ARTIFICIAL METAL-CHELATING OLIGOPEPTIDES FOR DUPLEX ASSEMBLY AS INORGANIC ANALOGUES OF DNA

A Thesis in
Chemistry

by
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ABSTRACT

Artificial oligopeptides with pyridyl-derived ligands have been synthesized that are analogous to peptide nucleic acid. Pyridine (py)- and 2,2′-bipyridine (bpy)-functionalized oligopeptides were prepared by traditional solid-phase methods. Spectrophotometric titrations with Cu$^{2+}$ and Fe$^{2+}$ show that the oligomers bind stoichiometric quantities of transition metals based on the number of pendant ligands. In the case of a bpy tripeptide, the titration stoichiometry and mass spectra indicate that the metal ions form interstrand crosslinks between two oligopeptides, creating duplex structures linked exclusively by metal ions. To increase product quantity, solution-phase protocols, similar to those used in dendrimer synthesis, were investigated. Using this synthetic methodology, two palindromic, heterofunctional artificial tripeptides were prepared. In the case of a py-bpy-py tripeptide, supramolecular, multi-metallic structures are created by chelation of metal ions with the central bpy moiety and coordination of inorganic complexes with the pendant py ligands. The spectroscopic and electrochemical signatures of these assemblies confirm the expected number of inorganic species in these assemblies. The preparation of an artificial tripeptide with 2,2′:6′,2″-terpyridine (tpy) and bpy (i.e., tpy-bpy-tpy) was prepared analogously to py-bpy-py, and these two tripeptides form a duplex in the presence of Cu$^{2+}$ ions, creating a complementary oligopeptide pair.
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LIST OF ABBREVIATIONS

ACN  acetonitrile
aeg  N-(2-aminoethyl)glycine
BOC  tert-butyloxycarbonyl
bpy  2,2'-bipyridine
Bz   benzoyl
CD   circular dichroism
CW   continuous-wave
DBU  1,8-diazabicyclo[5.4.0]undec-7-ene
DCC  tert-butyl N-(2-Boc-aminoethyl)-glycinate, N,N'-dicyclohexylcarbodiimide
DCM  dichloromethane
DETA diethylenetriamine
DhbtOH 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine
DIPCDI N,N'-diisopropylcarbodiimide
DIPEA N,N-diisopropylethylamine
DMF  N,N-dimethylformamide
DNA  deoxyribonucleic acid
EDC  1-[(3-dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride
EPR  electron paramagnetic resonance
ESI- MS negative ion electrospray ionization mass spectrometry
ESI+ MS positive ion electrospray ionization mass spectrometry
Et₂O  ethyl ether
EtOH  ethanol
Fmoc  9-fluorenylmethoxycarbonyl
G  glycine
HATU  \(\sigma-(7\text{-azabenzotriazol-1-yl})-N,N',N'',N'''\text{-tetramethyluronium hexafluorophosphate}\)
HBTU  \(2-(1H\text{-benzotriazole-1-yl})-1,1,3,3\text{-tetramethyluronium hexafluorophosphate}\)
HOBt  1-hydroxybenzotriazole
hp  hydroxypyridone
HPLC  high performance liquid chromatography
hq  hydroxyquinoline
iDNA  inorganic DNA
LDA  lithium diisopropylamide
LMCT  ligand-to-metal charge transfer
Lys  lysine
MALDI  matrix-assisted laser desorption ionization
Me  methyl
MeOH  methanol
MLCT  metal-to-ligand charge transfer
MM+  molecular mechanics
MS  mass spectrometry
NMR  nuclear magnetic resonance
OSu  succinimide
OtBu  tert-butyloxy
PAL  polyamide linker
pda  pyridine-2,6-dicarboxylate
PEG  polyethylene glycol
PNA  peptide nucleic acid/polyamide nucleic acid
PS  polystyrene
py  pyridine
PyBOP  (benzotriazol-1-yloxy)tritylrildinophosphonium hexafluorophosphate
spy  2,6-bis(ethylthiomethyl)pyridine
TBAF  tetrabutylammonium fluoride hydrate
TBAH  tetrabutylammonium hexafluorophosphate
TFA  trifluoroacetic acid
THF  tetrahydrofuran
TIS  triisopropylsilane
TLC  thin layer chromatography
TOF  time-of-flight
TOTU  $O\text{-}[\text{(ethoxycarbonyl)cyano}\text{methylenamino}]\text{-}N,N,N',N'$-tetramethyluronium tetrafluoroborate
tpy  2,2′:6′,2″-terpyridine
UV  ultraviolet
Vis  visible
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Chapter 1

Inorganic Functionalization of DNA and PNA Duplexes as Inspiration for iDNA

1.1 Introduction

The relationship between structure and function in biological systems gives rise to enormously complex chemical processes. The double-helix of deoxyribonucleic acid (DNA) is one of the most well characterized and best understood biological structures. Molecular recognition between DNA strands is achieved by specific hydrogen-bonding of nucleobase pairs: molecular geometry makes adenine (A) and thymine (T) or guanine (G) and cytosine (C) complementary, as shown in Figure 1-1. The overall stability of double-stranded (ds) DNA is the additive effect of many weak hydrogen bonds acting cooperatively. It is the specific sequence of A-T and G-C base pairs that is the code translated by a complex biomachinery for the production of proteins. This complex interplay between structure and function provides inspiration for the development of artificial, synthetic analogues. Such systems could have vast implications ranging from molecular information storage, bottom-up assembly of nanoscale materials, or novel pharmacological agents.

1.2 Peptide Nucleic Acids: Synthetic Analogues of DNA

The development of a pseudopeptide backbone functionalized with nucleobases was first reported in the early 1990s, and was originally designed to bind specifically to dsDNA. As a structural analogue of DNA, double- and triple-stranded helices can form
Figure 1-1. (Left) Double-stranded DNA helix with (Right) complementary nucleic acid base pairs (adenine (A) and thymine (T), and guanine (G) and cytosine (C)).
using hydrogen bonding between complementary nucleic acids.\textsuperscript{1} Nielsen, Buchardt, Egholm, and Berg named this family of molecules peptide nucleic acid or polyamide nucleic acid (PNA).\textsuperscript{2} Figure 1-2 compares the structures of the PNA and DNA backbones: the repeating \textit{N}-(2-aminoethyl)glycine (aeg) of PNA is isostructural with the sugar-phosphate subunit of DNA. Similar to peptides and proteins, the PNA monomers are tethered together by amide bonds, which allows for the incorporation of amino acids and nucleobases. In PNA, the aeg backbone is functionalized at the secondary amines with acetyl derivatives of the four natural DNA nucleobases.

Unlike sugar-phosphates, the aeg backbone is charge-neutral and resistant to enzymatic degradation. However, similar to DNA, oligopeptides can be synthesized by automated methods, making PNA preparation relatively straightforward. Thus, the ease of structural variability by automated methods and the stability of the oligopeptide scaffold have made PNA an attractive synthetic analogue of DNA for potential biomolecular and medical applications (\textit{e.g.}, nucleic acid sensors, genome mapping, antisense and antigene drug therapy).\textsuperscript{3}

Both dsPNA and dsDNA have helical structures, however these differ in pitch and width. For example, the dsPNA (PNA-PNA) duplex has a pitch of 18 base pairs and a width of 28 Å;\textsuperscript{4} the B-form of dsDNA (\textbf{Figure 1-1}) has a pitch of 10 base pairs and is 34 Å wide.\textsuperscript{5} The structural similarity of PNA also enables hydrogen bonding with complementary \textit{DNA} and \textit{RNA} to form the stable double- and triple-stranded structures. Double-stranded helices containing PNA (PNA-PNA, PNA-DNA, PNA-RNA, etc.) generally have melting temperatures that range from 45.5 to 72.3 °C.\textsuperscript{6-8} In the case of some PNA-DNA helices, an increase in melting temperature is observed when compared
Figure 1-2. Structure of DNA (3) and PNA (4) backbones.
to the respective dsDNA, suggesting that the helices formed with PNA are more stable. This stabilization has been attributed to an absence of electrostatic repulsions between the neutral aeg scaffold and the negatively-charged sugar-phosphate backbone.²

Inorganic complexes have been incorporated into both DNA and PNA to serve as probes of nucleic acid structure and as chemically reactive species. For example, metal complexes can provide spectroscopic and electrochemical signatures that enable the identification of nucleic acid sequences.⁹,¹⁰ Metal complexes are alternatively used for catalytic bond cleavage¹¹ and therapeutic drug design.¹² It should be emphasized that, in general, these complexes tether to individual DNA and PNA strands, but do not serve as interstrand crosslinks in dsDNA or dsPNA.

1.3 Incorporation of Metal Complex Crosslinks in Double-stranded DNA

In addition to hydrogen-bonded nucleic acids, it is feasible to use inorganic complexation as crosslinks between single strands of DNA. Schultz et al. were the first group to report the substitution of a metal complex in place of complementary nucleobases in a modified dsDNA.¹³ The inorganic complex selected for this investigation is shown in Figure 1-3. The pyridine 2,6-dicarboxylate (pda) ligand was incorporated into one single-stranded DNA chain, whereas pyridine (py) was appended to the complementary strand. In the presence of a tetracoordinate metal ion (e.g., M = Cu²⁺ or Zn²⁺), the ligands are expected to form a square planar complex ([M(pda)(py)]). It was shown that stable dsDNA helices formed even when a nucleic acid base pair was replaced with an artificial “metallobase”. In the case of a complementary 15-base pair duplex, substitution of dA:dT with [Cu(pda)(py)] in the middle of the sequence slightly
Figure 1-3. Structural representation of a DNA subunit in which the nucleobase pair is replaced with pyridine 2,6-dicarboxylate (pda) and pyridine (py) coordinated by a tetracoordinate metal ion.
decreased the melting temperature from 41.2 to 38.6 °C \((i.e., \Delta T_m = -2.6 \, ^\circ C)\). Since the metal-ligand bond strength is greater than a hydrogen bond, this observation is somewhat surprising. This result therefore implies that the substitution causes other structural effects that destabilize the helix.

To gain insight into the structure of the metal-substituted dsDNA helices, a self-complementary DNA sequence that contained pda and py moieties was prepared, 5′ CGC-GpdaA-TpyC-GCG 3′.\(^{14}\) After complexation with Cu\(^{2+}\), the x-ray crystal structure of the resultant dsDNA duplex revealed that the strands assembled in an anti-parallel, Z-form orientation and contained two \([\text{Cu(pda)(py)}]\) crosslinks. Analysis of the crystal structure showed that the two \([\text{Cu(pda)(py)}]\) species are Jahn-Teller distorted octahedral complexes and coordinate adjacent nucleobases in the axial positions. The lower melting point of the metallo-base-containing dsDNA is therefore attributed to a disruption in \(\pi\)-stacking of base pairs.

Schultz and co-workers further investigated the thermal stability of metal crosslinks in DNA by incorporating 2,6-bis(ethylthiomethyl)pyridine (spy) ligands within two complementary sequences, 5′ CAC-ATT-AspyT-GTT-GTA 3′ and 5′ TAC-AAC-AspyT-AAT-GTG 3′.\(^{15}\) Figure 1-4 compares the structures of pda (6) and spy (7); in the latter, the coordinating oxygen atoms in pda are replaced with thiomethyl groups. Based on hard-soft acid-base principles, the inherent affinity of the softer sulfur atoms towards late transition metals was expected to improve the thermal stability of the modified DNA duplex.\(^{16}\) To test this hypothesis, melting temperatures of the duplex in the presence of several metal ions (Ni\(^{2+}\), Pd\(^{2+}\), Pt\(^{2+}\), Cu\(^{2+}\), Ag\(^{+}\), Au\(^{3+}\)) were determined using UV-Vis spectroscopy. Of these, only Ag\(^{+}\) formed a metallobase pair within the DNA duplex, and
Figure 1-4. Structural comparison of pda and spy ligands.
gave rise to a substantially larger duplex melting temperature, $\Delta T_m = +19.1 \, ^\circ\text{C}$. Furthermore, the Ag$^+$-coordinated duplex had greater thermal stability ($T_m = 42.5 \, ^\circ\text{C}$) than duplexes with natural, complementary A-T and C-G nucleobase pairs ($T_m = 39.4$ and $41.1 \, ^\circ\text{C}$, respectively). These results indicate that the nature of both metal and ligand must be considered when designing metallobases, and with the appropriate choice, inorganic complexes can enhance the thermal stability of artificial DNA duplexes.

The incorporation of a 2,2$'$-bipyridine (bpy) ligand into a modified DNA sequence (5$'$ AGT-CG$\text{bpy}$-CGA-CT 3$'$) was concurrently reported by Tor and co-workers. Addition of tetracoordinate metals induces formation of [M(bpy)$_2$]$^{2+}$ metallobases. To test metal-induced crosslinking, the dimer (8) in Figure 1-5 was prepared to examine the binding of tetracoordinate metals to the bpy ligands. A series of spectrophotometric titrations was performed with the bpy dimer and three different metal ions: Cu$^+$, Pd$^{2+}$, and Ag$^+$. Isosbestic points were observed for each of the titrations in the region from 200 - 350 nm, which were attributed to the depletion of the uncoordinated dimer and the formation of new species. In the case of Cu$^+$, a weak band at 440 nm was attributed to the metal-to-ligand charge transfer (MLCT) band characteristic of [Cu(bpy)$_2$]$^+$ complexes. Monitoring the increase in absorption at a single wavelength enabled the construction of titration curves; analysis of these provided the stoichiometry of metal-to-dimer, which varied depending on metal.

The titrations were unable to conclusively determine how the metals bind to the strands (i.e., as inter- or intra-crosslinks). Electrospray ionization mass spectrometry (ESI MS) was therefore used to confirm the identities of the titration products. Whereas Cu$^+$ titrations produced only the monometallic species (Cu(8)), the Pd$^{2+}$ and Ag$^+$ ions
Figure 1-5. Bpy-functionalized metallobase dimer.
crosslinked the metallobase dimers to form dinuclear duplexes (Pd$_2$(8)$_2$ and Ag$_2$(8)$_2$). To better control the self-assembly of these structures into the desired metal-linked duplexes, a bpy metallobase was incorporated into a self-complementary DNA sequence, 5′ AGT-CG_bpy-CGA-CT 3′. Hydrogen-bonding between complementary nucleic acids enforced an anti-parallel orientation, thus restricting the structure to only one isomer while simultaneously aligning the bpy ligands with respect to one another. The single-strand DNA sequence was allowed to hybridize into its dsDNA structure and Cu$^{2+}$ was subsequently added. Comparison of the absorbance spectra before and after Cu$^{2+}$ addition indicated the formation of a [Cu(bpy)$_2$]$^{2+}$ complex. No differences in the absorbance spectra were observed when Cu$^{2+}$ ions were added to a control oligonucleotide that did not contain the bpy metallobase (5′ AGT-CGC-GAC-T 3′). Based on the sequence used, the [Cu(bpy)$_2$]$^{2+}$ complex would be expected to form a crosslink between the two DNA strands analogous to Schultz’s compounds.$^{13,14}$

To examine the effect of substitution and metal complexation on the stability of the duplex, the melting temperature of the unmetalated bpy-functionalized dsDNA was compared to dsDNA. In the absence of Cu$^{2+}$, both of these had the same melting temperature ($T_m = 56.5$ °C). The shallower slope of the melting curve for the bpy-modified DNA was attributed to unassociated bpy ligands.$^{17}$ Upon addition of one equivalent of Cu$^{2+}$, the melting temperature ($T_m = 64$ °C) and slope increase, indicating the formation of a more stable duplex. This enhanced stability was credited to formation of [Cu(bpy)$_2$]$^{2+}$ crosslinks within the core of the duplex, in dramatic contrast to the results reported by Schultz.$^{13}$ This difference is most likely a result of the greater binding affinity of Cu$^{2+}$ ions towards the bidentate bpy ligands vs monodentate pyridine, although
other factors, such as distortions to the helix and nucleic acid binding, most certainly play a role.

The thermal stability of a modified DNA duplex with a bis(py) metallobase crosslink was examined by Shionoya and co-workers; Figure 1-6 shows the structure of the bis(py) metallobase (9). Two complementary sequences containing py ligands, 5′ TTTTTTTTTTpyTTTTTTTTT 3′ and 5′ AAAAAApyAAAAAA 3′, were allowed to hybridize, and Ag⁺ was added to the resulting duplex. UV-Vis thermal denaturation was used to determine the effect of Ag⁺ ions on the stability of the duplex, and $T_m$ of the metalated duplex was higher than the duplex without bound Ag⁺ ions ($\Delta T_m = +6.8$ °C). However, both of these duplexes had lower melting temperatures than the control duplex containing a dT:dA nucleobase pair ($T_m = 46.5$ °C). This observation was similar to the results from Schultz, in which addition of metal ions to the ligand-functionalized DNA duplex increased its thermal stability by creating a metallobase crosslink, but the increase in stability was not as great as natural, complementary nucleobase pairs.

Taken together, of the results from Shionoya, Schultz, and Tor demonstrate that inorganic complexes can be employed as artificial base pairs in dsDNA. Conversely, dsDNA can be used to arrange inorganic complexes in specific locations with respect to one another. With this in mind, Shionoya et al. prepared artificial metallobase-containing DNA helices with one to five hydroxypyridone (hp) ligands. Two nucleic acids were incorporated into each of these sequences to enforce anti-parallel alignment. Addition of Cu²⁺ metal ions was monitored by UV-Vis absorption spectroscopy, and the increased absorption at 307 nm indicated the formation of [Cu(hp)₂] complexes. The calculated
Figure 1-6. Bis(py) metallobase.
structure of ds(5’ G(hp)₃C 3’) crosslinked by five Cu²⁺ ions predicts the assembly of a double-stranded helix in a right-handed orientation that was experimentally confirmed by circular dichroism (CD) spectroscopy.

The modeling suggests that the duplex DNA scaffold places the [Cu(hp)₂] complexes in close enough proximity to allow for electronic interaction. Each of the Cu²⁺ centers has an unpaired electron, so that their electronic coupling can be easily monitored using continuous-wave electron paramagnetic resonance (CW-EPR) spectroscopy. Thus, frozen aqueous solutions of each of the double-stranded structures were measured using CW-EPR at 1.5 K. The CW-EPR spectra for each of the duplexes dramatically varied based on the number of Cu²⁺ crosslinks. These data were understood on the basis of the spin state, S, of the respective helices. For example, Cu₂ (i.e., two, S = 1/2, Cu²⁺ ions) has a spin S = 1, Cu₃ has S = 3/2, etc.. In the case of Cu₂, the Cu²⁺–Cu²⁺ distance was estimated to be 3.7 ± 0.1 Å based on the spectral splitting. Pulsed EPR experiments indicated that the spins were aligned parallel and coupled ferromagnetically to their neighbors, confirming that the distance between Cu²⁺ was sufficiently small to allow for strong electronic coupling.

1.4 Metal-crosslinked PNA

As a structural analogue of DNA, incorporation of inorganic complexes into PNA has similar effects on structure and function of duplexes. The synthetic versatility of PNA enables the facile preparation of metalated structures of widely varying designs. As mentioned above, many different types of metal complexes have been incorporated onto the aeg backbone, adding chemical functionality that PNA would not normally possess.
These inorganic complexes include $[\text{Ru(bpy)}_3]^{2+}$ and ferrocene as spectroscopic and electrochemical sensors, $^9$ Zn$^{2+}$ chelators for probing DNA sequences, $^10$ and Zr$^{4+}$ complexes for sequence-selective DNA hydrolysis. $^11$ Although metal complexes have been incorporated onto single PNA strands, these were not used to form interstrand crosslinks analogous to Tor’s and Shionoya’s DNA structures. Achim and co-workers tested the effect of a metal complex crosslink (i.e., a metallolbase) on the stability of PNA duplexes. $^{23}$ Figure 1-7 shows the synthesis of an artificial bpy-PNA monomer (13); the bpy ligand is attached to the aeg backbone via an acetic acid at the 5 position and the primary amine is protected with a tert-butyloxycarbonyl (Boc). Analogous to Tor, $^{17}$ bpy ligands were inserted into two complementary PNA sequences (H-GTAG$bpy$TCACT-LysNH$_2$ and H$_2$N-Lys-CAT$bpy$AGTG-AH). Circular dichroism showed the modified PNA strands form a stable hydrogen-bonded duplex. Upon addition of Ni$^{2+}$, the CD spectrum showed spectral changes at 300 and ~320 nm that were attributed to formation of $[\text{Ni(bpy)}_2]^{2+}$ within the PNA duplex.

