Exploring Multiple Hydrogen-Bonding Interactions in the Design of Novel Molecular Architectures

THESIS SUBMITTED TO THE

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DOCTOR OF PHILOSOPHY

(IN CHEMISTRY)

By

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DECLARATION

I here by declare that the thesis entitled **"Exploring Multiple Hydrogen-Bonding Interactions in the Design of Novel Molecular Architectures"**, submitted for the Degree of Doctor of Philosophy in Chemistry to the University of Pune, has not been submitted by me to any other university or institution. This work has been carried out at Division of Organic Chemistry, National Chemical Laboratory, Pune under the supervision of Dr. G. J. Sanjayan (Research guide).

Date:
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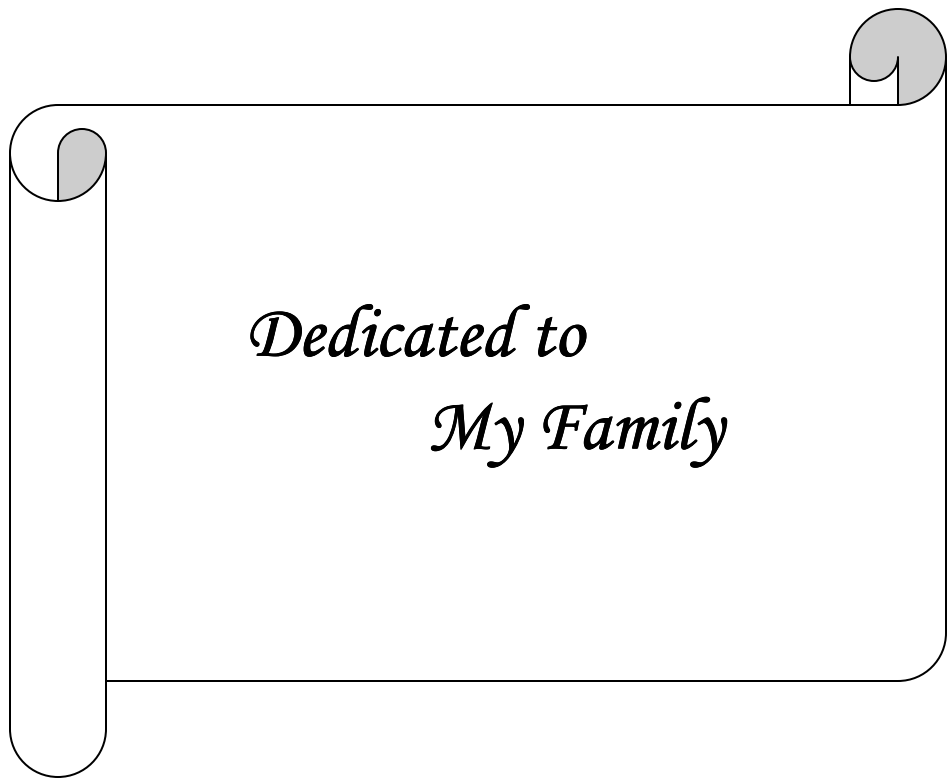National Chemical Laboratory   (Research student)
Pune

CERTIFICATE

Certified that the work incorporated in the thesis entitled **"Exploring Multiple Hydrogen-Bonding Interactions in the Design of Novel Molecular Architectures"**, submitted by **Ms. Panchami Prabhakaran** for the Degree of **Doctor of Philosophy** was carried out by the candidate under my supervision in the Division of Organic Chemistry, National Chemical Laboratory, Pune, India. Materials obtained from other sources have been duly acknowledged in the thesis.

Date: **Dr. G. J. Sanjayan**
Place: Pune   (Research Guide)

Dedicated to

My Family

Acknowledgement

This thesis is the result of five years of work whereby I have been accompanied and supported by many people. It is a pleasant aspect that I have now the opportunity to express my gratitude for all of them.

*It is my great privilege to express my deep gratitude to my research supervisor **Dr. G. J. Sanjayan** for the guidance, unflinching support, timely advices and encouragement during the course of my Ph.D work. He helped me to overcome many difficulties during the reaction chemistry with his stimulating suggestions.*

*I owe my gratitude to **Dr. Ganesh Pandey**, Head of the Organic Chemistry Division, and **Dr. S. Sivaram**, Director, for providing the infrastructure to work in this prestigious research institution.*

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CONTENTS

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Abbreviations

A
Å   Ångström
AA   Amino acid
AcOEt   Ethyl acetate
Ala   Alanine
Aib   Amino isobutyric acid

B
Boc   *tert*-Butyloxycarbonyl
Bn   Benzyl

C
CDCl$_3$ Chloroform-d
COSY   Correlated spectroscopy

D
d   doublet (NMR)
δ   Chemical shift (NMR)
DBG   Di-boc guanidine
DCC   *N, N'*-dicyclohexylcarbo
        dimide
DCM   Dichloromethane
DMF   Dimethyl formamide
DIEA   Diisopropyl ethylamine
DABCO 1,4-diazabicyclo [2.2.2]
        octane
DMSO   Dimethylformamide
DMS   Dimethylsulfate
4-DMAP 4-dimethyl aminopyridine

E
ESI   Electron Spray Ionization
Et   Ethyl

G
Gly   Glycine

H
H-bond Hydrogen bond
HMBC   Hetero Multiple Bond
        Correlation
HOBt   1-hydroxybenzotriazole

H
HSQC   Hetero Nuclear Single
        Quantum Coherence
Hz   Hertz

I
IHB   Intramolecular Hydrogen Bond

M
MALDI Matrix-Assisted Laser
        Desorption Ionization
m   Multiplet (NMR)
MS   Mass Spectrometry
Me   Methyl

N
NMR   Nuclear Magnetic Resonance
NOESY Nuclear Overhauser and
        Exchange Spectroscopy

P
Pro   Proline
Pd/C   palladium 10 % on activated
        Carbon

S
s   Singlet (NMR)
SEM   Scanning Electron Microscopy

T
TFA   Trifluroacetic acid
TBTU   O-benzotriazol-1-yl-N,N,
        N',N'-tetramethyluronium
        tetrafluoroborate
TEA   Triethyl amine
THF   Tetrahydrofuran
TBG   Tri-Boc Guanidine
TBAB   Tetrabutyl ammonium bromide
t   Triplet (NMR)
TOCSY   Total correlation spectroscopy

ABSTRACT

Name of the Candidate	**Panchami Prabhakaran**
Name of the Research Guide	**Dr. G. J. Sanjayan**
Synopsis of the thesis entitled	**EXPLORING MULTIPLE HYDROGEN-BONDING INTERACTIONS IN THE DESIGN OF NOVEL MOLECULAR ARCHITECTURES**

Chapter 1: Our approach for the pre-organization of hydrogen bonding codes in H-bonded duplexes *exclusively* based on intramolecular H-bonding is detailed. The hydrogen bonding propensities of **1** and **2** were investigated ^{1}H NMR, ESI-mass spectrometry and single crystal X-ray diffraction studies that supported our design strategy.

Fig. 1: Single crystal X-ray structures of various H-bonded duplexes

[Preorganizing Linear (Self-Complementary) Quadruple Hydrogen-Bonding Arrays Using Intramolecular Hydrogen Bonding as the Sole Force. **Prabhakaran, P.;** Puranik, V. G.; Sanjayan, G. J. *J. Org. Chem.* **2005**, *70*, 10067-10072.]

Chapter 1 also describes our attempts to synthesize a novel self-assembling system **1** starting from *N, N', N''*-Tri-Boc-guanidine (TBG) that led us to explore the

1
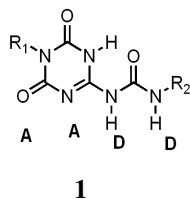
reaction chemistry of TBG with amines under various conditions affording *N*-substituted guanidines and amidinoureas; potentially important class of compounds with extensive applications in medicinal chemistry. The findings suggest that TBG can act as a readily available common starting material for the synthesis of various *N*-alkyl guanidines as well as *N*-alkyl-*N'*-substituted amidinoureas by simple manipulation of the reaction conditions.

N-Alkyl Guanidines   **N,N',N''-Tri-Boc-Guanidine (TBG)**   **N-Alkyl N'-Amidinoureas**
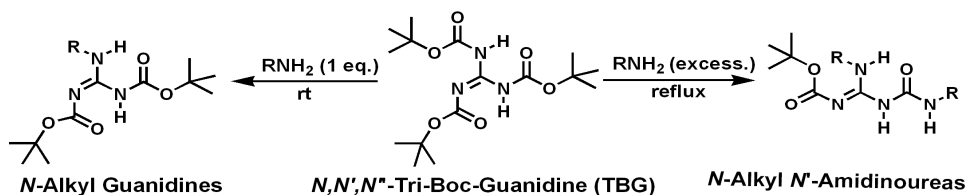
[*N,N',N''*-Tri-Boc-guanidine (TBG): a common starting material for both *N*-alkyl guanidines and amidinoureas. **Prabhakaran, P**.; Sanjayan, G. J.; *Tetrahedron Lett.* **2007,** *48*, 1725–1727.]

Chapter 2: This chapter deals with the design, synthesis and the conformational studies of α/β hybrid synthetic oligomers using natural and unnatural amino acids.

The first part of this chapter describes the design principles, synthesis and the conformational studies (both in solid and solution-state) of conformationally rigid α/β-hybrid peptides consisting of repeating anthranilic acid-L-proline building block. These oligomers adopt a compact, right-handed helical architecture with an unusual periodic (1→2) C9 helical turn networks.
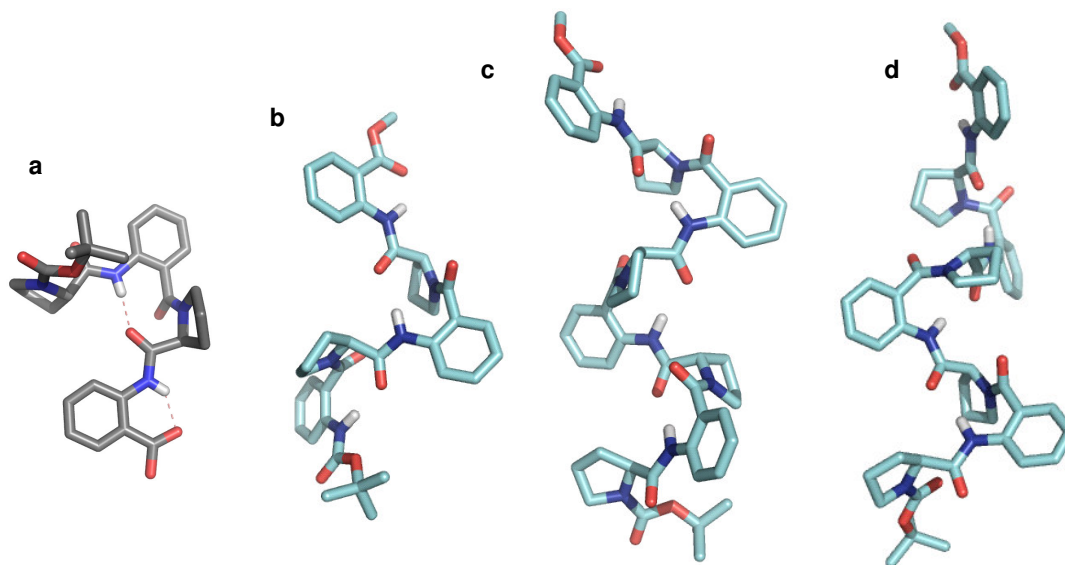
Fig. 2: Conformation of the hybrid oligomers with helical architectures. (a) Crystal structure of a tetrapeptide (b) Conformation of pentapeptide obtained at the HF/6-31G* level of ab initio MO theory studies. (c) and (d) conformations of octapeptide with *trans* and *cis* Pro1 carbamate bond by MO studies at the same approximation. Hydrogens other than H-bonding sites have been omitted for clarity.

The second part of this chapter describes results of our investigation in understanding the role of proline in stabilizing the C9 turn formation by substituting Pro with different amino acids.
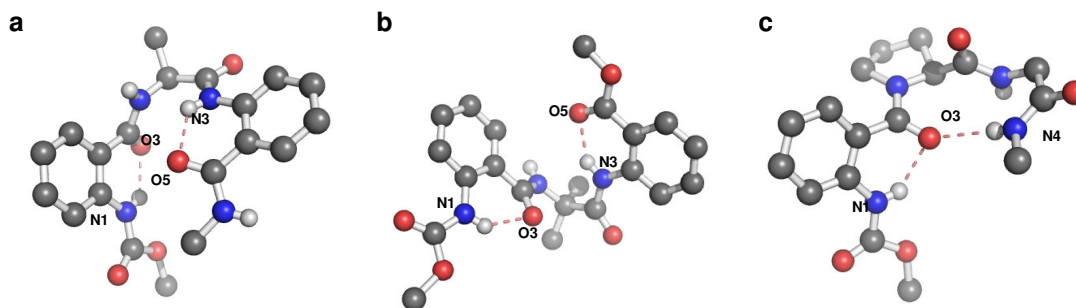
Fig. 3: The crystal structures of shorter analogs. Hydrogens, other than at the hydrogen bonding sites, have been omitted for clarity.

[Sequence-Specific Unusual (1→2)-type Helical Turns in α/β-Hybrid Peptides **Prabhakaran, P**.; Puranik, V. G.; Chetina, O.; Howard, J. A. K.; Rajamohanan, P. R.; Hofmann, H.-J.; Sanjayan, G. J.; (Communicated to *J. Am. Chem. Soc.*)

Chapter 3 describes our study towards the conformational features of oligomers with abiotic backbone. Naphthalene based homo-oligoamide backbones with diverse structural architectures were designed such that their conformational rigidity would be realized due to the electrostatic/ steric interactions in their close vicinity.
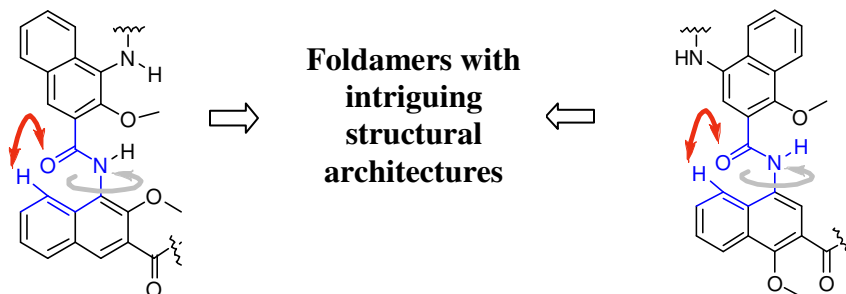
Fig. 4: Naphthalene homo-oligoamide-based foldamers developed in this study.
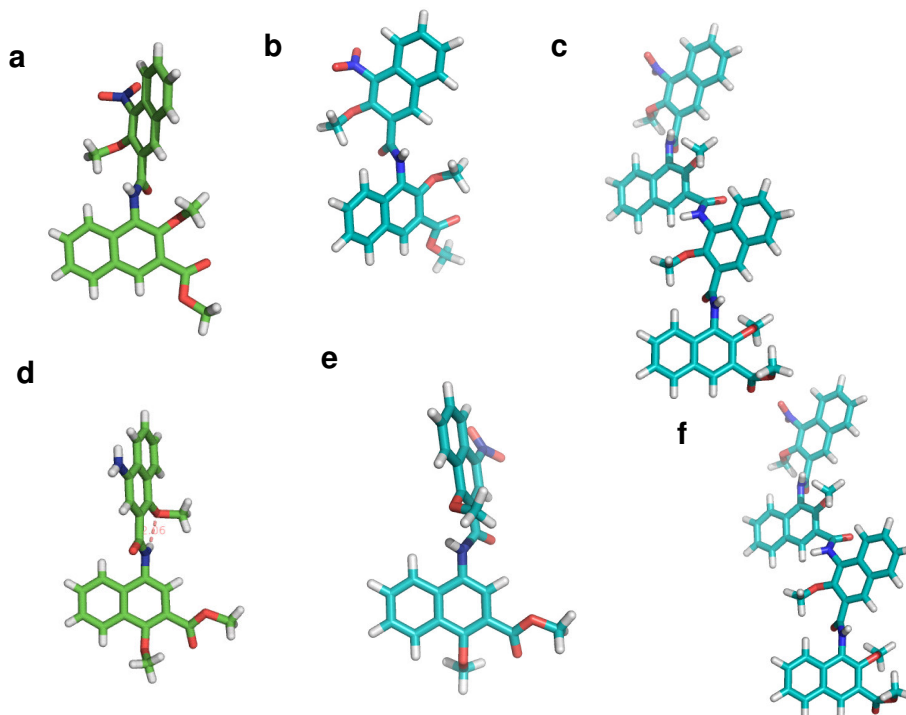
Fig. 5: Structural architecture of naphthalene homo-oligoamides obtained from X-ray crystallography (a, d) and from molecular modeling (b, c, e and f).

This chapter also describes the design strategy, synthesis and conformational studies of hybrid oligomers with co-facial aromatic building blocks.
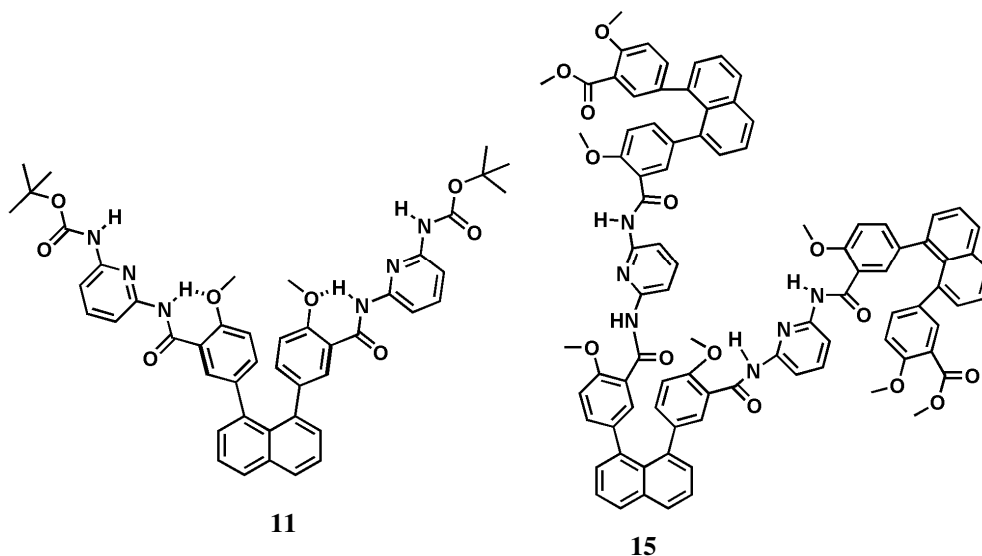
Fig. 6: Foldamers with 1,8-bis phenyl naphthalene unit as the cofacial building blocks.
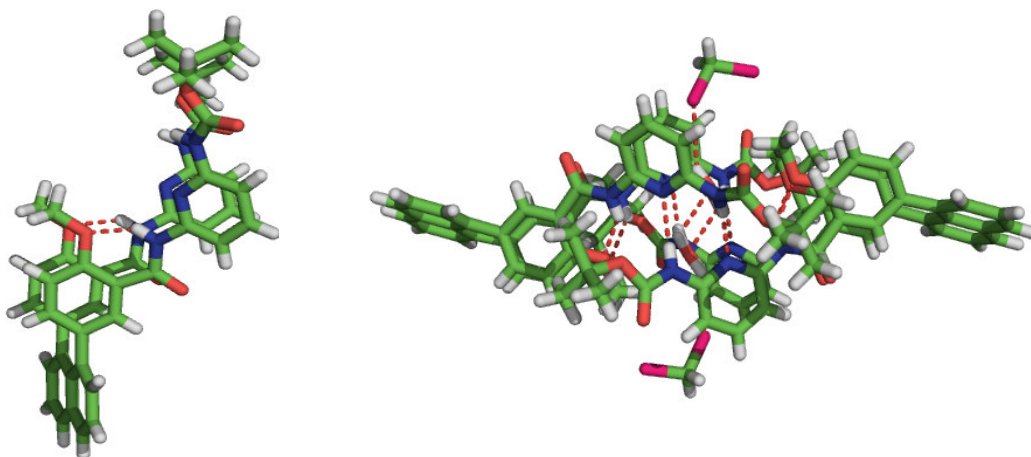
Fig. 7: (a) Crystal structure of **11** showing the co-facial structural architecture; (b) Duplex formation of **11** through H-bonding.

GENERAL REMARKS

- Unless otherwise stated, all the chemicals and reagents were obtained commercially.

- Required dry solvents and reagents were prepared using the standard procedures.

- All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel plates (Kieselgel $60F_{254}$, Merck) with UV, I_2 or ninhydrin solution as the developing reagents in the concerned cases.

- Column chromatographic purifications were done with 100-200 Mesh Silica gel or with flash silica gel (230-400 mesh) in special cases.

- Melting points were determined on a Buchi Melting Point B-540 and are uncorrected.

- IR spectra were recorded in nujol or $CHCl_3$ using Shimadzu FTIR-8400 spectrophotometer.

- NMR spectra were recorded in $CDCl_3$ on Ac 200 MHz, AV 400 MHz or DRX-500 MHz Bruker NMR spectrometers. All chemical shifts are reported in δ ppm downfield to TMS and peak multiplicities as singlet (s), doublet (d), quartet (q), broad (br), broad singlet (bs) and multiplet (m).

- Elemental analyses were performed on a Elmentar-Vario-EL (Heraeus Company Ltd.; Germany.

- Electron Scattered Ionization (ESI) Mass Spectrometric measurements were done with API QSTAR Pulsar mass Spectrometer and MALDI-TOF mass spectrometric measurements were done on Voyager-DE STR mass spectrometer.

- Quantum chemical calculations were performed employing the Guassian 03 program package (Guassian, Inc., Wallingford, CT 06492, USA)

- Single crystal X-ray data were collected on a *Bruker SMART APEX* CCD Area diffractometer with graphite monochromatized (Mo K_α = 0.71073Å) radiation at room temperature.

List of Publications:

1. Preorganizing Linear (Self-Complementary) Quadruple Hydrogen-Bonding Arrays Using Intramolecular Hydrogen Bonding as the Sole Force
   Prabhakaran, P.; Puranik, V. G.; Sanjayan, G. J. *J. Org. Chem.* **2005**, *70*, 10067-10072.

2. *N,N',N''*-Tri-Boc-guanidine (TBG): a common starting material for both N-alkyl guanidines and amidinoureas
   Prabhakaran, P.; Sanjayan, G. J. *Tetrahedron Lett.* **2007**, *48*, 1725–1727.

3. Sequence-Specific Unusual (1→2)-type Helical Turns in α/β-Hybrid Peptides
   Prabhakaran, P.; Puranik, V. G.; Rajamohanan, P. R.; Chetina, O.; Howard, J. A. K.; Hofmann, H.-J.; Sanjayan, G. J. (communicated to *J. Am. Chem. Soc.*)

4. Naphthalene Homo-oligoamides with Conformational Diversity
   Prabhakaran, P.; Puranik, V. G.; Rajamohanan, P. R.; Hofmann, H.-J; Sanjayan, G. J. (Manuscript ready for submission.)

5. Novel Foldamer Architectures from Co-facial Aromatic Building Blocks
   Prabhakaran, P.; Puranik, V. G.; Rajamohanan, P. R.; Sanjayan, G. J. (Manuscript under preparation)

6. Two-Dimensional Aromatic Amino Acids: Novel Building Blocks for Abiotic Foldamers
   Roy, A.; Ramesh, V. V.; Kendhale, A. M.; **Prabhakaran**, **P**.; Gonnade, R.; Puranik, V. G.; Sanjayan, G. J. (Manuscript under preparation)

CHAPTER 1

Pre-organization of Hydrogen-Bonding Codes in Quadruple H-bonded Molecular Duplexes using Intramolecular Hydrogen-Bonding as the Sole Force

In the Ist part of this chapter, a rational approach for pre-fixing multiple cooperative binding sites in an ideal spatial arrangement on a structurally rigid backbone exclusively by intramolecular hydrogen bonding, synthetic strategy and the investigation of the self-assembling propensities of these systems by ESI Mass spectrometry, NMR spectroscopy and single crystal X-ray diffraction studies are detailed. The second part of the chapter describes the interesting reaction chemistry of tri-Boc-guanidine with amines resulting in the selective formation of N-alkyl guanidines as well as N-alkyl-N'-substituted amidinoureas by simple manipulation of the reaction conditions.

Pre-organization of Hydrogen-bonding Codes in Quadruple H-bonded Molecular Duplexes using Intramolecular H-bonding as the Sole Force

The discovery of the Hydrogen Bond could have won someone the Nobel Prize, but it didn't.

-George A. Jeffrey, Wolfram Saenger-

1.1 Molecular self-assembly: a bottom-up approach

The concept of molecular self-assembly has attained significant interest in various fields of science like chemical biology, chemistry, and material science due to the oriented fabrication of supramolecular networks with wide range of applications.[1] Nature provided foundation behind this conception, as happened in many other cases. The base pairing in DNA, an example for the Nature's self-assembly, which helps it to exist in a doubly stranded structure, is indirectly responsible for the various biological activities as well as the existence and distinction of species on earth. According to Lawrence *et al*, "*self-assembly is a highly convergent synthetic protocol, one that is exclusively driven by non-covalent interactions. As a consequence, these same non-covalent interactions are wholly responsible for the structural integrity of the end product*".[2] Self-assembly is governed by weak interactions and hence reversible in nature. This can be considered as one of the advantage of this. For example, the processes of assembly and the disassembly of microtubules (tubulins as the subunits) in the cell mitosis are operated through the reversible nature of self-assembly. In the construction of supramolecular entities, various secondary forces such as metal-ligand (50-200 kJ/mol), H-bond (4-120 kJ/mol), dipole-dipole interaction (4-40 kJ/mol), π-π-stacking (1-20 kJ/mol) and van

der Waal's interaction (1-10 kJ/mol) have been encountered, resulting in the synthetic supramolecular architectures and materials of different sizes, shapes with interesting applications.[3]

Table 1.1: Characteristics of covalent and non-covalent synthesis.

	Covalent	**Non-covalent**
Building block	Atoms	Molecules/ions
Assembly	Molecule	Assembly
Force	Covalent	H-bond, Ionic bond, metal co-ordination, π-π-stacking
Kinetic stability	High	Low
Extrinsic factors	Secondary	Primary
Characteristics	--	Co-operativity

Among these secondary interactions, H-bond, although not the strongest secondary interaction, has been frequently used in the design of supramolecular assemblies due to its directionality, specificity and moderate strength.[4,5] It plays many major roles in our body in the processes such as antigen-antibody recognition, enzyme recognition, protein folding, DNA replication, α-helix and β-sheet formation etc. and also has an enormous impact on our daily life. The linear arrays of H-bonding sites found in nucleic acids can be encountered in synthetic molecules by proper arrangement of H-bonding sites by means of additional pre-organization force. Even though the H-bond energy for a single H-bond is not enough to hold the

molecules together, but its strength can be altered by the electron distribution and also by increasing the number of sites participating in H-bonding.[6]

1.2 Hydrogen-bonded duplexes

The design and synthesis of molecular duplexes with diversely positioned H-bonding acceptor (A) and donor (D) codes have emerged as an interesting area of research in the supramolecular synthesis.[6,7] Among these, H-bonded duplexes using multiple H-bonds ranging from doubly H-bonded[8] to duplexes which bind with eight H-bonds [9] are known so far (fig. 1.1).
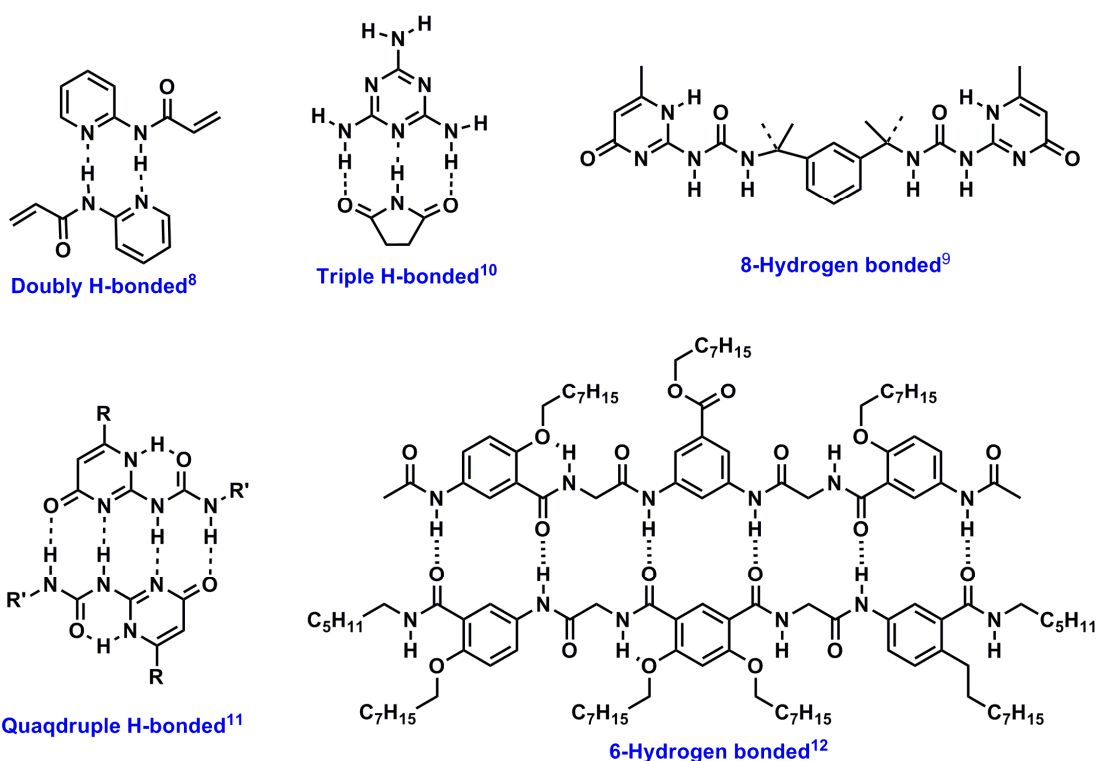
Fig. 1.1: Examples for H-bonded duplexes.

The number of molecular duplexes that can be obtained from a doubly H-bonded system is two. A triple H-bonded array can afford three duplexes and a quadruple H-bonded series would provide six different molecular duplexes. The number of complementary and self-complementary duplexes possible for a given number of hydrogen bonding sites; say n, can be calculated by the following equations.[12]

$$N = 2^{n-2} + 2^{(n-3)/2} \quad \text{When N = odd number} \quad\text{———————} \quad 1$$

$$N = 2^{n-2} + 2^{(n-2)/2} \quad \text{When N = even number} \quad\text{———————} \quad 2$$

Where,

N: number of duplexes

n: number of hydrogen bonding sites

1st term -number of complementary duplexes

2nd term - number of self-complementary duplexes

According to the equation, when n = 4, four complementary and two self-complementary arrangements are plausible (eq. 2). The possible arrangements are shown in fig. 1.2.[7b] But substantial difference in the stability is reflected with the change in the sequence of H-bonding codes having the same number of H-bonding sites.
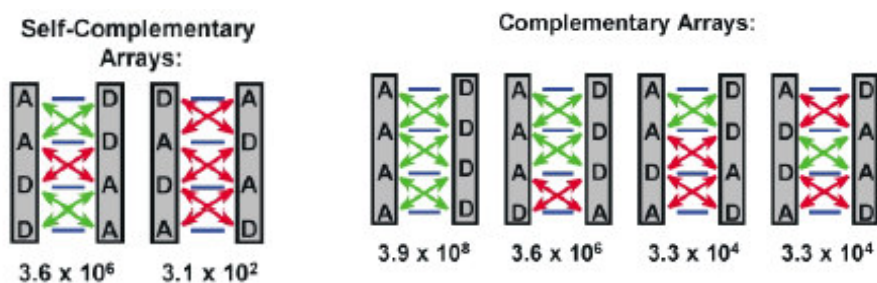
Fig. 1.2: Complementary and self-complementary arrangements in a quadruple H-bonded system. The arrows ↔ and ↔ indicate the secondary attractive and repulsive interactions, respectively. The value given below of each duplex indicates the dimerization constant, K_a for hetero and K_{dim} for homo arrays, respectively.

1.3 Factors affecting the stability and strength of the duplexes

Several factors are found to affect the stability of the duplexes. Since H-bond is sensitive to extrinsic factors, its stability alters with pH, temperature, nature of the solvent and also with concentration. Furthermore, arrays having same number of H-bonded sites with different arrangement (positioning) may have different stability profiles, even if they have same number of primary attractive interactions.

Table 1.2: The types of complementary and self-complementary H-bonded arrays possible for doubly, triply and quadruply H-bonded systems.

No of sites	Complementary arrays			Self-complementary arrays		
	No.	Type*	K_a (M^{-1})	No	Type	K_{dim}(M^{-1})
2	1	DD. AA	10^3	1	DA.AD	10^2
3	3	DDD.AAA	10^5-10^6	--	--	--
		DAD.ADA	10^2		--	--
		DDA.AAD	10^4		--	
4	4	DDDD.AAAA	3.9×10^8	2	DDAA.AADD	3.6×10^6
		DDDA.AAAD	3.6×10^6			
		AADA.DDAD	3.3×10^4		DADA.ADAD	3.1×10^2
		ADDA.DAAD	3.3×10^4			

*A stands for acceptor and **D** for donor, K_a is the association constant for complementary arrays and K_{dim} is dimerization constant for self-complementary arrays.

According to Jorgensen's concept, the differences in the stability among the H-bonded duplexes which is reflected in their dimerization constant[13] are caused by the operation of secondary interactions in addition to the primary attractive forces.[14] The primary interactions are attractive in nature and the secondary interactions can be either attractive or repulsive in nature. Thus, in a quadruple hydrogen bonded module, complementary **DDDD.AAAA** duplex has the highest stability (where all the secondary interactions are attractive, $K_{dim} = 3.9 \times 10^8 \, M^{-1}$) and the least stable one is **DADA.ADAD** (where all the secondary interactions are repulsive, $K_{dim} = 3.2 \times 10^2 \, M^{-1}$). According to Schneider rule, which was postulated after considering 58 H-bonded systems, the free energy value for the primary attractive interactions is about -8 kJ/mol and -2.9 and +2.9 kJ/mol for each attractive and repulsive secondary electrostatic interactions, respectively.[15] This is found to be in agreement with the calculated values for small molecules.

But in most of the cases, there are deviations from the calculated values. For example, quadruple **DADA** H-bonded duplexes **A** and **B** based on diamino triazine reported by Meijer *et al*[16] differ in their stability, though they are held together by identical arrays (fig. 1.3)
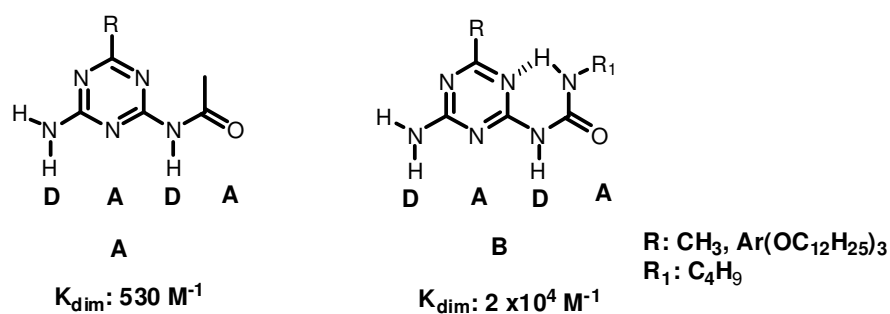
Fig. 1.3: Deviation from additivity rules based on secondary interactions.

This large difference indicates the limitations of the additivity rules based on the secondary interactions. In fig. 1.3, the molecule **B** pre-organizes the H-bonding code through an intramolecular H-bonding, a possibility which is clearly absent in **A**. The overall charge distribution in the molecule also plays an important role in determining the stability of the duplexes.

1.4 Forces encountered in the pre-organization of the H-bonding codes

The role of pre-organization is important in order to attain the co-planarity for effective H-bonding in H-bonded supramolecular synthesis.[17] The forces operating in the pre-organization can be electrostatic repulsion, covalent linkages, combination of covalent linkages and intramolecular H-bond etc.

1.4.1 Role of electrostatic repulsions

The electrostatic repulsion in molecule **A** (fig. 1.4), between the amide oxygen and the N-atom in the heterocyclic ring forces the amide bond to exist in an energetically unfavorable *syn* conformation (**B**) with a pre-organized DADA H-bonding code.[16]
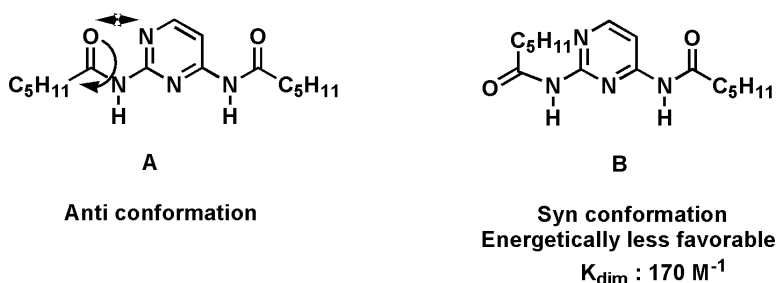
Fig. 1.4: Effect of electrostatic repulsion in pre-organization.

1.4.2 Covalent linkages

In doubly H-bonded and some triply H-bonded systems, the pre-organization can be effected merely with covalent linkages (fig. 1.5).[18]

ADA.DAD K_a : 78 M^{-1}
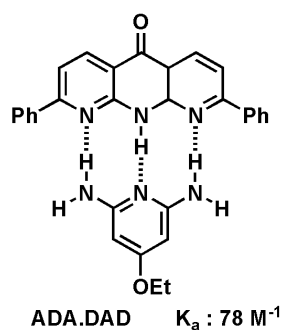
Fig. 1.5: Example for pre-organization effected using covalent bonds.

1.4.3 Combination of covalent linkages and intramolecular H-bond

In molecular duplexes containing four or more hydrogen bonding sites, it is impossible to keep the H-bonding face to be co-planar merely with the help of covalent force. Therefore, in addition to the covalent linkages, there should be some additional force which would combinely help the molecule in pre-organization in such a way that it can participate in effective hydrogen bonding to give a stable duplex. The intramolecular H-bond between A / D (where D is donor and A is the acceptor) from the side chain attached to heterocycles (fig. 1.6)[19] plays an additional force in the pre-organization of H-bond face. The delocalization of electron density further enhances the stability of the duplex (see section 1.3, page 6).

Davis et al. 1999[19a]   **Sanjayan et al. 2005**[19b]   **Schmuck et al. 1999**[19c]
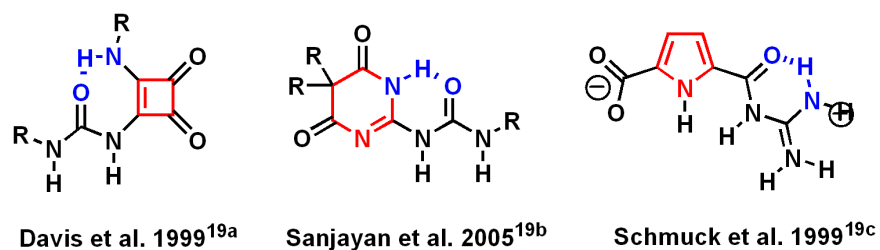
Fig. 1.6: Pre-organizing H-bond face with intramolecular H-bond and covalent bond.

1.5 Quadruple Hydrogen-bonded duplexes

Among the diverse H-bonded dimers, quadruple H-bonded duplexes have attained considerable interest because of the ease in the design as well as synthesis.[11] According to the equation **2** (section 1.2, page 4), there are four complementary and two self-complementary arrays possible for systems with quadruple H-bonding sites.

1.5.1 Self-complementary duplexes

For a quadruple H-bonded system, **DDAA** and **DADA** arrangements lead to the self-complementary duplexes (see fig. 1.2). The self-complementarity is required for a number of applications ranging from polymeric materials, container molecules and to supramolecular tubes.[20] Some selected examples of self-complementary quadruple H-bonded systems reported are Meijer's ureido-pyrimidinones (UPy) (fig.1.7 b),[11] Zimmermann's quinoline derived self-assembling motifs (fig. 1.7 c),[21] and Bing Gong's 3-aminobenzoic acid- derived hetero oligomers[22] (fig. 1.7 d).
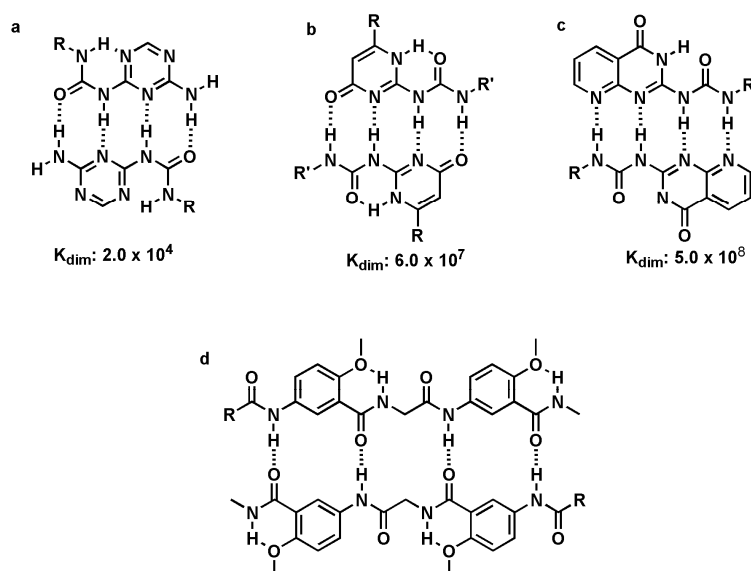
Fig. 1.7: Selected examples of self-complementary arrays.

1.5.2 Complementary arrays

Although self-complementarity is favored in some cases, it can impose restriction to the assembly of copolymers and the construction of certain class of supramolecular polymers.[23] In such cases, the complementary arrangement is more favored, although it involves more than one compound for duplex formation (fig.1.8).
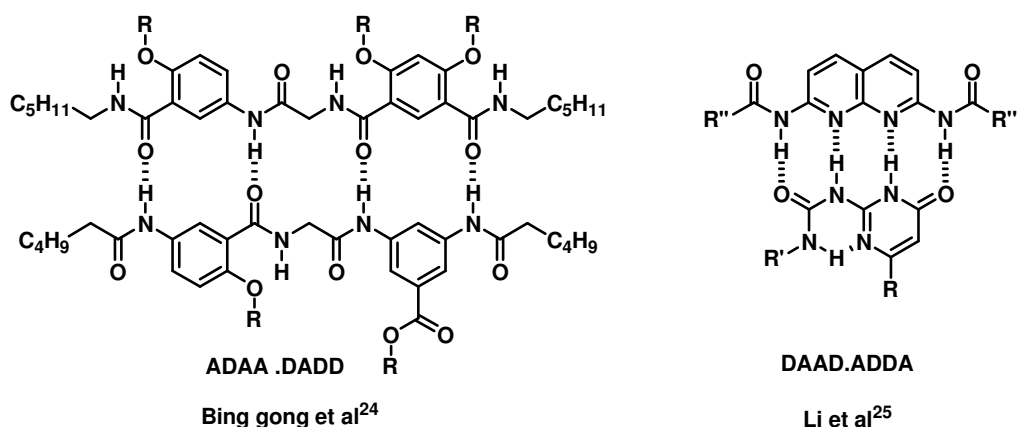
Fig. 1.8: Examples for complementary duplexes.

1.6 Application of hydrogen-bonded duplexes

The concepts of non-covalent synthesis for supramolecular entities may be utilized in the design of functional devices and supramolecular structures. Although the natural duplex DNA is unique in its function and properties, self-assembling synthetic entities have several advantages due to their ready availability in large quantities, much lower molecular weights, and compatibility with a wide variety of solvents compared to the former.[26] The synthetic duplexes find application in various disciplines like in nucleation and stabilization of β-sheets,[24] synthesis of supramolecular polymers,[23] templating the reaction pathway with increased selectivity,[27] and construction of artificial nanostructures.[28] The use of multiple H-bonding interactions in the design of supramolecular polymers with attractive features

such as high degree of polymerization, ease of synthesis, high degree of association constant has attracted the attention of chemists in self-assembly-mediated polymer synthesis.[23,7b]

(a) Templating β-sheet formation[24]

(b) Quadruple Hydrogen Bonding

Inducing conformational rigidity to flexible backbone

(c) Formation of supramolecular polymers[7b,23]

(d) Grubbs catalyst

Templating reaction pathway[27]

(e) Electronic communication[29]
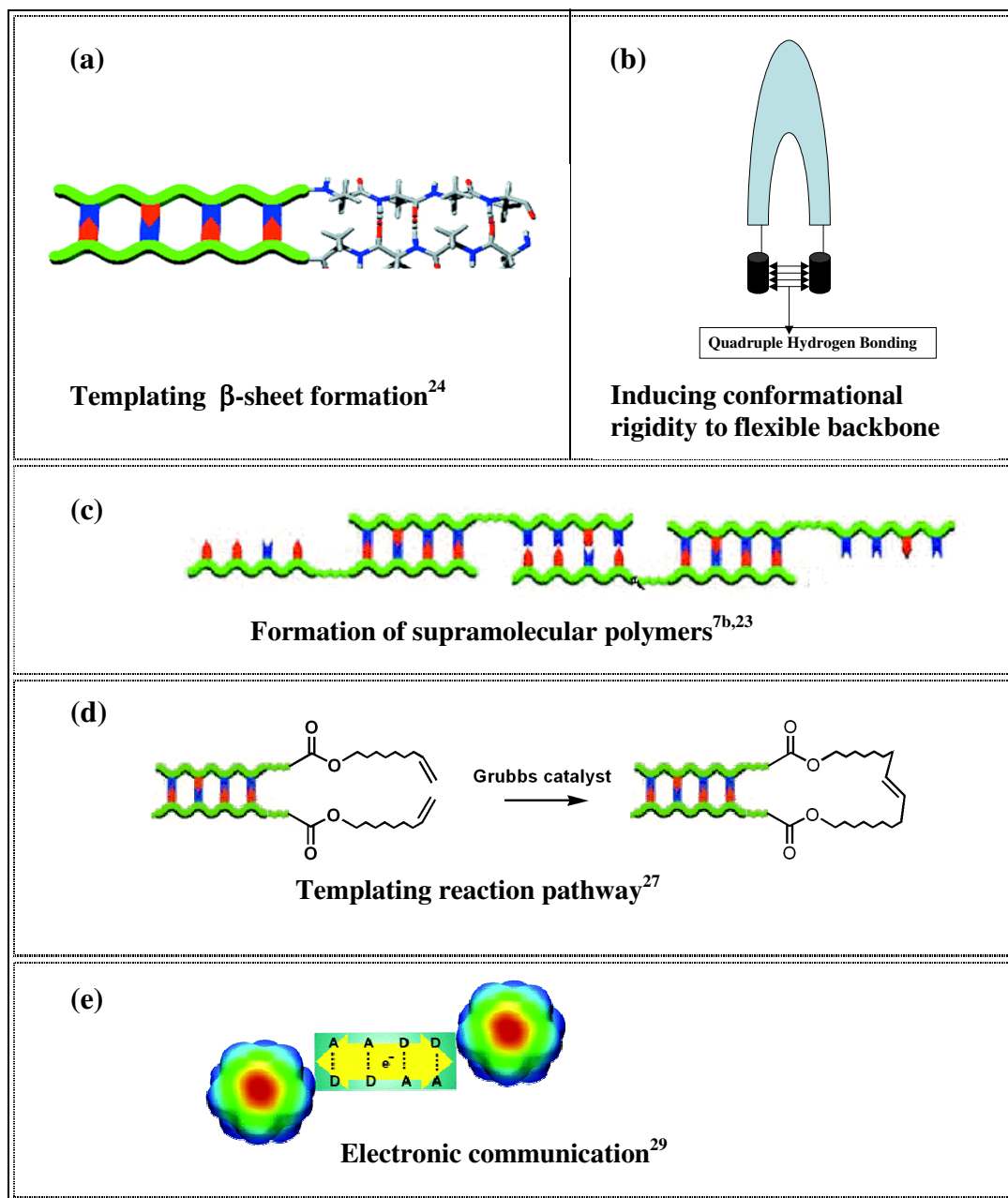
Fig. 1.9: Pictorial representation of various applications of quadruple H-bonded duplexes.

1.7 Analysis of H-bonding propensities in solution and solid-state

The self-assembling propensities of the hydrogen bonding duplexes have been analyzed in the solid-state and solution-state by powerful techniques like NMR, X-ray, and mass spectrometry. In the ^{1}H NMR spectra of H-bonded systems in a non-polar solvent like $CDCl_3$, NHs (donors) participating in the hydrogen bonding (both intra as well as intermolecular) get down field shifted.[8-12] Titration with a polar solvent like DMSO-d6 affects the chemical shift of intermolecular H-bonded NHs, whereas intramolecularly H-bonded NHs remain unaffected. Further, X-ray crystallography provides strong evidence of self-assembly in the solid-state.[9,19b] The formation of strong molecular duplexes can also be readily conformed by the observation of a dimer peak (2M+1) in mass spectrometry.[30]

1.8 Problem of prototropy

Even though considerable research efforts have been made to increase the stability of duplexes, existence of multiple conformers due to prototropy is a snag which decreases the stability further, than the calculated values.[11]
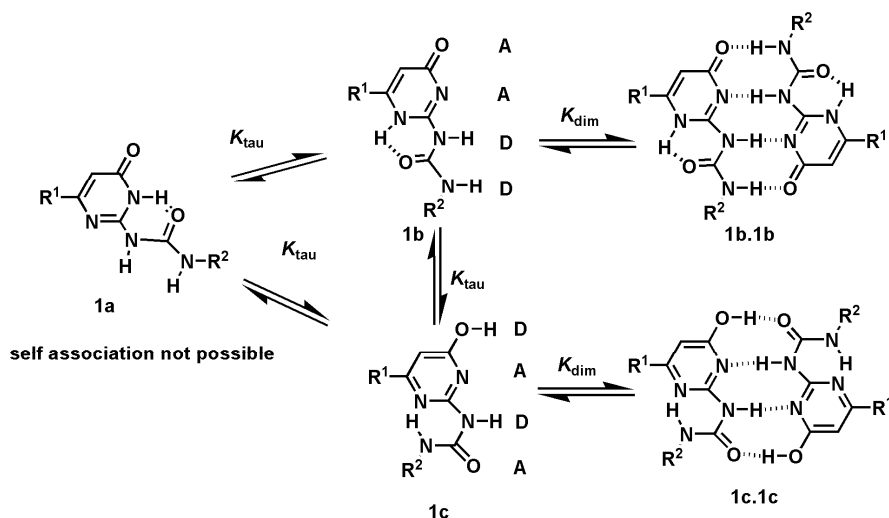
Fig. 1.10: The prototropy observed in ureido-pyrimidinone (UPy) based system.[11]

The molecule **1a** (fig. 1.10) has a H-bond face **ADD** that can not self-associate to form a duplex. But it can exist in two more tautomeric forms **1b** and **1c** which can further undergo self-association in solution using **AADD** and **ADAD** H-bond face to form the duplexes **AADD.DDAA** and **ADAD.DADA**, respectively. Since the tautomerism results in the most stable **(AADD)$_2$** and the least stable **(ADAD)$_2$** arrays, there is a considerable decrease in stability of the duplex than the expected value. The self-assembling system reported by Zimmermann based on quinoline, retains the same H-bond face resulting in the formation of duplexes with similar strength,[21] despite undergoing prototropy. Considerable work has recently been done to circumvent the problem of prototropy. For example, the dialkyl substituted pyrimidinedione system reported from our group could effectively avoid the formation of multiple conformers because of the degeneracy in the conformers (fig. 1.11).[19b]
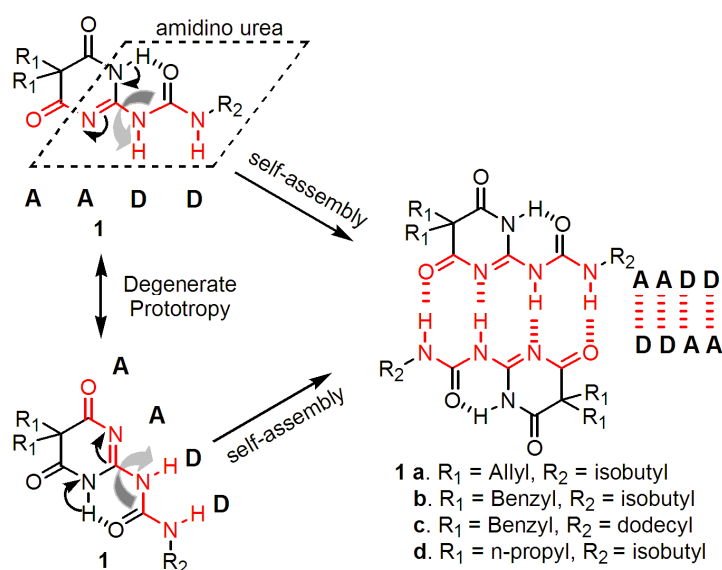
Fig. 1.11: Molecular duplexes with degenerate prototropy.[19b]

1.9 Objective of the present work

As described in section **1.4**, the pre-organization of the donor and acceptor sites on the H-bond face of self-assembling systems can be effected by the combined use of intramolecular H-bonding and covalent linkages. These covalent linkages usually form part of the heterocyclic ring on which the H-bonding codes are embedded and the intramolecular hydrogen bond can be between the hetero atom of the ring and the H-bond donor attached to the donor (fig. 1.12). [11, 16, 19, 21, 31]

Meijer et al. 1998[11]   Zimmerman et al. 1998[21]   Sanjayan et al. 2005[19b]

Meijer et al. 1998[16]   Davis et al. 1999[19a]   Schmuck et al. 1999[19c]

Gong et al. 2003[31]
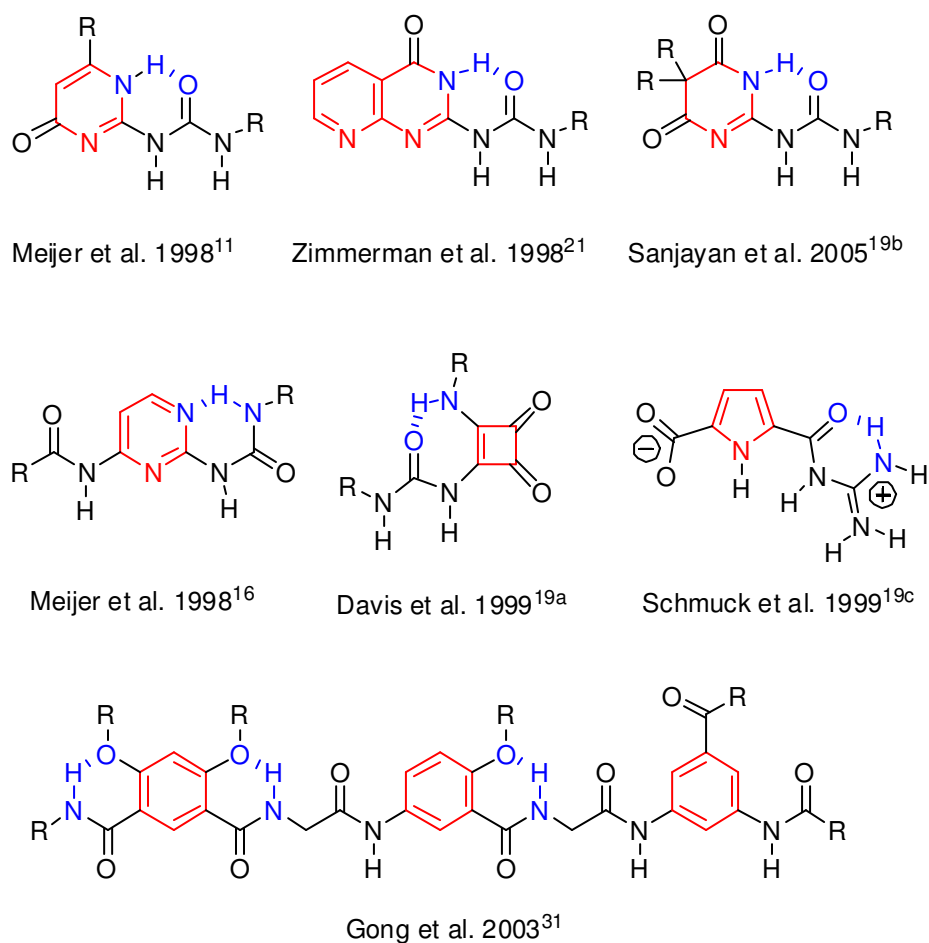
Fig. 1.12: Selected examples of self-complementary self-assembling modules that form molecular duplexes with covalent bonds (red) and intramolecular hydrogen bonds (blue).

