Solvent-Dependent Conformational Behaviour of Model Tetrapeptides Containing a Bicyclic Proline Mimetic

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Keywords: Conformational analysis / NMR spectroscopy / Peptidomimetics / Proline / Reverse turn

Two model tetrapeptides containing bicyclic analogues of either D- or L-proline were synthesised and their conformational properties were studied by NMR in different solvent systems and by molecular modelling techniques. Compound **1**, with the bicyclic D-proline mimetic in the *i*+1 position, generated a unique *trans* isomer, and the peptide showed a well organised structure, in accordance with the tendency of D-proline to act as a good turn inducer with respect to its enantiomer. Peptide **2** displayed structures equilibrating from type I,II to type VI β-turns, thus confirming the hypothesised relationship between the chirality of BGS/Bgs and proline enantiomers on nucleating compact turns. Moreover, such behaviour suggested a tool for peptidomimetic design of reverse turn peptides containing BGS/Bgs bicyclic proline mimetics, as the choice of chirality might influence the generation either of compact γ- and β-turns or of flexible equilibrating reverse turn structures. The effect of solvent on conformational behaviour was also studied.

Introduction

β-turns are structural motifs that play a crucial role in folding and generating compact structures in proteins and bioactive peptides,[1] and these templates have been extensively studied in peptidomimetic design. In particular, the development of conformationally restricted mimics of these structures has been pursued because, in the context of pharmacophore arrangement, they can allow two to four side chains of amino acids involved in biological interactions to be presented in a stereocontrolled fashion.[2] The majority of β-turn mimetics are based upon the replacement of the *i*+1 to *i*+2 central dipeptidic sequence of a turn with dipeptide isosters capable of retaining the intramolecular ten-membered ring hydrogen-bond.[3] Another approach involves the introduction of proline-mimetics, since, among the naturally occurring amino acids, proline is known to play a central role in the nucleation of reverse turn structures such as β-turns and β-hairpins, due to its ability to form *cis* peptide bonds and to undergo *cis*/*trans* isomerisation. A *cis* geometry of the proline amide bond causes the peptide backbone to fold in a type VI β-turn, in which proline occupies the *i*+2 position, while type I and type II β-turns show structures in which proline is in position *i*+1 and generates a *trans* amide bond with the preceding amino acid in position *i*. These structural properties of proline and its derivatives result in characteristic and unique constraints on the conformational spaces of peptide sequences containing proline or hydroxyproline.[4] Thus, peptidomimetic design of reverse turn analogues has also been focused on the creation of proline analogues, with the aim of modulation of the *cis*/*trans* ratio of acyl-Pro bonds, constraining the peptide bond conformation, and producing proline-like reverse turn inducers.[5] Among type VI β-turn mimetics, many proline-like molecules bearing bulky substituents have been developed in order to force the amide bond to assume a *cis* configuration, as reported by Lubell and co-workers in the case of 5-*tert*-butyl-proline.[6] During recent years we have developed a new class of bicyclic 3-aza-6,8-dioxabicyclo[3.2.1]octane scaffolds as γ/δ amino acids and named BTAas (**B**icycles from **T**artaric acid and **A**mino **a**cids) and BTKas (**B**icycles from **T**artaric acid and **K**eto-**a**mines).[7] These compounds are obtained from combinations of sugars or tartaric acid and amino carbonyl derivatives, and proved to be constrained dipeptide isosters when inserted in peptide chains. The sub-class of 7-*endo*-BTAa showed marked properties as reverse turn inducers in both cyclic[8] and linear[9] peptide sequences, acting as mimetics of *i*+1 to *i*+2 central dipeptidic sequences in a typical β-turn motif. Recently we described a new set of bicyclic compounds named BGS (**B**icycles from **G**lyceraldehyde and **S**erine derivatives, with *R* absolute configuration at C-4) or Bgs (with *S* absolute configuration at C-4), which act as constrained unnatural α-amino acids with marked similarities to proline, by virtue of the carboxyl moiety present in position 4 (Figure 1).[10] In particular, thanks to the structural asset of the bicyclic structure, a BGS amino acid could be con-

