This section deals with the synthesis of metal complexing ligands linked to polyamide *aminoethyl glycyl* backbone and their subsequent oligomerization on the Solid Phase. These polyamide oligomers were investigated for their metal binding properties.
3.1 Introduction

Nature uses simpler units in intricate ways and in numerous combinations to orchestrate complex processes that support and sustain life. This is exemplified in biopolymers such as proteins and polypeptides which participate in various cellular events like signalling cascades, biochemical pathways; polysaccharides that constitute structural units like cellulose, exoskeleton, cell walls; and nucleic acids which play the crucial role of storing and passing on the genetic information. In particular, comprehending the chemical origin of the properties of deoxyribonucleic acids (DNA) and emergence of complex functions from their sophisticated architectures has been an extremely tantalizing research area.¹ Nature, thus presents synthetic chemists with the challenge to construct or to mimic such assemblies. It was this endeavour which led to the emergence of the field of artificial DNA/DNA mimics, locked nucleic acid (LNA) and peptide nucleic acid (PNA).

Molecular recognition events in biological systems regulate various functions such as transport, catalysis, signal initiation and regulation, which are caused by the three-dimensional structural architectures adopted by the bio-polymers. The structural assembly which mediates the process of recognition by the receptors is driven by non-covalent forces such as hydrogen bonding, aromatic-stacking, solvophobic and van der Waals interactions.²

This section in the thesis presents studies on the synthesis of modified oligomers based on aminoethyl glycyl (aeg) backbone of PNA. It provides an overview of background literature for undertaking the research work and gives an account of recent advancements in the synthesis of metal complexating ligands.

3.1.1 Nucleic acids: chemical structure

Nucleic acids (DNA, RNA) form the basic hereditary material in all cells and are essential biopolymers of life. The double-helical structure of DNA was proposed by Watson and Crick in 1953 (Figure 1).³ DNA is a polymer made up of repeating nucleotide unit that consists of a nitrogenous base, a deoxyribose sugar and a phosphate residue. Alternating sugar-phosphate units constitute the backbone of DNA.
and each base adenine (A), thymine (T), guanine (G) and cytosine (C) is connected to sugar moiety via a $\beta$-glycosyl linkage.

The nitrogenous bases adenine (A), thymine (T), guanine (G) and cytosine (C) are paired by hydrogen bonds between complementary pairs (A:T, G:C) and these hold the two strands in a duplex form. The double helical structure of DNA is nature’s simplest and most elegant way of storing, retrieving and transferring the genetic information of a living organism. Ribonucleic acid (RNA) contains ribose instead of the deoxyribose sugar unit with the base composition of adenine (A), uracil (U), guanine (G) and cytosine (C).

**Base pairing via hydrogen bonding**

Watson and Crick recognized the the hydrogen-bonding capability of A:T and G:C base pairs, the consequent complementarity and the inherent advantage of such a scheme in the replication of this informational biopolymer through DNA model-building studies (Figure 2). The amine groups of the bases are potent H-bond donors. On the other hand, the $sp^2$-hybridized electron pairs on the oxygens of the base C=O groups and on the ring nitrogens are much better H-bond acceptors. The interactions
between acceptor:donor groups comprising hydrogen bonds are largely ionic in character.

![Image of base pairing](image)

**Figure 2.** Hydrogen-bonding interactions between (A) A-T and (B) G-C base pairs.

This base pairing pattern, known as **Watson-Crick pairing**, consists of two hydrogen bonds holding A:T base pair and three hydrogen bonds holding G:C base pair.\(^4\)

### 3.1.2 DNA modifications

Decades of intensive research in the development of oligonucleotides based therapeutics has yielded several synthetic oligonucleotides with various modifications to the deoxyribonucleotide unit. The objective of the several of these modifications was to achieve better stability under physiological conditions, longer *in vivo* half-life, enhanced cellular internalization and improved binding specificity, especially for the modulation of specific gene expressions *via* binding to complementary RNA and genomic DNA through antisense and antigene technology, respectively. Based on the incorporation of unnatural bases, modified sugars and altered phosphate backbone, the synthetic DNA mimics can be considered to have evolved in three generations (Figure 3).

‘First generation’ ONs, the best known example is phosphorothioate (PS)\(^5\) in which one of the non-bridging oxygen atoms in the phosphodiester bond is replaced by sulphur. These were shown to have cellular toxicity and found to exert slightly reduced affinity towards complementary RNA molecules in comparison to their corresponding phosphodiester oligodeoxynucleotides.

‘Second generation’ ONs are consists of nucleotides with alkyl modifications at the 2’ position of the ribose\(^6\) and have 2’-0-methyl and 2’-0-methoxy-ethyl RNA are the most important members of this generation of antisense ONs and are known to be less toxic than PS DNAs.
In order to improve properties such as target affinity, nuclease resistance and pharmacokinetics, the concept of conformational restriction has been widely utilised for enhanced binding affinity and biostability, paving way for the development of ‘third generation’ ONs encompassing variety of modified nucleotides.

These include several DNA and RNA analogs with modified phosphate linkages or riboses as well as nucleotides with completely different chemical moieties substituting the furanose ring.

Figure 3. Chemical modifications in the development of oligonucleotides.

‘Third generation’ ONs includes modified phosphate derivative N3'-P5' phosphoroamidates (NPs), in which the 3'-hydroxyl group of the 2'-deoxyribose ring is replaced by a 3'-amino group. Another first uniformly sugar-modified ONs is 2'-Deoxy-2'-fluoro-β-D-arabino nucleic acid (FANA), which is reported to induce RNase-H cleavage of a bound RNA molecule. The most proven class of chemically

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modified nucleotide is **Locked nucleic acid** (LNA), which contains a methylene bridge that connects the 2’-oxygen of the ribose with the 4’-carbon.\(^9\)

**Morpholino oligonucleotides** (MF) are nonionic DNA analogs in which ribose unit replaced by morpholino moiety and phosphoroamidate linkages instead of phosphodiester bonds in backbone.\(^10\) The replacement of the five-membered furanose ring by a six-membered ring lead to **Cyclohexene nucleic acid** (CeNA), which are characterized by a high degree of conformational rigidity of the oligomers.\(^11\) **Tricyclo-DNA** (tcDNA) exhibited enhanced binding to complementary sequences.\(^12\) **Peptide nucleic acid** (PNA) are most intensively studied DNA analogs besides phosphorothioate DNA and 2’-O-alkyl RNA.\(^13\) The present work is oriented towards the development of such repeating \(\textit{N}-(1\text{-aminoethyl})\)-glycyl \((\text{aeg})\) units having different metal complexing ligands.

### 3.1.3 Peptide nucleic acids (PNAs)

Peptide Nucleic Acid (PNA) is a DNA mimic resulting from the replacement of sugar-phosphate backbone by a pseudopeptide backbone and was introduced by Nielsen \textit{et al.} in 1991.\(^13\) The structure of PNA is remarkably simple. It consists of a repeating \(\textit{N}-(1\text{-aminoethyl})\)-glycyl \((\text{aeg})\) units linked by amide bonds (Figure 4). A methyl carbonyl linker connects natural as well as unusual nucleotide bases to the backbone at the amino nitrogens. The pseudopeptide (polyamide) backbone of PNA was originally designed to be a good structural mimic of the ribose-phosphate backbone of nucleic acids. PNA was proven to be an efficient DNA mimic with better DNA/RNA-recognition and triplex-forming properties.\(^14\)

![Figure 4. Structure of (A) PNA and (B) DNA, B = nucleobases.](image-url)
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PNA oligomers are better than the conventional antisense oligonucleotides, because of their high flexibility and absence of charge in the artificial backbone. They have longer life span in the cellular environment than any oligonucleotide and used to downregulate the target gene expression, being resistant to both nucleases and proteases. PNA hybridizes with complementary DNA/RNA sequences with superior thermal stability resulting from the overall decrease in electrostatic repulsion in DNA/RNA strands. Hence, they can successfully compete and eventually displace the natural complementary strand. In short, PNA has attracted wide attention in medicinal chemistry for development of gene therapeutics in antisense\textsuperscript{15} and antigene\textsuperscript{16} strategy and for diagnostics.

There is an increasing interest in modulating and expanding the recognition motifs of standard base pairs in PNA. Employing non-natural nucleobases in place of natural ones would help us in understanding of the recognition process in terms of various contributing factors such as hydrogen bonding, inter-nucleobase stacking \textit{etc}. Further, new recognition motifs may have potential applications in diagnostics and nanomaterial chemistry. 2,6-Diaminopurine (Figure 5A)\textsuperscript{17} offers increased affinity and selectivity for thymine/pseudoisocytosine (Figure 5B)\textsuperscript{18} and is an efficient mimic of protonated cytosine for triplex formation.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{modified-nucleobases.png}
\caption{Modified nucleobases used in the synthesis of PNA oligomers.\textsuperscript{17-21}}
\end{figure}

2-Aminopurine (Figure 5C) forms hydrogen bonds with U and T in reverse Watson-Crick fashion. The \textit{E}-base (Figure 5D) was rationally designed for
recognition of A-T base pair in the major groove and forms a stable triad with T in the central position. A wide variety of 5-substituted uracils (Figure 5E) were synthesized to study their ability for triplex formation. The G-clamp base (Figure 5F) was developed to build specific, additional bonding interactions with guanine. Unnatural heterocycles 3-nitropyrrrole (Figure 5G) and cyanuryl PNA (Figure 5H) were also synthesized.

### 3.1.4 Metal organic complexes

Metals are nature’s favourite class of guest molecules that are involved in stabilization of different three dimensional structures. These include Zn finger proteins, chlorophyll pigment, haemoglobin etc, which allow them to carry out vital functions like transcription process, photosynthesis and carry oxygen reversibly etc. Na\(^+-\)K\(^+\) pumps are crucial in maintaining electrolyte balance across cell membranes, metalloprotein ‘haemoglobin’ chooses Fe to carry out the metabolic respiration. The understanding of these recognition events has further deepened with the development of foldamers, where the interplay of basic forces and structural assembly process is often initiated by interactions with the guest molecule.

Jean Marie Lehn\(^{23}\) laid the foundation of supramolecular chemistry with his discovery of cryptands in the late 1980s.\(^{24}\) These complexes are found to possess various properties for example cation transport, anionic polymerization and removal of radioisotopes. Further, he designed novel inorganic and organic hybrids for various functions. Significant contributions towards the discovery of dynamers (dynamic polymers), [2 x 2] grid complexes, 2D- and 3D-cryptands were made by this finding. Metallofoldamers developed by Lehn et al.\(^{25}\) presents one of the many facets of the folding behaviour exhibited by synthetic oligomers by virtue of ion–dipole interactions, where Cs\(^+\) ions drive the helical assembly of the alternating naphthyridine-pyrimidine oligomer unit (Figure 6).
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![Diagram](image)

**Figure 6.** Naphthyridine–pyrimidine metallofoldamer reported by Lehn.\(^{25}\)

The same group reported Pb\(^{2+}\) ion binding pyridine-hydrazone oligomer that forms a helically folded complex (Figure 7).\(^{26}\)

![Diagram](image)

**Figure 7.** Pyridine–hydrazone metallofoldamer exhibited binding to Pb\(^{2+}\).\(^{26}\)

Various other helical assemblies have been designed by different groups for e.g. Moore *et al.*\(^{27}\) reported solvophobically driven folding of \(m\)-phenylene-ethynylene (\(m\)-PE) oligomers due to metal binding-induced helical conformation with participation of interacting cyano groups.

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3.1.5 Metallo-DNAs

In addition to being the carrier of genetic information, DNA molecule has also been recognized for its superior self-assembling properties. Sequences can be designed to assemble into different types of H-bonding induced architectures such as duplexes, triplexes, quadruplexes hairpins and a variety of branched nanostructures. The DNA strands can also arrange themselves into long nanowires. Several attempts have been made to exploit and extend these properties of DNA by synthetic modifications to the backbone and nucleobases for various applications in both biological and nanomaterials chemistry. Keeping the fundamental design of the DNA double helix intact, several groups have studied replacement of the natural base pairs of the DNA molecule with ligands that show pronounced affinity to bind to metal ions. Shionoya et al. (Figure 8) replaced the natural DNA base pairs with unnatural ones that can coordinatively bind metal ions mimicking the base pairs of DNA.

The strategy was used to assemble copper and mercury metal ions inside the DNA molecule to generate heterometallic wires by employing different metal binding ligands as substituents for the nucleobases. Some of the synthesized inorganic bases are depicted in Figure 9, which shows variety of binding selectivities. e.g. o-amino phenol binding to Pd$^{2+}$, o-hydroxy-quinone (Hq), catechol and bipyridyl (Bpy) binding to Cu$^{2+}$.

![Figure 8](image.png)

**Figure 8.** Cu$^{2+}$-mediated duplex formation between two artificial DNA strands in which hydroxypyridone nucleobases replace natural base pairs. $^{29}$
The pyridyl (Py), terpyridyl (Tpy), imidazolyl (Im) and S-methylpyridyl (Spy) bind preferably to Ag$^+$ metal ions.\textsuperscript{29}

### 3.1.6 Metallo-PNAs

Over the past few years, peptide nucleic acids (PNAs) have emerged as one of the most promising new type of molecules for the recognition of nucleic acids (DNA and RNA). The successful incorporation of artificial bases in DNA molecule prompted replacement in PNA also, since latter being conceptual DNA mimic. PNA backbone also provides a great deal of synthetic versatility and this feature paves an easy access to incorporate amino acid residues, non-natural bases and metal-binding ligands into it.\textsuperscript{30}

Nolte \textit{et al.}\textsuperscript{31} incorporated and studied the cellular uptake of octapeptide-cobalt complexes, which have 4 to 5 lysine residues and a guanidine group. The resulting complexes showed better results in the cellular uptake and its endosomal escape.
compared to ferrocenium complexes (Figure 10). Also in order to facilitate cellular imaging experiments, fluorescent di-rhenium organometallics have been attached to the PNA oligomers, which could successfully stain both cytoplasm and nucleus of HEK-293 cells simultaneously.32

![Synthesized cobaltocene peptides having lysines and guanidines](image)

**Figure 10.** Synthesized cobaltocene peptides having lysines and guanidines.31

To construct a hybrid of metal bound PNA with aeg backbone properties along with the co-ordination properties of transition metals, Achim et al. 33 incorporated three consecutive 8-hydroxyquinoline (hq)33 or bi-pyridine (bpy)34 units into PNA oligomers. It was observed that stabilization exerted by metal ions on terminally modified duplexes surpassed the effects exerted on the central modification. These modifications showed increased or decreased stability in presence of various metal ions. It was found that greater stabilization was exerted by the Cu$^{2+}$ or Ni$^{2+}$ metal ions, while Pd$^{2+}$ and Pt$^{2+}$ did not change the melting temperatures of the duplexes generated. Due to steric effect of these large ligands, more modifications in the oligomer backbone resulted in the decreased duplex melting temperatures and have shown high mismatch tolerance.

Several groups have reported insertion of pyridyl (py), ter-pyridyl (tpy) moieties into the PNA backbone (Figure 11).35 Also in order to evaluate the electrochemical behaviour of organometallic moieties within the PNA sequence, ferrocenyl click derivatives,36 chromium tricarboxyl,37 Fischer type carbene
complexes of tungsten\textsuperscript{38} and redox active ruthenium complexes\textsuperscript{39} were synthesized and have found great applications in spectroscopic analysis and bio-imaging areas.

Mokhir \textit{et al.}\textsuperscript{40} designed PNA oligomers that can bind specifically with metals which are available at higher concentrations in various organs\textsuperscript{41} as well as in some cancerous cells for \textit{e.g.} Zn\textsuperscript{2+}. In breast cancer tissues, zinc levels sometimes are known to increase by a factor of 72\%. With this rationale in mind several bi- and tridentate ligands \textit{e.g.} bis-(pyridine-2yl-methyl)amine (dpa)-PNA conjugates were designed and found to increase in the thermal stability of PNA.DNA/RNA duplexes (Figure 12). The modified terpyridyl PNA-Zn (II) complexes also have shown increased cellular uptake.\textsuperscript{42}

\textbf{Figure 11.} Structures of metal binding ligands attached to PNA backbone.\textsuperscript{33-36}
3.1.7 Synthetic methods and characterization of polyamide aeg oligomers

There are many methods to achieve synthesis of the oligomeric PNA strands. Solution phase synthesis using different coupling agents is not a preferred strategy for oligomerization of peptide mimics due to its tedious and time consuming procedure. The ease of handling and scale up procedures have made choice of solid phase peptide synthesis (SPPS) better compared to solution phase peptide synthesis (a general comparison has been discussed in Table 1).

3.1.7a Solid Phase Peptide Synthesis (SPPS)

Solid Phase Peptide Synthesis (SPPS) protocols are used for the synthesis of several peptides and oligomeric PNAs. The method allows easy access to incorporation of a large number of analogues which are useful in binding and elicit biological properties. There are several features that are beneficial in solid phase peptide synthesis like no loss of material during work-up as the peptide is never taken out of the reaction vessel. No purification of the intermediates is required during the synthesis, and operations are repetitive enabling automation.

Table 1. Solid Phase Peptide Synthesis (SPPS) vs Solution Phase Peptide Synthesis.

<table>
<thead>
<tr>
<th>Solid Phase Peptide Synthesis</th>
<th>Solution phase peptide synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time saving process.</td>
<td>Tedious process.</td>
</tr>
<tr>
<td>Practically possible for small as well as lengthy peptide sequences.</td>
<td>Practically difficult, as purification of polar intermediate peptides is not feasible.</td>
</tr>
<tr>
<td>Isolation and purification of intermediates is not needed.</td>
<td>Isolation and purification of intermediates is desirable for next step synthesis.</td>
</tr>
<tr>
<td>Excess of coupling reagent and monomers</td>
<td>Excess of coupling reagent and monomers are not desirable.</td>
</tr>
</tbody>
</table>
are needed.
- Racemization is not observed.
- Limited scope of protecting groups of side chains.
- Fast and good yielding synthesis.
- Racemization is observed in some cases.
- Several different groups can be employed as isolation of intermediates is needed.
- Slow process.

Peptides with different functionalities can be synthesised by proper choice of resins. A brief description used for the synthesis of peptide acids, peptide hydrazides and peptide carboxamides is described in Table 2.

**Table 2. Resins Used for Solid Phase Peptide Synthesis**

<table>
<thead>
<tr>
<th>Resin Structure</th>
<th>Protecting Group Used</th>
<th>Resin Cleavage Conditions</th>
<th>Final Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merrifield resin</td>
<td>Boc</td>
<td>HF/ TFA</td>
<td>Peptide acids</td>
</tr>
<tr>
<td>Wang resin</td>
<td>Fmoc</td>
<td>TFA</td>
<td>Peptide acids</td>
</tr>
<tr>
<td>Rink Amide resin</td>
<td>Fmoc</td>
<td>20% TFA/DCM</td>
<td>Peptide carboxamides</td>
</tr>
<tr>
<td>SASRIN resin</td>
<td>Fmoc</td>
<td>1% TFA/DCM</td>
<td>Peptide acids</td>
</tr>
</tbody>
</table>
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Resin Boc TFA/TFMSA Peptide carboxamides

Resin Fmoc 1% TFA/DCM Peptide carboxamides

Resin Oxime’s resin Boc NaOH, N₂H₄, NH₃, RNH₂ Peptide hydrazides

Resin Amino methyl resin Boc/Fmoc 20% TFA/DCM Peptide carboxamides

Resin PAM resin Boc TFA/TFMSA Peptide acids

Resin Trityl resin Fmoc 1-5% TFA/DCM Peptide acids

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Generally in solid phase peptide synthesis, either acid labile Boc-group\textsuperscript{44} or base labile Fmoc-group\textsuperscript{45} (Scheme 1) is used for N-protection. The C-terminus amino acid can be attached directly to the resin or through a linker. Other functional groups present on the side chains are protected orthogonally with Boc- and Fmoc- protecting groups.\textsuperscript{42} Fmoc chemistry is known for generating peptides of higher purity and in greater yield than t-Boc chemistry.

**Boc-chemistry**

\[ \text{MBHA resin} \]

\[
\begin{align*}
\text{A) Neutralization with} & \quad 20\% \text{DIPEA/DCM} \\
\text{B) Washing DCM, DMF} & \quad \text{Coupling} \\
\text{C) Coupling} & \quad \text{Rinkamide resin} \\
\text{D) Deprotection} & \quad 50\% \text{TFA/DCM} \\
\text{E) Neutralization} & \quad 5\% \text{DIPEA/DCM} \\
\text{F) Resin cleavage} & \quad \text{HF, or TFMSA, 1,2-Ethane dithiol thioanisole, TFA} \\
\end{align*}
\]

**Fmoc-chemistry**

\[
\begin{align*}
\text{A) Deprotection with} & \quad 20\% \text{piperidine/DCM} \\
\text{B) Washing DCM, DMF} & \quad \text{Coupling} \\
\text{C) Coupling} & \quad \text{NH}_2 \\
\text{D) Deprotection} & \quad 20\% \text{piperidine/DCM} \\
\text{E) Neutralization} & \quad 5\% \text{DIPEA/DCM} \\
\text{F) Resin cleavage} & \quad \text{TFA} \\
\end{align*}
\]

Scheme 1. General protocols for the synthesis of peptides via Boc (left) and Fmoc- groups (right).

The cleavage of Boc-group is carried out using TFA (trifluoroacetic acid) and the deprotection of Fmoc group is done under basic conditions using piperidine or

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diethylamine. After the synthesis the peptide up to the desired length, it is cleaved from the solid support, employing different deprotection conditions for different resins. The final cleavage of resin is achieved using a strong acid such as hydrogen fluoride (HF) or trifluoromethanesulphonic acid (TFMSA) in case of Boc protecting method and 20% TFA in DCM for the Fmoc protecting method (Scheme 2).

![Scheme 2. General strategies for Boc and Fmoc protecting groups.](image)

**Monitoring of coupling on resin**

Successful coupling of amino acid on the resin can be estimated by detecting the amount of unreacted amino groups on the resin.

**Kaiser’s test** is the most widely used qualitative test to detect the presence or absence of free amino groups (deprotection/coupling).

![Scheme 3. Proposed mechanism for Ninhydrin test: (a) Amine reacts with ninyhdrin to generate Schiff’s base; (b) Hydrolysis generates amine and aldehyde; (c) Amine reacts with another molecule of nindrin to generate colored compound, Rheumann’s purple.](image)

**where, L = Inorganic metal complexing ligands**

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It is used to monitor the completion of $t$-Boc deprotection and hence an efficient amide bond (peptide bond) formation. When free amines are present on surface of the resin, it reacts with ninhydrin to produce the purple coloured, Rheumann’s purple (Scheme 3). Kaiser’s test is negative upon completion of the coupling reaction and the resin beads remain colourless.\(^{47}\)

**Chloranil test** is highly sensitive towards unreacted primary as well as secondary amines present on the resin beads. Resin is taken in a small test tube, reacted with chloranil and acetone or acetaldehyde. For detection of primary amines, acetaldehyde is added and for secondary amines, acetone is added. The resin beads are left at room temperature for 5 min and colour is checked. When free amines are present on surface of the resin after deprotection, chloranil reacts with it and produces blue colored charge-transfer complex (Scheme 4). On the other hand, upon completion of the coupling reaction, the resin beads remain colourless.

![Scheme 4. Proposed mechanism for the formation of charge-transfer complex between chloranil and peptides.](image)

**3.1.7b Gel Filtration Chromatography (GFC)**

Gel Filtration Chromatography (GFC) is one of the separation techniques used widely in case of biomolecules. It separates molecules on the basis of size and molecular weight. The stationary phase consists of well-defined porous beads of different sizes having a fractionation range, which controls separation of molecules
based on variable molecular weights. Molecules of smaller molecular weights get
trapped inside the porous beads, and eluted in the end, while the high molecular
weight biomolecules do not enter the gel pores and are excluded/eluted first.\textsuperscript{48}

3.1.7c High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) is one of the
chromatographic techniques used to separate a complex mixture of compounds. It
enables purification and quantification of the individual components in the mixture. It
has wide application in analytical chemistry and biochemical research involving
separation of wide variety of organic biomolecules and in pharmaceutical industry.

The HPLC instrument typically includes a sample injector, pumps and a
detector all under computer control. The sample injector delivers the sample mixture
into the mobile phase stream, which is carried into the column and is under the control
of gradient mixer. The pump controls the flow and passes the solvents through the
column. The detector generates a signal proportional to the amount of sample
component emerging from the column, hence allowing quantitative analysis of the
sample components. Various detectors used in HPLC are UV/Vis, photodiode array
(PDA), refractive index, fluorescence etc. HPLC can also be used in a preparative
mode to collect the peak of interest for further characterization.

3.1.7d High Resolution-Mass Spectrometry (HR-MS)

Mass spectrometry (MS) is the science of displaying the spectra of masses of
the molecules. It is useful in determination of elemental composition, the masses, and
the chemical structures of molecules of various classes. It works by ionizing chemical
compounds to generate charged molecules or molecule fragments and measuring their
mass-to-charge ratios. A mass spectrometer instrument consists of four modules:

- **Inlet probe** for injecting samples.
- **Ionizer** for converting sample into positively or negatively charged ions.
- **Mass analyzer** sorts the ions by mass either by electric field or by magnetic
  field.
- **Detector and Amplifier** for converting charged ions into electric current.
The digitalized signal was further processed for getting the mass spectra of the compounds. The synthesized small peptide units purified by HPLC can be characterized by HR-MS. MS is commonly used in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds.\textsuperscript{49} It can be coupled to liquid chromatography and each peak eluted can be directly characterized by its mass.

**3.1.7e Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF)**

Matrix Assisted Laser Desorption Ionization (MALDI) was first introduced for proteins by M. Karas and F. Hillenkamp (1988) that allows determination of intrinsic molecular masses of nucleic acids.

MALDI-TOF is a key technology which employs ‘soft ionization’ method.\textsuperscript{50} The nucleic acid and protein samples are embedded in a crystalline matrix of a light absorbing molecules (e.g \(\alpha\)-cyano-\(p\)-hydroxycinnamic acid, nicotinic acid & sinapinic acid).\textsuperscript{51} This target is excited by a pulse from an ultraviolet laser beam that in high vacuum results in intact molecules of the sample becoming desorbed into the gas phase and ionized by the UV radiation to give (mostly) singly charged ions. The matrix assists in desorption and ionization of the analyte and molecular weight >500 kDa can be analyzed. The basic concept of TOF mass analyzer (Time Of Flight) is that the ions are separated based on the time taken by the ion to drift down the flight tube to the detector. Lighter ions have higher velocities than heavier ions and reach the detector first.

This technique is very much utilised for oligomers with high molecular weights.

