Incorporation of polypyridyl ligands into short peptides and generation of bis-mu-(oxo)dimetallic complexes

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Wayne State University,
DEDICATION

To a man who loves and supports me unconditionally and all my family
ACKNOWLEDGMENTS

First, I would like to thank a very special woman, my mother, for making me the person I am today. I would also like to thank the rest of my family, my father, my sister and my brother for their inspiration. I would like to honor my grandfather who passed away during my studies, and ask for his mercy not being able to do the last task for him. Next, I would like to thank my husband, Evren, for his love, extreme patience, support and encouragement.

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CHAPTER 1
SYNTHESIS OF THE NEW PYRIDYL- CONTAINING UNNATURAL AMINO ACID HPN

1.1 Introduction

1.1.1 Metal-Binding Motif Containing Unnatural Amino Acids

Research groups have been working on the design and the synthesis of unnatural amino acids possessing metal-binding sites that would increase metal cation selectivities and expand the range of metal coordination geometries. In the literature, construction of metal-binding motifs possessing unnatural amino acids that contain bidentate ligands such as 2,2-bipyridine 1, 2, 3 and 1,10-phenanthroline 4, 5 or tridendate ligand such as di(2-pyridylmethylene)amine 6 and di(quinoline-2-methylene)amine 7 are known.

Compounds 1-7 have high binding affinities for the metal cations due to chelation effect of the multidendate nitrogen ligands. The efficacy of these residues has been demonstrated in the previous studies. For simplistic semi-synthesis of two horse heart cytochrome c (cyt c) mutants containing chelating π-acceptor amino acids, 1, 2, 3 have been previously reported. 3 In order to form a folded, metal-independent ββα motif, 4 and 5 have been used. 4 Additionally, rhenium complexes of 6 and 7 have the suitable fluorescence properties to be used for in vitro microscopy studies. In order to prepare complementary fluorescent and radioactive peptides probes, synthons of 7 can be used. 5
Synthetic routes to prepare polypyridyl motifs containing amino acids 1-7 have been demonstrated. Synthesis of 4-substituted 2,2'-bipyridine derivative 1 (4Bpa) starts with the oxidation of 4-methyl-2,2'-bipyridine 8 to 4-formyl-2,2'-bipyridine 9 in the presence of selenium dioxide (Scheme 1-1). Ring closure of 9 with N-benzoyl glycine 10 gives the corresponding oxazolone. Finally, the desired product 1 is obtained after the reductive cleavage of the oxazolone as a racemic mixture. In order to obtain enantiomerically enriched product esterification of the α-amino acid is required. The methyl ester derivative of 1 can be hydrolyzed enantiomerically using commercially available alkaline protease.

Scheme 1-1. Synthesis of 4Bpa

Synthesis of 5-substituted 2,2'-bipyridine derivative 2 (5Bpa) starts with the reduction of 5-(ethoxycarbonyl)-2,2'-bipyridine to the corresponding alcohol followed by bromination to afford...
The key step to synthesize 2 (5Bpa) involves asymmetric alkylation of a commercially available glycine derivative 13 with 5-(bromomethyl)-2,2'-biyridine 12, in the presence of a chiral phase transfer catalyst.

Scheme 1-2. Synthesis of 5Bpa

The stereoselective synthesis of 6-substituted 2,2'-bipyridine 3 (6Bpa) involves the asymmetric alkylation of commercially available N-(diphenylmethylene)glycine tert-butyl ester 13 with 6-(bromomethyl)-2,2'-bipyridine 14 using the chiral phase transfer catalyst. Enantiomerically enriched product is obtained after crystallization from a hexane solution.

Scheme 1-3. Synthesis of 6Bpa

Synthesis of amino acids 4 and 5 starts with the alkylation of diethyl acetamidomalonate 17 with the corresponding chlorides 15 and 16. Hydrolysis of the ester and N-acetyl groups using 6N HCl gives the racemic mixture of fully unprotected α-amino acids. Esterification of α-amino acids allows enzymatic resolution. Stereoselective hydrolysis of the α-amino acid methyl esters was achieved using the enzyme alkaline protease.

Scheme 1-4. Synthesis of Fen and Neo
Synthesis of lysine-derived bis(pyridyl) amines 6 and 7 involves reductive amination of \( N\text{-}\alpha\text{-Fmoc-L-lysine} \) 18 with the corresponding aldehydes 19 and 20 in the presence of the NaBH(OAc)_3.

![Scheme 1-5. Synthesis of SAAC and SAACQ](image)

As shown Figure 1-1, all metal-binding units such as bidentate or tridentate polypyridyl ligands are directly attached to the unnatural amino acid. However, were no examples of unnatural amino acids possessing only a small portion of the metal-binding unit that could be further elaborated to different ligand structures. From this insight, our research team designed a series of amino acids, which have methyl pyridyl fragments as side chains. This fragment, which is a common fragment of the polydentate ligands N4Py, TPA, TLA and Bn-TPEN (Figure 1-2), will allow for the incorporation of covalently attached tetra- and pentadentate ligands into peptide chains in a divergent fashion.

![Figure 1-2. Tetradendate and pentadendate polypyridyl ligands](image)
In this series, the amino acid backbone was first connected to the ligand with a one-carbon linker 25. In order to better understand the influence of the distance between the amino acid side chain and the ligand, a three-carbon analog (CH₂-CH₂-CH₂ linker) 26 and an ether linker have been prepared (CH₂-O-CH₂ linker) 27. 7

**Figure 1-3.** Pyridyl fragment containing unnatural amino acids

### 1.2 Results

#### 1.2.1 Retrosynthetic Analysis

In order to incorporate tetra- and pentadentate metal-binding units into a peptide chain a three carbon analog of 25 (2-Hydroxymethyl-5-Pyridyl-Alanine, HPA), 26 (2-Hydroxymethyl-5-Pyridyl-Norvaline, HPN) was designed and synthesized. The amino acid HPN was synthesized using Sonogashira reaction of propargyl glycine ester derivative 32 with 5-bromo pyridine derivative 29 as a key step (Scheme 1-6).
1.2.2 Synthesis of (S)-tert-butyl 2-(diphenylmethyleneamino)pent-4-ynoate (32)

Intermediate 32 was prepared based upon a literature procedure, which was modified by changing the base, temperature and time. This procedure involves asymmetric alkylation of $N$-(diphenylmethylene)glycine tert-butyl ester 13 in the presence of a chiral phase transfer catalyst
(CPTC 38) (Equation 1-1). The optical rotation of 32 was measured and agreed well with the literature value (89\% ee).

![Equation 1-1. Alkylation of 13 with propargyl bromide in the presence of CPTC 38](image)

1.2.3 Synthesis of 5-Bromo-2-((tert-butyldimethylsilyloxy)methyl)pyridine (33)

Precursor 33 was synthesized according to a literature procedure.\(^9\) Lithium halogen exchange between n-BuLi and commercially available 2,5-dibromopyridine 37, followed by formylation with DMF gave the corresponding aldehyde. In situ reduction of this aldehyde with sodium borohydride gave intermediate 36. Protection of the alcohol functional group as a silyl ether with TBSCI in the presence of Et\(_3\)N gave the precursor 33 (Scheme 1-7).

![Scheme 1-7. Synthesis of 33](image)

1.2.4 Synthesis of (S)-tert-butyl 5-(6-((tert-butyldimethylsilyloxy)methyl)pyridin-3-yl)-2-(diphenylmethyleneamino)pent-4-ynoate (31)

Intermediate 31 was synthesized via a Sonogashira coupling reaction between precursors 32 and 33. The reaction was performed in the presence of two catalysts, CuI and Pd(PPh\(_3\))\(_4\). The base and solvent employed were Et\(_3\)N and THF, respectively (Equation 1-2). Purification at this stage proved difficult due to catalyst decomposition products that could not be
separated from the desired product, so the reaction yield was verified using an internal standard. Cyclooctadiene was used as the internal standard in the NMR experiment. The desired product was obtained with 75% yield. In order to verify the enantiomeric excess of the compound 31, racemic mixture of 31 was prepared using the racemic form of 32. Then, the imine functional group and TBS group were removed in one step with dilute citric acid solution and the resulting free amine 30 was reacted with a chiral isocyanide derivative α-methylbenzylisocyanate. At this stage, the enantiomeric excess of 30 was determined 93% identical to compound 32 within error, confirming that epimerization did not occur during the coupling or deprotection steps.

