Structure and conformation of the tripeptide: N-t-Boc–Prolyl– Phenylalanyl–Proline (Boc–Pro–Phe–Pro)

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The structure and conformation of the tripeptide N-t-Boc–Prolyl–Phenylalanyl–Proline (Boc–Pro-Phe–Pro) ($C_{24}H_{33}N_3O_6$) have been investigated with X-ray crystallographic and spectroscopic methods. Two conformations of Boc–Pro–Phe–Pro crystallized in the space group $P2_12_12_1$ with cell dimensions a = 11.912(1), b = 14.256(1), c = 30.402(3). The conformation of the backbone, the orientation of the aromatic side chain and the puckering modes for the pyrrolidine rings of these conformers differ significantly. The peptide bonds exist in the generally preferred *trans* conformation being slightly non-planar. These two conformations reflect α -helix- and collagentype prolines. The crystal structures of both the conformers are stabilized by two, although different intermolecular hydrogen bonds. An intermolecular bond between the carbonyl oxygen of the carboxy terminal (³Pro) and the amide proton (²Phe) is observed for both the conformers. The second intermolecular hydrogen bond for conformer 1 is between the hydrogen of the carboxy terminal (³Pro) and the carbonyl oxygen of the (²Phe) residue and the hydrogen of the carboxy terminal (³Pro) for conformer 2. NMR spectroscopic studies indicated the presence of stereoconformations due to the *cis* and *trans* amide bonds similar to other proline-containing peptides.

Introduction

Conformational studies on small proline-containing peptides are valuable in elucidating conformational preferences of larger peptides and proteins. Proline is an important imino acid of many proteins and neuropeptides and imposes certain conformational restraints on these biomolecules (Balasubramanian *et al.*, 1971; Ashida and Kakudo, 1974). In addition, the different puckering modes of the five-membered ring system of the pyrrolidine system is of conformational interest (Chacko *et al.*, 1983). In continuation of our interest in



conformationally restricted small peptides containing the prolyl residue (Milne *et al.*, 1992; 1993a,b) and as part of our structural and conformational studies on the μ -opioid receptor agonist, morphiceptin (Tyr-Pro-Phe-Pro-NH₂), its derivatives and fragments, (Oliver and Marshall, 1990), we report here the synthesis, conformational and spectroscopic properties of (tert-Butyloxycarbonyl-Prolyl-Phenylalanyl-Prolyl) (Boc-Pro-Phe-Pro) (see Scheme 1).

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Experimental

Synthesis

All reagents and solvents were of reagent grade and used without further purification. To a stirred solution of N-t-Boc-S-Phe (6 g, 22.615 mmol) and S-Pro-NH₂ (2.5815 g, 22. 615 mmol) in 1,2-dimethoxyethane (40 ml) at 0°C were added triethylamine (6, 58 ml, 47.49 mmol) and diethylphosphoryl cyanide (3, 77 ml, 24.85 mmol). After 1 h at 0°C and 4 h at room temperature the reaction mixture was diluted with ethyl acetate (250 ml) and washed successively with 5% hydrochloric acid (50 ml), aqueous sodium hydrogen carbonate (50 ml), and saturated brine (50 ml). Removal of the solvent in vacuo and column chromatography of the residue furnished the protected product as a colorless syrup (Rf = 0.52); chloroform-methanol (18:2). To a stirred solution of Boc-Phe-Pro-NaCl (Milne et al., 1993b) (0.84 g, 1.99 mmol) in dry dichloromethane (4 ml), trifluoroacetic acid (4 ml) was added dropwise while maintaining the temperature at 0°C. After 1 h at 0°C and 1 h at room temperature the reaction mixture was diluted with dry carbon tetrachloride (100 ml) and the solvents were removed in vacuo. The residue, a syrup, was dried under vacuum for 6 h. To a stirred solution of N-t-Boc-S-Pro (0.43 g, 1.99 mmol) and Phe-Pro-trifluoroacetate (0.84 g, 1.99 mmol) in dry 1, 2-dimethoxyethane (20 ml) at 0°C were added triethylamine (0, 58 ml, 4.18 mmol) and diethylphosphoryl cyanide (0, 33 ml, 2.19 mmol). After 1 h at 0°C and 4 h at room temperature the reaction mixture was diluted with ethyl acetate (100 ml) and washed successively with 5% hydrochloric acid (50 ml), aqueous sodium hydrogen carbonate (50 ml), and saturated brine (50 ml). Removal of the solvent in vacuo and column chromatography of the residue furnished the protected product as a colorless syrup (0.64 g) [Rf = 0.60; chloroform-methanol (18:2)].