**3.1.7f X-ray crystal structure determination**

X-ray crystallography is the ultimate method of structure characterization of organic, inorganic and biomolecules. The atoms arranged in the crystal lattice enables X-rays to diffract them in specific angles. After getting the diffraction pattern, electron density map of the structure is derived. This diffraction pattern helps in rendering the important information regarding position of the atoms, specific arrangements as well as chemical bonds and their disorders.\textsuperscript{52}
3.1.7. Determination of $pK_a$

The acid dissociation constant ($pK_a$) is an important physicochemical parameter of a substance, and its knowledge is of fundamental requisite in a wide range of applications in various research areas.\(^{53}\) In the pharmaceutical industry, $pK_a$ is an important factor for drug design and development. The acid–base property of any biomolecule is the key parameter in terms of determining solubility, absorption, distribution, metabolism and elimination.

There are several methods for the determination of dissociation constants like the traditional potentiometry and UV–Vis absorption spectrometry etc. In the present studies potentiometry method has been used. Potentiometric titration is a high-precision technique for determining the $pK_a$ values of substances. It is commonly used due its accuracy and the commercial availability of fast, automated instruments.

3.1.8 Metal binding studies of polyamide $aeg$ oligomers

The metal binding studies of polyamides is generally performed using optical spectroscopy. The present section focuses on detailed study of optical spectroscopy in the characterization, stoichiometry and binding constants of the metal-ligand complexes.

3.1.8a UV-Vis spectroscopy

Optical spectroscopy is one of the most widely used techniques for the study of stoichiometry and binding constants of metal-ligand complexes.\(^{54}\) For successful measurements, significant spectral change should occur during complex formation. In any spectrophotometric method, equivalence point is detected due to the difference in the molar absorptivities (at the wavelength selected) of the various species present in the mixture. The appearance of an absorbing species will give a concentration dependent change in absorbance resulting in two straight lines that intersect at the equivalence point. The selection of the analytical wavelength requires care, since at least three components are present that may absorb light: the original substance, the DNA/RNA/oligomer, metal salts and the resulting metal complex. The usual procedure is to select a wavelength at which only one component absorbs. The resulting spectral curves will pass through a common point of intersection, called an
isosbestic point, which shows presence of more than one species in the solution. (Figure 13).

![Representation of isosbestic point in UV-Vis spectroscopy.](image)

**Figure 13.** Representation of isosbestic point in UV-Vis spectroscopy.

For a successful spectrophotometric titration it is necessary that the measured species adhere roughly to Lambert-Beer's law, and the necessary precautions must be taken to maintain the relation $A = \varepsilon cl$. To avoid the effects caused by dilution on absorbance, titrant should have 10 times more concentration than the titrated solution.

**Job’s Method** was introduced by P. Job (1928) and is used to determine the stoichiometry of components. This method is known as the method of continuous variation and is used to derive quantitative binding relationships in chemical reactions. The total molar concentrations of two binding partners (*i.e.* metal and ligand) are held constant, but the relative mole fractions are varied. The absorption values from the complexation are plotted against the mole fractions of the two components. The maxima or minima on the plot correspond to the stoichiometry of the two binding species (Figure 14).

![Representation of maxima and minima in Job’s plot.](image)

**Figure 14.** Representation of maxima and minima in Job’s plot.
There are several conditions that must be considered for Job's method:

- The system must follow Lambert-Beer law within the concentration range
- The total mole fractions of two components should be held constant throughout the experiment
- Total absorption should be in the range of 0.1-1.0
- pH and ionic strength must be maintained constant

In the present work, studies on the interaction of monomers and oligomers with metal salts have been extensively investigated using UV spectroscopy.

3.1.8b Binding constant measurements

The extent of interactions between the two species / complexation is derived from binding constant values. Herein, few techniques employed for the calculation the binding constants have been comprehensively discussed.

Optical spectroscopy

The intrinsic binding constant, $K$, of the metal complex to monomer/polyamide oligomers can be determined from a Benesi-Hildebrand plot. It is often applied in one-to-one complex systems, such as charge-transfer complexes and host-guest molecular complexation. Benesi-Hildebrand equation has the following form for 1:1 binding systems (Eq. 1):

$$\frac{1}{\Delta A} = \frac{1}{\Delta A_{max}} + \left[\frac{1}{K[L]} \times \frac{1}{\Delta A_{max}}\right]$$  \hspace{1cm} (Eq. 1)

Benesi-Hildebrand equation has the following form for 1:2 binding systems (Eq. 2):

$$\frac{1}{\Delta A} = \frac{1}{\Delta A_{max}} + \left[\frac{1}{K[L]^2} \times \frac{1}{\Delta A_{max}}\right]$$  \hspace{1cm} (Eq. 2)

Where, $\Delta A = A - A_0$, $A$ is absorbance intensity of ligands in the presence of metal salts. $A_0$ is absorbance intensity of ligands in the absence of metal salts. $K$ is equilibrium/ binding constant for the reaction and $L$ is the ligand concentration. The binding constant ($K$) is determined from the intercept to slope ratio of Benesi-Hildebrand plot. The experiment involves measuring the change in the
absorption/emission spectra of the reaction before and after the formation of the product.

**Magnetic Resonance Spectroscopy (MRS)**

NMR is widely used for the binding studies. It throws light on the complexes in the solution. Difference in the chemical shifts of the ligand and metal complexes directly gives evidence in terms of binding as well as site of binding.

**Isothermal Titration Calorimetry (ITC)**

Calorimetry is a technique in which the heat of a reaction is measured. The commonly used method for measurement of enthalpy change associated with a binding interaction is isothermal titration calorimetry (ITC). It is utilised for almost any bimolecular binding interaction at a fixed and constant temperature. From the binding isotherms, the equilibrium-binding constant \( K_b = K_a \) and binding stoichiometry \( n \) can be determined.

**3.2 Present work: Rationale and Objective**

The primary idea behind replacing the natural bases linked to \( aeg \) backbone with ligands that have affinity towards metals, is to generate molecular assembly or molecular wires based on metal-ligand interactions (Figure 15).

![Figure 15. Metal complexes with designed ligands linked to aeg backbone.](image)

The properties of metal complexes can be tuned by changing metal ions and ligands such as: (1) thermodynamics and kinetics of complexation and decomplexation, (2) change in coordination numbers and geometries, (3) physical and chemical properties such as redox-, magnetic-, optical- and Lewis acidity, and (4)
rational control of assembling properties of the derived oligomers. With above mentioned characteristics in mind, 2-pyridyl benzimidazole (PBI) \textbf{1}, phenylenediamine (PDA) \textbf{2}, catechol (CAT) \textbf{3} were chosen as metal chelating ligands for incorporation of metal ions in \textit{aeg} backbone (Figure 16).

![Figure 16. Designed novel \textit{aeg} linked bidentate ligands.]

Amongst these, 2-pyridylbenzimidazole (PBI) \textbf{1} has venerable history in coordination chemistry. The choice of the ligands was made with a view of ease of synthesis, donor/acceptor properties of imidazole and pH triggered assembly and disassembly. Phenylenediamine (PDA) \textbf{2} was chosen due to its property of strong affinity towards palladium and gold ions. Catechol (CAT) \textbf{3} linked \textit{aeg} ligand was designed for its promising application in drug delivery as it is likely to be a good candidate to form stable complexes with boron. Thus, these peptidic catechols can potentially bind to boron and help deliver to the target tissues/cells. The designed ligands and oligomers are anticipated to form 2:1 metal complexes with different metals \textit{e.g.} PBI with Cu$^{2+}$, PDA with Pd$^{2+}$ and CAT with B$^-$ etc.

The designed \textit{aeg} linked ligands are bidentate ligands \textit{i.e.} each ligand has two binding sites. In case of PBI, two quaternary nitrogens are responsible for binding with copper, in PDA two aromatic amines are needed to bind with palladium and in CAT, boron is attached to both of the phenolic groups.

The rationally designed “metallo-oligomers” can form either parallel or antiparallel duplexes upon complexation with metal ions. They can also be useful in generating multimeatalic structures analogous to DNA based heterometallic nanowires by metal-coordination based self-assembly of modified aminoethyl glycine (\textit{aeg})
polyamides. Incorporation of metal ions into aminoethyl glycine (aeg) would result in stable complexes as well as a variety of metal based functions (Figure 17).

![Figure 17. Metal mediated duplex formation in modified ligand aminoethyl glycine (aeg) strands.](image)

The specific objectives of this work are:

- Synthesis of metal chelating aminoethyl glycine (aeg) linked ligands and their oligomerization.
- Study of the metal-complexation properties of ligand linked aminoethyl glycine (aeg) modified monomers and oligomers.

### 3.3 Results and Discussion

The retrosynthetic pathway (Scheme 5) for each of the target molecule suggests that 1,2-diaminoethane can act as prompt precursor for their synthesis. It can later be functionalized with different reagents and reacted with different substrates to achieve the target monomers. 2-Pyridylbenzimidazole (PBI) 1 aeg linked ligand would obtained from mono-N-alkylated-1,2-diaminoethane and 2-pyridylbenzimidazole, which in turn could be synthesized from pyridine-2-aldehyde and o-phenylenediamine.58 o-Phenylenediamine (PDA) 2 aeg linked ligand could be synthesized from mono-N-alkylated-1,2-diaminoethane and 3,4-diaminobenzoic

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acid. Catechol (CAT) 3 linked aeg ligand could be obtained from mono-\(N\)-alkylated-1,2-diaminoethane and 3,4-dihydroxyphenylacetic acid.

![Image of chemical structures](image)

**Scheme 5.** Retrosynthetic pathways towards the synthesis of target aeg linked ligands.

### 3.3.1 Synthesis of \(N\)-Boc-aminoethyl 2-pyridylbenzimidazole (PBI) glycinate

In this account, the synthesis of aeg linked ligands were carried out as literature\(^5\) reports starting from the readily available 1, 2-diaminoethane (Scheme 6). The monoprotected derivative of ethylenediamine was prepared by treating a large excess of 1,2-diaminoethane with (Boc)\(_2\)O in dioxane: water under high dilution conditions to minimize the formation of \(N^1, N^2\) di-Boc derivative. The formation of product 5 was confirmed by its spectral analysis which was in accordance to the literature data. Compound 5 was \(N\)-monoalkylated using ethylbromoacetate in

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acetonitrile to furnish compound 6 whose structure was confirmed by spectral analysis. Due to the instability of compound 6 at room temperature, it was immediately treated with chloroacetyl chloride in aqueous dioxan containing Na₂CO₃ to yield N-acyl compound 7 in good yield.

![Chemical diagram](image)

**Scheme 6.** Synthesis of N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 1.

Compound 7 was treated with 2-pyridylbenzimidazole (PBI) to furnish compound 8, whose structure was confirmed by the appearance of new peaks at δ 8.57, 8.49 (d, 2H, CH) ppm for aromatic protons in ¹H NMR spectrum. Final hydrolysis of compound 8 with 1N LiOH resulted in the desired monomer 1, obtained as a white solid in 90% yield. The formation of product 1 was confirmed by its spectral analysis. In ¹H NMR spectrum, the absence of signals due to ester group protons confirmed the formation of acid 1.

### 3.3.1a Synthesis of palladium complex with N-Boc-aminoethyl-2-pyridyl benzimidazole (PBI) glycinate

N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 was crystallized from a mixture of ethanol and dichloromethane (2:8), and its structure was confirmed by single crystal X-ray analysis (Figure 18). This was treated with different metal salts to synthesize the corresponding metal complexes. N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 was reacted with various palladium salts such as sodium tetrachloropalladate, palladium acetylacetonatoe, palladium nitrate and palladium-dba salt, in different molar ratios which resulted in either the formation of a precipitate or viscous liquid. It was found that treatment with palladium acetate in dry
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DMF resulted in formation of yellow crystals. The structure of N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8-Pd complex was determined by single crystal X-ray diffraction analysis.

![Diagram of 8-Pd complex formation](image)

**Figure 18.** ORTEP diagram of N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 and Pd complex.

The crystal analysis of N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8-Pd revealed following features:

- The complexation of metal involved two nitrogens, one each from the 2-pyridyl unit (2') and the benzimidazole ring (N3).
- Only 1:1 complex of metal and the N-Boc-aminoethyl (PBI) glycinate 8 was observed rather than the expected 2:1 complex.
- The orientation of the pyridyl nitrogen (2') that act as metal binding site in free ligand shows rotation by 90°.

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In the *trans* conformation, the pyridyl nitrogen (2') and the nitrogen atom (N$_1$) of benzimidazole attached to the *aeg* backbone are in the same plane, while in the *cis* conformation pyridyl nitrogen (2') and other nitrogen atom (N$_3$) of benzimidazole are in the same plane (Figure 19).

The original *trans* conformation of pyridyl nitrogen (2') and the N-benzimidazole moiety (N$_1$) switched to *cis* conformation for effective metal complexation.

In the ORTEP diagram of *N*-Boc-aminoethyl (PBI) glycinate 8, it is clear that carbonyl group is oriented towards the glycinate part, while in *N*-Boc-aminoethyl (PBI) glycinate 8-Pd projection of carbonyl group is towards the *Boc* group.

Similar reaction with other metal salts *i.e.* cobalt nitrate, copper nitrate, nickel nitrate, zinc nitrate did not result in formation of any crystals.

### 3.3.2 Synthesis of *N*-Boc-aminoethyl-α-phenylenediamine (PDA) glycinate

3,4-diaminobenzoic acid 10 was treated with CbzCl in NaOH to obtain the *bis*-N,N'-protected analogue 11 in 75% yield. The identity of compound 11 was confirmed by MALDI-MS, which showed a peak *m/z* at 420, indicating that both the amino groups are protected with Cbz group. Compound 6 was treated with the *bis*-N,N'-Cbz-protected benzoic acid 11, in the presence of EDC.HCl and HOBt, to furnish compound 12 in 50% yield. The structural identity of product 12 was confirmed by its spectral analysis (Scheme 7).

![Scheme 7. Synthesis of *N*-Boc-aminoethyl-α-phenylenediamine (PDA) glycinate 13.](image-url)
In $^1$H NMR spectrum, peaks at $\delta$ 7.35-7.24 ppm and in $^{13}$C NMR spectrum, peaks at $\delta$ 135.9, 128.7, 128.5 ppm were observed confirming the attachment of aromatic ring to the aeg backbone. The hydrolysis of compound 12 with LiOH (1N) resulted in the desired monomer 13, in 60% yield. The formation of product 13 was confirmed by its $^1$H NMR spectrum, which showed absence of ester ethyl group protons as expected for product 1.

### 3.3.3 Synthesis of N-Boc-aminoethyl-3,4-dihydroxyphenyl (CAT) glycinate

Compound 6 was treated with 3,4-dihydroxyphenyl acetic acid 14, in the presence of EDC.HCl and HOBr to furnish compound 15 in 65% yield. The characterization of product 15 was done by its $^1$H NMR spectrum (Scheme 8).

![Scheme 8. Synthesis of N-Boc-aminoethyl-3,4-dihydroxyphenyl (CAT) glycinate 3.](image)

The presence of peaks at $\delta$ 6.71-6.50 (m, 3H, CH) ppm confirmed the successful coupling of aromatic ring to the aeg backbone. The hydrolysis of compound 12 with LiOH (1N) resulted in the desired monomer 3, whose identity was confirmed by its $^1$H NMR spectrum, which showed the absence of signals due to ethyl ester group protons. Extensive efforts of crystallization culminated in the formation of crystals of compound 15 as white needles (Figure 19). The structure showed the orientation of carbonyl group towards the glycine ester moiety with respect to the phenolic hydroxyl groups.

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Figure 19. ORTEP diagram of compound 15.

In order to synthesize the catechol derived (CAT)$_6$ polyamide oligomer, compound 3 was subjected for solid phase peptide synthesis but failed to furnish the desired oligomer, due to presence of unprotected phenolic groups. Thus, a slightly modified synthetic protocol was employed, in which mono-Boc-ethylenediamine was alkylated using benzyl bromoacetate instead of ethyl bromoacetate. To synthesize product 18, catechol hydroxyls should be derivatized with base labile protecting group like 2-moc-ethylidene (acetal) (Scheme 9). The deprotection of benzyl group avoids base and hence would be suited for the present synthesis. The benzyl group of compound 18 could be removed under hydrogenolysis and would furnish title compound 19.

Scheme 9. Revised synthetic scheme for the synthesis of (CAT)$_6$ oligomer.
Compound 5 was N-alkylated using benzyl bromoacetate in acetonitrile to furnish compound 16 (Scheme 10). The formation of product 16 was confirmed by its spectral analysis. In $^1$H NMR spectrum, peaks $\delta$ 7.28-7.25 (benzyl) ppm and in $^{13}$C NMR spectrum, peaks at $\delta$ 60.6 (OCH$_2$) and 14.0 (CH$_3$) ppm were observed. Due to the instability of compound 16 at room temperature, it was further treated with 3,4-dihydroxyphenyl acetic acid 14, in presence of EDC.HCl and HOBr to furnish compound 17 in 65% yield. The hydroxyl groups of compound 17 were protected as its 2-Moc-ethylidene (Mocdene) acetal derivative 18, confirmed by the presence of peaks in $^1$H NMR spectrum, at $\delta$ 6.43 (t, $J = 5.5$ Hz, CH), 3.67 (s, OCH$_3$) and 2.89 (d, $J = 5.5$ Hz, CH$_2$) ppm and in $^{13}$C NMR spectrum, at $\delta$ 109.2 (CH), 51.7 (OCH$_3$), 48.5 (CH$_2$) and $\delta$ 168.3 ppm. The debenzylation of compound 18 using H$_2$/Pd-C resulted in the desired monomer 19, in 90% yield, which was confirmed by $^1$H NMR spectrum which also showed the absence of signals due to benzyl group protons.


3.3.4 Synthesis of N-Boc-aminoethyl 2-pyridylbenzimidazole (PBI)$_2$ (glycinate)$_2$

As the length of the aeg backbone increases, the resulting metal-linked duplex should become more rigid, giving rise to stronger electronic interactions between adjacent metal centers. Dimerization of the designed metal binding monomers can potentially introduce increasing electronic complexity which can be observed as changes in spectroscopy. With this rational, dimer of 2-pyridylbenzimidazole was

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synthesized. The synthesis of N-Boc-aminoethyl-2-pyridyl benzimidazole (PBI)$_2$ (glycinate)$_2$ 21 was carried out with the coupling of hydrochloride salt of primary amine 20 and acid derivative 1 in the presence of HBTU/DMF in 87% yield (Scheme 11).


Summary

This section has presented the design, synthesis and characterization of novel aeg-linked ligand compounds by the successful conjugation of 2-pyridylbenzimidazole (PBI), phenylenediamine (PDA) and catechol (CAT) moiety to aeg backbone. All metal complexing monomers have been derived from a common precursor unit, 1,2-diaminoethane. All the intermediates and new compounds have been characterized by $^1$H, $^{13}$C NMR spectroscopy and with other appropriate analytical data. Crystal structures of N-Boc-aminoethyl 3,4-dihydroxyphenyl (CAT) glycinate 15, N-Boc-aminoethyl (PBI) glycinate 8 and N-Boc-aminoethyl (PBI) glycinate 8-Pd were obtained. The crystal analysis of product 8 and 8-Pd suggested a conformational switching from trans to cis for effective complexation.

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3.4 Synthesis of the polyamide oligomers

The aminoethyl glycyl \((aeg)\) polyamide oligomers having metal complexing ligands \((22-28)\) were synthesized by manual solid phase peptide synthesis on the readily available 4-methyl-benzhydryl amine (MBHA) resin using standard \(t\)-Boc protocol from the \(C\)-terminus to the \(N\)-terminus. The resin after synthesis of polyamide oligomers was cleaved to yield the \(C\)-terminal amide peptide.

3.4.1 Solid phase method followed for polyamide oligomers

The hydrochloride salt of MBHA resin was neutralised with 50% DIPEA-DCM and the monomers were coupled as free acids using \textit{in situ} activation with 3 eq. of monomer, HBTU as a coupling reagent and DIPEA, HOBr as catalyst and recemization-suppressant respectively. Subsequently the resin bound \textit{Boc}-group was cleaved with 50% TFA/DCM before coupling the next amino acid. The deprotection and coupling reactions were monitored using Ninhydrin (Kaiser) and chloranil test. A positive color test after the coupling which indicates incomplete reaction and in such cases recoupling was carried out. To avoid deletion of sequences, a capping step with Ac\(_2\)O/DIPEA in DCM was performed. The polyamide oligomers \textbf{22-28} were cleaved from the resin at final stage using TFA and TFMSA.

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In order to complex metal ions on polyamide backbone using metal complexing monomers [2-pyridylbenzimidazole (PBI), phenylenediamine (PDA), catechol (CAT)] and obtain metal linked duplexes, target homo-oligomers (PBI)$_6$ 22, (PDA)$_6$ 23, (CAT)$_6$ 24 were designed (Figure 20). To study the effect of two different metal complexing units in the same molecule, hetero-oligomers of 2-pyridyl benzimidazole (PBI) and catechol (CAT) and those containing 2-pyridyl benzimidazole (PBI) and o-phenylenediamine (PDA) either in block units (PBI)$_3$-(PDA)$_3$ 25, (PBI)$_3$-(CAT)$_3$ 27 or in alternating units (PBI-PDA)$_3$ 26, (PBI-CAT)$_3$ 28 were designed (Figure 21).

![Figure 20. Structure of polyamide homo-oligomers (PBI)$_6$ 22, (PDA)$_6$ 23, (CAT)$_6$ 24.](image)

3.4.2 Cleavage of the oligomers from the solid support

The oligomers were cleaved from the solid support (MBHA resin), using trifluoromethanesulphonic acid (TFMSA) in the presence of trifluoroacetic acid (TFA) to yield aeg polyamide oligomers having amide at their C-termini. After cleavage, the polyamide oligomers obtained in solution were precipitated by addition...
of cold dry diethyl ether. Various polyamide oligomers synthesized for the present study are shown in Table 3.

![Polyamide Structures](image.png)

**Figure 21.** Structure of polyamide hetero-oligomers (PBI)$_3$-(PDA)$_3$ 25, (PBI-PDA)$_3$ 26, (PBI)$_3$-(CAT)$_3$ 27 and (PBI-CAT)$_3$ 28.

### 3.5 Purification and characterization of the polyamide oligomers

Purification of polyamides has been performed using gel filtration chromatography in which all the cleaved oligomers were passed through sephadex NAP column to remove low molecular weight impurities and their purities were ascertained by HPLC. MALDI-TOF mass spectrometric measurements were done on MDS-Sciex 4800 mass spectrometer.
3.5.1 High Performance Liquid Chromatography (HPLC)

The polyamide oligomers were subsequently purified by RP-HPLC on a semi-preparative C18 column (C18 column, acetonitrile:water system) in 95-99% purity and their purity were ascertained by analytical RP-HPLC. The HPLC retention time and mass data of the synthesized polyamide oligomers are given in Table 3 and their representative HPLC profiles are shown in Figure 22.

<table>
<thead>
<tr>
<th>Oligomers</th>
<th>HPLC (RT min)</th>
<th>Mol. Formula</th>
<th>$M_{\text{Calcd}}$/$M_{\text{Found}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(PBI)$_6$ 22</td>
<td>14.2</td>
<td>$C_{108}H_{105}N_{31}O_{12}$</td>
<td>2029.19/ 2052.048 [M+Na]$^+$</td>
</tr>
<tr>
<td>(PDA)$_6$ 23</td>
<td>4.11</td>
<td>$C_{66}H_{87}N_{25}O_{12}$</td>
<td>1422.5573/ 1445.036 [M+Na]$^+$</td>
</tr>
<tr>
<td>(CAT)$_6$ 24</td>
<td>11.0</td>
<td>$C_{72}H_{87}N_{19}O_{24}$</td>
<td>1518.5339/ 1541.479 (M+Na)$^+$</td>
</tr>
<tr>
<td>(PBI)$_3$ - (PDA)$_3$ 25</td>
<td>9.24</td>
<td>$C_{97}H_{96}N_{26}O_{12}$</td>
<td>1725.8735/ 1725.771</td>
</tr>
<tr>
<td>(PBI-PDA)$_2$ 26</td>
<td>11.9</td>
<td>$C_{97}H_{96}N_{26}O_{12}$</td>
<td>1725.8735/ 1748.844 [M+Na]$^+$</td>
</tr>
<tr>
<td>(PBI)$_3$ - (CAT)$_3$ 27</td>
<td>12.6</td>
<td>$C_{90}H_{96}N_{20}O_{18}$</td>
<td>1772.7273/ 1796.793 [M+Na]$^+$</td>
</tr>
<tr>
<td>(PBI-CAT)$_3$ 28</td>
<td>13.0</td>
<td>$C_{90}H_{96}N_{20}O_{18}$</td>
<td>1772.7273/ 1796.413 [M+Na]$^+$</td>
</tr>
</tbody>
</table>

*Molecular weights were calculated using chemdraw 12.0.

Figure 22. HPLC of polyamide oligomers (A) (PBI)$_6$ 22, (B) (PDA)$_6$ 23.
3.5.2 Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) characterization

The molecular weights of polyamide oligomers were confirmed by MALDI-TOF mass spectrometric analysis. The observed molecular weight along with the calculated molecular weight and the molecular formula of all the polyamide oligomers are given in Table 3. The MALDI-TOF data for the synthesized polyamide oligomers were shown in Figure 23. All the oligomers exhibited corresponding [M]$^+$ or [M + Na]$^+$ peaks.

![MALDI-TOF spectra](image)

**Figure 23.** MALDI-TOF of polyamide oligomers (A) (PBI)$_6$ 22, (B) (PDA)$_6$ 23, (C) (CAT)$_6$ 24 and (D) (PBI)$_3$-(PDA)$_3$ 25.
3.6 Molar Extinction Coefficients of polyamide oligomers

Determination of the exact concentration of the small amounts of peptides is always difficult. Initially the empty microfuge tubes are weighed and the solution containing HPLC purified peptides oligomers \((\text{PBI})_6 22, (\text{PDA})_6 23, (\text{CAT})_6 24, (\text{PBI})_2-(\text{PDA})_3 25, (\text{PBI-PDA})_3 26, (\text{PBI})_3-(\text{CAT})_3 27\) and \((\text{PBI-CAT})_3 28\) is transferred. The solvent is evaporated further, dried under vacuum for several hours and the peptide is stored over phosphorus pentoxide \((\text{P}_2\text{O}_5)\). The weight of the peptide is determined by deducting the empty weight from the recorded weight of the microfuge tubes after drying.

The synthesized polyamide oligomers were dissolved in precise amount of water to get accurate concentration (Figures 24-26). The molar extinction coefficients were determined from UV-Vis spectrophotometric method. According to the Lambert-Beer’s law, if the absorbing species has a molar concentration \(c\) and the sample thickness or path length is \(l\), the absorbance is given by

\[
A = \varepsilon cl
\]

where, \(\varepsilon\) is defined as the molar absorption coefficient or molar extinction coefficient. In order to calculate the molar extinction coefficient, known amounts of the de-ionized water was added and the absorbance spectra of the different concentrations of oligomeric solutions were recorded on UV-Vis spectrophotometer.