**Equation 1-2.** Synthesis of 31 via Sonogashira coupling reaction

1.2.5 Synthesis of (S)-2-amino-3-(6-(hydroxymethyl)pyridin-3-yl)pentanoic acid (28)

To obtain fully unprotected unnatural amino acid 28, several deprotection methods were attempted (Equation 1-3). Our approach was to get the desired product using the fewest possible steps with a high yield. For this purpose, we first examined cleavage of the imine and reduction of the alkyne to the alkane in the same reaction pot using catalytic hydrogenation.

**Equation 1-3.** Deprotection of compound 31
Trying a number of conditions, given in Table 1-1, compound 28 was not obtained. All trials were analyzed by TLC and NMR spectroscopy. Instead of cleaving imine and reducing alkyne in the same step, we decided to use two different steps. The first step was cleavage of the imine and second step was hydrogenation of the alkyne. Initially we tried to cleave the imine using NH₂OH.HCl in THF:H₂O, but this method did not give us the corresponding product. Eventually, we accomplished cleavage of the imine using 15% citric acid (aq) in THF to obtain the free amine. Hydrogenation of the alkyne with Pd(OH)₂/C in MeOH under 100 psi hydrogen pressure at room temperature gave the corresponding intermediate in up to 98% yield. The third and last deprotection step was the hydrolysis of the ester to the carboxylic acid using 6N HCl to get intermediate 28 (Scheme 1-8).

### Scheme 1-8. Synthesis of 28

1.2.6 (S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(6-((tert-butyldimethylsilyloxy)-methyl)pyridin-3-yl)pentanoic acid (26)

In order to furnish a derivative of HPN for solid phase synthesis, we prepared the Fmoc-derivative of the unnatural amino acid 28. In addition to the Fmoc- protection, we protected the alcohol functional group as a silyl ether to prevent acetylation during the solid phase peptide synthesis. Two possible pathways were considered to obtain the final product (Scheme 1-9).
Initially we tried to protect the alcohol in the first step and protect the amine in the second step. This pathway gave 26 with a low yield. This problem led us to consider an alternative route to generate 26. After protection of the amine group in the first step, we isolated intermediate 27 in up to 87% yield. Next, we developed appropriate conditions to protect the alcohol without cleaving the Fmoc- group which was challenging because Fmoc- is a base sensitive protecting group and most of the TBS- protection methods involve basic conditions. To determine the best reaction conditions we monitored several different bases, solvents, and temperatures (Table 1-2). Finally, we found that using pyridine as a base and dichloromethane as a solvent gave best results, with 70-80% isolated yield.

Table 1-2. Optimization of conversion of 28 to 26

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1.3 Discussion of Results

Unnatural amino acid 26, possessing a 2-pyridylmethylene group, which is a common fragment of several multidentate polypyridyl ligands, has been designed and synthesized. A three-carbon (CH₂-CH₂-CH₂) linker was attached between the α-carbon of the amino acid and the metal-binding unit to better understand the effect of the distance. After introduction of this amino acid into a peptide chain, the side chain of the amino acid could be transformed to many different multidentate ligands. Additionally, altering the location of the unnatural amino acid within the peptide chain could facilitate the incorporation of the ligands at various positions.

1.4 Conclusion

In summary, we have developed a synthetic route to prepare an enantioenriched unnatural amino acid using a Sonogashira coupling reaction as a key step. Even though propargylglycine 32 derivatives had been used in arylation reactions, for the first time we used 32 as a Sonogashira coupling partner. In the previously reported metal-binding units on the unnatural amino acids, metal-binding motifs are directly attached to the unnatural amino acid. In this research, however, we have only inserted a small portion of the binding motif which can be modified further to various ligands.
1.5 Experimental

1.5.1 General Considerations

All reagents were purchased from commercial suppliers and used as received. NMR spectra were recorded on a Varian FT-NMR Mercury-300, 400 or 500 MHz Spectrometer. Low-resolution mass spectra were recorded on a Waters ZQ2000 single quadrupole mass spectrometer using an electrospray ionization source, while high-resolution mass spectra were recorded on a Waters-Micromass LCT Premier XE time of flight mass spectrometer. IR spectra were recorded on a Nicolet FT-IR spectrophotometer. Optical rotations were obtained by using Autopol III Automatic Polarimeter. HPLC was performed on an Agilent 1200 Preparative Purification System equipped with a multi-wavelength detector. Column purifications were performed using silica gel flash chromatography unless mentioned otherwise. Compounds 13\textsuperscript{11,12}, 33\textsuperscript{9}, 38\textsuperscript{10} and Pd(PPh\textsubscript{3})\textsubscript{4}\textsuperscript{13} used in this report were synthesized according to previously reported literature procedures. All reactions were performed under ambient atmosphere unless otherwise noted. Anaerobic reactions were performed in Schlenk tubes. These reactions were deoxygenated by performing five vacuum-backfill cycles with Ar and were run under a constant purge of Ar. For anaerobic reactions, Et\textsubscript{3}N and DMF were distilled over CaH\textsubscript{2} while THF was deoxygenated by bubbling Ar through a submerged needle, before use. Toluene and dichloromethane were dried over 4Å molecular sieves, before use.

(S)-tert-butyl 2-(diphenylmethyleneamino)pent-4-ynoate (32)

![Chemical structure of 32](image)

Propargyl bromide 34 (3.15 mL, 28.4 mmol) was added dropwise to a mixture of N-(diphenylmethylene)glycine tert-butyl ester 13 (7 g, 23.7 mmol), CPTC 38 (0.681 g, 1.18 mmol), CsOH·H\textsubscript{2}O (39.8 g, 23.7 mmol) and Toluene/DCM (24.6 mL and 10.2 mL). This reaction mixture
was vigorously stirred at rt under an inert atmosphere, resulting in formation of orange solution. After 1 h, reaction mixture diluted with 150 mL water and extracted with EtOAc (3 \times 100 mL). Combined organic layers were dried (Na$_2$SO$_4$) and concentrated. The crude product was purified by silica gel chromatography (10% EtOAc:Hexanes) to give 32 (6.55 g, 93%) as a light green oil, $^1$H NMR and ESMS data matched with the literature data. [$\alpha$] = -96.8° (c = 1.0, CHCl$_3$)

(S)-tert-butyl 2-amino-5-(6-(hydroxymethyl)pyridin-3-yl)pent-4-ynoate (30)

\[
\begin{array}{c}
\text{NH}_2 \\
\text{Ot-Bu}
\end{array}
\]

Chemical Formula: C$_{15}$H$_{20}$N$_2$O$_3$
Molecular Weight: 276.33

(S)-tert-butyl 2-(diphenylmethyleneamino)pent-4-ynoate 32 (2.81 g, 8.44 mmol), 5-bromo-2-((tert-butyldimethylsilyloxy)methyl)pyridine 33 (3.03 g, 10.1 mmol), Pd(PPh$_3$)$_4$ (975 mg, 0.84 mmol), Cul (257 mg, 1.35 mmol), Et$_3$N (8.53 g, 84.4 mmol) and THF (85.0 mL) were combined in a sealed tube under nitrogen atmosphere in a dry box, resulting the formation of a dark brown solution. The sealed tube was heated at 60 °C. Over time, formation of white precipitate was observed. After 48 h, THF was removed under vacuum. The resulting residue was dissolved in water (50 mL) and extracted with EtOAc (3 \times 150 mL). The combined organic layer was dried over anhydrous Na$_2$SO$_4$ and concentrated. The crude product was purified by silica gel chromatography (0% to 10% EtOAc:hexanes) to give the product 31 as a yellow oil (3.75 g, 80%).

A mixture of yellow oil 31 (2.36 g, 4.24 mmol), 15% aqueous citric acid (14 mL) and THF (27 mL) was maintained at rt for 12 h. The organic layer was concentrated under vacuum and resulting residue were dissolved with 1N HCl (5 mL). The aqueous solution was extracted with diethyl ether (3 \times 75 mL). The aqueous layer was basified using Na$_2$CO$_3$ (aq., pH = 9-10) and extracted with EtOAc (3 \times 150 mL). The combined organic layer was dried over anhydrous Na$_2$SO$_4$ and concentrated to give 30 as a yellow oil (1.07 g, 91%). $^1$H NMR (400 MHz, CD$_3$OD) $\delta$
8.47 (s, 1H), 7.82 (d, J = 8.1 Hz, 1H), 7.50 (d, J = 8.1 Hz, 1H), 4.67 (s, 2H), 3.59-3.30 (m, 1H), 2.85-2.80 (dd, J = 16.2, 4.9 Hz, 2H). 147 (s, 9H); $^{13}$C NMR (100 MHz, CD$_3$OD) δ 174.0, 161.6, 151.8, 121.5, 120.4, 89.7, 82.8, 80.5, 65.3, 54.6, 28.3, 26.4; IR (thin film) 3342, 3293, 2977, 1916, 1732, 1701, 1594, 1556, 1487, 1459, 1420, 1393, 1338, 1302, 1238, 1157, 1064, 1028, 954, 846 cm$^{-1}$; [α] = −6.9° (c = 1.0, MeOH); LRMS (ESMS) calcd for C$_{15}$H$_{21}$N$_2$O$_3$ (M+H)$^+$ 277, found: 277.