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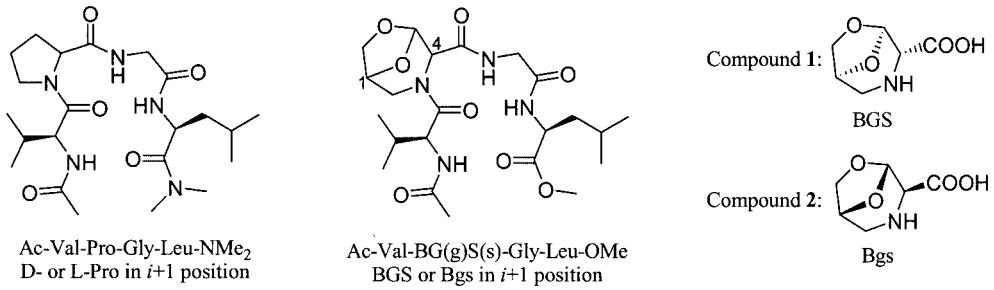

Figure 1. Bicyclic proline mimetic in a type II β-turn

sidered a bicyclic analogue of D-proline, while a Bgs showed similarity with L-proline. In order further to verify the proline-mimetic role of such bicyclic α-amino acids as turn inducers, we studied the conformational behaviour of model tetrapeptides containing BGS and Bgs scaffolds in their $i+1$ positions. The end-protected Val-D-Pro-Gly-Leu sequence, in which D-proline was to be found in the $i+1$ position and generated a type II β-turn, as described by Gellman et al. (Figure 1),[11] was used as reference peptide.

It is shown elsewhere how a proline chirality shift may affect the nucleation of β-hairpin structures both in parallel and in antiparallel strands.[11,12] In particular, Gellman has described the key role of D-proline in a heterochiral Val-Pro-Gly-Leu sequence for the generation of a hairpin by comparison with the corresponding all-L peptide. In line with these observations, we decided to test the stereochemical differences of BGS and Bgs amino acids when inserted into a model Val-BG(g)S(s)-Gly-Leu peptide. More precisely, a BGS scaffold was inserted in compound **1** as a D-proline mimetic, and a Bgs in compound **2** as an L-proline analogue (Figure 1). The conformational analysis of peptides was carried out by NMR spectroscopy and by molecular modelling.[13,14] As an additional aspect of the conformational study of these bicyclic proline-mimetics, we decided to test the influence of different solvents on the structural organisation of reverse turn peptides. The behaviour of amide protons was studied in $CDCl_3$, a relatively nonpolar solvent well suited for evaluation of intrinsic conformational propensities of small oligoamides. CD_3CN, as a moderate hydrogen-bond acceptor with enhanced solvating properties, was used to test the strength of intramolecular hydrogen-bonds of amide protons. In the case of $[D_6]$DMSO solutions, no intramolecular hydrogen-bond was detected spectroscopically, as this solvent is known to be a strong hydrogen-bonding competitor, thus disrupting the intramolecular interactions in flexible peptidomimetics.

Results and Discussion

The synthesis of the tetrapeptides **1** and **2** was achieved by solid-phase techniques through standard Fmoc methodology on HMBA [4-(hydroxymethyl)benzoic acid] resin, allowing nucleophilic cleavage to be performed at the end of the synthesis, thus providing the title peptides with their C-termini protected as methyl esters. Amino acid couplings were performed with DIPC (*N*,*N*′-diisopropylcarbodiimide)/HOBt (1-hydroxybenzotriazole) as activating mixture and in DMF as solvent. Completion of the coupling reactions was monitored by use of bromophenol blue as internal standard, as described by Krchnák et al.[15] In the case of valine coupling to the scaffold, reaction times were prolonged due to lowered reactivity at the nitrogen atom, as observed for similar compounds.[9] Despite the drastic conditions employed during the synthesis, the preparation of peptides afforded mixtures of the desired compound as the peptide ester and Ac-BG(g)S(s)-Gly-Leu-OMe as a by-product caused by incomplete coupling. Truncated and title compounds were separated by semi-preparative HPLC, and peptides **1** and **2** were characterised by analytical HPLC, ESI-MS and NMR spectroscopy.

Conformational Analysis

The conformational behaviour of compounds **1** and **2** was elucidated by their ^{1}H NMR features. We first performed ^{1}H NMR analysis to determine the lowest concentrations at which intermolecular hydrogen-bonding occurred. All data reported in this paper were obtained from samples at 300 K and 2 mM concentrations, conditions under which aggregation was not significant. NMR experiments were conducted with the aim of detecting intramolecular hydrogen-bonds by measuring the chemical shifts of NH protons and their temperature coefficients ($\Delta\delta/\Delta T$).[13] NOESY spectra were recorded to investigate both sequential and long-range NOEs as evidence of preferred conformations, and to give insights into stable reverse turn conformations. Moreover, molecular modelling calculations were used to rationalise NMR spectroscopic data relating to the hydrogen-bonding behaviour of compounds **1** and **2**.