To additionally confirm the Ni$^{2+}$ ions only coordinated to the bpy ligands (and not the aeg backbone), a spectrophotometric titration of the bpy-modified duplex was performed. The absorbance between 305 and 320 nm was observed to increase upon addition of Ni$^{2+}$. The titration curve generated by monitoring the increase in absorbance at 320 nm indicated a 1:1 stoichiometry of Ni$^{2+}$ ions to the artificial PNA duplex. No further increase was observed past the stoichiometric point. Taken together, the CD and UV-Vis absorption data suggested that one $[\text{Ni(bpy)}_2]^{2+}$ complex formed, creating a single interstrand crosslink residing in the core of the duplex.
Figure 1-7. Synthesis of artificial bpy PNA monomer: (i) O-[(ethoxycarbonyl)cyanomethylenamino]-N,N,N',N'-tetramethyluronium tetrafluoroborate (TOTU), DİPEA, DMF, 4 h, 44%; (ii) NaOH, EtOH, H₂O, 4 h, 60%.
The thermal stabilities of the modified PNA duplexes in the presence and absence of Ni$^{2+}$ was compared to several hydrogen-bonded PNA sequences. Table 1-1 contains the melting temperatures for this series of PNA duplexes. Comparison of these reveals that formation of the [Ni(bpy)$_2$]$^{2+}$ results in greater thermal stability than duplexes containing mismatched nucleic acid base pairs. For complementary bpy-modified PNA strands, Ni$^{2+}$ complexation substantially increases the melting point ($\Delta T_m = +8 \ ^\circ$C). However, replacement of a complementary hydrogen-bonded A-T base pair with a [Ni(bpy)$_2$]$^{2+}$ crosslink decreases the duplex stability ($\Delta T_m = -7.5 \ ^\circ$C). This decrease was attributed to steric hinderance of the 6 and 6′ bpy protons, which prevent the ligands from π-stacking,$^{24}$ although this explanation contrasts Tor’s observations using DNA duplexes.

In a subsequent report, an analogous artificial PNA monomer containing 8-hydroxyquinoline (hq) was prepared as shown in Figure 1-8.$^{24}$ The hq monomers (18) were incorporated into PNA duplexes (Figure 1-9; 20-25); for comparison, PNA sequences without hq (19, 26) and with mismatches (21-25) were also synthesized. Hydrogen-bonding between complementary nucleic acids enforced anti-parallel alignment and positioned the hq ligands across from each other. In the presence of Cu$^{2+}$ ions, square planar [Cu(hq)$_2$] complexes were expected to form interstrand crosslinks within the modified PNA duplex. Consistent with the previous study,$^{23}$ a dramatic increase in the melting point was observed when the completely complementary hq-modified PNA duplex (20) was presented with Cu$^{2+}$ ions ($\Delta T_m > 23 \ ^\circ$C). However, it is particularly interesting to note that the [Cu(hq)$_2$] crosslink imparts greater stability than a hydrogen-bonded A-T base pair ($\Delta T_m > +12 \ ^\circ$C), in contrast to the above bpy-modified PNA duplex. In both of these studies, 10-base pair PNA sequences were used, yet the
Table 1-1. Melting Temperatures (in °C) for Modified PNA Duplexes Monitored by the UV Absorbance at 260 nm

\[
\begin{align*}
H-\text{GTAGM} & \text{TC ACT-LysNH}_2 \\
H_2\text{N-Lys-CAT CNA GTGA-H} & \quad (14)
\end{align*}
\]

<table>
<thead>
<tr>
<th></th>
<th>M = A</th>
<th>M = bpy</th>
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<tbody>
<tr>
<td>N = T</td>
<td>66.5</td>
<td>47.3</td>
</tr>
<tr>
<td>N = T, + Ni(^{2+})</td>
<td>66.5</td>
<td>46.0</td>
</tr>
<tr>
<td>N = bpy</td>
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</tr>
<tr>
<td>N = bpy, + Ni(^{2+})</td>
<td>46.5</td>
<td>59</td>
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</table>
Figure 1-8. Synthesis of 8-hydroxyquinoline PNA monomer. (a) HCl, formaldehyde; (b) NaCN, DMSO, 90 °C; (c) HCl; (d) tert-butyl N-(2-Boc-aminoethyl)-glycinate, $N,N'$-dicyclohexylcarbodiimide (DCC), 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (DhbtOH); (e) NaOH, MeOH; (f) HCl.
Figure 1-9. Modified PNA (X_A and X_B) and 8-hydroxyquinoline functionalized PNA sequences (hq-X_n, n = A – E).
observed thermal stability of the [Cu(hq)$_2$] crosslinked duplexes is substantially greater than that of the [Ni(bpy)$_2$]$^{2+}$. This increase in thermal stability is attributed to the different geometries of the two metallolobases and their ability to π-stack with neighboring nucleobases. As mentioned above, the [Ni(bpy)$_2$]$^{2+}$ metallolobase was expected to be in a distorted square planar conformation, causing destabilization of the duplex. This effect was not anticipated or observed with the square planar [Cu(hq)$_2$] crosslink.

Spectrophotometric titrations of the PNA sequences with Cu$^{2+}$ ions were employed to determine the metal coordination stoichiometry for each of the hq-modified PNA duplexes at 25 and 95 °C. In each case, the shift of the ligand absorption band during the addition of Cu$^{2+}$ ions from 247 to 260 nm was monitored. The increase in absorption of the π-π$^*$ (320 nm) and MLCT (~400 nm) bands confirmed chelation of Cu$^{2+}$ at both temperatures. At 25 °C, the titration curve confirmed a 1:1 stoichiometry of Cu$^{2+}$ to the hq-modified PNA duplexes (i.e., [Cu(hq)$_2$], 0.5 Cu:1 PNA strand). However at 95 °C, a second inflection point at a 2:1 stoichiometry was observed, which was attributed to dehybridization of the PNA duplex and formation of two [Cu(hq)] complexes. The natural nucleobases also had a slight affinity for Cu$^{2+}$ ions at T = 95 °C, resulting in an increase in absorption beyond the 2:1 stoichiometric point.

Incorporation of metal complexes within DNA and PNA duplexes as substitutes for nucleic acid base pairs creates structures with thermal stabilities that depend on both the crosslinking inorganic species and the complementarity of the nucleobases. Each of the above studies relied on hydrogen-bonding to control their alignment and assembly. Together, all of these examples demonstrate the use of double-stranded scaffolds to direct the placement of inorganic complexes within these duplexes. Because of their
dependence on hydrogen bonding, these structures are limited to protic media and in some cases are susceptible to enzymatic degradation.

1.5 iDNA: Inorganic Analogues of DNA

Using these investigations and the structural features of DNA as inspiration, we have designed an artificial aeg analogue that relies entirely on metal coordination for duplex assembly. The stability and adaptability of the aeg backbone makes it an appropriate scaffold for these structures.\textsuperscript{25-27} Shown in Figure 1-10, the aeg backbone in our structures is fully substituted with ligands for metal chelation (metallobases); no nucleic acids are incorporated. The number, type, and sequence of ligands on the aeg backbone are synthetically controlled. Some of the ligands that have been appended to the aeg backbone are shown in the bottom of Figure 1-10, and vary in their binding affinity for metals. The synthetic methodology for preparation of some of these artificial metallobase aeg sequences is discussed in detail in Chapter 2.

These modified aeg strands are designed to bind metal ions and, in some cases, be joined together via chelation. The formation of single- or double-stranded structures depends on the coordination geometry of the metal and the denticities of the ligands. For example, using tetracoordinate metals (\textit{e.g.}, \(\text{Cu}^{2+}\), \(\text{Pt}^{2+}\), \(\text{Zn}^{2+}\), etc.), interstrand crosslinks can form when the ligands fulfill the necessary coordination geometry. As shown in Figure 1-11, two bidentate ligands (\textit{i.e.}, bpy) on separate strands, or conversely a monodentate (\textit{i.e.}, py) and tridentate (\textit{i.e.}, terpyridine, tpy) pair, bind a single tetracoordinate metal. Thus, in direct analogy to specific A-T and C-G base pairing in
Figure 1-10. (Top) Repeating oligopeptide backbone with (Bottom) incorporated metal-coordinating ligands. The bolded bonds on the ligands indicate their point of attachment to the backbone.
Figure 1-11. Metal-binding motif in artificial oligopeptides.
DNA, “complementary metallobases” are an alternative molecular recognition motif for assembly of double-stranded structures.

Molecular modeling can be used to predict the structures of these metal-linked assemblies. Figure 1-12 contains two artificial oligopeptide chains that are crosslinked by three Cu atoms. The metal complexes have a square planar geometry and the oligopeptide scaffold enforces their columnar arrangement within the core. This calculation predicts that the metal-to-metal distance in the assembly will be ~3.7 Å, and the chains will have a 19 metallobase pair helical pitch.

The results of this calculation suggest that the oligopeptide duplex arranges the metals in a predictable manner and in close enough proximity to facilitate electronic coupling. Our underlying hypothesis is that inorganic oligopeptide duplexes of this general design can be used as one-dimensional molecular wires with unique spectroscopic and electrochemical behaviors. To accomplish this, the work in this dissertation lays the synthetic foundation for the preparation and assembly of multimetallic and heterometallic artificial oligopeptide scaffolds.

In Chapter 2, the synthesis and characterization of two artificial oligopeptides, one with monodentate py and a second with bidentate bpy, are presented. Complementary metal complexes are used in spectrophotometric titrations to determine the binding stoichiometry of the py-functionalized oligopeptides. Conversely, the bpy oligopeptides are able to form double-stranded structures in the presence of metal ions, Cu$^{2+}$ and Fe$^{2+}$. The assembly of these complexes is schematically shown in Figure 1-13. The identities of the titration products were determined by NMR and mass spectrometry, and the spectroscopic and electrochemical properties of these duplexes are further
Figure 1-12. Model of artificial oligopeptide duplex assembled from square planar metal complexes.
Figure 1-13. (Top) Coordination of complementary metal complexes to a py-functionalized oligopeptide, and (Bottom) duplex assembly of a bpy tripeptide in the presence of metal ions.
investigated. The results led us to the conclusions that (a) metals bind to the ligands, not
the backbone, (b) the binding was stoichiometric regardless of size and charge of the
metal complex, and (c) the inability to grow crystals and broadening of the spectroscopic
and electrochemical signals was attributed to the formation of isomers.

To make larger quantities and to prevent isomers, alternative synthetic methods
were developed. We also sought to simultaneously incorporate more than one type of
ligand onto the peptide scaffold. Therefore, solution-phase synthetic protocols, similar to
those used in dendrimer synthesis, are described in Chapter 3 for the preparation of a py-
bpy-py oligopeptide by coupling two py monomers to the terminal positions of a central
bpy monomer. Figure 1-14 shows the synthetic scheme for the preparation of the py-
bpy-py artificial tripeptide (31). By sequential reactions of both metal ions and
complementary complexes, supramolecular coordination-induced structures are
assembled. Spectroscopic and electrochemical techniques were used to identify the
complexes formed in these assemblies. The multi-, heterometallic structures were
formed from a \([M(bpy)]_{n}^{2+}\) \((M = \text{Cu}^{2+}, \text{Zn}^{2+}, n = 2; M = \text{Fe}^{2+}, n = 3)\) crosslink at the
center of the tripeptide.

As an extension of this work, solution-phase synthesis was used to prepare a
complementary multi-ligand oligopeptide for the assembly of a duplex with py-bpy-py.
Chapter 4 discusses the preparation of a tpy-bpy-tpy tripeptide and its subsequent duplex
formation with py-bpy-py in the presence of Cu\(^{2+}\) ions. The synthesis of the
complementary tpy-bpy-tpy tripeptide (33) is shown in Figure 1-15. A series of
spectrophotometric titrations were performed, which confirm the assembly of
\([\text{Cu(bpy)}_2]^{2+}\) and \([\text{Cu(tpy)(py)}]^{2+}\) complexes. In addition, \(^1\text{H NMR}\) was used to confirm
Figure 1-14. Synthesis of py-bpy-py tripeptide.
Figure 1-15. Synthesis of tpy-bpy-tpy tripeptide.
coordination of Cu$^{2+}$ to the pendant ligands on the oligopeptide scaffold, and the electronic coupling of the Cu$^{2+}$ centers is investigated by EPR. Taken together, this data suggests that the Cu$^{2+}$ ions bind to the tripeptides forming a heterofunctional oligopeptide duplex.
1.6 References


Chapter 2

Artificial Oligopeptide Scaffolds for Stoichiometric Metal Binding

2.1 Introduction

The molecular structure of deoxyribonucleic acid (DNA) contains the instructions for the production of genes and provides a uniquely elegant template for self-replication in biological systems. The double helical structure of DNA was first described by Watson and Crick in the middle part of the previous century, and it is well-known that the molecular recognition between individual strands making up this duplex results from hydrogen bonding between complementary base pairs that decorate the sugar phosphate backbone. Using DNA as a model, we synthesize artificial oligomeric peptides that are designed to exhibit the conformational structure of DNA but which self-assemble upon transition metal chelation. The metal binding base pairs should enhance the stability and binding affinity of duplex structures. In comparison with DNA, the wider range of transition metal complexes (metals and ligands) available for use in these structures greatly increases the number of possible complementary components.1-3

While nucleic acids (and in some cases the phosphate backbone) in DNA bind metal ions, their chelation affinities are relatively weak, leading to stoichiometries and geometries that are not well-defined or controllable. Construction of synthetic analogues allows the incorporation of distinct, high affinity metal binding sites that circumvent these problems. To additionally avoid metal interaction with the sugar phosphates, we instead have chosen to utilize a peptidic backbone. The oligomer identified for our initial
studies of the inorganic helicates is identical to that used for peptide nucleic acids (PNAs). This peptide was chosen based on its (1) stability toward the enzymatic and chemical processes that can degrade the sugar-phosphate backbone of DNA and (2) easy adaptation toward large-scale automated synthesis. We utilize nitrogen containing heterocyclic ligands with predictable binding affinities for transition metal ions to covalently link to the PNA peptide backbone in place of nucleic acids, as shown in Figure 2-1. These artificial peptide oligomers therefore serve as scaffolds for the stoichiometric binding of metals in a geometrically defined arrangement.

We predict that when metal ions are used to link these artificial peptides, a structure similar to those calculated and shown in Figure 2-2 will result. In these synthetic model systems, molecular recognition and interchain linking between the new “base pairs” is based entirely on a coordination chemistry motif rather than hydrogen bonding. In addition to mimicking the helical structure of DNA, these multimetallic analogues mimic the information storage function of DNA, in that they are expected to exhibit unique optical and electronic properties that are strongly dependent on the peptide sequence and coordinated metals. The metal-decorated peptides therefore provide the basis for new motifs of functional self-assembled nanostructures for possible use as inorganic “bar codes” and may provide opportunities for the development of biocompatible transition metal pharmacological agents.

This chapter presents the synthesis and characterization of oligopeptides bearing pyridine (py) and 2,2′-bipyridine (bpy) ligands, demonstrates the ability of these to bind stoichiometric quantities of transition metal ions to form multimetallic single and double
Figure 2-1. Structure of the polyamide backbone showing positions of attachments of $x$ number of pendant ligands. Below are the two ligands used in this study, where $p$ indicates the point of attachment to the peptide backbone.
Figure 2-2. Side and top views of a calculated molecular model of a synthetic oligomeric helicate, formed by interstrand cross-linking of peptide strands by three metal ions.
stranded structures, and examines their electron paramagnetic resonance spectra and electrochemical properties.

2.2 Experimental

2.2.1 Chemicals

All materials were purchased from Aldrich and used as received unless otherwise noted. \(N,N\)-diisopropylethylamine (DIPEA, Avocado) was distilled over CaH\(_2\), and CH\(_2\)Cl\(_2\) (VWR) was dried on an activated alumina column. For all experiments, ultrapure water was used (Labconco Water Pro PS system, 18.2 MΩ). Fmoc-aeg-OtBu-HCl (34)\(^{18}\), 2,2′:6′,2″-terpyridine copper(II) diperchlorate (\(i.e., [Cu(tpy)(H\(_2\)O)][(ClO\(_4\))]\(_2\)\) (35)\(^{19}\), and bis(aquo) pyridine-2,6-dicarboxyl copper(II) (\(i.e., [Cu(pda)(H\(_2\)O)]\(_2\)\) (36)\(^{20,21}\) were prepared according to previously published procedures. Oligomers were synthesized on an Fmoc-PAL-PEG-PS resin (Applied Biosystems). A 4-molar excess of the monomers and amino acids was used during coupling reactions; the targeted oligomer was synthesized on a 0.1 mmol scale, as determined by the maximum loading level of the resin.

2.2.2 Instrumentation and Analysis

High performance liquid chromatography (HPLC) was performed on a Varian system equipped with two quaternary pumps (model 210), an autosampler (model 410), UV-visible detector (model 320), and fraction collector (model 701). Preparatory scale separations were performed with a 100 × 20 mm\(^2\) C18 column (S-5 \(\mu\)m, 12 nm, YMC,
Co.) and a 2 mL injection loop. Elution of the product was detected using the pyridine absorbance at 255 nm.

Positive ion electrospray ionization (ESI+) mass spectrometry was performed on a Mariner mass spectrometer (PerSeptive Biosystems). Theoretical mass spectrometry peaks and relative intensities were calculated using software available at http://www2.sisweb.com/mstools/isotope.htm. Elemental analyses were performed by Desert Analytics, Tuczon, Az.

Electrochemical data were collected with a CH Instruments model 660A potentiostat with a picoamp booster. Solutions were prepared from doubly distilled acetonitrile and thoroughly deoxygenated. Voltammetry was obtained using a 12.5 micron radius Pt working electrode, 2 mm diameter Pt wire counter electrode, and 22 gauge Ag wire quasi-reference electrode. The obtained voltammograms were manually corrected for uncompensated resistance.

Circular dichroism (CD) spectroscopy was performed using a Jasco J-810 spectropolarimeter with a quartz cell with an optical path length of 1 mm. Experiments were performed at room temperature with a bandwidth of 1 nm and wavelength increment of 1 nm.

All NMR spectra were obtained with either a Bruker 300 or 400 MHz spectrometer at room temperature. X-band EPR spectra were obtained using a 9.5 GHz Bruker eleXsys 500 spectrometer equipped with a liquid helium cryostat. All experiments were performed at 16 K, with a modulation frequency of 100 kHz and modulation amplitude of 5 G. For \([\text{Cu(bpy)}_2]_3(\text{PF}_6)_6\) and \([\text{Cu(pda)(py)})_6\), the microwave power was 8.23 mW; for \([\text{Cu(tpy)}(py)]_6(\text{ClO}_4)_{12}\), it was 0.15 mW.
Molecular structures were calculated using Hyperchem 6.0 using molecular mechanics (MM+) with atomic charge based electrostatic repulsions and a Polack-Ribiere conjugate gradient to a minimum energy gradient of 0.01 kcal/mol.

2.2.3 Synthesis

_Fmoc-aeg(py)-OH·HCl (30):_ A mixture of 4.057 g (10.2 mmol) of Fmoc-aeg-OrBu·HCl, 2.374 g of 1-[(3-dimethylamino)-propyl]-3-ethylcarbodiimide (EDC, 12.4 mmol), 2.200 g (12.7 mmol) of 4-pyridylacetic acid hydrochloride, 6.50 mL (37.3 mol) of DIPEA, and 300 mL of CH₂Cl₂ was stirred for 1 h under N₂. The yellow solution was extracted with H₂O (5 × 100 mL). The combined aqueous washings were extracted with CH₂Cl₂ (50 mL). The combined organic extracts were dried over anhydrous Na₂SO₄ and flash evaporated to give a yellow oil. The oil was stirred in an aqueous solution of 3 M HCl (150 mL). The acid was removed under reduced pressure to yield a yellow oil, which was dried for 16 h under vacuum. The pure product was obtained by recrystallization from CH₃CN, followed by vacuum drying for 16 h. Yield = 2.235 g (48.1%) (¹H NMR, 400 MHz, d₆-DMSO): 3.13 (q, J = 6 Hz, 1 H); 3.27 (q, J = 6 Hz, 1 H); 3.36 (t, J = 6 Hz, 1 H); 3.48 (t, J = 6 Hz, 1 H); 4.00 (s, 2 H); 4.17-4.23 (m, 2 H); 4.28-4.33 (m, 3 H); 7.30 (t, J = 7 Hz, 2 H); 7.40 (t, J = 7 Hz, 2 H); 7.47 (t, J = 6 Hz, 1 H); 7.67 (d, J = 7 Hz, 2 H); 7.88 (d, J = 7 Hz, 2 H); 8.82 (t, J = 6 Hz, 2 H). (ESI+) Calculated: (m + H)⁺ = 460.2. Found: (m + H)⁺ = 460.2. Elemental Analysis Calculated: C, 62.97%; H, 5.28%; N, 8.47%. Found: C, 63.17%; H, 5.12%; N, 8.76%.

