Design of a Helical Motif Using α,β-Dehydrophenylalanine Residues: Crystal Structure of Boc-Val-ΔPhe-Phe-Ala-Phe-ΔPhe-Val-ΔPhe-Gly-OCH_3, a 3_{10}-Helical Nonapeptide

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The first step in the de novo synthesis of protein mimics[1] is to make a careful choice of an amino acid sequence which generates the desired conformation, such as a helix. In this connection non-protein amino acids such as aminoisobutyric acid (Aib) and α,β-dehydrophenylalanine (ΔPhe) have also been used as conformation-determining residues.[2] Although extensive work using Aib residues has been reported,[3] the structural information on ΔPhe containing oligopeptides is rather limited. Experimental and theoretical conformational studies have indicated that ΔPhe strongly favors the formation of β-turn structures in small peptides.[4,7-9] Recently, the potential of ΔPhe residues in generating a helical motif has begun to be realized, and in small peptides up to pentamers, an incipient 3_{10}-helix has been observed.[4a,b] Therefore, it is of considerable interest to fully explore the possibility of using ΔPhe residues in designing polypeptide helices. We report here the crystal structure of a nonapeptide Boc-Val-ΔPhe-Phe-Ala-Phe-ΔPhe-Val-ΔPhe-Val-ΔPhe-Gly-OCH_3 (I) containing three ΔPhe residues, the longest α,β-dehydrooligopeptide whose crystal structure has been determined so far. Peptide I exhibits three full turns of a right-handed 3_{10}-helix, in the solid state. This clearly demonstrates the utility of ΔPhe as conformation-constraining residues in designing polypeptide helical modules.

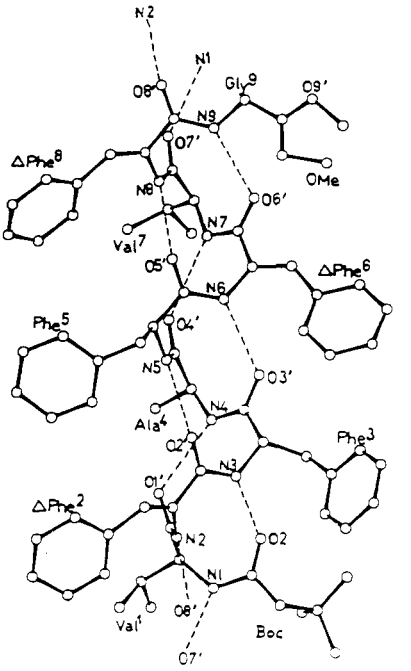

Figure 1. Molecular structure of Boc-Val^1-ΔPhe^2-Phe^3-Ala^4-Phe^5-ΔPhe^6-Val^7-ΔPhe^8-Gly^9-OMe. View perpendicular to the 3_{10}-helix axis. Dashed lines indicate intramolecular 4 → 1 and intermolecular head to tail hydrogen bonds.

Table I. Backbone Torsion Angles

residue	ϕ^a	ψ^a	ω
Val^1	−70.1[b]	−29.0	174.0
ΔPhe^2	−58.8	−23.9	176.6
Phe^3	−68.0	−16.3	175.4
Ala^4	−59.8	−22.8	175.9
Phe^5	−60.7	−16.8	167.7
ΔPhe^6	−46.4	−25.1	−179.6
Val^7	−68.6	−15.4	170.1
ΔPhe^8	−54.6	−35.1	−172.7
Gly^9	154.1		

[a] $\langle\phi\rangle$ = −60.9 and $\langle\psi\rangle$ = −23.0 (excluding Gly^9). [b] C5–N1–C1A–C1′.