Folding Behaviour of Compound 1 in Chloroform and Acetonitrile

The ^{1}H NMR spectroscopic data of compound **1** in $CDCl_3$ solution showed two sets of proton resonances. The population of the major isomer was greater than 96% and was attributable to the *trans* configuration at the Val−scaffold amide bond, as indicated by the strong NOESY peak between Val H-α and BGS H-2ax. The predominance of this isomer indicated that the BGS compound was exactly mimicking the *trans*-proline commonly found in the $i+1$ position of a type II β-turn (Figure 2, a).

For the major isomer of **1**, the Gly NH resonance was observed at 7.39 ppm, suggesting a moderate amount of internal hydrogen-bonding, while the other NH resonances appeared at 6.76 and 6.89 ppm (Table 1). Temperature coefficient values ($\Delta\delta/\Delta T$) suggested equilibration of the Gly and Val amide protons between hydrogen-bonded and non-hydrogen-bonded states, while the Leu NH showed behaviour typical of hydrogen-bonded amide protons, having a low temperature coefficient (−1.5 ppb/K) and a chemical shift value higher than 6.6 ppm. (Table 1).[13] NOESY analysis of **1** provided further insight into the conformation in solution. Two important NOEs were observed between protons on non-adjacent residues, defining a type II β-turn conformation (Figure 2, a). Specifically, NOESY cross-peaks were found between the Leu amide proton and both BGS H-4 and Gly NH. Moreover, Gly NH participated in a seven-membered ring hydrogen-bond with Val CO. The formation of this γ-turn was supported by the chemical shift value of Gly NH, and by the NOE contact between BGS H-4 and Gly NH.

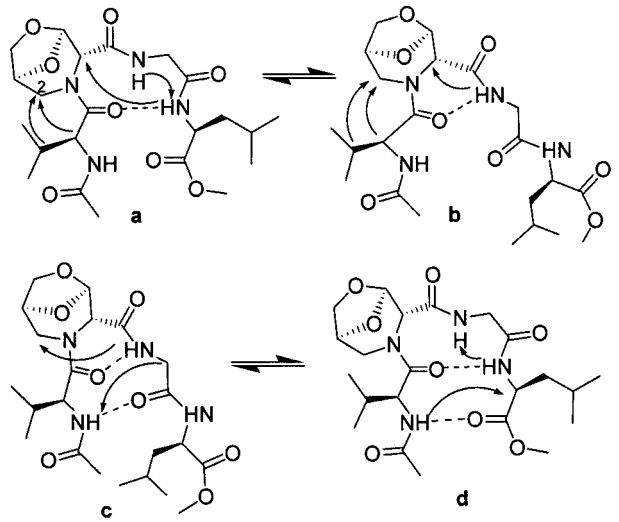

Figure 2. Hydrogen-bonded structures for peptide **1** in $CDCl_3$ (**a**, **b**) and CD_3CN (**c**, **d**); the arrows indicate significant NOE contacts

Table 1. Temperature-dependent ^1H NMR spectroscopic data for amide protons of peptide **1**; chemical shifts (δ) are reported in ppm, and temperature coefficients ($\Delta\delta/\Delta T$) in ppb/K

Amide proton	$CDCl_3$		CD_3CN	
	δ	$\Delta\delta/\Delta T$	δ	$\Delta\delta/\Delta T$
Val NH	6.76	−4	7.14	−5.5
Gly NH	7.39	−5	7.75	−3.5
Leu NH	6.89	−1.5	6.82	−1.5

Experiments carried out in CD_3CN showed marked differences, suggesting that the presence of a more competitive solvent induced peptide **1** to become more organised. ^1H NMR spectra of **1** in CD_3CN solution showed only one set of proton resonances, attributable to the *trans* isomer (Table 1), as confirmed by the strong NOESY peak between Val H-α and BGS H-2ax. The greater tendency of Val NH and Gly NH to show hydrogen-bonded character suggested the presence of an organised turn structure in which two seven- and 11-membered ring hydrogen-bonds existed (Figure 2, c). This hypothesis was confirmed by medium NOESY cross-peaks between Gly H-α and Val NH, and between Gly NH and BGS H-2ax. Moreover, two NOEs were observed between protons on non-adjacent residues, defining a minimal β-hairpin conformation (Figure 2, d). Specifically, a strong NOESY cross-peak between Leu NH and Gly NH, and a small one between Val NH and Leu H-α were found. Thus, Val NH was involved in two equilibrating hydrogen-bonds with the carbonyl groups of the Leu and Gly residues, respectively. The conformational behaviour of **1** was clearly determined by the nature of the solvent, since compound **1** showed a preference for a more compact structure in CD_3CN solution, in which the γ-turn was stabilised by seven- and 11-membered ring hydrogen-bonds, and the β-turn generated a family of conformations in which the tetrapeptide folded in a β-hairpin structure.

