Poly-*N*-methylated α-peptides: synthesis and X-ray structure determination of β-strand forming foldamers

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The first high resolution X-ray structure determination of poly-*N*-methylated α-peptides, a class of peptides widely used in biomedical research, is described; it shows that these molecules adopt a β-strand conformation.

Peptides containing single or multiple *N*-methylated amino acids are of high current interest in the area of medicinal chemistry.[1] Several natural products, *e.g.* vancomycin, cyclosporin, actinomycine D, and lead compounds with good proteolytic stability and improved pharmacokinetic properties are based on *N*-methyl amino acid containing substances.[2] Short poly-*N*-methylated peptides or peptides containing alternating *N*-methyl amide bonds and normal amide bonds have been especially successful as inhibitors of amyloidosis,[3] *i.e.* the process of protein aggregation thought to be, at least partially, responsible for Alzheimer's disease, type II diabetes, Parkinsonism, and prion diseases, to name but a few.[4]

Despite the excessive interest in the biological activity of peptides containing *N*-methyl amino acids, far less attention has been given to detailed structural investigations of such entities. In pioneering work by Goodman *et al.* on polymers of *N*-methylalanine, it was suggested that these molecules adopt a helical conformation.[5] These, and other,[6] studies were performed using low resolution methods, such as circular dichroism (CD) spectroscopy, one dimensional ^{1}H-NMR spectroscopy, and theoretical calculations. Furthermore, some studies were performed in trifluoroacetic acid (TFA) due to the poor solubility of polymeric *N*-Me-Ala in other solvents. As TFA was later shown to degrade polymeric *N*-Me-Ala,[7] these studies seem to have been mainly disregarded. In contrast to the helical structure of *N*-methylated peptides suggested by Goodman, most present work, especially in the biological literature, assumes that peptides containing *N*-methyl amino acids prefer an extended conformation.[3] The rationale for this is a report by Marraud *et al.*, which investigated the conformational preference for homo- and heterochiral dipeptides with one *N*-methylated amide bond.[8] In this paper it was suggested that homochiral dipeptides with an internal *N*-methylated amide bond prefer a *cis*-amide form, giving the peptide β-turn characteristics, while heterochiral dipeptides mainly exist in the *trans*-amide form.

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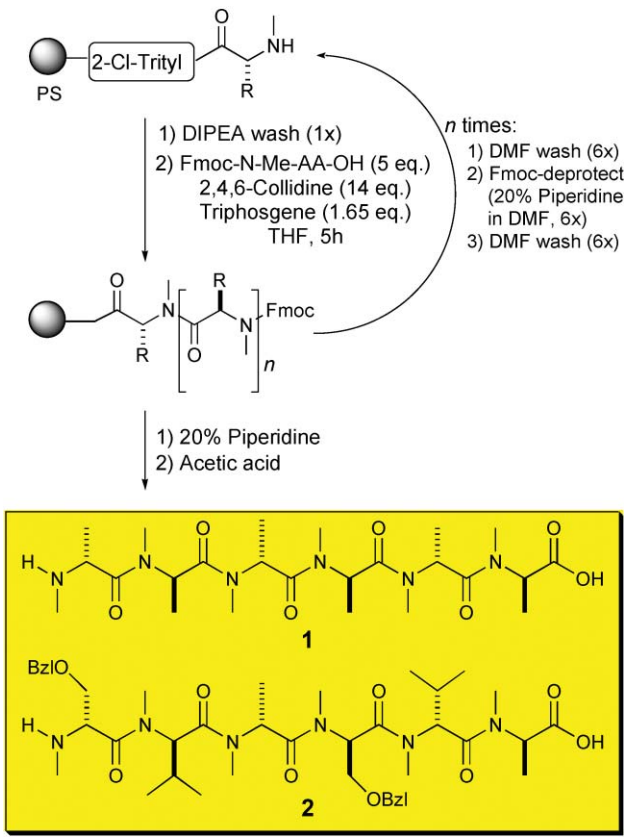
To the best of our knowledge, no high resolution structural study exists which can help to clarify the unresolved issues of the structural influence of *N*-methylated amino acids on the structure of these widely used poly-peptides. Herein, we report the synthesis of oligomers of poly-*N*-methylated peptides and their structural investigation by CD-spectroscopy and X-ray diffraction.