(S)-tert-butyl 2-amino-3-(6-(hydroxymethyl)pyridin-3-yl)pentanoate (29)

A mixture of (S)-tert-butyl 2-amino-5-(6-(hydroxymethyl)pyridin-3-yl)pent-4-ynoate (30) (1.12 g, 4.06 mmol), Pd(OH)$_2$/C (112 mg, 5% w/w) and MeOH (25 mL) was stirred at rt under H$_2$ (100 psi) for 3 h. The reaction mixture was filtered through a celite bed to remove Pd/C and the filtrate was concentrated to give 29 (1.10 g, 98%) as a white solid. $^1$H NMR (400 MHz, CD$_3$OD) δ 8.26 (s, 1H), 7.66 (dd, J = 8.1, 1.6 Hz, 1H), 7.44 (dd, J = 8.1, 1.6 Hz, 1H), 4.61 (s, 2H), 2.64-2.60 (m, 2H), 1.69-1.53 (m, 5H), 1.41 (s, 9H); $^{13}$C NMR (100 MHz, CD$_3$OD) δ 175.9, 159.8, 149.3, 138.8, 137.8, 121.9, 82.2, 65.3, 55.5, 35.2, 33.1, 28.3, 28.1; IR (KBr) 3353, 2976, 2931, 2863, 1726, 1601, 1572, 1479, 1460, 1393, 1368, 1251, 1155, 1068, 846 cm$^{-1}$; [α] = +3.6° (c = 1.0, MeOH); LRMS (ESMS) calcd for C$_{15}$H$_{25}$N$_2$O$_3$ (M+H)$^+$ 281, found: 281.

(S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(6-(hydroxymethyl)pyridin-3-yl)pen-tanoic acid (26)
A mixture of (S)-tert-butyl 2-amino-3-(6-(hydroxymethyl)pyridin-3-yl)propanoate 29 (1.10 g, 3.9 mmol) and 6N HCl (4 mL) was refluxed for 12 h. The reaction mixture was combined with H₂O (10 mL) and extracted with diethylether (2 × 50 mL). The aqueous layer was concentrated under high vacuum to obtain a crude product. The crude product was dissolved in H₂O (10 mL) and concentrated under high vacuum (twice) to remove excess HCl. The reaction mixture was concentrated to give the product 28 as a yellow solid (1.02 g, 88%).

A mixture of the yellow solid 28 (300 mg, 1.01 mmol), aqueous Na₂CO₃ (pH = 8-9), Fmoc-OSu and 1,4-dioxane (2 mL) was maintained at rt. After 12 h, the reaction mixture was acidified with 15% aqueous citric acid solution and the aqueous layer was extracted with EtOAc (3 × 150 mL). The combined organic layer was dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified by silica gel chromatography (0% to 10% MeOH:CH₂Cl₂) to give the corresponding product 27 as a white solid (385 mg, 85%).

A mixture of the white solid 27 (248 mg, 0.56 mmol), TBSCI (500 mg, 3.33 mmol), pyridine (482 mg, 6.11 mmol), and CH₂Cl₂ (5mL) was maintained at rt under a nitrogen atmosphere. After 12 h, the reaction mixture was diluted with CH₂Cl₂ (5 mL) and washed with water (3 × 50 mL). The aqueous layer was extracted using EtOAc (3 × 150 mL). The combined organic layer was dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified by silica gel chromatography (0% to 10% MeOH:CH₂Cl₂) to give 26 as a white solid (233 mg, 76%). ¹H NMR (400 MHz, CD₃OD) δ 8.29 (s, 1H), 7.77 (dd, J = 7.3, 3.2 Hz, 2H), 7.66 (m, 3H), 7.48 (d, J = 7.3 Hz, 1H), 7.36-7.27 (m, 4H), 4.89 (s, 2H), 4.35 (m, 2H), 4.20 (t, J = 6.9 Hz, 2H), 2.67 (m, 2H), 1.88 (m, 1H), 1.72 (m, 3H), 0.95 (s, 9H), 0.13 (s, 6H); ¹³C NMR (100 MHz, CD₃OD) δ 174.4, 157.5, 155.6, 144.2, 143.9, 143.6, 142.8, 141.4, 139.1, 127.6, 126.9, 125.1, 122.7, 119.8, 66.7, 63.1, 53.7, 31.4, 30.9, 27.0, 25.2, 18.1, –6.5; IR (thin film) 3318, 3018, 2953, 2929, 2857, 2360, 2342, 1716, 1610, 1533, 1450, 1399, 1259, 1174, 1106, 1084, 1052, 839, 780, 758, 467 cm⁻¹; [α] = +16.9° (c = 1.0, CHCl₃); HRMS (ESMS) calcd for C₃₂H₄₆N₂O₅SiNa (M+Na)+ 583.2604, found: 583.2598.

Determination of enantiomeric excess (ee): Enantiomers of compound 30 was not separable on a Chiracel-OD column. Hence 30 was converted to diastereomeric N-terminal α-
methylbenzylisocyanates using the procedure described below. Ratios of resulting diastereomers were determined by $^1$H NMR analysis using integration of peaks at 8.47 and 8.43 ppm for 30.
CHAPTER 2
SYNTHESIS OF PEPTIDE-LIGAND CONJUGATES ON SOLID-PHASE

2.1 Introduction

2.1.1 General Insight to the Solid Phase Strategy

Solid phase peptide synthesis (SPPS) was developed by Robert Bruce Merrifield in 1963. The general principle of SPPS is based on the repetition of coupling-deprotection cycles to form amide bonds between the two different amino acid units on a resin. Merrifield (chloromethyl) 41, Wang (p-benzyloxy benzyl alcohol) 42 and Rink amide 4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl-phenoxy-acetamido-norleucyl-MBHA resin 43 are frequently used in SPPS (Figure 2-1).

![Figure 2-1. Commonly used resins in SPPS](image)

During the coupling process, first, the free $N$-terminus of the resin is coupled onto a single $N$-protected amino acid unit. Then, this unit is deprotected, revealing a new $N$-terminal amine to which a further amino acid may be attached. In solution phase peptide synthesis, each coupling step requires isolation of intermediates. However, in SPPS all reagents and byproducts are easily eliminated by filtration. In addition to the ease of isolation methods, this approach speeds up the process. In SPPS, excess amount of amino acids are used to ensure complete coupling reactions in each step. This might cause side reactions or polymerization if amino acid units are not protected. For SPPS commonly used protecting groups are $t$-Boc- 44, Fmoc- 45, Cbz- 46 and Alloc- 47 (Figure 2-2).
In this thesis, for our solid phase synthesis we used Fmoc- protected amino acids. Fmoc- is a base sensitive protecting group. Fmoc- cleavage requires an amine. The mechanism for Fmoc-deprotection in the presence of piperidine is demonstrated below (Figure 2-3).

In SPPS, the carbonyl group of the amino acid is activated before the coupling reactions. Carbodiimides such as dicyclohexylcarbodiimide 48 (DCC), diisopropylcarbodiimide 49 (DIC) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide 50 (EDC) are often used as activating reagents. Due to the high reactivity of the carbodiimides, racemization can occur during the peptide synthesis. To solve the racemization problem, a secondary activating reagent such as 1-hydroxy-benzotriazole 51 (HOBr), 1-hydroxy-7-aza-benzotriazole 52 (HOBt), or N,N-dimethylpyridine 53 (DMAP) is required. These activating reagents (Figure 2-4) speed up the reaction and suppress byproduct (N-acyl urea) formation.
The reaction mechanism for coupling reaction in the presence of a carbodiimide and a secondary activating reagent is illustrated below (Figure 2-5).

