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Structure and conformation of the sodium chloride salt of N-t-Boc–Phenylalanyl–Proline (Boc–Phe–Pro·NaCl) and the dihydrate of N-t-Boc–Tyrosyl–Proline (Boc–Tyr–Pro·$2H_2O$)

P. J. Milne,[1] D. W. Oliver,[2]* P. H. Van Rooyen,[3] and H. M. Roos[3]

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The structure and conformation of the salt of N-t-Boc–Phenylalanyl–Proline (Boc–Phe–Pro·NaCl) ($C_{19}H_{26}N_2O_5NaCl$) (compound 2) and the dihydrate of N-t-Boc–Tyrosyl–Proline (Boc–Tyr–Pro·$2H_2O$) ($C_{19}H_{30}O_8N_2$) (compound 1) have been investigated with X-ray crystallographic and spectroscopic methods. Boc–Phe–Pro·NaCl crystallized in an extended trans conformation in the space group $P2_1$ with cell dimensions a = 7.961 (3), b = 10.045(2), and c = 13.495(4). One intermolecular hydrogen bond and one intramolecular hydrogen bond was observed for the dipeptide salt. Boc–Tyr–Pro·$2H_2O$ crystallized in an extended trans conformation in the space group $P2_1$ with cell dimensions a = 7.964(1), b = 10.011(1), and c = 13.853(2). Six intermolecular hydrogen bonds were observed for Boc–Tyr–Pro·$2H_2O$. The conformation of both dipeptides reflect collagen-type of proline-compounds. The puckering mode of the pyrrolidine ring of the proline residues can be described as an approximate C_2 half-chair symmetry having an A conformation with the Cγ atom located exo and Cβ atom located endo relative to the carboxamide group, i.e., $^{\gamma}_{\beta}$ T. *Cis-trans* isomerism was observed in the NMR spectra of both dipeptides with a predominance for the extended side chain for the phenylalanyl and tyrosyl residues, respectively.

KEY WORDS: Dipeptides; proline; NMR; X-rays.

Experimental

All reagents and solvents were of reagent grade and used without further purification.

Synthesis

(A) Synthesis of Boc–Phe–Pro·NaCl. To a stirred solution of N-t-Boc–S–Phe (6 g, 22.615 mmol) and S–Pro–NH_2 (2.5815 g, 22.615 mmol) in 1,2-dimethoxyethane (40 ml) at 0°C, triethylamine (6.58 ml, 47.49 mmol) and diethylphosphoryl cyanide (3.77 ml, 24.85 mmol) were added. 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). Recrystallization from chloroform-n-hexane

[1] School of Pharmacy, University of Port Elizabeth, Box 1600, Port Elizabeth 6000, Republic of South Africa.
[2] Department of Pharmacology, Potchefstroom University of C.H.E., Potchefstroom 2520, Republic of South Africa.
[3] Department of Chemistry, University of Pretoria, Pretoria, 0001, Republic of South Africa.
* To whom correspondence should be addressed.

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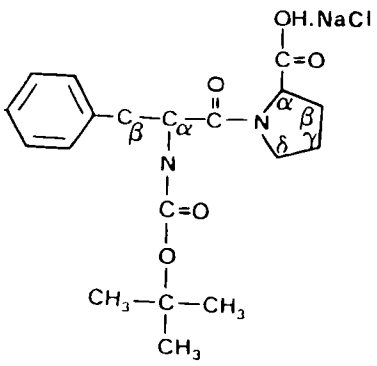
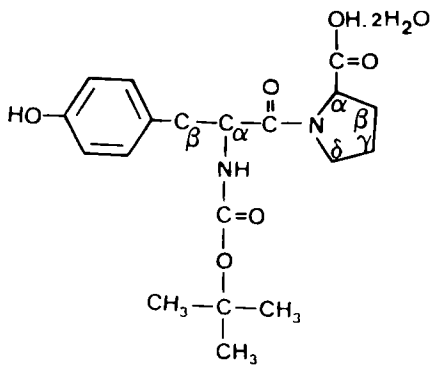
yielded Boc–Phe–Pro·NaCl (7.530 g, 79%, m.p. 68–70°C).

(B) Synthesis of Boc–Tyr–Pro·2H₂O. To a stirred solution of N-t-Boc–S–Tyr (2 g, 7.109 mmol) and S–Pro–NH_2 (0.81 g, 7.109 mmol) in 1,2-dimethoxyethane (40ml) at 0°C, triethylamine (2.07 ml, 14.93 mmol) and diethylphosphoryl cyanide (1.18 ml, 7.81 mmol) were added. 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* = 0.32; chloroform–methanol (18:2). Crystallization from chloroform–diethyl ether yielded Boc–Tyr–Pro·2H₂O (2.15 g, 73%, m.p. 99.5–100.5°C).



Boc-Phe-Pro·NaCl



Boc-Tyr-Pro·2H₂O

Scheme 1.

X-ray analysis

Diffraction quality crystals of Boc–Phe–Pro·NaCl and Boc–Tyr–Pro·2H₂O were obtained by standard crystallization methods. All diffraction measurements were obtained at room temperature and data were collected with an Enraf-Nonius CAD4 diffractometer with Mo-*K*α radiation (graphite monochromator, λ = 0.7107 Å). Accurate unit cell parameters were obtained by least-squared methods from the position of 25 selected centered reflections for each crystal. There was no significant crystal decay and intensities were corrected for absorption, as well as for Lorentz and polarisation effects. An empirical method for absorption correction was applied.[22] Standard intensity checks and orientation control were carried out.

