SYNTHESIS OF CYCLIC HYBRID PEPTOID AND PEPTIDE BASED INHIBITORS FOR β-AMYLOID FIBRILLAR AGGREGATION

A thesis submitted in partial fulfilment for
the degree of

Master of Science

as a part of the

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(Chemical Science)

by

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Dedicated to my parents

NEW CHEMISTRY UNIT,
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CERTIFICATE

I hereby certify that the work described in this thesis entitled "**Synthesis of cyclic hybrid peptoid and peptide based inhibitors for β-amyloid fibrillar aggregation**" has been carried out by Mr. K. Rajasekhar under my supervision at the Bioorganic Chemistry Laboratory, New Chemistry Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India and that it has not been submitted elsewhere for the award of any degree or diploma.

Dr. T. Govindaraju
(Research Supervisor)

DECLARATION

I hereby declare that the matter embodied in this thesis "**Synthesis of cyclic hybrid peptoid and peptide based inhibitors for β-amyloid fibrillar aggregation**" is the result of investigations carried out by me under the supervision of **Dr. T. Govindaraju** at the New Chemistry Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India and that it has not been submitted elsewhere for the award of any degree or diploma.

In keeping with the general practice in reporting the scientific observations, due acknowledgement has been made whenever the work described is based on the findings of other investigators. Any omission that might have occurred due to oversight or error in judgement is regretted.

K. Rajasekhar

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K. Rajasekhar

PREFACE

The thesis entitled "**Synthesis of cyclic hybrid peptoid and peptide based inhibitors for β-amyloid fibrillar aggregation**" is divided into 3 chapters as follows

Chapter 1: Introduction

Brief overview of chemical structures and properties of peptides and peptidomimics, explains strategies used for their synthesis and their applications in medicinal chemistry.

Chapter 2: *N*-Alkyl and *N*-acyl substituent enforced differential cyclization of hybrid peptoid monomers

Describes an elegant methodology for the synthesis of six and twelve membered cyclic hybrid peptoids from *N*-(2-aminoethyl)glycine functionalized monomers. Differential cyclization based on α*N*-substitution of *N*-(2-aminoethyl)glycine backbone and the asymmetric conformation adapted by the cyclic twelve membered hybrid peptoids due to intramolecular hydrogen bonding is discussed.

Chapter 3: Synthesis of peptide based potential inhibitors for β-amyloid fibrillar aggregation

This Chapter explains the synthesis of hybrid peptide molecules with thymine and barbiturate functionalities using automated solid phase peptide synthesis. Synthesis of β-amyloid fragments to use as controls in the fibrillar aggregation studies is described.

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List of Figures

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Abbreviations

Aβ	Amyloid beta peptide
AD	Alzheimer's disease
aeg	*N*-(2-aminoethyl)glycine
Boc	*N-tert*-butoxycarbonyl
Calcd	Calculated
CD	Circular Dichroism
DCM	Dichloromethane
DIPEA/DIEA	Diisopropylethylamine
DMF	N, N-Dimethylformamide
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
Fmoc	9-Fluronylmethoxycarbonyl
HATU	O-(7-Azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate
HBTU	O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate
HMBC	Heteronuclear multiple bond correlation spectroscopy

HOBt	1-Hydroxybenzotriazole
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum correlation spectroscopy
IR	Infrared
LCMS	Liquid chromatography mass spectroscopy
MALDI-TOF	Matrix assisted laser desorption/ ionisation-time of flight
mg	Milligram
mmol	Millimoles
nm	Nanometre
NMR	Nuclear magnetic resonance
ppm	Parts per million
PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
PNA	Peptide nucleic acid
SPPS	Solid phase peptide synthesis
TEA/Et_3N	Triethylamine
TFA	Trifluoroacetic acid
TLC	Thin layer chromatography

Chapter 1

INTRODUCTION

Chapter 1

Introduction

1.1 Peptides

Proteins form the very basis of life, the term protein is derived from the Greek word *proteios,* which means standing in front. Proteins regulate a variety of activities in all living organisms, from replication of DNA to transport of oxygen across the body. Proteins are responsible for regulating cellular machinery and consequently the phenotype of an organism. Proteins accomplish their task by three-dimensional tertiary and quaternary interactions with various substrates such as DNA, RNA and other proteins. By understanding the structure of the protein, we can probe for its function and potentially apply the new knowledge to various genome and proteome projects, such as mapping the functions of proteins in metabolic pathways and deducing evolutionary relationships and proteome network which is useful in system biology. Peptides[1] are short fragments of proteins consists of amino acids linked by amide bonds.[2] Peptides can be normally differentiated from proteins by the number of amino acids present in a given chain. Generally a peptide constitutes a minimum of two amino acid residues or a maximum of 50 amino acid residues per chain. Smallest known peptide is a dipeptide followed by tripeptide, tetrapeptide etc.

1.1.1 Amino acids

Amino acids are generally referred as aminoalkanoic acids[3] owing to the presence of both amino and carboxyl groups in the same molecule. The history of amino acid discovery dates back to early 19th century and the first amino acid discovered was asparagine by French chemists Nicolas Vauquelin and Pierre Jean Robiquet in 1806.[4] Amino acids under physiological conditions exists as zwitter ions (both positive and negative charges) and can

be represented as H_3N^+- $(CR_1R_2)_n$ - COO^- (n = 1, are referred to α amino acids, R_1 = H and varying R_2, n = 2 are β amino acids and n = 2 with αβ- unsaturation are known as dehydro amino acids). Nearly 500 different amino acids[2] are available in nature, of which only few are utilized by the living systems. Analysis of a vast number of proteins has revealed that, they are mainly composed of 20 standard amino acids (Table 1.1). The 20 standard amino acids found in the living systems are termed as proteinogenic amino acids and the rest as non-proteinogenic amino acids. All the proteinogenic amino acids are α-amino acids (except proline, which is imino acid) and bear *L*-configuration (except glycine which is achiral).

Table 1.1 Naturally occurring amino acids and their isoelectric point (pI) values.

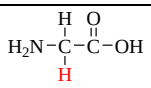
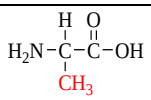
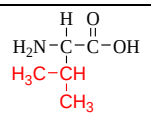
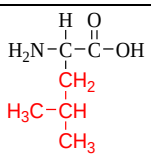
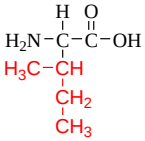
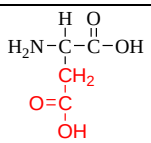
Classification	**Amino acid**	**Three letter code**	**One letter code**	**Structure**	**Molecular weight**	**Isoelectric point (pI)**
Aliphatic side chain amino acids (Hydrophobic)	Glycine	Gly	G		75.07	5.97
	Alanine	Ala	A		89.09	6.00
	Valine	Val	V		117.15	5.96
	Leucine	Leu	L		131.17	5.98
	Isoleucine	Ile	I		131.17	6.02
Acidic amino acids	Aspartic acid	Asp	D		133.10	2.77

Table 1.1 Naturally occurring amino acids and their isoelectric point (pI) values (continued).

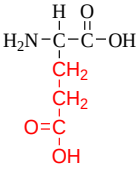
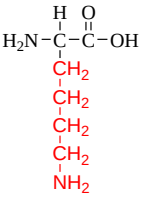
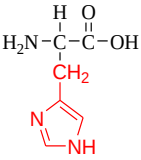
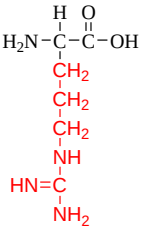
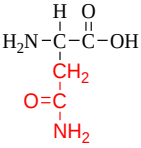
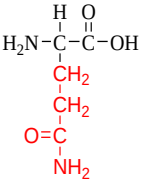
Classification	Amino acid	Three letter code	One letter code	Structure	Molecular weight	Isoelectric Point (pI)
Acidic amino Acids (Hydrophilic)	Glutamic acid	Glu	E		147.13	3.22
Basic amino acids (Hydrophilic)	Lysine	Lys	K		146.19	9.74
	Histidine	His	H		155.16	7.59
	Arginine	Arg	R		174.20	10.76
Amides of Acidic amino acids	Aspargine	Asn	N		132.12	5.41
	Glutamine	Gln	Q		146.15	5.65

Table 1.1 Naturally occurring amino acids and their isoelectric point (pI) values (Continued).

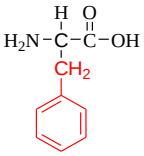
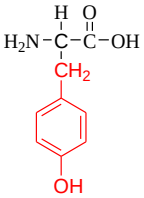
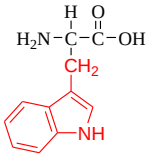
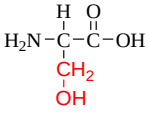
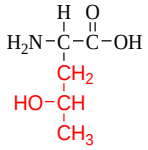
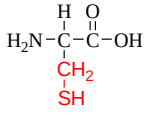
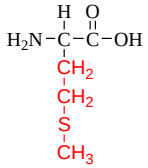
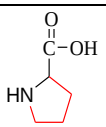
Classification	Amino acid	Three letter code	One letter code	Structure	Molecular weight	Isoelectric Point (pI)
Aromatic amino acids	Phenyl alanine	Phe	F		165.19	5.48
	Tyrosine	Try	Y		181.19	5.66
	Tryptophan	Trp	W		204.23	5.89
Hydroxy-containing amino acids	Serine	Ser	S		105.09	5.58
	Threonine	Thr	T		119.12	5.60
Sulphur-containing amino acids	Cysteine	Cys	C		121.15	5.07
	Methionine	Met	M		149.21	5.74

Table 1.1 Naturally occurring amino acids and their isoelectric point (pI) values (Continued).

Classification	Amino acid	Three letter code	One letter code	Structure	Molecular weight	Isoelectric Point (pI)
Imino acid	Proline	Pro	**P**		115.13	6.30

Biosynthesis pathways for all the 20 amino acids is not found in humans, only 11 of the 20 amino acids are synthesized in the body and for the rest we depend on food supplements. Deficiency of amino acids can lead to disorders like depression,[5] immune deficiency, indigestion and chronic fatigue. The net charge on the zwitter ionic amino acids is affected by pH of surrounding environment and can become more positively or negatively charged due to loss or gain of protons (H^+). The pH value at which amino acid is electrically neutral and does not move to either positive electrode or negative electrode on gel electrophoresis is known as isoelectric point (pI). The isoelectric point of an amino acid is specific and depends on the nature of side chains present. The isoelectric points of all proteinogenic amino acids are enlisted in Table 1.1.

1.1.2 Peptide bond

A peptide bond (amide bond) is a covalent chemical bond formed between carboxyl group of one amino acid and an amino group of second amino acid of same or different type with the release of a water molecule. Peptide bond has a rigid planar structure and the nitrogen atom is largely sp^2 hybridized due to conjugation with carbonyl moiety. Any distortion from planarity results in an increase in energy due to a loss of the stabilizing effect of the resonance. The peptide bond can exist in *cis* and *trans* conformations to maintain the planarity and stability.

In a *cis* conformation, α-carbons are on the same side of the peptide bond. Where as in *trans* conformation, α-carbons of both amino acids are on opposite sides of the peptide bond, with no steric interactions. Due to steric constraint peptide bond prefers the *trans* conformation over *cis* conformation (Figure 1.1).

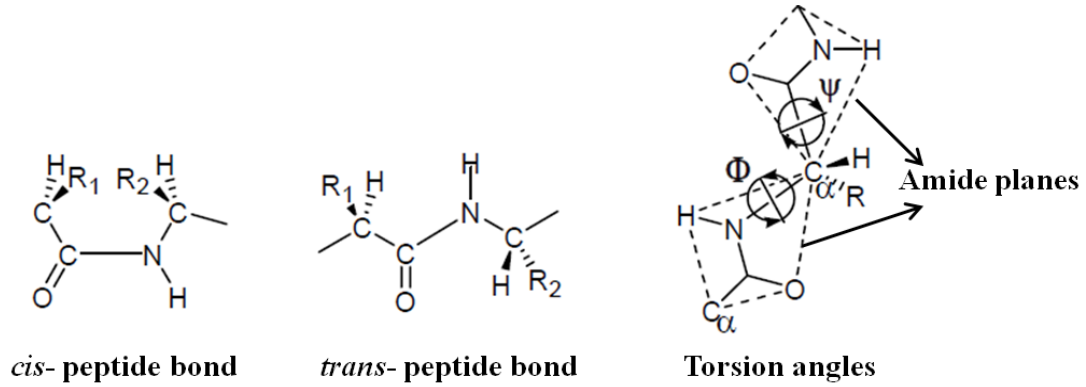


Figure 1.1 Conformations of peptide bond and torsion angles.

The rigid plane formed by the *trans* conformation of peptide bond is known as amide plane and consequence of this rigidity is that, the rotation of polypeptide backbone is only possible about the α-carbon. The conformation of the peptide backbone can be described by torsion angles (or dihedral angles) Φ and Ψ. These two angles are defined by the degree of rotation about the αC-N bond (Φ) and the αC-C bond (Ψ) respectively (Figure 1.1). The dihedral angles Φ and Ψ define the conformation around the amide bond and are very helpful in structural analysis of proteins.

1.1.3 Synthesis of peptides

Peptides are oligomers of amino acids and the synthesis of a given polypeptide can involve tens to hundreds of synthetic steps. Polypeptides synthesis involves repeated amide bond formation by coupling reactions. The reaction efficiency of any coupling reaction is always less than 100% and hence there is a gradual decrease in yields in every successive peptide bond formation. With increase in chain length secondary interactions become prominent

leading to a folded structure, and multiple functional groups in amino acids interfere with the coupling reaction which further decreases the coupling efficiency. To combat this, protected amino acids are employed with solid-phase synthetic methodologies for effective peptide synthesis. Protecting groups are auxiliary functional groups that mask a specific functional group in order to make it inert to peptide coupling reaction conditions that might lead to undesirable transformations. General protecting groups and their deprotection procedures are shown in table 1.2.

The process of peptide bond formation between two amino acids starts with activation of –COOH group using activating agents (Figure 1.3) like EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide), HBTU (O-Benzotriazole-N,N,N’,N’-tetramethyl-uronium-hexafluoro-phosphate), HATU (*O*-(7-azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate), TATU (O-(7-Azabenzotriazole-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate), PyBOP (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate) to form active ester of tBoc- or Fmoc-protected amino acid. Then slow addition of non-nucleophilic base (DIPEA) followed by carboxyl protected amino acid leads to a peptide bond formation as shown in Figure 1.3.

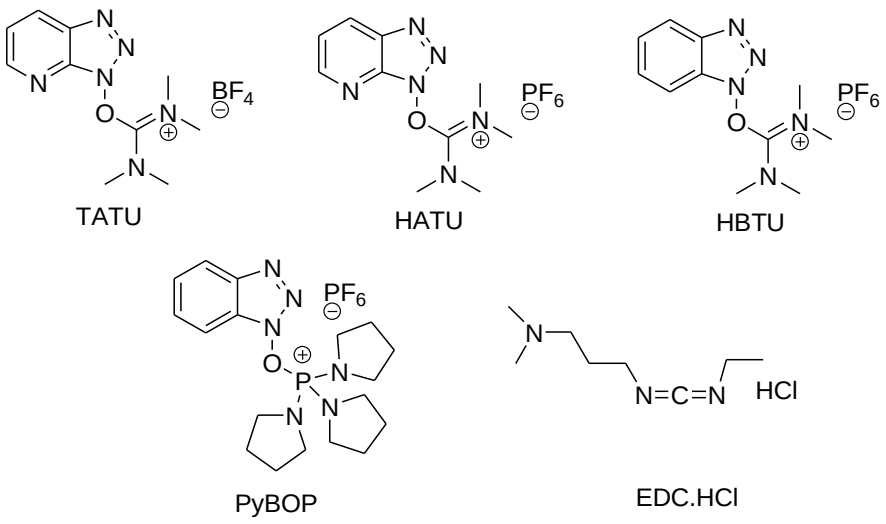


Figure 1.2 Molecular structures of coupling reagents.

Table 1.2 α-Amino and α-Carboxylic acid protecting groups.

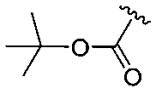
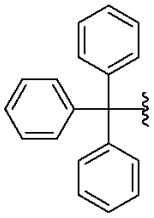
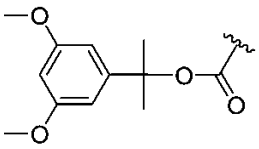
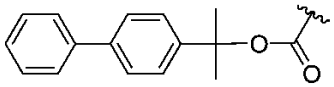
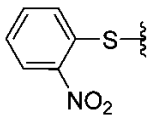
Name and Structure	Removal conditions	Stability to the removal of
tert-Butyloxycarbonyl (Boc) 	1) 25-50% TFA-DCM 2) 4 M HCl in dioxane 3) 2 M $MeSO_3H$ in dioxane 4) 1 M TMS-Cl, 1 M phenol-DCM	Fmoc, Z,[a] Trt, Alloc, *p*NZ
Trityl (Trt) 	1) 1% TFA-DCM 2) 0.1 M HOBt-TFE 3) 0.2% TFA, 1% H_2O-DCM 4) 3% TCA-DCM	Fmoc, Alloc
3,5-Dimethoxyphenylisoproxycarbonyl (Ddz) 	1-5% TFA-DCM	Fmoc, Alloc
2-(4-Biphenyl)isopropoxycarbonyl (Bpoc) 	0.2-0.5%-TFA	Fmoc, Alloc
2-Nitrophenylsulfenyl (Nps) 	1) Diluted solutions of HCl-$CHCl_3$-AcOH 2) 2-Mercaptopyridine-AcOH-MeOH, DMF or DCM 3) Ni Raney column in DMF	Fmoc

Table 1.2 α-Amino and α-Carboxylic acid protecting groups (continued).

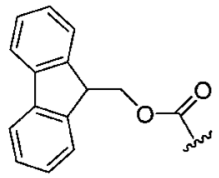
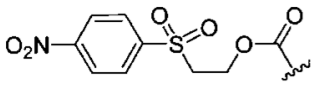
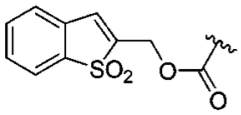
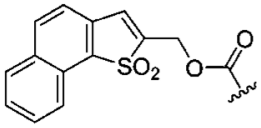
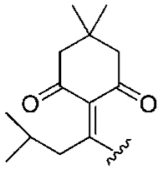
Name and Structure	Removal conditions	Stability to the removal of
9-Fluorenylmethoxycarbonyl (Fmoc) 	Solid phase: 1) 20% piperidine-DMF 2) 1-5% DBU-DMF 3) morpholine-DMF (1:1) 4) 2% HOBt, 2% hexamethyleneimine, 25% *N*-methylpyrrolidine in DMSO-NMP (1:1)	Boc, Z,[a] Trt, Alloc, *p*NZ [a]
2-(4-Nitrophenylsulfonyl) ethoxycarbonyl (Nsc) 	1) 20% of piperidine-DMF or DMF-dioxane (1:1) 2) 1% DBU-DMF or DMF-dioxane (1:1)	Boc, Trt, Alloc
(1,1-Dioxobenzo[b]thiophene-2-yl)methyloxycarbonyl (Bsmoc) 	1) 2-5% piperidine-DMF 2) 2% TAEA-DCM	Boc, Trt, Alloc
(1,1-Dioxonaphtho[1,2-b]thiophene-2-yl)methyloxycarbonyl (α-Nsmoc) 	1) 2-5% piperidine-DMF 2) 2% TAEA-DCM	Boc, Trt, Alloc
1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde) 	2% $N_2H_4 \cdot H_2O$-DMF	Boc, Fmoc, Z,[a] Trt, Alloc

Table 1.2 α-Amino and α-Carboxylic acid protecting groups.

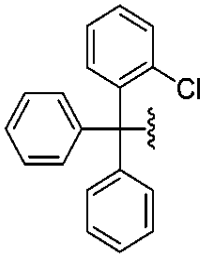
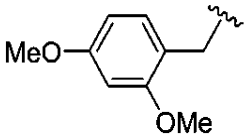
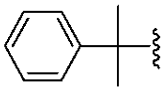
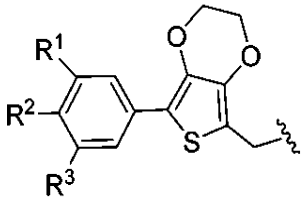
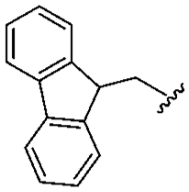
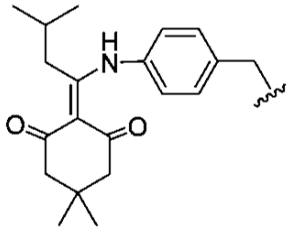
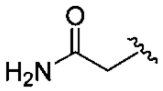
Name and Structure	Removal conditions	Stability to the removal of
tert-Butyl (^{t}Bu) 	90% TFA-DCM (solid phase and solution) or 4 M HCl in dioxane (solution)	Fmoc, Z,[a] Trt Alloc, *p*NZ,
2-Chlorotrityl (2-Cl-Trt) 	1% TFA-DCM	Fmoc, Alloc
2,4-Dimethoxybenzyl (Dmb) 	1% TFA-DCM	Fmoc, Alloc
2-Phenylisopropyl (2-Ph^{i}Pr) 	4% TFA-DCM	Fmoc, Alloc
5-Phenyl-3,4-ethylenedioxythenyl (Phenyl-EDOTn)  R^1=R^2=R^3= OMe; R^1=R^2= OMe, R^3= H; R^1=R^2= H, R^3= OMe or R^1=R^2=R^3= H.	0.01%-0.5% TFA-DCM and scavengers	Fmoc

Table 1.2 α-Amino and α-Carboxylic acid protecting groups.

Name and Structure	Removal conditions	Stability to the removal of
9-Fluorenylmethyl (Fm) 	15% DEA or 20% piperidine-DMF or DCM	Boc, Trt, Alloc
4-(N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino)benzyl (Dmab) 	2% hydrazine-H_2O-DMF (1:1)	Boc, Fmoc, Trt,
Methyl (Me) and Ethyl (Et)	LiOH, NaOH or KOH	Boc, Z
Carbamoylmethyl (Cam) 	NaOH or Na_2CO_3-DMF-H_2O	Boc, Fmoc [a] Z^b

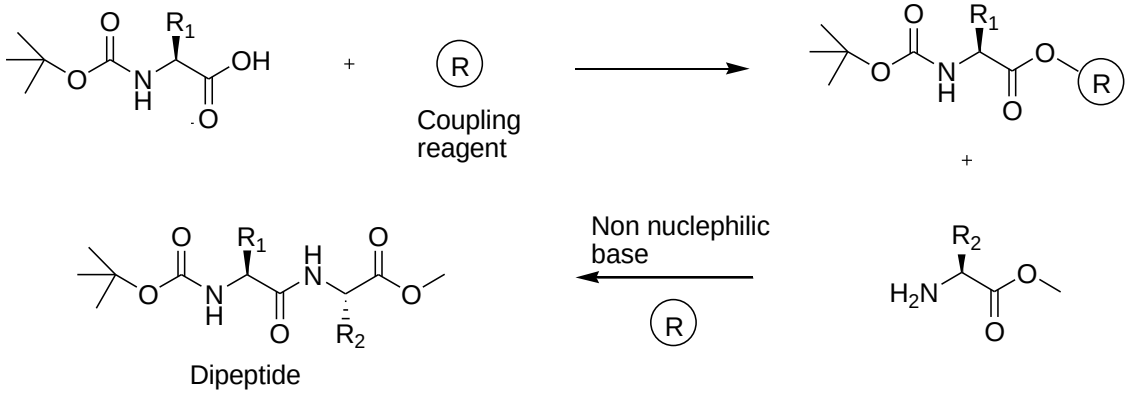


Figure 1.3 General procedure for peptide coupling.[6]

Solid-phase peptide synthesis (**SPPS**) was developed by R.B. Merrifield in 1962 for the facile synthesis of polypeptides.[6] In SPPS the first amino acid is attached to a solid support by a readily cleavable covalent bond, so that the desired peptide after the completion of synthesis can be cleaved from the solid support. The solid support used in SPPS must be stable and inert under reaction conditions. The commonly used solid phases are polystyrene or polyethylene glycol acrylamide (PEGA) resin beads. The first amino acid residue is immobilized on the solid support, leaving only one of the termini (generally $–NH_2$) free for coupling reactions with the next amino acid (suitably protected) and the coupling is carried out using standard peptide synthesis conditions. Protecting group of the coupled second amino acid is cleaved followed by capping and coupling cycles are repeated until the desired peptide sequence is obtained. If each of these reactions had to be carried out in solution phase, this would be an inefficient process both in terms of material (i.e. reagents and solvents) and labour. In SPPS, excess reagents are mixed with the solid beads then washed to remove excess reagents, leaving the newly elongated peptide still bound to the solid bead. SPPS follows the steps of deprotection of loaded first amino acid – coupling – deprotection – coupling, finally cleavage of the desired peptide from the solid beads, the reactions are highly repetitive, and can thus be performed in automated fashion.

1.1.4 Characterization of peptides

The peptides obtained through SPPS are purified using high performance liquid chromatography (HPLC) and analysed using matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS) or electron spray ionization mass spectrometry (ESI-MS) or high resolution mass spectrometry (HRMS). Peptides in solution generally undergo folding to form secondary structures, they can exist either in a random conformation (highly disordered) or in stereoregular conformations such as α-helix (either

right-handed or left-handed), β-sheet secondary structures.[3] The stereoregular conformations are stabilised by strong intra and intermolecular hydrogen bonding and have different dihedral angles due to the distorted *trans* amide bonds. Determination of conformation of the peptides is of great interest as the conformation of peptides influence their biological activity in living systems.

Various spectroscopic techniques like Infrared (IR) spectroscopy, X- ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, circular dichroism (CD) etc. are extensively used for characterization of peptides.

- **IR spectroscopy:** It is one of the most important and useful tool for determining the functional groups in organic molecules. The characteristic amide bond stretching frequencies of the peptide bond depend on the conformation, therefore IR data provide important information on secondary interactions. IR spectra can be interpreted to detect conformational changes that occur when solution parameters such as solvent polarity and ionic strength are changed, leading to a disruption or enhancement of hydrogen bond interactions reflecting in amide bond stretching frequencies (Table 1.2).

Table 1.3 IR features for α helix, β-sheet and random coil conformation.

Conformations	***v*** **(cm^{-1})**
N—H stretching frequency of the amide group	*v* = 3360–3260
trans-amides	*v* = 1250, 1550
cis-amides	*v* = 1350, 1500
α helix conformation	*v* = 620, 1650
β sheet conformation	*v* = 700, 1630(s), 1690(w)
Random (disordered) conformations	*v* = 650, 1660

*s = strong, w = weak

- **X- ray crystallography:** Peptides are crystallized in solution phase and their structural conformation and molecular packing can be determined by X- ray diffraction. It provides complete information about the orientation of the side chains and intra and intermolecular interactions among peptide molecules. The major drawback is that not all peptides or proteins form crystals and more over there is no proof to say that the obtained structure is the active conformation of peptide showing desired activity in solution. Solving the crystal structure of peptides is also not an easy process**.**
- **NMR spectroscopy:** NMR spectroscopy is a basic tool in the hands of organic chemists for determining the purity and integrity of synthesised compound. To determine conformation of peptides, 2D NMR techniques like NOESY, ROESY, COSY, TOCSY, HMBC etc are used, where NOESY is the most important technique providing precise information about the spatial correlation between the protons. Spatial interaction of protons for respective amino acids provides information of folding behaviour of peptides. NMR spectroscopy is extensively used to solve complex protein structures in solution which give actual conformation of active peptide/protein, structure of bovine serum albumin was completely solved by NMR spectroscopy.[8]
- **Circular dichroism (CD):** CD spectrometer is an instrument that measures the intensity of differential absorption of left circularly polarised light relative to that of right-circularly polarised light over a range of wavelengths. The intensity of the CD absorption depends upon the spatial relationship between the chromophore and groupings at the chiral centre of a molecule. The CD spectrum can be interpreted in terms of absolute configuration. The sign of a particular CD feature corresponds to a specific absolute configuration of the molecule, for the chiral centre nearest to the

chromophore responsible for that CD feature. Information on conformation can be obtained for amino acids and secondary structures of peptides and proteins which are in brief listed in Table 1.3.

Table 1.4 CD features for α-helix, β-sheet and random coil conformation.

Peptide conformations	**Random coil**	**α-Helix**	**β-Sheet**
Positive CD maximum	218 nm (weak)	191 nm (very weak)	195 nm (strong)
Zero CD	211, 234, 250 nm	202, 250 nm	207, 250 nm
Negative CD maximum	197 nm (strong) 240 nm (very weak)	208, 222 nm (strong)	217 nm (Medium)

1.1.5 Applications of peptides

Peptides are omnipresent in every living cell and play a crucial role in fundamental physiological and biochemical functions of life. Peptides are being used as therapeutic agents in diverse areas such as neurology, endocrinology and haematology. Peptide based drugs for cancer,[9] HIV,[10] tuberculosis,[11] and diabetes are available in market. Peptides as antimicrobial agents is an area of interest and several natural antimicrobial peptides are isolated includes dermcidin,[12] cathelicidin, defensin[13] from humans, cecropin, moricin, melitlin from insects, indolicidin (cattle), drosomycin (fruit fly). Synthetic modification of available antimicrobial peptides and development of new peptides for better stability and activity is an active area of research. Cyclic peptides compared to their linear counter parts show better bioactivity. They have reduced number of conformations in solutions due to structural constrainment in backbone induced by cyclization, antimicrobial cyclic peptides have better binding and cell

penetration efficiency. Development of peptide based materials has been an active area of research for the past one decade. Peptide based self-assembly plays an important role in the discovery of new biomaterials with a wide range of applications in nanotechnology and biotechnology.[14,15] Peptide self-assembly in nature is very common as seen in α-helix and β–sheet and other higher order structures of peptides, inspired by nature self-assembling peptides have been designed to form different modular self assembled structures such as nanofibers,[16] nanovesicles,[17] nanobelts,[18] and nanotubes.[19] Peptide amphiphiles[20] are one such examples where the β-sheet forming peptide is modified with long alkyl chains to self assemble into nanosheets, nanotubes, micelles and vesicles by tuning either the concentration of the peptide or solvent composition. Peptide nanostructures have mostly been used for applications in drug delivery,[21] gene delivery[22] and antimicrobial agent[23] development. Formation of amyloid fibrils[24] is a fundamental example of polypeptide self-assembly into ordered nanoscale fibrillar structures.

Peptide molecules unfortunately, show high conformational flexibility and a low *in vivo* stability which hampers their application as tool in medicinal and molecular biology. Peptides conformation depends on the surrounding environment which often leads to a conformational equilibrium. High flexibility of natural peptides is due to the multiple conformations that are energetically possible for each residue of amino acids. This leads to the fact that short polypeptides can hardly form any stable 3D structures in solution. In addition, it is doubtful whether the solid state conformations determined by X-ray analysis are identical to those occurring in solution. Despite their high bioactivity as drugs, their usage as commercial drugs is limited because they are rapidly degraded by proteases *in vivo* (poor bioavailability), and are frequently immunogenic. This drawback of natural peptides has lead to the development of peptide mimics for biomedical applications.[2]

1.2 Peptidomimitics

Peptidomimitics are molecules, which mimic natural peptides and thus produce the same biological effects as their natural analogues. They typically arise either from modification of an existing peptide, or by designing similar systems that mimic them. Irrespective of the approach, the altered chemical structure is designed to adjust the molecular properties such as, stability and biological activity. They are conformationally restricted and metabolically more stable compared to their natural counterparts.

