ESI

Supramolecular double helix from capped γ-peptide

Suman kumar Maity, Sibaprasad Maity, Poulami Jana and Debasish Haldar*

Department of Chemical Sciences, Indian Institute of Science Education and Research
Kolkata, Mohanpur, West Bengal 741252, India.

Fax: +913325873020; Tel: +913325873119;
E-mail: deba_h76@yahoo.com; deba_h76@iiserkol.ac.in

Table of contents

<table>
<thead>
<tr>
<th>Table/figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig. S1, ESI</td>
<td>2</td>
</tr>
<tr>
<td>Fig. S2, ESI</td>
<td>3</td>
</tr>
<tr>
<td>Table 1</td>
<td>4</td>
</tr>
<tr>
<td>Fig. S3, ESI</td>
<td>5</td>
</tr>
<tr>
<td>Fig. S4, ESI</td>
<td>6</td>
</tr>
<tr>
<td>Fig. S5, ESI</td>
<td>7</td>
</tr>
<tr>
<td>Fig. S6, ESI</td>
<td>8</td>
</tr>
<tr>
<td>Fig. S7, ESI</td>
<td>9</td>
</tr>
<tr>
<td>Fig. S8, ESI</td>
<td>10</td>
</tr>
<tr>
<td>Fig. S9, ESI</td>
<td>11</td>
</tr>
<tr>
<td>Figure S1</td>
<td>12</td>
</tr>
<tr>
<td>Experimental</td>
<td>13-18</td>
</tr>
<tr>
<td>Figure S2</td>
<td>19</td>
</tr>
<tr>
<td>Figure S3</td>
<td>20</td>
</tr>
<tr>
<td>Figure S4</td>
<td>21</td>
</tr>
<tr>
<td>Figure S5</td>
<td>22</td>
</tr>
</tbody>
</table>

*Corresponding author.
Fig. S1, ESI: Part of the concentration dependent $^1$H NMR spectra of peptide 2 in CDCl$_3$ downfield shift of the amide protons with increasing concentration. The fill square for Maba 2 NH, fill circle for Maba 1 NH and square for urea NH.
Fig. S2, ESI: Part of variable temperature $^1$H NMR spectra of peptide 2 in CDCl$_3$ showing upfield shifts of amide protons upon heating. The fill square for Maba 2 NH, fill circle for Maba 1 NH and square for urea NH.
Table 1: Characteristic $^1$H NMR parameters for Peptides 1 and 2 (chemical shifts $\delta$)$^a$

<table>
<thead>
<tr>
<th>Residues</th>
<th>NH</th>
<th>$\Delta\delta^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boc NH</td>
<td>6.6</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>(6.68)</td>
<td>(0.76)</td>
</tr>
<tr>
<td>Urea NH</td>
<td>6.3</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>(6.2 )</td>
<td>(0.36)</td>
</tr>
<tr>
<td>Maba(2) MH</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(8.42)</td>
<td>(0.70)</td>
</tr>
</tbody>
</table>

$^a$Chemical shift values of NH proton resonances for peptides 1 and 2 in CDCl$_3$. Values in parentheses correspond to peptide 2.

$^b$ $\Delta\delta$ is the chemical shift difference for NH protons in CDCl$_3$ and 10% (CD$_3$)$_2$SO/CDCl$_3$ for peptide 1 and 2.
Fig. S3, ESI: NOESY spectrum of peptide 2 in CDCl₃ at 50 mM concentration exhibits NOE intensities which are responsible for intermolecular interaction between aromatic protons (inside the red circle).
Fig. S4, ESI: CD spectrums of peptides (a) 1 and (b) 2 in CHCl₃.
Fig. S5, ESI: (a) ORTEP diagram of peptide 1 with atomic numbering scheme. Ellipsoids are shown as 30% probability. (b) The two molecules are actually very similar except the orientations of the cyclohexyl groups attached to N2/N5 (left), and when these are removed, a least squares fit is close to perfect (right).
Fig. S6, ESI: These dimers of peptide 1 are in turn connected to neighboring dimers through the N1-H7…O7 and N4-H4…O3 hydrogen bonds into two-dimensional layers, viewed here along a axis. Atoms colour: C black, H gray, N blue and O red. Hydrogen bonds are shown as dotted lines.
Fig. S7, ESI: ORTEP diagram of peptide 2 with atomic numbering scheme. Ellipsoids are shown as 50% probability. The PLATON/SQUEEZE program was used on the raw data to remove the disorder CHCl$_3$ solvent molecule.
Fig. S8, ESI: The view down the b-axis illustrates the packing of the duplexes and the solvent molecules (CHCl₃) filling voids in the crystal lattice. Atoms colour: C black, H gray, N blue, O red and Cl green. Hydrogen bonds are shown as dotted lines.
Fig. S9, ESI: Plot of NH chemical shifts of peptides 2 at varying concentrations of sulfamethoxazole added in CDCl₃ solutions.

