On the Oxy Analogues to the 4 → 1 Intramolecularly Hydrogen-Bonded Peptide Conformations[1]

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ABSTRACT: The occurrence of the oxy analogue to the type II′ 4 → 1 intramolecularly hydrogen-bonded nonhelical peptide conformation, recently proposed for *t*-BOC-Gly-L-Pro-OH in the solid state by Deber on the basis of infrared absorption evidence, has been disproved by x-ray diffraction analysis. This type of folding is also absent in solvents of moderate or high polarity. The latter conclusion is in agreement with Deber's results. However, in solvents of low polarity this intramolecularly hydrogen-bonded form could account for the strong negative Cotton effect near 230 nm observed in the circular dichroism spectrum.

The presence of the oxy analogues to the ten-membered ring 4 → 1 intramolecularly hydrogen-bonded nonhelical peptide conformations (also referred as to β turns, β loops, β bends, β twists, U folds, hairpin bends, $N_4H_4\cdots O_1C_1$ hydrogen-bonded conformations, or C_{10} ring forms) has been recently proposed in the solid state by Deber[3a] when the sequences Gly-L-Pro, L-Pro-Gly, and L-Pro-D-Pro occur in the two residues at the C terminus of the polypeptide chain. In *N-tert*-butyloxycarbonyl (*t*-BOC) dipeptides experimental evidence for these types of folding was obtained from the observation in the infrared absorption spectra of a ~30 cm^{-1} shift to lower frequency of the urethane (carbamate) carbonyl band, due to hydrogen-bond formation.

However, as Deber correctly pointed out,[3a] "x-ray crystallography remains the method of choice for substantiation of the postulated intramolecularly hydrogen-bonded structure". In fact, purely on the basis of the infrared absorption study, it was impossible to rule out unequivocally intermolecular hydrogen-bonding effects in the crystals of these acids as the source of the shifted carbonyl frequencies.

In this paper we report the infrared absorption and x-ray diffraction analyses of *t*-BOC-Gly-L-Pro-OH in the solid state. Its infrared absorption and circular dichroism properties in solvents of different polarity are also discussed. The conclusions were facilitated by comparison with the data obtained for *t*-BOC-Gly-L-Pro-OMe.

The structure proposed by Deber[3a] for *t*-BOC-Gly-L-Pro-OH, deduced from infrared absorption spectra in the solid state (oxy analogue to the type II′ 4 → 1 intramolecularly hydrogen-bonded nonhelical peptide conformation),[3b-11] is illustrated in Figure 1.

Experimental Section

Synthesis of Peptides. *t*-BOC-Gly-L-Pro-OMe was synthesized from *t*-BOC-Gly-OH and HCl·H-L-Pro-OMe via the mixed anhydride method,[12] as described in ref 13: mp 63–65 °C, after recrystallization from ethyl acetate–petroleum ether; $[\alpha]^{22}D$ −27° (*c* 1; methanol).

t-BOC-Gly-L-Pro-OH[14-17] was prepared by alkaline hydrolysis in an aqueous/dioxane mixture of *t*-BOC-Gly-L-Pro-OMe: mp 143–144 °C, after recrystallization from ethyl acetate-petroleum ether; $[\alpha]^{22}D$ −79.6° (*c* 2.5; methanol).

Infrared Absorption. Infrared absorption spectra were recorded using a Beckman Model IR 9 spectrophotometer. For the solid state measurements the KBr disk technique was employed. For the solution measurements demontable cells with path length ranging from 10 to 0.005 cm and calcium fluoride windows were used. Deuteriochloroform (99.8% *d*) was purchased from Merck, Darmstadt, purified according to the procedure described by Shields et al.,[18] and stored under nitrogen in the dark. The band positions are accurate to ±1 cm^{-1}.

Circular Dichroism. Circular dichroic spectra were recorded using a Cary Model 61 circular dichroic spectrophotometer. The spectra were obtained using cylindrical fused quartz cells of 0.5, 1, and 10 mm path lengths. Dry prepurified nitrogen was employed to keep the instrument oxygen free during the experiments. A complete baseline was recorded for every measurement using the same cell in which the sample solution had been replaced with pure solvent. Solutions of 10^{-2} to 10^{-3} M peptide were prepared by placing the weighed peptide in a volumetric flask and adding the appropriate solvent. The circular dichroic data represent average values from at least four recordings. The calibration was based upon $[\theta]_{290}$ = 7.840 deg cm^2 $dmol^{-1}$ for a purified sample of camphorsulfonic-10-*d* acid (Fluka, Buchs) in 0.1% aqueous solution.[19] The Lorentz refractive index correction was not applied.[19] The solvents used were double distilled water and spectrograde cyclohexane and chloroform (Merck, Darmstadt).