Fixing of the hydrogen bonding sites on a hydrogen bonding platform for the cooperative binding solely based on intramolecular H-bonding is an attractive idea for the construction of novel molecular architectures. In an attempt to meet the goal of prefixing the H-bonding codes in the monomer unit of the duplex using intramolecular H-bonding as the sole force, we designed a series of self-assembling modules **1** and **2** whose linear quadruple hydrogen-bonding arrays were embedded on an amidinourea backbone. It was envisaged that these designed constructs can form molecular duplexes, whose structural pre-organization would be solely effected by two intramolecular hydrogen bondings.

1.10 Design principle

In an attempt to pre-organizing the H-bonding codes exclusively using intramolecular H-bonding as the force, we designed the molecular constructs **1a-e** and **2a-b,** based on amidinourea backbone. It was anticipated that the primary amino group in **1** and **2** could prefix the H-bonding face due to its possibility of participating in two intramolecular hydrogen bonding interactions, as depicted in fig. 1.13.
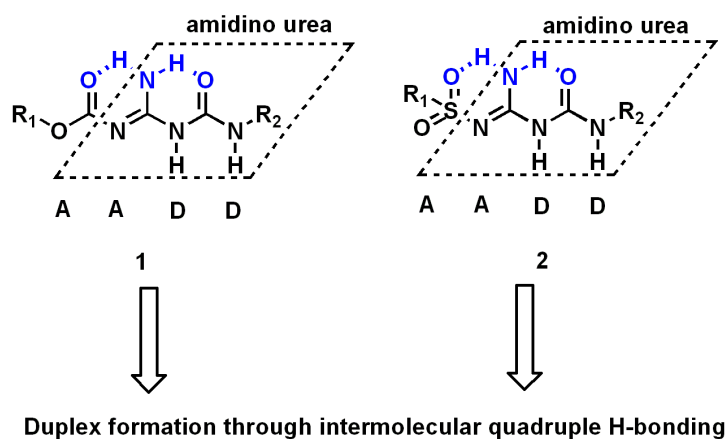
Fig. 1.13: Design principle for **1** and **2**.

As envisaged, structural analyses especially by single crystal X-ray diffraction studies showed that the pre-organization of the linear H-bonding arrays on amidinourea framework was effected with the help of two intramolecular H-bondings (*vide infra*).

1.11 Synthesis

The di-boc guanidine **3**[32] served as the common synthon for the preparation of the self-assembling monomer units **1** and **2**. *N*,*N*'-bis-boc guanidine **3** was reacted with primary amines in THF under reflux condition for 12 h yielding the carbamate protected compounds **1a,c,d,e**. Herein, the amine reacts with the *insitu* generated isocyanate to form an urea linkage.[33] The ^{t}Boc deprotection of **1a** on short exposure to DCM/TFA (1:1, v/v) and subsequent reaction with ethyl chloroformate yielded a similar analog, *N*-ethyl carbamate derivative **1b**. In order to evaluate the potential of sulfonamides in the pre-organization as well as in duplex formation, sulphonated amidinoureas **2a,b** (fig. 1.13) were synthesized by tosylation of the corresponding free amidinoureas obtained from **1a,e**, by deprotecting their ^{t}Boc group by short exposure to TFA/DCM (1:1, v/v).

Scheme 1.1: Synthesis of the quadruple self-assembling units **1a-e** and **2a,b**.


```
                              i    1 a. R1 = tBu, R2 = Bn, 65%
                             →        b. R1 = Et,   R2 = Bn, 61%    ← ii, iii
                                      c. R1 = tBu, R2 = iBu, 56%
                                      d. R1 = tBu, R2 = 2-pyridyl, 71%
                                      e. R1 = tBu, R2 = dodecyl, 58%

                                   2 a. R1 = p-Tol, R2 = Bn, 60%   ← ii, iv
                                     b. R1 = p-tol, R2 = dodecyl, 65%  ii, iv
```
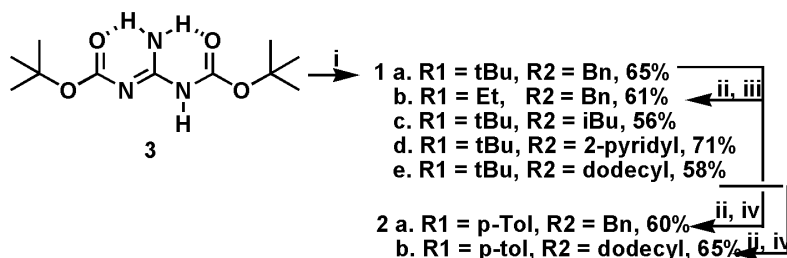
Reagents and conditions: (i) R$_2$NH$_2$, THF, reflux, 12h. (ii) TFA/DCM (1:1, v/v), 30 min. (iii) ClCO$_2$Et, Et$_3$N, DCM, 12h. (iv) p-Tos-Cl, Et$_3$N, DCM, 12h.

1.12 Structural investigation

The self-assembling propensities of **1a-e** and **2a,b** were investigated by X-ray crystallography, NMR studies and ESI mass spectrometry.

1.12.1 NMR Studies

Self-assembling modules **1** and **2** were readily soluble in non-polar solvents such as CDCl$_3$ which suggested that the NH protons were solvent shielded (fig.1.14).[34] However, detailed solution-state NMR studies, including the determination of stability constants could not be undertaken due to the existence of multiple conformations,[35] as evidenced by the broad proton resonances.
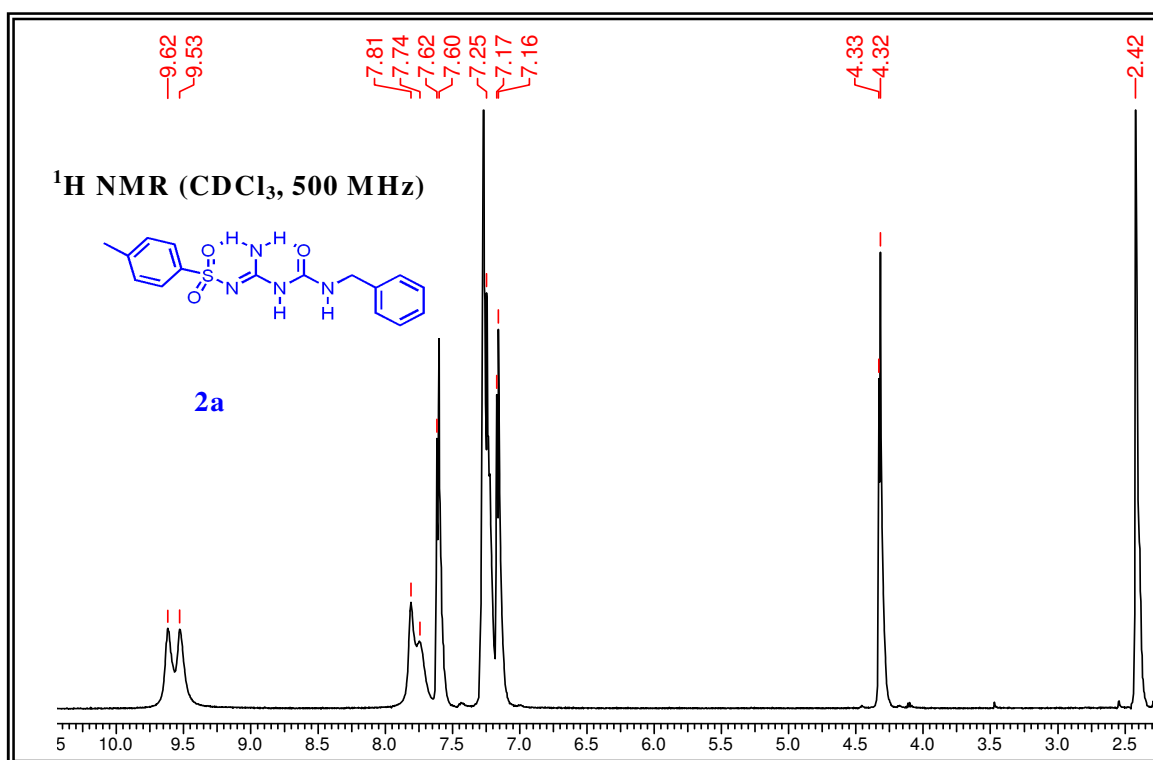
Fig. 1.14: ^{1}H NMR spectrum of **2a** in CDCl$_3$. The down field shifts of NHs indicate their participation in H-bonding.

1.12.2 ESI mass spectrometry

The formation of discrete dimers **1.1** and **2.2** was confirmed by ESI mass spectrometry (fig. 1.15). In addition to the molecular ion peaks $(M+H^+)$ ascribable due to the monomers **1** and **2**, ESI spectra showed sizable peaks corresponding to the dimers **1.1** (M_2+H^+) and **2.2** (M_2+H^+).
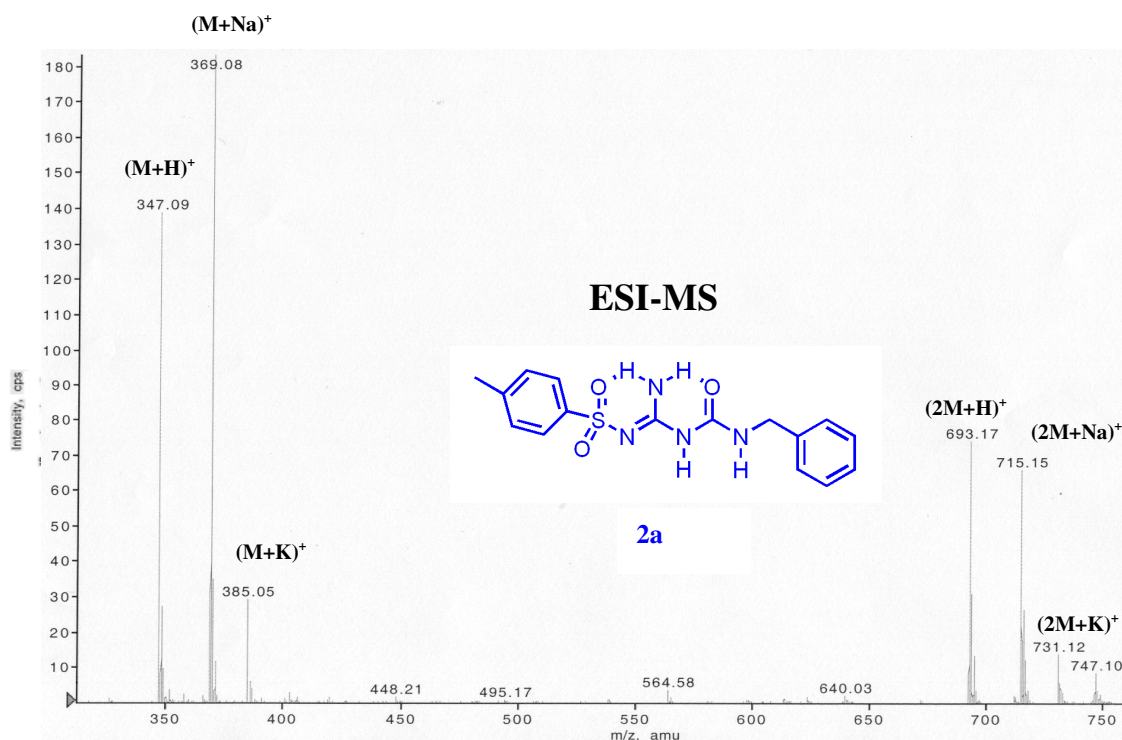
Fig. 1.15: The formation of duplex (**2a** as an example) by ESI-Mass spectrometry.

1.12.3 Single crystal X-ray diffraction studies

The crystallization of **1a-d** and **2a,b** was done either by the slow evaporation of a mixture of solvents or by the diffusion method.[36] In order to gain more insights into the self-assembling propensies of these self-assembling modules, a detailed crystal structure study of a diverse set of differentially substituted analogs was undertaken.
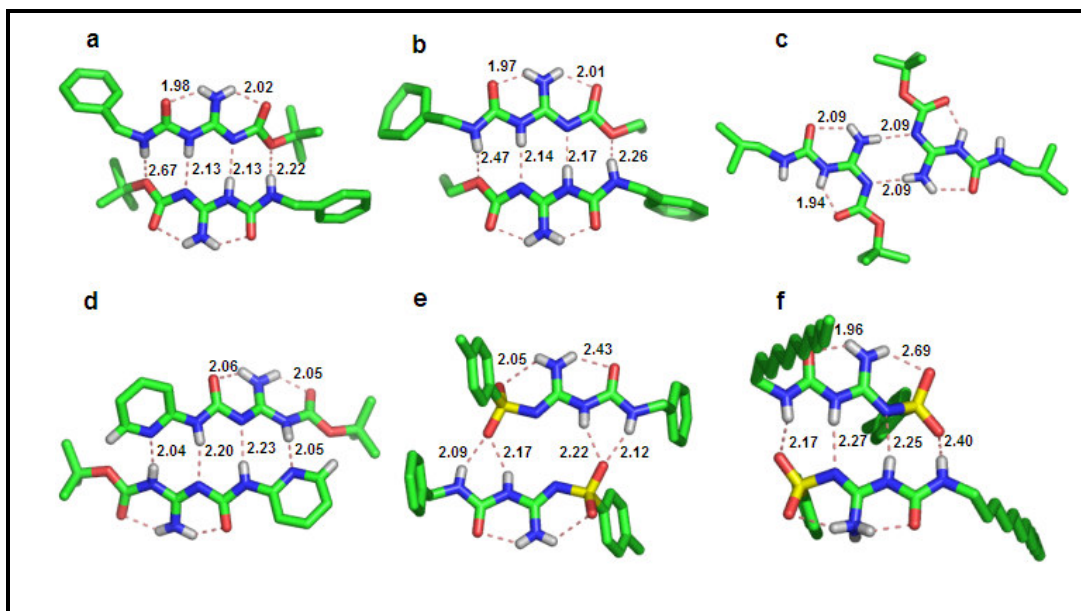
Fig. 1.16: Single crystal X-ray structures of dimers **a** (**1.1a**), **b** (**1.1b**), **c** (**1.1c**), **d** (**1.1d**), **e** (**2.2a**), and **f** (**2.2b**). Hydrogen bonding is highlighted in dashes (salmon colored), above which hydrogen bond distances (N–H····N and N–H····O) are displayed in Å. All hydrogens, other than at the hydrogen bonding sites, have been deleted for clarity. This figure was made using PyMOL.[37]

An exhaustive description about their pre-organization of H-bonding codes, the mode of arrangement of H-bonding codes, the factors encountered in the duplex formation and further assembly in the crystal lattice is given below. Comparison of the crystal structures of **1a-d** and **2a,b** revealed the following facts.

➢ All of the self-assembling constructs **1** and **2**, irrespective of the nature of substituents, underwent duplex formation through intermolecular hydrogen-bonding, although a substantial difference existed in the mode of their assembly.

➢ In all the cases, except in **1c,** the *programmed* double intramolecular hydrogen bonding, characterized by the graph set S(6),[38] was indeed proved to be

effective in pre-organizing the self-complementary AADD-type hydrogen bonding codes for duplex formation.

➢ Although, all of these self-assembling modules **1** and **2** were expected to dimerize through quadruple hydrogen bonding due to their similar AADD-type hydrogen bonding codes, substituents exerted a profound role in dictating the mode of hydrogen bonding in the duplex formation.

Table 1.3: The mode of arrangement in the duplex formed from **1a-d** and **2a,b**.

Name of the compound	Mode of arrangement of H-bonding codes	Duplex formed
1a	AADD	AADD.DDAA
1b	AADD	AADD.DDAA
1c	AD	AD.AD
1d	ADAD	ADAD.DADA
2a	AADD	Bifurcated
2b	AADD	AADD.DDAA

The key role of substituents in influencing the mode of hydrogen bonding in duplex formation is clearly evident in the comparison of crystal structures of **1a-d**. The closely resembling analogs **1a** and **1b**, differing only in their carbamate substituents, show the same trend in the duplex formation (**1.1a** and **1.1b**) through quadruple AADD-type intermolecular hydrogen bonding, as expected. The N-

isobutyl substituted analog **1c** formed duplex **1.1c** through N–H····N-type intermolecular double hydrogen-bonded interaction. The non-formation of a *quadruply* hydrogen-bonded duplex **1.1c** is presumably due to steric reasons since the formation of such a duplex, as in the case of **1.1a,b**, would have involved the closer positioning of the bulky N-isobutyl and *O-tert*-butyl substituents of the individual self-assembling strands.[39] The intermolecular hydrogen bonding interactions [graph set C(4)][3] in the duplexes **1a-d** are of medium strength[4] since the hydrogen bonding D–H····A distances [d(N–H····X); where X = N or O) are in the range 2.09-2.67 Å]. However, the S(6)-type intramolecular hydrogen bondings in the series **1a-d** remained relatively stronger[4] [d(N····O$_{avg}$) = 2.01 Å].

In an effort to investigate the influence of an additional acceptor atom on the N-substituent in modulating the hydrogen bonding codes in duplex formation, **1d** was synthesized. Surprisingly, **1d**, having a 2-pyridyl N-substituent,[40] formed ADAD-type quadruple hydrogen-bonded duplex; a mode of hydrogen bonding that is in stark contrast to what is observed in **1a-c**. It is noteworthy that heterocycle-based ADAD type self-assembling motifs whose linear hydrogen bonding arrays were pre-organized by a combination of covalent linking and intramolecular H-bonding have been reported in literature.[7] An interesting feature of the self-assembling constructs **1a-c** is the deployment of both carbamoyl oxygens (carbonyl and ether oxygens) in hydrogen-bonding interactions, a situation that is not commonly observed in self-assembling systems.[11,19] In the self-assembling constructs **1a-c**, the carbamoyl carbonyl oxygen atoms have been found to be involved in intramolecular hydrogen bonding, thereby pre-organizing the linear array of hydrogen bonding codes, while

the ether oxygen atom (of the carbamoyl moiety) takes part in self-assembly through intermolecular hydrogen-bonding interactions. However, the contribution of the intermolecularly hydrogen-bonding ether oxygen atom (of the carbamoyl moiety) towards the stability of the dimers and self-assembled ensembles would be minimal due to its reduced hydrogen-bonding acceptor potential.[41] Indeed, extensive studies have revealed that ester and lactone oxygens (both sp^3 and sp^2 oxygen atoms) are very poor hydrogen-bonding acceptors due to their reduced hydrogen-bonding acceptor potential, which in turn is due to their reduced Brønsted basicity.[42]

Sulfonamides have been used as flexible and polar peptidomimetic isosteres of carboxamides.[43] Further interest in this class of carboxamide isosteres stems from the fact that the more acidic N-H of sulfonamides may give rise to stronger hydrogen bonds.[44] In order to evaluate the potential of sulfonamides in our design strategy, the compounds **2a,b** were synthesized. Analysis of the crystal structures revealed that hydrogen bond-aided pre-organization as well as duplex formation was indeed realized, independent of the nature of the substituents, although the mode of hydrogen bonding was significantly affected, as happened in the case of **1a-d**. The dodecyl substituted **2b** formed duplex through AADD-type quadruple hydrogen bonding, as expected, whereas the analogous benzyl-substituted **2a** failed to dimerize in a similar manner, presumably due to the crystal-packing forces that may be influencing the adopted conformations and orientations. Instead, **2a** underwent dimerization through urea-type hydrogen bonding,[45,46] with the graph set C(4) [$R^1_2(6)$].[38] Remarkably, all the quadruply H-bonded duplexes (**1.1a,b,d,e** and **2.2a,b**) underwent further self-assembly using the not-so-common 4-membered ring intermolecular hydrogen

bonding interaction,[47] forming extended sheet-like supramolecular networks (fig. 1.17).
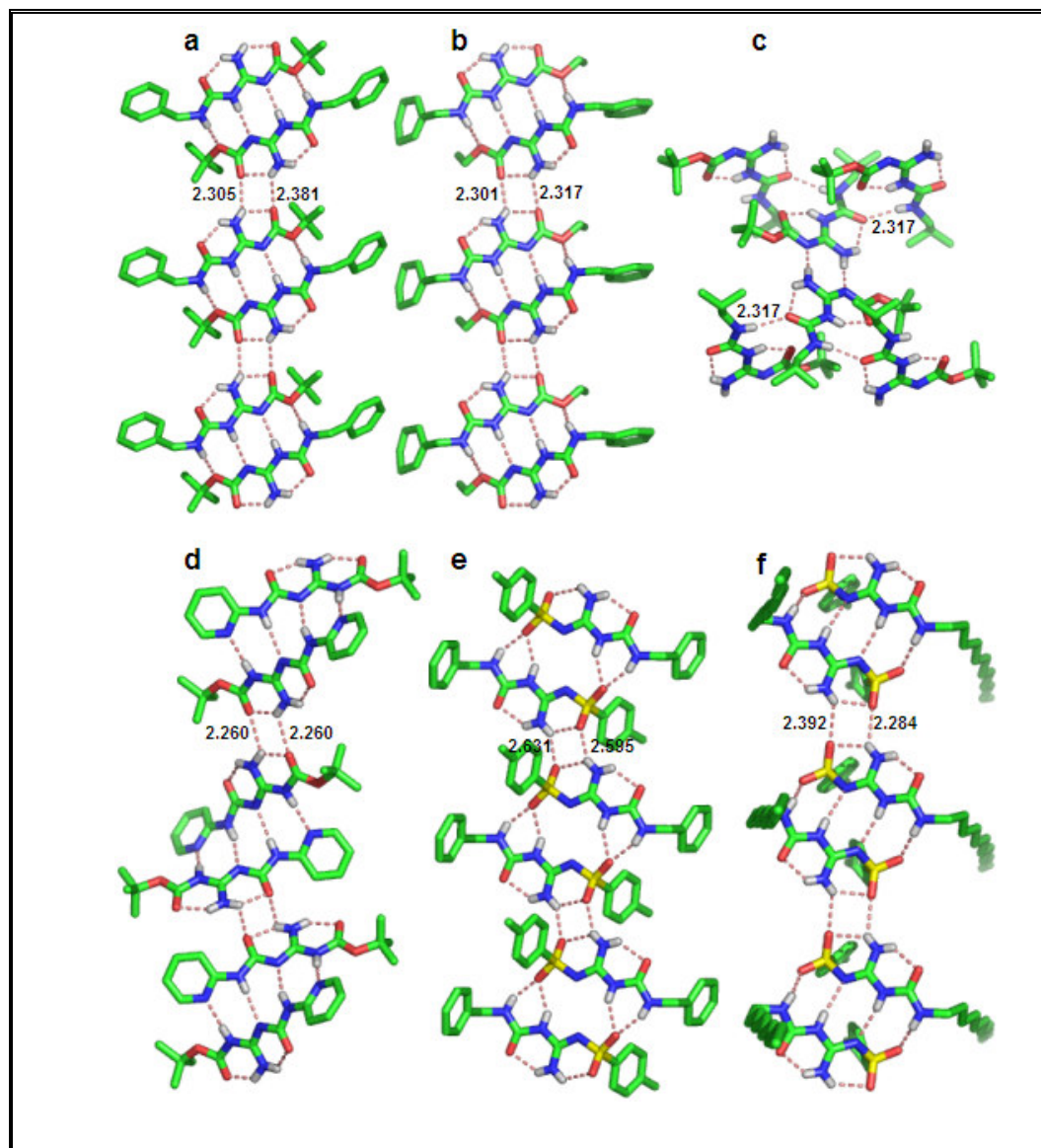

Fig. 1.17: One-dimensional packing in the crystal lattice.

Four-membered ring hydrogen bond-mediated self-assembly has been reported in certain nitro anilines,[47] wherein both the hydrogen bond acceptor oxygen atoms of nitro group bind a single proton, usually from a secondary aromatic amine, to form a

$[R^2_2(4)]$–type[38] four membered hydrogen-bonded network. Interestingly, the dimer **1.1c** that lacks predisposition of its N- and O substituents as in the other cases (**1a**,**b**,**d** and **2a**,**b**) self-assembles in a different fashion, affording a pillar-type supramolecular ensemble (fig 1.17c). The relatively longer hydrogen bonds $[d(\text{N-H-O}_{avg}) = 2.355$ Å$]$, in the four-membered ring intermolecular hydrogen bonding suggests that additional interactions, presumably from the cooperative hydrophobic interactions of the *N*-urea and *o*-carbamate substituents of the adjacent duplexes, might be involved in stabilizing these extended self-assembled networks. Indeed, such cooperative hydrophobic interactions are known to play a crucial role in aiding the self-assembly of peptide/protein β-sheets containing substantial amounts of hydrophobic amino acid residues.[48]

1.13 Conclusion

The ability of the self-assembling constructs **1** and **2**, both of them based on the amidinourea motif, to form molecular dimers whose structural pre-organization has been effected solely by intramolecular hydrogen bonding was studied by various techniques. The molecular dimers **1.1a**-**d** and **2.2a**,**b** underwent further self-assembly, affording supramolecular hydrogen-bonded networks. The ease with which the amidinourea motif could be manipulated to yield a diverse set of molecular dimers and their high propensity for crystallization are noteworthy. This novel way for facilitating the hydrogen bond-directed synthesis of molecular dimers can be extended for the pre-organization of much larger linear hydrogen-bonding arrays such that the construction of organic assemblies akin to those of nanoscale biological complexes may be realized.

Reactions of N,N',N''-tri-Boc Guanidine with Amines: Some Observations

"The idea of electrons, the stability of bonds between unlike atoms by special ionic character, as determined by the difference in electronegativity of the atoms of elements, actually introduced a clarification of chemical properties of inorganic substances that was quite extreme for its time. As a matter of fact, it still is."

-Linus Pauling-

1.14 Attempted synthesis of molecular duplexes with degenerate prototropy

The problem of prototropy observed in H-bonded duplexes considerably reduces the dimerization constant and renders their structural investigations tricky (see section 1.8). Towards this, we designed the molecule **4**, capable of displaying degenerate prototropy as we have described earlier with a different system.[19b] In this context, we planned an efficient synthetic strategy for the synthesis of **4** starting from tri-Boc guanidine (TBG) **1**, introduced first by Murray Goodman, a pioneer peptide chemist,[49] as a versatile guanidinylating reagent for the synthesis of *N*-substituted guanidines **3a** by its reaction with alcohols under Mitsunobu conditions.[50] A related trifluoro sulfonyl analog **2**, which is found to be one of the most powerful guanidinylating reagents so far, has also been introduced by Goodman's group for the efficient synthesis of substituted guanidines, under extremely mild conditions by its reaction with amines.[32] Herein, the reaction proceeds with the attack of amines at the

amidine carbon, knocking out the trifluoro sulfonamide moiety and cleanly furnishing

the protected guanidines **3b**.
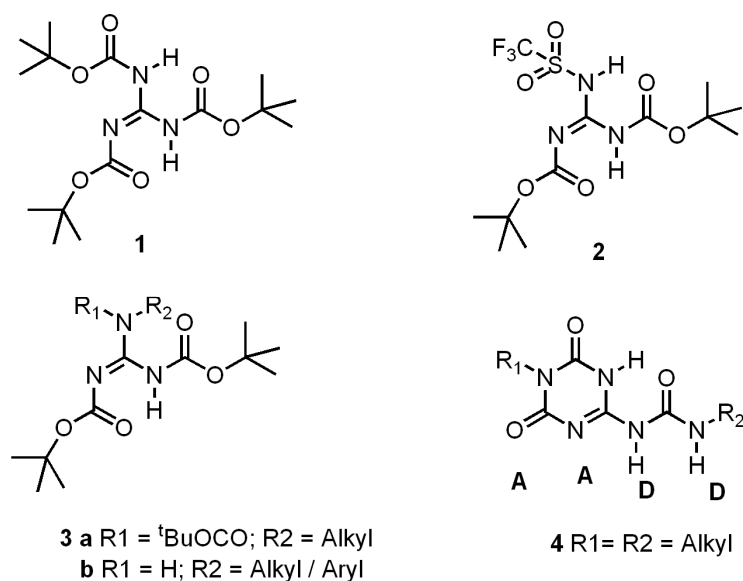
Fig. 1.18: Molecular structures of **1**, **2**, **3** and **4**.

It has already been mentioned in the previous section that di-Boc guanidine react with

amines to form *N*-alkyl amidinoureas[51] through the *in situ* generated intermediacy of

an isocyanate **6**.[33]
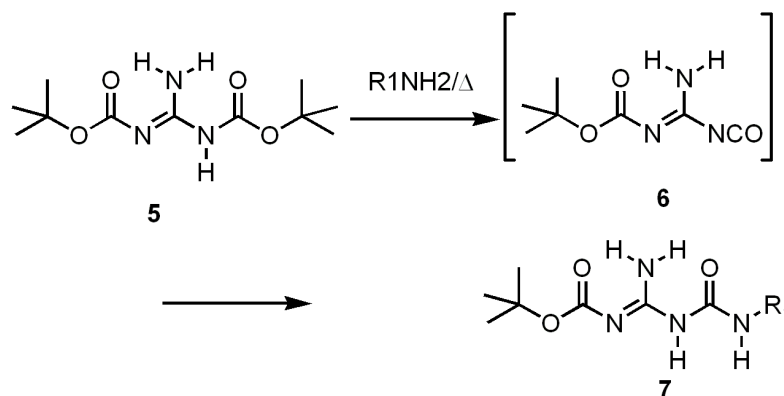
Fig. 1.19: Mechanism of reaction of di-Boc guanidine with amines.

Based on the reaction of di-Boc guanidine **5**, we anticipated that tri-Boc guanidine will also behave in the same way, eventually furnishing **11** through the intermediates **8** and **10**, which in turn could be subjected to cyclization to form the quadruple hydrogen bonded self-assembling system **4**.

Scheme 1.2: Reaction of TBG **1** with amines.
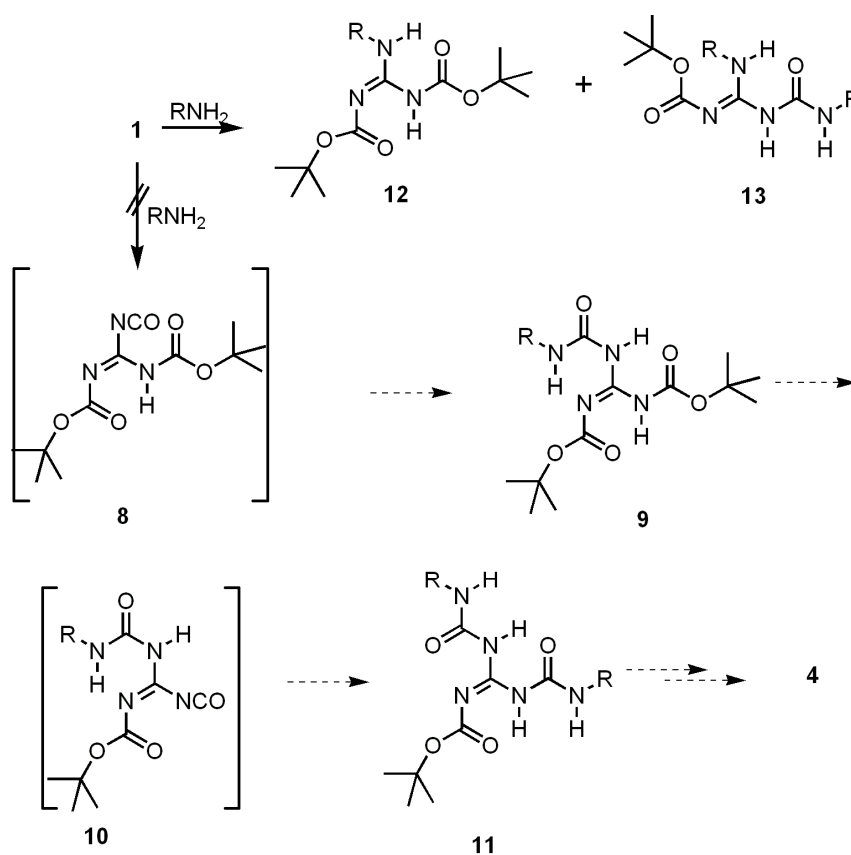
Unexpectedly, TBG reacted with primary amines at room temperature in an entirely different fashion affording *N*-substituted, *N',N''*-di-Boc guanidine **12**, and under reflux condition resulted in amidinourea derivative **13**. It should be noted that amidinoureas find considerable applications in medicinal chemistry;[52] for instance in

treating irritable bowel syndrome, gastrointestinal, spasmolytic and cardiovascular disorders and parasitic infestations. Furthermore, amidinourea has also been shown to be the essential structural feature of the potent antibacterial agent TAN-1057A-D,[53] a naturally occurring potent antibiotic dipeptide-amidinourea isolated from the bacteria *Flexibacter* sp. PK-74 and PK-176 (fig. 1.20).
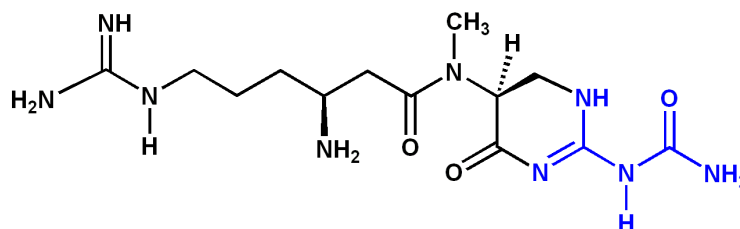
Fig. 1.20: The structure of TAN-1057-D (amidinourea backbone is shown in blue color.

Moreover, it has been noted that long chain *N*-substituted amidinoureas find industrial application as non-toxic and non-polluting stabilizers and dispersants (multifunctional) for middle distillate fuels.[54] The methods so far known in the literature for the preparation of amidinourea include, reaction of guanidines with isocyanates,[55] hydrogenation of 5-amino-3-amino-l,2,4-oxadiazoles,[56] reaction of carbamic esters with guanidine,[57] the hydrolysis of cyanoguanidines under strongly acidic condition,[58] and reaction of acyl-*S* methyl isothiourea with amines.[59]

Considering the importance of the work, we decided to explore further the reaction chemistry of TBG with various amines under different conditions.

1.15 Results and Discussion

A summarized view of the reactions performed under different conditions with the isolated yields is given in table 1.4.

Table 1.4: Reaction of TBG **1** with amines under different conditions

Entry	Amine	TBG: Amine (eq.)	Base /LA	Solvent, Temp.	12 (%)	13 (%)
1	Benzyl amine	1:1	- -	THF, rt	98	--
2	"	1:1	- -	THF, reflux	38	30
3	"	1:4	- -	THF, reflux	--	90
4	"	1:3	TMA	Toluene, reflux	20	30
5	Isobutyl amine	1:1	- -	THF, rt	60	--
6	"	1:1	- -	THF, reflux	50	22
7	"	1:3	- -	THF, reflux	--	86
8	"	1:1	DBU	THF, rt	61	--
9	"	1:1	DBU	DMSO, 50 $^{\circ}$C	40	40
10	Dodecyl amine	1:1	- -	THF, rt	97	--
11	"	1:3	- -	THF, reflux	--	91
12	Cyclohexyl amine	1:1	- -	THF, rt	52	--
13	"	1:3	- -	THF, reflux	--	60
14	Isopropyl amine	1:1	- -	THF, rt	40	--
15	"	1:4	--	THF, reflux	--	52
16	Aniline	1:1	--	THF, rt	--[a]	--[a]
17	Diethyl amine	1:1	--	THF, rt	--[a]	--[a]

LA: Lewis acid, TMA : Trimethyl aluminium; [a] intractable mixture of products were obtained.

The reactions of TBG **1** with amines under different conditions were investigated in order to understand the reaction pattern. The results obtained as described in the table 1.4 suggest that, reaction of **1** with amines can be manipulated to afford either *N*-substituted guanidines **12** or *N*-alkyl amidinoureas **13** under relatively mild reaction conditions. Unlike the reaction of di-Boc guanidine **5** with amine[51] that affords substituted amidinoureas **7** through the isocyanate mechanism, TBG reacted with primary amines at room temperature resulting in the formation of corresponding *N*-alkylated di-Boc guanidines, **12** (entry 1 and 5, table 1.4), in good yield. Enhancement in the rate of the reaction as well as the yield was examined by carrying out the reaction at elevated temperature and also by the addition of external base (DBU). When primary amines were reacted with TBG **1** under reflux condition in 1:1 ratio, both *N*-substituted guanidines **12** and amidinoureas **13** were obtained (entry 2 and 6, table 1.4). It is noteworthy that the use of excess amines under reflux condition dramatically increased the yield of **13** and the amidinoureas were the only products isolated under this condition (entry 3 and 7, table 1.4). In the presence of strong base like DBU, the reaction of TBG with isobutyl amine in equimolar ratio under ambient condition proceeded with no significant difference in the yield of substituted guanidine **12** (compare entries 5 and 8, table 1.4). However, raising the reaction temperature had a minor effect in the product distribution (compare entries 6 and 9, table 1.4). In order to understand the reaction pattern of aromatic amines, TBG was reacted with aniline (entry 16, table 1.4). However, this reaction led to the formation of intractable mixture of products. Similar fate was met with secondary amine as well (entry 17, table 1.4). Finally, in an effort to intimidate TBG to react with amines to

afford **11**, which could have been cyclo-condensed to furnish the self-assembling system **4**, TBG was reacted with excess benzyl amine in the presence of trimethyl aluminium (TMA). However, this reaction led to the formation of a mixture *N*-substituted guanidine and amidinourea (entry 4, table 1.4).

1.16 Proposed mechanism for the reaction

These observations suggested that the *N*-substituted guanidines **12** were formed first by the nucleophilic attack of amines at the highly electrophilic quarternary carbon of TBG that is flanked by three carbamate groups. Further reaction of **12**, presumably proceeding through the isocyanate intermediate **14**, afforded the *N*-substituted amidinoureas **13** (fig.1.21).
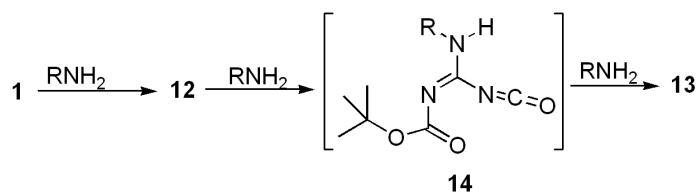
Fig. 1.21: Proposed mechanism of reaction of TBG **1** with amines

The formation of **12** at room temperature and **13** at elevated temperature strongly suggested that the formation of **12** *precedes* **13** and that even strong bases like DBU failed to intimidate TBG to form isocyanate intermediate **8** that could have led to the formation of **9** (scheme 1.2). The difference in the reaction when compared with di-Boc guanidine may be due to the increased electrophilicity at the quaternary carbon of guanidine moiety due to the additional Boc group.

1.17 Conclusion

This work demonstrated that TBG reacts in an unusual manner with amines under various conditions, which is in stark contrast to the manner by which its predecessor di-Boc guanidine **5** undergoes reaction. Our finding suggests that TBG can act as an excellent readily available and common starting material for the selective synthesis of both *N*-alkyl guanidines and amidinoureas in good yield by its reaction with primary aliphatic amines by manipulating the reaction conditions. Thus, our novel methodology finds application in selectively obtaining potentially important *N*-alkyl guanidine and amidinourea backbones.

1.18 Experimental section

Single Crystal X-ray Crystallographic Studies

The X-ray data of **1a-d** and **2a,b** were collected on a SMART APEX CCD single crystal X-ray diffractometer with omega and phi scan mode and different number of scans and exposure time for different crystals using λ MoK$_\alpha$ = 0.71073 Å radiation, at T = 293 (2) K with oscillation / frame -0.3°, maximum detector swing angle = -30.0°, beam center = (260.2, 252.5), in plane spot width = 1.24. All the data were corrected for Lorentzian, polarization and absorption effects using SAINT and SADABS programs. The crystal structures were solved by direct method using SHELXS-97 and the refinement was performed by full matrix least squares of F^2 using SHELXL-97.

Crystal Data for 1a ($C_{14}H_{20}N_4O_3$): M = 292.34, Crystal dimensions 0.51 x 0.34 x 0.19 mm, quadrant data acquisition, total scans = 4, total frames = 2424, exposure / frame = 20.0 sec / frame, θ range = 2.06 to 25.0°, completeness to θ of 25.0° is 99.9%, monoclinic, space group P2_1/c, a = 10.1570 (4), b = 10.0700 (4), c = 31.991 (1) Å, β = 103.547 (1)°, V = 3181.0 (2) Å^3, Z = 8, D$_c$ = 1.221 mg m^{-3}, μ (Mo K$_\alpha$) = 0.088 mm^{-1}, 29226 reflections measured, 5564 unique [I>2σ(I)], R = 0.0657, wR2 = 0.1621

Crystal Data for 1b ($C_{12}H_{16}N_4O_3$): M = 264.29, Colorless crystal, approximate size 0.23 x 0.22 x 0.15 mm, multi scan data acquisition, total scans = 3, total frames = 1818, exposure / frame = 7.0 sec / frame, θ range = 1.84 to 23.50°, completeness to θ of 23.50° is 99.8%, monoclinic, space group C2/c, a = 22.528 (4), b = 10.072 (2), c = 24.147 (5) Å, β = 101.265 (4) °, V = 5373.4 (18) Å^3, Z = 8, D$_c$ = 1.307 mg m^{-3}, μ (Mo

K_α) = 0.096 mm^{-1}, 11503 reflections measured, 3968 unique [I>2σ(I)], R = 0.0804, wR2 = 0.1386.

 Crystal Data for 1c (C$_{11}$H$_{22}$N$_4$O$_3$): *M* = 258.33, crystal size 0.48 x 0.16 x 0.01 mm, multi scan data acquisition, total scans = 5, total frames = 3030, exposure / frame = 20.0 sec / frame, θ range = 2.28 to 25º, completeness to θ of 25º is 99.9%, monoclinic, space group P2$_1$/c, *a* = 11.650 (1), *b* = 15.143 (2), *c* = 8.922(1) Å, β = 108.742 (3)º, V = 1490.7 (3) Å^3, *Z* = 4, D$_c$ = 1.151 mg m^{-3}, μ (Mo K$_\alpha$) = 0.085 mm^{-1}, 7412 reflections measured, 2620 unique [I>2σ(I)], R = 0.1065, wR2 = 0.2864.

 Crystal Data for 1d (C$_{12}$H$_{17}$N$_5$O$_3$): *M* = 279.30, crystal dimensions 0.25 x 0.17 x 0.16 mm, multi scan data acquisition, total scans = 5, total frames = 3030, exposure / frame = 20.0 sec / frame, θ range = 2.16 to 25.00º, completeness to θ of 25º is 99.7%, triclinic, space group P1̄, *a* = 9.4903 (6), *b* = 11.1867 (7), *c* = 16.240 (1) Å, α = 84.059 (1)º, β = 78.766 (1)º, γ = 82.300 (1)º, V = 1670.5 (2) Å^3, *Z* = 2, D$_c$ = 1.196 mg m^{-3}, μ (Mo K$_\alpha$) = 0.087 mm^{-1}, 17167 reflections measured, 5877 unique [I>2σ(I)], R = 0.0629, wR2 = 0.1558. There are two molecules along with disordered n-hexane molecule as a solvent of crystallization 2[(C$_{12}$H$_{17}$N$_5$O$_3$). 0.5(C$_6$H$_{14}$)].

 Crystal Data for 2a (C$_{16}$H$_{18}$N$_4$O$_3$S): *M* = 346.40, colorless crystal, approximate size 0.487 x 0.176 x 0.062 mm, quadrant data acquisition, total scans = 4, total frames = 2424, exposure / frame = 10.0 sec / frame, θ range = 2.19 to 25.0º, completeness to θ of 25.0º is 99.9%, monoclinic, space group C*c*, *a* = 15.7330 (8), *b* = 11.6432 (6), *c* = 19.019 (1) Å, β = 102.185 (1)º, *V* = 3405.5 (3) Å^3, *Z* = 4, D$_c$ = 1.351 mg m^{-3}, μ (Mo K$_\alpha$) = 0.212 mm^{-1}, 14425 reflections measured, 5966 unique [I>2σ(I)], R = 0.0505,

wR2 = 0.1221.

Crystal Data for 2b ($C_{21}H_{36}N_4O_3S$): M = 424.60, colorless crystal, approximate size 0.52 x 0.10 x 0.05 mm, multi scan data acquisition., total scans = 5, total frames = 3030, exposure / frame = 10.0 sec / frame, θ range = 1.55 to 25°, completeness to θ of 25° is 99.7%, triclinic, space group P$\bar{1}$, a =10.2504 (6), b = 13.3758 (8), c = 17.822 (1) Å, α = 90.941 (1)°, β = 94.799 (1)°, γ = 99.773 (1)°, V = 2398.5 (2) Å^3, Z = 2, D$_c$ = 1.176 mg m^{-3}, μ (Mo K$_\alpha$) = 0.162 mm^{-1}, 26145 reflections measured, 8428 unique [I>2σ(I)], R = 0.0939, wR2 = 0.2446.

Experimental procedures:

[Amino-(3-Benzyl-ureido)-methylene]-carbamic acid *tert*-butyl ester 1a:

A mixture of *N,N'*-di-boc-guanidine (1.3 g, 5 mmol, 1 equiv.) and benzyl amine (1.6 mL, 15 mmol, 3 equiv.) in dry THF (10 mL) was refluxed with stirring for 12 h. The solvent was evaporated under reduced pressure and the residue was subjected to purification by column chromatography (50% pet. ether/ethyl acetate, R$_f$: 0.7) to yield a colorless sticky compound which was crystallized from chilled chloroform to yield **1a** (65%). mp 110 ^{o}C; IR (CHCl$_3$) ν (cm^{-1}): 3396, 3269, 3013, 2980, 2934, 1647, 1547, 1508, 1454, 1302, 1244, 1148; ^{1}H NMR (500 MHz, CDCl$_3$) δ: 8.65 (br, 3H), 7.33-7.26 (m, 5H), 6.25 (bs, 1H), 4.36 (d, J = 5.5 Hz, 2H), 1.47 (s, 9H); ^{13}C NMR (125 MHz, CDCl$_3$) δ: 158.3, 138.6, 128.5, 127.2, 82.0, 43.7, 28.0; ESI Mass: 293.04 (M$^+$+1), 586.02 (2M$^+$+2); Anal. Calcd. for $C_{14}H_{20}N_4O_3$: C, 57.52; H, 6.90; N, 19.16. Found: C, 57.28; H, 7.10; N 19.32.

[Amino-(3-benzyl-ureido)-methylene]-carbamic acid ethyl ester 1b:
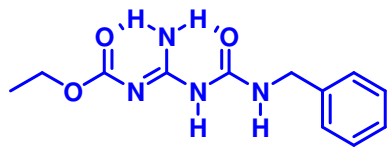
To a 1:1 mixture of TFA and DCM (10 mL) was added **1a** (300 mg, 1.0 mmol) at room temperature. After stirring for 30 min., the reaction mixture was stripped off the volatiles under reduced pressure and the waxy residue was dissolved in DCM (5 mL). Triethyl amine (0.43 mL, 3.1 mmol, 3 equiv.) was added to the above reaction mixture, followed by the addition of ethylchloroformate (0.1 mL, 1.0 mmol, 1 equiv.). After stirring for overnight, the reaction mixture was diluted with DCM (10 mL), washed successively with saturated bicarbonate solution and water and the organic was layer separated and dried over anhydrous Na_2SO_4. Evaporation of DCM under reduced pressure, followed by purification of the crude product by column chromatography (50% petroleum ether/ ethyl acetate, R_f: 0.5) gave **1b** as colorless solid (61%) which could be crystallized by diffusion of pet. ether into a solution of the compound in ethyl acetate. mp: 139-140 ^{o}C; IR (CHCl$_3$) ν (cm^{-1}): 3384, 3288, 3018, 2401, 2361, 1672, 1626, 1547, 1370, 1288, 1231, 1140; ^{1}H NMR (500 MHz, CDCl$_3$) δ: 10.67-8.80 (br, 3H), 7.34-7.25 (m, 5H), 6.41 (bs, 1H), 4.39 (d, J = 5.5 Hz, 2H), 3.99 (bs, 2H), 1.18 (bs, 3H); ^{13}C (125 MHz, CDCl$_3$) δ: 161.7, 160.4, 157.1, 138.1, 128.7, 127.4, 61.3, 43.7, 14.3; ESI Mass: 265.20 (M$^+$ +1), 529.39 (2M$^+$ +1); Anal. Calcd for $C_{12}H_{16}N_4O_3$: C, 54.54; H, 6.10; N, 21.20. Found: C, 54.38; H, 6.22; N, 21.28.

[Amino-(3-isobutyl-ureido)-methylene]-carbamic acid *tert*-butyl ester 1c:

Following the same procedure for synthesis of compound **1a**, and using isobutyl amine (0.2 mL, 1.8 mmol, 3 equiv.), **1c** was synthesized. Purification was effected by
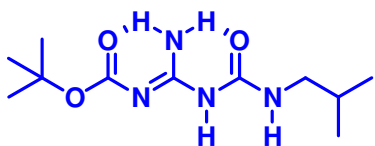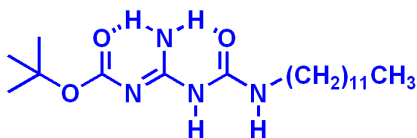
column chromatography (60% petroleum ether/ ethyl acetate, R_f: 0.4) to yield **1c** (56%) as a waxy compound that could be crystallized from methanol to yield needle shaped crystals. mp: 128-130 °C; IR (CHCl$_3$) ν (cm^{-1}): 3395, 3018, 2964, 1690, 1641, 1308, 1215, 1145; ^{1}H NMR (500 MHz, CDCl$_3$) δ: 12.08-8.41 (br, 3H), 5.57 (bs, 1H), 3.04 (br, 2H), 1.77 (m, 1H), 1.48 (s, 9H), 0.92 (d, *J* = 6.1 Hz, 6H); ^{13}C (125 MHz, CDCl$_3$) δ: 158.5, 81.7, 47.2, 28.6, 28.1, 20.0; ESI Mass: 259.04 (M$^+$+1), 518.02 (2M$^+$+2); Anal. Calcd for C$_{11}$H$_{22}$N$_4$O$_3$: C, 51.16; H, 8.52; N, 21.7. Found: C, 51.32; H, 8.78; N, 21.59.

[Amino-(pyridin-2-ylcarbamoylimino)-methyl]-carbamic acid *tert*-butyl ester 1d:
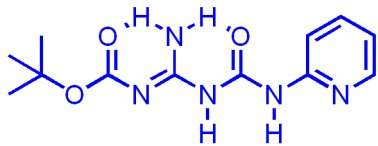
Following the same procedure for synthesis of compound **1a**, and using N-,N'-di-boc-guanidine (164 mg, 0.6 mmol, 1 equiv.) and 2-aminopyridine (179 mg, 1.9 mmol, 3 equiv.), **1d** was synthesized. Purification was effected by column chromatography (70% pet. ether/ ethyl acetate, R_f: 0.4) to yield **1d** (71%) as a waxy compound that could be crystallized from a mixture of ethyl acetate / DCM and pet. ether. mp: 158 °C; IR (CHCl$_3$) ν (cm^{-1}): 3394, 3219, 3175, 3016, 2984, 1724, 1655, 1605, 1578, 1526, 1433, 1394, 1369, 1304, 1258, 1236, 1150; ^{1}H NMR (500 MHz, CDCl$_3$) δ: 12.09 (bs, 1H), 10.81 (s, 1H), 9.51 (s, 1H), 8.67 (s, 1H), 8.25 (d, *J* = 4.2 Hz, 1H), 7.95 (d, *J* = 7.9 Hz, 1H), 7.66 (m, 1H), 6.95 (m, 1H), 1.53 (s, 9H); ^{13}C (125 MHz, CDCl$_3$) δ: 163.5, 159.9, 154.4, 153.4, 146.7, 138.3, 117.7, 115.5, 82.7, 28.2; ESI Mass: 280.19 (M$^+$+1), 559.41 (2M$^+$+1); Anal. Calcd for C$_{12}$H$_{17}$N$_5$O$_3$: C, 51.61; H, 6.14; N, 25.07. Found: C, 51.44; H, 6.32; N, 25.14.

[Amino-(3-dodecyl-ureido)-methylene]-carbamic acid *tert*-butyl ester 1e:

Following the same procedure for synthesis of compound **1a**, and using *N, N'*-di-boc-guanidine (1 g, 3.9 mmol, 1 equiv.) and dodecyl amine (2.6 mL, 11.6 mmol, 3 equiv.) **1e** was synthesized. Purification was effected by column chromatography (75% petroleum ether/ ethyl acetate, R_f: 0.8) to yield **1e** (58%) as a waxy compound. IR (CHCl$_3$) ν (cm^{-1}): 3394, 3018, 2928, 2854, 1647, 1543, 1302, 1238, 1215, 1147; ^{1}H NMR (200 MHz, CDCl$_3$) δ: 8.53-7.50 (br, 2H), 5.84 (bs, 1H), 4.73 (bs, 1H), 3.20 (q, *J* = 6.6 Hz, 2H), 1.49-1.25 (m, 29H), 0.88 (t, *J* = 6.7 Hz, 3H); ^{13}C (50 MHz, CDCl$_3$) δ: 179.1, 158.1, 156.9, 79.6, 39.9, 31.6, 29.7, 29.6, 29.5, 29.3, 28.2, 28.1, 26.8, 22.6, 14.0; ESI Mass: 371.40 (M$^+$ +1), 741.79 (2M$^+$ +1).

N-[amino-3-benzyl-ureido)-methylene]-4-methyl-benzenesulfonamide 2a:
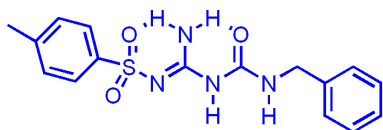
2a was synthesized from **1a** (300 mg, 1.0 mmol, 1 equiv.), following the same procedure for synthesis of compound **1b**, and using p-toluenesulfonylchloride (196 mg, 1.0 mmol, 1 equiv.). Purification was done by column chromatography (60% pet. ether/ethyl acetate, R_f: 0.7) to yield **2a** as a white solid (60%) which could be crystallized from chilled ethyl acetate. mp: 180-181 ^{o}C; IR (nujol) ν (cm^{-1}): 3377, 3271, 3229, 2924, 2854, 1711, 1633, 1582, 1549, 1456, 1261, 1224, 1155, 1142; ^{1}H NMR (500 MHz, CDCl$_3$) δ: 9.62 (s, 1H), 9.53 (s, 1H), 7.81 (s, 1H), 7.74 (bs, 1H), 7.61 (d, *J* = 7.9 Hz, 2H), 7.26-7.23 (m, 5H), 7.17 (d, *J* = 7.9 Hz, 2H), 4.32 (d, *J* = 5.7 Hz, 2H), 2.42 (s, 3H); ^{13}C (125 MHz, CDCl$_3$) δ: 156.8, 154.7, 142.8, 138.8, 137.6, 129.2, 128.3, 127.4, 127.1, 125.8, 43.5, 21.3; ESI Mass: 347.09 (M$^+$+1), 693.17 (2M$^+$+1); Anal. Calcd for

$C_{16}H_{18}N_4O_3S$: C, 55.49; H, 5.24; N, 16.18; S, 9.25. Found: C, 55.43; H, 5.33; N, 16.04; S, 8.94.

