

***Design, Synthesis and Study of Hybrid Cyclic
Peptides and Peptide Nucleic Acids from Delta
Amino Acids***

विद्या वाचस्पति की उपाधि की अपेक्षाओं की आंशिक पूर्ति में प्रस्तुत
शोध प्रबंध

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requirements of the degree of Doctor of Philosophy**

द्वारा / By

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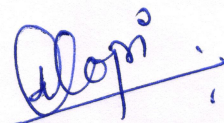
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Dedicated to
Teachers, Family, and Friends

CERTIFICATE

This is to certify that the work incorporated in the thesis entitled “**Design, Synthesis and Study of Hybrid Cyclic Peptides and Peptide Nucleic Acids from Delta Amino Acids**” submitted by **Souvik Panda Mahapatra (ID No. 20172015)** carried out by the candidate at the Indian Institute of Science Education and Research (IISER), Pune, under my supervision. The work presented here or any part of it has not been included in any other thesis submitted previously for the award of any degree or diploma from any other University or Institution.

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I declare that this written submission represents my idea in my own words and where others' ideas have been included; I have adequately cited and referenced the original sources. I declare that I have acknowledged collaborative work and discussions wherever such work has been included. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that violation of the above will be cause for disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

The work reported in this thesis is the original work done by me under the guidance of Prof. Hosahudya N. Gopi.

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Abbreviations

Ac₂O = Acetic anhydride

ACN = Acetonitrile

AcOH = Acetic acid

Aib = α -Amino isobutyric acid

Aic = 4-amino isocaproic acid

aq. = Aqueous

Bn = Benzyl

Boc = tert-Butoxycarbonyl

(Boc)₂O = Boc anhydride

^tBu = tertiary Butyl

Calcd. = Calculated

CCDC no. = Cambridge Crystallographic Data Centre number

CD = Circular Dichroism

CIF = Crystallographic Information File

CP = Cyclic Peptide

DCC = N, N' -Dicyclohexylcarbodiimide

DCM = Dichloromethane

DIPEA = Diisopropylethyl Amine

DMAP = 4-Dimethylaminopyridine

DMF = Dimethylformamide

DMSO = Dimethylsulfoxide

DNA = Deoxyribonucleic acid

EDC.HCl = (3-Dimethylamino-propyl)-ethyl-carbodiimide Hydrochloride.

Et = Ethyl

EtOAc = Ethyl acetate

EtOH = Ethanol

FE-SEM = Field Emission Scanning Electronic Microscopy

Fmoc = 9-Fluorenylmethoxycarbonyl

Fmoc-OSu = N-(9-Fluorenylmethoxycarbonyloxy) succinimide

g = gram

h = hours

H-bond = Hydrogen bond

HATU = Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium

HBTU = 2-(1H-benzotriazol-1-yl)-1, 1, 3, 3-tetramethyluronium hexafluorophosphate

HCl = Hydrochloric acid

HOBt = Hydroxybenzotriazole

IBX = 2-Iodoxybenzoic acid

IR = Infrared Spectroscopy

LAH = Lithium Aluminium Hydride

M = Molar

MALDI-TOF/TOF = Matrix-Assisted Laser Desorption/Ionization-Time of Flight

Me = Methyl

MeOH = Methanol

mg = Milligram

min = Minutes

μL = Micro liter

mL = Milliliter

mM = MilliMolar

mmol = millimoles

MS = Mass Spectroscopy

N = Normal

nM = NanoMolar

NHS = N-hydroxy succinimide

NMP = N-methyl pyrrolidone

NMR = Nuclear Magnetic Resonance

NOE = Nuclear Overhauser Effect

ORTEP = Oak Ridge Thermal-Ellipsoid Plot Program

PFM = Piezoresponse Force Microscopy

PG = Protecting Group

PNA = Peptide Nucleic Acid

ppm = Parts per million

Py = Pyridine

R_f = Retention factor

RNA = Ribonucleic acid

RP- HPLC = Reversed Phase-High Performance Liquid Chromatography

R_t = Retention time

RT = Room Temperature

ROESY = **R**otating-frame nuclear **O**verhauser **E**ffect correlation **S**pectroscopy

SCPNs = Self-assembled Cyclic Peptide Nanotubes

TEM = Transmission Electron Microscopy

TFA = Trifluoroacetic acid

THF = Tetrahydrofuran

TOCSY = Total Correlation Spectroscopy

UV = Ultraviolet–Visible Spectroscopy

Abstract

Self-assembling cyclic peptides are important building blocks for constructing synthetic nanotubes. They can be primarily classified into D, L-cyclic peptides, cyclic β -peptides, cyclic α , γ -peptides, and cyclic peptides incorporating δ - or ϵ -amino acids based on their composition. Our research focuses on the design of novel cyclic peptides derived from $\beta(\text{O})$ - δ^5 -amino acid residues, consisting of a hybrid structure of crown ether and peptide macrocycles. These peptides exhibit unique self-assembly, forming hollow sub-nanotubular structures with unidirectional hydrogen bonds. These cyclic dipeptides, adopting non-centrosymmetric space groups, display significant anisotropy, suggesting potential macroscopic dipole extensions. Furthermore, their inherent macrodipole orientation inspires investigation into their piezoelectric properties, opening new avenues for diverse applications. Additionally, we have synthesized size-variant macrocycles featuring $\beta(\text{O})$ - δ^5 -amino acid residues, which also fold into self-assembled sub-nanotubes in solution and gas phase. These supramolecular size-variant sub-nanotubes act as anion-selective transporters. The ion channel activity and selective ion transport activity of these cyclic peptides may have great potential in the construction of functional artificial transmembrane transporters. We further explored these δ -amino acids to design novel Peptide Nucleic Acids (PNAs), aiming to enhance solubility, binding affinity, and specificity to DNA/RNA. We have synthesized new PNAs and conducted UV- T_m studies to assess their thermal stability and binding affinity against ssDNA and ssRNA. Remarkably, the new PNAs bind to both ssDNA and ssRNA similarly to the aeg peptide nucleic acids. The advantage of these new δ -amino acid-based peptide nucleic acids over the aegPNAs is that they are easy to synthesize and scale up using solid-phase methods. Additionally, compared to aeg peptide nucleic acids, the new PNAs are highly soluble in aqueous solution. These new peptide nucleic acids may serve as alternatives to existing aeg-peptide nucleic acids.

Overall, we designed and studied new cyclic peptides and peptide nucleic acids from $\beta(\text{O})$ - δ^5 -amino acid residues. The new hybrid crown ether-cyclic peptide mimetics and peptide nucleic acids described here can serve as piezo-electronic materials, transmembrane channel-forming candidates, and DNA/RNA-binding proteolytically stable biomolecules.

Publications

1. **Souvik Panda Mahapatra**, Saikat Pahan, Souvik Roy, DRGKoppalu R. Puneeth Kumar, and Hosahudya N. Gopi. “Exploring Macroscopic Dipoles of Designed Cyclic Peptide Tubular Assemblies to Harvest Piezoelectric Properties.” *Angew. Chem. Int. Ed.* **2024**, e202409969.
2. **Souvik Panda Mahapatra**, Rahi Masoom Reja, and Hosahudya N. Gopi. “Designing Self-Assembling Cyclic β (O)- δ^5 -Peptide Nanotubes for Biomimetic Anion Selective Transmembrane Ion Channel Functionality”. *Manuscript under revision*.
3. **Souvik Panda Mahapatra**, and Hosahudya N. Gopi. “Optimizing DNA/RNA Binding Selectivity through the Synthesis and Evaluation of Modified Peptide Nucleic Acid”. *Manuscript Under Preperation*
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5. Saikat Pahan, Sanjit Dey, Gijo George, **Souvik Panda Mahapatra**, DRGKoppalu R. Puneeth Kumar, and Hosahudya N. Gopi. “Design of Chiral β -Double Helices from γ -Peptide Foldamers”. *Angew. Chem. Int. Ed.* **2024**, 63, e2023163.
6. DRGKoppalu R. Puneeth Kumar, Zahid Manzoor Bhat, Sanjit Dey, Souvik Roy, **Souvik Panda Mahapatra**, Saikat Pahan, Musthafa Ottakam Thotiyl, and Hosahudya N. Gopi. “Foldamer Nanotubes Mediated Label-Free Detection of Protein-Small Molecule Interactions.” *Chem. Eur. J.* **2023**, 29, e2023004.
7. Souvik Roy, Aman Chaturvedi, Sanjit Dey, DRGKoppalu R. Puneeth Kumar, Saikat Pahan, **Souvik Panda Mahapatra**, Pankaj Mandal, and Hosahudya N. Gopi. “Anion Tuned Structural Modulation and Nonlinear Optical Effects of Metal-Ion Directed 3_{10} -Helix Networks.” *Chem. Eur. J.* **2023**, 29, e2023031.
8. Saikat Pahan, **Souvik Panda Mahapatra**, Souvik Roy, Manjeet Singh, and Hosahudya N. Gopi. “Engineering β -Sheet Mimetics: Unveiling β -Double Helices and C_{14} -Helices via Backbone Modulation.” *Manuscript Under Revision*.
9. Sanjit Dey, Souvik Roy, DRGKoppalu R. Puneeth Kumar, Sachin A. Nalawade, Manjeet Singh, **Souvik Panda Mahapatra**, and Hosahudya N. Gopi “Metal Directed Hierarchical Superhelices from Hybrid Peptide Foldamers.” *Manuscript Under Preparation*.

Chapter 1

General Introduction of Cyclic Peptide Self-assembly and Exploration of their Supramolecular Tubular Structures

Chapter 1

1. 1. Introduction:

Peptides, compounds comprising two or more amino acid residues linked by amide bonds, occupy a middle ground between small molecules and larger biological entities like proteins or antibodies.¹ They offer distinct advantages over conventional small molecule therapies, including high selectivity, potency, specificity for biological targets, minimal side effects, and low tissue accumulation.² Notably, peptides exhibit lower immunogenicity compared to proteins or antibodies.^{1b} Their therapeutic potential spans a wide range of biological activities, from antimicrobial and antihypertensive to antioxidant, anticancer, antidiabetic, and anti-inflammatory effects, garnering interest across pharmaceutical, cosmeceutical, and nutraceutical sectors.³ The use of peptides in therapy traces back to the 1920s with the groundbreaking administration of insulin, transforming the management of type I diabetes and paving the way for peptides as viable therapeutic agents.⁴ Subsequently, the synthesis of hormones like oxytocin and vasopressin further propelled interest in peptides for clinical applications.⁵ Despite initial enthusiasm, research on peptides faced setbacks amid the prominence of small molecule discoveries. However, a resurgence of interest occurred in the 1980s, driven by biotechnology firms, leading to a steady increase in approved peptide therapeutics since then.⁶

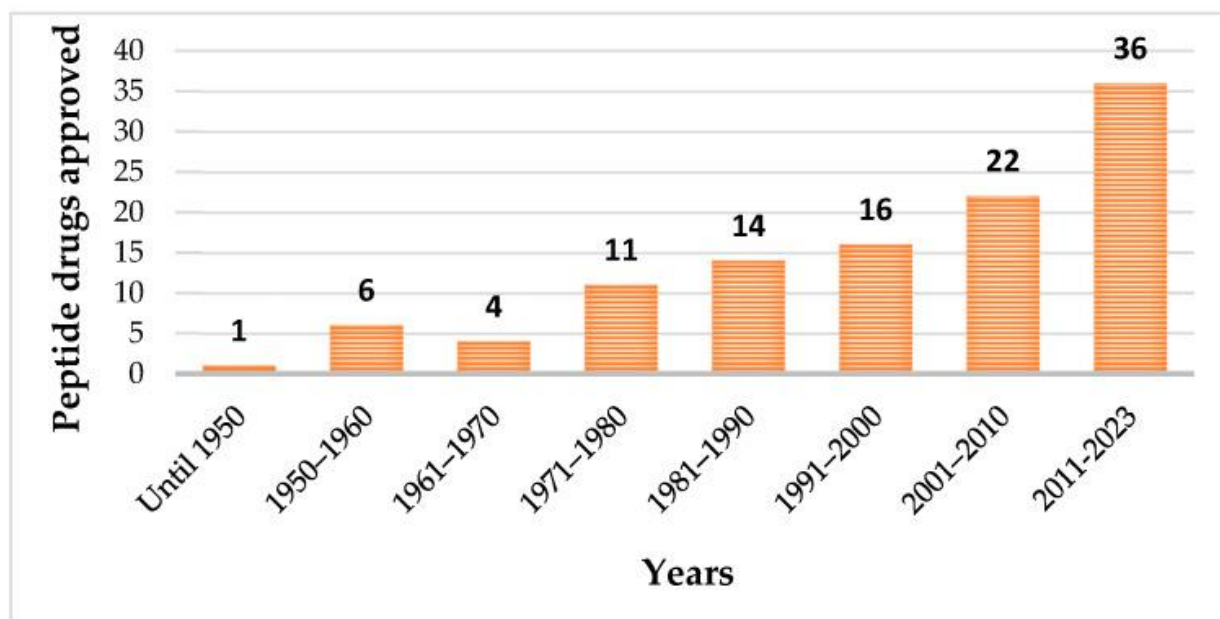


Figure 1: The progression of authorized peptide therapeutics throughout time.⁷

Chapter 1

As of May 2023, regulatory authorities have approved 114 peptides as therapeutic agents, encompassing pharmaceuticals and diagnostic tools, illustrating the growing recognition of peptides' clinical potential.⁷ Among the approved peptides as therapeutic agent, cyclic peptides represented 46% of all approved peptides. That's why cyclic peptides gained considerable attention as therapeutic tool nowadays.⁸

1.2. Cyclic Peptide as Therapeutic Tool:

The advent of cyclic peptides was catalyzed by the discovery of gramicidin S, an antibiotic unearthed by Gause and Brazhnikova during World War II, which saw widespread use in Soviet military hospitals, sparking a revolution in cyclic peptide research.⁹ Noteworthy examples include telavancin, dalbavancin, and oritavancin, which are semi-synthetic cyclic lipoglycopeptide antibiotics.¹⁰ Additionally, anidulafungin, belonging to the echinocandin class of antifungals, and lanreotide, pasireotide, and romidepsin, utilized in anticancer therapies, exemplify the diverse applications of cyclic peptides. Linaclotide, derived from an enterotoxin for gastrointestinal disorders, further underscores their versatility.¹¹

Rezafungin, an antifungal analog of anidulafungin, represents the latest addition to the roster of cyclic peptide approvals, gaining FDA approval in 2023.¹² Notably, its oral administration and extended half-life of 30 hours mark a significant advancement in pharmacokinetic attributes compared to other peptide drugs.

Another significant application of peptides lies in drug delivery, owing to their favorable hydrophilicity/hydrophobicity ratio and intra- and intermolecular interactions facilitated by weak non-covalent bonds. These properties enable peptides to self-organize into nanostructures, which exhibit high drug loading capacity, protection, and responsiveness to external stimuli.¹³ Recent advancements include the development of new cyclic peptides with nano-spherical structures capable of forming stable complexes with short-interfering RNA (siRNA), showcasing promise in nucleic acid delivery for cancer treatment.¹⁴

Furthermore, cyclic peptides such as vancomycin, teicoplanin, and ristocetin, initially known for their therapeutic actions as macrocyclic antibiotics, have found applications as chiral stationary phases in chromatography.¹⁵ This application, pioneered by Armstrong et al. in 1994, exploits the

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macrocyclic structure and high number of stereogenic centers in these peptides to enable enantioseparation and formation of inclusion complexes, enhancing chiral recognition.¹⁶

1.3. Source of Cyclic Peptides:

- **Natural Source:** Cyclic peptides can be sourced from terrestrial and marine organisms.¹⁷ Terrestrial origins encompass animal venoms,¹⁸ plants,¹⁹ microorganisms,²⁰ among others, yielding antibiotics like vancomycin,²¹ daptomycin,²² and teixobactin.²³ Marine sources, including tunicates,²⁴ sponges,²⁵ algae,²⁶ bacteria,²⁷ fungi,²⁸ and various invertebrates, offer peptides with diverse bioactivities, particularly anticancer and antimicrobial properties. Notably, ziconotide, derived from marine sources, stands as the first FDA-approved marine peptide drug in 2004.²⁹
- **Chemical Synthesis:** Chemical synthesis serves as a significant avenue for obtaining peptides in sufficient quantities for extensive biological assays.³⁰ It allows for the generation of structurally diverse analogs and derivatives, facilitating structure–activity relationship studies. Synthetic strategies encompass various molecular modifications, including incorporation of non-proteinogenic amino acids, acetylation of N-termini, glycosylation, and lipidation, aimed at enhancing pharmacokinetic properties and biological activities.³¹

1.4. Peptide Cyclization Strategies:

Cyclization can manifest in four distinct manners, depending on the composition of constituent amino acids: head-to-tail cyclization, head-to-side chain cyclization, side-chain-to-tail cyclization, and side-chain-to-side-chain cyclization (See Figure 2).³² This step is widely recognized as one of the most formidable challenges in peptide synthesis. Various peptide cyclization methods have been developed to address this challenge, each with its own merits and drawbacks.³³

Traditionally, cyclization is executed in solution using coupling agents. However, this method is plagued by issues such as C-terminal epimerization, oligomerization, and low coupling efficiency, particularly influenced by the ring size and peptide sequence. Additionally, the necessity of fully protecting the linear peptide for cyclization renders it poorly soluble in organic solvents.³⁴ Strategies to enhance coupling efficiency include conducting reactions at high dilution to prevent oligomerization and utilizing additives or activation sites like glycine (Gly) or proline (Pro)

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residues to mitigate C-terminal epimerization.³⁵ Nevertheless, these approaches have limitations, necessitating the exploration of alternative methods to achieve chemo-selective bond formation and reduce the likelihood of C-terminal epimerization without the need for full protection of side chains.

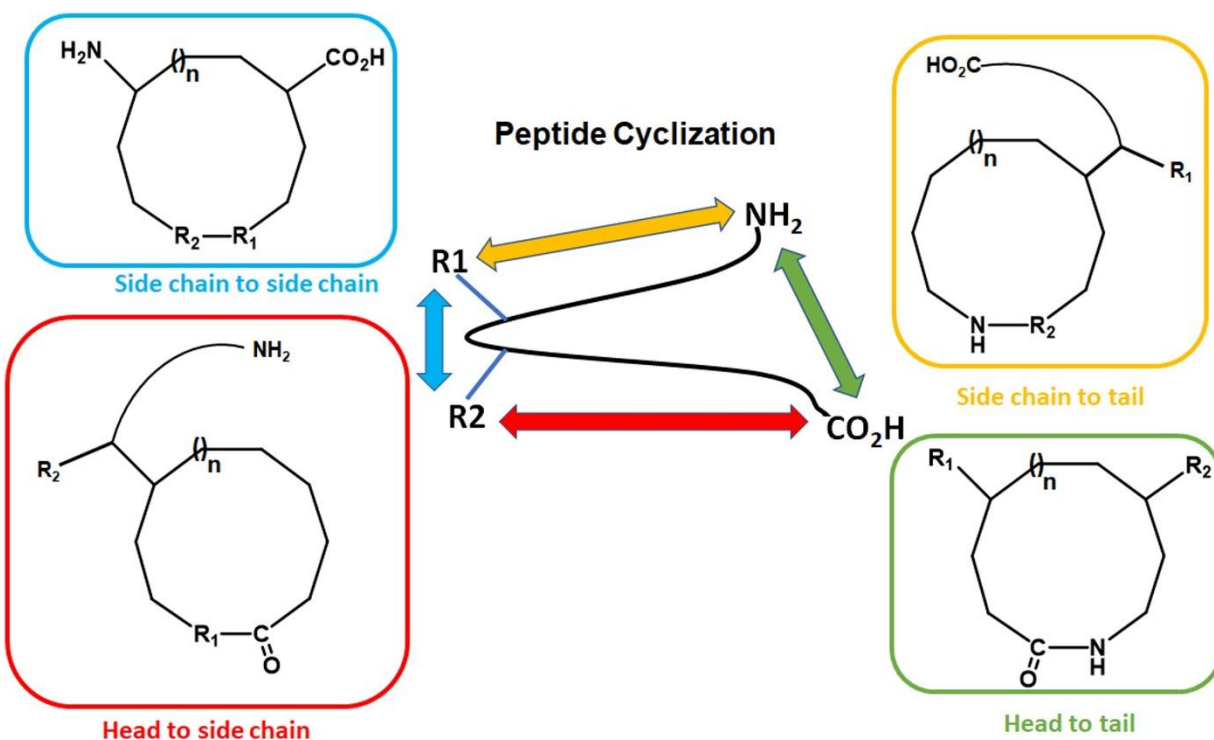


Figure 2: Type of Peptide Cyclization Methods.

There are several peptide cyclization chemical and enzymatic procedures present in literature. They can be classified as below (Figure 3).

- **Native Chemical Ligation (NCL):** One notable technique is Native Chemical Ligation (NCL), which involves a chemo-selective ligation between a cysteine (Cys) residue and a thioester peptide, forming an intermediate that rearranges to create a native peptide bond.³⁶
- **NCL-desulfurization:** It is a variation of NCL. It involves removing the thiol group post-reaction and replacing it with alanine or other amino acid residues.³⁷
- **Serine (Ser)/Threonine (Thr) ligation approach:** It involves a selective chemical bond formation between a peptide with a C-terminal glycolaldehyde ester and another peptide containing Ser, Thr, or Cys at the N-terminal end.³⁸ While this method represented progress

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in chemo-selective ligation, it faced challenges, particularly with slow reaction rates when the N-terminal end contained Thr or Ser. Additionally, the conversion of the resulting ring into an amide bond did not occur efficiently. To address these issues, an alternative version of Ser/Thr ligation was proposed, employing a salicylaldehyde ester at the C-terminal instead of glycolaldehyde ester.³⁹ This modification aimed to enhance the efficiency of the reaction. The utility of Ser/Thr ligation for peptide cyclization has been demonstrated in the synthesis of various compounds such as daptomycin,⁴⁰ cyclomontanin B,⁴¹ mahafacyclin B.⁴²

- **The Staudinger ligation method:** It involves the reaction between a C-terminal phosphinothioester and an N-terminal azide, resulting in the formation of an iminophosphorane. This intermediate subsequently undergoes hydrolysis to yield an amide bond.⁴³
- **The KAHA ligation method:** It involves the creation of an amide bond through a potent and selective reaction between N-alkylhydroxylamines and C-terminal peptide α -keto carboxylic acids. This specific reaction between these groups results in the formation of natural amide bonds without the need for additional reagents, yielding only carbon dioxide and water as byproducts. This approach was utilized to synthesize the peptide antibiotic gramicidin.⁴⁴
- **Enzyme-mediated Cyclization:** It represents another viable strategy, capitalizing on the high chemoselectivity of enzymes. This feature ensures cyclization reactions occur without generating undesired byproducts, and enzymes can catalyze these reactions efficiently with minimal enzyme-to-substrate ratios.⁴⁵ Furthermore, enzymes are typically non-toxic and cost-effective. Examples of enzymes employed for peptide cyclization include Butelase 1,⁴⁶ subtiligase.⁴⁷ Notably, Butelase 1 facilitated the cyclization of bacteriocins AS-48 and uberolysin.⁴⁸
- **CyClick strategy:** One notable innovation is the CyClick strategy, which involves the creation of a cyclic imine between the N-terminus of a peptide and the C-terminus of an aldehyde group. In this process, the proximity of the amide to the imine facilitates a nucleophilic attack by the amide nitrogen on the imine, resulting in the formation of a 4-imidazolidinone linked to the cyclized peptide.⁴⁹ This method is highly selective and does not yield any by-products, leading to consistently high yields.

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- **On-resin Cyclization:** This synthetic approach involves on-resin cyclization, where the linear peptide is first attached to a resin.⁵⁰ This method leverages the phenomenon termed "pseudo-dilution," which favors intramolecular reactions over intermolecular ones. Consequently, this setup reduces the occurrence of undesired side reactions such as epimerization and oligomerization. Moreover, any by-products formed can be readily removed through filtration and washing processes. Successful application of this strategy includes the synthesis of cyclic peptide stylostatin⁵¹ and teixobactin analogs.⁵²
- **Cyclization through gold Catalysis:** A highly effective approach was pioneered by Vanjari et al. involving the cyclization of propargylated peptides through imine formation catalyzed by gold. This innovative method enables peptide cyclization without the necessity for protective groups and within a remarkably short timeframe (typically ranging from 30 to 60 minutes). The gold complex interacts with the triple bond of the propargyl group, following which a free amine from the peptide serves as a nucleophile, attacking the gold-activated alkyne. This reaction operates via a regioselective Markovnikov addition mechanism.⁵³
- **Cyclization via formation of a triazene bond:** In a novel approach reported by Nwajiobi et al. in 2022 a distinctive method was introduced involving the incorporation of para-aminophenylalanine into a linear peptide via solid-phase peptide synthesis (SPPS). Following this, HCl and sodium nitrite are added, and subsequently, the pH is dramatically altered to 7.5 to yield a diazonium ion arene. Secondary amines, such as Pro or monomethyllysine, undergo reaction with this ion to form a triazene bond. Notably, the reaction boasts a quantitative yield and completes within a brief duration of 5 minutes, without yielding any by-products. This chemoselective reaction specifically targets secondary amines, occurring in a single step and devoid of the need for reagents.⁵⁴

Selective chemical reactions and the newer cyclization techniques represent potent alternatives for synthesizing cyclic peptides, offering distinct advantages over traditional solution-based cyclization methods. However, it's crucial to acknowledge that these synthetic approaches also come with their own set of constraints. For instance, they often rely on the presence of specific functional groups within the amino acid residues to facilitate cyclization.

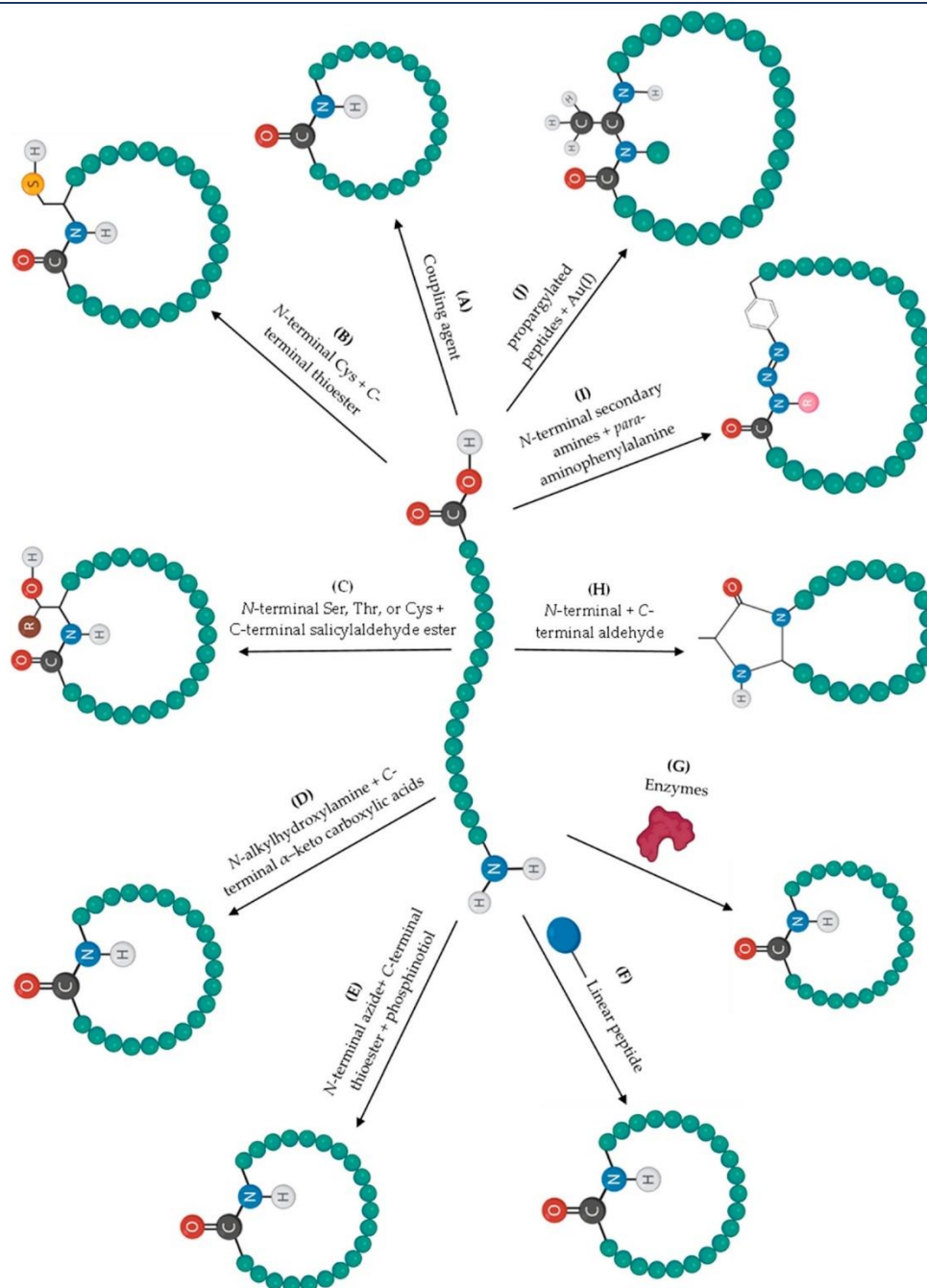


Figure 3: It illustrates a variety of methods for peptide cyclization: (a) Cyclization in solution; (b) Native chemical ligation for cyclization; (c) Ser/Thr ligation approach for cyclization; (d) KAHA ligation approach for cyclization; (e) Staudinger's ligation approach for cyclization; (f) On-resin cyclization; (g) Enzyme-mediated cyclization; (h) CyClick cyclization; (i) Cyclization via formation of a triazene bond; (j) Cyclization of propargylated peptides through imine formation mediated by gold.⁷

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1.5. Importance of Cyclic Peptides:

Peptide cyclization has become a useful tactic for improving the advantages of linear peptides and mitigating their disadvantages. A peptide's spatial vibrations are reduced when it is cyclized, which lessens the molecule's tendency to alter shape. Furthermore, cyclization increases the surface area that can be contacted by biological targets, improving the affinity and selectivity of binding.⁵⁵ Cyclization can enhance peptides' pharmacokinetic qualities in addition to their pharmacological ones. By making the structure more rigid, the peptide may interface with transport proteins more easily and enter cells through passive diffusion or active transport. This decreases the energy barrier needed for the peptide to adapt to the membrane environment. As a result, cyclization can improve membrane permeability and peptide absorption.⁵⁶

In addition, cyclic peptides have higher metabolic stability than linear peptides. Because they lack terminal amine and carboxylic acid groups, they are resistant to degradation by exopeptidases and by endopeptidases because they are unable to access the cleavage site.⁵⁷ Although cyclization has the potential to improve peptide pharmacokinetics, it is important to recognize that many cyclic peptides have faced considerable challenges due to poor pharmacokinetic characteristics, which frequently result in phase I/II trial failure.⁵⁸ However, molecular alterations can be added by chemical synthesis to produce variants with improved properties.⁵⁹ The unique properties of cyclic peptides continue to draw researchers interested in studying them in spite of these factors. The wide range of authorized cyclic peptides used in treatments for different illnesses serves as proof of this. Detailed discussion of favorable properties of cyclic peptides are discussed below.

1.6. Characteristic Properties of Cyclic Peptides:

Given their variety of beneficial qualities, it is not surprising that cyclic peptides are used as ligands by natural systems to accomplish essential biological tasks. Strong binding abilities, stability, evolution-adaptability, simplicity of synthesis, and, in some cases, cell membrane penetration are a few of these. Numerous characteristics, including binding affinity, specificity, and protease resistance, are mostly derived from the circular shape of these peptides. Furthermore, in certain peptides, cyclization has been shown to improve membrane permeability. The discussion that follows highlights the benefits of peptide cyclization as well as the underlying chemical mechanisms.

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1.6.1. Stability:

Cyclic peptides are generally more proteolytically stable than linear peptides due to several key factors. Their rigid, closed-loop structure reduces the flexibility of the peptide backbone, making it harder for proteolytic enzymes to recognize and bind to the peptide, thereby limiting enzymatic access to cleavage sites.⁶⁰ Additionally, the lack of free N- and C-termini, common targets for proteases in linear peptides, decreases potential cleavage points. The cyclic nature of these peptides further limits their conformational freedom, preventing proteases from easily accessing and cleaving peptide bonds. Furthermore, cyclic peptides often exhibit enhanced binding affinity to biological targets due to their pre-organized structure, leading to more selective interactions and reduced susceptibility to proteases. These characteristics contribute to their greater stability in biological environments, making them attractive candidates for drug development.

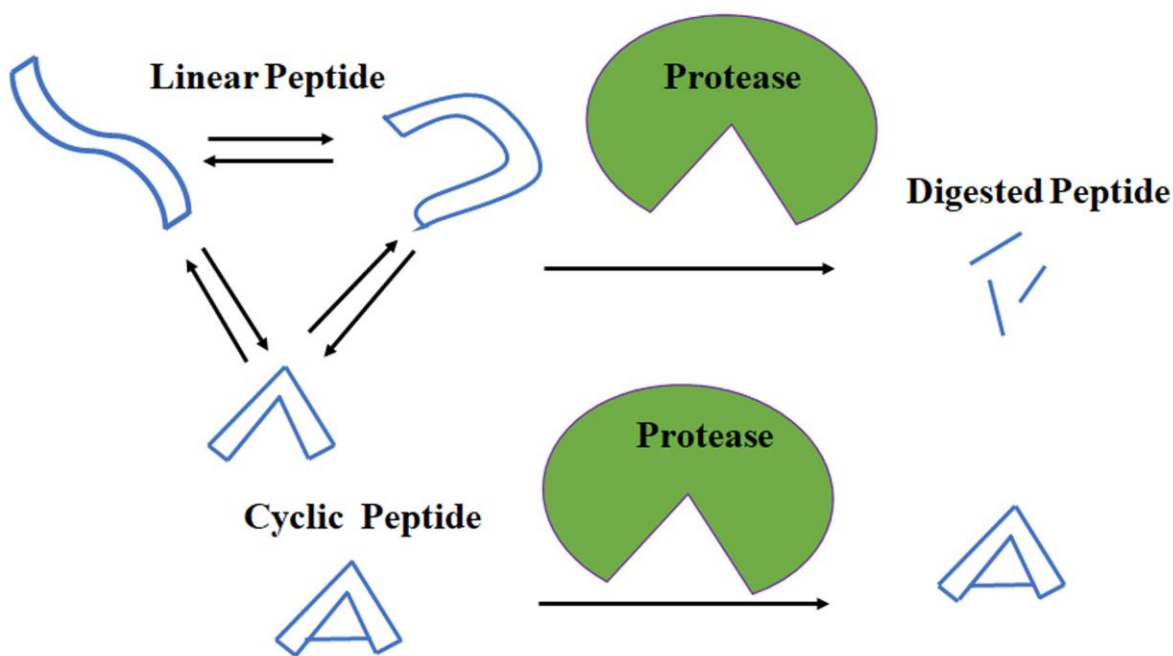


Figure 4: Effect of Cyclization on binding stability.

1.6.2. Binding Affinity and Specificity:

Cyclic peptides exhibit a superior ability to bind to targets with greater affinity compared to their linear counterparts, as evidenced by numerous comparisons between the two forms. Cyclic peptides regularly outperform their linear counterparts in phage display library screens, providing

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a strong indication of their improved binding. Devlin et al.'s 1990 phage display selection was one of the first to show this tendency. A random library screen of linear 15-mer peptides against streptavidin in this work produced a large number of peptides with two cysteines at random amino acid positions, indicating that the cysteines were most likely isolated in the disulfide-cyclized form.⁶¹ Subsequent in vitro selection tests yielded similar results, including phage display selections of bicyclic peptides against a wide range of targets. The bulk of isolated ligands were discovered to be bicyclic peptides, despite imperfect chemical cyclizations on phage producing combinations of cyclic and linear peptides.⁶²

The cyclization-induced increase in affinity can be ascribed to entropic variables. Cyclic peptides attach to targets with a lower entropic penalty than their linear counterparts because of their decreased flexibility in solution (see Figure 5). One additional element that contributes to the increased binding affinity of cyclic peptides is their restricted conformational range. Peptides that bind as folded structures, like α -helices or β -strands, are more likely to have binding events because cyclization stabilizes the conformation and increases the amount of secondary structure in solution. The design of inhibitors for protein-protein interactions (PPIs) involving α -helices has made strategic use of this idea. Cyclization, sometimes called stapling, is the method used to produce and stabilize peptides based on helical sequences.⁶³

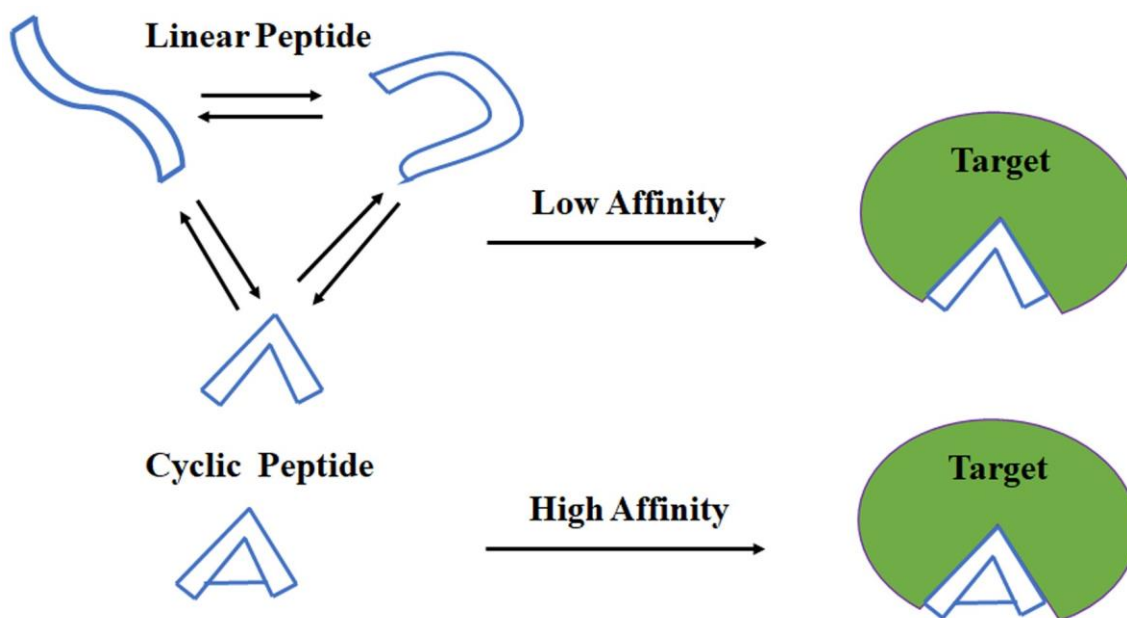


Figure 5: Effect of Cyclization on binding affinity and specificity.

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Furthermore, most naturally occurring or artificially produced high-affinity cyclic peptides made using display methods have excellent target selectivity that is on par with that of monoclonal antibodies. The limited conformation of cyclic peptides, which restricts the number of conformations that can be available for binding to non-targets, is the source of this selectivity. Analyzing the X-ray structures of protein-peptide complexes reveals the remarkable target selectivity of cyclic peptides. These structures often show perfect complementarity in charge and shape over large interaction surfaces (generally more than 500 Å²).⁶⁴

1.6.3. Membrane Permeability:

The desolvation of polar groups such as hydrogen (H)-bond donors within peptide bonds poses an energy barrier when peptides cross the nonpolar portion of a membrane. To overcome this difficulty, nature has cleverly used cyclization to encourage the creation of intramolecular hydrogen bonds in peptides. In the process, solvation is decreased and part of the polar surface area is hidden, which increases the possibility of membrane permeability (see Figure 6). In linear peptides, intramolecular hydrogen bonding is also possible, but it is relatively less preferred entropically.

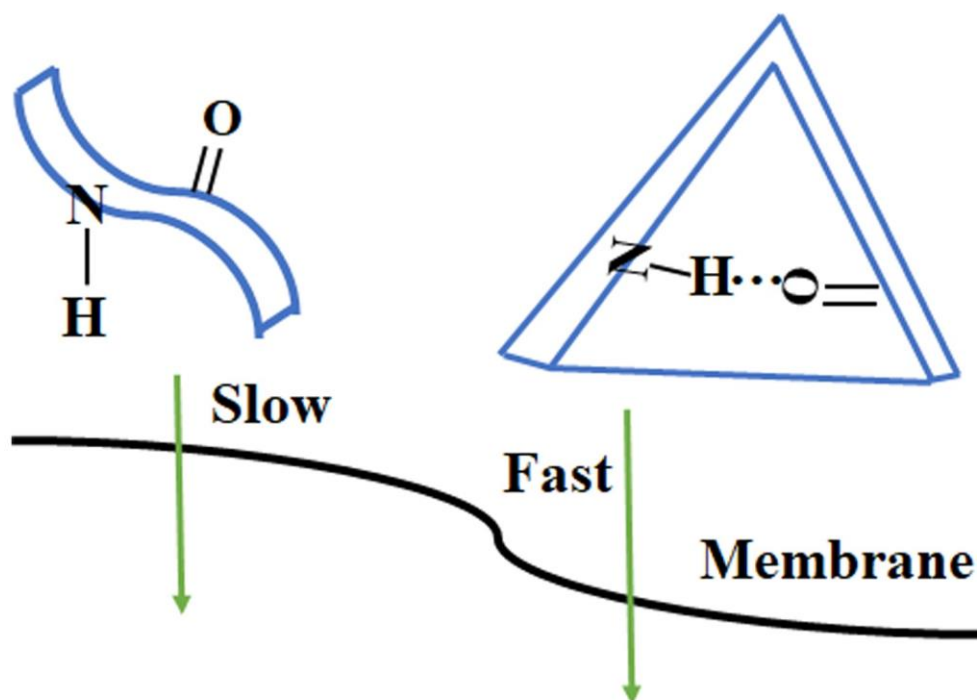


Figure 6: Effect of Cyclization on membrane permeability.

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The effects of intramolecular hydrogen bonding shielding have been thoroughly studied in the instance of cyclosporine, an 11-amino-acid cyclic peptide drug that is rather big in size yet is well-known for its oral bioavailability and cellular permeability. Cyclosporine N α -methylates seven of its eleven amide bonds in order to effectively cross membranes. The four other amide bond hydrogen donors form intramolecular hydrogen bonds in order to hide the donor and acceptor groups from the hydrophobic environment once they enter the lipid bilayer.⁶⁵

Cyclosporine modifies its conformation upon interacting to a target, using some of its polar groups to facilitate hydrogen bonding interactions. Cyclosporine's membrane permeability is reduced when it is linearized, highlighting the importance of its cyclic structure in the creation of intramolecular hydrogen bonds.⁶⁶ It has been shown that other naturally occurring cyclic peptides exhibit the same polar group concealing behavior as cyclosporine. It has also been purposefully done to design additional cyclic peptides with improved oral absorption and membrane permeability via intramolecular hydrogen bonding.⁶⁷

1.7. Self-Assembly and Supramolecular Chemistry of Cyclic Peptide:

A subfield of chemistry known as supramolecular chemistry studies chemical systems that have spatially ordered molecules as a result of noncovalent interactions.⁶⁸ Numerous mechanisms are involved in these interactions, such as van der Waals forces, hydrophobic contacts, metal coordination, hydrogen bonding, π - π interactions, and electrostatic interactions. Since its introduction by Moore and Winmill in 1912, hydrogen bonding has become one of the most often used noncovalent interactions in supramolecular chemistry.⁶⁹ Arrays of many hydrogen bonds show greater strength and directionality, while individual hydrogen bonds might not be strong enough to promote the creation of organized supramolecular structures.⁷⁰

Similar hydrogen bonding arrays are seen in many biological structures found in nature, including DNA's double helix and proteins' α -helices and β -sheets.⁷¹ These naturally occurring building blocks have inspired supramolecular chemists in the construction of supramolecular assemblies. These building blocks, which are simple to synthesize, derive, functionalize, and are biocompatible, have drawn a lot of interest in the last few decades from both basic research and real-world applications.

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Cyclic peptides (CPs) that self-assemble are bioinspired supramolecular building blocks that create supramolecular cyclic peptide nanotubes (SCPNs) by hydrogen bonding resembling a β -sheet.^{70b} These nanotubes provide a flexible pathway for building a wide variety of nanotubular shapes. Cyclic peptides' hydrophobic side chains efficiently protect hydrogen bonds from water molecules during the construction of nanotubes, which guarantees the remarkable durability of SCPNs in water and opens up a range of applications, including the creation of supramolecular assemblies and the investigation of biological processes.

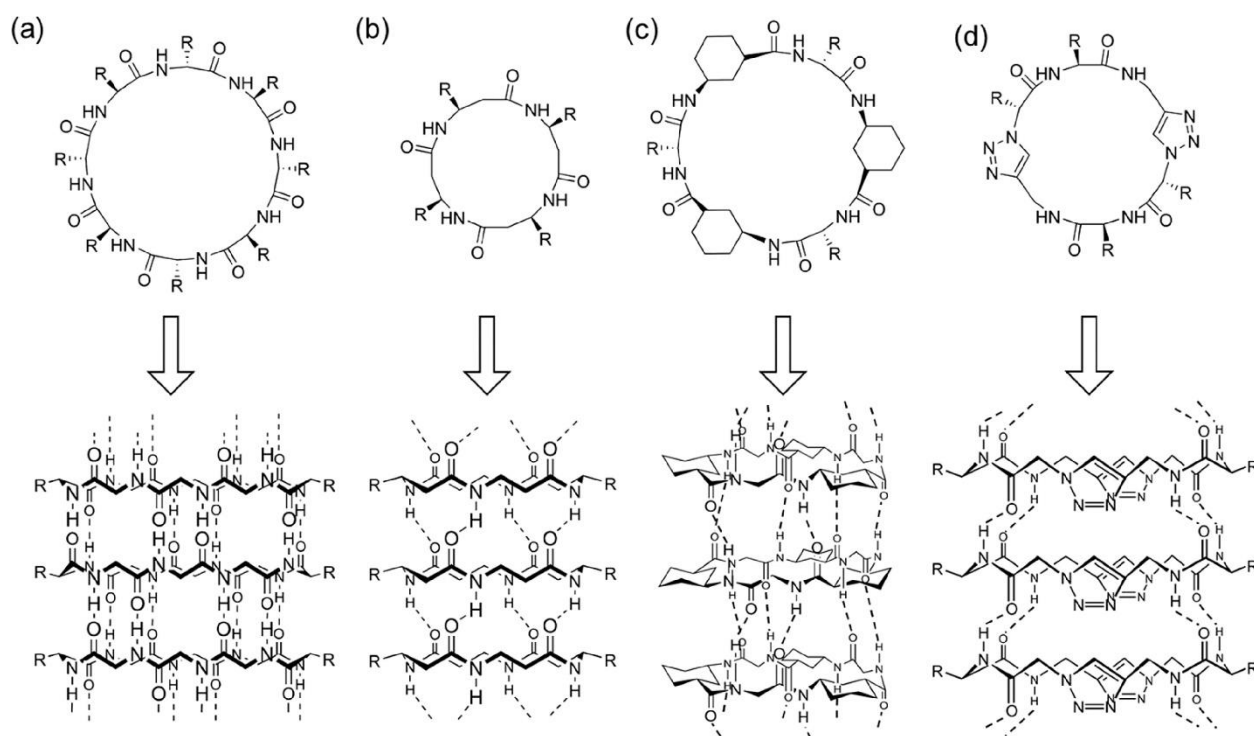


Figure 7: Different categories of cyclic peptides form supramolecular cyclic peptide nanotubes (SCPNs) via hydrogen bonding, including: (a) cyclic α -alt(D,L)-peptide, (b) cyclic β -peptide, (c) cyclic α,γ -peptide, and (d) cyclic peptide incorporating ϵ -amino acids.^{70e}

We give a thorough overview of the many forms of self-assembling cyclic peptides in this succinct introduction, including information on their structures, physical attributes, self-assembling traits, and synthesis methods. Additionally, we go over methods for creating and modifying cyclic peptides, such as conjugating them externally with polymers and modifying them internally. Peptide assemblies have found extensive use in bioapplications and functional materials, including drug delivery systems and ion transfer channels, thanks to polymer conjugation to cyclic peptides.

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The goal is to provide a thorough overview of the quickly developing field of supramolecular polymers that are produced by the self-assembly of cyclic peptides.

1.7.1. Tubular Assemblies of Cyclic Peptides:

Cyclic peptides that self-assemble are essential building blocks for creating artificial nanotubes. There are now several types of cyclic peptides that can form these structures: cyclic α -alt(D,L)-peptides, cyclic β -peptides, cyclic α,γ -peptides, and cyclic peptides with δ - or ϵ -amino acids that are different in terms of their chemical makeup. They all take on flattened ring-like conformations with the side chains of amino acids extending outward in a pseudo-equatorial orientation, notwithstanding the variances in their composition. The peptide backbone's amide and carbonyl protons align perpendicular to the ring plane at the same time. The ability to generate hydrogen bonds of the β -sheet type between adjacent peptide subunits on both sides of the ring structures aids in the development of these artificial nanotubes.

1.7.1.1. Tubular Assemblies of Cyclic α -alt(D,L)-Peptides:

A theoretical investigation carried out in 1974 by De Santis and colleagues suggested that cyclic peptides with an even number of alternating D- and L-amino acid residues could adopt conformations of the β -type, where the NH and CO bonds are orientated in opposite directions and almost parallel to the symmetry axis.⁷² Consequently, it was anticipated that cylindrical structures would arise as a result of the stacking of cyclic peptide rings in parallel or antiparallel configurations, stabilized by hydrogen-bond and van der Waals interactions. In the late 1980s, Lorenzi and associates made an initial attempt at experimental validation of these predictions (Figure 8).⁷³

Cyclo-[(D-Val-L-Val)_n], where $n = 2, 3, 4$], cyclo-[(D-Leu-L-Leu)₃], and cyclo-[(D-Phe-L-Phe)₃] are examples of cyclic oligopeptides that they studied. The amide band at 3270–3290 cm^{-1} in the infrared (IR) data indicates the presence of strong hydrogen bonds, and it was discovered that these peptides were insoluble in the majority of organic solvents.⁷⁴ The first well-characterized peptide nanotube made of a cyclic α -alt(D,L)-peptide was described by Ghadiri et al. in 1993. Two glutamic acids were combined to create the octapeptide cyclo-[(L-Gln-D-Ala-L-Glu-D-Ala)₂], which prevents subunit interaction in basic aqueous solution. By using molecular modeling, TEM, ED, and FT-IR spectroscopy, it was determined that acidification caused the production of rod-

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shaped crystals. These analyses revealed organized tubular structures with 7.5 Å internal diameters and 4.73 Å intervals between ring-shaped subunits due to antiparallel backbone–backbone hydrogen bonding.⁷⁵

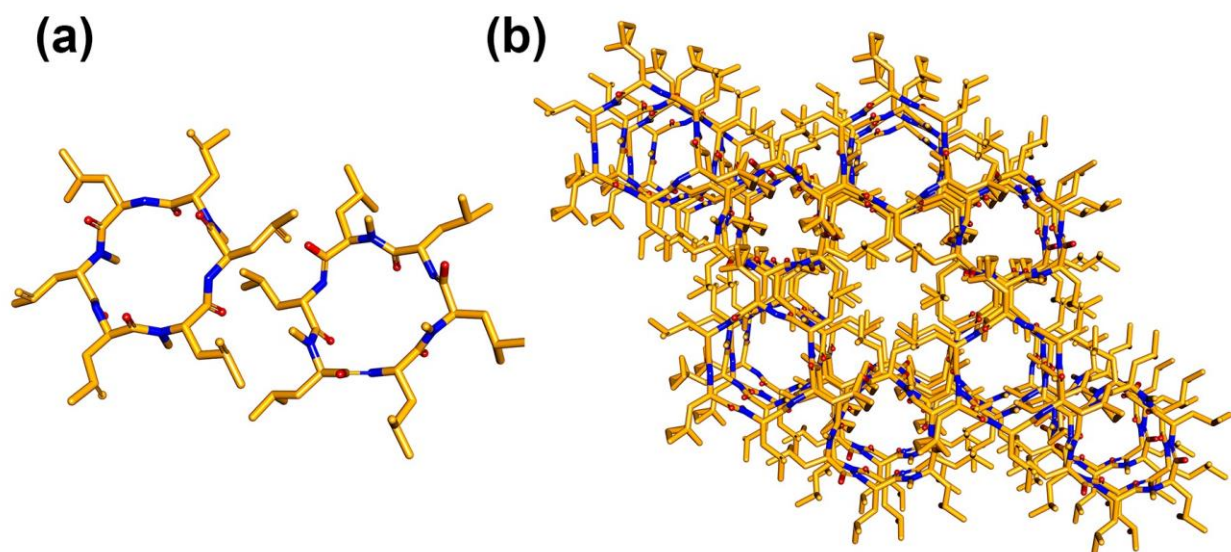


Figure 8: (a) X-ray analysis: Asymmetric unit composed of dimeric cyclo(-D-Leu-L-MeLeu-D-Leu-L-MeLeu-D-Leu-L-MeLeu-) (b) Packing pattern of cyclo(-D-Leu-L-MeLeu-D-Leu-L-MeLeu-D-Leu-L-MeLeu-) exhibited nanotubular channel.⁷³

Cyclic peptide sequences have a rational design that may lead to more ordered self-assembled structures. By creating a cyclic peptide that can switch from 1D to 2D self-assembly in water, Montenegro et al. illustrated this idea (See Figure 9). Their asymmetric cyclic sequence, cyclo-[D-Leu-L-Trp-D-Leu-L-His-D-Glu-L-Gln-DHis-L-Glu-], has separate hydrophobic and hydrophilic domains, containing residues that were sensitive to pH.⁷⁶ Amphiphilic nanotubes formed in solution as a result of hydrogen bonding interactions between peptide rings. These nanotubes subsequently arranged into a nanotubular bilayer to protect their hydrophobic portions from the surrounding aqueous environment. Significantly, deprotonated glutamate residues and neutral histidine residues on the nanotubes' highly anionic surface at physiological pH prevented individual layers from aggregating and instead promoted the formation of big, flat nanosheets with high micrometer-scale dimensions. These nanosheets were astonishingly thin, with a thickness of only 3.2 nm, considering their size. These nanosheets also exhibited dynamic behavior, being able to change from one state of pH to another and back again into 1D nanotubes.

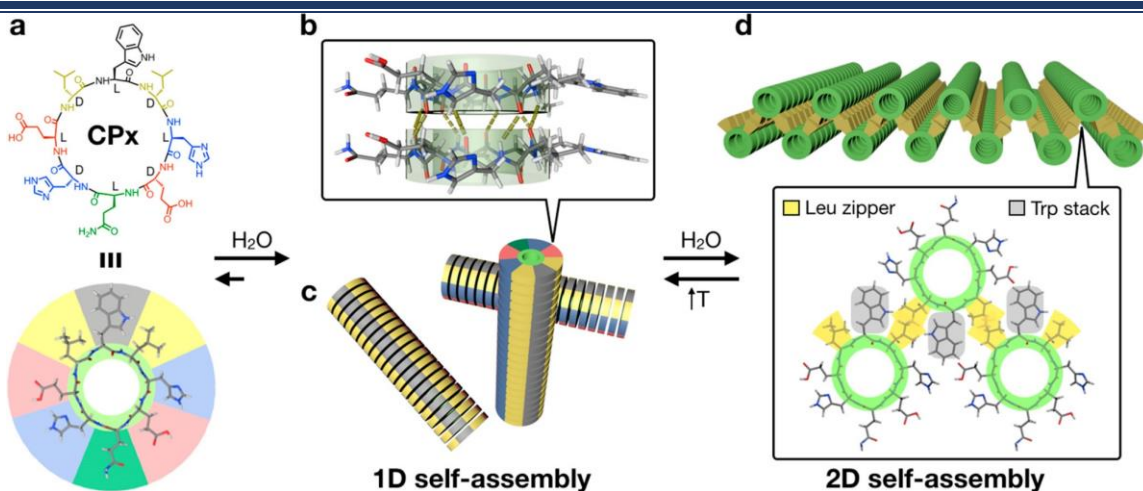


Figure 9: Proposed model for the sequential 1D-to-2D self-assembly of cyclic peptide -[D-Leu-L-Trp-D-Leu-L-His-D-Glu-L-Gln-DHis-L-Glu-].(a) Chemical structure (b) The formation of amphiphilic nanotubes is shown, facilitated by multiple hydrogen-bonding interactions between the peptide rings, (c,d) These amphiphilic nanotubes further organize into a 2D nanosheet assembly. Reproduced with permission from Copyright American Chemical Society.⁷⁶

1.7.1.2 Arrangements of Cyclic β -Peptides in Tubular Formations:

It has been shown that cyclic β -peptides, which are made up of a particular amount of β -amino acids, can generate SCPNs that resemble those made by cyclic α -alt(D,L)-peptides. While producing synthetic unnatural amino acids may present difficulties, β -peptides' resistance to enzymatic degradation and their advantageous structural features highlight their prospective advantages over cyclic α -alt(D,L)-peptides.

Seebach and colleagues pioneered the synthesis and characterization of cyclic β -peptides in 1997.⁷⁷ Using 3-amino butanoic acids, they created and produced three stereoisomeric cyclic β -tetrapeptides. Powder diffraction was used to identify the crystal structures of these three isomers (Figure 10). Four parallel intermolecular hydrogen bonds gave rise to tubular structures in all three isomers. All the isomers, however, took on unique conformations. With C₂ symmetry, the (all-S)-derivative was shaped like a flat parallelogram (Figure 10a). The two tetrahedral C atoms formed bridges between its amide planes, which were positioned within the sides. It's interesting to note that all four methyl groups were in pseudoequatorial positions, but the four C=O and the four N-H bonds were mutually antiperiplanar and directed in the same direction (Figure 11a).

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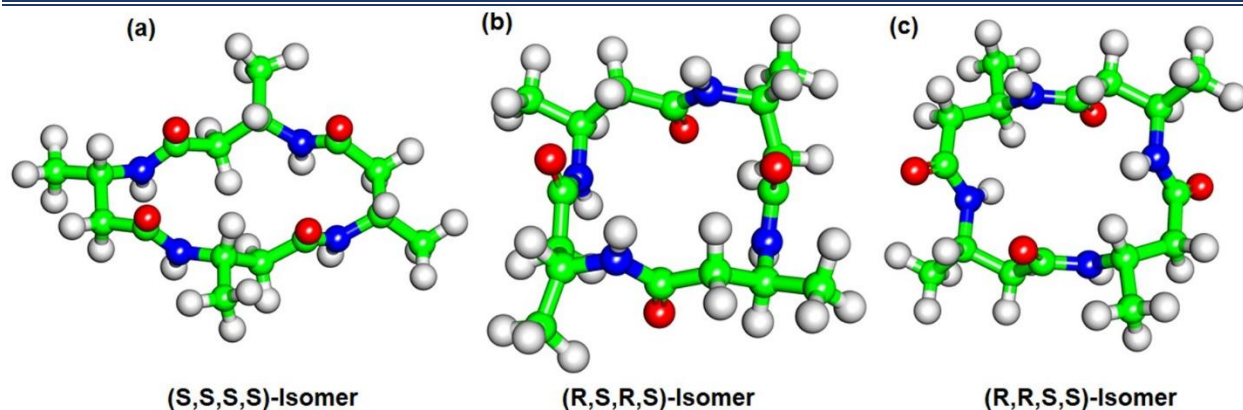
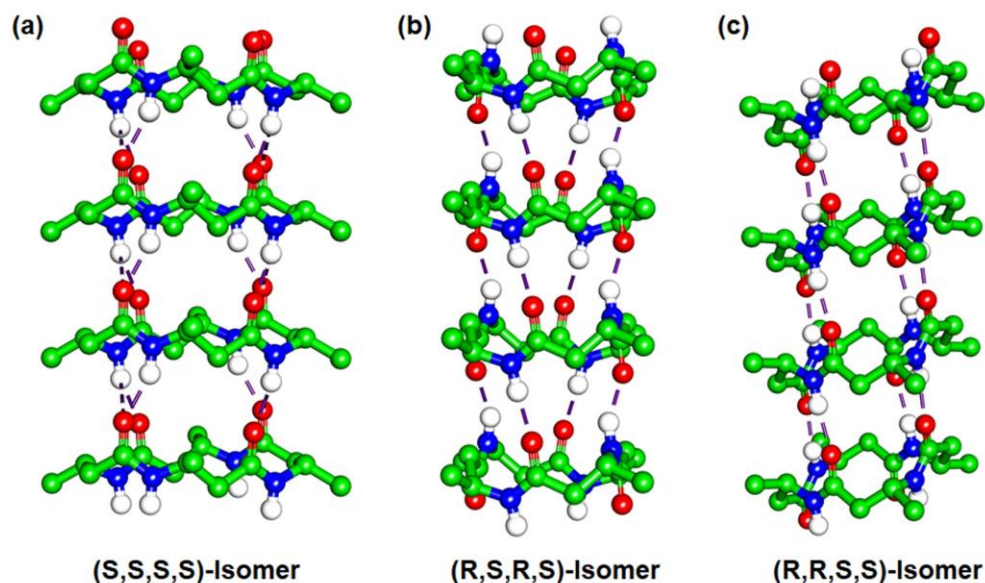


Figure 10: Structure of Cyclic Tetrapeptide from powder diffraction data. (a) All (S,S,S,S)-Isomer, (b). (R,S,R,S)-Isomer (c). (R,R,S,S)-Isomer.

As a result, a macrodipole was formed along the nanotube by the parallel stacking of the peptide rings (Figure 12a). Similar to the conformation of cyclic α -alt(D,L)-peptides, the (R,S,R,S)-isomer displayed a flat square shape (Figure 10b) with S4 symmetry, where opposite pairs of C=O links and N–H bonds directed in the same direction (Figure 11b). The peptide rings in this instance stacked parallel to one another as well (Figure 12b), but no macrodipole was anticipated. Regarding the (R,R,S,S)-isomer, Figure 10c depicts its cisymmetric rectangular shape, with parallel C=O and N–H bonds on adjacent sides. The peptide rings were oriented differently in relation to the stacking orientation than other isomers (Figure 12c). Because of their nonlinear hydrogen bonding, all three isomers displayed tubular stacking (Figure 11).



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Figure 11: Intermolecular Hydrogen bonding pattern of (a) a (S,S,S,S)-Isomer, (b) b (R,S,R,S)-Isomer (c) c (R,R,S,S)-Isomer.

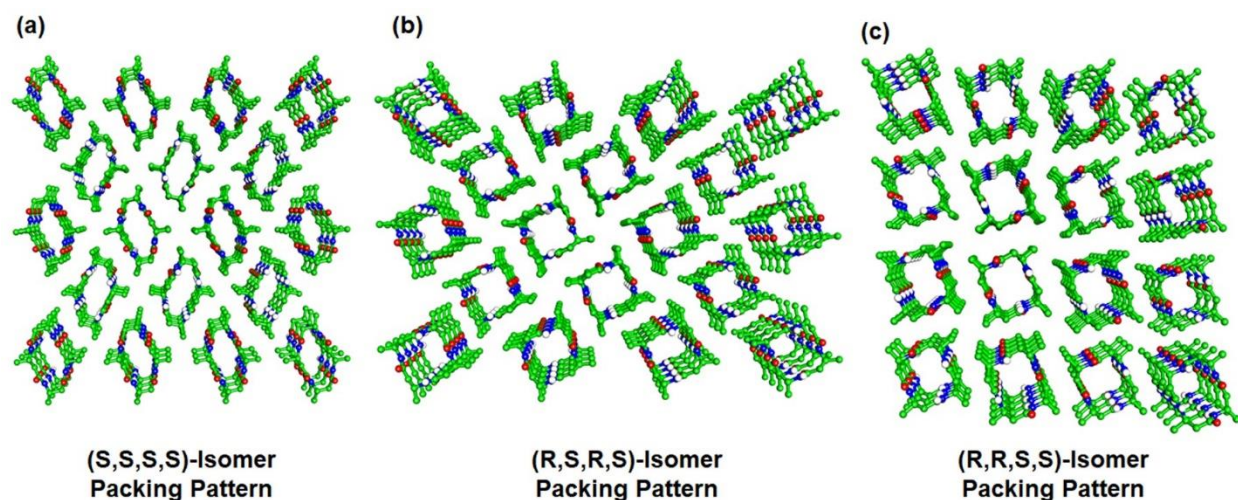


Figure 12: Packing pattern in self-assembly: (a) All (S,S,S,S)-Isomer, (b). (R,S,R,S)-Isomer (c). (R,R,S,S)-Isomer.

It is clear that the ability of cyclic β -peptides to stack is very flexible. The flexibility of the rings to adopt conformations that are good for stacking is ascribed to the presence of extra CH_2 units. As such, cyclic β -peptides are not subject to the same constraints as their α -equivalents.

It is anticipated that the stacking of homochiral cyclic β -peptides will produce a macrodipole, which will impact conductance in multiple ways, such as current rectification and voltage gating. Better performance as artificial transmembrane ion channels could result from this consequence. Ghadiri and associates investigated ion transport functions in cyclic β -peptides that form channels. They looked at $\text{cyclo}[-(\beta^3\text{-HTrp})_4-]$, $\text{cyclo}[-(\beta^3\text{-HTrp-}\beta\text{-HLeu})_2-]$, and $\text{cyclo}[-(\beta\text{-HLeu})_4-]$ as homochiral cyclic β -tetrapeptides.⁷⁸ Ghadiri's group suggested that these cyclic β -tetrapeptides would take on an alternate C_4 symmetrical conformation when embedded in a lipid membrane environment, yielding an internal diameter of 2.6–2.7 Å, as opposed to the C_2 symmetrical conformation that Seebach had previously observed. Consequently, the K^+ ion transport rate of the cyclic peptide $\text{cyclo}[-(\beta^3\text{-HTrp})_4-]$ was found to be 1.9×10^7 ions per second, which was similar to the K^+ transport rate previously reported for the cyclic α -alt(D,L)-peptide $\text{cyclo}[(\text{L-Trp-D-Leu})_3\text{-L-Gln-D-Leu-}]$.⁷⁹ It's crucial to remember that in order to determine the exact relevance of the current macrodipole, cyclic β -peptides and cyclic α -alt(D,L)-peptides with comparable internal

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diameters and chemical structures must be directly compared. Recently, Kimura et al. presented a novel class of cyclic β -peptides composed of amino acids produced from sugar (SAAs). Because they have many hydroxyl groups, these β -SAAs with a pyranose ring as a side chain improve the solubility of the corresponding cyclic β -peptides. In addition, the pyranose ring is expected to promote a planar structure in the cyclic peptide, which will enable molecular stacking and tubular formation. Also, Cyclic β -peptides containing 3-, 4-, and 6-acetylated β -glycosamino acid units were synthesized and analyzed.⁸⁰ These peptides will form nanotubular structures and remain well-dissolved in organic solvents such as pyridine, DMSO, DMF, TFE, and HFIP. Acetyl groups were then removed, resulting in the formation of water-soluble cyclic β -peptides and nanotubes with protein sites of attraction, particularly for lectins.⁸¹ Interestingly, it was found that when the cyclic hexa- β -peptide was dissolved in water with a polyethylene glycol (PEG) chain, it formed a polypseudorotaxane.⁸²

Nonetheless, the unique macrodipole present in the nanotubes generated by cyclic β -peptides implies that they could function more effectively as synthetic transmembrane ion channels. However, there is currently a lack of research on their uses in this context.

1.7.1.3 Arrangements of Cyclic α,γ -Peptides in Tubular Form:

Granja's group discovered and produced a novel class of self-assembling cyclic peptides with distinct structural properties and internal voids.⁸³ An even number of cyclic γ -amino acids and alternating α -amino acids make up these peptides. These peptides adopt planar ring-like conformations, similar to cyclic α -alt(D,L)-peptides and cyclic β -peptides, that promote the creation of nanotubes through interactions between hydrogen bonds between molecules. Most cyclic peptide nanotubes, such as cyclic α -alt(D,L)-peptides and cyclic β -peptides, have unchangeable hydrophilic interior canals that only permit the passage of polar molecules, as was previously described. On the other hand, cyclic α,γ -peptides produce a partially hydrophobic cavity because one of the cycloalkane methylenes projects into the interior when cyclic γ -residues are included.

Simple chemical adjustments can be used to further modify this cavity, providing precise control over the nanotube's transport properties. The alternating arrangement of α and γ amino acids in the flat ring-like conformation places all of the γ -amino acid's amide groups on one face (α -face) and

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all of its NH and CO groups on the other. The cyclic peptide rings are predicted to alternate orientations due to differences in $\text{CO}\cdots\text{HN}$ spacings between the α -face and γ -face. This will produce two different hydrogen bonding patterns, one of which involves selective α - α and γ - γ face stacking in an antiparallel arrangement. Recent studies, however, have shown that a parallel configuration made possible by interactions between the γ - and α -faces is more stable.⁸⁴

Using β -sheet-like hydrogen bonding arrangements, the Granja group has synthesized new -Acp-based, Cyclic Peptides⁸⁵ that, despite their huge diameters, readily assume flat conformations (Figure 13a) and form very stable dimers (Figure 13b). The capacity of dimers to organize into nanotubular solid-state arrays, as seen in crystals derived from a tetrachloroethane solution, was verified by crystallographic analysis (Figure 13c). It's interesting to note that these crystals also had rings of dichloroacetic acid molecules, which were probably created by the solvent oxidizing in response to light. This novel crystallization technique produced nanotubes that could find use in gas storage and separation technologies, among other areas.

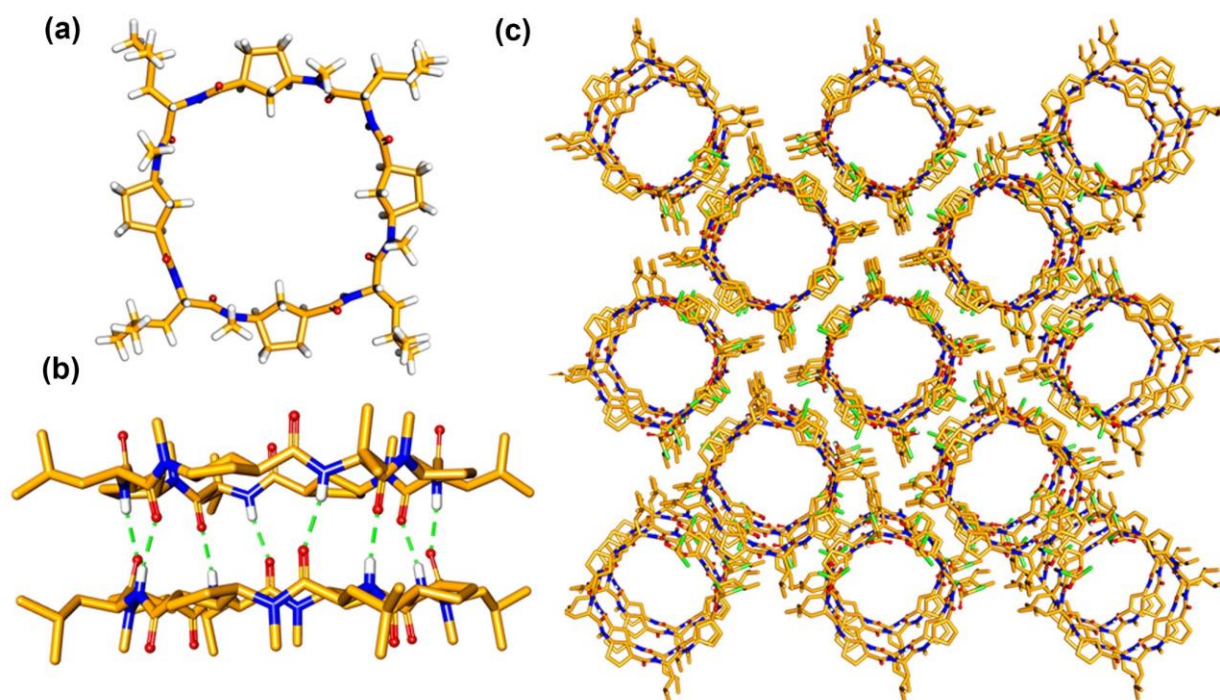


Figure 13: (a) X-ray analysis: molecular crystal structure, (b) Hydrogen bonding pattern in dimeric unit (c) Packing in three dimensions exhibited nanotubular channel.

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1.7.1.4 Arrangements of Cyclic δ -Peptides in Tubular Form:

In the beginning, Dory and associates studied cyclic peptides, which contain δ -amino acids.⁸⁶ It was noticed that a cyclic tripeptide with trans orientation in the vinyl group and α,β -unsaturated δ -amino acid residues crystallized in bundles of nanotubes (Figure 14a). X-ray single crystal analysis was used to identify the exact nanotubular structure. With all three CO and all three NH bonds pointing in the same direction, the cyclic peptide ring has a stiff triangle form (Figure 14b). As a result, a similar to cyclic β -peptides, a macrodipole is formed along the nanotube by the parallel alignment of the peptide rings (Figure 14c). But because of their flexibility, β,γ -unsaturated δ -amino acid residues in cyclic tetrapeptides and tripeptides showed undesirable packing. As a result, the cyclic tetrapeptide was unable to produce nanotube, and the cyclic tripeptide's crystal structure revealed that only two of the three amides were involved in the stacking of macrocycles, which had a rectangular shape because of one β -turn.

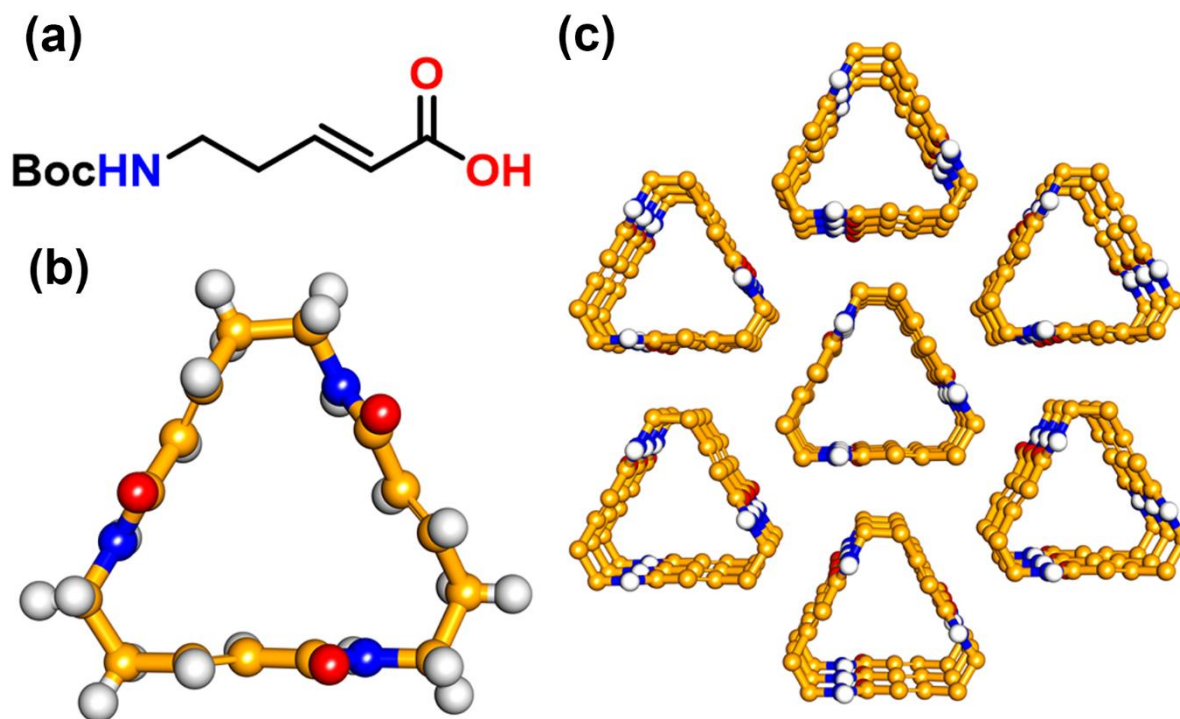


Figure 14: (a) Building Block: α,β -unsaturated δ -amino acid (b) crystal structure of cyclic α,β -unsaturated δ -amino acid containing tripeptide (c) Packing pattern of cyclic α,β -unsaturated δ -amino acid containing tripeptide exhibited nanotubular structure.

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Later, research teams under the direction of Zhao and Dory developed simple techniques to limit the structural flexibility of the previously stated cyclic tetrapeptide, which was made up of residues of β,γ -unsaturated δ -amino acid. They were able to do this by the use of hydrogen-bonded polymer or liquid crystal, which produced hexagonal hollow nanotubes.⁸⁷ Trans-4-aminocyclohexanecarboxylic acid (δ -Ach) is an important building component for creating cyclic peptides that contain δ -amino acid residues, just as γ -Ach is used to create cyclic peptides with γ -amino acid residues.

Even though δ -Ach is stiff enough to take on a planar ring-like conformation, cyclic peptides made entirely of δ -Ach residues cannot form a closed cyclic structure because of the 180° angle created by the bonds between the amino group–C δ and the carboxylic acid–C α . Rather, to create cyclic peptides that can form nanotubes, they must be coupled with α -residues. Therefore, in the creation of nanotubes with a greater diameter and hydrophobic interior characteristics, the δ -residue predominantly serves as a stiff extension. It can be added to a cyclic α -alt(D,L)-peptide after each α -amino acid to create a new cyclic peptide called cyclic D,L- α,δ -peptide. Because of their simplicity, N-methylated cyclic peptides were first studied.⁸⁸

We have carefully examined the stability differences between the parallel and antiparallel contacts of these cyclic D,L- α,δ -peptides that form dimers. Additionally, a pseudo-enantiomeric form of the previously stated dodecapeptide was created. While the two pseudo-enantiomers can arrange themselves in a parallel configuration to create heterodimers, each pseudo-enantiomer has the ability to generate homodimers on its own in an antiparallel fashion. According to experimental investigations, the heterodimers are roughly $1.80 \text{ kcal mol}^{-1}$ more stable than the homodimers. The relative location of the axial protons of the cyclohexyl moieties is most likely the cause of this variation in stability, according to computational simulations. Stated differently, the parallel heterodimer exhibits increased stability because the steric barrier between the cyclohexyl moieties allows the heteromer to form shorter hydrogen bonds.

1.7.1.5 Arrangements of Cyclic ϵ -Peptides in Tubular Form:

A similar method can be applied to the engineering of cyclic D,L- α,ϵ -peptides. In order to create a cyclic D,L- α,ϵ -tetrapeptide, a new 1,4-disubstituted-1,2,3-triazole ϵ -amino acid was first synthesized using copper-catalyzed azide–alkyne cycloaddition (CuAAC) (Figure 15).^{89,90}

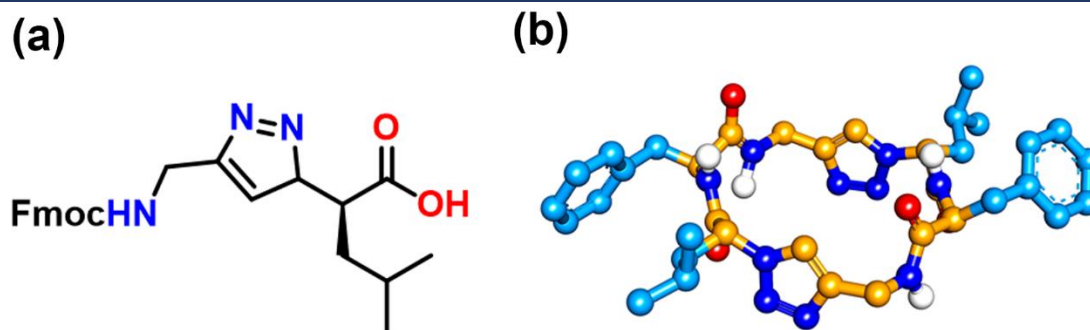


Figure 15: (a) **Building Block:** 1,4-disubstituted-1,2,3-triazole ϵ -amino acid; (b) crystal structure of cyclic D,L- α,ϵ -tetrapeptide (solvent ethanol molecule is omitted)

^1H NMR spectroscopy indicated that this peptide displayed the expected intermolecular aggregation behavior in CDCl_3 , driven by hydrogen bonding. The results showed relatively high association constants ($>104 \text{ M}^{-1}$). Direct proof of the nanotubular structure was obtained by X-ray crystallography, which demonstrated that peptide subunits took on a flat, ring-shaped conformation and stacked parallel to create tubular assemblies (Figure 16). Remarkably, three of the four amide bonds in the ring backbone contributed to an ethanol-mediated bridging hydrogen link, and the fourth amide bond produced a network of intermolecular hydrogen interactions.

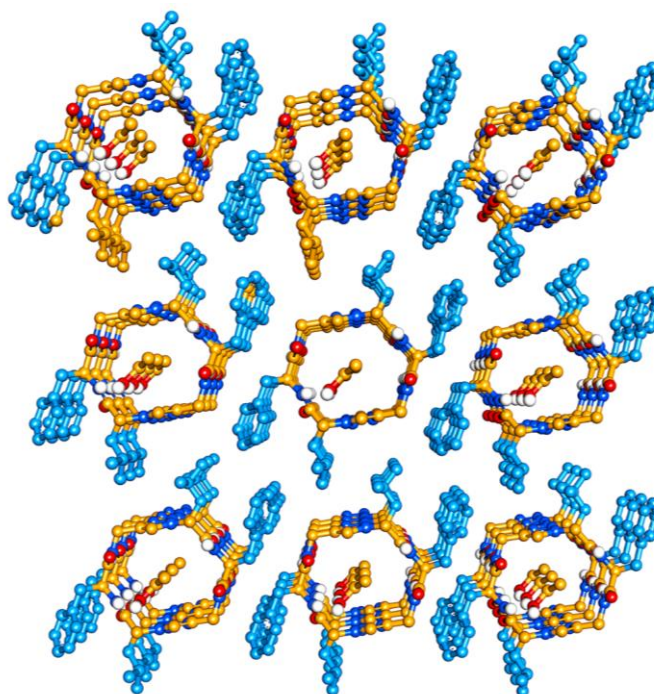


Figure 16: Packing pattern of cyclic D,L- α,ϵ -tetrapeptide exhibited nanotubular structure.

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1.8. Design and Customization of Cyclic Peptides for specific purposes:

The amount and makeup of amino acids in the cyclic peptide determine the diameter of the inner space in SCPNs. Table 1 shows that within the range of cyclic peptides previously discussed, the inner chamber's diameters can be accurately tuned from 1 to 30 Å, allowing them to be used as nanocarriers or nanochannels for various target molecules. The side chains of the amino acids affect the surface properties of SCPNs. As a result, it is easy to create SCPNs with certain characteristics by carefully planning peptide sequences. Moreover, covalent modification of the corresponding cyclic peptides allows for the fine-tuning of the exterior and interior characteristics of SCPNs.

Table 1. Summary of Internal Diameters Across Various Cyclic Peptides reported in literature.^{70e}

Type	No of Amino Acids	Internal Diameter in Reported examples
α-alt(D,L)-Cyclic Peptide	8	7-8
	10	10
	12	13
β-Cyclic Peptide	3	-
	4	2.6-2.7
	6	5.1
α,γ-Cyclic Peptide	4	1
	6	7
	8	10
	10	13
	12	17
D,L-α,δ-Cyclic Peptide	12	21
	16	30

1.8.1. Internal Modification and Functionalization:

Modification of the internal characteristics of the SCPN is possible through the projection of functional groups into its interior space through the incorporation of γ -, δ -, and ϵ -amino acids into peptide sequences. As an example, a hydroxyl group was synthesized into the cavity of a cyclic tetrapeptide, which resulted in higher association constants than other peptides of a comparable kind.⁹¹ Chemical modification further allows tuning of cavity properties, such as incorporating a

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pyridine moiety to trap various guests inside the structure.⁹² Similarly, modification of cyclic γ -Acas can finely control nanotube transport properties.⁹³ Moreover, the utilization of ϵ -amino acids promotes the creation of pH-responsive nanotubes, opening up new possibilities for the development of intelligent transmembrane channels.⁹⁴ Alternative approaches, such as the use of cyclic α -alt(L,L,L,D) peptides, also allow for the internal functionalization of nanotubes, which has promise for improvements in a number of areas, including separation technologies and synthetic ion channels.⁹⁵ All things considered, these methods present chances to create nanotubular structures with the required internal characteristics, opening up new directions for study and use.

1.8.2. External Modification and Functionalization:

In addition to determining the exterior properties of SCPNs, the side chains of amino acids facing outward serve as reactive sites for further chemical modification. These alterations allow for the effective alignment of functional groups since they do not obstruct the cyclic peptides' ability to self-assemble. For instance, attaching naphthalenediimide (NDI) moieties to cyclic peptides directed aromatic ring stacking, leading to the formation of electronically delocalized nanotubes.⁹⁶ Strategies using external components like Zn-porphyrins successfully controlled the arrangement of aromatic moieties within dimeric assemblies, offering potential applications in optical and electronic devices.⁹⁷ Peptide backbone interactions generate heterodimers that facilitate the insertion of various functional groups, resulting in the creation of innovative functional materials that can facilitate the flow of electrons or energy.⁹⁸ These developments pave the way for precisely arranging functional moieties in nanomaterials to create customized materials with desired characteristics.

1.9. Applications of Self-assembling Cyclic Peptides in Biology:

In the field of biochemical sciences, cyclic peptide nanotubes (CPNTs) play crucial roles as antibacterial agents and as essential constituents of lipid bilayers that facilitate ion channel activities. Utilizing CPNTs to create nanomaterials has also been the focus of recent research projects. These include the creation of stimuli-responsive supramolecular materials and sensing devices. This section highlights the various uses of N-alkylated cyclic peptides and cyclic peptide nanotubes.

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1.9.1. Ion Channels:

Cyclic peptides (CPs) are a good choice for ion channels because of their ability to break through membranes and incorporate into lipid bilayers. The potential of cyclic peptides to form "channels" that can: (i) allow small molecules to pass through the narrow space of their internal core; (ii) interact with and embed into lipid membranes due to particular amino acid combinations, causing structural disruptions; or (iii) interact uniquely with native peptide or lipid components in a biological setting, thereby influencing specific processes, has been explored because of their ability to assemble into elongated structures. Investigating these behaviors has involved various approaches. For instance, Ghadiri and colleagues investigated the administration of peptide nanotube assemblies to phosphatidylcholine liposome models with varying internal and external pH levels. They have performed fluorescent spectroscopy with a marker to track the pH gradient's collapse and attributed it to transmembrane channel development. Ghadiri and colleagues demonstrated efficient proton transfer by monitoring changes in fluorescence intensity in a solution containing large unilamellar vesicles (LUVs) with a pH-sensitive dye.⁹⁹

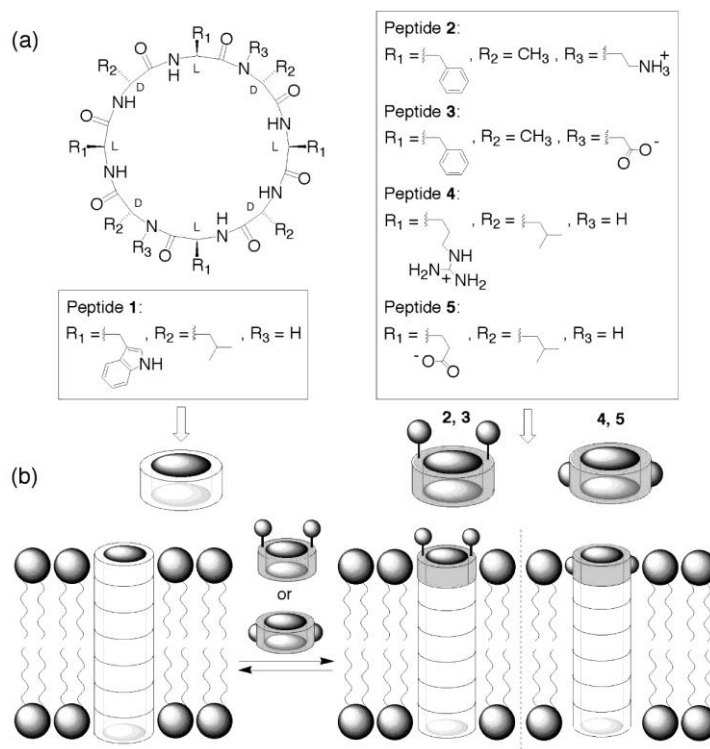
Interestingly, they discovered that not all CPs could be incorporated into lipid bilayers. For example, cyclo[-(L-Gln-D-Leu)₄-] could not insert into lipid bilayers, whereas cyclo[-(L-Trp-D-Leu)₃-L-Gln-D-Leu-] did. Furthermore, Ghadiri et al. used CPNTs to demonstrate the transport of K⁺ and Na⁺ across membranes.⁹⁹

Additionally, endogenous transmembrane proteins like α -hemolysin can have their ion transport function modulated by cyclic peptides. Sañchez-Quesada et al. found that N-substituted cyclic peptides and CPs with net positive or negative charges can lodge into the lumen of the α -hemolysin-barrel channel, changing the pore characteristics.¹⁰⁰ Anion selectivity is increased by adding cationic cyclic peptides, and cation-selective transmembrane pores are produced by adding negatively charged CPs.¹⁰⁰ N-alkyl-CPs can also be used as capping agents to accomplish this modulation. Larger pore diameters are necessary for the selective transmembrane transport of organic molecules like glutamic acid and glucose¹⁰¹, which depends on the length of the amino acid chain that forms the cyclic peptide.

Granja and Ghadiri assessed the capacity of a 10-residue cyclic peptide, cyclo[-(L-Trp-D-Leu)₄-L-Gln-D-Leu-], partitioned in LUVs and having an internal diameter of 10 Å, to transport D-glucose. In a similar way, the identical peptide promoted glutamic acid's transmembrane

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trafficking from LUVs. Enzyme-coupled assays were used to track these activities and identify variations in the NADPH or NADH absorption bands.¹⁰² Moreover, the Ghadiri group created a specialized technique using selective heteromeric supramolecular assembly to create functional single channels with altered ion conductance, charge selectivity, and rectification (Figure 17). In this procedure, the naturally occurring hollow transmembrane tubular structure, which is produced by cyclic-D,L- α -peptides self-assembling in lipid bilayers—is altered by including "cap" subunits of cyclic peptides that are specifically bound to the entrance of the channel assembly.¹⁰³ Granja and Ghadiri evaluated the D-glucose transport ability of a 10-residue cyclic peptide, cyclo[-(L-Trp-D-Leu)₄-L-Gln-D-Leu-], with an internal diameter of 10 Å, partitioned in LUVs. Similarly, the same peptide facilitated the transmembrane transport of glutamic acid from LUVs. These processes were monitored using enzyme-coupled assays to detect changes in NADPH or NADH absorption bands.¹⁰² Further Ghadiri group developed a specialized method to craft functional single channels with adjusted ion conductance, charge selectivity, and rectification through selective heteromeric supramolecular assembly (Figure 17). This process involves modifying the spontaneously formed hollow transmembrane tubular structure, generated by the self-assembly of cyclic-D,L- α -peptides within lipid bilayers, by integrating designer cyclic peptide "cap" subunits that selectively bind at the channel assembly's entrance.¹⁰³



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Figure 17: (a) Scheme of the cyclic D,L- α -peptides, (b) Peptide 1 autonomously organizes within lipid membranes, constructing the fundamental structure of transmembrane channels. Peptides 2 to 5 function as auxiliary subunits, forming caps that lead to the formation of diverse channel assemblies with modified ion conductivity characteristics.¹⁰³ Reproduced with permission from Copyright American Chemical Society.¹⁰³

1.9.2. Antimicrobial Treatments:

In recent years, the healthcare system has faced a significant hurdle with the rise of bacteria which have become resistant to traditional antibiotics due to widespread use among the public.¹⁰⁴ There's growing concern that these medications will progressively lose their effectiveness, prompting a shift in research towards finding alternative treatments to replace outdated clinical therapies. This problem has been addressed by cyclic peptide nanotubes, which have demonstrated strong antibacterial activity against both Gram-positive and Gram-negative bacteria. Their attractiveness as a workable solution to counteract antibiotic resistance is further strengthened by the fact that their structural stiffness offers improved proteolytic stability.

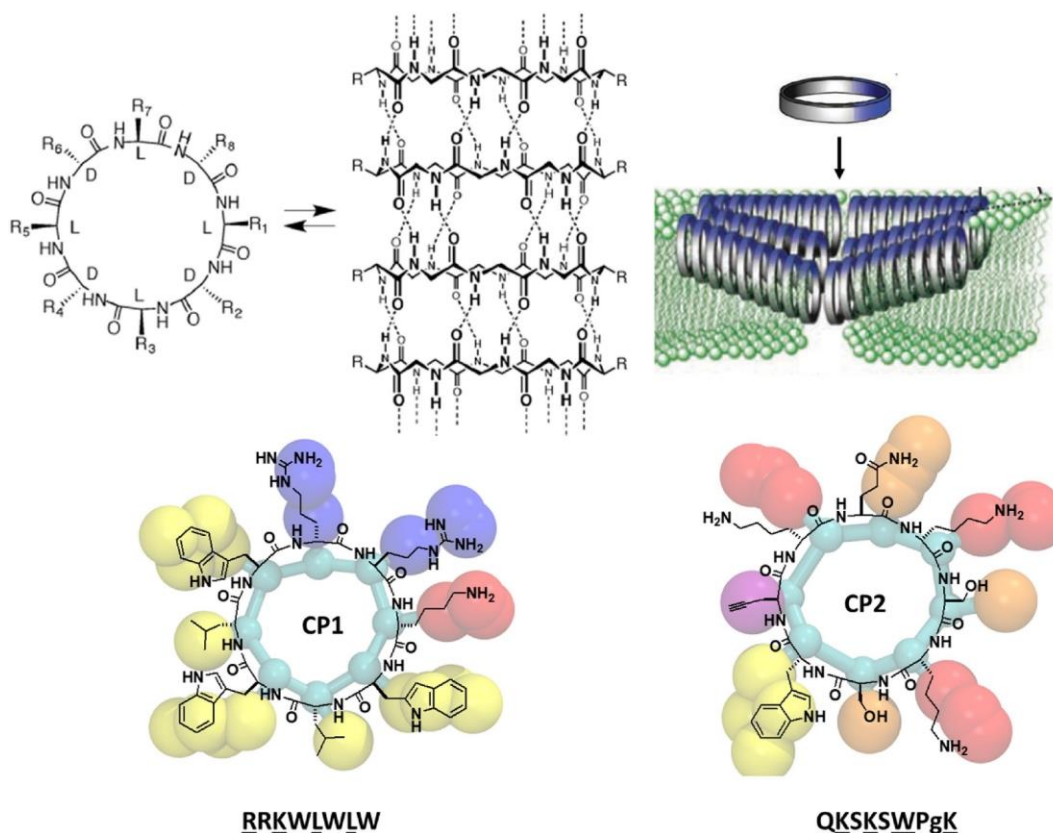
The study of structure-activity interactions in cyclic peptide nanotubes (SCPNs) was first proven by Lopez et al., who also defined SCPNs as a new class of antibacterial drugs.¹⁰⁵ They demonstrated how amphiphilic cyclic α -alt(D,L)-peptides, which are made up of three consecutive hydrophilic amino acids and alternating L-Trp and D-Leu segments, worked as ion transporters in lipid bilayer models and might have integrated into bacterial membranes. Cyclo-[D-Lys-L-Glu-D-Arg-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-] is a particular cyclic peptide that has been shown to be effective against a range of Gram-positive and negative bacterial strains. According to Lopez et al., the process entailed membrane insertion, breaking down transmembrane ion channels, and raising permeability, which resulted in a "carpet-like" mechanism that killed the bacteria. Additional cyclic peptide modifications to build a compound library clarified the effect of charge and hydrophobicity on activity. For example, the antibacterial properties were eliminated when histidine was added in place of lysine, and the inclusion of glutamic acid reduced activity because of electrostatic repulsion with the negative charges in the membrane. This demonstrates how rational design may improve these materials' efficacy in a variety of antibacterial models.

Applying screening procedures that are often used for small molecule drugs to macromolecular entities demonstrates how broadly applicable and biocompatible these approaches are outside of

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clinical settings. Claro et al. have modelled the interactions between several cyclic α -alt(D,L)-peptides and antimicrobial membrane targets using logic-based prediction techniques, such as computational simulations.¹⁰⁶ They contend that understanding of membrane/antimicrobial agent interactions can be improved by combining the analysis of biophysical characterisation and coarse-grained molecular dynamics (CG-MD) (Figure 18).

In order to determine the effect of negatively charged membrane density, the researchers tested two peptides: L-Glu-D-Lys-L-Ser-D-Lys-L-Ser-D-Trp-L-propylargylglycine-D-Lys (which boasts improved solubility due to three Lys residues) and D-Arg-L-Arg-D-Lys-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp (known for antimicrobial activity with an amphipathic sequence and three basic residues). The use of DSC, ATR-FTIR, and CG-MD methods validated earlier hypotheses that hydrophobic peptide residues are essential for causing major harm to Gram-positive bacteria. The results indicate that, in most cases, formed peptides align mostly parallel to membrane surfaces.



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Figure 18: It shows how D,L- α -CPs self-assemble into nanotubes with an antiparallel β -sheet structure and suggests a model for how they interact with lipid bilayers. The sequences and in-plane and three-dimensional structures of the study's two peptides, CP1 and CP2, are shown below. D-residues are highlighted in the sequence, lysine residues are displayed in red, arginine is displayed in blue, and the alkyne moiety is displayed in purple. The SCNP residues that remain are shown in yellow, while "Pg" stands for propargylglycine.¹⁰⁶ Reproduced with permission from Copyright Elsevier.¹⁰⁶

1.9.3. Gene Delivery:

Gene therapy has become a powerful and adaptable method for reprogramming genetic material to cure a wide range of illnesses, including cancer, viral infections, and hereditary problems.¹⁰⁷

This entails altering the genetic coding within cell nuclei or turning off mutative genes to positively impact the regulation of typical cellular processes.¹⁰⁸ Gene therapy faces several challenges even if it shows great potential. Its ability to preserve payload integrity, elude immune responses, and target cells precisely depends largely on the vector composition. Up until now, the majority of nanotubular vectors utilized for genetic transfer have been somewhat big and formed loaded vectors by intermolecular interaction between genetic material and amino acid moieties. Because the creation of supramolecular structures is hampered by repulsive electrostatic interactions, peptide nanotubes—which are made up of densely packed unimers—face difficulties. This poses a significant challenge for the complexation of charged genetic payloads. Only a few number of cyclic peptide types have been investigated thus far for potential use in gene transfer.

Li et al. created a non-assembling cationic cyclic octapeptide, 1(Figure 19), and by molecular modeling, they altered it with a new arginine analogue.¹⁰⁹ The purpose of this alteration was to overcome the lysine residues' charge repulsion and improve interaction with neighboring peptide backbones. According to the theory, this alteration would stabilize assembly and cause unique tubular structures to form in solution at pH 7.4. TEM and dynamic light scattering (DLS) demonstrated that complexation with calf thymus DNA did not compromise nanoparticle integrity. There was potential with this concept for a carrier. Green fluorescent protein (GFP) reporter labeling was also employed in transfection efficiency tests, which showed that the transfection of HeLa and HEK-293 cell lines by SCPNs was on par with transfection using commercially

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available polyethylene imine. Crucially, this transfection was significantly less harmful and happened without the assistance of active endocytosis pathways.

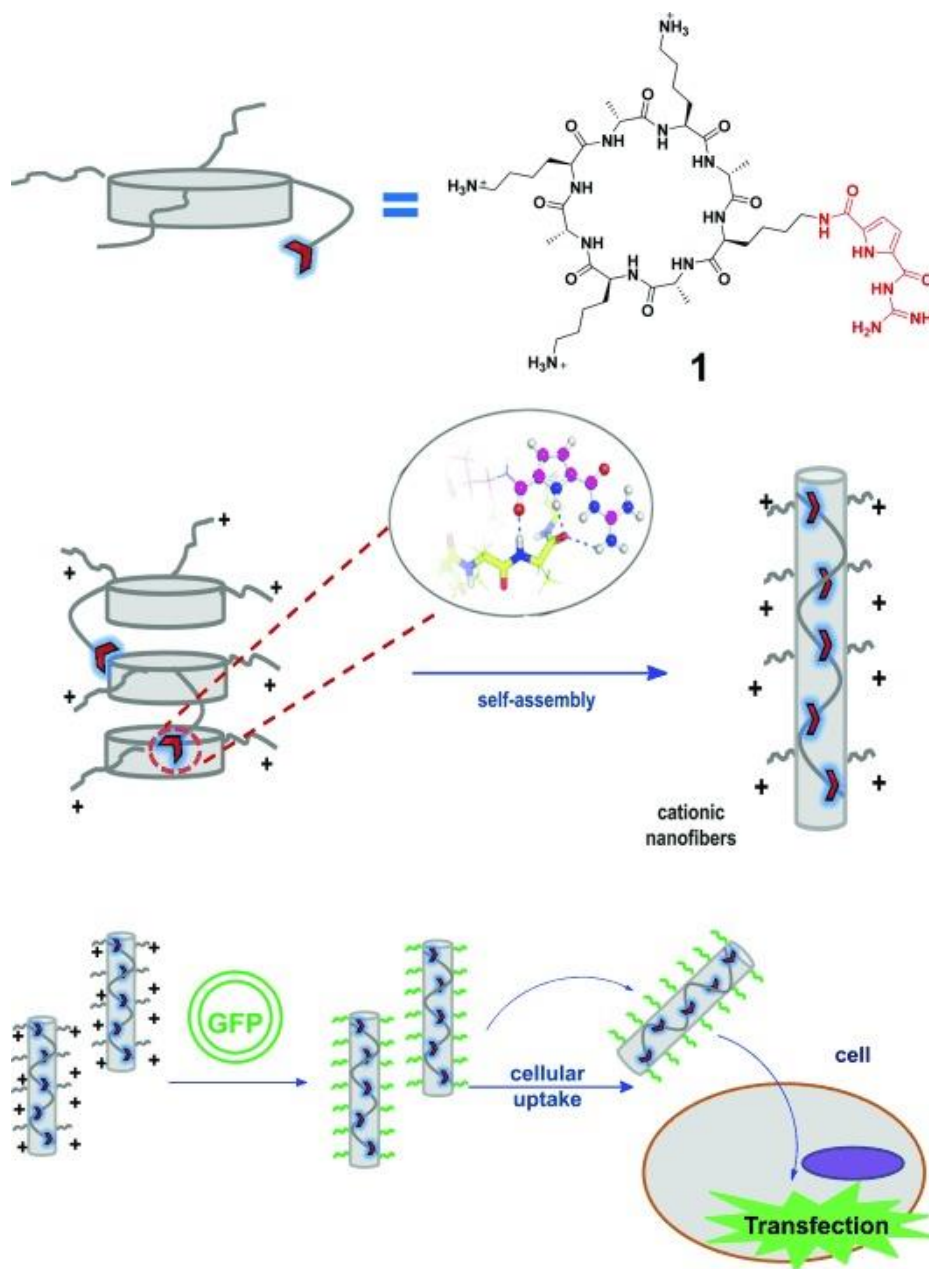


Figure 19: The chemical structure of cationic cyclic octapeptide is described, as is an illustration of how to assemble cationic nanotubes and attach to ctDNA. Additionally, the procedure of fluorescent labeling using GFP to track cellular absorption and transfection efficiency is demonstrated. Reproduced with permission from Copyright Wiley.¹⁰⁹

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1.9.4. Anti-Cancer Drug Delivery:

Since cyclic peptides can reversibly form tubular structures, its primary application has been as a possible drug delivery vehicle for anticancer medications. These materials are attractive for this purpose because of their supramolecular composition, high aspect ratio, and synthetic flexibility. Creating nanoparticles that can surmount biological obstacles such circulation, retention, dispersion, cellular uptake, and clearance is a major difficulty for researchers trying to develop artificial materials for *in vivo* use. When designing nanoparticles, size, shape, and surface chemistry are essential components.¹¹⁰ Furthermore, cyclic peptides' large aspect ratio has drawn interest as a potential characteristic to lengthen circulation times and promote cellular uptake.¹¹¹

Consequently, cyclic peptides' elongated structures are becoming more and more attractive as a means of augmenting the bioavailability and effectiveness of hydrophobic, poorly soluble anticancer agents—which are frequently toxic and nonselective, resulting in unwanted side effects. Facilitating transport across the cell membrane and releasing cargo in intracellular compartments for processing is a fundamental idea of efficient medication delivery. *In vitro*, the tubosome nanoparticles have shown encouraging outcomes for anticancer therapy. Yang and colleagues created amphiphilic tubisomes that exhibited controlled release properties by enclosing doxorubicin within their core and modifying a UV-responsive polymer to behave as the hydrophobic arm of the polymer.¹¹² The design of cyclic peptide-polymer conjugates is shown in Figure 20. These conjugates assemble form cylindrical micelle-like particles that can deconstruct upon NMBA photocleavage by a combination of hydrogen bonding and hydrophobic effects. After loading 10.3 weight percent of doxorubicin into the central cavity and incubating with MDA-MB-231 cells for 20 minutes under UV light, a cytotoxic profile comparable to the free drug was seen. Even though the cytotoxicity did not improve, the nanoparticle's responsiveness would be useful, especially given its time-dependent release behavior and burst release pattern that made it suitable for some kinds of therapies.

