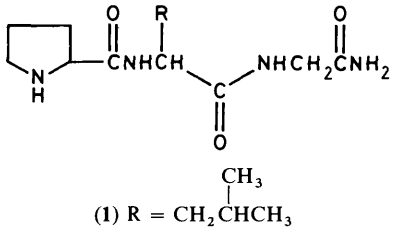
Crystal-state Structures of Boc-Pro-Leu-Gly-NH_2 Hemihydrate and Two Lactam-restricted Analogues

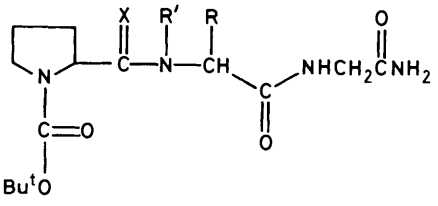
Giovanni Valle, Marco Crisma, and Claudio Toniolo *
Biopolymer Research Centre, C.N.R., Department of Organic Chemistry, University of Padova, 35131 Padova, Italy
Kuo-Long Yu and Rodney L. Johnson *
Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455, U.S.A.

A crystal-state structural analysis of Boc-Pro-Leu-Gly-NH_2 (**2a**) hemihydrate and two conformationally restricted analogues that incorporate a γ- or δ-lactam modification at the –Leu-Gly– sequence, (**3**) and (**4**) respectively, has been performed by *X*-ray diffraction. In all three compounds the conformation of the tertiary amide group of the N^{α}-protecting urethane moiety is *cis* and the prolyl residue is semi-extended. The basic conformational feature of the published structure of H-Pro-Leu-Gly-NH_2 (**1**), the ten-membered ring type-II β-bend structure at the *C*-terminus, is preserved in the N^{α}-t-butyloxycarbonyl derivative (**2a**). However, the γ- and δ-lactam modifications induce drastic changes in the backbone torsion angles of the –Leu-Gly– sequence, eventually promoting the onset of extended conformations.

The *C*-terminal tripeptide amide of oxytocin, L-Pro-L-Leu-Gly-NH_2 (**1**), has been shown to possess activity in a wide variety of *in vivo* and *in vitro* neuropharmacological assay systems.[1] The pharmacological profile of (**1**) suggests that it is capable of modulating dopamine receptors. The basic conformational feature of the *X*-ray diffraction structure of (**1**) hemihydrate is a type-II β-bend conformation[2–8] at the *C*-terminus that is stabilized by an intramolecular hydrogen bond connecting the Gly carboxamide *anti*-NH donor and the Pro carbonyl acceptor.[9]

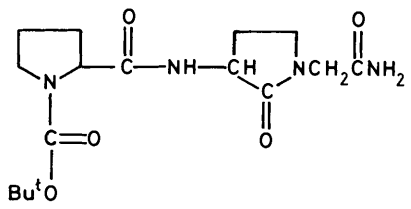


(1) R = CH_2CHCH_3 (CH$_3$)

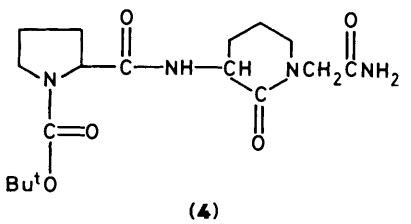


(**2**) **a**; X = O, R = $CH_2CH(CH_3)_2$, R′ = H
b; X = O, R = CH_3, R′ = H
c; X = O, R = $CH(CH_3)_2$, R′ = H
d; X = O, RR′ = $CH_2CH_2CH_2$
e; X = S, R = $CH_2CH(CH_3)_2$, R′ = H

In the present study we have solved, by *X*-ray diffraction, the molecular and crystal structures of the N^{α}-t-butyloxycarbonyl synthetic precursor of (**1**), Boc-L-Pro-L-Leu-Gly-NH_2 (**2a**) hemihydrate and of two conformationally restricted analogues incorporating either the γ- or δ-lactam modification[10,11] at the –Leu-Gly– sequence, namely 2-{(3S)-3-[(*S*)-1-t-butyloxycarbonylprolylamino]-2-oxopyrrolidino}acetamide (**3**) and 2-{(3*S*)-3-[(*S*)-1-t-butyloxycarbonylprolylamino]-2-oxo-piperidino}acetamide (**4**), respectively. The popularity of conformationally constrained amino acids in the synthesis of analogues of bioactive peptides is well documented.[12] The crystal-state structure of (**2a**) is compared with those already published for the analogues (**2b**—**d**) having either an Ala,[13] Val,[14] or Pro[15] residue, respectively, in place of the leucyl residue of (**2a**). A comparison is also made with Boc-L-Proψ(CSNH)-L-Leu-Gly-NH_2[16] (**2e**) in which a thioamide function serves as an isosteric peptide bond replacement.[17]



(**3**)



(**4**)

Experimental

Materials.—The synthesis of Boc-L-Pro-L-Leu-Gly-NH_2 (**2a**) hemihydrate was carried out as described by Mizoguchi *et al.*,[18] m.p. 127—129 °C (from acetone–diethyl ether); $[\alpha]_D$ −72.6° (*c*, 1.05 in MeOH) [lit.,[18] m.p. 137—139 °C (from EtOH–H_2O); $[\alpha]_D$ −72° (MeOH)].