The molecular structure[5] of I is illustrated in Figure 1. The backbone torsion angles are listed in Table I. All peptide units are trans. As a result of seven consecutive overlapping type III β-turns[6] formed by seven 4 → 1 intramolecular hydrogen bonds, the peptide adopts a right-handed 3_{10}-helical conformation with three complete helical turns. The carbonyl oxygen of the Boc group is the first acceptor in the helix. Viewed down the helix axis the side chains assume the energetically favorable slightly staggered arrangement, deviating from the ideal 3_{10}-helical conformation where the side chains are completely eclipsed.[11] At Gly^9 the helix is unwound to an extended structure with ϕ = 154.1°. Unwinding of the helix at the C-terminus is not unusual in helical peptide structures.[3e,12,13]

The molecules pack as continuous helical rods along the *b* axis in the crystal structure by head to tail hydrogen bonding. The head to tail region meets in good register, so that two intermolecular hydrogen bonds are formed. There are no lateral hydrogen

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(1) (a) Richardson, J. S.; Richardson, D. C. *Trends Biochem. Sci.* **1989**, *14*, 304. (b) Mutter, M. *Trends Biochem. Sci.* **1988**, *13*, 260. (c) DeGrado, W. F.; Wasserman, Z. R.; Lear, J. D. *Science* **1989**, *243*, 622. (d) Ghadiri, M. R.; Soars, C.; Choi, C. *J. Am. Chem. Soc.* **1992**, *114*, 825. (e) Karle, I. L.; Flippen-Anderson, J. L.; Sukumar, M.; Uma, K.; Balaram, P. *J. Am. Chem. Soc.* **1991**, *113*, 3952. (f) Ho, S. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **1987**, *109*, 6751.

(2) DeGrado, W. F. *Adv. Protein Chem.* **1988**, *39*, 51.

(3) (a) Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747. (b) Bosch, R.; Jung, G.; Schmitt, H.; Winter, W. *Biopolymers* **1985**, *24*, 960. (c) Bavoso, A.; Benedetti, E.; Di Blasio, B.; Pavone, V.; Toniolo, C.; Bonora, G. M. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1988. (d) Karle, I. L.; Flippen-Anderson, J. L.; Uma, K.; Balaram, P. *Biochemistry* **1989**, *28*, 6696. (e) Marshall, G. R.; Hodgkin, E. E.; Langs, D. A.; Smith, G. D.; Zabrocki, J.; Leplawy, M. T. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 487.

(4) (a) Ciajolo, M. R.; Tuzi, A.; Pratesi, C. R.; Fissi, A.; Pieroni, O. *Biopolymers* **1990**, *30*, 911. (b) Bhandary, K. K.; Chauhan, V. S. *Biopolymers*, in press. (c) Chauhan, V. S.; Bhandary, K. K. *Int. J. Pept. Protein Res.* **1992**, *39*, 223. (d) Patel, H. C.; Singh, T. P.; Chauhan, V. S.; Kaur, P. *Biopolymers* **1990**, *29*, 509. (e) Ciajolo, M. R.; Tuzi, A.; Pratesi, C. R.; Fissi, A.; Pieroni, O. *Int. J. Pept. Protein Res.* **1991**, *38*, 539. (f) Singh, T. P.; Haridas, M.; Chauhan, V. S.; Kumar, A.; Viterbo, D. *Biopolymers* **1987**, *26*, 819.

(5) Peptide I was synthesised by using standard procedures. Colorless crystals of I ($C_{66}H_{77}N_9O_{12}$, FW 1188.39) were obtained by slow evaporation of the peptide dissolved in an acetone–isopropyl alcohol mixture at 4 °C. X-ray intensity data with Cu Kα radiation (λ = 1.5418 Å) at 20 °C: *a* = 11.392 (2), *b* = 17.767 (3), *c*, = 17.117 (3) Å, β = 109.1 (1)°, *Z* = 2, monoclinic space group $P2_1$. The structure was solved by direct methods.[10] Least-squares refinement using 5625 reflections (θ < 70°) which had $|F_o| > 3\sigma(|F_o|)$ resulted in an agreement factor *R* = 0.055 and R_w = 0.065. The carbon of the OCH_3 group was found to be disordered between two positions with occupancies 0.55 and 0.45. This kind of OCH_3 end group disorder is not uncommon.[1e] A water molecule having low occupancy (~0.37) was also located.

