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N-(*tert*-Butoxycarbonylglycyl-α,β-dehydrophenylalanylglycylphenylalanyl)-4-nitroaniline

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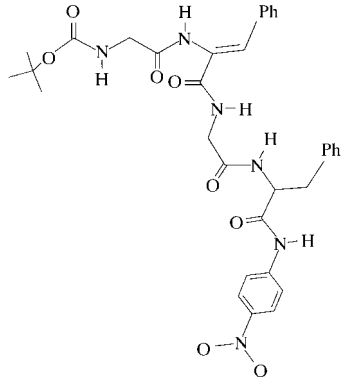
In the crystal structure of the tetrapeptide Boc^0–Gly^1–ΔPhe^2–Gly^3–Phe^4–*p*-NA (*p*-NA is *para*-nitroaniline), $C_{33}H_{36}N_6O_8$, there are two independent molecules differing in conformation in the asymmetric part of the unit cell. All the amino acids in the peptide are linked *trans* to each other. The torsion angles in the main chain of both molecules are close to the values of the type β-II turn. Two intramolecular and three intermolecular N—H···O hydrogen bonds stabilize the conformation of each of the molecules.

Comment

The α,β-dehydroamino acid residues have been found to occur naturally in several microbial peptides and antibiotics (Noda *et al.*, 1983; Spatola, 1983). Their presence in peptides confers increased resistance to enzymatic degradation and for this reason many highly active analogues of bioactive peptides have been designed and synthesized (Fisher *et al.*, 1981; Costa *et al.*, 1983; Sharma & Chauhan, 1988). In particular, α,β-dehydrophenylalanine (ΔPhe) residues exhibit preferential secondary structural features both in the solid state and in solution. Determination of the crystal and molecular structure of many ΔPhe-containing peptides has provided evidence that ΔPhe is a strong inducer of β-bends (Venkatachalam, 1968) in short sequences with a single ΔPhe residue (Główka *et al.*, 1987; Główka, 1988; Aubry *et al.*, 1991) and of 3_{10}-helical structures in long sequences (Rajashankar *et al.*, 1992; Padmanabhan & Singh, 1993; Rajashankar, Ramakumar, Jain & Chauhan, 1995; Rajashankar, Ramakumar, Mal *et al.*, 1995; Jain *et al.*, 1997). Additionally, the final conformation of any ΔPhe peptide depends upon the number and position of ΔPhe residues as well as the nature of the amino acids flanking them (Rajashankar *et al.*, 1996).

We present here the crystal structure of a tetrapeptide containing one Δ^ZPhe between two flexible glycine residues and one phenylalanine, *i.e.* Boc^0–Gly^1–ΔPhe^2–Gly^3–Phe^4–*p*-NA (*p*-NA is *para*-nitroaniline), (I). In the independent part of the unit cell, there are two independent molecules (*A* and *B*) differing in conformation (Fig. 1). The bond lengths and angles for the two molecules are the same within 5σ (Table 1).

The $C_\alpha{=}C_\beta$ (C8—C9 and C8′—C9′) distances for the ΔPhe residue in *A* and *B* agree well with the standard double-bond distance observed in structures containing ΔPhe (*e.g.* Główka, 1988). The conjugation of the aromatic ring with the $C_\alpha{=}C_\beta$ bond is extended to the N—C_α (N2—C8 and N2′—C8′) and C_α—C (C8—C16 and C8′—C16′) bonds. A shortening of about 0.35 Å for N—C_α and 0.55 Å for C_α—C is observed with respect to the corresponding bonds in the saturated Phe^4 unit (N4—C19, N4′—C19′, C19—C27 and C19′—C27′). The steric contacts between the side-chain and main-chain atoms of the ΔPhe residue are partly relaxed by rearrangement of the bond angles at C_α and C_β atoms. For example, the N—$C_\alpha{=}C_\beta$ (N2—C8—C9 and N2′—C8′—C9′) angles are increased from the value of 120° by *ca* 4°, whereas the $C_\beta{=}C_\alpha$—C (C9—C8—C16 and C9′—C8′—C16′) angles are reduced by *ca* 2°. Similar effects are observed in Boc–Val–ΔPhe–ΔPhe–ΔPhe–Val–OMe (Jain *et al.*, 1997).



(I)

All the amino acids in the peptide are linked *trans* to each other with a deviation of ω from 180° of less than *ca* 6° [with the exception of 11.8 (2)° for ω^4 for the *A* molecule]. The torsion angles in the main chains of both molecules and the (Φ/Ψ) of Gly^1 and Δ^ZPhe^2 approximate the values assigned to the amino acid residues in the corners of a type II β-turn. The intramolecular hydrogen bonds between the amide (N3 and N3′) of Gly^3 and the carboxyl (O2 and O2′) of the Boc groups are similar to those observed in Boc–Gly–ΔPhe–Gly–OMe (Główka, 1988). The torsion angles χ^1 [−7.4 (4) and 9.2 (4)°], $\chi^{2,1}$ [−27.9 (4) and 29.5 (4)°] and $\chi^{2,2}$ [155.0 (2) and −154.1 (2)°] of the ΔPhe residue suggest that the side chains in the *A* and *B* molecules are planar.

