially chosen to study the BDE of the Re-NH\(_2\) bond because the negative charge on the reactant complex could facilitate formation of ReO(SH)_3 and NH\(_2^1\)\(^-\) in the PES scan. However, the Mülliken charge of the NH\(_2\) moiety changes only slightly from -0.33 to -0.39 as the equilibrium bond length is increased by 6.5 Å. Apparently, the NH\(_2^1\)\(^-\) is a sufficiently poor leaving group in the gas phase that a different pathway is followed in the PES scans. However, in the ligands used for potential Re(V) radiopharmaceuticals, the negative charge on the leaving group is often distributed over an amide or a primary/secondary amine group. To test the effect of changing
the leaving group, a PES scan for the Re\(\text{–N(H)CHO}\) bond of [ReO(SH)\(_3\)(N(H)CHO)]\(^{1-}\) was performed. The PBE0/6-31G(d,p):LANL2TZ BDE of the Re\(\text{–N(H)CHO}\) bond predicted by the scan is 87.7 kcal/mol, which is 6.4 kcal/mol higher than the BDE calculated at the PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d,p):LANL2TZ level (Figure 3-5). Thus, the discrepancy between these two calculational levels again is observed. However, the net Mülliken charge for the [N(H)CHO]\(^{1-}\) group is -0.69 after the bond is elongated by 3.5 Å. Also, the BDE predicted by Eqn. 3.2 (84.1 kcal/mol) differs by less than 3 kcal/mol from that obtained from the PES scan (81.3 kcal/mol). The improved consistency in BDE between the two different approaches is encouraging with respect to applying the PES scan method to larger systems that allow better delocalization of the negative charge. In cases for which the Re\(\text{–NH}_2\) bond is more representative for the system of interest, implicit solvent effects may be included to facilitate the desired heterolytic cleavage, which is discussed in more detail, vide infra.

![Figure 3-5. Re\(\text{–N(H)CHO}\) BDE curves for [ReO(SH)\(_3\)(N(H)CHO)]\(^{1-}\).](image-url)
Effect of solvent

A common way in which solvent effects are taken into account is to calculate SPEs with an implicit solvent model on gas-phase geometries.\textsuperscript{40,41} For the Re–NH\textsubscript{3} bond in trans-ReO(SH)\textsubscript{2}(NH\textsubscript{2})(NH\textsubscript{3}), the Re–SH bond in [ReO(SH)\textsubscript{3}(NH\textsubscript{2})\textsuperscript{1−}] and the Re–N(H)CHO bond in [ReO(SH)\textsubscript{3}(N(H)CHO)]\textsuperscript{1−}, the IEF-PCM BDE curves obtained at the PCM-PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d,p):LANL2TZ level of theory were compared to the BDEs generated in the gas phase at the corresponding calculational level, as shown in Figure 3-6. As expected, for the neutral species, the solvent has little effect on the BDE. In fact, the implicit water BDE (BDE\textsubscript{water}) of the Re–NH\textsubscript{3} bond is only 1.6 kcal/mol lower than its gas-phase counterpart. On the contrary, the presence of solvent decreases the gas-phase BDEs by 26.5 kcal/mol and 23.5 kcal/mol for the Re–SH and Re–N(H)CHO bonds, respectively. For the charged species, we observe a smaller solvent effect when the charge is better delocalized, as expected. That is, the solvent stabilizes SH\textsuperscript{1−} and [N(H)CHO]\textsuperscript{1−}, for which the charge is more localized, by 67.9 kcal/mol and 64.4 kcal/mol, respectively, whereas the solvent stabilizes [ReO(SH)\textsubscript{3}(NH\textsubscript{2})\textsuperscript{1−}, ReO(SH)\textsubscript{2}(NH\textsubscript{2}), [ReO(SH)\textsubscript{3}(N(H)CHO)]\textsuperscript{1−} and ReO(SH)\textsubscript{3} are 52.4 kcal/mol, 14.5 kcal/mol, 47.4 kcal/mol and 8.3 kcal/mol, respectively. In addition, the PCM-PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d,p):LANL2TZ BDE\textsubscript{water} obtained from the PES scans and those obtained from Eqn. 3.1 and 3.2 have better agreement (within 2 kcal/mol) than do the corresponding gas-phase BDEs (Table 3-3). Overall, the trend in relative bond strength is not changed when implicit
solvent is included in the calculations, but the solvent affects the BDEs of the Re–NH$_3$, Re–SH and Re–N(H)CHO bonds differently. As a result, the strength of the Re–NH$_3$ bond becomes more competitive with respect to that of the other bonds in the presence of solvent, and the differences in the BDEs among the Re–NH$_3$, Re–SH and Re–N(H)CHO bonds become much smaller.

Figure 3-6. BDE curves for the Re–N(H)CHO, Re–SH and Re–NH$_3$ bonds in the presence of implicit solvent.

Table 3-3. Comparison of BDEs from infinite separation (Eqn. 3.1 and 3.2) and PES scans in the presence of implicit solvent.$^a$

<table>
<thead>
<tr>
<th>Model</th>
<th>Bond</th>
<th>$\text{BDE}_{\text{water}}$ from PES scan$^b$ (kcal/mol)</th>
<th>$\text{BDE}_{\text{water}}$ from infinite separation (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{trans}$-ReO(SH)$_2$(NH)$_2$(NH$_3$)</td>
<td>Re–NH$_3$ (C$_1$)</td>
<td>32.8</td>
<td>32.7$^c$</td>
</tr>
<tr>
<td>$[^{[\text{ReO(SH)}_3(\text{NH}_2)]^{1-}}$</td>
<td>Re–SH</td>
<td>41.9</td>
<td>43.9$^d$</td>
</tr>
<tr>
<td>$[^{[\text{ReO(SH)}_3(\text{N(H)CHO})]}^{1-}$</td>
<td>Re–N(H)CHO</td>
<td>57.8</td>
<td>58.7$^d$</td>
</tr>
</tbody>
</table>

$^a$PCM-PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d,p):LANL2TZ data. The point group is given in parentheses after the bond. $^b$BDE$_{\text{water}}$ at 3.5 Å from the equilibrium bond length. $^c$BDE$_{\text{water}}$ calculated using Eqn. 3.1. $^d$BDE$_{\text{water}}$ calculated using Eqn. 3.2.
To verify that the use of the above approach is reasonable, relaxed PES scans were performed for the Re–SH bond of [ReO(SH)₃(NH₂)]¹⁻ in the presence of implicit water. In Figure 3-7, at the PCM-PBE0/6-311+G(d)/LANL2TZ/(PCM-)PBE0/6-31G(d,p):LANL2TZ level, the Re–SH BDE_{water} values calculated with and without reoptimization are 39.6 kcal/mol and 41.9 kcal/mol, differing by only 2.3 kcal/mol after the Re–SH bond is elongated by 3.5 Å. Also, the Re–SH BDE_{water} curves agree well among the different calculational levels. Although the difference in the Re–SH BDE_{water} values among the various levels of theory is as large as 15 kcal/mol in the gas phase, the PCM-CCSD/cc-pVDZ-(pp)//PCM-PBE0/6-31G(d):LANL2DZ BDE_{water} differs from those at the other levels of theory by 5.7 to 7.8 kcal/mol. The difference among the PCM-PBE0/6-31G(d):LANL2DZ, PCM-PBE0/6-31G(d,p):LANL2TZ and PCM-PBE0/6-311+G(d)/LANL2TZ//PCM-PBE0/6-31G(d,p):LANL2TZ Re–SH BDE_{water} values is smaller than 3 kcal/mol at 3.5 Å. When the reactants and products in Eqn. 3.2 were reoptimized in the presence of implicit solvent, the Re–SH BDE_{water} is found to be 39.8 kcal/mol, only 0.2 kcal/mol higher than that calculated from the PCM-PES scan.

Relaxed PES scans were also performed for the Re–NH₂ bond of [ReO(SH)₃(NH₂)]¹⁻ in the presence of implicit solvent to determine whether inclusion of solvent leads to the desired heterolytic cleavage. As shown in Figure 3-7, the PCM-PBE0/6-311+G(d)/LANL2TZ//PCM-PBE0/6-31G(d,p):LANL2TZ PES scan results in a BDE_{water} of the Re–NH₂ bond about 24 kcal/mol lower than that of the PCM-CCSD/cc-pVDZ-(pp)//PCM-PBE0/6-31G(d):LANL2DZ level.
and about 18 kcal/mol lower than that of the other two DFT levels. Furthermore, at
the PCM-PBE0/6-31G(d):LANL2DZ and PCM-PBE0/6-31G(d,p):LANL2TZ
levels, the BDE\textsubscript{water} values from the PCM-PES scans are about 9 kcal/mol lower
than those from Eqn. 3.2, whereas at the PCM-PBE0/6-311+G(d)/LANL2TZ//PCM-PBE0/6-31G(d,p):LANL2TZ and
PCM-CCSD/cc-pVDZ-(pp)//PCM-PBE0/6-31G(d):LANL2DZ levels, the
deviations in the two BDE\textsubscript{water} values are 0.5 kcal/mol and 3.9 kcal/mol,
respectively. Even in the presence of implicit solvent, heterolytic dissociation of
the Re–NH\textsubscript{2} bond is not achieved in the PES scans at the
PCM-PBE0/6-31G(d):LANL2DZ and PCM-PBE0/6-31G(d,p):LANL2TZ levels.
Diffuse functions may be critical for the desired cleavage because at the
PCM-PBE0/6-311+G(d)/LANL2TZ//PCM-PBE0/6-31G(d,p):LANL2TZ level,
the net Mülliken charge of the NH\textsubscript{2} moiety changes from -0.39 to -0.97 as the
equilibrium bond length is increased by 3.5 Å, whereas at the
PCM-PBE0/6-31G(d):LANL2DZ and the PCM-PBE0/6-31G(d,p):LANL2TZ levels, the net Mülliken charge of the NH\textsubscript{2} moiety changes from -0.3 to -0.6. In our
earlier work\textsuperscript{18}, diffuse functions were also found to be particularly important for the
Re–NH\textsubscript{2} (and Re–PH\textsubscript{2}) bond with respect to reproducing the benchmark
thermochemical data. Their importance is further demonstrated here by the 25
kcal/mol decrease in BDE when the cc-pVDZ-(pp) basis set is replaced by the
aug-cc-pVDZ-(pp) basis set (Figure 3-7). That is, the
PCM-CCSD/aug-cc-pVDZ-(pp)//PCM-PBE0/6-31G(d):LANL2DZ BDE\textsubscript{water} is
81.8 kcal/mol from the PES scan (3.5 Å) compared to the
PCM-CCSD/cc-pVDZ-(pp)//PCM-PBE0/6-31G(d):LANL2DZ BDE$_{\text{water}}$ of 107.1 kcal/mol. The former value is essentially identical to the corresponding value of 81.9 kcal/mol from Eqn. 3.2, and both of these values are in much better agreement with the corresponding PCM-PBE0/6-311+G(d)/LANL2TZ//PCM-PBE0/6-31G(d,p):LANL2TZ values (Table 3-3 and Figure 3-7).

![Graph](image)

Figure 3-7. Re–NH$_2$ and Re–NH$_2$ BDE curves for [ReO(SH)$_3$(NH$_2$)$_2$]$^{1+}$ in the presence of implicit solvent.

Finally, in the presence of implicit solvent, with or without reoptimization, the PES scans recover 90 to 98% of the BDE$_{\text{water}}$ values at 2 Å beyond the equilibrium bond length (except for Re-NH$_2$ at some of the levels). This result is particularly
advantageous with respect to elongating the bonds of the chelate rings in multidentate systems.

**ReO-222-MAMA**

A number of monoamine-monoamide dithiol (MAMA) ligands have been synthesized because they are easily derivatized with various functional groups and form neutral, stable and relatively hydrophilic Re(V)/Tc(V) complexes that are potentially useful for targeted radiotherapy or SPECT imaging.\(^{19,42}\) To test whether the Re–S/N bonds in such rigid, multidentate systems can be elongated sufficiently to provide reliable information with respect to relative bond strengths, the Re–S/N bonds in ReO-222-MAMA (Figure 3-8) were studied using the relaxed PES scan method. The PBE0/6-311+G(d):LANL2TZ/PBE0/6-31G(d,p):LANL2TZ BDEs are found to be 94.8 kcal/mol, 92.9 kcal/mol, 73.2 kcal/mol and 49.5 kcal/mol for the Re–S\(_1\), Re–NC(O), Re–S\(_2\) and Re–NHR\(_2\) bonds, respectively. All four BDEs are higher than those of the corresponding bonds in *trans*-ReO(SH)\(_2\)(NH\(_2\))(NH\(_3\)), [ReO(SH)\(_3\)(NH\(_2\))]\(^{1–}\) and [ReO(SH)\(_3\)(N(H)CHO)]\(^{1–}\), which is not surprising because the five-membered chelate rings of ReO-222-MAMA are expected to improve the stability of the original 5-coordinate complex but to decrease the stability of the resultant 4-coordinate subunit by increasing the steric constraints. Due to the constraints of the chelate rings, the Re–NHR\(_2\) bond was elongated to only 1.4 Å beyond the equilibrium bond distance, as opposed to the 2 Å elongation for the Re–S\(_1\) and Re–NC(O) bonds. According to the results of the PES scans for the Re–NH\(_3\) bond of the *trans*-ReO(SH)\(_2\)(NH\(_2\))(NH\(_3\)) complex, about 80% of the total BDE is
usually recovered at this elongation distance. Likewise, the Re–S₂ bond was elongated by only 1.5 Å due to the formation of a non-physical S₂⋯H–N(amine) hydrogen bond beyond this distance. According to the PES scan for the Re–SH bond of the [ReO(SH)₃(NH₂)]¹⁻ complex, 75 to 80% of the total BDE is usually recovered at the 1.5 Å elongation distance, so the Re–S₂ BDE is likely to be higher than 90 kcal/mol at 2 Å.

