CHAPTER 2

Conformationally rigid two-dimensional abiotic foldamer building blocks
2.1 Foldamer-An overview

Folding and assembly of biomolecules are two of the most significant characteristics observed in the area of biomolecular science. The DNA-duplex formation till date symbolizes one of the most elegant and superlative examples of both self-assembly and folding in biomacromolecules.\(^1\) Nature employs the twenty amino acid-set to generate a collection of biomolecules with diverse structures and functions. Structural and conformational analyses of biopolymers reveal that most of the biological events result from their stable compact conformation, stabilized by a set of non-covalent interactions.\(^2\) As proposed by Linderstrom-Lang,\(^3\) proteins have structures at several different levels (Fig. 2.1). The primary structure of a protein consists of the order in which amino acids are linked to one another by peptide bonds. The secondary structure involves the way by which chain of amino acids twists or folds to form either α-helical, β-sheet or a variety of other possible arrangements.\(^4\) An assembly of secondary structure is referred to as the tertiary structure, and is responsible for the bioactivity of proteins. If a protein consists of more than one chain, the shape in which those separate chains bind together by non-covalent interaction is referred to as the quaternary structure.\(^5\) The combined shape of the secondary, tertiary and the quaternary structure, if there is one, makes up the conformation of a protein.\(^6\)

The three-dimensional conformations of biopolymers may have information-rich surfaces, which in fact are responsible for different biological processes.\(^7\) Biological machines play a multitude of roles in the living organisms such as molecular recognition, information storage, biocatalysis (enzymes), transmission of signals (hormones) etc. These mysterious events have always been a fascination to chemists and biochemists, and in an attempt to mimic these bio-
machineries, both structurally and functionally with man-made constructs, small molecules were designed and developed.

Fig. 2.1 The linear sequence of amino acids (primary structure) folds into helices or sheets (secondary structure) which pack into a globular or fibrous domain (tertiary structure). Some individual proteins self-associate into complexes (quaternary structure).

In this context, an area of research that has attained considerable attention in recent years is foldamers which are synthetic oligomers that mimic the conformational features of biopolymer. The term “foldamer” coined by Prof.
Gellman describes *any oligomer with a strong tendency to adopt a specific compact conformation*. The term *compact* is often used to describe the tertiary structure of proteins. Because there are very few synthetic polymers that display a specific tertiary structure, the primary step in designing a foldamer is therefore to identify new backbones with well-defined secondary structural preferences. By utilizing diverse synthetic tools, this ‘bottom up’ approach may help to engineer new frameworks that may be molded to mimic the structure and functions of the biopolymers. The scope and feasibility of this concept is reflected in the exponential growth from its foundation in the early 20th century to the present stage, as would be evident from the recent literature. Although foldamer chemistry started with the modification of natural α-peptides with their β- and γ-counterparts, a wide range of backbones have been developed and reported later.

2.2 Classification of foldamers

Based on the nature of the backbone, foldamers can be classified into two broad categories: biotic (or bio-inspired foldamers) and abiotic (or synthetic foldamers). Wherein most biotic foldamers chemically resemble proteins (Fig. 2.2), the majority of the abiotic foldamers include aromatic rings like Amb (2-aminomethyl benzoic acid), Adb (3-amino-4,6 dimethoxy benzoic acid), anthranilic acid (2-amino benzoic acid) etc. in the backbone (Fig. 2.3). Recent advancements relating to bio-inspired foldamers include elucidating the sequence specificity and stability of various secondary structures in solution and generating tertiary structures like helix bundles. Abiotic foldamer research has mainly been focused on designing the basic building block to modulate the structure of oligomers. Conformationally constrained building blocks which have obvious
edge over the flexible counterparts have been utilized in biotic as well as abiotic foldamers.

**Fig. 2.2** Selected examples of biotic (bio-inspired) foldamers.
Fig. 2.3 Selected examples of abiotic foldamers.
2.3 Diversity at the building block level

Intensive research in the field of peptidomimetics during the last decade has been proven fruitful with researchers in quest of alternative building blocks that can mimic the structure and function of peptides. It has been well established that a slight change in the backbone or the side chain of these residues generate a myriad of structures which is still counting. With ever-increasing number of building blocks available in the armory of foldamer design, many unseen structures and functions are waiting to be discovered. Given below, under appropriate sub-headings, are some of the selected building blocks used to generate diverse class of foldamers.