X-ray analysis

Colorless crystals of Boc-Pro-Phe-Pro ($C_{24}H_{33}$ -N₃O₆) crystallized from chloroform-n-hexane in the space group $P2_12_12_1$. The crystal selected for data collection had the dimensions $0.42 \times 0.34 \times 0.38$ mm. Reflections (5929) were collected with their indices being $h \ 0: 15, k \ 0: 18, l \ 0: 38$.

All diffraction measurements were performed at room temperature and data collected with an Enraf-Nonius CAD4 diffractometer using graphite monochromated Cu- K_{α} radiation. The lattice constants were obtained from a least squares fit of 25 centered reflections (27°

Table 1. Crystal data of Boc-Pro-Phe-Pro

Space group $P2_12_12_1$
a = 11.912(1) Å
b = 14.256(1) Å
c = 30.402(3) Å
$V = 5162.8 \text{ Å}^3$
Z = 8
$D = 1.182 \text{ g cm}^{-3}$
$\lambda \operatorname{Cu} - K_{\alpha} = 1.5418 \text{ Å}$
$\mu \operatorname{Cu} - K_{\alpha} = 6.22 \ \mathrm{cm}^{-1}$
F(000) = 1968
Scan type $(\omega: 2\theta) = 1:1$
Scan range $(\theta)^\circ = 3 \le \theta \le 78$
Scan speed (variable, deg. min^{-1}) = Max 5.49
Scan angle $(\omega + 0.34 \tan \theta)^\circ = 0.40$
Aperture size (mm) = 1.3×4.0
$R_{w} = 0.051$
$R = 0.073$ for 4956 unique observed reflections $(I > 2\sigma I)$
Residual electron density (eÅ ³):
Maximum = 0.27
Minimum = -0.32

 $\leq \theta \leq 47^{\circ}$) and are listed with other relevant crystal data in Table 1. The data were corrected for Lorentz and polarization effects, and for absorption, using an empirical method involving ψ -scans. Intensity checks were carried out every hour and an orientation control every 200 reflections. The minimum and maximum transmission factors were 0.9709 and 0.9989 (0.9841 average). Three standard reflections were used to check orientation and crystal stability at regular intervals, and the decay during data collection was 8% (corrected). The structure was solved by direct methods and refined anisotropically using a blocked full matrix least-squares method ($1/\sigma^2$ (F_o) weights) with SHELX76 (Sheldrick, 1976).

All hydrogen atoms were placed in calculated positions and were included in the refinement with a common isotropic temperature factor that converged to U =0.129(4). Fractional coordinates and equivalent thermal factors for Boc-Pro-Phe-Pro are listed in Table 2. The relevant torsion angles are listed in Table 3.

Spectroscopic analyses

¹H Proton (300 MHz) and ¹³C carbon (75 MHz) spectra were recorded on a Bruker AM-300 spectrometer, with DMSO as solvent and TMS as internal standard. Hetcor and Cosy spectra were recorded to assist with the ¹H and ¹³C assignments.

Infrared spectra were recorded on a Perkin-Elmer Model 1600 FTIR spectrophotometer as KBr disks.

