Conformations of Diastereoisomeric Peptides. *N*-(*tert*-Butyloxycarbonyl)-L-prolyl-D-proline and Its Methyl Ester in the Solid State and in Solution[1]

Ettore Benedetti,*[2a] Benedetto Di Blasio,[2a] Vincenzo Pavone,[2a] Carlo Pedone,[2a] Claudio Toniolo,[2b] and Gian Maria Bonora[2b]

Istituto Chimico, Università di Napoli, 80134 Napoli, Italy, and Centro di Studi sui Biopolimeri, CNR, Istituto di Chimica Organica, Università di Padova, 35100 Padova, Italy.
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ABSTRACT: A conformational analysis of *t*-Boc-L-Pro-D-Pro-OH and *t*-Boc-L-Pro-D-Pro-OMe was performed in the solid state and in solution. The crystal and molecular structures of the two dipeptides were found to be close. Both peptides exhibit a cis tertiary urethane bond and a trans tertiary amide bond. In the structure of the free acid the molecules are held together through an intermolecular O—H···O=C (urethane) hydrogen bond; i.e., the oxy analogue of the *trans*-II 4 → 1 intramolecularly hydrogen-bonded peptide conformation appears to be absent. In solvents of high polarity solvated species largely predominate. In the free acid, even in a solvent of low polarity at high dilution, the extent of intramolecularly hydrogen-bonded forms, if any, should be small.

The conformational versatility of regularly alternating high molecular weight linear D,L-peptides is well established.[3,4] These polypeptides are of interest not only because of their novel conformational possibilities, associated with their stereosequence, but also as models for the transmembrane ion-conducting channel-forming peptide antibiotic gramicidin A.[5,6] More recently, a limited number of investigations of the structure of low molecular weight linear D,L-peptides[7–11] and depsipeptides[9,12–14] have been carried out and, in some cases, the results compared to those of the L,L-diastereoisomers to obtain information on the effect of α-carbon chirality on peptide conformation. In general, these studies have dealt with N-unsubstituted α-amino acid residues, the *N*-alkyl-α-amino acids being considered only in the depsipeptide field.

We are currently performing a systematic analysis of the conformation of proline derivatives[15–17] and homooligoprolines (to the tetrapeptide), the latter having all possible chiral sequences, various N-protecting groups, and different capabilities of forming hydrogen bonds, i.e., the presence or absence of the hydroxyl group on the C-terminal carboxylic acid moiety.

Seven structures of proline derivatives and homooligoprolines have been solved so far by X-ray diffraction by our group and other groups.[15–23] They include (i) *t*-Boc-L-Pro-OH,[15] (ii) *t*-Aoc-L-Pro-OH,[16] (iii) Ac-L-Pro-NH_2,[17–19] (iv) Ac-L-Pro-NHMe,[20] (v) Z-(L-Pro)$_2$-OH,[21] (vi) *t*-Aoc-(L-Pro)$_3$-OH,[22] and (vii) *t*-Boc-(L-Pro)$_4$-OBzl.[23] Conformational regions A and F,[24] cis urethane and trans amide and peptide conformations,[25] pyrrolidine conformations A and B,[26] and intermolecularly hydrogen-bonded forms involving urethane, amide, and peptide carbonyls as acceptors have been shown to occur in the aforementioned compounds. The conformational properties of all-L oligoprolines have been calculated[27–30] and molecular models of poly(L-proline) examined,[31] taking into account not only the main-chain φ, ψ, and ω torsion angles[25] but also the pyrrolidine conformation.[26]

The purpose of the present study is to carry out a detailed conformational analysis, both in the solid state and in solution, using infrared (IR) absorption and X-ray diffraction, of the two dipeptides *t*-Boc-L-Pro-D-Pro-OH and *t*-Boc-L-Pro-D-Pro-OMe (*t*-Boc stands for *tert*-butyloxycarbonyl and OMe for methoxy). On the basis of IR absorption evidence Deber has proposed for the un-ionized acid derivative in the solid state the oxy analogue of the *trans*-II 4 → 1 intramolecularly hydrogen-bonded peptide conformation; in chloroform solution, however, no ordered conformation was detected.[32] The ester dipeptide is a solid material at room temperature, a unique property among the *N*-*tert*-butyloxycarbonyl and methoxy dipeptides formed by two *N*-alkyl-α-amino acid residues.[33–35]

Experimental Section

Synthesis of Peptides. The synthesis and characterization of *t*-Boc-L-Pro-OH[36] and Ac-L-Pro-OH[37] (where Ac is acetyl) have already been reported.

Ac-L-Pro-OMe was prepared by reacting acetyl chloride with HCl·H-L-Pro-OMe and *N*-methylmorpholine in chloroform: oil; $[\alpha]^{21}_D$ −99.1° (*c* 1, methanol); R_{f1} 0.70 (TLC, SiO_2, Merck, 9:1 chloroform–ethanol). Anal. Calcd for $C_8H_{13}NO_3$: C, 56.1; H, 7.6; N, 8.2. Found: C, 55.3; H, 7.5; N, 8.1.

t-Boc-L-Pro-L-Pro-OMe,[35] *t*-Boc-L-Pro-D-Pro-OMe, and *t*-Boc-L-Pro-L-Pro-OBzl[38–40] (where OBzl is benzyloxy) were synthesized from *t*-Boc-L-Pro-OH and the pertinent proline ester hydrochloride in chloroform in the presence of *N*-methylmorpholine and isobutyl chloroformate.[41]

t-Boc-L-Pro-L-Pro-OMe: oil; $[\alpha]^{21}_D$ −97.1° (*c* 1, methanol); R_{f1} 0.80. Anal. Calcd for $C_{16}H_{26}N_2O_5$: C, 58.9; H, 8.0; N, 8.6. Found: C, 58.2; H, 7.9; N, 8.5.

t-Boc-L-Pro-D-Pro-OMe: mp 99–100 °C, after recrystallization from ethyl acetate–petroleum ether; $[\alpha]^{21}_D$ 49.2° (*c* 1, methanol); R_{f1} 0.80. Anal. Calcd for $C_{16}H_{26}N_2O_5$: C, 58.9; H, 8.0; N, 8.6. Found: C, 58.3; H, 8.1; N, 8.7.

t-Boc-L-Pro-L-Pro-OBzl: oil; $[\alpha]^{21}_D$ −93.6° (*c* 0.98, methanol); R_{f1}, 0.80. Anal. Calcd for $C_{22}H_{30}N_2O_5$: C, 65.6; H, 7.5; N, 7.0. Found: C, 64.8; H, 7.4; N, 6.9.

t-Boc-L-Pro-L-Pro-OH[35,38,39,42–45] was prepared by catalytic (Pd/C) hydrogenation in *tert*-butyl alcohol of *t*-Boc-L-Pro-L-Pro-OBzl: mp 188–189 °C, after recrystallization from hot ethyl acetate–petroleum ether; $[\alpha]^{21}_D$ −110.15° (*c* 1, methanol); R_{f1} 0.20, R_{f2} 0.65 (TLC, SiO_2, Merck, 6:2:2 *n*-butyl alcohol–water–acetic acid). Anal. Calcd for $C_{15}H_{24}N_2O_5$: C, 57.7; H, 7.7; N, 9.0. Found: C, 57.1; H, 7.7; N, 8.9.

