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Peptoid Residues and β-Turn Formation

MARIO RAINALDI,[a] VITTORIO MORETTO,[a] MARCO CRISMA,[a] EVARISTO PEGGION,[a] STEFANO MAMMI,[a] CLAUDIO TONIOLO[a]* and GIORGIO CAVICCHIONI[b]

[a] Biopolymer Research Centre, CNR, Department of Organic Chemistry, University of Padova, 35131 Padova, Italy
[b] Department of Pharmaceutical Sciences, University of Ferrara, 44100 Ferrara, Italy

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Abstract: A set of terminally protected tripeptoids containing a residue of either *N*-methylglycine or *N-iso*butylglycine in position $i+1/i+2$ were synthesized and tested for intramolecularly H-bonded β-turn formation. By exploiting FT-IR absorption and ^{1}H NMR techniques, their folding tendencies were compared with those of a variety of reference peptides. The amount of β-turn induction and the relative extent of the various types of intramolecularly H-bonded β-turn conformers were determined in chloroform solution.

Keywords: conformational analysis; hydrogen bond; infrared absorption; nuclear magnetic resonance; peptoids; β-turn

INTRODUCTION

In recent years, peptide backbone modifications have attracted a great deal of attention, particularly in the fields of drug discovery and biocompatible materials [1–13]. In this connection, the preferred conformation of peptoid [14] (*N*-alkylated glycyl or 'shifty' [15]) residues, in which the side chain on the C^{α}-atom is moved onto the adjacent nitrogen, have been extensively investigated [14,16–37]. Most of the published works in this area have dealt with sequences very rich in peptoid and Pro residues, and/or significantly long. The main result extracted from these studies is the identification of a variety of long-range conformations characterized by either *cis* or *trans* ω torsion angles (tertiary amide bonds), but all requiring rather extended sets of φ, ψ torsion angles for the peptoid residues [16–29], therefore exploring the E and F regions of the Ramachandran space [38]. In particular, an *i*BuGly (*N-iso*butylglycine, also termed Nleu) residue was shown by Goodman and coworkers [22–29] to be an excellent Pro surrogate in collagen-like triple helical structures.

Conversely, only a few investigations have focused on the effect of one or two peptoid residues on the stability of the short-range β-turn conformations (Figure 1) [39–41]. The results of recent conformational energy calculations [30–33] indicate that a peptoid residue markedly restricts the conformational space available to the main chain. In *N*-acetylated dipeptide methylamides the major type of β-turns can still be formed in the presence of one or two peptoid residues as long as the intramolecular 1←4 C=O$\cdots$ H–N H-bond exists. More specifically, type-II and type-VI β-turns, the latter with a central *cis* tertiary peptide bond, are distinctly preferred over the other β-turn types. The remarkable stability of the type-VI β-turn emphasizes the importance of the *cis* ω torsion angle in sequences containing peptoid residues. Not surprisingly, the type-I β-turn, typical of an L-L homochiral dipeptide sequence, is considerably destabilized in sequences containing the achiral *N*-alkylated glycyl residues.

* Correspondence to: Professor Claudio Toniolo, Biopolymer Research Centre, CNR, Department of Organic Chemistry, University of Padova, via Marzolo 1, 35131 Padova, Italy;
e-mail: claudio.toniolo@unipd.it

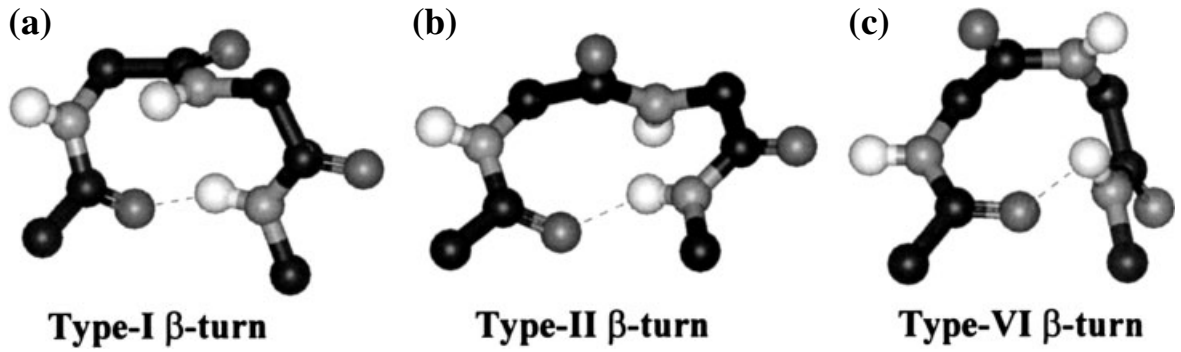

Figure 1 Backbone conformation and intramolecular H-bonding in type-I (**a**), type-II (**b**), and type-VI (**c**) β-turns.