The structures were solved by Patterson and direct methods.[10,23] Refinement was by full matrix least-squares methods, using σ^{-2} (F_{obs}) weights.[10] All the nonhydrogen atoms for 1 and 2 were refined anisotropically. The hydrogen atoms were fixed in either experimentally determined or idealized positions, and included in the refinement with common isotropic thermal parameters. Atomic scattering factors were taken from the literature.[25]

Perspective views of the molecules, prepared with ORTEP,[24] are represented in Figs. 1 and 2, illustrating the crystallographic numbering schemes used.

Colorless crystals of Boc–Phe–Pro·NaCl ($C_{19}H_{26}N_2O_5NaCl$) were crystallized from chloroform-n-hexane in the space group $P2_1$. The crystal selected for data collection had the dimensions 0.32 × 0.36 × 0.52 mm. Reflections (2444) were collected with their indices being *h* 0:11, *k* 0:14, *l*-19:19. The minimum and maximum transmission factors were 0.890 and 1.000, respectively (0.960 average), and are listed with other relevant crystal data in Table 1. Fractional coordinates and equivalent thermal factors for Boc–Phe–Pro·NaCl are listed in Table 2. The relevant torsion angles and fractional coordinates of the hydrogen atoms are listed in Table 3 and Table 4, respectively.

Colorless crystals of Boc–Tyr–Pro·2H₂O ($C_{19}H_{30}N_2O_8$) were crystallized from chloroform–diethyl ether in the space group $P2_1$. The crystal selected for data collection had the dimensions 0.10 × 0.38 × 0.40 mm. Reflections (3581) were collected with their

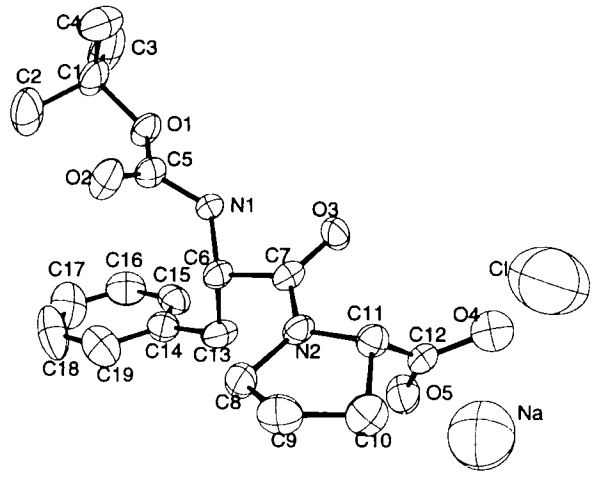


Fig. 1. ORTEP drawing of Boc–Phe–Pro·NaCl.

indices being h 0:11, k 0:14, l-19:19. The minimum and maximum transmission factors were 0.8230 and 1.000, respectively (0.918 average), and are listed with other relevant crystal data in Table 1. Fractional coordinates and equivalent thermal factors for Boc–Tyr–Pro·$2H_2O$ are listed in Table 5. The relevant torsion angles and fractional coordinates of the hydrogen atoms are listed in Table 6 and Table 7, respectively. Ellipsoids were at a 50% level.

The a, b, and c dimensions of the two cells were very close. The beta angles are 91.62° for Boc–Phe–Pro·NaCl and 90.64° for Boc–Tyr–Pro·$2H_2O$. As suggested by the cell parameters and the atomic positions, the two structures were isomorphous. The replacement of the two water molecules by NaCl, and

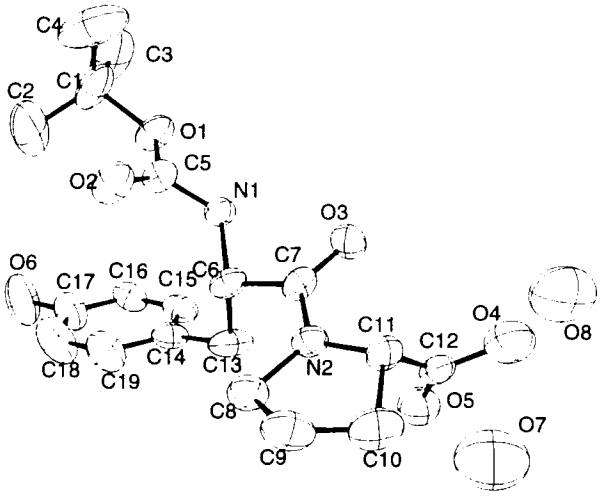


Fig. 2. ORTEP drawing of Boc–Tyr–Pro·$2H_2O$.

