Enforcing Solution Phase Nanoscopic Aggregation in a Palindromic Tripeptide

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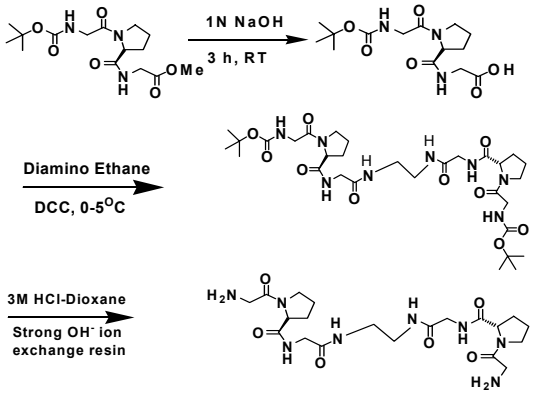

Scheme 1: Synthesis of $(GPG)_2$ diaminoethane (**3**)

General: All reactions were performed in nitrogen atmosphere. Solvents like Dichloromethane, N,N-Dimethylformamide were distilled following the standard procedure prior to use. Ethylene diamine, triethylamine were purchased from s.d. fine chemical ltd. Mumbai India and distilled before use. N.N'-dicyclohexylcarbodiimide , N-hydroxybenzotriazole were purches from spectrochem PVT. LTD.Mumbai India and used without further purification. ^{1}H and ^{13}C NMR were recorded on JEOL-JNM LAMBDA 400 model operating at 400, 100 MHz respectively**.** Mass spectra were recorded at Regional Sophisticated Instrumentation center Lucknow, India on a JEOL SX 102/DA-6000 mass spectrometer data system using Argon / Xenon (6kV, 10mA) as the FAB gas. Elemental (C, H, N) analyses were done on Perkin-Elmer 240-C automatic elemental analyzer. Solvent were evaporated using rotary evaporator under reduce pressure. Melting temperatures were determined on and are uncorrected. (Navyug India ltd. With 2×35 W coils)

Synthesis of *t*-butyloxycarbonyl-glycyl-prolyl-glycyl-methyl ester (1): To Boc-GP-OH, (3.53 g, 13 mmol, 1 eq.) in dichloromethane (30 ml) at 0-5° C(In an ice bath), GlyOMe HCl (1.63 g, 13 mmol, 1eq.), triethyl amine (2 ml, 14.3 mmol, 1.1 equiv) and DCC (3 g, 14.3 mmol, 1.1 equiv) were added and stirred for 8 h. Precipitated dicyclohexylurea was filtered. The organic layer was washed with 10% $NaHCO_3$ solution (2 x 20 ml), 1N HCl (2 x 20 ml) and with saturated brine solution (2 x 20 ml). Organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to get the crude product, which was recrystallized from 2-butanonone (3.4 g, 78.8%). Rf = 0.7 (6% methanol in dichloromethane). mp: 181°C. FAB MS: (M+1) = 344; ^{1}H NMR: (400MHz $CDCl_3$, 25^{0}C, TMS) δ(ppm) 1.44 (s, ^{t}Boc 9H); 1.89 (m, 4 H, Pro γ H); 2.37 (appeared as br s, 4 H, Pro β H); 3.38-3.60 (m, 2H Proδ H); 3.70(s, 3H, -OCH_3); 3.89-4.05 (m, 4H, Gly); 4.62(m, 1H, Pro α H); 5.46(br s, ^{t}Boc N-H); 7.36 (br s, -NH). ^{13}C NMR (100MHz, $CDCl_3$, 25^{0}C, TMS) δ(ppm) 24.71, 27.53, 28.30, 41.13, 43.01, 46.28, 52.17, 59.84, 79.76, 155.83, 168.83, 170.12, 171.13; Anal. Calcd. for $C_{15}H_{25}N_3O_6$; C, 52.47; H, 7.34; N, 12.26; found C, 52.25; H, 7.43, N,12.13.