Z-L-Pro-L-Pro-OH[46–48] (where Z is benzyloxycarbonyl) was prepared by alkaline hydrolysis in an aqueous methanol solvent mixture of Z-L-Pro-L-Pro-OMe:[49] mp 191–192 °C, after recrystallization from hot ethyl acetate–petroleum ether; $[\alpha]^{21}_D$ −91.6° (*c* 1, methanol), $[\alpha]^{21}_D$ −80.3° (*c* 1.04, dimethylformamide); R_{f1} 0.10, R_{f2} 0.70. Anal. Calcd for $C_{18}H_{22}N_2O_5$: C, 62.4; H, 6.4; N, 8.1. Found: C, 62.0; H, 6.3; N, 8.0.

t-Boc-L-Pro-D-Pro-OH was prepared by alkaline hydrolysis in an aqueous methanol solvent mixture of *t*-Boc-L-Pro-D-Pro-OMe: mp 199–200 °C, after recrystallization from hot ethyl acetate–petroleum ether; $[\alpha]^{21}_D$ 45.7 (*c* 1, methanol); R_{f1} 0.20, R_{f2} 0.85. Anal. Calcd for $C_{15}H_{24}N_2O_5$: C, 57.7; H, 7.7; N, 9.0. Found: C, 57.2; H, 7.6; N, 8.9.

Infrared Absorption. Infrared absorption spectra were recorded with a Perkin-Elmer Model 580 spectrophotometer. For the solution measurements a 10-cm cell was employed at low

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Table I
Crystal Data for t-Boc-L-Pro-D-Pro-OH and t-Boc-L-Pro-D-Pro-OMe

	acid dipeptide	ester dipeptide
molecular formula	$C_{15}H_{24}N_2O_5$	$C_{16}H_{26}N_2O_5$
mol wt	312.37	326.40
crystal system	monoclinic	orthorhombic
space group	$P2_1$	$P2_12_12_1$
Z, molecules/unit cell	2	4
a, Å	6.486 (7)	6.664 (8)
b, Å	15.046 (9)	15.567 (10)
c, Å	8.682 (8)	17.439 (11)
β, deg	106.45 (8)	90.00
V, Å^3	812.5	1809.2
ρ_{exptl},[a] g·cm^{-3}	1.28	1.20
ρ_{calcd}, g·cm^{-3}	1.277	1.198
radiation	Cu Kα (λ 1.5418 Å)	Cu Kα (λ 1.5418 Å)
no. of independent refl	1618	918
temp, °C	23 (ambient)	23 (ambient)

[a] By flotation.

concentrations (~10^{-4} M), whereas cells with path lengths of 0.2, 0.1, and 0.05 mm were used for measurements at high concentrations (~10^{-2} M).

Trimethyl phosphate (TMP), deuteriochloroform (99.8% D), methanol (MeOH), and deuterium oxide (99.9% D) were purchased from Merck, Darmstadt. For the solid-state measurements the KBr disk technique was employed. The band positions are accurate to ±1 cm^{-1}.

X-ray Diffraction. Samples of t-Boc-L-Pro-D-Pro-OH and t-Boc-L-Pro-D-Pro-OMe, both in the form of colorless needles, were crystallized from ethyl acetate solution and used as such for the X-ray investigation. The CAD4 Enraf-Nonius diffractometer of the Centro di Metodologie Chimico Fisiche of the University of Naples, equipped with PDP-8/E and PDP-11/34 digital computers, was used for the data collection, structure determination, and refinement. The SDP package of crystallographic programs (structure determination programs) was employed. The refined unit cell parameters and the orientation matrix for data collection were determined by a least-squares fitting of the setting values of 20 and 25 centered high-angle reflections for t-Boc-L-Pro-D-Pro-OH and t-Boc-L-Pro-D-Pro-OMe, respectively, using Cu Kα radiation. Crystallographic data are reported in Table I.

Data collection for t-Boc-L-Pro-D-Pro-OH and t-Boc-L-Pro-D-Pro-OMe was carried out by using an ω–2θ scan mode with a range of $(1.00 + 0.15 \tan \theta)°$ for the peak measurements; background counts were taken at each end of each scan. A crystal–counter distance of 368 mm was used with a counter entrance aperture of 4 mm. The tube placed between the goniometer head and the detector was evacuated by a vacuum pump.

Prescans were made with a speed of 4°/min. Reflections with a net intensity $I < 0.5\sigma(I)$ were flagged as "weak"; those having $I \geq 0.5\sigma(I)$ were measured at a lower speed (in the range 1–4°/min, depending on the value of $\sigma(I)/I$). The maximum time allowed for the scan was set to 60 s. Two intensity-control reflections were recorded every 60 min of X-ray exposure time; no significant change in their intensity was observed during data collection. Orientation checks were made with respect to the scattering vectors of two strong reflections every 200 reflections; reorientation was made with 20 high-angle reflections if displacement exceeded the calculated value by 0.2°.

A total of 1653 and 1985 reflections were collected in the range 1–140° of 2θ for t-Boc-L-Pro-D-Pro-OH and t-Boc-L-Pro-D-Pro-OMe; of these reflections 1618 and 918, respectively, had a net intensity greater than $3\sigma(I)$. All reflections were corrected for Lorentz and polarization factors; no absorption corrections were applied.

The structures were solved by using the direct methods program MULTAN included in the SDP package. The 232 and 192 largest E's with $E \geq 1.40$ and $E \geq 1.62$ and a total of 2000 and 1554 $\sum_2$ relationships for t-Boc-L-Pro-D-Pro-OH and t-Boc-L-Pro-D-Pro-OMe, respectively, were used. From the eight sets of phases obtained for t-Boc-L-Pro-D-Pro-OH, the set with the highest combined figure of merit led to an E map from which the positions of all the nonhydrogen atoms were recovered. For t-Boc-L-Pro-D-Pro-OMe 32 sets of phases were produced. The E map, corresponding to the set of phases having the highest figures of merit, again led to the determination of the positional parameters of all nonhydrogen atoms.

The structures were then refined by using a least-squares procedure to final R values of 0.042 and 0.076 for t-Boc-L-Pro-D-Pro-OH and t-Boc-L-Pro-D-Pro-OMe, respectively, with anisotropic temperature factors for C, N, and O atoms and isotropic temperature factors for hydrogen atoms in their calculated stereochemically expected positions. In both cases the parameters of the hydrogen atoms were kept fixed with the isotropic temperature factor for each hydrogen equal to that of the carrier atom. Atomic scattering factors for all atoms were calculated from Cromer and Waber.[50] For both compounds all reflections with $F_o^2 > 3\sigma(F_o^2)$ were considered in the least-squares refinement and a weight $w = 1/\sigma^2(F_o)$ was employed. Refinement was ended when the maximum shift in the atomic coordinates and anisotropic thermal parameters were less than $^1/_5$ and $^1/_3$ of the corresponding standard deviations for t-Boc-L-Pro-D-Pro-OH and t-Boc-L-Pro-D-Pro-OMe, respectively. All calculations were carried out on a PDP-11/34 digital computer using the SDP system.

The final atomic parameters for both structures are presented in Table II.