The solid-phase synthesis of poly-*N*-methylated peptides is notoriously difficult.[9] However, the recent introduction of the triphosgene coupling reagent for these purposes by Jung *et al.* now allows poly-*N*-methylated peptide sequences to be synthesized in good yields.[10]

Utilizing the triphosgene methodology on a hyper-acid labile 2-chlorotrityl resin and Fmoc-protected *N*-methyl amino acids, prepared from commercial Fmoc-amino acids *via* oxazolidinone formation and reduction,[11] we were able to obtain the poly-*N*-methylated hexapeptides **1** and **2** in 47% and 29% yield, respectively, after cleavage and purification by reversed-phase HPLC, Scheme 1.



Scheme 1

To gain a first indication of the structure of these poly-*N*-methylated peptides we initially measured their CD-spectra, Fig. 1. The CD-spectrum of peptide **1** in methanol is characterized by a negative Cotton effect around 220 nm, a zero crossing at 205 nm, and a weaker maximum below 200 nm. A slight red-shift occurs for the minimum when the solvent is changed to water, while the intensity of the absorptions remains similar. The CD-spectra of peptide **2** could only be measured in methanol, due to poor solubility of this protected peptide in water. The overall shape of this spectrum is similar to that of **1**, although the negative absorption is now significantly stronger and shifted to 228 nm; the stronger absorption could suggest that the different side chains in **2** force the molecule to adopt a more stable secondary structure than the simple Ala-based peptide **1**. It should also be noted that these spectra look very similar to those reported by Goodman *et al.* for polymeric *N*-Me-alanine, and assigned to represent a helical conformation.[5,6]

Single crystals of **1** and **2**, and also of the pentamer (*N*-Me-L-Ala)$_5$ synthesized separately according to Scheme 1 in 43% yield after HPLC purification, were grown from MeOH/water by slow evaporation. All peptides were crystallized in the zwitterionic form. For **1** and (*N*-Me-L-Ala)$_5$, there was a water molecule bridging the terminal amino nitrogen of one molecule with the terminal carboxylate group of another molecule. Fig. 2 shows the molecular conformations determined in crystals by X-ray diffraction analysis for (*N*-Me-L-Ala)$_6$ (**1**), *all-N*-Me-(Ser(OBz)-Val-Ala-Ser(OBz)-Val-Ala) (**2**), and (*N*-Me-L-Ala)$_5$; the observed backbone torsional angles are summarized in Table 1.

Although these poly-*N*-methylated peptides can be seen as acyclic proline analogs, or as more congested peptoid-like molecules, none of the investigated biopolymers with *all-N*-methyl amino acids adopts a helix-like secondary structure, like poly-prolines[12] or *N*-substituted glycines (peptoids)[13] are known to do. Instead, both poly-*N*-methyl peptides form an extended structure with two repeated conformations and all amide bonds in the *trans*-conformation. Likewise, none of the main-chain conformations found for any of the peptides are close to the values of an ideal β-sheet or to the theoretical values proposed by Goodman[5b] for polymeric *N*-Me-Ala. The *N*-Me-Ala hexa- and penta-peptides adopt two distinct main-chain conformations with ϕ/ψ values of about −134°/+65° and −67°/+141°. The first main-chain conformation causes a dihedral angle between the two