![Figure 3-8](image_url)

Figure 3-8. The ReO-222-MAMA complex investigated via relaxed PES scans along the Re–N/S bonds.

The effect of implicit solvent on the BDEs of the Re-N/S bonds in the ReO-222-MAMA complex was also examined, and the PCM-PBE0/6-311+G(d):LANL2TZ//PCM-PBE0/6-31G(d,p):LANL2TZ BDE_{water} values of the Re–S₁, Re–NC(O), Re–S₂ and Re-NHR₂ bonds were found to be 68.8 kcal/mol, 84.3 kcal/mol, 56.3 kcal/mol and 51.6 kcal/mol, respectively. Compared with the gas-phase BDEs, the Re–S₁ BDE_{water} decreases by about 26 kcal/mol in the presence of solvent. For the Re–S₂ bond, the higher percentage of the total BDE_{water} recovered at 1.5 Å in the presence of solvent counteracts the solvent effect, to some
extent, so that the decrease is about 17 kcal/mol. The Re–NC(O) BDE\textsubscript{water} is about 9 kcal/mol lower than the gas-phase BDE. That the solvent has a smaller influence on the Re–NC(O) bond is likely because the built-up negative charge on the amide can be better delocalized throughout the ReO-222-MAMA complex as the bond is elongated. As expected, the BDE\textsubscript{water} of the Re–NHR\textsubscript{2} bond is essentially the same as its gas-phase BDE, and this bond appears to be more competitive in the presence of solvent compared to the other bonds. The slight increase (2.1 kcal/mol) in the BDE\textsubscript{water} is also due to the higher percentage of the total BDE\textsubscript{water} recovered at 1.4 Å in the presence of solvent. In general, the drops in BDE\textsubscript{water} values for the Re–S\textsubscript{1}/S\textsubscript{2} and Re–NC(O) bonds of ReO-222-MAMA are smaller than those for the corresponding bonds in [ReO(SH)\textsubscript{3}(NH\textsubscript{2})]\textsuperscript{1–} and [ReO(SH)\textsubscript{3}(N(H)CHO)]\textsuperscript{1–}. This result can again be rationalized by the greater capacity for delocalization of the negative charge in the leaving groups of ReO-222-MAMA. Overall, bond elongations for ReO-222-MAMA in the presence of solvent reproduce the relative thermodynamic stabilities of the Re–S/N bonds in \textit{trans}-ReO(SH)\textsubscript{2}(NH\textsubscript{2})(NH\textsubscript{3}), [ReO(SH)\textsubscript{3}(NH\textsubscript{2})]\textsuperscript{1–} and [ReO(SH)\textsubscript{3}(N(H)CHO)]\textsuperscript{1–}.

**Conclusions**

The application of the relaxed PES scan method to study BDEs of Re(V) nitrogen and sulfur bonds is reported in this work using \textit{trans}-ReO(SH)\textsubscript{2}(NH\textsubscript{2})(NH\textsubscript{3}), [ReO(SH)\textsubscript{3}(NH\textsubscript{2})]\textsuperscript{1–} and [ReO(SH)\textsubscript{3}(N(H)CHO)]\textsuperscript{1–} as model complexes. The results show that in the gas phase the BDE curves begin to level off at 2 Å and plateau at 3.0 to 3.5 Å beyond the equilibrium bond length,
regardless of the choice of method/basis set combination. The inclusion of implicit
solvent enhances the agreement in BDE_{water} values among the various calculational
levels and between the PES scan and infinite separation approaches, and shortens
the distance at which the BDE_{water} curves plateau. Also, elongating the Re-donor
atom bonds by 1.4 to 2 Å in the rigid, multidentate system ReO-222-MAMA is
sufficient to yield reliable bond strength trends. Moreover, PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d,p):LANL2TZ, the calculational
level recommended in our earlier work, is suitable for the non-equilibrium
structures encountered along the PES scan pathway. For the model complexes
investigated in this work, the relative BDEs at infinite separation (Eqn. 3.1 and 3.2)
are Re–NH₂ > Re–N(H)CHO > Re–SH > Re–NH₃.

This trend coincides with the results for the PES scans that lead to the appropriate (heterolytically-cleaved)
products.

References


http://www.gaussian.com/g_tech/g_ur/k_scf.htm (2014/12/10),


43. We recognize that the observed BDE trend is affected by the trans influence, among other factors and is unlikely to be general.
Chapter 4


4.1 Introduction

Although trans-ReO(SH)$_2$(NH$_2$)(NH$_3$), [ReO(SH)$_3$(NH$_2$)]$^{1-}$ and [ReO(SH)$_3$(N(H)CHO)]$^{1-}$ model complexes are suitable for method calibration (Chapter 3), they are not suitable models for evaluating the chelate effect. In order to evaluate the chemical stability of the various coordination systems formed in Re-cyclized octreotide analogues, model complexes that contain chelate rings similar to those in the Re-peptide analogues should be examined using the method recommended in the previous studies.$^{1,2}$

In this chapter, we report the results of the relaxed PES scans performed for the Re–S/N bonds in different isomers of the model complexes ReO[SDPhe-Cys-Tyr][Cys] and ReO[DPhe-Cys-Tyr][Cys]. ReO[SDPhe-Cys-Tyr][Cys] was used to elucidate the coordination environment of the [ReO]$^{3+}$ in Re-SDPhe-TATE (Chapter 2); ReO[DPhe-Cys-Tyr][Cys] was used
to represent the coordination environment of Re-TATE, the analogue with the 29 nM IC$_{50}$ value but poor stability in PBS buffer.$^{3,4}$ The chemical stability of the 5- and 6-coordinate isomers of each of the model complexes can be compared, and additionally, the stabilities of the NS$_3$ and the N$_2$S$_2$ coordination systems can be compared.

Performing these electronic structure calculations provides a computational method for predicting the chemical stability of new Re-cyclized octreotide analogues. Additionally, the low energy equilibrium structures of ReO[SDPhe-Cys-Tyr][Cys] and ReO[DPhe-Cys-Tyr][Cys] provide geometry parameters and atomic partial charges for Re(V) force-field development (Chapter 5).

4.2 Computational Details

In the model complexes ReO[SDPhe-Cys-Tyr][Cys] (Chapter 2) and ReO[DPhe-Cys-Tyr][Cys], the terminal amines and carboxylates were capped with acetyl groups and methylamides, respectively. The [ReO]$^{3+}$ core is coordinated to the deprotonated backbone amide of Cys$^2$, Cys$^7$ (ReO[SDPhe-Cys-Tyr][Cys] only) or Tyr$^3$ and the thiolates (two in ReO[DPhe-Cys-Tyr][Cys]; three in ReO[SDPhe-Cys-Tyr][Cys]) to represent three different coordination possibilities, N2, N7 (ReO[SDPhe-Cys-Tyr][Cys] only) and N3. A variety of trigonal bipyramidal, square pyramidal and octahedral (only for N3-deprotonated isomers) starting geometries were screened at the HF/6-31G(d):LANL2DZ level of theory; the local minima with relative energies smaller than 50 kcal/mol were reoptimized.
at the PBE0/6-31G(d):LANL2DZ level of theory. The orientation of the R-groups was varied to identify the lowest energy structures. Vibrational frequencies were computed to verify the optimized structures correspond to minima. Correction terms were extracted from the vibrational frequency calculations to obtain enthalpies and Gibbs free energies. All the geometry optimizations and vibrational frequency calculations were performed using the Gaussian 03 or 09 software packages. The keyword “int=ultrafine” was used for all DFT calculations.

Relaxed PES scans were performed to obtain Re–S/N BDEs for one 5-coordinate and one 6-coordinate ReO[SDPhe-Cys-Tyr][Cys] isomer (N2-1 and N3-1) without symmetry restrictions (C1 point group). At each step, the relevant bond length was fixed, while the remaining geometric parameters were optimized. Initially, the calculations were carried out at the PBE0/6-31G(d):LANL2DZ level of theory. In these scans, the chemical bond being evaluated was elongated by 2 Å starting from the equilibrium bond length, in increments of 0.2 Å. The PES scans were repeated for the 5-coordinate ReO[SDPhe-Cys-Tyr][Cys] isomer (N2-1) at the PBE0/6-31G(d,p):LANL2TZ level of theory. In these scans, the Re–S/N bonds were elongated by 3 Å starting from the equilibrium bond length, in increments of 0.5 or 1 Å.

4.3 Results and Discussion

4.3.1 Calculated equilibrium structures

A total of eight low energy isomers were found for the model complex ReO[SDPhe-Cys-Tyr][Cys], representing different arrangements of the peptide
following N2, N3 and N7 deprotonation for Re-SDPhe-TATE (Chapter 2). Only N2- and N3-deprotonated possibilities were examined for the model complex ReO[DPhe-Cys-Tyr][Cys]. For this reason, only six low energy isomers were found for ReO[DPhe-Cys-Tyr][Cys] (Table 4-1 and Figure 4-1). The parameter \( \tau \) given in Table 4-1 is used to distinguish distorted square pyramidal and trigonal bipyramidal coordination geometries.  

For ReO[DPhe-Cys-Tyr][Cys], the N2-deprotonated structures are also found to be the thermodynamically most stable. The syn and anti N2-deprotonated diastereoisomers adopt 5-coordinate square pyramidal geometries with the apical oxo group aligned in the same or the opposite direction as the R-groups of DPhe\(^1\) and Tyr\(^3\). The basal DPhe\(^1\) amine is trans to the thiolate of Cys\(^2\), and the basal Cys\(^7\) thiolate is trans to the backbone nitrogen of Cys\(^2\). The syn and anti isomers differ by 5.5 kcal/mol in total energy and enthalpy, but are essentially identical in Gibb’s free energy at the PBE0/6-31G(d):LANL2DZ level of theory. Figure 4-1 shows ball-and-stick diagrams of the coordination spheres of the lowest energy isomers of ReO[DPhe-Cys-Tyr][Cys].
Table 4-1. Geometrical arrangements and relative energies of the low energy isomers of ReO[DPhe-Cys-Tyr][Cys].

<table>
<thead>
<tr>
<th>Geometry description</th>
<th>Molecular geometry (τ)</th>
<th>cis/trans isomerism</th>
<th>τ</th>
<th>∆E$_{298}$ (kcal/mol)$^a$</th>
<th>∆H$_{298}$ (kcal/mol)$^a$</th>
<th>∆G$_{298}$ (kcal/mol)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2-1 (anti)$^b$</td>
<td>Distorted square pyramid</td>
<td>N1,S2 $trans$ N2,S7 $trans$</td>
<td>0.30</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>N2-2 (syn)</td>
<td>Square pyramid</td>
<td>N1,S2 $trans$ N2,S7 $trans$</td>
<td>0.07</td>
<td>5.5</td>
<td>5.5</td>
<td>1.1</td>
</tr>
<tr>
<td>N3-1</td>
<td>Distorted octahedron</td>
<td>N1,S2 $cis$ N3,S7 $cis$ oxo, N2 $trans$</td>
<td>N/A</td>
<td>40.3</td>
<td>39.9</td>
<td>39.5</td>
</tr>
<tr>
<td>N3-2</td>
<td>Distorted octahedron</td>
<td>N1,S2 $trans$ N3,S7 $cis$ oxo, N2 $cis$</td>
<td>N/A</td>
<td>35.8</td>
<td>35.0</td>
<td>35.0</td>
</tr>
<tr>
<td>N3-3</td>
<td>Distorted octahedron</td>
<td>N1,S2 $trans$ N3,S7 $trans$ oxo, N2 $trans$</td>
<td>N/A</td>
<td>38.0</td>
<td>37.7</td>
<td>36.5</td>
</tr>
<tr>
<td>N3-4</td>
<td>Distorted octahedron</td>
<td>N1,S2 $cis$ N3,S7 $cis$ oxo, N2 $cis$</td>
<td>N/A</td>
<td>35.5</td>
<td>35.3</td>
<td>34.7</td>
</tr>
</tbody>
</table>

$^a$The energetics were obtained at the PBE0/6-31G(d):LANL2DZ level of theory. $^b$The gas-phase total energy and the correction terms for enthalpy and Gibbs free energy are -2817.50839, 0.70437 and 0.57263 hartrees, respectively.

The N3-deprotonated ReO[DPhe-Cys-Tyr][Cys] isomers also have similar conformations to the N3-deprotonated ReO[SDPhe-Cys-Tyr][Cys] isomers, adopting distorted octahedral geometries with three adjacent 5-membered chelate rings. Similarly to what was observed for the N3-deprotonated ReO[SDPhe-Cys-Tyr][Cys] isomers, the backbone nitrogen of Cys$^2$ shows
increased sp\(^3\) character from donating electrons to the [ReO]\(^{3+}\) core in the N3-deprotonated ReO[DPhe-Cys-Tyr][Cys] isomers. The Re–N2 bond length of the latter isomers is 2.3±0.1 Å, about 10\% longer than that of the Re–N3 bond (2.08±0.08 Å) and about 5\% longer than the Re–N1 bond (2.20±0.03 Å), concurrently the N2–C(O) bond (1.46±0.03 Å) is about 8\% longer than the average amide bond (1.351±0.004 Å).