Folding Behaviour of Tetrapeptide 2 in Chloroform and Acetonitrile

Two sets of proton resonances in a 2:1 ratio were observed for **2** in $CDCl_3$. We attributed this doubling of resonances to slow rotation about the tertiary C−N amide bond of the scaffold. The proton resonances of both conformers were fully assigned by COSY, TOCSY, and NOESY measurements. In the minor conformer, relatively strong NOEs were observed between Bgs H-2ax and Val H-α. These data established the rotational state about the tertiary amide bond of the minor isomer as the *trans* configuration (Figure 3). The minor conformer of **2** did not show a well organised structure, as the amide proton chemical shifts indicated non-hydrogen-bonded situations and the corresponding temperature coefficients suggested the existence of equilibrating structures. In the major *cis*-amide conformer of compound **2**, Gly NH displayed chemical shifts typical of hydrogen-bonded structures. A NOESY cross-peak between Gly NH and Val H-α supported the existence of an intramolecular ten-membered ring hydrogen-bond between Gly NH and the acetyl group, which indicated the existence of a type VI β-turn-like conformation in which the *cis*-Bgs amino acid was shifted to the $i+2$ position of the turn. Moreover, the Leu NH chemical shift value and the presence of an additional NOE between Leu NH and Bgs H-4 suggested the existence of a γ-turn stabilised by a seven-membered ring hydrogen-bond between Leu NH and the Bgs carbonyl group at the C-7 position (Figure 3, left). A more organised structure was also observed for peptide **2** on changing from $CDCl_3$ to CD_3CN as solvent. Two isomers were still found, but the *trans*/*cis* ratio was 4:3, suggesting that the solvating effect of CD_3CN lowered the energy barrier between the two isomers and increased the stabilisation of the *trans* amide. The amide proton chemical shift values were found to be downfield with respect to the $CDCl_3$ values, as a consequence of greater solvation effect of CD_3CN, though still non-competitive relative to hydrogen-bonding.

In fact, NOESY spectra of **2** showed some cross-peaks between protons on non-adjacent residues. These medium and weak NOEs were indicative of an equilibrium between more equivalent conformations (Table 2).

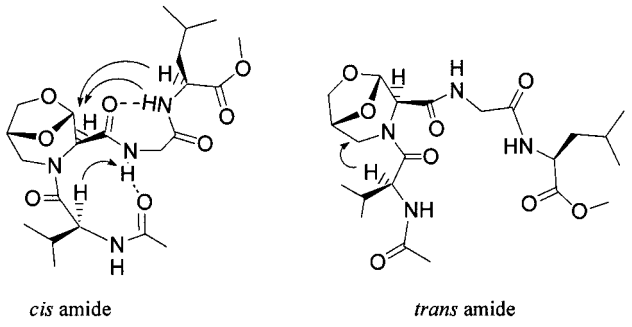

Figure 3. Preferred conformations for *cis* and *trans* amides of peptide **2** in $CDCl_3$ solution; the arrows indicate significant NOE contacts

Table 2. Chemical shifts and temperature-dependent ^1H NMR spectroscopic data for amide protons of peptide **2**; chemical shifts (δ) are reported in ppm, and temperature coefficients ($\Delta\delta/\Delta T$) in ppb/K

		$CDCl_3$		CD_3CN	
	Amide proton	δ	$\Delta\delta/\Delta T$	δ	$\Delta\delta/\Delta T$
cis Isomer	Val NH	6.04	−3.3	6.88	−5.0
	Gly NH	7.75	−4.5	7.69	−4.0
	Leu NH	6.84	−4.0	7.23	−4.0
trans Isomer	Val NH	6.26	−5.5	6.82	−4.5
	Gly NH	6.75	−7.1	7.04	−3.5
	Leu NH	6.30	−3.7	6.90	−4.0