### N-[amino-3-dodecyl-ureido)-methylene]-4-methyl-benzenesulfonamide 2b:

Compound **2b** was synthesized from **1e** (500 mg, 1.4 mmol, 1 equiv.), following the same procedure for synthesis of compound **2a** and using p-toluene sulfonyl chloride (258 mg, 1.4 mmol, 1 equiv.). Purification was effected by column chromatography (75% petroleum ether/ethyl acetate, R_f: 0.7) to yield **2b** as a white solid (65%) which could be crystallized from chilled ethyl acetate. mp: 116-117 $^{\circ}$C; IR (CHCl$_3$) ν (cm^{-1}): 3402, 3315, 3202, 2928, 2854, 2361, 2331, 1708, 1630, 1553, 1466, 1261, 1225, 1151, 1138, 1086, 856, 758, 669; ^{1}H NMR (500 MHz, CDCl$_3$) δ: 9.62 (s, 1H), 9.56 (s, 1H), 7.84 (s, 1H), 7.67 (d, *J* = 7.9 Hz, 2H), 7.27 (bs, 1H), 7.21 (d, *J* = 8.5 Hz, 2H), 3.17 (q, *J* = 6.7 Hz, 6.1 Hz, 2H), 2.45 (s, 3H), 1.59 (m, 2H), 1.51-1.47 (m, 2H), 1.27-1.22 (m, 16H), 0.86 (t, *J* = 6.7 Hz, 3H); ^{13}C (500 MHz, CDCl$_3$) δ: 157.2, 154.8, 142.9, 139.3, 129.5, 126.0, 40.1, 31.9, 29.6, 29.6, 29.5, 29.3, 29.2, 29.2, 26.8, 22.7, 21.6, 14.1; ESI Mass: 425.37 (M$^+$+1), 849.71 (2M$^+$+1); Anal. Calcd for $C_{21}H_{36}N_4O_3S$: C, 59.40; H, 8.55; N, 13.2; S, 7.55. Found: C, 59.66; H, 8.48; N, 13.12; S, 7.50.

PART B: *N,N',N''*-tri-Boc-guanidine was prepared according to the literature procedure, by reacting guanidine hydrochloride with Boc-anhydride.[50]

N,N'-bis (*tert*-butyloxycarbonyl)-*N''*-benzyl guanidine, 12a[32,60]

To a solution containing *N,N',N''*-tri-Boc-guanidine (200 mg, 0.6 mmol, 1equiv.) in 10 mL THF, benzyl amine (0.06 mL, 0.6 mmol, 1equiv.) was added and stirred

overnight at rt. The solvent was stripped off under reduced pressure to get the product
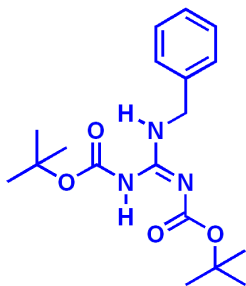
which on purification by column chromatography (9:1 pet. ether/ethyl acetate, R_f: 0.7) to yield **12a** (190 mg, yield: 98%) mp: 98-100 ^{o}C. Crystallized the compound from a solution mixture of ethyl acetate and pet ether. IR (CHCl$_3$) ν (cm^{-1}) 3329,

3288, 3007, 2982, 2930, 1720, 1634, 1414, 1367, 1329, 1252, 1229, 1157, 1132,

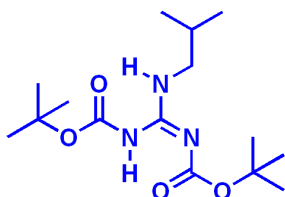1057; ^{1}H NMR (200 MHz, CDCl$_3$) δ: 11.55 (s, 1H), 8.62 (s, 1H), 7.37-7.29 (m, 5H),

4.66-4.64 (d, J = 5.21 Hz, 2H), 1.53 (s, 9H), 1.49 (s, 9H); ^{13}C (50 MHz, CDCl$_3$) δ:

163.6, 156.1, 153.2, 137.3, 128.8, 127.8, 83.2, 79.4, 45.1, 28.3, 28.1; ESI Mass:

350.20 (M+H)$^+$, 372.20 (M+ Na)$^+$; Anal. calcd for C$_{18}$H$_{27}$N$_3$O$_4$: C, 61.87; H, 7.79; N,

12.03. Found: C, 61.74; H, 7.89; N, 12.15.

N, N'-bis (*tert*-butyloxycarbonyl)-*N''*-isobutyl guanidine, 12b[61]

The above procedure for the synthesis of **12a** was repeated with isobutyl amine (200 mg, 0.6 mmol, 1 equiv.) and the crude product obtained after the complete evaporation of

THF was purified by 100-200 mesh silica gel column chromatography (9:1 pet ether/

ethyl acetate R_f: 0.8) to get **12b** (105 mg, yield: 60 %) as a solid compound which can

be crystallized from a mixture of ethyl acetate and pet ether. mp: 96-98 ^{o}C; IR

(CHCl$_3$) ν (cm^{-1}) 3329, 3288, 3152, 3018, 2984, 2968, 1722, 1638, 1634, 1416, 1367,

1331, 1306, 1215, 1136, 1055; ^{1}H NMR (200 MHz, CDCl$_3$) δ: 11.51 (bs, 1H), 8.40

(bs, 1H), 3.28-3.22 (m, 2H), 1.85-1.78 (m, 1H), 1.50(s, 9H), 1.49 (s, 9H) 0.97-0.94 (d,

J = 6.54 Hz, 6H); ^{13}C (50 MHz, CDCl$_3$) δ: 163.7, 156.3, 153.4, 83.0, 79.2, 48.3, 28.3,

28.1, 27.8, 20.2; ESI Mass: 316.22 $(M+H)^+$, 338.21 $(M+Na)^+$; Anal. Calcd for $C_{15}H_{29}N_3O_4$: C, 57.12; H, 9.27; N, 13.32. Found: C, 57.28; H, 9.16; N, 13.40.

N, N'-bis (*tert*-butyloxycarbonyl)-*N''*-cyclohexyl guanidine, 12c[32]
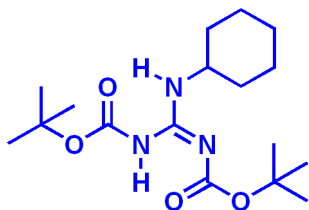
The above procedure for the synthesis of **12a** was repeated cyclohexyl amine and the crude product obtained after the complete evaporation of THF was purified by column chromatography (9:1 pet ether/ethyl acetate, R_f: 0.7), white sold. 110 mg, 58%, IR (CHCl$_3$) ν (cm^{-1}) 3325, 3277, 3018, 2984, 2936, 2858, 1719, 1634, 1614, 1418, 1367, 1340, 1215, 1151, 1132, 1057, 1028; ^{1}H NMR (500 MHz, CDCl$_3$) δ: 11.54 (s, 1H), 8.33-8.31 (d, *J* = 6.64 Hz, 1H), 4.06-4.01 (m, 1H), 1.95-1.20 (m, 12H), 1.51 (s, 9H), 1.49 (s, 9H); ^{13}C (125 MHz, CDCl$_3$) δ: 163.9, 155.3, 153.3, 82.9, 79.0, 48.6, 32.8, 28.3, 28.1, 25.5, 24.4; ESI Mass: 342.14 $(M+H)^+$, 364 $(M+Na)^+$; Anal. Calcd. for $C_{17}H_{31}N_3O_4$: C, 59.80; H, 9.15; N, 12.31. Found: C, 59.88; H, 9.23; N, 12.42.

N, N'-bis (*tert*-butyloxycarbonyl)-*N''*-dodecyl guanidine, 12d

The crude product obtained after the complete evaporation of THF was purified by column chromatography (9.1 pet ether/ethyl acetate, R_f: 0.8), white sold. 230 mg, 97%, IR (CHCl$_3$) ν (cm^{-1}) 3335, 2928, 2856, 1719, 1636, 1618, 1418, 1369, 1339, 1215, 1157, 1134, 1053; ^{1}H NMR (500 MHz, CDCl$_3$) δ: 11.51 (s, 1H), 8.30 (m, 1H), 3.42-3.38 (m, 2H), 1.66-1.26 (m, 20H), 1.51 (s, 9H), 1.50 (s, 9H), 0.90-0.87 (t, *J* = 6.93 Hz, 7.41 Hz, 3H); ^{13}C (125 MHz, CDCl$_3$) δ: 163.7, 156.1, 153.3, 83.0, 79.2, 41.0, 31.9, 29.6, 29.5, 29.3, 29.2, 29.0, 28.3, 28.1, 26.7, 22.7, 14.1;

ESI Mass: 428.19 (M+H)$^+$, 450.14 (M+Na)$^+$; Anal. Calcd for C$_{23}$H$_{45}$N$_3$O$_4$: C, 64.60; H, 10.61; N, 9.83. Found: C, 64.52; H, 10.48; N, 9.75.

N, *N'*-bis (*tert*-butyloxycarbonyl)-*N''*-isopropyl guanidine, 12e
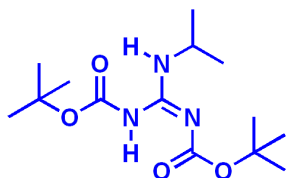
Purification of the compound was done with column chromatography (eluent : 9:1 pet ether/ethyl acetate, R$_f$: 0.6), White solid, 75 mg, 45%; ^{1}H NMR (400 MHz, CDCl$_3$) δ:

11.50 (s,1H), 8.20-8.18 (d, *J* = 7.16 Hz, 1H), 4.35-4.27 (m, 1H), 1.49 (s, 9H), 1.48 (s, 9H), 1.19-1.18 (d, *J* = 8.21 Hz , 6H); ^{13}C (125 MHz, CDCl$_3$) δ: 163.8, 155.2, 153.3, 82.9, 79.0, 28.3, 28.1, 22.7; ESI Mass: 302.35 (M+H)$^+$, 324.33 (M+Na)$^+$; Anal. Calcd. for C$_{14}$H$_{27}$N$_3$O$_4$: C, 55.79; H, 9.03; N, 13.94. Found: C, 55.86; H, 9.14; N, 13.82.

[Benzylamino-(3-benzyl-ureido)-methylene]-carbamic acid *tert*-butyl ester, 13a[33]
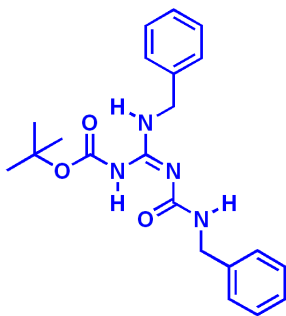
To a solution of N,N',N''-tri-Boc-guanidine (200 mg, 0.6 mmol, 1equiv.) in 10mL THF, added benzyl amine (0.24 mL, 2.2 mmol, 4 equiv.). The RB was fitted with a condenser and refluxed for overnight. The crude product was purified by column chromatography (9:1 pet ether/ethyl acetate, R$_f$: 0.6),

yielded **13a** (191 mg, 90%). mp: 96 ^{o}C; ^{1}H NMR (200 MHz, CDCl$_3$) δ: 11.55 (bs, 1H), 8.41 (bs, 1H), 7.33 (m, 10H), 5.56 (bs, 1H), 4.54 (b, 2H), 4.43-4.40 (d, *J* = 6.10 Hz, 2H), 1.47 (s, 9H); ESI Mass: 383.21 (M+H)$^+$, 405.20 (M+Na)$^+$; Anal. Calcd for C$_{21}$H$_{26}$N$_4$O$_3$: C, 65.95; H, 6.85; N, 14.65. Found: C, 66.06; H, 6.94; N, 14.48.

[Isobutylamino-(3-isobutyl-ureido)-methylene]-carbamic acid *tert*-butyl ester, 13b:

The procedure for the synthesis of **13a** was repeated with isobutyl amine (200

mg, 0.55 mmol, 4 equiv.) to get the product **13b**. After the reaction the THF was completely evaporated and the product was purified by column chromatography (9:1 pet. ether/ ethyl acetate R_f: 0.6), 150 mg, yield: 86%; mp 210-212 ^{o}C; IR (CHCl$_3$) ν (cm^{-1}) 3454, 3340, 3001, 2963, 1715, 1634, 1605, 1510, 1416, 1369, 1234, 1217, 1151; ^{1}H NMR (200 MHz, CDCl$_3$) δ: 12.21 (bs, 1H), 8.09 (bs, 1H), 5.24 (bs, 1H), 3.19-3.12 (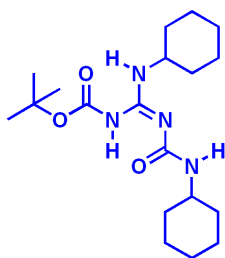m, 2H), 3.06-3.00 (m, 2H), 1.95-1.73 (m, 1H), 1.61 (bs, 1H), 1.49 (s, 9H), 0.96-0.92 (m, 12H); ^{13}C (50 MHz, CDCl$_3$) δ: 164.7, 154.3, 153.4, 82.3, 48.1, 47.5, 28.8, 28.1, 20.2; ESI Mass: 315.24 (M+H)$^+$, 337.23 (M+Na)$^+$; Anal. Calcd for C$_{15}$H$_{30}$N$_4$O$_3$: C, 57.30; H, 9.62; N, 17.82. Found: C, 57.22; H, 9.78; N, 17.76.

[cyclohexylamino-(3-cyclohexyl-ureido)-methylene]-carbamic acid *tert*-butyl ester, 13c :

The crude product obtained was purified by column chromatography (eluent: 9:1 pet ether/ethyl acetate, R_f: 0.6), Colorless thick liquid, 112 mg, 55%, IR (CHCl$_3$) ν (cm^{-1}) 3333, 3018, 2932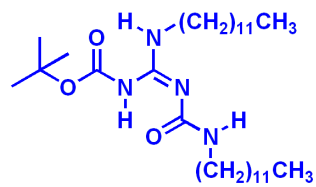, 2846, 1717, 1630, 1597, 1497, 1418, 1215, 1151; ^{1}H NMR (500 MHz, CDCl$_3$) δ: 12.23 (s, 1H), 7.94-7.92 (d, *J* = 7.13 Hz, 1H), 5.13-5.12 (d, *J* = 5.45 Hz, 1H), 3.84-3.77 (m, 1H), 3.58-3.50 (m, 1H), 1.94-1.08 (m, 24H), 1.45 (s, 9H); ^{13}C (50 MHz, CDCl$_3$) δ: 164.2, 153.4, 82.0, 48.7, 33.5, 32.7, 28.1, 25.6, 25.0, 24.6; ESI Mass: Calcd for C$_{19}$H$_{34}$N$_4$O$_3$: C, 62.27; H, 9.35; N 15.29. Found: C, 62.15; H, 9.18; N, 15.38.
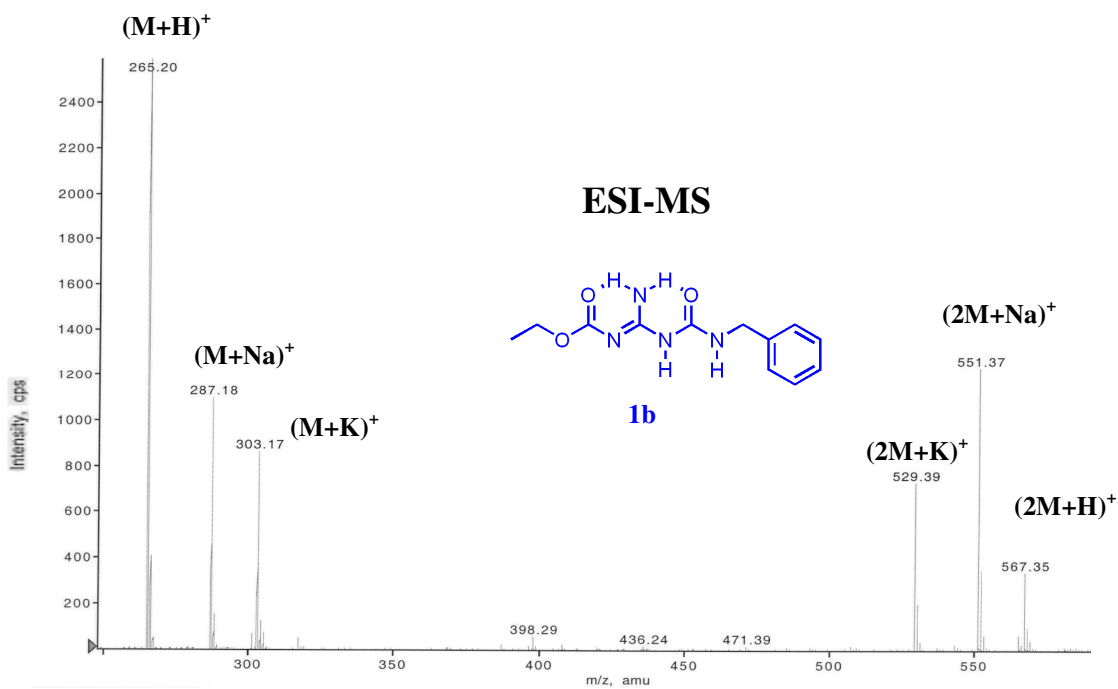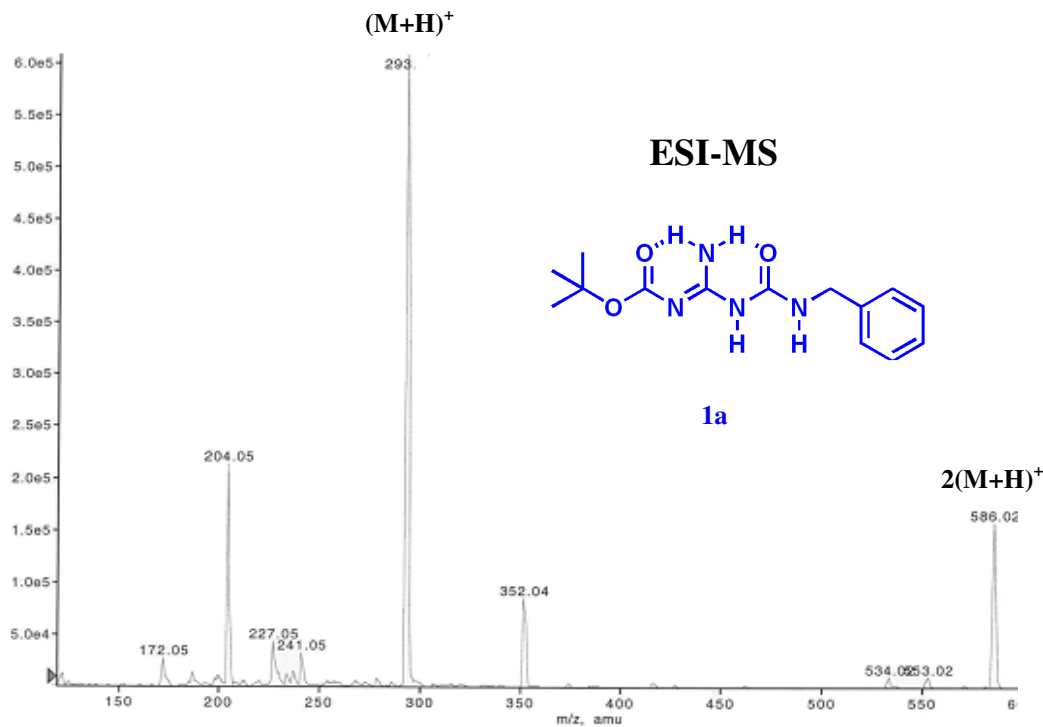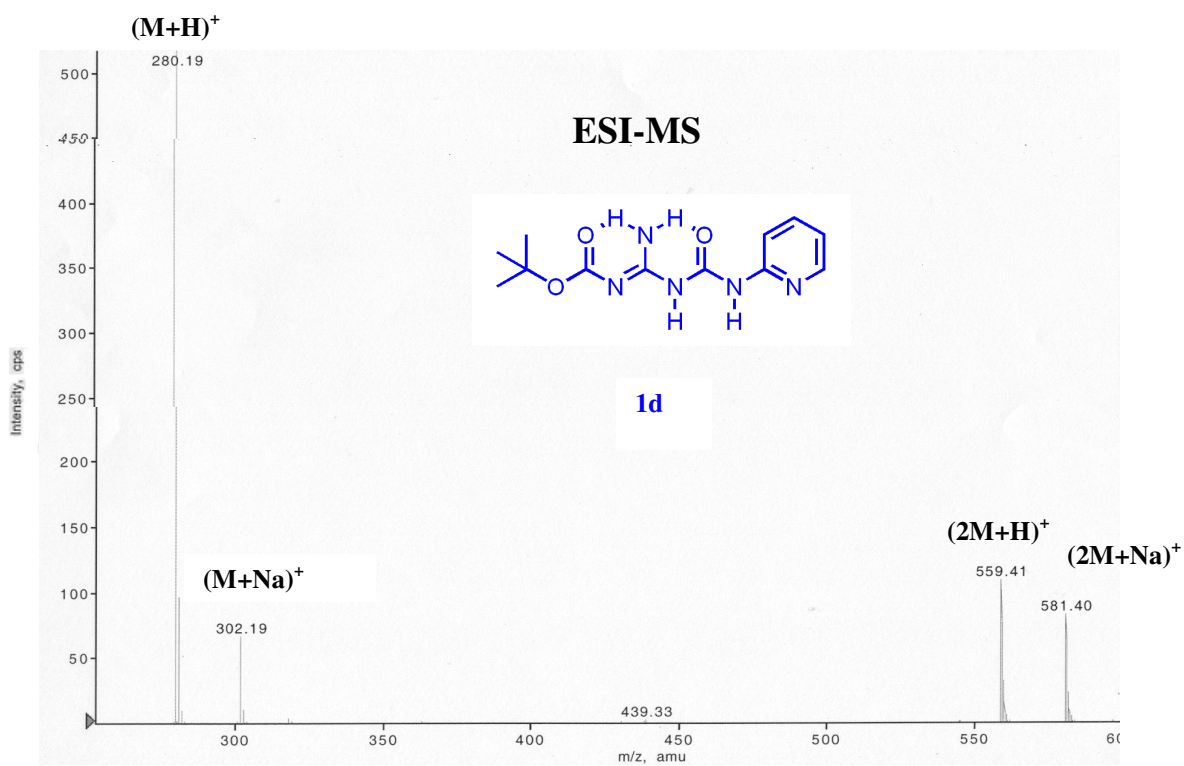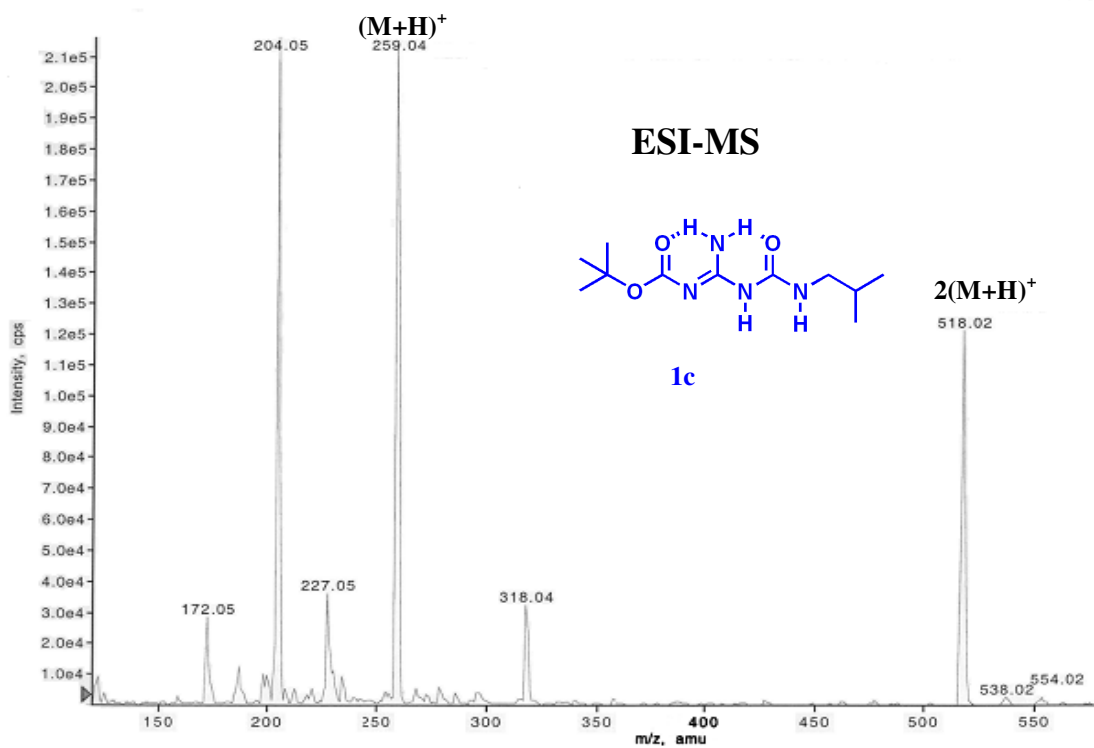
[dodecylamino-(3-dodecyl-ureido)-methylene]-carbamic acid *tert*-butylester, 13d
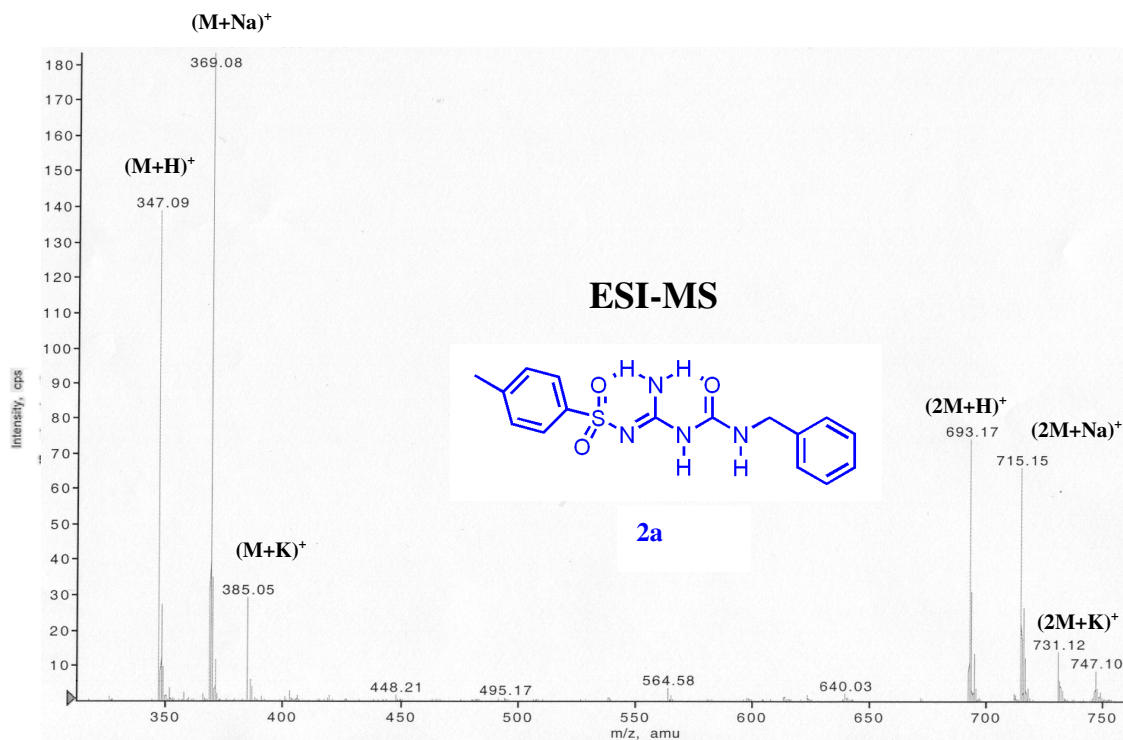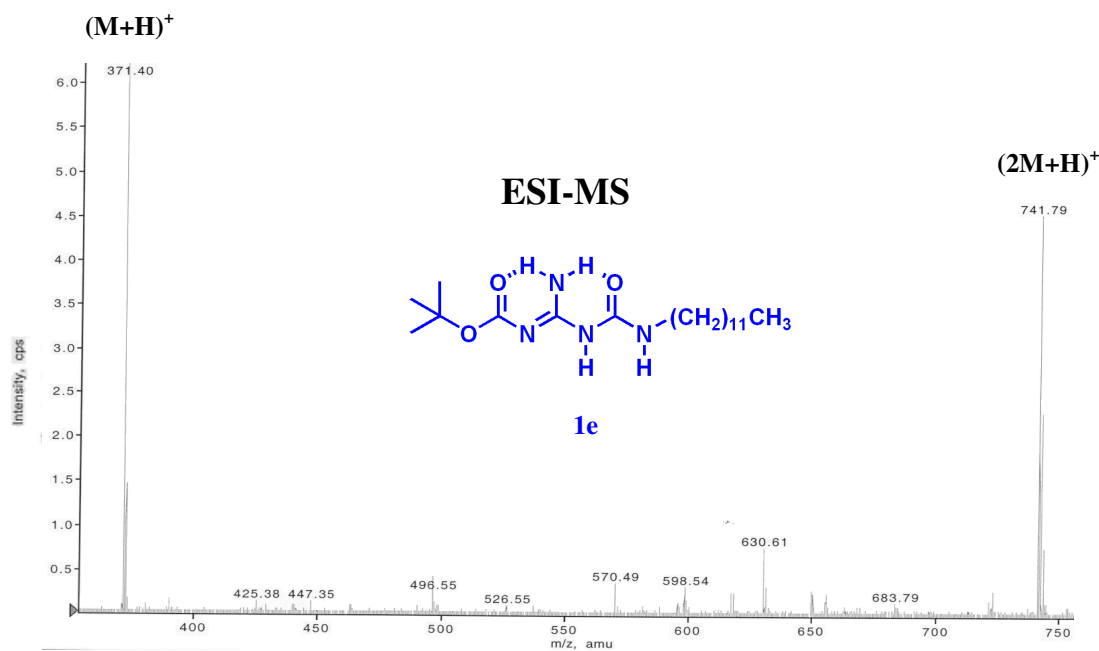
(eluent: 9:1 pet ether/ethyl acetate, R_f: 0.6), Colorless thick liquid, 271 mg, 91%, IR (CHCl$_3$) ν (cm^{-1}) 3447, 3342, 3018, 2928, 2854, 1717, 1636, 1605, 1506, 1418, 1215, 1148; ^{1}H NMR (500 MHz, CDCl$_3$) δ: 12.18 (S, 1H), 7.98 (bs, 1H), 5.18 (bs, 1H), 3.39-3.28 (m, 2H), 3.19-3.15 (m, 2H), 1.65-1.26 (m, 40H), 1.47 (s, 9H), 0.90-0.87 (t, *J* = 6.48 Hz, 7.32 Hz , 6H); ^{13}C (125 MHz, CDCl$_3$) δ: 164.9, 154.1, 153.4, 82.1, 40.6, 40.1, 31.9, 30.0, 29.6, 29.59, 29.5, 29.4, 29.3, 29.26, 29.1, 28.4, 28.1, 27.0, 26.9, 26.8, 22.7, 14.1; ESI Mass: 539.12, (M+H)$^+$, 561.05 (M+Na)$^+$; Anal. Calcd for C$_{31}$H$_{62}$N$_4$O$_3$: C, 69.10; H, 11.60; N, 10.40. Found: C, 69.20; H, 11.66; N, 10.52.

[isopropylamino-(3-isopropyl-ureido)-methylene]-carbamic acid *tert*-butylester, 13e

(eluent: 9:1 pet ether/ethyl acetate, R_f: 0.5), Colorless thick liquid, 52%, ^{1}H NMR (400 MHz, CDCl$_3$) δ: 12.18 (s, 1H), 7.84-7.83 (bs, 1H), 5.05-5.03 (d, *J* = 6.21 Hz, 1H), 4.13-4.05 (m, 1H), 3.92-3.84 (m, 1H), 1.46 (s, 9H), 1.17-1.14 (m, 12H); ^{13}C (100 MHz, CDCl$_3$) δ: 162.2, 153.3, 82.1, 42.2, 41.6, 28.1, 23.0, 22.6; ESI Mass: 287.50 (M+H)$^+$; Anal. Calcd for C$_{13}$H$_{26}$N$_4$O$_3$: C, 54.52; H, 9.15; N, 19.56. Found: C, 54.40; H, 9.02; N, 19.77.

(M+H)⁺

ESI-MS

2(M+H)⁺

1a

(M+H)⁺

ESI-MS

(M+Na)⁺

(M+K)⁺

(2M+Na)⁺

(2M+K)⁺

(2M+H)⁺

1b

(M+H)⁺

ESI-MS

1e

(2M+H)⁺

(M+Na)⁺

(M+H)⁺

ESI-MS

(M+K)⁺

(2M+H)⁺

(2M+Na)⁺

(2M+K)⁺

2a

(M+H)⁺

(M+Na)⁺

ESI-MS

(M+K)⁺

(2M+H)⁺

2b

(2M+Na)⁺
(2M+K)⁺

-TOF MS: 0.08 min (10 scans) from PPY-TBGBn-RT-1a

(M+H)⁺
350.20

ESI-MS

(M+Na)⁺
372.20

12a

(M+K)⁺
388.17

+TOF MS: 0.08 min (10 scans) from PPY-TBGISO-RT-1a

ESI-MS

(M+H)⁺ 316.22

260.18

(M+Na)⁺ 338.21

12b

m/z, amu

ESI-MS

230.1127

(M+H)⁺ 342.1368

286.1263

186.1568

242.1779

231.1170

(M+Na)⁺ 343.1389

(M+K)⁺ 364.1155

380.0618

12c

m/z, amu

ESI-MS

(M+H)⁺
428.1860

372.1948

(M + Na)⁺
450.1438

12d

ESI-MS

246.3024

(M+H)⁺
302.3515

146.2587

190.2506

(M+Na)⁺
324.3313

102.2652

202.3264

286.3760

340.3054

12e

(M+H)⁺

ESI-MS

367.1745

311.1633

312.1678

267.2101

368.1786

(M + Na)⁺

389.1353

13c

m/z, amu

(M+H)⁺

539.1162

ESI-MS

540.1172

519.1335

(M+Na)⁺

561.0495

13d

m/z, amu

ESI-MS

13e

1H NMR (CDCl₃, 500 MHz)

1a

¹H NMR (CDCl₃, 500 MHz)

1b

¹H NMR (CDCl₃, 500 MHz)

1c

—12.09 —10.81 —9.51 —8.67 —8.25 —7.96 —7.68 —7.27 —6.95

CDCL3

—1.53

¹H NMR (CDCl₃, 500 MHz)

1d

\#

┌─┐
│#│
└─┘

12 11 10 9 8 7 6 5 4 3 2 1 0

\#   Hexane trapped in the crystal lattice (seen in crystal structure as well)

—8.53 —7.50 —5.84 —4.73 —3.24 —3.21 —3.18 —3.14 —1.49 —1.45 —1.25 —0.91 —0.88 —0.84

¹H NMR (CDCl₃, 200 MHz)

1e

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 0.0

9.62
9.53

7.81
7.74
7.62
7.60
7.25
7.17
7.16

4.33
4.32

2.42

¹H NMR (CDCl₃, 500 MHz)

2a

9.62
9.56

7.84
7.68
7.66

7.27
7.22
7.20

3.18
3.17
3.16
3.14

2.45

1.61

1.51
1.49

1.27
1.24

0.89
0.88
0.87

¹H NMR (CDCl₃, 500 MHz)

2b

¹H NMR (200 MHz, CDCl₃)

—11.55
—8.62
CDCL3
7.33
7.27
4.66
4.64
1.53
1.49

12a

—11.51
—8.40
CDCL3
—7.27
3.28
3.22
1.50
1.49
0.97
0.94

¹H NMR (200 MHz, CDCl₃)

12b

11.54

CDCL3

8.31

7.27

4.05
4.03

1.93
1.69
1.68
1.67
1.51
1.49
1.44
1.41
1.25
1.23

¹H NMR (500 MHz, CDCl₃)

12c

11 10 9 8 7 6 5 4 3 2 1 0

11.51

CDCL3

8.30

7.27

3.42
3.40
3.38

1.51
1.50
1.45
1.30
1.29
1.28
1.26
0.90
0.88
0.87

¹H NMR (500 MHz, CDCl₃)

12d

11 10 9 8 7 6 5 4 3 2 1 0

^{1}H NMR (400 MHz. CDCl$_3$)

12e

^{1}H NMR (200 MHz, CDCl$_3$)

13a

¹H NMR (200 MHz, CDCl₃)

13b

¹H NMR (500 MHz, CDCl₃)

13c

¹H NMR (500 MHz, CDCl₃)

13d

¹H (400MHz, CDCl₃)

13e

158.26
138.60
128.54
127.19
CDCl3
82.01
77.00
43.71
28.02

^{13}C (CDCl$_3$, 500 MHz)

1a

160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 1

128.33
126.97
43.49
27.82

DEPT-135 (CDCl$_3$, 125 MHz)

1a

150 100 50 0

161.73
160.43
157.07
138.07
128.67
127.44
CDCL3
77.00
61.29
43.73
14.31

^{13}C (CDCl$_3$, 125 MHz)

1b

128.67
127.23
61.29
43.71
14.31

DEPT-135 (CDCl$_3$, 125 MHz)

1b

¹³C (CDCl₃, 125 MHz)

1c

158.52 — 81.72 — 77.00 (Chloroform-d) — 47.18 — 28.59 — 28.13 — 19.99

DEPT-135 (CDCl₃, 125 MHz)

1c

46.23 — 27.66 — 27.13 — 19.03 — 0.00

CDCl3

—163.54
—159.96
—153.38
—146.68
—138.31
—117.72
—115.49
—82.72
—77.00
—28.19

¹³C (CDCl₃, 125 MHz)

1d

#

160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

—146.41
—138.05
—117.45
—115.22
—27.91

DEPT-135 (CDCl₃, 125 MHz)

1d

150   100   50   0

the peaks at 14.1, 22.6and 31.6 are due to n-hexane (solvent of crystallization; could be observed in crystal structure as well).

179.13

158.12
156.89

CDCl3

79.58
77.00

39.86
29.57
29.50
28.06
26.84
22.62
14.03

¹³C (CDCl₃, 50 MHz)

1e

150   100   50   0

39.87
31.87
29.71
29.59
29.31
28.08
26.86
22.64
14.07

DEPT-135 (CDCl₃, 50 MHz)

1e

140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

156.82
154.65
142.76
138.81
137.61
129.24
128.29
127.39
127.10
125.79
77.00
43.51
21.28

^{13}C (CDCl$_3$, 125 MHz)

2a

160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

129.74
128.82
127.90
127.64
126.28
44.02
21.82

DEPT-135 (CDCl$_3$, 50 MHz,)

2a

150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

157.24
154.81
142.92
139.26
129.47
126.04
77.00
40.12
31.88
29.61
29.33
29.20
26.80
22.66
21.55
14.10

¹³C (CDCl₃, 125 MHz)

2b

DEPT-135 (CDCl₃, 125 MHz)

129.47
126.04
21.56
14.11

40.12
31.89
29.61
29.15
26.81
22.67

2b

^{13}C NMR (50 MHz, CDCl$_3$)

12a

DEPT-135 (50 MHz, CDCl$_3$)

12a

—163.65
—156.28
153.35

82.97
79.18

—48.25

28.29
28.05
27.83
20.18

¹³C (50 MHz, CDCl₃)

12b

150   100   50

—48.26

28.30
28.06
27.84

—20.20

DEPT-135 (50 MHz, CDCl₃)

12b

60   55   50   45   40   35   30   25   20   15   10   5

CDCl3

—163.88
—155.25
—153.32

—82.86
—79.03
—77.00

—48.55

—32.77
—28.33
—28.10
—25.50
—24.40

^{13}C (125 MHz, CDCl$_3$)

12c

70 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20

—48.55

—32.78
—28.32
—28.10
—25.50
—24.40

DEPT-135 (125 MHz, CDCl$_3$)

12c

60 55 50 45 40 35 30 25 20 15 10 5

^13^C (125 MHz, CDCl₃)

163.66, 156.09, 153.33, 82.97, 79.18, 77.00, 40.99, 31.90, 29.62, 29.46, 29.24, 28.95, 28.30, 28.07, 22.66, 14.10

12d

DEPT-135 (125 MHz, CDCl₃)

40.99, 31.90, 29.61, 29.55, 29.45, 29.32, 29.24, 28.95, 28.29, 28.06, 26.84, 22.67, 14.10

12d

^{13}C (100 MHz, CDCl$_3$)

12e

DEPT-135 (100 MHz, CDCl$_3$)

12e

^{13}C (50 MHz, CDCl$_3$)

13b

DEPT-135 (50 MHz, CDCl$_3$)

13b

164.23
153.38

CDCl3
81.96
77.26
77.00
76.74

48.70

33.48
32.68
28.05
25.62
25.03
24.63

¹³C (125 MHz, CDCl₃)

13c

170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

48.70

33.48
32.67
28.06
25.62
25.04
24.64

DEPT-135 (125 MHz, CDCl₃)

13c

75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0

^{13}C (125 MHz, CDCl$_3$)

13d

DEPT-135 (125 MHz, CDCl$_3$)

13d

CDCl3

—164.21
—153.27

—82.05
—77.00

—42.19
—41.59

—28.07
—23.04
—22.58

^{13}C (100 MHz, CDCl$_3$)

13e

150   100   50

—42.19
—41.59

—28.07
—23.03
—22.57

DEPT-135 (100 MHz, CDCl$_3$)

13e

140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

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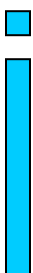
CHAPTER 2

Hybrid Foldamers with a Combination of Natural and Unnatural Amino acids: Design, Synthesis and Conformational Studies

This chapter is devoted to the study of novel hybrid foldamers with constrained amino acid building blocks. The design strategy, synthesis and investigation of their conformational organization both in solution as well as in the solid-state are detailed.

Hybrid Foldamer with Unusual (1→2)-type C9 Helical Turns: Design, Synthesis and Conformational Studies of Oligomers with Proline-Anthranilic acid (Pro-Ant) Repeating Sequence

The chemist finds illustration, inspiration, and stimulation in natural processes, as well as confidence and reassurance since they are proof that such highly complex systems can indeed be achieved on the basis of molecular components.

- Jean-Marie Lehn-

2.1 Introduction to the field of foldamers

Conformational studies of biological polymers revealed that most of the biological events result from their stable compact conformation stabilized by non-covalent interactions especially H-bonding. These three-dimensional conformations may include 'information rich surfaces' which in turn are actually responsible for the various biological processes.[1] Biological machines play myriad of roles in living beings such as molecular recognition, information storage, biocatalysts (enzymes), transmission of signals (hormones) etc. According to Linderstrøm Lang,[2] there are four levels of protein structure (fig. 2.1): primary structure represents the sequence of amino acids, secondary structure corresponds to conformation resulting from these sequences (examples are helices, β-sheets and turns).[3] The tertiary structure of peptides and proteins which is responsible for the bioactivity contains many secondary structural elements such as helix, β-sheet and turns.[4] The quaternary structure comprises different peptide strands held together by non-covalent interactions.[5] The peptide backbones undergo folding and defolding, a process governed by molecular recognition and self-assembly, which occurs in fraction of seconds.
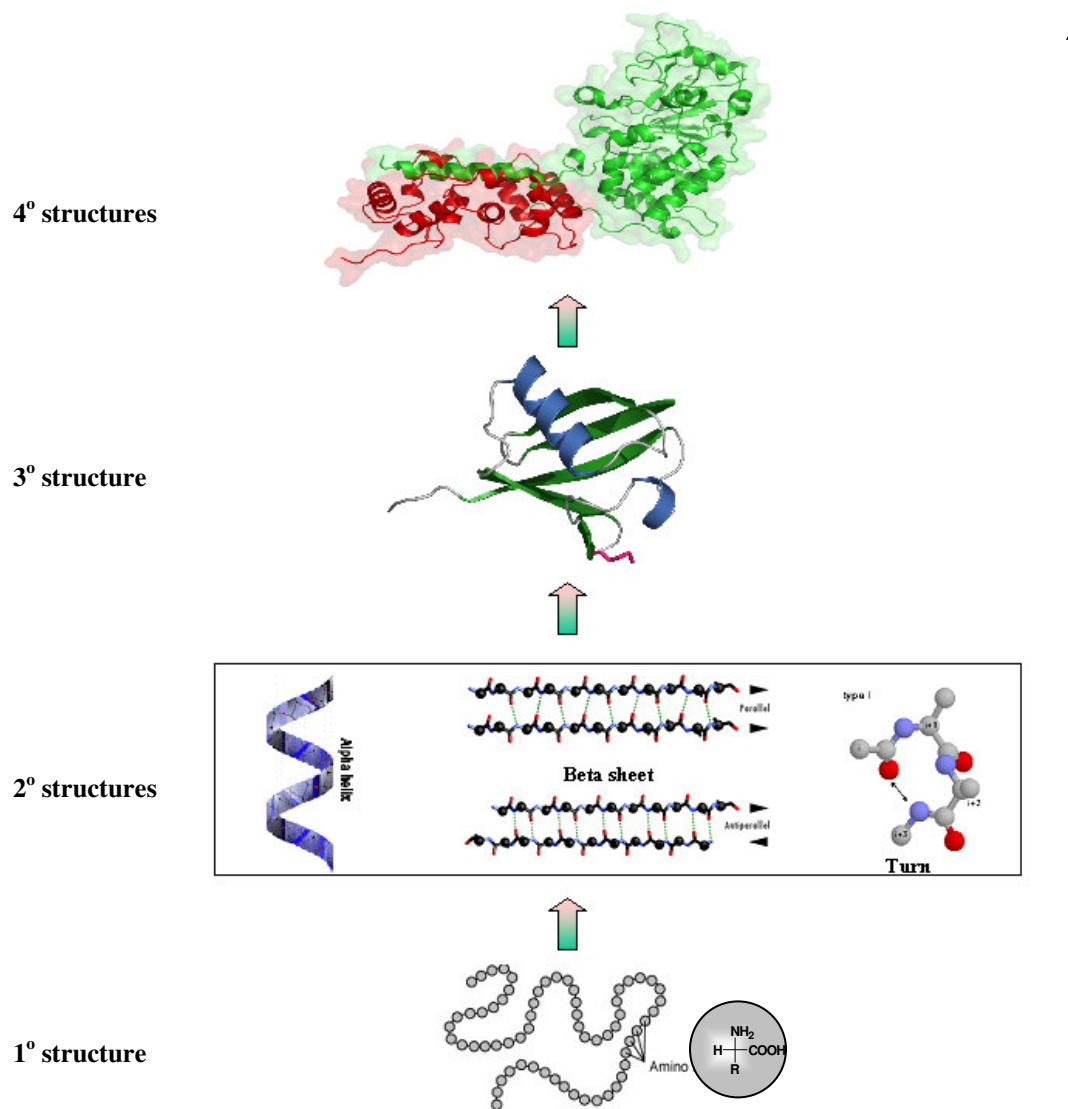
4° structures

3° structure

2° structures

1° structure

Fig. 2
structu

folding

promoting the stability of the folded conformation,[7] occurrence of various biological processes (especially molecular recognition)[8] and information regarding the exact relationship between the sequence and a particular conformation[9] are not yet fully understood. Even though many peptides exhibit biological activity,[10] their use as pharmaceutical agents is limited because of their poor bio-availability, low cell permeability and poor *in-vivo* stability owing to their conformational flexibility (resulting in multiple receptor selectivity) and susceptibility towards various proteolytic enzymes.[11]

In the quest of rectifying the above mentioned undesirable effects and also in an attempt to explore the nature's puzzling events especially the folding mechanism, chemists endeavored in the construction of molecular frameworks with unnatural backbones, mimicking both the structure and functions of conventional peptides/proteins. This led to the flourishing area of *peptidomimetics*[12]: an area of research which mainly focuses on the development of small molecules which mimic the biological properties of peptides. A related area of research that has attained considerable attention in recent years is foldamers that deals with unnatural synthetic oligomers mimicking conformational features of biopolymers.[13,14]

Gellman coined the term foldamer which is defined as "***any polymer with a strong tendency to adopt a specific compact conformation***".[15] Modern polymer chemistry, molecular biology, and supramolecular chemistry have considerably contributed to the growth of foldamers from its foundation in the early 20th century to the present stage.[16]

2.2 Classification of foldamers

Foldamers are generally categorized into biotic and abiotic (fully non-natural), based on the backbone type in comparison to natural analogs.[17] Biotic foldamers contain aliphatic backbone with amide or urea as the linkage in the backbone. Selected examples[18-29](fig. 2.2) are: β-,[18] γ-,[19] δ-,[20] oligoureas,[21] azapeptides,[22] aminoxy-peptides,[23] and sugar derived peptides[24]. These oligomers have attained considerable impact in foldamer chemistry not only because of their conformational resemblance, but also because of their improved pharmacokinetic profiles.[30]
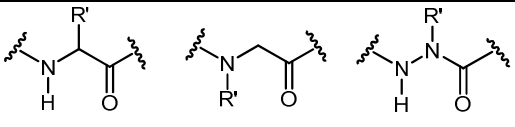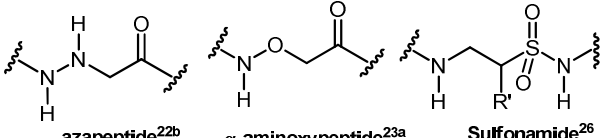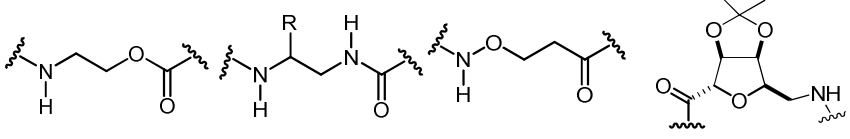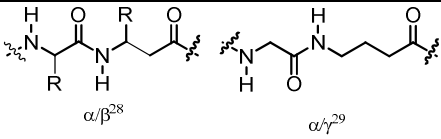
Parent backbone	Backbone modification			
α-peptide	α-peptide / Peptoid[25] / azapeptide[22a]			
β-peptide	azapeptide[22b] / α-aminoxypeptide[23a] / Sulfonamide[26]			
γ- and δ-peptides	carbamate[27] / oligoureas[21] / β-aminoxypeptide[23b] / carbopeptoid[24]			
α/β, α/γ	α/β[28] / α/γ[29]			

Fig. 2.2: Examples for biotic foldamers.

In natural peptides, the major helical conformations observed are 3_{10}- and α-helices with ten and thirteen membered intramolecular H-bond (IHB) network, respectively.[31] Some of the patterns of IHB observed in β- and γ- peptides, in comparison to α-peptides, are represented in fig. 2.3.
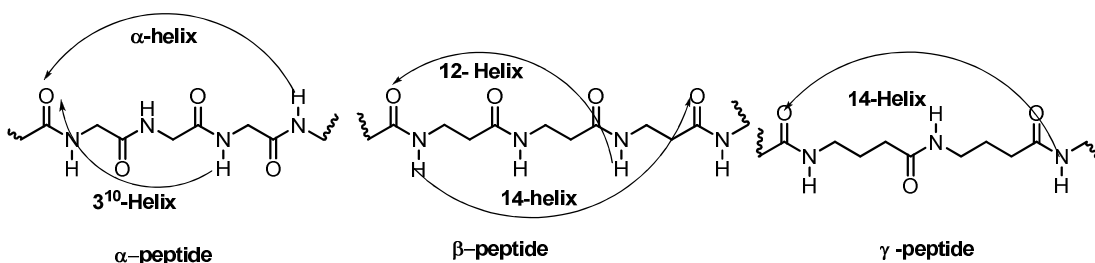
Fig. 2.3: Various H-bonding patterns observed in α-, β- and γ-peptides.

The secondary structures adopted by the biotic foldamer are determined by various structural factors such as substitution pattern within the backbone, conformational constraints and planarity of the amide bond (fig. 2.4).[32]
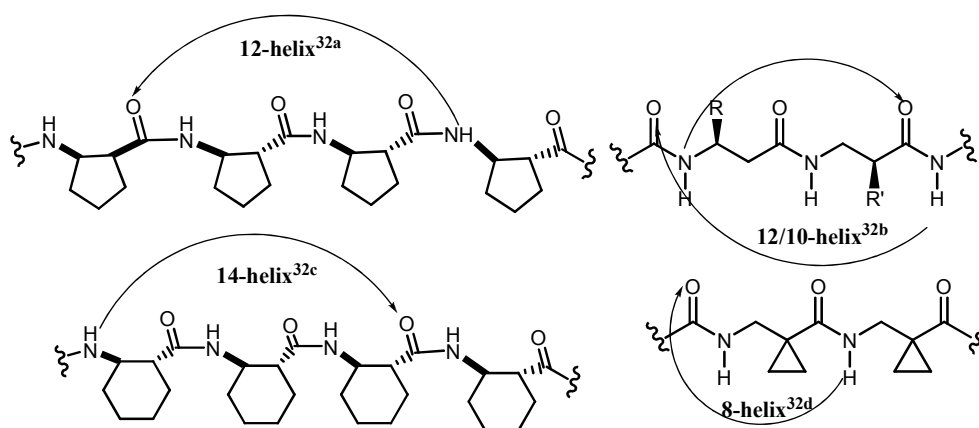
Fig. 2.4: Effect of substitution on the backbone of foldamers.

Various changes observed in the IHB pattern of biotic foldamers with substitution/sequence alteration / ring constraints are given in table 2.1

Table 2.1: Various IHB patterns possible for biotic foldamers.

Backbone	Amino acid substitution pattern	Number of atoms involved in IHB
α_n (3_{10}-helix)	$C\alpha$	10
α_n (α-helix)	$C\alpha$	13
β_n (12- helix)	Cyclic C2,3 cyclopentane ring	12
β_n (14- helix)	C2, C3 or Cyclic C2,3 cyclopentane ring	14
β_n (10/12-helix)	Alternative C2 and C3	10, 12
β_n (12/10-helix)	Alternative C3 and C2	12, 10
γ_n	C4	14
γ_n	C2,3,4	14
$\alpha\beta$ (11-helix)	$C\alpha$/C3 or $C\alpha$/Cyclic C2,3 cyclopentane ring	11
$\alpha\beta$(14,15-helix)	$C\alpha$/C3 or $C\alpha$/Cyclic C2,3 cyclopentane ring	14, 15
$\alpha_2\beta$	$C\alpha$/C3 or $C\alpha$/Cyclic C2,3 cyclopentane ring	10, 11, 11
$\alpha_2\beta$	$C\alpha$/C3	14
$\alpha\beta_2$	$C\alpha$/C3 or $C\alpha$/Cyclic C2,3 cyclopentane ring	11, 11, 12
$\alpha\beta_2$	$C\alpha$/C3 or $C\alpha$/Cyclic C2,3 cyclopentane ring	15

Oligomers typically with aromatic backbone belong to the class of abiotic foldamers and these class of oligomers attract considerable attention mainly because

of their predictable conformations and high conformational stability (fig. 2.5).[33-38] In majority of the cases reported in the literature, the backbones adopt helical structural architecture mainly because of the intramolecular H-bond directional effects (mediated by hetero atoms such as O, N etc.).[33-37] Selected examples are oligoamides,[33] oligoureas,[34] pyridine oligoamide,[35] oligo-*m*-phenylethynylenes,[36] oligohydrazides[37] etc.

Oligourea[34a]

Oligo-ureidophthalimide[34b]

Pyridine oligoamide[35]   **Oligo-m-phenylethynylenes**[36]   **H-bonded duplex**[38]
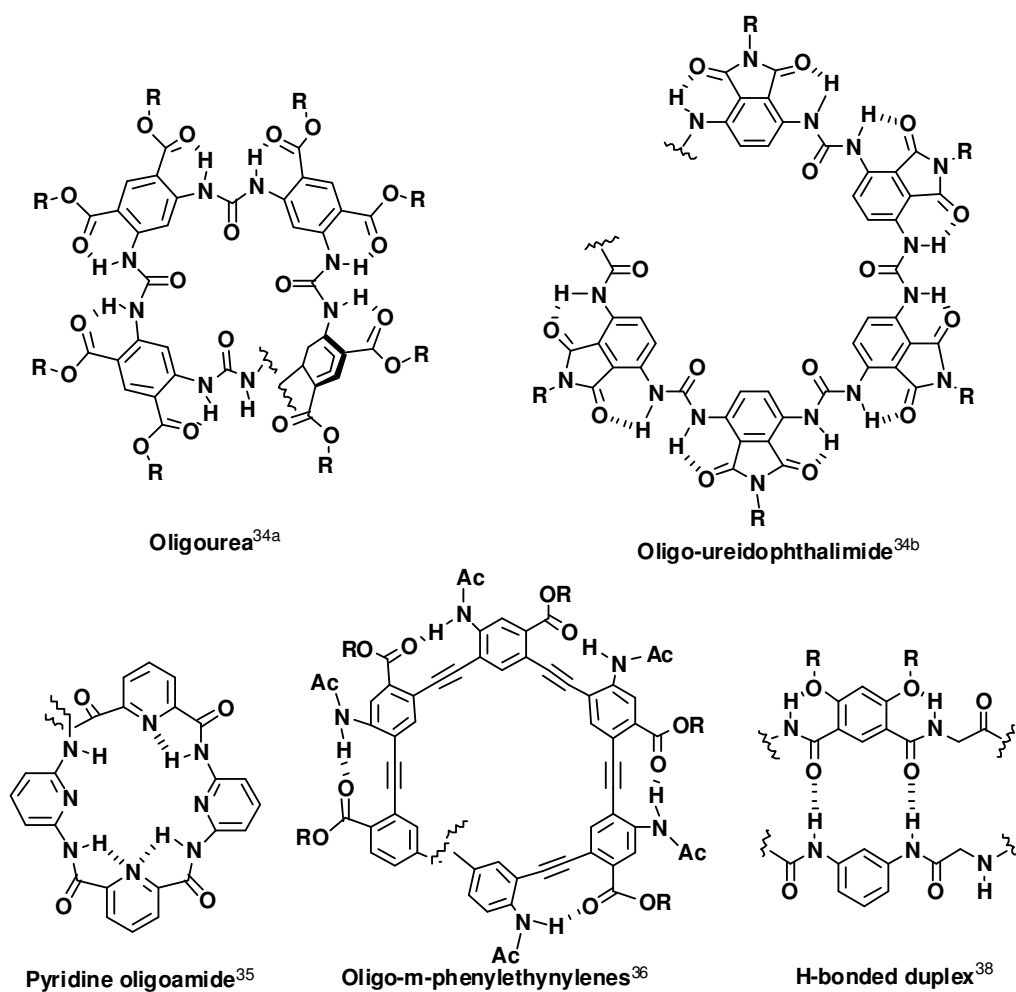
Fig. 2.5: Foldamers with abiotic backbones.