In addition to the carbodiimides another class of commonly used activating reagents are triazols involving phosphonium salts, such as 2-(1H-7-aza-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate 54 (HATU) O-benzotriazole-1-yl-N,N,N’N’-tetramethyluronium hexafluoro-phosphate 55 (HBTU) and O-(6-chloro-1-hydrobenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate 56 (HCTU) (Figure 2-6).
The reaction mechanism for coupling reaction in the presence of HATU 54 is illustrated below (Figure 2-7).

![Coupling reaction mechanism in the presence of HATU](image)

Figure 2-7. Coupling reaction mechanism in the presence of HATU

2.1.2 Peptide-Ligand Conjugates

Peptides with metal complexes in their side chains and peptide–metal complex conjugates have been used to increase or control binding affinities and peptide conformations. Peptide-metal complex conjugates have been studied commonly by many research groups because of their biological importance. For instance, peptide-ligand conjugates can be used in directed drug delivery, particularly to tumor cells. In recent years, several studies have demonstrated effective tumor targeting using peptide-ligand conjugates. Peptide-ligand conjugates can be used to deliver metal complexes to specific locations in cells. Metal-peptide conjugates have been used to catalyze organic transformations as artificial enzymes. In addition, incorporation of metal-binding motifs into the polyamide framework of polypeptides is useful in de novo design of metalloproteins.

In order to produce metal-binding peptides, different synthetic methods have been developed. Among those methods solid phase methodology has been extensively used due to ease of creation of the larger peptides. Using solid phase, metal complexes can be attached to variable
positions in the synthetic peptide chain such as the side chain,\textsuperscript{15b, 19} \( N \)-terminus,\textsuperscript{16b} or as part of the peptide backbone itself\textsuperscript{20} (Figure 2-8).

Figure 2-8. Attachment of metal-binding units on peptides

Perhaps the most attractive strategy among these three alternatives is to attach metal-binding complexes to side chains through a metal-binding motif containing amino acid. Incorporation of the ligand anywhere within the primary sequence of a synthetic peptide chain, rather than just on the \( N \)-terminus or through conjugation with Cys or Lys residues, is possible with this approach.

Despite major synthetic efforts directed toward producing peptide-metal ligand conjugates, there were no reports of divergent syntheses for incorporation of multidentate metal-binding units within a peptide chain. In the previously reported methods to prepare peptide-metal conjugates, the first step is the preparation of multidentate ligand containing unnatural amino acids. Then, these unnatural amino acids with desirable inherent metal-binding features can be inserted into peptides. In order to obtain various ligands on a peptide chain using this method, preparation of an unnatural amino acid with different ligands is required involving additional synthetic steps. From this insight, our research team designed a series of amino acids, which have a pyridyl-2-methyl fragment as a side chain (discussed in Chapter 1). This fragment, which is a common fragment of N4Py, TPA, TLA and
Bn-TPEN, will allow for the incorporation of covalently attached tetra- and pentadentate ligands into a short peptide chain in a divergent fashion.

Incorporation of polynitrogen ligands into model dipeptide has been established in our laboratory.\textsuperscript{3,21} In order to extend our peptide-ligand conjugates library, our research group synthesized different dipeptides 60-62 either using solution phase or solid phase methodology (Figure 2-9).

![Figure 2-9. Polypyridyl ligands possessing model dipeptides](image)

### 2.2 Results

#### 2.2.1 Retrosynthetic Analysis

In order to produce peptide-ligand conjugates, previously we reported the preparation of a three-carbon analog of HPA 25, (Fmoc-HPN(OTBS)-OH) HPN 26. We attached HPN and HPA into various positions of tetra- and pentapeptides and used 2-hydroxymethyl-pyridyl position as an anchor point to elaborate the metal-binding unit (Figure 2-10).
2.2.2 Synthesis of Peptide-Ligand Conjugates Using Solid Phase

Intermediates 65a-d were prepared starting either from Fmoc-Gly-Wang or Fmoc-Gly-Rink amide resin depending on which C-terminal was desired. In the case of C-terminal carboxylic acids, we used Wang resin and in the case of generation of C-terminal amides, we used Rink amide resin. Starting from Fmoc-Gly bound to Rink amide resin, the sequence began by deprotection of the Fmoc group, coupling with Fmoc amino acids 25 or 26 using reagent HBTU. In order to avoid coupling reactions of unreacted resin with the new coupling reagent, unreacted reagents trapped with the solution of Ac$_2$O followed by Fmoc deprotection and coupling process was continued until desired tetrapeptides (66a and 66b) or pentapeptides (66c and 66d) were obtained. N-acetylation with Ac$_2$O gave resin-bound peptides. Next, the silyloxy ether groups of peptides were converted into the corresponding chlorides using the conditions shown in Scheme 2-1. Using dipicolylamine, two tetra- and two pentapeptide derivatives were obtained after cleavage from the resin with TFA/H$_2$O (95:5). HPLC analysis showed that products obtained from the resin were of > 80% purity. These three steps, TBS cleavage, chlorination and N-alkylation proceeded in high yield. Purities of the peptides
(63a-d) indicate that epimerization did not occur to any significant extent under the reaction conditions used to attach ligands into peptides.

Scheme 2-1. Incorporation of metal-binding units (TPA) into peptides using SPPS.\(^a\)

In order to ensure the completion of the reaction small amount of resin was cleaved by TFA. NMR and mass spectra of crude product were acquired. For optimization of the coupling conditions, HOBT/DCC and HBTU/i-Pr\(_2\)EtN were tried (Table 2-1).

\(^a\) Conditions: (i) 20\% piperidine, rt, 20 min (two cycles); (ii) Fmoc-AA-OH, HBTU, i-Pr\(_2\)EtN, DMF, rt, 3-4 h; (iii) Ac\(_2\)O, i-Pr\(_2\)EtN, DMF, rt, 1 h; (iv) TBAF, AcOH, THF rt, 1 h (two cycles); (v) LiCl, TsCl, i-Pr\(_2\)EtN, MeCN, rt, 6 h; (vi) NHR\(_2\), i-Pr\(_2\)EtN, MeCN, Nal, 55 °C, 24 h; (vii) TFA/H\(_2\)O (95:5), rt, 1 h.
Table 2-1. Optimization of the coupling conditions

<table>
<thead>
<tr>
<th>Resin</th>
<th>Coupling cocktail</th>
<th>Ratio of the reagents</th>
<th>Coupling efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang</td>
<td>DCC/HOBT</td>
<td>1:3:3</td>
<td>Modest</td>
</tr>
<tr>
<td>Wang</td>
<td>HBTU/i-Pr₂EtN</td>
<td>1:3:3</td>
<td>Good</td>
</tr>
<tr>
<td>Rink amide</td>
<td>HBTU/i-Pr₂EtN</td>
<td>2:3:3</td>
<td>Good</td>
</tr>
<tr>
<td>Rink amide</td>
<td>HBTU/i-Pr₂EtN</td>
<td>2:2:2</td>
<td>Good</td>
</tr>
</tbody>
</table>

2.3 Discussion of Results

After preparation of amino acids 25 and 26, these amino acids were introduced into the peptide chain. Transformation of the silyloxy functionality into a chloride leaving group, followed by N-alkylation with a secondary amine such as bis(2-dipicolyl)amine could be used to attach various ligands to the peptide in a divergent fashion. In addition, changing the position of the unnatural amino acid within the peptide chain could facilitate the insertion of ligands at various positions. Moreover, availability of amino acids with different linkers, between the α-position and 2-(hydroxymethyl)pyridyl group, would afford extra flexibility in designing peptide-ligand conjugates. Structural, positional and metal-binding ligand varieties of peptide-ligand conjugates are expected to accelerate the synthesis of libraries of metal-binding peptides.

2.4 Conclusion

In conclusion, in this chapter, we have demonstrated a divergent strategy for attaching polypyridyl ligands to peptides. This methodology is highly divergent because after introducing the metal-binding motif containing amino acid anywhere in the primary chain, a variety of ligands could be inserted by changing the secondary amines.
2.5 Experimental

2.5.1 General Considerations

All peptides were synthesized on a 0.12-0.40 mmol scale by solid phase peptide synthesis methods using N\textsuperscript{\alpha}-9-fluorenylmethyloxy-carbonyl (Fmoc) amino acids with HBTU-activated ester chemistry in a Peptide Synthesis Vessel (ChemGlass CG-1860). Burrell Model 75 Wrist Action Lab Shaker was used for shaking the reaction mixture. Commercially available starting materials and reagents were purchased from ChemPep.Inc., AnaSpec.Inc. and Chem Impex.