At the PBE0/6-31G(d):LANL2DZ level of theory, the N3-deprotonated ReO[DPhe-Cys-Tyr][Cys] isomers are similar in energy (within 5 kcal/mol) and are about 35 kcal/mol higher in energy (on average) than the N2-deprotonated ReO[DPhe-Cys-Tyr][Cys] isomers. However, according to the experimental NMR data for Re-TATE, the [ReO]\(^{3+}\) core is coordinated to the Tyr\(^3\) amide rather than the Cys\(^2\) amide.\(^3\) There are several possible explanations for why formation of the N3-deprotonated Re-peptide complex could be kinetically favored: 1) the Tyr\(^3\) amide is in closer proximity to react with [ReO]\(^{3+}\) than the Cys\(^2\) amide, 2) the Tyr\(^3\) amide proton is more acidic than the Cys\(^2\) amide proton, and/or 3) the Tyr\(^3\) amide proton is more accessible for proton transfer than the Cys\(^2\) amide proton.
Figure 4-1. Ball-and-stick diagrams for N2-1 to N3-4 of ReO[DPhe-Cys-Tyr][Cys]. Only the core of the coordination system is shown for clarity. Geometries were obtained at the PBE0/6-31G(d):LANL2DZ level of theory.
4.3.2 PES scans

The PES scans performed for the trans-ReO(SH)_2(NH_2)(NH_3), [ReO(SH)_3(NH_2)]^{1-} and [ReO(SH)_3(N(H)CHO)]^{1-} model complexes suggested that reliable equilibrium and non-equilibrium geometries can be obtained at the PBE0/6-31G(d):LANL2DZ level of theory. Also, about 90% of the total BDE was recovered by elongating the Re–S/N bonds by 2 Å in the gas phase. Considering the size of ReO[SDPhe-Cys-Tyr][Cys] (79 atoms; 418 electrons), the PES scans were first performed for isomers N2-1 and N3-1 of ReO[SDPhe-Cys-Tyr][Cys] at the PBE0/6-31G(d):LANL2DZ level of theory rather than the higher PBE0/6-31G(d,p):LANL2TZ level of theory recommended for geometry optimizations.\(^1,2\) As shown in Figure 4-2, smooth BDE curves were observed for Re–S2 and Re–S7 but not for Re–N2 and Re–S1. The BDEs for Re–S2 and Re–S7 are 76.9 kcal/mol and 72.6 kcal/mol, respectively, at 2 Å. It is impossible to estimate BDEs for Re–N2 and Re–S1 with the current data because the bonds could not be elongated far enough at the PBE0/6-31G(d):LANL2DZ level of theory. The carbonyl oxygen atom of SDPhe\(^1\) rearranged and coordinated to Re when the Re–N2 bond was elongated by 1.4 Å; the H\(_a\) of SDPhe\(^1\) rearranged and coordinated to Re when the Re–S1 bond was elongated by 2 Å.
The PBE0/6-31G(d):LANL2DZ PES scans performed for the Re–S1 bond for ReO[SDPhe-Cys-Tyr][Cys] N3-1 yielded a smooth BDE curve and the BDE of the Re–S1 bond is 49.3 kcal/mol at 2 Å (Figure 4-3), which is about 50 kcal/mol lower than the BDEs of the Re–S2/S7 bonds in ReO[SDPhe-Cys-Tyr][Cys] N2-1. At the lower PBE0/6-31G(d):LANL2DZ level of theory, the observed magnitude of the bond weakening may be over-emphasized, but the trend is likely to be reliable. The coordination of the sixth ligand (Cys² amide) may have weakened the other Re coordination bonds, including the Re–S1 bond, due to the valence buffer effect.⁹

As shown in Figure 4-3, the Re–N2, Re–S2, Re–N3 and Re–S7 bonds were only elongated by 1.6 Å, 1.2 Å, 1.4 Å and 1.2 Å, respectively, before non-physical intramolecular interactions/distortions disrupted the BDE curves. For the Re–N2 PES scan, the BDE curve started to level off at around 1 Å, but then rose again at 1.6 Å due to excessive deformation of the chelate rings. For the Re–S2 scan, the Cys² thiolate bonded to the SDPhe¹ carbonyl carbon when the Re–S2 bond was
elongated by 1.2 Å. For the Re–N3 scan, the Tyr\(^3\) amide (deprotonated) formed a hydrogen bond with the Cys\(^2\) amide proton when the Re–N3 bond was elongated by 1.4 Å. Finally, for the Re–S7 scan, the Cys\(^2\) amide proton transferred to the Cys\(^7\) thiolate when the Re–S7 bond was elongated by 1.2 Å.

Figure 4-3. Re–S/N BDEs obtained at the PBE0/6-31G(d):LANL2DZ level of theory for the 6-coordinate N3-1 of ReO[SDPhe-Cys-Tyr][Cys].

PES scans for the Re–N2 and Re–S7 bonds in ReO[SDPhe-Cys-Tyr][Cys] were repeated at the PBE0/6-31G(d,p):LANL2TZ level of theory (Figure 4-4) to verify that the PBE0/6-31G(d):LANL2DZ equilibrium and non-equilibrium geometries are reliable. The PES scan for the Re–S7 bond again generated a smooth BDE curve and the Re–S7 BDE is estimated to be 71.1 kcal/mol at 2 Å, which is essentially the same as the Re–S7 BDE obtained at the PBE0/6-31G(d):LANL2DZ level of theory (72.6 kcal/mol). After the Re–S7 bond is elongated by 3 Å, the PBE0/6-31G(d,p):LANL2TZ BDE is 83.0 kcal/mol. About 86% of the Re–S7 BDE is recovered by elongating the bond by 2 Å, which is in
acceptable agreement with the about 90% recovery at 2 Å for the 
trans-ReO(SH)$_2$(NH$_2$)(NH$_3$), [ReO(SH)$_3$(NH$_2$)]$^{1-}$ and [ReO(SH)$_3$(N(H)CHO)]$^{1-}$ model complexes.$^2$

![Figure 4-4](image)

Figure 4-4. Re–S/N BDEs obtained at the PBE0/6-31G(d,p):LANL2TZ level of theory for the 5-coordinate N2-1 of ReO[SDPhe-Cys-Tyr][Cys].

The interaction between the carbonyl oxygen and Re observed when the Re–N2 bond was elongated by 1.4 Å for the PBE0/6-31G(d):LANL2DZ scan was not observed for the PBE0/6-31G(d,p):LANL2TZ scan. The Re–N2 BDEs are 84.2 kcal/mol and 93.8 kcal/mol at 2 Å and 3 Å, respectively, about 12 kcal/mol higher (on average) than that of the Re–S7 bond. The smaller basis set is known to over-emphasize electrostatic effects in the gas phase, which may have resulted in the different rearrangement of the peptide on bond elongation at these two different levels of theory.
4.4 Summary

The structures of two model complexes, ReO[SDPhe-Cys-Tyr][Cys] and ReO[DPhe-Cys-Tyr][Cys], were optimized at the PBE0/6-31G(d):LANL2DZ level of theory in the gas phase. N2-deprotonated isomers, as opposed to N3- or N7-deprotonated isomers, are thermodynamically most stable for both model complexes. Although PBE0/6-31G(d):LANL2DZ relaxed PES scans were shown to generate reliable geometries for the \( \text{trans-ReO(SH)}_2(\text{NH}_2)(\text{NH}_3) \), \([\text{ReO(SH)}_3(\text{NH}_2)]^{-}\) and \([\text{ReO(SH)}_3(\text{N(H)CHO})]^{-}\) model complexes, PBE0/6-31G(d,p):LANL2TZ scans appear to be required for the larger, more structurally relevant ReO[SDPhe-Cys-Tyr][Cys] and ReO[DPhe-Cys-Tyr][Cys] model complexes.

4.5 Future Studies

N7-deprotonated isomers of ReO[DPhe-Cys-Tyr][Cys] should be examined, although they are expected to have similar relative enthalpies as N3-deprotonated ReO[DPhe-Cys-Tyr][Cys] isomers, based on the observations from ReO[SDPhe-Cys-Tyr][Cys]. Better energetics for the ReO[DPhe-Cys-Tyr][Cys] isomers can be obtained at the PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d,p):LANL2TZ level of theory in the presence and absence of implicit solvent. Relaxed PES scans need to be performed for the 6-coordinate isomers of ReO[SDPhe-Cys-Tyr][Cys] and the 5- and 6-coordinate isomers of ReO[DPhe-Cys-Tyr][Cys] at the
PBE0/6-31G(d,p):LANL2TZ and PBE0/6-311+G(d):LANL2TZ// PBE0/6-31G(d,p):LANL2TZ levels of theory. Solvent effects should be investigated by performing PES scans for some of the Re–S/N bonds in the presence of implicit water, because the inclusion of implicit water shortened the distance at which the BDE curves plateaued and ensured heterolytic splitting of the Re–NH$_2$ bond in our previous studies (Chapter 3).$^2$

Partial atomic charges can be obtained for the N2- and N3-deprotonated equilibrium structures of ReO[SDPhe-Cys-Tyr][Cys] and ReO[DPhe-Cys-Tyr][Cys]. The partial charges, as well as the geometric parameters, can be used for Re(V) force field parameter development.

### 4.6 References


5. Due to the size of the model complex, systematic variation of all the dihedral angles can be prohibitively expensive.


Chapter 5

Modeling Disulfide-cyclized and Re-cyclized Octreotide Derivatives Using Molecular Dynamics (MD) Simulations and Quantum Chemical Methods

5.1 Introduction

The ability to model the disulfide-cyclized and Re(V)-cyclized octreotide analogues with a reliable computational method can save a lot of laboratory work and time in pursuing the best design of integrating Re(V) into the peptide. Disulfide-cyclized octreotide and some of its analogues have been modeled with computational methods, such as distance geometry and MD simulations, using force fields developed for studying biological systems. Mattern et al. reported the conformational analysis of a series of octreotide analogues containing stereochemical changes in the sixth and eighth amino acids.\(^1\) In addition, the C-terminus was changed from a carboxyl group to an amide. In Mattern’s study, NMR studies and computer simulations were combined to give reliable estimations of the influence of the stereochemistry within these residues on conformation and binding affinity. However, their computer simulations were carried out without
explicit solvent molecules, which may compromise the accuracy of the 3D conformations as well as the accessible space of the side chains. In Interlandi’s study, octreotide and another analogue of somatostatin (SOM230 [c-[(2-amino-ethyl-carbamoyl)-Hyp\(^1\)-Phg\(^2\)-DTrp\(^3\)-Lys\(^4\)-(4-O-benzyl)-Tyr\(^5\)-Phe\(^6\)]) were investigated by MD simulations with explicit water molecules.\(^2\) (Hyp is (2S,4R)-4-Hydroxyproline or hydroxyproline; Phg is phenylglycine) The equilibrium between the two main conformers observed in the computer simulations is consistent with previous crystallographic studies\(^3\) and NMR studies.\(^4\)

However, due to the lack of parameters for transition metals in the force fields that are commonly used for studying biological systems, modeling Re(V)-cyclized octreotide analogues requires providing additional parameters to the existing force fields. Comba et al. have developed a set of rhenium(V) parameters for the MOMEc97 force field, including parameters for bond stretching, bond bending and torsional angle functions, but no parameters were developed to account for non-bonded interactions\(^5\). We believe taking non-bonded interactions into account is necessary for modeling our system where intramolecular and intermolecular hydrogen-bonding interactions play an important role in keeping the peptide in its active receptor binding conformation.

Hybrid electronic structure methods are also approaches that may be used for modeling Re(V)-cyclized octreotide analogues. Hybrid methods are widely used for modeling large biomolecules due to their higher computational efficiency.\(^6\) The ONIOM method\(^7\) is the hybrid method available in the Gaussian 03 and 09 software

93
To perform ONIOM calculations, the system is defined as 2 or 3 regions (layers), and each layer is treated with methods of different accuracy. The definition of the layers (partitioning scheme) is critical for success in using hybrid methods.

In this work, the conformation of disulfide-cyclized Tyr$^3$-octreotate (Tyr$^3$-TATE) is modeled by MD simulations with explicit water molecules and the effect of the R-groups on the backbone conformation of disulfide-cyclized octreotide and derivatives is investigated using DFT methods. The results of this work give insight into designing new Re(V)-cyclized octreotide analogues. Possible partitioning schemes that may be used for modeling Re(V)-cyclized octreotide analogues using the ONIOM method are explored. In addition, considerations for developing rhenium(V) parameters for the AMBER force field are discussed. Finally, future MD simulations and quantum chemical calculations to be performed for Re(V)-cyclized octreotide analogues are proposed based on the results to date.

5.2 Computational Details

5.2.1 MD simulations

The initial conformation for the runs with Tyr$^3$-TATE was previously solved by NMR in aqueous solution$^9$, where the amine terminus and amine in the side chain of Lys$^5$ are protonated; a carboxylate group is at the C-terminus. A chloride ion was added to the system as the counter ion when generating parameter and topology files with the LeAP program of the AMBER program package$^{10}$. The MD
Simulations were performed with the Sander program of the AMBER program package. The AMBER ff99 force field was used. The solvent was treated explicitly using the TIP3P model of water. The water box was a truncated octahedron with the closest distance between the peptide and the surface of the octahedron being 12 Å. Periodic boundary conditions were applied. A step size of 0.5 fs was implemented throughout the simulations. The MD simulations followed a simulated annealing procedure: in the first step, the system was pre-equilibrated for 2000 steps at 298 K under constant volume conditions (NVT) followed by a 100,000 step constant pressure simulation to converge the density of the system to approximately 1.0 g/mL (NPT). Then, to search the accessible space thoroughly, a NVT simulation was performed to raise the temperature from 298 K to 1000 K with 100,000 steps using Langevin dynamics with a collision frequency (gamma Ln) of 5.0 ps⁻¹, followed by another 100,000 steps at 1000 K to allow the system to fully equilibrate. To sample conformations at 1000 K, a 2,000,000 step NVT simulation was performed following the equilibration, and at intervals of 2000 steps one independent structure was saved at 1000 K. Finally, with only 200 steps under constant volume conditions, the temperature of the 1000 saved structures was quenched to 300 K, followed by 2000 steps at 300 K for equilibration. All the MD simulation structures were visualized with the VMD program.¹¹

**5.2.2 Electronic structure calculations**

Electronic structure calculations have been performed for disulfide-cyclized octreotide derivatives to study how each side chain of the peptide affects the
conformation of the peptide backbone. The initial conformations used for the
electronic structure calculations with disulfide-cyclized peptides were generated
from the NMR structures deposited in the Protein Data Bank (PDB): 1SOC, 2SOC,
1YL8, 1YL9, and the three dimensional NMR structures solved by Dannoon et al. To
assess the effect of the side chains on the backbone conformation, the side chains of
DPhe, Phe, DTrp, Lys, and Thr were initially replaced with methyl groups, but the side
chains of the two cysteines were unchanged to keep the peptide cyclized, resulting
in DAla-Cys-Ala-DAla-(Ala)2-Cys-Ala(ol) (DAla1.4-Ala3.5.6-TIDE). Then, each side
chain following the order of Phe, DTrp, Lys, Thr, DPhe, was added back to the
peptide backbone. The peptide termini were kept neutral initially, but were
subsequently changed to zwitterionic form: the N-terminus being protonated and
the C-terminus being a carboxylate group. All the structures of the
disulfide-cyclized octreotide derivatives were optimized at the B3LYP/6-31G(d)
level of theory.