Table 1. Crystallographic data acquisition and refinement details

	Boc–Tyr–Pro·$2H_2O$	Boc–Phe–Pro·NaCl
Emprical formula	$C_{19}H_{26}N_2O_6 \cdot 2H_2O$	$C_{19}H_{26}N_2O_5 \cdot 0.7NaCl$
Molecular weight	414.45	403.33
Crystal dimension, mm	0.10 × 0.38 × 0.40	0.32 × 0.36 × 0.52
Space group	$P2_1$	$P2_1$
Cell dimensions		
a, Å	7.964(1)	7.961(3)
b, Å	10.011(1)	10.045(2)
c, Å	13.853(2)	13.495(4)
α,°	90	90
β,°	90.64(1)	91.62(3)
γ,°	90	90
Z	2	2
Volume, Å^3	1104(1)	1078(1)
D(calc), g cm^{-3}	1.25	1.24
μ, cm^{-1}	0.61	1.44
Radiation (λ, Å)	Mo$K\alpha$, 0.7107	Mo$K\alpha$, 0.7107
T, °C	24	22
F(000)	444	4427
Scan type (ω$^-$ 2θ)	1:1	1:1
Scan range (θ°)	3≤θ≤30	3≤θ≤30
Zone collected:		
h	0, + 11	0, + 11
k	0, + 14	0, + 14
l	−19, + 19	−19, + 19
Max. scan speed (deg min^{-1})	5.49	5.49
Max. scan time, sec.	60	60
Scan angle (ω + 0.34 tan θ)°	0.47	0.51
Aperture size (mm)	1.3 × 4.0	1.3 × 4.0
Reflections collected	3581	3492
Decay, %	−0.4	−1.0
EAC correction factor:		
Maximum	1.000	1.000
Minimum	0.823	0.890
Average	0.918	0.960
Unique refl. used	2006 (>2σ(I))	2444 (>3σ(I))
R_{int}	0.019	0.064
Parameters refined	283	263
Max. positional shift/esd	0.040	0.088
Residual electron density (eÅ^3):		
Maximum	0.332	0.472
Minimum	−0.479	−0.401
U_{iso}(H), Å^2	0.104(6)	0.076(4)
Refinement of H-atoms[a]	All fixed	All fixed
R	0.082	0.069
R_w, [$w = \sigma^{-2}(F_o)$]	0.045	0.047

[a] Fixed in calculated positions and included in the refinement with a common isotropic thermal parameter, or fixed in experimentally determined positions with a common isotropic thermal parameter, that was also refined.

Table 2. Fractional atomic coordinates ($\times 10^4$) and equivalent thermal factors ($\times 10^3$ Å^2) for Boc–Phe–Pro·NaCl

Atom	*x/a*	*y/b*	*z/c*	U_{eq}[a]
C(1)	−855(8)	−2928(7)	6191(4)	54(1)
C(2)	−1534(8)	−2315(8)	5227(4)	75(2)
C(3)	−2049(10)	−3969(7)	6534(5)	90(2)
C(4)	913(9)	−3409(8)	6094(5)	84(2)
O(1)	−845(4)	−1939(5)	7002(2)	42(1)
C(5)	57(6)	−804(6)	6940(4)	36(1)
O(2)	717(5)	−417(5)	6194(2)	51(1)
N(1)	96(4)	−183(5)	7829(3)	32(1)
C(6)	504(6)	1228(6)	7856(3)	33(1)
C(7)	1853(6)	1490(6)	8664(3)	35(1)
O(3)	2111(4)	704(5)	9350(2)	42(1)
N(2)	2695(5)	2648	8607(3)	34(1)
C(8)	2693(7)	3583(6)	7766(3)	43(1)
C(9)	4287(6)	4396(7)	7985(4)	50(1)
C(10)	4509(6)	4394(6)	9124(4)	49(1)
C(11)	3875(6)	3019(6)	9413(3)	35(1)
C(12)	2914(7)	3110(6)	10382(4)	41(1)
O(4)	3811(5)	2737(6)	11208(3)	76(1)
O(5)	1468(4)	3515(5)	10421(2)	50(1)
C(13)	−1072(6)	2035(6)	8123(4)	46(1)
C(14)	−2567(6)	1637(7)	7475(4)	43(1)
C(15)	−3803(6)	807(6)	7838(4)	48(1)
C(16)	−5131(7)	331(7)	7212(5)	61(2)
C(17)	−5229(7)	731(9)	6253(5)	72(2)
C(18)	−4055(8)	1558(9)	5882(4)	81(2)
C(19)	−2687(7)	2059(7)	6503(4)	61(2)
Na	10172(5)	5510(6)	9079(3)	86(1)
Cl	7439(5)	2638(6)	10941(3)	168(2)

[a] $U_{eq} = 1/3\ \Sigma_i\ \Sigma_j\ U_{ij} a^*_i a^*_j\ (a_i \cdot a_j)$.

Table 3. The relevant torsion angles of Boc−Phe−Pro·NaCl

	C2−C1−O1−C5	−60.140 (.690)
	C3−C1−O1−C5	−177.299 (.497)
	C4−C1−O1−C5	61.823 (.697)
	C1−O1−C5−O2	10.567 (.789)
	C1−O1−C5−N1	−169.589 (.467)
	O1−C5−N1−C6	−160.215 (.439)
	O2−C5−N1−C6	19.625 (.772)
ϕ_1	C5−N1−C6−C7	−131.490 (.491)
	C5−N1−C6−C13	110.584 (.523)
	N1−C6−C7−O3	−20.444 (.701)
ψ_1	N1−C6−C7−N2	162.108 (.404)
	C13−C6−C7−O3	98.739 (.575)
	C13−C6−C7−N2	−78.710 (.566)
χ_1^1	N1−C6−C13−C14	−50.053 (.613)
	C7−C6−C13−C14	−169.326 (.471)
	C6−C7−N2−C8	−12.050 (.689)
ω_1	C6−C7−N2−C11	173.660 (.397)
	O3−C7−N2−C8	170.503 (.450)
	O3−C7−N2−C11	−3.786 (.667)
	C7−N2−C8−C9	−160.857 (.466)
χ_2^4	C11−N2−C8−C9	13.711 (.500)
	C7−N2−C11−C10	−177.196 (.441)
ϕ_2	C7−N2−C11−C12	−59.830 (.533)
θ	C8−N2−C11−C10	7.759 (.507)
	C8−N2−C11−C12	125.124 (.458)
χ_2^3	N2−C8−C9−C10	−29.520 (.564)
χ_2^2	C8−C9−C10−C11	34.755 (.562)
χ_2^1	C9−C10−C11−N2	−25.721 (.521)
	C9−C10−C11−C12	−142.352 (.481)
	N2−C11−C12−O4	146.261 (.464)
	N2−C11−C12−O5	−35.466 (.732)
	C10−C11−C12−O4	−99.899 (.575)
	C10−C11−C12−O5	78.373 (.676)
χ_1^2	C6−C13−C14−C15	102.366 (.641)
χ_1^3	C6−C13−C14−C19	−75.296 (.719)
	C13−C14−C15−C16	−174.301 (.557)
	C19−C14−C15−C16	3.380 (.923)
	C13−C14−C19−C18	174.415 (.593)
	C15−C14−C19−C18	−3.279 (.937)
	C14−C15−C16−C17	−2.363 (.942)
	C15−C16−C17−C18	1.328 (1.052)
	C16−C17−C18−C19	−1.341 (1.118)
	C17−C18−C19−C14	2.322 (1.013)
	Calculations complete	