Numerous approaches have been reported in literature to prepare peptidomimics, among them amino acid modification and backbone modifications are the two important methodologies employed in large.[1]

- **Amino acid modifications** - The β-substitution[25] of the naturally occurring amino acids gives a new class of peptidomimics. β-Subistution of proteinogenic amino acids hinders the free rotation around the $C_\beta - C_\gamma$ bond resulting in the conformational constrainment and enhanced biological activity. For example, short peptides binding to δ-opioid receptor was successfully altered by exchanging phenylalanine by its β-methylated analogue leading to enhanced binding.[26]

- **Backbone Modification** - Various structural modifications performed on peptide backbone can be grouped into three main categories. First is the exchange of individual groups, like replacement of α-CH group by nitrogen to form azapeptides,[27] the change from amide to ester bond to get depsipeptides[28] and exchange of the carbonyl function by a $-CH_2$ group. The second category of backbone modification involves the extension of the backbone by one or two $-CH_2$ groups resulting in polypeptides built from β, γ-amino acids respectively.[1] The third and most widely

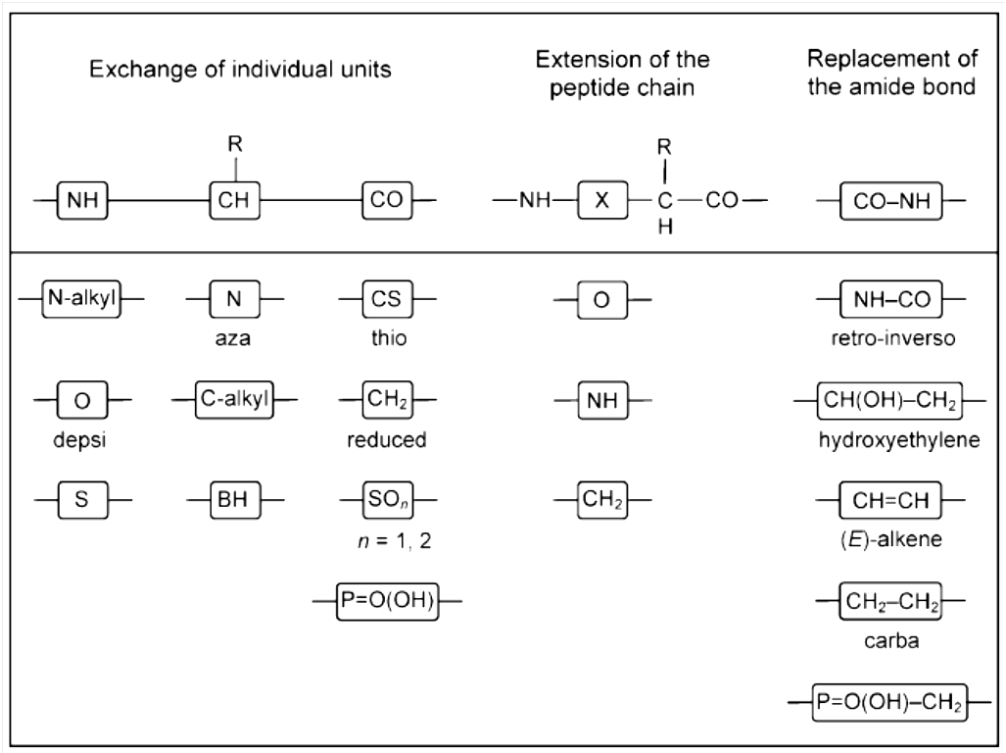


Figure 1.4 Some of the common modifications to the peptide backbone.[1]

used modification is the amide bond inversion, yielding a retro-inverso peptidomimetic.[29] Amide bonds are replaced by alkene[30] and other functional groups to develop different types of peptidomimecs. Figure 1.4 summarizes different strategies used in literature to modify the peptide backbone.

1.2.1 Types of peptidomimics

In literature large number of peptidomimics are reported, out of which few important mimics are discussed below.

1.2.1.1 Peptoids[31]

Peptoids are mimics of natural α-peptides in which the side chain is attached to the backbone amide nitrogen instead of the α-carbon (Figure 1.5). These oligomers of *N*-substituted glycine are an attractive scaffold for biological applications as they can be generated using a straightforward, modular synthesis that allows the incorporation of a wide variety of

functionalities. Peptoids were first developed by Reyna Simon, Paul Bartlett and Daniel Santi.[31] Ronald N. Zuckermann has extensively worked in developing peptoids for different applications. Peptoids have been evaluated as tools for studying biomolecular interactions, also hold significant promise for therapeutic applications owing to their enhanced stability towards the protelysis and increased cellular permeability.

1.2.1.2 D-Peptides

D-Peptides are peptides made of D-amino acids and rarely occur in living organisms. D-Peptides have similar but mirrored properties to the L-peptides. D-Peptides are less susceptible to degradation by proteolysis owing to the fact that the enzymes are less active towards the D-peptides.[32] D-Peptide based drugs can therefore be taken orally and are effective for a longer period of time. D-peptides are easy to synthesize, when compared to many other drugs and in some cases D-peptides also have a low immunogenic response.

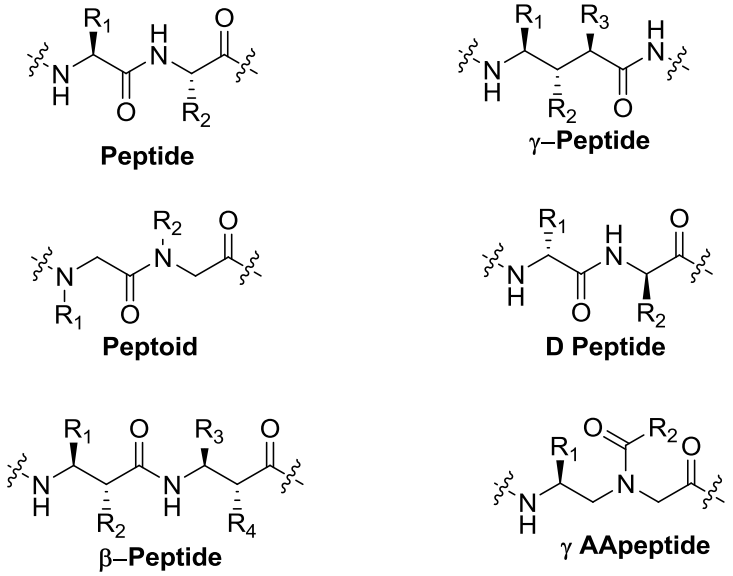


Figure 1.5 Different classes of peptidomimics.

1.2.1.3 β-Peptides[33]

β-Peptides consist of β-amino acids, which have their amino group ($-NH_2$) bonded to the β carbon unlike the α carbon as in the natural amino acids. β-Peptides in general do not appear in nature and the only naturally occurring β amino acid is β-alanine. Human peptidases are inefficient to recognize and cleave peptides containing β-amino acids. Substitution of α-amino acids with β-amino acids in peptide of interest, show good stability, affinity and activity. These results hold promising for development of new peptide-based therapeutics. Similarly γ-peptides consist of γ-amino acids and are inactive against proteolysis.[34]

1.2.1.4 γ-AApeptides

They are derived from γ-chiral peptide nucleic acid (PNA) backbone (*N*-(2-aminoethyl)glycine).[35] They are termed γ-AApeptides because they are comprised of γ-substituted-*N*-acylated-*N*-amino-ethyl amino acids (Figure 1.5). In this unit, one side chain is connected to the γ-C in relation to carbonyl group, and the other side chain is linked to the central *N* through acylation. γ-AApeptides are able to project an identical number of side chains as natural peptides of the same length; as such, they have great potential to be developed for peptide mimics. Compared to conventional peptides, γ-AApeptides are much superior in both their limitless potential for modification and their inherent resistance to biodegradation.

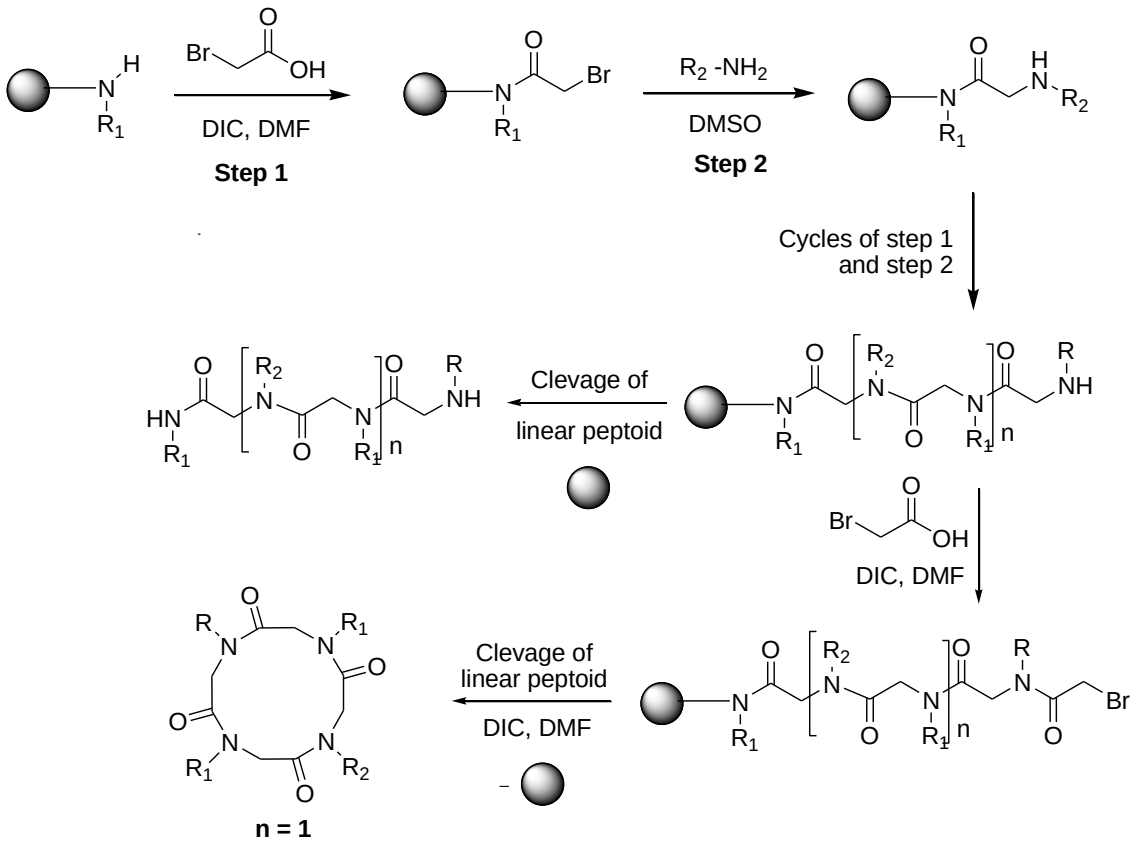
1.2.1.5 Cyclic peptidomimics

In linear peptidomimics the desired active conformation is not obtained due to flexible backbone and lack of secondary interactions as seen in natural peptides.[36] Therefore cyclization of linear peptidomimics is carried out to obtain conformationally stable and active molecules.[37] Cyclic peptidomimics compared to their linear counterparts have enhanced cell

permeability, improved resistance to enzymatic degradation and are presumed to bind more tightly to their protein targets because of their more restricted conformational flexibility. Therefore development of cyclic peptidomimics based therapeutics is an active area of research in pharmaceutical companies. Cyclic analogies like cyclic peptoids,[38] cyclic β-peptides[39] and cyclic γ-AApeptides are derived from their corresponding linear peptide mimics and show improved bioactivity, greater resistance to enzymatic degradation compared to their linear counterparts.

1.2.2 Synthesis of peptidomimitics

Peptidomimitics are mostly synthesised by SPPS (Section 1.1.3), each monomer with respective protecting group is subjected to subsequent steps of deprotection- coupling-deprotection - coupling to obtain the desired oligomer. General synthetic procedure for peptoids was developed by Ronald N Zuckermann (scheme 1.1).[40] In this synthetic process free amine on the resin is coupled to bromoacetic acid using coupling reagent DIC (N,N'-diisopropylcarbodiimide) in DMF. Followed by *N*-alkylation of alkyl amine to the resin bond bromoacetic acid, both these steps are repeated till we get the desired linear peptoid. The linear peptoid can be cyclized to obtain cyclic peptoids (Scheme 1.1). Synthesis of cyclic analogues is a difficult process, cyclization suffers from poor yields and reaction has to be performed under dilute conditions (0.1 - 1 mM). Polymerization of oligomers is major side reaction during cyclization, number of combinations obtained is huge. To overcome the problem of polymerization and other side reactions cyclization of peptidomimics are performed under dilute conditions. The cyclization can be head to tail (*N* terminal and *C* terminal), head to side chain, side chain to tail or side chain to side chain. Head to tail cyclization is performed using different coupling reagents like HBTU, HATU, TATU and pyBOP to obtain the product in good yields. Other reactive moieties like alkenes, alkynes,



Scheme 1.1 Synthesis of linear and cyclic peptoids.

Boronic acid, thiols, alkyl halides etc are introduced into the oligomers as side chains and are cyclised to obtain desired cyclic molecules.

1.2.3 Applications of peptidomimitics

Peptide based molecules are very important class of drugs in pharmaceutical industry and many peptide based drugs are available in market. These drugs are very selective and specific towards their targets and hence show better activity. Peptide based drugs suffer from low *in vivo* stability and bioavailability. Therefore peptide based mimics are developed as their substitutes. Michael S. Kay *et al.*[41] reported D-peptides for inhibition of HIV entry into the cells, Wuyuan Lua *et al.*[42] developed D-peptide inhibitors of the p53–MDM2 for therapy of malignant tumours. Molecular recognition is another area where peptidomimics are used as

chemical tools to study complex biomolecular interactions. Peptoid–protein interactions were first demonstrated by Zuckermann *et al.*, where the authors examined the high-affinity binding of peptoid dimers and trimers to G-protein coupled receptors.[43] There are many different interactions between peptide mimics and protein, and these interactions can induce enhancement, inhibition, cellular uptake or delivery. From a library of peptoid hexamers, Kodadek *et al.*[44] identified three peptoids with high binding affinities for the coactivator CREB-binding protein (CBP) *in vitro* which is involved in the transcription. Many examples for binding of peptide mimics to metal ions and forming conformationally ordered structures can be seen in literature, which is a characteristic property of proteins. PNA (peptide nucleic acids) are hybrid peptide backbone acylated to nucleobases. They are extensively studied for their interactions with DNA and RNA molecules,[45] for development of drugs for cancer. The most important characteristic property of cyclic peptidomimics is their antimicrobial activity.

1.2.3.1 Antimicrobial properties of cyclic Peptidomimics

Antimicrobial peptides[46] (AMPs) are found in living organisms and are potent, broad spectrum antibiotics which demonstrate their potential as novel therapeutic agents. The mechanism of action for most AMPs is breaking down the bacterial cytoplasmic membrane. Antimicrobial peptides have been demonstrated to kill gram-negative and gram-positive bacteria, mycobacteria, enveloped viruses, fungi and even transformed cancerous cells. Low in vivo stability of peptides has lead to development of antimicrobial peptidomimics.

De Riccardis *et al.*[47] have investigated the antimicrobial activities of five new cyclic cationic hexameric α-peptoids, comparing their efficacy with the linear cationic and the cyclic neutral counterparts. The synthesized peptoids have been assayed against clinically relevant bacteria and fungi, these studies concluded that cyclic peptoids showed better activity when compared to their linear and natural analogies. Jianfeng Cai *et al.*[33] reported the

design and synthesis of cyclic γ-AApeptides which showed broad-spectrum activity against fungus and multi-drug resistant gram-positive and gram-negative bacteria pathogens.

1.3 Present work

In the current project we have designed a strategy of using *N*-(2-aminoethyl) glycine as the basic peptoid backbone and functionalizing it by *N*-acyl and *N*-alkyl linkers. These *N*-alkyl and *N*-acyl hybrid peptoid monomers were expected to cyclize to form macrocyclic rings. Serendipitously we found that *N*-acyl monomer lead to 12-membered cyclic hybrid peptoid and *N*-alkyl monomer to 6-member cyclic hybrid peptoid after cyclization. We confirmed our observation by synthesising a series of similar molecules and tried to understand the conformation arrangement using NMR spectroscopy and energy minimization model. In another related project we are working towards developing peptide based inhibitors for amyloid fibrils and subsequently to find potential drugs for Alzheimer's disease.

1.4 References

(1) Grauer, A.; König, B. Peptidomimetics – A Versatile Route to Biologically Active Compounds. *Eur. J. Org. Chem.* **2009**, *30*, 5099-5111.

(2) Laszlo, O.; Walker, J. M.; (**2008**) Peptide - based Drug Design, Humana Press.

(3) Barret, G. C.; Elmore,D. T. (**2004**) Amino Acids and Peptides, Cambridge University Press.

(4) Samuel, B. S. (**1913**) The general characteristics of Proteins, Longmans press.

(5) Uchino, T.; Snyderman, S.; Lambert, M.; Qureshi, I.; Shapira, S.; Sansaricq, C.; Smit, L. E.; Jakobs, C.; Matsuda, I. Molecular basis of phenotypic variation in patients with argininemia. *Hum. Genet.* 1995, *96*, 255-260.

(6) Isidro-Llobet, A.; Álvarez, M.; Albericio, F. Amino acid-protecting groups. *Chem. Rev*. **2009**, *109*, 2455; (b) Merrifield, R. B. Solid phase peptide synthesis. I. the synthesis of a tetra peptide. *J. Am. Chem. Soc.* **1963**, *85*, 2149-2154

(7) Acharya, K. R.; Lloyd, M. D. The advantages and limitations of protein crystal structures. *Trends in Pharmacological Sciences* **2005**, *26*, 10-14.

(8) Fielding, L.; Rutherford, S.; Fletcher, D. Determination of protein–ligand binding affinity by NMR: observations from serum albumin model systems. *Magn. Reson. Chem*. **2005**, *43*, 463-470.

(9) Hatakeyama, S.; Sugihara, K.; Shibata, T. K.; Nakayama, J.; Akama, T. O.; Tamura, N.; Wong, S.-M.; Bobkov, A. A.; Takano, Y.; Ohyama, C.; Fukuda, M.; Fukuda, M. N. Targeted drug delivery to tumor vasculature by a carbohydrate mimetic peptide. *Proc. Natl. Acad. Sci.* **2011**, *23*, 2567-2572.

(10) Agopian, A.; Gros, E.; Aldrian-Herrada, G.; Bosquet, N.; Clayette, P.; Divita, G. A New generation of peptide-based inhibitors targeting HIV-1 reverse transcriptase conformational flexibility. *J. Biol. Chem.* **2009**, *284*, 254-264.

(11) Jeong, E. H.; Son, Y. M.; Hah, Y. S.; Choi, Y. J.; Lee, K. H.; Song, T.; Kim, D. R. RshA mimetic peptides inhibiting the transcription driven by a Mycobacterium tuberculosis sigma factor SigH. *Biochem. Biophys. Res. Commun.* **2006**, *339*, 392-398.

(12) Todorov, P. T.; Deacon, M.; Tisdale, M. J. Structural analysis of a tumor - produced sulfated glycoprotein capable of initiating muscle protein degradation. *J. Biol. Chem.* **1997**, *272*, 12279-12288.

(13) Tran, D.; Tran, P.; Roberts, K.; Ösapay, G.; Schaal, J.; Ouellette, A.; Selsted, M. E. Microbicidal properties and cytocidal selectivity of rhesus macaque theta defensins. *Antimicrob. Agents Chemother.* **2008**, *52*, 944-953.

(14) Slocik, J. M.; Naik, R. R. Probing peptide - nanomaterial interactions. *Chem. Soc. Rev.* **2010**, *39*, 3454-3463.

(15) Kim, S.; Kim, D.; Jung, H. H.; Lee, I.-H.; Kim, J. I. L.; Suh, J.-Y.; Jon, S. Bio-Inspired design and potential biomedical applications of a novel class of high-affinity peptides. *Angew. Chem. Int. Ed.* **2012**, *51*, 1890-1894.

(16) Zhang, S.; Gelain, F.; Zhao, X. Designer self-assembling peptide nanofiber scaffolds for 3D tissue cell cultures. *Cancer Biology* **2005**, *15*, 413-420.

(17) Santoso, S.; Hwang, W.; Hartman, H.; Zhang, S. Self-assembly of surfactant-like peptides with variable glycine tails to form nanotubes and nanovesicles. *Nano Lett.* **2002**, *2*, 687-691.

(18) Cui, H.; Muraoka, T.; Cheetham, A. G.; Stupp, S. I. Self-assembly of giant peptide nanobelts. *Nano Lett.* **2009**, *9*, 945-951.

(19) (a) Granja, J. R.; Ghadiri, M. R. Channel-mediated transport of glucose across lipid bilayers. *J. Am. Chem. Soc.* **1994**, *116*, 10785-10786; (b) Khazanovich, N.; Granja, J. R.; McRee, D. E.; Milligan, R. A.; Ghadiri, M. R. Nanoscale tubular ensembles

with specified internal diameters: Design of a self-assembled nanotube with a 13-.ang. pore. *J. Am. Chem. Soc.* **1994**, *116*, 6011-6012.

(20) (a) Hartgerink, J. D.; Beniash, E.; Stupp, S. I. Peptide-amphiphile nanofibers: A versatile scaffold for the preparation of self-assembling materials. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5133- 5138; (b) Hamley, I. W. Self-assembly of amphiphilic peptides. *Soft Matter* **2011**, *7*, 4122-4138.

(21) Vivès, E.; Schmidt, J.; Pèlegrin, A. Cell-penetrating and cell-targeting peptides in drug delivery. *Annu. Rev. Immunol.* **2008**, *1786*, 126-138.

(22) Simeoni, F.; Morris, M. C.; Heitz, F.; Divita, G. Insight into the mechanism of the peptide-based gene delivery system MPG: implications for delivery of siRNA into mammalian cells. *Nucleic Acids Res.* **2003**, *31*, 2717-2724.

(23) Jenssen, H.; Hamill, P.; Hancock, R. E. W. Peptide antimicrobial agents. *Clin. Microbiol. Rev.* **2006**, *19*, 491-511.

(24) Cinar, G.; Ceylan, H.; Urel, M.; Erkal, T. S.; Deniz Tekin, E.; Tekinay, A. B.; Dâna, A.; Guler, M. O. Amyloid inspired self-assembled peptide nanofibers. *Biomacromolecules* **2012**, *13*, 3377-3387.

(25) Jiao, D.; Russell, K. C.; Hruby, V. J. Locally constrained tyrosine analogues with restricted side chain dynamics. *Tetrahedron* **1993**, *49*, 3511-3520.

(26) Haskell-Luevano, C.; Toth, K.; Boteju, L.; Job, C.; Castrucci, A. M. d. L.; Hadley, M. E.; Hruby, V. J. β-Methylation of the Phe7 and Trp9 melanotropin side chain pharmacophores affects ligand−receptor interactions and prolonged biological activity. *J. Med. Chem.* **1997**, *40*, 2740-2749.

(27) Cheguillaume, A.; Lehardy, F.; Bouget, K.; Baudy-Floc'h, M.; Le Grel, P. Submonomer solution synthesis of hydrazinoazapeptoids, a new class of pseudopeptides . *J. Org. Chem.* **1999**, *64*, 2924-2927.

(28) Susmuth, R.; Muller, J.; von Dohren, H.; Molnar, I. Fungal cyclo oligomer depsipeptides: From classical biochemistry to combinatorial biosynthesis. *Nat. Prod. Rep.* **2011**, *28*, 99-124.

(29) Dürr, H.; Goodman, M.; Jung, G. Retro-inverso amide bonds between trifunctional amino acids. *Angew. Chem. Int. Ed.* **1992**, *31*, 785-787.

(30) Meinander, K.; Weisell, J.; Pakkala, M.; Tadd, A. C.; Hekim, C.; Kallionpaa, R.; Widell, K.; Stenman, U.-H.; Koistinen, H.; Narvanen, A.; Vepsalainen, J.; Luthman, K.; Wallen, E. A. A. Pseudopeptides with a centrally positioned alkene-based disulphide bridge mimetic stimulate kallikrein-related peptidase 3 activity. *MedChemComm.* **2013**, *4*, 549-553.

(31) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. Efficient method for the preparation of peptoids [oligo(N-substituted glycines)] by submonomer solid-phase synthesis . *J. Am. Chem. Soc.* **1992**, *114*, 10646-10647; (b) Simon, R. J.; Kania, R.S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D.A.; Banville, S.; Wang, L.; Rosenberg, S. Peptoids: A modular approach to drug discovery. *Proc. Natl. Acad. Sci. U. S. A.* **1992**, *89*, 9367–9371.

(32) Van Regenmortel, M. H. V.; Muller, S. D-peptides as immunogens and diagnostic reagents. *Curr. Opin. Biotechnol.* **1998**, *9*, 377-382.

(33) Godballe, T.; Nilsson, L. L.; Petersen, P. D.; Jenssen, H. Antimicrobial β-peptides and α-peptoids. *Chem. Biol. Drug Des* **2011**, *77*, 107-116.

(34) Seebach, D.; Beck, A. K.; Bierbaum, D. J. The world of β- and γ-peptides comprised of homologated proteinogenic amino acids and other components. *Chem Biodivers.* **2004**, *1*, 1111-1239.

(35) Wu, H.; Niu, Y.; Padhee, S.; Wang, R. E.; Li, Y.; Qiao, Q.; Bai, G.; Cao, C.; Cai, Design and synthesis of unprecedented cyclic γ-AApeptides for antimicrobial development. *J. Chem. Sci*. **2012**, *3*, 2570.

(36) Bogdanowich-Knipp, S. J.; Chakrabarti, S.; Siahaan, T. J.; Williams, T. D.; Dillman, R. K. Solution stability of linear vs. cyclic RGD peptides. *J. Pept. Res*. **1999**, *53*, 530-541.

(37) Young, T. S.; Young, D. D.; Ahmad, I.; Louis, J. M.; Benkovic, S. J.; Schultz, P. G. Evolution of cyclic peptide protease inhibitors. *Proc. Natl. Acad. Sci.* **2011,** *3*, 1-5.

(38) Shin, S. B. Y.; Yoo, B.; Todaro, L. J.; Kirshenbaum, K. Cyclic peptoids. *J. Am. Chem. Soc.* **2007**, *129*, 3218-3225.

(39) Price, J.L.; Horne, W. S.; Gellman, S. H. Discrete Heterogeneous quaternary structure formed by α/β-peptide foldamers and α-peptides *J. Am. Chem. Soc.* 2007, *129*, 6376- 6377.

(40) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H., Efficient method for the preparation of peptoids [oligo(N-substituted glycines)] by submonomer solid-phase synthesis. *J. Am. Chem. Soc.* **1992,** *114*, 10646-10647.

(41) Welch, B. D.; VanDemark, A. P.; Heroux, A.; Hill, C. P.; Kay, M. S. Potent D-peptide inhibitors of HIV-1 entry. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 16828-16833.

(42) Liu, M.; Li, C.; Pazgier, M.; Li, C.; Mao, Y.; Lv, Y.; Gu, B.; Wei, G.; Yuan, W.; Zhan, C.; Lu, W. Y.; Lu, W. D-Peptide inhibitors of the p53–MDM2 interaction for targeted molecular therapy of malignant neoplasms. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 14321-14329.

(43) Miller, S. M.; Simon, R. J.; Ng, S.; Zuckermann, R. N.; Kerr, J. M.; Moos, W. H. Proteolytic studies of homologous peptide and N-substituted glycine peptoid oligomers. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2657-2662.

(44) Lim, H.-S.; Archer, C. T.; Kodadek, T. Identification of a peptoid inhibitor of the proteasome 19s regulatory particle. *J. Am. Chem. Soc.* **2007**, *129*, 7750-7751.

(45) Govindaraju, T.; Kumar, V. A.; Ganesh, K. N. (SR/RS)-Cyclohexanyl PNAs: conformationally preorganized PNA analogues with unprecedented preference for duplex formation with RNA. *J. Am. Chem. Soc.* **2005**, *127*, 4144-4145.

(46) Christopher, D. F.; Jan, A. H.; Robert, E. W. H.; Gisbert, S. Designing antimicrobial peptides: form follows function. *Nature Rev. Drug Discov.* **2011**, *11*, 37-51.

(47) Comegna, D.; Benincasa, M.; Gennaro, R.; Izzo, I.; De Riccardis, F. Design, synthesis and antimicrobial properties of non-hemolytic cationic α-cyclopeptoids. *Biorg. Med. Chem.* **2010**, *18*, 2010-2018.

Chapter 2

N-Alkyl and *N*-acyl substituent enforced differential cyclization of hybrid peptoid monomers

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2.1 Introduction

Peptides are important class of biomolecules, made of L-amino acids linked by amide bonds. They present in every living cell and play a crucial role in fundamental physiological and biochemical functions of life. Their characteristic properties, specificity and selectivity are often dependent on the well-defined three-dimensional structure. Peptides in solution undergo three-dimensional folding, stabilized by non covalent interactions to give a stable and active conformation. The two and three-dimensional active conformation of peptides is strongly influenced by its surrounding environments (pH, temperature etc.) which often lead to a conformational equilibrium.[1] This high degree of conformational flexibility is due to multiple conformations that are energetically possible for each residue of amino acid. Linear peptides fail to attain ordered conformation or stable tertiary structures, this ambiguous behaviour of peptides has been addressed through cyclization and crosslinking, which is known to provide proper conformational stability. Cyclization constrains the peptide backbone, inducing ordered, stable and active conformation.[2] Cyclic peptides are found in nature as antimicrobial peptides,[3] lasso peptides,[4] knottins[5] and certain classes of antibiotics. Different synthetic approaches have been pursued to achieve cyclization of peptide chain like head to tail (C-terminus to N-terminus), head to side chain, side chain to tail and side chain to side chain cyclization (Chapter 1). Cyclic peptide constrained structure provides higher stability and cell penetration efficiency. However, they suffer from low *in vivo* stability (proteolysis). The development of linear and cyclic peptide mimics with stable and ordered conformation showing bioactivity is an area of immense interest.

A new class peptidomimics[6] with modified peptide backbone were derived, capable of withstanding hydrolytic peptidases and proteases.[7] Linear peptidomimics like peptoids have

shown high stability *in vivo* compared to their natural counterparts, as the *N*-substituted glycine backbone is less immunogenic and more stable.[8] However the desired active conformation is not obtained in linear peptidomimics due to flexible backbone and lack of secondary interactions as seen in natural peptides. To overcome this issue cyclic peptidomimics were developed; they are known to have enhanced cell permeability, improved resistance to enzymatic degradation and are presumed to bind more tightly to their protein targets because of restricted conformational flexibility. Cyclic peptoid,[10] cyclic *β*-peptoid,[11] γ-AApeptides[12] are few important examples of cyclic peptidomimics reported in literature. Development of cyclic peptidomimics based therapeutic is an area of interest for pharmaceutical companies.

Despite of having a huge potential application in the field of medicine and biology, cyclic peptidomimics use is limited by their difficult synthetic procedures. The conventional synthesis of cyclic peptidomimics involves complicated synthetic routes with low yields, posing a great synthetic challenge. Numerous procedures for the synthesis of small and medium sized rings are found in the literature,[13] the most challenging aspect of cyclic peptidomimic synthesis is the final ring-closure step. Well established solid phase synthesis protocols are employed for synthesis of linear peptidomimics, followed by cyclization. Generally head-to-tail (C-terminus to N-terminus) cyclization is performed, using coupling reagents like HBTU, HATU,[11] pyBOP[10] etc. to obtain the product in moderate yields. There also exist few other synthetic strategies reported in literature for cyclization, Yong Uk Kwon *et al.*[14] synthesised cyclic peptoids by ring closure metathesis (RCM) of modified linear peptoid chain, using ruthenium based catalyst. Kevin J. Luebke *et al.*[15] used the concept of boronic acids forming esters with diols in aqueous media, to synthesis cyclic peptidomimics. The yields obtained in cyclization of longer oligomers (5-20 monomer units) are generally moderate and cyclization of short oligomers (2-4 monomer units) suffers from poor yields.

Increase in ring size of cyclic peptidomimics enhances the flexibility, leading to increased number of conformations and thus reducing its bioactivity. Therefore the synthesis of relatively small or medium size cyclic peptidomimitics is preferred.

2.2 Objective of the work

To develop facile and efficient synthesis of small and medium sized cyclic peptoids to use them as antimicrobial agents. Small rings have better conformational stability compared to larger ring with huge number of changing conformations. High conformational stability of small or medium sized rings enhances the target binding affinity and cell permeability. We used achiral *N*-(2-aminoethyl)glycine (*aeg*) backbone as basic unit and performed α-*N* substitution. These monomers were subjected to oligomerization and subsequent cyclization to obtain small and medium sized rings in efficient way.