(6) (a) Venkatachalam, C. M. *Biopolymers* **1968**, *8*, 1425. (b) Rose, G. D.; Gierasch, L. M.; Smith, G. V. *Adv. Protein Chem.* **1985**, *37*, 1.

(7) Ajo, D.; Casarin, M.; Granozzi, G. *J. Mol. Struct.* **1982**, *86*, 297.

(8) (a) Uma, K.; Chauhan, V. S.; Kumar, A.; Balaram, P. *Int. J. Pept. Protein Res.* **1988**, *31*, 349. (b) Chauhan, V. S.; Uma, K.; Kaur, P.; Balaram, P. *Biopolymers* **1989**, *28*, 763. (c) Bach, A. C.; Gierasch, L. M. *Biopolymers* **1986**, *25*, S175.

(9) (a) Bharadwaj, A.; Jaswal, A.; Chauhan, V. S. *Tetrahedron* **1992**, *48*, 2691. (b) Gupta, A.; Bharadwaj, A.; Chauhan, V. S. *J. Chem. Soc., Perkin Trans. 2* **1990**, 1911.

(10) Sheldrick, G. M. In *Crystallographic Computing 3*; Sheldrick, G. M., Kruger, C., Goddard, R., Eds.; Oxford University Press: New York, 1985; pp 175.

(11) (a) Benedetti, E.; Blasio, B. D.; Pavone, V.; Pedone, C.; Toniolo, C.; Crisma, M. *Biopolymers* **1992**, *32*, 453. (b) Toniolo, C.; Benedetti, E. *Trends Biochem. Sci.* **1991**, *16*, 350.

(12) Karle, I. L.; Flippen-Anderson, J. L.; Sukumar, M.; Balaram, P. *Proteins: Struct., Funct., Genet.* **1992**, *12*, 324.

(13) Soman, K. V.; Karimi, A.; Case, D. A. *Biopolymers* **1991**, *31*, 1351.

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bonds between helices. The crystal structure has alternating sheets of aromatic residues (Phe, ΔPhe) and aliphatic residues (Val, Ala) parallel to the *ab* plane and separated by half translation along the *c* direction. Hydrophobic sheets have been observed in other peptide crystal structures also.[14] Adjacent helices are parallel to each other. Head to tail hydrogen bonding is commonly observed in short helical structures containing Aib or ΔPhe residues, packed in either parallel to antiparallel fashion.[3a,4a,b,15]

The high propensity of ΔPhe residues for helix formation is clearly demonstrated as the three ΔPhe residues present in the sequence overcome the effect of the remaining five residues, most of which are known to be poor α-helix formers.[16] The nonapeptide sequence containing three ΔPhe residues shows seven consecutive overlapped type III β-turns. Hence, there is no one to one correspondence between the number of β-bends and the number of ΔPhe residues.[4a] It is worth noting that even though ΔPhe[2] and ΔPhe[6] are contiguously separated by three saturated residues, viz. Phe[3]-Ala[4]-Phe[5], the 3_{10}-helical nature is retained. Thus, the utility of ΔPhe residues in designing polypeptide helices for the eventual design of protein mimics stands established.

Whether dehydro residues can also be used in designing α-helices and the role of the number and positioning of the dehydro residues in relation to the non-dehydro residues for defining the polypeptide conformation need to be further investigated by carrying out more peptide syntheses and structural studies.

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Supplementary Material Available: Synthesis and X-ray experimental details and tables of positional and thermal parameters, bond lengths, and bond angles for peptide I (27 pages); table of observed and calculated structure factors (24 pages). Ordering information is given on any current masthead page.

(14) Gorbitz, C. H.; Etter, M. C. *Int. J. Pept. Protein Res.* **1992**, *39*, 93.
(15) Karle, I. L. *Biopolymers* **1989**, *28*, 1.
(16) Chou, P. Y.; Fasman, G. D. *Biochemistry* **1974**, *13*, 222.