the replacement of the OH-group in the tyrosine residue by a H-atom in the phenylalanine residue has not affected the packing of the dipeptide in any major way.

The Na and Cl positions in Boc−Phe−Pro·NaCl were only partially occupied, and refinement of the site occupancy factor for these ions yielded a value of 0.699. In contrast the site occupancy factors for both oxygen atoms in the water molecules of Boc−Tyr−Pro·$2H_2O$ converged to 1 upon refinement of these values.

Spectroscopic analyses

^{1}H Proton (300 MHz) and ^{13}C (75 MHz) spectra were recorded on a Bruker AM-300 spectrometer, with DMSO-d_6 as solvent and TMS as internal standard. Hector and Cosy spectra were recorded to assist with the ^{1}H and ^{13}C assignments. Infrared spectra were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer as KBr disks. Fast atom bombardment (FAB) mass spectra of both Boc−Phe−Pro·NaCl and Boc−Tyr−Pro·$2H_2O$ dissolved in DMSO with 3-nitrobenzyl alcohol as matrix were obtained on a VG-7070E spectrometer.

Results and discussion

X-ray analysis

Figures 1 and 2 shows the ORTEP drawing of Boc−Phe−Pro·NaCl and Boc−Tyr−Pro·$2H_2O$ com-

Table 4. Coordinates of the hydrogen atoms ($\times 10^4$) for Boc−Phe−Pro·NaCl

	x/a	*y/b*	*z/c*	U_{iso} ($\times 10^3$)
H(2A)	−645(8)	−1653(8)	4892(4)	76(4)
H(2B)	−2672(8)	−1776(8)	5378(4)	76(4)
H(2C)	−1822(8)	−3127(8)	4726(4)	76(4)
H(3A)	−2148(10)	−4743(7)	5979(5)	76(4)
H(3B)	−3283(10)	−3576(7)	6672(5)	76(4)
H(3C)	−1510(10)	−4376(7)	7212(5)	76(4)
H(4A)	849(9)	−4164(8)	5525(5)	76(4)
H(4B)	1505(9)	−3817(8)	6757(5)	76(4)
H(4C)	1639(9)	−2574(8)	5840(5)	76(4)
HN(1)	−169(4)	−720(5)	8500(3)	76(4)
H(6)	946(6)	1519(6)	7139(3)	76(4)
H(8A)	2764(7)	3061(6)	7069(3)	76(4)
H(8B)	1588(7)	4207(6)	7756(3)	76(4)
H(9A)	5356(6)	3939(7)	7647(4)	76(4)
H(9B)	4138(6)	5401(7)	7711(4)	76(4)
H(10A)	3767(6)	5170(6)	9452(4)	76(4)
H(10B)	5813(6)	4522(6)	9346(4)	76(4)
H(11)	4887(6)	2313(6)	9523(3)	76(4)
HO(4)	4810(5)	2041(6)	11257(3)	76(4)
H(13A)	−823(6)	3082(6)	8019(4)	76(4)
H(13B)	−1356(6)	1853(6)	8889(4)	76(4)
H(15)	−3750(6)	519(6)	8609(4)	76(4)
H(16)	−6057(7)	−344(7)	7496(5)	76(4)
H(17)	−6254(7)	387(9)	5778(5)	76(4)
H(18)	−4145(8)	1848(9)	5112(4)	76(4)
H(19)	−1780(7)	2746(7)	6212(4)	76(4)

Table 5. Fractional atomic coordinates ($\times 10^4$) and equivalent thermal factors ($\times 10^3$ Å^2) for Boc−Tyr−Pro·2H$_2$O