2.3 Foldamer design

      Foldamer molecular frameworks with a wide range of backbones have been designed and synthesized, as evident from the recent literature.[13-16] The backbone of molecules restricts the flexibility and often determines their behavior in solvents. The side chains can be fine-tuned to modulate their solubility, solvophobic properties and backbone conformation to great extent. The methods for generation of biomimetic (peptidomimetic) foldamers involve mimicking the peptide primary structure by modification of backbone, side chains, introduction of constraints and by the use of amide bond isosteres and isoelectronic exchange (fig. 2.6).[18-29]
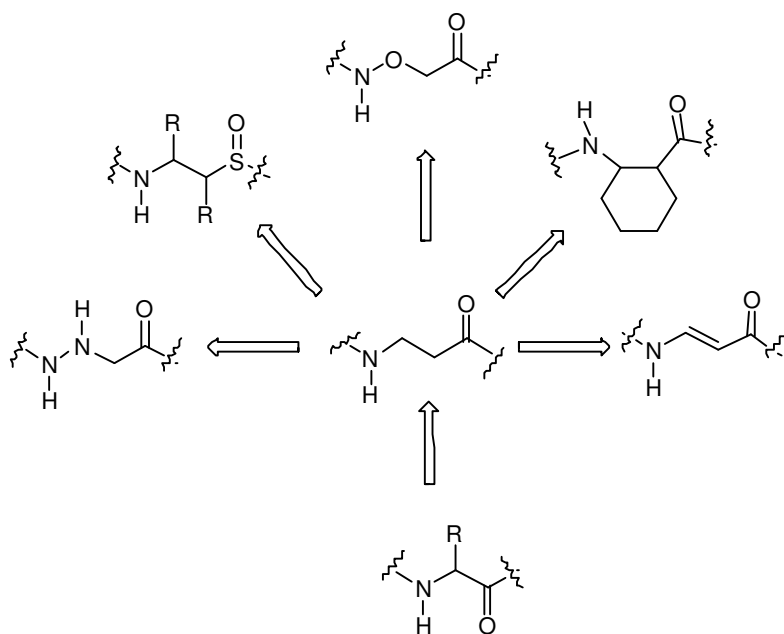
Fig. 2.6: Evolution of biotic foldamers.

      The strategies utilized for the biotic foldamer design is also applicable to abiotic foldamer design, However, in addition to the aforesaid strategies, the following secondary interactions such as aromatic π-π-stacking, metal co-ordination,

solvophobic interactions and steric effects are also being utilized in the design of abiotic foldamers.[13,16] Among these, H-bonding and aromatic π-π-stacking are the major driving forces explored in constructing these potentially interesting frameworks. In abiotic helical foldamers, the curvature can be modulated by altering the substitution pattern (on the aryl rings), H-bond donor/ acceptor sites, and the aromatic rings.[39]

2.3.1 Solvophobic interactions

To use solvophobic effect for the foldamer design, it is required to create solubility contrast between the backbone and side chains (solvophobic/solvophilic sites) along the primary sequence, which in principle, can modulate the folding↔defolding processes. The solvophobic behavior of proteins was extended to synthetic analogs by Moore *et al.* by introducing polar side chains on non-polar backbone of oligo-*m*-PE. It was demonstrated that polar solvents like acetonitrile promote a compact helical conformation where as non-polar solvents like chloroform, favor a random coil conformation (fig. 2.7A).[40] Interestingly, cholic acid based aliphatic foldamer reported by Zhao *et al.* undergo hydrophobically driven helix↔random coil transition and favors a helical conformation in non-polar solvent (fig. 2.7B).[41] In general, biotic oligomers are not suitable to employ these non-directional cooperative forces in the foldamer design strategy because of their conformational flexibility. However, this non-directional interaction has been extensively explored in the case of oligomers with aromatic backbone.

A   **B**

(Tg = triethylene glycol)
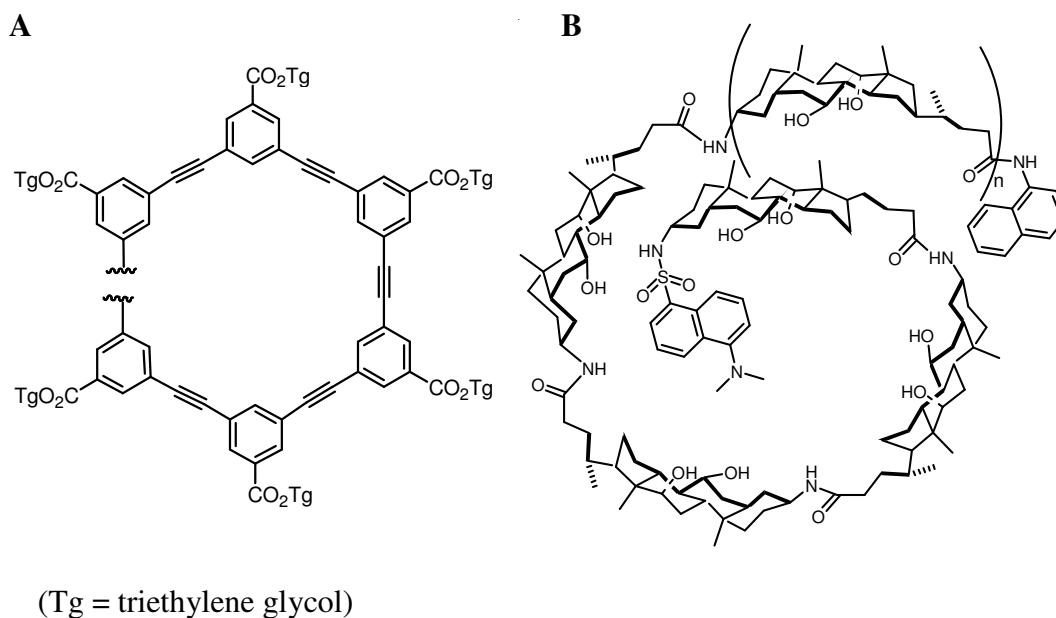
Fig. 2.7: Foldamers that display solvophobic property-induced conformational changes.

2.3.2 Hydrogen bonding interactions

Because of the directionality and specificity, H-bond plays an important role in the foldamer design to stabilize intrinsically flexible oligomeric strands into an ordered structure.[18-29] As mentioned in section 2.2, in the case of aliphatic/biotic foldamers, the formation of stable secondary structures stabilized by intramolecular H-bonding requires the pre-organization of the conformation so that the sequentially remote H-bonding sites can be positioned in close proximity. This can be achieved by restricting the conformational space of the amino acid residues in the sequence by imposing restriction on the dihedral angles and by using intrinsically constrained amino acids.[42] In the case of aromatic foldamers, the stabilization through IHB occurs between adjacent monomeric units which can either be backbone-backbone[43] (fig.

2.8A), side chain-side chain [36] (fig. 2.8B) or side chain-backbone[44] interactions (fig. 2.8C) and their propensity of H-bonding is readily predictable. Although in most of the abiotic foldamers, IHBs pre-organize the secondary structure into helical conformation (fig.2.8 A-C), it may also promote linear conformations[30g] as shown in fig. 2.87D.

Fig. 2.8: Design strategy based on H-bonding in abiotic foldamers.
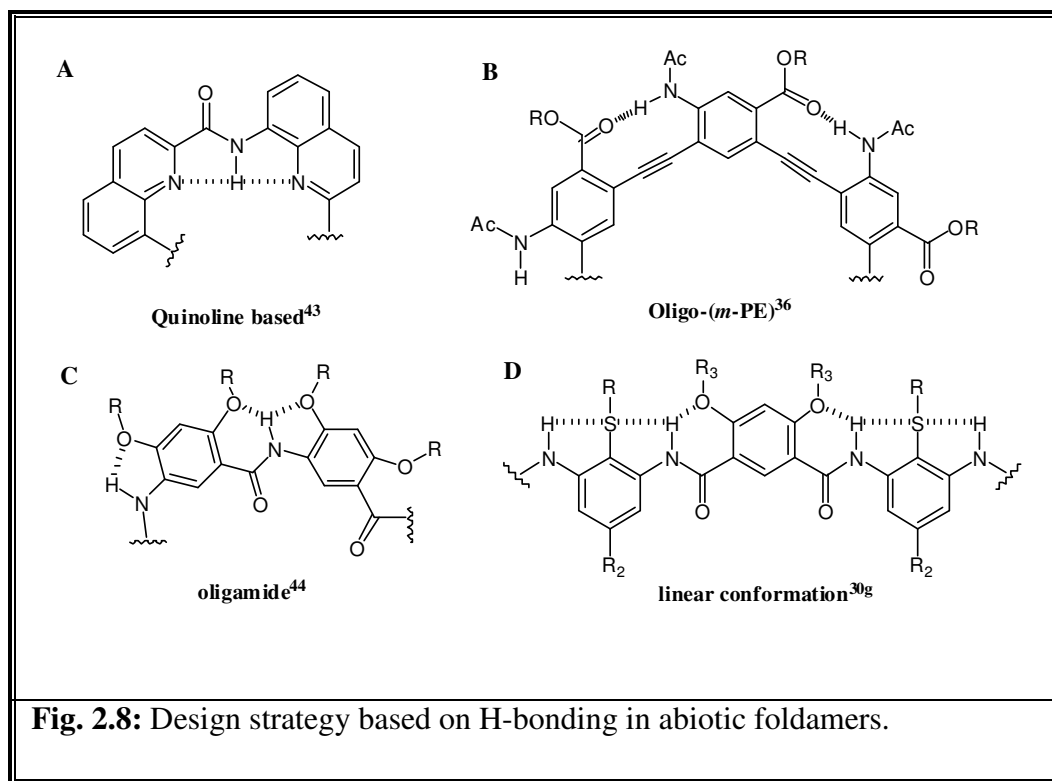
2.3.3 π-π stacking interactions

In addition to the above mentioned driving forces, intramolecular aromatic stacking interactions (π-π interactions) provide additional conformational stabilization in helical structure formation.[33-37] This secondary interaction cannot itself promote a specific conformation owing to the lack of directionality. However,

the role of π-π stacking interactions becomes critical in those cases, where local conformational organization is not complete. For example, in the oligomer shown in fig 2.7 A (section 2.3.1), the conformation is further stabilized by aromatic interactions, in conjunction with solvophobic interactions as evidenced from NMR studies.[40]

2.3.4 Acceptor-donor interactions

The concept of donor-acceptor interactions between the aromatic units as the driving force for the molecule to exist in a stable conformation was employed by Lokey and Iverson and the resultant 'aedamer' (aromatic electron donor-acceptor oligomers) resulted from such molecular backbone exists in a "pleated secondary structure" (fig. 2.9).[45]

Fig. 2.9: 'Aedamer' with acceptor donor interactions.[45]
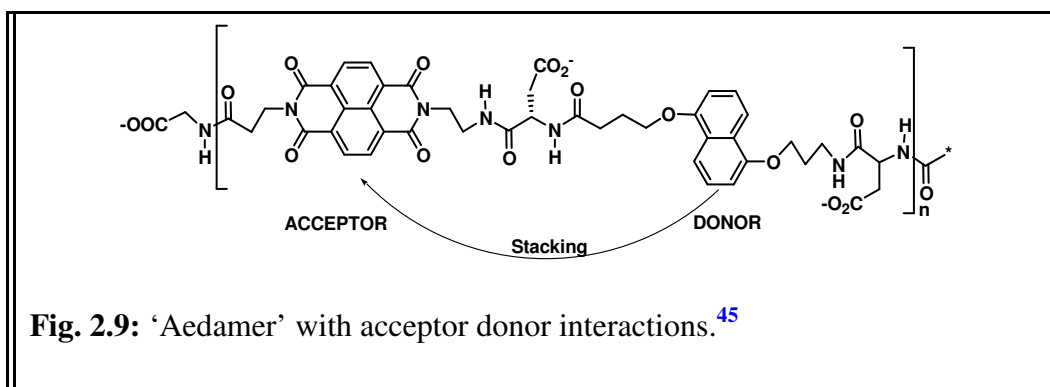
2.3.5 Metal coordination

One of the major disadvantages of hydrogen bonding is its limited strength in polar solvents. The stability of metal coordination compared to H-bonding provides an alternative way to achieve stable conformations even in highly polar solvents and these metal templated helical structures are termed as 'helicates'.[46] In order to have

coordination with the metal, the monomeric strand in the ligand part should possess appropriate metal binding sites. Metals and ammonium ions are frequently employed as coordination motifs for ligands[47] (fig.2.10).

Fig. 2.10: Metal coordination as the driving force in the backbone folding.[47c]
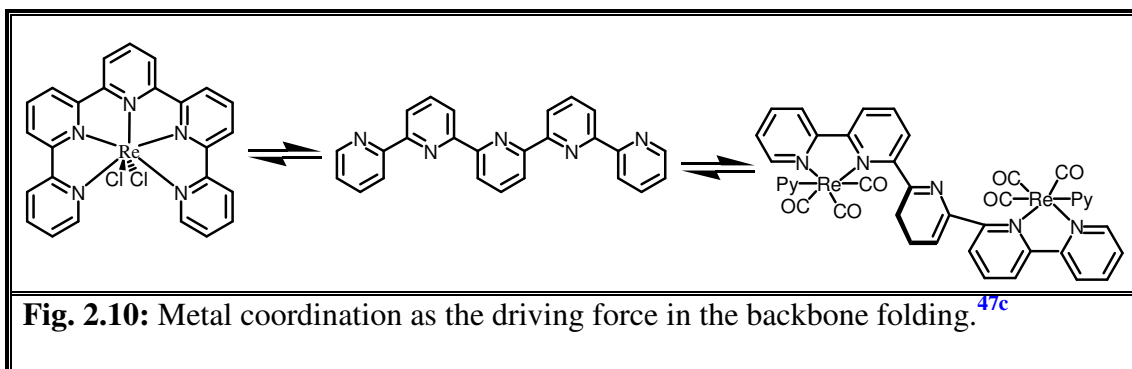
2.4 Methods of Characterization

In addition to providing structural identity, NMR studies furnish information about the nature of H-bonding interactions (intra *vs* inter) by evaluating the chemical shift changes upon dilution / titration.[36] Furthermore, nOe studies (dipolar coupling) provide strong evidence for the conformation of synthetic oligomers in solution-state.[36] In order to probe the conformation of achiral oligomers, introduction of chiral units into the foldamer backbones has been explored.[48] Single X-ray crystallography is a powerful tool for the investigation of solid-state conformation of oligomers, although the need to have crystals suitable for the data collection often hampers its utility.[49] CD and NMR techniques can be used to monitor the conformational changes in response to external stimuli like pH, temperature, and light.[50] A list of various methods used for the study of foldamers and the information obtained from

each method is tabulated below.

Table 2.2: General approaches in the conformational studies of foldamers.

Method	spectroscopic/ non spectroscopic	Information
1D NMR	spectroscopic	structure, aggregation, H-bonding, stacking
2D NMR	"	conformation in solution
IR	"	aggregation, H-bonding
UV-VIS, CD	"	chromophore interactions
Fluorescence[51]	"	chromophore interactions
X-ray crystallography	non-spectroscopic	solid state arrangement of atoms
Electron microscopy[52]	"	morphology and chain packing in solid state

In addition to these techniques, other methods employed for the selective studies are VPO, calorimetry and EPR.[53]

2.5 Foldamer applications

2.5.1 Bioactivity

As mentioned in section 2.1, one of the promising goals in the foldamer research is to mimic the biopolymers both structurally and functionally.[19-23] Efforts in mimicking the functional features of nature's biomolecular machinery with man-made constructs resulted in exploring the antibacterial and antimicrobial activities of β-peptides and peptoids.[54] The antimicrobial β-peptides reported by Gellman *et. al* were highly potent and specific towards bacteria, showing excellent activity against four species (including both Gram positive and Gram negative organisms) and minimum hemolytic activity against human red cell.[55] DeGrado and co-workers

described a different class of β-peptides that could destroy *Escherichia coli*, with varying cell selectivities.[56] Seebach *et. al* reported a series of β-peptides with limited antibiotic activity.[57] The helical amphipathic peptoids (*N*-alkyl-glycine oligomers) reported by Barron *et al* could act as mimics of magainin antibacterial peptides [58]

2.5.2 Material applications

Because of its dynamic nature, foldamers are suited for the design of stimuli responsive materials.[59] For example, the photoswitchable *trans*-azobenzene-incorporated amphiphilic oligo-(*m*-phenylenes) show considerable promise for developing photo responsive materials.[59] Chiral foldamers which can undergo inversion of helicity regulated by external stimuli are suitable for data storage and for developing optical devices. [60]

2.5.3 Molecular recognition

The inherently weak non-covalent interactions in foldamers provide tunability, facilitating the molecule to adopt flexibility, to some extent, and adaptability which would help them to act as better synthetic receptors in comparison with the rigid macrocycles and cavitands. Foldamers with circular or helical conformations may act as molecular containers and cavitands which can take part in molecular recognition events with small guest molecules.[61] A foldamer with quinoline pyridine hybrid backbone acts as a molecular container for water molecules as reported by Huc *et al*.[62] The H-bonded helical aryl oligoamides reported by Li *et .al*. bind ammonium ions, saccharides and fullerenes.[63] The hydrophobic interior cavity of the oligo-*m*(PE) reported by Moore *et al.* could encapsulate organic guest

molecules.[64] Recently, a novel foldamer that stabilizes polymeric chain of water clusters have been reported from our group.[65]

2.5.4 Catalytic activity

The synthesis of biomimetic catalysts, especially enzymes, is an important area of research in supramolecular chemistry and molecular recognition science. Synthetic helical backbones can provide active sites for the selective catalysis of various reactions.[66] Owing to their structural and functional similarity to the natural biomolecules, H-bonded foldamers appear promising for this purpose. Methylation of dimethylaminopyridine promoted by oligo-(*m*-PE) as reported by Moore *et al*,[66b] N-oxidation of the peripheral pyridine ring in oligo pyridine-dicarboxamide,[66c] and crown ether mimics[51] are few examples of foldamers as biocatalysts.

2.6 Objective of the present work

Although foldamer chemistry started with the modification of natural α-peptides with β- and γ-peptides and peptoids (peptidomimetic foldamers), there are innumerable examples of foldamers with a variety of backbone leading to structural architectures with interesting applications. Nonetheless, foldamers with biomimetic backbones draw considerable attention because of their close functional similarity to their natural counterparts.[18-30] Hybrid oligomers with α/β, α/γ and β/γ peptide building blocks have also attained interest in recent years mainly because of the innumerable possibilities to augment the conformational space available for the foldamer design.[67]

The work described in this chapter aspires at synthesizing hybrid oligomers

with constrained backbones. In our design strategy, we selected two constrained amino acids, proline (Pro; a highly constrained natural α-amino acid) and anthranilic acid (Ant; a rigid unnatural β-aminoacid) anticipating that the intrinsic constraints from the individual amino acid residues would help in the conformational pre-organization of the oligomers to exist in a favorable secondary structure. We have also investigated the effect of substituting Pro in the hetero oligomer sequence with alanine (Ala) and α-amino isobutyric acid (Aib),[68] the amino acids that are known to promote helical conformation in α-peptides.

2.7 Design strategy

Rigidification of the backbone to minimize the conformational freedom by using constrained amino acids is an unambiguous priciple in the foldamer chemistry field.[69] It is has been reported by Hamilton *et al.* that homo oligomers derived from anthranilic acid (Ant; a constrained β-amino acid), adopt an extended sheet conformation featuring repeating 6-membered ring H-bonding network.[70] Among the natural amino acids, proline is exceptional because of its constrained dihedral angles, lack of H-atom to form intramolecular H-bond in its homo-oligomers and the high prevalence of *cis/trans* isomerization of the imidic peptidic bond.[71] Even though it is present in many helices such as Collagen and poly(Pro-II), in general, it is considered as a helix and sheet breaker.[72] In addition to these, Pro is known to affect the conformation of the amino acid preceding it in polypeptides.[73] Keeping these facts in mind, we envisaged that a foldamer resulting from the combination of these two amino acids (Pro and Ant) should adopt a compact structural architecture owing to

the intrinsic conformational restriction of the individual residues.

Constrained Foldameric Backbone
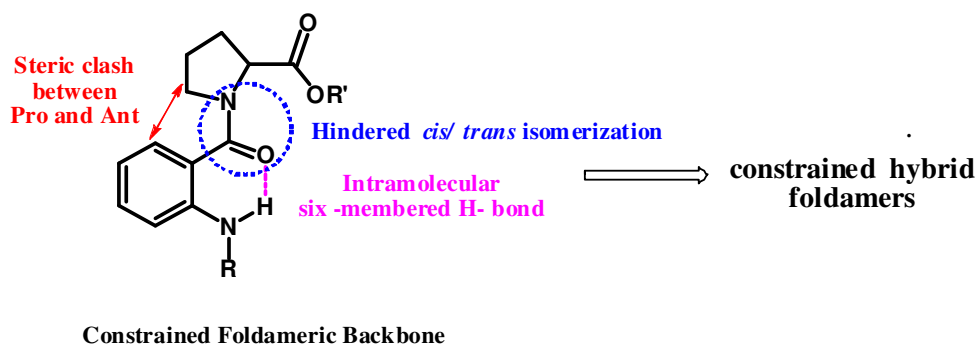
Fig. 2.11: Design principle of the hybrid foldamer (Ant-Pro)$_n$.

2.8 Synthesis

Although our initial strategy was to prepare oligomers with Ant at the N-terminal to arrest the possible *cis-trans* isomerization of Pro residue, the facile intramolecular cyclization of Ant-Pro-OMe/OBn (free amine), apparently due to the closer positioning of the amino and ester termini of the building block, yielding cycloanthranilyl proline[74] restricted our strategy to start with Boc-Ant-Pro-OMe/OBn. Therefore, we started the synthesis with reverse building block Pro-Ant-OMe. Thus starting from the dipeptide Boc-Pro-Ant-OMe **1a** using 'segment doubling strategy'[75] we could furnish the higher oligomers **2a** and **3** (Scheme 2.1) using TBTU (*O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate) as a coupling agent and DIEA (*N,N*-diisopropylethylamine) as the base. Coupling of dipeptide acid **1b** and the corresponding amine hydrochloride **1c** afforded the tetrapeptide **2a** in 78% yield after chromatographic purification. Similarly, octapeptide **3** was prepared by

coupling the tetrapeptide acid **2b** with corresponding amine hydrochloride **2c**. Introduction of Ant at the N-terminal could be undertaken at a later stage as demonstrated in the case of **2d** by coupling amine hydrochloride **2c** with Boc-Ant-OH to furnish the pentapeptide **2d**.

Scheme 2.1: Synthesis of oligomers **2** and **3**.
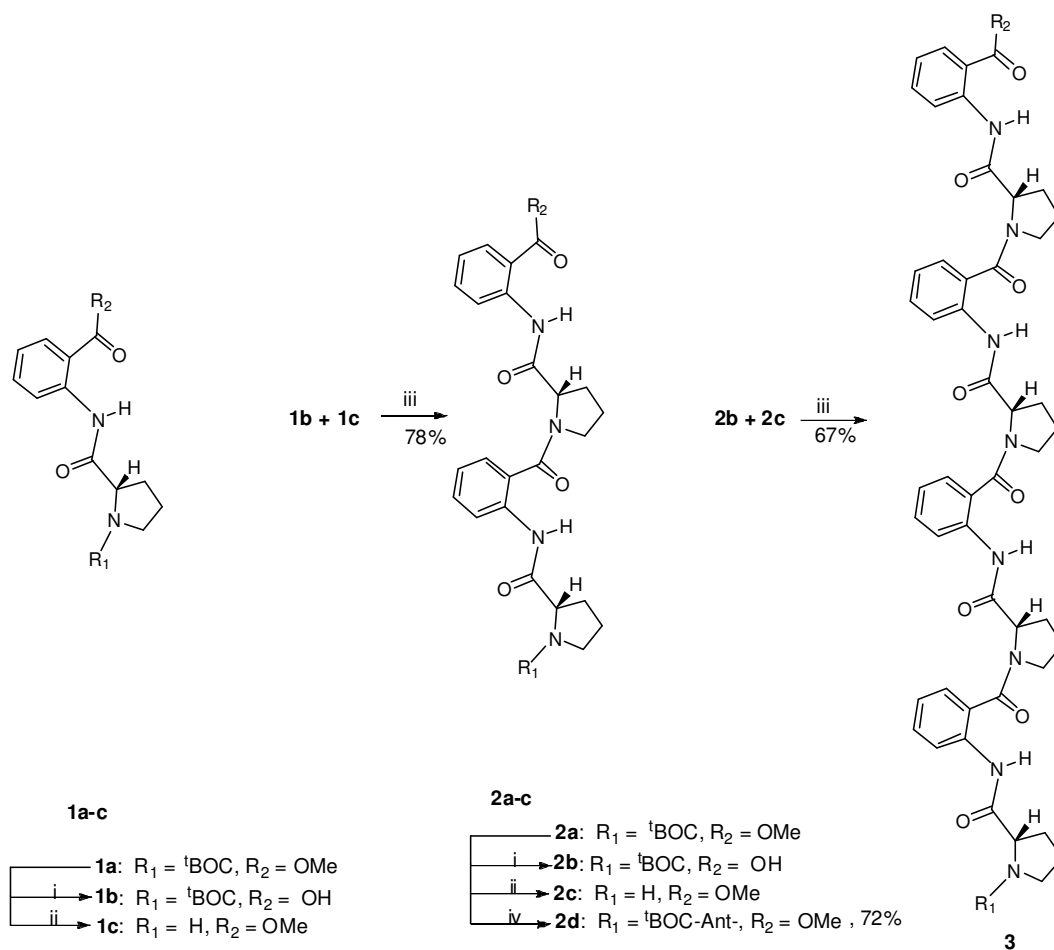
Reagents and conditions: (i) LiOH, MeOH, rt, 12 h; (ii) dry HCl (gas), ether, 0^0C, 10 min; (iii) TBTU, DIEA, MeCN, rt, 12 h. (iv) Boc-Ant-OH, TBTU, DIEA, MeCN, rt, 12h

2.9 Conformational analyses

Secondary structural analyses were accomplished by extensive 2D NMR, X-ray diffraction and HF/6-31G* level of *ab initio* MO theory studies.[76]

2.9.1 Single crystal X-ray diffraction studies

Extensive efforts to crystallize the oligomers resulted in the formation of crystals of **2b** from a solvent mixture of methanol containing few drops of PEG-200. Analysis of the crystal data revealed the presence of an unexpected (1→2)-type 9-membered-ring (N2…O5) pseudo β-turn[77] in **2b**, connecting the amide NH of the Ant2 residue and the carbonyl of the Pro3 residue, which we call as Ant-Pro C9 turn (fig. 2.12)
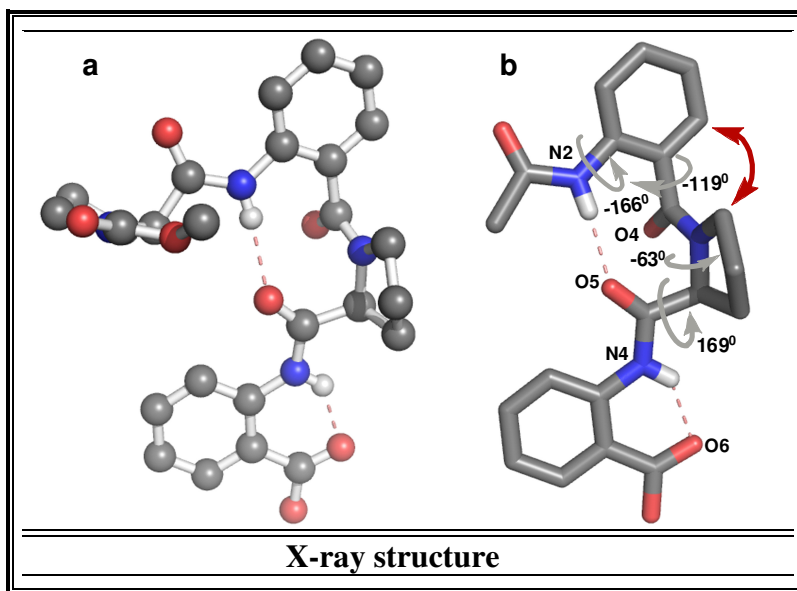

Fig. 2.12: Single crystal X-ray structure of the Boc-(Pro-Ant)$_2$-OH **2b** signifying the unusual pseudo β-turn.[77] (a) ball and stick representation (without ᵗBOC group). (b) annotated crystal structure (selected region) in caped stick representation. Hydrogens, other than at the hydrogen bonding sites, have been deleted for clarity.

Surprisingly, the formation of Ant-Pro C9 turn in **2b** was at the cost of the S(6)-type[78] 6-membered ring H-bonding (N2…O4, figure 2.12b) which is, otherwise, almost always observed in *N*-acyl anthranilamides.[70,79] Apparently, the steric clash (marked in double headed arrow in figure 2.12b) between the Ant2 aryl ring and the 5-membered proline ring (Pro3) [80] forces the H-bonding sites (Ant2 amide NH and the Pro3 amide CO) to come close in space resulting in the formation of a stronger H-bond in the Ant-Pro C9 turn [H-bond geometric parameters: bond distance $d(N…O)$ = 2.9 Å, $d(H…O)$ = 2.1 Å, bond angle (N–H...O) = 168°, and the planarity of the hydrogen bond torsion angle (N–H$^{...}$O=C) = -150°]. A fall out of this development is, interestingly, the weakening of the S(6)-type[78] 6-membered ring H-bonding interaction in Ant2[73] (N2…O4), [H-bond geometric parameters: bond distance $d(N…O)$ = 3.1 Å, $d(H…O)$ = 2.7 Å, bond angle (N–H...O) = 112°, and the planarity of the hydrogen bond torsion angle (N–H$^{...}$O=C) = 84°]. On the contrary, the C-terminal Ant4 residue in **2b**, having the perfect geometries for S(6)-type[78] intramolecular H-bonding interaction [H-bond geometric parameters: bond distance $d(N…O)$ = 2.6 Å, $d(H…O)$ = 1.9 Å, bond angle (N–H...O) = 138°, and the planarity of the hydrogen bond torsion angle (N–H$^{...}$O=C) = 5°], retained the characteristic 6-membered H-bonding usually found in *N*-acylated anthranilamides,[70,79] obviously due to the lack of steric clash with Pro, which its predecessor residue Ant2 experienced. It is noteworthy that the close proximity of the Ant2 amide NH and the Pro3 carbonyl groups, as evident from the crystal structure of **2b** [$d(N2…O5)$ = 2.9 Å] would readily explain why Ant-Pro-esters (free amines) have high propensity to undergo

cyclization yielding cycloanthranilylproline.[74] Reverse turns are characterized by the ϕ, ψ dihedral angles of the amino acid residues involved in the turn region (i+1 and i+2 sites).[81] The C9 turn in **2b** features the following dihedral angles: (ϕ_{Ant2} = -166°, ψ_{Ant2} = -101°, ϕ_{Pro3} = -63°, ψ_{Pro3} = +169°), wherein the Pro dihedral angles are typical of its semi-extended conformation, usually described.[73a]

2.9.2 NMR studies

We undertook extensive NMR studies to provide insights into the solution-state conformation of the oligomers (CDCl$_3$/CD$_2$Cl$_2$, 500 MHz). The signal assignments were made unambiguously using a combination of two-dimensional COSY, HSQC, HMBC, TOCSY and NOESY experiments. Details of the peak assignments with tables and spectra are provided in the experimental section of this chapter. The oligomers **2a**, **2d** and **3** were readily soluble in nonpolar organic solvents (>>100 mM in CDCl$_3$) at ambient temperature suggesting that the polar hydrogen-bonding groups are strongly solvent-shielded, preventing the formation of polymeric aggregates.[82] Inspection of the ^{1}H NMR spectra of the oligomers **1a, 2a**, and **3** revealed the existence of *cis-trans* isomerization[71] as evidenced from multiple signals from the ^{t}Boc, α-H of Pro1 and the NH of the Ant2. This is presumably caused by the N-terminal Pro residue wherein the amide bond connecting the pyrrolidine ring and ^{t}BOC group is free to rotate; a known property associated with polypeptides featuring Pro residue at the N-terminal.[71] However, the *cis-trans* isomerism could be effectively arrested by the introduction of an Ant residue at the N-terminal, as demonstrated in the case of **2d,** which gives rise to well-dispersed single set of sharp

signals in the ^{1}H NMR and ^{13}C spectra.

The (1→2)-type 9-membered-ring pseudo β-turn,[77] observed in the solid-state of **2b**, was unambiguously confirmed in its closer analog **2a** in the solution-state, by the observed characteristic dipolar couplings (nOes) from the 2D NOESY NMR spectra of **2a** (fig. 2.13).
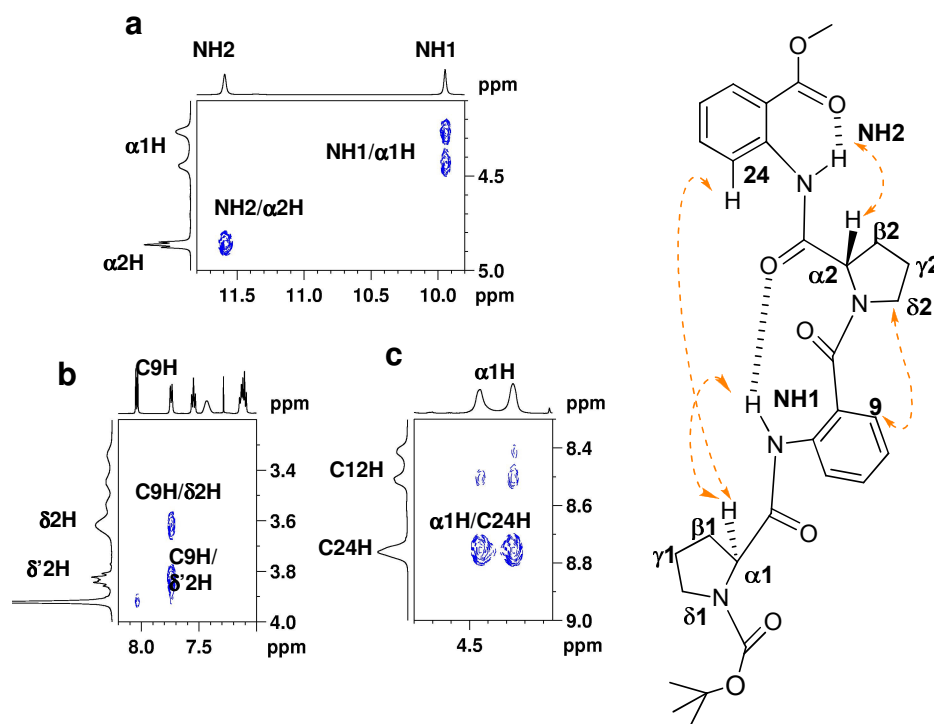
Fig. 2.13: 2D Extracts of **2a** (500 MHz, CDCl$_3$). (a) αH *vs* NH region; (b) αH *vs* Ar-H region; (c) δH *vs* Ar-H region.

Analysis of the crystal structure of **2b** had suggested that the most characteristic nOe that is quintessential to support the (1→2)-type 9-membered-ring pseudo β-turn[77] conformation would be the requirement of a diagnostic long range inter-residual dipolar coupling between αH of Pro1 and the aromatic protons of Ant4 [Pro1-αH *vs* Ant4-C24H, fig 2.13c, distance observed in the crystal structure of **2b**

d(αH-C24H = 2.47 Å)]. Although these residues are far apart, separated by two residues, the peculiar 9-membered-ring pseudo β-turn conformation requires the peptide backbone to considerably fold back resulting in the short distance, as evident from the crystal structure of **2b**. Other characteristic nOes that supported the folded conformation were the dipolar interactions between the Aryl-NH and the αH of Pro preceding it (NH1 *vs* α1H , NH2 *vs* α2H, fig. 2.13a) and the interactions between Pro δ-protons and their respective preceding aromatic protons (C9H vs δ2H, fig. 2.13b). Thus, the NMR studies suggest that the folded conformation observed in the crystal structure of **2b** was reflected in the solution-state also, as evidenced from the characteristic nOes observed for its closer analog **2a**.

Analysis of the 2D NOESY data of **2d** also revealed the existence of inter-residual nOe between αH of Pro2 residue and C31 Ar-H of Ant5 residue, suggestive of the prevalence of pseudo β-turn structure (fig. 2.14b). Further, the αH of Pro2 is found to have nOe with the amide NH of Ant1 (fig. 2.14b). Long-range inter-residual nOes were also observed between the β-protons of Pro2 and C31 Ar-H of Ant5 residue (fig. 2.14d). It is noteworthy that the observed strong nOes between Pro αH and the amide NH of the subsequent Ant residue (α1H *vs* NH2, α2H *vs* NH3, fig. 2.14a) is clearly suggestive of their closer positioning, which was again revealed in the crystal structure of **2b**. Other selected inter-residual nOes that supported the folded conformations were between the Pro β-protons and the amide NHs of the subsequent Ant residues (β1H *vs* NH2, β2H *vs* NH3, fig. 2.14c) and between the Pro δ-protons

and the amide NHs of the preceding Ant residues (δ1H *vs* NH1, δ2H *vs* NH2, fig. 2.14e). The Pro δ-protons also showed dipolar coupling to their respective preceding aromatic protons (δ1H *vs* C4H, δ2H *vs* C16H, fig. 2.14f), since the peculiar 9-membered-ring pseudo β-turn conformation required their closer positioning, as suggested from the crystal structure of **2b**.
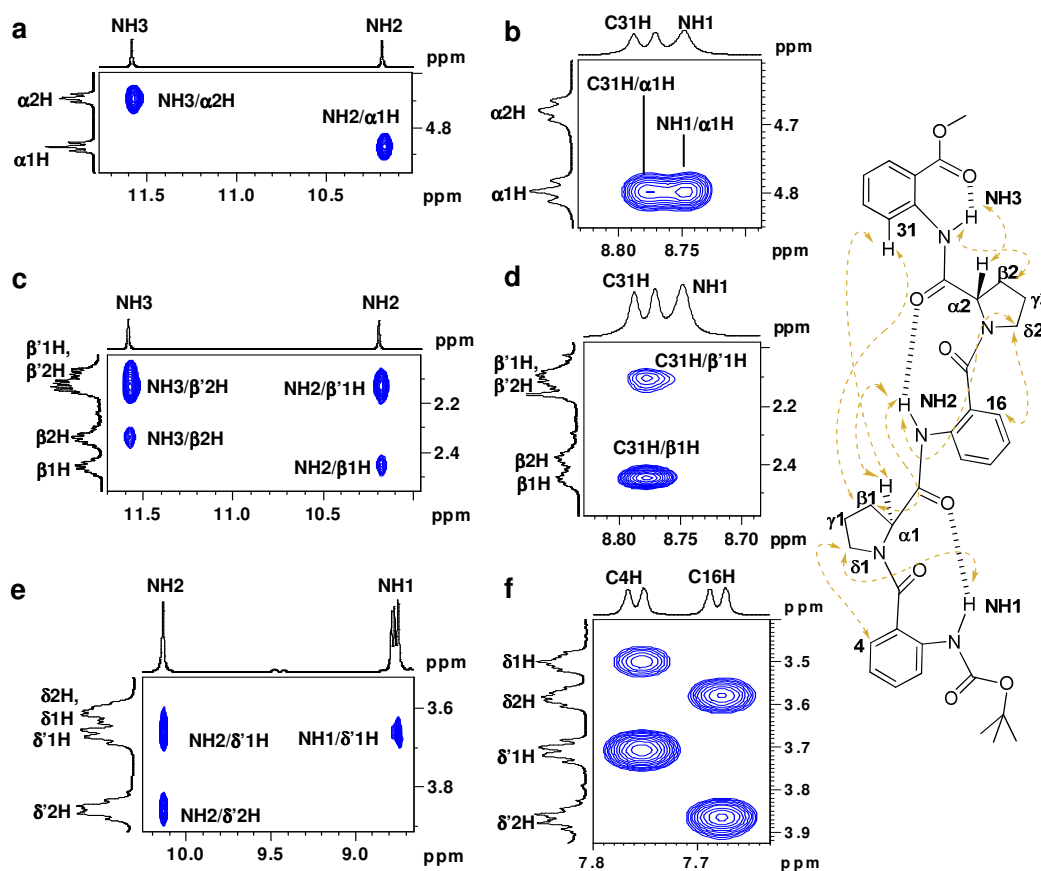
Fig. 2.14: Selected nOe extracts from the 2D NOESY data of **2d** (CDCl$_3$ / CD$_2$Cl$_2$, 500 MHz). (a) αH *vs* NH region (CDCl$_3$); (b) αH *vs* Ar-H region (CD$_2$Cl$_2$); (c) βH *vs* NH region (CDCl$_3$); (d) βH *vs* Ar-H region (CD$_2$Cl$_2$); (e) δH *vs* N-H region (CD$_2$Cl$_2$); (f) δH *vs* Ar-H region (CDCl$_3$). *Note*: For improved spectral dispersion in the aryl region, NMR studies were performed in CD$_2$Cl$_2$.

Interestingly, similar nOe patterns could also be observed for **2d** in different solvents ($CDCl_3$ and CD_2Cl_2), suggestive of similar conformational features in these solvents, although slight chemical shift dispersion was observed (fig. 2.14). It is noteworthy that analogous dipolar coupling patterns were observed in the oligomers **2a** (fig. 2.13), and **3** (page 171, experimental section) suggestive of similar conformational features for these oligomers in the solution-state, although rotamer formation was observed in these oligomers because of the *N*-terminal "free" Pro residues, as mentioned previously.[71]

Solvent titration and dilution experiments are particularly useful for differentiating the nature of hydrogen bonding interactions (inter *vs* intra) in the solution-state, wherein intermolecular hydrogen bonding interactions (solvent exposed NHs) are relatively more vulnerable to environment effects.[83] Further experimental support for the prevalence of intramolecular hydrogen bonding interactions in oligomers **2a, 2d**, and **3**, as observed in the solid-state structure of **2b** which was also substantiated by the observed dipolar couplings, came from DMSO titration and $CDCl_3$ dilution studies of the oligomers (fig. 2.15). Notably, all the amide NHs of the oligomers showed negligible shift ($\Delta\delta$ NH: < 0.15 ppm) when solutions of **2a**, **2d**, and **3** in $CDCl_3$ were titrated gradually with [D_6] DMSO (5 µl on each addition, totaling ten points). The same trend was observed in solvent dilution study as well, as demonstrated in the case of the pentapeptide **2d**, wherein the shift was observed to be marginal ($\Delta\delta$ NH: < 0.1 ppm). Thus, the solvent titration / dilution studies further supported the involvement of intramolecular hydrogen bonding

interactions in the oligomers **2** and **3**.
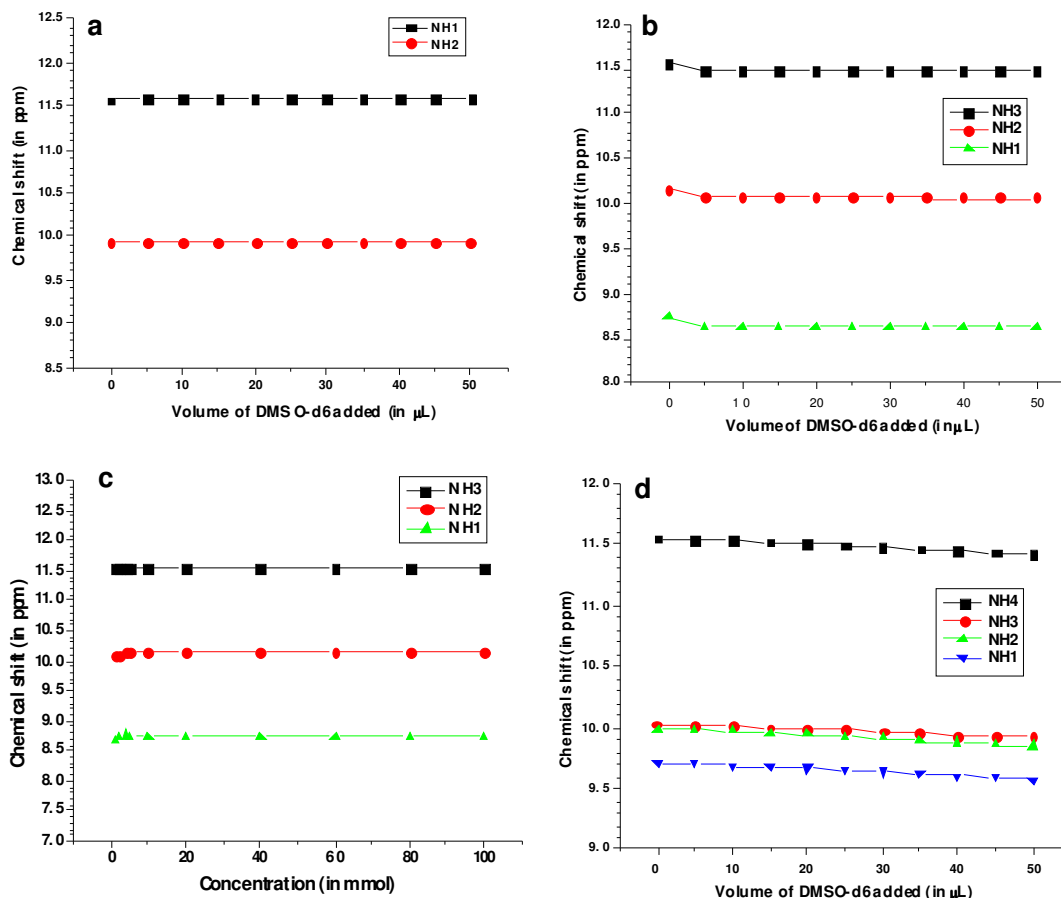
Fig. 2.15: [D_6] DMSO NMR titration / CDCl$_3$ dilution plots of the oligomers. (a) [D_6] DMSO titration plot of tetrapeptide **2a**; (b) [D_6] DMSO titration plot of pentapeptide **2d**; (c) CDCl$_3$ dilution plot of pentapeptide **2d**; (d) [D_6] DMSO titration plot of octapeptide **3**. *Note*: The assignments of the amide NHs should be from the N-terminal of the peptides, according to the molecular structure given in scheme 2.1

2.9.3 Theoretical studies

The difficulty in crystallizing other oligomers **2d** and **3** prompted us to visualize their structural architecture through *ab initio* MO theory studies.[76] It is should be noted that computational investigations using *ab initio* MO theory continue

to be of considerable utility in the structural understanding of synthetic oligomers, in particular of structurally rigid oligomers wherein structural/conformational alternatives are limited due to intramolecular stabilizing forces such as hydrogen bonding.[76] The (1→2)-type 9-membered-ring pseudo β-turn conformation, as clearly evident in the crystal structure of **2b**, was revealed in the pentapeptide **2d** as well, according *to ab initio* MO theory at the HF/6-31G* level (fig. 2.16a). Due to the presence of two Ant-Pro building blocks, **2d** periodically displays a right handed helical structural architecture, formed through intramolecular H-bonding[84]. As seen in the crystal structure of **2b**, the Ant residues involved in the C9-turn formation is deprived of effective 6-membered ring H-bonding interaction because of the misalignment of its H-bonding sites, although the C-terminal Ant residue clearly retains such an interaction owing to the absence of a successive Pro residue. It is noteworthy that the turn structures and the intramolecular hydrogen bonding interactions suggested in the HF/6-31G* level structure (fig. 2.16a) were strongly supported by solution-state NMR studies.

Structural investigations of **3** using *ab initio* MO theory at the HF/6-31G* level suggested two conformations (fig. 2.16 b,c); both differing in the orientation of the N-terminal carbamate carbonyls.[71] A helix type with only 9-membered hydrogen-bonded rings arising from nearest-neighbor (1→2) interactions was not found in α/β-hybrid peptides until now. Mixed helices are excluded in the present case due to the absence of the amide hydrogen in the proline residue. Thus, only one hydrogen-bonded ring type is possible. The alternative with only 11-membered rings is

obviously prevented by the rigid planar structure of the Ant residues.
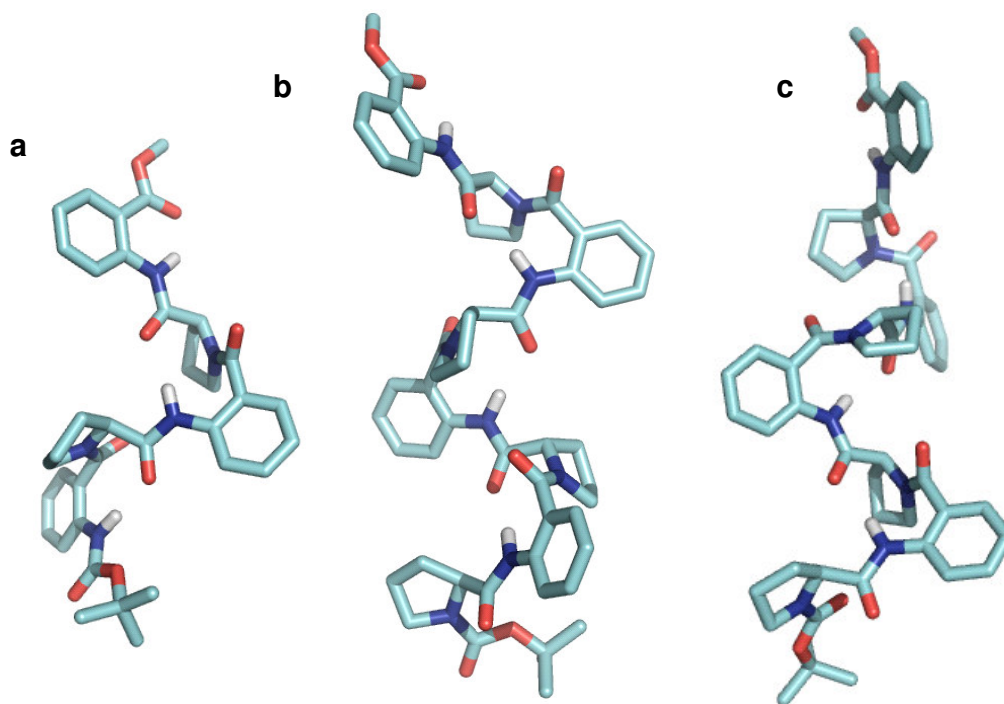
Fig. 2.16: Structural architecture of oligomers at the HF/6-31G* level of *ab initio* MO theory displaying C9 helical turns. (a) conformation of pentapeptide **2d**; (b) conformation of octapeptide **3** with *trans* Pro1 carbamate bond; (c) conformation of octapeptide **3** with *cis* Pro1 bond. Hydrogens, other than at the hydrogen bonding sites, have been omitted for clarity.

Indeed, the possibility of conformational alternatives was also clearly reflected in the solution-state as evident from multiple signals mainly arising from the N-terminal Pro residues.[71] As mentioned previously, such a conformational flipping could be effectively circumvented by the introduction of an N-terminal Ant residue, as demonstrated in **2d**. The octapeptide **3**, having the diagonally placed similar residues in the right handed helical framework, clearly replicates the turn structures observed in the crystal structure of its shorter analog **2b**. The hydrogen bonding

parameters of the C9 pseudo β-turn in **3** (H-bond geometric parameters: bond distance $d(N\ldots O)_{avg}$ = 3.18 Å, $d(H\ldots O)_{avg}$ = 2.18 Å, bond angle $(N–H...O)_{avg}$ = 168°] are reasonably comparable with that of the crystal structure of **2b** [H-bond geometric parameters: bond distance $d(N\ldots O)$ = 2.9 Å, $d(H\ldots O)$ = 2.1 Å, bond angle (N–H...O) = 168°)], suggesting the reliability of the structures obtained using ab initio MO theory at HF/6-31G* level.

2.10 Role of Pro in the C9 turn formation

After getting insight into the conformational features of **2b**, we turned our attention to investigate the role of Pro in the C9 turn formation. Oligomers **4** and **5**, featuring Ala and Aib residues as substitutes of Pro were synthesized. Whereas Ala was chosen as a confomationally flexible analog of Pro, Aib was a chosen as a conformationally constrained analog albeit with a different dihedral angle preference.[68] Also we studied a model tripeptide **6** wherein, the Ant3 in -Ant-Pro-Ant- sequence was substituted by glycine; an amino acid known for its flexible dihedral angle tolerance.[85]

2.10.1 Synthesis

The dipeptide ester **9** obtained by the coupling of Boc-Ant-OH with Ala-OBn was subjected to hydrogenolysis to yield the free acid **9a** which was subsequently coupled with Ant-OMe to furnish the tripeptide **10.** In order to probe their solid state conformaion, the non-crystalline ester **10** was converted to its corresponding methyl amide **4** by reacting with methanolic methyl amine (scheme 2.2)

Scheme 2.2: Synthesis of **4**.
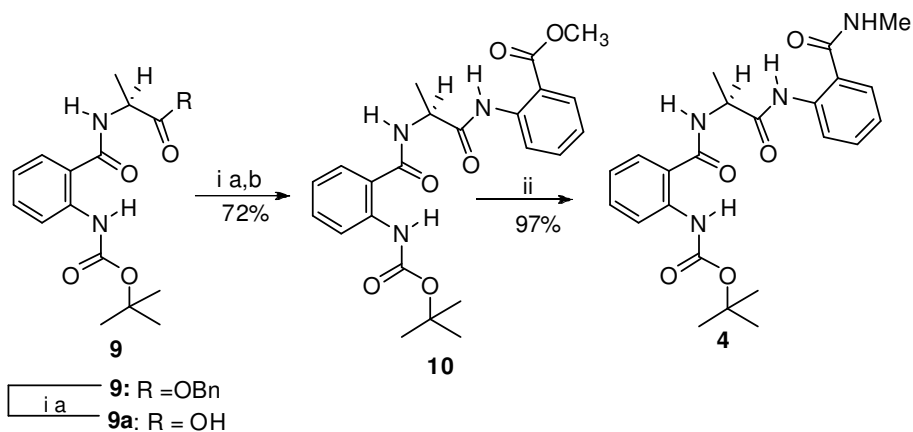
Reagents and Conditions: (i) (a) Pd/C, H$_2$, AcOEt, rt, 6 h; (b) Ant-OMe, DCC, DMAP, rt, 12h; (ii) methanolic methylamine rt, 6 h.

Compound **12** required for the synthesis of Aib incorporated tripeptide **5,** was obtained by reacting *o*-nitrobenzoyl chloride (generated by reacting *o*-nitrobenzoic acid with oxalyl chloride and cat. amount of DMF) with Aib. The acid **12** on coupling with Ant-OMe using DCC as the coupling agent furnished **13**. Reduction of nitro group followed by *in situ* Boc protection yielded **5** (scheme 2.3).