In isomer **2-*cis***, a particularly striking observation was the difference between the chemical shifts of Val NH in chloroform and in acetonitrile solutions. In $CDCl_3$, the chemical shift of this amide proton occurred in a region characteristic of non-hydrogen-bonded amide protons, while in CD_3CN solution Val NH appeared substantially downfield from this region. In contrast, the Gly NH chemical shift moved upfield. This behaviour suggested the presence of two families of structures with similar energies. The first structure resembled the doubly hydrogen-bonded pattern as observed in $CDCl_3$ (Figure 4, a), while the second indicated an 11-membered ring hydrogen-bond between Val NH and the Gly carbonyl group (Figure 4, b), thus indicating the partial hydrogen-bonded character of Val NH. The **2-*trans*** conformer also showed two equilibrating populations of structures, as evinced by $\Delta\delta/\Delta T$ coefficients and chemical shift values, together with observed NOESY peaks (Figure 4, c−d). NMR spectroscopic data clearly indicated the existence of a β-hairpin-like structure with ten- and 14-membered ring hydrogen-bonds, in equilibrium with a preferred open-turn structure featuring a strong NOE contact between Bgs H-4 and Gly NH, which supported the out-of-turn orientation of such an amide proton.

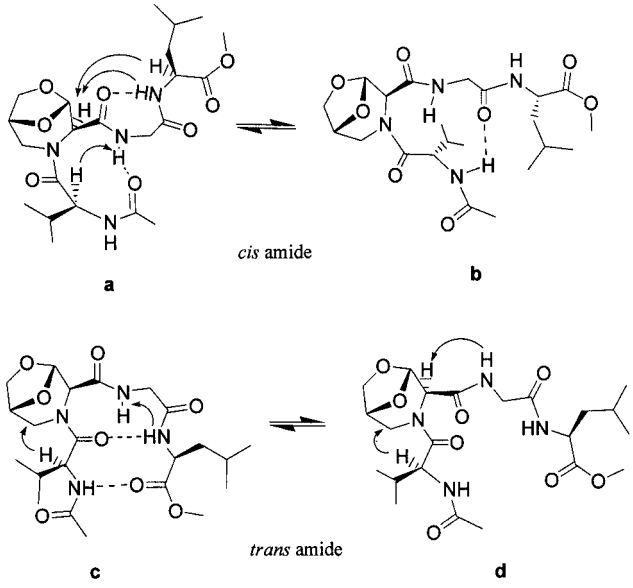

Figure 4. Equilibrating structures for *cis* and *trans* amides of peptide **2** in CD_3CN solution; the arrows indicate significant NOE contacts

Molecular Modelling of 1 and 2

Molecular modelling calculations were carried out with the aid of the AMBER*[16] force field to gain further insights into the conformational space accessible to peptides **1** and **2**, with use of a full unconstrained Monte Carlo conformational search.[17] Moreover, a mixed dynamic simulation by the MC/SD approach[18] as described by Marshall et al. for azaproline-containing peptides,[19] was performed in order to investigate the conformations available in the vicinity of local minima, and to verify the flexibility of the structures found in the conformational search. Such dynamic simulation was also used to assess the *cis*/*trans* ratios of peptides **1** and **2**. The Monte Carlo investigation into the *trans* isomer of compound **1** was carried out by starting from the 7+11-membered ring doubly hydrogen-bonded structure (Figure 2, c), and resulted in a global minimum structure ($E = -240$ kJ/mol) with the 11-membered ring hydrogen-bond being maintained between Val NH and Gly carbonyl group (Figure 5, left).

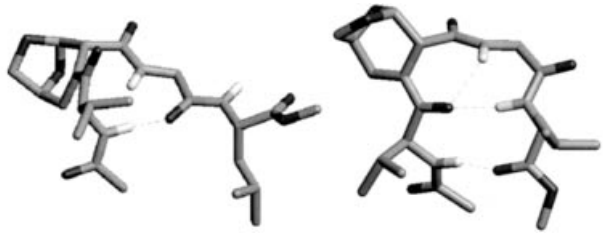

Figure 5. Preferred conformations for compound **1** obtained from Monte Carlo conformational search

