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Synthesis of Totally Chiral, Multiple Armed, Poly Glu and Poly Asp Scaffoldings on Bifunctional Adamantane Core

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Abstract: Three successive generations of peptidic scaffoldings consisting of two, six and fourteen chiral (all L) centres and four, eight and sixteen carbomethoxy groups, respectively, at the periphery with adamantane nucleus as the central core have been constructed by linking the two halves of corresponding Asp/Glu dendrons by 1,3 - bifunctional adamantane unit. Energy minimization and ^{1}H NMR studies have shown these scaffoldings to adopt increasingly globular and compact architecture with each succeeding generation. © 1997, Published by Elsevier Science Ltd. All rights reserved.

A key requirement for a protein molecule to become functional is to fold into a dense, well-defined, globular conformation.[1] We report herein a dendrimer-based[2] approach for the design of globular protein mimics using glutamic (Glu) and aspartic (Asp) acids as building blocks. The strategy involves the linking of pre-assembled Glu/Asp dendrons on a judiciously selected hydrophobic core so as to generate a spherical structural domains having multiple reactive arms for anchoring various types of ligands. These may have importance in the context of designing ion channel mimetics,[3] diagnostic reagents[4] and vaccines.[5]

Modelling studies led to the choice of a bifunctional adamantane core[6] to link succeeding generations of Glu and Asp bis-dendritic scaffoldings so programmed as to adopt increasingly compact 3-D globular conformation. The experimental outcome (*vide infra*) was dramatic and the expected profile (Figure 1) could be reached with third generation Glu dendron.

The synthetic strategy utilized for the construction of two-directional peptidic dendrimers reported here involves, first, the construction of unidirectional poly Glu or poly Asp dendron[7] using a convergent growth approach which after deprotection at the focal point (-NH_2 end) is coupled to the 1,3-adamantane dicarbonyl core leading to two-directional dendritic peptides.

1,3-Adamantane bis-Asp-OMe (**1a**) and bis-Glu-OMe (**1b**) (Figure 2) peptides - the first generation and the simplest of the two-directional peptidic scaffoldings in this series - were prepared by condensation of 1,3-adamantane dicarbonyl chloride with 2 equivalents of Asp/Glu-diOMe in dry CH_2Cl_2 in the presence of triethylamine. The tetrabranched 1,3-adamantane scaffoldings **1a** and **1b**, secured in excellent yields, were fully characterized by spectral and analytical data.[8] N-Deprotection of tetrabranched Asp and Glu dendron, followed by condensation with 1,3-adamantane dicarbonyl chloride afforded the second-generation adamantyl Asp-OMe (**2a**) and Glu-OMe (**2b**) (Figure 2) scaffoldings. As anticipated, the yields of **2a** and **2b**, whose spectral and analytical data[8] were in excellent agreement with that expected, were modest.