Fast atom bombardment (FAB) mass spectrum of

	x/a	y/b	z/c	$U_{ m eq}{}^a$		x/a	y/b	z/c	$U_{ m eq}$
C(1A)	2144(10)	1438(6)	6191(3)	136(3)	C(1B)	2689(7)	-2096(4)	9258(2)	101(2)
C(2A)	919(8)	1420(7)	6308(3)	197(4)	C(2B)	2384(7)	-2326(5)	8784(2)	148(3)
C(3A)	2667(10)	527(5)	6296(2)	221(5)	C(3B)	1642(7)	-2159(5)	9545(2)	134(3)
C(4A)	2219(10)	1697(6)	5717(2)	237(5)	C(4B)	3618(8)	-2657(5)	9431(3)	170(3)
O(5A)	2713(3)	2081(3)	6468(1)	91(1)	O(5B)	2950(3)	-1098(2)	9280(1)	84(1)
C(6A)	2449(6)	3008(4)	6466(2)	79(2)	C(6B)	3789(5)	-705(4)	9024(2)	75(2)
O(7A)	1813(3)	3402(3)	6226(1)	90(1)	O(7B)	4426(3)	-1144(3)	8798(1)	106(1)
N(8A)	3063(4)	3417(3)	6789(2)	77(1)	N(8B)	3761(3)	229(3)	9068(1)	65(1)
C(9A)	3064(6)	4435(4)	6833(2)	106(2)	C(9B)	4626(4)	819(4)	8872(2)	78(2)
C(10A)	3778(7)	4606(6)	7233(3)	141(3)	C(10B)	4618(4)	1679(4)	9156(2)	78(2)
C(11A)	4636(6)	3802(6)	7222(3)	143(3)	C(11B)	3414(4)	1744(3)	9308(2)	66(1)
C(12A)	3953(5)	2962(5)	7049(2)	92(2)	C(12B)	3064(4)	727(3)	9379(2)	54(1)
C(13A)	3552(5)	2362(4)	7437(2)	86(2)	C(13B)	1807(4)	610(3)	9282(2)	56(1)
O(14A)	4248(3)	1894(3)	7630(1)	114(1)	O(14B)	1419(3)	613(3)	8905(1)	71(1)
N(15A)	2455(3)	2408(3)	7546(1)	64(1)	N(15B)	1164(3)	573(2)	9642(1)	50(1)
C(16A)	2051(4)	2004(3)	7957(2)	65(1)	C(16B)	-58(3)	571(3)	9611(1)	51(1)
C(17A)	1546(4)	2751(4)	8259(2)	71(1)	C(17B)	-539(4)	112(3)	10027(1)	61(1)
C(18A)	2331(6)	3551(5)	8336(2)	85(2)	C(18B)	-1808(4)	84(3)	10033(2)	59(1)
C(19A)	3477(6)	3449(5)	8415(2)	104(2)	C(19B)	-2382(5)	513(4)	10358(2)	80(2)
C(20A)	4165(9)	4206(6)	8474(2)	142(3)	C(20B)	-3568(6)	454(5)	10374(2)	111(2)
C(21A)	3708(11)	5092(8)	8469(4)	169(4)	C(21B)	-4120(6)	-45(5)	10060(2)	114(3)
C(22A)	2562(11)	5222(8)	8398(4)	181(5)	C(22B)	-3539(6)	-489(5)	9725(2)	103(2)
C(23A)	1897(8)	4440(6)	8333(2)	137(3)	C(23B)	-2387(4)	-419(4)	9721(2)	71(1)
C(24A)	1127(5)	1290(4)	7850(2)	73(2)	C(24B)	-455(3)	1584(3)	9582(1)	51(1)
O(25A)	127(3)	1520(3)	7846(1)	106(1)	O(25B)	-163(2)	2173(2)	9854(1)	64(1)
N(26A)	1446(3)	399(3)	7759(1)	69(1)	N(26B)	-1162(3)	1813(2)	9251(1)	51(1)
C(27A)	2600(5)	38(4)	7753(2)	102(2)	C(27B)	-1469(4)	1230(3)	8859(1)	64(1)
C(28A)	2453(5)	-950(5)	7556(3)	136(3)	C(28B)	-2196(5)	1859(4)	8597(2)	87(2)
C(29A)	1379(5)	-952(5)	7320(2)	132(2)	C(29B)	-1874(5)	2841(4)	8708(1)	85(2)
C(30A)	647(5)	-262(4)	7569(2)	85(2)	C(30B)	-1502(4)	2794(3)	9191(1)	59(1)
C(31A)	-85(5)	-799(4)	7888(2)	77(2)	C(31B)	-2455(4)	3102(3)	9494(1)	55(1)
O(32A)	-762(3)	-1350(3)	7747(1)	114(1)	O(32B)	-2657(3)	3937(2)	9536(1)	78(1)
O(33A)	88(3)	-641(3)	8336(1)	110(1)	O(33B)	-3041(3)	2413(3)	9708(1)	89(1)