2.3.1 β-Peptides

β-amino acid is one of the most utilized building blocks for peptidomimetic foldamers. It has an additional methylene group in between the amine and acid counterparts. Although minute, it opens up a new dimension in the structural architecture of the designed molecule. The chemical similarity with α-peptides and higher proteolytic stability in vitro and in vivo has inspired many researchers to design well-defined secondary and quaternary structures using β-peptide as a scaffold. The field of β-peptide foldamers has been established by the research groups of Seebach and Gellman. Some of the frequently used β-amino acid building blocks include 2-aminocyclopentanecarboxylic acid (ACPC), 2-aminocyclohexanecarboxylic acid (ACHC) and 2-amino benzoic acid (anthranilic acid) (Fig. 2.4 A). A foldamer reported by our group that displays an unusual periodic pseudo β-turn network of 9-membered ring...
hydrogen-bonded network formed in the forward direction of the sequence by 1→2 amino acid interactions both in solid-state as well as in solution is depicted in Fig. 2.4B.

![Diagram of hydrogen-bonded network](image)

Fig. 2.4 Selected examples of β-peptide building blocks (A) and an oligomer featuring anthranilic acid as one of the building blocks (B).

2.3.2 γ-Peptides

γ-Peptides are the higher homologues of β-Peptides. In spite of receiving less attention, oligomers of γ-peptides often display versatility as compared to that of β-counterparts. The conventional β-turn secondary structure (C₁₀ turn) with an αα segment can be expanded to C₁₂ in a αγ counterpart. For instance, 1-(aminomethyl)-cyclohexaneacetic acid, “gabapentin” (Gpn) (Fig 2.5A) is a conformationally constrained γ-amino acid, extensively investigated by Balaram’s group in recent years for designing peptides with diverse secondary structures. Gellman’s group developed a cyclically constrained chiral γ-amino acid building block, featuring side chains, which was used for developing αγ
heterogeneous peptides, which showed clear preference for C12 H-bond pattern in solid as well as in the solution state (Fig 2.5B).

![Fig. 2.5 Selected examples of γ-peptide building blocks (A) and an αγ oligomer developed from alanine and cyclically constrained chiral γ-amino acid building blocks (B).](image)

2.3.3 δ-Peptides

δ-Peptides are the isosteric replacements of dipeptide units. β-turn is a common structural feature of proteins associated with the dipeptide fragment. Obviously, most of the work in the δ-peptide family has been intended to create a β-turn mimic. Most of the research in this area involves carbopeptoid backbones and the idea of using carbohydrate (sugar) amino acids for peptidomimetics has gained immense popularity in the recent past. The conformational preference of sugar residues incorporated into peptide chains were initially exploited for the rational design of a β-turn mimetic. Eventually, it was understood that appropriately linked sugar amino acids can serve as a dipeptide replacement. The research groups of Fleet, Chakraborty, Fuchs and many others have put an extensive effort in generating diverse class of sugar amino acids (SAAs) with varying preferences for secondary structure formation. Selected examples of such
building blocks and a peptidomimetic containing 3,4-dideoxy furanoid sugar amino acid are shown in Fig. 2.6.

Fig. 2.6 Selected examples δ-peptide building blocks (A) and a peptide featuring furanoid amino-acid (B).

2.3.4 Aminoxy acids

In contrast to the amino acids devoid of backbone oxygen, the N-O bond in aminoxy acids demonstrates unusual torsional characteristics because of the repulsion between the lone pairs of electrons on the CO and O atoms. As a result, the backbone of aminoxy peptides is endowed with extra rigidity. Oligomers comprising α-, β- and γ-aminoxy acids have been shown to form several well-defined secondary structures. Introduction of α-aminoxy acid in a peptide chain strongly favors secondary structures stabilized by 8-membered H-bond featuring i→i+3 C=O···NH (N-O turn, Fig. 2.7) – which is not very common. As compared to α-aminoxy peptides, the β-counterpart have an extra carbon atom in the backbone, which offers a greater variation in the substitution pattern. Interestingly, a peptide containing alternating L-aminoxy valine and D-alanine has
been shown to adopt a secondary structure wherein γ-turn can be initiated by a succeeding α-N-O turn (Fig. 2.7).45