Experimentally, it was found that in $CDCl_3$ solution the terminally protected -Xxx-MeGly- (MeGly is *N*-methyl glycine, alias sarcosine) sequence is usually highly folded in intramolecularly H-bonded β-turn conformations, the total amount of which is strongly dependent on Xxx [34–36]. Indeed, the -Pro-MeGly- sequence exhibits about 95% β-turn, this percentage being reduced to ca. 75% and 35% in the -Ala-MeGly- and the -Gly-MeGly- sequences, respectively.

In this work a systematic conformational investigation was performed in solution by the combined application of FT-IR absorption and ^{1}H NMR techniques to assess the β-turn propensity of Boc (*tert* -butyloxycarbonyl) N^{α}-protected and OMe (methoxy) *C*-protected tripeptoid sequences containing MeGly or *i*BuGly residues inserted at the $i+1$ and/or $i+2$ positions (the two peptoid residues correspond to the Ala and Leu protein amino acids, respectively). A comparison was also made with the results obtained with tripeptides containing Pro or other protein amino acids (Gly, Ala, Leu) or with tripeptoids characterized by the Piv (pivaloyl or *tert* -butylcarbonyl) N^{α}-blocking group, a *C*-terminal primary amide function, or the shifty analogues [15] MeAla and *i*BuAla. The N^{α}-acylated -Pro-Leu-Gly-NH_2 tripeptide amide, the prototypical sequence examined here, corresponds to the *C*-terminal tail of the hormone oxytocin that is enzymatically degraded to H-Pro-Leu-Gly-NH_2, the melanocyte inhibiting factor (MIF) possessing activity in a variety of neuropharmacological assay systems [42,43].

MATERIALS AND METHODS

Characterization of Peptides

The melting points were determined using a Leitz (Wetzlar, Germany) model Laborlux 12 apparatus and are not corrected. Optical rotations were measured using a Perkin-Elmer (Norwalk, CT) model 241 polarimeter equipped with a Haake (Karlsruhe, Germany) model D thermostat. Thin-layer chromatography was performed on Merck (Darmstadt, Germany) Kieselgel $60F_{254}$ pre-coated plates using the following solvent systems: 1 (toluene–EtOH 7 : 1), 2 (EtOAc–petroleum ether–EtOH 20 : 10 : 2), 3 (CH_2Cl_2–toluene–MeOH 17 : 1 : 2). The chromatograms were examined by UV fluorescence or developed by chlorine–starch–potassium iodide or the ninhydrin chromatic reaction as appropriate. All the compounds (Table 1) were obtained in a chromatographically homogeneous state. The mass spectra were recorded using a Mariner ESI-TOF (Perseptive Biosystem, Foster City, CA) mass spectrometer.

IR Absorption

The solid-state IR absorption spectra (determined in a film deposited on a KBr disk) were obtained with a Perkin-Elmer model 580 B spectrophotometer equipped with a Perkin-Elmer model 3600 IR data station. The solution spectra were obtained using a Perkin-Elmer model 1720 X FT-IR spectrophotometer, nitrogen flushed, equipped with a sample-shuttle device, at 2 cm^{-1} nominal resolution, averaging 100 scans. Cells with pathlengths of 0.1, 1.0 and 10 mm (with CaF windows) were used. Spectrograde deuterochloroform (99.8%, d) was purchased from Merck. Solvent (baseline) spectra were recorded under the same conditions.

^{1}H Nuclear Magnetic Resonance

NMR experiments were performed on Bruker (Karlsruhe, Germany) AM 400 and Avance DRX 400 spectrometers. The measurements were carried out at 298 K on 10 mM sample solutions in

Table 1 Physical Properties and Analytical Data for the Newly Synthesized Peptides

Compound	Peptide	Mp (°C)	$[\alpha]_D^{20}$[a]	TLC			Mass spectrometry (DA)			IR (cm^{-1})
				R_f1	R_f2	R_f3	Calcd.	$[M+H]^+$	$[M+Na]^+$	
1	Boc-Pro-Pro-Gly-OMe	Oil	−129.6	0.35	0.25	0.60	383.20	384.23	406.21	3309, 1754, 1694, 1675
2	Boc-Pro-D-Pro-Gly-OMe	Oil	41.8	0.30	0.20	0.65	383.20	384.23	406.21	3330, 1756, 1693, 1664
3	Boc-Pro-MeGly-Gly-OMe	Oil	−35.4	0.30	0.15	0.60	357.19	358.22	380.19	3314, 1756, 1695, 1667
4	Piv-Pro-MeGly-Gly-OMe	126–127	0.4 11.9[b]	0.25	0.15	0.60	341.19	342.22	364.20	3304, 1755, 1663
5	Boc-MeGly-MeGly-Gly-OMe	134–135	—	0.25	0.15	0.55	331.19	332.20	354.18	3313, 1754, 1694, 1680
6	Boc-Pro-*i*BuGly-Gly-OMe	Oil	−21.9	0.40	0.50	0.75	399.98	400.26	422.24	3314, 1757, 1695, 1665
7	Boc-Pro-*i*BuGly-Gly-NH_2	83–85	−23.4	0.15	0.80	0.50	384.23	385.25	407.24	3317, 3206, 1671
8	Boc-Pro-MeAla-Gly-OMe	Oil	−34.3	0.35	0.40	0.60	371.20	372.24	394.21	3326, 1756, 1690, 1680
9	Boc-Pro-D-MeAla-Gly-OMe	Oil	17.4	0.35	0.40	0.70	371.20	372.24	394.22	3330, 1757, 1690, 1671
10	Boc-Pro-*i*BuAla-Gly-OMe (**I**)	Oil	−39.5	0.40	0.65	0.75	413.25	414.28	436.29	3324, 1756, 1690, 1670
11	Boc-Pro-*i*BuAla-Gly-OMe (**II**)	Oil	−4.3	0.35	0.65	0.75	413.25	414.29	436.31	3330, 1757, 1694, 1670
12	Boc-Pro-Leu-Gly-OMe	120–121	−92.3	0.35	0.60	0.65	399.98	400.25	422.24	3294, 1758, 1700, 1656
13	Boc-Pro-Gly-Gly-OMe	Oil	−35.2	0.25	0.20	0.50	343.17	344.20	366.17	3316, 1754, 1694, 1673
14	Boc-Ala-MeGly-Gly-OMe	Oil	−21.9	0.25	0.30	0.50	331.17	332.19	354.18	3319, 1752, 1693, 1648