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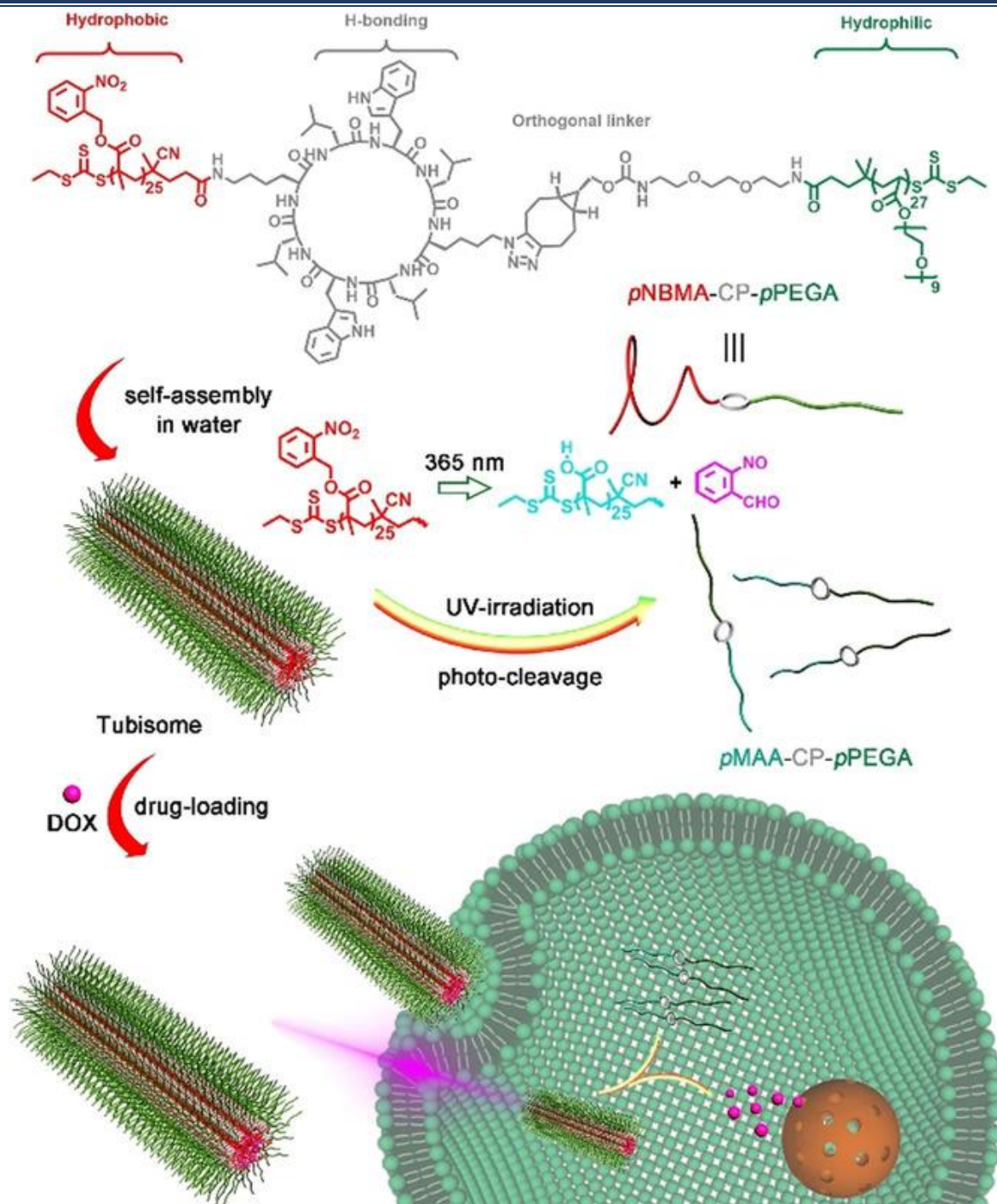


Figure 20: The technique of trigger-responsive assembly into tubisomes, which allows the anticancer medication doxorubicin to be delivered into cells efficiently, and the chemical structure of the asymmetric conjugate 157 are illustrated. Reproduced with permission from Copyright Wiley.¹⁰⁹

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1.9.5. Ion Sensor:

The potential of cyclic peptides (CPs) into self-assembled nanostructure underscores their suitability as candidates for ion sensors. Utilizing self-assembled monolayers (SAMs) composed of eight-residue alpha-cyclodextrin peptides (α -CPs) integrated with cyclic voltammetry, Motesharei and Ghadiri investigated the interaction of redox ion complexes in solution with nanotubes. Based on their redox activity, their investigations showed that both positively and negatively charged $[\text{Fe}(\text{CN})_6]^{3-}$ and $[\text{Ru}(\text{NH}_3)_6]^{3+}$ complexes may penetrate through the nanotube channels. On the other hand, following addition, the bigger $[\text{Mo}(\text{CN})_8]^{4-}$ anion showed no redox activity. Furthermore, the system's selectivity was verified by adding a combination of $[\text{Fe}(\text{CN})_6]^{3-}$ and $[\text{Mo}(\text{CN})_8]^{4-}$ to the SAM, which confirmed the system's preferred interaction with certain ions.¹¹³

1.10. Conclusion:

Since their initial discovery in the mid-1970s, self-assembling cyclic peptides have proven to be versatile components for constructing a diverse array of functional supramolecular nanomaterials. The flexibility offered by peptide synthesis grants access to a vast spectrum of functionalities, both on the outer and inner rings of these peptides. This adaptable design feature allows for the incorporation of desired functionalities onto either the outer shell or the inner channel of a nanotube upon self-assembly. Moreover, the wide variety of available peptides enables the creation of an extensive range of functional materials. Over the past decade, the integration of polymers with cyclic peptides has introduced new opportunities for these materials. In addition to enhancing functionality, polymer conjugation has significantly enhanced the solubility of these nanostructures, leading to exciting prospects, particularly in the realms of biology and medicine. Thus far, cyclic peptides have demonstrated their ability to form transmembrane channels, exhibit antimicrobial properties, and serve as promising vectors for drug delivery.

Significant advancements in the synthesis and characterization of self-assembled cyclic peptide nanotubes (SCPNs) have simplified the production and manipulation of these systems. Consequently, they are increasingly sought after for research and commercialization purposes. This recent progress is expected to stimulate further applications of SCPNs and make them more accessible to the broader scientific community.

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1.11. References:

1. a) B. G. de la Torre, F. Albericio, *Molecules* **2020**, 25, 2293; b) Y. Zhang, H. Zhang, D. Ghosh, R. O. Williams, *Int. J. Pharm.* **2020**, 587, 119491.
2. a) F. Albericio, H. G. Kruger, *Future Med. Chem.* **2012**, 4, 1527–1531; b) M. Akbarian, A. Khani, S. Eghbaltpour, V. N. Uversky, *Int. J. Mol. Sci.* **2022**, 23, 1445.
3. a) K. Anjum, S. Q. Abbas, N. Akhter, B. I. Shagufta, S. A. A. Shah, S. S. U. Hassan, *Chem. Biol. Drug Des.* **2017**, 90, 12–30; b) H. Zhang, S. Chen, *RSC Chem. Biol.* **2022**, 3, 18–31; c) J. Venkatesan, S. Anil, S. K. Kim, M. S. Shim, *Mar. Drugs* **2017**, 15, 143; d) S. Ghosh, T. Sarkar, S. Pati, Z. A. Kari, H. A. Edinur, R. Chakraborty, *Front. Mar. Sci.* **2022**, 9, 832957.
4. a) D. Bentia, M. V. Saceleanu, A. A. Marinescu, A. V. Ciurea, *Acta Endocrinol* **2021**, 17, 406–411; b) H. Ueno, W. Zhang, M. Nakazato, *Pharmacol. Ther.* **2022**, 239, 108187.
5. J. L. Lau, M. K. Dunn, *Biorg. Med. Chem.* **2018**, 26, 2700–2707.
6. M. Muttenthaler, G. F. King, D. J. Adams, P. F. Alewood, *Nat. Rev. Drug Discov.* **2021**, 20, 309–325.
7. L. Costa, E. Sousa, C. Fernandes, *Pharmaceuticals* **2023**, 16, 996.
8. V. D'Aloisio, P. Dognini, G. A. Hutcheon, C. R. Coxon, *Drug Discov. Today* **2021**, 26, 1409–1419.
9. a) Y. M. Gall, M. B. Konashev, *Hist. Philos. Life Sci.* **2001**, 23, 137–150; b) G. F. Gause, M. G. Brazhnikova, *Nature* **1944**, 154, 703.
10. K. P. Klinker, S. J. Borgert, *Clin. Ther.* **2015**, 37, 2619–2636.
11. a) D. Aguilar-Zapata, R. Petraitiene, V. Petraitis, *Clin. Infect. Dis.* **2015**, 61, S604–S611; b) S. Jain, S.; J. Zain, *J. Blood Med.* **2011**, 2, 37–47.
12. Y. Y. Syed, *Drugs* **2023**, 83, 833–840.
13. Z. Du, B. Fan, Q. Dai, L. Wang, J. Guo, Z. Ye, N. Cui, J. Chen, K. Tan, R. Li, *Giant* **2022**, 9, 100082.
14. B. Panigrahi, R. K. Singh, U. Suryakant, S. Mishra, A. A. Potnis, A. B. Jena, R. G. Kerry, H. Rajaram, S. K. Ghosh, D. Mandal, *Eur. J. Pharm. Sci.* **2022**, 171, 106125.
15. R. Berkecz, D. Tanács, A. Péter, I. Ilisz, *Molecules* **2021**, 26, 3380.
16. D. W. Armstrong, Y. Tang, S. Chen, Y. Zhou, C. Bagwill, J. R. Chen, *Anal. Chem.* **1994**, 66, 1473–1484.

Chapter 1

17. a) K. Mustafa, J. Kanwal, S. Musaddiq, S. Khakwani, Springer International Publishing: Cham, Germany, **2020**; 75–97; b) I. Ahmed, M. Asgher, F. Sher, S. M. Hussain, N. Nazish, N. Joshi, A. Sharma, R. Parra-Saldívar, M. Bilal, H. M. N. Iqbal, *Mar. Drugs* **2022**, *20*, 208.
18. S. D. Robinson, E. A. B. Undheim, B. Ueberheide, G. F. King, *Expert Rev. Proteom.* **2017**, *14*, 931–939.
19. N. L. Daly, D. T. Wilson, *Biochem. Soc. Trans.* **2021**, *49*, 1279–1285.
20. X. Wang, M. Lin, D. Xu, D. Lai, L. Zhou, *Molecules* **2017**, *22*, 2069.
21. O. V. Kisil, T. A. Efimenko, O. V. Efremenkova, *Antibiotics* **2021**, *10*, 1254.
22. M. Debono, M. Barnhart, C. B. Carrell, J. A. Hoffmann, J. L. Occolowitz, B. J. Abbott, D. S. Fukuda, R. L. Hamill, K. Biemann, J. Herlihy, *Antibiot.* **1987**, *40*, 761–777.
23. L. L. Ling, T. Schneider, A. J. Peoples, A. L. Spoering, I. Engels, B. P. Conlon, A. Mueller, T. F. Schäberle, D. E. Hughes, S. Epstein, *Nature* **2015**, *517*, 455–459.
24. K. M. White, R. Rosales, S. Yildiz, T. Kehrer, L. Miorin, E. Moreno, S. Jangra, M. B. Uccellini, R. Rathnasinghe, L. Coughlan, *Science* **2021**, *371*, 926–931.
25. S. Agrawal, A. Adholeya, S. K. Deshmukh, *Front. Pharmacol.* **2016**, *7*, 333.
26. W. J. Xu, X. J. Liao, S. H. Xu, J. Z. Diao, B. Du, X. L. Zhou, S. S. Pan, *Org. Lett.* **2008**, *10*, 4569–4572.
27. A. Pesic, H. I. Baumann, K. Kleinschmidt, P. Ensle, J. Wiese, R. D. Süßmuth, J. F. Imhoff, *Mar. Drugs* **2013**, *11*, 4834–4857.
28. F. Y. Du, P. Zhang, X. M. Li, C. S. Li, C. M. Cui, B. G. Wang, *J. Nat. Prod.* **2014**, *77*, 1164–1169.
29. M. McIntosh, L. J. Cruz, M. W. Hunkapiller, W. R. Gray, B. M. Olivera, *Arch. Biochem. Biophys.* **1982**, *218*, 329–334.
30. Y. Hamada, T. Shioiri, *Chem. Rev.* **2005**, *105*, 4441–4482.
31. X. Jing, K. Jin, *Med. Res. Rev.* **2020**, *40*, 753–810.
32. C. J. White, A. K. Yudin, *Nat. Chem.* **2011**, *3*, 509–524.
33. C. Bechtler, C. Lamers, *RSC Med. Chem.* **2021**, *12*, 1325–1351.
34. a) H. Y. Chow, Y. Zhang, E. Matheson, X. Li, *Chem. Rev.* **2019**, *119*, 9971–10001; b) M. Z. Wu, S. Z. Liu, X. Z. Cheng, W. Z. Ding, T. Zhu, B. Chen, B. Chin, *Chem. Lett.* **2016**, *27*, 1731–1739.

Chapter 1

35. X. Jing, K. Jin, *Med. Res. Rev.* **2020**, *40*, 753–810.
36. a) P. E. Dawson, T. W. Muir, I. Clark-Lewis, S. B. H. Kent, *Science* **1994**, *266*, 776–779;
b) J. S. Zheng, H. N. Chang, J. Shi, L. Liu, *China Chem.* **2012**, *55*, 64–69.
37. L. Z. Yan, P. E. Dawson, *J. Am. Chem. Soc.* **2001**, *123*, 526–533.
38. C. F. Liu, J. P. Tam, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 6584–6588.
39. X. Li, H. Y. Lam, Y. Zhang, C. K. Chan, *Org. Lett.* **2010**, *12*, 1724–1727.
40. H. Y. Lam, Y. Zhang, H. Liu, J. Xu, C. T. T. Wong, C. Xu, X. Li, *J. Am. Chem. Soc.* **2013**, *135*, 6272–6279.
41. C. T. T. Wong, H. Y. Lam, X. Li, *Org. Biomol. Chem.* **2013**, *11*, 7616–7620.
42. J. F. Zhao, X. H. Zhang, Y. J. Ding, Y. S. Yang, X. B. Bi, C. F. Liu, *Org. Lett.* **2013**, *15*, 5182–5185.
43. a) B. L. Nilsson, L. L. Kiessling, R. T. Raines, *Org. Lett.* **2000**, *2*, 1939–1941; b) E. Saxon, J. I. Armstrong, C. R. Bertozzi, *Org. Lett.* **2000**, *2*, 2141–2143.
44. a) J. W. Bode, R. M. Fox, K. D. Baucom, *Angew. Chem. Int. Ed.* **2006**, *45*, 1248–1252; b) T. Fukuzumi, L. Ju, J. W. Bode, *Org. Biomol. Chem.* **2012**, *10*, 5837–5844.
45. R. Wills, V. Adebomi, M. Raj, *ChemBioChem* **2021**, *22*, 52–62.
46. G. K. T. Nguyen, S. Wang, Y. Qiu, X. Hemu, Y. Lian, J. P. Tam, *J. Nat. Chem. Biol.* **2014**, *10*, 732–738.
47. D. Y. Jackson, J. P. Burnier, J. A. Wells, *J. Am. Chem. Soc.* **1995**, *117*, 819–820.
48. X. Hemu, Y. Qiu, G. K. T. Nguyen, J. P. Tam, *J. Am. Chem. Soc.* **2016**, *138*, 6968–6971.
49. a) R. Wills, V. Adebomi, C. Spancake, R. D. Cohen, M. Raj, *Tetrahedron* **2022**, *126*, 133071; b) V. Adebomi, R. D. Cohen, R. Wills, H. A. H. Chavers, G. E. Martin, M. Raj, *Angew. Chem. Int. Ed.* **2019**, *58*, 19073–19080.
50. J. N. Lambert, J. P. Mitchell, K. D. Roberts, *J. Chem. Soc. Perkin Trans.* **2001**, *5*, 471–484.
51. C. Rosenbaum, H. Waldmann, *Tetrahedron Lett.* **2001**, *42*, 5677–5680.
52. S. A. H. Abdel Monaim, E. J. Ramchuran, A. El-Faham, F. Albericio, B. G. de la Torre, *J. Med. Chem.* **2017**, *60*, 7476–7482.
53. R. Vanjari, D. Panda, S. Mandal, G. B. Vamisetti, A. Brik, *J. Am. Chem. Soc.* **2022**, *144*, 4966–4976.
54. O. Nwajiobi, A. K. Verma, M. Raj, *J. Am. Chem. Soc.* **2022**, *144*, 4633–4641.

Chapter 1

55. M. Akbarian, A. Khani, S. Eghbaltpour, V. N. Uversky, *Int. J. Mol. Sci.* **2022**, *23*, 1445.
56. H. Zhang, S. Chen, *RSC Chem. Biol.* **2022**, *3*, 18–31.
57. J. S. Choi, S. H. Joo, *Biomol. Ther.* **2020**, *28*, 18–24.
58. C. Lamers, *Future Drug Discov.* **2022**, *4*, FDD75.
59. M. Muttenthaler, G. F. King, D. J. Adams, P. F. Alewood, *Nat. Rev. Drug Discov.* **2021**, *20*, 309–325.
60. B. Khatri, V. R. Nuthakki, J. Chatterjee, *Methods Mol. Biol.* **2019**, *2001*, 17–40.
61. J. J. Devlin, L. C. Panganiban, P. E. Devlin, *Science* **1990**, *249*, 404–406.
62. K. Deyle, X. D. Kong, C. Heinis, *Acc. Chem. Res.* **2017**, *50*, 1866–1874.
63. a) L. D. Walensky, G. H. Bird, *J. Med. Chem.* **2014**, *57*, 6275–6288; b) P. M. Cromm, J. Spiegel, T. N. Grossmann, *ACS Chem. Biol.* **2015**, *10*, 1362–1375; c) D. A. Guarracino, J. A. Riordan, G. M. Barreto, A. L. Oldfield, C. M. Kouba, D. Agrinoni, *Chem. Rev.* **2019**, *119*, 9915–9949.
64. A. K. Malde, T. A. Hill, A. Iyer, D. P. Fairlie, *Chem. Rev.* **2019**, *119*, 9861–9914.
65. K. M. Corbett, L. Ford, D. B. Warren, C. W. Pouton, D. K. Chalmers, *J. Med. Chem.* **2021**, *64*, 13131–13151.
66. D. A. Price, H. Eng, K. A. Farley, G. H. Goetz, Y. Huang, Z. Jiao, A. S. Kalgutkar, N. M. Kablaoui, B. Khunte, S. Liras, C. Limberakis, A. M. Mathiowetz, R. B. Ruggeri, J. M. Quan, Z. Yang, *Org. Biomol. Chem.* **2017**, *15*, 2501–2506.
67. M. R. Naylor, A. T. Bockus, M. J. Blanco, R. S. Lokey, *Curr. Opin. Chem. Biol.* **2017**, *38*, 141–147.
68. J. M. Lehn, *Polym. Int.* **2002**, *51*, 825–839.
69. T. S. Moore, T. F. Winmill, *J. Chem. Soc. Trans.* **1912**, *101*, 1635–1676.
70. a) E. Krieg, M. M. C. Bastings, P. Besenius, B. Rybtchinski, *Chem. Rev.* **2016**, *116*, 2414–2477; b) I. W. Hamley, *Angew. Chem. Int. Ed.* **2014**, *53*, 6866–6881; c) K. Sato, M. P. Hendricks, L. C. Palmer, S. I. Stupp, *Chem. Soc. Rev.* **2018**, *47*, 7539–7551; d) X. Gao, H. Matsui, *Adv. Mater.* **2005**, *17*, 2037–2050; e) Q. Song, Z. Cheng, M. Kariuki, S. C. L. Hall, S. K. Hill, J. Y. Rho, S. Perrier, *Chem. Rev.* **2021**, *121*, 13936–13995.
71. C. K. McLaughlin, G. D. Hamblin, H. F. Sleiman, *Chem. Soc. Rev.* **2011**, *40*, 5647–5656.
72. P. De Santis, S. Morosetti, R. Rizzo, *Macromolecules* **1974**, *7*, 52–58.

Chapter 1

73. M. Saviano, L. Zaccaro, A. Lombardi, C. Pedone, B. Di Blasio, X. Sun, G. P. A. Lorenzi, *J. Inclusion Phenom. Mol. Recognit. Chem.* **1994**, 18, 27–36.
74. L. Tomasic, G. P. Lorenzi, *Helv. Chim. Acta* **1987**, 70, 1012–1016.
75. M. R. Ghadiri, J. R. Granja, R. A. Milligan, D. E. McRee, N. Khazanovich, *Nature* **1993**, 366, 324–327.
76. I. Insua, J. Montenegro, *J. Am. Chem. Soc.* **2020**, 142, 300–307.
77. D. Seebach, J. L. Matthews, A. Meden, T. Wessels, C. Baerlocher, L. B. McCusker, *Helv. Chim. Acta* **1997**, 80, 173–182.
78. T. D. Clark, L. K. Buehler, M. R. Ghadiri, *J. Am. Chem. Soc.* **1998**, 120, 651–656.
79. F. Fujimura, T. Hirata, T. Morita, S. Kimura, Y. Horikawa, J. Sugiyama, *Biomacromolecules* **2006**, 7, 2394–2400.
80. T. Hirata, F. Fujimura, Y. Horikawa, J. Sugiyama, T. Morita, S. Kimura, *Biopolymers* **2007**, 88, 150–156.
81. F. Fujimura, Y. Horikawa, T. Morita, J. Sugiyama, S. Kimura, *Biomacromolecules* **2007**, 8, 611–616.
82. T. Hirata, F. Fujimura, S. Kimura, *Chem. Commun.* **2007**, 1023–1025.
83. M. Amorín, L. Castedo, J. R. Granja, *J. Am. Chem. Soc.* **2003**, 125, 2844–2845.
84. M. Calvelo, A. Lamas, A. Guerra, M. Amorín, R. Garcia-Fandino, J. R. Granja, *Chem. Eur. J.* **2020**, 26, 5846–5858.
85. R. J. Brea, L. Castedo, J. R. Granja, *Chem. Commun.* **2007**, 3267–3269.
86. D. Gauthier, P. Baillargeon, M. Drouin, Y. L. Dory, Y. L. Dory, *Angew. Chem. Int. Ed.* **2001**, 40, 4635–4638.
87. S. Leclair, P. Baillargeon, R. Skouta, D. Gauthier, Y. Zhao, Y. L. Dory, *Angew. Chem. Int. Ed.* **2004**, 43, 349–353. (b) D. Bélanger, X. Tong, S. Soumaré, Y. L. Dory, Y. Zhao, *Chem. Eur. J.* **2009**, 15, 4428–4436.
88. M. Calvelo, A. Lamas, A. Guerra, M. Amorín, R. Garcia-Fandino, J. R. Granja, *Chem. Eur. J.* **2020**, 26, 5846–5858.
89. S. W. Horne, C. D. Stout, M. R. Ghadiri, *J. Am. Chem. Soc.* **2003**, 125, 9372–9376.
90. J. H. van Maarseveen, W. S. Horne, M. R. Ghadiri, *Org. Lett.* **2005**, 7, 4503–4506.

Chapter 1

91. C. Reiriz, M. Amorín, R. García-Fandiño, L. Castedo, J. R. Granja, *Org. Biomol. Chem.* **2009**, 7, 4358–4361.
92. N. Rodríguez-Vázquez, R. García-Fandiño, M. J. Aldegunde, J. Brea, M. I. Loza, M. Amorín, J. R. Granja, *Org. Lett.* **2017**, 19, 2560–2563.
93. S. S. Burade, T. Saha, N. Bhuma, N. Kumbhar, A. Kotmale, P. R. Rajamohanan, R. G. Gonnade, P. Talukdar, D. D. Dhavale, *Org. Lett.* **2017**, 19, 5948–5951.
94. S. M. Darnall, C. Li, M. Dunbar, M. Alsina, S. Keten, A. B. Helms, T. Xu, *J. Am. Chem. Soc.* **2019**, 141, 10953–10957.
95. D. Wang, L. Guo, J. Zhang, R. W. Roeske, L. R. Jones, Z. Chen, C. Pritchard, *J. Pept. Res.* **2001**, 57, 301–306.
96. W. S. Horne, N. Ashkenasy, M. R. Ghadiri, *Chem. Eur. J.* **2005**, 11, 1137–1144.
97. L. P. Hernández-Eguía, R. J. Brea, L. Castedo, P. Ballester, J. R. Granja, *Chem. Eur. J.* **2011**, 17, 1220–1229.
98. R.J. Brea, L. Castedo, J. R. Granja, M. A. Herranz, L. Sánchez, N. Martín, W. Seitz, D. M. Guldi, *Proc. Natl. Acad. Sci. U. S. A.* **2007**, 104, 5291–5294.
99. M. R. Ghadiri, J. R. Granja and L. K. Buehler, *Nature*, **1994**, 369, 301–304.
100. M. R. Ghadiri, H. Bayley, O. Braha, *J. Am. Chem. Soc.* **2000**, 122, 11757–11766.
101. J. R. Granja, M. R. Ghadiri, *J. Am. Chem. Soc.* **1994**, 116, 10785–10786.
102. J. Sa´nchez-Quesada, H. S. Kim, M. R. Ghadiri, *Angew. Chem, Int. Ed*, **2001**, 40, 2503–2506.
103. J. Sa´nchez-Quesada, M. P. Isler, M. R. Ghadiri, *J. Am. Chem. Soc.* **2002**, 124, 10004–10005
104. U. Theuretzbacher, *J. Glob. Antimicrob. Resist.* **2013**, 1, 63–69.
105. S. Fernandez-Lopez, H. S. Kim, E. C. Choi, M. Delgado, J. R. Granja, A. Khasanov, K. Kraehenbuehl, G. Long, D. A. Weinberger, K. M. Wilcoxen, M. R. Ghadiri, *Nature* **2001**, 412, 452–455.
106. B. Claro, E. González-Freire, M. Calvelo, L. J. Bessa, E. Goormaghtigh, M. Amorín, J. R. Granja, R. Garcia-Fandiño, M. Bastos, *Colloids Surf. B* **2020**, 196, 111349.
107. Y. K. Sung, S. W. Kim, *Biomater. Res.* **2019**, 23, 8.
108. B. Shi, M. Zheng, W. Tao, R. Chung, D. Jin, D. Ghaffari, O. C. Farokhzad, *Biomacromolecules*, **2017**, 18, 2231–2246.

Chapter 1

109. M. Li, M. Ehlers, S. Schlesiger, E. Zellermann, S. K. Knauer, C. Schmuck, *Angew. Chem. Int. Ed.* **2016**, 55, 598–601.
110. C. Battistella, H. A. Klok, *Macromol. Biosci.* **2017**, 17, 1700022.
111. X. Zhu, C. Vo, M. Taylor, B. R. Smith, *Mater. Horiz.* **2019**, 6, 1094–1121.
112. J. Yang, J. I. Song, Q. Song, J. Y. Rho, E. D. H. Mansfield, S. C. L. Hall, M. Sambrook, F. Huang, S. Perrier, *Angew. Chem. Int. Ed.* **2020**, 59, 8860–8863.
113. K. Motesharei, M. R. Ghadiri, *J. Am. Chem. Soc.* **1997**, 119, 11306–11312.

Chapter 1

1D to 2D Self Assembly of Cyclic Peptides



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Membrane targeting antimicrobial cyclic peptide nanotubes - an experimental and computational study

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Chapter 2

***Design, Synthesis and Characterization of Crown ether
Mimetic Cyclic Peptides from Delta Amino Acid***

2.1. Introduction:

Cyclic peptides play a crucial role in contemporary science and medicine due to their robust stability, precise specificity, and significant therapeutic potential. These distinctive characteristics make them indispensable in drug discovery, biotechnology, and a wide array of therapeutic applications, paving the way for enhanced treatments and groundbreaking innovations across numerous disciplines.¹ One effective strategy for enhancing the benefits of linear peptides and mitigating their drawbacks is peptide cyclization. Cyclization decreases a peptide's spatial vibrations, making the molecule more stable and less prone to shape changes. This structural rigidity increases the surface area available for biological target interaction, thereby improving binding affinity and selectivity.² Additionally, cyclization enhances pharmacokinetic properties by facilitating easier interaction with transport proteins, which improves membrane permeability and absorption.³ Moreover, cyclic peptides exhibit greater metabolic stability compared to linear peptides, as they lack terminal groups susceptible to degradation by exopeptidases and endopeptidases.⁴ Although some cyclic peptides face challenges with poor pharmacokinetic characteristics, leading to trial failures, chemical synthesis can introduce modifications to improve these properties. Researchers remain deeply interested in cyclic peptides due to their unique advantages, as evidenced by the range of approved cyclic peptides used to treat various infections and diseases. To enhance metabolic stability and explore self-assembling cyclic peptide nanostructures, researchers incorporate unnatural amino acids such as β -, γ -, δ -, and ϵ -amino acids and their compositions.⁵

The main focus of nanotechnology revolves around creating and analyzing structures at the nanoscale level, as well as using these nanomaterials to design functional molecules and devices.⁶ Among various nanostructures, nanotubes stand out due to their versatile applications, including transportation, molecular separation, catalysis, optics, electronics, antimicrobial properties, and drug delivery systems.⁷ Various nanotubular structures have been developed, including those based on cyclodextrins and lipids, graphite and different inorganic complexes.⁸ Over the past few decades, significant advancements have been achieved in the realm of covalently bonded nanostructures. Recently, attention has shifted towards non-covalent interactions⁹, leading to the development of self-assembled nanostructures such as hollow rods, stacked rings, helically truncated structures, and truncated wedges.⁸

Peptides, due to their spontaneous self-assembling properties, have garnered considerable interest. Not all cyclic peptides can self-assembled to tubular structure.¹⁰ However, designed cyclic peptides, can self-assemble into nanotubes through hydrogen bonding interactions. The assembly of these structures involves stacking cyclic peptides in a flat conformation with side chains projecting outward and the amide backbone perpendicular to the ring. The diameter of these nanotubes can be controlled by the number of amino acids involved in the cyclization process. Consequently, cyclic peptides are highly regarded for constructing various nanotubes tailored for specific functions.¹⁰

Self-assembling cyclic peptides are important building blocks for constructing synthetic nanotubes. They can be primarily classified into D, L-cyclic peptides, cyclic β -peptides, cyclic α , γ -peptides, and cyclic peptides incorporating δ - or ϵ -amino acids based on their composition.¹¹ In our introduction chapter we have discussed tubular self-assembly of cyclic peptides derived from natural and unnatural amino acids. In the existing literature, δ -amino acid-based peptides are significantly less discussed in comparison to those composed of β - and γ -amino acids. So, in this work we have explored modified delta amino acid, β -oxy- δ^5 -amino acids as building block for the construction of cyclic peptides. Before entering to our work, we would like to have discuss existing literature on the delta amino acids containing foldamers.

2.2. Foldamers Containing δ - Amino Acids:

In the existing literature, δ -amino acid-based peptides are significantly less discussed in comparison to those composed of β - and γ -amino acids. However, Hofmann and his colleagues introduced a theoretical framework outlining various helical structures that homo-oligomers of δ -amino acids can adopt. They proposed that the 10-helix (comprising 10-membered hydrogen bonds) is the most stable helical structure, followed by 14, 16, and 8-membered hydrogen bonded helices.¹² Additionally, they identified the potential formation of β -turn conformations using δ -amino acid building blocks. Chakraborty and Sharma, among others, have investigated diverse hybrid helical structures formed by combining pyranose- or furanose-based carbo amino and furan-based δ -amino acids. Chakraborty's group used constrained furanoid sugar amino acids to create different reverse turns in β -hairpins.¹³ Sharma and his group demonstrated the formation of mixed 11/13 helices from α/δ hybrid peptides, employing both NMR experiments and theoretical calculations.¹⁴ Furthermore, Huc and his colleagues reported the existence of helical structures in

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single crystals derived from quinolone-based δ -amino acids.¹⁵ They also generated polar cyclic peptide nanotubes (CPNs) from cyclic tripeptides that incorporated an α,β -unsaturated δ -amino acid residue with a trans geometry of the vinyl group.¹⁶ Balaram and his co-workers showcased the stability of these helical structures by substituting Gly-Gly residues with δ -aminovaleric acid (δ -Ava).¹⁷ Recently, Cobb group explored a novel α,δ -hybrid peptide foldamer structure, the 13/11-helix, confirmed through XRD and NMR.¹⁸ Moreover, by replacing two adjacent α -residues with ornithines, they developed a catalyst that mimics an aldolase, accelerating the retroaldol reaction of methodol. The straightforward design process to introduce distinct reactive groups into these α,δ -foldamer scaffolds, coupled with their unique structure featuring a deep helical groove, bodes well for the development of functional α,δ -foldamers. The Huc group synthesized aromatic oligoamide foldamers with various δ -amino acids, finding that helix stability is influenced by the balance of rigid and flexible units. Their work demonstrates that combining different δ -amino acids can precisely control helix characteristics, even accommodating multiple sp³ centers.¹⁹

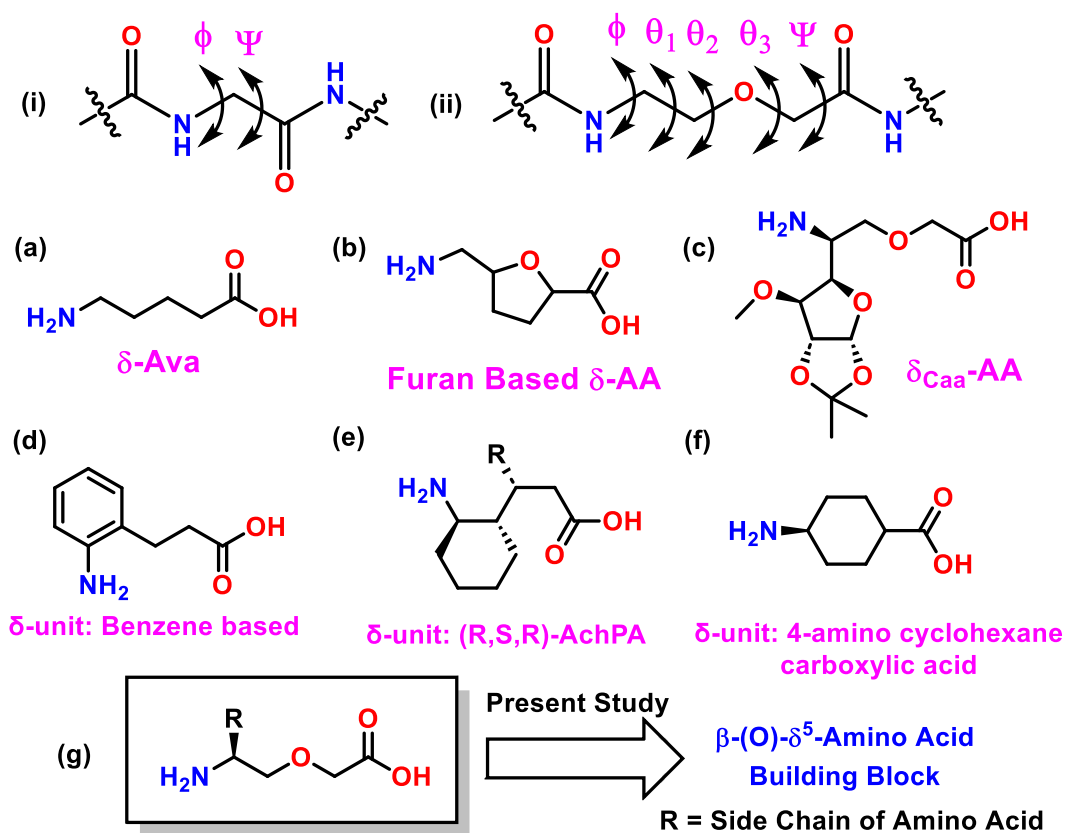


Figure 1: (i)-(ii) Torsion angle parameters of α and δ -amino acids, (a-f) different δ -amino acids containing building block explored in literature, (g) present study of β -oxy- δ^5 -amino acids.

In literature, δ -amino acids are well-explored for designing various helical foldamer structures. However, cyclic peptides containing δ -amino acids are not as extensively reported.

2.3. Past Studies related $\beta(\text{O})$ - δ^5 -AA building block:

We recently investigated the hydrogelation properties of hybrid peptides incorporating δ -amino acids.²⁰ Unlike dipeptides with a complete carbon backbone, δ^5 -amino acids with an oxygen atom at the β -position demonstrated high solubility in organic solvents and the ability to form gels in aqueous buffers. The straightforward synthesis and solubility of these new δ -amino acids ($\beta(\text{O})$ - δ^5 -amino acids), combined with their similarity to α -dipeptides, led us to systematically examine their structural properties by incorporating $\beta(\text{O})$ - δ^5 -amino acids into α -peptides, α,γ^4 -hybrid peptides, and in 1:1 alternating α - and $\beta(\text{O})$ - δ^5 -amino acids. When $\beta(\text{O})$ - δ^5 -amino acids were integrated into α,γ^4 -hybrid peptides, a novel helix structure featuring both 12- and 13-membered hydrogen bonds emerged. Interestingly, instead of the anticipated 13-helix, α,δ^5 -hybrid peptides exhibited a mixed 13/11-helix configuration.²¹ These observations highlighted the versatile nature of $\beta(\text{O})$ - δ^5 -amino acids, suggesting their potential in designing proteolytically stable peptidomimetics.

Our previous research has thus developed easily synthesizable building block ($\beta(\text{O})$ - δ^5 -amino acids) that can self-assemble into superior hydro-gelators. When mixed with α residues, they form proteolytically stable foldamers. This success inspires us to further utilize this dipeptide mimetic building block to create cyclic peptide molecules.

2.4. Aim and Rationale of Present work:

The length of dipeptides composed of δ^5 -amino acids is equivalent to the length of tetra- and tri-peptides made from α - and β -amino acids, respectively (Figure 2a). We aim to cyclize our $\beta(\text{O})$ - δ^5 -amino acid dipeptides to mimic the cyclic peptides formed by tetra- and tri-peptides of α - and β -amino acids (Figure 2c). In the crystal structure of Boc-NH- $\beta(\text{O})$ - δ^5 -Val- $\beta(\text{O})$ - δ^5 -Leu-COOH, we observed that both the N-terminus and C-terminus are in close proximity (Figure 2b). This motivated us to design and synthesize polar cyclic peptides and study their self-assembled structures.

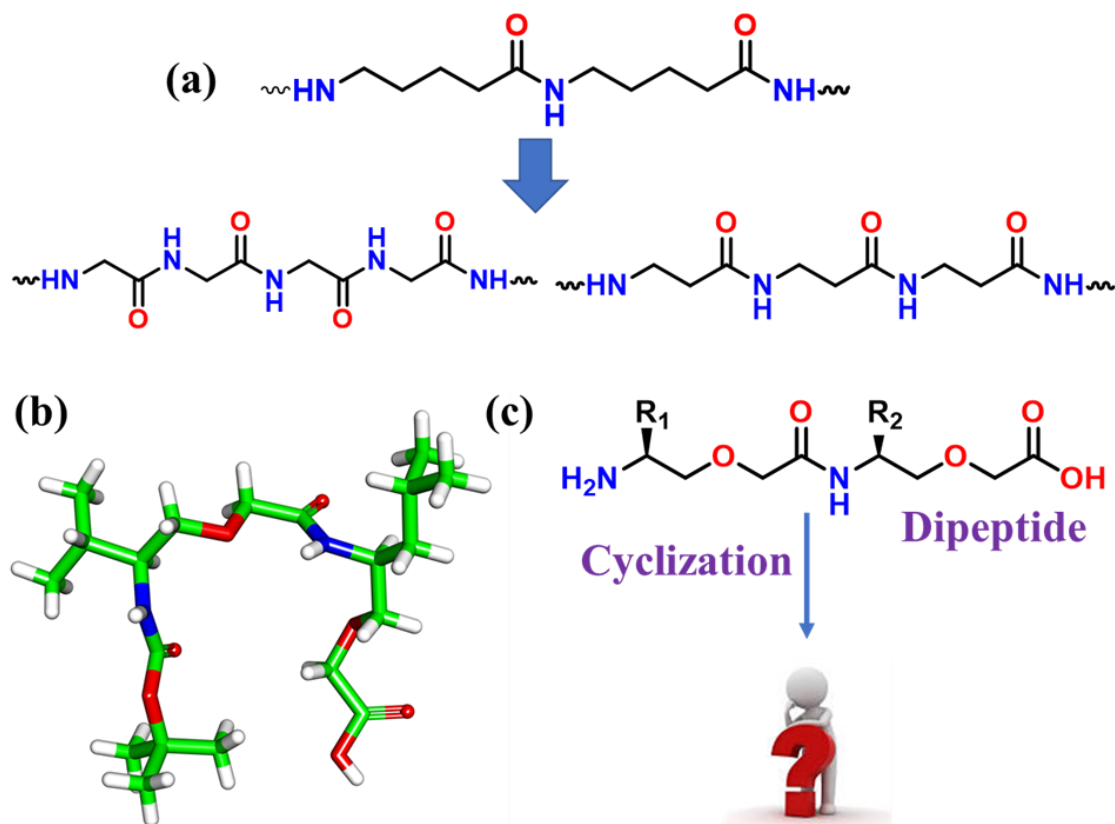
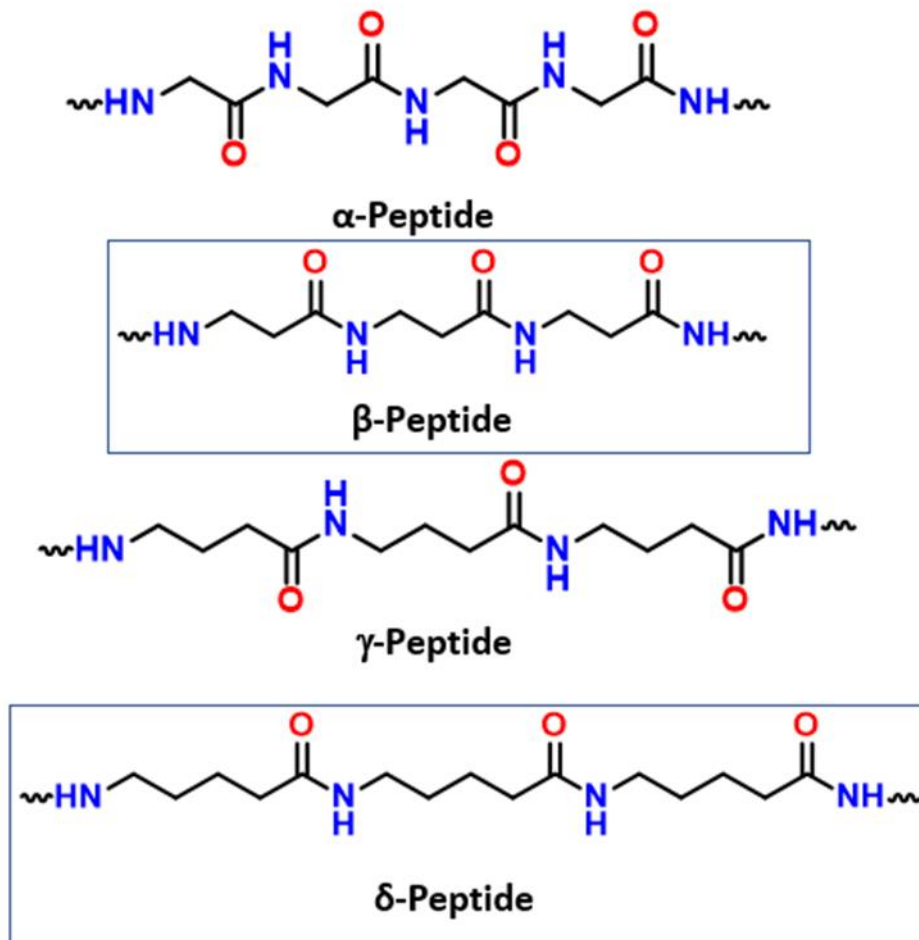


Figure 2: (a) Dipeptide of δ -amino acids is equivalent to the length of tetra- and tri-peptides made from α - and β -amino acids, (b) Crystal structure of Boc-NH- $\beta(\text{O})$ - δ^5 -Val- $\beta(\text{O})$ - δ^5 -Leu-COOH (c) Design of cyclization of linear dipeptides of $\beta(\text{O})$ - δ^5 AAs.

Most cyclic peptide nanotubes (CPNs) formed from non-canonical amino acids typically adopt an anti-parallel β -sheet structure, resulting in non-polar characteristics due to the hydrogen bonds projected between the stacks.²² Conversely, polar CPNs are less explored in scientific literature, despite their significant potential in organic nano-electronics. Producing polar nanotubes is particularly challenging, except through cyclic β -peptides.²³ CPNs derived from homo-oligomers of β -amino acids have been less studied, possibly due to the solubility issues associated with cyclic β -peptides. Since δ -amino acids are higher homologues of β -amino acids, possessing an even number of backbone atoms, it is hypothesized that cyclic peptides made from δ -amino acids could form polar nanotubes by projecting amide NH groups on one face of the cyclic peptide and carbonyl groups on the opposite face. However, the exploration of CPNs containing δ -amino acids is limited, primarily due to the difficulties in synthesizing δ -amino acids. Scheme 1 illustrates the

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alternating projection of amide bonds in α - and γ -peptides and the projection of all carbonyl and amide NH groups on the same side in peptides containing β - and δ -amino acids.



Scheme 1: Projection of all carbonyl and amide NH groups in peptides α -, β -, γ - and δ -amino acids.

Here we focus on investigating whether $\beta(\text{O})$ - δ^5 -amino acids can be used to design self-assembling cyclic peptides. Notably, cyclic peptides of $\beta(\text{O})$ - δ^5 -amino acids are hybrids of crown ethers and conventional cyclic peptides (Figure 3). This inspires us to further explore these dipeptide mimetic building blocks for the synthesis of self-assembled polar tubular structures. From our design, we anticipate that our cyclic peptides will function as crown ether mimetics, exhibiting the characteristics of both cyclic peptides and crown ether moieties. The design of our work is illustrated in Figure 3 below.

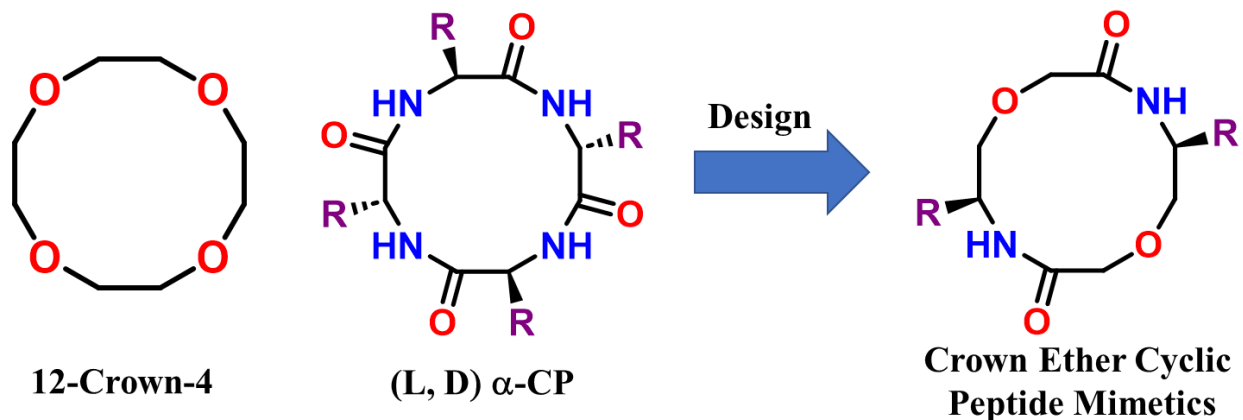


Figure 3: Design of crown ether cyclic peptide mimetics.

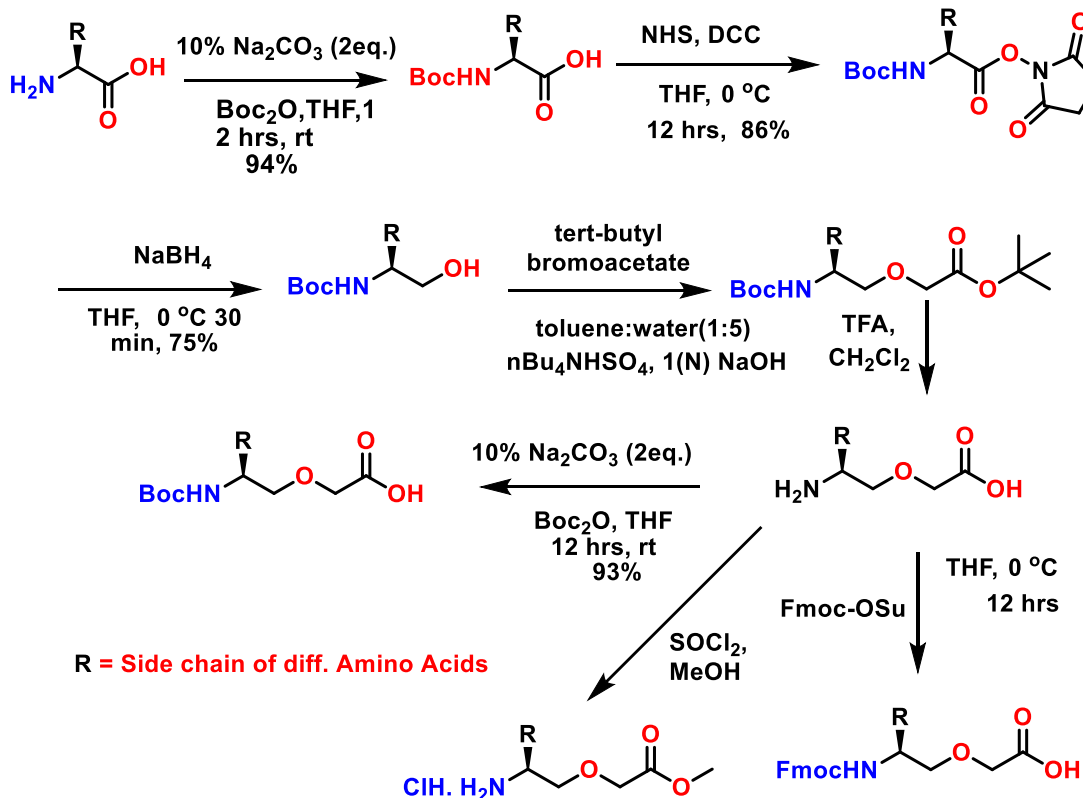
2.5. Results and Discussion:

2.5.1. Design and Synthesis of Cyclic Peptides (C1-C7):

The designed cyclic dipeptides **C1-C7** are shown in Scheme 2. The amino acids and linear peptides were synthesized as reported earlier.²⁰ The synthesis scheme of $\beta(\text{O})$ - δ^5 -amino acids ($\beta(\text{O})$ - δ^5 AA) are shown in below Scheme 2. Boc-NH- $\beta(\text{O})$ - δ^5 AA-COOH and NH₂- $\beta(\text{O})$ - δ^5 AA-COOMe building blocks are used for solution phase peptide synthesis. Initially, linear dipeptides were synthesized through the traditional solution-phase condensation approach. After that C-terminal ester group was hydrolyzed using 1(N) NaOH and N terminal Boc group was deprotected by (1:1) mixture of (TFA:DCM). Both side free amine and acid was then kept for macrocyclization using a high dilution method. The macrocyclization process is detailed in the experimental section. All peptides were purified using column chromatography and confirmed by mass spectral analysis. The length of cyclic dipeptides of δ^5 -amino acids is equivalent to tetra- and tri-peptides of α - and β -amino acids, respectively.

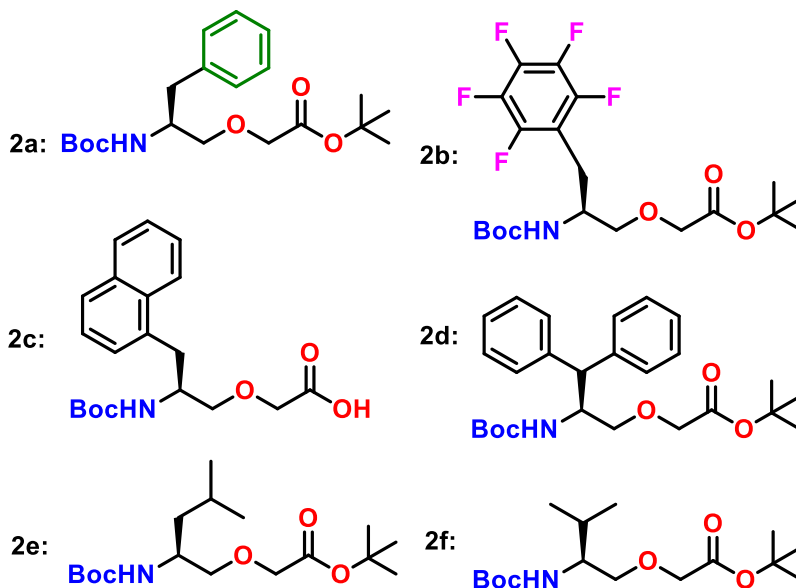
We have designed a total of seven cyclic dipeptides, incorporating both aliphatic and aromatic amino acids to explore various properties of these molecules.²⁴ To address solubility issues, we included more polar oxygen atoms, ensuring our cyclic peptides dissolve in organic solvents. This solubility facilitates the design and execution of different experiments to compare their self-assembled nanostructures.

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Scheme 2: Synthesis Pathway of $\beta(O)$ - δ^5 AA building blocks.

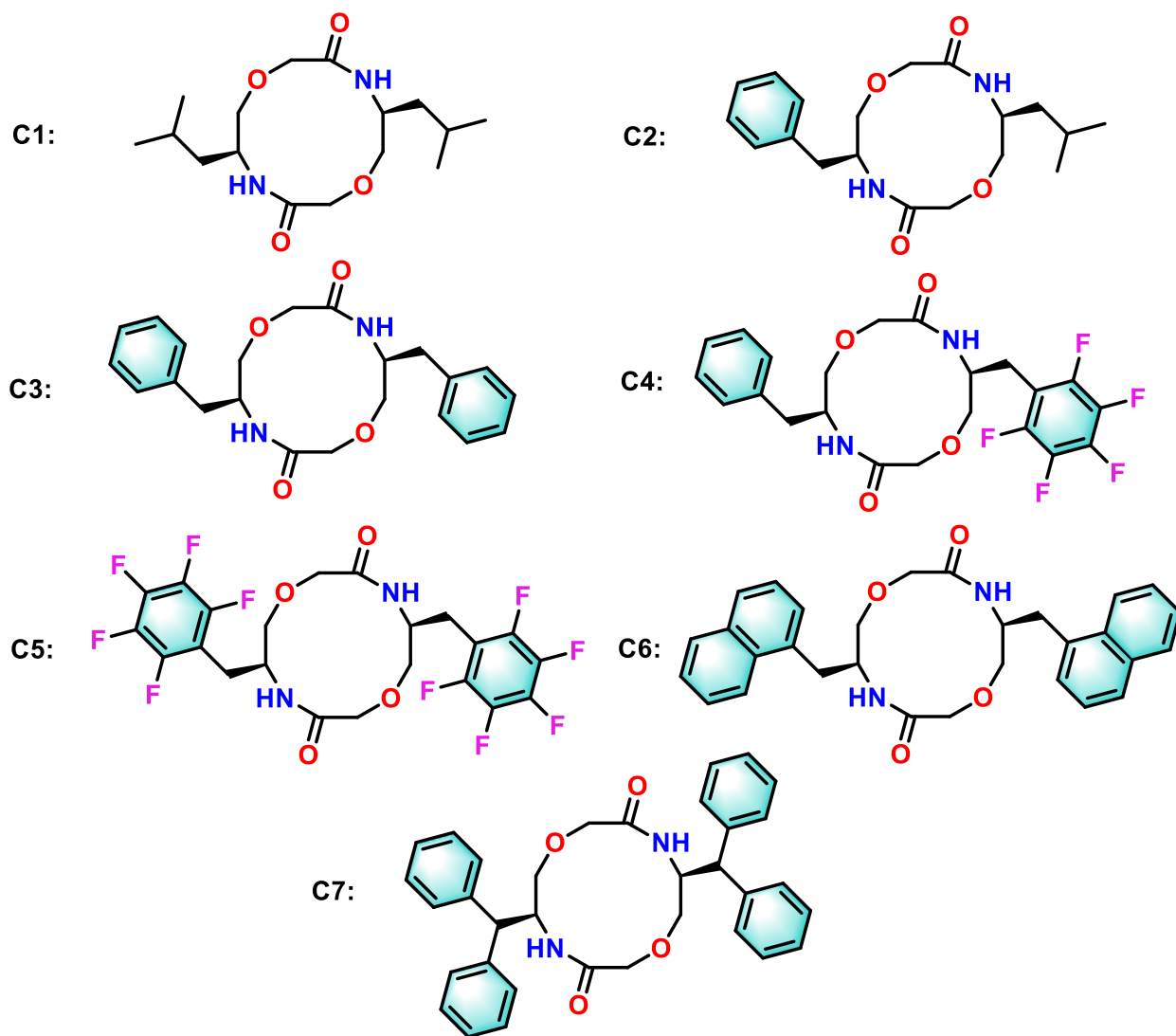
We have synthesized different $\beta(O)$ - δ^5 AA containing building blocks and characterized them with HRMS, ^1H , ^{13}C and SCXRD data. Synthesized building blocks are shown in below scheme 3.



Scheme 3: Synthesized Building blocks of different $\beta(O)$ - δ^5 Amino Acids.

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The details of synthesis are described in experimental section. We have successfully synthesized cyclic dipeptides **C1-C7** and synthesizes cyclic peptides are attached in below Scheme 4.



Scheme 4: Sequence of the Cyclic Peptides **C1-C7**.

2.5.2. Crystal Structures Analysis:

We have got single crystal structures of two building block $\beta(O)$ - δ^5 Amino Acids **2b** and **2c**. Crystal structure descriptions of these building block are described below.

2.5.2.1. Crystal Structures Analysis of $\beta(O)$ - δ^5 Amino Acid building block **2b** & **2c**:

The $\beta(O)$ - δ^5 Amino Acid building blocks gave single crystals after the slow evaporation of its

solution in methanol/water. The crystal structure of **2b** is shown in Figure 4i and the crystal structure of **2c** is shown in Figure 4ii. Both of the molecules are crystallized in the $C2$ space group.

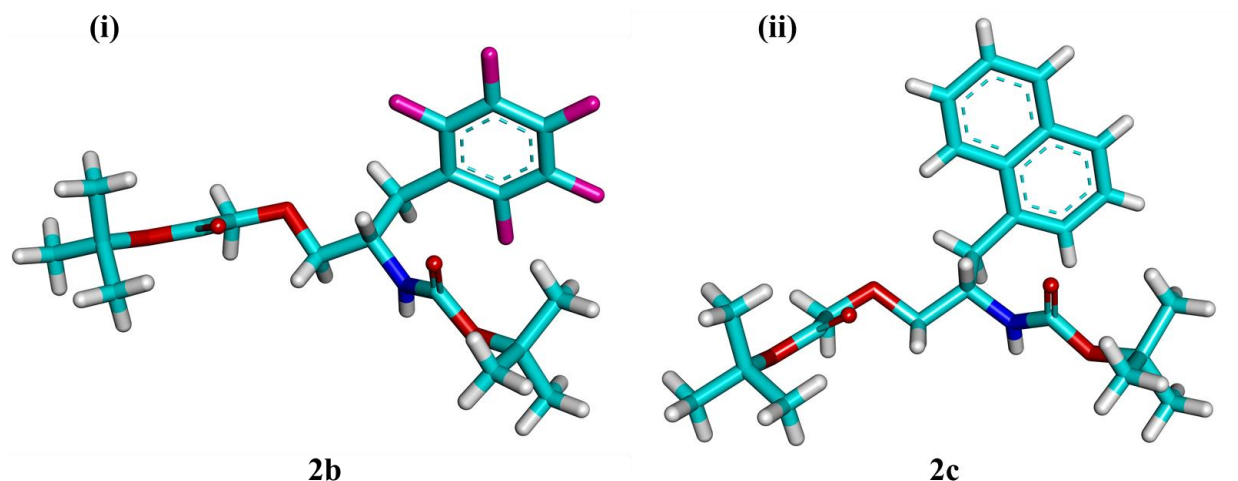


Figure 4: Single crystal structures of two building block $\beta(O)$ - $\delta 5$ Amino Acids **2b** and **2c**.

2.5.2.2. Crystal Structures Analysis of Peptide **C3**:

The cyclic peptide **C3** gave single crystals after the slow evaporation of its solution in methanol/water. The crystal structure of **C3** is shown in Figure 5a. The molecule crystallized in the $P2_1$ space group. The cyclic peptide adopted a polar structure, with both amide NH protons projecting in one direction and carbonyl groups projecting in the opposite direction. Both amide NH groups are involved in bifurcated H-bonding with a water molecule, and the same water molecule is involved in H-bonding with the CO groups of other peptides (Figure 5c). A water molecule connects two cyclic peptides, continuing along the vertical direction. Even though there is no direct H-bonding involved between the cyclic peptides, the top view shows the tubular assembly of **C3** (Figure 5b). The pore diameter was found to be 3.95 Å, and the two cyclic peptides were separated by 6.75 Å.

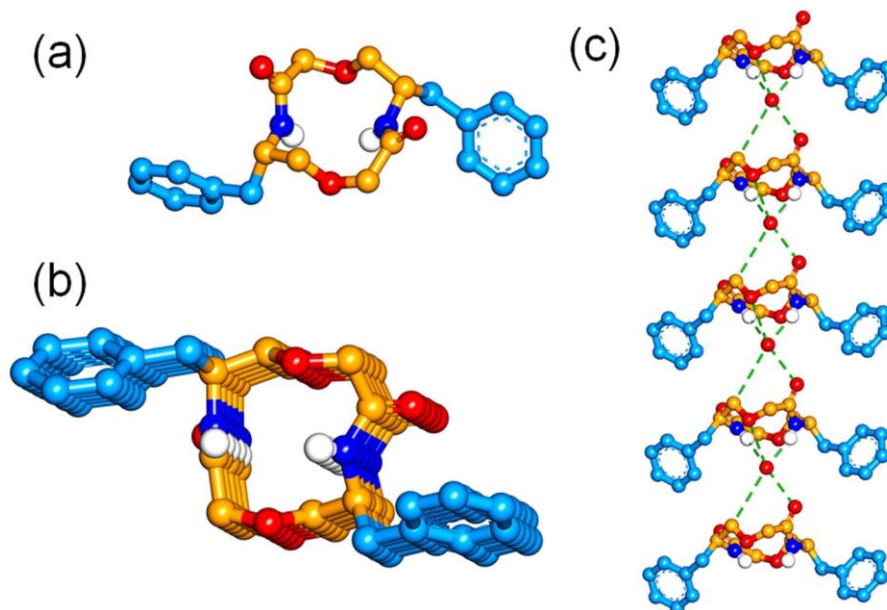


Figure 5. (a) Crystal Structure of **C3**, (b) Top view of **C3**, (c) Intermolecular H-bonding driven **C3** assembly (water assisted).

Torsion Angles Parameters of Peptide **C3**:

Peptide CP1	ϕ (deg)	θ_1 (deg)	θ_2 (deg)	θ_3 (deg)	ψ (deg)
$\beta(\text{O})$ - $\delta 5$ -Phe 1	-85.01	-69.45	161.85	-80.04	-26.77
$\beta(\text{O})$ - $\delta 5$ -Phe 2	-114.16	-65.64	150.61	-73.47	-56.67

Table 1: Torsion Angle parameters of **C3**.

Hydrogen-Bond Parameters of Peptide **C3**:

We have updated all potential hydrogen bonds in the cyclic peptide **C3**. Using single crystal data, we generated and listed all possible hydrogen bond parameters below **Table 2**.

D-H...A	d(D-H)	d(H...A)	d(D...A)	$\angle(\text{DHA})$
N(1)-H(1)...O#1	0.88	2.06	2.880	154.9
C(2)-H(2A)...O(2)#2	0.99	2.49	3.198	128.1
N(2)-H(2)...O#1	0.88	2.42	3.256	157.6

Table 2: Hydrogen bond parameters of **C3**.

Symmetry transformations used to generate equivalent atoms: #1 $x-1,y,z$ #2 $-x+1,y-1/2,-z+1$

2.5.2.3. Crystal Structures Analysis of Peptide C4:

The crystal structure of **C4** is shown in Figure 6a. The peptide crystallized in monoclinic $P2_1$. Similar to **C3**, **C4** also adopts a polar cyclic peptide conformation where the amide NH groups project in the same direction and the CO groups in the opposite direction. Careful analysis revealed that one of the carbonyls is involved in bifurcated H-bonding with both amide NH groups of the other cyclic peptide (Figure 6c). Interestingly, the other carbonyl group is involved in $\pi \rightarrow \pi$ interaction with the pentafluorophenyl group. We observed $\pi \rightarrow \pi$ interactions between the pentafluorophenyl rings and not between the phenyl and pentafluorophenyl rings. The pore diameter of the tubular assemblies was found to be 3.85 Å. The inter-distance between the cyclic peptides in the tubular assemblies was found to be 5.217 Å. In comparison, the distance between the two cyclic peptides in **C3** is smaller than in **C4** (Figure 6b).

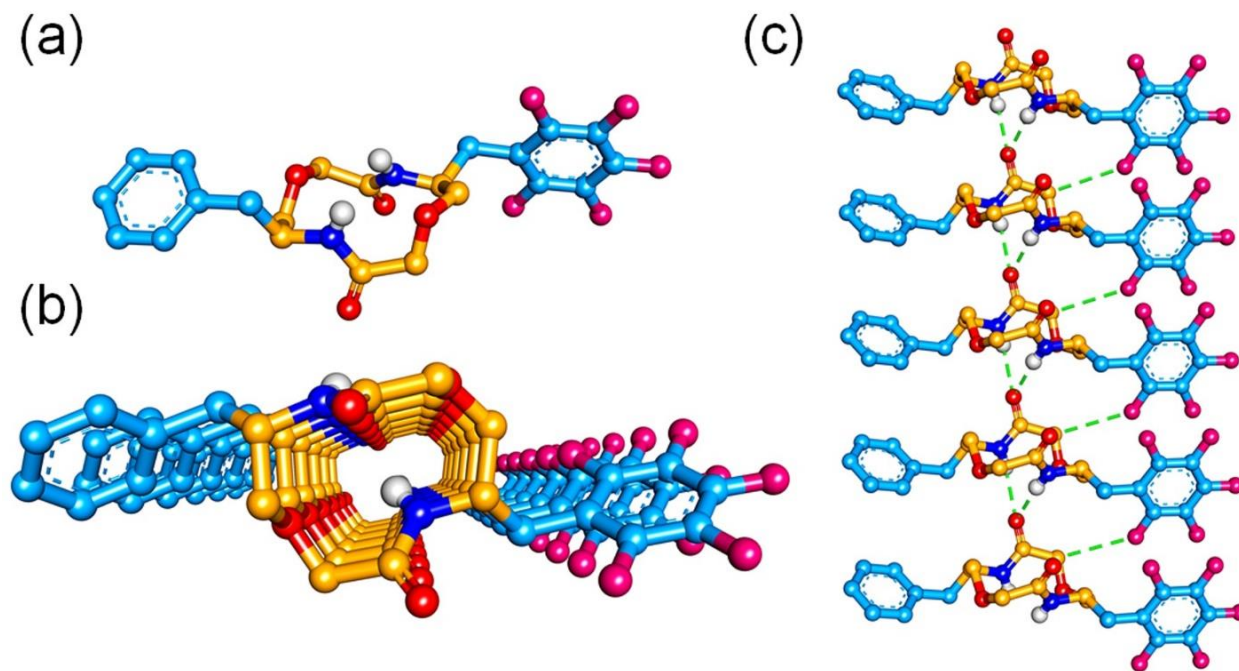


Figure 6. (a) Crystal Structure of **C4**, (b) Top view of **C4**, (c) Intermolecular H-bonding driven **C4** assembly.

Torsion Angles Parameters of Peptide C4:

Peptide C4	ϕ (deg)	θ_1 (deg)	θ_2 (deg)	θ_3 (deg)	ψ (deg)
$\beta(\text{O})$ - $\delta 5$ -PheF5	-150.53	36.36	63.66	-120.98	-8.85
$\beta(\text{O})$ - $\delta 5$ -Phe 2	-75.59	-68.22	147.53	-75.99	-45.73

Table 3: Torsion Angle parameters of **C4**.

Hydrogen-Bond Parameters of Peptide C4:

We have updated all potential hydrogen bonds in the cyclic peptide **C4**. Using single crystal data, we generated and listed all possible hydrogen bond parameters below **Table 4**.

D-H...A	d(D-H)	d(H...A)	d(D...A)	$\angle(\text{DHA})$
N(1)-H(1A)...O(3)#1	0.86	2.26	3.097	164.8
N(2)-H(2)...O(3)#1	0.86	2.39	3.137	145.4
C(3)-H(3A)...F(4)#2	0.97	2.39	3.101	129.9
C(8)-H(8A)...F(1)#3	0.97	2.58	3.342	135.3
C(8)-H(8A)...F(2)#4	0.97	2.60	3.411	140.9
C(7)-H(7A)...F(3)#4	0.97	2.62	3.165	116.0

Table 4: Hydrogen bond parameters of **C4**.

Symmetry transformations used to generate equivalent atoms: #1 x, -1+y, z #2 1-x, -0.5+y, 1-z
#3 x, 1+y, z #4 2-x, 0.5+y, 1-z

2.5.2.4. Crystal Structures Analysis of Peptide C5:

Single crystals of **C5** were obtained after the slow evaporation of its solution in aqueous methanol, yielding the structure shown in Figure 1c. The molecule crystallized with the *C2* space group. In comparison to **C3** and **C4**, this cyclic peptide adopted a perfect tubular assembly (Figure 7b). The two carbonyl groups of the cyclic peptide projected in the same direction and were involved in H-bonding with the amide NH groups of the other cyclic peptide (Figure 7c). This H-bonding interaction led to a tubular assembly, with all H-bonds projected in the same direction. Additionally, these structures were stabilized by CH---O H-bonds between the amide CO of one

cyclic peptide and the CH₂ of the pentafluorobenzyl group of the other cyclic peptide in assembly. The average distance between the two cyclic peptides and the pore size in the tubular assemblies of **C5** were found to be 3.5 Å and 4.2 Å, respectively. The comparison of the three cyclic peptides revealed that **C5** is packed more closely with a relatively larger pore size compared to **C4** and **C3**. This is probably due to the electron-withdrawing nature of the penta-fluorophenyl groups.

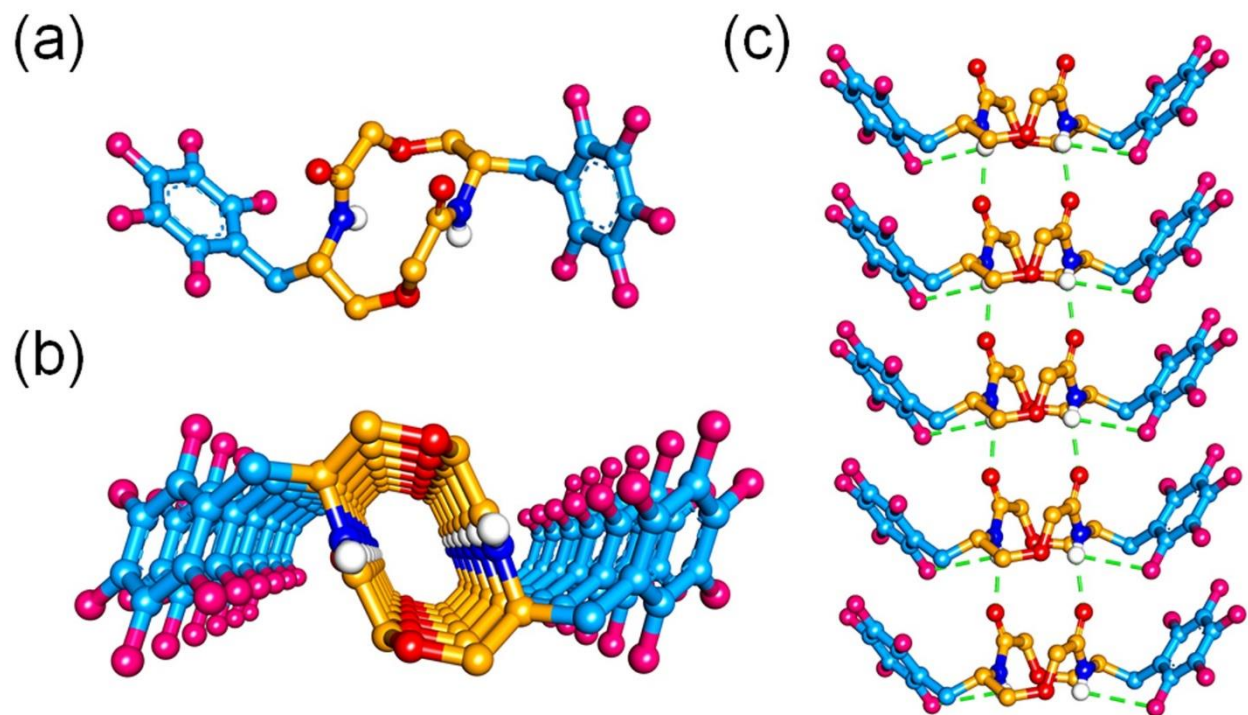


Figure 7. (a) Crystal Structure of **C5**, (b) Top view of **C5**, (c) Intermolecular H-bonding driven **C5** assembly.

Torsion Angles Parameters of Peptide **C5**:

Peptide C5	ϕ (deg)	θ_1 (deg)	θ_2 (deg)	θ_3 (deg)	ψ (deg)
$\beta(\text{O})$ - $\delta 5$ -PheF5 1	-119.97	36.52	54.53	-121.70	-11.12
$\beta(\text{O})$ - $\delta 5$ -PheF5 2	-119.97	36.52	54.53	-121.70	-11.12

Table 5. Torsion Angle parameters of **C5**.

Hydrogen-Bond Parameters of Peptide C5:

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(1)-H(1)...O(1)#1	0.86	2.16	2.94	151
C(3)-H(3AB)...F(2)#2	0.97	2.63	3.25	121.8

Table 6: Hydrogen bond parameters of **C5**.

Symmetry transformations used to generate equivalent atoms: #1 $x, -1+y, z$ #2 $-0.5+x, -0.5+y, z$.

2.5.2.5. Crystal Structures Analysis of Peptide C7:

The cyclic peptide **C7** gave single crystals after the slow evaporation of its solution in methanol/water. The crystal structure of **C7** is shown in Figure 7a. The molecule crystallized in the P_1 space group. The cyclic peptide adopted a polar structure, with both amide NH protons projecting in one direction and carbonyl groups projecting in the opposite direction. Both amide NH groups are involved in bifurcated H-bonding with a water molecule, and the same water molecule is involved in H-bonding with the CO groups of other peptides (Figure 7b). A water molecule connects two cyclic peptides, continuing along the vertical direction.. The pore diameter was found to be 3.95 Å, and the two cyclic peptides were separated by 6.75 Å.

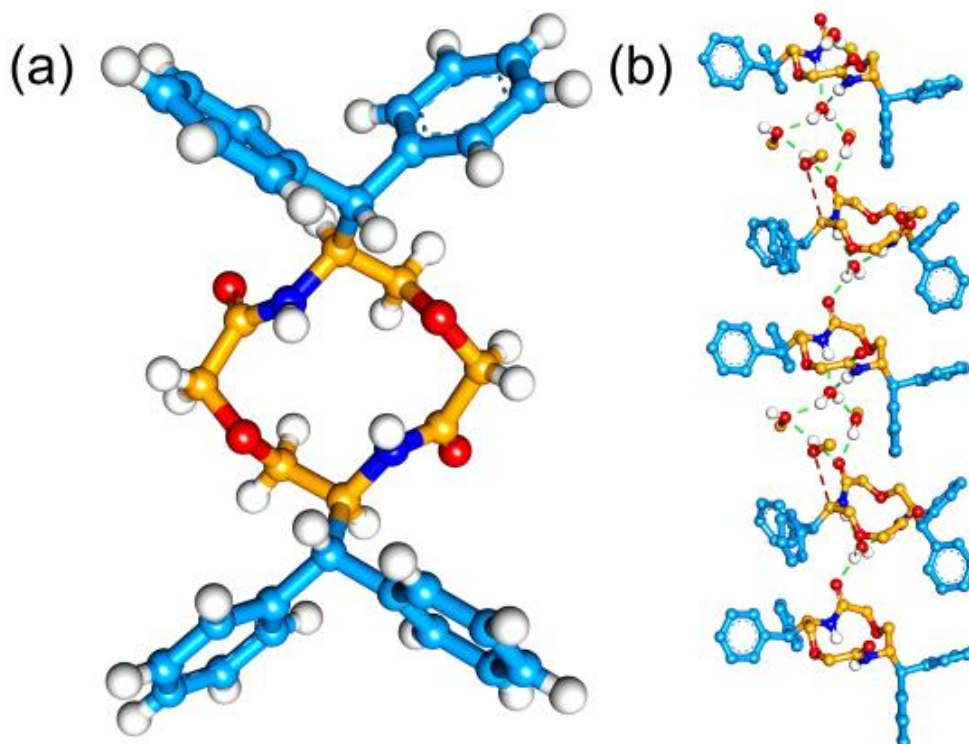


Figure 7. (a) Crystal Structure of **C7**, (c) Intermolecular H-bonding driven **C7** assembly (water assisted).

Torsion Angles Parameters of Peptide **C7**:

Peptide C7	ϕ (deg)	θ_1 (deg)	θ_2 (deg)	θ_3 (deg)	ψ (deg)
Molecule A					
$\beta(\text{O})\text{-}\delta^5\text{-Diphe 1}$	-99.54	-69.46	157.22	-77.59	-40.6
$\beta(\text{O})\text{-}\delta^5\text{-Diphe 2}$	-95.54	-72.09	159.25	-73.47	-40.75
Molecule B					
$\beta(\text{O})\text{-}\delta^5\text{-Diphe 1}$	-111.44	-67.62	151.02	-77.74	-38.57
$\beta(\text{O})\text{-}\delta^5\text{-Diphe 2}$	-101.98	-68.34	154.57	-79.34	-28.49

Table 5. Torsion Angle parameters of **C7**.

Hydrogen-Bond Parameters of Peptide **C7**:

Donor(D)	Acceptor(A)	D....A(Å)	DH....A(Å)	NH....O(deg)
N4'	O1W	2.852	1.984	169.03
N3'	O1W	2.954	2.112	159.95
N1'	O2W	2.909	2.065	160.55
N2'	O2W	2.842	2.013	156.34

Table 8: Hydrogen bond parameters of **C7**.

2.5.3. Solution Structure Analysis:

The ^1H NMR analysis of **C1**, **C3**, **C5**, **C6** and **C7** shows a single set of protons, suggesting the symmetric nature of molecules; however, other peptides **C2** and **C4** showed a distinct ^1H NMR pattern of both residues. Further, we have carried out DMSO- d_6 titration of these cyclic peptides

to observe the protons involved for hydrogen bonding and temperature dependent ^1H NMR to check the stability of these cyclic peptides.

2.5.3.1. DMSO- d_6 Titration of Peptide C1, C2 and C3:

To identify the protons involved in hydrogen bonding, we conducted a DMSO- d_6 titration experiment on cyclic peptides. We observed significant shifts in the chemical shift positions of all amide NH protons as the DMSO- d_6 concentration increased, strongly indicating that the backbone amide NH and CO groups participate in intermolecular hydrogen bonding between the cyclic peptide units. However, the shifts in the chemical shift positions of all amide NH protons in **C2** and **C3** were less pronounced compared to **C1**. These results suggest that while DMSO disrupts intermolecular hydrogen bonds between NH and carbonyl groups, the increasing aromaticity enhances non-covalent interactions in CPs. Consequently, the shifts are less in aromatic CPs compared to aliphatic CPs.

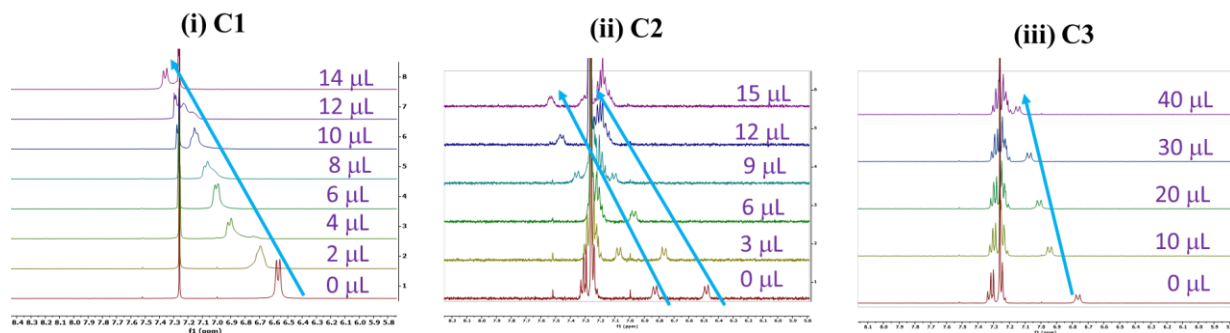
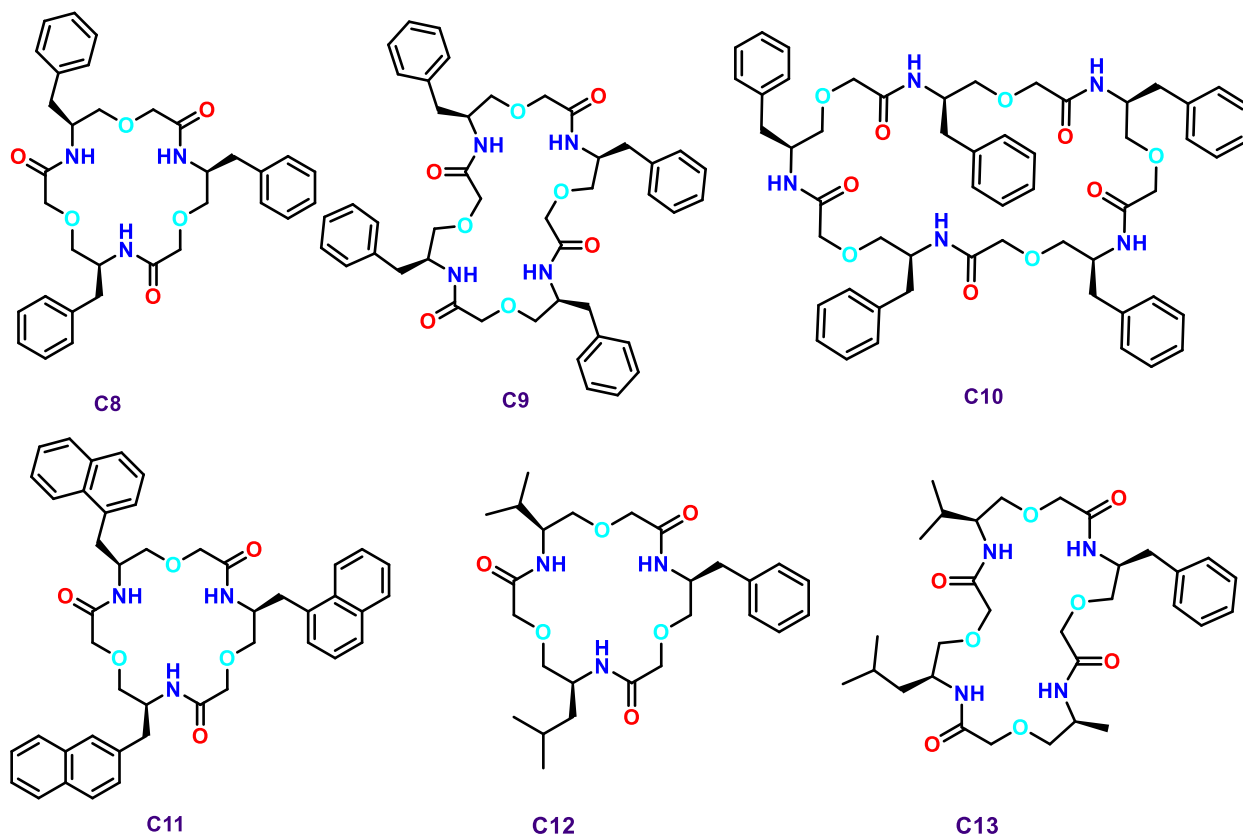


Figure 8: DMSO- d_6 titration of ^1H NMR Experiment of **C1-C3**.

2.5.4. Design of C8 to C13:

In the previous section, we showcased that cyclic dipeptides featuring $\beta(\text{O})$ - δ^5 -amino acid residues can be synthesized in solution phase peptide synthesis protocol. We characterized our molecules and observed they can assemble into unique hollow sub-nanotubular structures, distinguished by unidirectional hydrogen bonds. Building on this foundation, the current study focuses on the synthesis of macrocycles of varying sizes, incorporating $\beta(\text{O})$ - δ^5 -amino acids, to explore bigger macrocycles. We have successfully synthesized macrocycles containing residues ranging from 2 to 5 δ -amino acids.

we designed seven peptides. These peptides are detailed in Scheme 5 and serve as the basis for our comparative analysis.



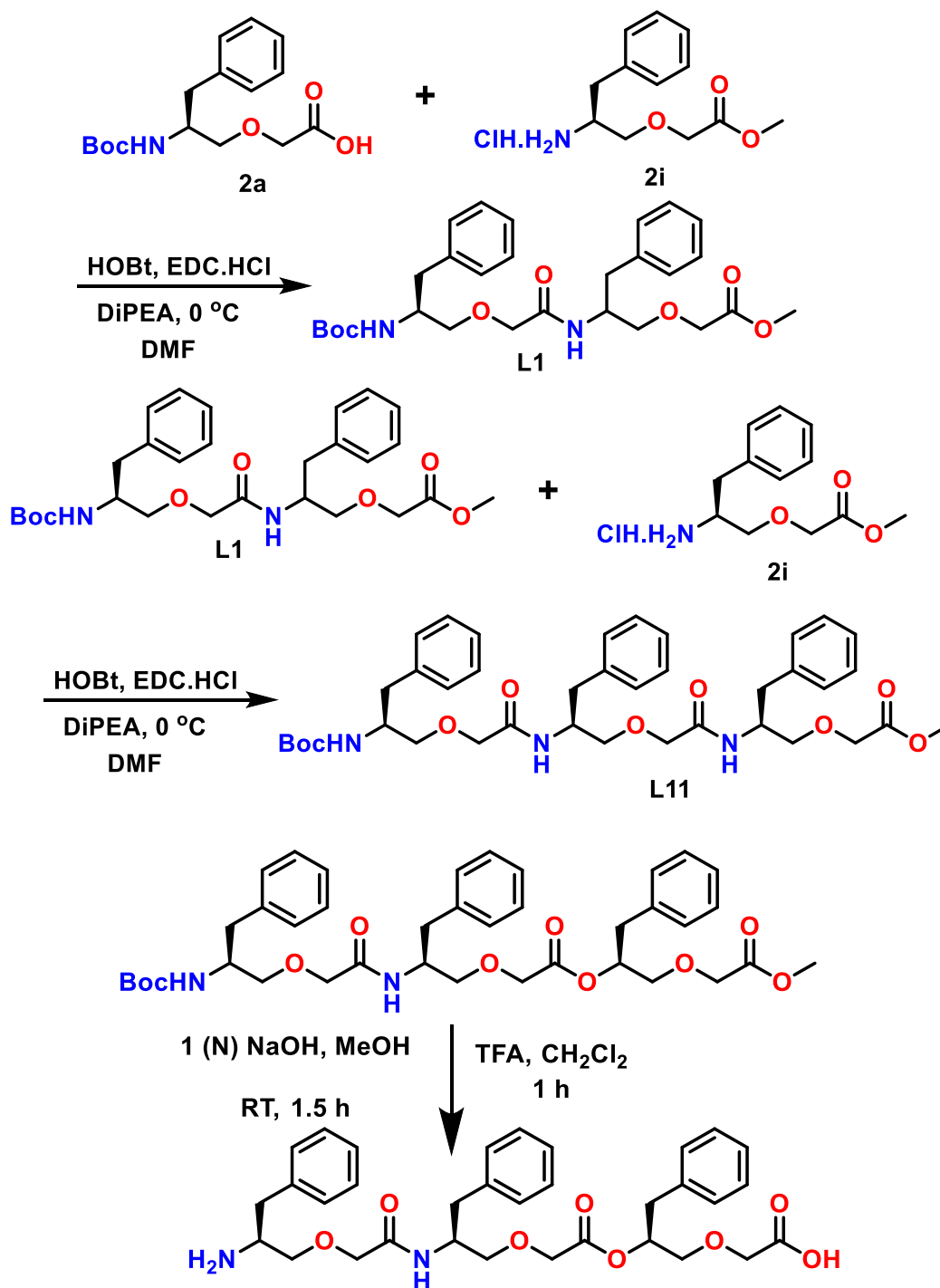
Scheme 5: Sequence of the Cyclic Peptides **C8-C13**.

2.5.4.1. Synthesis of C18 to C13:

In the previous section, we detailed the synthesis of all cyclic peptides using a solution-phase peptide synthesis protocol, which yielded high yields for all cyclic dipeptides. However, as we aimed to synthesize peptides with longer sequences, we encountered challenges with the conventional solution-phase method. The synthesis route became more intricate and time-consuming for higher oligomers. To address these challenges and improve both the efficiency and yield, we developed a solid-phase peptide synthesis protocol for synthesizing higher oligomers. In this approach, we employed 2-chloro-tritylchloride resin to synthesize linear peptides of extended lengths. Subsequently, a solution-phase macrocyclization was conducted using a high dilution method to produce the desired cyclic peptides. The synthesis protocols for both solid and solution

phase methods are depicted below in Scheme 6 and Scheme 7. Macrocyclization method is described in Scheme 8.

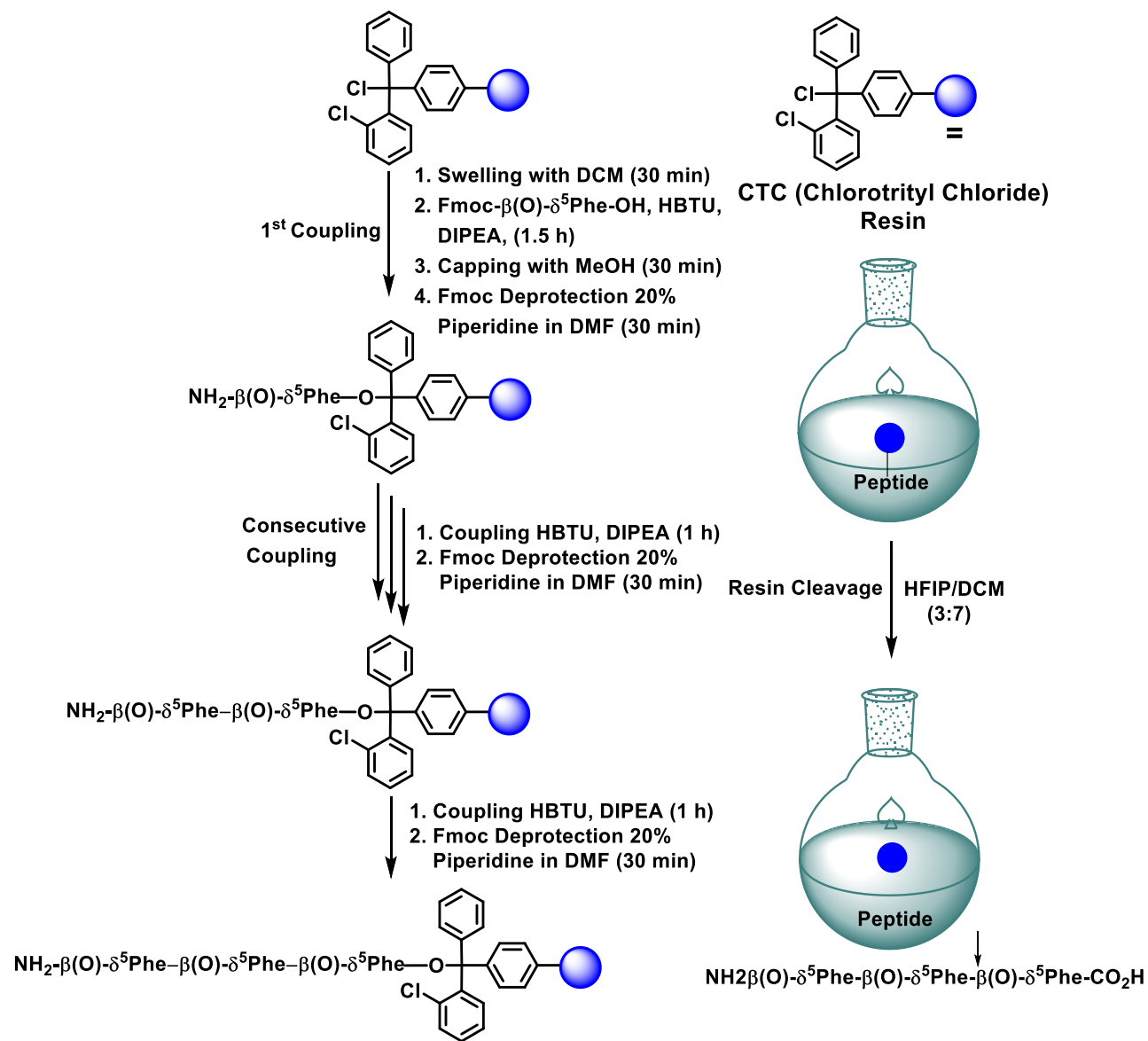
2.5.4.2. Solution Phase Synthesis Protocol:



Scheme 6: Synthesis of Linear tripeptide of **C8** precursor.

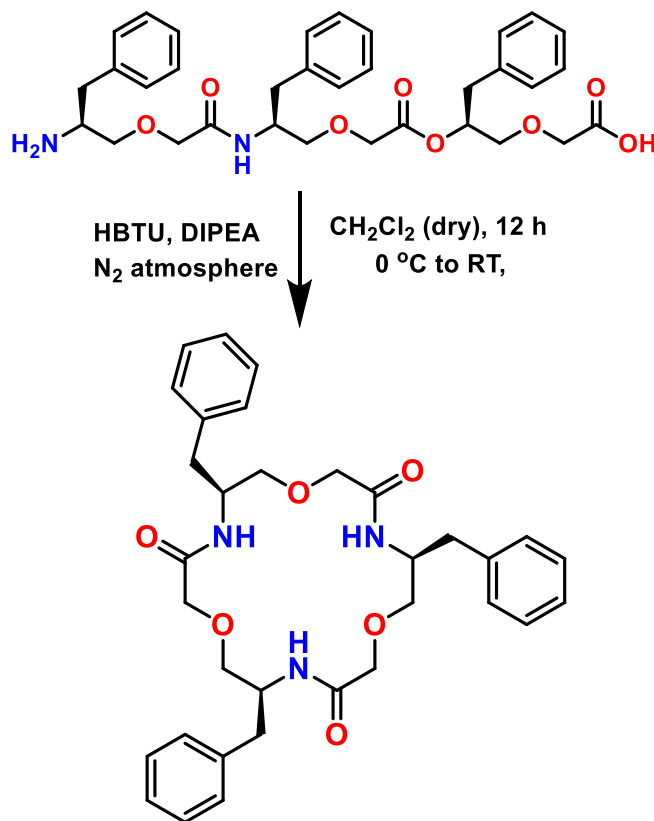
2.5.4.3. Solid Phase Synthesis Protocol:

CTC resin peptide synthesis involves anchoring the first Fmoc-protected amino acid to the CTC resin, followed by iterative cycles of Fmoc deprotection and amino acid coupling to build the peptide sequence. Finally, the peptide is cleaved from the resin using a cleavage cocktail, purified, and analyzed for purity and yield. Here, we have discussed the synthesis route of precursor **C8** cyclic peptide. Similarly, cyclic peptides **C8-C13** are also synthesized in similar protocol discussed below.



Scheme 7: Synthesis of Linear tripeptide of **C8** precursor.

2.5.4.4. Peptide Macrocyclization Protocol:



Scheme 8: Peptide **C8** Macrocyclization Protocol

2.5.4.5. Reverse Phase HPLC Purification:

Following synthesis, all peptides were purified through reverse-phase column chromatography using a MeOH/H₂O solvent system and a C₁₈ column, ensuring high purity and quality of the synthesized peptides.

2.5.4.6. Crystal Structure Analysis of **C8**:

In pursuit of unequivocal structural insights, we subjected a series of cyclic peptides to crystallization using diverse solvent combinations, aiming to obtain single crystal structures. Notably, among the three cyclic peptides investigated, **C8** exhibited the formation of high-quality single crystals suitable for X-ray diffraction through the slow evaporation of its solution in toluene/CHCl₃. Despite repeated attempts, **C9-C13** failed to yield single crystals of X-ray diffraction quality. The X-ray diffraction analysis of **C8**, depicted in Figure 9, disclosed a C3

symmetric molecular arrangement, characterized by an average pore diameter of approximately 5.35 Å. Importantly, **C8** manifests a hybrid composition, combining features of both crown ether and peptide macrocycles.

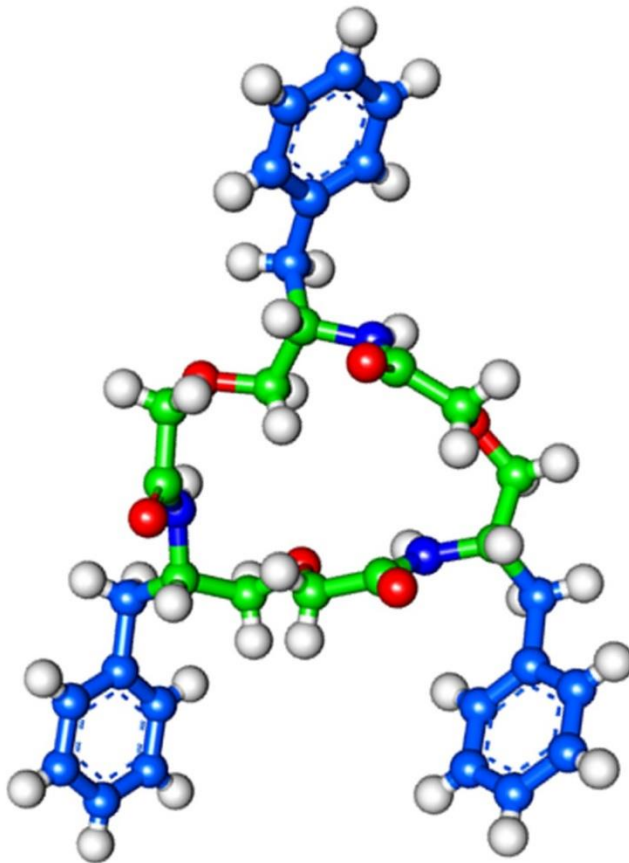


Figure 9: Single Crystal structure of **C8**

Significantly, the cyclic peptides (CPs) assembled into tubular structures through intermolecular hydrogen bonding in a parallel β -sheet configuration. The hydrogen bonding direction within the tubular assembly was unidirectional, portraying the polar nature of the tubular **C8** assembly (Figure 10a). The conformational analysis of the cyclic peptide **C8** focused on the $\beta(\text{O})$ - δ^5 -amino acid, represented by backbone torsion angles $\phi(\text{N}-\text{C}_\delta)$, $\theta_1(\text{C}_\delta-\text{C}_\gamma)$, $\theta_2(\text{C}_\gamma-\text{O}_\beta)$, $\theta_3(\text{O}_\beta-\text{C}_\alpha)$, and $\psi(\text{C}_\alpha-\text{C}=\text{O})$. The corresponding torsion angle parameters are detailed in Table 5, while Table 6 provides intermolecular hydrogen bond parameters. The average inter-distance between stacked cyclic peptides was approximately 5.17 Å.

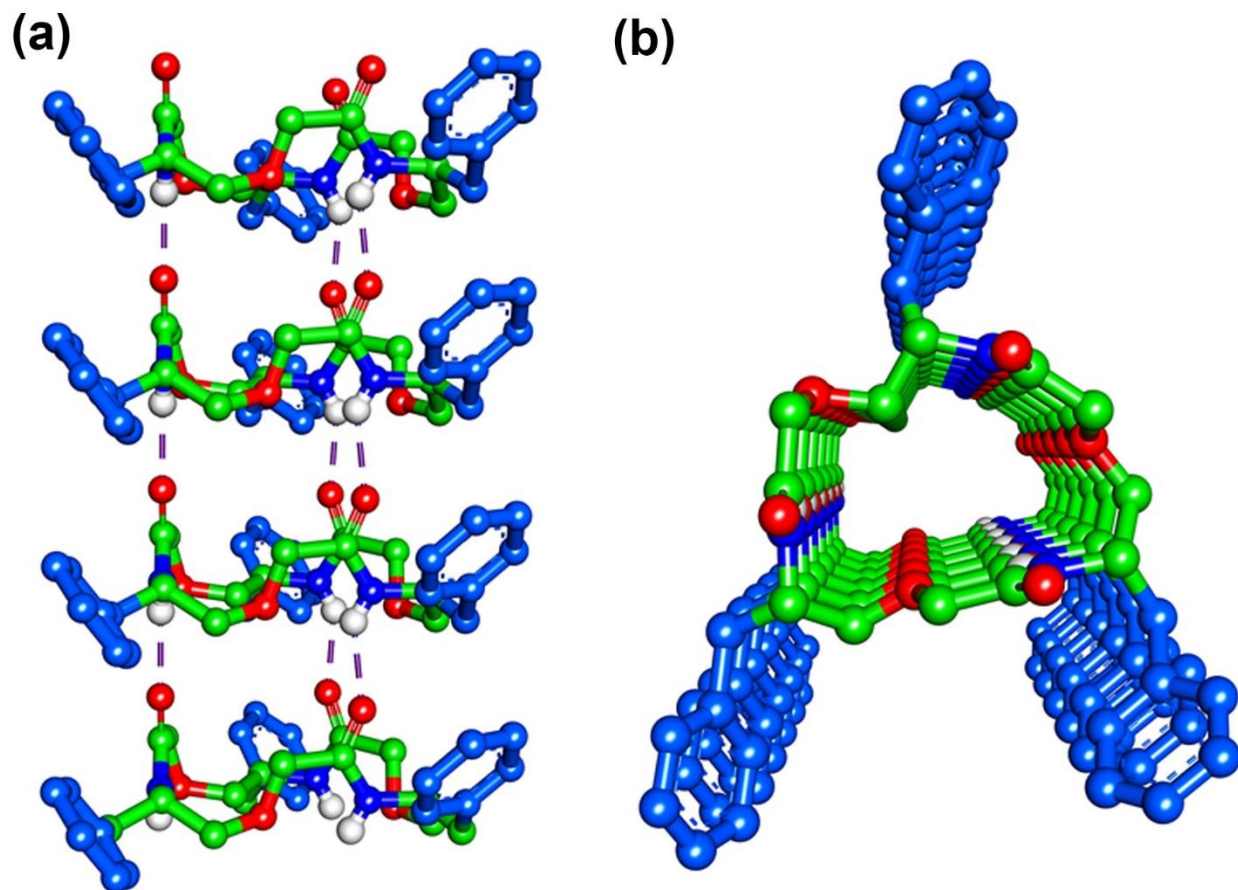


Figure 10: (a) Parallel β -sheet type of hydrogen bonding in **C8**, (b) Top View of self-assembled **C8**

Torsion Angles Parameters of Peptide C8:

All backbone torsional angle parameters of compound **C8** are described in below Table 9.

Peptide C8	ϕ_1 (deg)	θ_1 (deg)	θ_2 (deg)	θ_3 (deg)	ψ (deg)
$\beta(\text{O})$ - $\delta 5$ -Phe 1	-120.04	45.8	66.99	161.38	-12.34
$\beta(\text{O})$ - $\delta 5$ -Phe 2	-129.24	42.06	63.88	-161.80	-17.58
$\beta(\text{O})$ - $\delta 5$ -Phe 3	-92.50	173.60	-91.10	-79.55	-13.84

Table 9: Torsion angle parameters of **C8**.

Hydrogen Bond Parameters of Peptide C8:

All intermolecular hydrogen bond parameters including bond angle and bond distances of compound **C8** are described in Table 10.

Donor(D)	Acceptor(A)	D....A(Å)	DH....A(Å)	NH....O(deg)
C8				
N3	O1	2.914	2.173	144.29
N2	O5	2.903	2.147	146.50
N1	O3	2.916	2.129	151.71

Table 10: Hydrogen Bond parameters of **C8**.

2.5.4.7. Solution Structure Analysis of C12:

As **C8** having symmetric molecule having all same building block, it is very difficult to differentiate each phe unit. For that, we have synthesized cyclic tri-peptide with all different amino acid residues (valine, leucine, phenylalanine). The ^1H NMR of the peptide **C12** in CDCl_3 (3 mM) revealed the well dispersed NH and C^δH in solution. The two-dimensional ROESY spectrum of **C12** is shown in Figure 11 and 12. The amino acid type and sequential connectivity of the residues were established using ROESY and TOCSY spectra. The observed key NOEs are shown in below mentioned figures. The assigned spectra suggested that the strong NOEs were observed between $\text{NH}(i) \leftrightarrow \text{C}^\gamma\text{H}(i)$ and weak NOEs observed between $\text{NH}(i) \leftrightarrow \text{C}^\alpha\text{H}(i+2)$ residue.

2.5.4.8. ROESY Spectra of C12:

The through space correlations were assigned using rotating frame nuclear Overhauser effect spectroscopy (ROESY) for establishing the three-dimensional structure and assignment of homonuclear correlations. The two-dimensional ROESY spectrum is shown in below figures.

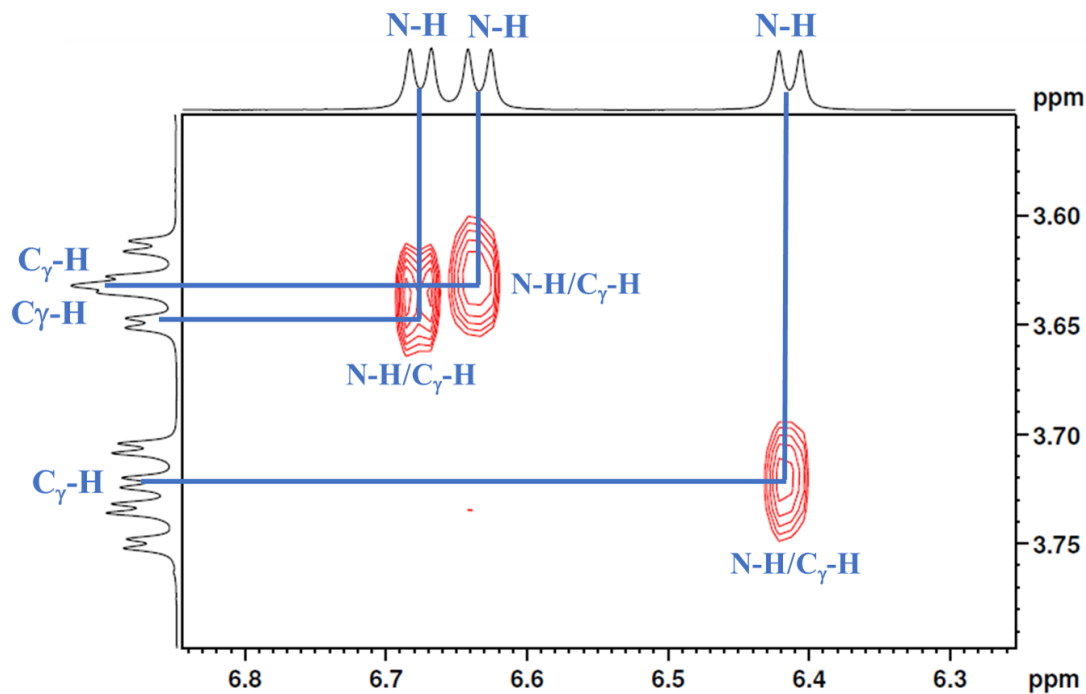


Figure 11: NMR characterization of **C8** in CDCl_3 . ROESY Spectra shows $\text{NH}(i) \leftrightarrow \text{C}^\gamma\text{H}(i)$ interaction.