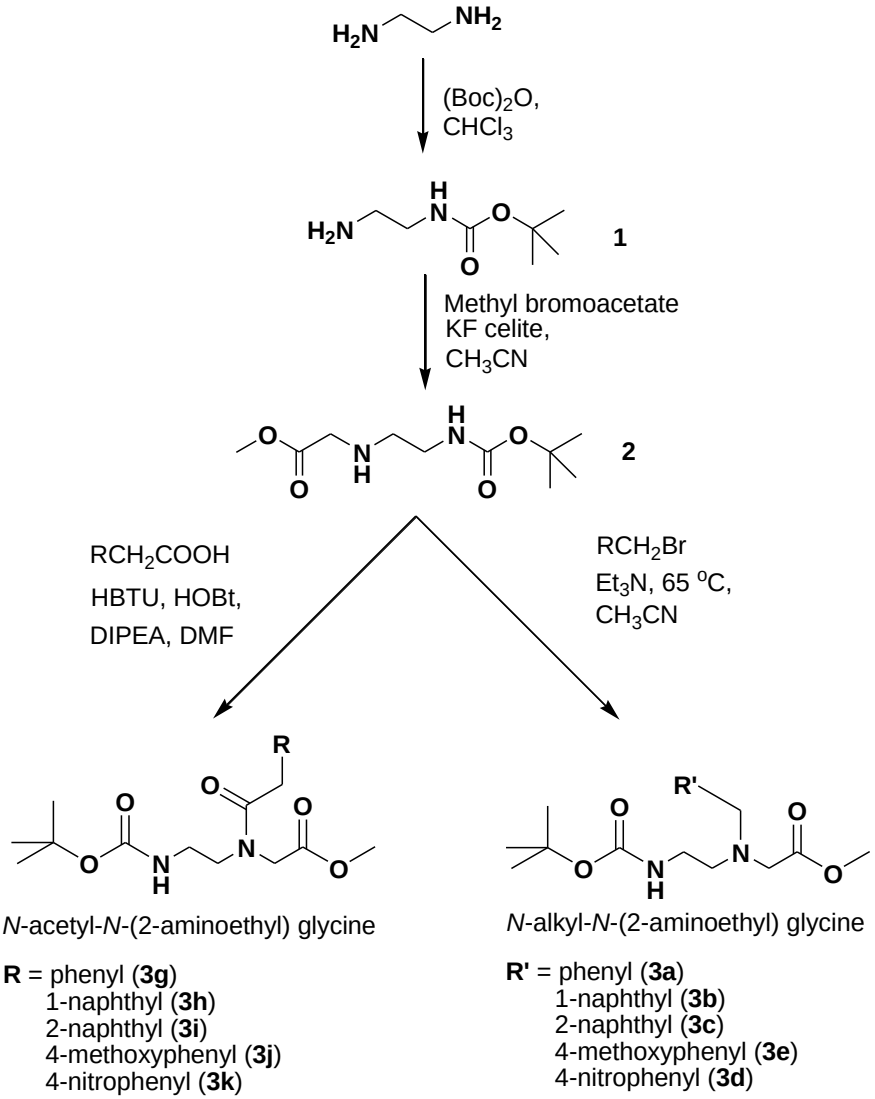
2.3 Design strategy

Application of linear and cyclic peptidomimics as antimicrobial agents is an area of immense interest, mimics like peptoids, azapeptides,[16] depsipeptides,[17] D-peptides,[18] β-peptides[19] etc. in both linear and cyclic form are used as antimicrobial agents. γ-AApeptides are recent addition to peptidomimics, they are composed of γ-substituted *N*-(2-aminoethyl)glycine monomeric units, showing enhanced antibacterial activity. In literature, *N*-(2-aminoethyl)glycine has been extensively used as a basic backbone unit for synthesis of peptide nucleic acids (PNA).[20] Here we have used similar *N*-(2-aminoethyl)glycine[21] as basic backbone unit to prepare *N*-acyl-*N*-(2-aminoethyl)glycine and *N*-alkyl-*N*-(2-aminoethyl)glycine hybrid peptoid monomers. The oligomerization of these hybrid peptoid monomer units gave rise to new class of linear peptidomimics. Serendipitously we observed that these monomer units undergo differential cyclization (either six or twelve membered rings) under similar reaction conditions. Universality of the reaction was confirmed by synthesizing a series of analogous *N*-alkyl and *N*-acyl substituted *N*-(2-aminoethyl)glycine monomer units, followed by

cyclization. Synthesised cyclic hybrid peptoids have asymmetric and stable conformation in solution.

2.4 Synthesis of cyclic hybrid peptoids

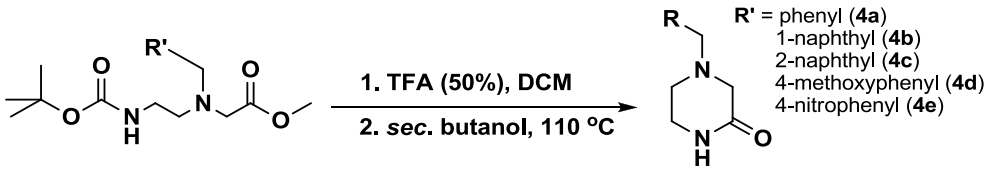
The *N*-(2-aminoethyl)glycine (*aeg*) backbone was synthesised from ethylene diamine. The selective mono-Boc–protection of ethylene diamine was achieved by dropwise addition of di-tert-butyldicarbonate in chloroform under ice cold conditions to obtain *N*-tert-butyloxycarbonyl-1,2-ethylenediamine (**1**) (Scheme 1). *N*-Alkylation of **1** was performed with bromomethyl acetate using KF celite[22] as non nucleophilic base to obtain *N*-methyl-*N*-(2-Boc-aminoethyl)glycinate (**2**).



Scheme 2.1 Synthesis of *N*-(2-aminoethyl)glycine (*aeg*) hybrid peptoid monomers.

Then the backbone (**2)** was coupled to acetic acid derivatives through standard peptide coupling protocols using HBTU to obtain respective *N*-acyl-*N*-(2-aminoethyl)glycine monomers. *N*-Alkylation of backbone (**2)** was performed with bromomethyl derivatives using triethylamine as base to obtain *N*-alkyl-*N*-(2-aminoethyl)glycine monomers. Both *N*-alkyl and *N*-acyl monomers were independently subjected to *t*Boc-deprotection using 50% TFA in DCM followed by cyclization at 110 °C in *sec*-butanol containing 1% acetic acid.[23]

N-Alkyl-*N*-(2-aminoethyl)glycine showed intramolecular cyclization to form six membered ring (Figure 2.1) whereas, *N*-acyl-*N*-(2-aminoethyl)glycine hybrid peptoid monomer underwent intermolecular coupling followed by cyclization leading to the formation of twelve membered ring (Figure 2.2). To understand the generality of differential cyclization of monomers with varying functionalities we have synthesised a library of six and twelve member cyclic hybrid peptoids starting with various aromatic functionalities attached to *N*-(2-aminoethyl)glycine through acyl and methyl groups. Initially simple benzyl unit (**3a, 3g**) were used and then α and β naphthalene units (**3b, 3h** and **3c, 3i** respectively) were used to demonstrate that ring cyclization is independent of type of aromatic moiety and position of attachment. Monomers having electron withdrawing group (4-nitrophenyl, **3k** and **3e**) and donating group (4-methoxyphenyl, **3e** and **3j**) were synthesised to study the influence of electronic effects on cyclization of monomers. Interestingly, no changes were observed in the differential six or twelve member ring formation from *N*-alkyl and *N*-acyl substituted peptide monomers respectively. This observation emphasized the generality of the effect of *N*-alkyl and *N*-acyl substituent on the cyclization of the hybrid peptoid monomers. Further, we have synthesized monomer **3f** with ethyl linker and cyclized under the similar reaction conditions. Cyclization of monomer **3f** gave six membered ring and this further confirmed our assumption that, the absence of carbonyl moiety (flexibility and free rotation of *N*-alkyl



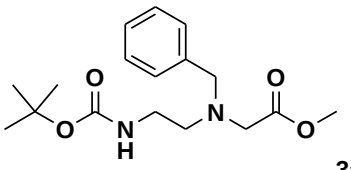
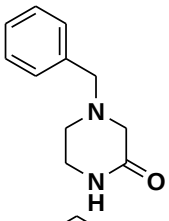
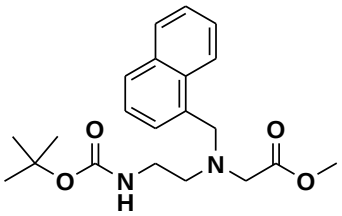
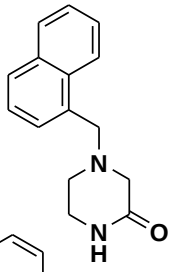
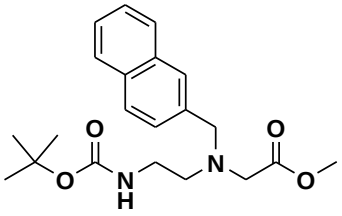
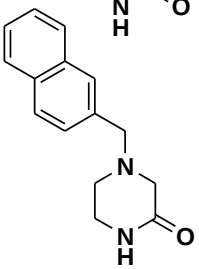
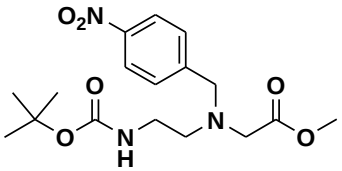
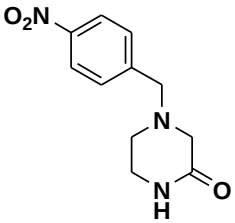
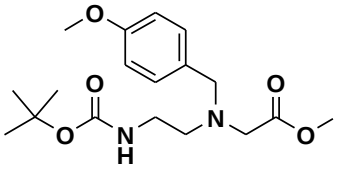
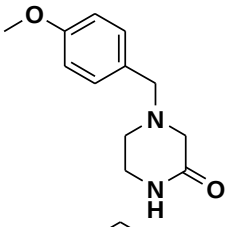
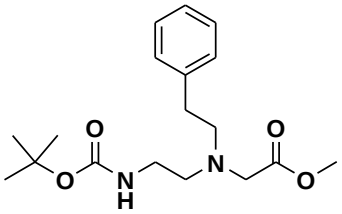
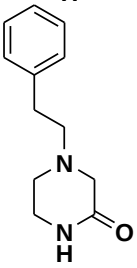
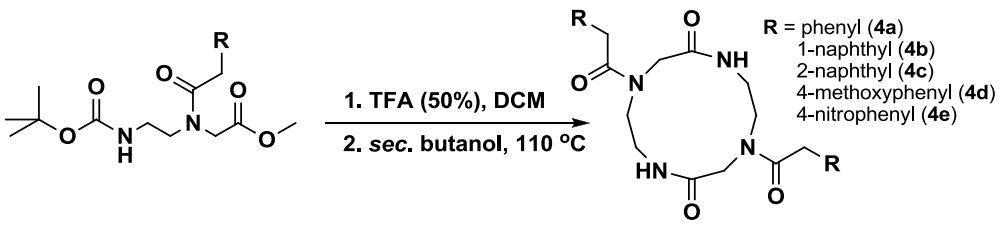
Reactant	Product	Yield
 3a	 4a	73%
 3b	 4b	69%
 3c	 4c	71%
 3d	 4d	79%
 3e	 4e	80%
 3f	 4f	43%

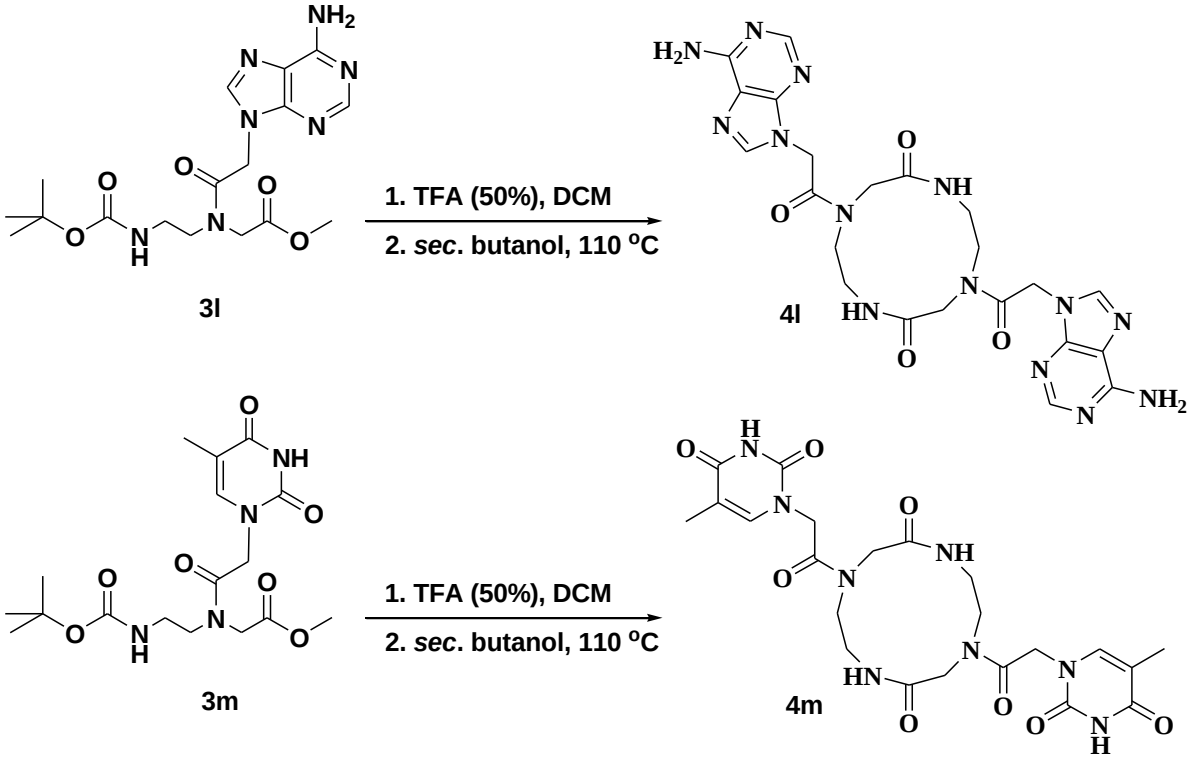
Figure 2.1 Cyclization of *N*-alkyl-*N*-(2-aminoethyl)glycine monomer units.



Reactant	Product	Yield
3g	4g	72%
3h	4h	68%
3i	4i	76%
3j	4j	68%
3k	4k	82%

Figure 2.2 Cyclization of *N*-acyl-*N*-(2-aminoethyl)glycine monomer units.

substituent) favours the formation of six member rings and the presence of carbonyl group (free rotation of *N*-acyl substituent is restricted) favours the formation of twelve member rings. Similar trend was observed for the cyclization of adenine (**3l**) and thymine (**3m**) acetic acid substituted *N*-(2-aminoethyl)glycine monomers (Scheme 2.2).[24] These molecules were synthesised to study their interactions with single strand DNA as functional molecules.



Scheme 2.2 Adenine (**3l**) and thymine (**3m**) acetic acid substituted *N*-(2-aminoethyl)glycine monomers cyclization to form twelve membered cyclic *aeg*-hybrid peptoids **4l** and **4m** respectively.

2.5 Results and discussion

Cyclic *aeg*-hybrid peptoids were characterized by nuclear magnetic resonance spectroscopy (^{1}H NMR, ^{13}C NMR, DEPT, HSQC and HMBC), IR spectroscopy and energy minimized model to determine the structure.

2.5.1 ^{1}H NMR and ^{13}C NMR for cyclic hybrid peptoids

NMR studies conformed the *N*-alkyl and *N*-acyl enforced cyclization of *N*-alkyl-*N*-(2-aminoethyl)glycine and *N*-acyl-*N*-(2-aminoethyl)glycine hybrid peptoid monomers to six and twelve membered cyclic *aeg*-hybrid peptoids. It is well known in case of natural cyclic peptides and cyclic peptidomimics that they are conformationally[25] constrained and rarely exists in symmetric cyclic forms. The protons which are seen in the NMR spectrum of an uncyclized form of peptide are hard to correlate with the protons observed in the cyclized form of the same peptide. Symmetric cyclic dipeptides (CDP) like Gly-Gly shows two different peaks corresponding to two amide protons in ^{1}H NMR spectrum, owing to its asymmetric conformation. Similar spectral features can be expected in larger cyclic peptides[26] or their mimics. Our cyclic hybrid peptoids were differentiated as six and twelve membered rings based on NMR and mass analysis. ^{1}H-NMR spectra of monomer units (eg., **3d** and **3k)** clearly showed a *singlet* peak at 1.5 *ppm* (nine protons) which corresponds to *tert*-butyl group and a *singlet* peak at 3.7 *ppm* (three protons) for -OMe of methyl ester. We have recorded the ^{1}H-NMR spectra of **3d** and **3k** after Boc–deprotection followed by cyclization. Surprisingly, we did not find peaks of amino and -OMe group indicating the possible cyclization of respective monomers. In Figure 2.4, the ^{1}H NMR of **4d** in *$CDCl_3$*, showed four peaks (*2, 3, 5* and *7*) in region from 2.4 *ppm* to 4 *ppm* corresponding to protons of four -CH_2 groups, one broad peak at 6.83ppm corresponding to amide proton (H_1) and *a doublet* for four aromatic protons (HRMS mass - 236.1055, $[M+H]^+$) confirming six membered cyclic structure of **4d**.

In case of ^{1}H NMR of molecule **4k** in *DMSO d_6*, the splitting pattern was completely different, presence of two amide protons (H_{12} and H_{18}) indicates the existence of either trimer or a cyclic dimer. Presence of trimer was ruled out by mass analysis (HRMS mass =

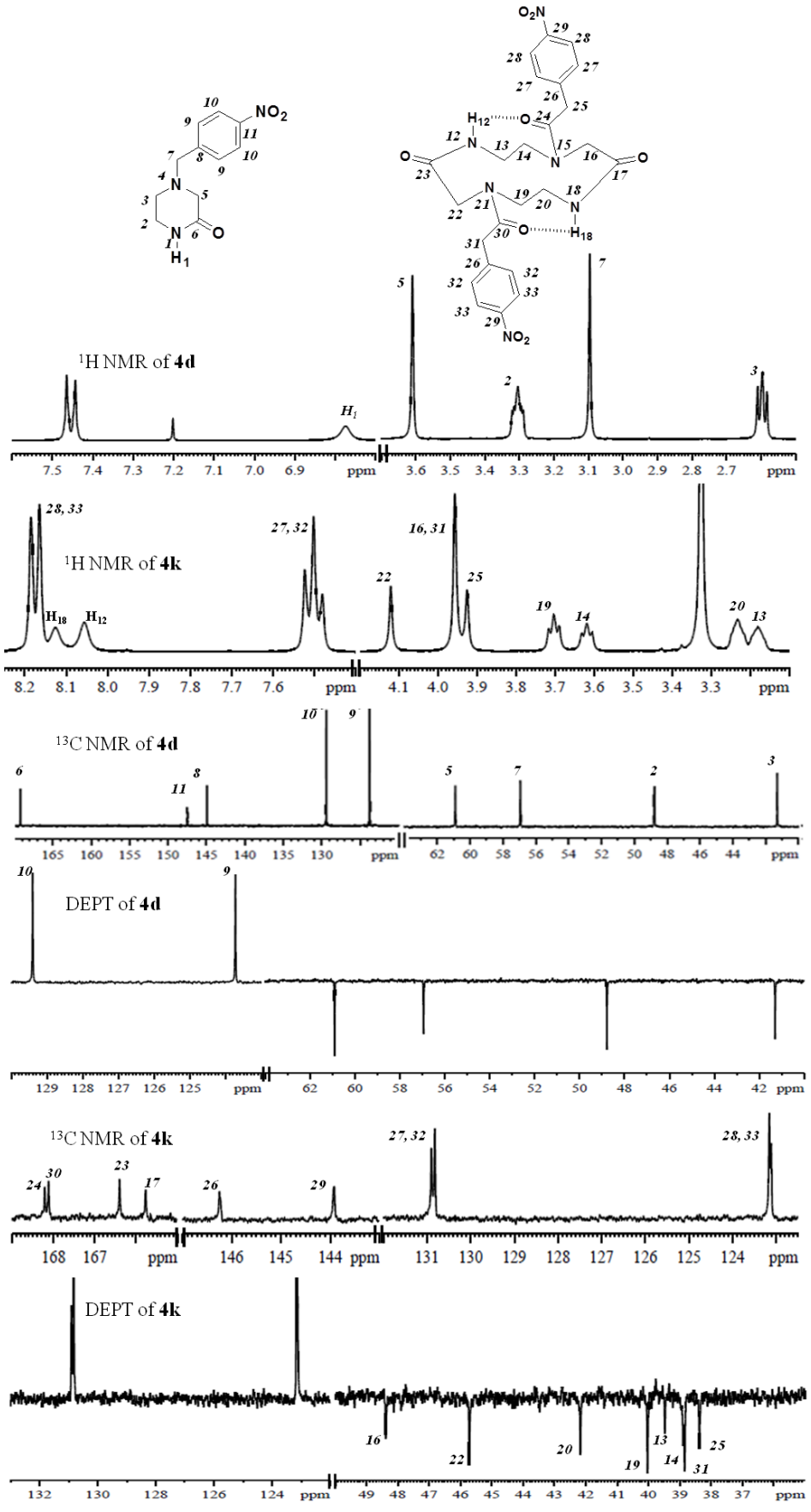


Figure 2.3 Comparison of ^{1}H NMR and ^{13}C NMR spectra of molecules **4d** and **4k**.

549.1664, $[M+Na]^+$), confirming cyclic dimer (twelve membered ring) structure of **4k**. Seven peaks in region from 3 *ppm* to 4.3 *ppm* (*13, 14, 16, 20, 22, 25* and *31*) correspond to protons of eight -CH_2 groups, indicating that molecule **4k** exist as conformationally constrained asymmetric cyclic dimer (12-member ring). The asymmetric twelve membered cyclic structure of **4k** was further confirmed by ^{13}C NMR and DEPT-135 (distortionless enhancement by polarization transfer) spectral data. In molecule **4d** four peak (*2, 3, 5* and *7*) for -CH_2 groups and one peak for carbonyl (*6*) was observed. In case of **4k**, eight peaks (*13, 14, 16, 20, 22, 25* and *31*) for corresponding -CH_2 groups, four peaks (*17, 23, 24* and *30*) for four carbonyls and two peaks for amide protons was observed. 4-nitrophenyl moiety have four set of equivalent aromatic carbons (two –CH and two quaternary carbons) and **4d** showed all the four peaks (*8, 9, 10* and *11*) in ^{13}C NMR and corresponding two peaks (-CH) in DEPT-135. Whereas **4k** showed six set of aromatic peaks (*26, 27, 28, 29, 32* and *33*) in ^{13}C NMR, of which two are from quaternary carbon (*26* and *29*) and other peaks from four –CH carbons. The four –CH carbon signals were also observed in DEPT-135 spectrum. This implies that the two 4-nitrophenyl aromatic rings in **4k** experience differential chemical environment due to asymmetric conformation of twelve membered ring of **4k**.

2.5.2 Temperature and concentration dependent NMR spectroscopy

We have investigated the hydrogen bonding patterns of amide bonds in **4k** with the help of temperature dependent ^1H-NMR studies.[27] Temperature dependent NMR spectra showed an upfield shift (0.6 *ppm*) of amide protons by increasing the temperature from 25°C to 95°C indicating the presence of hydrogen bonding. The hydrogen bonding in **4k** can be either inter or intramolecular and intermolecular hydrogen bonding is more common in macrocyclic peptides and peptoids.[10] The nature of hydrogen bonding (intra or inter molecular) was validated through a series of concentration dependent NMR and IR studies. ^1H NMR of **4k** was recorded at various concentrations (3.8 mM, 1.9 mM, 0.95 mM, 0.47 mM and 0.23 mM)

in *DMSO* d_6. The concentration dependent ^{1}H NMR spectra did not show any change in the

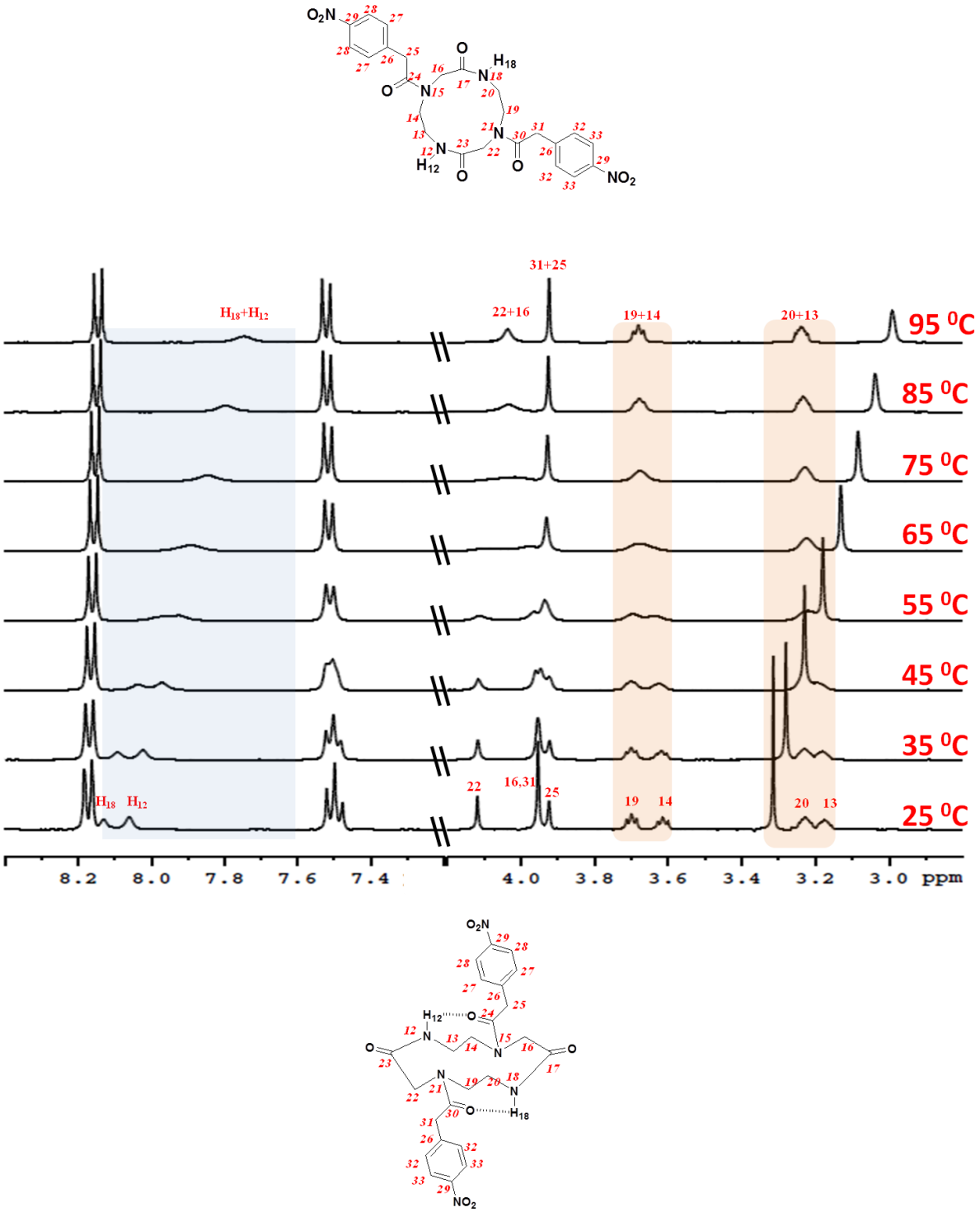


Figure 2.4 Temperature dependent^{1}H NMR spectra of molecule **4k** (in *DMSO* d_6).

position of amide proton signals with the varying concentration (dilution) of **4k** from 3.8 mM to 0.23 mM, which revealed existence of intramolecular hydrogen bonding interactions,

which is generally not expected in cyclic peptides. The strength of intramolecular hydrogen bonding for amide protons can be estimated by using temperature coefficient ($\Delta\delta_{NH}/\Delta T$) data.[28] Temperature coefficient is the slope of a plot of amide proton chemical shift observed against temperature in ^{1}H NMR. The temperature coefficient values obtained for amide protons H_{12} and H_{18} are -4.46 *ppb* and -5.7 *ppb*. The obtained temperature coefficient values indicate that the strength of hydrogen bonding is moderate and amide protons are less shielded from the solvent.[29] Proton H_{12} exhibited relatively lower hydrogen bond strength compared to proton H_{18}. The amide protons H_{12} and H_{18} at higher temperature merge to form a single broad peak with a net upfield shift of 0.33 *ppm* and 0.39 *ppm* respectively (Figure 2.4). Similarly protons *13* and *20* that appear as two broad *multiplets* (at 25 °C) merge to form a broad singlet with a marginal downfield shift (0.02 *ppm*) as the temperature increase from 25 °C to 65 °C which ultimately transformed into broad multiplet at 95 °C. Similar spectral changes were observed in case of protons of all other -CH_2 groups (*14* & *19*, *31* & *25*, and *22* & *16*). At 45 °C, the *singlet* corresponding to four -CH_2 protons at 3.95 *ppm* split into two peaks indicating that all these protons are not equivalent and may correspond to protons from two different sets of -CH_2 groups (*16* and *31*).

Recently, F. De Riccardis *et al.* have shown that eighteen membered cyclic peptoid change its conformation form an asymmetric cyclic form to a symmetric form with the addition of sodium picrate.[30] Na^+ acts as guest and induces S_6 symmetry into the molecule, this phenomena was studied and confirmed by concentration depended NMR spectroscopy. In our case molecule **4k** with increase in temperature showed merging of related peaks, indicating that **4k** is changing conformation from an asymmetric, constrained form to highly symmetric form (C_2 symmetry). ^{1}H NMR spectrum of **4k** at 95 °C showed proper splitting pattern and proton count corresponding to symmetric twelve member ring. ^{13}C NMR and DEPT data at 75 °C have further strengthened the observation.

2.5.3 IR spectroscopy

The presence of intramolecular hydrogen bonding in molecule **4k** was confirmed through IR measurements at different concentrations by monitoring the change in the peak position corresponding to amide stretching (3300 cm^{-1}). A broad peak at 3280 cm^{-1} was observed corresponding to the hydrogen bonded amide and showed no change with decrease of concentration from 2 mM to 0.5 mM confirming the presence of intramolecular hydrogen bonding in the molecule (Figure 2.5).

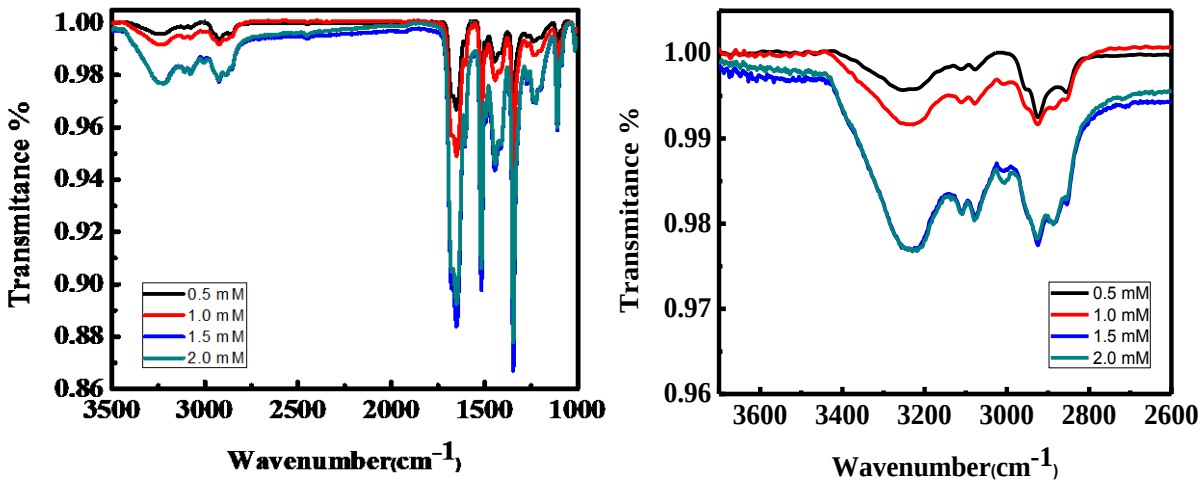


Figure 2.5 IR spectra showing intramolecular hydrogen bonding in molecule **4k**.