Atom	*x/a*	*y/b*	*z/c*	U_{eq}[a]
C(1)	−1028(12)	−3095	6284(6)	67(2)
C(2)	−1722(13)	−2402(12)	5395(6)	106(3)
C(3)	−2232(12)	−4134(11)	6689(6)	98(3)
C(4)	651(14)	−3709(12)	6102(8)	124(4)
O(1)	−835(6)	−2196(9)	7139(3)	51(1)
C(5)	134(8)	−1084(10)	7067(5)	40(2)
O(2)	828(6)	−725(9)	6323(3)	58(1)
N(1)	144(6)	−457(10)	7915(4)	37(1)
C(6)	592(9)	988(10)	7924(5)	41(2)
C(7)	1908(8)	1239(10)	8696(4)	36(2)
O(3)	2134(6)	490(9)	9376(3)	46(1)
N(2)	2745(7)	2423(9)	8640(3)	38(1)
C(8)	2750(9)	3315(11)	7782(4)	50(2)
C(9)	4355(9)	4160(11)	7994(5)	61(2)
C(10)	4570(9)	4161(11)	9082(5)	63(2)
C(11)	3870(8)	2806(10)	9410(4)	41(2)
C(12)	2971(9)	2922(10)	10340(5)	40(2)
O(4)	3798(8)	2568(10)	11164(4)	91(2)
O(5)	1523(6)	3341(9)	10390(3)	58(1)
C(13)	−1059(9)	1794(10)	8191(5)	57(2)
C(14)	−2478(9)	1420(10)	7504(5)	43(2)
C(15)	−3762(9)	627(10)	7850(5)	47(2)
C(16)	−5030(8)	162(10)	7231(5)	46(2)
C(17)	−5028(9)	504(12)	6280(5)	54(2)
C(18)	−3780(10)	1333(12)	5948(6)	70(2)
C(19)	−2489(10)	1763(11)	6549(5)	60(2)
O(6)	−6187(6)	52(10)	5621(3)	78(2)
O(7)	10184(7)	5320(10)	9061(4)	81(2)
O(8)	7426(7)	2459(10)	10862(5)	99(2)

[a] $U_{eq} = 1/3\ \Sigma_i\ \Sigma_j\ U_{ij}\ a^*_i a^*_j\ (\mathbf{a}_i \cdot \mathbf{a}_j)$.

plete with the numbering scheme, respectively. The X-ray data appears in Table 1 and the torsion angles of the backbone and the relevant side chains appear in Tables 3 and 6.

The amide bond torsion angles (ω_1 = 173.7) (ω_1 = 172.6) indicate a near *trans* conformation for the dipeptides. The positive values of (ψ_1 = 162.1) (ψ_1 = 164.3) indicate that the dipeptides belong to the collagen-type of proline compounds.[1,11] We previously reported that the tripeptide (Boc−Pro−Phe−Pro) adopted both α-helix type and collagen-type conformations by crystallizing into two distinctly different conformers[8].

The puckering mode of the proline residues of Boc−Phe−Pro·NaCl (χ_2^1 = −25.7) and Boc−Tyr−Pro·2H$_2$O (χ_2^1 = −23.1) can be described as approximate C_2 half-chair symmetry having an A conformation with the Cγ atom positioned exo and Cβ endo relative to the carboxamide group, i.e., ${}^{\gamma}_{\beta}$T.[1,2-14] This conformation was also observed for the pyrrolidine rings of the cyclodipeptide, cyclo(Phe−Pro)[15] (χ_2^1 = −28.2) and the ^{1}Pro residue of conformer 2 of the tripeptide Boc−Pro−Phe−Pro^8 (χ_1^1 = −28.8).

A distinctly different puckering mode (${}^{\beta}_{\gamma}$T; C_s-envelope) (B conformation)[2,16] was adopted by the ^{1}Pro [(conformer 1) (χ_1^1 = 26.4)], ^{3}Pro [(conformer 1) (χ_3^1 = 28.4)] and ^{3}Pro [(conformer 2) (χ_3^1 = 24.4)] residues of Boc−Pro−Phe−Pro^8 as well as Boc−L-Pro (χ_2^1 = 31.0)[12], L-Pro (χ_2^1 = 33.6)[17] and Boc−Tyr−Pro·2H$_2$O (χ_2^1 = 26.6)[9] (intermediate between C_s and C_2).[1,13,14] Two molecular hydrogens bonds were observed in the crystal structure for Boc−Phe−Pro·NaCl (N1--HN1---O3; 2.557 A°, angle = 86.8°; intramolecular) and (N1--NH1---O5; 1.967 A°; angle = 87.5°; intermolecular). The distance between the Na- and Cl-atoms was, 2.862(5) A°. The following distances were also observed (Na--O5, 2.873 A°; Na--O3, 2.838 A°; Cl--HN1, 2.815 A° and Cl--HO4, 2.230 A°). Six molecular hydrogens bonds of the intermolecular type were observed in the crystal structure for the dihydrate of Boc−Tyr−Pro (Table 8).

Crystal water is involved in hydrogen bonds to the carboxyl terminals (O7−H7A--O3), (O7−H7B--