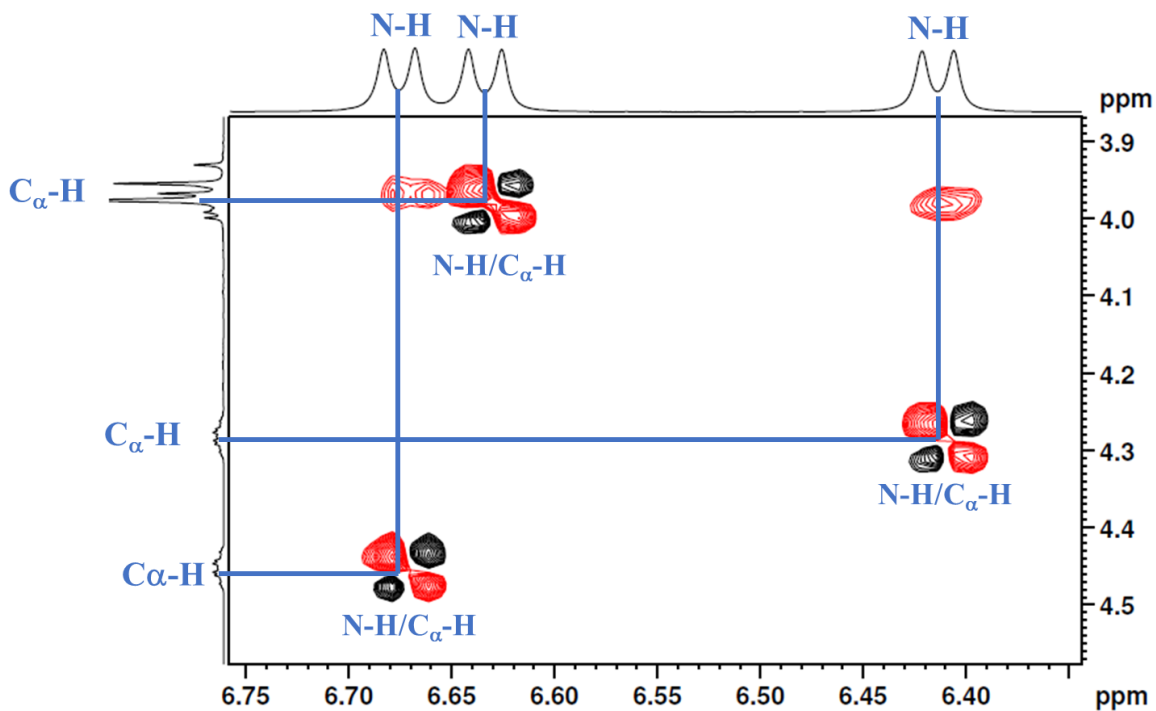


Figure 11: NMR characterization of **C8** in CDCl_3 . ROESY Spectra shows $\text{NH}(i) \leftrightarrow \text{C}^\alpha\text{H}(i)$ interaction.

2.6. Conclusion:

In summary, we are reporting cyclic peptide library derived from $\beta(\text{O})\text{-}\delta^5$ -amino acids, which acts as a hybrid between crown ethers and peptide macrocycles. Additionally, the cyclic peptide composed of carbon analogs of $\beta(\text{O})\text{-}\delta^5$ -amino acids was found to be insoluble in most organic solvents in literature. The insertion of an oxygen atom into the backbone significantly influences the solubility of cyclic peptides derived from δ -amino acids. The study demonstrates that the cyclic peptides formed from the $\beta(\text{O})\text{-}\delta^5$ -amino acid oligomer self-assembles into hollow tubular structures with different pore diameter of approximately 3 Å to 6 Å. These structures are stabilized by intermolecular hydrogen bonds. Unlike cyclic peptides derived from α -amino acids, those from $\beta(\text{O})\text{-}\delta^5$ -amino acids are stabilized by unidirectional hydrogen bonds. NMR analysis further supports the existence of tubular assemblies of these peptides in solution.

Thus, the newly designed polar crown ether peptide macrocycles present new opportunities in the development of cyclic peptide-based biomaterials and transmembrane ion channels.

2.7. Experimental Section:

2.7.1. Materials and Methods:

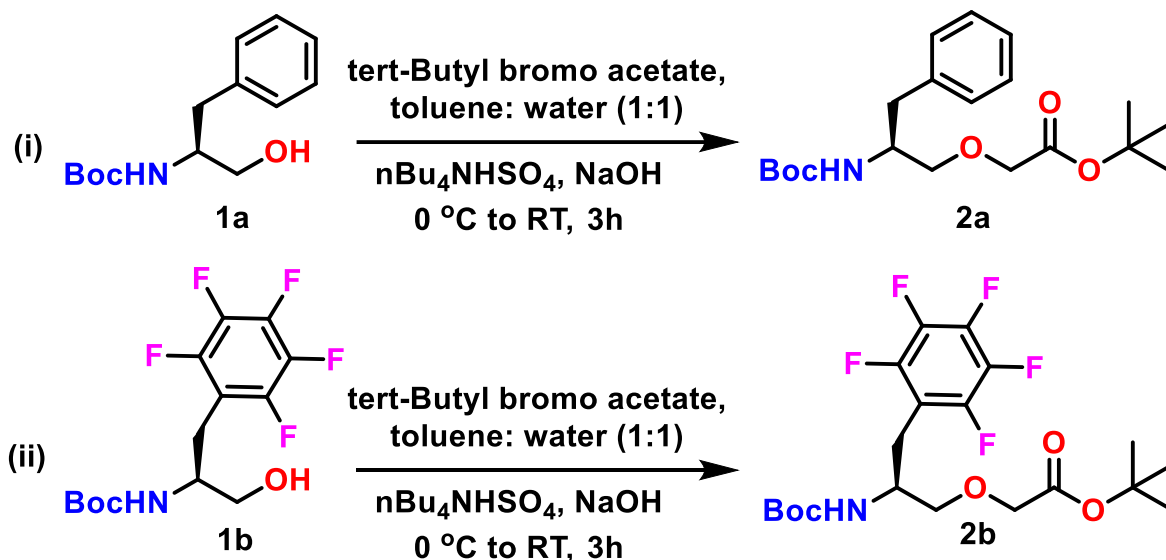
All coupling reactions were carried out under a nitrogen atmosphere. We sourced various amino acids, Boc anhydride, HBTU, HOBt, as well as, Trifluoroacetic acid (TFA), N,N-Diisopropylethylamine (DIPEA), DMF, EtOAc, Hexane, THF, from different commercial suppliers. Before use, we distilled Ethyl acetate and Hexane. Silica gel (100-200 mesh) was employed for column chromatography. We monitored reactions using analytical thin-layer chromatography (TLC) and verified results under UV light and ninhydrin or iodine staining. All final cyclic peptides were purified by reverse phase HPLC. NMR spectra (^1H at 400 MHz and 600MHz and ^{13}C at 101 MHz) were recorded using deuterated solvents with CDCl_3 as the internal standard. Chemical shifts are reported in parts per million (ppm, δ) relative to tetramethyl silane (δ 0.00). ^1H NMR splitting patterns were identified as singlet (s), doublet (d), broad doublet (bd), triplet (t), doublet of doublets (dd), and quintet (qn). Coupling constants (J) are reported in Hertz (Hz). Mass spectra were acquired using a MALDI-TOF instrument. Additionally, X-ray data were collected at low temperatures (150 K) using a Bruker APEX (II) DUO CCD diffractometer with

both Mo K α ($\lambda = 0.71073 \text{ \AA}$) and Cu K α radiation ($\lambda = 1.54178 \text{ \AA}$), which had been monochromated with graphite.

2.7.2. Synthesis Procedures and Schemes

Synthesis of N-Boc- β (O)- δ^5 AA-OtBu

N-Boc- β (O)- δ^5 -Amino Acid was synthesized following a previously established procedure starting from N-Boc-amino alcohol.²⁰ In a nutshell, a solution comprising 30 mL of a 1:1 mixture of water and toluene was prepared. In this solution, sodium hydroxide (290 mmol) was dissolved under cold conditions. Subsequently, *tert*-butylbromoacetate (15 mmol) was slowly introduced into the biphasic reaction mixture at 0 °C. Following this, a solution containing N-Boc-amino alcohol (22.5 mmol) in 10 mL of toluene was also slowly added to the reaction mixture at 0 °C. The reaction mixture was stirred at room temperature for approximately 3 hours. Once the reaction was completed (confirmed by TLC), the organic layer was extracted with diethyl ether (40 mL $\times$ 2). The combined organic layer was washed with brine (40 mL $\times$ 2) and then dried over anhydrous sodium sulfate. The organic layer was subsequently concentrated under reduced pressure to yield a white, gummy product. The crude *tert*-butyl-protected N-Boc- β (O)- δ^5 -Amino Acid product was purified using column chromatography with an EtOAc/pet ether solvent system.

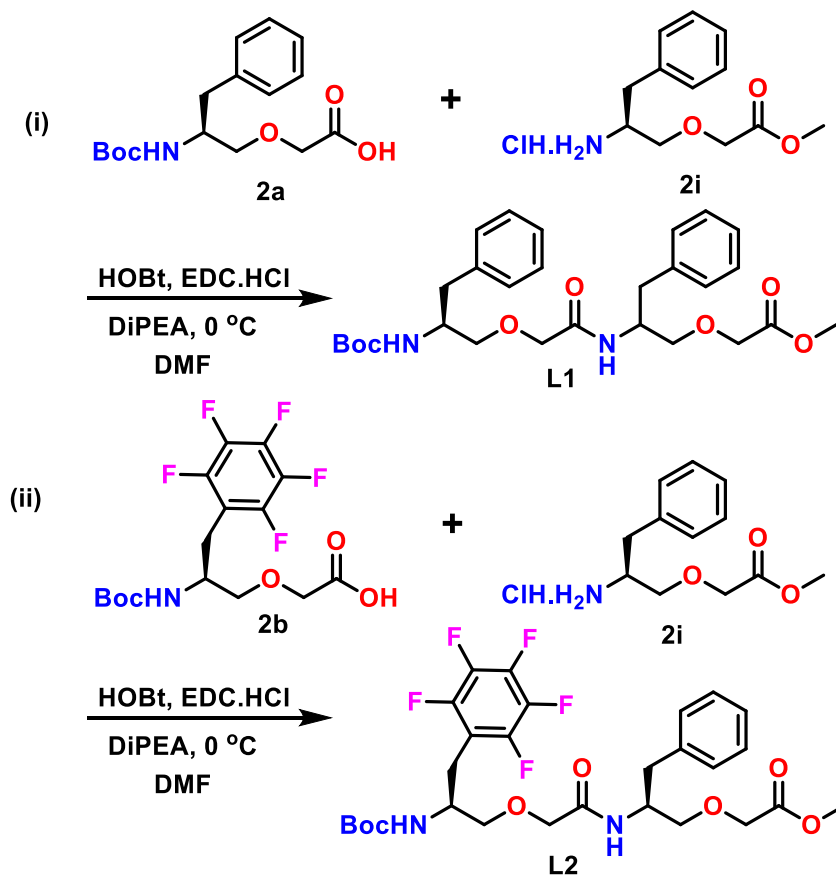


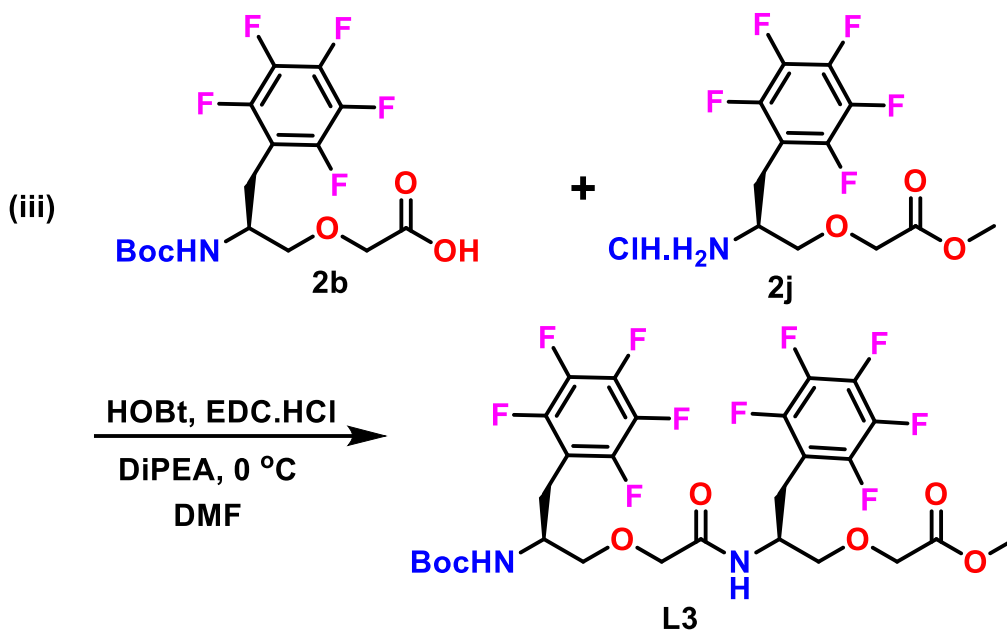
Scheme 8: Synthesis of N-Boc- β (O)- δ^5 -Amino Acids (i) 2a, and (ii) 2b.

Similarly, we have synthesized all the building blocks containing delta amino acid (2c-2f).

General Procedure for the Solution Phase Synthesis of Dipeptides (L1–L3):

The process began by dissolving 1.54 gram (5 mmol) of N-Boc- β (O)- δ^5 -Phe-OH in 3 mL of DMF under a nitrogen atmosphere. In this solution, 960 mg (5 mmol) of EDC.HCl, 675 mg (5 mmol) of HOBt, and 1.74 mL (10 mmol) of DiPEA were added at 0 °C. The reaction mixture was stirred for approximately 15 minutes. Following that, 1.42 grams (5.5 mmol) of ClH₃N- β (O)- δ^5 -Phe-OMe were introduced, and the mixture was stirred for about 12 hours. After confirming the completion of the reaction using TLC, 15 mL of a brine solution was added, and the compound was extracted with ethyl acetate (EtOAc) three times using 20 mL portions each time. The combined organic layers were successively washed with 10% HCl (three times, 20 mL each), 10% Na₂CO₃ (three times, 20 mL each), and brine (three times, 20 mL each). The combined organic layer was then dried using anhydrous Na₂SO₄. Afterward, the solvent was evaporated under reduced pressure to obtain the dipeptide BocNH- β (O)- δ^5 -Phe- β (O)- δ^5 -Phe-OMe (**L1**). Dipeptide **L2**, **L3** were synthesized by similar **L1** Synthesis strategy. Synthesis of **L1**, **L2**, and **L3** scheme are below depicted.





Scheme 9: Synthesis of linear dipeptides (i) L1, (ii) L2, (iii) L3.

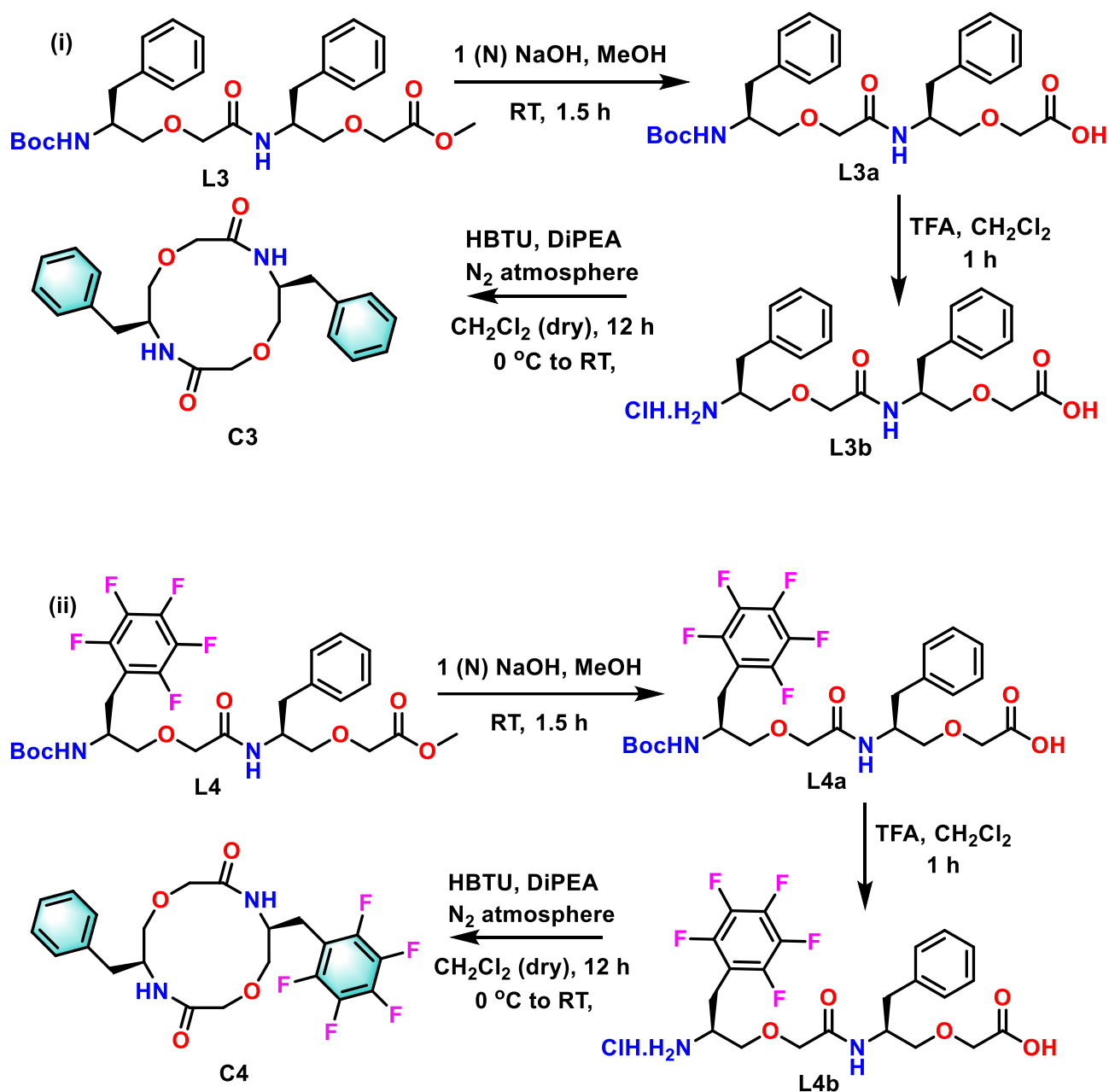
Similarly, we have synthesized all the linear dipeptides containing delta amino acids.

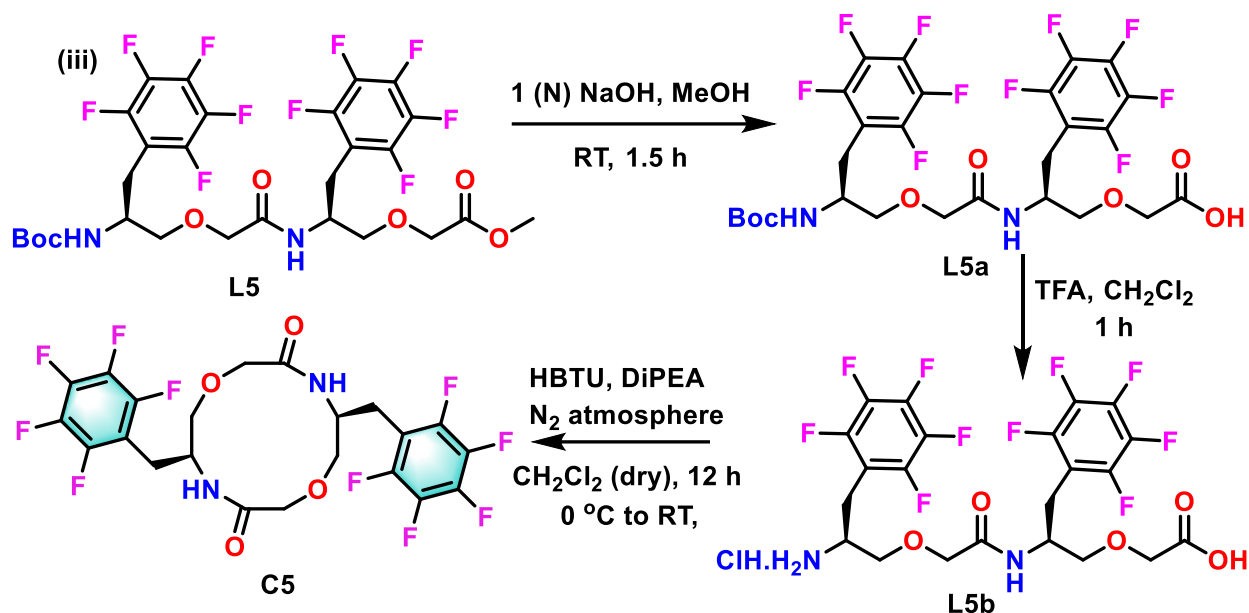
General Procedure for the Solution Phase Synthesis of Cyclic Peptides (C1-C7):

Subsequently, the dipeptide (**L1**) BocNH- β (O)- δ^5 -Phe- β (O)- δ^5 -Phe-OMe was hydrolyzed using 1N NaOH in MeOH, and the reaction mixture was stirred for 1.5 hour. After confirming the completion of the reaction with TLC, MeOH was evaporated under vacuum. The residue was acidified with 10% HCl and extracted with EtOAc three times using 20 mL portions each time. The combined organic layer was washed with brine three times using 20 mL portions each time. The combined organic layer was then dried over anhydrous Na₂SO₄. Afterward, the solvent was evaporated under reduced pressure to obtain the dipeptide (**L1a**) BocNH- β (O)- δ^5 -Phe- β (O)- δ^5 -Phe-OH. The Boc protecting group was then removed using a TFA/DCM mixture. After the reaction was completed, TFA was evaporated under reduced pressure to obtain (**L1b**) ClH₃N- β (O)- δ^5 -Phe- β (O)- δ^5 -Phe-OH, which was then subjected to macrocyclization. The macrocyclization of the linear peptide was carried out using a high dilution technique. Both sides of the deprotected peptide, at a concentration of 1 mg per 10 mL, were dissolved in dry DCM under a nitrogen atmosphere and cooled to 0 °C. To the reaction mixture, HBTU (3 equivalents) and DiPEA (10 equivalents) were added at 0 °C. The reaction mixture was stirred for about 12 hours. After the reaction was completed, DCM was evaporated under reduced pressure, and the

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compound was extracted with EtOAc (three times, 20 mL each). The combined organic layer was washed with 10% HCl (three times, 20 mL each), 10% Na₂CO₃ (three times, 20 mL each), and brine (three times, 20 mL each). The combined organic layer was dried over anhydrous Na₂SO₄. Subsequently, the solvent was evaporated under reduced pressure to obtain the crude cyclic peptide C3. C4, C5 also were synthesized by same protocol and discussed below.





Scheme 10: Synthesis of Cyclic Peptide (i) **C3**, (ii) **C4**, and (iii) **C5**.

We have synthesized all cyclic dipeptides **C1-C7** in same synthesis protocol discussed above.

HPLC Purification:

This crude peptides **C1-C13** were then dissolved in MeOH and purified by reverse-phase high-performance liquid chromatography (RP-HPLC) using a C₁₈ column and a MeOH/H₂O gradient solvent system.

2.7.3. Characterization of Peptides:

(a) Solution Phase Synthesized C1-C7 CPs' Boc protected Building Block:

2a: N-Boc-β(O)-δ⁵-Phe-O^tBu

¹H NMR (400 MHz, Chloroform-*d*) δ 7.32 – 7.26 (m, 2H), 7.25 – 7.17 (m, 3H), 5.17 (d, *J* = 8.2 Hz, 1H), 4.04 – 3.83 (m, 3H), 3.45 (dd, *J* = 3.9, 1.6 Hz, 2H), 2.98 – 2.82 (m, 2H), 1.48 (s, 9H), 1.42 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ 169.76, 155.60, 138.42, 129.62 (2C), 128.51 (2C), 126.42, 81.99, 79.31, 71.72, 69.09, 51.98, 37.82, 28.53 (3C), 28.25 (3C). HRMS (ESI) calculated for C₂₀H₃₃NO₅ [M+H]⁺ *m/z* 366.2280 found 366.2278.

2b: N-Boc-β(O)-δ⁵-Phe(F5)-O^tBu

^1H NMR (400 MHz, Chloroform-*d*) δ 5.20 (d, J = 9.1 Hz, 1H), 4.06 – 3.98 (m, 1H), 3.98 (d, J = 2.8 Hz, 2H), 3.66 – 3.56 (m, 2H), 3.05 (dd, J = 13.8, 9.3 Hz, 1H), 2.96 (dd, J = 13.9, 5.1 Hz, 1H), 1.49 (s, 9H), 1.34 (s, 9H). HRMS (ESI) calculated for $\text{C}_{20}\text{H}_{27}\text{F}_5\text{NO}_5$ $[\text{M}+\text{H}]^+$ m/z 456.1809 found 456.1808.

2c: N-Boc- β (O)- δ^5 -Nap-O^tBu

^1H NMR (400 MHz, Chloroform-*d*) δ 8.35 (d, J = 8.5 Hz, 1H), 7.84 (d, J = 8.1 Hz, 1H), 7.75 – 7.72 (m, 1H), 7.55 (t, J = 7.6 Hz, 1H), 7.49 – 7.45 (m, 1H), 7.39 (d, J = 6.1 Hz, 2H), 5.43 (d, J = 8.0 Hz, 1H), 4.11 – 4.05 (m, 1H), 4.00 (d, J = 16.3 Hz, 1H), 3.86 (d, J = 16.4 Hz, 1H), 3.58 – 3.49 (m, 1H), 3.44 – 3.37 (m, 2H), 3.27 (dd, J = 17.6, 6.2 Hz, 1H), 1.49 (s, 9H), 1.46 (s, 9H). ^{13}C NMR (101 MHz, CHLOROFORM-*D*) δ 169.76, 155.76, 134.73, 133.98, 132.49, 128.62, 127.96, 127.31, 126.29, 125.71, 125.52, 124.37, 82.02, 79.27, 71.41, 69.03, 51.39, 34.96, 28.56 (3C), 28.25 (3C).

2d: N-Boc- β (O)- δ^5 -Diphe-O^tBu

^1H NMR (400 MHz, Chloroform-*d*) δ 7.36 – 7.31 (m, 4H), 7.28 – 7.24 (m, 4H), 7.16 (q, J = 7.3 Hz, 2H), 4.95 (d, J = 9.7 Hz, 1H), 4.57 (t, J = 10.5 Hz, 1H), 4.30 (d, J = 10.9 Hz, 1H), 3.92 (d, J = 16.2 Hz, 1H), 3.81 (d, J = 16.1 Hz, 1H), 3.55 (dd, J = 9.5, 3.1 Hz, 1H), 3.32 (dd, J = 9.4, 2.9 Hz, 1H), 1.46 (s, 9H), 1.31 (s, 9H). ^{13}C NMR (101 MHz, CDCl_3) δ 169.61, 155.60, 142.53, 141.83, 128.80 (2C), 128.73 (2C), 128.43 (2C), 126.73 (2C), 126.49 (2C), 81.81, 79.28, 72.05, 69.54, 52.99, 52.74, 28.40 (3C), 28.24 (3C).

2e: N-Boc- β (O)- δ^5 -Leu-O^tBu

^1H NMR (400 MHz, Chloroform-*d*) δ 4.84 (d, J = 8.8 Hz, 1H), 3.95 (s, 2H), 3.76 (bs, 1H), 3.53 (dd, J = 9.2, 3.7 Hz, 1H), 3.50 – 3.46 (m, 1H), 1.69 – 1.62 (m, 1H), 1.47 (s, 9H), 1.43 (s, 9H), 1.40 – 1.31 (m, 2H), 0.91 (dd, J = 6.6, 1.6 Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 169.79, 155.73, 81.81, 79.12, 73.99, 69.21, 48.66, 41.16, 28.54 (3C), 28.25 (3C), 24.94, 23.04, 22.52.

2f: N-Boc- β (O)- δ^5 -Val-O^tBu

^1H NMR (400 MHz, Chloroform-*d*) δ 4.92 (d, J = 8.9 Hz, 1H), 3.94 (d, J = 5.3 Hz, 2H), 3.70 – 3.64 (m, 1H), 3.46 (q, J = 3.9 Hz, 2H), 1.90 (dt, J = 13.7, 6.8 Hz, 1H), 1.47 (s, 9H), 1.44 (s, 9H), 0.94 (dd, J = 10.9, 6.8 Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 169.82, 156.11, 81.83, 79.12, 71.99, 69.22, 55.70, 29.68, 28.56 (3C), 28.27 (3C), 19.62, 18.92.

2g: N-Boc-β(O)-δ⁵-Ala-O^tBu

¹H NMR (400 MHz, Chloroform-*d*) δ 4.98 (s, 1H), 3.95 (s, 2H), 3.88 – 3.70 (m, 1H), 3.50 – 3.44 (m, 2H), 1.47 (s, 9H), 1.42 (s, 9H), 1.19 (d, *J* = 6.7 Hz, 3H).

¹³C NMR (101 MHz, CDCl₃) δ 169.81, 155.59, 81.89, 79.19, 74.75, 68.97, 46.31, 28.52 (3C), 28.23 (3C), 17.91.

(b) Solution Phase Synthesized C1-C7 CPs' precursor linear dipeptide:

L1: N-Boc-β(O)-Leu-β(O)-δ-Leu-OMe

¹H NMR (400 MHz, Chloroform-*d*) δ 6.97 (d, *J* = 8.7 Hz, 1H), 5.02 (d, *J* = 9.2 Hz, 1H), 4.10 (dt, *J* = 10.0, 3.6 Hz, 1H), 4.04 (t, *J* = 3.1 Hz, 2H), 3.89 (t, *J* = 2.8 Hz, 2H), 3.78 (s, 1H), 3.70 (s, 3H), 3.55 (dd, *J* = 9.3, 3.9 Hz, 1H), 3.49 – 3.44 (m, 2H), 3.41 (dd, *J* = 9.4, 4.0 Hz, 1H), 1.58 (dq, *J* = 12.9, 6.4 Hz, 2H), 1.51 – 1.39 (m, 2H), 1.37 (s, 9H), 1.35 – 1.18 (m, 2H), 0.86 (dt, *J* = 6.5, 3.0 Hz, 12H). ¹³C NMR (101 MHz, CDCl₃) δ 171.21, 169.11, 155.57, 79.03, 74.22, 73.22, 70.76, 67.79, 51.89, 48.52, 46.80, 40.85, 40.44, 28.37 (3C), 24.84 (2C), 22.98, 22.77, 22.46, 22.20.

L2: N-Boc-β(O)-Phe-β(O)-δ-Leu-OMe

¹H NMR (400 MHz, Chloroform-*d*) δ 7.30 – 7.26 (m, 2H), 7.23 (d, *J* = 1.5 Hz, 1H), 7.21 – 7.18 (m, 3H), 5.34 (d, *J* = 9.0 Hz, 1H), 4.18 – 4.13 (m, 2H), 4.07 (d, *J* = 16.9 Hz, 2H), 3.93 (d, *J* = 14.0 Hz, 2H), 3.74 (s, 3H), 3.64 (dd, *J* = 9.2, 4.0 Hz, 1H), 3.51 (ddd, *J* = 9.4, 8.2, 3.5 Hz, 2H), 3.40 (dd, *J* = 9.4, 3.6 Hz, 1H), 2.90 (dd, *J* = 7.6, 4.6 Hz, 2H), 1.64 – 1.53 (m, 2H), 1.40 (s, 9H), 1.37 – 1.28 (m, 1H), 0.96 (d, *J* = 0.9 Hz, 3H), 0.94 (d, *J* = 0.8 Hz, 3H). ¹³C NMR (101 MHz, CHLOROFORM-*D*) δ 171.63, 169.05, 168.89, 138.09, 129.32 (2C), 128.60 (2C), 126.58, 73.33, 72.06, 70.82, 68.40, 67.83, 52.20, 49.94, 47.03, 41.85, 40.59, 28.48 (3C), 25.05, 24.29, 22.72.

L3: N-Boc-β(O)-δ⁵-Phe-β(O)-δ⁵-Phe-OMe

¹H NMR (400 MHz, Chloroform-*d*) δ 7.54 (d, *J* = 7.6 Hz, 1H), 7.30 – 7.26 (m, 3H), 7.24 (d, *J* = 3.5 Hz, 2H), 7.22 – 7.15 (m, 5H), 5.44 (d, *J* = 9.2 Hz, 1H), 4.22 (tt, *J* = 5.3, 2.8 Hz, 1H), 4.17 (d, *J* = 17.0 Hz, 1H), 4.02 (d, *J* = 17.0 Hz, 2H), 3.93 – 3.84 (m, 2H), 3.71 (s, 3H), 3.54 – 3.46 (m, 2H), 3.44 – 3.33 (m, 2H), 3.07 (dd, *J* = 13.3, 5.6 Hz, 1H), 2.90 (d, *J* = 9.2 Hz, 1H), 2.88 – 2.84 (m, 2H), 1.37 (s, 9H). HRMS (ESI) calculated for C₂₈H₃₉N₂O₇ [M+H]⁺ *m/z* 515.2757 found 515.2753.

L4: N-Boc-β(O)-δ-F5- β(O)-δ-F-OMe

¹H NMR (400 MHz, Chloroform-*d*) δ 7.55 – 7.47 (m, 1H), 7.28 (dd, *J* = 6.6, 4.3 Hz, 2H), 7.24 (s, 2H), 7.21 – 7.16 (m, 1H), 5.57 (d, *J* = 9.7 Hz, 1H), 4.23 – 4.16 (m, 2H), 4.07 – 3.91 (m, 4H), 3.77 (s, 3H), 3.63 (dd, *J* = 9.3, 3.5 Hz, 1H), 3.52 (dd, *J* = 10.0, 6.3 Hz, 2H), 3.37 (dd, *J* = 9.2, 3.2 Hz, 1H), 3.05 (dd, *J* = 13.3, 5.4 Hz, 1H), 2.97 (t, *J* = 7.4 Hz, 2H), 2.88 – 2.81 (m, 1H), 1.32 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ 172.13, 168.76, 155.53, 144.48 (2C), 141.24, 138.10, 137.46(2C), 129.61(2C), 128.61 (2C), 126.63, 112.20, 79.77, 73.32, 71.05, 70.92, 67.55, 52.36, 50.61, 50.09, 37.02, 28.33(3C), 25.63. ¹⁹F NMR (377 MHz, Chloroform-*d*) δ -143.15, -156.65, -162.71. HRMS (ESI) calculated for C₂₈H₃₄F₅N₂O₇ [M+H]⁺ *m/z* 605.2286 found 605.2280.

L5: N-Boc-β(O)-δ-F5- β(O)-δ-F5-OMe

¹H NMR (400 MHz, Chloroform-*d*) δ 7.56 (d, *J* = 8.4 Hz, 1H), 5.57 (d, *J* = 9.7 Hz, 1H), 4.40 – 4.32 (m, 1H), 4.23 (d, *J* = 17.1 Hz, 1H), 4.11 – 3.86 (m, 4H), 3.79 (s, 3H), 3.67 (ddd, *J* = 9.6, 6.3, 3.4 Hz, 2H), 3.60 – 3.52 (m, 2H), 3.18 – 3.11 (m, 1H), 3.05 – 2.95 (m, 3H), 1.34 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ 171.88, 168.97, 155.51, 146.82 (4C), 144.44 (2C), 136.28 (4C), 111.93 (2C), 79.78, 73.29, 72.32, 70.80, 67.57, 52.43, 50.09, 48.37, 28.31 (3C), 25.44, 24.92. ¹⁹F NMR (377 MHz, Chloroform-*d*) δ -143.15, -156.65, -162.71. HRMS (ESI) calculated for C₂₈H₂₉F₁₀N₂O₇ [M+H]⁺ *m/z* 695.1815 found 695.1809.

L6: N-Boc-β(O)-δ-Nap-β(O)-δ-Nap-OMe

¹H NMR (400 MHz, Chloroform-*d*) δ 8.61 (d, *J* = 8.5 Hz, 1H), 8.24 (d, *J* = 8.5 Hz, 1H), 8.06 (d, *J* = 7.0 Hz, 1H), 7.86 (dt, *J* = 8.2, 2.3 Hz, 2H), 7.77 – 7.75 (m, 2H), 7.63 – 7.58 (m, 1H), 7.55 (t, *J* = 7.7 Hz, 1H), 7.51 – 7.47 (m, 2H), 7.44 – 7.39 (m, 4H), 5.79 (d, *J* = 9.1 Hz, 1H), 4.41 (dt, *J* = 8.9, 4.6 Hz, 1H), 4.28 (s, 1H), 4.24 (s, 1H), 4.04 – 3.99 (m, 2H), 3.94 – 3.79 (m, 3H), 3.74 (s, 3H), 3.60 – 3.52 (m, 2H), 3.42 – 3.38 (m, 2H), 3.34 – 3.26 (m, 2H), 1.44 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ 172.42, 169.61, 155.67, 134.59, 134.45, 134.05, 134.02, 132.56, 132.39, 128.89, 128.67, 128.03, 127.57, 127.47, 126.57, 126.34, 125.92, 125.81, 125.60, 125.46, 124.77, 123.93, 123.87, 79.40, 71.74, 70.91, 70.77, 67.50, 52.49, 51.14, 50.37, 34.74, 34.32, 28.60 (3C).

L7: N-Boc-β(O)-Diphe-β(O)-δ-Diphe -OMe

¹H NMR (400 MHz, Chloroform-*d*) δ 7.40 – 7.28 (m, 12H), 7.25 – 7.14 (m, 8H), 7.13 – 7.09 (m, 2H), 5.06 – 4.96 (m, 2H), 4.55 – 4.43 (m, 2H), 4.12 (d, *J* = 16.8 Hz, 1H), 3.98 – 3.87 (m, 3H), 3.54

(d, $J = 2.3$ Hz, 3H), 3.50 – 3.45 (m, 2H), 3.29 (dd, $J = 9.3, 2.7$ Hz, 1H), 2.91 (dd, $J = 9.3, 3.7$ Hz, 1H), 1.35 (s, 9H). ^{13}C NMR (101 MHz, CDCl_3) δ 171.22, 169.22, 155.58, 142.19, 141.91, 141.76, 141.76, 128.98 (2C), 128.89 (2C), 128.65 (2C), 128.52 (2C), 128.43 (2C), 128.36 (2C), 128.04 (2C), 127.70 (2C), 127.67, 126.96, 126.88, 126.51, 79.35, 71.80, 71.72, 70.55, 68.13, 53.07, 52.89, 52.34, 52.01, 51.00, 28.42 (3C).

(c) Solution Phase Synthesized Cyclic Peptide C1-C7:

C1:

^1H NMR (400 MHz, Chloroform- d) δ 6.57 (d, $J = 9.7$ Hz, 2H), 4.19 (s, 1H), 4.17 (t, $J = 2.3$ Hz, 1H), 4.15 (s, 1H), 4.13 (dd, $J = 5.7, 2.0$ Hz, 1H), 3.76 (dd, $J = 9.6, 1.7$ Hz, 2H), 3.69 (s, 1H), 3.65 (s, 1H), 3.33 (d, $J = 2.6$ Hz, 1H), 3.31 (d, $J = 2.6$ Hz, 1H), 1.67 – 1.62 (m, 2H), 1.57 (dd, $J = 9.7, 3.8$ Hz, 2H), 1.43 – 1.35 (m, 2H), 0.95 (d, $J = 4.0$ Hz, 6H), 0.93 (d, $J = 4.0$ Hz, 6H). ^{13}C NMR (101 MHz, CHLOROFORM- D) δ 169.50 (2C), 74.64 (2C), 71.19 (2C), 46.67 (2C), 40.09 (2C), 25.08 (2C), 23.02 (2C), 22.32 (2C).

C2:

^1H NMR (400 MHz, Chloroform- d) δ 7.33 – 7.29 (m, 2H), 7.26 – 7.20 (m, 3H), 6.85 (d, $J = 9.3$ Hz, 1H), 6.50 (d, $J = 9.5$ Hz, 1H), 4.32 – 4.20 (m, 2H), 4.20 – 4.11 (m, 2H), 3.74 (d, $J = 1.8$ Hz, 1H), 3.72 (d, $J = 1.9$ Hz, 1H), 3.69 (d, $J = 2.2$ Hz, 1H), 3.65 (d, $J = 1.8$ Hz, 1H), 3.36 (dd, $J = 9.6, 2.6$ Hz, 1H), 3.25 (dd, $J = 10.0, 2.7$ Hz, 1H), 2.92 (d, $J = 8.0$ Hz, 2H), 1.69 – 1.62 (m, 2H), 1.45 – 1.37 (m, 1H), 0.96 (d, $J = 1.3$ Hz, 3H), 0.94 (d, $J = 1.3$ Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 169.55, 169.33, 137.49, 129.39 (2C), 128.75 (2C), 126.89, 74.53, 72.44, 71.46, 71.00, 49.52, 46.87, 40.04, 37.18, 25.09, 23.04, 22.31.

C3:

^1H NMR (400 MHz, Chloroform- d) δ 7.35 – 7.28 (m, 5H), 7.26 – 7.21 (m, 5H), 6.78 (d, $J = 9.2$ Hz, 2H), 4.34 – 4.27 (m, 2H), 4.20 (s, 1H), 4.16 (s, 1H), 3.71 (d, $J = 1.9$ Hz, 1H), 3.69 (s, 1H), 3.68 (s, 1H), 3.65 (s, 1H), 3.29 (dd, $J = 9.8, 2.7$ Hz, 2H), 2.94 (d, $J = 8.0$ Hz, 4H). ^{13}C NMR (101 MHz, CHLOROFORM- D) δ 169.37 (2C), 137.42 (2C), 129.36 (4C), 128.79 (4C), 126.94 (2C), 72.36 (2C), 71.23 (2C), 49.65 (2C), 37.11 (2C). HRMS (ESI) calculated for $\text{C}_{22}\text{H}_{27}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$ m/z 383.1971 found 383.1979. HRMS (ESI) calculated for $\text{C}_{22}\text{H}_{27}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$ m/z 383.1971 found 383.1979.

C4:

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^1H NMR (400 MHz, Chloroform-*d*) δ 7.35 – 7.29 (m, 2H), 7.26 (s, 1H), 7.26 – 7.22 (m, 2H), 6.95 (d, J = 9.7 Hz, 1H), 6.57 (d, J = 9.0 Hz, 1H), 4.33 (p, J = 7.6, 6.6 Hz, 2H), 4.20 (d, J = 14.5 Hz, 1H), 4.12 (d, J = 15.4 Hz, 1H), 3.86 (dd, J = 10.1, 1.7 Hz, 1H), 3.74 – 3.58 (m, 3H), 3.42 (dd, J = 10.1, 2.8 Hz, 1H), 3.27 (dd, J = 9.7, 2.7 Hz, 1H), 3.13 – 3.05 (m, 1H), 2.96 (dd, J = 8.2, 2.6 Hz, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 169.44, 169.01, 146.29, 144.69, 141.08, 139.46, 138.28, 136.6, 137.13, 129.15 (2C), 128.70 (2C), 126.87, 111.23, 73.20, 72.09, 71.51, 70.53, 49.68, 47.81, 36.71, 24.83. ^{19}F NMR (565 MHz, Chloroform-*d*) δ -144.02, -156.40, -162.92. HRMS (ESI) calculated for $\text{C}_{22}\text{H}_{22}\text{F}_5\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$ m/z 473.1498 found 473.1501.

C5:

^1H NMR (600 MHz, Chloroform-*d*) δ 6.78 (d, J = 9.6 Hz, 2H), 4.38 – 4.31 (m, 2H), 4.16 (s, 1H), 4.14 (s, 1H), 3.81 (dd, J = 10.0, 1.7 Hz, 2H), 3.67 (d, J = 15.0 Hz, 2H), 3.38 (dd, J = 9.9, 2.8 Hz, 2H), 3.11 (dd, J = 14.1, 10.1 Hz, 2H), 2.98 (dd, J = 14.2, 5.2 Hz, 2H). ^{13}C NMR (151 MHz, CDCl_3) δ 169.29 (2C), 146.36, 144.79, 141.22, 139.56, 138.45, 136.78, 111.36, 111.25, 111.23, 111.11, 73.25(2C), 71.11(2C), 48.21(2C), 24.73 (2C). ^{19}F NMR (377 MHz, Chloroform-*d*) δ -143.04, -155.22, -161.75. HRMS (ESI) calculated for $\text{C}_{22}\text{H}_{16}\text{F}_{10}\text{N}_2\text{O}_4\text{Na}$ $[\text{M}+\text{Na}]^+$ m/z 585.0847 found 585.0847.

C6:

^1H NMR (600 MHz, Chloroform-*d*) δ 8.22 (d, J = 8.5 Hz, 2H), 7.87 (dd, J = 8.1, 1.3 Hz, 2H), 7.78 (dd, J = 7.5, 2.0 Hz, 2H), 7.58 (ddd, J = 8.4, 6.8, 1.4 Hz, 2H), 7.51 (ddd, J = 8.0, 6.8, 1.1 Hz, 2H), 7.46 – 7.39 (m, 4H), 6.99 (d, J = 9.0 Hz, 2H), 4.53 – 4.49 (m, 2H), 4.26 (s, 1H), 4.24 (s, 1H), 3.72 – 3.68 (m, 4H), 3.51 (dd, J = 13.8, 5.9 Hz, 2H), 3.36 – 3.31 (m, 4H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 169.48 (2C), 134.07 (2C), 133.49 (2C), 132.19 (2C), 129.00 (2C), 127.89 (2C), 127.65 (2C), 126.62 (2C), 125.98 (2C), 125.56 (2C), 123.83 (2C), 72.14 (2C), 71.26 (2C), 49.05 (2C), 34.29 (2C).

C7:

^1H NMR (600 MHz, Chloroform-*d*) δ 7.35 (d, J = 7.2 Hz, 4H), 7.31 – 7.22 (m, 12H), 7.17 (ddd, J = 9.0, 5.0, 1.6 Hz, 4H), 6.61 (d, J = 10.0 Hz, 2H), 4.88 (ddt, J = 11.9, 10.0, 1.9 Hz, 2H), 4.30 (d, J = 12.1 Hz, 2H), 3.93 (d, J = 15.0 Hz, 2H), 3.52 (d, J = 9.7 Hz, 2H), 3.48 (d, J = 15.0 Hz, 2H), 3.18 (dd, J = 9.6, 2.5 Hz, 2H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 169.55 (2C), 141.68 (2C), 141.26

(2C), 129.01 (4C), 128.76 (4C), 128.26 (4C), 128.09 (4C), 127.13 (2C), 126.89 (2C), 72.48 (2C), 71.04 (2C), 51.74 (2C), 50.88 (2C).

(d) Solid Phase Synthesized C8-C13 CPs' Fmoc protected delta amino acid Building Block:

S1: N-Fmoc- β (O)- δ^5 -Phe-OH

^1H NMR (400 MHz, Chloroform-*d*) δ 7.75 (d, J = 7.6 Hz, 2H), 7.55 (d, J = 7.2 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.34 – 7.27 (m, 4H), 7.22 (d, J = 6.8 Hz, 3H), 5.40 (d, J = 8.2 Hz, 1H), 4.51 – 4.28 (m, 2H), 4.18 (d, J = 7.3 Hz, 1H), 4.16 – 3.98 (m, 3H), 3.52 (s, 2H), 2.90 (d, J = 9.9 Hz, 2H). COOH proton did not appear in CDCl_3

^{13}C NMR (101 MHz, CDCl_3) δ 174.07, 156.34, 144.05 (2C), 141.46 (2C), 137.71, 129.47 (2C), 128.70 (2C), 127.83 (2C), 127.21 (2C), 126.76, 125.27 (2C), 120.12 (2C), 72.24, 68.26, 66.90, 52.36, 47.37, 37.65.

S2: N-Fmoc- β (O)- δ^5 -Leu-OH

^1H NMR (400 MHz, Chloroform-*d*) δ 7.75 (d, J = 7.5 Hz, 2H), 7.59 (d, J = 7.5 Hz, 2H), 7.41 – 7.37 (m, 2H), 7.30 (td, J = 7.5, 1.2 Hz, 2H), 5.03 (d, J = 8.9 Hz, 1H), 4.42 (d, J = 6.9 Hz, 2H), 4.21 (t, J = 6.6 Hz, 1H), 4.10 (s, 2H), 4.01 – 3.81 (m, 2H), 3.53 (d, J = 4.9 Hz, 1H), 1.81 – 1.46 (m, 1H), 1.45 – 1.14 (m, 2H), 1.00 – 0.73 (m, 6H). COOH proton did not appear in CDCl_3

^{13}C NMR (101 MHz, CDCl_3) δ 173.30, 156.63, 144.02 (2C), 141.48 (2C), 127.81 (2C), 127.18 (2C), 125.19 (2C), 120.10 (2C), 74.58, 68.36, 66.70, 49.35, 47.44, 40.70, 24.87, 23.13, 22.27.

S3: N-Fmoc- β (O)- δ^5 -Val-OH

^1H NMR (400 MHz, Chloroform-*d*) δ 9.25 (s, 1H), 7.76 (d, J = 7.5 Hz, 2H), 7.60 (dt, J = 13.0, 6.4 Hz, 2H), 7.39 (td, J = 7.2, 2.3 Hz, 2H), 7.31 (td, J = 7.5, 1.2 Hz, 2H), 5.32 (d, J = 9.1 Hz, 1H), 4.60 – 4.37 (m, 2H), 4.28 – 3.95 (m, 3H), 3.81 – 3.63 (m, 1H), 3.59 – 3.26 (m, 2H), 1.97 – 1.63 (m, 1H), 0.95 (dd, J = 10.4, 6.8 Hz, 6H).

^{13}C NMR (101 MHz, CDCl_3) δ 174.59, 156.96, 144.05 (2C), 141.42 (2C), 127.76 (2C), 127.15 (2C), 125.20 (2C), 120.05 (2C), 72.11, 68.09, 66.83, 56.41, 47.37, 29.47, 19.48, 18.89.

S4: N-Fmoc- β (O)- δ^5 -Nap-OH

^1H NMR (400 MHz, Chloroform-*d*) δ 8.31 (d, J = 8.5 Hz, 1H), 7.85 (d, J = 7.2 Hz, 1H), 7.80 – 7.68 (m, 3H), 7.67 – 7.42 (m, 4H), 7.39 (td, J = 7.1, 3.7 Hz, 4H), 7.30 (t, J = 7.5 Hz, 2H), 5.80 (d, J = 7.9 Hz, 1H), 4.39 (td, J = 9.8, 8.7, 5.6 Hz, 2H), 4.26 – 3.97 (m, 4H), 3.56 – 3.45 (m, 2H), 3.45 – 3.13 (m, 2H). COOH proton did not appear in CDCl_3

^{13}C NMR (101 MHz, CDCl_3) δ 174.52, 156.49, 144.06 (2C), 141.43 (2C), 134.06, 134.02, 132.33, 128.86, 127.93 (2C), 127.81 (2C), 127.63, 127.19 (2C), 126.49, 125.87, 125.53, 125.31, 124.13, 120.10 (2C), 71.69, 68.07, 66.95, 51.78, 47.35, 34.86.

(e) Solid Phase Synthesized Cyclic Peptide C8-C13:

C8:

^1H NMR (600 MHz, Chloroform-*d*) δ 7.32 (t, J = 7.5 Hz, 6H), 7.27 (s, 2H), 7.25 (s, 1H), 7.21 – 7.19 (m, 6H), 6.63 (d, J = 9.8 Hz, 3H), 4.45 (tt, J = 12.6, 4.2 Hz, 3H), 3.91 (s, 3H), 3.91 (s, 3H), 3.60 (dd, J = 9.7, 2.9 Hz, 3H), 3.40 (dd, J = 9.7, 2.8 Hz, 3H), 3.00 (dd, J = 13.5, 6.8 Hz, 3H), 2.91 (dd, J = 13.5, 9.4 Hz, 3H).

^{13}C NMR (101 MHz, CDCl_3) δ 168.12 (3C), 137.21 (3C), 129.23 (6C), 128.81 (6C), 127.00 (3C), 70.80 (3C), 70.44 (3C), 49.16 (3C), 37.92 (3C).

C9:

^1H NMR (400 MHz, Chloroform-*d*) δ 7.31 – 7.27 (m, 6H), 7.26 – 7.20 (m, 6H), 7.19 – 7.14 (m, 8H), 6.86 (d, J = 8.8 Hz, 4H), 4.42 – 4.32 (m, 4H), 3.97 – 3.82 (m, 8H), 3.51 – 3.38 (m, 8H), 2.88 (h, J = 6.5, 6.0 Hz, 8H).

^{13}C NMR (151 MHz, CDCl_3) δ 169.40 (4C), 137.15 (4C), 129.13(8C), 128.77 (8C), 126.93 (4C), 72.48 (4C), 71.09 (4C), 50.02 (4C), 37.27 (4C).

C10:

^1H NMR (600 MHz, Chloroform-*d*) δ 7.29 – 7.27 (m, 6H), 7.26 (d, J = 0.8 Hz, 4H), 7.24 – 7.20 (m, 5H), 7.17 – 7.13 (m, 10H), 6.97 (d, J = 9.1 Hz, 5H), 4.48 – 4.37 (m, 5H), 3.89 (d, J = 14.9 Hz, 5H), 3.84 (d, J = 14.9 Hz, 5H), 3.40 (dd, J = 9.7, 4.4 Hz, 5H), 3.34 (dd, J = 9.8, 5.8 Hz, 5H), 2.84 (qd, J = 13.7, 7.2 Hz, 10H).

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^{13}C NMR (151 MHz, CDCl_3) δ 169.38 (5C), 137.26 (5C), 129.28 (10C), 128.89 (10C), 127.07 (5C), 72.52 (5C), 70.81 (5C), 49.52 (5C), 37.44 (5C).

C11:

^1H NMR (600 MHz, Chloroform-*d*) δ 8.25 (d, J = 8.5 Hz, 3H), 7.86 (dd, J = 8.2, 1.3 Hz, 3H), 7.79 (d, J = 8.2 Hz, 3H), 7.58 (ddd, J = 8.3, 6.8, 1.3 Hz, 3H), 7.50 (ddd, J = 8.0, 6.8, 1.1 Hz, 3H), 7.43 (dd, J = 8.2, 6.9 Hz, 3H), 7.32 (dd, J = 7.0, 1.2 Hz, 3H), 6.86 (d, J = 8.6 Hz, 3H), 4.66 – 4.62 (m, 3H), 3.94 (d, J = 4.1 Hz, 6H), 3.60 (dd, J = 8.5, 5.1 Hz, 6H), 3.39 (dd, J = 9.6, 2.9 Hz, 3H), 3.27 (dd, J = 13.8, 9.8 Hz, 3H).

^{13}C NMR (151 MHz, CDCl_3) δ 168.61 (3C), 134.16 (3C), 133.60 (3C), 132.28 (3C), 129.11 (3C), 128.07 (3C), 127.80 (3C), 126.77 (3C), 126.14 (3C), 125.59 (3C), 123.77(3C), 70.82 (3C), 49.10 (3C), 34.93 (3C), 29.84 (3C).

C12:

^1H NMR (400 MHz, Chloroform-*d*) δ 7.31 (dd, J = 8.0, 6.4 Hz, 2H), 7.24 (d, J = 7.5 Hz, 1H), 7.20 – 7.16 (m, 2H), 6.65 (d, J = 9.1 Hz, 1H), 6.61 (d, J = 9.7 Hz, 1H), 6.39 (d, J = 9.3 Hz, 1H), 4.43 (td, J = 9.1, 6.6 Hz, 1H), 4.26 (dtd, J = 8.9, 6.2, 3.2 Hz, 1H), 4.01 – 3.96 (m, 1H), 3.96 – 3.92 (m, 6H), 3.71 (ddd, J = 11.3, 9.6, 2.6 Hz, 2H), 3.61 (ddd, J = 9.6, 8.0, 2.8 Hz, 2H), 3.42 (ddd, J = 15.5, 9.5, 2.5 Hz, 2H), 2.96 (dd, J = 13.3, 6.5 Hz, 1H), 2.88 (dd, J = 13.3, 8.9 Hz, 1H), 1.97 – 1.90 (m, 1H), 1.52 (ddd, J = 14.9, 8.9, 6.3 Hz, 2H), 1.44 – 1.37 (m, 1H), 1.04 (dd, J = 10.1, 6.8 Hz, 6H), 0.97 (dd, J = 6.5, 3.9 Hz, 6H).

^{13}C NMR (151 MHz, CDCl_3) δ 168.25, 168.15, 168.04, 137.20, 129.11, 128.76, 126.96, 72.70, 71.03, 70.64, 70.62, 70.52, 70.42, 53.01, 49.17, 46.30, 40.84, 37.96, 30.22, 25.11, 22.88, 22.49 (2C), 19.66 (2C).

C13:

^1H NMR (400 MHz, Chloroform-*d*) δ 7.30 (dd, J = 8.0, 6.4 Hz, 2H), 7.25 – 7.16 (m, 3H), 6.97 (d, J = 8.9 Hz, 1H), 6.77 (d, J = 8.6 Hz, 1H), 6.65 (d, J = 9.4 Hz, 1H), 6.61 (d, J = 9.3 Hz, 1H), 4.45 – 4.36 (m, 1H), 4.30 (d, J = 7.9 Hz, 1H), 4.22 – 4.14 (m, 2H), 4.05 – 4.00 (m, 2H), 3.98 – 3.87 (m, 6H), 3.59 (ddd, J = 13.5, 8.7, 3.3 Hz, 4H), 3.47 (ddd, J = 24.9, 9.8, 4.9 Hz, 4H), 2.92 (d, J = 7.6

Hz, 2H), 1.87 (q, $J = 6.9$ Hz, 1H), 1.61 (m, 2H), 1.45 (dd, $J = 8.9, 5.6$ Hz, 1H), 0.98 – 0.91 (m, 12H), 0.88 (d, $J = 6.2$ Hz, 3H).

2.7.4. Crystal Structure Information:

(a) Crystallographic Information of Peptide C3:

Crystals of peptide **C3** were grown by slow evaporation of methanol/ water solution. A single crystal ($1 \times 0.74 \times 0.082$ mm) was mounted on loop with a small amount of the paraffin oil. The X-ray data were collected at 150 K temperature on a APEX(II) DUO CCD diffractometer using Mo $K\alpha$ radiation ($\lambda = 0.71073$ Å), ω -scans ($2\theta = 50.124$), for a total of 16990 independent reflections. Space group P 21, $a = 6.7691$, $b = 14.044$, $c = 11.743$ Å; $\alpha = 90$, $\beta = 105.364$, $\gamma = 90$; $V = 1076.4$ Å³; Monoclinic, $Z = 2$ for chemical formula $C_{22}H_{28}N_2O_5$; $\rho_{\text{calcd}} = 1.235$ gcm⁻³, $\mu = 0.088$ mm⁻¹, $F(000) = 428$, The structure was obtained by direct methods using SHELXS-97.^[1] The final R value was 0.0394 ($wR_2 = 0.0890$) 3794 observed reflections ($F_0 \geq 4\sigma(|F_0|)$) and 267 variables, $S = 1.042$.

(b) Crystallographic Information of Peptide C4:

Crystals of peptide **C4** were grown by slow evaporation of methanol/ water solution. A single crystal ($0.2 \times 0.15 \times 0.1$ mm) was mounted on loop with a small amount of the paraffin oil. The X-ray data were collected at 150 K temperature on a APEX(II) DUO CCD diffractometer using Mo $K\alpha$ radiation ($\lambda = 0.71073$ Å), ω -scans ($2\theta = 50.050$), for a total of 24072 independent reflections. Space group P 21, $a = 11.806$, $b = 5.2174$, $c = 17.099$ Å; $\alpha = 90$, $\beta = 104.425$, $\gamma = 90$; $V = 1020$ Å³; Monoclinic, $Z = 2$ for chemical formula $C_{22}H_{21}F_5N_2O_4$; $\rho_{\text{calcd}} = 1.538$ gcm⁻³, $\mu = 0.136$ mm⁻¹, $F(000) = 488$, The structure was obtained by direct methods using SHELXS-97.^[1] The final R value was 0.0880 ($wR_2 = 0.2234$) 3590 observed reflections ($F_0 \geq 4\sigma(|F_0|)$) and 298 variables, $S = 1.031$.

(c) Crystallographic Information of Peptide C5:

Crystals of peptide **C5** were grown by slow evaporation of methanol/ water solution. A single crystal ($1.2 \times 0.83 \times 0.093$ mm) was mounted on loop with a small amount of the paraffin oil. The X-ray data were collected at 150 K temperature on a APEX(II) DUO CCD diffractometer using Mo $K\alpha$ radiation ($\lambda = 0.71073$ Å), ω -scans ($2\theta = 50.048$), for a total of 13514 independent reflections. Space group C 2, $a = 14.915$, $b = 5.1446$, $c = 14.460$ Å; $\alpha = 90$, $\beta = 91.735$, $\gamma = 90$; $V = 1109.1$ Å³;

Monoclinic, $Z = 2$ for chemical formula $C_{22} H_{16} F_{10} N_2 O_4$; $\rho_{\text{calcd}} = 1.684 \text{ gcm}^{-3}$, $\mu = 0.171 \text{ mm}^{-1}$, $F(000) = 568$, The structure was obtained by direct methods using SHELXS-97.^[1] The final R value was 0.0674 ($wR2 = 0.1171$) 1941 observed reflections ($F0 \geq 4\sigma (|F0|)$) and 172 variables, $S = 1.011$.

(d) Crystallographic Information of Monomer (N-Boc- β (O)- δ^5 -PheF5-O^tBu):

Crystals of monomer **N-Boc- β (O)- δ^5 -PheF5-O^tBu** were grown by slow evaporation of methanol/ water solution. A single crystal ($0.21 \times 0.14 \times 0.11 \text{ mm}$) was mounted on loop with a small amount of the paraffin oil. The X-ray data were collected at 150 K temperature on a APEX(II) DUO CCD diffractometer using Mo $K\alpha$ radiation ($\lambda = 1.54178 \text{ \AA}$), ω -scans ($2\theta = 133.754$), for a total of 16184 independent reflections. Space group C 2, $a=39.919$, $b=5.3792$, $c=10.430 \text{ \AA}$; $\alpha = 90$, $\beta = 93.848$, $\gamma = 90$; $V = 2234.6 \text{ \AA}^3$; Monoclinic, $Z = 4$ for chemical formula $C_{20} H_{26} F_5 N O_5$; $\rho_{\text{calcd}} = 1.354 \text{ gcm}^{-3}$, $\mu = 1.074 \text{ mm}^{-1}$, $F(000) = 952$, The structure was obtained by direct methods using SHELXS-97.^[1] The final R value was 0.0316 ($wR2 = 0.0955$) 3890 observed reflections ($F0 \geq 4\sigma (|F0|)$) and 286 variables, $S = 0.810$.

(e) Crystallographic Information of Monomer (N-Boc- β (O)- δ^5 -Nap-O^tBu):

Crystals of **N-Boc- β (O)- δ^5 -PheF5-O^tBu** were grown by slow evaporation of methanol/ water solution. A single crystal ($0.15 \times 0.1 \times 0.09 \text{ mm}$) was mounted on loop with a small amount of the paraffin oil. The Xray data were collected at 150 K temperature on a APEX(II) DUO CCD diffractometer using Mo $K\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$), ω -scans ($2\theta = 56.768$), for a total of 31323 independent reflections. Space group C 2, $a= 31.384$, $b= 5.3071$, $c = 13.710 \text{ \AA}$; $\alpha = 90$, $\beta = 91.574$, $\gamma = 90$; $V = 2282.7 \text{ \AA}^3$; Monoclinic, $Z = 4$ for chemical formula $C_{24} H_{33} N O_5$; $\rho_{\text{calcd}} = 1.209 \text{ gcm}^{-3}$, $\mu = 0.084 \text{ mm}^{-1}$, $F(000) = 896$, $R_{\text{int}} = 0.0771$. . The structure was obtained by direct methods using SHELXS-97. The final R value was 0.0383 ($wR2 = 0.0935$) 5685 observed reflections ($F0 \geq 4\sigma (|F0|)$) and 277 variables, $S = 1.022$

(f) Crystallographic Information of Peptide C8:

Crystals of **C8** were grown by slow evaporation of chloroform/toluene solution. A single crystal ($0.15 \times 0.1 \times 0.09 \text{ mm}$) was mounted on loop with a small amount of the paraffin oil. The Xray data were collected at 150 K temperature on a APEX(II) DUO CCD diffractometer using Mo $K\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$), ω -scans ($2\theta = 50.1$), for a total of 95619 independent reflections. Space

group $P2_12_12_1$, $a = 5.159$, $b = 17.291$, $c = 33.33$ Å; $\alpha = 90$, $\beta = 90$, $\gamma = 90$; $V = 2973$ Å³; Monoclinic, $Z = 4$ for chemical formula $C_{33}H_{39}N_3O_6$; $\rho_{\text{calcd}} = 1.282$ gcm⁻³, $\mu = 0.088$ mm⁻¹, $F(000) = 1224$, The structure was obtained by direct methods using SHELXS-97. The final R value was 0.1024 ($wR_2 = 0.1927$) 5254 observed reflections ($F_0 \geq 4\sigma(|F_0|)$) and 381 variables, $S = 0.991$.

2.7.5. ORTEP Diagrams:

a) ORTEP Diagram of C3:

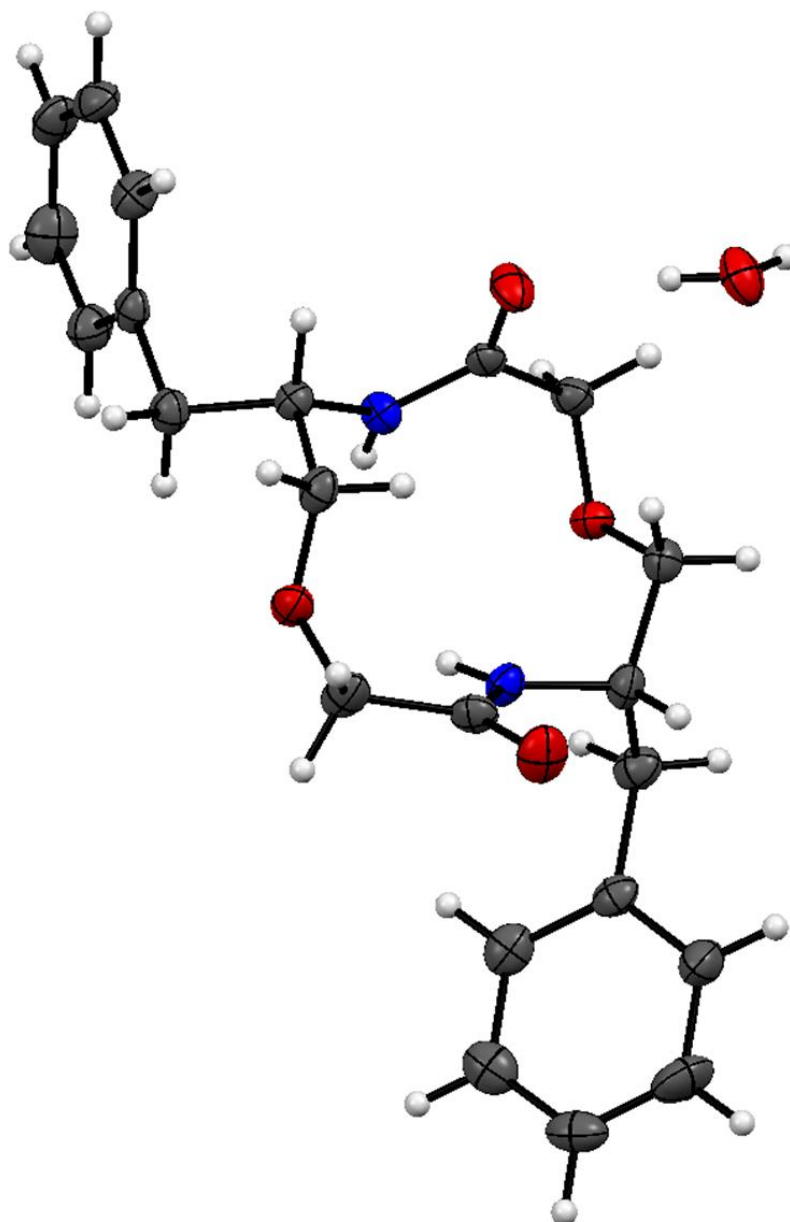


Figure 12: ORTEP diagram of C3 (CCDC no. 2337014). Ellipsoids are drawn at 50% probability.

b) ORTEP Diagram of C4:

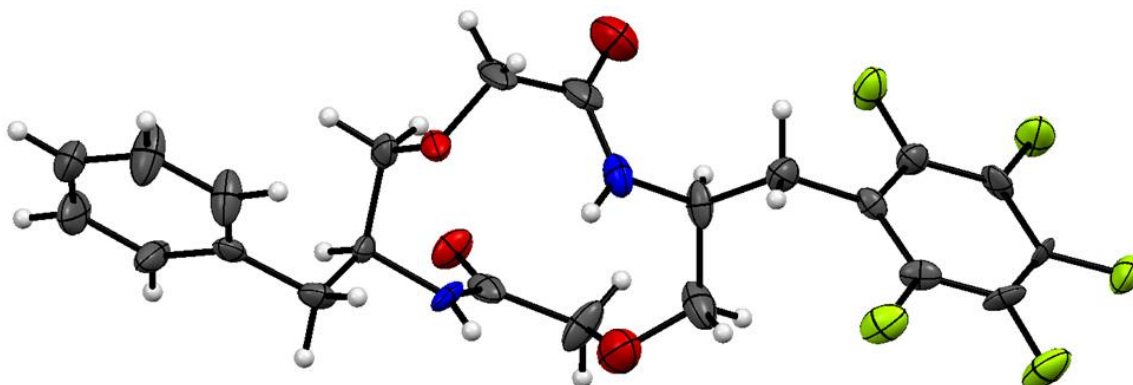


Figure 13: ORTEP diagram of C4 (CCDC no. 2337018). Ellipsoids are drawn at 50% probability.

c) ORTEP Diagram of C5:

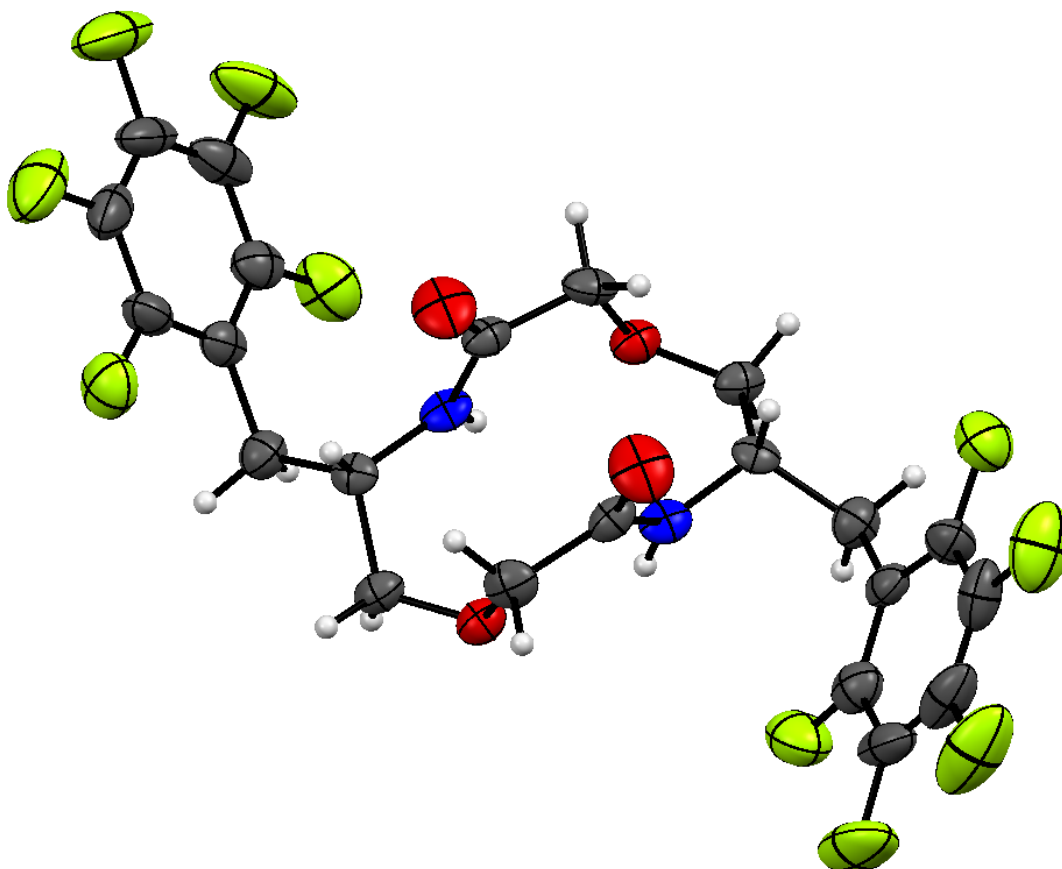


Figure 14: ORTEP diagram of C5 (CCDC no. 2337020). Ellipsoids are drawn at 50% probability.

d) ORTEP Diagram of 2b: (N-Boc- β (O)- δ^5 -PheF5-OtBu)

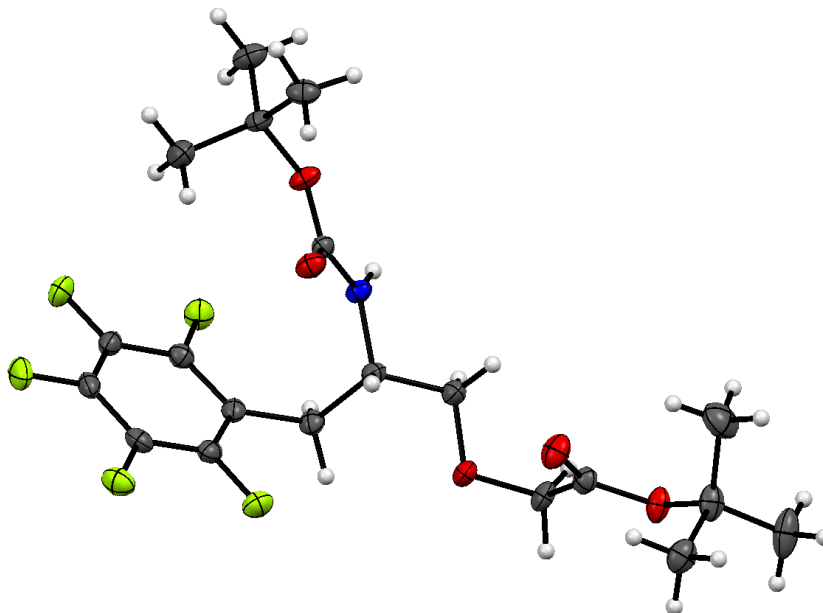


Figure 15: ORTEP diagram of N-Boc- β (O)- δ^5 -PheF5-OtBu (CCDC no. 2337022). Ellipsoids are drawn at 50% probability.

(e) ORTEP Diagram of 2b: (N-Boc- β (O)- δ^5 -Nap-OtBu)

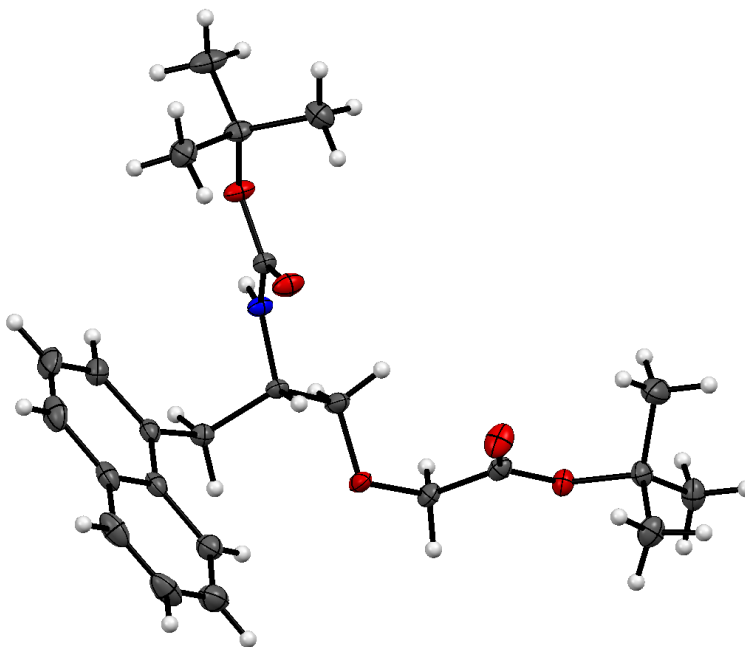


Figure 16: ORTEP diagram of N-Boc- β (O)- δ^5 -Nap-OtBu Ellipsoids are drawn at 50% probability.

(e) ORTEP Diagram of C8:

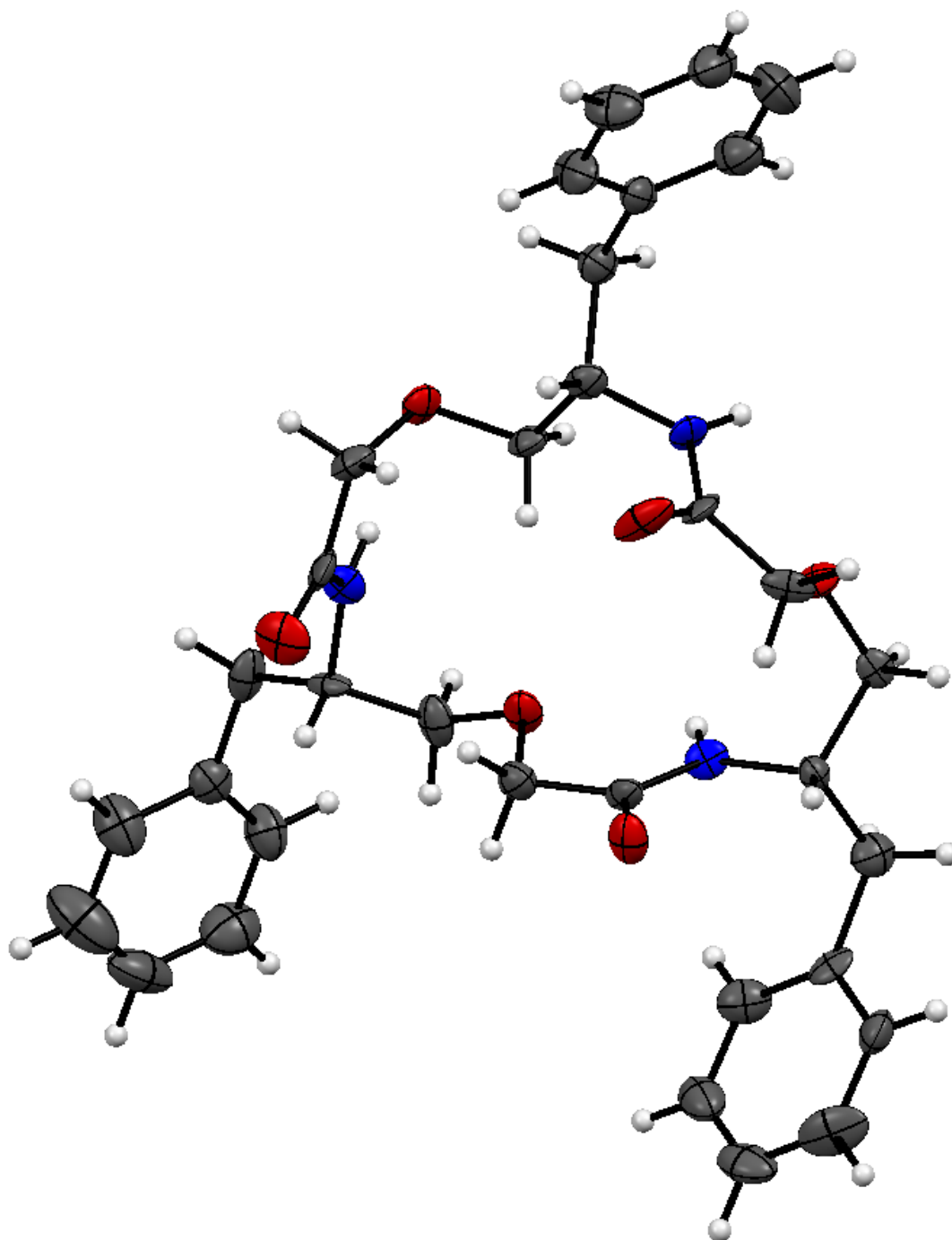


Figure 17: ORTEP diagram of **C8**. Ellipsoids are drawn at 50% probability.

2.8. References:

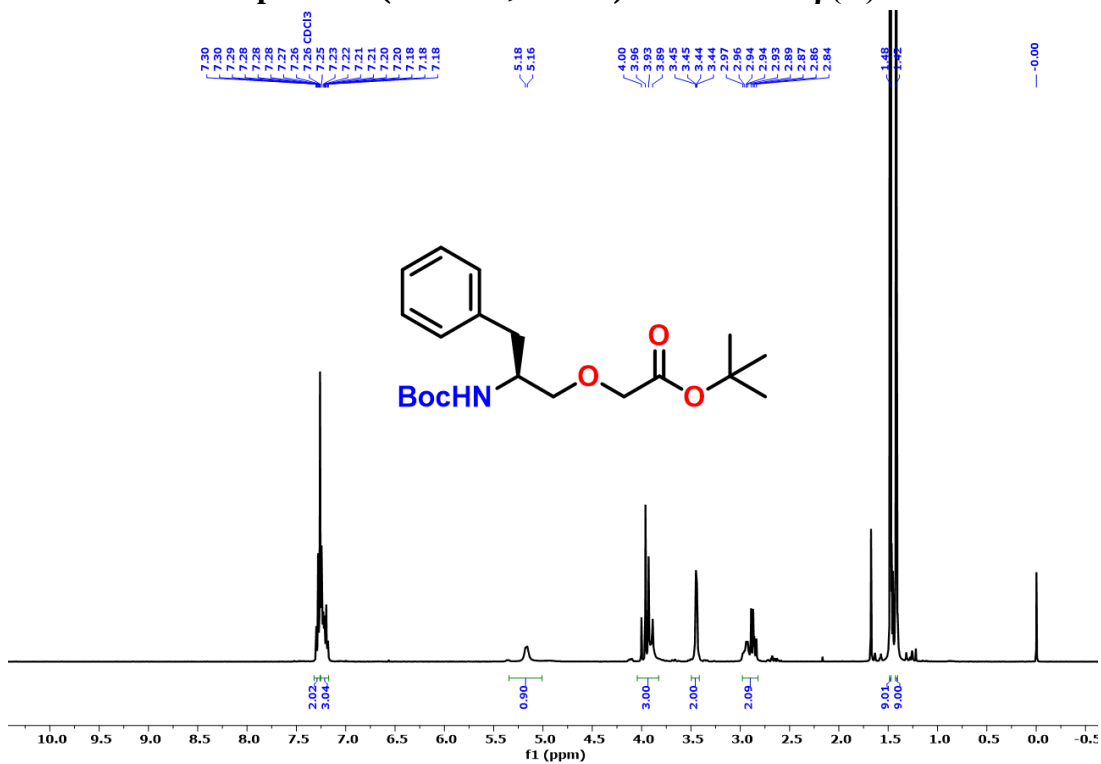
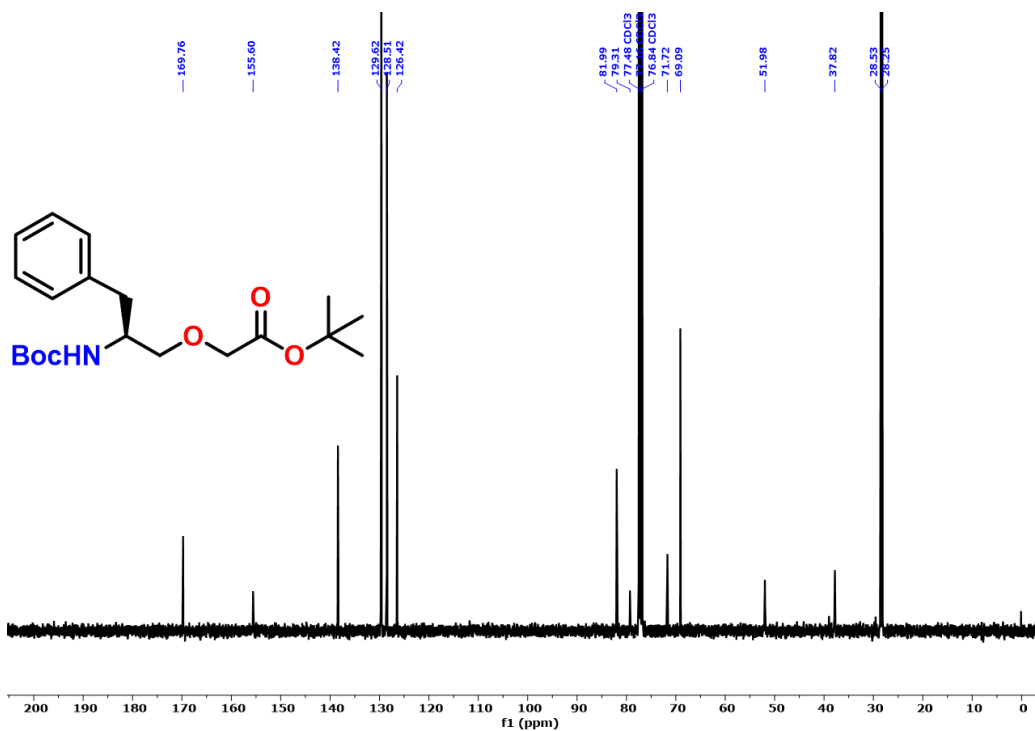
1. V. D'Aloisio, P. Dognini, G. A. Hutcheon, C. R. Coxon, *Drug Discov. Today* **2021**, 26, 1409–1419.
2. M. Akbarian, A. Khani, S. Eghbaltpour, V. N. Uversky, *Int. J. Mol. Sci.* **2022**, 23, 1445.
3. H. Zhang, S. Chen, *RSC Chem. Biol.* **2022**, 3, 18–31.
4. J. S. Choi, S. H. Joo, *Biomol. Ther.* **2020**, 28, 18–24.
5. Q. Song, Z. Cheng, M. Kariuki, S. C. L. Hall, S. K. Hill, J. Y. Rho, S. Perrier, *Chem. Rev.* **2021**, 121, 13936–13995.
6. a) G. M. Whitesides, J. Mathias, C. Seto, *Science* **1991**, 254, 1312; b) C. A. Mirkin, *Small* **2005**, 1, 14.
7. a) C. R. Martin, P. Kohli, *Nat. Rev. Drug Discovery*. **2003**, 2, 29; b) E. Gazit, *Chem. Soc. Rev.* **2007**, 36, 1263.
8. a) A. Casini, B. Woods, M. Wenzel, *Inorg. Chem.* **2017**, 56, 14715; b) D. Bong, T. D. Clark, J. R. Granja, M. R. Ghadiri, *Angew. Chem. Int. Ed.* **2001**, 40, 988; c) R. Rui Qiao, P. C. Ke, *J. Am. Chem. Soc.* **2006**, 128, 13656.
9. Special issue on supramolecular chemistry and self-assembly, *Science* **2002**, 295, 2395.
10. a) R. J. Brea, J. R. Granja, Dekker Encyclopedia of Nanoscience and Nanotechnology, Marcel Dekker Inc. New York (USA), **2004**, 3439. b) R. J. Brea, C. Reiriz, J. R. Granja, *Chem. Soc. Rev.* **2010**, 39, 1448.
11. R. Chapman, M. Danial, M. L. Koh, K. A. Jolliffe, S. Perrier, *Chem. Soc. Rev.* **2012**, 41, 6023.
12. C. Baldauf, R. Gunther, H.-J. Hofmann, *J. Org. Chem.* **2004**, 69, 6214.
13. a) T. K. Chakraborty, S. Roy, S. K. Kumar, A. C. Kunwar, *Tetrahedron Lett.* **2005**, 46, 3065; b) T. K. Chakraborty, S. Roy, D. Koley, S. K. Dutta, A. C. Kunwar, *J. Org. Chem.* **2006**, 71, 6240; c) T. K. Chakraborty, A. Arora, S. Roy, N. Kumar, S. Maiti, *J. Med. Chem.* **2007**, 50, 5539; d) A. Siriwardena, K. K. Pulukuri, P. S. Kandiyal, S. Roy, O. Bande, S. Ghosh, J. M. G. Fernandez, F. A. Martin, J. M. Ghigo, C. Beloin, K. Ito, J. R. Woods, R. S. Ampapathi, T. K. Chakraborty, *Angew. Chem. Int. Ed.* **2013**, 52, 10221.

Chapter 2

14. G. V. M. Sharma, B. S. Babu, K. V. S. Ramakrishna, P. Nagendar, A. C. Kunwar, P. Schramm, C. Baldauf, H. J. Hofmann, *Chem. Eur. J.* **2009**, *15*, 5552.
15. H. Jiang, J. M. Léger, I. Huc, *J. Am. Chem. Soc.* **2003**, *125*, 3448.
16. D. Gauthier, P. Baillargeon, M. Drouin, Y. L. Dory, *Angew. Chem. Int. Ed.* **2001**, *40*, 4635.
17. A. Banerjee, A. Pramanik, S. Bhattacharjya, P. Balaram, *Biopolymers* **1996**, *39*, 769.
18. Q. Lin, H. Lan, C. Ma, R. T. Stendall, K. Shankland, R. A. Musgrave, P. N. Horton, C. Baldauf, H. J. Hofmann, C. P. Butts, M. M. Müller, A. J. A. Cobb, *Angew. Chem. Int. Ed.* **2023**, *62*, e202305326.
19. D. Bindl, P. K Mandal, I. Huc, *Chem. Eur. J.* **2022**, *28*, e202200538.
20. R. M. Reja, R. Patel, V. Kumar, A. Jha, H. N. Gopi, *Biomacromolecules* **2019**, *20*, 1254–1262.
21. R. M. Reja, V. Kumar, G. George, R. Patel, D. R. Puneeth Kumar, S. Raghothama, H. N. Gopi, *Chem. Eur. J.* **2020**, *26*, 4304-4309.
22. Z. Du, B. Fan, Q. Dai, L. Wang, J. Guo, Z. Ye, N. Cui, J. Chen, K. Tan, R. Li, *Giant* **2022**, *9*, 100082.
23. M. McIntosh, L. J. Cruz, M. W. Hunkapiller, W. R. Gray, B. M. Olivera, *Arch. Biochem. Biophys.* **1982**, *218*, 329–334.
24. S. Panda Mahapatra, S. Pahan, A. Chatterjee, S. Roy, D. R. Puneeth Kumar, H. N. Gopi, *Angew. Chem. Int. Ed.* **2024**, e202409969.

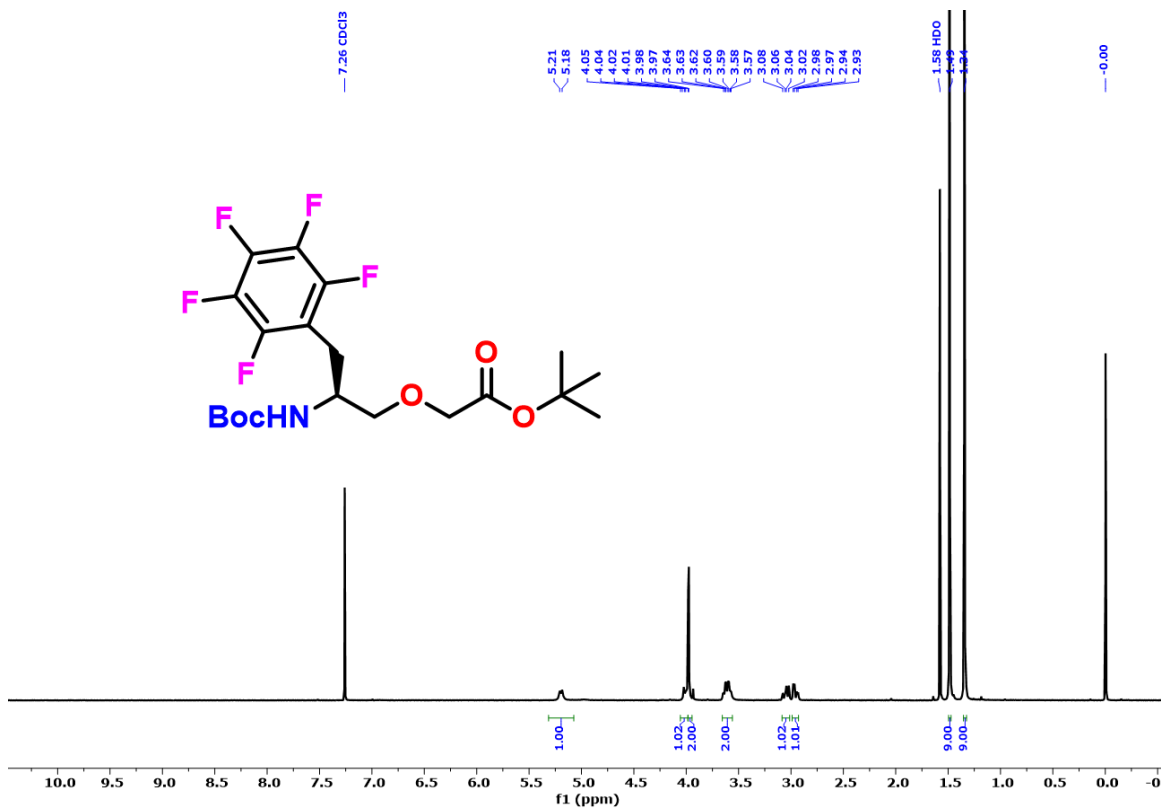
Note: Characterization data and full spectral charts for all compounds can also be found in the Supporting information of the following publication. S. Panda Mahapatra, S. Pahan, A. Chatterjee, S. Roy, D. R. Puneeth Kumar, H. N. Gopi, *Angew. Chem. Int. Ed.* **2024**, e202409969.