Table 2. Fractional coordinates ($\times 10^4$) and equivalent thermal factors ($\times 10^3$ Å²) for Boc-Pro-Phe-Pro

 ${}^{a}U_{eq} = \frac{1}{3} \Sigma_{i} \Sigma_{j} U_{ij} a_{i}^{*} a_{j}^{*} (a_{i} \cdot a_{j}).$

Boc-Pro-Phe-Pro dissolved in DMSO with 3-nitrobenzyl alcohol as matrix was obtained on a VG-7070E spectrometer.

Results and discussion

X-ray analysis

The X-ray data (Table 2) indicated the crystallization of two conformations for Boc-Pro-Phe-Pro as shown in Fig. 1. The two conformers of Boc-Pro-Phe-Pro differ significantly with respect to their three dimensional conformational orientation for the backbone around the following torsion angles (Conformer 1: ψ_1 , = -9.3; $\phi_2 = -123.3$; $\psi_2 = 86.9$; Conformer 2: ψ_1 = 144.8; $\phi_2 = -85.2$; $\psi_2 = 128.6$) and the orientation of the phenylalanyl side chains (Conformer 1: X_2^1 = $-53,2; X_2^2 = -42,2;$ Conformer 2: $X_2^1 = 179,2; X_2^2 =$ -120,4) (see Fig. 2 and Table 3 for comparison). The amide bonds (ω_1 and ω_2) are for both conformers in a near trans conformation with conformer 1 slightly more nonplanar ($\omega_2 = -167.6$) than conformer 2 ($\omega_2 =$ -175.6). The orientation of the aromatic ring for conformer 1 is folded $(X_2^1 = -53,2)$ towards the pyrrolidine ring of the ¹Pro residue in contrast to conformer 2 where the aromatic ring is in a trans orientation $(X_2^1 =$ 179.2). The pyrrolidine rings of the two conformers of Boc-Pro-Phe-Pro crystallized in different puckering modes (see Table 3 for torsion angles). The conformation of the pyrrolidine rings of ¹Pro (conformer 1), ³Pro (conformer 1) and ³Pro (conformer 2) show general similarity and are consistent with other reported data of pro-

		Boc-Pro-Phe-Ph	ro
Torsion angle ^a		Conformer 1	Conformer 2
C6A(6B)-N8A(8B)-C12A(12B)-C13A(13B)	φı	-81.7(7)	-65.2(6)
N8A(8B)-C12A(12B)-C13A(13B)-N15A(15B)	ψ_1	-9.3(8)	144.8(4)
C12A(12B)-C13A(13B)-N15A(15B)-C16A(16B)	ω_1	-168.2(5)	173.0(4)
N8A(8B)-C12A(12B)-C11A(11B)-C10A(10B)	\mathbf{x}_{1}^{1}	26.4(7)	-28.8(5)
C12A(12B)-C11A(11B)-C10A(10B)-C9A(9B)	χ_1^2	-36.6(8)	37.3(5)
C11A(11B)-C10A(10B)-C9A(9B)-N8A(8B)	χ_1^3	31.9(8)	-30.6(5)
C10A(10B)-C9A(9B)-N8A(8B)-C12A(12B)	χ_1^4	-15.9(7)	13.1(5)
C9A(9B)—N8A(8B)—C12A(12B)—C11A(11B)	θ	-6.9(7)	9.9(5)
C13A(13B)-N15A(15B)-C16A(16B)-C24A(24B)	ϕ_2	-123.3(5)	-85.2(5)
N15A(15B)-C16A(16B)-C24A(24B)-N26A(26B)	ψ_2	86.9(6)	128.6(4)
C16A(16B)-C24A(24B)-N26A(26B)-C30A(30B)	ω_2	-167.6(5)	-175.6(4)
N15A(15B)-C16A(16B)-C17A(17B)-C18A(18B)	χ_2^1	-53.2(6)	179.2(4)
C16A(16B)-C17A(17B)-C18A(18B)-C19A(19B)	χ^2_2	-42.2(9)	-120.4(5)
C24A(24B) - N26A(26B) - C30A(30B) - C31A(31B)	ϕ_3	-86.6(7)	-81.6(5)
N26A(26B)-C30A(30B)-C29A(29B)-C28A(28B)	χ_3^1	28.4(6)	24.4(5)
C30A(30B) - C29A(29B) - C28A(28B) - C27A(27B)	χ_3^2	-30.7(7)	-32.0(5)
C29A(29B)-C28A(28B)-C27A(27B)-N26A(26B)	χ_{3}^{3}	20.7(7)	26.1(6)
C28A(28B)-C27A(27B)-N26A(26B)-C30A(30B)	χ_3^4	-2.3(6)	-10.3(5)
C27A(27B)-N26A(26B)-C30A(20B)-C29A(29B)	θ	-15.9(6)	-8.8(5)

Table 3. Torsion angles of the backbone and side chains of Boc-Pro-Phe-Pro

^a atom designation of conformer 1, with conformer 2 in brackets.



Fig. 1. ORTEP view of conformers 1 and 2 of Boc-Pro-Phe-Pro.



Fig. 2. Comparison of the crystal conformations of conformer 1 and conformer 2 of Boc-Pro-Phe-Pro.

lyl residues (Kartha et al., 1974; Benedetti et al., 1974; Kamwaya et al., 1981). Their puckering modes can be described as approximate C_s (envelope) symmetry having a B conformation with the C^{γ} atoms located endo and C^{β} atoms located exo relative to the carboxamide group, i.e., ${}^{\beta}_{\gamma}T$. In contrast the pyrrolidine ring of ¹Pro (conformer 2) assumes a half-chair conformation A which can be described as a ${}^{\gamma}_{\beta}T$ conformation, with the C^{β} atom positioned *endo* and C^{γ} positioned *exo* relative to the carboxamide group (Haasnoot et al., 1981; Ashida and Kakudo, 1974). This conformation was also observed for the pyrrolidine ring of the cyclodipeptide, cyclo (Phe-Pro) (Mazza et al., 1984) This is in contrast to the conformation observed for the pyrrolidine rings of closely related cyclodipeptides, cyclo (Tyr-Pro) (Milne et al, 1992) and cyclo (Phe-fluoro-Pro), (Ciarkowski et al., 1990). The significant difference in value of ψ_1 for the two conformers (conformer 1: -9,3; conformer 2: 144.8) is of importance. The negative value indicates that conformer 1 belongs to the α -helix type of proline compounds, whereas conformer 2 approaches collagen-type prolines (Ashida and Kakudo, 1974; Hospital et al., 1979). This conformational flexibility even in the solid state may support the importance of proline in biological systems. The crystal packing for both conformers of Boc-Pro-Phe-Pro is stabilized by two intermolecular hydrogen bonds (Table 4) for each conformer. No intramolecular hydrogen bond was observed. Both conformers show an intermolecular hydrogen bond between the carbonyl oxygen of the carboxy terminal (³Pro) and the amide proton (²Phe):

 Table 4. Intermolecular hydrogen bonding schemes: D-donor, H-hydrogen, A-acceptor

D-H···A	D-A	Н∙∙∙А	Angle $D-H\cdots A^a$
Conformer 1			
N15A—H17A · · · O32A O33A—H33A · · · O7A	2.829 Å 2.961 Å	1.858 Å 2.130 Å	147.5° 131.8°
Conformer 2 N15B-H17B····O32B O33B-H33B····O25B	2.949 Å 2.917 Å	1.936 Å 2.298 Å	154.5° 114.5°

^a The O33A-H33A · · · O7A and O33B-H33B · · · O25B hydrogen bond angles are quite small, this may be due to the fact that the hydrogens were refined in calculated positions. The D-A distances, which may give a more significant indication in this case, are within acceptable limits: 2.961 Å and 2.917 Å for conformer 1 and conformer 2, respectively.