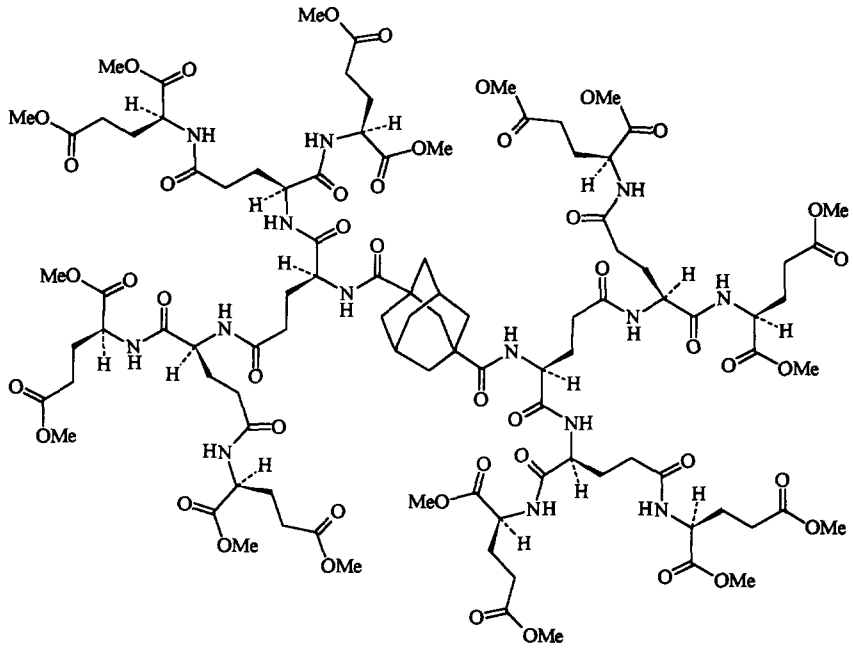

Figure 1. **Glu_4-Glu_2-Glu-[ADM]-Glu-Glu_2-Glu_4**

A similar protocol (N-deprotection, acylhalide condensation) from octabranched Asp and Glu dendron, gave the third-generation scaffoldings (**3a**) and (**3b**), (Figure 2). The condensation, monitored by tlc was considerably slower compared with previous cases. The third-generation bis-Asp-OMe scaffolding (**3a**) was obtained as a syrup in low yields (~10%). The corresponding Glu analog (**3b**) however gave satisfactory yields (~33%) and as a glassy solid which melted, without decomposition, at 140-143°C. The lower yield in Asp

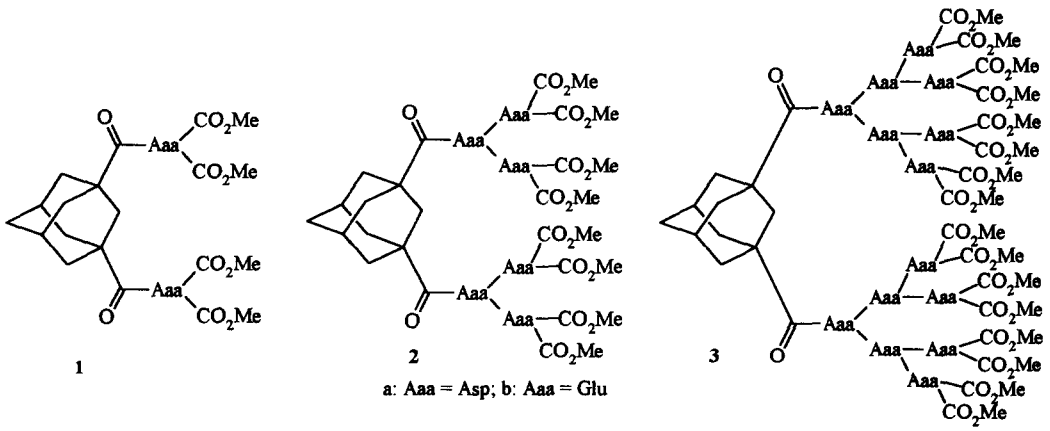

Figure 2

dendrimer may be attributed to comparatively more steric congestion. The dendrimer scaffoldings were purified by column chromatography (silica gel, $CHCl_3$/MeOH as eluents) and purity checked by HPLC on reverse phase column (C_{18}) using CH_3CN/H_2O (60:40) as the mobile phase. The purified scaffoldings gave spectral data[8] in excellent agreement with the assigned structures.

Interestingly, the NH and the $C^{\alpha}H$ resonances at the three levels of Glu-OMe branching in **3b** could easily be distinguished by the order of their appearance in the ^1H NMR spectrum.. Comparison of the ^1H NMR spectra of **1b**, **2b** and **3b** clearly showed that these resonances shift to lower field with each successive addition of glutamic acid layer. The behaviour of NH protons in **3b** was studied by variable - temperature ^1H NMR in order to gain insight into this molecule's folding profile.The chemical shifts of NH protons in **3b** measured with 1mM solution in DMSO-d_6 over the temperature range 293-343 K showed that the fourteen amide protons appear as seven pairs of doublets spread over a range of δ 7.4-8.4. In all cases, the chemical shifts were found to vary linearly with temperature. The relatively small (-2.77 ppb/K) temperature dependence of NH_a - the first level and the innermost amide protons - is strongly indicative of their involvement in intramolecular hydrogen bonding and shielding from the solvent. While a large majority of the amide protons in **3b** were found to be solvent exposed as evident from their comparatively higher temperature coefficients (-4.31 to - 4.85 ppb/K), the NH_b proton - belonging to the second level of Glu branching - showed a border-line $d\delta/dT$ value of -3.35 indicating modest shielding from the solvent.[9]

The adoption of increasingly globular architecture by **1b**, **2b** and **3b** - the three successive generations of Glu scaffoldings - was indicated by molecular modelling and energy minimization studies.[10] Thus, while **1b** shows the open, W-shaped architecture, the subsequent generation **2b** and **3b** adopt compact spheroidal shapes.

A feature of Glu/Asp dendritic scaffoldings is their remarkable solubility behavior. Thus, whereas Glu_3 and Glu_7 dendrons are quite insoluble in water, the adamantane supported bisdendritic scaffoldings **2b** and **3b** slowly dissolve in warm water giving a clear solution. This property may be attributed to the overall globular nature of **2b** and **3b** with polar hydrophilic outer surface enclosing a hydrophobic interior core. Further work related to the functional properties of peptide dendritic scaffoldings is in progress.