Scheme 2.3: Synthesis of **5**.
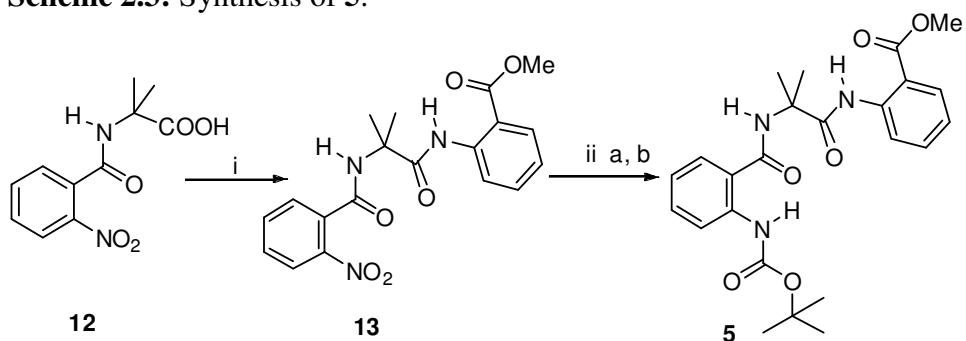
Reagents and conditions: (i) Ant-OMe, DCC, DMAP, rt, 12 h; (ii) (a) Pd/C, H$_2$, MeOH, rt, and then (b) (Boc)$_2$O.

Dipeptide **8** (scheme 2.4) was prepared by coupling Bo-Ant-OH with Pro-OBn using DCC as the coupling agent. Hydrogenolysis of **14** followed by coupling with glycine benzyl ester p-toluenesulfonate using DCC as the coupling agent yielded **15** and subsequent reaction with methanolic methylamine furnished the model tripeptide **6** with an amidated C-terminal.

Scheme 2.4: Synthesis of **6**.
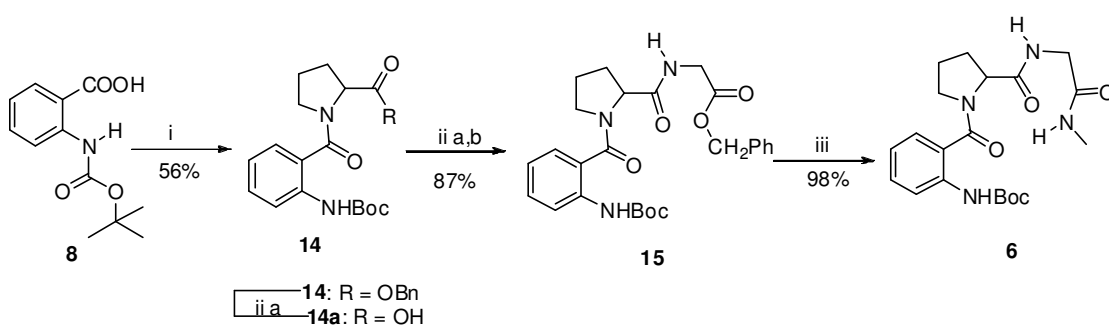
Reagents and conditions: (i) Pro-OBn, DCC, DCM, 12h; (ii)(a) Pd/C, H$_2$, MeOH, rt, 10 h; (b) DCC, Gly-OBn.PTSA, TEA, DCM, rt, 12 h; (iii) methanolic methylamine, 6 h, rt.

2.10.2 Solid-state conformational analyses

Interestingly, crystal structure studies revealed the absence of any C9 turn formation in the oligomers **4**, and **5** containing Pro substitutes (fig. 2.17 a,b). The single crystal X-ray diffraction studies of both **4** and **5** revealed that the amide NHs of the Ant1 residue are clearly involved in strong intramolecular 6-membered-ring hydrogen bonding [H-bond geometric parameters for Ant1 in **4**: bond distance d(N…O) = 2.7 Å, d(H…O) = 2.0 Å, bond angle (N–H...O) = 137°), and for Ant1 in **5:** bond distance d(N…O) = 2.69 Å, d(H…O) = 2.0 Å, bond angle (N–H...O) = 135°].

It may be recalled that owing to the C9 turn, the S(6)-type[78] interaction was quite weaker in **2b**, as evident from its H-bond geometric parameters [bond distance $d(N…O) = 3.1$ Å, $d(H…O) = 2.7$ Å, bond angle (N–H...O) = 112°]. Nevertheless, the C-terminal Ant3 residues in **4** and **5** retained the geometries for S(6)-type[78] intramolecular H-bonding interaction [H-bond geometric parameters for Ant3 in **4**: bond distance $d(N…O) = 2.66$ Å, $d(H…O) = 2.02$ Å, bond angle (N–H...O) = 130°), and for Ant3 in **5**: bond distance $d(N…O) = 2.65$ Å, $d(H…O) = 1.93$ Å, bond angle (N–H...O) = 139°)], as observed in the case of 2b [bond distance $d(N…O) = 2.6$ Å, $d(H…O) = 1.9$ Å, bond angle (N–H...O) = 138°)]. Comparison of the ϕ, ψ dihedral angles of the α-amino acid residues in **4**, **5** and **2b** reveals a realistic picture of the requirement of α-amino acid residues with suitable dihedral angle constraints in the formation of the C9 turn. As evident from the crystal structure investigations of **2b**, the ϕ, ψ dihedral angles of the Pro residue involved in the C9 turn formation are: $\phi_{Pro3} = -63°$, $\psi_{Pro3} = +169°$, which differ considerably from the dihedral angles of the Ala and Aib residues in **4**, and **5**, respectively: $\phi_{ala} = -103^0$, $\psi_{ala} = -3^0$, $\phi_{aib} = -52^0$, $\psi_{aib} = -39^0$. Interestingly, the model peptide **6** having an Ant residue preceding Pro-Gly sequence, a dipeptide motif well-known for its ability to induce type-II β-turns in peptides and proteins,[86] also did not show any sign of folded conformation, which suggest that issues other than steric and dihedral angles might also be involved in the stabilization of the unusual $(1 \rightarrow 2)$-type 9-membered-ring pseudo β-turn conformation described herein. As in the cases of **4**, and **5**, Ant1 in **6** was found to be clearly involved in the six-membered H-bonding interactions; [bond distance $d(N…O) =$

2.79 Å, d(H…O) = 2.13 Å, bond angle (N–H...O) = 133°)]. Further, the carbonyl of

Ant1 also participates in type-II β-turn formation (fig. 2.17 c).[86]
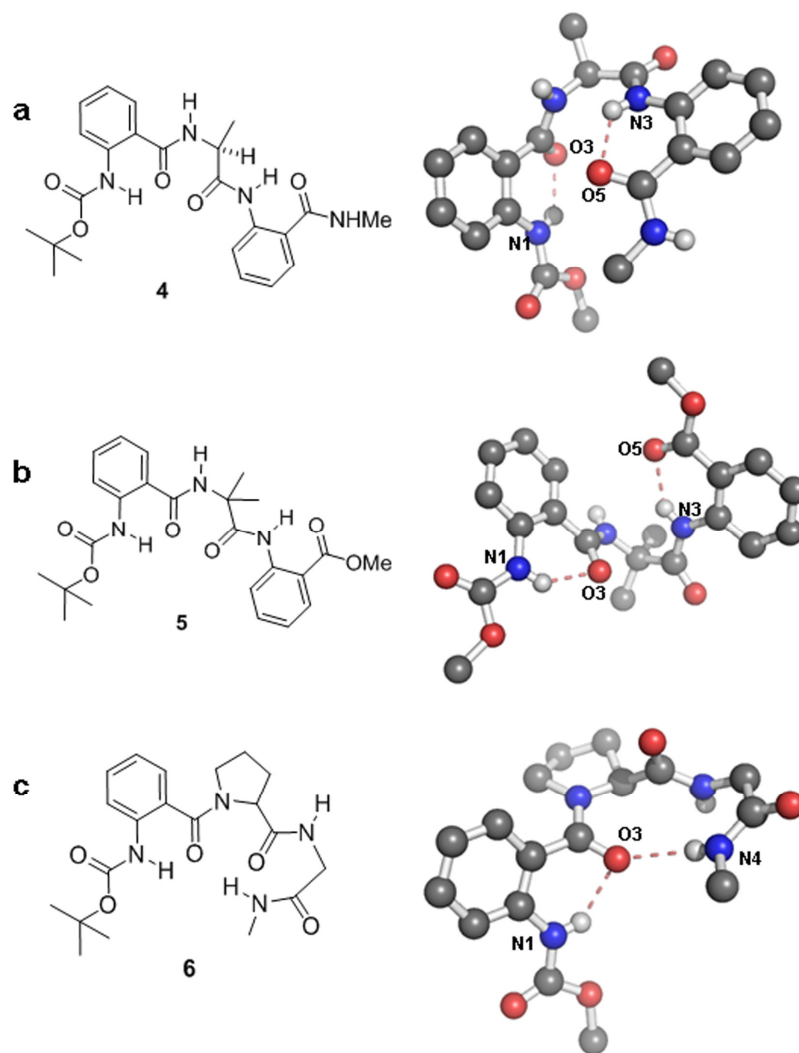
Fig. 2.17: Crystal structures of short analogs **4** and **5** (right) along with their molecular structures (left). Hydrogens, other than at the hydrogen bonding sites, have been omitted for clarity in the crystal structures.

It is clear from the crystal structures of **4**, **5** and **6** that the amide carbonyl of

the central amino acids are inaccessible for an intramolecular hydrogen bonding

leading to a folded conformation as in the case of Pro incorporated oligomers **2**-**3**. Instead, they facilitate intermolecular H-bonding (fig. 2.18)

Fig. 2.18: Self-assembly observed in **4** and **5**.
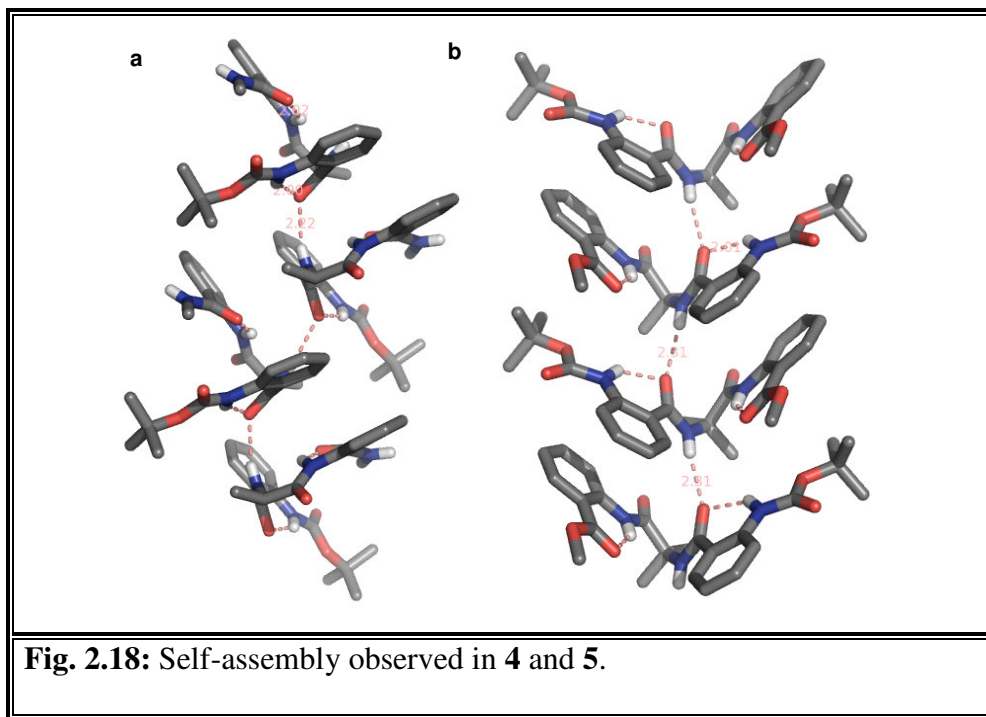
2.10.3 Dilution and titration experiments of 4-6

In order to distinguish the hydrogen bonding interactions (inter *vs* intra), we undertook dilution / titration experiments in $CDCl_3$. In all the cases, the NH of Ant which is involved in strong S(6)-type intramolecular hydrogen bonding underwent negligible shift ($\Delta\delta$ NH: < 0.2 ppm) with addition of DMSO-d6; a polar solvent that competes for hydrogen bonding.[83] On the contrary, the amide NHs of the central amino acids (Ala, and Aib) in **4** and **5** underwent significant shift ($\Delta\delta$ NH_{ala} = 0.67 ppm, $\Delta\delta$ NH_{aib} = 0.25 ppm), upon DMSO titration, suggesting their role in intermolecular hydrogen bonding (fig. 2.19a,b). The formation of turn structure in **6,**

as evident from its crystal structure, was supported by dilution / titration studies (fig. 2.19c,d)
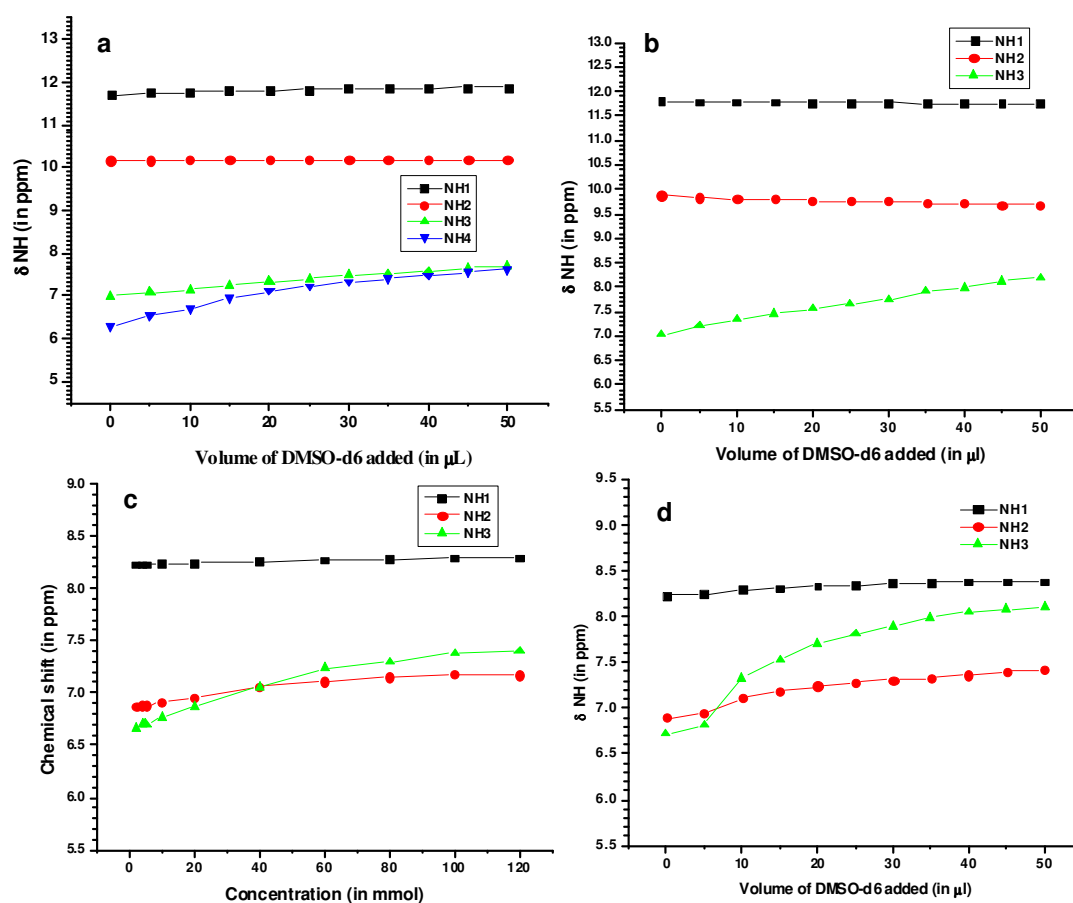
Fig. 2.19: [D_6] DMSO NMR titration / CDCl$_3$ dilution plots of the **4**, **5** and **6** (a) [D_6] DMSO titration plot of **4**; (b) [D_6] DMSO titration plot of **5**; (c) CDCl$_3$ dilution plot of **6**; (d) [D_6] DMSO titration plot **6**. *Note*: The assignments of the amide NHs are given in the experimental section.

2.11 Conclusion

In conclusion, hetero oligomers comprising Pro-Ant motifs, repeating at regular intervals, have been shown to be adopting right-handed helical structural architecture, displaying an unusual (1→2)-type 9-membered-ring pseudo β-turn

conformation. The structural architecture of these hetero oligomers is in stark contrast to the conformation reported for oligo-anthranilamides, which assume extended sheet structure.[70] The intrinsic conformational bias of the individual amino acids Pro and Ant helps the oligomer backbone adopt the folded conformation, as evident from the results of investigations of the oligomers and their analogs. The requirement of Pro as an essential amino acid for the formation of such a structural architecture suggests that steric and dihedral angle constraints play key roles in the stabilization of the folded conformation.[87] The findings disclosed herein have the potential to design novel conformationally restricted structures using steric and dihedral angle constraints, and would help augment the conformational space available for synthetic oligomer design with diverse backbone structures. Investigations on the sequential preferences clearly suggest the requirement of Ant and Pro residues for effecting the formation of the unusual (1→2)-type 9-membered-ring pseudo β-turn conformation described herein. The ease of synthesis and the simplicity of the backbone are attractive features of these hybrid oligomers whose structural architecture could be modulated by altering the chirality[88] of Pro residues and by incorporating functional side chains on the Ant and Pro residues[89] would enable these helical oligomers to tailor their structural features for possible applications involving protein recognitions.[90]

2.12 Experimental section

<u>**Crystal Data:**</u> Data for the compounds **4-6** were collected at T = 293 K, on SMART APEX CCD Single Crystal X-ray diffractometer using Mo-K$_\alpha$ radiation (λ = 0.7107 Å), crystal data of **2b** were collected at 120 K on a Bruker SMART CCD 6000 diffractometer using the same radiation. The structures were solved by direct methods using SHELXTL. All the data were corrected for Lorent, polarisation and absorption (except 2b) effects. SHELX-97 (ShelxTL) was used for structure solution and full matrix least squares refinement on F^2. Hydrogen atoms were included in the refinement in the riding mode. The refinements were carried out using SHELXL-97.

Crystal data for 2b: Prismatic crystals of **2b** were obtained from a mixture of methanol with PEG-200. A small piece (0.24 x 0.17 x 0.07 mm) of a large prism has been used for the data collection. $C_{29}H_{34}N_4O_7$, 0.5 H_2O, M = 559.61, monoclinic, space group P 2_1, a = 9.6923(5), b = 9.4608 (4), c = 15.6706 (7) Å , β = 93.158 (2)°, V = 1434.76 (12) Å^3, F(000) = 594, Z = 2, D$_c$ = 1.295 mg m^{-3}, μ = 0.094 mm^{-1} (Mo-K$_\alpha$, λ = 0.71073 Å), T = 120 (2)K. 17934 reflections (1.30 $\leq$ θ $\leq$ 29.0°) were collected (ω-scan, 0.3°/frame) yielding 4027 unique data (R$_{merg}$ = 0.1064). Final wR$_2$ (F^2) = 0.0949 for all data (375 refined parameters), conventional R (F) = 0.0411 for 5539 reflections with I $\geq$ 2σ, GOF = 0.960.

Crystal data for 4: Single crystals of **4** were grown by slow evaporation of the mixture of ethyl acetate and pet. ether. Colorless needle of approximate size 0.41 x 0.28 x 0.08 mm, was used for data collection. Multiscan acquisition. Total scans = 3, total frames = 1271, Oscillation / frame -0.3°, exposure / frame = 15.0 sec / frame, θ

range = 2.51 to 25.00º, completeness to θ of 25.0º is 99.9 %. SADABS correction applied, $C_{23}H_{28}N_4O_5$, M = 440.49. Crystals belong to Monoclinic, space group $P2_1/n$, a =15.219 (1), b = 9.4069 (6), c = 16.268 (1) Å, β = 101.440 (1)º. V = 2282.6 (3) Å^3, Z = 4, D_c = 1.282 mg m^{-3}, μ (Mo-K$_\alpha$) = 0.092 mm^{-1}, 11233 reflections measured, 4016 unique [I>2σ(I)], R value 0.0433, wR2 = 0.1039.

Crystal data for 5: Colorless plate crystals of the compound were grown by slow evaporation of methanol. Crystal size 0.52 x 0.20 x 0.17 mm, was used for data collection. multiscan data acquisition. Total scans = 4, total frames = 2199, Oscillation / frame -0.3º, exposure / frame = 15.0 sec / frame, θ range = 2.39 to 25.00º, completeness to θ of 25.0º is 100.0 %. SADABS correction applied, $C_{24}H_{29}N_3O6$, M = 455.50. Crystals belong to Orthorhombic, space group $Pna2_1$, a = 10.1637 (6), b = 15.6505 (8), c = 15.0116 (9) Å, V = 2387.9 (2) Å^3, Z = 4, D_c = 1.267 mg m^{-3}, μ (Mo-K$_\alpha$) = 0.092 mm^{-1}, 19877 reflections measured, 4192 unique [I>2σ(I)], R value 0.0354, wR2 = 0.0821.

Crystal data for 6: Single crystals of the compound were grown by slow evaporation of the solution mixture of methanol and DCM. Colorless plate of approximate size 0.22 x 0.18 x 0.09 mm was used for data collection. Multiscan data acquisition. Total scans = 3, total frames = 1271, Oscillation / frame -0.3º, exposure / frame = 15.0 sec / frame, θ range = 2.40 to 25.00º, completeness to θ of 25.0º is 100.0 %. SADABS correction applied, $C_{20}H_{28}N_4O_5$, M = 404.46. Crystals belong to the Orthorhombic, space group $Pbca$, a = 10.867 (1) Å, b = 16.478 (3) Å, c = 24.053 (2) Å, V = 4306.9 (10) Å^3, Z = 8, D = 1.248 gcm^{-3}, μ = 0.091 mm^{-1}, $F(000)$= 1728, 20702 reflections

measured, 3796 unique [I>2σ(I)], R = 0.0768. Rw = 0.1355

tert-Butyl 2-(2-(methoxycarbonyl)phenylcarbamoyl)pyrrolidine-1-carboxylate 1a:

A solution containing Boc-proline (4 g, 18.6 mmol, 1 equiv.) and Ant-OMe (2.8 g, 18.6 mmol, 1 equiv.) in dry acetonitrile (30 mL) was cooled to 0 ^{o}C. To this was added TBTU (7.16 g, 22.4 mmol, 1.2 equiv.) followed by DIEA (4.8 mL, 27.8 mmol, and 1.5 equiv.) and the reaction mixture was stirred at 0 ^{o}C for 10 minutes and at room temperature for 12 h. The reaction mixture was stripped off the solvent under reduced pressure, the residue was taken in dichloromethane and the organic layer was washed sequentially with potassium hydrogen sulfate solution, saturated sodium bicarbonate and water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which on purification by column chromatography (80:20 pet. ether/ethyl acetate, R_f: 0.3) afforded **1a** as a waxy liquid (5.8 g, 90%). $[\alpha]^{25}_D$: -127.3 (*c* = 0.11, CHCl$_3$); IR (CHCl$_3$) ν (cm^{-1}): 3269, 2984, 2941, 2908, 2878, 1738, 1703, 1587, 1529, 1450, 1371, 1240, 1047; ^{1}H NMR (200 MHz, CDCl$_3$) δ: 11.57$_{rotamer}$ & 11.47 (1H), 8.77-8.73 (d, *J* = 8.3 Hz, 1H), 8.02 (d, *J* = 7.58 Hz, 1H), 7.53 (t, *J* = 7.35 Hz, 1H), 7.08 (t, *J* = 7.54 Hz, 1H), 4.44$_{rotamer}$ & 4.31 (1H), 3.89 (s, 3H), 3.68 (m, 2H), 2.18 (m, 2H), 1.91 (m, 2H), 1.49$_{rotamer}$ & 1.33 (9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ: 172.4, 168.2, 154.3, 141.2, 140.9, 134.6, 131.0, 130.7, 122.7, 120.3, 120.1, 115.3, 80.3, 62.7, 62.1, 52.4, 52.1, 47.2, 46.8, 31.5, 30.5, 28.4, 28.2, 24.4, 23.8; ESI Mass: 349.09 (M+H)$^+$, 371.07 (M+Na)$^+$, 387.05 (M+K)$^+$;

Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05.; H, 6.94.; N, 8.04. Found: C, 62.20.; H, 7.02.; N, 7.92.

2-(1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxamido)benzoic acid 1b:

To a solution of **1a** (2 g, 5.7 mmol, 1 equiv.) in methanol (15 mL) was added LiOH·H_2O (0.49 g, 11.5 mmol, 2 equiv.) in water (6 mL) and the reaction mixture was stirred for 12 hours. The solvent was stripped off under reduced pressure and the product was dried in P_2O_5 vacuum desiccator overnight. This crude product was taken for the next reaction without further purification.

Methyl 2-(pyrrolidine-2-carboxamido)benzoate, hydrochloride salt 1c:

To an ice cold solution of **1a** (2.2 g, 6.3 mmol) in dry ether (10 mL) dry HCl gas was passed for ten minutes. The solvent was stripped off under reduced pressure and the residue was dried in KOH desiccator overnight. The crude product was used for the next step without further purification.

***tert*-Butyl 2-(2-(2-(2-(methoxycarbonyl)phenylcarbamoyl)pyrrolidine-1-carbonyl) phenyl carbamoyl)pyrrolidine-1-carboxylate 2a:**

  The acid **1b** (1.6 g, 4.8 mmol, 1 equiv.) was coupled with amine **1c** (1.36 g, 4.8 mmol, 1 equiv.) using TBTU (1.84 g, 5.7 mmol, 1.2 equiv.) and DIEA (2 mL, 12 mmol, 2.5 equiv.). Work up, as described in the case of **1a**, followed by column chromatographic purification (eluent: pet.ether/ethyl acetate: 40:60, R_f: 0.6) yielded **2a** (2.1 g, 78%) as a fluffy solid, mp: 68-70 ^{o}C, $[\alpha]^{26}_D$: -130 (*c* = 1, CHCl$_3$); IR (CHCl$_3$) ν (cm^{-1}) 3271, 3016, 2980, 1693, 1682, 1589, 1526, 1452, 1393, 1267, 1215,

1164, 1089; ^{1}H NMR (400 MHz, CDCl$_3$) δ: 11.59 (s, 1H), 9.94 (s, 1H), 8.76 (bs, 1H), 8.50$_{rotamer}$ & 8.41 (1H), 8.04-8.02 (d, J = 6.91 Hz, 1H), 7.75-7.73 (d, J = 7.75 Hz, 1H), 7.55 (m, 1H), 7.43 (bs, 1H), 7.13-7.07 (m, 2H), 4.86 (m, 1H), 4.43$_{rotamer}$ & 4.26 (1H), 3.92 (s, 3H), 3.83-3.79 (m, 1H), 3.63-3.33 (m, 3H), 2.46-2.39 (m, 1H), 2.21-2.13 (m, 2H), 2.10-2.02 (m, 2H), 1.91-1.87 (m, 2H), 1.81 (m, 1H), 1.50$_{rotamer}$ & 1.36 (9H); ^{13}C NMR (125 MHz, CDCl$_3$) δ: 171.6, 170.7, 169.7, 168.7, 154.0, 141.2, 136.7, 134.6, 134.3, 131.4, 130.8, 128.0, 122.7, 121.7, 120.4, 115.2, 79.9, 62.2, 61.5, 52.4, 50.5, 47.16, 46.65, 31.39, 30.22, 30.01, 28.3, 25.4, 24.3, 23.8; ESI-MS m/z: 565.47, (M+H)$^+$, 587.46 (M+Na)$^+$, 603.44; Anal. Calcd for C$_{30}$H$_{36}$N$_4$O$_7$: C, 63.82.; H, 6.43.; N, 9.92. Found: C, 63.98.; H, 6.46.; N, 9.82.

2-(1-(2-(1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxamido)benzoyl) pyrrolidine-2-carboxamido) benzoic acid 2b:

  Compound **2a** (0.8 g, 1.4 mmol, 1 equiv.) was subjected to ester hydrolysis with 2N LiOH solution in MeOH. The progress of the ester hydrolysis was monitored by the TLC and after the complete consumption of ester (12 h), the solvent was evaporated under vacuum, and the free acid was liberated by treating with aq. potassium hydrogen sulfate solution followed by extraction with dichloromethane (2 x 25 mL). The residue obtained after evaporation of the solvent under vacuum was carried forward for the next reaction. Crystals, suitable for single crystal analysis, could be obtained by crystallizing a small amount of the residue from methanol containing few drops of PEG 200. mp: 180-181 ^{o}C; IR (CHCl$_3$) ν (cm^{-1}) 3211, 3015, 2980, 1686, 1589, 1526, 1452, 1398, 1296, 1217, 1163, 1088, 1045; ESI Mass: 551.05 (M+H)$^+$, 573.03 (M+Na)$^+$, 589.0 (M+K)$^+$.

Methyl 2-(1-(2-(pyrrolidine-2-carboxamido)benzoyl)pyrrolidine-2-carboxamido) benzoate, hydrochloride salt 2c:

To an ice cold solution of **2a** (1.1 g, 1.9 mmol) in dry ether (10 mL), dry HCl gas was passed for ten minutes. The solvent was stripped off under reduced pressure and the residue obtained was dried in KOH desiccator overnight. The crude product was used for the next step without further purification.

Methyl 2-(1-(2-(1-(2-(*tert*-butoxycarbonylamino)benzoyl)pyrrolidine-2-carboxamido)benzoyl) pyrrolidine-2-carboxamido)benzoate 2d:

Boc-Ant-OH **8** (0.24 g, 1 mmol, 1 equiv.) was coupled with **2c** (0.5 g, 1 mmol, 1 equiv.) using TBTU (0.38 g, 1.2 mmol, 1.2 equiv.) and DIEA (0.4 mL, 2.5 mmol, 2.5 equiv.). Work up, as described in the case of **1a**, followed by column chromatographic purification (eluent: pet ether/ethyl acetate: 40:60, R_f: 0.6) yielded the pentapeptide **2d** (0.49 g, 72%) as a fluffy solid, mp: 116-118 ^{o}C; $[\alpha]^{26}_D$: -108 ($c =$ 0.5, CHCl$_3$); IR (CHCl$_3$) ν (cm^{-1}) 3273, 3018, 2995, 1724, 1686, 1624, 1589, 1522, 1452, 1412, 1393, 1298, 1267, 1248, 1219, 1092, 1047; ^{1}H NMR (400 MHz, CDCl$_3$) δ: 11.57 (s, 1H), 10.17 (s, 1H), 8.75-8.73 (m, 2H), 8.52-8.49 (d, *J* = 8.04 Hz, 1H), 8.17-8.15 (d, *J* = 8.30 Hz, 1H), 8.04-8.02 (d, *J* = 6.96 Hz, 1H), 7.73-7.71 (d, *J* = 7.23 Hz, 1H), 7.66-7.64 (d, *J* = 7.50 Hz, 1H), 7.56-7.52 (m, 1H), 7.43-7.36 (m, 2H), 7.14-7.08 (m, 3H), 4.85-4.83 (m, 1H), 4.69-4.66 (m, 1H), 3.91 (s, 3H), 3.87-3.80 (m, 1H), 3.71-3.65 (m, 1H), 3.58-3.53 (m, 1H), 3.50-3.45 (m, 1H), 2.47-2.39 (m,1H), 2.37-2.30 (m,1H), 2.14-1.76 (m, 6H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ: 170.8, 170.6, 170.1, 169.6, 168.7, 153.1, 141.1, 137.6, 136.8, 134.6, 131.2, 130.9, 128.3, 127.7, 123.96, 123.1, 122.9, 122.8, 121.7, 121.4, 120.3, 120.1, 115.2, 80.1, 62.3, 61.9, 52.4, 50.7, 50.4, 30.0, 29.98, 28.3, 25.5, 25.4; ESI Mass: 684.16 (M+H)$^+$, 706.11

(M+Na)$^+$, 722.07 (M+K)$^+$; Anal. Calcd for C$_{37}$H$_{41}$N$_5$O$_8$: C, 64.99.; H, 6.04.; N, 10.24. Found: C, 65.10.; H, 6.18.; N, 10.16.

tert-Butyl 2-(2-(2-(2-(2-(2-(2-(methoxycarbonyl)phenylcarbamoyl) pyrrolidine -1-carbonyl) phenylcarbamoyl)pyrrolidine-1-carbonyl)phenylcarbamoyl) pyrrolidine-1-carbonyl) phenyl carbamoyl)pyrrolidine-1-carboxylate 3:

The acid **2b** (0.6 g, 1.1 mmol, 1 equiv.) was coupled with amine **2c** (0.55 g, 1.1 mmol, 1 equiv.) using TBTU (0.42 g, 1.3 mmol, 1.2 equiv.) and DIEA (0.48 mL, 2.7 mmol, 2.5 equiv.) and the reaction was allowed to proceed for 12 h, at room temperature. Work up, as described in the case of **1a**, followed by column chromatographic purification (eluent: ethyl acetate/ MeOH: 98:2, R$_f$: 0.3) afforded **3** (0.73 g, 67%) as a fluffy solid; mp: 145-146 ^{o}C; [α]^{24}$_D$: -60 (*c* = 0.2, CHCl$_3$); IR (CHCl$_3$) ν (cm^{-1}) 3271, 3124, 3016, 2980, 1693, 1682, 1626, 1589, 1528, 1454, 1416, 1296, 1276, 1215, 1165, 1092; ^{1}H NMR (400 MHz, CDCl$_3$) δ: 11.56 (s,1H), 10.12 (s, 1H), 10.03 (s, 1H), 9.81$_{rotamer}$ & 9.73 (s, 1H), 8.74 (d, *J* = 8.31 Hz, 1H), 8.51-8.35 (m, 3H), 8.07 (d, *J* = 7.71 Hz, 1H), 7.70-7.47 (m, 4H), 7.46-7.32 (m, 3H), 7.25-7.10 (m, 4H), 5.05-4.7 (m, 3H), 4.48 $_{rotamer}$ & 4.30 (1H), 3.94 (s, 3H), 3.9-3.2 (m, 8H), 2.5-1.6 (m, 16H), 1.46$_{rotamer}$ & 1.35 (9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ: 172.13, 171.5, 168.85, 168.8, 168.7, 154.1, 141.1, 136.7, 135.9, 134.7, 130.96, 130.92, 130.6, 127.2, 127.16, 127.1, 123.98, 123.9, 122.98, 122.9, 121.7, 121.6, 121.1, 120.9, 115.3, 62.0, 61.97, 61.37, 61.1, 50.31, 49.9, 47.2, 46.7, 31.3, 30.19, 30.01, 29.7, 24.9, 25.43, 25.2, 23.9; ESI Mass: 998.04 (M+H)$^+$, 1019.99 (M+Na)$^+$, 1035.95 (M+K)$^+$; Anal. Calcd for C$_{54}$H$_{60}$N$_8$O$_{11}$: C, 65.05.; H, 6.07.; N, 11.24. Found: C, 65.28.; H, 6.20.; N, 11.10.

Benzyl 2-(2-(*tert*-butoxycarbonylamino)benzamido)propanate 9:

To an ice cold solution of Boc-Ant-OH (2 g, 8.4 mmol, 1 equiv.) and alanine benzyl ester (1.51 g, 8.4 mmol, 1 equiv.) in dichloromethane (20 mL) was added DCC (2.09 g, 10 mmol, 1.2 equiv.) and DMAP (cat. amount). The reaction mixture was stirred at 0 ^{o}C for 10 minutes and for 12h at room temperature. The reaction mixture was filtered to remove DCU, diluted with more dichloromethane and the organic layer was washed sequentially with potassium hydrogen sulfate solution, saturated sodium bicarbonate and water. Drying and concentration in vacuum yielded the crude ester **9**, which was directly used for the next reaction without further purification.

2-(2-(*tert*-Butoxycarbonylamino)benzamido)propanoic acid 9a:

The dipeptide **9** (2 g, 5 mmol) in ethyl acetate was subjected to hydrogenolysis using 10 % Pd/C (0.2 g) and H$_2$ (60 psi). After completion, the reaction mixture was filtered over celite pad. The filterate on evaporation under reduced pressure yielded acid **9a** which was carried over for the next reaction without further purification.

Methyl 2-(2-(2-(*tert*-butoxycarbonylamino)benzamido)propanamido)benzoate 10:

A solution of dichloromethane (15 mL) containing the acid **9a** (1 g, 3.2 mmol, 1 equiv.), Ant-OMe (0.49 g, 3.2 mmol, 1 equiv.), DCC (0.8 g, 3.9 mmol, 1.2 equiv.) and DMAP (cat. amount) was stirred at room temperature for 12 h. The reaction mixture was filtered to remove DCU and diluted with more solvent. The organic layer was washed sequentially with potassium hydrogen sulfate solution, saturated sodium

bicarbonate and water. Drying and concentration in vacuum yielded the crude product which on purification by column chromatography (eluent: ethyl acetate/pet. ether: 20/80, R_f: 0.3) afforded **10** (1 g, 72 %); mp: 112-113 ^{o}C; ^{1}H NMR (400 MHz, CDCl$_3$) δ: 11.56 (s, 1H), 10.14 (s, 1H), 8.69-8.67 (d, *J* = 8.48 Hz, 1H), 8.38-8.36, (d, *J* = 8.49 Hz, 1H), 8.04-8.02 (d, *J* = 8.13 Hz, 1H), 7.66-7.64 (d, *J* = 7.62 Hz, 1H), 7.57-7.53 (t, *J* = 7.48 Hz, 1H), 7.46-7.42 (t, *J* = 8.06 Hz, 1H), 7.13-7.08 (m, 2H), 7.02-6.99 (t, *J* = 7.65 Hz, 1H), 4.88-4.81 (m, 1H), 3.88 (s, 3H), 1.65-1.63 (d, *J* = 6.98 Hz, 3H), 1.50 Hz (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ: 171.0, 168.7, 153.1, 140.9, 140.5, 134.7, 132.8, 130.9, 127.1, 123.1, 121.3, 120.4, 119.7, 119.1, 115.3, 80.2, 52.5, 50.8, 28.3, 18.97; ESI-MS: 442.49 (M+H)$^+$; 464.4 (M+Na)$^+$; 480.43 (M+K)$^+$.

tert-Butyl 2-(1-(2-(methylcarbamoyl)phenylamino)-1oxopropan-2-ylcarbamoyl) phenyl carbamate 4:

The ester **10** (0.5 g, 1.1 mmol) was taken in saturated methanolic methylamine solution (5 mL) and stirred at room temperature for 6 h. The solvent was removed under reduced pressure, and the product was purified by column chromatography furnishing **4** as a white solid (0.49 g, 97%); mp: 185-186 ^{o}C; $[\alpha]^{24}_D$: 110 (*c* 0.1, CHCl$_3$); IR (CHCl$_3$) ν (cm^{-1}): 3308, 3018, 1717, 1647, 1637, 1587, 1522, 1508, 1447, 1217, 1159, 1047, 1024; ^{1}H NMR (400 MHz, CDCl$_3$) δ: 11.70 (s, 1H), 10.16 (s, 1H), 8.60-8.58 (d, *J* = 8.04 Hz, 1H), 8.38-8.36 (d, *J* = 8.43 Hz, 1H), 7.69-7.67 (d, *J* = 6.89 Hz, 1H), 7.51-7.43 (m, 3H), 7.12-7.08 (m, 1H), 7.04-6.99 (m, 2H), 6.28 (bs, 1H), 4.84-4.77 (m, 1H), 2.97-2.95 (d, *J* = 4.78 Hz, 3H), 1.64-1.63 (d, *J* = 7.13 Hz, 3H), 1.51 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ: 170.9, 169.3, 168.6, 153.1, 140.3, 139.0, 132.7, 132.5, 127.2, 126.4, 123.2, 121.4, 121.4, 120.4, 119.7, 119.3, 80.2, 50.7,

28.3, 26.7, 19.0; ESI-MS: 463.5 (M+Na)$^+$; 479.55 (M+K)$^+$; Elemental analysis calculated for $C_{23}H_{28}N_4O_5$: C, 62.71; H, 6.41; N, 12.72. Found: 62.84, H, 6.52, N, 12.60.

2-Methyl-2-(2-nitrobenzamido)propanoic acid 12:

2-nitro benzoic acid (2 g, 11.9 mmol, 1 equiv.) was converted to the corresponding acid chloride using oxalyl choride (2 mL, 24 mmol, 2 equiv.) and cat amount of DMF in dichloromethane (10 mL) as the solvent. After 3h, the solvent was stripped off from the reaction mixture under vacuum and to the residue was added a solution containing α-amino isobutyric acid (Aib) (1.85 g, 17.9 mmol, 1.5 equiv.) and sodium hydroxide (1.2 g, 30 mmol, 2.5 equiv.) in water. The reaction mixture was vigorously stirred at room temperature for 30 min. The free acid generated on acidification with dil HCl, was extracted into ethyl acetate. Drying and concentration in vacuum yielded the crude acid **12**, which was directly used for the next reaction without further purification.

Methyl 2-(2-methyl-2-(2-nitrobenzamido)propanamido)benzoate 13:

The acid **12** (1 g, 4 mmol 1 equiv.), Ant-OMe (0.6 g, 4 mmol, 1 equiv.), DCC (0.98 g, 4.8 mmol, 1.2 equiv.) and DMAP (cat. amount) in CH_2Cl_2 was stirred at rt for 12 h. The reaction mixture was filtered to remove the DCU and the organic layer was washed sequentially with potassium hydrogen sulfate solution, saturated sodium bicarbonate and water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to get the crude product **13**. This was used for the next reaction with out further purification. ESI-MS: 387.09 (M+H)$^+$; 409.01 (M+Na)$^+$; 424.96 (M+K)$^+$.

Methyl 2-(2-(2-(*tert*-butoxycarbonylamino)benzamido-2-methylpropanamido) benzoate 5:

The compound **13** (0.5 g, 1.3 mmol, 1 equiv.) in ethyl acetate (10 mL) was subjected to hydrogenation of the nitro group using H_2, Pd-C (50 mg) followed by *in situ* Boc protection with (Boc)$_2$O (0.42 g, 1.9 mmol, 1.5 equiv.). The reaction mixture was filtered over celite pad, washed with MeOH, and evaporated to get the crude product. The pure compound was obtained by column purification (eluent: ethyl acetate/pet.ether: 20/80, R_f: 0.3); furnishing **5** as a white solid. mp: 175-176 ^{o}C; IR (CHCl$_3$) ν (cm^{-1}): 3315, 3263, 3018, 2926, 2845, 1176, 1701, 1589, 1539, 1364, 1254, 1236, 1142, 1095, 1065; ^{1}H NMR (400 MHz, CDCl$_3$) δ: 11.79 (s, 1H), 9.86 (s, 1H), 8.77-8.76 (d, *J* = 8.42 Hz, 1H), 8.3-8.01, (dd, *J* = 1.73 Hz, 6.51 Hz, 1H), 7.64-7.62 (dd, *J* = 1.26 Hz, 6.55 Hz, 1H), 7.59-7.55 (m, 1H), 7.46-7.42 (m, 1H), 7.12-7.07 (m, 1H, 7.05-7.01 (m, 2H), 3.82 (s, 3H), 1.80 (s, 6H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ: 173.4, 168.9, 168.7, 153.1, 141.6, 140.3, 134.8, 132.5, 130.9, 127.0, 122.6, 121.3, 120.3, 120.2, 119.8, 115.1, 80.2, 58.5, 52.4, 28.3, 25.1; ESI-MS: 455.10 (M+H)$^+$; 476.94 (M+Na)$^+$; 492.72 (M+K)$^+$; Elemental analysis calculated for C$_{24}$H$_{29}$N$_3$O$_6$: C, 63.28; H, 6.42; N, 9.22. Found: C, 63.34; H, 6.56; N, 9.20.

Benzyl 1-(2-(*tert*-butoxycarbonylamino)benzoyl)pyrrolidine-2-carboxylate 14:

To an ice cold solution of Boc-Ant-OH **8** (3 g, 12.7 mmol, 1 equiv.) and proline benzyl ester (2.6 g, 12.7 mmol, 1 equiv.) in dichloromethane (20 ml) was added DCC (3.13 g, 15.1 mmol, 1.2 equiv.) and DMAP (cat. amount). The reaction mixture was allowed to stir at 0 ^{o}C for 10 minutes and for 12 h at room temperature. The reaction mixture was filtered to remove DCU, diluted with more dichloromethane and the

organic layer was washed sequentially with potassium hydrogen sulfate solution, saturated sodium bicarbonate and water. The crude product obtained after the removal of solvent under reduced pressure was purified by column chromatography to afford **14** (3 g, 56%). White solid; mp: 95-96 ^{o}C; $[\alpha]^{24}{}_{D}$: -90 (c = 0.4, CHCl$_3$); IR (CHCl$_3$) ν (cm^{-1}) 3356, 3016, 2980, 1717, 1626, 1591, 1520, 1454, 1416, 1215, 1159, 1051, 1026; ^{1}H NMR(400 MHz, CDCl$_3$) δ: 8.41 (s,1H), 8.22-8.18 (d, J = 8.37 Hz, 1H), 7.41-7.34 (m, 7H), 7.05-6.98 (t, J = 7.57 Hz, 1H), 5.24 (s, 2H), 4.78-4.74 (m, 1H), 3.64-3.38 (m, 2H), 2.45-2.28 (m, 1H), 2.07-1.84 (m, 3H), 1.50 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ : 171.9, 168.9, 153.1, 137.4, 135.7, 131.0, 128.6, 128.3, 128.1, 127.3, 123.5, 121.6, 120.2, 80.3, 66.98, 59.1, 50.0, 29.3, 28.3, 25.3; ESI-MS: 425.21 (M+H)$^+$; 447. 23 (M+Na)$^+$; 463.24 (M+K)$^+$.

1-(2-(*tert*-butoxycarbonylamino)benzoyl)pyrrolidine-2-carboxylic acid 14a:

The dipeptide **14** (2 g, 4.7 mmol) in ethyl acetate was subjected to hydrogenolysis using 10 % Pd/C (0.2 g) and H$_2$ (60 psi). After completion, the reaction mixture was filtered over celite pad. The filterate on evaporation under reduced pressure yielded acid **14a** quantitatively which was carried over for the next reaction with out further purification.

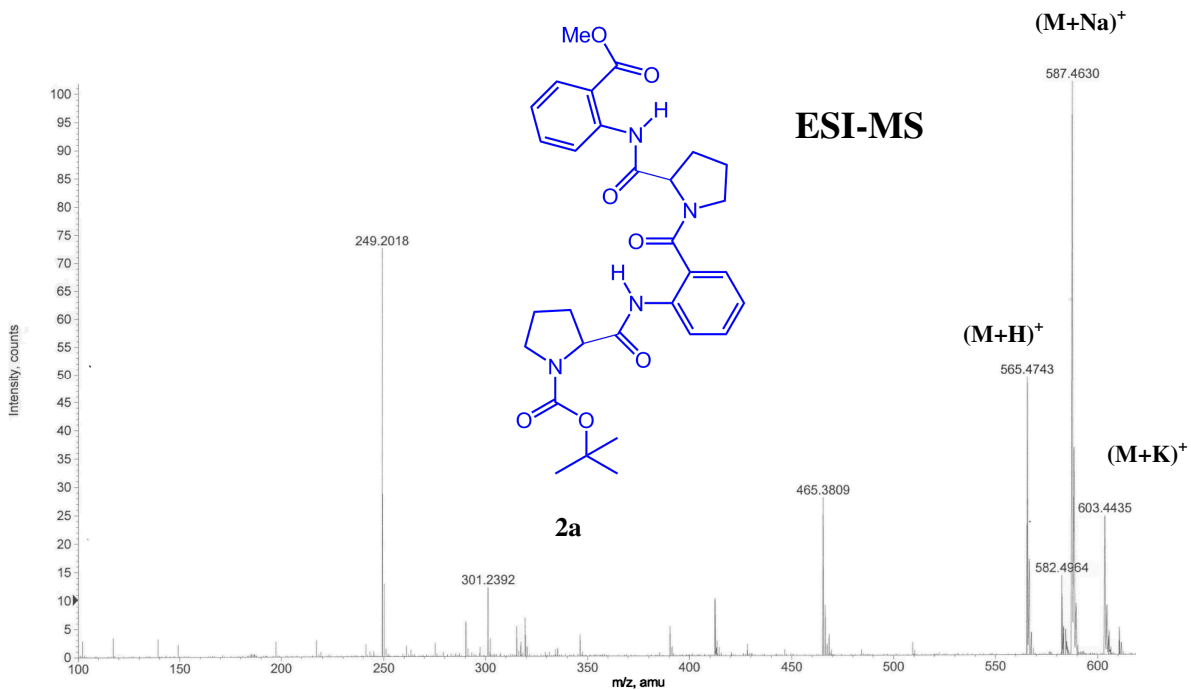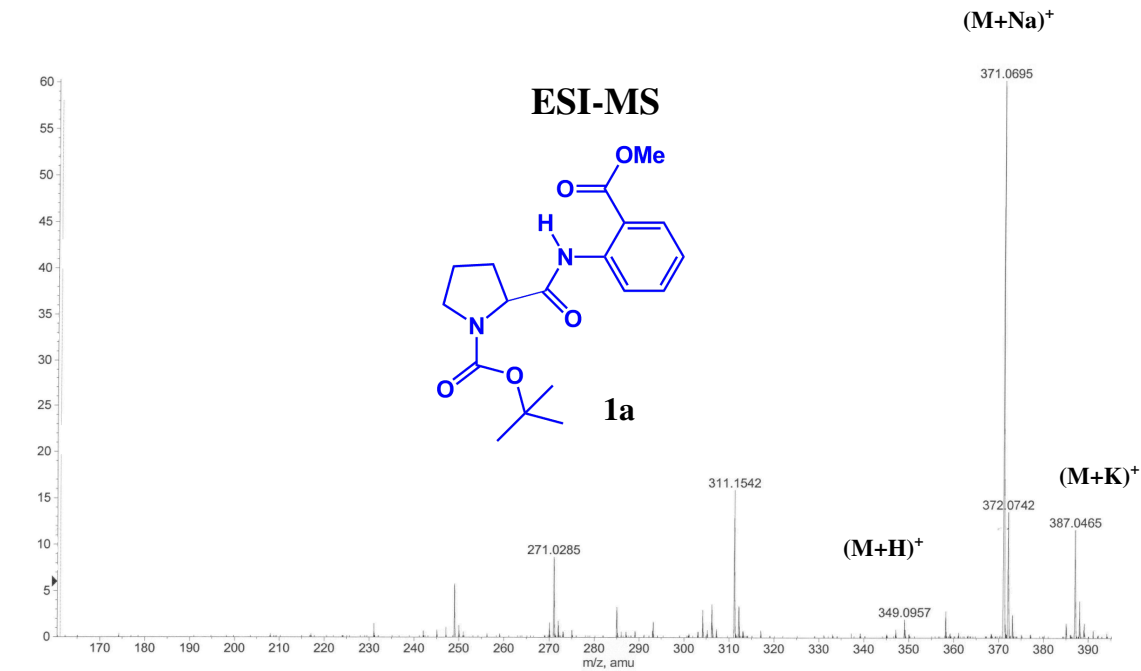### Benzyl 2-(1-(2-(*tert*-butoxycarbonylamino)benzoyl)pyrrolidine-2-carboxamido) acetate 15:

A solution containing the acid **14a** (1 g, 3 mmol, 1 equiv.) and glycine benzyl ester p-toluenesulfonate (1.13 g, 3 mmol, 1 equiv.) in dichloromethane (15 ml) was cooled to 0^{0}C. To this was added DCC (0.68 g, 3.3 mmol, 1.1 equiv.), followed by TEA (0.5
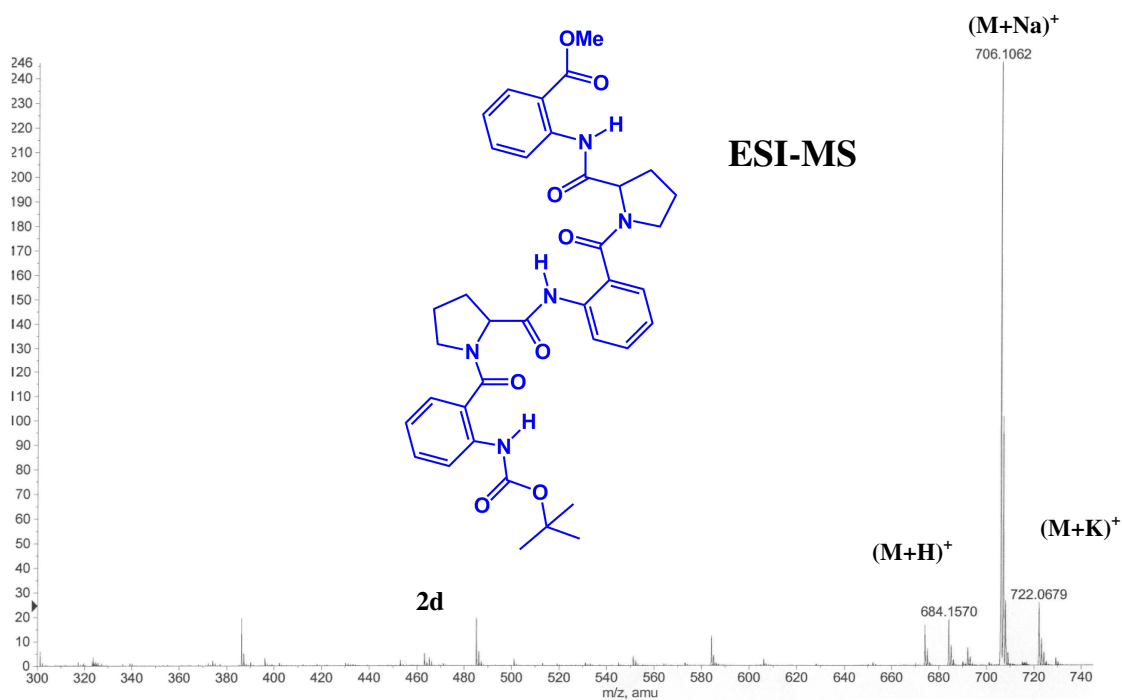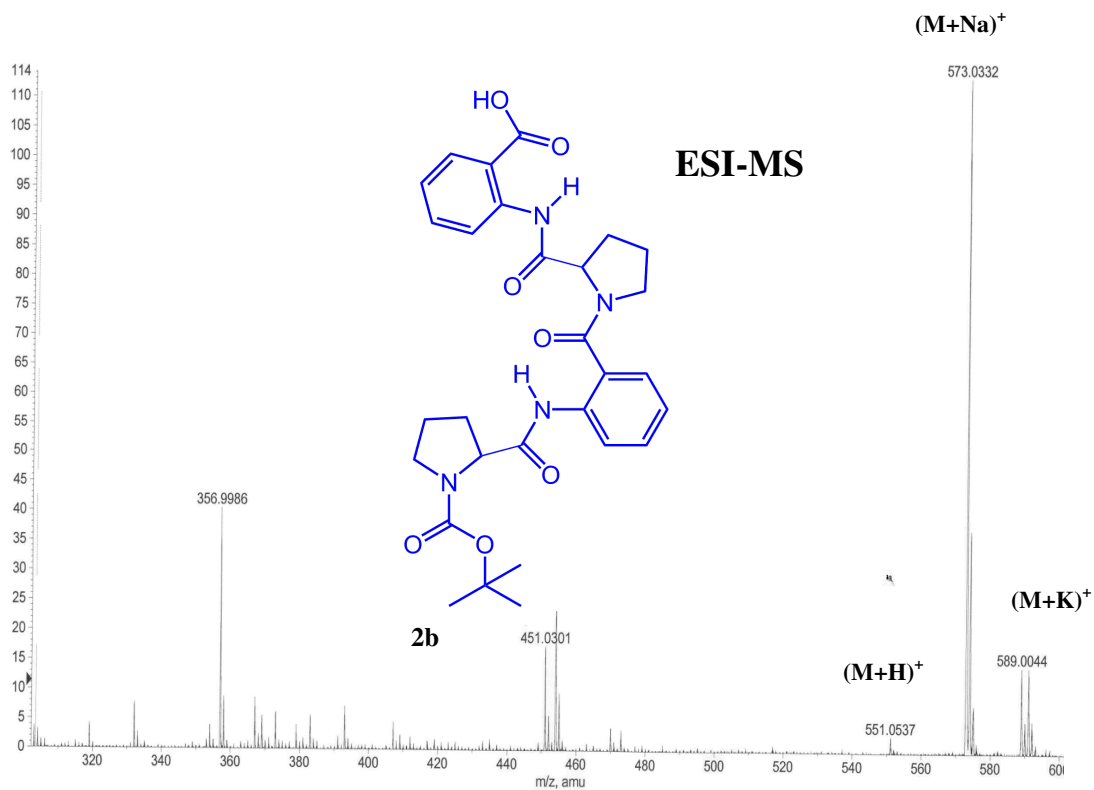
mL, 3.6 mmol, 1.2 equiv.). The reaction mixture was allowed to stir at 0 $^{\circ}$C for 10 min and then for 12 h at room temperature. The reaction mixture was filtered, diluted with more dichloromethane, and the organic layer was washed sequentially with aq. potassium hydrogen sulfate solution and then with sat. sodium bicarbonate solution and finally with water. Drying and evaporation of the solvent under reduced pressure gave the crude product which on purification by column chromatography (eluent: ethyl acetate/pet. ether: 60/40, R_f: 0.8) afforded **15** as a colorless waxy compound (1.25 g , 87%); $[\alpha]^{26}_D$: -121° (*c* 0.96, CHCl$_3$); IR (CHCl$_3$) ν (cm^{-1}): 3362, 3018, 2981, 1728, 1682, 1620, 1589, 1516, 1454, 1415, 1367, 1215, 1159, 1051, 1024; ^{1}H NMR (500 MHz, CDCl$_3$) δ: 8.36 (s, 1H), 8.13-8.12 (d, *J* = 8.40 Hz, 1H), 7.41-7.30 (m, 7H), 7.21 (b, 1H), 7.05-7.01 (t, *J* = 7.45 Hz, 1H), 5.18-5.17 (d, *J* = 6.87 Hz, 2H), 4.85-4.82 (t, *J* = 6.87 Hz, 1H), 4.13-4.10 (m, 2H), 3. 57-3.46 (m, 2H), 2.44-2.37 (m, 1H), 2.18-2.11 (m, 1H) 2.05-1.99 (m, 1H), 1.85-1.80 (m, 1H), 1.50 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ: 171.4, 170.0, 169.5, 153.0, 137.1, 135.1, 131.1, 128.5, 128.4, 128.3, 127.5, 123.8, 122.0, 120.8, 80.4, 67.1, 59.5, 50.5, 41.4, 28.2, 27.7, 25.3; ESI-MS: 482.39 (M+H)$^+$; 504.44 (M+Na)$^+$; 520.42 (M+K)$^+$; Elemental Analysis calculated for C$_{26}$H$_{31}$N$_3$O$_6$: C, 64.85; H, 6.49; N, 8.73. Found: C, 64.97; H, 6.56; N, 8.80.
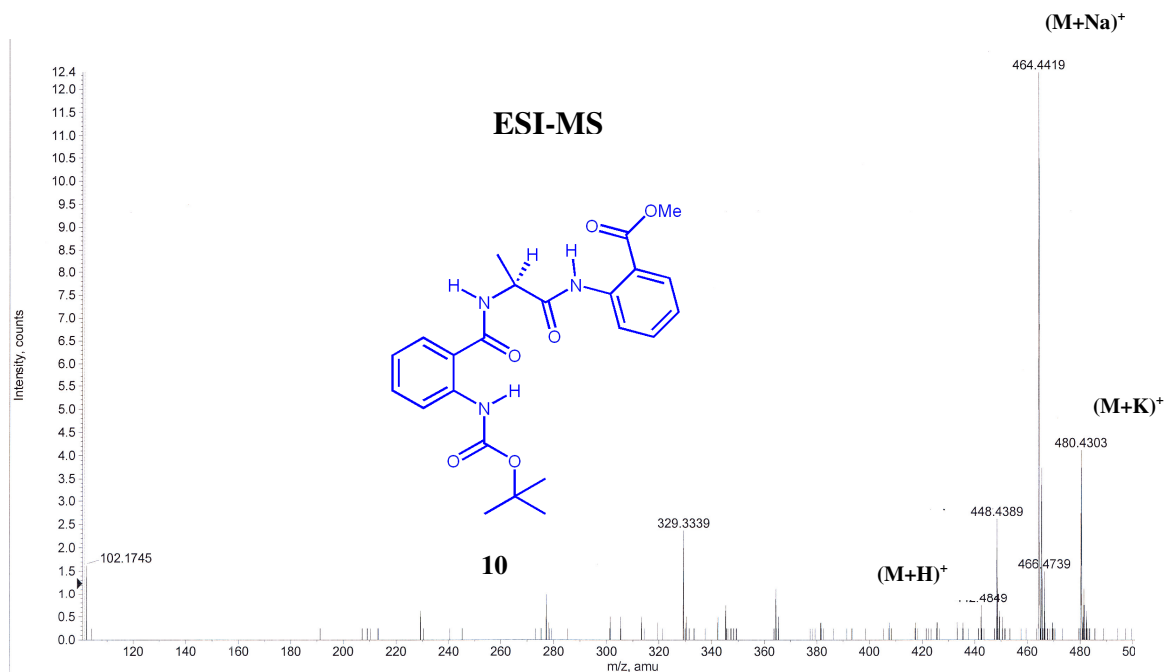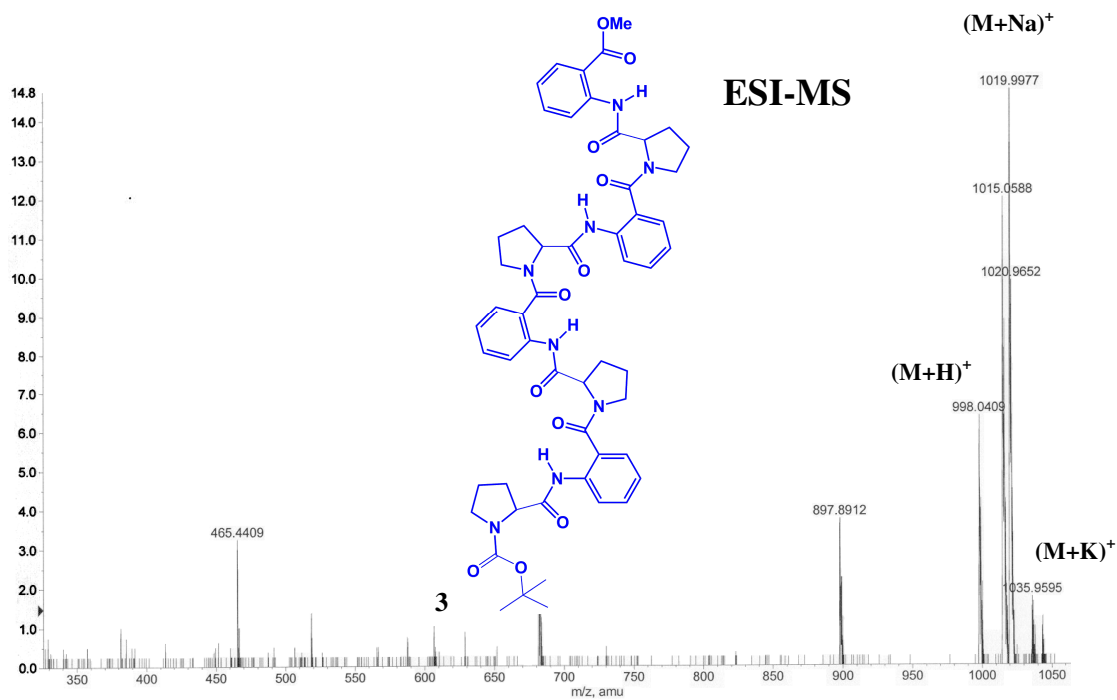
tert-Butyl 2-(2-(2-(methylamino)-2-oxoethylcarbamoyl)pyrrolidine-1-carbonyl) phenyl carbamate 6:
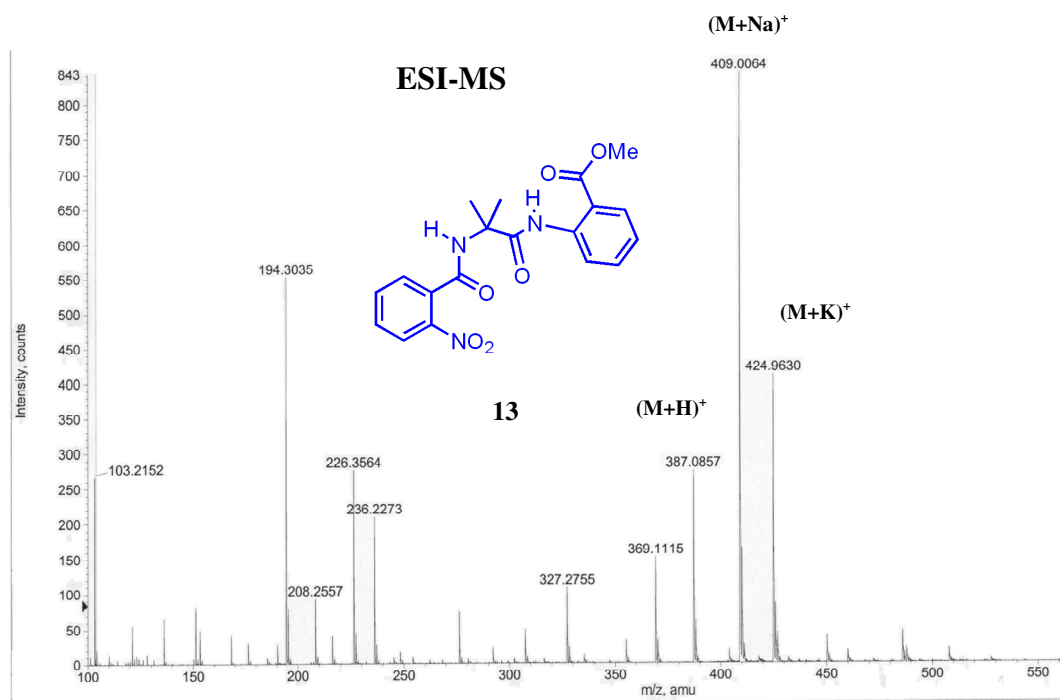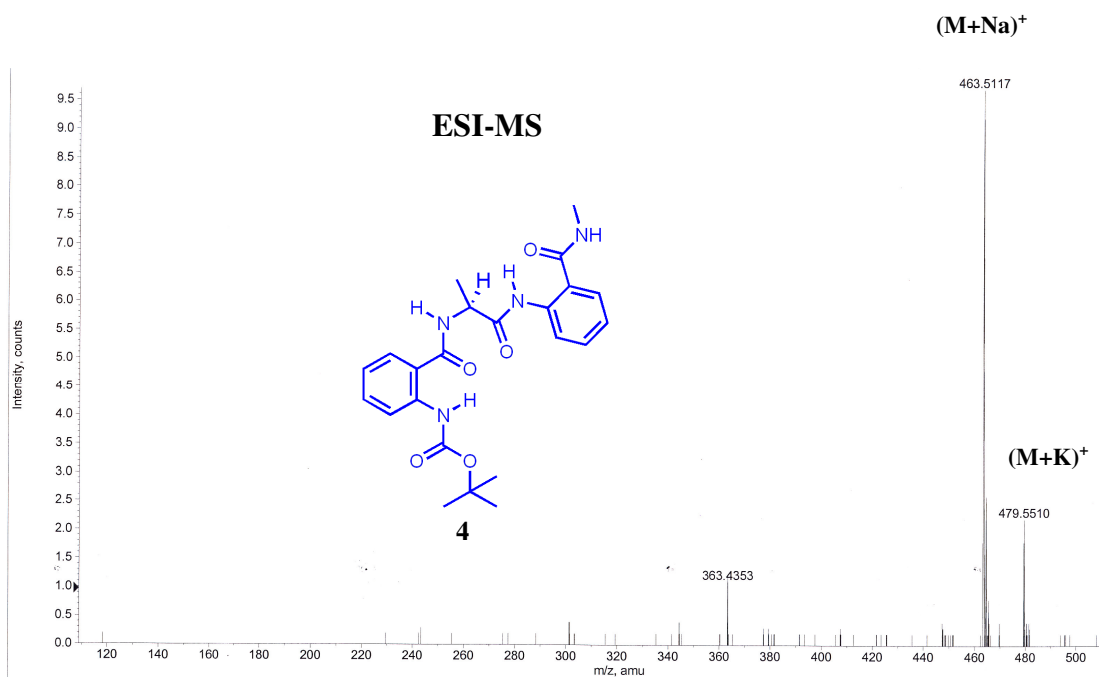
The tripeptide **15** (0.5 g, 1 mmol) in 5 ml methanolic methyl amine solution was stirred at room temperature for 6 hr. The reaction mixture was stripped off the solvent under reduced pressure and the residue obtained was purified by column chromatography (eluent: ethyl acetate/ pet. ether: 90/10, R_f: 0.3) to afford **6** (0.4 g,
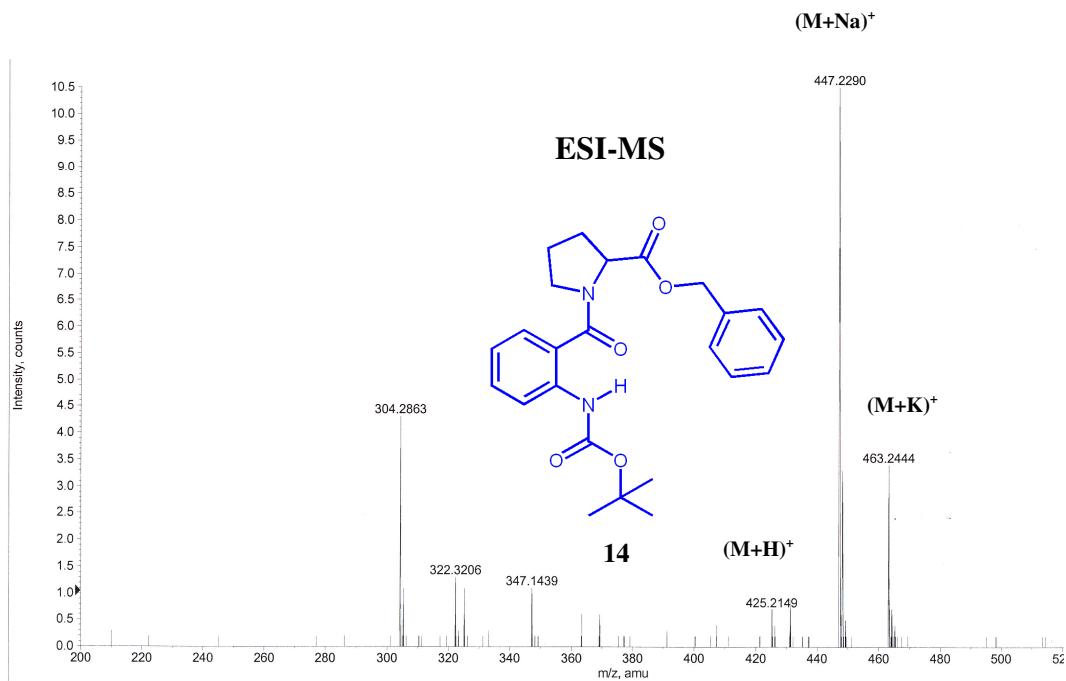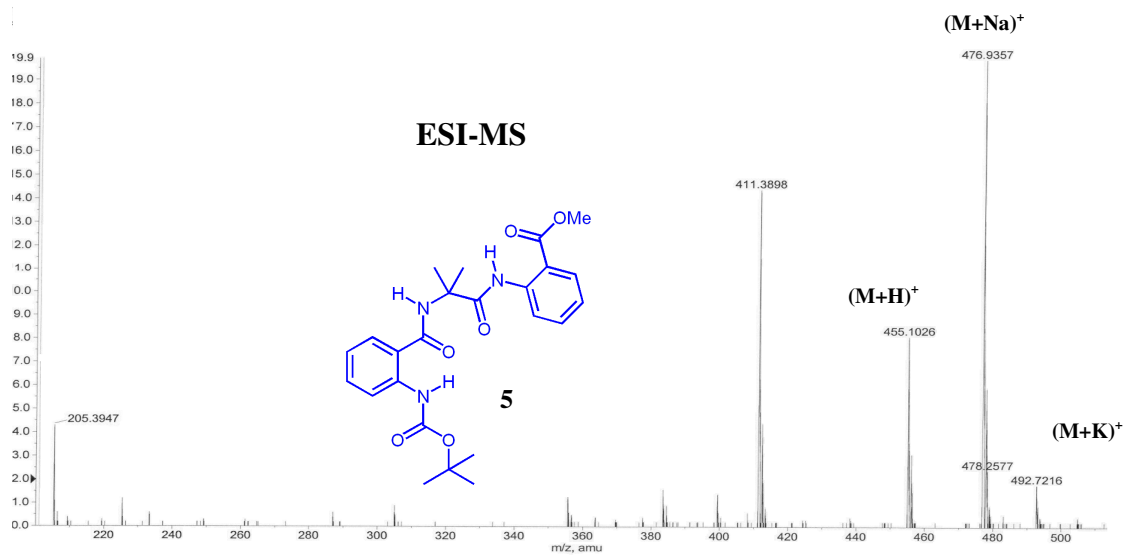
98%); $[\alpha]^{26}_D$: -50 (c = 0.2, CHCl$_3$); Colorless plate crystals were obtained from a solvent mixture of DCM and MeOH;. mp: 196-198 ^{o}C; IR (CHCl$_3$) ν (cm^{-1}): 3360, 3018, 1716, 1666, 1620, 1593, 1520, 1456, 1418, 1369, 1300, 1215, 1157, 1047, 1024; ^{1}H NMR (400 MHz, CDCl$_3$) δ: 8.26 (s, 1H), 8.13-8.12 (d, J = 8.24 Hz, 1H), 7.42-7.30 (m, 2H), 7.06-7.03 (t, J = 7.47 Hz, 1H), 6.96 (bs, 1H), 6.76 (bs, 1H), 4.54-4.51 (t, J = 7.25 Hz, 7.51 Hz, 1H), 4.24-4.19 (dd, J = 7.24 Hz, 1H), 3.82-3.77 (dd, J = 5Hz, 1H), 3.68-3.53 (m, 2H), 2.80-2.79 (d, J = 4.72 Hz, 3H), 2.32-2.17 (m, 2H), 2.14-2.05 (m, 1H), 1.93-1.84 (m, 1H), 1.51 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ: 172.3, 169.8, 169.7, 153.1, 137.1, 131.2, 127.3, 123.6, 122.0, 120.6, 80.6, 61.1, 50.7, 43.2, 29.2, 28.3, 26.2, 25.5; ESI-MS: 405.46 (M+H)$^+$; 427.50 (M+Na)$^+$; 443.43 (M+K)$^+$; Elemental Analysis calculated for C$_{20}$H$_{28}$N$_4$O$_5$: C, 59.39; H, 6.98; N, 13.85. Found: C, 59.50; H, 7.06; N, 13. 92.

(M+Na)⁺
573.0332

ESI-MS

356.9986

(M+K)⁺

2b
451.0301

(M+H)⁺
589.0044

551.0537

m/z, amu

(M+Na)⁺
706.1062

ESI-MS

(M+K)⁺

(M+H)⁺

2d
684.1570
722.0679

m/z, amu

ESI-MS

(M+Na)⁺

(M+H)⁺

(M+K)⁺

3

ESI-MS

(M+Na)⁺

(M+K)⁺

(M+H)⁺

10

(M+Na)⁺

463.5117

ESI-MS

(M+K)⁺

479.5510

363.4353

4

Intensity, counts

m/z, amu

(M+Na)⁺

409.0064

ESI-MS

194.3035

(M+K)⁺

424.9630

(M+H)⁺

226.3564

236.2273

387.0857

103.2152

369.1115

327.2755

208.2557

13

Intensity, counts

m/z, amu

ESI-MS

15

ESI-MS

6

^{1}H NMR (200 MHz, CDCl$_3$)

1a

^{1}H NMR (400 MHz, CDCl$_3$)

2a

¹H NMR (400 MHz, CDCl₃)

2d

¹H NMR (400 MHz, CDCl₃)

3

^{1}H (400 MHz, CDCl$_3$)

10

^{1}H (400 MHz, CDCl$_3$)

4

^{1}H (500 MHz, CDCl$_3$)

CDCl3

13

^{1}H (400 MHz, CDCl$_3$)

CDCl3

5

¹H NMR (400 MHz, CDCl₃)

14

¹H NMR (500 MHz, CDCl₃)

15

8.25
8.15
8.13
7.43
7.39
7.37
7.28
7.08
7.07
7.05
6.91
6.74
4.56
4.55
4.53
4.25
4.21
4.19
3.84
3.82
3.79
3.78
3.65
3.57
2.82
2.81
2.29
2.28
2.26
2.11
1.91
1.62
1.53

^{1}H (400 MHz, CDCl$_3$)

6

^{13}C NMR (100 MHz, CDCl$_3$)

1a

DEPT-135 (100 MHz, CDCl$_3$,)

1a

¹³C (100 MHz, CDCl₃)

171.62
170.73
168.75
141.24
136.74
134.59
131.39
130.82
128.04
122.77
120.36
115.22
79.91
62.24
61.53
52.37
50.57
47.17
46.65
31.43
30.01
28.32
25.41
24.27
23.82

2a

DEPT-135 (100 MHz, CDCl₃)

134.59
131.03
130.81
128.05
122.77
120.86
120.35
62.24
61.52
52.37
50.58
47.12
46.65
31.41
30.01
28.41
25.40
23.79

2a

^{13}C NMR (100 MHz, CDCl$_3$)

170.81
170.58
170.10
169.57
168.72
153.12
137.57
134.56
131.14
130.84
128.33
127.67
123.96
122.82
121.74
121.43
120.30
120.08
115.16

CDCl3

80.13
77.00
62.25
61.92
52.36
50.71
50.37
38.55
29.97
28.26
25.46

2d

150   100   50   0

DEPT-135 (100 MHz, CDCl$_3$)

134.61
131.19
130.91
128.36
127.71
123.01
122.90
121.82
120.34
120.13

62.33
61.97
52.45
50.75
50.42
30.05
28.32
25.51
25.42

2d

40 130 120 110 100 90 80 70 60 50 40 30 20 10 0

^{13}C (100 MHz, CDCl$_3$)

172.13
171.16
168.81
154.08
141.10
136.66
135.90
134.64
130.96
130.36
127.37
123.07
121.66
120.36
115.28
79.66
62.06
61.54
61.10
52.48
50.31
49.84
47.15
46.70
31.50
30.18
28.30
25.39
25.18

3

DEPT-135 (100 MHz, CDCl$_3$)

134.64
130.97
130.69
130.36
127.37
127.26
123.83
121.66
120.35
61.52
61.12
52.49
50.31
49.87
49.77
46.71
31.49
30.22
29.69
28.50
28.30
25.19
25.39

3

10

^{13}C (100 MHz, CDCl$_3$)

171.00, 168.68, 153.06, 140.89, 140.47, 134.71, 132.75, 130.90, 127.09, 123.05, 121.35, 120.39, 119.71, 119.11, 115.34, 80.23, 77.38, 77.06, 76.74, 52.45, 50.77, 28.33, 18.97

DEPT-135 (100 MHz, CDCl$_3$)

10

134.72, 132.75, 130.90, 127.10, 123.06, 121.35, 120.38, 119.70, 52.45, 50.78, 28.33, 18.96

Chloroform-d

170.85 169.34 168.59 153.07 140.33 138.97 132.65 132.52 127.19 126.41 123.23 121.40 120.41 119.71 119.27 80.22 77.31 77.00 76.68 50.66 28.31 26.69 19.03

^{13}C NMR (100 MHz, CDCl$_3$)

4

150   100   50

132.65 132.53 127.19 126.42 123.23 121.40 119.70 50.66 28.30 26.69 19.03

DEPT-135 (CDCl$_3$, 100 MHz)

4

140   130   120   110   100   90   80   70   60   50   40   30   20

¹³C (100 MHz, CDCl₃)

DEPT-135 (100 MHz, CDCl₃)

¹³C (100 MHz, CDCl₃)

DEPT -135 (100 MHz, CDCl₃)

171.36
170.00
169.54
153.02
137.12
135.09
131.05
128.54
128.43
128.30
127.52
123.84
121.96
120.78

CDCl3
80.38
77.32
77.00
76.69
67.10
59.54
50.47
41.40
28.24
27.70
25.26

¹³C (100 MHz, CDCl₃)

15

150   100   50

131.06
128.55
128.43
128.30
127.52
121.96
120.77

67.10
59.54
50.48
41.40
28.24
27.70
25.26

DEPT-135 (100 MHz, CDCl₃)

15

140 130 120 110 100 90 80 70 60 50 40 30 20

^{13}C (100 MHz, CDCl$_3$)

172.30
169.77
153.06
137.07
131.15
127.25
123.64
121.99
120.64
CDCl3
80.61
77.32
77.00
76.69
61.08
50.66
43.19
29.19
28.25
26.18
25.47

DEPT-135 (100 MHz, CDCl$_3$)

131.14
127.24
121.98
120.62
61.08
50.65
43.17
29.19
28.25
26.17
25.47

Table 1: Dilution study of pentapeptide 2d in CDCl₃ (concentration from 100 mmol to 1 mmol)

No	Concentration (in mmol)	δ_{NH3}	δ_{NH2}	δ_{NH1}
1	100	11.55	10.14	8.73
2	80	11.56	10.15	8.74
3	60	11.56	10.15	8.74
4	40	11.56	10.16	8.74
5	20	11.56	10.16	8.74
6	10	11.56	10.16	8.75
7	5	11.56	10.16	8.75
8	4	11.56	10.16	8.76
9	2	11.53	10.06	8.74
10	1	11.53	10.06	8.67
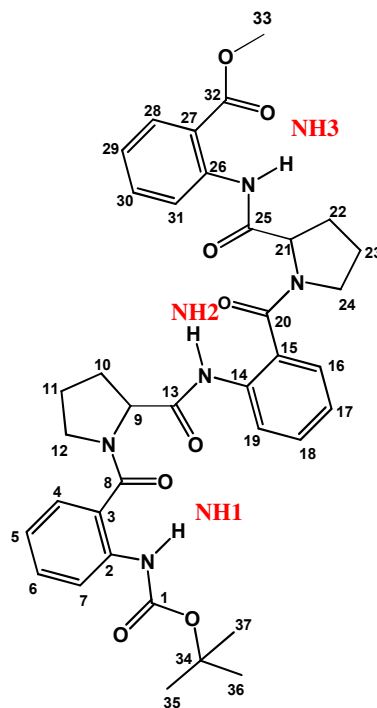
Table 2: Titration study of pentapeptide 2d in CDCl₃ (5 mmol) with DMSO-d6 (each addition with 5 µ lit)

No	$V_{DMSO\text{-}d6}$ (in µ lit)	δ_{NH3}	δ_{NH2}	δ_{NH1}
1	0	11.56	10.16	8.74
2	5	11.47	10.06	8.64
3	10	11.47	10.07	8.64
4	15	11.47	10.07	8.64
5	20	11.48	10.07	8.63
6	25	11.48	10.07	8.63
7	30	11.48	10.07	8.63
8	35	11.48	10.06	8.62
9	40	11.47	10.06	8.63
10	45	11.47	10.06	8.62
11	50	11.47	10.06	8.62

Table 3: Titration study of tetrapeptide 2a in CDCl₃ (5mmol) with DMSO-d6 (volume of DMSO-d6 added at each addition = 5 μl)

No	V DMSO-d6 (in μ lit)	δ_{NH2}	δ_{NH1}
1	0	11.57	9.93
2	5	11.58	9.93
3	10	11.58	9.93
4	15	11.58	9.93
5	20	11.58	9.93
6	25	11.59	9.93
7	30	11.59	9.94
8	35	11.59	9.94
9	40	11.59	9.94
10	45	11.59	9.94
11	50	11.59	9.95
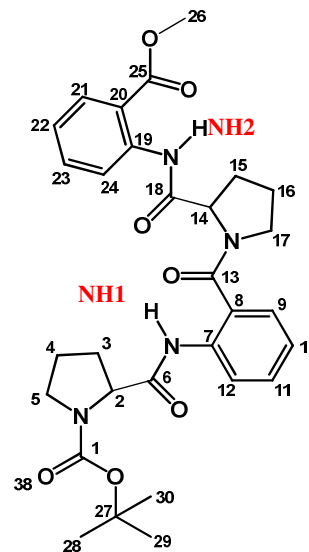
Table 4: Titration study of octapeptide 3 in CDCl₃ (5mmol) with DMSO-d6 (volume of DMSO-d6 added at each addition = 5 μl)

No	V DMSO-d6 (in μ lit)	NH4	NH3	NH2	NH1
1	0	11.55	10.03	9.99	9.71
2	5	11.54	10.02	9.98	9.71
3	10	11.53	10.01	9.97	9.69
4	15	11.52	10.0	9.95	9.68
5	20	11.51	9.99	9.94	9.67
6	25	11.49	9.98	9.92	9.65
7	30	11.48	9.97	9.91	9.64
8	35	11.46	9.95	9.89	9.62
9	40	11.44	9.94	9.88	9.61
10	45	11.43	9.93	9.86	9.59
11	50	11.42	9.92	9.85	9.58
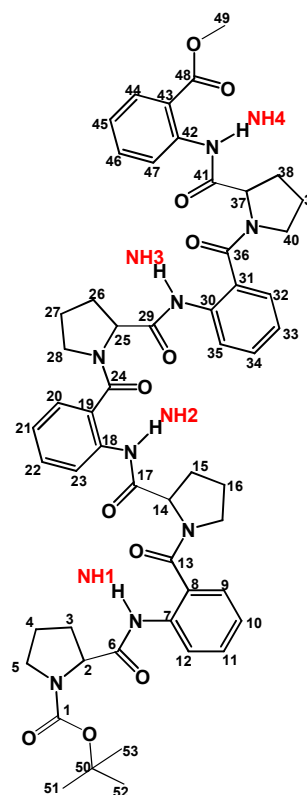
Table 5: Titration study of 4 in CDCl$_3$ with DMSO-d6 (0-50 μl)

No	V$_{DMSO-d6}$ (in μ lit)	δ_{NH1}	δ_{NH2}	δ_{NH3}	δ_{NH4}
1	0	11.70	10.16	6.99	6.28
2	5	11.74	10.16	7.08	6.54
3	10	11.76	10.17	7.14	6.70
4	15	11.79	10.17	7.24	6.93
5	20	11.81	10.17	7.33	7.12
6	25	11.83	10.17	7.39	7.22
7	30	11.84	10.17	7.48	7.33
8	35	11.85	10.17	7.52	7.40
9	40	11.86	10.17	7.57	7.48
10	45	11.87	10.17	7.65	7.55
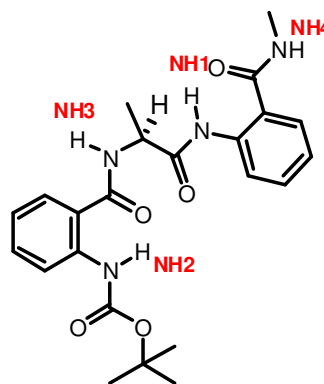
Table 6: Titration study of 5 in CDCl$_3$ with DMSO-d6 (0-50 μl)

No	V$_{DMSO-d6}$ (in μ lit)	δ_{NH1}	δ_{NH2}	δ_{NH3}
1	0	11.80	9.87	7.03
2	5	11.78	9.83	7.20
3	10	11.78	9.81	7.34
4	15	11.78	9.79	7.45
5	20	11.77	9.77	7.56
6	25	11.77	9.76	7.66
7	30	11.77	9.75	7.74
8	35	11.76	9.72	7.92
9	40	11.76	9.71	7.99
10	45	11.75	9.69	8.12
11	50	11.75	9.68	8.18
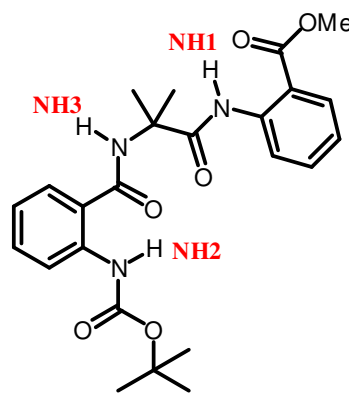
Table 7: Dilution Study of 6 (concentration from 120 mmol to 2 mmol)

Concentration (in mmol)	Chemical shift (in ppm)		
	δNH1	δNH2	δNH3
120	8.29	7.17	7.40
100	8.29	7.18	7.38
80	8.28	7.15	7.30
60	8.27	7.11	7.24
40	8.26	7.06	7.06
20	8.24	6.95	6.87
10	8.24	6.91	6.77
5	8.23	6.88	6.70
4	8.23	6.88	6.70
2	8.23	6.87	6.66
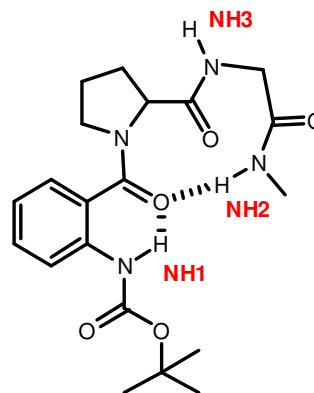
Table 8: Titration data of 6 in CDCl₃ with DMSO-d6 (0-50 µl)

Volume of DMSO-d6 added (in µl)	Chemical shift (in ppm)		
	δNH1	δNH2	δ NH3
0	8.23	6.89	6.72
5	8.24	6.95	6.82
10	8.29	7.11	7.33
15	8.31	7.18	7.53
20	8.33	7.24	7.71
25	8.34	7.28	7.81
30	8.36	7.31	7.90
35	8.37	7.33	7.99
40	8.38	7.36	8.05
45	8.38	7.40	8.08
50	8.38	7.42	8.11

Fig. 1: Partial COSY, TOCSY and HSQC spectra of pentapeptide **2d** (500 MHz, CDCl₃). For better view, aromatic and aliphatic regions are given separately.
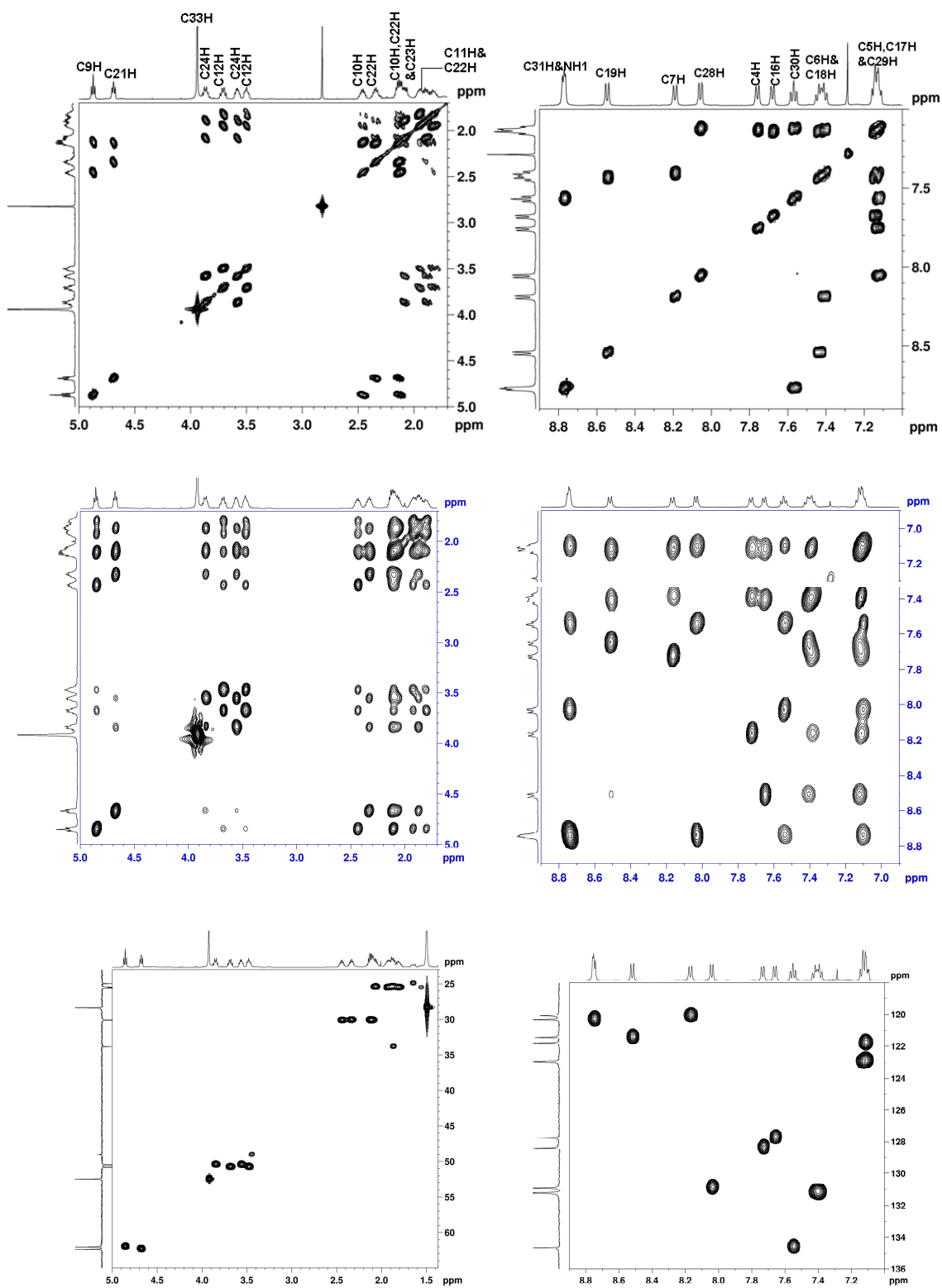
Fig. 2: Partial COSY, TOCSY and HSQC spectra of tetrapeptide **2a** (500 MHz, CDCl₃). For better view, aromatic and aliphatic regions are given separately.
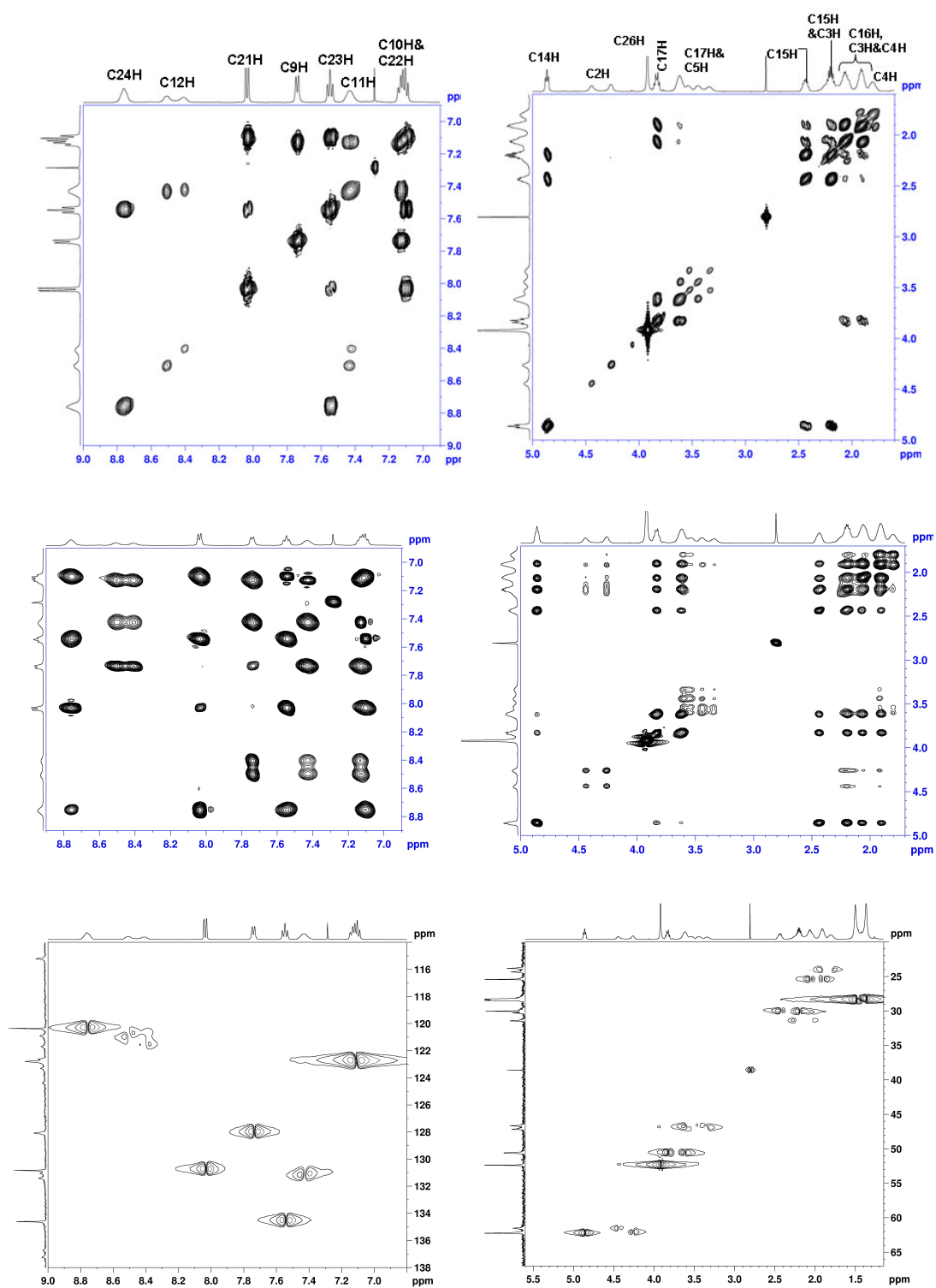
Fig. 3: Partial COSY, TOCSY and HSQC spectra of octapeptide **3** (500 MHz, CDCl₃). For better view, aromatic and aliphatic regions are given separately.
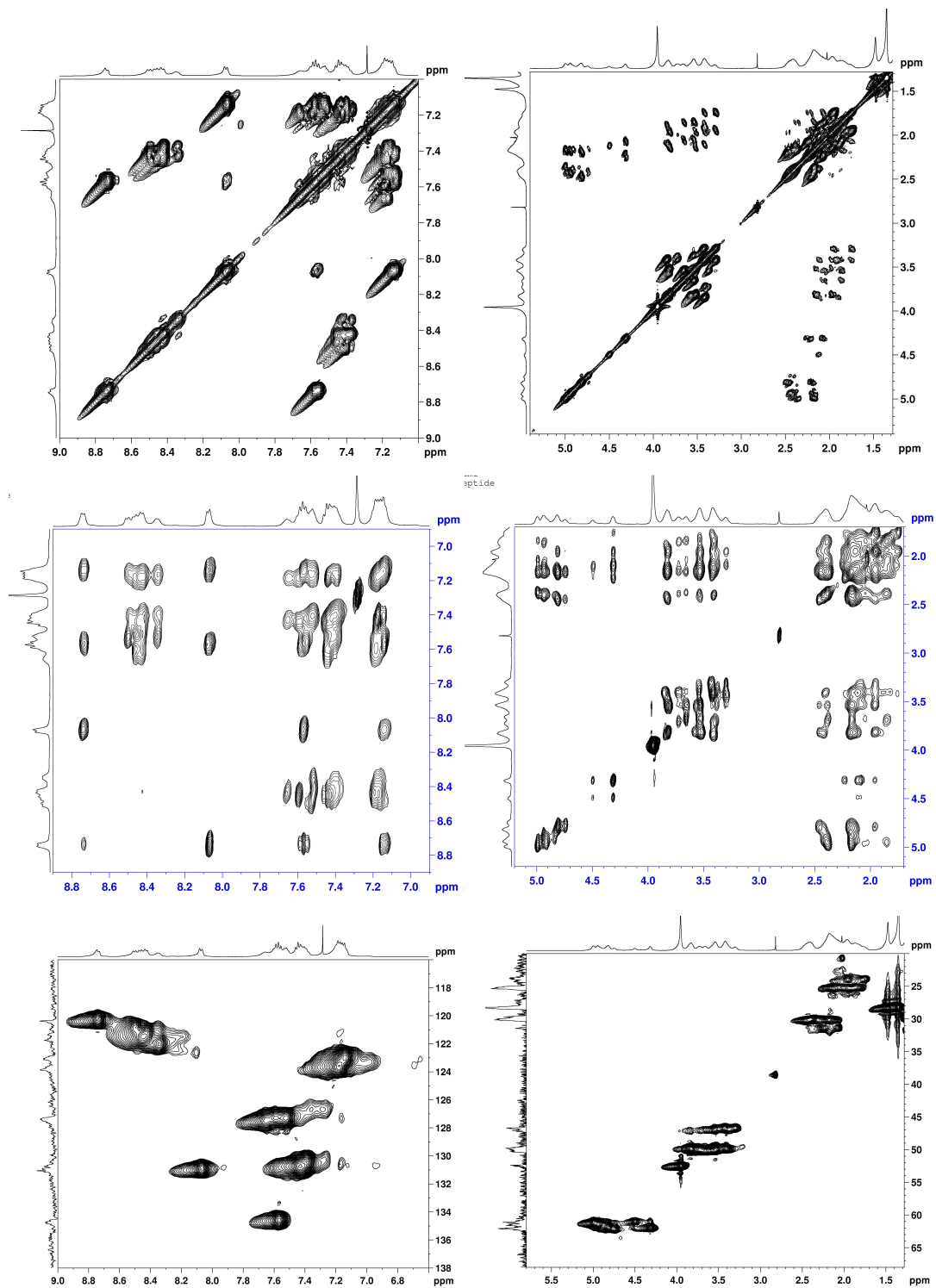
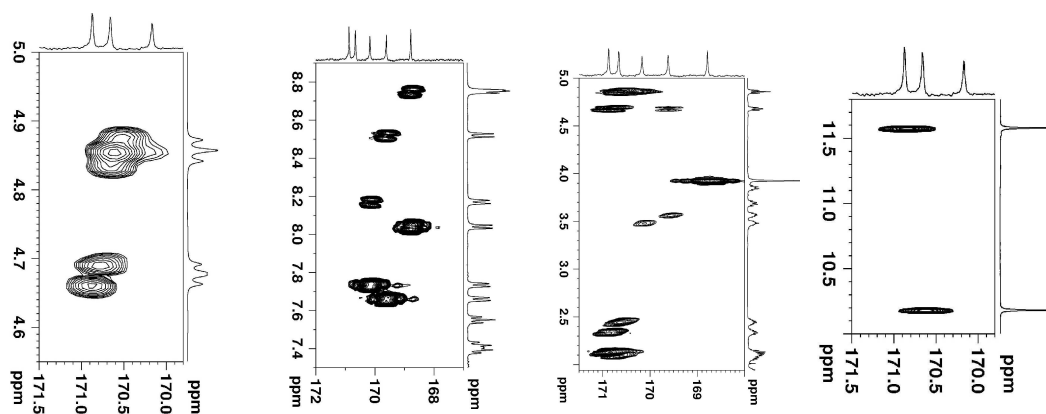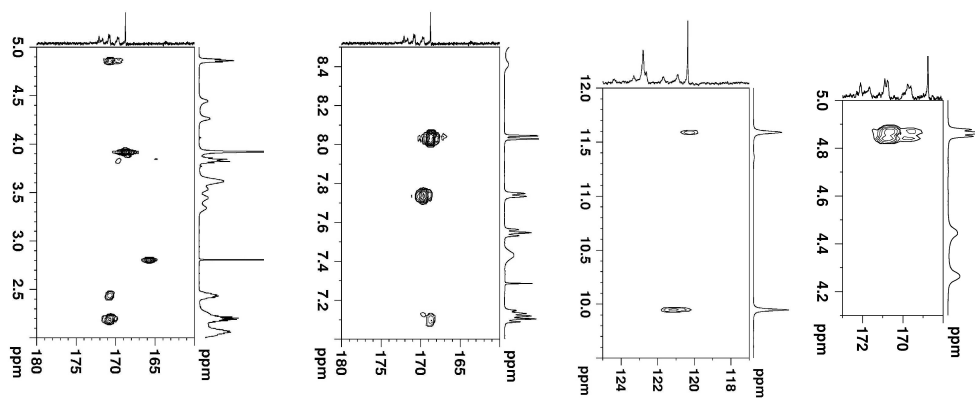**Fig. 4:** Partial HMBC (^{1}H-^{13}C) spectra of pentapeptide **2d** (500 MHz, CDCl$_3$).

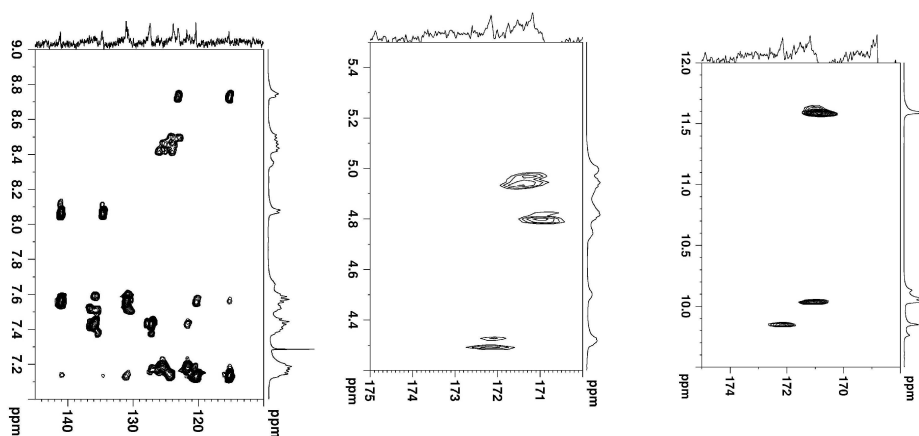**Fig. 5:** Partial HMBC (^{1}H-^{13}C) spectra of tetrapeptide **2a** (500 MHz, CDCl$_3$).

Fig. 6: Partial HMBC (^{1}H-^{13}C) spectra of octapeptide **3** (500 MHz, CDCl$_3$).