2.5.4 2D NMR spectroscopy and energy minimized model

The ^{1}H -^{13}C HSQC and HMBC spectra for molecule **4k** was recorded in *DMSO* d_6. HSQC spectra obtained were remarkably well dispersed, indicative of a non repetitive folded conformation. Other important information was the presence of only a single set of peaks for two protons bonded to a single carbon atom, implying exceptional conformational purity.[32] We assigned the correlation peaks and determined the protons bonded to different carbons in a sequence-specific manner. ^{1}H -^{13}C HMBC spectrum[34] showed through bond coupling peaks

for carbonyl of primary amide and triplet protons (*14* and*17*) are indicating the existence of amide bond.

Energy minimized structure for molecule **4k** showed intramolecular hydrogen bonding between primary amide proton and secondary amide carbonyl (H_{12} & $^{24}C{=}O$ and H_{18} & $^{30}C{=}O$). The two intramolecular hydrogen bonds present in single molecule exhibit different bond lengths (Figure 2.6), supporting the inference deduced through temperature coefficient values obtained from temperature dependent ^{1}H NMR spectroscopy. The minimized structure showed *trans* conformation for both the amide bonds, concluding that the molecule exist in low energy stable state.

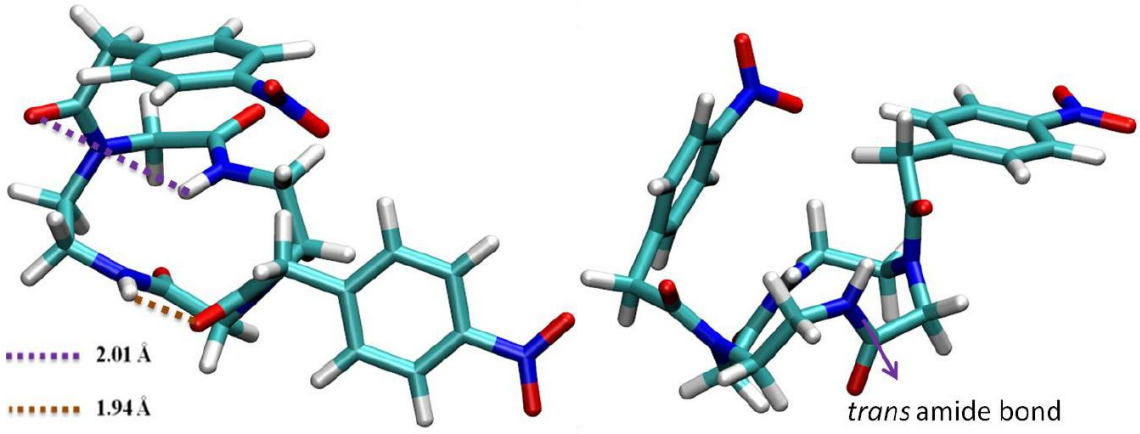


Figure 2.6 Energy minimized structure of molecule **4k** showing intramolecular hydrogen bonding and *trans* amide bonds.

2.6 Proposed mechanism

The phenomenon of differential cyclization can be understood from structural aspects of *N*-alkyl and *N*-acyl *N*-(2-aminoethyl)glycine monomers, the tertiary nitrogen is in pyramidal form substituted with flexible alkyl chains in case of *N*-alkyl-*N*-(2-aminoethyl)glycine monomers. Under reaction conditions the free amine attacks the ester without any hindrance from the tertiary nitrogen substituent in the backbone of linear *N*-(2-aminoethyl)glycine

chain, hence follow conventional Bladwin rules[35] of intramolecular cyclization and form six membered cyclic product. Whereas in case of *N*-acyl-*N*-(2-aminoethyl) glycine monomers, tertiary nitrogen involved in amide linkage is in conjugation with carbonyl (flexibility and free rotation restricted), forms amide plane which prevent bending of the linear *N*-(2-aminoethyl)glycine chain, and hence conformationally hinder the attacking of nucleophilic amine on to the electrophilic ester functionality, thus preventing intramolecular cyclization to form six membered cyclic product (**4a**-**4f**). Instead the free amine of one monomer attacks ester carbonyl on the other monomeric unit (intermolecular reaction) as the unhindered face energetically favoured which lead to the formation of dimer which is now flexible enough to undergo intramolecular cyclization to form twelve-membered cyclic products (**4g**-**4m**).

2.7 Conclusion

We have developed an elegant methodology for the synthesis of six and twelve membered cyclic hybrid peptoids from *N*-(2-aminoethyl)glycine functionalized monomers. The reaction yields are high compared to other cyclization procedures reported in literature for twelve-member cyclic products. The *N*-(2-aminoethyl)glycine backbone undergo differential cyclization based on α*N*- substitution. For biological activity such as antimicrobial activity cyclic peptide or peptidomimics should posses conformationally constrained structure, which is found in case of smaller rings compared to larger ring structures, hence these six and twelve membered cyclic hybrid peptoids can be studied for antimicrobial activity. Functionalization with extra charged groups can enhance their bioactivity. Further we are trying to make oligomers using solid phase synthesis for various biological applications from antimicrobial activity to inhibitors of amyloid aggregation.

2.8 Experimental section

General experimental procedure

All chemicals, solvents and analytical grade reagents were obtained from Sigma-Aldrich or Spectrochem and used as purchased without any further purification. Elemental analysis was carried out on Thermo scientific FLASH 2000 organic element analyzer. ^{1}H, ^{13}C NMR, DEPT, HMBC, HSQC were performed using Bruker AV–400 spectrometer with chemical shifts reported as parts per million (in $CDCl_3$ or *DMSO* d_6, with tetramethylsilane as internal standard). Mass spectra were obtained from Agilent 6538 UHD HRMS/Q-TOF spectrometer and LCMS-2020 Shimadzu spectrometer.

Synthesis of *N*-tert-butyloxycarbonyl-1,2-ethylenediamine (1): To a solution of ethylenediamine (50 mL, 750 mmol) in chloroform (300 mL), di-tert-butyldicarbonate (8.6 mL, 37.5 mmol) dissolved in chloroform (250 mL) was added dropwise using pressure equaliser at 0 °C. Reaction was monitored by thin layer chromatography and on completion of the reaction, reaction mixture was filtered. The filtrate was concentrated, extracted to organic layer, dried over anhydrous sodium sulfate and concentrated under vacuo to obtain colorless viscous liquid **1** in good yield (90 %). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.86 (s, 1H), 3.16-3.17 (m, 2H), 2.77-2.80 (m, 2H), 1.44 (s, 9H), 1.39 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.2, 78.25, 42.4, 40.8, 27.4.

Synthesis of *N*-(2-(*N*-tert-butyloxycarbonyl)aminoethyl)glycine methyl ester (2): To a solution of *tert*-butyl *N*-(2-aminoethyl)carbamate (**1**) (3 g, 18.72 mmol) in acetonitrile (25 mL), KF-celite (10 g, 131.04 mmols) was added and allowed to be stirred for 15 min. Bromomethyl acetate (1.42 mL, 14.97 mmol) dissolved in acetonitrile (20 mL) was added dropwise for 15 min. using pressure equaliser and then the reaction mixture was allowed to be stirred for 7 h. Reaction was monitored by thin layer chromatography and on completion the reaction mixture was filtered, filtrate was concentrated. The crude product was purified by column chromatography on

silica gel using 0.3% methanol in chloroform as an eluent to afford brown liquid product **2** in good yield (83 %); ^{1}H NMR (*CDCl*$_3$, 400 MHz) δ 4.98 (s, 1H), 3.72 (s, 3H), 3.41(s, 2H), 3.189-3.230 (m, 2H), 2.72-2.75 (t, 2H, *J* = 5.6 Hz), 1.60 (s, 1H), 1.44 (s, 9H); ^{13}C NMR (*CDCl*$_3$, 100 MHz) δ 172.9, 156.0, 51.8, 50.3, 48.7, 40.1, 28.4.

General synthetic procedure for *N*-alkyl-*N*-(2-aminoethyl)glycine monomers

To a solution of **2** (1.1 mmol) in acetonitrile (15 mL) was added TEA (2 mmol) at room temperature under argon atmosphere and stirred for 15 min. Then bromomethyl derivative was added and the reaction mixture was refluxed (81 °C) for 6 – 8 h. Reaction was monitored by thin layer chromatography, on completion of reaction solvent is dried and the crude product was purified on silica gel column chromatography.

***N*-(2-Boc-aminoethyl)-*N*-(benz-1-ylmethyl)glycine (3a):** Eluted with 5% ethyl acetate in hexane and obtained as colourless liquid; yield (68 %); ^{1}H NMR (*CDCl*$_3$, 400 MHz) δ 7.31 (s, 5H), 5.15 (s, 1H), 3.78 (s, 2H), 3.69 (s, 3H), 3.32 (s, 2H), 3.19 (d, *J* = 4.4 Hz, 2H), 2.76-2.81 (t, *J* = 6 Hz, 2H), 1.45 (s, 9H); ^{13}C NMR (*CDCl*$_3$, 400 MHz) δ 169.6, 160.8, 156.0, 135.7, 129.7, 129.3, 127.7, 79.5, 51.6, 47.6, 38.2, 37.3, 28.5; LCMS mass calcd: 322.1 $[M+H]^+$ found: 322.4.

***N*-(2-Boc-aminoethyl)-*N*-(naphth-1-ylmethyl)glycine (3b):** Eluted with 0.1% methanol in chloroform and obtained as liquid; yield (60 %); ^{1}H NMR (*CDCl*$_3$, 400 MHz) δ 7.74-7.81 (m, 3H), 7.43-7.50 (m, 3H), 7.40 (s, 1H), 5.02 (s, 1H), 3.81 (s, 2H), 3.27 (s, 2H), 3.12 (s, 2H), 2.71-2.76 (t, *J* = 6.2, 2H), 1.43 (s, 1H); ^{13}C NMR (*CDCl*$_3$, 400 MHz) δ 169.5, 162.4, 160.0, 140.1, 137.3 135.7, 129.1, 129.3, 129.7, 127.7,126.8, 79.9, 52.6, 44.6, 36.2, 35.3, 28.5; LCMS mass calcd: 372.2 $[M+H]^+$ found: 372.4.

***N*-(2-Boc-aminoethyl)-*N*-(naphth-1-ylmethyl)glycine (3c):** Eluted with 6% ethyl acetate in hexane and obtained as light brown semi solid; yield (61 %); ^{1}H NMR (*CDCl*$_3$, 400 MHz) δ

7.71-7.83 (m, 3H), 7.50 (s, 1H), 7.43-7.50 (m, 3H), 5.17 (s, 1H), 3.93 (s, 2H), 3.37 (s, 2H), 3.32 (s, 2H), 2.80-2.82 (t, *J* = 5.3, 2H), 1.43 (s, 1H); ^{13}C NMR (*CDCl*$_3$, 400 MHz) δ 169.5, 162.4, 160.0, 140.1, 137.3 135.7, 129.1, 129.3, 129.7, 127.7,126.8, 79.9, 52.6, 44.6, 36.2, 35.3, 28.5; LCMS mass calcd: 372.2 $[M+H]^+$ found: 372.4.

***N*-(2-Boc-aminoethyl)-*N*-((4-nitrobenz)-1-ylmethyl)glycine (3d):** Eluted with 0.1% methanol in chloroform and obtained as brownish liquid; yield (78 %); ^{1}H NMR (*CDCl*$_3$, 400 MHz) δ 7.21-7.24 (m, 2H), 6.84-6.69 (m, 2H),5.26 (s, 1H), 4.32 (s, 2H), 3.66 (s, 2H), 3.45 (s, 3H), 3.34-3.36 (t, 2H, J = 3.6 Hz), 3.21 (s, 2H), 1.45 (s, 9H); ^{13}C NMR (*CDCl*$_3$, 100 MHz) δ 168.4, 161.4, 132.5, 128.9, 126.6, 124.8, 79.8, 53.1, 44.5, 36.4, 36.5, 28.8; LCMS mass calcd: 368.1 $[M+H]^+$ found: 368.5.

***N*-(2-Boc-aminoethyl)-*N*-((4-methoxybenz)-1-ylmethyl)glycine (3e):** Eluted with 0.07% methanol in chloroform and obtained as a white solid; yield (76 %); ^{1}HNMR (*CDCl*$_3$, 400 MHz) δ 7.23-7.27 (m, 2H), 6.67-6.70 (m, 2H),6.02 (s, 1H), 4.31 (s, 2H), 3.66 (s, 2H), 3.45 (s, 3H), 3.34-3.39 (t, 2H, *J* = 4.3 Hz), 3.33 (s, 3H), 3.21 (s, 2H), 1.45 (s, 9H); ^{13}C NMR (*CDCl*$_3$, 100 MHz) 169.6, 161.4, 136.3, 128.8, 126.3, 123.2, 79.8, 53.1, 44.3, 38.3, 36.4, 36.5, 28.2; LCMS mass calcd: 353.2 $[M+H]^+$ found: 353.3.

***N*-(2-Boc-aminoethyl)-*N*-(benz-1-ylethyl)glycine (3f):** Eluted in chloroform and obtained as colourless liquid; yield 43%; ^{1}H NMR (*CDCl*$_3$, 400 MHz) δ 7.34-7.40 (m, 5H), 5.13 (s, 1H), 3.76 (s, 2H), 3.69 (s, 3H), 3.32 (s, 2H), 3.24-3.28 (t, 2H, *J* = 6.3 Hz) 3.19 (d, *J* = 4.4 Hz, 2H), 2.76-2.81 (t, 2H, *J* = 6 Hz), 1.45 (s, 9H); ^{13}C NMR (*CDCl*$_3$, 400 MHz) δ 169.6, 160.8, 156.0, 135.7, 129.7, 129.3, 127.7, 79.5, 51.6, 47.6, 38.2, 37.3, 28.5; LCMS mass calcd: 337.2 $[M+H]^+$ found: 337.8.

General synthetic procedure for *N*-acyl-*N*-(2-aminoethyl)glycine monomers

To a solution of acetic acid derivative (1 mmol) in DMF was added HBTU (1.2 mmol)

followed by HOBT (1.2 mmols) at 0 °C under argon atmosphere. Reaction mixture was stirred till a clear solution was obtained and then added **2** (1.1 mmol). Using a syringe DIPEA (3 mmol) was slowly added in portions to the reaction mixture and the reaction was stirred for 5–6 h at room temperature. Reaction was monitored by thin layer chromatography, on completion of reaction the reaction mixture was poured into water. The organic layer was separated, dried (Na_2SO_4) and evacuated. The crude product was purified by silica gel column chromatography.

***N*-(2-Boc-aminoethyl)-*N*-(benz-1-ylacyl)glycine (3g):** Eluted with 15% ethyl acetate in hexane and obtained as a white solid; yield (89 %); ^{1}H NMR (*$CDCl_3$*, 400 MHz) δ 7.20-7.33 (m, 5H), 5.38 (s, 1H), 4.00 (s, 2H), 3.76 (s, 2H), 3.75 (s, 3H), 3.49 (t, *J* = 5.6 Hz, 2H), 3.21 (q, *J* = 5.6 Hz, 2H), 1.45 (s, 9H); ^{13}C NMR (*$CDCl_3$*, 100 MHz) δ 169.6, 161.4, 156.0, 133.5, 128.6, 126.5, 125.8, 79.5, 51.1, 47.3, 37.4, 36.3, 27.2; LCMS mass calcd: 351.3 $[M+H]^+$ found: 351.4.

***N*-(2-Boc-aminoethyl)-*N*-(naphth-1-ylacyl)glycine (3h):** Eluted with 0.4 % methanol in chloroform and obtained as colourless liquid; yield (83%); ^{1}H NMR (*DMSO d_6*, 400 MHz) δ 7.81-7.90 (m, 4H) 7.75 (s, 1H), 7.45-7.52 (m, 2H), 7.38 (dd, *J* = 1.48 Hz, *J* = 8.4 Hz, 1H), 7.00 (t, *J* = 5.7 Hz, 1H), 4.06 (s, 2H), 3.91 (s, 2H), 3.63 (s, 3H), 3.44 (t, *J* = 6 Hz), 3.14 (q, *J* = 6 Hz, 2H), 1.39 (s, 9H); ^{13}C NMR (*DMSO d_6*, 100 MHz) δ 168.4, 161.9, 156.2, 132.1, 135.6, 128.9, 127.9, 126.5, 125.8, 124.2, 78.5, 52.1, 43.3, 37.2, 36.9, 28.8; LCMS mass calcd: 401.2 $[M+H]^+$ found: 401.6.

***N*-(2-Boc-aminoethyl)-*N*-(naphth-2-ylacyl)glycine (3i):** Eluted with 15 % ethyl acetate in hexane and obtained as white semi-solid; yield (75 %); ^{1}H NMR (*DMSO d_6*, 400 MHz) δ 7.85-7.93 (m, 4H) 7.73 (s, 1H), 7.46-7.51 (m, 2H), 7.36-7.39 (d, 1H, *J* = 1.53), 6.89-7.11 (t, *J* = 5.3 Hz, 1H), 4.06 (s, 2H), 3.91 (s, 2H), 3.63 (s, 3H), 3.42-3.56 (t, *J* = 6.1 Hz), 3.23-3.33 (q,

J = 6 Hz, 2H), 1.39 (s, 9H); ^{13}C NMR (*DMSO* d_6, 100 MHz) δ 169.6, 161.4, 156.0, 133.5, 132.6, 128.6, 126.9, 126.5, 125.8, 125.0, 79.5, 51.1, 47.3, 38.2, 36.3, 28.3; LCMS mass calcd: 401.2 $[M+H]^+$ found: 401.3.

***N*-(2-Boc-aminoethyl)-*N*-((4-nitrobenz)-1-yl acyl)glycine (3j):** Eluted with 0.2% methanol in chloroform and obtained as a brown solid; yield (68 %); ^{1}H NMR (*CDCl*$_3$, 400 MHz) δ 7.20-7.23 (m, 2H), 6.89-6.49 (m, 2H),5.26 (s, 1H), 4.32 (s, 2H), 3.66 (s, 2H), 3.45 (s, 3H), 3.34-3.39 (t, 2H, *J* = 4.3 Hz), 3.21 (s, 2H), 1.43 (s, 9H); ^{13}C NMR (*CDCl*$_3$, 100 MHz) δ 168.6, 160.4, 156.2, 132.5, 128.9, 126.6, 124.8, 79.8, 53.1, 44.3, 36.4, 36.5, 28.2; LCMS mass calcd: 395.1 $[M+H]^+$ found: 396.4.

***N*-(2-Boc-aminoethyl)-*N*-((4-methoxybenz)-1-yl acyl)glycine (3k):** Eluted with 0.05% methanol in chloroform and obtained as a white solid; yield (70 %); ^{1}H NMR (*CDCl*$_3$, 400 MHz) δ 7.23-7.27 (m, 2H), 6.67-6.70 (m, 2H), 6.02 (s, 1H), 4.31 (s, 2H), 3.66 (s, 2H), 3.45 (s, 3H), 3.34-3.39 (t, 2H, *J* = 4.3 Hz), 3.33 (s, 3H), 3.21 (s, 2H), 1.45 (s, 9H); ^{13}C NMR (*CDCl*$_3$, 100 MHz) δ 169.6, 161.4, 154.2, 136.3, 128.8, 126.3, 123.2, 79.8, 53.1, 44.3, 38.3, 36.4, 36.5, 28.2; LCMS mass calcd: 381.2 $[M+H]^+$ found: 381.3.

2-(*N*-(2-(tert-butoxycarbonyl) ethyl)-2-chloroacetamido) acetate (3k-1): To a solution of **2** (3 g, 12.9 mmol) in acetonitrile (100 ml), KF-celite (7.224 g, 90 mmol) was added and stirred for 10 min. Chloroacyl chloride (1.43 mL, 18.1 mmol) was added slowly using syringe for 10 minutes, reaction was allowed to stirr for 7 hours. Reaction was monitored by thin layer chromatography and on completion reaction mixture was filtered, filtrate was concentrated. The crude product was purified using column chromatography on silica gel using $CHCl_3$ as an eluent to afford a colourless liquid product in good yield (85 %); ^{1}H NMR (400 MHz, *CDCl*$_3$) δ 5.40 (s, 1H), 4.14 (s, 2H), 4.04 (s, 2H), 3.76 (s, 3H), 3.52-3.55 (t, *J* = 5.9 Hz, 2H), 3.26-3.30 (q, *J* = 6 Hz, 2H), 1.44 (s, 9H); ^{13}C NMR (*CDCl*$_3$, 100 MHz) 169.4,

161.4, 156.0, 79.5, 51.1, 47.3, 37.4, 36.3, 27.3; LCMS mass calcd: 309.7 $[M+H]^+$ found: 309.4.

***N*-(2-Boc-aminoethyl)-*N*-(adenin-9-ylacyl)glycine (3l):** To a solution of Adenine (3g, 8.11mmol) in DMF (25 ml), NaH (0.2 g, 8.11 mmol) was added and allowed to stirr the reaction mixture for 1 h. Then **3f-1** (3.02 g, 9.73 mmol) in DMF (5ml) was added to the above reaction mixture slowly. The reaction mixture was allowed to stirr overnight. Reaction mixture was filtered and was extracted with water and chloroform. Dried over Na_2SO_4 and evacuated. The crude product was purified using column chromatography on silica gel using $CHCl_3$: MeOH (97.3:2.7) as an eluent to afford a white solid product in good yield (72 %); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ 8.34 (s, 1H), 7.91 (s, 1H), 5.71 (s, 1H), 5.58 (s, 2H), 5.10 (s, 2H), 4.07 (s, 2H), 3.75 (s, 3H), 3.65 (t, *J* = 5.8 Hz, 2H), 3.40 (q, *J* = 5.8 Hz, 2H), 1.41 (s, 9H); ^{13}C NMR (*CDCl$_3$*, 100 MHz) 169.1, 160.0, 156.2, 154.4, 149.5, 143.7, 132.5, 128.9, 126.6, 124.8, 123.1, 79.8, 53.1, 44.8, 44.3, 36.4, 36.5, 28.2; LCMS mass found: 408.6 $[M+H]^+$ calcd: 408.2.

***N*-(2-Boc-aminoethyl)-*N*-(thymin-1-ylacyl)glycine (3m):** Eluted with 1.4% methanol in chloroform and obtained as a white powder; yield (73 %); ^{1}H NMR (*CDCl$_3$*, 400 MHz) δ 8.16 (s, 1H), 6.96 (s, 1H), 5.49 (s, 1H), 4.57 (s, 2H), 4.05 (s, 2H), 3.76 (s, 3H), 3.53 (t, *J* = 5.8 Hz, 2H), 3.34 (q, *J* = 5.92 Hz, 2H), 1.92 (d, *J* = 1.04 Hz, 3H), 1.45 (s, 9H); ^{13}C NMR (*CDCl$_3$*, 100 MHz) δ 170.8, 170.4, 167.6, 167.2, 164.4, 155.8, 151.0, 142.0, 108.2, 78.1,78.0, 49.2; LCMS mass calcd: 399.4 $[M+H]^+$ found: 399.2.

General synthetic procedure for cyclization To a solution of *N*-alkyl-*N*-(2-aminoethyl)glycine or *N*-acyl-*N*-(2-aminoethyl)glycine monomers (200 mg) was added TFA, DCM (v/v 1:1) (5 mL) and a few drops of triisopropylsilane (TIPS) at 0°C. Reaction mixture was stirred for 1 h, solvent is dried and the product obtained is dissolved in *sec*-butanol (10

mL), reflux the reaction mixture at 110 °C, under argon atmosphere for 10–12 h. Reaction was monitored by thin layer chromatography, on completion of reaction solvent is dried and the crude product was purified by silica gel column chromatography.

4-benzylpiperazin-2-one (4a): Eluted with 0.3% methanol in chloroform and obtained as white powder; yield 73%; M.P 163°C- 165°C; ^{1}H NMR (*CDCl$_3$*, 400 MHz) δ 7.24-7.32 (m, 5H), 6.67 (s, 1H) 3.57 (s, 2H), 3.32-3.35 (m, 2H), 3.15(s, 1H), 2.62-3.64 (t, 2H, *J* = 5.6 Hz); ^{13}C NMR (*CDCl$_3$*, 100 MHz) δ 169.7, 136.9, 129.0, 128.4, 127.5, 61.8, 56.9, 48.5, 41.4; DEPT-135 (*CDCl$_3$*, 100 MHz) δ 129.0, 128.4, 127.5, 61.8, 56.9, 48.5, 41.4; HRMS mass calculated for $C_{11}H_{14}N_2O_1$, 191.1179 $[M+H]^+$. found: 191.1167; Elemental analysis. found: C, 68.92; H, 7.32; N, 15.65.calcd: C, 69.45; H, 7.42; N, 14.73.

4-(naphthalen-1-ylmethyl)piperazin-2-one (4b): Eluted with 0.1% methanol in chloroform and obtained as white powder; yield 69%; M.P 172°C- 175°C; C^{1}H NMR (*CDCl$_3$*, 400 MHz) δ 7.87-7.90 (m, 3H), 7.81 (s, 1H), 7.73 (s, 1H), 7.46-7.52 (m, 3H), 3.16 (m, 2H), 2.95 (s, 2H), 2.57-2.60 (t, 2H, *J* = 5.68); ^{13}C NMR (*CDCl$_3$*, 100 MHz) δ 167.6, 135.1, 132.8, 132.3, 127.7, 127.5, 127.4, 127.1, 127.12, 126.0, 125.7, 60.8, 56.7, 48.4, 40.35; DEPT-135 (*CDCl$_3$*, 100 MHz) δ 127.7, 127.5, 127.4, 127.1, 127.12, 126.0, 125.7, 60.8, 56.7, 48.4, 40.35; HRMS mass calculated for $C_{15}H_{16}N_2O_1$, 241.1335 $[M+H]^+$. found: 241.1319; Elemental analysis. found: C, 74.81; H, 6.11; N, 11.32. calcd: C, 74.97; H, 6.71; N, 11.66.

4-(naphthalen-2-ylmethyl)piperazin-2-one (4c): Eluted with 0.12% methanol in chloroform and obtained as white crystalline powder; yield (69%); M.P 171°C- 173°C; ^{1}H NMR (*CDCl$_3$*, 400 MHz) δ 7.82-7.88 (m, 3H), 7.76 (m, 2H), 7.46-7.52 (m, 3H), 3.23 (m, 2H), 2.91 (s, 2H), 2.57-2.60 (t, 2H, *J* = 5.68); ^{13}C NMR (*CDCl$_3$*, 100 MHz) δ 167.6, 133.2, 132.4, 131.3, 127.7, 127.5, 127.4, 127.1, 127.12, 126.0, 125.7, 60.8, 56.7, 48.4, 40.35; DEPT-135 (*CDCl$_3$*, 100 MHz) δ 127.7, 127.5, 127.4, 127.1, 127.12, 126.0, 125.7, 60.8, 56.7,

48.4, 40.35; HRMS mass calculated for $C_{15}H_{16}N_2O_1$, 241.1335 $[M+H]^+$. Found: 241.1326; Elemental analysis. Found: C, 72.97; H, 6.22; N, 11.18.Calcd: C, 74.97; H, 6.71; N, 11.66.

4-(4-nitrobenzyl)piperazin-2-one (4d): Precipitated by diethyl ether and isolated as light brown powder; yield 71%; M.P 178°C- 180°C; ^{1}H NMR (*CDCl*$_3$, 400 MHz) δ 8.16-8.19 (d, 2H, *J* = 8.4 Hz), 7.50-7.52 (d, 2H, *J* = 8.8 Hz), 6.83 (s, 1H), 3.66 (s, 2H), 3.34-3.37 (m, 2H), 3.15 (s, 1h), 2.64-2.66 (t, 2H, *J* = 5.6 Hz); ^{13}C NMR (*CDCl*$_3$, 100 MHz) δ 169.2, 147.4, 144.9, 129.4, 123.7, 60.9, 56.9, 48.7, 41.2; DEPT-135 (*CDCl*$_3$, 100 MHz) δ 129.4, 123.7, 60.9, 56.9, 48.7, 41.2; HRMS calculated for $C_{11}H_{13}N_3O_3$, 236.1035 $(M+H)^+$. found: 236.1055; Elemental analysis. found: C, 56.11; H, 5.32; N, 17.68. Calcd: C, 56.16; H, 5.57; N, 17.86.

4-(4-methoxybenzyl)piperazin-2-one (4e): Eluted with 0.08 % methanol in chloroform and obtained as white crystalline powder; yield (69%); M.P 156°C- 159°C; ^{1}H NMR (*CDCl*$_3$, 400 MHz) δ 8.14-8.19 (d, 2H, *J* = 7.4 Hz), 7.50-7.52 (d, 2H, *J* = 8.8 Hz), 6.83 (s, 1H), 3.23 (m, 2H), 2.91 (s, 2H), 2.57-2.60 (t, 2H, *J* = 5.68); ^{13}C NMR (*CDCl*$_3$, 100 MHz) δ 167.2, 145.5, 144.3, 129.4, 123.7, 60.8, 56.7, 48.4, 40.35; DEPT-135 (*CDCl*$_3$, 100 MHz) δ 129.4, 123.7, 60.8, 56.7, 48.4, 40.35; HRMS mass calculated for $C_{12}H_{16}N_2O_2$, 221.1285 $[M+H]^+$. Found: 221.1251; Elemental analysis. found: C, 65.59; H, 7.63; N, 11.11. calcd: C, 65.43; H, 7.32; N, 12.72.

4-phenethylpiperazin-2-one (4f): Eluted in chloroform and obtained as brown powder; yield 43%; M.P 155°C- 159°C; ^{1}H NMR (*CDCl*$_3$, 400 MHz) δ 7.27-7.31 (m, 2H), 7.18-7.22 (m, 3H), 6.59 (s, 1H), 3.36-3.39 (m, 2H), 3.22 (s, 2H), 2.79-2.83 (m, 2H), 2.67-2.73 (m, 2H); ^{13}C NMR (*CDCl*$_3$, 100 MHz) δ 169.5, 139.58, 128.6, 128.4, 126.2, 56.8, 49.1, 41.2, 33.4, 29.6; DEPT-135 (*CDCl*$_3$, 100 MHz) δ 128.6, 128.4, 126.2, 56.8, 49.1, 41.2, 33.4, 29.6;

HRMS mass calculated for $C_{12}H_{16}N_2O_1$, 205.1335 $[M+H]^+$. found: 205.1336; Elemental analysis found: C, 71.56; H, 8.01; N, 13.56. calcd: C, 70.56; H, 7.90; N, 13.71.

4,10-(*N,N'*)-bis(benz-1-ylacyl) cyclo(*aeg-aeg*) (4g): Eluted with 0.8% methanol in chloroform and obtained as white powder; yield 72%; M.P 189°C- 196°C; ^{1}H NMR (*DMSO* d_6, 400 MHz) δ 7.44-7.56 (m, 10H), 7.25 (s, 1H), 4.46 (s, 2H), 4.31 (s, 2H), 4.01-4.04 (t, 2H, *J* = 5.2 Hz), 3.98 (s, 2H), 3.94 (s, 2H), 3.81-3.84 (t, 2H, *J* = 5.2), 3.50 (b, 2H), 3.35 (b, 2H); ^{13}C NMR (*DMSO* d_6, 100 MHz) δ 169.7, 168.1, 166.8, 134.2, 133.8, 128.9, 128.7, 128.5, 127.15, 49.15, 46.0, 42.7, 41.2, 40.8, 40.6, 38.5, 29.6; DEPT-135 (*DMSO* d_6, 100 MHz) δ 128.9, 128.7, 128.5, 127.15, 49.15, 46.0, 42.7, 41.2, 40.8, 40.6, 38.5, 29.6; HRMS mass calculated for $C_{20}H_{24}N_8O_8$, 459.2003 $[M+Na]^+$. found: 459.1945; Elemental analysis. found: C, 66.23; H, 6.45; N, 12.17. calcd: C, 66.19; H, 6.25; N, 12.87.