X-Ray Diffraction. Crystals of *t*-BOC-Gly-L-Pro-OH in the form of colorless plates were grown from acetonic solutions. A summary of crystal data is given in Table I. The data collection was carried out by counter techniques on a Datex-automated General Electric XRD-5 diffractometer using Ni-filtered Cu Kα radiation (λ 1.5418 Å). The structure, solved by direct methods, was refined with anisotropic thermal factors for all heavy atoms and isotropic thermal factors for all the hydrogen atoms. The final conventional *R* value was 0.076 for the 1445 measured reflections. Details of the fully refined molecular structure of *t*-BOC-Gly-L-Pro-OH will appear in a forthcoming paper.[20]

Results and Discussion

The infrared absorption spectra in the 1800–1600-cm^{-1} region of *t*-BOC-Gly-L-Pro-OH and its methyl ester in the solid state are shown in Figure 2. The curve of the free acid matches very well that previously reported by Deber.[3a] The main difference between the two spectra in Figure 2 rests in the position of the urethane carbonyl band which is found at 1721 cm^{-1} in the ester and at 1660–1670 cm^{-1} in the free acid. This large shift to lower frequency could be attributed either to intramolecular[3a] or to intermolecular hydrogen-bond formation. In addition, the amide carbonyl and urethane N–H groups are also hydrogen bonded, although not strongly, in both compounds, as suggested by their absorptions near 1645 (Figure 2) and 3415 cm^{-1} (not shown), respectively.[3a,21-24] Conversely, the positions of the band of the carboxylic acid at 1744 cm^{-1} and of the methyl ester at 1754 cm^{-1} indicate that these two C=O groups are not involved in hydrogen-bond formation.[3a]

The x-ray diffraction analysis of *t*-BOC-Gly-L-Pro-OH allowed us to solve the ambiguity of the conformational assignment made on the basis of its infrared absorption properties. The molecular structure is shown in Figure 3; bond distances, bond angles, and internal rotation angles are listed in Tables II–IV.

Without considering the methyl groups of the *tert*-butyl moiety and the carboxylic acid group, the conformation of the molecule can be grossly described as planar. In particular, the carboxylic acid group as well as the urethane and amide groups are very close to planarity. The pyrrolidine ring of the proline residue is puckered at the β-carbon atom which deviates by 0.51 Å from the best plane passing through the other atoms of the ring. For a detailed discussion of the problem of the puckering of the pyrrolidine ring in molecules containing

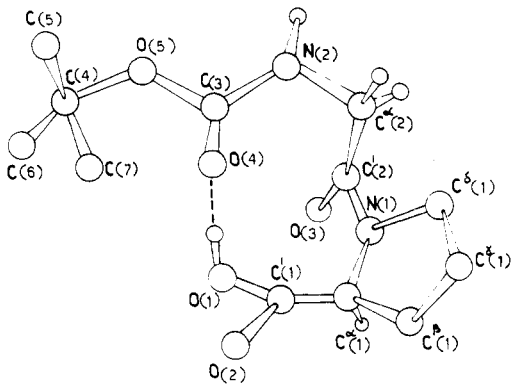

Figure 1. The oxy analogue to the type II′ 4 → 1 intramolecularly hydrogen-bonded nonhelical peptide conformation proposed by Deber[3a] for *t*-BOC-Gly-L-Pro-OH in the solid state.

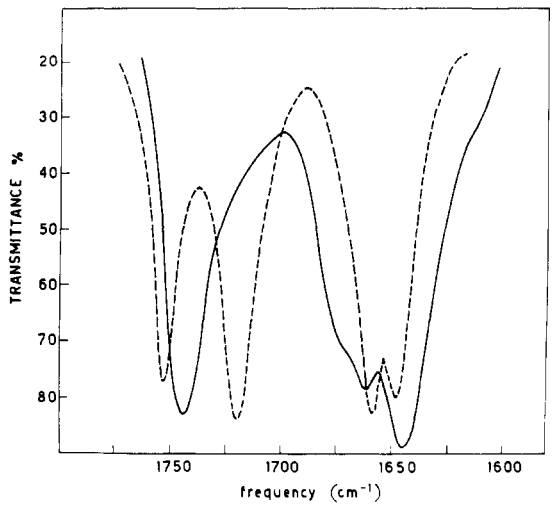

Figure 2. Infrared absorption spectra in the 1800–1600-cm^{-1} region of *t*-BOC-Gly-L-Pro-OH (solid line) and *t*-BOC-Gly-L-Pro-OMe (dashed line) in KBr pellets.

