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Protecting group assisted structural diversity: β-sheet to rhombus-shaped structure of a short aromatic γ-peptide

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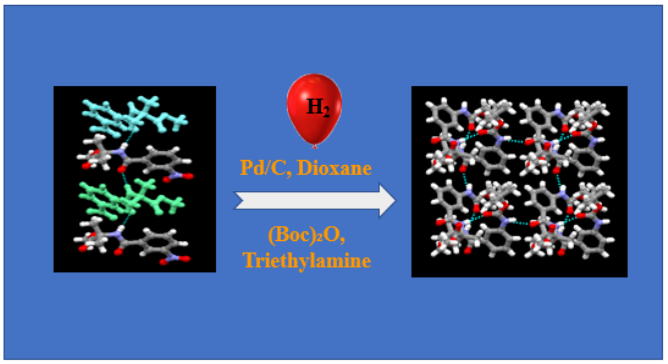
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Abstract

In this paper, the effect of the Boc-protecting group was investigated on the structure and self-assembly propensities of the hybrid dipeptide Mnba-Aib-OMe **1**. Dipeptide motif Mnba-Aib-OMe, **1** was converted into Boc-Maba-Aib-OMe, **2** by a one-pot nitro to amine reduction followed by incorporation of the Boc-group. The single crystal X-ray studies reveal that the dipeptide **1** self-associates to form a β-sheet structure. But modification of the nitro group into Boc-capped amine group converts the β-sheet structure into a dimeric unit, which further self assembles to form a supramolecular rhombus-shaped matrix.



Keywords: Protecting group, β-sheet, rhombus-shaped structure, γ-peptide, N-terminus modification.

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Introduction

Protective groups[1] play an important role during the synthesis of a complex molecule having lots of functionalities. In peptide chemistry, they have significant effect on the structure, function as well as the self-assembly pattern of short synthetic peptides[2]. Generally, incorporation of acid-labile Boc-protecting group increases the crystallinity[3] of a peptide. Not only that, it also increases the hydrophobicity of the peptide. It provides an additional hydrogen bond donor site into a peptide. Again, base-labile Fmoc-protecting group increases the gelatinous property and helps the peptides to be fluorescent.[4] Cbz, Troc, Alloc etc are other N-terminus protecting groups having their individual characteristic features.

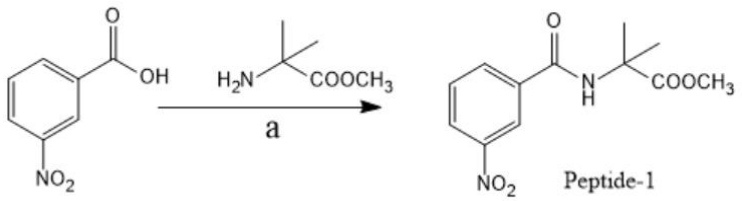
Conversion of secondary structure of a protein or peptide to other structure is highly important as it is associated with many human diseases like Alzheimer's disease, Parkinson's disease etc.[5] Therefore, modulation of secondary structure of a peptide or protein into other structure is of great interest. Because, these misfolding related diseases may be inhibited by the modulation of the secondary structure of the misfolded proteins or peptides.[6] The easiest method of modulation of peptide or protein secondary structure is minor chemical modification of functionalities of the related proteins or peptides.[7]

Intriguing this knowledge, we previously investigated the self-assembly propensities of a rigid dipeptide Boc-Maba-Aba-OMe by functional group modification. The N-terminus modification of the dipeptide leads to structural transformation from anti-parallel to parallel β-sheet secondary structure[8]. Now, we want to explore our experience with another dipeptide to generalize our observation. Herein, we have designed and synthesized a dipeptide motif Mnba-Aib-OMe, **1** consists of a conformationally constrained m-nitro benzoic acid (Mnba) and α-aminoisobutyric acid (Aib) residues. The aromatic pro-amino acid Mnba was introduce with the assumption that it will increase the conformational rigidity of the molecule and Aib residue will enhance the crystallinity of the peptide. The dipeptide motif Mnba-Aib-OMe, **1** was converted into Boc-Maba-Aib-OMe, **2** by a one-pot functional group modification technique. The single crystal X-ray studies reveal that the dipeptide **1** self-assembles to form an anti-parallel β-sheet structure. But modification of the nitro group into Boc-capped amine group converts the sheet-like structure into a dimeric unit, which further self assembles and forms a supramolecular rhombus-shaped structure.