The synthesis of 2-{(3*S*)-3-[(*S*)-1-t-butyloxycarbonylprolylamino]-2-oxopiperidino}acetamide (**4**) was carried out as previously described by us,[19] m.p. 209 °C (decomp); $[\alpha]_D$ −71.7° (*c*, 0.85 in MeOH).

The synthesis of 2-{(3*S*)-3-[(*S*)-1-t-butyloxycarbonylprolyl-

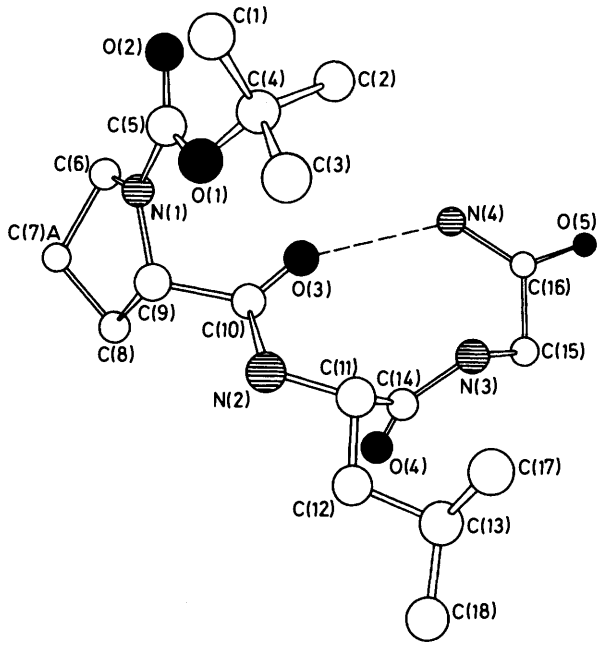


Figure 1. Molecular structure of Boc-L-Pro-L-Leu-Gly-NH_2 (**2a**) hemihydrate, molecule **A**, with the numbering of the atoms. The intramolecular hydrogen bond is indicated as a dashed line

amino]-2-oxopyrrolidino}acetamide (**3**) was carried out in the following manner. Methyl (3*S*)-3-[(*S*)-1-t-butyloxycarbonylamino]-2-oxopyrrolidinoacetate[19] (2 g, 7.34 mmol) was deprotected with 4M-HCl in dioxane (10 ml) at 0 °C for 20 min. The mixture was stripped of dioxane and HCl under reduced pressure and the resulting residue was dried overnight under vacuum. This material and Boc-Pro-OH (1.58 g, 7.34 mmol) were dissolved in dry DMF (15 ml) and cooled in a salt–ice bath. To this cooled solution were added, sequentially, diphenylphosphoryl azide (2.22 g, 8.81 mmol) and Et_3N (2.25 ml, 16.16 mmol). The mixture was stirred in a salt–ice bath for 2 days and then at room temperature for 6 h. The solvent was evaporated under reduced pressure and the residue which was obtained was partitioned between EtOAc (150 ml) and 10% citric acid (50 ml). The organic layer was washed sequentially with 1M-$NaHCO_3$, H_2O, and saturated NaCl aqueous (50 ml of each) and then dried ($MgSO_4$). Evaporation of the solution under reduced pressure afforded an ester intermediate as an oil. This oil was dissolved in methanolic ammonia (50 ml) and stirred at room temperature for 2 days. The solvent and excess of ammonia were removed under reduced pressure. Crystallization of the residue from MeOH–Et_2O gave (**3**) (1.21 g, 46.5%), m.p. 241.5 °C (decomp.) (Found: C, 54.4; H, 7.45; N, 15.7. $C_{16}H_{26}N_4O_5$ requires C, 54.22; H, 7.40; N, 15.81%); $[\alpha]_D$ −87.8° (*c*, 1.07 in MeOH); δ_H (300 MHz; $CDCl_3$) 1.47 (9 H, s, Boc CH_3), 1.80—1.95 (3 H, m, Pro γ-H_2, β-H), 2.10—2.30 (2 H, m, Pro β-H and 4-H), 2.40—2.55 (1 H, m, 4-H), 3.47 (1 H, d, *J* 16.9 Hz, C*H*$CONH_2$), 3.30—3.50 (3 H, m, Pro δ-H_2 and 5-H), 3.60—3.70 (1 H, m, 5-H), 3.95—4.05 (1 H, m, 3-H), 4.15—4.35 (1 H, m, Pro α-H), 4.52 (1 H, d, *J* 16.9 Hz, C*H*$CONH_2$), 5.43 (1 H, br s, 3-NH), and 7.25—7.45 (2 H, br s, $CONH_2$); δ_C (90 MHz; [$^2H_6$]Me_2SO) 23.00 (Pro γ-C), 25.47 (C-4), 27.66, 27.96 (Boc CH_3), 30.42 (Pro C-β), 44.0 (C-5), 45.43 (CH_2CONH_2), 46.27 (Pro C-δ), 49.52 (C-3), 59.49 (Pro C-α), 78.32 (Boc CO), 153.19 (Boc C=O), 169.07 ($CONH_2$), and 171.61 and 172.32 (Pro and 2-C=O).