4,10-(*N,N'*)-bis(naphth-1-ylacyl) cyclo(*aeg-aeg*) (4h): Precipitated using diethyl ether to obtain white crystalline powder; M.P 180°C- 184°C; ^{1}H NMR (*DMSO* d_6, 400 MHz) δ 8.14 (s, 1H), 8.07 (s, 1H), 7.91-7.96 (m, 4H), 7.81-7.83 (d, *J* = , 2H), 7.49-7.52 (m, 3H), 7.42-7.46 (m, 2H), 7.30-7.35 (m, 2H), 4.18-4.22 (m, 6H), 3.97 (s, 2H), 3.75-3.77(t , 2H, *J* =4.3 Hz), 3.64-3.62 (t, *J* = , 2H), 3.25 (b, 2H), 3.20 (b, 2H); (*DMSO* d_6, 100 MHz) δ 169.7, 169.4, 166.6, , 133.5, 132.4, 131.7, 131.3, 128.7, 128.6, 127.6, 127.5, 127.4, 127.0, 126.7, 126.5, 126.4,126.3, 126.0, 125.9, 49.2, 46.1, 42.7, 41.4, 41.6, 41.0, 40.69, 38.6; DEPT-135 (*DMSO* d_6, 100 MHz) δ 128.7, 128.6, 127.6, 127.5, 127.4, 127.0, 126.7, 126.5, 126.4,126.3, 126.0, 125.9, 49.2, 46.1, 42.7, 41.4, 41.6, 41.0, 40.69, 38.6; HRMS mass calculated for $C_{32}H_{32}N_4O_4$, 559.2316 $[M+Na]^+$. found: 559.2277; Elemental analysis. found: C, 71.83; H, 5.91; N, 10.68. calcd: C, 71.62; H, 6.01; N, 10.44.

4,10-(*N,N'*)-bis(naphth-2-ylacyl) cyclo(*aeg-aeg*) (4i): Eluted with 0.5% methanol in chloroform and obtained as white crystalline powder; yield 68%; M.P 183°C- 187°C; ^{1}H

NMR (*DMSO* d_6, 400 MHz) δ 7.77-7.48 (m, 3H), 7.68 (s, 1H), 7.45-7.48(m, 2H), 7.36-7.38 (d, 1H, *J* = 8.32 Hz), 6.74 (s, 1H), 6.63 (s, 1H) 4.28 (s, 2H), 4.14 (s, 2H), 3.93 (s, 2h), 3.88 (s, 2H), 3.81-3.84 (t, 2H, *J* = 4.92), 3.62-3.65 (t, 2H, *J* = 5.36), 3.35 (b, 2H), 3.11 (b, 2H); ^{13}C NMR (*DMSO* d_6, 100 MHz) δ 169.7, 169.4, 166.6, , 133.5, 132.4, 131.7, 131.3, 128.7, 128.6, 127.6, 127.5, 127.4, 127.0, 126.7, 126.5, 126.4,126.3, 126.0, 125.9, 49.2, 46.1, 42.7, 41.4, 41.6, 41.0, 40.69, 38.6; DEPT-135 (*DMSO* d_6, 100 MHz) δ 128.7, 128.6, 127.6, 127.5, 127.4, 127.0, 126.7, 126.5, 126.4,126.3, 126.0, 125.9, 49.2, 46.1, 42.7, 41.4, 41.6, 41.0, 40.69, 38.6; HRMS mass calculated for $C_{32}H_{32}N_4O_4$, 559.2316 $[M+Na]^+$. found: 559.2307; Elemental analysis. found: C, 71.94; H, 6.02; N, 10.34.calcd: C, 71.62; H, 6.01; N, 10.44.

4,10-(*N,N'*)-bis((4-methoxybenz)-1-yl acyl) cyclo(*aeg-aeg*) (4j): Eluted with 1.2 % methanol in chloroform and obtained as white crystalline powder; yield 68%; M.P 184°C-179°C; ^{1}H NMR (*DMSO* d_6, 100 MHz) δ 7.14-7.16 (s, 4H, *J* = 8.4 Hz), 6.84-6.87 (s, 4H, *J* = 8.8 Hz), 6.63 (s, 1H), 6.50 (s, 1H), 4.25 (s, 2H), 4.10 (s, 2H), 3.70 (s, 4H), 3.66 (s, 2H), 3.61-3.63 (t, 2H, *J* = 4.8 Hz), 3.36 (b, 2H), 3.16 (b, 2H); ^{13}C NMR (*DMSO* d_6, 100 MHz) δ 169.0, 157.7, 128.7, 128.5, 124.7, 54.2, 48.1, 45.1, 41.6, 40.1, 39.7, 39.3, 39, 37.5; HRMS mass calculated for $C_{26}H_{32}N_4O_6$, 519.2214 $[M+Na]^+$. found: 519.2167; Elemental analysis. found: C, 62.34; H, 6.50; N, 10.96. calcd: C, 62.89; H, 6.50; N, 11.28.

4,10-(*N,N'*)-bis((4-nitrobenz)-1-yl acyl) cyclo(*aeg-aeg*) (4k): Precipitated by diethyl ether and isolated as light brown powder; yield 82%; M.P 195°C- 200°C; ^{1}H NMR (*DMSO* d_6, 400 MHz) δ 8.184-8.186 (d, 4H, *J* = 0.8 Hz), 8.16 (s, 1H), 8.07 (s, 1H), 7.47-7.52 (m, 4H), 4.11 (s, 2H), 3.95 (s, 4H), 3.92 (s, 2H), 3.68-3.71 (t, 2H, *J* = 5.6 Hz), 3.6-3.62 (t, 2H, *J* = 5.6 Hz), 3.21 (b, 2H), 3.18 (b, 2H); ^{13}C NMR (*DMSO* d_6, 100 MHz) δ 168.2, 168.1, 166.4, 165.7, 146.2, 143.9, 130.9, 130.8, 123.16, 123.12, 48.3, 45.7, 42.1, 40.1, 40.0, 38.8, 38.3; DEPT-135 (*DMSO* d_6, 100 MHz) δ 130.9, 130.8, 123.16, 123.12, 48.3, 45.7, 42.1, 40.0, 39.47, 38.8,

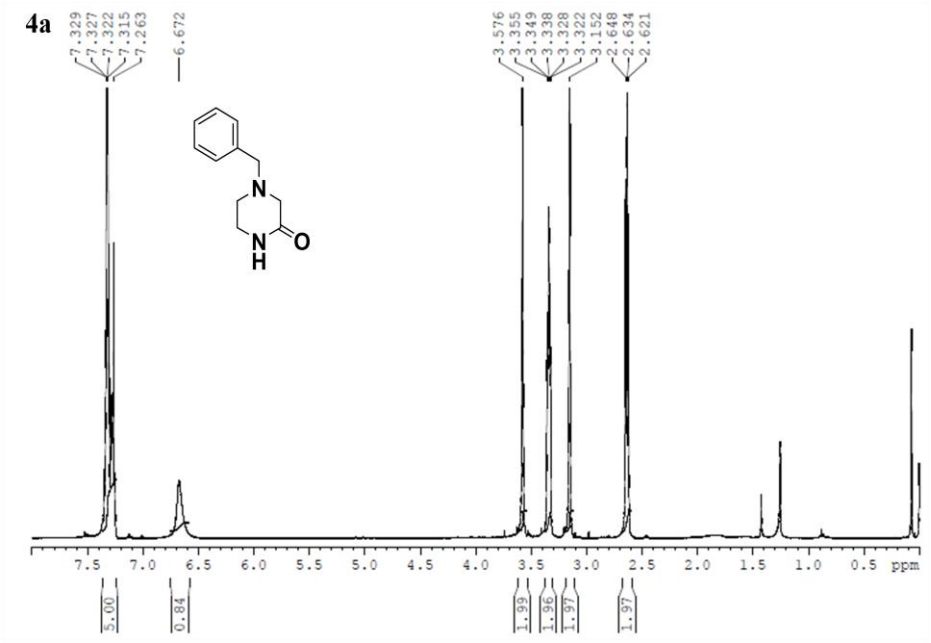
38.3; HRMS mass calculated for $C_{24}H_{26}N_6O_8$, 549.1710 $[M+Na]^+$. found: 549.1664; Elemental analysis. found: C, 54.35; H, 4.68; N, 15.83. calcd: C, 54.75; H, 4.98; N, 15.96.

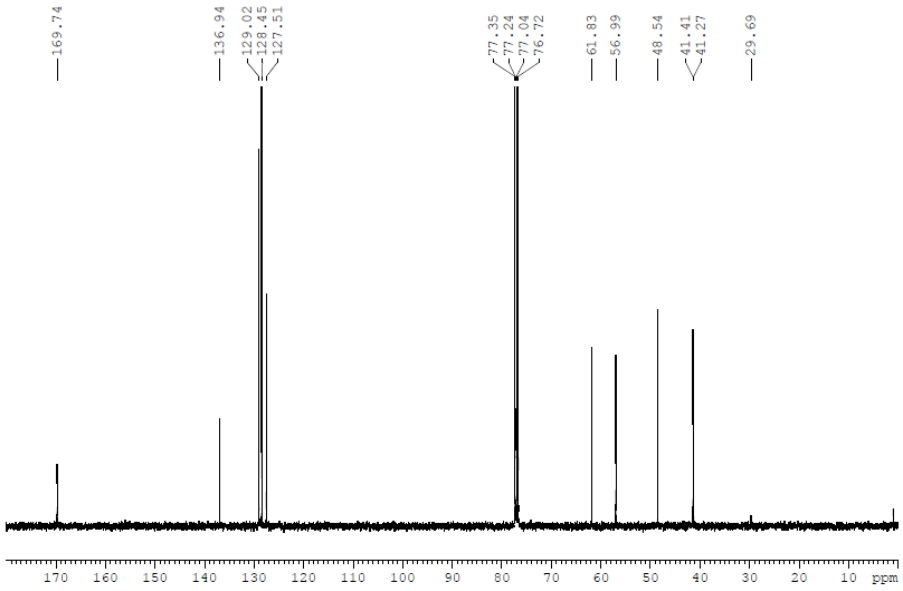
4,10-(*N,N'*)-bis(adenin-9-ylacyl) cyclo(*aeg-aeg*) (4l): Isolated as white powder; yield 78%; ^{1}H NMR (*DMSO* d_6, 400 MHz) δ 8.81 (b, 4H), 8.34 (s, 2H), 8.32 (s, 2H), 8.27 (s, 2H), 8.22 (s, 2H), 5.34-5.38 (d, *J* = , 4H), 4.27 (s, 2H), 4.01 (s, 2H), 3.81-3.84 (t, *J* = , 2H), 3.66-3.68 (t, *J* = , 2H), 3.42 (b, 2H), 3.26 (b, 2H); ^{13}C NMR (*DMSO* d_6, 100 MHz) δ 166.0, 165.3, 164.6, 164.5, 158.8, 158.5, 151.6, 149.1, 146.8, 144.1, 117.7, 114.7, 47.3, 45.8, 44.7, 44.6, 41.2, 40.1; DEPT-135 (*DMSO* d_6, 100 MHz) δ 146.7, 146.6, 144.1, 47.2, 45.8, 44.7, 44.6, 41.2, 39.8, 39.7, 39.3; HRMS mass calculated for $C_{22}H_{26}N_{14}O_4$, 573.2159 $[M+Na]^+$. found: 573.2110; Elemental analysis. found: C, 47.07; H, 4.73; N, 34.52. calcd: C, 48.00; H, 4.76; N, 35.62.

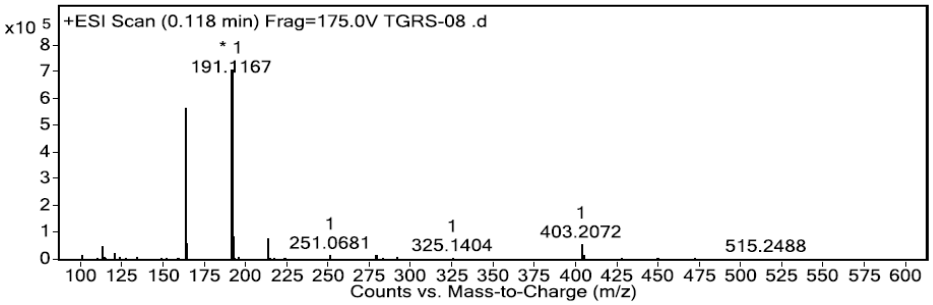
4,10-(*N,N'*)-bis(thymin-1-ylacyl) cyclo(*aeg-aeg*) (4m): Isolated as white powder; yield 72%; ^{1}H NMR (*DMSO* d_6, 400 MHz) δ 11.27 (s, 1H), 11.26 (s, 1H), 8.15 (s, 1H), 8.09 (s, 1H), 7.36 (s, 1H), 7.32 (s, 1H), 4.61 (s, 2H), 4.58 (s, 2H), 4.07 (s, 2H), 3.93 (s, 2H), 3.63-3.66 (t, 2H, *J* = 5.2), 3.58-3.60 (t, 2H, *J* = 4.8), 3.29 (b, 2H), 3.18 (b, 2H), 1.74 (s, 6H); ^{13}C NMR (*DMSO* d_6, 100 MHz) δ 166.1, 165.5, 165.4, 164.3, 151.0, 142.1, 142.0, 108.0, 48.1, 47.9, 47.1, 45.7, 41.0, 11.8; DEPT (*DMSO* d_6, 100 MHz) δ 142.1, 142.0, 48.1, 47.9, 47.1, 45.7, 41.0, 39.8, 39.7, 39.5, 11.8; HRMS mass calculated for $C_{20}H_{24}N_8O_8$, 555.1878 $[M+Na]^+$. found: 555.1891; Elemental analysis. found: C, 66.23; H, 5.15; N, 13.02. calcd: C, 66.19; H, 6.25; N, 12.87.

2.9 Appendix

^{1}H and ^{13}C NMR and Mass (HRMS) spectra.







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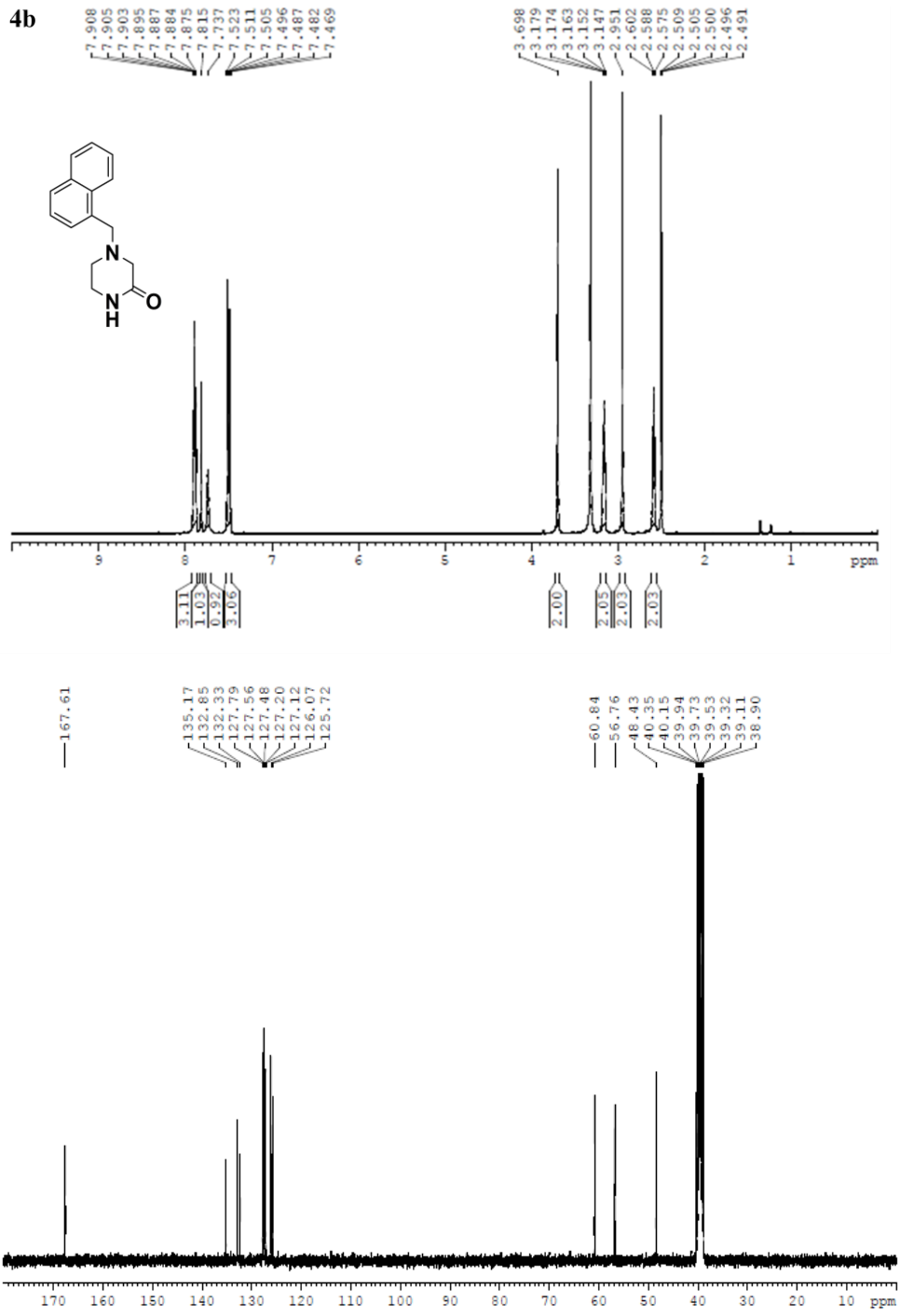
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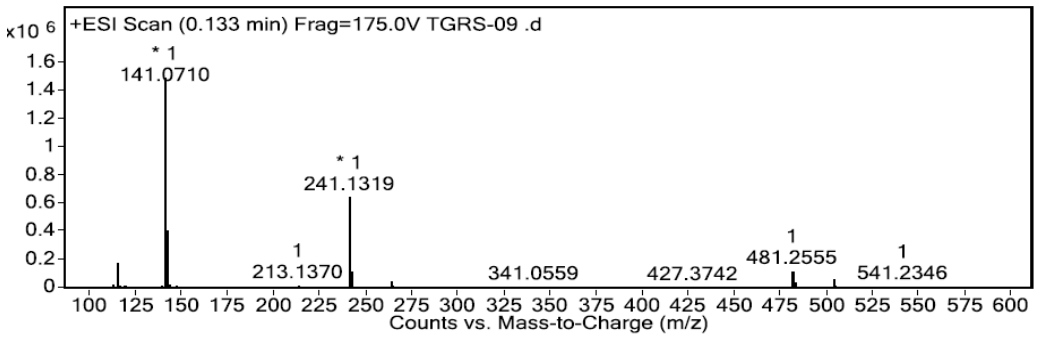
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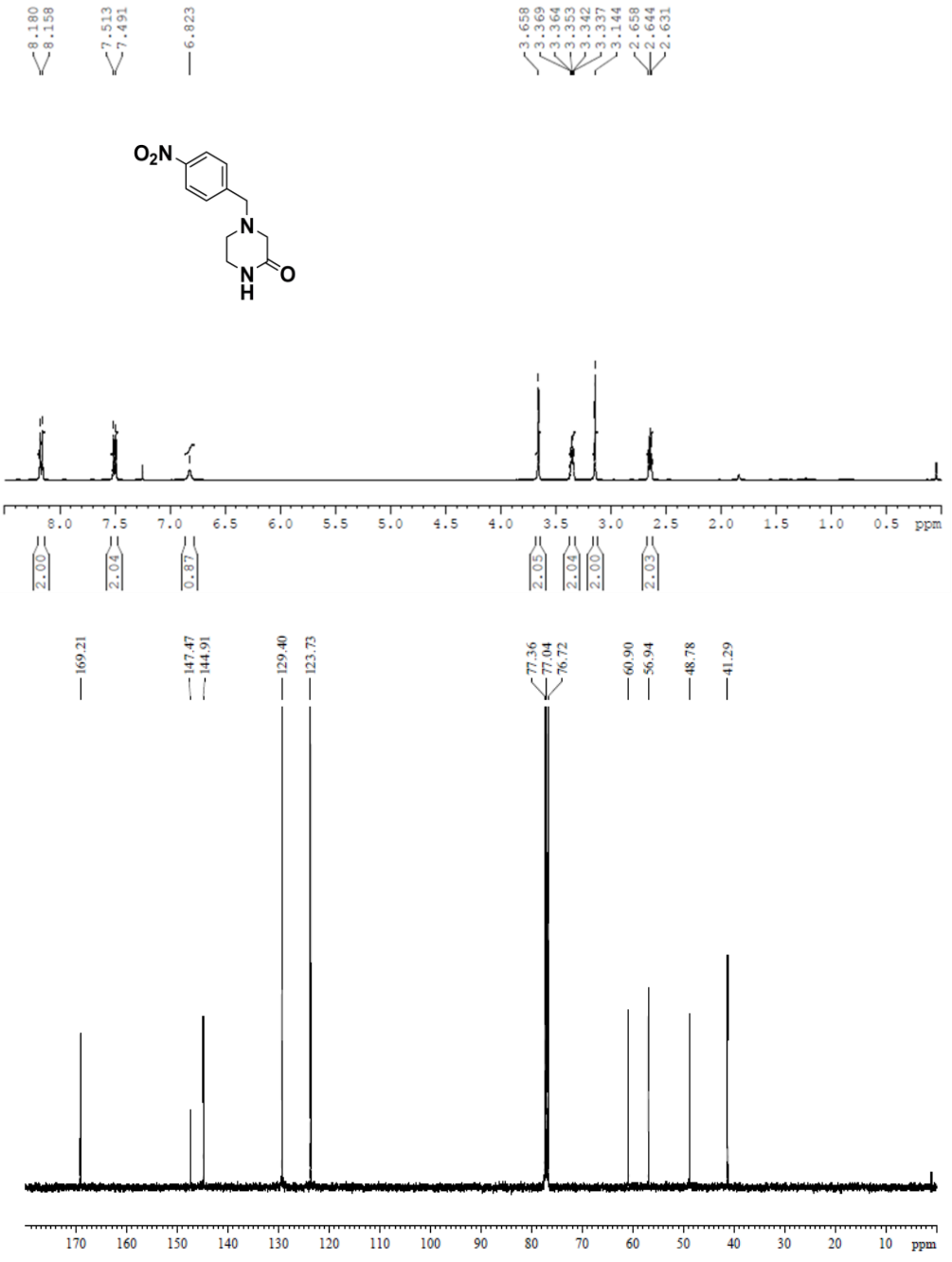
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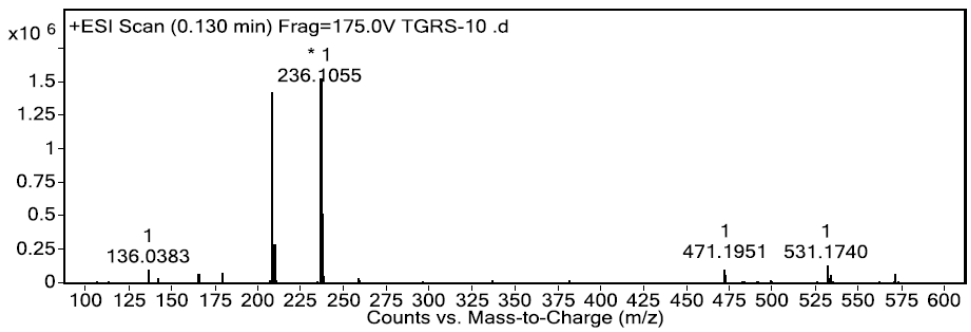
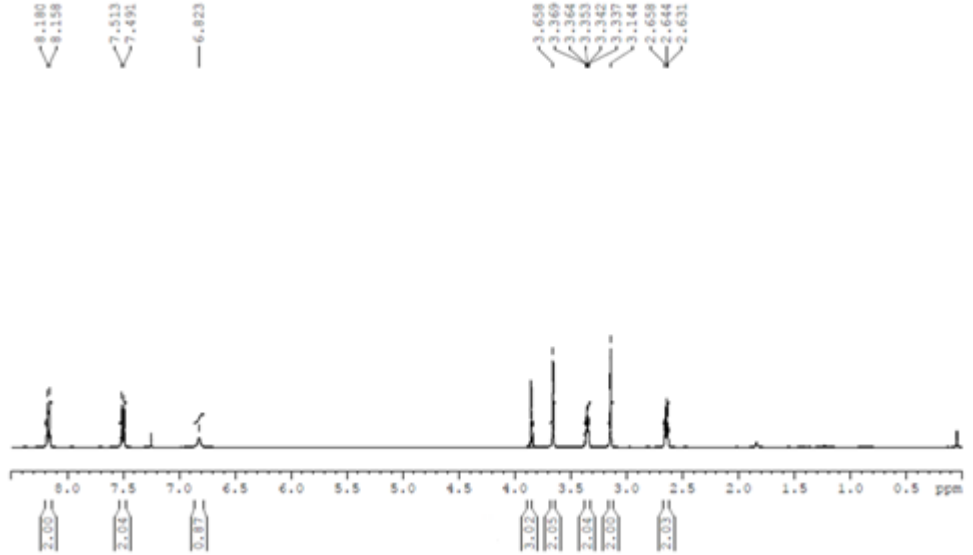
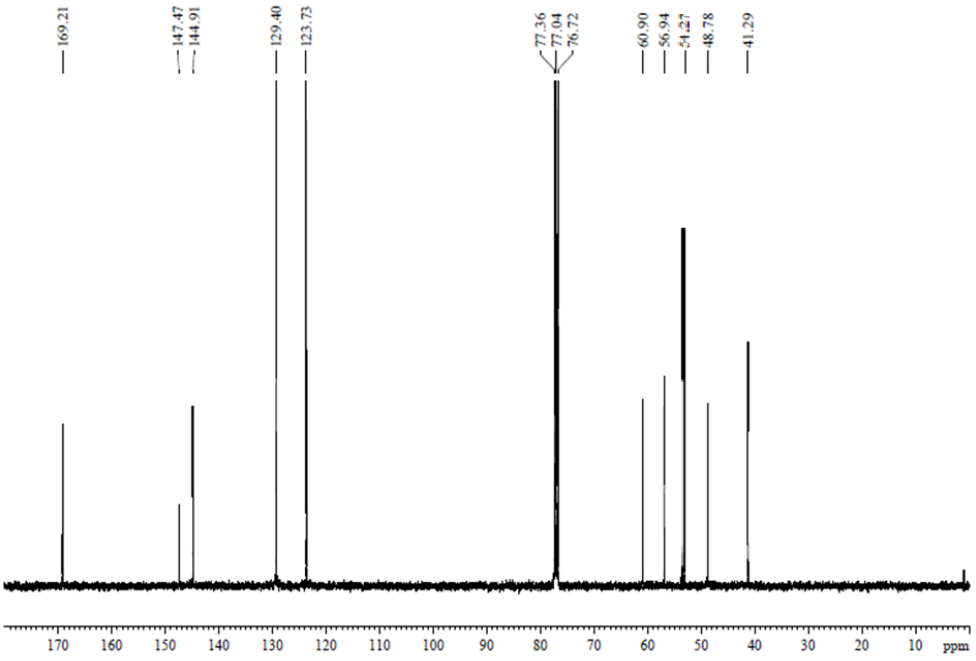
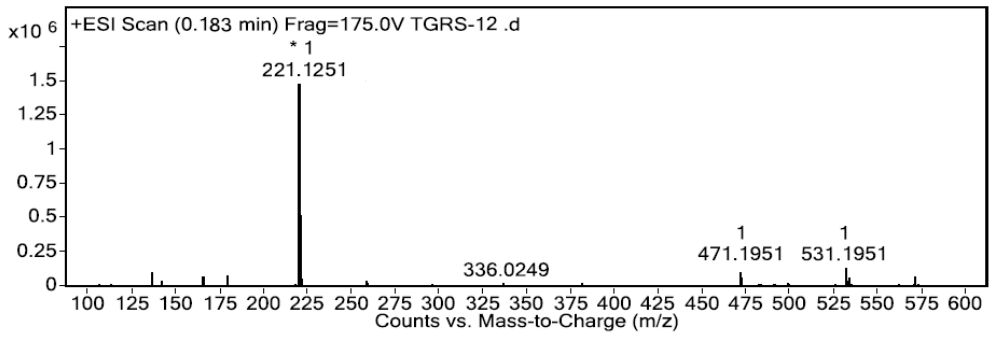
41.29
ppm

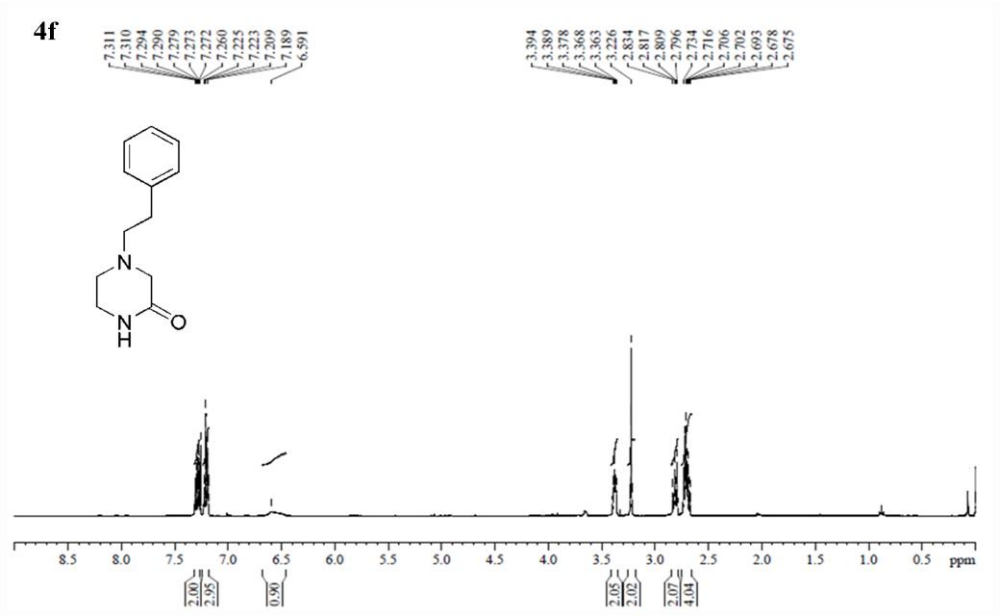

x10 6
+ESI Scan (0.130 min) Frag=175.0V TGRS-10 .d
* 1
236.1055
1
136.0383
1
471.1951
1
531.1740
Counts vs. Mass-to-Charge (m/z)

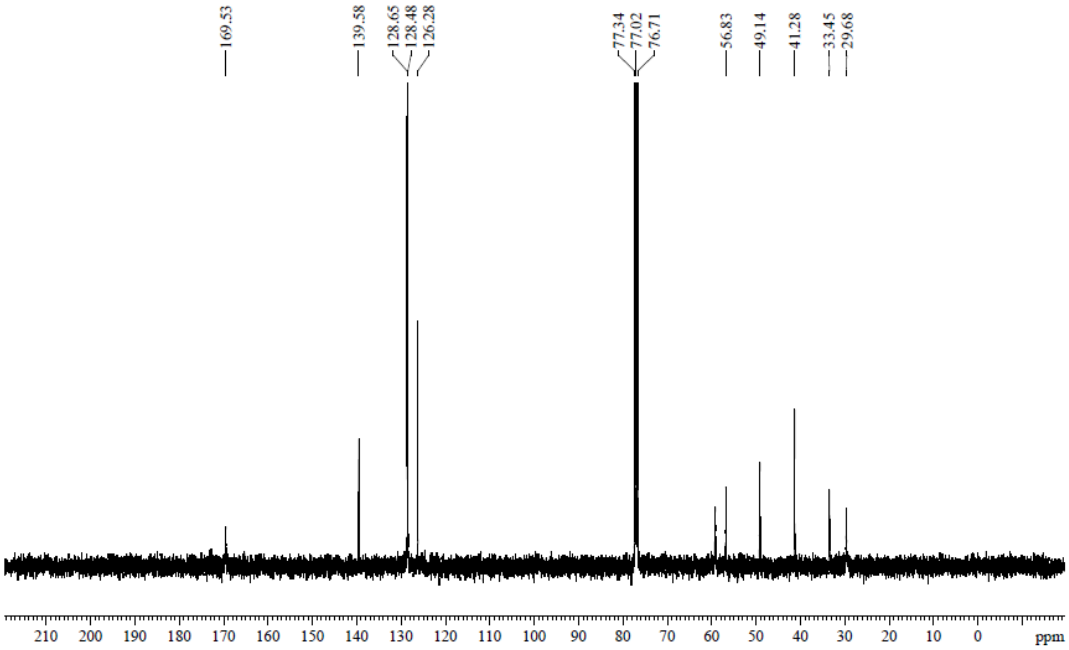
4e

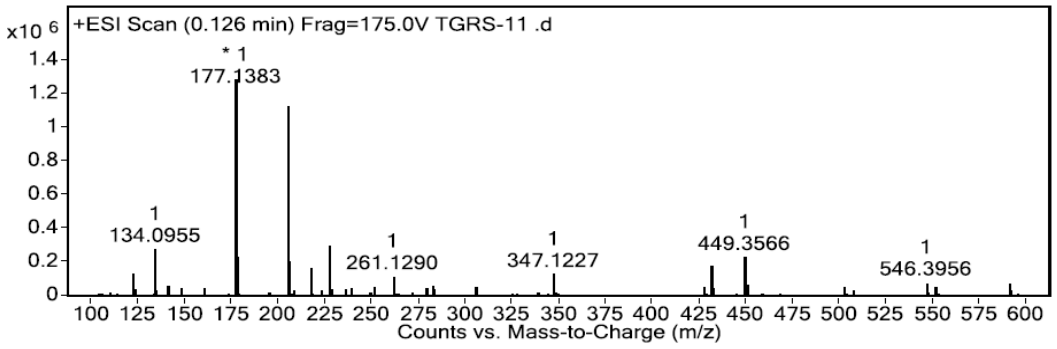

8.180
8.159
7.513
7.491
6.823
3.658
3.369
3.364
3.353
3.342
3.337
3.144
2.658
2.644
2.631
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
2.00
2.04
0.87
3.07
2.05
2.04
2.00
2.03