_Fmoc-aeg(bpy)-OH·TFA (37):_ Fmoc-aeg-OrBu·HCl (1.333 g, 3.08 mmol) was dissolved in CHCl₃ (100 mL) and washed with an aqueous solution of saturated NaHCO₃
(3 × 100 mL). The washings were extracted with CHCl₃ (50 mL). The organics were then dried over Na₂SO₄, and the solvent was removed under vacuum leaving a clear oil. A solution of 4′-methyl-2,2′-bipyridine-4-acetic acid⁹ (1.004 g, 4.40 mmol), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, Novabiochem, 2.236 g, 5.90 mmol), and DIPEA (1.015 mL, 5.84 mmol) in N,N-dimethylformamide (DMF, 100 mL) was prepared, added to the oil, and was stirred under N₂ for 2 h. The tert-butyl-protected product was then precipitated by addition of the solution to ice water (1.3 L). The precipitate was collected by filtration and dried in vacuo overnight, leaving a yellow solid. The solid was dissolved in a solution of 2.5% water in trifluoroacetic acid (TFA, 10 mL). The solution was stirred for 2 h and then precipitated from cold ethyl ether (Et₂O, 200 mL), giving the product as an off-white powder. Yield = 1.014 g (49.6%) (¹H NMR, 300 MHz, d₆-DMSO): 2.50 (s, 3 H); 3.17 (t, J = 6 Hz, 1 H); 3.28 (t, J = 6 Hz, 1 H); 3.38 (t, J = 6 Hz, 1 H); 3.49 (t, J = 6 Hz, 1 H); 3.84 (s, 1 H); 3.90-4.05 (m, 2 H); 4.18-4.38 (m, 4 H); 7.29 (t, J = 7 Hz, 2 H); 7.40 (t, J = 7 Hz, 2 H); 7.53 (m, 2 H); 7.65 (t, J = 8 Hz, 2 H); 7.87 (d, J = 8 Hz, 2 H); 8.37 (s, 2 H); 8.45-8.64 (m, 2 H). (¹³C NMR, 400 MHz, d₆-DMSO): 21.3, 36.7, 46.8, 47.8, 48.1, 65.5, 114.2, 117.1, 120.2, 122.3, 123.5, 123.8, 123.9, 125.2, 127.4, 127.7, 140.8, 145.3, 147.5, 148.5, 152.5, 156.5, 158.4, 158.8, 168.1, 169.2, 170.8. (ESI+) Calculated: (m + H)⁺ = 551.2. Found: (m + H)⁺ = 551.3.

Bz-(py)₆G-NH₂ (27). The oligomer was synthesized at room temperature on a Pioneer peptide synthesis system (Applied Biosystems) with DMF as the solvent. A solution of 20% piperidine in DMF (Applied Biosystems) was used for Fmoc-deprotection (5 min), and 0.5 M diisopropylcarbodiimide (DIPCDI, Applied Biosystems)
and 0.5 M DIPEA were used for the couplings (30 min). A capping cycle was performed after every coupling with 0.5 M benzoic anhydride (Avocado) and 0.5 M DIPEA (5 min).

Following synthesis, the resin was washed 3 × 10 mL with DMF, followed by 3 × 10 mL alternating 2-propanol and CH$_2$Cl$_2$. The oligomer was cleaved from the resin by stirring the resin with 10 mL of 2.5% H$_2$O, 2.5% triisopropylsilane (TIS) in TFA for 2 h. The mixture was filtered in 4 equal volumes through a glass frit into 4 × 40 mL of cold Et$_2$O. The solutions were mixed and kept at 0 °C for 1 h. The oligomer was collected by centrifugation as pale yellow pellets and washed 3 × 10 mL with Et$_2$O and dried under vacuum for 1 h.

Purification of the oligomer (the major product) was accomplished by preparatory scale HPLC using a gradient elution of 5% (0.1% TFA in CH$_3$CN):95% (0.1% TFA in H$_2$O) to 15% (0.1% TFA in CH$_3$CN):85% (0.1% TFA in H$_2$O) ramped over 13 min with a total flow of 20 mL/min and a fraction collection program. TFA and CH$_3$CN were removed from the collected fractions under reduced pressure, and H$_2$O was removed by lyophilization. Yield = 0.072 g (33%) ($^1$H NMR, 300 MHz, $d_6$-DMSO): 3.15-3.75 (m, 26 H); 3.75-4.30 (m, 24 H); 7.08 (s, 1 H); 7.21 (s, 1 H); 7.50 (m, 4 H); 7.71 (m, 14 H); 8.01 (m, 1 H); 8.30 (m, 3 H); 8.50 (m, 2 H); 8.70 (m, 12 H). HMQC, COSY, and HMBC NMR spectra are shown and discussed in detail in Appendix A. (ESI+) Calculated: (m + H)$^+$ = 1493.7, (m + Na)$^+$ = 1515.6. Found: (m + H)$^+$ = 1493.8, (m + Na)$^+$ = 1515.7.

Bz-(bpy)$_3$G-NH$_2$ (28). A 25 mL fritted polypropylene reservoir (Alltech) was used in the synthesis of the oligomer. The reservoir was heated to ~50 °C with electric heating tape (Barnstead/Thermolyne), and N$_2$ was bubbled through the DMF solution. The synthesis uses 20% piperidine in DMF for Fmoc-deprotection (15 min), and
solutions of 0.5 M HBTU and 0.5 M DIPEA for monomer and amino acid coupling (30 min), and 0.5 M benzoic anhydride and 0.5 M DIPEA in DMF for capping (5 min). Each coupling step was monitored for completion using a Kaiser test. After the desired number of cycles, the resin was washed 3 × 10 mL with DMF, followed by 3 × 10 mL CH₂Cl₂. The oligomer was cleaved from the resin by stirring the resin with 10 mL of 2.5% H₂O, 2.5% TIS in TFA for 2 h. The mixture was filtered in 4 equal volumes through a glass frit into 4 × 40 mL of cold Et₂O. The oligomer was collected as pale yellow pellets by centrifugation, and the pellets washed with 3 × 30 mL with Et₂O and dried under vacuum for 1 h.

The desired oligomer was separated from deletion sequences using an HPLC gradient elution of 10% (0.1% TFA in CH₃CN):90% (0.1% TFA in H₂O) ramped to 28% (0.1% TFA in CH₃CN):72% (0.1% TFA in H₂O) over 15 min at 20 mL/min. After fraction collection, the oligomer was lyophilized to give a pale pink solid. Yield = 1.2 mg (1.19%). (¹H NMR, 400 MHz, MeOH-d₄): 2.22-2.41 (m, 9 H); 3.38-4.32 (m, 26 H); 7.00-8.59 (m, 23 H). HMQC NMR spectrum is shown and discussed in detail in Appendix B. (ESI+) Calculated: (m + H)⁺ = 1109.5. Found: (m + H)⁺ = 1109.4.

### 2.2.4 Spectrophotometric Titrations

Bz-(py)₆G-NH₂ + [Cu(pda)(H₂O)₂] or [Cu(tpy)(H₂O)₂](ClO₄)₂. Titrations were performed using a Varian Cary 500 spectrophotometer. A solution of 27 was prepared in methanol (MeOH). The concentration of the oligomer (2.22 mM) was determined using its molar extinction coefficient (ε = 31860 M⁻¹ cm⁻¹) at 256 nm. This solution was titrated in 5, 10, and 20 µL increments into 2.5 mL of a 0.6608 mM [Cu(pda)(H₂O)₂]
methanolic solution at 4 min intervals between each addition. For each addition, the reference solution was diluted with an equal volume of MeOH to account for concentration effects. UV-visible absorption spectra were acquired for each iterative addition of oligomer. The titration product was precipitated by slow addition of ether, filtered, rinsed with ether, and reprecipitated from methanol. Titrations with [Cu(tpy)(H$_2$O)](ClO$_4$)$_2$ were performed analogously using a 2.804 mM methanolic solution containing a small amount (3.36 µM) H$_2$O. The titration product was isolated as for the [Cu(pda)] adduct.

\[
Fe(ClO_4)_2 + Bz-(bpy)_3G-NH_2.\]

Titrations were performed on a Varian Cary 50 spectrophotometer. A solution of 28 was prepared, and the concentration was determined to be 70.8 µM using its molar extinction coefficient ($\varepsilon = 6322.6$ M$^{-1}$ cm$^{-1}$) at 284 nm. A solution of 784 µM [Fe(ClO$_4$)$_2$] in MeOH was added in 2 µL increments to the oligomer solution in 5 min intervals, and UV-visible absorption spectra were obtained for each addition. The titration products were precipitated with NH$_4$PF$_6$, the supernatant was decanted, and the solid was rinsed with copious amounts of water.

\[
Cu(OAc)_2 + Bz-(bpy)_3G-NH_2.\]

This titration was performed similarly to that from the Fe(ClO$_4$)$_2$ experiment, except that a 734.5 µM 28 solution in MeOH was titrated with a solution of 9.29 mM Cu(OAc)$_2$ in MeOH in 20 µL increments at 15 min intervals. Spectra were corrected for the background absorbance of unbound Cu acetate. The titration product was isolated and purified as above. Elemental Analysis Calculated: C, 47.99%; H, 4.64%; N, 11.87%. Found: C, 47.72%; H, 4.96%; N, 12.04%.$^{24}$
2.3 Results and Discussion

2.3.1 Synthesis

Py and bpy were chosen as the initial target ligands for incorporation onto the peptide backbone because of their wide use in coordination chemistry for a variety of transition metals. To prepare peptides incorporating these pendant ligands, we use standard solid-phase synthesis methods. The peptide/ligand monomers were prepared according to the method shown in Figure 2-3 from their acetic acid derivatives, by adaptation of a literature procedure. Briefly, the ligands were reacted with Fmoc-protected N-[2-aminoethyl] glycine tert-butyl ester (Fmoc-aeg-OtBu·HCl) using N,N-diisopropylethylamine (DIPEA) and the coupling reagent shown in Figure 2-3. Amide coupling to the py ligand was accomplished with DIPEA and EDC at 25 °C for 1 h. The bpy analogue required the use of DIPEA and HBTU. For both ligands, the terminal acid was deprotected by acid cleavage of the tert-butyl to yield monomers 30 and 37 with overall yields 48% and 50%, respectively.

Oligomers were prepared from monomers 30 and 37 by solid-phase synthetic methods on a resin support (Fmoc-PAL-PEG-PS, Applied Biosystems). Oligomers were typically prepared using an automated peptide synthesizer (Applied Biosystems Pioneer System), which enables the facile preparation of sequences of varying length and composition. In some cases, resin-supported synthesis was performed by hand. For the sake of clarity, we detail the synthesis and metal binding of the two oligomer sequences shown in Figure 2-3, a py hexamer, Bz-(py)_6G-NH₂ (27), and bpy trimer, Bz-(bpy)_3G-NH₂ (28).
Figure 2-3. Synthesis of ligand-peptide monomers and structures of the oligomers; Bz = Benzoyl, G = Glycine.
The oligomers were cleaved from the resin using an excess quantity of 2.5% water and 2.5% triisopropylsilane (TIS) in trifluoroacetic acid (TFA), giving the oligomers shown in Figure 2-3. The products were separated from deletion sequences and reaction byproducts by reverse-phase high-performance liquid chromatography (HPLC), and the desired fraction was collected and lyophilized to yield the purified oligomer. For 27, the final yield of the hexamer was 33% (based on the loading of the resin) and purity was confirmed by NMR and mass spectrometry. The typical yield of 28 was 1.19%, with purity again confirmed by NMR and mass spectrometry. The significantly lower yield of the bpy oligomer is thought to arise from both steric hindrance and aggregation induced by π-stacking of the ligands, and our ongoing efforts seek to improve the overall yield for this and other multidentate ligand oligomers.27

2.3.2 Metal Coordination to Pyridine Hexamer

We next investigated the ability of the artificial peptide oligomers to bind transition metal ions. Since it is well-known that metal coordination is typically accompanied by the appearance of a peak in the UV-visible absorption spectrum, we used spectrophotometric titrations to monitor the complexation of Cu$^{2+}$ and Fe$^{2+}$ ions to the oligomers. We chose to strictly enforce one-to-one complexation to the pyridine hexamers (i.e., one metal to each pendant ligand) to confirm the availability of each of the ligands for chelation. Therefore, tetracoordinate metal ions bearing tridentate ligands (i.e., [Cu(pda)] and [Cu(tpy)]$^{2+}$) were used to preferentially bind to the pendant pyridine oligomers to fill their coordination shell.20,21 Figure 2-4A shows the difference spectra for the change in visible absorbance resulting from the titration of a methanolic solution
Figure 2-4. UV-visible absorption spectroscopy difference spectra for the titration of 27 with (A) [Cu(pda)] and (B) [Cu(tpy)]$^{2+}$. The insets contain plots of the peak absorbance as a function of relative molar ratio of metal ion to oligomer.
of 27 oligomer with [Cu(pda)(H₂O)]₂. The peak at 660 nm, associated with a [Cu(pda)(py)] absorption, increases during early additions of oligomer and reaches a maximum value. A negative change in absorbance at 840 nm is simultaneously observed and is attributed to the consumption of free [Cu(pda)] in solution. The titration curve for this experiment, plotted as the change in absorbance as a function of added oligomer, is shown in the Figure 2-4A inset. This curve indicates that the growth of the [Cu(pda)(py)] absorption peak begins to level at a maximum value of ~6 molar equivalents of [Cu(pda)] per mole of 27. These spectrophotometric data strongly suggest the formation of an oligomeric strand of Bz-(py)₆G-NH₂ with six bound [Cu(pda)] centers, (Bz-[Cu(pda)(py)]₆G-NH₂, 38).

To test the effect of ionic charge on the addition of complexes to the pyridine oligomers, 27 was also titrated with [Cu(tpy)](ClO₄)₂. Figure 2-4B shows the difference spectra collected during the titration of Bz-(py)₆G-NH₂ with [Cu(tpy)]²⁺, together with the titration curve (Figure 2-4B, Inset) that conclusively demonstrates the formation of the complex (Bz-[Cu(tpy)(py)]₆G-NH₂)(ClO₄)₁₂ (39). The Cu complexes in 39 each have a +2 charge, so that the overall charge of the metal-coordinated oligopeptide is +12, versus the charge-neutral 38 species. Importantly, stoichiometric complexation is observed despite the large positive charge, indicating that electrostatic repulsion between metal centers is unimportant for binding. The isolated solid products of these multimetallic structures are finely divided powders, and our attempts to grow crystallographic quality crystals have been fruitless to date. However, we have used molecular modeling to predict and understand these molecules, discussed further below.
2.3.3 Metal Coordination by Bpy Oligomer

The bpy ligands in 28 are bidentate towards transition metals and therefore provide a distinctly different binding motif than the (monodentate) py oligopeptide. Because of the presence of tridentate ligands coordinated to the tetracoordinate metal center, the [Cu(pda)] and [Cu(tpy)]\(^{2+}\) complexes used in the titrations of the py oligomer do not bind to the bidentate bpy ligands and were therefore not used to study binding to the bpy tripeptide. However, it is known that free Fe\(^{2+}\) ions form hexacoordinate complexes with bpy (i.e., [Fe(bpy)]\(^{3+}\)), whereas Cu\(^{2+}\) ions form the tetracoordinate complex [Cu(bpy)]\(^{2+}\). In the absence of other ligands, metal ions added to solutions containing 28 should coordinate to multiple bpy ligands to form inter- and/or intrachain linkages. Metal complexation could also induce the formation of undesired coordination polymers, which we prevent by use of solutions with low oligomer and metal concentration. Spectrophotometric titrations of dilute solutions of the Bz-(bpy)\(_3\)G-NH\(_2\) oligomer were performed with Fe\(^{2+}\) perchlorate (Fe(ClO\(_4\))\(_2\)) and Cu\(^{2+}\) acetate (i.e., Cu(OAc)\(_2\)). Figure 2-5A shows the change in visible absorbance during the addition of Fe(ClO\(_4\))\(_2\) to a methanolic solution of 28; the strong peak at 540 nm is the well-known metal-to-ligand charge transfer (MLCT) transition for [Fe(bpy)]\(^{2+}\). The titration curve in Figure 2-5B shows that the intensity of the MLCT peak increases upon addition of Fe\(^{2+}\) and levels off at a stoichiometric equivalence point of 1 mol of Fe\(^{2+}\):1 mol of 28.

Two possible structures would be consistent with this stoichiometric ratio: either one oligopeptide coordinated to a single Fe\(^{2+}\) ion, [Bz-[Fe(bpy)]\(_3\)]G-NH\(_2\)] \(\text{(i.e., [Fe(bpy)]\(_3\), calculated mass 1164.4 amu)}\), or two oligopeptides cross-linked by two Fe\(^{2+}\) ions to form [Bz-[Fe(bpy)]\(_3\)]G-NH\(_2\)]\(_2\) \(\text{(i.e., [Fe(bpy)]\(_3\)]\(_2\), calculated mass 2328.8 amu)}\).
Figure 2-5. UV-visible absorption spectroscopy difference spectra for the titration of 28 with (A) Fe(ClO$_4$)$_2$ and (C) Cu(OAc)$_2$. (B and D) Plots of the peak absorbance as a function of relative molar ratio of metal ion to oligomer for the Fe$^{2+}$ and Cu$^{2+}$ ions, respectively.
High-resolution mass spectrometry was used to identify which of these was the titration product, and a molecular ion was observed at 583.7424 $m/z$ (see Table 2-1). Both the $z = +2$ ion of [Fe(bpy)$_3$] and the $z = +4$ ion of [Fe(bpy)$_3$]$_2$ are expected to have peaks at this $m/z$ ratio. However, analysis of the observed isotopic splitting of the high-resolution molecular ion peak evidences sharp peaks with 0.25 $m/z$ separation, consistent with an ion of $z = +4$ charge. Therefore, the high-resolution mass spectrometry data leads to the conclusion that the titration product is an oligopeptide duplex linked by two bound Fe$^{2+}$ ions ([Fe(bpy)$_3$]$_2$, 40).$^{31}$

To unambiguously confirm that metal coordination can be utilized to induce duplex formation, we also titrated 28 with [Cu(OAc)$_2$],$^{32}$ which we reasoned would have a different stoichiometric equivalence point based on the tetracoordinate nature of Cu$^{2+}$. Visible absorption spectra were again collected during the titration, Figure 2-5C. These spectra contain a peak at 610 nm that grew and reached a maximum value in the titration curve in Figure 2-5D at a Cu$^{2+}$/oligomer equivalence point of ~1.6. This molar ratio is consistent with either a single bpy tripeptide binding 1.5 Cu$^{2+}$ ions to form [Bz-[Cu(bpy)$_2$]$_{1.5}$G-NH$_2$]$_2$ (i.e., [Cu(bpy)$_2$]$_{1.5}$, calculated mass 613.0 amu) or two bpy tripeptides linked by three Cu$^{2+}$ ions to make [Bz-[Cu(bpy)$_2$]$_3$G-NH$_2$]$_2$ (i.e., [Cu(bpy)$_3$]$_2$, calculated mass 1225.9 amu). Elemental analysis of the titration product, isolated in larger quantity (~3 mg) than the Fe analogue (<1 mg), confirmed its purity but could not differentiate between the two possible structures. However, analysis of the product using mass spectrometry revealed a $z = +2$ molecular ion peak (based on the isotopic splitting) at 1227.7 $m/z$ (see Table 2-2). This molecular ion peak is consistent with the calculated
Table 2-1. High-resolution mass spectrometry analysis of titration product of 28 with [Fe(ClO₄)₂]. Theoretical mass spectrometry peaks and relative intensities were calculated using software available at http://www2.sisweb.com/mstools/isotope.htm.

<table>
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Table 2-2. Mass spectrometry analysis of titration product of 28 with [Cu(OAc)₂]. Theoretical mass spectrometry peaks and relative intensities were calculated using software available at http://www2.sisweb.com/mstools/isotope.htm.

<table>
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</table>

* These peaks were not observed due to the lower resolution of the experiment. However, shoulders were observed for each of the listed peaks.
molecular weight of the \([\text{Cu}(bpy)_3]_2\) duplex structure, and taken together, these data strongly suggest the formation of an *oligopeptide duplex linked by three* \(\text{Cu}^{2+}\) *ions* (41).

In both the Fe and Cu bpy oligopeptide duplex cases, the isolated metal-containing molecules form finely divided powders, which likely results from the large number of possible structural isomers. **Figure 2-6** shows just two examples (for each of the metals) of the possible isomers that result from antiparallel vs parallel alignment of the oligopeptides, an asymmetry that arises from the sequence of the chain and the different termini. Many more structures are possible based on changes in the metal-ligand connectivity and the asymmetry of the bipyridine, which is attached only in the 4-position.\(^{33}\) Coupled together, the structural isomers and the small quantity of prepared metalated material have made crystallization of the metal-linked duplexes impossible to date. Our continuing efforts are focused on the preparation of large enough quantities of the oligopeptides to enable the isolation of a single isomer.