[a] c = 0.5 (MeOH).
[b] $[\alpha]_{436}^{20}$.

deuterochloroform (99.96%, d; Aldrich, Milwaukee, WI, USA). Tetramethylsilane was used as an internal standard. The complete assignment of the spin systems was achieved utilizing DQF-COSY [44,45] and/or CLEAN-TOCSY [46,47] spectra, while ROESY [48,49] and T-ROESY [50,51] experiments were used for the sequential assignment. All spectra were acquired by collecting 300–512 experiments, each one consisting of 56–256 scans in 2048 data points in t_2. The typical spin-locking time was 400 ms. Two-dimensional data were zero-filled in the second dimension to yield frequency-domain matrices of 2048 × 1024 real data points. Typically, gaussian (F2) and shifted squared sine-bell (F1) window functions were used prior to FT. For conformational analysis, T-ROESY peaks were classified as weak, medium and strong based on their relative integrated volume. For the complete assignment of Boc-MeGly-MeGly-Gly-OMe (**5**), HMQC [52] and CO-selective HMBC [53–55] experiments acquired on a Bruker Avance DMX 600 spectrometer were employed. An HMQC-TOCSY experiment on Boc-Pro-Leu-Gly-OMe (**12**) was acquired on the same instrument.

RESULTS AND DISCUSSION

Synthesis and Characterization of Peptides

The model tripeptoids were synthesized in solution using either a 2 + 1 or a 1 + 2 condensation approach (the latter for those containing the *i*BuGly and *i*BuAla peptoid residues). Peptide bond formation was achieved by use of the EDC/HOBt [56] or the HOAt *C*-activating method [57]. The H-*i*BuGly-Gly-OMe and racemic H-*i*BuAla-Gly-OMe dipeptoids were prepared via the submonomeric strategy [16] by treatment of BrAc-Gly-OMe (BrAc, monobromoacetyl) or racemic BrPr-Gly-OMe (BrPr, 2-bromopropionyl) with *iso*butylamine in toluene in the presence of Ag_2O [58]. Separation of the two diastereomeric tripeptoids (**I** and **II**) of Boc-Pro-D,L-*i*BuAla-Gly-OMe was achieved by reverse-phase preparative HPLC. The tripeptoid amide Boc-Pro-*i*BuGly-Gly-NH_2 was prepared by treatment of the corresponding ester with ammonia in DMF solution.

All tripeptoids and tripeptides were chemically pure and characterized by melting point determination (if solid materials), optical rotatory power, thin-layer chromatography (TLC) in three different solvent systems, mass spectrometry, solid-state IR absorption (Table 1) and ^{1}H NMR (data not reported).

Infrared Absorption Analysis

We based our FT-IR absorption conformational analysis of fully protected tripeptoids and related compounds in $CDCl_3$, a solvent with low propensity to participate in H-bonding (Figures 2–4, and Table 2). The very informative N–H stretching (amide A) mode absorbs in two regions, 3460–3405 cm^{-1} and 3350–3300 cm^{-1}, where bands associated with free (solvated) and H-bonded NH groups, respectively, are found [59–62]. The spectral patterns do not change with dilution from 10^{-2}M to 10^{-4}M, a clear indication that the nature of the observed H-bonds is intramolecular. Since most of our tripeptoids and tripeptides exhibit very strong absorptions at 3350–3310 cm^{-1} while the dipeptides Boc-Pro-Gly-OMe and Boc-MeGly-Gly-OMe show only a weak band in that region (spectra not reported), it is safe to conclude that the intramolecularly H-bonded species observed in compounds **1**–**14** are essentially of the β-turn [38–41] and not of the γ-turn [40,63] type. Indeed, although both types of turn structures absorb in the 3370–3310 cm^{-1} region [60,61], an N^{α}-protected dipeptide ester can form an intramolecularly H-bonded γ-turn, but it is too short to fold into a β-turn.