Table I
Crystallographic Data for *t*-BOC-Gly-L-Pro-OH

Molecular formula	$C_{12}H_{20}N_2O_5$
Molecular weight	272.28
Crystal system	Orthorombic
Space group	$P2_12_12_1$
Z, molecules/unit cell	4
Cell dimensions, Å	*a* = 5.743
	b = 23.849
	c = 10.440
Density experimental by flotation ($CHCl_3$-*n*-hexane)	1.26 g cm^{-3}
Density, calcd	1.264 g cm^{-3}
Radiation	Cu Kα, λ 1.5418 Å; Ni filtered
No. of independent reflections	1445
Temp, °C	23, ambient

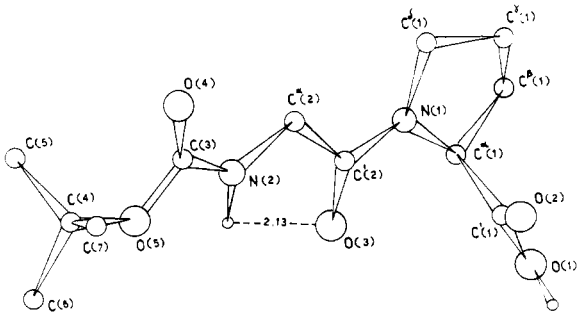

Figure 3. Molecular structure of *t*-BOC-Gly-L-Pro-OH.

proline residues, the reader is referred to ref 25. The carboxylic acid group assumes a conformation with respect to the C$^\alpha$(1)–N(1) bond which is neither synplanar nor synclinal, since the O(2)–C′(1)–C$^\alpha$(1)–N(1) internal rotation angle presents a value of −26° (almost halfway between 0 and −60°). The pyramidal character of the nitrogen atoms seems to be very small, if any, since the two ω values (rotation around the N(1)–C′(2) and N(2)–C(3) bonds) are −179° (ω_1) and −178° (ω_2) (trans configuration).

In the structure of *t*-BOC-Gly-L-Pro-OH two different types of hydrogen bond occur. The first is an intramolecular hydrogen bond between the urethane N(2)–$H_{N(2)}$ group and the amide carbonyl group C′(2)–O(3) (2.59 Å). The H···O(3) distance is 2.13 Å. This hydrogen bond then gives rise to the formation of a five-membered ring in the molecule. The highest tendency of the glycyl residue to give this intramolecularly hydrogen-bonded extended conformation has been explained by the fact that intramolecular nonbonded interactions introduced by the side-chain substituent in other amino acid residues induce a warping of these molecules.[26] The angles observed for the glycyl residue in the *t*-BOC-Gly-L-Pro-OH molecule are φ_2 = 172° and ψ_2 = 177°, very near to the optimal values (180°, 180°) for a five-membered ring 2 → 2 intramolecularly hydrogen-bonded peptide conformation.[9]

The second type of hydrogen bond is intermolecular between the hydroxyl group O(1)–$H_{O(1)}$ and the urethane carbonyl group C′(3)–O(4) of the molecule related by the symmetry elements in the crystal (Figure 4). The O(1)–O(4) distance between hydrogen-bonded atoms is 2.64 Å. This bond produces rows of hydrogen-bonded molecules extending along the *c* direction. The O(2) atom is not involved in hydrogen bonding.