### 2.3.4 Molecular Modeling

To better understand the molecular structures of the metal-coordinated pyridine hexapeptides and the metal-linked bpy tripeptide duplexes, we have turned to computational molecular modeling. In the cases of the former oligopeptides, because the metal complexes decorate a single peptide chain, we expected a large amount of disorder in the calculated structures. **Figure 2-7A** contains the energy minimized structure of 38, shown without the terminal benzoyl and glycine caps for clarity, from the side and top. In agreement with the crystal structures that we have obtained for the small molecule analogue \([\text{Cu}(pda)(py)]\) (see Appendix C), the bound Cu complexes each have a square
Figure 2-6. Schematic diagrams of bipyridine tripeptides linked by (A) three tetracoordinate \(\text{Cu}^{2+}\) ions and (B) two octahedral \(\text{Fe}^{2+}\) ions. Circles on chain termini indicate benzoyl caps.
Figure 2-7. Energy-minimized structures of (A) 38 and (B) 39 calculated using MM+ (Hyperchem 6.0) from (left) side view and (right) top. Cu-Cu distances are (A) \( d = 4.4 \, \text{Å} \) and (B) average \( \sim 20 \, \text{Å} \). Hydrogen atoms and oligomer termini are omitted for clarity.
planar geometry. The most striking feature of this calculated structure is the twist of the oligopeptide backbone around the column of Cu metal complexes, most likely a result of $\pi-\pi$ interactions of the pda ligands, with a helical pitch of 16-17 monomers. The oligopeptide strand constrains the Cu centers to a columnar arrangement with a regular spacing of ~4 Å.

In contrast, molecular modeling of 39 is shown in Figure 2-7B. The metal complexes are splayed apart with an average distance of 20 Å. We justify the differences between the calculated structures in Figure 2-7A and 2-7B based on the charge of the Cu complexes, where electrostatic repulsions between the $[\text{Cu(tpy)(py)}]^{2+}$ complexes prevents $\pi$-stacking of the aromatic ligands. The structures in Figure 2-7 predict that, in the solid form, the degree of electronic interaction between Cu centers is much larger in the charge-neutral 38 than in 39. These differences are not expected to exist in solutions of these multimetallic molecules.

We also performed calculations on the metal-linked bpy tripeptide duplexes to visualize the most stable possible isomers. Figure 2-8 contains the energy minimized, calculated structures (shown from the side and top) for the 40 and 41. In each of these calculated structures, the oligopeptide acts as a scaffold to hold the metal complexes in close proximity: for the Cu$^{2+}$ and Fe$^{2+}$ duplexes, the metal centers are separated by 4.8 and 11 Å, respectively. In comparison with the molecular structures in Figure 2-7, the spacing between the Cu atoms in the duplex shown in Figure 2-8 is similar to that predicted for 38. It is evident from the models that differing metal coordination geometries dramatically affect the orientation of the peptide chains: whereas the octahedral Fe$^{2+}$ complexes twist the peptide into a knotlike structure, the (distorted)
Figure 2-8. Energy-minimized structures of (A) 41 and (B) 40, calculated using MM+ (Hyperchem 6.0), from the side and top. Calculated Cu-Cu distances are $d = 4.8 \, \text{Å}$; Fe-Fe distance is $d = 11 \, \text{Å}$. Hydrogen atoms and oligomer termini are omitted for clarity.
square planar geometry of the Cu$^{2+}$ complexes allows the peptide strands to adopt a helical confirmation with a pitch of ~18 monomers.

### 2.3.5 Electron Paramagnetic Resonance Spectroscopy

Since electron paramagnetic resonance (EPR) spectroscopy is sensitive to the local environment and coupling between paramagnetic species, we next turned to analysis of the metalated oligopeptides to provide additional insight into their structures. Frozen EPR spectra were obtained in methanolic solutions at 16 K for each of the oligopeptides with bound Cu (ions or complexes) and are shown in Figure 2-9. Values of the EPR spectroscopy parameters and coupling constants are given in Table 2-3. The spectrum for the pyridine hexapeptide with six-coordinated [Cu(tpy)]$^{2+}$ complexes (i.e., 39; Figure 2-9, top) has the classic line shape for a Cu complex with a hyperfine coupling of $A_\parallel = 167 \times 10^{-4}$ cm$^{-1}$. While this spectrum would be expected for a single Cu complex, this observation for the hexametallic peptide implies that each of the [Cu(tpy)(py)]$^{2+}$ subunits is electronically identical and they must be separated by a distance of at least 6 Å. This spectrum is contrasted by that obtained for the charge neutral 38 oligopeptide, Figure 2-9, middle, in which the lines are broadened and the hyperfine coupling is reduced to ~130 $\times 10^{-4}$ cm$^{-1}$. These are consistent with weak interactions between Cu centers that give rise to some delocalization of the unpaired electrons. The spectra of the 38 is further complicated by the presence of (at least) a second set of peaks with lower intensity and similar $A_\parallel$, which implies that several types of Cu complexes exist in this structure with slightly different electronic environments. For both 38 and 39, the values of $g_\parallel > 2.1 > g_\perp > 2.0$ are typical for Cu complexes with a square planar geometry.\textsuperscript{36,37}
Figure 2-9. Frozen EPR spectra of 0.5 mM solutions of 39 (top), 38 (middle), and 41 (bottom).
Table 2-3. EPR Spectroscopy Parameters and Hyperfine Coupling Constants

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<th>$g_{\perp}$</th>
<th>$A_{\parallel} \times 10^{-4}$ (cm$^{-1}$)</th>
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<tr>
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The spectrum of the Cu-linked bpy trimer duplex (Figure 2-9, bottom) is similar to that of 38, except that it is even further broadened and lacks hyperfine coupling. The overall shape is again consistent with a structure containing Cu atoms with several electronic environments. The lack of hyperfine peaks may be due to very small coupling constants but is most likely due to multiple Cu environments both within one molecule and between the isomeric forms that are present. Taken together, the EPR spectra are consistent with the structures from molecular modeling in Figures 2-7 and 2-8. Based on these, 38 and 41 would be expected to rotate polarized light because of their helical structures. However, our measurements of the molecules’ circular dichroism spectra (not shown) did not contain evidence of optical activity for any of these multimetallic complexes. We rationalize this result based on the expected differences in the solution phase and frozen molecular structures of the single-strand peptide and note that the observation for the Cu-linked tripeptide duplex is again consistent with the presence of several geometric isomers.

2.3.6 Electrochemistry

The oligopeptide complexes and duplexes possess structural components (metal atoms and ligands) with unique oxidation and reduction properties. Each of the above oligopeptide sequences is expected to have a distinctive voltammetric signature based on the type and number of metal complex(es) linked to the peptide backbone. The voltammograms (not shown) of the py hexapeptides containing [Cu(pda)] (38) and [Cu(tpy)]^{2+} (39) complexes are distinctive only in the large amount of adsorption to the electrode surface and extremely slow heterogeneous kinetics for the Cu^{2+/1+} reduction (for
both the tpy and pda complexes), which completely obfuscate further quantitative analysis.

Voltammetry of the Cu- and Fe-linked bpy oligopeptide duplexes is however quantitatively informative; Figure 2-10 contains voltammograms acquired with a microdisk electrode in acetonitrile solutions containing either the 40 or 41. Supporting electrolyte was not added to these solutions to avoid contamination of the very small quantities of available material with salt; therefore slow potential scan rates (5 mV/s) and compensation for solution resistance were used to produce the voltammograms in Figure 2-10. In the case of the trimetallic Cu duplex (41), a sigmoidal wave attributed to the Cu$^{2+/1+}$ reduction was observed with a half wave potential of 0.22 V vs Ag QRE. Using the microelectrode limiting current at large overpotentials, and assuming that each of the three Cu atoms are reduced during the reaction, the diffusion coefficient of the Cu duplex is calculated to be $5 \times 10^{-7}$ cm$^2$/s.$^{38}$

The dimetallic Fe duplex (40) exhibited a voltammetric response at more positive potentials, where the [Fe(bpy)$_3$]$^{3+/2+}$ oxidation is expected to appear. However in contrast to the Cu duplex, the Fe duplex voltammetry possesses an oxidative signature that appears to have two sequential reactions: the first of these is smaller and occurs at a half wave potential of 0.98 V and the second larger wave at 1.2 V vs Ag QRE. Background cyclic voltammograms of a nonmetalated peptide oligomer do not possess any redox activity within this potential region. The appearance of two waves is unusual and could arise from strong electronic interactions between the two metal centers or from the existence of two structural isomers of 40. The former of these has been ruled out because similar coupling in the Cu duplex (which is expected to be far more ordered)
Figure 2-10. Cyclic voltammograms of 40 and 41, obtained using a 12.5 micron radius Pt working electrode at a scan rate of 5 mV/s, with backgrounds corrected for uncompensated resistance. Solutions contained 1 mM peptide duplex in acetonitrile.
voltammetry is not observed and our molecular modeling predicts a substantial 1.1 nm separation between the Fe centers. Additionally, it would be expected that strong electronic coupling would result in two waves of equal magnitude in current: the wave observed at lower potentials is about half the size of the larger wave. We therefore believe that the two peaks in the voltammetry are a result of two isomeric forms of the Fe duplex that contain Fe complexes with different degrees of conformational stabilization, which is the subject of ongoing studies with larger quantities of duplex material.

Using the currents obtained for the Fe duplex, the diffusion coefficients for the waves at 0.98 and 1.2 V are determined to be $5 \times 10^{-7}$ and $1 \times 10^{-6}$ cm$^2$/s, respectively. These values compare well with that calculated for the Cu duplex. In both cases, the solution phase diffusion coefficients are consistent with mass transport rates that are expected for structures of this size and charge. The voltammetry therefore demonstrates that these relatively simple, homometallic oligopeptide duplexes have distinguishable electrochemical signatures.

2.4 Conclusions

We have synthesized two new artificial oligopeptides with pendant nitrogen-containing ligands and used these to prepare peptidic scaffolds that bind transition metal ions in a stoichiometric manner. In the case of the bpy oligomers, the metal ions cross-link the strands to self-assemble structures into double-stranded oligopeptide duplexes with sequence-dependent electrochemical and spectroscopic properties. Our continuing efforts seek to obtain crystallographic confirmation of both the Fe$^{2+}$ and Cu$^{2+}$ duplex
structures, to expand the library of artificial peptides, and to examine detailed optical and
electronic properties of these supramolecular structures.
2.5 References


(19) Ohr, K. The Pennsylvania State University, unpublished results.


(24) Insufficient quantities of material were available to quantitatively determine the percentage of metal ion in the sample.


(27) Gilmartin, B. P.; Pantzar, L. The Pennsylvania State University, unpublished results.

(28) The slight rise in absorbance beyond the equivalence point is likely a result of either background absorbance of free [Cu(pda)] in solution, whose broad absorbance peak overlaps the [Cu(pda)(py)] transition, or the slow chelation kinetics.

(29) No polymerization products have been observed under dilute solution conditions; however these are expected to be large and insoluble, and the reaction solutions are filtered prior to precipitation of the desired material.


(31) Because of the very small quantities of this duplex that were produced, elemental analysis of this titration product was not possible.

(32) Cu$^{2+}$ ion complexation is kinetically slower than Fe$^{2+}$ binding, requiring longer equilibration times between iterative additions. The difference spectra show free Cu acetate ions ($\lambda > 650$ nm) because of a lower equilibrium binding constant.

(33) Ignoring possible rotamers of the bpy ligand around the ligand-peptide bond, which gives hundreds of geometric isomers, there are 12 possible tri-copper and 14 possible di-iron duplexes.


(37) Wei, N.; Murthy, N. N.; Karlin, K. D. “Chemistry of Pentacoordinate [LCu\textsuperscript{II}-Cl]\textsuperscript{+}


(39) Compared to the diffusion coefficient of ferrocene (radius \(\approx 2\) Å) in acetonitrile
\((\sim 10^{-5} \text{ cm}^2/\text{s})\) and using the voltammetrically determined diffusion coefficient \((D)\), the
Stokes-Einstein relationship \((D = k_B T/(6\pi \eta r_H))\) would predict a hydrodynamic radius
(which would include stabilizing counterions and solvent molecules) for the metal-linked tripeptide duplexes of \(\sim 4\) nm.
Chapter 3

Artificial Tripeptide Scaffolds for Self-Assembly of Heteromultimetallic Structures with Tunable Electronic and Magnetic Properties

3.1 Introduction

Peptide nucleic acids (PNAs) are structural mimics of deoxyribonucleic acid (DNA) that replace the sugar phosphate with a poly(aminoethylglycine) (aeg) backbone and have a great deal of synthetic versatility based on straightforward synthetic modifications that are not possible in natural DNA. For example, in addition to the incorporation of amino acid residues and nucleobases, nonnatural base pairs and metal-binding ligands have been inserted into the PNA backbone. These variations increase the number of structural motifs for the assembly of double-stranded PNA-PNA or PNA-DNA duplexes and enable the synthetic tunability of their physical properties.

Further synthetic modifications should enable a wider variety of biocompatible, peptide-based materials for applications in sensors and pharmaceuticals or as a new motif for self-assembly of nanostructures.

We have recently reported the synthesis of artificial peptides based on the PNA backbone which contained a homosubstituted polyamide chain with a single type of chelating ligand, either monodentate pyridine or bidentate bipyridine. These oligopeptides were shown to bind stoichiometric quantities of transition-metal ions, which resulted in the formation of multimetallic structures. Because the oligopeptides contained the same ligand, the resulting materials contained multiple copies of the same metal complex tethered in close proximity along a peptide scaffold. In those studies,
the metal-binding oligopeptides were prepared via solid-phase peptide synthesis, a common approach in which peptides are initiated and sequentially extended while tethered to a solid resin support. Isolation of the desired product from reactants and deletion sequences is relatively facile using this strategy. However, we observed in our earlier report that the artificial oligopeptide yield was typically poor; the optimized synthesis of a tripeptide containing three bipyridine ligands gave only 1.19% yield.\textsuperscript{16} We hypothesized that our yields were negatively affected by the loading of reactive groups on the resin support, the degree of solvation, and aggregation of longer or aromatic-containing sequences.\textsuperscript{18-20} To enable the study of these materials in a variety of electrochemical, spectroscopic, structural, and biophysical experiments, we have sought an alternative route toward the preparation of our ligand-containing artificial oligopeptides that would enable the rapid production of a larger quantity of the desired peptide scaffold.

Inspiration for a more efficient synthesis scheme was derived from the divergent synthetic approach for preparation of dendrimers in which the supramolecular structures are constructed from the core outward.\textsuperscript{21} One of the coupling chemistries for the growth of successive generations is the formation of amide linkages,\textsuperscript{22-24} and it has recently been shown that a variety of peptide dendrimers may be prepared in this manner.\textsuperscript{25} A similar solution-based synthetic approach, focusing on the sequential growth outward from the center of the peptide chain would therefore be a feasible and potentially advantageous way to overcome the limitations of resin-supported synthesis. We subsequently rely on an approach taken by chelation-based dendrimer syntheses\textsuperscript{26-31} to prepare supramolecular structures by linking together the peptide strands with metal coordination chemistry.
In this chapter we present a one-pot, solution-based synthesis of an artificial tripeptide with pendant metal-binding ligands in larger yields and on a relatively shorter time scale than typical solid-phase approaches. The central unit for the peptide is diethylenetriamine (DETA), protected at the terminal amines with 9-fluorenylmethoxycarbonyl (Fmoc), and to which a pendant 2,2′-bipyridine (bpy) is linked. Following Fmoc deprotection, coupling chemistry is simultaneously performed on both ends of the chain to link a pyridine (py)-modified aeg monomer (Fmoc-aeg(py)-OH·HCl).\textsuperscript{16,17} This study is the first report of multifunctional tripeptides that contain ligands with differing denticity and therefore reactivity toward metal complexation. Importantly, this approach also enables the facile preparation of peptides that are palindromic, eliminating one source of geometric isomers.\textsuperscript{16,17} By sequential exposure to different transition-metal ions or complexes, we demonstrate the use of this tripeptide to create a series of heterometallic structures, each of which has unique optical and electrochemical properties that are characteristic of both metal centers tethered to the peptide scaffold.

3.2 Experimental

3.2.1 Chemicals

All materials were purchased from Aldrich and used as received unless otherwise noted. Ultrapure water (Barnstead, 18.2 MΩ) was used for all experiments; 4′-methyl-2,2′-bipyridine-4-acetic acid (bpy-CH\textsubscript{2}CO\textsubscript{2}H) (\textsuperscript{42}),\textsuperscript{32} py-substituted aeg monomer (Fmoc-aeg(py)-OH·HCl) (\textsuperscript{30}),\textsuperscript{16,17} iodo(2,2′:6′,2″-terpyridine)platinum(II) iodine dihydrate (i.e.,
[Pt(tpy)]I·2H₂O) (43), and aqua(pyridine-2,6-dicarboxylato) copper(II) (i.e., [Cu(pda)(H₂O)]) (36) were prepared according to previously published procedures. Tetrabutylammonium hexafluorophosphate (TBAH; Apollo Scientific) was recrystallized three times from ethyl acetate (EtOAc, VWR).

3.2.2 Instrumentation and Analysis

Electrochemical measurements were performed using a CH Instruments potentiostat (model 660A) under an inert atmosphere in a glovebox (MBraun LabMaster 130) using either a 12.5 µm radius Pt or a 1.0 mm radius Pt working electrode, a Ag/Ag⁺ reference electrode, and a Pt counter electrode. The working electrode was polished using aqueous slurries of progressively finer grits of alumina (Buehler), rinsed with water and ethanol, and dried before use.

Nuclear magnetic resonance (NMR) spectra were obtained from either a Bruker AMX-360 MHz or a Bruker DPX-400 MHz spectrometer. Gradient HMQC spectra were obtained on a Bruker DRX-400 operating at 400.13 MHz for ¹H; data processing was performed on Topspin NMR software. X-band electron paramagnetic resonance (EPR) spectra were acquired with a 9.5 GHz Bruker eleXsys 500 spectrometer with a liquid He cryostat at 20 K using 8.22 mW power, 100 kHz modulation frequency, and 5 G modulation amplitude. UV-vis absorbance spectra were collected using a Varian Cary 50 spectrophotometer and a 1 cm path length quartz cuvette, and the spectra were manually corrected for background absorption using separately collected spectra of reference solutions.
Positive ion electrospray mass spectrometry (ESI+ MS) was performed at the Penn State Mass Spectrometry Facility using a Mariner mass spectrometer (Perseptive Biosystems). Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were acquired with a Waters Micromass MALDI micro MX spectrometer.

Molecular structures were calculated using Hyperchem 6.0 using molecular mechanics (MM+) with atomic charge based electrostatic repulsions, and a Polack-Ribiere conjugate gradient to a minimum energy gradient of 0.01 kcal/mol.

### 3.2.3 Synthesis

\( (\text{Fmoc})_2\text{DETA} \cdot \text{HCl} (44) \). A 100 mL solution of DETA (2.00 mL, 18.5 mmol) and \( N,N \)-diisopropylethylamine (DIPEA, 6.42 mL, 36.8 mmol) in dichloromethane (DCM, VWR) was stirred under \( \text{N}_2 \). To this was added 12.1 g of (9-fluorenylmethoxycarbonyl) succinimide (Fmoc-OSu; Novabiochem, 37.1 mmol) in 80 mL of DCM over 1 h, and the resulting solution was allowed to stir under \( \text{N}_2 \) for 16 h. The solution was then shaken vigorously with 50 mL of 1 M HCl, yielding a white precipitate which was isolated by vacuum filtration and dried in vacuo for 16 h. Yield: 4.77 g (44.3%). \( ^1\text{H NMR (400 MHz, } d_4\text{-MeOH}) \): \( \delta \) 3.07 (t, \( J = 4 \text{ Hz, } 4 \text{ H} \)); 3.36 (t, \( J = 4 \text{ Hz, } 4 \text{ H} \)); 4.15 (t, \( J = 6 \text{ Hz, } 2 \text{ H} \)); 4.34 (d, \( J = 5 \text{ Hz, } 4 \text{ H} \)); 7.26 (t, \( J = 7 \text{ Hz, } 4 \text{ H} \)); 7.36 (t, \( J = 7 \text{ Hz, } 4 \text{ H} \)); 7.58 (d, \( J = 7 \text{ Hz, } 4 \text{ H} \)); 7.76 (d, \( J = 7 \text{ Hz, } 4 \text{ H} \)). \( ^{13}\text{C NMR (400 MHz, } d_4\text{-MeOH}) \): \( \delta \) 13.2, 17.3, 18.7, 26.3, 55.8, 121.0, 126.1, 128.1, 128.8, 142.6, 145.2. ESI+ MS (m/z): calcd, \((\text{M} + \text{H})^+ = 548.3\); found, \((\text{M} + \text{H})^+ = 548.3\).