Crystal Data for Boc-L-Pro-L-Leu-Gly-NH_2 (**2a**) *Hemihydrate.*—$C_{18}H_{32}N_4O_5 \cdot 0.5H_2O$, M = 402.5. Monoclinic, a = 19.112(2), b = 9.931(1), c = 14.230(2) Å, β = 126.0(2)°, V = 2 185.1(56) Å^3, space group $C2$ [No. 5], Z = 4, D_c = 1.173 g cm^{-3}, μ = 0.48 cm^{-1} (Mo-K_α), final R value 0.058.

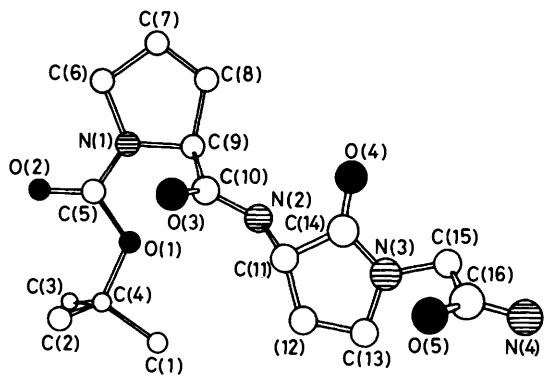


Figure 2. Molecular structure of the γ-lactam analogue of Boc-L-Pro-L-Leu-Gly-NH_2 (**3**) with numbering of the atoms

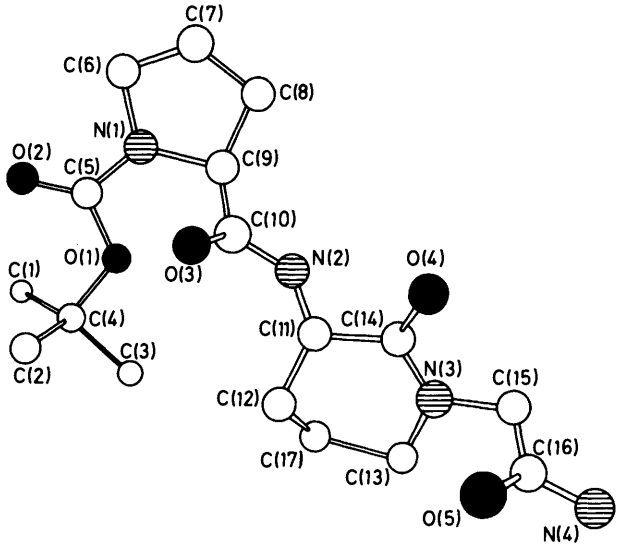


Figure 3. Molecular structure of the δ-lactam analogue of Boc-L-Pro-L-Leu-Gly-NH_2 (**4**) with numbering of the atoms

Crystal Data for 2-{(3S)-3-[(S)-1-*t-Butyloxycarbonylprolylamino*]-2-*oxopyrrolidino*}*acetamide* (**3**).—$C_{16}H_{26}N_4O_5$, M = 354.5. Monoclinic, a = 10.084(1), b = 14.270(2), c = 6.502(1) Å, β = 101.5(3)°, V = 916.9(10) Å^3, space group $P2_1$ [No. 4], Z = 2, D_c = 1.282 g cm^{-3}, μ = 0.60 cm^{-1} (Mo-K_α), final R value 0.046.

Crystal Data for 2-{(3S)-3-[(S)-1-*t-Butyloxycarbonylprolylamino*]-2-*oxopiperidino*}*acetamide* (**4**).—$C_{17}H_{28}N_4O_5$, M = 368.5. Monoclinic, a = 9.990(1), b = 14.575(2), c = 6.845(1) Å, β = 104.7(2)°, V = 964.0(9) Å^3, space group $P2_1$ [No. 4], Z = 2, D_c = 1.269 g cm^{-3}, μ = 0.58 cm^{-1} (Mo-K_α), final R value 0.061.

X-Ray Crystal Structure Determination of (**2a**) *Hemihydrate,* (**3**), *and* (**4**).—Colourless crystals of (**2a**) hemihydrate, (**3**), and (**4**) were grown by slow evaporation of acetone–diethyl ether, methanol–diethyl ether, and methanol–diethyl ether solutions, respectively. Philips PW 1100 diffractometer, θ-2θ scan mode up to θ = 25°; graphite-monochromated Mo-K_α radiation (λ = 0.710 69 Å); 2 798, 1 681, and 1 922 (1 771 independent)

Table 1. Torsion angles (°) common to Boc-L-Pro-L-Leu-Gly-NH_2 (**2a**) hemihydrate and its γ-lactam (**3**) and δ-lactam (**4**) analogues (with e.s.d.s in parentheses)