Thus, the hypothesis of the folding of the *t*-BOC-Gly-L-Pro-OH molecule in the solid state, with the formation of the ten-membered ring oxy analogue to the type II′ 4 → 1 intramolecularly hydrogen-bonded nonhelical peptide conformation, put forward on the basis of the infrared absorption results,[3a] has to be rejected. In fact, the low-frequency value of the urethane carbonyl vibration is perfectly explainable also in terms of the intermolecular hydrogen bond as found by the present x-ray diffraction analysis. Furthermore the five-membered ring structure involving the glycyl residue is responsible for the location of the urethane N–H and amide carbonyl bands at slightly lower frequencies than those of the respective groups in the free form.[24,26,27] An extended conformation was also suggested in the solid state for *N*-benzyloxycarbonylglycyl-L-proline on the basis of a preliminary x-ray diffraction study.[28]

In order to investigate the possible occurrence in solution of the oxy analogues to the 4 → 1 intramolecularly hydrogen-bonded peptide conformations in the case of *t*-BOC-Gly-L-Pro-OH we re-examined[3a] its infrared absorption properties in chloroform at various concentrations and compared them with those of *t*-BOC-Gly-L-Pro-OMe in identical experimental conditions. Also, we extended our study by analyzing the circular dichroic spectra of the two compounds in water and in cyclohexane solutions (*t*-BOC-Gly-L-Pro-OH has not been examined in pure cyclohexane, since it is not soluble in this solvent at concentrations suitable for circular dichroism measurements) (Figure 5). The interpretation of the results obtained is particularly difficult, since cis–trans isomerism around both glycylurethane[29,30] and amide X-Pro[17,31–36] bonds occurs. In addition, various types of intra-

Table II
Bond Distances (Å) for *t*-BOC-Gly-L-Pro-OH

C′(1)–O(1)	1.31	C′(2)–Cᵅ(2)	1.51
C′(1)–O(2)	1.20	Cᵅ(2)–N(2)	1.43
C′(1)–Cᵅ(1)	1.52	N(2)–C(3)	1.33
Cᵅ(1)–N(1)	1.45	C(3)–O(4)	1.22
Cᵅ(1)–Cᵝ(1)	1.51	C(3)–O(5)	1.34
Cᵝ(1)–Cᵞ(1)	1.50	O(5)–C(4)	1.47
Cᵞ(1)–Cᵟ(1)	1.52	C(4)–C(5)	1.50
N(1)–Cᵟ(1)	1.46	C(4)–C(6)	1.51
N(1)–C′(2)	1.33	C(4)–C(7)	1.49
C′(2)–O(3)	1.24		

Table III
Bond Angles (deg) for *t*-BOC-Gly-L-Pro-OH

O(1)–C′(1)–O(2)	124	O(3)–C′(2)–Cᵅ(2)	121
O(1)–C′(1)–Cᵅ(1)	111	C′(2)–Cᵅ(2)–N(2)	108
O(2)–C′(1)–Cᵅ(1)	124	Cᵅ(2)–N(2)–C(3)	124
C′(1)–Cᵅ(1)–N(1)	110	N(2)–C(3)–O(4)	125
C′(1)–Cᵅ(1)–Cᵝ(1)	111	N(2)–C(3)–O(5)	110
N(1)–Cᵅ(1)–Cᵝ(1)	103	O(4)–C(3)–O(5)	125
Cᵅ(1)–Cᵝ(1)–Cᵞ(1)	104	C(3)–O(5)–C(4)	122
Cᵝ(1)–Cᵞ(1)–Cᵟ(1)	105	O(5)–C(4)–C(5)	110
Cᵞ(1)–Cᵟ(1)–N(1)	103	O(5)–C(4)–C(6)	101
Cᵟ(1)–N(1)–Cᵅ(1)	113	O(5)–C(4)–C(7)	110
Cᵟ(1)–N(1)–C′(2)	126	C(6)–C(4)–C(5)	111
Cᵅ(1)–N(1)–C′(2)	121	C(6)–C(4)–C(7)	111
N(1)–C′(2)–O(3)	122	C(5)–C(4)–C(7)	113
N(1)–C′(2)–Cᵅ(2)	118		

molecularly and intermolecularly hydrogen-bonded forms could account for the observed spectral properties.[26,29,30] In particular, by infrared absorption and ^1H and ^{13}C nuclear magnetic resonance spectroscopies it has been shown that: (i) the equilibrium between the cis and trans forms of the glycylurethane group is shifted toward the cis form in more polar solvents (in this context it is worth noting that all oxy analogues to the 4 → 1 intramolecularly hydrogen-bonded peptide conformations for *t*-BOC-Gly-L-Pro-OH should have the glycyl urethane group in the trans configuration); and (ii) in various solvent systems, despite widely divergent solvent polarities, the Gly-Pro bond of the *t*-BOC-Gly-L-Pro-OH molecule has been found to be a mixture of conformers, with the trans bond always predominating to the extent of 70–80%.