\( (\text{Fmoc})_2\text{DETA(bpy)} \cdot \text{HCl} (29) \). A 25 mL solution containing 1.73 g of 44 (2.96 mmol) and 1.27 mL of DIPEA (7.30 mmol) in \( N,N \)-dimethylformamide (DMF, VWR)
was stirred under N\textsubscript{2}. To this was added 1.02 g of bpy-CH\textsubscript{2}CO\textsubscript{2}H (4.48 mmol), 2.26 g of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, Novabiochem, 5.96 mmol), and 75 mL of DMF. The yellow solution was allowed to stir under N\textsubscript{2} for 16 h and then poured into 1.3 L of 1 M HCl, giving a white precipitate. The solid was collected by vacuum filtration and dried under vacuum for 16 h. Yield: 2.17 g (92.2%). \textsuperscript{1}H NMR (360 MHz, \textit{d}\textsubscript{6}-DMSO): δ 2.46 (s, 3 H); 3.17 (t, \textit{J} = 6 Hz, 2 H); 3.24 (t, \textit{J} = 5 Hz, 2 H); 3.33 (t, \textit{J} = 6 Hz, 2 H); 3.42 (t, \textit{J} = 6 Hz, 2 H); 3.95 (s, 2 H); 4.18-4.22 (m, \textit{J} = 7 Hz, 2 H); 4.26 (d, \textit{J} = 6 Hz, 2 H); 4.32 (d, \textit{J} = 6 Hz, 2 H); 7.28 (t, \textit{J} = 8 Hz, 4 H); 7.38 (t, \textit{J} = 8 Hz, 4 H); 7.56-7.66 (m, \textit{J} = 8 Hz, 5 H); 7.84-7.87 (m, \textit{J} = 8 Hz, 5 H); 8.37 (s, 1 H); 8.39 (s, 1 H); 8.64 (d, \textit{J} = 6 Hz, 1 H); 8.70 (d, \textit{J} = 6 Hz, 1 H). \textsuperscript{13}C NMR (400 MHz, \textit{d}\textsubscript{6}-DMSO): δ 21.2, 30.8, 35.8, 46.1, 46.7, 47.9, 65.4, 120.1, 123.9, 124.5, 125.1, 127.0, 127.3, 127.6, 128.0, 140.7, 143.8, 144.8, 146.8, 147.1, 151.2, 156.4, 162.3, 168.8.

ESI+ MS (\textit{m}/\textit{z}): calcd, (M + H)+ = 758.3; found, (M + H)+ = 758.4.

Fmoc-aeg(py)-DETA(bpy)-aeg(py)-Fmoc (31). To a 20 mL solution containing 0.5273 g of 29 (0.664 mmol) in DMF was added 1.60 g of tetrabutylammonium fluoride trihydrate (TBAF-3H\textsubscript{2}O, 5.06 mmol), and the red solution was sonicated under N\textsubscript{2} for 15 min. A 3.43 g amount of 1-hydroxybenzotriazole (HOBt, 25.4 mmol) was added, forming a yellow solution which was stirred under N\textsubscript{2} for 30 s, and 0.804 g of 30 (1.62 mmol) and 0.312 g of 1-[(3-dimethylamino)propyl]-3-ethylcarbodiimide (EDC, 1.63 mmol) were subsequently added. This solution was stirred under N\textsubscript{2} for 1 h, and 20 mL of Et\textsubscript{2}O (VWR) and 40 mL of EtOAc were added. The solution was purified by extraction with 3 × 50 mL of 5% NaHCO\textsubscript{3} and 3 × 50 mL of H\textsubscript{2}O; the aqueous washings were discarded. Petroleum ether was then added to the organic layer, giving a white solid.
which was allowed to precipitate for 16 h. The mother liquor was decanted, and the precipitate was dispersed by sonication in petroleum ether, allowed to settle as a fine white precipitate which was collected by vacuum filtration, and dried in vacuo for 3 h. Yield: 197 mg (24.8%). \(^1\)H NMR (360 MHz, \(d_6\)-DMSO): \(\delta\) 2.39 (s, 3 H); 3.16 (t, \(J = 5\) Hz, 4 H), 3.32 (m, 18 H); 3.55 (s 4 H); 4.18-4.32 (m, 6 H); 7.12 (t, \(J = 7\) Hz, 1 H); 7.19 (t, \(J = 7\) Hz, 1 H); 7.30 (t, \(J = 7\) Hz, 4 H); 7.39 (t, \(J = 7\) Hz, 4 H); 7.66 (d, \(J = 7\) Hz, 4 H); 7.87 (d, \(J = 7\) Hz, 4 H); 7.98 (d, \(J = 7\) Hz, 4 H); 8.19 (s, 1 H); 8.27 (s, 1 H); 8.36-8.55 (m, 6 H). HMQC NMR spectrum of 31 is shown and discussed in detail in Appendix D. ESI+ MS (m/z): calcd, (M + H)+ = 1196.5; found, (M + H)+ = 1196.5.

\textit{Fmoc-aeg[Pt(tpy)(py)]-DETA(bpy)-aeg[Pt(tpy)(py)]-Fmoc(PF_6)_4} (45). To a solution containing 0.185 g of [Pt(tpy)]I·2H_2O (0.258 mmol), 49 mL of acetonitrile (ACN, VWR), and 14 mL of H_2O was added 91.0 mg of AgNO_3 (Acros, 0.536 mmol). The solution was stirred vigorously for 5 min and centrifuged to isolate precipitated AgI. The mother liquor was decanted, recentrifuged, and decanted to ensure that all AgI had been removed. The solution was then filtered into a flask containing 0.152 g of 31 (0.127 mmol) and the resulting solution stirred at 40 °C for 1 h. The ACN was then removed under reduced pressure and the product precipitated with saturated aqueous ammonium hexafluorophosphate (NH_4PF_6, Acros) to give an orange solid which was collected and recrystallized from acetone/H_2O. The sample was dried under vacuum for 16 h. Yield: 178 mg (53.3%). A 1.13 mM stock solution of 45 was prepared by dissolution of 178 mg of 45 in 60 mL of acetonitrile and subsequently used for the preparation of compounds 47-51.
To a 10 mL solution containing 0.101 g of tripeptide 31 (0.0840 mmol) in ACN was added a 3.1 mL amount of 8.91 mM [Fe(ClO$_4$)$_2$] (0.0280 mmol). The red solution was stirred under N$_2$ for 3 h, the solvent removed under reduced pressure, and a saturated aqueous NH$_4$PF$_6$ solution added to yield a red precipitate. The solid was collected by centrifugation, washed with H$_2$O, and dried under vacuum for 16 h. Yield: 91.9 mg (83.4%).

A 20 mL portion of the 1.13 mM solution of 45 (22.6 µmol) was added to a methanolic solution of 52.3 mM [Fe(ClO$_4$)$_2$] (144 µL, 7.53 µmol) and the resulting solution stirred for 16 h under N$_2$. The volume was reduced by rotary evaporation, and a red solid precipitated upon addition of aqueous NH$_4$PF$_6$. The solid was collected by centrifugation, rinsed, and dried under vacuum for 16 h. Yield: 57.4 mg (92.6%). $^1$H NMR (400 MHz, $d_6$-DMSO): δ 2.33 (m, 5 H); 2.66 (m, 3 H); 2.72 (s, 2 H); 2.88 (s, 3 H); 3.11-3.73 (br m, 130 H); 3.88-4.48 (br m, 18 H); 7.09-7.45 (br m, 30 H); 7.50-7.92 (br m, 48 H); 7.96 (t, $J$ = 6 Hz, 12H); 8.00-8.43 (m, 15 H); 8.47-8.77 (m, 48 H); 8.79-8.89 (m, 3H); 8.99 (d, $J$ = 6 Hz, 6 H).

A 20 mL portion of the 1.13 mM solution of 45 (0.0226 mmol) was added to a methanolic solution of 17.7 mM [Cu(OAc)$_2$]-H$_2$O (144 µL, 0.0112 mmol) and the resulting solution stirred for 16 h. The volume was reduced by rotary evaporation, and a light green colored solid was precipitated using a saturated aqueous solution of NH$_4$PF$_6$. The solid was collected by centrifugation, rinsed, and dried under vacuum for 16 h. Yield: 52.9 mg (83.5%). $^1$H NMR (400 MHz, $d_6$-DMSO): δ 2.33 (m, 3 H); 2.67 (m, 2 H); 2.89 (s, 1 H);
3.32 (br m, 51 H); 3.54 (m, 2 H); 4.01-4.41 (br m, 11 H); 7.12-7.42 (br m, 20 H); 7.55-7.90 (br m, 32 H); 7.98 (t, J = 6 Hz, 8 H); 8.51-8.75 (m, 32 H); 9.00 (d, J = 6 Hz, 4 H).

\[
[Zn(Fmoc-aeg[Pt(tpy)(py)]-DETA(bpy)-aeg[Pt(tpy)(py)]-Fmoc)_2](PF_6)_{10} \ (49)\n\]

A 20 mL portion of the 1.13 mM solution of 45 (0.0226 mmol) was added to a methanolic solution of 51.8 mM Zn(OAc)_2·H_2O (Strem, 634 µL, 0.0113 mmol) and the resulting solution stirred for 16 h. The volume was reduced by rotary evaporation, and saturated aqueous NH_4PF_6 was added to precipitate a yellow solid. The solid was collected by centrifugation, rinsed with H_2O, and dried under vacuum for 16 h. Yield: 55.8 mg (88.0%). ^1H NMR (400 MHz, d_6-DMSO): δ 2.32 (m, 3 H); 2.67 (m, 2 H); 2.89 (s, 1 H); 3.01-3.48 (br m, 72 H); 4.09-4.33 (br m, 14 H); 7.12-7.41 (br m, 20 H); 7.60-7.95 (br m, 32 H); 7.98 (t, J = 6 Hz, 8 H); 8.01-8.36 (m, 10 H); 8.50-8.73 (m, 32 H); 8.89-8.96 (m, 2 H); 9.00 (d, J = 7 Hz, 4 H).

\[
[Fe(Fmoc-aeg[Cu(pda)(py)]-DETA(bpy)-aeg[Cu(pda)(py)]-Fmoc)_3](PF_6)_2 \ (50)\n\]

A 10 mL solution containing 43.7 mg of 46 (0.0111 mmol) in ACN was added to 3.30 mL of a methanolic solution of 20.3 mM [Cu(pda)(H_2O)] (0.0669 mmol) and the resulting solution allowed to stir for 16 h. The volume was reduced by rotary evaporation and Et_2O added, yielding a maroon precipitate. The solid was collected by centrifugation, rinsed with H_2O, and dried under vacuum for 16 h. Yield: 48.6 mg (82.5%). ^1H NMR (400 MHz, d_6-DMSO): δ 2.32 (m, 6 H); 2.66 (m, 3 H); 2.73 (s, 3 H); 2.89 (s, 4 H); 3.02-3.59 (br m, 75 H); 4.01-4.48 (br m, 8 H); 6.76-7.47 (br m, 48 H); 7.56-7.74 (br m, 12 H); 7.77-7.98 (m, 12 H).
3.2.4 Spectrophotometric Titrations

**Formation of 45 by Titration of 31 with [Pt(tpy)](NO$_3$)$_2$.** The titration was performed using a Varian Cary 500 double-beam spectrophotometer. To a solution containing 0.879 mg of [Pt(tpy)]I·2H$_2$O (1.22 µmol) in 10 mL of 7:2 ACN/H$_2$O was added 0.622 mg of AgNO$_3$ (3.66 µmol). This solution was stirred vigorously for 5 min and centrifuged to isolate precipitated AgI.$^{36}$ The mother liquor was decanted, recentrifuged, decanted again, and finally filtered to ensure removal of all solid AgI. A solution of 31 was then prepared in ACN and filtered. The concentration of the oligopeptide (0.353 mM) was determined from its molar extinction coefficient ($\varepsilon = 15119$ M$^{-1}$ cm$^{-1}$) at 379 nm. This solution was titrated into 2.5 mL of the [Pt(tpy)]$^{2+}$ solution in 15 µL increments, and the solution was stirred at 50 °C for 5 min, followed by 5 min of stirring at room temperature after each addition.$^{17}$ To account for changes in absorption due to sample dilution, an equal volume of ACN was added to the reference cell (containing only the [Pt(tpy)]$^{2+}$) for each iterative addition of oligopeptide. Following the experiment, the oligopeptide/[Pt(tpy)]$^{2+}$ solution was back-titrated with [Pt(tpy)]$^{2+}$ to reach the equivalence point, and this solution was stirred at 50 °C for 15 min and at room temperature for 5 min.

**Formation of 47 by Titration of 45 with Fe(ClO$_4$)$_2$.** A 31.5 µM solution of the [Pt(tpy)]$^{2+}$-functionalized oligopeptide, compound 45, was prepared by serial dilution from the previous titration. A solution of 0.263 mM Fe(ClO$_4$)$_2$ was prepared in ACN and titrated into 2.5 mL of the [Pt(tpy)]$^{2+}$/oligopeptide solution in 10 or 100 µL increments. After each addition, the solution was allowed to stir for 15 min, and UV-vis absorbance spectra were obtained.
3.3 Results and Discussion

3.3.1 Synthesis of Heterofunctionalized Tripeptides

In our previous reports,\textsuperscript{16,17} the use of solid-phase peptide synthesis enabled the preparation of molecules with multiple ligands tethered to an oligopeptide backbone. To increase the synthetic yield, rapidly produce multifunctional peptides, and eliminate the asymmetry of the different \textit{N}- and \textit{C}-termini on the chain, we have developed an alternative approach to prepare these artificial oligopeptides. We have instead designed palindromic oligopeptides that are easily prepared in solution rather than requiring the use of a solid resin support. DETA, which contains a central secondary amine and two terminal primary amines, was chosen because of its adaptability to our previously developed synthetic methods; Figure 3-1 shows the method by which the central monomeric unit is prepared from DETA and a monocarboxylated bpy ligand. To covalently link the bpy ligand (\textit{42}) to the DETA secondary amine of DETA, it was first necessary to protect the two terminal amines by adding stoichiometric amounts of Fmoc-O\textsubscript{5}u and DIPEA to a stirring solution of DETA. Following protonation of the di-Fmoc protected DETA, \textit{42} was coupled to the secondary amine using HBTU.

Subsequent attachment of two pyridine-modified aeg monomers to either end of the DETA monomer (see Figure 3-1) is accomplished in a single-pot reaction in nearly quantitative yield. After TBAF deprotects the terminal amines, HO\textsubscript{5}t is added to quench free F\textsuperscript{−} in solution. To ensure complete amide coupling, 2.5 molar equiv of both Fmoc-aeg(py)-OH·HCl and EDC were used, a reduction relative to the (typical) 4-fold molar excess for single-site coupling reaction in solid-phase synthesis. The artificial tripeptide
Figure 3-1. Synthesis of the mixed-ligand tripeptide.
was prepared in >100 mg quantities (25% yield) with high purity using significantly less material (solvents, reagents, etc.) and in a relatively shorter time. Importantly, the resulting tripeptide (31) is both heterofunctional and symmetrically substituted along its backbone. While this approach can be used to prepare oligopeptides containing any two similarly substituted ligands, and extended to longer sequences by subsequent coupling reactions, in this study we chose to prepare and fully characterize tripeptides containing bpy and py ligands on the basis of their well-known coordination chemistry with several metallic species and to make connections to our earlier study.16

3.3.2 Spectrophotometric Titration of Tripeptide

Because the pendant ligands have different denticities on the tripeptide backbone, judicious choice of the order with which metal ions or complexes are added can create a library of heterometallic, multinuclear compounds. The two possible routes are shown in Figure 3-2. We reasoned that tetracoordinate metal ions bearing a tridentate ligand (i.e., [Pt(tpy)]^{2+} and Cu(pda)) should preferentially bind to the pyridine ligands to fulfill their preferred coordination geometry. Unligated metal ions (Fe^{2+}, Cu^{2+}, and Zn^{2+}) should alternatively bind to the pendant bpy ligands; site-specific coordination is expected both because the bpy ligands are the only vacant sites on the peptide and on the basis of the larger binding affinity of the bidentate (vs monodentate) ligands. To test this method, we first performed spectrophotometric titrations of tripeptide 31 with the complex [Pt(tpy)]^{2+}, which we have shown previously binds strongly and in stoichiometric quantities to pyridine-substituted oligopeptides.17 The [Pt(tpy)(py)]^{2+} complex has large extinction at distinct wavelengths in the UV region of the spectrum, making observation
Figure 3-2. Synthesis of heteromultimetallic tripeptides.
of complexation of \([\text{Pt(tpy)}]^2+\) to py straightforward.\textsuperscript{38} The difference spectra shown in **Figure 3-3A** were acquired during the titration of \([\text{Pt(tpy)}]^2+\) with tripeptide 31; the figure shows the increase in the intensity of the characteristic metal-to-ligand charge transfer (MLCT) absorbance at 348 nm following each iterative addition. The MLCT absorption increases and remains constant at a molar ratio of \(\sim 2\) equiv of \([\text{Pt(tpy)}]^2+\) per oligopeptide. This ratio is consistent with the stoichiometric addition of two \([\text{Pt(tpy)}]^2+\) ions to the peptide at each of the pyridine ligands, shown in **Figure 3-2** as compound 45.

In this dimetalated peptide, an unmetalated bpy ligand remains available for complexation to another metal ion. Titration of this molecule with additional transition-metal ions would therefore create a heterometallic structure. To be able to again monitor this by UV-vis absorption spectroscopy, we performed a second titration with Fe\(^{2+}\), which would be expected to strongly bind any uncoordinated bpy ligands. **Figure 3-3B** shows the difference spectra acquired during the titration of 45 with Fe\(^{2+}\); the iterative additions cause the evolution of spectra with increased absorption intensity and a peak at 538 nm, which is attributed to the well-known MLCT band of \([\text{Fe(bpy)}_3]^2+\).\textsuperscript{39} The inset of **Figure 3-3B** shows that the peak intensity at 538 nm increases and levels off at a molar ratio of \(\sim 0.3\) Fe\(^{2+}\) to 1 tripeptide. Because Fe\(^{2+}\) is known to preferentially form tris(bipyridine) complexes, this result implies that one Fe\(^{2+}\) ion cross-links three oligopeptide strands: the resulting heterometallic structure is a peptide-based, supramolecular structure containing six pendant \([\text{Pt(tpy)(py)}]^2+\) complexes, compound 47 (**Figure 3-2**).
Figure 3-3. UV-vis absorption difference spectra acquired during (A) the titration of [Pt(tpy)(H$_2$O)](NO$_3$)$_2$ with 31 and (B) the subsequent titration of that product (i.e., the [Pt(tpy)]$^{2+}$-coordinated oligopeptide) with Fe(ClO$_4$)$_2$. Solutions were in ACN and the spectra plotted as the change in absorption vs a reference solution containing a molar equivalent of metal ion in the absence of oligopeptide. Insets: Change in absorption for each iterative addition, at (A) 348 nm and (B) 538 nm, plotted against the molar ratio of metal to peptide or complex.
3.3.3 Formation of Heteromultimetallic Complexes

We utilized a similar strategy of sequential complexation with different metal ions and complexes to prepare a series of complexes. Spectrophotometric titration of 45 with Cu$^{2+}$ and Zn$^{2+}$ is difficult to observe because of their significantly smaller extinction coefficients. Therefore, we prepared the analogous heterometallic complexes of these metals by adding stoichiometric quantities of Zn$^{2+}$ and Cu$^{2+}$ to the diplatinated tripeptide 45. The resulting complexes, which each contain four [Pt(tpy)(py)]$^{2+}$ complexes tethered to two peptide strands that are cross-linked by either [Zn(bpy)$_2$]$^{2+}$ or [Cu(bpy)$_2$]$^{2+}$, were isolated by precipitation from an aqueous solution containing saturated NH$_4$PF$_6$, yielding compounds 48 and 49 as their hexafluorophosphate salts.

To test our ability to first coordinate the central bpy ligand (i.e., the alternative route in Figure 3-2), the tripeptide was reacted with Fe$^{2+}$ in a molar ratio of peptide to metal of 3:1 (following the experimentally determined stoichiometric quantity above), again producing the dark red color characteristic of [Fe(bpy)$_3$]$^{2+}$ complexes. This product was isolated and purified by precipitation with NH$_4$PF$_6$ before it was reacted with the complex [Cu(pda)(H$_2$O)], which we have previously demonstrated will stoichiometrically bind to pyridines on the oligopeptide backbone. The resulting complex 50 (in Figure 3-2), which contains six [Cu(pda)(py)] complexes tethered to peptide strands that are linked by a central [Fe(bpy)$_3$]$^{2+}$ complex, was precipitated by the addition of ether.

Validation of this method of preparation of heterometallic oligopeptides is gained by examination of their purity and identity using $^1$H NMR. The peaks in the $^1$H NMR spectra of the metalated complexes are broadened because of the increased $T_2$ relaxation times, a consequence of the large size of the molecules. In addition, the flexible nature of
the peptide backbone makes it possible for several solution conformations that give rise to slightly different environments for otherwise equivalent protons. While 2D NMR spectroscopic techniques are useful for determination of the metalated oligopeptide sequence, and in some cases structure, those experiments are complicated by the presence of paramagnetic metal ions in some of the materials in this chapter. We are however able to assess the purity of the synthesized materials by comparison of the number of aromatic vs aliphatic protons in the 1-D proton NMR spectra. For example, both complexes 48 and 50 contain paramagnetic Cu$^{2+}$; complexation to this metal center shifts the ligand protons far downfield so that they are not observed in the aromatic region of the spectrum. As a result, the $^1$H NMR spectrum of complex 48 does not contain peaks associated with the bpy ligand; for complex 50, the pyridyl protons are absent. These observations confirm stoichiometric complexation of Cu$^{2+}$ to the bpy and py ligands, respectively. In all cases, comparison of the number of aliphatic and aromatic protons is consistent with the number expected for the fully metalated complexes.