N15A(B)-H17A(B) ----O32A(B). However, different hydrogen bonds are observed for the hydrogen of the carboxy terminal (³Pro) of these conformers. The hydrogen bond for conformer 1 is between hydrogen (H33A) and the carbonyl of the N-t-Boc protecting group (O7A): O33A-H33A---O7A, in contrast to the hydrogen bond for conformer 2 between hydrogen (H33B) and carbonyl of the ²Phe residue (O25B): O33B-H33B-O25B. This difference in the intermolecular hydrogen bonds for these conformers of Boc-Pro-Phe-Pro may contribute and account for their different solid state conformations. The backbone conformation for conformer 2 of Boc-Pro-Phe-Pro is much closer to the conformation of the t-Amyloxycarbonyl-Pro-Pro-Pro (Kartha et al., 1974) then conformer 1. However, no hydrogen bonds were reported for this all-proline tripeptide with steric hinderince between the pyrrolidine rings being suggested as an important factor in the crystal conformation.

Spectroscopic analyses

The mass spectrum of Boc-Pro-Phe-Pro showed the expected parent ion peak at m/z 459. The infrared spectrum showed broad peaks at 3409.9 cm⁻¹ (OH, NH), 1676.7 cm⁻¹ (C = O's overlapping) and 1520.2 cm⁻¹ (aromatic vibrations). The NMR data of Boc-Pro-Phe-Pro clearly indicate the presence of conformational isomers (well known for proline containing peptides (Deslauriers *et al.*, 1979; Goodman and Mierke, 1989; Doi *et al.*, 1988) due to the *cis* and *trans* amide bonds of the two proline residues. Recently, several stereoconformations were proposed for morphiceptin (Tyr-

Carbon Atom	PPM
³ Pro-γ	23.0 $(t)^{a}$
'Pro-γ	24.3 (t)
$C(\underline{C}H_3)_3$	27.8 (q)
$^{1}Pro-\beta$	29.1 (t)
³ Pro-β	29.3 (t)
Phe- β	36.4 (t)
¹ Pro-δ	46.4 (t)
³ Pro–δ	46.7 (t)
Phe-a	51.8 (d)
^{1,3} Pro- α 's	59.5 (d)
$C(CH_3)_3$	78.3 (s)
Phe-Ar	126.1 (d)
Phe-Ar	127.9 (d)
Phe-Ar	129.1 (d)
Phe-Ar	137.9 (s)
0	
/	
Boc-C=O	153.2 (s)
Phe-C=O	169.8 (s)
¹ Pro-C=O	172.1 (s)
³ Pro-C=O	173.4 (s)
I	
OH	

 Table 5.
 ¹³C NMR data of Boc-Pro-Phe-Pro

^as = singlet; d = doublet; t = triplet; q = quartet.

Pro-Phe-Pro-NH₂) and its NMePhe analogue as well as for valine morphiceptin (Tyr-Pro-Phe-Val-NH₂) with the *trans* rotamers being the preferred conformation (Goodman and Mierke, 1989; Doi *et al.*, 1988.) The ¹H NMR spectrum of Boc-Pro-Phe-Pro showed several signals overlapping and accurate determination of all the parameters was not always possible due to the existence of the cis and trans equilibrium. The ¹³C NMR data of the major conformational isomer of Boc-Pro-Phe-Pro appear in Table 5. The carbon chemical shift values of the amino acid residues are in close agreement with the values obtained for the corresponding amino acid residue of the tetrapeptide, morphiceptin (Goodman and Mierke, 1989). Detailed NMR studies on the conformational characteristics of Boc-Pro-Phe-Pro are currently being undertaken.

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Structure factor data have been deposited with the British Library, Boston Spa, Wetherby, West Yorkshire, UK, as supplementary publication No. 63278 (22 pages)