Table 9: ¹H, ¹³C HSQC assignments for 2d

¹H (δ/ ppm)		¹³C (δ/ ppm)
8.75 (C31H)		120.33
8.52 (C19H)		121.45
8.17 (C7H)		120.11
8.04 (C28H)		130.90
7.73 (C4H)		128.37
7.66 (C16H)		127.72
7.55 (C30H)		134.64
7.40 (2H)	C6H	131.20
	C18H	131.18
7.12 (3H)	C5H	121.80
	C29H	122.90
	C17H	122.96
4.86 (C9H)		61.98
4.68 (C21H)		62.31
3.92 (C33H)		52.44
3.84, 3.56 (C24H)		50.42
3.70, 3.48 (C12H)		50.76
2.43, 2.13 (C10H)		30.03
2.33, 2.13 (C22H)		30.07
1.95, 1.82 (C11H)		25.51
2.09, 1.9 (C23H)		25.43
1.49 (C35H, C36H, C37H		28.31
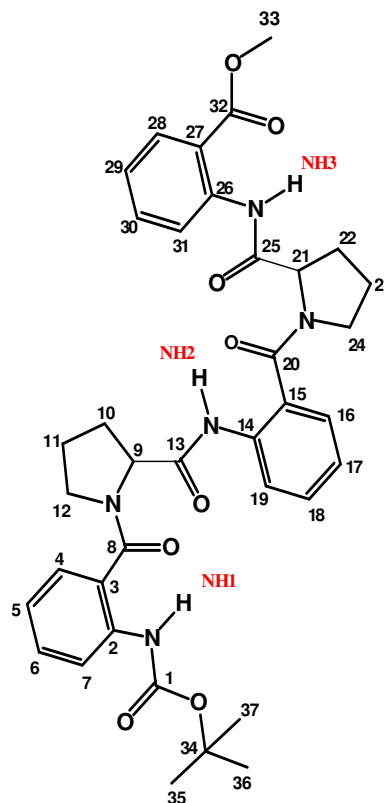
Table 10: ¹H, ¹³C HMBC assignments for 2d

¹H (δ /ppm)	¹³C (δ /ppm)
11.57 (NH3)	17.86, 134.62, 120.32
10.17 (NH2)	170.64, 136.72, 131.21, 123.94
8.77 (NH1)	,137.60, 123.1, 120.11,
8.75 (C31H)	168.77, 141.19, 134.62, 122.90, 115.20
8.52 (C19H)	169. 60, 136.72, 123.98, 122.96
8.17 (C7H)	170.15, 137.60, 123.12, 121.80
8.04 (C28H)	168.77, 141.19, 134.62, 115.20
7.73 (C4H)	170.15, 137.60, 131.20
7.66 (C16H)	169.60, 136.72, 131.18
7.55 (C30H)	141.19, 130.90, 120.33
7.40 (2H, C6H, C18H)	137.60, 136.72, 128.37, 127.71
7.13 (3H)	123.98, 123.12, 121.45, 120.33, 120.11, 115.20
4.86 (C9H)	170.64, 170.15, 50.76, 25.51, 30.03
4.68 (C21H)	170.86, 169.60, 50.42, 25.43
3.92 (C33H)	168.77
3.84, 3.56 (C24H)	169.60, 62.31, 30.05, 25.43,
3.70, 3.48 (C12H)	170.15, 61.98, 30.03, 61.98
2.43, 2.13 (2H, C10H)	170.64, 61.98, 50.76, 25.51
2.33, 2.13 (2H, C22H)	170.86, 62.31, 50.42, 25.43
1.95, 1.82 (2H,C11H)	61.98, 50.76, 30.03
2.09, 1.90 (2H, C23H)	62.31,50.42, 30.05

Table 11: ^{1}H, ^{13}C HSQC assignments for 2a	
^{1}H (δ /ppm)	^{13}C (δ /ppm)
8.76 (C24H)	120.36
8.50, 8.41 (C12H)	120.92
8.03 (C21H)	130.81
7.74 (C9H)	128.03
7.55 (C23H)	134.59
7.43 (C11H)	131.05
7.13 (C10H)	122.77
7.10 (C22H)	122.77
4.86 (C14H)	62.24
4.26 (C2H)	61.52
3.92 (C26H)	52.37
3.83, 3.62 (C17H)	50.56
3.62, 3.42 (C5H)	47.16
2.43, 2.20 (C15H)	30.01
2.19, 2.08 (C3H)	31.39
2.06, 1.90 C16H)	25.41
1.8, 1.90 (C4H)	23.82
1.49 (C28H, C29H, C30H)	28.34
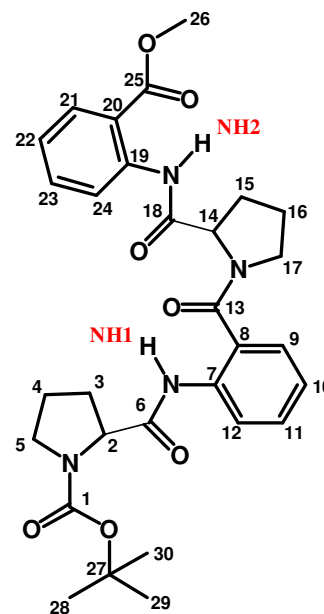
Table 12: ^{1}H, ^{13}C HMBC assignments for 2a	
^{1}H (δ /ppm)	^{13}C (δ/ ppm)
8.76 (C24H)	122.77, 115.22
8.50, 8.41 (C12H)	122.77
8.03 (C21H)	168.74, 141.24, 134.59, 120.36
7.74 (C9H)	169.67, 136.73, 131.38
7.55 (C23H)	115.22, 120.36, 122.77, 130.81, 141.24
7.43 (C11H)	128.03, 136.73
7.13 (C10H)	128.03, 136.73, 120.92, 122.78
7.10 (C22H)	115.22, 120.36, 130.82, 134.59, 141.24
4.86 (C14H)	170.73, 169.67, 50.56, 25.41
4.26 (C2H)	31.39, 23.82
3.92 (C26H)	168.74
3.83, 3.62 (C17H)	170.73, 169.67, 62.24, 30.01
3.62, 3.42 (C5H)	23.81, 31.39
2.43, 2.20 (C15H)	170.73, 62.24, 50.56
2.19, 2.08(C3H)	23.82, 47.16
2.06, 1.90 (C16H)	62.24, 50.56, 30.01
1.8, 1.9 (C4H)	31.39, 47.16
1.45 (9Hs, C28H, C29H, C30H)	79.91

Table 13: ^{1}H, ^{13}C HSQC assignments for 3

^{1}H (δ /ppm)	^{13}C (δ /ppm)
8.75 (C48H)	120.36
8.50 (C12H)	120.88
8.45 (C36H)	121.62
8.34 (C24H)	121.70
8.06 (C45H)	130.96
7.60 (C33H)	127.22
7.57 (C47H)	134.71
7.53 (C21H)	127.16
7.52 (C9H)	127.1
7.43 (C35H)	130.63
7.42 (C11H)	130.92
7.40 (C23H)	130.92
7.18 (C34H)	123.98
7.16 (C22H)	123.9
7.15 (C10H)	122.98
7.14 (C46H)	122.90
4.98 (C14H)	61.11
4.93 (C26H)	61.37
4.80 (C38H)	61.97
4.30 (C2H)	62.01
3.92 (C50H)	52.44
3.82, 3.40 (C29H)	49.88
3.81, 3.53 (C41H)	50.31
3.68, 3.53 (C17H)	47.18
2.44, 2.16 (C39H)	30.19
3.40, 3.33 (C5H)	46.71
2.40, 2.15 (C27H)	30.01
2.36, 2.17 (C15H)	29.65
2.22, 2.09 (C3H)	31.34
2.03, 1.84 (C16H)	24.88
1.95, 1.75 (C4H)	23.86
1.94, 1.8 (C40H)	25.43
1.9, 1.86 (C28H)	25.22
1.45-1.37 (C52H, C53H, C54H)	28.25
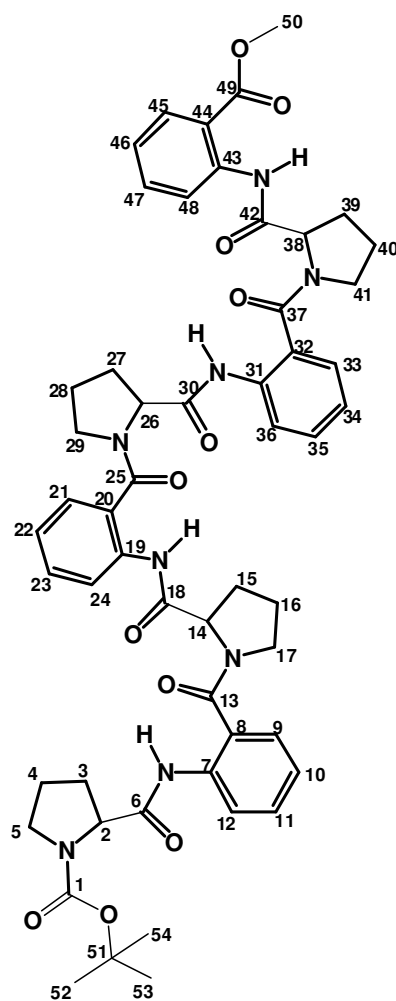
Table 14: : ^{1}H, ^{13}C HMBC assignments for 3	
^{1}H (δ/ppm)	**^{13}C (δ/ppm)**
11.59 (NH4)	170.89, 120.36, 115.24
10.12 (NH3)	121.62, 125.1
10.03 (NH2)	121.12, 125.97
9.93 (NH1)	124.16, 120.89
8.73 (C48H)	168.85, 141.18,
8.49 (C12H)	124.16, 122.96, 136.83
8.45 (C36H)	123.98, 125.1
8.34 (C24H)	123.99, 125.97
8.06 (C45H)	168.5, 141.18, 134.65, 120.31
7.6 (C33H)	168.83, 135.82, 130.63
7.60 (C33H)	168.8, 135.8, 130.6,
7.57 (C47H)	168.85, 141.18, 130.9, 120.31, 115.24
7.53 (C21H)	130.92, 135.81, 168.72
7.43 (C35H)	135.75, 127.22, 121.49, 121.70
7.42 (C11H)	121.16
7.40 (C23H)	134.80, 127.16
7.18 (C34H)	127.22, 121.62, 125.1
7.16 (C22H)	127.16, 120.8
7.14 (C46H)	115.28, 130.96, 120.36
4.98 (C14H)	24.88, 47.18
4.93 (C26H)	171.46, 30.01, 25.22
4.80 (C38H)	170.89, 25.43
4.30 (C2H)	23.86, 172.19, 31.34
3.92 (C50H)	168.85, 115.24
3.82, 3.40 (C29H)	30.01, 25.22
3.81, 3.53 (C41H)	25.43, 30.18
3.68, 3.53 (C17H)	29.65, 24.88
3.40, 3.33 (C5H)	31.34, 23.86
2.44, 2.16 (C39H)	170.65, 61.97, 50.31, 25.43
2.40, 2.15 (C27H)	49.11, 25.22
2.36, 2.17(C15H)	47.19
2.22, 2.09 (C3H)	46.71, 23.86
1.95, 1.75 (C4H)	46.71
1.94, 1.8 (C40H)	50.31, 30.18
1.9, 1.86 (C28H)	30.01, 49.88
1.45-1.37 (C52H, C53H, C54H)	77.2
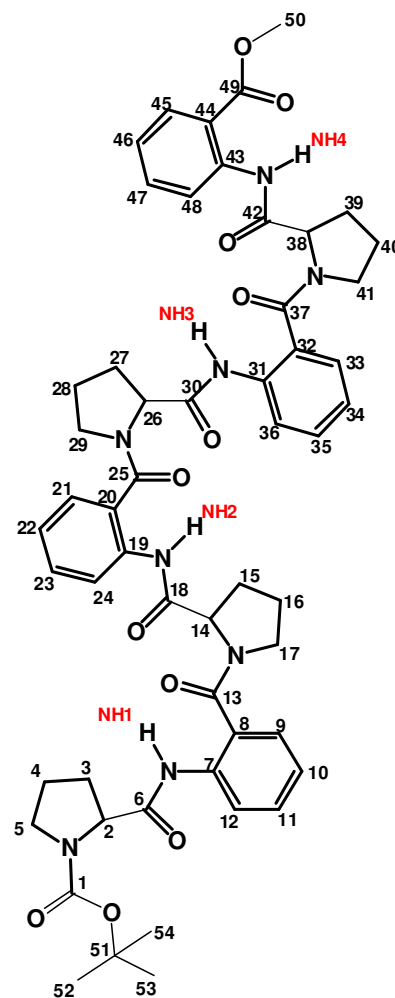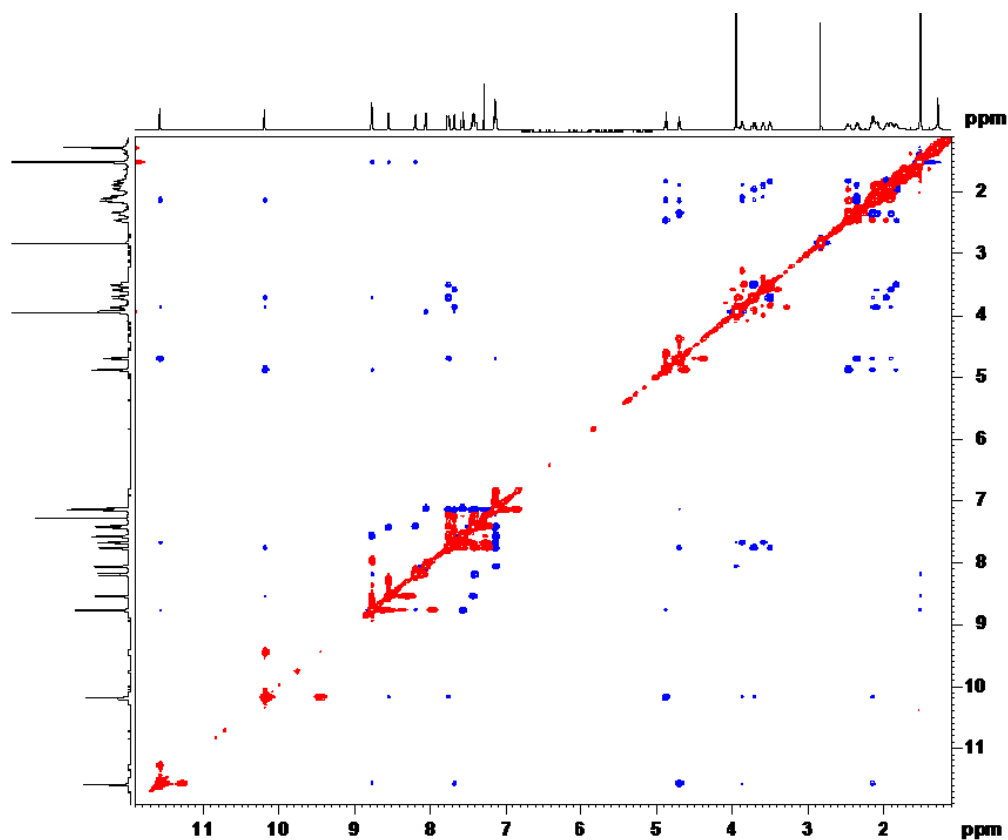
Fig. 7: 2D NOESY spectrum of **2d** (500 MHz, CDCl$_3$).
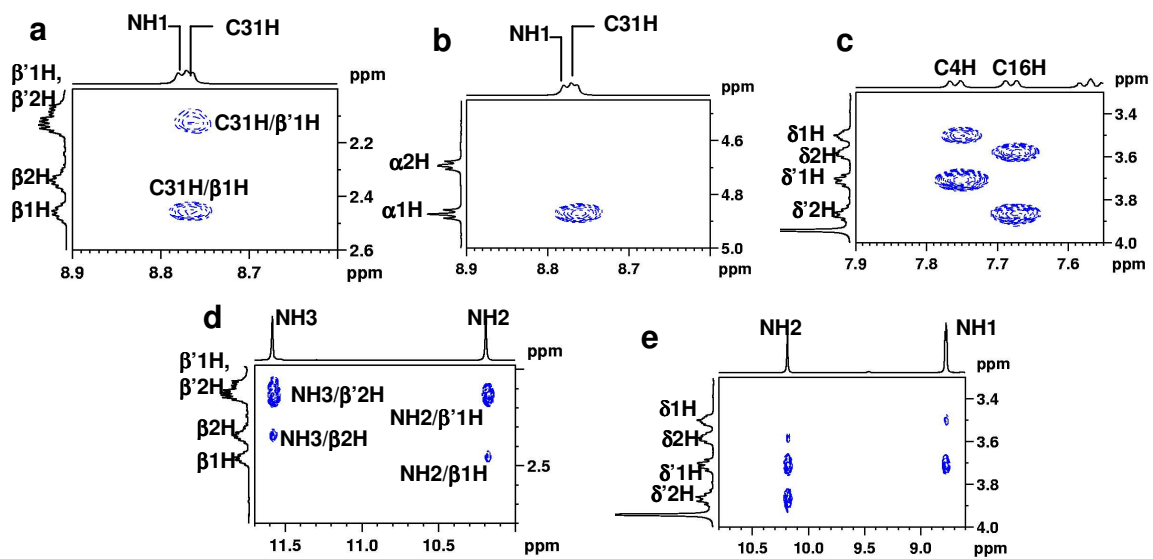
Fig. 8: 2D Extracts of **2d** (500 MHz, CDCl$_3$).

Fig. 9: 2D NOESY spectrum of **2d** (500 MHz, CD_2Cl_2).
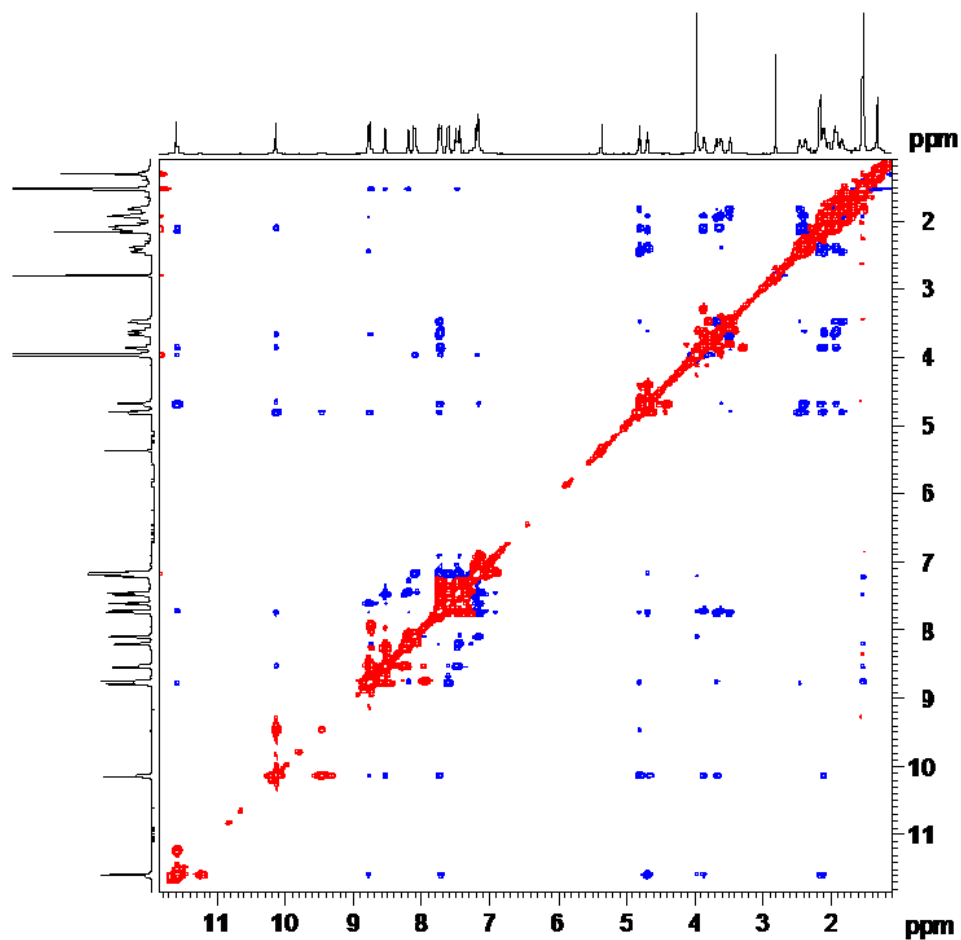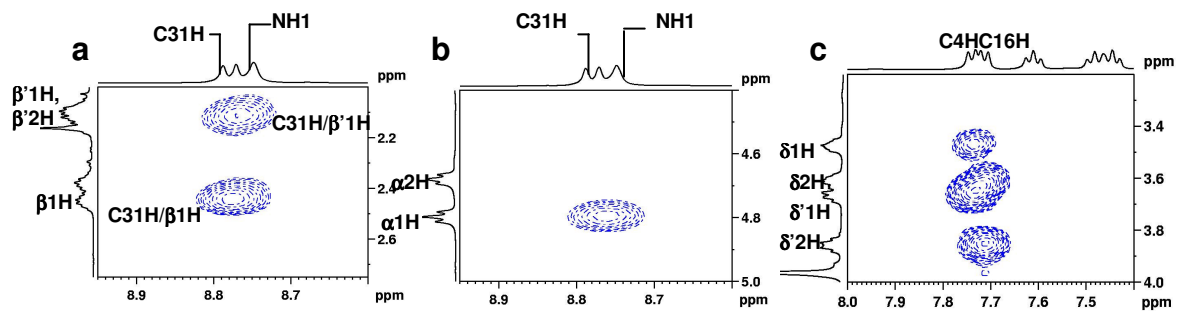

Fig. 10: 2D Extracts of **2d** (500 MHz, CD_2Cl_2).
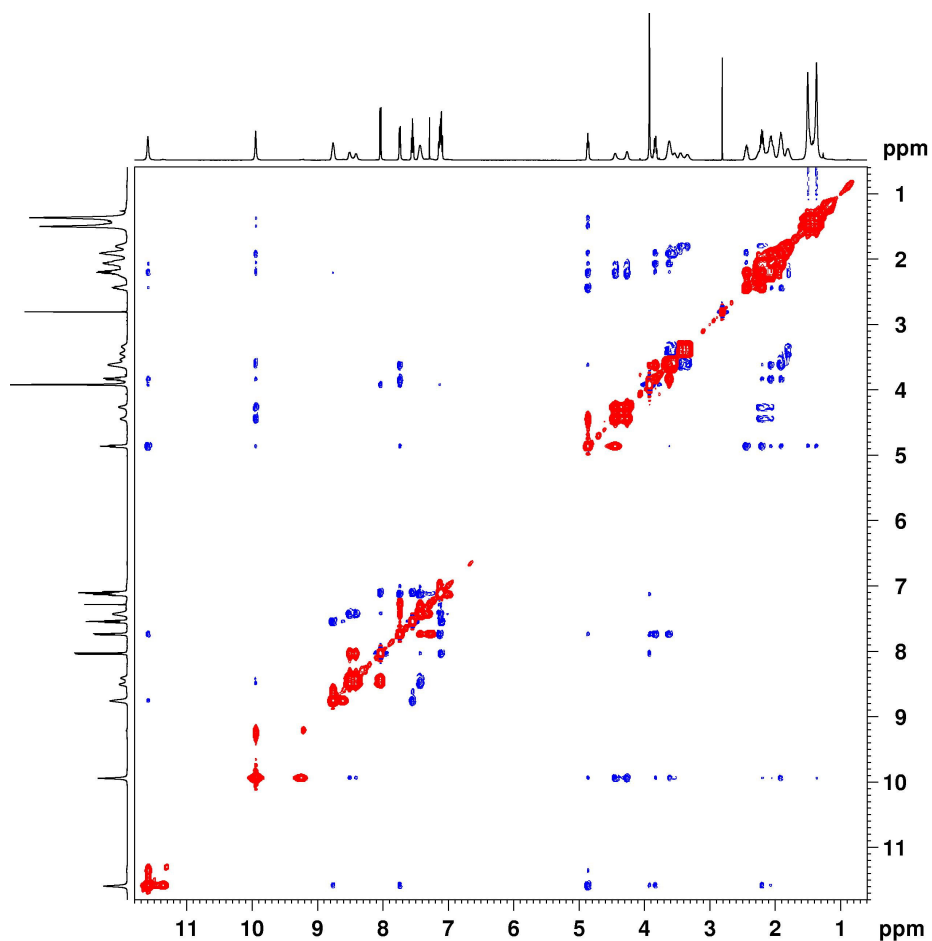
Fig. 11: 2D NOESY spectrum of **2a** (500 MHz, CDCl$_3$).
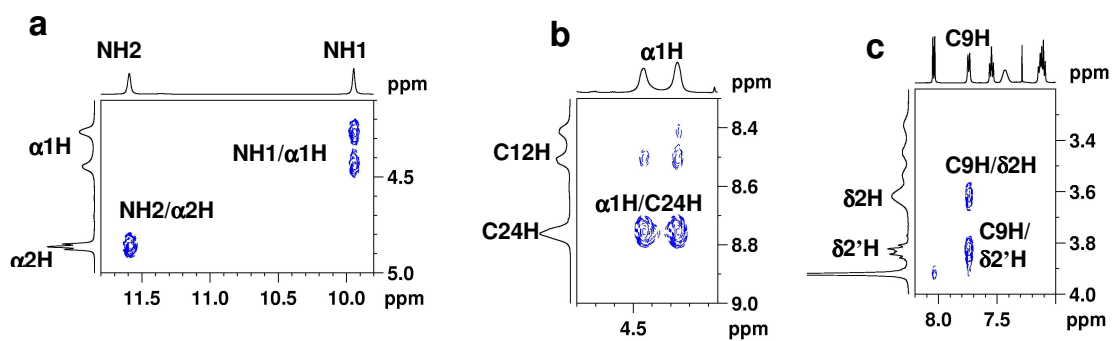

Fig. 12: 2D Extracts of **2a** (500 MHz, CDCl$_3$). (a) αH *vs* NH region; (b) αH *vs* Ar-H region; (c) δH *vs* Ar-H region.
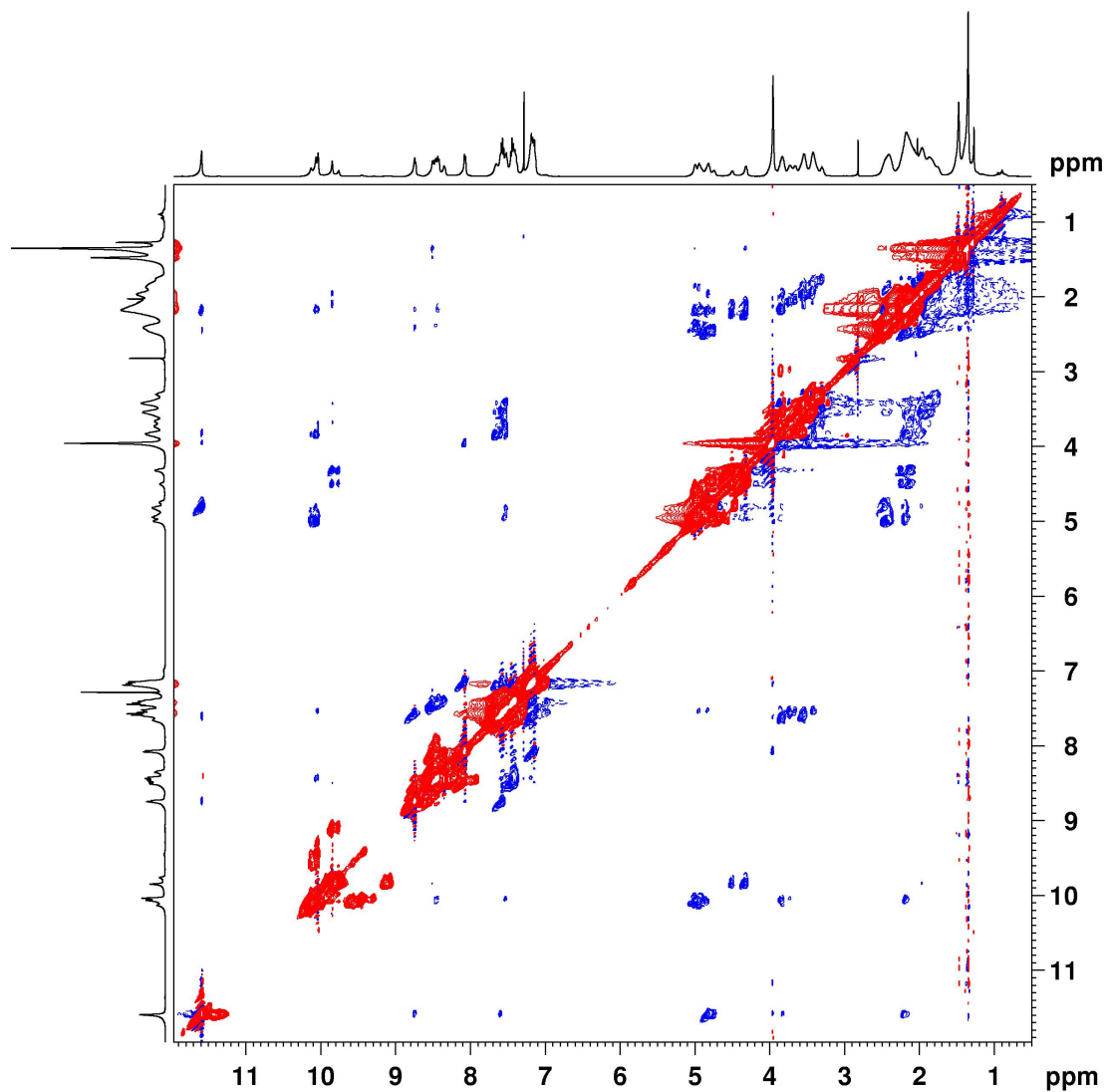
Fig. 13: 2D NOESY spectrum of **3** (500 MHz, CDCl₃).

2.13 References and Notes

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CHAPTER 3

Oligomers with Abiotic Backbone: Design, Synthesis and Conformational Studies

This chapter is devoted to the study of novel oligomer architectures on abiotic backbone, their design, synthetic strategy and the investigation of their conformational organization both in the solution as well as in the solid-state using powerful techniques. First part of the chapter describes about naphthalene homo-oligoamides with conformational pre-organization based on steric/electrostatic repulsive interactions whereas, the second part of this chapter signifies a novel hybrid oligomer with co-facial structural architecture using 1,8-bis(phenyl) naphthalene diacid and 2,6-diaminopyridine.

3.12 Experimental section

Single crystal X-ray crystallographic studies:

Crystal Data of 3 ($C_{25}H_{20}N_2O_7$): *M* = 460.43, Crystal dimensions 0.19 x 0.05 x 0.01, Multi run data acquisition. Total scans = 5, total frames = 2174, θ range = 2.39 to 25.00º, Orthorhombic, *P*na2$_1$, *a* = 27.786 (2), *b* = 4.5194 (8), *c* = 17.0553 (4) Å, *V* = 2141.7 (4) Å^3, *Z* = 4, D$_c$ = 1.428 mg m^{-3}, *μ* (Mo K$_\alpha$) = 0.106 mm^{-1}, 16929 reflections measured, 3772 unique [I>2σ(I)], R value 0.0350, wR2 = 0.0751.

Crystal Data of 7b ($C_{25}H_{22}N_2O_5$.CHCl$_3$): *M* = 549.81, Crystal dimensions 0.11 x 0.08 x 0.03, Multiscan data acquisition. Total scans = 4, total frames = 2424, θ range = 1.75 to 23.5º, completeness to θ of 23.5º is 100.0%, Triclinic, *P*ī, *a* = 10.1275 (13), *b* = 10.7976 (14), *c* = 11.7307 (14) Å, α = 96.883 (3)º, β = 94.267 (3)º, γ = 90.920 (3)º, *V* = 1269.6 (3) Å^3, *Z* = 2, D$_c$ = 1.438 mg m^{-3}, *μ* (Mo K$_\alpha$) = 0.402 mm^{-1}, 10361 reflections measured, 3749 unique [I>2σ(I)], R value 0.0705, wR2 = 0.1925

Experimental procedures:

Methyl-3-methoxy-4-nitro-2-naphthoate 2:
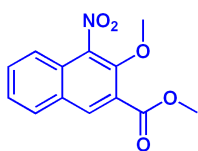
Methyl 3-hydroxy-4-nitro-2-naphthoate[25] (5 g, 20 mmol, 1 equiv.) in acetone (100 mL) was subjected to O-methylation using dimethylsulphate (3.9 mL, 40 mmol, 2 equiv.) and K$_2$CO$_3$ (5.6 g, 40 mmol, 2 equiv.). After stirring at room temperature for 12 h, the reaction mixture was filtered, and the filtrate was evaporated under reduced pressure. The residue obtained was taken in dichloromethane (150 mL), and washed with dil. HCl. Drying and purification by column chromatography (eluent: 15% AcOEt / pet.ether,

R_f: 0.4) yielded **2** as an yellow solid (5.12 g, 97%). mp: 72-73 ^{o}C; IR (CHCl$_3$) ν (cm$^-$^{1}): 3024, 2951, 1732, 1636, 1537, 1499, 1450, 1356, 1340, 1290, 1242, 1215, 1161, 1148, 1088; ^{1}H NMR (200 MHz, CDCl$_3$) δ: 8.60 (s, 1H), 7.99-7.94 (m, 1H), 7.72-7.60 (m, 3H), 4.03 (s, 3H), 4.02 (s, 3H); ^{13}C NMR (50 MHz, CDCl$_3$) δ: 164.6, 148.1, 142.7, 135.5, 130.9, 129.1, 129,0, 127.1, 126.8, 123.5, 120.8, 64.4, 52.7; ESI MS: 284.02 (M+Na)$^+$; Elemental Analysis calculated for C$_{13}$H$_{11}$NO$_5$: C, 59.77; H, 4.24; N, 5.36.; Found: C, 59.88; H, 4.32, N, 5.28.

3-Methoxy-4-nitro-2-naphthoic acid 2a:
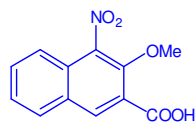
The ester **2** (2 g, 7.7 mmol) in methanol (10 mL) was subjected to ester hydrolysis using 2N LiOH solution. After completion of the reaction (12 h), the pale yellow precipitate obtained on acidification of the reaction mixture was filtered, washed with water till the pH was neutral and the residue obtained was dried in P$_2$O$_5$ desiccator yielding the acid **2a** quantitatively. This was used for further reaction without purification.

Methyl-4-amino-3-methoxy-2-naphthoate 2b:
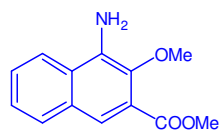
The amine 2**b** was obtained by the reduction of NO$_2$ group in **2** (2 g, 7.7 mmol) using H$_2$ (60 psi), Pd-C (90 mg) in AcOEt as the solvent. After the complete consumption of starting material (6 h), the reaction mixture was filtered over celite pad. The filtrate was evaporated and dried to get the amine **2b** which was taken for the next reaction without further purification.

Methyl-3-methoxy-4-(3-methoxy-4-nitro-2-naphthamido)-2-naphthoate 3:

To a solution containing acid **2a** (2 g, 8 mmol, 1 equiv.) and amine **2b** (1.87 mg, 8 mmol, 1 equiv) in dichloromethane (20 mL), was added DCC (2.0 g, 9.6 mmol, 1.2
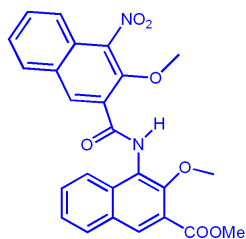
equiv.) and HOBt (cat. amount). The reaction mixture was allowed to stir at 0 °C for 10 min and for 12 h at room temperature. The DCU was filtered and the filtrate was washed sequentially with sat. solution of sodium bicarbonate, potassium hydrogen sulphate solution and water. The organic layer was dried over an. Na_2SO_4 and evaporated to get the crude product which on purification by column chromatography (eluent: AcOEt / pet.ether: 20/80, R_f: 0.3) afforded **3** as a yellow solid (2 g, 55%), which could be crystallized from a solution of ethyl acetate and pet. ether; mp: 177-178 °C; IR (CHCl$_3$) ν (cm^{-1}): 3365, 3018, 1728, 1674, 1628, 1537, 1506, 1358, 1298, 1213, 1045, 1001; ^{1}H NMR (500 MHz, CDCl$_3$) δ: 9.34 (s, 1H), 8.94 (s, 1H), 8.46 (s, 1H), 8.05-8.03 (d, *J* = 8.03 Hz, 1H), 7.93-7.91 (d, *J* = 8.03 Hz, 1H), 7.84-7.82 (d, *J* = 8.28 Hz, 1H), 7.76 (m, 2H), 7.67-7.51 (m, 3H), 4.28 (s, 3H), 4.01 (s, 3H), 3.94 (s, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ: 165.9, 163.3, 151.9, 146.8, 142.1, 135.8, 133.1, 132.6, 130.9, 129.9, 129.8, 129.5, 129.4, 129.0, 127.6,126.9, 126.1, 125.7, 125.2, 123.6, 123.3, 121.0, 65.1, 62.6, 52.5; ESI MS: 461.29 (M+H)$^+$, 483.30 (M+Na)$^+$, 499.28 (M+K)$^+$; Elemental Analysis calculated for $C_{25}H_{20}N_2O_7$: C, 65.21; H, 4.38; N, 6.08. Found: C, 65.28; H, 4.46; N, 6.02.

3-Methoxy-4-(3-methoxy-4-nitro-2-naphthamido)-2-naphthoic acid 3a:
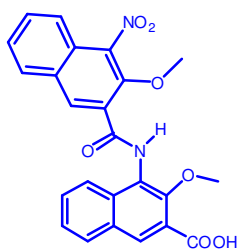
The compound **3** (0.9 g, 1.9 mmol) in dioxane (10 mL) was subjected to ester hydrolysis using 2N LiOH solution. After the completion, the pale yellow precipitate obtained on acidification of the reaction mixture was filtered, washed with water till the

pH was neutral and the residue obtained was dried in P_2O_5 desiccator to yield the acid

3a quantitatively. This was used for further reaction without purification.

Methyl 4-(4-amino-3-methoxy-2-naphthamido)-3-methoxy-2-naphthote 3b:
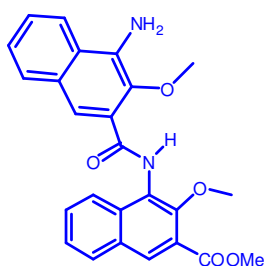
The dimer **3** (0.95 g, 2 mmol) was subjected to the reduction of NO_2 group using H_2 (60 psi), Pd-C (90 mg) in AcOEt as the solvent. After the complete consumption of starting material (6 h), the reaction mixture was filtered over celite pad. The filtrate was evaporated and dried to get the amine **3b** which was taken for the next reaction without further purification.

Methyl 3-methoxy-4-(3-methoxy-4-(3-methoxy-4-(3-methoxy-4-nitro-2-naphthamido) -2-naphthamido)-2-naphthamido)-2-naphthoate 4:
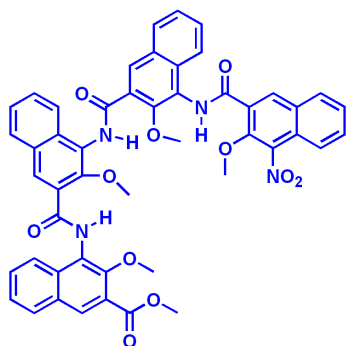
The acid **3a** (0.3 g, 0.7 mmol, 1 equiv.) was converted to the corresponding acid chloride **3c** using oxalyl chloride (0.1 mL, 2.1 mmol, 3 equiv.) and DMF (cat. amount) in dichloromethane as the solvent. After 3h, solvent and excess oxalyl chloride were removed under reduced pressure. The residue obtained was taken in dichloromethane (10 mL) and a solution containing amine **3b** (0.29 g, 0.7 mmol, 1 equiv.) and TEA (0.28 mL, 2.1 mmol, 3 equiv.) in dichloromethane (5 mL) was added and the reaction mixture was allowed to stir for 12 h at room temperature. The solvent was removed under reduced pressure. The crude product obtained was purified by column chromatography (chloroform/AcOEt: 90:10 R_f: 0.5) yielding **4** (0.23, 40 %); mp: 228-230 ^{o}C; IR (CHCl$_3$) ν (cm^{-1}): 3238, 3020, 1724, 1651, 1632,

1599, 1502, 1460, 1416, 1217, 1153, 1038, 1003; ^{1}H NMR (500 MHz, CDCl$_3$) δ: 9.84 (s, 2H), 9.45 (s, 1H), 9.03 (s, 1H), 9.98 (s, 1H), 8.92 (s, 1H), 8.46 (s, 1H), 8.11-8.04 (m, 3H), 7.96-7.87 (m, 4H), 7.79 (m, 2H), 7.71-7.49 (m, 7H), 4.33 (s, 3H), 4.18 (s, 3H), 4.17 (s, 3H), 4.00 (s, 3H), 3.93 (s, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ: 166.0, 164.5, 164.4, 163.3, 151.8, 151.2, 146.8, 142.2, 136.2, 133.9, 133.4, 132.9, 132.85, 132.8, 132.7, 131.2, 130.7, 130.6, 130.1, 129.9, 129.7, 129.3, 129.0, 128.9, 127.8, 127.1, 126.7, 126.5, 126.4, 126.1, 125.8, 125.3, 124.7, 124.6, 124.5, 124.7, 124.6, 124.5, 123.7, 123.3, 122.9, 121.1, 64.9, 63.1, 62.6, 52.5; ESI MS: 859.86 (M+H)$^+$; 881.89 (M+Na)$^+$; 897.76 (M+K)$^+$; Elemental Analysis calculated for C$_{49}$H$_{38}$N$_4$O$_{11}$: C, 68.52; H, 4.46; N, 6.52. Found: C, 68.62; H, 4.58; N, 6.46.

Methyl-1-methoxy-4-nitro-2-naphthoate 6:
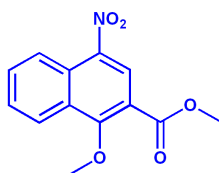
1-Hydroxy-2-naphthoic acid **5** (6 g, 31.9 mmol, 1equiv.) in acetone (150 mL) was subjected to simultaneous esterification and O-methylation using dimethyl sulphate (9.7 mL, 95.7 mmol, 3 equiv.) and K$_2$CO$_3$ (13.2 g, 95.7 mmol, 3 equiv.). After reflux for 8 h, the solid was removed by filtration and the residue obtained on evaporation of the filtrate was taken in dichloromethane. The organic layer was washed sequentially with sat. sodium bicarbonate, dil. HCl and water. The organic layer was dried over an. Na$_2$SO$_4$, filtered, and the filterate was subjected to careful nitration using Conc. HNO$_3$ at 0 ^{o}C. The progress of the reaction was monitored by TLC and after the completion of the reaction, the organic layer was washed with sat. sodium bicarbonate and then with water. The crude product obtained after the removal of solvent was purified by column chromatography (eluent: 15% AcOEt / pet.ether, R$_f$: 0.4) affording **6** as an

yellow solid (7.3 g, 88%). mp: 106 ^{o}C; IR (CHCl$_3$) ν (cm^{-1}): 3020, 2955, 1728, 1626, 1564,1524, 1506, 1445, 135, 1323, 1279, 1234, 1215, 1157, 1090; ^{1}H NMR (200 MHz, CDCl$_3$) δ: 8.77 (s, 1H), 8.70-8.65 (m, 1H), 8.46-8.41 (m, 1H), 7.89- 7.69 (m, 2H), 4.16 (s, 3H), 4.03 (s, 3H); ^{13}C NMR (50 MHz, CDCl$_3$) δ: 164.5, 162.8, 141.5, 131.6, 129.5, 128.1, 127.8, 126.5, 124.3, 123.5, 116.9, 64.0, 52.7; ESI-MS 261.07 (M+H)$^+$, 284.04 (M+Na)$^+$; Elemental Analysis calculated for C$_{13}$H$_{11}$NO$_5$: C, 59.77; H, 4.24; N, 5.36.; Found: C, 59.72; H, 4.28; N, 5.48.

1-Methoxy-4-nitro-2-naphthoic acid 6a:
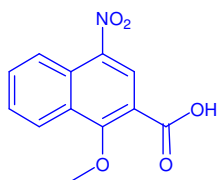
The ester **6** (2 g, 7.7 mmol) in methanol (10 mL) was subjected to ester hydrolysis using 2N LiOH solution. After the completion, the pale yellow precipitate obtained on acidification of the reaction mixture was filtered, washed with water till the pH was neutral and the residue obtained was dried in P$_2$O$_5$ desiccator to yield the acid **6a** quantitatively. This was used for further reaction without further purification.

Methyl-4-amino-1-methoxy-2-naphthoate 6b:
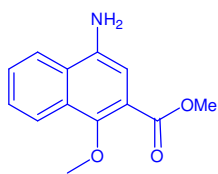
The compound **6** (2 g, 7.7 mmol) was subjected to the reduction of NO$_2$ group using H$_2$ (60 psi), Pd-C (90 mg) in AcOEt as the solvent. After the complete consumption of starting material (6 h), the reaction mixture was filtered over celite pad. The filtrate was evaporated and dried to get the amine **6b** which was taken for the next reaction without further purification.

Methyl-1-methoxy-4-(1-methoxy-4-nitro-2-naphthamido)-2-naphthoate 7:

To a solution containing acid **6a** (2 g, 8 mmol, 1 equiv.) and amine **6b** (1.88 g, 8
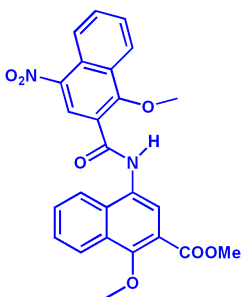
mmol, 1 equiv.) in acetonitrile (15 mL), was added HBTU (2.96 g, 9.2 mmol, 1.2 equiv.) followed by DIEA (1.6 mL, 9.2 mmol, 1.2 equiv.). The reaction mixture was stirred at room temperature for 12 h. The solvent was removed under reduced pressure, added dichloromethane and the organic layer was washed with sat. solution of sodium bicarbonate. The crude product obtained after removal of the solvent was purified by column chromatography (eluent: 20% AcOEt / 80% pet.ether, R_f: 0.3) affording **7** as an yellow solid (3 g, 85%); mp: 205-206 ^{o}C; IR (nujol) ν (cm^{-1}): 3274, 3018, 1716, 1643, 1550, 1516, 1458, 1377, 1229, 1155, 1085, 1003; ^{1}H NMR (500 MHz, CDCl$_3$) δ: 10.14 (s, 1H), 9.02 (s, 1H), 8.65-8.63 (m, 2H), 8.38-8.36 (d, J = 8.28 Hz, 2H), 8.02-8.00 (d, J = 8.53 Hz, 1H), 7.87-7.62 (m, 4H), 4.24 (s, 3H), 4.08 (s, 3H), 4.00 (s, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ: 165.9, 161.9, 159.1, 156.0, 143.4, 131.4, 130.1, 129.2, 129.0, 128.5, 128.34, 128.3, 128.2, 126.8, 126,.0, 124.6, 124.0, 123.6, 121.4, 120.9, 120.6, 119.1, 64.6, 63.5, 52.3; ESI MS: 461.03 (M+H)$^+$, 483.07 (M+Na)$^+$, 499.04 (M+K)$^+$; Elemental Analysis calculated for C$_{25}$H$_{20}$N$_2$O$_7$: C, 65.21; H, 4.38; N, 6.08. Found: C, 65.18; H, 4.44; N, 6.18.

**1-Methoxy-4-(1-methoxy-4-nitro-2-naphthamido)-2-naphthoic acid 7a:**
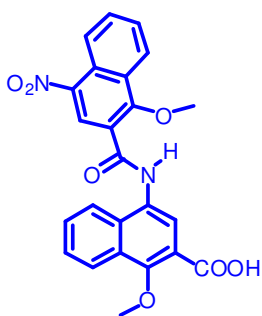
To a solution containing **7** (1 g, 2.2 mmol, 1 equiv.) in dioxane (10 mL) was added LiOH. H$_2$O (0.19 g, 4.4 mmol, 2 equiv.) in water (5 mL). After the completion of the reaction, the yellow precipitate obtained on acidification of the reaction mixture with dil. HCl was filtered, washed with water till the pH was

neutral and the residue obtained was dried in P_2O_5 desiccator to yield the acid **7a** quantitatively. This was used for the next reaction without further purification.

Methyl 4-(4-amino-1-methoxy-2-naphthamido)-1-methoxy-2-naphthoate 7b:
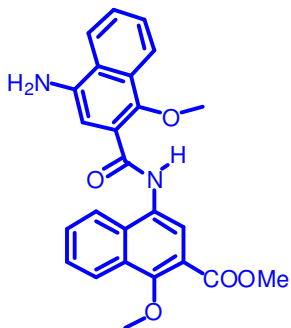
The compound **7** (1g, 2.2 mmol, 1 equiv.) was subjected to reduction of the NO_2 group using H_2 (60 psi), Pd-C (100 mg) in AcOEt as the solvent. After the complete consumption of starting material, the reaction mixture was filtered over celite pad. Filtrate was evaporated and dried to get the amine **7b** which was taken for the next reaction without further purification.
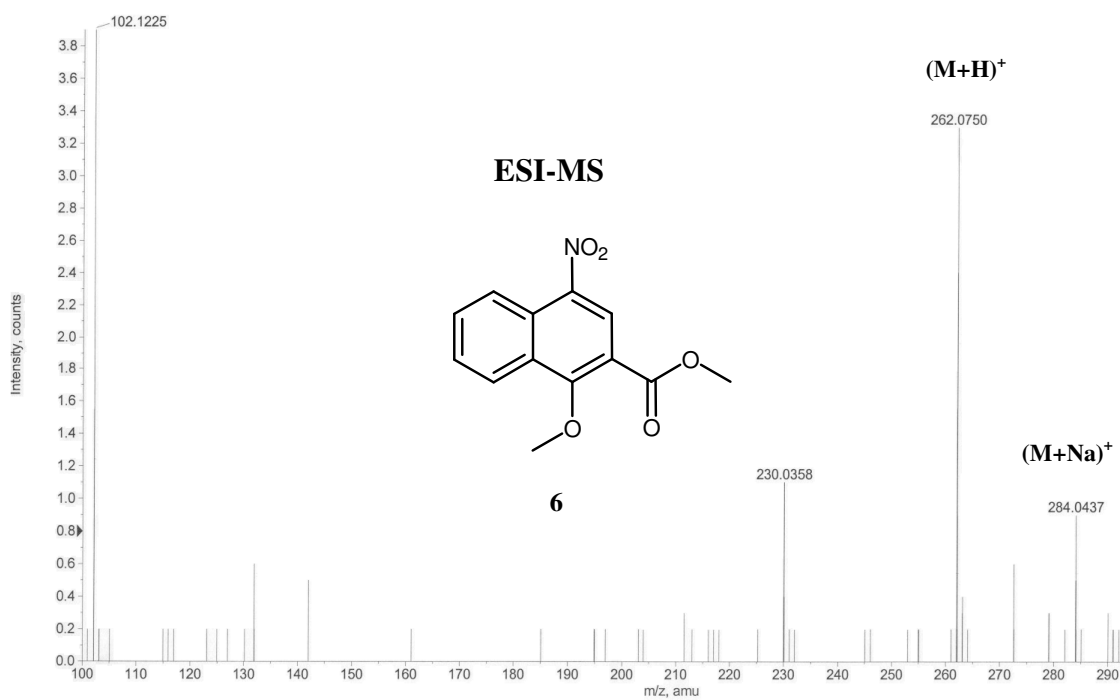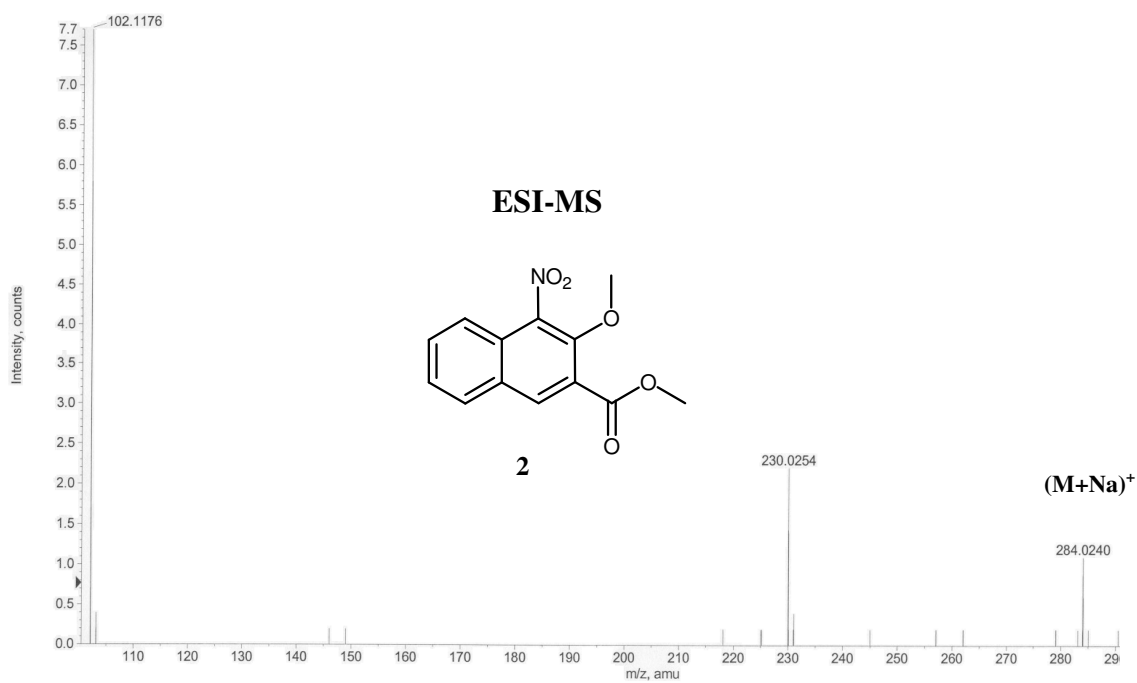
Methyl 1-methoxy-4-(1-methoxy-4-(1-methoxy-4-(1-methoxy-4-nitro-2-naphthamido)-2-naphthamido)-2-naphthamido)-2-naphthoate 8:
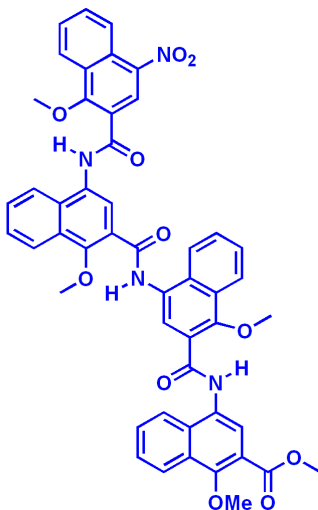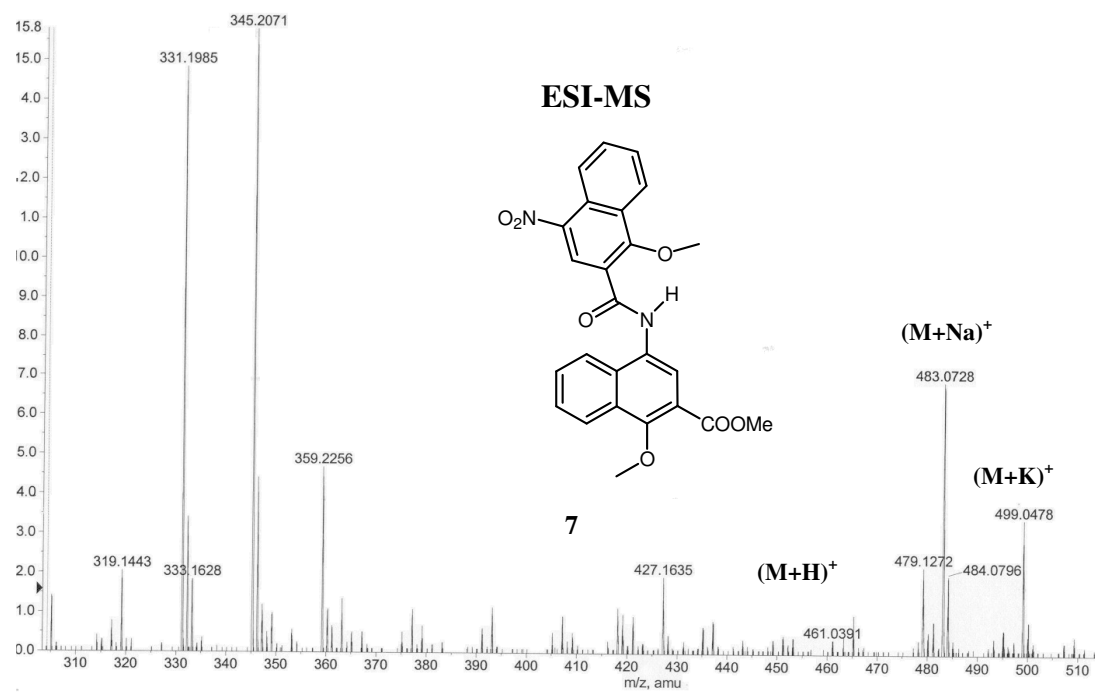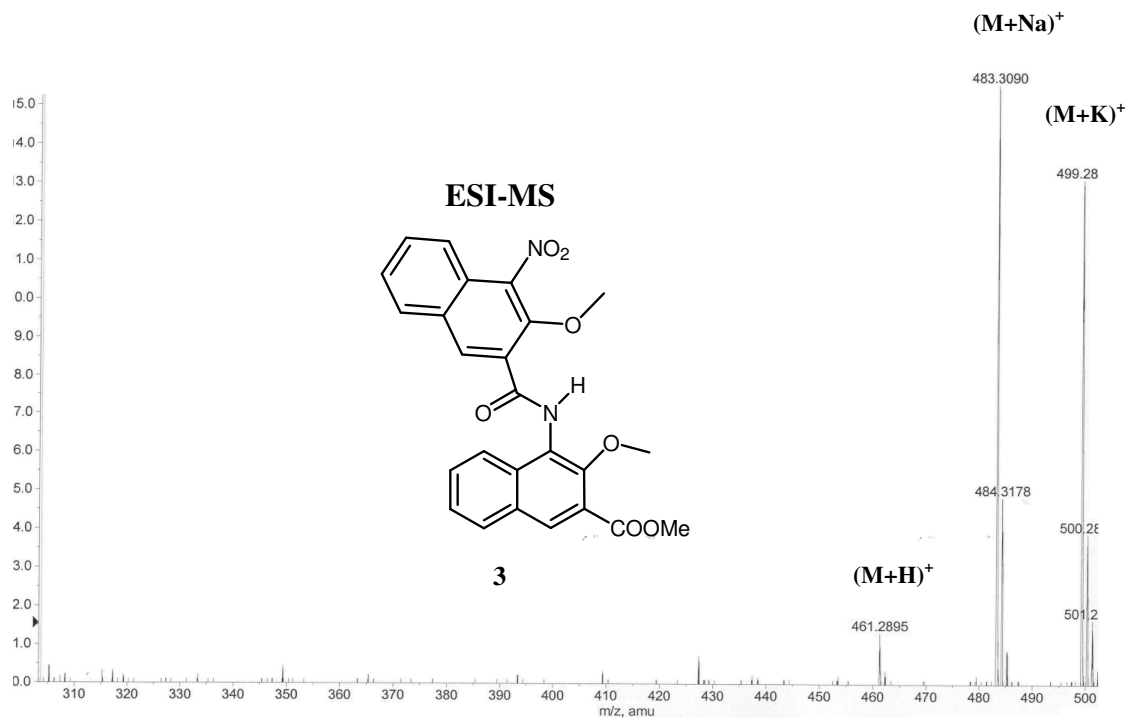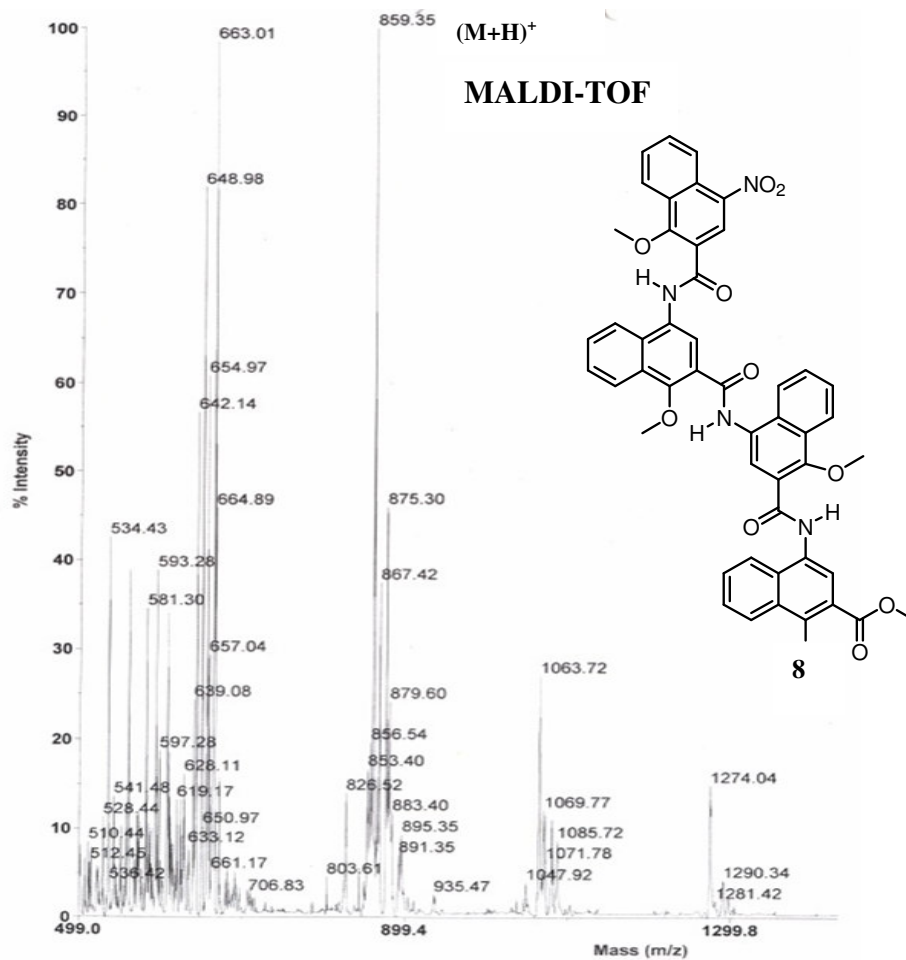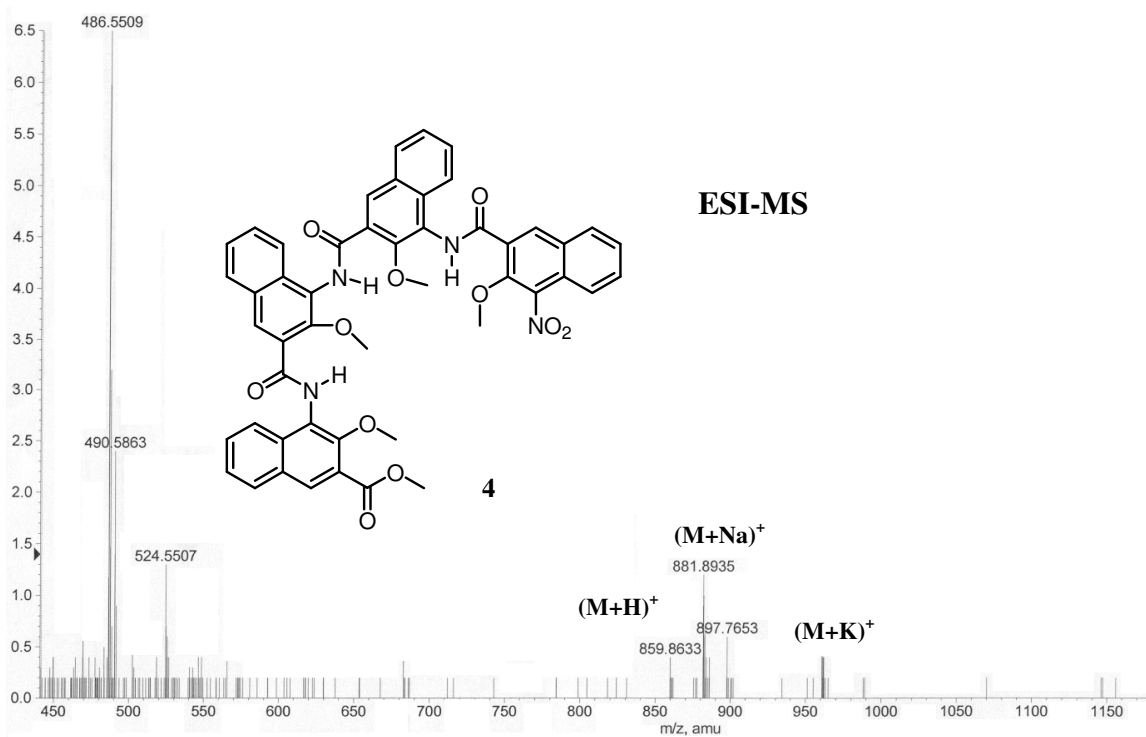

The acid **7a** (0.5 g, 1.1 mmol, 1 equiv.) and the amine **7b** (0.48 g, 1.1 mmol, 1 equiv.) were taken in acetonitrile (15 mL). HBTU (0.5 g, 1.3 mmol, 1.2 equiv.) and DIEA (0.23 mL, 1.3 mmol, 1.2 equiv.) were added and the reaction mixture was stirred at 60 ^{o}C for 12 h at room temperature. The crude product obtained after the work-up was purified by column chromatography (chloroform/AcOEt: 90:10 R_f: 0.5) yielding **8** as an yellow solid (43%). mp: 235-237 ^{o}C; IR (nujol) ν (cm^{-1}): 3234, 2922, 2852, 1722, 1639, 1462, 1377, 1312, 1227, 1151, 1086; ^{1}H NMR (400 MHz, CDCl$_3$) δ: 10.34 (s, 1H), 10.20 (s, 1H), 10.00 (s, 1H), 9.05 (s, 1H), 8.79 (s, 1H), 8.75 (s, 1H), 8.68-8.65 (m, 2H), 8.40-8.35 (m, 4H), 8.14-8.05 (m, 3H), 7.89-7.85 (t, J = 7.35 Hz, 1H), 7.80-7.70 (m, 6H), 7.67-7.63 (t, J = 7.61 Hz, 1H), 4.29 (s, 3H), 4.25 (s,

3H), 4.20 (s, 3H), 4.10 (s, 3H), 4.0 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ: 166.1, 163.8, 163.6, 162.4, 159.3, 155.6, 153.9, 153.2, 143.7, 139.3, 131.6, 130.5, 13.2, 129.9, 129.2, 129.1, 128.9, 128.8, 128.7, 128.4, 128.3, 127.3, 127.2, 126.7, 126.1, 124.6, 124.1, 123.8, 123.7, 122.4, 122.2, 122.1, 121.3, 121, 120.5, 119.3, 114.1, 64.6, 64.1, 64.0, 60.4, 52.3; MALDI-TOF: 859.35 (M+H)$^+$; Elemental Analysis calculated for C$_{49}$H$_{38}$N$_4$O$_{11}$: C, 68.52; H, 4.46; N, 6.52. Found: C, 68.72, H, 4.60, N, 6.30.