ONIOM calculations were performed for the N2-deprotonated anti isomer of
Re-SDPhe-TATE. Seven different partitioning schemes were examined, where the
high, middle (partitioning scheme 6 only) and low layers were optimized at the
PBE0/6-31G(d,p):LANL2TZ, PBE0/6-31G(d):LANL2DZ and UFF/PM6 levels of
theory, respectively. The starting geometry was generated by manually connecting
Tyr and Cys of the N2-deprotonated anti isomer of the
ReO[SDPhe-Cys-Tyr][Cys] model complex (N2-1) with DTrp4-Lys-Thr and
attaching Thr to the carboxylate of Cys, adopting a β-turn-like conformation.
The structures were fully optimized and vibrational frequencies were computed to verify that the stationary points found correspond to minima using the Gaussian03 or 09 program packages\textsuperscript{13}. The keyword “int=ultrafine” was used for all DFT calculations. The GaussView program was employed for the structure modification required to obtain desired starting geometries. The PyMOL program\textsuperscript{14} was used to perform RMSD analyses and to evaluate the backbone alignment of the disulfide-cyclized octreotide derivatives.

5.2.3 Computational resources

All MD simulations were done on a Mac desktop computer with 4 CPUs and 4 GB of memory. All electronic structure calculations were performed on a cluster of computer nodes from multiple vendors (Dell, IBM, and Advanced Clustering Technologies), all with multi-core Intel 64 architecture Xeon processors, or an SGI Altix BX2 NUMA architecture machine with 64 1.5 GHz Intel Itanium2 processors and 128 GB of shared memory.

5.3 Current Results and Discussion

5.3.1 MD simulations

After defining the size of the water-box, about 2000 water molecules were included in the Tyr\textsuperscript{3}-TATE system. The constant pressure equilibration resulted in a system density of about 0.99 g/ml. One thousand conformations were stored in a movie file at 1000 K. The RMSD shows that as the temperature increases, a larger conformational space is accessed. To date, thirty conformations were extracted and
cooled to 300 K. Quenching did not cause significant conformational changes. Among the 30 conformations trapped by fast cooling to 300 K, the β-turn and distorted helical conformations, as well as conformations between the two extremes, were observed (Figure 5-1). The positively charged side chain of Lys$^5$ swung between the indole ring of DTrp$^4$ and the carboxylate terminus. The orientation of the phenyl group of DPhe$^1$ relative to the disulfide bridge and the width between the two peptide termini varied as the backbone conformation changed.
Figure 5-1. Tyr$_3$-TATE Conformations observed in simulated annealing results. a. The C-terminus adopts a helical conformation; b. The C-terminus is parallel to the N-terminus; c. The phenyl group of DPhe1 is turned away from the disulfide bridge and the R-groups of DTrp4 and Lys5 are apart from each other; d. The phenyl group of DPhe1 is close to the disulfide bridge and the R-groups of DTrp4 and Lys5 are next to each other.

In general, the results of our MD simulations on Tyr$_3$-TATE, in which the conformation of the peptide changes from a β-turn-like conformation to a distorted helical conformation, show good agreement with previous NMR studies, indicating that the peptide exists in an equilibrium between β-turn and partially
helical structures. Dihedral angles $\Phi$ (C-N-C$_\alpha$-C) and $\psi$ (N-C$_\alpha$-C-N) are the dihedral angles conventionally used for describing peptide backbone conformations. As shown in Figure 5-2, the dihedral angle $\psi$ of Cys$^7$ changes from about $-70^\circ$ (distorted helical) to about $-140^\circ$ (β-turn) over time, indicating the conformational change at the C-terminus.

Figure 5-2. Trajectory of dihedral angle $\psi$ of Cys$^7$.

Particularly, it was encouraging to see the agreement with the NMR studies reported by Dannoon et al.,$^9$ in which the same peptide was studied. The difference between Tyr$^3$-TATE and octreotide is very important because in Tyr$^3$-TATE, the last residue has a negatively charged carboxylate group, whereas in octreotide, the last residue is a neutral alcohol. This difference made it imperative to include explicit water molecules in our system. With explicit water molecules, helpful information such as the number of water molecules that are in closest proximity to
the terminal carboxylate and how these water molecules interact with the negatively charged terminus may be obtained and then used in subsequent quantum chemical calculations. In the gas phase, the electrostatic interaction between the positively charged groups and the carboxylate group tends to be overestimated, which leads to a preference in adopting a helical conformation (Figure 5-1c). This problem may be solved by including a minimal number of water molecules around the charged groups for calculations that must be done in the gas phase.

5.3.2 Electronic structure calculations

5.3.2.1 DFT calculations

To date, in all published work, no electronic structure calculations have been performed to study octreotide analogues, which is probably because of the extremely long CPU time required for these calculations (20 days on average for the disulfide-cyclized peptides). Although the MD simulations provide very good initial structures with much less calculation time, the structures must be reoptimized to obtain relative energies, atomic charges and bond dissociation energies. Thus, we have carried out quantum chemical calculations on disulfide-cyclized and Re-cyclized analogues of octreotide and octreotate.

Initially, 17 conformations of DAla$^{1,4}$-Ala$^{3,5,6}$-TIDE were screened, but only the 6 lowest energy conformations were kept for the following study on the effect of the side chains on the octreotide backbone conformation. Three of the 6 adopted a helix-like conformation, whereas the other three adopted a β-turn-like conformation. 1SOC and 2SOC represent the initial backbone conformations taken
from two of the Protein Data Bank structures, and they are the examples discussed here for convenience. The side chains of Phe$^3$, DTrp$^4$, Lys$^5$, Thr$^6$ and DPhe$^1$ were added back to DAla$^1$-Ala$^{3,5,6}$-TIDE consecutively resulting a series of octreotide derivatives, DAla$^4$-Ala$^{3,5,6}$-TIDE, DAla$^4$-Ala$^{5,6}$-TIDE, Ala$^{3,5,6}$-TIDE, etc. Table 5-1 shows the dihedral angles of the peptide backbones and the relative energies of the octreotide derivatives studied. Each group of two that is separated by a blank line is composed of the same sequence, but the two entries in the same group adopt different conformations, which are classified as either “α” or “β”. The “α” indicates that the carboxylate terminus bends away from the amine terminus and forms a distorted helical structure; the “β” indicates the two termini are nearly parallel to each other and the conformation of the peptide resembles a β-turn conformation (Figures 5-1a and 5-1b). Here the dihedral angles $\Phi$ and $\psi$ are given in the order of the $\alpha$ carbons, beginning from the $N$-terminus. The relative energies were calculated using all six conformations of the same sequence. When the termini were kept neutral in charge, the β-turn conformations are the most stable ones, but after changing the alcohol group of Thr$^8$(ol) to a carboxylate group, 1SOC (β-turn-like) changed into a helical conformation and is no longer the lowest energy conformation. Figure 5-3 shows that after the hydrogen-bonding interaction between the DAla$^1$ carbonyl group and the Thr$^8$ alcohol group is broken, the carboxylate terminus turns away from the amine terminus and interacts with the positively charged lysine side chain. It is understandable that in the gas phase the zwitterionic form of a molecule will be higher in energy. For 2SOC (helix-like), after changing the C-terminus from neutral to negatively charged, the Thr$^8$ side
chain alcohol group interacts with the carbonyl group of Lys$^5$ and the C-terminal carboxylate group interacts with the alcohol group of the Thr$^6$ side chain, with the conformation remaining helix-like. However, this change caused the termini to move closer together, and once the amine terminus was protonated, the structural features of the $\beta$-turn in 2SOC dominated those of the helical conformation, as shown in Figure 5-4. Compared with the changes caused by the electrostatic interactions, the side chains of DPhe$^1$, Phe$^3$, DTrp$^4$, Lys$^5$ and Thr$^6$ have a relatively small influence on the backbone conformation, although some of the side chains are really bulky. Figure 5-5 shows that if the termini are kept neutral in charge, the peptide backbones overlap each other very well, no matter what combination of side chains is present. On the other hand, if the termini are charged, electrostatic interactions dominate the backbone conformation to a large extent.
Table 5-1. Dihedral angles and relative energies of octreotide and derivatives.

<table>
<thead>
<tr>
<th>Conformation</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
<th>C7</th>
<th>ΔE  (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1SOC DAla&lt;sup&gt;a&lt;/sup&gt;-Ala&lt;sup&gt;b&lt;/sup&gt;-TIDE</td>
<td>β</td>
<td>-133.3</td>
<td>84.8</td>
<td>-87.6</td>
<td>70.8</td>
<td>62.0</td>
<td>-118.6</td>
</tr>
<tr>
<td>2SOC DAla&lt;sup&gt;a&lt;/sup&gt;-Ala&lt;sup&gt;b&lt;/sup&gt;-TIDE</td>
<td>α</td>
<td>-80.4</td>
<td>66.5</td>
<td>-84.4</td>
<td>69.1</td>
<td>67.2</td>
<td>-98.2</td>
</tr>
<tr>
<td>1SOC DAla&lt;sup&gt;c&lt;/sup&gt;-Ala&lt;sup&gt;d&lt;/sup&gt;-TIDE</td>
<td>β</td>
<td>-137.6</td>
<td>84.2</td>
<td>-90.2</td>
<td>75.9</td>
<td>60.3</td>
<td>-120.9</td>
</tr>
<tr>
<td>2SOC DAla&lt;sup&gt;c&lt;/sup&gt;-Ala&lt;sup&gt;d&lt;/sup&gt;-TIDE</td>
<td>α</td>
<td>-80.1</td>
<td>66.6</td>
<td>-90.1</td>
<td>74.1</td>
<td>64.8</td>
<td>-101.2</td>
</tr>
<tr>
<td>1SOC DAla&lt;sup&gt;e&lt;/sup&gt;-Ala&lt;sup&gt;f&lt;/sup&gt;-TIDE</td>
<td>β</td>
<td>-146.9</td>
<td>84.4</td>
<td>-89.9</td>
<td>76.2</td>
<td>63.7</td>
<td>-109.9</td>
</tr>
<tr>
<td>2SOC DAla&lt;sup&gt;e&lt;/sup&gt;-Ala&lt;sup&gt;f&lt;/sup&gt;-TIDE</td>
<td>α</td>
<td>-80.2</td>
<td>66.3</td>
<td>-90.4</td>
<td>75.4</td>
<td>65.0</td>
<td>-102.5</td>
</tr>
<tr>
<td>1SOC DAla&lt;sup&gt;1&lt;/sup&gt;-Ala&lt;sup&gt;6&lt;/sup&gt;-TIDE</td>
<td>β</td>
<td>-145.6</td>
<td>88.3</td>
<td>-84.6</td>
<td>77.0</td>
<td>74.2</td>
<td>-81.7</td>
</tr>
<tr>
<td>2SOC DAla&lt;sup&gt;1&lt;/sup&gt;-Ala&lt;sup&gt;6&lt;/sup&gt;-TIDE</td>
<td>α</td>
<td>-80.1</td>
<td>64.5</td>
<td>-88.3</td>
<td>70.8</td>
<td>69.2</td>
<td>-91.3</td>
</tr>
<tr>
<td>1SOC DAla&lt;sup&gt;1&lt;/sup&gt;-TIDE</td>
<td>β</td>
<td>-148.1</td>
<td>86.3</td>
<td>-84.4</td>
<td>74.7</td>
<td>72.5</td>
<td>-83.5</td>
</tr>
<tr>
<td>2SOC DAla&lt;sup&gt;1&lt;/sup&gt;-TIDE</td>
<td>α</td>
<td>-79.9</td>
<td>73.5</td>
<td>-85.4</td>
<td>73.5</td>
<td>70.2</td>
<td>-90.3</td>
</tr>
<tr>
<td>1SOC DAla&lt;sup&gt;1&lt;/sup&gt;-TATE&lt;sup&gt;g&lt;/sup&gt;</td>
<td>α</td>
<td>-144.7</td>
<td>122.2</td>
<td>-136.0</td>
<td>49.1</td>
<td>82.2</td>
<td>-82.3</td>
</tr>
<tr>
<td>2SOC DAla&lt;sup&gt;1&lt;/sup&gt;-TATE&lt;sup&gt;g&lt;/sup&gt;</td>
<td>α</td>
<td>-86.3</td>
<td>77.2</td>
<td>-79.3</td>
<td>66.7</td>
<td>83.5</td>
<td>-64.8</td>
</tr>
<tr>
<td>1SOC TATE&lt;sup&gt;h&lt;/sup&gt;</td>
<td>α</td>
<td>-146.0</td>
<td>122.3</td>
<td>-136.0</td>
<td>48.8</td>
<td>82.3</td>
<td>-81.1</td>
</tr>
<tr>
<td>2SOC TATE&lt;sup&gt;h&lt;/sup&gt;</td>
<td>α</td>
<td>-125.2</td>
<td>121.0</td>
<td>-84.6</td>
<td>63.9</td>
<td>80.4</td>
<td>-66.0</td>
</tr>
<tr>
<td>1SOC N-terminus protonated TATE&lt;sup&gt;i&lt;/sup&gt;</td>
<td>α</td>
<td>-157.0</td>
<td>133.1</td>
<td>-157.3</td>
<td>110.6</td>
<td>55.3</td>
<td>-133.6</td>
</tr>
<tr>
<td>2SOC N-terminus protonated TATE&lt;sup&gt;i&lt;/sup&gt;</td>
<td>β</td>
<td>-66.8</td>
<td>134.2</td>
<td>-155.4</td>
<td>48.3</td>
<td>89.6</td>
<td>-66.4</td>
</tr>
<tr>
<td>1SOC NMR structure&lt;sup&gt;j&lt;/sup&gt;</td>
<td>β</td>
<td>-142.9</td>
<td>101.0</td>
<td>-128.2</td>
<td>92.3</td>
<td>66.5</td>
<td>-127.2</td>
</tr>
<tr>
<td>2SOC NMR structure&lt;sup&gt;j&lt;/sup&gt;</td>
<td>α</td>
<td>-80.6</td>
<td>104.1</td>
<td>-147.5</td>
<td>90.7</td>
<td>72.3</td>
<td>-128.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>C-terminus is negatively charged but N-terminus is neutral in charge. <sup>b</sup>C- and N-termini are both charged. <sup>c</sup>1SOC and 2SOC structures retrieved from the Protein Data Bank.
Figure 5-3. Conformational change caused by replacing the alcohol terminus with a carboxylate group. Hydrogen-white; carbon-grey; nitrogen-blue; oxygen-red; sulfur-yellow.