Crystal data*:* Crystal suitable for X-ray analysis was grown from $CHCl_3$ by slow evaporation method. Molecular formula $C_{15}H_{25}N_3O_6$, M= 343.4, Orthorhombic, Space group P 2121 21, a=5.9752(4) Å, b=9.0939(7) Å, c=32.0920(2) Å, α=90.00°, β=90°, γ=90.00°, d=1.308 g/cm^{-1}, T=100(2)K, Z=4, μ=0.101mm^{-1}, 11645 reflections were collected, 4294 reflection independent [R(*int*) = 0.0348], final R1=0.0475, wR2= 0.108 [I>2sigma(I)], R1=0.0539, wR2= 0.111 (all data). The structure was solved by direct methods and expanded using Fourier techniques. Non-hydrogen atoms were refined anisotropically, while H-atoms were included at geometrically idealized positions and were not refined. The final cycle of full-matrix least-squares refinement using SHELXL97 converged with unweighted and weighted agreement factors, R = 0.0475 and wR2= 0.111 (all data), respectively, and goodness of fit, S=1.06. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.33 and -0.19 e. Å^{-3}. Crystallographic data (excluding structure factors) for the structure reported in this paper has been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-249980. Copies of the data can be obtained free of charge on application to CCDC, 12, Union Road, Cambridge CB2 1EZ, UK (fax: +0044-1223-336-033; Email: deposit@ccdc.cam.ac.uk).

1. G. M. Sheldrick, SHELXL97, University of Göttingen, Germany, 1997.

Hydrolysis of *t*-butyloxycarbonyl-glycyl-prolyl-glycyl-methyl ester: BocGPG-OMe (1.5 g, 4.5 mmol, 1eq.) was dissolved in minimum volume of methanol and NaOH (0.22 g, 5.4 mmol, 1.2 eq.) as its 1N solution was added and was stirred for 2 h . Then, methanol was evaporated and 20 ml of water was added to it. The solution was acidified to pH 2-3 and extracted in ethyl acetate. Ethyl acetate was dried over anhydrous sodium sulfate, filtered and evaporated to get pure product (1.13 g, 78% yield). mp: 185°C, ^{1}H NMR: (400MHz $CDCl_3$, 25^{0}C, TMS) δ(ppm) 1.44(s, ^{t}Boc 9H); 1.90 (m, 4 H, Pro γ H); 2.37 (appeared as br s, 4 H, Pro β H); 3.38-3.60 (m, 2H Proδ H); 3.89-4.05 (m, 4H, Gly); 4.62(m, 1H, Pro α H); 5.5(br s, ^{t}Boc N-H); 7.26 (br s, -NH). ^{13}C NMR (100MHz, $CDCl_3$, 25^{0}C, TMS) δ(ppm)= 24.8, 25.49, 28.03, 41.48, 46.38, 49.3, 59.84, 60.11, 156.57,157.7, 168.65, 171.33; Anal. Calcd. for $C_{14}H_{23}N_3O_5$; C, 51.06; H, 7.04; N, 12.76; found 51.17; H, 6.98; N, 12.82

CD studies: Methanolic solution of **1** and **3** (0.5mM as final concentration) were used as fresh and after 15 days of incubation at 30° C. Far-UV CD measurements were performed with JASCO spectropolarimeter (J-810 Model) which is monitored by J-810 program that runs on Windows 95/NT and the curve was smoothened using Microcal Origin 6 software with a curve fitting program. 0.1 cm path length cuvettes were used for this purpose. The measurements were taken at 0.5nm wavelength intervals, 1nm spectral bandwidth and five sequential scans were recorded for each sample.

Transmission Electron Microscopy: Methanolic solution of **3** (1 mM) was incubated for 15 days at 30° C, then sonicated for 10 seconds and transferred onto a Formvar (Fluka, Switzerland) coated copper grids (SPI supplies, West Chester, USA, 200 mesh) and dried. These grids were negatively stained with 2% uranyl acetate, dried and subsequently examined under JEOL 2000FX-II electron microscope, at an operating voltage of 100 kv. Protected and fully deprotected GPG tripeptide failed to show any aggregation under standard experimental conditions applied for **3**.