Results and Discussion

Solid-State Conformational Analysis. A preliminary conformational analysis of t-Boc-L-Pro-D-Pro-OH and its methyl ester in the solid state was performed by IR absorption. A number of model compounds were also investigated to place the assignments on a sound basis. The spectral patterns of t-Boc-L-Pro-D-Pro-OH and its L,L-2-diastereoisomer are in agreement with those reported by Deber.[32]

The 1800–1700-cm^{-1} region of t-Boc-L-Pro-D-Pro-OH shows a band at 1747–1746 cm^{-1}, i.e., in the region of the carbonyl stretching absorption of free un-ionized carboxylic acid groups of N-protected N-alkyl-α-amino acids.[16,32,33,51,52] This conclusion is corroborated by the position of the corresponding absorption of t-Boc-L-Pro-OH (1740 cm^{-1}), t-Aoc-L-Pro-OH (where t-Aoc is *tert*-amyloxycarbonyl) (1754 cm^{-1}),[16] and Z-L-Pro-L-Pro-OH (1742 cm^{-1}), which are known to have a free acid C=O group in the solid state.[15,16,21] In the same spectral region t-Boc-L-Pro-D-Pro-OMe has a band at 1748 cm^{-1}, appropriate for a free methyl ester carbonyl.[16,32,33,51-53]

A strong tertiary urethane C=O stretching vibration is seen at about 1650 cm^{-1} for the acid dipeptide and at 1688 cm^{-1} for the methyl ester derivative. The position of the corresponding band in compounds containing a free urethane group is in the 1710–1680-cm^{-1} range;[16,32,33,51] in compounds containing a hydrogen-bonded urethane group, however, the band is apparent at 1670–1635 cm^{-1}.[16,32,33,51]

The tertiary amide C=O stretching absorption of t-Boc-L-Pro-D-Pro-OH should also contribute to the large band near 1650 cm^{-1}. Its assignment to the free amide group is readily given by the occurrence of a band in this spectral region for t-Boc-L-Pro-D-Pro-OMe (1644 cm^{-1}), t-Boc-L-Pro-L-Pro-OMe (1662 cm^{-1}), t-Boc-L-Pro-L-Pro-OBzl (1660 cm^{-1}), Z-L-Pro-L-Pro-OMe (1650 cm^{-1}),[49] and Ac-L-Pro-OMe (1641 cm^{-1}), all having an amide group but lacking acid hydrogens.

All assignments proposed above are in line with the IR absorption data of Z-L-Pro-L-Pro-OH (1742 and 1652 cm^{-1}), which is characterized by a hydrogen bond connecting the OH and urethane C=O groups.[21] The spectral patterns do not change on changing the solvent from which the solid materials are obtained.

Interestingly, a comparison between the IR absorption data of the two t-Boc L,D-dipeptides and their L,L-dia-

Table II
Final Atomic Parameters for *t*-Boc-L-Pro-D-Pro-OH and *t*-Boc-L-Pro-D-Pro-OMe[a]

A. *t*-Boc-L-Pro-D-Pro-OH

atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	0.0835 (8)	0.7733 (3)	−0.4111 (5)	0.0556 (17)	0.0064 (3)	0.0133 (5)	0.0061 (11)	0.0169 (15)	0.0038 (7)
C(2)	0.3414 (7)	0.6510 (4)	−0.3042 (5)	0.0353 (11)	0.0079 (3)	0.0167 (6)	0.0083 (10)	0.0152 (13)	−0.0052 (7)
C(3)	−0.0363 (7)	0.6359 (3)	−0.2898 (5)	0.0456 (13)	0.0064 (2)	0.0230 (7)	−0.0104 (10)	0.0271 (15)	−0.0086 (8)
C(4)	0.1459 (6)	0.6996 (3)	−0.2868 (4)	0.0267 (9)	0.0045 (2)	0.0102 (4)	0.0017 (7)	0.0060 (10)	−0.0010 (5)
O(1)	0.2261 (4)	0.7386 (2)	−0.1234 (3)	0.0226 (6)	0.0050 (1)	0.0108 (3)	0.0046 (5)	0.0054 (6)	−0.0020 (3)
C(5)	0.1059 (5)	0.7926 (2)	−0.0616 (4)	0.0223 (7)	0.0029 (1)	0.0122 (4)	0.0013 (6)	0.0081 (9)	0.0012 (4)
O(2)	−0.0810 (4)	0.8120 (0)	−0.1260 (3)	0.0220 (6)	0.0045 (1)	0.0159 (4)	0.0042 (5)	0.0023 (8)	−0.0006 (4)
N_1	0.2186 (4)	0.8217 (2)	0.0837 (3)	0.0231 (7)	0.0036 (1)	0.0128 (4)	0.0044 (5)	0.0039 (8)	−0.0019 (4)
C_1^{α}	0.4388 (5)	0.7929 (2)	0.1659 (4)	0.0203 (8)	0.0035 (1)	0.0144 (5)	0.0013 (7)	0.0034 (10)	−0.0009 (5)
C_1'	0.4379 (5)	0.6956 (2)	0.2174 (4)	0.0182 (7)	0.0037 (1)	0.0108 (4)	0.0011 (6)	0.0040 (9)	−0.0014 (5)
O_1	0.2989 (4)	0.6684 (2)	0.2783 (3)	0.0236 (5)	0.0049 (1)	0.0187 (4)	0.0009 (5)	0.0197 (7)	0.0005 (4)
C_1^{β}	0.5018 (7)	0.8520 (3)	0.3187 (7)	0.0371 (13)	0.0045 (2)	0.0206 (7)	0.0020 (9)	−0.0099 (17)	−0.0057 (7)
C_1^{γ}	0.3291 (7)	0.9210 (3)	0.2972 (5)	0.0405 (13)	0.0047 (2)	0.0172 (6)	0.0015 (9)	0.0028 (15)	−0.0049 (6)
C_1^{δ}	0.1291 (6)	0.8810 (2)	0.1809 (4)	0.0299 (10)	0.0032 (1)	0.0156 (5)	0.0033 (7)	0.0120 (12)	−0.0025 (5)
N_2	0.5965 (4)	0.6432 (2)	0.2023 (3)	0.0184 (6)	0.0032 (1)	0.0134 (3)	0.0003 (5)	0.0100 (7)	0.0002 (4)
C_2^{α}	0.6140 (5)	0.5525 (2)	0.2661 (4)	0.0205 (8)	0.0032 (1)	0.0136 (5)	0.0007 (6)	0.0039 (10)	0.0004 (5)
C_2'	0.4339 (5)	0.4934 (2)	0.1684 (4)	0.0213 (8)	0.0034 (1)	0.0162 (5)	0.0003 (6)	0.0075 (11)	0.0013 (5)
O_2	0.3508 (4)	0.5001 (2)	0.0283 (3)	0.0330 (7)	0.0057 (1)	0.0142 (4)	−0.0086 (6)	−0.0011 (9)	0.0013 (4)
O_T	0.3896 (4)	0.4298 (2)	0.2602 (3)	0.0337 (8)	0.0053 (1)	0.0186 (4)	−0.0106 (6)	−0.0033 (10)	0.0045 (4)
C_2^{β}	0.8326 (6)	0.5222 (3)	0.2488 (5)	0.0193 (8)	0.0041 (2)	0.0241 (7)	0.0035 (7)	0.0043 (13)	−0.0015 (6)
C_2^{γ}	0.8501 (6)	0.5719 (3)	0.1003 (5)	0.0211 (8)	0.0057 (2)	0.0217 (6)	0.0011 (7)	0.0152 (11)	−0.0055 (6)
C_2^{δ}	0.7616 (5)	0.6635 (3)	0.1182 (4)	0.0198 (7)	0.0046 (2)	0.0172 (5)	−0.0012 (7)	0.0160 (9)	−0.0008 (5)