Figure 5-4. Conformational change caused by replacing the terminal NH$_2$ with a [NH$_3$]$^{+}$. Hydrogen-white; carbon-grey; nitrogen-blue; oxygen-red; sulfur-yellow.
Figure 5-5. Comparison of the effects of charged termini and the other side chains on backbone conformation. a. Peptides with side chains of residues 1 to 6 and neutral termini. b. The cyan colored structure is the methyl substituted peptide and is shown for reference. The purple structure has a carboxylate group at the C-terminus, but its N-terminus is neutral in charge. The yellow structure adds one phenyl group to the first residue of the purple structure, so they have the same charge. The pink structure adds a proton to the yellow structure; in other words, both of its termini are charged.

Further, the side chains of DTrp$^4$ and Lys$^5$ were observed to affect the positions of each other. As shown in Figure 5-6a, the side chain ammonium (positively charged) of Lys$^5$ interacts with Thr$^6$ in the absence of the indole group of DTrp$^4$. The addition of the indole group prevents the Lys$^5$ side chain from interacting with Thr$^6$ as shown in Figure 5-6b. Similarly, the presence of the Lys$^5$ side chain affects the position of DTrp$^4$ as well. As shown in Figure 5-6c, the addition of the Lys$^5$ side chain causes the orientation of indole group to
change. These observations provide an explanation for the proposed Trp$_1$-Lys$_5$ pharmacophore.

Figure 5-6. The effect of R-groups on the position of the R-groups of neighboring residues. a. A DAla$_{1,4}$-Ala$_{5,6}$-TIDE conformer aligned to the corresponding DAla$_{1,4}$-Ala$_6$-TIDE conformer. b. A DAla$_{1,4}$-Ala$_6$-TIDE conformer aligned to the corresponding DAla$_1$-Ala$_6$-TIDE conformer. c. A DAla$_1$-Ala$_{5,6}$-TIDE conformer aligned to the corresponding DAla$_1$-Ala$_6$-TIDE conformer.

5.3.2.2 ONIOM calculations

As preliminary studies, seven partitioning schemes were screened for the N2-deprotonated Re-SDPhe-TATE anti isomer to determine the influence of the definition of the high and low layers on molecular structure and computational efficiency (Table 5-2). The partitioning expands the high layer until there is no change in the backbone conformation, Re-S/N BDEs, relative enthalpies and R-group orientations. More than one partitioning scheme may be useful for this work depending on the molecular properties sought from the
calculations. In all the partitioning schemes examined thus far, the ReO[NS$_3$]$^{1-}$ core, where the bond formation/dissociation occurs, is included in the high layer. For example, in partitioning Scheme 1, [ReO]$^{3+}$, SDPhe$^1$-Cys$^2$-Tyr$^3$ and Cys$^7$-Thr$^6$ were included in the high layer and optimized at the PBE0/6-31G(d,p):LANL2TZ level of theory; the rest of the atoms in the Re-peptide complex were optimized at the molecular mechanics (MM) level using the UFF method. Descriptions of partitioning Schemes 2-7 can be found in Table 5-2. The ONIOM calculations at the DFT//UFF level of theory completed for all the partitioning schemes except for 4 and 6 (PBE0/6-31G(d,p):LANL2TZ//UFF for Schemes 1-5 and 7; PBE0/6-31G(d,p):LANL2TZ//PBE0/6-31G(d):LANL2DZ//UFF for Scheme 6). The effect of electronic embedding (EE) was examined using Scheme 7. Electronic embedding incorporates the partial charges of the molecular mechanical layer (UFF layer) into the quantum mechanical Hamiltonian and is expected to provide a better description of the electrostatic interaction between the DFT and MM layers, although it increases the computational cost. However, no significant difference was observed with and without using electronic embedding at the PBE0/6-31G(d,p):LANL2TZ//UFF level of theory for partitioning Scheme 7 in terms of computational efficiencies and optimized geometries. The ONIOM (PBE0/6-31G(d,p):LANL2TZ//PM6) calculations were carried out for Schemes 3, 5 and 7, and completed for Schemes 3 and 7,
which suggests atoms linked by double bonds should be placed in the same layer. Partitioning Scheme 3 is recommended for subsequent ONIOM calculations for Re-cyclized octreotide analogues because the peptide backbone needs to be included in the high layer to avoid non-physical peptide bond configurations.
Table 5-2. Partitioning schemes investigated for ONIOM calculations for an isomer of Re-SDPhe-TATE.

<table>
<thead>
<tr>
<th>Partition schemes’ pictorial illustration</th>
<th>Description</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Partitioning scheme 1" /></td>
<td>High layer: [ReO]$^{5+}$, SDPhe$^1$-Cys$^2$-Tyr$^3$ and Cys$^7$-Thr$^6$ (PBE0/6-31G(d,p):LANL2TZ); Low layer: the rest (UFF).</td>
<td>cis Thr$^6$-Cys$^7$ peptide bond was observed.</td>
</tr>
<tr>
<td><img src="image2.png" alt="Partitioning scheme 2" /></td>
<td>High layer: [ReO]$^{5+}$, SDPhe$^1$-Cys$^2$-Tyr$^3$ and Cys$^7$ (PBE0/6-31G(d,p):LANL2TZ); Low layer: the rest (UFF).</td>
<td>cis Thr$^6$-Cys$^7$ peptide bond was observed.</td>
</tr>
<tr>
<td><img src="image3.png" alt="Partitioning scheme 3" /></td>
<td>High layer: ReO[NS$<em>3$]$^{1-}$ core, peptide backbone atoms up to C$</em>\beta$ atoms (PBE0/6-31G(d,p):LANL2TZ); Low layer: the rest (UFF or PM6).</td>
<td>All peptide bond configurations are normal.</td>
</tr>
<tr>
<td><img src="image4.png" alt="Partitioning scheme 4" /></td>
<td>High layer: ReO[NS$_3$]$^{1-}$ core (PBE0/6-31G(d,p):LANL2TZ); Low layer: the rest (UFF).</td>
<td>Non-physical connectivity change was observed and ONIOM calculation could not complete normally.</td>
</tr>
</tbody>
</table>
5.4 Conclusions

Tyr\textsuperscript{3}-TATE was studied to provide a reference for the Re(V)-cyclized analogues. DFT calculations in the gas phase suggest that the effect of R-groups on the backbone conformation of Tyr\textsuperscript{3}-TATE is relatively small compared to that of the charged termini. However, the R-groups can affect the position of the
side chains on the neighboring residues. MD simulations suggest that the backbone of Tyr$^3$-TATE changes between a β-turn-like conformation and a distorted helical conformation in the presence of explicit water. Different ONIOM partitioning schemes were examined for modeling Re-cyclized octreotide analogues, and the partitioning scheme where the ReO[NS$_3$]$^{1-}$ core and the backbone atoms up to the β carbons are included in the high layer is recommended for subsequent ONIOM calculations.

5.5 Future Studies

5.5.1 Develop a set of Re(V) parameters for the AMBER force field$^{15}$.

The Re(V) parameters developed by Comba et al.$^5$ can be used as the initial parameters for the bond stretching, bond bending and torsional angle functions for parameter refinements. The Lennard-Jones function can be used to account for van der Waals interactions. In addition, the rhenium model complexes ReO[SDPhe-Cys-Tyr][Cys] and ReO[DPhe-Cys-Tyr][Cys], which have been optimized using quantum chemical methods, can provide the required Merz-Singh-Kollman (MK) atomic charges (Chapter 4). The atomic charges can be read into the AMBER program and used to account for electrostatic interactions. Crystal structures of relevant rhenium compounds retrieved from the Cambridge Structural Database (CSD) can be used as benchmarks to refine and test the modified AMBER force field.
The parameterization of the AMBER force field is a challenge of this project. Although the bond stretching, bond bending and torsional angle functions of the MOMEC97 force field are basically the same as those of AMBER, still, parameters from two distinctive force fields are generally not transferable. Furthermore, because the MOMEC97 force field does not account for non-bonded interactions, initial parameters for the Lennard-Jones function as well as for the electrostatic interactions will have to be obtained first. This can be done, as long as an appropriate van der Waals radius for Re(V) is identified. In the literature, van der Waals radii of transition metals have been developed using various methods, but the data belongs to two categories. The radii of the first category are developed based on geometries of relevant crystal structures, and this type of radius is similar to the distance where the potential energy curve crosses the zero energy axis in the plot of potential energy versus distance. The radii of the second category are developed from theoretical calculations, and this type of radius corresponds to the equilibrium radius, where the repulsion equals the attraction. Since the rhenium van der Waals radius differs from different sources, and the MOMEC97 force field parameters need to be adjusted to fit in the AMBER force field, the parameterization is going to be a trial-and-error process. However, the Re-cyclized octreotide analogues that have been characterized experimentally can be used to refine our parameters, and the experience described in the literature with developing
Zinc(II)$^{17}$ Copper(II) and Indium(III)$^{18}$ parameters for the AMBER force field can serve as a guide.

5.5.2 **Perform MD simulations on Re(V)-cyclized octreotide analogues and Tyr$^3$-TATE.**

Once the Re(V) parameters for the AMBER force field are developed, the conformational space of the molecules of interest can be explored using a simulated annealing approach: a molecule is put in an explicit water model, then the system of analyte and solvent molecules is equilibrated under constant volume conditions for 1 ps followed by a constant pressure equilibration until the density of the system is approximately 1 g/mL. After that, the temperature is increased gradually to 1000 K over 50 ps so that the peptide has access to all spatial conformations. After the system is fully equilibrated at 1000 K, a number of independent structures should be saved and a quenching method should be applied to cool the saved structures to room temperature within 0.1 ps to trap the molecule in the conformation corresponding to local minima on the potential energy surface. Finally, cluster analysis or dihedral angle analysis can be performed to identify major conformers of each analogue.

Tyr$^3$-TATE has been studied with the AMBER program using the approach described above. Three Re(V)-cyclized octreotide analogues (Figures
2-1 and 2-2) should be first investigated with the modified force field: Re(V)-cyclized Tyr\textsuperscript{3}-TATE, the analogue that has nanomolar level sst\textsubscript{2} receptor binding affinity but the less stable N\textsubscript{2}S\textsubscript{2} coordination system\textsuperscript{19}; Re(V)-cyclized NAc-Cys\textsuperscript{1}-TATE, the analogue that has a more stable NS\textsubscript{3} coordination system but no sst\textsubscript{2} receptor binding affinity; Re(V)-cyclized SDPhe\textsuperscript{1}-TATE, the analogue that has the best binding affinity among the NS\textsubscript{3} analogues (Chapter 2).

The MD simulation results for Tyr\textsuperscript{3}-TATE can be used for reference in future studies, so that we will be able to compare the conformational differences or similarities between Re-cyclized octreotide analogues and Tyr\textsuperscript{3}-TATE.

5.5.3 Perform electronic structure calculations on representative conformers selected from MD simulations.

Representative conformers selected from MD simulations of the molecules of interest can be further investigated using a well-calibrated ONIOM method (carefully chosen partitioning scheme and level of theory), to obtain more reliable energetics for the various conformers. If the MD simulations suggest some water molecules have intimate interactions with the metal-peptide complex, those water molecules can be included explicitly in ONIOM calculations. Vibrational frequencies can be computed to confirm the desired
types of stationary points are identified. In addition, an electrostatic potential surface can be computed to show the charge distributions on the accessible surface of the metal-peptide complex and the disulfide-cyclized peptide. The Re–S/N BDEs can be determined to compare the stability of different coordination systems. The ONIOM Re–S/N BDEs calculated for Re-SDPhe-TATE can be compared with those calculated for the ReO[SDPhe-Cys-Tyr][Cys] model complex and provide insights into how reliable the truncated model complexes are for evaluation of chemical stability of Re-cyclized octreotide analogues. Finally, molecular orbital analysis can be performed to help understand the nature of the coordination bonds in the metal-peptide complexes.