2.6 Experimental Procedures and Tabulated Data for New Compounds

2.6.1 Solid Phase Peptide Synthesis

Fmoc-Gly-Rink Amide MBHA Resin (0.32 mmol/g) or Rink Amide MBHA Resin (0.75 mmol/g) was used to afford carboxyl terminus primary amides. Typical protocols for coupling a residue involved 30-90 min coupling cycles with 3-4 eq of amino acid. However, for the unnatural amino acids 25 and 26 only 2 eq were used. Activated esters were formed in situ using HBTU and \(i\)-Pr\(_2\)EtN in DMF. After coupling, Fmoc deprotection as well as washing steps, solutions were filtered manually through a CG-1860 Peptide Synthesis Vessel. The Fmoc deprotection was performed using 20% Piperidine/DMF with three cycles 10-20 min each. After constructing the desired length of a peptide chain, the \(N\)-terminal was acetylated using acetic anhydride (50 eq) and \(i\)-Pr\(_2\)EtN (50 eq) in DMF for 2 h. For peptides 63a-d after each coupling reaction, capping was performed using \(N\)-acetylation conditions. After each reaction step, the resin was washed with DMF, \(i\)-Pr\(_2\)EtN and CH\(_2\)Cl\(_2\). Kaiser test was used to monitor the coupling reactions and deprotection of the Fmoc-group. Standard SPPS steps were summarized in (Table 2-2).
Table 2-2. Standard Solid Phase Peptide Synthesis (0.1 mmol scale)

<table>
<thead>
<tr>
<th>Operation</th>
<th>Reagent</th>
<th>Volume</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fmoc- deprotection</td>
<td>20 % Piperidine/DMF</td>
<td>7 ml</td>
<td>3 × 15 min</td>
</tr>
<tr>
<td>Drain and wash</td>
<td>DMF</td>
<td>4 mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IPA</td>
<td>4 mL</td>
<td>2 min</td>
</tr>
<tr>
<td></td>
<td>CH₂Cl₂</td>
<td>4 mL</td>
<td></td>
</tr>
<tr>
<td>Coupling Gly or Ala</td>
<td>Fmoc-AA-OH (0.3 mmol)</td>
<td>2 mL DMF</td>
<td>1-2 h</td>
</tr>
<tr>
<td></td>
<td>HBTU (0.3 mmol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>i-Pr₂EtN (0.3 mmol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coupling HPN, HPA or HPMA</td>
<td>HBTU (0.2 mmol)</td>
<td>1.5 mL DMF</td>
<td>3-4 h</td>
</tr>
<tr>
<td></td>
<td>i-Pr₂EtN (0.2 mmol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capping</td>
<td>Ac₂O (5 mmol)</td>
<td>2.5 mL DMF</td>
<td>1 h</td>
</tr>
<tr>
<td></td>
<td>i-Pr₂EtN (5 mmol)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.6.2 General Procedure for Chlorination

Cleavage of the silyloxyether group of compounds 66a-d were performed by shaking a mixture of the corresponding intermediate with acetic acid (1 eq) and TBAF (4 eq) in THF at rt for 1 h. The cleavage procedure was repeated to ensure complete cleavage of the silyloxyether group. After applying the standard washing procedure, the resin was dried under vacuum. A mixture of the dried resin, flame dried LiCl (50 eq), MeCN and i-Pr₂EtN (15 eq) was purged under nitrogen atmosphere for 5-10 min and TsCl (10 eq) was added. The reaction mixture was shaken at rt for 6 h. The color of the resin changed from pale yellow to red. At least one water washing was included in the standard washing protocol. Finally, the resin was dried under vacuum.

2.6.3 General Procedure for N-Alkylation

A mixture of the resin containing the chlorinated adduct 64a-d, MeCN, i-Pr₂EtN (12 eq), sodium iodide (0.2 eq) and the corresponding secondary amine (10 eq) was heated at 55 °C for 24 h.
The reaction mixture was washed using the standard washing procedure and the peptides were cleaved from the resin by treatment with TFA/H₂O (95:5) for 2 h. The resin was filtered, washed with TFA, and the combined filtrate was concentrated to 1-2 mL and stirred with ether/hexanes. The supernatant was decanted and remaining residue was purified by preparative HPLC.

### Table 2-3. Reactions on the side chain (0.1 mmol scale)

<table>
<thead>
<tr>
<th>Operation</th>
<th>Reagent</th>
<th>Vol</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBS- Cleavage</td>
<td>TBAF (1M in THF, 0.4 mmol)</td>
<td>4 mL THF</td>
<td>2 × 1 h</td>
<td>RT</td>
</tr>
<tr>
<td></td>
<td>AcOH (0.1 mmol)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LiCl (5 mmol)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorination</td>
<td>TsCl (1 mmol)</td>
<td>4 mL CH₃CN</td>
<td>1 × 6 h</td>
<td>RT</td>
</tr>
<tr>
<td></td>
<td>i-Pr₂EtN (1.5 mmol)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Alkylation</td>
<td>Nal (0.02 mmol)</td>
<td>2 mL CH₃CN</td>
<td>1 × 24 h</td>
<td>55 °C</td>
</tr>
<tr>
<td></td>
<td>R₁R₂NH (1 mmol)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>i-Pr₂EtN (1.2 mmol)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(S)-2-Acetamido-N-((S)-1-((S)-1-(2-amino-2-oxoethylamino)-3-(6-((bis(pyridin-2-ylmethyl)amino)methyl)pyridin-3-yl)-1-oxopropan-2-ylamino)-1-oxopropan-2-yl)-3-(6-((bis(pyridin-2-ylmethyl)amino)methyl)pyridin-3-yl)propanamide (63a)

The crude product was purified by preparative HPLC. HPLC column: Phenomenex LUNA C18 (2) 100 Å 250 × 21.2 mm; Mobile phase: 0.01% aq NH₄OH and MeOH; Flow rate = 20 mL/min; Gradient elution 0-5 min 2% MeOH in aq NH₄OH, 5-20 min 98% MeOH in aq NH₄OH. Alternate method: 0-5 min 50% MeOH in aq NH₄OH and 5-20 min 95% MeOH in aq NH₄OH, RT = 16.7 min.¹H NMR (400 MHz, CD₃OD) δ 8.41 (d, J = 4.9 Hz, 4H), 8.30 (dd, J = 4.9, 1.6 Hz, 2H), 7.81-7.55 (m,
12H), 7.29-7.23 (m, 4H), 4.60-4.45 (m, 2H), 4.25- 4.15 (m, 1H), 3.91 (d, J = 17.0 Hz, 1H), 3.81-3.78 (m, 12H), 3.69 (d, J = 17.0 Hz, 1H) 3.35-3.30 (m, 1H), 3.10-3.05 (m, 2H), 2.85-2.75 (m, 1H), 1.86 (s, 3H), 1.19 (d, J = 7.3 Hz, 3H); 13C NMR (100 MHz CD3OD) δ 175.1, 174.1 173.4, 173.3, 173.1, 160.0, 158.5, 158.4, 150.4, 150.3, 149.5, 139.6, 139.5, 138.7, 133.5, 133.4, 124.8, 124.8, 124.5, 124.4 123.9, 61.0, 60.9, 60.8, 55.9, 55.4, 50.9, 43.2, 35.6, 34.8, 22.4, 17.5; IR (KBr) 3289, 3064, 2927, 2820, 1656, 1594, 1539, 1435, 1372,1283, 1239, 1126, 1050, 979, 766; [α] = −7.8° (c = 0.38, MeOH); HRMS (ESMS) calcd for C49H56N13O5 (M+H)+: 906.4527, found: 906.4530.