Figure 2 shows that the amide A region of the IR absorption spectra of the sequences -Pro-MeGly- (**3**) and -Pro-*i*BuGly- (**6**), with the peptoid residue in position $i + 2$, is dominated by a very intense band assigned to the H-bonded NH groups while only an extremely weak band is seen in the 3450–3400 cm^{-1} region. In these two tripeptoids the extent of H-bonded forms is only slightly lower than that originated by the heterochiral -Pro-D-Pro- (**2**) sequence, but significantly higher than that exhibited by the homochiral -Pro-Pro- (**1**) sequence. There is no appreciable difference between the amounts of folding induced by the two peptoid residues MeGly (**3**) and *i*BuGly (**6**).

From Figure 3 it is evident that shifting the *iso*butyl side chain from the C^{α}-atom (tripeptide **12**) to the N-atom (tripeptoid **6**) dramatically increases the amount of H-bonding. The folding tendency of the -Pro-Gly- (**13**) sequence is intermediate between those of the -Pro-*i*BuGly- (**6**) and -Pro-Leu- (**12**) sequences.

The effect of the incorporation of a peptoid residue in position $i + 1$ is illustrated in Figure 4. In this series of compounds the rank order for intramolecular H-bond formation is Pro (**3**) > MeGly (**5**) ≫ Ala (**14**).

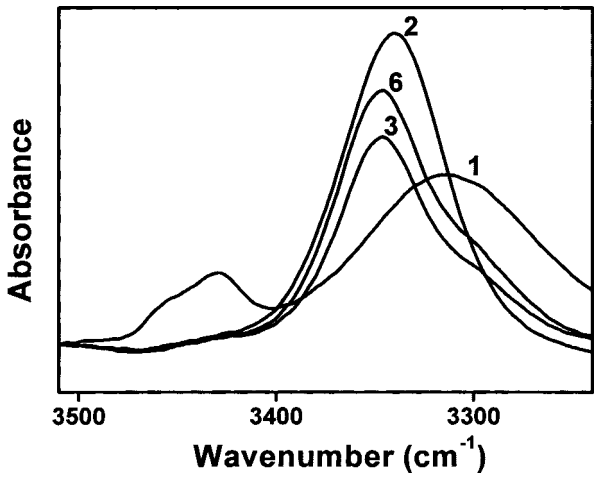

Figure 2 FT-IR absorption spectra (3500–3250 cm^{-1} region) of Boc-Pro-Pro-Gly-OMe (**1**), Boc-Pro-D-Pro-Gly-OMe (**2**), Boc-Pro-MeGly-Gly-OMe (**3**) and Boc-Pro-*i*BuGly-Gly-OMe (6) in $CDCl_3$ solution. Peptide concentration: 1×10^{-3} M.

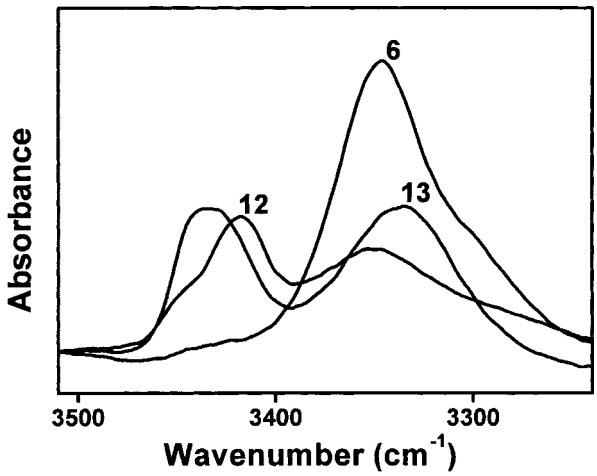

Figure 3 FT-IR absorption spectra (3500–3250 cm^{-1} region) of Boc-Pro-*i*BuGly-Gly-OMe (**6**), Boc-Pro-Leu-Gly-OMe (**12**) and Boc-Pro-Gly-Gly-OMe (**13**) in $CDCl_3$ solution. Peptide concentration: 1×10^{-3} M.

Other conclusions can be extracted from Table 2. (i) Replacement at the *N*-terminus of the more flexible Boc group (compound **3**) with the rigid Piv group (compound **4**) (the Piv-Pro sequence cannot undergo *cis* ⇌ *trans* isomerization) [35] decreases only slightly the relative intensity of the free NH band. (ii) The tripeptoid amide (**7**) is almost completely folded in two consecutive intramolecularly H-bonded forms, the moderately intense band at high wavenumbers (3484 cm^{-1}) being associated