Results and Discussion

The nitropeptide **1** was synthesized by conventional solution phase methodology using commercially available starting material 3-nitrobenzoic acid and 2-aminoisobutyric acid (Aib). C-terminus of Aib was protected as a form of methyl ester. 3-Nitrobenzoic acid was dissolved in dry DCM in an ice-water bath. H-Aib-OMe was isolated from the corresponding methyl ester hydrochloride by neutralization and subsequent extraction with ethyl acetate. It was then added to the reaction mixture, followed by immediate addition of dicyclohexyl carbodiimide (DCC) and HOBt. The reaction mixture was allowed to come to room temperature and stirred for 48 hrs. After the extraction and purification, peptide **1** was obtained as a yellowish-white solid with high yield (88%). The product was characterized by ^{1}H-NMR, ^{13}C-NMR, FT-IR and mass spectrometry (MS) analysis.

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Scheme 1. Reactions and conditions: (a) DCM, DCC/ HOBt, 0 ^{0}C, 48 h.

Peptide **1** was designed with the assumption that the incorporation of the hydrophobic 2-aminoisobutyric acid (Aib) will help the self-assembly process and increase the crystallinity of the molecule. The structure of the- nitropeptide **1** has been characterized by x-ray crystallography. Colorless monoclinic crystals of compound **1** suitable for X-ray diffraction studies were obtained from their methanol-water solution by slow evaporation.[10] From methanol-water solution, compound **1** crystallizes with one molecule in the asymmetric unit (Figure 1). Interestingly, the torsion angles around the m-nitrobenzoic acid residues (ψ1 = 156.50°) and Aib (ϕ2 = 50.69°, ψ2 = 50.70°) appears to play a critical role in pointing the overall backbone structure of peptide **1**. The torsion angles are listed in Table **1**. In higher order assembly, the compound **1** forms anti-parallel sheet like structure through intermolecular hydrogen bonding interactions along crystallographic a direction. The hydrogen bonding parameters are given in Table 2.

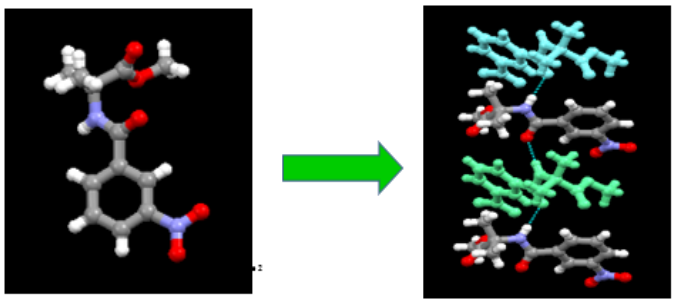
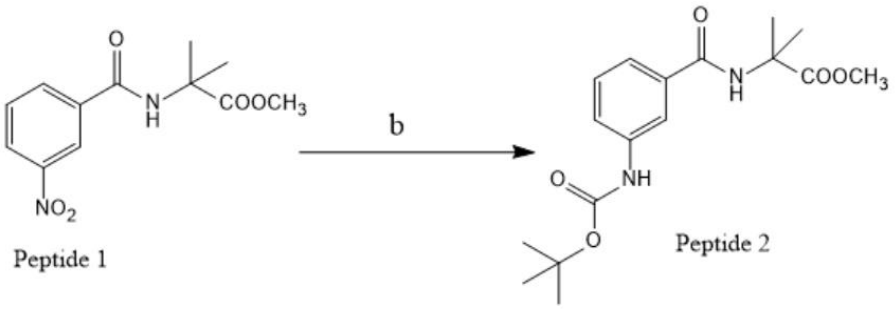


Fig. 1: (a) The molecular conformation of peptide **1** in the solid-state and (b) The solid-state structures of peptide **1** showing hydrogen bonded anti-parallel β-sheet structure. The intermolecular hydrogen bonds are represented as dotted lines.

N-terminus of peptide **1** was modified by a one-pot methodology. Peptide **1** was dissolved in dioxane solvent and the nitro group of the peptide backbone was reduced using hydrogen gas as a reducing agent and Pd/C as a catalyst. The reduction was completed within 6 hours. The completion of the reduction was monitored by TLC. After the completion of the reaction, hydrogen gas ballon was removed and without further extraction of the reaction mixture, ditertiarybutylpyrocarbonate and triethylamine were added into it. The reaction was completed within 6 hours. After the extraction and purification peptide **2** was obtained as a off-white solid with moderate yield (75%). Peptide **2** was fully characterized by ^{1}H-NMR, ^{13}C-NMR, FT-IR and mass spectrometry (MS) analysis.