5.6 References


Chapter 6

Conclusions and Future Studies

In summary, new $^{99m}$Tc/Re(V)-cyclized octreotide analogues, $^{99m}$Tc/Re-SDPhe-TATE were synthesized, characterized and evaluated in vitro. Four isomers were observed for $^{99m}$Tc/Re-SDPhe-TATE, whereas only two isomers were observed for previously reported $^{99m}$Tc/Re(V)-cyclized octreotide analogues.\textsuperscript{1-3} Electronic structure calculations and 2D NMR experiments suggest Re-SDPhe-TATE isomers have 5- or 6-coordinate NS\textsubscript{3} coordination systems. Isomers of $^{99m}$Tc/Re-SDPhe-TATE exhibited different in vitro stability and sst\textsubscript{2} receptor binding affinity. Compared to previously reported $^{99m}$Tc-cyclized octreotide analogues, $^{99m}$Tc-SDPhe-TATE isomers are less stable than the other NS\textsubscript{3} analogues but are more stable than the N\textsubscript{2}S\textsubscript{2} analogues\textsuperscript{1-3}. The sst\textsubscript{2} receptor binding assays showed Re-SDPhe-TATE isomers are the most potent NS\textsubscript{3} analogues examined thus far, but are less potent than Re-TATE.\textsuperscript{1-3}
Because a 0.4 to 1.1 minute difference in retention times was observed for the $^{99m}$Tc- and Re-SDPhe-TATE isomers by RP-HPLC analyses, the molecular structures of the $^{99m}$Tc-SDPhe-TATE isomers must be further characterized. Synthesizing $^{99}$Tc-SDPhe-TATE is one possible approach to further characterize the $^{99m}$Tc-SDPhe-TATE isomers and determine their structures.

Efforts were made to develop computational methods for studying the chemical stability and 3D molecular structures of Re(V)-cyclized octreotide analogues. Calculating the Re–S/N BDEs by performing relaxed PES scans for ReO[SDPhe-Cys-Tyr][Cys] and ReO[DPhe-Cys-Tyr][Cys] model complexes provides a method for evaluating the stability of Re(V)-cyclized octreotide analogues. PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d,p):LANL2TZ PES scans were shown to yield reliable BDEs for the Re–S/N bonds in the model complexes $^{\text{trans-}}$ReO(SH)$_2$(NH$_2$)(NH$_3$), $^{\text{[ReO(SH)$_3$(NH$_2$)]}^{1-}}$ and $^{\text{[ReO(SH)$_3$(N(H)CHO)]}^{1-}}$. Gas-phase PES scans are reliable for obtaining bond strength trends but often overestimate the difference among different Re–S/N bonds, suggesting solvent effects should be examined using implicit solvent.

PES scan studies for the ReO[SDPhe-Cys-Tyr][Cys] and ReO[DPhe-Cys-Tyr][Cys] model complexes should be continued. The recommended PBE0/6-311+G(d):LANL2TZ//PBE0/6-31G(d,p):LANL2TZ method yields reliable geometries and energetics but is very expensive for
larger models such as ReO[SDPhe-Cys-Tyr][Cys] and ReO[DPhe-Cys-Tyr][Cys]. Thus, efforts should be made to identify a reliable and cost-effective PES scan protocol for these systems.

Performing ONIOM calculations on representative conformations selected from MD simulations offers an approach for modeling the 3D molecular structures of Re(V)-cyclized octreotide analogues. MD simulations were performed on Tyr3-TATE to provide a reference for studying the peptide backbone conformations of Re(V)-cyclized octreotide analogues. A partitioning scheme was identified for modeling Re(V)-cyclized octreotide analogues using the ONIOM method.

The AMBER force field needs to be expanded with parameters for Re(V). Then, MD simulations and ONIOM calculations can be performed to predict 3D molecular structures of new Re(V)-cyclized octreotide analogues. In addition, the computational methods suitable for studying Re(V) complexes should be examined for their reliability for studying Tc(V) complexes.

References


Appendix I

The First Re(V)-cyclized Somatostatin Antagonist Peptide Analogue

7.1 Introduction

Radiolabeled agonist peptide analogues of somatostatin have been extensively investigated for diagnostic imaging and targeted radiotherapy for patients with neuroendocrine tumors.1-5 These peptides are all analogues of octreotide, [DPhe-c(Cys-Phe-DTrp-Lys-Thr-Cys)-Thr(ol)], a metabolically stablized analogue of native somatostatin. The most well-known derivatives of octreotide, Tyr3-octreotide and Tyr3-octreotate, have been radiolabeled with gamma-emitters such as 111In and 99mTc, positron-emitters such as 18F, 64Cu and 68Ga, and β-emiters such as 90Y and 177Lu, either directly or through a bifunctional chelator for imaging or treatment of somatostatin receptor (sst)-positive tumors.1,4-8
Agonists, upon binding to their receptor, readily internalize into tumor cells, which was believed crucial for efficient accumulation and retention of radioactivity in tumor cells. However, more recently, radiolabeled antagonist peptide analogues of somatostatin were shown to outperform agonists.\textsuperscript{9-12} Ginj et al. compared the somatostatin antagonist peptide analogue \(111\text{In}-\text{DOTA}-\text{4-NO}_2\text{-Phe-c(DCys-Tyr-DTrp-Lys-Thr-Cys)}-\text{DTyrNH}_2\) (\(111\text{In}-\text{DOTA-sst}_2\text{-ANT}\)) and agonist peptide analogue \(111\text{In}-\text{DTPA}-\text{DPhe-c(Cys-Tyr-DTrp-Lys-Thr-Cys)}-\text{Thr(OH)}\) (\(111\text{In}-\text{DTPA-Tyr}^3\text{-TATE}\)) and demonstrated that the former had higher uptake and longer retention in somatostatin receptor subtype 2 (sst\textsubscript{2})-positive tumor in mice.\textsuperscript{9} Furthermore, a pilot clinical study involving five patients with metastatic thyroid carcinoma or neuroendocrine tumors showed \(111\text{In}-\text{DOTA-sst}_2\text{-ANT} \) detected more tumor lesions than \(111\text{In}-\text{DTPA-octreotide} \) in the same patient.\textsuperscript{10} Wadas et al. reported preclinical studies on the comparison of \(64\text{Cu-CB-TE2A-sst}_2\text{-ANT} \) and \(64\text{Cu-CB-TE2A-Y3-TATE} \) as PET radiopharmaceuticals for imaging of sst\textsubscript{2}-positive tumors, demonstrating superior tumor-to-background contrast of the former.\textsuperscript{11} Fani et al. reported four PET radiometal-labeled antagonist analogues:
\(68\text{Ga/64Cu-NODAGA-p-Cl-Phe-c(DCys-Tyr-D-4-amino-Phe(carbamoyl)-Lys-Thr-Cys)}-\text{DTyrNH}_2\) (\(68\text{Ga/64Cu-NODAGA-sst}_2\text{-LM3}\)) and
$^{68}$Ga/$^{64}$Cu-CB-TE2A-p-Cl-Phe-c(DCys-Tyr-D-4-amino-Phe(carbamoyl)-Lys-Thr-Cys)-DTyrNH$_2$ ($^{68}$Ga/$^{64}$Cu-CB-TE2A-sst$_2$-LM3), and demonstrated that although the chelate has a large effect on the pharmacokinetics, all four radiolabeled antagonist analogues showed promise as candidates for clinical translation.\textsuperscript{12} The high tumor uptake, long tumor retention, and high tumor-to-normal tissue contrast of the radiolabeled antagonist analogues observed in various \textit{in vivo} studies are of particular importance for targeted therapeutic applications.

The chemical congener of technetium, rhenium, has two attractive therapeutic radionuclides: Re-186 and Re-188. Rhenium-186 has a half-life of 90 hours and emits $\beta^-$ particles with a maximum energy of 1.07 MeV, along with a 137 keV $\gamma$ ray (9\% abundance). Rhenium-188 has a half-life of 17 hours and emits $\beta^-$ particles with a 2.1 MeV maximum energy, along with a 155 keV $\gamma$ ray (15\% abundance). We previously synthesized a series of $^{99m}$Tc/Re(V)-cyclized somatostatin agonist peptide analogues and evaluated their \textit{in vitro} sst$_2$ affinity, chemical stability, and \textit{in vivo} biodistribution in normal mice.\textsuperscript{13} Among the peptides studied, those with N$_2$S$_2$ metal coordination (where $[^{99m}$TcO]$^{3+}$ or [ReO]$^{3+}$ was bound to two nitrogen and two sulfur atoms) retained modest SSRT2 affinity, yet exhibited chemical instability at the radiotracer level along with low and non-specific uptake in
SSTR2-expressing tissues. The instability was attributed to the unfavorable size of the chelate rings formed upon cyclization, as well as a neutral amine donor. Peptide sequence modifications to improve radiotracer stability were successful. However, the gain in stability was not sufficient to outweigh the simultaneous loss in receptor affinity.

Highly potent somatostatin antagonist peptide analogues provide an alternative route for developing $^{186/188}$Re-based SSRT2-targeting therapeutic agents. The goal of this work was to directly coordinate a $[\text{ReO}]^{3+}$ core to the somatostatin peptide analogue DOTA-sst$_2$-ANT, taking advantage of the thiolates and amides present in the peptide sequence, and to evaluate the SSRT2 affinity of the metal-peptide complex. The presence of the N-terminal DOTA group allows direct comparison against a bifunctional chelator-labeled peptide such as Lu-labeled DOTA-sst2-ANT.

### 7.2 Methods and Material

#### 7.2.1 General methods

Most reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO) and Fisher Scientific (Pittsburgh, PA) at the highest quality available.

N-alpha-(9-Fluorenylmethyloxycarbonyl)-S-p-methoxytrityl-D-cysteine
(Fmoc-DCys(Mmt)-OH) was purchased from Iris Biotech GmbH (Marktredwitz, Deutschland), and $^{125}$I-labeled Ala-Gly-c(Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Tyr-Thr-Ser-Cys)

([$^{125}$I]-Tyr$^{11}$-somatostatin-14]) was purchased from Perkin Elmer (Waltham, MA). All of the chemicals were used as received without further purification.

Liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) analysis and semi-preparative purification were performed on a Beckmann Coulter System Gold HPLC equipped with the 32 KARAT software package (Beckmann Coulter, Fullerton, CA), a 507e auto-injector, and a 168 diode array detector connected to a Thermo Finnigan TSQ7000 triple-quadrupole mass spectrometer (Thermo Finnigan, San Jose, CA). Analytical scale purifications were performed on an Ultra Fast Liquid Chromatograph Shimadzu HPLC. The mobile phase consisted of 0.1% trifluoroacetic acid (TFA) in H$_2$O (solvent A) and 0.1% TFA in acetonitrile (ACN) (solvent B). LC-ESI-MS analyses and analytical scale purifications were carried out on a Thermo Scientific (Pittsburgh, PA) BetaBasic C18 column (150 Å, 0.46 cm $\times$ 15 cm, 5 μm) at a 1 mL/min flow rate with in-house optimized linear gradients and UV detection at 214 and 280 nm. Semi-preparative purifications were performed on a Waters (Milford, MA) Prep Nova-Pak HR C18 column (60 Å, 1.9 cm $\times$ 30 cm, 6 μm) at flow rates up
to 10 mL/min, using in-house optimized linear gradients and UV detection at 225/235 and 280 nm. Inductively-coupled plasma mass spectrometry (ICP-MS) analyses were performed on a VG Axiom SC high-resolution ICP-MS (Thermo Fisher, Waltham, MA).

7.2.2 Synthesis of Lu-DOTA-sst₂-antagonist

The linear DOTA-sst₂-antagonist, DOTA-[4-NO₂-Phe-DCys-Tyr-DTrp-Lys-Thr-Cys-DTyrNH₂], was prepared with an Advanced ChemTech (Louisville, KY) 396 Omega multiple peptide synthesizer following conventional 9-fluorenylmethoxycarbonyl (Fmoc) solid-phase peptide synthesis protocols. Disulfide cyclization was achieved by stirring roughly 20 mg of the linear peptide in 2 mL of 2:1:1 dimethyl sulfoxide (DMSO):ACN:H₂O solution at room temperature for about 3 days. The DMSO was diluted to less than 10% by adding MilliQ water prior to lyophilization. The recovered solid and 3 molar equivalents of LuCl₃·6H₂O were dissolved in a 0.2 M ammonium acetate solution (pH 5.0) and heated for 30 minutes at 90 °C to label the peptide with [Lu]³⁺ via the DOTA chelate. The reaction solution was lyophilized, the crude product was purified using semi-preparative RP-HPLC, and the identity of the isolated product was confirmed by LC-ESI-MS.
7.2.3 Synthesis of Re(V)-cyclized DOTA-sst$_2$-antagonist (Re-DOTA-sst$_2$-ANT)

Conventional Fmoc solid-phase peptide synthesis protocols were followed and orthogonally-protected Cys residues (Fmoc-Cys(Mmt)-OH and Fmoc-DCys(Mmt)-OH) were incorporated during the synthesis of the linear peptide. The Cys residues were then selectively deprotected through repeat treatments with 1% TFA and 5% TIS in CH$_2$Cl$_2$. Complexation with Re(V) was achieved by reacting the on-resin peptide precursor with excess ReOCl$_3$(PPh$_3$)$_2$ in N-methyl-2-pyrrolidone (NMP) overnight. The Re(V)-cyclized peptide was then fully deprotected and cleaved from the resin using a non-standard cocktail (free of thiol scavengers). Semi-preparative and analytical scale RP-HPLC purifications were performed to isolate the Re-DOTA-sst$_2$-ANT product. The purity and identity of the product was confirmed by LC-ESI-MS, lyophilized, and stored frozen for use in subsequent studies.