Angle	(**2a**)	(**3**)	(**4**)
C(1)–C(4)–O(1)–C(5)	69.6(14)	−179.4(10)	62.6(13)
C(2)–C(4)–O(1)–C(5)	−54.3(14)	−59.8(14)	−65.0(13)
C(3)–C(4)–O(1)–C(5)	−173.0(10)	64.6(13)	178.8(9)
C(4)–O(1)–C(5)–O(2)	−19.2(17)	−6.3(17)	−5.5(16)
C(4)–O(1)–C(5)–N(1)	161.3(10)	173.0(10)	177.1(9)
O(1)–C(5)–N(1)–C(6)	−174.6(10)	170.5(10)	178.3(9)
O(1)–C(5)–N(1)–C(9)	−10.3(15)	−4.3(15)	−10.0(14)
O(2)–C(5)–N(1)–C(6)	5.9(19)	−10.1(18)	0.8(16)
O(2)–C(5)–N(1)–C(9)	170.2(11)	175.0(11)	172.5(10)
C(5)–N(1)–C(6)–C(7)	−164.2(14) / 151.6(16) [a]	−160.2(11)	165.8(11)
C(5)–N(1)–C(9)–C(8)	−173.8(11)	−179.2(10)	−177.5(10)
C(5)–N(1)–C(9)–C(10)	−55.7(14)	−59.7(14)	−57.4(13)
N(1)–C(6)–C(7)–C(8)	−40.1(17) / 30.5(20) [a]	−29.7(13)	17.4(14)
C(6)–C(7)–C(8)–C(9)	36.3(18) / −35.6(20) [a]	33.9(13)	−21.3(15)
C(7)–C(8)–C(9)–N(1)	−17.5(15) / 26.9(16) [a]	−23.5(11)	15.9(13)
C(7)–C(8)–C(9)–C(10)	−135.9(13) / 91.4(16) [a]	−143.1(10)	−103.7(12)
C(7)–C(6)–N(1)–C(9)	29.8(15) / −14.4(18) [a]	15.2(13)	−6.9(12)
C(8)–C(9)–N(1)–C(6)	−8.2(12)	5.6(12)	−5.1(11)
C(8)–C(9)–C(10)–O(3)	85.2(13)	76.4(13)	82.5(13)
C(8)–C(9)–C(10)–N(2)	−92.6(12)	−99.7(11)	−93.1(11)
N(1)–C(9)–C(10)–O(3)	−28.7(15)	−37.3(14)	−31.9(14)
N(1)–C(9)–C(10)–N(2)	153.6(9)	146.6(9)	152.5(9)
C(9)–C(10)–N(2)–C(11)	−179.5(9)	173.9(9)	177.8(9)
O(3)–C(10)–N(2)–C(11)	2.8(17)	−2.0(17)	2.4(17)
C(10)–N(2)–C(11)–C(12)	−170.9(10)	126.9(11)	87.9(13)
C(10)–N(2)–C(11)–C(14)	−50.5(13)	−115.7(11)	−143.3(10)
N(2)–C(11)–C(14)–O(4)	56.4(14)	47.8(15)	68.7(14)
C(12)–C(11)–C(14)–O(4)	61.7(14)	170.0(11)	−164.3(11)
N(2)–C(11)–C(14)–N(3)	125.7(10)	−131.7(9)	−115.6(11)
C(11)–C(14)–N(3)–C(15)	−178.2(9)	176.6(9)	−172.2(10)
O(4)–C(14)–N(3)–C(15)	4.0(17)	−2.8(18)	3.5(16)
C(14)–N(3)–C(15)–C(16)	107.5(12)	119.2(11)	110.2(11)
N(3)–C(14)–C(11)–C(12)	−116.2(10)	−9.5(11)	11.4(16)
N(3)–C(15)–C(16)–O(5)	151.5(11)	−31.7(14)	−30.7(15)
N(3)–C(15)–C(16)–N(4)	−30.2(14)	150.9(10)	150.3(9)

[a] The upper value refers to molecule **A** and the lower value to molecule **B**.

unique reflections for (**2a**), (**3**), and (**4**), respectively; 1 534, 1 355, and 1 068 reflections with $I > 3\sigma(I)$ considered observed for (**2a**), (**3**), and (**4**), respectively. The three structures were solved by MULTAN 80[20] and refined by block-diagonal least-squares with weight $w = 1$. The thermal parameters were anisotropic for all non-hydrogen atoms. The hydrogen atoms of (**2a**) hemihydrate were calculated and were not refined; the hydrogen atoms of (**3**) were found on a difference-Fourier map and refined isotropically; the hydrogen atoms of (**4**) were found on a difference-Fourier map and were not refined. All calculations were performed on the IBM 370/158 computer of the University of Padova using SHELX-76.[21]

Tables of fractional atomic co-ordinates, bond lengths, and bond angles for (**2a**) hemihydrate, (**3**), and (**4**) are available from the Cambridge Crystallographic Data Centre.*

* For details of deposition of material at the Cambridge Crystallographic Data Centre, see 'Instructions for Authors (1989)', *J. Chem. Soc., Perkin Trans. 2*, 1989, issue 1, p. xviii, paragraph 5.6.3.

Results and Discussion

The molecular structures of compounds (**2a**) hemihydrate, (**3**), and (**4**) with their atomic numbering schemes are shown in Figures 1—3, respectively. The torsion angles[22] common to the three structures are listed in Table 1.