On the basis of the position of the urethane carbonyl vibration of *t*-BOC-Gly-L-Pro-OH in the infrared absorption spectrum in chloroform (at 1708 cm^{-1}, as found by Deber;[2] not shown) and of the single negative Cotton effect near 205 nm in the circular dichroic spectrum in water (curve A in Figure 5), and in view of the strong similarities of the aforementioned spectra to the corresponding spectra of *t*-BOC-Gly-L-Pro-OMe (the circular dichroic spectrum of *t*-BOC-Gly-L-Pro-OMe in water is reported in curve C of Figure 5), we are sufficiently confident to conclude that in the case of *t*-BOC-Gly-L-Pro-OH the intramolecular hydrogen bond involving the OH group and urethane carbonyl is absent in these solvents. This interpretation of our experimental findings is in accord with Deber's conclusions.[3a]

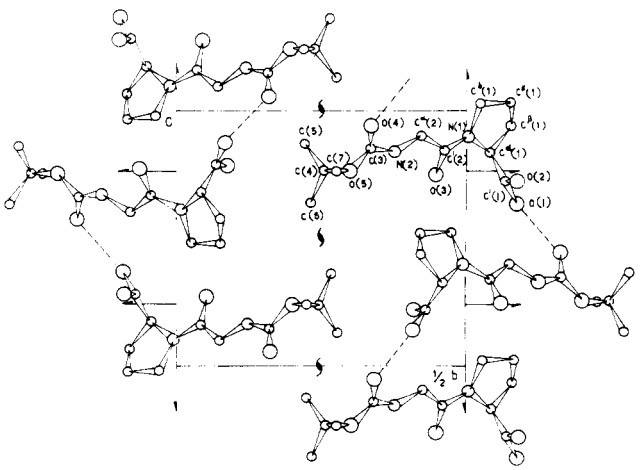

Figure 4. Mode of packing of the *t*-BOC-Gly-L-Pro-OH molecules projected down the *a* axis. The hydrogen bonds are indicated as dashed lines.

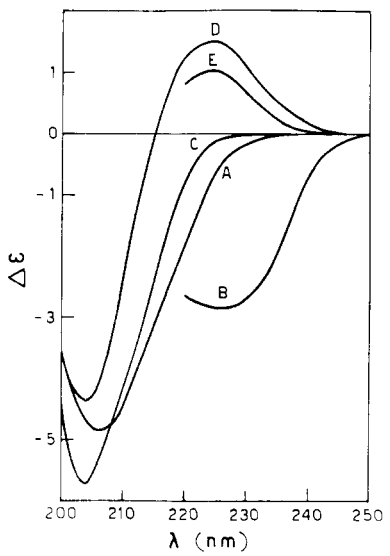

Figure 5. Circular dichroism spectra of *t*-BOC-Gly-L-Pro-OH in water (A) and in cyclohexane/chloroform 80:20, v/v (B), and of *t*-BOC-Gly-L-Pro-OMe in water (C), in cyclohexane (D), and in cyclohexane/chloroform 80:20, v/v (E). The concentrations were in the range 0.7–1.1 mg/ml.

It is significant that the circular dichroic spectra of *t*-BOC-Gly-L-Pro-OH and its methyl ester in a solvent mixture of lower polarity (cyclohexane/chloroform 80:20, v/v) are markedly different (compare curves B and E in Figure 5). Since results of theoretical calculations showed that the large

