ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/272569498

Structural characterization of folded and extended conformations in peptides containing γ amino acids with proteinogenic side chains: Crystal structures of γn, (αγ)n and γγδγ seque…

Article *in* New Journal of Chemistry · February 2015

DOI: 10.1039/C5NJ00132C

CITATIONS
3

READS
124

7 authors, including:

Madhusudana Reddy
Reva University
36 PUBLICATIONS **533** CITATIONS
SEE PROFILE

Krishnayan Basuroy
Deutsches Elektronen-Synchrotron
23 PUBLICATIONS **182** CITATIONS
SEE PROFILE

Dinesh Bhimareddy
French National Centre for Scientific Research
22 PUBLICATIONS **282** CITATIONS
SEE PROFILE

Vasantha Basavalingappa
Indian Institute of Science
12 PUBLICATIONS **105** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

One-Pot Protocol for the Conversion View project

Harvard _ post doc at RB Woodward View project

All content following this page was uploaded by Vasantha Basavalingappa on 08 September 2015.

The user has requested enhancement of the downloaded file.

CrossMark
click for updates

View Article Online
View Journal

NJC

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: M. M. B. Reddy, K. Basuroy, C. Siddappa, D. Bhimareddy, V. Basavalingappa, V. M. Achanna and P. Balaram, *New J.*

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

View Article Online
DOI: 10.1039/C5NJ00132C

Structural characterization of folded and extended conformations in peptides containing γ amino acids with proteinogenic side chains: Crystal structures of γ_n, $\alpha\gamma_n$ and γγδγ sequences.

Muthukurpalya Bhojegowd Madhusudana Reddy, Krishnayan Basuroy, Siddappa Chandrappa, Bhimareddy Dinesh, Vasantha Basavalingappa, Manjunath Achanna Venkatesha, Padmanabhan Balaram*

Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India

pb@mbu.iisc.ernet.in

Published on 18 February 2015. Downloaded by Indian Institute of Science on 04/03/2015 12:06:08.

Abstract

The crystal structures of nine peptides containing γ^4Val and γ^4Leu are described. The short sequences Boc-[γ^4(*R*)Val]$_2$-OMe **1,** Boc-[γ^4(*R*)Val]$_3$-NHMe **2** and Boc-γ^4(*S*)Val-γ^4(*R*)Val-OMe **3** adopt extended apolar, sheet like structures. The tetrapeptide Boc-[γ^4(R)Val]$_4$-OMe **4** adopts an extended conformation, in contrast to the folded C_{14} helical structure determined previously for Boc-[γ^4(R)Leu]$_4$-OMe. The hybrid αγ sequence Boc-[Ala-γ^4(*R*)Leu]$_2$-OMe **5** adopts an S-shaped structure devoid of intramolecular hydrogen bonds, with both α residues adopting local helical conformations. In sharp contrast, the tetrapeptides Boc-[Aib-γ^4(*S*)Leu]$_2$-OMe **6** and Boc-[Leu-γ^4(*R*)Leu]$_2$-OMe **7** adopt folded structures stabilized by two successive C_{12} hydrogen bonds. γ^4Val residues have also been incorporated into the strand segments of a crystalline octapeptide, Boc-Leu-γ^4(*R*)Val-Val-^{D}Pro-Gly-Leu-γ^4(*R*)Val-Val-OMe **8**. The γγδγ tetrapeptide containing γ^4Val and δ^5Leu residues adopts an extended sheet like structure. The hydrogen bonding pattern at γ residues corresponds to an apolar sheet, while a polar sheet is observed at the lone δ residue. The transition between folded and extended structures at γ residues involves a change of the torsion angle from the *gauche* to *trans* conformation about the C^β-C^γbond.

Introduction

Hydrogen bonded polypeptide structures have been extensively characterized in peptides and proteins, since the seminal work of Pauling and Corey introduced the α-helix and β-sheet to the literature of protein chemistry.[1] In conventional peptide sequences composed of α amino acids (α-peptides), a limited set of backbone hydrogen bonded motifs characterize the known elements of secondary structure. In the case of helices, the 3_{10} and α-helical structures, which

New Journal of Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C5NJ00132C

Published on 18 February 2015. Downloaded by Indian Institute of Science on 04/03/2015 12:06:08.

are stabilized by C_{10} (4→1, 10 atoms in the hydrogen bonded ring) and C_{13} (5→1, 13 atoms in the hydrogen bonded ring), are the most widely observed structures. The extended segments of α-peptide chains commonly associate into the canonical parallel and anti-parallel β-sheet structures,[2] although the less common anti-parallel α-sheet structure has also been recently advanced as an important structural element in the case of aggregation prone sequences, implicated in amyloidogenesis.[3] A key characteristic of the sheet structures is that the hydrogen bond donors and acceptors may lie far apart in a polypeptide sequence or are derived from different molecules, resulting in intermolecular association. The design of relatively short folded peptide sequences is implicitly based on the recognition that intramolecular hydrogen bonding is a dominant stabilizing interaction.

In recent years, the rapid development of the field of peptide *foldamers*[4] based largely on sequences containing the higher homologs of the α amino acids, primarily β and γ residues,[5] has refocused attention on the diversity of hydrogen bonded structures in polypeptides with unnatural backbones.[6] In the case of helices, hydrogen bonding patterns unprecedented in the structural chemistry of α-peptides have been revealed in the characterization of the C_{14} helix in β-peptides[7] and helices with mixed hydrogen bond directionality in hybrid αβ and αγ sequences.[8] The polar sheet structures in which hydrogen bond acceptors (C=O groups) and donors (NH groups) are aligned in the opposite sides of an extended strand have been observed in β peptide sequences.[9] As part of a program to systematically explore the conformational space and accessible regular structures for γ residues,[10] derived by backbone homologation of proteinogenic amino acids, we have structurally characterized by X-ray diffraction a number of short γ peptides and αγ sequences.

In this report, we describe the structural characterization of γ^4Val and γ^4Leu residues, (Fig. 1), in extended sheet structures and compare residue conformations in extended and helical structures. We also present the first structural characterization of a hybrid γγδγ tetrapeptide in an extended sheet conformation.

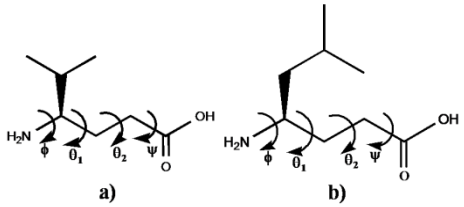

Fig. 1 Chemical structures of the γ residues, a) γ^4Val and b) γ^4Leu.

New Journal of Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C5NJ00132C

Results and discussion

The solid state conformations and hydrogen bonding patterns observed in crystals are presented for nine synthetic peptides containing γ residues. The sequences and the observed backbone torsion angles are summarized in Table 1. Hydrogen bond parameters are listed in supplementary Table S1.

Table 1 Backbone torsion angles (°) in the crystal structures of peptides.

Peptides	Residues	ϕ	θ_1	θ_2	θ_3	ψ
Boc-[γ^4(*R*)Val]$_2$-OMe **1**	γ^4(*R*)Val (1)	**-122.0**	**64.3**	**178.3**		**-143.6**
	γ^4(*R*)Val (2)	**-147.1**	**62.2**	**176.1**		**-167.6**
Boc-[γ^4(*R*)Val]$_3$-NHMe **2**	γ^4(*R*)Val (1)	**-122.6**	**66.2**	**-177.6**		**-147.1**
	γ^4(*R*)Val (2)	**-125.4**	**61.2**	**174.4**		**-123.1**
	γ^4(*R*)Val (3)	**-112.8**	**58.6**	**174.8**		**-125.6**
Boc-γ^4(*S*)Val-γ^4(*R*)Val-OMe **3**	γ^4(*S*)Val (1)	**115.7**	**-67.7**	**173.0**		**145.2**
	γ^4(*R*)Val (2)	**-136.5**	**59.5**	**-177.3**		**-167.6**
Boc-[γ^4(*R*)Val]$_4$-OMe **4**	γ^4(*R*)Val (1)	**-127.4**	**68.5**	**-179.4**		**-145.2**
	γ^4(*R*)Val (2)	**-116.9**	**68.3**	**178.4**		**-144.6**
	γ^4(*R*)Val (3)	**-117.8**	**65.7**	**176.9**		**-143.7**
	γ^4(*R*)Val (4)	**-148.2**	**63.9**	**174.0**		**-171.5**
Boc-[Ala-γ^4(*R*)Leu]$_2$-OMe **5** *Molecule-1*	Ala (1)	-64.5				-38.2
	γ^4(*R*)Leu (2)	**-141.4**	**54.2**	**-176.1**		**-147.6**
	Ala (3)	-65.9				-29.7
	γ^4(*R*)Leu (4)	**-133.8**	**60.8**	**170.6**		**135.3**
Molecule-2	Ala (1)	-61.6				-43.8
	γ^4(*R*)Leu (2)	**-137.2**	**55.3**	**-177.6**		**-151.8**
	Ala (3)	-67.5				-26.2
	γ^4(*R*)Leu (4)	**-144.2**	**61.7**	**165.9**		**153.6**
Boc-[Aib-γ^4(*S*)Leu]$_2$-OMe **6**	Aib (1)	59.8				46.9
	γ^4(*S*)Leu (2)	**123.8**	**-51.9**	**-64.1**		**133.7**
	Aib (3)	65.3				29.1
	γ^4(*S*)Leu (4)	**117.5**	**-54.9**	**-173.7**		**-164.1**
Boc-[Leu-γ^4(*R*)Leu]$_2$-OMe **7**	Leu (1)	-67.7				-35.4
	γ^4(*R*)Leu (2)	**-121.2**	**46.7**	**67.3**		**-117.5**
	Leu (3)	-63.1				-33.7
	γ^4(*R*)Leu (4)	**-101.7**	**60.1**	**-176.8**		**-175.2**
Boc-Leu-γ^4(*R*)Val-Val-^{D}Pro-Gly-Leu-γ^4(*R*)Val-Val-OMe **8**	Leu (1)	**-156.3**				**144.7**
	γ^4(*R*)Val (2)	**-128.8**	**71.8**	**-166.2**		**-142.8**
	Val (3)	**-167.1**				**141.7**
	^{D}Pro (4)	**58.9**				**-138.3**
	Gly (5)	**-83.2**				**-7.9**
	Leu (6)	**-78.3**				**134.5**
	γ^4(*R*)Val (7)	**-146.1**	**63.0**	**-179.9**		**-125.9**
	Val (8)	**-67.4**				**-39.2**
Boc-[γ^4(*R*)Leu]$_2$-δ^5(*R*)Leu-γ^4(*R*)Leu-OMe **9**	γ^4(*R*)Leu (1)	**-116.5**	**60.3**	**172.0**		**-130.8**
	γ^4(*R*)Leu (2)	**-109.0**	**60.4**	**176.5**		**-134.4**
	δ^5(*R*)Leu (3)	**-120.7**	**65.7**	**173.6**	**-64.4**	**121.2**
	γ^4(*R*)Leu (4)	**-139.5**	**57.3**	**-174.0**		**174.8**

Published on 18 February 2015. Downloaded by Indian Institute of Science on 04/03/2015 12:06:08.

New Journal of Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C5NJ00132C

Published on 18 February 2015. Downloaded by Indian Institute of Science on 04/03/2015 12:06:08.

Homooligomeric γ sequences

Di- and tri-peptides: Fig. 2 shows the structures of the peptides, Boc-[γ^4(*R*)Val]$_2$-OMe **1**, Boc-[γ^4(*R*)Val]$_3$-NHMe **2** and Boc-γ^4(*S*)Val-γ^4(*R*)Val-OMe **3**. In all three cases the molecules are extended with intermolecular hydrogen bonds linking adjacent chains which are aligned in parallel fashion.

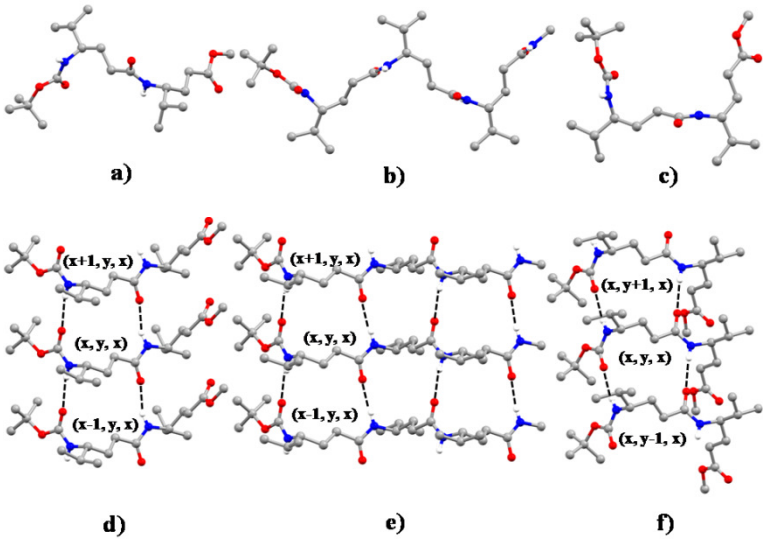

Fig. 2 Molecular conformations observed for short di- and tri-peptides in crystals: a) Boc-[γ^4(*R*)Val]$_2$-OMe **1**, b) Boc-[γ^4(*R*)Val]$_3$-NHMe **2** and c) Boc-γ^4(*S*)Val-γ^4(*R*)Val-OMe **3**. Molecules are packed in form an *apolar* sheet like structure in the crystals of d) Boc-[γ^4(*R*)Val]$_2$-OMe **1**, e) Boc-[γ^4(*R*)Val]$_3$-NHMe **2** and f) Boc-γ^4(*S*)Val-γ^4(*R*)Val-OMe **3**.

The observed conformational angles for the γ residues establish that both ϕ(N-C$^\gamma$) and ψ(C$^\alpha$-CO) correspond to extended conformations. The torsion angle θ_1(C$^\gamma$-C$^\beta$) corresponds to a folded, *gauche* conformation, while θ_2(C$^\beta$-C$^\alpha$) is *trans* and fully extended. In the observed sheet arrangement, alternating NH and CO groups point on opposite sides of the extended strand resulting in an *apolar* sheet arrangement, analogous to that observed in α polypeptides. *Tetrapeptide*: Fig. 3 shows the observed solid state conformation for Boc-[γ^4(*R*)Val]$_4$-OMe **4**. The molecule adopts an extended conformation, resulting in an extended version of the parallel *apolar* sheet structure, observed in di- and tripeptide sequences (**1** and **2**). Once again, θ_1(C$^\gamma$-C$^\beta$) adopts a *gauche* conformation, while the other three backbone torsion angles are extended at the γ residues.

New Journal of Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C5NJ00132C

Published on 18 February 2015. Downloaded by Indian Institute of Science on 04/03/2015 12:06:08.

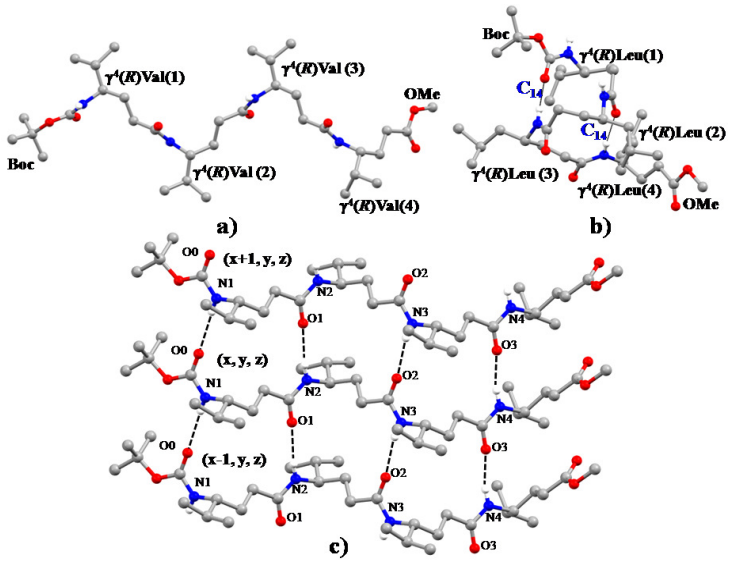

Fig. 3 a) Solid state conformation for the tetrapeptide, Boc-[γ^4(*R*)Val]$_4$-OMe **4**. b) Solid state conformation of the previously reported helical tetrapeptide, Boc-[γ^4(*R*)Leu]$_4$-OMe.[11] c) Molecules are packed in form an *apolar* sheet like structure in the crystals of Boc-[γ^4(*R*)Val]$_4$-OMe **4**.

Interestingly, in a previously report, the closely related γγγγ sequences, Boc-[γ^4(*R*)Leu]$_4$-OMe and its corresponding *enantiomer* Boc-[γ^4(*S*)Leu]$_4$-OMe, were shown to adopt folded helical structure in crystals.[11] Fig.3 compares the observed conformations for the two related γγγγ sequences. The sole difference observed in backbone torsion angles is that in the extended structure, Boc-[γ^4(*R*)Val]$_4$-OMe, $\theta_2(C^\beta\text{-}C^\alpha)$ adopts a *trans* conformation, while in the folded helical structure $\theta_2(C^\beta\text{-}C^\alpha)$ corresponds to a *gauche* conformation. The dramatic structural difference observed between the two closely related γ-tetrapeptides can be realized by a conformational switch (*gauche*⇌*trans*) about the $C^\beta\text{-}C^\alpha$bond. In a previous study using IR and NMR methods in $CHCl_3$ solution, we had indeed suggested folded C_{14} helical conformations for Boc-[γ^4(*R*)Val]$_4$-OMe.[12] Presumably, in this case the tetrapeptide adopts a predominantly folded conformation, in the *apolar* solvent $CHCl_3$, which promotes intramolecular hydrogen bond formation. Crystals obtained from the more strongly solvating medium, methanol, yield an extended conformation, suggesting that relatively small energy differences, exist between these conformations, resulting in pronounced solvent dependence.

Hybrid αγ sequences

Fig. 4a, 4c and 4d show the observed solid state conformation for three αγαγ tetrapeptides, Boc-[Ala-γ^4(*R*)Leu]$_2$-OMe **5**, Boc-[Aib-γ^4(*S*)Leu]$_2$-OMe **6** and Boc-[Leu-γ^4(*R*)Leu]$_2$-OMe **7**.

New Journal of Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C5NJ00132C

Published on 18 February 2015. Downloaded by Indian Institute of Science on 04/03/2015 12:06:08.

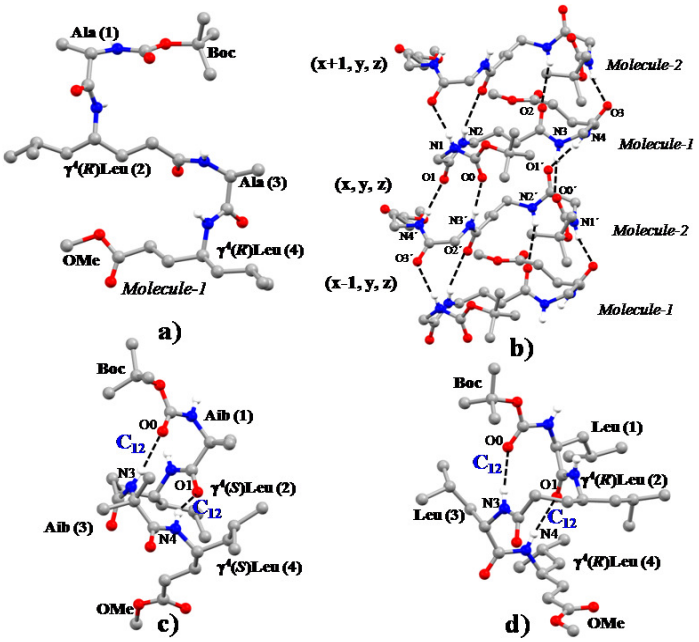

Fig. 4 Observed solid state conformation for three αγαγ tetrapeptides, a) Boc-[Ala-γ^4(*R*)Leu]$_2$-OMe **5**, c) Boc-[Aib-γ^4(*S*)Leu]$_2$-OMe **6** and d) Boc-[Leu-γ^4(*R*)Leu]$_2$-OMe **7**. b) Packing of molecules in the crystals of Boc-[Ala-γ^4(*R*)Leu]$_2$-OMe **5**.

In Boc-[Ala-γ^4(*R*)Leu]$_2$-OMe **5** an 'S' shaped structure is observed for the polypeptide backbone which is devoid of intramolecular hydrogen bonds. This folded arrangement is a consequence of the fact that both the α residues, Ala(1) and Ala(3) adopts helical α_R conformations, while the γ residues retain the conformation observed in the extended structures of homooligomeric γ peptides. Two independent molecules are observed in the crystallographic asymmetric unit, which are linked by intermolecular hydrogen bonds, as shown in Fig. 4b. The structure of tetrapeptide **5** raises the possibility of exploring partially folded states of polypeptide backbones which are not stabilized by intramolecular hydrogen bonds.

The peptides Boc-[Aib-γ^4(*S*)Leu]$_2$-OMe **6** and Boc-[Leu-γ^4(*R*)Leu]$_2$-OMe **7** fold into helical conformations stabilized by two successive C_{12} hydrogen bonds. These two structures may be classified as the backbone expanded analogues of the incipient 3_{10} helix, first characterized in the 1970s for tetrapeptides containing the conformationally constrained Aib (α-aminoisobutyric acid) residue.[13] Comparison of the observed structures of peptides **5** and **7** is instructive. In both cases, α and γ residues bear only a single substituent and may be considered as largely unconstrained. The dramatic difference in structures for the two tetrapeptides suggests that in these short sequences conformational choice may be mainly

New Journal of Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C5NJ00132C

determined by subtle environmental factors, primarily solvent interactions in solutions and crystal contact forces in the solid state. The sole difference in backbone conformational angles is observed for $\theta_2(C^{\beta}\text{-}C^{\alpha})$, which is *trans* for the residue 2, γ^4(*R*)Leu(2) and *gauche* for the γ^4Leu(2) residue in peptides **5** and **7**, respectively. Interestingly, in both the helical structures, **6** and **7**, θ_2 at the C-terminus γ residue is *trans*. In the case of this residue only the NH group contributes to the intramolecular hydrogen bonded pattern, leaving the conformational choice at this terminal residue open.

Facing γ residue in an antiparallel strands of a β hairpin

Previous studies on designed peptide hairpins have established that β residues can be readily accommodated at facing positions of antiparallel strands.[14]

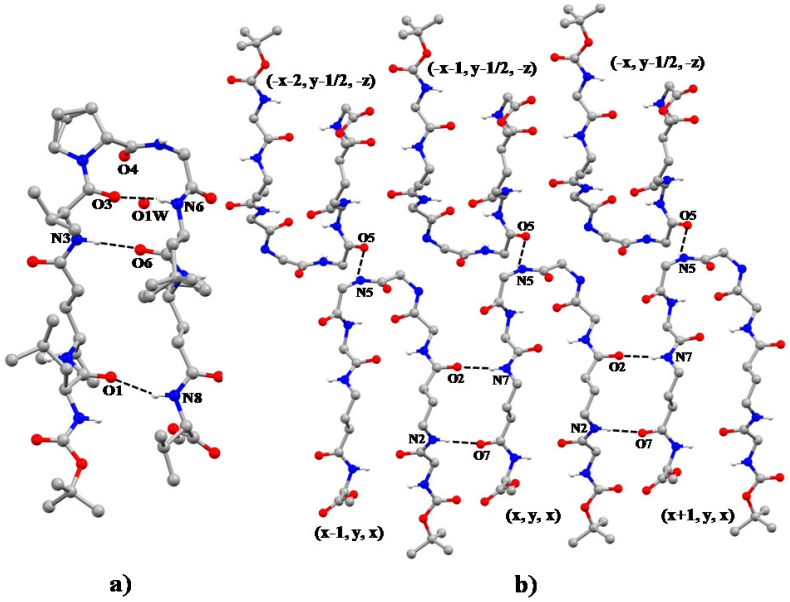

Fig. 5 a) The molecular conformation observed in crystals for Boc-Leu-γ^4(*R*)Val-Val-^{D}Pro-Gly-Leu-γ^4(*R*)Val-Val-OMe **8**. b) Packing of molecules reveals an anti-parallel sheet motif for the octapeptide **8**.

The β hairpin structure of an octapeptide containing the unsubstituted γ residue, γAbu (γ-aminobutyric acid) has been characterized in crystals for the peptides, Boc-Leu-Val-γAbu-Val-^{D}Pro-Gly-Leu-γAbu-Val-Val-OMe.[15] The reported torsion angles at the γAbu residues in two crystallographically independent molecules were: Molecule-1, γAbu(3), $\phi = -119°$, $\theta_1 = 107°$, $\theta_2 = 173°$and $\psi = -158°$; γAbu(8), $\phi = 94°$, $\theta_1 = -175°$, $\theta_2 = -65°$ and $\psi = 143°$, Molecule-2, γAbu(3), $\phi = -116°$, $\theta_1 = -173°$, $\theta_2 = 176°$ and $\psi = 106°$; γAbu(8), $\phi = 179°$, $\theta_1 = -171°$, $\theta_2 = -135°$ and $\psi = 132°$. In order to establish the ability of γ residues from proteinogenic amino acids to be positioned at the strand segment of hairpins, we determined the crystal structures of the octapeptide, Boc-Leu-γ^4(*R*)Val-Val-^{D}Pro-Gly-Leu-γ^4(*R*)Val-Val-

Published on 18 February 2015. Downloaded by Indian Institute of Science on 04/03/2015 12:06:08.

New Journal of Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C5NJ00132C

OMe **8**. Fig.5 shows the molecular conformation observed in crystals. The observed packing arrangement reveals an anti-parallel sheet motif. In the structure of the hairpin the ^{D}Pro-Gly segment adopt the hairpin facilitating type II´ β-turn[16], while the α residues with the exception of the C terminus Val(8) adopt extended conformation in the P_{II} region of ϕ-ψ space. The γ^4(*R*)Val(2) and γ^4(*R*)Val(7)have conformations identical to that observed in the short extended peptides, **1**, **2**, **3** and **4** with θ_1 adopting *gauche* conformation, while θ_2 is *trans*.

A hybrid γγδγ tetrapeptide

In an attempt to extend our study to the higher ω amino acids we determined the crystal structure of the tetrapeptide, Boc-γ^4(*R*)Leu-γ^4(*R*)Leu-δ^5(*R*)Leu-γ^4(*R*)Leu-OMe **9**. The observed molecular conformation corresponds to an extended polypeptide with molecules arranged parallel to one another linked by hydrogen bonds into sheet like structures.

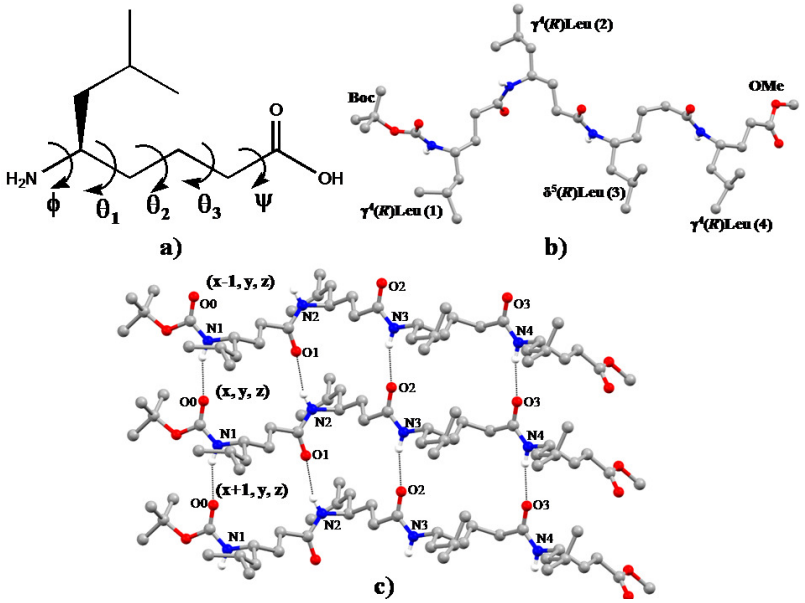

Fig. 6 a) Chemical structure of the δ residue, δ^5Leu. b) Molecular conformations observed for tetrapeptide, Boc-γ^4(*R*)Val-γ^4(*R*)Val-δ^5(*R*)Leu-γ^4(*R*)Val-OMe **9**, in crystals. c) Packing of molecules in crystals for the tetrapeptide reveals an overall apolar sheet like structure, while at the δ^5(*R*)Leu(3) a polar sheet hydrogen bonding pattern was observed.

Inspection of the packing diagrams in Fig.6 reveals that at the γ residues an *apolar* sheet pattern is observed, while at the lone δ residue a *polar* sheet hydrogen bonding pattern is seen. At the δ residue, adjacent NH groups point on one side of the sheet, while CO groups are oriented on the opposite side. The δ^5(*R*)Leu residue adopts a conformation in which ϕ and ψ are extended. The torsion angles about the central C-C bonds are 65.7°, 173.6° and −64.4°,

Published on 18 February 2015. Downloaded by Indian Institute of Science on 04/03/2015 12:06:08.

New Journal of Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C5NJ00132C

corresponding to a g^+, t, g^- conformation about the θ_1, θ_2 and θ_3 torsional variables. In a previously reported structure containing the unconstrained δ residue, δAva (δ-aminovaleric acid), the observed torsion angles are, $\phi = -127°$, $\theta_1 = 53°$, $\theta_2 = 64°$, $\theta_3 = 167°$ and $\psi = 77°$.[17] In that example, the δAva residue occupied the i+2 position in an expanded ^{D}Pro-δAva C_{13} turn which nucleated a hairpin structure, Boc-Leu-Val-Val-^{D}Pro-δAva-Leu-Val-Val-OMe. These observations suggest that δ residues can also be considered as monomers of choice in the design of new *foldamer* structures.

Conclusions

The results presented above, together with the growing number of studies on peptides containing unconstrained γ residues with proteinogenic sidechains[18] provides an opportunity to understand the nature of the conformational transitions that occurs upon the conversion of folded helices into extended sheet like structures. Fig.7 presents a cluster plot of the observed θ_1, θ_2 values for γ^4 residues with proteinogenic sidechains (primarily γ^4Val, γ^4Leu and γ^4Phe) in peptide crystal structures. In both helices and sheets, the ϕ, ψ values lie in the same region of conformational space ($\phi \approx -125°$, $\psi \approx -128°$ for helices; $\phi \approx -129°$, $\psi \approx -154°$ for sheets).

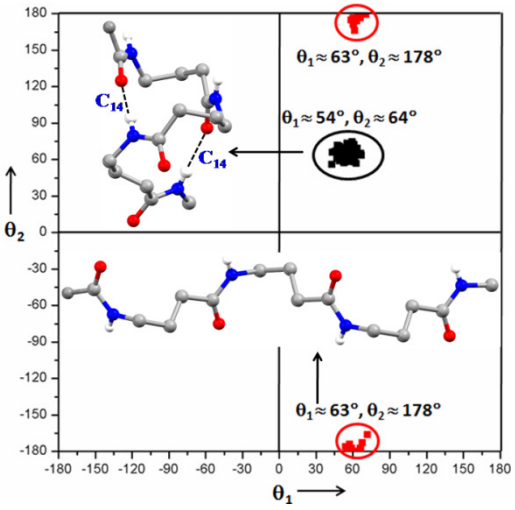

Fig. 7 A θ_1-θ_2 scatter plot for γ^4 residues with proteinogenic sidechains.

Two distinct clusters are observed in the θ_1-θ_2 plot corresponding to a transition of θ_2 from *gauche* (≈ 60°) to *trans* (≈ 180°). The use of γ residues, synthetically accessible from readily available proteinogenic α residues, may be exploited in the design of *foldamers* which mimic structures observed in proteins.

Published on 18 February 2015. Downloaded by Indian Institute of Science on 04/03/2015 12:06:08.

New Journal of Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C5NJ00132C

Published on 18 February 2015. Downloaded by Indian Institute of Science on 04/03/2015 12:06:08.

Experimental

Synthesis

General: The γ amino acids, Boc-γ^4(*R*)Val-OH, Boc-γ^4(*S*)Val-OH, Boc-γ^4(*R*)Leu-OH, and Boc-δ^5(*R*)Leu-OH were synthesized by previously described procedures.[19] Peptide synthesis was carried out using conventional solution-phase procedures.[20] The tert-butyloxycarbonyl(Boc) group was used for N terminal protection and C terminus was protected using a methyl ester. Deprotections were performed using 98% formic acid to remove the Boc group, whereas the methyl ester was removed by alkaline hydrolysis. The details of the synthesis are discussed below. The final peptides were purified by reversed-phase high-performance liquid chromatography (HPLC) on Jupitor Proteo C_{12} column (10-250 mm, 4 μ particle size) using methanol/water systems and monitored at 226 nm. Melting points were determined using Stuart melting point apparatus SMP10. Electrospray ionization mass spectra (ESI-MS) were obtained on a Bruker Daltonics Esquire-3000 instrument. ^{1}H NMR spectra were recorded on Bruker 500/700 MHz spectrometers. Characterization data for the peptides **1**-**9** are given in the Electronic Supplementary Information (ESI).†

X-ray crystallography

Crystals for the peptides, Boc-[γ^4(*R*)Val]$_2$-OMe **1**, Boc-[γ^4(*R*)Val]$_3$-NHMe **2**, Boc-[γ^4(*R*)Val]$_4$-OMe **4**, Boc-[Leu-γ^4(*R*)Leu]$_2$-OMe **7** and Boc-Leu-γ^4(*R*)Val-Val-^{D}Pro-Gly-Leu-γ^4(*R*)Val-Val-OMe **8** were assigned to the monoclinic space group $P2_1$ from systematic absences (0k0, k = odd). Boc-[Ala-γ^4(*R*)Leu]$_2$-OMe **5**, Boc-[Aib-γ^4(*S*)Leu]$_2$-OMe **6** and Boc-[γ^4(*R*)Leu]$_2$-δ^5(*R*)Leu-γ^4(*R*)Leu-OMe **9** crystallized in the triclinic space group *P*1. The dipeptide, Boc-γ^4(*S*)Val-γ^4(*R*)Val-OMe **3** crystallized in the monoclinic space group *C*2 and the space group assignment was done from the systematic absences (hkl, h+k = odd). Peptides **1**, **2**, **3**, **4** and **9** crystallized with one peptide molecule in the asymmetric unit and were free of solvent. The tetrapeptide **5** crystallized with two peptide molecules in the asymmetric unit. The peptides, **6**, **7** and **8** crystallized with one peptide molecule and co-crystallized solvent molecules [**6** (one dimethylformamide (DMF) molecule); **7** (two water molecules); **8** (one water molecule)] in the crystallographic asymmetric unit.

For peptides **1**, **3**, **4** and **8** X-ray data were collected on BRUKER AXS SMART APEXII ULTRA CCD (rotating anode X-ray generator) with CuKα (1.54178 Å) radiation and for peptides **2**, **5**, **6**, **7** and **9** X-ray data were collected on BRUKER AXS KAPPA APEXII CCD with MoKα (0.71073 Å) radiation. All the data sets were collected at room temperature (296K), in phi and omega scan type mode.

New Journal of Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C5NJ00132C

All peptides structures were solved by using iterative dual space direct methods in SHELXD.[21] After the initial solution methods, all the structures were refined against F^2 isotropically followed by full matrix anisotropic least-squares refinement using SHELXL-97.[22] In the case of peptide **1**, only two hydrogen atoms attached to N2 and C2G were located from the difference Fourier map and rest of the hydrogen atoms were fixed geometrically in idealized positions and allowed to ride with the C or N atoms to which they were bonded, in the final cycles of refinement. In the case of peptides **2** and **4**, all the hydrogen atoms were fixed geometrically in the idealized position and allowed to ride with the non-hydrogen atoms to which they were bonded. For peptides **3**, **5**, **6**, **7**, **8** and **9** a few hydrogen atoms bonded to respective C or N atoms were located from difference Fourier maps [**3** (N1, N2, C1A, C1B, C1G, C1D, C2B, C2G and C2D); **5** (N1, N2, N3, N4, C1A, C1G, C2A, C2B, C2G, C2D, C2E, C3A, C3B, C3G, C4G, C4D); **6** (N1, N3, N4); **7** (N1, N2, N3, N4, C1A, C1G, C2A, C2B, C2G, C2D, C2E, C3A, C3B, C3G, C4G, C4D); **8** (N1, N2, N3, N5, N6, N7, N8, C1A, C2A, C2B, C2G, C2D, C3A, C3B, C4A, C6A, C6B, C7A, C7B, C7G, C7D, C8A, C8B); **9** (N1, N2, N3, N4, C1B, C1G, C1D, C2G, C3A, C3B, C3G, C3D, C4G, C4D, C4E)] and rest of the hydrogen atoms were fixed geometrically in the idealized position and allowed to ride with the C or N atoms to which they were bonded, in the final cycles of refinement. For the octapeptide, **8**, positional disorder was observed at three atomic sites. One was at the site of C^{γ} position (C4G) of the ^{D}Pro (4) residue and other two at the $C^{\delta1}$ (C6D1) and $C^{\delta2}$ (C6D2) positions of Leu (6) residue sidechain. Positional disorder at different atomic sites of the crystal structure mentioned above was treated with partial occupancy and also with proper restraints and constraints to get chemically meaningful geometry of the disordered groups. Non-hydrogen atoms of the solvent molecules in peptides, **6**, **7** and **8** were also located from difference Fourier map. The details of the crystal data and structure refinement parameters for all the peptides mentioned above are given as the Supplementary Tables, ST1, ST2 and ST3.

CCDC deposition numbers for the peptides are 1038826 (**1**), 1038825 (**2**), 1038828 (**3**), 1038827 (**4**), 1038821 (**5**), 1038829 (**6**), 1038822 (**7**), 1038823 (**8**) and 1038824 (**9**) which contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

Acknowledgements

M.B.M.S.R., S.C., B.D., B.V., and M.A.V acknowledge the University Grants Commission (UGC), India for UGC-DSK Postdoctoral Fellowships.

Published on 18 February 2015. Downloaded by Indian Institute of Science on 04/03/2015 12:06:08.