Figure S1. Scheme 1: Reagent and condition: (a) Dry DCM, H-Maba-OMe, DCC, HOBt, 0°C. (b) MeOH, 2M NaOH. (c) DCU, Et₃N, DCC, HOBt.
Experimental Section

General Methods and Materials. Meta-aminobenzoic acid was purchased from Spectochem. HOBt (1-hydroxybenzotriazole) and DCC (dicyclohexylcarbodiimide) were purchased from SRL.

Peptide Synthesis. The peptides were synthesized by conventional solution-phase methods using fragment condensation strategy. The Boc group was used for N-terminal protection and the C-terminus was protected as a methyl ester. Couplings were mediated by dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt). Methyl ester deprotection was performed via the saponification method. All the intermediates were characterized by 500 MHz or 400 MHz 1H NMR and mass spectrometry. The final compounds were fully characterized by 500 MHz or 400 MHz 1H NMR spectroscopy, 13C NMR spectroscopy, mass spectrometry, and IR spectroscopy. The peptide 1, 2 were characterized by X-ray crystallography. The products were purified by column chromatography using silica (100-200-mesh size) gel as stationary phase and n-hexane–ethyl acetate mixture as eluent.

(a) Boc-Maba(1)-OH (3). A solution of m-aminobenzoic acid (4.1 g, 30 mmol) in a mixture of dioxane (60 mL), water (30 mL) and 1M NaOH (30 mL) was stirred and cooled in an ice-water bath. Di-tert-butylpyrocarbonate (7.0 g, 32 mmol) was added and stirring was continued at room temperature for 6 h. Then the solution was concentrated in vacuum to about 20-30 mL, cooled in an ice-water bath, covered with a layer of ethyl acetate (about 50 mL) and acidified with a dilute solution of KHSO₄ 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 and drier over anhydrous Na₂SO₄ and evaporated in a vacuum. The pure material was obtained as a white solid.

Yield: 5.42g (22.8 mmol, 76 %).

Melting point: 177°C.

1H NMR (DMSO-d₆, 400 MHz, δ in ppm): 12.87 [1H, s, COOH], 9.54 [1H, s, aromatic proton], 8.14 [1H, s, NH Maba(1)], 7.61-7.60 [1H, d, J = 7.32 Hz aromatic proton],
7.54-7.52 [1H, d, J = 7.32 Hz aromatic proton], 7.38-7.34 [1H, m, aromatic proton], 1.48 [9H, s, Boc protons]; $^1$C NMR (DMSO-$d_6$, 125 MHz, $\delta$ in ppm): 167.25, 152.73, 139.76, 131.76, 128.77, 122.87, 122.23, 118.74, 79.28, 28.05; FTIR ( in cm$^{-1}$, KBr): 3353, 2972, 2653, 1694, 1594, 1560, 1478, 1452, 1417, 1292, 1243, 1159, 1058.

Anal. Calcd for C$_{12}$H$_{15}$NO$_4$ (237.10): C, 60.75; H, 6.37; N, 5.90.

Found: C, 60.80; H, 6.35; N, 5.92.