Table IV
Internal Rotation Angles (deg) for *t*-BOC-Gly-L-Pro-OH

O(1)–C′(1)–Cᵅ(1)–N(1)	156	Cᵅ(1)–N(1)–Cᵟ(1)–Cᵞ(1)	−10	O(3)–C′(2)–Cᵅ(2)–N(2)	−4
O(1)–C′(1)–Cᵅ(1)–Cᵝ(1)	−90	Cᵝ(1)–Cᵞ(1)–Cᵟ(1)–N(1)	28	C′(2)–Cᵅ(2)–N(2)–C(3)	172
O(2)–C′(1)–Cᵅ(1)–N(1)	−26	Cᵝ(1)–Cᵅ(1)–N(1)–Cᵟ(1)	−12	Cᵅ(2)–N(2)–C(3)–O(4)	3
O(2)–C′(1)–Cᵅ(1)–Cᵝ(1)	88	Cᵝ(1)–Cᵅ(1)–N(1)–C′(2)	171	Cᵅ(2)–N(2)–C(3)–O(5)	−178
C′(1)–Cᵅ(1)–Cᵝ(1)–Cᵞ(1)	−89	Cᵞ(1)–Cᵟ(1)–N(1)–C′(2)	167	N(2)–C(3)–O(5)–C(4)	−177
C′(1)–Cᵅ(1)–N(1)–Cᵟ(1)	107	Cᵞ(1)–Cᵝ(1)–Cᵅ(1)–N(1)	29	O(4)–C(3)–O(5)–C(4)	2
C′(1)–Cᵅ(1)–N(1)–C′(2)	−70	Cᵟ(1)–N(1)–C′(2)–O(3)	−179	C(3)–O(5)–C(4)–C(5)	62
Cᵅ(1)–Cᵝ(1)–Cᵞ(1)–Cᵟ(1)	−36	Cᵟ(1)–N(1)–C′(2)–Cᵅ(2)	0	C(3)–O(5)–C(4)–C(6)	179
Cᵅ(1)–N(1)–C′(2)–O(3)	−2	N(1)–C′(2)–Cᵅ(2)–N(2)	177	C(3)–O(5)–C(4)–C(7)	−63
Cᵅ(1)–N(1)–C′(2)–Cᵅ(2)	177				

negative Cotton effect near 230 nm present in *N*-acetyl-L-proline in cyclohexane arises primarily from an intramolecular interaction of the polar hydrogen of the COOH group with the carbonyl amide group,[37,38] we cannot exclude the occurrence of OH···OC (urethane) intramolecularly hydrogen-bonded forms in the conformational equilibrium mixture of *t*-BOC-Gly-L-Pro-OH in solvents of low polarity.

The main conclusion of the present work concerns the demonstration by x-ray diffraction analysis of the absence of the type II′ 4 → 1 intramolecularly hydrogen-bonded nonhelical peptide conformation for *t*-BOC-Gly-L-Pro-OH in the solid state, in contrast to the suggestion put forward on the basis of its infrared absorption properties.[3a] Still there remains to explain the pattern of infrared band shifting for some other peptides, particularly those with the Pro-Gly (rather than the Gly-Pro) sequence.[3a] It is possible that each molecule presents an individual case. There may even be some dependence on the crystallization solvent, with less polar solvents promoting crystallization into more folded structures. Perhaps the pattern of infrared shifted bands[3a] correlates with crystal packing phenomena and may be rather independent of the stability of potential oxy analogues to the 4 → 1 intramolecularly hydrogen-bonded peptide conformations. Crystallographic data on some other peptides of these types are required, after which one may draw some more permanent conclusions on this problem. These studies are now in progress in our laboratories.

References and Notes

(1) This work is part 31 of the series; for part 30 see J. S. Balcerski, E. S. Pysh, G. M. Bonora, and C. Toniolo, *J. Am. Chem. Soc.*, in press.
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On the Oxy Analogues to the 3 → 1 Intramolecularly Hydrogen-Bonded Peptide Conformations[1]

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ABSTRACT: The possible occurrence of the oxy analogues to the 3 → 1 intramolecularly hydrogen-bonded peptide conformations (main feature of the γ turn), recently proposed in solution by several authors, has been investigated in a number of *N*-*tert*-butyloxycarbonyl-α-amino acids by x-ray diffraction, infrared absorption, proton magnetic resonance, and circular dichroism techniques. These folded conformations are absent in the solid state in all cases so far examined; however, they seem to be present in solution, the extent of the population of these forms in the conformational equilibrium mixtures being solvent, temperature, and structure dependent. In the solid state *N*-*tert*-butyloxycarbonyl-D-valine has the urethane –CONH– group in the cis configuration; this is the first time such a configuration has been found in the solid state for a secondary amide group in a linear compound.

The seven-membered ring 3 → 1 intramolecularly hydrogen-bonded peptide conformations have been recognized as an important feature of polypeptide secondary structure.[3] They characterize the γ turn, postulated by Némethy and Printz in 1972 on the basis of conformational energy calculations,[4] and recently proposed by several authors as occurring in the solid state and in solution.[5-10]

In this paper we describe a conformational analysis in the solid state and in solution, using infrared absorption, x-ray diffraction, proton magnetic resonance, and circular dichroism techniques, of a series of *N*-*tert*-butyloxycarbonyl(*t*-BOC)-α-amino acids (and α-amino methyl esters) to shed light on the possible existence of the oxy analogues to the 3 → 1 intramolecularly hydrogen-bonded peptide conformations. In these