The concentration is plotted against absorbance and a linear fitting gives the slope which is known as the molar extinction coefficient \((\varepsilon)\) of the oligomers solution. The representative plots for molar extinction coefficient \((\varepsilon)\) are mentioned in the Tables 4-10 below.

Conventionally, molar extinction coefficient \((\varepsilon)\) for the monomer units is calculated and for the higher oligomers, the value is calculated by the multiplication of the number of repeating monomer units in the same solvent. This method also helps in evaluating presence of any secondary structures.
Figure 24. Concentration vs absorbance plots for calculating molar extinction coefficient (ε) for polyamide oligomer (A) (PBI)$_6$ 22 (B) (PDA)$_6$ 23 (C) (CAT)$_6$ 24.

Table 4: Conc. vs absorbance plot at 302 nm for (PBI)$_6$ 22

<table>
<thead>
<tr>
<th>Conc. (µM)</th>
<th>Absorbance at 302 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.0</td>
<td>0.42</td>
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<tr>
<td>7.6</td>
<td>0.40</td>
</tr>
<tr>
<td>7.3</td>
<td>0.38</td>
</tr>
<tr>
<td>6.9</td>
<td>0.36</td>
</tr>
<tr>
<td>6.6</td>
<td>0.34</td>
</tr>
<tr>
<td>6.3</td>
<td>0.32</td>
</tr>
<tr>
<td>5.9</td>
<td>0.31</td>
</tr>
<tr>
<td>5.6</td>
<td>0.29</td>
</tr>
<tr>
<td>5.4</td>
<td>0.27</td>
</tr>
<tr>
<td>5.1</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Table 5: Conc. vs absorbance plot at 269 nm for (PDA)$_6$ 23

<table>
<thead>
<tr>
<th>Conc. (µM)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>38.0</td>
<td>0.28</td>
</tr>
<tr>
<td>36.1</td>
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<tr>
<td>34.3</td>
<td>0.25</td>
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<tr>
<td>32.6</td>
<td>0.24</td>
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<tr>
<td>31.0</td>
<td>0.22</td>
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<td>29.4</td>
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<td>27.9</td>
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<td>26.5</td>
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<td>25.2</td>
<td>0.18</td>
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<tr>
<td>23.9</td>
<td>0.17</td>
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Table 6: Conc. vs absorbance plot at 281 nm for (CAT)$_6$ 24

<table>
<thead>
<tr>
<th>Conc. (µM)</th>
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<td>0.026</td>
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<tr>
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<tr>
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<tr>
<td>1.28</td>
<td>0.021</td>
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<tr>
<td>1.22</td>
<td>0.019</td>
</tr>
<tr>
<td>1.16</td>
<td>0.018</td>
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</table>
Figure 25. Concentration vs absorbance plots for calculating molar extinction coefficient (ε) for polyamide oligomer (D) (PBI)₃-(PDA)₃ 25, (E) (PBI-PDA)₃ 26 (F) (PBI)₃-(CAT)₃ 27.

Table 7: Conc. vs absorbance plot at 302 nm for (PBI)₃-(PDA)₃ 25

<table>
<thead>
<tr>
<th>Conc. (µM)</th>
<th>Absorbance at 302 nm</th>
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</thead>
<tbody>
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</tr>
<tr>
<td>19.0</td>
<td>0.51</td>
</tr>
<tr>
<td>18.1</td>
<td>0.48</td>
</tr>
<tr>
<td>17.2</td>
<td>0.45</td>
</tr>
<tr>
<td>16.3</td>
<td>0.43</td>
</tr>
<tr>
<td>15.5</td>
<td>0.41</td>
</tr>
<tr>
<td>14.7</td>
<td>0.38</td>
</tr>
<tr>
<td>13.9</td>
<td>0.36</td>
</tr>
<tr>
<td>13.3</td>
<td>0.35</td>
</tr>
<tr>
<td>12.6</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Table 8: Conc. vs absorbance plot at 302 nm for (PBI-PDA)₃ 26

<table>
<thead>
<tr>
<th>Conc. (µM)</th>
<th>Absorbance at 302 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0</td>
<td>0.46</td>
</tr>
<tr>
<td>9.0</td>
<td>0.41</td>
</tr>
<tr>
<td>8.1</td>
<td>0.37</td>
</tr>
<tr>
<td>7.29</td>
<td>0.33</td>
</tr>
<tr>
<td>6.56</td>
<td>0.30</td>
</tr>
<tr>
<td>5.90</td>
<td>0.27</td>
</tr>
<tr>
<td>5.31</td>
<td>0.24</td>
</tr>
<tr>
<td>4.78</td>
<td>0.22</td>
</tr>
<tr>
<td>4.30</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Table 9: Conc. vs absorbance plot at 302 nm for (PBI)₃-(CAT)₃ 27

<table>
<thead>
<tr>
<th>Conc. (µM)</th>
<th>Absorbance at 302 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>0.141</td>
</tr>
<tr>
<td>4.75</td>
<td>0.131</td>
</tr>
<tr>
<td>4.51</td>
<td>0.123</td>
</tr>
<tr>
<td>4.29</td>
<td>0.116</td>
</tr>
<tr>
<td>4.07</td>
<td>0.109</td>
</tr>
<tr>
<td>3.86</td>
<td>0.102</td>
</tr>
<tr>
<td>3.67</td>
<td>0.099</td>
</tr>
<tr>
<td>3.49</td>
<td>0.091</td>
</tr>
<tr>
<td>3.31</td>
<td>0.085</td>
</tr>
<tr>
<td>3.15</td>
<td>0.081</td>
</tr>
</tbody>
</table>
The synthesized polyamide oligomers were solubilized in deionized water and their molar extinction coefficients are summarized in Table 11.

Table 11. Molar Extinction Coefficients (ε) for the polyamide oligomers

<table>
<thead>
<tr>
<th>Oligomer</th>
<th>Molar Extinction Coefficient (ε) (mM⁻¹ cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(PBI)₆ 22</td>
<td>53.9</td>
</tr>
<tr>
<td>(PDA)₆ 23</td>
<td>7.72</td>
</tr>
<tr>
<td>(CAT)₆ 24</td>
<td>16.7</td>
</tr>
<tr>
<td>(PBI)₃⁻ (PDA)₃ 25</td>
<td>27.6</td>
</tr>
<tr>
<td>(PBI-PDA)₃ 26</td>
<td>46.9</td>
</tr>
<tr>
<td>(PBI)₃⁻ (CAT)₃ 27</td>
<td>32.0</td>
</tr>
<tr>
<td>(PBI-CAT)₃ 28</td>
<td>25.0</td>
</tr>
</tbody>
</table>

In conclusion, it was seen that (PBI)₆ had the highest value of the molar extinction coefficient than all the other polyamide oligomers synthesized, perhaps due to its higher conjugation.
The polyamide oligomer \((\text{PBI-PDA})_3\) is found to have almost double the value of molar extinction coefficient in comparison to \((\text{PBI})_3-(\text{PDA})_3\), this may be attributed to stable dimeric assembly formed by the former; exactly the opposite was observed in case of oligomer \((\text{PBI-CAT})_3\), which has lesser molar extinction coefficient as compared to \((\text{PBI})_3-(\text{CAT})_3\). In general polyamide oligomers of 2-pyridylbenzimidazole (PBI) and phenylenediamine (PDA) possess comparatively larger molar extinction coefficient value than that of -pyridylbenzimidazole (PBI) and catechol (CAT) oligomers. The reason may be that PDA is devoid of one extra methylene carbon of the aminoethyl glycyol \((\text{aeg})\) chain, providing better conjugation.

3.7 Determination of pK\(_a\) of synthesized oligomers

Potentiometric titration is a high-precision technique for determination of pK\(_a\) values of compounds. It is commonly used due its accuracy and the commercial availability of fast, automated instruments. The dissociation constant of uncharged substances (hydrophobic organic molecules) in aqueous-organic mixtures is not only ruled by electrostatic interactions but also with specific solute-solvent interactions (solvation effects).

In order to determine aqueous pK\(_a\) values, synthesized polyamide oligomers were subjected to potentiometric titrations. The pH of oligomers (8-20 \(\mu\)M, 2 mL) in deionized water was first adjusted to 2.0-2.5 using HCl (50%). This solution was titrated with 2.5 \(\mu\)L aliquots of aq. NaOH (0.5 M). After each addition of NaOH solution aliquot, pH of the solution was recorded. The pH of the sample solution changes rapidly at the pK\(_a\) of the functional group. The pK\(_a\) values were derived from the first derivative of the plot of pH vs volume of NaOH (Figure 27).
Figure 27. Potentiometric pH titration of polyamide oligomers with NaOH (0.5 M) (A) (PBI)$_6$ 22, (B) (PDA)$_6$ 23, (C) (CAT)$_6$ 24, (D) (PBI)$_3$- (PDA)$_3$ 25, (E) (PBI-PDA)$_3$ 26, (F) (PBI)$_3$- (CAT)$_3$ 27, (G) (PBI-CAT)$_3$ 28.
The pKₐ values for the synthesized polyamide ligands are shown in Table 12.

Table 12. pKₐ values for the synthesized polyamide oligomers and reported derivatives

<table>
<thead>
<tr>
<th>Oligomer</th>
<th>pKₐ</th>
<th>Literature reports</th>
<th>pKₐ</th>
</tr>
</thead>
<tbody>
<tr>
<td>(PBI)₆ 22</td>
<td>5.7</td>
<td>Pyridine</td>
<td>5.2</td>
</tr>
<tr>
<td>(PDA)₆ 23</td>
<td>5.4</td>
<td>Benzimidazole</td>
<td>5.5</td>
</tr>
<tr>
<td>(CAT)₆ 24</td>
<td>5.7</td>
<td>3,4-Dihydroxybenzoic acid</td>
<td>4.5</td>
</tr>
<tr>
<td>(PBI)₃⁻ (PDA)₂ 25</td>
<td>5.6</td>
<td>o-Phenylenediamine</td>
<td>4.6</td>
</tr>
<tr>
<td>(PBI-PDA)₆ 26</td>
<td>5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(PBI)₃⁻ (CAT)₂ 27</td>
<td>4.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(PBI-CAT)₆ 28</td>
<td>4.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(PBI)₆ 22 has two nitrogens, one from the pyridine (N2') and other from the benzimidazole (N3) are quite acidic and in literature,⁵³ pKₐ values for the pyridine and benzimidazole has been reported to be 5.2 and 5.5, respectively. The presence of different nitrogens in the molecule with closer pKₐ values often leads to only single. However, it is to be noted that the pKₐ of 2-pyridylbenzimidazole may be slightly different when it is part of a larger peptide chain. The pKₐ for (PBI)₆ was observed to be 5.7 (Figure 28).

![Figure 28. Acid base equilibria of (PBI)₆ polyamide oligomer 22.](image)

In case of (PDA)₆ polyamide oligomers 23, both the 3,4-diamino groups are acidic and display a single transition. In literature,⁵³ pKₐ for the o-phenylenediamine has been reported to be 4.6 and pKₐ obtained for the (PDA)₆ is 5.4 (Figure 29). Thus, an increment of 0.8 pKₐ was observed for the oligomer.

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Figure 29. Acid base equilibria of (PDA)$_6$ polyamide oligomers 23.

(CAT)$_6$ polyamide oligomer 24, also posseses acidic 3,4-dihydroxy groups. The p$_{K_a}$ for the (CAT)$_6$ was observed to be 5.7 and literature$^{53}$ value of p$_{K_a}$ for the 3,4-dihydroxybenzoic acid has been reported to be 4.5 (Figure 30). Thus, an increment of 1.2 p$_{K_a}$ was observed for the (CAT)$_6$ oligomer.

Figure 30. Acid base equilibria of (CAT)$_6$ polyamide oligomers 24.

In conclusion, it is observed that both (PBI)$_6$ 22, (CAT)$_6$ 24 possess same values of p$_{K_a}$ (5.7) and (PDA)$_6$ 23 possess slightly lower value of p$_{K_a}$ (5.4). The oligomers (PBI)$_6$ 22, (CAT)$_6$ 24 are less acidic (larger p$_{K_a}$) as compared to (PDA)$_6$ 23. In case of hetero-oligomers it is difficult to state that observed p$_{K_a}$ is due to which functional group. Hetero-oligomers of 2-pyridylbenzimidazole (PBI) and phenylenediamine (PDA) possess comparatively larger p$_{K_a}$ values and hence are less acidic than those of 2-pyridylbenzimidazole (PBI) and catechol (CAT).

Summary

This section has demonstrated the synthesis of novel polyamide oligomers by incorporation of metal binding 2-pyridylbenzimidazole (PBI), phenylenediamine (PDA) and catechol (CAT) moieties utilizing the solid phase peptide synthesis (SPPS). It deals with the synthesis of polyamide oligomers having two units of different monomers either in block or alternative arrangements. All the synthesized

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polyamide oligomers were purified by High Pressure Liquid Chromatography (HPLC), and subsequently their molecular weights were confirmed by the MALDI-TOF analysis. Molar extinction coefficients ($\varepsilon$) were calculated from the calibration curve of absorbance versus concentration. \(pK_a\) of the synthesized polyamide oligomers were calculated according to the potentiometric methods.\textsuperscript{53}
3.8 NMR studies

In order to assess the binding characteristics of catechol with trimethylborate, NMR studies were undertaken as reported in the literature. Phenolic hydroxyl groups are acidic in nature and capable of binding with different metal ions. Catechols bind efficiently with boron ions and the complexation-decomplexation can be tuned with pH change. The postulated mechanism for catechol (CAT) linked *aeg* ligand and boron complexation is depicted in Scheme 13.

![Scheme 13](image)

**Scheme 13.** Postulated mechanism of catechol (CAT) linked *aeg* ligands with trimethyl borate.

The mechanism involves deprotection of the catechol groups with bases like triethylamine or diisopropyl ethylamine to furnish the phenoxide intermediate (A). The generated phenoxide intermediate (A) reacts with trimethyl borate to form intermediate (B), which subsequently reacts with another molecule of (A) to generate the desired the final complex (C).

3.8.1 NMR studies on ethyl-\(N\)-Boc-aminoethyl-3,4-dihydroxyphenyl (CAT) glycinate 15

Ethyl-\(N\)-Boc-aminoethyl-3,4-dihydroxyphenyl (CAT) glycinate 15 was first reacted with triethylamine to generate the phenoxide ions and was subsequently treated with stoichiometric amounts of trimethylborate to form the metal complexes. In \(^1\)H NMR spectra of ethyl-\(N\)-Boc-aminoethyl-3,4-dihydroxyphenyl (CAT) glycinate 15, signals for the phenolic hydroxyl group appeared at 8 8.80 in DMSO-\(d_6\) and after
complexation with boron, this signal disappeared and a new signal due to triethylamine salt appeared at $\delta$ 8.31 ppm (Figure 31).

![Diagram of molecular structures](image)

**Figure 31.** NMR studies of ethyl-$N$-Boc-aminoethyl-3,4-dihydroxyphenyl (CAT) glycinate 15 and trimethyl borate (A) $^1$H NMR, (B) $^{11}$B NMR in DMSO-$d_6$.

In $^{11}$B NMR, the signal for the trimethyl borate appeared at $\delta$ 18.53 ppm, whereas in the complex, it appeared upfield at $\delta$ 14.23 ppm. These results clearly indicate the binding of catechol groups with trimethyl borate, to form the 2:1 [Ethyl-$N$-Boc-aminoethyl-3,4-dihydroxyphenyl (CAT) glycinate 15:boron] complex 15a.

### 3.8.2 NMR studies on the benzyl $N$-Boc-aminoethyl-3,4-dihydroxy phenyl (CAT) glycinate, 17

Just as with the ethyl ester derivative, benzyl-$N$-Boc-aminoethyl-3,4-dihydroxyphenyl (CAT) glycinate 17 upon treatment with trimethyl borate provided a 2:1 complex. In $^1$H NMR spectra, resonances for the aromatic protons that appears at $\delta$ 6.59 of the benzyl-$N$-Boc-aminoethyl-3,4-dihydroxyphenyl (CAT) glycinate 17-boron complex in DMSO-$d_6$ appeared upfield at $\delta$ 6.30 ppm upon complexation. Consequently, the proton signals at $\delta$ 8.73 ppm (OH) coming from the phenolic hydroxyl group completely disappeared upon its complexation with boron (Figure 32).

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In conclusion, both the designed ethyl and benzyl-N-Boc-aminoethyl-3,4-dihydroxyphenyl (CAT) glycinate 17 and trimethyl borate in DMSO-d$_6$. 

Figure 32. $^1$H NMR studies of benzyl-N-Boc-aminoethyl-3,4-dihydroxyphenyl (CAT) glycinate 17 and trimethyl borate in DMSO-d$_6$.

Figure 33. Pictorial representation of ethyl and benzyl-N-Boc-aminoethyl-3,4-dihydroxyphenyl (CAT) glycinate 15 and 17 with boron ion.
3.9 High Resolution-Mass spectrometry (HR-MS) Studies

High Resolution-Mass Spectrometry (HR-MS) was used for additional characterization of the synthesized \textit{aeg} linked ligands (Figure 34). Both \textit{N}-Boc-aminoethyl (PBI) glycinate 8 and \textit{N}-Boc-aminoethyl (PBI)$_2$ (glycinate)$_2$ 21 were treated with metal salts. After stirring the stoichiometric mixture of the \textit{aeg} ligands and metal salts overnight, the complexes were purified on an alumina column. Electrospray Ionization-Time of Flight (ESI-TOF) mass spectra was recorded to confirm metal complexation.

3.9.1 HR-MS studies on ethyl-\textit{N}-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8

Ethyl-\textit{N}-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 was treated with copper chloride salt and its high resolution mass spectrum was recorded, which showed the presence of two types of molecular stoichiometry (Figure 35A) i.e. molecular ion peaks at \( m/z \) 579.0979 (Calculated: 579.0847) and 1060.6124 (Calculated: 1060.3635). The former and latter peak corresponded to molecular stoichiometry of 1:1 [ethyl-\textit{N}-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8: Cu$^{2+}$] and 2:1 [ethyl-\textit{N}-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8: Cu$^{2+}$], respectively.

![Figure 34. Structures of complexes of ethyl-\textit{N}-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 with various metal salts.](image)

In the same way, on treating ethyl-\textit{N}-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 with copper nitrate, the high resolution mass spectrum displayed a desired molecular ion peak at \( m/z \) 1087.4622 (Calculated: 1087.3825) corresponding
to molecular complex stoichiometry of 2:1 [ethyl-N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8: Cu$^{2+}$] (Figure 35B) and peak corresponding to 1:1 molecular stoichiometry was absent.

![Figure 35](image)

Figure 35. HR-MS spectra for ethyl-N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 with (A) copper chloride, (B) copper nitrate, (C) copper perchlorate and (D) nickel chloride.

Copper perchlorate complex with ethyl-N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 revealed high resolution molecular ion peaks at $m/z$ 643.1181 (Calculated: 643.1106) and 1124.3650 (Calculated: 1124.3846), respectively for molecular stoichiometry of 1:1 [ethyl-N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8: Cu$^{2+}$] and 2:1 [ethyl-N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8: Cu$^{2+}$] (Figure 35C). And for nickel chloride at $m/z$ 1055.3873 (Calculated: 1055.3692) corresponded to molecular stoichiometry of 2:1 [ethyl-N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8: Ni$^{2+}$] (Figure 35D) with no 1:1 molecular stoichiometry.
In conclusion, it was observed that (PBI) 8 binds with all the four metal salts \textit{i.e.} copper chloride, copper nitrate, copper perchlorate and nickel chloride and forms stable duplexes in either in 1:1 or 2:1 molecular stoichiometry (Figure 36).

![Figure 36](image)

**Figure 36.** Schematic representation of \textit{aeg} linked ethyl-\textit{N}-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 with copper and nickel metal salts.

3.9.2 HR-MS studies on ethyl-\textit{N}-Boc-aminoethyl-2-pyridylbenzimidazole (PBI)$_2$ (glycinate)$_2$ 21

In a similar fashion, \textit{N}-Boc-aminoethyl (PBI)$_2$ (glycinate)$_2$ 21 was also examined for metal complexation with all aforesaid metal salts, but only copper chloride yielded fruitful results (Figure 37).

![Figure 37](image)

**Figure 37.** Structure of \textit{N}-Boc-aminoethyl (PBI)$_2$ (glycinate)$_2$ 21 and copper chloride complex.

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*N-Boc-aminoethyl (PBI)$_2$ (glycinate)$_2$ 21* treated with copper chloride showed the presence of peak at $m/z$ 1730.6259 (Calculated: 1730.6399) suggesting single metal incorporation between ligands (Figure 38).

![HR-MS spectra of N-Boc-aminoethyl (PBI)$_2$ (glycinate)$_2$ 21 with copper chloride.](image)

**Figure 38.** HR-MS spectra of *N-Boc-aminoethyl (PBI)$_2$ (glycinate)$_2$ 21* with copper chloride.

In conclusion, both *N-Boc-aminoethyl (PBI)* glycinate 8 and *N-Boc-aminoethyl (PBI)$_2$ (glycinate)$_2$ 21* both displayed metal complexation. Though (PBI)$_2$ 21 showed metal complexation with all metal salts, but HR-MS data could only be obtained with copper chloride. A pictorial depiction of the plausible complexation is presented below in Figure 39.

![Schematic representation of N-Boc-aminoethyl (PBI)$_2$ (glycinate)$_2$ 21 with copper chloride.](image)

**Figure 39.** Schematic representation of *N-Boc-aminoethyl (PBI)$_2$ (glycinate)$_2$ 21* with copper chloride.

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3.10 UV-Vis spectroscopic studies

Since metal coordination is accompanied by the appearance of a peak in the UV-Vis absorption spectrum, spectrophotometric titrations were used as a probe to monitor the metal complexation of synthesized aeg linked ligands.

3.10.1 UV-Vis spectrophotometric titrations of (PBI) and (CAT) aeg linked ligands

UV-Vis titrations were performed by incremental addition of metal ions into a methanolic solution of methanolic solutions $N$-Boc-aminoethyl (PBI) glycinate 8 and $N$-Boc-aminoethyl (CAT) glycinate 17 of known concentrations.

3.10.1a UV-Vis spectrophotometric titrations of the $N$-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8

The binding studies of $N$-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 was carried out with different metal salts i.e. copper nitrate, nickel nitrate, palladium nitrate, lead nitrate, iron nitrate, zinc nitrate, cobalt nitrate, ruthenium trichloride, silver nitrate, gold chloride etc (Scheme 14). A change in the ultraviolet (UV) absorption upon complexation with $Cu^{2+}$/ $Ni^{2+}$ ions were used as quantitative structural probe to verify $Cu^{2+}$/ $Ni^{2+}$ mediated duplex formation. Thus, titration studies followed by UV-Vis spectroscopic studies were undertaken for $N$-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8.

![Scheme 14. Metal Complexation of the $N$-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8.](image)

$Cu(NO_3)_2$: The electronic spectra of $N$-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 in methanol showed maximum absorption ($\lambda_{\text{max}}$) at 308 nm. Upon addition
of aliquots of Cu(NO$_3$)$_2$, intensity of the peak at 308 nm reduces and a new red shifted absorption band ($\lambda_{\text{max}}$) at 324 nm (+16 nm) appears. This new absorption band along with two isosbestic points at 256 and 314 nm indicate the formation of Cu$^{2+}$- N-Boc-aminoethyl (PBI) glycinate 8 complex.

**Figure 40.** Changes in the absorption spectra of the N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 (25 µM) in methanol upon the addition of metal salts (A) Cu(NO$_3$)$_2$ (10 mM), (C) Ni(NO$_3$)$_2$ (10 mM), (E) CuCl$_2$ (2.5 mM) and (F) Zn(NO$_3$)$_2$ (10 mM). Plot of the change in absorbance at 308 and 345 nm as a function of molar ratio of metal to (PBI) 8 (B) Cu(NO$_3$)$_2$, (D) Ni(NO$_3$)$_2$.  

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The titration curve, plotted as the change in absorbance against as a function of (PBI) 8 concentration, is shown in Figure 40A & B. The plot of molar absorptivity at 308 nm vs equivalents of Cu\(^{2+}\) added shows a saturation ca. 2 equivalents of Cu(NO\(_3\))\(_2\) in methanol.

**Ni(NO\(_3\))\(_2\):** Similarly, titrations were carried out by adding the aliquots of Ni(NO\(_3\))\(_2\) into the N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 solution. This new absorption band at (\(\lambda_{max}\)) at 308 nm with two isosbestic points at 265 and 314 nm indicated the formation of Ni\(^{2+}\)-N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 complete with a saturation approx. with 2 equiv. of Ni(NO\(_3\))\(_2\) in methanol (Figure 40C & D).

**CuCl\(_2\):** N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 was also titrated with copper chloride and showed appreciable binding, with two isosbestic points at 256 and 315 nm (Figure 40E). Among copper salts, copper nitrate was carried out further due to its better binding results observed.

**Zn(NO\(_3\))\(_2\):** UV-Vis titration studies of N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 with zinc nitrate salt results in the weak binding (Figure 40F).

**AuCl\(_3\):** UV-Vis titrations were carried out with AuCl\(_3\) (10 mM), but unfortunately absorption change due to the monomer was completely masked by the gold absorption itself. So, in order to attain the equivalence point, titration was carried out at a lower concentration of AuCl\(_3\) (2.5 mM) (Figure 41).

**Figure 41.** Changes in the absorption spectra of the N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 (25 \(\mu\)M) in methanol upon the addition of metal salts (A) AuCl\(_3\) (10 mM) and (B) AuCl\(_3\) (2.5 mM).
Although an isobestic point was observed at 315 nm, the emergent absorption at 350 failed to saturate even at as high molar equivalence as 10 perhaps indicating transient binding of the metal to the ligands. However, no significant spectral change was observed.

UV-Vis titration results have been summarised in Table 13.

**Table 13. Summary of UV-Vis titrations for N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8**

<table>
<thead>
<tr>
<th>SI No.</th>
<th>Metal salts (2.5/10 mM)</th>
<th>Observation</th>
<th>Inflection points</th>
<th>Isosbestic points</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Copper nitrate</td>
<td>Binding</td>
<td>308 and 324</td>
<td>256 and 314</td>
</tr>
<tr>
<td>2.</td>
<td>Copper chloride</td>
<td>Binding</td>
<td>308 and 324</td>
<td>269 and 315</td>
</tr>
<tr>
<td>2.</td>
<td>Nickel nitrate</td>
<td>Binding</td>
<td>308 and 324</td>
<td>265 and 314</td>
</tr>
<tr>
<td>3.</td>
<td>Gold chloride</td>
<td>Weak binding</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Zinc nitrate</td>
<td>Weak binding</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*No appreciable binding with palladium nitrate, lead nitrate, iron nitrate, cobalt nitrate, ruthenium trichloride, silver nitrate, gold chloride

The continuous variation Job’s method provides information about binding stoichiometry between metal and N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 (Figure 42), which was examined by keeping the overall concentration constant and plotted against the mole fraction of Cu$^{2+}$/Ni$^{2+}$.