7.2.4 ICP-MS analysis

Purified and lyophilized peptides and metal-peptide complexes were dissolved in ultrapure water at a target concentration of 1 mg/mL, and ICP-MS analysis was employed to determine actual solution concentrations. Briefly, duplicate aliquots (10-12 µL) were transferred to pre-cleaned/pre-weighed
tubes, were weighed, and were then digested with 150 µL of concentrated HNO$_3$ for 1 h at 100 °C. After addition of ultrapure water to a ~5 mL volume and reweighing, aliquots of the digestates were further diluted (for metal-containing solutions only) and fortified with Be/Sc/Y/Tl internal standards of known concentration. Internal standards were also added to series of linearity standards prepared from MS-D multi-element (Re and S)/single-element (Lu) stock solutions (High Purity Standards, Charleston, SC) which contained S, Re and Lu at parts per billion levels. Mean results were used, as measured by signals from $^{32}$S for S, $^{175}$Lu for Lu, and both $^{185}$Re and $^{187}$Re for Re. Solvent and digestion blanks were similarly analyzed.

7.2.5 Cell culture

Rat pancreatic tumor cells (AR42J cells), which are known to express SSRT2, were maintained by serial passage in monolayers in a modified RPMI 1640 medium (GIBCO-Invitrogen, Carlsbad, CA) with 4.5 g/L glucose supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 50 µg/mL gentamycin. Cells were incubated in a humidified atmosphere of 5% CO$_2$ in air at 37 °C by the Cell and Immunobiology Core Facility at the University of Missouri-Columbia. Prior to IC$_{50}$ experiments, the cells were harvested by trypsinization with TrypLE Express (GIBCO-Invitrogen, Carlsbad, CA) and the trypsin was quenched with 1 mL of 1 mg/mL soybean trypsin inhibitor
(Sigma-Aldrich, St. Louis, MO) for every 1 mL TrypLE used. Subsequently, the cells were washed and concentrated to 20 million cells per mL using the binding buffer, RPMI 1640 medium with 0.25% bovine serum albumin (GIBCO-Invitrogen).

7.2.6 **IC<sub>50</sub> studies**

The somatostatin receptor binding affinities of Lu-DOTA-sst<sub>2</sub>-ANT and Re-DOTA-sst<sub>2</sub>-ANT were determined in AR42J cells from competitive binding assays with \(^{125}\)I-Tyr\(^{11}\)-somatostatin-14 following modified literature procedures.\(^{16}\) Briefly, triplicate aliquots of cells (2 million cells/aliquot) were incubated with \(^{125}\)I-Tyr\(^{11}\)-somatostatin-14 (~100,000 cpm) and increasing concentrations of Lu-DOTA-sst<sub>2</sub>-ANT (0.01 nM to 1 μM) or Re-DOTA-sst<sub>2</sub>-ANT (0.01 nM to 10 μM) at room temperature for 1 hour. Cell-bound radioactivity was recovered by centrifugation and aspiration of the incubation media. The cells were kept at 4 °C and washed three times with ice-cold binding buffer to remove any residual unbound radioactivity. The cell pellets were counted on a Wallac 1480 Wizard 3” automated gamma counter (PerkinElmer Life Sciences, Gaithersburg, MD) to determine the amount of \(^{125}\)I-Tyr\(^{11}\)-somatostatin-14 bound to the cells. The results were analyzed using GraphPad Prism 6.0\(^{17}\) to determine the concentrations of the compounds resulting half-maximal inhibition of \(^{125}\)I-Tyr\(^{11}\)-somatostatin-14 binding to the
receptors (IC\textsubscript{50} values). The IC\textsubscript{50} values are reported as the means of triplicate measurements.

### 7.3 Results and Discussion

#### 7.3.1 Synthesis and characterization of the metal-peptide complexes

The synthesis of Lu-DOTA-sst\textsubscript{2}-ANT was achieved by coordination of [Lu\textsuperscript{3+}] onto the DOTA chelate of disulfide-cyclized DOTA-sst\textsubscript{2}-ANT. The calculated and observed mass-to-charge ratios for disulfide-cyclized DOTA-sst\textsubscript{2}-ANT and Lu-DOTA-sst\textsubscript{2}-ANT are summarized in Table App.1-1. It should be noted that the disulfide bond formation took approximately three times longer than comparable agonist peptides\textsuperscript{14, 15}, possibly due to the presence of DCys in the antagonist peptide sequence.

Attempts to cyclize DOTA-sst\textsubscript{2}-antagonist with [TBA][ReOCl\textsubscript{4}] using previously described protocols\textsuperscript{14} resulted in poor reaction yields (typically < 3%) with numerous interfering impurities, thus an on-resin Re(V) cyclization protocol was employed. The Re starting material, ReOCl\textsubscript{3}(PPh\textsubscript{3})\textsubscript{2}, was selected because it does not require air-free conditions and is compatible with the peptide synthesizer reaction conditions. Selectively deprotecting the Cys/DCys residues allows the [ReO\textsuperscript{3+}] core to coordinate to the thiolates and preferred backbone amides free of interference from the carboxylates of the DOTA
chelate or the R-groups of the other residues (Figure App.1-1). Also, being on-resin may have reduced the formation of intermolecular disulfide bonds, which are more likely to occur if peptides move freely in solvent. This on-resin cyclization approach significantly increased the overall yield of Re-DOTA-sst2-ANT, though it resulted in the observance of the expected mass-to-charge ratio in multiple peaks within the chromatogram, making it difficult to accurately estimate the percent yield. Isolating the product also proved challenging. Multiple rounds of semi-preparative RP-HPLC purification were performed to isolate the Re-DOTA-sst2-ANT. The metal-peptide complex, when first dissolved and especially at higher concentrations (5-10 mg/mL), appeared to agglomerate, presumably resulting in aggregates with a range of repeating units with progressively more lipophilic nature (as evidenced by increasing retention times) yet the same overall mass-to-charge ratio. It also could be that a large number of apparent kinetic products were formed upon the Re-cyclization of DOTA-sst2-ANT. However, these peaks converted to just two peaks after equilibrating the product recovered from semi-prep RP-HPLC at room temperature in a 20: 80 solvent B:solvent A solution at a relatively low concentration (about 3 mg/mL) for 2 days. LC-MS analysis confirmed both isomers had a mass-to-charge ratio matching the calculated value (Table App.1-1). It is common for complexes having a [ReO]^{3+} core to have two diastereoisomers, syn and anti with respect to
the position of the R-group on the metal-bound residue relative to the Re-oxo bond.\textsuperscript{14,18,19} For Re-DOTA-sst\textsubscript{2}-ANT, the major isomer (about 85\%) eluted at 21.3 minutes, whereas the minor isomer (about 15\%) eluted at 26.3 minutes (Gradient: 15\% solvent B and 85\% solvent A to 30\% solvent B and 70\% solvent A over 30 minutes). Only the major isomer was purified for IC\textsubscript{50} studies. The presence of the chelate DOTA led to improved hydrophilicity of Re-DOTA-sst\textsubscript{2}-ANT (lower percentages of organic solvent for comparable retention time) compared to the Re(V)-cyclized octreotide analogues studied previously.\textsuperscript{14,15}

Table App.1-1. LC-MS characterization of reduced, disulfide-cyclized and metal-complexed peptides.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Calculated m/z (M+H)$^+$</th>
<th>Observed m/z (M+H)$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear DOTA-sst\textsubscript{2}-ANT</td>
<td>1544.7</td>
<td>1544.4</td>
</tr>
<tr>
<td>Disulfide-cyclized DOTA-sst\textsubscript{2}-ANT</td>
<td>1541.6</td>
<td>1541.5</td>
</tr>
<tr>
<td>Lu-DOTA-sst\textsubscript{2}-ANT</td>
<td>1714.7</td>
<td>1714.9</td>
</tr>
<tr>
<td>Re(V)-cyclized DOTA-sst\textsubscript{2}-ANT</td>
<td>1743.6</td>
<td>1743.8</td>
</tr>
</tbody>
</table>
7.3.2 *In vitro* sst2 receptor binding affinity

The IC\textsubscript{50} values of Lu-DOTA-sst\textsubscript{2}-ANT and Re-DOTA-sst\textsubscript{2}-ANT were determined from *in vitro* competitive receptor binding studies in sst\textsubscript{2}-expressing AR42J rat pancreatic tumor cells. Due to the small amounts of purified Lu-DOTA-sst\textsubscript{2}-ANT and Re-DOTA-sst\textsubscript{2}-ANT prepared, their stock solutions were analyzed by ICP-MS for S, Re and/or Lu content, which allowed accurate determination of the peptide solution concentrations. The control blanks analyzed also contained S, Lu and/or Re at or below the limit of detection. The IC\textsubscript{50} value of Lu-DOTA-sst\textsubscript{2}-ANT was determined to be 1.5 nM in live AR42J cells (Figure App.1-2a), which is consistent with the sst\textsubscript{2} affinity for Lu-DOTA-sst\textsubscript{2}-ANT reported by Cescato et al. (1.5 nM) in CCL39 cell membranes, a cell line stably expressing human sst\textsubscript{2}.\textsuperscript{20} The IC\textsubscript{50} value was lower for Re-DOTA-sst\textsubscript{2}-ANT (18 nM, Figure App.1-2b), yet still a reasonable affinity and comparable to the IC\textsubscript{50} value of Octreoscan\textsuperscript{TM} (22 nM) determined
in CCL39 cell membranes.\textsuperscript{21} The difference in sst\textsubscript{2} binding affinity is attributed to the distinct metal complexation approach. Coordinating the metal via a bifunctional chelator appears to result in a smaller change on the peptide conformation than by directly cyclizing the peptide around the metal. As suggested by the previous studies on \textsuperscript{99mTc/Re(V)}-cyclized \(\alpha\)-melanocyte-stimulating hormone (\(\alpha\)-MSH) analogues,\textsuperscript{22, 23} the integrated radiolabeling design may potentially lead to increased tumor uptake and retention. The sst\textsubscript{2} affinity of Re-DOTA-sst\textsubscript{2}-ANT is improved compared to that of Re(V)-cyclized Tyr\textsuperscript{3}-octreoate, the best binder among the Re(V)-cyclized octreotide analogues we previously reported.\textsuperscript{14, 15} Further, radiolabeled somatostatin antagonist analogues may show superior tumor-to-background contrast \textit{in vivo} compared to the corresponding agonist analogues because they are able to bind to a greater number of receptor sites despite some of them possessing lower \textit{in vitro} receptor affinities.\textsuperscript{11}

Figure App.1.\textsuperscript{2} IC\textsubscript{50} for Lu-DOTA-sst\textsubscript{2}-ANT and Re-DOTA-sst\textsubscript{2}-ANT determined \textit{in AR42J} cells from competitive binding assays with \textsuperscript{125I}-Tyr\textsuperscript{11}-somatostatin-14.
7.4 Conclusions

A Re(V)-cyclized somatostatin antagonist peptide analogue, Re-DOTA-sst$_2$-ANT, was synthesized using an on-resin Re(V)-cyclization protocol and characterized by LC-ESI-MS. The sst$_2$ affinity of Re-DOTA-sst$_2$-ANT was determined to be 18 nM from competitive binding assays.

7.5 References


17. Nonlinear regression was performed using GraphPad Prism version 6.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com


Appendix II

\(^1\text{H} \text{ and } ^{13}\text{C} \text{ chemical shifts}\)

Chemical shifts were referenced relative to DSS and were measured at 298 K. The chemical shifts are generally accurate to 0.01 ppm for \(^1\text{H}\) and 0.1 ppm for \(^{13}\text{C}\). \(^{13}\text{C}\) chemical shift values were indicated in the parentheses.

Table App.2-1. \(^1\text{H} \text{ and } ^{13}\text{C} \text{ chemical shifts of linear SDPhe-TATE}\)

<table>
<thead>
<tr>
<th>Residue</th>
<th>NH</th>
<th>(\alpha\text{CH} )</th>
<th>(\beta\text{CH} )</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDPhe(^1)</td>
<td>—</td>
<td>3.94 (38.4)</td>
<td>3.29 (37.0)</td>
<td>(\delta:7.45 (124.6); \epsilon:7.55 (124.2); \zeta:7.51 (122.6))</td>
</tr>
<tr>
<td>Cys(^2)</td>
<td>8.15</td>
<td>4.57 (50.8)</td>
<td>2.70, 2.77 (21.0)</td>
<td></td>
</tr>
<tr>
<td>Tyr(^3)</td>
<td>7.99</td>
<td>4.68 (^a)</td>
<td>2.95, 3.06 (31.8)</td>
<td>(\delta:7.11 (126.0); \epsilon:6.93 (110.9))</td>
</tr>
<tr>
<td>DTrp(^4)</td>
<td>8.16</td>
<td>4.79 (^a)</td>
<td>3.30, 3.41 (22.6)</td>
<td>(\delta1:7.38 (119.8); \epsilon:3.78 (113.8); \zeta2:7.68 (107.3); \zeta3:7.37 (114.7); \eta2:7.44 (117.3))</td>
</tr>
<tr>
<td>Lys(^5)</td>
<td>8.09</td>
<td>4.43 (49.0)</td>
<td>1.67, 1.86 (25.7)</td>
<td>(\gamma:1.15 (17.2); \delta1:1.69 (21.8); \epsilon1:3.04 (34.8); \zeta:7.62)</td>
</tr>
<tr>
<td>Thr(^6)</td>
<td>8.23</td>
<td>4.61 (54.0)</td>
<td>4.55 (62.9)</td>
<td>(\gamma2:1.43 (14.6))</td>
</tr>
<tr>
<td>Cys(^7)</td>
<td>8.32</td>
<td>4.86 (^a)</td>
<td>3.15, 3.17 (21.3)</td>
<td></td>
</tr>
<tr>
<td>Thr(^8)</td>
<td>8.12</td>
<td>4.56 (54.8)</td>
<td>4.44 (62.5)</td>
<td>(\gamma2:1.39 (14.6))</td>
</tr>
</tbody>
</table>

\(^a\)Chemical shifts of \(^{13}\text{C}_\alpha\) could not be assigned due to resonances overlapping with \(\text{H}_2\text{O}\) on the \(^1\text{H}\)-\(^{13}\text{C}\) HSQC spectrum. \(^b\)Two conformations were observed but all chemical shifts in this table correspond to the major conformation of the linear peptide. Less than 10% of the peptide form a minor conformation, which can not be assigned based on current data.
Table App.2-1. $^1$H and $^{13}$C chemical shifts of Re(V)-cyclized SDPhe$^1$-Tyr$^3$-octreotate isomer I.