(S)-2-Aacetamido-N-((S)-1-((S)-1-(2-amino-2-oxoethylamino)-5-(6-((bis(pyridin-2-ylmethyl)amino)methyl)pyridin-3-yl)-1-oxopentan-2-ylamino)-1-oxopropan-2-yl)-5-(6-((bis(pyridin-2-ylmethyl)amino)methyl)pyridin-3-yl)pentanamide (63b)

The crude product was purified by preparative HPLC HPLC column: Phenomenex LUNA C18 (2) 100 Å 250 × 21.2 mm; Mobile phase: 0.01% aq NH4OH and MeOH; Flow rate = 20 mL/min; Gradient elution 0-5 min 2% MeOH in aq NH4OH, 5-20 min 98% MeOH in aq NH4OH. Alternate method: 0-5 min 50% MeOH in aq NH4OH and 5-20 min 95% MeOH in aq NH4OH, RT = 17.1 min.1H NMR (400 MHz, CD3OD) δ 8.42-8.39 (m, 4H), 8.27-8.26 (m, 2H), 7.79-7.54 (m, 12H), 7.27-7.24 (m, 4H), 4.27-4.25 (m, 3H), 3.86-3.75 (m, 14H), 2.63-2.62 (m, 4H), 1.89 (s, 3H), 1.76-1.64 (m, 8H), 1.34 (d, J = 6.4 Hz, 3H); 13C NMR (100 MHz, CD3OD) δ 175.7, 174.7, 174.5, 174.3, 173.5, 173.5, 160.2, 157.6, 157.5, 149.5, 149.4, 149.5, 138.7, 138.6, 137.9, 137.8, 124.9, 124.6, 124.6, 123.9, 61.2, 61.2, 60.9, 60.8, 55.2, 55.1, 49.9, 43.3, 33.0, 32.2, 31.8, 28.4, 22.4, 17.4; IR (KBr) 3290, 3063, 2925, 2854, 1655, 1594, 1542, 1435, 1372, 1304, 1249, 1201, 1150, 1126, 1094, 1049, 1002, 980, 767, 654; [α] = +13.1° (c = 1.3, MeOH); HRMS (ESMS) calcd for C53H63N13O5 (M+H)+ 962.5153, found: 962.5144.
2-acetamido-N-((2S)-1-((2-((1-((2-amino-2-oxoethyl)amino)-3-(6-((dibenzylamino)methyl)pyridin-3-yl)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-1-oxopropan-2-yl)-3-(((benzyl(phenyl)-amino)methyl)pyridin-3-yl)propanamide (63c)

The crude product was purified by preparative HPLC. ZORBAX Extend C18 PrepHT 21.2 X 150 mm: Mobile phase: 0.01% aq NH₄OH and MeOH; Flow rate = 20 mL/min; Gradient elution 0-5 min 10% MeOH in aq NH₄OH, 5-25 min 90% MeOH in aq NH₄OH, RT = 19.8 min. ¹H NMR (400 MHz, CD₃OD) δ 8.42 (d, J = 4.9 Hz, 4H), 8.33 (d, J = 6.5 Hz, 2H), 7.78 (td, J = 7.7 and 1.6 Hz, 4H), 7.70 (d, J = 8.1 Hz, 1H), 7.63 (d, J = 8.1 Hz, 5H), 7.54 (d, J = 7.3 Hz, 2H), 7.27 (d, J = 4.9 Hz, 4H), 4.55 (m, 2H), 4.14 (m, 1H), 3.97-3.67 (m, 16H), 3.25-3.17 (m, 1H), 3.15 (dd, J = 14.2 and 6.1 Hz, 1H), 3.02 (dd, J = 13.4 and 9.7 Hz, 1H), 2.94 (dd, J = 8.5 and 6.1 Hz, 1H), 1.89 (s, 3H), 1.14 (d, J = 6.5 Hz, 3H); IR (KBr) IR (KBr) 3289, 3064, 2927, 2854, 1656, 1594, 1539, 1435, 1372, 1283, 1239, 1126, 1050, 979, 766; [α] = −15.5° (c = 0.47, MeOH); HRMS (ESMS) calc’d C₅₁H₅₉N₁₄O₆ (M+H)⁺ 963.4742, found: 963.4760.
2-acetamido-N-((2S)-1-((2-((1-((2-amino-2-oxoethyl)amino)-5-(6-((bis(pyridin-2-ylmethyl)amino)methyl)pyridin-3-yl)-1-oxopentan-2-yl)amino)-2-oxoethyl)amino)-1-oxopropan-2-yl)-5-(6-((bis-(pyridin-2-ylmethyl)amino)methyl)pyridin-3-yl)pentanamide (63d)

The crude product was purified by preparative HPLC. ZORBAX Extend C18 PrepHT 21.2 X 150 mm: Mobile phase: 0.01% aq NH₄OH and MeOH; Flow rate = 20 mL/min; Gradient elution 0-5 min 10% MeOH in aq NH₄OH, 5-25 min 90% MeOH in aq NH₄OH, RT = 20.6 min. ¹H NMR (400 MHz, CD₃OD) δ  8.44 (d, J = 4.1 Hz, 4H), 8.29 (m, 2H), 7.79 (td, J = 7.7 and 1.6 Hz, 4H), 7.63 (d, J = 8.1 Hz, 6H), 7.54 (d, J = 7.3 Hz, 2H), 7.27 (d, J = 4.9 Hz, 4H), 4.29 (dd, J = 7.7 and 3.7 Hz 2H), 4.22 (m, 1H), 3.87-3.82 (m, 16H), 2.62 (d, J = 7.3 Hz, 4H), 1.97 (s, 3H), 1.71-1.65 (m, 8H), 1.37 (d, J = 7.3 Hz, 3H); IR (KBr) 3291, 3064, 2927, 2854, 1656, 1594, 1539, 1435, 1372, 1283, 1239, 1126, 1050, 979, 766; [α] = –8.4° (c = 0.77, MeOH); HRMS (ESMS) calcd for C₅₅H₆₇N₁₄O₆ (M+H)+ 1019.5368, found: 1019.5356.
\[
X = \text{CH}_2
\]
Chemical Formula: C_{64}H_{93}N_{19}O_{6}

906.05
Chemical Formula: $C_{69}H_{52}N_{32}O_5$
946.35
Chemical Formula: C_{24}H_{34}N_{12}O_{6}
Exact Mass: 962.47
CHAPTER 3
SYNTHESIS OF DIMETALLIC COMPLEXES WITHIN THE TETRA- AND PENTAPEPTIDES

3.1 Introduction

Binuclear oxo or/and carboxylato bridged manganese complexes have been widely studied due to their relevance in biological systems such as the oxygen evolving complex (OEC) of photosystem II (PS II) and the manganese catalases (Mn Cat). In PS II, it is believed that a tetrameric manganese cluster, proposed in the S_1 and S_2 states in Kok’s S state cycle, catalytically oxidizes two molecules of water to molecular oxygen. In addition to their biological significance, high oxidation state manganese complexes have an intense interest in organic chemistry because of their potential uses in oxidation chemistry as oxidizing agents, catalysts and electro-catalysts. Earlier, Crabtree and Brudvig reported that a nonporphyrin di-μ-oxo dimanganese compound that catalyzes the highly regioselective oxygenation of saturated C-H bonds in ibuprofen and (4-methylcyclohexyl)acetic acid with >100 turnovers. More recently, in addition to the site-selective modification of apoptolidin A, a variety of group transfer reactions (such as acylation, phosphorylation, sulfenylation, thiocarbonylation) of polyol substrates with high enantio- and regio-selectivity were achieved in the presence of a peptide-based catalyst. We believe that μ-oxo-bridged dimetallic centers on a peptide-ligand conjugate have potential as catalysts or oxidants.

In this thesis, we report the generation of mixed valance bis(μ-oxo)dimanganese species [Mn^{III}L(μ-O)_2Mn^{IV}L] \( 63a'-63d' \) (where L = Ac-Ala(TPA)AlaAla(TPA)Gly-NH_2 \( 63a \), Ac-Nva(TPA)AlaNva(TPA)Gly-NH_2 \( 63b \), Ala(TPA)GlyAlaAla(TPA)Gly-NH_2 \( 63c \) and Ac-Nva(TPA)GlyAlaNva(TPA)Gly-NH_2 \( 63d \)) using tetrapeptides and pentapeptides containing two TPA units (Figure 3-1).
3.2 Results