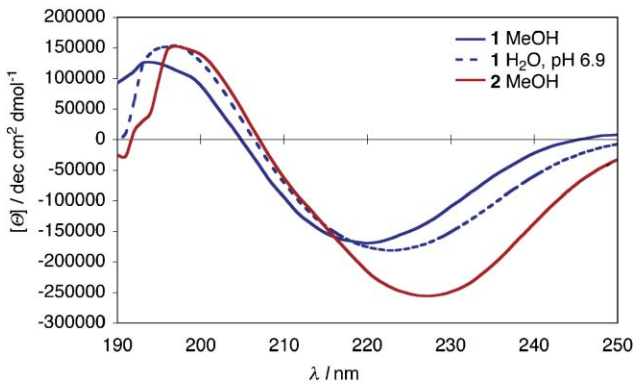


Fig. 1 Circular dichroism spectra of poly-*N*-Me-(Ala)$_6$ **1** in methanol solution (solid blue) and aqueous solution (pH 6.9, dotted blue) and poly-*N*-methylated hexapeptide **2** in methanol solution (solid red). All spectra were recorded at 0.1 μM conc. at 25.0 °C using a Jasco J-810 spectropolarimeter.

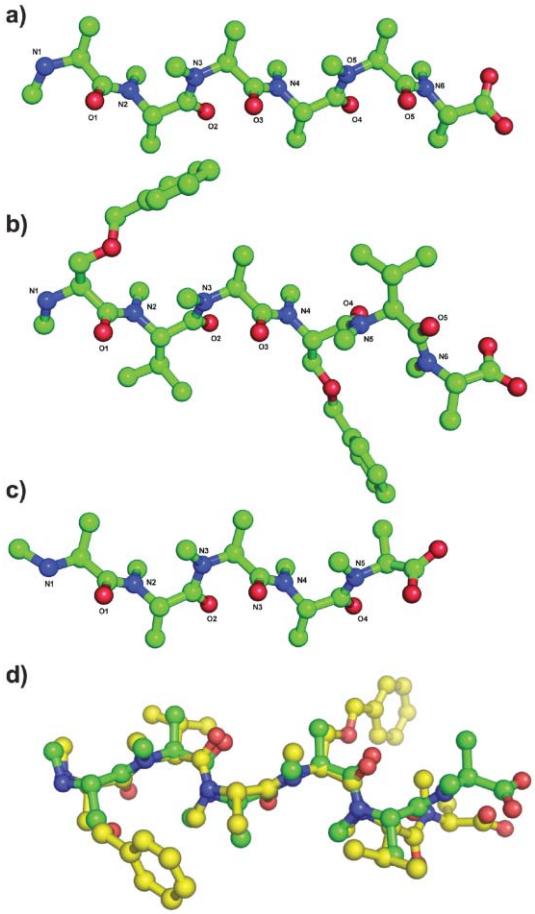


Fig. 2 Molecular conformations of a) (*N*-Me-L-Ala)$_6$ (**1**), b) *all-N*-Me-(Ser(OBz)-Val-Ala-Ser(OBz)-Val-Ala) (**2**), and c) (*N*-Me-L-Ala)$_5$ in the crystals. d) shows an overlay of **1** and **2**.

methyl groups (Me-*N*-C$_a$-Me) of about −68° and the second conformation keeps the two methyl groups on the same side with a dihedral angle of about 18°. Although the poly-*N*-methyl-L-alanine peptides do not form a helical structure, it is noteworthy that one of the observed backbone conformations resembles that of the poly-proline type II ($\phi = -75°$, $\psi = +145°$).[12] The backbone of the *N*-methylated peptide **2**, with different amino acid side chains, adopts a similar backbone conformation, Fig. 2d. Towards the

Table 1 Backbone torsional angles [°] for (*N*-Me-L-Ala)$_6$ (**1**), *all-N*-Me-(Ser(OBz)-Val-Ala-Ser(OBz)-Val-Ala) (**2**), and (*N*-Me-L-Ala)$_5$