169.21
147.47
144.91
129.40
123.73
77.36
77.04
76.72
60.90
56.94
54.27
48.78
41.29
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

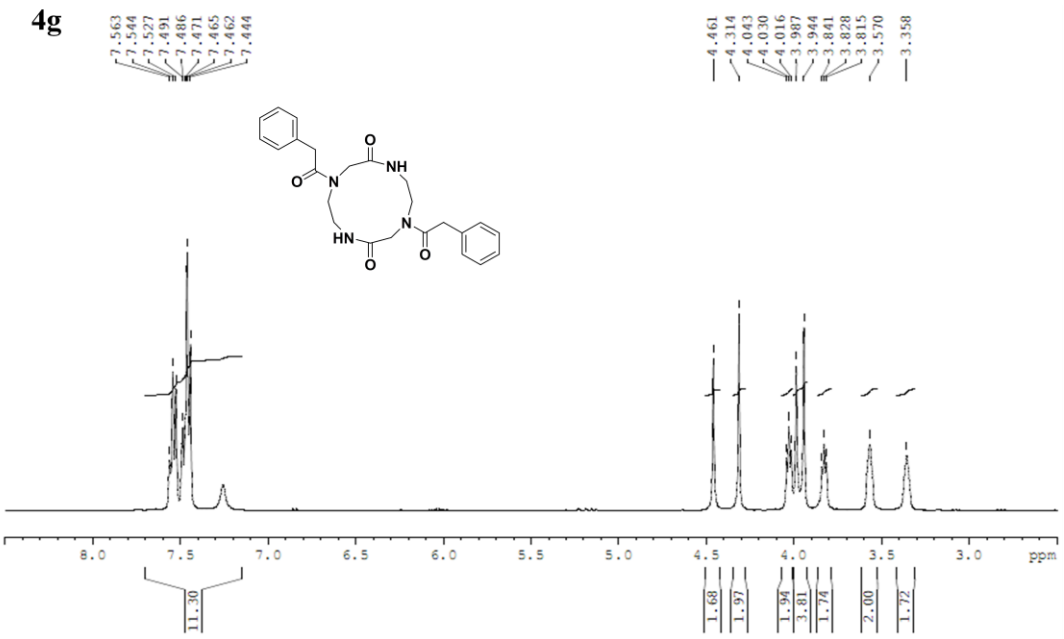

x10 6
+ESI Scan (0.183 min) Frag=175.0V TGRS-12 .d
* 1
221.1251
1.5
1.25
1
0.75
0.5
0.25
0
336.0249
1
471.1951
1
531.1951
100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550 575 600
Counts vs. Mass-to-Charge (m/z)

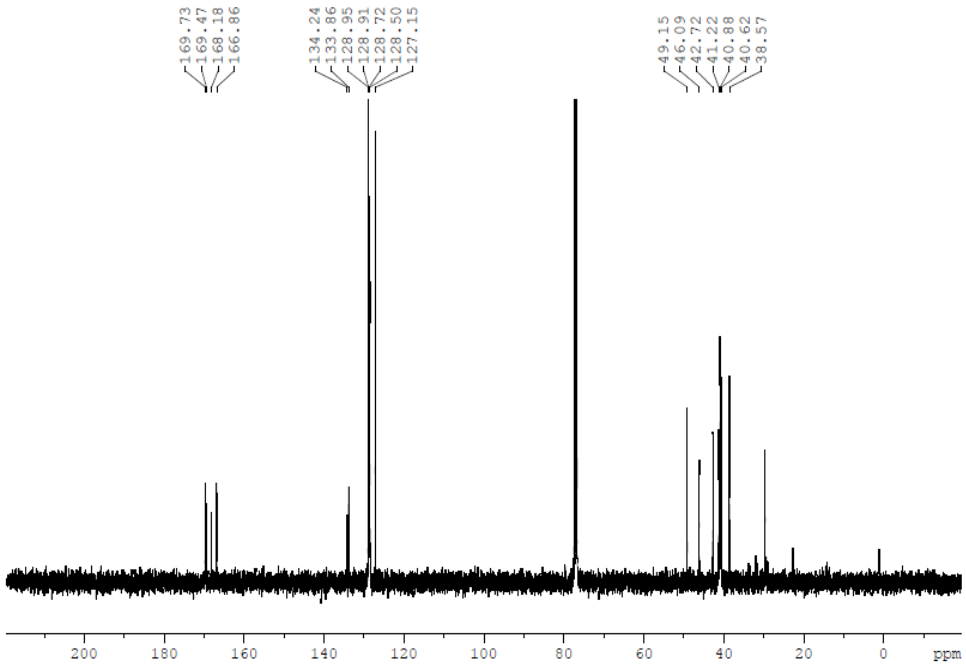

4f
7.311
7.310
7.294
7.290
7.279
7.273
7.272
7.260
7.225
7.223
7.209
7.189
6.591
3.394
3.389
3.378
3.368
3.363
3.226
2.834
2.817
2.809
2.796
2.734
2.716
2.706
2.702
2.693
2.678
2.675
N
N
H
O
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
2.00
2.95
0.90
2.05
2.02
2.07
4.04

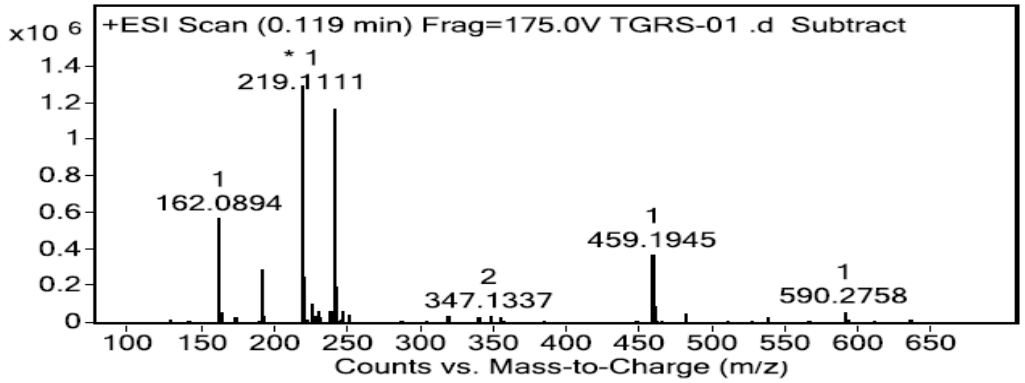

169.53
139.58
128.65
128.48
126.28
77.34
77.02
76.71
56.83
49.14
41.28
33.45
29.68
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

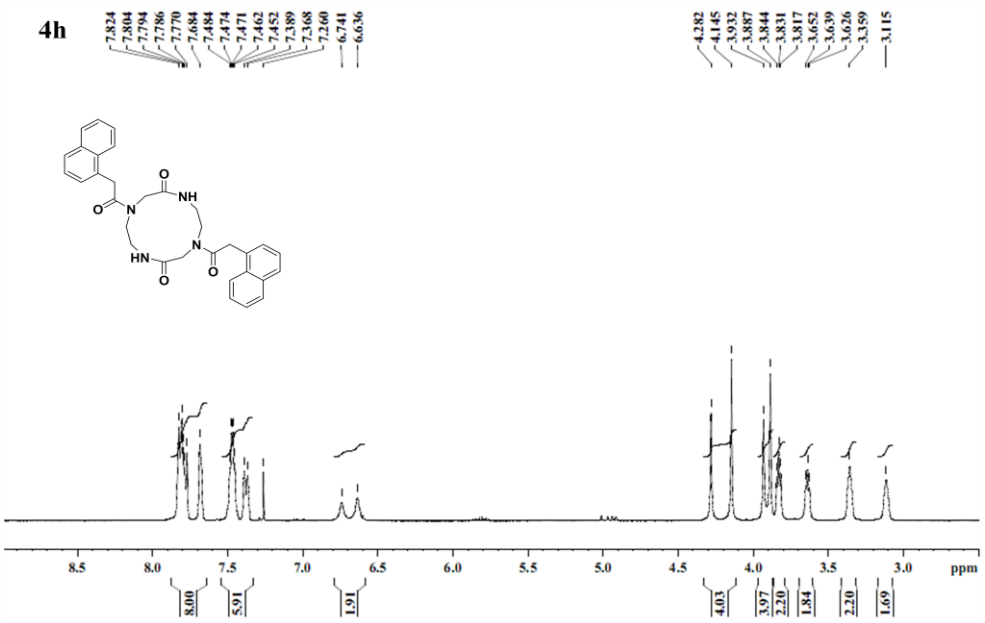

x10 6
+ESI Scan (0.126 min) Frag=175.0V TGRS-11 .d
1.4
1.2
1
0.8
0.6
0.4
0.2
0
* 1
177.1383
1
134.0955
1
261.1290
1
347.1227
1
449.3566
1
546.3956
100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550 575 600
Counts vs. Mass-to-Charge (m/z)

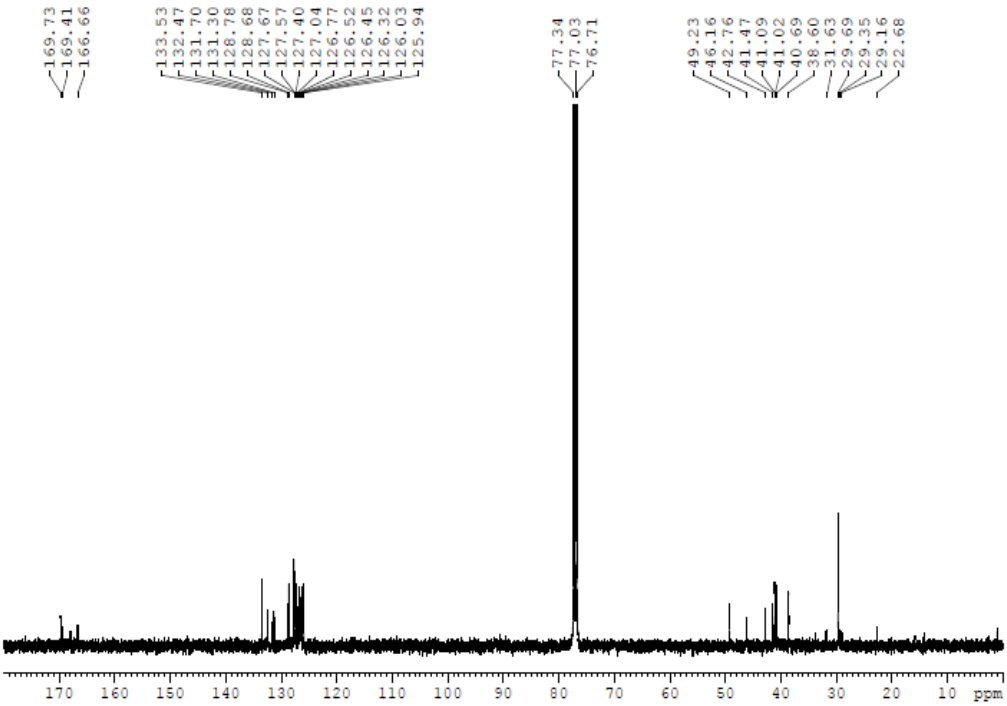
4g

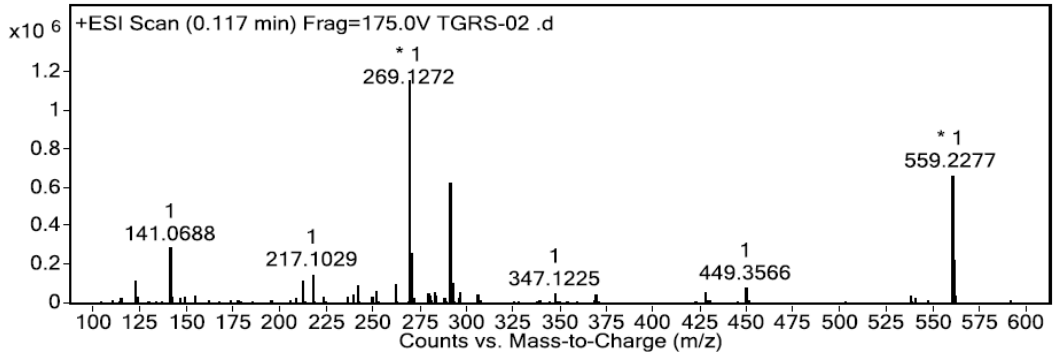

7.563
7.544
7.527
7.491
7.486
7.471
7.465
7.462
7.444
4.461
4.314
4.043
4.030
4.016
3.987
3.944
3.841
3.828
3.815
3.570
3.358
O
NH
N
HN
O
O
O
N
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm
11.30
1.68
1.97
1.94
3.81
1.74
2.00
1.72


169.73
169.47
168.18
166.86
134.24
133.86
128.95
128.91
128.72
128.50
127.15
49.15
46.09
42.72
41.22
40.88
40.62
38.57
200
180
160
140
120
100
80
60
40
20
0
ppm

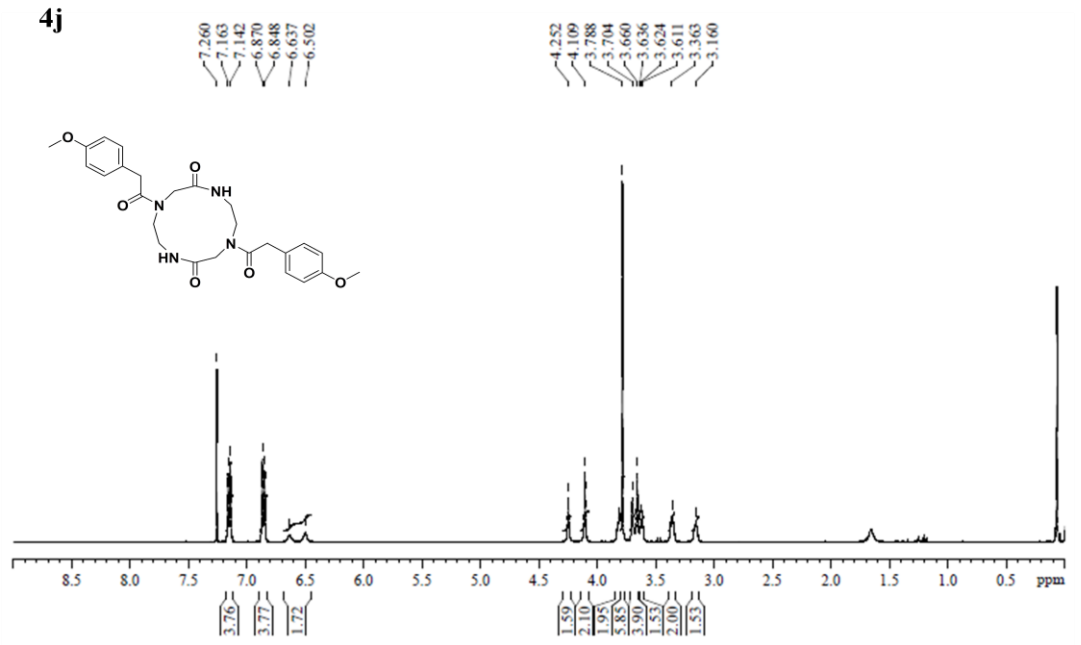

x10 6
+ESI Scan (0.119 min) Frag=175.0V TGRS-01 .d Subtract
* 1
219.1111
1
162.0894
1
459.1945
2
347.1337
1
590.2758
1.4
1.2
1
0.8
0.6
0.4
0.2
0
100
150
200
250
300
350
400
450
500
550
600
650
Counts vs. Mass-to-Charge (m/z)

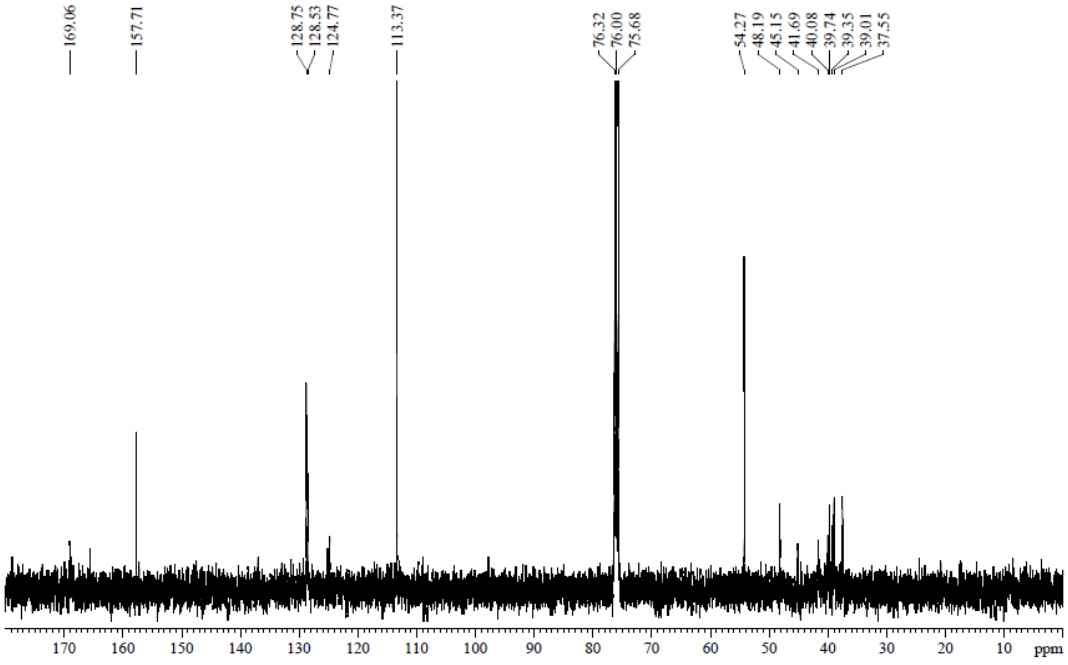

4h
7.824
7.804
7.794
7.786
7.770
7.684
7.484
7.474
7.471
7.462
7.452
7.389
7.368
7.260
6.741
6.636
4.282
4.145
3.932
3.887
3.844
3.831
3.817
3.652
3.639
3.626
3.359
3.115
NH
HN
8.00
5.91
1.91
4.03
3.97
2.20
1.84
2.20
1.69
ppm

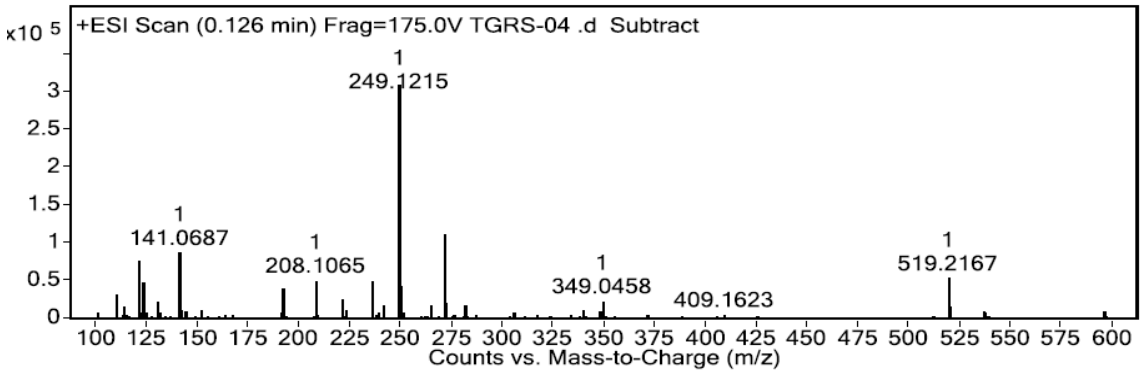

169.73
169.41
166.66
133.53
132.47
131.70
131.30
128.78
128.68
127.67
127.57
127.40
127.04
126.77
126.52
126.45
126.32
126.03
125.94
77.34
77.03
76.71
49.23
46.16
42.76
41.47
41.09
41.02
40.69
38.60
31.63
29.69
29.35
29.16
22.68
ppm

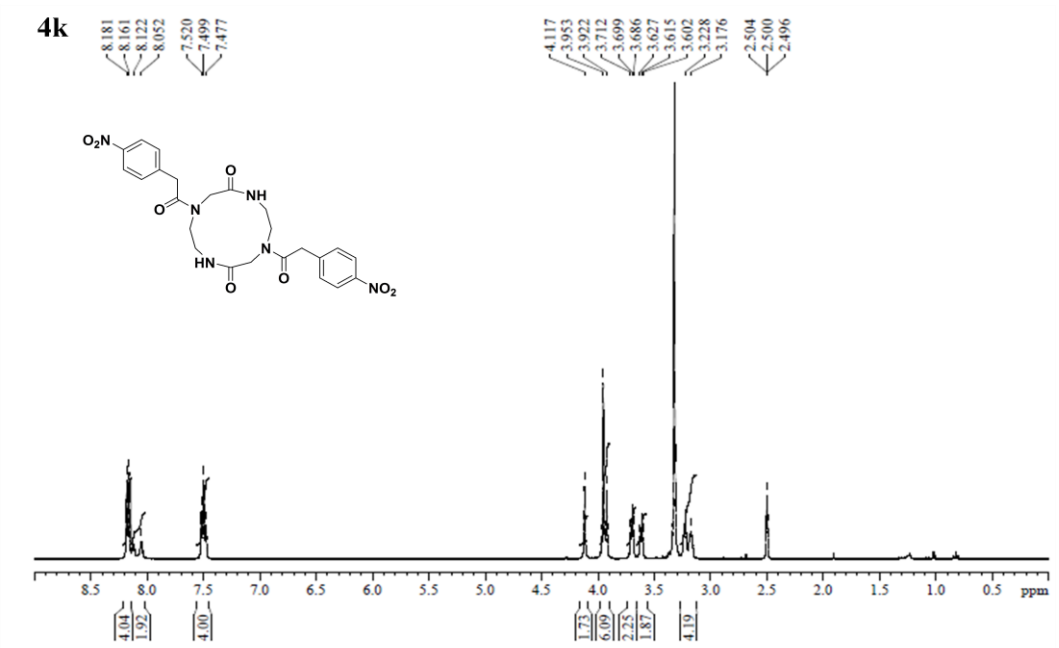

x10 6
+ESI Scan (0.117 min) Frag=175.0V TGRS-02 .d
* 1
269.1272
* 1
559.2277
1
141.0688
1
217.1029
1
347.1225
1
449.3566
Counts vs. Mass-to-Charge (m/z)

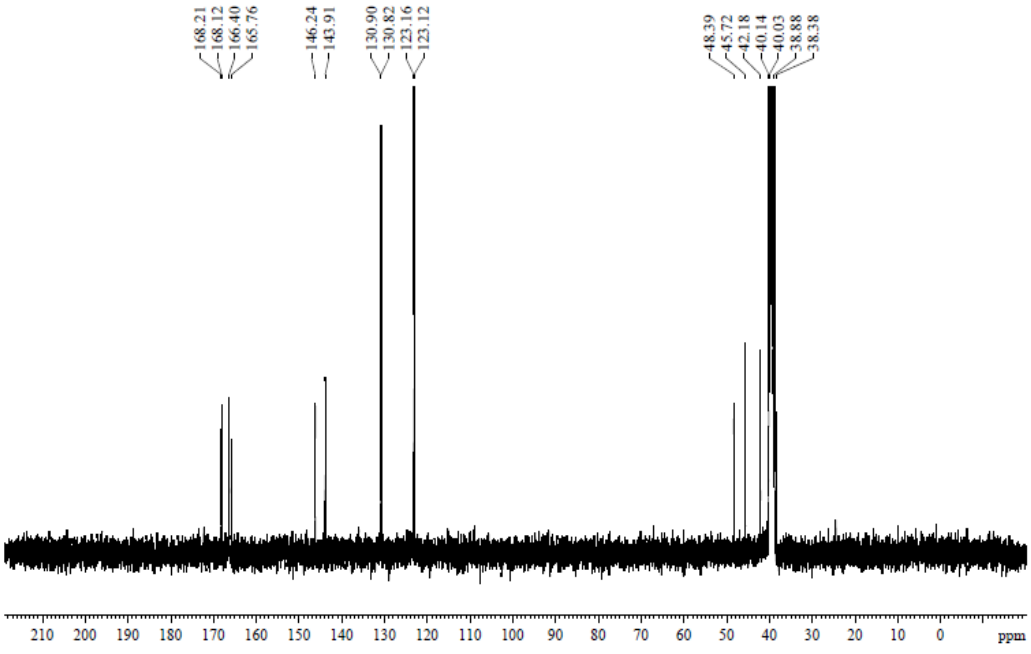
4j

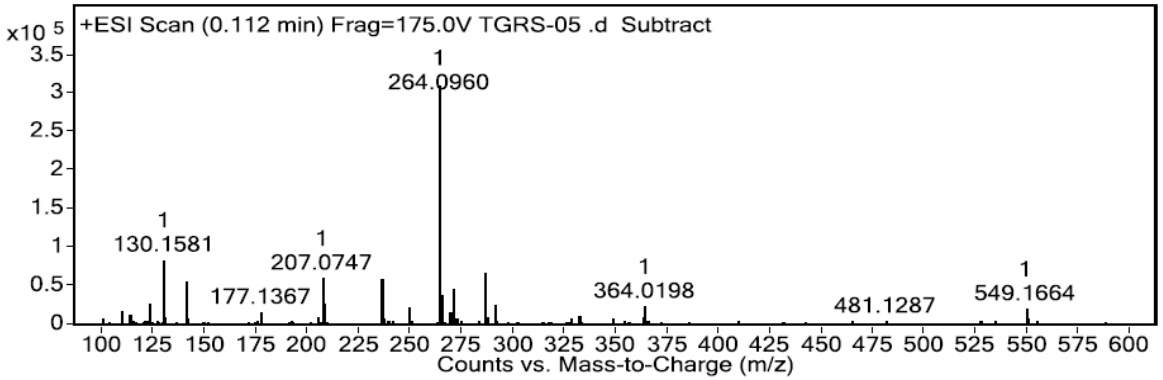

7.260
7.163
7.142
6.870
6.848
6.637
6.502
4.252
4.109
3.788
3.704
3.660
3.636
3.624
3.611
3.363
3.160
O
NH
N
HN
O
O
O
O
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
3.76
3.77
1.72
1.59
2.10
1.95
5.85
3.90
1.53
2.00
1.53


169.06
157.71
128.75
128.53
124.77
113.37
76.32
76.00
75.68
54.27
48.19
45.15
41.69
40.08
39.74
39.35
39.01
37.55
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

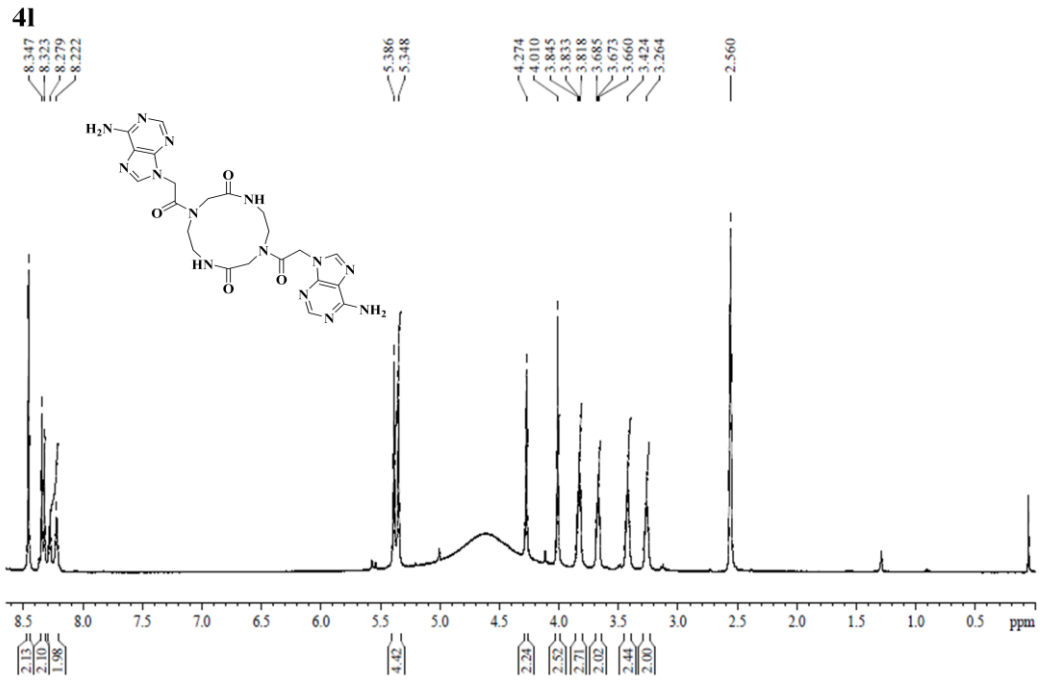

x10 5
+ESI Scan (0.126 min) Frag=175.0V TGRS-04 .d Subtract
1
249.1215
1
141.0687
1
208.1065
1
349.0458
409.1623
1
519.2167
3
2.5
2
1.5
1
0.5
0
100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550 575 600
Counts vs. Mass-to-Charge (m/z)

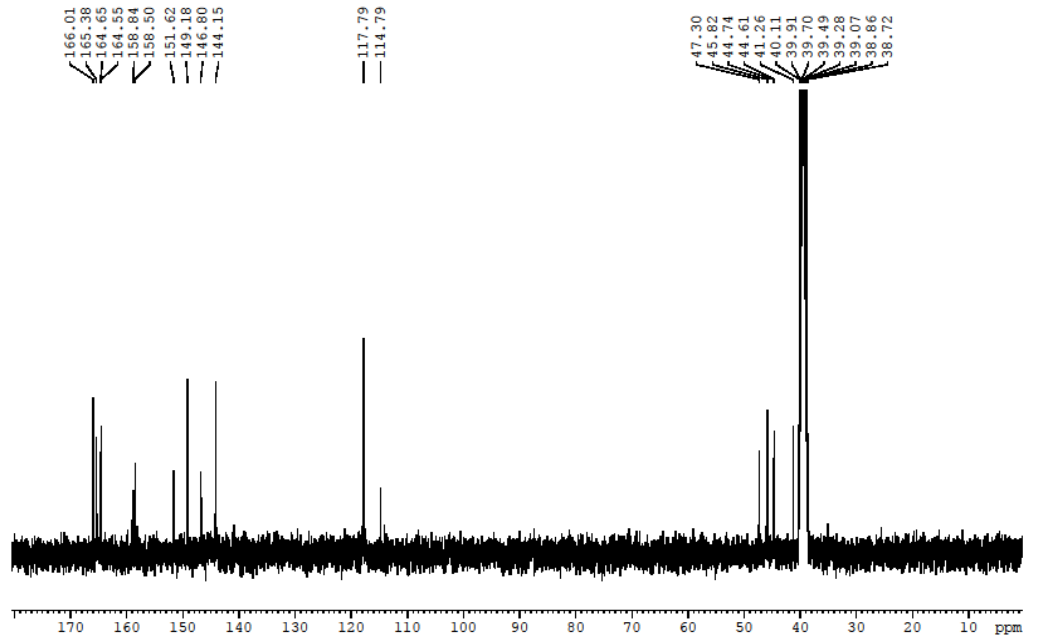

4k
8.181
8.161
8.122
8.052
7.520
7.499
7.477
4.117
3.953
3.922
3.712
3.699
3.686
3.627
3.615
3.602
3.228
3.176
2.504
2.500
2.496
O2N
NO2
NH
HN
ppm

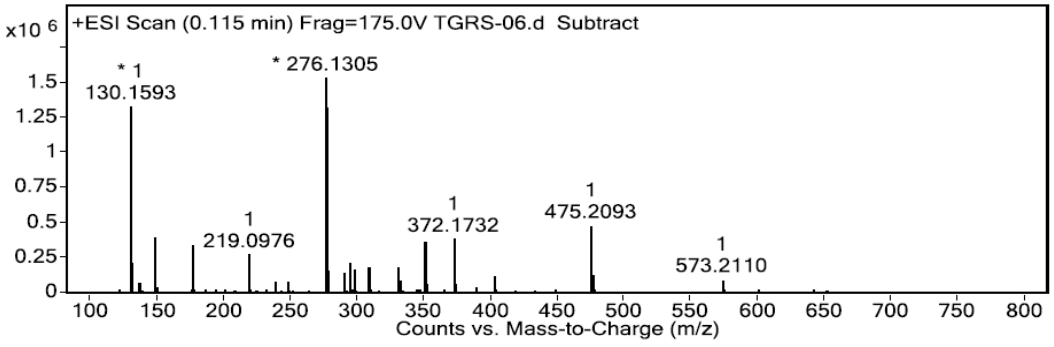

168.21
168.12
166.40
165.76
146.24
143.91
130.90
130.82
123.16
123.12
48.39
45.72
42.18
40.14
40.03
38.88
38.38
ppm


+ESI Scan (0.112 min) Frag=175.0V TGRS-05 .d Subtract
x10 5
264.0960
130.1581
207.0747
177.1367
364.0198
481.1287
549.1664
Counts vs. Mass-to-Charge (m/z)

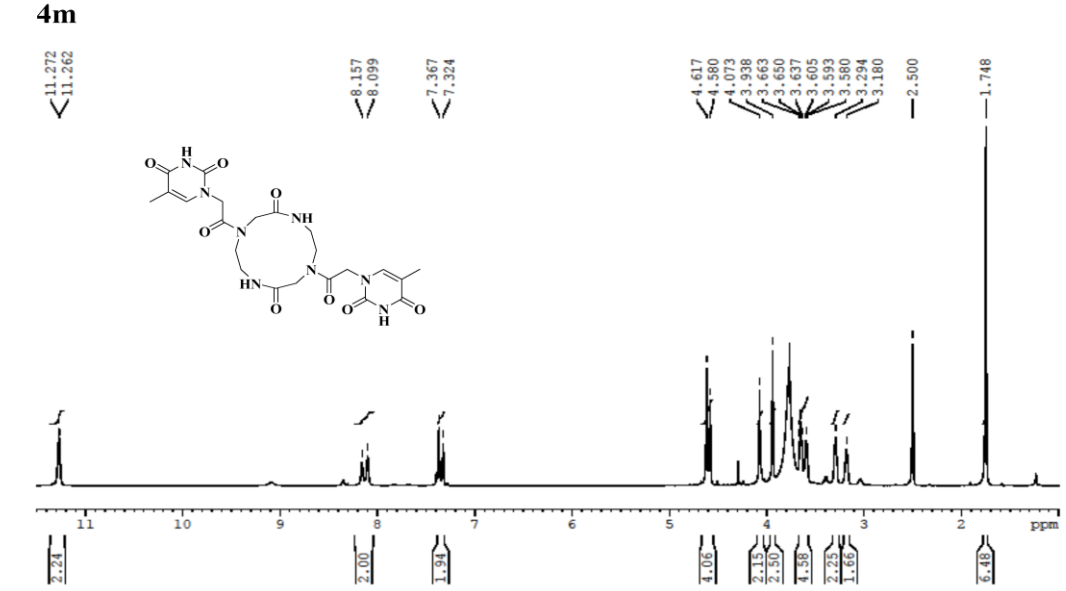
4l

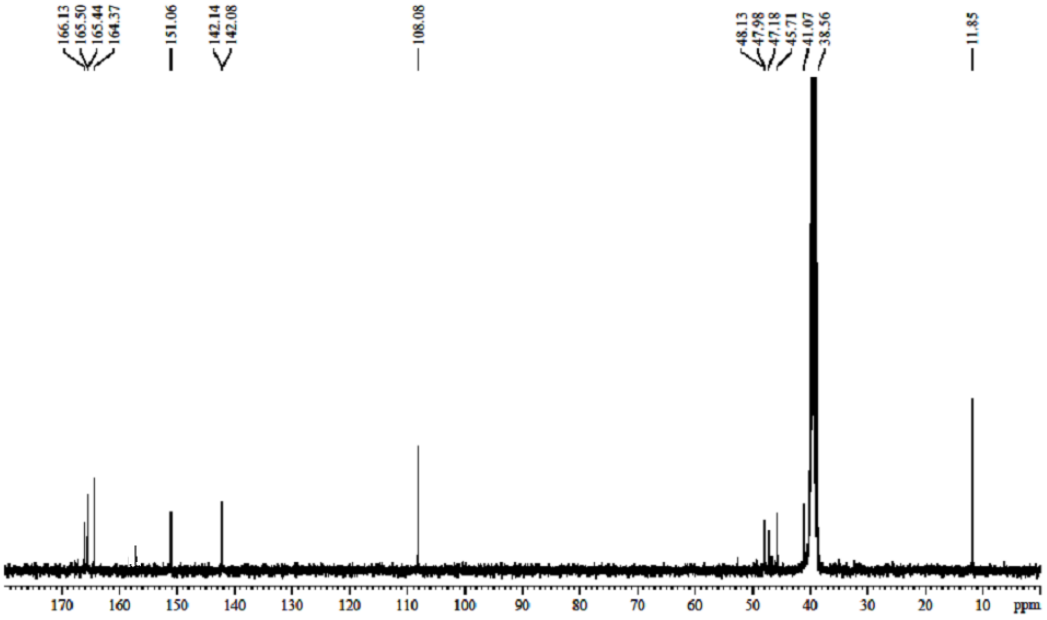

8.347
8.323
8.279
8.222
5.386
5.348
4.274
4.010
3.845
3.833
3.818
3.685
3.673
3.660
3.424
3.264
2.560
ppm

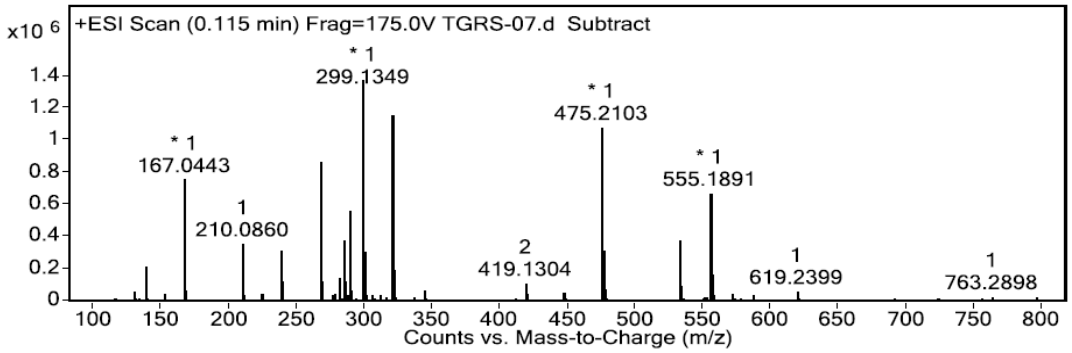

166.01
165.38
164.65
164.55
158.84
158.50
151.62
149.18
146.80
144.15
117.79
114.79
47.30
45.82
44.74
44.61
41.26
40.11
39.91
39.70
39.49
39.28
39.07
38.86
38.72
ppm


x10 6
+ESI Scan (0.115 min) Frag=175.0V TGRS-06.d Subtract
* 1
130.1593
* 276.1305
1
219.0976
1
372.1732
1
475.2093
1
573.2110
Counts vs. Mass-to-Charge (m/z)

4m


11.272
11.262
8.157
8.099
7.367
7.324
4.617
4.580
4.073
3.938
3.663
3.650
3.637
3.605
3.593
3.580
3.294
3.180
2.500
1.748
ppm
2.24
2.00
1.94
4.06
2.15
2.50
4.58
2.25
1.66
6.48


166.13
165.50
165.44
164.37
151.06
142.14
142.08
108.08
48.13
47.98
47.18
45.71
41.07
38.56
11.85
ppm


x10 6
+ESI Scan (0.115 min) Frag=175.0V TGRS-07.d Subtract
* 1
299.1349
* 1
475.2103
* 1
555.1891
* 1
167.0443
1
210.0860
2
419.1304
1
619.2399
1
763.2898
Counts vs. Mass-to-Charge (m/z)

2.10 References

(1) (a) Berg, B., Ellis, J., Dobson, C., Effects of macromolecular crowding on protein folding and aggregation *The EMBO Journal.* **1999**, *18*, 6927–6933. (b) Ojeda-May, P.; Garcia, M. E., Electric field-driven disruption of a native beta-sheet protein conformation and generation of a helix-structure *Biophys. J.* **2010**, *99*, 595- 604.

(2) (a) Aldrich, J. V.; Senadheera, S. N.; Ross, N. C.; Ganno, M. L.; Eans, S. O.; McLaughlin, J. P., The Macrocyclic peptide natural product cj-15, 208 is orally active and prevents reinstatement of extinguished cocaine-seeking behavior. *J. Nat. Prod.* 2013, DOI: 10.1021/np300697k (b) Thorstholm, L.; Craik, D. J., Unnatural amino acids for the study of chemokine receptor structure and dynamics *Drug Discovery Today: Technologies* **2012**, *9*, e13-e22.

(3) (a) Todorov, P. T.; Deacon, M.; Tisdale, M. J., Structural Analysis of a Tumor-produced Sulfated Glycoprotein Capable of Initiating Muscle Protein Degradation *J. Biol. Chem.* **1997**, *272*, 12279-12285.

(4) Knappe, T. A.; Linne, U.; Zirah, S. v.; Rebuffat, S.; Xie, X.; Marahiel, M. A., Isolation and Structural Characterization of Capistruin, a Lasso Peptide Predicted from the Genome Sequence of Burkholderia thailandensis E264. *J. Am. Chem. Soc.* **2008,** *130*, 11446-11454.

(5) Zarembinski, T. I.; Kim, Y.; Peterson, K.; Christendat, D.; Dharamsi, A.; Arrowsmith, C. H.; Edwards, A. M.; Joachimiak, A., Deep trefoil knot implicated in RNA binding found in an archaebacterial protein. *Proteins: Structure, Function, and Bioinformatics* **2003,** *50*, 177-183.

(6) (a) Barron, A. E.; Zuckermann, R. N., Bioinspired polymeric materials: in-between proteins and plastics *Curr. Opin. Chem. Biol.* **1999**, *3*, 681-687. (b) Kirshenbaum, K.;

Zuckermann, R. N.; Dill, K. A., Designing polymers which mimic biomolecules *Curr. Opin. Struct. Biol*. **1999**, *9*, 530-535.

(7) (a) Frackenpohl, J.; Arvidsson, P. I.; Schreiber, J. V.; Seebach, D. The outstanding biological stability of β- and γ-peptides toward proteolytic enzymes: An *in vitro* investigation with fifteen peptidases *ChemBioChem* **2001**, *2*, 445-455. (b) Schreiber, J.; Frackenpohl, J.; Moser,F.; Fleischmann, T.; Kohler, H. P.; Seebach, D. *ChemBioChem*. **2002**, *3*,424-432.

(8) (a) Gellman, S. H., Foldamers: A manifesto *Acc. Chem. Res.* **1998**, *31*, 173-180. (b) Price, J.L.; Horne, W. S.; Gellman, S. H., Discrete heterogeneous quaternary structure formed by α/β-peptide foldamers and α-peptides *J. Am. Chem. Soc.* **2007**, *129*, 6376-6377.

(9) Wipf, P., Synthetic Studies of Biologically Active Marine Cyclopeptides. *Chem. Rev.* **1995,** *95*, 2115-2134.

(10) Shin, S. B. Y.; Yoo, B.; Todaro, L. J.; Kirshenbaum, K., Cyclic Peptoids. J. Am. Chem. Soc. **2007**, 129, 3218-3225. (b) Fowler, S. A.; Stacy, D. M.; Blackwell, H. E., Design and synthesis of macrocyclic peptomers as mimics of a quorum sensing signal from Staphylococcus aureus Org. Lett. **2008**, 10, 2329-2332. (c) Seo, J.; Barron, A. E.; Zuckermann, R. N., Novel Peptoid Building Blocks: Synthesis of Functionalized Aromatic Helix-Inducing Submonomers. Org. Lett. **2010**, 12, 492-495.

(11) Roy, O.; Faure, S.; Thery, V.; Didierjean, C.; Taillefumier, C., Cyclic β-Peptoids. *Org. Lett.* **2008,** *10*, 921-924.

(12) Wu, H.; Niu, Y.; Padhee, S.; Wang, R. E.; Li, Y.; Qiao, Q.; Bai, G.; Cao, C.; Cai, J., Design and synthesis of unprecedented cyclic [gamma]-AApeptides for antimicrobial development. *Chem. Sci.* **2012,** *3*, 2570-2575.

(13) (a) White, C. J.; Yudin, A. K., Contemporary strategies for peptide macrocyclization *Nat. Chem*. **2011**, *3*, 509–524; (b) Pitsinos, E. N.; Vidali, V. P.; Couladouros, E. A., Diaryl Ether Formation in the Synthesis of Natural Products *Eur. J. Org. Chem.* **2011**, *17*, 1207–1222.

(14) Khan, S. N.; Kim, A.; Grubbs, R. H.; Kwon, Y.-U., Ring-Closing Metathesis Approaches for the Solid-Phase Synthesis of Cyclic Peptoids. *Org. Lett.* **2011,** *13*, 1582-1585.

(15) Chirayil, S.; Luebke, K. J., Cyclization of peptoids by formation of boronate esters. *Tetrahedron Lett.* **2012,** *53*, 726-729.

(16) Cheguillaume, A.; Lehardy, F.; Bouget, K.; Baudy-Floc'h, M.; Le Grel, P. *J. Org. Chem.* **1999**, *64*, 2924-2930; (b) Niu, Y.; Bai, G.; Wu, H.; Wang, R. E.; Qiao, Q.; Padhee, S.; Buzzeo, R.; Cao, C.; Cai, J., Cellular Translocation of a γ-AApeptide Mimetic of Tat Peptide. *Molecular Pharmaceutics* **2012,** *9*, 1529-1534.

(17) Susmuth, R.; Muller, J.; von Dohren, H.; Molnar, I., Fungal cyclooligomer depsipeptides: From classical biochemistry to combinatorial biosynthesis *Nat. Prod. Rep.* **2011**, *28*, 99-124.

(18) Van Regenmortel, M. H. V.; Muller, S., D-peptides as immunogens and diagnostic reagents *Curr. Opin. Biotechnol.* **1998**, *9*, 377-392.

(19) Godballe, T.; Nilsson, L. L.; Petersen, P. D.; Jenssen, H., Antimicrobial β-peptides and α-peptoids *Chem. Biol.Drug Des* **2011**, *77*, 107-119.

(20) Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H. *J. Am. chem. Soc.* **1992**, *114*, 9677. (b Nelson, K. E.; Levy, M.; Miller, S. L., Peptide nucleic acids rather than RNA may have been the first genetic molecule *Proc. Natl. Acad. Sci.* **2000,** *97*, 3868-3871.

(21) Nielsen, P.; Egholm, M.; Berg, R.; Buchardt, O., Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. *Science* **1991,** *254*, 1497-1500.

(22) Pace, V.; Martínez, F.; Fernández, M.; Sinisterra, J. V.; Alcántara, A. R., Effective Monoallylation of Anilines Catalyzed by Supported KF *Org. Lett.* **2007**, *9*, 2661-2668.

(23) Kaur, N.; Zhou, B.; Breitbeil, F.; Hardy, K.; Kraft, K. S.; Trantcheva, I.; Phanstiel Iv, O., A Delineation of Diketopiperazine Self-Assembly Processes: Understanding the Molecular Events Involved in Nϵ-(Fumaroyl)diketopiperazine of l-Lys (FDKP) Interactions. *Molecular Pharmaceutics* **2008,** *5*, 294-315.

(24) Dueholm, K. L.; Egholm, M.; Behrens, C.; Christensen, L.; Hansen, H. F.; Vulpius, T.; Petersen, K. H.; Berg, R. H.; Nielsen, P. E.; Buchardt, O., Synthesis of Peptide Nucleic Acid Monomers Containing the Four Natural Nucleobases: Thymine, Cytosine, Adenine, and Guanine and Their Oligomerization. *The Journal of Organic Chemistry* **1994,** *59*, 5767-5773.

(25) Deber, C. M.; Madison, V.; Blout, E. R., Why cyclic peptides? Complementary approaches to conformations. *Acc. Chem. Res.* **1976,** *9*, 106-113.

(26) Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N., Self-assembling organic nanotubes based on a cyclic peptide architecture *Nature* **1993**, *366*, 324-327; (b) Khazanovich, N.; Granja, J. R.; McRee, D. E.; Milligan, R. A.; and Ghadiri, M. R., Nanoscale tubular ensembles with specified internal diameters. design of a self-assembled nanotube with a 13-.ANG. pore *J. Am. Chem. Soc.* **1994**, *116*, 6011-6018. (c) Ghadiri, M. R.; Granja, J. R.; Buehler, L. K., Artificial transmembrane ion channels from self-assembling peptide nanotubes *Nature* **1994**, *369*, 301-318.

(27) Cordier, F.; Grzesiek, S., Temperature-dependence of protein hydrogen bond properties as studied by high-resolution NMR. *J. Mol. Biol.* **2002,** *317*, 739-752.

(28) Cierpicki. T.;Otlewski. J., Amide proton temperature coefficients as hydrogen bond indicators in proteins *J Biomol NMR*. **2001**, *21*, 249-255.

(29) Sefler, A. M.; Lauri, G.;Bartlett P. A., A convenient method for determining cyclic peptide conformation from 1D ^{1}H-NMR information *Int. J. Pept. Protein Res.* **1996**, *48*, 129-138.

(30) Maulucci, N.; Izzo, I.; Bifulco, G.; Aliberti, A.; De Cola, C.; Comegna, D.; Gaeta, C.; Napolitano, A.; Pizza, C.; Tedesco, C.; Flot, D.; De Riccardis, F., Synthesis, structures, and properties of nine-, twelve-, and eighteen-membered N-benzyloxyethyl cyclic [small alpha]-peptoids. *Chem. Commun.* **2008,** *0*, 3927-3929.

(31) Takahashi, R.; Noguchi, T., Criteria for determining the hydrogen-bond structures of a tyrosine side chain by fourier transform infrared spectroscopy: Density functional theory analyses of model hydrogen-bonded complexes of p-Cresol. J. Phys. Chem. B **2007,** *111*, 13833-13844.

(32) Huang, K.; Wu, C. W.; Sanborn, T. J.; Patch, J. A.; Kirshenbaum, K.; Zuckermann, R. N.; Barron, A. E.; Radhakrishnan, I., A threaded loop conformation adopted by a family of peptoid nonamers. *J. Am. Chem. Soc.* **2006,** *128*, 1733-1738.

(33) Armand, P.; Kirshenbaum, K.; Goldsmith, R. A.; Farr-Jones, S.; Barron, A. E.; Truong, K. T. V.; Dill, K. A.; Mierke, D. F.; Cohen, F. E.; Zuckermann, R. N.; Bradley, E. K., NMR determination of the major solution conformation of a peptoid pentamer with chiral side chains. *Proc. Natl. Acad. Sci.* **1998,** *95*, 4309-4314.

(34) Park, S. H.; Szleifer, I., Structural and dynamical characteristics of peptoid oligomers with achiral sliphatic side chains studied by molecular dynamics simulation. *The J. Phys. Chem. B* **2011,** *115*, 10967-10975.

(35) Baldwin, J. E., Rules for ring closure *J. Chem. Commun*. **1976**, 734-736.

Chapter 3

Synthesis of peptide based potential inhibitors for β-amyloid fibrillar aggregation

Chapter-3

Synthesis of peptide based potential inhibitors for β-amyloid fibrillar aggregation

3.1 Introduction

Misfolding of proteins and transformation from their soluble form into highly insoluble amyloid deposits can lead to neurodegenerative disorders or systemic amyloidosis. Amyloid refers to extracellular proteinaceous deposits consisting of fibrils. Fibrils are oligomers of misfolded proteins or peptide fragments which are characterized by a cross β-sheet structure.[1] Until now, over 25 diseases are identified and thought to be related to the (extracellular) deposition of amyloid. Well known examples of amyloidosis are Alzheimer's disease (Aβ-peptide), Parkinson's disease (α-synuclein), Huntington's disease (poly-Gln) and type 2 diabetes mellitus (amylin).[2] Extensive studies have been performed to elucidate the mechanism of these diseases and to reveal the secondary and supramolecular structure of amyloid peptides, which are self-assembled into fibrils. These fibrillar intermediates are thought to be the cytotoxic species.[3]

β-Amyloid peptide (Aβ 1-42) is derived from the amyloid precursor protein (APP) by proteolysis.[4] The assembly and aggregation behaviour of the proteolytic products have been regarded as crucial parts to understand the pathogenesis of Alzheimer's disease. Non-crystalline nature of the amyloidal peptide fibril makes it experimentally challenging for structural analysis. The structural evidence at the molecular level is vital to obtain the insight into the mechanisms underlying the assembly structures, such as amyloid peptide conformations, configurations, and environmental effects. For Aβ 1–42, a structural model was proposed as shown in Figure 3.1. Residues 1–10 could be attributed to the structurally disordered feature, 12–24 and 30–40 fragments adopt parallel β-sheets through hydrogen bonds between peptide backbones. Fragment 25–29 acts as a bend of the peptide main chain

that connect two β-sheets by side-chain interactions.[5] The Aβ 1–42 molecules features are parallel to each other, forming lamella structures. The arrangement of peptides is nearly perpendicular to the stripe axis. The separations between two neighbouring peptide chains were measured and were comparable with normal β-sheet spacing.[6]

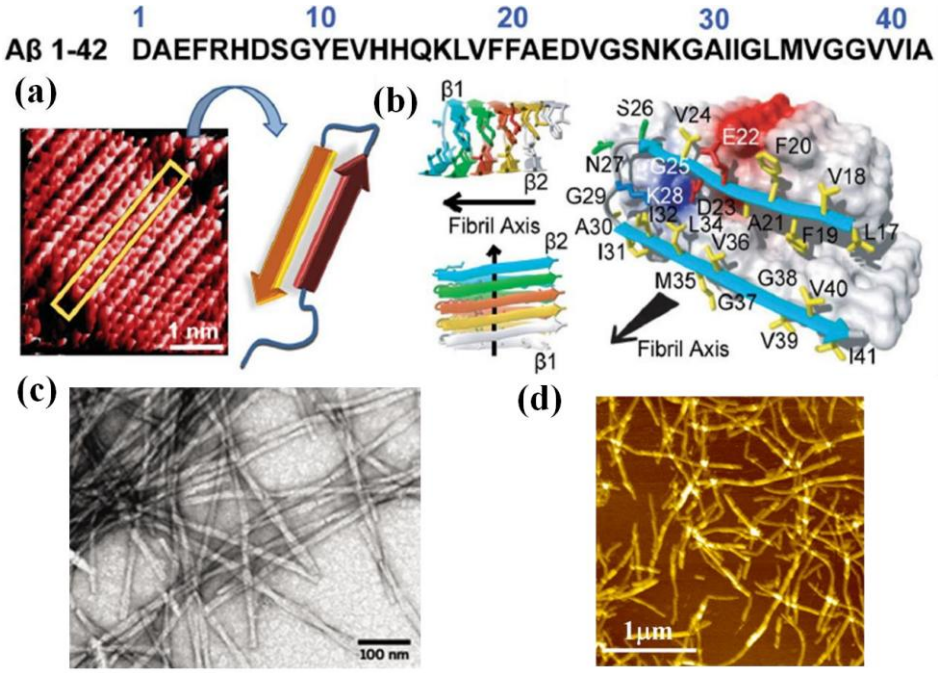


Figure 3.1 Structures of β-Amyloid peptide, (a) high resolution scanning tunnelling microscopy (STM) image of Aβ 1–42 assembly structure (b) proposed hairpin model for Aβ 1–42 (c) scanning electron microscopy (SEM) image of Aβ 1–42 fibrils and (d) atomic force microscopy (AFM) image of Aβ 1–42 fibrils.[6]

Several strategies have been used to develop potential inhibitors of peptide or protein aggregates by stabilizing the peptide or proteins in their native soluble conformations or destabilizing the altered amyloidogenic conformers.[7] Several inhibitors based on peptide and small organic molecules are reported in the literature.[8] Of which peptide based inhibitors are better candidates owing to their specificity towards the Aβ protein, where as non-peptidic inhibitors lacks Aβ specificity and their inhibiting activity is not well understood. The history of peptide based inhibitors for β-amyloid aggregation began with the pioneering work of Tjernberg, which led to the identification of β-amyloid self-recognition motif. They have synthesized 31 sequential pentapeptides and measured the affinity of radio labelled β-amyloid

(1-42) peptides towards these pentapeptides. They identified 2 distinct regions of β-amyloid that recognized complementary pentapeptides. They identified KLVFF pentapeptides as the smallest fragment with reasonable binding affinity to the self-recognition region of β-amyloid.[9] An immobilized artificial receptor, was used to measure the binding affinity of a large number of ligands including LVFFAE and KLVFFAE.[10] Initial studies suggested that ligand receptor complex prefer an anti-parallel arrangement over favoured parallel arrangement. Since then, the structure with KLVFFAE region has been explored with various experimental and computational techniques.[11] KLVFFAE is also the shortest region of β-amyloid that can form oligomers and fibrils by itself. Soto *et al.* showed that pentapeptide, LPFFD could interfere β-amyloid fibrillar aggregation in a sub-acute animal model of AD.[12]

Gordon *et al.* synthesised KLVFFAE and KLVFF and performed secondary modification of amino acids in the peptide sequence. N-methylation of alternate amino acids in KLVFFAE and KLVFF lead to enhanced β-amyloid inhibiting activity.[13] KLVFFAE has better binding affinity than KLVFF, but it is not used as inhibitor because of its self aggregating nature. The N-methylated analogue of KLVFFAE, were more potent and do not undergo self aggregation. They also found that N-methylated versions of KLVFF and KLVFFAE had better aqueous solubility and cell membrane permiablity.[14] In another approach, Findeis *et al*. used dextrorotary amino acids for synthesising KLVFF and this short peptide was more potent at inhibiting β amyloid (1-40) aggregation and cytotoxicity than KLVFF.[15] They also showed that fragments 15-20 and 16-22 of β-amyloid peptide have an anti-aggregation activity. These short peptide sequences crossed the blood-brain barrier showed therapeutic effect in animal models with Alzheimer disease (AD).

3.2 Objective of the work

The objective of this project is to synthesise peptide-hybrid molecules and use them as inhibitors of amyloid peptide aggregation. β-Amyloid (1-40) peptide fragments involved in

fibrils formation have been synthesised.[16] These peptides act as model system for Aβ 1–42 fibrils and can be used to study inhibitory activity of small peptide and organic molecules.

3.3 Design strategy

Peptide based β-amyloid inhibitors are efficient and specific compared to other classes of inhibitors. Peptide inhibitors mainly target the hydrophobic region (β-sheet) or the hydrogen bonding present in the amyloid peptides.[17] KLVFF is a well known example in literature as an inhibitor, it interferes with β-amyloid peptides β-sheet formation and prevent the fibrillization. Modifications at C or N termini with unnatural amino acids or organic moieties are explored to increase inhibition efficiency. Here we have synthesised peptide-organic hybrid systems, as β-amyloid inhibitors. We have synthesised four peptide-organic hybrid sequences with KLVFF and QKLVFFA as core peptide sequences which can act as efficient inhibitors (Figure 3.2). These core peptides are known as inhibitors, but the extent of inhibition is not effective enough to use them for practical applications. We modified the N-terminal of the peptide sequences with small organic molecules like thymine acetic acid and barbiturate acetic acid to obtain **Pep-1** (Bar-KLVFF), **Pep-2** (Thy-KLVFF), **Pep-3** (Bar-QKLVFFA) and **Pep-4** (Thy-QKLVFFA) (Figure 3.2). The peptide appended small organic have multiple hydrogen donor and acceptor sites, which can interfere with amyloid aggregation. Both the site specificity of peptide and hydrogen bond interfering ability of organic molecule can add up to show better efficiency compared to conventional peptide inhibitors.

We have also synthesised β-amyloid peptide core fragments involved in amyloid aggregation. These fragments can be incubated at 37 $^{\circ}$C to obtain fibrils similar to β-amyloid (1-40) aggregates and can be used as a model of β-amyloid system to study small organic molecules based inhibitors. The two core β-peptides synthesised are HHQKLVFFAED (Ab-1) and EVHHQKLVFFAEDVG (Ab-2) (Figure 3.3).

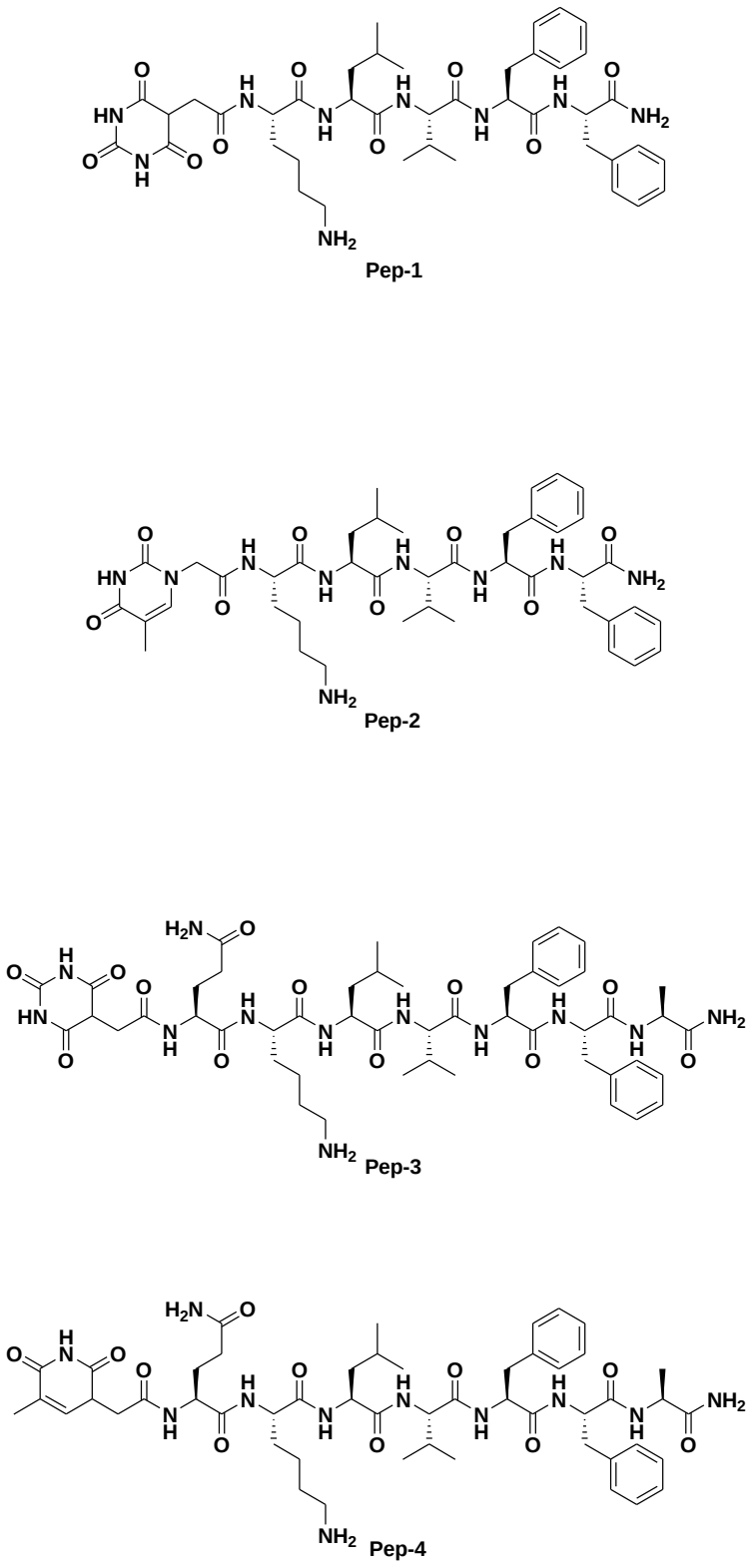
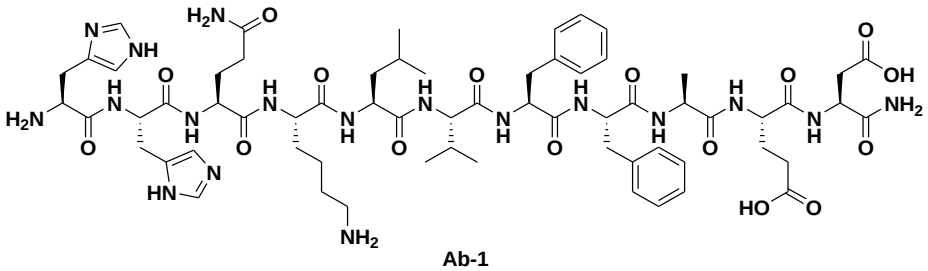
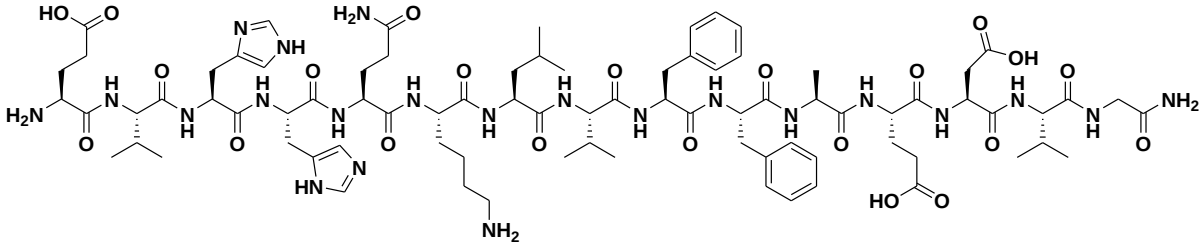


Figure 3.2 Structures of hybrid peptide with thymine and barbiturate.