It can be downloaded from the link attached : [Exploring Macroscopic Dipoles of Designed Cyclic Peptide Ordered Assemblies to Harvest Piezoelectric Properties](#)
[Supplementary Information](#)

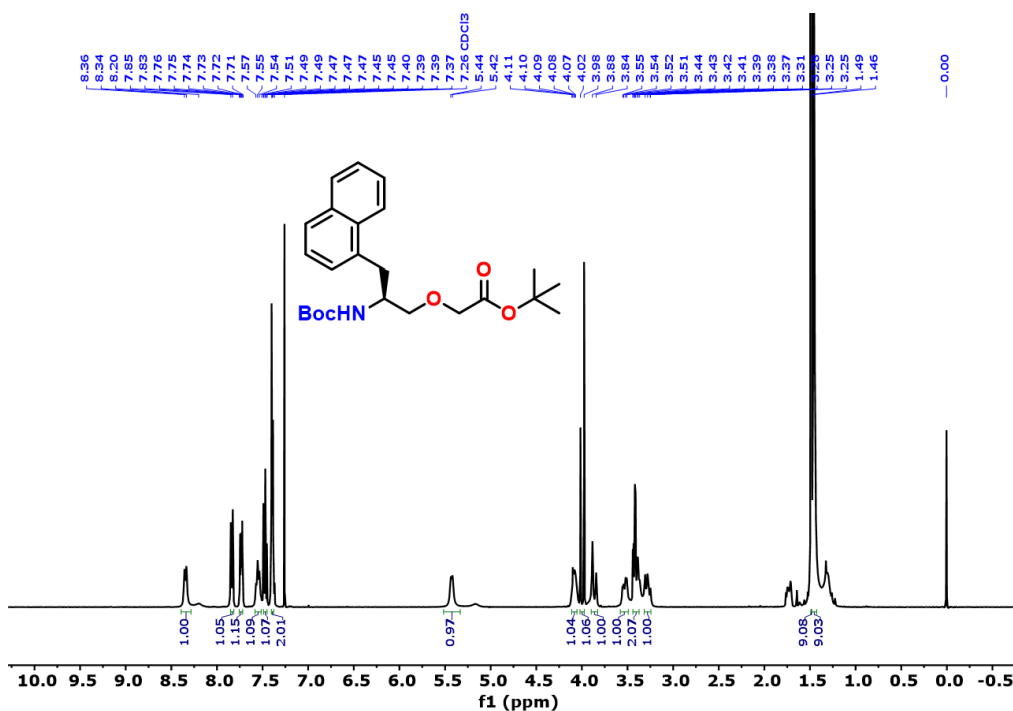
2.9. Appendix II: ^1H , ^{13}C , ^{19}F NMR and HRMS Spectrum: ^1H NMR Spectrum (400MHz, CDCl_3) of 2a: N-Boc- $\beta(\text{O})$ - δ^5 -Phe-O^tBu ^{13}C NMR Spectrum (101 MHz, CDCl_3) of 2a: N-Boc- $\beta(\text{O})$ - δ^5 -Phe-O^tBu

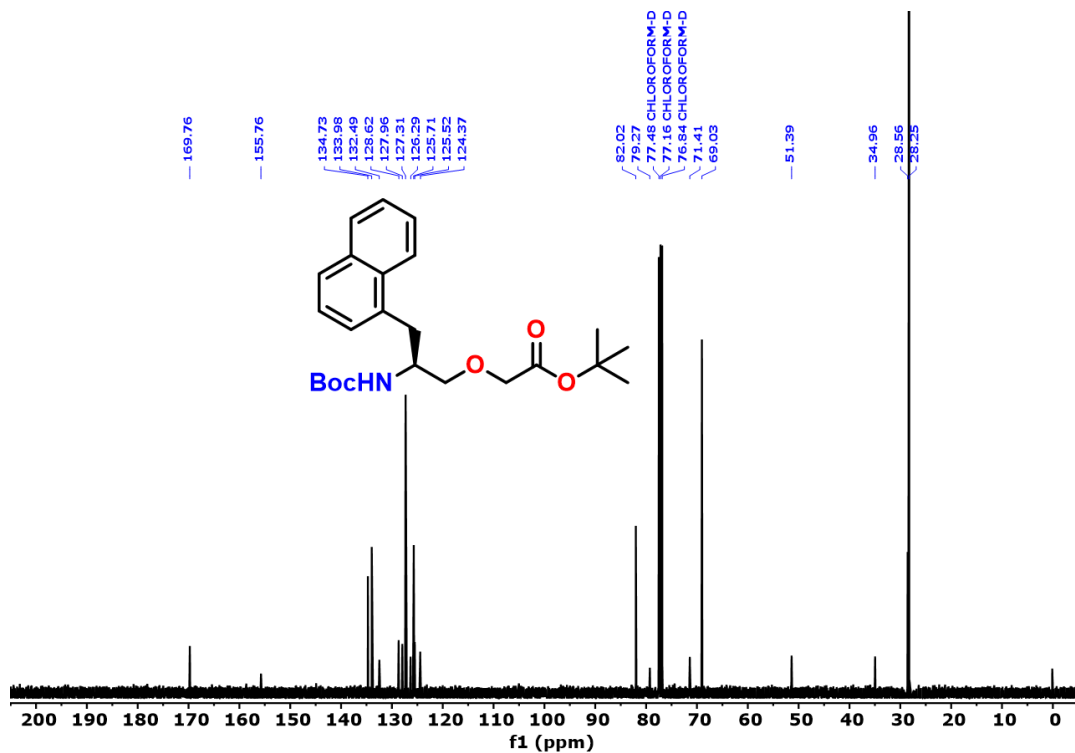
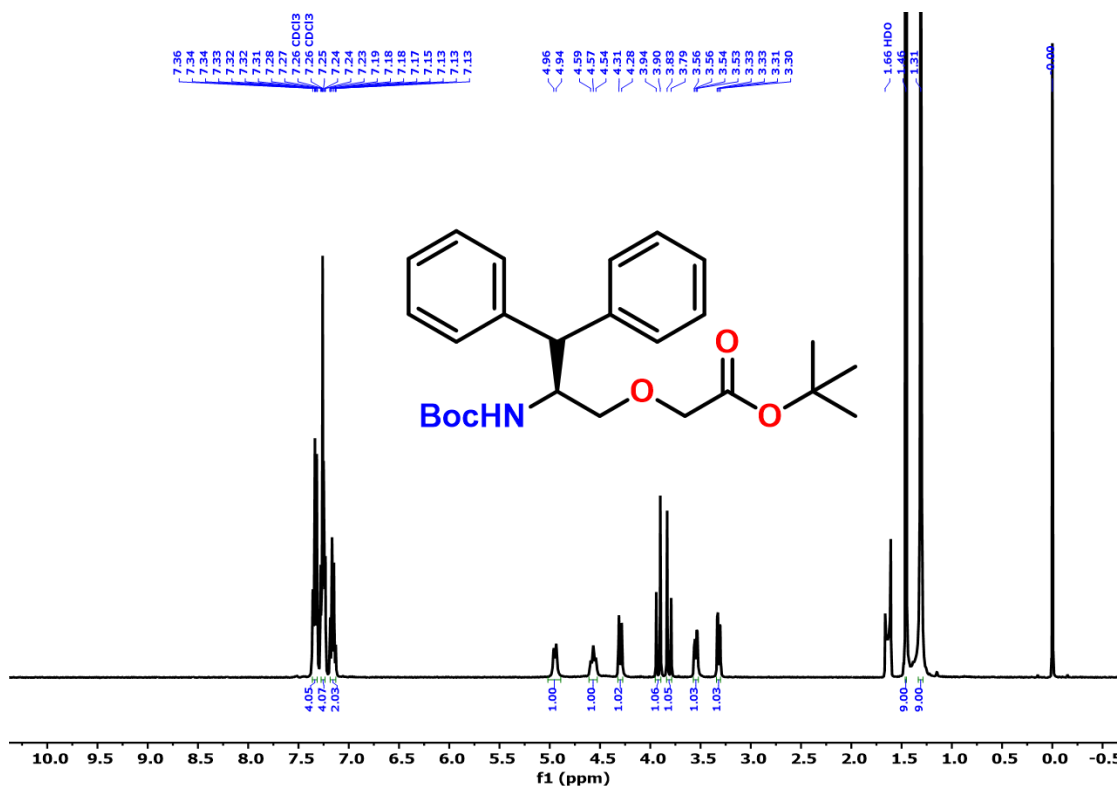
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^1H NMR Spectrum (400MHz, CDCl_3) of 2b: N-Boc- $\beta(\text{O})$ - δ^5 -PheF5-O'Bu



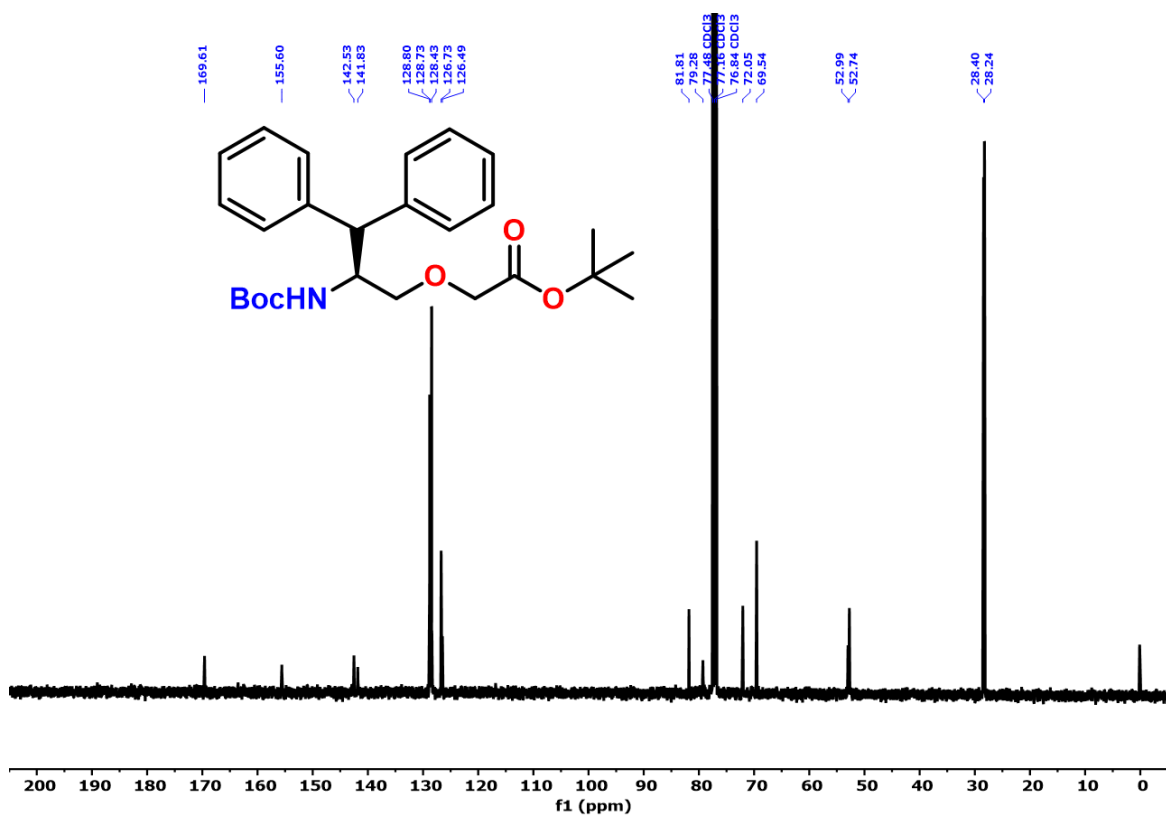
^1H NMR Spectrum (400MHz, CDCl_3) of 2c: N-Boc- $\beta(\text{O})$ - δ^5 -Nap-O'Bu



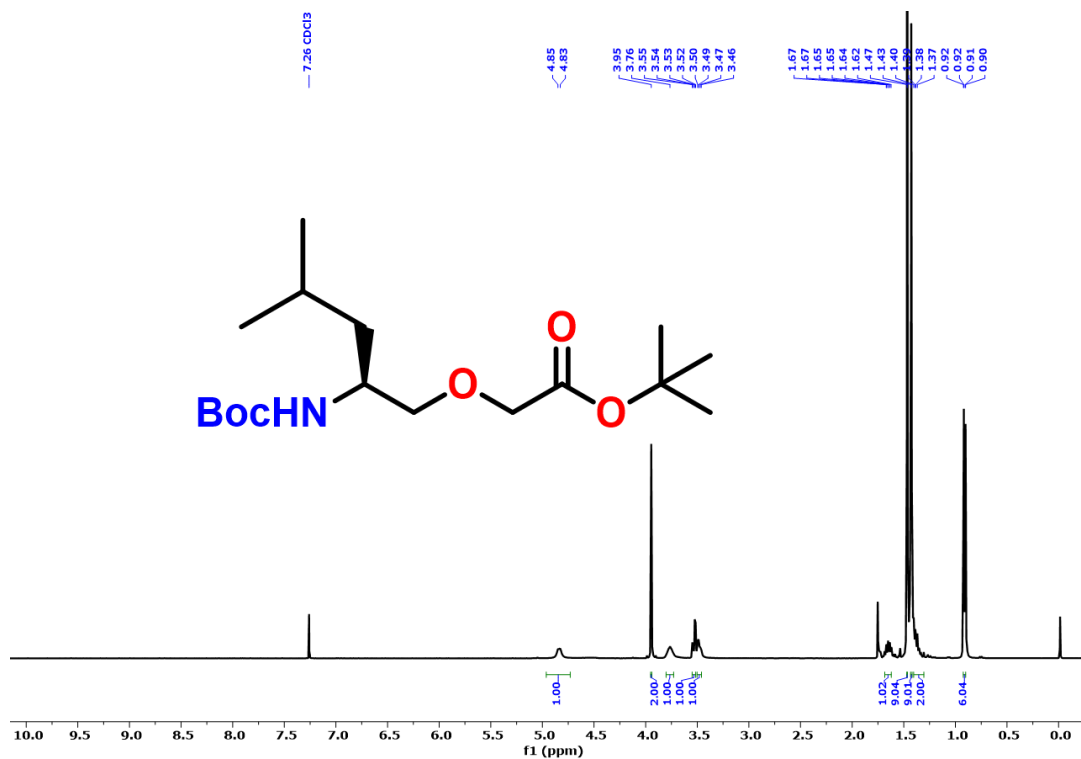
^{13}C NMR Spectrum (101 MHz, CDCl_3) of 2c: N-Boc- $\beta(\text{O})$ - δ^5 -Nap-O^tBu ^1H NMR Spectrum (400MHz, CDCl_3) of 2d: N-Boc- $\beta(\text{O})$ - δ^5 -Diphe-O^tBu

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^{13}C NMR Spectrum (101 MHz, CDCl_3) of 2d: N-Boc- $\beta(\text{O})$ - δ^5 -Diphe-O^tBu

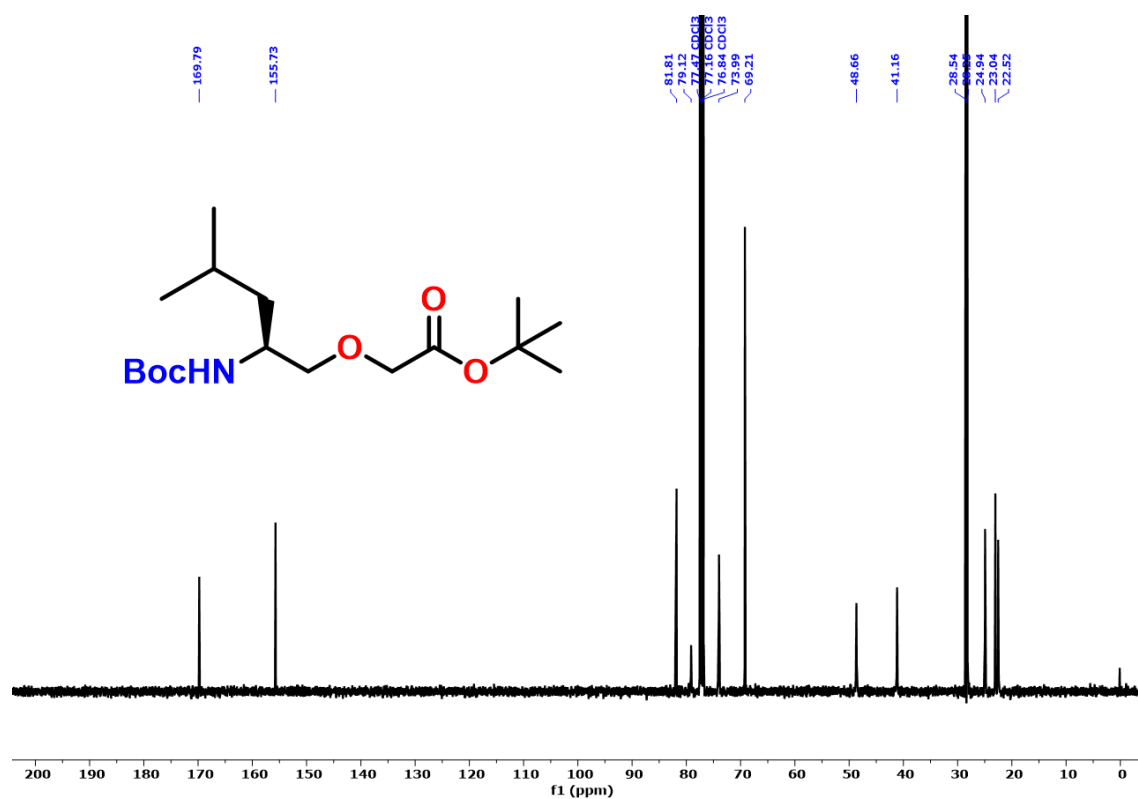


^1H NMR Spectrum (400MHz, CDCl_3) of 2e: N-Boc- $\beta(\text{O})$ - δ^5 -Leu-O^tBu

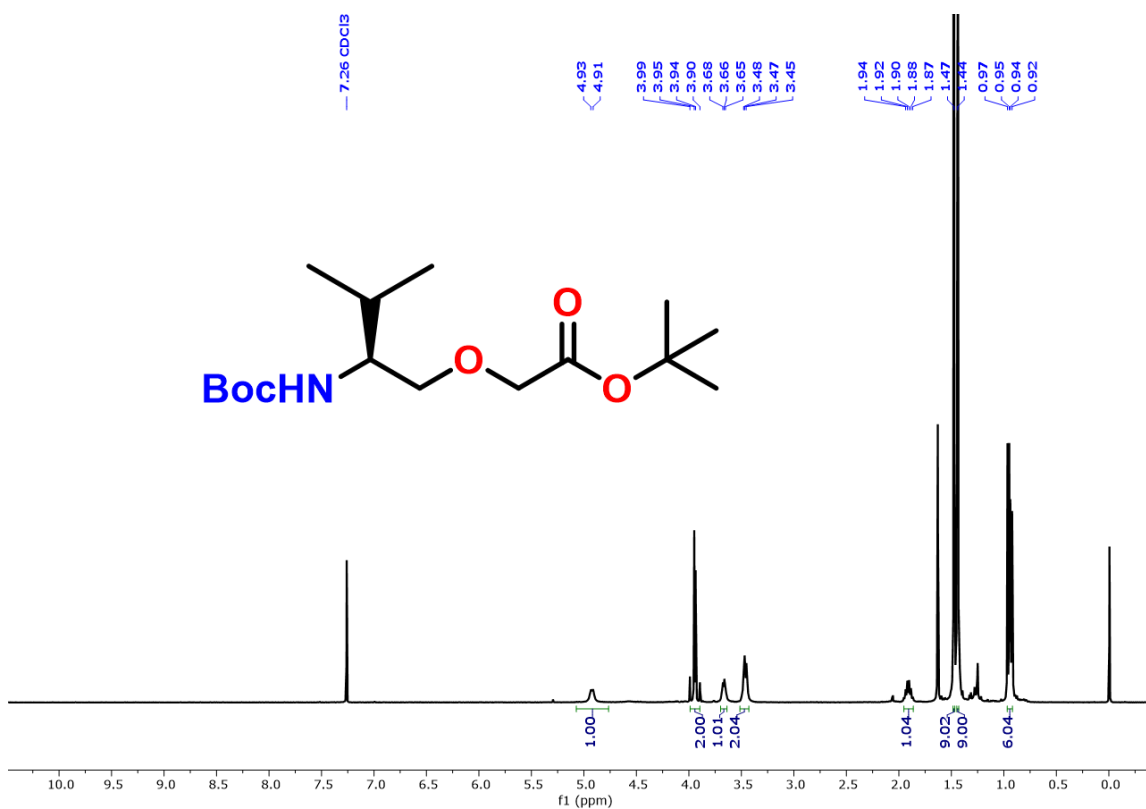


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^{13}C NMR Spectrum (101 MHz, CDCl_3) of 2e: N-Boc- $\beta(\text{O})$ - δ^5 -Leu-O^tBu

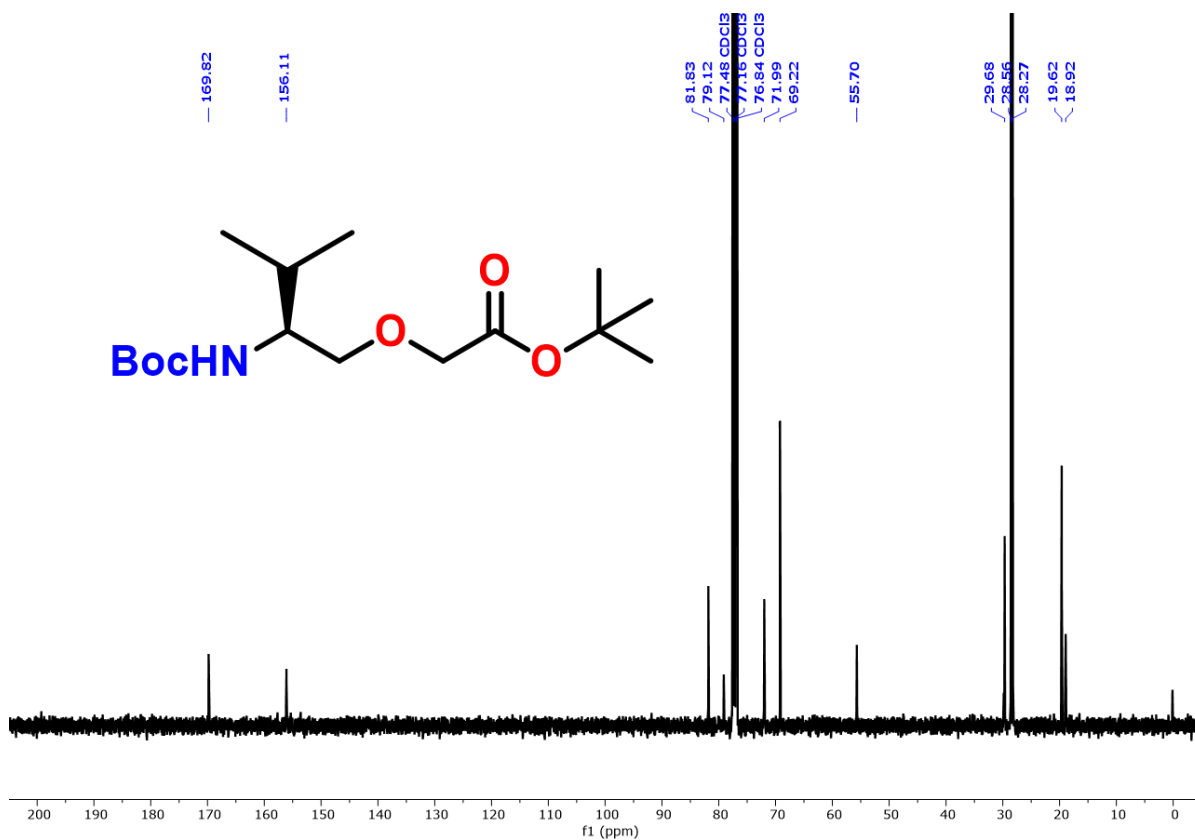


^1H NMR Spectrum (400MHz, CDCl_3) of 2f: N-Boc- $\beta(\text{O})$ - δ^5 -Val-O^tBu

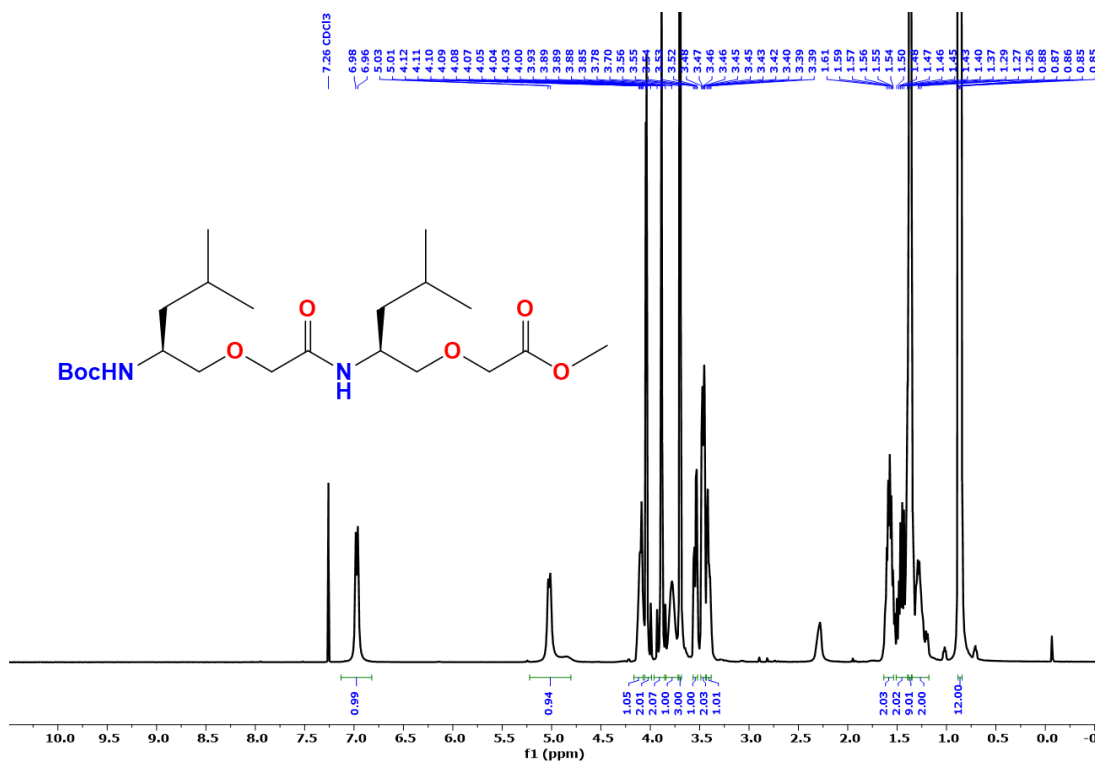


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^{13}C NMR Spectrum (101 MHz, CDCl_3) of 2f: N-Boc- $\beta(\text{O})$ - δ^5 -Val-O^tBu

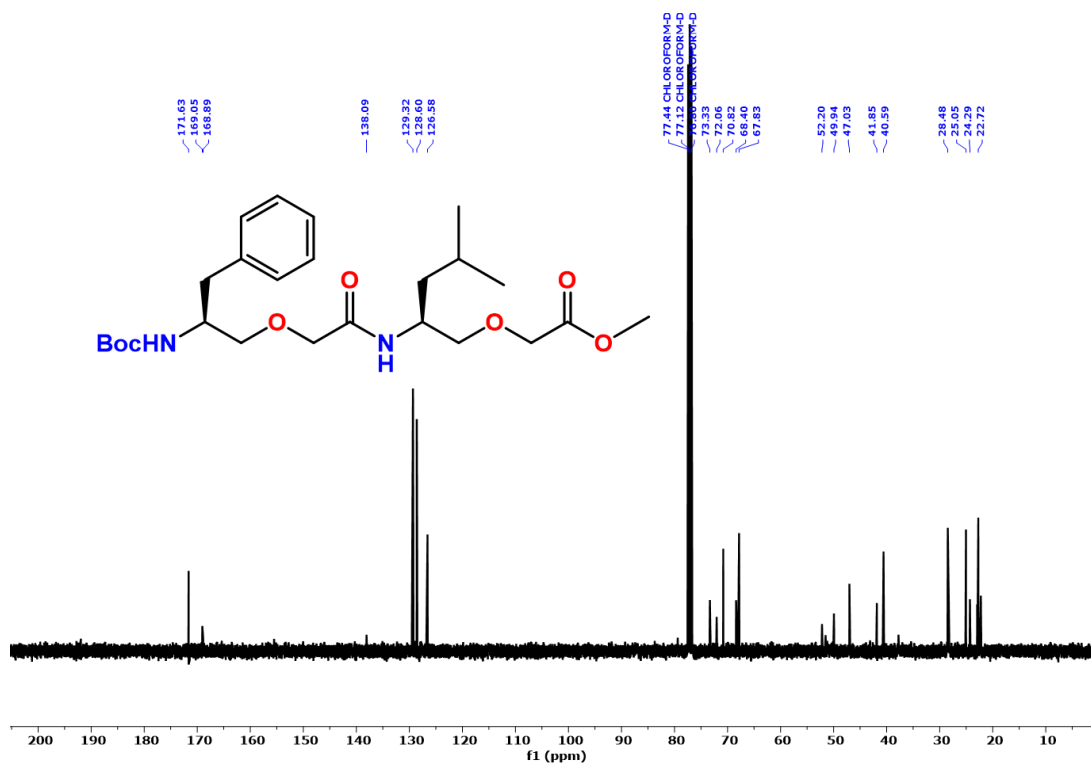


^1H NMR Spectrum (400MHz, CDCl_3) of L1: N-Boc- $\beta(\text{O})$ - δ^5 -Leu- $\beta(\text{O})$ - δ^5 -Leu-OMe

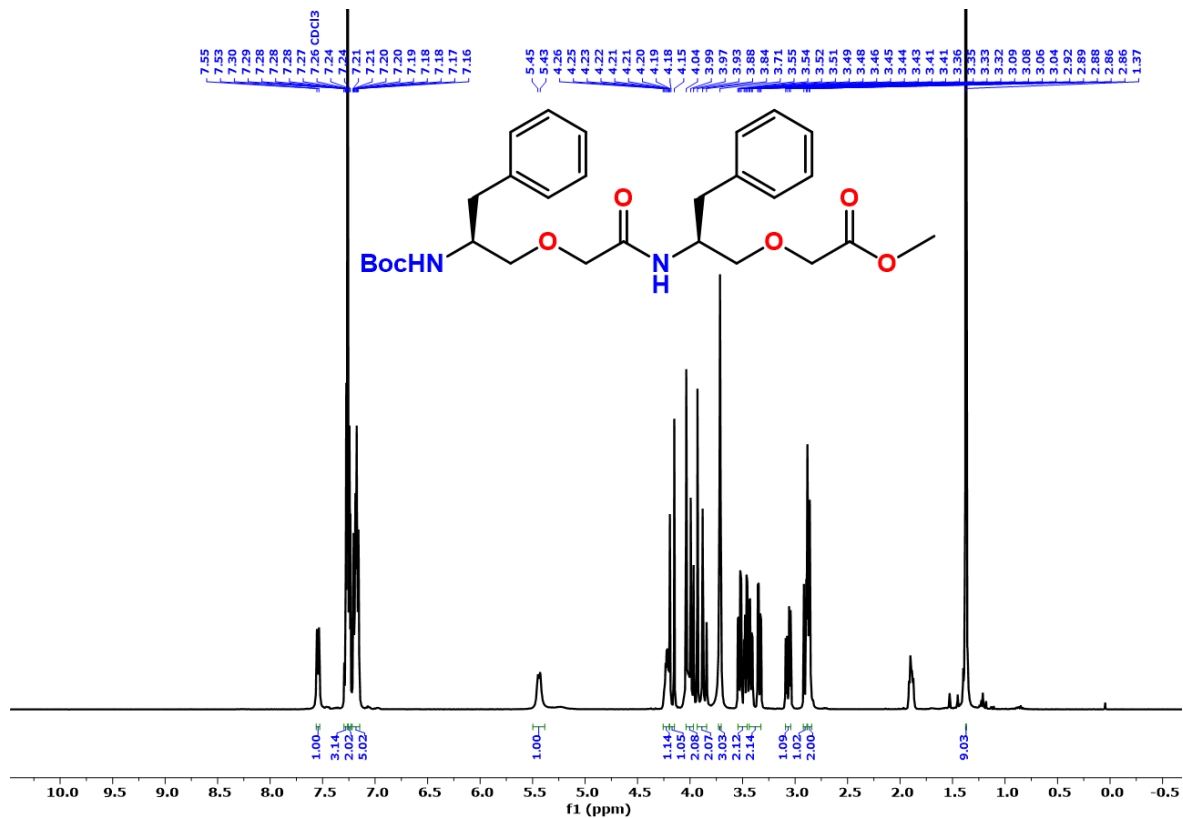


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^{13}C NMR Spectrum (101 MHz, CDCl_3) of L2: N-Boc- $\beta(\text{O})$ - δ^5 -Phe- $\beta(\text{O})$ - δ^5 -Leu-OMe

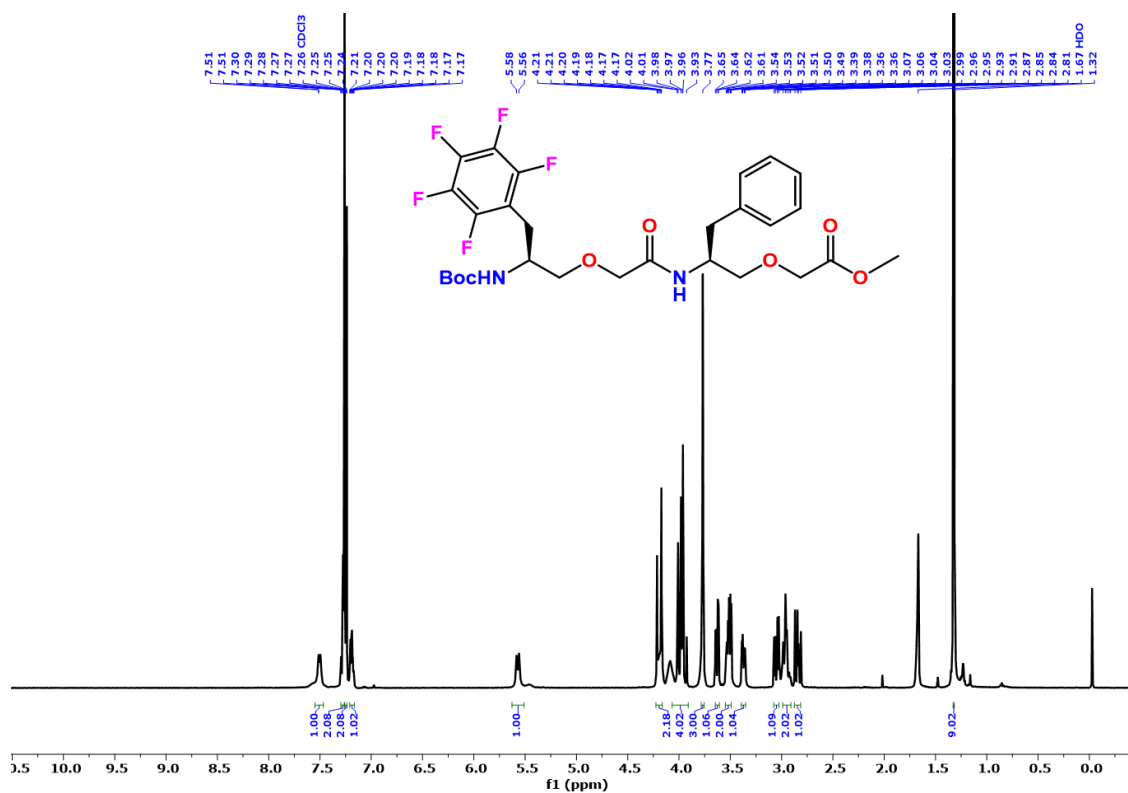


^1H NMR Spectrum (400 MHz, CDCl_3) of L3: N-Boc- $\beta(\text{O})$ - δ^5 -Phe- $\beta(\text{O})$ - δ^5 -Phe-OMe

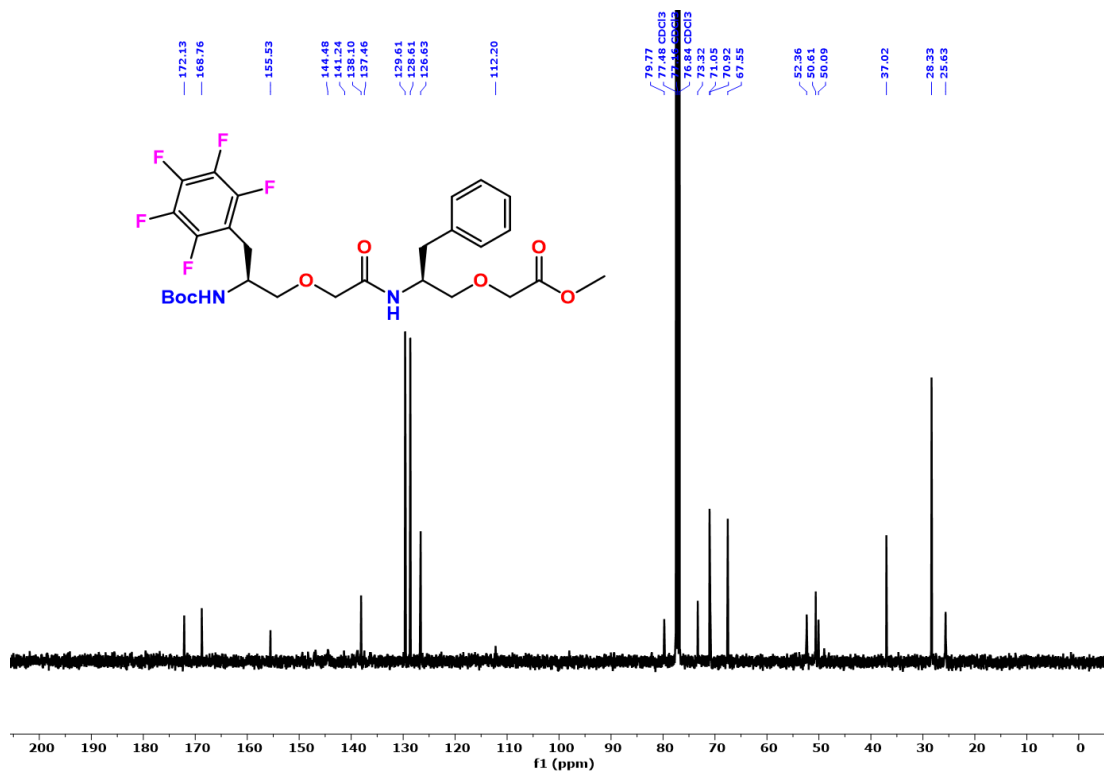


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^1H NMR Spectrum (400MHz, CDCl_3) of L4: N-Boc- $\beta(\text{O})$ - δ^5 -PheF5- $\beta(\text{O})$ - δ^5 -Phe-OMe

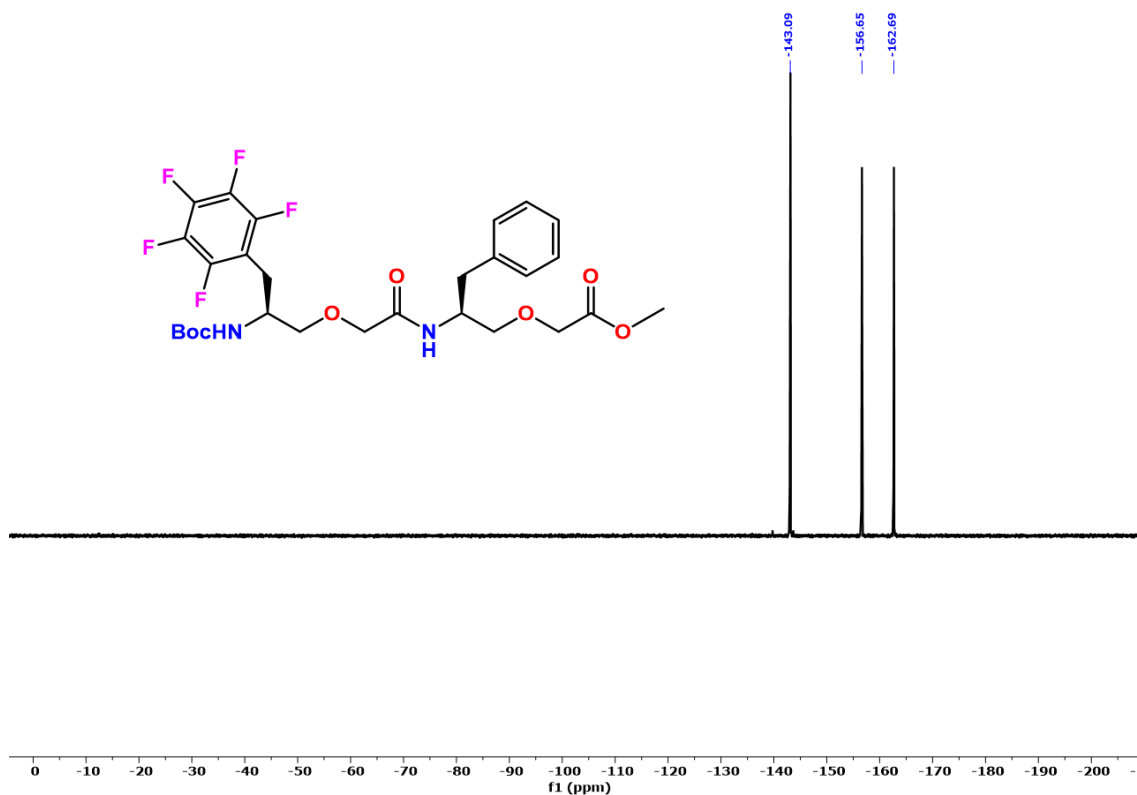


^{13}C NMR Spectrum (101 MHz, CDCl_3) of L4: N-Boc- $\beta(\text{O})$ - δ^5 -PheF5- $\beta(\text{O})$ - δ^5 -Phe-OMe

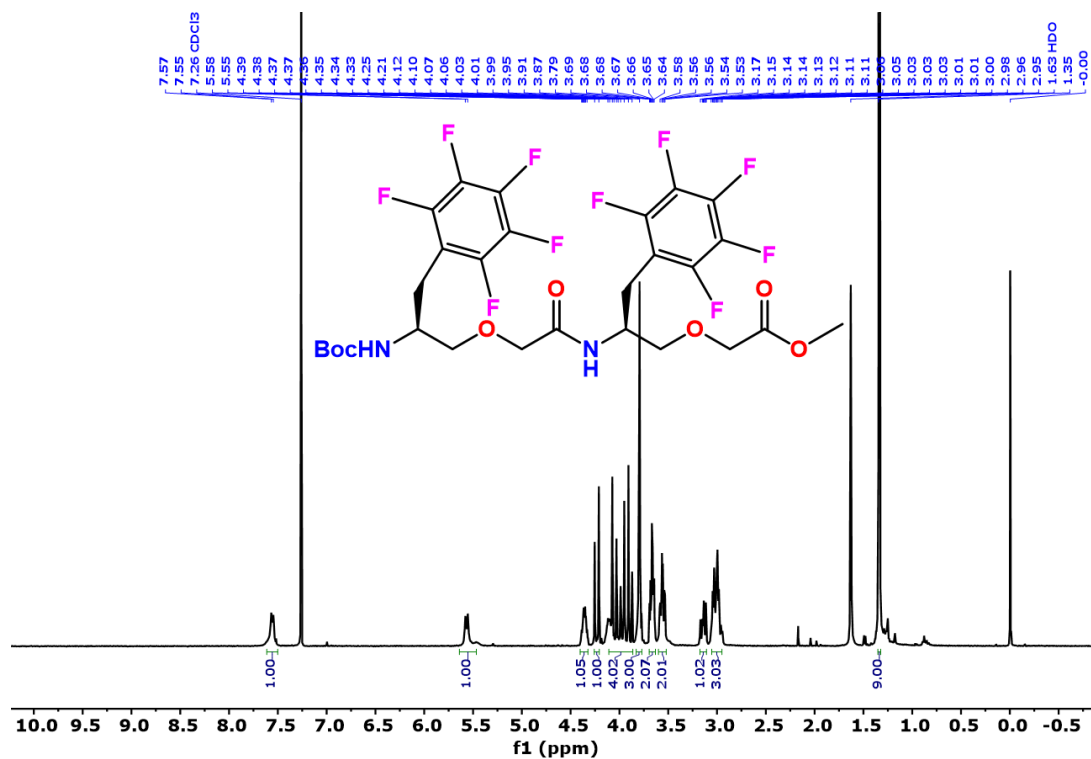


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^{19}F NMR Spectrum of L4: N-Boc- $\beta(\text{O})$ - δ^5 -PheF5- $\beta(\text{O})$ - δ^5 -Phe-OMe

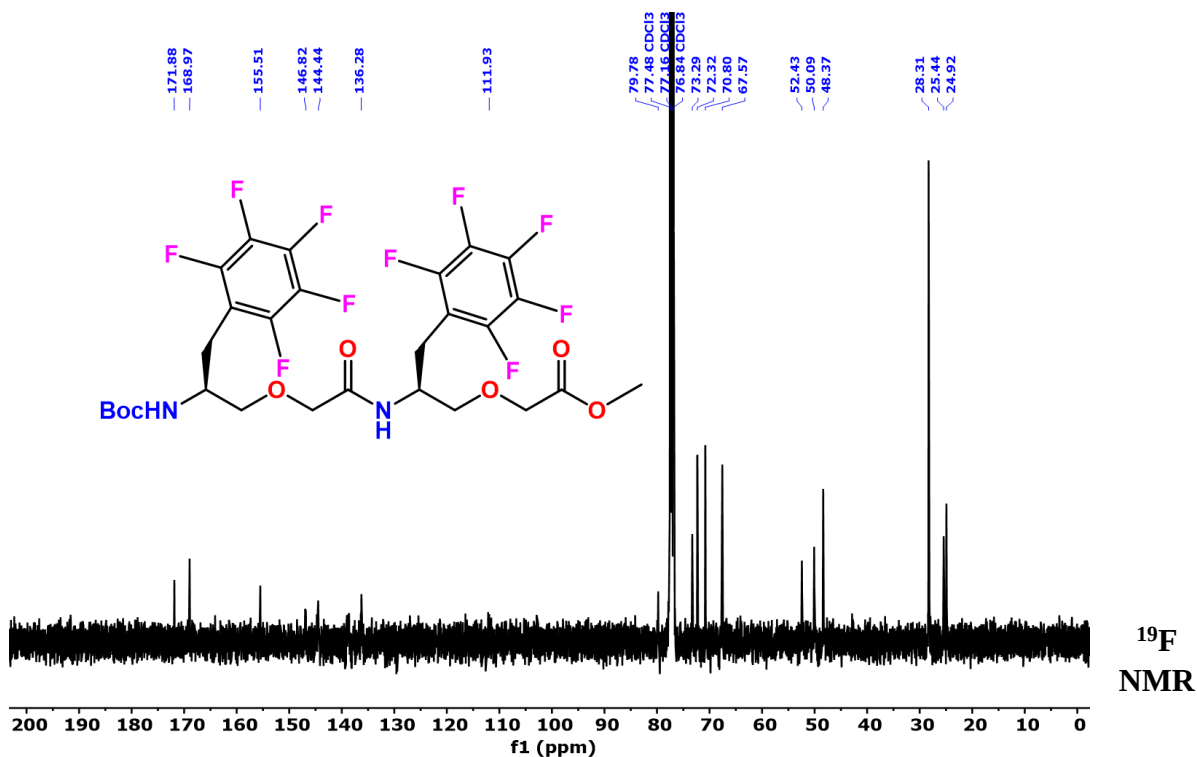


^1H NMR Spectrum (400MHz, CDCl_3) of L5: N-Boc- $\beta(\text{O})$ - δ^5 -PheF5- $\beta(\text{O})$ - δ^5 -PheF5-OMe



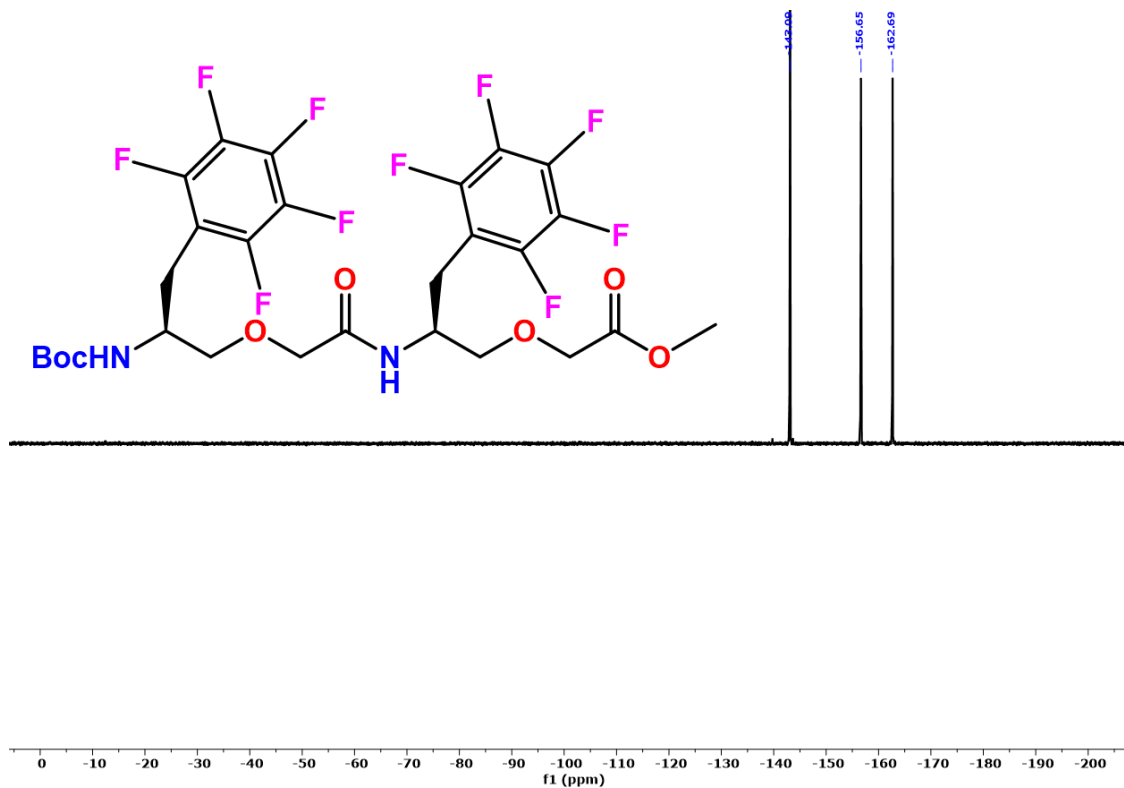
Chapter 2

^{13}C NMR Spectrum (101 MHz, CDCl_3) of L5: N-Boc- $\beta(\text{O})$ - δ^5 -PheF5- $\beta(\text{O})$ - δ^5 -PheF5-OMe



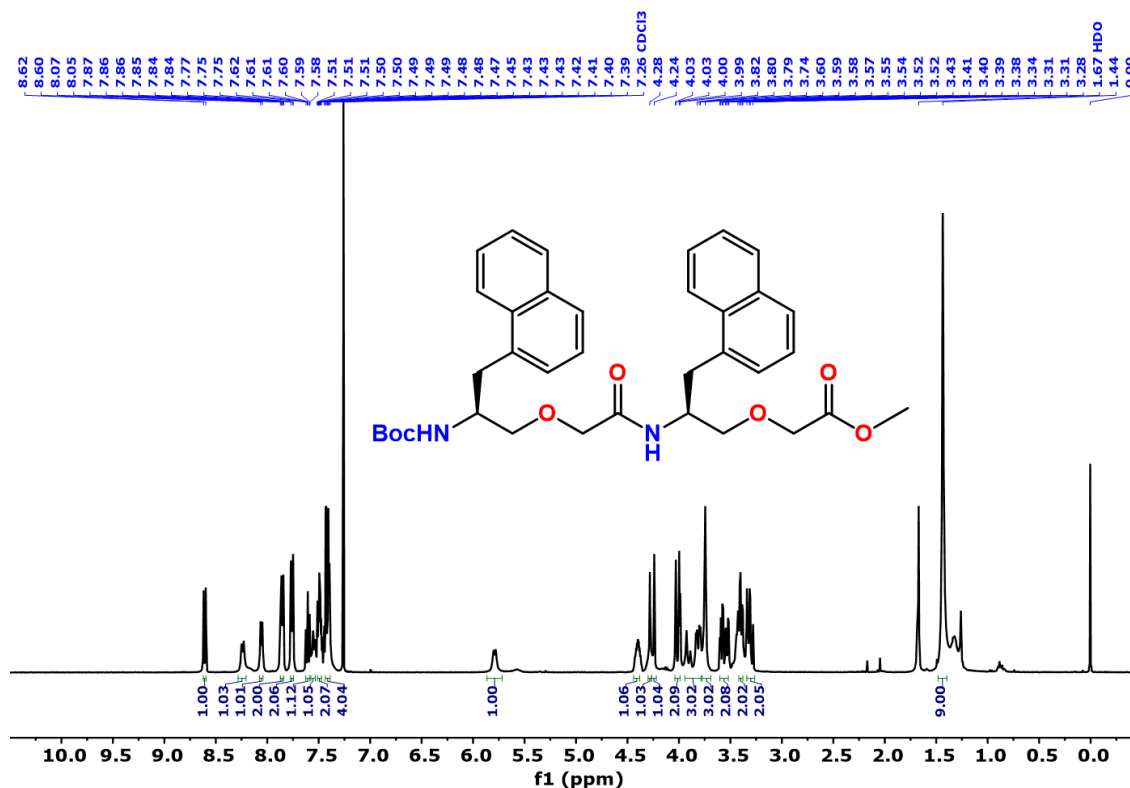
^{19}F
NMR

Spectrum of L5: N-Boc- $\beta(\text{O})$ - δ^5 -PheF5- $\beta(\text{O})$ - δ^5 -PheF5-OMe

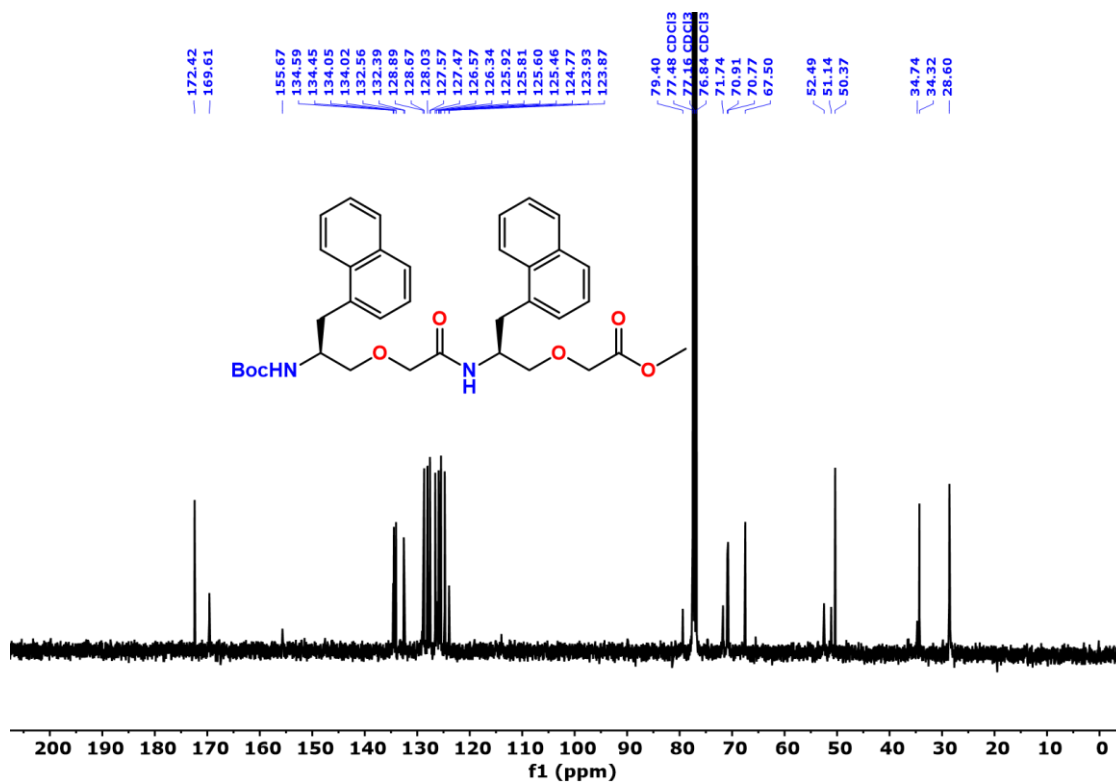


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^1H NMR Spectrum (400MHz, CDCl_3) of L6: N-Boc- $\beta(\text{O})$ - δ^5 -Nap- $\beta(\text{O})$ - δ^5 -Nap-OMe

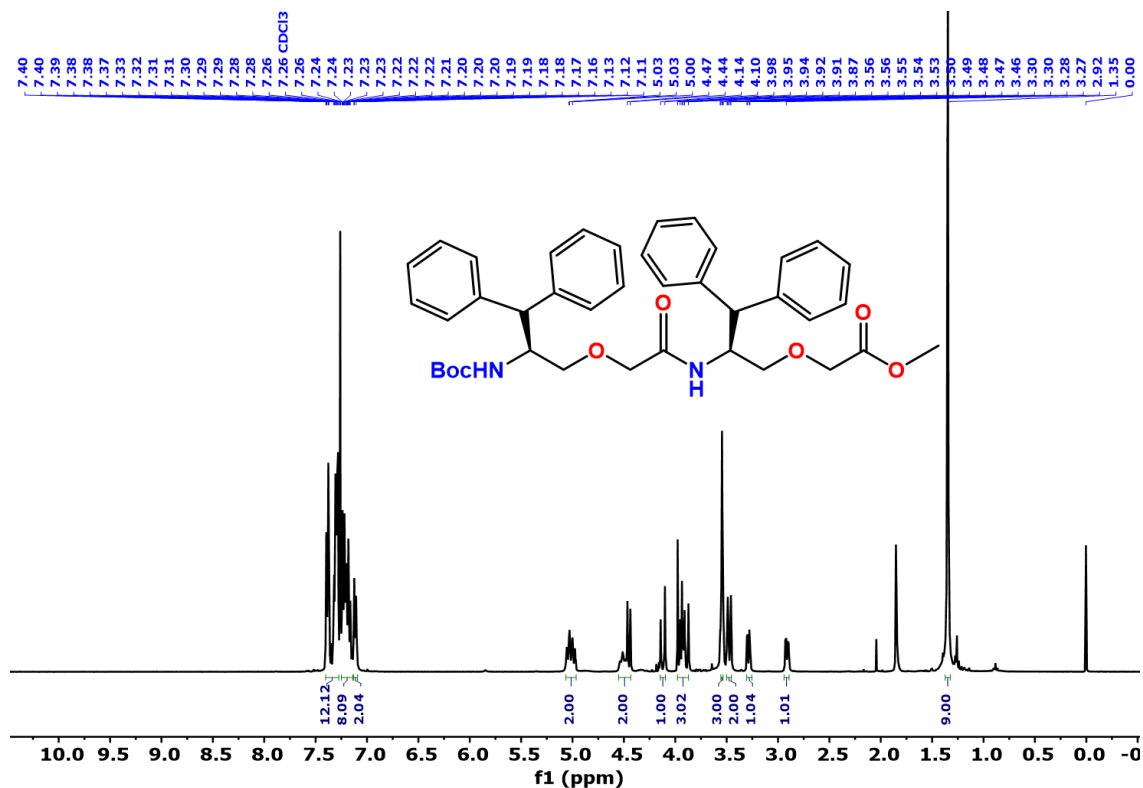


^{13}C NMR Spectrum (101 MHz, CDCl_3) of L6: N-Boc- $\beta(\text{O})$ - δ^5 -Nap- $\beta(\text{O})$ - δ^5 -Nap-OMe

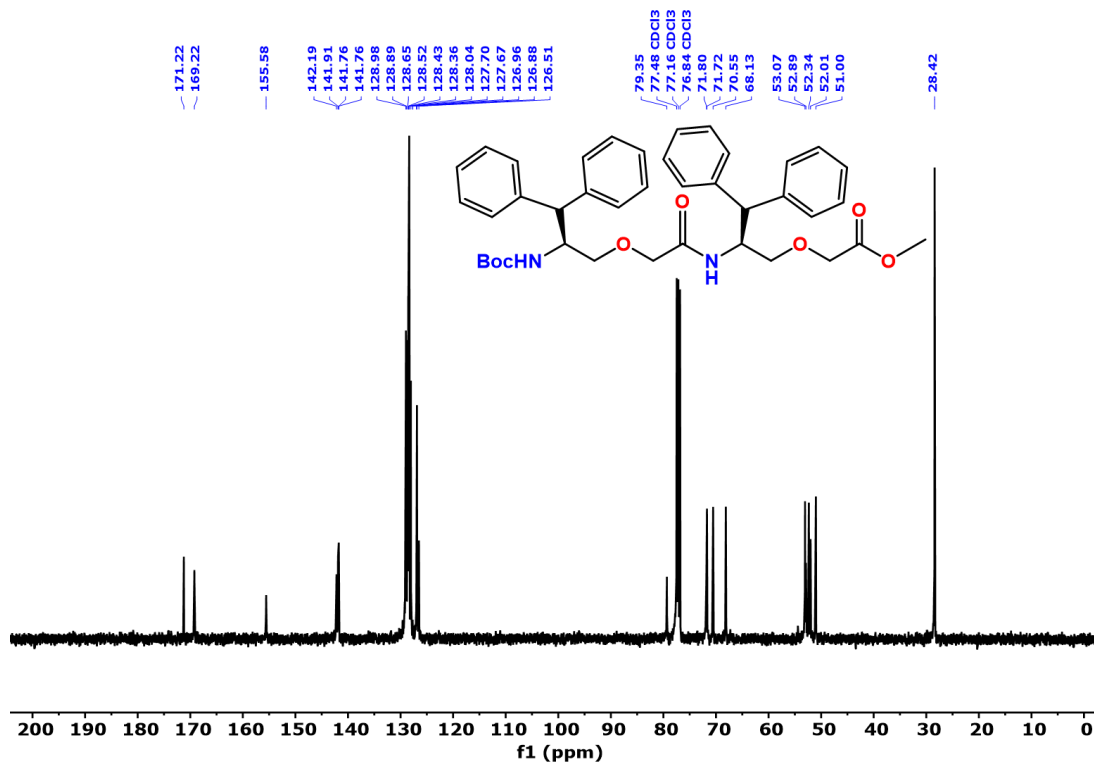


Chapter 2

^1H NMR Spectrum (400MHz, CDCl_3) of L7: N-Boc- $\beta(\text{O})$ - δ^5 -Diphe- $\beta(\text{O})$ - δ^5 -Diphe-OMe

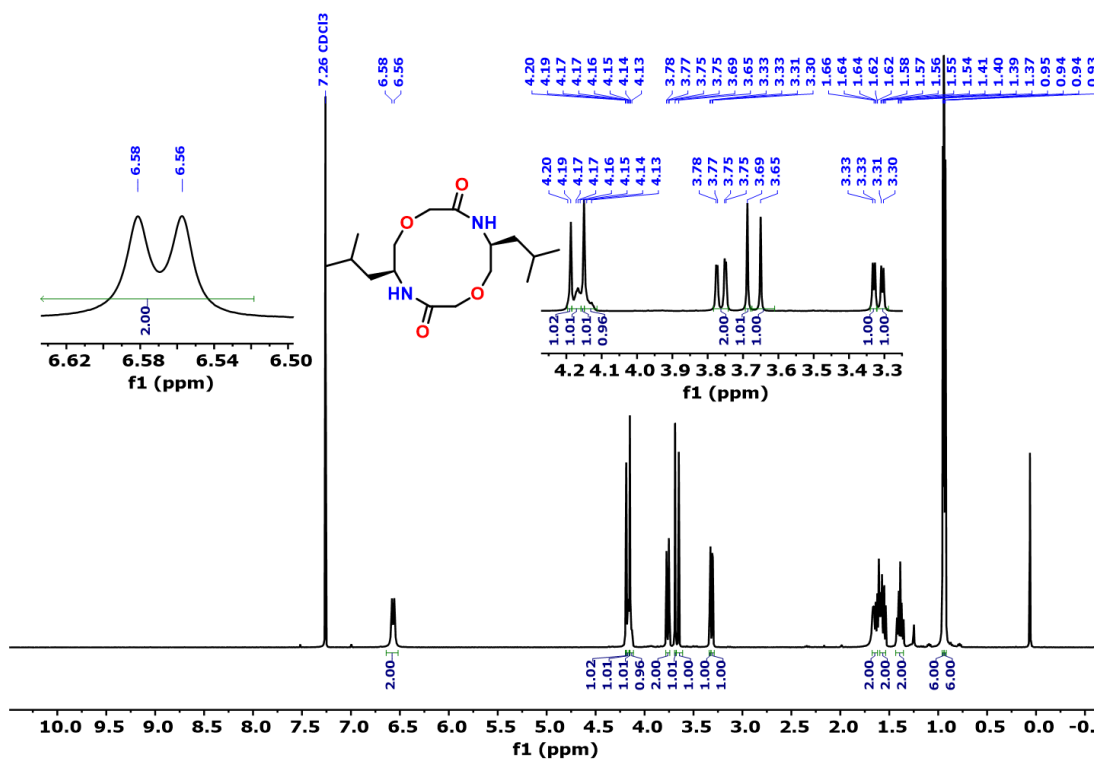


^{13}C NMR Spectrum (101 MHz, CDCl_3) of L7: N-Boc- $\beta(\text{O})$ - δ^5 -Diphe- $\beta(\text{O})$ - δ^5 -Diphe-OMe

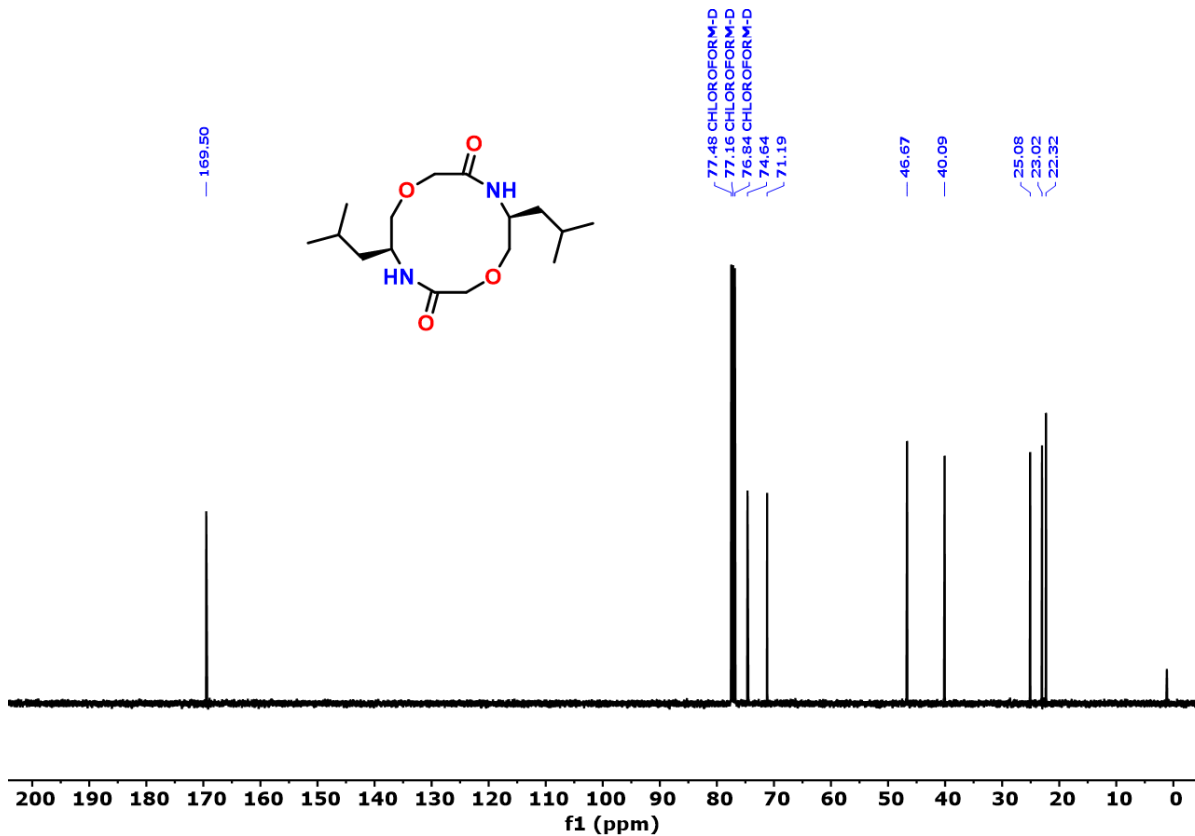


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^1H NMR Spectrum (400 MHz, CDCl_3) of C1

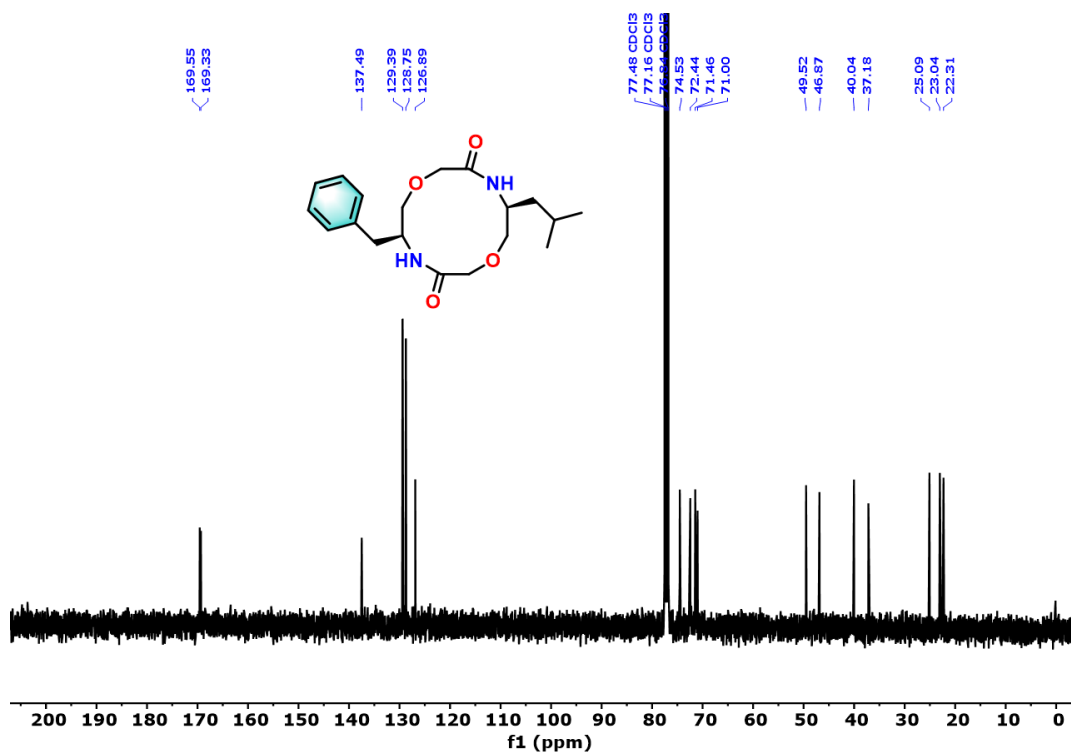


^{13}C NMR Spectrum (101 MHz, CDCl_3) of C1

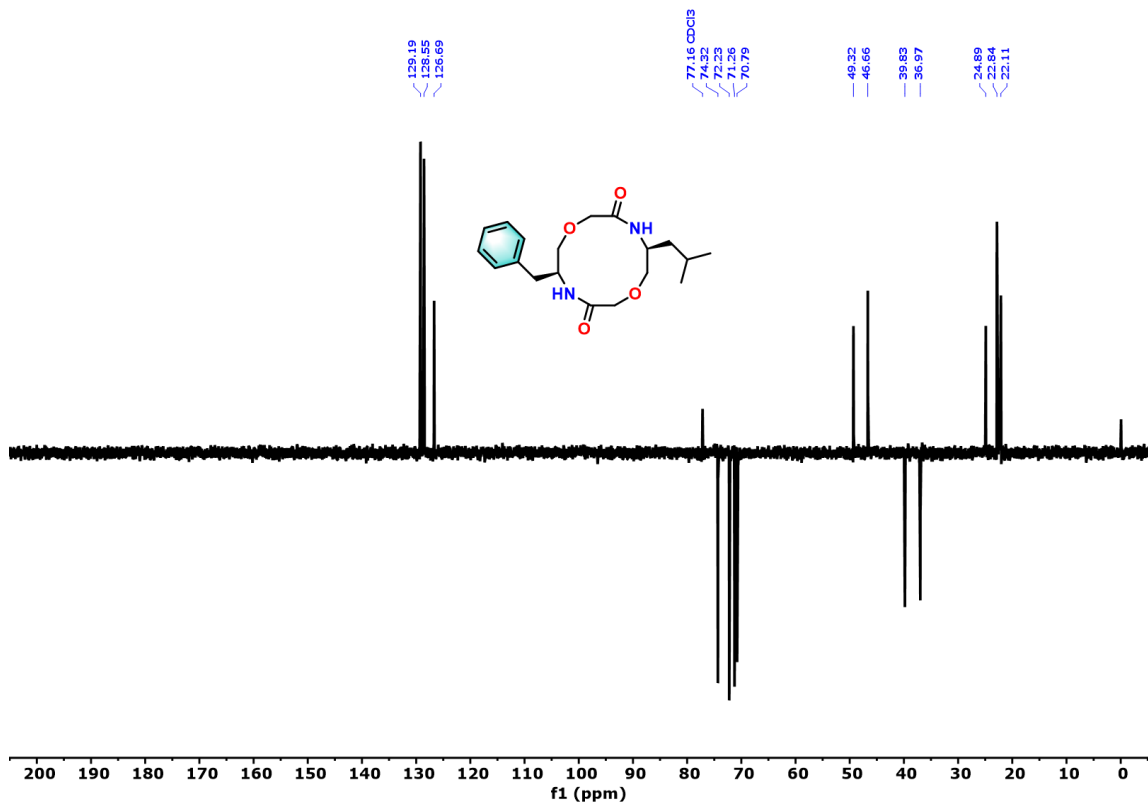


Chapter 2

^{13}C NMR Spectrum (101 MHz, CDCl_3) of C2

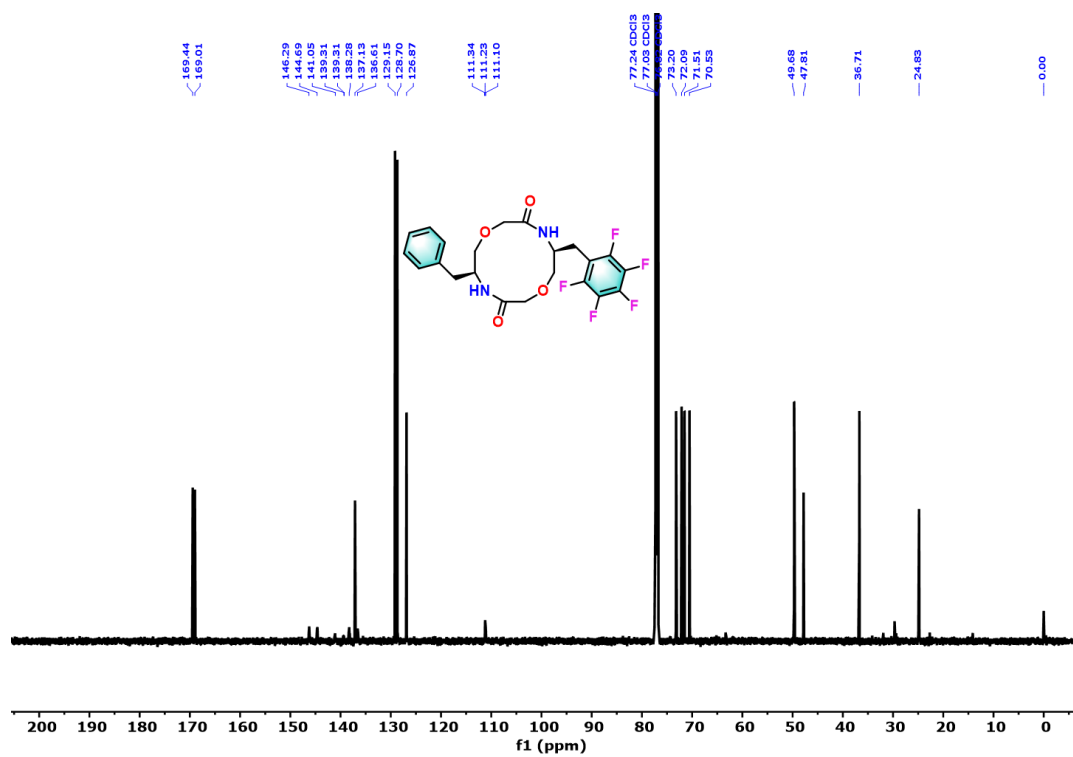


DEPT-135 NMR Spectrum of C2 (151 MHz)

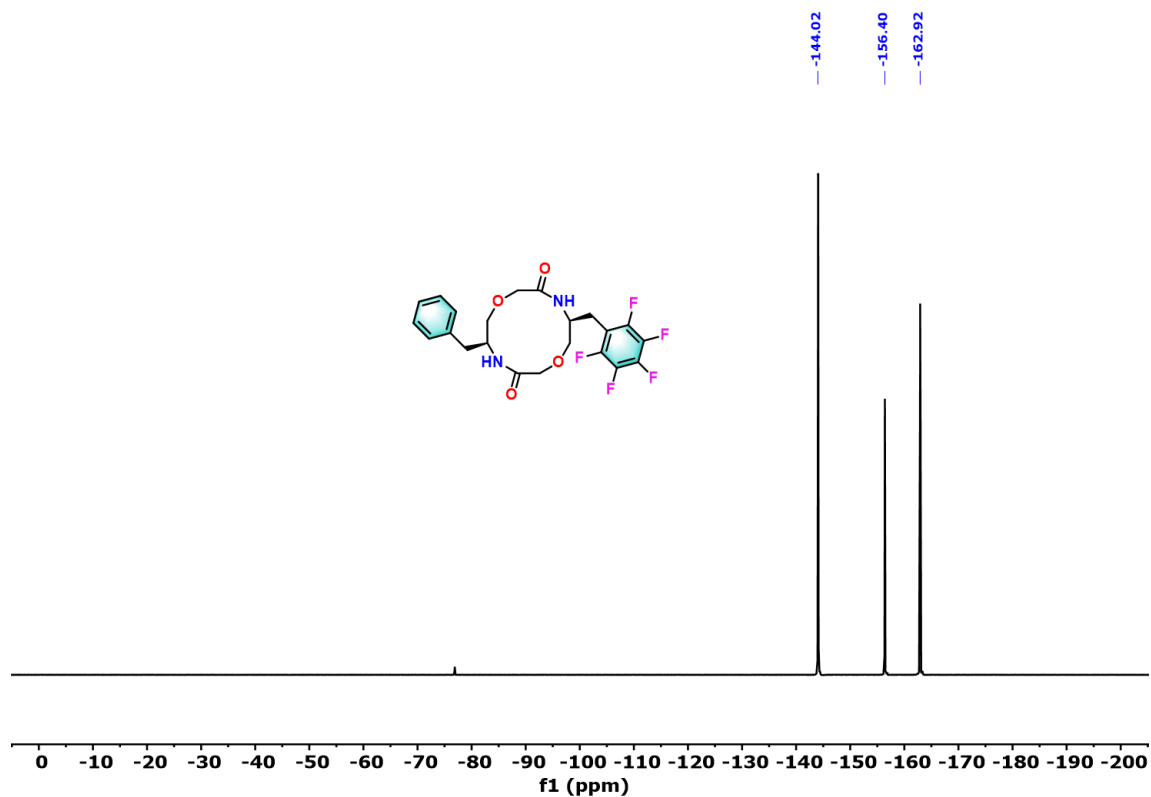


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^{13}C NMR Spectrum (101 MHz, CDCl_3) of C4

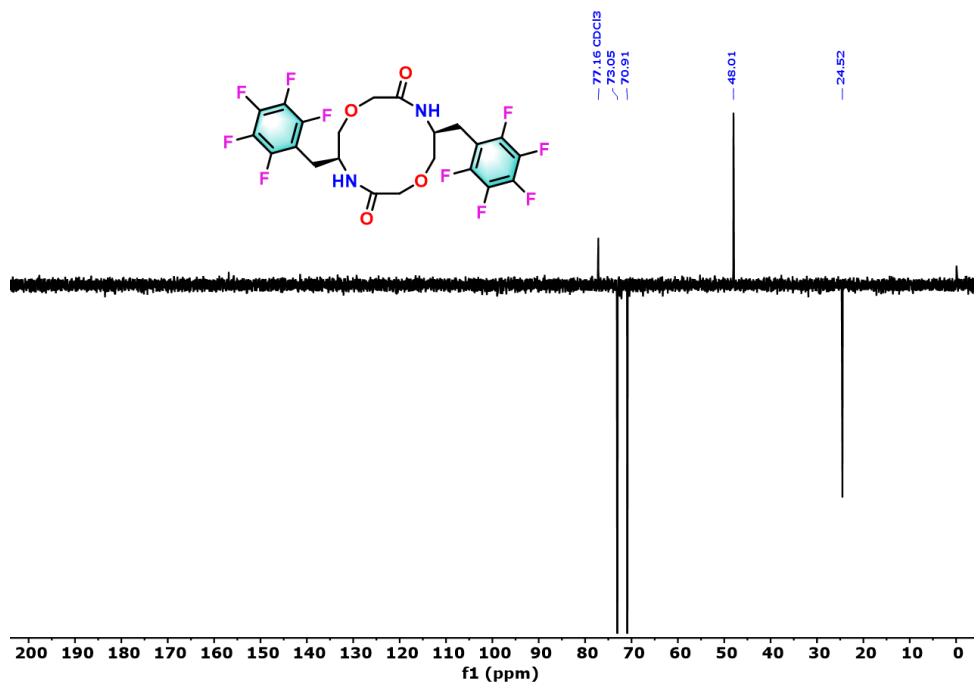


^{19}F NMR Spectrum of C4 (151 MHz):

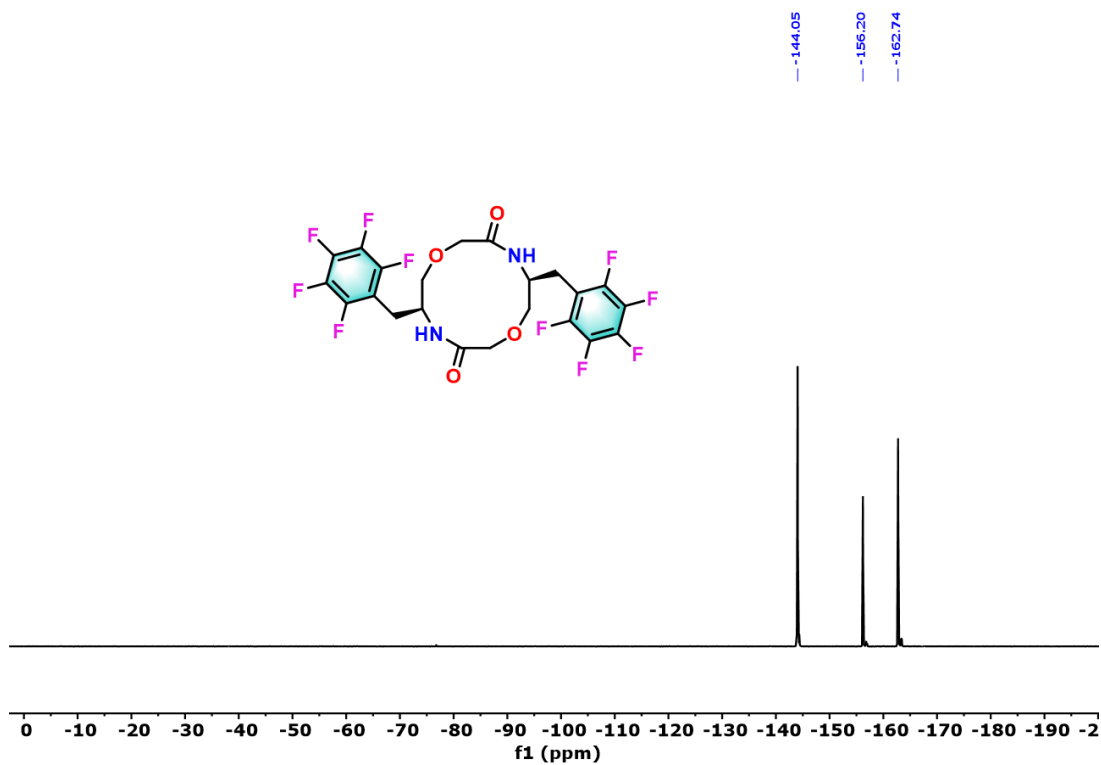


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DEPT-135 NMR Spectrum of C5 (151 MHz):

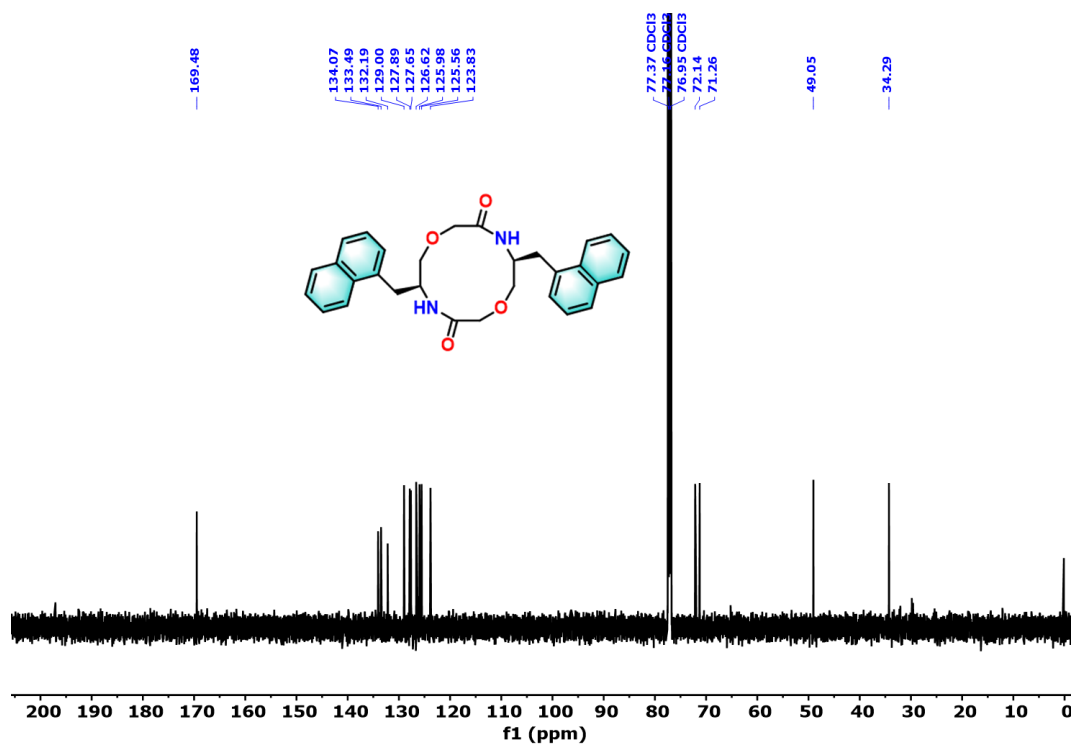


¹⁹F NMR Spectrum of C5 (151 MHz):

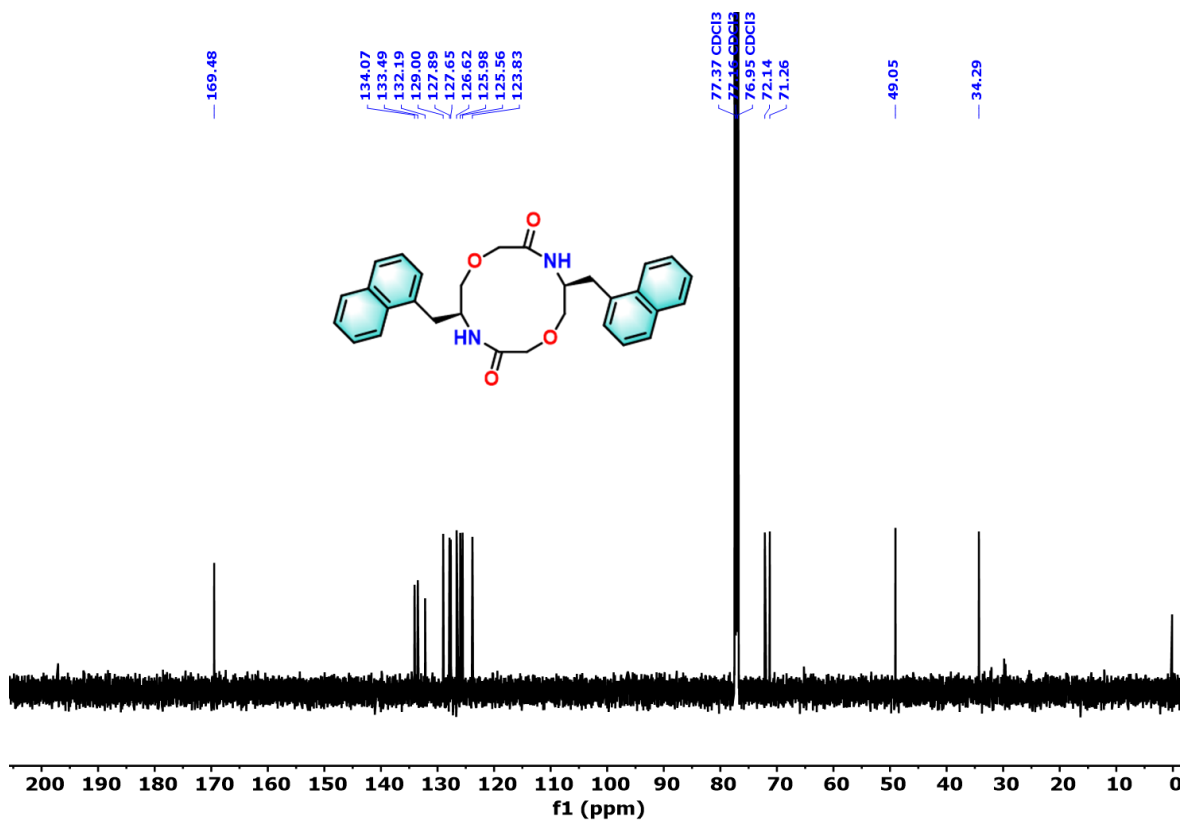


Chapter 2

^1H NMR Spectrum (400 MHz, CDCl_3) of C6

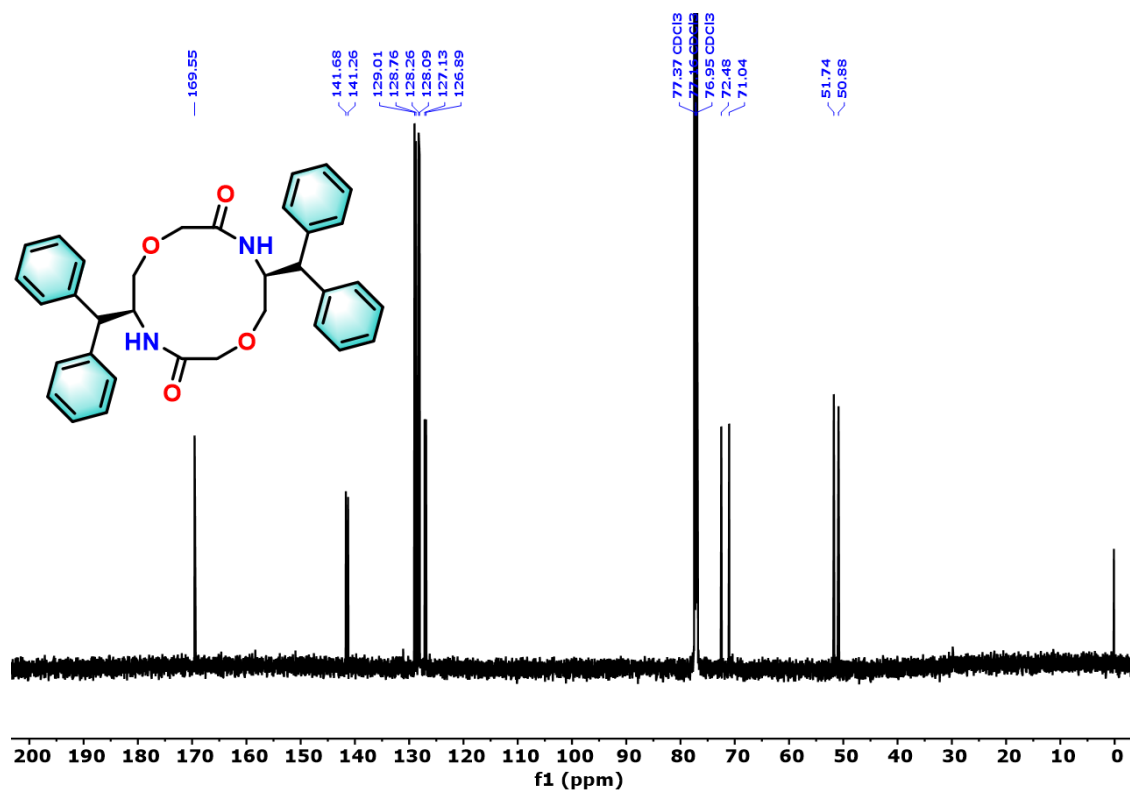


^{13}C NMR Spectrum (101 MHz, CDCl_3) of C6

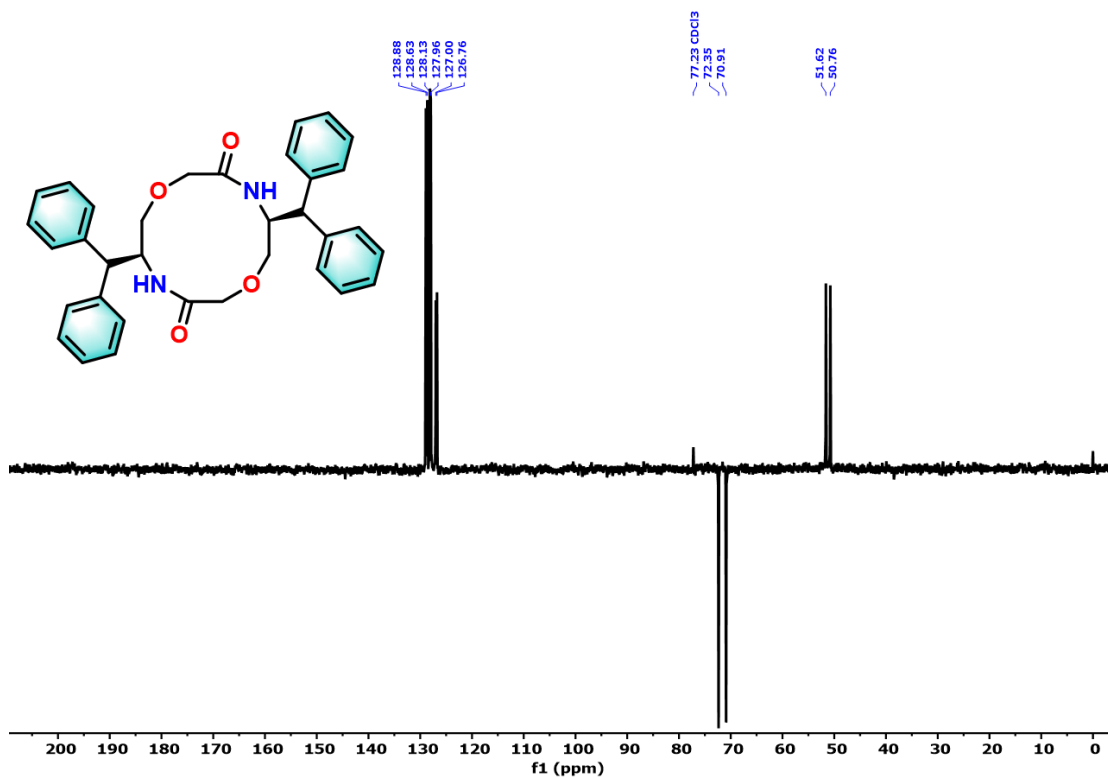


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^{13}C NMR Spectrum (101 MHz, CDCl_3) of C7

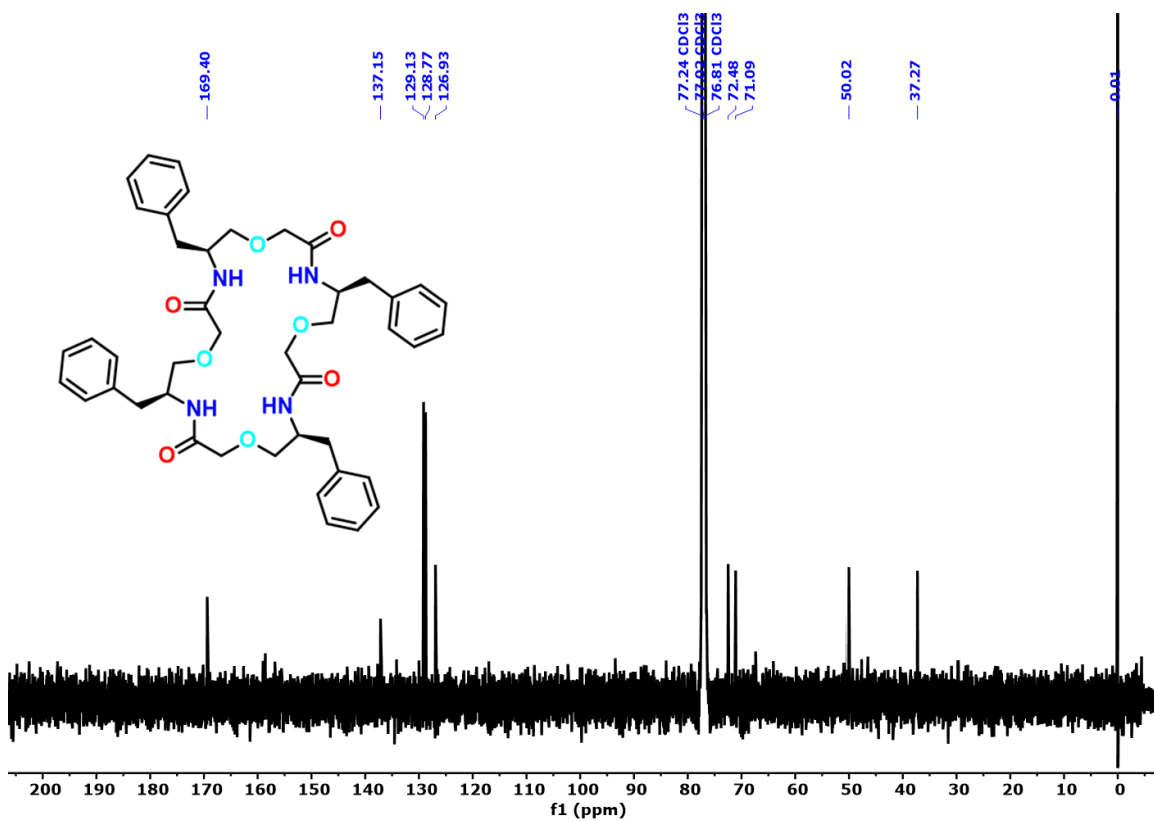


DEPT-135 NMR Spectrum of C7 (151 MHz)

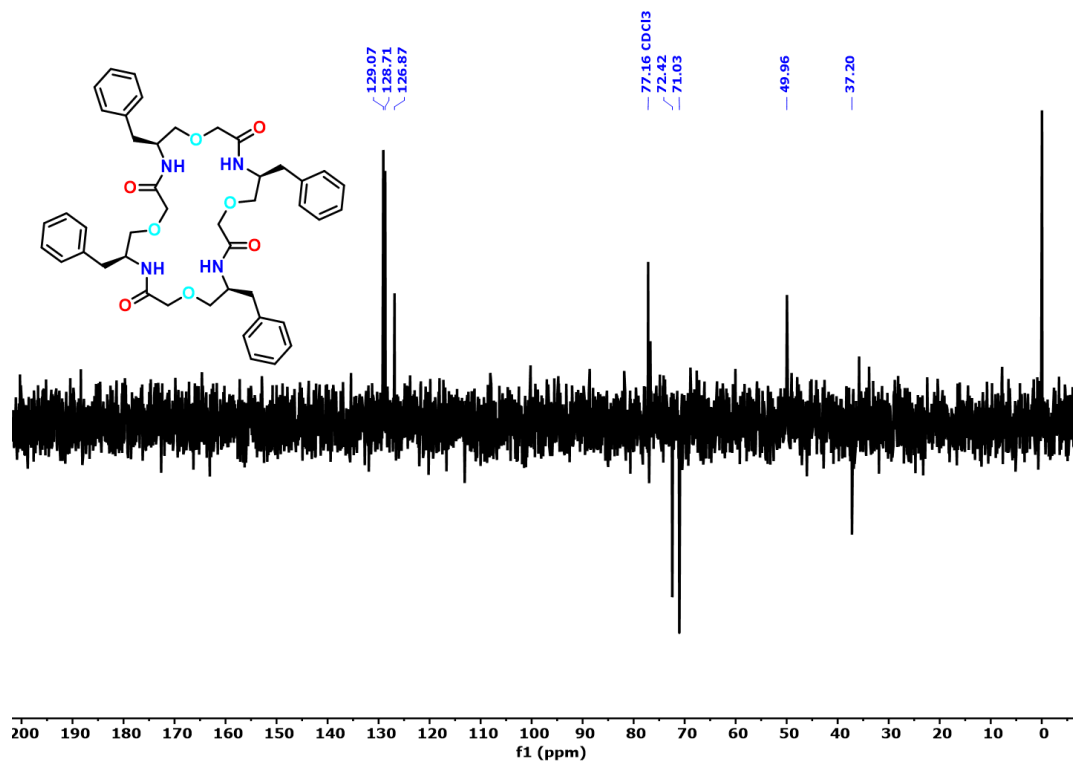


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^{13}C NMR Spectrum (101 MHz, CDCl_3) of C9

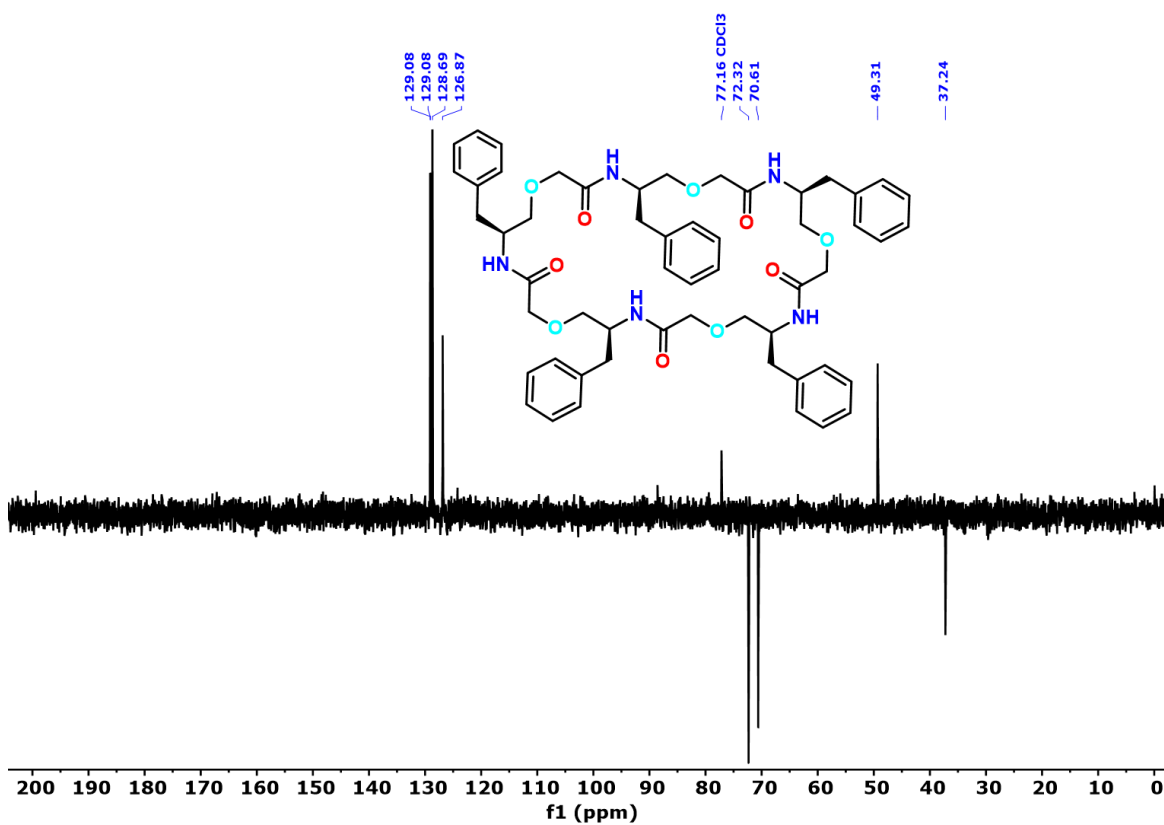


DEPT-135 NMR Spectrum of C9 (151 MHz)

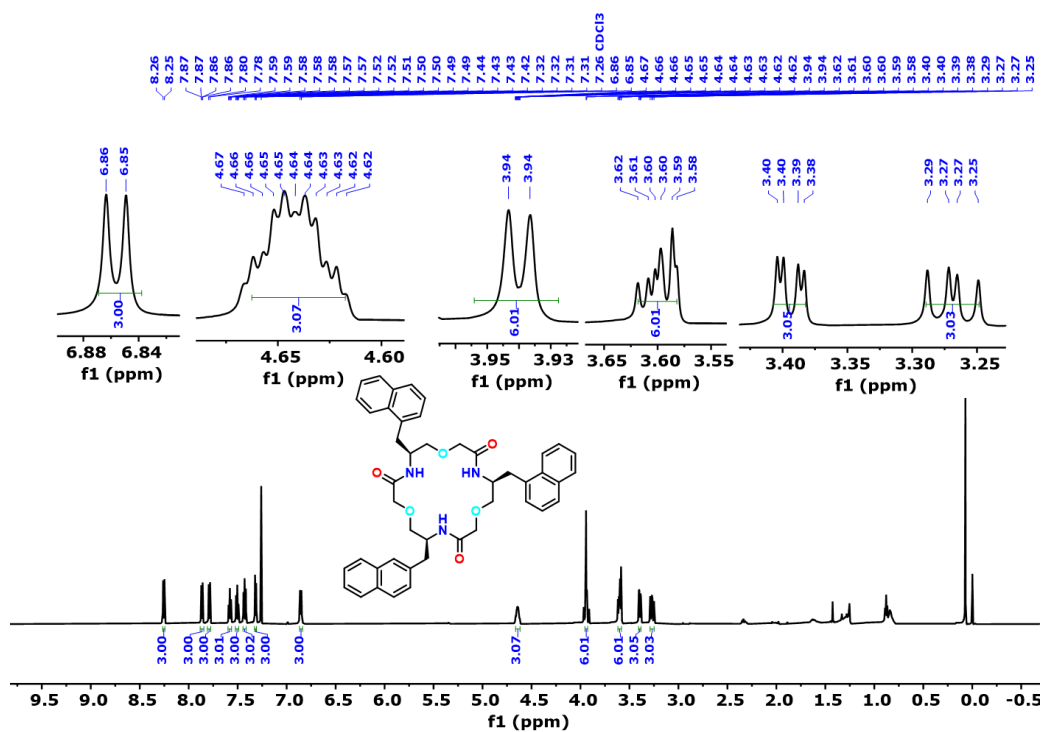


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DEPT-135 NMR Spectrum of C10 (151 MHz)

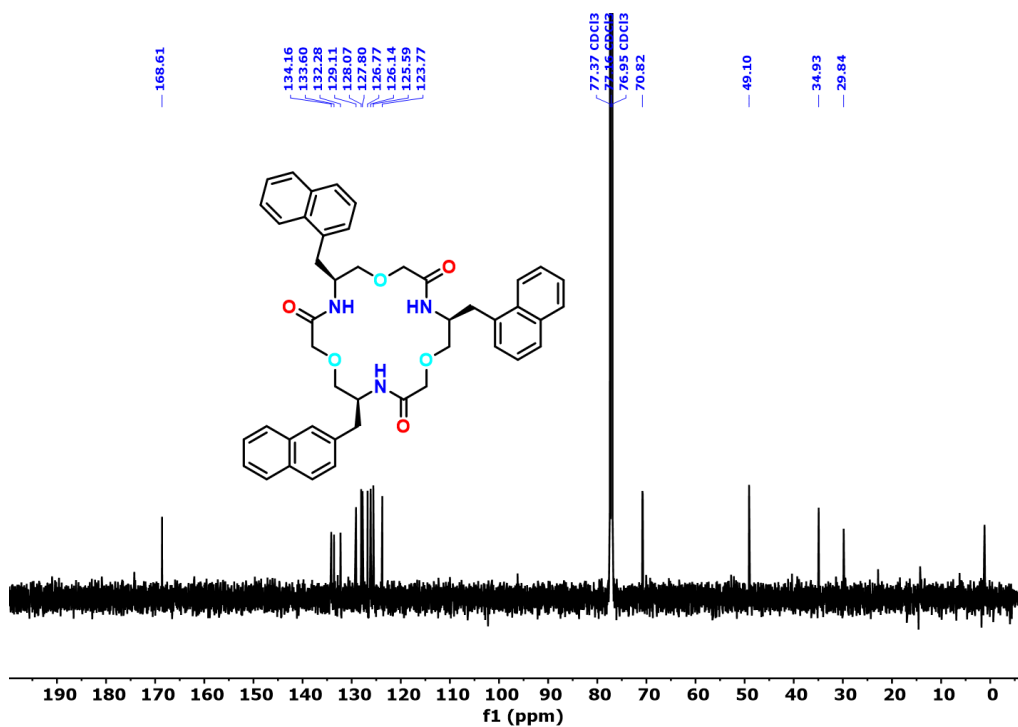


¹H NMR Spectrum (400 MHz, CDCl₃) of C11

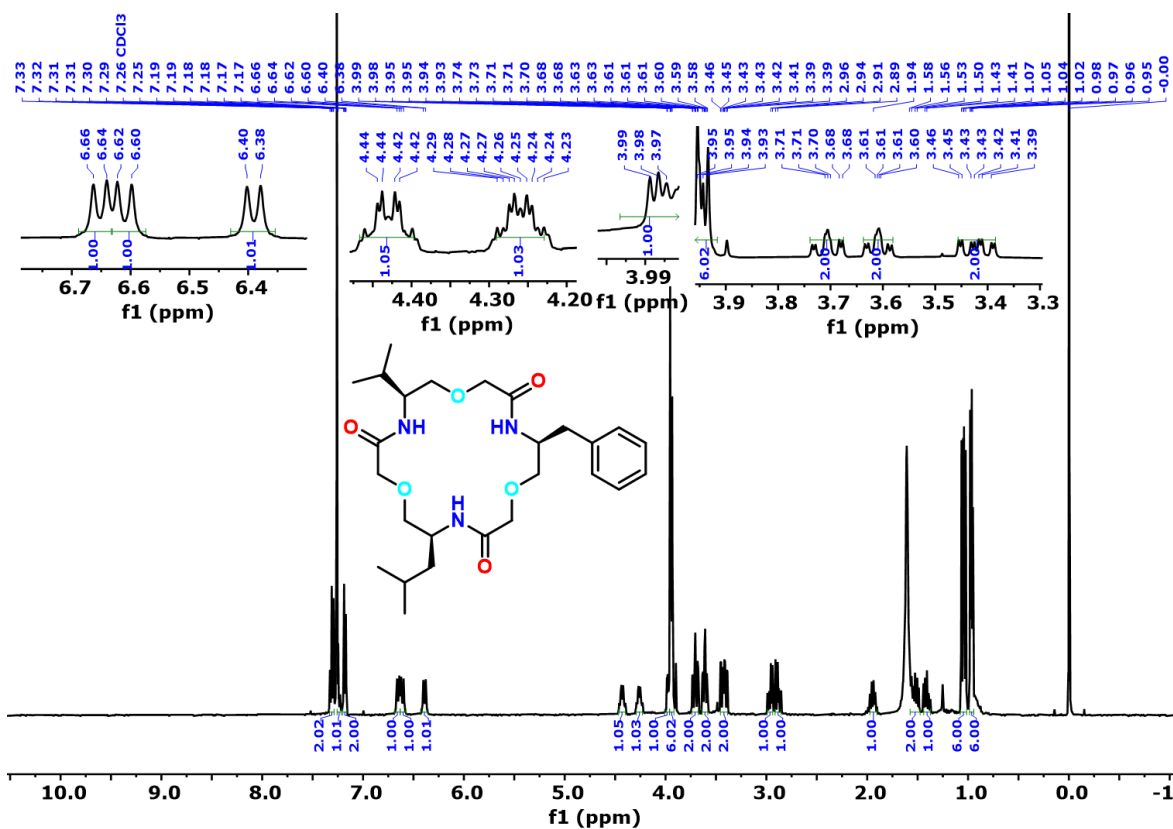


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^{13}C NMR Spectrum (101 MHz, CDCl_3) of C11

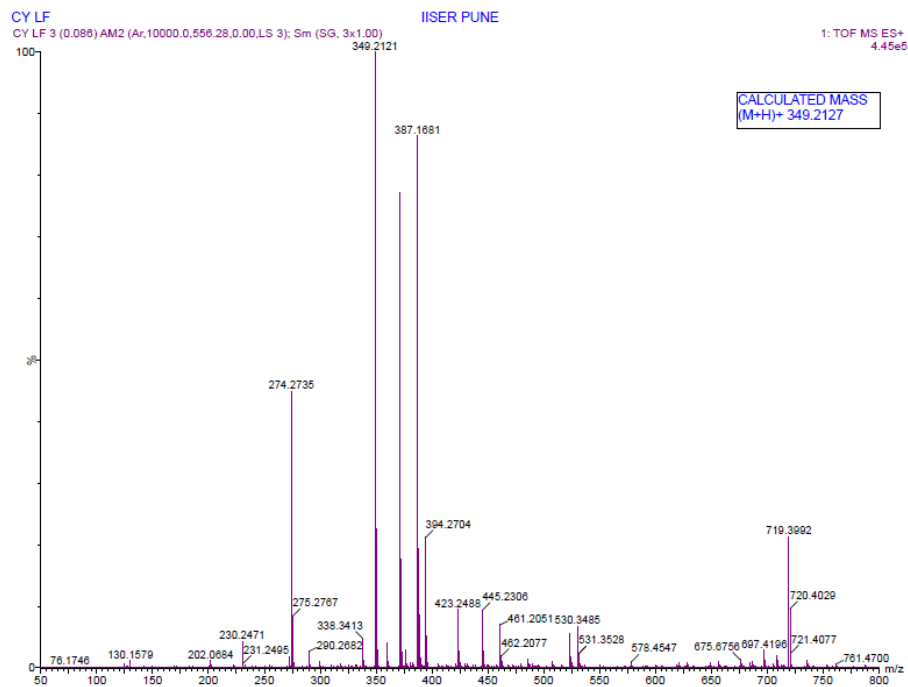


^1H NMR Spectrum (400 MHz, CDCl_3) of C12

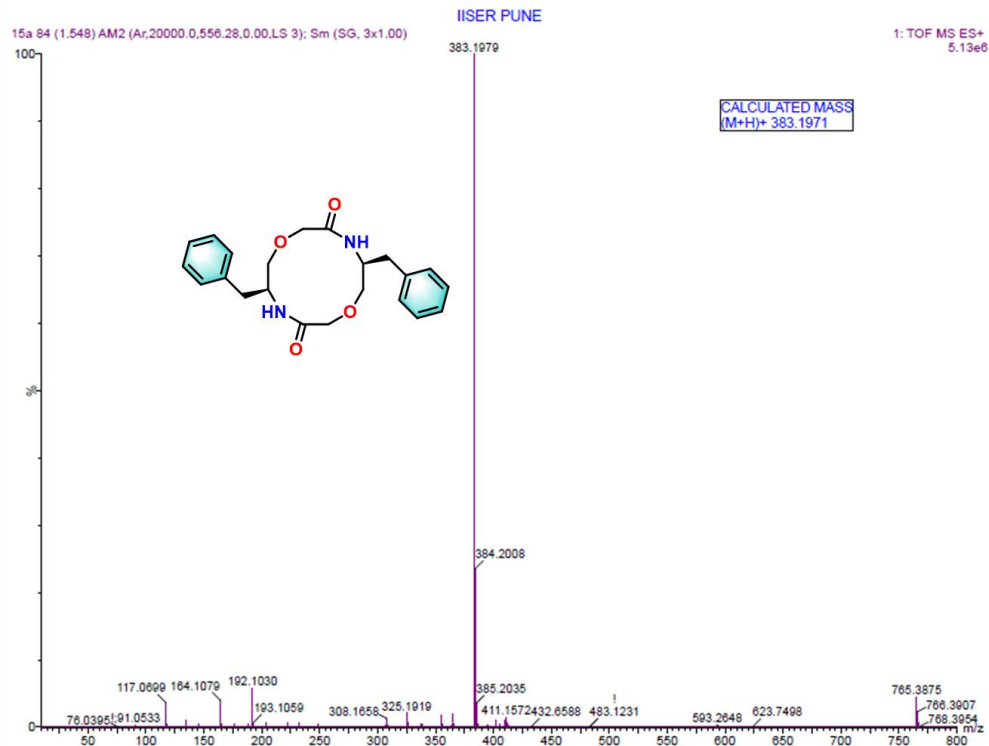


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HRMS Spectrum of Compound C2