Such a hydrogen-bond existed in 31% of the structures found within the range up to 6 kJ/mol above the global minimum energy. The 7+11-structure was present in 12.4%

of cases and the 10+14-hairpin-like conformation in 13.6% (Figure 2, d and Figure 5, right), with the latter having an energy about 1 kcal/mol higher than the former (E = −236 kJ/mol). The equal abundance of the two structures was consistent with the assumption of an equilibrium between the two conformers, as evinced by NMR spectroscopic data. MC/SD dynamic simulation on compound **1** predicted 7% of the *cis*-BGS isomer, in accordance with NMR spectroscopic data indicating the predominance of the *trans* isomer. Moreover, the 7+11-membered ring hydrogen-bonded structures proved to be more stable than their 10+14-counterparts, and emphasized the differences between them with respect to Monte Carlo calculation. In particular, the 11-membered ring hydrogen-bonded conformation was the most populated, in accordance with the NMR spectroscopic data. The population of hydrogen-bonded structures of Figure 5 was found at lower percentages than in the Monte Carlo calculation, confirming the high flexibility of peptide **1** and agreeing with the presence of equilibrating structures. All the calculations were performed explicitly in chloroform as solvent, suggesting that in CD_3CN such trends should be maintained or even emphasized.

Molecular modelling on compound **2** was carried out independently on the two isomers. Monte Carlo conformational searches on the *trans* isomer were conducted by starting from the 10+14-membered ring hydrogen-bonded structure (see Figure 4, c). The global minimum (E = −241 kJ/mol) showed strict similarities with the starting structure, but was poorly populated (8%) among the 301 structures found within the range up to 6 kJ/mol above the global minimum energy. The overall result involved a predominance of open-turn structures, and the tendency towards high flexibility was confirmed, in agreement with NMR spectroscopic data. The stochastic dynamic on the *trans* isomer starting from the global minimum conformer found in Monte Carlo revealed a 2:1 ratio of *trans*/*cis* amides, in contrast with NMR spectroscopic data. Moreover, it was demonstrated that the 10+14-membered ring hydrogen-bonded structure was not stable, and open structures were found at high percentages. A Monte Carlo conformational search on the *cis*-amide was conducted by starting from the structure with a ten-membered ring hydrogen-bond between Gly NH and the acetyl group, as found in NMR experiments in CD_3CN (Figure 4, a). The global minimum found (E = −234 kJ/mol) was similar to the starting structure, with a hydrogen-bond frequency of 27.6% among the 489 structures found (Figure 6, left).

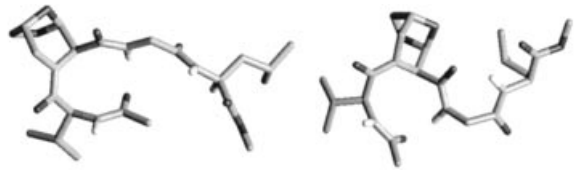

Figure 6. Type VI β-turn-like conformations for *cis*-amide **2**

Moreover, a γ-turn structure with a seven-membered ring hydrogen-bond between Leu NH and Bgs CO was found (16.2%), in accordance with the NMR spectroscopic data showing a NOE contact between Leu NH and Bgs H-4 (see Figure 4, a and Figure 6, right). However, the majority of structures found were of open-turn character, thus suggesting a flexible structure. Confirmation was supplied of the Bgs shift to the i+2 position to generate a type VI β-turn-like conformation, stabilised by the ten-membered ring hydrogen-bond between Gly NH and the acetyl group. The existence of such VI β-turn-type geometry was also supported by a NOE contact[20] between Val H-α and Gly NH, which was present in 97.3% of the conformers. NOESY interaction between the Val and Gly amide protons (41.3%) demonstrated the equilibrating nature of *cis*-peptide **2** from the type VI β-turn-like geometry to the conformation with Val NH involved in an 11-membered ring hydrogen-bond with Gly CO (Figure 4, a−b). MC/SD simulation on the *cis* amide generated a 1:1 ratio of the two isomers, with better agreement with the NMR spectroscopic data in CD_3CN. Of the MC/SD structures, 41.4% showed a NOE contact between Leu NH and Bgs H-4, confirming the existence of a γ-turn stabilised by a seven-membered ring hydrogen-bond between Leu NH and the Bgs CO (see Figure 4, a). Moreover, in analogy with the *trans* amide, the occurrence of many open structures suggested the presence of equilibrating populations of conformers.