Table 6. The relevant torsion angles of Boc−Tyr−Pro·2H_2O

	C2−C1−O1−C5	−57.428 (.899)
	C3−C1−O1−C5	−178.063 (.675)
	C4−C1−O1−C5	66.628 (.893)
	C1−O1−C5−O2	3.048 (1.137)
	C1−O1−C5−N1	−178.931 (.629)
	O1−C5−N1−C6	−158.849 (.677)
	O2−C5−N1−C6	19.098 (1.180)
$Ø_1$	C5−N1−C6−C7	−130.819 (.756)
	C5−N1−C6−13	111.567 (.787)
	N1−C6−C7−O3	−21.353 (1.088)
ψ_1	N1−C6−C7−N2	164.298 (.661)
	C13−C6−C7−O3	95.358 (.896)
	C13−C6−C7−N2	−78.991 (.876)
χ_1^1	N1−C6−C13−C14	−54.413 (.889)
	C7−C6−C13−C14	−172.506 (.697)
	C6−C7−N2−C8	−14.740 (1.154)
ω_1	C6−C7−N2−C11	172.577 (.675)
	O3−C7−N2−C8	170.785 (.739)
	O3−C7−N2−C11	−1.898 (1.127)
	C7−N2−C8−C9	−160.005 (.736)
χ_2^4	C11−N2−C8−C9	12.951 (.810)
	C7−N2−C11−C10	179.348 (.708)
ϕ_2	C7−N2−C11−C12	−60.488 (.909)
θ	C8−N2−C11−C10	5.968 (.842)
	C8−N2−C11−C12	126.132 (.744)
χ_2^3	N2−C8−C9−C10	−26.867 (.889)
χ_2^2	C8−C9−C10−C11	31.594 (.888)
χ_2^1	C9−C10−C11−N2	−23.083 (.838)
	C9−C10−C11−C12	−143.022 (.736)
	N2−C11−C12−O4	145.291 (.744)
	N2−C11−C12−O5	−35.670 (1.109)
	C10−C11−C12−O4	−99.183 (.895)
	C10−C11−12−O5	79.855 (.997)
χ_1^2	C6−C13−C14−C15	107.157 (.879)
χ_1^3	C6−C13−C14−C19	−68.665 (1.081)
	C13−C14−C15−C16	−174.781 (.758)
	C19−C14−C15−C16	1.213 (1.272)
	C13−C14−C19−C18	176.716 (.830)
	C15−C14−C19−C18	.919 (1.324)
	C14−C15−C16−17	−1.007 (1.249)
	C15−C16−C17−C18	−1.339 (1.278)
	C15−C16−C17−O6	177.534 (.779)
	C16−C17−C18−C19	3.520 (1.394)
	O6−C17−C18−C19	−175.424 (.831)
	C17−C18−C19−C14	−3.337 (1.385)
	Calculations complete	

Table 7. Coordinates of the hydrogen atoms (×10^4) for Boc−Tyr−Pro·2H_2O

	x/a	*y/b*	*z/c*	U_{iso} (×10^3)
H(2A)	−2761(13)	−1815(12)	5655(6)	104(6)
H(2B)	−2127(13)	−2980(12)	4776(6)	104(6)
H(2C)	−711(13)	−1746(12)	5189(6)	104(6)
H(3A)	−2598(12)	−4909(11)	6187(6)	104(6)
H(3B)	−3314(12)	−3533(11)	6862(6)	104(6)
H(3C)	−1734(12)	−4584(11)	7341(6)	104(6)
H(4A)	94(14)	−4523(12)	5699(8)	104(6)
H(4B)	1311(14)	−4101(12)	6726(8)	104(6)
H(4C)	1522(14)	−3178(12)	5650(8)	104(6)
HN(1)	−163(6)	−978(10)	8573(4)	104(6)
H(6)	1073(9)	1293(10)	7232(5)	104(6)
H(8A)	2859(9)	2755(11)	7119(4)	104(6)
H(8B)	1639(9)	3932(11)	7749(4)	104(6)
H(9A)	4194(9)	5167(11)	7729(5)	104(6)
H(9B)	5435(9)	3712(11)	7657(5)	104(6)
H(10A)	5880(9)	4252(11)	9281(5)	104(6)
H(10B)	3871(9)	4971(11)	9399(5)	104(6)
H(11)	4848(8)	2078(10)	9540(4)	104(6)
HO(4)	5057(8)	2962(10)	11223(4)	104(6)
H(13A)	−814(9)	2853(10)	8137(5)	104(6)
H(13B)	−1414(9)	1554(10)	8921(5)	104(6)
H(15)	−3783(9)	366(10)	8606(5)	104(6)
H(16)	−6013(8)	−470(10)	7508(5)	104(6)
H(18)	−3810(10)	1654(12)	5204(6)	104(6)
H(19)	−1489(10)	2368(11)	6262(5)	104(6)
HO(6)	−7171(6)	−344(10)	6069(3)	104(6)
HO(7A)	9054(53)	5696(61)	9392(43)	104(6)
HO(7B)	10680(72)	4606(56)	9569(36)	104(6)
HO(8A)	7235(69)	2359(86)	11633(11)	104(6)
HO(8B)	6191(34)	2631(84)	10564(35)	104(6)

O5) and the hydroxyl group of the proline residue (O8−H8B--O4), (O4−H4--O8). The role of the crystal water in stabilizing the dipeptide in this conformation is not clear and the X-ray structure of the anhydrous compound could assist in determining its influence.

Spectroscopic analyses

The mass spectra of Boc−Phe−Pro·NaCl and Boc−Tyr−Pro·2H_2O show a parent ion peak at *m/z* 362 and *m/z* 378, respectively, i.e., the expected Boc-dipeptide form. The characteristic phenylalanyl side chain cleavage yielding the fragmentation m/z 91 $[C_7H_7]^+$ and the tyrosyl side chain cleavage yielding the fragmention *m/z* 107 $[C_7H_7O]$ is the highest observed fragment ion in the mass spectra.[18]

The NMR data of Boc−Phe−Pro·NaCl (Fig. 3) and Boc−Tyr−Pro·2H_2O (Fig. 4) indicate the presence of cis-trans isomerism well known for proline containing peptides.[19] A predominance of the extended conformation 67% for the Phe side chain and an extended conformation 66% for the tyrosyl side chain in solution, were estimated using (Pachler's analysis).[20] This is very similar to the observed solution conformations for the cyclic dipeptides cyclo(Phe−-Fluoro−Pro) 72%[21] and cyclo(Tyr−Pro) 64%[7] as well as for the dipeptide Boc−Tyr−Pro·H_2O 63%.[9]