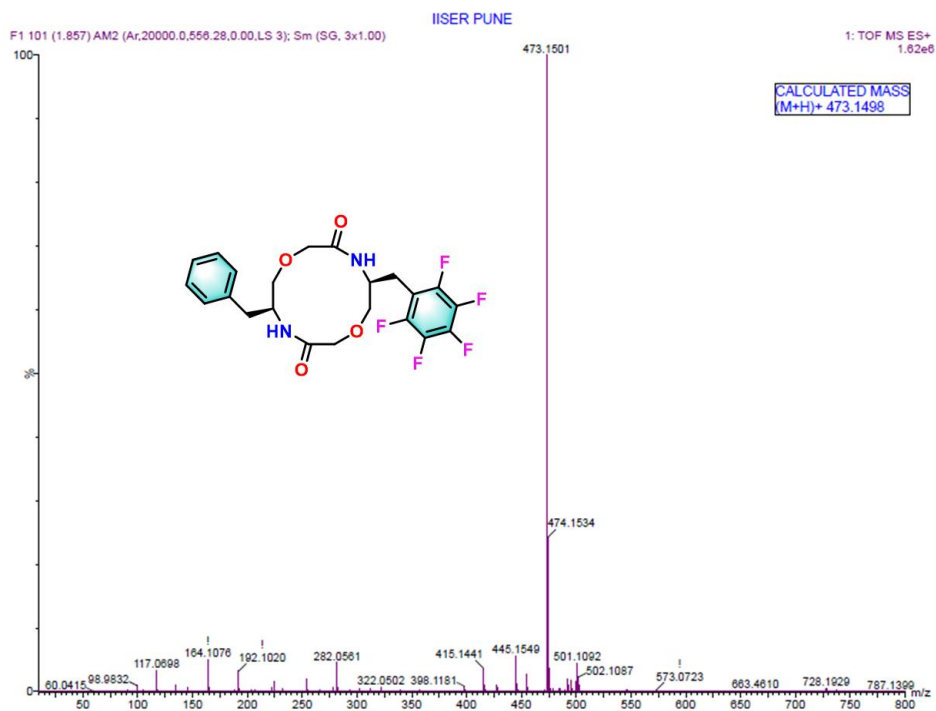


HRMS Spectrum of Compound C3

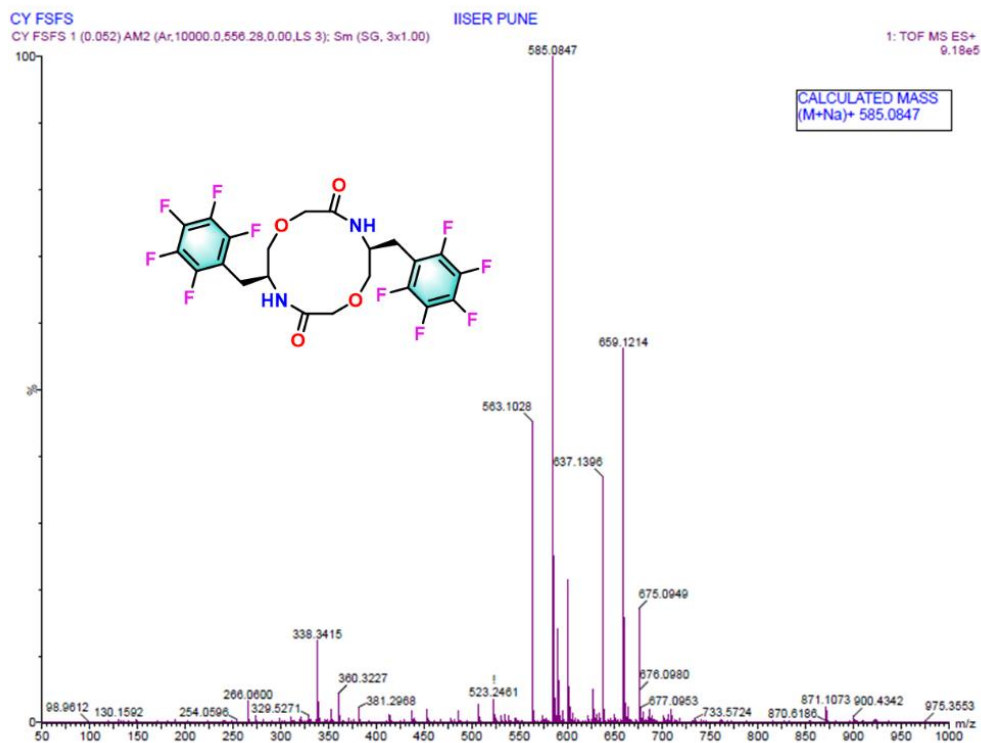


HRMS Spectrum of Compound C4

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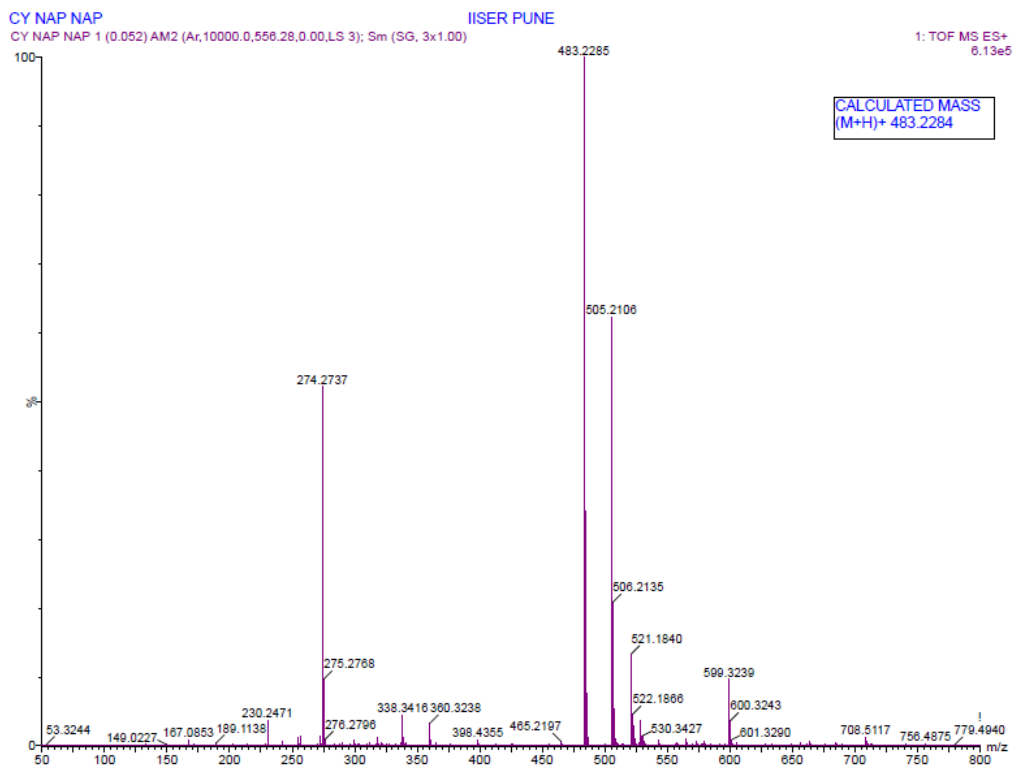


HRMS Spectrum of Compound C5

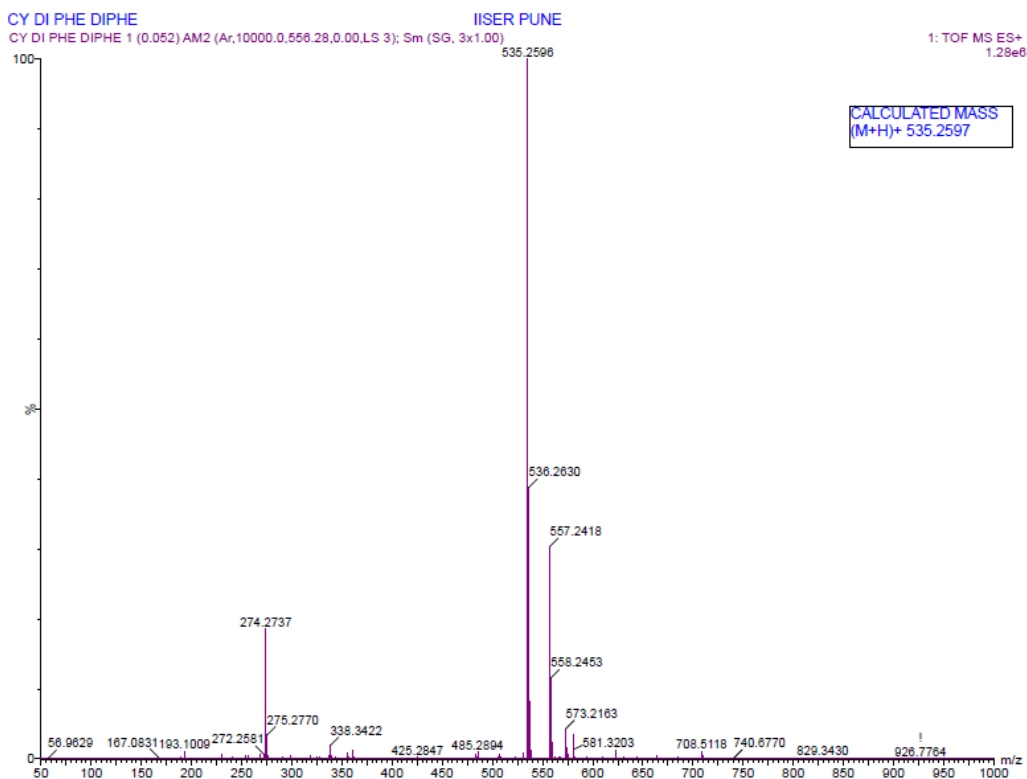


HRMS Spectrum of Compound C6

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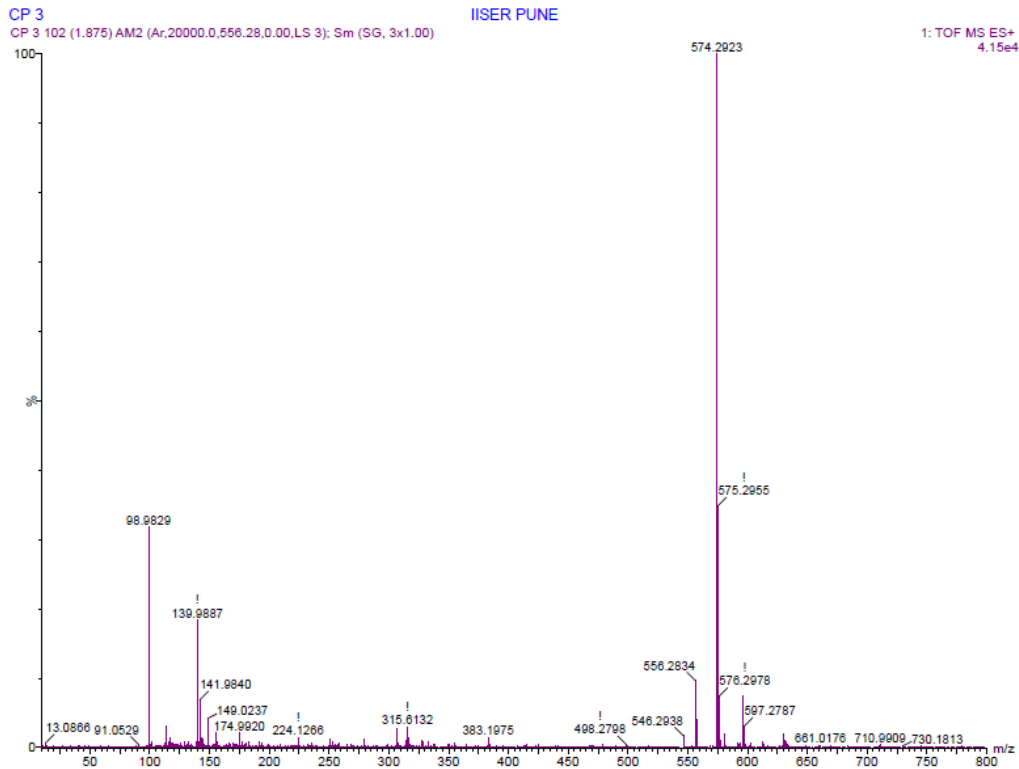


HRMS Spectrum of Compound C7

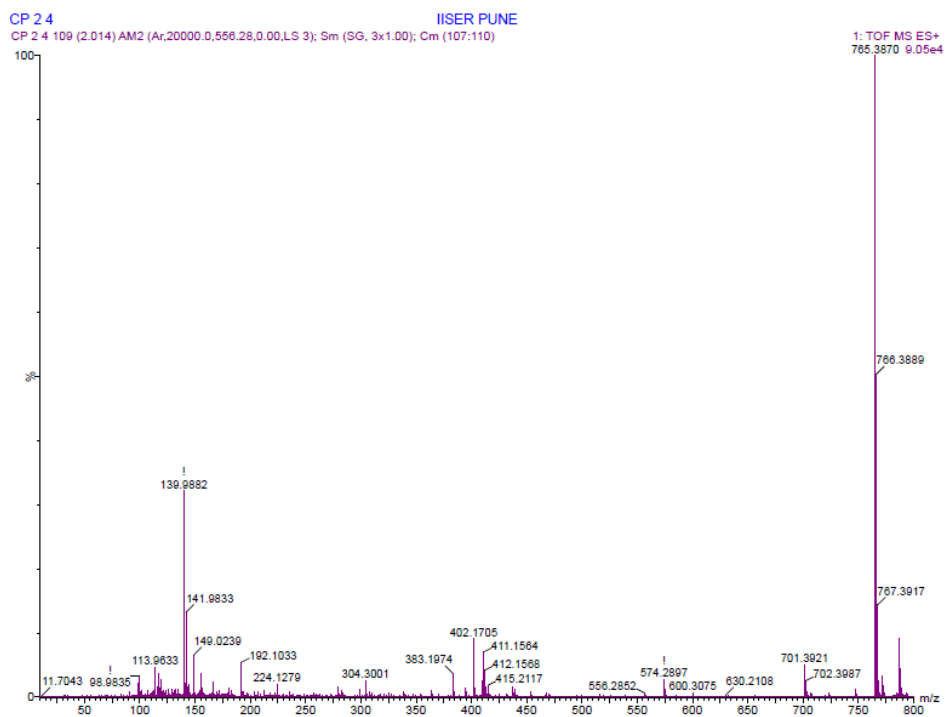


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HRMS Spectrum of Compound C8



HRMS Spectrum of Compound C9

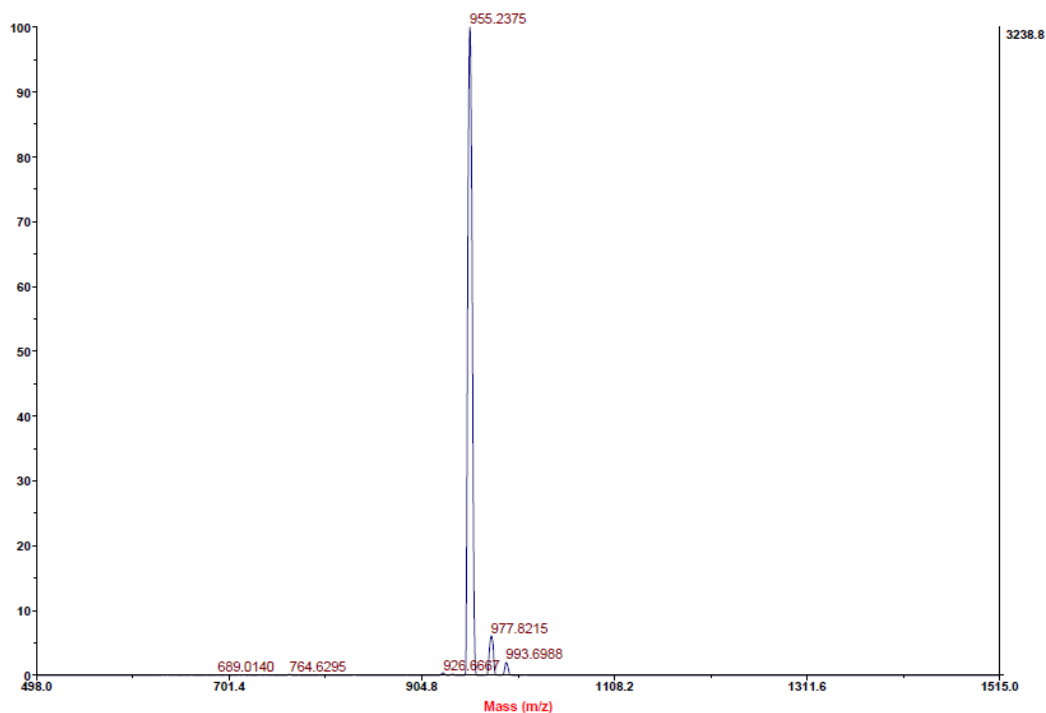


Chapter 2

MALDI/TOF Spectrum of Compound C10

Spectrum Report

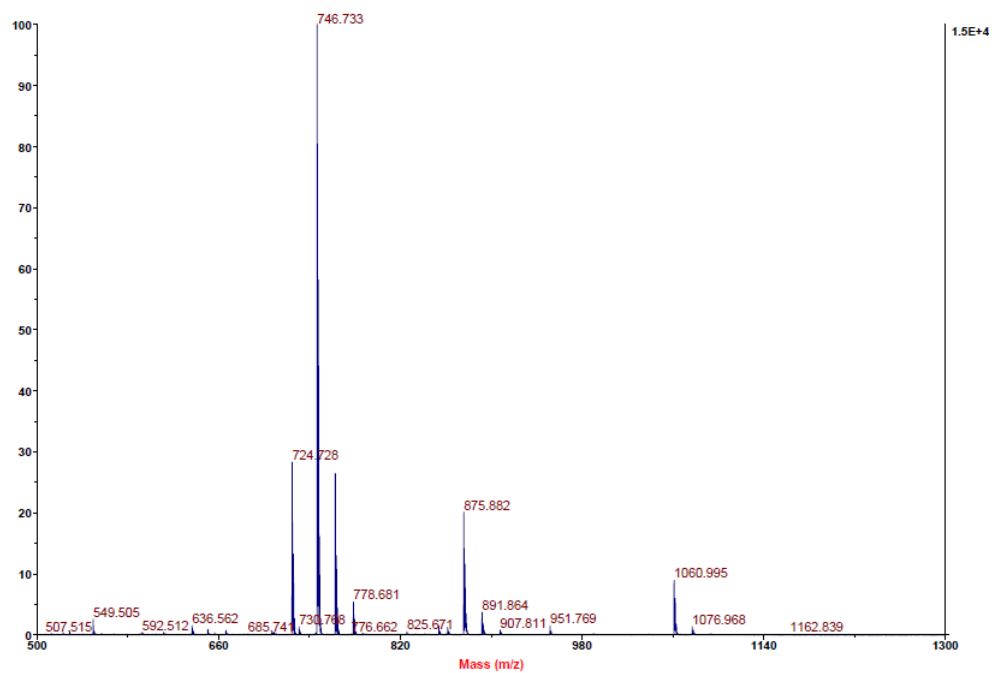
Final - Shots 2000 - IISER-96-1-2022; Label B4



MALDI/TOF Spectrum of Compound C11

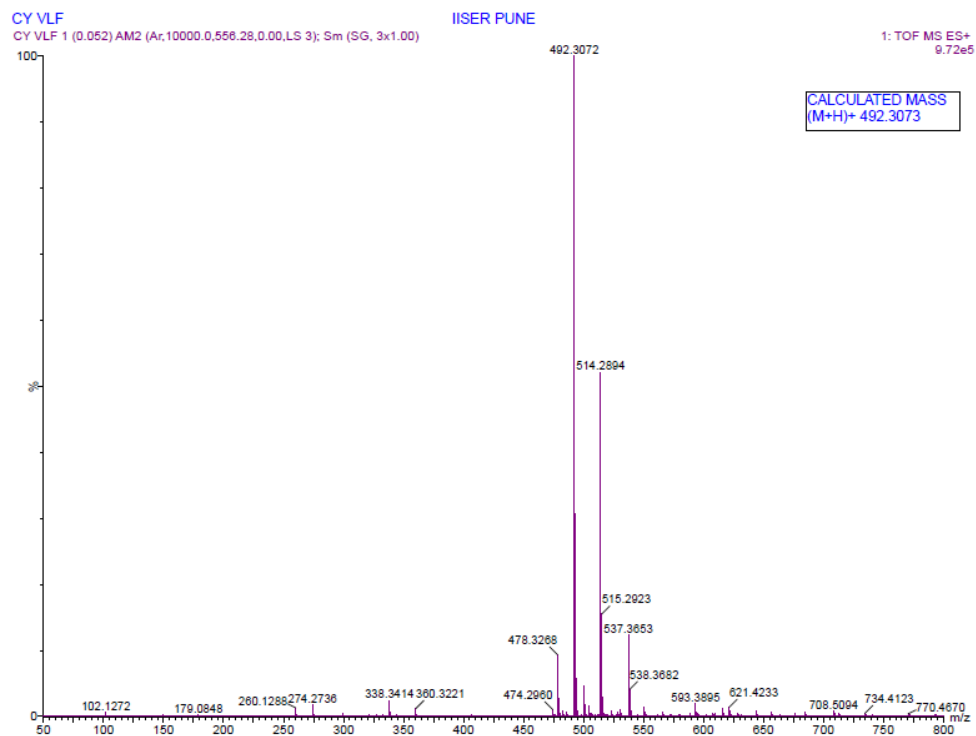
Spectrum Report

Final - Shots 1000 - IISER-96-1-2018; Label F7



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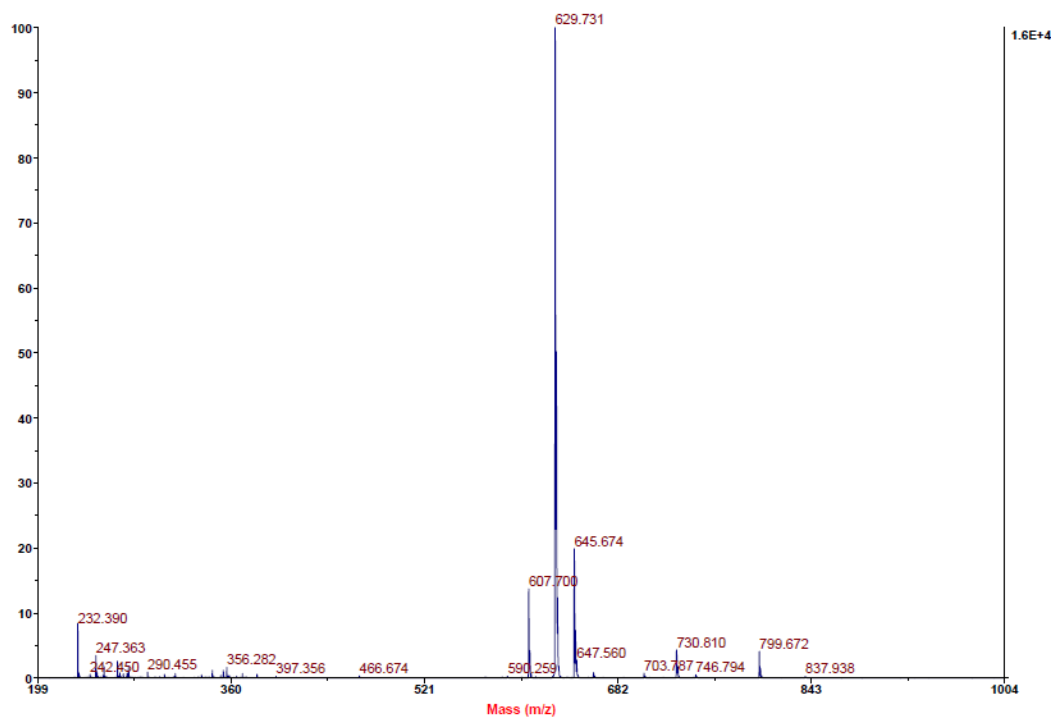
HRMS Spectrum of Compound C12



MALDI/TOF Spectrum of Compound C13

Spectrum Report

Final - Shots 500 - IISER-96-2-2018; Label E12



Chapter 3

**Exploring Macroscopic Dipoles of Designed Cyclic
Peptide Tubular Assemblies to Harvest Piezoelectric
Properties**

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3.1. Introduction:

3.1.1. Piezo-electronic Materials:

Piezoelectricity is a phenomenon that showcases the interaction between the electrical and mechanical states of a material. When a piezoelectric material is subjected to mechanical deformation, such as compression, it generates an electric current and charges its surfaces. Conversely, applying an electric field causes the material to deform. This effect was discovered by the Curie brothers in 1880^[1], who investigated how crystal structures influence piezoelectric phenomena and predicted the relationship between voltage and stress in these materials^[2]. In 1941, natural biomaterials were observed to possess polarization properties^[3], and in the 1950s, Fukada identified shear piezoelectricity in various biopolymers, including cellulose and collagen^[4]. The remarkable piezoelectric properties of Lead-Zirconate-Titanate (PZT) ceramics, discovered in 1954^[5], established PZT as a crucial material for piezoelectric applications. Initially, these materials were used in macroscale electromechanical transducers for military and marine purposes^[6]. The development of microelectromechanical systems (MEMS) accelerated the study and application of piezoelectric materials^[7], which are now extensively used in energy harvesters, sensors, transformers, actuators, piezotronics, and more^[8]. Despite extensive research on piezoelectric ceramics such as barium titanate, PZT, zinc oxide, and molybdenum disulfide, their brittleness and non-biological nature limit their use in biological systems.

3.1.2. Biodegradable Metabolite Piezo-electronic Materials:

Piezoelectricity is an excellent feature that can be harvested from materials with non-centrosymmetric crystals.^[9] Many inorganic crystalline materials, including barium titanate,^[10] and lead zirconate titanate,^[11] have been shown to display strong piezoelectricity. In contrast to inorganic crystalline materials, many self-assembled biomolecules such as collagen,^[12] elastin,^[13] and viruses^[14] have exhibited modest piezoelectric properties, which cannot be used to design devices. Over the years, there has been a quest to design biocompatible piezoelectric materials for biomedical applications.^[15]

Traditional piezoelectric materials, such as ZnO and AlN, often lack biocompatibility, limiting their use in wearable electronics. To address this issue, bioinspired organic piezoelectric materials have emerged as promising alternatives due to their biocompatibility, biodegradability, and robust

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mechanical properties. Various bioorganic materials, including bone, wood, silk, and amino acids, exhibit piezoelectric properties and are potential candidates for energy harvesting applications. Among these, supramolecular self-assembled amino acids and peptides are particularly notable for their high piezoelectric polarization.

3.1.3. Organic Peptide and amino acid based Piezo-electronic Materials:

In contrast to natural biomolecules, many synthetic biomaterials with tailor-made self-assembling properties have shown better piezoelectric properties. In their pioneering work, Gazit and co-workers, and others, examined the piezoelectric properties of self-assembled peptides,^[16] peptide nanotubes,^[17] and amino acids.^[18] Synthetic biomaterials have attracted considerable attention due to their tailor-made self-assembling properties. The studies revealed that the large surface dipole moment emerging from self-assembled nanostructures is crucial for piezoelectric properties.^[19] Smaller dipole moments of crystals also result in smaller piezoelectric coefficients. These materials, composed of amino acids, are biocompatible, biodegradable, and chemically transformable. Their properties are significantly influenced by their self-assembly processes. Therefore, understanding these processes and their resulting properties is crucial for both fundamental research and practical applications.

Piezoelectricity has been observed in various peptides, such as β glycine^[20], gamma (γ) glycine^[21], diphenylalanine (FF)^[22], Fmoc-FF^[23] etc. Among these, FF peptides have been the subject of the most extensive research as piezoelectric biomaterials. Few examples are described below. Glycine, an amino acid, exists in three polymorphic phases (α , β , and γ), with the β -phase showing the highest piezoelectric shear coefficient. However, the β -phase is unstable under ambient conditions and can transition to the α - or γ -phase. Experiments have shown that devices made with γ -glycine crystals can produce significant voltage under mechanical stress.^[20,21] In 2019, researchers found that aromatic amino acids like L-phenylalanine and L-tyrosine form non-centrosymmetric structures with strong hydrogen-bonded assemblies. L-tyrosine, in particular, has high thermal stability and mechanical strength, making it suitable for stable power generation in devices.^[24] These discoveries highlight the potential of amino acid-based piezoelectric materials for wearable electronic applications. In 2020, researchers demonstrated interactions between rigid aromatic 4,4'-bipyridine (4,4'-Bpy) and nonaromatic amino acid derivative N-acetyl-L-alanine (AcA). They observed transitions between H-aggregation, J-aggregation, and X-aggregation stacking modes in

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these hybrid systems. These stacking modes enhance chirality, red-shift emission, thermal resistance, and mechanical properties. The non-centrosymmetric nature of these crystals contributes to their piezoelectric and ferroelectric properties.^[25] Ultrashort peptides, particularly the aromatic dipeptide diphenylalanine (FF), form self-assembled structures with notable piezoelectric properties. FF nanotubes, exhibiting a non-centrosymmetric crystal structure, demonstrated high piezoelectric coefficients. Despite challenges in uniformly orienting nanotubes for large-scale manufacturing, methods have been developed to align FF microrods using external electric fields, achieving significant piezoelectric performance. In 2016, Yang et al. aligned FF microrods, producing a high piezoelectric coefficient and a PEH device with notable voltage and current output. In 2018, Lee et al. synthesized horizontally aligned FF nanotubes, creating a PEH device with impressive output, capable of powering LCD panels through simple finger presses.^[26] More recently, the group engineered piezoelectricity in collagen-mimicking peptide assemblies. They developed piezoelectric generators using FF-derived peptides with proline (Pro) or hydroxyproline (Hyp) termini, named Pro-Phe-Phe (P-F-F) and Hyp-Phe-Phe (Hyp-F-F).^[27] These tripeptides formed helical sheet-like structures with strong mechanical strength and high piezoelectric response, particularly Hyp-F-F due to robust H-bonding. These findings illustrate the potential of molecular engineering in peptide nanotechnology for piezoelectric applications.

3.1.4. Cyclic Peptide based Piezo-electronic Materials:

Gazit and his team developed a dipeptide based on cyclo glycine-tryptophan (cyclo-GW), which exhibits impressive piezo-electronic property due to its supramolecular β -sheet hydrogen-bonded structure and herringbone edge-to-face aromatic interactions.^[28] Single crystals of cyclo-GW were obtained by dissolving the dipeptide in a methanol/water solvent mixture and heating it to 80°C, followed by slow cooling to room temperature over 30 days to form larger crystals. X-ray diffraction analysis revealed β -sheet hydrogen-bonding patterns and herringbone packing interactions in the cyclo-GW crystals. The non-centrosymmetric monoclinic crystal structure suggests potential piezoelectric properties. Additionally, Gazit and colleagues explored tryptophan-based aromatic dipeptides for biocompatible PEH applications. These peptides exhibit strong hydrogen-bonded structures with notable rigidity and thermal stability, alongside unique semiconducting, mechanical, and piezoelectric properties. Cyclo-phenylalanine-tryptophan (cyclo-FW) and cyclo-tryptophan-tryptophan (cyclo-WW) were synthesized, featuring

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diketopiperazine rings that promote hydrogen-bonded interactions. Cyclo-FW crystallizes in an orthorhombic space group, displaying promising piezoelectric properties.^[28]

3.2. Rationale of present work:

Similar to the self-assembled nanotubes of Phe-Phe dipeptide^[29] and other peptides,^[30] cyclic peptides derived from alternating L- and D- α -amino acids,^[31] β -amino acids,^[32] alternating α,γ -amino acids,^[33] and δ -amino acids^[34] self-assembled into tubular assemblies. The overall dipoles of cyclic peptides derived from alternating L- and D-amino acids H-bonds are nullified due to the alternating directionality of the H-bonds in the tubular assemblies. In contrast, cyclic β -peptides and δ -peptides showed unidirectional H-bonds in their tubular assemblies, which yield a macrodipole along the central axis.^[35] Kimura and colleagues recently demonstrated the piezoelectric properties of cyclic peptides derived from β -amino acids.^[36] They demonstrated relatively high piezoelectric coefficient from cyclic β -peptides with unidirectional H-bonds compared to cyclic peptides with alternating H-bond directionality. Beyond the cyclic β -peptides, no efforts have been made in the literature to design cyclic peptides with unidirectional H-bonds to explore the piezoelectric properties. Recently, we have examined the helical conformations of hybrid peptides composed of β -oxy- δ^5 -amino acids and their biocompatibility.^[37] In contrast to the δ -amino acids with complete carbon backbone, the flexible β -oxy δ^5 -amino acids and peptides are highly soluble in various organic solvents due to the presence of “O” in the backbone. These interesting properties motivated us to synthesize the cyclic dipeptides of these amino acids and examine self-assembling and piezo-electronic properties. Here we report the tubular assemblies of a series of cyclic dipeptides in single crystals with unidirectional H-bonds, assembly in solution, and piezoelectric properties. The piezoelectric property depends on the packing and macrodipole.^[38] The cyclic peptides displayed higher piezoelectric coefficient compared to the linear peptides. The piezoelectric coefficients displayed by cyclic peptides reported here are much higher than the reported linear and cyclic peptides. These new cyclic peptides consisting of both amide and ether linkages in the backbone provide a unique opportunity to design new piezoelectric materials.

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3.3. Objective of present work:

In the previous chapter, we detailed our approach to designing self-assembled cyclic peptides, highlighting the critical role of unidirectional hydrogen bonds dictated by the amino acid sequence in forming tubular assemblies of cyclic β -peptides and δ -peptides. These assemblies exhibit a macrodipole along their central axis, a feature pivotal to their piezoelectric behaviour. Molecular packing intricacies further influence these properties, underscoring the significance of molecular polarity in dictating piezo-electronic behaviour. To augment the piezoelectric properties of our cyclic peptides, we strategically introduced aromatic amino acids into cyclic δ -peptides and compared their piezo-electronic performance with aliphatic amino acid containing cyclic δ -peptides. Additionally, we enhanced molecular polarity by substituting hydrogen atoms with electronegative fluorine atoms in the phenyl rings of phenylalanine. This modification increases the molecule's dipole moment, fostering the creation of a macroscopic dipole moment aligned in a specific direction upon self-assembly of these modified peptides. Our objective in developing these tailored peptides is to explore and contrast their piezo-electronic characteristics relative to their molecular polarity. This endeavour contributes to advancing the field of biomolecular piezoelectric materials, with the potential to uncover novel peptide-based materials endowed with enhanced piezo-electronic functionalities.

3.4. Results and Discussion:

3.4.1. Design and Synthesis of Peptides (C1-C7):

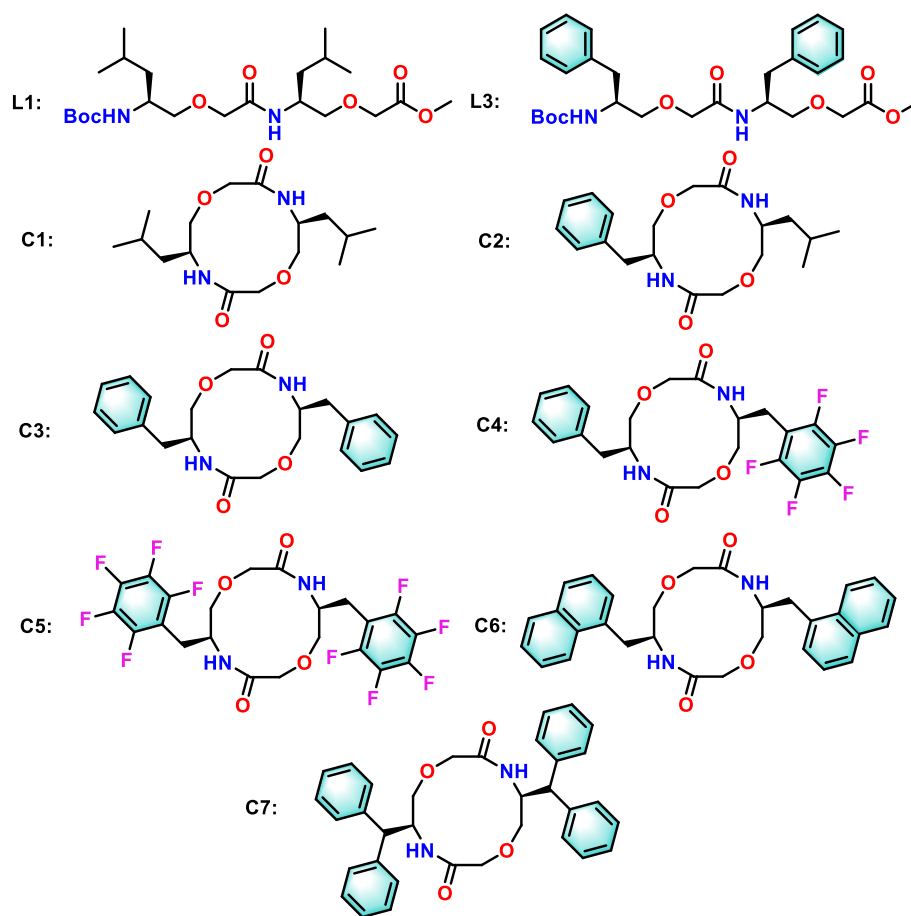
The designed cyclic dipeptides **C1-C7** and linear dipeptides **L1-L3** are depicted in Scheme 1, with detailed descriptions of the amino acids and the synthesis of both linear and cyclic peptides provided in the preceding chapter.^[39] **L1** and **L3** consist of linear dipeptides composed of aliphatic and aromatic amino acids, respectively. **C1** is entirely aliphatic cyclic peptide, whereas **C2** incorporates one aliphatic and one aromatic β -oxy- δ^5 -amino acids containing cyclic peptide. Moving forward, **C3** to **C7** exclusively feature aromatic β -oxy- δ^5 -amino acids containing cyclic peptide. Notably, **C6** and **C7** incorporate Naphthyl and Diphenyl containing aromatic β -oxy- δ^5 -amino acids which are notably more electron-rich.

In the case of **C4**, we introduced a pentafluorophenyl ring containing β -oxy- δ^5 -amino acids acid, whereas **C5** incorporates both pentafluorophenyl ring containing aromatic β -oxy- δ^5 -amino acids.

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The rationale behind this study is to compare the piezoelectronic properties in tubular packing between electron-rich and electron-deficient systems. Additionally, the incorporation of electronegative fluorine atoms is expected to enhance charge separation, thereby increasing the dipole moment. Our aim is to thoroughly examine and compare these properties within tubular-packed systems. This research contributes to advancing our understanding of how molecular electronic properties can be tailored through amino acid sequence and substitution, potentially leading to the development of novel materials with enhanced piezo-electronic functionalities.

Scheme 1:



Scheme 1: Sequence of the Peptides, Linear Dipeptide **L1** and **L3**, Cyclic Dipeptides **C1-C7**.

3.4.2. Self-assembly of Cyclic Peptides:

In the previous chapter, we have shown that these cyclic peptides can self-assemble parallelly packed tubular structure. Different non-covalent interaction like hydrogen bonding, polar-pi

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interactions, electrostatic interactions etc. are involved in the self-assembly of cyclic peptides. In this section we discussed all the interaction involved in crystal packing.

3.4.2.1. Interactions involved in Tubular Self-assembly of C3:

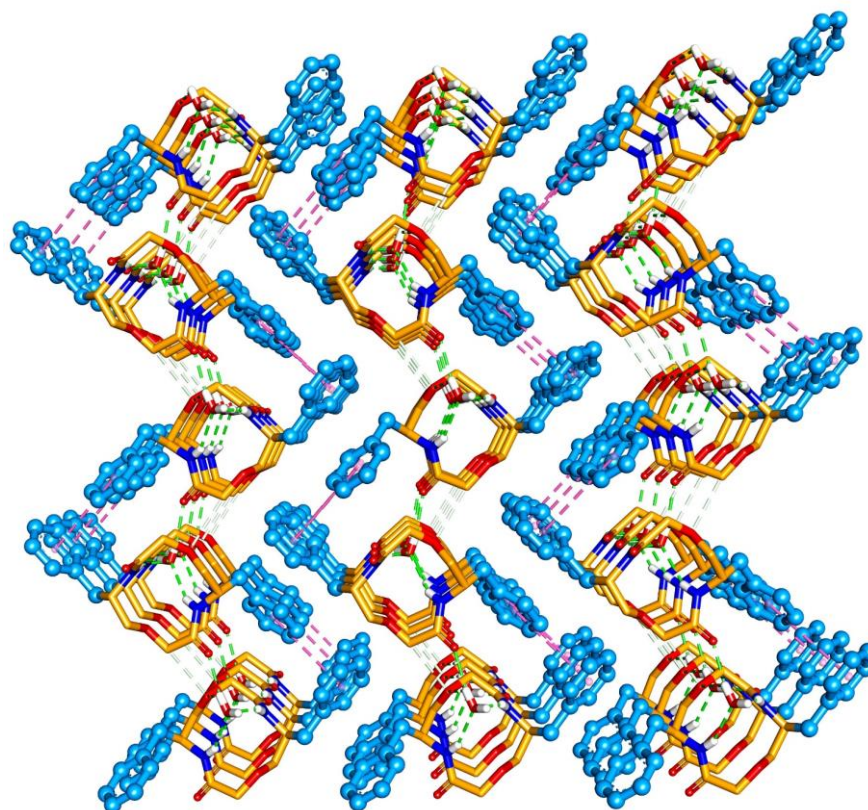


Figure 1: Overall Packing of tubular self-assembled C3 molecules and their interactions.

The self-assembled structures of C3 display four distinct interaction types, which can be categorized as follows:

- (i).... Intermolecular Hydrogen Bonding between Water molecule and amide unit ($\text{H}_2\text{O} \cdots \text{HN-CO}$) and ($\text{HOH} \cdots \text{OC-NH}$).
- (ii).... Intermolecular Hydrogen Bonding between Water molecule and β -O atom ($\text{HOH} \cdots \text{O}_\beta$).
- (iii).... Pi-Pi Stacking Interaction.
- (iv).... Intermolecular Hydrogen bonding between two macrocycles ($\text{C}_\alpha\text{H} \cdots \text{O}_\beta$).

3.4.2.2. Interactions involved in Tubular Self-assembly of C4:

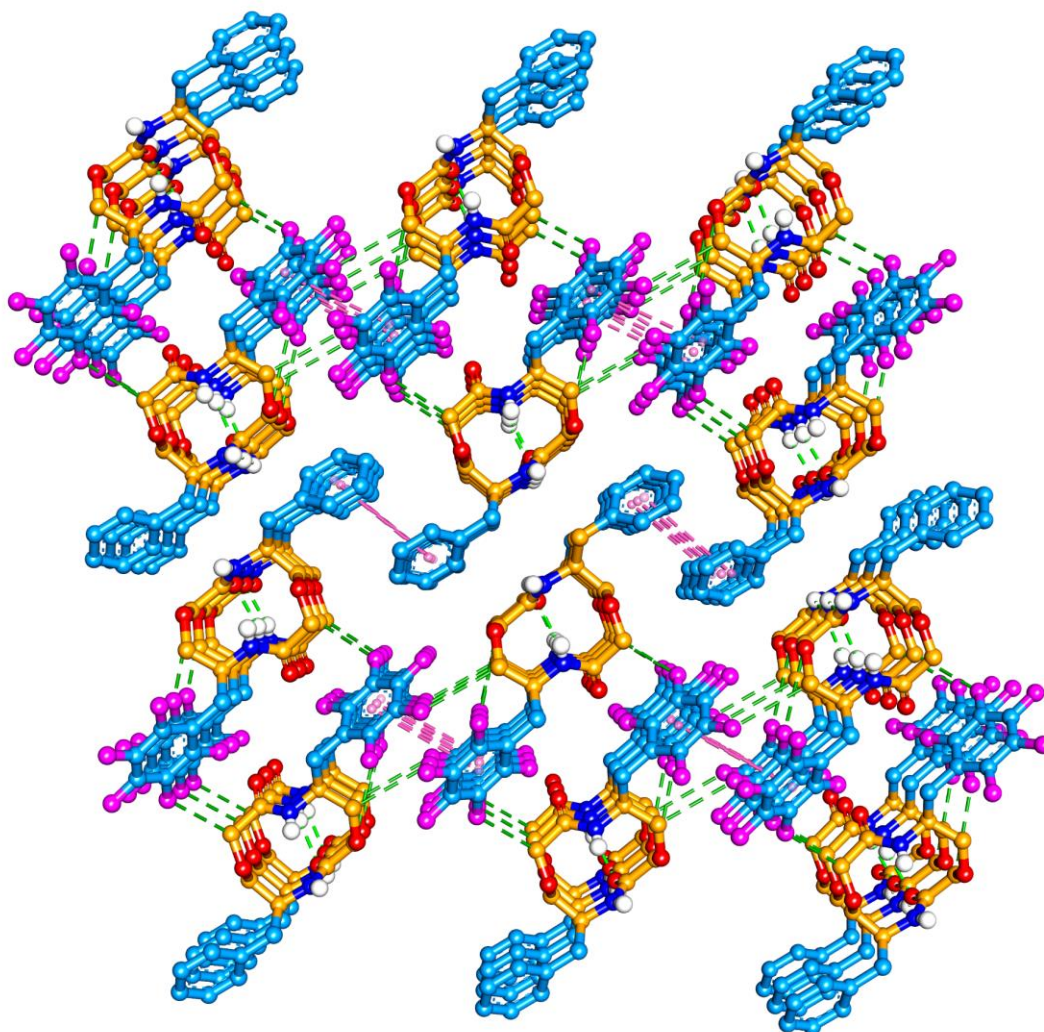


Figure 2: Overall Packing of tubular self-assembled **C4** molecules and their interactions.

The self-assembled structures of **C4** display four distinct interaction types, which can be categorized as follows:

- (i).... Intermolecular Hydrogen Bonding between carbonyl O and amide NH ($\text{CO} \cdots \text{HN}$).
- (ii).... Pi-Pi Stacking Interaction.
- (iii).... Intermolecular Hydrogen bonding between F(ortho) and $\alpha\text{-CH}_2$ unit, ($\text{C}_\alpha\text{H} \cdots \text{F}_{\text{ortho}}$).
- (iv).... Intermolecular Hydrogen bonding between F(meta) and $\alpha\text{-CH}_2$ unit, ($\text{C}_\alpha\text{H} \cdots \text{F}_{\text{ortho}}$) and F(meta) and $\gamma\text{-CH}_2$ unit, ($\text{C}_\gamma\text{H} \cdots \text{F}_{\text{meta}}$)

3.4.2.3. Interactions involved in Tubular Self-assembly of C5:

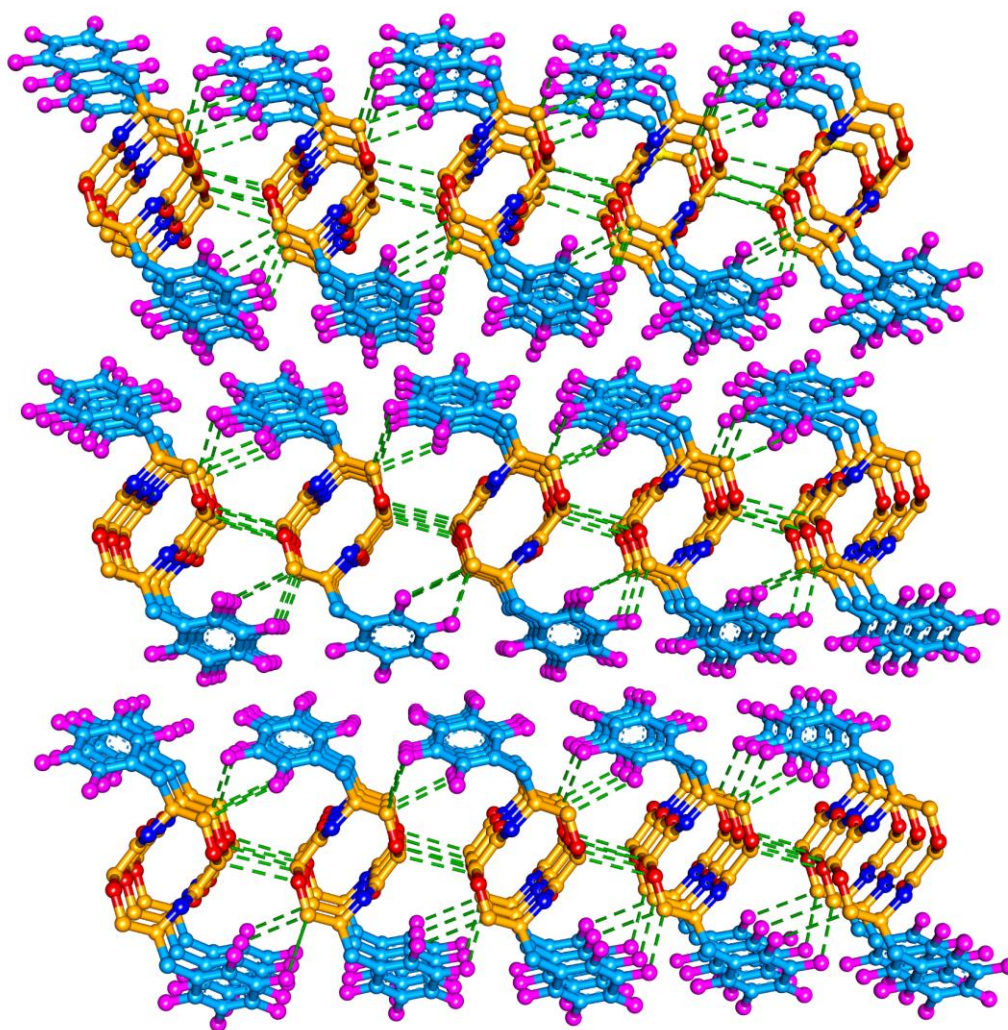


Figure 3: Overall Packing of tubular self-assembled C5 molecules and their interactions.

The self-assembled structures of C5 display four distinct interaction types, which can be categorized as follows:

- (i).... Intermolecular Hydrogen Bonding between carbonyl O and amide NH ($\text{CO} \cdots \text{HN}$).
- (ii).... Intermolecular Hydrogen bonding between F(meta) and $\gamma\text{-CH}_2$ unit, ($\text{C}_\alpha\text{H} \cdots \text{F}_{\text{meta}}$).
- (iii).... Intermolecular Hydrogen bonding between F(ortho) and $\gamma\text{-CH}_2$ unit, ($\text{C}_\gamma\text{H} \cdots \text{F}_{\text{ortho}}$).
- (iv).... Intermolecular Hydrogen bonding between two macrocycles ($\text{C}_\alpha\text{H} \cdots \text{O}_\beta$).

3.4.2.4. Interactions involved in Tubular Self-assembly of C7:

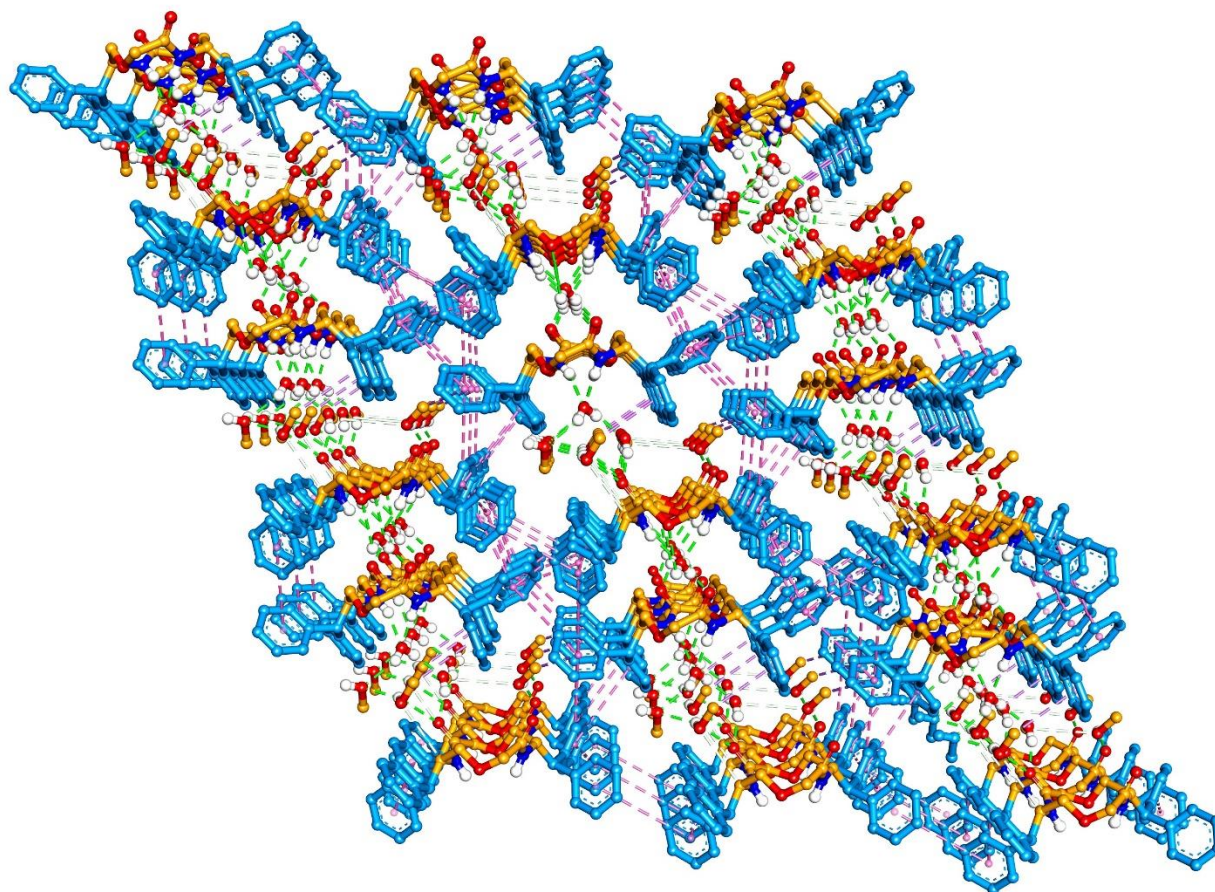


Figure 4: Overall Packing of tubular self-assembled C7 molecules and their interactions.

The self-assembled structures of C7 display four distinct interaction types, which can be categorized as follows:

- (i).... Intermolecular Hydrogen Bonding between Water molecule and amide unit ($\text{H}_2\text{O} \cdots \text{HN-CO}$) and ($\text{HOH} \cdots \text{OC-NH}$).
- (ii).... Intermolecular Hydrogen Bonding between Carbonyl O atom proton of methanol ($\text{CH}_3\text{OH} \cdots \text{O=C}$).
- (iii).... Pi-Pi Stacking and CH-Pi Interaction.
- (iv).... Intermolecular Hydrogen bonding between $\text{C}_8\text{-H}$ and Oxygen atom of methanol ($\text{C}_8\text{H} \cdots \text{O}$).

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3.4.2.5. Other Interaction involved in Self-assembly of C4:

In pentafluoro-benzene ring of **C4**, the fluorine atoms are electron-withdrawing, causing the remaining electron density to be richer around the fluorine atoms and leaving the central region of the benzene ring electron-deficient. This electron deficiency creates a π -hole within the benzene ring. The lone pairs of a carbonyl group can interact with this π -hole, as these lone pair orbitals are directed towards the benzene ring's π orbitals. This interaction between the carbonyl group and the π -hole of the fluorinated aromatic ring is well-documented in the literature and is referred to as an $O\cdots\pi$ interaction.^[40] The interaction is illustrated in below figure.

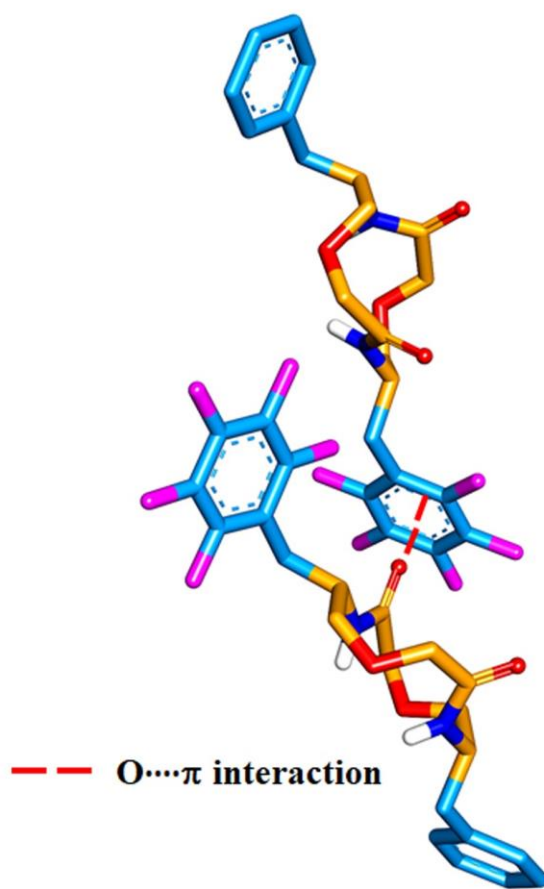


Figure 5: $O\cdots\pi$ interaction in Self-assembly of **C4**

3.4.2.6. Hirshfeld surface analysis for Self-assembled CPs Interactions:

Hirshfeld surface analysis^[41] was conducted to investigate all non-covalent molecular interactions of **C3**, **C4**, **C5** and **C7** using Crystal Explorer 3.1 software. The analysis utilized the single-crystal

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X-ray crystallographic information file (CIF) to visualize various interactions present on the Hirshfeld surface. These interactions were depicted through 3D color mapping images, including normalized contact distance (d_{norm}), shape index, and curvedness. The surface colors representing strong interactions were depicted in red, medium in blue, and weak in white. The shape index, a dimensionless quantity, indicated the local shape of convex and concave regions on the surface, with values ranging between 1.0 (most convex) and -1.0 (most concave), while values were fixed at 0 for minimal surface regions. Curvedness measured the degree of curvature of the Hirshfeld surface, with a general range of -4.0 to 0.4. Additionally, a 2D fingerprint plot was generated, illustrating the distances of atoms nearest to the interior (d_i) and exterior (d_e) of the Hirshfeld surface. Different contours in the plot depicted various types of molecular interactions and contact information. The Hirshfeld surface images were generated with an iso-value of a weight function of 0.5 au. Summary of the observed Hirshfeld analysis data is presented in below figures.

(i) C3:

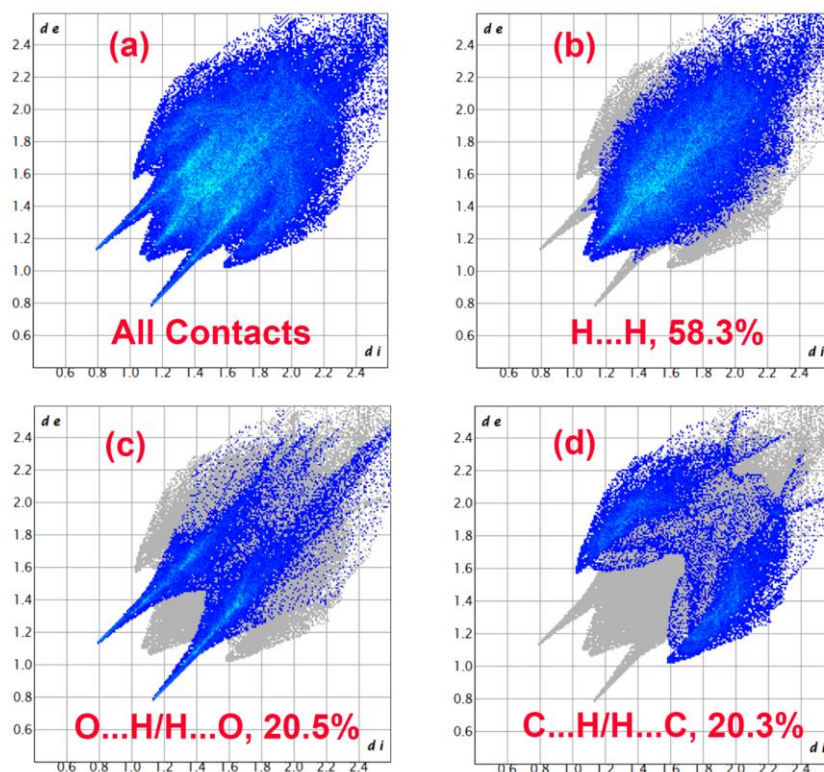


Figure 6: (a-d) The 2D fingerprint plot (d_e vs d_i) of C3 at 296 K.

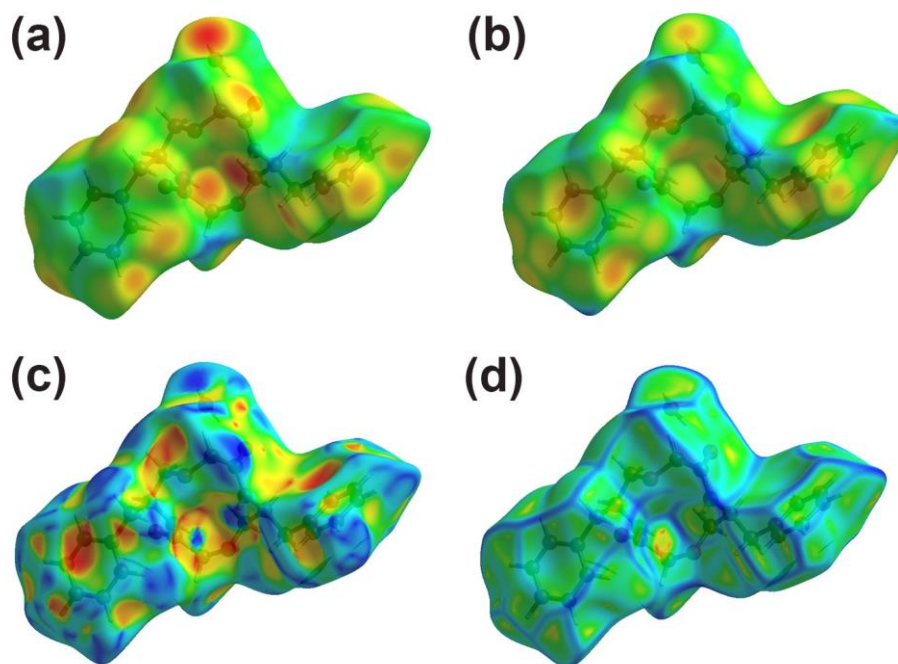


Figure 7. The 3D color mapping Hirshfeld surface analysis of **C3** (296 K) shows (a) d_i , (b) d_e , (c) shape index and (d) curvedness.

(ii) **C4:**

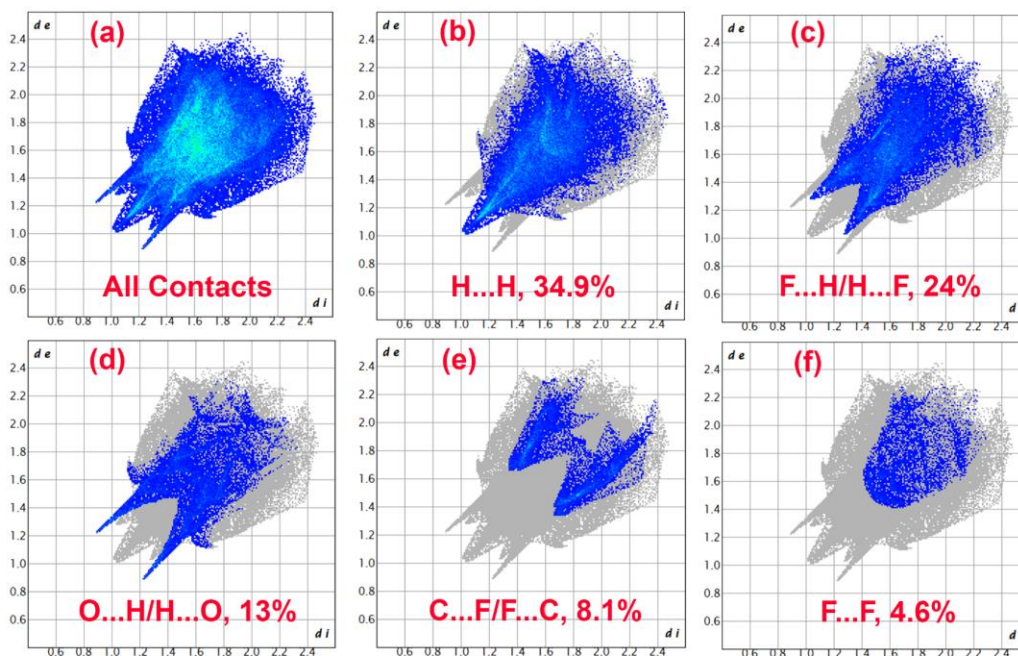


Figure 8: (a) The 2D fingerprint plot (d_e vs d_i) of **C4** at 296 K (a-f).

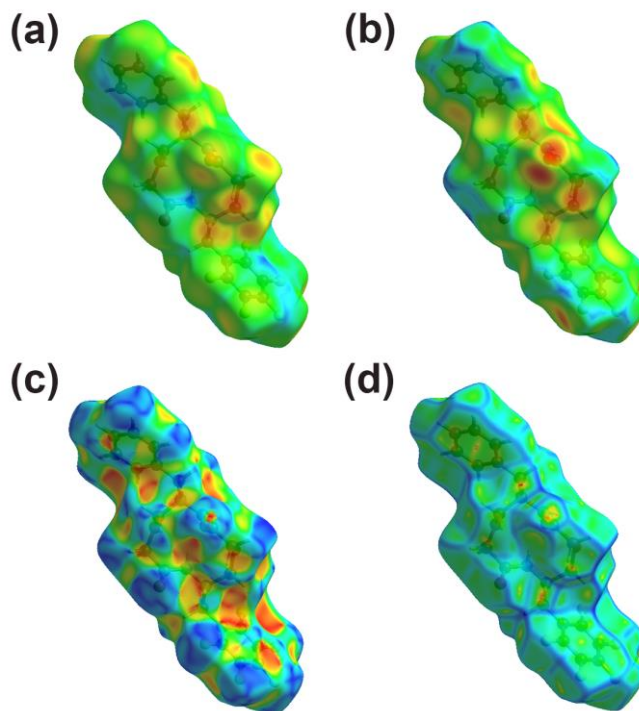


Figure 9: The 3D color mapping Hirshfeld surface analysis of **C4** (296 K) shows (a) d_i , (b) d_e , (c) shape index and (d) curvedness.

(iii) **C5:**

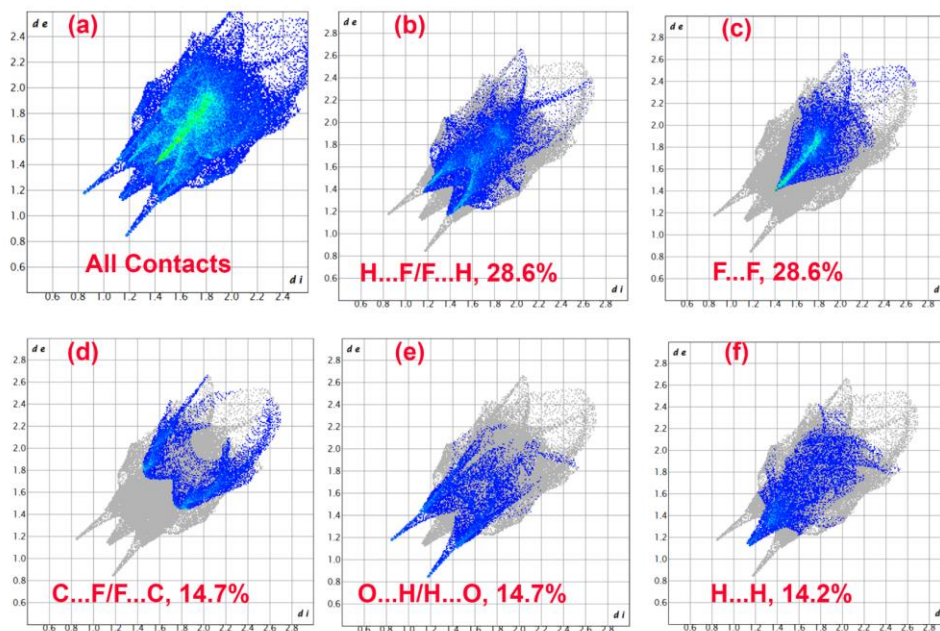


Figure 10: (a-f) The 2D fingerprint plot (d_e vs d_i) of **C5** at 296 K

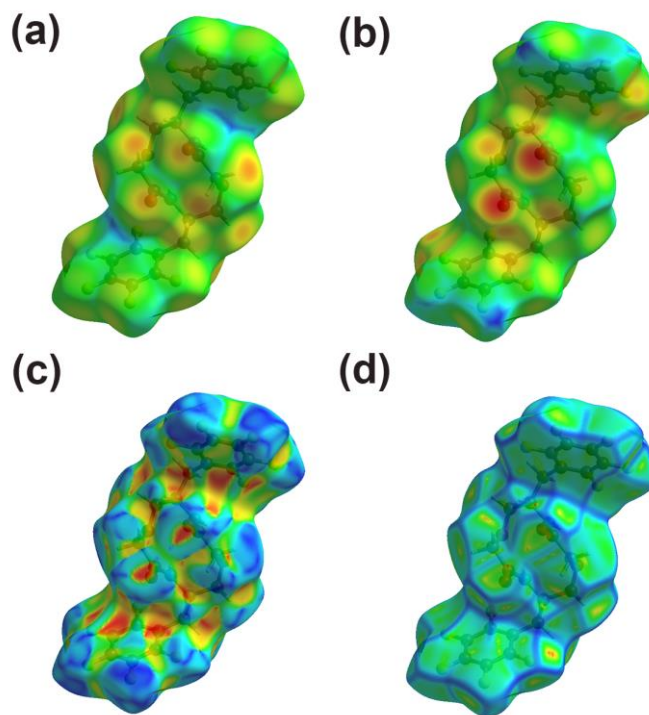


Figure 11. The 3D color mapping Hirshfeld surface analysis of **C5** (296 K) shows (a) d_i , (b) d_e , (c) shape index and (d) curvedness.

(iii) **C7:**

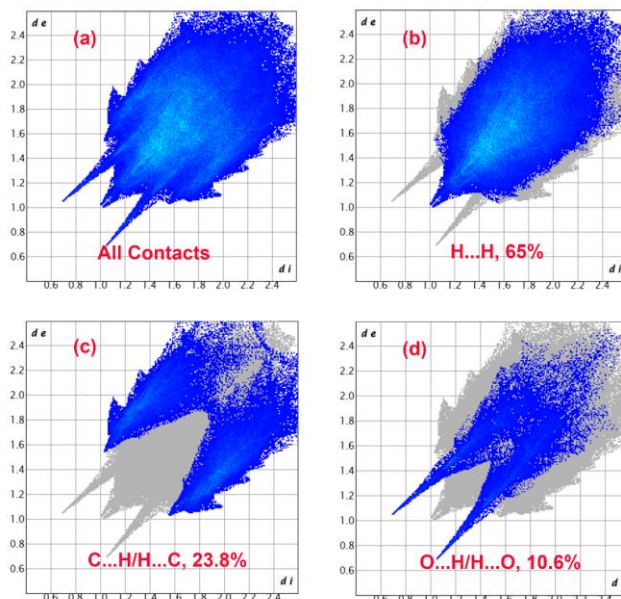


Figure 12: (a) The 2D fingerprint plot (d_e vs d_i) of **C7** at 296 K (a-f).

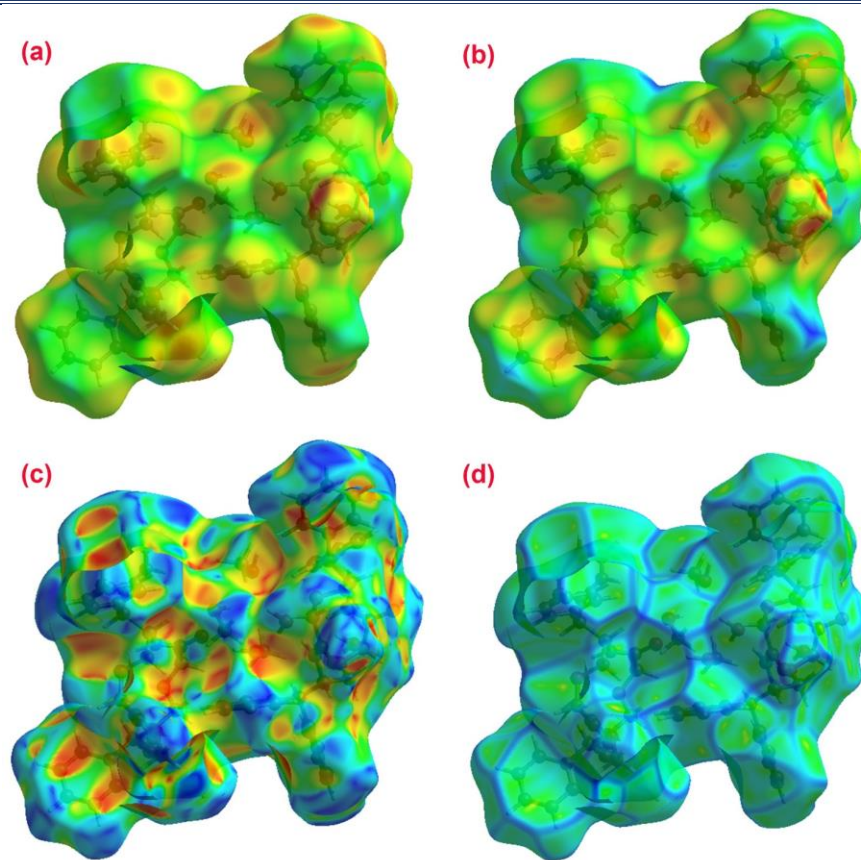


Figure 13. The 3D color mapping Hirshfeld surface analysis of **C7** (296 K) shows (a) d_i , (b) d_e , (c) shape index and (d) curvedness.

3.4.2.7. FE-SEM and HR-TEM Analysis for Self-assembled CPs Interactions:

Further, to understand the morphology of the self-assembled cyclic peptides, we subjected some of them to FE-SEM and HR-TEM analysis. The solutions of cyclic peptides **C3** to **C5** in methanol/water (1 mg/ml) were incubated and examined for their self-assembly. Both FE-SEM and HR-TEM analysis revealed a sheet-shaped supramolecular assembly. The FE-SEM and HR-TEM images are shown in Figure 14 below. Lateral interaction of the peptide nanostructures may also occur due to electrostatic and hydrogen bonding interactions mentioned above. Interaction of the peptides in both vertical and lateral direction helps to build long-range molecular self-assembled nano-structure, that eventually observed as sheet-shaped or plate shaped nanostructure in FE-SEM images. These secondary interactions resulted in the formation of a beta sheet type structure which stacks through intermolecular hydrogen bonding and other electrostatic interactions, that will also help in elongating peptide nanostructures.

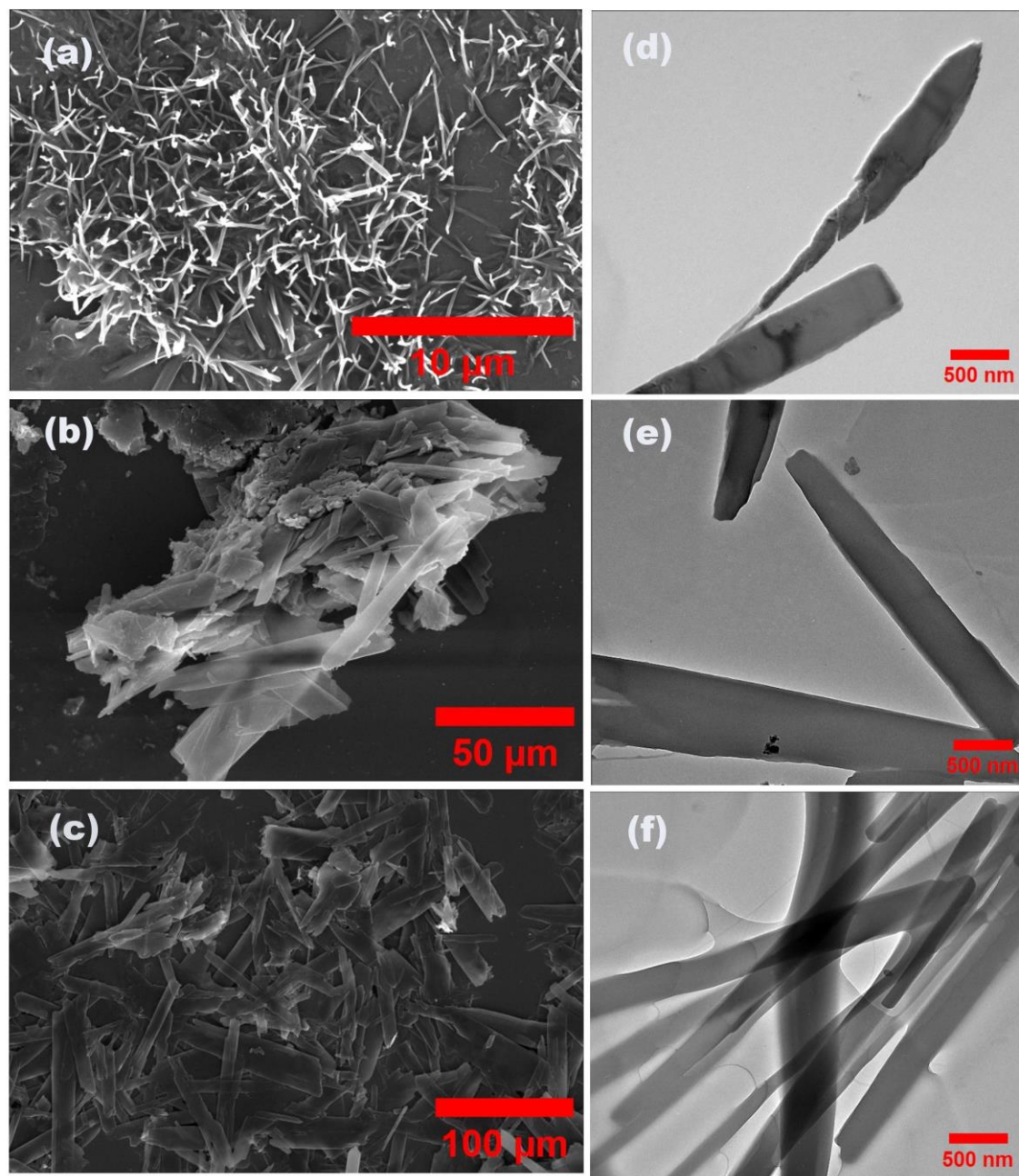


Figure 14. FE-SEM images of (a) C3, (b) C4, and (c) C5 and HR-TEM images (d) C3, (e) C4, and (f) C5 after 24 hours incubation in MeOH/H₂O her

3.4.2.8. DLS Study for Self-assembled CPs Interactions:

To understand whether these cyclic peptides can self-assemble in solution, we subjected them to dynamic light scattering (DLS) analysis in polar aprotic acetonitrile and chloroform. The DLS analysis of equimolar concentration of each cyclic peptide suggested that they self-assembled into

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approximately 100 nm aggregates after being kept in solution for about an hour. The DLS spectrum of **C3**, **C4** and **C5** cyclic peptides are attached in below Figure 17. In contrast, no significant self-aggregation was observed in polar protic solvent methanol. These results further support the H-bond driven supramolecular assembly of cyclic peptides. In the case of methanol it interferes in H-bonding between the cyclic peptides (Figure 15 and 16). The order of observed aggregation in apolar solvent was found to be **C5** > **C4** > **C3**.

(a) Solvent Dependent DLS Experiment:

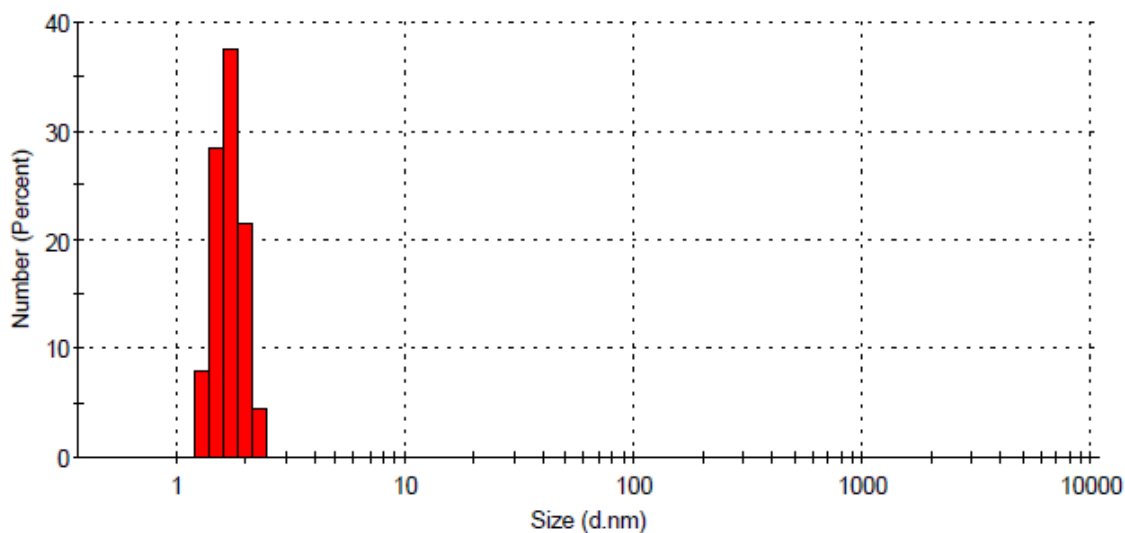


Figure 15: DLS study Of **C3** in Methanol

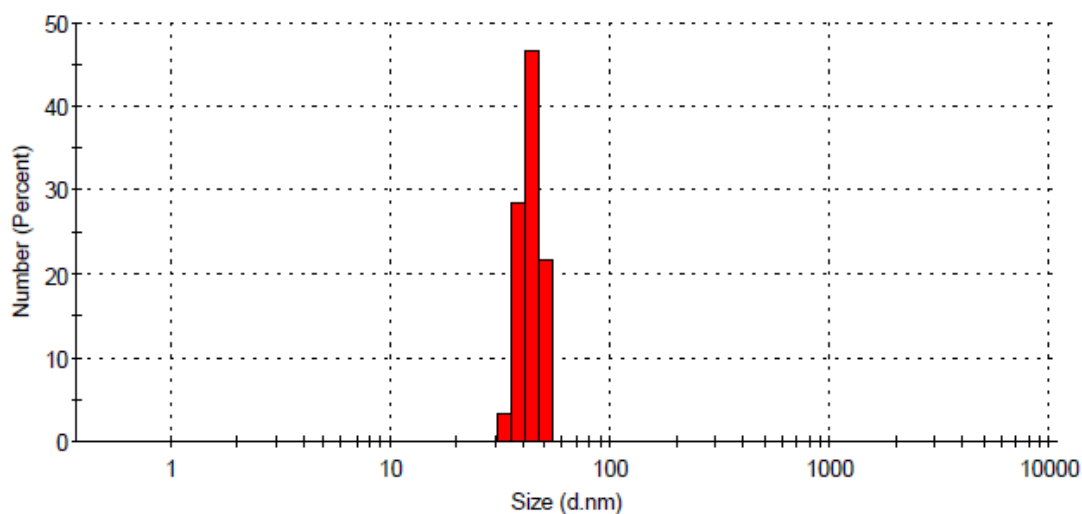


Figure 16: DLS study Of **C3** in Acetonitrile

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(b) DLS Study of Cyclic Peptides in Acetonitrile:

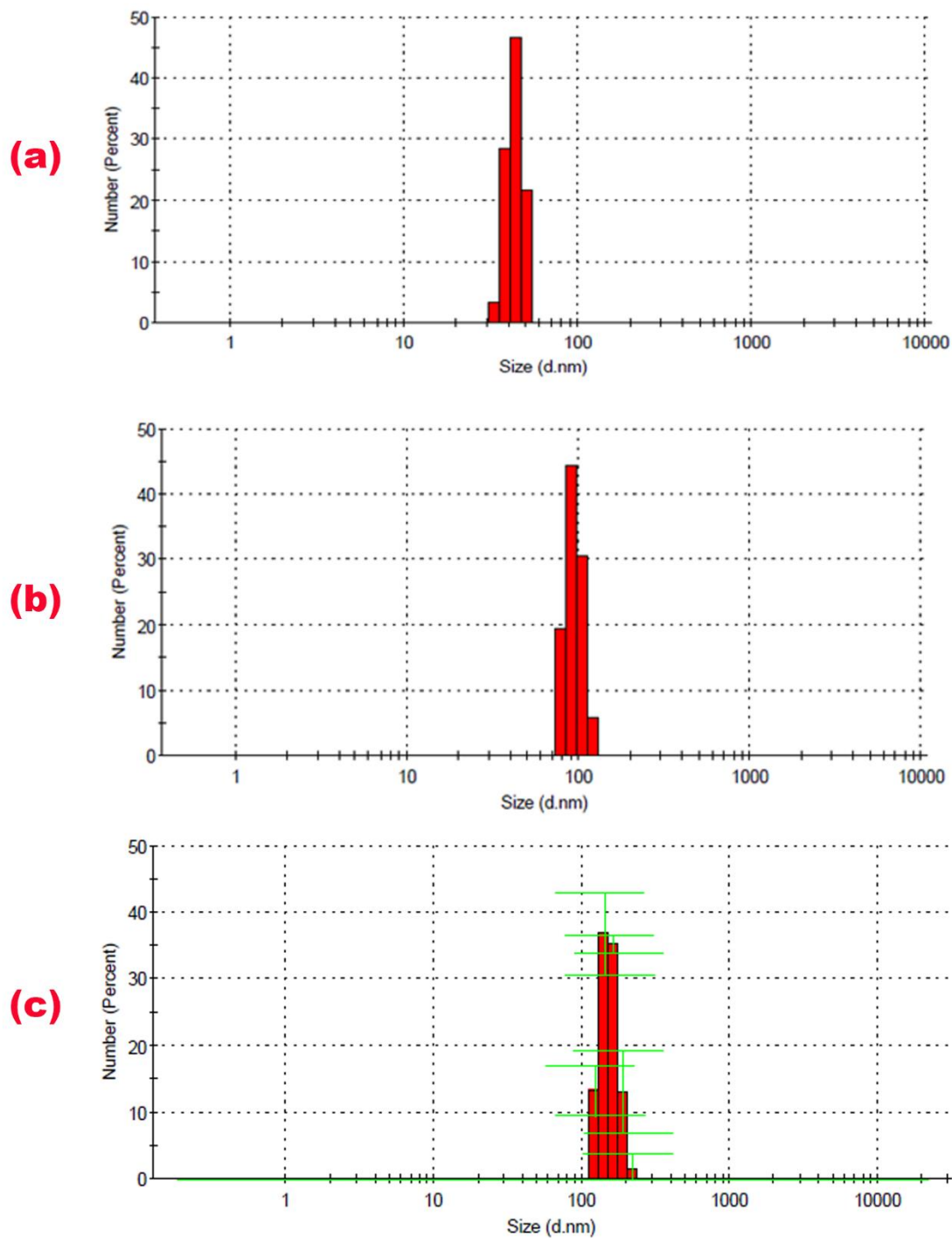


Figure 17: DLS study of (a) C3, (b) C4, and (c) C5 in Acetonitrile.

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3.4.2.9. UV-Vis Spectrum and Circular Dichroism Experiment:

(a). UV-Vis Spectrum:

To determine if there is any excitonic coupling between the aromatic side chains of three cyclic peptides, we recorded their concentration-dependent UV spectra. The experimental results show no shift in the characteristic peaks, raising the question of whether excitonic coupling is present between the chromophores. Vibrational features in the 240-280 nm region, likely corresponding to C-H vibrations of the peripheral benzene ring, are most prominent in **C3**, intermediate in **C4**, and absent in **C5**.

The concentration-dependent UV spectra for all three compounds are attached below.

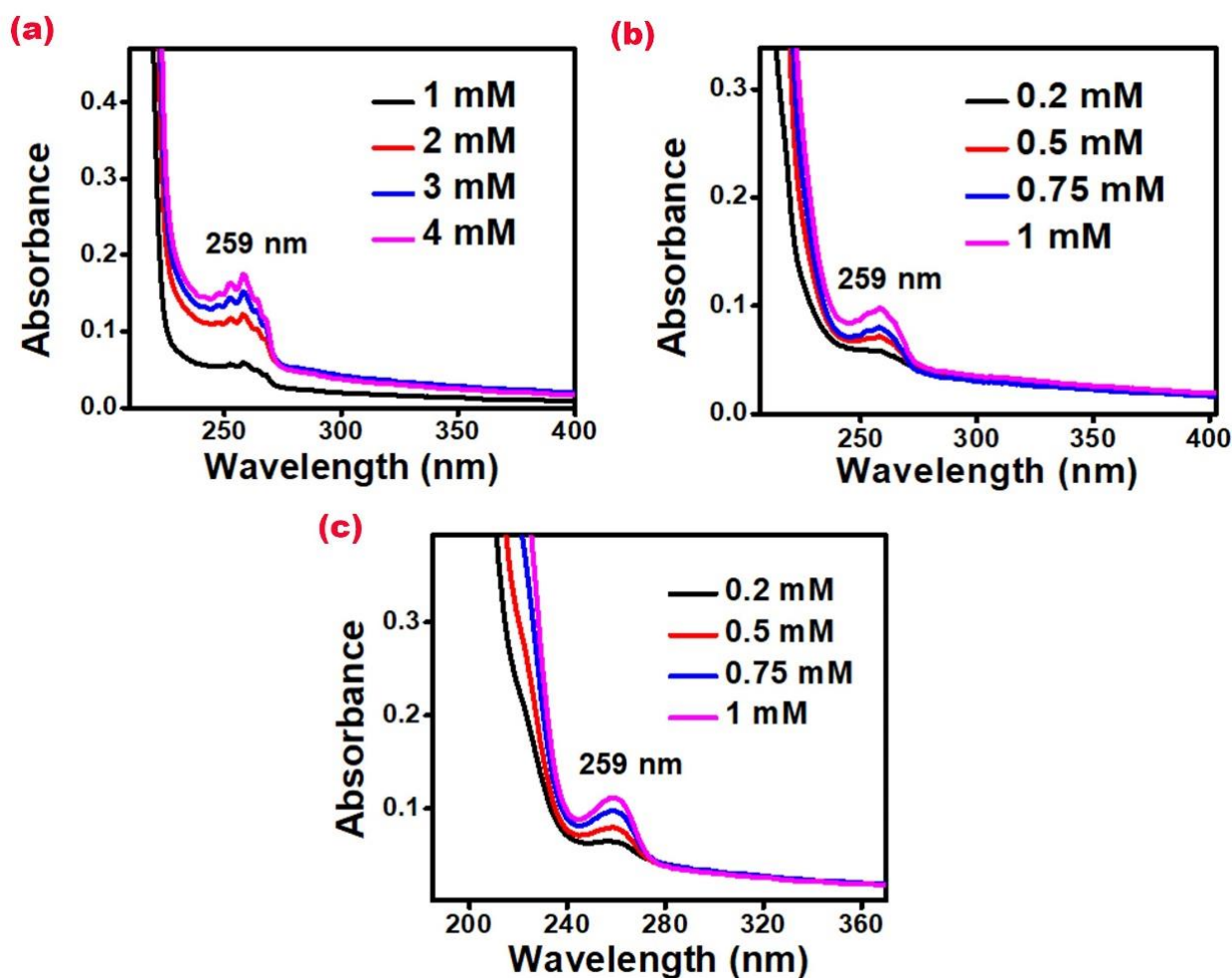


Figure 18: Concentration dependent UV-Vis Spectrum of (a) **C3**, (b) **C4**, and (c) **C5** in Acetonitrile.

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(b). Circular Dichroism Spectrum:

Further we have recorded circular dichroism experiment of all three compounds. The CD spectra of all three compounds reveal similar characteristic peaks: two negative peaks at 198 nm and 222 nm, attributed to $\pi\text{---}\pi^*$ transition interactions and $n\text{---}\pi^*$ interactions, respectively, and a broad positive peak at 246 nm, indicative of aromatic side chains.^[42] We've also recorded concentration-dependent CD spectra for these cyclic peptides, observing that there's no shift in the characteristic peaks, only a change in intensity. These experiments suggest that there is no such significant excitonic coupling between these two pentafluorophenyl rings. Concentration Dependent CD Spectrum of **C3**, **C4**, and **C5** in Acetonitrile are attached below.

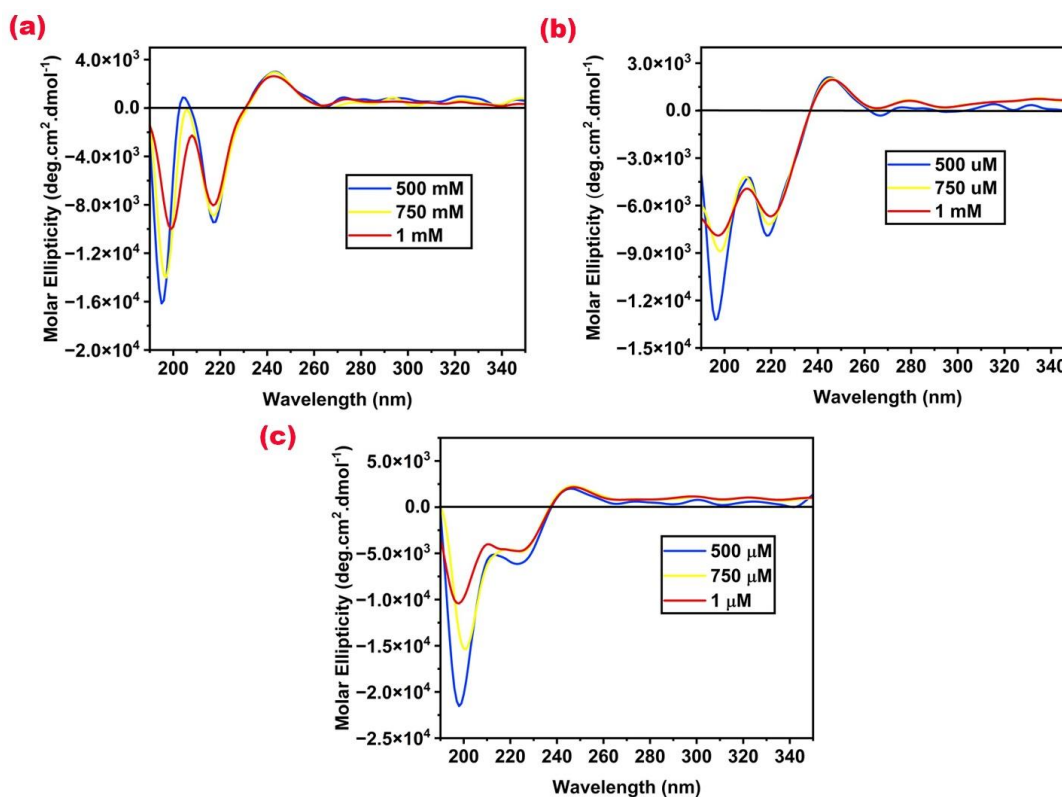


Figure 19: Concentration dependent CD Spectrum of (a) **C3**, (b) **C4**, and (c) **C5** in Acetonitrile.

These two experiments suggest that self-assembly in tubular packing is primarily governed by intermolecular hydrogen bonding. Consequently, there is no observed significant exciton coupling between the penta-fluorophenyl rings. In case **C3**, the inter-distance between two phenyl rings is 6.769 Å, whereas in **C4**, this distance is maintained at 5.217 Å, consistent with the inter-distance between the phenyl rings and pentafluorophenyl rings. **C5** exhibits a shorter inter-distance between

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the pentafluorophenyl rings, measuring 5.145 Å. These intermolecular distances indicate a far proximity of the aromatic rings, suggesting very weak pi-pi stacking interactions.

Furthermore, the average distance between cyclic peptide units decreases from **C3** (6.75 Å) to **C4** (5.22 Å) to **C5** (3.5 Å), indicating a more compact molecular arrangement in **C5**, likely driven by increased intermolecular hydrogen bonding. This suggests that self-assembly in tubular packing is primarily governed by intermolecular hydrogen bonding.

3.4.2.10. IR Experiment:

We have recorded the IR Spectrum of three cyclic peptides **C3**, **C4**, and **C5**. The IR studies showed N–H stretching frequencies in the region at 3200-3300 cm^{-1} indicating involvement of amide N–H protons in strong intermolecular H-bonding. The C=O stretching frequencies in the amide I region at 1650-1662 cm^{-1} and in the amide II region at 1501-1503 cm^{-1} are indicative of similarity with the β -sheet type of framework. The IR Spectrum of **C3**, **C4**, and **C5** are attached below.

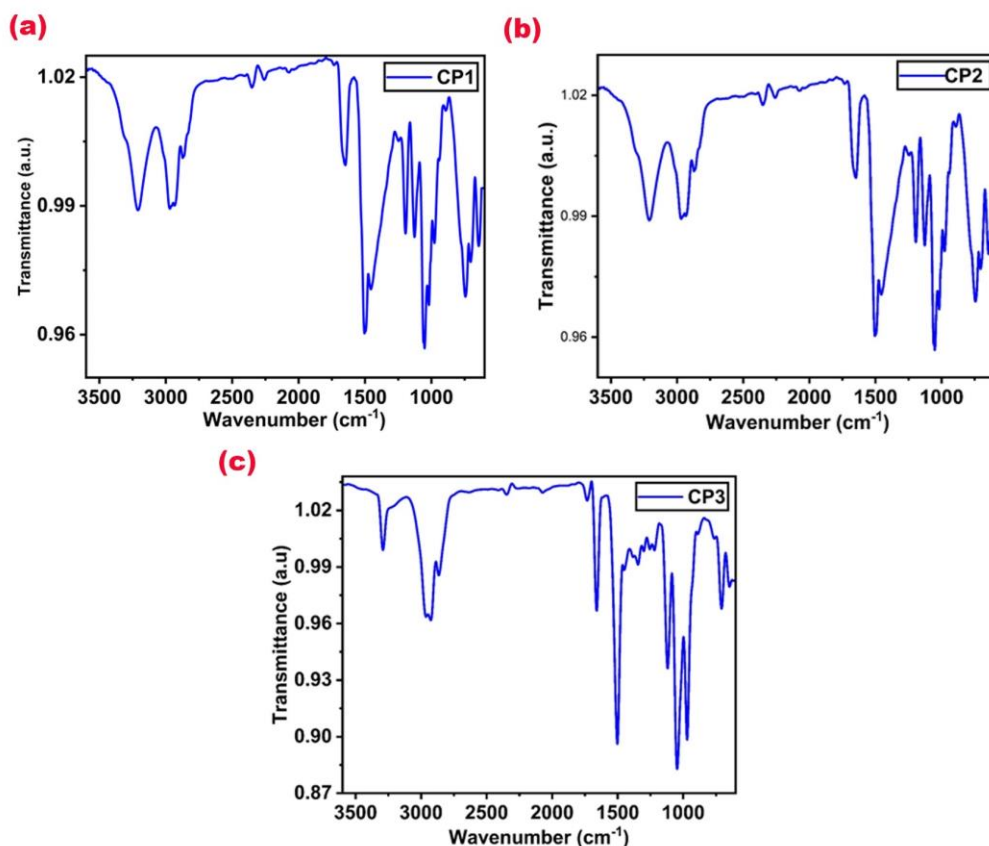


Figure 20: IR Spectrum of (a) **C3**, (b) **C4**, and (c) **C5**.

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3.4.3. Piezoresponse Force Microscopy Characterization:

The tubular assembly of the noncentrosymmetric cyclic peptides with unidirectional H-bond-associated macrodipole motivated us to examine the piezoelectric and ferroelectric properties of these cyclic peptides using piezoresponse force microscopy (PFM).^[43] Peptides at a concentration of 5 millimolar were used for drop-casting onto an ITO plate. The thickness of the resulting thin film was measured using PFM (Piezoelectric Force Microscopy) and was found to be between 0.1 μm and 1 μm (see Experimental section). The relative piezoelectric coefficients were quantified in all cyclic peptides using PFM. The PFM measurements enabled the direct visualization of the domain structure and orientation in vertical directions. PFM images were captured at a resonance frequency of 300 ± 40 KHz, with PFM hysteresis obtained under an applied bias of ± 25 V. Figure 21 and Figure 22 depict the vertical PFM amplitude and phase signals of three cyclic peptides drop-casted thin films. It is clear that the ferroelectric domain structures appear to be arranged in a strip-like pattern, with domain walls separating adjacent domains, as indicated by the minimum amplitude signal.^[44] The phase patterns align with the corresponding amplitude images, showing that the domains are oriented in upward and downward directions. Further, to understand the ferroelectric domains in the drop-casted cyclic peptides thin films, piezoresponse force spectroscopy was conducted at a single point ($10 \times 10 \mu\text{m}^2$), using Dual AC Resonance Tracking (DART) mode PFM. The distinctive phase hysteresis and a butterfly-shaped amplitude loop for **CPs** obtained from the PFM measurements are shown in Figure 23. The near 180° phase difference between opposite signals confirmed the inverse switching polarization behavior. These results confirm the reversible ferroelectric domain structure of the **CPs** thin films. While **L1** and **C1**, composed of aliphatic amino acids in their linear and cyclic peptide forms respectively, did not exhibit a significant piezoelectric response, both Linear Aromatic **L3** and aromatic cyclic peptides **C2-C7** displayed distinct PFM hysteresis characteristics. We further calculated the piezoelectric coefficients (d_{33}) for all cyclic peptides (See experimental section).^[45] The calculated d_{33} values for **L3**, **C2**, **C3**, **C4**, **C5**, **C6**, and **C7** were found to be 50 ± 1 pm/V, 53 ± 1 pm/V, 60 ± 4 pm/V, 64 ± 4 pm/V, and 76 ± 6 pm/V, 85 ± 2 pm/V, 90 ± 3 pm/V respectively. These values were found to be higher than the values reported for many linear and cyclic peptides. Among these peptides, **C7**, which features diphenyl side chains, demonstrated the highest piezoelectric coefficient. This suggests that as aromaticity in tubular packing increases, the piezoelectric properties are enhanced.

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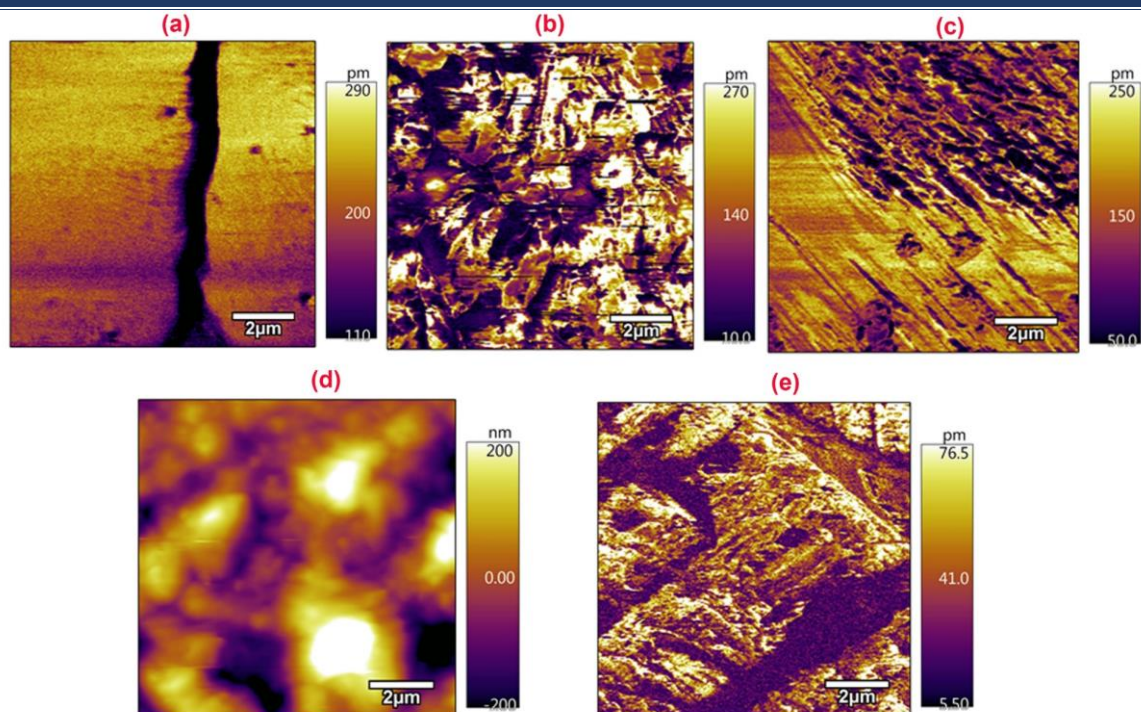


Figure 21: Diagram representing PFM images showing 3D-vertical amplitude of (a) C3, (b) C4, (c) C5, (d) C6, (e) C7.

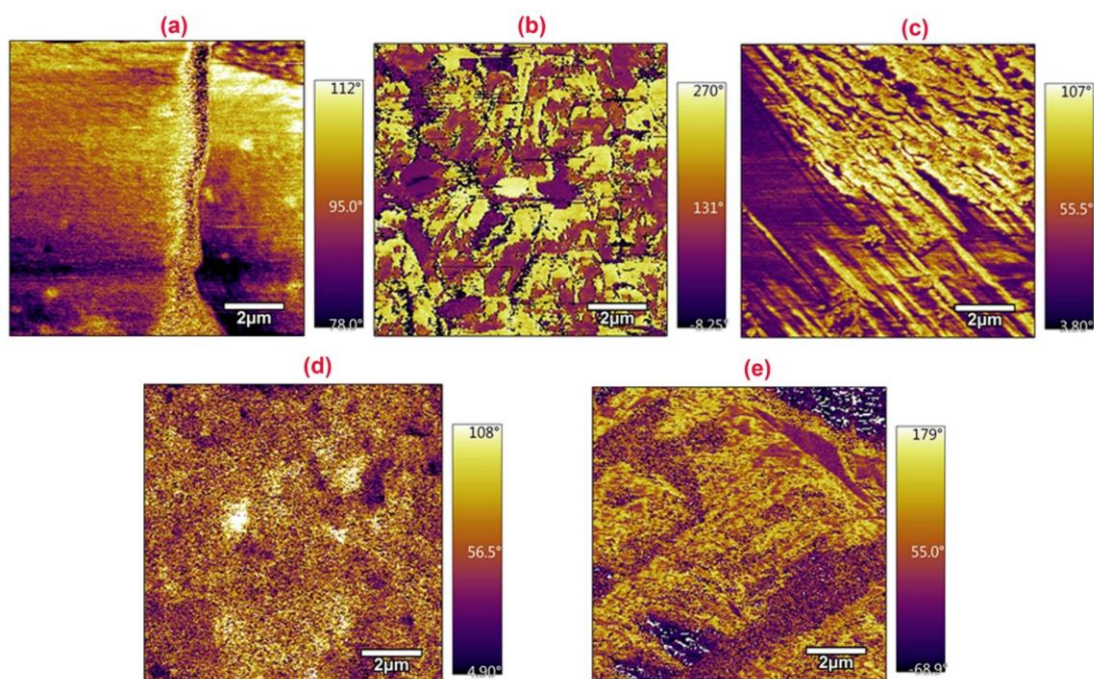


Figure 22: Diagram representing PFM images showing 3D-vertical phase of (a) C3, (b) C4, (c) C5, (d) C6, (e) C7.

