Acta Crystallographica Section C
Crystal Structure Communications
ISSN 0108-2701

Design of peptides with α,β-dehydro residues: pseudo-tripeptide *N*-benzyloxycarbonyl–ΔLeu–L-Ala–L-Leu–OCH_3

Jyoti Makker, Sharmistha Dey, Pravindra Kumar and Tej P. Singh*

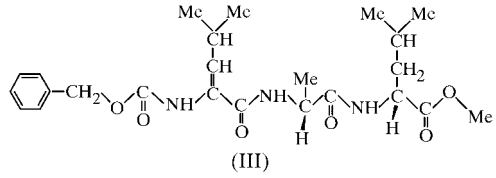
Department of Biophysics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110 029, India
Correspondence e-mail: tps@aiims.aiims.ac.in

Received 11 June 2001
Accepted 7 December 2001
Online 12 March 2002

The title peptide *N*-benzyloxycarbonyl–ΔLeu–L-Ala–L-Leu–OCH_3 [methyl *N*-(benzyloxycarbonyl)-α,β-dehydroleucyl-L-alanyl-L-leucinate], $C_{24}H_{35}N_3O_6$, was synthesized in the solution phase. The peptide adopts a type II′ β-turn conformation which is stabilized by an intramolecular 4 → 1 N—H···O hydrogen bond. The crystal packing is stabilized by two intermolecular N—H···O hydrogen bonds.

Comment

The conformational preferences of amino acid side chains govern the folding of peptides. These preferences differ from one amino acid to another, as observed in protein crystals (Chandrasekaran & Ramachandran, 1970; Janin *et al.*, 1978; Bhat *et al.*, 1979) and oligopeptides (Benedetti *et al.*, 1983), and as determined theoretically by means of conformational energy computations (Zimmerman *et al.*, 1977; Vasquez *et al.*, 1983). Short-range interactions involving the atoms of the side chains with the atoms of the backbone, as well as the atoms of the two neighbouring peptide units, determine the conformational preferences in peptides. Thus, the peptides can adopt a large number of conformations in order to gain preferred side-chain–backbone and side-chain–side-chain interactions. This makes the design strategy rather weak and impractical. In order to develop an effective design tool, it is necessary to restrict the number of preferred conformations to a minimum. This can be achieved through introduction of well defined steric constraints with α,β-dehydro residues. So far, it has been shown that the dehydro residues, such as dehydrophenylalanine (ΔPhe), dehydroleucine (ΔLeu) and dehydro-α-aminobutyric acid (ΔAbu), induce a type II β-turn conformation when placed at the (*i*+2) position (Singh & Narula, 1996). However, the conformational contributions of these dehydro residues when placed at the (*i*+1) position have not yet been fully defined. In order to establish the design rules with α,β-dehydro residues at the (*i*+1) position, a tripeptide, *N*-benzyloxycarbonyl(Cbz)–ΔLeu–L-Ala–L-Leu–OCH_3, (III), was synthesized and its three-dimensional structure determined by X-ray diffraction.



(III)

The structure of peptide Cbz–ΔLeu–L-Ala–L-Leu–OCH_3 shows that the side chain of the ΔLeu residue adopts the expected geometry, with the vinyl H atom on the same side of the adjacent carbonyl group. The C1*A*—C1*B* bond length of 1.322 (5) Å is in agreement with the average value of 1.323 (2) Å quoted for this bond (Benedetti, 1977). The planarity imposed by the α,β-double bond should promote an electronic delocalization, with shrinking of the C1*A*—N1 and C1*A*—C1*P* bonds, and lengthening of the carbonyl double bond (Table 1). The C1*A*—N1 and C1*A*—C1*P* bond lengths of 1.413 (4) and 1.500 (4) Å, respectively, are in accordance with the expected values reported for dehydro residues (Singh & Narula, 1996). The C1*P*—O1*P* bond length of 1.217 (4) Å seems to be only slightly influenced by electronic effects. The bond angles N1—C1*A*—C1*P*, N1—C1*A*—C1*B* and C1*A*—C1*B*—C1*G* in the ΔLeu residue deviate from the standard value of 120° (Table 1). The opening of the C1*A*—C1*B*—C1*G* angle helps in releasing the constraints caused by the changes introduced in an amino acid as a result of dehydrogenations at the C1*A* and C1*B* atoms.