Bond lengths and bond angles observed for compounds (**2a**), (**3**), and (**4**) are in agreement with previously described results for the geometry of the Boc-urethane[23,24] and amide[25] groups, Pro,[26–31] Leu,[31–33] and Gly residues, γ-[34] and δ-lactam[25,34] ring structures, and the peptide unit.[35] In particular: (*a*) unfavourable interactions between the bulky t-butyl group and spatially proximate atoms, especially the carbonyl oxygen O(2), result in alterations of several bond angles of the Boc moiety of (**2a**), (**3**), and (**4**) relative to values observed in unhindered compounds, *e.g.* the values of the C(4)–O(1)–C(5) bond angle is 121.9(7)° for (**2a**), 121.1(7)° for (**3**), and 120.9(6)° for (**4**), significantly larger than the average value of the corresponding angle in esters, 117.4(16)°.[36] (*b*) The bond angles of the amide group of the lactam moieties are determined mainly by the ring size, some of them deviating markedly from the 120° value. The C(13)–N(3)–C(14), N(3)–C(14)–C(11), N(3)–C(14)–O(4), and C(11)–C(14)–O(4) values for the γ-lactam (**3**) are 114.4(6), 108.2(6), 125.3(7), and 126.5(7)°, respectively, while the C(13)–N(3)–C(14) value for the δ-lactam (**4**) is 126.1(9)°.

The tertiary urethane bond of the Boc-Pro moiety of the three compounds adopts the relatively common *cis* conformation, the O(1)–C(5)–N(1)–C(9) (ω_0) torsion angle being −10.3(15)° for (**2a**), −4.3(15)° for (**3**), and −10.0(14)° for (**4**). In addition, the C(4)–O(1) bond is in the usual *trans* arrangement relative to the C(5)–N(1) bond, the C(4)–O(1)–C(5)–N(1) torsion angle being 161.3(10)° for (**2a**) 173.0(10)° for (**3**) and 177.1(9)° for (**4**). This feature, accompanied by the *cis* arrangement of the O(1)–C(5) bond relative to the N(1)–C(9) bond, allows us to classify the urethane moiety of the three peptides as type *a*.[23,24]

The Leu side-chain conformation of (**2a**) is the second most common conformation of this γ-branched residue, $t(g^+, t)$,[31–33] the χ^1[N(2)–C(11)–C(12)–C(13)], $\chi^{2,1}$[C(11)–C(12)–C(13)–C(17)], and $\chi^{2,2}$[C(11)–C(12)–C(13)–C(18)] torsion angles being −180.0(9), 65.4(13), and −170.8(11)°, respectively.

Two crystallographically independent molecules (**A** and **B**) with an occupancy of 50:50 are found in the crystals of (**2a**), the only difference between them being in the position of the C(7) atom, *i.e.* the C^γ atom of the Pro residue. The C(7)**A** atom and the C(7)**B** atom also exhibit large thermal vibrations perpendicular to the pyrrolidine ring. This heterocyclic ring has an approximate C_s (envelope) symmetry (conformation A) in molecule **A**, and a C_2 (half-chair) symmetry (conformation B) in molecule **B**, the ring-puckering parameters[37] being q_2 = 0.390(25) Å for **A** and 0.350(25) Å for **B**, and φ_2 = 26.7(22)° for **A** and −130.3(23)° for **B**.[26–31]

The pyrrolidine ring of (**3**) shows an approximate C_2 symmetry (conformation B) with q_2 = 0.322(14) Å and φ_2 = 46.8(23)°, while that of (**4**) has a C_2 symmetry (conformation A) with q_2 = 0.189(16) Å, and φ_2 = 87.9(34)°.

The γ-lactam ring conformation of (**3**) is an envelope with q_2 = 0.163(13) Å and φ_2 = −146.7(88)°, while the δ-lactam ring conformation of (**4**) is a half-chair with q_2 = 0.158(13) Å, φ_2 = 96.5(44)°, and q_3 = 0.483(14) Å.

The backbone conformation of (**2a**) is folded at the –Leu-Gly– sequence. The φ,ψ sets of values for the Leu [−50.5(13), 125.7(10)°] and Gly [107.5(12), −30.2(14)°] residues are not too far from those expected for an ideal type-II β-bend.[2–8] A 4→1 intramolecular hydrogen bond is seen between the Gly carboxamide *anti*-NH donor and the Pro carbonyl acceptor. The N(4)···O(3) (*x,y,z*) intramolecular separation, 2.952(12) Å, agrees well with the average value determined from a large number of peptide structures.[38,39]

The incorporation of γ- and δ-lactam modifications, as in (**3**)

Table 2. Backbone torsion angles (°), β-bend type, and intramolecular hydrogen-bonding N ··· X distance (Å) for tripeptides (**1**), (**2a—e**), (**3**), and (**4**)