atom	x	y	z	β, Å^2	atom	x	y	z	β, Å^2
H(1)C(1)	0.025	0.745	−0.530	5.60	H(1)C_1^{γ}	0.302	0.938	0.410	4.90
H(2)C(1)	0.222	0.815	−0.408	5.60	H(2)C_1^{γ}	0.375	0.981	0.244	4.90
H(3)C(1)	−0.041	0.815	−0.388	5.60	H(1)C_1^{δ}	0.034	0.844	0.247	4.04
H(1)C(2)	0.304	0.618	−0.421	5.97	H(2)C_1^{δ}	0.025	0.932	0.110	4.04
H(2)C(2)	0.390	0.599	−0.212	5.97	HC_2^{α}	0.616	0.553	0.394	3.39
H(3)C(2)	0.476	0.695	−0.294	5.97	H(1)C_2^{β}	0.966	0.539	0.353	4.13
H(1)C(3)	−0.095	0.607	−0.407	5.20	H(2)C_2^{β}	0.837	0.450	0.231	4.13
H(2)C(3)	−0.164	0.670	−0.257	5.20	H(1)C_2^{γ}	1.017	0.575	0.099	4.45
H(3)C(3)	0.025	0.583	−0.202	5.20	H(2)C_2^{γ}	0.757	0.539	−0.006	4.45
HC_1^{α}	0.547	0.803	0.090	3.59	H(1)C_2^{δ}	0.885	0.707	0.189	3.54
H(1)C_1^{β}	0.516	0.812	0.426	4.84	H(2)C_2^{δ}	0.690	0.693	0.002	3.54
H(2)C_1^{β}	0.661	0.883	0.334	4.84	HO_T	0.257	0.407	0.201	4.69

B. *t*-Boc-L-Pro-D-Pro-OMe

atom	*x*	*y*	*z*	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	0.2036 (22)	0.1692 (8)	0.0651 (6)	0.0377 (48)	0.0118 (9)	0.0037 (4)	−0.0039 (42)	0.0061 (28)	−0.0016 (12)
C(2)	−0.0150 (17)	0.2767 (8)	0.1265 (6)	0.0356 (34)	0.0101 (7)	0.0049 (4)	0.0055 (31)	−0.0017 (24)	0.0024 (11)
C(3)	0.3504 (19)	0.2867 (9)	0.1409 (7)	0.0392 (38)	0.0121 (9)	0.0064 (5)	−0.0068 (37)	−0.0025 (29)	0.0031 (13)
C(4)	0.1781 (15)	0.2265 (7)	0.1314 (5)	0.0269 (27)	0.0030 (6)	0.0035 (3)	0.0006 (25)	0.0008 (20)	0.0013 (9)
O(1)	0.1484 (10)	0.1771 (4)	0.2021 (3)	0.0259 (17)	0.0068 (3)	0.0041 (2)	0.0078 (14)	0.0022 (12)	−0.0001 (6)
C(5)	0.2783 (17)	0.1192 (6)	0.2268 (5)	0.0361 (30)	0.0053 (5)	0.0046 (4)	0.0078 (23)	−0.0031 (22)	−0.0015 (8)
O(2)	0.4408 (11)	0.1032 (5)	0.1942 (5)	0.0367 (21)	0.0112 (5)	0.0073 (4)	0.0202 (19)	0.0067 (17)	−0.0027 (8)
N_1	0.2203 (12)	0.0823 (5)	0.2913 (5)	0.0322 (23)	0.0051 (4)	0.0050 (3)	0.0092 (18)	−0.0032 (18)	0.0001 (7)
C_1^{α}	0.0374 (15)	0.1038 (6)	0.3323 (5)	0.0328 (29)	0.0050 (5)	0.0043 (4)	−0.0001 (24)	−0.0027 (21)	−0.0004 (8)
C_1'	0.0556 (15)	0.1908 (5)	0.3701 (5)	0.0297 (27)	0.0040 (4)	0.0035 (3)	0.0007 (19)	0.0036 (20)	0.0016 (7)
O_1	0.2093 (9)	0.2136 (4)	0.4020 (4)	0.0195 (14)	0.0053 (3)	0.0055 (3)	−0.0001 (14)	−0.0025 (13)	−0.0006 (6)
C_1^{β}	0.0164 (21)	0.0321 (6)	0.3921 (7)	0.0608 (49)	0.0043 (5)	0.0064 (5)	−0.0069 (28)	0.0006 (31)	0.0015 (9)
C_1^{γ}	0.2309 (25)	0.0073 (7)	0.4050 (7)	0.0695 (53)	0.0057 (5)	0.0074 (6)	0.0048 (32)	−0.0131 (35)	0.0026 (11)
C_1^{δ}	0.3310 (19)	0.0151 (7)	0.3318 (7)	0.0489 (44)	0.0053 (5)	0.0076 (6)	0.0123 (28)	−0.0055 (30)	0.0005 (11)
N_2	−0.1094 (11)	0.2416 (4)	0.3719 (4)	0.0213 (18)	0.0042 (3)	0.0041 (3)	0.0001 (15)	−0.0023 (14)	−0.0001 (6)
C_2^{α}	−0.1031 (14)	0.3211 (6)	0.4145 (5)	0.0208 (23)	0.0057 (4)	0.0033 (3)	0.0032 (20)	0.0016 (17)	−0.0009 (8)
C_2'	0.0366 (13)	0.3857 (6)	0.3739 (6)	0.0185 (22)	0.0050 (4)	0.0065 (5)	0.0042 (19)	0.0010 (20)	−0.0029 (9)
O_2	0.0602 (11)	0.3899 (5)	0.3075 (4)	0.0338 (21)	0.0081 (4)	0.0063 (3)	−0.0049 (18)	0.0043 (16)	0.0014 (7)
O_T	0.1110 (11)	0.4389 (4)	0.4248 (5)	0.0289 (19)	0.0065 (4)	0.0087 (4)	−0.0041 (17)	0.0012 (17)	−0.0032 (7)
C_2^{β}	−0.3255 (14)	0.3554 (7)	0.4104 (6)	0.0170 (24)	0.0068 (5)	0.0065 (5)	0.0036 (21)	0.0044 (20)	−0.0001 (10)
C_2^{γ}	−0.3930 (15)	0.3177 (6)	0.3372 (6)	0.0172 (23)	0.0060 (5)	0.0075 (5)	0.0017 (21)	0.0005 (22)	0.0026 (10)
C_2^{δ}	−0.3081 (15)	0.2283 (7)	0.3337 (6)	0.0202 (24)	0.0072 (6)	0.0062 (5)	−0.0033 (24)	−0.0051 (22)	−0.0013 (10)
C(6)	0.2329 (21)	0.5051 (8)	0.3856 (10)	0.0325 (36)	0.0071 (6)	0.0161 (10)	0.0029 (27)	0.0054 (40)	0.0070 (16)

atom	*x*	*y*	*z*	β, Å^2	atom	*x*	*y*	*z*	β, Å^2
H(1)C(1)	0.232	0.194	0.015	7.87	$H(2)C_1^{\gamma}$	0.253	−0.047	0.429	8.85
H(2)C(1)	0.087	0.124	0.058	7.87	$H(1)C_1^{\delta}$	0.481	0.034	0.337	7.34
H(3)C(1)	0.329	0.124	0.074	7.87	$H(2)C_1^{\delta}$	0.330	−0.040	0.304	7.34
H(1)C(2)	−0.021	0.320	0.080	7.24	HC_2^{α}	−0.056	0.314	0.472	3.97
H(2)C(2)	−0.031	0.319	0.174	7.24	$H(1)C_2^{\beta}$	−0.415	0.333	0.453	5.18
H(3)C(2)	−0.143	0.244	0.125	7.24	$H(2)C_2^{\beta}$	−0.337	0.420	0.410	5.18
H(1)C(3)	0.378	0.325	0.092	7.89	$H(1)C_2^{\gamma}$	−0.380	0.359	0.289	5.16
H(2)C(3)	0.482	0.261	0.155	7.89	$H(2)C_2^{\gamma}$	−0.524	0.289	0.332	5.16
H(3)C(3)	0.320	0.333	0.183	7.89	$H(1)C_2^{\delta}$	−0.301	0.203	0.279	6.66
HC_1^{α}	−0.083	0.107	0.296	4.85	$H(2)C_2^{\delta}$	−0.402	0.186	0.363	6.66
$H(1)C_1^{\beta}$	−0.060	0.053	0.443	7.81	H(1)C(6)	0.285	0.548	0.425	11.21
$H(2)C_1^{\beta}$	−0.071	−0.019	0.375	7.81	H(2)C(6)	0.136	0.542	0.351	11.21
$H(1)C_1^{\gamma}$	0.296	0.055	0.442	8.85	H(3)C(6)	0.339	0.483	0.354	11.21

[a] The form of the anisotropic thermal parameter is $\exp\{-[\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + \beta_{12}hk + \beta_{13}hl + \beta_{23}kl]\}$.