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Scheme 2. Reaction and condition: (b) Dioxarane, Pd/C, H_2, RT, 6 h then $(BOC)_2O$, Et_3N, RT, 6 h.

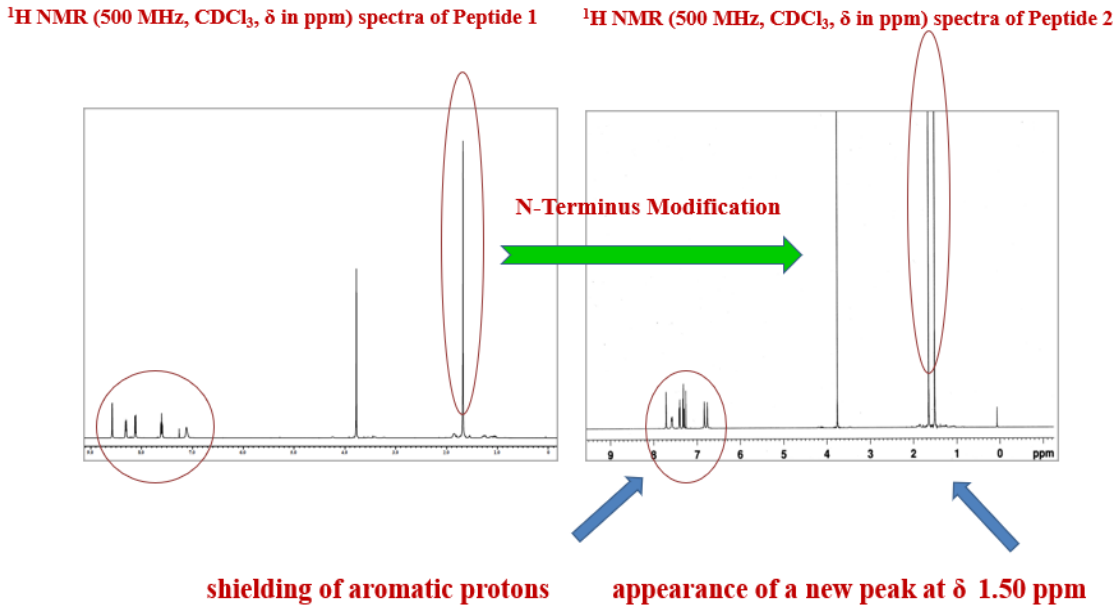


Figure 2. Conformation of one-pot N-terminus modification reaction by ^{1}H NMR spectroscopy**.**

Colourless monoclinic crystals of peptide **2** suitable for X-ray diffraction studies were acquired from ethylacetate solvent by slow evaporation technique.[13] From the crystal structure of peptide **2,** it is clear that, due to internal rigidity, a kink-like structure was adopted by the peptide molecule (Fig. 3a). Two molecules of peptide **2** are interlinked by mutually hydrogen bonding between Boc C=O and Aib(2) N-H. Thus, two molecules of peptide **2**, form a dimeric unit along the crystallographic a-axis (Fig. 3b). The dimer is further stabilized by a T-shaped π-stacking interaction (C–C distance 4.36 Å). These types of four dimeric unit of peptide **2** are interlinked through four intermolecular hydrogen bonds between N1–H1…O3 to form a supramolecular rhombus-shaped matrix in higher order packing along the crystallographic a- and b- directions (Fig. 4). There is another T-shaped π-stacking interaction with C–C distance 4.59 Å in the matrix where the benzene rings are mutually perpendicular to each other. The intermolecular hydrogen bonding parameters of peptide **2** are given in table 2.

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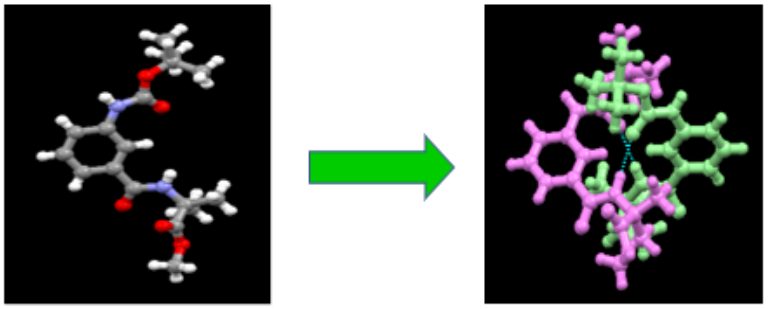


Figure 3. (a) The molecular conformation of peptide **2** in the solid state and (b) the hydrogen bonded dimer. Hydrogen bonds are shown as dotted lines.

Table 1. Important torsion angles of dipeptide **1** and **2**.

Peptide	ϕ_1	ψ_1	ϕ_2	ψ_2
1	170.06	156.5	50.69	50.70
2	23.6	22.9	-57.1	147.4

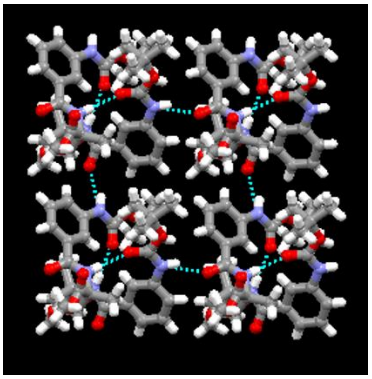


Figure 4. The intermolecular hydrogen bonded supramolecular matrix structure of peptide **2**. Hydrogen bonds are shown as dotted lines.

Table 2. Hydrogen bonding parameters of peptide[a] 1 and 2

Peptide	D-H…A	H…A (Å)	D…A (Å)	D-H…A (°)
1	N2-H2A…O3	2.008	2.868	177.55
2	N1–H1B…O3	2.17	2.96	153
	N2–H2C…O2	2.14	2.97	163

[a]Symmetry equivalent: A= -1/2+x, 1/2-y, -1/2+z; B= 3/2-x, -1/2+y, 1/2-z; C= 2-x, y, 1/2-z

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Conclusions

We have designed and successfully synthesized a dipeptide motif Mnba-Aib-OMe, **1** and crystallographically investigated that dipeptide **1** self-assembled into an anti-parallel β-sheet structural scaffold. The N-terminus of the dipeptide was modified by a smooth nitro to amine reduction under hydrogen atmosphere in the presence of Pd/C catalyst followed by incorporation of the Boc-group in the same reaction pot. The modified dipeptide **2** self-associates to fabricate a dimeric unit, which further self assembles to form a supramolecular rhombus-shaped matrix.

Thus, we have been able to change the secondary structure of a dipeptide by N-terminus modification.

Experimental Section

General. 3-Nitrobenzoic acid, 2-amino isobutyric acid, DCC (dicyclohexylcarbodiimide) and HOBt (N-hydroxybenzotriazole) were purchased from Sigma-aldrich. The dipeptide **1** was synthesized by conventional solution phase methodology using a racemization free fragment condensation strategy. The coupling reaction was done by using DCC (dicyclohexylcarbodiimide) and HOBt (N-hydroxybenzotriazole)**.** The final compounds were fully characterized by 500 MHz ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy, FT-IR spectroscopy and mass spectrometry.

NMR experiments

All NMR studies were carried out on a Bruker AVANCE 500 MHz spectrometer at 278 K. Compound concentrations were in the range 1–10 mM in $CDCl_3$.

Mass spectrometry

Mass spectra were recorded on a Q-Tof Micro YA263 high resolution (Waters Corporation) mass spectrometer by positive mode electrospray ionization.

FT-IR Spectroscopy

All reported solid-state FT-IR spectra were obtained with a Perkin Elmer Spectrum RX1 spectrophotometer with the KBr disk technique.

Synthesis of Mnba-Aib-OMe (1). 3-Nitrobenzoic acid (0.84 g, 5 mmol) was dissolved in 20 mL dry DCM in an ice-water bath. H-Aib-OMe was isolated from 1.17 g (10 mmol) of the corresponding methyl ester hydrochloride by neutralization and subsequent extraction with ethyl acetate and ethyl acetate extract was concentrated to 10 mL. It was then added to the reaction mixture, followed by immediate addition of 1.44 g (7 mmol) dicyclohexyl carbodiimide (DCC) and 0.95 g (7 mmol) of HOBt. The reaction mixture was allowed to come to room temperature and stirred for 48 hrs. After that, DCM was evaporated and the residue was dissolved in ethyl acetate (60 mL) and dicyclohexyl urea (DCU) was filtered off. The organic layer was washed with 2 (M) HCl (3 ×50 mL), brine (2 × 50 mL), 1(M) sodium carbonate (3 × 50 mL) and brine (2 × 50 mL) and dried over anhydrous sodium sulfate. The solution was evaporated under vacuum to obtain dipeptide **1** as a yellowish-white solid. The product was purified by silica gel (60-120 mesh) using n- hexane-ethyl acetate (3:1) as eluent. Yield: 1.17 g (4.4 mmol, 88%).

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^{1}H NMR (500 MHz, $CDCl_3$, δ in ppm): 8.59 [1H, S, Mnba(1) CH], 8.33- 8.31 [1H, d, *J* 8Hz, Mnba(1) CH], 8.11 8.03 [1H, d, *J* 8 Hz, Mnba(1) CH], 7.64-7.67 [1H, m, Mnba(1) CH], 7.11 [1H, s, Aib(2) NH,], 3.86 [3H, s, OCH_3], 1.71 [6H, s, Aib Cβ H].
^{13}C NMR (125 MHz, $CDCl_3$, δppm): 175.17, 166.32, 152.67, 138.79, 135.27, 129.27, 128.47, 123.36, 55.81, 54.69, 26.77.
Mass spectra: $[M+Na]^+$: 289.09, (actual 289.08). $[M+K]^+$: 306.295 (actual 305.35).
FT-IR Spectra (in cm^{-1}): 713, 1352, 1527, 1631, 1734, 3233.

One-pot N-terminus modification. 0.27 g (1 mmol) of peptide **1** was dissolved in dioxane (10 mL) and was treated with 120 mg of Pd/C. Hydrogen gas was supplied into the solution through balloon. The reaction mixture was stirred under hydrogen atmosphere for about 6 hours. The completion of the reduction was monitored by TLC. After the completion of the reaction, hydrogen gas was removed. Then, ditertiarybutylpyrocarbonate (0.22 g, 1 mmol) and triethylamine (2 mL) were added into it and the stirring was continued at room temperature for another 6 h. The mixture was diluted with dioxane (25 mL) and it was filtered through sintered funnel using celite bed and celite bed was washed with dioxane (3x25 mL). Then, the solution was concentrated under vacuum to about 15-20 mL, cooled in an ice-water bath, covered with a layer of ethyl acetate (about 30 mL), and acidified with a dilute solution of $KHSO_4$ to pH 2-3 (Congo red). The aqueous phase was extracted with ethyl acetate, and this operation was done repeatedly. The ethyl acetate extracts were pooled, washed with water, dried over anhydrous Na_2SO_4, and evaporated under vacuum to obtain the dipeptide **2** as a off-white solid. Purification was done on a silica gel column (100-200 mesh) using ethyl acetate: hexane (3:1) as the eluent. Yield: 0.25 g (7.5 mmol, 75%).

^{1}H NMR (500 MHz, $CDCl_3$, δ in ppm): 7.71 [1H, S, Maba(1) CH], 7.57-7.59 [1H, d, *J* 8 Hz, Maba(1) CH], 7.40-7.42 [1H, d, *J* 8 Hz, Maba(1) CH], 7.30-7.33 [1H, m, Maba(1) CH], 6.83 [1H, s, Aib(2) NH,], 6.76 [1H, s, Maba(1) NH], 3.76 [3H, s, OCH3], 1.65 [6H, s, Aib Cβ H], 1.50 [9H, s, Boc CH_3].
^{13}C NMR (125 MHz, $CDCl_3$, δ in ppm): 175.17, 166.32, 152.67, 138.79, 135.27, 129.27, 121.47, 121.36, 116.97, 80.78, 56.81, 52.69, 28.28, 24.77.
Mass spectra: [M+Na]+ : 359.15, (actual 359.07).
FT-IR Spectra (in cm^{-1}): 1232, 1492, 1553, 1634, 1735, 2850, 2935, 2981, 3322, 3371.

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Supplementary Material

Detailed experimental procedures and ^{1}H NMR spectra, ^{13}C NMR spectra, Mass spectra and FT-IR spectra associated with all the compounds reported in this article are available as supplementary information.

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11. Crystallographic data: Peptide **1**: $C_{12}H_{14}N_2O_5$, Mw=266.26, monoclinic, space group P21/n, a = 7.3142(3), b = 20.7629(8), c = 9.1666(3) Å, α = 90°, β = 109.800(5)°, γ = 90° V= 1309.78Å^3, Z = 4, dm = 1.410 Mgm^{-3}. Intensity data were collected with MoKα radiation at room temperature using Bruker APEX-2 CCD diffractometer. Data were processed using the Bruker SAINT package and the structure solution and refinement procedures were performed using SHELX97.[14] The non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms were included in geometric positions and given thermal parameters equivalent to 1.2 times those of the atom to which they were attached. The final R values were for peptide **1**: R1 = 0.0503 and wR2 0.1777 for 1917 data with I> 2σ(I). The data have been deposited at the Cambridge Crystallographic Data Centre with reference number CCDC 1402681[9].
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