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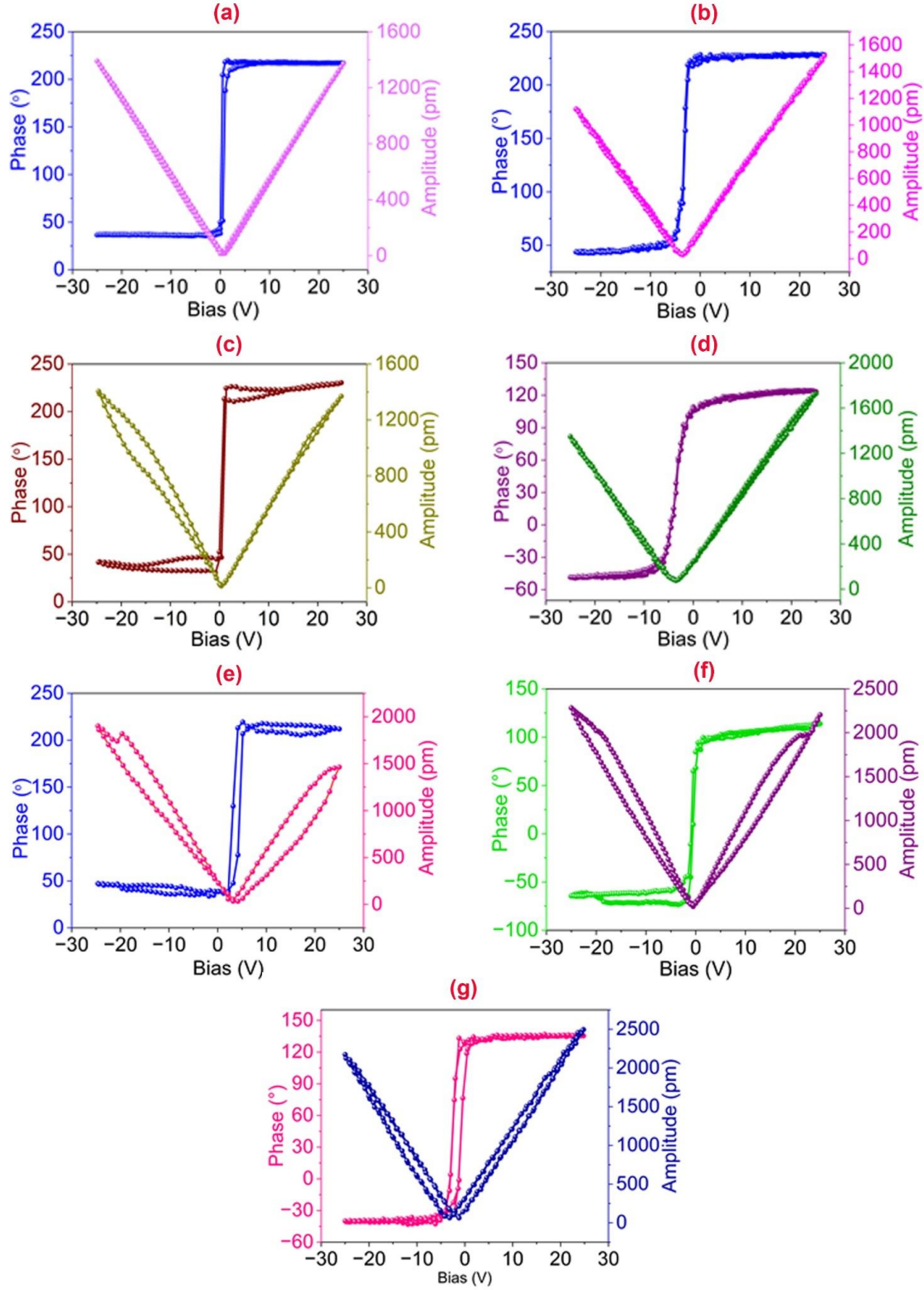


Figure 23: Local PFM phase versus bias and amplitude versus bias hysteresis loop for a selected point on the thin film of (a) L3, (b) C2, (c) C3, (d) C4, (e) C5, (f) C6, (g) C7.

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3.4.4. DFT Calculations for measurement of Dipole Moment:

Among these peptides, **C7**, which features diphenyl side chains, demonstrated the highest piezoelectric coefficient, indicating that increased aromaticity in tubular packing enhances piezoelectric properties. However, we also observed that incorporating an electronegative fluorine atom as a pentafluorophenyl ring in **C4** and **C5** resulted in a higher piezoelectric coefficient compared to **C3**. To understand this phenomenon, we conducted Density Functional Theory (DFT) calculations to analyze the impact of these modifications.^[46] We compared DFT calculated dipole moment data for these three cyclic peptides **C3-C5** and details discussion are below.

At first, we calculated dipole moments of the individual monomers and their comparison with the observed d_{33} values. To provide a more accurate assessment and comment on the macrodipole component contributing to d_{33} , we conducted DFT calculations not only on a single monomeric species, but on an ensemble of molecules, and compared their vectorial distribution. For this purpose, one molecule was selected from the crystal packing arrangements for each of **C3**, **C4**, and **C5**. Additionally, four molecules (arranged in two columns and two rows) and six molecules (arranged in three columns and two rows) were also chosen (Figure 24). The magnitude and direction of dipole moments for each system are shown in Figure 24 and Table 1. Notably, in all three systems, as the number of molecules increases, the magnitude of the dipole moment also increases. However, the magnitude of the increment is highest in **C5** (6.27 folds), followed by **C4** (5.27 folds) and **C3** (4.75 folds) (Figure 24 & Table 1). This indicates that, upon aggregation, the magnitude of the dipole moment experiences a significant boost, resulting in much higher d_{33} values in these systems. In **C5**, this increment is the highest, supporting the fact that **C5** exhibits the maximum d_{33} value.

System	Single molecule	Four molecules	Six molecules
C3	2.86 D	5.72 D	13.59 D
C4	4.01 D	14.94 D	21.13 D
C5	5.76 D	25.06 D	36.10 D

Table 1: Magnitude of dipole-moment vectors of all three molecules in monomer and aggregated systems.

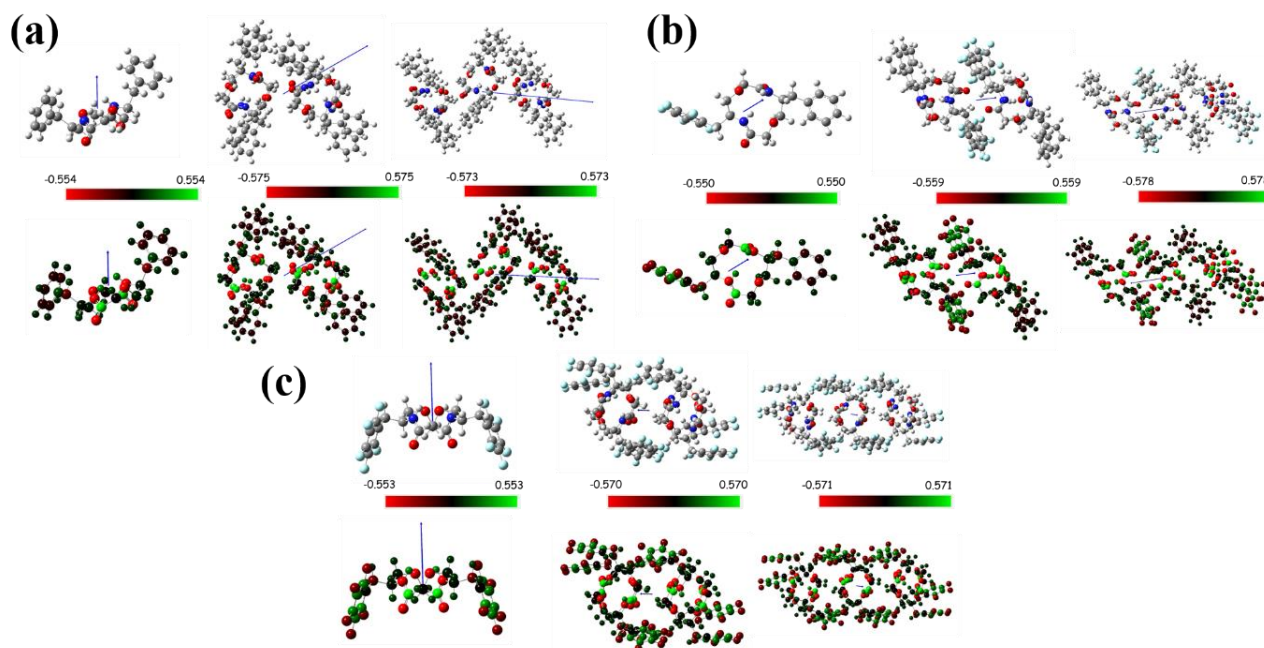


Figure 24: Dipole-moment vector direction (shown as arrows) and Mulliken atomic charge maps for (a) **C3**, (b) **C4**, and (c) **C5** for single molecule, four molecules, and six molecules. obtained from DFT optimization with B3LYP functional and 6-31 G(d,p) basis set.

To understand the observed trend mentioned above, we also analysed the Mulliken atomic charge map of our molecular systems. In this map, the green color represents positive atomic charge, while the red color represents a negative atomic charge. It is important to note that a higher atomic charge will have a greater impact on the total observed dipole moment, while neutral atoms will have a minimal impact. In **C3**, both in the monomer and higher molecular systems, the dipole moment will mainly be contributed by the core ring. In **C4**, the contribution from one fluoro-substituted peripheral benzene ring will also occur. Interestingly, for **C5**, this contribution will be the highest, as it contains highly electronegative fluoro-substituted benzene rings from both ends (Figure 24). Another factor that will come into the picture is the direction factor- which will be mainly governed by the components of the dipole-moment vector along three directions. If we consider the monomers of all three molecules, the **C3** monomer adopts an almost symmetrical arrangement (computed dihedral angle of the peripheral benzene rings are 103.98° & 104.05° with the core ring), resulting in an almost vertical dipole-moment vector direction with the core ring

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and its magnitude are distributed well in three different directions (Figure 25). In case of, the **C4** monomer, although the magnitude of dipole moment is higher comparatively due to the additional pentafluoro-substituted benzene ring, its asymmetrical molecular arrangement (computed dihedral angle of the rings are 78.80° & 85.64° with the core ring) leads to a much tilted dipole-vector direction with its magnitude are again well-distributed in three different directions (Figure 25). Interestingly, both the magnitude and direction factors favour the case of the **C5** molecule, as it possesses two strongly contributing pentafluoro-substituted rings and a symmetrical molecular arrangement (computed dihedral angle of both the rings is 102.38° with the core ring), resulting in the vertical-upward directed dipole-moment vector with the core-ring and its magnitude prevailing in a single direction (dipole moment magnitude= 5.76 D; direction-wise x-component= 0, y-component= 0, z-component= -5.76) (Figure 25). The same principle applies to aggregated systems as well. For example, when we consider systems with six molecules, unlike the **C3** & **C4** case, the dipole moment vector magnitude in the case of **C5** is again oriented in a single direction (dipole moment magnitude = 36.09 D; direction-wise x-component = 0.01, y-component = 36.09, z-component = 0.004), with the direction almost vertically upward with the central ring (Figure 26). We anticipate that as the system size increases, the direction will remain the same while the magnitude continues to increase. However, in the case of **C3** & **C4**, the direction will depend on the crystal packing of the system as well, as in both cases, they exhibit an antiparallel brick-like stacking of neighbouring columns.

Notes on ‘The origin of the macrodipole showing the PFM response’:

We observed that the overall dipole moment magnitudes of the studied systems increase significantly with an increase in packing size (or number of molecules). This is mainly due to the contribution of the core ring. We conducted Mulliken atomic charge mapping of the systems, which indicated a higher positive charge on the amide-carbonyl carbon and a higher negative charge on the oxygen atoms and amide nitrogen atoms. In **C3**, the presence of two essentially neutral benzene rings means that the total contribution comes from the core ring only. In **C4**, a pentafluoro-substituted benzene ring contributes to one end; in **C5**, this contribution is from both ends. The antiparallel brick-like crystal packing in the case of **C3** & **C4** also contributes to the total dipole moment magnitude when considering the macro-dipole moment. However, along with the additional contribution from one extra pentafluoro-substituted benzene ring, its dipole-moment

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magnitude along a specific direction denies any vectorial cancellation, leading to the highest dipole-moment magnitude in C5.

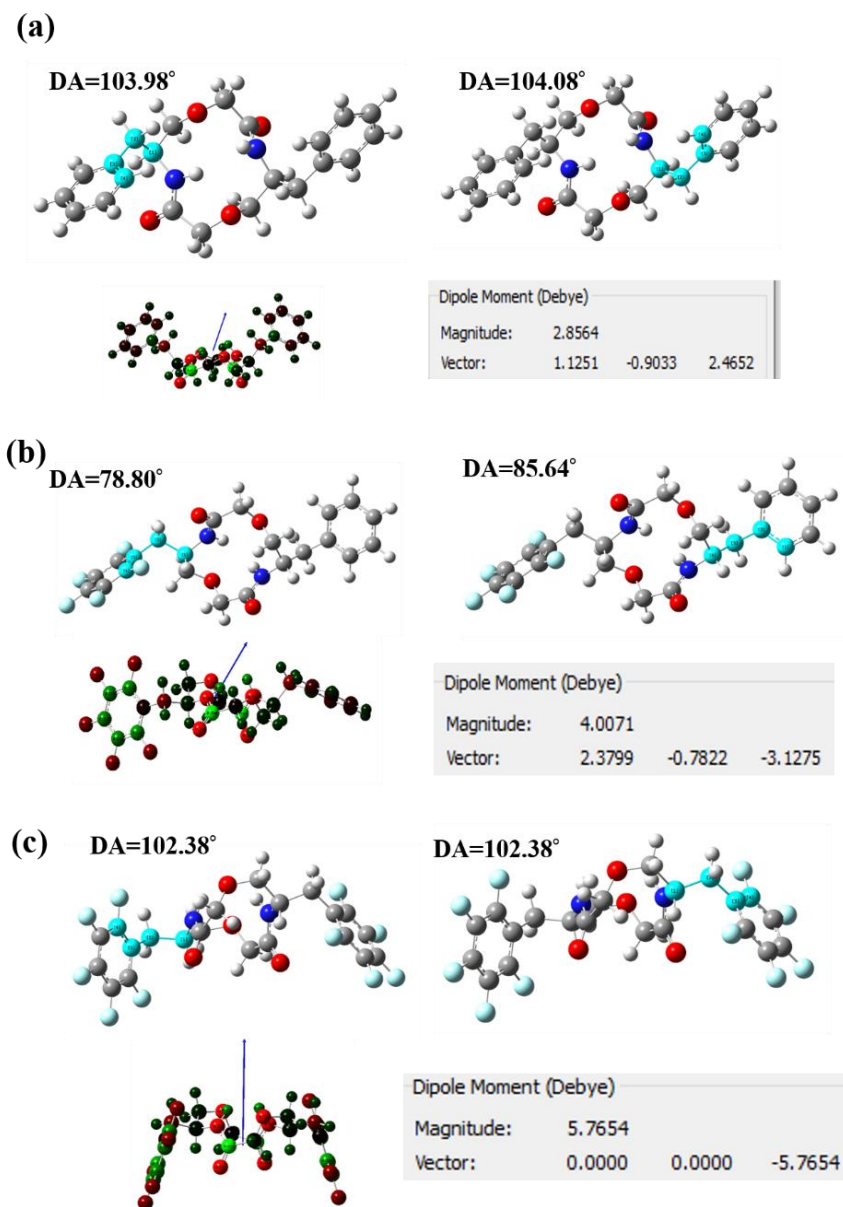


Figure 25: Dihedral angles (abbreviated as DA, associated atoms taken under calculation are marked in cyan color) with peripheral substituted/unsubstituted benzene rings with the core moieties for (a) C3, (b) C4 & (c) C5. Distribution of dipole moment vector's magnitude in x (first entry), y (second entry) & z (third entry)- directions are also enlisted in respective sections.

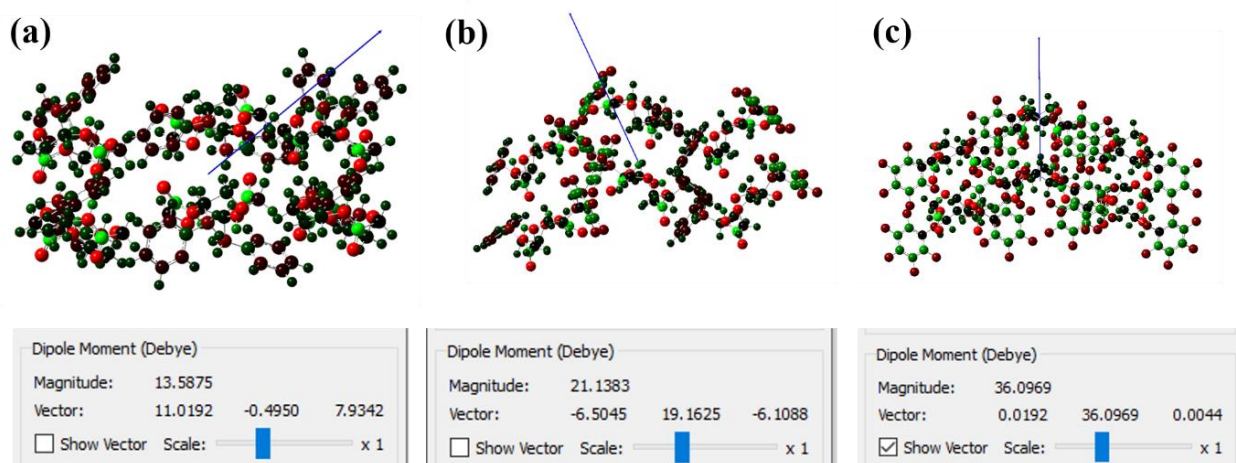


Figure 26: Mulliken atomic charge map and distribution of dipole moment vector's magnitude in x (first entry), y (second entry) & z (third entry)-directions of (a) **C3**, (b) **C4** & (c) **C5** molecules.

3.5. Conclusion:

We have demonstrated the tubular assemblies from a new class of cyclic peptides derived from δ^5 -amino acids. These peptides consist of both amide and ether bonds in the backbone. The peptides spontaneously self-assembled into tubes with unidirectional H-bonds. Irrespective of the H-bonding between the cyclic peptides, all three peptides displayed tubular assemblies with unidirectional H-bonds. Among these peptides, **C5** with electron-withdrawing penta-fluorophenyl groups, tightly packed into tubular assembly, showed a higher dipole moment compared to cyclic peptides **C1-C4**. Also, the non-centrosymmetric structures with unidirectional H-bonds motivated us to examine the ferro and piezoelectric domain switching analysis of these cyclic peptides. Among all these peptides, **C7**, which features diphenyl side chains, demonstrated the highest piezoelectric coefficient. This suggests that as aromaticity in tubular packing increases, the piezoelectric properties are enhanced. Among the cyclic peptides, **C7** showed a better piezoelectric coefficient (d_{33}) of 90 ± 3 pm/V. The piezoelectric coefficient values displayed by these are commendable with respect to the applied voltage. The macrodipole emerged from the spontaneous tubular assemblies of these cyclic peptides can be explored to design piezo- and ferroelectric materials and other devices.

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3.6. Experimental Section:

3.6.1. Materials and Method:

All coupling reactions were carried out under a nitrogen atmosphere. We sourced various amino acids, Boc anhydride, HBTU, HOBt, as well as, Trifluoroacetic acid (TFA), N,N-Diisopropylethylamine (DIPEA), DMF, EtOAc, Hexane, THF, from different commercial suppliers. Before use, we distilled Ethyl acetate and Hexane. Silica gel (100-200 mesh) was employed for column chromatography. We monitored reactions using analytical thin-layer chromatography (TLC) and verified results under UV light and ninhydrin or iodine staining. All final cyclic peptides were purified by reverse phase HPLC. NMR spectra (^1H at 400 MHz and 600MHz and ^{13}C at 101 MHz) were recorded using deuterated solvents with CDCl_3 as the internal standard. Chemical shifts are reported in parts per million (ppm, δ) relative to tetramethyl silane (δ 0.00). ^1H NMR splitting patterns were identified as singlet (s), doublet (d), broad doublet (bd), triplet (t), doublet of doublets (dd), and quintet (qn). Coupling constants (J) are reported in Hertz (Hz). Mass spectra were acquired using a MALDI-TOF instrument. Additionally, X-ray data were collected at low temperatures (150 K) using a Bruker APEX (II) DUO CCD diffractometer with both Mo $\text{K}\alpha$ ($\lambda = 0.71073 \text{ \AA}$) and Cu $\text{K}\alpha$ radiation ($\lambda = 1.54178 \text{ \AA}$), which had been monochromated with graphite.

3.6.2. Peptide Synthesis:

The designed cyclic dipeptides **C1-C7** are shown in Scheme 1. The amino acids and linear peptides and cyclic dipeptides **C1-C7** were synthesized as reported last chapter.^[47] Initially, the linear dipeptides were synthesized through the traditional solution-phase condensation approach. After that C-terminal ester group was hydrolysed using 1N NaOH and the N-terminal Boc group was deprotected by 1:1 mixture of TFA and DCM. The free amine and carboxylic acid was then kept for macrocyclization using a high dilution method. The macrocyclization process is detailed in the experimental section. All peptides were purified using column chromatography and confirmed by mass spectral analysis.

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3.6.3. FE-SEM Study:

To examine the self-assembly of the peptides, we initially dissolved the peptides methanol/water at a concentration of 1 mg/mL to create a primary stock solution. Subsequently, the stock solutions were incubated at room temperature. After that peptide solution was vigorously vortexed for approximately 1 minute and then subjected to centrifugation. The resulting filtrate was ultimately applied as 2.5 μ L droplets onto a silicon vapor plate (SiO_2/Si substrate), which had been thoroughly cleaned beforehand. This process was repeated at various time. It's important to note that freshly prepared solutions were used for all experiments. The samples were subsequently analyzed using the FE-SEM ZEISS ULTRA PLUS instrument to capture the final images.

We have updated the scale bar of the recorded SEM images. The updated images now include measurements of the length and breadth of the sheets, as shown in the figure below.

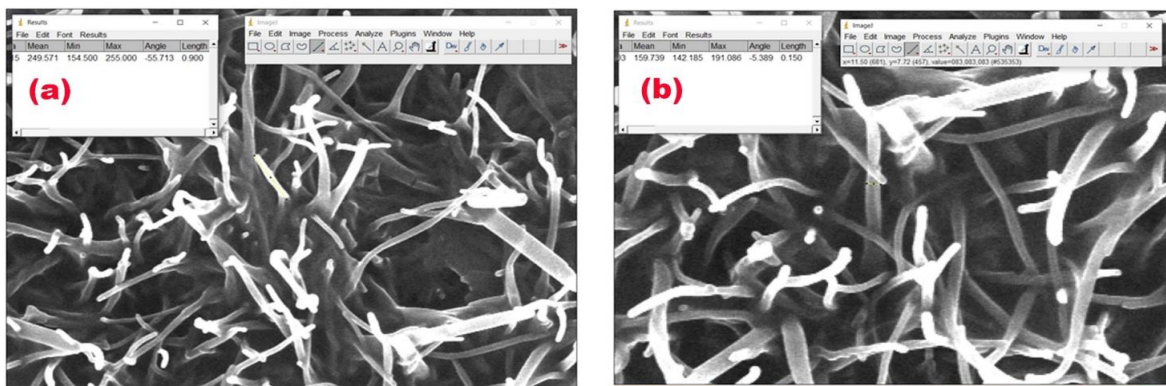


Figure S27: Scalebar Mentioned FE-SEM Images of C3.

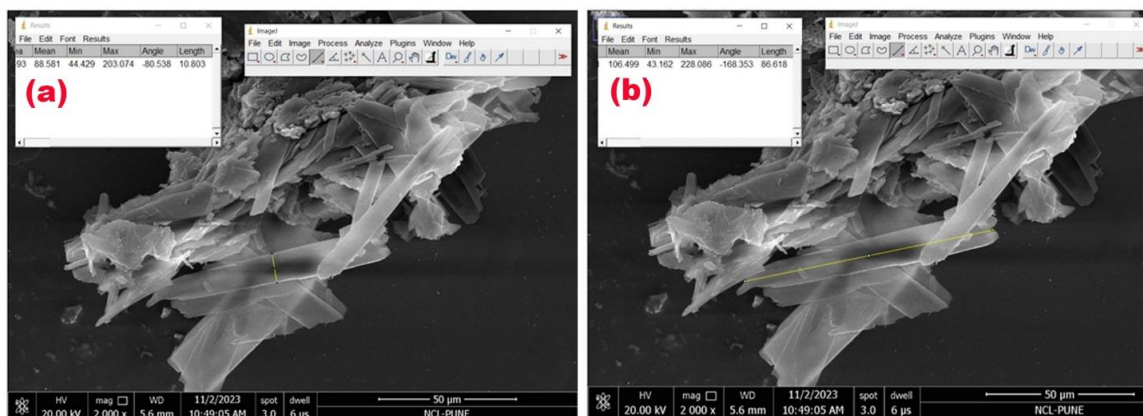


Figure 28: Scalebar Mentioned FE-SEM Images of C4.

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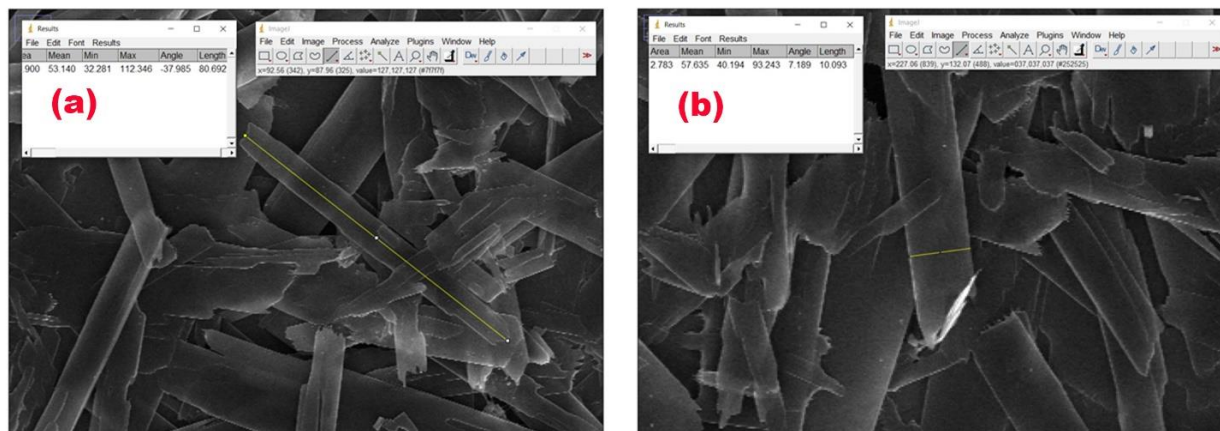


Figure 29: Scalebar Mentioned FE-SEM Images of C5

3.6.4. HR-TEM Study:

HR-TEM Samples were prepared in the similar way of FE-SEM analysis and samples were drop casted (4 μ L) on carbon coted copper grid and dried at room temperature and imaged it. Samples were drop casted on TEM grids to check the stability of the peptide nanotubes. Drop casted samples were examined under Bruker High-Resolution Transmission Electron Microscope to take the final images.

3.6.5. DLS Study:

The DLS (Dynamic Light Scattering) experiment was employed to analyze the size distribution of peptide sub-nanotubes in two different Solvent like Polar Protic methanol and Polar Aprotic acetonitrile. The experiment used a 90° scattering angle and followed the same sample preparation process as the HR-TEM samples. A control sample consisting of Methanol and acetonitrile in the same ratio as the prepared sample was included. The Zeta Sizer Nanoseries ZS90 instrument was used to conduct this experiment.

3.6.6. PFM Analysis and d_{33} Calculation:

The displacement of the tip happened due to the deformation of ferroelectric samples under an applied electric field. The hysteresis curves exhibit piezoelectric effect in its thin film. According to the definition of the converse piezoelectric effect, the piezoelectric coefficient d_{33} can be calculated from the slope of the amplitude versus bias curve at low voltage range.^[48] The observed

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piezoelectric coefficients (d_{33}) of **C3**, **C4**, **C5** in thin film are the average value of slope at multiple points of corresponding amplitude vs Bias curve respectively. The points taken for determining piezoelectric coefficient values are highlighted in figure **30**. The piezoelectric coefficient values are obtained in a range.

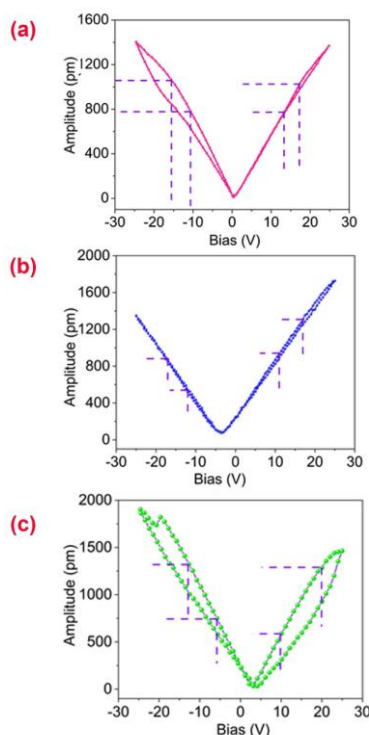


Figure 30: Multiple points are taken in Amplitude vs Bias plot to calculate d_{33} for **(a) CP1, (b) CP2, (c) CP3**.

In order to understand “The polarization switching in terms of the domain orientation” we have performed PFM microscopy at 0 V and applying ($\pm$) 25V and compared their domain orientation. Our molecule is neutral organic, and we are limited to applying a maximum of ± 25 V with our available PFM facility. After recording PFM images at 0 V bias, we applied a +25 V bias to the same area and collected additional PFM images. Again, we have chosen another area ($10 \times 10 \mu\text{m}^2$) and recorded PFM image initially at 0 V bias and then we applied -25 V bias to the same area ($10 \times 10 \mu\text{m}^2$). We have seen the changes in the domain patterns in both the cases (See below figure no 31 and 32) but complete switching all over the area is not observed. It may require higher voltage at longer scan to observe distinct complete switching for such kind of neutral organic molecules.

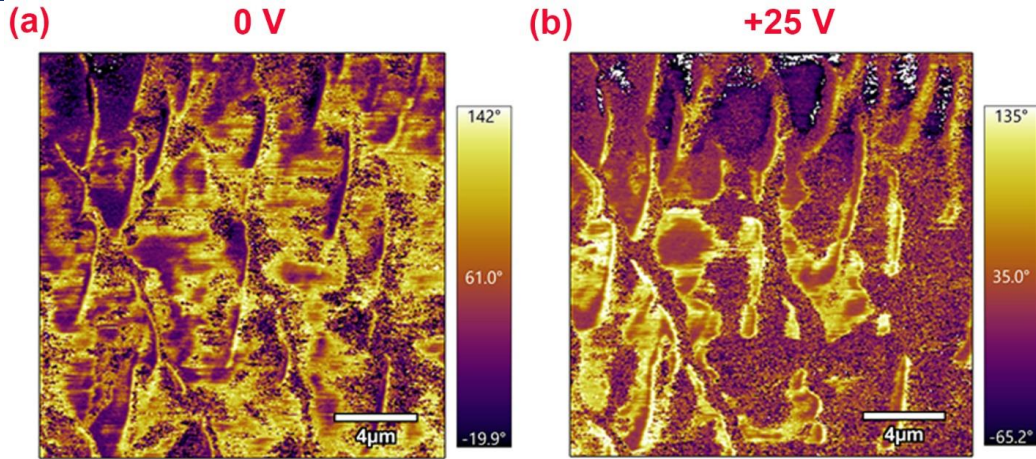


Figure 31: Diagram representing 3D-vertical phase of C5 at (a) 0 V bias and (b) +25 V bias.

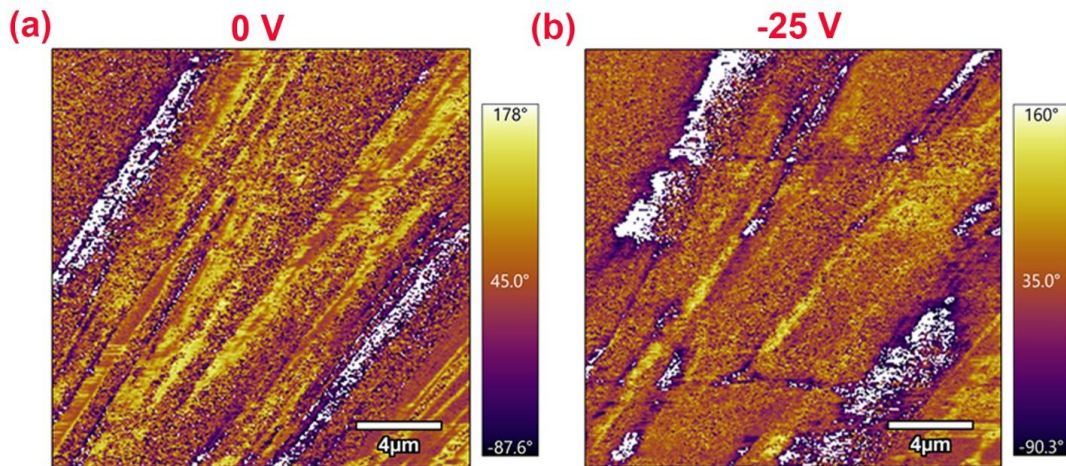


Figure 32: Diagram representing 3D-vertical phase of C5 at (a) 0 V bias and (b) -25 V bias.

3.6.7. Piezoresponse Force Microscopy Measurement:

Piezo and ferroelectric characteristics of crystal film on an ITO-coated glass surface were investigated using PFM measurements conducted with the Bio-AFM system from Asylum Research's MFP-3D family. DART PFM experiments utilized AC-240TM-R3 cantilever probes with a spring constant range of 0.3 to 4 N/m and a tip radius of 28 nm, coated with Ti/Pt, to assess the piezo response of the crystal films (with an RMS roughness of approximately 200 nm). PFM data was acquired through DART-PFM experiments, where an AC voltage was applied to the conductive AFM tip while the bottom electrode remained grounded. PFM images were captured at a resonance frequency of 300 ± 40 KHz, with PFM hysteresis obtained under an applied bias of

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± 25 V. Additionally, PFM phase and amplitude contrast were recorded in areas measuring about 10 X 10 micrometers, using Dual AC Resonance Tracking (DART) mode PFM. The d_{33} values were determined by measuring the amplitude signal at the first contact resonance of the cantilever while varying the tip bias voltage. We drop-cast each compound at the same concentration and allowed them to dry slowly on ITO-coated glass to get crystalline thin film. This process resulted in the growth of micro-crystals, influenced by the molecular packing of the peptides, under identical conditions. We conducted experiments at multiple points, alternating the voltage from +25 V to -25 V. The topographical images are provided below.

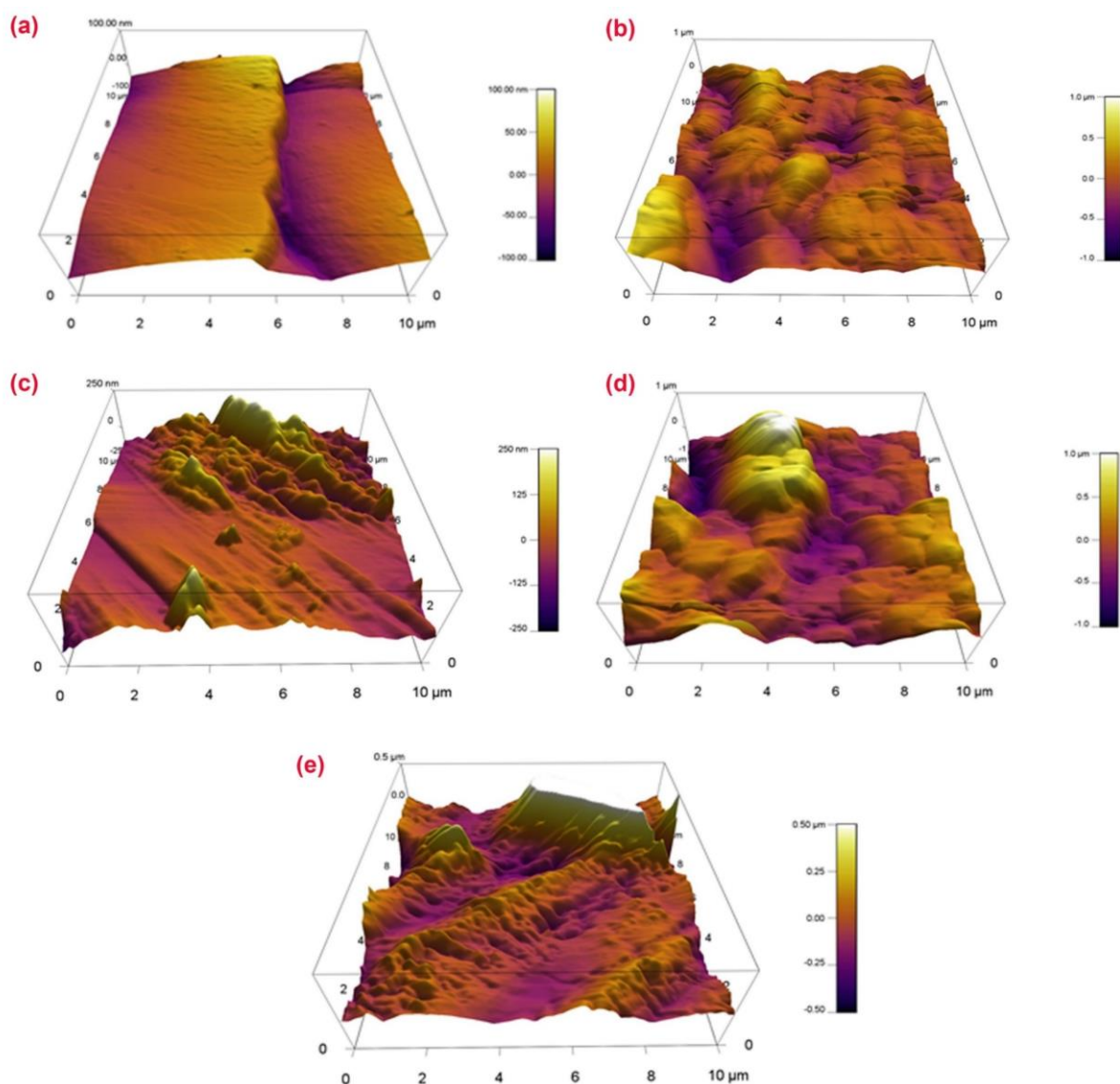


Figure 31: Topography image of (a) C3, (b) C4, (c) C5, (d) C6, (e) C7.

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3.6.8. DFT Calculations for measurement of Dipole Moment:

Density Functional Theory (DFT) Calculations: Single molecule dipoles were calculated from gas phase molecular electronic structures solved using the Gaussian9 suite of programs with the hybrid DFT B3LYP functional and 6-31 G(d,p) basis set.^[46] We optimized electronic structures from crystal structures.

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3.7. References:

- [1] J. Curie, P. Curie, *Bulletin de la Société Minéralogique de France* 1880, 3, 90
- [2] M. J. Schulz, M. J. Sundaresan, J. McMichael, D. Clayton, R. Sadler, B. Nagel, *J. Intell. Mater. Syst. Struct.* **2003**, 14, 693-705.
- [3] A. Martin, *Proc. Phys. Soc.* **1941**, 53, 186-189.
- [4] E. Fukada, I. Yasuda, *Jpn. J. Appl. Phys.* **1964**, 3, 117.
- [5] B. Jaffe, R. S. Roth, and S. Marzullo, *J. Appl. Phys.* **1954**, 25, 809-810.
- [6] W. Heywang, K. Lubitz, W. Wersing Springer Science & Business Media, **2008**, 114.
- [7] P. Muralt, "Recent progress in materials issues for piezoelectric MEMS," *J. Am. Ceram. Soc.* **2008**, 91, 1385–1396.
- [8] Z. L. Wang, J. Song, *Science* **2006**, 312, 242–246.
- [9] a) S. Mondal, P. Tanari, S. Roy, S. Bhunia, R. Chowdhury, A. K. Pal, A. Datta, B. Pal, C. M. Reddy, *Nat Commun* **2023**, 14, 6589; b) M. Owczarek, K. A. Hujsak, D. P. Ferris, A. Prokofjevs, I. Majerz, P. Szklarz, H. Zhang, A. A. Sarjeant, C. L. Stern, R. Jakubas, S. Hong, V. P. Dravid, J. F. Stoddart, *Nat Commun* **2016**, 7, 13108.
- [10] a) C. Zhao, H. Wu, F. Li, Y. Cai, Y. Zhang, D. Song, J. Wu, X. Lyu, J. Yin, D. Xiao, J. Zhu, S. J. Pennycook, *J. Am. Chem. Soc.* **2018**, 140, 15252–15260; b) A. Sood, M. Desseigne, A. Dev, L. Maurizi, A. Kumar, N. Millot, S. S. Han, *Small* **2023**, 19, e2206401.
- [11] a) J. Li, W. Qu, J. Daniels, H. Wu, L. Liu, J. Wu, M. Wang, S. Checchia, S. Yang, H. Lei, R. Lv, Y. Zhang, D. Wang, X. Li, X. Ding, J. Sun, Z. Xu, Y. Chang, S. Zhang, F. Li, *Science* **2023**, 380, 87–93; b) M. D. Nguyen, E. P. Houwman, M. Dekkers, G. Rijnders, *ACS Appl. Mater. Interfaces* **2017**, 9, 9849–9861.
- [12] a) E. Fukada, I. Yasuda, *Jpn. J. Appl. Phys.* **1964**, 3, 117; b) J. Kwon, H. Cho, *Commun Biol* **2022**, 5, 1229.

Chapter 3

- [13] Y. Liu, H. L. Cai, M. Zeliskoc, Y. Wang, J. Sun, F. Yan, F. Ma, P. Wang, Q. N. Chen, H. Zheng, X. Meng, P. Sharma, Y. Zhang, J. Li, *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, E2780–E2786.
- [14] B. Y. Lee, J. Zhang, C. Zueger, W. J. Chung, S. Y. Yoo, E. Wang, J. Meyer, R. Ramesh, S. W. Lee, *Nat. Nanotechnol.* **2012**, *7*, 351–356.
- [15] R. Wang, J. Sui, X. Wang, *ACS Nano* **2022**, *16*, 17708–17728.
- [16] a) S. Bera, S. Guerin, H. Yuan, J. O'Donnell, N. P. Reynolds, O. Maraba, W. Ji, L. J. W. Shimon, P. A. Cazade, S. A. M. Tofail, D. Thompson, R. Yang, E. Gazit, *Nat Commun* **2021**, *12*, 2634; b) H. Yuan, P. Han, Z. Tao, B. Xue, Y. Guo, D. Levy, W. Hu, Y. Wang, Y. Cao, E. Gazit, R. Yang, *ACS Appl. Mater. Interfaces* **2022**, *14*, 6538–6546; c) V. Basavalingappa, S. Bera, B. Xue, J. O'Donnell, S. Guerin, P. A. Cazade, H. Yuan, E. U. Haq, C. Silien, K. Tao, L. J. W. Shimon, S. A. M. Tofail, D. Thompson, S. Kolusheva, R. Yang, Y. Cao, E. Gazit, *ACS Nano* **2020**, *14*, 7025–7037.
- [17] a) Z. X. Gan, X. L. Wu, X. B. Zhu, J. C. Shen, *Angew. Chem. Int. Ed.* **2013**, *52*, 2055; *Angew. Chem.* **2013**, *125*, 2109; b) K. Ryan, J. Beirne, G. Redmond, J. I. Kilpatrick, J. Guyonnet, N. V. Buchete, A. L. Kholkin, B. J. Rodriguez, *ACS Appl. Mater. Interfaces* **2015**, *7*, 12702–12707; c) J. H. Lee, K. Heo, K. Schulz-Schönhausen, J. H. Lee, M. S. Desai, H. E. Jin, S. W. Lee, *ACS Nano* **2018**, *12*, 8138–8144.
- [18] Y. Wang, S. Liu, L. Li, H. Li, Y. Yin, S. R. Lazar, S. Guerin, W. Ouyang, D. Thompson, R. Yang, K. Cai, E. Gazit, W. Ji, *J. Am. Chem. Soc.* **2023**, *145*, 15331–15342.
- [19] S. Guerin, A. Stapleton, D. Chovan, R. Mouras, M. Gleeson, C. McKeown, M. R. Noor, C. Silien, F. M. F. Rhen, A. L. Kholkin, N. Liu, T. Soulimane, S. A. M. Tofail, D. Thompson, *Nat. Mater.* **2018**, *17*, 180–186.
- [20] S. Guerin, A. Stapleton, D. Chovan, *Nature Materials* **2017**, *17*, 180–186.
- [21] A. Heredia, V. Meunier, I. K. Bdikin, J. Gracio, N. Balke, S. Jesse, A. Tselev, P. K. Agarwal, B. G. Sumpter, S. V. Kalinin, A. L. Kholkin, *Adv. Funct. Mater.* **2012**, *22*, 2996–3003.
- [22] V. Nguyen, R. Zhu, K. Jenkins, R. Yang, *Nat. Commun.* **2016**, *7*, 13566.

Chapter 3

- [23] K. Ryan, J. Beirne, G. Redmond, J. I. Kilpatrick, J. Guyonnet, N. V. Buchete, A. L. Kholkin, B. J. Rodriguez, *ACS Appl. Mater. Interfaces* **2015**, *7*, 12702-12707.
- [24] W. Ji, B. Xue, Z. A. Arnon, H. Yuan, S. Bera, Q. Li, D. Zaguri, N. P. Reynolds, H. Li, Y. Chen, S. Gilead, S. Rencus-Lazar, J. Li, R. Yang, Y. Cao, E. Gazit, *ACS Nano* **2019**, *13*, 14477.
- [25] W. Ji, B. Xue, S. Bera, S. Guerin, Y. Liu, H. Yuan, Q. Li, C. Yuan, L. J. W. Shimon, Q. Ma, E. Kiely, S. A. M. Tofail, M. Si, X. Yan, Y. Cao, W. Wang, R. Yang, D. Thompson, J. Li, E. Gazit, *ACS Nano* **2020**, *14*, 10704.
- [26] a) A. Kholkin, N. Amdursky, I. Bdikin, E. Gazit, G. Rosenman, *ACS Nano* **2010**, *4*, 610; b) V. Nguyen, R. Zhu, K. Jenkins, R. Yang, *Nat. Commun.* **2016**, *7*, 13566; c) K. Jenkins, S. Kelly, V. Nguyen, Y. Wu, R. Yang, *Nano Energy* **2018**, *51*, 317; d) J. H. Lee, K. Heo, K. S. Schonbagen, J. H. Lee, M. S. Desai, H. E. Jin, S. W. Lee, *ACS Nano* **2018**, *12*, 8138.
- [27] S. Bera, S. Guerin, H. Yuan, J. O. Donnell, N. P. Reynolds, O. Maraba, W. Ji, L. J. W. Shimon, P. A. Cazade, S. A. M. Tofail, D. Thompson, R. Yang, E. Gazit, *Nat. Commun.* **2021**, *12*, 2634.
- [28] K. Tao, B. Xue, Q. Li, W. Hu, L. J. W. Shimon, P. Makam, M. Si, X. Yan, M. Zhang, Y. Cao, R. S. Yang, J. B. Li, E. Gazit, *Mater. Today* **2019**, *30*, 10.
- [29] a) M. Reches, E. Gazit, *Science* **2003**, *300*, 625–627; b) E. Gazit, *Chem. Soc. Rev.* **2007**, *36*, 1263–1269; c) L. Adler-Abramovich, D. Aronov, P. Beker, M. Yevnin, S. Stempler, L. Buzhansky, G. Rosenman, E. Gazit, *Nature Nanotech* **2009**, *4*, 849–854; d) X. Yan, P. Zhu, J. Li, *Chem. Soc. Rev.* **2010**, *39*, 1877–1890; e) A. S. Tayi, A. Kaeser, M. Matsumoto, T. Aida, S. I. Stupp, *Nat. Chem.* **2015**, *7*, 281–294.
- [30] D. T. Bong, T. D. Clark, J. R. Granja, M. R. Ghadiri, *Angew. Chem. Int. Ed.* **2001**, *40*, 988–1011; *Angew. Chem.* **2001**, *113*, 1016–1041; b) M. F. Perutz, J. T. Finch, J. Berriman, A. Lesk, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5591–559; c) J. Couet, J. D. J. S. Samuel, A. Kopyshv, Svetlana Santer, M. Biesalski, *Angew. Chem. Int. Ed.* **2005**, *44*, 3297–3301, *Angew. Chem.* **2005**, *117*, 3361–3365; d) R. Ni, W. S. Childers, K. I. Hardcastle, A. K. Mehta, D. G. Lynn *Angew. Chem. Int. Ed.* **2012**, *51*, 6635–6638; *Angew. Chem.* **2012**, *124*, 6739; e) S. V. Jadhav, R. Misra, H. N. Gopi, *Chem. Eur. J.* **2014**, *20*, 16523–16528; f) F. Thomas, N. C. Burgess, A. R. Thomson, D. N. Woolfson, *Angew. Chem. Int. Ed.* **2016**, *55*, 987–991; *Angew. Chem.* **2016**, *128*, 999–1003; g) D.

Chapter 3

R. Puneeth Kumar, Z. M. Bhat, S. Dey, S. Roy, S. Panda Mahapatra, S. Pahan, M. O. Thotiyl, H. N. Gopi, *Chem. Eur. J.* **2023**, 29, e2023004.

[31] a) P. De Santis, S. Morosetti, R. Rizzo, *Macromolecules* **1974**, 7, 52–58; b) M. R. Ghadiri, J. R. Granja, R. A. Milligan, D. E. McRee, N. Khazanovich, *Nature* **1993**, 366, 324–327; c) M. R. Silk, B. Mohanty, J. B. Sampson, M. J. Scanlon, P. E. Thompson, D. K. Chalmers, *Angew. Chem. Int. Ed.* **2019**, 58, 596–601; *Angew. Chem.* **2019**, 131, 606–611; d) I. Insua, J. Montenegro, *J. Am. Chem. Soc.* **2020**, 142, 300–307.

[32] a) D. Seebach, J. L. Matthews, A. Meden, T. Wessels, C. Baerlocher, L. B. McCusker, *Helv. Chim. Acta* **1997**, 80, 173–182; b) T. D. Clark, L. K. Buehler, M. R. Ghadiri, *J. Am. Chem. Soc.* **1998**, 120, 651–656; c) F. Fujimura, T. Hirata, T. Morita, S. Kimura, Y. Horikawa, J. Sugiyama, *Biomacromolecules* **2006**, 7, 2394–2400; d) F. Novelli, M. Vilela, A. Pazó, M. Amorín, J. R. Granja, *Angew. Chem. Int. Ed.* **2021**, 60, 18838–18844; *Angew. Chem.* **2021**, 133, 18986–18992.

[33] a) M. Amorín, L. Castedo, J. R. Granja, *J. Am. Chem. Soc.* **2003**, 125, 2844–2845; b) M. Calvelo, A. Lamas, A. Guerra, M. Amorín, R. Garcia-Fandino, J. R. Granja, *Chem. Eur. J.* **2020**, 26, 5846–5858.

[34] a) D. Gauthier, P. Baillargeon, M. Drouin, Y. L. Dory, *Angew. Chem. Int. Ed.* **2001**, 40, 4635–4638; *Angew. Chem.* **2001**, 113, 4771–4774; b) S. Leclair, P. Baillargeon, R. Skouta, D. Gauthier, Y. Zhao, Y. L. Dory, *Angew. Chem. Int. Ed.* **2004**, 43, 349–353; *Angew. Chem.* **2004**, 116, 353–357; c) A. Lamas, A. Guerra, M. Amorin, J. R. Granja, *Chem. Sci.* **2018**, 9, 8228–8233.

[35] Q. Song, Z. Cheng, M. Kariuki, S. C. L. Hall, S. K. Hill, J. Y. Rho, S. Perrier, *Chem. Rev.* **2021**, 121, 13936–13995.

[36] Y. Tabata, S. Mitani, H. Uji, T. Imai, S. Kimura, *Polym J* **2019**, 51, 601–609.

[37] R. M. Reja, V. Kumar, G. George, R. Patel, D. R. Puneeth Kumar, S. Raghothama, H. N. Gopi, *Chem. Eur. J.* **2020**, 26, 4304–4309.

[38] W. Ji, H. Yuan, B. Xue, S. Guerin, H. Li, L. Zhang, Y. Liu, L. J. W. Shimon, M. Si, Y. Cao, W. Wang, D. Thompson, K. Cai, R. Yang, E. Gazit, *Angew. Chem. Int. Ed.* **2022**, 61, e2022012; *Angew. Chem.* **2022**, 134, e202201234.

Chapter 3

- [39] S. Panda Mahapatra, S. Pahan, A. Chatterjee, S. Roy, D. R. Puneeth Kumar, H. N. Gopi, *Angew. Chem. Int. Ed.* **2024**, e202409969.
- [40] T. Korenaga, H. Tanaka, T. Ema, T. Sakai, T. Korenaga, *J. Fluor. Chem.* **2003**, 122, 201–205.
- [41] P. R. Spackman, M. J. Turner, J. J. McKinnon, S. K. Wolff, D. J. Grimwood, D. Jayatilakab, M. A. Spackman, *J. Appl. Cryst.* **2021**, 54, 1006–1011.
- [42] a) L. Adler-Abramovich, M. Reches, V. L. Sedman, S. Allen, S. J. B. Tendler, E. Gazit, *Langmuir* **2006**, 22, 1313–1320; b) M. Gupta, A. Bagaria, A. Mishra, P. Mathur, A. Basu, S. Ramakumar, V. S. Chauhan, *Adv. Mater.* **2007**, 19, 858–861.
- [43] S. V. Kalinin, A. Rar, S. Jesse, *IEEE transactions on ultrasonics, ferroelectrics, and frequency control* **2006**, 53, 2226–2252.
- [44] C. T. Nelson, P. Gao, J. R. Jokisaari, C. Heikes, C. Adamo, A. Melville, S. H. Baek, C. M. Folkman, B. Winchester, Y. Gu, Y. Liu, K. Zhang, E. Wang, J. Li, L. Q. Chen, C. B. Eom, D. G. Schlom, X. Pan, *Science* **2011**, 334, 968–971.
- [45] A. Jalalian, A. M. Grishin, X. L. Wang, Z. X. Cheng, S. X. Dou, *Appl. Phys. Lett.* **2014**, 104, 103112.
- [46] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, *Gaussian 09, Revision A.02 Gaussian 09, Inc., Wallingford CT*, **2009**.
- [47] R. M. Reja, R. Patel, V. Kumar, A. Jha, H. N. Gopi, *Biomacromolecules* **2019**, 20, 1254–1262.

Chapter 3

[48] A. Jalalian, A. M. Grishin, X. L. Wang, Z. X. Cheng, S. X. Dou, *Appl. Phys. Lett.* **2014**, *104*, 103112.

Note: Characterization data and full spectral charts for all compounds can also be found in the Supporting information of the following publication. S. Panda Mahapatra, S. Pahan, A. Chatterjee, S. Roy, D. R. Puneeth Kumar, H. N. Gopi, *Angew. Chem. Int. Ed.* **2024**, e202409969.

It can be downloaded from the link attached : [Exploring Macroscopic Dipoles of Designed Cyclic Peptide Ordered Assemblies to Harvest Piezoelectric Properties Supplementary Information](#)

Chapter 4

Exploring Self-Assembling Cyclic β -(O)- δ^5 -Peptide Sub-Nanotubes for Anion Selective Transmembrane Ion Channels

4.1. Introduction:

4.1.1. Introduction to Supramolecular Self-assembled Foldamers Design:

Within the natural world, proteins and peptides coordinate a wide range of physiologically relevant processes, such as molecular recognition, catalysis, and electron transport, mainly because of their unique, stable, and well-organized three-dimensional structures. Growing knowledge about the complex interactions between protein/peptide structure and function has led to a enormous efforts in drug development to create peptide-based compounds that can bind to important biological targets with exceptional selectivity and affinity.¹ However, there are significant obstacles to the therapeutic application of native peptides due to their inherent limitations. Their vulnerability to conformational flexibility, which compromises the accuracy and specificity of peptide-target binding, and their susceptibility to destruction by a variety of peptidases in physiological settings are the main causes for worry. These difficulties have prompted scientists to work together to develop peptidomimetics with increased biostability and structural rigidity.

A current principle states that a peptidomimetic compound will have higher affinity and selectivity when it interacts with a macromolecular target because it will have a predefined secondary structure that mimics the biologically active conformation of a flexible native peptide. This is due to the expectation that a prearranged molecule will experience a lower entropic cost during complex formation. As a result, one common approach in peptidomimetic design is to construct artificial oligomeric structures with stiff and well-defined conformations. Research on peptidomimetic foldamers² has produced a wealth of interesting compounds that can bind to a variety of substrates, such as proteins,³ RNA,⁴ carbohydrates,⁵ and lipids.⁶ Remarkably, these compounds often demonstrate affinities comparable to, or even surpassing, those exhibited by natural peptides or proteins.

The creation of novel molecules with distinctive architectures and practical uses is an area that synthetic chemists are constantly drawn to. Peptides are unique among biomolecules because they are made up of twenty distinct amino acids, which can create a variety of environments, including polar, nucleophilic, electrophilic, hydrophobic, neutral, acidic, basic etc. This adaptability makes it possible to create and synthesize unique molecules with remarkable qualities. The amide (NH-CO) group in peptides is self-complementary, which promotes intermolecular hydrogen bonding

and the creation of three-dimensional supramolecular structures that are anatomically and functionally similar to natural systems.

The potential of cyclic peptides in particular to function as therapeutics, ion channels, and ion sensor templates has attracted a lot of attention. Cyclic peptides have been shown in recent research to be able to produce nanotubes, which increases their potential use as novel biomaterials. It's interesting to note that the majority of studies on protein secondary structure preferences to date have concentrated on cyclic peptides made of natural amino acids. Few synthesized cyclic peptides that contain unnatural amino acid units have been reported. On the other hand, unusual non-amino acid structural elements, such as thiazole and oxazoline amino acids, are frequently present in naturally occurring cyclic peptides from marine and terrestrial microorganisms and have strong biomedical applications.⁷

Our goal is to create a new class of cyclic peptides by incorporating unnatural amino acid units into the ring structures of cyclic peptides based on natural amino acids, with inspiration from nature. The characteristics of the integrated hybrid unit influence the design of these hybrid macrocycles. The work presented here focuses on our efforts to create cyclic peptides with specific purposes in mind, such as the construction of cyclic peptide-based ion channels and the production of self-assembled nanotubes. Our hybrid peptide macrocycle design approach is property-directed, which means that the intended characteristics or uses of the generated cyclopeptides are the main factors influencing the selection of hybrid units.

4.1.2. Development of Ion Channel and Anion Selective Ion Channel:

Ion channels and carriers are specialized proteins that help move ions and bigger organic molecules across cell membranes.⁸ Enabling the targeted and effective translocation of ions, metabolites, and biopolymers across cellular membranes is an essential component in preserving cell viability.⁹ Numerous mechanisms, including ion channels, pores, carriers, passive diffusion, are involved in this transportation process. Ion channels are essential for precisely controlling ion flow and selectivity via lipid bilayers among various processes.¹⁰ Numerous biological processes, such as ion flux, signal transduction, and molecule transport, are regulated by natural ion channels and pores. The phospholipid membrane contains these functional channels and pores, which are gaps that are designed to strategically minimize the natural repulsion between water-soluble molecules and the hydrophobic lipid environment.¹¹

Anions are needed for many biological functions, such as the regulation of chloride levels, which are necessary for maintaining pH balance and cell osmotic pressure, both of which are necessary for life. It follows that the evolution of specialized protein channels and pumps by nature to transport charged molecules across ion-resistant lipid bilayers is not surprising. Ion transport proteins have different structures, but they all have three-dimensional binding sites that are carefully engineered to attach to target ions in a topologically and electrically particular manner. Research into the creation of synthetic ionophores as potential remedies has been prompted by diseases like Best's disease and cystic fibrosis that arise from the malfunction of these transport proteins and the ensuing ion imbalance.¹² These artificial substances are designed to make it easier for ions—especially anions—to pass through lipid membranes. However, equal degrees of affinity and selectivity are necessary for synthetic ionophores to effectively replace natural transport systems.

When it comes to ion and water transport across membranes, nature has created complex, highly adapted structures that provide a significant challenge to chemists trying to create synthetic equivalents.¹³ Nonetheless, the development of artificial ion channels and carriers can benefit greatly from an understanding of the basic principles employed by nature, including size exclusion, charge repulsion, ion hopping, hydrogen bonding, ion pairing, and hydrophobic/hydrophilic interactions.¹⁴ Promising possibilities for anion transport are those compounds that exhibit hydrogen bonding, halogen bonding, or anion- π interactions with anions.¹⁵ Cyclic structures with a well-defined cavity that makes ion recognition easier, such as calixarenes¹⁶ calixpyrroles¹⁷ Catenane¹⁸ and peptides¹⁹, are particularly fascinating.

4.2. Previous Studies:

In order to construct functional ion channels or pores, one essential structural need is the capacity of molecules to combine into tubular structures. Pore-forming molecules need to be long enough to cross the membrane, stable enough to stay in an open state, partially soluble in water, and have favorable partition coefficients and assembly properties in the lipid bilayer. A perfect fit for this application would have regulated dimensions, chemical functionalization, and form, all of which could be achieved with a simple synthetic method.²⁰ Cyclic peptides that self-assemble are essential components for creating artificial nanotubes. Based on their composition, they can be mainly categorized as cyclic α -alt(D,L)-peptides, cyclic β -peptides, cyclic α,γ -peptides, and cyclic

peptides integrating δ - or ϵ -amino acids.²¹ Several instances of ion channels based on self-assembled cyclic peptide nanotubes have been reported.

4.2.1. Ion Channel model based on L, D- α -Amino Acid containing Cyclic Peptide Nanotubes:

Through theoretical calculations, De Santis et al.- proposed in 1974 that peptides consisting of an even number of alternating conformationally equivalent β -type dihedral angles of D- and L-amino acid residues could form a closed cyclic structure through interactions between backbones and hydrogen bonds.²² Owing to these peptides' limited solubility, the experimental data were equivocal. Twenty years later, in 1993, Ghadiri and colleagues capitalized on this theoretical suggestion, demonstrating how pH fluctuation can generate a nanotubular structure.²³ The octapeptide cyclo-[(L-Gln-D-Ala-L-Glu-D-Ala)₂] was the source of the first well-researched and characterized CPNs. Further, the Ghadiri group created a specialized technique using selective heteromeric supramolecular assembly (Figure 1) to create functional single channels with altered ion conductance, charge selectivity, and rectification. This process entails incorporating designer cyclic peptide subunits that bind selectively at the entrance of the channel assembly into the naturally occurring hollow transmembrane tubular structure that is produced by the self-assembly of cyclic-D,L- α -peptides within lipid bilayers.²⁴

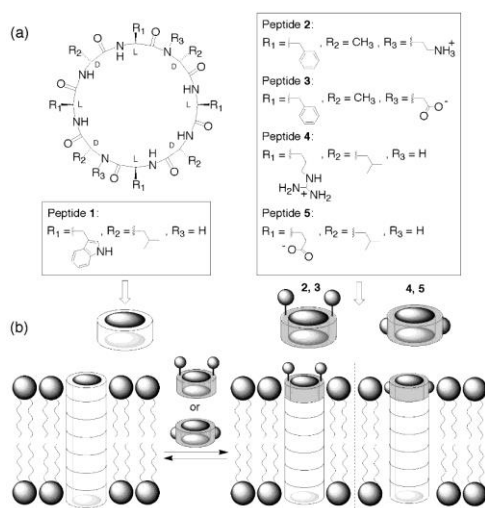


Figure 1: (a) Scheme of the cyclic D,L- α -peptides (b), Peptide 1 autonomously organizes within lipid membranes, constructing the fundamental structure of transmembrane channels. Peptides 2 to 5 function as auxiliary subunits, forming caps that lead to the formation of diverse channel

assemblies with modified ion conductivity characteristics.²⁴ Reproduced with permission from Copyright American Chemical Society.²⁴

4.2.2. Ion Channel model based on β^3 -Amino Acid containing Cyclic Peptide Nanotubes:

The side chains of homochiral β^3 -CPs extend outward from the peptide ring plane, just like those of their D,L- α -CPs counterparts. But the extra carbon in β^3 -Amino acid residues causes the amide and carbonyl groups to align clearly in one direction along the nanotube's longitudinal axis. This arrangement produces a discernible β -helix like macrodipole moment, which may influence the nanotube's conductance via processes including voltage gating and current rectification. The ion transport properties of cyclic β -peptides were also studied in order to support this notion. The anticipated inner diameter of tetrameric β^3 -CPs in a flat ring C4 configuration (2.6-2.7 Å) suggests the potential formation of water channels within the membrane's tubular superstructure. To investigate this, hydrophobic β^3 -CPs, namely CP1, CP2, and CP3, which include tryptophan and/or leucine residues, were synthesized to assess their transport capabilities (Figure 2).²⁵

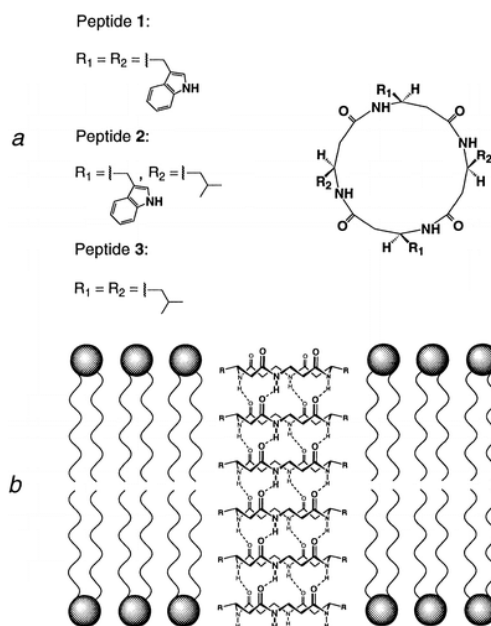


Figure 2: (a) The molecular structure of the cyclic β^3 -peptide subunits 1-3, depicted in a planar ring-shaped configuration. (b) Hypothetical model of transmembrane channels self-assembled from cyclic β^3 -peptides 1-3. The channel complex is shown with anticipated parallel stacking of

the rings and significant hydrogen bonding between subunits.²⁵ Reproduced with permission from Copyright American Chemical Society.²⁵

Conductance measurements in planar BLMs definitively demonstrated the formation of distinct ion channels by CP1 and CP2. In a 500 mM KCl solution, CP1 displayed a conductance of 56 pS, corresponding to a transport rate of 1.9×10^7 ions/s. Variations in conductance levels and channel gating were observed, attributed to differences in the number of CP subunits, assembly-disassembly processes, and/or ring flexibility. However, the current rectification behavior of ion channels formed by β -peptides has not yet been confirmed. It is possible that more robust and rigid tubular structures could better leverage the macrodipole moments of these constructs for the development of voltage-gated ion channels.

4.2.3. Ion Channel model based on α,γ -Amino Acid containing Cyclic Peptide Nanotube:

The structural attributes of the previously discussed D,L- α -CP and β^3 -CP flat rings naturally limited additional chemical modifications within the associated nanotube cavities. To address this challenge while upholding the self-assembly principles of cyclic peptide nanotubes, a solution was found in CPs that incorporated cis-3-aminocycloalkancarboxylic acid (γ -Aca) residues paired with (R)-Amino acids as of suitable chirality. Various modifications and potential applications of several γ -Aca scaffolds have been extensively investigated, leading to a diverse range of tubular structures and nanomaterials. Promising computational results led to the synthesis and membrane evaluation of three hydrophobic CPs (CP1-CP3; Figure 3). Control tests with self-quenched LUVs $\Rightarrow$ CF showed a detergent effect for CP3, while CP1 and CP2 had no leakage. The pH-sensitive LUVs $\Rightarrow$ CF assay confirmed concentration-dependent proton transport for CP1 and CP2, validating the predicted water string relay mechanism.²⁶

Planar BLM ion transport tests showed that CP1's limited diameter and partial hydrophobicity prevented ions from moving through it, while CP2 showed ion-conducting current. CP2 ions were transported at a rate of about 10^7 ions/s in symmetrical alkali metal ion solutions. Its mean open time was marginally shorter than that of D,L-R-CPs, and its total ion transport efficiency was five times lower. Up to 120 mV, the conductance was ohmic, suggesting that dual cation incorporation might occur at higher voltages. The order $\text{Cs}^+ > \text{K}^+ > \text{Na}^+$ showed minimal cation selectivity,

although the Na^+ conductance was 30% higher than predicted, most likely because of the dehydration penalty and effective ion size.

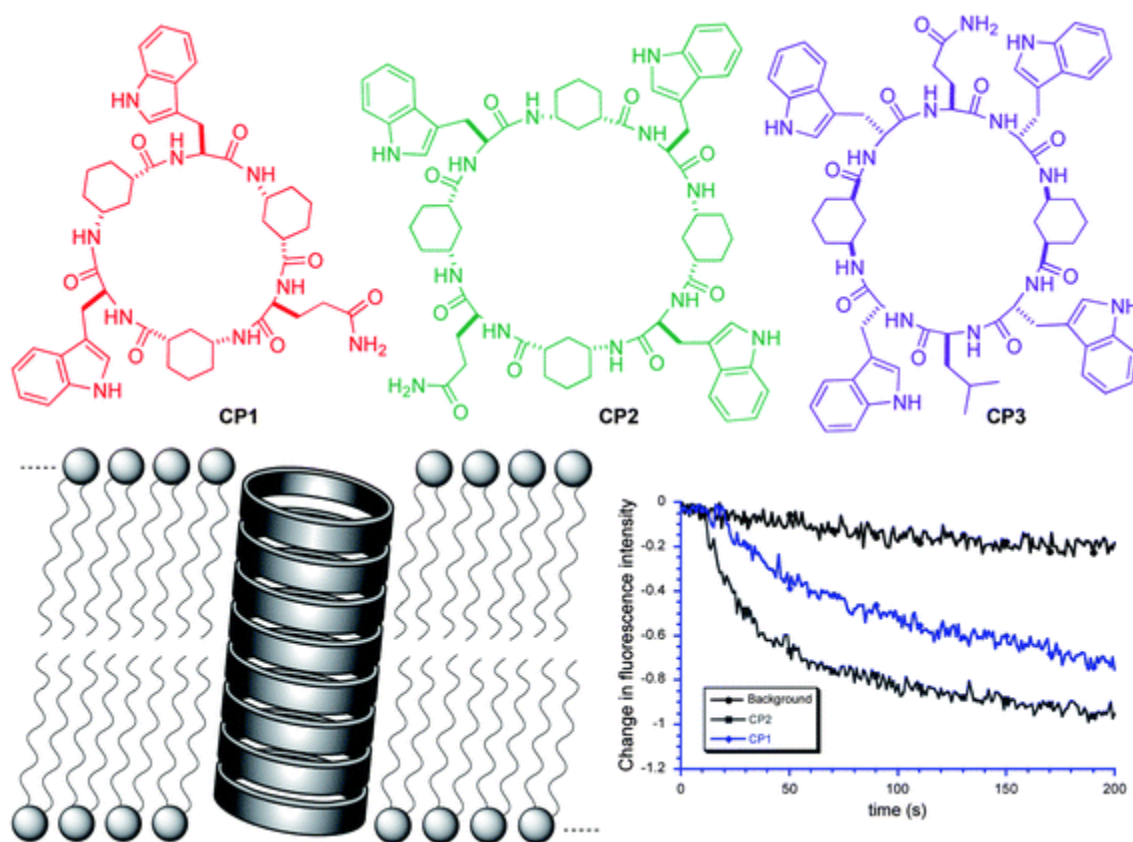


Figure 3: (a) Structures of the cyclic peptides (CPs) analyzed, each containing a varying number of γ -amino acids. (b) Diagram of a transmembrane ion channel formed by the stacking of CPs. (c) Normalized UV fluorescence data from liposomes containing 5(6)-carboxyfluorescein, either without CPs (control) or with CP1 or CP2, indicating fluorescence decrease due to the collapse of an initial transmembrane pH gradient.²⁶

4.4. Aim of the Current Work:

The majority of cyclic peptide nanotubes (CPNs) reported usually have an anti-parallel β -sheet structure. The hydrogen bond orientation inside the stacks makes these CPNs primarily non-polar. Polar CPNs, however, have not received much attention in the literature to far, even though organic nano-electronics holds great promise for them. Particularly difficult to access are polar nanotubes, of which cyclic β -peptides are an exception.

Less research has been done on CPNs derived from homo-oligomers of β -amino acids, most likely because of problems with cyclic β -peptides' solubility. It is postulated that similar to β -amino acids, cyclic peptides derived from δ -amino acids may produce polar CPNs because they have an even number of backbone atoms. This would entail the cyclic peptide's amide NH groups projecting on one face and carbonyl groups on the other. Nevertheless, the difficulties in manufacturing δ -amino acids have restricted research on CPNs containing these amino acids.

The same-side projection of all carbonyl groups and amide NH groups in peptides containing β - and δ -amino acids, as well as the alternating projection of amide bonds in α - and γ -peptides, have already been covered. In the preceding chapter, we highlighted various properties of $\beta(\text{O})$ - δ^5 -amino acids and demonstrated their ability to form polar self-assembled tubular structures. We also observed the presence of cavities within these self-assembled cyclic peptides based on $\beta(\text{O})$ - δ^5 -amino acids. Interestingly, our design combines the features of crown ethers with those of regular cyclic peptides. Our objective is to investigate these cavities within the self-assembled tubular structures as potential templates for ion transport and to assess their ion selectivity.

4.5. Results and Discussions:

4.5.1. Design of Cyclic Peptides:

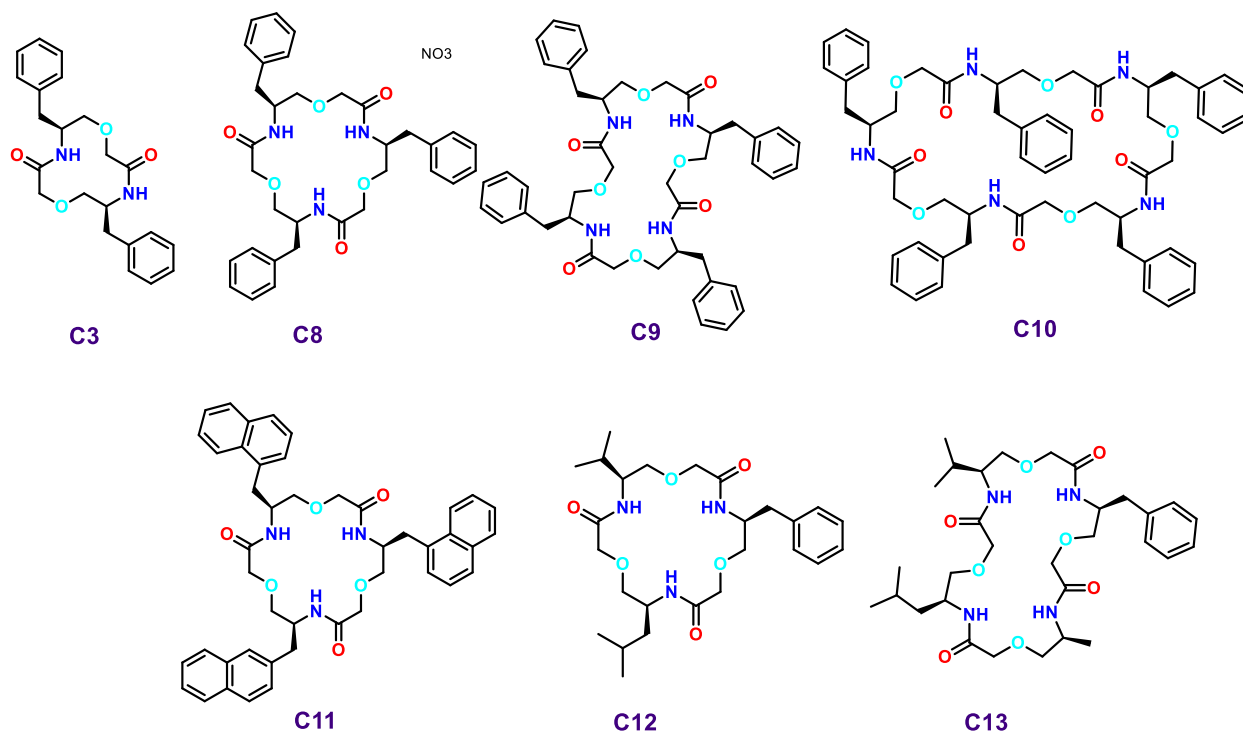
In the previous chapter, we demonstrated that cyclic dipeptides featuring $\beta(\text{O})$ - δ^5 -amino acid residues can autonomously assemble into unique hollow sub-nanotubular structures characterized by unidirectional hydrogen bonds. These cyclic dipeptides, which adopt non-centrosymmetric space groups, display significant anisotropy, suggesting their potential for macroscopic dipole extensions.²⁷

Building on this foundation, the current study focuses on synthesizing macrocycles of varying sizes incorporating $\beta(\text{O})$ - δ^5 -amino acids to explore their self-assembly behaviors in solution phase. We have successfully synthesized macrocycles containing residues ranging from 2 to 5 $\beta(\text{O})$ - δ^5 -amino acids. Additionally, we assessed these macrocycles for their ion channel activity and examined their selective transport behaviors.

To further investigate the influence of hydrophobicity and cavity size of cyclic peptides on their self-assembly properties, we designed seven peptides, detailed in Scheme 1, serving as the basis

for our comparative analysis. Among these, **C3** represents aromatic cyclic dipeptides, **C8**, **C11**, and **C12** represent cyclic tripeptides, **C9** and **C12** are cyclic tetrapeptides, and **C10** is a cyclic pentapeptide. All of these peptides are composed of $\beta(O)-\delta^5$ -amino acids. Notably, **C12** and **C13** are aliphatic cyclic peptides, whereas **C3**, **C8**, **C9**, **C10**, and **C11** are aromatic peptide macrocycles.

This library of peptides will provide insight into our design and enable a comparative analysis of their self-assembly into tubular structures for ion channel activity, helping us understand the role of specific amino acids in designing effective self-assembled ion channels.



Scheme 1: Synthesized Cyclic Peptide **C3**, **C8-C13**.

4.5.2. Synthesis of Cyclic Peptides:

In the previous chapter, we detailed the synthesis of all cyclic peptides using a solution-phase peptide synthesis protocol, which yielded high yields for all cyclic dipeptides. However, as we aimed to synthesize peptides with longer sequences, we encountered challenges with the conventional solution-phase method. The synthesis route became more intricate and time-consuming for higher oligomers.

To address these challenges and improve both the efficiency and yield, we developed a solid-phase peptide synthesis protocol for synthesizing higher oligomers. In this approach, we employed 2-chloro-tritylchloride resin to synthesize linear peptides of extended lengths. Subsequently, a solution-phase macrocyclization was conducted using a high dilution method to produce the desired cyclic peptides. The synthesis protocols for both method are described in detailed in previous chapter. Following synthesis, all peptides were purified through reverse-phase column chromatography using a MeOH/H₂O solvent system and a C₁₈ column, ensuring high purity and quality of the synthesized peptides.

4.5.3. Crystal Structure Analysis of C8:

In pursuit of unequivocal structural insights, we subjected a series of cyclic peptides to crystallization using diverse solvent combinations, aiming to obtain single crystal structures. Notably, among all cyclic peptides investigated, **C8** exhibited the formation of high-quality single crystals suitable for X-ray diffraction through the slow evaporation of its solution in toluene/CHCl₃. Despite repeated attempts, **C9-C13** failed to yield single crystals of X-ray diffraction quality. The X-ray diffraction analysis of **C8**, depicted in Figure 4, 5, and 6, disclosed a C₃ symmetric molecular arrangement, characterized by an average pore diameter of approximately 5.26 Å. Importantly, **C8** manifests a hybrid composition, combining features of both crown ether and peptide macrocycles.

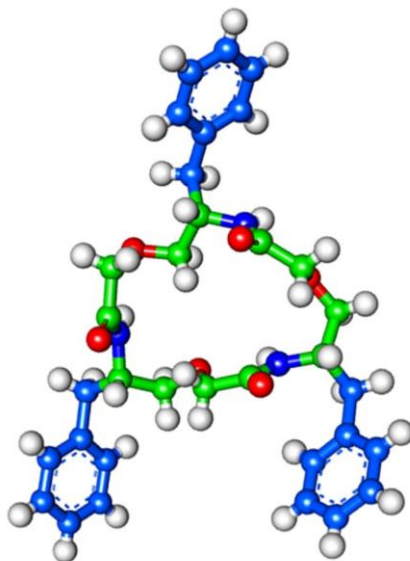


Figure 4: (a) Single Crystal structure of **C8**

Significantly, the cyclic peptides (CPs) assembled into tubular structures through intermolecular hydrogen bonding in a parallel β -sheet configuration. The hydrogen bonding direction within the tubular assembly was unidirectional, portraying the polar nature of the tubular **C8** assembly (Figure 5a). The conformational analysis of the cyclic peptide **C8** was discussed in previous chapter. The average inter-distance between stacked cyclic peptides was approximately 5.14 Å. Moreover, lateral stabilization of each CP was achieved through CH---O interactions, involving O_β with $C_\alpha H$ of an adjacent CP molecule, as depicted in the overall crystal packing arrangement (Figure 6).

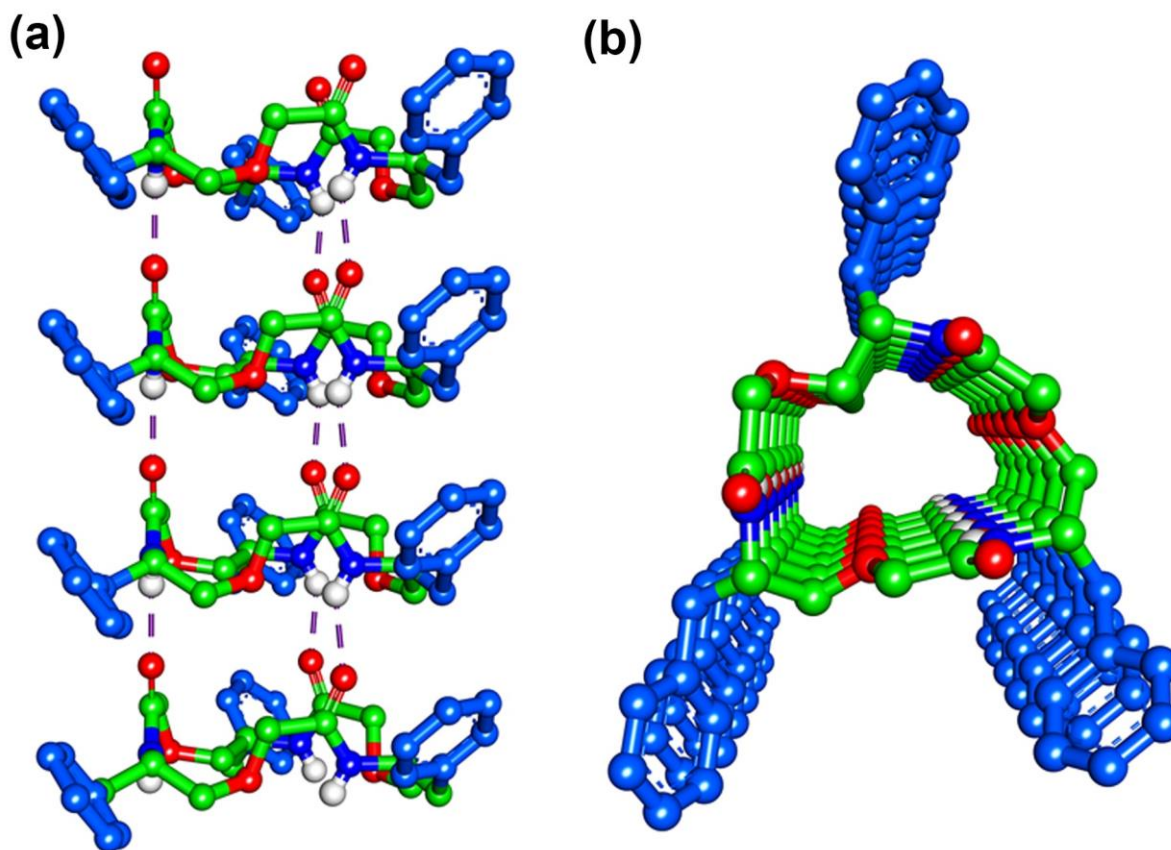


Figure 5: (a) Parallel β -sheet type of hydrogen bonding in **C8**, (b) Top View of self-assembled **C8**

The unique crystal structure of our self-assembled system motivates us to explore all possible interactions involved in this polar tubular packing. From the crystallographic packing, it is evident that **C8** contributes a cavity to the self-assembled system. The overall packing of the tubular structure of **C8** is described below.

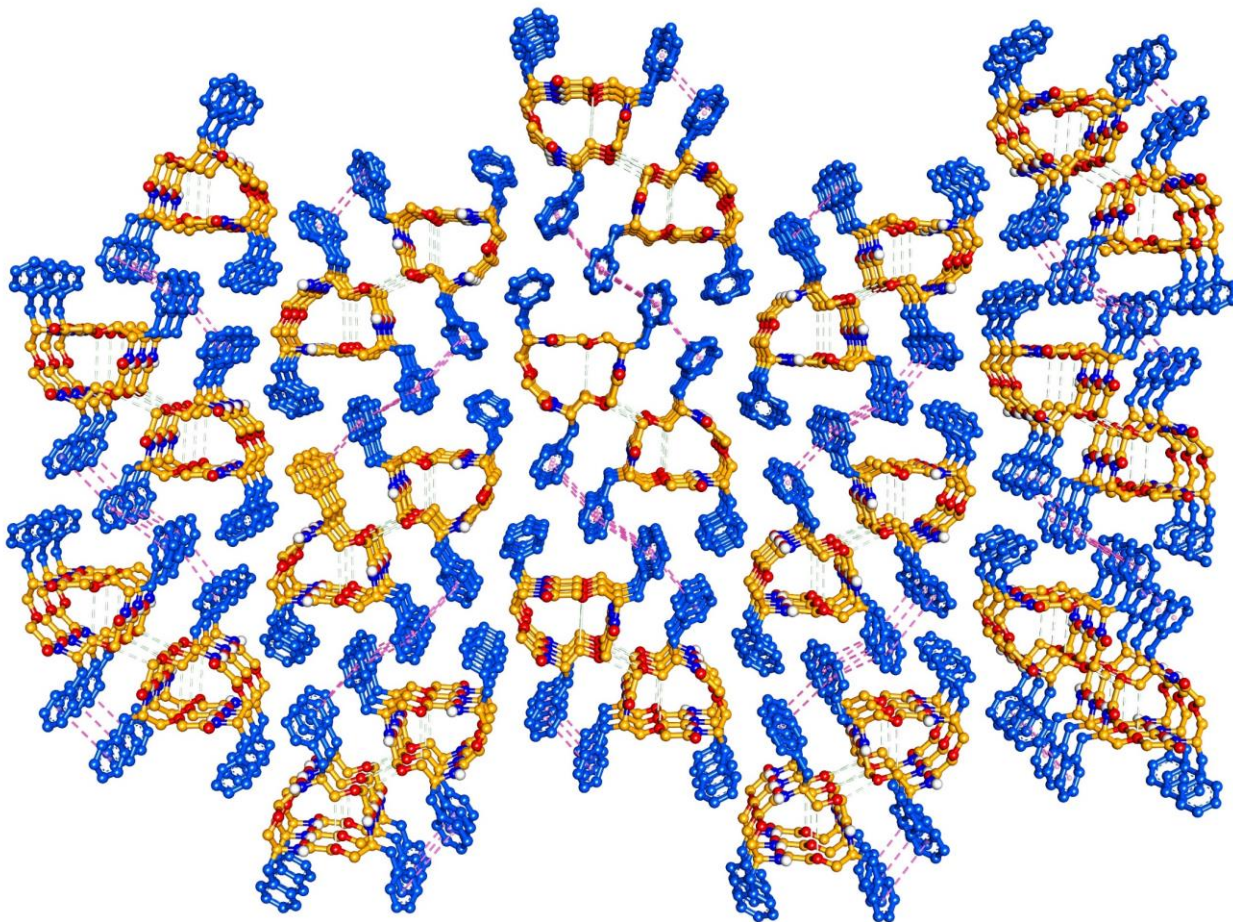


Figure 6: Overall packing of tubular self-assembled **C8** molecules and their interactions throughout assembly.

The self-assembled structures of **C8** display four distinct interaction types, which can be categorized as follows:

- (i).... Intermolecular Hydrogen Bonding between carbonyl O and amide NH ($\text{CO} \cdots \text{HN}$).
- (ii).... Intramolecular Hydrogen bonding between β -O atom and γ -CH₂ unit, ($\text{C}_\gamma\text{H} \cdots \text{O}_\beta$).
- (iii).... Hydrophobic Pi-Pi T-shaped Stacking and CH-Pi Interaction.
- (iv).... Intermolecular Hydrogen bonding between two macrocycles ($\text{C}_\alpha\text{H} \cdots \text{O}_\beta$).

Self-assembled structure is favoured by several non-covalent interactions like intermolecular hydrogen bonding, stacking interactions between aromatic chromophores.

4.5.4. Hirshfeld Surface Analysis of C8:

Hirshfeld surface analysis²⁸ was conducted to investigate all non-covalent molecular interactions of **C8** using Crystal Explorer 3.1 software. The analysis utilized the single-crystal X-ray crystallographic information file (CIF) to visualize various interactions present on the Hirshfeld surface. These interactions were depicted through 3D color mapping images, including normalized contact distance (d_{norm}), shape index, and curvedness. The surface colors representing strong interactions were depicted in red, medium in blue, and weak in white. The shape index, a dimensionless quantity, indicated the local shape of convex and concave regions on the surface, with values ranging between 1.0 (most convex) and -1.0 (most concave), while values were fixed at 0 for minimal surface regions. Curvedness measured the degree of curvature of the Hirshfeld surface, with a general range of -4.0 to 0.4. Additionally, a 2D fingerprint plot was generated, illustrating the distances of atoms nearest to the interior (d_i) and exterior (d_e) of the Hirshfeld surface. Different contours in the plot depicted various types of molecular interactions and contact information. The Hirshfeld surface images were generated with an iso-value of a weight function of 0.5 au. Summary of the observed Hirshfeld analysis data is presented in below figures.

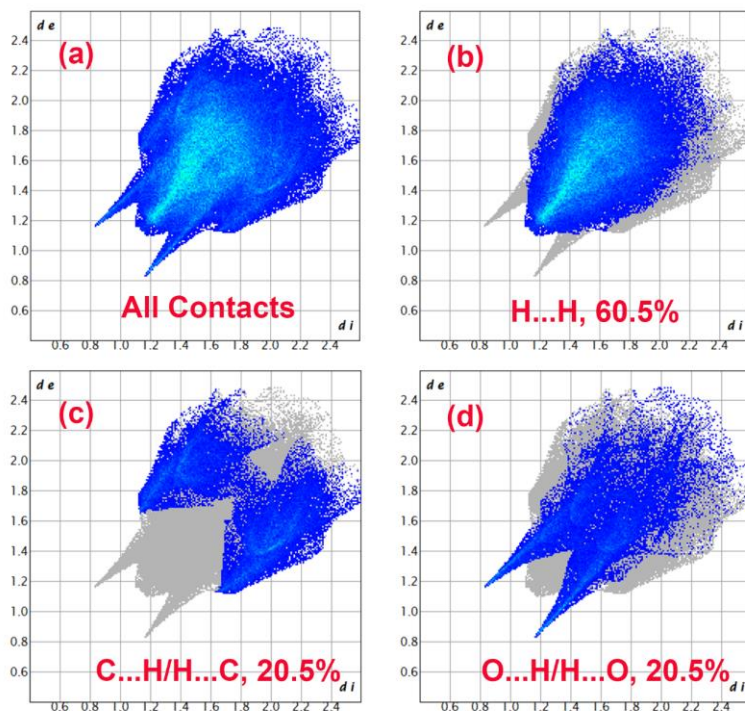


Figure 7: (a-d) The 2D fingerprint plot (d_e vs d_i) of **C8** at 296 K.

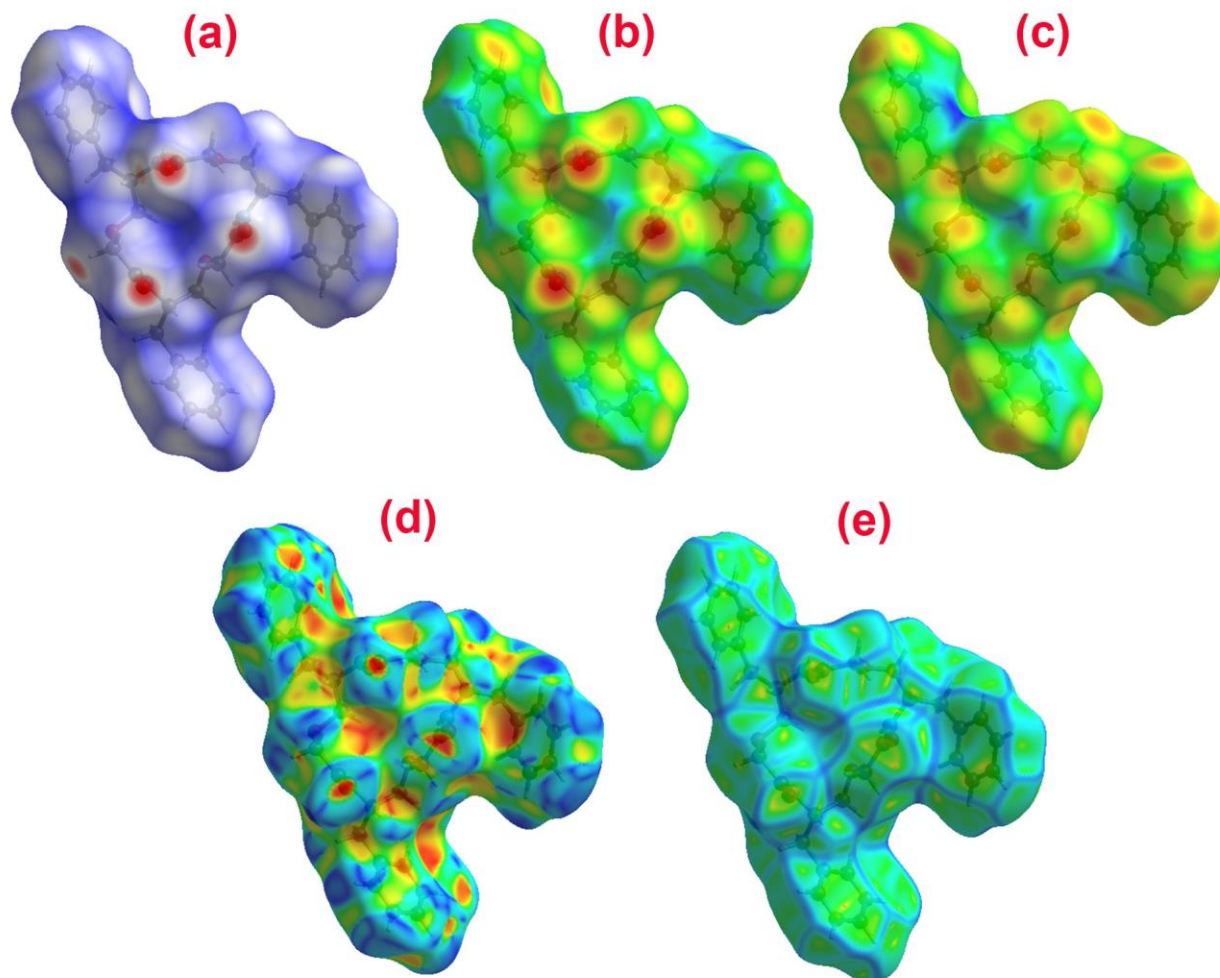


Figure 8. The 3D color mapping Hirshfeld surface analysis of **C8** (296 K) shows (a) d_{norm} , (b) d_{e} , (c) d_{i} , (d) shape index and (e) curvedness.

4.5.5. Solution Structure Analysis of C8:

4.5.5.1. SEM and TEM Analysis of C8:

To further understand the morphology of the self-assembled cyclic peptides, we subjected them to FE-SEM and HR-TEM analysis. Solutions of cyclic peptide **C8** in methanol/water (1 mg/ml) was incubated and examined for their self-assembly. Both FE-SEM and HR-TEM analyses revealed a sheet-shaped supramolecular assembly.

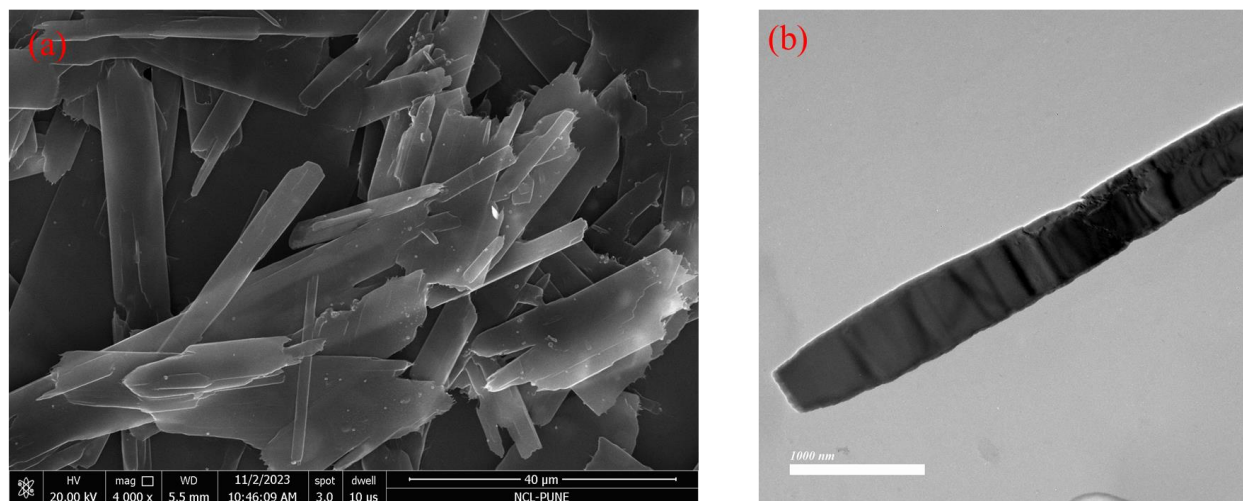


Figure 9: The images of **C8** obtained from (a) FE-SEM (b) HR-TEM are shown.

4.5.6. Transmembrane Ion Channel Activity:

Valinomycin has been widely employed as a transmembrane ion channel, with its ion transport mechanism being extensively studied. Despite our designed cyclic peptides **C3** and **C8-C13** being relatively short, we sought to determine their ability to mediate ion transport across membranes. For this purpose, we prepared liposomes containing 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) using established protocols, and conducted a membrane transport assay in HEPES buffer. A pH gradient was maintained with 0.5 N NaOH, and we added increasing concentrations of all individual cyclic peptides to the solution, monitoring fluorescence over time. As the concentration of the peptides increased, fluorescence emission also increased (Figure 10). We then lysed the liposomes with Triton X-100. The fluorescence emission data, analyzed using the Hill equation, provided EC_{50} values of 31.8 μM and 105 μM for **C8** and **C12**, respectively (see details in experimental section).

Cyclic dipeptide **C3** exhibits no activity due to the small cavity size in its tubular assembly. We anticipated that increasing aromaticity would enhance transmembrane activity. However, we observed minimal activity with **C9**, **C10**, and **C11**. These highly hydrophobic aromatic cyclic peptides did not dissolve well and precipitated immediately upon addition to the vesicle solution. Maybe the non-planar backbone of cyclic tetrapeptide **C9** and cyclic pentapeptide **C10** likely prevents them from adopting an ideal tubular structure, resulting in negligible ion transport

activity. Conversely, cyclic tripeptide **C8** demonstrated better activity, prompting us to conduct further experiments to investigate its ion selectivity.

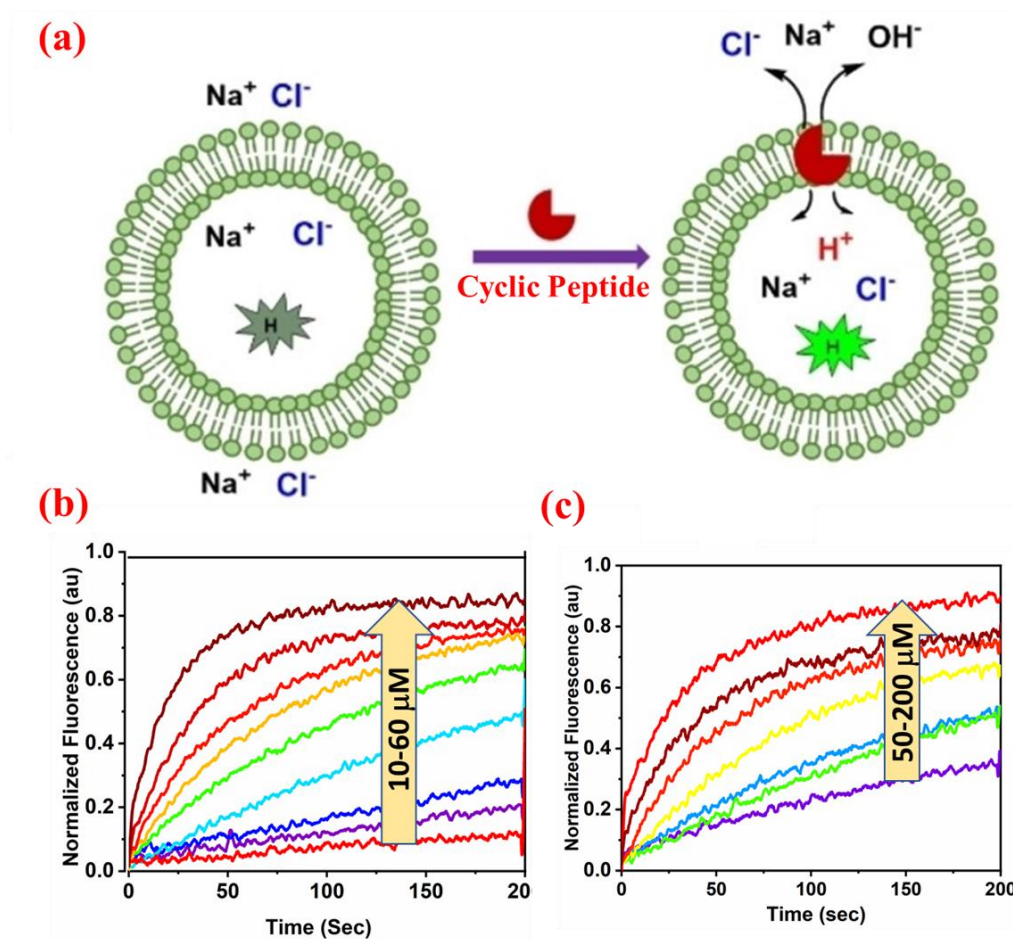


Figure 10: (a) Diagram²⁹ illustrating the ion transport activity of peptides **C8** and **C12**. The ion transport activity is shown to be dependent on concentration for (b) Cyclic Peptide **C8** and (c) Cyclic Peptide **C12**.

The differing ion transport activities of compounds when using NaCl salts led us to explore the ion selectivity of the most active compound **C8**. We started by examining the anion selectivity across EYPC-LUVs using HPTS, varying both the internal and external Na_nX (where n represents anion valency, $\text{X}_n^- = \text{F}^-, \text{Cl}^-, \text{Br}^-, \text{I}^-, \text{NO}_3^-, \text{AcO}^-, \text{ClO}_4^-$) salts. The results showed significant differences in HPTS fluorescence intensity with changes in the salt medium inside and outside the vesicles. This indicates that our channel-forming molecules preferentially select anions in the order of $\text{Cl}^- > \text{NO}_3^- > \text{F}^- > \text{Br}^- > \text{AcO}^- > \text{I}^- > \text{ClO}_4^-$ as shown in Figure 11. This data indicates that the

cavity is ideally suited for chloride ions. However, as the size of the anions increases, such as with I^- and ClO_4^- , the fit becomes imperfect, resulting in significantly reduced activity.

Additionally, we assessed the role of cations in the ion transport process across EYPC-LUVs using HPTS by varying both the internal and external MCl ($\text{M}^+ = \text{Li}^+, \text{Na}^+, \text{K}^+, \text{Rb}^+, \text{Cs}^+$) salts. The fluorescence intensity was monitored separately under a pH gradient of 0.8 ($\text{pH}_{\text{in}} = 7.0$ and $\text{pH}_{\text{out}} = 7.8$) by adding 0.5 M NaOH to the external buffer solution. No change in HPTS fluorescence intensity was observed with different cations, indicating that alkali metal ions do not play a role in the transport process. This suggests that our peptide is not selective towards cations but demonstrates selectivity for anions. Notably, it exhibits better ion channel activity with chloride ions.

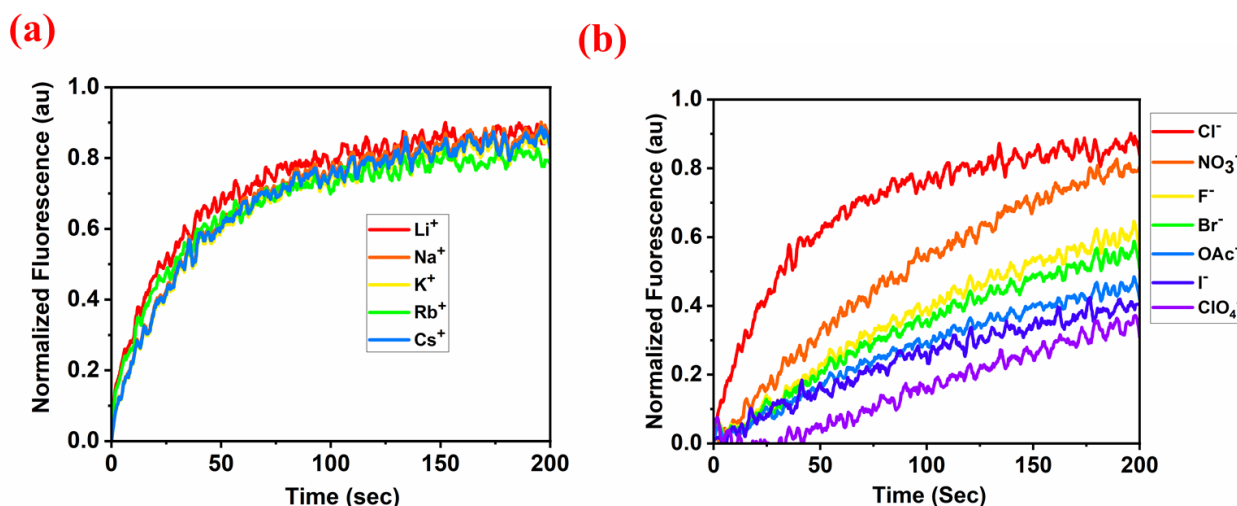


Figure 11: (a) Cation and (b) anion selectivity of **C8** across EYPC-LUVs, measured using HPTS.

To explore whether these peptides disrupt membranes rather than transport ions, a calcein leakage assay was performed (see experimental section). Notably, no fluorescence emission was detected even at high peptide concentrations, indicating that these peptides likely serve as ion transporters. Given their size, it is reasonable to conclude that these peptides function mainly as transmembrane channels.

4.5.7. ESI-MS Spectrum of X^- ($\text{X} = \text{Cl}^-, \text{Br}^-$) bound Complex of **C8**:

The formation of the X^- ($\text{X} = \text{Cl}^-, \text{Br}^-$) ion bound complex of **C8** in solution phase was confirmed from ESI-MS studies. For that, a solution of **C8** (10 μM) was prepared in acetonitrile and into that

solution 3.0 equivalents of tetramethylammonium halide salt like (TBAF, TBACl, TBABr) were added. Then the ESI-MS spectrum was recorded in negative (-ve) mode. While analyzing the mass spectrum data (attached in experimental section), the observed mass is exactly matched with complex mass and significant complex-mass only obtained after complexation. This proved that our design peptide can able to make complex that can be identified in solution phase.