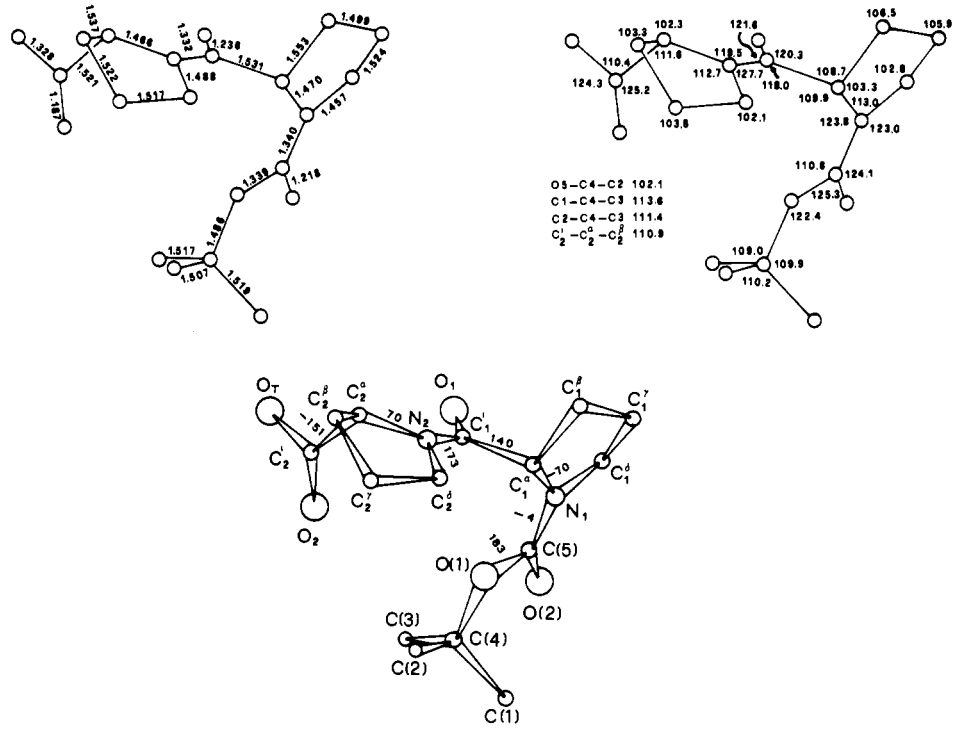

Figure 1. Molecular structure of *t*-Boc-L-Pro-D-Pro-OH showing bond lengths, bond angles, and relevant torsion angles.

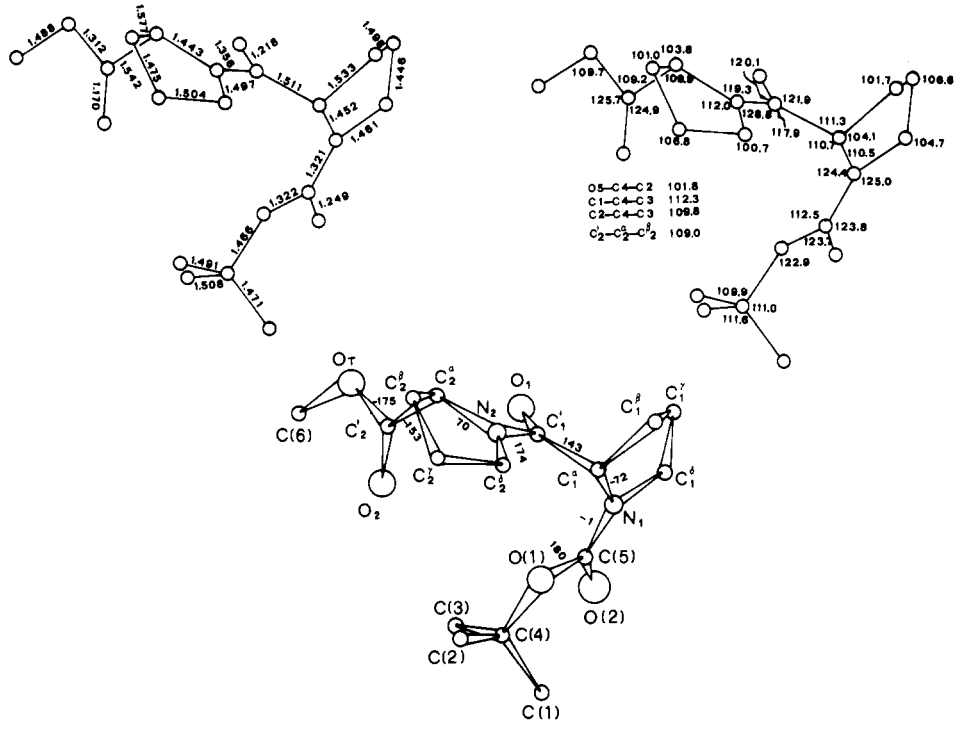

Figure 2. Molecular structure of *t*-Boc-L-Pro-D-Pro-OMe showing bond lengths, bond angles, and relevant torsion angles.

stereoisomers (and the L,L-benzyl ester analogue) has indicated that (i) the urethane C=O group of the acid L,D-dipeptide (near 1650 cm^{-1}) is hydrogen-bonded, whereas that of its L,L-diastereoisomer (at about 1685–1690 cm^{-1}) is free,[32] (ii) the amide C=O group of the acid L,D-dipeptide (near 1650 cm^{-1}) if free, whereas that of its L,L-diastereoisomer (1606–1608 cm^{-1}) is hydrogen-bonded,[32] and (iii) the urethane and amide C=O groups of the methyl ester L,D-dipeptide (1688 and 1644 cm^{-1}) experience a different environment from that of the ester L,L-dipeptides (1700–1705 cm^{-1} and 1650–1662 cm^{-1}, respectively) (this finding should probably be associated with their different physical states (see above)).

From the IR absorption data it is possible to conclude that a hydrogen bond occurs in the solid state between the OH hydrogen and the urethane carbonyl oxygen in *t*-Boc-L-Pro-D-Pro-OH. However, this technique cannot determine unambiguosly whether the hydrogen bonds are (i) intramolecular, forming the oxy analogue of the *trans*-II 4 → 1 intramolecularly hydrogen-bonded peptide structure, as proposed by Deber,[32] or (ii) intermolecular, forming a dimeric or a polymeric structure.