Molecule Residue	ϕ	ψ	ω
1			
N-Me-L-Ala (2)	−131.58	62.40	−171.02
N-Me-L-Ala (3)	−64.94	143.02	−176.78
N-Me-L-Ala (4)	−136.17	61.80	−177.26
N-Me-L-Ala (5)	−69.90	134.77	−170.97
2			
N-Me-L-Val (2)	−132.43	75.31	−182.83
N-Me-L-Ala (3)	−78.63	140.16	−178.96
N-Me-L-Ser-O-Bzl (4)	−116.91	101.23	−181.78
N-Me-L-Val (5)	−128.08	65.37	−189.14
(*N*-Me-L-Ala)$_5$			
N-Me-L-Ala (2)	−139.92	63.75	−180.73
N-Me-L-Ala (3)	−66.95	145.41	−172.44
N-Me-L-Ala (4)	−136.33	72.88	−174.38

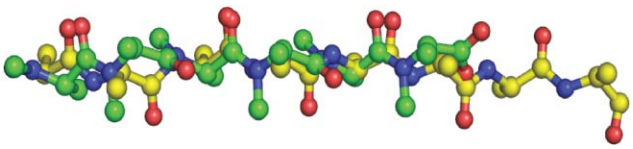


Fig. 3 Overlay of **1** (green) and an α-peptide in an ideal anti-parallel β-strand conformation (yellow), showing that poly-*N*-methylated peptides are capable of hydrogen bonding to natural α-peptides (overlay of the carbonyl carbon of amide groups 1, 3, and 5).

C-terminus, the conformation deviates from the structure of **1**, which is possible due to the crystal packing in the solid state to facilitate the intermolecular interactions between the carboxylate group and the neighboring *N*-terminal amino group.

The extended conformations found for both poly-*N*-methylated α-peptides **1** and **2** allow for hydrogen bonding between these molecules and a peptide in the β-strand conformation, Fig. 3.

The overlay shown in Fig. 3 clearly demonstrates the potential of *N*-methylated peptides as inhibitors of continued β-sheet growth, as characteristic of amyloidosis formation. The *N*-methylated peptides are capable of hydrogen bonding to the α-peptide, but further β-sheet formation is inhibited by the *N*-methyl substituent.[14] In the poly-*N*-methylated peptides investigated here, only every second residue has dihedral angles that allow for an interaction with the sheet conformation of an α-peptide; this finding helps to explain why peptides with alternating *N*-methyl amino acid residues were found to be more potent inhibitors of Aβ-aggregation than peptides containing *all-N*-methyl-amino acid residues.[3b] It should be noted that the interaction between poly-*N*-methylated peptides and α-peptides in the sheet conformation is not sterically inhibited, instead the lower binding observed for *all-N*-Me-peptides over peptides with alternating *N*-Me substituents probably reflects the fact that only every second amide group is capable of contributing to hydrogen bond formation with the sheet.

In conclusion, we have synthesized three representative poly-*N*-methylated α-peptides and been able to determine their structures in the solid-state by X-ray diffraction. All peptides were found to adopt an extended conformation, in which the carbonyl functionalities of the amide bonds are oriented in a way that allows interaction with α-peptides in a strand-conformation. This finding is of utmost importance, considering the wide use of *N*-methyl amino acids in the development of peptides and other substances for use as lead structures in pharmaceutical research and in materials science, as it for the first time provides concrete evidence for the fact that poly-*N*-methylated α-peptides are capable of adopting an extended conformation. This insight also rationalizes why α-peptides with alternating *N*-methyl amino acids are potent inhibitors of amyloidosis formation, and will help in the design of more potent anti-amyloidosis agents, as well as other sheet-containing molecular constructs.†