Compd.	Residue 1			Residue 2[c]			Residue 3			β-Bend type[d]	Intramol. H-bonding N ··· X distance[e]
	ω_0[a]	φ	ψ	ω_1[b]	φ	ψ	ω_2	φ	ψ		
(**1**)			152.9	171.0	−61.2	127.8	−179.9	71.8	*f*	II	3.04
(**2a**)	−10.3	−55.7	153.6	−179.5	−50.5	125.7	−178.2	107.5	−30.2	II	2.95
(**2b**)	−12.6	−54.3	148.0	−171.0	−63.5	−23.4	176.8	−75.7	−7.2	I	3.00
(**2c**)	0.1[g]	−66.9	165.6	−175.1	−156.3	146.2	176.7	−166.0	169.2		
	3.9[g]	−78.2	152.8	175.9	−138.6	154.8	173.4	−166.7	159.2		
(**2d**)	−2.2	−60.9	156.3	−178.8	−64.9	−23.0	177.9	−88.8	6.1	I	3.07
(**2e**)	−4.5	−73.6	154.2	169.0	−77.0	131.8	−178.0	71.3	15.6	II	3.56
(**3**)	−4.3	−59.7	146.6	173.9	−115.7	−131.7	176.6	119.2	150.9		
(**4**)	−10.0	−57.4	152.5	177.8	−143.3	−115.6	−172.2	110.2	150.3		

[a] Tertiary urethane bond. [b] Peptide bond; in (**2e**) thiopeptide bond. [c] Leu in (**1**), (**2a**), and (**2e**); Ala in (**2b**); Val in (**2c**); Pro in (**2d**); γ-lactam in (**3**); δ-lactam in (**4**). [d] Involving residues 2 and 3. [e] X = S in (**2e**); in all other cases X = O. [f] Not given in ref. 9. [g] Upper values, molecule **A**; lower values, molecule **B**.

and (**4**), respectively, induces dramatic changes in the backbone torsion angles of the –Leu-Gly– sequence, eventually producing extended conformations. The φ,ψ sets of values for the 3-amino-2-pyrrolidone [−115.7(11)°, −131.7(9)°] and Gly[119.2(11)°, 150.9(10)] residues of (**3**) are reasonably close to those of the 3-amino-2-piperidone[40] [−143.3(10)°, −115.6(11)°] and Gly [110.2(11)°, 150.3(9)°] residues of (**4**). It should be noted that the ψ torsion angle of the two amino lactam moieties is about the endocyclic C(11)–C(14) bond. Conformational energy calculations of (*S*)-3-amino-2-pyrrolidone and (*S*)-2-amino-2-piperidone derivatives have recently been performed by Freidinger *et al.*[10] and Madison and Kopple.[41] The low-energy conformers found by Madison and Kopple for the five-membered lactam are slightly puckered with ψ near −140°, while one of the two low-energy conformers found by Freidinger *et al.* for the six-membered lactam is a half-chair with ψ = −108°.

In all three compounds the Pro residue is semi-extended [polyproline (II)-type conformation] with φ,ψ torsion angles of −55.7(14), 153.6(9)° for (**2a**), −59.7(14), 146.6(9)° for (**3**), and −57.4(13), 152.5(9)° for (**4**). In addition, the ω_1[C(9)–C(10)–N(2)–C(11)] and the ω_2 [C(11)–C(14)–N(3)–C(15)] torsion angles of the peptide (or lactam) moieties are all *trans*, −179.5(19) and −178.2(9)° for (**2a**), 173.9(9) and 176.6(9)° for (**3**), and 177.8(9) and −172.2(10)° for (**4**).

In the crystal the molecules of (**2a**) hemihydrate pack by forming rows of dimers along the *y*-direction. Dimers, generated by (amide)NH ··· O=C(urethane) intermolecular hydrogen bonds [the N(4) ··· O(2) (1 − *x*, *y*, 1 − *z*) separation is 3.004(12) Å[38,39]], are interconnected by (Gly)NH ··· O=C-(Leu) hydrogen bonds [the N(3) ··· O(4) (3/2 − *x*, 1/2 + *y*, 2 − *z*) separation is 2.928(12) Å]. The water molecule is involved in four hydrogen bonds.[42] The first pair of contacts involving the Gly C=O groups of two symmetry-related molecules as acceptors and a second pair of contacts involving the Leu NH groups of two additional symmetry-related molecules as donors are observed. The O_w ··· O(5) (1 − *x*, *y*, 1 − *z*) distance is 2.756(10) Å,[43,44] while the O_w ··· N(2) (*x* − 1/2, 1/2 + *y*, *z* − 1) distance is 2.965(10) Å. This complex intermolecular hydrogen-bonding scheme is completely at variance with that reported for the N^{α}-deblocked tripeptide (**1**).[9]

The crystallographic parameters of (**3**) and (**4**) are quite similar. In fact, with the obvious exception of certain parts of the lactam rings, the atomic co-ordinates are almost identical, so that the two structures can be considered almost isomorphic. As a consequence, it is not surprising to find not only a close similarity in the overall conformation but identical modes of packing of the molecules in the crystals as well. The molecules of both (**3**) and (**4**) form rows extending along the *x*,*z* diagonal, being interconnected by (amide)NH ··· O=C(urethane) hydrogen bonds [the N(4) ··· O(2) (*x* − 1, *y*, *z* − 2) distance is 2.917(8) Å[38,39] for (**3**) and the O(2) ··· N(4) (*x* − 1, *y*, *z* − 2) distance is 2.970(9) Å for (**4**)] and (amide)NH ··· O=C(peptide) hydrogen bonds [the N(4) ··· O(3) (*x* − 1, *y*, *z* − 1) distance is 2.919(7) Å for (**3**) and the O(3) ··· N(4) (*x* − 1, *y*, *z* − 1) distance is 2.938(9) Å for (**4**)]. Additional rows of molecules seen along the *z*-direction, characterized by (peptide)NH ··· O=C(amide) intermolecular hydrogen bonds [the N(2) ··· O(5) (*x*, *y*, 1 + *z*) separation is 2.836(7) Å for (**3**) and the O(5) ··· N(2) (*x*, *y*, 1 + *z*) separation is 2.925(10) Å for (**4**)].