Table III
Internal Rotation Angles (Deg) for t-Boc-L-Pro-D-Pro-OH and t-Boc-L-Pro-D-Pro-OMe

angle	acid	ester	angle	acid	ester
O_T-C_2'-C_2^α-N_2	−151	−153	$C_2\beta$-$C_2\gamma$-$C_2\delta$-N_2	−32	−36
O_2-C_2'-C_2^α-N_2	32	32	$C_2\gamma$-$C_2\delta$-N_2-$C_2\alpha$	13	17
C_2'-C_2^α-N_2-C_1'	70	70	O(2)-C(5)-N_1-$C_1\delta$	−1	−3
C_2^α-N_2-C_1'-C_1^α	173	174	O_1-C_1'-C_1^α-$C_1\beta$	69	74
C_2^α-N_2-C_1'-O_1	−3	−2	N_2-C_1'-C_1^α-$C_1\beta$	−107	−102
N_2-C_1'-C_1^α-N_1	140	143	O_1-C_1'-N_2-$C_2\delta$	174	177
O_1-C_1'-C_1^α-N_1	−43	−41	O_2-C_2'-C_2^α-$C_2\beta$	−81	−81
C_1'-C_1^α-N_1-C(5)	−70	−72	O_T-C_2'-C_2^α-$C_2\beta$	95	94
C_1^α-N_1-C(5)-O(2)	175	176	C_2'-C_2^α-$C_2\beta$-$C_2\gamma$	88	89
C_1^α-N_1-C(5)-O(1)	−4	−1	C_2'-C_2^α-N_2-$C_2\delta$	−107	−110
N_1-C(5)-O(1)-C(4)	−177	180	C(4)-O(1)-C(5)-O(2)	4	3
C(5)-O(1)-C(4)-C(1)	59	57	O(1)-C(5)-N_1-$C_1\delta$	−179	179
C(5)-O(1)-C(4)-C(2)	176	176	C(5)-N_1-$C_1\delta$-$C_1\gamma$	−160	169
C(5)-O(1)-C(4)-C(3)	−66	−68	C(5)-N_1-C_1^α-$C_1\beta$	175	168
$C_1\delta$-N_1-C_1^α-$C_1\beta$	−9	−12	$C_1\gamma$-$C_1\beta$-C_1^α-C_1'	−127	−91
N_1-C_1^α-$C_1\beta$-$C_1\gamma$	−10	28	$C_1\delta$-N_1-C_1^α-C_1'	107	108
C_1^α-$C_1\beta$-$C_1\gamma$-$C_1\delta$	25	−36	C_1^α-C_1'-N_2-$C_2\delta$	−10	−7
$C_1\beta$-$C_1\gamma$-$C_1\delta$-N_1	−29	29	C_1'-N_2-C_2^α-$C_2\beta$	−171	−174
$C_1\gamma$-$C_1\delta$-N_1-C_1^α	24	−10	C_1'-N_2-$C_2\delta$-$C_2\gamma$	−164	−162
$C_2\delta$-N_2-C_2^α-$C_2\beta$	12	7	C(6)-O_T-C_2'-C_2^α		−175
N_2-C_2^α-$C_2\beta$-$C_2\gamma$	−32	−28	C(6)-O_T-C_2'-O_2		0
C_2^α-$C_2\beta$-$C_2\gamma$-$C_2\delta$	40	40			

To ascertain unambiguously the structure of t-Boc-L-Pro-D-Pro-OH and that of t-Boc-L-Pro-D-Pro-OMe in the solid state we have examined the two dipeptides by X-ray diffraction.

The molecular structures of t-Boc-L-Pro-D-Pro-OH and t-Boc-L-Pro-D-Pro-OMe are shown in Figures 1 and 2. Bond lengths and bond angles are also given, together with the torsion angles[25] defining the conformation of the main chain for both peptides. The complete sets of torsion angles are listed in Table III.

The geometries of the two molecules show some differences due to the fact that in the methyl ester dipeptide the atoms present larger temperature factors, indicative of their larger thermal motion. Since no correction of the geometrical parameters has been applied, the bond lengths and bond angles in the two dipeptides are slightly different. The values of the bond lengths and bond angles given for the un-ionized acid dipeptide, which, in general, shows smaller estimated standard deviations (0.004 Å and 0.4°, respectively), are in agreement with literature data on proline residues,[26,54,55] the peptide unit,[56,57] and t-Boc urethanyl derivatives.[58] As an example, the angle involving the nitrogen atoms show the same trend encountered in other peptides having cis or trans X-Pro bonds.

The overall conformation of the two dipeptides is very close. In fact, with the exception of the torsion angles of the pyrrolidine ring of the L-proline residue, the differences observed in the values of corresponding conformational angles are not larger than 4°. The conformational similarity is reflected in the nearly identical bent shape of the two molecules. Consequently, the unit cell dimensions are closely related: in fact the *a* axes (Table II) in both crystals are nearly equal, the *c* axis of the acid (8.68 Å) is nearly half of the *c* axis of the ester (17.44 Å), due to the change in the crystal system and space group, and the *b* axis of the acid (15.04 Å) is slightly shorter than the *b* axis of the ester (15.57 Å), due to the presence of the additional methylene group.

Both peptides show a cis urethane bond and a trans peptide bond. The occurrence of a tertiary urethane group in the cis conformation has recently been shown to be rather frequent and minimum-energy calculations have demonstrated a much lower difference in the energy between the cis and trans urethane bond, if compared to the difference between the cis and trans amide bonds.[58] In addition, this trend (cis OCO–Pro bond and trans Pro–Pro bond) is that always observed in crystals of the all-L *N*-urethane homooligoprolines[21-23] investigated so far.

The φ_1 and ψ_1 torsion angles of the L-proline residues (−70, 140° and −72, 143° for the acid and the ester dipeptide, respectively) correspond to one of the calculated minimum-energy regions (region F in the letter code introduced by Zimmermann et al.[24]) for Ac-L-Pro-NHMe. The sets of φ_2 and ψ_2* (for these compounds the ψ_2* notation corresponds to the N_2–C_2^α–C_2'–O_T internal rotation angle) torsion angles for the D-proline residues (+70, −151° and +70, −153° for the acid and ester dipeptide, respectively) fall in the corresponding F* region of enantiomeric amino acid residues.

The N-blocking t-Boc group presents in both structures the same conformational parameters, within the ranges recently summarized for a series of *N*-t-Boc amino acids and oligopeptides.[58] In particular, in both molecules the C(4)–O(1) bond is in the usual trans arrangement relative to the C(5)–N_1 bond; this feature, accompanied by the cis conformation of the urethane group, allows one to classify the urethane moiety of these two molecules as type a.[16,58]

The pyrrolidine ring of both proline residues in the ester derivative exhibits approximate C_2 symmetry with C^β-*exo* and C^γ-*endo*[54] (conformation B, according to the nomenclature of Balasubramanian et al.,[26] provided that reflection is made in the values of the torsion angles of the D residue). In the un-ionized acid molecule, however, the pyrrolidine ring of the L-proline residue has an approximate C_s symmetry with C^γ-*exo* (conformation A), while the pyrrolidine ring of the D-proline residue presents again an approximate C_2 symmetry with C^β-*exo* and C^γ-*endo* (conformation B). This finding confirms the great flexibility of this ring system;[30] in fact, in the two molecules, for which the same overall conformation is observed, one of the pyrrolidine rings adopts a conformation (A) different from that (B) present in the other three pyrrolidine rings, probably as a result of packing forces. In both the acid and ester crystals the intermolecular interactions involving the atoms of the annular system are all above 3.5 Å. On the contrary, several short intermolecular interactions would occur if the L-proline residue in the un-ionized acid structure had a pyrrolidine ring in an approximate C_2 symmetry with C^β-*exo* and C^γ-*endo* (conformation B).