(b) Boc-Maba(1)-Maba(2)-OMe ($4^b$): 5.2 g (22.0 mmol) of Boc-Maba-OH was dissolved in 25 mL dry DCM in an ice-water bath. H-Maba-OMe was isolated from 9.3 g (50 mmol) of the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate and ethyl acetate extract was concentrated to 10 mL. It was then added to the reaction mixture, followed immediately by 5.0 g (24 mmol) dicyclohexylcarbodiimide (DCC) and 3.2 g (24 mmol) of HOBT. The reaction mixture was allowed to come to room temperature and stirred for 48 h. DCM was evaporated and the residue was dissolved in ethyl acetate (60 mL) and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2M HCl (3 × 50 mL), brine (2 × 50 mL), 1M sodium carbonate (3 × 50 mL) and brine (2 × 50 mL) and dried over anhydrous sodium sulfate; and evaporated in a vacuum to yield compound 2 as a white solid. The product was purified by silica gel (100-200 mesh) using n hexane – ethyl acetate (5:1) as eluent.

Yield: 5.55 g (14.36 mmol, 64%).

Melting point: 140$^{\circ}$-141$^{\circ}$C.

$^1$H NMR (CDCl$_3$, 500 MHz, $\delta$ in ppm): 8.17 [1H, s, aromatic proton], 8.14 [1H, s, aromatic proton], 8.06-8.03 [1H, d, J = 8.31 Hz aromatic proton], 7.97 [1H, s, NH Maba(1)], 7.83-7.81 [1H, d, J = 7.50 Hz aromatic proton], 7.57-7.55 [1H, d, J = 8.50 Hz aromatic proton], 7.49-7.41 [3H, m, aromatic proton], 6.68 [1H, s, NH Maba(2)], 3.93 [3H, s, OCH$_3$] 1.53 [9H, s, Boc protons]; $^1$C NMR (CDCl$_3$, 125 MHz, $\delta$ in ppm): 166.76, 165.76, 152.75, 138.95, 138.19, 135.38, 130.77, 129.38, 129.09, 125.49, 124.91, 121.82, 121.65, 121.32, 117.03, 80.97, 28.27; FTIR ( in cm$^{-1}$, KBr): 3297, 2929, 1725, 1697, 1661, 1611, 1592, 1489, 1443, 1367, 1321, 1288, 1263, 1216, 1160, 1100, 1066.
 Anal. Calcd for C_{20}H_{22}N_{2}O_{5} (370.15): C, 64.85; H, 5.99; N, 7.56.

Found: C, 64.82; H, 6.00; N, 7.60.

TOF MS m/z 393.01 [M + Na]+; Mealed: 370.15.

(c) Boc-Maba(1)-Maba(2)-OH (5): To 3.7g (10.00 mmol) of compound 2, 25 mL MeOH and 2M 15 mL NaOH were added and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred. After 10 h, methanol was removed under vacuum; the residue was dissolve in 50 mL of water, and washed with diethyl ether (2 × 50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1M HCl and it was extracted with ethyl acetate (3 × 50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated under vacuum to obtained compound 5 as a white solid.

Yield: 3.1 g (8.6 mmol, 87%).

Melting point: 200°-202°C.

^1^H NMR (DMSO-\(d_6\), 400 MHz, δ in ppm): 12.98 [1H, br, COOH ], 10.41 [1H, s, NH maba(1)], 9.57 [1H, s, NH Maba(2)], 8.42 [1H, s, aromatic proton], 8.06-8.01 (2H, m, aromatic protons], 7.67 [1H, d, aromatic proton], 7.58 [1H, d, aromatic proton], 7.55 [1H, d, aromatic proton], 7.49-7.41 [2H, m, aromatic protons], 1.49 [9H, s, Boc protons]; ^13^C NMR (DMSO-\(d_6\), 125 MHz, δ in ppm): 167.21, 165.92, 152.81, 139.77, 139.44 135.50, 131.21, 121.86, 128.64, 124.41, 124.36, 121.29, 121.07, 117.65, 79.30, 28.11. FTIR (cm\(^{-1}\), in KBr): 3354, 2977, 2361, 1702, 1649, 1590, 1542, 1489, 1409, 1304, 1243, 1172, 1065.

Anal. Calcd for C_{19}H_{20}N_{2}O_{5} (356.14): C, 64.04; H, 5.66; N, 7.86.

Found: C, 64.00; H, 5.68; N, 7.87.