4.5.8. NMR Titration of X^- ($X = Cl^-, Br^-$) bound Complex of C8:

Inspired by significant results from vesicle leakage and mass complex studies, which revealed a pre-organized anion-binding cavity in **C8**, we examined the binding affinities of these receptors for different anions ($X = Cl^-, Br^-, I^-$) using 1H NMR spectroscopic titration experiments in $CDCl_3$. Upon adding tetrabutylammonium (TBA) chloride salts to the macrocycle **C8** significant chemical shift perturbations were observed in the protons of amide (Figure 12). This suggests that halide ions bind to the receptors through amide proton. However, when excess TBABr, TBAI was progressively added to $CDCl_3$, no notable perturbations were detected in the macrocycle 1H NMR spectra (as illustrated in Figure 13 and 14). This suggests that the macrocyclic and interlocked hosts bind more specifically to chloride ions, as these ions better fit the cavity.

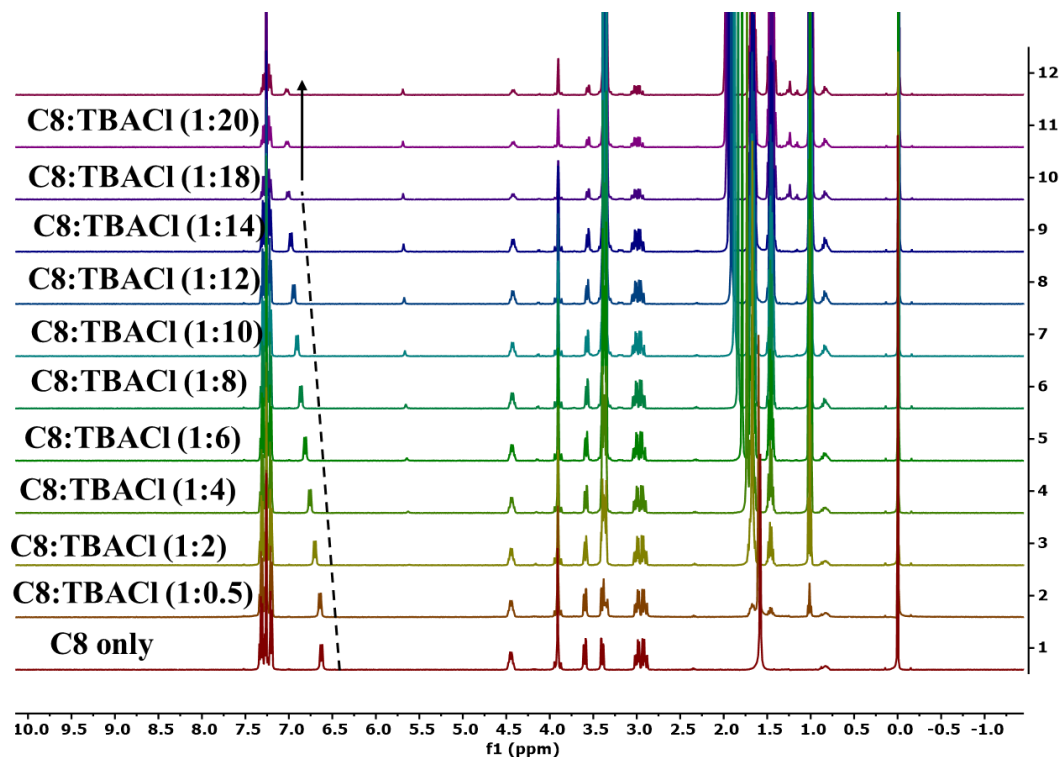


Figure 12: NMR Titration of **C8** with varying concentration ratio of TBACl.

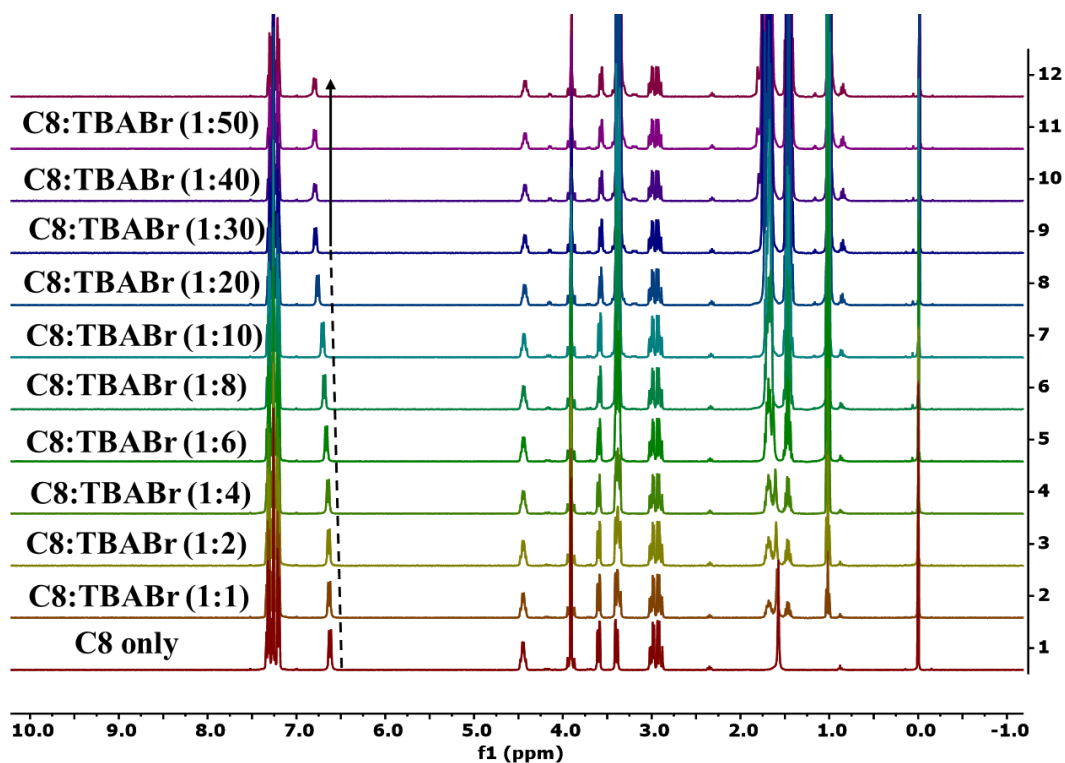


Figure 13: NMR Titration of C8 with varying concentration ratio of TBABr.

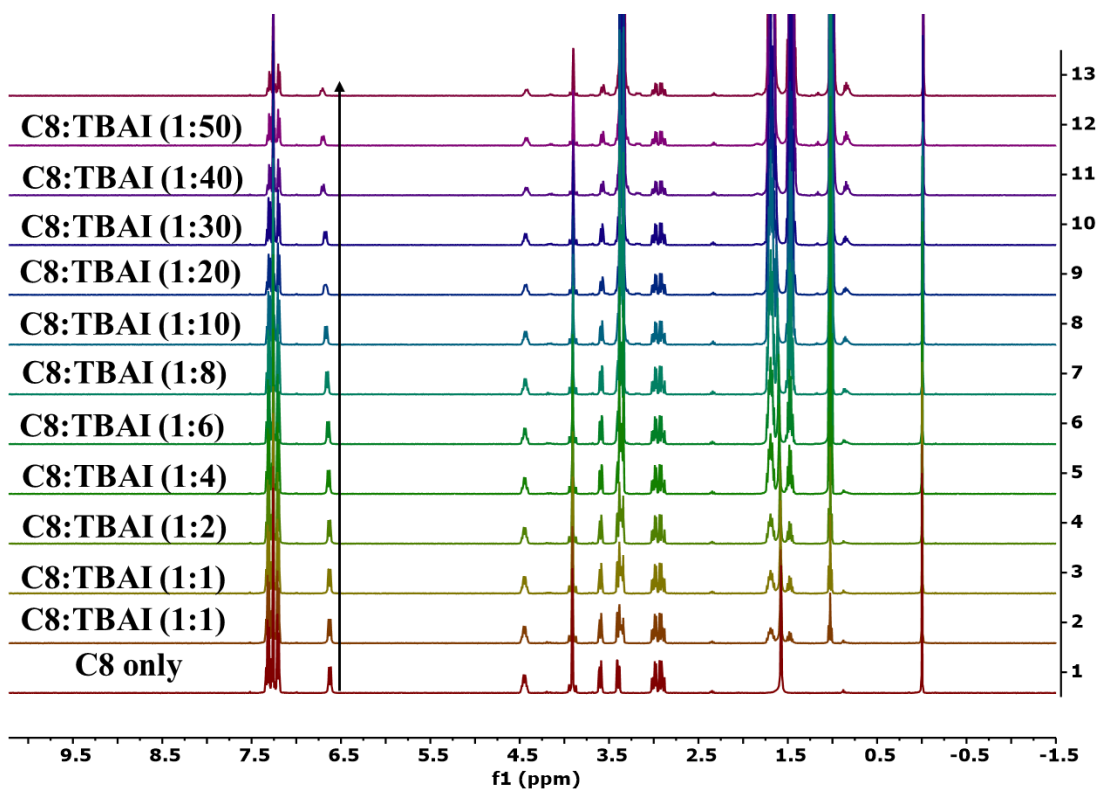


Figure 14: NMR Titration of C8 with varying concentration ratio of TBAI.

4.5.9. 2D-DOSY NMR Experiment of **C8** and $[\text{C8}+\text{Cl}]^-$ complex:

To examine the aggregation behavior and complex formation with chlorides, we conducted 2D-DOSY NMR experiments for both the free receptor **C8** and its anionic complex in a CDCl_3 solution. Initially, the 2D-DOSY NMR spectrum for a 5.0 mM solution of the cyclic peptide **C8** was recorded, yielding a diffusion coefficient of $9.40 \times 10^{-10} \text{ m}^2/\text{s}$ (Figure 15). Upon adding 3.0 equivalents of TBACl to the same 5.0 mM receptor solution, the diffusion coefficient decreased to $8.24 \times 10^{-10} \text{ m}^2/\text{s}$ (Figure 16). This reduction in the diffusion coefficient, from $9.40 \times 10^{-10} \text{ m}^2/\text{s}$ to $8.24 \times 10^{-10} \text{ m}^2/\text{s}$, upon the addition of Cl^- ions, indicates that chloride ions bind to **C8**. The decrease in the diffusion coefficient suggests an increase in supramolecular aggregation within the complex.

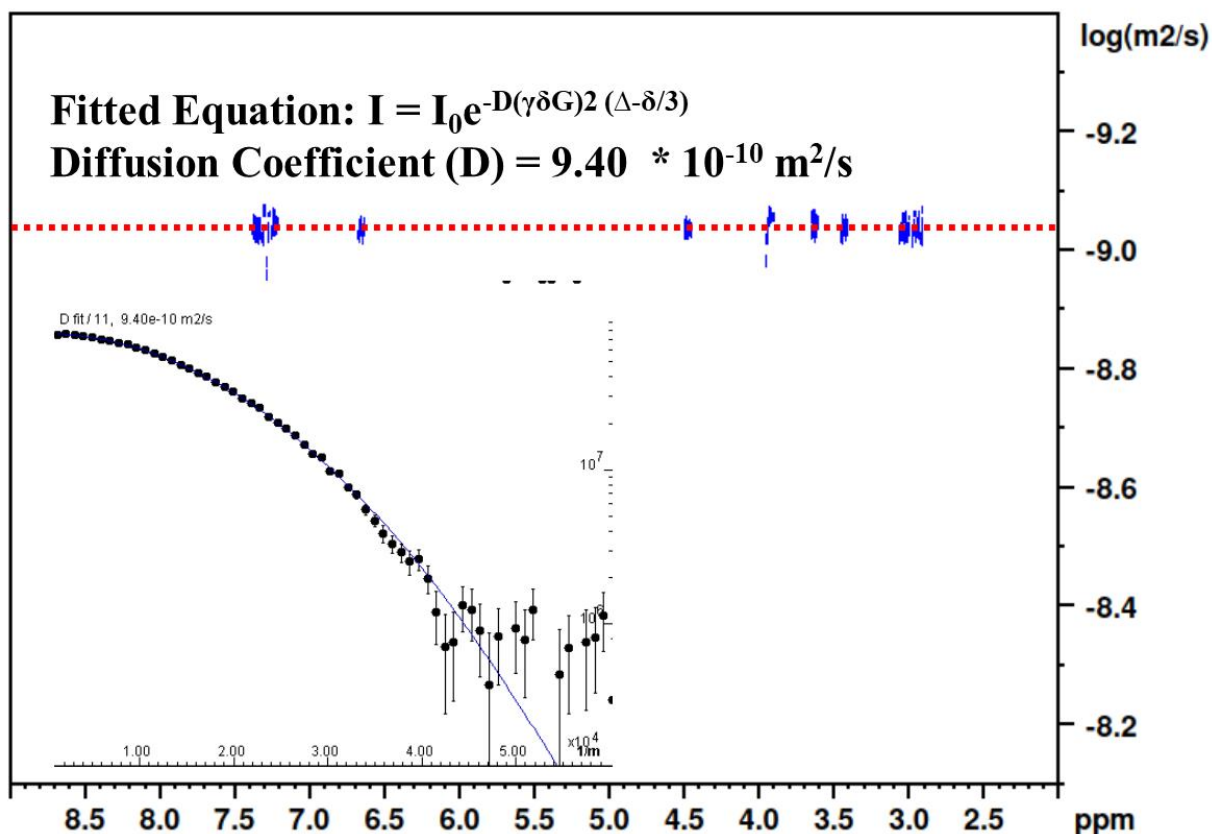


Figure 15: 2D-DOSY NMR experiments for free receptor **C8**.

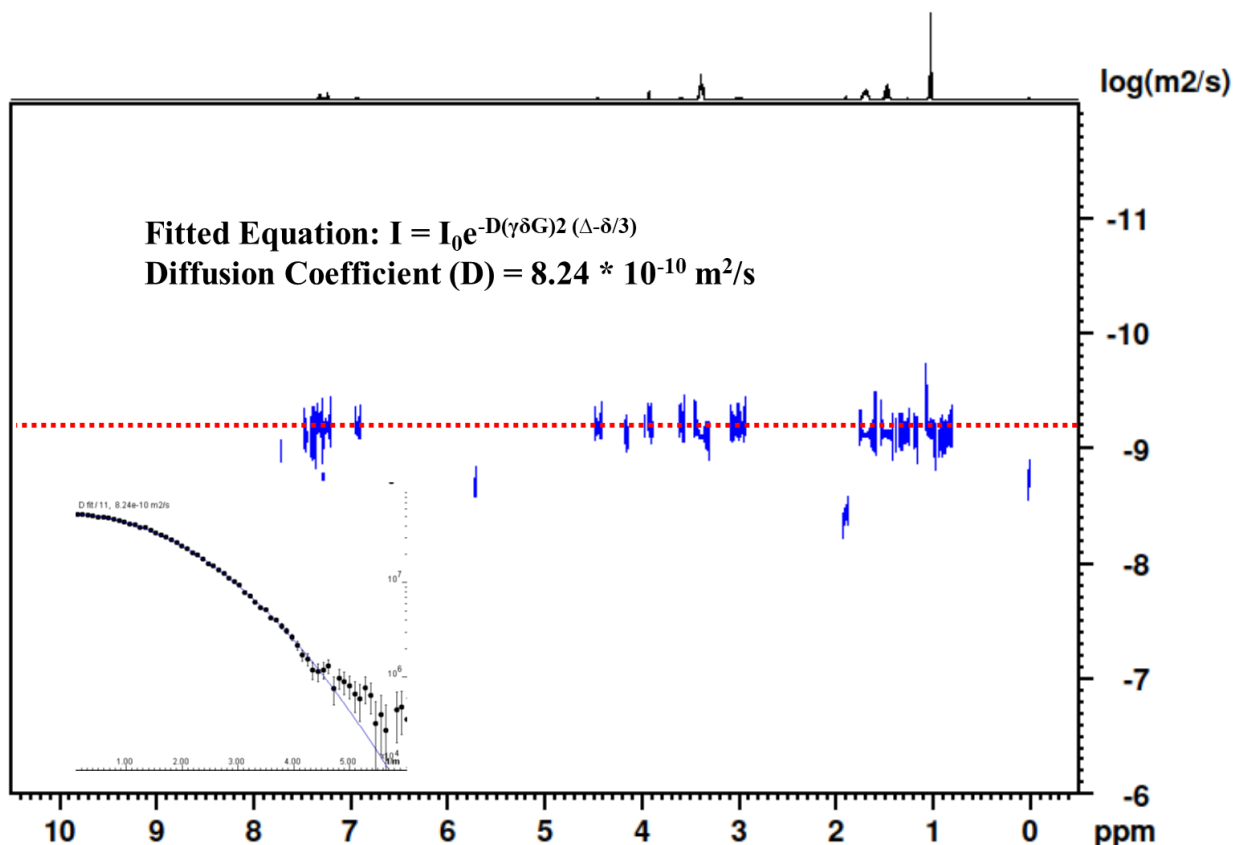


Figure 16: 2D-DOSY NMR experiments for both the free receptor **C8** and its anionic complex in a CDCl_3 solution.

4.6. Conclusion:

In summary, we report a cyclic peptide library derived from $\beta(\text{O})\text{-}\delta^5$ -amino acid, which effectively mimics a hybrid of crown ether and peptide macrocycles. Our findings demonstrate that the cyclic peptide, originating from the oligomer of $\beta(\text{O})\text{-}\delta^5$ -amino acid, self-assembles into hollow tubular structures with a pore diameter of approximately $5.25 \text{ \AA}$. Among these size-variant hybrid peptide macrocycles, the cyclic tripeptide exhibits superior tubular assembly, as confirmed by crystal structure analysis. These tubular structures are stabilized by intermolecular hydrogen bonds, but unlike those derived from α -amino acids, the $\beta(\text{O})\text{-}\delta^5$ -amino acid cyclic peptides are stabilized by unidirectional hydrogen bonds. The solubility of cyclic peptides formed from δ -amino acids is significantly influenced by the oxygen atom in their backbone, as evidenced by the discovery that the cyclic peptide made up of the carbon analogs of delta amino acid was insoluble in the majority of organic solvents. Assays for vesicle leakage showed that these cyclic peptides further assemble

into tubular structures within lipid bilayers, operating as transmembrane ion channels. Ion selectivity experiments indicated that while these peptides are not selective for cations, they exhibit a preference for anions, especially chloride ions. NMR titration experiments showed a higher binding affinity for chloride ions compared to other halides, which was further supported by mass complex analysis.

Our comprehensive study, encompassing vesicle leakage experiments, solution-phase NMR, and mass complex experiments, confirms that the tubular assembly and exposed amide groups are crucial for anion binding. This foldameric design of tubular assemblies demonstrates their potential as highly effective anion-selective templates.

In conclusion, our innovative design of polar crown ether peptide macrocycles offers promising new approaches for developing biomaterials based on peptide macrocycles and artificial transmembrane ion channels, paving the way for advancements in the field of biomolecular engineering.

4.7. Experimental Section:

4.7.1. Materials and Methods:

All reactions were conducted using solvents that were distilled and dried, obtained from Sigma-Aldrich and Merck, and oven-dried glassware. Proton NMR (400 MHz/600 MHz) and carbon-13 NMR (101 MHz/151 MHz), as well as DOSY (400 MHz) spectra, were acquired in CDCl₃ on a Bruker NMR instrument. The NMR spectra were processed using Bruker's TopSpin and MestreNova NMR software. Chemical shifts are reported in δ units (parts per million/ppm) relative to TMS as an internal standard. Using a time-of-flight (TOF) analyzer, HRMS were acquired in both positive and negative ion ESI modes. On pre-coated plates (0.20 mm, silica gel 60 F254, sigma-aldrich), TLC was performed. Column chromatography was carried out using silica gel (100–200 mesh). The coupling reagents, including 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), and 2-Chloro-1-methylpyridinium iodide (CMPI), as well as solvents such as Diisopropylethylamine (DIPEA), Tetrahydrofuran (THF), Methanol (MeOH), and acetonitrile, were procured from Sigma-Aldrich and were utilized in peptide coupling reactions in solution phase. Following neutralization, the workup process involved washing the combined organic layer with aqueous sodium bicarbonate,

1N HCl, water, and brine, followed by drying with anhydrous sodium sulfate and evaporating the solvent under reduced pressure. HPLC was used to purify all the final molecules. Circular dichroism (CD) analyses were performed on a JASCO J-1500 CD spectrometer using a 2 mm path length cell. The spectra were generated by accumulating 3 scans at a scan speed of 100 nm/min, with a resolution of 1.0 nm, a band-width of 5.0 nm, and a 1-second response time. The resulting spectra were smoothed (5) and plotted using Origin Pro 6.0 software.

Constraints were derived from volume integrals obtained from the NOESY spectra using a two-spin approximation and a reference distance of 1.8 Å for the geminal protons. The upper and lower bounds of the distance constraints were set by increasing and decreasing the derived distance by 10%. In ion transport studies, egg-yolk phosphatidylcholine (EYPC) was obtained from Avanti Polar Lipids, as a solution (25 mg/mL in CHCl₃) and as a solid, respectively. Additionally, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethane sulfonic acid (HEPES) buffer, 8-hydroxypyrene-1,3,6-trisulfonate (HPTS) dye, Triton X-100, NaOH, and inorganic salts were purchased in molecular biology grade from Sigma. The HEPES buffer solution was prepared using Milli-Q water, which was filtered and autoclaved to eliminate microbial contaminants. This step was necessary as some buffers, such as HEPES, are not resistant to high temperatures. Acetonitrile was sourced from Sigma-Aldrich. Fluoromax-4 with an injector port and a magnetic stirrer was used to record fluorescence spectra. A Helmer pH meter was used to monitor pH, and Origin 8.5 3.51 software was used to handle data from fluorescence investigations.

4.7.2. General Procedure for the Synthesis of Peptides:

The synthesis and characterisation of cyclic peptides in both solid and solution phases was thoroughly discussed in the previous chapter.

4.7.3. Ion Channel Experiments:

4.7.3.1. Ion Transport Activity:

A saline solution was created by using autoclaved water containing 100 mM of NaCl and 10 mM of HEPES. The initial pH of the solution was below 7.0, and to adjust it to around 7.0, an appropriate amount of NaOH solution (0.5 M) was introduced. Subsequently, a 1 mM HPTS

solution was prepared from solid HPTS using the previously mentioned buffer solution. The stock solution of the transporter for the HPTS assay was formulated using HPLC-grade Acetonitrile.

4.7.3.2. Preparation of EYPC-LUVs $\Rightarrow$ HPTS:

Initially, 1 mL of EYPC lipid solution (25 mg/mL in chloroform) was placed in a 10 mL round-bottomed flask. The chloroform within the lipid solution was then removed by a gradual stream of nitrogen gas while rotating the round-bottomed flask, resulting in a thin lipid film. Any residual chloroform in the lipid film was further eliminated by subjecting it to high vacuum drying for approximately 5 hours. The thin lipid film was hydrated with an HPTS solution (1 mM HPTS, 10 mM HEPES, 100 mM NaCl, pH 7) and then subjected to vortexing five times (2 minutes each time) with 10-minute intervals, with the entire process taking around 1 hour.

Following hydration, the vesicle suspension underwent 15 freeze-thaw cycles, followed by 19 extrusions (an odd number) through a 100 nm polycarbonate membrane. The extravesicular dye was separated from the vesicles using size exclusion column chromatography (employing Sephadex G-50 gel) and eluted with a buffer solution (10 mM HEPES, 100 mM NaCl, pH 7).

After collecting the vesicles from the column, they were diluted to 6 mL using the aforementioned buffer (10 mM HEPES, 100 mM NaCl, pH 7) to achieve a concentration of approximately 5.5 mM of EYPC-LUVs $\Rightarrow$ HPTS, assuming no lipid loss occurred throughout the process. The composition of the vesicles was maintained with 1 mM HPTS, 10 mM HEPES, 100 mM NaCl, pH 7 inside and 10 mM HEPES, 100 mM NaCl, pH 7 outside.

4.7.3.3. Description of the HPTS assay for the study of ion transport:

In a pristine cuvette, 1975 μ L of buffer solution (10 mM HEPES, 100 mM NaCl, pH 7) and 25 μ L of vesicle solution containing HPTS were combined, yielding a lipid concentration of approximately 65 μ M. This mixture was then placed in a fluorescence instrument equipped with a magnetic stirrer. The fluorescence emission intensity (I_t) of the HPTS dye was recorded at $\lambda_{em} = 510$ nm (with $\lambda_{ex} = 450$ nm) for a duration of 350 seconds, considering the instrument's start time as $t = 0$ s for each reading.

At $t = 20$ s, 20 μ L of 0.5 M NaOH solution was introduced into the same cuvette to establish a pH gradient (Δ pH = 0.8) between the intra and extra-vesicular environments. Subsequently, at $t = 100$

s, 20 μL solutions of transporters in Acetonitrile at various concentrations were added. At $t = 300$ s, 10% Triton X-100 (25 μL) was introduced to disrupt all vesicles and eliminate the pH gradient.

For vesicle preparation, 25 mg of EYPC lipid (Egg PC, Formula Weight = 770.123) was utilized, resulting in a 6 mL stock solution/suspension of vesicles with a concentration of approximately 5.4 mM, assuming minimal lipid loss during the preparation process. Fluorescence experiments were conducted by suspending 25 μL of this stock solution in 2 mL of buffer solution, yielding a final lipid concentration of approximately 65 μM .

The fractional emission intensity (in percentage), I_F (Figure 17) was calculated after normalizing all the data using the following equation 1.

$$I_F = [(I_t - I_0)/(I_\infty - I_0)] \times 100 \quad \text{Eq. 1}$$

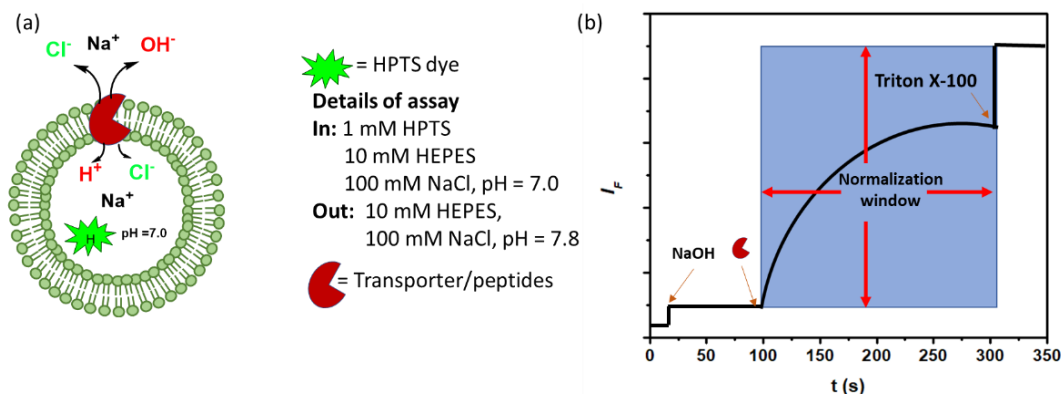


Figure 17: (a) Illustration of fluorescence assay for evaluating ion transport activity using EYPC-LUVs and HPTS. (b) Graph representing ion transport kinetics, highlighting normalization range.²⁹

4.7.3.4. HPTS Assay for Checking Ion Selectivity:

Autoclaved water with a strength of 100 mM MX (MX = NaCl, NaBr, NaI, NaNO₃, NaOAc, LiCl, KCl, RbCl, and CsCl) and 10 mM HEPES was used to generate various buffer solutions. NaOH solution (0.5 M) was added to the solutions to bring their pH levels down to 7.0. Using acetonitrile of HPLC quality, the stock solution for the ion selectivity experiment was made from solid transporter material.

4.7.3.5. Preparing Vesicles for HPTS Assay Ion Selectivity:

The identical process previously described was used to create vesicles for the ion selectivity experiment.

4.7.3.6. A Clarification of the HPTS Cation Selectivity Assay:

25 μL of the HPTS-encapsulated vesicles solution and 1975 μL of the buffer solution (10 mM HEPES, 100 mM MCl, pH 7, where $\text{M}^+ = \text{Na}^+, \text{Li}^+, \text{K}^+, \text{Rb}^+, \text{and } \text{Cs}^+$) were combined and put in a clean cuvette that was then put in a fluorescence spectrometer with a magnetic stirrer. The intensity of the HPTS dye's fluorescence emission was measured for 350 seconds at an emission wavelength of 510 nm (excited at 450 nm). For every reading, the instrument's start time was set to $t = 0$ s. The cuvette was filled with 20 μL of 0.5 M NaOH solution at $t = 20$ s in order to produce a pH gradient ($\Delta\text{pH} = 0.8$) between the vesicles' outside and interior. Next, 20 μL of the transporter solution in acetonitrile was added at $t = 100$ s. 25 μL of 10% Triton X-100 was added at $t = 300$ s in order to lyse the vesicles and remove the pH gradient. Equations 1 and 2 were used to standardize all of the data.

4.7.3.8. A Clarification of the pH Dissipation Trends Checking HPTS Assay:

1975 μL of the buffer solution (10 mM HEPES, 100 mM NaX, pH 7; where $\text{X}^- = \text{F}^-, \text{Cl}^-, \text{Br}^-, \text{I}^-, \text{NO}_3^-, \text{ClO}_4^-, \text{and } \text{AcO}^-$), 25 μL of the HPTS trapped vesicles solution, and a fluorescence equipment with a magnetic stirrer were placed in a clean cuvette. The HPTS dye's fluorescence emission intensity was measured for 350 seconds at $\lambda_{\text{em}} = 510$ nm (where $\lambda_{\text{ex}} = 450$ nm). The instrument's start time was taken into consideration for each reading, with $t = 0$ s. Subsequently, at $t = 20$ s, a pH gradient ($\Delta\text{pH} = 0.8$) between the intra and extra vesicular media was created in the same cuvette by adding 20 μL of 0.5 M NaOH solution. At $t = 100$ s, a 20 μL solution of the transporter in acetonitrile was then added. 10% triton X-100 (25 μL) was added at $t = 300$ s to completely kill all of the vesicles and eliminate the pH gradient. Equations 1 and 2 in the Supplementary Data were used to standardize all the data.

4.7.3.9. Activity Dependent on Dose:

The ion transport activities of all the peptides were evaluated at various concentrations. Specifically, the ion transport activities of P1 and P3 at different concentrations are shown below. The concentration profile data were analyzed at $t = 300$ s to determine the effective concentration,

Chapter 4

EC_{50} , which is the concentration needed to achieve 50% ion efflux activity, using the Hill equation (Eq. 2).

$$Y = Y_{\infty} + (Y_0 - Y_{\infty}) / [1 + (c/EC_{50})^n] \quad \text{Eq. 2}$$

where, Y_0 = Fluorescence intensity immediately prior to the addition of the channel-forming molecule (at $t = 0$ s), Y_{∞} = Fluorescence intensity at high compound concentration. c = Concentration of a molecule that forms channels., and n = Hill coefficient (*i.e.*, The number of molecules needed for the transportation of a single ion).

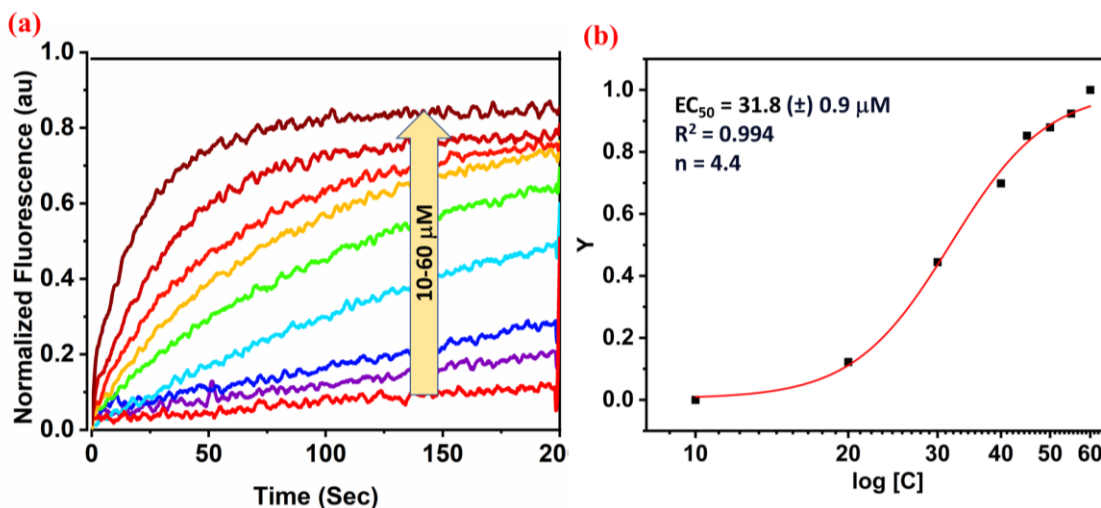


Figure 18: The ion transport activity of cyclic peptide **C8**, measured at concentrations ranging from 10 to 60 μM with NaCl salt across EYPC–LUVs and using HPTS, is shown. Additionally, the Hill plot for Peptide **C8** at $t = 290$ seconds is presented.

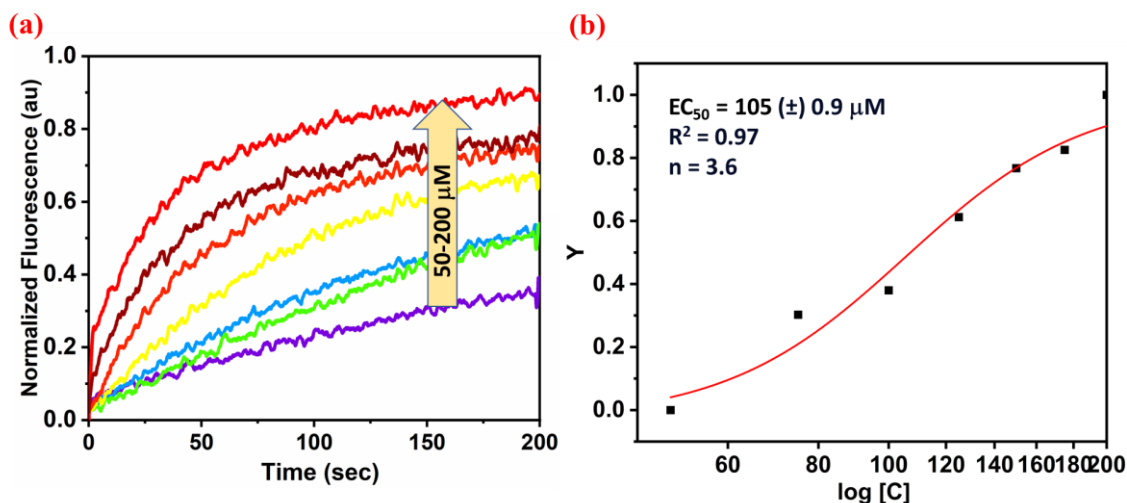


Figure 19: The ion transport activity of cyclic peptide **C12**, measured at concentrations ranging from 10 to 60 μM with NaCl salt across EYPC–LUVs and using HPTS, is shown. Additionally, the Hill plot for Peptide **C8** at $t = 290$ seconds is presented.

4.3.9. Calcein Leakage Assay Experiment:

Preparation of Buffer Solutions: The Calcein-HEPES buffer was created by dissolving solid calcein at a concentration of 50 mM in a solution containing 10 mM NaCl and 10 mM HEPES. To dissolve the mixture, concentrated NaOH was added in small amounts, and the pH was adjusted to 7.0. For the extravesicular buffer, 100 mM NaCl and 10 mM HEPES were dissolved in MilliQ water, and the pH was adjusted to 7.0 using 0.5 M NaOH.

4.3.10. Preparation of EYPC-LUVs $\Rightarrow$ calcein:

25 mg of EYPC were dissolved in 1 ml of CHCl_3 to form a thin lipid film, which was subsequently vacuum-expelled for four hours. The resultant lipid film was periodically vortexed (4-5 times) for an hour while being hydrated with 0.5 ml of a buffer solution (10 mM HEPES, 10 mM NaCl, 50 mM calcein, pH 7.0). After that, a freeze-thaw cycle utilizing liquid nitrogen and a 55 $^{\circ}\text{C}$ water bath was carried out at least 20 times. The resulting vesicle solution was then run through an Avanti polycarbonate membrane with 19 nm pores, making sure that the number of passes was odd, to create vesicles with an average diameter of 100 nm. Size exclusion chromatography (Sephadex G-50) was used with a 10 mM HEPES buffer containing 100 mM NaCl, pH 7.0, to exclude any extracellular dye. After that, the concentrated vesicles were diluted to a final volume of 3 ml, which produced EYPC-LUVs $\Rightarrow$ calcein. About 5.0 mM EYPC lipid was present in the final composition, along with an extravesicular solution containing 10 mM HEPES and 100 mM NaCl at pH 7.0 and an intravesicular solution containing 10 mM HEPES, 10 mM NaCl, and 50 mM calcein.

4.3.11. Determination of Ion Leakage by Calcein Assay:

1980 μL of HEPES buffer (10 mM HEPES, 100 mM NaCl, pH 7.0) and 20 μL of EYPC-LUVs $\Rightarrow$ Calcein were added to a dry, clean fluorescence cuvette. After that, starting at $t = 0$ s, the cuvette was put in a fluorescence spectrometer that had a magnetic stirrer for gentle mixing. At an emission wavelength of 515 nm (excitation at 495 nm), the fluorescence emission intensity of calcein was constantly observed. The peptides were injected at the appropriate concentrations at t

= 50 s, and 25 μ L of 10% Triton X-100 was added at $t = 300$ s to lyse the vesicles and disturb the pH gradient. Next, using Eq. S1, the gathered data were standardized to show the percentage change in fluorescence intensity over time..

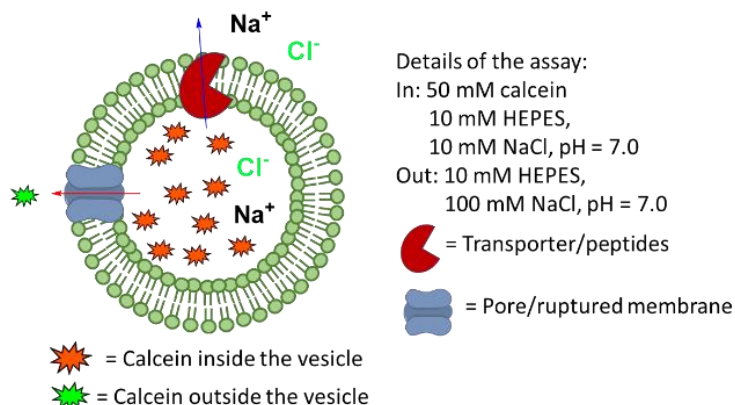


Figure 20: Diagram showing calcein release from EYPC-LUVs $\Rightarrow$ calcein when exposed to an ion transporter compared to a pore-forming or membrane-disrupting agent.²⁹

4.7.4. Diffusion-ordered NMR Spectroscopy (DOSY):

DOSY in NMR has emerged as a valuable technique for investigating non-covalent interactions between multiple molecules in solution. The rate of diffusion is linked to the size of the molecule, with larger molecules displaying slower diffusion rates due to increased hydrodynamic radius. NMR spectroscopy enables the measurement of translational diffusion through the use of pulsed-field gradients (PFG). These gradients induce variations in the magnetic field within the NMR sample, causing changes in the precession frequencies of specific signals, which are dependent on the effective magnetic field.

The signal evolution under PFG is corrected by applying an identical PFG after a 180° radio frequency pulse, known as refocusing. However, this refocusing is only effective if there is no diffusion occurring in the sample. Translational diffusion results in magnetization decay, represented by the equation:

$$I = I_0 e^{-D(\gamma \delta G)^2 (\Delta - \delta/3)}$$

The intensity measured after applying the refocusing gradient is denoted by I , while I_0 represents the intensity without any gradient applied. The diffusion coefficient is indicated by D , and the

gyromagnetic ratio is symbolized by γ . The gradient strength and its duration are represented by G and δ , respectively, and the time interval between the two gradients is denoted by Δ .

Using the known diffusion constants (D), the formula weights (FW) of molecules or systems can be determined through the relationship $\log D \propto \log FW$.

This estimated formula weight provides a good approximation of the number of interacting molecules and their proportions. The spin-echo diffusion method with PFG has been widely used to investigate intermolecular hydrogen bonding, ion pairing, aggregation, π - π stacking in supramolecular chemistry, and transition metal catalysts. DOSY has also been applied to determine the formula weights of polymers and the structures of organometallic complexes in solution. Additionally, DOSY is valuable for determining the presence or absence of intermolecular interactions or molecular aggregation.

Free molecules exhibit a specific diffusion rate, which decreases when they interact with other molecules, resulting in an increase in the hydrodynamic radius. This technique is particularly effective for examining protein-ligand interactions, where the diffusion coefficient of a small ligand changes significantly upon binding to a large protein molecule. Due to the rapid decay in the diffusion filter, small free molecules with fast diffusion can be eliminated from the spectrum, while molecules binding to large biomolecules remain due to their considerably slower diffusion rates.

Similar to relaxation studies, changes in intermolecular interactions are monitored by observing alterations in molecular size (hydrodynamic radius) after interactions between two or more molecules. Smaller molecules exhibit a more noticeable change compared to larger molecules, as the sum of the hydrodynamic radii is significantly altered after the interaction. In our study, we have measured the diffusion coefficient of cyclic peptide **C8** in acetonitrile and compared its binding coefficient with TBACl containing $[\mathbf{C8}+\text{Cl}]^-$ complex.

4.7.5. SEM and TEM Analysis:

To investigate the self-assembly of the peptides, we first prepared a primary stock solution by dissolving the peptides in a methanol/water mixture at a concentration of 1 mg/mL. The stock solutions were then incubated at room temperature. Following incubation, the peptide solution was vigorously vortexed for about 1 minute and centrifuged. The resulting supernatant was applied in

2.5 μL droplets onto a thoroughly cleaned silicon vapor plate (SiO_2/Si substrate). This procedure was repeated at different time intervals. For all experiments, freshly prepared solutions were used. Finally, the samples were analyzed using the FE-SEM ZEISS ULTRA PLUS to capture the images. HR-TEM samples were prepared using a method similar to that for FE-SEM analysis. Specifically, 4 μL of the peptide solution was drop-cast onto carbon-coated copper grids and allowed to dry at room temperature. This step was performed to assess the stability of the peptide nanotubes. The dried samples on the TEM grids were then examined using the Bruker High-Resolution Transmission Electron Microscope to obtain the final images.

4.8. References:

1. R. E. Babine, S. L. Bender, *Chem. Rev.* **1997**, 97, 1359-1472.
2. a) S. H. Gellman, *Acc. Chem. Res.* **1998**, 31, 173-180; b) D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes, J. S. Moore, *Chem. Rev.* **2001**, 101, 3893-4011; c) C. M. Goodman, S. Choi, S. Shandler, W. F. DeGrado, *Nat. Chem. Biol.* **2007**, 3, 252-262.
3. a) K. Gademann, M. Ernst, D. Hoyer, D. Seebach, *Angew. Chem. Int. Ed.* **1999**, 38, 1223-1226; b) J. D. Sadowsky, M. A. Schmitt, H. S. Lee, N. Umezawa, S. Wang, Y. Tomita, S. H. Gellman, *J. Am. Chem. Soc.* **2005**, 127, 11966-11968; c) N. Koglin, C. Zorn, R. Beumer, C. Cabrele, C. Bubert, N. Sewald, O. Reiser, A. G. Beck-Sickinger, *Angew. Chem. Int. Ed.* **2003**, 42, 202-205; d) J. A. Kritzer, J. D. Lear, M. E. Hodsdon, A. Schepartz, *J. Am. Chem. Soc.* **2004**, 126, 9468-9469.
4. M. A. Gelman, S. Richter, H. Cao, N. Umezawa, S. H. Gellman, T. M. Rana, *Org. Lett.* **2003**, 5, 3563-3565.
5. S. Choi, D. J. Clements, V. Pophristic, I. Ivanov, S. Vemparala, J. S. Bennett, M. L. Klein, J. D. Winkler, W. F. DeGrado, *Angew. Chem. Int. Ed.* **2005**, 44, 6685-6689.
6. a) E. A. Porter, X. Wang, H. S. Lee, B. Weisblum, S. H. Gellman, *Nature* **2000**, 404, 565; b) Y. Hamuro, J. P. Schneider, W. F. DeGrado, *J. Am. Chem. Soc.* **1999**, 121, 12200-12201; c) R. F. Epand, T. L. Raguse, S. H. Gellman, R. M. Epand, *Biochemistry* **2004**, 43, 9527-9535.
7. D. Ranganathan, *Acc. Chem. Res.* **2001**, 34, 919-930.
8. a) J. T. Davis, O. Okunola, R. Quesada, *Chem. Soc. Rev.* **2010**, 39, 3843; (b) P. Lauser, *Angew. Chem. Int. Ed.* **1985**, 24, 905.
9. W. D. Stein, *Academic Press: San Diego, CA*, **1990**.
10. B. Hille, *Sinauer: Sunderland, MA*, **1992**.
11. B. Eisenberg, *Acc. Chem. Res.* **1998**, 31, 117-124.
12. a) J. T. Davis, P. A. Gale, R. Quesada, *Chem. Soc. Rev.* **2020**, 49, 6056-6086; b) X. Wu, E. N. W. Howe, P. A. Gale, *Acc. Chem. Res.* **2018**, 51, 1870-1879; c) L. E. Bickerton, T. G. Johnson, A. Kerckhoffs, M. J. Langton, *Chem. Sci.* **2021**, 12, 11252-11274; d) A. Barba-Bon, M. Nilam, A. Hennig, *ChemBioChem* **2020**, 21, 886-910.
13. A. L. Sisson, M. R. Shah, S. Bhosale, S. Matile, *Chem. Soc. Rev.* **2006**, 35, 1269-1286.

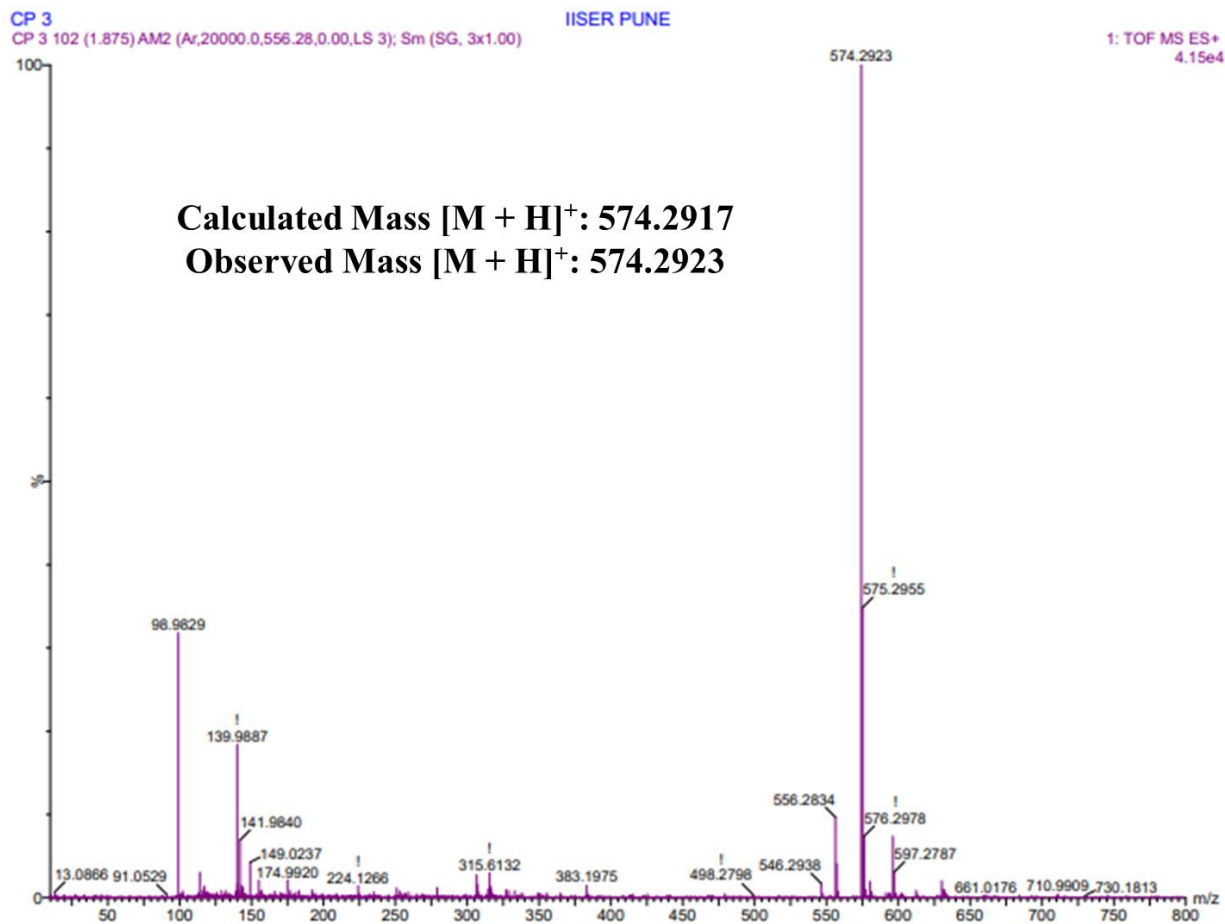
14. a) J. K. W. Chui, T. M. Fyles, *Chem. Soc. Rev.* **2011**, *41*, 148–175; b) T. M. Fyles, *Chem. Soc. Rev.* **2007**, *36*, 335–347; c) S. Matile, A. Vargas Jentzsch, J. Montenegro, A. Fin, *Chem. Soc. Rev.* **2011**, *40*, 2453–2474.
15. a) A. Vargas Jentzsch, A. Hennig, J. Mareda, S. Matile, *Acc. Chem. Res.* **2013**, *46*, 2791–2800; b) A. V. Jentzsch, D. Emery, J. Mareda, S. K. Nayak, P. Metrangolo, G. Resnati, N. Sakai, S. Matile, *Nat. Commun.* **2012**, *3*, 905; c) C. R. Yamnitz, S. Negin, I. A. Carasel, R. K. Winter, G. Gokel, *Chem. Commun.* **2010**, *46*, 2838–2840.
16. a) V. Sidorov, F. W. Kotch, G. Abdrakhmanova, R. Mizani, J. C. Fettinger, J. T. Davis, *J. Am. Chem. Soc.* **2002**, *124*, 2267–2278; b) J. L. Seganish, P. V. Santacrose, K. J. Salimian, J. C. Fettinger, P. Zavalij, J. T. Davis, *Angew. Chem. Int. Ed.* **2006**, *45*, 3334–3338.
17. a) D. S. Kim, J. L. Sessler, *Chem. Soc. Rev.* **2015**, *44*, 532–546; b) H. Li, H. Valkenier, L. W. Judd, P. R. Brotherhood, S. Hussain, J. A. Cooper, O. Jurcek, H. A. Sparkes, D. N. Sheppard, A. P. Davis, *Nat. Chem.* **2016**, *8*, 24; c) C. C. Tong, R. Quesada, J. L. Sessler, P. A. Gale, *Chem. Commun.* **2008**, 6321–6323.
18. H. M. Tay, T. G. Johnson, A. Docker, M. J. Langton, P. D. Beer, *Angew. Chem. Int. Ed.* **2023**, *62*, e202312745.
19. a) T. Suga, S. Osada, H. Kodama, *Bioorg. Med. Chem.* **2012**, *20*, 42–46; b) H. Behera, N. Madhavan, *J. Am. Chem. Soc.* **2017**, *139*, 12919–12922.
20. a) D. Bong, T. Clark, J. Granja, M. Ghadiri, *Angew. Chem. Int. Ed.* **2001**, *40*, 988–1011; b) R. Chapman, M. Danial, M. L. Koh, K. A. Jolliffe, S. Perrier, *Chem. Soc. Rev.* **2012**, *41*, 6023–6041.
21. Q. Song, Z. Cheng, M. Kariuki, S. C. L. Hall, S. K. Hill, J. Y. Rho, S. Perrier, *Chem. Rev.* **2021**, *121*, 13936–13995.
22. P. De Santis, S. Morosetti, *Macromolecules* **1974**, *7*, 52–58.
23. M. R. Ghadiri, J. R. Granja, R. A. Milligan, D. E. McRee, N. Khazanovich, *Nature* **1993**, *366*, 324–327.
24. J. Sánchez-Quesada, M. P. Isler, M. R. Ghadiri, *J. Am. Chem. Soc.* **2002**, *124*, 10004–10005.
25. T. D. Clark, L. K. Buehler, M. R. Ghadiri, *J. Am. Chem. Soc.* **1998**, *120*, 651–656.
26. R. G. Fandiño, M. Amorín, L. Castedo, J. R. Granja, *Chem. Sci.* **2012**, *3*, 3280.
27. S. Panda Mahapatra, S. Pahan, A. Chatterjee, S. Roy, D. R. Puneeth Kumar, H. N. Gopi, *Angew. Chem. Int. Ed.* **2024**, e202409969.

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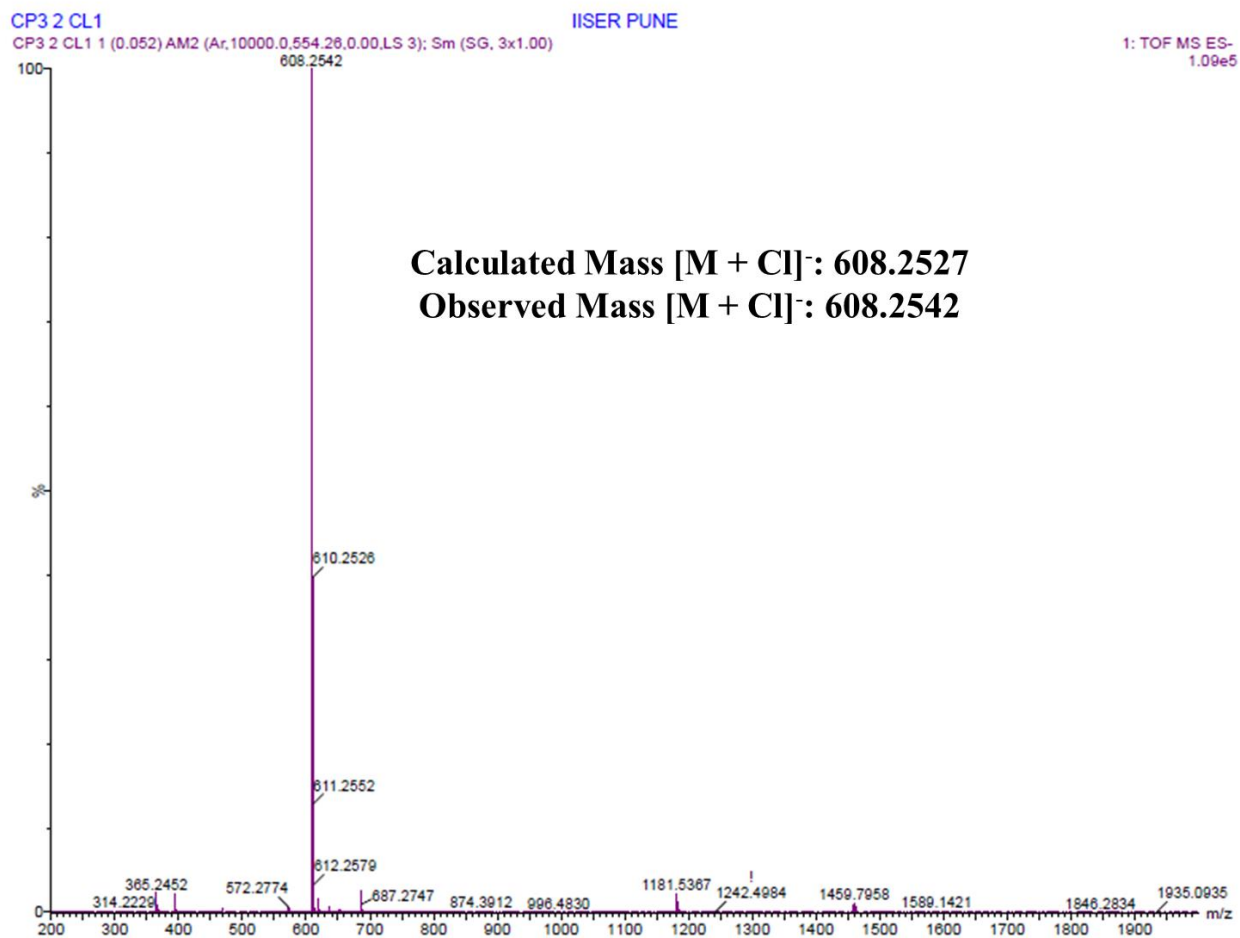
28. P. R. Spackman, M. J. Turner, J. J. McKinnon, S. K. Wolff, D. J. Grimwood, D. Jayatilakab, M. A. Spackman, *J. Appl. Cryst.* **2021**, 54, 1006–1011.
29. S. Pahan, S. Dey, G. George, S. Panda Mahapatra, D. R. Puneeth Kumar, H. N. Gopi, *Angew. Chem. Int. Ed.* **2024**, 63, e202316309.

4.9. Appendix IV: HRMS Spectrum of [C8+anion]⁻ complex:

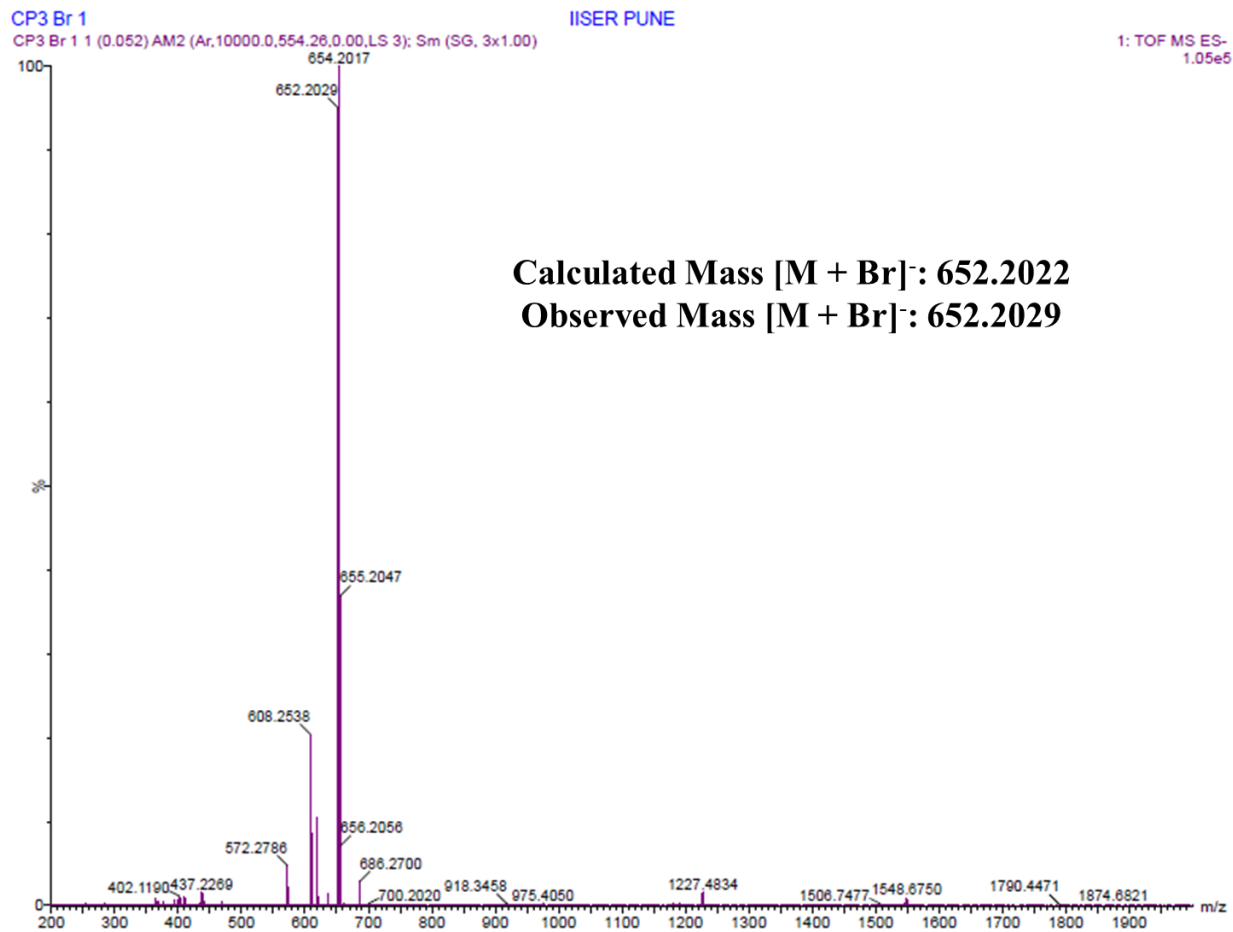
(a) HRMS Spectrum of C8:



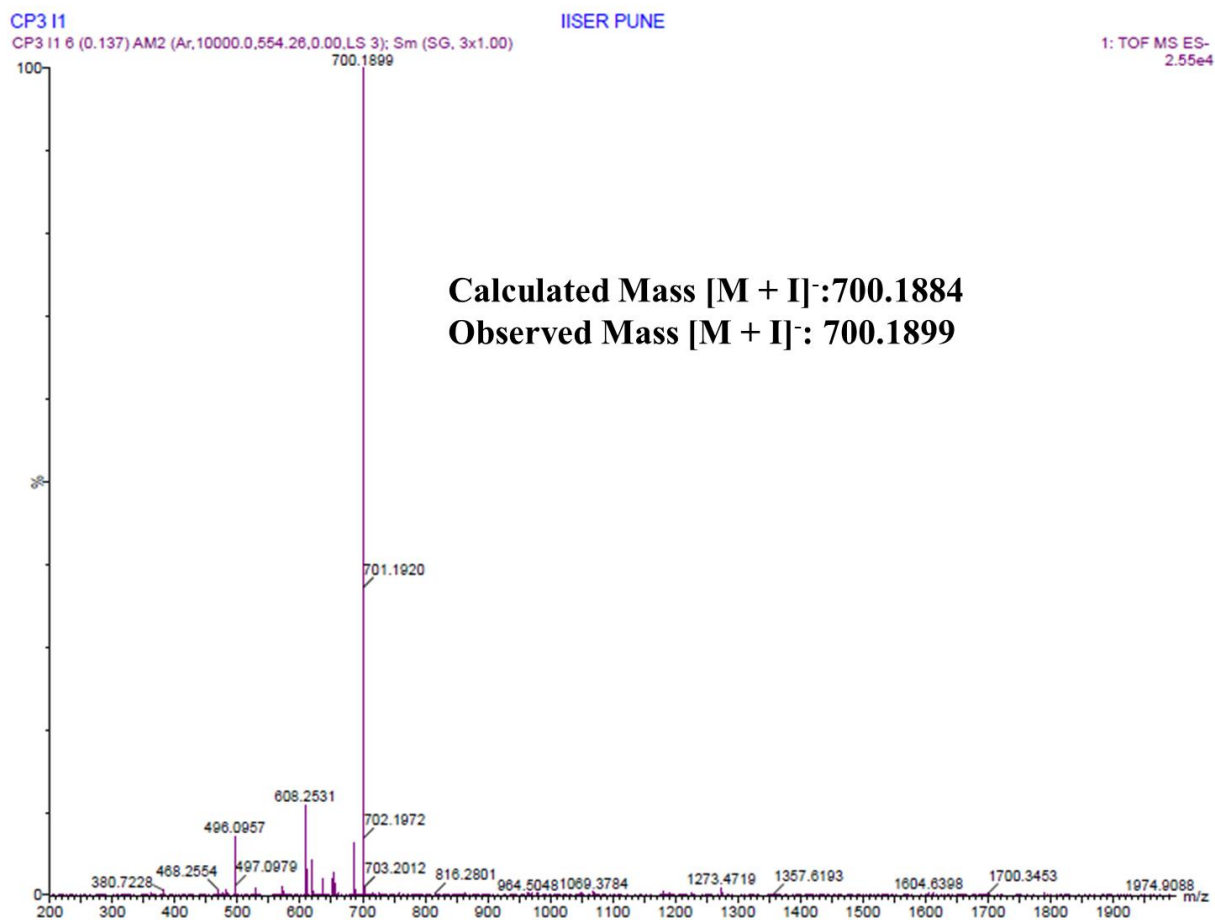
(b) HRMS Spectrum of $[C8 + Cl]^-$ complex:



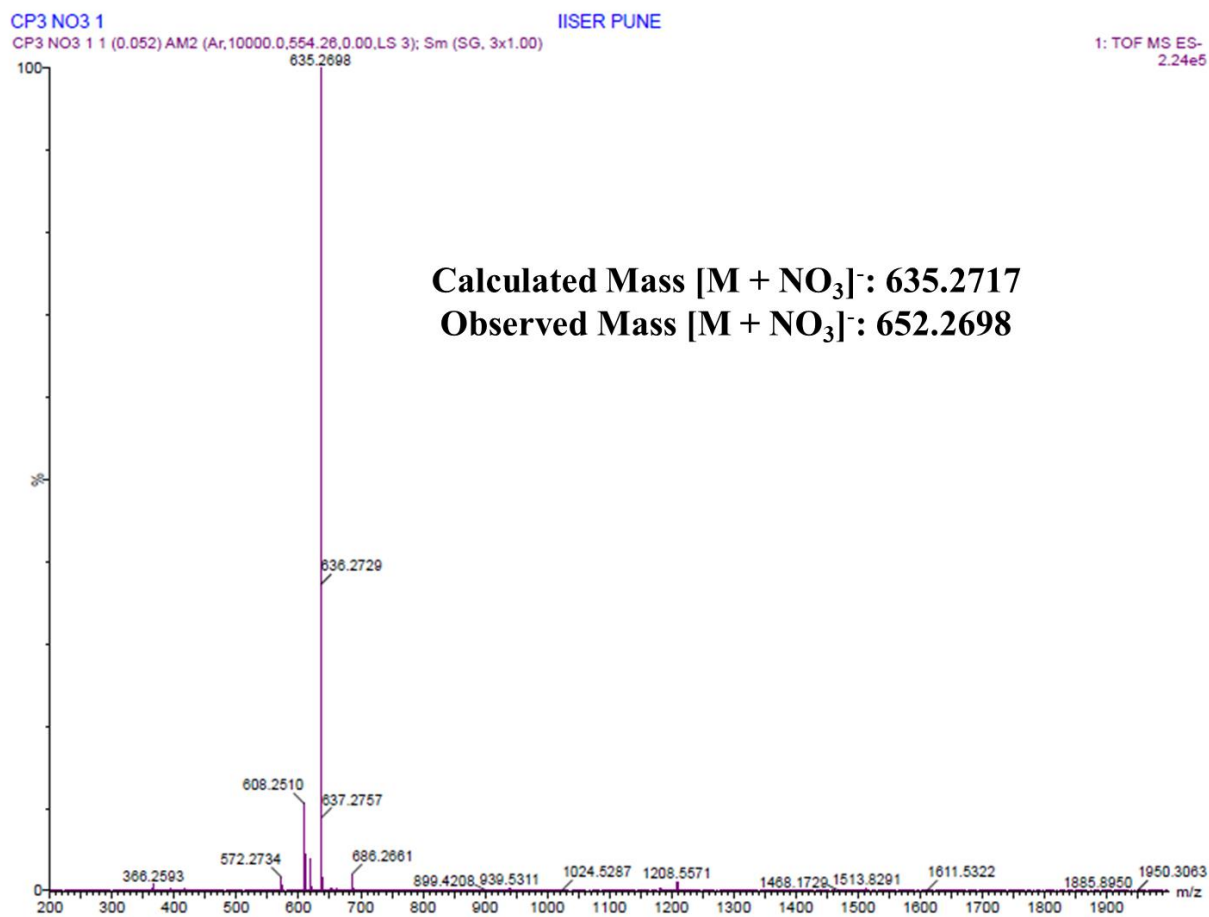
(c) HRMS Spectrum of $[C8 + Br]^-$ complex:



(d) HRMS Spectrum of [C8 + I]⁻ complex:



(e) HRMS Spectrum of $[C8 + NO_3]^-$ complex:



Chapter 4

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Self-Assembling Cyclic β -Peptide Nanotubes as Artificial Transmembrane Ion Channels



Author: Thomas D. Clark, Lukas K. Buehler, M. Reza Ghadiri

Publication: Journal of the American Chemical Society

Publisher: American Chemical Society

Date: Feb 1, 1998

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Modulating Ion Channel Properties of Transmembrane Peptide Nanotubes through Heteromeric Supramolecular Assemblies



Author: Jorge Sánchez-Quesada, Markus P. Isler, M. Reza Ghadiri

Publication: Journal of the American Chemical Society

Publisher: American Chemical Society

Date: Aug 1, 2002

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Chapter 5

**Design of Delta Amino Acid Based Peptide Nucleic
Acid and Examine Their Specificity towards DNA and
RNA**

5.1. Introduction:

The basic genetic material present in practically all living things is called DNA, and it contains the instructions needed to synthesize proteins.^[1] In 1953, Watson and Crick discovered DNA's double helix structure (Figure 1). DNA is a polymer made up of nucleotide units that repeat and are made up of a phosphate group, a deoxyribose sugar, and a nitrogenous base. The main chemical components of DNA are (i) the nitrogenous bases adenine (A), thymine (T), guanine (G), and cytosine (C), which point toward the center of the helix, and (ii) the sugar-phosphate backbone, which forms the structure of each strand and is positioned on the outside of the helix. Every base is joined to a sugar through a β -glycosyl bond. The two strands are held together by hydrogen bonds formed between complementary base pairs (A:T ; G:C). (Figure 1). The molecular structure of DNA features a double-stranded helix of uniform diameter with a right-handed twist. In contrast, RNA contains ribose instead of deoxyribose sugar and includes adenine (A), uracil (U), guanine (G), and cytosine (C) as its bases.

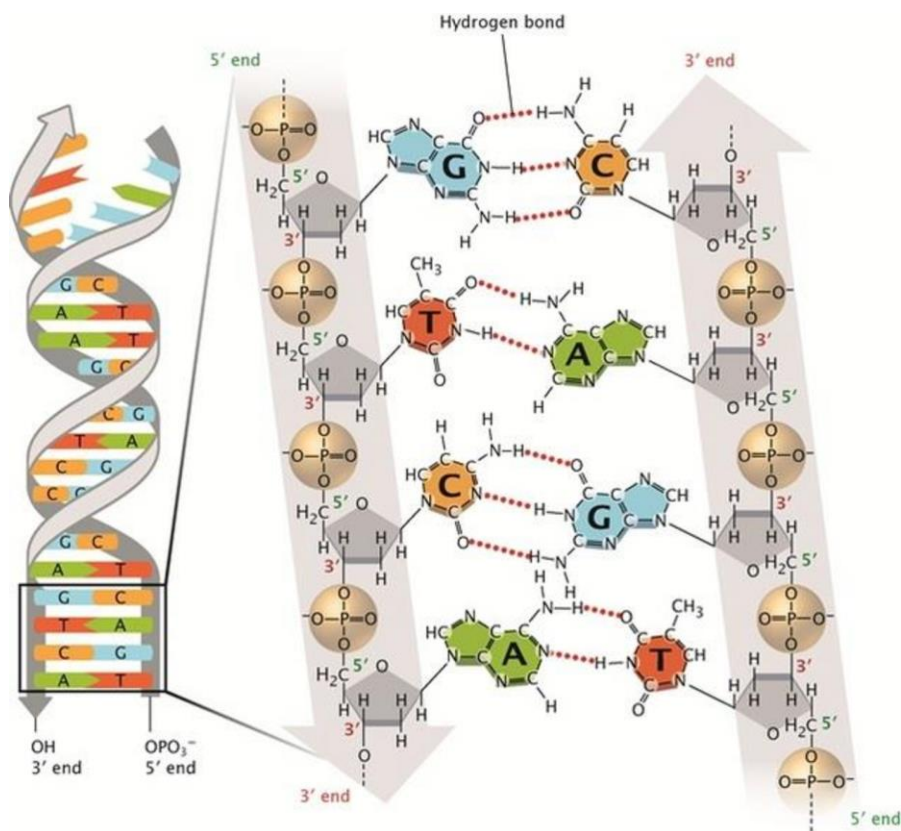


Figure1: Double-helical Structure of DNA.^[1,2]

The elegant and effective way that living things store, retrieve, and transfer their genetic information is exemplified by the DNA double helix.^[3] A crucial feature of the DNA double helix is the specificity and reversibility of hydrogen bonds between complementary nucleobases, which allow the strands to accurately unwind and reassemble. DNA's distinct qualities and intricate structure have enthralled chemists ever since its double helical form was discovered. Understanding DNA's structure, properties, and uses in the biological, physicochemical, and material sciences has been the subject of extensive research.^[4] For synthetic and structural organic chemists, creating DNA analogues that can detect particular DNA and RNA sequences has emerged as a useful and intellectually engaging project. For hybridization-based biological applications, the capacity to identify DNA and RNA sequences using complementary oligonucleotides is crucial to biotechnology. The diagnostic protocols and commonly used experimental techniques are used to study this recognition.

5.1.1. Base pairing and duplex structure of DNA:

In 1953, Watson and Crick introduced a revolutionary duplex structure for DNA.^[1] The DNA molecule consists of two helical strands coiled around the same axis, with each polymer chain comprising nucleotides linked by phosphate bonds between the 3' and 5' hydroxyl groups of the sugar components. The heterocyclic bases are bonded to the sugars at the C1' position through β -glycosidic linkages. These two strands run in opposite directions, one oriented 3'-5' and the other 5'-3', forming an antiparallel, right-handed double helix. The spacing between adjacent bases in the DNA duplex is 3.4 Å, and the angle between successive base planes is 36 degrees. This configuration results in the helical unit repeating every 10 residues, producing a helical turn of 34 Å. The water content significantly influences the DNA structure: high water content results in the B-DNA form, while low water content leads to the A-DNA form, with variations in parameters such as base tilts and helix pitch between these forms. A notable feature of the DNA duplex structure is the complementary base pairing that holds the two strands together. The bases, specifically the purines and pyrimidines, engage in hydrogen bonding, with sp^2 hybridized carbonyl groups serving as hydrogen bond acceptors and NH groups as donors. Adenine (A) pairs with thymine (T), and guanine (G) pairs with cytosine (C) through hydrogen bonds. This specific base pairing, known as Watson-Crick hydrogen bonding, is dependent on the nucleobases being in the keto form, ensuring the NH groups function as hydrogen bond donors.^[1]

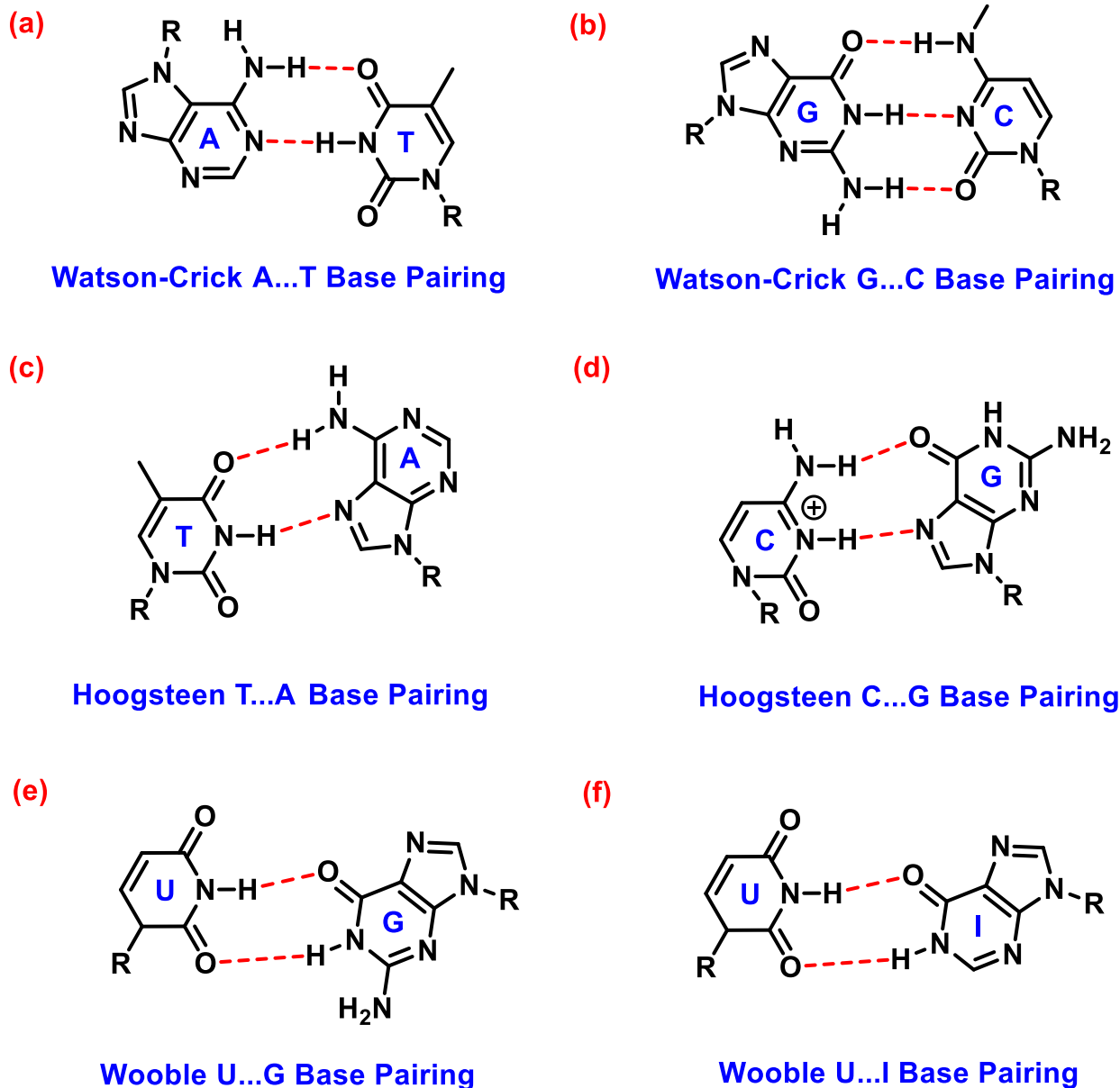


Figure 2: Different Type of Base Pairing in DNA Nucleobases (a-b) Watson-Crick, (c-d) Hoogsteen, (e-f) Wobble base pairing.

In addition to Watson-Crick base pairing, other significant base pairing modes in DNA and RNA include Hoogsteen^[5] and Wobble^[6] base pairing (Figure 2). Hoogsteen base pairs involve hydrogen bonding between adenine and thymine (or protonated cytosine and guanine) with a distinct orientation of the nucleobases compared to Watson-Crick pairs, playing a role in triplex formation. Wobble base pairs, such as guanine and uracil (G:U), occur during protein synthesis, specifically in the interactions between mRNA and tRNA (codon-anticodon interactions). X-ray

studies of oligodeoxynucleotides have also revealed various mismatched base pairs and unusual hydrogen bonding patterns.^[7]

5.1.2. Secondary structures of RNA:

Although RNA is typically single-stranded, it has the capacity to form intricate and unusual structures due to chain folding, which results from intramolecular base pairing. For instance, t-RNA exhibits base-paired stems, bulges, and open loops, ultimately adopting an L-shaped structure when fully folded. The formation of a B-type helix is not feasible for RNA because the steric hindrance from the 2'-OH group destabilizes the B-helix. However, this 2'-OH group can be accommodated in the A-helix, giving RNA greater structural diversity compared to DNA.

At low ionic strength, RNA forms a right-handed, antiparallel double helix with 11 base pairs per turn. In this configuration, the sugar adopts a C3'-endo pucker conformation, and other helical parameters align with those of A-DNA (Figure 3). When the salt concentration exceeds 20%, A-RNA transitions to an A'-RNA form, characterized by 12 base pairs per helical turn. A-RNA duplexes feature a very deep major groove and a rather shallow minor groove, both displaying typical Watson-Crick base-pairing properties.^[1]

However, the 2'-OH group in RNA nucleotides makes the molecule more prone to degradation, as it can act as a nucleophile and attack the adjacent phosphate group.^[8] This leads to RNA being more labile than DNA, particularly in alkaline conditions, where the 2'-OH group deprotonates, resulting in faster degradation of RNA compared to DNA.

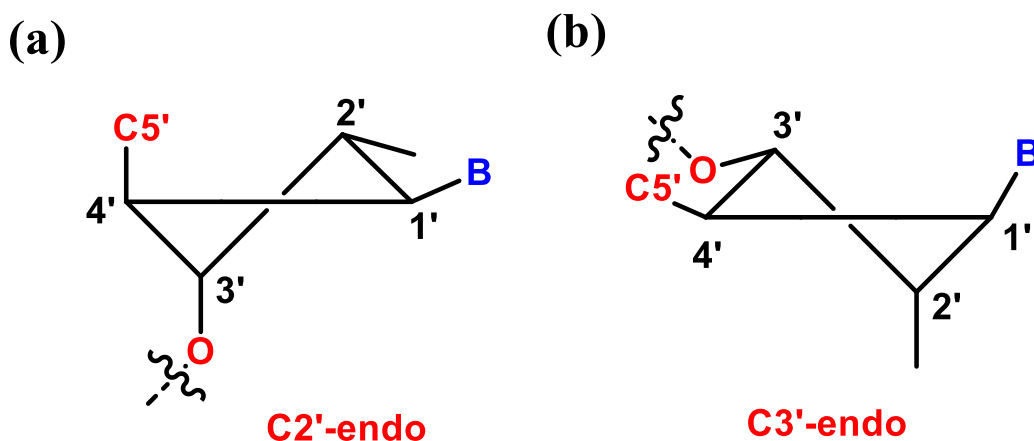


Figure 3: Configuration of C2'-endo and C3'-endo sugar pucker.

5.1.3. Peptide Nucleic Acid (PNA):

Peptide Nucleic Acids (PNAs) are artificial analogs of DNA, where the 2'-deoxyribose sugar ring in DNA is substituted with a 2-aminoethyl-glycine backbone. The traditional phosphodiester bonds in DNA are replaced by amide linkages in PNAs, and the nucleobases are attached to this backbone through a methylene carbonyl linker. A distinguishing feature of PNAs is their charge neutrality, which significantly impacts their behavior and properties.^[9]

Initially developed by Peter Nielsen in 1991 as a triplex-forming oligonucleotide intended to recognize duplex DNA as a third strand, PNAs have evolved into highly versatile DNA mimics due to their unique characteristics. Structurally, PNAs exhibit a six-atom spacing between adjacent nucleobases in their backbone, akin to natural DNA, and a two-atom spacing between the backbone and nucleobases, allowing effective binding to complementary DNA or RNA.

PNAs are not classified as peptides or nucleic acids, making them resistant to proteases and nucleases, with negligible toxicity, although they do not activate RNase H. They replicate the properties of DNA, including Watson-Crick base pairing with complementary DNA or RNA and the formation of triplex structures with DNA duplexes. Hybrids of PNA and PNA demonstrate higher thermal stability (melting temperature, T_m) compared to DNA and DNA hybrids, and a complementary PNA strand can invade a DNA duplex, displacing one of the strands in a process known as strand invasion. The enhanced stability of PNA and PNA duplexes is due to the neutral nature of the PNA backbone, which eliminates the electrostatic repulsion between the anionic phosphate charges present in DNA and DNA duplexes. Furthermore, mismatches in sequence have a more significant effect on the thermal stability of PNA and PNA duplexes compared to DNA duplexes. These superior properties have garnered considerable interest from medicinal chemists and molecular biologists, leading to the development of PNAs for gene therapeutics, diagnostics, and material science applications.^[9c-9h]

5.1.3.1 PNA Duplex Structure:

The X-ray structures of PNA:PNA, PNA:DNA, and PNA:RNA duplexes, along with NMR spectroscopy analysis, have uncovered several intriguing characteristics of PNA complexes. The structural distinctions between PNA and DNA manifest in the π - π stacking interactions between nucleobases, the profile and dimensions of the resulting helix, and the nature of the major and

minor grooves (Figure 4). DNA duplexes typically form a B-helix with a diameter of 20 Å and a pitch of 10 base pairs per turn. RNA duplexes, on the other hand, generally adopt an A-form helix, characterized by a diameter of 20 Å, a pitch of 11 base pairs per turn, and bases that are tilted by approximately 20 degrees relative to the helix axis.

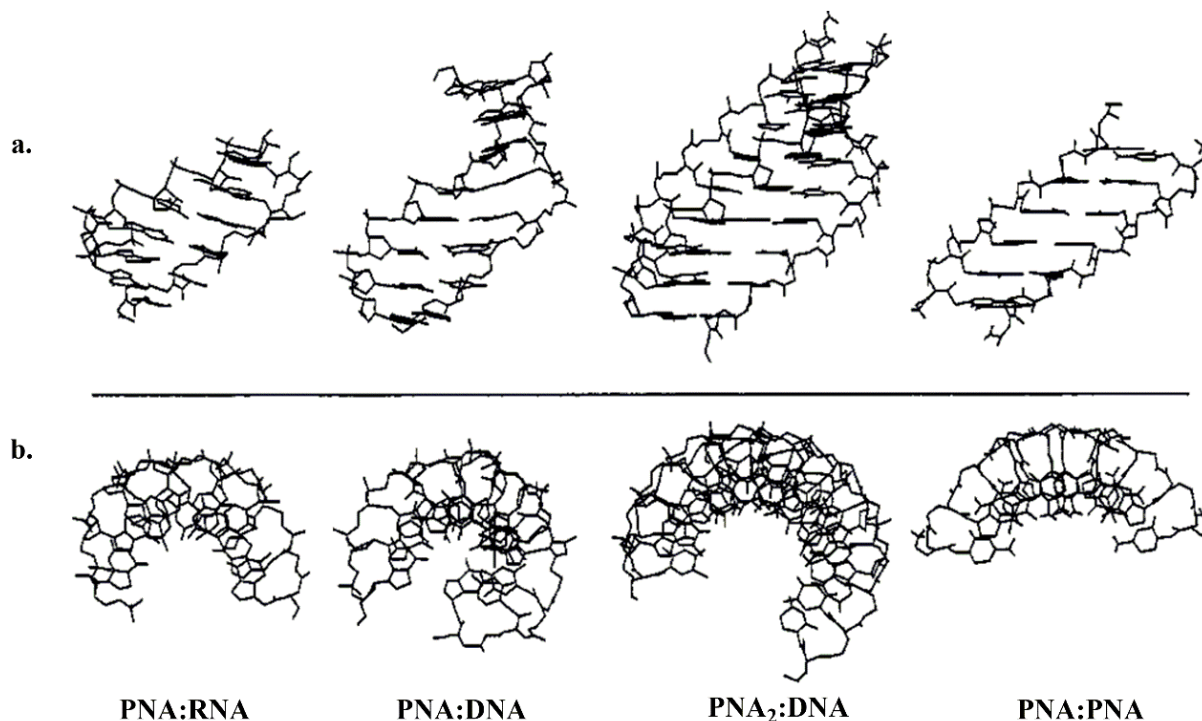


Figure 4: The configuration of different PNA complexes, viewed from above (top perspective) and from the side (lateral perspective)¹⁰

In contrast, PNA duplexes assume a P-helix structure, which is notable for its wide duplex diameter of 28 Å, a greater helical displacement of base pairs, and a large helical pitch of 18 base pairs. This implies that PNA duplexes (PNA/cRNA) form a structure that combines features of both DNA and RNA duplexes. PNA can pair with complementary DNA or RNA strands in both parallel and antiparallel orientations, adhering to Watson-Crick base pairing rules (Figure 5). In a parallel duplex, the N-terminus of PNA aligns with the 5'-terminus of DNA/RNA, whereas in an antiparallel duplex, the C-terminus of PNA aligns with the 5'-terminus of DNA (Figure 5).¹¹ Antiparallel duplexes typically exhibit higher thermal stability compared to their parallel counterparts.

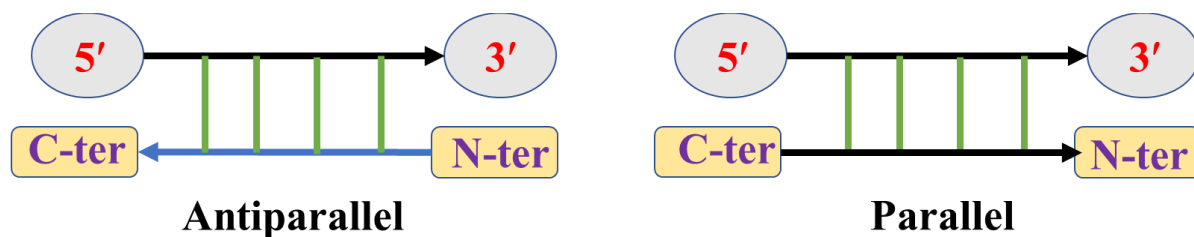


Figure 5: Different orientation of PNA and DNA Duplex.

5.1.3.2. PNA Triplex Structure:

Polypyrimidine PNA can hybridize with complementary polypurine DNA to create PNA2 triplex structures (Figure 4).^[12] Within this triple helix, one PNA strand engages in Watson-Crick hydrogen bonding with the DNA, while the other PNA strand interacts via Hoogsteen hydrogen bonding. For a single polypyrimidine DNA strand in a PNA2 triplex, both PNA strands can align either parallel or antiparallel to the DNA strand (Figure 4). However, when two different polypyrimidine PNA strands form a triple helix, the strand that engages in Watson-Crick bonding must be in an antiparallel orientation, whereas the Hoogsteen-bonding strand should be parallel relative to the DNA. The stability of these triplexes is influenced by the selectivity of the PNA duplex, with the third strand contributing moderately to this selectivity. The ability to form polypyrimidine triplexes facilitates the strand invasion process, a distinctive characteristic of Peptide Nucleic Acids.

5.1.4. Applications of PNA in Biological and material science:

(a) PNA as a possible antisense agent:

PNA has a number of unique characteristics that make it a great option for gene therapy, such as low toxicity, the capacity to invade strands in a novel way, and resistance to hydrolytic enzymes like nucleases and proteases. These characteristics set PNA apart from other synthetic nucleic acid analogs as a superior gene therapy agent. PNA is used in therapeutic applications in molecular biology using two main approaches: antigene and antisense.^[13]

(b) Translation Inhibition:

Antisense PNAs bind to mRNA, which is created by transcription of DNA, to form PNA duplexes, which obstruct the translation process. This binding stops protein synthesis by interfering with translation.^[14] Translation inhibition can be achieved by both pyrimidine-rich triplex-forming sequences and duplex-forming mixed-PNA sequences, as shown by in vitro translation experiments utilizing rabbit reticulocyte lysates.^[15] In particular, *Arabidopsis thaliana*'s ability to survive in cold climates depends on the suppression of protein translation mediated by AtGCN1.^[15b]

(c) Transcription Inhibition:

PNAs have the ability to prevent transcription by a number of different mechanisms, such as triplex formation, strand invasion, strand displacement, and duplex creation.^[15] The phase T3 polymerase activity is blocked by the simple 8-mer PNA-T8, whose inhibitory properties have been effectively demonstrated by Peter E. Nielson et al.^[16]

(d) Replication inhibition:

PNA can inhibit DNA replication by binding to duplex DNA, preventing the synthesis of daughter DNA. This occurs when PNA bonds with complementary DNA strands or forms duplexes with single-stranded DNA. Taylor et al. found that PNA effectively stops the replication of extrachromosomal mitochondrial DNA, which is often single-stranded. Despite support for PNA-based antigene and antisense therapies, no PNA drugs are in clinical trials for these applications. PNA strands with a C-C and C-N backbone tend to adopt random shapes, requiring preorganization to form stable duplexes with DNA or RNA. Introducing conformational constraints, such as substitutions at α , β , and γ positions or cyclized units, enhances PNA's binding affinity and selectivity for complementary DNA sequences.^[17]

5.1.5. Chemical Modification of PNA:

A PNA single strand with a C-C and C-N-only backbone has a tendency to take on a random shape. In order to create well-organized duplexes with either DNA or RNA, PNA needs to go beyond an entropy barrier. Pre-organizing the random structure of the PNA backbone can improve PNA's selective binding to DNA or RNA. In order to achieve this, different conformational constraints may be introduced, ranging from straightforward substitutions at the α , β , and γ locations to more

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intricate cyclized units along the backbone.^[18] The affinity and selectivity of PNA for its complementary DNA sequences are greatly increased by this chiral preorganization. (Figure 6).

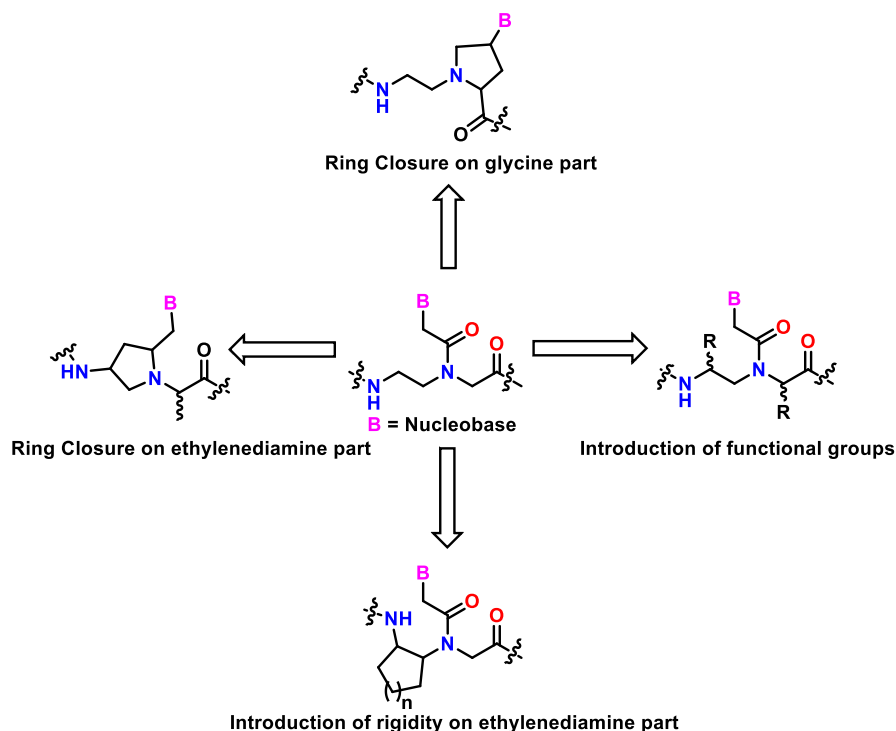


Figure 6: Method of modification in backbone of PNA building blocks.

5.2. Objectives and Rationale of the Work:

Various modifications have been investigated to overcome the limitations of Peptide Nucleic Acids (PNA). Researchers, Nielsen and coworkers, have explored different approaches, such as extending the PNA backbone with diverse substituents, developing a PNA ester backbone, and enhancing backbone rigidity through ring strain.^[18] Several short PNA oligomers with chemical modifications resembling those of DNA have been documented.^[19] Modifications at the α , β , and γ positions are common, particularly at α and γ . Studies indicate that adding a chiral center increases the rigidity of the PNA backbone. Moreover, substituting the C- γ position with L-lysine sidechains has been shown to improve PNA solubility and stabilize PNA hybrid duplexes by aligning the PNA backbone into right-handed double helices with complementary DNA

Despite these advancements, the structural and conformational factors that enable effective hybridization with complementary nucleic acids are still not fully understood. To investigate how

backbone architecture affects the binding of PNA to complementary DNA/RNA, we propose to synthesize a PNA analogue featuring an ether backbone, with nucleobases linked via an acetyl group to the chiral C- γ carbon (Figure 7). We would like to compare the hybridization properties of these modified PNA oligomers to those of the standard aeg PNA, and aiming to elucidate the fundamental principles behind DNA/RNA binding by polyamide analogues.

The specific objectives are: (a) To develop a modified PNA monomer incorporating a heteroatom and substituting at the γ -C position. (b) To synthesize the modified PNA oligomer alongside a standard aeg PNA oligomer using solid-phase peptide synthesis. (c) To perform biophysical evaluations of the synthesized PNA oligomer through circular dichroism (CD) (d) To explore the formation of duplexes and triplexes involving the modified PNA with complementary RNA or DNA.

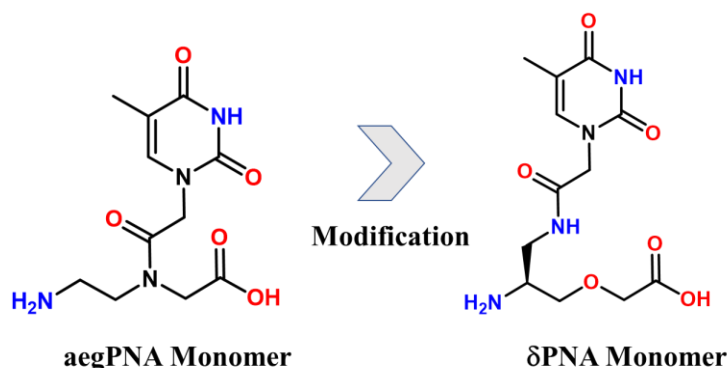


Figure 7: Modification and design of PNA building block.

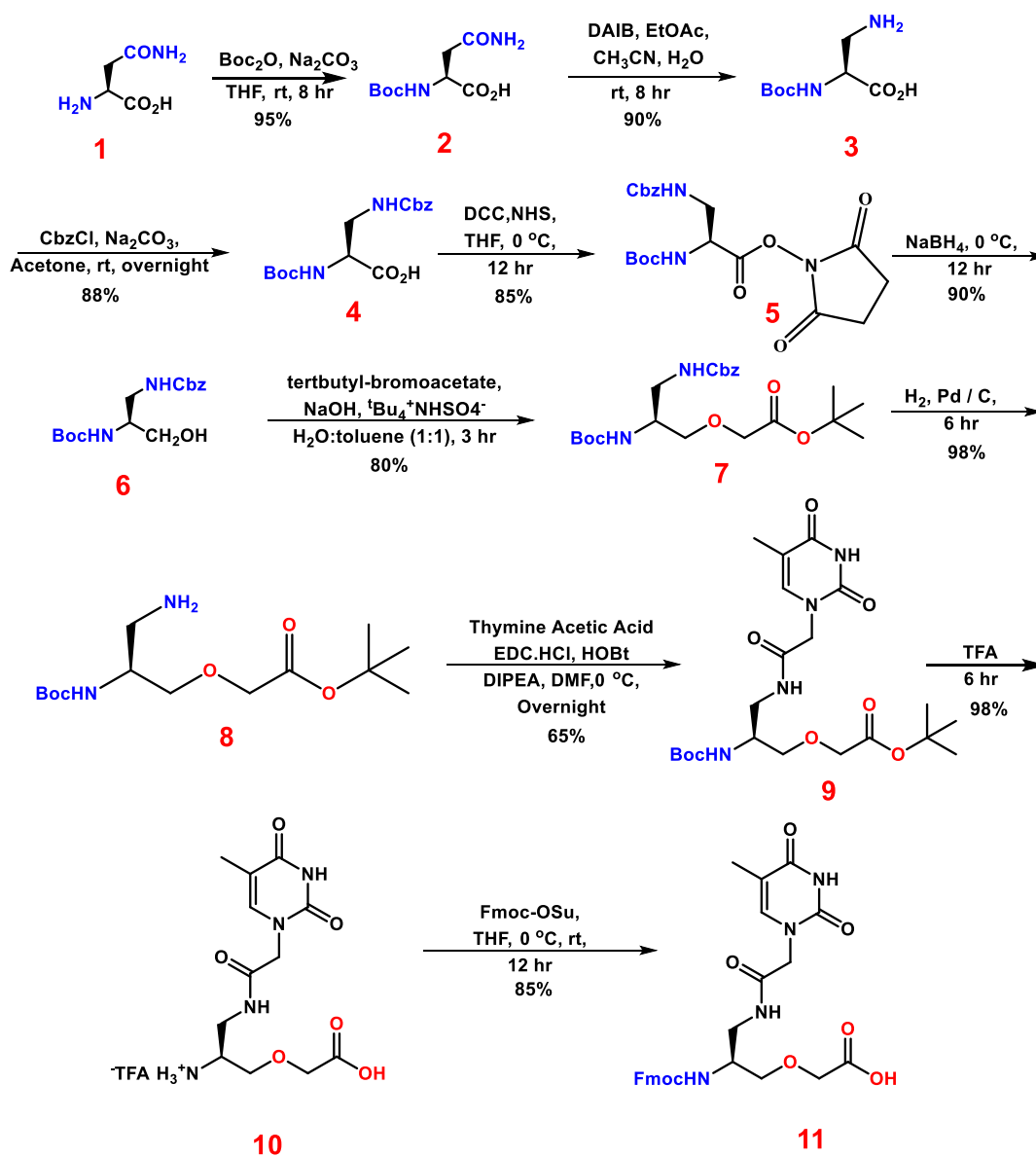
5.3. Results and Discussion:

5.3.1. Synthesis of PNA Building Block:

The synthesis of the modified δ PNA monomer 11 begins with the commercially available L-Asparagine (1). The initial step involves protecting L-Asparagine (1) with Boc_2O in the presence of THF and 9% Na_2CO_3 , yielding compound 2. Compound 2 then undergoes a Hofmann rearrangement to form the side-chain free amine compound 3. The free amine (3) is subsequently protected with Cbz-Cl, resulting in compound 4 where side chain amine is Cbz protected. Compound 4 is converted to its active ester form using DCC and NHS, producing activated ester compound 5. The active ester (5) is reduced with sodium borohydride to yield primary alcohol 6.

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The primary alcohol (6) reacts with tert-butyl bromoacetate in the presence of aqueous NaOH and toluene, and in the presence of phase transfer catalyst tetrabutylammonium hydrogen sulfate, to form compound 7. Compound 7 is then reduced with H₂ and Pd/C to generate the side-chain free amino derivative 8. The amino derivative (8) undergoes coupling with thymine acetic acid using EDC.HCl and HOBt as coupling reagent and DIPEA as a base, yielding compound 9. The terminal Boc and tert-butyl groups in compound 9 are deprotected using 98% TFA, resulting in compound 10. Finally, compound 10 reacts with Fmoc-OSu in the presence of Na₂CO₃ and THF, producing the modified δ PNA monomer 11. This meticulous sequence of reactions ensures the precise incorporation of thymine into the PNA backbone, resulting in the desired modified PNA monomer.



Scheme1: Synthesis Strategy of modified PNA building block

5.3.2. Synthesis of PNA oligomers using SPPS:

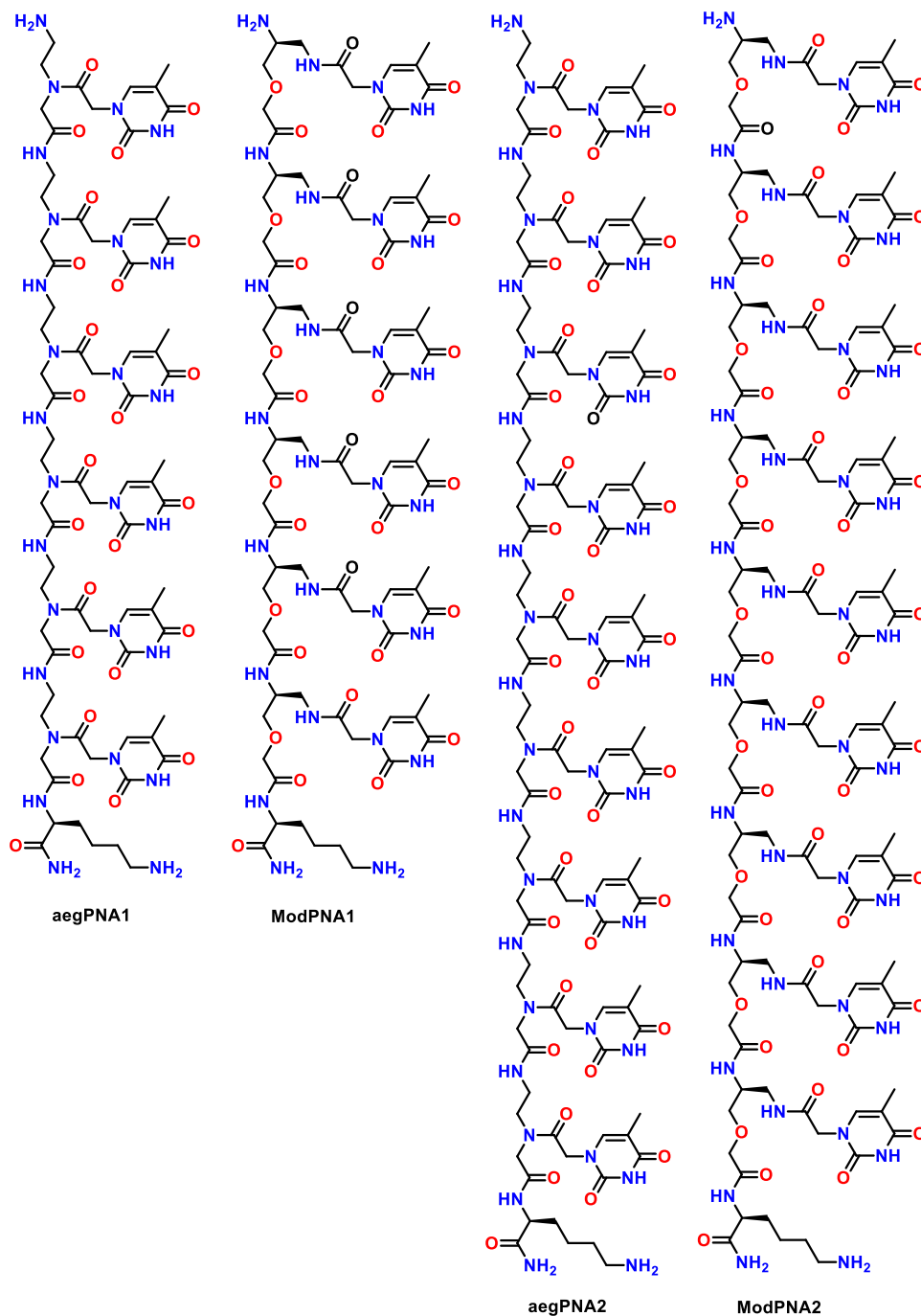
Solid Phase Peptide Synthesis (SPPS) was employed to synthesize PNA oligomers, starting with the attachment of the Lys-Fmoc monomer to the resin support to address solubility issues. The synthesis proceeded from the C-terminus to the N-terminus, utilizing monomeric units with protected amino and carboxylic acid functionalities. The Fmoc coupling strategy was intentionally chosen for its superior yields and reduced side product formation compared to the Boc strategy.

We selected the rink amide resin, specifically [4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy resin], as the solid support. After swelling the resin with DMF and DCM, the Fmoc group was deprotected using a 20% piperidine in DMF solution. Monomer coupling was facilitated by in situ activation using the DIC/Oxyma coupling reagent, with reactions conducted at 60°C in NMP solution and assisted by microwave heating. For the C-terminal spacer, we incorporated commercially available orthogonally protected (Fmoc) L-lysine, which was conjugated to the resin via an amide bond. The designed sequence was synthesized on a small scale (0.04 mmol/g), starting with the lysine residue, followed by the coupling of either aeg building blocks or modified delta building block δ PNA sequence building blocks.