The two Gly residues adopt a conformation known as a polyglycine helix (Walton, 1981), with Φ and Ψ close to 80 and −150°, respectively. The relative inclination of the planes of the two Gly units is 37.5 (3)° for *A* and 31.5 (3)° for *B*. The torsion angles for the Boc group (ω^0 and Φ^0) in both molecules (*A* and *B*) correspond to a *trans–trans* conformation. This makes it possible for O2 (Boc) atoms to take part in an intramolecular N3—H3*D*···O2 hydrogen bond (Table 2). The carbonyl oxygen of ΔPhe^2 takes part in another intramolecular hydrogen bond with the amide group of *p*-NA. Those

two intramolecular hydrogen bonds stabilize the conformation of both molecules. Each of the molecules is connected to three others by N—H···O hydrogen bonds of medium strength.

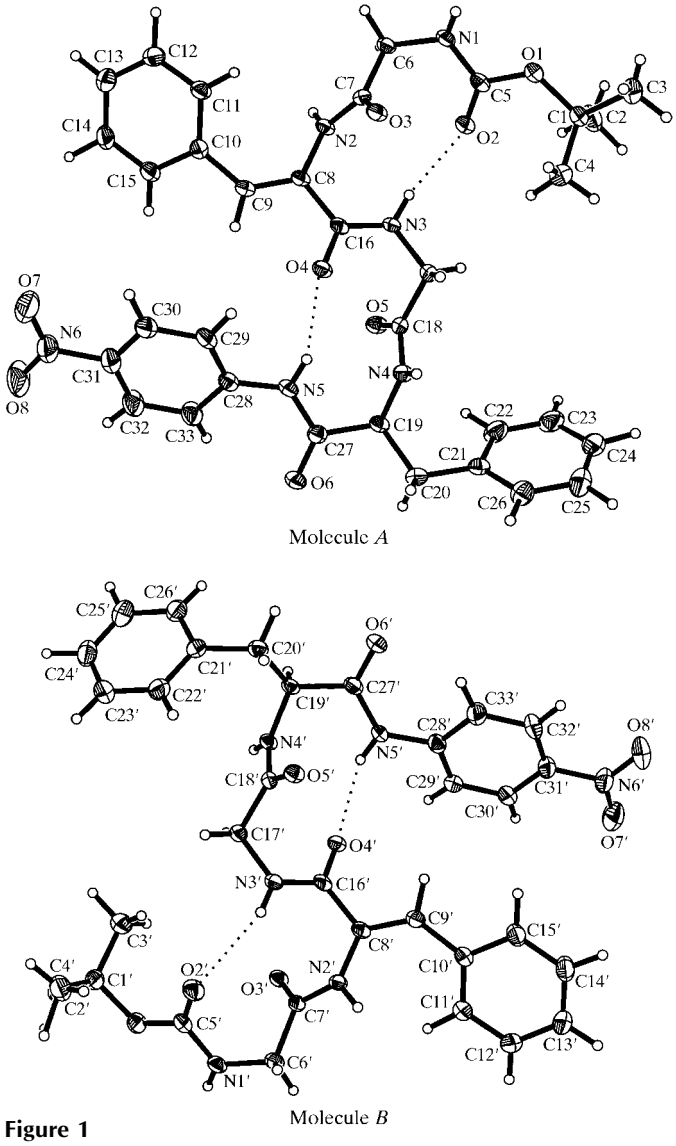


Figure 1
The molecular structure (molecules *A* and *B*) of Boc–Gly–ΔPhe–Gly–Phe–*p*-NA. Displacement ellipsoids are shown at the 50% probability level. Dashed lines indicate intramolecular hydrogen bonds.

Experimental

Boc–Gly–ΔPhe–Gly–Phe–*p*-NA was synthesized according to the method of Makowski *et al.* (2001). Crystals suitable for X-ray structure analysis were grown from an ethyl acetate–diethyl ether (1:1)/hexane solution.

Crystal data

$C_{33}H_{36}N_6O_8$
M_r = 644.68
Triclinic, *P*1
a = 10.328 (2) Å
b = 11.605 (2) Å
c = 14.796 (3) Å
α = 70.00 (3)°
β = 86.37 (3)°
γ = 76.19 (3)°
V = 1617.9 (5) Å^3
Z = 2
D_x = 1.323 Mg m^{-3}
Mo *Kα* radiation
Cell parameters from 29 reflections
θ = 5–12°
μ = 0.096 mm^{-1}
T = 105 (2) K
Plate, yellow
0.5 × 0.4 × 0.3 mm

Data collection

Kuma KM-4 diffractometer
ω scans
11299 measured reflections
5804 independent reflections
5429 reflections with $I > 2\sigma(I)$
R_{int} = 0.029
θ_{max} = 26.06°
$h = -12 \rightarrow 12$
$k = -13 \rightarrow 12$
$l = -16 \rightarrow 16$
2 standard reflections
every 50 reflections
intensity decay: 0.34%

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.032
$wR(F^2)$ = 0.105
S = 1.018
5804 reflections
847 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.0814P)^2 + 0.1142P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.011
$\Delta\rho_{max}$ = 0.27 e Å^{-3}
$\Delta\rho_{min}$ = −0.37 e Å^{-3}

Table 1
Selected geometric parameters (Å, °).

N2—C8	1.421 (3)	N2′—C8′	1.413 (3)
N4—C19	1.454 (3)	N4′—C19′	1.454 (3)
C8—C9	1.339 (4)	C8′—C9′	1.339 (4)
C8—C16	1.489 (4)	C8′—C16′	1.488 (4)
C19—C20	1.529 (4)	C19′—C20′	1.531 (4)
C19—C27	1.537 (4)	C19′—C27′	1.543 (4)
C9—C8—N2	124.1 (2)	C9′—C8′—N2′	124.3 (2)
C9—C8—C16	118.1 (2)	C9′—C8′—C16′	117.5 (2)
N2—C8—C16	117.6 (2)	N2′—C8′—C16′	117.8 (2)

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

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
N1—H1*A*···O3′^{i}	0.90	2.06	2.898 (3)	154
N2—H2*D*···O5′^{ii}	0.90	2.04	2.879 (3)	156
N3—H3*D*···O2	0.90	2.27	3.053 (3)	145
N4—H4*D*···O4′^{iii}	0.90	2.06	2.917 (3)	160
N5—H5*A*···O4	0.90	2.09	2.917 (3)	153
N5—H5*A*···N4	0.90	2.26	2.724 (3)	112
N1′—H1′*A*···O3^{iv}	0.90	2.16	2.965 (3)	149
N2′—H2′*D*···O5^{v}	0.90	1.83	2.693 (3)	159
N3′—H3′*D*···O2′	0.90	2.46	3.215 (3)	141
N4′—H4′*D*···O4^{vi}	0.90	2.05	2.873 (3)	151
N5′—H5′*A*···O4′	0.90	2.13	2.964 (3)	153
N5′—H5′*A*···N4′	0.90	2.28	2.737 (3)	111

Symmetry codes: (i) *x*, *y*, 1 + *z*; (ii) *x*, *y* − 1, 1 + *z*; (iii) 1 + *x*, *y* − 1, 1 + *z*; (iv) *x*, *y*, *z* − 1; (v) *x*, 1 + *y*, *z* − 1; (vi) *x* − 1, 1 + *y*, *z* − 1.

The structure is non-centrosymmetric since both molecules have the same configuration (*S* on C19 and C19′). Refinement in the centrosymmetric $P\overline{1}$ space group was attempted and gave an *R* value of *ca* 12%. In the absence of any significant anomalous scatterers, the Friedel equivalents were merged and the absolute configuration set by reference to that of natural phenylalanine. H atoms were treated as riding with N—H = 0.90 Å and C—H = 0.96 Å.

Data collection: *Kuma Diffraction Software* (Kuma, 1998); cell refinement: *Kuma Diffraction Software*; data reduction: *Kuma Diffraction Software*; program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1997); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *SHELXTL* (Sheldrick, 1990); software used to prepare material for publication: *SHELXL*97.

Table 3
Important torsion angles (°).

Angle	Molecule *A*	Molecule *B*
Φ^0 C6—N1—C5—O1	−169.8 (2)	164.0 (2)
ω^0 C1—O1—C5—N1	−179.1 (2)	−176.1 (2)
Φ^1 C5—N1—C6—C7	53.9 (3)	−52.7 (3)
ω^1 C8—N2—C7—C6	177.4 (2)	−178.3 (2)
Ψ^1 N1—C6—C7—N2	−143.8 (2)	149.3 (2)
Φ^2 C7—N2—C8—C16	−59.3 (3)	49.9 (3)
χ^1 N2—C8—C9—C10	−7.6 (4)	9.2 (5)
$\chi^{2,1}$ C8—C9—C10—C11	−27.8 (4)	29.5 (5)
$\chi^{2,2}$ C8—C9—C10—C15	155.1 (2)	−154.2 (3)
ω^2 C17—N3—C16—C8	−178.0 (2)	175.3 (3)
Ψ^2 N2—C8—C16—N3	−19.2 (3)	29.0 (3)
Φ^3 C16—N3—C17—C18	55.1 (2)	−55.6 (3)
ω^3 C19—N4—C18—C17	−175.9 (2)	173.9 (2)
Ψ^3 N3—C17—C18—N4	−135.0 (2)	130.1 (2)
Φ^4 C18—N4—C19—C27	−91.3 (2)	61.4 (3)
ω^4 C28—N5—C27—C19	168.2 (2)	175.0 (2)
Ψ^4 N4—C19—C27—N5	16.8 (3)	20.7 (3)

Supplementary data for this paper are available from the IUCr electronic archives (Reference: GD1118). Services for accessing these data are described at the back of the journal.

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