Optical microscopy: Congo red Solution (4μM saturated solution in 80% ethanol/H_2O) was added to aged solution of **3** and incubated for 6 hours at room temperature. 50 μL of this incubated solution was transferred to a glass slide and air dried and viewed under polarizing optical microscope (AX10 Lab, Zeiss) with cross-polarized light (500x). Images were obtained and processed by using Image-Pro Plus software.

Atomic force microscopy: The samples were imaged with an atomic force microscope (Molecular Imaging, USA), operated under Acoustic AC mode (AAC) with the aid of cantilever (NSC12(c), MikroMasch). The force constant was 0.6 N/m, while the resonant frequency used was 150 kHz. The images were taken in air at room temperature, with a scan speed of 1.5-2.2 lines/sec. Data acquisition was performed by Pico Scan 5 software and the analysis was done with the aid of visual SPM. 10 μL of 15 days aged solution of **3** (1 mM) was transferred to a freshly cleaved mica piece. Mica piece was dried for 30 min at room temperature, followed by AFM imaging.

Molecular Dynamics (MD) simulations of GPG peptides: Initial structure of an individual GPG peptide was generated from the crystal structure internal parameters (*this communication*). In the first simulation, four GPG peptides were randomly placed near four corners of a box and solvated with ~ 6100 methanol molecules (Total: 18,632 atoms). The solvated complex was energy minimized using steepest descent and conjugate gradient methods. Minimization and MD simulations were carried out using periodic boundary conditions with GROMACS suite[1] (http://www.gromacs.org) at 300 K. A time step of 2 fs was used. In order to give sufficient time for the solvent molecules to adjust around the solute peptides, positional restraints were applied on peptide atoms during the first 1 ns of the simulation. Restraints were removed for the rest of 15 ns production run. In minimization and molecular dynamics, a twin-range cut-off was used to calculate the long-range interactions; 10 Å for Lennard-Jones interactions without shift or switch functions and 18 Å for electrostatic interactions. Non-bonded list was updated every 10 steps. The algorithm LINCS[2] was used to constrain bond lengths involving hydrogen atoms. GROMOS96 force field parameters[1,3] were used for methanol and peptide molecules. A second simulation was performed with the same number of peptides with a different starting structure. In this simulation, the GPG peptides were arranged to form an aggregate in which each peptide made at least one hydrogen bond with its neighbor. After minimization and 1 ns simulation with positional restraints on peptides, 3 ns simulation was carried out to see the stability of this aggregate. Non-bonded parameters and other details are the same as that of the first simulation.

1. Lindahl, E.; Hess, B.; van der Spoel, D., *J. Mol. Mod.* **2001**, *7* 306.
2. Hess, B.; Bekker, H.; Berendsen, H.J.C.; Fraaije, J.G.E.M. *J. Comp. Chem.* **1997**, *18*, 1463.
3. Berendsen, H.J.C.; van der Spoel, D.; van Drunen, R. *Comp. Phys. Comm.* **1995**, *91*, 43..

Table:1 Hydrogen bond distances in Å and angle <DHA in degrees

D-H...A	d(H...A)	d(D...A)	<(DHA)
N(1)-H(1)...O(4)#1	2.28	2.8436(19)	122.8
N(3)-H(3)...O(2)#1	1.95	2.7662(19)	158.5

Symmetry operations: #1 -x+1, y+1/2, -z+3/2

Table 2 Selected torsional angles

C(6)-N(1)-C(5)-O(1)	-179.61(13)	ω_1
C(5)-N(1)-C(6)-C(7)	164.14(15)	ϕ_1
N(1)-C(6)-C(7)-N(2)	177.41(13)	ψ_1
C(11)-N(2)-C(7)-C(6)	-179.51(14)	ω_2
C(7)-N(2)-C(11)-C(12)	-74.49(18)	ϕ_2
N(2)-C(11)-C(12)-N(3)	146.45(14)	ψ_2
C(13)-N(3)-C(12)-C(11)	173.16(14)	ω_3
C(12)-N(3)-C(13)-C(14)	-103.72(19)	ϕ_3
N(3)-C(13)-C(14)-O(6)	7.5(2)	ψ_3