The carbon chemical shift values are in close agreement with the values observed for the corresponding amino acid residues of morphiceptin,[19] cyclo-

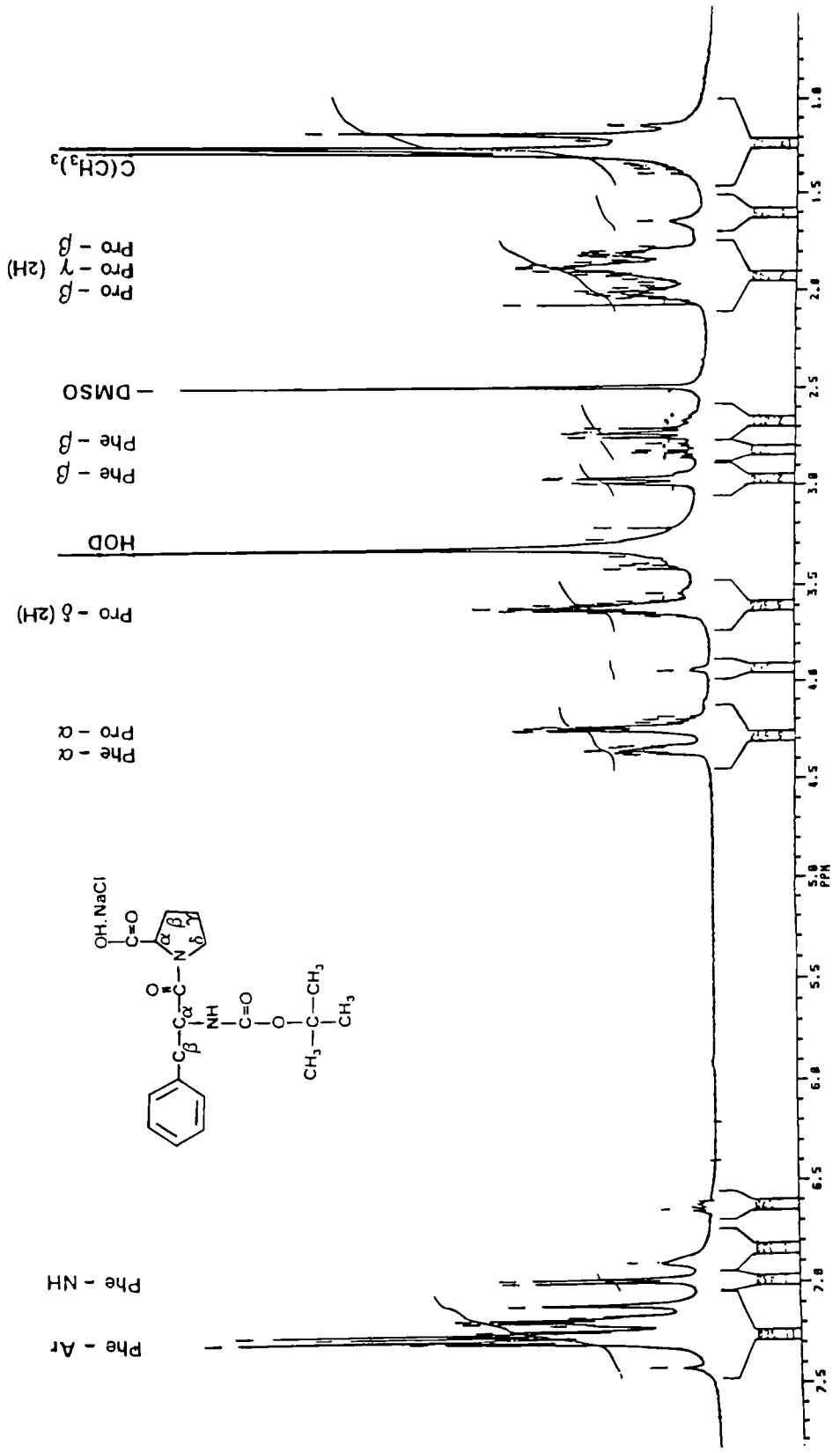


Fig. 3. ^{1}H-NMR spectrum of Boc–Phe–Pro·NaCl

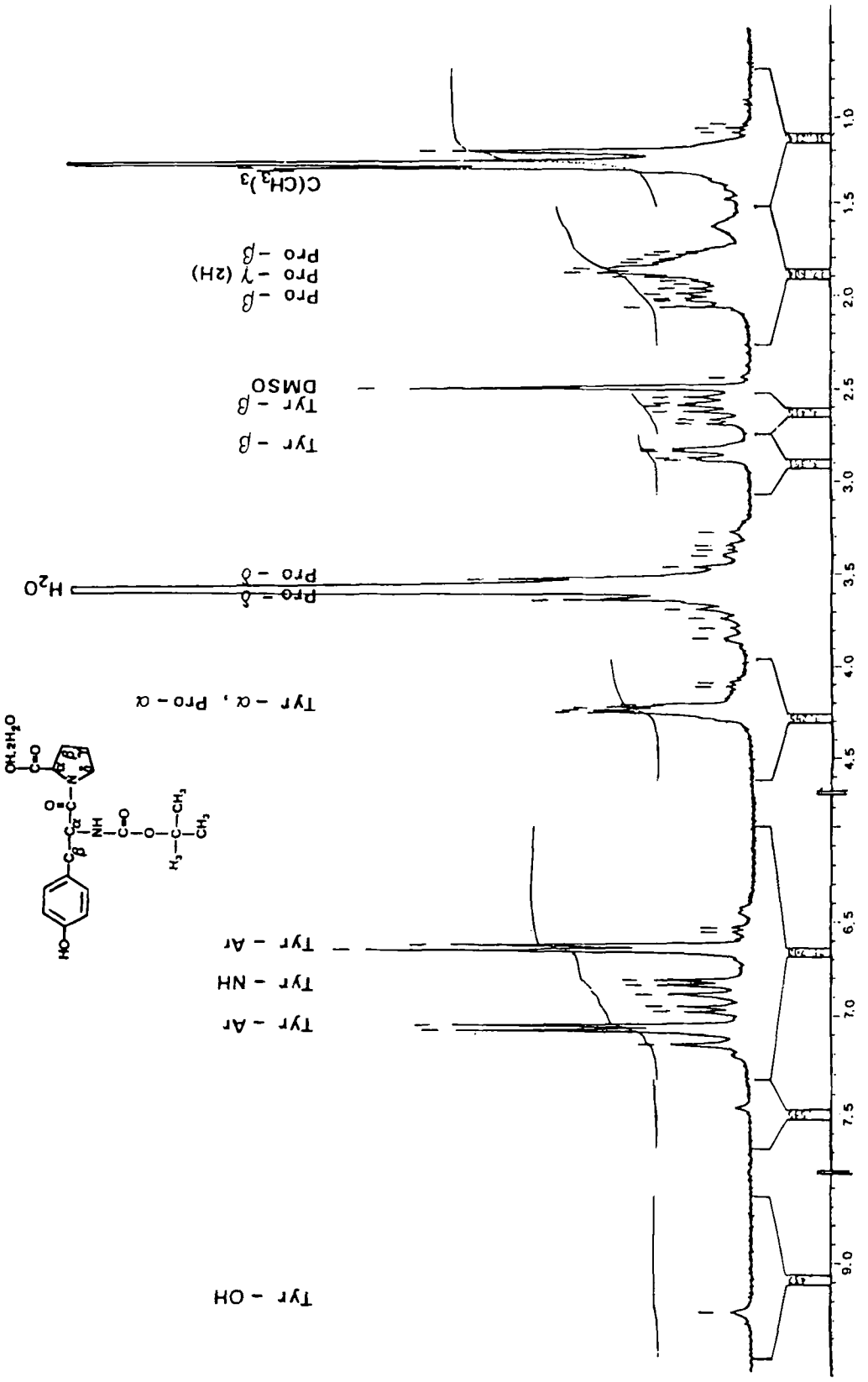


Fig. 4. ^{1}H-NMR spectrum of Boc–Tyr–Pro·2H$_2$O

Table 8. Hydrogen bonding scheme for Boc−Tyr−Pro·2H_2O

D-H---A[a]	D−A	H---A	Angle D−H---A
O6−H6A---O2	2.693 A°	1.680 A°	154.1°
N1−H1---O5	2.966 A°	1.934 A°	118.1°
O7−H7A---O3	2.866 A°	1.971 A°	137.7°
O8−H8B---O4	2.927 A°	2.089 A°	132.6°
O7−H7B---O5	1.898 A°	1.826 A°	177.6°
O4−H4---O8	2.927 A°	2.022 A°	139.4°

[a] D—donor, H—hydrogen, A—acceptor.

Table 9. ^{13}C NMR data of Boc−Phe−Pro·NaCl

Carbon Atom	PPM
Pro-γ	24.4(t)[a]
C(CH_3)$_3$	28.1(q)
Pro-β	29.1(t)
Phe-β	36.3(t)
Pro-δ	46.6(t)
Phe-α	53.7(d)
Pro-α	59.5(d)
$C(CH_3)_3$	77.9(s)
Phe−Ar	126.1(d)
Phe−Ar	127.9(d)