3.3.4 Electronic Absorption Spectroscopy

We designed the multimetallic oligopeptides with the expectation that they would have optical, electronic, and magnetic properties that are characteristic of each of the metal complexes from which they are constructed. Each sequence should therefore have unique signatures that may be “read out” using spectroscopic and electrochemical techniques. Therefore, UV-vis absorption spectroscopy was used to additionally confirm the identity of our complexes and conversely to measure the sequence-specific electronic states available in the multimetallic peptides. The spectra for solutions of each of the
metalated oligopeptide complexes were obtained and compared to those for their small-molecule components, shown in Figure 3-4. The spectra for complexes 47-49 (Figure 3-4A through 3-4C) contain distinctive peaks at 333 and 347 nm that also appear in the spectrum for [Pt(tpy)(py)]^{2+}. These peaks are known to result from the MLCT, confirming that 47-49 all contain bound [Pt(tpy)(py)]^{2+} moieties. The extinction of the [Pt(tpy)(py)] MLCT absorption in compound 47 is ~1.5x greater than that in 48 and 49; this difference is expected because of its larger number of Pt complexes.

The spectra for compounds 47 and 50 (Figure 3-4A and 3-4D) also have visible absorption peaks centered at a wavelength of 537 nm that correspond to the MLCT band observed for [Fe(bpy)]_3^{2+}, confirming the presence of this species in these structures. While the extinction coefficient for this band in [Fe(bpy)]_3^{2+} complexes is large, the size of the MLCT peak in Figure 3-4A is overshadowed by the [Pt(tpy)(py)]^{2+} transitions, which are present in a larger quantity than the peptide scaffold. In the case of 50, the weak absorption peak observed at 740 nm is due to the ligand-to-metal charge transfer (LMCT) band of [Cu(pda)(py)]. As expected, there are no observable peaks in the UV-vis region of the spectrum for 49 that correspond to the colorless [Zn(bpy)]^{2+} complex. In all cases, the electronic absorption spectra of compounds 47-50 are consistent with the linear combinations of the spectra obtained from their small-molecule components using the stoichiometry of the relative ratios of the metal complexes tethered by the peptide backbone.
Figure 3-4. UV-vis absorbance spectra of heterometallic assemblies and respective small-molecule components: (A) compound 47 (---) with [Fe(Me$_2$bpy)$_3$]$^{2+}$ (―) and [Pt(tpy)(py)]$^{2+}$ (•••); (B) compound 48 (---) with [Cu(Me$_2$bpy)$_2$]$^{2+}$ (―) and [Pt(tpy)(py)]$^{2+}$ (•••); (C) compound 49 (---) with [Zn(Me$_2$bpy)$_2$]$^{2+}$ (―) and [Pt(tpy)(py)]$^{2+}$ (•••); (D) compound 50 (---) with [Fe(Me$_2$bpy)$_3$]$^{2+}$ (―) and [Cu(pda)(py)] (•••). All spectra were normalized for complex concentration in DMF solutions. The insets contain expanded regions of the visible part of the spectrum.
3.3.5 Electron Paramagnetic Resonance Spectroscopy

Two of the metalated oligopeptides contain paramagnetic metals, which gave characteristic shifts in the $^1$H NMR spectra (vide supra): 48 and 50 each contain Cu$^{2+}$ complexes with unpaired electrons whose environments can also be probed using electron paramagnetic resonance (EPR) spectroscopy. Figure 3-5 contains EPR spectra obtained from frozen DMF solutions of each of these complexes. The larger signal-to-noise ratio in the spectra of complex 50 vs 48 arises from the larger number of bound Cu$^{2+}$ centers per oligopeptide: for 48, there is a single Cu$^{2+}$, whereas complex 50 contains six Cu$^{2+}$ complexes bound to each tripeptide triplex. In addition to the magnitudes of the signals, the shapes of the EPR signals are quite different for these two metalated materials. Each of the spectra have the $g$ values and hyperfine splitting (Table 3-1) that are characteristic of Cu$^{2+}$ complexes with square planar geometry ($g _{||}$ > 2.1 > $g _{⊥}$ > 2.0; $A_{||}$ is in the range of (158-200) × 10$^{-4}$ cm$^{-1}$).$^{43,44}$ However, the smaller hyperfine splitting in 50 is indicative of an oligopeptide containing Cu$^{2+}$ complexes that are (at least weakly) electronically coupled; analysis of this spectrum gives parameters that are similar to those that we have previously reported for a series of pyridine-substituted oligopeptides containing six bound [Cu(pda)(py)] complexes.$^{16,17}$ The similarity between the spectrum for that homometallic hexamer and the EPR spectra shown here for 50 indicates that the central [Fe(bpy)$_3$]$^{2+}$ complex has little or no effect on the electronic environment of the pendant Cu$^{2+}$ complexes. The diminution of hyperfine splitting in the spectrum leads to the conclusion that, even in frozen solution, the Cu complexes are only weakly coupled, which likely arises from favorable van der Waals forces that result from $\pi$-$\pi$ stacking of the aromatic rings of the pda ligand.$^{17}$ Although the signal is less well defined, the EPR
Figure 3-5. X-band EPR spectra of frozen solutions of (A) 0.85 mM 48 and (B) 0.22 mM 50 in DMF.
Table 3-1. EPR Parameters and Hyperfine Coupling Constants for Oligopeptide Assemblies 48 and 50

<table>
<thead>
<tr>
<th></th>
<th>Cu center</th>
<th>Cu/oligopeptide complex</th>
<th>$g_\perp$</th>
<th>$g_\parallel$</th>
<th>$A_{\parallel} \times 10^{-4}$ (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>[Cu(bpy)$_2$$^{2+}$]</td>
<td>1</td>
<td>2.08</td>
<td>2.24</td>
<td>165</td>
</tr>
<tr>
<td>50</td>
<td>[Cu(pda)(py)]</td>
<td>6</td>
<td>2.09</td>
<td>2.27</td>
<td>131</td>
</tr>
</tbody>
</table>
spectrum of complex 48 (Figure 3-5A) has larger hyperfine splitting, indicative of an electronically isolated Cu$^{2+}$ complex. The shape of this spectrum is nearly identical to that reported for [Cu(bpy)$_2$]$^{2+}$, which is distorted due to the presence of both the cis- and trans- isomers of coordinated H$_2$O ligands. The sensitivity of EPR to detect small changes in the bonding environment is in agreement with the earlier $^1$H NMR results that showed that complex 48 (as well as the other tripeptides) contain coordinated H$_2$O ligands. Finally, the similarity between the EPR spectrum in Figure 3-5A and that of the small-molecule analogue implies that the presence of the four pendant [Pt(tpy)(py)]$^{2+}$ complexes causes no discernible distortion in the EPR spectrum, again indicating a lack of coupling between the metallic complexes.

3.3.6 Electrochemistry

Analogous to the above spectroscopic methods, unique voltammetric signatures are expected for each of the multimetalated peptides on the basis of the number and type of bound metal complexes. Several of the metals used to decorate the tripeptide backbone have experimentally accessible redox states, so electrochemical methods are used to further characterize these materials. Moreover, electronic communication between adjacent complexes should be readily discernible on the basis of the waveshapes and formal potentials in the voltammetry.

We used microelectrode voltammetry to measure the solution-phase redox behavior of each of the molecules because of the greater flux to these electrode surfaces, which reduces the differences in the voltammograms due to heterogeneous electron-transfer rates. All reactions were carried out in air-free environments to reduce the
chemical irreversibility of some of the complexes in the presence of dioxygen. Cyclic voltammograms for 47-49, normalized to the concentration of the metalated materials, are shown in Figure 3-6: all three of the voltammograms contain two large, sigmoidally shaped reductions with half-wave potentials ($E_{1/2}$) of -0.6 and -1.1 V. These waves are attributed to the two sequential tpy-centered reductions (tpy$^{0/1-}$ and tpy$^{1/-2-}$) and are not observed for complex 50. In the voltammogram of compound 47, the current of the first reduction wave ($E_{1/2} = -0.60$ V) is 50% larger than observed for compounds 48 and 49, which is a result of 47 containing six pendant [Pt(tpy)(py)]$^{2+}$ moieties vs four in compounds 48 and 49. In all three compounds, the observed currents for the second tpy reduction ($E_{1/2} = -1.1$ V) are larger than those for the tpy$^{0/1-}$ reaction. Since in the small molecule the tpy reductions are both electrochemically reversible, one-electron reactions, the difference in magnitude in the limiting currents in the voltammograms in Figure 3-6 is not due to a change in the redox properties of the molecule. Instead, the enhanced currents in the second wave are attributed to an overlapping reduction reaction that is associated with the bpy ligands that are also tethered to the tripeptide. In the small-molecule analogues, the first bpy-centered reduction occurs at an $E_{1/2}$ of -1.1 V and is slightly shifted depending on the metal to which it is coordinated (see Table 3-2).

Examination of the voltammograms at anodic potentials (Figure 3-6 inset) indicates clear differences in the oxidation reactions for 47-49 that are a result of the variation in the metal forming the [M(bpy)$_n$]$^{2+}$ peptide cross-link. Complex 47 possesses an oxidative wave at an $E_{1/2}$ of +0.96 V that is attributed to the Fe$^{3+/2+}$ oxidation. Alternatively, the voltammogram of 48 contains an electrochemically irreversible wave, typical for Cu$^{2+/1+}$ reactions because of a conformational change from square planar to
Figure 3-6. Cyclic voltammograms of 47 (---), 48 (—), and 49 (----), performed with a 25 µm diameter Pt working electrode, a Pt wire counter electrode, and a Ag/Ag⁺ reference electrode. All solutions contained 0.25 M TBAH in DMF using a potential scan rate of 50 mV/s. The currents are normalized for the concentration of the complex.
Table 3-2. Summary of Electrochemical Data for 47-49

<table>
<thead>
<tr>
<th>compound</th>
<th>$E^\circ$ (V vs SCE)$^b$</th>
<th>$D \times 10^6$ (cm$^2$/s)</th>
<th>$r_H$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe$^{3+/2+}$</td>
<td>Cu$^{2+/1+}$</td>
<td>tpy$^{0/1-}$</td>
</tr>
<tr>
<td>47</td>
<td>+0.96</td>
<td>-0.59</td>
<td>-1.16</td>
</tr>
<tr>
<td>48</td>
<td>+0.36</td>
<td>-0.62</td>
<td>-1.15</td>
</tr>
<tr>
<td>49</td>
<td>-0.64</td>
<td>-1.12</td>
<td></td>
</tr>
<tr>
<td>[Fe(bpy)$_3$]$^{2+}$</td>
<td>+0.95</td>
<td>-1.10</td>
<td>-1.30</td>
</tr>
<tr>
<td>[Pt(tpy)(py)]$^{2+}$</td>
<td>-0.65</td>
<td>-1.12</td>
<td></td>
</tr>
<tr>
<td>[Cu(bpy)$_2$]$^{2+}$</td>
<td>+0.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Cu(pda)(py)]</td>
<td>+0.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ All experiments performed in DMF solutions containing 0.25 M TBAH. $^b$ Formal potentials determined either from the half-wave potential (~$E_{1/2}$) in microelectrode cyclic voltammograms (Figure 3-6) or the average peak potential in macroelectrode voltammograms (not shown). $^c$ Calculated from the diffusion coefficients using eq 3.2. $^d$ Not determined because of the electrochemical and chemical irreversibility of these reactions.
tetrahedral during this redox reaction.\textsuperscript{49} No oxidation wave is observed in \textit{49}, which is consistent with the known lack of oxidative redox chemistry.

Analysis of the relative magnitudes for each of the redox reactions for a particular heterometallic tripeptide provides a means to assess the relative quantities of the two coordinated metal complexes. For example, careful examination of the cyclic voltammogram for complex \textit{47} indicates that the limiting current ($i_{\text{lim}}$) for the \([\text{Fe(bpy)}_3]^{3+/2+}\) reaction is 2.3 $\mu$A; for the first tpy reduction of \([\text{Pt(tpy)(py)}]^{2+}\) $i_{\text{lim}}$ is 15.7 $\mu$A.\textsuperscript{50} These values are used to quantitatively evaluate the solution transport of these materials using the expression for the limiting current at a microelectrode:\textsuperscript{47}

$$i_{\text{lim}} = 4nF\text{r}DC$$  \hspace{1cm} (eq 3.1)

where $n$ is the number of electrons exchanged in the redox reaction, $F$ is Faraday’s constant (96485 C/mol), $r$ is the radius of the microelectrode (cm), $D$ is the molecular diffusion coefficient (cm$^2$/s), and $C$ is the molecular concentration (mol/cm$^3$). Since the \([\text{Fe(bpy)}_3]^{2+}\) and \([\text{Pt(tpy)(py)}]^{2+}\) are linked by the tripeptide scaffold, they possess the same diffusion coefficient. The difference in the amount of observed current for the two reactions (both of which are known to be one-electron processes) is due to the difference in the number of metal complexes per oligopeptide. On the basis of the ratio of the limiting currents, complex \textit{47} contains 1 \([\text{Fe(bpy)}_3]\) molecule:6.8 \([\text{Pt(tpy)(py)}]\) molecules, consistent with the molar ratio of 1:6 that is confirmed separately. The slight difference may arise from an attenuated Fe oxidative current as a result of its location at the core of this peptide supramolecular structure or slight enhancement of the tpy reduction because of small amounts of O$_2$ or H$_2$O in the sample, both of which contribute to the background currents at these potentials. Alternatively, since the multimetallic structure has an overall
charge of +14, its mass transport to the positively charged anode may be slowed by electrostatic repulsion. However, the voltammograms contain no evidence for adsorption of these molecules to the electrode surface, so the differences in current are not due to anomalous behavior. Similar analysis of the two metal complexes that comprise each of compounds 48-50 is limited because of the electrochemical irreversibility (for the Cu complexes) or lack of a redox wave (for Zn).

The limiting currents observed for the first tpy reduction for compounds 47-49 (Figure 3-6) are used with eq 3.1 to calculate the diffusion coefficients for these compounds, and these are also given in Table 3-2. The data show that the calculated diffusion coefficients for compounds 48 and 49 are approximately equivalent, and larger than that of the oligopeptide triplex 47. To understand these data, we used the energy-minimized structures in Figure 3-7 to compare the relative molecular sizes. These molecular models show that even though the metal-coordinated peptides are highly flexible in solution, the diameter of the Fe-linked peptide triplex is ~4 times greater than that of the small-molecule analogue [Fe(Me₂bpy)₃]²⁺. Comparison of the electrochemical data shows that the multimetallic structures diffuse 4-5 times slower than their small-molecule analogues (Table 3-2, see Figure 3-8 for small molecule cyclic voltammetry). The relationship between the molecular size and measured diffusion coefficient is given by the Stokes-Einstein relationship

\[ D = \frac{k_B T}{6 \pi \eta r_H} \]  

(eq 3.2)

where \( k_B \) is Boltzmann’s constant, \( T \) is the absolute temperature, \( \eta \) is the solution viscosity, and \( r_H \) is the hydrodynamic radius of the molecule. Thus, the measured \( D \) values are used to calculate the hydrodynamic radii for each of the multimetallic peptides,
Figure 3-7. Energy-minimized (MM+) molecular models of (A) [Fe(Me₂bpy)₃]²⁺, (B) compound 48, and (C) compound 47, calculated using Hyperchem 6.0. The scale bar indicates 2.0 nm.
Figure 3-8. Cyclic voltammetry of 11.28 mM [Cu(Me₂bpy)₂]²⁺ (---), 4.95 mM [Fe(Me₂bpy)₃]²⁺ (----), 10.02 mM [Zn(Me₂bpy)₂]³⁺ (-----), 9.91 mM [Cu(pda)(py)] (●●●), and 1.01 mM [Pt(tpy)(py)]²⁺ (-----) performed with a 3.0 mm radius Pt working electrode, a Pt wire counter electrode, and a Ag/Ag⁺ reference electrode. Potentials are converted to SCE using an internal reference. All solutions contained 0.25 M TBAH in DMF using a potential scan rate of 50 mV/s. All scans normalized for concentration.
which are also given in Table 3-2 with the radii calculated for the small-molecule analogues. The calculated $r_H$ values are systematically larger than those predicted by molecular modeling (Figure 3-7), which is commonly observed in electrochemical measurements because diffusion of the molecule is accompanied by solvent and counterion molecules. However, the relationship between $r_H$ and $D$ for the Cu- or Zn-linked peptide duplexes, compared to the Fe-linked peptide triplex and the small molecules, is consistent with the smaller molecular dimensions in Figure 3-7B, in support of the understanding of their connectivity to the peptide scaffold.

3.4 Conclusions

A heterofunctional metal-binding tripeptide has been synthesized using solution-phase synthetic protocols similar to those used in dendrimer synthesis. This oligopeptide was prepared in substantially greater quantity and in much less time than our previous syntheses performed on solid resin supports. Stoichiometric metal coordination was confirmed by spectrophotometric titrations, $^1$H NMR, EPR, and electrochemistry. We are currently using this same synthetic methodology for the preparation of a tripeptide ‘complement’ to 31, which will ultimately be used in the constructing of double-stranded assemblies.
3.5 References


(41) Some of the spectra indicate the presence of H$_2$O, which is expected to be highly coordinating for Cu$^{2+}$, Pt$^{2+}$, and Zn$^{2+}$, which typically form bis(aquo) complexes. Those protons are taken into consideration for the NMR data analysis.


(50) Because of the slight hysteresis in the current for the forward and reverse scans, the limiting current for the reactions is taken as the average of these two.
Chapter 4

Heterofunctional Oligopeptides for Duplex Assembly by Stoichiometric Metal Chelation

4.1 Introduction

We have previously shown that supramolecular heterometallic assemblies may be constructed from an artificial tripeptide by its sequential reaction with metals ions and complexes.\(^1\) For example, using the tripeptide pyridine-(2,2′-bipyridine)-pyridine (pbp, 51, Figure 4-1), coordination of the inorganic complex [Pt(tpy)]\(^{2+}\) to the pendant pyridine (py) ligands followed by reaction with Cu\(^{2+}\) ion resulted in the formation of a pentanuclear, heterometallic structure. Coordination selectivity relied on geometry of the pendant ligands on the tripeptide and on the relative coordinative saturation of the incoming metal. Using tetracoordinate metals, two tripeptides were crosslinked by one [M(bpy)\(_2\)]\(^{2+}\) (bpy = 2,2′-bipyridine) complex; hexacoordinate metals form a crosslink between three tripeptides (e.g., [M(bpy)\(_3\)]\(^{2+}\)).

Our ongoing efforts seek to construct inorganic supramolecular structures that are analogous to the DNA double-stranded helicate.\(^2\) The pbp tripeptide can be used for their assembly, but requires an artificial oligopeptide sequence that is ‘complementary’. In consideration of four-coordinate metals, a complementary sequence would contain a bidentate ligand (e.g., bpy) flanked by two tridentate ligands (e.g., 2,2′:6′,2″-terpyridine, tpy), which saturates the chelation geometry and drives the formation of metal-linked double-stranded structures. Shown in Figure 4-1, a tripeptide sequence of tpy-bpy-tpy (tbt, 33) satisfies this requirement.
Figure 4-1. Structures of the py-bpy-py (pbp) and tpy-bpy-tpy (tbt) tripeptides.
The solution-phase synthetic methods developed in Chapter 3 provide a route for the preparation of artificial metal-binding oligopeptides containing many more types of ligands to increase the library of multifunctional oligopeptide sequences. The ease and yield of this approach makes it attractive for the synthesis of the complementary tbt oligopeptide. To accomplish this, this chapter first presents the synthesis of a tpy-functionalized monomer for use in oligopeptide coupling chemistry. This monomer is then used in the solution-phase synthetic method to prepare an Fmoc protected tbt tripeptide. Fmoc deprotection using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in the presence of 1-octanethiol is employed to give the tripeptide shown in Figure 4-1.

As we have shown previously, mixing the two tripeptides with Cu$^{2+}$ should lead to chelation-induced crosslinking. A series of experiments are undertaken to monitor this reaction and to identify and characterize the products. Spectrophotometric titrations monitor the reaction stoichiometry, and vary depending on the order in which the reactants are combined. The products of these reactions are examined using NMR and EPR spectroscopies.

4.2 Experimental

4.2.1 Chemicals

All materials were purchased from Aldrich and used as received unless otherwise noted. Tetrahydrofuran (THF, VWR) was dried on an activated alumina column. For all experiments, ultrapure water was used (Barnstead, 18.2 MΩ). 4′-methyl-2,2′:6′,2″-terpyridinyl (52), Fmoc-aeg-OrBu·HCl (34), (Fmoc)$_2$DETA(bpy)·HCl (29), and Fmoc-
aeg(py)-DETA(bpy)-aeg(py)-Fmoc (31) were prepared according to previously published procedures.