Notes and references

† X-Ray intensity data were measured at room temperature on a Bruker-Nonius 1kCCD diffractometer, by using MoKα radiation (λ = 0.71073 Å). All structures were solved by direct methods using SIR92[15] and the non-hydrogen atoms were refined anisotropically against F^2, with full-matrix least squares methods by using SHELXL-97.[16] All hydrogen atoms were positioned geometrically and refined as riding. Crystal data for: **1** (*N*-Me-L-Ala)$_6$ ($C_{24}H_{44}N_6O_7\cdot 3H_2O$): M_r = 582.70, triclinic, space group *P*1 (No. 1) , a = 5.9757(3), b = 9.9960(8), c = 13.5831(10) Å, α = 98.898(3)° (1), β = 93.638° (4), γ = 102.759° (4), V = 777.8(1) Å^3, Z = 1, D_x = 1.244 Mg m^{-3}, μ = 0.096 mm^{-1}. θ range 2.82–26.02°, 6936 reflections collected, 3017 independent reflections, 2689 observed ($I > 2\sigma(I)$), R_1 = 0.0534, wR_2 = 0.1319 for 362 parameters, GOF = 1.101. **2** ($C_{42}H_{64}N_6O_9$): M_r = 799.01, orthorhombic, space group $P2_12_12_1$ (No. 19), a = 6.1923(2), b = 18.8230(8), c = 38.868(2) Å, V = 4530.4(1) Å^3, Z = 1, D_x = 1.169 Mg m^{-3}, μ = 0.082 mm^{-1}. θ range 1.05–19.73°, 8407 reflections collected, 2365 independent reflections, 1825 observed ($I > 2\sigma(I)$), R_1 = 0.0668, wR_2 = 0.1952 for 516 parameters, GOF = 1.099. (*N*-Me-L-Ala)$_5$ ($C_{20}H_{37}N_5O_6\cdot H_2O$): M_r = 461.56, triclinic, space group *P*1 (No. 1) , a = 5.9382(4), b = 6.7703(7), c = 15.524(2) Å, α = 97.13° (1), β = 92.39° (1), γ = 99.13° (1), V = 610.2(1) Å^3, Z = 1, D_x = 1.256 Mg m^{-3}, μ = 0.095 mm^{-1}. θ range 0.998–26.373°, 5136 reflections collected, 2353 independent reflections, 2153 observed ($I > 2\sigma(I)$), R_1 = 0.0488, wR_2 = 0.1251 for 290 parameters, GOF = 1.016. CCDC 278107, 278108 and 279547. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b513277k

1 (*a*) F. Haviv, T. D. Fitzpatrick, R. E. Swenson, C. J. Nichols, N. A. Mort, E. U. Bush, G. Diaz, G. Bammert, A. Nguyen, H. N. Nellans, D. J. Hoffman, E. S. Johnson and J. Greer, *J. Med. Chem.*, 1993, **36**, 363; (*b*) D. P. Failie, G. Abbenante and D. R. March, *Curr. Med. Chem.*, 1995, **2**, 654; (*c*) R. Schmidt, A. Kalman, N. N. Chung, C. Lemieux, C. Horvath and P. W. Schiller, *Int. J. Pept. Protein Res.*, 1995, **46**, 47; (*d*) W. L. Cody, J. X. He, M. D. Reily, S. J. Haleen, D. M. Walker, E. L. Reyner, B. H. Stewart and A. M. Doherty, *J. Med. Chem.*, 1997, **40**, 2228.

2 (*a*) J. M. Ostresh, G. M. Husar, S. Blondelle, B. Dorner, P. A. Weber and R. A. Houghten, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 11138; (*b*) S. M. Miller, R. J. Simon, S. Ng, R. N. Zuckermann, J. M. Kerr and W. H. Moos, *Drug Dev. Res.*, 1995, **35**, 20.