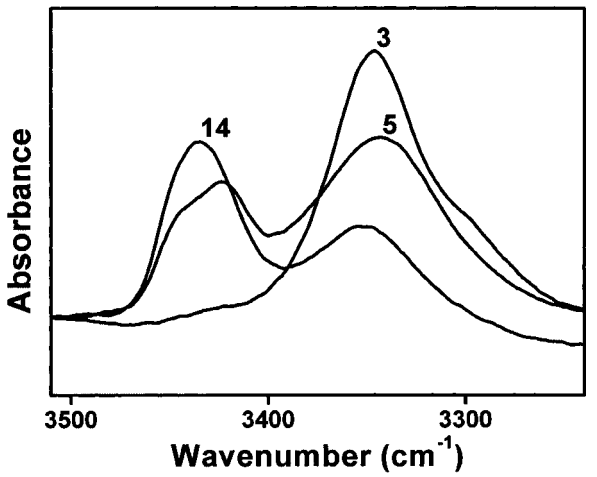

Figure 4 FT-IR absorption spectra (3500–3250 cm^{-1} region) of Boc-Pro-MeGly-Gly-OMe (**3**), Boc-MeGly-MeGly-Gly-OMe (**5**) and Boc-Ala-MeGly-Gly-OMe (**14**) in $CDCl_3$ solution. Peptide concentration: 1×10^{-3}M.

with one (free) of the two NH groups of the primary amide function [59]. Thus, modification of the tripeptoid ester (**6**) to a tripeptoid amide affects only marginally the extent of H-bonding at the -Pro-*i*BuGly- sequence. (iii) The Ala shifty analogues **8** and **9** show only a modest reduction in the amount of intramolecular H-bond formation compared with tripeptoids **3** and **6** containing MeGly and *i*BuGly, respectively, in the $i+2$ position. (iv) While isomer **II** of the *i*BuAla tripeptoid (**11**) is even more folded than its *i*BuGly (**6**) counterpart, although slightly, the spectrum of isomer **I** (**10**) is quite complex with a very broad band encompassing the 3450–3250 cm^{-1} region characterized by an absorption of moderate intensity (shoulder) at 3405 cm^{-1}, a maximum at 3347 cm^{-1} and an additional, distinct shoulder at 3301 cm^{-1} (isomers **I** and **II** correspond to the L- and D-*i*BuAla peptoid residues, respectively; for these configurational assignments, *vide infra*).

Nuclear Magnetic Resonance Analysis

Of the 14 compounds presented here, eight were selected for detailed NMR analysis: the parent peptide (**12**), all the compounds containing *N*-alkylated glycines (except **14**), and the two diastereomers **I** (**10**) and **II** (**11**). With the exception of the parent peptide, these analogues all contain either one tertiary urethane and one tertiary amide, or two tertiary amides (compound **4**). Therefore, four stereoisomers are possible in all cases, because of the various

Table 2 Infrared Absorption Data in the N-H Stretching Region for the Model Tripeptoids

Compound	Model tripeptoid	Absorption (cm^{-1}) maxima[a]
1	Boc-Pro-Pro-Gly-OMe	*3458*, 3430, **3314**
2	Boc-Pro-D-Pro-Gly-OMe	**3338**
3	Boc-Pro-MeGly-Gly-OMe	*3420*, **3327**
4	Piv-Pro-MeGly-Gly-OMe	*3420*, **3316**
5	Boc-MeGly-MeGly-Gly-OMe	*3446*, 3423, **3342**
6	Boc-Pro-*i*BuGly-Gly-OMe	*3425*, **3327**
7	Boc-Pro-*i*BuGly-Gly-NH_2	3484, *3412*, **3335**
8	Boc-Pro-MeAla-Gly-OMe	*3428*, **3327**
9	Boc-Pro-D-MeAla-Gly-OMe	*3454*, *3425*, **3332**
10	Boc-Pro-*i*BuAla-Gly-OMe (**I**)	3405, **3347**, 3301
11	Boc-Pro-*i*BuAla-Gly-OMe (**II**)	**3328**
12	Boc-Pro-Leu-Gly-OMe	*3450*, 3418, 3350
13	Boc-Pro-Gly-Gly-OMe	3434, 3335
14	Boc-Ala-MeGly-Gly-OMe	**3434**, 3350

[a] values in italic are very weak bands; values in bold are very strong bands; other values are strong bands.

combinations of *cis* and *trans* conformations. We envisage that isomerization at the urethane bond involves the (CO)-N linkage while the (CO)-O linkage should be freely rotating and thus conformationally averaged [64]. As conformational averaging at the tertiary urethane (CO)-N bond is expected to be faster than that of a tertiary amide bond, specific isomers with a *cis* conformation at the urethane bond were not always detected. In these cases the two situations in which a single *trans* urethane conformation is present or there is fast exchange between *trans* and *cis* urethane conformations on the NMR time scale cannot be distinguished. At any rate, a single signal for the *tert* -butyl protons is always observed.