Solvent Effects on the Transition States for Nucleophilic Additions to Substituted Acetaldehydes

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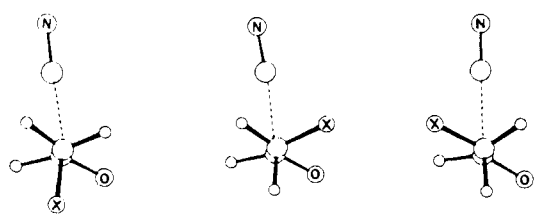
The origin of the π-facial selectivity in nucleophilic additions to carbonyl groups has been of considerable recent interest both experimentally[1] and theoretically,[2-4] and several models have been proposed to explain the experimental observations.[5] A series of careful theoretical studies of the addition to substituted acetaldehydes in the gas phase has recently been reported using both cyanide ion[2,4] and lithium hydride as nucleophiles.[3,4] However, in practice, all nucleophilic additions to carbonyl groups are carried out in solution. The addition of a molecule of lithium hydride across a C=O group in the gas phase would appear to have little to do with nucleophilic additions of hydride ions in solution. Here, the reaction need not proceed via a concerted addition, and it is likely to involve ion pairs or free ions. The addition of cyanide ion is a fundamentally simpler process, and the gas-phase calculations may well have a bearing on the process in solution.

Wong and Paddon-Row[2] have noted that there are important electrostatic effects in the addition of cyanide ion to substituted acetaldehydes. The electrostatic interactions will be mediated by solvents, and the relative energies of the several conformationally different transition states may vary significantly on going from the gas phase to solution. The Onsager reaction field model[6] as incorporated into ab initio MO theory using a spherical cavity for the solute has been found to be remarkably successful in reproducing the effects of solvents on the relative energies of conformers.[7] Therefore, we have applied it to the study of cyanide ion additions using the RHF/6-31G* level of theory.[8]

The anti, +sc, and −sc transition-state conformers were examined by geometry optimization at the RHF/6-31G* level for ε = 1 (gas phase), ε = 7.2 (a weakly polar solvent such as dimethoxyethane or tetrahydrofuran (ε = 7.6)), and ε = 78.5 (a highly polar solvent; any value over ~50 would give the same result) where X = CH_3, F, CN, and SiH_3. The relative energies

Table I. Effect of Solvents on the Relative Energies (kcal/mol) of Transition States

X	ε	anti	−sc	+sc
CH_3	1	0.62	***0.00***	1.42
	7.2	***0.00***	0.82	1.20
	78.5	***0.00***	1.09	1.19
F	1	***0.00***	3.67	0.49
	7.2	0.80	2.99	***0.00***
	78.5	1.06	2.91	***0.00***
SiH_3	1	0.85	***0.00***	3.26
	7.2	***0.00***	1.19	3.57
	78.5	***0.00***	1.64	3.61
CN	1.0	***0.00***	2.53	1.41
	7.2	0.03	0.22	***0.00***
	78.5	0.30	***0.00***	0.03



(1) Danishefsky, S. J.; Langer, M. *J. Org. Chem.* **1985**, *50*, 3672. Cheung, C. K.; Tseng, L. T.; Lin, M. H.; Srivastava, S.; le Noble, W. J. *J. Am. Chem. Soc.* **1986**, *108*, 1598. Fraser, R. R.; Stanciulescu, M. *J. Am. Chem. Soc.* **1987**, *109*, 1580. Lodge, E. P.; Heathcock, C. H. *J. Am. Chem. Soc.* **1987**, *109*, 3353. Mukherjee, D.; Wu, Y.-D.; Froczek, F. R.; Houk, K. N. *J. Am. Chem. Soc.* **1988**, *110*, 3328. Cieplak, A. S.; Tait, B. D.; Johnson, C. R. *J. Am. Chem. Soc.* **1989**, *111*, 8447. Okada, K.; Tomita, S.; Oda, M. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 459. Mehta, G.; Kahn, F. A. *J. Am. Chem. Soc.* **1990**, *112*, 6140. Halterman, R. L.; McElvoy, M. *J. Am. Chem. Soc.* **1990**, *112*, 6690. Heathcock, C. H. *Aldrichimica Acta* **1990**, *23*, 99. DiMaio, G.; Migneco, L. M.; Vecchi, E. *Tetrahedron* **1990**, *46*, 6053. Biesemans, M.; van de Woude, H.; van Hove, L. *Bull. Soc. Chim. Belg.* **1990**, *99*, 29. Nagao, Y.; Goto, M.; Ochiai, M. *Chem. Lett.* **1990**, 1507. Lange, J. H. M.; Klunder, A. J. H.; Zwanenburg, B. *Tetrahedron* **1991**, *47*, 1495. Li, H.; Mehta, G.; Padma, S.; le Noble, W. J. *J. Org. Chem.* **1991**, *56*, 2006. Hahn, J. M.; le Noble, W. J. *J. Am. Chem. Soc.* **1992**, *114*, 1916. Paquette, L. A.; Underiner, T. L.; Gallucci, J. C. *J. Org. Chem.* **1992**, *57*, 86. Mehta, G.; Praveen, M. *Tetrahedron Lett.* **1992**, *33*, 1759. Gmeiner, P.; Bollinger, B. *Ann. Chem.* **1992**, 273. Senda, Y.; Okamura, K.; Kuwahara, M.; Ide, M.; Itoh, H.; Ishiyama, J.-I. *J. Chem. Soc., Perkin. Trans. 2* **1992**, 799.
(2) Wong, S. S.; Paddon-Row, M. N. *Aust. J. Chem.* **1991**, *44*, 765. Wong, S. S.; Paddon-Row, M. N. *J. Chem. Soc., Chem. Commun.* **1991**, 327; **1990**, 456.
(3) Wu, Y.-D.; Tucker, J. A.; Houk, K. N. *J. Am. Chem. Soc.* **1991**, *113*, 5027. Wu, J.-D.; Houk, K. N. *J. Am. Chem. Soc.* **1987**, *109*, 908.
(4) (a) Frenking, G.; Köhler, K. F.; Reetz, M. T. *Tetrahedron* **1991**, *47*, 9005. (b) Frenking, G.; Köhler, K. F.; Reetz, M. *Angew. Chem.* **1991**, *103*, 1167. (c) Coxon, J. M.; McDonald, D. Q. *Tetrahedron* **1992**, *48*, 3353.
(5) Cherest, M.; Felkin, H.; Prudent, N. *Tetrahedron Lett.* **1968**, 2199. Cherest, M.; Felkin, H. *Tetrahedron Lett.* **1968**, 2205. Ahn, N. T.; Eisenstein, O *Nouv. J. Chem.* **1977**, *1*, 61. Ahn, N. T. *Top. Curr. Chem.* **1980**, *88*, 1. Cieplak, A. S. *J. Am. Chem. Soc.* **1981**, *103*, 4540.
(6) Onsager, L. *J. Am. Chem. Soc.* **1936**, *56*, 1486.
(7) (a) Wong, M. W.; Frisch, M.; Wiberg, K. B. *J. Am. Chem. Soc.* **1991**, *113*, 4776. (b) Wong, M. W.; Wiberg, K. B.; Frisch, M. J. *J. Am. Chem. Soc.* **1992**, *114*, 523, 1645.
(8) The reaction field model used herein, i.e., a spherical cavity and considering just the dipole, is the simplest implementation. It, however, remains the only model for which analytical gradients (ref 7a) and second derivatives (Wong, M. W.; Wiberg, K. B.; Frisch, M. J. *J. Chem. Phys.* **1991**, *95*, 8991) are available. In view of the success of this model in other cases (ref 7), the present calculations would be expected to correctly reproduce the trends of relative energies with changing the dielectric constant.

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