New Journal of Chemistry Accepted Manuscript

Published on 18 February 2015. Downloaded by Indian Institute of Science on 04/03/2015 12:06:08.

Notes and references

View Article Online
DOI: 10.1039/C5NJ00132C

1. L. Pauling, R. B. Corey and H. R. Branson, *Proc. Natl. Acad. Sci. U.S.A.*, 1951, **37**, 205; L. Pauling and R. B. Corey, *Proc. Natl. Acad. Sci. U.S.A*., 1951, **37**, 251.
2. G. N. Ramachandran, C. Ramakrishnan and V. Sasisekharan, *J. Mol. Biol.* 1963, **7**, 95; J. S. Richardson, *Adv. Prot. Chem.*, 1981, **34**, 167.
3. G. Hopping, J. Kellock, R. P. Barnwal, P. Law, J. Bryers, G. Varani, B. Caughey and V. Daggett, *eLife* 2014, 3:e01681. (DOI: 10.7554/eLife.01681.)
4. S. H. Gellman, *Acc. Chem. Res*. 1998, *31*, 173.
5. D. Seebach, A. K. Beck and D. J. Bierbaum, *Chem. Biodiv*. 2004, **1**, 1111; L. K. A. Pilsl, and O. Reiser, *Amino Acids* 2011, **41**, 709; F. Bouillère, F. Thétiot-Laurent, C. Kouklovsky and V. Alezra, *Amino Acids* 2011, **41**, 687; R. P. Cheng, S. H. Gellman and W. F. DeGrado, *Chem. Rev.* 2001, **101**, 3219; G. Guichard and I. Huc, *Chem. Commun*. 2011, **47**, 5933; T. A. Martinek and F. Fülöp, *Chem. Soc. Rev*. 2012, **41**, 687.
6. P. G. Vasudev, S. Chatterjee, N. Shamala, and P. Balaram, *Chem. Rev*. 2011, **111**, 657.
7. D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell and S. H. Gellman, *J. Am. Chem. Soc*. 1999, **121**, 6206.
8. K. Basuroy, K. Vasantham and P. Balaram, *Org. Lett.* 2014, **16**, 4614.
9. J. M. Langenhan and S. H. Gellman, *J. Org. Chem.* 2003, **68**, 6440.
10. K. Basuroy, B. Dinesh, N. Shamala and P. Balaram, *Angew. Chem. Int. Ed.* 2013, **52**, 3136.
11. K. Basuroy, B. Dinesh, M. B. Madhusudana Reddy, S. Chandrappa, S. Raghothama, N. Shamala and P. Balaram. *Org. Lett*. 2013, **15**(18), 4866.
12. B. Dinesh, V.Vinaya, S.Raghothama and P.Balaram, *Eur. J. Org. Chem.* 2013, 3590.
13. N. Shamala, R.Nagaraj and P. Balaram, *Biochem. Biophys. Res. Commun*. 1977, **79**, 292; R. Nagaraj, N. Shamala and P. Balaram, *J. Am. Chem. Soc.* 1979, **101**, 16.
14. I. L. Karle, H. N. Gopi and P. Balaram, *Proc. Natl. Acad. Sci. U.S.A.,* 2002, **99**, 5160.
15. R. S. Roy, H. N. Gopi, S. Raghothama, I. L. Karle and P. Balaram, *Chem. Eur. J*. 2006, **12**, 3295.
16. S. K. Awasthi, S. Raghothama and P. Balaram, *Biochem. Biophys. Res. Commun*. 1995, **216,** 375; I. L. Karle, C. Das and P. Balaram, *Proc. Natl. Acad. Sci. U.S.A.*, 2000, **97**, 3034; J. Venkatraman, S. C. Shankaramma and P. Balaram, *Chem. Rev.* 2001, **101**, 3131; H. E. Stanger and S. H. Gellman, *J. Am. Chem. Soc.* 1998, **120***,* 4236; S. J. Maynard, A. M. Almeida, Y. Yoshimi and S. H. Gellman*, J. Am. Chem. Soc.* 2014, **136**, 16683.
17. R. Rai, P. G. Vasudev, K. Ananda, S. Raghothama, N. Shamala, I. L. Karle and P. Balaram, *Chem. Eur. J.* 2007, **13**, 5917.
18. C. J. Craig, J. L. Goodman and A. Schepartz, *Chem. Bio. Chem.* 2011, **12**, 1035; J. P. Miller, M. S. Melicher and A. Schepartz, *J. Am. Chem. Soc.* 2014, **136**, 14726; P. S. Wang, C. J. Craig and A. Schepartz, *Tetrahedron* 2012, **68**, 4342.
19. K. Pluncinska and B. Liberek, *Tetrahedron*1987, **43**, 3509; M. Smrcma, P. Maler, E. Majerov, T. A. Guerassina and M. A. Eissenstat, *Tetrahedron*1997, **53**, 12867; B. Dinesh, K. Basuroy, N. Shamala and P. Balaram, *Tetrahedron* 2012, **68**, 4374.
20. M. Bodanszky and A. Bodanszky, The Practice of Peptide Synthesis; Springer-Verlag: Berlin, 1984

New Journal of Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C5NJ00132C

21. T. R. Schneider and G. M. Sheldrick, *Acta Cryst*. 2002, **D58**, 1772.
22. G. M. Sheldrick, SHELXL-97, A program for crystal structure refinement, University of Göttingen: Göttingen, 1997; G. M. Sheldrick, *Acta Cryst.* 2008, **A64**,112.

Published on 18 February 2015. Downloaded by Indian Institute of Science on 04/03/2015 12:06:08.

New Journal of Chemistry Accepted Manuscript

View publication stats