Conclusion

Our conformational results have provided a tool for the peptidomimetic design of reverse turn mimics relating to the choice of BGS and Bgs bicyclic proline-mimetics. The Bgs scaffold, as demonstrated by analysis of peptide **2**, is good for generating structures in which a type I,II to type VI β-turn switch is desirable, while the BGS scaffold, present in the i+1 position of compound **1,** has a marked tendency to promote either γ- or β-turn-like hydrogen-bonded structures. This is in agreement with the hypothesised relationship between the chirality of BGS/Bgs and proline enantiomers. In fact, the D-proline mimetic BGS compound, once inserted in peptide **1** in the i+1 position, generated a unique *trans* isomer, and the peptide became well organised, in accordance with reported data pointing to D-proline as the better nucleator, with respect to its enantiomer, of compact turn structures. All NMR and computational data suggested that peptide **2** had a minor tendency to generate organised structures with respect to tetrapeptide **1**. Moreover, the presence of two isomers for peptide **2** was an indication that there is no precondition for a strong organisation of a peptide framework. ^{1}H NMR spectroscopic data suggested that the folding pattern of **1** and **2** in CD_3CN are qualitatively similar to those occurring in chlorocarbon solvents. However, this more interactive solvent appeared to promote more compact structures, in which β-turn and seven-membered-ring structures were stabilised by a minimal β-hairpin

and by a 11-membered-ring hydrogen-bonded structure, respectively.

Experimental Section

General Remarks: Fmoc-protected BGS and Bgs amino acids were synthesised as reported.[10] HPLC purifications were performed with an HPLC system fitted with a semipreparative C-18 10 μm, 250 × 10 mm, reversed-phase column with H_2O/CH_3CN eluent buffered with 0.1% TFA, and were characterised by ESI-MS, 2D NMR and HPLC on a system with an analytical C-18 10 μm, 250 × 4.6 mm, reversed-phase column.

Chemistry: Peptides **1** and **2** were prepared by solid-phase techniques on a HMBA-AM polystyrene resin. A DIPC/HOBt carboxylic-activating mixture was used throughout the synthesis, and DMF was used as solvent. Coupling at the scaffold amine function was prolonged to up to three days in order to achieve completion. Final acetylation was performed with Ac_2O in DMF with catalytic 4-dimethylaminopyridine. All amide couplings were monitored with internal bromophenol blue as colorimetric indicator.[15] Nucleophilic cleavage from the resin was achieved by transesterification, with a suspension of the resin in a 9:1 MeOH/triethylamine mixture being heated at 50 °C overnight.[21] Crude peptides containing truncated Ac−BG(g)S(s)−Gly−Leu−OMe were purified by semi-preparative HPLC with 10−90% acetonitrile/55 min as gradient.

NMR Methods: NMR spectra were performed on a Bruker Avance 400 spectrometer operating at 400 MHz for ^1H. The spectra were obtained in 2 mM $CDCl_3$ or CD_3CN solutions, in which aggregation was not significant. One-dimensional ^1H NMR spectra for determination of temperature coefficients were obtained at 280−320 K with increments of 5 K. Sample temperatures were controlled with the variable-temperature unit of the instrument. Complete proton resonance assignments were carried out with the aid of COSY, TOCSY, HSQC and NOESY experiments.

Computational Methods: Molecular mechanics calculations were carried out on a SGI IRIX 6.5 workstation, with use of MacroModel (v6.5) molecular modelling software,[22] AMBER* as a force field[16] and the implicit chloroform GB/SA solvation system.[23] Monte Carlo conformational searches[17] were carried out without imposition of any constraint and with inclusion of amide bonds in the rotatable bonds. Ring-closure was defined for the six- and seven-membered rings of BGS and Bgs scaffolds. 2000 structures were generated and minimised until the gradient was less than 0.05 kJ/Å·mol by the TNCG gradient implemented in MacroModel.[24] All conformers with an energy 6 kcal/mol above the global minimum conformer were discarded. The *cis* and *trans* isomers of peptide **2** were analysed separately. A Monte Carlo/Stochastic Dynamics (MC/SD) hybrid simulation algorithm[18] was used to assess the *cis*/*trans* ratios of peptides **1** and **2**. AMBER* was used as force field, as implemented in Macromodel (v6.5). A time step of 0.75 fs was used for the stochastic dynamics part of the algorithm. The MC simulation used random torsional rotations between ±60° and ±180° for all rotatable bonds except for the amide ones, for which

Table 3. Proton and carbon chemical shifts of **1** (major conformer) in $CDCl_3$ and CD_3CN at 300 K