Phe−Ar	129.2(d)
Phe−Ar	138.1(s)
Boc−C=O	155.2(s)
Phe−C=O	170.3(s)
Pro−C=O	173.5(s)

[a] s = singlet; d = doublet; t = triplet; q = quartet.

Table 10. ^{13}C-NMR data of Boc−Tyr−Pro·2H_2O

Carbon Atom	PPM
Pro-γ	24.46 (t)[a]
C(CH_3)$_3$	28.14 (q)
Pro-β	29.05 (t)
Tyr-β	35.50 (t)
Pro-δ	46.57 (t)
Tyr-α	54.09 (d)
Pro-α	59.51 (d)
$C(CH_3)_3$	77.90 (s)
Tyr−Ar	114.84 (d)
Tyr−Ar	128.14 (s)
Tyr−Ar	130.18 (d)
Tyr−Ar	155.28 (s)
Boc−C=O	155.98 (s)
Tyr−C=O	170.57 (s)
Pro−C=O	173.57 (s)

[a] s = singlet; d = doublet; t = triplet; q = quartet.

(Tyr−Pro)[7] and Boc−Tyr−Pro·H_2O.[9] The ^{13}C data is shown in Table 9 and Table 10, respectively.

Supplementary material. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-1003/5134 and CCDC-1003/5135. Copies of available material can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail: teched@chemcrys.cam.ac.uk).

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