4.2.2 Instrumentation and Analysis

High performance liquid chromatography (HPLC) was performed on a Varian system equipped with two quaternary pumps (model 210), an autosampler (model 410), UV-visible detector (model 320), and fraction collector (model 701). Preparatory scale separations were performed with a 100 × 20 mm² C18 column (S-5 µm, 12 nm, YMC, Co.) and a 2 mL injection loop. Elution of the product was monitored using the Fmoc absorbance at 301 nm.

Positive ion electrospray mass spectrometry (ESI+ MS) was performed at the Penn State Mass Spectrometry Facility using a Mariner mass spectrometer (Perseptive Biosystems). Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were acquired with a Waters Micromass MALDI micro MX spectrometer using an α-cyano-4-hydroxycinnamic acid matrix.

All NMR spectra were obtained with Bruker CDPX-300, AMX-360, or DPX-400 MHz spectrometers at room temperature. X-band EPR spectra were obtained using a 9.5 GHz Bruker eleXsys 500 spectrometer equipped with a liquid helium cryostat. All experiments were performed at 20 K, with 10.25 mW power, a modulation frequency of 100 kHz, and modulation amplitude of 5 G.

UV-Vis absorbance spectra were collected using a Varian Cary 500 spectrophotometer and a 1 cm path length quartz cuvette against a solvent background.
4.2.3 Synthesis

4′-Acetic acid-2′,6′-terpyridinyl hydrochloride (53). Under inert atmosphere, a solution of 3.80 mL 2,2,6,6-tetramethylpiperidine (21.7 mmol) in dry THF was stirred in an acetone/dry ice bath. A 12.0 mL portion of a 1.6 M solution of methyllithium (19.2 mmol) in ethyl ether (Et₂O) was added and was stirred for 15 min. A 4.25 g quantity of 52 (17.2 mmol) was dissolved in a minimal amount dry THF and added using an air-tight syringe. The resulting maroon solution was stirred for 2 h and the acetone/dry ice bath was removed. After the solution reached room temperature, it was bubbled with CO₂ (g) for 1 h, over which time the solution turned a turquoise color. The solution was centrifuged leaving a dark brown solid, and the orange mother liquor was decanted. The crude solid was dissolved in ~50 mL of 1 M HCl, and 500 mL ethanol (EtOH) was added. The solution was concentrated by rotary evaporation until green crystals formed. The crystals were collected, washed with EtOH, and dried in vacuo. Yield = 4.02 g (71.4%) (¹H NMR, 400 MHz, d₆-DMSO): 3.96 (s, 2 H); 7.89 (t, J = 6 Hz, 2 H); 8.46 (t, J = 8 Hz, 2 H); 8.63 (s, 2 H); 8.94 (m, 4 H). ESI+ MS (m/z): Calculated (M + H)⁺ = 292.1; Found (M + H)⁺ = 292.1.

Fmoc-aeg(tpy)-OH·TFA (32). A solution of 0.954 g 53 (2.91 mmol), 0.606 g 1-[(3-dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDC, VWR, 3.16 mmol), and 0.653 g 1-hydroxybenzotriazole (HOBt, VWR, 4.83 mmol) was prepared in 200 mL CH₂Cl₂. The solution was stirred for 15 min and then 1.19 g Fmoc-aeg-OtBu·HCl (2.76 mmol) and 2.02 mL N,N-diisopropylethylamine (DIPEA, 11.6 mmol) was added. The orange solution was stirred for 16 h and extracted with H₂O (5 x 100 mL); the aqueous portions were combined and subsequently extracted with 25 mL
CH$_2$Cl$_2$. The CH$_2$Cl$_2$ solutions were combined and dried over Na$_2$SO$_4$. The solvent was removed by evaporation under reduced pressure. The sample was purified by column chromatography using a silica column and an acetone mobile phase. Elution was monitored using a hand-held UV lamp, and the first fraction was collected, dried, and redissolved in 10 mL 2.5% H$_2$O in trifluoroacetic acid (TFA). The green solution was allowed to stir for 2 h and subsequently added to 250 mL Et$_2$O, giving a flocculent white precipitate. The sample was collected and dried under vacuum. Yield = 1.70 g (84.7%) ($^1$H NMR, 300 MHz, $d_6$-DMSO): 3.11 (q, $J = 6$ Hz, 1 H); 3.26 (q, $J = 6$ Hz, 1 H); 3.37 (t, $J = 6$ Hz, 1 H); 3.51 (1, $J = 6$ Hz, t H); 3.89 (s, 1 H); 4.02 (s, 1 H); 4.07 (s, 1 H); 4.18-4.42 (m, 4 H); 7.28 (t, $J = 7$ Hz, 2 H); 7.38 (t, $J = 7$ Hz, 2 H); 7.55 (m, $J = 7$ Hz, 2 H); 7.64 (d, $J = 5$ Hz, 2 H); 7.86 (d, $J = 8$ Hz, 2 H); 8.11 (m, 2 H); 8.37 (d, $J = 8$ Hz, 2 H); 8.68 (m, 4 H). ($^{13}$C NMR, 400 MHz, $d_6$-DMSO): 46.8, 47.7, 48.1, 65.6, 117.2, 120.2, 121.7, 123.0, 125.1, 127.1, 127.6, 139.2, 140.8, 143.9, 147.9, 153.5, 156.3, 156.5, 158.5, 158.8, 169.7, 170.9, 171.3. ESI+ MS (m/z): Calculated (M + H)$^+$ = 614.2; Found (M + H)$^+$ = 614.4.

*aeg(tpy)-DETA(bpy)-aeg(tpy)* (33). To a stirring solution of 0.498 g 29 (0.627 mmol) in 20 mL $N,N$-dimethylformamide (DMF), 1.62 g tetrabutylammonium fluoride trihydrate (TBAF, 5.13 mmol) was added. The red solution was stirred under N$_2$ and sonicated for 15 min. After addition of 3.49 g HOBt (25.8 mmol), the yellow solution was stirred for 30 s and added 1.16 g 32 (1.59 mmol) and 0.326 g EDC (1.70 mmol). The solution was stirred under N$_2$ for 16 h and added 20 mL Et$_2$O and 40 mL ethyl acetate (EtOAc). The solution was extracted with 5% NaHCO$_3$ (3 x 50 mL) and H$_2$O (3 x 50 mL). The organics were collected and added petroleum ether, which gave a yellow
precipitate. The mother liquor was decanted and the solid was dried under vacuum. The solid was purified by preparatory HPLC using a linear gradient of 5% (0.1% TFA in CH$_3$CN):95% (0.1% TFA in H$_2$O) ramped to 56% (0.1% TFA in CH$_3$CN):44% (0.1% TFA in H$_2$O) over 12 min with a flow rate of 10 mL/min and a fraction collection program. The TFA and CH$_3$CN were removed by rotary evaporation and H$_2$O was removed by lyophilization leaving the di-Fmoc protected tripeptide as a yellow solid (0.0980 g, 0.0651 mmol). The identity of the product was confirmed by mass spectrometry (ESI+ MS (m/z): Calculated (M + H)$^+$ = 1504.7; Found (M + H)$^+$ = 1504.7). The solid was suspended in 10 mL toluene and 226 µL 1-octanethiol (1.30 mmol). The suspension was added 39.0 µL DBU (0.261 mmol)$^3$ and sonicated to dissolve the solid; the solution was stirred for 2.5 h under N$_2$ producing a yellow film. The mother liquor was decanted, and the film was dried in vacuo and dissolved in ~10 mL CH$_3$CN. The solution was added to 200 mL Et$_2$O giving a yellow precipitate. Yield = 0.0504 g (7.6%) ($^1$H NMR, 300 MHz, CDCl$_3$): 2.38 (s, 3 H); 2.74 (d, $J$ = 9 Hz, 4 H); 3.35-3.54 (m, 16 H); 7.07 (m, 1 H); 7.30 (m, 4 H); 7.63 (m, 1 H); 7.82 (t, $J$ = 7 Hz, 4 H); 8.17 (m, 2 H); 8.29 (m, 4 H); 8.42 (m, 1 H); 8.60 (m, 9 H). ($^{13}$C NMR, 400 MHz, d$_6$-DMSO): 18.8, 20.8, 21.3, 23.3, 25.9, 28.2, 30.0, 30.7, 66.3, 120.3, 120.8, 122.2, 124.3, 125.2, 131.0, 135.3, 140.7, 143.8, 149.1, 149.8, 154.6, 155.1, 158.1, 161.8, 169.8, 176.8, 188.5, 191.5, 195.3. MALDI-TOF MS (m/z): Calculated (M + Na)$^+$ = 1082.5; Found (M + Na)$^+$ = 1082.4.

$^{aeg(py)}$-DETA(bpy)-$^{aeg(py)}$ ($^{51}$). To a 0.352 g amount of $^{31}$ (0.294 mmol) in 10 mL dry THF was added 1.02 mL 1-octanethiol (5.88 mmol). To the suspension was added 176 µL DBU (1.18 mmol), and the mixture was stirred under N$_2$ for 3 h, during
which time a film formed. The mother liquor was decanted and the film was washed with THF and dried under vacuum. The film was dissolved in ~10 mL MeOH and added to 250 mL Et₂O giving a white precipitate. The precipitate was collected and dried in vacuo. Yield = 0.127 g (57.3%) (¹H NMR, 400 MHz, CDCl₃): 2.42 (s, 3 H); 2.69 (m, 4 H); 2.86 (t, J = 6 Hz, 4 H); 3.30 (t, J = 6 Hz, 4 H); 3.34-3.50 (m, 10 H); 3.62 (s, 4 H); 6.99 (m, 2 H); 7.12 (m, 4 H); 7.39 (m, 1 H); 7.72 (m, 1 H); 8.24 (q, J = 5 Hz, 4 H); 8.48 (m, 2 H). (¹³C NMR, 400 MHz, CDCl₃): 19.2, 23.7, 26.4, 28.8, 32.2, 37.9, 48.3, 54.1, 117.7, 120.0, 124.7, 126.4, 140.1, 140.8, 145.4, 147.0, 148.2, 148.7, 149.5, 158.5, 163.5, 165.7, 176.6, 187.7. MALDI-TOF MS (m/z): Calculated (M + Na)⁺ = 774.4; Found (M + Na)⁺ = 774.5.

4.2.4 Spectrophotometric Titrations

*Titration of [Cu(ClO₄)₂]·6H₂O with 51 (Cu + 51).* A solution of 30.1 µM [Cu(ClO₄)₂]·6H₂O (0.0752 µmol) was prepared in DMF. A second solution of 250 µM 51 (0.250 µmol) was prepared in 1.0 mL DMF and added in 10 µL increments to the Cu solution. After each addition, the solution was stirred in a water bath (~55 °C) for 5 min and after cooling to room temperature, a UV-visible absorbance spectrum was obtained.

*Titration of 51 and [Cu(ClO₄)₂]·6H₂O with 33 (51/Cu + 33).* A solution of 10.0 µM 51 (0.0250 µmol) and 30.0 µM [Cu(ClO₄)₂]·6H₂O (0.0749 µmol) in 2.5 mL DMF was prepared. The solution was heated in a ~55 °C water bath and after 15 min, no change in the UV-visible absorbance spectrum was observed. A separate solution of 249 µM 33 (0.249 µmol) in 1.0 mL DMF was prepared and titrated in 10 µL increments to the 51/Cu solution. The solution was heated for 5 min in a ~55 °C water bath after each
addition. After cooling to room temperature, UV-visible absorbance spectra were obtained. \( ^1H \text{ NMR, 400 MHz, } d_6\text{-DMSO): 2.12 (s, 6 H); 2.63 (m, 8 H); 3.25 (m, 8 H); 3.32 (m, 20 H); 3.48 (m, 8 H); 3.55 (m, 6 H); 3.97 (s, 2 H); 6.63 (s, 4 H); 6.83 (s, 4 H).}

**Titration of [Cu(ClO_4)_2]·6H_2O with 33 (Cu + 33).** To a 30.1 µM [Cu(ClO_4)_2]·6H_2O (0.0752 µmol) solution in DMF was added 10 µL increments of 249 µM 33 (0.249 µmol) prepared in 1.0 mL DMF. The solution was stirred in a water bath (~55 °C) for 5 min after each addition. The solution was cooling to room temperature and UV-visible absorbance spectra were obtained.

**Titration of 33 and [Cu(ClO_4)_2]·6H_2O with 51 (33/Cu + 51).** A solution of 10.0 µM 33 (0.0250 µmol) and 29.9 µM [Cu(ClO_4)_2]·6H_2O (0.0748 µmol) in 2.5 mL DMF was prepared. The solution was heated in a ~55 °C water bath for 5 min and a UV-visible absorbance spectrum was obtained against DMF. This was repeated until no change in the spectrum was observed (15 min total). A separate solution of 250 µM 51 (0.250 µmol) in 1.0 mL DMF was prepared and titrated in 10 µL increments to the 33/Cu solution. After each iterative addition, the solution was heated for 5 min in a ~55 °C water bath. After cooling to room temperature, UV-visible absorbance spectra were obtained. \( ^1H \text{ NMR, 400 MHz, } d_6\text{-DMSO): 2.12 (s, 6 H); 2.63 (m, 8 H); 3.17 (m, 8 H); 3.32 (m, 20 H); 3.48 (m, 8 H); 3.55 (m, 6 H); 3.81 (s, 2 H); 6.63 (s, 4 H); 6.87 (s, 4 H).}

**Titration of 33 and 51 with Cu(ClO_4)_2·6H_2O (33/51 + Cu).** A solution of 761 µM [Cu(ClO_4)_2]·6H_2O (7.61 µmol) was prepared in 10.0 mL DMF. This solution was titrated in 10 µL increments into 2.5 mL of a solution of 10.2 µM 33 (0.0254 µmol) and 10.1 µM 51 (0.0253 µmol) in DMF. The solution was heated to ~55 °C in a water bath for 5 min after each addition. The solution was allowed to cool to room temperature and a UV-
visible absorbance spectrum was obtained. \((^1\text{H NMR, 400 MHz, } d_6\text{-DMSO): 2.11 (s, 6 H); 2.65 (m, 8 H); 3.25 (m, 8 H); 3.32 (m, 20 H); 3.48 (t, } J = 6 \text{ Hz, 8 H); 3.55 (m, 6 H); 3.97 (s, 2 H); 6.63 (s, 4 H); 6.83 (s, 4 H).}

### 4.3 Results and Discussion

#### 4.3.1 Synthesis of Complementary Oligopeptides

The pbp tripeptide was synthesized from a central, symmetric bpy monomer that was reacted with 2.5 equivalents of a pyridine-functionalized monomer according to the scheme shown in Figure 3-1.\(^1\) A pyridine monomer was covalently linked to each of the two primary amines using standard amide coupling; the resulting tripeptide \((31)\) is therefore palindromic in sequence. We next turned to the synthesis of the complementary (for tetracoordinate metals) oligopeptide sequence, which would contain two tridentate ligands on either side of the bpy ligand. To accomplish this, tpy was chosen as the tridentate ligand because of its use in prior reports\(^{1,2,7,8}\) and its well-known coordination affinity for several metal ions.\(^{9-12}\) The tpy monomer was prepared by a standard amide coupling reaction of the acetic acid-derived tpy ligand \((53)\) with an Fmoc-protected aeg backbone subunit, Fmoc-aeg-OrBu\(\cdot\)HCl. The tert-butyl group was removed in the presence of an acid, which afforded \(32\) (see Figure 4-2) in 84.7% yield. The identity and purity of the monomer were confirmed by mass spectrometry and NMR, respectively.

Using the synthetic methodology developed for the preparation of the pbp tripeptide, the complementary tbt tripeptide was synthesized using the tpy monomer as
Figure 4-2. Synthesis of the tpy ligand (53) and Fmoc-aeg(tpy)-OH·TFA (32).
shown in Figure 4-3. Following deprotection of the two Fmoc protecting groups on the bpy monomer ((Fmoc)$_2$DETA(bpy)-HCl) using TBAF and HOBT, 32 was added in excess together with EDC.$^{1,6}$ Preparatory-scale HPLC was used for separation of the mixture, as it was most efficient for purification of the tripeptide on this reaction scale (< 200 mg). The Fmoc termini were subsequently cleaved using DBU and 1-octanethiol (see Figure 4-3)$^3$ yielding the desired product (33). This same deprotection reaction was performed with 31 giving the Fmoc deprotected tripeptide 51 in 57.3% yield. Mass spectrometry was used for identification of the two complementary tripeptides and the purity was confirmed by $^1$H and $^{13}$C NMR.

4.3.2 Spectrophotometric Titrations of Complementary Tripeptides

The creation of a double-stranded assembly from 33 and 51 requires the presence of tetracordinate metal ions to create the crosslinking complexes [M(bpy)$_2$]$^{2+}$ and [M(tpy)(py)]$^{2+}$. We have previously shown that Cu$^{2+}$ ions induce duplex formation of a self-complementary, bpy-functionalized tripeptide.$^2$ The [Cu(bpy)$_2$]$^{2+}$ metallobase formation was monitored by spectrophotometric titration: the increase in absorbance of the ligand-to-metal charge transfer (LMCT) band leveled off as the molar ratio of Cu$^{2+}$ to the oligopeptide reached 3:2. An analogous experimental method was used to monitor the Cu$^{2+}$ complexation by the pbp and tbt tripeptides in a series of experiments.

Spectrophotometric titrations were also performed to determine the role of reaction sequence on metal binding. For example, the absorbance spectra shown in Figure 4-4A were obtained during the titration of 51 into a solution of Cu$^{2+}$ (Cu + 51). To better visualize these changes, the spectra were replotted in Figure 4-4B as the
Figure 4-3. Synthesis of the tpy-bpy-tpy tripeptide.
Figure 4-4. (A) Spectrophotometric absorbance spectra acquired during the titration of 5I (250 µM) into a solution Cu(ClO$_4$)$_2$ (30.1 µM), (B) the difference spectra obtained by manual subtraction of the original Cu(ClO$_4$)$_2$ solution spectrum from each of the titration spectra, and (C) the difference spectra from the titration of 5I/Cu with 33 (249 µM). Insets contain the increase in absorption versus molar ratio.
change in the absorbance versus the original tripeptide solution (i.e., difference spectra). Over the course of the titration, the absorbance increases in the UV region of the spectrum. A titration curve was generated by monitoring the increase in absorbance at one wavelength (Figure 4-4B inset). It is apparent that the absorbance at 380 nm does not level off at a set molar ratio. This is observed for all wavelengths in this titration, which at least in part reflects some weak binding of the pyridines and primary amine termini. However, since uncomplexed pbp oligopeptide also absorbs light of these wavelengths, the increase in absorbance is most likely due to the increasing concentration of added pbp.

Therefore, the solution was back-reacted with Cu$^{2+}$ to reach a relative molar ratio of 3 Cu$^{2+}$: 1 pbp tripeptide. After allowing this solution to stir, it was then titrated with a solution containing the tbt tripeptide ($51/Cu + 33$): the difference spectra for this reaction are shown in Figure 4-4C. The absorbance of the solution increases over UV wavelengths, such as shown in the figure inset for the absorbance at 350 nm. There is an apparent inflection in the titration curve at a molar ratio of ~ 0.8; the continued increase beyond this point is again attributed to uncomplexed 33 in solution that also absorbs at these wavelengths.

The opposite experiment was also performed by titrating 33 into a solution containing Cu$^{2+}$ ($Cu + 33$); the difference spectra for this reaction are given in Figure 4-5A. The spectral peak shapes are strikingly similar to those in Figure 4-4C, but the critical difference is that the absorbance during the titration increases and levels at a molar ratio of 3 Cu$^{2+}$: 1 tbt tripeptide. It has previously been shown that Cu will bind to tpy ligands with the fourth coordination site occupied by a solvent molecule, so that the
Figure 4-5. UV-visible difference spectra of (A) 33 (249 µM) titrated into a solution of Cu(ClO₄) (30.1 µM) and (B) titration of 33/Cu with 51 (250 µM). Insets contain the change in absorbance versus molar ratio.
data in **Figure 4-5A** suggest that the three equivalents of Cu$^{2+}$ are bound by tpy ligands as well as forming [Cu(bpy)$_2$]$^{2+}$ complexes. The solution containing 3 Cu$^{2+}$: 1 tbt oligopeptide was then titrated with a solution containing pbp tripeptide (33/Cu + 51). **Figure 4-5B** contains the difference spectra acquired during this titration, in which the absorbance again increases but does not level similar to **Figure 4-4B**.

To prevent the background absorbance of uncomplexed oligopeptide, the titrations were performed by instead using a solution containing equimolar quantities of 33 and 51. This was titrated with aliquots of a solution containing Cu(ClO$_4$)$_2$ (33/51 + Cu), and the UV-visible absorption spectra that were obtained following each addition of Cu$^{2+}$ are shown in **Figure 4-6A**. Multiple peaks are observed in this region of the spectrum, and are primarily associated with $\pi-\pi^*$ aromatic ligand and d-d metal transitions. The intensities of these peaks change as Cu$^{2+}$ is added, indicative of chelation by the ligands. These spectra were replotted as difference spectra in **Figure 4-6B**. As shown in the titration curve, (inset **Figure 4-6B**), the absorbance increases upon addition of Cu$^{2+}$, and levels off at a value of 0.1 when three equivalents of Cu$^{2+}$ have been added. The final UV-visible absorbance spectra for each of the titrations containing both tripeptides and three stoichiometric equivalents of Cu$^{2+}$ are shown in **Figure 4-7**. The differences in the absorption intensities may indicate the formation of different species, however each of these spectra show the signatures from those observed in each of the individual titrations, confirming the formation of [Cu(bpy)$_2$]$^{2+}$ and [Cu(tpy)(py)]$^{2+}$.