![Chemical structures](image)

**Fig. 2.7** α- and β-aminoxy acids and the characteristic N–O turns and a peptide containing L-aminoxy valine.45

2.3.5 Azapeptides

When the β^3^-carbon of a β-peptide is replaced by a trivalent nitrogen atom, we obtain an azapeptide. This little change brings about a marked difference in the H-bonding preferences of the backbone. Similarly, as for the aminoxy peptides, the structure is organized by short range turn-like H-bonded rings. Azapeptide homo-oligomers tend to achieve strand structures that rely on a framework of hydrazino turns (Fig. 2.8A).46 The β^3^-peptide foldamer shown in Fig. 2.8B prove that the backbone organization consistently depends on a framework of consecutive eight-membered hydrogen-bonded pseudocycles.47 These findings were also supported by solid and solution state studies.

![Chemical structures](image)

**Fig. 2.8** An azapeptide moiety and hydrazino turn (A)46 and an aza- β^3^-peptides foldamer (B).47
2.3.6 Oligoureas

Oligoureas are obtained when the \( \gamma \)-carbon in the \( \gamma \)-peptide family is replaced with nitrogen. The urea functionality in itself is known to be one of the strongest H-bond mainstays, and this is augmented by the likelihood of bifurcated H-bonding and consequently restricted flexibility of the backbone. Recent studies reveal that oligoureia sequences can adopt a 14-helix (Fig. 2.9B) suggestive of the \( \gamma \)-H14 helix. The folding of these structures is robust enough to allow further applications. Also, given below is an example of an oligoureia (Fig. 2.9C), wherein the hydrogen-bonding between the heteroatom on the aromatic ring and the backbone NH has been utilized to a great extent in the design of foldamers with pre-disposed conformation.

![Fig. 2.9 A urea moiety (A) and oligoureas (B, C).](image)

2.3.7 Peptoids

Peptoids or N-substituted glycines are a class of peptidomimetic compounds wherein the nitrogen atom of the peptide bond is protected. Application of peptoids in biology is immense. The group of Taillefumier and Edwards first reported the synthesis and structural investigations of a linear and cyclic \( \alpha/\beta \)-alternating peptoids. Given is an example of peptoid foldamer reported by Kirshenbaum’s group (Fig. 2.10B) to explore the possibilities to augment conformational ordering of N-substituted glycine oligomers which
describes the use of N-aryl side chains as an instrument to enforce the presence of trans-amide bonds, thereby ensuring structural order.

Fig. 2.10 Comparison of peptide and peptoids structures (A), and a peptoid foldamer (B) reported by Kirshenbaum’s group.52

2.3.8 Foldamers based on heterocyclic skeleton

Foldamers based on heterocyclic skeleton have regularly been used to generate well defined secondary structures.53 Our efforts in this direction culminated in the development of a foldamer having a structural feature that adopts co-facial architecture (Fig. 2.11A), using diamino-pyridine as one of the building blocks.53d Stable helical conformations, even in a polar solvent like methanol were feasible with aromatic oligoamides derived from 1,10-phenanthroline diacid and O-phenylenediamine as reported by Chen and co-workers (Fig. 2.11B).54 Based on extensive spectroscopic studies, these conformations have been shown to be stabilized through a combination of intramolecular hydrogen-bondings and aromatic π−π stacking interactions. Also given is an example of aromatic oligomer reported by Hamilton and co-workers that is composed of anthranilic acid and 2,6-pyridine dicarboxylic acid55 (Fig. 2.11C). A drastic change in conformation from linear to helical was observed upon incorporation of pyridine-2, 6-dicarboxylic acid unit.
Fig. 2.11 Selected examples of foldamers which use heterocycles as building blocks.