A perspective view of the title molecule is shown in Fig. 1. Selected torsion angles are given in Table 1. The peptide adopts a type II′ β-turn conformation characterized by torsion angles φ_1 = 47.9 (4)°, ψ_1 = −137.2 (3)°, φ_2 = −87.2 (3)° and

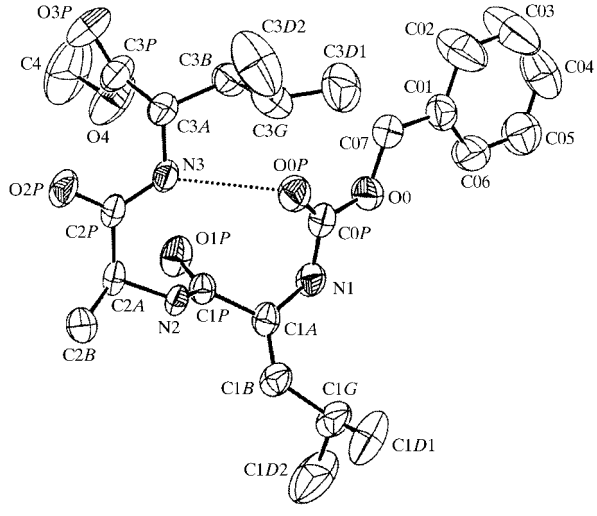


Figure 1
A perspective view of the title peptide. Displacement ellipsoids are drawn at the 50% probability level.

  DOI: 10.1107/S0108270101016468

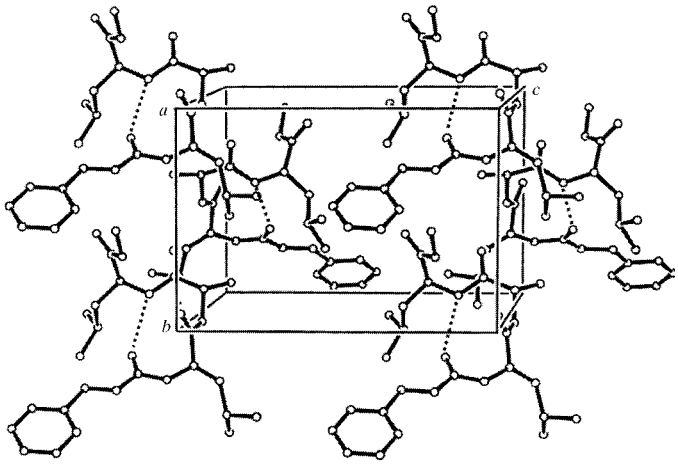


Figure 2
The molecular packing viewed along the *a* axis.

ψ_2 = 10.2 (4)°, and an intramolecular N3—H3···O0P 4 → 1 hydrogen bond [N3···O0*P* = 3.181 (7) Å and N3—H3···O0*P* = 151°]. The side-chain conformation of the ΔLeu residue, with χ_1^1 = −0.4 (6), $\chi_1^{2,1}$ = 111.1 (5) and $\chi_1^{2,2}$ = −127.3 (5)°, is different from that found in the saturated leucine residue. The two sets of values of the side-chain torsion angles in Leu are −60, −60 and 180°, and 180, 180 and −60°, with the first combination being observed more frequently. It may be mentioned here that this is the first complete sequence with a dehydro residue at the (*i*+1) position which shows the formation of a type II′ β-turn conformation. In an earlier structure with the sequence *N*-Ac–ΔPhe–L-Val–L-Val–OCH_3, the peptide adopted an open conformation (Narula *et al.*, 1991), presumably due to the presence of branched β-carbon residues in the sequence. The structure of the present peptide indicates that peptides with a dehydro residue at the (*i*+1) position tend to form a type II β-turn conformation in the presence of a non-β-branched residue at the (*i*+2) position.

The packing of the molecules in the crystal is shown in Fig. 2 and details of the hydrogen bonds are given in Table 2. Two intermolecular hydrogen bonds are formed, involving the NH and CO groups of screw-related ΔLeu and Ala residues. The packing is also stabilized by hydrophobic regions involving the benzene ring of the Cbz group, the side chain of the ΔLeu residue and the terminal OCH_3 group.

Experimental

Compound (I), Cbz–ΔLeu–OH, was synthesized by the condensation of 3-methyl-2-oxopentanoic acid (0.9 g, 7.8 mmol) with benzyl carbamate (1.4 g, 9.4 mmol) and *p*-toluenesulfonic acid (0.27 g, 9.4 mmol) in dry benzene. The reaction mixture was refluxed for 8 h at 373 K using a Dean–Stark water remover. The solution was then extracted with saturated sodium bicarbonate. The extracts were neutralized by adding concentrated hydrochloric acid dropwise to yield a white solid, which was filtered off and recrystallized from benzene. The solid product of Cbz–ΔLeu–OH was obtained in a yield of 67%. For the synthesis of NH_3–L-Ala–L-Leu–OCH_3 trifluoroacetate solvate, (II), isobutyl chloroformate (IBCF; 2.14 ml, 16 mmol) and *N*-methylmorpholine (NMM; 1.75 ml, 16 mmol) were added to a precooled solution (263 K) of Boc–L-Ala–OH (3.0 g, 16 mmol) in dichloromethane (DCM) and the resulting solution stirred for 20 min. To this, a precooled solution of L-Leu–OCH_3·HCl (2.75 g, 19 mmol) in DCM and NMM (2.1 ml, 19 mmol) was added and stirred for 3 h at 263 K and then at room temperature overnight. L-Amino acids were used in all the syntheses. The resulting solution was evaporated, taken up in ethyl acetate and washed three times with 10% sodium bicarbonate and three times with 5% citric acid and water, dried over anhydrous sodium sulfate and then evaporated to yield Boc–L-Ala–L-Leu–OCH_3, which was dissolved in 1:1 (*v*/*v*) DCM–TFA (trifluoroacetic acid) and stirred for 1 h at room temperature. The resulting solution was concentrated *in vacuo* and triturated with dry ether to give (II) [yield 3.51 g (10.6 mmol), 66.7%; m.p. 388 K]. For the synthesis of Cbz–ΔLeu–L-Ala–L-Leu–OCH_3, (III), triethylamine (TEA; 0.11 ml, 0.8 mmol) and IBCF (0.1 ml, 0.8 mmol) were added to a precooled solution of (I) (200 mg, 0.8 mmol) in tetrahydrofuran and the resulting solution stirred for 20 min at 263 K. To this, a precooled solution of (II) in TEA (320 mg, 0.96 mmol) was added and stirring continued for 3 h at 283 K and then at room temperature overnight. The final solution was worked up as outlined for (II) and the final compound was recrystallized from an ethyl acetate–petrol solution (yield: 180 mg).

Crystal data

$C_{24}H_{35}N_3O_6$	D_x = 1.185 Mg m^{-3}
M_r = 461.55	Cu $K\alpha$ radiation
Monoclinic, $P2_1$	Cell parameters from 25 reflections
a = 10.2550 (15) Å	θ = 0–25°
b = 9.5090 (14) Å	μ = 0.70 mm^{-1}
c = 13.4550 (14) Å	T = 293 (2) K
β = 99.715 (11)°	Prism, colourless
V = 1293.9 (3) Å^3	0.3 × 0.2 × 0.1 mm
Z = 2	

Table 1
Selected geometric parameters (Å, °).

N1—C1*A*	1.413 (4)	C1*P*—N2	1.341 (4)
C1*A*—C1*B*	1.322 (5)	N2—C2*A*	1.451 (4)
C1*A*—C1*P*	1.500 (4)		
O0*P*—C0*P*—N1	125.2 (3)	N1—C1*A*—C1*P*	115.5 (3)
C0*P*—N1—C1*A*	121.6 (3)	O1*P*—C1*P*—N2	123.1 (3)
C1*B*—C1*A*—N1	123.3 (3)	O1*P*—C1*P*—C1*A*	121.1 (3)
C1*B*—C1*A*—C1*P*	120.9 (3)	N2—C1*P*—C1*A*	115.7 (3)
C0*P*—N1—C1*A*—C1*P*	47.9 (4)	N2—C2*A*—C2*P*—N3	10.2 (4)
N1—C1*A*—C1*B*—C1*G*	−0.4 (7)	C2*P*—N3—C3*A*—C3*P*	−60.3 (4)
C1*A*—C1*B*—C1*G*—C1*D*2	−127.3 (5)	N3—C3*A*—C3*B*—C3*G*	−73.0 (4)
C1*A*—C1*B*—C1*G*—C1*D*1	111.1 (5)	C3*A*—C3*B*—C3*G*—C3*D*2	−77.1 (5)
N1—C1*A*—C1*P*—N2	−137.2 (3)	C3*A*—C3*B*—C3*G*—C3*D*1	160.1 (4)
C1*P*—N2—C2*A*—C2*P*	−87.2 (3)	N3—C3*A*—C3*P*—O3*P*	149.4 (5)

Table 2
Hydrogen-bonding geometry (Å, °).

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
N1—H1···O1*P*^{i}	0.86	2.08	2.904 (4)	159
N2—H2···O2*P*^{ii}	0.86	2.01	2.848 (4)	165
N3—H3···O0*P*	0.86	2.40	3.181 (7)	151

Symmetry codes: (i) $2-x, \frac{1}{2}+y, -z$; (ii) $1-x, \frac{1}{2}+y, -z$.

Data collection

Enraf–Nonius CAD-4 diffractometer
ω–2θ scans
Absorption correction: empirical *via* ψ scans (*SDP*; Enraf–Nonius, 1979)
T_{min} = 0.845, T_{max} = 0.932
2887 measured reflections
2731 independent reflections
2363 reflections with $I > 2\sigma(I)$
R_{int} = 0.051
θ_{max} = 75.0°
$h = 0 \rightarrow 12$
$k = 0 \rightarrow 11$
$l = -16 \rightarrow 16$
3 standard reflections
frequency: 60 min
intensity decay: none

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.056
$wR(F^2)$ = 0.162
S = 1.20
2731 reflections
294 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.1P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.006
$\Delta\rho_{max}$ = 0.21 e Å^{-3}
$\Delta\rho_{min}$ = −0.20 e Å^{-3}
Absolute structure: Flack (1983)
Flack parameter = 0.0 (4)

H atoms were refined as riding, with C—H distances in the range 0.93–0.98 Å.

Data collection: *SDP* (Enraf–Nonius, 1979); cell refinement: *SDP*; data reduction: *SDP*; program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1997); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *PLUTON* (Spek, 1999); software used to prepare material for publication: *SHELXL*97.

The authors thank the Council of Scientific and Industrial Research, New Delhi, for financial support.

Supplementary data for this paper are available from the IUCr electronic archives (Reference: DE1174). Services for accessing these data are described at the back of the journal.

References

Benedetti, E. (1977). *Peptides*, edited by M. Goodman & J. Meinhofer, p. 257. New York: John Wiley and Sons Inc.
Benedetti, E., Morelli, G., Nemethy, G. & Scheraga, H. A. (1983). *Int. J. Pept. Protein Res.* **22**, 1–15.
Bhat, T. N., Sasisekaran, V. & Vijayan, M. (1979). *Int. J. Pept. Protein Res.* **13**, 170–180.
Chandrasekaran, R. & Ramachandran, G. N. (1970). *Int. J. Pept. Protein Res.* **2**, 223–233.
Enraf–Nonius (1979). *Structure Determination Package*. Enraf–Nonius, Delft, The Netherlands.
Flack, H. D. (1983). *Acta Cryst.* A**39**, 876–881.
Janin, J., Wodak, S., Levitt, M. & Margret, B. (1978). *J. Mol. Biol.* **125**, 357–386.
Narula, P., Khandelwal, B. & Singh, T. P. (1991). *Biopolymers*, **31**, 987–992.
Sheldrick, G. M. (1997). *SHELXL*97 and *SHELXS*97. University of Göttingen, Germany.
Singh, T. P. & Narula, P. (1996). *Prog. Biophys. Mol. Biol.* **66**, 141–165.
Spek, A. L. (1999). *PLUTON*. University of Utrecht, The Netherlands.
Vasquez, M., Nemethy, G. & Scheraga, H. A. (1983). *Macromolecules*, **16**, 1043–1049.
Zimmerman, S. S., Pottle, M. S., Nemethy, G. & Scheraga, H. A. (1977). *Macromolecules*, **10**, 1–9.