At least two isomers are seen in the spectra of all the compounds (Table 3). The most populated conformations are *trans*–*trans* and *trans*–*cis*, which in the case of compounds **4** and **7** are the only isomers detected in the NMR spectra. For compound **4** this result is a consequence of the presence of a Piv moiety which keeps the Piv-Pro tertiary amide bond in the *trans* conformation for steric reasons [35]. The presence of only two isomers for compound **7** is justified on the basis of conformational preference (*vide infra*). Diastereomers **I** (**10**) and **II** (**11**) contain barely detectable traces of a third isomer in the NH region of their spectra while more isomers are found as flexibility is increased: three in compounds **3** and **6** (the former contains also traces of a fourth isomer) and four (only the NH proton was detected and integrated at 3% for the fourth isomer) in compound **5**. The third (and also the fourth) isomer is ascribed to the *cis* conformation around the Boc-X bond. Also the two isoforms detected for compound **12** are attributed to different conformations at the tertiary urethane bond, in slow exchange. This assignment was obtained *via* an HMCQ-TOCSY experiment performed at 600 MHz and 283 K. At this field and temperature the exchange rate between the two isoforms is slow enough to allow the distinction of two Pro($i+1$) α proton resonances, while at 400 MHz and 298 K the exchange rate is intermediate and one broad peak is observed. The δ protons overlap even at 600 MHz. Two sets of resonances are observed for the β and γ carbons at each proton frequency (Figure 5). Their position and relative intensity indicate that one corresponds to the *trans* and one corresponds to the *cis* form of the Boc-Pro bond [65].

The assignment of the *trans* peptide bonds was deduced from the presence of sequential ROESY peaks between the α proton of residue $i+1$ and the *N*-side chain of residue $i+2$. An $\alpha\alpha(i+1, i+2)$ peak, indicative of a *cis* conformation, was clearly found only between Pro($i+1$) and residue $i+2$ in isomer B of compounds **3**, **4**, **6** and **10**. The same peak is obscured by overlap in isomer B of compound **5**, while it was not detected in isomer B of compounds **7** and **11**, possibly because of the

Table 3 NMR Data for the Model Tripeptoids

Compound	Peptide	Isomer	Urethane (amide) bond	Peptide bond	$\Delta\delta_{\alpha\alpha}\, i+2$ (ppm)	Conformation
3	Boc-Pro-MeGly-Gly-OMe	A (72%)	Trans	Trans	1.30	β-turn II
		B (19%)	Trans	Cis	0.50	β-turn VI
		C (9%)	Cis	Trans	0.30	Open
4	Piv-Pro-MeGly-Gly-OMe	A 80%)	Trans	Trans	1.45	β-turn II
		B (20%)	Trans	Cis	0.58	β-turn VI
5	Boc-MeGly-MeGly-Gly-OMe	A (66%)	Trans	(Trans)	~0	β-turn
		B (21%)	Trans	Cis	~0	β-turn VI
		C (10%)	Cis		~0	Open
6	Boc-Pro-iBuGly-Gly-OMe	A (76%)	Trans	Trans	1.38	β-turn II
		B (18%)	Trans	Cis	0.34	β-turn VI
		C (6%)	Cis	Trans		Open
7	Boc-Pro-*i*BuGly-Gly-NH_2	A (95%)	Trans	Trans	1.11	β-turn II + I′
		B (5%)	Trans	(Cis)	0.22	β-turn VI
10	Boc-Pro-L-iBuAla-Gly-OMe (**I**)	A (73%)	Trans	Trans		β-turn II
		B (27%)	Trans	Cis		β-turn VI
11	Boc-Pro-D-iBuAla-Gly-OMe (**II**)	A (91%)	Trans	Trans		β-turn II
		B (9%)	Trans	Cis		β-turn VI
12	Boc-Pro-Leu-Gly-OMe	A (77%)	Trans	Trans		β-turn II
		B (23%)	Cis	Trans		Open

low population of these isomers. The $\alpha\alpha(i+1, i+2)$ peak between Pro and Leu was not detected in the case of compound **12**, even though the second isomer integrates at 23% of the total. This result is in agreement with the previous conclusion that this bond is not involved in *trans-cis* isomerism in compound **12**. The assignment of the *trans–cis* (rather than *cis–trans*) conformation to isomer B of compounds **5**, **7** and **11** was based on comparison and on the observation that the greatest differences in chemical shift on going from isomer A to isomer B were confined to residues $i+2$ and, in part, $i+3$, thus suggesting that the second bond, and not the first one, is modified by the isomerization.

The absence of chirality in Boc-MeGly-MeGly-Gly-OMe (**5**) and its increased flexibility complicated rather than simplified the assignment. In fact, this was the only compound in which all the methylene protons of MeGly and Gly were isochronous (*vide infra*), causing more severe overlap in this region. The complete assignment of the most abundant isomer was achieved by means of heteronuclear spectroscopy utilizing CO-selective HMBC experiments.

The considerations that follow are based on qualitative assessment of the possible conformations. Molecular modelling studies using computer simulations were deemed unnecessarY in view of the simplicity of the molecules that could be easily studied by building CPK models.