3 (*a*) E. Hughes, R. M. Burke and A. J. Doig, *J. Biol. Chem.*, 2000, **275**, 25109; (*b*) D. J. Gordon, K. L. Sciarretta and S. C. Meredith, *Biochemistry*, 2001, **40**, 8237; (*c*) A. J. Doig, E. Hughes, R. M. Burke, T. J. Su, R. K. Heenan and J. Lu, *Biochem. Soc. Trans.*, 2002, **30**, 537; (*d*) D. J. Gordon, R. Tappe and S. C. Meredith, *J. Pept. Res.*, 2002, **60**, 37; (*e*) A. Kapurniotu, A. Schmauder and K. Tenidis, *J. Mol. Biol.*, 2002, **315**, 339; (*f*) D. T. S. Rijkers, J. W. M. Höppener, G. Posthuma, C. J. M. Lips and R. M. J. Liskamp, *Chem. Eur. J.*, 2002, **8**, 4285; (*g*) M. Cruz, J. M. Tusell, D. Grillo-Bosch, F. Albericio, J. Serratosa, F. Rabanal and E. Giralt, *J. Pept. Res.*, 2004, **63**, 324.

4 J. M. Mason, N. Kokkoni, K. Stott and A. J. Doig, *Curr. Opin. Struct. Biol.*, 2003, **13**, 526.

5 (*a*) M. Goodman and M. Fried, *J. Am. Chem. Soc.*, 1967, **89**, 1264; (*b*) J. E. Mark and M. Goodman, *J. Am. Chem. Soc.*, 1967, **89**, 1267; (*c*) M. Goodman, F. Chen and F. R. Prince, *Biopolymers*, 1973, **12**, 2549.

6 (*a*) W. L. Mattice, *Macromolecules*, 1973, **6**, 855; (*b*) Y. Imanishi, K. Kugimiya and T. Higashimura, *Polymer*, 1975, **16**, 350.

7 J. Urban, T. Vaisar, R. Shen and M. S. Lee, *Int. J. Pept. Protein Res.*, 1996, **47**, 182.

8 B. Vitoux, A. Aubry, M. T. Cung and M. Marraud, *Int. J. Pept. Protein Res.*, 1986, **27**, 617.

9 M. Teixidó, F. Albericio and F. Giralt, *J. Pept. Res.*, 2005, **65**, 153.

10 (*a*) B. Thern, J. Rudolph and G. Jung, *Angew. Chem., Int. Ed.*, 2002, **41**, 2307; (*b*) B. Thern, J. Rudolph and G. Jung, *Tetrahedron Lett.*, 2002, **43**, 5013; (*c*) N. Sewald, *Angew. Chem., Int. Ed.*, 2002, **41**, 4661.

11 (*a*) D. Ben-Ishai, *J. Am. Chem. Soc.*, 1957, **79**, 5736; (*b*) R. M. Freidinger, J. S. Hinkle, D. S. Perlow and B. H. Arison, *J. Org. Chem.*, 1983, **48**, 77; (*c*) S. Zhang, T. Govender, T. Norström and P. I. Arvidsson, *J. Org. Chem.*, 2005, **70**, 6918; (*d*) for a review see: L. Aurelio, R. T. C. Brownlee and A. B. Hughes, *Chem. Rev.*, 2004, **104**, 5823.

12 A. A. Adzhubei and M. J. E. Sternberg, *J. Mol. Biol.*, 1993, **229**, 472.

13 (*a*) K. Kirshenbaum, A. E. Barron, R. E. Goldsmith, P. Armand, E. K. Bradley, K. T. V. Truong, K. A. Dill, F. E. Cohen and R. N. Zuckerman, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 4305; (*b*) For a recent overview see: J. A. Patch, K. Kirsenbaum, S. L. Seurynck and R. N. Zuckermann,Versatile Oligo(*N*-Substituted) Glycines: The Many Roles of Peptoids in Drug Discovery, in *Pseudo-peptides in Drug Discovery*, (Ed. P. E. Nielsen), Wiley-VCH, Weinheim, 2004, pp. 1–31.

14 A. J. Doig, *Chem. Commun.*, 1997, 2153.

15 A. Altomare, M. C. Burla, G. Camalli, G. Cascarano, C. Giacovazzo, A. Gualiardi and G. Polidori, *J. Appl. Crystallogr.*, 1994, **27**, 435.

16 G. M. Sheldrick, *SHELXL-97*, A program for crystal structure refinement, University of Göttingen, Göttingen (Germany), 1997.