Various stoichiometric mixtures of N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8:metal salts in varying molar ratios (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100) were prepared keeping concentration of N-Boc-aminoethyl (PBI) glycinate 8 as 100 µM in methanol. The intersection point in the Job’s plot was found to be at 0.50, which indicates binding stoichiometry 1:1 for the complexes N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 with both copper nitrate and nickel nitrates.
Figure 42. UV-Vis absorption spectra of N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8 with metal salts in molar ratios of (a) 0:100 (b) 10:90 (c) 20:80 (d) 30:70 (e) 40:60 (f) 50:50 (g) 60:40 (h) 70:30 (i) 80:20 (j) 90:10 (k) 100:0; (A) UV spectra and (B) Job’s plot with Cu(NO$_3$)$_2$ (C) UV spectra and (D) Job’s plot with Ni(NO$_3$)$_2$.

Benesi-Hildebrand (BH) equation, is highly useful in determining binding constants for 1:1 and 1:2 [(Metal:ligand) or (Host:guest) or (DNA/RNA:peptides)] systems. For N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8, Job’s plot indicated the binding stoichiometry of 1:1 and it was subsequently fitted in the BH equation. In this equation, concentration was plotted against the change in the metal concentration. After selecting proper binding model, BH equation results in the straight line, which is observed in graphs.

The binding constant (K) was determined from the intercept to slope ratio of the Benesi–Hildebrand plot and the values calculated were 6.71 x 10$^3$ and 1.82 x 10$^3$ [M]$^{-1}$ for Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$, respectively, which indicate the comparable strength of binding with both the metals (Figure 43, inset).
Figure 43. Benesi Hildebrand’s plots (A) UV-Vis absorption spectra of \( \text{N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8} \) with (A) Cu(NO\(_3\))\(_2\) and (B) Ni(NO\(_3\))\(_2\). Arrows indicate decrease in absorption at 308 nm from 0 to 2.5 mM metal concentration. The Benesi Hildebrand plots are represented as insets.

In conclusion, \( \text{N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8} \) strongly binds in 1:1 stoichiometry with methanolic solution of Cu(NO\(_3\))\(_2\) and Ni(NO\(_3\))\(_2\), while no appreciable binding is observed with zinc, cobalt and gold metal salts.

These binding models are only proposed models and at present no direct evidence is available. Based on the present investigations using UV-Vis spectroscopy (1:1), single crystal X-ray diffraction studies (1:1), HRMS (2:1) and ITC analyses, it can be concluded that \( \text{N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8} \) predominantly forms both 2:1 and 1:1 complexes with metal salts (Figure 44).

Figure 44. Proposed model of \( \text{N-Boc-aminoethyl-2-pyridylbenzimidazole (PBI) glycinate 8} \) with copper and nickel nitrates.
3.10.1b UV-Vis spectrophotometric titrations of $N$-Boc-aminoethyl-catechol (CAT) glycinate 17

Metal binding studies of the synthesized $N$-Boc-aminoethyl-catechol (CAT) glycinate 17 with various metal salts were done (Scheme 15).

![Scheme 15](image.png)

**Scheme 15.** Metal complexation of $N$-Boc-aminoethyl-catechol (CAT) glycinate 17.

The electronic spectra of $N$-Boc-aminoethyl-catechol (CAT) glycinate 17 in methanol showed maximum absorption ($\lambda_{\text{max}}$) at 283 nm. UV-Vis experiments of $N$-Boc-aminoethyl-catechol (CAT) glycinate 17 were studied with different metal ions ($\text{Cu}^{2+}$, $\text{Ni}^{2+}$, $\text{Zn}^{2+}$, $\text{Ru}^{3+}$, $\text{Pd}^{2+}$, $\text{Fe}^{3+}$, $\text{Ag}^+$, $\text{Au}^{3+}$, $\text{Tl}^{3+}$, $\text{Ln}^{3+}$) as their nitrate/chloride salts.

**Phenyloboronic acid:** $N$-Boc-aminoethyl-catechol (CAT) glycinate 17 was examined for its metal binding studies with phenylboronic acid and exhibited significant spectral change in the absorbance spectra (Figures 45).

![Figure 45](image.png)

**Figure 45.** Changes in the absorption spectra of $N$-Boc-aminoethyl-catechol (CAT) glycinate 17 (50 $\mu$M) in methanol upon the addition of phenylboronic acid (2.5 mM).

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The methanolic solution of \(N\)-Boc-aminoethyl-catechol (CAT) glycinate 17 showed maximum absorption (\(\lambda_{\text{max}}\)) at 283 nm and upon adding aliquots of phenylboronic acid (2.5 mM), the intensity of the peak at 283 nm decreases and intensity of new peak at 328 nm increases. Since, isosbestic points were not clearly indicative, important information regarding stoichiometry could not be obtained.

Metal salts \(i.e.\) copper nitrate, nickel nitrate, ruthenium trichloride and ferric nitrate, exhibited visible change in the absorbance spectra. Ruthenium trichloride showed absorbance change in the 550 nm that can be attributed to the formation of ruthenium nanoparticles. UV-Vis titration spectra for Cu\(^{2+}\), Ni\(^{2+}\), Zn\(^{2+}\), Ru\(^{3+}\) are displayed below (Figure 46).

![Figure 46. Changes in the absorption spectra of \(N\)-Boc-aminoethyl-catechol (CAT) glycinate 17 (50 \(\mu\)M) in methanol upon the addition of metal salts (A) Cu(NO\(_3\))\(_2\) (10 mM), (B) Ni(NO\(_3\))\(_2\) (10 mM), (C) Zn(NO\(_3\))\(_2\) (10 mM) and (D) RuCl\(_3\) (10 mM).](image)

Similar results were also observed for silver nitrate (Figure 47). However, no significant spectral change was observed by the addition of palladium nitrate, thallium nitrate and lanthanum nitrate.

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Summary of metal binding for the $N$-Boc-aminoethyl-catechol (CAT) glycinate 17 is displayed in Table 14.

**Table 14.** Summary of UV-Vis titration results for the $N$-Boc-aminoethyl-catechol (CAT) glycinate 17.

<table>
<thead>
<tr>
<th>Metal salts (10 mM)</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylboronic acid</td>
<td>Binding</td>
</tr>
<tr>
<td>Copper nitrate</td>
<td>Weak binding</td>
</tr>
<tr>
<td>Nickel nitrate</td>
<td>Weak binding</td>
</tr>
<tr>
<td>Ruthenium trichloride</td>
<td>Ru-Nanoparticles</td>
</tr>
<tr>
<td>Ferric nitrate</td>
<td>Weak binding</td>
</tr>
<tr>
<td>Gold chloride</td>
<td>Weak binding</td>
</tr>
<tr>
<td>Silver nitrate</td>
<td>Ag-Nanoparticles</td>
</tr>
</tbody>
</table>

*palladium nitrate, thallium nitrate and lanthanum nitrate did not show any significant spectral change.

In conclusion, (CAT) 17 showed binding with phenylboronic acid, whereas other metal salts did not exhibit appreciable binding.
3.10.2 UV-Vis spectrophotometric titrations of (PBI) Dimer

UV-Vis titrations were performed by incremental addition of metal ions into a methanolic solution of methanolic solutions N-Boc-aminoethyl (PBI)₂ glycinate₂ 21 of known concentrations.

3.10.2a UV-Vis spectrophotometric titrations of N-Boc-aminoethyl 2-pyridylbenzimidazole (PBI)₂ (glycinate)₂ 21

(PBI)₂ structure possesses two pyridyl-benzimidazole units linked to aeg-backbone, providing total of two sites for metal complexation (Scheme 16).

![Scheme 16. Schematic representation of metal complexation of the N-Boc-aminoethyl 2-pyridylbenzimidazole (PBI)₂ (glycinate)₂ 21.](image)

**Cu(NO₃)₂:** The electronic spectra of N-Boc-aminoethyl 2-pyridylbenzimidazole (PBI)₂ (glycinate)₂ 21 obtained from on UV-Vis Spectrophotometric titration carried out in methanol showed maximum absorption at \( \lambda_{\text{max}} \) 308 nm and shows inflection point approximately at 2 equivalents of Cu(NO₃)₂ in methanol. Similar to (PBI) 8, upon addition of aliquots of Cu(NO₃)₂, a new red shifted absorption band at \( \lambda_{\text{max}} \) 321 nm (13 nm) was observed at the expense of reduction of intensity of the peak at 308 nm. Isosbestic points at 266 and 316 nm indicated the formation of Cu²⁺-N-Boc-aminoethyl 2-pyridylbenzimidazole (PBI)₂ (glycinate)₂ 21 complex (Figure 48).

**Ni(NO₃)₂:** For Ni(NO₃)₂, same value of red shifted absorption band was noted \( \lambda_{\text{max}} \) at 321 nm (+13 nm), but different isosbestic points at 261 and 317 nm, indicating the formation of complex.

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Summary of metal binding for the N-Boc-aminoethyl 2-pyridylbenzimidazole (PBI)$_2$ (glycinate)$_2$ 21 is displayed in Table 15.

**Table 15.** Summary of UV-Vis titration for the N-Boc-aminoethyl 2-pyridylbenzimidazole (PBI)$_2$ (glycinate)$_2$ 21.

<table>
<thead>
<tr>
<th>Metal salts (2.5 mM)</th>
<th>Observation</th>
<th>Inflection points</th>
<th>Isosbestic points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nickel nitrate</td>
<td>Binding</td>
<td>308 and 321</td>
<td>266 and 316</td>
</tr>
<tr>
<td>Copper nitrate</td>
<td>Binding</td>
<td>308 and 321</td>
<td>261 and 317</td>
</tr>
</tbody>
</table>

Using Job’s method, the concentrations of metal salts and N-Boc-aminoethyl 2-pyridylbenzimidazole (PBI)$_2$ (glycinate)$_2$ 21 were examined by keeping the overall concentration constant. The Job’s plot (Figure 49) indicated an intersection point around 0.40, suggesting binding stoichiometry of 2:3 for N-Boc-aminoethyl 2-
pyridylbenzimidazole (PBI)$_2$ (glycinate)$_2$ 21:Cu(NO$_3$)$_2$ complex. In comparison, N-Boc-aminoethyl 2-pyridylbenzimidazole (PBI)$_2$ (glycinate)$_2$ 21: Ni(NO$_3$)$_2$ complex showed intersection point around 0.60 in Job’s plot indicating a binding stoichiometry to be 3:2.

![Figure 49. UV-Vis absorption spectra of N-Boc-aminoethyl 2-pyridylbenzimidazole (PBI)$_2$ (glycinate)$_2$ 21 with metal salts in molar ratios of (a) 0:100 (b) 10:90 (c) 20:80 (d) 30:70 (e) 40:60 (f) 50:50 (g) 60:40 (h) 70:30 (i) 80:20 (j) 90:10 (k) 100:0; (A) UV spectra and (B) Job’s plot with Cu(NO$_3$)$_2$; (C) UV spectra and (D) Job’s plot with Ni(NO$_3$)$_2$.](image)

Benesi Hildebrand equation holds good fitting values only in case of either 1:1 or 1:2 binding models. However, Job’s plot for N-Boc-aminoethyl 2-pyridylbenzimidazole (PBI)$_2$ (glycinate)$_2$ 21 with Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$ yielded the binding stoichiometry 2:3 [N-Boc-aminoethyl 2-pyridylbenzimidazole (PBI)$_2$ (glycinate)$_2$ 21: Cu(NO$_3$)$_2$], and 3:2 [N-Boc-aminoethyl 2-pyridylbenzimidazole (PBI)$_2$ (glycinate)$_2$ 21: Ni(NO$_3$)$_2$], respectively. To avoid any ambiguity, BH equation (1:2 binding model) was used for calculating the binding constants and results in the straight line, which is observed in graphs below (Figure 50A & B, inset).
Figure 50. Benesi Hildebrand’s plots (A) UV-Vis absorption spectra of N-Boc-aminoethyl 2-pyridylvbenzimidazole (PBI)$_2$ (glycinate)$_2$ 21 with (A) Cu(NO$_3$)$_2$ and (B) Ni(NO$_3$)$_2$. Arrows indicate decrease in absorption at 308 nm from 0 to 2.5 mM metal concentration. The Benesi Hildebrand plots are represented as insets.

The calculated values of binding constant (K) were found to be $2.24 \times 10^4$ [M]$^{-1}$ for Cu(NO$_3$)$_2$ and $3.18 \times 10^4$ [M]$^{-1}$ for Ni(NO$_3$)$_2$ in 1:2 binding models (Table 16), which implies better and strong binding in comparison to (PBI) 8.

Table 16. Calculation of binding constants for (PBI)$_2$ (glycinate)$_2$ 21 using UV-Vis spectroscopy

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Metal salts</th>
<th>UV-Vis K (M)$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(PBI)$_2$ 21</td>
<td>Cu(NO$_3$)$_2$</td>
<td>$2.24 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>Ni(NO$_3$)$_2$</td>
<td>$3.18 \times 10^4$</td>
</tr>
</tbody>
</table>

The present investigations into the metal binding ability of N-Boc-aminoethyl 2-pyridylvbenzimidazole (PBI)$_2$ (glycinate)$_2$ 21 shows that it binds to both Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$. However unlike the monomeric units (PBI) 8, the binding stoichiometries of the dimer N-Boc-aminoethyl 2-pyridylvbenzimidazole (PBI)$_2$ (glycinate)$_2$ 21 is different for its complexes with the two metal ions. In view of the results obtained from the above studies, the geometry of the complexes and considering the tetracoordinate nature of Cu$^{2+}$, hexacoordinate nature of Ni$^{2+}$ the following plausible model models as depicted in Figure 51 can be proposed.
In the model proposed here, \( N\text{-Boc-aminoethyl 2-pyridylbenzimidazole (PBI)}_2 \) (glycinate)_2 21 and copper, the tetracoordinate character of \( \text{Cu}^{2+} \) has been fulfilled by the attachment of two bidentate ligands. Thus, two \( N\text{-Boc-aminoethyl 2-pyridylbenzimidazole (PBI)}_2 \) (glycinate)_2 21 strands are held together by three copper metal ions. In case of nickel, the hexacoordinate character of \( \text{Ni}^{2+} \) could be fulfilled by the attachment of three bidentate ligands. Therefore, three \( N\text{-Boc-aminoethyl 2-pyridylbenzimidazole (PBI)}_2 \) (glycinate)_2 21 units are likely to be held together in either parallel or in antiparallel manner by two nickel metal ions.

**Figure 51.** Proposed model for \( N\text{-Boc-aminoethyl 2-pyridylbenzimidazole (PBI)}_2 \) (glycinate)_2 21 (A) with \( \text{Cu(NO}_3)_2 \) and (B) \( \text{Ni(NO}_3)_2 \).

Figure 51 shows only two examples (for both copper and nickel metal ions) of the possible isomers that results from antiparallel vs parallel alignment of polyamide oligomers inside the formed duplexes.
3.10.3 UV-Vis spectrophotometric titrations of polyamide homo-oligomers

This section discusses the metal binding properties of synthesized polyamide homo-oligomers (PBI)$_6$ 22, (PDA)$_6$ 23 and (CAT)$_6$ 24 with diverse metal salts.

3.10.3a UV-Vis spectrophotometric titrations of 2-pyridyl-benzimidazole (PBI)$_6$ oligomer, 22

(PBI)$_6$ structure provides total of six sites for metal complexation (Figure 52).

![Figure 52. Structure of 2-pyridylbenzimidazole (PBI)$_6$ oligomer 22.](image)

UV-Vis spectrophotometric titrations were performed in water, where 2-pyridylbenzimidazole (PBI)$_6$ oligomer 22 displayed maximum absorption ($\lambda_{max}$) at 302 nm.

**Cu(NO$_3$)$_2$:** On addition of Cu(NO$_3$)$_2$, spectrum reveals a hyperchromic shift ($\lambda_{max}$) at 324 nm (+16 nm) upon binding with and two isosbestic points at 258 and 315 nm, with inflection point ~ 3 equivalents of Cu(NO$_3$)$_2$ in water (Figure 53A & B).

**Ni(NO$_3$)$_2$:** Similarly, addition of Ni(NO$_3$)$_2$ exhibited a new red shifted absorption band ($\lambda_{max}$) at 324 nm (+16 nm). This new absorption band along with two isosbestic points at 256 and 312 nm indicated the formation of Ni$^{2+}$-2-pyridylbenzimidazole (PBI)$_6$ oligomer 22 complex (Figure 53). The saturation point was not clearly visible (Figure 53D), indicative of a very weak binding of 2-pyridylbenzimidazole (PBI)$_6$ oligomer 22 with Ni(NO$_3$)$_2$. 

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Figure 53. Changes in the absorption spectra of 2-pyridylbenzimidazole (PBI)$_6$ oligomer 22 (8-10 µM) in water upon the addition of metal salts (A) Cu(NO$_3$)$_2$ (7.5 mM). (C) Ni(NO$_3$)$_2$ (10 mM). Plot of the change in absorbance at 308 and 323 nm as a function of molar ratio of metal to peptides (B) Cu(NO$_3$)$_2$ and (D) Ni(NO$_3$)$_2$.

**AuCl$_3$:** In case of Au$^{3+}$, with increasing concentration a gradual decrease in the absorbance at 302 nm was observed, but unfortunately, since the absorption changes of the ligand was completely masked by the gold absorption itself, meaningful interpretation of this result was not possible (Figure 54).

Figure 54. Changes in the absorption spectra of 2-pyridylbenzimidazole (PBI)$_6$ oligomer 22 (8-10 µM) in water upon the addition of AuCl$_3$ (10 mM).
UV-Vis titration experiments of 2-pyridylbenzimidazole (PBI)$_6$ oligomer 22 with several metal ions like Zn$^{2+}$, Pt$^{3+}$, Ag$^+$, Tb$^{3+}$, Eu$^{3+}$, Cd$^{2+}$, Pt$^{2+}$, Pb$^{2+}$, Ho$^{3+}$, Mn$^{3+}$ etc were undertaken. No significant spectral changes were observed by the addition of any of these metal salts (Table 17), suggesting lack of complexation to (PBI)$_6$ 22.

<table>
<thead>
<tr>
<th>Metal salts (2.5 mM)</th>
<th>Observation</th>
<th>Inflection points</th>
<th>Isosbestic points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper nitrate</td>
<td>Binding</td>
<td>302 and 324</td>
<td>258 and 315</td>
</tr>
<tr>
<td>Nickel nitrate</td>
<td>Binding</td>
<td>302 and 324</td>
<td>256 and 312</td>
</tr>
<tr>
<td>Gold chloride</td>
<td>Binding</td>
<td>302 and 350</td>
<td>293 and 312</td>
</tr>
<tr>
<td>Ruthenium chloride</td>
<td>very weak binding</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Palladium nitrate</td>
<td>Very weak binding</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*silver nitrate, cadmium nitrate, lead nitrate, holmium nitrate, manganese acetate, iron nitrate, zinc nitrate, europium nitrate, terbium nitrate did not show metal binding.

To determine the stoichiometry of binding, Job’s plot of 2-pyridylbenzimidazole (PBI)$_6$ oligomer 22 (100 µM in water) with Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$ were obtained. The intersection point was obtained around 0.60, which indicated a binding stoichiometry 3:2 for the complexes (Figure 55). However, Job’s plot for 2-pyridylbenzimidazole (PBI)$_6$ oligomer 22 with Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$ yielded the different binding stoichiometry 2:3 [2-pyridylbenzimidazole (PBI)$_6$ oligomer 22: Cu(NO$_3$)$_2$], and 1:2 [2-pyridylbenzimidazole (PBI)$_6$ oligomer 22: Ni(NO$_3$)$_2$], respectively.
Figure 55. UV-Vis absorption spectra of 2-pyridylbenzimidazole (PBI)_6 oligomer 22 with metal salts in molar ratios of (a) 0:100 (b) 10:90 (c) 20:80 (d) 30:70 (e) 40:60 (f) 50:50 (g) 60:40 (h) 70:30 (i) 80:20 (j) 90:10 (k) 100:0; (A) UV spectra and (B) Job’s plot with Cu(NO₃)₂; (C) UV spectra and (D) Job’s plot with Ni(NO₃)₂.

Benesi Hildebrand equation was used for calculating the binding constants and resulted in the straight line, which is shown in graphs (Figure 56A & B, inset).

Figure 56. Benesi Hildebrand’s plots for 2-pyridylbenzimidazole (PBI)_6 oligomer 22 (A) UV-Vis absorption spectra of with (A) Cu(NO₃)₂ and (B) Ni(NO₃)₂. Arrows indicate decrease in absorption at 302 nm from 0 to 2.5 mM metal concentration. The Benesi Hildebrand plots are represented as insets.
The calculated binding constant (K) values are $5.19 \times 10^3$ [M]$^{-1}$ and $2.61 \times 10^3$ [M]$^{-1}$ for Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$, respectively. These values are comparable with those of the monomer (PBI) 8.

In conclusion, 2-pyridylbenzimidazole (PBI)$_6$ oligomer 22, showed binding with copper, nickel and gold metal ions.

3.10.3b UV-Vis spectrophotometric titrations of o-phenylenediamine polyamide oligomer (PDA)$_6$ 23

As per the reports, the $o$-phenylenediamines are found to binds better with palladium, gold, mercury, cadmium etc. With this rationale in mind $o$-phenylenediamine oligomer (PDA)$_6$ 23 (Figure 57) consisting of $o$-phenylenediamines attached to aeg-backbone were titrated with dissimilar metal salts.

![Figure 57. Structure of o-phenylenediamine oligomer (PDA)$_6$ 23.](image)

$o$-Phenylenediamine oligomer (PDA)$_6$ 23 was studied for its complexation behaviour towards diverse metal ions i.e copper nitrate, nickel nitrate and gold chloride (Figure 57).

Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$: The absorption spectra of aqueous solution of $o$-phenylenediamine oligomer (PDA)$_6$ 23 displays two absorption peaks ($\lambda_{max}$) at 269 nm and 271 nm. UV-Vis titration experiments of $o$-phenylenediamine oligomer (PDA)$_6$ 23 with Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$ were carried out at lower concentration (2.5 mM) which resulted insignificant spectral change (Figure 58A & B). Concentration of the metal salts were increased to 7.5 mM in order to shift the equilibrium towards metal complexation. The titrations also performed with Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$ at higher concentration (7.5 mM), but displayed no significant spectral change (Figure 58C & D).
Figure 58. Changes in the absorption spectra of o-phenylenediamine oligomer (PDA)$_6$ 23 (8-10 µM) in water upon the addition of metal salts (A) Cu(NO$_3$)$_2$ (2.5 mM), (B) Ni(NO$_3$)$_2$ (2.5 mM), (C) Cu(NO$_3$)$_2$ (7.5 mM), (D) Ni(NO$_3$)$_2$ (7.5 mM), (E) AuCl$_3$ (2.5 mM) and (F) AuCl$_3$ (7.5 mM).

AuCl$_3$: Similar results were seen in the titration of o-phenylenediamine oligomer (PDA)$_6$ 23 with gold chloride at both lower (2.5 mM) as well as higher concentrations (7.5 mM) (Figure 59E & F). Complexation of other metal ions like Zn$^{2+}$, Cd$^{2+}$, Pd$^{2+}$, Hg$^{2+}$ as their nitrate/perchlorate salts were also explored. However, no significant spectral change was observed by the addition of these metal salts (Figure 59).
Figure 59. Changes in the absorption spectra of o-phenylenediamine oligomer (PDA)$_6$ 23 (8-10 µM) in water upon the addition of metal salts. (A) Pd(NO$_3$)$_2$ (2.5 mM), (B) Zn(NO$_3$)$_2$ (2.5 mM), (C) Cd(NO$_3$)$_2$ (2.5 mM) and (D) Hg(ClO$_4$)$_2$ (2.5 mM).

UV-Vis experiments of o-phenylenediamine oligomer (PDA)$_6$ 23 were done with Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$ under basic conditions also (Figure 60), but no significant spectral changes were observed.

Figure 60. Changes in the absorption spectra of o-phenylenediamine oligomer (PDA)$_6$ 23 (8-10 µM) in water upon the addition of metal salts followed by NaOH (0.5 M) (A) Cu(NO$_3$)$_2$ (0.5 mM) and (B) Ni(NO$_3$)$_2$ (0.5 mM).

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In summary, \( o \)-phenylenediamine oligomer (PDA)\( \textsubscript{6} \) 23 exhibited poor or negligible complexation towards various metal ions such as like Zn\(^{2+} \), Cd\(^{2+} \), Pd\(^{2+} \), Hg\(^{2+} \), Cu\(^{2+} \), Au\(^{3+} \), and Ni\(^{2+} \).

3.10.3c UV-Vis spectrophotometric titrations of catechol (CAT)\( \textsubscript{6} \) Oligomer 24

Catechol (CAT)\( \textsubscript{6} \) oligomer 24 (Figure 61) consisting of catechol units attached to \( aeg \)-backbone were titrated with various metal salts.

![Figure 61. Structure of catechol (CAT)\( \textsubscript{6} \) oligomer 24.](image)

The electronic spectra of aqueous solution of catechol (CAT)\( \textsubscript{6} \) oligomer 24 displays absorption (\( \lambda_{\text{max}} \)) at 281 nm. UV-Vis titration experiments of catechol (CAT)\( \textsubscript{6} \) oligomer 24 with Cu(NO\( \textsubscript{3} \))\( \textsubscript{2} \) and Ni(NO\( \textsubscript{3} \))\( \textsubscript{2} \) (Figure 62).

![Figure 62. Changes in the absorption spectra of catechol (CAT)\( \textsubscript{6} \) oligomer 24 (8-10 \( \mu \)M) in water upon the addition of metal salts (A) Cu(NO\( \textsubscript{3} \))\( \textsubscript{2} \) (10 mM) and (B) Ni(NO\( \textsubscript{3} \))\( \textsubscript{2} \) (10 mM).](image)

Similarly, UV-Vis spectrophotometric titrations were also carried out at higher concentration (10 mM) but resulted in no significant spectral change (Figure 63).
In summary, catechol (CAT)₆ oligomer 24 exhibited poor complexation towards metal ions such as like Cu²⁺, Au³⁺ and Ni²⁺.

### 3.10.4 UV-Vis spectrophotometric titrations of polyamide hetero-oligomers

*Hetero*-oligomers having different metal complexing ligands e.g. PBI/PDA or PBI/CAT expands the repertoire of metallo-polyamides. Hence, polyamide *hetero*-oligomers (PBI)₃-(PDA)₃ 25, (PBI-PDA)₃ 26, (PBI)₃-(CAT)₃ 27 and (PBI-CAT)₃ 28 were synthesized and titrated with different metal salts to investigate their metal binding properties.

#### 3.10.4a UV-Vis spectrophotometric titrations of (PBI)₃-(PDA)₃ oligomer 25

UV-Vis titration experiments of (PBI)₃-(PDA)₃ oligomer 25 (Figure 64) were studied with different metal ions (Zn²⁺, Ru³⁺, Pd²⁺, Pt³⁺, Co²⁺, Cd²⁺, Pb²⁺, Pd²⁺) as their nitrate/chloride salts. UV-Vis spectrum of aqueous solution of (PBI)₃-(PDA)₃ oligomer 25 shows absorbance (λ<sub>max</sub>) at 302 nm.

![Figure 64. Structure of (PBI)₃-(PDA)₃ oligomer 25.](image)
Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$: Upon addition of Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$, the new absorption band appears ($\lambda_{\text{max}}$) at 325 nm (+13 nm) and 324 nm (+12 nm) respectively, both exhibiting isosbestic points at 256 and 312 nm that indicated the formation of Cu$^{2+}$-[(PBI)$_3$-(PDA)$_3$ oligomer 25] and Ni$^{2+}$-[(PBI)$_3$-(PDA)$_3$ oligomer 25] complexes (Figure 65).

It is worthy to note that synthesized aeg linked oligomers exhibits differential binding patterns with various metal salts. The synthesized o-phenylenediamine (PDA)$_6$ oligomer 23 did not show any strong binding for nickel nitrate salt, whereas hetero-oligomer (PBI)$_3$-(PDA)$_3$ 25 exhibited strong binding.

AuCl$_3$: Surprisingly, with an increase in the Au$^{3+}$ concentration the absorbance ($\lambda_{\text{max}}$) at 302 nm gradually decreased, but the absorbance was completely masked by the absorbance of gold itself. So, in order to obtain the clear isosbestic point, it was titrated with lesser amount (2.0 µL of 10 mM solution) and it revealed two isosbestic...
points at 286 and 332 nm suggesting the formation of \( \text{Au}^{3+} \)-[(PBI)\(_3\)-(PDA)\(_3\)] oligomer 25 complex (Figure 66).

**Figure 66.** Changes in the absorption spectra of (PBI)\(_3\)-(PDA)\(_3\) oligomer 25 (8-10 \( \mu \)M) in water upon the addition of metal salts (A) \( \text{AuCl}_3 \)(10 mM) (B) \( \text{AuCl}_3 \)(10 mM).

In comparison to (PBI)\(_6\) 22 (Figure 67A &B), (PBI)\(_3\)-(PDA)\(_3\) oligomer 25 (Figure 65C & D) binds better with similar concentration of nickel nitrate (10 mM). So, it is possible that the octahedral geometry of nickel salts could be stabilized nicely by either two PBI units and one PDA unit or *vice versa.*

**Figure 67.** Changes in the absorption spectra of (PBI)\(_6\) oligomer 22 (8-10 \( \mu \)M) in water upon the addition of metal salts (A) \( \text{Ni(NO}_3\)\(_2\) (7.5). Plot of the change in absorbance at 302 and 345 nm as a function of molar ratio of metal to peptides (B) \( \text{Ni(NO}_3\)\(_2\).

UV-Vis spectral change of (PBI)\(_3\)-(PDA)\(_3\) oligomer 25 were studied in the presence of metal ions like zinc nitrate, ruthenium trichloride, palladium nitrate,
cobalt nitrate, lead nitrate, potassium tetrachloroplatinate and did not exhibit significant spectral change (Table 18).

**Table 18. Summary of UV-Vis titrations for the (PBI)\textsubscript{3}-(PDA)\textsubscript{3} oligomer 25.**

<table>
<thead>
<tr>
<th>Metal salts</th>
<th>Observation</th>
<th>Inflection points</th>
<th>Isosbestic points</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2.5/7.5/10 mM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper nitrate</td>
<td>Binding</td>
<td>302 and 325</td>
<td>258 and 315</td>
</tr>
<tr>
<td>Nickel nitrate</td>
<td>Binding</td>
<td>302 and 324</td>
<td>256 and 312</td>
</tr>
<tr>
<td>Gold chloride</td>
<td>Weak binding</td>
<td>302</td>
<td>286 and 332</td>
</tr>
</tbody>
</table>

*zinc nitrate, ruthenium trichloride, palladium nitrate, cobalt nitrate, lead nitrate, potassium tetrachloroplatinate

Determination of binding stoichiometry by Job’s plot (100 µM in water) gave intersection point ~ 0.60 shows 3:2 binding stoichiometry for [(PBI)\textsubscript{3}-(PDA)\textsubscript{3} \textbf{25}]: Cu(NO\textsubscript{3})\textsubscript{2}]\textsuperscript{2-}, implying that the duplex is assembled from two oligomeric strands linked by three copper metal ions (Figure 68).

**Figure 68.** UV-Vis absorption spectra of (PBI)\textsubscript{3}-(PDA)\textsubscript{3} \textbf{25} with metal salts in molar ratios of (a) 0:100 (b) 10:90 (c) 20:80 (d) 30:70 (e) 40:60 (f) 50:50 (g) 60:40 (h) 70:30 (i) 80:20 (j) 90:10 (k) 100:0; (A) UV spectra and (B) Job’s plot with Cu(NO\textsubscript{3})\textsubscript{2} (C) UV spectra and (D) Job’s plot with Ni(NO\textsubscript{3})\textsubscript{2}.

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Job’s plot of Ni(NO$_3$)$_2$ and (PBI)$_3$-(PDA)$_3$ oligomer 25 exhibits intersection point at 0.67, shown at 1:2 binding stoichiometry for [(PBI)$_3$-(PDA)$_3$ oligomer 25: Ni(NO$_3$)$_2$], suggests that two oligomeric strands are bound with four nickel metal ions.

UV-Vis spectroscopic studies confirmed that (PBI)$_3$-(PDA)$_3$ oligomer 25 binds better to the Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$ than other metal salts. The calculated binding constants were found to be $7.42 \times 10^4$ and $3.34 \times 10^3 [M]^{-1}$ for Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$, respectively (Figure 69).

![Figure 69. Benesi Hildebrand’s plots (A) UV-Vis absorption spectra of (PBI)$_3$-(PDA)$_3$ oligomer 25 with (A) Cu(NO$_3$)$_2$ and (B) Ni(NO$_3$)$_2$. Arrows indicate decrease in absorption at 302 nm from 0 to 2.5 mM metal concentration. The Benesi Hildebrand plots are represented as insets.](image)

In conclusion, (PBI)$_3$-(PDA)$_3$ oligomer 25, showed binding with copper, nickel and gold metal ions.

3.10.4b UV-Vis spectrophotometric titrations of (PBI-PDA)$_3$ oligomer 26

Inspired by the metal binding results from the hetero-oligomer (PBI-PDA)$_3$ 25, alternately linked (PBI-PDA)$_3$ oligomer 26 was also checked for its complexation studies. The UV-Vis absorbance spectra of (PBI-PDA)$_3$ oligomer 26 shows absorbance ($\lambda_{max}$) at 303 nm in water (Figure 70).

![Figure 70. Structure of (PBI-PDA)$_3$ oligomer 26.](image)
Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$: Addition of Cu(NO$_3$)$_2$ solution led to red shifts revealing absorbance ($\lambda_{\text{max}}$) at 324 nm (+11 nm) and isosbestic points at 254 and 315 nm indicating the formation of Cu$^{2+}$-(PBI-PDA)$_3$ oligomer 26 complex. So was the observation for Ni(NO$_3$)$_2$, but with isosbestic points at 255 and 315 nm. Similar, results regarding its better binding with Ni(NO$_3$)$_2$ can also be observed for (PBI-PDA)$_3$ oligomer 26 (Figure 71).

Figure 71. Changes in absorption spectra of (PBI-PDA)$_3$ oligomer 26 (8-10 µM) in water upon the addition of metal salts (A) Cu(NO$_3$)$_2$ (7.5 mM) and (C) Ni(NO$_3$)$_2$ (7.5 mM). Plot of the change in absorbance at 302 and 345 nm as a function of molar ratio of metal to peptides (B) Cu(NO$_3$)$_2$ and (D) Ni(NO$_3$)$_2$.

AuCl$_3$: Spectroscopic behaviour of (PBI-PDA)$_3$ oligomer 26 upon complexation with Au$^{3+}$, showed a fall in absorbance at 302 nm was noted and presence of two isosbestic points at 288 and 333 nm indicated formation of Au$^{3+}$-[(PBI-PDA)$_3$ oligomer 26] complex (Figure 72).
Figure 72. Changes in absorption spectra of (PBI-PDA)$_3$ oligomer 26 (8-10 µM) in water upon the addition of AuCl$_3$ (10 mM).

UV-Vis titration results for the (PBI-PDA)$_3$ oligomer have been summarized in Table 19.

Table 19. Summary of UV-Vis titrations for the (PBI-PDA)$_3$ oligomer 26.

<table>
<thead>
<tr>
<th>Metal salts (7.5/10 mM)</th>
<th>Observation</th>
<th>Inflection points</th>
<th>Isosbestic points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper nitrate</td>
<td>Binding</td>
<td>303 and 324</td>
<td>254 and 315</td>
</tr>
<tr>
<td>Nickel nitrate</td>
<td>Binding</td>
<td>303 and 324</td>
<td>255 and 315</td>
</tr>
<tr>
<td>Gold chloride</td>
<td>Weak binding</td>
<td>303</td>
<td>288 and 333</td>
</tr>
</tbody>
</table>

In the Job’s continuous variation method (100 µM in water), intersection point was attained ~0.60 showing 3:2 stoichiometry for (PBI-PDA)$_3$ oligomer 26: Cu(NO$_3$)$_2$ complex. The two oligomeric strands are linked by three copper metal ions. In comparison Ni(NO$_3$)$_2$ exhibits intersection point ~0.67, which shows the 1:2 stoichiometry wherein two oligomeric strands are linked by four nickel metal ions (Figure 73).
Figure 73. UV-Vis absorption spectra of (PBI-PDA)$_3$ oligomer 26 with metal salts in molar ratios of (a) 0:100 (b) 10:90 (c) 20:80 (d) 30:70 (e) 40:60 (f) 50:50 (g) 60:40 (h) 70:30 (i) 80:20 (j) 90:10 (k) 100:0; (A) UV spectra and (B) Job’s plot with Cu(NO$_3$)$_2$; (C) UV spectra and (D) Job’s plot with Ni(NO$_3$)$_2$.

The binding constant (K) calculated from Benesi-Hildebrand’s equation were found $1.58 \times 10^3$ and $2.1 \times 10^3$ [M]$^{-1}$ for Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$, respectively (Figure 74).

Figure 74. Benesi Hildebrand’s plots (A) UV-Vis absorption spectra of (PBI-PDA)$_3$ oligomer 26 with (A) Cu(NO$_3$)$_2$ and (B) Ni(NO$_3$)$_2$. Arrows indicate decrease in absorption at 302 nm from 0 to 2.5 mM metal concentration. The Benesi Hildebrand plots are represented as insets.

In conclusion, (PBI-PDA)$_3$ oligomer 26, showed binding with copper, nickel and gold metal ions.

3.10.4c UV-Vis spectrophotometric titrations of (PBI)$_3$-(CAT)$_3$ Oligomer 27

Polyamide hetero-oligomers with sequence (PBI)$_3$-(CAT)$_3$ oligomer 27 (hexamer unit) showed absorbance ($\lambda_{max}$) at 303 nm in water (Figure 75).
Cu(NO$_3$)$_2$: The electronic spectra of (PBI)$_3$-(CAT)$_3$ oligomer 27 upon addition of Cu(NO$_3$)$_2$ revealed binding pattern similar to that of Cu$^{2+}$-(PBI-PDA)$_3$ 26 complex. Titrations with Ni(NO$_3$)$_2$ exhibited a similar red shift, but a slightly more shifted isosbestic points at 260 and 313 nm indicating the formation of Ni$^{2+}$-(PBI)$_3$-(CAT)$_3$ oligomer 27 complex (Figure 76). These changes in the absorption spectra indicated complex formation between (PBI)$_3$-(CAT)$_3$ oligomer 27 and metal ions (Cu$^{2+}$/Ni$^{2+}$).

![Figure 75. Structure of (PBI)$_3$-(CAT)$_3$ oligomer 27.](image)

![Figure 76. Changes in the absorption spectra of (PBI)$_3$-(CAT)$_3$ oligomer 27 (8-10 μM) in water upon the addition of metal salts (A) Cu(NO$_3$)$_2$ (7.5 mM) and (C) Ni(NO$_3$)$_2$ (7.5 mM).](image)

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Plot of the change in absorbance at 303 and 345 nm as a function of molar ratio of metal to peptides (B) Cu(NO$_3$)$_2$ and (D) Ni(NO$_3$)$_2$.

The continuous variation method to determine molecular stoichiometry, showed intersection point ~0.60 proving 3:2 stoichiometry for (PBI)$_3$-(CAT)$_3$ oligomer 27:Cu(NO$_3$)$_2$. The two oligomeric strands are possibly linked by three copper metal ions. (PBI)$_3$-(CAT)$_3$ oligomer 27:Ni(NO$_3$)$_2$ indicated intersection point at 0.67, which indicates a binding stoichiometry of 1:2 (Figure 77).

![Figure 77. UV-Vis absorption spectra of (PBI)$_3$-(CAT)$_3$ oligomer 27 with metal salts in molar ratios of (a) 0:100 (b) 10:90 (c) 20:80 (d) 30:70 (e) 40:60 (f) 50:50 (g) 60:40 (h) 70:30 (i) 80:20 (j) 90:10 (k) 100:0; (A) UV spectra and (B) Job’s plot with Cu(NO$_3$)$_2$; (C) UV spectra and (D) Job’s plot with Ni(NO$_3$)$_2$.](image)

The binding constant (K), which denotes the strength of binding, were found to be 1.45 x 10$^4$ and 5.16 x 10$^3$ [M]$^{-1}$ in 1:2 binding model for Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$, respectively (Figure 78).
Figure 78. Benesi Hildebrand’s plot (A) UV-Vis absorption spectra of \( (PBI)_3-(CAT)_3 \) oligomer 27 with (A) Cu(NO\(_3\))\(_2\) and (B) Ni(NO\(_3\))\(_2\). Arrows indicate decrease in absorption at 302 nm from 0 to 2.5 mM metal concentration. The Benesi Hildebrand plots are represented as insets.

UV-Vis titration results have been summarized in Table 20.

<table>
<thead>
<tr>
<th>Metal salts (7.5 mM)</th>
<th>Observation</th>
<th>Inflection points</th>
<th>Isosbestic points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper nitrate</td>
<td>Binding</td>
<td>303 and 324</td>
<td>255 and 314</td>
</tr>
<tr>
<td>Nickel nitrate</td>
<td>Binding</td>
<td>303 and 324</td>
<td>260 and 313</td>
</tr>
</tbody>
</table>

In conclusion, \( (PBI)_3-(CAT)_3 \) oligomer 27 binds strongly with copper and nickel metal salts.

3.10.4d UV-Vis spectrophotometric titrations of \( (PBI-CAT)_3 \) Oligomer 28

The electronic spectrum of alternately linked \( (PBI)_3-(CAT)_3 \) oligomer 28 in aqueous medium featured absorption (\( \lambda_{\text{max}} \)) at 305 nm, which on addition of Cu(NO\(_3\))\(_2\) shifted to (\( \lambda_{\text{max}} \)) at 324 nm (+11 nm) with isosbestic points at 265 and 317 nm, this indicates the formation of Cu\(^{2+}\)-(PBI-CAT)\(_3\) – 28 complex (Figure 79).

Figure 79. Structure of \( (PBI)_3-(CAT)_3 \) oligomer 28.
A similar red shift was observed with isosbestic points at 255 and 317 nm for the formation of Ni$^{2+}$-(PBI)$_3$-(CAT)$_3$ oligomer 28 complex (Figure 80).

Figure 80. Changes in the absorption spectra of (PBI)$_3$-(CAT)$_3$ oligomer 28 (8-10 µM) in water upon the addition of metal salts (A) Cu(NO$_3$)$_2$ (2.5 mM) and (C) Ni(NO$_3$)$_2$ (2.5 mM). Plot of the change in absorbance at 305 and 350 nm as a function of molar ratio of metal to peptides (B) Cu(NO$_3$)$_2$ and (D) Ni(NO$_3$)$_2$.

UV-Vis titration results have been summarized in Table 21.

<table>
<thead>
<tr>
<th>Metal salts (2.5 mM)</th>
<th>Observation</th>
<th>Inflection points</th>
<th>Isosbestic points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper nitrate</td>
<td>Binding</td>
<td>305 and 324</td>
<td>265 and 317</td>
</tr>
<tr>
<td>Nickel nitrate</td>
<td>Binding</td>
<td>305 and 324</td>
<td>255 and 317</td>
</tr>
</tbody>
</table>

The Job’s plot (100 µM in water) indicated the intersection point at 0.60, proving binding stoichiometries to be 3:2 for (PBI)$_3$-(CAT)$_3$ oligomer 28:Cu(NO$_3$)$_2$. whereas, the intersection point at 0.67 for (PBI)$_3$-(CAT)$_3$ oligomer 28:Ni(NO$_3$)$_2$ was indicative of 1:2 stoichiometry (Figure 81).
Figure 81. UV-Vis absorption spectra of (PBI)$_3$-(CAT)$_3$ oligomer 28 with metal salts in molar ratios of (a) 0:100 (b) 10:90 (c) 20:80 (d) 30:70 (e) 40:60 (f) 50:50 (g) 60:40 (h) 70:30 (i) 80:20 (j) 90:10 (k) 100:0; (A) UV spectra and (B) Job’s plot with Cu(NO$_3$)$_2$; (C) UV spectra and (D) Job’s plot with Ni(NO$_3$)$_2$.

The binding constant (K) values obtained from Benesi-Hildebrand plot was $7.87 \times 10^3$ and $4.54 \times 10^4$ [M]$^{-1}$ for Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$, respectively (Figure 82).

Figure 82. Benesi Hildebrand’s plot (A) UV-Vis absorption spectra of (PBI)$_3$-(CAT)$_3$ oligomer 28 with (A) Cu(NO$_3$)$_2$ and (B) Ni(NO$_3$)$_2$. Arrows indicate decrease in absorption at 305 nm from 0 to 2.5 mM metal concentration. The Benesi Hildebrand plots are represented as insets.

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In short, \((\text{PBI})_6\) 22-oligomer and mixed oligomers were found to bind strongly with copper and nickel metal salts. These oligomers exhibited intersection point ~0.6 which confirmed 3:2 binding stoichiometry with \(\text{Cu(NO}_3\text{)}_2\). With this in mind, binding model was proposed in which two oligomer strands are held together with the three copper metal ions (Figure 83).

\[
\text{Figure 83. Schematic diagram of polyamide oligomers binding with (A) Cu(NO}_3\text{)}_2 \text{ and (B) Ni(NO}_3\text{)}_2.}
\]

The polyamide hetero-oligomers showed 2:1 binding stoichiometry with \(\text{Ni(NO}_3\text{)}_2\), which is indicative of the two oligomer units to be held together with four nickel metal ions.

### 3.11 Isothermal Titration Calorimetry (ITC)

Having established that \(aeg\) linked ligands bind better to \(\text{Cu(NO}_3\text{)}_2 \& \text{Ni(NO}_3\text{)}_2\) than other metal salts, ITC was used to investigate the equilibrium thermodynamics of \(aeg\) linked ligand-metal complex formation.

In the experiments, the heat changes are directly measured upon the addition of small volumes of metal salts \(\text{Cu(NO}_3\text{)}_2 \& \text{Ni(NO}_3\text{)}_2\) to the reaction cell containing the polyamide homo-oligomer \((\text{PBI})_6\) 22 in aqueous solution. Integration of each peak after the addition of titrant yielded the calorimetric binding enthalpy \((\Delta H)\) as a function of the concentration of polyamide homo-oligomer \((\text{PBI})_6\) 22. In control experiments, the enthalpy of the dilution of polyamide homo-oligomer \((\text{PBI})_6\) 22 was
determined and subtracted from the total change in enthalpy of the formation of the (PBI)$_6$ oligomer 22: metal complex (Figure 84).

![Figure 84. ITC figures in water for (PBI)$_6$ oligomer 22 with Cu(NO$_3$)$_2$.](image)

A non-linear, least squares minimization software program (Origin 7.0 from Microcal Inc.) was used to fit the data and generate the titration curve using one site binding model for (PBI)$_6$ oligomer 22: metal complex. The free-energy ($\Delta G$) change during complex formation was obtained using standard thermodynamic relationships from above data. The calculated values of binding constants are $6.65 \times 10^4$ for Cu(NO$_3$)$_2$ and $9.59 \times 10^4 \text{[M]}^{-1}$ for Ni(NO$_3$)$_2$.

Similar ITC analysis was carried out for (PBI)$_8$ in methanolic solution and the values of binding constants are found to be $4.57 \times 10^5$ for Cu(NO$_3$)$_2$ and $2.55 \times 10^5 \text{[M]}^{-1}$ for Ni(NO$_3$)$_2$, respectively.

In ITC the calculated values of binding constants for polyamide hetero-oligomer (PBI)$_3$- (PDA)$_3$ 25 were found to be $1.0 \times 10^5$ and $1.17 \times 10^4 \text{[M]}^{-1}$ for Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$, respectively (Figure 83).
ITC data for polyamide hetero-oligomer (PBI-PDA)_3 26 indicated the values of binding constants to be $3.07 \times 10^5$ and $1.06 \times 10^4$ [M]$^{-1}$ Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$, respectively. The polyamide oligomer (PBI)$_3$-(CAT)$_3$ 27 determined binding constants were $1.06 \times 10^5$ and $8.83 \times 10^4$ [M]$^{-1}$ for Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$. 

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Figure 85. ITC figures in water for (A) (PBI)$_3$- (PDA)$_3$ oligomer 25 with Ni(NO$_3$)$_2$, (B) (PBI-PDA)$_3$ oligomer 26 with Cu(NO$_3$)$_2$, (C) (PBI)$_3$-(CAT)$_3$ oligomer 27 with Cu(NO$_3$)$_2$. (D) (PBI-CAT)$_3$ 28 in water with Cu(NO$_3$)$_2$. 
respectively. The alternative oligomers (PBI-CAT), 28 gave a values of binding constants of $1.44 \times 10^5$ and $2.21 \times 10^4$ [M]$^{-1}$ for Cu(NO$_3$)$_2$ and Ni(NO$_3$)$_2$, respectively.

The various thermodynamic parameters thus obtained at 273 K are listed in Table 22. Under the conditions of complexation, the value of $\Delta G$ is negative favouring association. The complexation process is exothermic and entropy-driven. The ITC data confirm in a quantitative manner that there are 3 binding sites on the synthesized aeg ligands and millimolar concentrations of the metal salts (copper nitrate and nickel nitrate).

### Table 22. ITC thermodynamic parameters describing interaction of oligomers with metal salts.

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Metal salts</th>
<th>Conc. (mM)</th>
<th>$\Delta H$ (kCal/mol)</th>
<th>$T\Delta S$ (kCal/mol)</th>
<th>$\Delta G$ (kCal/mol)</th>
<th>K (M$^{-1}$)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>(PBI$^2$) 8</td>
<td>Cu(NO$_3$)$_2$</td>
<td>0.02</td>
<td>-4.86 $\times$ 10$^2$</td>
<td>-4.76 $\times$ 10$^2$</td>
<td>-9.2</td>
<td>4.57 $\times$ 10$^5$</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Ni(NO$_3$)$_2$</td>
<td>0.02</td>
<td>7.56 $\times$ 10$^3$</td>
<td>7.57 $\times$ 10$^3$</td>
<td>-10.0</td>
<td>2.55 $\times$ 10$^5$</td>
<td>4.8</td>
</tr>
<tr>
<td>(PBI$^6$) 22</td>
<td>Cu(NO$_3$)$_2$</td>
<td>0.02</td>
<td>-21.4</td>
<td>-14.6</td>
<td>-6.99</td>
<td>6.65 $\times$ 10$^4$</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Ni(NO$_3$)$_2$</td>
<td>0.02</td>
<td>-24.8</td>
<td>-18.03</td>
<td>-6.77</td>
<td>9.59 $\times$ 10$^4$</td>
<td>3</td>
</tr>
<tr>
<td>(PBI$_3$)-(PDA)$_3$ 25</td>
<td>Cu(NO$_3$)$_2$</td>
<td>0.02</td>
<td>-3.62</td>
<td>3.22</td>
<td>-6.84</td>
<td>1.0 $\times$ 10$^3$</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Ni(NO$_3$)$_2$</td>
<td>0.02</td>
<td>-3.11</td>
<td>2.44</td>
<td>-5.55</td>
<td>1.17 $\times$ 10$^4$</td>
<td>3</td>
</tr>
<tr>
<td>(PBI-PDA)$_3$ 26</td>
<td>Cu(NO$_3$)$_2$</td>
<td>0.02</td>
<td>-58.5</td>
<td>-50.96</td>
<td>-7.54</td>
<td>3.07 $\times$ 10$^5$</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Ni(NO$_3$)$_2$</td>
<td>0.02</td>
<td>-170.4</td>
<td>-164.79</td>
<td>-5.61</td>
<td>1.06 $\times$ 10$^4$</td>
<td>3</td>
</tr>
<tr>
<td>(PBI$_3$)-(CAT)$_3$ 27</td>
<td>Cu(NO$_3$)$_2$</td>
<td>0.05</td>
<td>-20.1</td>
<td>-13.32</td>
<td>-6.78</td>
<td>1.06 $\times$ 10$^3$</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Ni(NO$_3$)$_2$</td>
<td>0.05</td>
<td>-3.42</td>
<td>3.34</td>
<td>-6.76</td>
<td>8.83 $\times$ 10$^4$</td>
<td>3</td>
</tr>
<tr>
<td>(PBI-CAT)$_3$ 28</td>
<td>Cu(NO$_3$)$_2$</td>
<td>0.05</td>
<td>-72.4</td>
<td>-65.24</td>
<td>-7.14</td>
<td>1.44 $\times$ 10$^3$</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Ni(NO$_3$)$_2$</td>
<td>0.05</td>
<td>-43.8</td>
<td>-36.65</td>
<td>-7.14</td>
<td>2.21 $\times$ 10$^5$</td>
<td>3</td>
</tr>
</tbody>
</table>

* From these experimentally determined parameters, the free energy of binding ($\Delta G$) and the entropy change ($\Delta S$) are obtained using the standard thermodynamic relationship $\Delta G = -RT\ln K = \Delta H - T\Delta S$.

The synthesized aeg linked ligands bind to metal salts with binding constants in the range of $10^4$-$10^5$ strength. The synthesized aeg linked ligands in polyamides exhibit better efficiencies with copper nitrate as compared to nickel nitrate. The comparison of binding constants for polyamide oligomers calculated from both UV-Vis spectroscopy and ITC analysis are shown in Table 23. It suggests that binding constants obtained from the two different methods shown variation. Several authors$^{62}$

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have also reported similar trends and the proper reason is not known. ITC values about 10 times higher than that of UV-Vis.

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Metal salts</th>
<th>Binding constants (UV-Vis) K (M)(^{-1})</th>
<th>Binding constants (ITC) K (M)(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(PBI) 8</td>
<td>Cu(NO(_3))(_2)</td>
<td>6.71 x 10(^3)</td>
<td>4.57 x 10(^5)</td>
</tr>
<tr>
<td></td>
<td>Ni(NO(_3))(_2)</td>
<td>1.82 x 10(^3)</td>
<td>2.55 x 10(^5)</td>
</tr>
<tr>
<td>(PBI) 22</td>
<td>Cu(NO(_3))(_2)</td>
<td>5.19 x 10(^3)</td>
<td>6.65 x 10(^4)</td>
</tr>
<tr>
<td></td>
<td>Ni(NO(_3))(_2)</td>
<td>2.61 x 10(^3)</td>
<td>9.59 x 10(^4)</td>
</tr>
<tr>
<td>(PBI-PDA) 26</td>
<td>Cu(NO(_3))(_2)</td>
<td>1.34 x 10(^4)</td>
<td>1.0 x 10(^5)</td>
</tr>
<tr>
<td>25</td>
<td>Ni(NO(_3))(_2)</td>
<td>3.34 x 10(^3)</td>
<td>1.17 x 10(^4)</td>
</tr>
<tr>
<td>(PBI-PDA) 26</td>
<td>Cu(NO(_3))(_2)</td>
<td>1.58 x 10(^3)</td>
<td>3.07 x 10(^5)</td>
</tr>
<tr>
<td></td>
<td>Ni(NO(_3))(_2)</td>
<td>2.1 x 10(^3)</td>
<td>1.06 x 10(^4)</td>
</tr>
<tr>
<td>(PBI-CAT) 27</td>
<td>Cu(NO(_3))(_2)</td>
<td>1.45 x 10(^4)</td>
<td>1.06 x 10(^5)</td>
</tr>
<tr>
<td>28</td>
<td>Ni(NO(_3))(_2)</td>
<td>5.16 x 10(^3)</td>
<td>8.83 x 10(^4)</td>
</tr>
<tr>
<td>(PBI-CAT) 28</td>
<td>Cu(NO(_3))(_2)</td>
<td>7.87 x 10(^3)</td>
<td>1.44 x 10(^5)</td>
</tr>
<tr>
<td></td>
<td>Ni(NO(_3))(_2)</td>
<td>4.54 x 10(^4)</td>
<td>2.21 x 10(^5)</td>
</tr>
</tbody>
</table>

In summary, all the polyamide oligomers 22-28 bind with Cu(NO\(_3\))\(_2\) better than with Ni(NO\(_3\))\(_2\).

### 3.12 Co-ordination chemistry of metal complexes

The incorporation of metals in the artificial nucleobases in the backbone resulted in the generation of metal complexes of specific geometry. The artificial nucleosides have either monodentate (one), bidentate (two) or tridentate (three) donor atoms for generating transition metal complexes. Shionoya et al.\(^{63}\) found that bidentate ligand 2-hydroxypyridone forms square planar complexes with Cu\(^{2+}\) (Hp, Figure 86A), tridentate ligand 2,6-bis(methylthiomethyl)pyridine forms octahedral complexes with Ag\(^+\) (Spy, Figure 86B), monodentate ligand pyridyl forms linear complexes with Ag\(^+\) (Py, Figure 86C), bidentate ligand catechol exhibits distorted tetrahedral complexes with B\(^{3+}\) (Cat, Figure 86D) etc inside the formed duplexes.

William et al.\(^{35a}\) also synthesized homo-substituted polyamide chain with chelating ligands e.g. monodentate pyridine or bidentate bipyridine, resulted in the generation of multimetallic structures upon co-ordination with transition metal ions.
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The synthesized phenyl terpyridyl ligand (φ-tpy, Figure 86E) and studied its complexation with Co\(^{3+}\)/Fe\(^{3+}\) in octahedral geometry.

In a similar way, Achim et al.\(^6\) synthesized the bipyridyl ligand and studied complexes of different geometries with different metal ions. It formed tetrahedral complexes with Cu\(^{2+}\) whereas both tetrahedral and octahedral complexes with Ni\(^{2+}\).

![Diagram of coordination geometries of the reported ligands](image)

**Figure 86.** Co-ordination geometries of the reported ligands.\(^6\), \(^3\)\(^5\)\(a\)

In view of these facts, the synthesized bidentate ligands was seen to exhibits 2:1 [ligand: metal] complexes with different metals in specific geometries *e.g.* PBI, 1 forms square planar complexes with Cu\(^{2+}\), PDA, 2 forms square planar complexes with Pd\(^{2+}\) and CAT, 3 forms distorted tetrahedral complexes with B\('\) *etc.* (Figure 87).
Achim et al.\textsuperscript{64} have studied the different behaviour exhibited by the adjacent bipyridyl ligands substituted in the PNA oligomers. They found that adjacent bipyridyl contributed towards more strong duplexes due to the supramolecular chelate effect. This effect comes into light where several bipyridyl moieties are in close proximity. With nickel salts they observed the formation of two different geometries of metal complexes; one with square planar geometry [Ni(Bpy)\textsubscript{2}] and other with octahedral geometry [Ni(Bpy)\textsubscript{3}]. By means of electron paramagnetic resonance (EPR), they confirmed the formation of different metal complexes with copper salts also; square planar geometry of [Cu(Bpy)\textsubscript{2}] and octahedral geometry of [Cu(Bpy)\textsubscript{3}] complexes.

Similar observations were found in our designed bidentate 2-pyridyl benzimidazole ligand (PBI), which is also analogous to bipyridyl ligand. 2-Pyridyl benzimidazole ligand (PBI) may form either square-planar or octahedral complexes with transition metal ions such as Cu\textsuperscript{2+}, Ni\textsuperscript{2+} etc. In case of N-Boc-aminoethyl (PBI) glycinate \textbf{8}, it can only form complexes in square planar geometry and in stoichiometry of either 1:1 [PBI:Pd\textsuperscript{2+}] or 2:1 [PBI:Cu\textsuperscript{2+}/Ni\textsuperscript{2+}]. On the other hand in case of N-Boc-aminoethyl (PBI)\textsubscript{2} (glycinate)\textsubscript{2} \textbf{21}, it can form complexes in square planar geometry with Cu\textsuperscript{2+} as well as octahedral geometry Ni\textsuperscript{2+} metal ions.

\textbf{Figure 87.} Co-ordination geometries of the synthesized \textit{aeg} linked ligands.
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Figure 88. Plausible co-ordination geometries of the aeg linked ligands in the formed metal mediated duplexes.

On the other hand in case of N-Boc-aminoethyl (PBI)_2 (glycinate) staggered 21, it can form complexes in square planar geometry with Cu^{2+} as well as octahedral geometry Ni^{2+} metal ions. Due to supramolecular chelate effect exerted by polyamide homo-oligomer (PBI)_6 oligomer 22, it is possible that complexes would form either with square planar geometry (Figure 88A) or octahedral geometry (Figure 88B) with Cu^{2+} metal ions. on the other hand with nickel metal ions, complexes of octahedral geometry is more stable (Figure 88C).

Similar results are expected from polyamide hetero-oligomers which has three 2-pyridylbenzimidazole ligand (PBI) units and more likely to show supramolecular chelate effect. Based on all these facts various binding models have been proposed.

3.13 Binding models

To summarise, copper and nickel metal salts reveal possible binding pattern as depicted pictorially below (Figure 89).
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![Diagram](Diagram1.png)

**Figure 89.** Pictorial representation of possible binding mode for 2-pyridylbenzimidazole (PBI) monomer 8, dimer 21 and oligomers 22.

In contrast, PDA did not result in appreciable binding with any metal salts investigated (Figure 90).

![Diagram](Diagram2.png)

**Figure 90.** Pictorial representation of possible binding mode for (PDA) oligomers 23.

Catechol monomer, however showed binding with boron ions at slightly basic pH, but no binding with different metal salts (Figure 91).
Figure 91. Pictorial representation of possible binding mode for (CAT) monomer 17 and oligomers 24.

It was observed that phenylenediamine oligomer was not binding to any of the metal salts, but hetero-oligomers of these two units exhibited better binding (Figure 92).

Figure 92. Pictorial representation of possible binding mode for 2-pyridylbenzimidazole (PBI) and o-phenylenediamine (PDA) hetero-oligomers.

So was the case of catechol oligomers, with no binding to any of the metal salts, but hetero-oligomers of these two units exhibited better binding (Figure 93).
Figure 93. Pictorial representation of possible binding mode for 2-pyridylbenzimidazole (PBI) and catechol (CAT) hetero-oligomers.

3.14 Conclusions

In conclusion, successful synthesis of 2-pyridylbenzimidazole (PBI) ortho-phenylenediamine (PDA), catechol (CAT) monomers and its oligomer on the SPPS were achieved. This thesis presented the facile construction of supramolecular structures having multiple complexes tethered to 2-pyridylbenzimidazole (PBI), ortho-phenylenediamine (PDA), catechol (CAT) oligopeptide scaffolds. The studies here provide the necessary foundation to enable characterization of the multimetallic structures and application to design molecular motifs of increased complexity and function. Based on the denticities of the ligands attached to aeg backbone, it could be highly useful in designing metal sensors.

In case of (PBI)$_6$ oligomer and polyamide hetero-oligomers, the metal ions cross-link the strands to self assemble structures into double stranded oligopeptide duplexes with sequence dependent spectroscopic properties. It was observed that ortho-phenylenediamine (PDA) and catechol (CAT) oligomers did not exhibit metal complexation. It may be possible that presence of ring nitrogen or any hetero atom effects the binding properties of the designed ligands. In the literature, best rational designs are pyridyl, bipyridyl, terpyridyl, phenyl terpyridyl, hydroxyquinoline, 2-hydroxyypyridone, which shows the presence of either one or two hetero atoms. Due to this possibility ortho-phenylenediamine (PDA) and catechol (CAT) oligomers are incapable of binding strongly.
It is also observed that catechol (CAT) monomer exhibited binding with boron ions under slightly basic pH. Whereas synthesized hetero oligomer of two different units like PBI/PDA or PBA/CAT shown strong binding affinities towards nickel nitrate. Binding constants obtained from ITC and UV-Vis is shown to have comparable binding strength of these synthesized aeg ligands.
3.15 Experimental Section

3.15.1 General remarks: All reagents and chemicals were of laboratory or analytical grade obtained from commercial sources and were used without further purification unless mentioned. Thin layer chromatography (TLC) was done on precoated silica gel 60 F_{254} plates (Merck). TLCs were visualized under UV light, iodine and/or ninhydrin spray followed by heating up to 110°C with heat gun. Silica gel 60-120 and 100-200 mesh (Merck) was used for routine column chromatography with ethyl acetate/petroleum ether or dichloromethane/methanol mixture as elution solvent depending upon the compound polarity and chemical nature. All solvents were distilled under an inert atmosphere with appropriate desiccant.

$^1$H NMR spectra were routinely recorded at 200 MHz on a Bruker AC-200 instrument controlled by an Aspect 2000 computer. $^{13}$C NMR and $^{13}$C-DEPT spectra (at 50 MHz) were recorded on the same instrument. The spectra were analyzed using ACD spec-viewer software from ACD labs. For some compounds, NMR spectra were also recorded on 400 MHz JEOL spectrometer; and data processed Cambridge Soft’s MestReNova software. All chemical shifts are referenced with respect to TMS as internal standard and are expressed in $\delta$-scale (ppm). Mass spectra were obtained by ESI-MS technique on AP-QSTAR spectrometer. MALDI analysis were done on MDS-SCIEX 4800 MALDI TOF/TOF instrument (Applied Biosystems). High Resolution Mass Spectrometry (HRMS) was recorded on waters SYNAPT G2 MS system. Melting points of the samples were determined in open capillary tubes using Büchi Melting Point M-560 apparatus and are uncorrected. IR spectra were recorded on an Infrared Fourier Transform Spectrophotometer using chloroform. Peptide purification was carried out on High Pressure Liquid Chromatography (HPLC). HPLC system is of Dionex ICS-3000 series attached with PDA detector and SP (single pump) made. Analytical HPLC was performed using a LiChrospher 100 RP-18e 5 $\mu$M (250 mm x 10 mm) column from Merck. Preparative HPLC was carried out on a LiChrospher RP-18e 5 $\mu$M (250 mm x 10 mm). UV-vis spectrophotometric titrations were done on Perkin Elmer 950 spectrophotometer. All spectra presented for UV are drawn by Origin 8 software.
3.15.2 Procedures and Spectral Data

**tert-butyl (2-aminoethyl)carbamate (5)**

![Structural formula of tert-butyl (2-aminoethyl)carbamate (5)]

To an ice-cold solution of 1,2-diaminoethane (20 g, 0.33 mmol) in dichloromethane (500 mL), Boc₂O in dichloromethane (500 mL) was slowly added over a period of 3-4 h. The reaction mixture was stirred at rt for 24 h. After completion of the reaction, solvent was evaporated and the precipitated N',N''-di-Boc derivative was removed by filtration. The corresponding N'-mono-Boc derivative was obtained by repeated extraction of the filtrate in dichloromethane. The organic layer was separated, dried over anhydrous Na₂SO₄, filtered, and concentrated to give the crude product N'(Boc)-1,2-diaminoethane, 5 (3.45 g).

**Yield**

50%; colorless viscous oil; Rₓ = 0.18 (EtOAc: MeOH; 1:1).

**Mol. Formula**

C₇H₁₆N₂O₂

**IR (CHCl₃)**

νₓmax (cm⁻¹) = 3449, 3379, 3018, 2978, 2932, 2870, 2400, 1701, 1508, 1392, 1367, 1216, 1169.

**¹H NMR**

δₓ (ppm) = 5.36 (br, 1H, NH), 3.00 (q, J = 5.4 Hz, 2H, NHCH₂), 2.63 (dt, J = 5.9 Hz, 2H, NHCH₂), 1.28 (s, 9H, (CH₃)₃C).

**¹³C NMR**

δₓ (ppm) = 158.1 (C), 78.8 (CH₃)₃C, 43.1 (NHCH₂), 41.6 (NHCH₂), 28.2 (CH₃)₃C.

**Elemental analysis**

Calcd for C₇H₁₆N₂O₂: C, 52.48; H, 10.07.

Found: C, 52.55; H, 10.16.

**Ethyl N-(2-Boc-aminoethyl)-glycinate (6)**

![Structural formula of Ethyl N-(2-Boc-aminoethyl)-glycinate (6)]

To an ice-cold solution of N'-(Boc)-1,2-diaminoethane 5 (4.0 g, 25 mmol) in acetonitrile (80 mL), triethylamine (8.6 mL, 61.8 mmol) was slowly added. After stirring at room temperature for 20 min, the solution of ethylbromoacetate (2.8 mL, 25
mmol) in acetonitrile (100 mL) was added. The reaction was stirred at room temperature for 12 h. After completion of the reaction, it was extracted with EtOAc (3 x 50 mL). The organic layer was washed with brine (10 mL), dried over anhydrous Na₂SO₄, and the solvent was evaporated under vacuum. The crude residue was purified by silica gel column chromatography (50% pet-ether/1:1) to furnish product 6 (4.3 g).

Yield 67%; colorless oil; Rᵣ = 0.5 (EtOAc: Pet-ether; 1:1).

Mol. Formula C₁₁H₂₂N₂O₄

IR (CHCl₃) νmax (cm⁻¹) = 3338, 2977, 2935, 1694, 1515, 1455, 1391, 1366, 1247, 1160, 1027.

¹H NMR δH (ppm) = 5.23 (br, 1H, NH), 4.09 (q, J = 7.2 Hz, 2H, CH₂), 3.12 (t, J = 5.7 Hz, 2H, NHCH₂), 2.65 (t, J = 5.6 Hz, 2H, NHCH₂), 1.34 (s, 9H, (CH₃)₃C), 1.18 (t, J = 7.2 Hz, 2H, CH₃) ppm.

¹³C NMR δC (ppm) = 172.3 (C), 155.9 (C), 78.9 (CH₃)₂C, 60.6 (OCH₂), 50.2 (CH₂), 48.6 (NHCH₂), 39.9 (NHCH₂), 28.2 (CH₃)₂C, 14.0 (CH₂CH₃) ppm.

Elemental analysis Calcd for C₁₁H₂₂N₂O₄: C, 53.64; H, 9.00. Found: C, 53.73; H, 9.09.


Ethyl-2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-chloroacetyl)-glycinate (7)³¹

To a solution of ethyl-N-(2-Boc-aminoethyl)-glycinate 6 (2.6 g, 11.0 mmol) in dioxan (60 mL), 10% aqueous Na₂CO₃ (75 mL) and chloroacetyl chloride (1.3 mL, 11.5 mmol) was slowly added with stirring. After stirring for 1 h at room temperature, dioxan was evaporated under reduced pressure. The aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na₂SO₄, filtered and the solvent was evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography (50% pet-ether/1:1) to furnish product 6 (4.3 g).
pressure. The crude residue was purified by silica gel column chromatography (30% pet-ether/ EtOAc) to afford product 7 (2.7 g).

Yield 80%; colorless oil; $R_f = 0.4$ (EtOAc: pet ether; 3:7).

**Mol. Formula** C$_{13}$H$_{23}$ClN$_2$O$_5$

**IR** (CHCl$_3$) 
$\nu_{\text{max}}$ (cm$^{-1}$) = 3366, 2980, 1741, 1700, 1657, 1509, 1454, 1398, 1368, 1247, 1208, 1164, 1025.

**$^1$H NMR**
(CDCl$_3$, 200 MHz) 
$\delta$ (ppm) = 5.54 (br, 1H, NH), 4.05 (s, 2H, CH$_2$), 4.06 (q, $J = 7.3$ Hz, 2H, CH$_2$), 3.92 (s, 2H, CH$_2$), 3.40 (t, $J = 5.8$ Hz, 2H, NHCH$_2$), 3.14 (q, $J = 5.6$ Hz, 2H, NHCH$_2$), 1.30 (s, 9H, (CH$_3$)$_3$C), 1.15 (t, $J = 7.2$ Hz, 2H, CH$_3$).

**$^{13}$C NMR**
(CDCl$_3$, 50 MHz) 
$\delta$ (ppm) = 169.2 (C), 167.2 (C), 155.8 (C), 79.3 (CH$_3$)$_2$C, 61.2 (CH$_2$), 49.1 (NHCH$_2$), 48.5 (CH$_2$), 40.5 (CH$_2$), 38.2 (NHCH$_2$), 27.9 (CH$_3$)$_2$C, 13.7 (CH$_3$).

**Elemental analysis**
Calcd for C$_{13}$H$_{23}$ClN$_2$O$_5$: C, 48.37; H, 7.18. 

**MALDI -TOF**
Calcd for C$_{13}$H$_{23}$ClN$_2$O$_5$: 361.883 (M+K)$^+$
Found: 361.718.

**Ethyl-2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(2-(pyridin-2-yl)-1H-benzo [d] imidazol-1-yl) acetamido)acetate (8)**

To a suspension of NaH (330 mg, 14 mmol) in dry DMF (7 mL) at 0 °C under N$_2$ atmosphere, 2-pyridylbenzimidazole 9 (1.36 g, 7.0 mmol) in dry DMF (2 mL) was added. The reaction mixture was stirred for 15 min and compound (2.48 g, 7.7 mmol) in DMF (2 mL) added into it. The resulting reaction mixture was heated at 100 °C for 24 h and cooled to room temperature. After completion reaction mixture was cooled

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and extracted with EtOAc (3 x 50 mL). The organic layer was washed with brine (10 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (20% pet-ether/EtOAc) to afford product 8 (2.34 g).

Yield 70%; white solid; \( R_f = 0.57 \) (EtOAc: pet ether; 1:1).

Melting Point 142.9-145.8°C

Mol. Formula C₂₅H₃₁N₅O₅

IR (CHCl₃) \( v_{\text{max}} \) (cm\(^{-1}\)) = 3743, 3678, 3647, 3619, 3339, 2978, 2362, 1741, 1704, 1666, 1589, 1511, 1449, 1394, 1368, 1338, 1250, 1210, 1171, 1096, 1028.

\(^1\)H NMR (CDCl₃, 200 MHz) \( \delta_H \) (ppm) = 8.57 (m, 1H, CH), 8.49 (d, \( J = 8.2 \) Hz, 2H, CH), 7.83-7.76 (m, 2H, CH), 7.37-7.27 (m, 5H, CH), 5.79 (s, 2H, CH₂), 5.63 (s, 1H, NH), 4.10 (q, \( J = 7.3 \) Hz, 2H, CH₂), 3.98 (s, 2H, CH₂), 3.63-3.61 (m, 2H, NHCH₂), 3.38-3.34 (m, 2H, NHCH₂), 1.40 (s, 9H, (CH₃)₃C), 1.17 (t, \( J = 7.3 \) Hz, 2H, CH₃).

\(^13\)C NMR (CDCl₃, 50 MHz) \( \delta_C \) (ppm) = 169.9 (C), 167.9 (C), 156.0 (C), 148.4 (C), 137.0 (C), 136.9 (C), 124.3 (C), 123.7 (C), 122.9 (C), 119.3 (C), 109.8 (C), 80.0 (CH₃)₃C, 61.5 (CH₂), 48.9 (NHCH₂), 48.8 (CH₂), 46.8 (CH₂), 38.6 (NHCH₂), 28.3 (CH₃)₃C, 13.9 (CH₃).

Elemental analysis
Calcd for C₂₅H₃₁N₅O₅: C, 62.36; H, 6.49.
Found: C, 62.42; H, 6.54.

HRMS (ESI) Calcd for C₂₅H₃₁N₅O₅: 482.2403 (M+H)⁺
Found: 482.2404.

2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(pyridin-2-yl)-1H-benzo[d]imidazol-1-yl) acetamido)acetic acid (1)

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To a solution of ester 8 (0.2 g, 0.5 mmol) in MeOH at 0 °C, 10% aqueous LiOH (3 mL) was added. The resulting reaction mixture was stirred for 6 h at room temperature, and after that MeOH was removed under reduced pressure. The aqueous layer was washed with diethylether (2 x 10 mL), acidified with 1N KHSO₄ (8 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layer was washed with brine (10 mL), filtered and dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford product 1 (0.17 g).

**Yield**
90%; white solid; $R_f = 0.16$ (EtOAc).

**Melting Point**
149.6-154.0°C

**Mol. Formula**
C₂₃H₂₇N₅O₅

**IR (CHCl₃)**
$\nu_{\text{max}}$ (cm⁻¹) = 3837, 3742, 3677, 3647, 3616, 3565, 3020, 2360, 1834, 1703, 1650, 1515, 1458, 1424, 1395, 1366, 1215, 1173.

**¹H NMR**
(Methanol-D₄, 200 MHz)
δ_H (ppm) = 8.76-8.70 (m, 1H, CH), 8.34-8.28 (m, 1H, CH), 7.99-7.91 (m, 1H, CH), 7.78-7.75 (m, 1H, CH), 7.60-7.35 (m, 5H, CH), 5.89 (s, 2H, CH₂), 5.73 (s, 1H, NH), 4.13 (s, 2H, CH₂), 3.74-3.63 (m, 2H, NHCH₂), 3.54-3.42 (m, 2H, NHCH₂), 1.47 (s, 9H, (CH₃)₃C).

**¹³C NMR**
(Methanol-D₄, 50 MHz)
δ_C (ppm) = 172.7 (C), 170.3 (C), 158.6 (C), 151.7 (C), 150.4 (C), 142.7 (C), 138.5 (C), 138.4 (C), 125.6 (C), 125.2 (C), 125.1 (C), 124.5 (C), 120.1 (C), 111.8 (C), 80.8 (CH₃)₃C, 50.8 (CH₂), 50.4 (NHCH₂), 39.6 (CH₂), 39.3 (NHCH₂), 28.9 (CH₃)₃C.

**Elemental analysis**
Calcd for C₂₃H₂₇N₅O₅: C, 60.92; H, 6.00.
Found: C, 60.99; H, 6.08.

**HRMS (ESI)**
Calcd for C₂₃H₂₇N₅O₅: 454.2089 (M+H)+
Found: 454.2091.

Ethyl-2-(3,4-bis(((benzloxy)carbonyl)amino)-N-(2-((tert-butoxycarbonyl)amino)ethyl) benzamido) acetate (12)
To a 0 °C cooled solution of compound 11 (4.2 g, 10.0 mmol) and compound 6 (2.46 g, 10.0 mmol) in anhydrous DMF (10 mL), DCC (3.09 g, 15.0 mmol) was slowly added into it. The reaction was stirred at room temperature for 24 h. After completion reaction mixture formed dicyclohexylurea was filtered and filtrate was extracted with EtOAc (3 x 50 mL). The combined organic layer was washed with brine (10 mL), dried over anhydrous Na₂SO₄ and evaporated under vacuum. The crude residue was purified by silica gel column chromatography (30% pet-ether/EtOAc) to afford product 12 (3.24 g).

Yield 50%; orange semi-solid; \( R_f = 0.33 \) (EtOAc: pet ether; 1:1).

Melting Point 92.8-94.9 °C

Mol. Formula C₃₄H₄₆N₄O₉

IR (CHCl₃) \( \nu_{\text{max}} \) (cm⁻¹) = 3842, 3743, 3619, 3328, 2979, 2362, 1705, 1620, 1515, 1458, 1369, 1308, 1202, 1040.

\(^1\)H NMR (CDCl₃, 200 MHz) \( \delta_H \) (ppm) = 7.35-7.24 (m, 13H, \( CH \)), 5.17 (s, 4H, \( CH_2 \)), 4.21-4.14 (s, 4H, \( CH_2 \)), 3.37-3.26 (m, 4H, NHCH₂), 1.36 (s, 9H, (CH₃)₃C) 1.26 (t, \( J = 7.3 \) Hz, 2H, \( CH_3 \)).

\(^13\)C NMR (CDCl₃, 50 MHz) \( \delta_C \) (ppm) = 171.8 (C), 169.5 (C), 156.2 (C), 154.2 (C), 135.9 (C), 128.7 (C), 128.5 (C), 79.7 (CH₃)₃C, 67.5 (CH₂), 61.5 (CH₂), 50.5 (NHCH₂), 47.8 (CH₂), 38.5 (NHCH₂), 28.5 (CH₃)₃C, 14.2 (CH₂).

Elemental analysis Calcd for C₃₄H₄₆N₄O₉: C, 65.71; H, 4.79.

Found: C, 65.82; H, 4.86.

HRMS (ESI) Calcd for C₃₂H₃₆N₄O₉: 671.2692 (M+Na)⁺

Found: 671.2689.

2-(3,4-bis((benzyloxy)carbonyl)amino)-N-(2-((tert-butoxycarbonyl)amino)ethyl) benzamido) acetic acid (13)

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To a 0 °C cooled solution of ethyl ester 12 (0.2 g, 0.30 mmol) in MeOH, aqueous 10% LiOH (2 mL) was added. The resulting reaction mixture was stirred for 4 h at room temperature, and after that MeOH was removed under reduced pressure. The aqueous layer was washed with diethylether (2 x 10 mL), acidified with 1N KHSO₄ (8 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layer was washed with brine (10 mL), filtered and dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford product 13 (0.12 g).

Yield 60%; orange solid; Rₜ = 0.3 (EtOAc).

Mol. Formula C₃₂H₃₆N₄O₉

IR (CHCl₃) ν max (cm⁻¹) = 3743, 3618, 3351, 2975, 2361, 1688, 1641, 1598, 1519, 1462, 1396, 1368, 1311, 1239, 1170, 1057.

Elemental analysis Calcd for C₃₂H₃₆N₄O₉: C, 61.93; H, 5.85.

Found: C, 62.02; H, 5.92.

HRMS (ESI) Calcd for C₃₂H₃₆N₄O₉: 643.2379 (M+Na)⁺

Found: 643.2371.

Ethyl-2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(3,4-dihydroxyphenyl)acetamido) acetate (15)

To a 0 °C cooled solution of 3,4-dihydroxyphenylacetic acid 14 (0.84 g, 5.0 mmol) in anhydrous DMF (10 mL), EDC.HCl (1.24 g, 6.5 mmol) and HOBT (0.88 g, 6.5 mmol) was added. The solution of amine (1.23 g, 5.0 mmol) in anhydrous DMF (5 mL) was added into the reaction mixture. The reaction was stirred at room temperature for 24 h. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3
x 50 mL). The organic layer was washed with brine (10 mL), dried over anhydrous Na₂SO₄ and the solvent was evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography (30% pet-ether/EtOAc) to afford product 15 (2.34 g).

Yield 50%; white solid; \( R_f = 0.41 \) (EtOAc: pet ether; 1:1).

Melting Point 127.8-133.7°C

Mol. Formula C₁₉H₂₈N₂O₇

IR (CHCl₃) \( \nu_{\text{max}} (\text{cm}^{-1}) = 3749, 3610, 3393, 3020, 1734, 1699, 1602, 1555, 1523, 1473, 1421, 1215, 1045, 929. \)

\(^1\text{H NMR}\) (Methanol-D₄, 200 MHz) \( \delta \text{H (ppm)} = 6.71-6.50 \) (m, 3H, CH), 4.18 (q, J = 7.3 Hz, 2H, CH₂), 4.07 (s, 2H, CH₂), 3.65 (m, 2H, NHCH₂), 3.52-3.46 (m, 2H, NHC), 80.6 (CH), 19.3 (C), 146.7 (C), 145.5 (C), 127.4 (C), 121.4 (C), 117.2 (C), 116.5 (C), 80.6 (CH₃)₂C, 62.5 (CH₂), 41.4 (NHCH₂), 40.4 (CH₂), 39.6 (NHCH₂), 28.9 (CH₃)₂C, 14.5 (CH₃).

\(^1^3\text{C NMR}\) (Methanol-D₄, 50 MHz) \( \delta \text{C (ppm)} = 175.2 \) (C), 171.4 (C), 158.5 (C), 146.7 (C), 145.5 (C), 127.4 (C), 121.4 (C), 117.2 (C), 116.5 (C), 80.6 (CH₃)₂C, 62.5 (CH₂), 41.4 (NHCH₂), 40.4 (CH₂), 39.6 (NHCH₂), 28.9 (CH₃)₂C, 14.5 (CH₃).


2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(3,4-dihydroxyphenyl)acetamido)acetic acid (3)

To a 0 °C cooled solution of ethyl ester 15 (0.2 g, 0.50 mmol) in MeOH, aqueous 10% LiOH (2 mL) was added. The resulting reaction mixture was stirred for 4 h at
room temperature, and after that MeOH was removed under reduced pressure. The aqueous layer was washed with diethylether (2 x 10 mL), acidified with 1N KHSO₄ (8 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layer was washed with brine (10 mL), filtered and dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford product 3 (0.093 g).

**Yield**
50%; white solid; Rₜ = 0.16 (EtOAc).

**Melting Point**
103.3-108.4°C

**Mol. Formula**
C₁₇H₂₄N₂O₇

**IR (CHCl₃)**
νₘₐₓ (cm⁻¹) = 3422, 2925, 2855, 1605, 1495, 1460, 1377, 1157, 1082, 1030, 759, 728.

**¹H NMR**
δₓ (ppm) = 6.52-6.50 (m, 3H, CH), 3.87 (s, 2H, CH₂), 3.46 (s, 2H, CH₂), 3.02-2.99 (m, 2H, NHCH₂), 2.95-2.92 (m, 2H, NHCH₂), 1.25 (s, 9H, (CH₃)₃C).

**¹³C NMR**
δₓ (ppm) = 175.1 (C), 173.5 (C), 158.5 (C), 146.6 (C), 145.4 (C), 133.1 (CH), 130.1 (CH), 127.2 (CH), 121.5 (CH), 117.2 (CH), 116.5 (CH), 80.6 (CH₃)₃C, 54.9 (CH₂), 50.1 (NHCH₂), 40.3 (CH₂), 39.6 (NHCH₂), 28.8 (CH₃)₃C.

**Elemental analysis**
Calcd for C₁₇H₂₄N₂O₇: C, 55.43; H, 6.57. Found: C, 55.52; H, 6.62.

**Benzyl-N-(2-Boc-aminoethyl)-glycinate (16)**

To an ice-cold solution of N⁹-(Boc)-1,2-diaminoethane 5 (9.47 g, 59 mmol) in acetonitrile (80 mL), triethylamine (8.6 mL, 61.8 mmol) was slowly added. After stirring at room temperature for 20 min, the solution of benzylbromoaacetate (9.29 mL, 59 mmol) in acetonitrile (100 mL) was added. The reaction was stirred at room temperature for 12 h. After completion of the reaction, it was extracted with EtOAc (3 x 50 mL). The organic layer was washed with brine (10 mL), dried over anhydrous Na₂SO₄, and the solvent was evaporated under vacuum. The crude residue was
purified by silica gel column chromatography (50% pet-ether/ EtOAc) provided product 16 (12.7 g).

**Yield** 70%; colorless oil; \( R_f = 0.66 \) (EtOAc: pet-ether; 1:1).

**Mol. Formula** \( C_{16}H_{24}N_2O_4 \)

**IR (CHCl₃)** \( \nu_{\text{max}} (\text{cm}^{-1}) = 3335, 2974, 2934, 1738, 1697, 1509, 1455, 1391, 1365, 1248, 1164. \)

**\(^1\)H NMR** \( \delta_H (\text{ppm}) = 7.28-7.25 \) (m, 5H, CH), 5.28 (br, 1H, NH), 5.09 (s, 2H, CH₂), 3.38 (s, 2H, CH₂), 3.14-3.11 (m, 2H, NHCH₂), 2.66 (t, \( J = 5.9 \) Hz, 2H, NHCH₂), 1.37 (s, 9H, \((\text{CH}_3)_3C\)).

**\(^{13}\)C NMR** \( \delta_C (\text{ppm}) = 172.1 \) (C), 155.9 (C), 135.3 (CH), 128.4 (CH), 128.1 (CH), 78.8 (\((\text{CH}_3)_3C\)), 66.3 (CH₂), 50.2 (CH₂), 48.5 (NHCH₂), 39.9 (NHCH₂), 28.2 (\((\text{CH}_3)_3C\)).

**Elemental analysis** Calcd for \( C_{16}H_{24}N_2O_4 \): C, 62.32; H, 7.84. 

Found: C, 62.39; H, 7.91.

**HRMS (ESI)** Calcd for \( C_{16}H_{24}N_2O_4 \): 309.1815 (M+H)+

Found: 309.1816.

**Benzyl-2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(3,4-dihydroxyphenyl)acetamido)acetate (17)**

To a 0 °C cooled solution of 3,4-dihydroxyphenylacetic acid 14 (0.51 g, 3.0 mmol) in anhydrous DMF (10 mL), DCC (0.93 g, 4.5 mmol) was added. The solution of compound 16 (0.93 g, 3.0 mmol) in anhydrous DMF (5 mL) was added into the reaction mixture. The reaction was stirred at room temperature for 24 h. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 x 50 mL). The organic layer was washed with brine (10 mL), dried over anhydrous Na₂SO₄ and the
solvent was evaporated under reduced pressure. The crude residue was purified by silica gel chromatography (30% pet-ether/ EtOAc) to afford product 17 (0.93 g).

**Yield**  
70%; white solid; $R_f = 0.38$ (EtOAc: pet-ether; 1:1).

**Melting Point**  
102-104°C

**Mol. Formula**  
C$_{24}$H$_{30}$N$_{2}$O$_{7}$

**IR (CHCl$_3$)**  
$\nu_{\text{max}}$ (cm$^{-1}$) = 3743, 3647, 3618, 3335, 2977, 2938, 2362, 1741, 1691, 1631, 1518, 1450, 1363, 1280, 1251, 1170, 1114, 1041.

**$^1$H NMR**  
δ$_H$ (ppm) = 7.19-7.16 (m, 5H, CH), 6.54-6.45 (m, 3H, CH$_2$), 5.31 (br, 1H, NH), 4.99 (s, 2H, CH$_2$), 4.92 (s, 1H, CH$_2$), 3.95 (s, 2H, CH$_2$), 3.47 (s, 2H, CH$_2$), 3.14-3.13 (m, 2H, NHCH$_2$), 3.00 (t, $J = 6.1$ Hz, 2H, NHCH$_2$), 1.26 (s, 9H, (CH$_3$)$_3$C).

**$^{13}$C NMR**  
δ$_C$ (ppm) = 175.2 (C), 171.2 (C), 158.5 (C), 146.6 (C), 145.5 (C), 137.3 (CH), 129.7 (CH), 129.5 (CH), 127.4 (C), 121.4 (C), 117.2 (C), 116.5 (C), 80.6 (CH$_3$)$_3$C, 68.1 (CH$_2$), 50.2 (NHCH$_2$), 41.3 (CH$_2$), 40.4 (CH$_2$), 39.6 (NHCH$_2$), 28.9 (CH$_3$)$_3$C.

**Elemental analysis**  
Calcd for C$_{24}$H$_{30}$N$_{2}$O$_{7}$: C, 62.87; H, 6.59.  
Found: C, 62.92; H, 6.64.

**HRMS (ESI)**  
Calcd for C$_{24}$H$_{30}$N$_{2}$O$_{7}$: 481.1950 (M+Na)$^+$  

**Benzyl-2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(2-(2-methoxy-2-oxoethyl)benzo-[d] [1,3]dioxol-5-yl) acetamido)acetate (18)**

To a solution of compound 17 (0.2 g, 0.43 mmol) in acetonitrile (5 mL), DMAP (79.9 mg, 0.655 mmol) was added under N$_2$ atmosphere. The reaction mixture was stirred
for 10 min at room temperature, followed by the slow addition of methyl propiolate (40 µL, 0.47 mmol) into it. After stirring for 30 min, solvent was evaporated and the crude residue was purified by silica gel column chromatography (30% pet-ether/EtOAc) to afford product 18 (0.19 g).

Yield 80%; yellow syrup; \( R_f = 0.71 \) (EtOAc: pet ether; 1:1).

**Mol. Formula**

C\(_{28}\)H\(_{34}\)N\(_2\)O\(_9\)

**IR (CHCl\(_3\))**

\( \nu_{\text{max}} \) (cm\(^{-1}\)) = 3893, 3860, 3743, 3677, 3647, 3618, 3395, 2944, 2830, 2361, 1740, 1695, 1640, 1497, 1446, 1395, 1361, 1245, 1169, 1101, 1026.

**\(^1\)H NMR**

\( \delta_H \) (ppm) = 7.31-7.27 (m, 5H, CH), 6.70-6.61 (m, 3H, CH), 6.43 (t, \( J = 5.5 \) Hz, 1H, CH), 5.51 (br, 1H, NH), 5.10 (s, 2H, CH\(_2\)), 4.00 (s, 1H, CH\(_2\)), 3.67 (s, 3H, OCH\(_3\)), 3.61 (s, 2H, CH\(_2\)), 3.42 (t, \( J = 5.9 \) Hz, 2H, NHCH\(_2\)), 2.89 (d, \( J = 5.5 \) Hz, 2H, CH\(_2\)), 1.39 (s, 9H, (CH\(_3\))\(_3\))C.

**\(^{13}\)C NMR**

\( \delta_C \) (ppm) = 171.7 (C), 169.6 (C), 168.3 (C), 155.7 (C), 146.9 (C), 145.6 (C), 134.9 (C), 128.3 (CH), 128.1 (CH), 127.9 (CH), 121.8 (CH), 109.2 (CH), 108.0 (CH), 107.6 (CH), 79.2 (CH\(_3\))\(_3\)C, 66.7 (CH\(_2\)), 51.7 (OCH\(_3\)), 48.9 (NHCH\(_2\)), 48.5 (CH\(_2\)), 39.6 (NHCH\(_2\)), 39.1 (CH\(_2\)), 38.4 (CH\(_2\)), 28.0 (CH\(_3\))\(_3\)C.

**Elemental analysis**

Caled for C\(_{28}\)H\(_{34}\)N\(_2\)O\(_9\): C, 61.98; H, 6.32.

Found: C, 62.01; H, 6.45.

**HRMS (ESI)**

Caled for C\(_{28}\)H\(_{34}\)N\(_2\)O\(_9\): 565.2161 (M+Na)

Found: 565.2167.

2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(2-methoxy-2-oxoethyl)-benzo[d][1,3]dioxol-5-yl)acetamido)acetic acid (19)

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2013 PhD thesis: T. Kaur, University of Pune
To a solution compound 18 (2.2 g, 4.05 mmol) in dry MeOH (10 mL), 10% Pd/C (0.2 g) was added and stirred under H₂ atmosphere. After stirring for 12 h at room temperature, the reaction mixture was filtered through celite. The solvent was evaporated and the crude product was purified by silica gel chromatography (100% EtOAc) to furnish the pure product 19 (1.65 g).

Yield 90%; white solid; Rf = 0.6 (EtOAc: pet-ether; 1:1).

Melting Point 110-123°C

Mol. Formula C₂₁H₂₉N₂O₉

IR (CHCl₃) νₘₐₓ (cm⁻¹) = 3743, 3618, 3394, 2977, 2362, 1736, 1705, 1640, 1497, 1444, 1400, 1361, 1244, 1168, 1101, 1039.

¹H NMR δₜ (ppm) = 6.71-6.65 (m, 3H, CH), 6.47 (t, J = 5.0 Hz, 1H, CH), 5.46 (br, 1H, NH), 4.00 (s, 1H, CH₂), 3.73 (s, 3H, OCH₃), 3.65 (s, 2H, CH₂), 3.50-3.46 (m, 2H, NHCH₂), 3.24-3.20 (m, 2H, NHCH₂), 2.94 (d, J = 5.0 Hz, 2H, CH₂), 1.42 (s, 9H, (CH₃)₃C).

¹³C NMR δₜ (ppm) = 173.0 (C), 172.3 (C), 168.7 (C), 156.2 (C), 147.3 (C), 146.0 (C), 128.1 (CH), 122.2 (CH), 109.5 (CH), 108.4 (CH), 107.9 (CH), 79.9 (CH₃)C, 52.1 (OCH₃), 49.6 (NHCH₂), 49.0 (CH₂), 40.2 (CH₂), 39.9 (NHCH₂), 39.4 (CH₂), 38.7 (CH₂), 28.3 (CH₃)₃C.

Elemental analysis Calcd for C₂₁H₂₉N₂O₉: C, 55.75; H, 6.24.

Found: C, 55.83; H, 6.32.

HRMS (ESI) Calcd for C₂₁H₂₉N₂O₉: 475.1692 (M+Na)⁺
Chapter 3

Ethyl-2,2-dimethyl-4,10-dioxo-8,14-bis(2-(2-(pyridin-2-yl)-1H-benzo[d]imidazol-1-yl) acetyl) -3-oxa-5,8,11,14-tetraazahexadecan-16-oate (21)

To 0 °C cooled solution of compound 8 (4.8 g, 10 mmol) in dioxan (5 mL), HCl in dioxan (4M, 10 mL) was added and stirred under N₂ atmosphere. After stirring for 2 h at room temperature, the solvent was evaporated under reduced pressure. The formed hydrochloride salt 20 was washed with toluene (2 x 5 mL). It was dried.

To a 0 °C cooled solution of 2-pyridylbenzimidazole (PBI) aeg acid 1 (0.875 g, 1.93 mmol) and hydrochloride salt 20 (1.0 g, 1.93 mmol) in anhydrous DMF (10 mL), HBTU (0.88 g, 2.32 mmol) was added. DIPEA (0.671 µL, 3.864 mmol) was added into it. The reaction was stirred at room temperature for 24 h. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 x 50 mL). The organic layer was washed with brine (10 mL), dried over anhydrous Na₂SO₄ and the solvent was evaporated under reduced pressure. The crude residue was purified by silica gel chromatography (5% DCM/ Methanol) to afford product 21 (1.29 g).

Yield 87%; white solid; Rₜ = 0.6 (Methanol: DCM; 1:9).

Melting Point 143-146°C

Mol. Formula C₄₃H₄₉N₁₀O₇

IR (CHCl₃) νₘₐₓ (cm⁻¹) = 3746, 3679, 3642, 3623, 3342, 2971, 2359, 1735, 1645, 1572, 1501, 1442, 1373, 1365, 1323, 1242, 1201, 1161, 1067, 1018.

¹H NMR (CDCl₃, 200 MHz) δₜ (ppm) = 8.60-8.40 (m, 5H, CH), 8.28-8.26 (m 1H, CH), 7.76-7.70 (m, 5H, CH), 6.14 (s, 1H), 5.70-5.67 (m, 2H, CH), 5.38 (s, 1H, NH), 5.23 (s, 1H, NH), 4.18 (q, J = 7.3 Hz, 2H, CH₂), 3.86 (s, 2H, CH₂), 3.74 (s, 2H, CH₂), 3.35 (s, 2H, CH₂),

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3.15.3 Solid Phase Peptide Synthesis (SPPS)

3.15.3a Functionalization of the MBHA [(4-methyl benzhydryl) amine] resin

The resin (4-methylbenzhydrylamine) MBHA.HCl, from Novabiochem, [catalog number 855000, 100-200 mesh]) (100 mg) was taken in sintered vessel (25 mL) and rinsed with 5 mL of dry DCM and filtered. The process was repeated 3 to 4 times and the resulting resin was kept for 2 h in DCM (10 mL) for swelling. The solvent was removed and rinsed 3 times with dry DMF and kept 2 h in dry DMF (10 mL) for swelling before the first coupling. The resin neutralisation was done with 20% DIPEA/DCM.

The resin was swollen overnight in DCM before couplings cycle. The resin was neutralized with 20% DIPEA/DCM and after that subsequent steps were repeated.

- Wash with DCM (3 x 5 mL), MeOH (3 x 5 mL) and DMF with (3 x 5 mL).
- Coupling reaction with monomer, DIPEA, HOBt and HBTU (3 eq.) in DMF (1 mL).
- Test for completion of coupling reaction (chloranil test), colorless beads.

\[ \delta_C (\text{ppm}) = 169.7 (C), 169.4 (C), 169.3 (C), 167.7 (C), 156.0 (C), 150.3 (CH), 150.4 (CH), 150.3 (CH), 150.1 (CH), 149.5 (C), 149.3 (C), 149.4 (C), 148.4 (C), 142.2 (C), 137.4 (CH), 137.1 (CH), 136.8 (CH), 124.4 (CH), 124.0 (CH), 123.7 (CH), 119.9 (CH), 108.6 (CH), 110.1 (CH), 79.7 (CH\_3)\_C, 61.7 (CH\_2), 49.2 (NH\_CH\_2), 48.5 (CH\_2), 48.0 (CH\_2), 46.7 (CH\_2), 46.1 (NH\_CH\_2), 28.4 (CH\_3)\_C, 14.1 (CH\_3). \]

**Elemental analysis**

Calcd for C\textsubscript{43}H\textsubscript{48}N\textsubscript{10}O\textsubscript{7}: C, 63.22; H, 5.92.

Found: C, 63.28; H, 5.98.

**HRMS (ESI)**

Calcd for C\textsubscript{43}H\textsubscript{48}N\textsubscript{10}O\textsubscript{7}: 817.3786 (M+H)^+.

Found: 817.3798.
• Wash with DMF (3 x 5 mL), and DCM with (3 x 5 mL).
• Deprotection of t-Boc group with 50% TFA/DCM (3 x 5 mL).
• Wash with DCM (3 x 5 mL), DMF (3 x 5 mL) and DCM with (3 x 5 mL).
• Test for complete deprotection (chloranil test), blue beads.
• Neutralization with 5% DIPEA/DCM (3 x 5 mL).
• Wash with DCM (3 x 5 mL) and DMF with (3 x 5 mL).
• Repeat of the coupling reaction in NMP for better yield.
• This cycle was repeated for every monomer.

3.15.3b Coupling tests (Kaiser’s/Chloranil test)
These cycles were repeated for every amino acid. The coupling and deprotection reactions were monitored by a combination of Kaiser’s (ninhydrin) test and chloronil test. In case of negative test after coupling the re-coupling was performed with same aminoacid followed by capping of the unreacted amino groups using Ac2O, pyridine & DCM (1:1:1), in case coupling does not go to completion even after re-coupling.

3.15.3c Kaiser’s test
Kaiser’s was used to monitor the t-Boc/Fmoc deprotection and coupling reactions of glycine (or basically primary amines) in the solid phase peptide synthesis using three solutions.

Solution A: Ninhydrin (5.0 g) dissolved in ethanol (100 ml)
Solution B: Phenol (80.0 mg) dissolved in ethanol (20 ml)
Solution C: KCN (2 ml, 0.001 M aqueous solution) added to 98 ml pyridine

• Few beads of resin to be tested were taken in a test tube and washed 3 times with ethanol.
• 3-4 drops of each of the three solutions described above were added to it
• The test tube was heated to 120 °C for 4-6 min

The successful deprotection was indicated by blue resin beads while colourless beads indicate the completion of coupling step.

3.15.3c Chloranil test
A few beads of resin were taken in a glass test tube (5 mL capacity) and were washed with methanol followed by toluene. To this three drops of saturated chloranil solution in toluene and 200 µl of acetone were added. The mixture was shaken for 2-3 minutes. Blue or green color is observed on the resin beads if free amines are present.

### 3.15.4 Synthesis of polyamide oligomers incorporating 2-pyridylbenzimidazole (PBI) \textit{aeg} monomer, \textit{o}-phenylenediamine (PDA) \textit{aeg} monomer and 3,4-dihydroxyphenyl (CAT) \textit{aeg} monomer

The modified polyamide monomers were built into polyamide oligomers using the standard procedure on MBHA resin (initial loading value = 0.67 meq/g) using HBTU/HOBt/DIPEA in DMF/NMP as coupling reagents. The polyamide oligomers were cleaved from the resin using a TFA/TFMSA mixture and then precipitated with diethyl ether and air-dried. The oligomers were purified by reversephase HPLC (C18 column) and were characterized by MALDI-TOF mass spectrometry. The overall yields of the raw products were 55-88%.

### 3.15.5 Cleavage of the PNA oligomers from the resin

The dry peptide-resin (10 mg) was taken in a sample vial and thioanisole (20 µL) and 1,2-ethanedithiol (8 µL) were added. It is kept at 0 °C for 10 min in an ice-bath, which further was treated with trifluoroacetic acid (TFA, 120 µL) and again kept it in an ice-bath for 10 min and then trifluoromethane sulphonic acid (TFMSA, 16 µL) was added into it. The resulting mixture was kept for 2 h by gentle shaking. The mixture was filtered through a sintered funnel and the resin was washed with TFA (3 x 1 mL). The filtrate was collected in round-bottom flask and evaporated under reduced pressure. Diethyl ether was chilled and added into it for precipitation. The off-white precipitate obtained was centrifuged. The re-precipitation procedure was done to obtain crude peptide. The crude peptide was dissolved in water and further purified on HPLC.

### 3.15.6 Purification and Characterization

**3.15.6a Gel Filtration Chromatography (GFC)**

The crude peptides obtained after ether precipitation were dissolved in deionized water (~0.5 ml) and loaded onto a gel filtration G10 Sephadex column with a void volume of 1 mL. The presence of the peptide was detected by measuring the
absorbance at 254/302 nm. The fractions containing the peptides were pooled together and freeze-dried. The purity of the cleaved crude peptide was determined by analytical RP-HPLC on a C18 column.

3.15.6b High Performance Liquid Chromatography (HPLC)

All the cleaved polyamide oligomers were initially subjected to gel filtration over sephadex NAP column to remove low molecular weight impurities. The purity of the obtained polyamide oligomers were checked on analytical RP-HPLC (C18 column, acetonitrile:water system) and was found to be more than 80-85% purity. These were subsequently purified by RP-HPLC on a semi-preparative C18 column to give polyamide oligomers in 95-99% purity as ascertained by analytical RP-HPLC. The representative HPLC profiles for oligomers are shown in Figure 20. An isocratic elution method with 10% CH$_3$CN in 0.1% TFA/H$_2$O was used with flow rate 1.5 mL/min (linear gradient from A to B in 20 min) and the eluent was monitored at 254 nm.

The purity of the polyamide oligomers was further assessed by RP-C18 analytical HPLC column (25×0.2 cm, 5 µm) with gradient elution: A to 100% B in 20 min; A= 0.1% TFA in CH$_3$CN:H$_2$O (5:95); B= 0.1% TFA in CH$_3$CN:H$_2$O (1:1) with flow rate 1 mL/min. The purities of the hence purified oligomers were found to be > 90%.

3.15.7 MALDI-TOF Characterization

MALDI-TOF mass spectra were obtained on either Voyager-Elite instrument (PerSeptive Biosystems Inc., Farmingham, MA) equipped with delayed extraction or on Voyager-De-STR (Applied Biosystems) instrument. Sinapinic acid and α-cyano-4-hydroxycinnamic acid (CHCA) both were used as matrix for peptides of which CHCA was found to give satisfactory results. A saturated matrix solution was prepared with typical dilution solvent (50:50:0.1 Water:MeCN:TFA) and spotted on the metal plate along with the oligomers. The metal plate was loaded to the instrument and the analyte ions are then accelerated by an applied high voltage (15-25 kV) in reflector mode, separated in a field-free flight tube and detected as an electrical signal at the end of the flight tube. HPLC purified peptides were characterized through this
method and were observed to give good signal to noise ratio, mostly producing higher molecular ion signals.

3.15.8 Determination of $pK_a$

The pH of polyamide oligomers 22-28 (50 mM, 1 mL) in deionized water was adjusted to 2.0 using conc. HCl. This solution was titrated with 2.5 µL aliquots of 0.5M aq. NaOH. After each addition of NaOH solution, pH was recorded after the reading reached a stable value (1 min). The $pK_a$ values were derived from the first derivative of the plot of pH vs volume of NaOH.

3.15.9 X-ray crystal structure determination

X-ray diffraction data for all the crystallized compounds were collected at $T = 296$ K, on SMART APEX CCD Single Crystal X-ray diffractometer using Mo-Kα radiation ($\lambda = 0.7107$ Å) to a maximum θ range of 25.00°. Crystal to detector distance was 6.05 cm, 512 x 512 pixels / frame and other conditions used are oscillation / frame (-0.3°), maximum detector swing angle (–30.0°), beam center (260.2, 252.5) and in plane spot width (1.24). SAINT integration and SADABS correction were also applied. The structures were solved by direct methods using SHELXTL. All the data were corrected for Lorentzian, polarisation and absorption effects. SHELX-97 (ShelxTL) was used for structure solution and full matrix least squares refinement on F2. Hydrogen atoms were included in the refinement as per the riding model. The refinements were carried out using SHELXL-97.
3,4-dihydroxyphenyl (CAT) aeg monomer 15

![Chemical Structure](image)

**Table 24.** Crystal data and structure refinement for compound 15.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C₁₉H₂₄N₂O₇</td>
</tr>
<tr>
<td>Formula weight</td>
<td>392.40</td>
</tr>
<tr>
<td>Temperature</td>
<td>296(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P-1</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>a = 7.621(6) Å, b = 9.069(8) Å, c = 17.016(14) Å</td>
</tr>
<tr>
<td></td>
<td>α = 89.819(15)°, β = 79.348(15)°, γ = 80.997(15)°</td>
</tr>
<tr>
<td>Volume</td>
<td>1141.2(16) Å³</td>
</tr>
<tr>
<td>Z</td>
<td>2</td>
</tr>
<tr>
<td>Density (Calcd)</td>
<td>1.142 mg/m³</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>0.088 mm⁻¹</td>
</tr>
<tr>
<td>F(000)</td>
<td>416</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.335 x 0.232 x 0.105 mm³</td>
</tr>
<tr>
<td>Theta range for data collection</td>
<td>1.22 to 28.28°</td>
</tr>
<tr>
<td>Index ranges</td>
<td>-10&lt;=h&lt;=9, -12&lt;=k&lt;=12, -22&lt;=l&lt;=22</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>19181</td>
</tr>
</tbody>
</table>
2-Pyridylbenzimidazole (PBI) \textit{aeg} monomer 8

\begin{table}
\centering
\caption{Crystal data and structure refinement for compound 8.}
\begin{tabular}{ll}
\hline
Empirical formula & $C_{50}H_{62}N_{10}O_{6}$ \\
Formula weight & 899.10 \\
Temperature & 296(2) K \\
Wavelength & 0.71073 Å \\
Crystal system & Monoclinic-C \\
Space group & C2/c \\
Unit cell dimensions & $a = 14.953(5)$ Å \hspace{1em} $\alpha = 90^\circ$. \\
& $b = 15.681(5)$ Å \hspace{1em} $\beta = 97.472(6)^\circ$. \\
& $c = 22.700(8)$ Å \hspace{1em} $\gamma = 90^\circ$. \\
Volume & 5277(3) Å\textsuperscript{3} \\
\hline
\end{tabular}
\end{table}
Z  4
Density (Calcd)  1.132 mg/m³
Absorption coefficient  0.076 mm⁻¹
F(000)  1920
Crystal size  0.435 x 0.332 x 0.135 mm³
Theta range for data collection  1.81 to 28.42°.
Index ranges  -19<=h<=19, -20<=k<=20, -30<=l<=30
Reflections collected  25008
Independent reflections  6616 [R(int) = 0.0337]
Completeness to theta = 28.28°  99.6 %
Absorption correction  Semi-empirical from equivalents
Refinement method  Full-matrix least-squares on F²
Data / restraints / parameters  6616 / 0 / 321
Goodness-of-fit on F²  1.035
Final R indices [I>2sigma(I)]  R1 = 0.0568, wR2 = 0.1406
R indices (all data)  R1 = 0.1238, wR2 = 0.1706
Extinction coefficient  0.0008(2)
Largest diff. peak and hole  0.409 and -0.140 e.Å⁻³

2-Pyridylbenzimidazole (PBI) aeg monomer and Pd (8-Pd complex)
Table 26. Crystal data and structure refinement for compound 8-Pd.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C$<em>{54}$H$</em>{68}$N$_5$O$_9$Pd</td>
</tr>
<tr>
<td>Formula weight</td>
<td>844.26</td>
</tr>
<tr>
<td>Temperature</td>
<td>296(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P21/c</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>3224(2) Å$^3$</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
</tr>
<tr>
<td>Density (Calcd)</td>
<td>1.739 mg/m$^3$</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>1.171 mm$^{-1}$</td>
</tr>
<tr>
<td>F(000)</td>
<td>1632</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.207x 0.77x 0.26 mm$^3$</td>
</tr>
<tr>
<td>Theta range for data collection</td>
<td>1.81 to 28.32°</td>
</tr>
<tr>
<td>Index ranges</td>
<td>-24&lt;=h&lt;=25, -18&lt;=k&lt;=18, -16&lt;=l&lt;=15</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>52475</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>8004 [R(int) = 0.0996]</td>
</tr>
<tr>
<td>Completeness to theta = 28.28°</td>
<td>99.5%</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>Semi-empirical from equivalents</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on F$^2$</td>
</tr>
<tr>
<td>Data / restraints / parameters</td>
<td>8004 / 0 / 404</td>
</tr>
<tr>
<td>Goodness-of-fit on F$^2$</td>
<td>4.369</td>
</tr>
<tr>
<td>Final R indices [I&gt;2sigma(I)]</td>
<td>R1 = 0.1326, wR2 = 0.2904</td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>R1 = 0.1799, wR2 = 0.2943</td>
</tr>
<tr>
<td>Extinction coefficient</td>
<td>0.0054(7)</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>8.795 and -1.064 e.Å$^{-3}$</td>
</tr>
</tbody>
</table>
3.15.10 UV-Vis spectrophotometric titrations

UV-Vis titrations were carried out on Perkin Elmer 950 instrument. Titrations were performed using either methanolic solutions of known concentrations of monomer or water solutions of polyamide oligomers and Cu(NO$_3$)$_2$/Ni(NO$_3$)$_2$. The UV spectra were recorded between 250-500 nm as a function of metal concentration. The cuvette was filled with monomer/polyamide oligomers (5-20 µM) in methanol/water, respectively. The metal salts (2.5-10 mM, 40 µl) were added into it with the help of pipette. The ΔAbs were corrected by doing the blank correction using the double beam spectrophotometer using only the metal salts. Data obtained were plotted in the Origin 8 software.

**Table 27.** Calculation of binding constants using Benesi-Hildebrand equation.

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Metal Salts</th>
<th>Models</th>
<th>Binding constants [M]$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(PBI) 8</td>
<td>Cu(NO$_3$)$_2$</td>
<td>1:1</td>
<td>6.71 x 10$^3$</td>
</tr>
<tr>
<td></td>
<td>Ni(NO$_3$)$_2$</td>
<td>1:1</td>
<td>1.82 x 10$^3$</td>
</tr>
<tr>
<td>(PBI)$_2$ 21</td>
<td>Cu(NO$_3$)$_2$</td>
<td>1:1</td>
<td>2.24 x 10$^4$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:2</td>
<td>3.82 x 10$^4$</td>
</tr>
<tr>
<td></td>
<td>Ni(NO$_3$)$_2$</td>
<td>1:1</td>
<td>3.18 x 10$^4$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:2</td>
<td>3.84 x 10$^4$</td>
</tr>
<tr>
<td>(PBI)$_6$ 22</td>
<td>Cu(NO$_3$)$_2$</td>
<td>1:1</td>
<td>2.9 x 10$^3$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:2</td>
<td>5.19 x 10$^3$</td>
</tr>
<tr>
<td></td>
<td>Ni(NO$_3$)$_2$</td>
<td>1:1</td>
<td>4.64 x 10$^2$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:2</td>
<td>2.61 x 10$^3$</td>
</tr>
<tr>
<td>(PBI)$_3$- (PDA)$_3$ 25</td>
<td>Cu(NO$_3$)$_2$</td>
<td>1:1</td>
<td>7.42 x 10$^4$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:2</td>
<td>1.34 x 10$^4$</td>
</tr>
<tr>
<td></td>
<td>Ni(NO$_3$)$_2$</td>
<td>1:1</td>
<td>1.36 x 10$^4$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:2</td>
<td>3.34 x 10$^3$</td>
</tr>
<tr>
<td>(PBI-PDA)$_2$ 26</td>
<td>Cu(NO$_3$)$_2$</td>
<td>1:1</td>
<td>6.38 x 10$^4$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:2</td>
<td>1.58 x 10$^3$</td>
</tr>
<tr>
<td></td>
<td>Ni(NO$_3$)$_2$</td>
<td>1:1</td>
<td>7.39 x 10$^4$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:2</td>
<td>2.1 x 10$^3$</td>
</tr>
</tbody>
</table>

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3.15.11 Isothermal Titration Calorimetry (ITC)

Isothermal titration calorimetric studies were done on MicroCal iTC200 instrument. Experiments were carried out at 20 °C. The sample cell was filled with monomer/polyamide oligomers (20 µM) in methanol/water, respectively. The metal salts (0.30 mM, 40 µl) were loaded into the syringe. The injection volumes were 1 µl each, injection time, a 120 s delay between each injection and stirring speed 1000 rpm. The integrated peaks of the heat p have been plotted as a function of molar ratio. With MicroCal origin, binding isotherms have been fitted to a one-site binding or sequential site binding model, giving values of the enthalpy of binding (ΔH_ITC), entropy (ΔS_ITC) and the binding constant (K_ITC). The blank corrections were carried out by doing the titrations of the metal salts with the blank water.
3.16 References


48. Gel Filtration; Principles and Methods, Amersham Biosciences


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### 3.16 Appendix F: Characterization data of synthesized compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Description</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 5</td>
<td>$^1$H NMR, $^{13}$C NMR, DEPT-NMR</td>
<td>359</td>
</tr>
<tr>
<td>Compound 6</td>
<td>$^1$H NMR, $^{13}$C NMR, DEPT-NMR, FT-IR, MALDI-MS</td>
<td>360-361</td>
</tr>
<tr>
<td>Compound 7</td>
<td>$^1$H NMR, $^{13}$C NMR, DEPT-NMR, FT-IR, MALDI-MS</td>
<td>362-363</td>
</tr>
<tr>
<td>Compound 8</td>
<td>$^1$H NMR, $^{13}$C NMR, DEPT-NMR, FT-IR, HR-MS</td>
<td>364-365</td>
</tr>
<tr>
<td>Compound 1</td>
<td>$^1$H NMR, $^{13}$C NMR, DEPT-NMR, FT-IR, HR-MS</td>
<td>366-367</td>
</tr>
<tr>
<td>Compound 12</td>
<td>$^1$H NMR, $^{13}$C NMR, DEPT-NMR, FT-IR, HR-MS</td>
<td>368-369</td>
</tr>
<tr>
<td>Compound 13</td>
<td>HR-MS</td>
<td>370</td>
</tr>
<tr>
<td>Compound 15</td>
<td>$^1$H NMR, $^{13}$C NMR, DEPT-NMR</td>
<td>371</td>
</tr>
<tr>
<td>Compound 3</td>
<td>$^1$H NMR, $^{13}$C NMR, DEPT-NMR</td>
<td>372</td>
</tr>
<tr>
<td>Compound 16</td>
<td>$^1$H NMR, $^{13}$C NMR, DEPT-NMR, FT-IR, HR-MS</td>
<td>373-374</td>
</tr>
<tr>
<td>Compound 17</td>
<td>$^1$H NMR, $^{13}$C NMR, DEPT-NMR, FT-IR, HR-MS</td>
<td>375-376</td>
</tr>
<tr>
<td>Compound 18</td>
<td>$^1$H NMR, $^{13}$C NMR, DEPT-NMR, FT-IR, HR-MS</td>
<td>377-378</td>
</tr>
<tr>
<td>Compound 19</td>
<td>$^1$H NMR, $^{13}$C NMR, DEPT-NMR, FT-IR, HR-MS</td>
<td>379-380</td>
</tr>
<tr>
<td>Compound 21</td>
<td>$^1$H NMR, $^{13}$C NMR, DEPT-NMR, FT-IR, HR-MS</td>
<td>381-382</td>
</tr>
<tr>
<td>Polyamide oligomers</td>
<td>HPLC and MALDI-TOF spectra</td>
<td>383-384</td>
</tr>
<tr>
<td>Compound 8</td>
<td>UV-Vis spectra</td>
<td>385</td>
</tr>
<tr>
<td>Compound 17</td>
<td>UV-Vis spectra</td>
<td>386</td>
</tr>
<tr>
<td>Polyamide oligomers 22</td>
<td>UV-Vis spectra</td>
<td>387-390</td>
</tr>
<tr>
<td>Polyamide oligomers 25</td>
<td>UV-Vis spectra</td>
<td>390-391</td>
</tr>
<tr>
<td>Polyamide oligomers</td>
<td>ITC Figures</td>
<td>392-393</td>
</tr>
</tbody>
</table>
**Chapter 3**

**H NMR**

![H NMR spectrum](image)

**C NMR**

![C NMR spectrum](image)

**DEPT**

![DEPT spectrum](image)

*tert*-butyl (2-aminoethyl)carbamate (5)

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**Chapter 3**

**H NMR**

![H NMR spectrum](image)

**C NMR**

![C NMR spectrum](image)

**DEPT**

![DEPT spectrum](image)

**Ethyl N-(2-Boc-aminoethyl)-glycinate (6)**

---

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Chapter 3

FT-IR

MALDI-Spectra

285.401

Calculated Mass: 285.172
Observed Mass: 285.401 (M+K)^-

Ethyl N-(2-Boc-aminoethyl)-glycinate (6)
Ethyl 2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-chloroacetyl)-glycinate (7)
Chapter 3

FT-IR

MALDI-Spectra

Ethyl-2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-chloroacetyl)-glycinate (7)

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Ethyl 2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(2-(pyridin-2-yl)-1H-benzo[d]imidazol-1-yl)acetamido)acetate (8)
Ethyl-2-((N-(2-((tert-butoxycarbonyl)amino)ethyl))imidazol-1-yl)acetamido)acetate (8)

**Calcd Mass:** 482.2403
**Found Mass:** 482.2404 [M+H]^+
\[
\text{\textsuperscript{1}H NMR}
\]

\[
\begin{array}{c}
\text{8.76} \\
\text{8.74} \\
\text{8.34} \\
\text{8.30} \\
\text{7.99} \\
\text{7.78} \\
\text{7.75} \\
\text{7.60} \\
\text{7.39} \\
\text{7.38} \\
\text{7.37} \\
\text{7.35} \\
\text{5.89} \\
\text{5.73} \\
\text{5.01} \\
\text{4.45} \\
\text{4.13} \\
\text{3.70} \\
\text{3.50} \\
\text{3.45} \\
\text{3.39} \\
\text{3.35} \\
\text{3.23} \\
\text{3.20} \\
\end{array}
\]

\[
\text{\textsuperscript{13}C NMR}
\]

\[
\begin{array}{c}
\text{172.68} \\
\text{170.30} \\
\text{158.57} \\
\text{151.29} \\
\text{150.71} \\
\text{150.38} \\
\text{142.66} \\
\text{138.54} \\
\text{125.61} \\
\text{125.23} \\
\text{125.09} \\
\text{124.52} \\
\text{120.11} \\
\text{111.85} \\
\text{80.79} \\
\text{70.83} \\
\text{50.83} \\
\text{50.42} \\
\text{49.15} \\
\text{47.87} \\
\text{39.63} \\
\text{39.28} \\
\text{28.88} \\
\end{array}
\]

\[
\text{DEPT}
\]

\[
\begin{array}{c}
\text{152.82} \\
\text{140.98} \\
\text{138.05} \\
\text{127.62} \\
\text{125.96} \\
\text{122.64} \\
\text{114.28} \\
\text{50.96} \\
\text{31.31} \\
\end{array}
\]

2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(2-(pyridin-2-yl)-1H-benzo[d]imidazol-1-yl)acetamido)acetic acid (1)
Chapter 3

FT-IR

Calcd Mass: 454.2089
Found Mass: 454.2091 [M+H]^+

HRMS-Spectra

2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(2-(pyridin-2-yl)-1H-benzo[d]imidazol-1-yl)acetamido)acetic acid (1)
$^1$H NMR

$^{13}$C NMR

DEPT

Ethyl-2-(3,4-bis((benzyloxy)carbonyl)amino)-N-(2-((tert-butoxycarbonyl)amino)ethyl) benzamido)acetate (12)

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**Chapter 3**

**FT-IR**

![FT-IR spectrum](image)

**HRMS-Spectra**

Calcd Mass: 671.2692  
Found Mass: 671.2689 [M+Na]^+

**Ethyl-2-(3,4-bis((benzyloxy)carbonyl)amino)-N-(2-((tert-butoxycarbonyl)amino)ethyl) benzanido)acetate (12)**
HRMS-Spectra

Calcd Mass: 643.2379
Found Mass: 643.2371 [M+Na]^+

2-(3,4-bis((benzyloxy)carbonyl)amino)-N-(2-((tert-butoxycarbonyl)amino)ethyl) benzamido) acetic acid (13)
**Chapter 3**

**$^1$H NMR**

![$^1$H NMR spectrum](image)

**$^{13}$C NMR**

![$^{13}$C NMR spectrum](image)

**DEPT**

![DEPT spectrum](image)

**Ethyl-2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(3,4-dihydroxyphenyl)acetamido) acetate (15)**

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2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(3,4-dihydroxyphenyl)acetamido)acetic acid (3)
**Chapter 3**

**H NMR**

![H NMR Spectrum](image)

**C NMR**

![C NMR Spectrum](image)

**DEPT**

![DEPT Spectrum](image)

**Benzyl N-(2-Boc-aminoethyl)-glycinate (16)**
Chapter 3

FT-IR

[Graph showing FT-IR spectrum]

HRMS-Spectra

[Graph showing HRMS spectrum]

Calcd Mass: 309.1815
Found Mass: 309.1816 [M+H]^+ 

Benzyl N-(2-Boc-aminoethyl)-glycinate (16)
Chapter 3

H NMR

C NMR

DEPT

Benzyl-2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(3,4-dihydroxyphenyl)acetamido) acetate (17)
Benzyl-2-((N-((tert-butoxycarbonyl)amino)ethyl)-2-(3,4-dihydroxyphenyl)acetamido) acetate (17)

**FT-IR**

**HRMS-Spectra**

Calcd Mass: 481.1950
Found Mass: 481.1947 [M+Na]⁺
**Chapter 3**

**H NMR**

![H NMR spectrum](image)

**C NMR**

![C NMR spectrum](image)

**DEPT**

![DEPT spectrum](image)

Benzyl 2-((N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(2-(2-methoxy-2-oxoethyl)benzo[d][1,3]dioxol-5-yl)acetamido)acetate (18)

---

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FT-IR

[Graph of FT-IR spectrum]

Chapter 3

HRMS-Spectra

Calcd Mass: 565.2161
Found Mass: 565.2167 [M+Na]^+

Benzyl-2-((N-((tert-butoxycarbonyl)amino)ethyl)-2-(2-(2-methoxy-2-oxoethyl)benzo[d] [1,3]dioxol-5-yl)acetamido)acetate (18)
$^{1}$H NMR

$^{13}$C NMR

DEPT

2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(2-(2-methoxy-2-oxoethyl)benzo[d][1,3]dioxol-5-yl)acetamido)acetic acid (19)

2013 PhD thesis: T. Kaur, University of Pune
2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(2-methoxy-2-oxoethyl)benzo[d][1,3]dioxol-5-yl)acetamido)acetic acid (19)

Calcd Mass: 475.1692
Found Mass: 475.1689 [M+Na]+
Ethyl-2,2-dimethyl-4,10-dioxo-8,14-bis(2-(2-(pyridin-2-yl)-1H-benzo[d]imidazol-1-yl) acetyl)-3-oxa-5,8,11,14-tetraazaheptadecan-16-oate (21)
Ethyl-2,2-dimethyl-4,10-dioxo-8,14-bis(2-(2-(pyridin-2-yl)-1H-benzo[d]imidazol-1-yl)acetyl)-3-oxa-5,8,11,14-tetraazahexadecan-16-oate (21)

Calcd Mass: 817.3786
Found Mass: 817.3798 [M+H]^+
Figure 94. (A) HPLC of oligomers (PBI)$_6$ 22. (B) MALDI-TOF of (PBI)$_6$ 22. (C) HPLC of oligomer (PDA)$_6$ 23. (D) MALDI-TOF of oligomer (PDA)$_6$ 23. (E) HPLC of oligomer (CAT)$_6$ 24. (F) MALDI-TOF of (CAT)$_6$ 24.
Figure 95. (A) HPLC of oligomer (PBI)$_3$-(PDA)$_3$ 25. (B) MALDI-TOF of oligomer (PBI)$_3$-(PDA)$_3$ 25. (C) HPLC of oligomer (PBI-PDA)$_3$ 26. (D) MALDI-TOF of (PBI-PDA)$_3$ 26. (E) HPLC of oligomer PBI)$_3$-(CAT)$_3$ 27. (F) MALDI-TOF of oligomer PBI)$_3$-(CAT)$_3$ 27. (F) HPLC of oligomer (PBI-CAT)$_3$ 28 (G) MALDI-TOF of oligomer (PBI-CAT)$_3$ 28.
UV-Vis spectrophotometric titrations of 2-pyridylbenzimidazole (PBI) aeg 8

Figure 96. Changes in the absorption spectra of the 2-pyridylbenzimidazole (PBI) aeg 8 (25 µM) in methanol upon the addition of metal salts (A) Cu(NO$_3$)$_2$ (2.5 mM). (C) Ni(NO$_3$)$_2$ (2.5 mM). Plot of the change in absorbance at 308 and 345 nm as a function of molar ratio of metal to (PBI) 8 (B) Cu(NO$_3$)$_2$ (D) Ni(NO$_3$)$_2$. (E) Zn(NO$_3$)$_2$ (2.5 mM). (F) Co(NO$_3$)$_2$ (10 mM).
UV-Vis spectrophotometric titrations of benzyl-N-Boc-aminoethyl-3,4-dihydroxyphenyl (CAT) glycinate 17

**Figure 97.** Changes in the absorption spectra of benzyl-N-Boc-aminoethyl-3,4-dihydroxyphenyl (CAT) glycinate 17 (8-10 µM) in methanol upon the addition of metal salts (A) Pd(NO$_3$)$_2$ (10 mM). (B) Fe(NO$_3$)$_3$ (10 mM). (C) Ln(NO$_3$)$_3$ (10 mM). (D) Tl(NO$_3$)$_3$ (10 mM).
UV-Vis spectrophotometric titrations of 2-pyridylbenzimidazole (PBI)$_6$ oligomer 22

Figure 98. Changes in the absorption spectra of 2-pyridylbenzimidazole (PBI)$_6$ oligomer 22 (8-10 µM) in water upon the addition of metal salts (A) Cu(NO$_3$)$_2$ (2.5 mM), (C) Cu(NO$_3$)$_2$ (10 mM). Plot of the change in absorbance at 302 and 345 nm as a function of molar ratio of metal to peptides (B) Cu(NO$_3$)$_2$ and (D) Cu(NO$_3$)$_2$. (E) Pd(NO$_3$)$_2$ (2.5 mM). (F) RuCl$_3$ (2.5 mM).

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UV-Vis spectrophotometric titrations of 2-pyridylbenzimidazole (PBI)$_6$ oligomer 22

**Figure 99.** Changes in the absorption spectra of 2-pyridylbenzimidazole (PBI)$_6$ oligomer 22 (8-10 µM) in water upon the addition of metal salts (A) (PPh$_3$)$_2$PtCl$_2$ (2.5 mM), (B) Eu(NO$_3$)$_2$ (2.5 mM), (C) Pb(NO$_3$)$_2$ (2.5 mM), (D) Co(NO$_3$)$_2$ (2.5 mM), (E) Mn(OAc)$_3$ (2.5 mM), (F) AgNO$_3$ (2.5 mM).
UV-Vis spectrophotometric titrations of 2-pyridylbenzimidazole (PBI)$_6$ oligomer 22

**Figure 100.** Changes in the absorption spectra of 2-pyridylbenzimidazole (PBI)$_6$ oligomer 22 (8-10 µM) in water upon the addition of metal salts (A) Tb(NO$_3$)$_2$ (2.5 mM). (B) Zn(NO$_3$)$_2$ (2.5 mM). (C) Cd(NO$_3$)$_2$ (2.5 mM). (D) Ho(NO$_3$)$_2$ (2.5 mM).
**UV-Vis spectrophotometric titrations of hetero-(PBI)$_3$ - (PDA)$_3$ oligomer 25**

Figure 101. Changes in the absorption spectra of (PBI)$_3$ - (PDA)$_3$ oligomer 25 (8-10 µM) in water upon the addition of metal salts (A) Cu(NO$_3$)$_2$ (2.5 mM), (C) Cu(NO$_3$)$_2$ (10 mM). Plot of the change in absorbance at 302 and 345 nm as a function of molar ratio of metal to peptides (B) and (D) Cu(NO$_3$)$_2$. 

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UV-Vis spectrophotometric titrations of *hetero-(PBI)₃ - (PDA)₃* oligomer 25

**Figure 102.** Changes in the absorption spectra of (PBI)₃ - ((PDA)₃ oligomer 25 (8-10 µM) in water upon the addition of metal salts (A) Pd(NO₃)₂ (2.5 mM), (B) Cd(NO₃)₂ (2.5 mM), (C) RuCl₃ (2.5 mM), (D) Pb(NO₃)₂ (2.5 mM), and (E) Co(NO₃)₂ (2.5 mM).
Isothermal Titration Calorimetry (ITC) analysis of synthesized aeg linked ligands with nickel nitrate

Figure 103. ITC figures in water (A) (PBI) 8 with Cu(NO$_3$)$_2$ (B) (PBI) 8 with Ni(NO$_3$)$_2$ (C) 2-pyridylbenzimidazole (PBI)$_6$ oligomer 22 with Ni(NO$_3$)$_2$, (D) (PBI)$_3$ (PDA)$_3$ oligomer 25 with Ni(NO$_3$)$_2$.
Isothermal Titration Calorimetry (ITC) analysis of synthesized aeg linked ligands with nickel nitrate

Figure 104. ITC figures in water (A) (PBI-PDA)$_3$ 26 in water Ni(NO$_3$)$_2$ (B) (PBI)$_3$-(CAT)$_3$ 27 with Ni(NO$_3$)$_2$. (C) (PBI-CAT)$_3$ 28 with Ni(NO$_3$)$_2$.

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