Conclusions

Table 2 summarizes the backbone torsion angles, the β-bend type, and the intramolecular hydrogen-bonding N ··· O (or N ··· S) distance for tripeptides (**1**), (**2a—e**), (**3**), and (**4**). The crystal-state structure of the *C*-terminal tripeptide amide of oxytocin, Pro-Leu-Gly-NH_2 (**1**), is characterized by a polyproline (II)-type (semi-extended) conformation at the *N*-terminal part and a type-II β-bend[2–8] at the *C*-terminal part.[9] The reported backbone torsion angles are: ψ_1 = 152.9°, φ_2 = −61.2°, ψ_2 = 127.8°, and φ_3 = 71.8°. The two peptide linkages are *trans*. A weak intramolecular hydrogen bond (the N ··· O distance is 3.04 Å), involving the Gly carboxamide *anti*-NH and Pro carbonyl groups, determines the overall compact structural feature of the molecule.

The structure of the N^{α}-t-butyloxycarbonyl derivative (**2a**) hemihydrate is remarkably similar to that of its parent compound. Again, the polyproline (II)-type conformation [φ_1,ψ_1 = −55.7(14), 153.6(9)°] and a slightly distorted type-II β-bend [φ_2,ψ_2 = −50.5(13), 125.7(10)°; φ_3,ψ_3 = 107.5(12), −30.2(14)°] are seen at the *N*- and *C*-termini of the peptide chain. The observed N ··· O distance of 2.952(12) Å confirms that intramolecularly hydrogen-bonded β-bend is one of the favourite conformations for peptides having amidated *C*-terminal groups. Interestingly, the similarity of the structures of (**1**) and (**2a**) should be attributed to the *cis* conformation adopted by the tertiary urethane moiety of (**2a**) which does not make the Boc carbonyl group available as an acceptor for an additional intramolecular hydrogen bond.

Apparently, the sequence of (**2a**) does not readily accommodate modifications without a concomitant structural change. In fact, although the [$^1\psi^2$, CSNH][16] isosteric analogue (**2e**) is folded exactly as its oxygenated counterpart (**2a**),[17] the replacement of the central Leuresidue by an Ala (**2b**)[13] or a Pro (**2d**)[15] residue was found to induce the onset of a different type of β-bend (type-I)[2–8] at the *C*-terminus, with a 180° rotation in

the Xaa-Gly peptide group so that the carbonyl oxygen is directed into the plane of the ten-membered ring. An apparently more severe change, such as the replacement of the γ-branched Leu residue by the β-branched Val residue [tripeptide (**2c**)], generates extended chains at the *C*-terminus packed similarly to antiparallel β-sheets.[14] On these bases, it is not surprising that a side-chain to main-chain cyclization such as that characterizing the γ-lactam in (**3**) or the δ-lactam in (**4**) would induce a dramatic structural change, while keeping the ω_2 torsion angle frozen in the *trans* conformation.[10,11] The resulting conformation at the *C*-terminus of both (**3**) and (**4**) not only is extended but it is somewhat unusual in having negative signs for both the φ,ψ torsion angles of the lactam residues and positive signs for both the Gly φ,ψ torsion angles. In all the Boc-Pro tripeptides discussed here [(**2a**—**d**) (**3**), and (**4**)] the Boc-Pro tertiary urethane bond is *cis* and the Pro residue is semi-extended [polyproline (II)-type conformation].

Acknowledgements

This work was supported in part by a grant (NS 20036) to R. L. J. from the National Institutes of Health.