<table>
<thead>
<tr>
<th>Residue</th>
<th>NH</th>
<th>$\alpha$CH</th>
<th>$\beta$CH</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDPhe$^1$</td>
<td>—</td>
<td>4.64(52.3)</td>
<td>3.15, 3.56 (37.2)</td>
<td>$\delta$:7.37(124.3); $\varepsilon$:7.37(125.4); $\zeta$:7.31(122.6)</td>
</tr>
<tr>
<td>Cys$^2$</td>
<td>5.41 (62.5)</td>
<td>3.35, 3.38 (39.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyr$^3$</td>
<td>7.92</td>
<td>4.31 (52.1)</td>
<td>2.95,3.04 (31.2)</td>
<td>$\delta$:7.19(126.6); $\varepsilon$:6.93(111.5)</td>
</tr>
<tr>
<td>DTrp$^4$</td>
<td>7.71</td>
<td>4.61 (49.8)</td>
<td>3.07, 3.19 (21.5)</td>
<td>$\delta^1$:7.00(120.4); $\varepsilon^1$:10.13; $\varepsilon^3$:7.41(114.2); $\zeta^2$:7.53(107.7); $\zeta^3$:7.17(115.2); $\eta^2$:7.28(117.8)</td>
</tr>
<tr>
<td>Lys$^5$</td>
<td>8.08</td>
<td>4.15 (49.0)</td>
<td>1.61, 1.84 (24.1)</td>
<td>$\delta$:1.54 (21.5); $\varepsilon$:2.84 (34.5); $\zeta$:7.47</td>
</tr>
<tr>
<td>Thr$^6$</td>
<td>7.25</td>
<td>4.27 (55.3)</td>
<td>4.36 (62.3)</td>
<td>$\gamma$:2.12 (14.7)</td>
</tr>
<tr>
<td>Cys$^7$</td>
<td>7.98</td>
<td>5.36</td>
<td>4.19, 4.37 (30.5)</td>
<td></td>
</tr>
<tr>
<td>Thr$^8$</td>
<td>7.42</td>
<td>4.28</td>
<td>4.32 (63.6)</td>
<td>$\gamma$:2.12 (14.7)</td>
</tr>
</tbody>
</table>

$^a$Chemical shifts of $^{13}$C$_\alpha$ could not be assigned due to intermediate timescale chemical exchange.

Table App.2-2. $^1$H and $^{13}$C chemical shifts of Re(V)-cyclized SDPhe$^1$-Tyr$^3$-octreotate isomer 2.

<table>
<thead>
<tr>
<th>Residue</th>
<th>NH</th>
<th>$\alpha$CH</th>
<th>$\beta$CH</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDPhe$^1$</td>
<td>4.49</td>
<td>2.84,3.82(37.6)</td>
<td>$\delta$:7.51(124.2); $\varepsilon$:7.51(125.0); $\zeta$:7.45(122.3)</td>
<td></td>
</tr>
<tr>
<td>Cys$^2$</td>
<td>7.77</td>
<td>5.24(66.6)</td>
<td>3.64, 3.90(40.4)</td>
<td></td>
</tr>
<tr>
<td>Tyr$^3$</td>
<td>4.50(51.0)</td>
<td>2.93,3.05(31.8)</td>
<td>$\delta$:7.15(126.3); $\varepsilon$:6.94(111.1)</td>
<td></td>
</tr>
<tr>
<td>DTrp$^4$</td>
<td>7.74</td>
<td>4.63</td>
<td>3.14,3.26(21.3)</td>
<td>$\delta^1$:7.12(120.1); $\varepsilon^1$:10.18; $\varepsilon^3$:7.54(114.0); $\zeta^2$:7.57(107.5); $\zeta^3$:7.24(114.9); $\eta^2$:7.34(117.5)</td>
</tr>
<tr>
<td>Lys$^5$</td>
<td>8.11</td>
<td>4.27</td>
<td>1.88,1.92</td>
<td>$\delta$:1.62(21.6); $\varepsilon$:2.93(34.5); $\zeta$:7.71</td>
</tr>
<tr>
<td>Thr$^6$</td>
<td>7.36</td>
<td>4.34(55.4)</td>
<td>4.27(63.6)</td>
<td>$\gamma$:2.13 (14.3)</td>
</tr>
<tr>
<td>Cys$^7$</td>
<td>8.02</td>
<td>5.39</td>
<td>4.25,4.42</td>
<td></td>
</tr>
<tr>
<td>Thr$^8$</td>
<td>7.59</td>
<td>4.31</td>
<td>4.37(62.3)</td>
<td>$\gamma$:2.13 (14.8)</td>
</tr>
</tbody>
</table>

$^a$Chemical shifts of $^{13}$C$_\alpha$ could not be assigned due to resonances overlapping with H$_2$O on the $^1$H-$^{13}$C HSQC spectrum. $^b$Chemical shifts of $^{13}$C$_\alpha$ could not be assigned due to intermediate timescale chemical exchange.
Table App.2-4. $^1$H and $^{13}$C chemical shifts of Re(V)-cyclized SDPhe$^1$-Tyr$^3$-octreotate isomer 4.

<table>
<thead>
<tr>
<th>Residue</th>
<th>NH</th>
<th>$\alpha$CH</th>
<th>$\beta$CH</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDPhe$^1$</td>
<td>4.56$^a$</td>
<td>3.04, 3.64(37.4)</td>
<td></td>
<td>$\delta$:7.47(124.1); $\epsilon$:7.41(125.3); $\zeta$:7.42(122.4)</td>
</tr>
<tr>
<td>Cys$^2$</td>
<td>5.46$^b$</td>
<td>3.38, 3.55$^b$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyr$^3$</td>
<td>7.17</td>
<td>4.45(51.9)</td>
<td>3.04, 3.16(30.9)</td>
<td>$\delta$:7.24(126.3); $\epsilon$:7.01(111.3)</td>
</tr>
<tr>
<td>DTyr$^4$</td>
<td>7.86</td>
<td>4.62$^e$</td>
<td>3.14, 3.30(21.3)</td>
<td>$\delta$:1.70(21.2); $\epsilon$:1.19; $\zeta$:7.54(114.1); $\zeta$:7.66(107.5); $\zeta$:7.28(115.0); $\eta$:7.37(117.5)</td>
</tr>
<tr>
<td>Lys$^5$</td>
<td>8.05</td>
<td>4.18$^b$</td>
<td>1.70$^b$</td>
<td>$\gamma$:1.04(17.3); $\delta$:1.59(21.7); $\epsilon$:2.93(34.7); $\zeta$:7.53</td>
</tr>
<tr>
<td>Thr$^6$</td>
<td>7.23</td>
<td>4.26(55.9)</td>
<td>4.43(62.2)</td>
<td>$\gamma$:2.13$^b$</td>
</tr>
<tr>
<td>Cys$^7$</td>
<td>7.95</td>
<td>5.37$^b$</td>
<td>4.34(29.6)</td>
<td></td>
</tr>
<tr>
<td>Thr$^8$</td>
<td>7.52</td>
<td>4.46(54.5)</td>
<td>4.43(63.2)</td>
<td>$\gamma$:2.13$^b$</td>
</tr>
</tbody>
</table>

$^a$Chemical shifts of $^{13}$C$_{\alpha}$ could not be assigned due to resonances overlapping with H$_2$O on the $^1$H-$^{13}$C HSQC spectrum. $^b$Chemical shifts of $^{13}$C$_{\alpha}$ could not be assigned due to intermediate timescale chemical exchange.
Figure App.2-1. Pictures of ReO[SDPhe-Cys-Tyr][Cys] isomers 1-8. Geometries optimized at the PBE0/6-31G(d):LANL2DZ level of theory.
Table App.2-5. Calculated NMR chemical shifts of the N2- and N3-deprotonated isomers of ReO[SDPhe-Cys-Tyr][Cys].

<table>
<thead>
<tr>
<th></th>
<th>N2-2</th>
<th>N2-1</th>
<th>N3-1</th>
<th>N3-2</th>
<th>N3-3</th>
<th>N3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2HA</td>
<td>3.68</td>
<td>3.80</td>
<td>1.85</td>
<td>3.02</td>
<td>2.91</td>
<td>2.19</td>
</tr>
<tr>
<td>C2HB2</td>
<td>1.66</td>
<td>2.43</td>
<td>0.53</td>
<td>1.72</td>
<td>1.30</td>
<td>1.60</td>
</tr>
<tr>
<td>C2HB3</td>
<td>2.08</td>
<td>2.18</td>
<td>1.23</td>
<td>2.05</td>
<td>1.71</td>
<td>1.22</td>
</tr>
<tr>
<td>Y3HA</td>
<td>3.10</td>
<td>1.83</td>
<td>3.28</td>
<td>1.52</td>
<td>4.18</td>
<td>2.47</td>
</tr>
<tr>
<td>Y3HB2</td>
<td>2.11</td>
<td>1.81</td>
<td>2.99</td>
<td>2.06</td>
<td>2.31</td>
<td>2.85</td>
</tr>
<tr>
<td>Y3HB3</td>
<td>0.71</td>
<td>2.25</td>
<td>1.59</td>
<td>0.40</td>
<td>2.13</td>
<td>1.28</td>
</tr>
<tr>
<td>F1HA</td>
<td>2.36</td>
<td>3.03</td>
<td>4.91</td>
<td>3.47</td>
<td>2.67</td>
<td>1.79</td>
</tr>
<tr>
<td>F1HB2</td>
<td>1.85</td>
<td>1.24</td>
<td>2.03</td>
<td>2.59</td>
<td>2.14</td>
<td>2.24</td>
</tr>
<tr>
<td>F1HB3</td>
<td>2.40</td>
<td>1.81</td>
<td>2.10</td>
<td>1.86</td>
<td>2.15</td>
<td>1.68</td>
</tr>
<tr>
<td>C7HA</td>
<td>3.17</td>
<td>3.14</td>
<td>3.39</td>
<td>3.27</td>
<td>2.92</td>
<td>3.27</td>
</tr>
<tr>
<td>C7HB2</td>
<td>2.47</td>
<td>2.28</td>
<td>2.50</td>
<td>2.71</td>
<td>2.80</td>
<td>2.60</td>
</tr>
<tr>
<td>C7HB3</td>
<td>2.68</td>
<td>2.48</td>
<td>3.11</td>
<td>2.83</td>
<td>2.52</td>
<td>3.44</td>
</tr>
</tbody>
</table>

*Chemical shifts were calculated referencing to the protons of tetramethylsilane (TMS).
Table App.2-6. Effect of changing the position of the R-group and the capped terminal group on the calculated chemical shift.\(^a\)

<table>
<thead>
<tr>
<th>Calculational level: CSGT-PCM-PBE0/6-311+G(d):LANL2TZ/PBE0/6-31G(d):LANL2DZ</th>
<th>Solvent: H(_2)O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical shift (ppm)(^b)</td>
<td>(\Delta H = 4.8) kcal/mol</td>
</tr>
<tr>
<td>C2HA</td>
<td>3.24</td>
</tr>
<tr>
<td>C2HB2</td>
<td>2.35</td>
</tr>
<tr>
<td>C2HB3</td>
<td>1.71</td>
</tr>
<tr>
<td>Averaged C2HB</td>
<td>2.03</td>
</tr>
<tr>
<td>Y3HA</td>
<td>2.32</td>
</tr>
<tr>
<td>Y3HB2</td>
<td>1.90</td>
</tr>
<tr>
<td>Y3HB3</td>
<td>2.49</td>
</tr>
<tr>
<td>Averaged Y3HB</td>
<td>2.20</td>
</tr>
<tr>
<td>SDF1HA</td>
<td>2.86</td>
</tr>
<tr>
<td>SDF1HB2</td>
<td>1.26</td>
</tr>
<tr>
<td>SDF1HB3</td>
<td>2.43</td>
</tr>
<tr>
<td>Averaged SDF1HB</td>
<td>1.84</td>
</tr>
<tr>
<td>C7HA</td>
<td>3.27</td>
</tr>
<tr>
<td>C7HB2</td>
<td>2.75</td>
</tr>
<tr>
<td>C7HB3</td>
<td>2.11</td>
</tr>
<tr>
<td>Averaged C7HB</td>
<td>2.43</td>
</tr>
</tbody>
</table>

\(^a\)Data shown in this table are associated with N\(_2\)-1 and one local minimum that has the same ligand arrangement as N\(_2\)-1 but different R-group/capped terminal group orientations.  
\(^b\)Chemical shifts were calculated referencing to the protons of tetramethylsilane (TMS).

Figure App.2-2. Selected occupied MOs of the ReO(SH)\(_3\)(NH\(_2\)) lowest-energy structure.
VITA

Yawen Li, the daughter of Mr. Dongsheng Li and Mrs. Ping Jiang, was born on June 14th, 1985 in Dalian, China. She received her B. E. in Biomedical Engineering from Beihang University in Beijing in 2008. In 2009, she enrolled in the Chemistry Graduate Program at University of Missouri and joined Dr. Jurisson’s group. She is married to Mr. Shengkui Gao in St. Louis, MO, in 2014. She will earn her PhD degree in Chemistry in May, 2015 from University of Missouri, and will begin her postdoctoral research at University of Washington in Seattle in Dr. D. Scott Wilbur’s research group.