The fully modified PNA homo-oligomers was synthesized using the Fmoc approach to investigate its DNA and RNA recognition capabilities. Additionally, a commercially available Fmoc-protected aegPNA monomer was used to produce an unmodified aeg PNA homo-oligomer, **aegPNA1** and **aegPNA2**, serving as a control sequence. This unmodified oligomer was employed to compare the hybridization and discrimination characteristics against the modified **δ PNA1** and **δ PNA2** and to evaluate stability upon binding with complementary ssDNA/ssRNA. In **aegPNA1**, six thymine nucleobases and one lysine building block are utilized, while in **aegPNA2**, nine thymine nucleobases and one lysine building block are incorporated. Similarly, in **δ PNA1**, six delta-thymine nucleobases and one lysine building block are used, whereas **δ PNA2** contains nine delta-thymine nucleobases and one lysine building block. For binding experiments, we acquired complementary adenine-containing DNA and RNA molecules from a commercial source. The sequences of the commercially obtained DNA and RNA are provided below table 1.

DNA/RNA Code	DNA/RNA Sequence
DNA1:	5'-AAAAAA-3'
DNA2:	5'-AAAAAAAA-3'
RNA:	5'-AAAAAAAA-3'

Table 1: Details of used DNA and RNA for experiment



Scheme2: Synthesized aeg and δ PNA molecules

5.3.3. The cleavage of PNA oligomers from the solid support:

The PNA oligomers were separated from the solid support, specifically the L-lysine derivatized Rink Amide resin, using a cocktail solution of trifluoroacetic acid (TFA, 500 μ L) and triisopropylsilane (TIPS, 50 μ L). TIPS acted as scavengers to capture free radicals produced during the cleavage process. The resulting PNA oligomers, featuring an L-lysine amide at their C-terminus, required an optimal cleavage period of two hours for both the oligomers and the lysine side chain protecting groups. Following cleavage, the resin was removed by filtration, and the filtrate was washed twice with TFA. Residual TFA was then evaporated with DCM under low pressure until minimal amounts remained. The mixed filtrate was precipitated using cold, dry diethyl ether, and the resulting peptide was isolated by centrifugation of the precipitate. Finally, the precipitate was dissolved in 400 μ L of deionized water and loaded onto a Sephadex G25 column for final purification.

5.3.4. Purification of the PNA oligomers using HPLC:

Upon analysis using analytical HPLC (C_{18} column, acetonitrile: water system), the initially synthesized crude PNA oligomers exhibited a purity exceeding 80%. These oligomers were then subjected to further purification through RP-HPLC on a semi-preparative C_{18} column. HPLC traces of all these **aegPNAs** and **δ PNAs** are attached below figure XX.

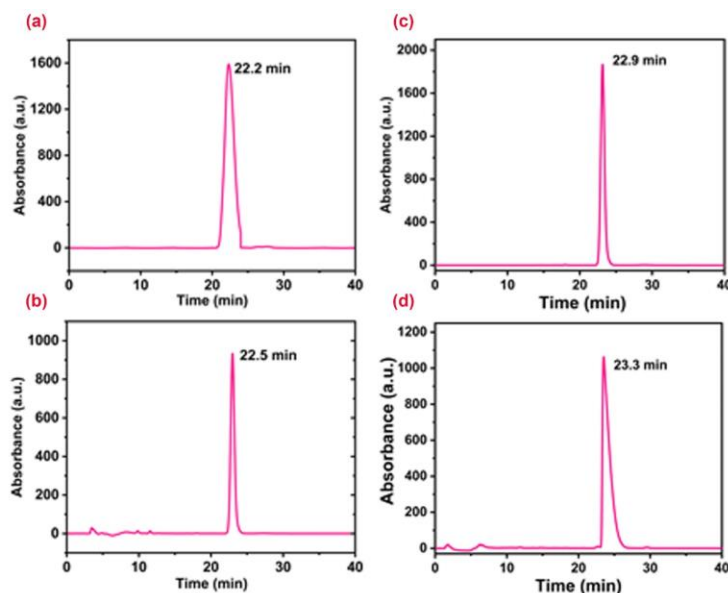


Figure 8: HPLC Traces of Synthesized PNA molecules.

The purification process was carried out using a low-pressure gradient pump with two solvent systems: Solvent A (95% H₂O, 4.9% CH₃CN, 0.1% TFA) and Solvent B (50% H₂O, 49.9% CH₃CN, 0.1% TFA). The flow rate was set at 1.5 mL/min over a 40-minute run, with eluent monitoring at 260 nm and 220 nm. This method yielded PNA oligomers with high purity, as confirmed by analytical RP-HPLC trace. For DNA/RNA oligos, purification was performed using denaturing polyacrylamide gel electrophoresis, and the purity was further validated by RP-HPLC using a triethylammonium acetate/acetonitrile solvent system.

5.3.5. Comparative Analysis of Circular Dichroism Spectroscopy:

We further investigated these δ -amino acid-containing, novel Peptide Nucleic Acids (δ PNAs) to enhance their solubility, binding affinity, and specificity to DNA/RNA. To assess the binding capability of our newly synthesized PNAs with complementary single-stranded DNA (ssDNA) and single-stranded RNA (ssRNA), we used Circular Dichroism (CD) Spectroscopy. The complementary ssDNA/ssRNA was paired with PNA strands at various stoichiometric concentrations and annealed in a 10 mM phosphate-buffered saline solution (pH 7.0) to form duplexes or triplexes. To ensure proper binding with complementary ssDNA and ssRNA, we annealed our designed δ PNAs with the complementary strands and cooled the mixture at 4°C to confirm duplex or triplex formation. To validate our concept, we compared the binding of our designed δ PNAs with that of the already established aegPNAs. Below, we have discussed all possible duplex or triplex formations of PNA (both aegPNA and dPNA) in detail.

(a) CD analysis of Duplex formation of aegPNA1 and δ PNA1 with complementary ssDNA1:

We have annealed **aegPNA1** with complementary 6-mer adenine containing DNA sequence **ssDNA1** and **δ PNA1** with complementary 6-mer adenine containing DNA sequence **ssDNA1** at (1:1) stoichiometric concentrations and annealed in a 10 mM phosphate-buffered saline solution (pH 7.0) to form duplexes and compared their characteristics signature CD signal.

In the CD spectra (Figure 9), we observe four distinct bands. There is a highly intense negative band at 205 nm, a moderate intensity positive band at 218 nm, another moderate intensity negative band at 249 nm, and a final positive maximum band at 268 nm. Comparing the CD spectra of **aegPNA1** and **δ PNA1** with complementary **ssDNA1**, a similar characteristics CD signature is observed suggesting that our designed **δ PNA1** may behave similar property like **aegPNA1**.

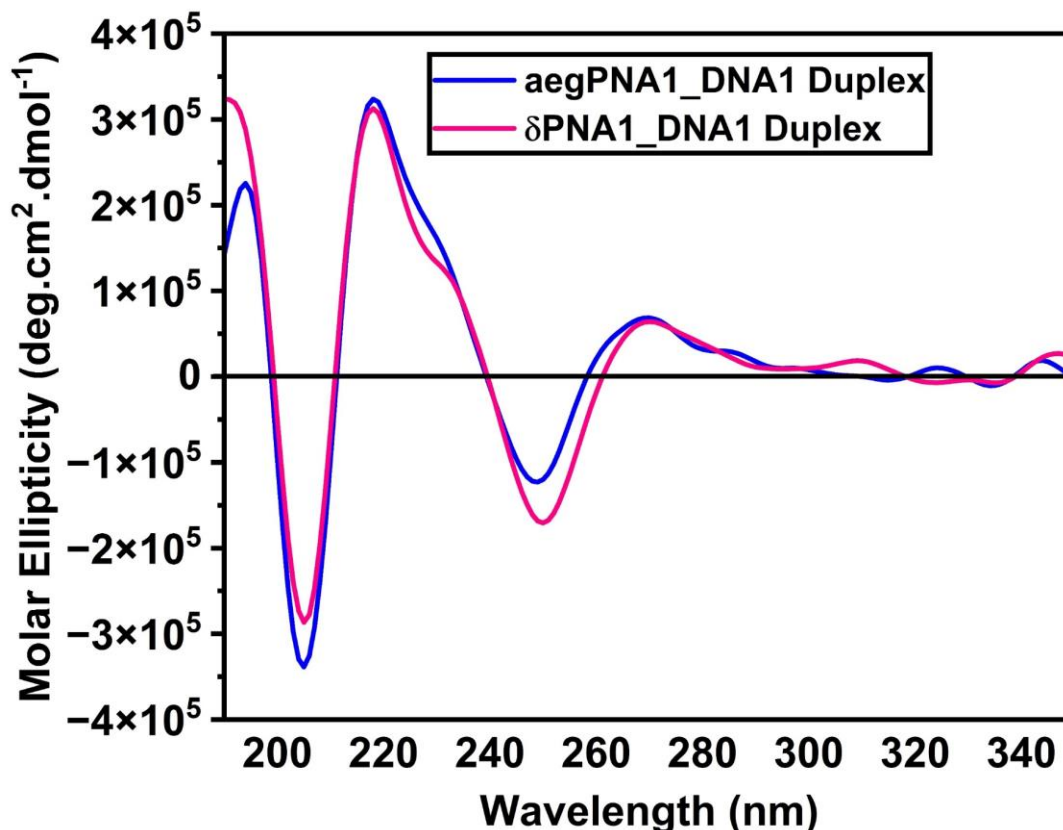


Figure 9: CD Spectra of duplex formation of **aegPNA1** and **δPNA1** with complementary **ssDNA1**

(b) CD analysis of Duplex formation of aegPNA2 and δPNA2 with complementary ssDNA2:

We have annealed **aegPNA2** with complementary 9-mer adenine containing DNA sequence **ssDNA2** and **δPNA2** with complementary 6-mer adenine containing DNA sequence **ssDNA2** at (1:1) stoichiometric concentrations and annealed in a 10 mM phosphate-buffered saline solution (pH 7.0) to form duplexes and compared their characteristics signature CD signal.

In the CD spectra (Figure 10), we observe four distinct bands. There is a highly intense negative band at 205 nm, a moderate intensity positive band at 217 nm, another moderate intensity negative band at 249 nm, and a final positive broad maximum band at 270 nm. Comparing the CD spectra of **aegPNA2** and **δPNA2** with complementary **ssDNA2**, a similar characteristics CD signature is observed suggesting that our designed **δPNA2** may behave similar property like **aegPNA2**.

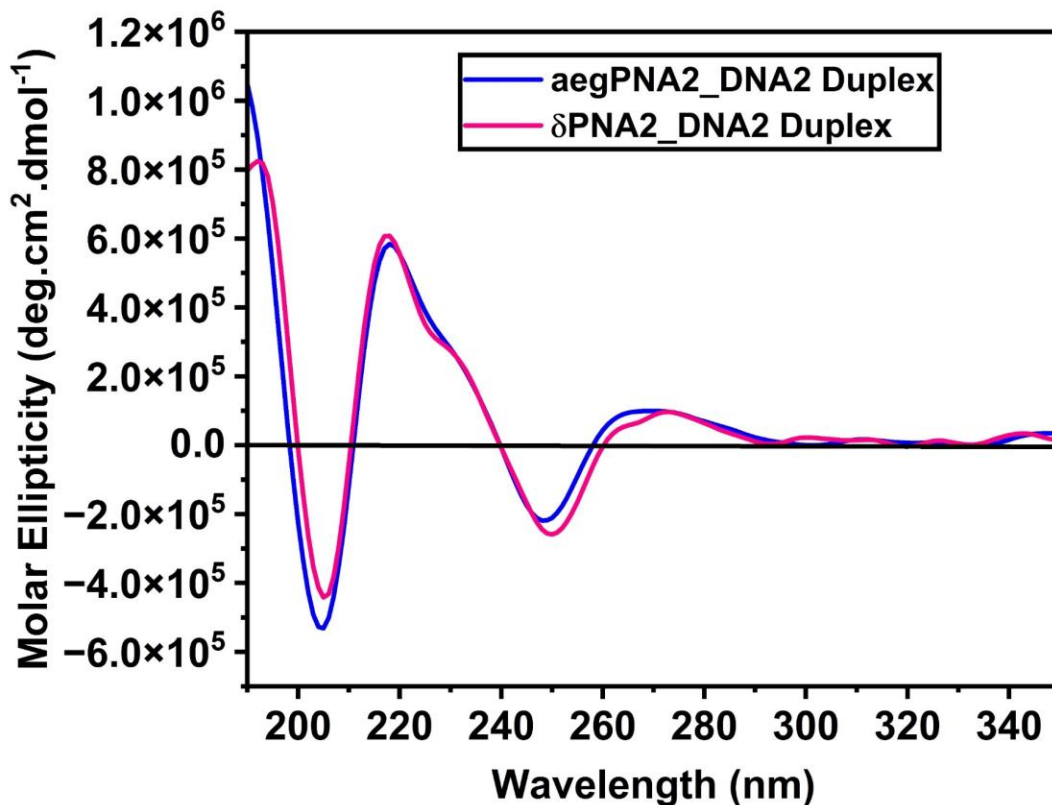


Figure 10: CD Spectra of duplex formation of **aegPNA2** and **δPNA2** with complementary ssDNA2

(c) CD analysis of Triplex formation of aegPNA1 and δPNA1 with complementary ssDNA1:

We have annealed **aegPNA1** with complementary 6-mer adenine containing DNA sequence **ssDNA1** and **δPNA1** with complementary 6-mer adenine containing DNA sequence **ssDNA1** at (2:1) stoichiometric concentrations and annealed in a 10 mM phosphate-buffered saline solution (pH 7.0) to form duplexes and compared their characteristics signature CD signal.

In the CD spectra (Figure 11), we observe four distinct bands. There is a highly intense negative band at 205 nm, a moderate intensity positive band at 217 nm, another moderate intensity negative band at 248 nm, and a final positive broad maximum band at 268 nm. Comparing the CD spectra of **aegPNA1** and **δPNA1** with complementary **ssDNA1**, a similar characteristics CD signature of triplex state is observed suggesting that our designed **δPNA1** may behave similar property like **aegPNA1**.

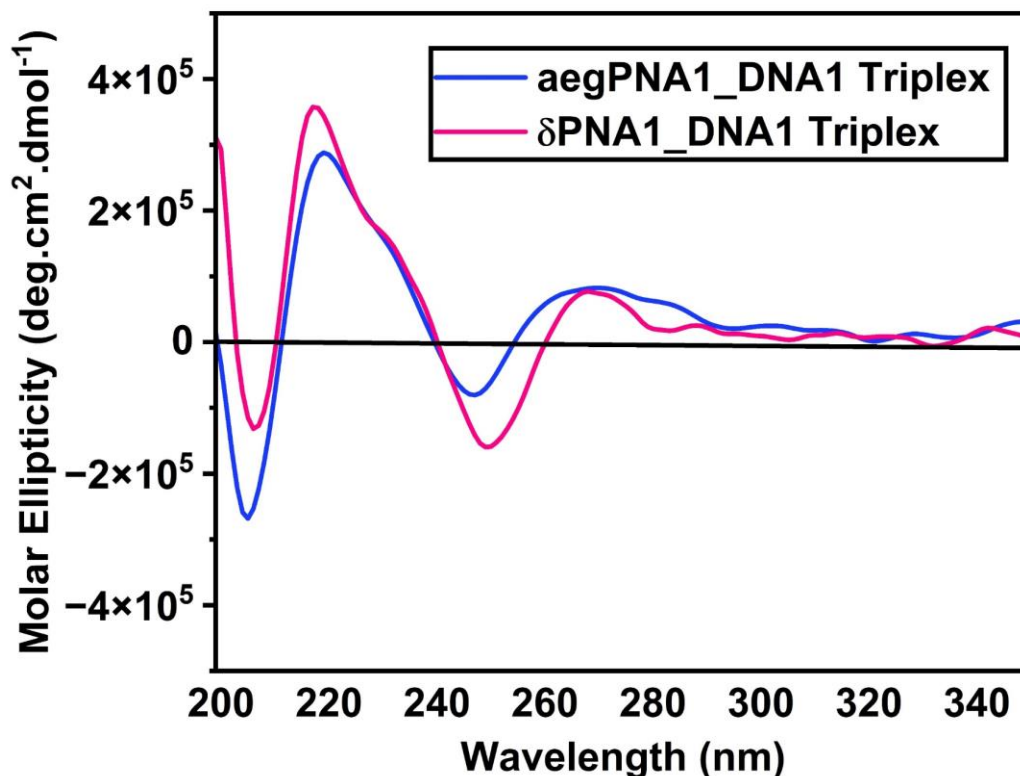


Figure 11: CD Spectra of triplex formation of **aegPNA1** and **δPNA1** with complementary ssDNA1

(d) CD analysis of Triplex formation of aegPNA2 and δPNA2 with complementary ssDNA2:

We have annealed **aegPNA2** with complementary 9-mer adenine containing DNA sequence **ssDNA2** and **δPNA2** with complementary 6-mer adenine containing DNA sequence **ssDNA2** at (2:1) stoichiometric concentrations and annealed in a 10 mM phosphate-buffered saline solution (pH 7.0) to form triplexes and compared their characteristics signature CD signal.

In the CD spectra (Figure 12), we observe four distinct bands. There is a highly intense negative band at 205 nm, a moderate intensity positive band at 217 nm, another moderate intensity negative band at 248 nm, and a final positive broad maximum band at 268 nm. Comparing the CD spectra of **aegPNA2** and **δPNA2** with complementary **ssDNA2**, a similar characteristics CD signature of triplex state is observed suggesting that our designed **δPNA2** may behave similar property like **aegPNA2**.

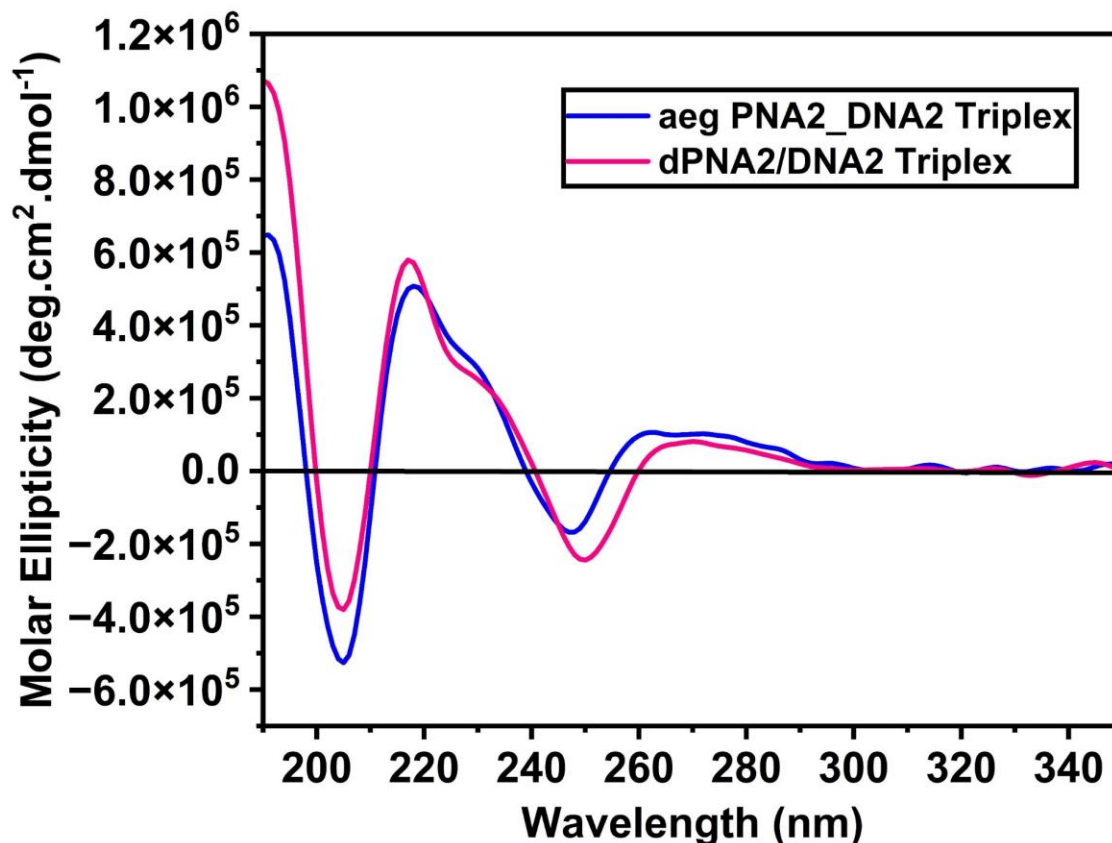


Figure 12: CD Spectra of triplex formation of **aegPNA1** and **δPNA1** with complementary ssDNA1

(e) CD analysis of Duplex formation of aegPNA2 and δPNA2 with complementary ssRNA:

We have annealed **aegPNA2** with complementary 9-mer adenine containing RNA sequence **ssRNA** and **δPNA2** with complementary 9-mer adenine containing RNA sequence **ssRNA** at (1:1) stoichiometric concentrations and annealed in a 10 mM phosphate-buffered saline solution (pH 7.0) to form duplexes and compared their characteristics signature CD signal.

In the CD spectra (Figure 13), we observe four distinct bands. There is a highly intense negative band at 204 nm, a moderate intensity positive band at 220 nm, another moderate intensity negative band at 247 nm, and a final intense positive maximum band at 264 nm. Comparing the CD spectra of **aegPNA2** and **δPNA2** with complementary **ssRNA**, a similar characteristics CD signature is observed suggesting that our designed **δPNA2** may behave similar property like **aegPNA2**.

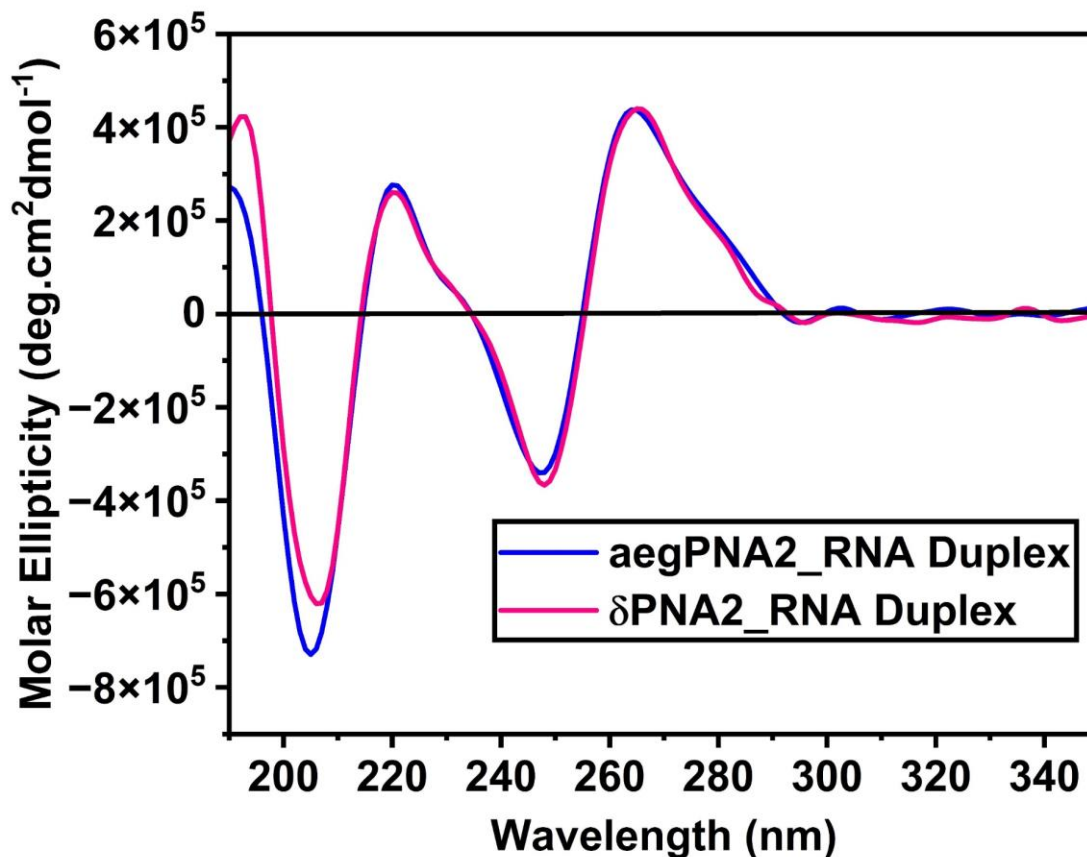


Figure 13: CD Spectra of duplex formation of **aegPNA2** and **δPNA2** with complementary **ssRNA**

(f) CD analysis of Triplex formation of aegPNA2 and δPNA2 with complementary ssRNA:

We have annealed **aegPNA2** with complementary 6-mer adenine containing RNA sequence **ssRNA1** and **δPNA2** with complementary 6-mer adenine containing RNA sequence **ssRNA1** at (2:1) stoichiometric concentrations and annealed in a 10 mM phosphate-buffered saline solution (pH 7.0) to form duplexes and compared their characteristics signature CD signal.

In the CD spectra (Figure 14), we observe four distinct bands. There is a highly intense negative band at 204 nm, a moderate intensity positive band at 220 nm, another moderate intensity negative band at 247 nm, and a final intense positive maximum band at 264 nm. Comparing the CD spectra of **aegPNA2** and **δPNA2** with complementary **ssRNA**, a similar characteristics CD signature is observed suggesting that our designed **δPNA2** may behave similar property like **aegPNA2**.

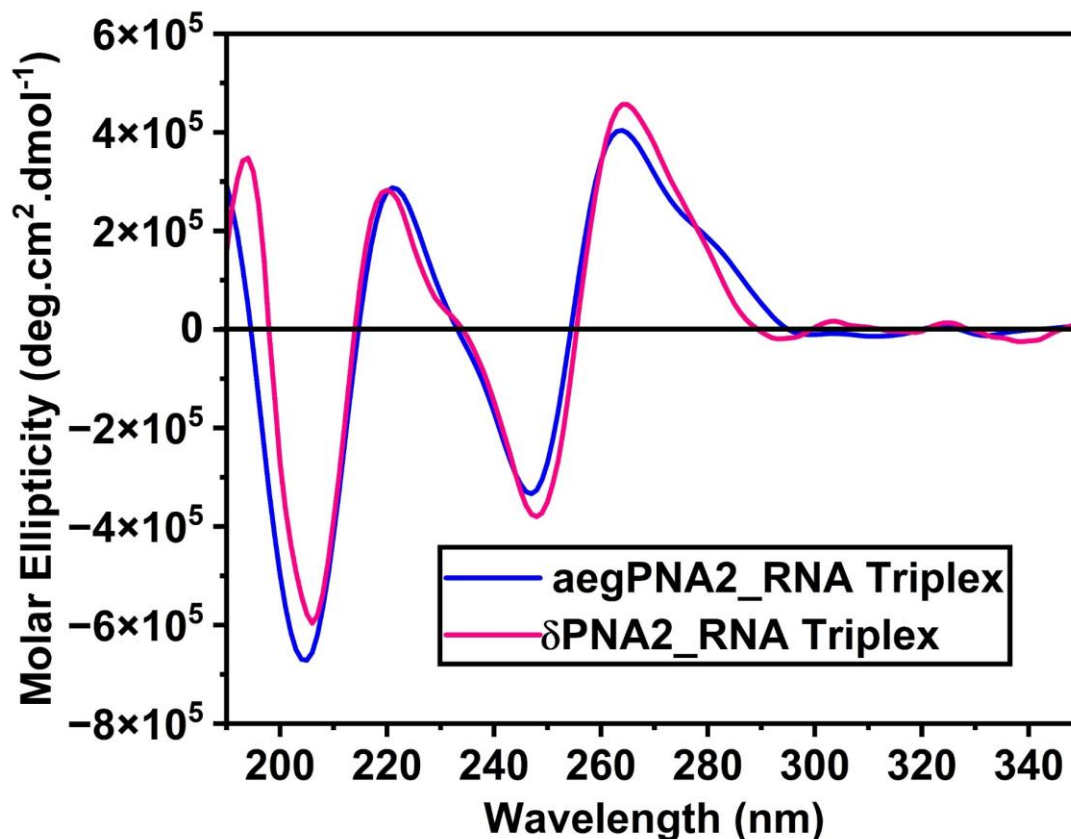


Figure 14: CD Spectra of triplex formation of **aegPNA2** and **δPNA2** with complementary **ssRNA**

These experiments demonstrated that our newly designed δ PNAs have a binding affinity for complementary ssDNA/ssRNA sequences, comparable to the well-established aegPNAs. Motivated by these findings, we proceeded to assess the thermal stability of these complexes. We conducted temperature-dependent CD experiments to evaluate the stability of the duplexes and triplexes and to measure their melting temperatures. The thermal stability of all the discussed duplexes and triplexes was determined through these temperature-dependent CD experiments.

(g) Temperature Dependent CD analysis of Duplex formation of aegPNA2 and δ PNA2 with complementary ssDNA2:

We observed similar characteristic CD signals for both **aegPNA2** and **δPNA2** with complementary **ssDNA2** across the temperature range from 15°C to 75°C. However, as the temperature increased, the intensity of the CD signals decreased in both cases. This suggests that the non-covalent interactions (like hydrogen bonding and pi-pi stacking interactions) between PNA and DNA weaken with rising temperature, leading to a reduction in signal intensity.

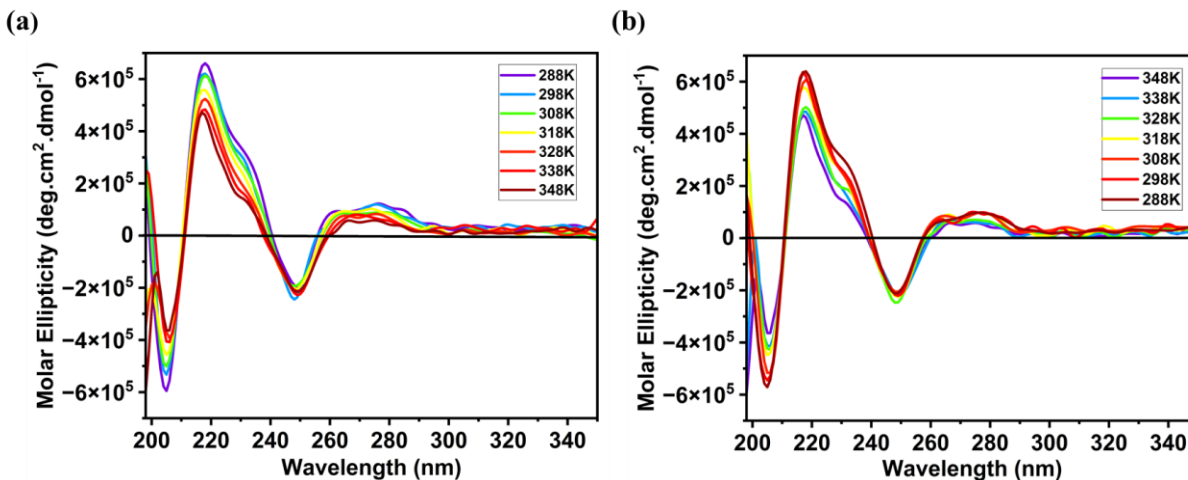


Figure 15: Temperature dependent CD Spectra of duplex formation of (a) δ PNA2 and (b) aegPNA2 and with complementary ssDNA2

(h) Temperature Dependent CD analysis of Triplex formation of aegPNA2 and δ PNA2 with complementary ssDNA2:

We observed similar characteristic CD signals for both aegPNA2 and δ PNA2 with complementary ssDNA2 across the temperature range from 15°C to 75°C. However, as the temperature increased, the intensity of the CD signals decreased in both cases. This suggests that the non-covalent interactions (like hydrogen bonding and pi-pi stacking interactions) between PNA and DNA weaken with rising temperature, leading to a reduction in signal intensity.

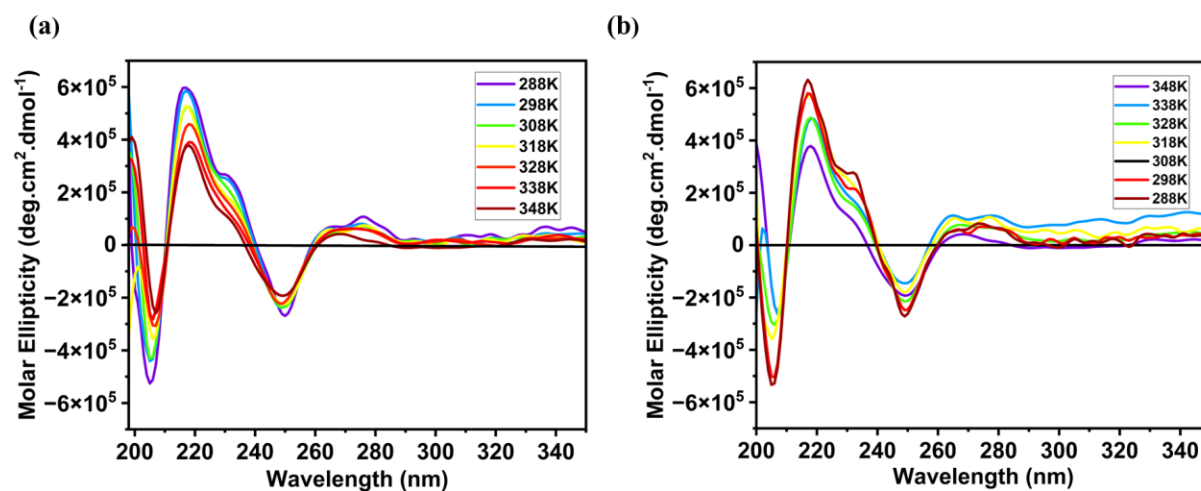


Figure 16: Temperature dependent CD Spectra of duplex formation of (a) δ PNA2 and (b) aegPNA2 and with complementary ssDNA

(g) Temperature Dependent CD analysis of Duplex formation of aegPNA2 and δ PNA2 with complementary ssRNA:

We observed similar characteristic CD signals for both **aegPNA2** and **δ PNA2** with complementary **ssRNA2** across the temperature range from 15°C to 75°C. However, as the temperature increased, the intensity of the CD signals decreased in both cases. This suggests that the non-covalent interactions (like hydrogen bonding and pi-pi stacking interactions) between PNA and DNA weaken with rising temperature, leading to a reduction in signal intensity.

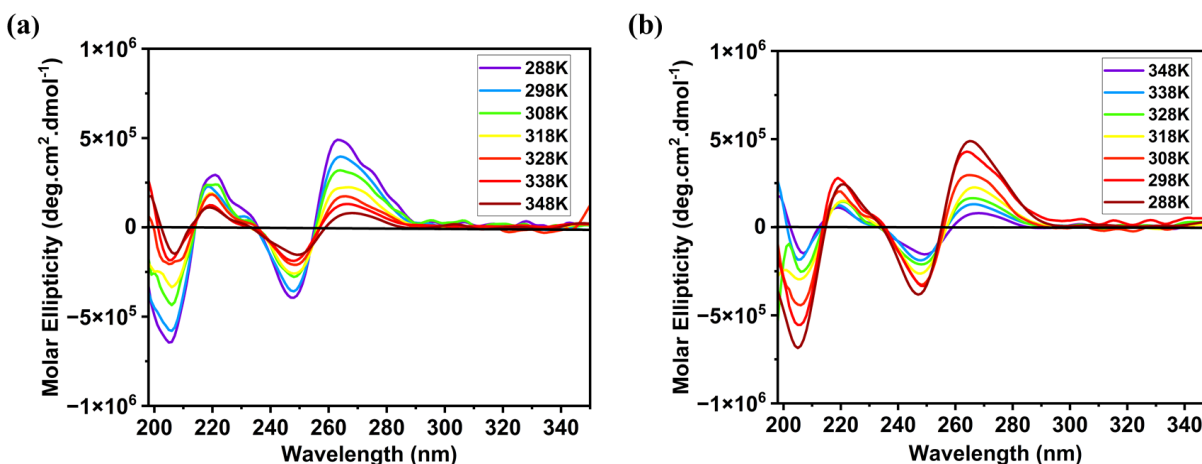


Figure 17: Temperature dependent CD Spectra of Duplex formation of (a) δ PNA2 and (b) aegPNA2 and with complementary ssRNA

(h) Temperature Dependent CD analysis of Triplex formation of aegPNA2 and δ PNA2 with complementary ssRNA:

We observed similar characteristic CD signals for both **aegPNA2** and **δ PNA2** with complementary **ssRNA2** across the temperature range from 15°C to 75°C. However, as the temperature increased, the intensity of the CD signals decreased in both cases. This suggests that the non-covalent interactions (like hydrogen bonding and pi-pi stacking interactions) between PNA and RNA weaken with rising temperature, leading to a reduction in signal intensity.

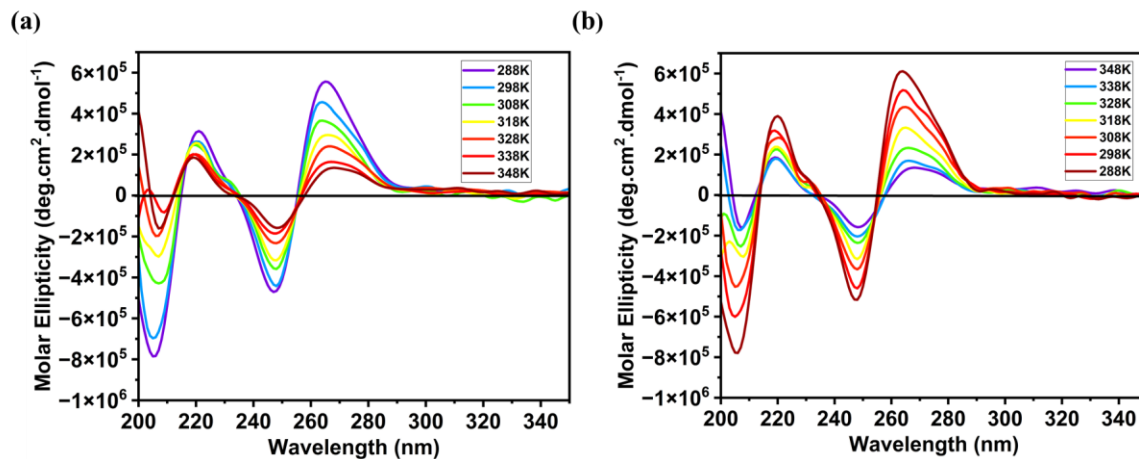


Figure 18: Temperature dependent CD Spectra of Triplex formation of (a) δ PNA2 and (b) aegPNA2 and with complementary ssDNA

These temperature-dependent CD experiments allowed us to determine the melting points of the corresponding complexes. We normalized all the graphs and applied a sigmoidal fit using the Boltzmann formula in Origin software to obtain the melting temperatures (T_m), which we have reported after averaging out triplicate data points. While a more accurate UV- T_m experiment could provide a better method for calculation, we measured the T_m through temperature-dependent CD due to the available facilities. Further theoretical investigation could help refine our calculations and provide detailed binding parameters and T_m values. The figure is attached below figure 19 .

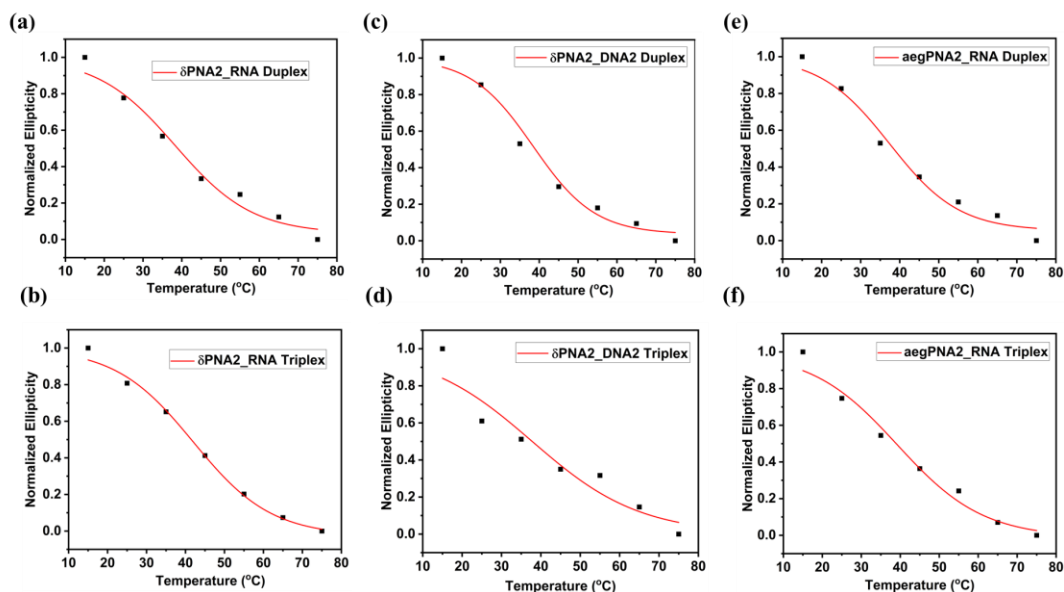


Figure 19: Melting Curve of PNA and DNA/RNA Complexes. Duplex of (a) δ PNA2 and RNA, (b) δ PNA2 and DNA2, (c) aegPNA2 and RNA. Triplex of (d) δ PNA2 and RNA, (e) δ PNA2 and DNA2, (f) aegPNA2 and RNA.

Table 2: Melting Temperature T_m for (aeg or δ)PNA/(DNA or RNA) Duplex or Triplex

Complex	Duplex T_m	Triplex T_m
δ PNA2_DNA2	35 °C	37 °C
δ PNA2_RNA	38 °C	42 °C
aegPNA2_RNA	38 °C	40 °C

Based on the initial data, it is clear that both aeg and δ -PNA building blocks exhibit similar behavior, as indicated by the comparable CD signatures of their complexes and similar melting data. However, further experiments and theoretical studies are necessary to fully substantiate these findings.

5.4. Conclusion:

We explored these δ -amino acids to design novel Peptide Nucleic Acids (PNAs), aiming to enhance solubility, binding affinity, and specificity to DNA/RNA. We have synthesized new PNAs and conducted UV- T_m studies to assess their thermal stability and binding affinity against ssDNA and ssRNA. Remarkably, the new PNAs bind to both ssDNA and ssRNA similarly to the aeg peptide nucleic acids. The advantage of these new δ -amino acid-based peptide nucleic acids over the aegPNAs is that they are easy to synthesize and scale up using solid-phase methods. Additionally, compared to aeg peptide nucleic acids, the new PNAs are highly soluble in aqueous solution. These new peptide nucleic acids may serve as alternatives to existing aeg-peptide nucleic acids.

5.5. Experimental Section

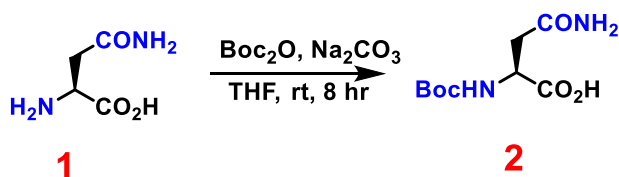
5.5.1. Materials and Methods:

All coupling reactions were performed under a nitrogen atmosphere. Various amino acids, Boc anhydride, HBTU, HOBt, Trifluoroacetic acid (TFA), N,N-Diisopropylethylamine (DIPEA),

DMF, Ethyl acetate (EtOAc), Hexane, and THF were procured from different commercial suppliers. Ethyl acetate and Hexane were distilled before use. Silica gel (100-200 mesh) was utilized for column chromatography. Analytical thin-layer chromatography (TLC) was used to monitor reactions, with results verified under UV light and stained with ninhydrin or iodine. All final cyclic peptides were purified using reverse-phase HPLC. NMR spectra (^1H at 400 MHz and 600 MHz, and ^{13}C at 101 MHz) were recorded with deuterated solvents, using CDCl_3 as the internal standard. Chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (δ 0.00). ^1H NMR splitting patterns were identified as singlet (s), doublet (d), broad doublet (bd), triplet (t), doublet of doublets (dd), and quintet (qn). Coupling constants (J) are reported in Hertz (Hz). Mass spectra were obtained using a MALDI-TOF instrument. We purchased the DNA and RNA samples from the commercial supplier Mylab.

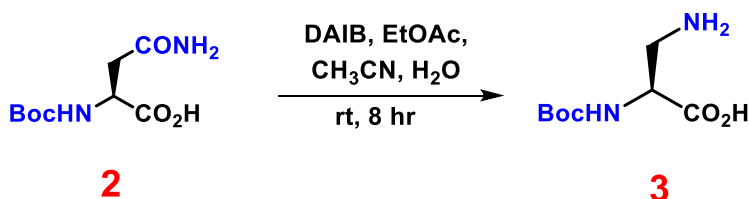
5.5.2. δ PNA Building Block Synthesis Protocol:

a) Step 1:



In an ice-bath supported round bottom flask, a solution of Boc_2O (10.3 mL, 45 mmol) in THF was added to a mixture containing L-asparagine (5.94 g, 45 mmol) and 10% Na_2CO_3 solution. The reaction mixture was stirred at room temperature for 8-9 hours. THF was then completely removed under reduced pressure. The resulting crude residue was acidified with 10% HCl to a pH of 3-4 and extracted with ethyl acetate (3 x 60 mL). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered, and concentrated to yield compound 2 as a white solid.

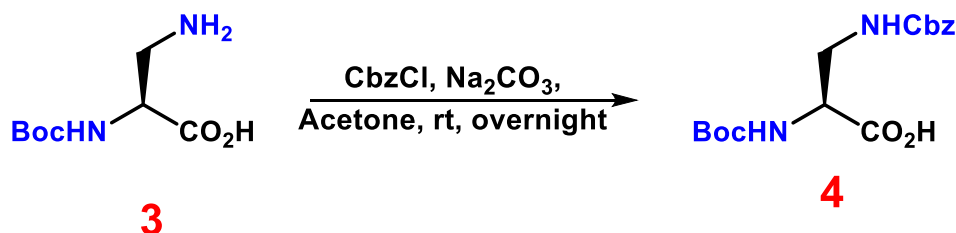
b) Step 2:



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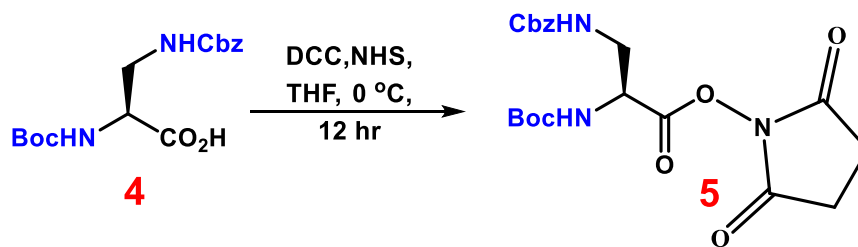
A mixture of Boc-L-asparagine (**2**) (6.15 g, 25 mmol), ethyl acetate (30 mL), acetonitrile (30 mL), water (15 mL), and iodobenzene diacetate (9.83 g, 30 mmol) was cooled and stirred at 15 °C for 30 minutes. The temperature was then held at 20 °C for 4 hours, followed by cooling to 0 °C and filtration. The solid residue was washed with ethyl acetate and dried under vacuum to yield compound **3**.

c) Step 3:



A solution of 10% aqueous NaHCO₃ (2.55 g, 30 mmol) was added to an ice-cold solution of compound **3** (2.173 g, 10 mmol) in acetone (30 mL) and stirred for 15 minutes at 0 °C. Benzyl chloroformate (4.036 mL, 12 mmol) in a 50% toluene solution was then added, and the reaction mixture was stirred overnight at room temperature. The acetone was evaporated, and the aqueous layer was washed with diethyl ether (3 × 50 mL). The aqueous layer was then acidified to pH 3.5-4 using a saturated aqueous KHSO₄ solution and extracted with ethyl acetate (3 × 60 mL). The combined organic extracts were dried over anhydrous Na₂SO₄, filtered, and concentrated to yield compound **4** as a sticky oil.

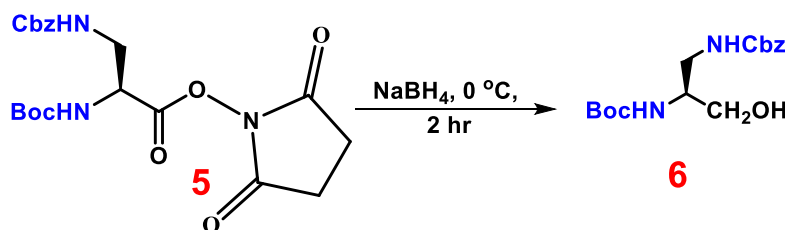
d) Step 4:



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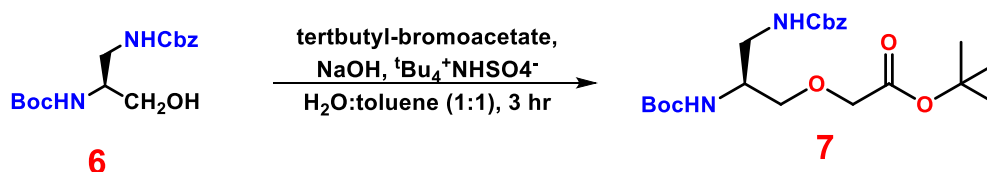
Compound **4** (3.38 g, 10 mmol) was dissolved in THF, and N-Hydroxysuccinimide (NHS, 2.3 g, 20 mmol) was added to the solution. Next, the coupling reagent DCC (2.47 g, 12 mmol) was introduced, and the mixture was maintained at 0 °C. The reaction was stirred for 12 hours, then washed and filtered using a sintered funnel to yield compound **5**.

e) Step 5:



Compound **5** was dissolved in THF, followed by the addition of NaBH₄ (1.3 g, 34 mmol) dissolved in water, in a ratio of 5 equivalents. The mixture was then stirred for 2 hours at 0 °C, resulting in the formation of the protected alcohol, compound **6**.

f) Step 6:



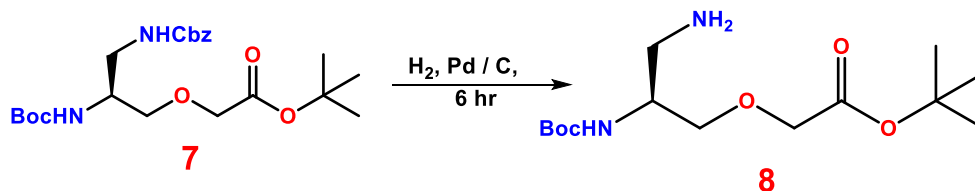
At 0 °C, a mixture of 1N NaOH in water (10 mL) and toluene (15 mL) was prepared, followed by the addition of a phase transfer catalyst tetrabutyl ammoniumhydrogen sulfate (tBu₄NHSO₄, 68 mg, 0.2 mmol). Tertbutyl bromoacetate (0.32 mL, 1.2 mmol) was then introduced and the mixture stirred for 15 minutes. A solution of compound **6** (0.64 g, 2 mmol) in toluene was added, and the mixture was stirred for 3 hours. Subsequently, a 10% HCl solution was added, and the mixture was extracted three times with ethyl acetate. The combined organic layer was washed with brine, dried over Na₂SO₄, and concentrated to yield compound **7**.

¹H NMR (400 MHz, Chloroform-d) δ 7.35 (d, J = 2.4 Hz, 3H), 7.34 – 7.27 (m, 2H), 5.84 – 5.70 (m, 1H), 5.41 – 5.23 (m, 1H), 5.15 – 5.08 (m, 2H), 3.94 (s, 2H), 3.82 (s, 1H), 3.63 (dd, J = 9.1, 3.8 Hz, 1H), 3.52 (dd, J = 9.1, 5.1 Hz, 1H), 3.43 (td, J = 13.7, 12.3, 6.2 Hz, 2H), 1.47 (s, 9H), 1.43 (s,

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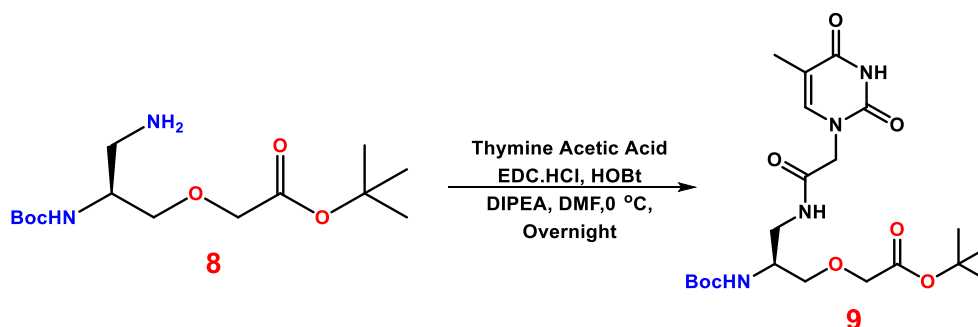
9H). ^{13}C NMR (101 MHz, Chloroform- d) δ 170.06, 157.26, 155.97, 136.83, 128.57 (2C), 128.09 (2C), 128.04, 82.33, 79.70, 71.38, 68.58, 66.73, 50.34, 42.80, 28.49 (3C), 28.23 (3C). **Mass (MALDI/TOF)** calculated for $\text{C}_{22}\text{H}_{34}\text{N}_2\text{O}_7$, 438.2366 obtained mass 438.2385.

g) Step 7:



A solution of compound **7** (2 g, 4.4 mmol) in methanol was treated with a catalytic amount of Pd/C (0.4 g) under an ice bath and hydrogen atmosphere. The mixture was stirred for 6 hours. Afterward, the solution was filtered through methanol, and the filtrate was concentrated to obtain compound **8**. **Mass (MALDI/TOF)** calculated for $\text{C}_{17}\text{H}_{28}\text{N}_2\text{O}_5$, 304.1998 obtained mass 304.1999.

h) Step 8:



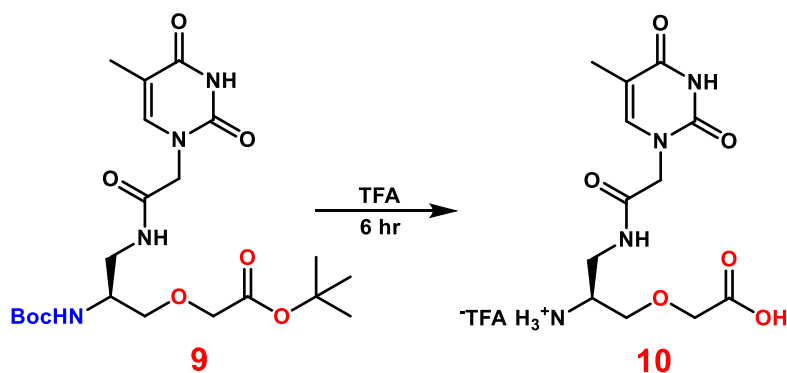
Thymine acetic acid (133 mg, 0.723 mmol) was dissolved in DMF (4 mL) at 0°C under a nitrogen atmosphere and stirred for 15 minutes. To this solution, a mixture of HOBT (98 mg, 0.723 mmol) and $\text{EDC}\cdot\text{HCl}$ (138 mg, 0.723 mmol) was added, followed by the dropwise addition of DIPEA (0.3 mL, 2 mmol). After 5 minutes, compound **8** (220 mg, 0.723 mmol) in DMF was introduced into the round-bottom flask and the mixture was stirred for 8 to 10 hours. The reaction was then extracted with a 10% HCl solution and extracted three times with ethyl acetate (3×30 mL). The

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combined organic extracts were washed with 10% Na₂CO₃ and brine, dried over Na₂SO₄, and concentrated to yield compound **9** as a white solid. Further we have purified compound **9** by column chromatography using ethyl acetate/pet ether solvent system to get pure compound **9**.

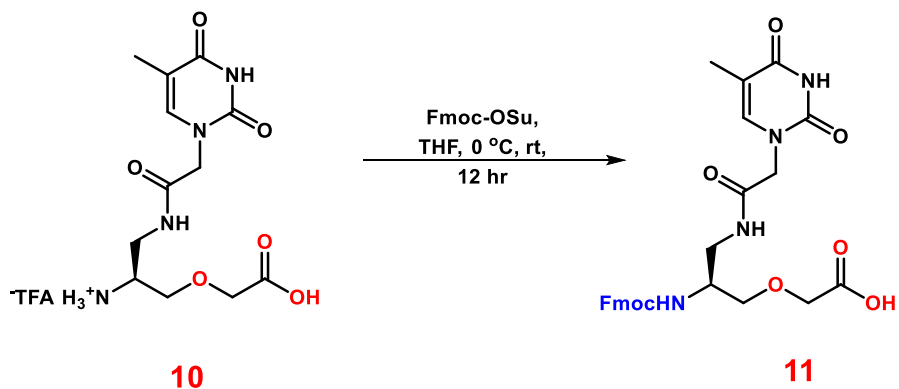
¹H NMR (600 MHz, DMSO-*d*₆) δ 11.27 (s, 1H) δ 8.13 (t, *J* = 5.8 Hz, 1H), 7.39 (d, *J* = 1.4 Hz, 1H), 6.61 (d, *J* = 8.5 Hz, 1H), 4.27 (q, *J* = 16.3 Hz, 2H), 3.95 (s, 2H), 3.74 – 3.45 (m, 1H), 3.38 (dd, *J* = 6.5, 2.9 Hz, 1H), 3.29 – 3.20 (m, 1H), 3.07 (dt, *J* = 12.9, 6.1 Hz, 1H), 1.75 (s, 3H), 1.42 (s, 9H), 1.38 (s, 9H). **¹³C NMR (151 MHz, DMSO-*d*₆)** δ 169.89, 167.71, 164.97, 155.72, 151.47, 142.73, 108.52, 81.34, 78.44, 71.03, 68.58, 65.39, 50.25, 49.73, 28.66 (3C), 28.20(3C), 12.36. **Mass (MALDI/TOF)** calculated for C₂₁H₃₄N₄O₈, 470.2377 obtained mass 470.2385.

i) Step 9:



At 0 °C, TFA (6 mL) was added to compound **9**, and the mixture was stirred for 6 hours. The TFA was then removed by evaporation with dichloromethane, repeated several times. The resulting oily residue was precipitated with diethyl ether, and the solid obtained was compound **10**.

j) Step 10:

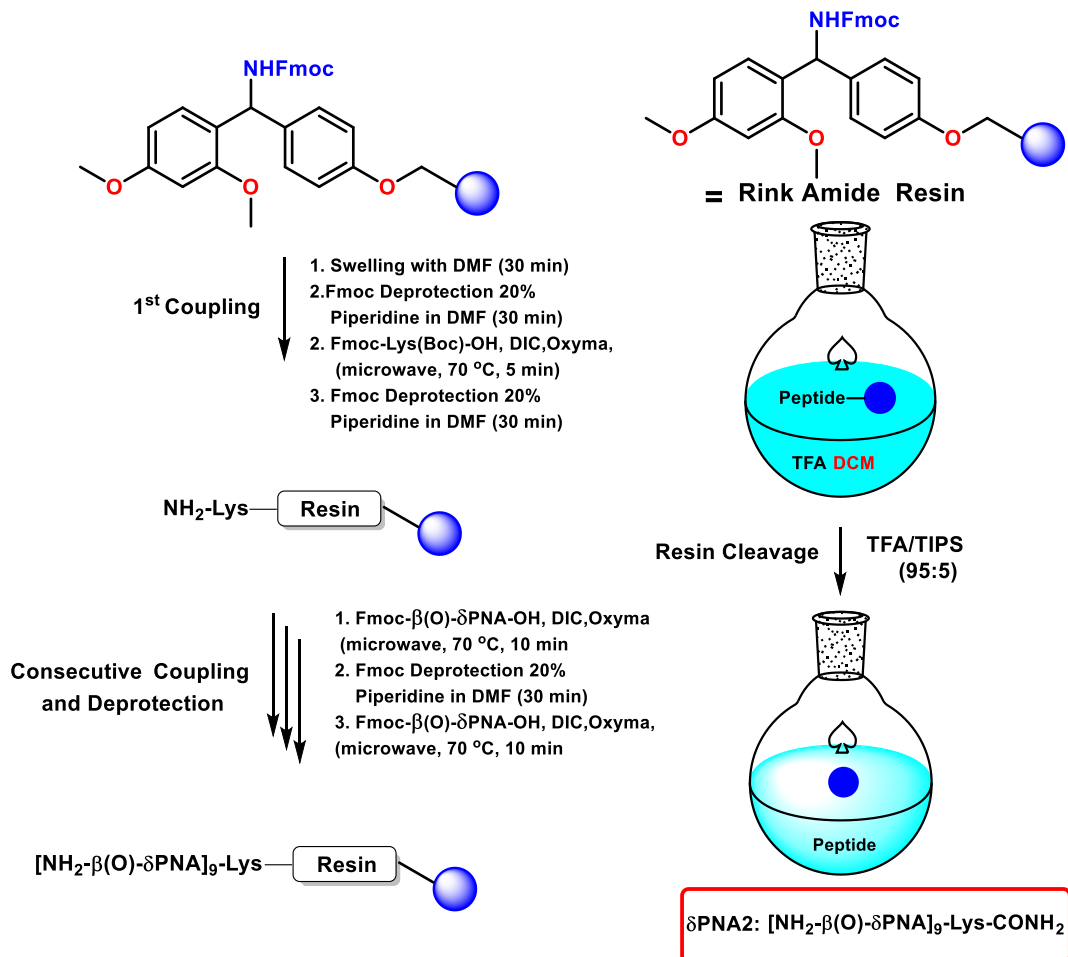


Compound **10** (0.944 g, 2 mmol) was dissolved in THF at 0 °C, and the pH was adjusted to around 9 using a 10% Na₂CO₃ solution. After 5 minutes, a THF solution of Fmoc-OSu (0.606 g, 1.8 mmol) was added, and the mixture was stirred for 12 hours. The reaction was then quenched with a 10% HCl solution and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, and concentrated. The resulting residue was treated with ethyl acetate and petroleum ether, decanted, and dried under high vacuum to yield a white solid, compound **11**. Further we have purified compound **11** by column chromatography using ethyl acetate/pet ether solvent system to get pure compound **11**.

¹H NMR (400 MHz, DMSO-*d*₆) δ 12.67 (s, 1H), 11.26 (s, 1H), 8.26 (s, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.71 (d, *J* = 7.5 Hz, 2H), 7.40 (q, *J* = 6.4, 5.3 Hz, 3H), 7.33 (t, *J* = 7.4 Hz, 3H), 4.28 (d, *J* = 4.3 Hz, 4H), 4.23 (d, *J* = 7.0 Hz, 1H), 4.01 (s, 2H), 3.70 (d, *J* = 7.4 Hz, 1H), 3.44 (d, *J* = 5.8 Hz, 2H), 3.32 (s, 2H), 1.73 (s, 3H). **¹³C NMR (151 MHz, DMSO-*d*₆)** δ 172.01, 167.54, 164.80, 156.13, 151.25, 144.08 (2C), 142.59, 140.91 (2C), 127.90 (2C), 127.36 (2C), 125.51 (2C), 120.33 (2C), 108.31, 70.67, 67.85, 65.76, 50.54, 49.57, 46.89, 40.06, 12.09. **Mass (MALDI/TOF)** calculated for C₂₇H₂₈N₄O₈, 536.1907 obtained mass 536.1955.

5.5.3. PNA Building Synthesis Protocol:

We have synthesized all PNA molecules in solid phase peptide synthesis protocol in Biotage Alstra instrument. We have taken the rink amide resin, specifically 4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy resin, as the solid support. After swelling the resin with DMF, the Fmoc group was deprotected using a 20% piperidine in DMF solution. Monomer coupling was facilitated by in situ activation using the DIC/Oxyma coupling reagent, with reactions conducted at 70°C in NMP solution and assisted by microwave heating. For the C-terminal spacer, we incorporated commercially available orthogonally protected (Fmoc) L-lysine, which was conjugated to the resin via an amide bond. The designed sequence was synthesized on a small scale (0.04 mmol/g), starting with the lysine residue, followed by the coupling of either aeg building blocks or modified delta building block δPNA sequence building blocks. We bought aeg containing Fmoc protected building block from commercial sources (BLD Pharma). We have provided details synthesis protocol of δPNA2 in below Scheme 3. All other PNA molecules described here were synthesized similar protocol. After synthesizing we have cleaved our compound from the resin using TFA and triisopropylsilane cocktail mixture as (95:5) ratio.



Scheme 3: Solid Phase Synthesis Protocol and resin cleavage schematic diagram.

5.5.4. Circular Dichroism Spectroscopy Measurement:

CD spectra of the prepared samples were recorded using a JASCO J-715 spectropolarimeter. Measurements were taken for the control single strands and PNA or PNA complexes in a solution containing 10 mM NaCl and 10 mM sodium phosphate buffer at pH 7.0. The temperature of the circulating water was maintained at 4°C, below the melting temperature of the strands. The homooligomer of delta PNA and aeg PNA was combined with ssDNA or ssRNA in the appropriate ratios to form duplex or triplex structures. CD spectra were recorded from 350 to 195 nm with 3 scans accumulated using a 1 cm cell, 0.1 nm resolution, 2 mdeg sensitivity, 1.0 nm bandwidth, 50 nm/min scan speed, and 2 sec response time. Each CD experiment was conducted twice to ensure reproducibility.

5.6. References:

- [1]. a) J. D. Watson, F. H. C. Crick, *Nature* **1953**, *171*, 737-738.
- [2]. L. Pray, *Nature Education* **2008**, *1*, 100.
- [3]. S. T. Crooke, *Nat. Biotechnol.* **1992**, *10*, 882-886.
- [4]. P. E. Nielsen, *Acc. Chem. Res.* **1999**, *32*, 624-630.
- [5]. K. Hoogsteen, *Acta. Cryst.* **1963**, *65*, 907.
- [6]. F. H. C. Crick, *J. Mol. Biol.* **1966**, *19*, 548-555.
- [7]. N. C. Seeman, J. M. Rosenberg, A. Rich, *Proc. Natl. Acad. Sci. USA*, **1976**, *73*, 804-807.
- [8]. W. A. Velema, E. T. Koo, *Nat. Rev. Chem.* **2020**, *4*, 22-37.
- [9]. a) P. E. Nielsen, M. Egholm, R. H. Berg, O. Buchardt, *Science* **1991**, *254*, 1497-1500; b) H. Qrum, P. E. Nielsen, M. Egholm, R. H. Berg, O. Buchardt, C. Stanley, *Nucleic acid Res.* **1993**, *21*, 5332- 5336. c) C. Thiede, E. Bayerdoerffer, R. Blasczyk, B. Wittig, A. Neubauer, *Nucleic Acid Res.* **1996**, *24*, 983-984; d) D. B. Demers, E. T. Curry, M. Egholm, A. C. Sozer, *Nucleic Acid Res.* **1995**, *23*, 3050-3055; e) P. E. Nielsen, M. Egholm, R. H. Berg, O. Buchardt, *Nucleic Acid Res.* **1993**, *21*, 197-200; f) L. Good, P. E. Nielsen, *Antisense Nucleic Acid Drug Dev.* **1997**, *7*, 431. g) De Mesmaeker, A. et al: *Curr. Open. Struct. Biol.* 1995, *5*, 343. h) E. Uhlmann, A. Peyman, G. Breipohl, D. W. Will, *Angew. Chem. Int. Ed.* **1998**, *37*, 2796-2823.
- [10]. a) S. C. Brown, S. A. Thomson, J. M. Veal, D. G. Davis, *Science* **1994**, *265*, 777-780; b) H. Rasmussen, J. S. Kastrup, J. N. Nielsen, J. M. Nielsen, P. E. Nielsen, *Nat. Struct. Biol.* **1997**, *4*, 98-101; c) M. Leijon, A. Graeslund, P. E. Nielsen, O. Buchardt, B. Norden, S. M. Kristensen, M. Eriksson, *Biochemistry* **1994**, *22*, 9820-9825; (d) M. Eriksson, P. E. Nielsen, *Nat. Struct. Biol.*, **1996**, *3*, 410-413; f) L. Bets, J. A. Josey, J. M. Veal, S. R. Jordan, *Science* **1995**, *270*, 1838-1841.
- [11]. E. Uhlmann, D. W. Will, G. Breipohl, D. Langner, A. Rytte, *Angew. Chem. Int. Ed.* **1996**, *35*, 2632-2635.
- [12]. a) G. Felsenfeld, R. D. Davies, A. Rich, *J. Am. Chem. Soc.* **1957**, *79*, 2023-2024; b) T. Le Deon, L. Perrouault, D. Praseuth, N. Habhoub, J. L. Decout, N. T. Thuong, J. Lhomme, C. Helene,

Nucleic Acid Res. **1987**, 15, 7749-7760; d) C. Helene, J. J. Toulene, *Oligonucleotides: Antisense Inhibitors of Gene Expression*, McMillan Press, London, **1989**; e) H. E. Moser, P. B. Dervan, *Science* **1987**, 238, 645-650. (e) G. Felsenfeld, A. Rich, *Biochem. Biophys. Acta.* **1957**, 26, 457-468; f) P. E. Nielsen, M. Egholm, R. H. Berg, O. Buchardt, *Science* **1991**, 254, 1497-1501; g) P. E. Nielsen, M. Egholm, O. J. Buchardt, *Mol. Recogn.* **1994**, 7, 165-170.

[13]. a) P. P. Chan, P. M. Glazer, *J. Mol. Med.* **1997**, 75, 267-282; b) E. Uhlmann, A. Peyman, *Chem. Rev.* **1990**, 90, 543-584.

[14]. A. Ray, B. Norden, *FASEB J.* **2000**, 14, 1041-1060.

[15]. a) H. Knudsen, P. E. Nielsen, *Nucleic Acids Res.* **1996**, 24, 494-500; b) L. Wang, H. Li1, C. Zhao, S. Li1, L. Kong, W. Wu, W. Kong, Y. Liu, Y. Wei, J. K. Zhu, H. Zhang, *Plant Cell Environ.* **2017**, 40, 56–68.

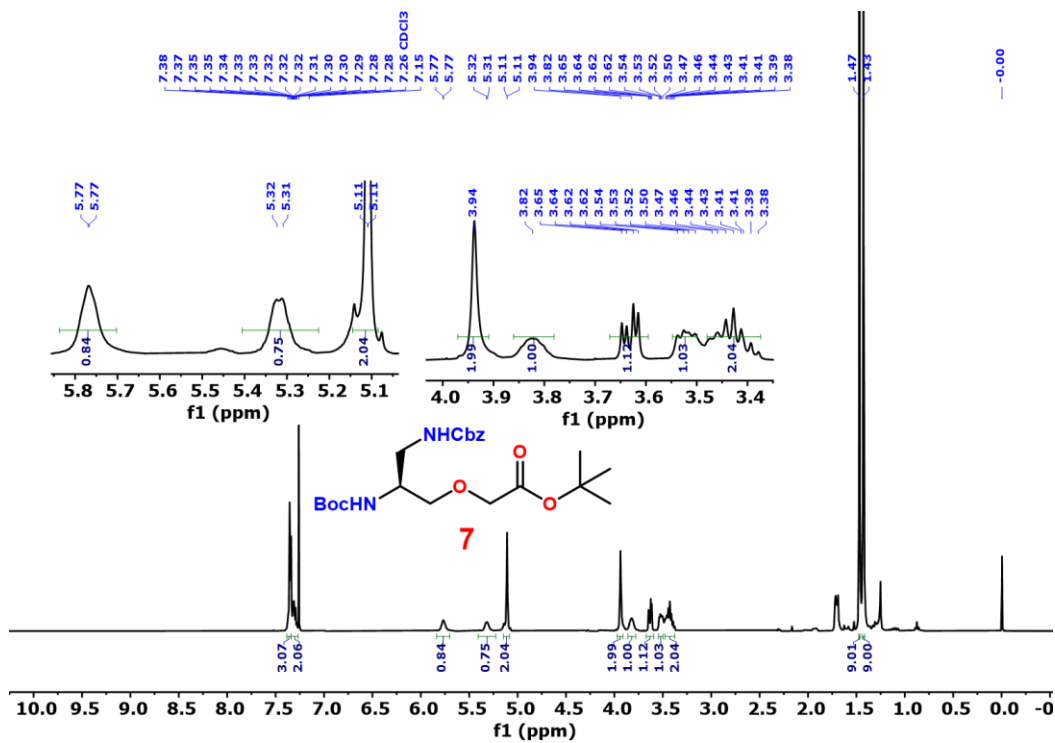
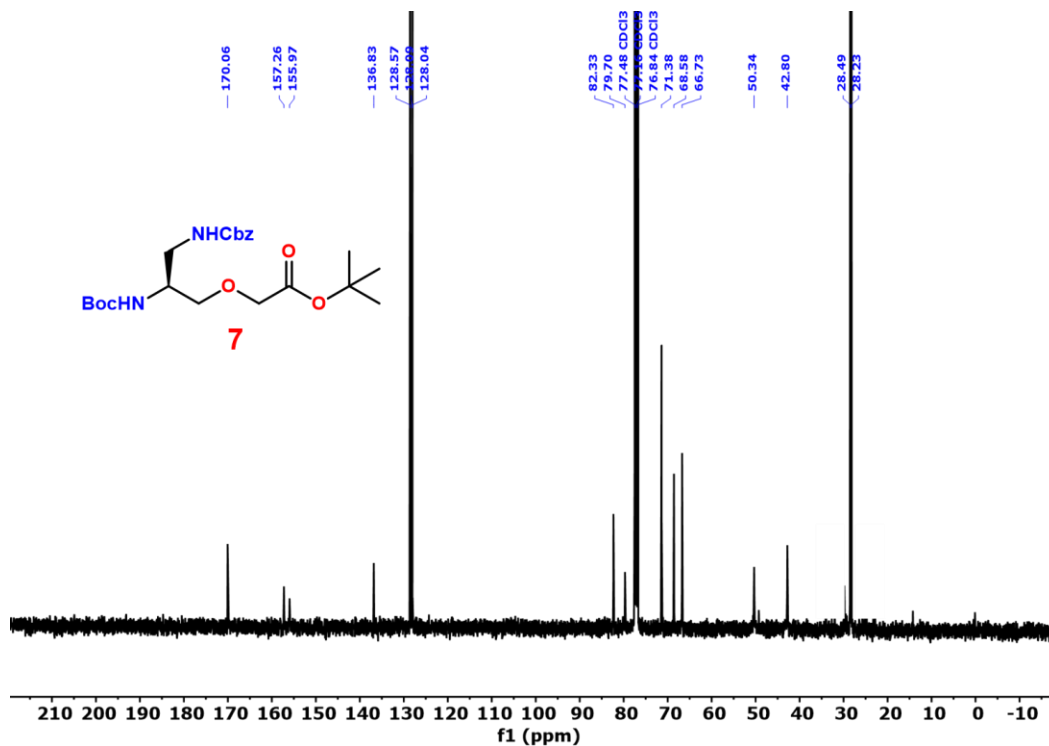
[15]. Y. Huang, S. Dey, X. Zhang, F. Sonnichsen, P. Garner, *J. Am. Chem. Soc.* **2004**, 126, 4626-4640.

[16]. P. E. Nielsen, M. Egholm, O. Buchardt, *Gene.* **1994**, 149, 139-145.

[17]. R. W. Taylor, P. F. Chinnery, D. M. Turnbull, R. N. Lightowlers, *Nature Genet* **1997**, 15, 212-215.

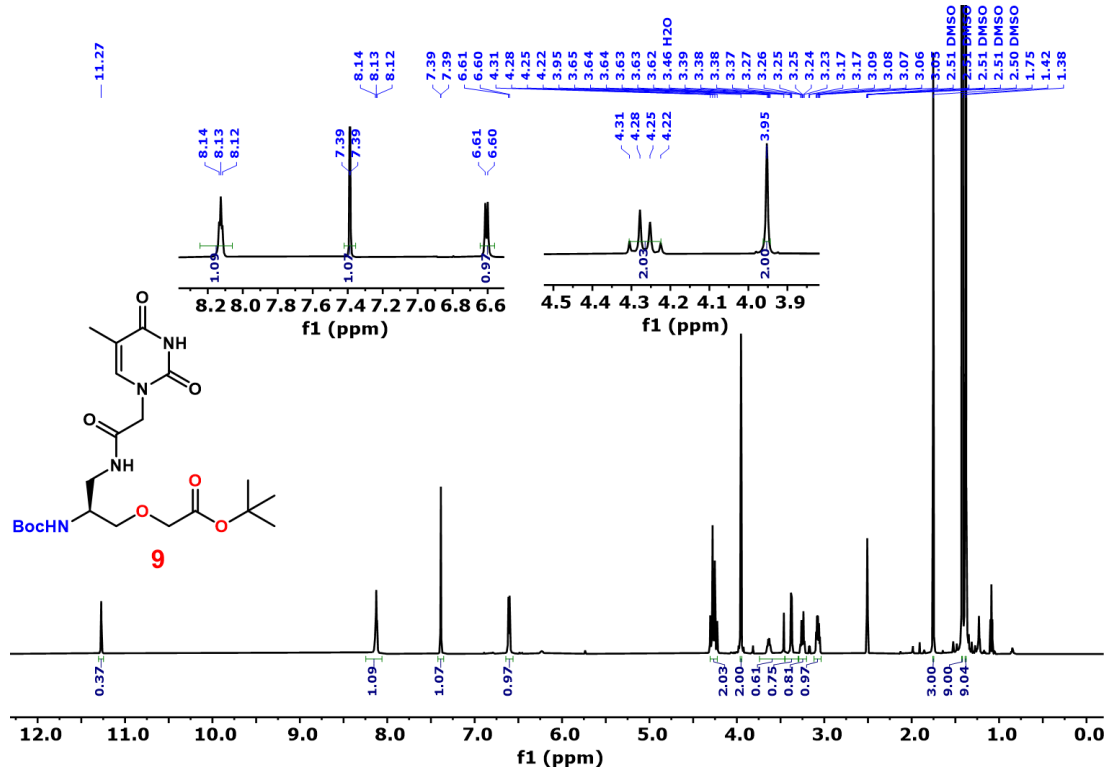
[18]. R. W. Taylor, P. F. Chinnery, D. M. Turnbull, R. N. Lightowlers, *Nature Genet.* **1997**, 15, 212-215. G. M. Sheldrick, *Acta Cryst.* **2008**, A64, 112-122.

[19]. a) P. E. Nielsen, *Curr. Opin Biotechnol.* **1999**, 10, 71-75; b) V. A. Kumar, K. N. Ganesh, *Acc. Chem. Res.* **2005**, 38, 404-412.

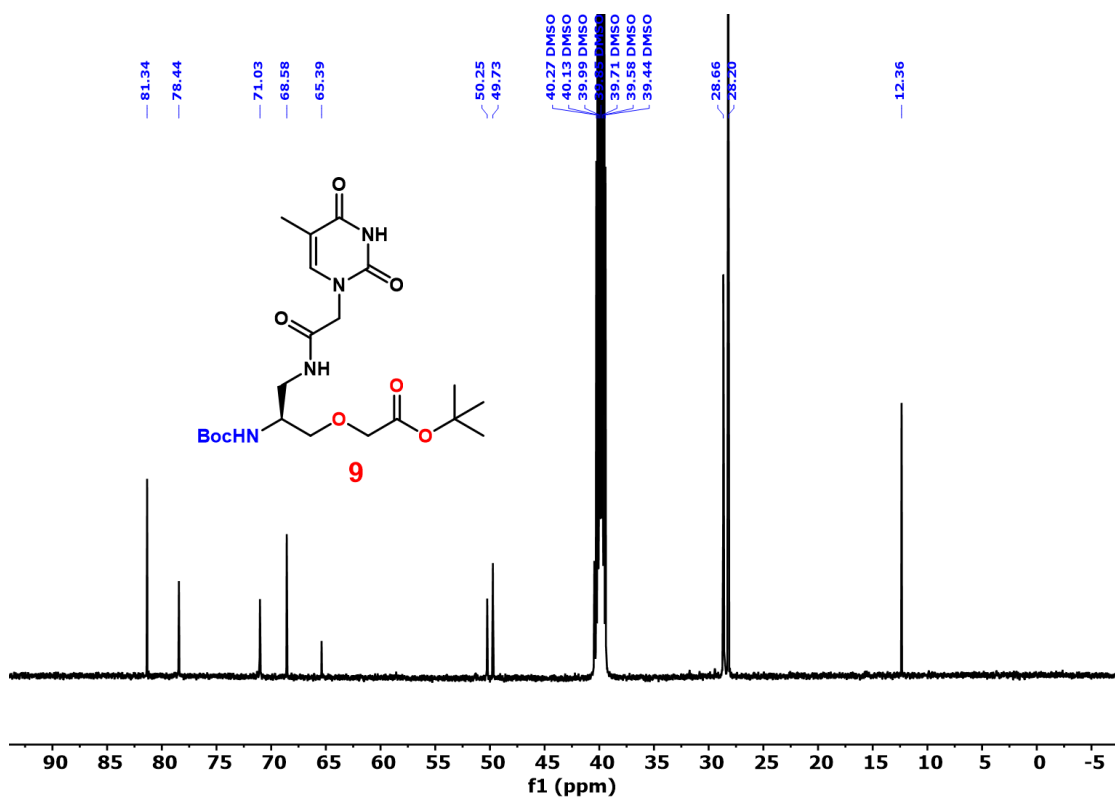
5.7. Appendix V: ^1H , ^{13}C NMR and Mass Spectrum:a) ^1H NMR Spectrum of Compound 7b) ^{13}C NMR Spectrum of Compound 7

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c) ^1H NMR Spectrum of Compound 9

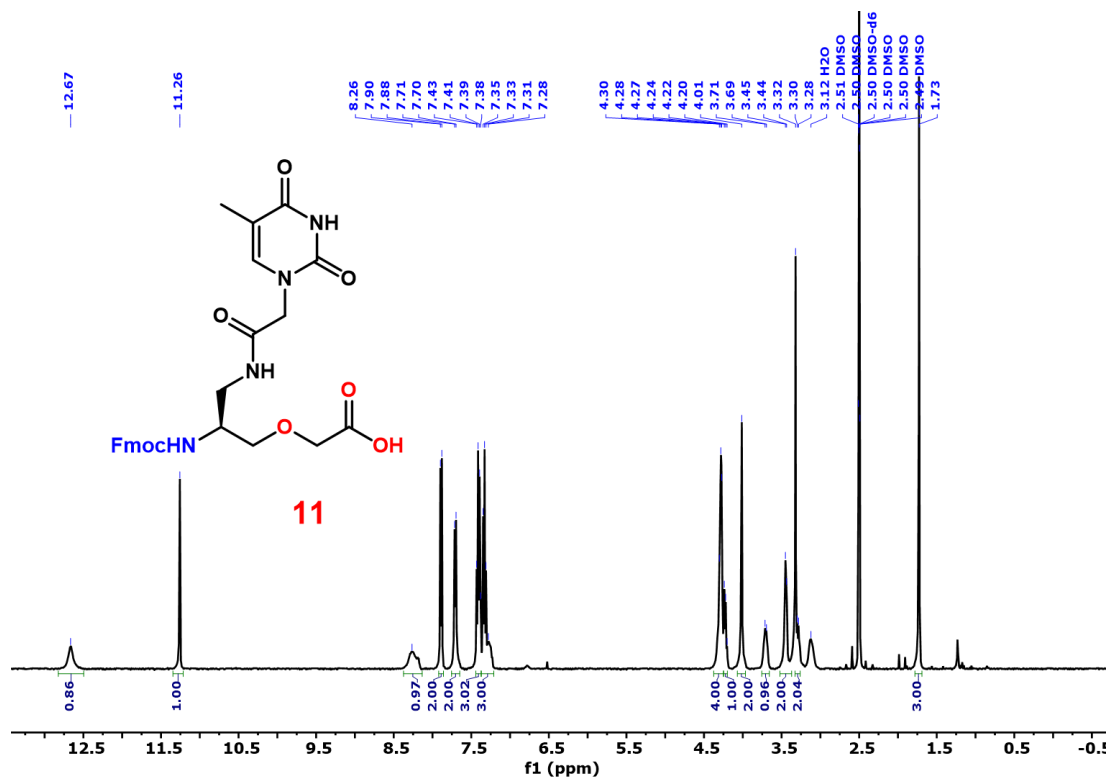


d) ^{13}C NMR Spectrum of Compound 9

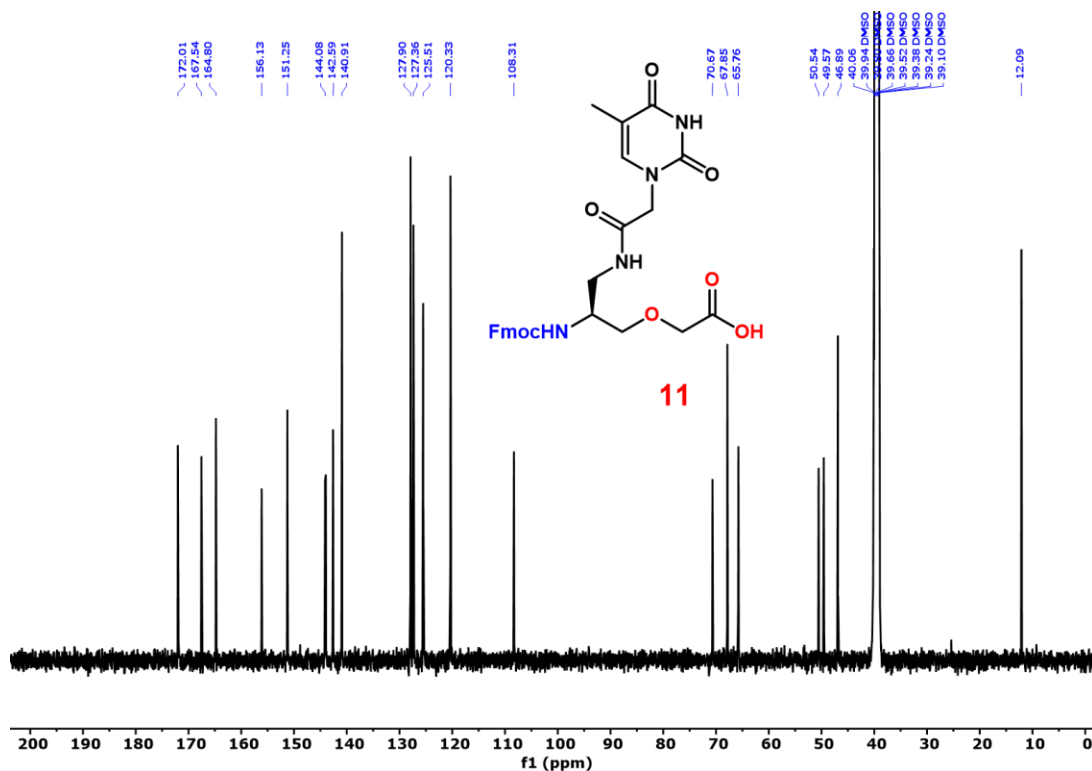


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e) ^1H NMR Spectrum of Compound 11

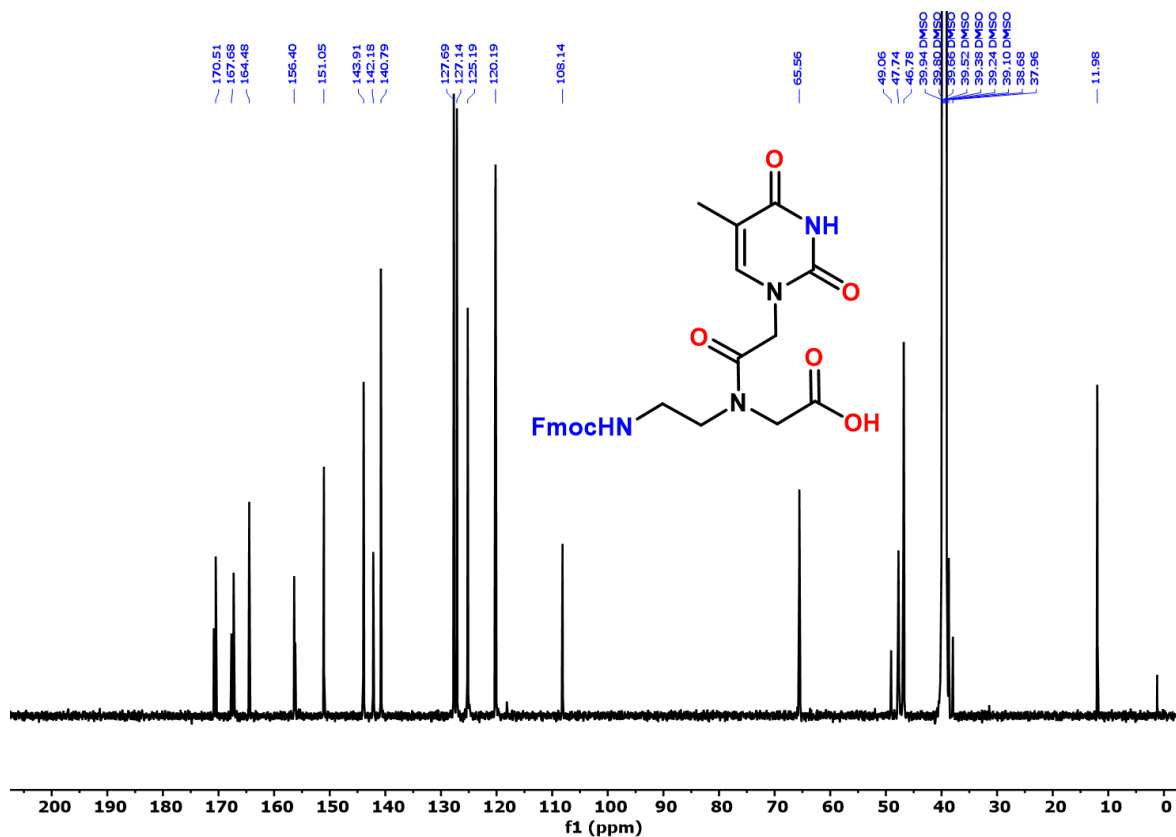


f) ^{13}C NMR Spectrum of Compound 11

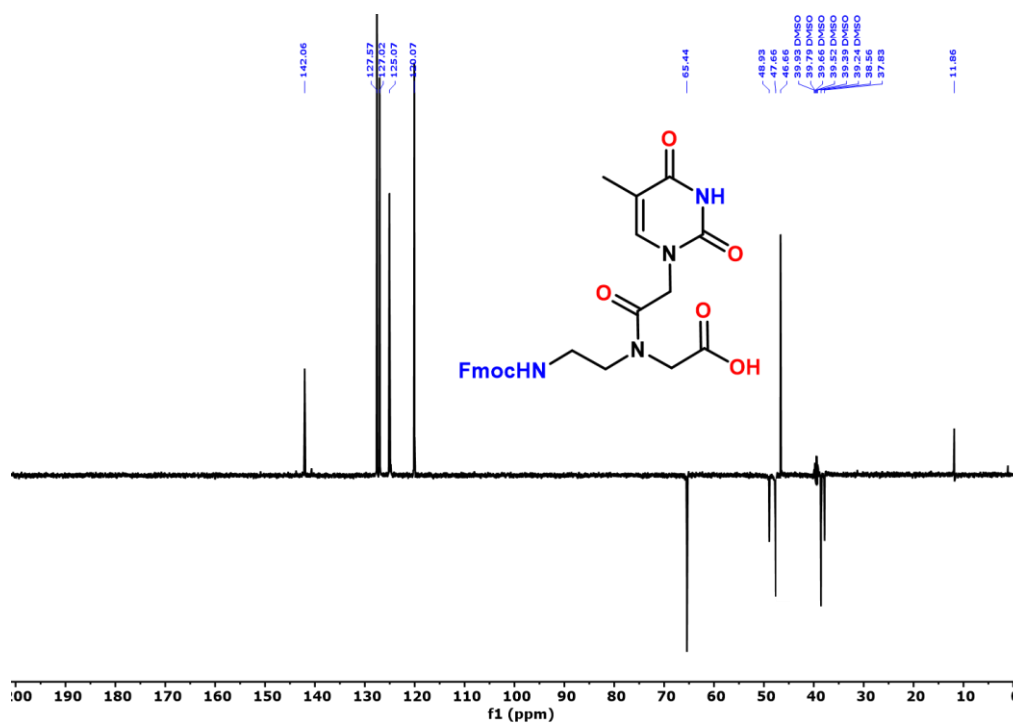


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i) ^{13}C NMR Spectrum of Compound FmocNH-aegThy-COOH

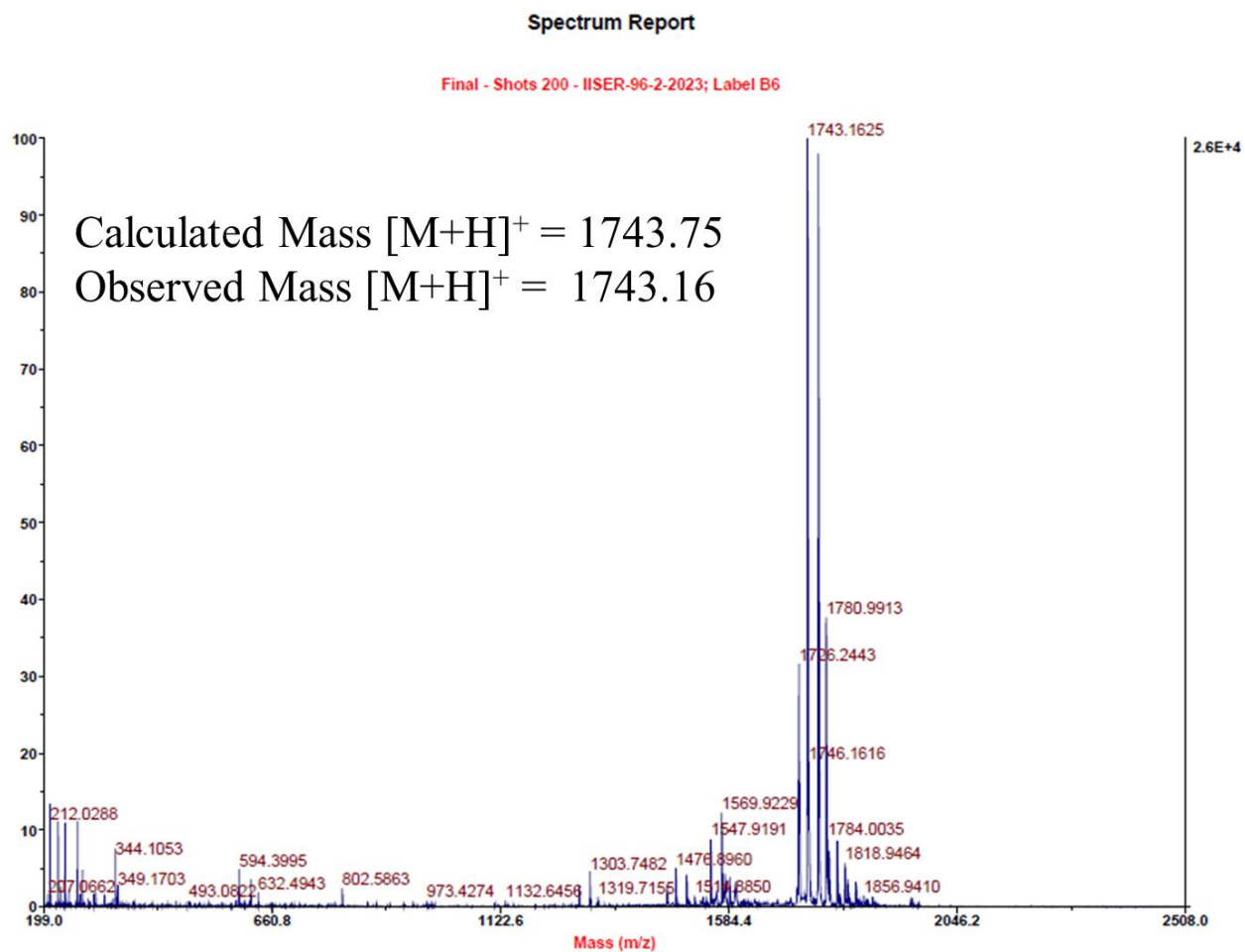


i) DEPT-135 NMR Spectrum of Compound FmocNH-aegThy-COOH

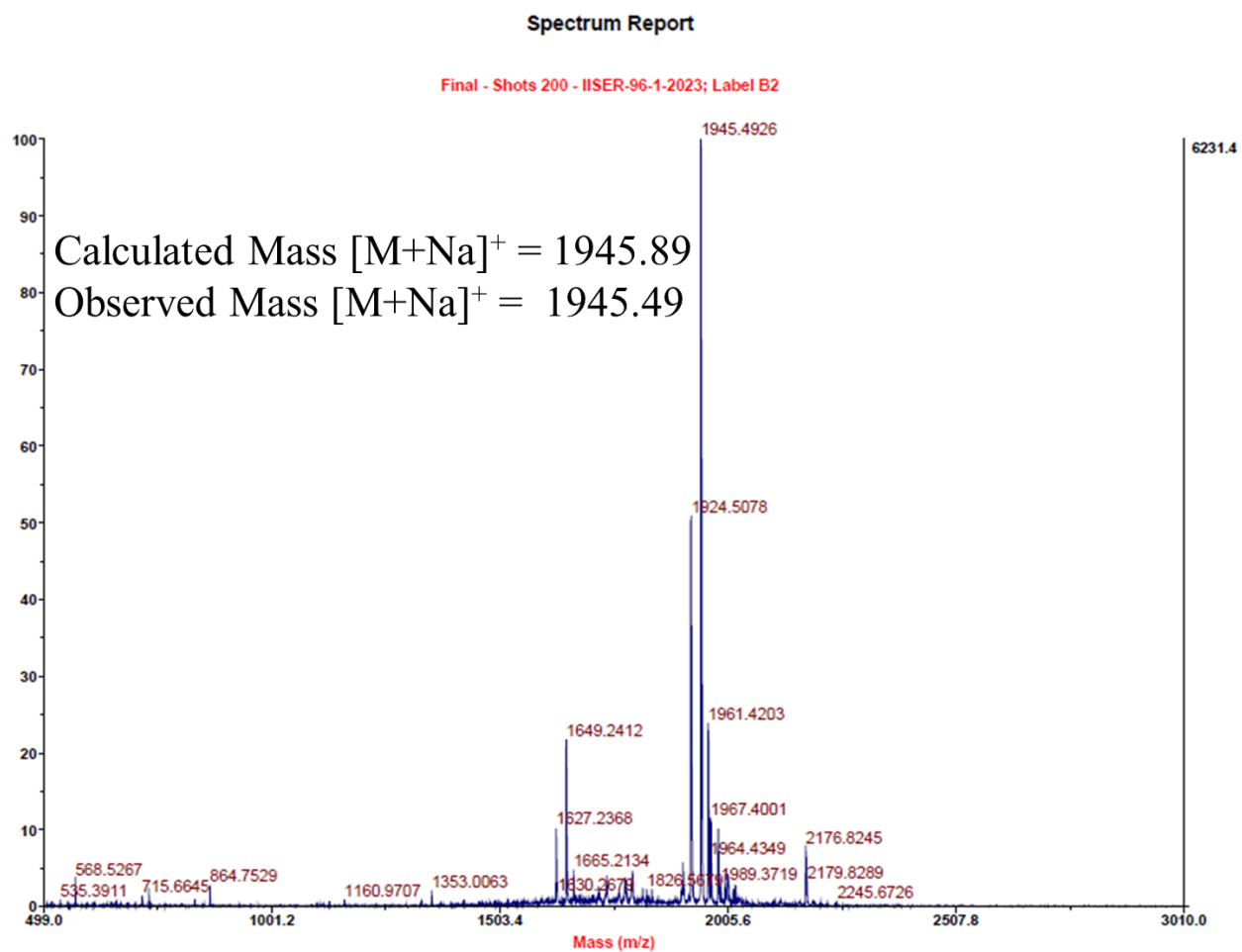


MALDI/TOF Spectra of PNA Molecules

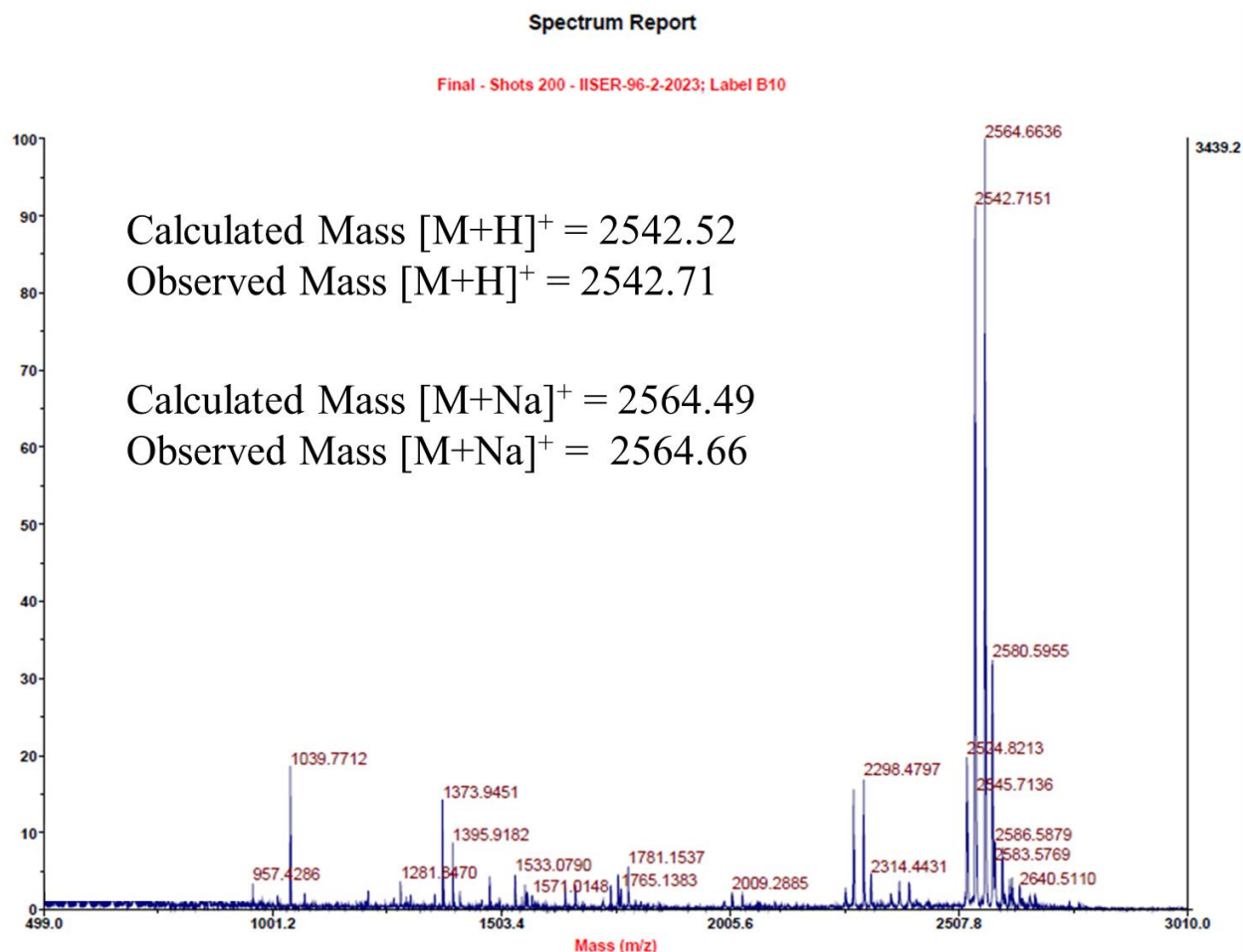
(a) MALDI/TOF Spectra of aegPNA1:



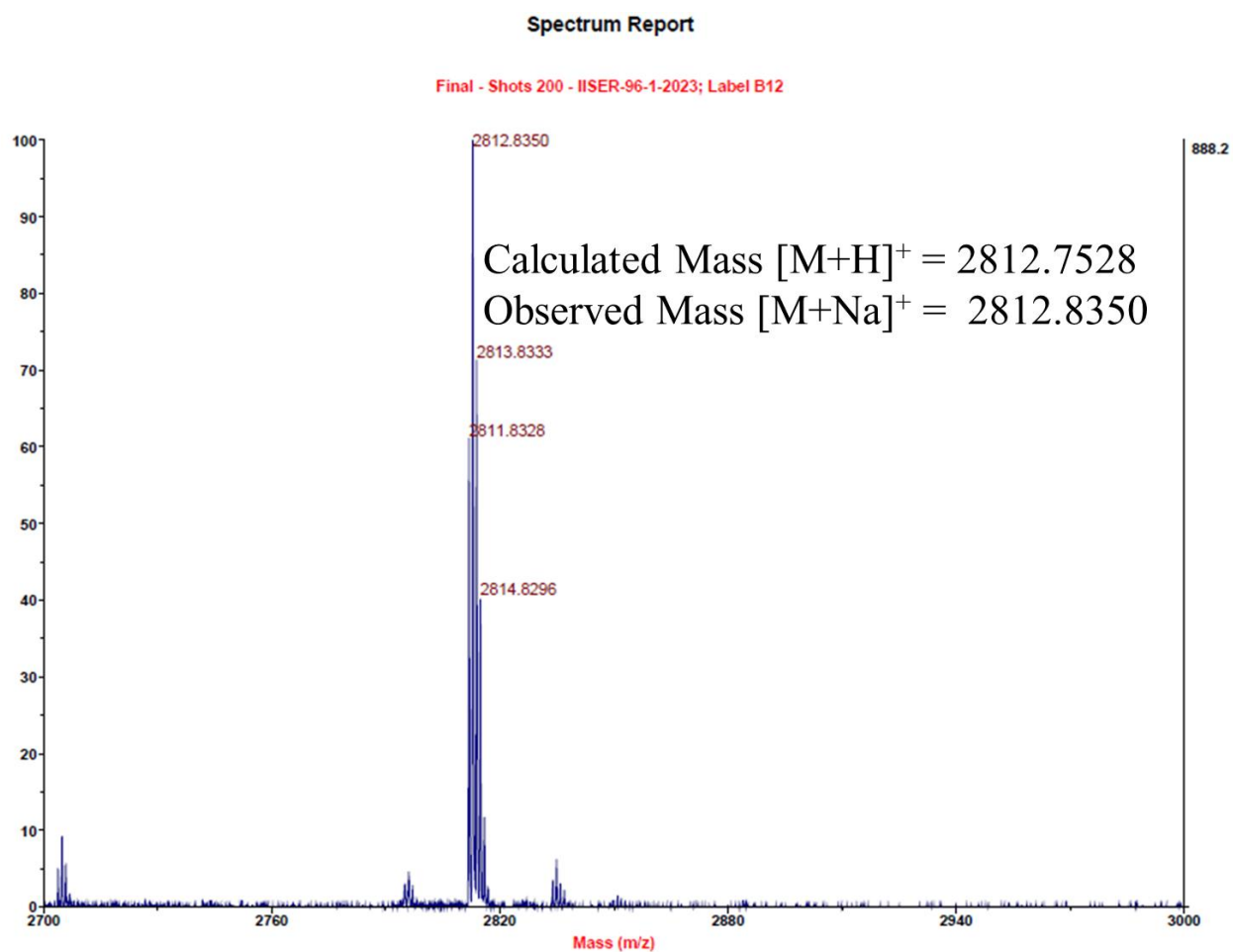
(b) MALDI/TOF Spectra of δ PNA1:



(c) MALDI/TOF Spectra of aegPNA2:



(d) MALDI/TOF Spectra of δ PNA2:



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