3.2.1 UV-vis Analysis of [MnIII(L(μ-O))2MnIVL]

In this work, peptide-ligand conjugates were designed in such a way that two TPA units on the peptide skeleton can be used as a ligand to generate an oxo-bridged dimetallic center. In order to better understand the influence of the distance between the amino acid side chain and the ligand, a one-carbon linker (CH₂63a and 63c) and a three-carbon linker ((CH₂)₃63b and 63d) were prepared. We also considered that the distance between the amino acid chain and the ligand would affect the stability of the metal complex. In the literature, there are extensively studied examples of manganese complexes containing [Mn₂(μ-O)₂]X⁺, [Mn₂(μ-O)₂(μ-carboxylato)]X⁺, and [Mn₂(μ-O)(μ-carboxylato)₂]X⁺.²⁸ Over the last decade, oxo-bridged complexes of transition metals (M = Fe or Cu) [M₂(μ-O)₂]²⁺ or ³⁺ complexes have also been prepared. However, to the best of our knowledge there are no reported examples of bis-μ-oxo-bridged dinuclear centers attached to a peptide. Because of the lability of oxo-bridged diiron or dicopper(III) species, we decided to generate more stable bis-μ-oxo-bridged dimanganese complexes, based on a report that (TPA)₂Mn₂(μ-O₂)³⁺ 21’ can be used as a catalyst for wood pulp bleaching, due to its stability in aqueous solution under acidic conditions pH = 3.5 and temperatures higher than 80 °C.²⁹
To generate bis-μ-oxo-bridged dinuclear Mn(III, IV) complexes $[\text{Mn}_2(\mu-O_2)L]^{2+} \: 63a', \: 63b', \: 63c'$ and $63d'$ (Figure 3-2), 1 equiv of concentrated peptide was treated with 2 equiv of Mn(ClO$_4$)$_2$·6H$_2$O followed by addition of 50-200 equiv of H$_2$O$_2$ in water, which furnished green species. The UV-vis spectra of $63a'$, $63b'$, $63c'$ and $63d'$, displayed absorption bands at $\lambda_{\text{max}}$ = 440, 560 and 660 nm which are similar to that of $[\text{Mn}_2(\mu-O_2)(\text{TPA})]^{2+} \: 21'$ (Figure 3-3). Three lower energy transitions to an oxo→Mn$^{IV}$ and two Mn$^{IV}$ d-d transitions were assigned for complex $21'$. To form complexes $63a'$, $63b'$, $63c'$ and $63d'$, excess amount of peroxide is required. However, excess amount of concentrated H$_2$O$_2$ causes oxygen evolution. In order to destroy the unreacted H$_2$O$_2$, after the addition of the H$_2$O$_2$, reactions were allowed to continue for 5-10 min, then quenched with the addition of catalase. With the addition of the catalase, species do not decay as rapidly by time, as judged by UV-vis spectroscopy.

![Figure 3-3](image)

**Figure 3-3.** UV-vis spectra of $[\text{Mn}^{IIIL}(\mu-O_2)\text{Mn}^{IVL}]$ species. $\lambda_{\text{max}}$ = 440, 560 and 660 nm.$^a$ The UV-vis spectrum of the species was recorded 12 h after the generation of the species $21'$, $63a'$, $63b'$, $63c'$ and $63d'$.

In order to study the kinetics of the species generated, we optimized conditions to avoid oxygen evolution. Oxygen evolution causes inconsistent data collection due to the formation of bubbles. Using different concentrations of H$_2$O$_2$ (40 to 600 mM) or Mn$^{II}$-L (0.4-1.6 mM) complexes,

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$^a$ 2.5 mM, 100 μL Mn$^{II}$-L (L = 21); 1.25 mM, 100 μL Mn$^{III}$-L (L = 63a, 63b, 63c and 63d); 300 mM, 20 μL H$_2$O$_2$
generation of the species was followed by the UV-vis spectroscopy. This spectrum shows that
dimetallic complexes \(63a', 63b', 63c'\) and \(63d'\) can endure longer than dimetallic complex \(21'\) under
the same reaction conditions which suggests that the peptide is able to enhance stability of the
dimanganese complexes. We also recorded time vs. absorbance plots of the dimetallic complexes
\(21', 63a', 63b', 63c'\) and \(63d'\) (Figure 3-4 and Figure 3-5) every 1 min for 6 h. Time vs. absorbance
plots clearly indicated that depending on the concentrations of the ligands, formation and decay of the
complexes \(21', 63a', 63b', 63c'\) and \(63d'\) occur at different rates. However, it was not possible to
establish the kinetic order of the reaction since we could not get linear correlations. Looking at the
time vs. absorbance graphs, we can conclude that distance between the ligand and the peptide
backbone or the distance between the two metal binding units has an effect on the stability of the
complexes \(63a', 63b', 63c'\) and \(63d'\).

![Time versus absorbance graph of \([\text{Mn}^{III}L(\mu-O)_{2}\text{Mn}^{IV}L]\) species.]

\[\text{Figure 3-4. Time versus absorbance graph of } [\text{Mn}^{III}L(\mu-O)_{2}\text{Mn}^{IV}L] \text{ species.}\]

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\(^b\) 0.84 mM, 100 \(\mu\)L \(\text{Mn}^{II}-L\) (\(L = 21\)); 0.42 mM, 100 \(\mu\)L \(\text{Mn}^{III}-L\) (\(L = 63a, 63b, 63c\) and \(63d\)); 300 mM, 20 \(\mu\)L \(\text{H}_2\text{O}_2\)
3.2.2 EPR Analysis of [Mn$^{III}$L(μ-O)$_2$Mn$^{IV}$L]

For further identification of the species generated, the EPR spectra of these species were recorded. The EPR spectrum of complex $21'$ was known in the literature.$^{31}$ Nonetheless, we recorded it again in order to compare similarity of spectra of $63a'$, $63b'$, $63c'$ and $63d'$, with $21'$. The EPR spectrum of $21'$ showed a typical sixteen $^{55}$Mn hyperfine pattern at $g = 2.003$ as reported earlier. As shown in Figure 3-6, the EPR spectra of $63a'$, $63b'$, $63c'$ and $63d'$ had some different patterns. In order to determine these different patterns, the EPR spectrum of Mn$^{II}$-L and Mn$^{II}$-H$_2$O$_2$ were also recorded. These experiments showed that Mn$^{II}$-L and Mn$^{II}$-H$_2$O$_2$ species were not EPR silent. Due to difficulty to prepare samples of the Mn(III, IV) complexes which do not contain trace amounts of Mn$^{II}$-L and Mn$^{II}$-H$_2$O$_2$ impurities, the EPR spectra of $63a'$, $63b'$, $63c'$ and $63d'$ had extra coupling patterns.

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$^c$ 2.5 mM, 100 μL Mn$^{III}$-L (L = $21$); 1.25 mM, 100 μL Mn$^{III}$-L (L = $63a$, $63b$, $63c$ and $63d$); 300 mM, 20 μL H$_2$O$_2$
3.2.3 ESI-MS Analysis of [Mn^{III}L(\mu-O)_{2}Mn^{IV}L]

The formation of Mn(III, IV) species was also evidenced by ESI-MS monitoring. After addition of H_{2}O_{2} to the solution of Mn^{II}+L mass spectra for complexes \textit{63a'}, \textit{63b'}, \textit{63c'} and \textit{63d'} were recorded in 1 hr. Complexes \textit{21'}, \textit{63a'}, \textit{63b'}, \textit{63c'} and \textit{63d'} give rise to peaks, m/z = 240.7239, 349.1031, 367.7907, 368.1101 and 386.7977 respectively corresponding to the trication (Figure 3-7, Figure 3-8, Figure 3-9, Figure 3-10, Figure 3-11). The ion exhibited an isotope pattern that matched the calculated molecular formula within 5 ppm.

\footnote{Samples were frozen in liquid N\textsubscript{2} 3 hr after mixing. Temperature, 116 K; microwaves 2.00 mW at 9.34 GHz, modulation amplitude 2.00 G for HPA and 4.00 G for HPN and receiver gain 20,000.}
Figure 3-7. High Resolution TOF-MS Spectrum of $[\text{Mn}^{III}\text{L}(\mu-\text{O})_2\text{Mn}^{IV}\text{L}]$.

Figure 3-8. High Resolution TOF-MS Spectrum of $[\text{Mn}^{III}\text{L}(\mu-\text{O})_2\text{Mn}^{IV}\text{L}]$.

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$^e$ m/z = 240.7239 (right) and calculated m/z = 240.7241 (left).

$^f$ m/z = 349.1031 (right) and calculated m/z = 349.1036 (left).
Figure 3-9. High Resolution TOF-MS Spectrum of $[\text{Mn}^{III}L(\mu-O)_2\text{Mn}^{IV}L]$ 63b.\(^g\)

Figure 3-10. High Resolution TOF-MS Spectrum of $[\text{Mn}^{III}L(\mu-O)_2\text{Mn}^{IV}L]$ 63c.\(^h\)

\(^g\) 367.7907 (right) and calculated m/z = 367.7911 (left).