(d) Boc-Maba(1)-DCU (I): 1.06 g (4.5 mmol) compound Boc-Maba-OH was dissolved in 15 mL dry DCM on an ice-water bath. 0.9 g (4.5 mmol) DCC was added to the solution. 0.62 mL (4.5 mmol) of triethyl amine was added to 1.0 g (4.5 mmol)
dicyclohexylurea in 15 mL dry DCM. This solution was added to the previous solution. The reaction mixture was allowed to come to room temperature and stirred for 48 h. DCM was evaporated under vacuum and ethyl acetate was added to the solid mass. Dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCL (3 × 50 mL), brine (2 × 50 mL), then 1 M sodium carbonate (3 × 30 mL) and brine (2 × 30 mL) and dried over anhydrous sodium sulfate and evaporated under vacuum to yield the desired compound as a white solid. Purification was done by silica gel column (100-200 mesh size) and ethyl acetate and hexane (1:3) as eluent.

Yield: 1.7 g (3.9 mmol, 89%).

Melting point: 185°-186°C

H NMR (CDCl3, 400 MHz, δ in ppm): 7.50 [2H, d, aromatic protons], 7.30-7.16 [2H, m, aromatic protons], 6.60 [1H, s, NH Maba(1)], 6.30 [1H, b, NH DCU], 4.06 [1H, m, CH cyh], 3.49 [1H, m, CH cyh], 3.07-3.10 [2H, m, CH cyh], 2.07-1.76 [4H, m, cyh], 1.60-1.51 [6H, m, cyh], 1.51 [9H, s, Boc protons], 1.3-1.12 [5H, m, cyh], 1.12-1.06 [1H, m, cyh], 0.95-0.87 [2H, m, cyh]; 13C NMR (CDCl3, 125 MHz, δ in ppm): 170.69, 154.12, 152.52, 138.83, 137.50, 129.13, 120.81, 120.32, 116.50, 80.73, 57.09, 49.62, 32.16, 30.64, 28.22, 26.12, 24.49; FTIR (KBr, in cm⁻¹): 3280, 3055, 2933, 2855, 2359, 2341, 1726, 1687, 1628, 1490, 1412, 1366, 1235, 1164, 1060.


Found: C, 67.70; H, 8.45; N, 9.45.

TOF MS m/z 466.09 [M + Na]⁺; Mcalc: 443.28.

(e) Boc-Maba(1)-Maba(2)-DCU (2): 0.46 g (1.29 mmol) compound Boc-Maba-Maba-OH was dissolved in 15 mL dry DCM on an ice-water bath. 0.226 g (1.29 mmol) DCC was added to the solution. 0.18 mL (1.29 mmol) of triethyl amine was added to 0.29 g (1.29 mmol) dicyclohexylurea in 15 mL dry DCM. This solution was added to the previous solution. The reaction mixture was allowed to come to room temperature and stirred for 48 h. DCM was evaporated under vacuum and ethyl acetate was added to the solid mass. Dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCL (3 × 50 mL), brine (2 × 50 mL), then 1 M sodium carbonate (3
× 30 mL) and brine (2 × 30 mL) and dried over anhydrous sodium sulfate and evaporated under vacuum to yield the desired compound as a white solid. Purification was done by silica gel column (100-200 mesh size) and ethyl acetate and hexane (1:3) as eluent.

Yield: 0.6 g (1.06 mmol, 82%).

Melting point: 196°-197°C.

1H NMR (CDCl₃, 500 MHz, δ in ppm): 8.42 [1H, s, NH Maba(2)], 7.98 [1H, s, aromatic proton], 7.94-7.93 [1H, d, aromatic proton], 7.74 [1H, s, aromatic proton], 7.47-7.38 [3H, d, aromatic protons], 7.31-7.30 [1H, d, aromatic proton], 6.68 (1H, s, NH Maba(1)), 6.20 (1H, b, NH DCU), 4.12-4.3 [1H, m, C α H cyh], 3.49 [1H, m, C α H cyh], 2.01-1.99 [2H, m, cyh], 1.85-1.77 [5H, m, cyh], 1.53 [9H, s, Boc protons], 1.33-1.16 [8H, m, cyh], 0.91-0.87 [4H, m, cyh]; 13C NMR (CDCl₃, 125 MHz, δ in ppm): 169.87, 165.84, 154.25, 152.87, 138.75, 138.56, 137.34, 135.47, 129.29, 129.00, 122.39, 122.31, 122.01, 121.88, 118.80, 117.18, 56.29, 49.90, 32.02, 31.87, 30.66, 29.64, 29.60, 29.30, 28.26, 26.05, 25.36, 25.22, 24.71, 24.59, 22.64, 14.06; FTIR (in cm⁻¹, KBr): 3297, 2932, 2855, 2358, 2341, 1644, 1614, 1539, 1338, 1312, 1245, 1161, 1081, 1056.