2.4 Applications of foldamer

Due to their structural tunability and stability, foldamers can be potential candidates for diverse applications. Helical foldamers with hollow cavity can find applications in host-guest chemistry, drug delivery, catalysis, chemical transformation etc. β-sheet foldamers can provide insights into various β-sheet-mediated diseases. Due to its dynamic nature, foldamers are suited for the design of stimuli-responsive materials that can respond to temperature, chemicals, pH, light, etc. For instance, the photoswitchable trans-azobenzene-incorporated amphiphilic oligo-(m-phenylenes) shows considerable promise for developing photo-responsive materials. Some of the promising foldamers that act as molecular receptors, sensors, biomimetics, catalysts etc. are given in Fig. 2.12.
2.5 Objective of the present work and design strategy

Alteration of the amino acid order and torsional parameters of the individual units have been shown to have a marked influence on the overall conformation and structural architecture (shape) of biopolymers. In the last decade, a sizable amount of work has been done in this area of foldamers to obtain novel molecular architectures with wide range of applications (vide supra). In this regard, the synthetic oligomers may provide an excellent starting point for the elaboration of peptide mimics that could be developed only with difficulties on the basis of small-molecule scaffolds. Herein, our endeavor was to develop building blocks which may lead to foldamers that would be structurally different and unique in their conformation. Although there are innumerable unnatural amino
acids and other building blocks reported in the literature, aromatic building blocks with two-dimensional orientations of the chain propagating groups appended on conformationally rigid framework suitable for foldamer generation have not yet been explored. Positioning of the chain propagating groups on a rigid aromatic framework can be expected to show a marked influence on the overall shape of the oligomers containing such building blocks. Furthermore, such a strategy could furnish synthetic oligomers with dazzling structural architectures.\(^{63}\)

![Design strategy to synthesize conformationally rigid two-dimensional foldamer building blocks.](image)

Fig. 2.13 Design strategy to synthesize conformationally rigid two-dimensional foldamer building blocks.

Considering the high conformational rigidity (because of the presence of spiro-annulated rings that restricts the rotation of aryl rings) and the ease of synthesis, we anticipated that 1,1'-spirobiindane building block (Fig. 2.13) would be a useful basic unit in the design and development of synthetic oligomers with unique structural architectures. Moreover, the introduction of H-bond directing alkoxy groups on the aromatic rings would exert directional effect for H-bonding interactions and hence would control the conformational feature of the oligomers. Furthermore, introducing the chain propagating groups, such as amino (NH\(_2\)) and carboxylic acid (CO\(_2\)H), ortho to the alkoxy groups followed by coupling would result in the formation of oligomers.
2.6 Synthesis of two-dimensional building blocks

2.6.1 Conformationally rigid two-dimensional amino acid

The spirobiindane-based amino acid building block 1 was synthesized starting from\(^{63b}\) spirobiindane bis-ether 4 (Scheme 1), obtained in quantitative yield by the exhaustive methylation of the known spirobiindanol 3, was subjected to Friedel–Crafts acylation-haloform reaction sequence to introduce the carboxyl group on the aromatic framework. This procedure for the installation of the carboxyl group on the aryl rings ortho to alkoxy groups was preferred over a possible metal directed \((O\)-lithiation\)-one-step carboxylation procedure, due to the anticipated difficulties associated with the latter procedure, particularly when working on a larger scale. The nitro derivative 6, obtained by the careful nitration of 5, was subjected to haloform reaction to afford the nitro acid 7, which after catalytic nitro reduction readily furnished the novel amino acid 1 in an overall yield of 16% starting from 3.

Scheme 2.1 Synthesis of building block 1

Reagents and conditions: (i) CH\(_3\)SO\(_3\)H, rt, 96 h, 67%; (ii) Me\(_2\)SO\(_4\), K\(_2\)CO\(_3\), acetone, reflux, 8 h, 96%; (iii) CH\(_3\)COCl, SnCl\(_2\), DCM, -10 °C, 1.5 h, 70%; (iv) HNO\(_3\), H\(_2\)SO\(_4\), CH\(_3\)COOH, 2 min, 60%; (v) NaOCl (4%), NaOH (50%), dioxane, 12 h, 69%; (vi) HCOONH\(_4\), Pd-C, MeOH, reflux, 6 h, 62%.
2.6.2 Conformationally rigid two-dimensional abiotic foldamer building block

Scheme 2.2 Synthesis of building block 2

Reagents and conditions: (i) CH$_3$COCl, SnCl$_4$, DCM, rt, 24h, 94%; (ii) NaOCl (4%), NaOH, dioxane, 24h, 76%; (iii) K$_2$CO$_3$, acetone, reflux, 8h, 90%; (iv) HNO$_3$, Ac$_2$O, 0 °C, 1.5 h, 50%; (v) H$_2$, Pd-C, 60 psi, 8h; (vi) LiHMDS, Boc$_2$O, THF, rt, 3h, 45%; (vii) (COCl)$_2$, DCM, DMF(cat), 3h; (viii) Et$_3$N, THF, reflux, 5h, 90%.

The foldamer building block 2 was synthesized starting from bisphenol A by one-step acid-mediated rearrangement followed by exhaustive methylation of the spirobiindanol to obtain 3 (Scheme 2.2). To get the bis-acid 9, the bis-alkoxy derivative 3 was first subjected to Friedel-Craft’s acylation to deliver bis-acylated building block 8, followed by the haloform reaction. The amine counterpart 13 was prepared starting from commercially available p-cresol which on O-methylation and nitration afforded the di-nitro analog 11 in 50% yield. On catalytic hydrogenation, 11 afforded reduced product 12 in quantitative yield, which was subjected to mono Boc protection of one of the amine functionalities in presence of one equivalent of Boc-anhydride and LiHMDS to furnish the amine.
counterpart 13 in 45% yield. Bis-acid chloride derivative was obtained by reacting the bis-acid 9 with oxalyl chloride, which was then reacted with two equivalents of mono-amine 13 to obtain the foldamer building block 2 in 90% yield.

2.7 Single crystal X-ray diffraction studies of building block 1

Extensive efforts to grow crystals of the conformationally restricted aromatic amino acid building block culminated in the formation of crystals of 1 (Fig. 2.14A). Investigations reveal that in the spirobiindane based unnatural aromatic amino acid 1, the amine and acid groups are oriented in such a way that they lie in two different planes, reversing the growth of the backbone by 83.5° (Fig. 2.14B). Furthermore, intra-residual hydrogen bonding between the chain propagating amino and carboxylic acid functionalities and the adjacent methoxy groups help the backbone to attain additional rigidity, as anticipated.

![Fig. 2.14 Crystal structure of 1 exhibiting the two-dimensional orientation of the chain propagating groups (A), and the ring bearing planes at an angle of 83.5° (B).](image)

2.8 Conclusion

Summarizing our results, we have developed two novel conformationally rigid foldamer building blocks, wherein the chain propagating groups, embedded
on the aryl rings, are projected in an anti-periplanar arrangement (two-dimensional arrangement). Oligomers containing such building blocks are expected to have overwhelming ‘conformational ordering’, facilitating ease of characterization. Structural investigations of \( \text{I} \) by single-crystal X-ray studies provided clear evidence for the two-dimensional orientation of the chain propagating groups. The strategy disclosed herein for the construction of conformationally restricted building blocks would be useful for the construction of oligomers displaying novel molecular architectures with conformations distinctly different from those classically observed.
2.9 Experimental Section

Crystal data for 1: C_{24}H_{29}NO_{4}, $M = 395.48$, Pale yellow colored crystals, approximate size 0.50 x 0.48 x 0.07 mm, Multiscan data acquisition. Total scans = 3, total frames = 1818, Oscillation / frame -0.3°, exposure / frame = 15.0 sec / frame, 0 range = 2.24 to 25.00°, completeness to 0 of 25.00 ° is 99.8 %. Crystals belong to Monoclinic, space group P 21/n, $a = 10.881 \, (7), \, b = 17.932 \, (11), \, c = 11.015 \, (7) \, \text{Å}, \, \beta = 104.754 \, (10)°, \, V = 2078 \, (2) \, \text{Å}^3, \, Z = 4, \, D_c = 1.264 \, \text{mg m}^{-3}. \mu (\text{MoK}α) = 0.085 \, \text{mm}^{-1}, \, T = 297(2) \, \text{K}, \, 14637 \, \text{reflections measured, 3648 \, [R(int) = 0.0488] \, unique \, [I>2\sigma(I)], \, R \, value \, 0.0912, \, wR2 = 0.2283.}

5-Acyl-6, 6'-dimethoxy-3, 3', 3', 3'-tetramethyl-1, 1'-spirobiindane 5:

To a stirred solution containing 6,6'-Dimethoxy 3,3',3',3'-tetramethyl-1,1'-spirobiindane 4^{6b} (5 g, 14.8 mmol, 1 equiv.) in dichloromethane (50 mL) at -10 °C, acetyl chloride (1.27 mL, 17.8 mmol, 1.2 equiv.) was added drop wise for 10 minutes, followed by SnCl$_4$ (3.48 mL, 29.7 mmol, 2 equiv.). The reaction mixture was allowed to stir at -10 °C for one and half hour, diluted with dichloromethane (50 mL), acidified with dil. HCl, and the product was extracted into the organic layer. Drying and purification by column chromatography furnished 5. Yield: 3.72 g (66%); mp: 116-118 °C; IR (CHCl$_3$) ν (cm$^{-1}$): 3299, 3236, 3020, 2958, 1668, 1602, 1485, 1465, 1404, 1217, 1155; $^1$H NMR (200 MHz, CDCl$_3$) δ: 7.58 (s, 1H), 7.09 (d, $J = 8.33$ Hz, 1H), 6.84-6.79 (dd, $J_1 = 2.40$ Hz, $J_2 = 5.94$ Hz, 1H), 6.37 (s, 1H), 6.30(d, $J = 4.27$ Hz, 1H), 3.74 (s, 3H), 3.70 (s, 3H), 2.60 (s, 3H), 2.40-2.21 (q, 4H), 1.39(d, $J = 1.39$ Hz, 6H), 1.33(d, $J = 1.64$ Hz, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ: 200.06, 159.45, 159.31, 156.75, 151.19, 144.62, 144.61, 127.84, 123.79, 122.59, 113.57, 108.97, 107.12, 59.49, 59.39, 58.14, 55.70, 55.45.
5-Acyl-5'-nitro-6,6'-dimethoxy-3,3,3',3'-tetramethyl-1,1'-spirobiindane 6:
To a solution of 5-Acyl-6, 6'-dimethoxy-3, 3', 3'-tetramethyl-1, 1'-spirobiindane 5 (3.5 g, 9.25 mmol, 1 equiv.) in AcOH (50 mL), H2SO4 (5 mL) was added drop wise followed by the drop wise addition of HNO3 (14 mL) and stirred for two minutes. The solid residue was then filtered, washed with water and was purified by column chromatography furnishing 6 as yellow solid. Yield: 2.35 g (60%), m.p: 215-217 °C; IR (CHCl3) v (cm⁻¹): 3326, 3245, 3217, 3018, 2958, 2866, 1670, 1604, 1521, 1521, 1217, 1126, 1010; ¹H NMR (200 MHz, CDCl3) δ: 7.64 (s, 1H), 7.57 (s, 1H), 6.42 (s, 1H), 6.30 (s, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 2.60 (s, 3H), 2.44-2.17 (m, 4H), 1.42-1.40 (d, J = 2.53 Hz, 6H), 1.35-1.33 (d, J = 4.67 Hz, 6H); ¹³C NMR (100 MHz, CDCl3 & DMSO-D₆ (5%)) δ: 199.30, 158.86, 156.12, 154.55, 152.59, 144.02, 143.96, 139.06, 127.82, 123.40, 118.88, 108.47, 106.30, 58.65, 58.28, 58.03, 56.29, 55.30, 42.72, 42.64, 31.27, 31.06, 30.95, 29.79, 29.63; LC-MS: 446.22 (M+Na)⁺; Anal. Calcd. for C₂₅H₂₉NO₅: C, 70.90; H, 6.90; N, 3.31; Found: C, 71.02; H, 6.77; N, 3.54.

5-Carboxy-5'-nitro-6,6'-dimethoxy-3,3,3',3'-tetramethyl-1,1'-spirobiindane 7:
To 5-Acyl-5'-nitro-6,6'-dimethoxy-3,3,3',3'-tetramethyl-1,1'-spirobiindane 6 (1.7 g, 4 mmol, 1 equiv.), NaOCl (4% W/V) (80 ml) was added drop wise over a period of 5 minutes followed by the addition NaOH (50% W/V, 40 ml). The reaction mixture was then heated to 70 °C and stirred for 12 h, allowed to come down to room temperature and 5% HCl was added drop wise until pH = 1, extracted with ethyl acetate, dried over anhydrous Na₂SO₄ and was purified by
column chromatography. Yield: 1.17 g (69%); m.p >300°C; IR (Nujol) ν (cm⁻¹): 3296, 3242, 2923, 1733, 1616, 1458, 1377, 1220, 1130, 1049; ¹H NMR (200 MHz, CDCl₃) δ: 8.05 (s, 1H), 7.64 (s, 1H), 6.40 (bs, 2H), 3.94 (s, 3H), 3.79 (s, 3H), 2.46-2.21 (m, 4H), 1.43-1.42 (d, J = 1.26 Hz, 6H). 1.36-1.35 (d, J = 1.52 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃) δ: 171.1, 156.8, 156.5, 152.3, 149.0, 144.2, 143.3, 139.3, 122.3, 118.8, 109.0, 106.7, 58.99, 58.57, 58.13, 56.83, 55.87, 42.84, 31.59, 30.35; LC-MS: 426.08 (M+H)+; Anal. Calcd. for C₂₄H₂₇NO₆: C, 67.75; H, 6.40; N, 3.29; Found: C, 67.62; H, 6.15; N, 3.60.