\(^h\) 368.1101 (right) and calculated m/z = 368.1108 (left).
3.3 Discussion of Results

In this chapter, we showed incorporation of oxo-bridge dimetallic centers within the peptide-ligand conjugates. These complexes were characterized by UV-vis spectroscopy, EPR spectroscopy and high resolution mass spectrometry. After generation of the complexes, we tried to establish the reaction mechanism; however, formation of a heterogeneous reaction solution did not allow us to study the kinetics of the reaction.

3.4 Conclusion

In conclusion, for the first time we showed the generation of bis-\(\mu\)-oxo-bridged dinuclear metallic centers based on TPA on a synthetic peptide. In addition, we showed that putting two ligands on a peptide backbone increased the stability of these species compared to parent TPA complex 21\textsuperscript{1} under the identical reaction conditions. Different linkers on the peptide chain and distance between the metal binding units also play a crucial importance on the stability of this species. Time vs.

\textsuperscript{1} m/z = 386.7977 (right) and calculated m/z = 386.7983 (left).
absorbance plots noticeably signified that changing the ligand concentrations, formation and decay rates of the complexes 21, 63a, 63b, 63c and 63d can be modified. Kinetic and mechanistic studies are still underway in our laboratory and will be published in due course.

3.5 Experimental

3.5.1 General considerations

UV-vis spectra were acquired on a TECAN Infinite M200 micro-plate reader. EPR spectra were acquired using a Bruker X-band EPR spectrometer. Mass spectra were recorded in positive ion mode using Waters ZQ2000 single quadrupole mass spectrometer using an electrospray ionization source or Waters-Micromass LCT Premier XE time of flight mass spectrometer equipped with an electrospray ionization source (ESI). Compound 21 was synthesized according to previously reported literature procedures. H2O2 and Mn(ClO4)2·6 H2O were used as received from the commercial suppliers.

3.5.2 Characterization Data for [MnIII(L(μ-O))2MnIVL]

**UV-vis Data**

To a 1.25 mM, 100 μL solution of MnII-L (L = 63a, 63b, 63c and 63d), 300 mM, 20 μL H2O2 was added. UV-vis spectra were recorded after 12 h.

**EPR Data**

Solutions of 63a, 63b, 63c and 63d or TPA (CH3CN:CH3OH), 500 μL, 2 mM was concentrated under vacuum and the remaining residue was mixed with 1000 μL, 2 mM solution of Mn(ClO4)2·6H2O (in H2O), 50 μL CH3CN and 120 μL, 1667 mM solution of H2O2. Samples were frozen in liquid N2 after mixing. The key parameters used for the experiment are as follows: center field 3400 G, frequency 9.34 GHz, modulation frequency 50 kHz, time constant 40.96 msec and conversion time 163.84 msec.
Figure 3-12. X- Band EPR Spectrum for complex 21\(^{\text{j}}\).

To a 1000 \(\mu\text{L}\), 2 mM solution of \(\text{Mn(ClO}_4\text{)}_2\cdot6\text{H}_2\text{O}\) 120 \(\mu\text{L}\), 1667 mM solution of \(\text{H}_2\text{O}_2\) was added. The sample was frozen in liquid \(\text{N}_2\) 1 hr after mixing and EPR spectrum was recorded.

Figure 3-13. X- Band EPR Spectrum for complex \(\text{Mn}^{\text{II}}+\text{H}_2\text{O}_2\)\(^{\text{k}}\).

500 \(\mu\text{L}\), 2 mM solution of \text{Ala(tpa)AlaAla(tpa)Gly-NH}_2\ L_1\ \text{(in CH}_3\text{CN:CH}_3\text{OH)}\ was concentrated under vacuum and the remaining residue was mixed with 1000 \(\mu\text{L}\), 2 mM solution of

\(^{\text{j}}\) Sample was frozen in liquid \(\text{N}_2\) 30 min. after mixing. Temperature 116 K; microwaves 2.00 mW at 9.34 GHz; modulation amplitude 2.00 G and receiver gain 20 000.

\(^{\text{k}}\) Sample was frozen in liquid \(\text{N}_2\) 1 hr after mixing. Temperature 116 K; microwaves 1.00 mW at 9.34 GHz; modulation amplitude 2.00 G and receiver gain 20 000.
Mn(ClO$_4$)$_2$•6H$_2$O (in H$_2$O), 50 μL CH$_3$CN. Sample was frozen in liquid N$_2$ 1 hr after mixing and EPR spectrum was recorded.

**Figure 3-14.** X- Band EPR Spectrum for complex Mn$^{II}$-L (L = 63b).$^1$

**Mass Spectrometry Data**

Mass spectra were recorded in positive ion mode using Waters ZQ2000 single quadrupole mass spectrometer using an electrospray ionization source or Waters-Micromass LCT Premier XE time of flight mass spectrometer equipped with an electrospray ionization source (ESI). The key parameters used for the experiment are as follows: capillary voltage 3.3 kV, sample cone voltage 40.0 V, desolvation temperature 350 °C, source temperature 120 °C, cone gas flow 200 L/hr, desolvation gas flow 800 L/hr. The instrument was equilibrated with MeCN before injecting sample.

Solution of ligands 63a, 63b, 63c, 63d 500 μL, 2 mM (CH$_3$CN:CH$_3$OH) were concentrated under vacuum and the remaining residue was mixed with 1000 μL, 2 mM solution of Mn(ClO$_4$)$_2$•6H$_2$O (in H$_2$O), 50 μL CH$_3$CN and 120 μL, 1667 mM solution of H$_2$O$_2$. 10 μL of the resulting solution was injected in Waters ZQ2000 single quadrupole mass spectrometer every 5 min for 1 h. The dominant molecular ion was observed at m/z 349.1031 for 63a′ (calc’d m/z 349.1036), 367.7911 for 63b′ (calc’d m/z 367.7907), m/z 368.1101 for 63c′ (calc’d m/z = 368.1108) and m/z = 386.7977 for 63d′ and (calc’d m/z 386.7983).

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$^1$ Temperature 116 K; microwaves 1.00 mW at 9.34 GHz; modulation amplitude 2.00 G and receiver gain 20 000.
REFERENCES


Template for Attaching Four Peptides: An Approach to Artificial Iron(II)-Containing Peroxidases.  


ABSTRACT

INCORPORATION OF POLYPYRIDYL LIGANDS INTO SHORT PEPTIDES AND GENERATION OF BIS-μ-(OXO)DIMETALLIC COMPLEXES

by

SELMA ATES ULKU

May 2011

Advisor: Jeremy J. Kodanko, Ph.D.
Major: Chemistry
Degree: Master of Science

An unnatural amino acid HPN, possessing a 2-pyridylmethylene group, which is a common fragment of several multidendante polypyridyl ligands, has been designed and synthesized.

We have demonstrated a divergent strategy for attaching polypyridyl ligands into peptides. Pyridyl-containing amino acids were introduced into peptide chains. Transformation of the silyloxy functionality into a chloride leaving group, followed by N-alkylation with a secondary amine such as bis(2-dipicolyl)amine was used to attach various ligands to the peptide in a divergent fashion. In addition, changing the position of the unnatural amino acid within the peptide chain could facilitate the insertion of ligands at various positions.

Finally, we showed the generation of bis-μ-oxo-bridged dinuclear metallic centers based on TPA on a synthetic peptide. In addition, we demonstrated that putting two ligands on a peptide backbone increased the stability of these species compared to parent TPA complex 21′ under the identical reaction conditions. Different linkers on the peptide chain and distance between the metal-binding units also play a crucial importance on the stability of this species. Time vs. absorbance plots noticeably signified that changing the ligand concentrations, formation and decay rates of the complexes 21′, 63a′, 63b′, 63c′ and 63d′ can be modified. Dimetallic
complexes were characterized by UV-vis spectroscopy, EPR spectroscopy and high resolution mass spectrometry.
AUTOBIOGRAPHICAL STATEMENT

SELMA ATES ULKU

Ms. Ulku was born in Ankara, Turkey. She received her B.Sc. degree in Chemistry in 2003 from Ankara University, Ankara, Turkey. She worked as a graduate research assistant at Ankara University between 2003 and 2005. She completed her first M.Sc. studies in Chemistry at Ankara University in January 2006. She has been studying towards her second M.Sc. degree at the Chemistry Department of Wayne State University as a graduate teaching & research assistant since August 2007. She has coauthored the following publications:


