

AMINO ACID AND PEPTIDE BASED GELS

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ABSTRACT

AMINO ACID AND PEPTIDE BASED GELS

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Self-assembling peptides and amino acid derivatives are becoming an emerging field especially in last decades. Especially for their usage in biomedical applications, tissue engineering and drug delivery, extensive research are being done. Large variety of peptides with different physical and chemical properties were acquired by using different amino acids and their sequences. As a more easily synthesized and bio-inspired alternative materials to the compounds used nowadays for organic electronic devices, gel forming peptides are getting more attention. Design, synthesis and applications of these peptides in biomedical materials and organic electronic devices is a challenging topic that requires alternative structures to get better and easily applied systems. Not only self-assembling peptides, also low molecular weight gelators of amino acid derivatives were developed quite for a wide range of applications.

In the first part of this study, conductive peptide-based gels were developed through chemical peptide synthesis, utilizing artificial amino acids. To enhance the conductivity of self-assembling peptides, different artificial amino acids were developed that provide well-ordered structures with enhanced charge mobility through π - π stacking of aromatic units. By using these artificial amino acids different

peptide sequences that are able to self-assemble were synthesized and new conductive bio-inspired materials were developed with higher conductivity values than the literature data.

In the second part of this study, amino acid based low molecular weight gelators were discovered, even some of them have the lower molecular weight among other amino acid based gelators found in the literature. These newly developed materials are able to form gel in a wide range of solvents and thus, utilized for different applications such as dye removal and drug release studies.

Keywords: Peptide, Amino Acid, Self-assembly, Conductive Gel, Drug Release

ÖZ

AMİNO ASİT VE PEPTİT TEMELLİ JELLER

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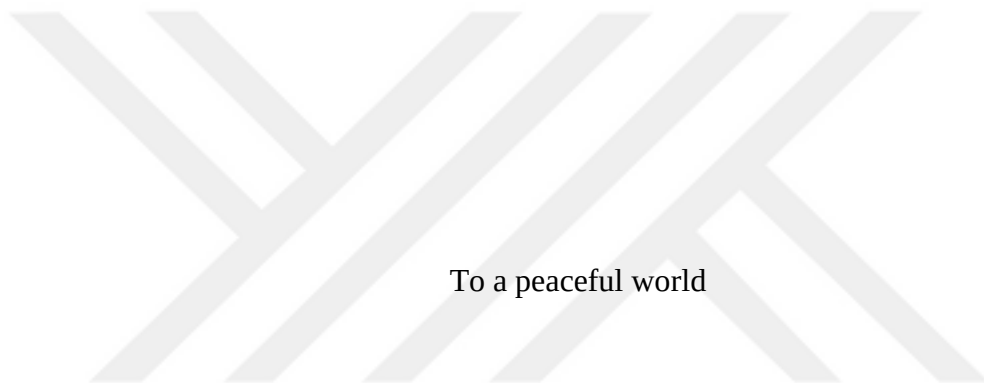
Kendiliğinden yapılanabilen peptitler ve amino asit türevleri son yirmi yılda yeni geliştirilen bir alandır. Özellikle, biyomedikal uygulamalar, doku mühendisliği ve ilaç taşınımı alanlarında kullanımları için kapsamlı araştırmalar yapılmaktadır. Çeşitli amino asitler ve bunların dizilimleri kullanılarak farklı fiziksel ve kimyasal özelliklere sahip çok çeşitli peptitler elde edilmiştir. Günümüzde organik elektronik cihazlar için kullanılan bileşiklere alternatif olarak daha kolay sentezlenebilen ve biyo-esinlenilmiş malzemeler olan jelleşebilen peptitler giderek daha çok dikkat çekmektedir. Daha iyi ve kolay uygulanabilen sistemlere ihtiyaç duyan peptitlerin biomedikal malzemeler ve organik elektronik cihazlar için tasarım, sentez ve uygulamaları zorlayıcı bir konudur. Sadece kendiliğinden yapılanabilen peptitler değil, ayrıca amino asit türevi düşük molekül ağırlıklı jelatörler geniş skalada uygulamalar için çokça geliştirilmiştir.

Bu çalışmanın ilk kısmında, yapay amino asitler kullanılarak ve kimyasal peptit sentezi ile iletken peptit bazlı jeller geliştirilmiştir. İletkenliği iyileştirmek için aromatik halkaların π - π istiflenmesi ile artırılmış yük mobilitesine sahip düzenli yapılar sağlayabilen farklı yapay amino asitler geliştirilmiştir. Bu yapay amino asitleri kullanarak, kendiliğinden yapılabilen peptitler sentezlenmiş ve literatürdeki

verilere göre daha yüksek iletkenlik deęerine sahip yeni iletken biyo-esinlenilmiř malzemeler geliřtirilmiřtir.

Bu alıřmanın ikinci kısmında ise, amino asit bazlı dūřuk molekūl aęırlıklı jelatōrler keřfedilmiř olup, hatta bazıları literatūrdeki amino asit bazlı jelatōrlere gōre daha dūřuk molekūl aęırlıęına sahiptirler. Bu yeni geliřtirilen malzemeler ok geniř bir ōzūcū skalasında jelleřebilmektedirler ve boya uzaklařtırma ve ila salınımı alıřmaları gibi farklı uygulamalarda kullanılabilirler.

Anahtar Kelimeler: Peptit, Amino Asit, Kendilięinden Yapılanabilme, İletken Jel, İla Salınımı



To a peaceful world

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LIST OF ABBREVIATIONS

1,2-DCE	1,2-Dichloroethane
1,2-DME	1, 2-Dimethoxyethane
AC	Alternating current
AFM	Atomic force microscopy
Boc	<i>tert</i> -butoxycarbonyl
BPO	Benzoyl peroxide
Cbz	Benzyloxycarbonyl
CD	Circular dichroism
COD	1,5-Cyclooctadiene
DBU	1,8-Diazabicyclo(5.4.0)undec-7-ene
DCM	Dichloromethane
DIC	<i>N,N'</i> -Diisopropylcarbodiimide
DIEA	<i>N,N</i> -Diisopropylethylamine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DTT	1,4-Dithiothreitol
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDC.HCl	<i>N</i> -(3-Dimethylaminopropyl)- <i>N'</i> -ethylcarbodiimide hydrochloride
Fmoc	Fluorenylmethoxycarbonyl
FT-IR	Fourier transform infrared spectroscopy

HATU	1-[Bis(dimethylamino)methylene]-1 <i>H</i> -1,2,3-triazolo[4,5- <i>b</i>]pyridinium 3-oxid hexafluorophosphate
HBTU	<i>N,N,N',N'</i> -Tetramethyl-O-(1 <i>H</i> -benzotriazol-1-yl)uronium hexafluorophosphate
HOBt	Hydroxybenzotriazole
HPLC	High Pressure liquid chromatography
HRMS	High resolution mass spectrometry
LMW	Low molecular weight
MBHA	4-Methylbenzhydrylamine hydrochloride
MD	Molecular dynamics
MTBE	Methyl <i>tert</i> -butyl ether
Mtt	4-Methyltrityl
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NBS	<i>N</i> -Bromosuccinimide
NDI	Naphthalenediimide
NMP	<i>N</i> -methyl Pyrrolidinone
Pbf	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
PyAOP	(7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
PyBrOP	Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate
Q-TOF	Quadrupole time of flight

RDF	Radial distribution functions
SEM	Scanning electron microscopy
SPPS	Solid phase peptide synthesis
TBDMS	<i>tert</i> -Butyldimethylsilyl ether
TCEP	Tris(2-carboxyethyl)phosphine
TEM	Transmission electron microscopy
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIPS	Triisopropylsilane
TLC	Thin layer chromatography
TMS	Tetramethylsilane
Tris	Tris(hydroxymethyl)aminomethane
Trt	Trityl
UV-Vis	Ultraviolet-Visible
XRD	X-ray diffraction

CHAPTER 1

DESIGN, SYNTHESIS AND ANALYSIS OF CONDUCTIVE PEPTIDE GELS

1.1 Introduction

In this section, the general properties of peptides and their self-assembly properties were addressed, at first. Then, the β -sheet forming peptides found in literature were explained in detail. After giving information about the conductivity of biologically active self-assembling peptides, the conductive peptides found in literature and the methods developed by researchers to increase the conductivity of self-assembling peptides were discussed particularly. Then, a general information about solid phase peptides synthesis is given. At the end of this part, the aim and the method used in this study to enhance conductivity of self-assembling peptides were addressed.

1.1.1 Peptides

Proteins and peptides are structurally similar to each other. They both are made up of the binding of amino acids to each other with peptide bonds which are also known as amide bonds.^{1,2} They have important roles in biological functions. Short peptide fragments can be produced by the digestion of proteins by enzymes. Generally the short sequences that contains amino acids between 2 to 50 are accepted as peptides, whereas the longer sequences are named as proteins. In some instances, researchers use the term oligopeptide to define peptide sequences with 2 to 20 amino acids. However, polypeptide term can be used both for longer peptide sequences or proteins

alternatively. Because of their huge structures, proteins tend to form complex conformations as secondary, tertiary and quaternary structures and become biologically active, whereas most peptide sequences could not be classified depending on complex conformations. Nevertheless, there are well known peptide sequences that form nanostructures such as nanotubes, nanofibers, nanotapes, nanorods and vesicles.^{3,4}

1.1.2 Self-Assembly of peptides

Well-ordered nanostructures of organic and inorganic molecules attract the interest of many researchers because of their potentials in biological and chemical applications.⁵⁻⁷ Peptides are the most promising organic molecules for different applications among others because of their chemical diversity, biocompatibility and similarity to proteins.⁴ Drug delivery, vaccine engineering, tissue engineering, regenerative medicine, biosensing, wound healing, photonics and smart electronic are some of the application areas that self-assembling peptides are quite preferable materials.⁸⁻¹³ Self-assembly is the spontaneous organization of molecules into well-ordered systems with the presence of free-energy of electrostatic interactions, van der Waals forces, hydrogen bonding and π - π stacking. Arrangement of molecules is not only dependent of these attractive and repulsive forces inside the molecular composition but also to the properties of medium in which assembly occurs such as solvent, pH, ionic strength, temperature and co-assembling molecules.^{10,14} The variety of side chains of amino acids in peptide sequences provide these type of interactions within the molecule itself and with the environment. For example, tyrosine and tryptophan are amino acids containing aromatic units on their side chains, thus these aromatic units may interact with the aromatic unit on another peptide through π - π interactions. Moreover, the presence of negative charges on carboxylates of glutamic acid or aspartic acid may interact with positive charges of amine containing amino acids with electrostatic interactions at defined pH values.

As these type of interactions become favorable within peptides, well-ordered structures are obtained through self-assembly. The interactions between peptide-peptide and peptide-solvent are the main factor that affect the resultant secondary structure. β -sheet and α -helix are some of the important secondary structures that could be obtained by self-assembly of peptides.⁴ β -sheet formation can be in parallel fashion or in antiparallel fashion in which the *N*-terminus of a strand interact with *C*-terminus of the second peptide strand to self-assemble (Figure 1.1). The distance between each peptide strand is known as 4.7-4.8 Å.^{15,16}

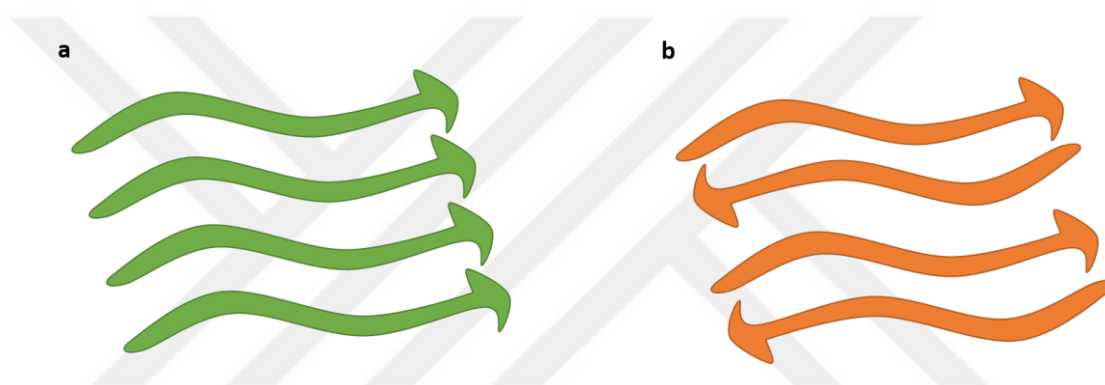


Figure 1.1. **(a)** β -sheet formation in parallel fashion **(b)** β -sheet formation in antiparallel fashion.

As a result, several nanostructures such as nanofibers, nanotubes and nanotapes could be attained that can be proven with microscopy techniques such as transmission electron microscopy, scanning electron microscopy and atomic force microscopy.^{4,14,17} As the number of these nanostructures raises in the medium, they entangle with each other and results with the immobilization of solvent. This continuous structure with macroscopic dimensions is referred as gel by Flory.¹⁸

1.1.2.1 Beta-sheet forming peptides

β -sheet formation may occur with different size of peptides starting from two amino acids up to hundreds of amino acids containing polypeptides.^{4,19–22} Tubular nanostructures are obtained by using L-Phe-L-Phe (FF) dipeptide¹⁹ and L-Phe-L-Trp (FW) dipeptide²⁰ whereas sheet-like membrane systems or spherical vehicles could be obtained depending on the number of lysine and leucine moieties in poly(L-Lysine)-b-poly(L-Leucine) copolypeptide.²²

Researches expanded much efforts for the discovery of new β -sheet forming tripeptides and their nanostructural analysis.^{23–25} Moreover, Tuttle and coworkers analyzed the self-assembly possibility of 8000 gene-encoded tripeptides by using computational methods and found-out that 4 of them forms hydrogels: L-Lys-L-Tyr-L-Phe (LYF), L-Lys-L-Tyr-L-Tyr (LYY), L-Lys-L-Phe-L-Phe (LFF), L-Lys-L-Tyr-L-Trp (LYW).²⁶ These indications were supported with experimental results and the fibrillar formations were observed in TEM images.

One of the well-known β -sheet forming peptide (Ala-Glu-Ala-Glu-Ala-Lys-Ala-Lys)₂ (AEK16) is (Figure 1.2) a region of a yeast protein Zuotin which contains both hydrophobic (alanine) and hydrophilic amino acids (glutamic acid and lysine) in alternating order in its sequence.²⁷ Circular dichroism studies proved the β -sheet formations in pH 7.4 phosphate buffer with NaCl addition.

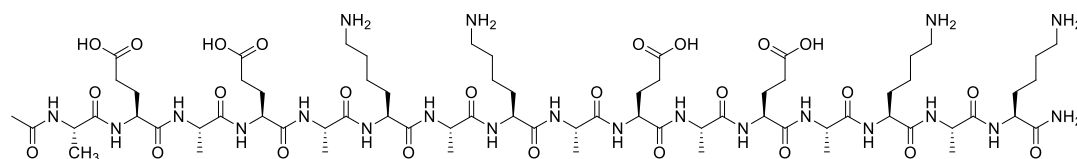


Figure 1.2. Structure of β -sheet forming peptide AEK16.

After the discovery of AEK16, Zhang and his coworkers developed a new β -sheet forming peptide (Phe-Lys-Phe-Glu)₃ (FKE12) which is able to form gel at pH 7.4

with the addition of salts (Figure 1.3).²⁸ This peptide sequence is also contains both hydrophobic and hydrophilic moieties in alternative order. The replacement of alanine with phenylalanine provide π - π stacking, thus gelation ability increased. When they analyzed the rheological behavior of this peptide at different pH values, they observed the gel formation at pH values between 7 and 10. This newly developed β -sheet forming peptide pave the way for the discovery of novel peptide sequences and their potential chemical and biological applications.^{3,8,29–31}

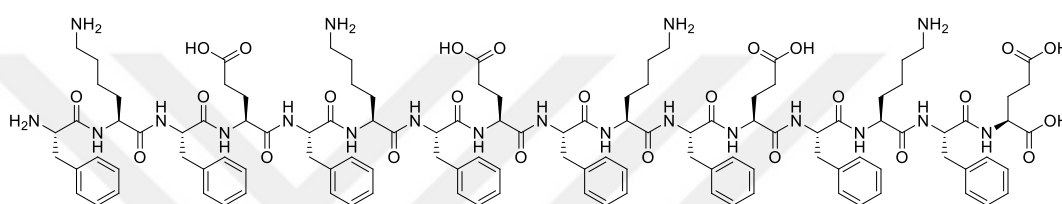


Figure 1.3. Structure of β -sheet forming peptide FKE12.

EAK16 and RAD16 peptides that are able to assemble in β -sheet secondary structure were developed by Zhang and coworkers with the amino acid sequences Ac-AEAEAKAKAEAEAKAK-NH₂ and Ac-RARADADARARADADA-NH₂ respectively that can be used in wound healing, tissue regeneration and tissue implantation.²⁷ Other well-known β -sheet forming and commercially available both in powder and hydrogel form peptide is RADA16 peptide (Figure 1.4) with a sequence of Ac-RADARADARADARADA-NH₂.^{32,33} This peptide also contains hydrophilic and hydrophobic amino acids in alternating order in the sequence.

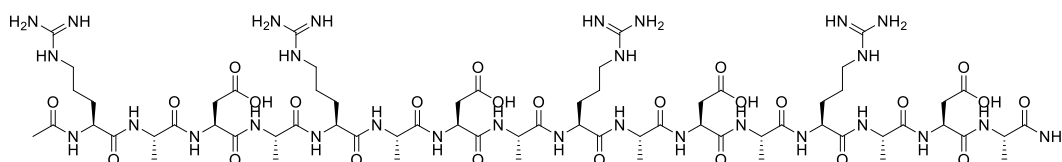


Figure 1.4. Structure of β -sheet forming peptide RADA16.

RADA16 peptide is able to form gel by assembling as β -sheet in anti-parallel fashion at different pH values such as 2.0, 4.8 and 7.4 which increases its potential to be used in different applications.^{32,34,35} Commercially available RADA16 hydrogel, PuraMatrix is used by researchers for different applications such as wound healing³⁶, neurite outgrowth and synapse formation³⁷, tissue repair³⁸ and controlled drug release.³⁹⁻⁴¹ RATEA16 with amino acid sequence Ac-RATARAEEARATARAEEA-NH₂ is also another hydrogel forming peptide that can be used in similar applications.³³

Aggeli and her coworkers developed 11 amino acid containing four new peptides that assemble in anti-parallel fashion to form tapes, ribbons, fibrils or fibers depending on the sequence and pH of the medium.^{42,43} Initially, they demonstrated the importance of one net positive or negative charge on the peptide sequence for the stabilization of fibrillar structures by synthesizing P₁₁-2 (Ac-Gln-Gln-Arg-Phe-Gln-Trp-Gln-Phe-Glu-Gln-Gln-NH₂) and P₁₁-3 peptide (Ac-Gln-Gln-Arg-Phe-Gln-Trp-Gln-Phe-Gln-Gln-Gln-NH₂).⁴³ They achieved the gelation of P₁₁-3 independently from pH. Then, they ensured the pH control on nematic gel formation by direct electrostatic forced between side chain carboxylates of glutamic acid or ammoniums of ornithine with using glutamic acid in peptide P₁₁-4 (Ac-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH₂) and ornithine (Orn) in peptide P₁₁-5 (Ac-Gln-Gln-Orn-Phe-Orn-Trp-Orn-Phe-Gln-Gln-Gln-NH₂). Thus new pH sensitive β -sheet forming peptides with reversible switching ability between fluid and gel phase were attained. As represented in Figure 1.5, P₁₁-4 is able to form nematic fluid at pH values lower than 6.8 and then gel at pH 2. At higher pH values, negative charges on carboxylate repel each other and result with isotropic solution. Conversely, P₁₁-5 is found in the nematic fluid form at pH value 7.8 and become gel at higher pH values. For this peptide, positive charges of ornithine repel each other at lower pH values and prevent the self-assembly. After finding out that the both peptides are in isotropic phase at a common pH region 7.2-7.4, they achieved the gelation of these peptides as cross- β -structure at pH 7.3 (Figure 1.6).^{44,45} As a result, these peptides assemble in alternating order to form tapes, in which phenylalanine and tryptophan interact

with hydrophobic and π - π interactions within peptide strands, glutamic acid and ornithine residues ensure strong columbic and complementary electrostatic interactions. Moreover, the presence of glutamine, glutamic acid and ornithine residues increase the hydrophilicity of one surface of β -sheet which could be important for the rational molecular designs of self-assembling peptides for tissue engineering.⁴⁶ Moreover, in a recent study P₁₁-4 peptide was used in its assembled form for bone regeneration in an *in vivo* study and the results are quite promising to be used in tissue engineering.⁴⁷



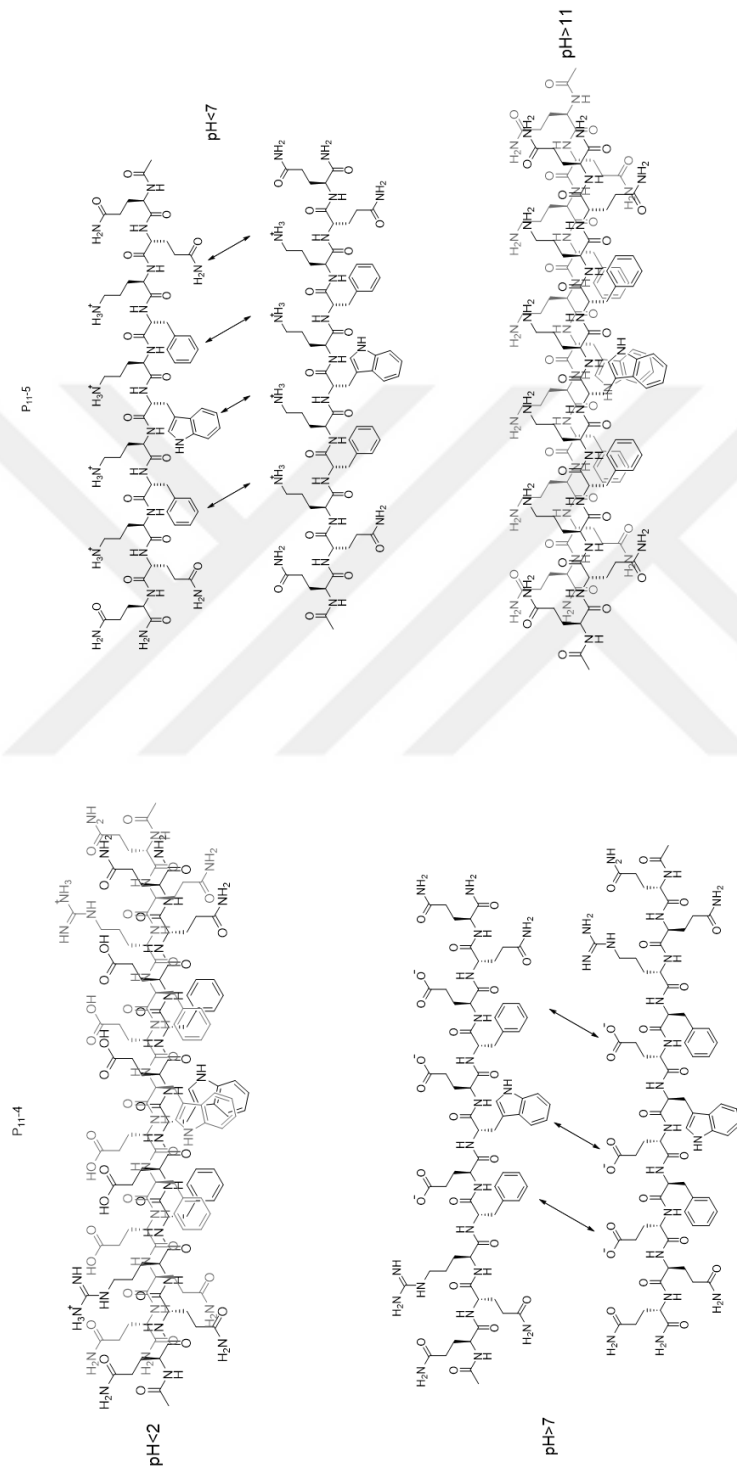


Figure 1.5. P₁₁₋₄ self-assemble and form hydrogel at pH values lower than 2 and peptide strands repel each other at pH values higher than 7.0. P₁₁₋₅ self-assemble and form hydrogel at pH values at high pH values, whereas peptide strands repel each other at pH values lower than 7.0.

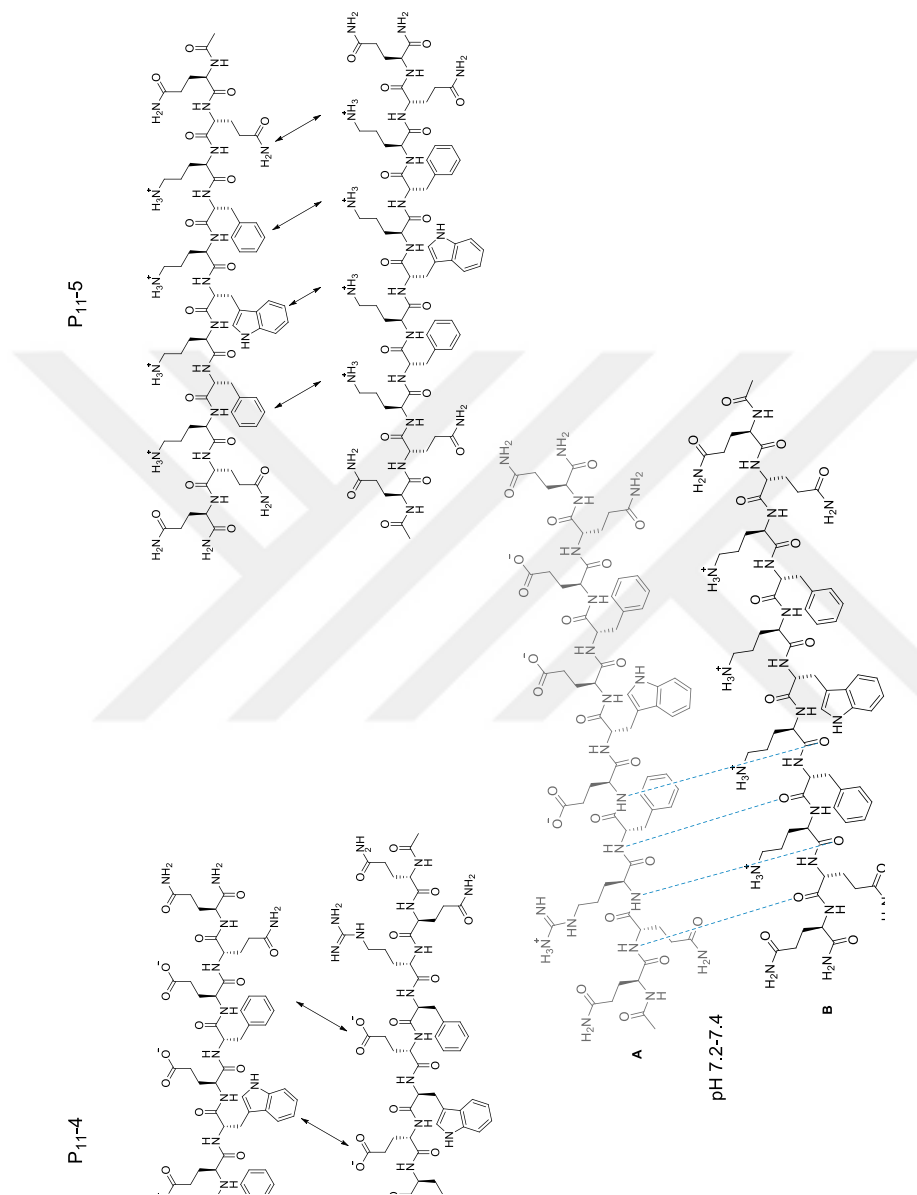


Figure 1.6. Both P₁₁₋₄ and P₁₁₋₅ peptides are found in isotropic phase at pH 7.2-7.4. They can form co-assembled peptides at this pH interval when they are mixed.

In 1974, De Santis and coworkers suggested that the even number of alternating D- and L- amino acid containing peptides could be stacked with backbone-backbone hydrogen bonding interactions to form tubular structures depending on their theoretical calculations.⁴⁸ Around 20 years later, Ghadiri and colleagues achieved the self-assembly of a cyclic octapeptide, cyclo-[(Gln-D-Ala)₄].⁴⁹ After protonation, crystallization occurs in tubular structure with hundreds nanometer long and with 7-8 Å internal diameter. The alternating order of D- and L- amino acids in cyclic octapeptide results with a flat ring shape, thus the intramolecular transannular side chain-side chain and side chain-back bone interactions are minimized.^{50,51} The presence of anti-parallel intermolecular hydrogen bonds force the self-assembly in tubular β-sheet structure as showed in Figure 1.7. They used FT-IR, ¹H-NMR and high resolution X-ray crystallography to prove the anti-parallel β-sheet formation. Also, three new nanotube forming peptides were also developed as cyclo-[(Gln-D-Val)₄], cyclo-[(Gln-D-Leu)₄] and cyclo-[(Gln-D-Phe)₄]. Different derivatives of peptide nanotubes could have applications in molecular electronics, catalysis, sensors, development of photo-responsive materials and colloids, drugs and biomedical devices.⁵²⁻⁵⁴

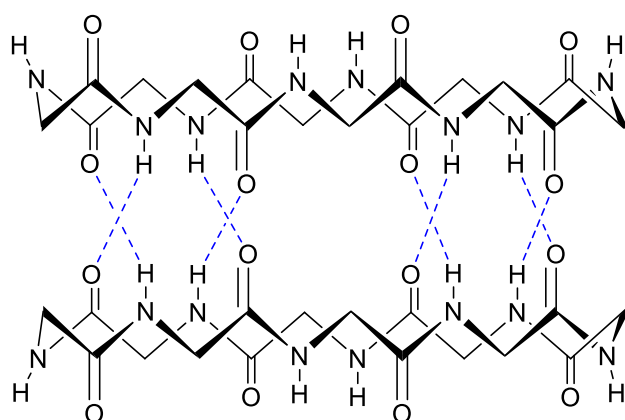


Figure 1.7. The self-assembly of cyclic D-, L- octapeptide. Hydrogen bonding occurs in anti-parallel fashion intermolecularly. Side chains are not shown for clarity.

Beside the even number of alternating D-, L- amino acid containing peptides that form crystalline structures after self-assembly, cyclic dipeptides have self-assembly ability to form fibrillar structures and thus gels (Figure 1.8). These type of gelator peptides, firstly discovered by Hanabusa and his coworkers and they act as organogelators.⁵⁵ The gelation of organic solvents including vegetable oils provide possible applications, e.g. oil-spill recovery. Cyclo-(L-Phe-L-Gly), Cyclo-(L-Phe-L-Ser), Cyclo-(L-Phe-L-Cys), Cyclo-(L-Phe-L-Glu), Cyclo-(L-Phe-L-His) and Cyclo-(L-Phe-L-Lys) dipeptides⁵⁶ and many other derivatives⁵⁷ were then found to form hydrogels in water, in which Cyclo-(L-Phe-L-Gly) is the lowest molecular weight hydrogelator. The hydrogen bond donor and acceptor property of cyclic dipeptides promote self-assembly in fibrillar structure.⁵⁸⁻⁶⁰ The presence of aromatic units or aliphatic chains on the side chains of amino acids increase the possibility of gelation in different kind of solvents. There are wide range of application areas of self-assembled cyclic dipeptides including energy storage and bionano-architectonics.⁶¹

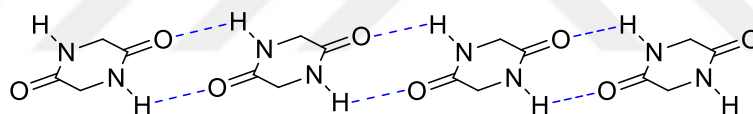


Figure 1.8. The self-assembly of cyclic dipeptides. Side chains are neglected for clarity.

1.1.3 Conductive peptides

In recent years, there is a demand for environmentally friendly and easily fabricated organic semiconductors.⁶² These requirements led researchers to create electronically enhanced self-assembling peptide nanostructures which are eco-friendly, easy to synthesize and flexible morphologically and functionally. In addition to environmental concerns, biocompatibility is another important concern

for the biological applications of organic electronics.⁶³ Self-assembling peptides have an inherent bioinspired nature, thus they can be a bridge between semiconductor world and biological systems for the development of fundamental biology and the research of health care.⁶² Inorganic semiconductors generally contains toxic metals and other organic π -conjugated polymeric semiconductors generally have complicated synthesis and they have weak oxidation stability or sustainability, whereas self-assembling peptides overcome these drawbacks as bioderived molecules. The non-covalent interactions of π -electrons offer researchers to control the assemblies for well-developed systems.⁶⁴ Moreover, the diversity of amino acids provides solubility in aqueous medium for several applications by maintaining self-assembly.

In a study, researchers calculated the charge transport of both linear L-Phe-L-Phe and cyclic L-Phe-L-Phe form.⁶⁵ They showed that the hole mobility is higher than the electron mobility for the cyclic peptide, whereas electron migration is easier for the linear L-Phe-L-Phe. Depending on the result, the charge migration has high conformational dependency. The calculated band-gaps are comparable for the conventional inorganic materials that can be used in nanomaterial applications,⁶⁵ nevertheless device fabrication is an important obstacle that should be overcome.⁶⁶ The self-assembly of L-Phe-L-Phe dipeptide is similar to the Alzheimer's amyloid- β fibrils, and many other researchers mimic proteins found in biological systems that show conductivity for new bioelectronic systems.^{65,67-71}

In another study conducted by Amdursky, the conductivities of L-Phe-L-Phe and L-Phe-L-Trp were measured by using conductive probe atomic force microscopy and the tryptophan containing peptide showed 5 times better conductivity, nevertheless the obtained conductivities are degree of 10^{-9} Siemens.⁷² Moreover, a peptide sequence inspired by mammalian elastic tissues with a sequence of $\text{NH}_2\text{-Gly-Phe-Pro-Arg-Phe-Ala-Gly-COOH}$ forms fibrillar structures in aqueous conditions.⁷⁰ The conductivity of this peptide both in monomer and assembled form was measured by using semiconductor parameter analyzer. The self-assembled peptide has a conductivity value of 10^{-10} S whereas the peptide monomer showed a conductivity

of 10^{-11} S. It was concluded that the interactions that cause self-assembly, especially the π - π interactions of aromatic units improves the conductivity.

Although theoretical calculations represent that the conductivity of peptides can be enhanced by modifying peptide sequences, experimental data clearly showed that the side chains of natural amino acids are not sufficient to provide nanostructures with good conductivity.⁷³ In order to attain bioinspired molecules with acceptable semiconductor properties, different strategies are developed and employed by researchers. Most preferable method to obtain efficient charge transport via self-assembled peptides is to incorporate aromatic units in to the middle of the β -sheet forming peptide, its N-terminus, or one of its amino acids' side chain.^{64,74,75} First example of oligothiophene-peptide conjugate was developed by Bauerle and his coworkers by bonding oligothiophene unit to silk inspired COOH-GAGAG-NH₂ peptides.⁷⁶ Afterward they used different peptide sequences containing alternating threonine and valine, however they did not prove the presence of effective π - π stacking possibly because of the long alkyl chains on thiophene units.^{77,78} Moreover the formation of fibrillar structures is driven by addition of methanol to dichloromethane solution of oligothiophene conjugated peptide which is a drawback for biological applications.

Tovar and his group have great contributions to the synthesis and analysis of semiconductor peptides.^{74,79-81} The first peptide bearing internal π -system incorporated study was reported in 2008.⁷⁴ In this system dithiophene units appear in the middle of a ten amino acid containing β -sheet forming peptide (Figure 1.9). They observed 1-D fibrillar formation both in aqueous and physiologically relevant environments. Although the distance between each aromatic unit was calculated as 5 Å, they proved the electrical communication among dithiophene units with CD and fluorescence spectroscopy.

To improve the π - π interactions between aromatic units to a more extended level, aligned monodomain gel formation could be used as a method. This method was developed by Stupp and his coworkers, and known as noodle formation.⁸² After the

exposure of aqueous peptide solution to heat, it was drawn in to a salty medium such as phosphate buffered saline solution manually and noodle like gel formation was observed. SEM images verify the directionally aligned nanostructure formation as shown in Figure 1.10.

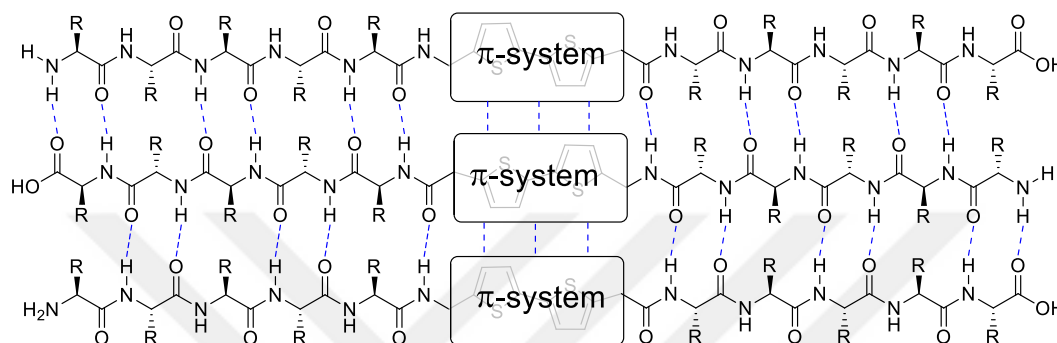


Figure 1.9. Self-assembled β -sheet forming peptide with internal π -system incorporation and π - π stacking.

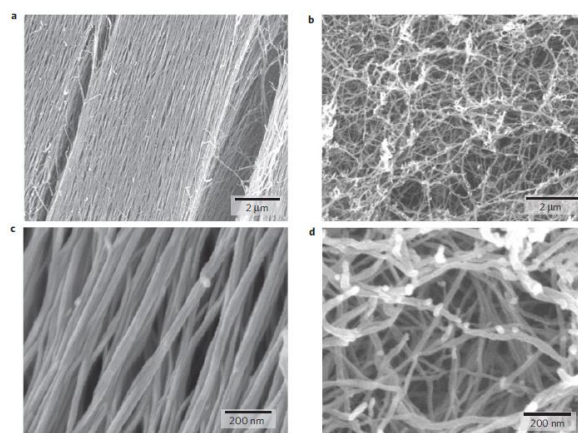


Figure 1.10. **a** and **c** show SEM images of aligned peptide nanofibers achieved by noodle formation method, **b** and **d** shows the isotropic fibrillar formations without same method. Reprinted by permission from the licensor: Springer Nature, Nature Materials, A self-assembly pathway to aligned monodomain gels, Shuming, Zhang et. al., COPYRIGHT (2010).

To attain aligned macroscopic monodomain optoelectronic nanostructures, noodle formation was implemented for the quarterthiophene or 1,4-distyrylbenzene incorporated β -sheet forming peptides with slight modifications in protocol.⁷⁹ For the quarterthiophene containing aligned system, the hole mobility was measured as $0.03 \pm 0.005 \text{ cm}^2 \text{ V}^{-1}\text{s}^{-1}$ in parallel direction and as $0.0014 \pm 0.0001 \text{ cm}^2 \text{ V}^{-1}\text{s}^{-1}$ in perpendicular direction by using semiconductor parameter analyzer. Interestingly, randomly oriented hydrogel did not show any transport anisotropy. The peptide sequence dependency of aromatic unit conjugated peptide hydrogels in terms of mechanical, electrical and photophysical properties were also investigated.^{83–85} COOH-DVV- quarterthiophene-DVV-COOH peptide hydrogel showed the lowest sheet resistance among other hydrogels formed with peptide sequences DII, DGG, DFF and DAA.⁸⁴ Moreover, illumination with halogen lamp lowers the sheet resistance of DGG containing peptide significantly. Tovar and his colleagues also designed field-effect transistor system to calculate the hole mobility values of different peptide sequences with π -conjugation.⁸⁵ The highest mobility value was calculated as $0.017 \pm 0.006 \text{ cm}^2 \text{ V}^{-1}\text{s}^{-1}$ for COOH-DGG-quarterthiophene-DGG-COOH peptide hydrogel from the I-V curves. According to this study, the three order of magnitude differences of hole mobility values of different peptide sequences indicates the importance of peptide sequence. Moreover, for the control peptide nanostructures, that did not contain quarterthiophene unit, did not exhibit field-effect current which represents the importance of π -systems to achieve enhanced conductivity in peptide systems.

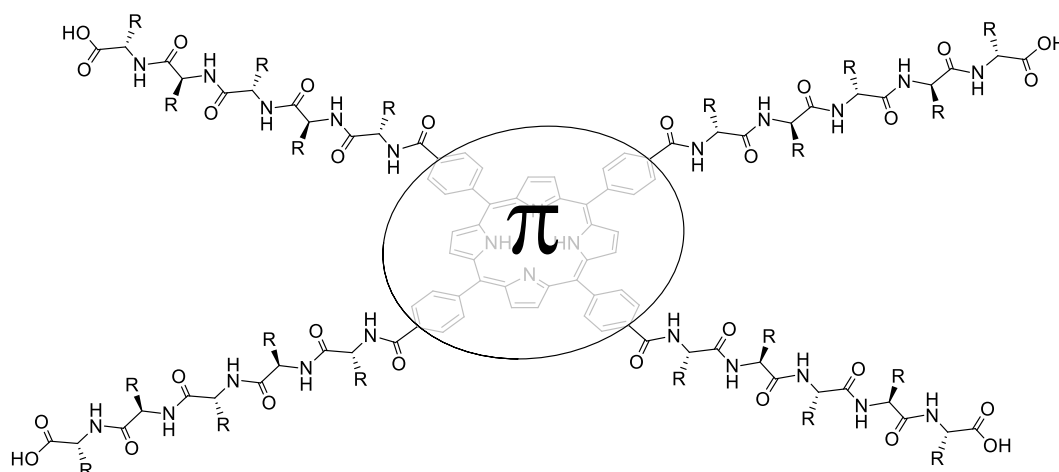


Figure 1.11. Meta-substituted porphyrin conjugated self-assembling peptide tetramer.

In another study, they developed a synthetic route to obtain multivalent peptides connected with an aromatic core (Figure 1.11).⁸⁶ By using different aromatic cores such as benzotrithiophene, decacyclene triimide, and meso-substituted porphyrin, trimer and tetramer peptide were synthesis. Their fibrillar formations were proven with TEM imagining after self-assembly. The charge mobility of these new nanostructures were measured by using field effect transistor. The meso-substituted porphyrin containing tetramer peptide has the highest hole mobility among other multivalent π -conjugated peptides as $7.2 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. The resultant low charge mobility value is because of the crowded non-conducting peptide domains. They hinders the charge hopping mechanism among porphyrin groups within each nanostructure.

Parquette and his coworkers investigated the self-assembly of naphthalene diimide containing dipeptides, and found out that the position of naphthalene diimide and the presence of fluorenyl unit alters the nanostructures as helical fibers, twisted ribbons or nanobelt.⁸⁷ Fmoc-Lys-Lys(NDI) peptide (Figure 1.12) can assemble itself in anti-parallel fashion and forms nanobelt structures. According to their designed self-

assembly model, long-range charge migration can occur intermolecularly with π -delocalization among n-type aromatic units.⁷⁵

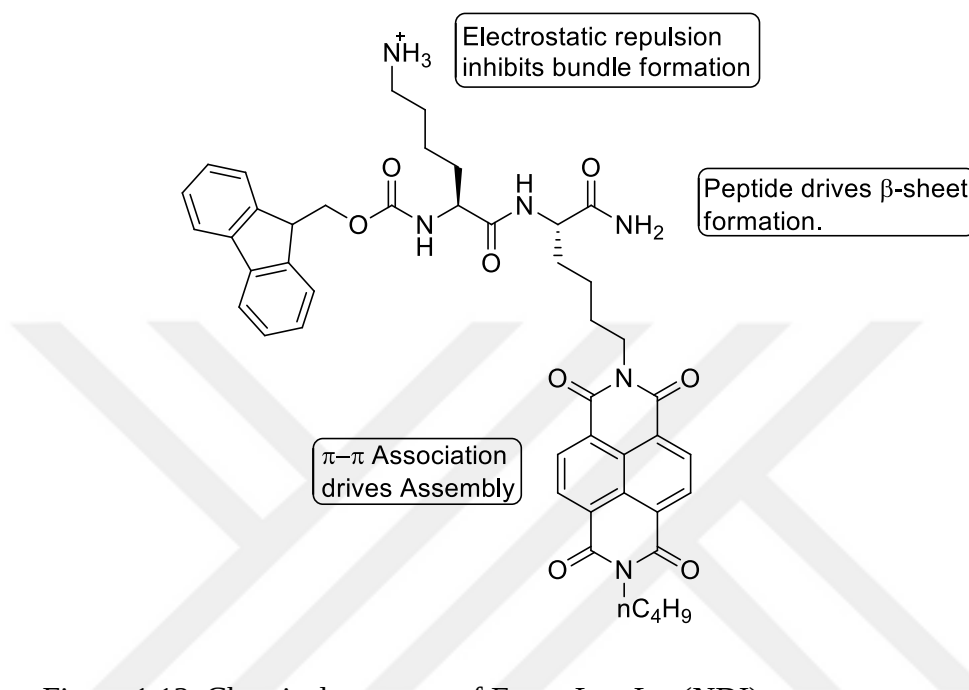


Figure 1.12. Chemical structure of Fmoc-Lys-Lys(NDI).

In another study, enzyme triggered self-assembly was used for the gelation of Fmoc-Leu-Leu-Leu tripeptide in π - β nanotubular structure as shown in Figure 1.13.^{16,88} The π - π stacking between fluorenyl units was proven by using fluorescence spectroscopy and depending on the molecular dynamics simulations the distance between fluorenyl units is between 3.6-3.8 Å. By using complex impedance spectroscopy, they measured the sheet resistance as 0.1 M Ω /sq in air and 500 M Ω /sq in vacuum.

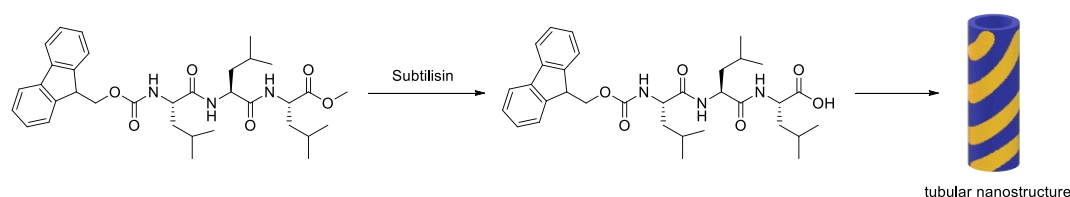


Figure 1.13. Enzyme-triggered ester hydrolysis of Fmoc-Leu-Leu-Leu-OMe and self-assembly of Fmoc-Leu-Leu-Leu-OH to tubular nanostructure. Yellow color represents Fmoc moiety.

As mentioned before, cyclic even number of alternating D-, L- amino acid containing peptides also assemble in anti-parallel fashion to form β -sheet, thus peptide nanotubes. In a study, the peptide nanotubes of (L-Gln-D-Ala)₄, (L-Gln-D-Leu)₄ and (L-Gln-D-Phe)₄ were found to be poor tunneling conductors by using complex band structure approach.¹⁵ It was claimed that, using better stacked aromatic rings could improve the conductivity of peptide nanotubes to be used as molecular wires. Ghadiri and his colleagues used four cationic 4,5,8-naphthalenetetracarboxylic diimide residue in a eight amino acid containing cyclic peptide to trigger self-assembly by redox reaction (Figure 1.14).⁷³ Their designed assembled nanotube model represented that the side chain NDI residues bend to form effective π - π stacking with a distance of 3.6 Å which is crucial for effective charge-transfer among a long range. Moreover, new methodologies were developed for the deposition of natural amino acid containing peptide nanotubes on gold electrode and it was found that highly efficient electron transfer takes place by hopping mechanism at the low voltage regime.⁸⁹

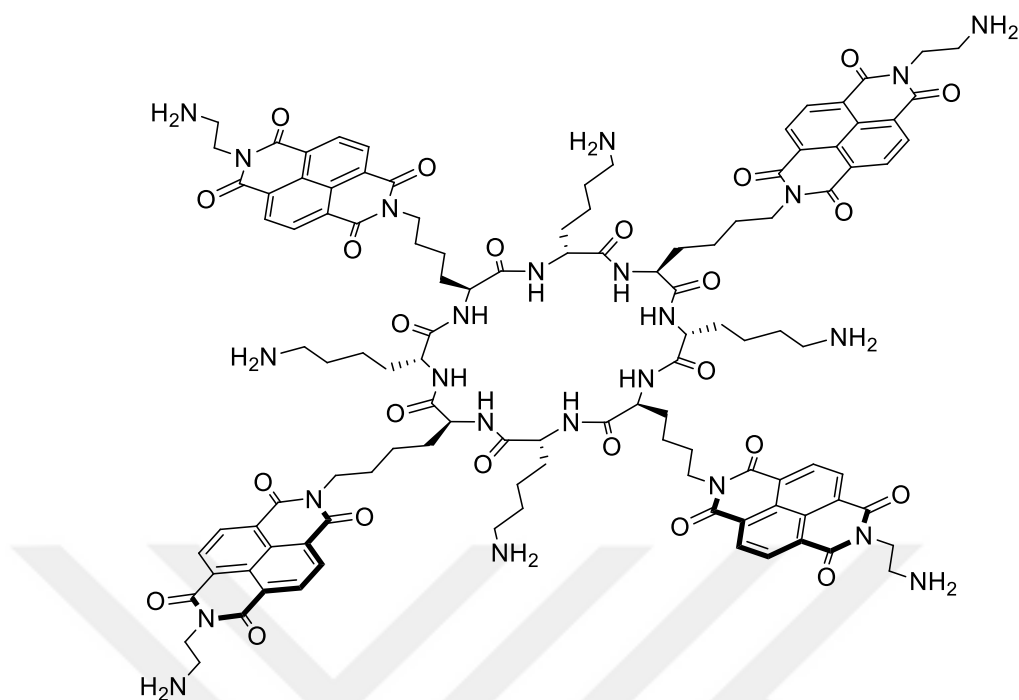


Figure 1.14. Chemical structure of NDI containing cyclic peptide.

Beside above peptides that forms peptide nanotubes, cyclic tri- β peptides are able to form nanotube structures.⁹⁰ Kimura and his coworkers synthesized cyclic tri- β peptides that contains one or three benzyl units on the side chains of β -amino acids, and after the self-assembly process the conductance values were measured as 0.05 and $0.17 \times 10^{-9} \text{ S}$ respectively.⁹¹ In addition, the control peptide that lacks benzyl unit on the side chain has a conductance as low as $2.3 \times 10^{-14} \text{ S}$ indicating the importance of aromatic units for the long range charge transfer.^{91,92}

Another method to enhance conductivity is to use both electron rich and electron poor aromatic units on β -sheet forming peptide assemblies.⁹³⁻⁹⁵ Tovar *et. al.* synthesized oligothiophene containing self-assembling peptides, with a NDI residue located peripherally on the side chain of lysine moiety in this case.⁹³ The oligothiophene moiety acts as donor unit and its excitation results with electron transfer to the acceptor unit NDI (Figure 1.15). The self-assembled peptide

nanostructure used in this study has a 100 times higher charge-separation lifetime when compared to the donor-acceptor π -peptide hybrid isotropic solution.

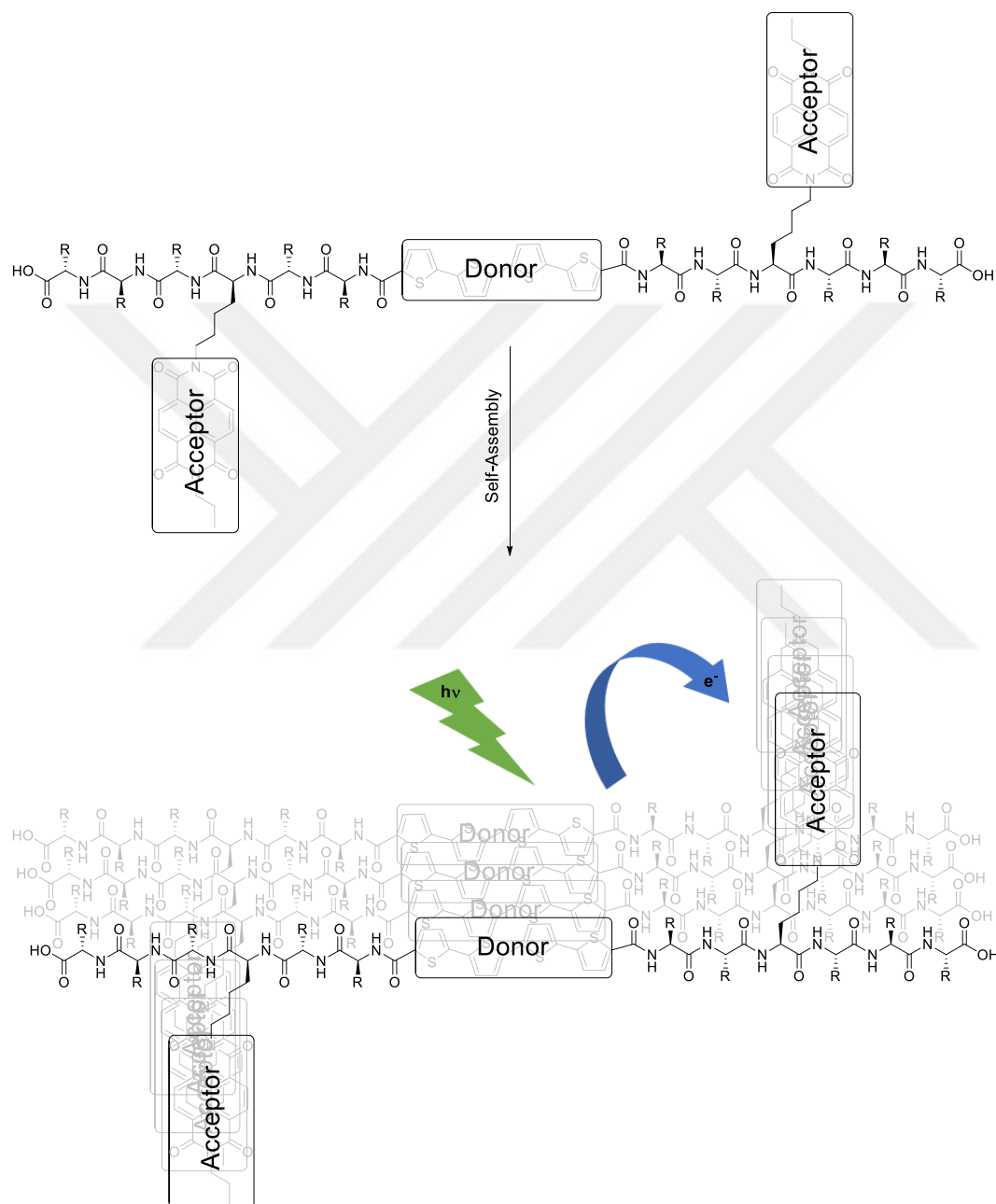


Figure 1.15. Self-assembly of donor-acceptor π -peptide hybrid and its electron transfer after excitation.

Beside using donor and acceptor groups on same peptide sequence as a hybrid structure, Güler and his colleagues synthesized two different peptide sequences that can able to co-assemble not only with hydrogen bonds and electrostatic interactions, but also with charge-transfer complex formation of their donor and acceptor moieties on their N-terminus (Figure 1.16).⁹⁴ The self-assembly of pyrene containing p-type peptide and NDI containing n-type peptide was achieved individually at different conditions. The co-assembly of both peptides was done by mixing p-type peptide solution at pH 5 and n-type peptide solution at pH 8 in 1:1 ratio. The electrical measurements of all peptide nanostructures were performed by using semiconductor parameter analyzer. The co-assembled peptide assembly has a conductivity value of 1.65×10^{-5} S/cm which is around 2400 and 10 times more conductive than n-type and p-type self-assembled peptide films, respectively.

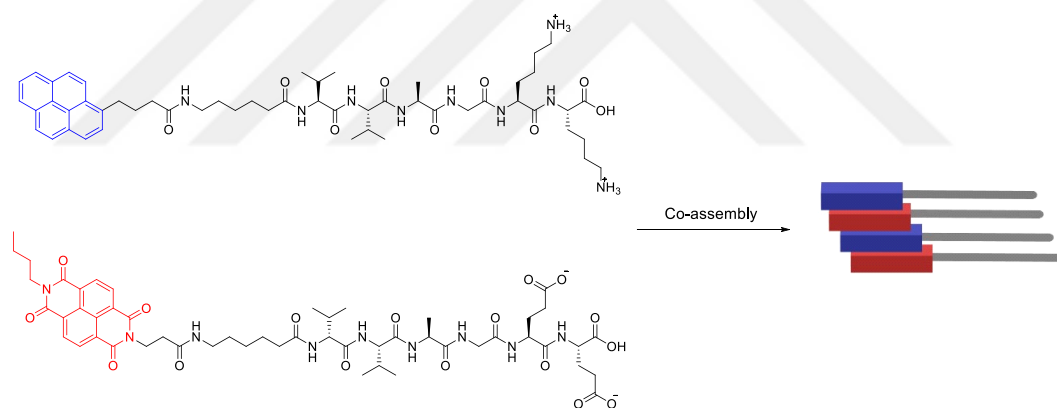


Figure 1.16. Co-assembly of p-type and n-type peptides in alternating order.

In addition, cross-linking between peptides in self-assembled form used to develop mechanical properties of gels and thus electronic properties.⁹⁶ Moreover, device fabrication is also an important challenge for researchers. It was reported that applying the annealing process to peptide solution coated surface increases the value of measured conductivity.⁹⁷ Beside the utility of β -sheet forming peptides to develop

new materials for biological and electronic applications, α -helix is also a preferred secondary structure to design new conductive materials.^{98–100} It was found that the oligothiophene containing α -helix forming peptide has a hole mobility in the order of $10^{-7} \text{ cm}^2/\text{V.s.}$ ⁹⁸

1.1.4 Solid phase peptide synthesis

Solid Phase Peptide Synthesis (SPPS) is a highly practical and wisely developed method for the preparation of peptides in changing lengths by Merrifield in early 1963.¹⁰¹ He was considered worthy for the Nobel Prize for developing this new synthesis methodology by using a solid matrix in 1984. In SPPS, an insoluble polymeric resin is used as a solid support in order to grow peptides on it. Most importantly, the complicated isolation procedures of peptide intermediates is eliminated with this methodology. The waste solvent, reagent and by-products can be removed with a simple filtration and washing procedure. Thus the SPPS method is highly speedy and easy in practical manner. Nevertheless, researches and companies developed SPPS method in many way to decrease chemical use, time of reaction, side product formation and so on.¹⁰² Therefore, automated and microwave assisted instrumentations are in demand in recent years.

General SPPS cycle is shown in Figure 1.17 and Scheme 1.1. There are different types of resins found in the market to be used depending on the peptide sequence and researchers aim.¹⁰³ Resin may or may not be loaded with first amino acid. First amino acid loading is differs according to the type of resin used. After loading first amino acid to the resin or using directly the commercially available amino acid loaded resin, one may start to the peptide synthesis cycle. SPPS starts from the C-terminus of an amino acid and ends up with the N-terminus of the synthesized peptide.¹⁰⁴ Amino acids that will be bounded to one another contains protecting units. One of the protecting unit is a temporary group bounded to the amino group of resin

bounded amino acid that should be cleaved at the beginning of each cycle, whereas the other protecting group should be permanent up to the cleavage of peptide from resin. This permanent protecting group is bounded to the functional units of side chains of amino acids to prevent any undesired reaction to occur during the peptide synthesis because of the reagents that would be used. The deprotection of temporary protecting group was done by a deprotection reagent. Immediately after the removal of this group, activation of next amino acid should be done with a coupling reagent to attain reactive ester derivative of aforementioned amino acid. The activated amino acid derivative is then added to the first amino acid loaded resin. The course of the reaction could be checked easily and quickly with qualitative colorimetric tests. Nevertheless, this method may be misleading in some cases since it depends on naked-eye control. After the completion of coupling reaction, peptide synthesis cycle start again with deprotection step. The cycle diminishes when the desired peptide sequence is synthesized. Herein, cleavage reagent should be added on peptide-bounded resin to cleave not only the peptide from resin, but also the permanent side chain protections of amino acids.

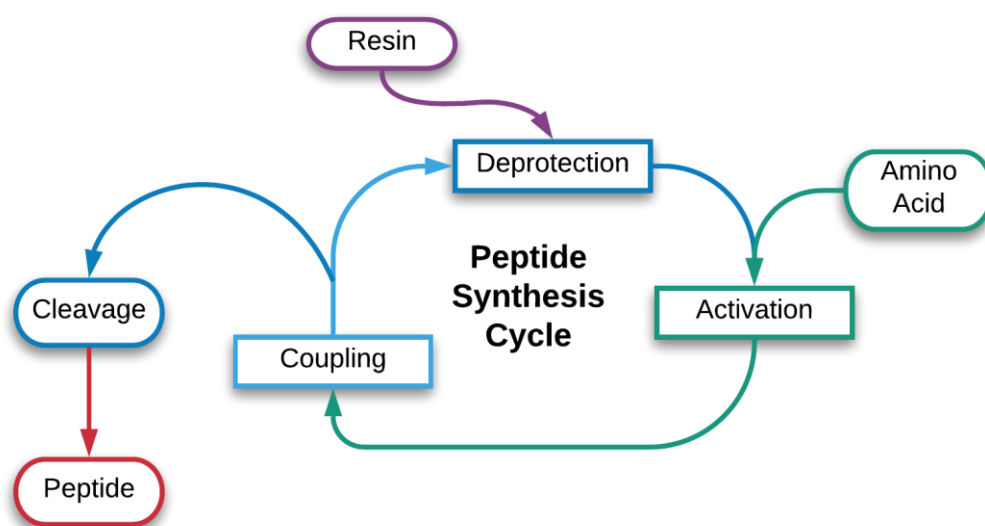
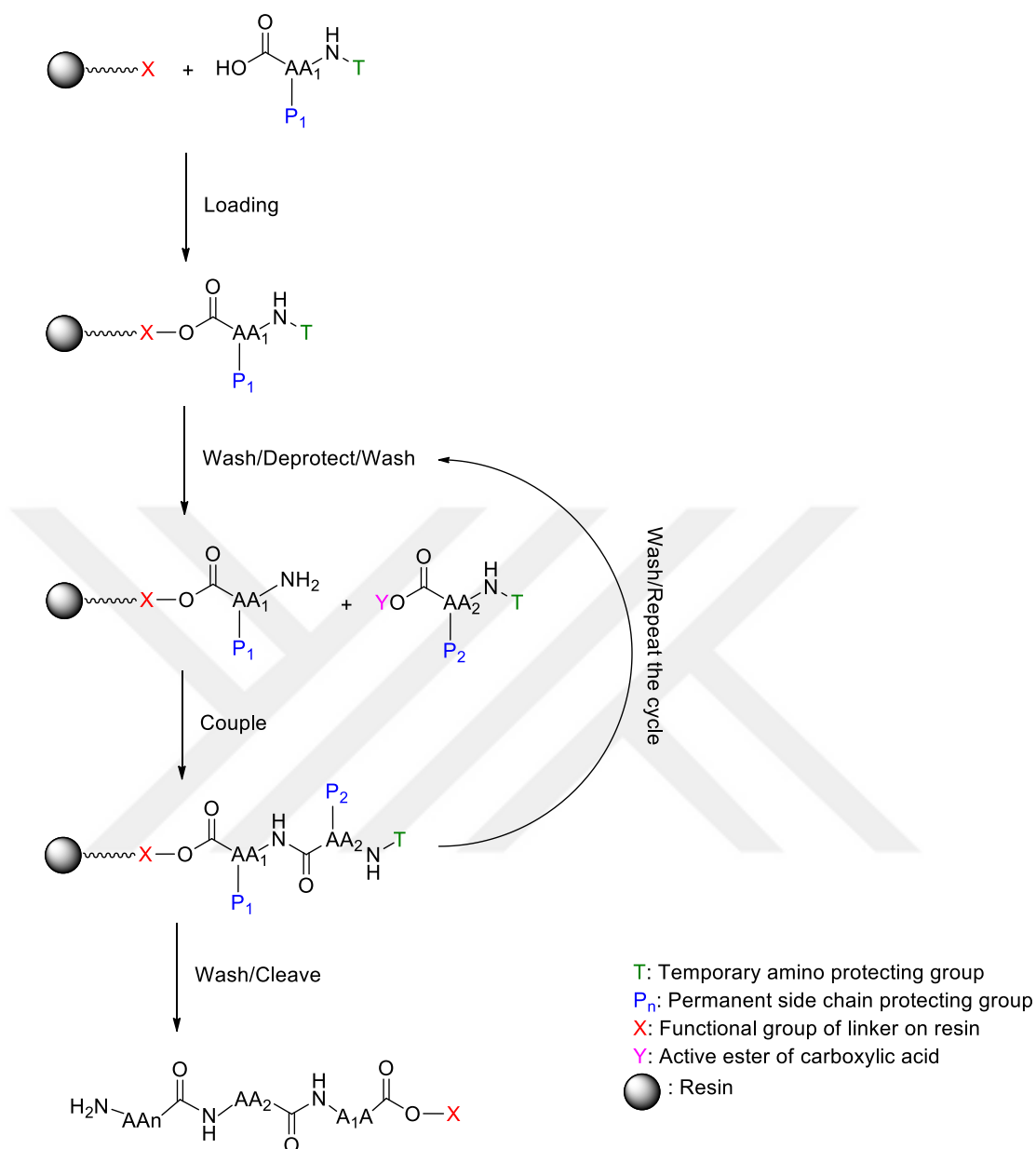


Figure 1.17. Standard Solid Phase Peptide Synthesis Cycle.

The SPPS method developed by Merrifield depends on the Boc/Benzyl chemistry in which the temporary protecting groups of amino groups are *tert*-butoxycarbonyl (Boc) and the permanent side chain protections are benzyl based protecting groups. In this method, the deprotection of Boc unit should be done with trifluoroacetic acid (TFA) in every cycle which causes harsh conditions for some peptide sequences that can result with undesired modifications. Moreover, at the end of the peptide synthesis the peptide should be cleaved from resin with hydrofluoric acid. In parallel with high toxicity of HF, special equipments are necessary to be used. Besides the speed coupling times of Boc-SPPS method, researchers developed 9-Fluorenylmethoxycarbonyl (Fmoc) amino protecting group¹⁰⁵ and then Fmoc/*t*Bu chemistry for SPPS.¹⁰⁶ By using this method, milder conditions are achieved. The temporary amino protecting group Fmoc could be removed with 20% (v/v) piperidine solution in *N,N*-dimethylformamide (DMF) and coupling reactions are performed in DMF after activating the next amino acid by using coupling reagents such as HBTU, HATU, EDC, DIC, PyBOP, PyBrOP and their other uranium/aminium salts, carbodiimides and phosphonium salts, respectively. After synthesizing desired peptide sequence, cleavage of peptide from resin and the removal of acid labile side chain protections are done with 95% TFA solution which is called cleavage solution. The cleavage cocktail should also contains scavengers and react with the cleaved highly reactive side chain protections to prevent unwanted peptide modifications. The isolation of crude peptide is generally done by settling in ether. Moreover, simple and affordable glassware is used in Fmoc-SPPS method. Nevertheless, there are always obstacles that have to be overcome to achieve the synthesis of especially the biologically relevant peptides.^{107,108}



Scheme 1.1. General Protocol for Solid Phase Peptide Synthesis.

1.1.5 Aim of the study

As mentioned before, natural amino acids' side chains are not sufficient to create electrically conductive systems as other organic π -conjugated polymers. However,

conducting polymers have complicated synthesis and isolation protocols as well as solubility problem in aqueous medium. As a consequence, researchers focus on aromatic unit conjugated self-assembled peptides to develop new conductive materials for organic electronics, tissue engineering and other biomedical applications. β -sheet forming peptides are quite preferable to create new conductive systems since it is possible to attain a long-range charge transfer among π - π stacked aromatic units in 1-D systems. Although researchers claim that the distance between aromatic units is calculated as 3.4 Å, when they are bounded to side chain of amino acid or N-terminus of self-assembled β -sheet forming peptides, the optimum distance between each peptide strand is known as 4.7-5.0 Å for β -sheet secondary structure. This distance is too long for an effective π - π stacking among aromatic units.

In order to acquire enhanced conductivity by using self-assembled peptides (Figure 1.19 and Figure 1.20), we have designed four new artificial amino acids that lowers the distance between aromatic units in β -sheet structure to attain effective π - π stacking (Figure 1.18), thus improved charge transfer along the self-assembled peptides.¹⁵ The lysine derived artificial amino acids contain two parallel aromatic units on their side chains. Terpyridine and pyrene moieties were chosen for this study as aromatic groups. 2,2':6',2''-terpyridines are simply known as terpyridine were discovered in early 1930s and continue to be used in many applications as sensor for clinical chemistry and biochemistry because of its superior binding properties to metals.¹⁰⁹ Terpyridine is known to act as a tridentate ligand to form complexes with metal ions such as Mn(II), Fe(II), Ni(II), Zn(II), Pt(II) and Ru(II) which might be used to increase conductivity in self-assembled peptides.¹⁰⁹⁻¹¹¹ In the literature, metal-bonded terpyridine containing cyclic beta peptides were discovered to form self-assembled nanostructures.¹¹² Moreover, the study of Yaghi and coworkers represented that it is possible to apply SPPS to synthesize metal-coordinated terpyridine containing peptides.¹¹³ Furthermore, the electron deficient terpyridine moiety could be used together with electron rich pyrene moiety in self-assembled systems to obtain donor-acceptor type systems.

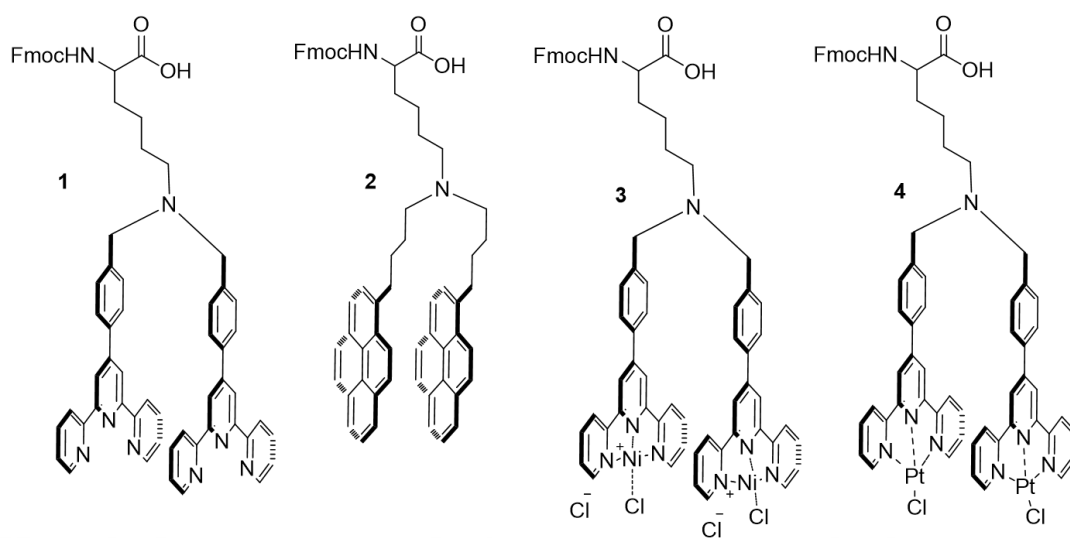


Figure 1.18. Designed artificial amino acids **(1)** Bis-terpyridine derivative of Fmoc-L-Lysine-OH, **(2)** Bis-pyrene derivative of Fmoc-L-Lysine-OH, **(3)** Ni-coordinated **(1)** and **(4)** Pt-coordinated **(1)**.

In this study, the synthesis of designed artificial amino acids were performed, at first. Then, self-assembling peptides that contain artificial amino acids were synthesized by using solution phase and solid phase peptide synthesis method. Not only 9, 11 and 17 number of amino acids containing conductive β -sheet forming peptides that is able to form hydrogels were attained, also 8 amino acid containing nanotube forming cyclic peptides was tried to form self-assembled nanostructures. Moreover, self-assembling cyclic dipeptides were used as template to obtain conductive peptides that contains artificial amino acids. To compare the conductivities, peptides that do not contain artificial amino acids, mono-terpyridine containing cyclic dipeptide and Pt-coordinated bis-terpyridine containing cyclic peptides were also synthesized and gelation was done. TEM imaging, CD and IR spectroscopy were utilized to prove self-assembly and nanostructure formations. The electrical measurements were done collaboratively with Prof. Dr. Sait Eren San, Assist. Prof. Gökhan Bakan and Assoc. Prof. Gökem Günbaş.

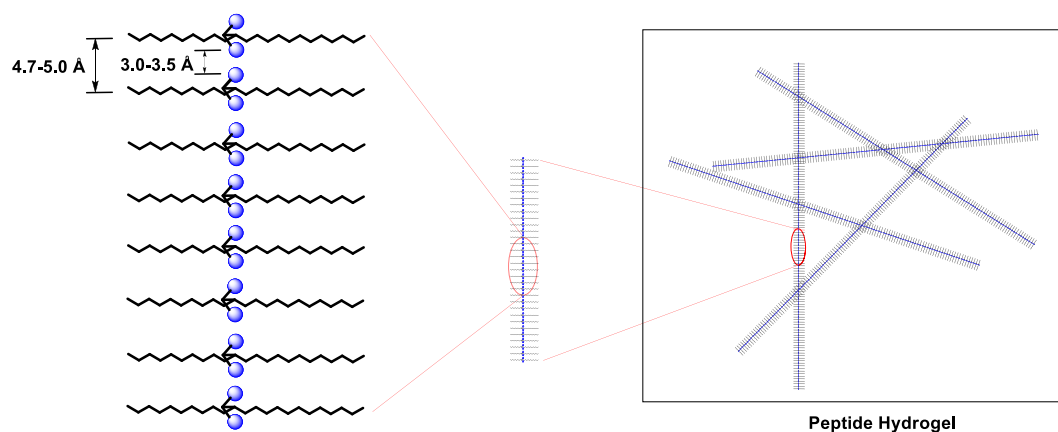


Figure 1.19. Representative image of reduced distances between aromatic units incorporated in β -sheet forming peptides, fibril formations and entanglement of fibrils.

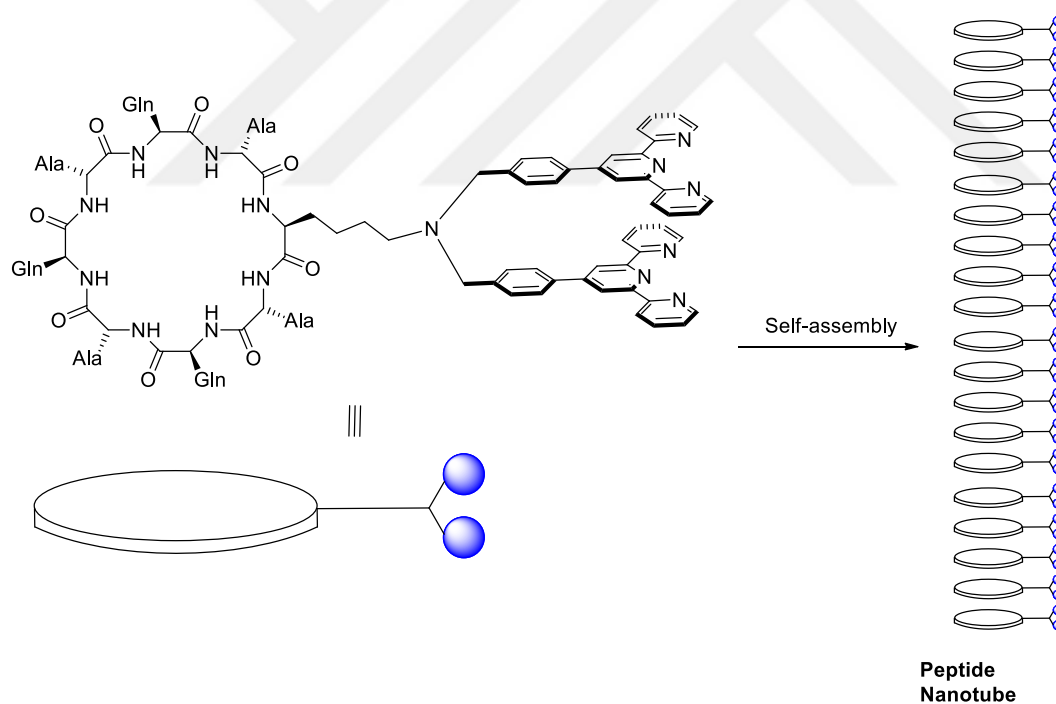
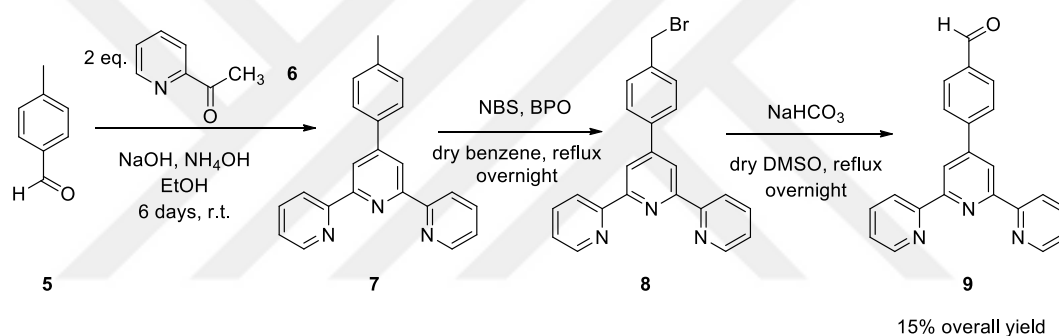


Figure 1.20. Representative image of 1-D molecular wire obtained by the self-assembly of cyclic octapeptide.

1.2 Results and Discussion

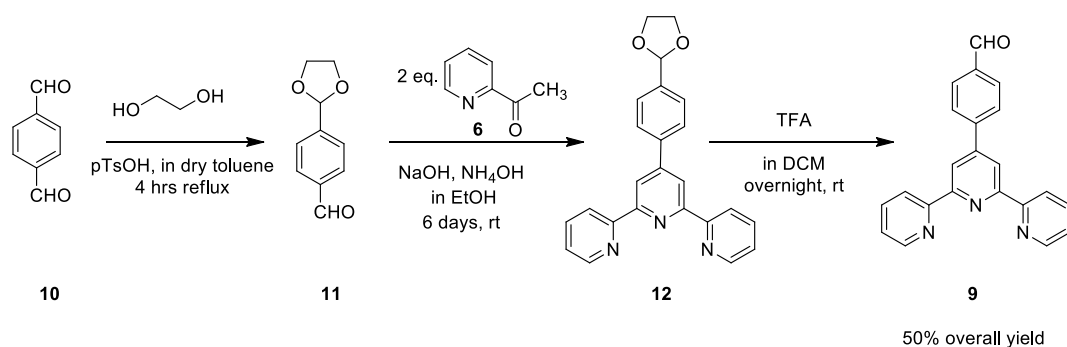
1.2.1 Synthesis of artificial amino acid 1

For the synthesis of artificial amino acid **1**, we started with the synthesis of 4'-(methylphenyl)-2,2':6',2''-terpyridine (**7**), then 4'-(4-Bromomethylphenyl)-[2,2':6',2'']terpyridine (**8**) and 4'-(4-Formylphenyl)-[2,2':6',2'']terpyridine (**9**) afterward, depending on the literature procedures (Scheme 1.2).¹¹⁴ However, the overall yield of **9** is quite low as 15%.



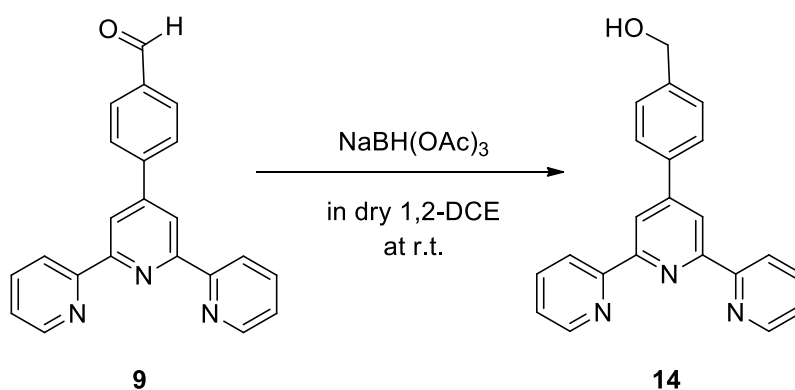
Scheme 1.2. Literature route for the synthesis of **9**.

Thus, a new synthesis path was designed and the overall yield was elevated to 50% as shown in Scheme 1.3. Starting from terephthalaldehyde (**10**), the protection of one aldehyde with ethylene glycol was performed depending on literature procedure.¹¹⁵ After that, the synthesis of 4'-(4-(1,3-dioxolan-2-yl)phenyl)-2,2':6',2''-terpyridine (**12**) was achieved by using 2-acetyl pyridine (**6**). The removal of acetal unit was performed by using TFA to obtain **9**.¹¹⁶



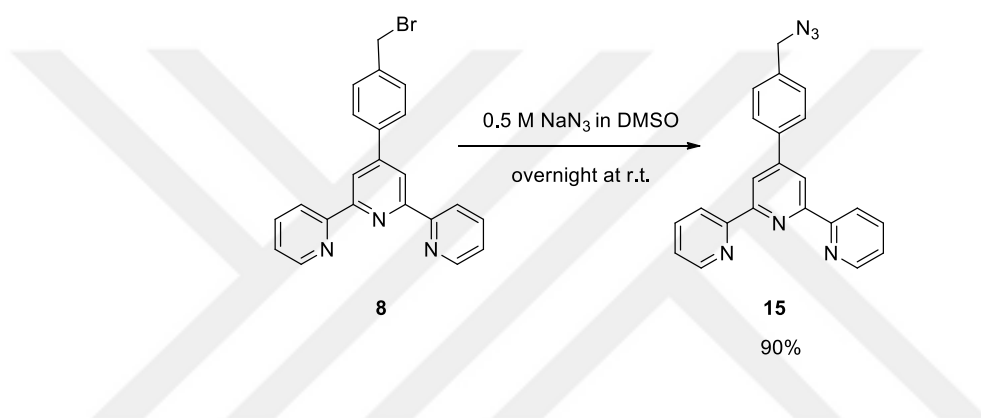
Scheme 1.3. New synthesis route for the synthesis of **9**.

To obtain **1**, reductive amination was performed between one equivalent of Fmoc-L-Lysine-OH (**13**) and two equivalents of **9** by using $\text{NaBH}(\text{OAc})_3$ as reducing agent, based on the literature procedure.¹¹⁷ It was observed that the imine formation was not achieved even after overnight stirring at room temperature. The reducing agent addition resulted with the conversion of **9** to (4-([2,2':6',2''-terpyridin]-4'-yl)phenyl)methanol (**14**) without any desired product formation (Scheme 1.4). Then, the imine formation was tried in the presence of acid catalyst, but the presence of basic nitrogens prevent the reaction to occur.

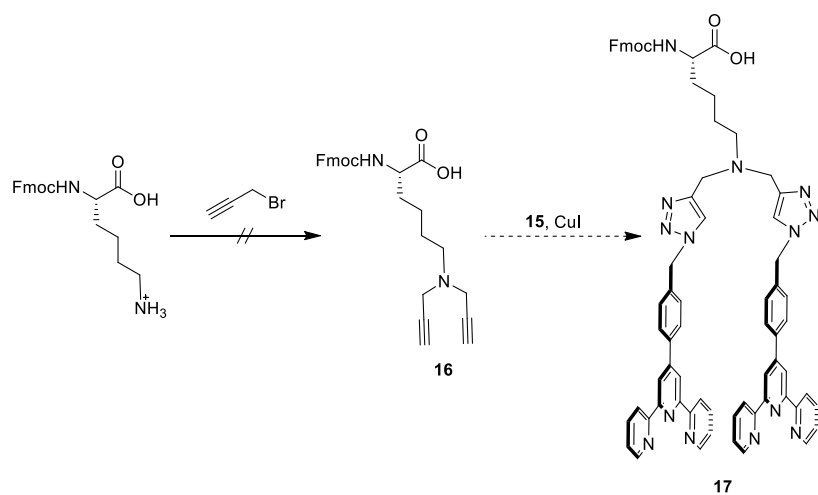


Scheme 1.4. Reduction of **9** to **14**.

It was decided that the non-flexible structure of **9** prevent amination with **13**, especially for the binding of second **9**. We decided to perform bis-propargylation of Fmoc-L-Lys-OH.TFA with propargyl bromide¹¹⁸ to make the product react with 4'-(4-(azidomethyl)phenyl)-2,2':6',2''-terpyridine (**15**) (Scheme 1.5) and obtain double click product as shown in Scheme 1.6 to bind terpyridine moieties to amine with longer linker groups. However, the bis-propargylation reaction was not achieved even in different conditions.

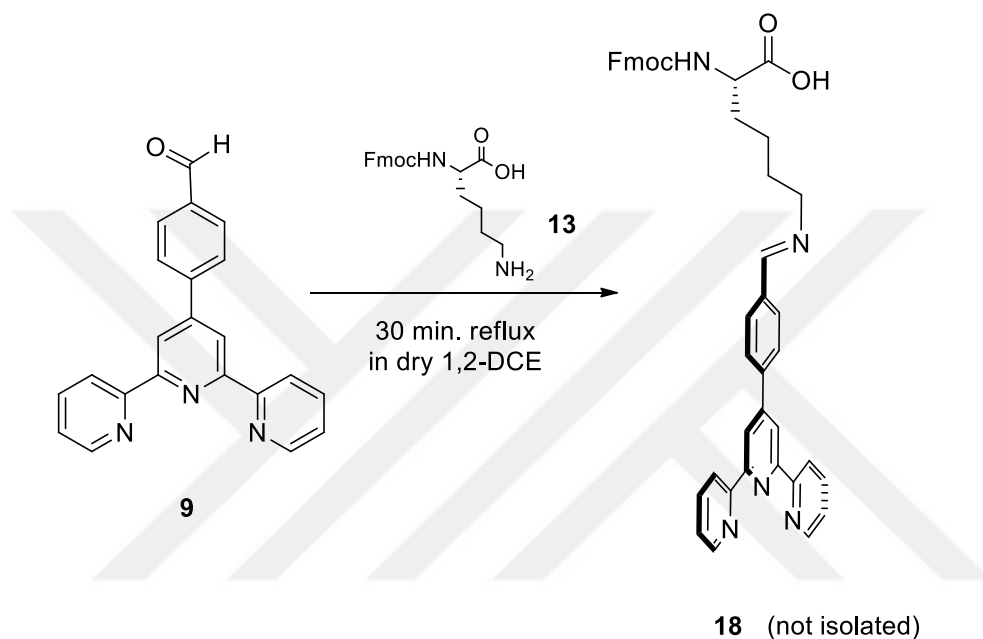


Scheme 1.5. Azide (**15**) synthesis from **8**.



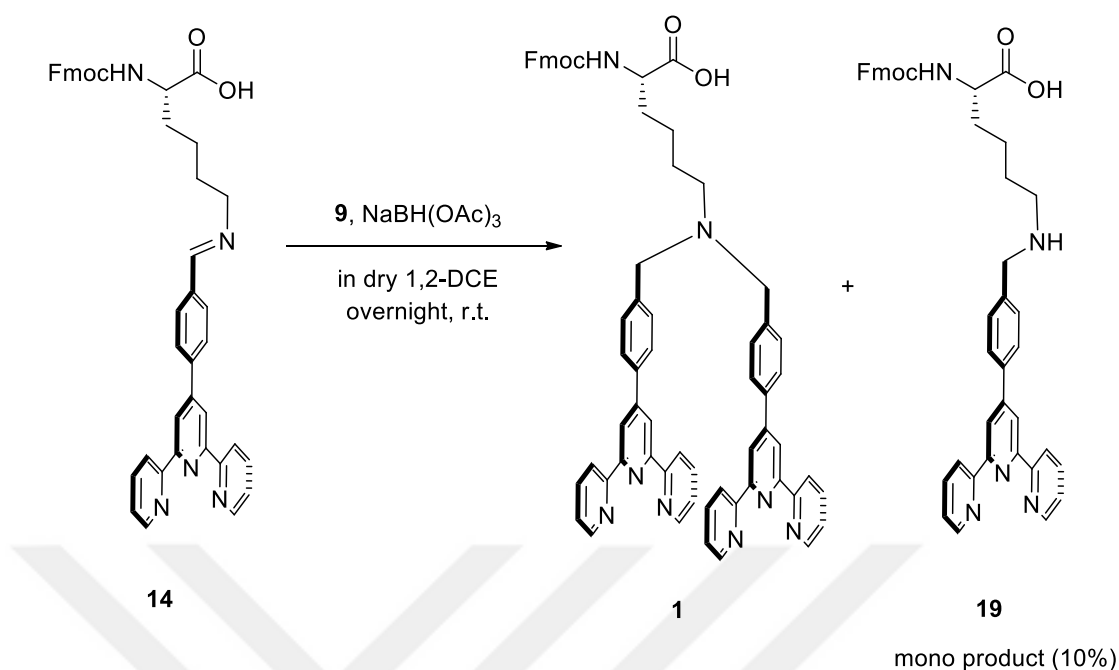
Scheme 1.6. Bis-propargylation of Fmoc-L-Lys-OH.TFA to yield **16** and **17** with click reaction.

Then, it was decided to perform reductive amination again. At last, 10 to 30 minutes reflux was done in dry 1,2-DCE for the imine (**18**) formation with one equivalent of **9** (Scheme 1.7). Then, the addition of 1.25 equivalent $\text{NaBH}(\text{OAc})_3$ was done for reduction.



Scheme 1.7. The reaction of **9** and **13** to form imine under reflux conditions.

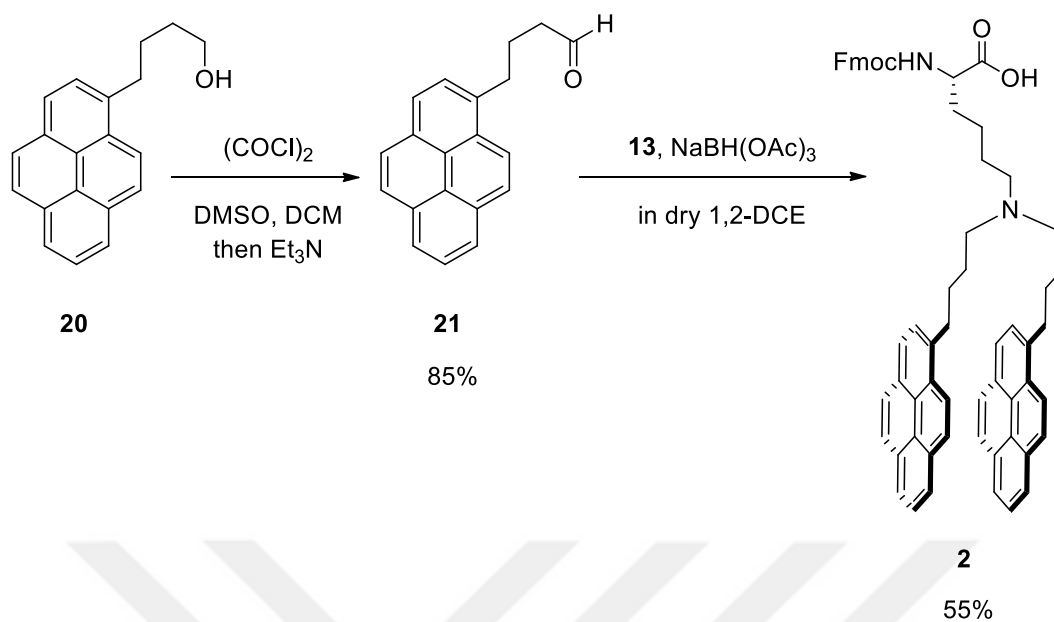
The second equivalent of **9** was added in to the reaction medium afterward with $\text{NaBH}(\text{OAc})_3$ in 0 °C to obtain **1** (Scheme 1.8). The isolated crude product contains not only bis-adduct **1**, but also mono-adduct **19** with 10% equivalency depending on the NMR analysis. The isolation of **19** and purification of **1** were not achieved by using preparative TLC with silica gel and column chromatograph with stationary phases; silica gel, basic and neutral alumina. The purification of **1** only be achieved by using semi-preparative reverse phase HPLC only in little amounts for structural identification with NMR and HRMS. Thus the synthesis of peptides was performed without further purification.



Scheme 1.8. In situ synthesis of bis-adduct **1** and mono-adduct **19**.

1.2.2 Synthesis of artificial amino acid **2**

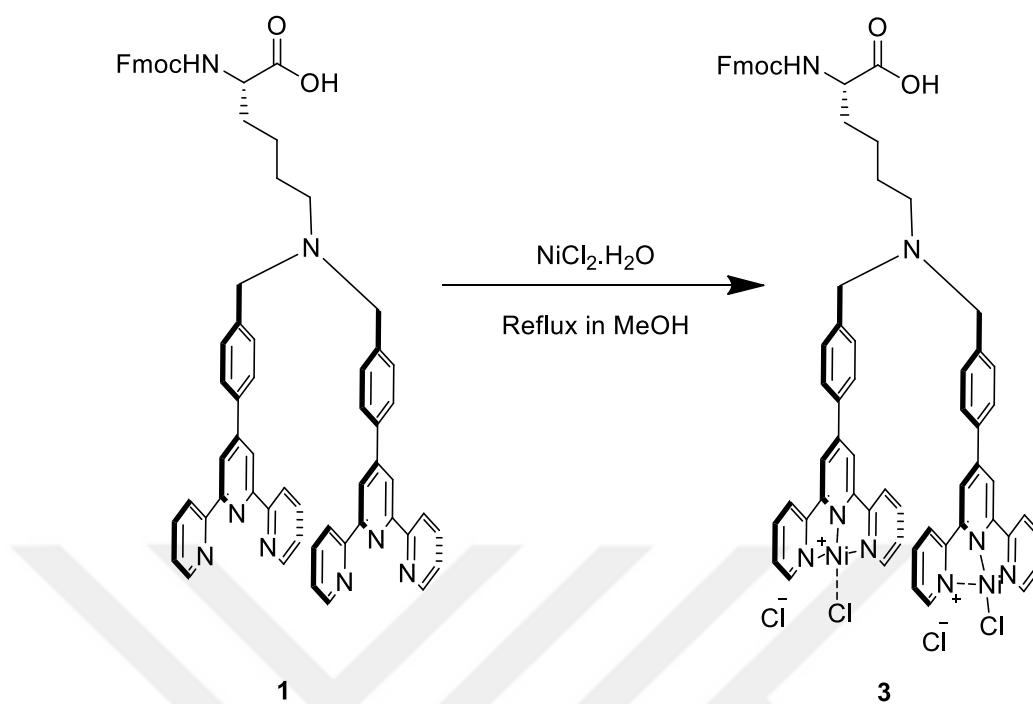
In order to synthesize artificial amino acid **2**, we started with the commercially available 1-pyrenebutanol (**20**). Swern oxidation was performed to obtain 1-pyrenebutanal (**21**) on the literature (Scheme 1.9).¹¹⁹ Artificial amino acid **2** was synthesized successfully with modifications on literature procedure.¹¹⁷ The imine formation was achieved at room temperature with overnight stirring at equal amount of **21** and **13**. To speed reaction heat was applied, however the pyrene moiety decomposes at high temperatures. Thus, the addition of one equivalent reducing agent, then remaining aldehyde and the other one equivalent of NaBH(OAc)_3 was done in an order within two days to increase the yield. The isolation of **2** was achieved by applying column chromatography with silica gel.



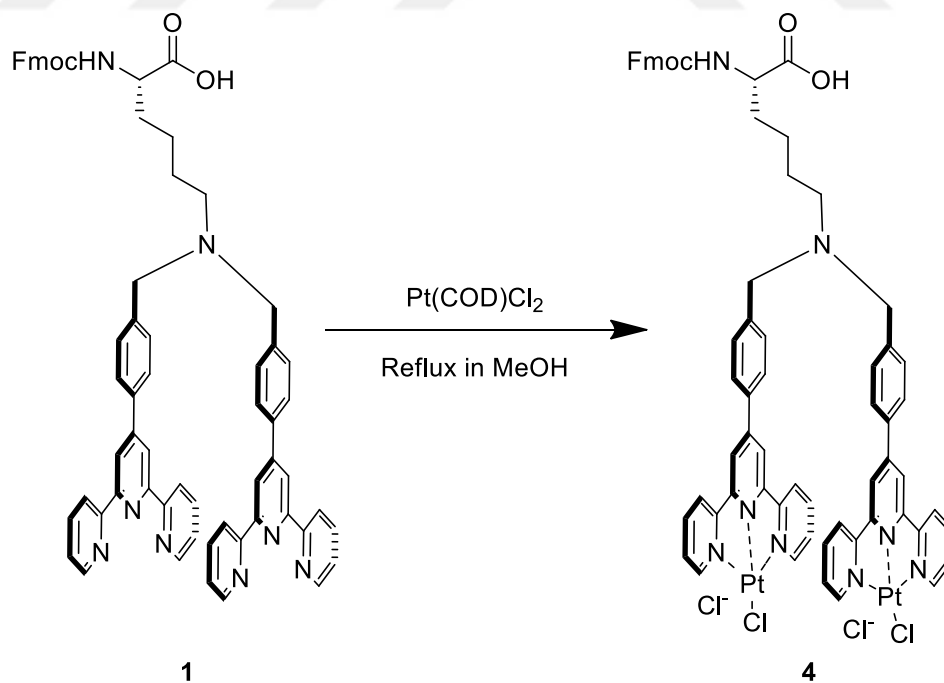
Scheme 1.9. The Swern oxidation to obtain **21** and then reductive amination to get bis-adduct **2**.

1.2.3 Synthesis of artificial amino acid 3 and 4

For the synthesis of metal coordinated bis-terpyridine containing artificial amino acid **1**, NiCl_2 (Scheme 1.10) and $\text{Pt}(\text{COD})\text{Cl}_2$ (Scheme 1.11) were used.¹¹³ The nickel and platinum coordinated products precipitates in high yield by cooling after overnight reflux in MeOH.



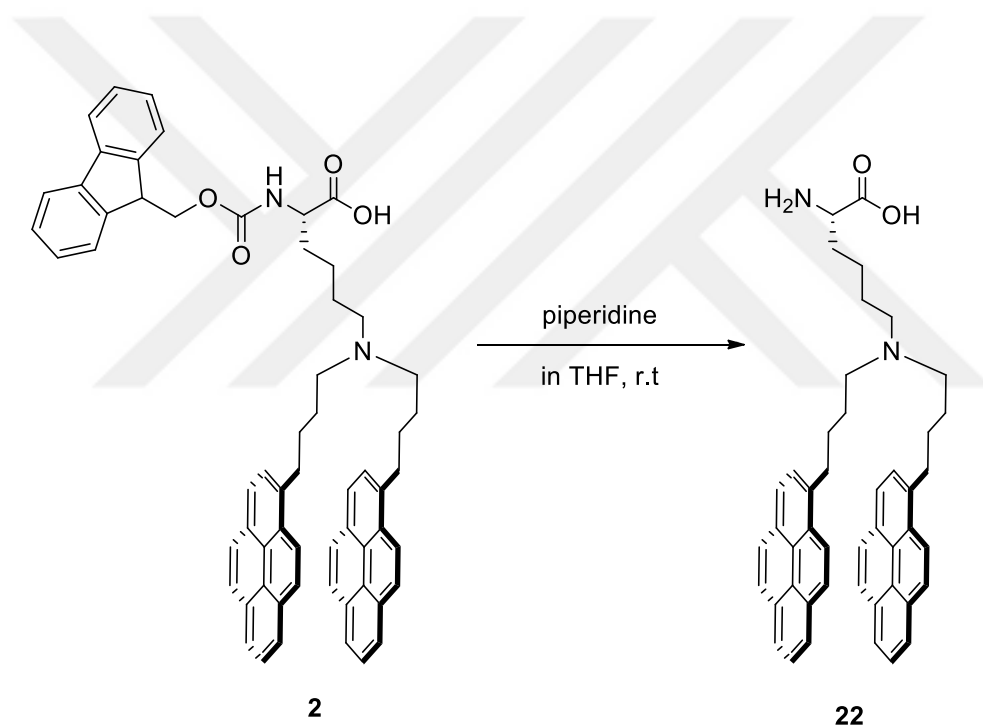
Scheme 1.10. Ni(II) coordination to artificial amino acid **1**.



Scheme 1.11. Pt(II) coordination to artificial amino acid **1**.

1.2.4 Π - Π stacking between aromatic units of artificial amino acid

In the designed artificial amino acids, the aromatic units should interact intramolecularly to attain π - π stacking which is necessary to attain long range charge migration effectively after self-assembly. To prove the π - π interactions among pyrene moieties in artificial amino acid **2**, fluorescence microscopy was used. Same concentrations of 1-pyrenebutanol (**20**) and Fmoc-cleaved derivative of **2** (**22**) was analyzed. The removal of Fmoc unit (Scheme 1.12) is necessary to eliminate the interference of its fluorescence emission with the emission of pyrene.



Scheme 1.12. Fmoc removal of **2** with piperidine.

In the fluorescence spectroscopy analysis (Figure 1.21), **20** shows two peaks at 379 nm and 398 nm when excited at 343 nm. The fluorescence emission intensities diminished at 379 nm and 398 nm, whereas a broad new peak with a maxima at 481

nm was appeared for **22**. This results are consistent with the literature¹²⁰ and proves the π - π stacking between pyrene moieties on the artificial amino acid.

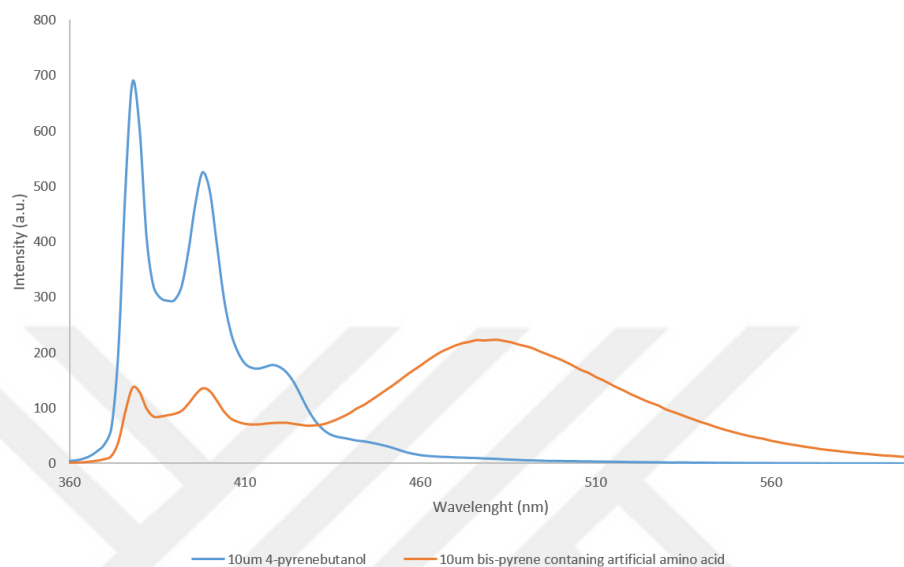


Figure 1.21. Fluorescence emission spectrum of **20** and **22**.

1.2.5 Design and synthesis of β -sheet forming conductive peptides

(FKFE)₂, (FKFE)₄, RADA-16 and cyclo-(Qa)₄ peptides are well-known peptides that have ability to self-assemble in β -sheet secondary structures.^{28,32,33} Additionally, P₁₁-4 and P₁₁-5 are complementary peptides that can assemble in alternating order at pH between 7.2-7.4.^{44,45} Thus, we decided to synthesize these β -sheet forming peptides with slight modifications and by incorporating artificial amino acids in to the middle of peptides. The sequences of synthesized β -sheet forming peptides is shown in Table 1.1 and Table 1.2. All β -Sheet forming peptides were synthesized using general SPPS protocol. All the coupling and Fmoc removal procedures were followed in DMF. *N,N,N',N'*-Tetramethyl-O-(1*H*-benzotriazol-1-yl)uronium

hexafluorophosphate (HBTU) was used as coupling reagent, and diisopropylethylamine (DIEA) as base. Natural amino acids are used as 5.5 equivalent to resin. Kaiser test was used to check the completion of the reaction when necessary. The cleavage of Fmoc units was performed by the addition 20 v/v% piperidine/DMF solutions. The cleavage cocktail contains 95% TFA, 2.5% milliQ water and 2.5% TIPS. The resin cleaved peptide solution was decanted in diethyl ether. The purification of all peptides was done by using *semiprep* RP-HPLC. The structures was justified using mass spectroscopy. The chosen resin, the amount of artificial amino acids used and other specifications will be indicated in the related parts.

Table 1.1. List of synthesized β -Sheet forming peptides.

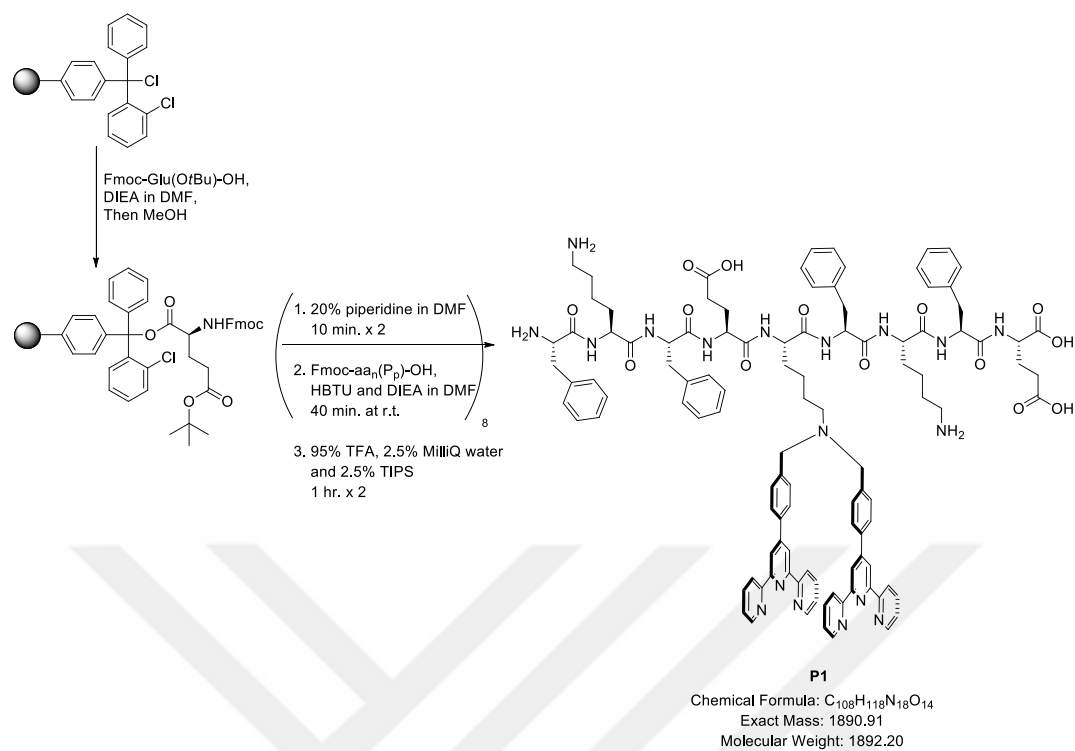
Entry	β -Sheet forming Peptide Sequence
P1	FKFE-1-FKFE
P2	FKFEFKFE-1-FKFEFKFE
P3	RADARADA-1-RADARADA-NH ₂
P4	RADARADA-2-RADARADA-NH ₂
P5	RADARADA-3-RADARADA-NH ₂
P6	RADARADA-K-RADARADA-NH ₂
P7	CYCLIC-QaQa-1-aQa
P8	CYCLIC-QaQaQaQa

Table 1.2. List of synthesized complementary β -Sheet forming peptides.

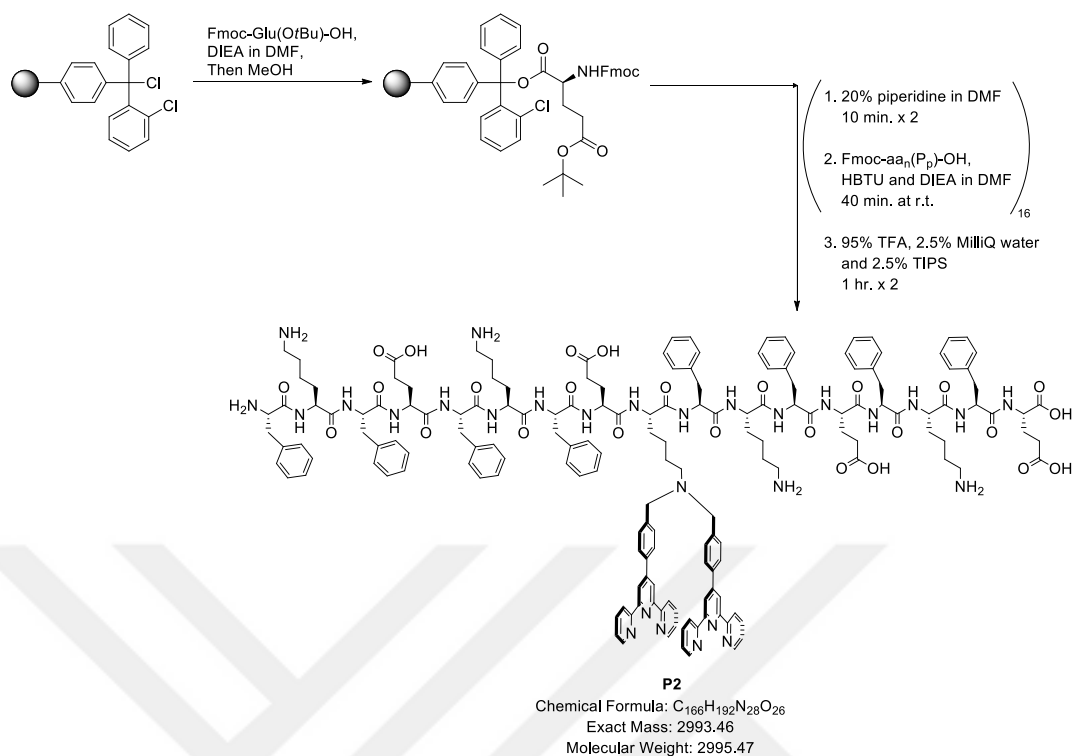
Entry	Complementary β -Sheet Forming Peptides Sequence
P9	QQRFEWEFEQQ-NH ₂
P10	Ac-QQRFEWEFEQQ-NH ₂
P11	QQKFKWKFKQQ-NH ₂
P12	Ac-QQRFE-1-EFEQQ-NH ₂
P13	Ac-QQKFK-1-KFKQQ-NH ₂
P14	Ac-QQRFE-2-EFEQQ-NH ₂
P15	Ac-QQKFK-2-KFKQQ-NH ₂

1.2.5.1 Synthesis of FKFE-1-FKFE peptide (P1) and FKFEFKFE-1-FKFEFKFE peptide (P2)

SPPS was used for the synthesis of **P1** (Scheme 1.13) and **P2** (Scheme 1.14) at room temperature. 2-chlorotrityl chloride resin was used as the solid support. The first amino acid (Glu) was bonded to resin with loading protocol. Amount of substitution was calculated using UV-Vis spectroscopy. Three equivalent of crude **1** was added for the coupling of artificial amino acid. The coupling of **1** was checked with Kaiser Test and continued overnight.



Scheme 1.13. SPPS of **P1** by using 2-chlorotrityl chloride resin and Fmoc chemistry. The coupling reaction of **1** continued overnight.

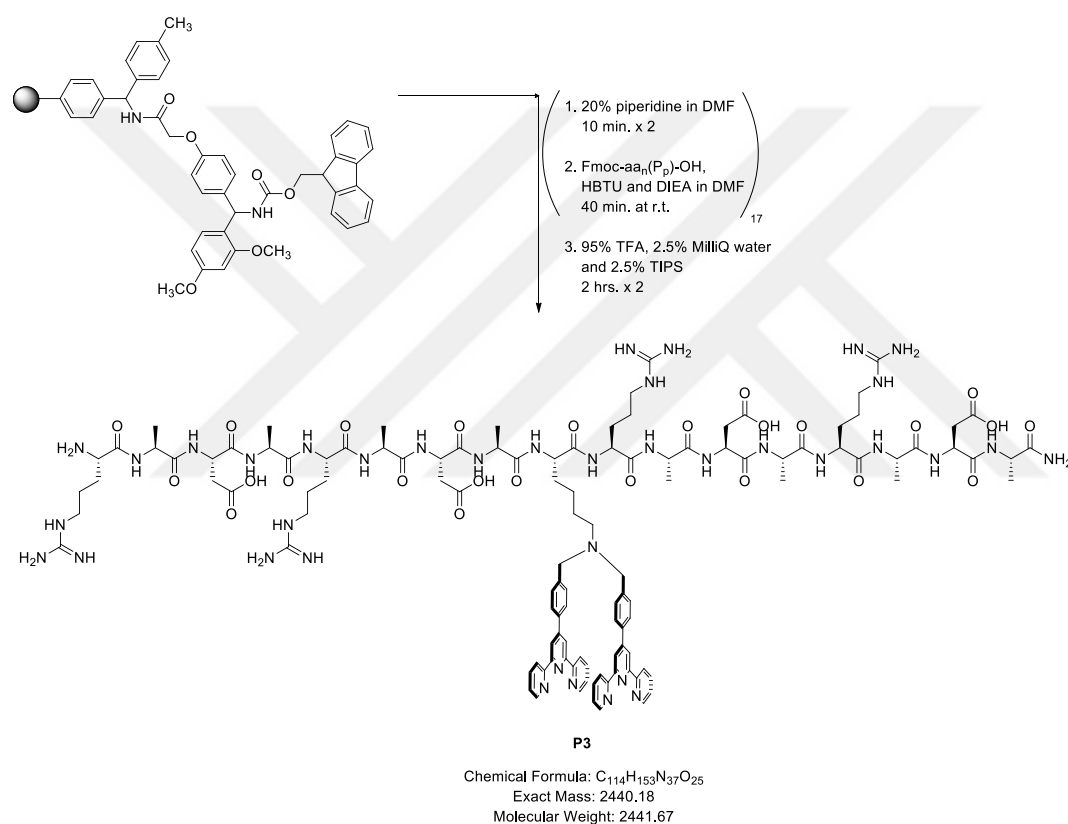


Scheme 1.14. SPPS of **P2** by using 2-chlorotrityl chloride resin and Fmoc chemistry. The coupling reaction of **1** continued overnight.

1.2.5.2 Synthesis of RADA-16 derivatives; **P3**, **P4**, **P5** and **P6**

P3, **P4**, **P5** peptides were synthesized by using SPPS (Scheme 1.15) and with incorporating artificial amino acids **1**, **2** and **3** in to the middle of peptide sequence, respectively. **P6** peptide contains lysine amino acid in the middle of sequence as a negative control for electrical measurements. All peptides were synthesized by using Rink Amide MBHA resin. This resin contains Fmoc unit at the end of the linker group, thus the synthesis starts directly by cleaving Fmoc with 20% piperidine/DMF solution and the coupling reaction is followed as standard SPPS procedure. For the coupling of **1**, **2** and **3**, three equivalent artificial amino acid were used and the completion of reactions were controlled with Kaiser Test. The reactions were

allowed to stay overnight. For the cleavage of peptides from resin, the peptide bounded resin was suspended in cleavage cocktail for two hours in each time due to the presence of 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) protecting groups at the side chains of arginine amino acids. The resin cleaved peptides has amide unit in its C-terminus because of using Rink Amide MBHA resin. The terminal amide group provides stability of peptides for longer times.



Scheme 1.15. SPPS of **P3**. The coupling reaction of **1** continued overnight.

The structures of **P4** and **P6** can be seen in Figure 1.22 and Figure 1.23.

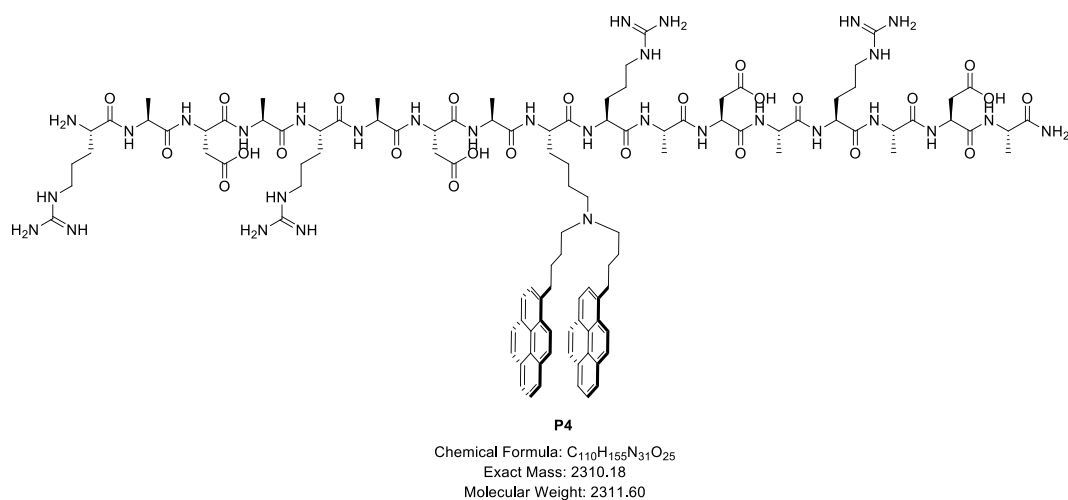


Figure 1.22. Structure of RADARADA-2-RADARADA-NH₂ peptide (**P4**).

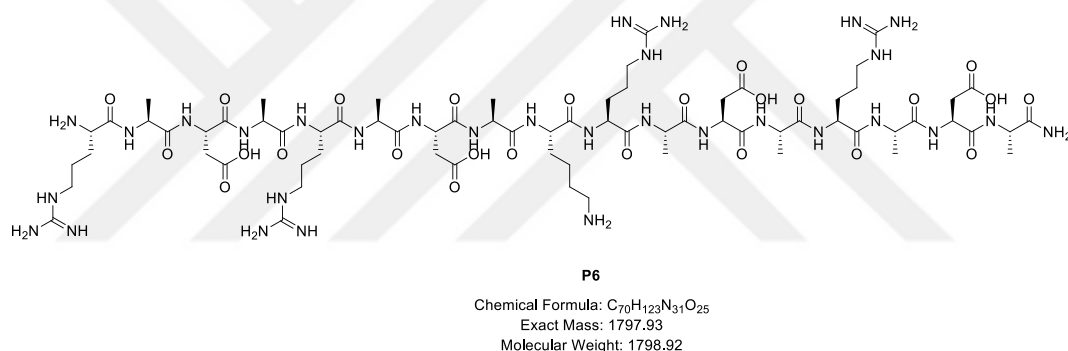
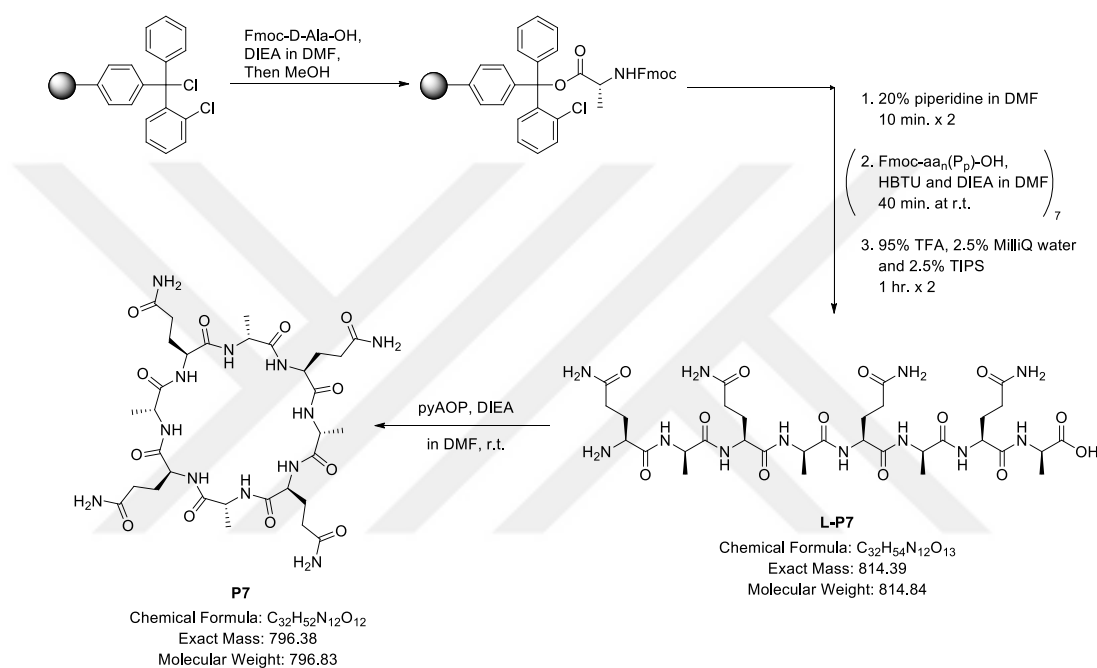


Figure 1.23. Structure of RADARADAKRADARADA-NH₂ peptide (**P6**).

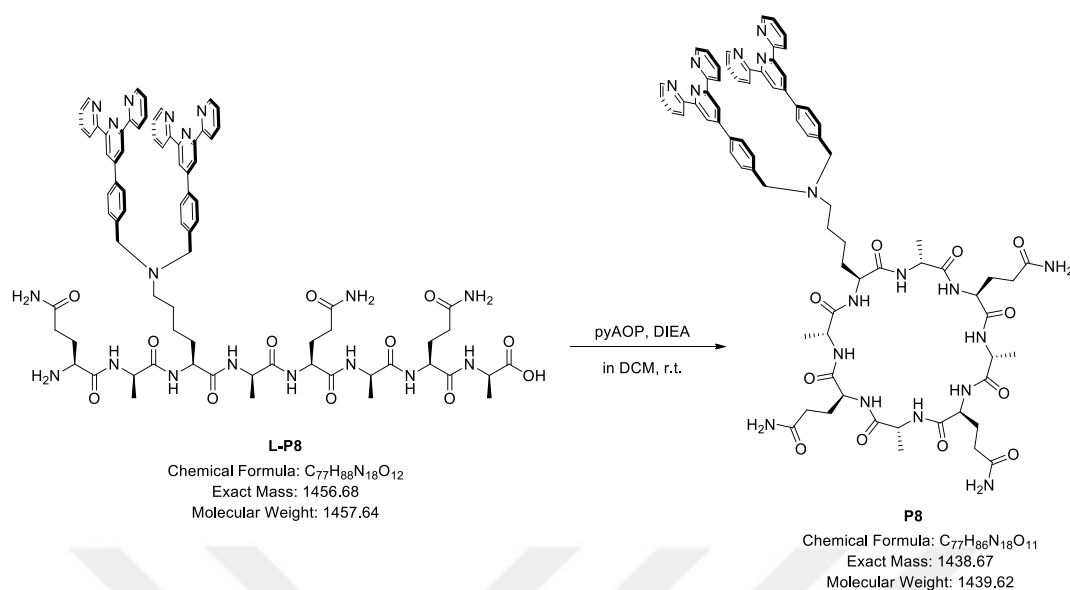
Unfortunately, during the cleave step of **P5** from resin, which contains the Ni(II) coordinated bis-terpyridines, the highly acidic medium causes the removal of nickel coordination from terpyridine moieties. The removal of nickel was seen with naked eye since the green color of resin observed after coupling of **3** disappears when the addition of cleavage cocktail was done. The mass spectroscopy analysis also resulted with the same mass data as **P3**. In a study, Yaghi and his coworkers¹¹³ used 5% TFA in 1,2-DCE to cleave metal-organic arrays from resin, which is not a sufficient amount of acid for the removal of side chain protections in our case (Scheme 1.16).

reactions of **L-P7** and **L-P8** were performed in solution phase by utilizing coupling reagent; PyAOP.¹²¹ The reaction mediums were too dilute to prevent the head-to-tail coupling of linear peptides and other undesired reactions that could be occur with the side chains' functional groups. The completion of reactions were controlled with analytical RP-HPLC analysis.



Scheme 1.17. SPPS of **L-P7** and solution phase cyclisation to yield **P7**.

The solution phase cyclisation of **L-P8** is represented in Scheme 1.18.



Scheme 1.18. Solution phase cyclization of **L-P8** to yield **P8**.

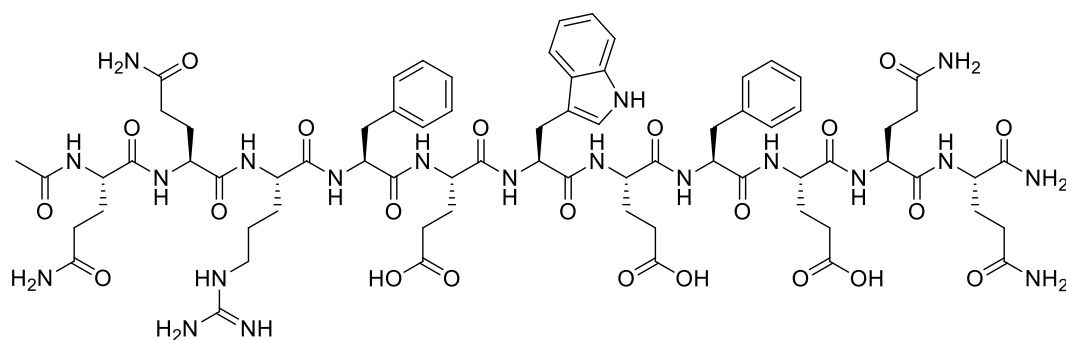
1.2.5.4 Synthesis of complementary peptides **P9**, **P10** and **P11**

P9, **P10** and **P11** were synthesized with microwave-assisted SPPS protocol by using Rink Amide MBHA resin (Scheme 1.19) as negative control for electrical measurements. The Fmoc removal and coupling reactions occurs in a short period of time compared to the standard SPPS protocols that occur at room temperature. Shorter coupling times decreases the formation of side reactions and thus increases yield by applying microwave-assisted SPPS protocol. After synthesizing desired peptide sequences, the cleavage of peptide from resin is followed as in earlier. Unfortunately, the peptides that contain glutamic acid or glutamine on their N-terminus can undergo pyroglutamate formation reaction in acidic medium (Scheme 1.20) during the cleavage step as a side reaction. The mass analysis of **P11** crude peptide (Figure 1.26) represented pyroglutamate formation at the N-terminus with the molecular ion peak 17 g/mol lower than the mass of **P11** (Figure 1.24), which was resulted with the elimination of $-NH_3$ unit. Analytical RP-HPLC analysis showed that the pyroglutamate formation is 27% compared to the main product peak

P9
 Chemical Formula: $C_{70}H_{96}N_{20}O_{21}$
 Exact Mass: 1552.7059
 Molecular Weight: 1553.6326

The reaction mechanism for the acid-catalyzed cyclization of N-allyl-L-glutamine to N-allyl-L-proline is shown below. The process begins with the protonation of the terminal amino group of the side chain by H_3O^+ . This is followed by the nucleophilic attack of the side chain nitrogen on the carbonyl carbon of the main chain, forming a tetrahedral intermediate. Subsequent loss of a water molecule and proton transfer leads to the formation of the five-membered ring of the proline derivative, with the final step showing the loss of an ammonia molecule.

47



P10

Chemical Formula: $C_{72}H_{98}N_{20}O_{22}$

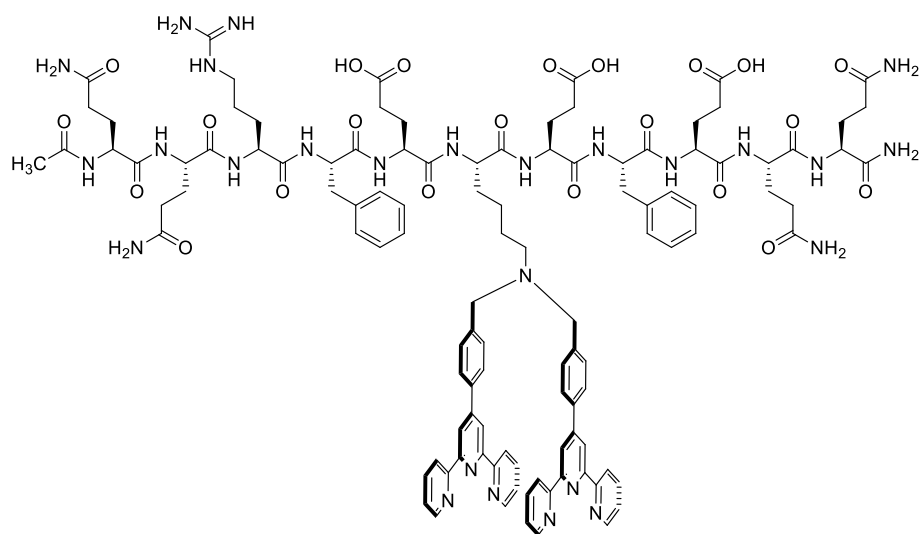
Exact Mass: 1594.7165

Molecular Weight: 1595.6693

Figure 1.27. Chemical structure of **P10**.

1.2.5.5 Synthesis of complementary peptides **P12** and **P13**

The complementary peptides **P12** (Figure 1.28) and **P13** (Figure 1.29) were synthesized with microwave-assisted SPPS method by using Rink Amide MBHA resin. However, the coupling of artificial amino acid **1** was done as 3 equivalent to resin without applying microwave. The coupling reaction was allowed to bubble under nitrogen at room temperature for 3 hours. The completion of reaction was checked by Kaiser Test. After the cleavage of peptide from resin, the analytical and *semiprep* RP-HPLC were performed before mass analysis.



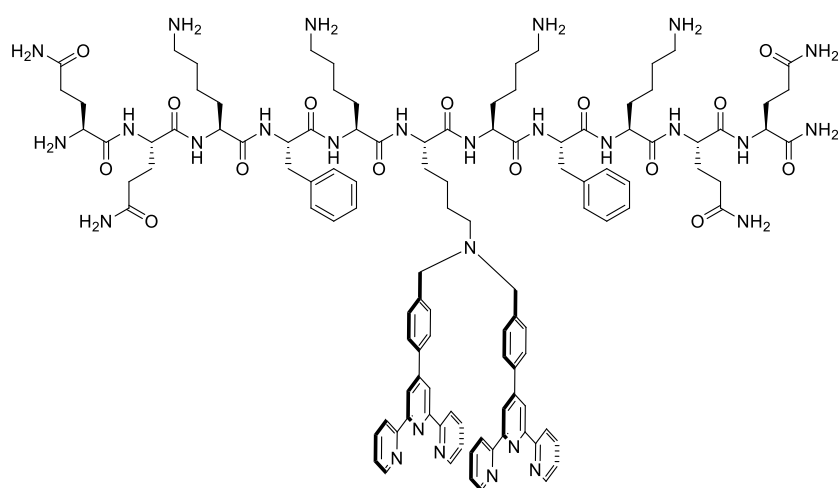
P12

Chemical Formula: $C_{111}H_{130}N_{26}O_{22}$

Exact Mass: 2178.99

Molecular Weight: 2180.38

Figure 1.28. Chemical structure of **P12** synthesized by microwave-assisted SPPS method.



P13

Chemical Formula: $C_{112}H_{143}N_{27}O_{15}$

Exact Mass: 2106.1257

Molecular Weight: 2107.5057

Figure 1.29. Chemical structure of **P13** synthesized by microwave-assisted SPPS method.

1.2.5.6 Synthesis of complementary peptides **P14** and **P15**

Firstly, the synthesis of **P14** (Scheme 1.21) and **P15** (Figure 1.30) were tried by performing standard SPPS protocol at room temperature. However, we did not observe any main peaks in analytical RP-HPLC analysis. It was previously experienced that the pyrene moiety decomposes at long reaction times and at higher temperatures. Thus, microwave-assisted SPPS method were used for the synthesis of these peptides with slight modifications in the protocol to prevent the decomposition of pyrene moieties. Rink Amide MBHA resin was used as solid support. Standard microwave-assisted protocol (20 W, max. 75 °C) was applied for the reactions of the amino acids with 5.5 equivalency that was coupled before artificial amino acid **2**. For the coupling and Fmoc removal of **2**, the reaction proceeded at room temperature with continuous bubbling under nitrogen gas for 6 hours and 10 minutes, respectively. The coupling of remaining amino acids and Fmoc removal reactions were done at 20 W and maximum 45 °C. The completion of coupling reactions of **2** and other remaining amino acids were checked by Kaiser Test. Analytical RP-HPLC and mass analysis proves the product formation in higher yield for **P14** and **P15**.

1.2.6 Design and synthesis of low molecular weight cyclic dipeptides as conductive gels

Cyclic dipeptides and their derivatives are known to form self-assembled nanostructures with the H-bond donor and acceptor ability of the peptide backbone, thus gelation occurs.^{58,59} One of the well-known cyclic dipeptides that can able to assemble itself is cyclo-(L-Tyr-L-Lys) peptide.⁵⁹ Also, the presence of Fmoc unit on the side chain of lysine make the molecule to form gel in different kind of solvents. Thus, it was decided that to use cyclo-(L-Tyr-L-Lys) peptide as template to attain conductive peptide assemblies. The designed cyclic dipeptides with terpyridine and pyrene moieties are shown in Figure 1.31 and Figure 1.32, respectively.

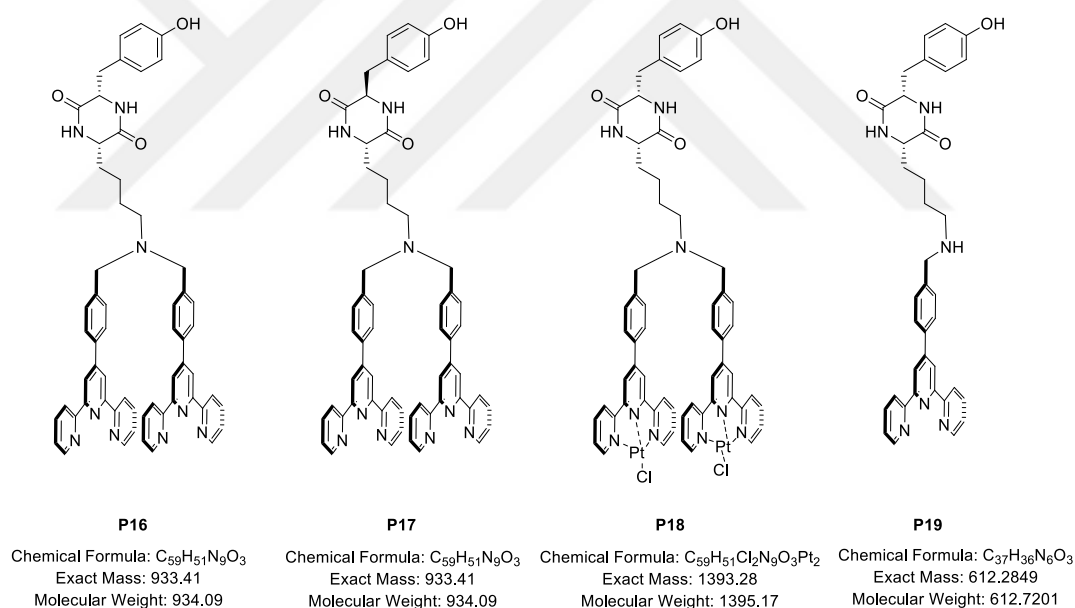


Figure 1.31. Designed conductive dipeptides with terpyridine moieties.

As a cyclic peptide, **P16** (cyclo-(L-Tyr-**1**)) contains artificial amino acid **1** instead of L-Lysine. In **P17** (cyclo-(D-Tyr-**1**)) D-Tyrosine was used as amino acid instead of its L-enantiomer to evaluate the effect of using a different diastereomer on gel

formation and conductivity. Moreover, **P18** (cyclo-(L-Tyr-4)) is the bis-platinum coordinated derivative of **P16** to observe the effect of metal coordination on conductivity. Lastly, **P19** was designed to investigate and compare the influence of utilizing bis-adduct on the conductivity of peptide assemblies.

In addition, cyclic dipeptides with artificial amino acid **2** which were designed to attain conductive peptide gels are **P20** (cyclo-(L-Tyr-2)) and **P21**.

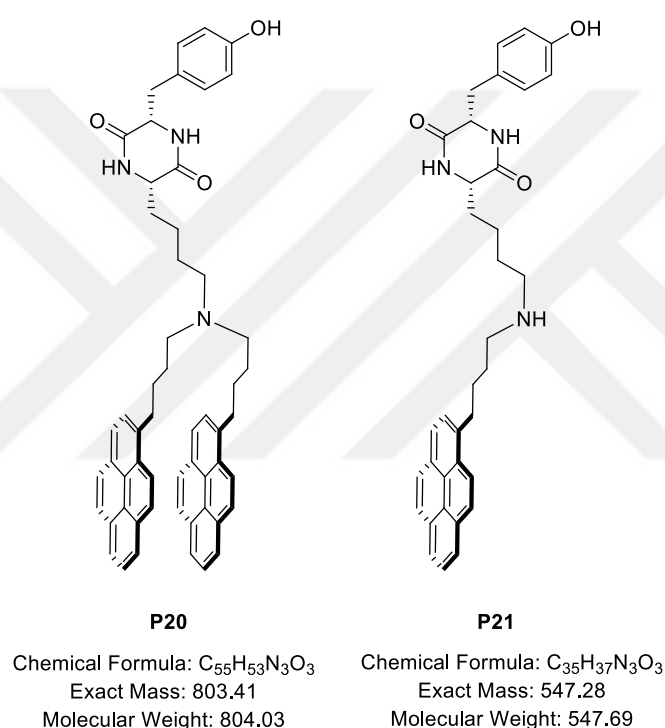
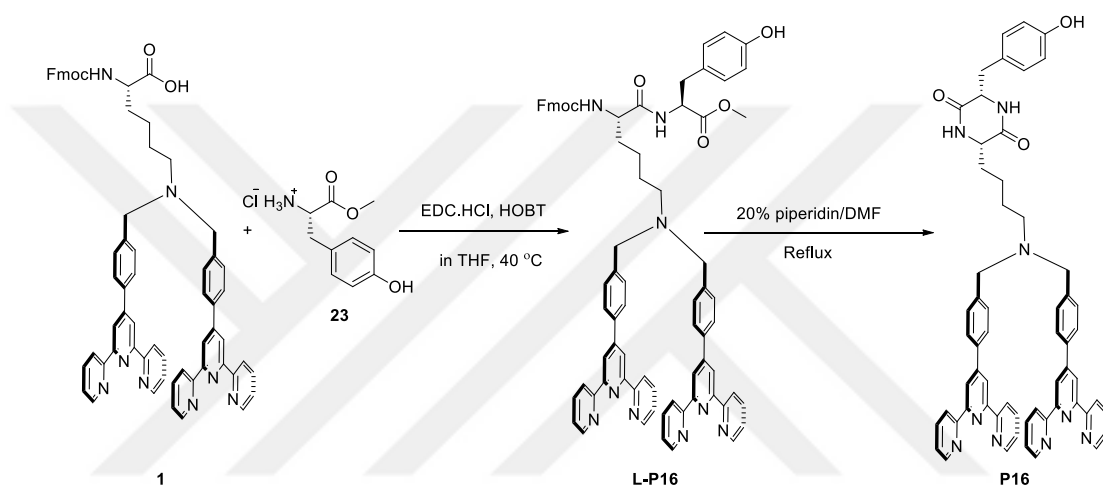


Figure 1.32. Designed cyclic dipeptides with pyrene moieties

1.2.6.1 Synthesis of cyclo-(L-Tyr-1) (**P16**) and cyclo-(D-Tyr-1) (**P17**)

The synthesis of **P16** and **P17** was achieved mainly by following literature procedure as shown in Scheme 1.22.⁵⁹ After performing methyl ester formation of L-Tyrosine

and D-Tyrosine, they were coupled with artificial amino acid **1** in THF/DMF with coupling reagent *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC.HCl) and hydroxybenzotriazole (HOBT) to yield Fmoc protected linear form of **P16** (**L-P16**) and **P17** (**L-P17**). After removing of Fmoc unit in 20% piperidine/DMF solution under reflux, cyclization of dipeptide occurs spontaneously.

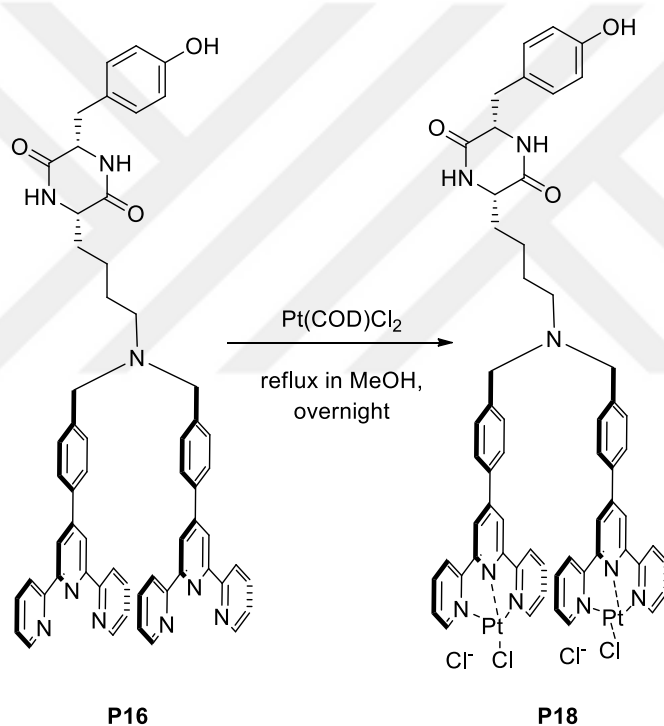


Scheme 1.22. Coupling of artificial amino acid **1** with L-Tyr-OMe.HCl (**23**) and cyclization of linear Fmoc protected dipeptide to yield **P16**.

After acquiring crude **P16** and **P17** by precipitating in diethyl ether, *semiprep* RP-HPLC was performed for the purification of peptides. The structures were proven with ^1H NMR spectroscopy, as well as analysis with mass spectroscopy.

1.2.6.2 Synthesis of bis-platinum ion coordinated cyclic dipeptide **P18**

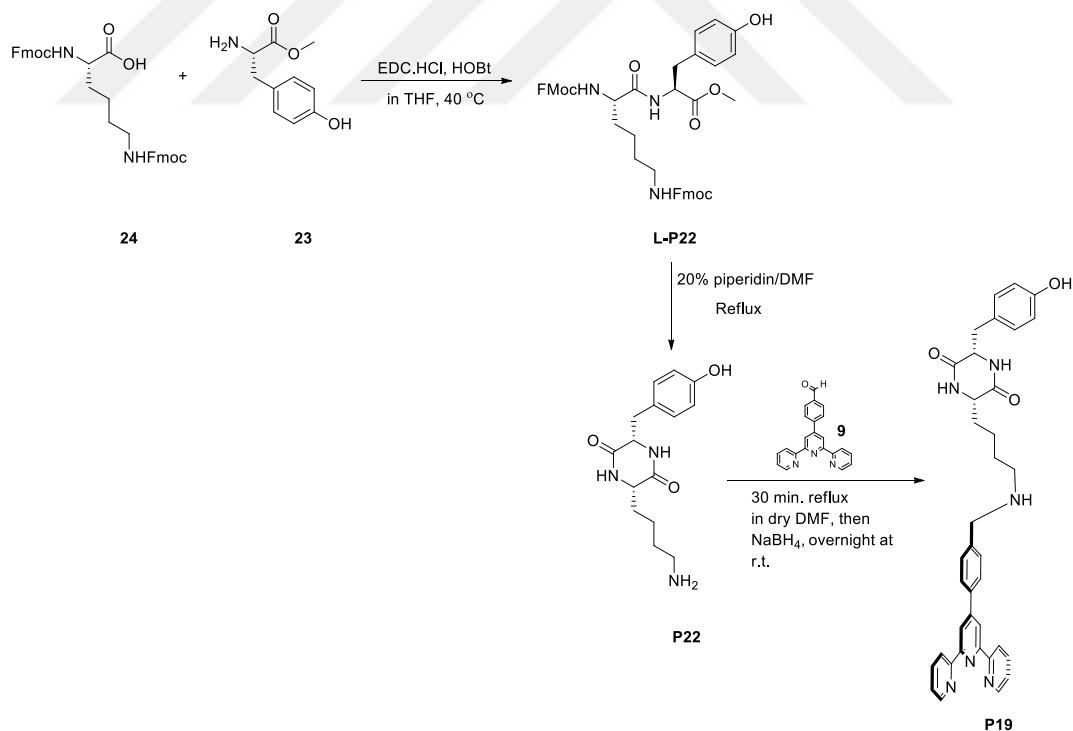
As the precipitation of **L-P16** was done in 0.1 M HCl, the platinum ions could be eliminated from artificial amino acid **4**. Thus, it was decided that Pt coordination should be performed directly on peptide **P16** after purification by following a standard procedure¹¹³ as shown in Scheme 1.23. Then, the **P16** peptide was removed from reaction medium as orange precipitate by centrifugation. The mass analysis proved the bis-platinum coordination of terpyridine moieties.



Scheme 1.23. Bis-Platinum ion coordination of **P16** to result **P18**.

1.2.6.3 Synthesis of mono-terpyridine containing peptide **P19**

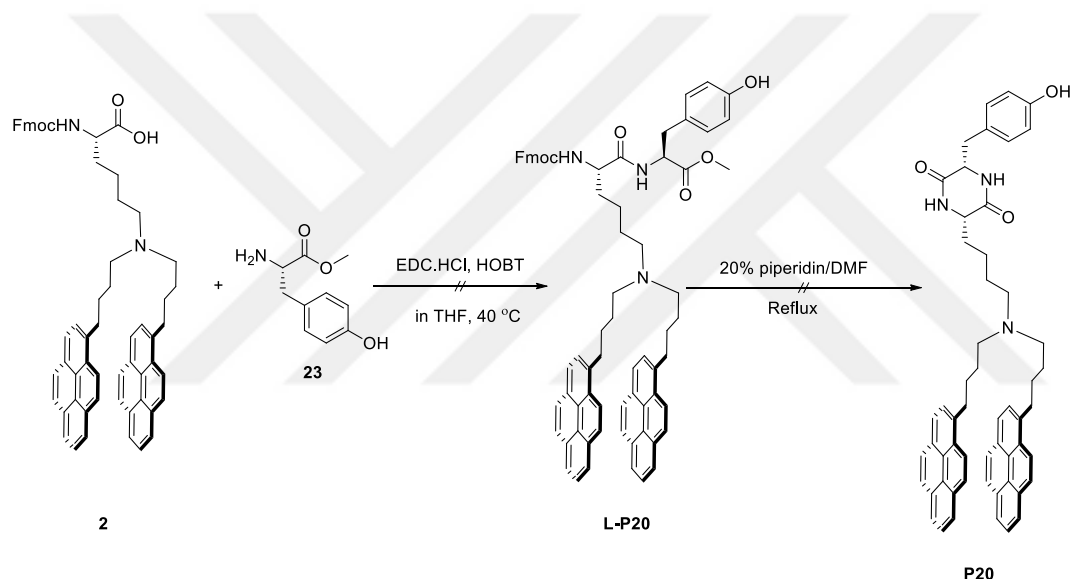
As mentioned before, the mono-terpyridine containing Fmoc-Lysine derivative (**19**) is not isolated from the reaction medium that the synthesis of **P19** could not be followed as the synthesis route of **P16**. As another method to obtain **P19** (Scheme 1.24), cyclo-(L-Lys-L-Tyr) (**P22**) that can be used as negative control for electrical measurements was synthesized with the cyclization of linear dipeptide (**L-P22**) by the removal of Fmoc moieties following the coupling of Fmoc-L-Lys(Fmoc)-OH (**24**) with L-Tyr-OMe (**23**). Then, the reductive amination was performed directly on peptide with **9** as aldehyde and NaBH₄ as reducing agent. The imine formation was achieved by refluxing **P22** and **9** in DMF for half an hour. Then the addition of NaBH₄ was done at 0 °C. The peptide was precipitated with DCM and purified with semipreparative RP-HPLC.



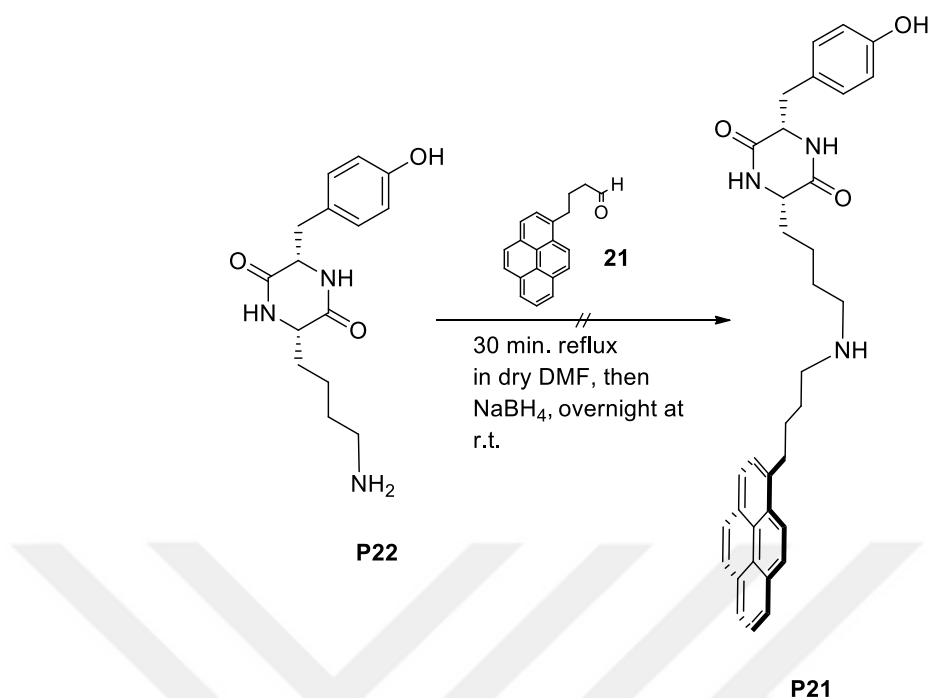
Scheme 1.24. Alternative synthesis route for **P19**.

1.2.6.4 Synthesis of pyrene containing cyclic dipeptides **P20** and **P21**

Same synthesis route was followed to attain **P20** (Scheme 1.25) and **P21** as the synthesis of **P16**. Unfortunately, the heating in coupling and cyclization steps resulted with decomposition of pyrene moieties which prevent us to obtain any main product. For the synthesis of **P21**, the synthesis route of **P19** was applied (Scheme 1.26), however the imine formation was not achieved at room temperature because of the low solubility of **P22**, and the heating for imine formation again resulted with the decomposition of pyrene.



Scheme 1.25. Solution phase synthesis route for **P20**.



Scheme 1.26. Synthesis of **P21** with the reductive amination of **P22** and **21**.

1.2.7 Self-assembly and gel formation of synthesized peptides

Different methods were utilized to form self-assembly of synthesized peptides such as pH adjustment, salt addition, using buffer or co-solvents systems.

1.2.7.1 Gelation of **P1** and **P2**

Both peptides were tried to form gel in different concentrations. 1 wt% of **P1** and **P2** was prepared in milliQ water. Sonication was applied to force self-assembly, however no gelation was observed. Then, 1 mg of **P1** and **P2** was dissolved in 50 mM NaCl solution for gelation, separately. Ultrasonication was applied, but gelation did not occur. The concentration of peptides were increased and gelation was tried

as 2 wt% and 3 wt% in 50 mM NaCl solution again. After ultrasonication, the solutions were allowed to stand. Gelation was not observed for 2 wt% gels, but 3 wt% solutions became viscous which may be an indication for self-assembly formation. However, the inversion test was not positive for that samples, thus it is not possible to accept these samples as gel. Depending on the literature, it was discovered that same concentration of FKFEFKFE peptide forms stiff gels.²⁹ It was concluded that the presence of artificial amino acid **1** in the middle these peptide sequences prevent peptides to form strong β -sheet secondary structures.

1.2.7.2 Gelation of P3, P4 and P6

Gelation of **P3**, **P4** and **P6** was tried in milliQ water as 0.5 wt% at first. Gel like structures were obtained in the vials after 15 minutes ultasonication and 45 minutes standing at room temperature. The self-assembly and gelation of RADA-16 peptides occurs better at higher pH values.³² Since *semiprep* RP-HPLC purification of peptides resulted with TFA salt formation of peptides, the pH values are around 2.5-3.0 after lyophilisation of peptides.



Figure 1.33. **P3** gel in pH 7.5 Tris.HCl buffer. The sample did not flow by inversion of the vial.

Depending on this information, the gelation was repeated in 20 mM pH 7.5 Tris.HCl buffer as 4 wt% samples for each peptide and stiffer gels were obtained which they gave positive results at inversion test (Figure 1.33).

1.2.7.3 Self-assembly of cyclo-octapeptides **P7** and **P8**

The self-assembly formation of **P7** and **P8** was applied as in literature which should result with microcrystalline precipitates in TFA. Vapor diffusion method was used for self-assembly and crystalline formation.⁵¹ The peptides were dissolved in TFA and the solution vials were suspended in a beaker filled with water. Microcrystalline precipitates were observed for **P8** when checked with a lens and microscopy (Figure 1.34), whereas **P7** peptides formed precipitate without any crystalline appearance after 72 hours.



Figure 1.34. Microscope image of microcrystals of **P8** obtained after self-assembly.

1.2.7.4 Gelation of complementary peptides

P10 is the complementary of **P11**, which they can assemble in alternating order to form β -sheet secondary structure at pH between 7.2-7.4 as in literature.^{44,45} 3.3 mM solution of each peptide was prepared in milliQ water at same volume. The pH adjustment of peptides was done with the addition of 0.5 and 0.25 M NaOH. **P10** formed gel like structure when dissolved in milliQ water, since it is able to assemble itself in acidic medium. As the pH increased with NaOH addition, the sample became more solution like. Inversely, the addition of NaOH make the **P11** peptide solution more viscous. Peptides solutions at pH values 7.2-7.4 were mixed together by pipetting after vortexing, then the gel formation was observed spontaneously and tested with inversion method.

P12 and **P13** peptides are complementary to each other. The solutions of peptides were prepared in the same manner and similar observations were attained for the appearances of samples as above. The gel formation was achieved again after mixing two pH adjusted peptide solutions and tested with inversion method (Figure 1.35).

The bis-pyrene containing complementary peptides **P14** and **P15** was slightly soluble in milliQ water. Nevertheless, the pH adjustment to 7.2-7.4 was done. Unfortunately, the mixing of these two suspensions did not form gel and remained as suspension, in which the pyrene moieties on peptides decreases the solubility of peptides in water fairly.



Figure 1.35. The gelation of mixed **P12-P13** after pH adjustment.

Moreover, the gelation of a bis-terpyridine containing peptide with its complementary that contains bis-pyrene moiety was tested to obtain donor-acceptor type systems (Figure 1.36). When 3.3 mM **P12** was mixed with 3.3 mM **P15** after pH adjustment, it was resulted with a suspension instead of gelation. Since the solubility of **P14** is higher than **P15**, same concentration of **P13** and **P14** was mixed at pH 7.2-7.4 and a gel like sample was obtained which is not passed from inversion test. Then, the gelation of these donor-acceptor complementary peptides was repeated as a concentration of 6.6 mM for each peptide and gelation was achieved depending on inversion test with a softer gel appearance.

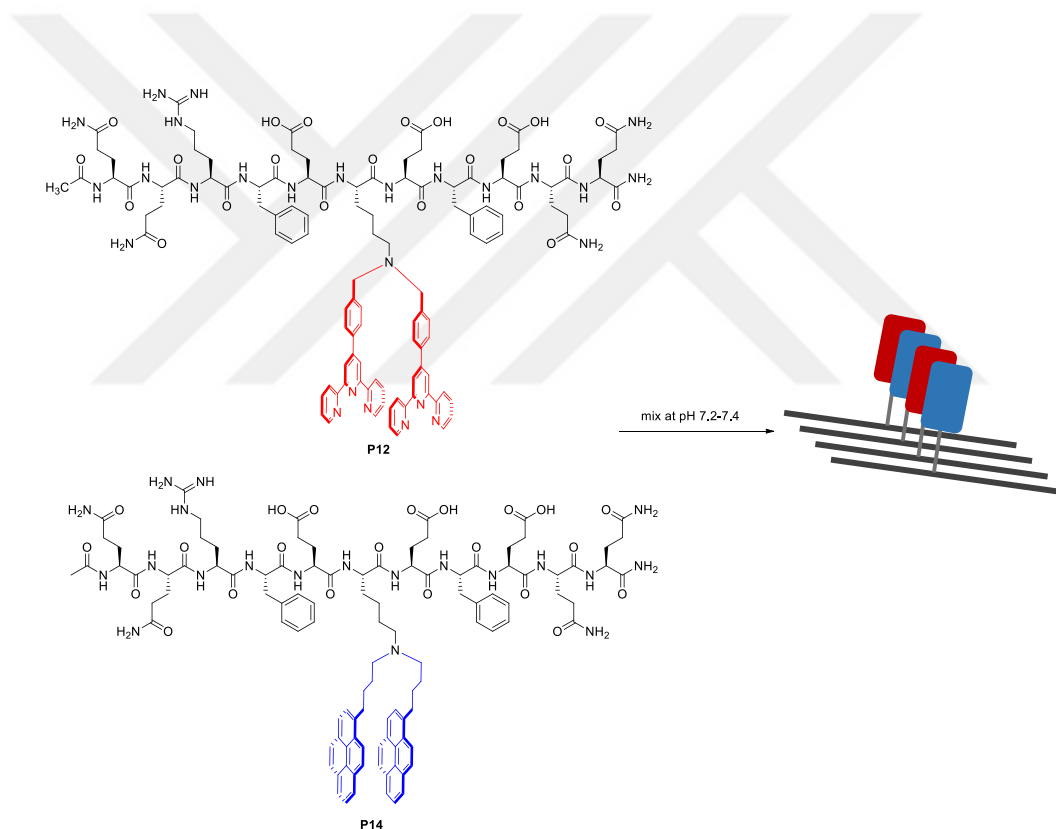


Figure 1.36. Representative picture of co-assembly of donor-acceptor type peptides **P12** and **P14**.

1.2.7.5 Gelation of cyclic dipeptides

The presence of big hydrophobic units in these short cyclic peptides decreases the solubility in water or buffer solutions fairly. Because of the low solubility of **P16**, **P17**, **P18** and **P19** in aqueous medium, co-solvent system was used for the gelation of these peptides. In this method, the sample is dissolved in a minimum amount of solvent which is a good solvent for the dissolution of sample. Then, the addition of other solvent which is not a good solvent for the dissolution of sample was done up to the gelation. All these peptides are quite soluble in DMSO. Thus, the defined amount of peptides were dissolved in DMSO and then pH 7.0 phosphate buffer addition was done slowly on the peptide solutions. The addition of buffer on **P16** solution causes gel like formation firstly, but suddenly resulted with the precipitation. The mixture was then heated in water bath to 80-90 °C up to the dissolution. After observing the complete dissolution, the solution was exposed to ultrasonication at 60 °C for a minute, and gel formation was observed with a positive result in inversion test (Figure 1.37a). The gelation of **P18** was achieved by applying same method. Nevertheless, the both gels are likely to partially precipitate, if they are allowed to stand too much time at room temperature. As an alternative method for the gelation of **P18**, the defined amount of peptide was dissolved in dry DMSO by heating directly in 60 °C ultrasonic bath and the buffer addition was applied to the hot solution to observe gelation without any precipitate formation (Figure 1.37b). The gel was in orange color for **P18** since it is a platinum complex. For the gelation of **P19**, heating is not necessary after buffer addition since a clear gel formation was observed directly. Its higher solubility in buffer, makes gel formation easier for **P19**. Unfortunately, the gelation of **P17** was not observed because of its different stereochemistry. This prevents the self-assembly of **P17** in the same medium with the other cyclic dipeptides. In addition, the gelation of **P22** was done directly by dissolving in DMSO and applying ultrasonication for 2 minutes at room temperature as described in literature.⁵⁹



Figure 1.37. Gel formations of **(a) P16 (b) P18** by using co-solvent system.

1.2.8 Characterization of self-assembly and microstructures of gels

The secondary structures of self-assembled peptides were defined using circular dichroism spectroscopy (CD) and IR spectroscopy. The microstructures that cause gelation were characterized with electron microscopy.

1.2.8.1 Characterization of P1 gel

As mention in gelation part, proper gels were not obtained for **P1**, although viscous solutions observed. Nevertheless, TEM imaging was done for **P1** sample and we did not observe acceptable indication for self-assembly, although very short fibril like structures are shown in Figure 1.38.

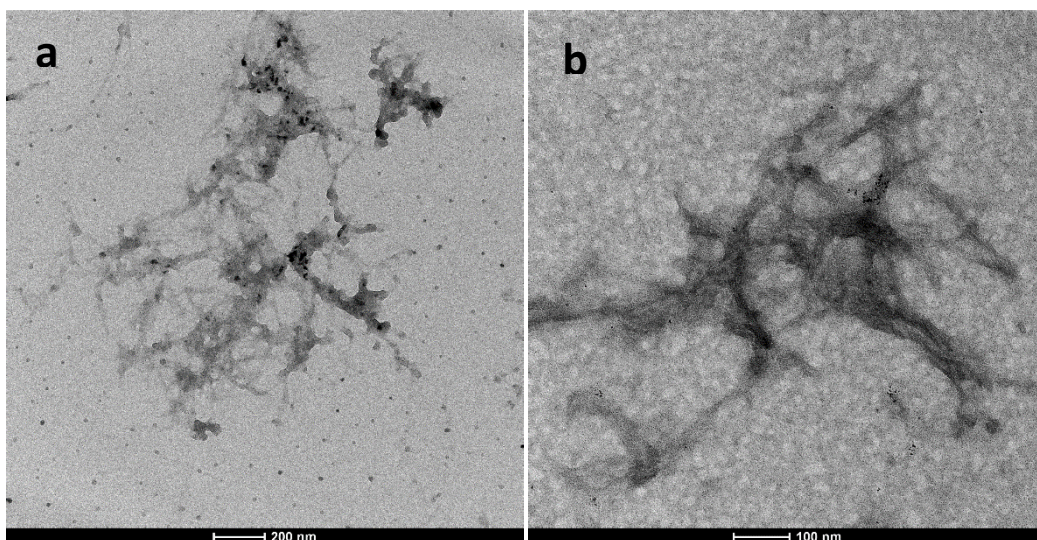


Figure 1.38. TEM images of **P1** sample (Scale bar: **(a)** 200 nm and **(b)** 100 nm)

1.2.8.2 Characterization of P3, P4 and P6 gels

The CD spectrums of diluted gel of **P3**, **P4** and **P6** are represented in Figure 1.39, respectively.

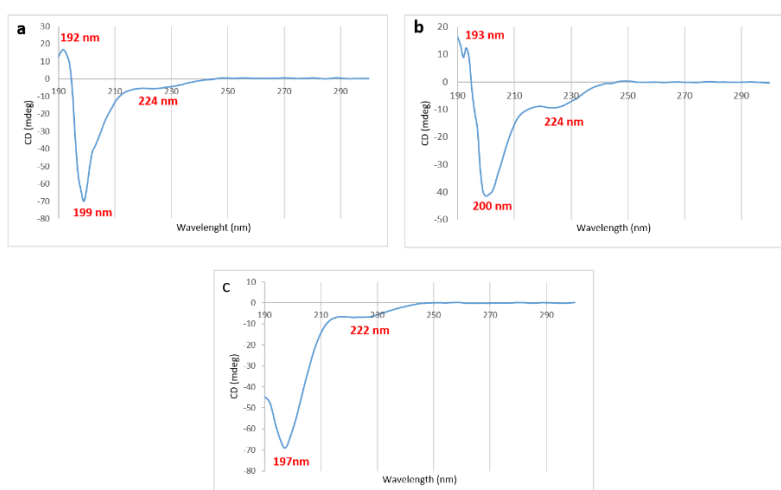


Figure 1.39. CD spectrum of self-assembled **(a) P3**, **(b) P4** and **(c) P6**.

In CD spectroscopy, the secondary structures of peptides and proteins show typical spectra for α -helix, β -sheet and random coil. In general, there should be a maxima at 195 nm and a minima at 220 nm for β -sheet in CD spectra. However, it is known that the presence of π - π stacking for β -sheet forming peptides causes a shift toward lower wavelength.⁶⁶ Furthermore, an extra peak formation in CD spectrum as a result of π - π stacking among aromatic units was explained in literature.²⁹ For both self-assembled peptides **P3** and **P4**, they showed a maxima at 192 nm and 193 nm, respectively. The presence of minima at 200 nm is also consistent with the literature. Moreover, the π - π stacking among aromatic units in the self-assembled structures create an extra minima at 224 nm. The interpretation of CD spectra proves the β -sheet formation as well as π - π stacking for **P3** and **P4**. When the CD spectrum of **P6** is evaluated, the random-coil formation was observed mainly because of the presence of only minima at 197 nm. However, the presence of a small negative peak at 222 nm indicate the presence of β -sheet structures in the medium, also.

IR analysis (Figure 1.40) was also done to prove the β -sheet secondary structure formations in the gel systems of **P3**, **P4** and **P6**. Normally, the amide I peak of amide carbonyl is shown between 1650-1690 cm^{-1} . When self-assembly occurs as β -sheet secondary structure, the amide I band is at 1620-1630 cm^{-1} which is a primary indication of β -sheet formation for IR spectroscopy. The IR spectra of **P3** and **P4** are consistent with this information, representing the β -sheet formations with amide I bands at 1620 cm^{-1} for both and amide II bands at 1522 cm^{-1} and 1527 cm^{-1} , respectively. For the self-assembled peptide **P6**, the obtained IR spectrum do not indicate a β -sheet formation which is mainly relevant with its CD spectrum. The amide I band is shown at 1645 cm^{-1} which is the amide carbonyl peak for a compound that contains amide in its structure.

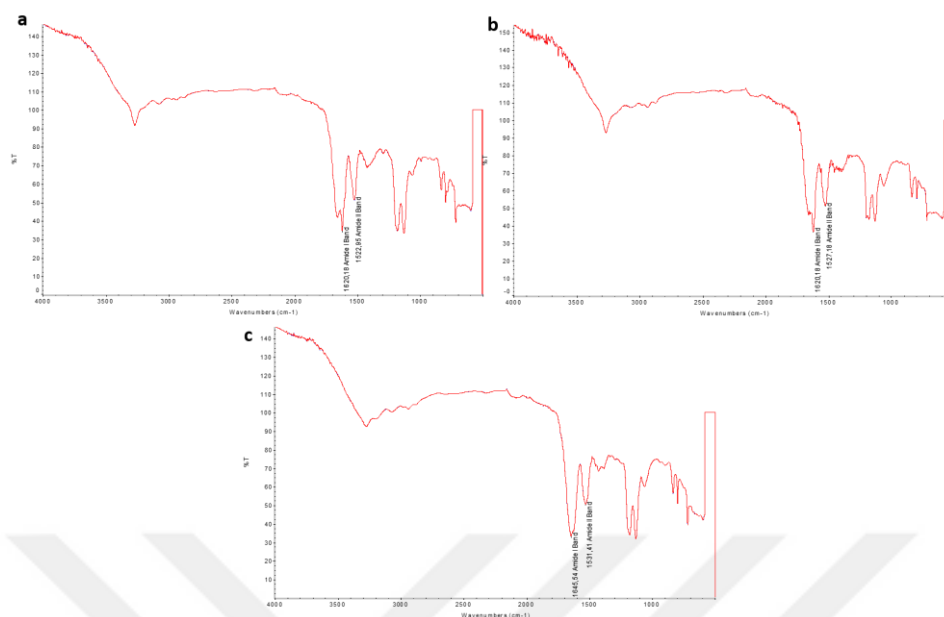


Figure 1.40. IR Spectrum of (a) **P3**, (b) **P4** and (c) **P6** after self-assembly.

To characterize microstructures of **P3**, **P4** and **P6** formed after gelation TEM imaging was done by staining the self-assembled peptides solutions with uranyl acetate (Figure 1.41). Highly uniform fibrillar formations were observed for all three peptide assemblies and it was concluded that the gelation took place due to the presence of these fibrous nanostructures. All these fibrils have a wide of around 8 nm and several micrometers in length. Since the linear length of an amino acid is accepted as 4.0 Å,¹²² peptides with 17 amino acids should have a length of 7 nm. Thus, each fibril do not interact with other fibrils and found only as a single peptide length as the width of the formed microstructure. This type of single molecule thick systems can be classified as tapes, and their entanglement generally resulted with viscoelastic solutions instead of a stiff gel formation which explains the soft gel appearance of **P3**, **P4** and **P6** samples after self-assembly.

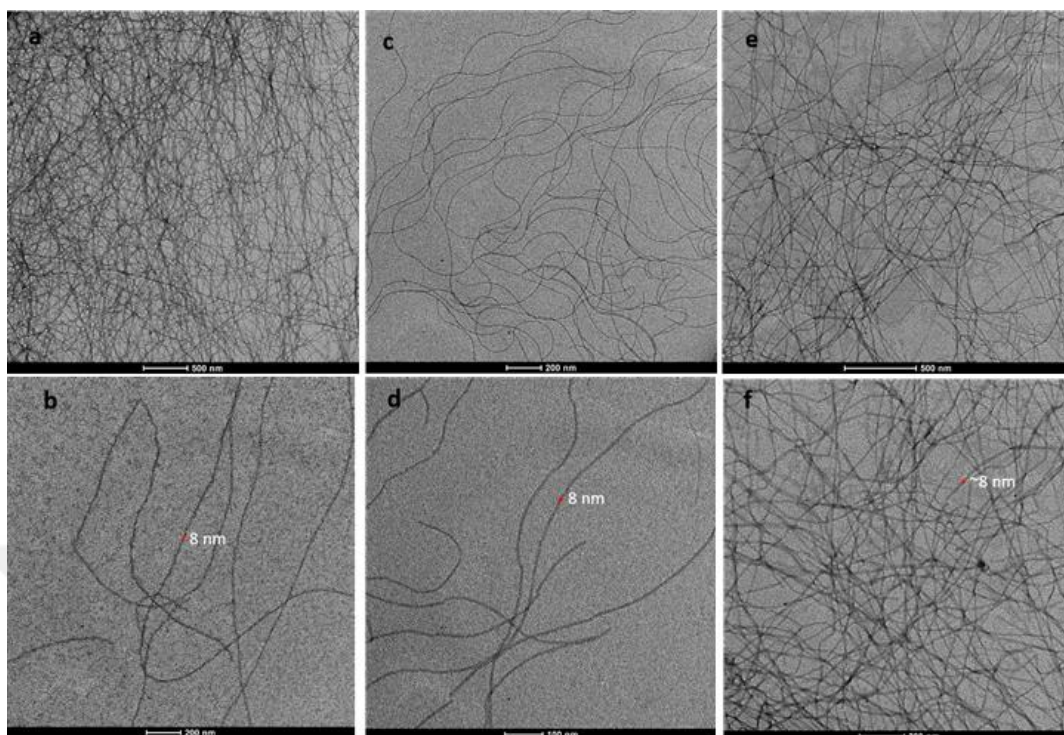


Figure 1.41. TEM images of microstructures of **(a)** and **(b)** **P3** (Scale bar: 500 nm and 200 m), **(c)** and **(d)** **P4** (Scale bar: 200 nm and 100 m), **(e)** and **(f)** **P6** (Scale bar: 500 nm and 200 m).

Although the self-assembly was obtained for all three peptides, a computational study of Cormier and his coworkers³⁵ represented that the self-assembly of RADA-16 peptides occurs in parallel fashion but with an angle of 35°. In other words, the first amino acid of a peptide interact with the third amino acid of second strand which prevents the effective π - π stacking of terpyridine or pyrene moieties between each peptide strand in this study (Figure 1.42). This finding is relevant with the low intensity of π - π stacking peaks in CD spectra of **P3** and **P4**.

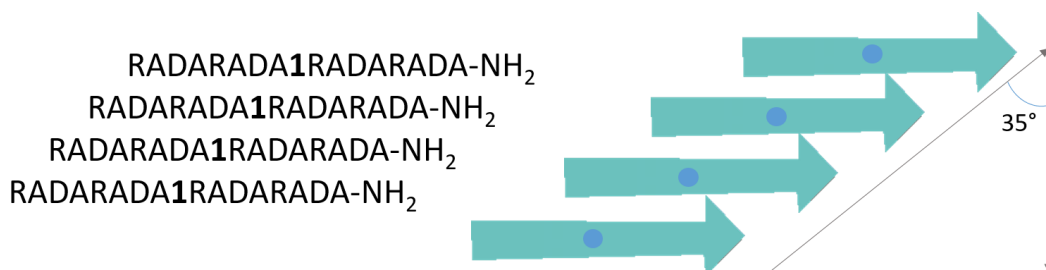


Figure 1.42. The proposed self-assembly of **P3**.

1.2.8.3 Characterization of **P7** and **P8** microstructures

After the vapor diffusion crystallization of **P7** and **P8**, precipitate formation was observed for both peptide. The characterization of these samples were enlightened by using FT-IR (Figure 1.43) and Scanning Electron Microscopy (SEM) (Figure 1.44). According to the IR spectrum of **P8** crystals, the wavenumber of amide I, amide II and NH bands are in correlation with the literature data. Moreover, the morphology and dimensions of **P8** crystals was analyzed by SEM imaging. **P8** peptide formed microcrystalline structures after self-assembly with variable thicknesses from 100 nm to 1.20 μm and several micrometers length (1-100 μm) which indicates the nanotube formation and they came together to form bunches of nanotubes.

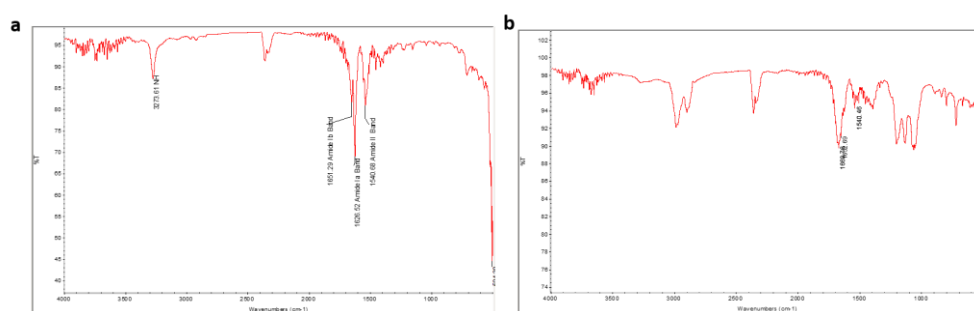


Figure 1.43. FT-IR spectrum of (a) **P8** crystals and (b) **P7** solids.

Beside the satisfying IR spectrum and SEM images of **P8** crystals, the amide and NH bands did not observed properly in the IR spectrum of **P7** solid. Additionally, **P7** peptide did not assemble after vapor diffusion and has as an amorphous appearance in SEM image. It was interpreted that **P7** started to assembled but the medium may be too dilute for the growth of crystals with self-assembly.

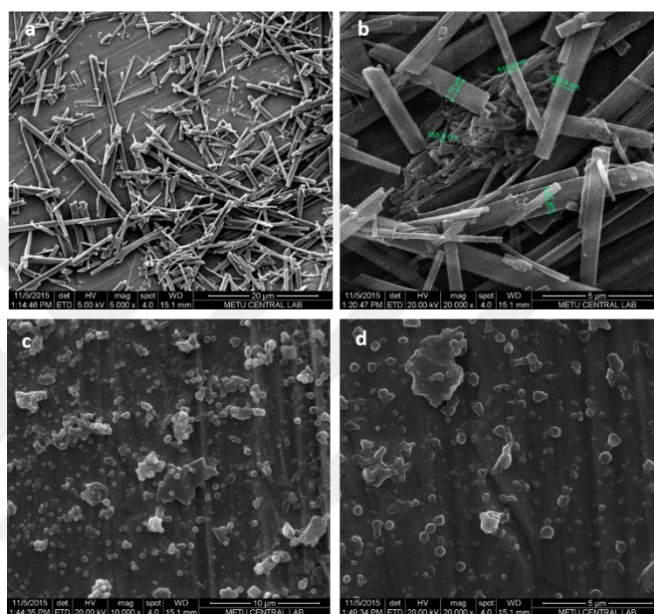


Figure 1.44. SEM image of (a) and (b) **P8** (Scale bar: 20 μm and 5 μm , (c) and (d) **P7** (Scale bar: 10 μm and 5 μm) after vapor diffusion method for self-assembly.

1.2.8.4 Characterization of complementary peptide gels

After the gelation of **P12** and **P13** together as the complementary of each other, the β -sheet formation was proven with FT-IR (Figure 1.45) and CD spectroscopy (Figure 1.46), whereas the nanostructural formations were enlightened with TEM imaging (Figure 1.47). Amide I and amide II bands wavenumbers are 1626 cm^{-1} and 1540 cm^{-1} , respectively which indicates the β -sheet formation occurred as a result of

self-assembly. Moreover, CD analysis represent the peaks for β -sheet formation as well as π - π stacking of terpyridine moieties with a maxima at 192 nm and two minima at 217 nm and 222 nm. TEM images represents the nanofibrous morphology which is obtained by self-assembly. The linear length of an 11 amino acid containing peptide is around 4 nm. These nanofibrils could be classified as tapes since each fibril has a single peptide thickness. Also, each tape has length of several micrometers. Similarly, Figure 1.48 shows the fibrillar formations due to the self-assembly of complementary peptide **P13** and **P14**. The thickness of a fibril is around 10 nm and the length is in several micrometers.

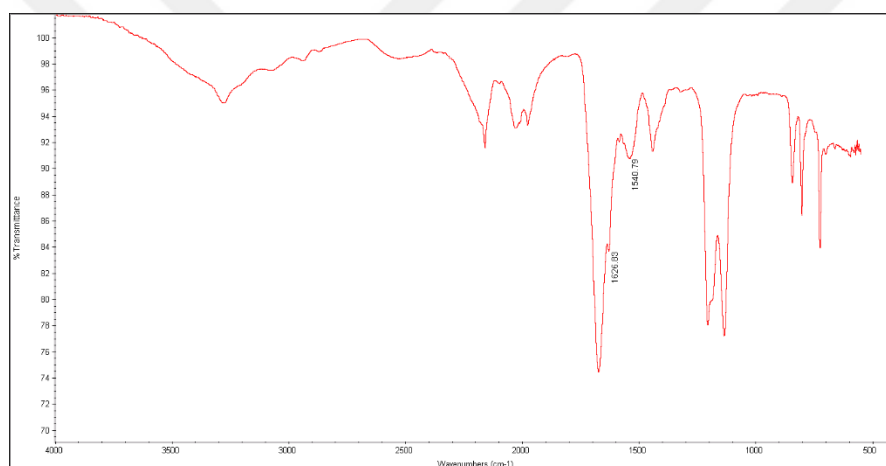


Figure 1.45. FT-IR spectrum of self-assembled **P12-P13**.

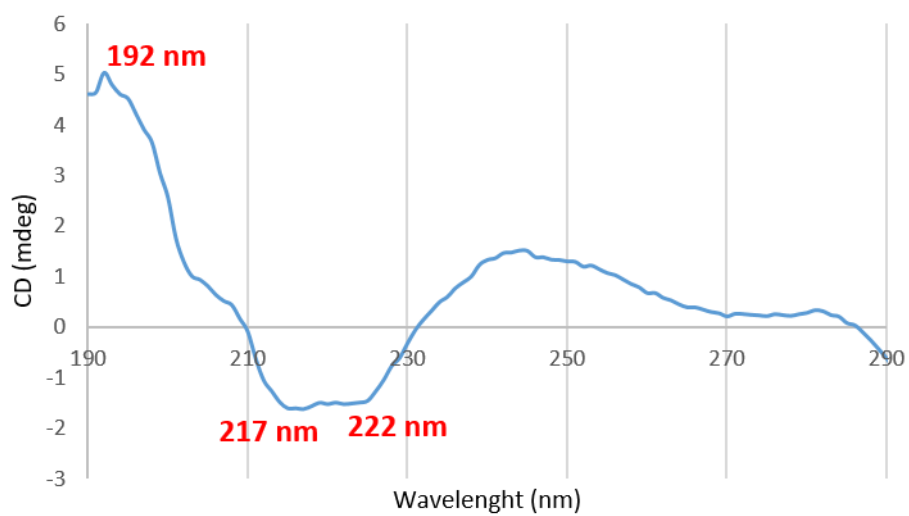


Figure 1.46. CD spectrum of co-assembled peptides **P12-P13**.

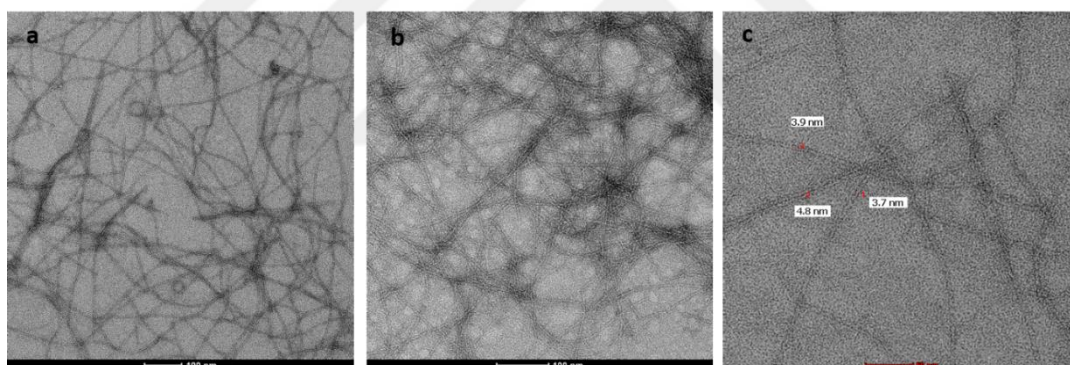


Figure 1.47. TEM images of co-assembled **P12-P13** with a scale bar **(a), (b)** 200 nm and **(c)** 50 nm.

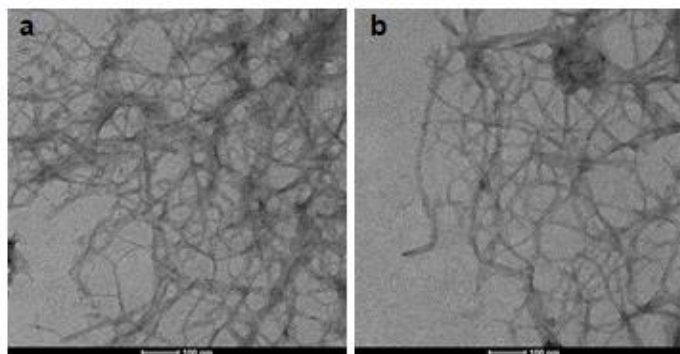


Figure 1.48. TEM images of co-assembled **P13-P14** with a scale bar **(a)** and **(b)** 100 nm.

1.2.8.5 Characterization of cyclic dipeptide gels

Aggregation of cyclic dipeptides **P16** and **P19** was studied by UV-Vis spectroscopy (Figure 1.49 and Figure 1.50). Besides, **P16** xerogel was analyzed by powder XRD spectroscopy (Figure 1.51). The images of microstructures were visualized by TEM imaging (Figure 1.52). The UV-Vis spectroscopy analysis for both peptides were followed in DMSO and buffer to compare the behavior of unassembled and assembled peptides, respectively. Both **P16** and **P19** peptide showed absorptions between 260 to 350 nm as a characteristic of terpyridine moiety. The minor red shift of maxima from DMSO to buffer can be attributed to differences of solvent polarities as well as self-assembly of **P16** and **P19**. Although the red shifts seen to be insignificant, there is a decrease in intensity of peak and broadening of absorption bands indicates the J type π - π interaction among aromatic units.

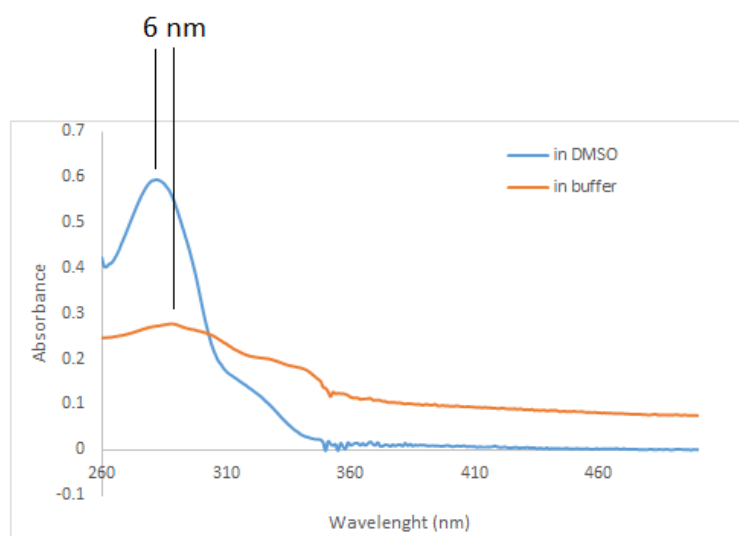


Figure 1.49. UV-Vis spectra of **P16** in DMSO (unassembled) and in buffer (assembled).

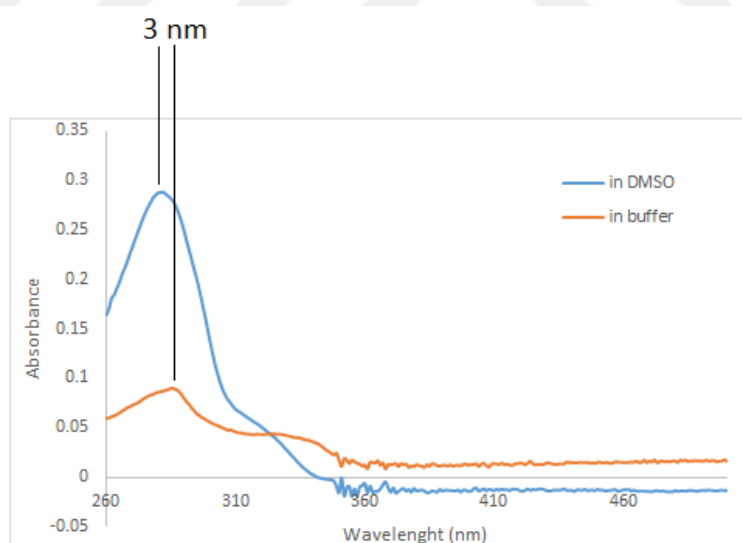


Figure 1.50. UV-Vis spectra of **P19** in DMSO (unassembled) and in buffer (assembled).

Although the resolution is bad in powder XRD spectrum due to the little amount of **P16** xerogel obtained by the lyophilisation of **P16** gels, the 2θ peak at 26.6° corresponds to a distance of $3.3 \text{ \AA}$ which is the distance between two π - π stacked aromatic units. Powder XRD and UV-Vis spectra of self-assembled **P16** are coherent with each other.

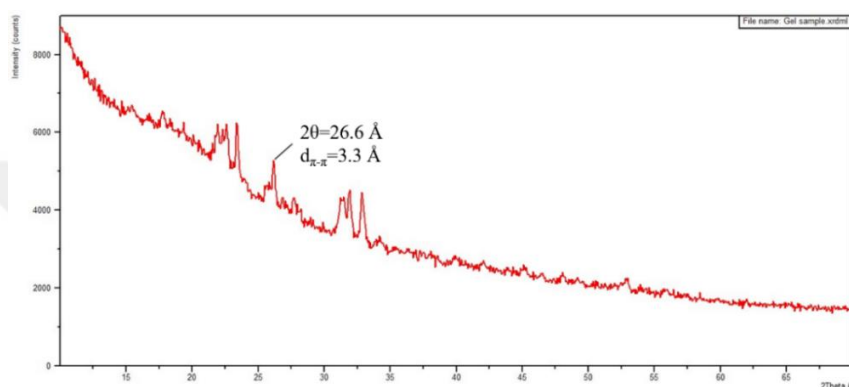


Figure 1.51. Powder XRD spectrum of **P16** xerogel.

In the TEM image of **P16** gel, uniform nanofibrillar structures are seen in microns long in length. The thickness of fibrils varies from 10 nm to 40 nm. The thicknesses indicates the fibril and fiber formation due to self-assembly since these values are much higher than a linear length of a cyclic dipeptide. Moreover, the constitution of fibers in helical shape can be easily seen as the thickness of fibers increases as in Figure 1.52c. The TEM image of **P19** gel represents the construction of nanofibers due to self-assembly. Shorter rod-like fibers are obtained in the case of self-assembly of **P19** with a thickness of around 20 nm to 30 nm. Similarly, Figure 1.53 contains the TEM images of self-assembled **P18** in which the fibrillar formations can be seen easily with a thickness of around 15 nm.

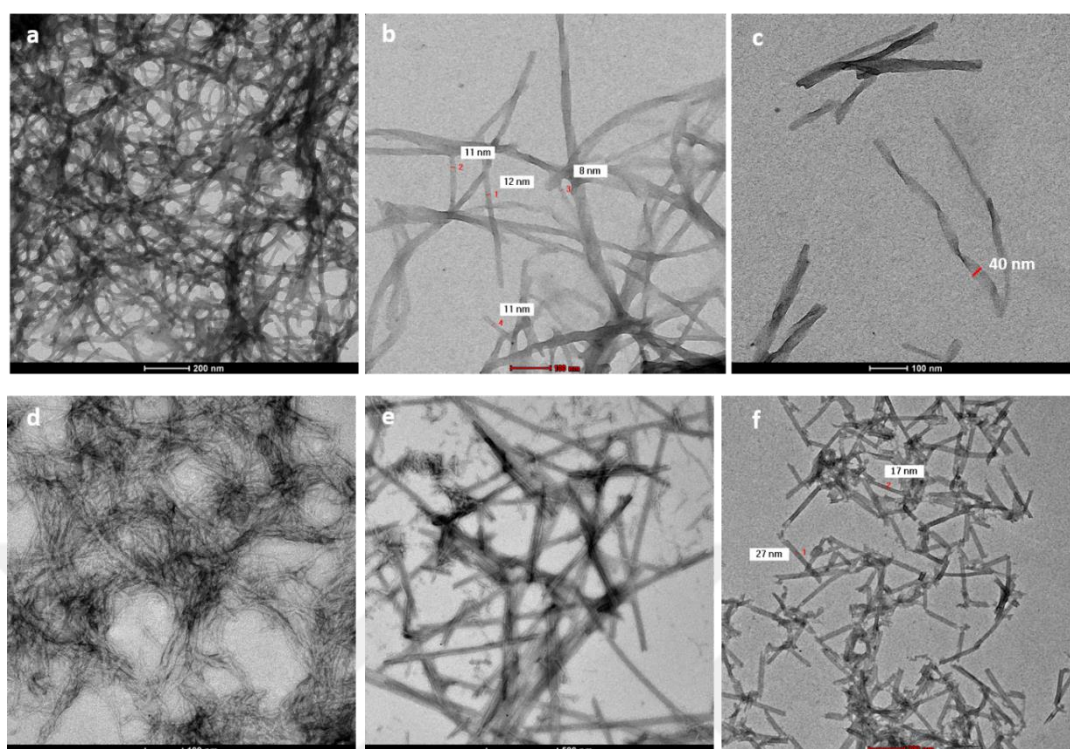


Figure 1.52. TEM images of dried **(a)**, **(b)** and **(c)** **P16** gel (Scale bar: 200 nm, 100 nm and 100 nm, respectively), **(d)**, **(e)** and **(f)** **P19** gel (Scale bar: 100 nm, 500 nm and 200 nm, respectively).

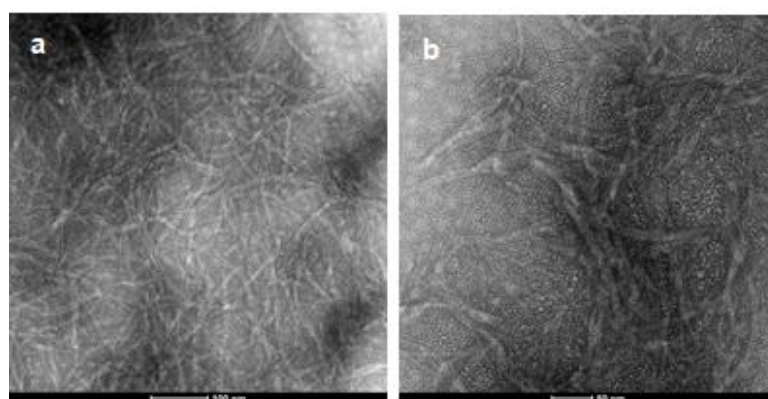


Figure 1.53. TEM images of dried **P18** gel (Scale bar: 100 nm, 50 nm respectively)

1.2.9 Electrical measurements of self-assembled peptides

All the electrical measurements are performed collaboratively with Prof. Dr. Sait Eren San, Asisst. Prof. Gökhan Bakan and Assoc. Prof. Görkem Günbaş. Mainly, I-V curves are obtained by using semiconductor parameter analyzer and their conductivities were calculated depending on these I-V curves.

1.2.9.1 Electrical measurements of P3, P4 and P6 gels

As mentioned before, an effective π - π could not be attained between aromatic moieties of RADA-16 type peptides. Nevertheless, in a collaborative study with Prof. Dr. Sait Eren San, below I-V curves were obtained with some deviations from linearity (Figure 1.54). The acid cleaned glass was coated with the gels of **P3**, **P4** and **P6** and allowed to dry at room temperature. Then the surfaces was coated with silver electrode by using thermal evaporation method. Unfortunately, this study remained unfinished.

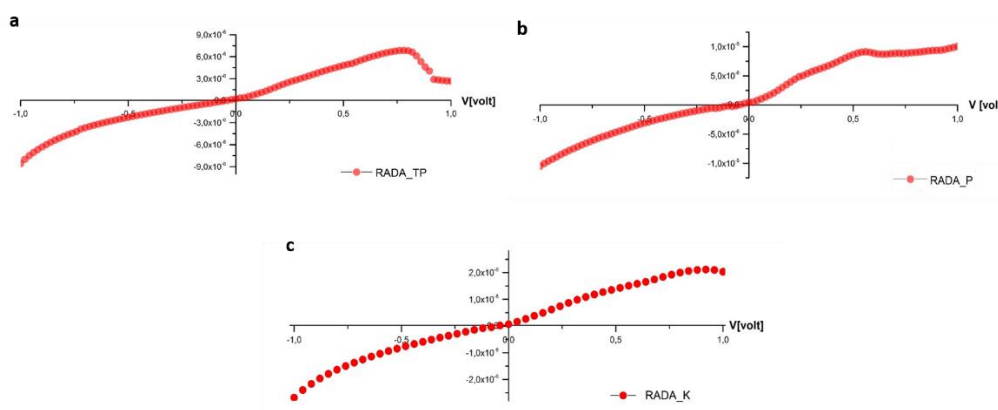


Figure 1.54. I-V curves of (a) **P3**, (b) **P4** and (c) **P6** xerogels.

Then, to measure the conductivity of **P3**, **P4** and **P6** peptides, 4-point probe method was used. The gels were dropped on cleaned glass with a 1 mm thickness and 7 mm diameter. The resistance of hydrogels were measured and the conductivity values were calculated by using Equation 1.1. The distance between electrodes is 1.27 mm. The calculated conductivity value for 4 wt% gels of **P3**, **P4** and **P6** are 3.3×10^{-4} S/cm, 6.7×10^{-5} S/cm and 3.3×10^{-5} S/cm, respectively. According to these results, **P3** and **P4** have 10 and 2 times higher conductivities than negative control hydrogel **P6**, respectively. Beside the self-assembly configuration of RADA-16 type peptides, the calculated conductivity values are comparable with other peptides. The reason of relatively high conductivity values may be related with the conducting the measurements in gel for, not as xerogel. Nevertheless, the conductivity value of 20 mM tris.HCl gelating buffer was found as 2.0×10^{-6} S/cm which is 165 times lower than **P3** gel conductivity. Moreover, the conductivity of a self-assembled quintetthiophen was measured as around 1×10^{-5} S/cm by Stupp and coworkers¹²³ which proves the enhancement of conductivity in this study. Although the 4-point probe method is not a highly sensitive method, the results are sufficient to be the proof of concept.

$$\frac{1}{\sigma} = \rho = R \frac{t \times d}{L}$$

Equation 1.1. The equation used to calculate resistivity and conductivity in which ρ is resistivity, σ conductivity, R is resistance, t is the thickness of xerogel, d is the diameter of sample and L is width of channel spacing.

1.2.9.2 Electrical measurements of P10-P11, P12-P13 and P14-P13 complementary peptide gels

At first, temperature dependent AC conductivity measurement was conducted by Prof. Dr. Sait Eren San. The conductivity of **P12-P13** co-assembled peptides increases as the temperature increases which represent that the assembled peptide have semiconductor property as shown in Figure 1.55. Then, the I-V curve of **P12-P13** co-assembled peptides were obtained both for the hydrogel and xerogel forms (Figure 1.56). The I-V curve of hydrogel deviate from linearity highly, revealing that the measurements should be conducted for the dried gels. The hole mobility value was calculated depending on the I-V curve as $0.0104 \text{ cm}^2/\text{V.s}$. Tovar and coworkers achieved the self-assembly of quarterthiophene conjugated β -sheet forming peptides as directionally aligned by using noodle formation method and measure the hole mobility of their system as $0.03 \pm 0.005 \text{ cm}^2/\text{V.s}$.⁷⁹ by using semiconductor parameter analyzer. These results are comparable. Even the nanofibrils of **P12-P13** co-assembly is not directionally aligned to same direction, the calculated hole mobility value indicates the favorable effect of artificial amino acids that decreases the distance between aromatic units in β -sheet secondary structure.

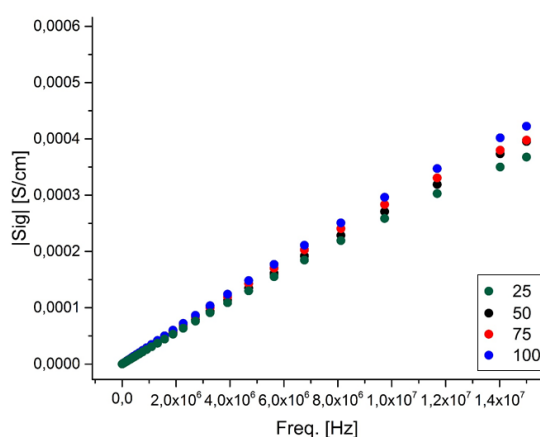


Figure 1.55. AC conductivity measurement of **P12-P13** co-assembled peptide.

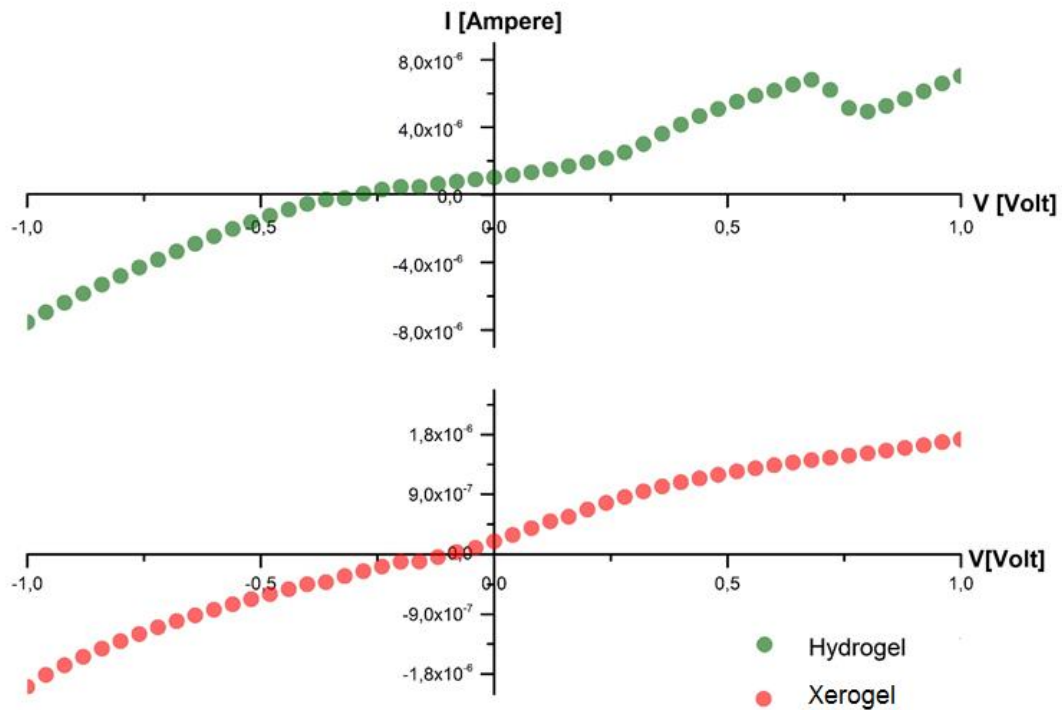


Figure 1.56. The I-V curves for the co-assembled **P12-P13** as hydrogel and xerogel.

To compare the conductivities of all co-assembled peptides, a collaborative study with Assoc. Prof. G rkem G nbař was conducted. After coating glass surfaces with gold by using shadow mask, freshly prepared gels were applied on surfaces by drop casting method. The devices was allowed to dry at room temperature for one day. Each glass contains 20 devices and I-V curves are obtained for each device by using semiconductor parameter analyzer. The thickness of xerogel was calculated by taking average of ten thickness value measured from different parts of the glass with profilometer. The conductivity value then calculated by using the resistivity value obtained, channel width and length. The more detailed explanation of device preparation and conductivity calculations is done in next section. For the electrical measurements of **P10-P11** co-assembled peptides (3 mM), devices with 10 micrometer length was used, and the average thickness of xerogel was measured as

3.46 micrometer as average. The samples have I-V curves deviated from linearity. The obtained resistance values changes from 9 MΩ to 3 GΩ and the calculated conductivity values ranges from 9.63×10^{-8} S/cm to 3.21×10^{-6} S/cm for 17 devices as shown in Figure 1.57. The average conductivity of **P10-P11** co-assembled peptide was calculated as 4.56×10^{-7} S/cm by neglecting the lowest three conductivity value.

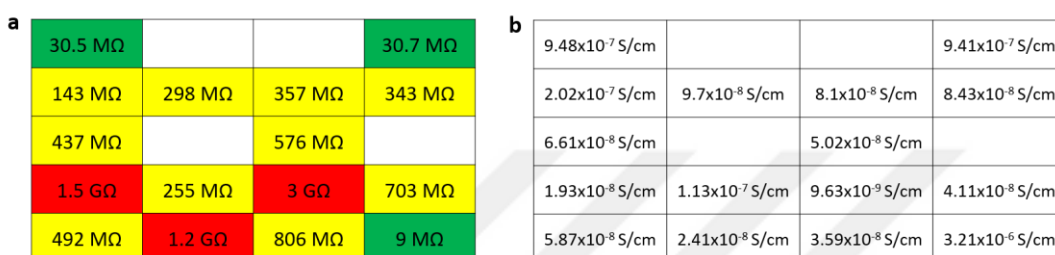


Figure 1.57. **(a)** Resistance map **(b)** conductivity map of **P10-P11** co-assembled peptide. Green color shows the lowest conductivities whereas red colored values are the highest resistance values.

For the co-assembled peptides **P12-P13** (3 mM), resistance map and conductivity map of 20 devices is represented in Figure 1.58. The devices have 10 micrometer length and 1000 micrometer width. The average thickness of xerogels was measured as 1.67 micrometer. The devices showed non-linear I-V curves. The resistance values was calculated from the linear regions of I-V curves with variable values ranging from 1.5 MΩ to 979 MΩ. Also, the conductivity values changes from 6.12×10^{-8} S/cm to 3.99×10^{-5} S/cm and the average conductivity was obtained as 1.68×10^{-5} S/cm by neglecting the three devices with high resistance values. Depending on these measurements, bis-terpyridine containing complementary peptides has 37 times higher conductivity value than tryptophan containing negative control peptides. Moreover, the conductivity of co-assembled **P12-P13** is same conductivity value with the peptide based donor-acceptor system found in literature.⁹⁴

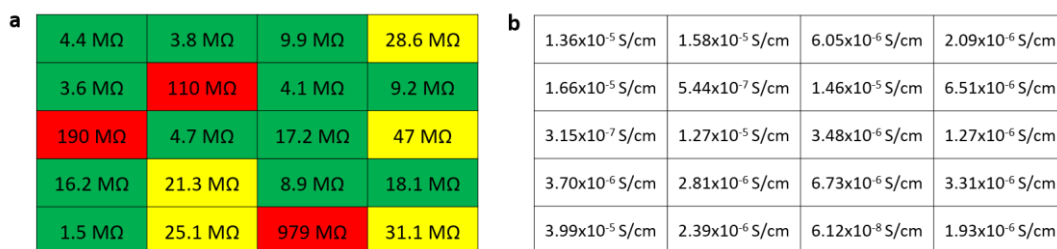


Figure 1.58. **(a)** Resistance map **(b)** conductivity map of **P12-P13** co-assembled peptide. Green color shows the lowest conductivities whereas red colored values are the highest resistance values.

The I-V curves of **P14-P13** co-assembled peptides (6 mM) was also measured to evaluate donor-acceptor type systems. Devices with 20 micrometer channel length was used and the average thickness of xerogel is 0.9 micrometer. The attained I-V curves are non-linear and the resistance values were calculated from the linear regime of curves. Figure 1.59 demonstrate the resistance and conductivity map of **P14-P13** co-assembled peptides which contains both pyrene and terpyridine moieties.

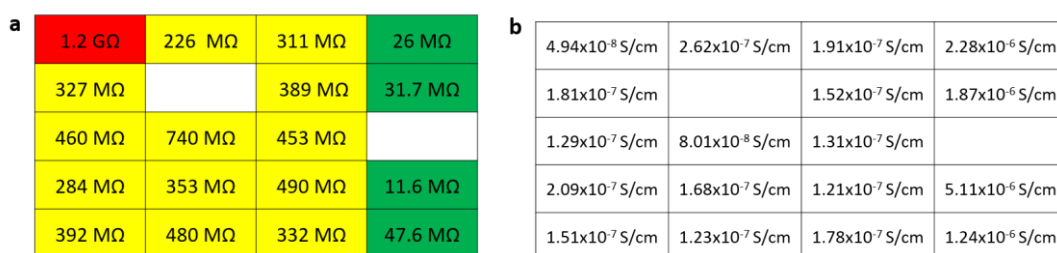


Figure 1.59. **(a)** Resistance map **(b)** conductivity map of **P14-P13** co-assembled peptide. Green color shows the lowest conductivities whereas red colored values are the highest resistance values.

The resistance values changes from 11.6 M Ω to 1.2 G Ω , thus conductivity value varies from 8.01×10^{-8} S/cm to 5.11×10^{-6} S/cm for 18 devices. The calculated

average conductivity value for 17 devices is found as 7.4×10^{-7} S/cm. This value is comparable with the negative control peptide co-assembly since the terpyridine and pyrene moieties do not overlap with each other to provide a strong charge transfer complex. This inference is consistent with the soft and viscous appearance of **P14-P13** gel.

1.2.9.3 Electrical measurements of cyclic dipeptides

The electrical measurements of cyclic dipeptide gels **P16** (5 mM), **P19** (1 mM) and **P22** (1 mM) were conducted in a collaborative study with Assist. Prof. Gökhan Bakan. Shadow masks with 10 micrometer and 20 micrometer channel spacing were used for metal electron coating (Figure 1.60). Gold electrodes were coated with thermal evaporation method, whereas aluminum, tin and copper electrodes were coated with electron beam evaporation method. Preliminary, all these metal electrodes were studied to decide which electrode to use. The gold electrodes give I-V curves with slight deviation from linearity for a long range of voltage. To prevent the destruction of self-assembly of peptides during the gold coating in thermal evaporator, it was decided to coat glass directly with gold electrodes before applying the gels on the surface. The device fabrication procedure is shown in Figure 1.61. After obtaining I-V curves by using semiconductor parameter analyzer, resistances were calculated from the slope of curves. The thickness of each device was measured as an average value with atomic force microscopy (AFM). Then the resistivity and conductivity values were calculated by using below formula (Equation 1.2).

$$\frac{1}{\sigma} = \rho = R \frac{t \times W}{L}$$

Equation 1.2. The equation used to calculate resistivity and conductivity in which ρ is resistivity, σ conductivity, R is resistance, t is the thickness of xerogel, W is the channel length and L is width of channel spacing.

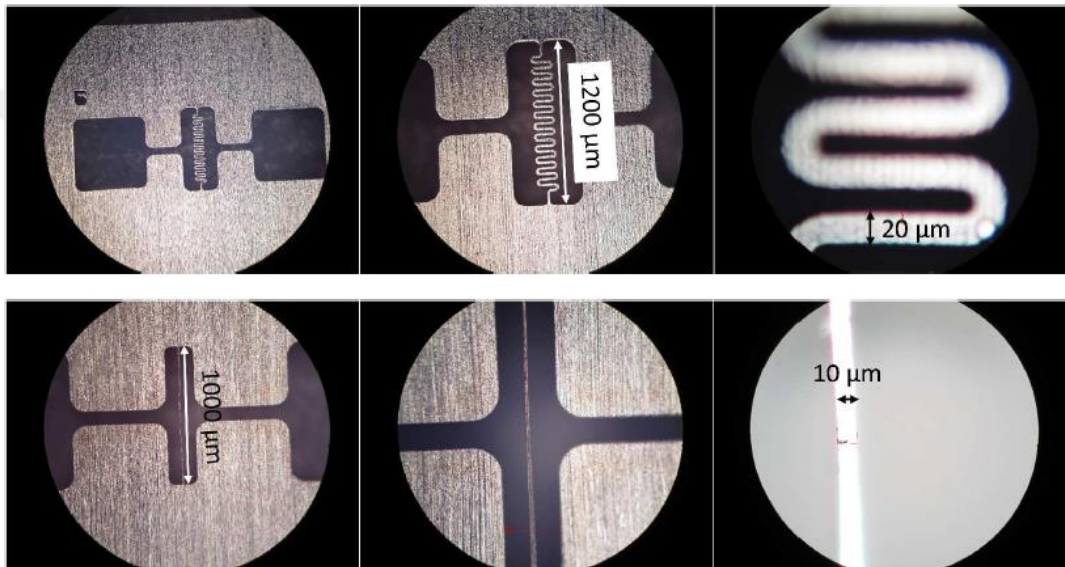


Figure 1.60. Microscope images of 20 μm and 10 μm channel spacing with a width of 3.75 mm and 1.0 mm, respectively.

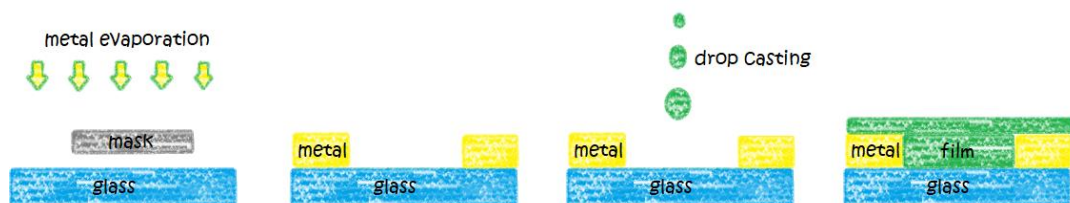


Figure 1.61. Process followed for device fabrication.

The conductivity values of self-assembled **P16**, **P19** and **P22** peptides were calculated with above method. The sample microscope image of a gel coated device is demonstrated below (Figure 1.62). Figure 1.63 represents the resistance and conductivity map of bis-terpyridine contained cyclic peptide, **P16**. The resistance ranges from 12 k Ω to 163 k Ω and the calculated highest conductivity value is 9.7×10^{-4} S/cm. The average conductivity of 19 devices is 3.9×10^{-4} S/cm with a standard deviation 60% standard deviation.

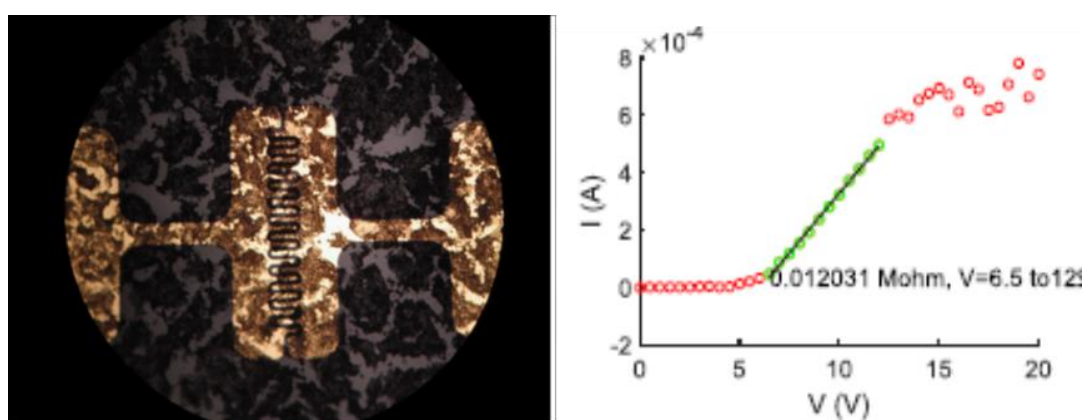


Figure 1.62. Sample microscope image of a **P16** gel coated device and its I-V curve. (The linear region used to calculate resistance is represented in green color.)

a

#1: 53 kΩ	#6: 37 kΩ	#11: 26 kΩ	#16: 34 kΩ
#2: 35 kΩ	#7: 112 kΩ	#12: 45 kΩ	#17: 12 kΩ
#3: 29 kΩ	#8: 64 kΩ	#13: 50 kΩ	#18: 27 kΩ
#4: 70 kΩ	#9: 163 kΩ	#14: 39 kΩ	#19: 22 kΩ
#5:	#10: 57 kΩ	#15: 60 kΩ	#20: 28 kΩ

b

#1: 2.8×10 ⁻⁴ S/cm	#6: 2.9×10 ⁻⁴ S/cm	#11: 5.9×10 ⁻⁴ S/cm	#16: 9.7×10 ⁻⁴ S/cm
#2: 3.5×10 ⁻⁴ S/cm	#7: 1.4×10 ⁻⁴ S/cm	#12: 2.3×10 ⁻⁴ S/cm	#17: 9.2×10 ⁻⁴ S/cm
#3: 4.9×10 ⁻⁴ S/cm	#8: 3.1×10 ⁻⁴ S/cm	#13: 2.5×10 ⁻⁴ S/cm	#18: 5.0×10 ⁻⁴ S/cm
#4: 1.5×10 ⁻⁴ S/cm	#9: 9.7×10 ⁻⁵ S/cm	#14: 4.2×10 ⁻⁴ S/cm	#19: 5.1×10 ⁻⁴ S/cm
#5:	#10: 2.6×10 ⁻⁴ S/cm	#15: 2.8×10 ⁻⁴ S/cm	#20: 4.5×10 ⁻⁴ S/cm

Figure 1.63. **(a)** Resistance and **(b)** conductivity map of **P16** after self-assembly.

In the same manner, the conductivity of mono-terpyridine containing cyclic dipeptide, **P19** gels were obtained. Sample image of a **P19** gel coated device after drying and its I-V curves is shown in Figure 1.64. The resistance values ranges from 0.2 M Ω to 16.7 M Ω (Figure 1.65). The highest conductivity is 2.0 x10⁻³ S/cm. Average conductivity is calculated from 17 devices as 2.7 x10⁻⁴ S/cm with a standard deviation of 86%.

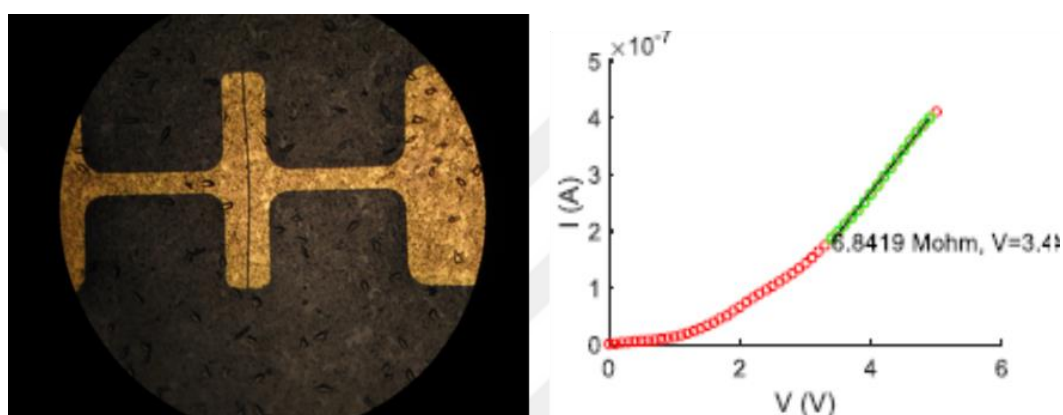


Figure 1.64. Sample microscope image of a **P19** gel coated device and its I-V curve. (The linear region used to calculate resistance is represented in green color.)

a

#1: 1.4 MΩ	#6: 3.6 MΩ	#11: 8.6 MΩ	#16: 2.6 MΩ
#2: 1.1 MΩ	#7: 4.0 MΩ	#12: 5.5 MΩ	#17: 2.4 MΩ
#3: 6.1 MΩ	#8:	#13: 6.8 MΩ	#18: 1.8 MΩ
#4:	#9: 16.7 MΩ	#14: 1.5 MΩ	#19:
#5: 1.2 MΩ	#10: 2.7 MΩ	#15: 0.2 MΩ	#20: 1.1 MΩ

b

#1: 4.2×10 ⁻⁴ S/cm	#6: 6.7×10 ⁻⁵ S/cm	#11: 5.8×10 ⁻⁵ S/cm	#16: 1.3×10 ⁻⁴ S/cm
#2: 5.9×10 ⁻⁴ S/cm	#7: 6.4×10 ⁻⁵ S/cm	#12: 6.7×10 ⁻⁵ S/cm	#17: 1.1×10 ⁻⁴ S/cm
#3: 3.7×10 ⁻⁵ S/cm	#8:	#13: 4.1×10 ⁻⁵ S/cm	#18: 1.5×10 ⁻⁴ S/cm
#4:	#9: 2.1×10 ⁻⁵ S/cm	#14: 2.1×10 ⁻⁴ S/cm	#19:
#5: 3.3×10 ⁻⁴ S/cm	#10: 2.2×10 ⁻⁴ S/cm	#15: 2.0×10 ⁻³ S/cm	#20: 1.7×10 ⁻⁴ S/cm

Figure 1.65. **(a)** Resistance and **(b)** conductivity map of **P19** after self-assembly.

The conductivity of **P22** which do not consist of any terpyridine moiety, was measured as a negative control. Although many of the devices was destroyed after

applying gel of **P22**, the average conductivity of **P22** self-assembled peptide was calculated as 9.7×10^{-8} S/cm (Figure 1.66).

a	#1: 100 GΩ	#6: broken	#11: broken	#16: broken
	#2: 90 GΩ	#7: broken	#12: broken	#17: 35 GΩ
	#3: short	#8: broken	#13: 38.1 GΩ	#18: broken
	#4: broken	#9: broken	#14: broken	#19: 7 GΩ
	#5: 20.1 GΩ	#10: broken	#15: broken	#20: 2.5 GΩ

b	#1:	#6:	#11:	#16:
	#2:	#7:	#12:	#17: 4.8×10^{-8} S/cm
	#3:	#8:	#13: 5.3×10^{-9} S/cm	#18:
	#4:	#9:	#14:	#19: 1.6×10^{-7} S/cm
	#5: 2.0×10^{-8} S/cm	#10:	#15:	#20: 2.5×10^{-7} S/cm

Figure 1.66. **(a)** Resistance and **(b)** conductivity map of **P22** after self-assembly.

As a result, the conductivity of **P16** and **P19** peptide gels are comparable with each other even the **P16** peptide has two parallel terpyridine moieties. Unfortunately, the **P16** peptide solubility is lower than **P19** and the peptide became precipitated partially during the drying of gel on gold coated glass and cause a bulk resistivity by forming rough films on the surface of glass substrate. As shown in microspore images of sample devices (Figure 1.62a and Figure 1.64a), the **P16** gel dried on surface without a film like formation and crack formation can be seen on images, whereas the **P19** gel dried on surface by forming more smooth films because of its higher solubility in gelation medium. Nevertheless, **P16** and **P19** gels show 4020 and 2780 times higher conductivity than the negative control **P22** gel, respectively. Moreover, the conductivity of **P16** and **P19** gels have 23 and 16 times enhanced conductivity than the peptide based donor-acceptor system found in literature.⁹⁴

The conductivity measurements of **P18** was conducted by Assoc. Prof. Dr. Görkem Günbaş. Unfortunately, the obtained I-V curves (Appendix E) deviate from linearity enormously that the conductivity value did not calculated.

1.2.10 Summary of synthesis, gelation and conductivity of designed peptides

Table 1.3 summarizes the synthesis, gelation and electrical measurement experiments of all designed peptides that intent to enhance conductivity of peptide based gels. As mentioned before, Nickel coordinated bis-terpyridine artificial amino acid (**3**) containing peptide was not synthesized because of the removal of Nickel ion coordination due to the presence of concentrated acid used in cleave step. Moreover, the synthesis of bis-pyrene and mono-pyrene containing cyclic peptides were not achieved since the pyrene moiety is highly temperature sensitive. The self-assembly of cyclo-octapeptide **P7** was not succeeded depending on the SEM imaging and the gelation of D-tyrosine containing cyclic dipeptide **P17** did not occur in the same gelation medium with **P16**. All synthesized peptides were enlighten with mass spectroscopy. The gelation was checked with inversion test and the secondary structures and microstructures were investigated with IR, UV-Vis and CD spectroscopy and TEM imaging. Lastly, the conductivity of gel forming peptides were measured with semiconductor parameter analyzer. The obtained I-V curves for **P18** deviate from linearity enormously that the conductivity could not be calculated. Chart 1.1 represents the compared conductivity values for complementary peptides and cyclic dipeptides. Calculated conductivity values of terpyridine containing peptide assemblies are comparable with or higher than literature data that aim to enhance conductivity, even 1500 times.^{79,88,94,123} These results let us to conclude that using two parallel aromatic units to decrease the distance between aromatic units in self-assembled systems enhances the conductivity. Nevertheless, the conductivity of peptides can be enhanced by improving device fabrication as well as using diluted self-assembled peptides to form less rough films which may decrease the bulk resistivity, doping method or using n-type and p-type aromatic units together in these reduced distance systems.⁸⁴ Improvements in stabilities and conductivities of these systems can make self-assembled peptides favorite materials for applications in which conductive polymers are used.

Table 1.3. Synthesis, Gelation and Conductivity of Designed Peptides.

PEPTIDE	SYNTHESIS	GELATION/SELF- ASSEMBLY	CONDUCTIVITY
P1	+	Viscous Solution	<i>not tested</i>
P2	+	Viscous Solution	<i>not tested</i>
P3	+	Soft Gel	3.3×10^{-4} S/cm
P4	+	Soft Gel	6.7×10^{-5} S/cm
P5	-	<i>not tested</i>	<i>not tested</i>
P6	+	Soft Gel	3.3×10^{-5} S/cm
P7	+	Precipitate	<i>not tested</i>
P8	+	Microcrystals	<i>not tested</i>
P9	+	Gel with P11	<i>not tested</i>
P10	+	Gel with P11	4.56×10^{-7} S/cm
P11	+	Gel w/ P10	4.56×10^{-7} S/cm
P12	+	Gel w/ P13	1.68×10^{-5} S/cm
P13	+	Gel w/ P12 and Soft Gel w/ P14	1.68×10^{-5} S/cm and 7.4×10^{-7} S/cm
P14	+	Gel w/ P13	7.4×10^{-7} S/cm
P15	+	Suspension w/ P14 and P12	<i>not tested</i>
P16	+	Gel	3.9×10^{-4} S/cm
P17	+	-	<i>not tested</i>
P18	+	Gel	<i>not calculated</i>
P19	+	Gel	2.7×10^{-4} S/cm
P20	-	<i>not tested</i>	<i>not tested</i>
P21	-	<i>not tested</i>	<i>not tested</i>
P22	+	Gel	9.7×10^{-8} S/cm

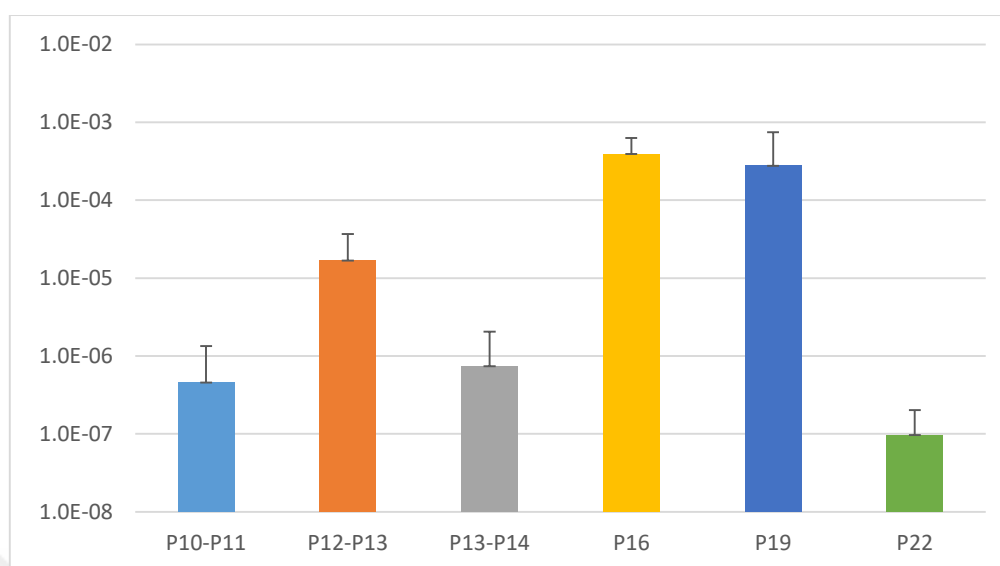


Chart 1.1. Bar graph for conductivities of complementary peptides and cyclic dipeptides. (Peptide sequence of **P10** is Ac-QQRFWEFEQQ-NH₂; **P11** is QQKFKWKFKQQ-NH₂; **P12** is Ac-QQRFE-1-EFEQQ-NH₂; **P13** is Ac-QQKFK-1-KFKQQ-NH₂; **P14** is Ac-QQRFE-2-EFEQQ-NH₂; **P16** is cyclo-(L-Tyr-1); **P19** is cyclo-(L-Tyr-19); and **P22** is cyclo-(L-Lys-L-Tyr).)

1.3 Experimental

1.3.1 Materials

All reagents are commercially available and used without any further purification. For chemical reactions, reagents and solvents; 2-acetylpyridine, *p*-tolualdehyde, teraphthalaldehyde, NaBH(OAc)₃, oxalyl chloride, 1-pyrenebutanol, Et₃N, Pt(COD)Cl₂, TIPS, ethanol, ethyl acetate and DMF were purchased from Sigma Aldrich; NH₄OH, NaOH, benzene, BPO, DMSO, sodium bicarbonate, toluene, *p*-toluenesulfonic acid, chloroform, sodium azide, 1,2-DCE, THF and HCl from Merck; N-bromosuccinimide, DCM, acetonitrile from Carlo Erba Reagents; piperidine and NaBH₄ from Acros Organics; DIEA from Fisher Chemical and TFA from Fluka. All amino acid derivatives and coupling reagents used in SPPS were purchased from Chem-Impex International Inc.

1.3.2 Synthesis of 4'-(methylphenyl)-2,2':6',2''-terpyridine (7)

In a 100 ml round bottom flask, 6.0 mmol *p*-tolualdehyde (**5**) (0.71 ml) and 12.0 mmol 2-acetylpyridine (**6**) (1.35 mL) were mixed in 25 ethanol. Then, 1 ml of 26% NH₄OH and 0.5 gram of NaOH previously dissolved in 1.6 ml distilled H₂O were added dropwisely on above solution. As the base addition done, the solution immediately started to be suspension with a color of firstly green and then yellow-orange. The reaction was allowed to stir vigorously without closing the flask at room temperature. The addition of extra 1 ml 26% NH₄OH was done every day throughout six days. After completion of reaction, 50 ml of distilled water was added on reaction medium to make the product precipitate well enough. The precipitate was taken with filtration and the product was washed with extra 150 ml distilled H₂O for

purification. After that, the off-white solid was allowed to dry at room temperature. (Yield: 80%)

^1H NMR (400 MHz, CDCl_3) δ 8.66 (4H, m), 8.61 (1H, m), 8.59 (1H, m), 7.28 (2H, ddd, J = 7.5, 4.8, 1.1 Hz), 7.25 (2H, d, J = 8.1 Hz), 2.36 (3H, s).

1.3.3 4'-(4-Bromomethylphenyl)-[2,2':6',2'']terpyridine (8)

2.00 mmol of **7** (647 mg) was dissolved in 26 mL of dry benzene in a 50 ml round bottom flask. Then 2.700 mmol (480.5 mg) N-bromosuccinimide (NBS) and 0.801 mmol (194 mg) benzoyl peroxide (BPO) were added on above solution. The mixture was refluxed at 90 °C for overnight by stirring under N_2 atmosphere. After the completion of reaction, the brown solution was filtered and washed with distilled H_2O . The aqueous phase was extracted with ethyl acetate. The combined organic layer was dried over MgSO_4 and then concentrated under vacuum. The column chromatography was applied to purify **8** with the eluent 1:10 THF/Hexane. White solids was attained. (Yield: 45%)

^1H NMR (400 MHz, CDCl_3) δ 8.66 (4H, m), 8.62 (1H, m), 8.60 (1H, m), 7.85 – 7.79 (4H, m), 7.49 – 7.44 (2H, m), 7.29 (2H, ddd, J = 7.5, 4.8, 1.1 Hz), 4.50 (2H, s).

1.3.4 4'-(4-Formylphenyl)-[2,2':6',2'']terpyridine (9)

2.85 mmol **8** was dissolved in 5.7 ml of DMSO which was dried molecular sieve before. After the addition of 1.425 gr NaHCO_3 , the reaction mixture was heated to 100 °C for 15 hours under N_2 atmosphere. Then, 30 ml of distilled H_2O was added to precipitate **9**. The crude product was purified over silica gel to obtain white solid. (Yield: 40%)

¹H NMR (400 MHz, CDCl₃) δ 10.04 (1H, s), 8.70 (2H, s), 8.67 (1H, dd, *J* = 1.7, 0.8 Hz), 8.66 (1H, dd, *J* = 1.7, 0.8 Hz), 8.62 (1H, m), 8.60 (1H, m), 8.01 – 7.93 (4H, m), 7.83 (2H, td, *J* = 7.7, 1.8 Hz), 7.30 (2H, ddd, *J* = 7.5, 4.8, 1.2 Hz)

1.3.5 4-(1,3-Dioxolan-2-yl)benzaldehyde (**11**)

In a 10 ml round, 6.00 mmol of teraphthalaldehyde (**10**) (805 mg) is dissolved in 2.4 ml dry toluene. Then, 6.00 mmol ethylene glycol (0.336 ml) and 12 mg p-toluenesulfonic acid were added on above solution. The mixture was refluxed with a Dean-Stark trap to remove produced H₂O from reaction medium for 4 hours under N₂ atmosphere. After 4 hours the reaction mixture was washed with Na₂CO₃ solution for two times even the reaction was not completed to prevent the double protection of **10**. The organic phases were combined and dried over MgSO₄. Then the solvent was removed under vacuum. The crude product was purified by column chromatography and glassy crystals was obtained. (Eluent: 1:3 Ethylacetate/Hexane, Yield: 70%)

¹H NMR (400 MHz, CDCl₃) δ 9.97 (1H, s), 7.86 – 7.81 (2H, m), 7.59 (2H, d, *J* = 8.2 Hz), 5.81 (1H, s), 4.08 – 3.99 (4H, m).

1.3.6 4'-(4-(1,3-dioxolan-2-yl)phenyl)-2,2':6',2''-terpyridine (**12**)

3.50 mmol of **11** (624 mg) was mixed with 7.00 mmol of 2-acetylpyridine (**6**) (0.785 ml) in 15 ml of ethanol. Then, 0.6 ml of 26% NH₄OH and 0.285 gr NaOH previously dissolved in 1 ml of distilled water were added on above solution. The yellow suspension formed immediately. The reaction was allowed to stir vigorously open to air at room temperature. 0.6 ml of 26% NH₄OH addition was done every day for six days. After completion, 30 ml of distilled water was added to form the precipitate of

12 as yellow solid. The product was washed with extra water for purification. (Yield: 75%)

^1H NMR (400 MHz, CDCl_3) δ 8.67 (3H, s), 8.66 – 8.65 (1H, m), 8.61 (2H, d, J = 7.9 Hz), 7.87 (2H, d, J = 8.3 Hz), 7.82 (2H, td, J = 7.8, 1.8 Hz), 7.56 (2H, d, J = 8.2 Hz), 7.29 (2H, ddd, J = 7.4, 4.8, 1.0 Hz), 5.84 (1H, s), 4.12 – 4.00 (4H, m).

1.3.7 4'-(4-Formylphenyl)-[2,2':6',2'']terpyridine (**9**) with acetal removal

To remove the acetal protection of **12**, 3 mmol of **12** was dissolved in CHCl_3 . The addition of 18 mmol TFA (1.4 ml) was done. After overnight stirring, the reaction mixture was washed with Na_2CO_3 solution to neutralize the reaction medium. The organic phase was dried over MgSO_4 and concentrated under vacuum to obtain a yellow precipitated. The product was used without further purification. (Yield: 97%)

^1H NMR (400 MHz, CDCl_3) δ 10.04 (1H, s), 8.70 (2H, s), 8.67 (1H, dd, J = 1.7, 0.8 Hz), 8.66 (1H, dd, J = 1.7, 0.8 Hz), 8.62 (1H, m), 8.60 (1H, m), 8.01 – 7.93 (4H, m), 7.83 (2H, td, J = 7.7, 1.8 Hz), 7.30 (2H, ddd, J = 7.5, 4.8, 1.2 Hz)

1.3.8 4'-(4-(Azidomethyl)phenyl)-2,2':6',2''-terpyridine (**15**)

170 mg of **8** was dissolved in 2.5 ml of 0.5 M NaN_3 solution prepared in DMSO. The reaction was allowed to stir for overnight at room temperature. After 3 ml of distilled H_2O addition, extraction was done with 2.5 ml DCM for 5 times to get the precipitated product in organic phase. The combined organic phases were dried over MgSO_4 . DCM was removed under vacuum. The product was used without further purification. (Yield: 90%)

^1H NMR (400 MHz, CDCl_3) δ 8.67 (4H, s), 8.61 (2H, d, $J = 7.4$ Hz), 7.86 (2H, d, $J = 8.1$ Hz), 7.81 (2H, t, $J = 7.6$ Hz), 7.39 (2H, d, $J = 8.1$ Hz), 7.29 (2H, m), 4.36 (2H, s).

1.3.9 Artificial amino acid **1**

1.00 mmol **9** (337 mg) and 1.00 mmol Fmoc-L-Lys-OH (**13**) (368 mg) was refluxed in 1,2-DCE at 70 °C for 30 minutes for the imine (**18**) formation under N_2 atmosphere. After decreasing the temperature of reaction vessel to room temperature, the addition of 1.25 mmol (265 mg) $\text{NaBH}(\text{OAc})_3$ was done in small portions slowly in ice bath at 0 °C. The reaction mixture was allowed to stir at room temperature for overnight under N_2 atmosphere. Then the addition of second equivalent 1.00 mmol **9** (337 mg) was done at room temperature. The addition of 1.25 mmol (265 mg) $\text{NaBH}(\text{OAc})_3$ was performed in small portions slowly in ice bath at 0 °C, again. The reaction mixture was allowed to stir for overnight under N_2 at room temperature. The reaction was checked by TLC. (Eluent: 1:6 MeOH/DCM) After the aldehyde **9** was run out, the reaction mixture was washed with 40 ml of distilled water. The aqueous phase was extracted with 40 ml DCM for 3 times. The combined organic phases washed with brine and then dried over MgSO_4 . The solvent was removed under vacuum. According to the ^1H NMR of crude product, the mixture contains 10% **15** in the medium. Nevertheless, the crude **1** was used for peptide synthesis without further purification. For NMR analysis, **1** was purified with *semiprep* RP-HPLC.

^1H NMR (400 MHz, D_2O) δ ppm 8.72 (4H, d, $J = 8.0$ Hz), 8.78 (4H, d, $J = 5.3$ Hz, 4H), 8.64 (4H, s), 8.33-8.22 (4H, m), 7.97 (4H, d, $J = 8.2$ Hz), 7.74 (5 H, dd, $J = 7.0, 5.9$ Hz), 7.69 (4H, d, $J = 8.2$ Hz), 7.50-7.44 (2H, m), 7.41-7.34 (2H, m), 7.12 (2H, t, $J = 7.5$ Hz), 7.04 (2H, dd, $J = 14.4, 7.4$ Hz), 4.54 (4H, s), 4.14 (1H, m), 4.10-4.03 (2H, m), 3.94 (1H, s), 3.17-3.05 (2H, m), 1.91-1.73 (4H, m), 1.43-1.25 (3H, m)
HRMS: calculated for $[\text{M}-\text{H}]^-$ $\text{C}_{65}\text{H}_{54}\text{N}_8\text{O}_4$, 1009.4268; found 1009.4280.

1.3.10 1-pyrenebutanal (**21**)

In a 100 ml round bottom flask, 0.452 ml of oxalyl chloride was mixed with 9.8 ml dry DCM. The mixture was cooled to -78 °C. Then, previously mixed 1.02 ml dry DMSO and 1.51 ml dry DCM was added on above solution. The solution was allowed to stir for 10 minutes in -78 °C under N₂ atmosphere. In a different flask, 274.4 mg 1-pyrenebutanol (**20**) (1.000 mmol) was dissolved in a mixture of 1.02 ml dry DMSO and 1.51 ml dry DCM. The mixture was added on oxalyl chloride solution dropwisely at -78 °C and allowed to stir for 1 hour under N₂ atmosphere. After that, 2.53 ml Et₃N was added on reaction medium. The reaction was allowed to warm for overnight by stirring. 20 ml of distilled H₂O was added to the reaction medium and the organic phase was separated in an extraction funnel. The aqueous phase extracted with DCM for two times. The combined organic layer was dried over MgSO₄ and removed under vacuum. The crude product was purified with column chromatography. (Eluent: 1/1 DCM:Hexane) A yellow product was obtained. (Yield: 85%)

¹H NMR (400 MHz, CDCl₃) ppm 9.74 (1H, t, *J* = 1.3 Hz), 8.23 (1H, d, *J* = 9.3 Hz), 8.11 (2H, dd, *J* = 7.6, 3.6 Hz), 8.06 (2H, dd, *J* = 8.5, 3.7 Hz), 7.97 (2H, s), 7.92 (1H, t, *J* = 7.6 Hz), 7.77 (1H, d, *J* = 7.8 Hz), 3.36-3.30 (2H, m), 2.50 (2H, td, *J* = 7.1, 1.1 Hz), 2.14 (3H, p, *J* = 7.2 Hz)

1.3.11 Artificial amino acid 2

0.25 mmol 1-pyrenebutanal (**21**) and 0.25 mmol Fmoc-L-Lysine-OH were mixed in 4.5 ml dry 1,2-DCE and allowed to stir for imine formation along the night under N₂ atmosphere. After overnight stirring at room temperature, the suspension became clearer solution. Then, the addition of 0.3 mmol NaBH(OAc)₃ addition was done at 0 °C. The reaction was allowed to stir overnight at room temperature under N₂. After that, the addition of second equivalent of **21** (0.25 mmol) and 0.31 mmol

NaBH(OAc)₃ were performed in ice bath. The reaction was stirred for overnight under N₂ atmosphere. The reaction solution was extracted with distilled water. The aqueous phase was washed with DCM for 3 times. The combined organic phase was dried over MgSO₄ and removed under vacuum. The crude **2** was purified by column chromatography. (Eluent: 1:12 MeOH/DCM) (Yield: 55%)

¹H NMR (400 MHz, CDCl₃) δ 8.09 – 7.95 (m, 11H), 7.91 – 7.84 (m, 6H), 7.66 (t, *J* = 8.5 Hz, 4H), 7.60 (dd, *J* = 13.3, 7.6 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 7.26 – 7.20 (m, 2H), 6.09 (d, *J* = 5.6 Hz, 1H), 4.35 (p, *J* = 10.6 Hz, 2H), 4.23 – 4.14 (m, 2H), 3.13 (t, *J* = 7.1 Hz, 4H), 2.74 (m, 4H), 2.65 – 2.51 (m, 2H), 1.96 – 1.10 (m, 14H). HRMS: calculated for [M+H]⁺ C₆₁H₅₆N₂O₄, 881.4240; found 881.4252.

1.3.12 Artificial amino acids **3** and **4**

Around 0.25 mmol (~253 mg) of crude artificial amino acid **1** was dissolved in 7.5 ml methanol. For the synthesis of **3**, 0.52 mmol NiCl₂ and for the synthesis of **4**, 0.52 mmol Pt(COD)Cl₂ was added on above mixture and stirred under reflux for 12 hours. The reaction mixtures were cooled to room temperature and filtered. The residues were washed with pure methanol and allowed to dry at room temperature. Green and orange crystals were obtained for **3** and **4**, respectively. The products were directly used for peptide synthesis without further purification. HRMS: calculated for **3** [M+H]⁺ C₆₅H₅₄Cl₂N₈Ni₂O₄²⁺, 1197.2341; found 1197.2236.

1.3.13 Artificial amino acid **22**

0.060 mmol **2** (53 mg) was dissolved in 1 ml THF and 100 µl piperidine was added. The reaction was allowed to stir for overnight. The solvent was removed under vacuum and the solid was washed with hexane for 5 times to remove cleaved Fmoc

moiety. The obtained product was directly used for the fluorescence spectroscopy analysis.

1.3.14 Cyclo-(1-L-Tyr) (P16) and Cyclo-(1-D-Tyr) (P17)

0.25 mmol **1** (1.0 gr) was dissolved in 1.5 ml THF/DMF (1:1) mixture and stirred for 30 minutes in ice bath under N₂ atmosphere. Later, 0.30 mmol EDC.HCl (58 mg) and 0.30 mmol HOBT (41 mg) was added in above solution and stirred for another 1 hour. Then, 0.30 mmol L-Tyr-OMe.HCl or D-Tyr-OMe.HCl (69 mg) was added. The reaction mixture was stirred at 0 °C for 1 hour and then, 24 hours at 40 °C. The product was precipitated in 30 ml 0.1 M HCl, filtered and dried at room temperature to attain **L-P16** or **L-P17**. The 50 mg crude peptide was dissolved in 0.5 ml piperidine/DMF (20% v/v) solution and stirred at 50 °C for 3 hours. Then, the crude product was precipitated in 12 ml diethyl ether. Precipitate was washed with 5 ml diethyl ether for 3 times. The crude product was purified with *semiprep* RP-HPLC.

¹H NMR (400 MHz, DMSO) δ 8.67 – 8.63 (5H, m), 8.62 (2H, m), 8.60 (2H, m), 8.59 (2H, s), 8.08 (4H, td, *J* = 7.8, 1.7 Hz), 7.92 (4H, d, *J* = 8.3 Hz), 7.58 (5H, d, *J* = 8.3 Hz), 7.55 (4H, ddd, *J* = 7.5, 5.1, 1.1 Hz), 6.94 (2H, d, *J* = 8.5 Hz), 6.64 (2H, d, *J* = 8.5 Hz), 4.51 (4H, s), 4.23 – 4.18 (1H, m), 3.76 – 3.68 (1H, m), 3.16 (1H, dd, *J* = 14.2, 3.5 Hz), 3.09 – 3.01 (2H, m), 2.72 (1H, dd, *J* = 13.9, 4.7 Hz), 1.67 – 1.56 (2H, m), 1.56 – 1.46 (2H, m), 1.12 – 1.05 (1H, m), 0.76 – 0.70 (1H, m).

1.3.15 Bis-platinum coordinated cyclo-(1-L-Tyr) (P18)

4.0 mg of purified **P16** (4.3×10^{-3} mmol) was dissolved in 130 μL methanol and after adding 1.7 mg of Pt(COD)Cl₂ (9.0×10^{-3} mmol), the mixture was refluxed for 12 hours. The reaction allowed to cool to room temperature and then precipitate was

filtered. The residue was washed with cold methanol to obtain **P18**. The product was directly used for gelation experiment. HRMS: calculated for **P18** $[M+2H]^{2+}$ $C_{59}H_{51}Cl_2N_9O_3Pt_2$, 697.1383; found 697.1609.

1.3.16 Cyclo-(L-Lys-L-Tyr) (P22)

1.7 mmol Fmoc-L-Lys(Fmoc)-OH (1.0 gr) was dissolved in 10 ml THF/DMF (1:1) mixture and stirred for 30 minutes in ice bath under N_2 atmosphere. Later, 2.04 mmol EDC.HCl (391 mg) and 2.04 mmol HOBT (274 mg) was added in above solution and stirred for another 1 hour. Then, 2.04 mmol L-Tyr-OMe.HCl (469 mg) was added. The reaction mixture was stirred at 0 °C for 1 hour and then, 24 hours at 40 °C. The product was precipitated in 170 ml 0.1 M HCl, filtered and dried at room temperature to attain **L-P22**. Then, crude peptide was dissolved in 5 ml piperidine/DMF (20% v/v) solution and stirred at 50 °C for 3 hours. The crude product was precipitated in 50 ml diethyl ether. Precipitate was washed with 10 ml diethyl ether for 3 times. The crude product was purified by washing with acetic acid for 3 times.

^1H NMR (400 MHz,) δ 8.13 (1H, d, J = 1.3 Hz), 8.02 (1H, d, J = 1.5 Hz), 7.64 (2H, s), 6.97 (2H, d, J = 8.4 Hz), 6.68 (2H, d, J = 8.4 Hz), 4.13 (1H, m), 3.63 (1H, m), 3.07 (1H, dd, J = 13.6, 3.6 Hz), 2.76 (1H, dd, J = 13.6, 4.7 Hz), 2.64 (2H, m), 1.38 – 1.27 (2H, m), 1.19 – 1.08 (1H, m), 0.85 (d, J = 7.6 Hz), 0.91 – 0.77 (3H, m).

1.3.17 Mono-terpyridine containing cyclic dipeptide P19

0.5 mmol **P22** and 0.5 mmol **9** were mixed together in 10 ml dry DMF. The mixture was heated to 70 °C for 30 minutes under N_2 atmosphere. The reaction mixture was cooled to 0 °C in an ice bath and the addition of 0.625 mmol $NaBH_4$ (23.8 mg) was

done in small portions. The flask was allowed to stir at room temperature for overnight under inert atmosphere. The crude product was precipitated with the addition of 30 ml DCM. The residue was washed with 20 ml DCM. **P19** was then purified by *semiprep* RP-HPLC.

^1H NMR (400 MHz,) δ 8.87 (4H, m), 8.83 (2H, s), 8.32 (2H, t, $J = 7.8$ Hz), 8.15 (2H, d, $J = 8.1$ Hz), 7.78 (4H, d, $J = 7.5$ Hz), 7.06 (2H, d, $J = 8.4$ Hz), 6.78 (2H, d, $J = 8.4$ Hz), 4.35 (2H, s), 4.33 – 4.29 (1H, m), 3.80 (1H, t, $J = 5.4$ Hz), 3.28 (1H, dd, $J = 14.3, 3.4$ Hz), 3.01 – 2.92 (2H, m), 2.86 (1H, dd, $J = 14.0, 4.6$ Hz), 1.59 – 1.48 (2H, m), 1.25 – 1.14 (1H, m), 0.98 – 0.80 (3H, m).

1.3.18 General solid phase peptide synthesis protocol

1.3.18.1 1st Amino acid loading to 2-chlorotrityl chloride resin

0.5 mmol of resin was swelled in DMF for 20 minutes in the reaction vessel. Then, 0.75 mmol of Fmoc-protected amino acid was weighed and dissolved in 1.5 ml DMF and 652 μl DIEA. The solution was added on resin slowly with a Pasteur pipette. It was allowed to stay for overnight. The resin was washed with DMF and then DCM quietly. Resins was dried under vacuum. 5 mg dried resin was weighed in an eppendorf tube and 1 ml of 20 v/v% piperidine/DMF was added on it. It was float in ultrasonic bath for 20 minutes for the cleavage of Fmoc unit. 100 μl of solution was taken and diluted to 10 ml with DMF. The absorbance of solution at 310 nm was analyzed in UV-Vis spectroscopy for three times to calculate the substitution of resin with below formula (Equation 1.3). The remaining resin was swollen in DMF again to cap with 1 ml methanol for 10 minutes. Then, the resin was washed with DMF.

$$\text{Substitution} = \frac{101 \times \text{Abs}}{7.8 \times \text{mg of resin}}$$

Equation 1.3. Equation to calculate substitution of resin depending on the absorbance at 301 nm.

1.3.18.2 Cleavage of Fmoc moiety

In order to couple amino acids to the resin amide resin or to previous amino acid Fmoc cleavage should be performed, at first. For the 0.1 mmol resin, 10 ml of 20% piperidine/DMF solution was added on to the swollen resin. After 10 minutes, the solution was removed with vacuum filtration and another 10 ml piperidine solution was added. 10 minutes later, the piperidine solution was removed and the resin was washed with DMF for purification.

1.3.18.3 Coupling of amino acids

For 0.10 mmol of resin, 0.55 mmol of Fmoc-protected amino acids was weighed in a vial and dissolved in 1 ml of 0.5 M HBTU solution prepared previously in DMF. For the activation, 200 µl DIEA was added and the solution was shaken for 30 seconds. The activated amino acid solution was added on resin slowly with Pasteur pipette. The reaction was allowed to stand for 40 minutes by stirring occasionally with a glass rod. The coupling reaction can be checked with Kaiser Test by taking small amount of resin beads in to a test tube, if necessary. The taken beads should be washed with ethanol before performing test. After the completion of reaction, resins were washed with DMF to remove the excess reagents and by-products.

For the coupling of artificial amino acids, 0.30 mmol of artificial amino acid, 0.55 ml 0.5 M HBTU solution and 109 μ l DIEA was used. If the artificial amino acid was not dissolved in HBTU solution, minimum amount of DMF was added up to dissolution. The reaction was allowed to stand at least 3 hours up to 16 hours. The completion of reaction was checked with Kaiser Test. To continue to synthesize peptide before the completion of previous coupling reaction, capping of free amino on resin should be performed with acetic anhydride.

1.3.18.4 Acetylation of N-terminus peptides

For the acetylation of 0.1 mmol peptide bonded to resin, a solution of 200 μ l acetic anhydride and 200 μ l pyridine was prepared in 4 ml DMF. The solution was added in reaction vessel and allowed to stay at room temperature for 20 minutes. Then, the resin was washed with DMF.

1.3.18.5 Cleavage of peptide from resin

The resin was washed with DCM fairly and allowed to dry by suction filtration. For the cleavage of 0.1 mmol peptide from resin, 6 ml of cleavage cocktail was prepared with a composition of 95% TFA, 2.5% TIPS and 2.5% MilliQ water. The solution was added slowly on resins with a Pasteur pipette and it was allowed to stay for 1 hour or 2 hour depending on the sequence. The solution was collected dropwisely on 15 ml cold diethyl ether for precipitation. Resin was washed with extra 1 ml of TFA. The ether solution was removed from crude peptide by centrifugation. Peptide was washed with 10 ml cold diethyl ether for 3 times and allowed to dry at room temperature. The peptide profile was analyzed with analytical RP-HPLC and

purification was performed with *semiprep* RP-HPLC. Then mass spectroscopy analysis was performed to verify the synthesized peptide.

1.3.19 Microwave-assisted solid phase peptide synthesis

CEM Discover Bio - Manual Microwave Peptide Synthesizer was used for the microwave-assisted SPPS. After loading first amino acid to 2-chlorotrityl chloride resin or directly with Rink Amide MBHA resin, Fmoc cleavage was conducted by adding 5 ml of 20% piperidine/DMF solution for 0.1 mmol of peptide synthesis. The mixture was exposed to 20 Watt microwave with a maximum temperature of 75 °C only for 1 minute with N₂ bubbling. The solution was removed and another 5 ml piperidine solution was added. The reaction was exposed to microwave for 3 minutes, this time. Then, the resin was washed with 10 ml DMF for 5 times and continued with coupling reaction. 5.5 fold of Fmoc-protected amino acid (0.55 mmol) and 0.50 mmol HBTU (190 mg) was dissolved in 4 ml of 0.3 M DIEA solution prepared in DMF. After complete dissolution, the solution was added on resin and the reaction was followed for 5 minutes at 20 Watt and maximum 75 °C with N₂ bubbling. The resin was washed with 10 ml DMF for 5 times. The peptide synthesis continued with Fmoc cleavage and repeat as above. For the coupling of artificial amino acids (3 fold used), the reaction was not exposed to microwave and conducted at room temperature with N₂ bubbling. After the coupling of **2**, the following Fmoc removal and coupling reactions were done at 20 Watt with maximum temperature of 45 °C to prevent the decomposition of pyrene moiety. Afterward, the acetylation and cleavage of peptide from resin procedures were followed as mentioned above.

1.3.20 Cyclization of P7 and P8

47.2 μmol **L-P7** and **L-P8** was dissolved in 65 ml DMF. Then, 143.3 μmol pyAOP and 287 μmol Et_3N (40.0 μl) were added on above solution and allowed to stir for 3 hours at room temperature. The completion of reactions were checked with RP-HPLC. The solvent was removed under vacuum and the peptides were suspended in 14 ml water/acetonitrile/TFA (1:1:0.001) solution. The peptides were collected by centrifugation and washed with above solution twice. The suspension of peptides was obtained in water were lyophilized. **P7** and **P8** peptides were directly used for self-assembly experiment.

1.3.21 Analytical and semi-preperative RP-HPLC analysis

Reverse phase high pressure liquid chromatograph analysis were performed by using Dionex UltiMate 3000 HPLC instrument. The two component eluent system was used. The eluents consist of MilliQ water with 0.1% TFA and acetonitrile with 0.08% TFA. For analytical analysis Thermo Scientific AcclaimTM 120 C18 column (46 x 150 mm, 3 μm) with 0.5 ml/min was employed. The semi-preparative RP-HPLC was conducted with Thermo Scientific Hypersil Gold C18 column (250 x 10 mm, 5 μm) with 2 ml/min or 3 ml/min. 20 mg crude peptide was dissolved in 0.5 ml MilliQ water with TFA, if dissolves or same amount of peptide was dissolved in 0.4 ml acetic acid and diluted with 0.4 ml MilliQ water for purification. Fractions with 0.5 to 1.0 ml volume were collected in separate test tubes and analyzed with RP-HPLC one by one to determine their purities. The combined pure peptide was frozen in liquid nitrogen and lyophilized by Telstar Cryodos Freeze Dryer. The specified analytical and semi-preparative methods applied for each peptide is indicated in APPENDIX D.

1.3.22 Mass analysis

The freshly prepared peptide solutions (1 mg/ml) was characterized on Agilent 6530 Q-TOF mass spectrometer or Waters Synapt G1 mass spectrometer both equipped with ESI source.

1.3.23 Gelation experiments

Gelation experiments of each peptide was explained in below sections.

1.3.23.1 Gelation method for P1 and P2

To prepare 3 wt% gels, 3 mg of purified peptides were weighted in 1.5 ml vials and dissolved in 100 μ l 20 mM NaCl solution. The solutions were sonicated in ultrasonic bath for 5 minutes and allowed to stand for 5 minutes at room temperature.

1.3.23.2 Gelation method for P3, P4 and P6

To prepare 4 wt% gels, 4 mg of purified peptides were weighted in 1.5 ml vials and dissolved in 100 μ l 20 mM pH 7.5 Tris.HCl buffer and sonicated in Kudos ultrasonic bath at 53 kHz for 5 minutes and allowed to stand for 30 minutes at room temperature.

1.3.23.3 Gelation method for complementary peptides

The purified peptides were weighed in 2 ml Eppendorf tubes and dissolved in 0.5 ml MilliQ water to have a concentration of 3.1 mM. The pH of each peptide solution was determined with micro pH electrode. By adding 0.50 M and 0.25 M NaOH solution in microliter portions, the pH of each peptide solution was adjusted to 7.2 to 7.4. The pH adjusted complementary peptides were mixed by adding one solution to another by pipetting. For the gelation of **P14-P13**, 6 mM of peptide solutions were used and same procedure was followed.

1.3.23.4 Gelation method for cyclic dipeptides

The weighted purified peptides were dissolved in dry DMSO in 2 ml Eppendorf tubes and the addition of same volume of 0.1 M pH 7.0 phosphate buffer was done to make the concentration 5 mM. The samples were heated at 80-90 °C up to complete dissolution and sonicated in Kudos ultrasonic bath at 60 °C for 30 seconds to 1 minute to obtain gel. The gelation of purified **P22** was performed by directly sonicating the DMSO solution of peptide in ultrasonic bath for 2 minutes.

1.3.24 Self-assembly method for P7 and P8

10 mg of cyclic octapeptide was dissolved in 1 ml neat TFA in an Eppendorf tube. The open Eppendorf tube was floated in a 30 ml distilled water filled beaker for 48 to 72 hours. Then, TFA was removed by centrifugation and the precipitates were washed with 1 ml of distilled water for 3 times and allowed to dry open to air.

1.3.25 Fluorescence spectroscopy analysis

10 μ M solutions of **20** and **22** were prepared in DCM. Their fluorescence emission spectrum at excitation wavelength 343 nm was attained by using Agilent Technologies Cary Eclipse Spectrophotometer.

1.3.26 Transmission electron microscopy imaging

The microstructure formations after self-assembly was imaged with FEI Tecnai G2 Spirit BioTwin. Gels were diluted 50-fold or 100-fold with water by pipetting and applied on Cu grid. After waiting for 2 minutes, excess solution on Cu grid was removed. Then, the surface was stained with 2% uranyl acetate solution. The excess of stain was removed by dipping grids on pure water droplets. The grids was allowed to dry at least 4 hours.

1.3.27 Scanning electron microscopy imaging

SEM imaging was conducted by using Philips FEI Quanta 400F. The dried samples of **P7** and **P8** were suspended in water and dropped on sample holder. They were allowed to dry at room temperature. Then, SEM imaging was performed.

1.3.28 Circular dichroism spectroscopy analysis

The gels were diluted to 200 μ M with MilliQ water. The analysis was done from 260 nm to 190 nm with 3 scans for each sample at a scan rate of 50 nm/min with a 1 nm

step interval and measured in mdeg. Quartz cuvette was used with a path length of 1.0 mm and the CD analyses were conducted with Jasco J-815 CD Spectrometer.

1.3.29 Infrared spectroscopy analysis

The prepared gels were dropped on the crystal surface as 10 μ l volume and allowed to dry at least half an hour. Attenuated total reflectance was used and the analyses were performed as 32 scans with Thermo Scientific Nicolet iS10 instrument.

1.3.30 UV-Vis spectroscopy analysis

The 5 mM gels of **P16** and **P19** was diluted to 20 μ M with pH 7 buffer. Same concentration of unassembled peptide solutions was also prepared in DMSO to compare absorbance of self-assembled and unassembled peptides. Varian Cary 100 Bio UV-Vis Spectrophotometer was used for the analysis.

1.3.31 Powder X-ray diffraction measurements

P19 gel was lyophilized to obtain xerogel. XRD measurements were conducted by Assoc. Prof. Ferdi Karadaş by using X'Pert³ MRD with Cu K α X-ray radiation (λ =1.540598 Å).

1.3.32 Electrical measurements

For the 4-point probe measurements of **P3**, **P4** and **P6**, 10 μ l gels were directly deposited on a clean glass surface and resistance value was measured.

The electrical measurements of cyclic dipeptide gels and their conductivity calculations was conducted by Assist. Prof. Gökhan Bakan. For the electrical measurements of complementary peptide gels, we have collaborated with Assoc. Prof. Görkem Günbaş. For the electrical measurement of them, peptide gels were coated on gold electrode deposited glass substrates. The shadow masks with channel spacing 10 μm and 20 μm were purchased from Ossila Inc. Gold electrodes were deposited on cleaned 1.5 x 2.0 cm^2 glass surface with thermal evaporation method at a pressure of 5×10^{-6} Torr with a evaporation rate of 1-2 $\text{\AA}/\text{s}$. The final thickness of gold is around 50 nm. Then, 100 μl gels were coated on glass substrates by drop casting method and allowed to dry open to air at least one day. **P10-P11** (3 mM), **P12-P13** (3 mM), **P8** (5 mM), **P18** (5 mM), **P19** (5 mM) and **P22** (1 mM) gels were applied on devices with 10 μm channel spacing, while **P14-P13** (6 mM) and **P16** (1 mM) gels were coated on 20 μm channel space having devices. Electrical measurements were conducted with Keitley semiconductor parameter analyzer with a microprobe station. The tungsten needles were placed on contact pads. The contact resistances are quite negligible. The voltage applied to one pad was fixed to 0 V, and other pad was exposed to voltage from -20 V to 20 V to obtain I-V curves. The thickness of each device for cyclic dipeptides were measured one by one with AFM, whereas the thickness of complementary peptides were calculated by measuring the thickness of xerogel on glass from 10 different points with Dektak profilometer. The resistivity and conductivity of films were calculated depending on the Equation 1.2.

CHAPTER 2

LOW MOLECULAR WEIGHT ORGANOGELOTORS AND THEIR APPLICATIONS

2.1 Introduction

In this section, the structural properties of natural α -amino acids are discussed and a general information of about some side chain protecting groups of amino acids used in solid phase peptide synthesis are given. After mentioning about low molecular weight gelators and self-assembly phenomenon, amino acid derived low molecular weight gelators and their applications are discussed. Then, fluoride ion sensitive gelators and chemosensors, and drug release studies of gelators following multi-component gelators are explained. At the end, the general information about this study is addressed.

2.1.1 Amino acids and their structural properties

Amino acids contain amine, carboxyl unit and a specific side chain. There are hundreds of α -, β -, γ - and δ - amino acids in the nature.¹²⁴ Nevertheless, only 20 naturally occurring α -amino acids are found in genetic code. These 20 amino acids are denoted both with one letter notation and three letter code. These amino acids can be classified depending on their molecular weight, polarity, pH and/or side chain group type. Taylor¹²⁵ creates a Venn diagram (Figure 2.1) to represent chemical, physical and structural properties of amino acids.

Alanine, leucine, isoleucine, valine and proline contain aliphatic units.¹²⁶ Methionine can also be considered in the same category. Lysine, arginine, glutamic acid and glutamine are amphipathic amino acids since they both contain hydrophobic and hydrophilic units. Not all hydrophobic side chains are aliphatic, but also aromatic units are found in amino acid structures. Although, phenylalanine, tryptophan, histidine and tyrosine are aromatic amino acids, phenylalanine and tryptophan are more hydrophobic. Aromatic amino acids take part in π - π stacking in proteins.¹²⁷ Polar amino acids can be categorized in three parts: negatively charged, positively charged and neutral ones.¹²⁶ Aspartate and glutamate are negatively charged ones, whereas lysine and arginine are positively charged. Histidine, asparagine, glutamine, tyrosine, serine and threonine are neutral polar amino acids. Unlike phenylalanine, tyrosine is partially hydrophobic since it contains a hydroxyl unit bonded to aromatic ring. It can participate in π - π stacking with other aromatic rings. Furthermore, hydroxyl units present in tyrosine, serine and threonine are reactive and make them interact with other non-carbon atoms. Lipases and proteases contain serine in their active sites. Intracellular serine, threonine and tyrosine can be phosphorylated in proteins by protein kinases. Besides, the lower molecular weight amino acids can also be classified as small amino acids. These class of amino acid includes glycine, alanine, cysteine, proline, serine and threonine.

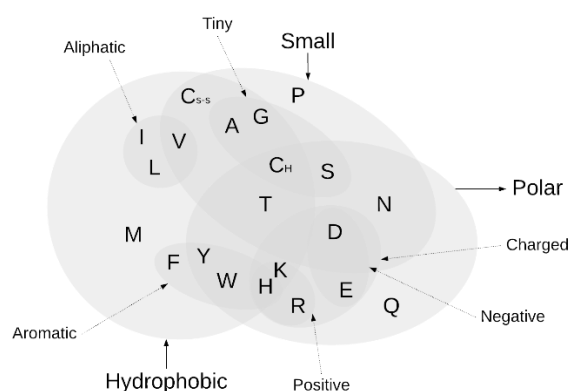
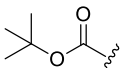
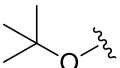
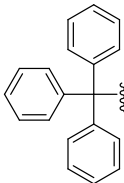
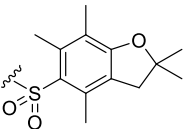
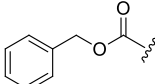
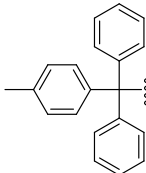


Figure 2.1. Venn diagram of amino acids.

2.1.2 Amino acid protecting groups

Amino acids are versatile building blocks for peptide synthesis. Backbone amino groups and side chains, if they contain functional units, should be protected with orthogonal protecting groups.¹²⁸ These protecting groups belong to different classes and can be removed with distinctive mechanisms. Thus, orthogonal protecting groups can be removed in any order, independent of the presence of rest. There are two main methods for solid phase peptide synthesis: Fmoc strategy and Boc strategy. Solid phase peptide synthesis occurs from C-terminus to N-terminus, thus, carboxylic acid is not protected, whereas amino group is protected with either 9-fluorenylmethoxycarbonyl (Fmoc) or *tert*-butyloxycarbonyl (Boc) group. In the Fmoc strategy, since this unit is base sensitive, the side chains should not be protected with base sensitive groups. Under these circumstances, side chains are generally protected with acid sensitive groups such as, *tert*-butyloxycarbonyl (Boc), *tert*-butoxy (OtBu), trityl (Trt), 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf), benzyloxycarbonyl (Cbz), 4-methyltrityl (Mtt) groups (Table 2.1).^{128,129} Boc, OtBu, Trt, and Pbf are common protecting groups used in Fmoc-based solid phase peptide synthesis, whereas Cbz and Mtt are rarely used to provide selective cleavage on an amino acid side chain.

Table 2.1. Certain acid sensitive side chain protecting groups of amino acids.

Boc	OtBu	Trt	Pbf	Cbz	Mtt
					

2.1.3 Self-assembly and low molecular weight gels

Low molecular weight (LMW) gels are attained from atom economic molecules and have attracted significant research attention over the past two decades due to their numerous potential applications, including drug delivery, oil-spill recovery, crystal growth, ion sensing, smart electronics, stimuli-responsive materials, catalysis, crystal growth, biomaterial replacement, vaccine engineering, and data storage.^{10,130–135} LMW gels are completely reversible and the gelators are very potent for several applications since generally form gels below 2 wt%.^{130,131,136–139} In contrast to macromolecular and inorganic gelators, characteristics such as the intermolecular hydrogen bonding, π - π stacking of aromatic units, hydrophobic effect, and van der Waals forces lead to the self-assembly of low molecular weight gelators (LMW) in well-ordered fibrous structures, tapes, sheets, α -helix etc.^{10,130–134,140,141} The entanglement of these secondary structures (Figure 2.2) eventually results in the immobilization of the solvent, which can be referred to as a gel.¹³⁶ Not only the interactions between gelator-gelator, but also the gelator-solvent specific and nonspecific interactions have main importance for secondary structure formation.

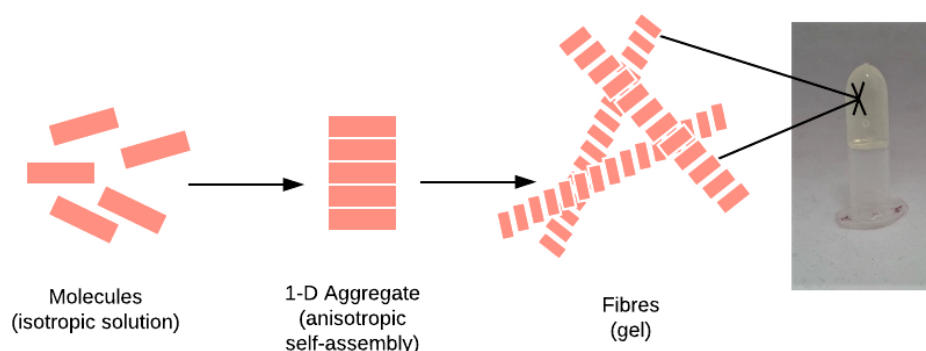


Figure 2.2. Schematic representation of molecular gel self-assembly.

In 1861, Thomas Graham define gel as: “while the rigidity of the crystalline structure shuts out external impressions, the softness of the gelatinous colloid partakes of fluidity, and enables the colloid to become a medium for liquid diffusion, like water itself.”¹⁴² Almost a hundred years later, Flory reported that ‘One may infer from this universal characteristic of those systems regarded as gels that they must possess a continuous structure of some sort, the range of continuity of the structure being of macroscopic dimensions.’¹⁸ In recent years, for LMW gelators it is accepted that the gelation sits on a knife-edge between solubility and precipitation.^{143,144} Thus, both gelator-solvent interactions and gelator-gelator interactions should be at suitable level for gel formation. Otherwise, strong gelator-solvent interactions cause solution formation, whereas strong gelator-gelator interactions rise up with simply dissolution.

The known lowest molecular weight organogelators are dimethyl urea, diethyl urea and dipropyl urea with the molecular weights of 88.1 g/mol, 116.2 g/mol and 144.2 g/mol respectively.¹⁴⁵ However, this organogelators can only be gelate in silicone oil, toluene and carbontetrachloride which limits their application potentials.

2.1.4 Amino acid derived low-molecular weight gelators

Amino acids are versatile compounds for self-assembled structures and, therefore, their derivatives have found various applications as LMW gelators.^{55,56,58,59,146–150} For example, benzyl urea derivative of histidine (Figure 2.3) is able to form organogel in acetonitrile, ethyl acetate, nitromethane and nitrobenzene as well as being able to form hydrogel in water.¹⁴⁶ Moreover, with the addition of cobalt (II) chloride metallogel formation is observed.

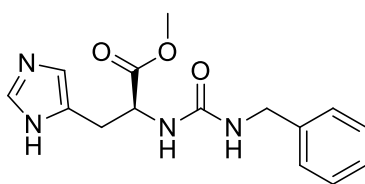


Figure 2.3. Benzyl urea derivative of Histidine.

As an application, long alkyl chain derivatives of phenyl alanine, tyrosine and tryptophan was used as injectable gel in sunflower oil and their anti-Alzheimer drug releases was investigated.¹⁴⁷ Tyrosine derivative (Figure 2.4) showed low-burst release of anti-Alzheimer drugs, therefore it was accepted as a promising system for parental delivery of these type of drugs.

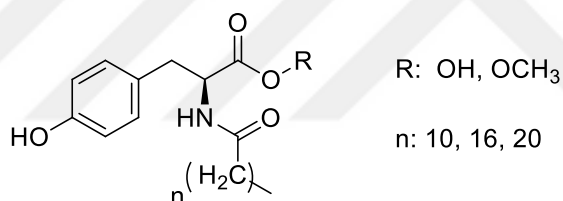


Figure 2.4. Long alkyl chain containing tyrosine derivative.

Basak and coworkers¹⁴⁸ synthesize long alky chain containing phenylalanine or alanine derivatives and observed that they forms gel in apolar solvents such as *n*-octane. Also, the gelation in diesel, kerosene and pump oil shows that these type of amino acid derivatives can be used in oil-spill recovery applications. In another study, it was proven that Fmoc-L-Lysine(Fmoc)-OH (Figure 2.5) is able to form organogel in chlorinated solvents and methanol as well as hydrogel at different pH values.¹⁴⁹ Cell viability, drug release and dye removal applications of this lysine derivative was also studied. Different concentrations of Fmoc-L-Lysine(Fmoc)-OH

did not showed toxicity to human immortalized keratinocyte cells. Drug release studies proved the gel is suitable for sustained drug release. Dye removal applications demonstrated that almost 75% of methyl red can be removed with the hydrogel of Fmoc-L-Lysine(Fmoc)-OH from water.

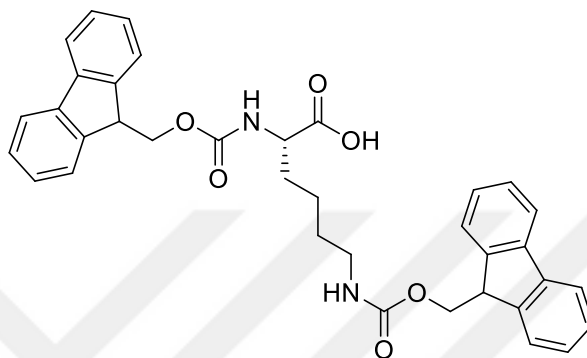


Figure 2.5. Organo- and Hydro-gelator Fmoc-L-Lysine(Fmoc)-OH.

Cyclic dipeptide derivatives are also examined as LMW gelators.^{55,56,58,59} Kleinsmann and coworkers discover the gelation ability of cyclo-L-Phenylalanine-Glycine (Figure 2.6) in water which was the lowest molecular weight gelator (204.2 g/mol) until now with a minimum gelation concentration 1 wt %.⁵⁶ Beside this, cyclo-L-Phenylalanine-L-Cysteine is the most efficient hydrogelator since its minimum gelation concentration is as low as 0.25 wt%.

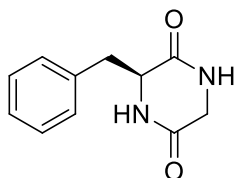
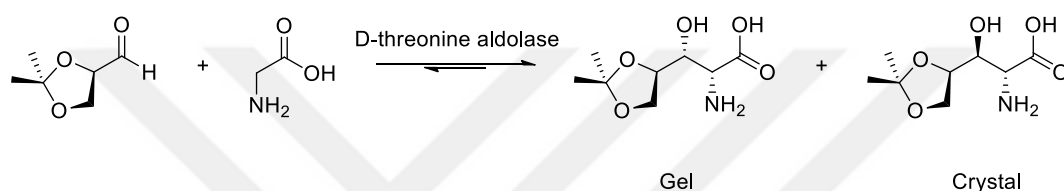


Figure 2.6. Cyclo-L-Phenylalanine-Glycine.

The amino acid based lowest molecular weight organogelator (205.2 g/mol) until now was synthesized with an enzymatic reaction.¹⁵⁰ The enzymatic aldol reaction of glycine with 2,3-*O*-isopropylidenglyceraldehyde was performed to obtain optically active β -hydroxy- α -amino acid (Scheme 2.1). One of the diastereomer crystallized in the medium, whereas the other one gelled with the solvent. The gelator forms gel in a wide range of solvents with very low gelation concentration, however, gelation occurs at 3 °C generally and solution transition was observed above 12 °C.



Scheme 2.1. Enzymatic aldol reaction of Glycine and 2,3-*O*-isopropylidenglyceraldehyde.

2.1.5 Multi-component low molecular weight gels

Beside the gelators form gel directly in different type of solvents, there are also many examples of multi-component LMW gels in the literature (Figure 2.7).¹⁵¹ In some cases, two components are necessary to form a gel, whereas in other cases two different components, each having gelation ability on its own, are mixed in order to obtain a gel that results from the assembly of the two components in an alternating fashion. Additionally, a non-gelling additive can be used to increase the lifetime stability of a gel and/or to enhance its mechanical properties.

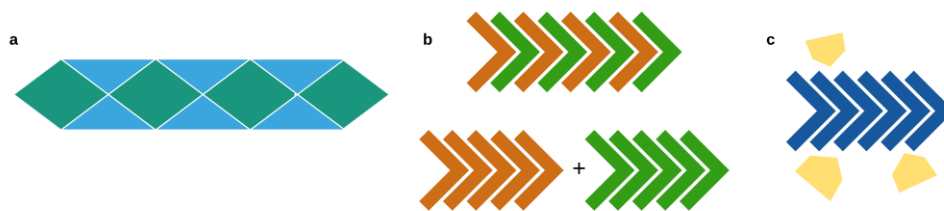


Figure 2.7. **(a)** Two-component gel in which both components are necessary to form gel **(b)** Two gelator component can form co-gel or self-sorting gel **(c)** Gelator and non-gelling additive in which addition of non-gelling additive affects the gel properties.

As an example to the effect of non-gelling additive to the gel properties, Foster and coworkers showed that the yield stress of bis-urea derivative gel enhances with the addition of one equivalent of carbamazepine as a non-gelling additive.¹⁵² As a subclass of non-gelling component containing gels, chemo-responsive gels acts as a readout for the existence in the medium. These type of chemical stimulus can be redox reactions^{143,153}, anions^{143,154}, enzymes^{143,155} and pH.^{143,156,157} N-(2-hydroxyalkyl)-L-Valine amphiphiles were proven to form hydrogel at pH values greater than 11.5.¹⁵⁶ Moreover, L-Lysine long alkyl chain derivative forms hydrogel in water (Figure 2.8).¹⁵⁷ Acid addition increases gel formation ability 12 times. Similarly, inorganic salts addition accelerates gel formation due to the reduction of electrostatic repulsion between pyridinium groups with higher gel strength.

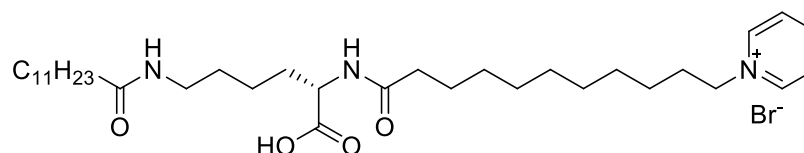
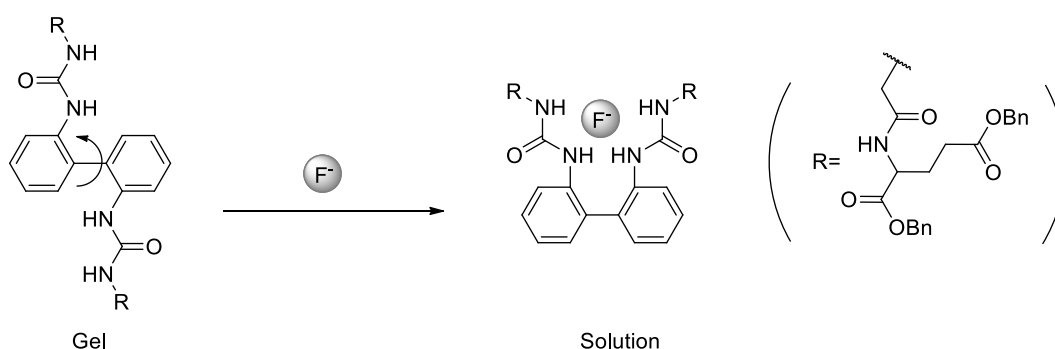


Figure 2.8. Long alkyl chain derivative of L-Lysine gelator that gel properties enhances in acidic medium.

2.1.6 Fluoride ion sensitive molecules

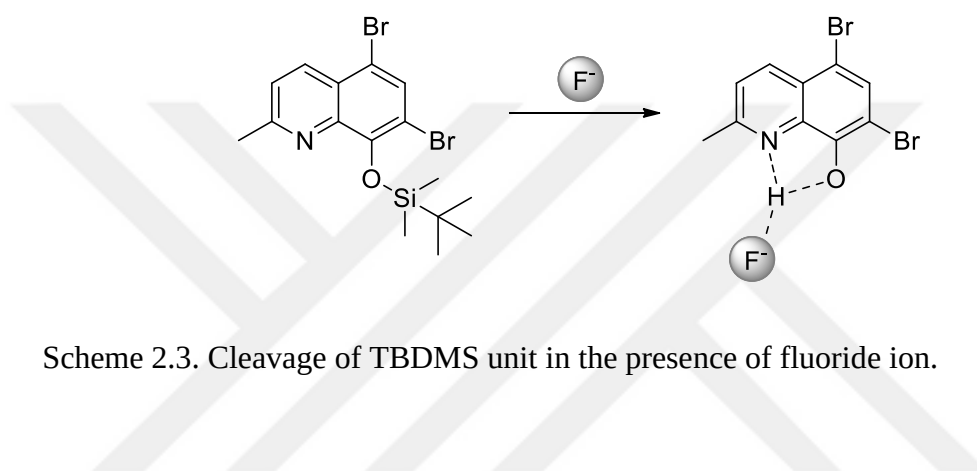
The design of fluoride-sensitive molecules is the focus of research efforts on the part of many groups, notably due to the implications of such molecules for dental care and for the treatment of osteoporosis.^{158–160} However, overexposure to fluoride ions can cause kidney problems and fluorosis. The World Health Organization has declared the appropriate amount of fluoride ions in drinking water to be 0.5–1.0 mg/L.¹⁶¹ In the literature, stimuli-responsive organogels have been used to selectively detect fluoride ions by forming charge-transfer complexes.^{162–164} Lin and coworkers proposed a gelator which forms charge-transfer complex with fluoride ion selectively in the gel form.¹⁶³ A colorimetric detection is likely to be useful since the gel-gel transition occurs with a color change. In another study, L-Glu-Gly dipeptide 2,2'-diaminobisphenyl derivative was synthesized as an organogelator.¹⁶⁴ Selectively, with the addition of fluoride ion, gel to solution transition observed by forming a charge-transfer complex (Scheme 2.2).



Scheme 2.2. Gel to solution transition by forming charge-transfer complex of L-Glu-Gly dipeptide derivative with fluoride ion.

Further, many chemosensor systems have been investigated with regards to their ability to sense fluoride ions using the fluorescence and colorimetric properties of

materials.^{158,160} Some of these studies depend on the cleavage of the silicon-oxygen bond of silyl ethers to promote fluoride ions (Scheme 2.3).^{165–169} Boa and his colleagues synthesized a naked-eye and fluorescent chemosensor.¹⁶⁹ With the cleavage of TBDMS unit in the presence of fluoride ion, a green solution was observed with naked-eye because of excited-state proton transfer. Moreover, blue to green color transition was obtained under UV light.



Scheme 2.3. Cleavage of TBDMS unit in the presence of fluoride ion.

2.1.7 Drug release

Molecular gels are used frequently in drug delivery and drug release studies.¹⁴³ In one of the approaches, gel formation occurs in the drug dissolved solvent and then drug release occurs passively by diffusion of drug (Figure 2.9). Besides passive delivery, stimuli-responsive systems are used to deliver therapeutic agent dynamically. In the third approach, the therapeutic motif is integrated into the gel forming system; thus, the whole system becomes a therapeutic molecular gel. Drug release occurs with degradation of self-assembled molecule.

Amino acid-based systems are very potent since they are typically biocompatible molecules. Different concentrations of Fmoc-L-Lysine(Fmoc)-OH (Figure 2.5) showed no toxicity toward human immortalized keratinocyte cells within 5 days.¹⁴⁹ Furthermore, Kirby-Bauer experiments indicated that the Fmoc-L-Lysine(Fmoc)-

OH and its co-assembled form with Fmoc-L-Leucine shows promising results for drug delivery of antimicrobial agents both for Gram positive and Gram negative bacteria. Thus, the gel is suitable for sustained drug release.

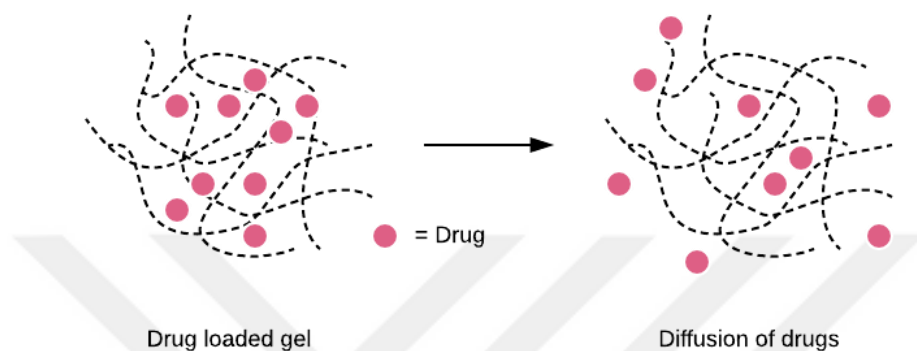


Figure 2.9. Passive drug release by diffusion.

Friggeri and coworkers¹⁷⁰ performed *in vitro* drug release studies of quinolone derivatives incorporated with gelator *N,N'*-dibenzoyl-L-cystine (Figure 2.10). It was shown that the release of amino and hydroxyl unit containing drugs are seven times different from each other. This explains the importance of drug-gelator interactions in drug release systems.

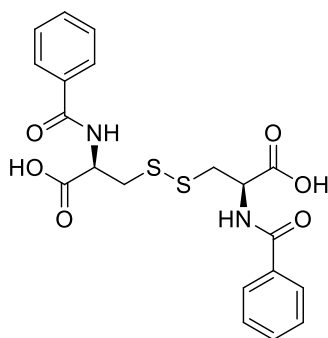


Figure 2.10. Molecular structure of *N,N'*-dibenzoyl-L-cystine.

The first *in vivo* drug release study with molecular gels was conducted by Liang and coworkers.¹⁷¹ Napthalene-D-Phe-D-Phe dipeptide derivative (Figure 2.11) hydrogel showed valuable results for controlled release of ¹²⁵I isotope.

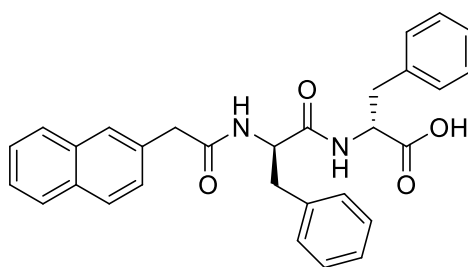
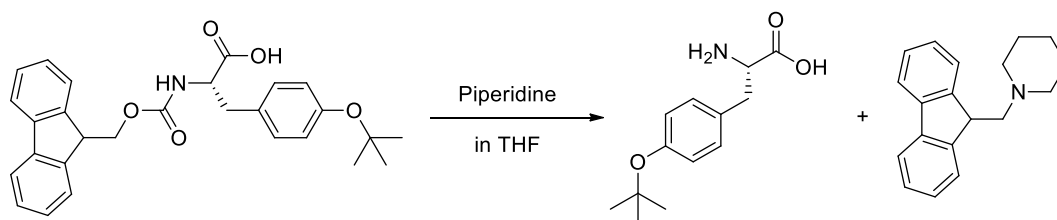


Figure 2.11. Molecular structure of Napthalene-D-Phe-D-Phe gelator.

2.1.8 Aim of the study

In this study, new amino acid based low molecular weight gelators was expected to be discovered with several potential applications such as fluoride ion detection, dye removal and drug release for treatments.

Reaction in Scheme 2.4 was initially performed to obtain unprotected tyrosine to be used in another study and this led us to the discovery of this new organogelator L-Tyr(*t*Bu)-OH.



Scheme 2.4. Fmoc cleavage of Fmoc-L-Tyr(*t*Bu)-OH.

After the serendipitous discovery of gelation of L-Tyr(*t*Bu)-OH in THF/piperidine solution, gelation in different type of organic solvents was investigated. Also, the effect of additives was studied. Characterization of microstructures and gel behaviors were also studied to understand the self-assembly phenomenon of this organogelator better. Molecular dynamic studies were conducted by Dr. Antoine Marion. Then, L-Tyr(TBDMS)-OH was synthesized as a fluoride ion sensitive gel and its gel to solution transitions with fluoride was investigated. After emerging the outstanding gelation properties of low molecular weight L-Tyr(*t*Bu)-OH in a wide range organic solvent, other 13 natural amino acid derivatives which contains various protecting groups on their side chains were synthesized and their gelation abilities were studied. Remaining 8 amino acid that have no protecting group on their side chain were directly used for gelation tests. Later, antimicrobial drug release studies of gel forming amino acid derivatives and cytotoxicity experiments were performed by Prof. Shreeparna Baneerje.

2.2 Results and Discussion

2.2.1 Spontaneous gelation of L-Tyr(*t*Bu)-OH

It is synthetically straightforward to produce L-Tyr(*t*Bu)-OH in excess amount from Fmoc-L-Tyr(*t*Bu)-OH in one step.¹⁷² It is also an inexpensive commercially available compound. Gelation occurs spontaneously during the reaction (Scheme 2.4). During the cleavage of the fluorenylmethyloxycarbonyl (Fmoc) group with piperidine in THF, gelation occurred in 15 minutes. After removing the by-product, gelation with pure L-Tyr(*t*Bu)-OH was also successfully achieved under identical conditions. This indicates that gelation process independently happens from the by-product.

The other enantiomer D-Tyr(*t*Bu)-OH shows same gelation properties as expected. However, no gel formation was observed in the case of racemic Tyr(*t*Bu)-OH at minimum gelation concentration. Thus the gelator should be enantiomerically pure for self-assembly. Additionally, gel formation does not occur for either L-Tyr-OH, L-Phe-OH and L-Tyr(Me)-OH (Figure 2.12). We observed that the *tert*-butyl moiety plays a significant role in the gelation process, since gel formation does not occur for either L-Tyr-OH, L-Phe-OH and L-Tyr(Me)-OH.

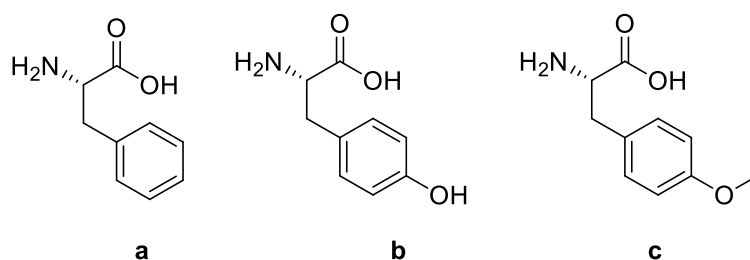


Figure 2.12. Molecular structures of (a) L-Phe-OH (b) L-Tyr-OH (c) L-Tyr(Me)-OH.

2.2.2 Additive screening for gelation

It is observed that the presence of one equivalent of piperidine in gelation medium decreases the minimum gelation concentration in THF. The effects of different additives, in this case bases and alcohols, on gelation is demonstrated in Table 2.2. Inversion test was used to determine the formation of the gels.

Table 2.2. Gelation Results of L-Tyr(*t*Bu)-OH with Different Additives in THF.

Additives	Conc. (wt/v%)	Appearances
-	1.1 ^a	Gel
Piperidine	0.45 ^a	Gel
Diisopropylamine	1	Gel
Diethylamine	1	Gel
DBU	1	Solution
Imidazole	2	Gel
2-ethylhexylamine	1	Solution
Triethylamine	1	Suspension
NaOH	0.25 ^a	Gel
Methanol	2	Suspension
Ethanol	2 ^a	Gel
Isopropanol	2 ^a	Gel
<i>n</i> -Butanol	1.5 ^a	Gel
2-ethylhexanol	0.75 ^a	Gel

^aMinimum gelation concentration.

Without using any additive L-Tyr(*t*Bu)-OH forms a gel in THF with a minimum concentration of 1.1 wt/v%. Amazingly, the minimum gel concentration decrease to 0.45 wt/v% in the presence of piperidine as additive. Even though the gel appearances are weaker, gelation is also observed in the existence of other bases, such as diisopropylamine, diethylamine and imidazole. However, the usage of DBU and triethylamine as additive causes a clear solution and a suspension formation, respectively. According to this result, the gel formation is triggered with donor-acceptor hydrogen bond ability of additives. The short-alkyl-chain-containing alcohols such as methanol, ethanol and isopropanol were also considered, to further test this hypothesis. Nevertheless, as additive they have no favorable role in the gelation process. On the other hand, the minimum gelation concentration lowers with the van der Waals interactions between organogelator and additive, since the addition of 2-ethylhexanol does show important effect on gelation. Moreover, the addition of one equivalent of NaOH resulted in a minimum gelation concentration as low as 0.25 wt/v% in THF, beside these organic bases and alcohols. These results indicate that hydrogen bond network formation is not the necessarily main role of additives, but the deprotonation of the carboxylic acid with the amine-type additives allows the resulting carboxylate group to better involve in the hydrogen bond network. Amine-type additives act also as base in this systems. This is also coherent with the observation of higher minimum gelation concentrations when the short-alkyl-chain-containing alcohols were used as additives, since alcohols are weakly acidic compounds.

2.2.3 Solvent screening for gelation

In order to further investigate the gelation of L-Tyr(*t*Bu)-OH, wide variety of solvents with or without the addition of the non-gelling agent, 2-ethylhexanol, piperidine or NaOH were used. As shown in Table 2.3, L-Tyr(*t*Bu)-OH is able to form gel with a wide variety of organic solvents at remarkably low concentrations.

Among the analyzed conditions, DMF appears to be the best gelation solvent for L-Tyr(*t*Bu)-OH, with an ability to gel at 0.1 wt/v% without using any additive. Further, when 2-ethylhexanol used as a solvent, it demonstrates promising gelation results with a minimum gelation concentration as low as 0.2 wt/v%. The presence of a non-gelling agent merely changes the appearance of the gel from opaque to transparent and does not have an important effect on the minimum gelation concentration. Similarly, toluene, hexane, and 1,2-dichloroethane also seem to be good solvents for gelation merely by adding piperidine. When the solvents *tert*-butylmethylether and 1,2-dimethoxyethane is used solution formation could be observed at the defined concentrations without additives, but presence of piperidine lowers the gelation concentration. Interestingly, cellulose thinner, isopropylalcohol, and *n*-butylalcohol, when used as solvents, induce clear gel formation without piperidine, whereas piperidine addition causes the formation of solution. Since the gelation occurs with sunflower oil, L-Tyr(*t*Bu)-OH is an organogelator with promising potential applications in the field of drug delivery.^{173–175} Similarly, it could also be functional to use L-Tyr(*t*Bu)-OH organogelator in oil-spill recovery applications.^{132,148} Although L-Tyr(*t*Bu)-OH is able to form gel both in many polar and apolar solvent, gelation in water, methanol, ethyl acetate, acetone and acetic acid is not observed even the piperidine addition is in the question.

Table 2.3. Gelation Results of L-Tyr(*t*Bu)-OH in Different Solvents With and Without Non-gelling Agent. (The values are given in wt/v%.)

Solvent ^a	Additive	Conc. (wt/v%)	Appearance
2-ethylhexanol	-	0.2 ^b	Gel
2-ethylhexanol	Piperidine	0.2 ^b	Clear Gel
Water	Piperidine	1	Suspension
Water	2-ethylhexanol	1	Suspension

Table 2.3 (cont'd)

Chloroform	Piperidine	1	Solution
Chloroform	2-ethylhexanol	1	Solution
Toluene	-	1	Solution
Toluene	Piperidine	0.3 ^b	Gel
Toluene	2-ethylhexanol	1.5	Gel
Toluene	NaOH	1	Precipitate
MTBE	-	0.7 ^b	Gel
MTBE	Piperidine	0.45 ^b	Gel
MTBE	2-ethylhexanol	1.5	Gel
MTBE	NaOH	1	Precipitate
Methanol	Piperidine	1	Solution
1,2-DME	-	0.3 ^b	Gel
1,2- DME	piperidine	0.2 ^b	Gel
1,2- DME	NaOH	0.4 ^b	Gel
DMF	-	0.1	Gel
Acetonitrile	-	0.3 ^b	Gel
Acetonitrile	piperidine	0.2	Solution
Acetonitrile	NaOH	0.2	Precipitate
Ethyl acetate	piperidine	1	Suspension
Hexane	-	0.75	Solution
Hexane	piperidine	0.75	Gel
Hexane	NaOH	1	Precipitate
1,2-DCE	-	1	Solution
1,2-DCE	piperidine	0.2 ^b	Gel
1,2-DCE	NaOH	1	Precipitate
Acetic acid	piperidine	1	Solution
Acetone	piperidine	1	Solution
Isopropanol	-	0.5 ^b	Gel
Isopropanol	piperidine	0.4	Solution

Table 2.3 (cont'd)

<i>n</i> -Butanol	-	0.5 ^b	Gel
<i>n</i> -Butanol	piperidine	0.4	Solution
<i>n</i> -Butanol	NaOH	0.4	Solution
Sunflower oil	-	1	Precipitate
Sunflower oil	piperidine	1	Gel
Sunflower oil	NaOH	1	Precipitate
Olive oil	piperidine	1	Suspension
Cellulose Thinner	-	1	Clear Gel
Cellulose Thinner	piperidine	1	Solution
Cellulose Thinner	NaOH	1	Precipitate
Diesel	-	2	Solution
Diesel	piperidine	2	Gel
Diesel	NaOH	2	Precipitate

^aMTBE: *tert*-Butylmethylether; 1,2-DME: 1,2-Dimethoxyethane; DMF: *N,N*-Dimethylformamide; ACN: Acetonitrile; 1,2-DCE: 1,2-Dichloroethane; ^bMinimum gelation concentration.

2.2.4 Characterization of microstructures

To understand the microstructural organization and molecular packing at atomic scale of L-Tyr(*t*Bu)-OH in gel state transmission electron microscope (TEM) imaging (Figure 2.13 and Figure 2.14), X-ray powder diffraction (XRD) measurements (Figure 2.15), and molecular dynamics (MD) simulations were performed. In the TEM images of the L-Tyr(*t*Bu)-OH in THF with the addition of piperidine show formation of nanofibers with an estimate width of 40 nm and several micrometers in length.

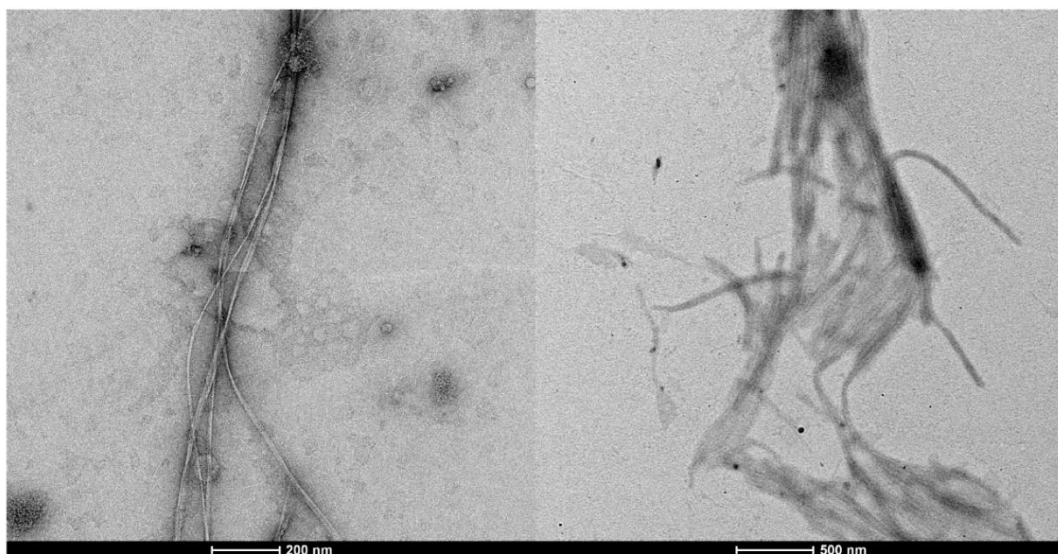


Figure 2.13. TEM images of L-Tyr(*t*Bu)-OH in THF/piperidine diluted with miliQ water. (Scale bar: 200 nm and 500 nm).

Moreover, fibrillar microstructures was also seen in TEM image when the gel was prepared by using NaOH as additive (Figure 2.14).

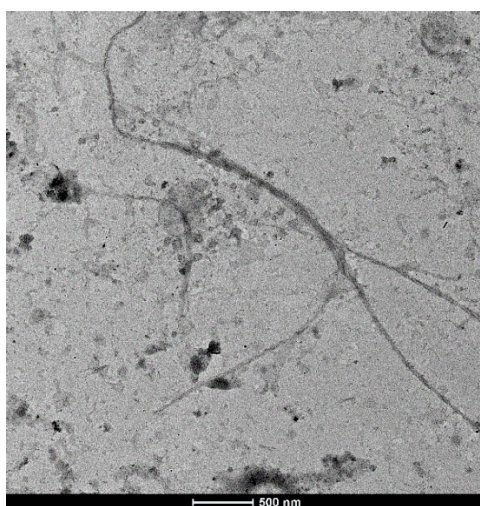


Figure 2.14. TEM image of L-Tyr(*t*Bu)-OH in THF/NaOH diluted with miliQ water. (Scale bar: 500 nm).

As reported in Figure 2.15 that the XRD patterns for the xerogels prepared with and without piperidine addition are nearly identical. Thus, similar XRD patterns indicate that the piperidine does not included in molecular packing.

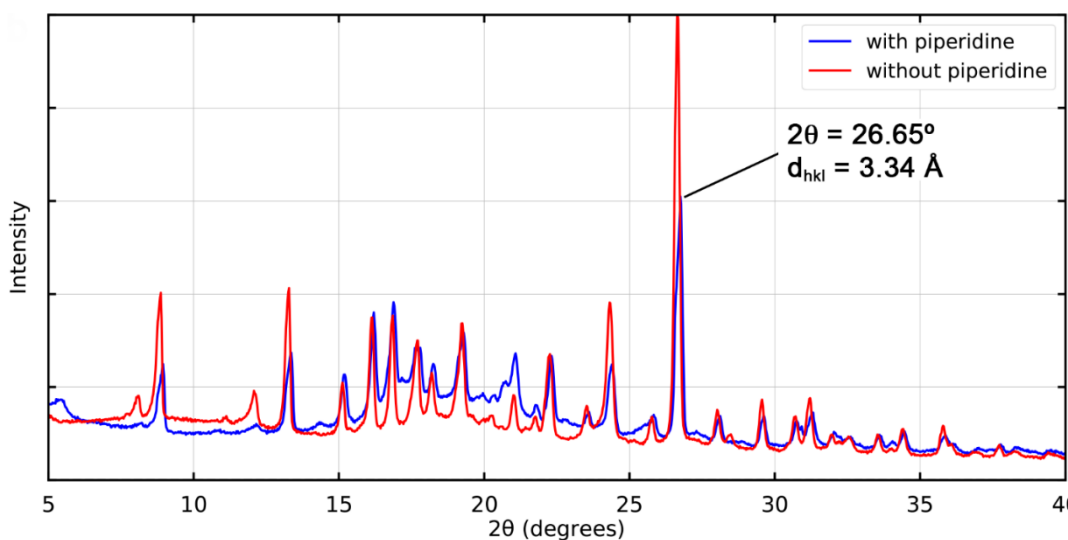


Figure 2.15. XRD pattern of xerogels with and without piperidine.

Furthermore, spontaneous aggregate formation of L-Tyr(*t*Bu)-OH in THF was obtained in MD simulation and this aggregate formation was stabilized by strong hydrogen bond interactions between the carboxylate and ammonium groups of the gelator (Figure 2.16a). The assembly in question does not break once formed during the present simulation, thus it is highly stable. When an analogous simulation with including piperidine molecules was conducted, a spontaneous self-assembly, but with a significantly discrete interaction motif (Figure 2.16b). In the second system, L-Tyr(*t*Bu)-OH and piperidine molecules assembles in alternating order, in which the carboxylate part of the former is participated in the interactions merely to hold the molecules together. However, the less dense network of hydrogen bonds results with a less stable assembly. Since the self-assembly motifs with and without

piperidine are significantly different from each other, one would predict to yield significantly different XRD patterns for such structure. Nevertheless, depending on the nearly identical XRD patterns of xerogels of L-Tyr(*t*Bu)-OH with and without piperidine (Figure 2.15), we conclude that piperidine does not have a structural role in self-assembly and thus gel formation.

The formation of long networks of interacting molecules is highlighted in the subsequent microsecond-long MD simulation of pure L-Tyr(*t*Bu)-OH. Formation of a series of parallel packed fiber-like structures shown in Figure 2.16 is in agreement with the TEM images.

As highlighted in Figure 2.17c and further detailed in Figure 2.16, the composition of each fiber consist of a strong and compact network of hydrogen bonds of the hydrophilic core, while the interactions among fibers is ensured with the hydrophobic side chains. It is also noted that the branching is ensured with the sporadic occurrence of hydrogen bonds between the fibers, which are likely to involve in the overall stability of the molecular assembly.

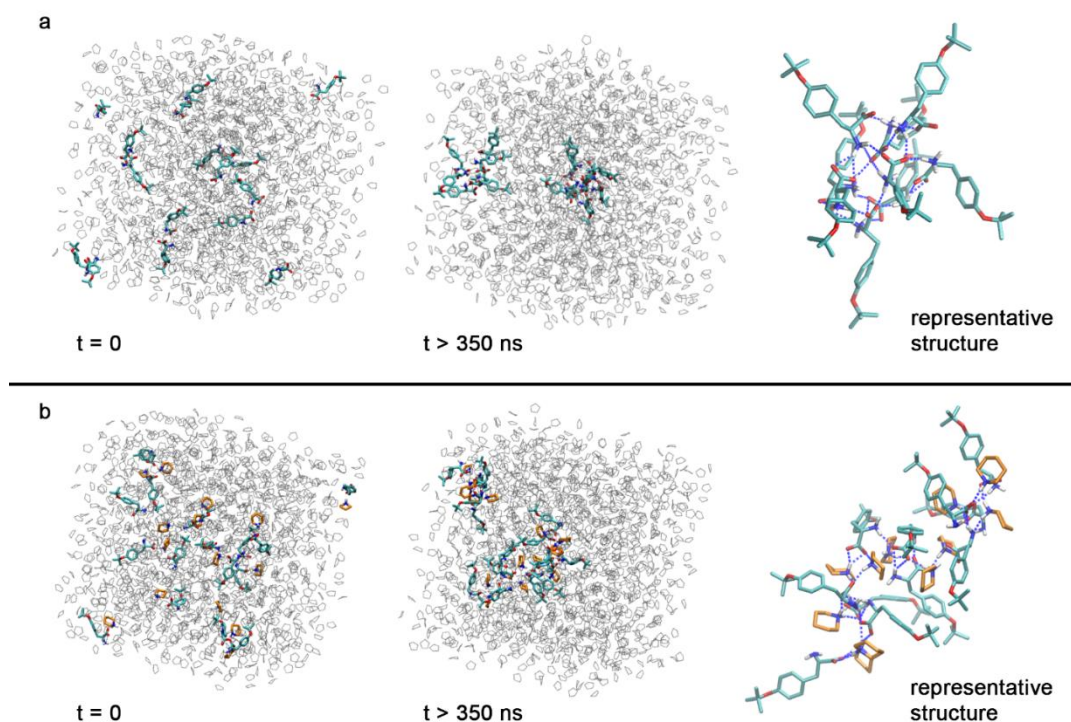


Figure 2.16. Representative configurations along the molecular dynamics simulation of L-Tyr(*t*Bu)-OH without **(a)** and with piperidine **(b)** in THF. Both in **a** and **b**, the first two panels represent the simulation box at the beginning of the simulation and after a given period of time, showing the self-assembly of the solutes, respectively. Grey color represents THF molecules without hydrogen atoms, whereas carbon atoms of L-Tyr(*t*Bu)-OH and piperidine are shown in cyan and orange colors, respectively. The hydrogen atoms of amine and ammonium groups are depicted only. Dashed blue lines show the hydrogen bond interactions.

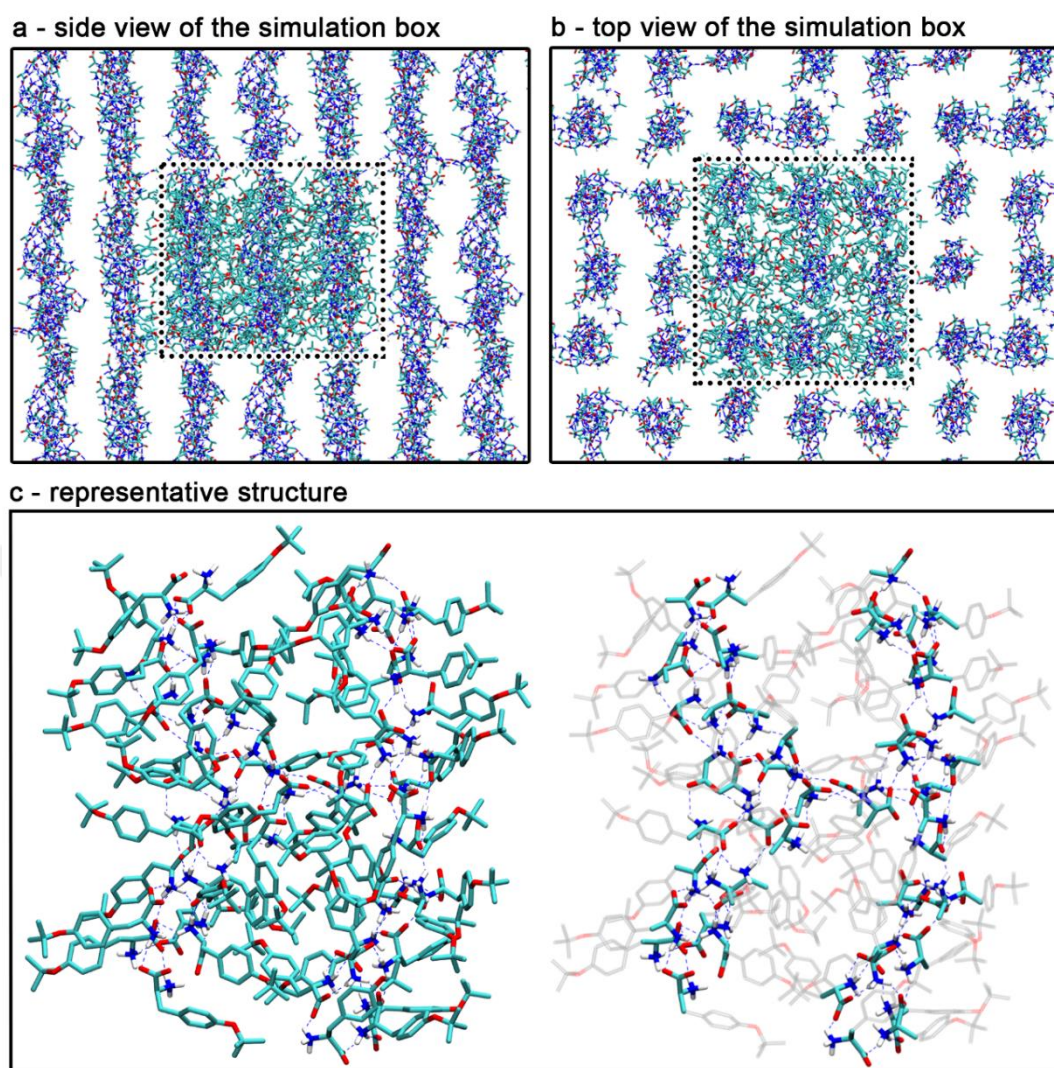


Figure 2.17. Representative image of the fiber-like structure gained after one microsecond MD simulation of pure L-Tyr(*t*Bu)-OH. **(a, b)** demonstrate a side and top view of the simulation box with its periodic images, respectively. The dotted lines border the main unit cell. Carbon atoms of back bone and side chains of each molecule are colored in cyan. To highlight the distinct assemblies of linear structure, the backbone atoms which form the core of each fiber are represented only. A representation for the interaction of two fibers is shown in **(c)**, where the right-hand side highlights the interactions in the core of the fibers only. Dashed blue lines represent hydrogen bonds and show the network of fibers in which the individual molecules forms by interacting and while bridging fibers together.

The radial distribution functions (RDF) analysis (Figure 2.18) for specific pairs of atoms along the dynamics showed a series of well-defined peaks centered at distances consistent with the diffractions seen in the XRD pattern.

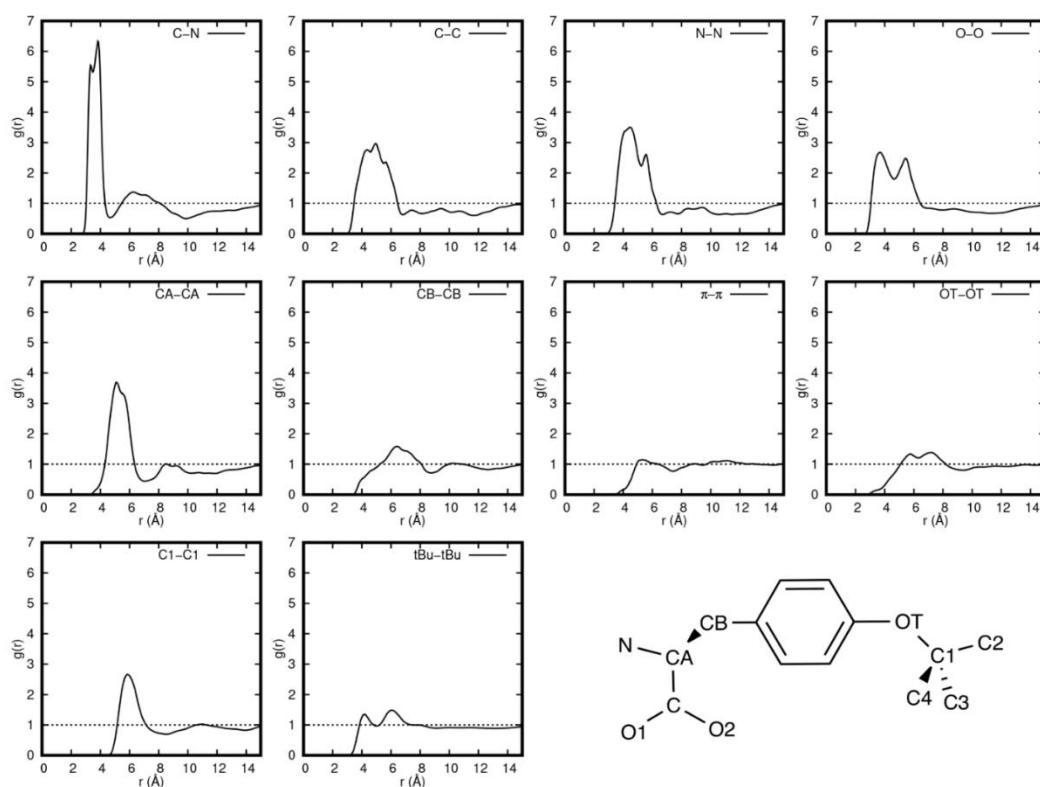


Figure 2.18. Radial distribution functions (RDFs) for selected pairs of atom of pure L-Tyr(tBu)-OH, as calculated from the last 300 ns of the 1 μ s-long simulation. The non-hydrogen atoms are labeled in the inset at the bottom-right to depict the nomenclature used.

It was revealed in that there is no evidence of stable π - π stacking interaction among aromatic rings of L-Tyr(tBu)-OH. The lack of π - π stacking interaction is most likely due to the large steric hindrance caused by *tert*-Butyl groups. Since a carbon-nitrogen RDF strongly peaks at a distance of 3.33 Å, the sharp and intense peak shown at 2 θ

= 26.65° (dhkl = 3.34 Å) in the XRD pattern can be referred to the tight interaction observed between ammonium and carboxylate groups throughout the simulation, instead of the π - π stacking interaction among aromatic rings of L-Tyr(*t*Bu)-OH. The fairly intense peak at around 6 Å in the C1-C1 RDF depict the interactions of the side chains giving rise to a well-organized packing. Besides, the distributions C-C and N-N pairs in hydrophilic part feature highly structured peaks which suggests the repetition of several sets of interatomic distances. The high concentration of diffraction peaks in the XRD pattern at angles corresponding to distances shorter than 7 Å is coherent with this deduction.

The driving forces that trigger gel formation is similar to those responsible for reverse micelle formation. The polar part of L-Tyr(*t*Bu)-OH tend to interact with each other because of the hydrophobic character of solvent, thus the hydrophobic *tert*-butyl groups point toward the solvent molecule in an isolated core. In this case, however, linear network formation is favored since the small hydrophilic amino acid backbone assemble highly directional with dipolar character of the interacting groups rather than spherical shaped. As anticipated by MD simulations (Figure 2.17), van der Waals interactions and hydrogen bonds trigger the interconnection of fibers and thus, branching between linear network. As a result of the formation of tree-dimensional bundles, it is postulated that gelation is observed. In summary, the intense network of hydrogen bonds between ammonium and carboxylate groups of L-Tyr(*t*Bu)-OH is crucial to form gel. Although, there are a number of amino acid-derived gelator reported in the literature, which includes substitution either on the carboxyl group or amine group, there was thus far no report of a natural amino acid-derived organogelator containing free -NH₂ and -COOH groups. Moreover, to inhibit the π - π interactions among aromatic units, *tert*-butyl moiety plays a vital role by providing steric hindrance. This is further explained the incapability of L-Tyr(OMe)-OH to generate a gel. Precipitation occurs instead of gelation in THF, since the methyl group is too small to prevent the π - π interactions.

2.2.5 Gel behaviors of L-Tyr(*t*Bu)-OH and effect of additive on gel

The gel behaviors of L-Tyr(*t*Bu)-OH was investigated by conducting rheological measurements. Before analyzing strain-controlled behaviors of gels, strain-sweep analysis was conducted to detect the linear viscoelastic regime and yield strain (Figure 2.19). Firstly, the G' values show a rough plateau, then start to decrease after $\gamma_0 = 0.27\%$ and $\gamma_0 = 0.1\%$ for additive free and additive containing gel, respectively. Thus, the organogels were partially destructed and began to flow at higher strain values. The non-destructive frequency sweep experiments were conducted at constant strain values determined by strain-sweep experiments (Figure 2.20). The 1 wt/v % gel prepared in 2-ethylhexanol showed a large difference between dynamic storage modulus G' and loss modulus G'' at all frequencies between 1 to 100 Hz indicating that the organogel heavily exhibit elastic character as a soft matter (Figure 2.20a). Piperidine added gel was also analyzed to prove the favorable effect of non-gelling additive on the gel (Figure 2.20b). Although, the piperidine additives did not involve in the self-assembly process, the rheological results clearly indicated that non-gelling additives like piperidine improves the gel properties (Figure 2.20c). The piperidine containing organogel have greater difference between G'' and G' than the additive free organogel, thus it can be concluded that the gel properties of soft material enhanced with the existence of non-gelling additive.

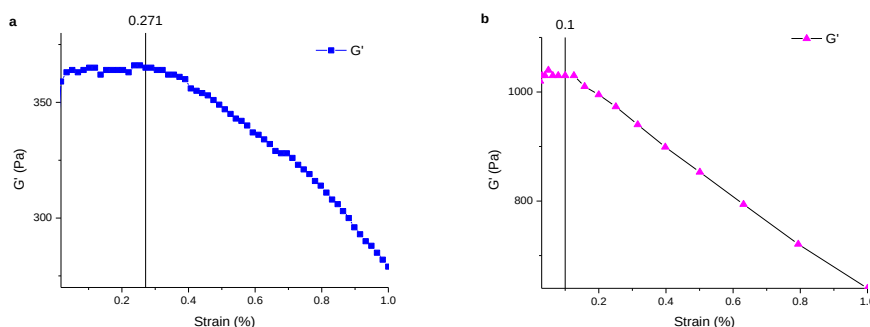


Figure 2.19. Strain-sweep analysis of (a) 1 wt/v% L-Tyr(*t*Bu)-OH in 2-ethylhexanol (b) 1 wt/v% L-Tyr(*t*Bu)-OH in 2-ethylhexanol with piperidine

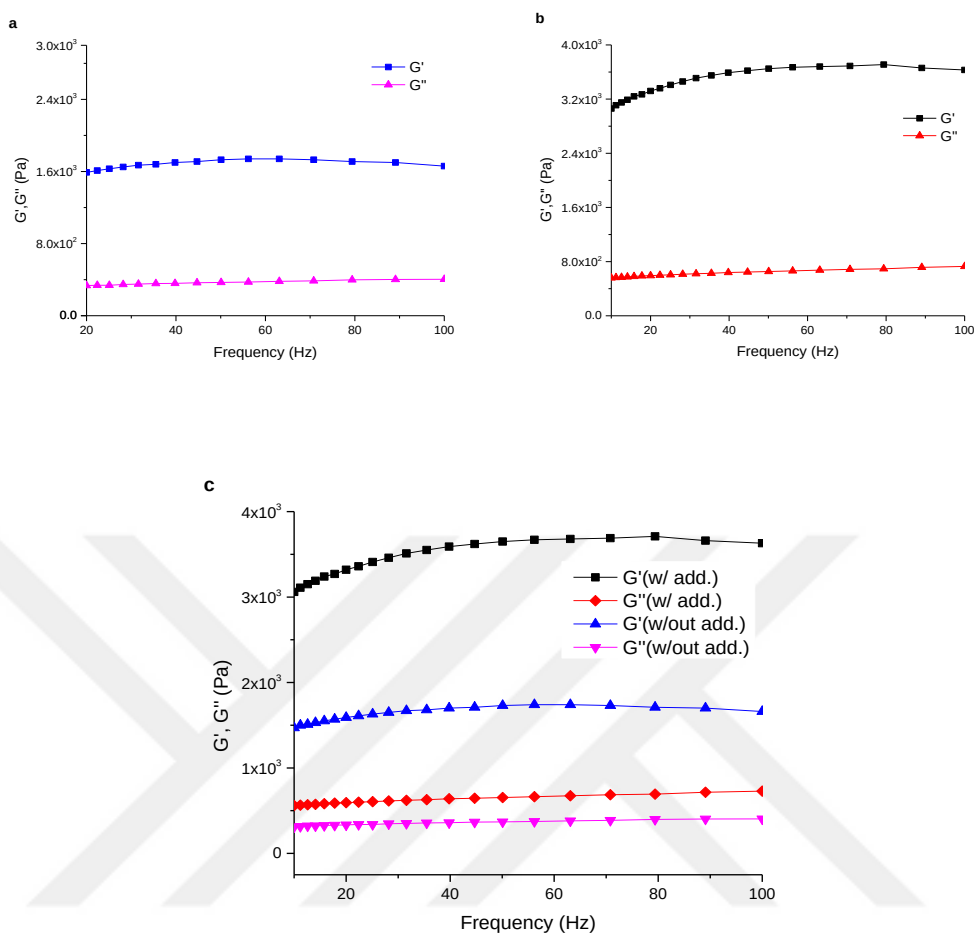
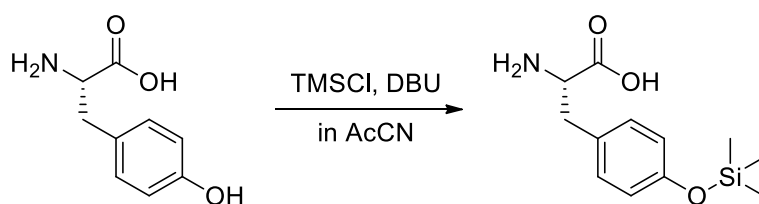


Figure 2.20. Viscoelastic behavior of **(a)** 1 wt/v% L-Tyr(*t*Bu)-OH in 2-ethylhexanol **(b)** 1 wt/v% L-Tyr(*t*Bu)-OH in 2-ethylhexanol with piperidine, **(c)** shows the overlapped (a) and (b).

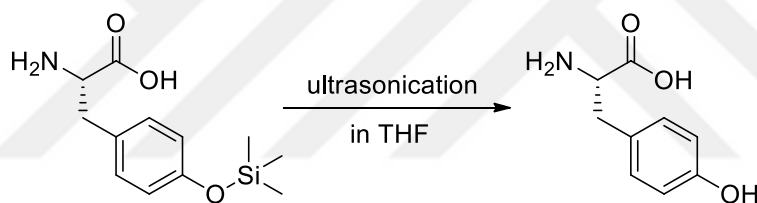
2.2.6 Synthesis and gelation of L-Tyr(TMS)-OH

The structural similarity between *O-tert*-butyl unit and trimethylsilyl unit led us to synthesize L-Tyr(TMS)-OH molecule to investigate its gelation and possible applications for fluoride ion detection. L-Tyr(TMS)-OH was synthesized successfully (Scheme 2.5).



Scheme 2.5. Synthesis of L-Tyr(TMS)-OH.

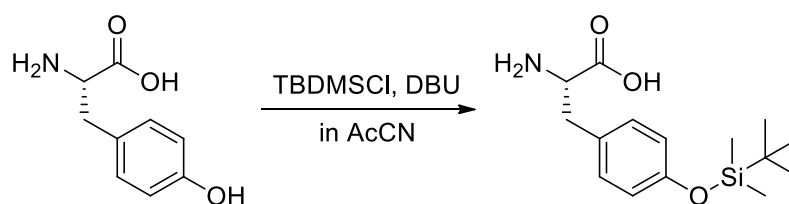
After the purification of L-Tyr(TMS)-OH, gelation studies in THF was conducted and 1 wt/v % gel was obtained with the help of ultrasonication at first 30 seconds. However, longer exposure to ultrasonic bath induce the cleavage of labile trimethylsilyl unit (Scheme 2.6).



Scheme 2.6. Removal of trimethylsilyl unit with ultrasonication.

2.2.7 Synthesis and gelation of L-Tyr(TBDMS)-OH

In order to obtain stable silyl unit containing tyrosine derivative, L-Tyr(TBDMS)-OH was synthesized (Scheme 2.7). The synthesis was achieved based on the literature synthesis of L-DOPA(TBDMS)₂-OH with some modifications.¹⁷⁶



Scheme 2.7. Synthesis of L-Tyr(TBDMS)-OH.

After performing purification, gelation study was conducted in several conditions. Inversion test represented that the gelation occurs with the help of ultrasonication in THF and 2-ethylhexanol with a minimum gelation concentration of 1 wt/v% for both solvents. Moreover, gelation of L-Tyr(TBDMS)-OH in 25% ethanol/water mixture was achieved with a minimum gelation concentration of 0.2 wt/v%. The gel has a clear appearance, whereas opaque gels were obtained in THF and 2-ethylhexanol.

2.2.8 Microstructures of L-Tyr(TBDMS)-OH gel

The fibril formations due to self-assembly of L-Tyr(TBDMS)-OH was imaged by using transmission electron microscopy (TEM).

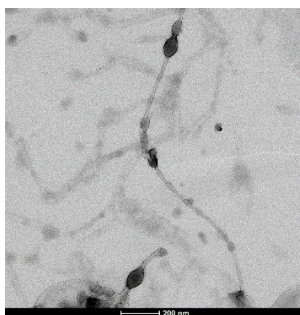


Figure 2.21. TEM image of L-Tyr(TBDMS)-OH in THF/piperidine diluted with miliQ water. (Scale bar: 200 nm).

Even the excess stain was remained on the grid, the several micrometer length fibrillar microstructures was seen in TEM image (Figure 2.21).

2.2.9 Gel behavior of L-Tyr(TBDMS)-OH

Rheological experiments was conducted to prove the soft viscoelastic property of material. At first, strain-sweep analysis was performed up to $\gamma_0 = 10.0\%$ (Figure 2.22a). Even though a plateau was not observed properly, a slight decrease in storage modulus occurred at greater amplitudes then $\gamma_0 = 2\%$. By determining the γ_0 as 2%, frequency-sweep analysis was done as shown in Figure 2.22b. The difference between dynamic storage modulus and loss modulus represents the viscoelastic behavior of soft matter. Thus, the material can be qualified as organogel.

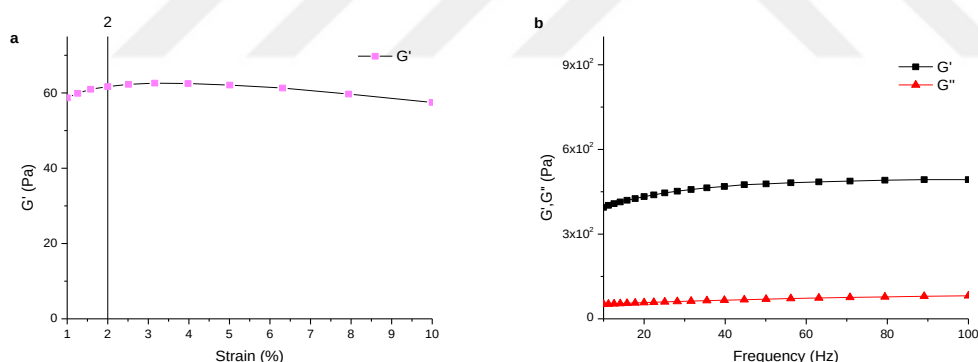
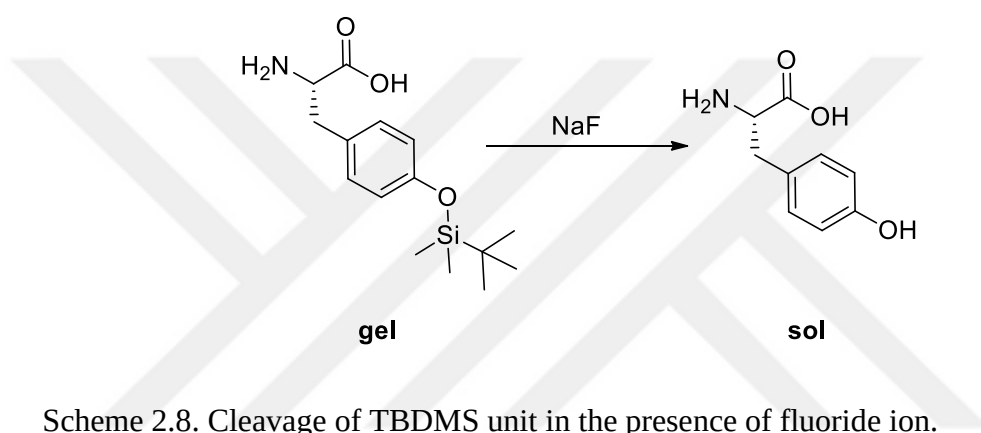


Figure 2.22. **(a)** Strain-sweep analysis and **(b)** viscoelastic property of 1 wt/v% L-Tyr(TBDMS)-OH in 2-ethylhexanol with piperidine.

2.2.10 Fluoride ion response of L-Tyr(TBDMS)-OH gel

The fluoride ion triggers the cleavage of the Si-O bond that the L-Tyr(TBDMS)-OH is sensitive to the existence of fluoride ion. Thus, gel to solution transition can be achieved irreversibly by forming L-Tyr-OH by cleaving Si-O bond of L-Tyr(TBDMS)-OH in the presence of fluoride ion (Scheme 2.8) since the latter is not able to form gel in organic solvents as discussed previously.



Scheme 2.8. Cleavage of TBDMS unit in the presence of fluoride ion.

The addition of 0.2, 0.3 and 0.5 ppm of sodium fluoride to gels of L-Tyr(TBDMS)-OH in 2-ethylhexanol, end up with complete gel to solution transition within 44 h, 18 h, and 1 h, respectively (Figure 2.23). Depending on the declaration of The World Health Organization about the appropriate amount of fluoride in drinking water being 0.5–1.0 ppm¹⁶¹, it takes less than one hour to detect the fluoride ion in drinking water above 0.5 ppm. Therefore, it can be suggested that L-Tyr(TBDMS)-OH is a promising gelator with a potential to be used for the detection of fluoride ions.

To further investigate its sensitivity to fluoride ion, spectrophotometric experiments were conducted. However, we did not observed fluorescence emission at different wavelengths for L-Tyr(TBDMS)-OH and L-Tyr-OH. We can concluded that

TBDMS unit has no distinctive effect then the hydroxyl unit on the aromatic ring to result with red or blue shift in fluorescence spectra.

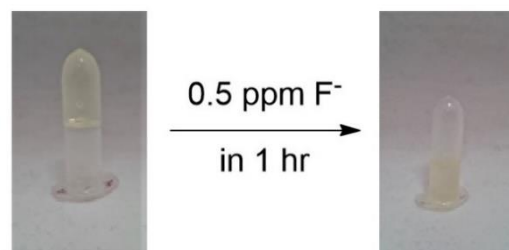


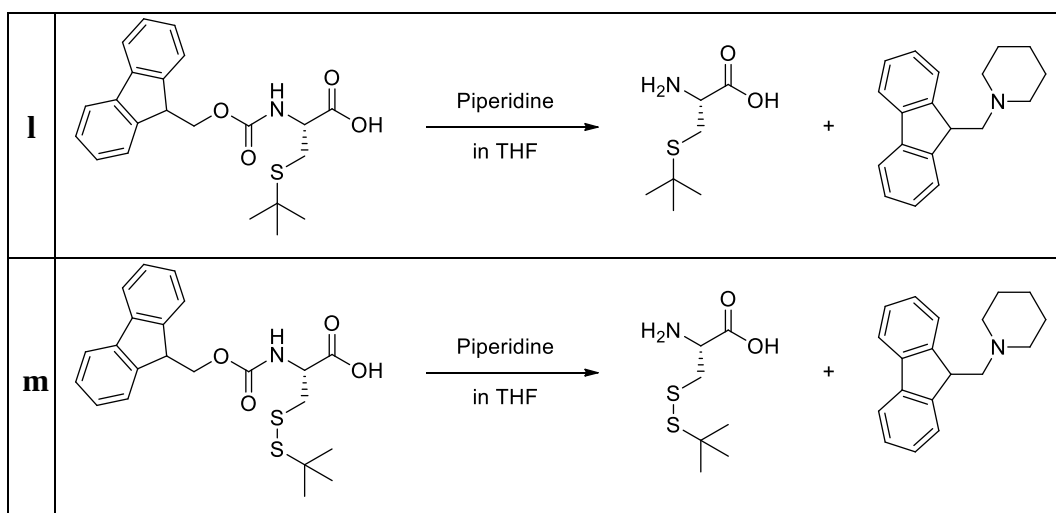
Figure 2.23. Complete gel to solution transition of 1 wt/v % L-Tyr(TBDMS) in 2-ethylhexanol in the presence of 0.5 ppm fluoride ion.

2.2.11 Synthesis of other protected amino acids

In order to investigate the gelation ability of amino acid derivatives other than L-Tyr(*t*Bu)-OH, the synthesis of 11 side chain protected amino acids was performed starting from their Fmoc protected derivatives of amino groups (Scheme 2.9). To do so, the procedure that was used to synthesize L-Tyr(*t*Bu)-OH was followed identically. All amino acid derivatives were obtained with a yield greater than 90% and their chemical structures were proved by ^1H NMR spectroscopy. Scheme 2.9 shows the synthesis of L-Trp(Boc)-OH, L-Asp(O*t*Bu)-OH, L-Glu(O*t*Bu)-OH, L-Asn(Trt)-OH, L-Gln(Trt)-OH, L-Ser(*t*Bu)-OH, L-Thr(*t*Bu)-OH, L-Lys(Boc)-OH, L-His(Trt)-OH, L-Arg(Pbf)-OH, L-Cys(Trt)-OH, L-Cys(*t*Bu)-OH and L-Cys(StBu)-OH amino acid derivatives respectively.

a	Reaction scheme a: A fluorenylmethyl carbamate derivative with a 2-tert-butyl-1H-indol-3-yl group reacts with piperidine in THF to yield a fluorenylmethyl piperidine and a 2-tert-butyl-1H-indol-3-yl carbamate derivative.
b	Reaction scheme b: A fluorenylmethyl carbamate derivative with a 2-tert-butyl-1H-indol-3-yl group reacts with piperidine in THF to yield a fluorenylmethyl piperidine and a 2-tert-butyl-1H-indol-3-yl carbamate derivative.
c	Reaction scheme c: A fluorenylmethyl carbamate derivative with a 2-tert-butyl-1H-indol-3-yl group reacts with piperidine in THF to yield a fluorenylmethyl piperidine and a 2-tert-butyl-1H-indol-3-yl carbamate derivative.
d	Reaction scheme d: A fluorenylmethyl carbamate derivative with a 2-tert-butyl-1H-indol-3-yl group reacts with piperidine in THF to yield a fluorenylmethyl piperidine and a 2-tert-butyl-1H-indol-3-yl carbamate derivative.
e	Reaction scheme e: A fluorenylmethyl carbamate derivative with a 2-tert-butyl-1H-indol-3-yl group reacts with piperidine in THF to yield a fluorenylmethyl piperidine and a 2-tert-butyl-1H-indol-3-yl carbamate derivative.
f	Reaction scheme f: A fluorenylmethyl carbamate derivative with a 2-tert-butyl-1H-indol-3-yl group reacts with piperidine in THF to yield a fluorenylmethyl piperidine and a 2-tert-butyl-1H-indol-3-yl carbamate derivative.

g	Reaction scheme g: A fluorenylmethyl carbamate derivative (with a tert-butyl ester and a chiral auxiliary) reacts with piperidine in THF to yield a fluorenylmethyl piperidine and a chiral auxiliary derivative.
h	Reaction scheme h: A fluorenylmethyl carbamate derivative (with a long chain and a chiral auxiliary) reacts with piperidine in THF to yield a fluorenylmethyl piperidine and a chiral auxiliary derivative.
i	Reaction scheme i: A fluorenylmethyl carbamate derivative (with a benzimidazole group and a chiral auxiliary) reacts with piperidine in THF to yield a fluorenylmethyl piperidine and a chiral auxiliary derivative.
j	Reaction scheme j: A fluorenylmethyl carbamate derivative (with a guanidine group and a chiral auxiliary) reacts with piperidine in THF to yield a fluorenylmethyl piperidine and a chiral auxiliary derivative.
k	Reaction scheme k: A fluorenylmethyl carbamate derivative (with a thioether group and a chiral auxiliary) reacts with piperidine in THF to yield a fluorenylmethyl piperidine and a chiral auxiliary derivative.



Scheme 2.9. Synthesis of **(a)** L-Trp(Boc)-OH, **(b)** L-Asp(OtBu)-OH, **(c)** L-Glu(OtBu)-OH, **(d)** L-Asn(Trt)-OH, **(e)** L-Gln(Trt)-OH, **(f)** L-Ser(*t*Bu)-OH, **(g)** L-Thr(*t*Bu)-OH, **(h)** L-Lys(Boc)-OH, **(i)** L-His(Trt)-OH, **(j)** L-Arg(Pbf)-OH, **(k)** L-Cys(Trt)-OH, **(l)** L-Cys(*t*Bu)-OH, **(m)** L-Cys(*St*Bu)-OH

2.2.12 Spontaneous gelation of amino acid derivatives in reaction medium

For each Fmoc protected derivatives of amino acids, 0.14 M solutions were prepared in dry THF and then piperidine was added. The gelation results are listed in Table 2.4 .L-Asn(Trt)-OH, L-Arg(Pbf)-OH and L-Cys(Trt)-OH remained as clear solution and no gelation was observed even after overnight stirring. The common reason of solution formation could be the too bulky structure of side chain protecting groups, Trt and Pbf. Nevertheless, the gelation of L-Gln(Trt)-OH in the reaction medium after overnight stirring was pointed out even it contains Trt group on its side chain. Likewise, gel formation of L-Trp(Boc)-OH and L-Cys(*St*Bu)-OH needed overnight stirring. Gelation of L-Ser(*t*Bu)-OH and L-Lys(OtBu)-OH was taken place readily in 3 minutes and 10 minutes, respectively. Moreover, L-Asp(OtBu)-OH, L-Thr(*t*Bu)-OH and L-Cys(*t*Bu)-OH formed gels in reaction medium only in 30 minutes, whereas L-Glu(OtBu)-OH needed around 70 minutes to become gel in the reaction

medium. Interestingly, after the addition of piperidine in L-His(Trt)-OH solution, opaque and very stiff gel formation was observed spontaneously. After destructing the gel, the precipitate formation occurred as the reaction proceed. It is important to keep in mind that, gelation time in reaction medium not only depends on the gel formation ability of organogelator but also the reaction rate and rate of stirring.

2.2.13 Solvent screening for the gelation of amino acid derivatives

After purification of all amino acid derivatives, gelation experiments in different type of solvents and solvent-additive combinations were conducted by applying inversion test. Table 2.4 shows the molecular weight of amino acid derivatives, the gelation in reaction medium, THF/piperidine, THF/NaOH, MeOH/H₂O, NMP and DMSO. THF with piperidine additive is chosen since the medium is similar with the reaction medium. The NaOH usage as additive instead of piperidine reduce the minimum gelation concentration of L-Tyr(*t*Bu)-OH in THF from 0.45 wt/v% to 0.25 wt/v%, so THF/NaOH was also used to test the gelation of other amino acid derivatives. As a serendipity, it was discovered that L-Tyr(TBDMS)-OH is able to form gel in MeOH/H₂O and EtOH/H₂O mixture which can pave the way for the formation of hydrogel like systems. Thus, gelation in MeOH/H₂O mixture was also studied for other amino acids. Moreover, the gelation studies were conducted in 1-methyl-2-pyrrolidinone (NMP), dimethylsulfoxide (DMSO), sun flower oil and olive oil since these solvents are biocompatible that can be used in biological experiments such as drug release studies. The gelation of L-Ser(*t*Bu)-OH and L-Cys(*St*Bu)-OH in sunflower oil open a gate for applications on living cells. Moreover, L-His(Trt)-OH was discovered to form gel in MeOH/Acetone mixture serendipitously with gelation concentration 0.8 wt/v%.

2.2.14 Comparison of molecular weights of gelators

Low molecular weight gelators are atom economic that, it is important to develop new gelators with different application potentials. In the literature, the lowest molecular weight hydrogelator is a cyclic dipeptide with a molecular weight of 203.2 g/mol and the lowest molecular weight organogelator is a glycine derivative with 204.2 g/mol molecular weight.^{56,150} In this study, we were able to synthesize twelve new organogelator, in which five of them have a molecular weight lower than the literature data (Table 2.4), namely, L-Glu(OtBu)-OH (203.2 g/mol), L-Asp(OtBu)-OH (189.2 g/mol), L-Cys(*t*Bu)-OH (177.3 g/mol), L-Thr(*t*Bu)-OH (175.2 g/mol) and L-Ser(*t*Bu)-OH (161.2 g/mol). Especially, the synthesis of glycine derivative needs enzymatic reactions to obtain the organogelator in the literature, however these newly synthesized organogelators are not only more atom economic but also easy to synthesize them starting from commercially available materials only in one step.

Table 2.4. Molecular Weights of Amino Acid Derivatives and Their Gelation Tests in Different Solvents and Solvent-Additive Combinations.

Amino acid Derivatives	MW (g/mol)	Gelation during reaction	Gel in THF/pip. (wt/v%)	Gel in THF/NaOH (wt/v%)	Gel in MeOH:H ₂ O (wt/v%)	Gel in NMP (wt/v%)	Gel in DMSO (wt/v%)	Gel in sunflower oil (wt/v%)
L-Tyr(<i>t</i> Bu)-OH	237.3	+ (in 15 min.)	0.45 ^a	0.25 ^a	Solution	1	1	2 ^a
L-Trp(Boc)-OH	304.3	+ (overnight)	2 (gel like)	Suspension	Solution	Suspension	Suspension	Suspension
L-Gln(Trt)-OH	388.5	+ (overnight)	1	1	1.25	Suspension	Solution	Suspension
L-Asn(Trt)-OH	374.4	-	<i>nt</i>	<i>nt</i>	<i>nt</i>	Solution	Solution	<i>nt</i>
L-Glu(<i>Ot</i> Bu)-OH	203.2	+ in 70 min.	1	Suspension	Solution	1	2	Suspension
L-Asp(<i>Ot</i> Bu)-OH	189.2	+ in 30 min.	3	Suspension	Solution	3	2	Suspension
L-His(Trt)-OH	397.5	+ immediately	Suspension	Solution	0.8	Suspension	Suspension	Suspension
L-Ser(<i>t</i> Bu)-OH	161.2	+ in 5 min.	3	2	Solution	3	1 ^a	3 ^a

Table 2.4 (cont'd)

L-Thr(<i>t</i> Bu)-OH	175.2	+ in 30 min.	3	Suspension	1.25	3 (gel like)	3	Suspension
L-Cys(Trt)-OH	363.5	-	<i>nt</i>	<i>nt</i>	<i>nt</i>	<i>nt</i>	<i>nt</i>	<i>nt</i>
L-Arg(Pbf)-OH	425.2	-	<i>nt</i>	<i>nt</i>	<i>nt</i>	<i>nt</i>	<i>nt</i>	<i>nt</i>
L-Lys(Boc)-OH	246.3	+ in 5 min.	3	Solution	Solution	2	1 ^{<i>a</i>}	Suspension
L-Cys(<i>t</i> Bu)-OH	177.3	+ in 30 min.	2	<i>nt</i>	Solution	<i>nt</i>	2	6 ^{<i>a</i>}
L-Cys(<i>St</i> Bu)-OH	209.3	+ (overnight)	1	<i>nt</i>	<i>nt</i>	<i>nt</i>	2	5 ^{<i>a</i>}

a: Minimum gelation concentration. *nt*: not detected.

2.2.15 Characterization of microstructures of L-Ser(*t*Bu)-OH gel

Since the L-Ser(*t*Bu)-OH is the known lowest molecular weight amino acid based organogelator, its microstructural organization was analyzed with TEM imaging. Figure 2.24 shows the nanofibrillar formations with a width of around 20 nm. The length of fibrils are short relatively to L-Tyr(*t*Bu)-OH.

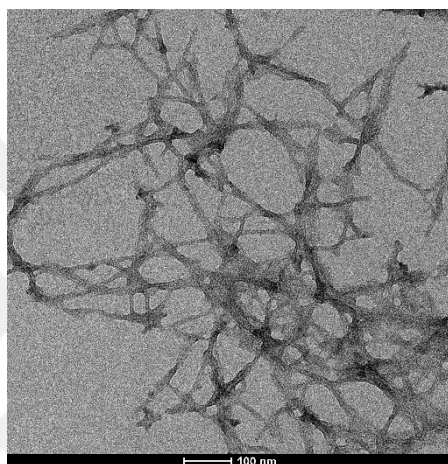


Figure 2.24. TEM image of L-Ser(*t*Bu)-OH in DMSO diluted with milliQ water. (Scale bar: 100 nm).

2.2.16 Gel behavior of L-Ser(*t*Bu)-OH

Soft viscoelastic property of the gel was proven with rheological measurements. Depending on the strain-sweep analysis, the γ_0 value was determined as 2%. Then, frequency-sweep analysis was done as shown in Figure 2.25. The difference between dynamic storage modulus and loss modulus represents the viscoelastic behavior of soft matter. Thus, the material can be qualified as gel.

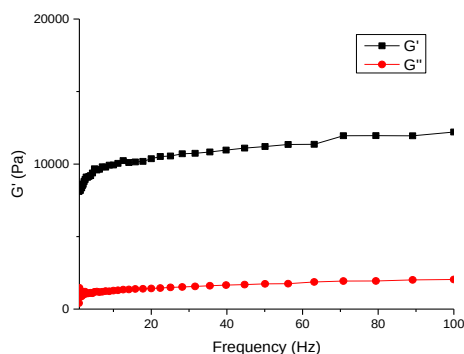


Figure 2.25. Viscoelastic property of 3 wt/v% L-Ser(*t*Bu)-OH gel in DMSO.

2.2.17 Gelation studies of L-Valine, L-Leucine, L-Isoleucine and L-*tert*-Leucine

After concluding the favorable effect of hydrophobic unit on side chain with molecular dynamics studies and gelation studies of protected amino acids, the possible gelation ability of L-Valine, L-Leucine, L-Isoleucine and L-*tert*-Leucine (Figure 2.26) was also investigated.

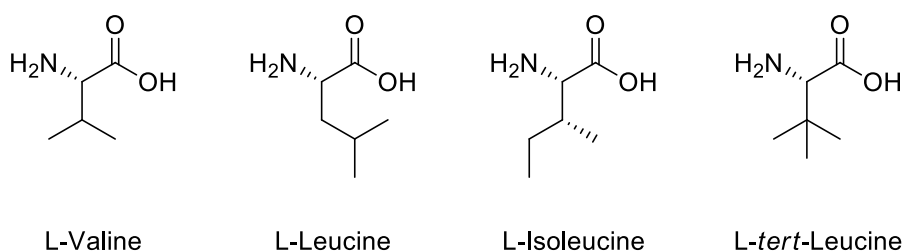


Figure 2.26. Molecular structures of hydrophobic side chain containing amino acids, L-Valine, L-Leucine, L-Isoleucine and L-*tert*-Leucine.

Commercially available amino acids were used for gelation studies. Although the all side chains of above amino acids are shown to be suitable for gelation, no gel formation was observed in THF, NMP and EtOH/H₂O, and amino acids remained in solvents undissolved. Since the amino acids are found as zwitterions, it was thought that the piperidine addition in THF and NMP would increase their solubility to become gel. Nevertheless, even after the addition of piperidine, amino acids remained undissolved in the medium.

2.2.18 Dye removal study of L-Tyr(*t*Bu)-OH, L-Ser(*t*Bu)-OH and L-Glu(*Ot*Bu)-OH organogelators

As an application, gelators are developed and analyzed to remove waste dye from water by absorbing dye molecules by the help of the interactions with gelator. To investigate the dye removal ability of chosen organogelators L-Tyr(*t*Bu)-OH, L-Glu(*Ot*Bu)-OH and L-Ser(*t*Bu)-OH, methyl red was used. At first, gels were prepared in DMSO. By adding water solution of methyl red on different concentrations of gels, the effect of water on gels was observed. Unfortunately, the water added gels started to decompose immediately. Then the DMSO solution of methyl red was prepared and added on fresh gels of three amino acid derivatives in DMSO. The decomposition of 1 wt/v% L-Glu(*Ot*Bu)-OH and 3 wt/v% L-Ser(*t*Bu)-OH gels happened again, whereas the 1 wt/v % and 2 wt/v % L-Tyr(*t*Bu)-OH containing gels, 2 wt/v% L-Glu(*Ot*Bu)-OH and 4 wt/v% L-Ser(*t*Bu)-OH gels remained undisrupted even after overnight standing. Although it is more effective to study the dye removal from water because of environmental reasons, the removal of methyl red from DMSO was analyzed by using L-Tyr(*t*Bu)-OH as organogelator (Figure 2.27). Different concentrations of gel was freshly prepared and after adding methyl red solutions, aliquots was taken from the dye solution at three different time intervals and analyzed by UV-Vis spectrophotometry.

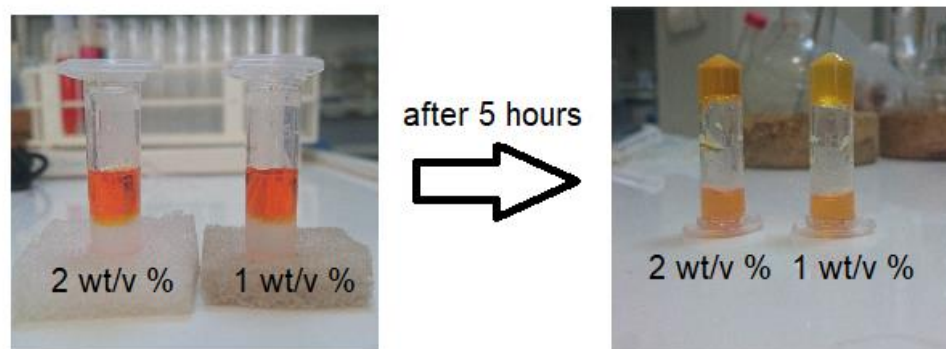


Figure 2.27. Color change of 1 wt/v% and 2 wt/v% gels after 5 hours.

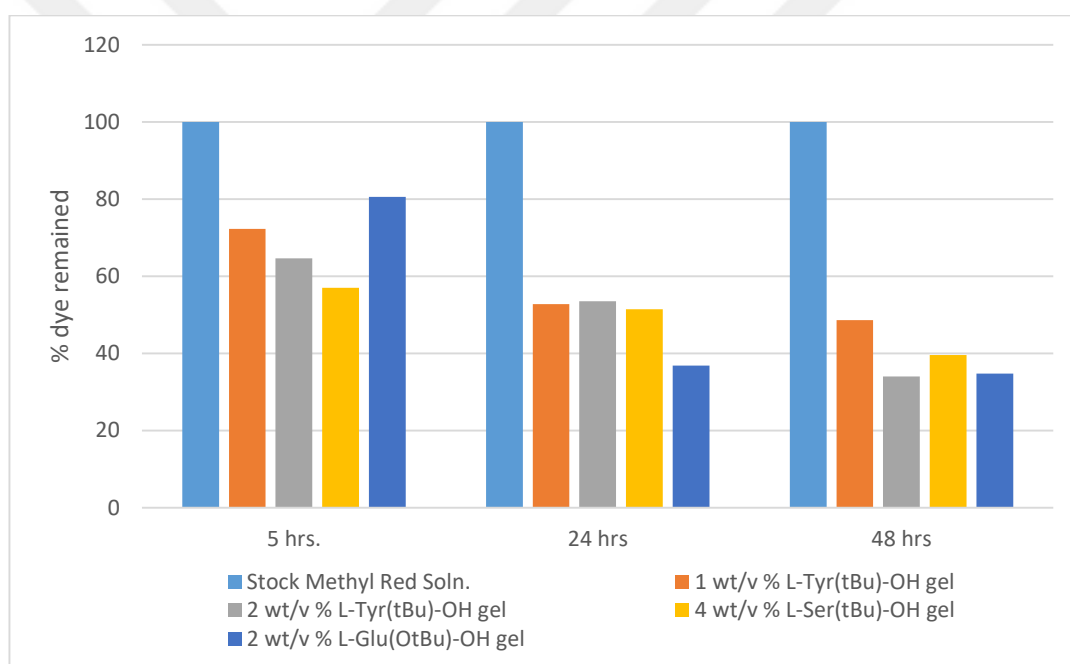


Chart 2.1. Percent absorbance of 0.5 mM Methyl Red Solution, Methyl Red solution on 1 wt/v% L-Tyr(*t*Bu)-OH gel, 2 wt/v% L-Tyr(*t*Bu)-OH gel, 4 wt/v% L-Ser(*t*Bu)-OH and 2 wt/v% L-Glu(*Ot*Bu)-OH gel respectively after 5, 24 and 48 hours at 430 nm.

According to the spectroscopic analysis 51%, 60%, 65% and 66% of dye removed from the medium by using 1 wt/v% L-Tyr(*t*Bu)-OH, 4 wt/v% L-Ser(*t*Bu)-OH, 2 wt/v% L-Glu(*Ot*Bu)-OH and 2 wt/v% L-Tyr(*t*Bu)-OH respectively in 48 hours (Chart 2.1). Even after 24 hours, 2 wt/v% L-Glu(*Ot*Bu)-OH removed 63% of the dye from medium.

2.2.19 Biological Studies

In order to investigate the drug release abilities of organogels, antibacterial activity of drug-loaded and unloaded gels and then the toxicity of gels were analyzed in Sreeparna Banerjee's research laboratory by Sinem Ulasan.

2.2.19.1 Drug release studies of protected amino acid derivative organogels

Kirby Bauer assay was applied both for gram negative bacteria *E. coli* and gram positive bacteria *S. aureus*. Chloramphenicol was chosen as antibacterial agent. DMSO and chloramphenicol are used as negative control and positive control respectively. After incubating gels with bacteria grown agar plates for overnight, clear zones was measured to determine the drug release from gels. The agar plates for drug-loaded and unloaded gels of L-Glu(*Ot*Bu)-OH, L-Thr(*t*Bu)-OH, L-Asp(*Ot*Bu)-OH, L-Tyr(*t*Bu)-OH, L-Ser(*t*Bu)-OH, L-Lys(Boc)-OH and L-Tyr(TBDMS)-OH for gram negative bacteria are demonstrated in Figure 2.28.

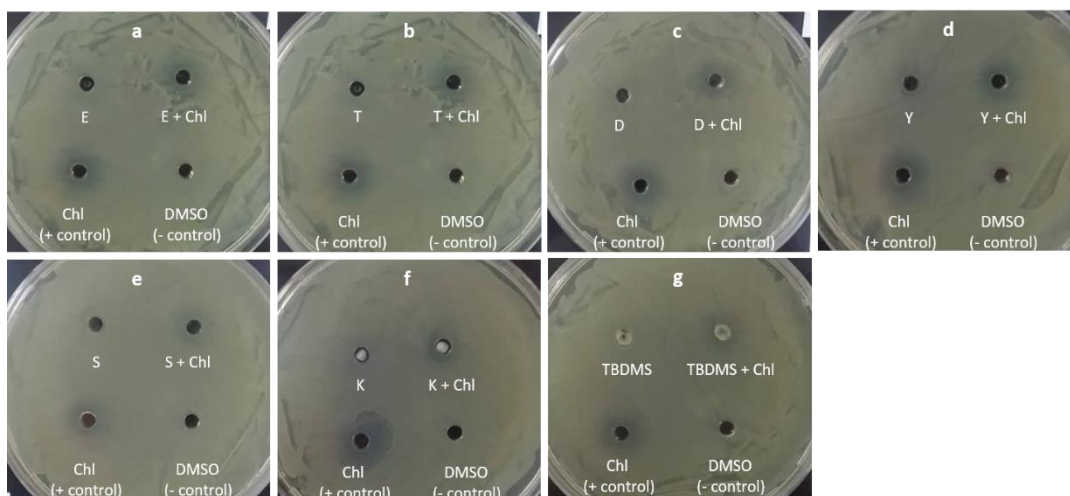


Figure 2.28. Images show the zone of inhibitions of positive control Chloramphenicol (Chl) and negative control DMSO for all agar plates whereas, **a** shows unloaded L-Glu(OtBu)-OH gel (E) and drug-loaded L-Glu(OtBu)-OH gel (E+Chl), **b** shows unloaded L-Thr(*t*Bu)-OH gel (T) and drug-loaded L-Thr(*t*Bu)-OH gel (T+Chl), **c** shows unloaded L-Asp(OtBu)-OH gel (D) and drug-loaded L-Asp(OtBu)-OH gel (D+Chl), **d** shows unloaded L-Tyr(*t*Bu)-OH gel (Y) and drug-loaded L-Tyr(*t*Bu)-OH gel (Y+Chl), **e** shows unloaded L-Ser(*t*Bu)-OH gel (S) and drug-loaded L-Ser(*t*Bu)-OH gel (S+Chl), **f** shows unloaded L-Lys(Boc)-OH gel (K) and drug-loaded L-Lys(Boc)-OH gel (L+Chl) and **g** shows unloaded L-Tyr(TBDMS)-OH gel (TBDMS) and drug-loaded L-Tyr(TBDMS)-OH gel (TBDMS+Chl) for *E. coli*.

The drug release measurements in *E. coli* grown medium for seven different amino acid derivatives were shown in Table 2.5.

Table 2.5. Detected Inhibition Zones in Centimeter for Each Kirby Bauer Assay With *E. coli*.

Organogelator	Gel	Gel+Chl	Chl (+ control)	DMSO (- control)
E	<i>n.d.</i>	1.0 cm	1.1 cm	<i>n.d.</i>
T	<i>n.d.</i>	0.9 cm	1.1 cm	<i>n.d.</i>
D	<i>n.d.</i>	0.7 cm	1.1 cm	<i>n.d.</i>
Y	<i>n.d.</i>	0.8 cm	1.1 cm	<i>n.d.</i>
K	<i>n.d.</i>	0.5 cm	1.1 cm	<i>n.d.</i>
TBDMS	<i>n.d.</i>	0.5 cm	1.1 cm	<i>n.d.</i>
S	<i>n.d.</i>	0.4 cm	1.1 cm	<i>n.d.</i>

Similar Kirby Bauer experiments was also conducted for gram positive bacteria *S. aureus*. Results for drug-loaded and unloaded organogels are represented in Figure 2.29 and Table 2.6 for *S. aureus*. DMSO was used as negative control since the gels were prepared in DMSO. Drug unloaded organogels in DMSO did not inhibit the bacteria growth by itself. The positive control 40 μ molar Chloramphenicol inhibit bacterial growth with a zone of 1.1 cm and 1.5 cm for *E. coli* and *S. aureus* grown plates respectively, whereas inhibition zones for drug-loaded organogels are smaller than for the positive controls. The fewer drug release is not only related to the encapsulation in a gel structure but also the interaction of antibacterial agent with organogelator. Although each organogelator resulted with different inhibition zone, it was proved that all seven amino acid derived organogelators have potential to be used as a vehicle for the delivery of antibacterial agents. Moreover, lowest molecular weight organogelator L-Ser(*t*Bu)-OH resulted with minimum release of chloramphenicol after overnight incubation for both experiments.

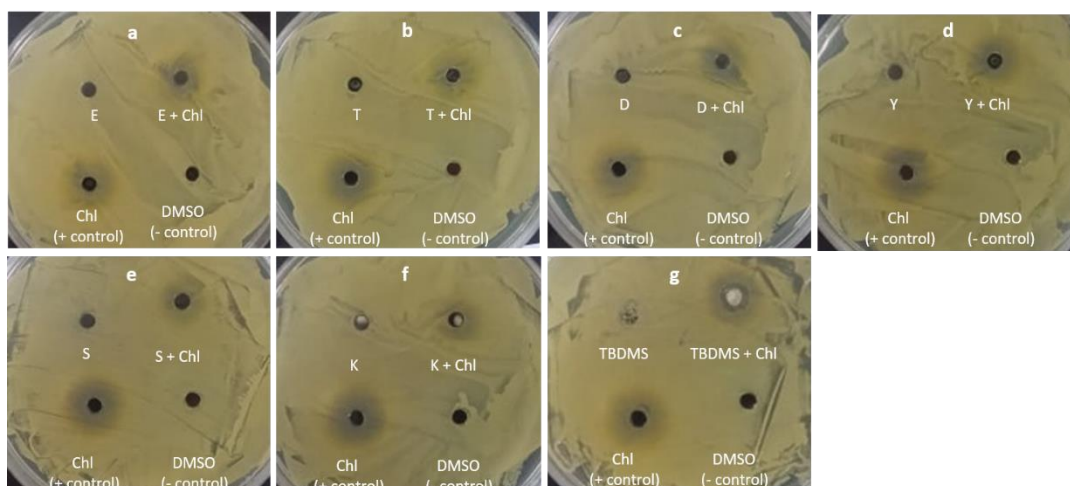


Figure 2.29 Images show the zone of inhibitions of positive control Chloramphenicol (Chl) and negative control DMSO for all agar plates whereas, **a** shows unloaded L-Glu(OtBu)-OH gel (E) and drug-loaded L-Glu(OtBu)-OH gel (E+Chl), **b** shows unloaded L-Thr(tBu)-OH gel (T) and drug-loaded L-Thr(tBu)-OH gel (T+Chl), **c** shows unloaded L-Asp(OtBu)-OH gel (D) and drug-loaded L-Asp(OtBu)-OH gel (D+Chl), **d** shows unloaded L-Tyr(tBu)-OH gel (Y) and drug-loaded L-Tyr(tBu)-OH gel (Y+Chl), **e** shows unloaded L-Ser(tBu)-OH gel (S) and drug-loaded L-Ser(tBu)-OH gel (S+Chl), **f** shows unloaded L-Lys(Boc)-OH gel (K) and drug-loaded L-Lys(Boc)-OH gel (L+Chl) and **g** shows unloaded L-Tyr(TBDMS)-OH gel (TBDMS) and drug-loaded L-Tyr(TBDMS)-OH gel (TBDMS+Chl) for *S. aureus*.

Table 2.6. Detected inhibition zones in centimeter for each Kirby Bauer assay with *S. aureus*.

Organogelator	Gel	Gel+Chl	Chl (+ control)	DMSO (- control)
E	<i>n.d.</i>	1.2 cm	1.5 cm	<i>n.d.</i>
T	<i>n.d.</i>	1.3 cm	1.5 cm	<i>n.d.</i>
D	<i>n.d.</i>	1.4 cm	1.5 cm	<i>n.d.</i>
Y	<i>n.d.</i>	1.4 cm	1.5 cm	<i>n.d.</i>

Table 2.6 (cont'd)

TBDMS	<i>n.d.</i>	1.1 cm	1.5 cm	<i>n.d.</i>
S	<i>n.d.</i>	0.9 cm	1.5 cm	<i>n.d.</i>
K	<i>n.d.</i>	0.9 cm	1.5 cm	<i>n.d.</i>

2.2.19.2 Drug release studies for different concentrations of L-Ser(*t*Bu)-OH gels

Even its very small structure, drug-loaded L-Ser(*t*Bu)-OH organogels showed very promising results to be used as vehicle for antibacterial agents. Further, the drug release of different concentrations of L-Ser(*t*Bu)-OH organogels was also conducted both for *E. coli* and *S. aureus* to see the relation between drug release and gel concentration. Figure 2.30 and Table 2.7 represent the Kirby-Bauer experiment results for gram negative bacteria *E. coli*. As shown in the Table 2.7, drug release from organogels diminishes as the gel concentration increases, even no drug release was observed from 4 wt/v% to 7 wt/v% gels. Similar experiments was also done for gram positive bacteria *S. aureus* and same trend was obtained (Figure 2.31, Table 2.8). However, the release of drug was observed starting from 1 wt/v% gel to 6 wt/v% gel. According to these results, by changing the gel concentrations of drug-loaded vehicle systems controlled drug release durations can be acquired since the gels itself did not represent toxicity even for 7 wt/v% gel both for *E. coli* and *S. aureus*.

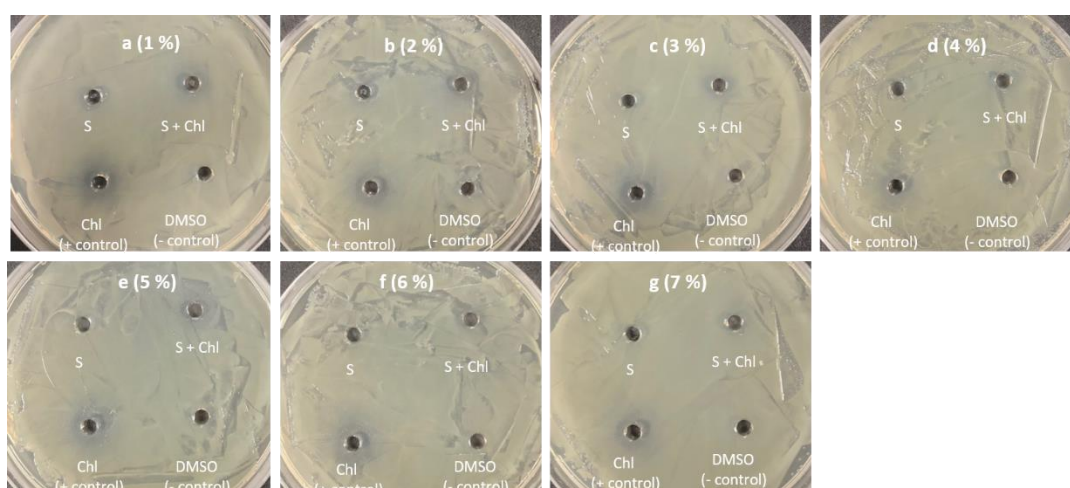


Figure 2.30. Images show the zone of inhibitions of positive control Chloramphenicol (Chl) and negative control DMSO for all agar plates whereas, **a** shows unloaded L-Ser(*t*Bu)-OH gel (S) and drug-loaded L-Ser(*t*Bu)-OH gel (S+Chl) for 1 wt/v% gel, **b** shows unloaded L-Ser(*t*Bu)-OH gel (S) and drug-loaded L-Ser(*t*Bu)-OH gel (S+Chl) for 2 wt/v% gel, **c** shows unloaded L-Ser(*t*Bu)-OH gel (S) and drug-loaded L-Ser(*t*Bu)-OH gel (S+Chl) for 3 wt/v% gel, **d** shows unloaded L-Ser(*t*Bu)-OH gel (S) and drug-loaded L-Ser(*t*Bu)-OH gel (S+Chl) for 4 wt/v% gel, **e** shows unloaded L-Ser(*t*Bu)-OH gel (S) and drug-loaded L-Ser(*t*Bu)-OH gel (S+Chl) for 5 wt/v% gel, **f** shows unloaded L-Ser(*t*Bu)-OH gel (S) and drug-loaded L-Ser(*t*Bu)-OH gel (S+Chl) for 6 wt/v% gel, **g** shows unloaded L-Ser(*t*Bu)-OH gel (S) and drug-loaded L-Ser(*t*Bu)-OH gel (S+Chl) for 7 wt/v% gel for *E. coli*.

Table 2.7. Detected inhibition zones in centimeter for each Kirby Bauer assay with *E. coli*.

L-Ser(<i>t</i> Bu)-OH	Gel	Gel+Chl	Chl (+ control)	DMSO (- control)
1%	<i>n.d.</i>	0.9 cm	1.1 cm	<i>n.d.</i>
2%	<i>n.d.</i>	0.7 cm	1.1 cm	<i>n.d.</i>
3%	<i>n.d.</i>	0.6 cm	1.1 cm	<i>n.d.</i>

Table 2.7 (cont'd)

4%	<i>n.d.</i>	<i>n.d.</i>	1.1 cm	<i>n.d.</i>
5%	<i>n.d.</i>	<i>n.d.</i>	1.1 cm	<i>n.d.</i>
6%	<i>n.d.</i>	<i>n.d.</i>	1.1 cm	<i>n.d.</i>
7%	<i>n.d.</i>	<i>n.d.</i>	1.1 cm	<i>n.d.</i>

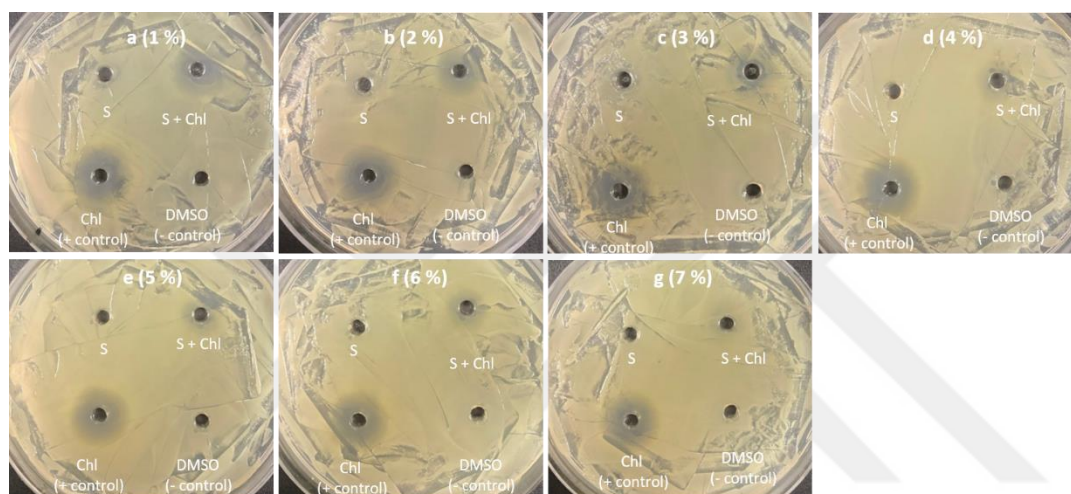


Figure 2.31. Images show the zone of inhibitions of positive control Chloramphenicol (Chl) and negative control DMSO for all agar plates whereas, **a** shows unloaded L-Ser(*t*Bu)-OH gel (S) and drug-loaded L-Ser(*t*Bu)-OH gel (S+Chl) for 1 wt/v% gel, **b** shows unloaded L-Ser(*t*Bu)-OH gel (S) and drug-loaded L-Ser(*t*Bu)-OH gel (S+Chl) for 2 wt/v% gel, **c** shows unloaded L-Ser(*t*Bu)-OH gel (S) and drug-loaded L-Ser(*t*Bu)-OH gel (S+Chl) for 3wt/v % gel, **d** shows unloaded L-Ser(*t*Bu)-OH gel (S) and drug-loaded L-Ser(*t*Bu)-OH gel (S+Chl) for 4 wt/v% gel, **e** shows unloaded L-Ser(*t*Bu)-OH gel (S) and drug-loaded L-Ser(*t*Bu)-OH gel (S+Chl) for 5wt/v % gel, **f** shows unloaded L-Ser(*t*Bu)-OH gel (S) and drug-loaded L-Ser(*t*Bu)-OH gel (S+Chl) for 6 wt/v% gel, **g** shows unloaded L-Ser(*t*Bu)-OH gel (S) and drug-loaded L-Ser(*t*Bu)-OH gel (S+Chl) for 7wt/v % gel for *S. aureus*.

Table 2.8. Detected inhibition zones in centimeter for each Kirby Bauer assay with *S. aureus*.

L-Ser(<i>t</i>Bu)-OH	Gel	Gel+Chl	Chl (+ control)	DMSO (- control)
1%	<i>n.d.</i>	1.4 cm	1.5 cm	<i>n.d.</i>
2%	<i>n.d.</i>	1.2 cm	1.5 cm	<i>n.d.</i>
3%	<i>n.d.</i>	1.0 cm	1.5 cm	<i>n.d.</i>
4%	<i>n.d.</i>	0.9 cm	1.5 cm	<i>n.d.</i>
5%	<i>n.d.</i>	0.6 cm	1.5 cm	<i>n.d.</i>
6%	<i>n.d.</i>	0.5 cm	1.5 cm	<i>n.d.</i>
7%	<i>n.d.</i>	<i>n.d.</i>	1.5 cm	<i>n.d.</i>

2.2.19.3 Cell viability/MTT assay

Cell viability of L-Ser(*t*Bu)-OH vs. L-Ser-OH is tested by the MTT assay. Here, we investigated the effect of the modification of the gelator that may have cause cells to be more susceptible to death. MTT reagent diffuses into the active and functional mitochondria of a living cell, giving a bright blue color in the visible range. At 570 nm, it absorbs the visible light which enables the one to determine death percentage since the absorption is proportional to number of the living cells. The range of concentrations is very broad as 1 μ L to 5mM, so that we could see variations of cell death according to gelator concentration (Figure 2.32). For very low concentrations such as 1 μ M-50 μ M, the toxicity level is less significant. However, from 100 μ M to 5 mM, the toxicity suddenly increases. Results suggest that, modification of L-Ser-OH with *tert*-butyl appears to show a significant toxic effect in gelation concentrations such as 3 wt/v% (186 μ M).

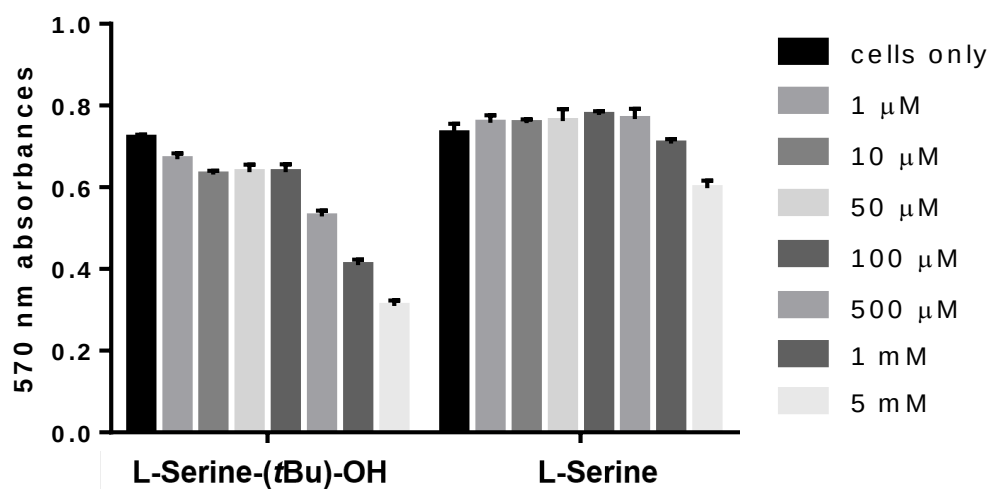


Figure 2.32. Cell viability/MTT assay results showing percentage of the living cells vs. concentration of the solutions used.

2.2.19.4 Cell compatibility

After obtaining the results from cell viability assay, cell compatibility assays was performed in order to comment about the toxicity of the solvent itself instead of the gelator in the cell culture solution. The properties of the gelator in solution versus in the gel form can be different because of the interactions of the modified serine with the cells in the three dimensional network will be different. Also the solvent used for gelation process should be taken into consideration. The solvent provides the three dimensional network where the gelator and the cells interact. The HCT-116 cell line is derived from colon tissue needs to adhere to the matrix of the gel to stay alive. Hence, level of cell adhesion gives a perspective to comment about the possible proliferation of the cells.

One can comment about wellness of the cell adhesion to the surface from the morphology of the cells and their position with respect to each other. HCT-116 cells, once start attaching to the matrix, appears to be in spherical shape. However, the

more they start to adhere to the surface to survive, the more they deviate from the form and elongate in shape. In addition, a gathering of the cells closer to each other can be seen to carry cell-cell interactions. When the compatibility assay is done with the DMSO-based gel, the cells couldn't start elongating and growing in addition to staying away from each other, during live monitoring (Figure 2.33). Also to mention that gel environment looked the significantly different environment is probably due to the use of DMSO as gelation solvent.

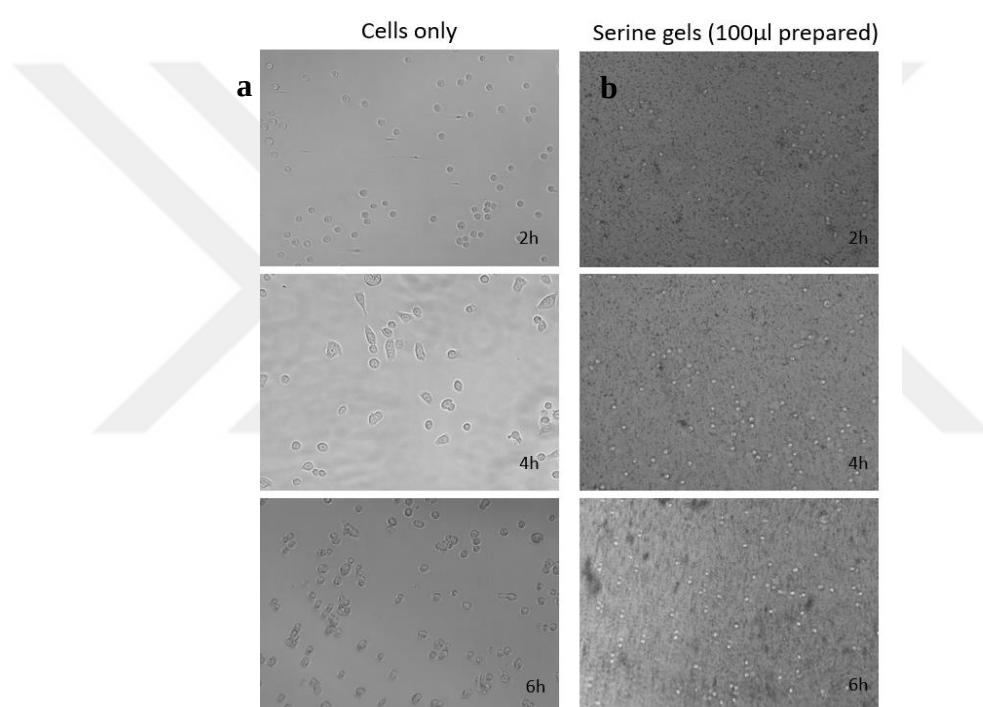


Figure 2.33. Cell compatibility assay for DMSO-based gel from 2 to 6 hours. **(a)** Image of cells only in cell culture medium. **(b)** Image of cells added to the organogel containing medium.

When the same compatibility assay was performed in sunflower oil-based organogel, it looks like the matrix of the gel was well suited for cells to attach. Even though the elongation could not be seen yet, this results indicates a possible proliferation in a 6

hour timeline. As well as the cells looked similar to an early stage of adhesion to continue proliferation, the matrix seemingly mimics a natural environment better compared to DMSO based gel (Figure 2.34) which can gives a chance for the sunflower oil based L-Ser(*t*Bu)-OH gels to be for drug release in HCT-116 cells.

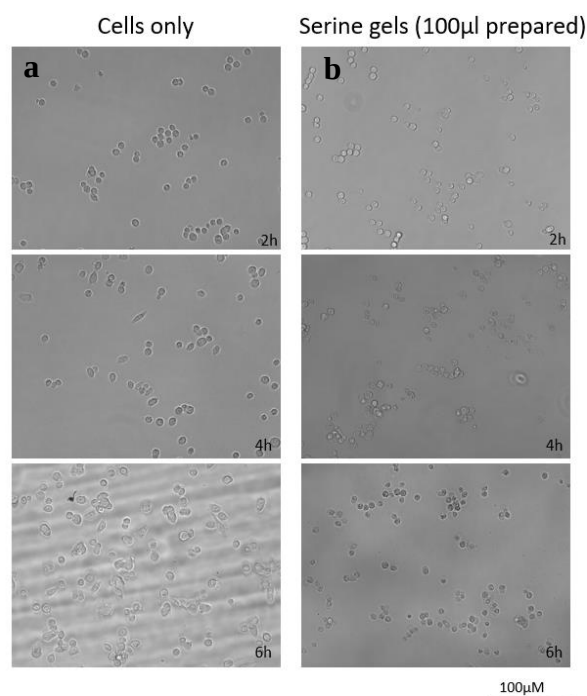
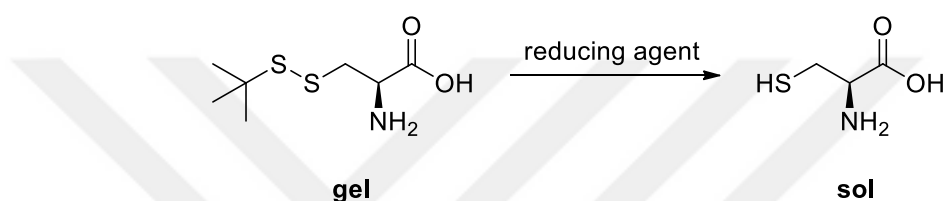


Figure 2.34. Cell compatibility assay in sunflower oil-based gel from 2 to 6 hours. **(a)** Image of cells only in cell culture medium. **(b)** Image of cells added to the organogel containing medium.

However, the additional research is needed to perform to investigate its cytotoxicity in longer times intervals as well as apoptosis assays, including variety of cell lines. Vegetable oil based organogels are highly promising since they offer an alternative to conventional organic solvents to be used as the continuous phase and may permit the creation of non-cytotoxic organogels which makes them suitable for *in vivo* applications of the water insoluble materials.

2.2.20 Gel to solution transition of L-Cys(*St*Bu)-OH containing gels

The presence of reducing agents such as TCEP and DTT cause the cleavage of S-S bond of L-Cys(*St*Bu)-OH (Scheme 2.10). When this reaction was performed in the gel form, gel to solution transition was observed not only with TCEP or DTT addition, but also with the addition of L-Cystein-OH which mimics L-Glutathione found in living cells especially in the cancer cells at higher concentrations.



Scheme 2.10. Cleavage of S-S bond in the presence of a reducing agent.

The addition of 50 ppm TCEP solution in 1 wt/v% L-Cys(*St*Bu)-OH gel in THF the complete gel to solution transition was observed in less than 2 hours (Figure 2.35). As the TCEP concentration increases to 150 ppm and 200 ppm, gel to solution transitions happened in 40 minutes and 20 minutes respectively for 1.5 wt/v% L-Cys(*St*Bu)-OH gel in THF. It is also possible to observe transition with 7 ppm of TCEP, but in longer durations. Additionally, 300 ppm of DTT and L-Cystein-OH addition on 2 wt/v% gel in DMSO cause to complete solution transition in 2 hours and 3.5 hours respectively. To make this finding more suitable for biological applications, the reaction was done both in 5 wt/v% gel in sunflower oil and 6 wt/v% gel in olive oil with the addition of 500 ppm of L-Cystein-OH solution in DMSO. Since DMSO is not miscible with sunflower oil and olive oil, the reaction did not take place even in one week. To make the L-Cystein-OH mix with gels in oil, its solution in propane glycol was prepared. Thus, the complete gel to solution transition

of gels in sunflower oil and olive oil was observed with the addition of 500 ppm of L-Cystein-OH at around 24 hours.

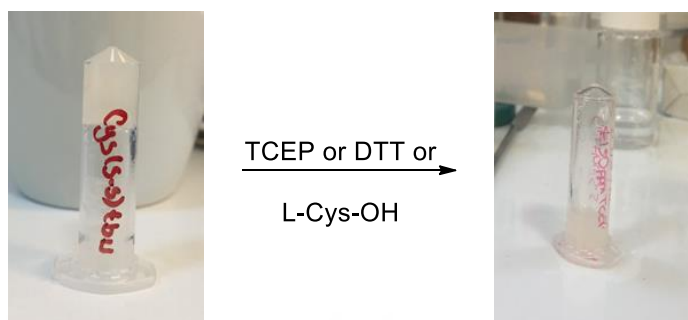


Figure 2.35. Complete gel to solution transition of L-Cys(StBu) gel in the presence of TCEP, DTT or L-Cys-OH.

This results suggest that a controlled drug release can be ensured with the drug encapsulated L-Cys(StBu)-OH gels especially in cancer cells that contains L-Glutathione in higher concentrations not only with the diffusion of drug, but also with the cleavage of S-S bond found in the structure of organogelator.

2.3 Experimental

2.3.1 Materials

All reagents are commercially available and used without any further purification. For chemical reactions and gelation processes protected amino acids Fmoc-L-Tyr(*t*Bu)-OH, Fmoc-D-Tyr(*t*Bu)-OH, L-Tyr-OH, L-Tyr(OMe)-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Gln(Trt)-OH, Fmoc-L-Asn(Trt)-OH, Fmoc-L-Glu(O*t*Bu)-OH, Fmoc-L-Asp(O*t*Bu)-OH, Fmoc-L-His(Trt)-OH, Fmoc-L-Ser(*t*Bu)-OH, Fmoc-L-Thr(*t*Bu)-OH, Fmoc-L-Cys(Trt)-OH, Fmoc-L-Arg(Pbf)-OH, Fmoc-L-Cys(*t*Bu)-OH, Fmoc-L-Cys(StBu)-OH, DTT and unprotected amino acids L-Valine, L-Leucine, L-Isoleucine were purchased from Chem-Impex International Inc.; *tert*-Butyldimethylchlorosilane from TCI Chemicals; DMF, 1,2-DME, imidazole, methanol and ethanol from Sigma Aldrich; THF, Toluene, MTBE, 1,2-DCE, *n*-BuOH, diisopropylamine, DMSO and DBU from Merck; ACN, triethylamine from Carlo Erba Reagents; Piperidine and 2-ethylhexylamine from Acros Organics; hexane and isopropylamine from Lab Scan; diethylamine from Riedel-de Haen; 2-ethylhexanol from Veskim.

For biological assays gram negative bacteria *E. coli* and gram positive bacteria *S. aureus* was purchased from ATCC; human colon cancer cell line HCT-116 from Deutsche Sammlung von Mikroorganismen und Zellkulturen; all cell culture supplements from Biological Industries (Beit Haemek, Israel); plasmocin from Invivogen and MTT from Sigma Aldrich

2.3.2 General Fmoc cleavage procedure for Fmoc-protected amino acid derivatives

L-Tyr(*t*Bu)-OH, D-Tyr(*t*Bu)-OH, L-Trp(Boc)-OH, L-Gln(Trt)-OH, L-Asn(Trt)-OH, L-Glu(O*t*Bu)-OH, L-Asp(O*t*Bu)-OH, L-His(Trt)-OH, L-Ser(*t*Bu)-OH, L-Thr(*t*Bu)-OH, L-Cys(Trt)-OH, L-Arg(Pbf)-OH, L-Lys(Boc), L-Cys(*t*Bu) and L-Cystine(*t*Bu), were synthesized based on the literature procedure¹⁷² with some alterations in purification method. To a solution of 1.1 mmol of Fmoc-protected amino acid derivative in 8 ml of THF, 0.54 ml (5.5 mmol) of piperidine was added. After stirring for 3-16 hours at room temperature, solvent was removed under vacuum. Remaining white solid was washed with 200 ml of hexane in 10 portions and allowed to dry open to air to obtain white solids. Yellow solids were attained for L-Asp(O*t*Bu)-OH and L-Cys(Trt)-OH. All proton and carbon NMR spectra are present in Appendix F.

L-Tyr(*t*Bu)-OH: ¹H NMR (400 MHz, CD₃OD) δ 7.36 – 7.12 (2H, m), 7.12 – 6.87 (2H, m), 3.77 (1H, dd, *J* = 8.8, 4.3 Hz), 3.29 (1H, dd, *J* = 8.8, 4.3 Hz), 2.99 (dd, *J* = 14.6, 8.8 Hz), 1.36 (9H, s); ¹³C NMR (100 MHz, CD₃OD) δ 173.9 (1C), 155.9 (1C), 132.3 (1C), 131.1 (2C), 125.6 (2C), 79.6 (1C), 57.6 (1C), 37.6 (1C), 29.2 (9C). Purity measured by HPLC (Appendix C): 98.9 %.

L-Trp(Boc)-OH: ¹H NMR (400 MHz, CD₃OD) δ 8.02 (1H, d, *J* = 7.9 Hz), 7.64 (1H, d, *J* = 7.7 Hz), 7.51 (1H, s), 7.22 (1H, m), 7.19 – 7.11 (1H, m), 3.76 (1H, dd, *J* = 9.4, 4.1 Hz), 3.35 (1H, dd, *J* = 14.9, 3.7 Hz), 3.02 (1H, dd, *J* = 15.2, 9.4 Hz), 1.57 (9H, s).

L-Gln(Trt)-OH: ¹H NMR (400 MHz, CD₃OD) δ 7.25 – 7.05 (15H, m), 3.36 (1H, t, *J* = 6.0 Hz), 2.57 – 2.34 (2H, m), 1.90 (2H, dd, *J* = 13.0, 5.9 Hz).

L-Asn(Trt)-OH: ¹H NMR (400 MHz, CD₃OD) δ 7.32 – 7.17 (15H, m), 3.69 (1H, dd, *J* = 8.6, 3.6 Hz), 3.03 – 2.99 (1H, m), 2.72 (1H, dd, *J* = 16.5, 8.7 Hz).

L-Glu(OtBu)-OH: ^1H NMR (400 MHz, CD_3OD) δ 3.48 (1H, dt, $J = 6.3, 2.3$ Hz), 2.41 – 2.30 (2H, m), 2.06 – 1.88 (2H, m), 1.35 (9H, s).

L-Asp(OtBu)-OH: ^1H NMR (400 MHz, CD_3OD) δ 3.80 (1H, dd, $J = 8.4, 3.7$ Hz), 2.93 (1H, dd, $J = 17.9, 3.6$ Hz), 2.75 (1H, dd, $J = 17.8, 8.4$ Hz), 1.48 (9H, s).

L-His(Trt)-OH: ^1H NMR (400 MHz, CD_3OD) δ 7.40 (10 H, m), 7.25 – 7.13 (6H, m), 6.87 (1H, s), 3.84 (1H, dd, $J = 8.1, 4.0$ Hz), 3.16 (1H, dd, $J = 15.2, 3.7$ Hz), 2.97 (1H, dd, $J = 15.3, 8.2$ Hz).

L-Ser(tBu)-OH: ^1H NMR (400 MHz, CD_3OD) δ 3.84 (1H, dd, $J = 8.6, 2.5$ Hz), 3.69 (1H, dd, $J = 10.3, 5.1$ Hz), 3.67 – 3.63 (1H, m), 1.24 (9H, s).

L-Thr(tBu)-OH: ^1H NMR (400 MHz, CD_3OD) δ 4.31 (1H, m), 1.34 – 1.28 (4H, m), 1.22 (9H, s).

L-Cys(Trt)-OH: ^1H NMR (400 MHz, CD_3OD) δ 7.56 – 7.41 (7H, m), 7.40 – 7.27 (6H, m), 7.24 (2H, m), 3.12 (1H, dd, $J = 8.6, 4.4$ Hz), 2.71 (1H, dd, $J = 12.3, 4.3$ Hz), 2.53 (1H, dd, $J = 12.4, 8.5$ Hz).

L-Arg(Pbf)-OH: ^1H NMR (400 MHz, CD_3OD) δ 3.46 (1H, t, $J = 6.1$ Hz), 3.20 (2H, m), 3.02 (2H, s), 2.59 (3H, s), 2.53 (3H, s), 2.10 (3H, s), 1.77 – 1.68 (4H, m), 1.47 (6H, s).

L-Lys(Boc)-OH: ^1H NMR (400 MHz, CD_3OD) δ 3.31 (1H, dd, $J = 98.2, 92.8$ Hz), 2.94 (2H, m), 1.87 – 1.63 (2H, m), 1.41 (4H, m), 1.33 (9H, s).

L-Cys(tBu)-OH: ^1H NMR (400 MHz, CD_3OD) δ 3.53 (1H, dd, $J = 9.7, 3.6$ Hz), 3.14 (1H, dd, $J = 13.7, 3.6$ Hz), 2.78 (1H, dd, $J = 13.7, 9.7$ Hz), 1.27 (9H, s).

L-Cys(StBu)-OH: ^1H NMR (400 MHz, CD_3OD) δ 3.73 (1H, dd, $J = 10.1, 3.4$ Hz), 3.29 (1H, dd, $J = 14.3, 3.4$ Hz), 2.86 (1H, dd, $J = 14.2, 10.1$ Hz), 1.28 (9H, s).

2.3.3 Synthesis of L-Tyr(TBDMS)-OH

L-Tyr(TBDMS)-OH was synthesized based on the synthesis of L-DOPA(TBDMS)₂-OH found in literature with slight modifications.¹⁷⁶ TBDMS-Cl (1.20 g, 8.28 mmol) dissolved in anhydrous MeCN (12.5 ml) was added on previously weighted L-Tyr-OH (0.100 g, 5.05 mmol). The mixture was cooled in an ice-water bath for 10 minutes. After that, the addition of DBU (1.24 ml, 8.28 mmol) was done dropwisely to the reaction mixture in 10 minutes. The reaction mixture was stirred for 4 hours in ice bath and additionally for 20 hours at room temperature. After the completion of reaction, the solvent was removed under vacuum. Methanol addition was done, to obtain impurities as precipitate. After filtration, solvent was removed under vacuum. The crude product was washed with water then, allowed to dry. Lastly, it was washed with ethyl acetate to obtain pure product as off-white solid. The proton and carbon NMR is shown in Appendix F.

¹H NMR (400 MHz, CD₃OD) δ 7.00 (2H, d, J = 8.5 Hz), 6.63 (2H, dd, J = 6.6, 1.9 Hz), 3.55 (1H, dd, J = 8.7, 4.3 Hz), 3.05 (1H, dd, J = 14.6, 4.2 Hz), 2.75 (1H, dd, J = 14.6, 8.7 Hz), 0.80 (9H, s), 0.00 (6H, s); ¹³C NMR (100 MHz, CD₃OD) δ 174.0 (1C), 156.0 (1C), 131.6 (2C), 130.0 (1C), 121.5 (2C), 57.6 (1C), 37.5 (1C), 26.2 (3C), 19.0 (1C), 4.31 (2C). HRMS: calculated for [M+H]⁺ C₁₅H₂₆NO₃Si, 296.1682; found 296.1696. Purity measured by HPLC: 98.3%.

2.3.4 General gelation procedure

In order to prepare 1% (w/v) gel; 10 mg of amino acid derivatives was weighed and placed into an Eppendorf tube. 1.0 ml solvent was added on organogelators. The vial was placed into ultrasonic bath for 4 minutes. The temperature of ultrasonic bath is around 40 °C. Then 10.0 μ l additive was added only for the additive containing gels. Eppendorf tube was again placed into ultrasonic bath for 4–10 minutes. Gel

formation occurs spontaneously or up to 1 hour generally, however depending on the solvent used and gel concentration gelation may occur in 48 hours. Gel formations were proven by applying inversion test. Kudos ultrasonic bath was used for gelations with 53 kHz frequency. The bath is full with distilled water only up to operating level for most efficient ultrasonication.

2.3.5 Transmission electron microscopy imaging

The fibril formations due to self-assembly was imaged with FEI Tecnai G2 Spirit BioTwin CTEM microscope. 1 wt/v% gel of L-Tyr(*t*Bu)-OH was prepared freshly in THF/NaOH and THF/piperidine following the gelation procedure whereas 1 wt/v% gels of L-Tyr(TBDMS)-OH prepared only in THF/piperidine. Additionally, 3 wt/v% L-Ser(*t*Bu)-OH was used for sample preparation. After diluting 50-fold with water, the solutions were applied on Cu grid. Excess solutions were removed after 2 minutes and grids were stained with 2% uranyl acetate solution for all samples.

2.3.6 Rheological measurements

1 wt/v% gels of L-Tyr(*t*Bu)-OH were prepared in 2-ethylhexanol with and without piperidine addition freshly for the rheological measurements. Similarly, 1 wt/v % gel of L-Tyr(TBDMS) was also prepared to investigate its rheological behavior. For the rheological measurements of L-Ser(*t*Bu)-OH, 3 wt/v% gel in DMSO was prepared. Physica MCR 301, Anton Paar was used. At first, the linear viscoelastic regimes of deformations of the organogels were determined by a strain-sweep experiment. With a limit of linearity of G' , the strain values that used for the strain-controlled analyses were assigned. Then, the visco-elastic character of the samples was examined by dynamic storage modulus G'' and loss modulus G' . Frequency sweep was scanned from 0.1 to 100 Hz by using a constant target strain determined before.

2.3.7 Powder X-ray diffraction measurements

XRD measurements were conducted using X'Pert³ MRD with Cu K α X-ray radiation ($\lambda = 1.540598 \text{ \AA}$). Gels of L-Tyr(*t*Bu)-OH in THF and THF/piperidine were prepared as 3 wt/v% and allowed to dry in air for overnight and then vacuum was applied to obtain xerogels.

2.3.8 Molecular Dynamic Simulations

All molecular dynamics simulations and analysis were performed using the Amber16/AmberTools17 program package.³ Simulations in THF were performed for two systems: system 1 with only L-Tyr(*t*Bu)-OH molecules in their zwitterionic form (YTZ residues; net charge = 0) and system 2 containing an equimolar mixture of L-Tyr(*t*Bu)-OH molecules with deprotonated carboxylic group (YTB residues; net charge = -1) and protonated piperidine molecules (PPH residues; net charge = +1). GAFF parameters⁴ were used for THF and PPH molecules, while the parameters for YTB and YTZ were adapted from the ff14SB force field.⁵ The derivation of atomic point charges was performed for each newly defined residue following the RESP strategy⁶ based on electrostatic potentials calculated at the HF/6-31G* level with Orca⁷ on a Merz-Singh-Kollman grid accordingly with Amber force field standards. Parameters and library files are available as Supporting Information. Cubic simulation boxes with initial edges length of 56 $\text{\AA}$ were built with the packmol program.¹⁰ System 1 contained 16 YTZ and 1300 THF molecules, while system 2 was made of 16 YTB, 16 PPH, and 1300 THF molecules.

2.3.9 Fluoride ion response of gels of L-Tyr(TBDMS)-OH

After the formation of gel in 2-ethylhexanol as 1 wt/v%, 10 μl sodium fluoride solution was dropped on gel from the stock solution (5 mg NaF in 1 mL water) to

analyze the effect of 0.5 ppm NaF. Then, it was allowed to stay without stirring. Complete gel to solution transition was observed with naked eye and inversion test after the indicated times passed.

2.3.10 Dye Removal

All gels were prepared depending on the above gelation procedure freshly as 0.5 ml in eppendorf tubes. The gelation solvent is DMSO. 0.5 ml of 0.5 mM methyl red solution in DMSO was added on each gel slowly. After allowing to stand for 5 hours, 24 hours and 48 hours, 30 microliter aliquots were taken from each sample and diluted to 1 ml with DMSO. UV-Visible spectroscopy was used to measure the absorbance values for each sample at 430 nm. To calculate the percent amount of dye removed from the medium by gels, 0.5 mM methyl red solution was also diluted in same amount to measure its absorbance value at 430 nm.

2.3.11 Kirby Bauer test for antibacterial activity

Antibacterial activity of the drug-loaded and unloaded gels was determined using Kirby Bauer assay. Agar plates containing MH Broth were prepared under sterile conditions and autoclaved prior to use. Gram negative bacteria *E. coli* (ATCC 3789) and gram positive bacteria *S. aureus* (ATCC 29213) cultures were grown overnight at 37 °C and 160 rpm. 80 µl from each of these cultures were spread-plated on MH Agar plates. Then, 2 mm diameter holes were opened in the plates, 25 µl of drug-loaded and unloaded gels were injected inside the holes. The plates were incubated overnight at 37 °C and clear zones were measured.

2.3.12 Cell viability and MTT assay

Cells were cultured in T25 flasks and transferred to 96-well plates after the confluency was reached. HCT-116 cells were allowed to attach for 24 hours followed by treatment with L-Ser-(*t*Bu)-OH so that the final concentrations were 1 μ M, 10 μ M, 50 μ M, 100 μ M, 500 μ M, 1 mM and 5 mM. L-Ser-(*t*Bu)-OH was dissolved directly in the cell culture medium and no vehicle was included. As a control, untreated cells were also included. The cells were treated for 24 hours and the viability was determined following the MTT assay procedure. Shortly, after 24 hours of treatment, cells were incubated in culture medium containing MTT for 4 hours. The quantity of formazan, presumably directly proportional to the number of viable cells, is measured by recording changes in absorbance at 570 nm using a plate reading spectrophotometer. The experiment was conducted using 4 technical replicates.

2.3.13 Cell compatibility assay

3 wt/v% and 4 wt/v% L-Ser(*t*Bu)-OH solutions respectively in DMSO and sunflower oil are filtered with 13 mm Whatman syringe filter prior to gelation. The 96-well plates are coated with the sterile solution and waited 24 hours until they formed gels in plates. After that, HCT-116 cells that are previously grown in T25 flasks are added to the gel coated wells. Cell adhesion to the matrix is monitored by FLoid™ Cell Imaging Station under white channel in 2, 4 and 6 hours.

2.3.14 S-S cleavage of L-Cys(*St*Bu)-OH containing gel

Defined concentrations of gels were prepared by the help of ultrasonic bath and 10 μ l of the reducing agent in the defined ppm value was added in to the gel medium with a pipette.

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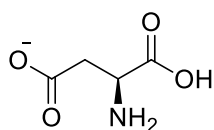
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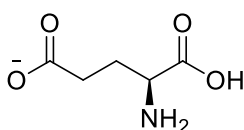
APPENDICES

A. Amino Acids And Their One- And Three- Letter Notations

Amino acids with negatively charged side chains.

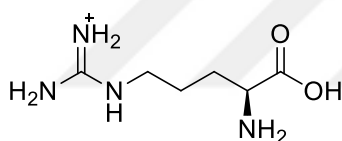


Aspartic acid
(Asp, D)

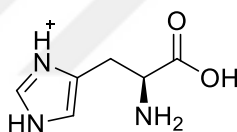


Glutamic acid
(Glu, E)

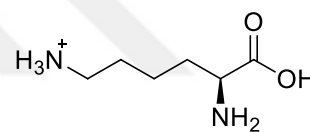
Amino acids with positively charged side chains.



Arginine
(Arg, R)

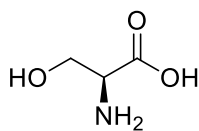


Histidine
(His, H)

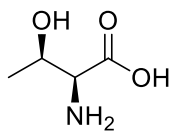


Lysine
(Lys, L)

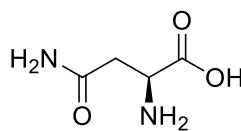
Amino acids with polar uncharged side chains.



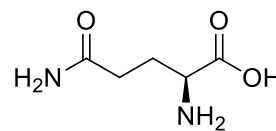
Serine
(Ser, S)



Threonine
(Thr, T)

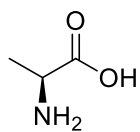


Asparagine
(Asn, N)

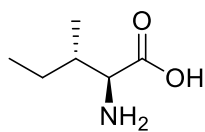


Glutamine
(Gln, Q)

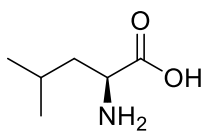
Amino acids with hydrophobic side chains.



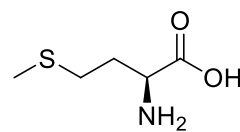
Alanine
(Ala, A)



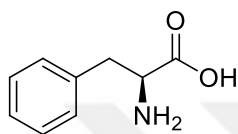
Isoleucine
(Ile, I)



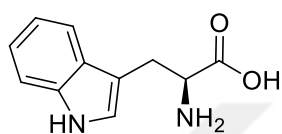
Leucine
(Leu, L)



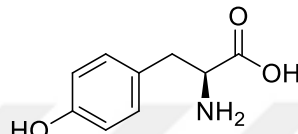
Methionine
(Met, M)



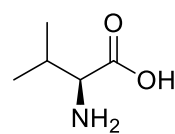
Phenylalanine
(Phe, F)



Tryptophan
(Trp, W)

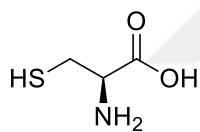


Tyrosine
(Tyr, Y)

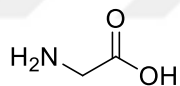


Valine
(Val, V)

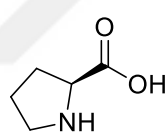
Amino acids with special cases.



Cysteine
(Cys, C)



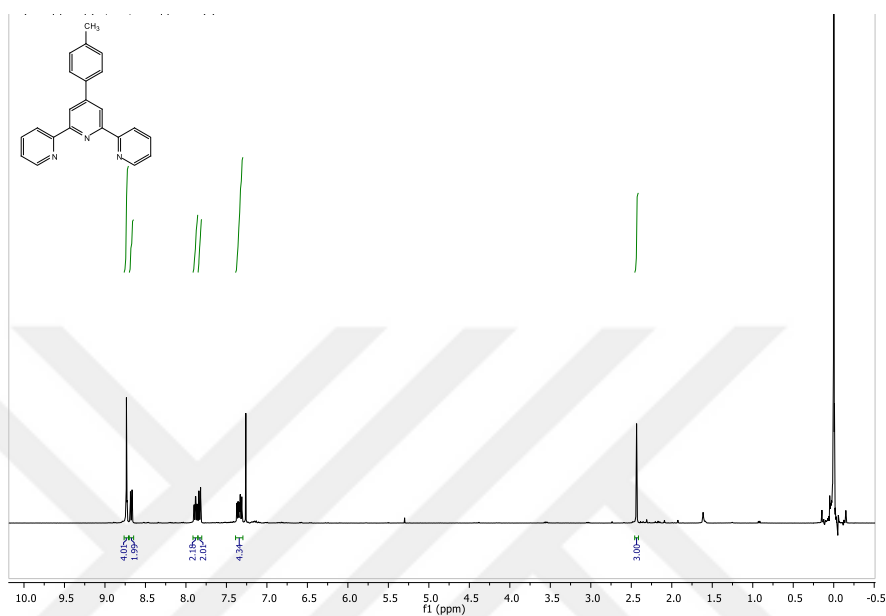
Glycine
(Gly, G)



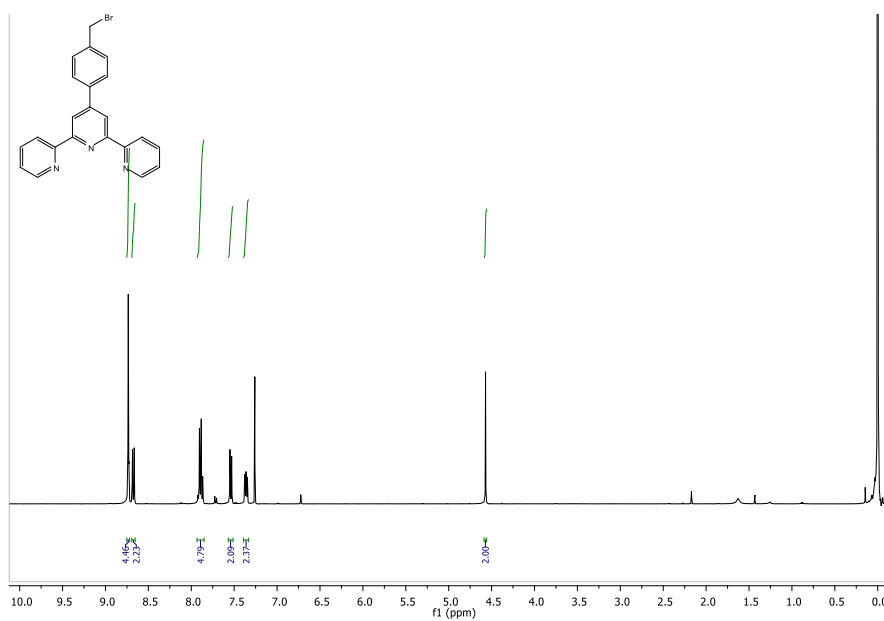
Proline
(Pro, P)

B. NMR Spectra of Chapter 1

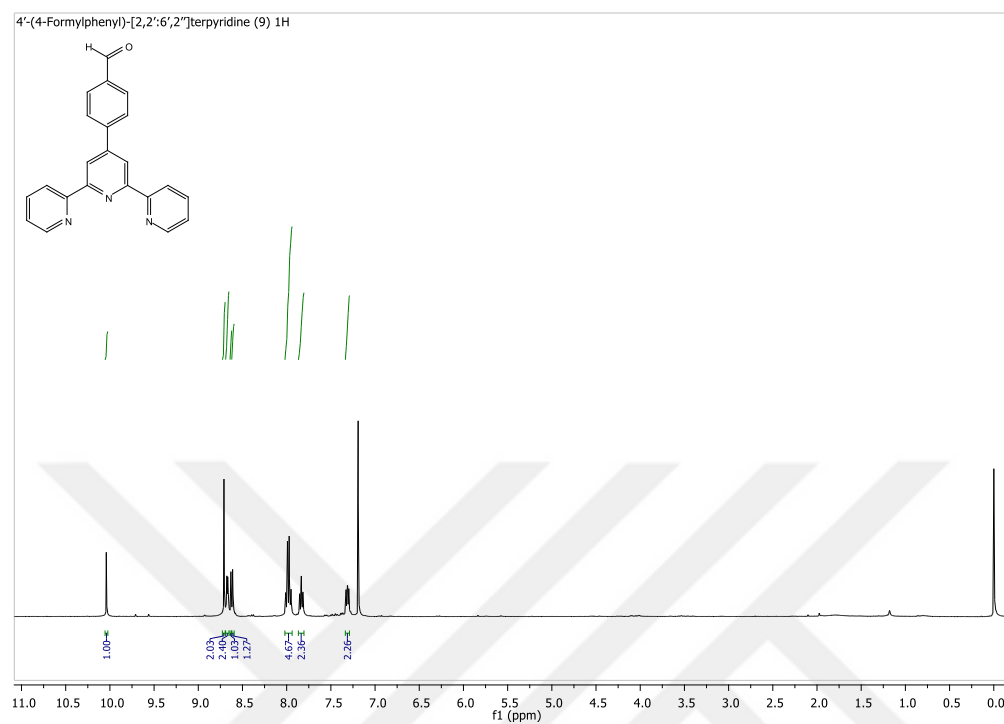
^1H NMR spectrum of 4'-(methylphenyl)-2,2':6',2''-terpyridine (**7**) in CDCl_3 .



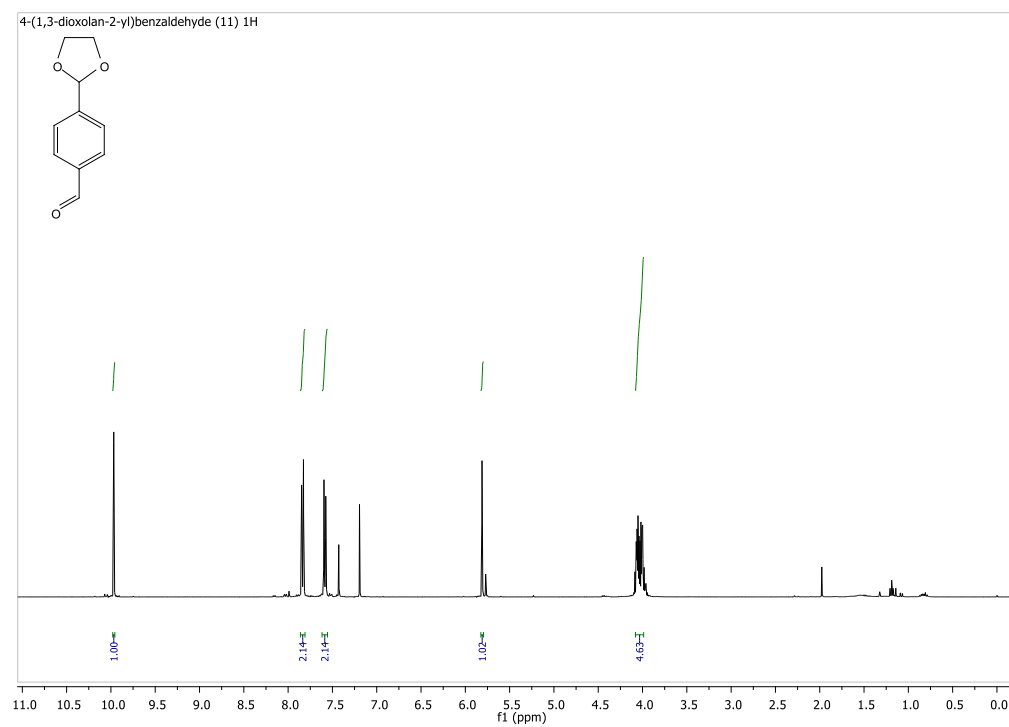
^1H NMR spectrum of 4'-(4-Bromomethylphenyl)-[2,2':6',2'']terpyridine (**8**) in CDCl_3 .



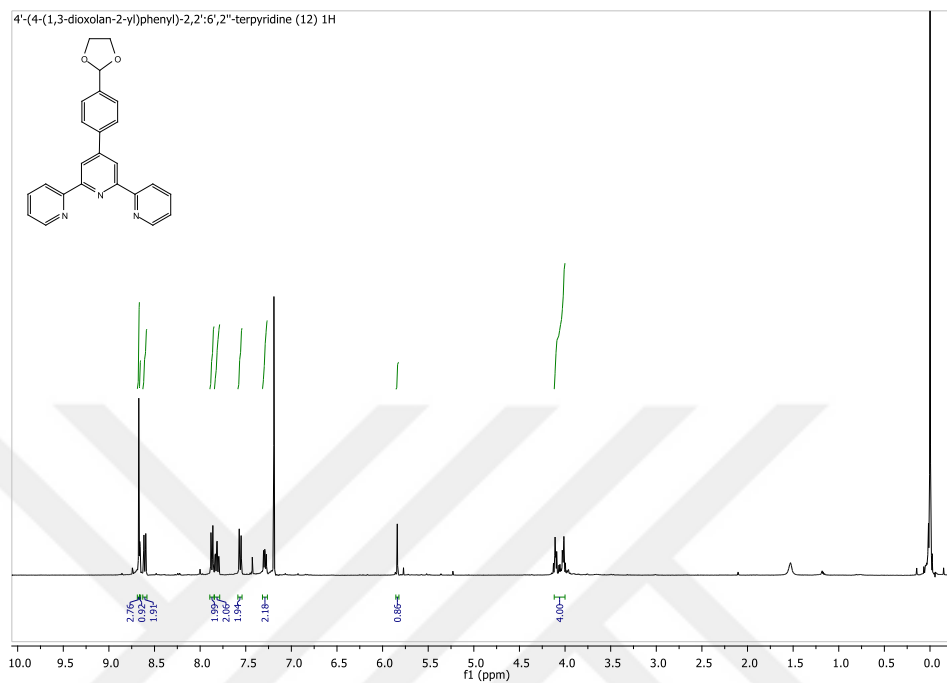
^1H NMR spectrum of 4'-(4-Formylphenyl)-[2,2':6',2'']terpyridine (**9**) in CDCl_3 .



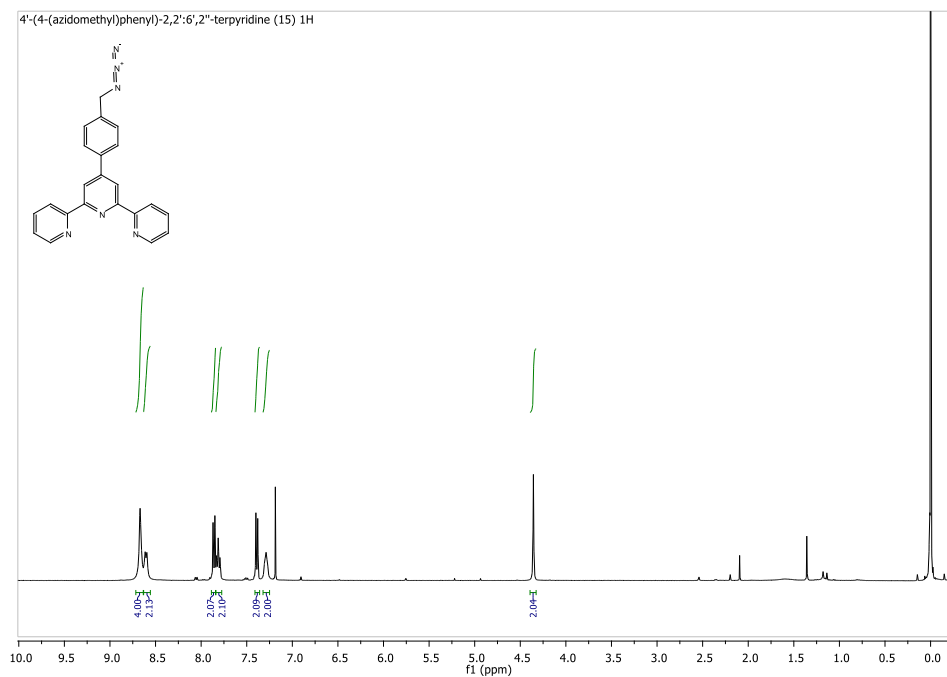
^1H NMR spectrum of 4-(1,3-dioxolan-2-yl)benzaldehyde (**11**) in CDCl_3 .



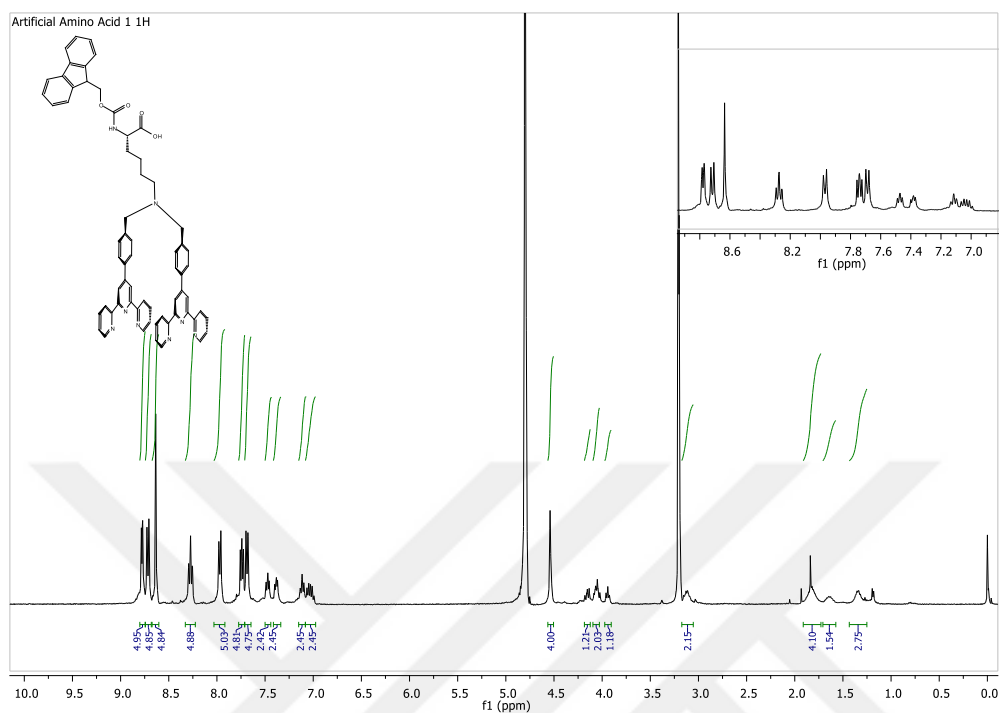
^1H NMR spectrum of 4'-(4-(1,3-dioxolan-2-yl)phenyl)-2,2':6',2''-terpyridine (**12**) in CDCl_3 .



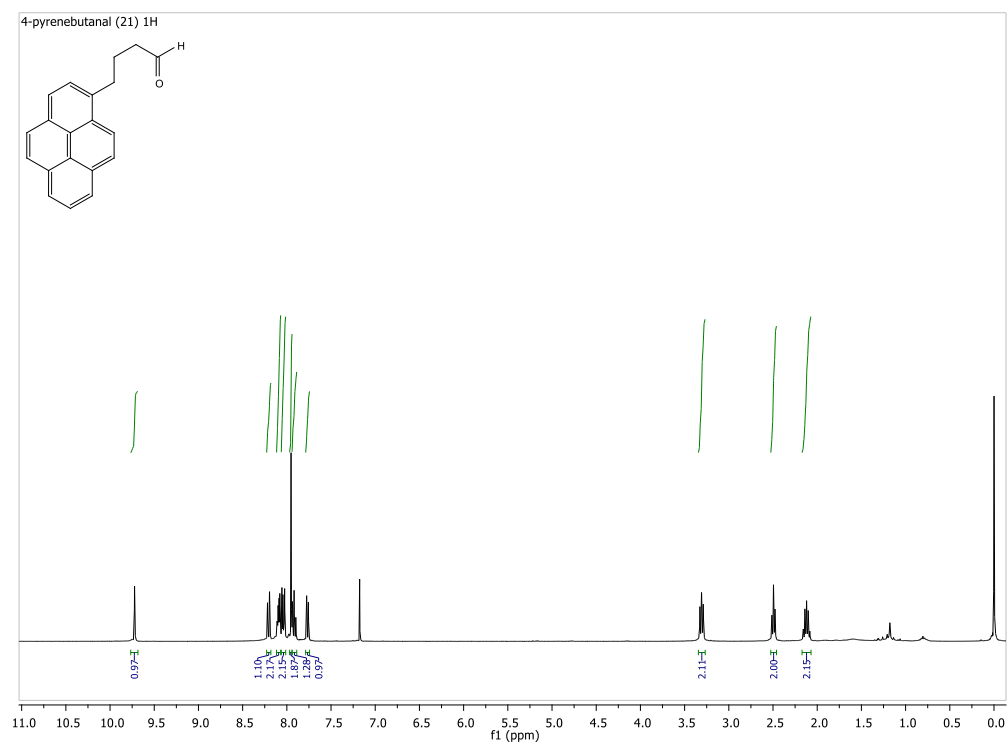
^1H NMR spectrum of 4'-(4-(azidomethyl)phenyl)-2,2':6',2''-terpyridine (**15**) in CDCl_3 .



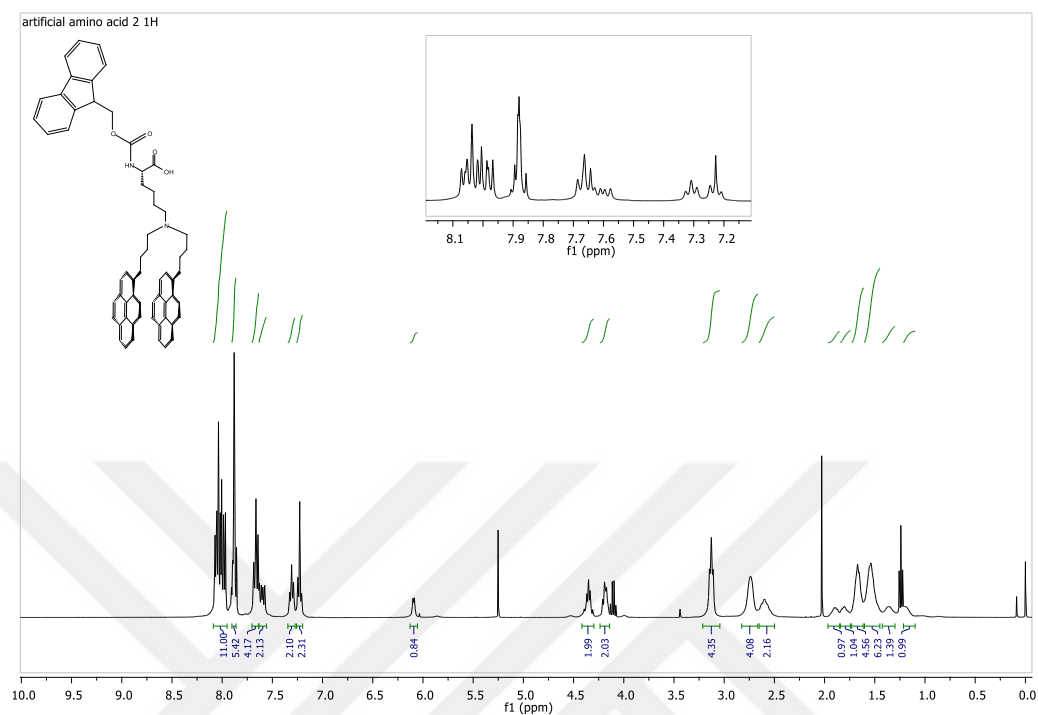
^1H NMR spectrum of artificial amino acid **1** in CD_3OD .



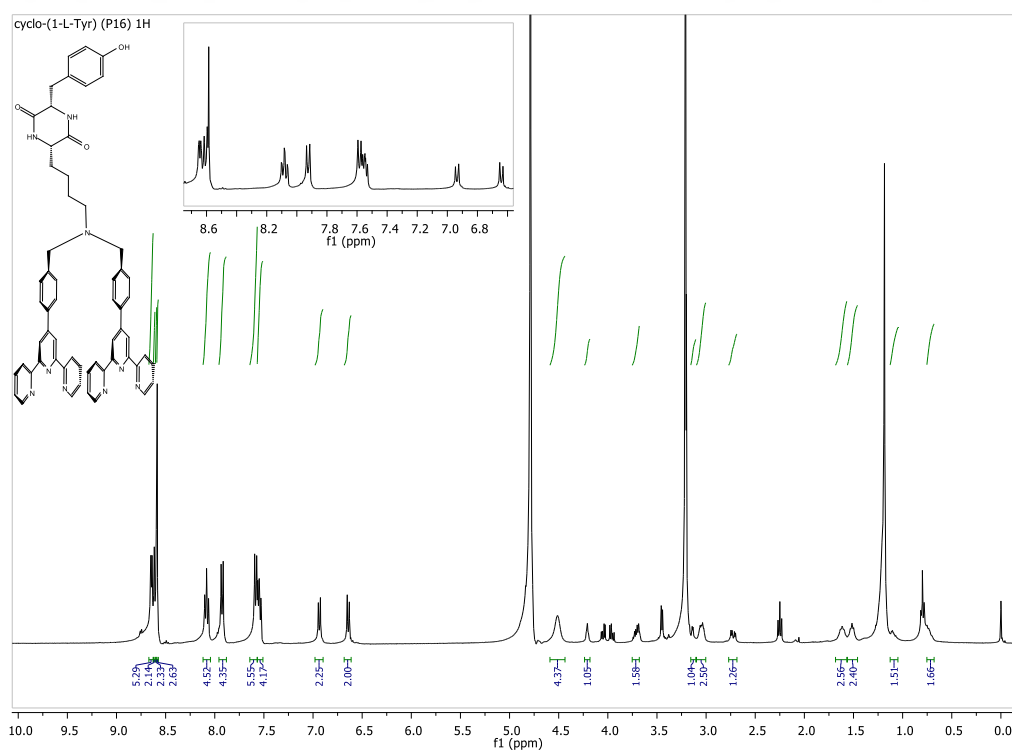
^1H NMR spectrum of 1-pyrenebutanal (**21**) in CDCl_3 .



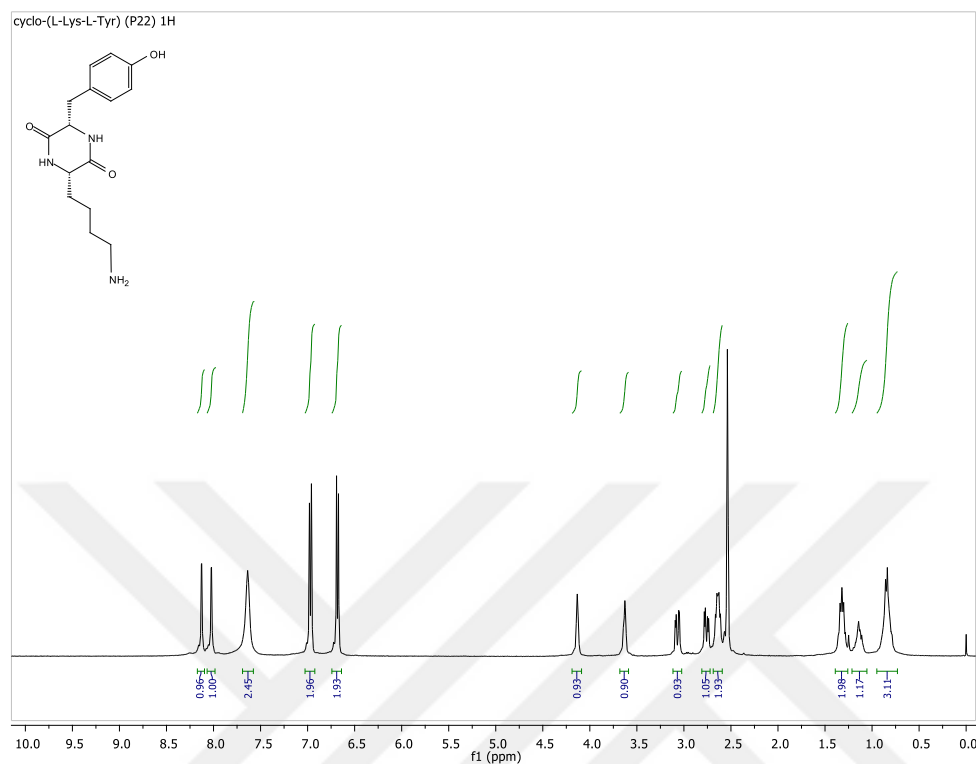
^1H NMR spectrum of artificial amino acid **2** in CDCl_3 .



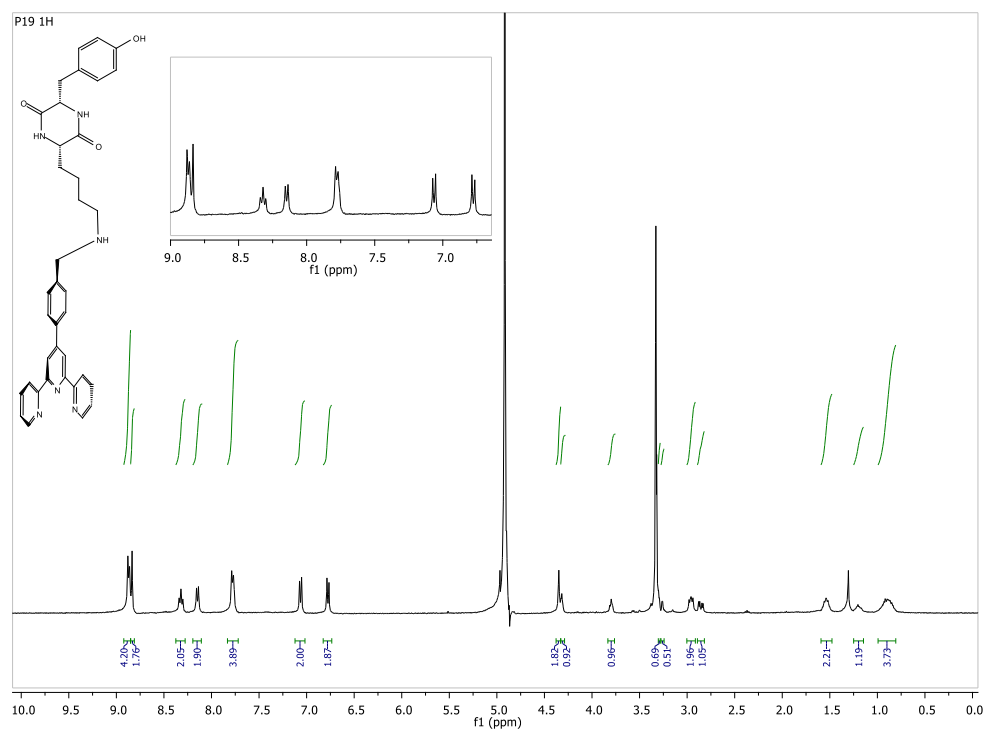
^1H NMR spectrum **P16** in CD_3OD .



^1H NMR spectrum **P22** in DMSO- d_6 .

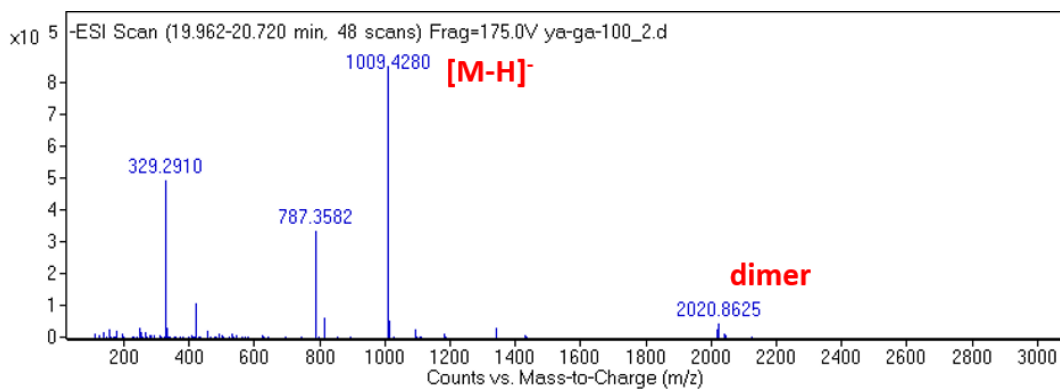


^1H NMR spectrum **P19** in CD_3OD .

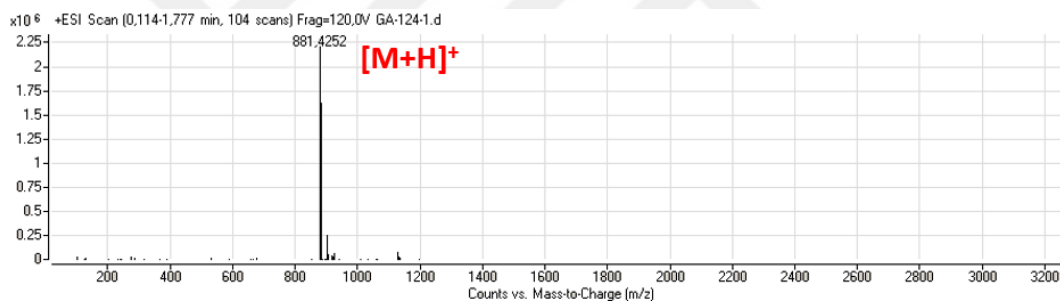


C. Mass Analysis of Artificial Amino Acids

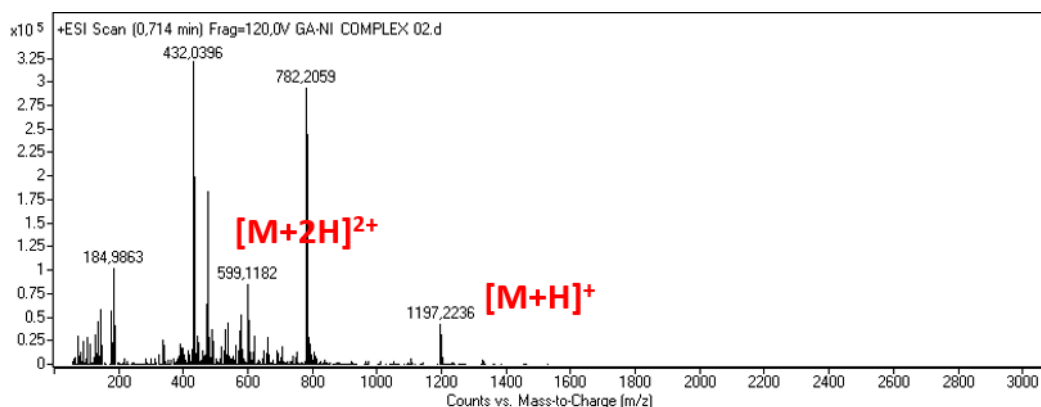
Mass analysis of **1** at negative ionization.



Mass analysis of **2** at positive ionization.

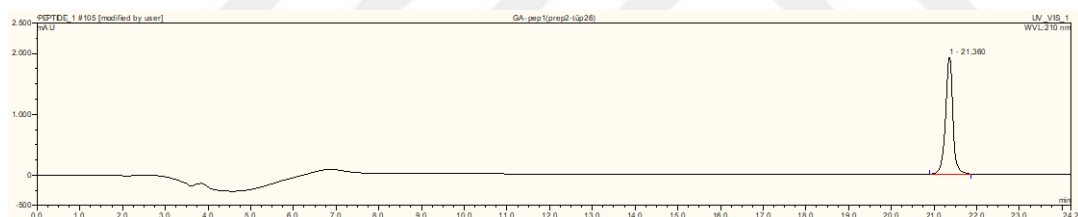
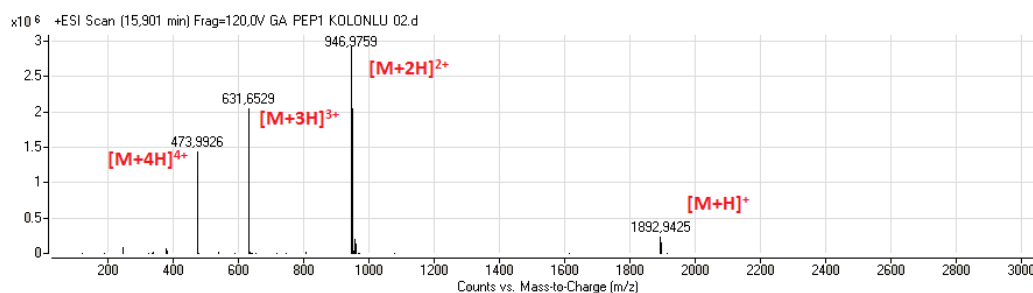


Mass analysis of **3** at positive ionization.



D. HPLC and Mass Analysis of Peptides

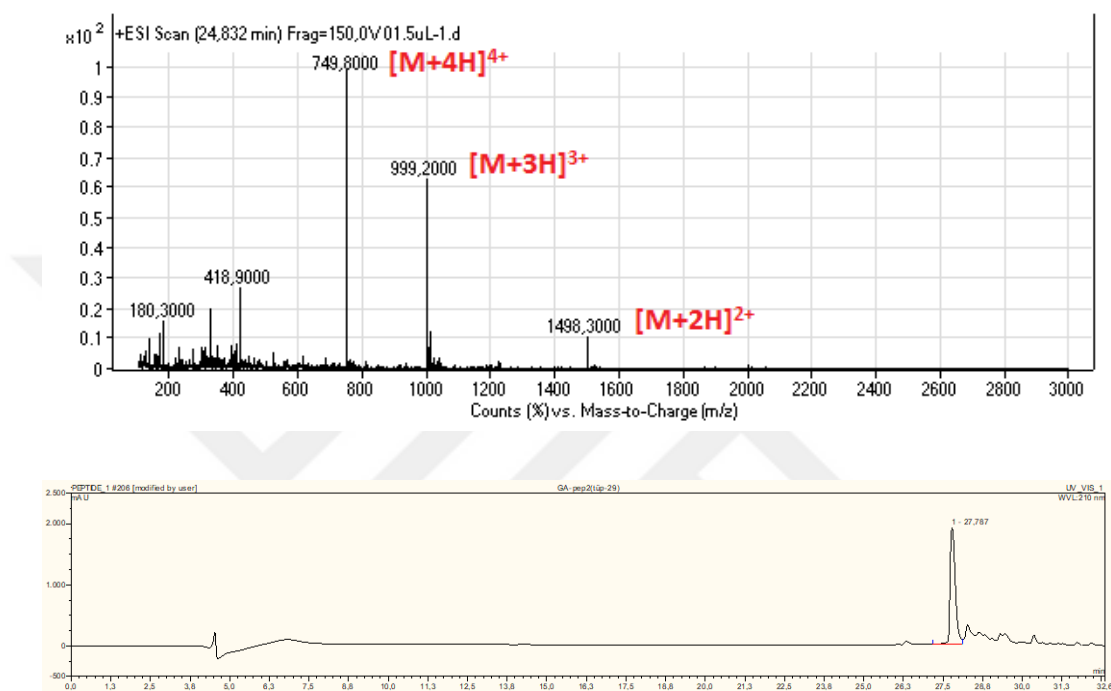
Peptide	Sequence	[M+H] ⁺ calculated	[M+H] ⁺ observed	Retention time (210 nm)
P1	FKFE-1-FKFE	1891.91	1892.94	21.4



Analytical HPLC method: 10% (ACN with 0.08% TFA) to 60% (ACN with 0.08% TFA) in 30 minutes. Flow rate: 0.5 ml/min

Semi-preparative HPLC method: 1% (ACN with 0.08% TFA) for 10 min. then, 1% to 60% (ACN with 0.08% TFA) in 150 minutes. Flow rate: 3.0 ml/min

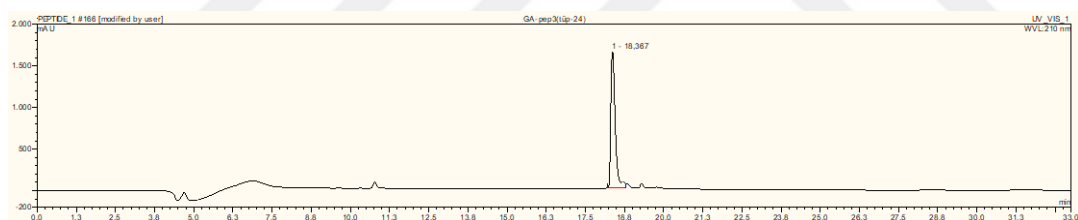
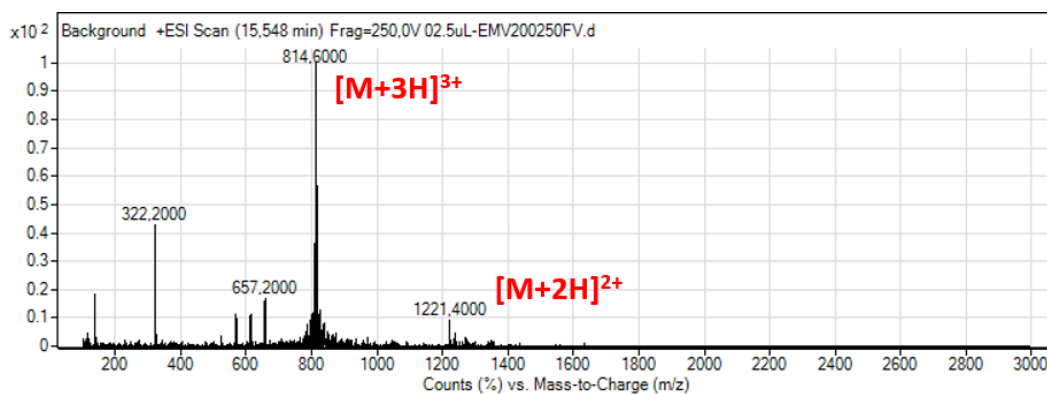
Peptide	Sequence	[M+2H] ²⁺ calculated	[M+2H] ²⁺ observed	Retention time (210 nm)
P2	FKFEFKFE-1-FKFEFKFE	1498.24	1498.30	27.8



Analytical HPLC method: 10% (ACN with 0.08% TFA) to 50% (ACN with 0.08% TFA) in 30 minutes. Flow rate: 0.5 ml/min

Semi-preparative HPLC method: 1% (ACN with 0.08% TFA) for 10 min. then, 1% to 40% (ACN with 0.08% TFA) in 150 minutes. Flow rate: 3.0 ml/min

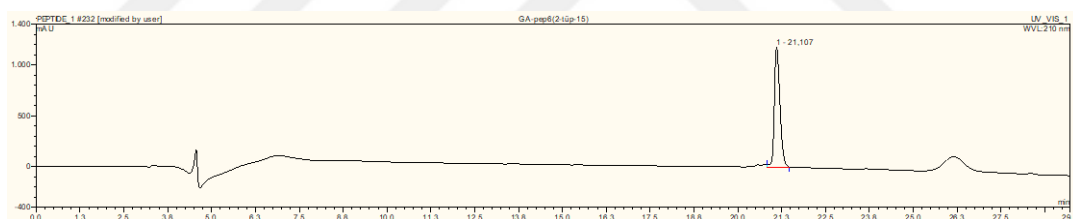
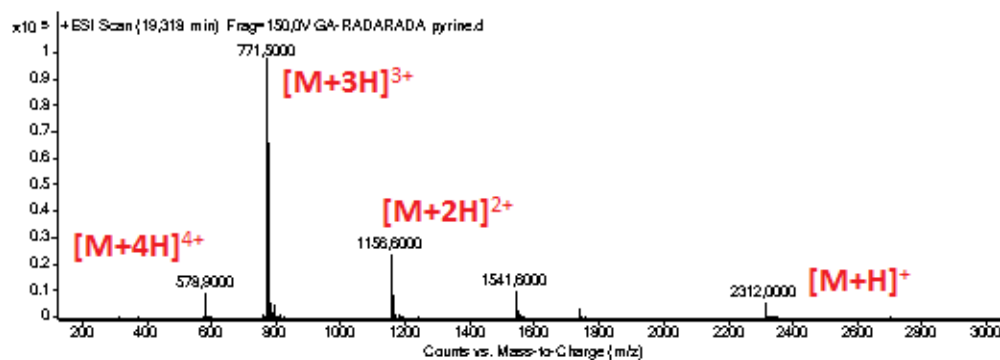
Peptide	Sequence	$[M+2H]^{2+}$ calculated	$[M+2H]^{2+}$ observed	Retention time (210 nm)
P3	RADARADA-1- RADARADA-NH ₂	1221.09	1221.40	18.4



Analytical HPLC method: 10% (ACN with 0.08% TFA) to 50% (ACN with 0.08% TFA) in 30 minutes. Flow rate: 0.5 ml/min

Semi-preparative HPLC method: 1% (ACN with 0.08% TFA) for 10 min. then, 1% to 40% (ACN with 0.08% TFA) in 150 minutes. Flow rate: 3.0 ml/min

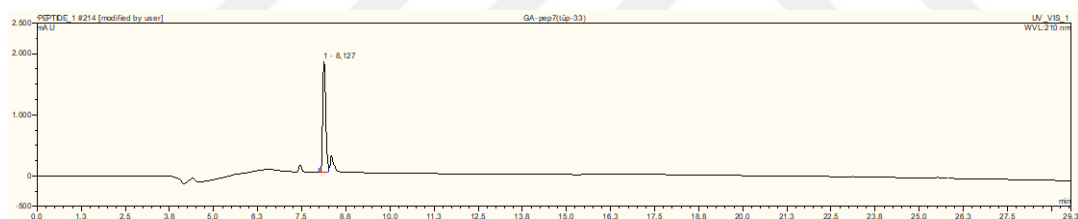
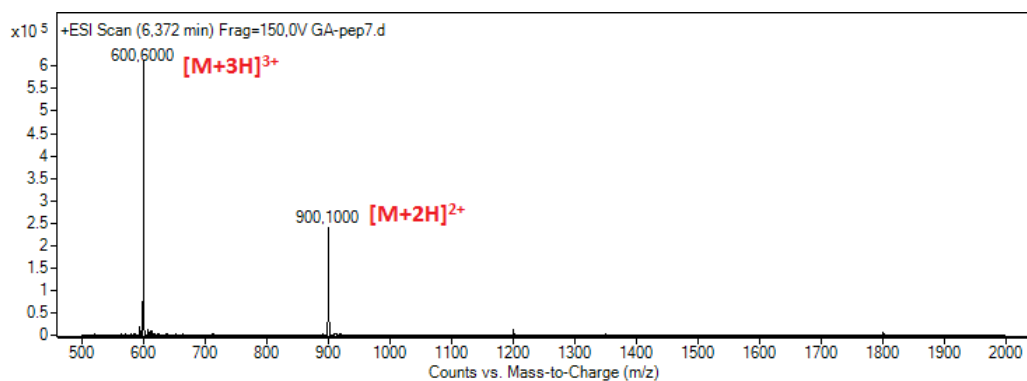
Peptide	Sequence	[M+H] ⁺ calculated	[M+H] ⁺ observed	Retention time (210 nm)
P4	RADARADA-2- RADARADA-NH ₂	2311.17	2312.00	21.1



Analytical HPLC method: 10% (ACN with 0.08% TFA) to 90% (ACN with 0.08% TFA) in 30 minutes. Flow rate: 0.5 ml/min

Semi-preparative HPLC method: 1% (ACN with 0.08% TFA) for 10 min. then, 1% to 60% (ACN with 0.08% TFA) in 150 minutes. Flow rate: 3.0 ml/min

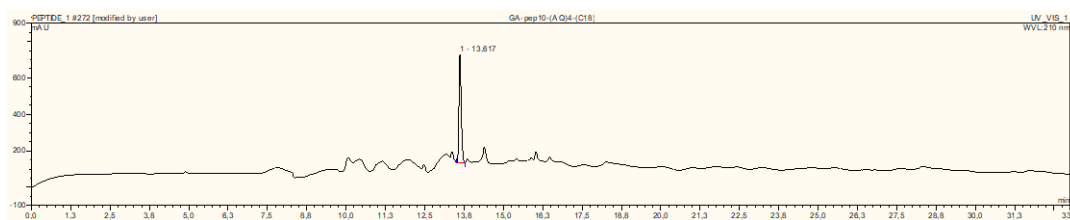
Peptide	Sequence	$[M+2H]^{2+}$ calculated	$[M+2H]^{2+}$ observed	Retention time (210 nm)
P6	RADARADAKRADARADA- NH ₂	900.46	900.10	8.1



Analytical HPLC method: 10% (ACN with 0.08% TFA) to 90% (ACN with 0.08% TFA) in 30 minutes. Flow rate: 0.5 ml/min

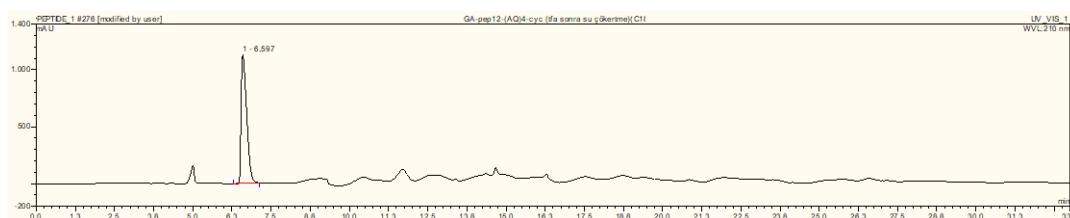
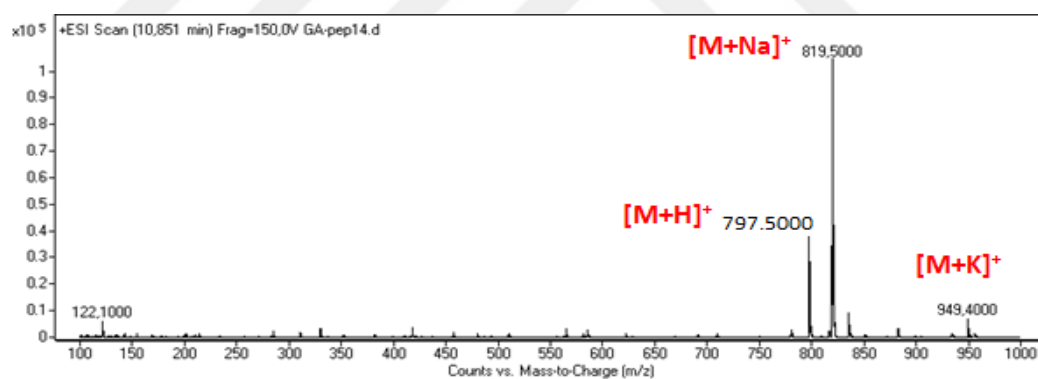
Semi-preparative HPLC method: 1% (ACN with 0.08% TFA) for 10 min. then, 1% to 25% (ACN with 0.08% TFA) in 120 minutes. Flow rate: 3.0 ml/min

L-P7: HPLC chromatogram (retention time: 13.6)



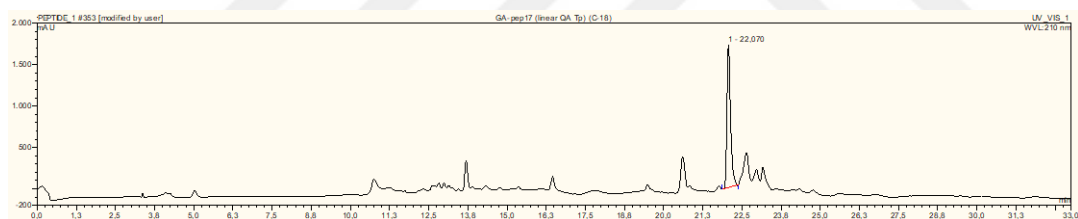
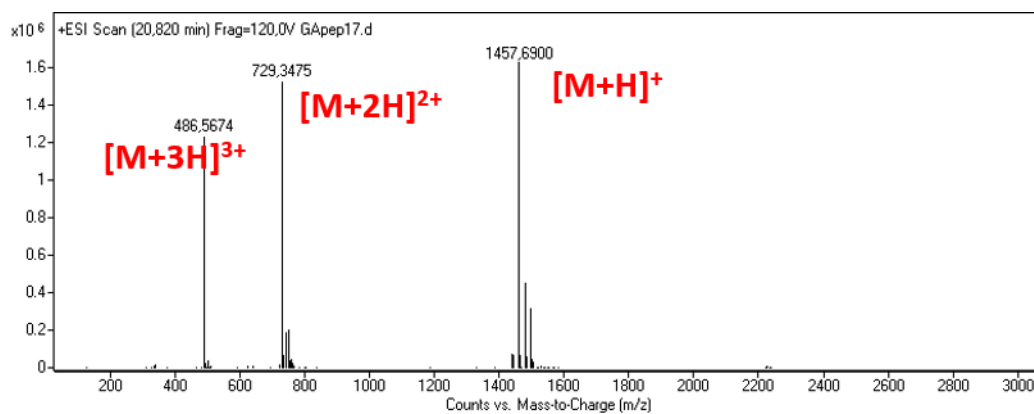
Analytical HPLC method: 1% (ACN with 0.08% TFA) to 50% (ACN with 0.08% TFA) in 30 minutes. Flow rate: 0.5 ml/min

Peptide	Sequence	[M+H] ⁺ calculated	[M+H] ⁺ observed	Retention time (210 nm)
P7	Cyclo-QaQaQaQa	797.38	797.50	6.6



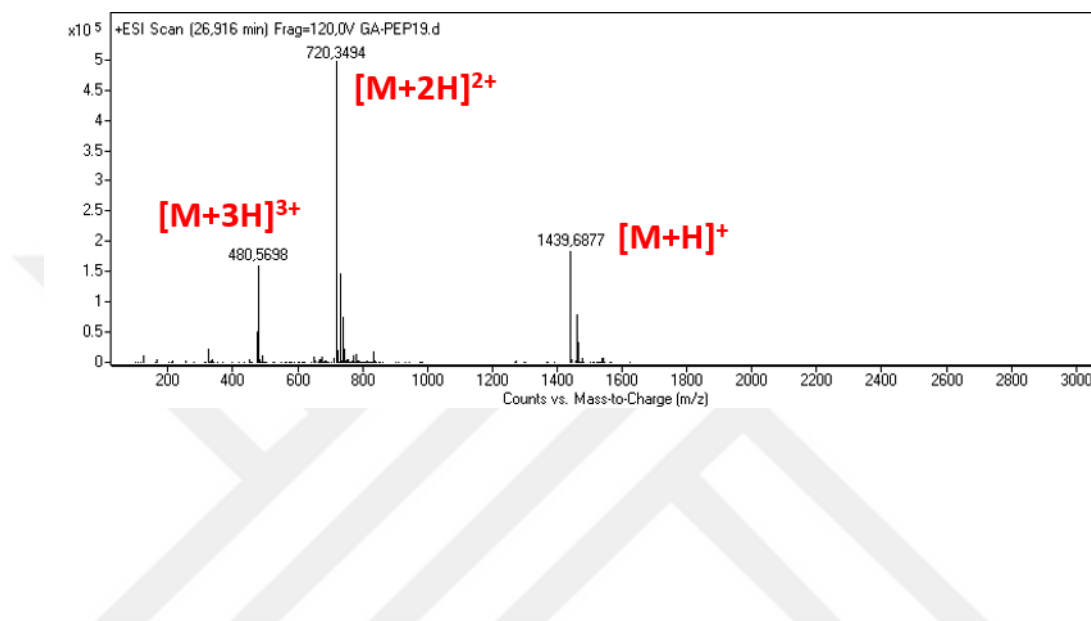
Analytical HPLC method: 1% (ACN with 0.08% TFA) to 50% (ACN with 0.08% TFA) in 30 minutes. Flow rate: 0.5 ml/min

Peptide	Sequence	[M+H] ⁺ calculated	[M+H] ⁺ observed	Retention time (210 nm)
L-P8	QaQa-1-aQa	1457.68	1457.69	22.1

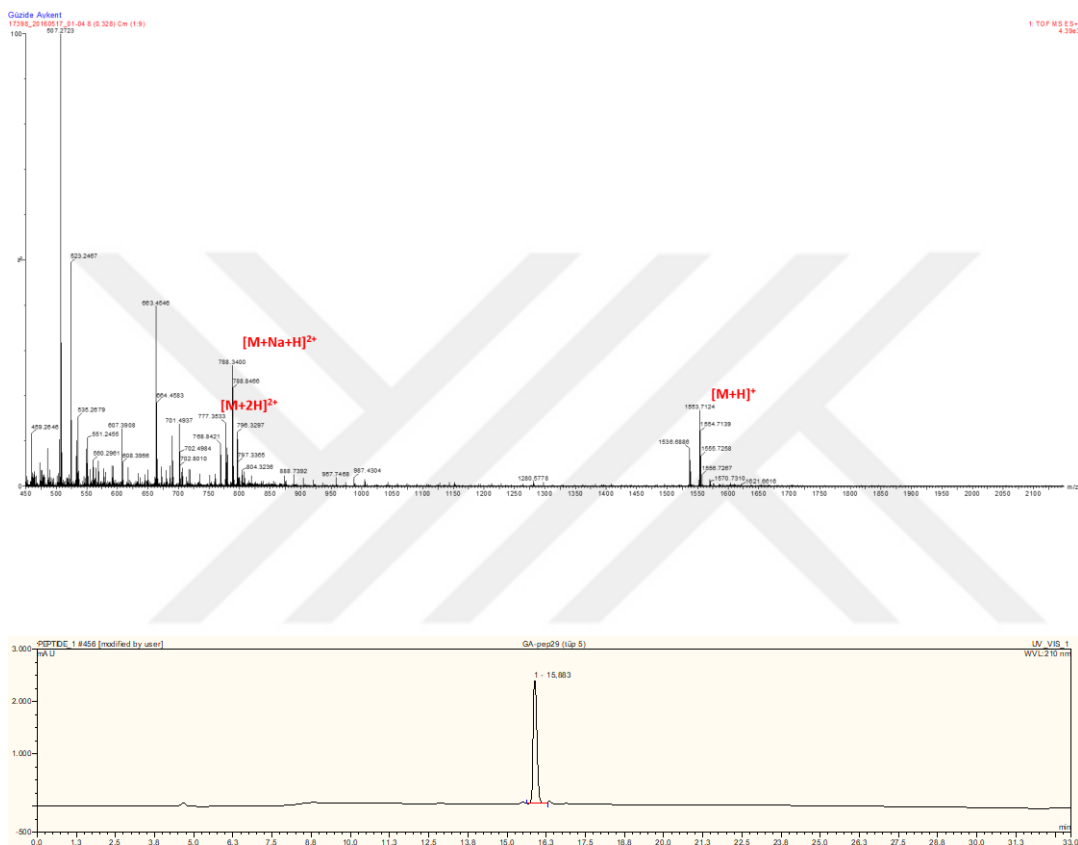


Analytical HPLC method: 1% (ACN with 0.08% TFA) to 50% (ACN with 0.08% TFA) in 30 minutes. Flow rate: 0.5 ml/min

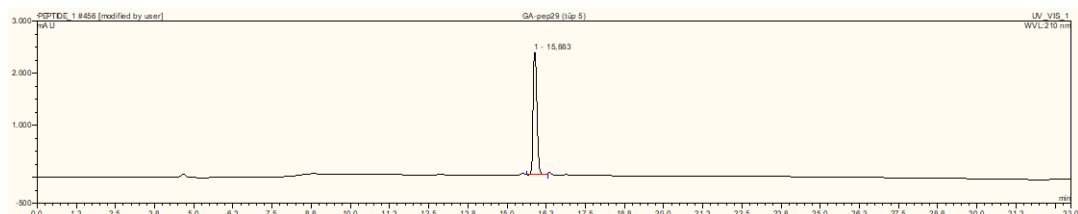
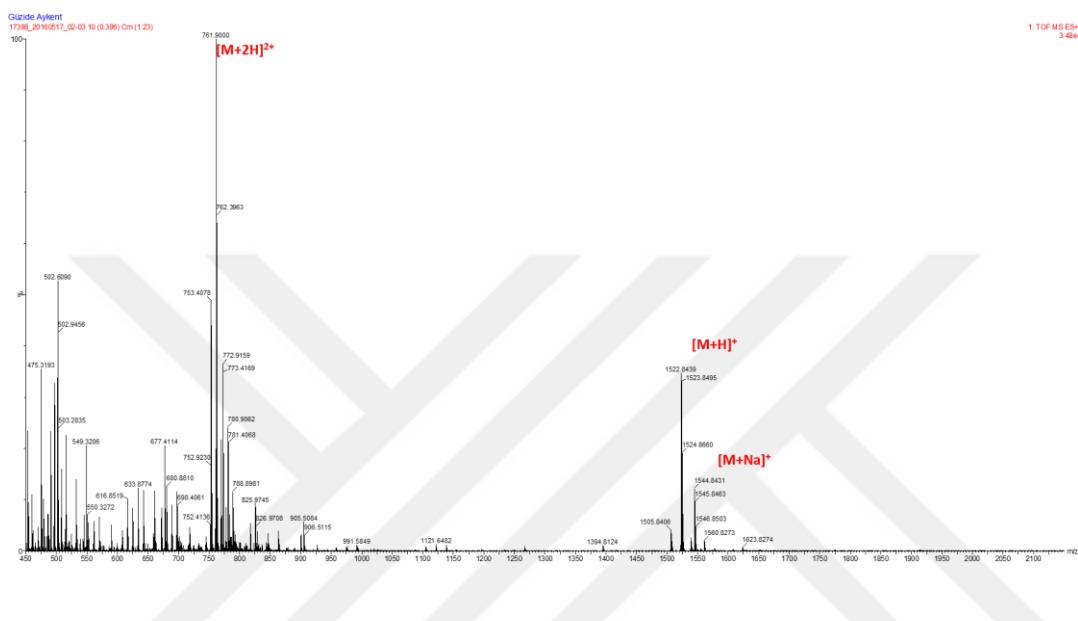
Peptide	Sequence	$[M+H]^+$ calculated	$[M+H]^+$ observed	Retention time (210 nm)
P8	Cyclo-QaQa-1-aQa	1439.67	1439.69	-



Peptide	Sequence	[M+H] ⁺ calculated	[M+H] ⁺ observed	Retention time (210 nm)
P9	QQRFEWEFEQQ-NH ₂	1553.71	1553.71	15.9



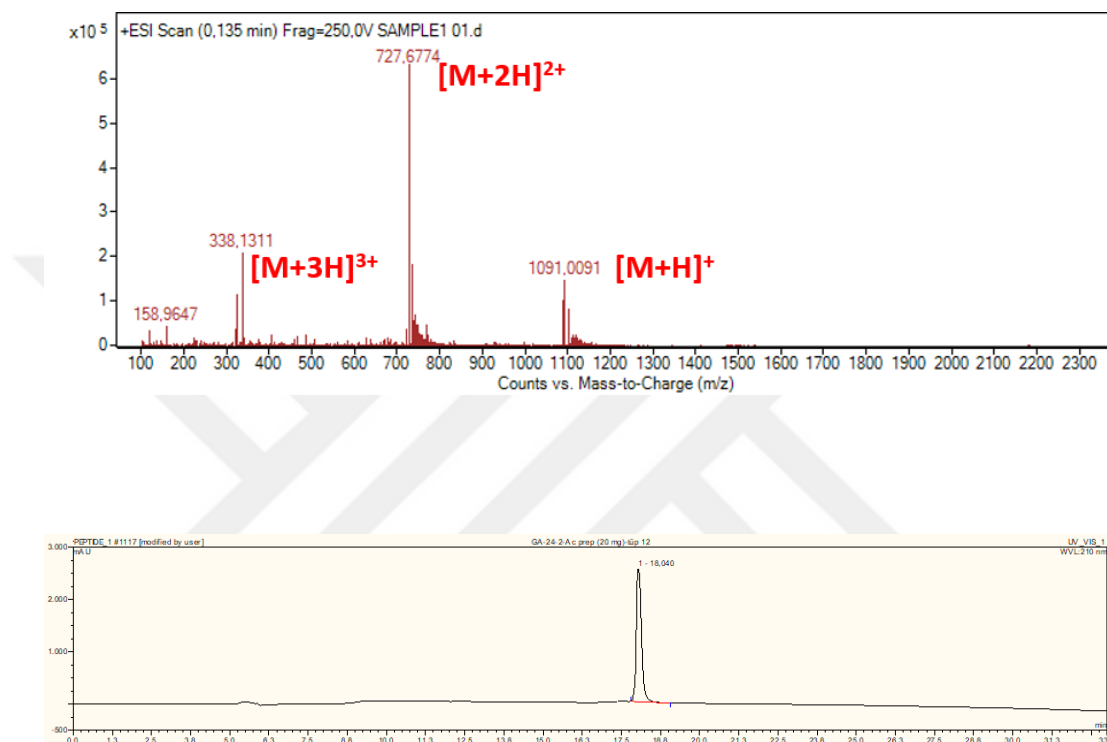
Peptide	Sequence	[M+H] ⁺ calculated	[M+H] ⁺ observed	Retention time (210 nm)
P11	QKFKWKFKQQ-NH ₂	1522.86	1522.84	15.9



Analytical HPLC method: 1% (ACN with 0.08% TFA) to 75% (ACN with 0.08% TFA) in 30 minutes. Flow rate: 0.5 ml/min

Semi-preparative HPLC method: 1% (ACN with 0.08% TFA) for 10 min. then, 1% to 40% (ACN with 0.08% TFA) in 150 minutes. Flow rate: 3.0 ml/min

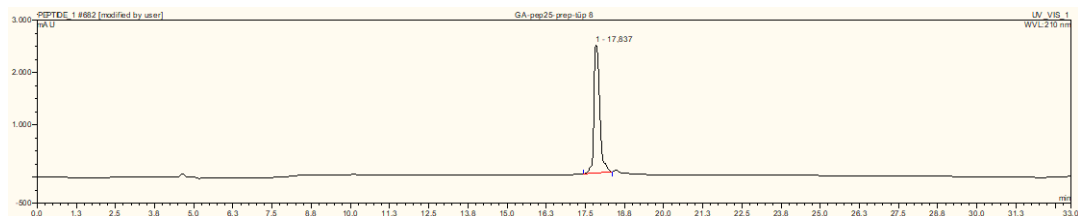
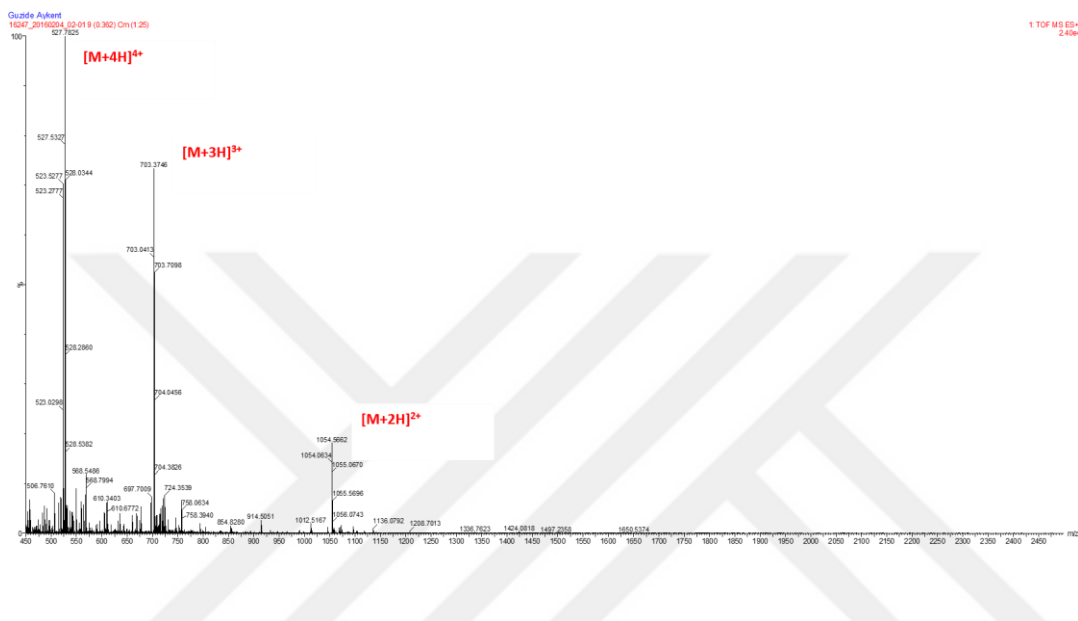
Peptide	Sequence	$[M+2H]^{2+}$ calculated	$[M+2H]^{2+}$ observed	Retention time (210 nm)
P12	Ac-QQRFE-1-EFEQQ-NH ₂	1091.19	1091.01	18.0



Analytical HPLC method: 1% (ACN with 0.08% TFA) to 90% (ACN with 0.08% TFA) in 30 minutes. Flow rate: 0.5 ml/min

Semi-preparative HPLC method: 1% (ACN with 0.08% TFA) for 10 min. then, 1% to 40% (ACN with 0.08% TFA) in 150 minutes. Flow rate: 2.0 ml/min

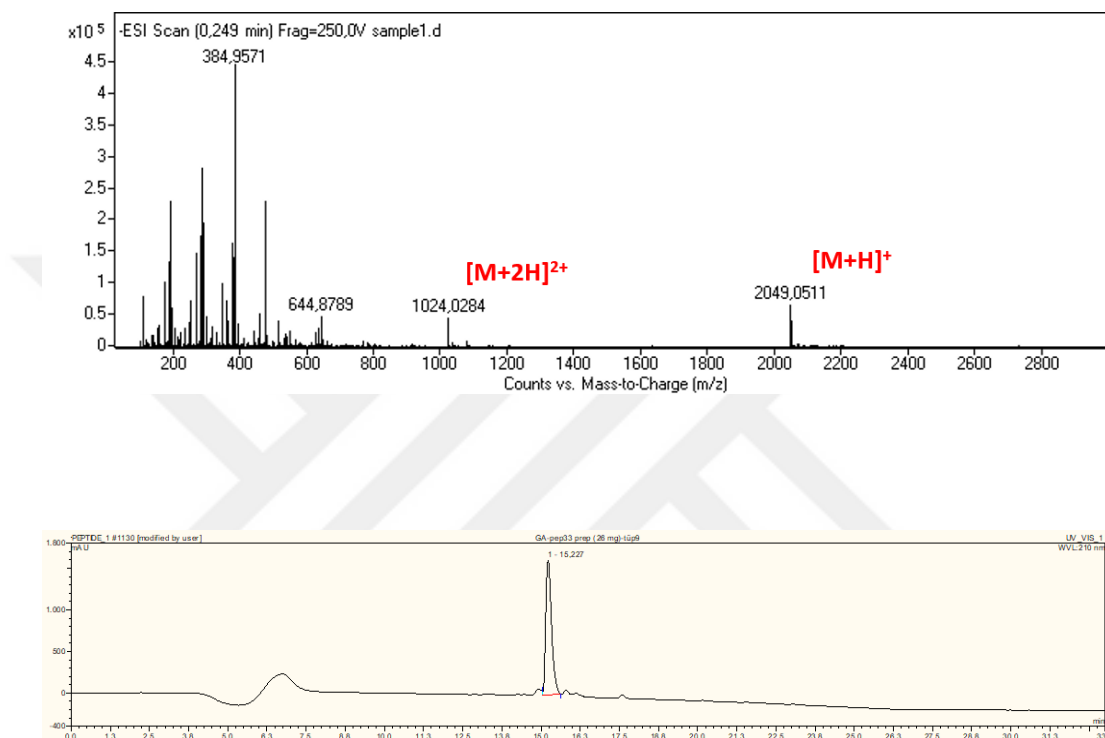
Peptide	Sequence	[M+H] ⁺ calculated	[M+H] ⁺ observed	Retention time (210 nm)
P13	QQKFK-1-KFKQQ-NH ₂	1054.75	1054.57	17.8



Analytical HPLC method: 1% (ACN with 0.08% TFA) to 75% (ACN with 0.08% TFA) in 30 minutes. Flow rate: 0.5 ml/min

Semi-preparative HPLC method: 1% (ACN with 0.08% TFA) for 10 min. then, 1% to 40% (ACN with 0.08% TFA) in 150 minutes. Flow rate: 3.0 ml/min

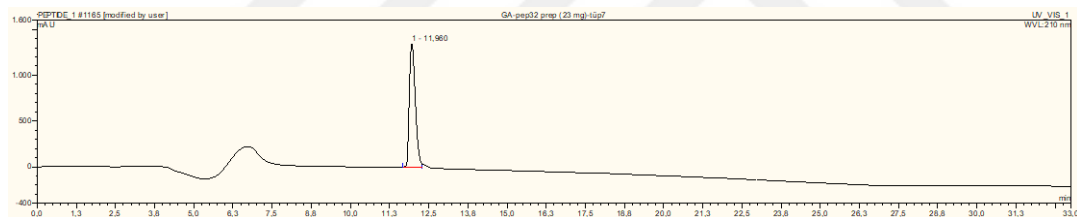
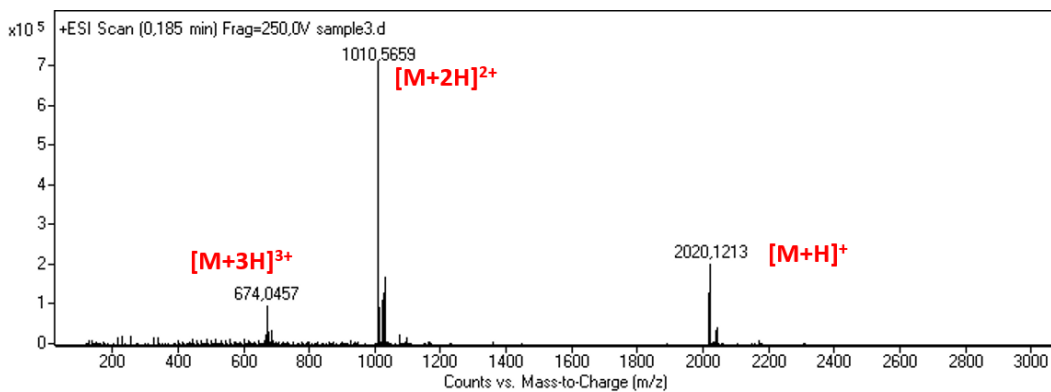
Peptide	Sequence	[M-H] ⁻ calculated	[M-H] ⁻ observed	Retention time (210 nm)
P14	Ac-QQRFE-2-EFEQQ-NH ₂	2049.31	2049.05	15.2



Analytical HPLC method: 40% (ACN with 0.08% TFA) to 90% (ACN with 0.08% TFA) in 20 minutes then, 90% (ACN with 0.08% TFA) for 10 minutes. Flow rate: 0.5 ml/min

Semi-preparative HPLC method: 1% to 30% (ACN with 0.08% TFA) for 20 min. then, 30% to 80% (ACN with 0.08% TFA) in 90 minutes. Flow rate: 2.0 ml/min

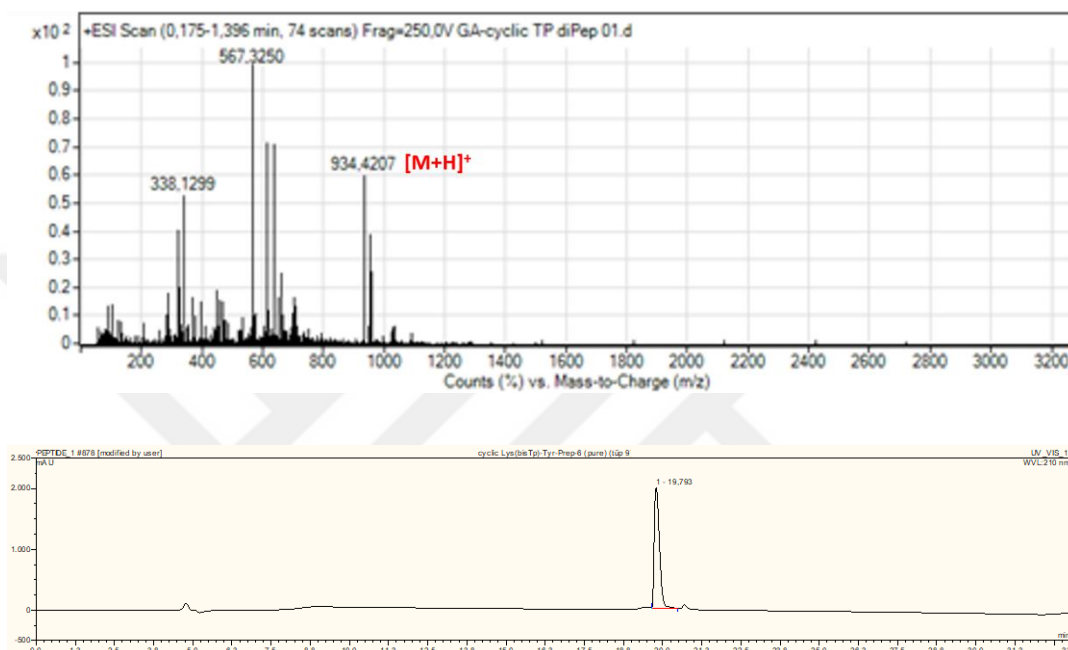
Peptide	Sequence	$[M+H]^+$ calculated	$[M+H]^+$ observed	Retention time (210 nm)
P15	Ac-QQKFK-2-KFKQQ-NH ₂	2020.48	2020.12	12.0



Analytical HPLC method: 40% (ACN with 0.08% TFA) to 90% (ACN with 0.08% TFA) in 20 minutes then, 90% (ACN with 0.08% TFA) for 10 minutes. Flow rate: 0.5 ml/min

Semi-preparative HPLC method: 1% to 30% (ACN with 0.08% TFA) for 20 min. then, 30% to 70% (ACN with 0.08% TFA) in 120 minutes. Flow rate: 2.0 ml/min

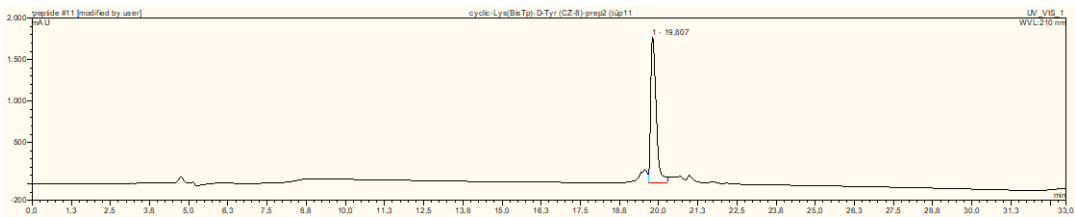
Peptide	Sequence	[M+H] ⁺ calculated	[M+H] ⁺ observed	Retention time (210 nm)
P16	Cyclo-(L-1-L-Tyr)	934.41	934.42	19.8



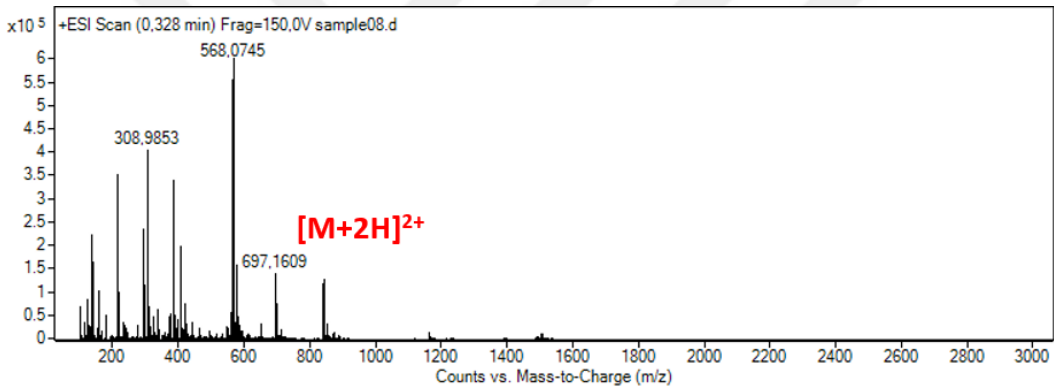
Analytical HPLC method: 1% (ACN with 0.08% TFA) to 75% (ACN with 0.08% TFA) in 30 minutes. Flow rate: 0.5 ml/min

Semi-preparative HPLC method: 1% (ACN with 0.08% TFA) for 10 min. then, 1% to 50% (ACN with 0.08% TFA) in 90 minutes. Flow rate: 2.0 ml/min

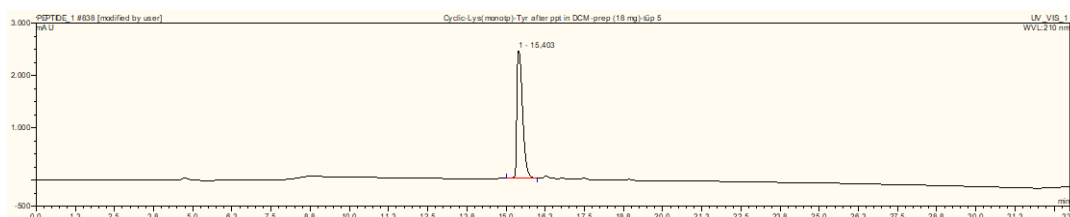
P17: HPLC chromatogram (retention time: 19.8; same method as above.



Peptide	Sequence	$[M+2H]^{2+}$ calculated	$[M+2H]^{2+}$ observed	Retention time (210 nm)
P18	Cyclo-(L-4-L-Tyr)	697.64	697.16	



Peptide	Sequence	[M+H] ⁺ calculated	[M+H] ⁺ observed	Retention time (210 nm)
P19	Cyclo-(L-19-L-Tyr)			15.4

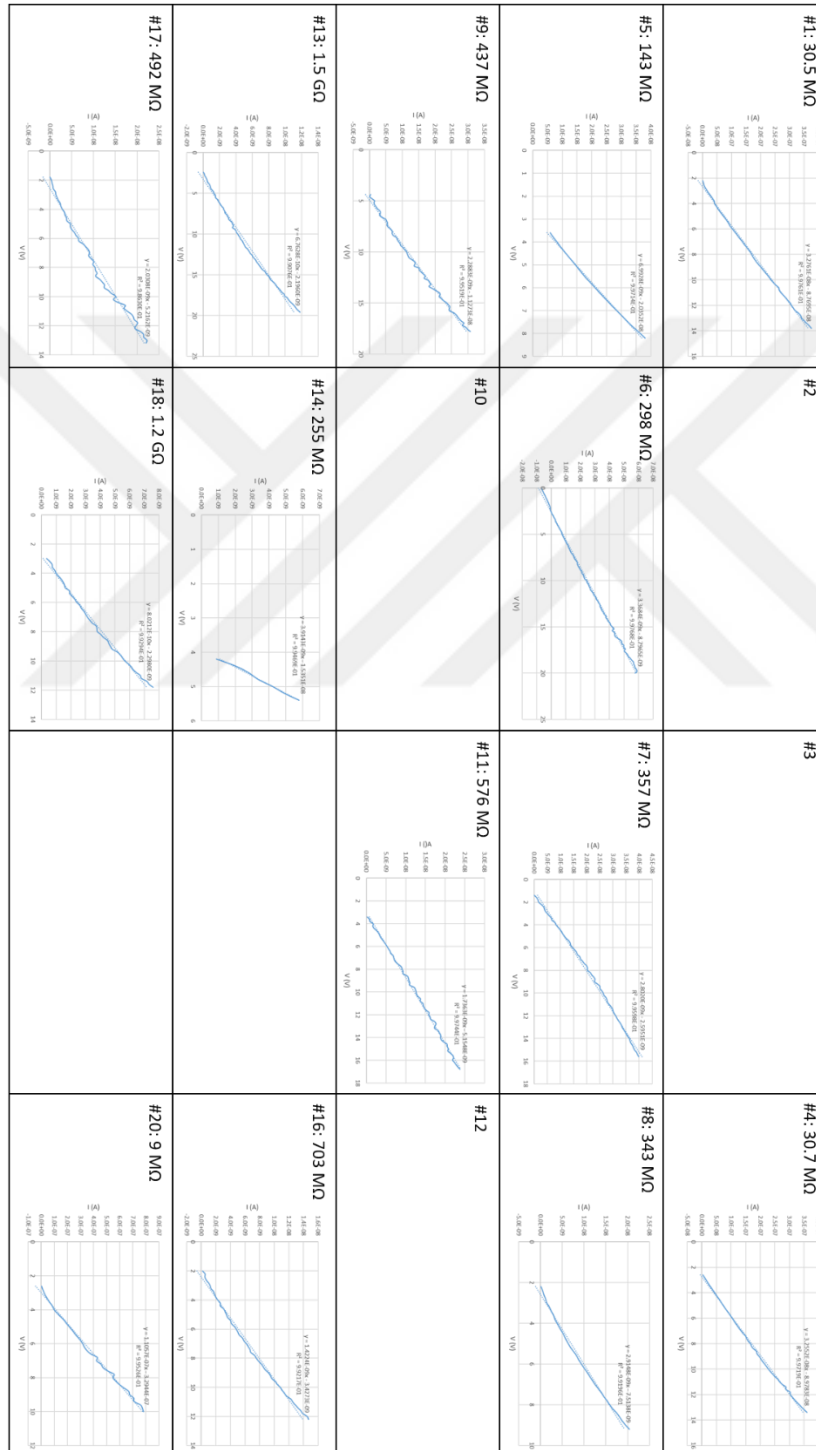


Analytical HPLC method: 1% (ACN with 0.08% TFA) to 90% (ACN with 0.08% TFA) in 30 minutes. Flow rate: 0.5 ml/min

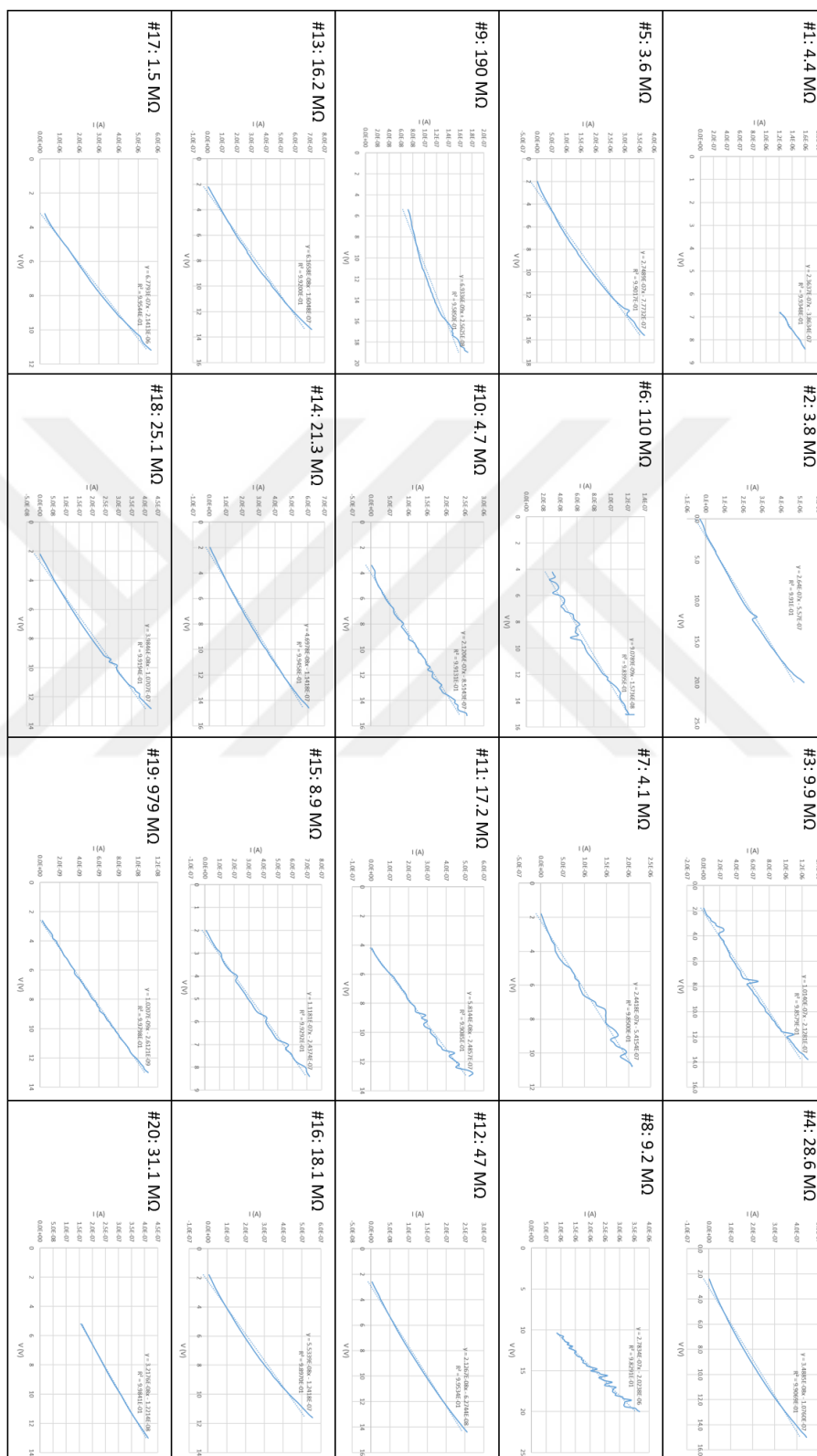
Semi-preparative HPLC method: 1% (ACN with 0.08% TFA) for 10 min. then, 1% to 60% (ACN with 0.08% TFA) in 90 minutes. Flow rate: 2.0 ml/min

E. I-V Curves of Peptide Gels

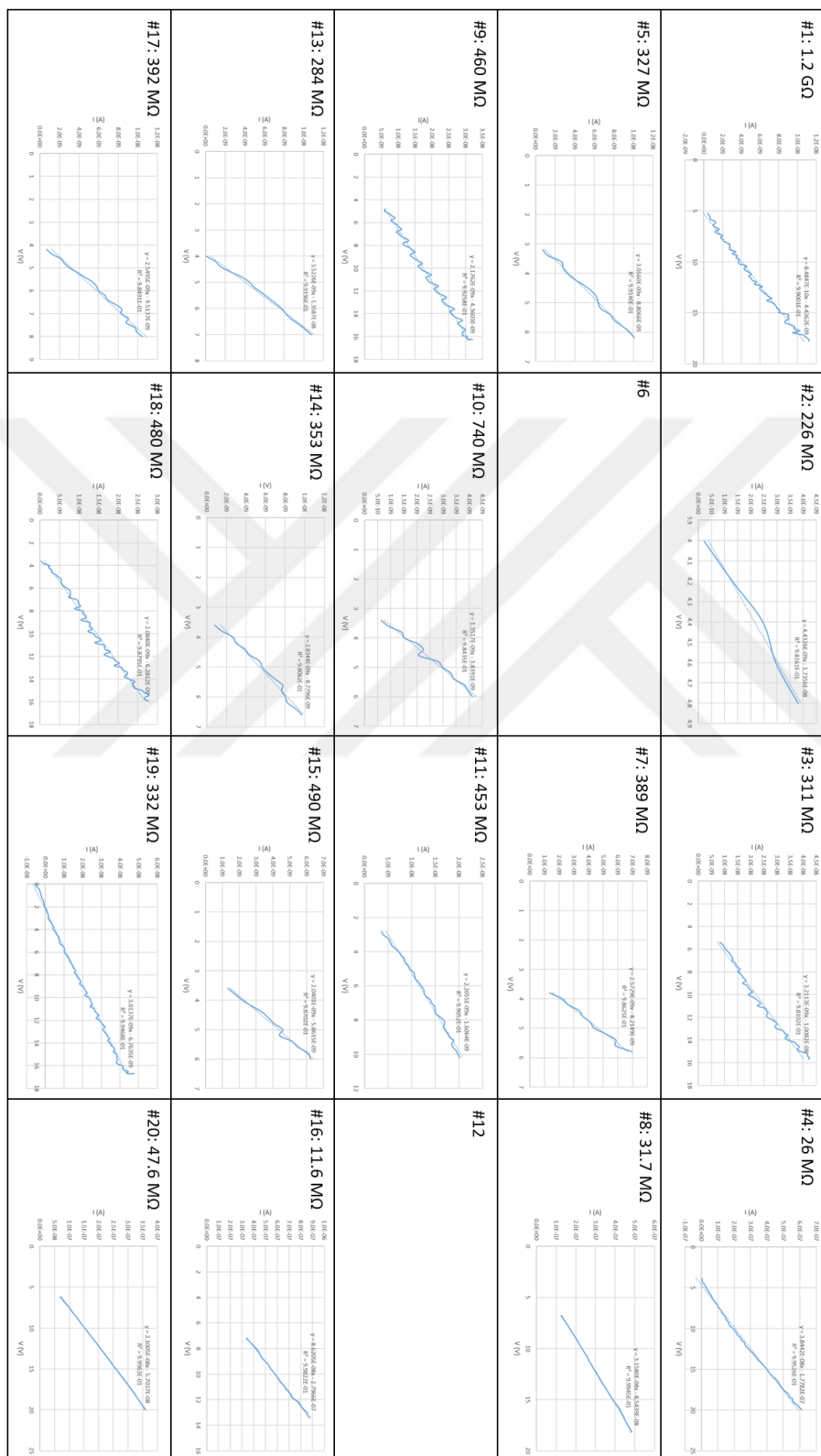
I-V curve map of **P9-P11** gel. The linear regimes are only shown in graphs.



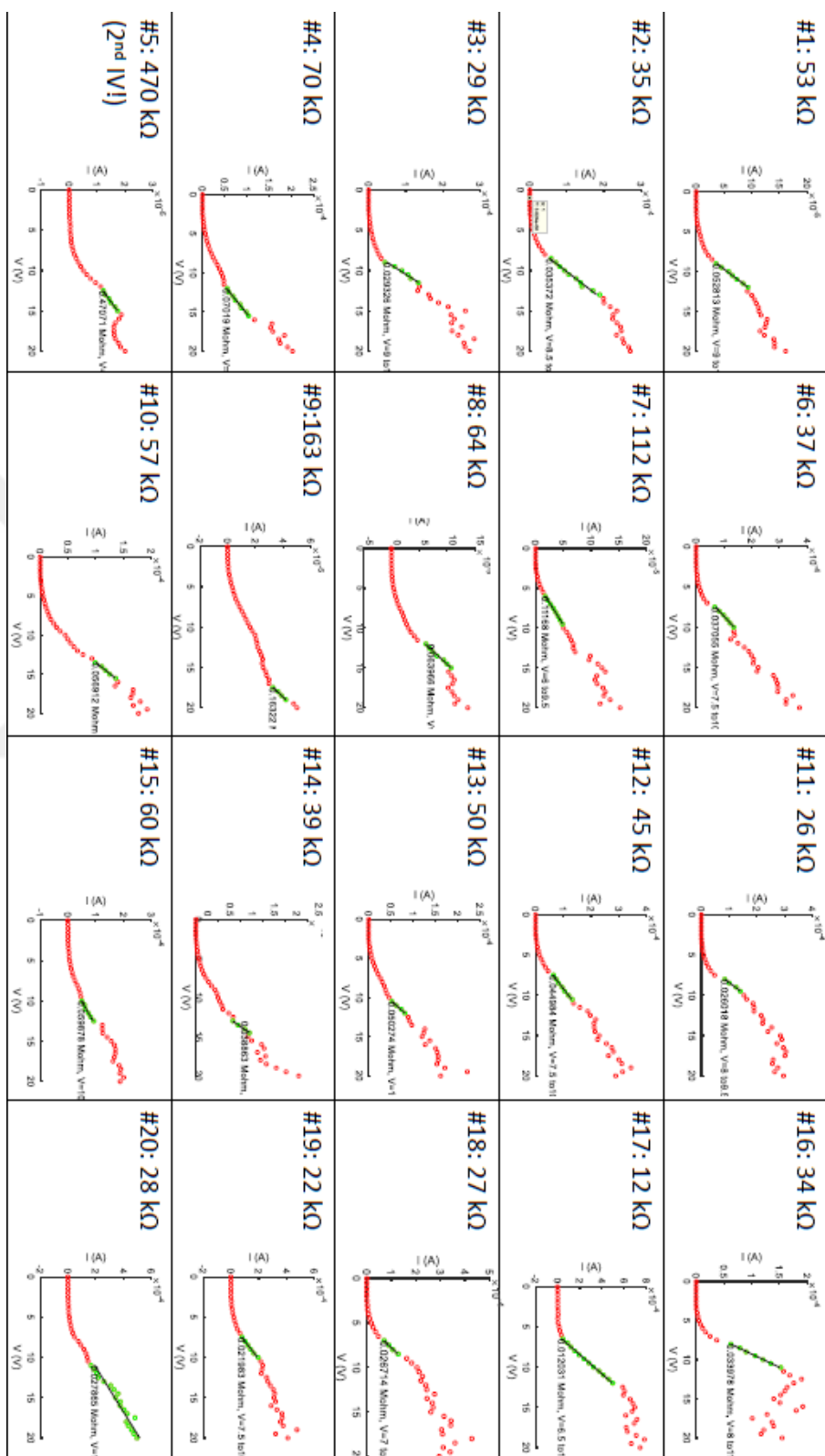
I-V curve map of **P12-P13** gel. The linear regimes are only shown in graphs.



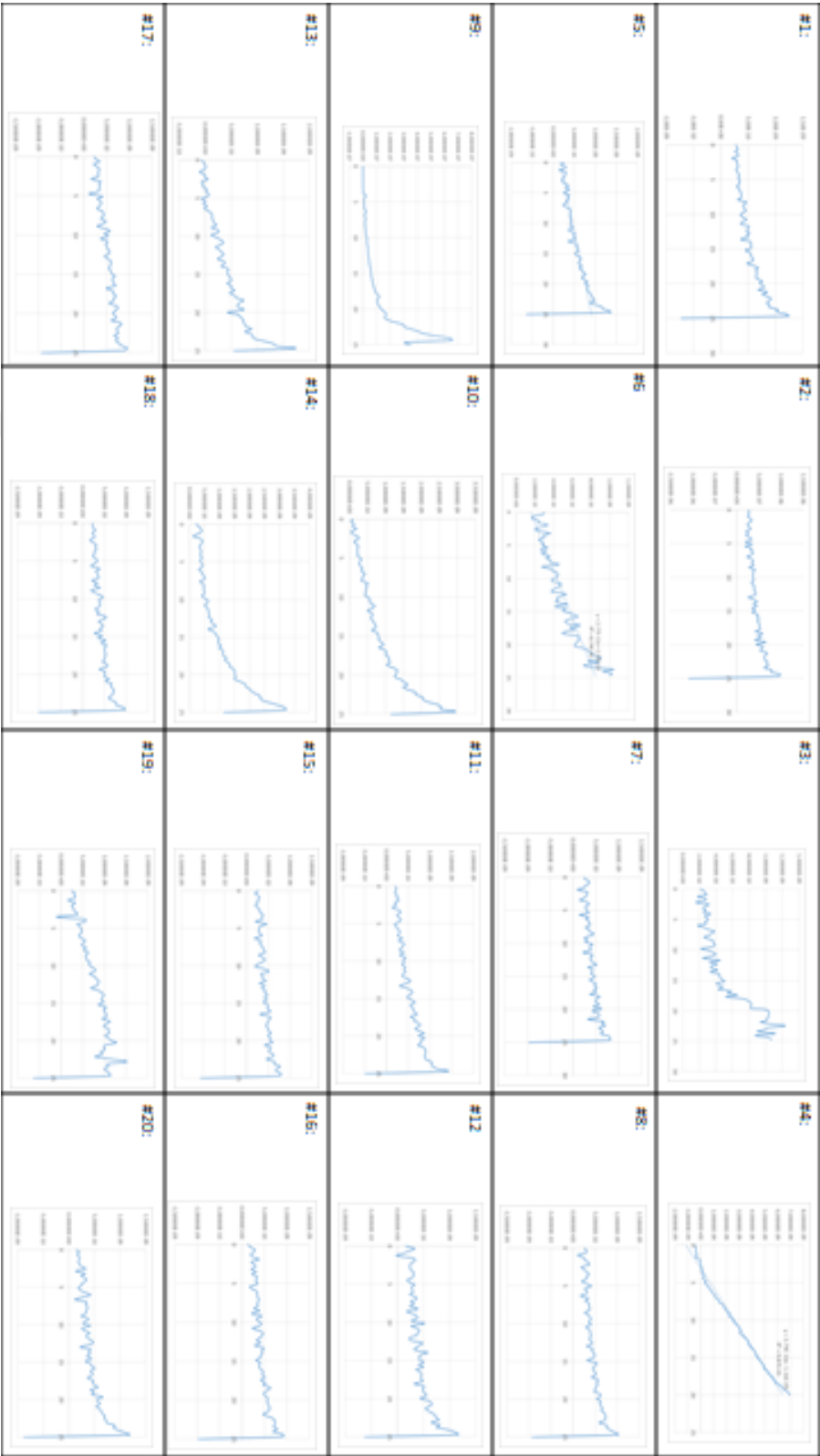
I-V curve map of **P14-P12** gel. The linear regimes are only shown in graphs.



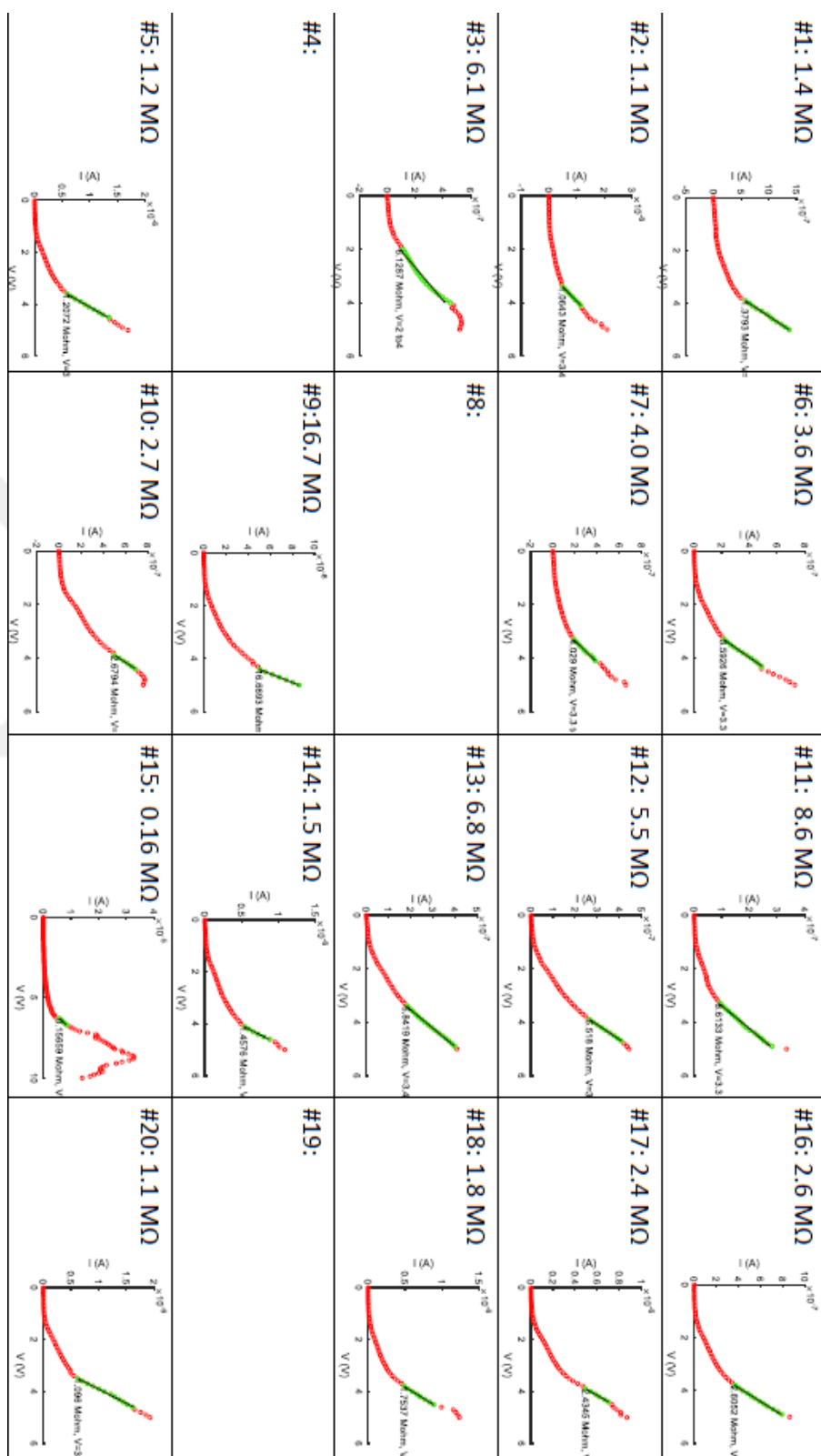
I-V curve map of **P16** gel.



I-V curve map of **P18** gel.

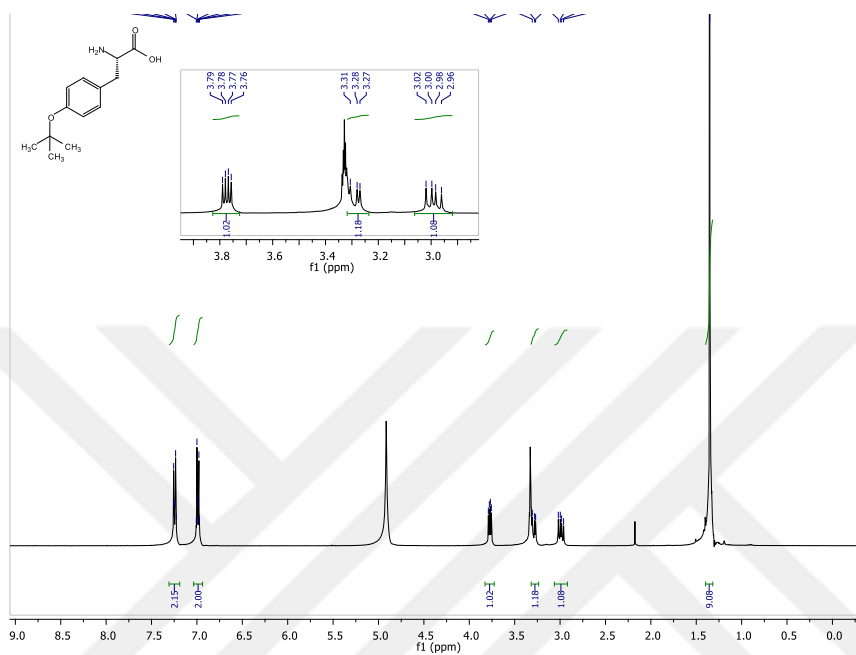


I-V curve map of **P19** gel.

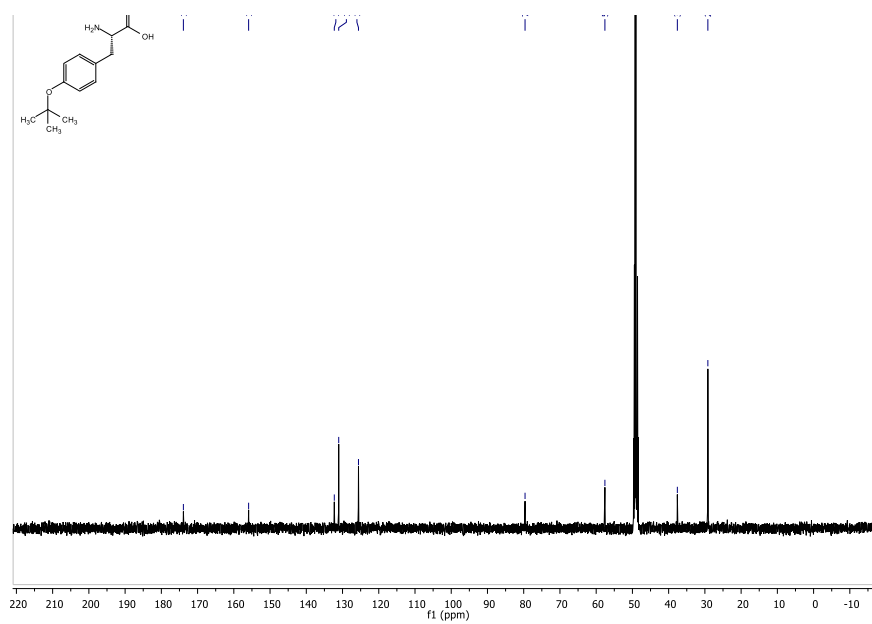


F. NMR Spectra of Chapter 2

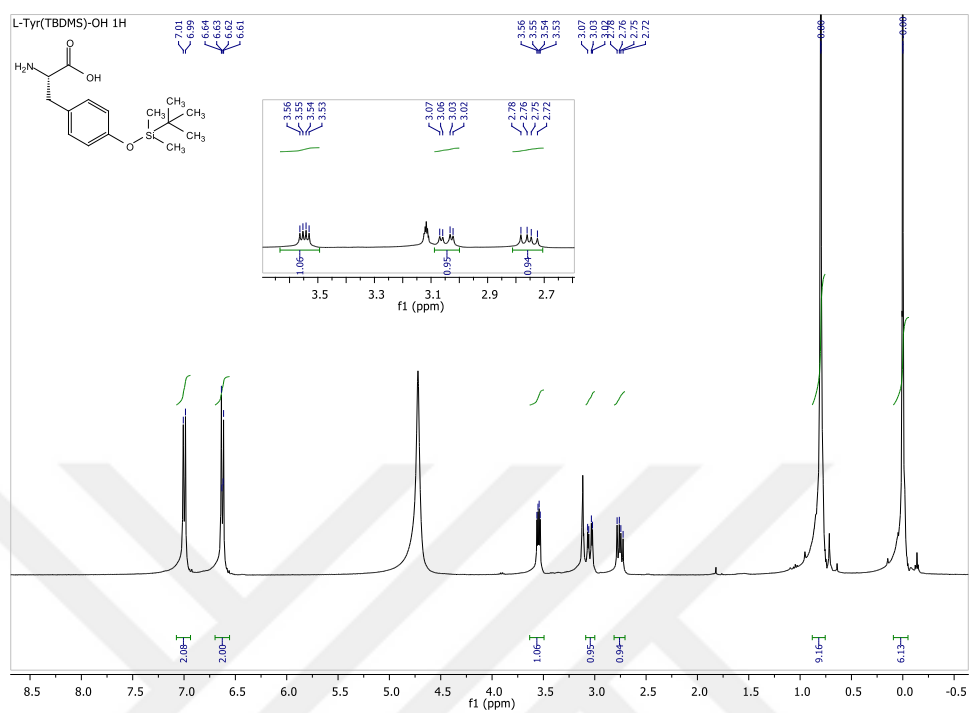
^1H NMR spectrum of L-Tyr(tBu)-OH in CD_3OD .



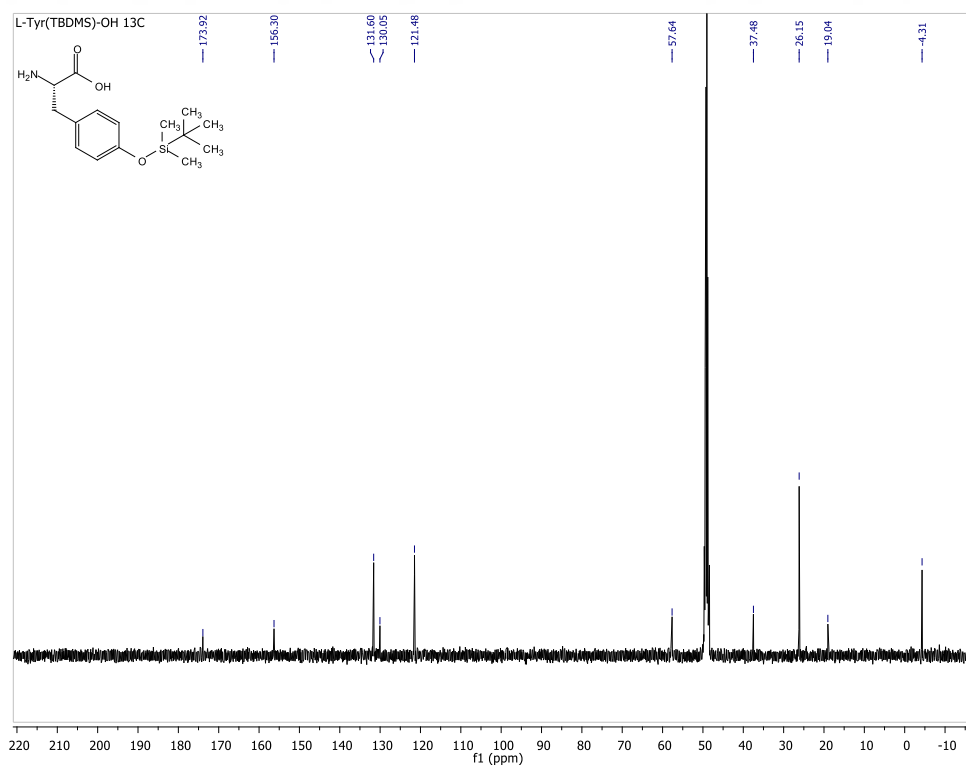
^{13}C NMR spectrum of L-Tyr(tBu)-OH in CD_3OD .



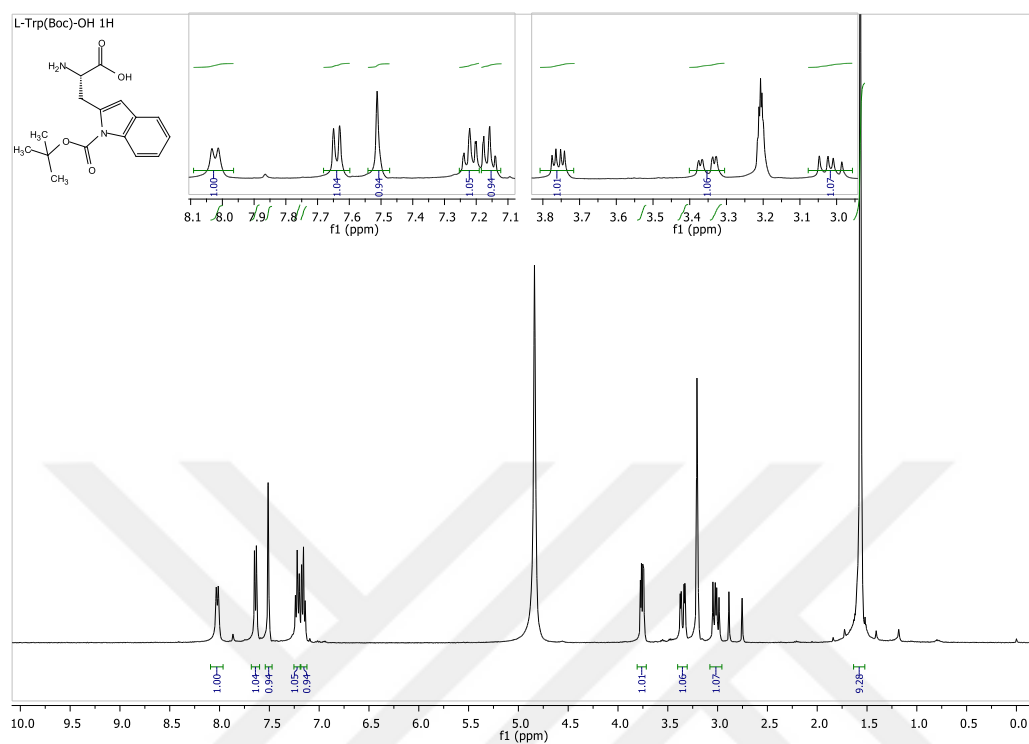
^1H NMR spectrum of L-Tyr(TBDMS)-OH in CD_3OD .



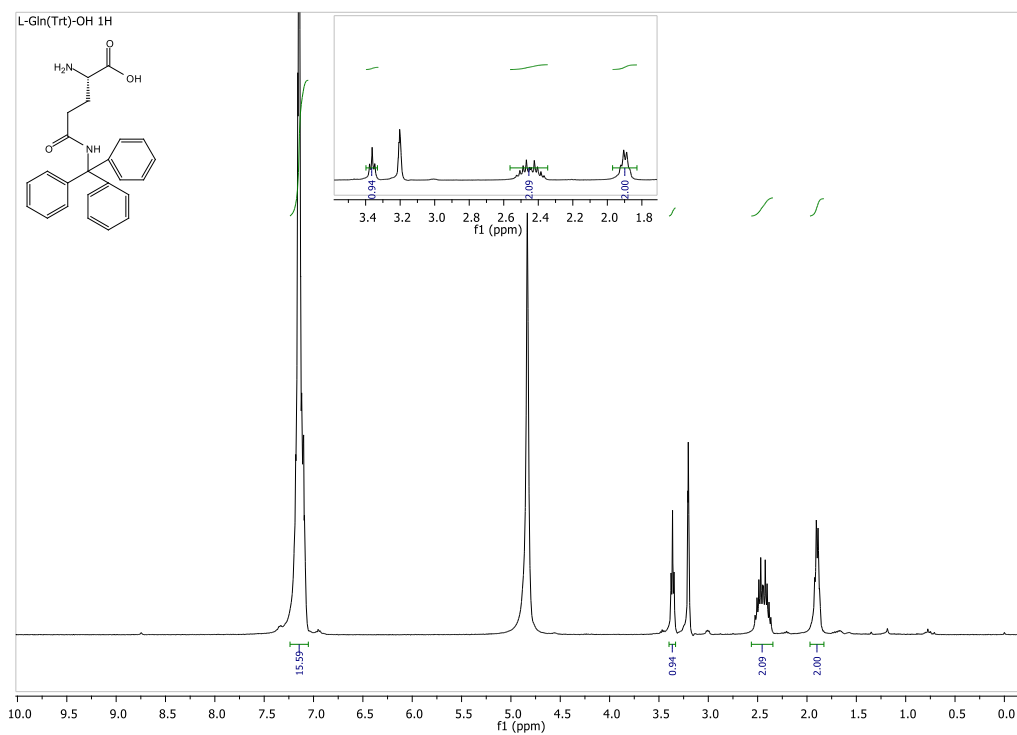
^{13}C NMR spectrum of L-Tyr(TBDMS)-OH in CD_3OD .



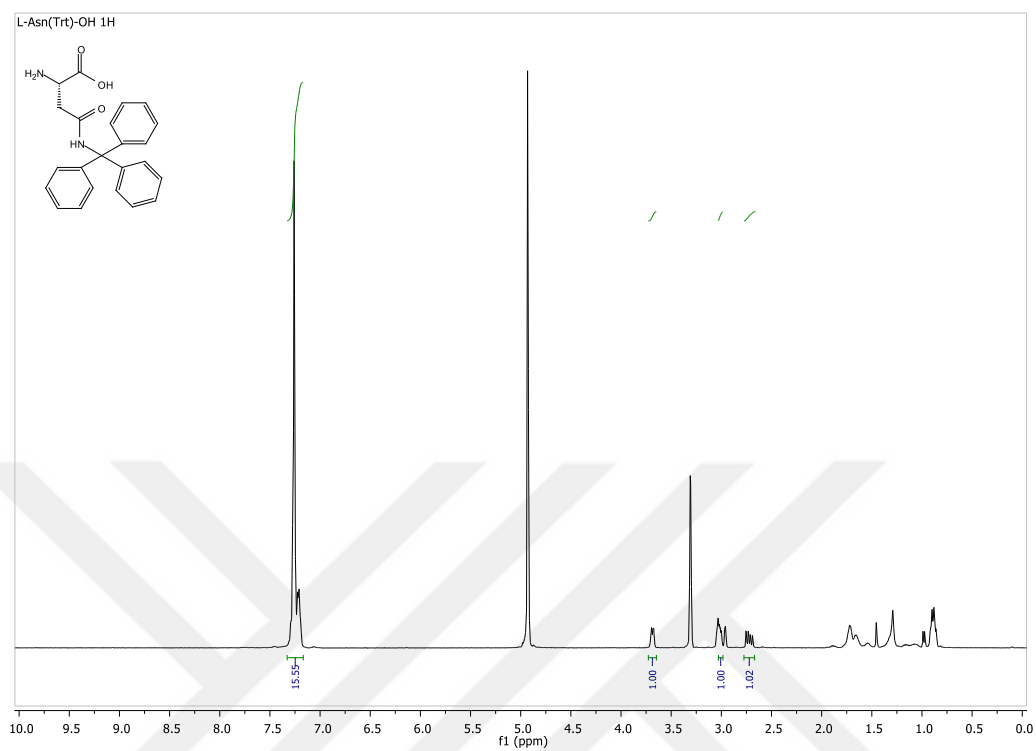
^1H NMR spectrum of L-Trp(Boc)-OH in CD_3OD .



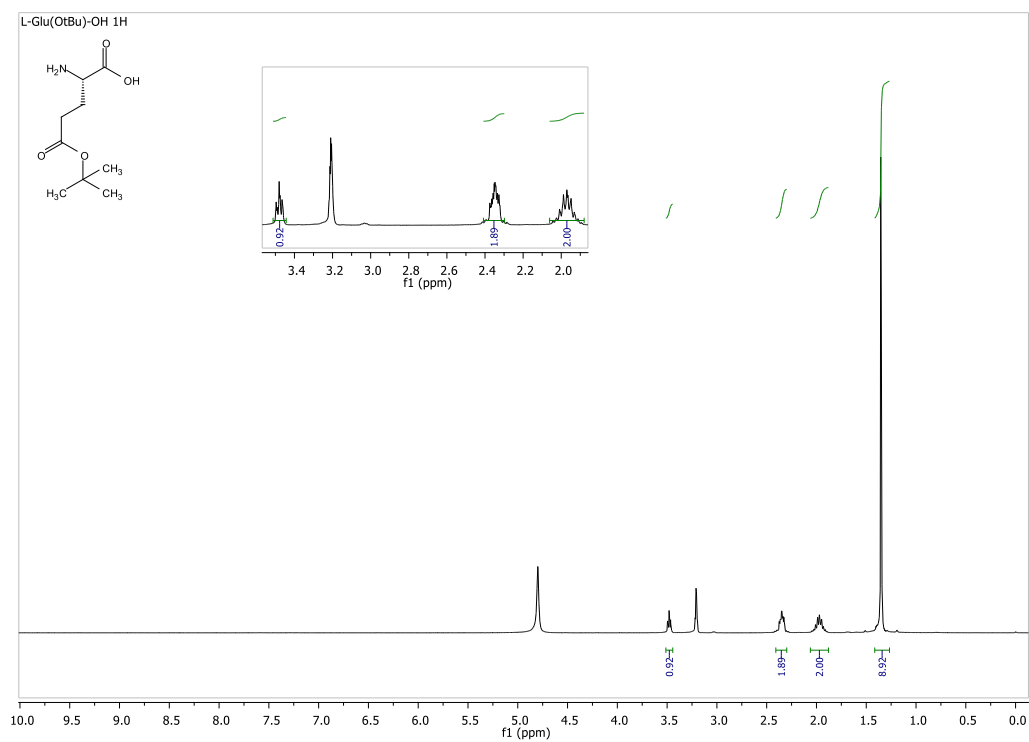
^1H NMR spectrum of L-Gln(Trt)-OH in CD_3OD .



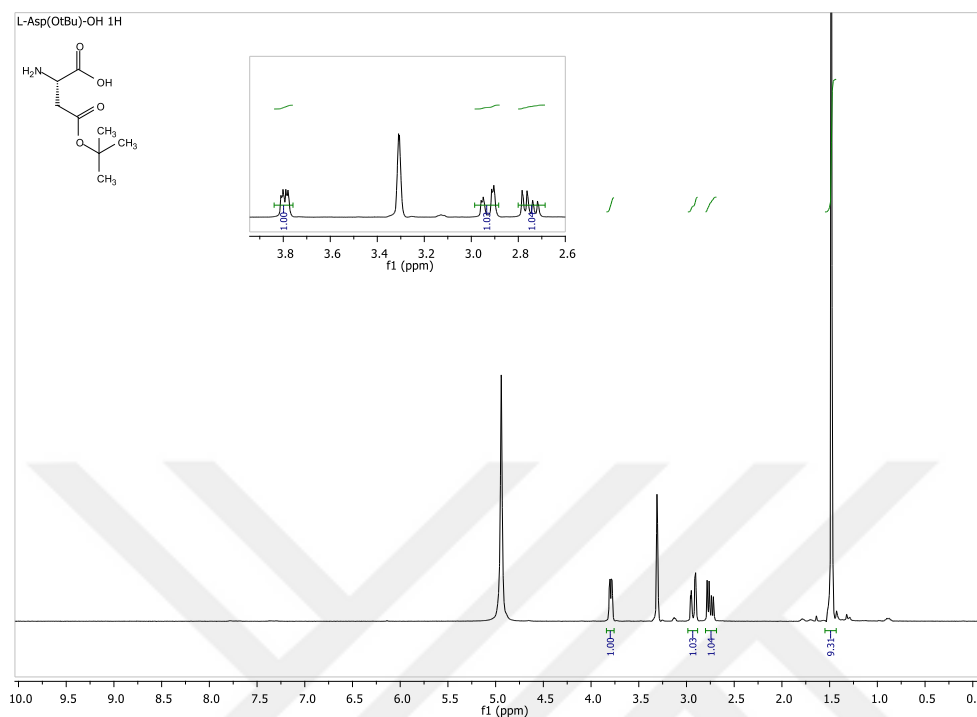
^1H NMR spectrum of L-Asn(Trt)-OH in CD_3OD .



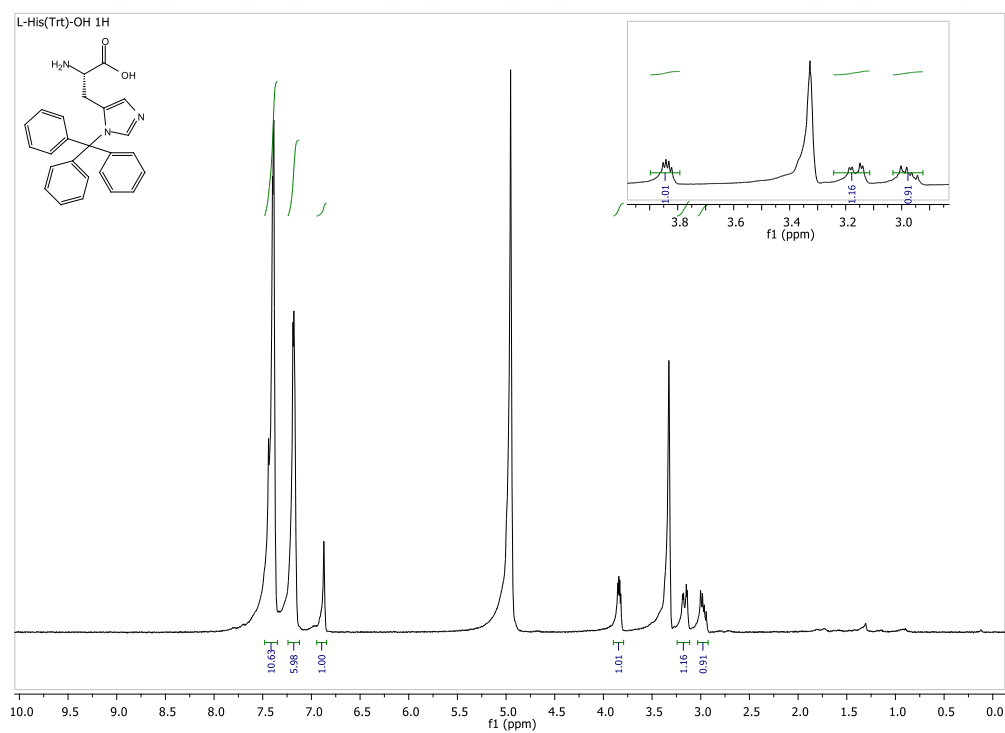
^1H NMR spectrum of L-Glu(OtBu)-OH in CD_3OD .



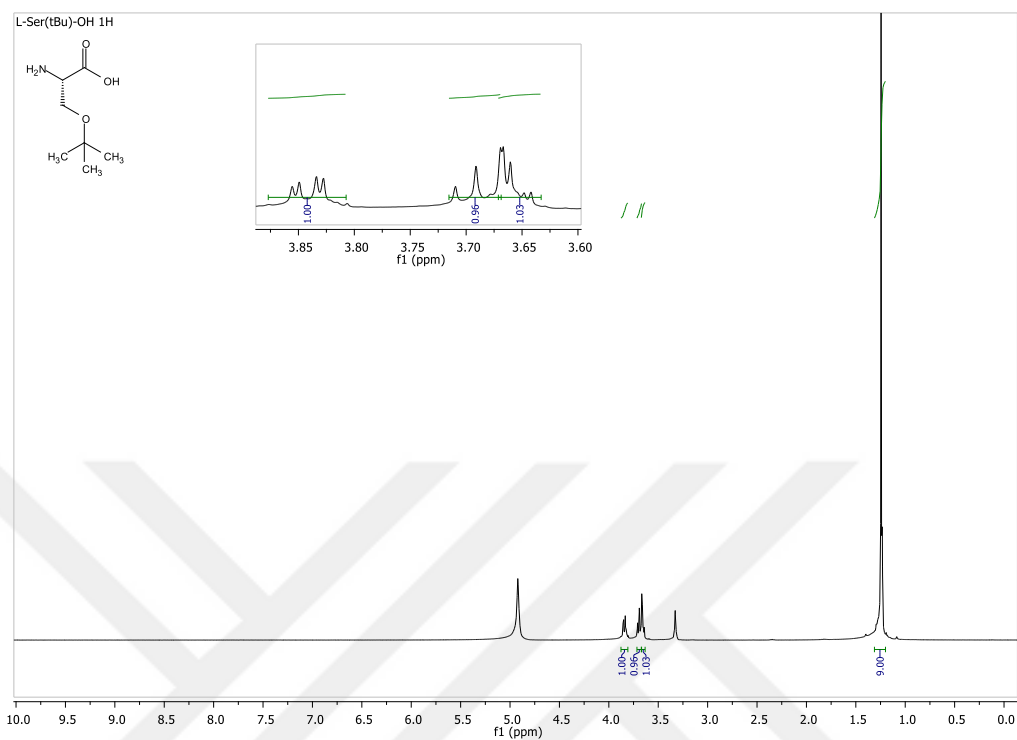
^1H NMR spectrum of L-Asp(OtBu)-OH in CD_3OD .



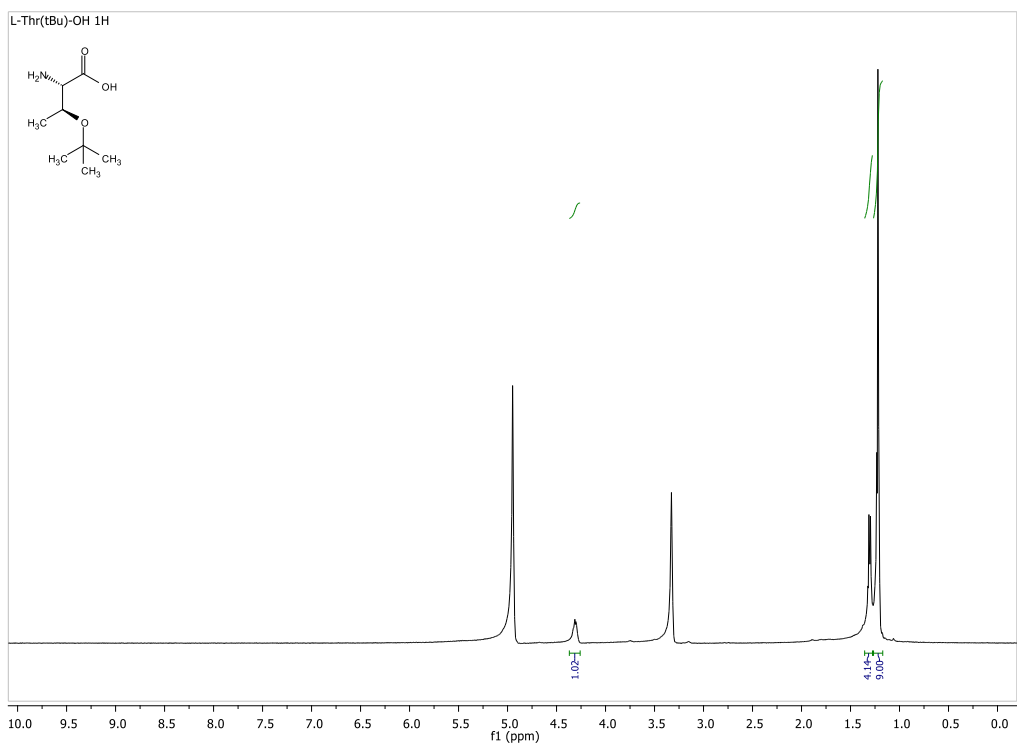
^1H NMR spectrum of L-His(Trt)-OH in CD_3OD .



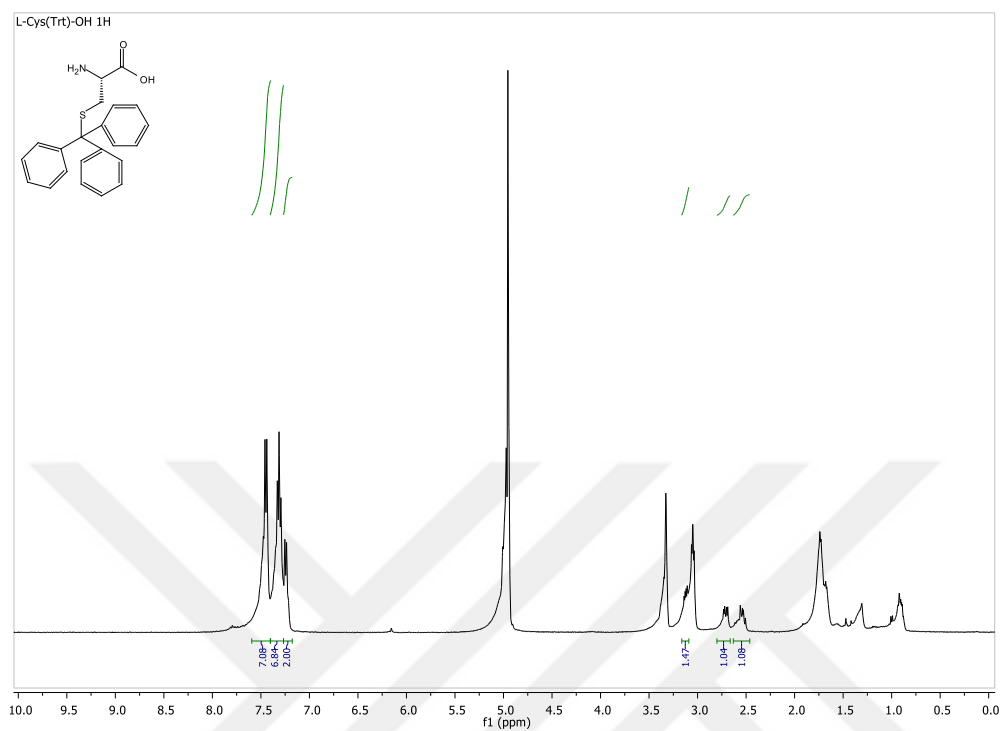
^1H NMR spectrum of L-Ser(*t*Bu)-OH in CD_3OD .



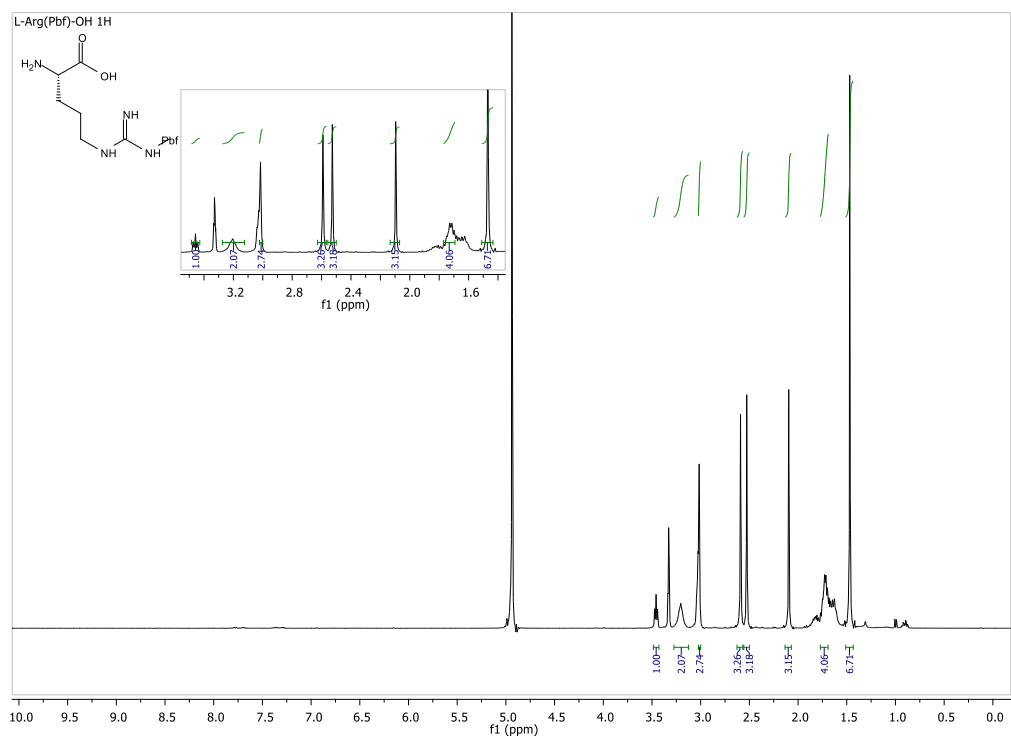
^1H NMR spectrum of L-Thr(*t*Bu)-OH in CD_3OD .



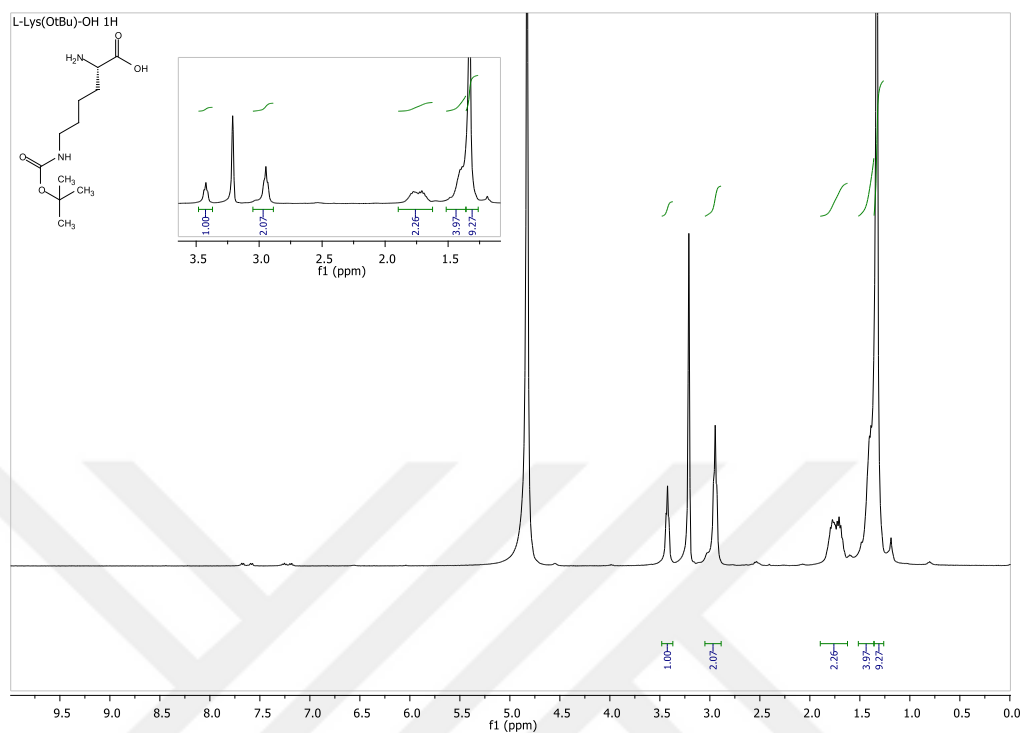
^1H NMR spectrum of L-Cys(Trt)-OH in CD_3OD .



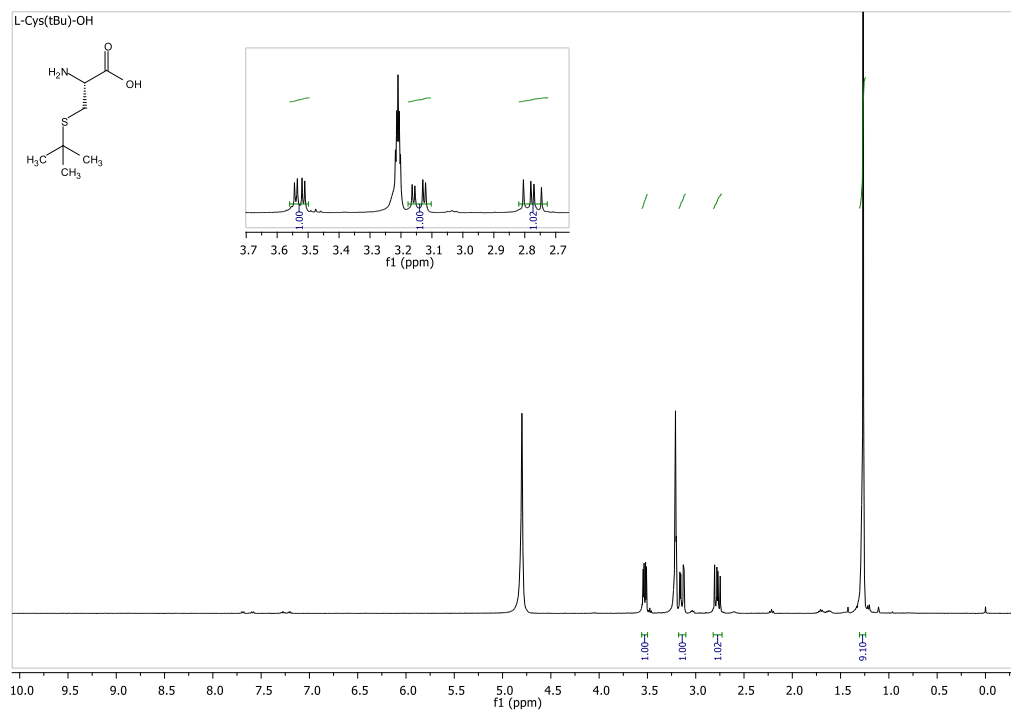
^1H NMR spectrum of L-Arg(Pbf)-OH in CD_3OD .



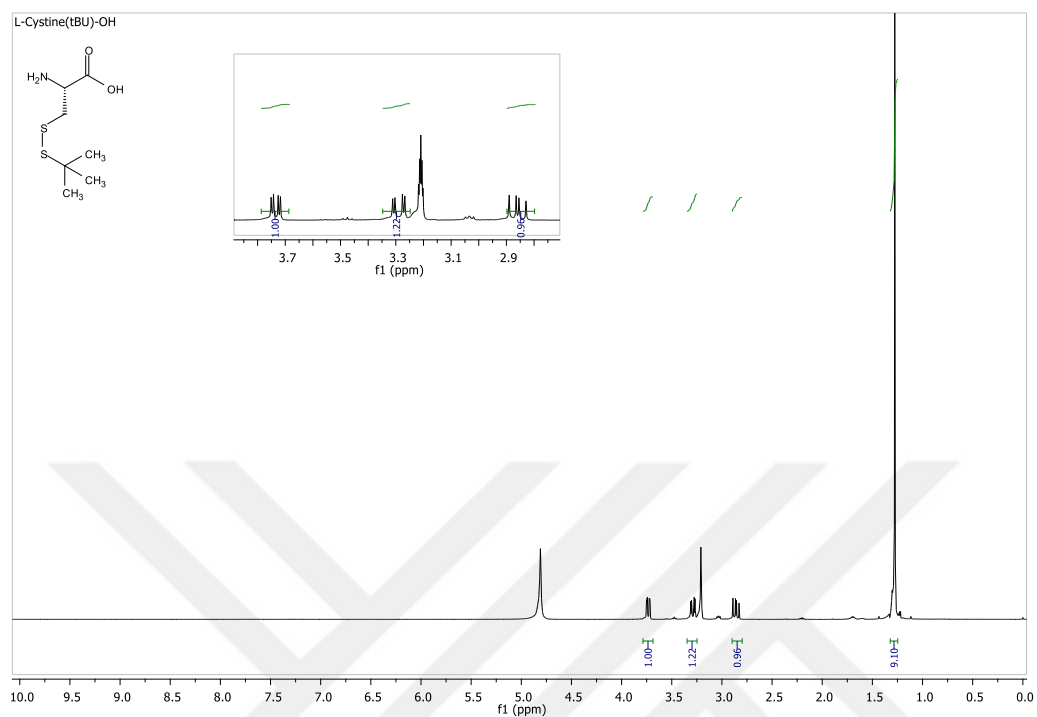
^1H NMR spectrum of L-Lys(OtBu)-OH in CD_3OD .



^1H NMR spectrum of L-Cys(tBu)-OH in CD_3OD .

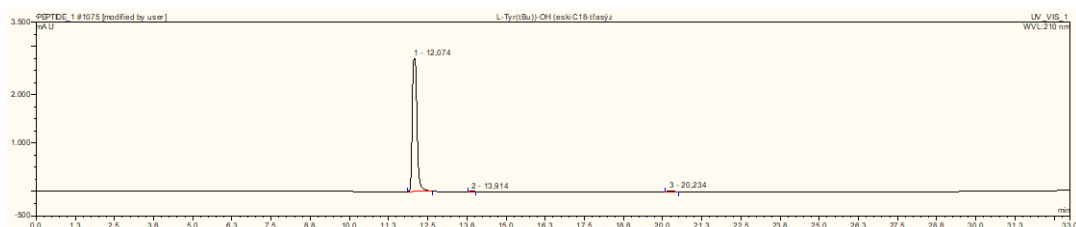


^1H NMR spectrum of L-Cys(StBu)-OH in CD_3OD .



G. HPLC Spectra of Amino Acid Derivatives (Chapter 2)

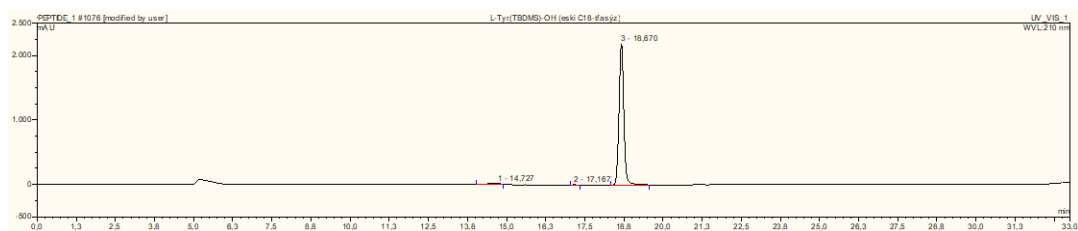
HPLC chromatogram of L-Tyr(tBu)-OH (210 nm).



Relative percentage of L-Tyr(tBu)-OH from HPLC chromatogram. Retention time of L-Tyr(tBu)-OH is 12.07 min.

No.	Ret.Time	Height	Area	Rel.Area
	min	mAU	mAU*min	%
1	12,07	2,757,099	488,526	98,91
2	13,91	11,286	1,372	0,28
3	20,23	22,998	4,027	0,82
Total:		2,791,382	493,926	100

HPLC chromatogram of L-Tyr(TBDMS)-OH (210 nm).



Relative percentage of L-Tyr(TBDMS)-OH from HPLC chromatogram. Retention time of L-Tyr(TBDMS)-OH is 18.67.

No.	Ret.Time	Height	Area	Rel.Area
	min	mAU	mAU*min	%
1	14,73	13,214	6,656	1,69
2	17,17	1,232	0,183	0,05
3	18,67	2,187,603	386,698	98,26
Total:		2,202,049	393,537	100