Found: C, 68.32; H, 7.54; N, 9.95.

TOF MS m/z 585.12 [M + Na]+; Mcalcd: 562.32.

Abbreviation we used:

Cyh = cyclohexane.

Maba = m-aminobenzoic acid

DCU = dicyclohexyl urea

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 mmol in CDCl₃ and
(CD$_3$)$_2$SO.

**FT-IR spectroscopy**

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

**Mass spectrometry**

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

**X-ray Crystallography**

Single crystal X-ray analysis of peptide 1 and 2 was recorded on a Bruker high resolution X-ray diffractometer instruments. Intensity data were collected with MoK$\alpha$ radiation for peptide 1 at 296 K and MoK$\alpha$ radiation for peptide 2 at 100 K 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. For peptide 1, the non-hydrogen atoms were refined with anisotropic thermal parameters. For peptide 2, the non-hydrogen atoms were refined with isotropic thermal parameters due to closely space Cl atoms in the solvent molecule. The PLATON/SQUEEZE program was used on the raw data to generate a new dataset that removed the scattering contribution of disorder solvent molecule. The hydrogen atoms were included in geometric positions and given thermal parameters. The data have been deposited at the Cambridge Crystallographic Data Centre with reference number CCDC 832275 and 832274 for peptides 1 and 2 respectively.
Figure S2: $^1$H NMR (400 MHz, DMSO-$d_6$) spectra of Boc-Maba(1)-OH 3.
Figure S3: $^{13}$C NMR (125 MHz, DMSO-$d_6$) spectra of Boc-Maba(1)-OH 3.
Figure S4: $^1$H NMR (500 MHz, CDCl$_3$) spectra of Boc-Maba(1)-Maba(2)-COOCH$_3$ 4.
Figure S5: $^{13}$C NMR (125 MHz, CDCl$_3$) spectra of Boc-Maba(1)-Maba(2)-COOCH$_3$ 4.
Figure S6: $^1$H NMR (400 MHz, DMSO-$d_6$) spectra of Boc-Maba(1)-Maba(2)-COOH 5.
Figure S7: $^{13}$C NMR (125 MHz, DMSO-$d_6$) spectra of Boc-Maba(1)-Maba(2)-COOH 5.
Figure S8: $^1$H NMR (400 MHz, CDCl$_3$) spectra of Boc-Maba(1)-DCU 1.
Figure S9: $^{13}$C NMR (100 MHz, CDCl$_3$) spectra of Boc-Maba(1)-DCU 1.
Figure S10: $^1$H NMR (500 MHz, CDCl$_3$) spectra of Boc-Maba(1)-Maba(2)-DCU 2.
Figure S11: $^{13}$C NMR (125 MHz, CDCl$_3$) spectra of Boc-Maba(1)-Maba(2)-DCU 2.
Figure S12: FTIR spectra of Boc-Maba(1)-COOH in solid state.
Figure S13: FTIR spectra of Boc-Maba(1)-Maba(2)-COOMe in solid state.
Figure S14: Mass spectra of Boc-Maba(1)-Maba(2)-COOME 4.
Figure S15: FTIR spectra of Boc-Maba(1)-Maba(2)-COOH in solid state.
Figure S16: FTIR spectra of Boc-Maba(1)-DCU 1 in solid state.
Figure S17: Mass spectra of Boc-Maba(1)-DCU 1.
Figure S18: FTIR spectra of Boc-Maba(1)-Maba(2)-DCU in solid state.
Figure S19: Mass spectra of Boc-Maba(1)-Maba(2)-DCU 2.