Conformation of isomer A. The presence of sequential ROESY peaks between the α proton of Pro$(i+1)$ [or MeGly$(i+1)$] and the *N*-side chain of the following residue is not only specific for the *trans* conformation at that bond, but also hints at the orientation of the peptide bond relative to the Pro ring: the carbonyl oxygen must point away from the Pro α proton, in a position characteristic of a type-II β-turn. This conformation is supported by the ROESY cross peaks between the N-side chain of residue $i+2$ and Gly$(i+3)$ NH, detected in compounds **3** –**7** with higher intensity than the corresponding sequential $\alpha(i+2)$-NH$(i+3)$ peak. The $\alpha(i+1)$-NH$(i+3)$ peak, characteristic for β-turns, was found with weak intensity in compounds **3**, **4**, **7** and **12**. A cross peak between the Boc protons and the Gly$(i+3)$ NH proton was detected, at various intensities, in all compounds except **6**. Notably, this peak is present for isomer A and not for isomer B in compound **12**. The observation of type-II, and not type-I, β-turns may derive from the possible 'D-behaviour' of the achiral $i+2$ residue and is also a consequence of the presence of an *N*-alkylated amino acid in that position. Indeed, a type-I β-turn conformation would be strained by steric hindrance

between the *N*-alkyl chain and the Pro ring. A further indication of the presence of type-II β-turns is the chemical shift difference between the two α protons in residue $i+2$ which is always very high (>1.1 ppm) for all isomers A (Table 3). A high difference has been recently connected to the presence of type-II β-turns in dipeptides and dipeptoids [34]. The only tripeptoid that does not display this feature is compound (**5**). The reason for this behavior is its complete lack of chirality making the two protons equivalent.

The impossibility of forming a type-I β-turn helped in the assignment of the L- and D-*i*BuAla residues in diastereomeric compounds **I** (**10**) and **II** (**11**), respectively. From model building it is easy to see that in a type-II β-turn the *N*-alkyl chain of an L-*i*BuAla residue is closer in space to the α-proton than to the α-methyl group; the opposite is true for a D-*i*BuAla residue. A strong cross peak between the α-proton and the *N*-alkyl chain was detected only for diastereomer **I** (**10**) which is identified as that containing the L-*i*BuAla residue. This cross peak is illustrated in Figure 6 together with other peaks that allowed the distinction between isomers A and B of compound **10**. The diagnostic peaks are between the α proton of the Pro residue and either the N-*i*Bu chain or the α proton of the *i*BuAla residue.

The relative amount of isomer A is very high in compound (**7**), where the possibility of a second H-bond, between the terminal NH_2 group and (possibly) Pro($i+1$)-CO can provide increased stabilization. In this case the second H-bond could define a type-I′ β-turn, the most probable one that can follow consecutively a type-II β-turn [66]. Also diastereomer **II** (**11**) displays a very high content of this isomer. This result is in line with the known stability of type-II β-turns in compounds containing Pro-D-Xxx sequences [35,36] which are enhanced here by the presence of an alkyl chain on the nitrogen atom.

Conformation of isomer B. The information on this conformer is more scarce, given its relatively modest abundance. The conclusions are therefore tentative. An αN($i+1$, $i+3$) cross peak between Pro($i+1$) and Gly($i+3$) was clearly detected only in compounds **4** and **6**. Together with the presence of a *cis* conformation at the Pro-N(alkyl) Gly bond, this correlation is indicative of a type-VI β-turn. These NMR results can be generalized to isomer B of all the compounds if the FT-IR absorption spectra (Table 2) are taken into account. In fact, the position and the intensities of the N-H stretching bands indicate that the NH groups of all the compounds studied by NMR [except for compound **7** which contains a terminal amide] are extensively involved in H-bonds. In a *trans-cis* arrangement of the urethane and peptide bonds, this is possible only if the compound adopts a type-VI β-turn conformation.

A special case is represented by compound **12** in which isomer B has a *cis–trans* conformation and the IR absorption spectrum shows a high percentage of free amide groups. The Boc-CO group in this isomer does not point toward Gly-NH which is therefore not involved in H-bonding. The resulting conformation is therefore open.

Conformations of other isomers. These isomers were detected at low relative amounts (maximum 10%) and their conformation was not defined in detail. It is likely that the presence of a *cis* conformation at the Boc-X bond results in an open conformation, as discussed above for isomer B in compound **12**. The limited amounts of free NH groups detected in the IR absorption spectra are likely to be due to these isomers.

CONCLUSIONS

In 1992 Zuckermann and coworkers [14,16] introduced a new concept in the search for bioactive molecules: the side chain on the α-amino acid α-carbon is shifted by one position along the peptide backbone to the next nitrogen atom to generate an *N*-substituted oligoglycine. The advantages of this new structure (peptoid) are enhanced metabolic stability, greater variability in functional groups and the absence of chirality.