ESI-MS

2

230.0254

(M+Na)⁺

284.0240

(M+H)⁺

262.0750

ESI-MS

6

230.0358

(M+Na)⁺

284.0437

ESI-MS

(M+Na)⁺ 483.3090

(M+K)⁺ 499.28

484.3178

500.28

501.2

(M+H)⁺ 461.2895

3

ESI-MS

345.2071

331.1985

359.2256

319.1443 333.1628

427.1635

(M+H)⁺

461.0391

479.1272 484.0796

(M+Na)⁺ 483.0728

(M+K)⁺ 499.0478

7

ESI-MS

(M+Na)⁺

(M+H)⁺

(M+K)⁺

4

MALDI-TOF

(M+H)⁺

8

\..\PPY-NAP14A-CHCA_0001.dat
Acquired: 14:34:00, September 17, 2007

CDCL3

8.60
7.99
7.94
7.72
7.70
7.64
7.63
7.60
7.27

4.03
4.02

^{1}H (200 MHz, CDCl$_3$)

2

9   8   7   6   5   4   3   2

CDCL3

8.77
8.70
8.66
8.65
8.45
8.42
7.85
7.81
7.75
7.75
7.71
7.71
7.27

4.16
4.03

^{1}H (200 MHz, CDCl$_3$)

6

9   8   7   6   5   4   3   2

^{1}H (500 MHz, CDCl$_3$)

3

^{1}H (500 MHz, CDCl$_3$)

7

¹H (500 MHz, CDCl₃)

4

¹H (400 MHz, CDCl₃)

8

¹³C (50 MHz, CDCl₃)

DEPT-135 (50 MHz, CDCl₃)

164.52
162.83
141.46
131.60
129.46
128.11
127.84
126.52
124.34
123.53
116.92
77.63
77.00
76.36
63.98
52.71

^{13}C (50 MHz, CDCl$_3$,)

6

170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

131.59
127.83
126.52
124.34
123.52
63.97
52.71

DEPT-135 (50 MHz, CDCl$_3$)

6

140 130 120 110 100 90 80 70 60 50 40 30 20 10

¹³C (125 MHz, CDCl₃)

165.88, 163.27, 151.93, 146.84, 135.84, 133.08, 130.94, 129.52, 129.40, 128.99, 127.58, 126.10, 125.66, 123.26, 120.99, CDCl3 77.26, 77.00, 76.76, 65.05, 62.58, 52.50

DEPT-135 (125 MHz, CDCl₃)

135.85, 133.08, 130.94, 129.52, 129.40, 128.99, 127.57, 126.09, 120.98, 65.05, 62.57, 52.50

165.88
161.91
159.07
155.97
143.41
131.36
128.99
128.34
128.29
126.78
125.98
124.64
124.02
123.60
120.88

77.32
77.00
76.68

64.55
63.52

52.33

¹³C (100 MHz, CDCl₃)

O₂N

O

N
H

COOMe

7

150   100   50

131.36
128.99
128.29
126.78
125.97
124.63
124.01
123.59
120.87
120.63

64.55
63.52

52.34

DEPT-135 (100 MHz, CDCl₃)

O₂N

O

N
H

COOMe

7

140 130 120 110 100 90 80 70 60 50 40 30 20 10

^{13}C (100 MHz, CDCl$_3$)

166.04, 164.45, 163.28, 151.23, 146.79, 136.16, 130.05, 129.89, 129.65, 129.32, 129.03, 128.86, 126.44, 126.06, 123.72, 121.08, 77.31, 77.00, 76.68, 64.94, 63.12, 62.59, 52.46

4

DEPT-135 (100 MHz, CDCl$_3$)

135.96, 131.04, 129.86, 129.13, 128.84, 128.67, 127.61, 126.47, 126.23, 125.85, 123.51, 122.72, 120.88, Chloroform-d 77.00, 64.74, 62.93, 62.40, 52.26

4

^{13}C (100 MHz, CDCl$_3$)

166.10, 163.81, 163.63, 162.42, 159.30, 155.57, 153.90, 153.22, 143.68, 139.28, 129.86, 128.86, 128.30, 127.27, 126.68, 126.14, 124.58, 124.12, 123.80, 123.67, 120.97, 120.52, 114.06

Chloroform-d

77.32, 77.00, 76.69, 64.63, 64.09, 64.01, 63.54, 60.38, 52.31

8

DEPT-135 (100 MHz, CDCl$_3$)

131.36, 128.85, 128.77, 128.27, 126.66, 126.13, 124.55, 124.09, 123.76, 123.64, 122.59, 120.46
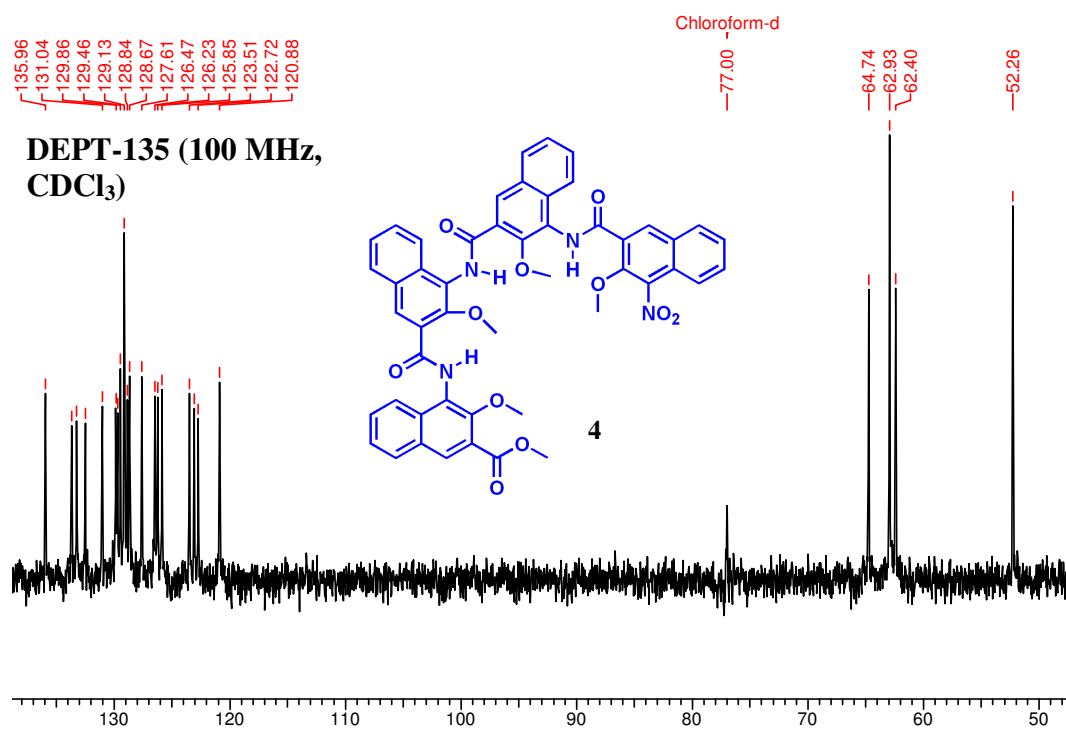
64.61, 64.07, 63.99, 63.53, 52.31

8

Dilution and titration study of 3 in CDCl₃

Table 1: Dilution study

No	Concentration (in mmol)	δ_{NH}
1	120	9.34
2	100	9.34
3	80	9.34
4	60	9.34
5	40	9.34
6	20	9.34
7	10	9.35
8	5	9.35
9	4	9.35
10	2	9.35

Table 2: Titration study

No	$V_{DMSO-d6}$ (in µL)	δ_{NH}
1	0	9.34
2	5	9.26
3	10	9.31
4	15	9.33
5	20	9.35
6	25	9.37
7	30	9.41
8	35	9.44
9	40	9.47
10	45	9.48
11	50	9.50

Dilution and titration study of 7 in CDCl₃

Table 3: Dilution study

No	Concentration (in mmol)	δ_{NH}
1	120	10.14
2	100	10.14
3	80	10.15
4	60	10.15
5	40	10.15
6	20	10.15
7	10	10.15
8	5	10.16
9	4	10.16
10	2	10.16

Table 4: Titration study

No	$V_{DMSO-d6}$ (in µL)	δ_{NH}
1	0	10.16
2	5	10.08
3	10	10.10
4	15	10.13
5	20	10.14
6	25	10.15
7	30	10.17
8	35	10.18
9	40	10.19
10	45	10.21
11	50	10.23

Table 5: Titration study of 4 in CDCl$_3$ (V$_{DMSO-d6\ added}$ = 0-50 μ lit)

V$_{DMSO-d6}$ (in μl)	Chemical shift in ppm		
	δ_{NH1} *	δ_{NH2} *	δ_{NH3}
0	9.84	9.83	9.45
5	9.84	9.84	9.46
10	9.84	9.84	9.47
15	9.85	9.85	9.50
20	9.86	9.86	9.51
25	9.89	9.86	9.60
30	9.94	9.88	9.71
35	9.94	9.88	9.73
40	9.97	9.90	9.78
45	9.98	9.90	9.79
50	9.98	9.90	9.79

*
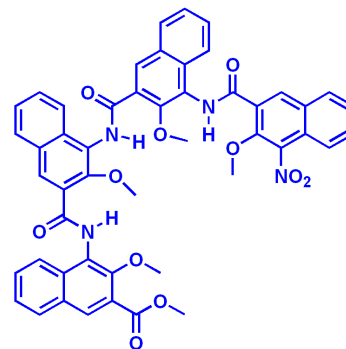
Table 6: Titration study of 8 in CDCl$_3$ (V$_{DMSO-d6\ added}$ = 0-50 μ lit)

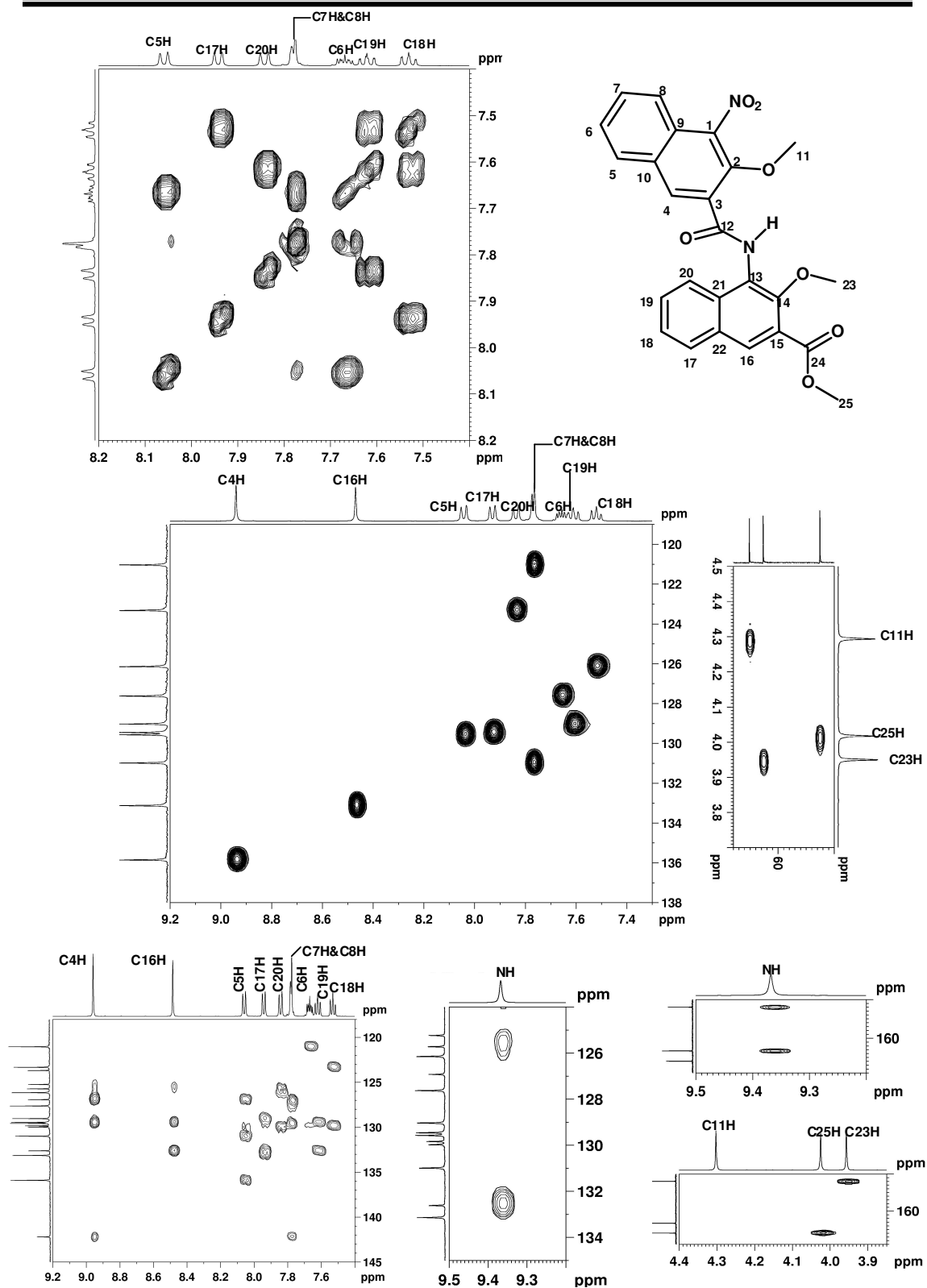| V$_{DMSO-d6}$ (in μl)	Chemical shift in ppm		
	δ_{NH1}	δ_{NH2}	δ_{NH3}
0	10.34	10.20	10.0
5	10.34	10.20	10.0
10	10.34	10.20	10.0
15	10.34	10.21	10.02
20	10.37	10.27	10.12
25	10.38	10.28	10.14
30	10.39	10.30	10.16
35	10.40	10.31	10.19
40	10.40	10.32	10.20
45	10.41	10.34	10.23
50	10.42	10.35	10.24
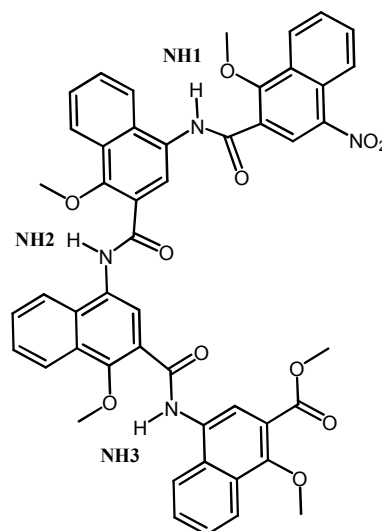
Fig. 1: Partial 2D COSY, HSQC and HMBC spectra of **3** (500 MHz, CDCl$_3$)
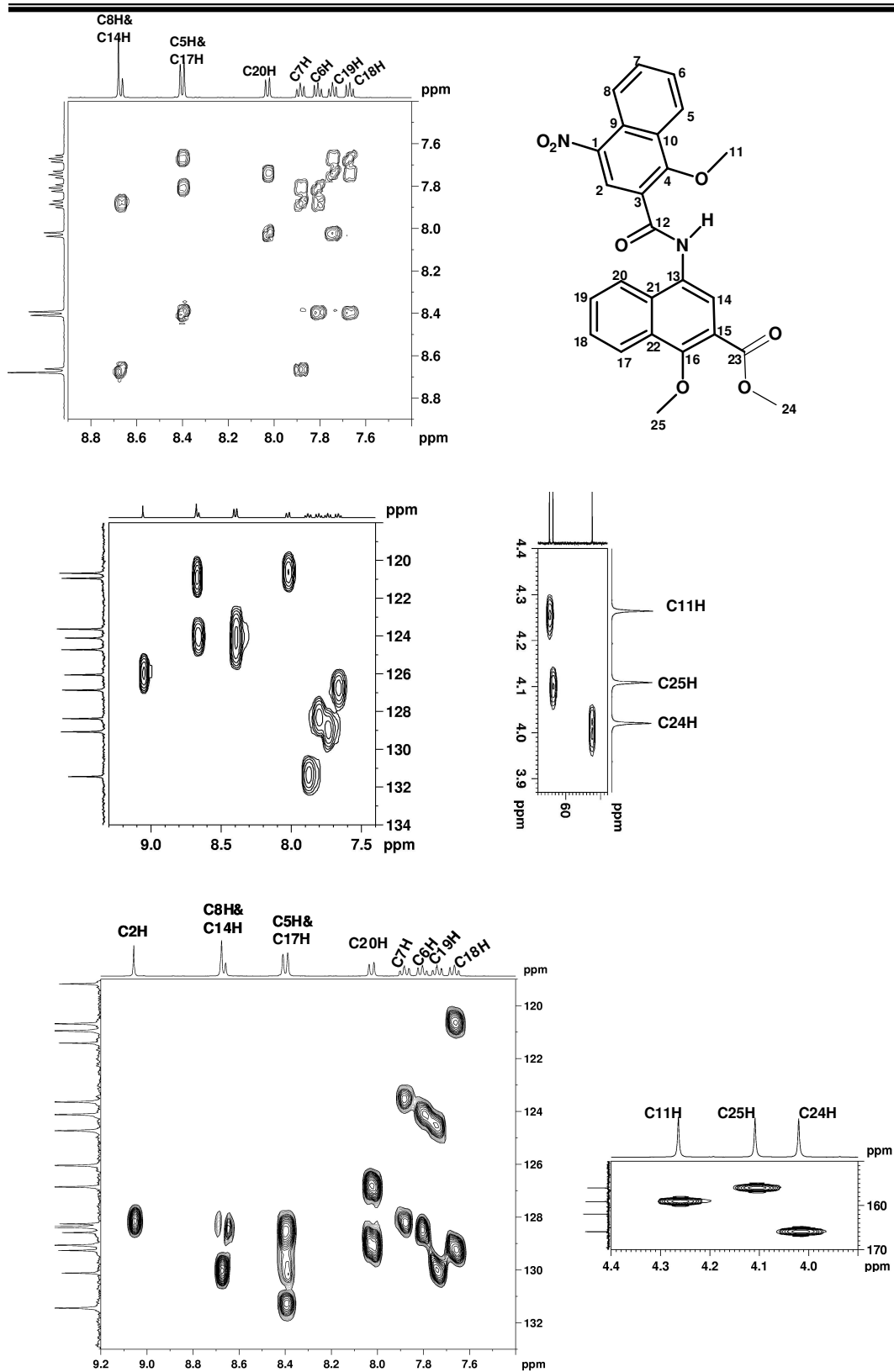
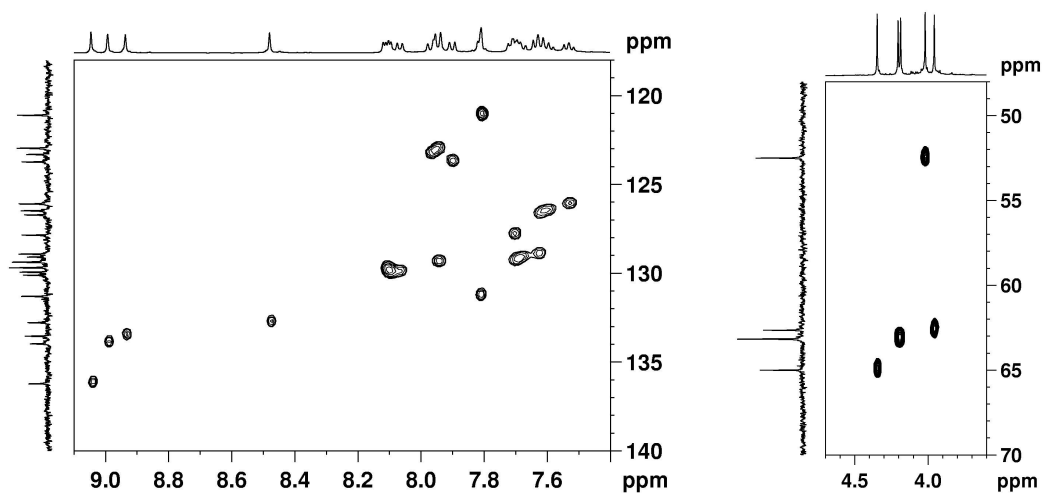**Fig. 2**: Partial 2D COSY, HSQC and HMBC spectra of **3** (500 MHz, CDCl$_3$)
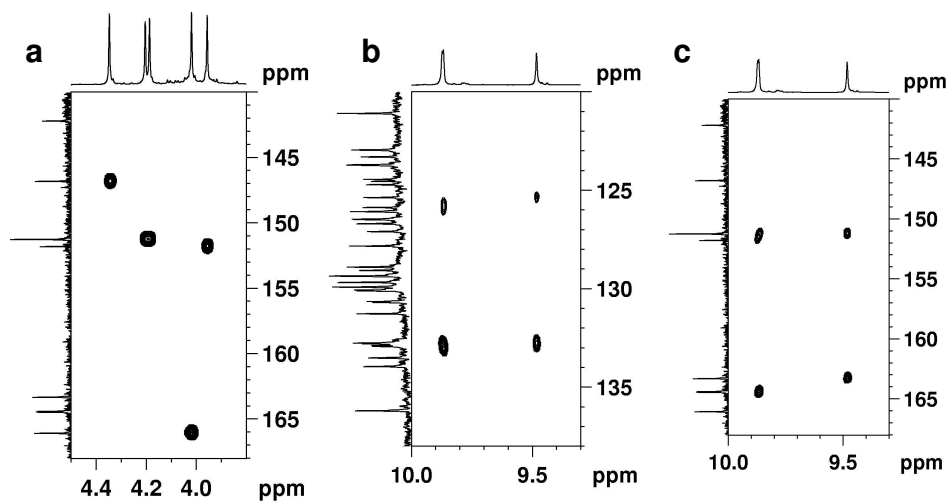
Fig. 3: Partial HSQC spectra of **4** (500 MHz, CDCl₃)

Fig. 4: Partial HMBC spectra of **4** (500 MHz, CDCl₃).

Table 7:
¹H, ¹³C HSQC assignments for **3**

¹H (δ/ppm)		¹³C (δ/ppm)
8.95 (C4H)		135.62
8.48 (C16H)		132.86
8.06 (C5H)		129.36
7.94 (C17H)		129.70
7.84 (C20H)		127.35
7.77 (2H)	C7H	123.04
	C8H	120.76
7.67 (C6H)		130.72
7.62 (C19H)		128.76
7.52 (C18H)		125.87
4.30 (3H, C11H)		64.83
4.02 (3H, C25H)		52.27
3.95 (3H, C23H)		62.37
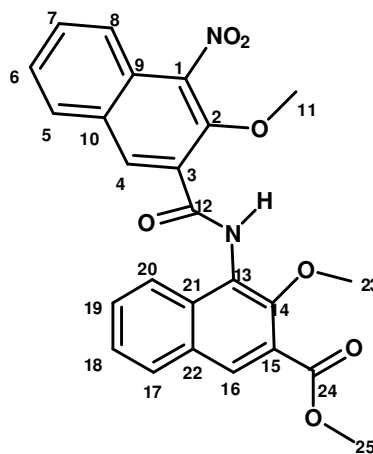
Table 8:
¹H, ¹³C HMBC assignments for **3**

¹H (δ/ppm)	¹³C (δ/ppm)
9.36 (NH)	163.03, 151.69, 132.32, 124.94
8.95 (C4H)	163.03, 146.61, 126.65, 124.94
8.48 (C16H)	165.64, 151.69, 132.32
8.06 (C5H)	126.65, 130.72, 141.91
7.94 (C17H)	132.32, 132.86, 128.76
7.84 (C20H)	129.70, 125.43, 125.87
7.67 (C6H)	132.32,
7.62 (C19H)	132.32, 129.17
7.52 (C18H)	129.70, 123.04,
4.30 (3H, C11H)	146.61
4.02 (3H, C25H)	165.64
3.95 (3H, C23H)	151.69

Table 9:

¹H, ¹³C HSQC assignments of **7**

¹H (/ppm)		¹³C (/ppm)
9.02 (C2H)		125.98
8.65 (C14H)		120.88
8.64 (C8H)		124.02
8.37	C5H	128.28, 126.78
	C17H	
8.01 (C20H)		120.64
7.85 (C7H)		131.36
7.78 (C6H)		128.99
7.72 (C19H)		120.64
7.64 (C18H)		126.78
4.24 (3H, C11H)		64.55
4.08 (3H, C25H)		63.52
4.00 (3H, C24H)		62.33
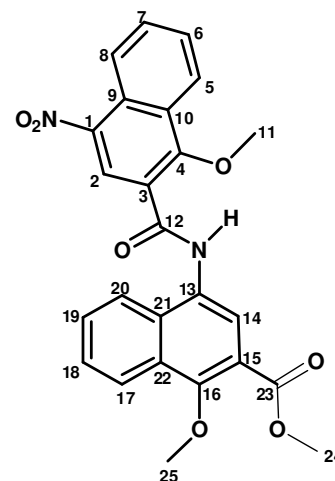
Table 10:

¹H, ¹³C HMBC assignments for **7**

¹H (/ppm)		¹³C (/ppm)
10.14 (NH)		161.91, 130.06, 120.88
9.02 (C2H)		161.91, 159.07, 128.17
8.65 (C14H)		165.88, 155.97, 130.06
8.64 (C8H)		121.35, 128.29, 128.34
8.37	C5H	159,07, 131.36, 128.17
	C17H	155.97, 130.06, 128.99
8.01 (C20H)		129.19, 126.78
7.85 (C7H)		128.17, 124.64
7.78 (C6H)		128.34, 124.02
7.72 (C19H)		130.06, 124.64
7.64 (C18H)		120.64, 129.19
4.24 (3H, C11H)		159.07
4.08 (3H, C25H)		155.97
4.00 (3H, C24H)		165.88

PART II

Crystal Data for 5 ($C_{28}H_{24}O_6$): M = 456.47, Single crystals of the compound were grown by slow evaporation of a mixture of ethyl acetate and pet. ether. Crystal dimensions 0.38 x 0.11 x 0.08, Quadrant data acquisition. Total scans = 4, total frames = 2424, θ range = 2.17 to 25.00º, completeness to θ of 25.00º is 99.8 %, Triclinic, $P\bar{1}$, a = 8.0132 (5), b = 10.2043 (6), c = 15.3713 (9) Å, α = 86.8620 (10), β = 75.9320 (10), γ = 67.0470 (10), V = 1121.54(12) Å^3, Z = 2, D_c = 1.352 mg m^{-3}, μ (Mo K$_\alpha$) = 0.095 mm^{-1}, 10926 reflections measured, 3953 unique [I>2σ(I)], R value 0.0572, wR2 = 0.1396.

Crystal Data for 11 ($C_{46.50}H_{47.50}Cl\,N_6\,O_{8.50}$): M = 861.86. Single crystals of the **11** were grown by slow evaporation of a mixture of methanol and DCM. Colorless plate of approximate size 0.33 x 0.23 x 0.07 mm was used for data collection quadrant data acquisition. Total scans = 4, total frames = 2424, θ range = 1.87 to 25.00º, completeness to θ of 25.0º is 99.8 %. Crystals belong to Triclinic, space group $P\bar{1}$, a = 12.0667 (8) Å, b = 14.506 (1) Å, c = 15.091 (1) Å, α = 92.767 (7)º, β = 109.028 (1)º, γ = 105.108 (6)º, V = 2385.0 (3) Å^3, Z = 2, D = 1.200 gcm^{-3}, μ = 0.137 mm^{-1}, 23258 reflections measured, 8380 unique [I>2σ(I)], R = 0.0935. Rw = 0.2298.

Experimental procedures

1,8-diaminonapthalene **1**, and 3-formyl-4-methoxyphenyl boronic acid **7** were obtained from Aldrich. 1,8-diiodonapthalene **2**[21] and the naphthalene diboronic acid **3**[22] were prepared according to the literature procedures. Suzuki couplings of **3** with **4**, and **2** with **7** to yield **5**, and **8**, respectively, were carried out following a standard

reported procedure.[23]

1,8-Bis-(4-methoxy-3-methylcarboxyphenyl)-naphthalene 5:
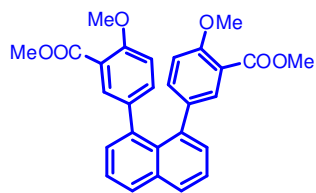
The suzuki coupling of **3** with **4** yielded **5** following a recent procedure as follows. A reaction mixture in PEG-400 (6 mL) containing naphthalene diboronic acid **3** (0.2 g, 0.9 mmol, 1 equiv.), Methyl-5-bromo-2-methoxy-benzoic acid methyl ester **4** (0.68 g, 2.7 mmol, 3 equiv.), $Pd(OAc)_2$ (12 mol%), DABCO (24 mol%), TBAB (0.1 equiv.), K_2CO_3 (8 equiv.) was sealed and heated at 110 ^{o}C for 15 h. After cooling to room temperature, the product was extracted into DCM (100 mL), and the crude compound obtained was purified by column chromatography (eluent: AcOEt / pet.ether: 25/75; R_f: 0.3) to yeld **5** (0.19 g, 45%). mp: 158-160 ^{o}C; IR (CHCl$_3$) ν (cm^{-1}): 3020.3, 2951, 2841, 1724, 1717, 1611, 1501, 1437, 1308, 1277, 1238, 1217, 1182, 1086, 1026; ^{1}H NMR (200 MHz, CDCl3) δ: 7.98-7.93 (dd, J = 1.37 Hz, 2H), 7.59-7.51 (m, 2H), 7.46-7.39 (m, 4H), 7.08-7.03 (dd, J = 2.28 Hz, 1H), 6.98-6.93 (dd, J = 2.43 Hz, 1H), 3.85-3.81 (m, 12 H); ^{13}C NMR (50 MHz, CDCl3) δ: 166.5, 166.0, 157.6, 157.4, 138.7, 135.4, 135.2, 135.1, 134.9, 134.4, 133.6, 132.6, 131.0, 130.7, 128.9, 125.2, 118.6, 111.4, 111.3, 56.3, 56.1, 51.9, 51.8; ESI-MS: 457.35 (M+H)$^+$, 479.28 (M+Na)$^+$, 496.26 (M+K)$^+$; Elemental Analysis calculated for $C_{28}H_{24}O_6$: C, 73.67; H, 5.30; Found: C, 73.82; H, 5.42.
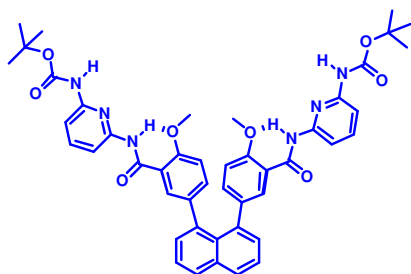
1,8-Bis-(3-formyl-4-methoxy-phenyl)-naphthalene[24]
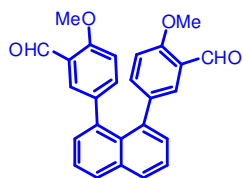
Following the same procedure for the preparation of **5**, the suzuki coupling of **2** (0.4 g, 1.5 mmol, 1 equiv.) with **7** (0.57

g, 3.1 mmol, 3 equiv.) yielded **8** (60%). [13]C (50 MHz, CDCl$_3$) δ: 189.3, 159.7, 138.1, 137.3, 135.5, 135.2, 130.6, 129.4, 128.95, 125.1, 123.2, 110.7, 55.7; ESI-MS: 397.31 (M+H)$^+$, 419.30 (M+Na)$^+$, 435.28 (M+K)$^+$;

**Synthesis of short oligomer 11:** To a stirred solution of the diacid **6** (1g, 2.3 mmol, 1 equiv.) in dry DCM (15 ml), was added 2 drops of DMF followed by the drop wise addition of oxalyl choride (1.2 ml, 9.2, 4 equiv.). After 3 h at room temperature, solvent and excess oxalyl chloride were removed from the reaction mixture under reduced pressure to get the crude acid chloride which was directly reacted with a reaction mixture containing mono-boc-2,6-diaminopyridine (9.2 mmol, 4 equiv.) and TEA (9.2 mmol, 4 equiv.) in DCM (10 ml) was added and the reaction mixture was allowed to stir overnight at rt. Work-up followed by column chromatographic purification afforded **11** (eluent: 80% dichlorometahane / pet.ether, R$_f$:0.4) as a white solid (1.41 g, 75%) which could be crystallized from a solution of dichloromethane and MeOH. mp: 172 ^{o}C (decom.); IR (CHCl$_3$) ν (cm^{-1}): 3421, 3356, 3018, 1730, 167, 1587, 1506, 1497, 1456, 1369, 1305, 1217, 1153, 1053, 1028; ^{1}H NMR (CDCl$_3$, 400 MHz) δ: 10.12, (s, 2H), 8.48 (bs, 2H), 8.08-8.06 (m, 2H), 7.98-7.96, *J* = 8.28 Hz, 2H), 7.77- 7.73 (m, 6H), 7.58-7.54 (t, *J* = 7.54 Hz, 2H), 7.40-7.38 (d, *J* = 7.04 Hz, 2H), 7.18-7.16 (dd, *J* = 2.26 Hz, *J* = 6.29 Hz, 2H), 6.69-6.67 (d, *J* = 8.80 Hz, 2H), 3.73 (s, 6H), 1.38 (s, 18H); ^{13}C NMR (CDCl$_3$, 100 MHz) δ: 163.1, 155.4, 152.5, 150.8, 150.1, 140.9, 138.7, 136.7, 135.6, 135.3, 133.2, 130.6, 130.0, 128.8, 125.2, 119.4, 110.6, 108.9, 107.7, 81.1, 56.1, 28.1; ESI MS: 810.96 (M+H)$^+$, 832.94 (M+Na)$^+$; 848.95 (M+K)$^+$ Elemental Analysis

calculated for $C_{46}H_{46}N_6O_8$: C, 68.13; H, 5.72; N, 10.36. Found: C, 68.26; H, 5.84; N, 10. 44.

Synthesis of 14: The compound **11** (0.5 g, 0.6 mmol) was subjected to the Boc
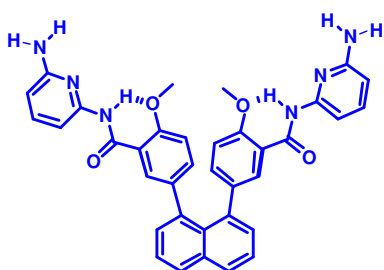
deprotection using TFA/DCM. After complete consumption of **11**, the solvent was stripped off from the reaction mixture. The residue was neutralized with sat. sodium bicarbonate solution and the white

precipitate obtained was filtered, washed with water and the residue was dried in KOH desiccator. This was then taken for the further reaction without any purification.

Synthesis of mono acid 12: The bis-ester **5** (0.6 g, 1.3 mmol, 1 equiv.) was subjected
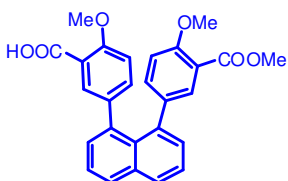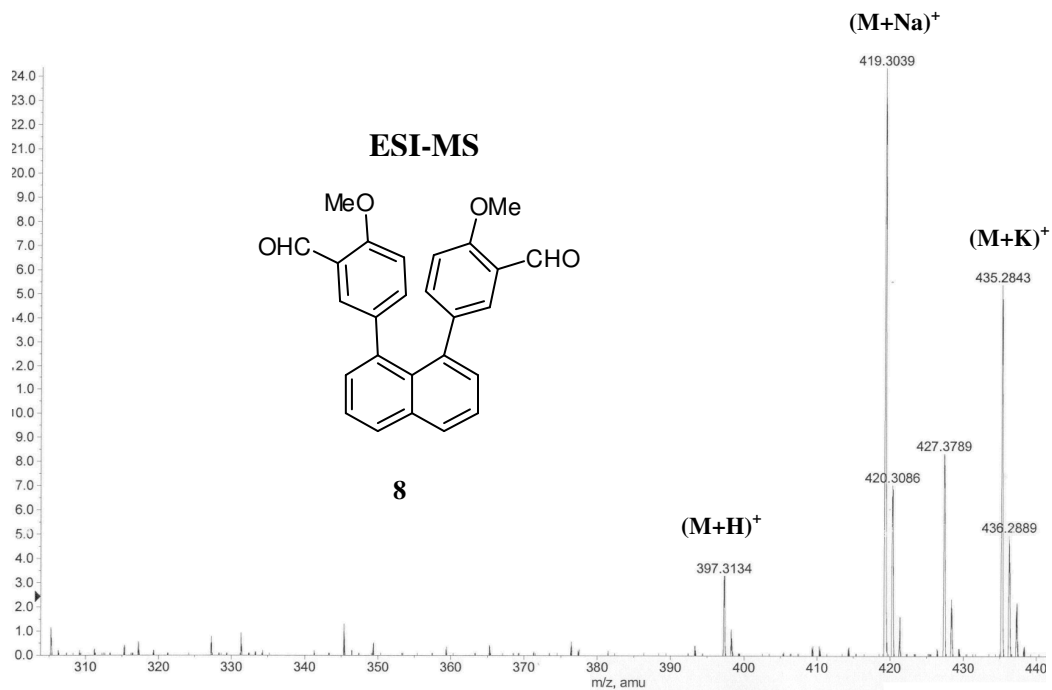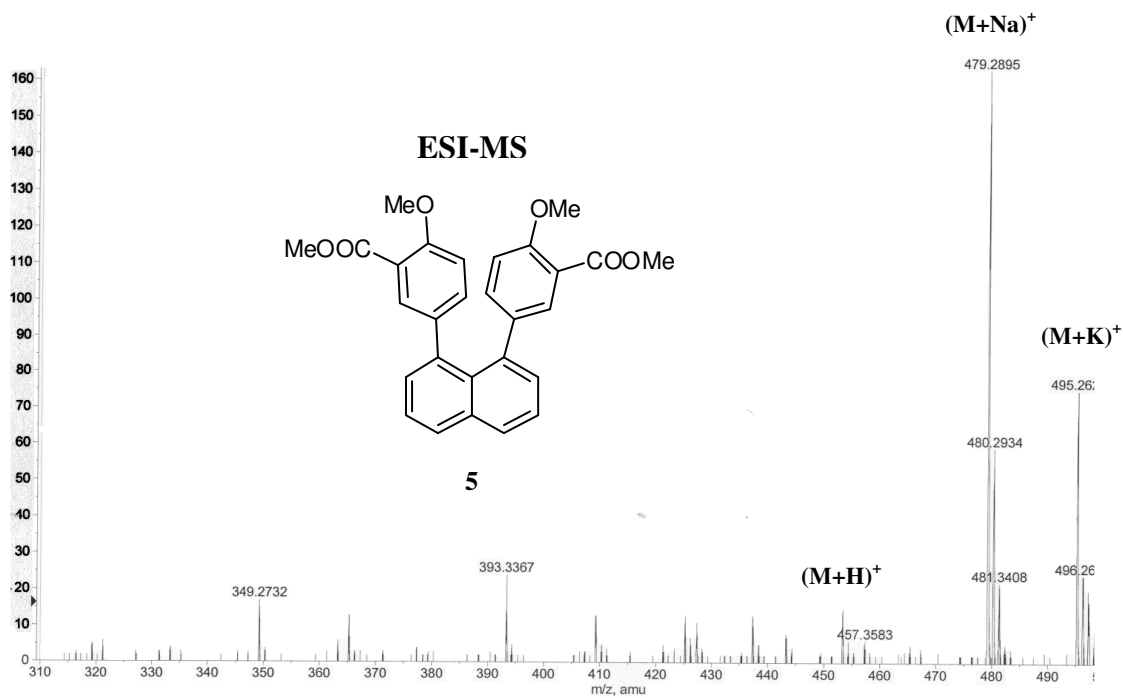

to careful saponification using $LiOH.H_2O$ (56 mg, 1.3 mmol, 1 equiv.) dissolved in $MeOH-H_2O$ (9:1 mL). After stirring for 6 h, the reaction mixture was stripped off the solvent under

reduced pressure. The residue was suspended in water and washed with DCM to remove any unreacted bis ester **5**. The aqueous layer was neutralized with dil. HCl to get an off-white precipitate taken in DCM, which was taken in DCM and filtered to remove the undissolved material which is presumably the bis acid **6**. The filterate was evaporated to get the crude acid which was used for the next reaction without further purification.

Synthesis of 15: The mono acid **12** (0.36 g, 0.8 mmol, 3 equiv.) was converted to the corresponding acid chloride **13** using oxalyl chloride (4 equiv.) and cat. amount of DMF in DCM (15 mL) as the solvent. After stirring the reaction mixture for 3h at room temperature, the solvent and excess oxalyl chloride were removed under
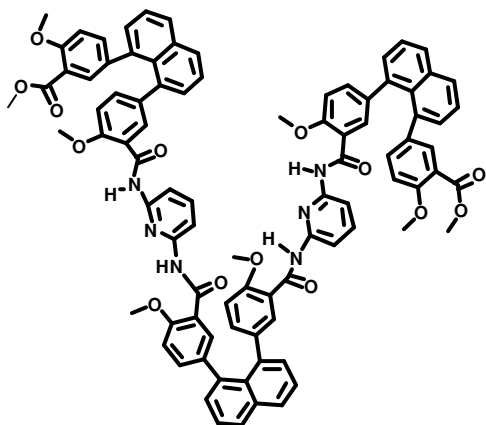
reduced pressure to get the crude acid chloride which was taken in a solution containing the bis-amine **14** (0.166 g, 0.27 mmol, 1 equiv.) and TEA (4 equiv.). The reaction mixture was allowed to stir for 12 h at rt. The insoluble product was filtered off and washed with DCM to get an off-white residue (62%) which could not be further purified owing to insolubility in various organic solvents. mp: > 295 ^{o}C, IR (nujol) ν (cm^{-1}): 3321, 3159, 2954, 2922, 2852, 2725, 1716, 1703, 1670, 1458, 1377, 1305, 1242, 1169, 1153, 1078, 1016; MALDI TOF: 1460.85 (M+H)$^{+}$, 1483.75 (M+1+Na)$^{+}$, 1497 (M+K)$^{+}$.

ESI-MS

5

(M+Na)⁺ 479.2895

(M+K)⁺ 495.26

(M+H)⁺

480.2934

481.3408 496.26

457.3583

349.2732

393.3367

m/z, amu

ESI-MS

8

(M+Na)⁺ 419.3039

(M+K)⁺ 435.2843

427.3789

420.3086

436.2889

(M+H)⁺

397.3134

m/z, amu

\...\PPY-SUZ-MONO-DHB_0001.dat
Acquired: 14:24:00, September 17, 2007

F:\...\PPY-SUZHIG-D-DHB_0001.dat
Acquired: 15:31:00, September 11, 2007

8.45
8.45
8.42
8.41
7.88
7.87
7.84

CDCL3

7.27
7.12
7.09
7.05

¹H (200MHz, CDCl₃)

2

├─ 9.5 9.0 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 ─┤

7.98
7.97
7.94
7.93
7.59
7.55
7.51
7.43
7.42
7.40
7.39
7.26
6.94
6.93
6.59
6.54
6.49

3.85
3.82
3.81

¹H NMR (200 MHz, CDCl₃)

MeO   OMe

MeOOC   COOMe

5

├─ 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 ─┤

¹H NMR (200 MHz, CDCl₃)

8

¹H NMR (400 MHz, CDCl₃)

11

^{13}C NMR (50 MHz, CDCl$_3$)

166.41, 157.26, 138.5, 135.30, 134.99, 132.53, 130.57, 128.80, 125.10, 118.50, 111.15, 77.63, 77.00, 76.37, 56.02, 51.79

5

DEPT-135 (50 MHz, CDCl$_3$)

135.10, 133.55, 132.64, 130.67, 128.90, 125.21, 111.39, 111.26, 56.26, 56.12, 51.89

5

^{13}C NMR (50 MHz, CDCl$_3$)

8

189.33, 159.86, 138.07, 137.33, 135.54, 135.22, 130.55, 128.95, 125.11, 123.17, 110.70, 77.63, 77.00, 76.36, 55.66

DEPT-135 (50 MHz, CDCl$_3$)

189.33, 137.33, 130.54, 128.94, 125.10, 110.70, 55.66

8

163.11
155.39
152.49
150.79
150.14
140.86
138.69
136.67
135.59
135.29
133.22
130.56
128.80
125.17
119.37
110.56
108.85
107.72
81.09
77.35
77.03
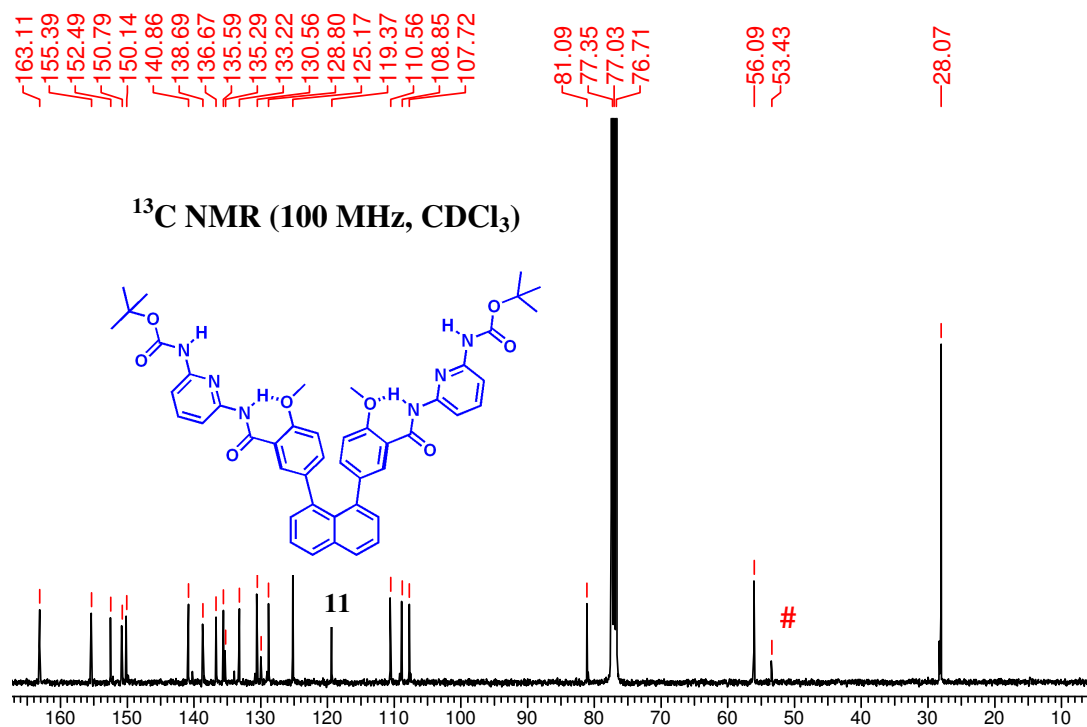76.71
56.09
53.43
28.07

¹³C NMR (100 MHz, CDCl₃)

11

#

the peak at 53.43 ppm corresponds to DCM in the crystal lattice

140.88
135.61
133.22
130.57
128.81
125.19
110.57
108.85
107.73
56.09
28.07

DEPT-135 (100 MHz, CDCl₃)

11
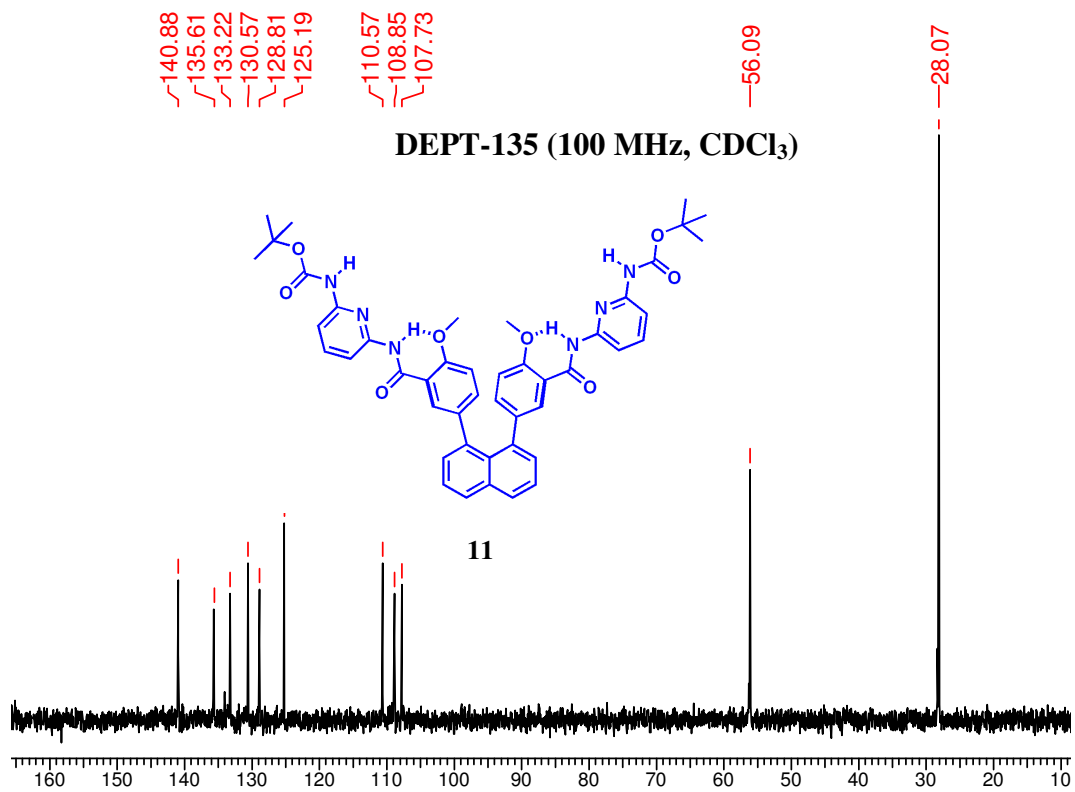
Table 11: Titration study of 11 in CDCl$_3$ (V$_{DMSO-d6\ added}$ = 0-50 μ lit)

No	V$_{DMSO-d6}$ (in μl)	δ_{NH2}
1	0	10.05
2	5	10.05
3	10	10.05
4	15	10.05
5	20	10.05
6	25	10.05
7	30	10.05
8	35	10.05
9	40	10.05
10	45	10.05
11	50	10.05
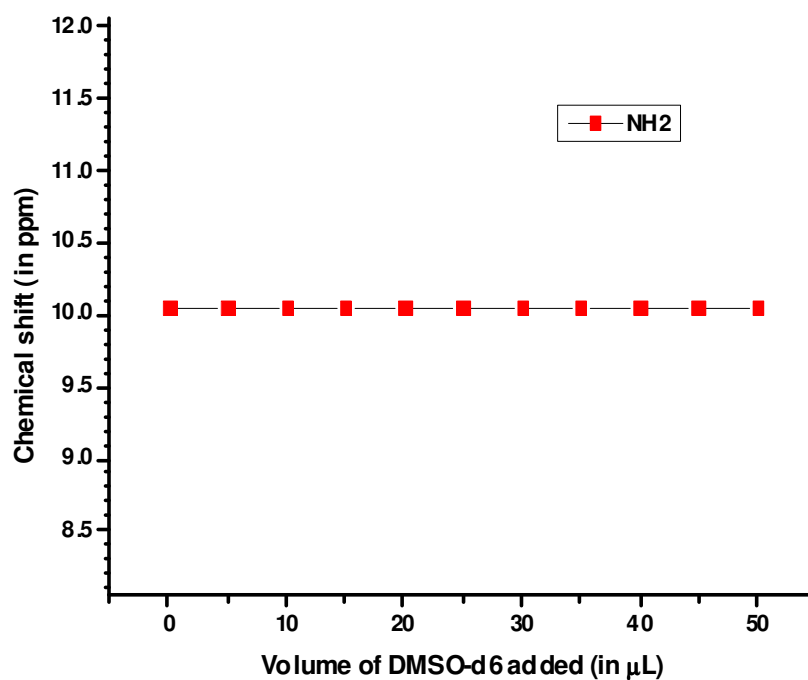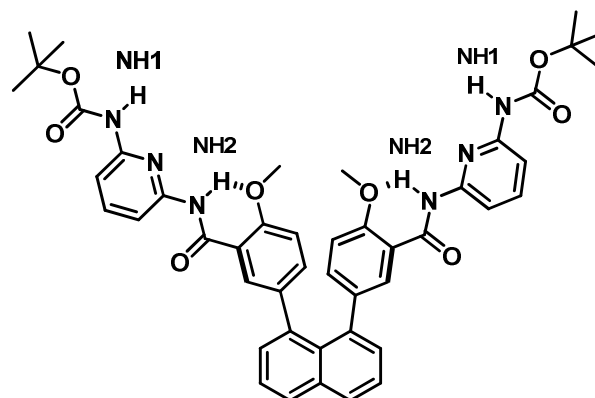
3.13 References and notes

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