Further confirmation of Cu$^{2+}$ coordination to the pendant ligands on the tripeptides was examined using 1-D proton NMR spectrometry. In Chapter 3, the
Figure 4-6. (A) Spectrophotometric titration of an equimolar solution of 33 (10.2 µM) and 51 (10.1 µM) with Cu(ClO$_4$)$_2$ (761 µM) and (B) respective difference spectra. Inset shows the change in absorption at 380 nm for each iterative addition versus the molar ratio of Cu to the tripeptide.
Figure 4-7. Comparison of absorbance spectra of titrated products from Cu titrated with 
$33$ and $51$ (9.71 µM) (---), Cu and $33$ titrated with $51$ (9.62 µM) (-----), and Cu and $51$
titrated with $33$ (9.62 µM) (-----).
formation of a \([\text{Cu(bpy)}_2]^2+\) crosslink was confirmed by the disappearance of the bpy peaks in the \(^1\text{H}\) NMR spectrum due to the extreme downfield shift after coordinating the paramagnetic metal.\(^1\) Similarly, the formation of three \(\text{Cu}^{2+}\) interstrand crosslinks in a tbt/pbp duplex would eliminate all of the ligand peaks from the aromatic region of the \(^1\text{H}\) NMR spectrum. Spectra were obtained for the titration products from \(33/\text{Cu} + 51\), \(51/\text{Cu} + 33\), and \(33/51 + \text{Cu}\). As anticipated, no peaks were observed in the aromatic region of these spectra with the exception of the amine peaks from the backbone (6.6 and 6.8 ppm). The disappearance of the ligand peaks, along with the presence of the aliphatic peaks from the backbone, confirm coordination of \(\text{Cu}^{2+}\) ions to each of the pendant ligands. The results from this experiment strongly support the formation of three interstrand crosslinks in a tbt/pbp duplex.

### 4.3.3 Electron Paramagnetic Resonance Spectroscopy

The \(\text{Cu}^{2+}\) ions that are used to crosslink \(33\) and \(51\) are paramagnetic, which allows for the extent of their spin coupling, and therefore proximity within the duplexes, to be assessed by electron paramagnetic resonance (EPR) spectroscopy. The EPR spectrum shown in **Figure 4-8** was obtained at 20 K from the duplex prepared during the spectrophotometric titration of \(33\) and \(\text{Cu}^{2+}\) with \(51\). The EPR spectrum contains line shapes indicative of hyperfine splitting that are slightly broadened. Broadening is attributed to weak electronic coupling between \(\text{Cu}^{2+}\) centers. The quantitative data obtained from these experiments are listed in **Table 4-1**, and are consistent with four-coordinate \(\text{Cu}^{2+}\) complexes with a square planar geometry (\(g_{\parallel} > 2.1 > g_{\perp} > 2.0\) with \(A_{\parallel} = 158 – 201 \times 10^{-4} \text{ cm}^{-1}\)).\(^{13,14}\)
Figure 4-8. X-band EPR spectra of frozen solution from the titration of $33$ and Cu($\text{ClO}_4$)$_2$ with $51$. 

Field (G)

2000 2500 3000 3500 4000
**Table 4-1.** EPR Spectroscopy Parameters and Hyperfine Coupling Constants for the Titration of $\text{33/Cu}$ with $5I$ and Small Molecules

<table>
<thead>
<tr>
<th></th>
<th>$g_\perp$</th>
<th>$g_\parallel$</th>
<th>$A_\parallel \times 10^{-4} \text{ cm}^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>$\text{33/Cu + 5I}$</strong></td>
<td>2.063</td>
<td>2.270</td>
<td>161</td>
</tr>
<tr>
<td><strong>$[\text{Cu(Me_2Bpy)}_2]^{2+}$</strong></td>
<td>2.059</td>
<td>2.275</td>
<td>154</td>
</tr>
<tr>
<td><strong>$[\text{Cu(tpy)(py)}]^{2+}$</strong></td>
<td>2.047</td>
<td>2.260</td>
<td>154</td>
</tr>
<tr>
<td><strong>$2 [\text{Cu(tpy)(py)}]^{2+}; 1 [\text{Cu(Me_2Bpy)}_2]^{2+}$</strong></td>
<td>2.050</td>
<td>2.263</td>
<td>156</td>
</tr>
</tbody>
</table>
The spectral features from the duplex were compared to those of the individual small molecules by acquiring EPR spectra of \([\text{Cu(bpy)}_2]^{2+}\) and \([\text{Cu(tpy)(py)}]^{2+}\). In addition, the spectrum of a 2:1 mixture of \([\text{Cu(tpy)(py)}]^{2+}/[\text{Cu(Me}_2\text{bpy)}_2]^{2+}\) was also obtained; these spectra are shown in Figure 4-9. Similar to the duplex spectrum, the three small molecule spectra have hyperfine splitting patterns and EPR parameters that confirm their square planar geometry (Table 4-1). Addition of the \([\text{Cu(tpy)(py)}]^{2+}\) and \([\text{Cu(Me}_2\text{bpy)}_2]^{2+}\) spectra in a 2:1 ratio showed only slight differences with the spectrum obtained from the 2:1 mixture (Figure 4-9C). This calculated EPR spectrum was compared to that of the duplex as shown in Figure 4-10. These spectra, and the parameters from Table 4-1, suggest that the duplex has features that are not explained by the sum of the small molecule analogues. A significant shift in the \(g\)-values was observed for the duplex, as well as a double peak in the trough of the EPR spectrum. The shift in the \(g\)-values is attributed to distortions in geometry of the metallobases compared to those of the free inorganic complexes. Studies have shown that changes in the metal center geometry causes a shift in the \(g\)-values.\(^{15,16}\) It is anticipated that confining these inorganic complexes within the core of a duplex would cause some geometric distortions, which may explain this shift in the \(g\)-values.

4.4 Conclusions

A pair of artificial metal-binding tripeptides has been synthesized and used to coordinate \(\text{Cu}^{2+}\) ions. The observed stoichiometry depends on the sequence of addition, but under the proper conditions, the spectrophotometric data suggest that the two strands are crosslinked by three metal ions. The disappearance of aromatic peaks from \(^1\text{H}\) NMR
Figure 4-9. Frozen X-band EPR spectra at 20 K of (A) [Cu(Me₂Bpy)₂]²⁺ (1 mM), (B) [Cu(tpy)(py)]²⁺ (1 mM), and (C) a mixture of [Cu(Me₂Bpy)₂]²⁺ (0.333 mM) and [Cu(tpy)(py)]²⁺ (0.667 mM). Dashed line in spectrum C is the calculated spectrum of 2:1 [Cu(tpy)(py)]²⁺/[Cu(Me₂Bpy)₂]²⁺ from A and B.
Figure 4-10. Comparison of frozen X-band EPR spectrum from titration of 33 and Cu$^{2+}$ with 51 (——) with the calculated spectrum of 2:1 \([\text{Cu(tpy)(py)}]^2+/[\text{Cu(Me}_2\text{Bpy)}_2]^2+\) (••••).
spectra as a result of a paramagnetic shift indicates that Cu$^{2+}$ ions bind to the ligands rather than the oligopeptide backbone. Structural parameters obtained from EPR spectroscopy are consistent with the formation of four-coordinate complexes. By comparison with the spectra obtained from the small molecules analogues, the broadening of the EPR lines in the duplex suggest that the [Cu(tpy)(py)]$^{2+}$ and [Cu(bpy)$_2$]$^{2+}$ metallobases are weakly coupled within the core of the duplex. The synthetic methodologies discussed here are currently being investigated for the development of longer complementary oligopeptide sequences, and several metal-ligand combinations are being considered for use in the assembly of similar double-stranded structures.
4.5 References


Appendix A

2D NMR Data for Bz-(py)$_6$G-NH$_2$

**Figure A-1.** HMQC NMR spectrum of Bz-(py)$_6$G-NH$_2$ (27).
Figure A-2. Expanded alkyl region of HMQC NMR spectrum of Bz-(py)₆G-NH₂ (27).

Figure A-3. Expanded aromatic region of HMQC NMR spectrum of Bz-(py)₆G-NH₂ (27).
Figure A-4. (A) DQF-COSY NMR spectrum of Bz-(py)$_6$G-NH$_2$ (27) with (B) aromatic region expanded for clarity.
Figure A-5. Gradient-selected HMBC NMR spectrum of Bz-(py)$_6$G-NH$_2$ (27).
Figure A-6. Expanded alkyl regions of HMBC NMR spectrum of Bz-(py)$_6$G-NH$_2$ (27).
Figure A-7. Expanded aromatic region of HMBC NMR spectrum of Bz-(py)$_6$G-NH$_2$ (27).
Confirmation of the purity for 27 was obtained using 2-D NMR techniques. The benzoyl peaks were observed at 7.79 – 7.84, 7.40 – 7.53, and 7.48 – 7.56 ppm in the DQF-COSY spectrum shown in Figure A-4. These peaks correspond to the carbon peaks at 126, 128, and 131 ppm as seen in the gradient-selected HMQC spectrum (Figures A-1 and A-3). The peaks corresponding to the pyridine ring are observed in the DQF-COSY spectrum at 8.56 – 8.76 and 7.53 – 7.77 ppm. These protons correlate to carbons at 142.5 (carbons α to the pyridine nitrogen) and 127 ppm (respectively) in the HMQC spectrum. The HMQC spectrum contains carbon peaks at 38.3 – 38.6 ppm that correlate to protons at 3.79 – 4.13 ppm. The gradient-selected HMBC spectrum (Figures A-5, A-6, and A-7) shows that these correlate to the pyridine carbons at 127 ppm in the HMQC spectrum; these are therefore assigned to the methylene carbons next to the pyridine rings. The pyridine carbons at 142.5 ppm in the HMQC spectrum can therefore be assigned to the carbons that reside next to the nitrogens in the pyridine rings. The HMQC spectrum also shows protons at 8.4 – 8.5, 8.2 – 8.3, 8.0, 7.4, 7.2, and 7.1 ppm that do not correlate to carbon peaks; these are assigned to the oligopeptide amide protons. The DQF-COSY spectrum shows a correlation between the proton resonances at 7.2 and 7.1 ppm, which are assigned to the terminal amide. This is further supported by the alkyl region of the spectrum by assignment of the protons at 3.60 – 3.74 ppm to the single methylene adjacent to the terminal amide. This proton region correlates strongly to the carbon peak at 41.6 ppm in the HMQC spectrum, while the remaining alkyl carbon peaks have differing chemical shifts. The remaining carbon peaks at 36.2 – 37.5, 38.3 – 38.6, 46.3 – 47.9, and 48.3 – 50.7 ppm in the HMQC spectrum (Figure A-2) are assigned to
the oligopeptide backbone, as they correspond to the proton peaks at 3.18 – 3.60, 3.79 – 4.13, 3.31 – 3.64, and 3.86 – 4.25 ppm, respectively.
Appendix B

2D NMR Data for Bz-(bpy)$_3$G-NH$_2$

Figure A-8. HMQC NMR spectrum of Bz-(bpy)$_3$G-NH$_2$ (28).
Figure A-9. Expanded aromatic region of HMQC NMR spectrum of Bz-(bpy)$_3$G-NH$_2$ (28).
The peaks in the HMQC spectrum of 28 (Figure A-8) were difficult to interpret because of the small amount of material that was prepared (an interpretable COSY spectrum was also not obtained due to the low concentration). However, peak assignments were made by analysis of the HMQC spectrum of the analogous Bz-(py)₆G-NH₂ (27, Figures A-1, A-2, and A-3). The alkyl regions of the two compounds are similar because their poly[N-(2-aminoethyl)glycine] backbones. The protons from the single methylene adjacent to the terminal amide were observed at 3.79 – 3.85 ppm in the HMQC spectrum; these correlate to a carbon peak at 43 ppm. The remaining proton peaks from the aeg backbone were observed at 3.47 – 3.53, 3.05 – 3.08, 3.61 – 3.67, and 4.05 – 4.15 ppm. These correlate to carbon peaks at 38.5, 41, 50, and 51 ppm, respectively. The protons from the methyl on the bipyridine were observed at 2.34 - 2.48 ppm, and correlate to a carbon peak at 20.2 ppm. The benzoyl protons from 28 were observed at 7.37 – 7.42, 7.76 – 7.82, and 7.18 – 7.26 ppm, and correlate to carbon peaks at 129, 128, and 126 ppm, respectively (Figure A-9). Although the bipyridine rings have six different protons, only three different chemical shifts are observed because of their similarity in position. The proton peaks at 8.36 – 8.45, 7.12 – 7.32, and 7.93 – 8.16 ppm correspond to the bipyridine protons, and are correlated to carbon peaks at 149, 126, and 123.5 ppm (respectively) in the HMQC spectrum. The carbon peak at 149 ppm is tentatively assigned to the α-carbon next to the bipyridine nitrogen. It should be noted that the HMQC spectrum of 28 contains proton regions that are broadened by the existence of rotamers and the slightly different chemical shifts of the oligopeptide repeat units. This broadening is not as pronounced as it is with 27, which is likely due to the shorter length of this oligopeptide and the lower concentration of the solution.
Appendix C

Crystallographic Data for [Cu(pda)(py)]

A blue block shaped crystal of [Cu(pda)(py)] (C12 H8 Cu N2 O4) with approximate dimensions 0.17 x 0.26 x 0.35 mm, was used for the X-ray crystallographic analysis. A total of 1850 frames were collected with a scan width of 0.3° in ω and an exposure time of 10 seconds/frame. The total data collection time was about 8 hours. The integration of the data using a Monoclinic unit cell yielded a total of 3499 reflections to a maximum θ angle of 28.28° (0.90 Å resolution), of which 1322 were independent, completeness = 99.0 %, R_int = 0.0104, R_sig = 0.0139 and 1291 were greater than 2σ(I).

The final cell constants: a = 7.816(4) Å, b = 13.613(6) Å, c = 10.078(5) Å, α = 90°, β = 91.653(8)°, γ = 90°, volume = 1071.9(8) Å³, are based upon the refinement of the XYZ-centroids of 2876 reflections above 20σ(I) with 2.992° < θ < 28.228°. Analysis of the data showed negligible decay during data collection. Data were corrected for absorption effects using the multiscan technique (SADABS). The ratio of minimum to maximum apparent transmission was 0.6712.

The structure was solved and refined using the Bruker SHELXTL (Version 6.1) Software Package, using the space group C2/c, with Z = 4 for the formula unit, C12 H8 Cu N2 O4. The final anisotropic full-matrix least-squares refinement on F² with 89 variables converged at R1 = 2.64 %, for the observed data and wR2 = 7.19 % for all data. The goodness-of-fit was 1.137. The largest peak on the final difference map was 0.346 e⁻/Å³ and the largest hole was -0.523 e⁺/Å³. Based on the final model, the calculated density of the crystal is 1.907 g/cm³ and F(000) amounts to 620 electrons.

The crystal structure of [Cu(pda)(py)] was obtained from the Penn State Department of Chemistry X-ray Crystallography Facility; funding was provided by the National Science Foundation (NSF CHE-0131112).
Figure A-10. X-ray crystal structure and unit cell of [Cu(pda)(py)].
Table A-1. Sample and crystal data for [Cu(pda)(py)]

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<tr>
<td>Wavelength</td>
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<td>Crystal size</td>
<td>0.35 x 0.26 x 0.17 mm</td>
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<tr>
<td>Crystal habit</td>
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<td>Crystal system</td>
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<tr>
<td>Space group</td>
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<tr>
<td></td>
<td>b = 13.613(6) Å, (\beta = 91.653(8)^\circ)</td>
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<td></td>
<td>c = 10.078(5) Å, (\gamma = 90^\circ)</td>
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<td>Density (calculated)</td>
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<tr>
<td>Absorption coefficient</td>
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<td>F(000)</td>
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Table A-2. Data collection and structure refinement for [Cu(pda)(py)]

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<tr>
<td>Generator power</td>
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<td>Data collection method</td>
<td>phi and omega scans</td>
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<td>Theta range for data collection</td>
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<td>Index ranges</td>
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Table A-3. Atomic coordinates and equivalent isotropic atomic displacement parameters (Å²) for [Cu(pda)(py)]

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<th>z</th>
<th>U(eq)</th>
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<td>0.0165(4)</td>
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<td>C2</td>
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U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.
Table A-4. Bond lengths (Å) for [Cu(pda)(py)]

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<td>C2-H2</td>
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<td>C3-C4</td>
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<tr>
<td>C4-O1</td>
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<tr>
<td>C5-C6</td>
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<td>C6-C7</td>
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<td>Cu1-N1</td>
<td>1.895(2)</td>
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<td>Cu1-O1</td>
<td>2.0120(13)</td>
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<td>N1-C3#1</td>
<td>1.3333(17)</td>
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#1 -x+1,y,-z+1/2

C1-C2         | 1.3969(19)  |
C2-C3         | 1.386(2)    |
C3-N1         | 1.3333(17)  |
C4-O2         | 1.231(2)    |
C5-N2         | 1.3472(18)  |
C5-H5         | 0.9500      |
C6-H6         | 0.9500      |
C7-H7         | 0.9500      |
Cu1-N2        | 1.947(2)    |
Cu1-O1#1      | 2.0120(13)  |
N2-C5#1       | 1.3472(18)  |
Table A-5. Bond angles (°) for [Cu(pda)(py)]

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<th>Angle (°)</th>
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Symmetry transformations used to generate equivalent atoms: #1 -x+1,y,-z+1/2
Table A-6. Torsion angles (°) for [Cu(pda)(py)]

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Table A-7. Anisotropic atomic displacement parameters (Å²) for [Cu(pda)(py)]

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<td>0.0085(8)</td>
<td>0.0131(9)</td>
<td>0.000</td>
<td>-0.0010(7)</td>
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<td>N2</td>
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<td>0.0098(8)</td>
<td>0.0130(8)</td>
<td>0.000</td>
<td>0.0002(6)</td>
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<tr>
<td>O1</td>
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<td>0.0093(5)</td>
<td>0.0151(5)</td>
<td>0.0001(4)</td>
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<tr>
<td>O2</td>
<td>0.0221(6)</td>
<td>0.0140(5)</td>
<td>0.0154(5)</td>
<td>-0.0003(4)</td>
<td>-0.0054(4)</td>
<td>-0.0025(4)</td>
</tr>
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</table>

The anisotropic atomic displacement factor exponent takes the form: \(-2\pi^2 [h^2a^2 U_{11} + \ldots + 2hk a^* b^* U_{12}]\)
Table A-8. Hydrogen atom coordinates and isotropic atomic displacement parameters (Å²) for [Cu(pda)(py)]

<table>
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<tr>
<th></th>
<th>x/a</th>
<th>y/b</th>
<th>z/c</th>
<th>U</th>
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<td>H6</td>
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<tr>
<td>H7</td>
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<td>0.0189</td>
<td>0.2500</td>
<td>0.023</td>
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</table>
Appendix D

2D NMR Data for Fmoc-aeg(py)-DETA(bpy)-aeg(py)-Fmoc

Figure A-11. $^1$H NMR spectrum of 3I.
Figure A-12. (A) Expanded aliphatic and (B) aromatic regions of the gradient HMQC NMR spectrum of 31.
Purity of 31 was confirmed using HMQC NMR spectrometry. The $^1$H NMR spectrum (Figure A-11) reveals a singlet at 2.4 ppm, which corresponds to the methyl protons on the bipyridine ring. These protons correlate to a single carbon at 21 ppm in the gradient HMQC spectrum (Figure A-12A). The integration of this $^1$H singlet is compared to that for the peaks between 6.4 – 8.7 ppm (36 protons total, Figure A-12B). This value accounts for both the amide and aromatic protons that are present in 31, and confirms the identity and purity of the tripeptide. The alkyl protons from 31 are observed at 2.6 – 2.75 ppm (4 H), 2.75 – 2.9 ppm (3 H), 3.2 – 3.45 ppm (4 H), 3.6 – 4.0 ppm (5 H), and 4.15 – 4.4 ppm (5 H). Nine additional protons at 2.5 ppm and 3.0 – 3.2 ppm are partially obscured by solvent peaks, but are clearly differentiated from solvent in the HMQC spectrum. Finally, the peaks at 4.15 – 4.4 ppm correspond to the methylene protons between the oxygen and the fluorenyl groups; these proton peaks correlate to a carbon at 65 ppm in the HMQC spectrum. The protons next to the nitrogen on the pyridine ring are observed at 8.6 – 8.7 ppm. These correlate to a carbon peak in the HMQC spectrum at 144 ppm.
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