5-Carboxy-5'-amino-6,6'-dimethoxy 3,3',3',3'-tetramethyl-1,1'-spirobiindane 1:
5-Carboxy-5'-nitro-6,6'-dimethoxy-3,3',3',3'-tetramethyl-1,1'-spirobiindane 8 (1g, 2.35 mmol, 1equiv) in methanol (30 ml) was subjected to the reduction of nitro group by transfer hydrogenation using ammonium formate (0.74 g, 11.7 mmol, 5 equiv) and Pd-C (10%, 0.10 g) After reflux for 6 hours, the reaction mixture was filtered over celite pad, washed with methanol (3 x 10 ml) and on purification by column chromatography gave amine 1. Yield: 0.57 g (61%); mp: 289-292 °C; IR (Nujol) ν (cm⁻¹): 3444, 3330, 3120, 2954, 2925, 2854, 2725, 1718, 1610, 1461, 1377, 1149; ¹H NMR (200 MHz, CDCl₃) δ: 8.01 (s, 1H), 6.77 (s, 1H), 6.45 (s, 1H), 6.20 (s, 1H), 3.92 (s, 3H), 3.73 (s, 3H), 2.39-2.12 (m, 4H), 1.41-1.31(m, 12H); ¹³C NMR (100 MHz, DMSO-d₆) δ: 167.85, 158.25, 156.37, 146.78, 144.30, 143.61, 137.41, 136.49, 124.22, 120.71, 107.52, 107.04, 105.64, 59.37, 59.10, 57.64, 56.13, 55.69, 42.93, 42.28, 31.77, 31.45, 30.69, 30.28; LC MS mass: 396.12 (M+H)+; Anal. Calcd. for C₂₄H₂₇NO₆: C, 72.89; H, 7.39, N, 3.54; Found: C, 72.55; H, 7.21; N, 3.78.

tert-butyl (3-amino-2-methoxy-5-methylphenyl)carbamate 13:
1 molar LiHMDS solution in THF (2.62 ml, 2.62 mmol) was added drop-wise to a
solution of compound 12\textsuperscript{(b)} (0.20 g, 1.31 mmol) in 5 mL THF at room temperature and was stirred for 10 min. To the resulting mixture a solution of Boc-anhydride (0.28 g, 1.31 mmol) in THF (5 mL) was added slowly for 5 min. Reaction mixture was then allowed to stir at room temperature for 3 h, and volatiles were evaporated. The residue was purified by column chromatography. Yield: 0.15 g (45%), mp: 117-119 °C, IR (CHCl\textsubscript{3}) v (cm\textsuperscript{-1}): 3428, 3019, 2400, 1725, 1619, 1535, 1463, 1368, 1215, 1158, 993, 758, 669; \textsuperscript{1}H NMR (400 MHz,CDCl\textsubscript{3}): \delta 7.33 (S. 1H), 6.94 (S, 1H), 6.26 (S, 1H), 3.73 (S, 3H), 3.69 (bs, 1H), 2.22 (S, 3H), 1.53 (S, 9H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \delta: 152.7, 138.7, 134.7, 133.4, 131.6, 110.9, 109.3, 80.3, 59.3, 28.3, 21.4. HRMS: C\textsubscript{13}H\textsubscript{20}N\textsubscript{2}O\textsubscript{3} Calcd. 252.1474, Found: 253.1574 (M+H)+, 275.1366 (M+Na)+.

di-tert-butyl (((6,6′-dimethoxy-3,3,3′,3′-tetramethyl-2,2′,3,3′-tetrahydro-1,1′-spirobi[indene]-5,5′-dicarbonyl)bis(azanediyl))bis(2-methoxy-5-methyl-3,1-phenylene))dicarbanilate 2:

To a solution of spirobiindane based bis-acid 9 (0.9 g, 2.12 mmol) in DCM (15 ml) containing dry DMF (cat.), oxalyl chloride (1.06 g, 0.73 ml, 8.45 mmol) was added drop-wise at 0 °C, and the reaction mixture was allowed to stir at room temperature for 3 h. The volatiles were stripped off and to a solution of mono-amine 13 (1.16 g, 4.6 mmol) in anhy. DCM (25 ml) and Et\textsubscript{3}N (1.06 g, 1.46 ml, 10.5 mmol) was added drop-wise at 0 °C. To the resulting mixture crude bis-acid chloride dissolved in anhy. DCM was added drop-wise maintaining 0 °C, which was then stirred at room temperature for 12 h. Purification of the crude material by column chromatography furnished 2 as a white solid. Yield: 1.7 g (90%), mp: 117-119 °C, IR (CHCl\textsubscript{3}) v (cm\textsuperscript{-1}): 3422, 3354, 3019, 2400, 1731, 1604, 1504, 1215, 1156, 988, 756, 699; \textsuperscript{1}H NMR (400 MHz,CDCl\textsubscript{3}): \delta 10.44 (S, 2H), 8.20 (S, 2H), 7.66 (bs, 2H), 6.45 (S, 2H), 3.94 (S, 6H), 3.79 (S, 6H), 2.46-
2.42 (d, 2H, J = 13.3 Hz), 1.54 (S, 18 H), 1.48 (S, 6H), 1.39 (S, 6H); $^1$C NMR (100 MHz, CDCl$_3$) δ: 163.3, 157.4, 155.5, 152.6, 145.6, 135.6, 135.1, 131.4,
130.8, 126.2, 121.4, 115.7, 114.5, 106.5, 106.8, 80.6, 60.4, 59.2, 58.4, 56.2, 43.2,
31.5, 30.3, 28.2, 21.8; LC MS mass: 915.61 (M+Na)$^+$; Anal. Calcd. for
C$_{51}$H$_{64}$N$_{16}$O$_{16}$: C, 68.59; H, 7.22; N, 6.27. Found: C, 68.70; H, 6.98; N, 6.51.
Chapter 2

(M+Na)^+ 435.2945

(M+K)^+ 415.2016

(M+H)^+ 373.2416

(M+Na)^+ 457.2714

ESI-MS

ESI-MS

Chapter 2

ESI-MS

![Diagram of chemical structures with ESI-MS spectra](image)

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Chapter 2

HRMS

\[ \text{C}_{13} \text{H}_{21} \text{O}_{3} \text{N}_2 = 253.1547 \]
\[ 0.3984 \text{ ppm} \]

\[ \text{C}_{13} \text{H}_{20} \text{O}_{3} \text{Na} = 275.1366 \]
\[ -0.0619 \text{ ppm} \]

LCMS

\[ (\text{M}+\text{Na})^+ \]
\[ 915.61 \]
Chapter 2

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

![NMR spectrum of compound 5](image)

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

![NMR spectrum of compound 6](image)
Chapter 2

Chloroform-d

1H NMR (CDCl₃, 200 MHz)

# Dichloromethane

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

$^1$H NMR (CDCl$_3$, 400 MHz)
Chapter 2

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

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

$^{13}$C NMR (CDCl$_3$ & DMSO-D$_6$, 100 MHz)

DEPT-135 (CDCl$_3$ & DMSO-D$_6$, 100 MHz)
Chapter 2

$^{13}$C NMR (DMSO-$d_6$, 100 MHz)

**DEPT-135 (DMSO-$d_6$, 100 MHz)**
$^{13}$C NMR (CDCl$_3$, 100 MHz)

\[ \text{H}_2\text{N} \quad \text{NHBoc} \]

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

\[ \text{H}_2\text{N} \quad \text{NHBoc} \]
\[ ^{13}C \text{NMR (CDCl}_3, \text{ 100 MHz)} \]

\[ \text{DEPT-135 (CDCl}_3, \text{ 100 MHz)} \]

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2.10 References and notes


(2) A. Karshikoff, Non-covalent interactions in proteins, Imperial College Press, United Kingdom, 2006.