	^1H ($CDCl_3$)	^{13}C ($CDCl_3$)	^1H (CD_3CN)
BGS H-1	4.73	70.6	4.71
BGS H-2	4.16−3.79	48.8	3.96−3.78
BGS H-4	4.65	60.6	4.47
BGS H-5	5.98	99.7	5.85
BGS H-7	3.91	67.3	3.96−3.78
Gly NH	7.39		7.75
Leu NH	6.89		6.82
Val NH	6.76		7.14
Gly H-α	4.20−3.69	43.3	3.84−3.69
Leu H-α	4.59	51.0	4.35
Leu H-β	1.66−1.60	41.5	1.57
Leu H-γ	1.67	25.1	1.62
Leu H-δ	0.95−0.91	23.3−22.0	0.9
Val H-α	4.29	56.1	4.29
Val H-β	2.1	30.4	2.03
Val H-γ	1.14−1.04	19.5	1.04
$-OCH_3$	3.7	52.5	3.68
CH_3CO	2.1	23.2	2.11

Table 4. Proton and carbon chemical shifts of **2** in $CDCl_3$ and CD_3CN at 300 K

	2 − ***cis***			**2** − ***trans***		
	^1H ($CDCl_3$)	^{13}C ($CDCl_3$)	^1H (CD_3CN)	^1H ($CDCl_3$)	^{13}C ($CDCl_3$)	^1H (CD_3CN)
Bgs H-1	4.67	71.9	4.62	4.67	71.9	4.65
Bgs H-2	4.49−3.34	44.4	4.31−3.21	4.17−3.93	48.6	4.09−3.78
Bgs H-4	4.37	61.7	4.48	4.86	59.0	4.69
Bgs H-5	6.12	107	5.91	5.86	111	5.74
Bgs H-7	3.87	67.6	3.79−3.64	4.03−3.81	67.0	3.97−3.71
Gly NH	7.75		7.69	6.75		7.04
Leu NH	6.84		7.23	6.3		6.90
Val NH	6.04		6.88	6.26		6.82
Gly H-α	4.00−4.06	44.9	3.83	3.9−4.1	43.3	3.86
Leu H-α	4.67	51.2	4.45	4.65	51.2	4.40
Leu H-β	1.55	42.0	1.62	1.55	42.0	1.61
Leu H-γ	1.66	25.2	1.63	1.66	25.2	1.63
Leu H-δ	0.95	18.3	0.94	0.99	19.7	0.96
Val H-α	4.36	55.7	4.36	4.75	54.3	4.56
Val H-β	2.1	30.7	1.99	2.1	30.7	2.05
Val H-γ	1.05−1.08	18.0−19.9	0.93	0.95	22.6	0.94
$-OCH_3$	3.7	52.4	6.67	3.76	52.7	3.68
CH_3CO	2.03	23.1	2.10	2.06	23.2	2.10

the random rotations were between ±90° and ±180°. No rotations were applied to the bonds of BGS and Bgs scaffolds. The total simulation was 2000 ps, and samples were taken at 1 ps intervals, yielding 2000 conformations for analysis. The MC/SD algorithm gave percentage values weighted by the energetic content of structures found, according to Boltzmann's law. In order to verify the convergence of calculations, both Monte Carlo and MC/SD simulations were carried out on structures as depicted in Figures 2−4, always giving a unique result for each isomer of peptide **1** and **2**, and thus indicating good convergence.

Ac−Val−BGS−Gly−Leu−OMe (1): Compound **1** was obtained in 13% yield after HPLC purification. The pure peptide showed a HPLC peak at t_R = 20.3 min (91% HPLC purity) with 0% acetonitrile/5 min, 0−10% acetonitrile/5 min, then 10−90% acetonitrile/20 min as gradient and an ESI-MS peak of *m*/*z* = 485.3 (40) [M^+ + 1]. ^1H and ^{13}C NMR spectroscopic data of major conformer are shown in Table 3.

Ac−Val−Bgs−Gly−Leu−OMe (2): Compound **2** was obtained in 18% yield after HPLC purification. The pure peptide showed a HPLC peak at t_R = 19.9 min (84% HPLC purity) with 0% acetonitrile/5 min, 0−10% acetonitrile/5 min, then 10−90% acetonitrile/20 min as gradient and an ESI-MS peak of *m*/*z* = 485.3 (85) [M^+ + 1]. ^1H and ^{13}C NMR spectroscopic data of *cis* and *trans* amides are shown in Table 4.

Acknowledgments

The authors thank the Università degli Studi di Firenze, the Università degli Studi di Milano, FIRB 2001 and COFIN 2002−2003 for financial support. Prof. Laura Belvisi is acknowledged for useful comments about computational analysis. Ms Brunella Innocenti and Mr Maurizio Passaponti are acknowledged for technical support.

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Received May 21, 2004