References

1 R. K. Mishra, S. P. Chiu, and C. P. Mishra, *Methods Find. Exptl. Clin. Pharmacol.*, 1983, **5**, 203.
2 C. M. Venkatachalam, *Biopolymers*, 1968, **6**, 1425.
3 P. N. Lewis, F. A. Momany, and H. A. Scheraga, *Biochim. Biophys. Acta*, 1973, **303**, 211.
4 P. Y. Chou and G. D. Fasman, *J. Mol. Biol.*, 1977, **115**, 135.
5 P. Y. Chou and G. D. Fasman, *Biophys. J.*, 1979, **26**, 367.
6 C. Toniolo, *CRC Crit. Rev. Biochem.*, 1980, **9**, 1.
7 J. S. Richardson, *Adv. Protein Chem.*, 1981, **34**, 167.
8 G. D. Rose, L. M. Gierasch, and J. A. Smith, *Adv. Protein Chem.*, 1985, **37**, 1.
9 L. L. Reed and P. L. Johnson, *J. Am. Chem. Soc.*, 1973, **95**, 7523.
10 R. M. Freidinger, D. F. Veber, R. Hirschmann, and L. M. Paege, *Int. J. Pept. Protein Res.*, 1980, **16**, 464.
11 R. M. Freidinger, D. S. Perlow, and D. F. Veber, *J. Org. Chem.*, 1982, **47**, 104.
12 A. F. Spatola, in 'Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins,' ed. B. Weinstein, Dekker, New York, 1983, vol. 7, p. 267.
13 T. Kojima, I. Tanaka, and T. Ashida, *Acta Crystallogr., Sect. B*, 1982, **38**, 221.
14 T. Ashida, T. Kojima, I. Tanaka, and T. Yamane, *Int. J. Pept. Protein Res.*, 1986, **27**, 61.
15 I. Tanaka, T. Ashida, Y. Shimonishi, and M. Kakudo, *Acta Crystallogr., Sect. B*, 1979, **35**, 110.
16 IUPAC–IUB Commission on Biochemical Nomenclature, *Eur. J. Biochem.*, 1984, **138**, 9.
17 R. Bardi, A. M. Piazzesi, C. Toniolo, O. E. Jensen, T. P. Andersen, and A. Senning, *Tetrahedron*, 1988, **44**, 761.
18 T. Mizoguchi, K. Shigezawa, and N. Takamura, *Chem. Pharm. Bull., (Tokyo)*, 1970, **18**, 1465.
19 K.-L. Yu, G. Rajakumar, L. K. Srivastava, R. K. Mishra, and R. L. Johnson, *J. Med. Chem.*, 1988, **31**, 1430.
20 P. Main, S. J. Fiske, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq, and M. M. Woolfson, 'MULTAN 80. A System of Computer Programs for the Automatic Solution of Crystal Structures from *X*-Ray Diffraction Data,' University of York and University of Louvain, 1980.
21 G. M. Sheldrick, 'SHELX-76. Program for Crystal Structure Determination,' University of Cambridge, 1976.
22 IUPAC–IUB Commission on Biochemical Nomenclature, *J. Mol. Biol.*, 1970, **52**, 1.
23 E. Benedetti, A. Ciajolo, B. Di Blasio, V. Pavone, C. Pedone, C. Toniolo, and G. M. Bonora, *Int. J. Pept. Protein Res.*, 1979, **14**, 130.
24 E. Benedetti, C. Pedone, C. Toniolo, G. Némethy, M. S. Pottle, and H. A. Scheraga, *Int. J. Pept. Protein Res.*, 1980, **16**, 156.
25 P. Chakrabarti and J. D. Dunitz, *Helv. Chim. Acta*, 1982, **65**, 1555.
26 R. Balasubramanian, A. V. Lakshminarayanan, M. N. Sabesan, G. Tegoni, K. Venkatesan, and G. N. Ramachandran, *Int. J. Protein Res.*, 1971, **3**, 25.
27 T. Ashida and M. Kakudo, *Bull. Chem. Soc. Jpn.*, 1974, **47**, 1129.
28 D. L. F. DeTar and N. P. Luthra, *J. Am. Chem. Soc.*, 1977, **99**, 1232.
29 C. M. K. Nair and M. Vijayan, *Indian Inst. Sci.*, 1981, **63c**, 81 (*Chem. Abstr.*, 1982, **96**, 69376b).
30 E. Benedetti, A. Bavoso, B. Di Blasio, V. Pavone, C. Pedone, C. Toniolo, and G. M. Bonora, *Biopolymers*, 1983, **22**, 305.
31 T. Ashida, Y. Tsunogae, I. Tanaka, and T. Yamane, *Acta Crystallogr., Sect. B*, 1987, **43**, 212.
32 E. Benedetti, G. Morelli, G. Némethy, and H. A. Scheraga, *Int. J. Pept. Protein Res.*, 1983, **22**, 1.
33 R. O. Gould, A. M. Gray, P. Taylor, and M. D. Walkinshaw, *J. Am. Chem. Soc.*, 1985, **107**, 5921.
34 L. Norskov-Lauritsen, H.-B. Bürgi, P. Hofmann, and H. R. Schmidt, *Helv. Chim. Acta*, 1985, **68**, 76.
35 E. Benedetti, in 'Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins,' ed. B. Weinstein, Dekker, New York, 1982, vol. 6, p. 105.
36 W. B. Schweizer and J. D. Dunitz, *Helv. Chim. Acta*, 1982, **65**, 1547.
37 D. Cremer and J. A. Pople, *J. Am. Chem. Soc.*, 1975, **97**, 1354.
38 C. Ramakrishnan and N. Prasad, *Int. J. Protein Res.*, 1971, **3**, 209.
39 R. Taylor, O. Kennard, and W. Versichel, *Acta Crystallogr., Sect. B*, 1984, **40**, 280.
40 G. Valle, M. Crisma, C. Toniolo, R. L. Johnson, and K.-L. Yu, *Acta Crystallogr.*, in the press.
41 V. Madison and K. D. Kopple, *J. Am. Chem. Soc.*, 1980, **102**, 4855.
42 C. H. Yang, J. N. Brown, and K. D. Kopple, *Int. J. Pept. Protein Res.*, 1979, **14**, 12.
43 I. D. Brown, *Acta Crystallogr., Sect. B*, 1976, **32**, 24.
44 J. Mitra and C. Ramakrishnan, *Int. J. Pept. Protein Res.*, 1977, **9**, 27.

Received 18*th March* 1988; *Paper* 8/00928