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Structure of *tert*-Butoxycarbonylglycylglycyl-L-phenylalanine Ethyl Ester, $C_{20}H_{29}N_3O_6$

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Abstract. $M_r = 407·46$, monoclinic, $P2_1$, $a = 10·959$ (4), $b = 9·098$ (3), $c = 11·714$ (4) Å, $\beta = 101·41$ (2)°, $V = 1144·9$ (7) Å^3, $Z = 2$, $D_m = 1·180$ (1), $D_x = 1·182$ Mg m^{-3}, λ(Cu $K\alpha$) = 1·5418 Å, $\mu = 0·6915$ mm^{-1}, $T = 293$K. Final $R = 0·064$ for 1848 independent reflections. Although no intramolecular hydrogen bonds are present, the molecule assumes a β-bend conformation around the glycylglycyl residue. NMR spectra of the compound indicated that this bent conformation may also exist preferentially in solution.

Introduction. The crystal structure of the protected tripeptide *tert*-butoxycarbonylglycylglycyl-L-phenylalanine ethyl ester (Boc-Gly-Gly-Phe-OC_2H_5) is reported here as part of a program of X-ray studies of crystalline peptides.

The program is particularly aimed at exploring the possible geometrical features of enkephalins [L-tyrosylglycylglycyl-L-phenylalanyl-L-methionine (Tyr-Gly-Gly-Phe-Met) and L-tyrosylglycylglycyl-L-phenylalanyl-L-leucine (Tyr-Gly-Gly-Phe-Leu)] with morphine-like activities (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975). In the crystal structures of the analogues Tyr-Gly-Gly-Phe (Prangé & Pascard, 1979) and Tyr-Gly-Gly-Phe-Leu (Smith & Griffin, 1978), both molecules adopted β-bend conformations around the Gly-Gly residue with an intramolecular 4→1 hydrogen bond [(Tyr)C=O ···HN(Phe)]. The X-ray study of the present crystal was performed in order to investigate whether the Gly-Gly-Phe sequence takes a β-bend conformation or not.

Experimental. Synthesized by liquid-phase method, crystallized from ethyl acetate/*n*-hexane mixture, 0·4 × 0·3 × 0·7 mm, lattice parameters determined by least squares from 2θ values of high-angle reflections, intensity data collected on a Rigaku four-circle diffractometer, graphite-monochromated Cu $K\alpha$, $\sin\theta/\lambda = 0·588$ Å^{-1}; of 2086 reflections measured by ω–2θ continuous-scan mode, 1848 with $I \geq 2\sigma(I)$ were subsequently used for structure refinement; Lorentz and polarization corrections were applied, but absorption ignored; structure solved by direct methods with *MULTAN* 78 (Main, Hull, Lessinger, Germain, Declercq & Woolfson, 1978).

On the E map calculated from the phase set having the highest combined figure of merit, 26 of the 29 non-H atoms were obtained reasonably. A difference Fourier synthesis revealed the three remaining atoms corresponding to the ethyl ester. These atoms had relatively low electron densities on a Fourier map, compared with the other atoms. Refinement carried out by block-diagonal least squares, all H atoms, except those bonded to the ethyl ester, obtained on a difference Fourier map, and included in the refinement, final $R = 0·064$, $R_w = 0·060$, function minimized $\sum w(|F_o| - |F_c|)^2$ with $w = 1·0$ for $F_o \leq 40·0$, and $w = 1·0/[1·0 + 0·250\,(F_o - 40·0)]$ for $F_o > 40·0$, atomic scattering factors taken from *International Tables for X-ray Crystallography* (1974), $F(000) = 436$, all numerical calculations made on an ACOS-900 computer at the Computation Center of Osaka University using *The Universal Crystallographic Computing System* (1979).

^{1}H NMR spectra were measured on a Varian XL-200 (200 MHz, FT mode) spectrometer equipped with a variable-temperature accessory. Samples were adjusted to 0·1 *M* for $CDCl_3$ solution.