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6. Adamantane skeleton functionalized with activated carboxylate groups at 1,3-bridge head positions appeared particularly attractive for supporting dendrons because of the anticipation that these centers would, not only provide most appropriate geometry to mimic a tetrahedral nucleus but the amide side chains carrying dendrons would, in most likelihood prefer to adopt an anti conformation (Karle, I.; Ranganathan D.; Haridas, V. *J. Am. Chem. Soc.* **1996**, *118*, 10916; Ranganathan, D.; Haridas, V.; Madhusudanan K. P.; Roy, R.; Nagaraj, R.; John, G. B.; Sukhaswami, M. B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1105) particularly suitable for attaining globular conformation.
7. Using a common strategy, the Asp7 and Glu7 dendrons were assembled in a two-step sequence involving first, the coupling of N-protected L-Asp/Glu with L-Asp/ Glu-diOMe, to afford the tetra-branched Asp/Glu tripeptides in quantitative yields as tetramethyl esters which, in the second step were converted, via sequence, hydrolysis to the tetracarboxylic acid, and condensation with Asp/Glu-diOMe to the octabranched, third generation dendrons Asp-Asp_2-Asp_4 and Glu-Glu_2-Glu_4, containing seven chiral centers and eight terminal carbomethoxy groups, in excellent yields. Vapour pressure osmometry experiments conducted with 4mM and 40mM solutions of hexadeca carboxylic acid (sticky solid) of **3b** showed that it remains as a monomeric entity in aqueous solution.
8. Selected characteristic data for scaffoldings **1-3**.
1a: Yield 98%; M.p. 165-166°C; $[\alpha]_D^{26}$ = +50.96 (c = 2.17 in $CHCl_3$) ; ^1H NMR (90 MHz, $CDCl_3$) δ = 1.55-2.15 (12H, m), 2.22 (2H, s), 2.88 (4H, m), 3.72, 3.77 (6H, 6H, s, s), 4.80 (2H, m), 6.72 (2H, d, J=7.5 Hz); MS: m/z 511 $(MH)^+$. **1b**: Yield 99%; M.p. 135-136°C; $[\alpha]_D^{26}$ = +7.97 (c = 2.19 in $CHCl_3$); ^1H NMR (90 MHz, $CDCl_3$) δ = 1.63-2.56 (22H, m), 3.67, 3.78 (6H, 6H, s, s), 4.60 (2H, m), 6.41 (2H, d, J=7.5 Hz); MS: m/z 539 $(MH)^+$. **2a**: Yield 59%; syrup; $[\alpha]_D^{26}$ = +42.25 (c = 2.60 in $CHCl_3$); ^1H NMR (90 MHz, $CDCl_3$) δ = 1.47-2.33 (14H, m), 2.61-3.05 (12H, m), 3.72, 3.77 (12H, 12H, s, s), 4.10 (2H, m), 4.82 (4H, m), 7.23 (2H, d, J=7.5 Hz), 7.70 (4H, dd); MS: m/z 1027, $(MH)^+$, 1049 $(M + Na)^+$. **2b**: Yield 67%; white foamy hygroscopic solid; $[\alpha]_D^{26}$ = -12.82 (c = 2.30 in $CHCl_3$); ^1H NMR (90 MHz, $CDCl_3$) δ = 1.44-2.61 (38H, m), 3.66, 3.77 (12H, 12H, s, s), 4.31 (2H, m), 4.61 (4H, m), 6.63 (2H, d, J=7.5 Hz), 7.55 (2H, d, J=7.5 Hz), 7.90 (2H, d, J=7.5 Hz); MS: m/z 1111 $(MH)^+$, 1133 $(M + Na)^+$. **3a**: Yield 10%; syrup; ^1H NMR (90 MHz, $CDCl_3$) δ = 1.45-2.35 (14H, m), 2.62 (8H, m), 2.95 (12H, m), 3.41 (8H, m), 3.80 (48H, m), 4.04 (6H, m), 4.82 (8H, m), 5.65 (8H, br), 6.85 (4H, br), 8.88 (2H, d, J=7.5 Hz). **3b**: Yield 33%; M.p. 140-143°C; $[\alpha]_D^{26}$ = -20.99 (c = 1.35 in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$ + 2% DMSO-d_6) δ = 1.60-2.73 (70H, m), 3.62-3.77 (48H, m), 4.04 (2H, m), 4.22 (4H, m), 4.68 (4H, m), 4.80 (2H, m), 4.91 (2H, m), 6.13 (2H, d, J=7.5 Hz), 7.68 (2H, d, J=7.5 Hz), 7.75 (2H, d, J=7.5 Hz), 8.04 (2H, d, J=7.5 Hz), 8.11 (2H, d, J=7.5 Hz), 8.22 (2H, d, J=7.5 Hz), 8.46 (2H, d, J=7.5 Hz); ^{13}C NMR δ = 26.49, 26.62, 26.93, 27.14, 28.06, 29.78, 30.01, 30.40, 32.00, 40.87, 51.41, 51.59, 51.67, 51.73, 51.82, 52.80, 52.87, 52.93, 53.03, 53.19, 171.75, 172.61, 172.66, 172.86, 172.97, 173.50, 173.56, 173.67, 174.03, 174.18, 174.39, 176.05; MS: m/z 2388 $(M + Cs)^+$.
9. The notion that the globular architecture in **3b** would lead to differential shielding of NH protons in successive layers of glutamic acid attached to the hydrophobic core and that this would be reflected in differential exchange rate of amide protons, was confirmed by NMR D_2O exchange experiments conducted with third-generation scaffolding **3b** in H_2O/D_2O mixtures. NMR exchange studies were carried out with **3b** in H_2O/D_2O mixtures with D_2O varying from 0, 10, 20, 30, 50 %. At zero mixing time, the data showed that the order of exchange of amide protons was $NH_{(c)} > NH_{(b)} > NH_{(a)}$ where a, b, c represent the first, second and third level Glu branchings attached to the adamantane core. Same conclusions were reached by studying the amide NH exchange in 1:1 H_2O/D_2O mixture as a function of time.
10. The models for Glu scaffoldings were generated using Biosym version 2.3.5 package on a Silicon Graphics IRIS Crimson Elan Work station. The energy minimization was done with INSIGHT and DISCOVER program packages (Biosym Technologies, San Diego, CA), using CVFF force field.

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