Ab-1



Ab-2

Figure 3.3 Structures of β-amyloid peptide core fragments.

3.4 Results and discussion

3.4.1 Synthesis of peptides: Peptides were synthesised using solid phase chemistry protocols (Chapter 1). An automated peptide synthesizer (Syro II, MultisynTech) has been used to synthesis desired peptide sequences. First amino acid is immobilized on a solid support by a readily cleavable linkage, the desired peptide at the end is cleaved from the solid support. Here we have used Fmoc chemistry on Rink amide resin as a solid support for synthesis of peptides. Protected amino acids are used to prevent oligomerization and undesired product formation during the course of peptide synthesis, the side chain functional groups present in amino acids was protected by different orthogonal protection groups.

The resin is taken in reaction vessel and soaked in DCM for swelling (2 h) with optimal vortexing. Once the swelling is completed, resin is washed with DMF, followed by addition of 40% piperidine in DMF to deprotect Fmoc-group to obtain reactive free $–NH_2$ group on the resin. The first amino acid (4 equivalents) is coupled to the resin using coupling

reagent HBTU and non-nucleophilic base, DIPEA for 1 h with periodic vortexing. Unreacted reagents are washed off by multiple washing cycles. Then the next amino acid is deprotected (Fmoc-deprotection) with 40% piperidine in DMF, for 15 min. Cycles of coupling and deprotection were continued till the desired peptide sequence is obtained (Figure 3.4).

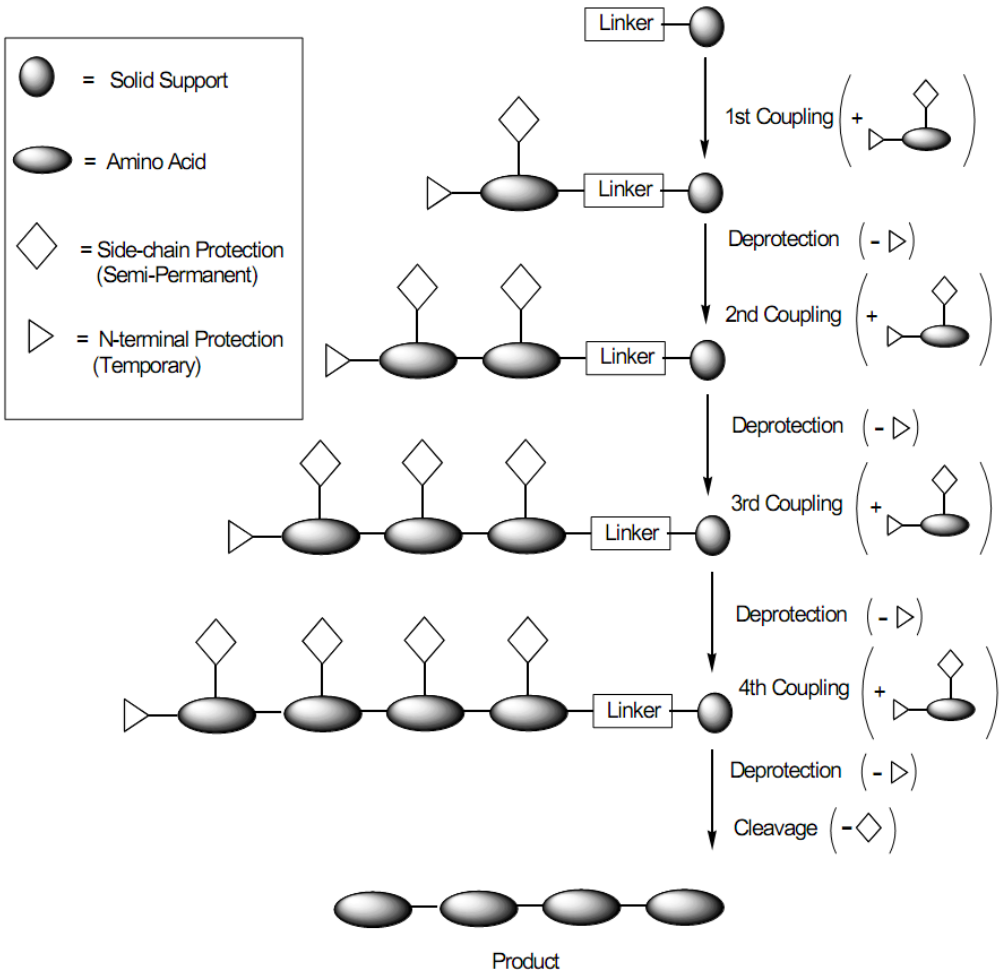
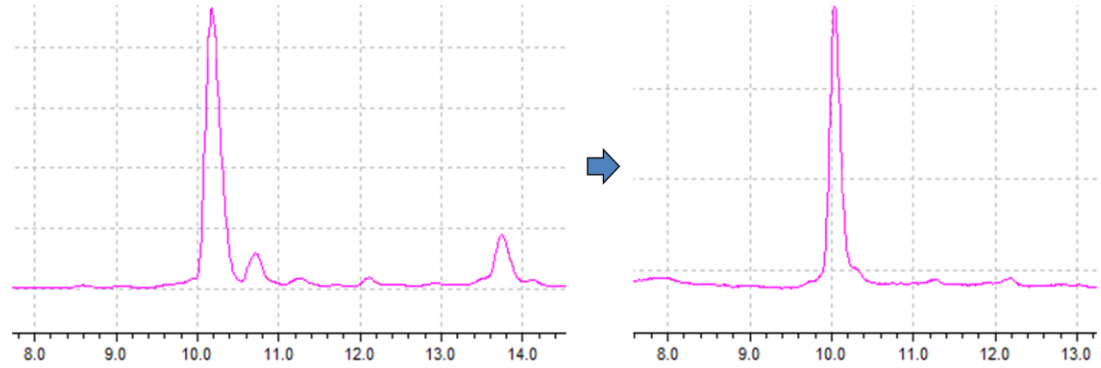


Figure 3.4 General scheme for the solid phase peptide synthesis (SPPS).

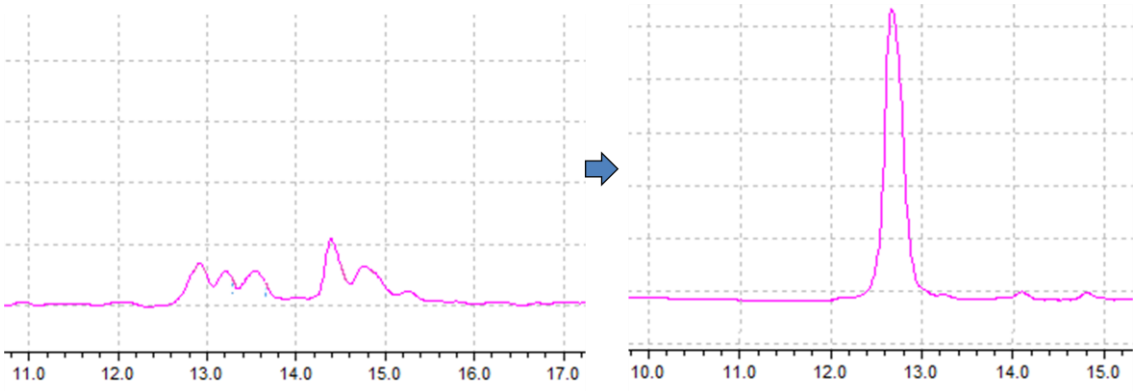
In case of hybrid peptide molecules (**Pep-1**, **Pep-2**, **Pep-3** and **Pep-4**), after the Fmoc deprotection of the final amino acid thymine acetic acid or barbiturate acetic acid is added with coupling reagent (HBTU) and base (DIPEA). Once the peptides are synthesised, they are cleaved from the resin using a cocktail made up of 95% TFA, 2% TIPS in DCM. Cleaving cocktail cleaves the peptide from resin and also deprotect all side chain protecting groups of amino acids to obtain the desired free hybrid peptide sequence. The resin is

separated from cocktail solution through filtration and cold diethyl ether was added followed by incubation at 0 °C for 1 h. The peptides suspensions were centrifuged, the supernatant was decanted and the crude product was collected.

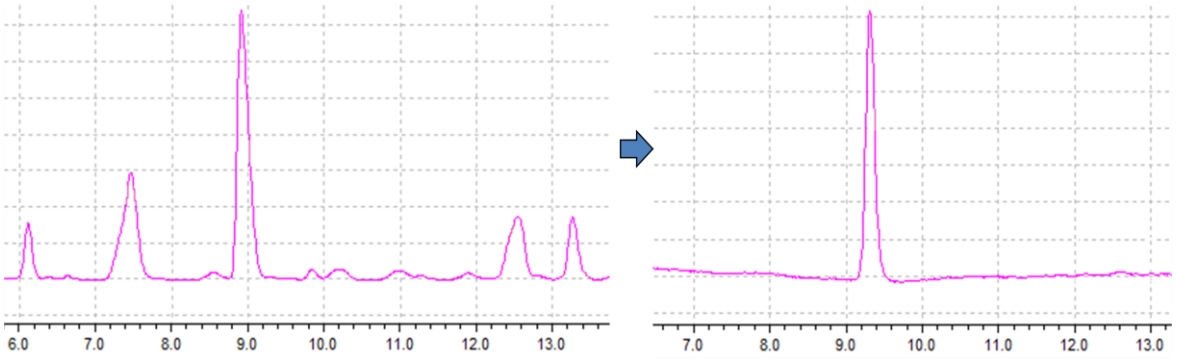
3.4.2 Purification of peptides: The hybrid peptides were purified by reverse phase high performance liquid chromatography (HPLC) using a binary gradient of water and acetonitrile as eluent. The peptides were dissolved in minimum amount of acetonitrile, water or mixture of both solvents and in cases where peptide was insoluble a small amount of methanol was used. The solution was filtered through millipore filters (0.45 μm) to remove undissolved particulate matter. Preparative HPLC traces for before (left) and after (right) purification of **Pep-1**, **Pep-2**, **Pep-3**, **Pep-4**, **Ab-1** and **Ab-2** are given below. The purified peptide fractions were further analyzed by LCMS, the pure peptide fraction was freeze dried and stored at 0°C.



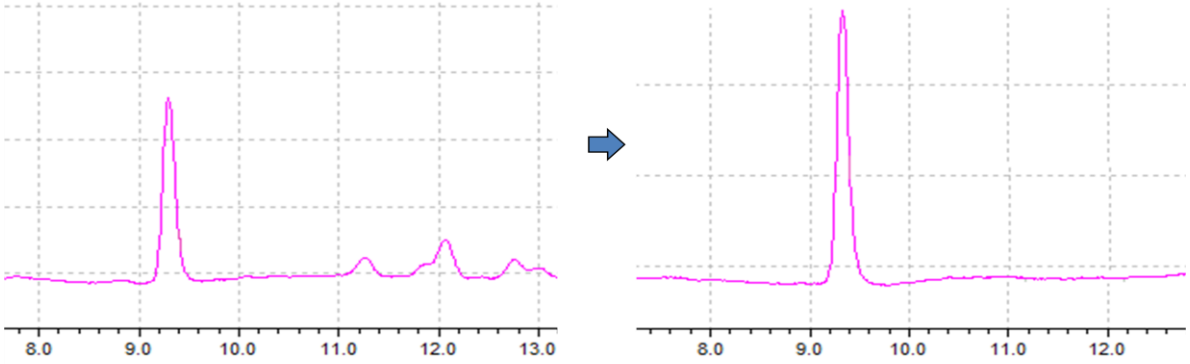
Pep-1



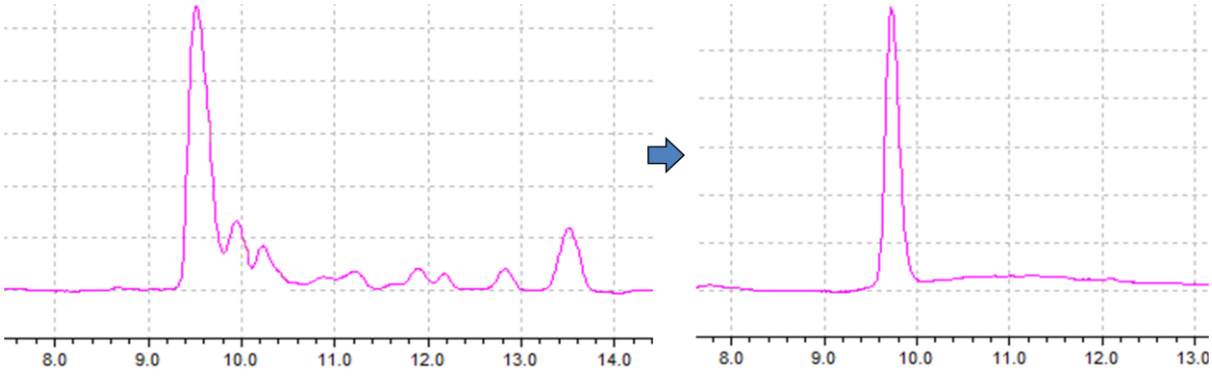
Pep-2



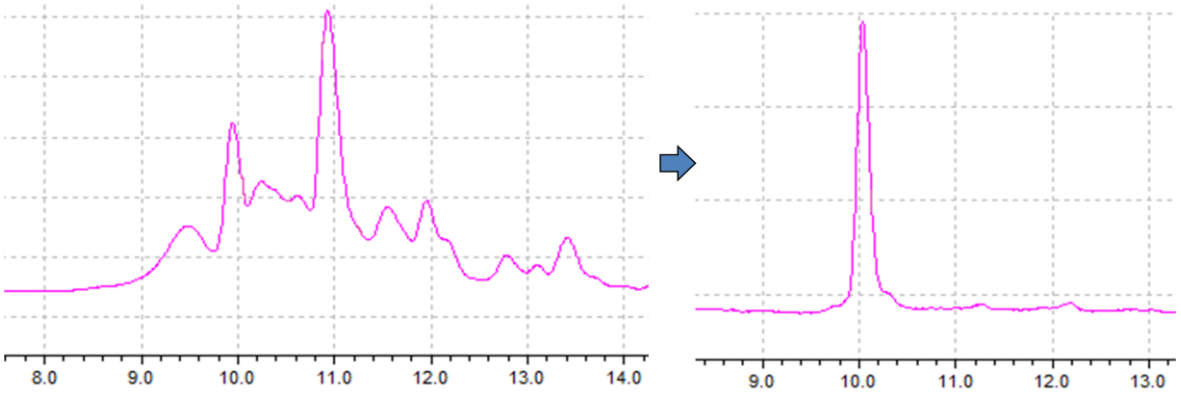
Pep-3



Pep-4



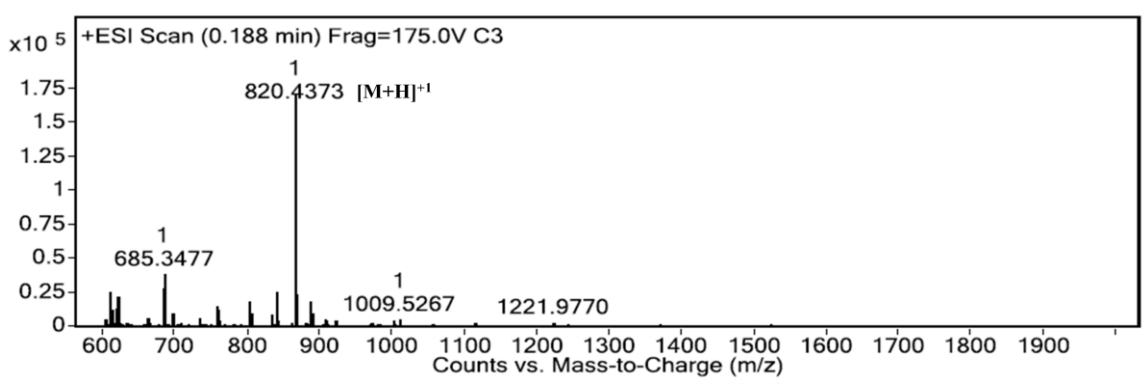
Ab-1



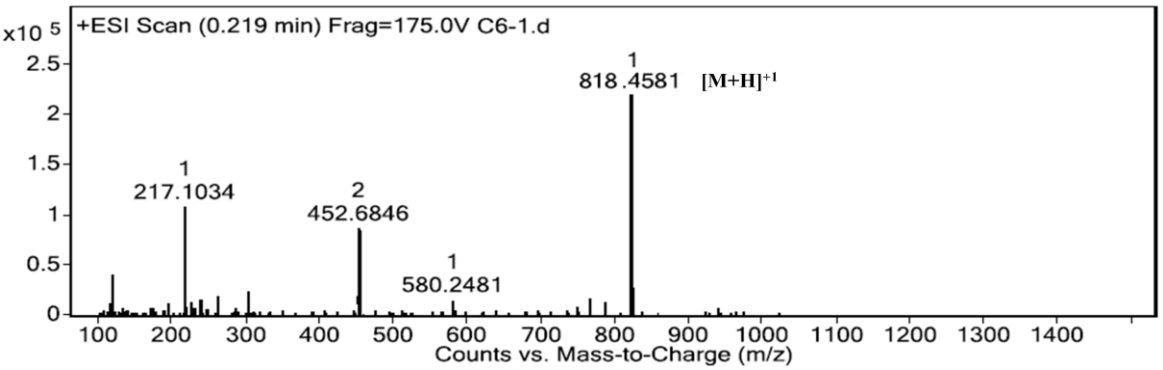
Ab-2

3.4.3 Characterization of peptides: The integrity of peptides obtained were further characterized using high resolution mass spectroscopy (HRMS). HRMS data for **Pep-1**, **Pep-2**, **Pep-3**, **Pep-4**, **Ab-1** and **Ab-2** are given below.

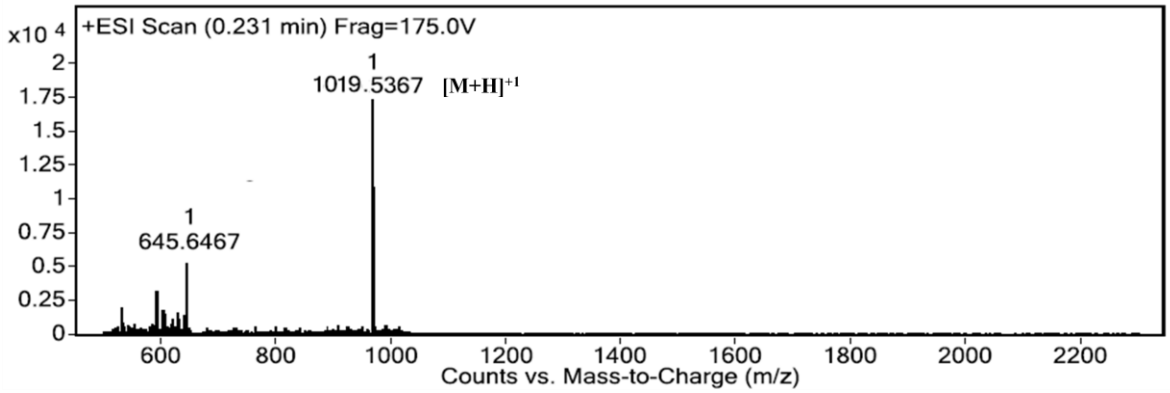
Exact mass of **Pep-1** $[M+H]^{+1}$= 820.4373 obtained mass = 820.4371



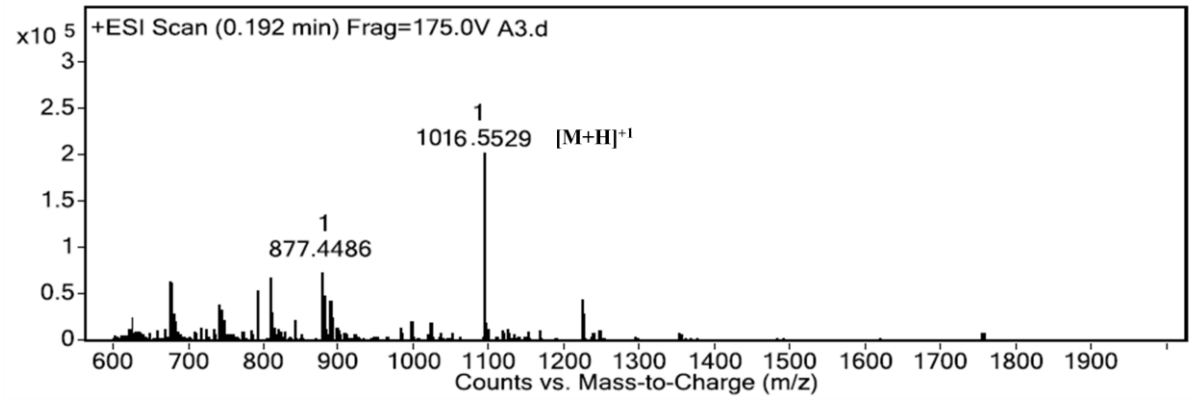
Exact mass of **Pep-2** $[M+H]^{+1}$= 818.4559 obtained mass = 818.4581



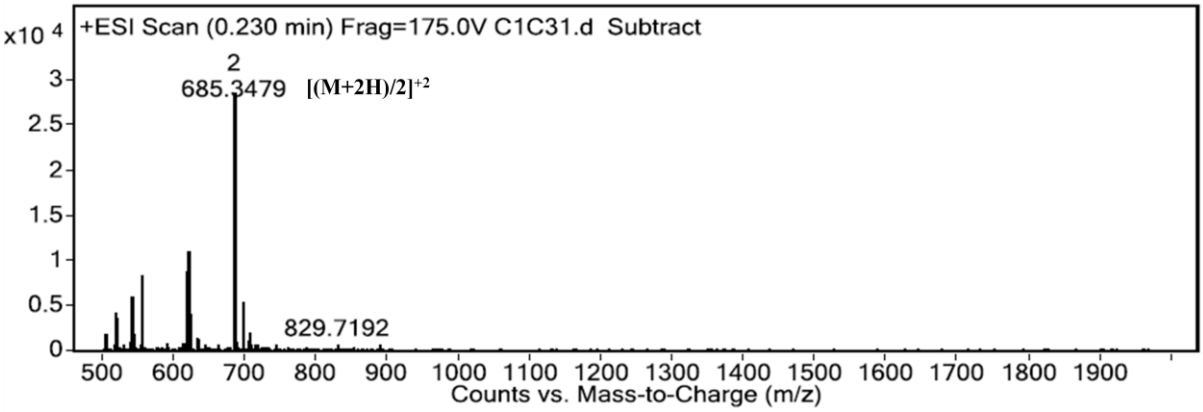
Exact mass of **Pep-3** $[M+H]^{+1}$= 1019.5309 obtained mass = 1019.5367



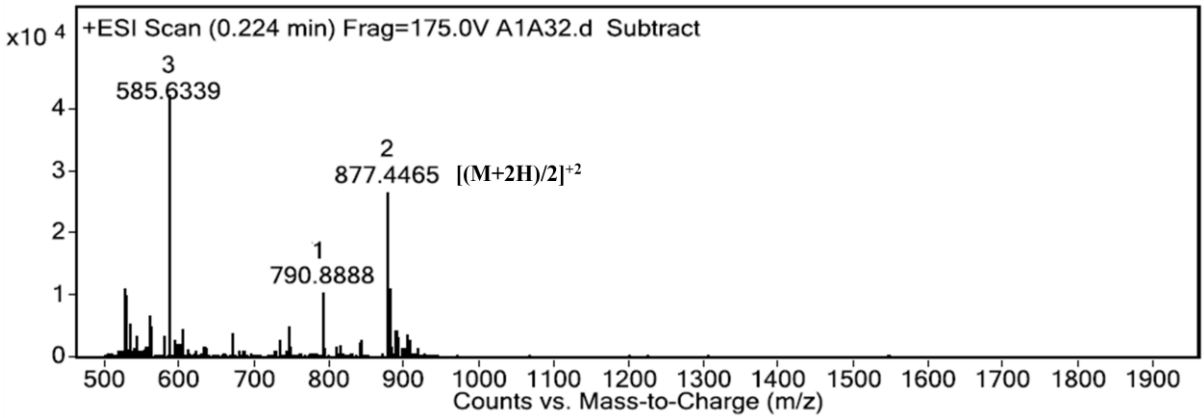
Exact mass of **Pep-4** $[M+H]^{+1}$= 1016.5564 obtained mass = 1016.5529



Exact mass of **Ab-1** $[(M+2H)/2]^{+2}$= 685.3466 obtained mass = 685.3479



Exact mass of **Ab-2** $[(M+2H)/2]^{+2}$= 877.4445 obtained mass = 877.4465



3.5 Conclusion

We have synthesised hybrid peptide molecules with thymine and barbiturate using automated solid phase peptide synthesiser. These molecules are to be studied as inhibitors for β amyloid (1-42) fibrillar aggregation. The β-amyloid fragments were synthesised to use as controls in the fibrillar aggregation studies. These peptides can be used as model systems for studying small peptide and organic molecule based inhibitors.

3.6 Experimental section

Amino acids and coupling reagents were obtained from Novabiochem, other chemicals, solvents and analytical grade reagents were obtained from Sigma-Aldrich and used as purchased without any further purification. Peptides were synthesized by automated peptide synthesizer Syro II from Multisyntech. Purification of peptides was performed with LC-8A Shimadzu preparative HPLC. Mass spectra were obtained from Agilent 6538 UHD HRMS/Q-TOF spectrometer and LCMS-2020 Shimadzu spectrometer.

3.7 References

(1) Goldsbury, C.; Baxa, U.; Simon, M. N.; Steven, A. C.; Engel, A.; Wall, J. S.; Aebi, U.; Müller, S. A. Amyloid structure and assembly: Insights from scanning transmission electron microscopy. *J. Struct. Bio.* **2011,** *173*, 1-13.

(2) Chiti, F.; Dobson, C. M., Protein misfolding, functional amyloid, and human disease. *Annu. Rev. Biochem*, **2006**, *75*, pp 333-366.

(3) Xue, W. F.; Hellewell, A. L.; Hewitt, E. W.; Radford, S. E., Fibril fragmentation in amyloid assembly and cytotoxicity: When size matters. *Prion*, **2010,** *4*, 20-25.

(4) Lynn, D. G.; Meredith, S. C., Review: Model peptides and the physicochemical approach to β-amyloids. *J. Struct. Bio.* **2000,** *130*, 153-173.

(5) Petkova, A. T.; Ishii, Y.; Balbach, J. J.; Antzutkin, O. N.; Leapman, R. D.; Delaglio, F.; Tycko, R., A structural model for Alzheimer's β-amyloid fibrils based on experimental constraints from solid state NMR. *Proc. Natl. Acad. Sci.* **2002,** *99*, 16742-16747.

(6) Lührs, T.; Ritter, C.; Adrian, M.; Riek-Loher, D.; Bohrmann, B.; Döbeli, H.; Schubert, D.; Riek, R., 3D structure of Alzheimer's amyloid-β (1–42) fibrils. *Proc. Natl. Acad. Sci.* **2005,** *102*, 17342-17347.

(7) Findeis, M. A., Approaches to discovery and characterization of inhibitors of amyloid β-peptide polymerization. *Biochim Biophys Acta.* **2000,** *1502*, 76-84.

(8) Wang, C.; Yang, A.; Li, X.; Li, D.; Zhang, M.; Du, H.; Li, C.; Guo, Y.; Mao, X.; Dong, M.; Besenbacher, F.; Yang, Y.; Wang, C., Observation of molecular inhibition and binding structures of amyloid peptides. *Nanoscale* **2012,** *4*, 1895-1909.

(9) Tjernberg, L. O.; Näslund, J.; Lindqvist, F.; Johansson, J.; Karlström, A. R.; Thyberg, J.; Terenius, L.; Nordstedt, C., Arrest of amyloid fibril formation by a pentapeptide ligand. *J. Biol. Chem.* **1996,** *271*, 8545-8548.

(10) Tjernberg, L. O.; Lilliehöök, C.; Callaway, D. J. E.; Näslund, J.; Hahne, S.; Thyberg, J.; Terenius, L.; Nordstedt, C., Controlling amyloid β-peptide fibril formation with protease-stable ligands. *J. Biol. Chem.* **1997,** *272*, 12601-12605.

(11) Klimov, D. K.; Thirumalai, D., Dissecting the assembly of Aβ 16–22 amyloid peptides into antiparallel β-sheets. *Structure* **2003,** *11*, 295-307.

(12) Soto, C.; Sigurdsson, E. M.; Morelli, L.; Kumar, R. A.; Castano, E. M.; Frangione, B. β-sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis: Implications for Alzheimer's therapy. *Nat. Med.* 1998, *4*, 822–826.

(13) Gordon, D. J.; Tappe, R.; Meredith, S. C., Design and characterization of a membrane permeable N-methyl amino acid-containing peptide that inhibits Aβ 1–40 fibrillogenesis. *J Pept Res.* **2002,** *60*, 37-55.

(14) Gordon, D. J.; Sciarretta, K. L.; Meredith, S. C., Inhibition of β-Amyloid (40) fibrillogenesis and disassembly of β-amyloid (40) fibrils by short β-amyloid congeners containing N-methyl amino acids at alternate residues. *Biochemistry*, **2001,** *40*, 8237-8245.

(15) Chalifour, R. J.; McLaughlin, R. W.; Lavoie, L.; Morissette, C.; Tremblay, N.; Boule, M.; Sarazin, P.; Stea, D.; Lacombe, D.; Tremblay, P.; Gervais, F., Stereoselective interactions of peptide inhibitors with the β-amyloid peptide. *J. Biol. Chem.* **2003,** *278*, 34874-34881.

(16) Balbach, J. J.; Ishii, Y.; Antzutkin, O. N.; Leapman, R. D.; Rizzo, N. W.; Dyda, F.; Reed, J.; Tycko, R., Amyloid fibril formation by Aβ16-22, a seven-residue fragment of the Alzheimer's β-amyloid peptide, and structural characterization by solid state NMR. *Biochemistry* **2000,** *39*, 13748-13759.

(17) Hetenyi, C.; Kortvelyesi, T.; Penke, B., Mapping of possible binding sequences of two β-sheet breaker peptides on β-amyloid peptide of Alzheimer's disease. *Biorg. Med. Chem.* **2002,** *10*, 1587-1593.