Discussion. The final coordinates of the non-H atoms are given in Table 1.*

The bond lengths and angles of the non-H atoms are given in Fig. 1. Most of the bond lengths and angles in the structure are in agreement with those of similar oligopeptides. A few bonds in the ethyl ester moiety, however, are abnormally short. All these bonds are associated with atoms having high thermal parameters.

A perspective view of the molecule is shown in Fig. 2. The torsion angles that define the main-chain and side-chain conformations are listed in Table 2. As is shown in Fig. 2, the main chain of this peptide is folded

* Lists of structure factors, anisotropic thermal parameters for non-H atoms, and coordinates and isotropic thermal parameters for H atoms have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 38155 (12 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

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Table 1. *Positional* (× 10⁴) *and equivalent isotropic thermal parameters of non*-H *atoms with e.s.d.'s in parentheses*

	x	y	z	B_{eq}(Å^2)*
O(1)	6696 (3)	2925 (5)	771 (3)	5·6 (2)
O(2)	6944 (4)	5399 (5)	967 (4)	6·4 (2)
O(3)	9710 (3)	6982 (4)	−1016 (3)	5·0 (2)
O(4)	12482 (4)	6525 (5)	1776 (3)	5·3 (2)
O(5)	12786 (6)	2075 (6)	3626 (4)	9·1 (3)
O(6)	13516 (6)	3587 (7)	5068 (4)	10·2 (3)
N(1)	7583 (4)	4142 (5)	−474 (4)	4·7 (2)
N(2)	10077 (4)	4830 (5)	−45 (4)	3·7 (2)
N(3)	11726 (4)	4380 (5)	2276 (3)	4·4 (2)
C(1)	5735 (7)	1087 (10)	1644 (8)	8·5 (4)
C(2)	4757 (6)	3564 (10)	1338 (8)	7·8 (4)
C(3)	6762 (9)	3205 (13)	2827 (7)	9·7 (5)
C(4)	5973 (5)	2749 (9)	1675 (5)	6·2 (3)
C(5)	7060 (5)	4265 (7)	476 (5)	5·1 (2)
C(6)	7965 (5)	5419 (7)	−1020 (5)	5·0 (2)
C(7)	9340 (5)	5806 (6)	−683 (4)	3·7 (2)
C(8)	11420 (4)	5013 (6)	226 (4)	3·9 (2)
C(9)	11913 (4)	5388 (6)	1504 (4)	3·9 (2)
C(10)	12169 (5)	4623 (7)	3520 (4)	5·0 (3)
C(11)	12848 (7)	3261 (8)	4055 (5)	7·8 (4)
C(12)	14254 (17)	2359 (15)	5692 (8)	19·3 (12)
C(13)	14772 (19)	2642 (20)	6603 (13)	21·2 (12)
C(14)	11088 (6)	5037 (9)	4139 (5)	6·1 (3)
C(15)	10541 (5)	6482 (8)	3740 (5)	5·4 (3)
C(16)	11122 (6)	7804 (9)	4085 (6)	6·6 (3)
C(17)	10615 (7)	9143 (10)	3647 (6)	7·4 (4)
C(18)	9486 (7)	9161 (10)	2895 (6)	7·2 (3)
C(19)	8905 (7)	7874 (11)	2547 (6)	7·2 (3)
C(20)	9401 (6)	6550 (10)	2946 (5)	6·5 (3)

$^*B_{eq} = \frac{4}{3}(a^2B_{11} + b^2B_{22} + c^2B_{33} + 2acB_{12}\cos\beta)$.

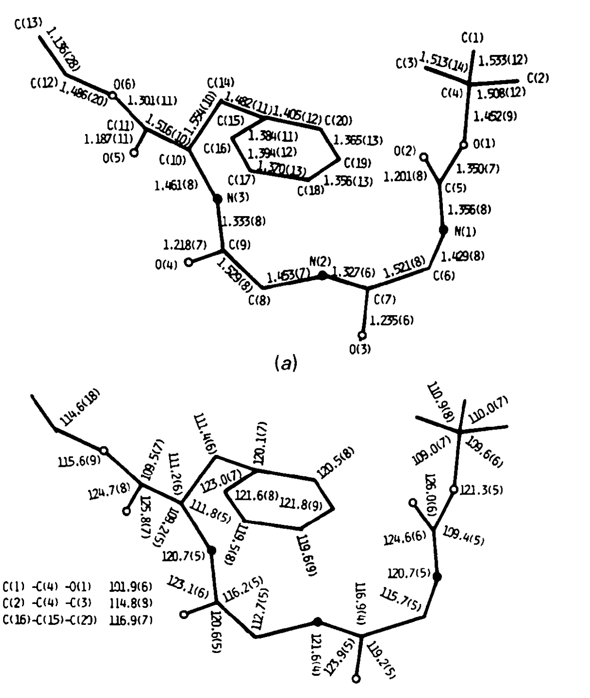

Fig. 1. (*a*) Bond lengths (Å) and (*b*) bond angles (°).

Table 2. *Selected torsion angles* (°)

C(1)–C(4)–O(1)–C(5)	177·1 (6)	C(8)–C(9)–N(3)–C(10):ω_2	179·5 (5)
C(4)–O(1)–C(5)–N(1)	−172·6 (5)	C(9)–N(3)–C(10)–C(11):φ_3	−132·4 (6)
O(1)–C(5)–N(1)–C(6):ω_0	175·3 (5)	N(3)–C(10)–C(11)–O(6):ψ_3	163·4 (6)
C(5)–N(1)–C(6)–C(7):φ_1	97·6 (6)	C(10)–C(11)–O(6)–C(12)	−179·1 (10)
N(1)–C(6)–C(7)–N(2):ψ_1	8·6 (7)	C(11)–O(6)–C(12)–C(13)	−175·5 (16)
C(6)–C(7)–N(2)–C(8):ω_1	173·6 (5)	N(3)–C(10)–C(14)–C(15):χ_1	−66·0 (7)
C(7)–N(2)–C(8)–C(9):φ_2	109·3 (6)	C(10)–C(14)–C(15)–C(16):χ_2	−75·1 (9)
N(2)–C(8)–C(9)–N(3):ψ_2	62·2 (7)		

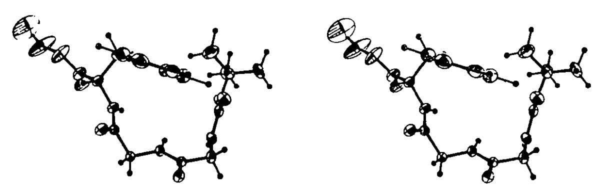

Fig. 2. A stereoscopic view of Boc-Gly-Gly-Phe-OC_2H_5.

at the Gly-Gly site into the β-turn type (I′) (Ashida, Yamane & Tanaka, 1980), which is similar to those of the related peptides Tyr-Gly-Gly-Phe and Tyr-Gly-Gly-Phe-Leu. Although the intramolecular 4→1 (C=O ···HN) hydrogen bonds stabilize the β-turn conformation in these peptides, such hydrogen bonds are absent in this crystal. Therefore, it appears that the protected Gly-Gly-Phe sequence favors the β-turn conformation intrinsically.

In order to investigate whether such a β-turn conformation exists in solution or not, we measured ¹H NMR spectra of the compound in $CDCl_3$ solution; the assignment of proton resonances was made by homonuclear decoupling, spin multiplicities and with respect to related reports (Fournie Zaluski, Prangé, Pascard & Roques, 1977; Garbay-Jaureguiberry, Roques, Oberlin, Anteunis & Lala, 1976). However, the peak assignments of Gly protons are tentative. The experimental data and the possible φ angles are given in Table 3, in which the φ values were estimated from the equations $J_{HN\alpha} = 7{\cdot}9\cos^2\theta - 1{\cdot}5\cos\theta + 1{\cdot}3\sin^2\theta$ ($\theta = |\varphi - 60°|$) for the main-chain conformation of the Phe residue (Ramachandran, Chandrasekaran & Kopple, 1971), $\sum J_{HN\alpha 2} = 6{\cdot}0\cos^2\varphi - 1{\cdot}5\cos\varphi + 12{\cdot}5\sin^2\varphi$ for that of the Gly residue (Kopple, Go, Logan & Savrda, 1972), and from the most probable values of the (φ, ψ) map (Ramachandran, Ramakrishnan & Sasisekharan, 1963). Among the possible values for the φ angles, the first set in the last column of Table 3 is in agreement with a Gly–Gly β bend. The second set, which corresponds nearly to an antiparallel β-pleated-sheet structure, is excluded because the observed temperature variation for the NH proton (δNH/d*T*) is too large for such a conformation (Fournie Zaluski *et al.*, 1977; Khaled, Long, Thompson, Bradley, Brown & Urry, 1977).

Table 3. ^{1}H *NMR data at* 298 K *and possible values of the torsion angle* φ

Residue	Proton	Chemical shift (p.p.m.)	Coupling constant (Hz)	δNH/dT ($\times 10^{-3}$p.p.m. K^{-1})	φ(°)
Gly	α_1	3·80	5·5 ($J_{NH-\alpha}$)		±68, −128
	α_2				
	NH	5·34		6·0	
Gly	α_1	3·93	5·5 ($J_{NH-\alpha_1}$)		±63, −133
	α_2	3·92	5·0 ($J_{NH-\alpha_2}$)		
	NH	7·02		8·0	
Phe	α	4·82	8·0 ($J_{NH-\alpha}$)		−94, −146
	β_1	3·12	6·0 ($J_{\alpha-\beta_1}$)		
	β_2	3·04	6·5 ($J_{\alpha-\beta_2}$)		
	NH	6·81		9·0	
Boc	CH_3	1·45			
Ethyl ester	CH_2	1·22			
	CH_3	4·14			

Estimated errors of chemical shift and coupling constant are ± 0·02 p.p.m. and ±0·2 Hz respectively. The values of δNH/dT were measured from the variation of the NH proton at 273, 283, 288, 293, 298 and 303 K.

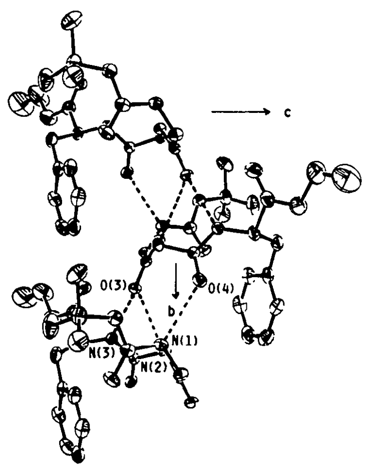

Fig. 3. The molecular arrangement of Boc-Gly-Gly-Phe-OC_2H_5 viewed along the *a* axis. The hydrogen bonds are shown by dotted lines.

The molecular arrangement projected along the *a* axis is shown in Fig. 3, in which the dotted lines represent hydrogen bonds. The folded Gly-Gly moiety of the molecule is arranged around the 2_1 screw axis parallel to the *b* axis; the molecule is linked with the neighboring molecules by three hydrogen bonds between peptide amino and carboxyl groups [N(1)···O(4) 2·822 (6), H(1)···O(4) 1·93 (6) Å, ∠N(1)–H(1)···O(4) 169 (6)°; N(2)···O(3) 2·865 (5), H(2)···O(3) 2·15 (7) Å, ∠N(2)–H(2)···O(3) 136 (6)°; N(3)···O(3) 2·916 (6), H(3)···O(3) 1·99 (6) Å, ∠N(3)–H(3)···O(3) 162 (6)°].

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Structure of *o*-Nitroaniline Hydrochloride, $C_6H_7N_2O_2^+.Cl^-$

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Abstract. $M_r = 174·6$, orthorhombic, *Pbca*, $a = 7·878$ (2), $b = 7·940$ (2), $c = 23·73$ (1) Å, $V = 1484·3$ (8) Å^3, $Z = 8$, $D_m = 1·560$, $D_x = 1·562$ (1) Mg m^{-3}, λ(Mo $K\alpha$) = 0·71069 Å, $\mu = 0·46$ mm^{-1}, $T = 295$ K. Final $R = 0·07$ for 1621 observed reflexions. Layers of anilinium ions are held

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