However, it is reasonable to foresee that this structural modification will produce significant local and overall conformational changes in the peptide molecule. Although this problem would have been extensively tackled as far as the long-range and cooperative effects are concerned (helical conformations) [14,16–37], the short-range conformational variations (stability of β-turns and their nature, *cis* $\rightleftharpoons$ *trans* isomerism about the tertiary amide bond) induced by a peptoid residue have not been adequately investigated. In the present work we have systematically addressed this latter question by analysing the solution conformational preferences of a series of terminally protected tripeptoids characterized by an *N*-alkylated glycyl residue either in position $i+1$ or $i+2$ of the potential β-turn and the results were compared with those of a large set of selected peptides. The main conclusion that we have extracted from our analysis is that shifting the

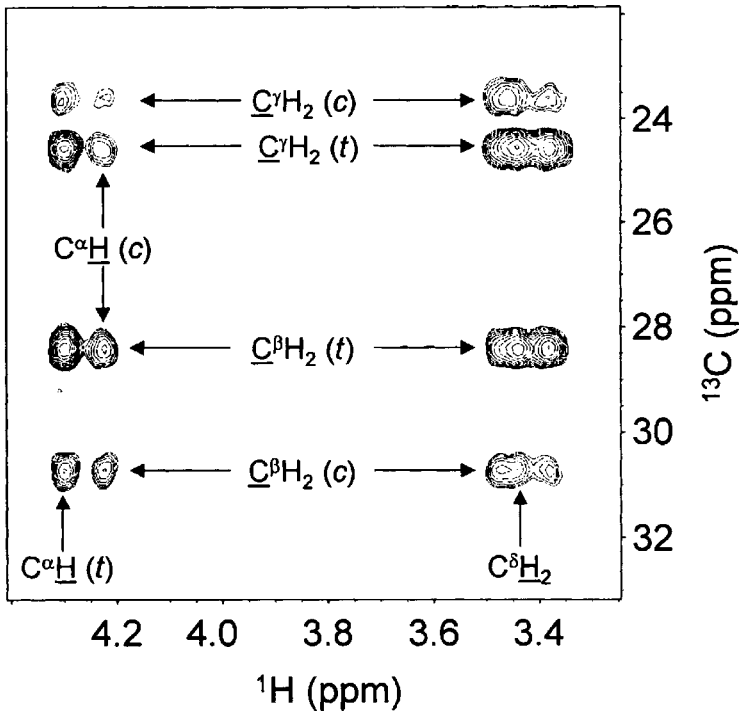

Figure 5 Selected region of an HMQC-TOCSY spectrum of Boc-Pro-Leu-Gly-OMe (**12**) in $CDCl_3$ solution. Peptide concentration: 1×10^{-2}M.

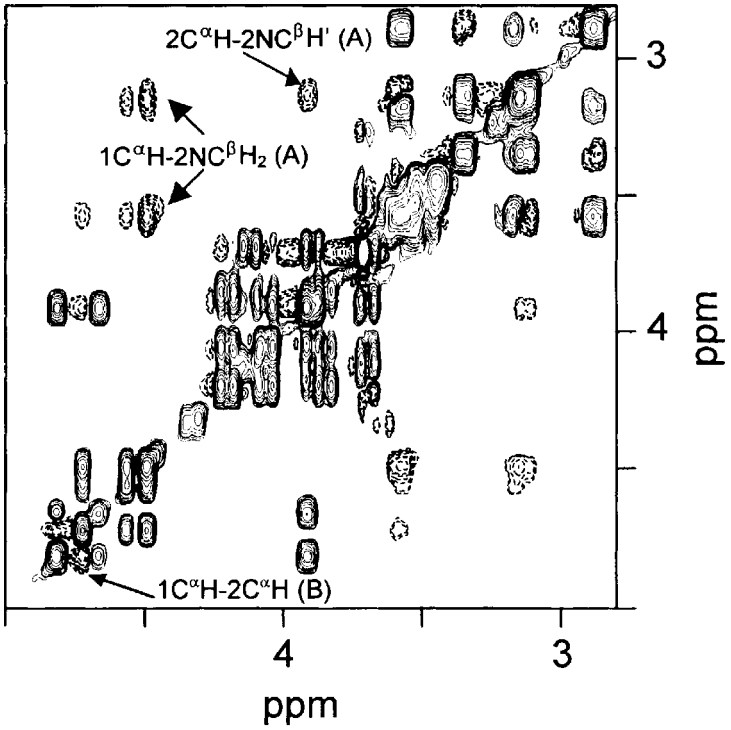

Figure 6 Portion of a ROESY spectrum in $CDCl_3$ solution illustrating the assignment of the two isomers A and B of Boc-Pro-L-*i*BuAla-Gly-OMe (**10**). Dashed lines correspond to negative peaks.

amino acid side chain from the C^{α} to the adjacent N atom has a profound influence on the preferred local peptide conformation. The two most altered parameters are the amount of β-turn (dramatically increased) and the type of β-turn (significantly enhanced fractions of type-II and type-VI β-turns). The following can be related: (i) the increased population of type-II β-turn to the achiral nature of the peptoid residue in position i+2, which might produce a mimic of a heterochiral -L-D- sequence, and to the steric destabilization of type-I β-turn in peptides with an *N*-alkylated residue at position $i+2$ and (ii) the increased population of type-VI β-turn to the tertiary amide group joining residues $i+1$ and $i+2$, known to easily undergo isomerization to the *cis* form.

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