The acid and ester groups assume a conformation with

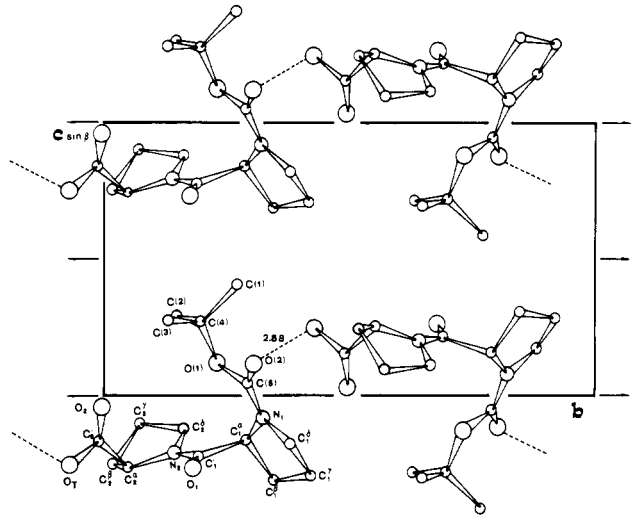

Figure 3. Mode of packing of *t*-Boc-L-Pro-D-Pro-OH as projected down the *a* axis. The hydrogen bonds are indicated as dashed lines.

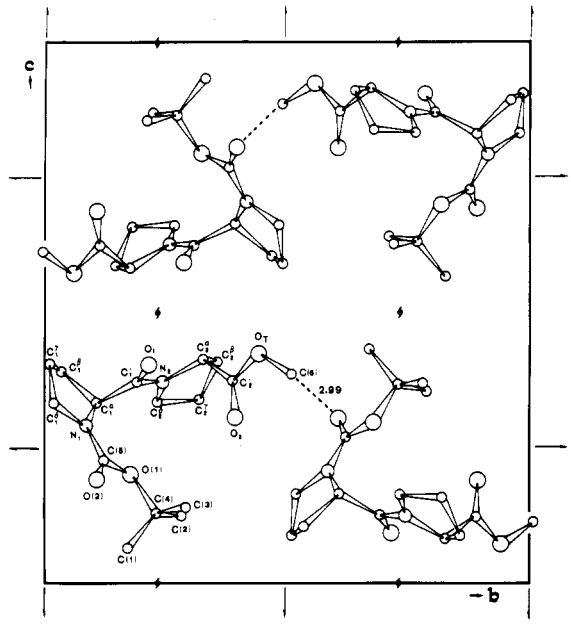

Figure 4. Mode of packing of *t*-Boc-L-Pro-D-Pro-OMe as projected down the *a* axis. The short CH_3···O distance is indicated as a dashed line (see text).

respect to the C^{α}–N_2 bond which is halfway between the syn-planar and synclinal conformations, since the O_2–C_2'–C_2^{α}–N_2 torsion angle presents a value of 32° in both crystals.[59]

In the structure of the ester dipeptide no hydrogen bond occurs because of the lack of hydrogen-bonding donors. In the structure of the un-ionized acid the hydroxyl group of the carboxylic acid is intermolecularly hydrogen-bonded to the carbonyl oxygen of the urethane group (the O···O distance is 2.68 Å, i.e., close to the most probable range for O–H···O hydrogen bonds in the crystal state with a carbonyl group as hydrogen acceptor[60]), forming long rows of molecules along the *b* axis (Figure 3). The same type of rows, even if no hydrogen bond is present, is observed in the mode of packing of the molecules in the structure of the ester dipeptide along the same axis (Figure 4). Packing in the other directions in both crystals is achieved by van der Waals interactions among atoms belonging to molecules of neighboring rows. The present structural analysis clearly shows that, at least in some instances, the role of hydrogen bonds is rather secondary in directing crystal packing.

To summarize, in *t*-Boc-L-Pro-D-Pro-OH in the solid state the oxy analogue of the *trans*-II 4 → 1 intramolecularly hydrogen-bonded peptide form, proposed by Deber[32] for this compound on the basis of an IR absorption investigation, appears to be absent. A comparison with the structure of Z-L-Pro-L-Pro-OH[21] indicates that (i) the conformational sequence of the urethane and amide bonds (ω angles) is identical (cis, trans), (ii) in both N-protected dipeptides the disposition of the urethane moiety is of type a, according to the classification proposed by Benedetti et al.,[16,58] (iii) the sets of φ, ψ angles of all proline residues are close (conformational regions F or F*),[24] (iv) the type of puckering of the two pyrrolidine rings partially diverges in the sense that in Z-L-Pro-L-Pro-OH both rings exhibit the same conformation (type A), while in *t*-Boc-L-Pro-D-Pro-OH different conformations (A, B)[26,54] are present, and (v) the intermolecular O–H···O hydrogen bond has the urethane carbonyl as the acceptor in both compounds, despite their different chiral sequences (L,L vs. L,D). Clearly, it would be of interest to also have in hand the structure of *t*-Boc-L-Pro-L-Pro-OH, which has the same chiral sequence as Z-L-Pro-L-Pro-OH and the same N-protecting group as *t*-Boc-L-Pro-D-Pro-OH; in this context, it is worth recalling that, according to the IR absorption data, in *t*-Boc-L-Pro-L-Pro-OH it is the amide carbonyl and not the urethane carbonyl that is the hydrogen-bonding acceptor. This study is currently in progress in our laboratories.

As far as *t*-Boc-L-Pro-D-Pro-OMe is concerned, its conformation and intermolecular interactions are similar (apart from the puckering of one pyrrolidine ring) to those of the corresponding un-ionized acid. Consequently, it is reasonable that also their IR absorption data (apart from the position of the intense urethane C=O stretching band, due to the behavior of this carbonyl as a hydrogen-bonding acceptor in the un-ionized acid dipeptide) and physical (melting point) properties would be close. Conversely, as stated above, all the N-protected L,L-dipeptide esters are oily products and exhibit urethane and amide IR absorptions at higher frequencies than those of *t*-Boc-L-Pro-D-Pro-OMe.

Solution Conformational Analysis. The possible formation of intramolecular or solute–solute intermolecular hydrogen bonds in *t*-Boc-L-Pro-D-Pro-OH was investigated by IR absorption in solvents of divergent polarity and hydrogen-bonding properties and at different concentrations, using the ester derivative, analogues, and model compounds to interpret the experimental findings (Tables IV and V).

In the strongly hydrogen-bonding acceptor (TMP) (Table IV) all *t*-Boc-L-Pro-D-Pro-OH molecules are solvated, showing bands at 1741 (free C=O group of a COOH moiety with a O—H···O=P hydrogen bond), 1696 (free urethane C=O group from an *N*-*t*-Boc, *N*-alkyl-α-amino acid residue), and 1659 cm^{-1} (free Pro-Pro C=O group).[16,51] The locations of the urethane and amide bands of the three ester dipeptides and the urethane bands of *t*-Boc-L-Pro-OMe[16] and *t*-Boc-L-Pro-Sar-OMe[51] are consistent with the above interpretation. The type of N-protecting group and stereosequence do not seem to have any influence on the spectra.

In D_2O, which can act as both hydrogen-bonding donor and acceptor, *t*-Boc-L-Pro-D-Pro-OH and its diastereoisomer *t*-Boc-L-Pro-L-Pro-OH behave differently, in the sense that only the latter is readily soluble. Clearly (Table IV), all its carbonyl groups are strongly solvated, as indicated

Table IV
Infrared Absorption Data (cm^{-1}) for t-Boc-L-Pro-D-Pro-OX (X = H, Me), Their Analogues, and Model Compounds in Solvents of High Polarity (Concentration 3×10^{-2} M) in the 1800–1600-cm^{-1} Region

compd	TMP			D_2O		
t-Boc-L-Pro-D-Pro-OH	1741	1696	1659	[a]		
t-Boc-L-Pro-D-Pro-OMe	1747	1697	1660	1732	1650	1622
t-Boc-L-Pro-L-Pro-OH	1739	1697	1661	1710	1651	1624
t-Boc-L-Pro-L-Pro-OMe	1746	1699	1661	1727	1656	1624 sh[b]
t-Boc-L-Pro-L-Pro-OBzl	1744	1698	1660	[a]		
Z-L-Pro-L-Pro-OH	1738	1708	1660	[a]		
t-Boc-L-Pro-OH	1742	1697		1705	1650	

[a] Not soluble. [b] sh = shoulder.

Table V
Infrared Absorption Data (cm^{-1}) for t-Boc-L-Pro-D-Pro-OX (X = H, Me), Their Analogues, and Model Compounds in Deuteriochloroform in the 1800–1630-cm^{-1} Region

compd	concn 3×10^{-1} M				concn 3×10^{-2} M				concn 5×10^{-4} M		
t-Boc-L-Pro-D-Pro-OH	1749	1723	1693	1657	1754		1695	1656	1754	1693	1662 sh[b]
t-Boc-L-Pro-D-Pro-OMe	[a]				1742		1691	1658	[a]		
t-Boc-L-Pro-L-Pro-OH	1746	1719	1684	1659	1755	1719 sh[b]	1686	1660 sh[b]	1756	1685	1660 sh[b]
t-Boc-L-Pro-L-Pro-OMe	[a]				1743		1689	1659	[a]		
t-Boc-L-Pro-L-Pro-OBzl	[a]				1741		1688	1659	[a]		
Z-L-Pro-L-Pro-OH	1746	1720 sh[b]	1696	1657	1756	1720 sh[b]	1697	1659	1758	1695	1660 sh[b]
t-Boc-L-Pro-OH	1753	1722	1691	1650 sh[b]	1759	1721	1692		1758	1690	
Ac-L-Pro-OH	1757	1723		1635	1756	1723		1638	[a]		
Ac-L-Pro-OMe	[a]				1744			1640	[a]		

[a] Not examined. [b] sh = shoulder.

by the occurrence of the three bands at 1710 (bonded acid C=O group),[16,51,61] 1651 (bonded urethane C=O group from an N-t-Boc, N-alkyl-α-amino acid residue),[16,33,51] and 1624 cm^{-1} (bonded Pro-Pro C=O group).[51] Below 1700 cm^{-1} the spectrum of t-Boc-L-Pro-L-Pro-OH in D_2O is similar to those of the two dipeptide methyl esters and above 1640 cm^{-1} to that of t-Boc-L-Pro-OH.

In a solvent of low polarity, deuteriochloroform, at high concentration (3×10^{-1} M) bands at 1749–1746, 1723–1719, 1696–1684, and 1659–1657 cm^{-1} are visible for the three acid dipeptides (Table V). They are attributed to free C=O groups of the acid moieties,[51,62,63] bonded C=O groups of the acid moieties associated as dimers and/or polymers,[51,62,63] free tertiary urethane C=O groups,[51] and free Pro-Pro C=O groups.[64] These assignments are substantiated by (i) the bands shown by the model compounds t-Boc-L-Pro-OH and Ac-L-Pro-OH under identical experimental conditions, (ii) the bands shown by the three ester dipeptides, lacking acid hydrogens (at 3×10^{-2} M concentration), and (iii) their spectra in more dilute solutions, characterized by the disappearance of the 1723–1719-cm^{-1} absorption and the constancy of the position of urethane and peptide C=O absorptions. However, since in the acid dipeptides the ratio of intensities of the bands at 1690 and 1660 cm^{-1} decreases with increasing concentration, it cannot be excluded that at high concentration in deuteriochloroform hydrogen-bonded urethane C=O groups also contribute to the observed absorption at 1660 cm^{-1}; in any case, the dominant type of association which seems to occur under these experimental conditions, involving the acid C=O groups as the hydrogen-bonding acceptors, is similar in all three acid dipeptides but different from that found in the solid state.[51] The highest tendency to form this type of association is shown by t-Boc-L-Pro-OH, the lowest by t-Boc-L-Pro-D-Pro-OH. The experimental data and conformation assignments for t-Boc-L-Pro-D-Pro-OH and its diastereoisomer in deuteriochloroform at high concentration agree with those reported by Deber.[32]

It may be concluded that in deuteriochloroform nonaggregated and aggregated species coexist in t-Boc-L-Pro-D-Pro-OH and the two other acid dipeptides, the amount of aggregation increasing with increasing concentration.[51] Even at 5×10^{-4} M concentration the percentage of intramolecularly hydrogen-bonded species[32,65,66] appears to be minor.[51]

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Triple Helix of *Schizophyllum commune* Polysaccharide in Dilute Solution. 3. Hydrodynamic Properties in Water

Toshio Yanaki,[1] Takashi Norisuye,* and Hiroshi Fujita

Department of Macromolecular Science, Osaka University, Toyonaka, Osaka, 560 Japan.
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ABSTRACT: A native sample of a *Schizophyllum commune* polysaccharide (schizophyllan) and its sonicated fragments, ranging in weight-average molecular weight (M_w) from 10^5 to 6×10^6 g mol^{-1}, were studied by ultracentrifugation and viscometry in water at 25 °C, in which schizophyllan has been found to exist as a triple helix. Measurements of intrinsic viscosity [η] and limiting sedimentation coefficient s_0 showed that the schizophyllan triple helix is almost perfectly rigid up to $M_w = 5 \times 10^5$ g mol^{-1} but acquires flexibility for higher M_w. The data in the lower molecular weight range were analyzed by Yamakawa's theory for [η] and Yamakawa-Fujii's theory for s_0 of a long rigid cylinder, with the result that M_L (the molar mass per unit cylinder length) = 2150 ± 150 nm^{-1} and d (the diameter) = 2.6 ± 0.4 nm. This M_L gives the pitch of the triple helix per β-1,3-D-glucose residue a value of 0.30 ± 0.02 nm, which agrees closely with the reported pitches for lentinan and a β-1,3-D-xylan in the crystalline region. The d value 2.6 nm is consistent with the diameter of the model triple helix for schizophyllan. With M_L = 2150 ± 150 nm^{-1} and d = 2.6 ± 0.4 nm, the persistence length that allows the theoretical curves of s_0 and [η] for wormlike cylinders to fit the present data was found to be 200 ± 30 nm. This value indicates that the schizophyllan triple helix is stiffer than that of native collagen, a known triple-helical biopolymer.

In part 1[2] of this series, we concluded that schizophyllan, [β-1→3-Glu-β-1→3-Glu-(β-6←1-Glu)-β-1→3-Glu]$_n$, an extracellular polysaccharide produced by *Schizophyllum commune*,[3,4] is dispersed in 25 °C water as a triple helix similar to that proposed by Atkins et al.[5] for a crystalline β-1,3-D-xylan and that the triple helix is rigid for weight-average molecular weights M_w lower than 5×10^5 g mol^{-1} but has a certain flexibility for higher M_w. These conclusions were drawn from the following observations: (1) the M_w of the samples in water were approximately 3 times those in dimethyl sulfoxide, in which schizophyllan is dispersed as a single randomly coiled chain; (2) the exponents in the Mark-Houwink-Sakurada [η] (intrinsic viscosity) vs. M_w relation in water were about 1.7 and 1.2 below and above $M_w = 5 \times 10^5$ g mol^{-1}, respectively; and (3) intrinsic viscosities in water for M_w lower than 5×10^5

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