Influence of Side Chain Restriction and NH···π Interaction on the β-Turn Folding Modes of Dipeptides Incorporating Phenylalanine Cyclohexane Derivatives

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Abstract: We have synthesized the model dipeptides Piv-L-Pro-c_6Phe-NH^{i}Pr, incorporating each of the two *cis* cyclohexane analogues of phenylalanine: (*S*,*S*)- and (*R*,*R*)-1-amino-2-phenylcyclohexanecarboxylic acid. Their structural analysis has been carried out in solution by ^{1}H NMR and FTIR absorption spectroscopy and in the solid state by X-ray diffraction. In weakly polar chlorinated solvents, the (*S*,*S*)c_6Phe-containing dipeptide mainly accommodates a type I β-turn, whereas the (*R*,*R*) residue shows a greater propensity to βII-folding. This behavior does not differ significantly from that exhibited by the analogous dipeptides containing L- and D-Phe. However, the L-Pro-L-Phe sequence has been shown to undergo a βI-to-βII transition in the presence of a strong solvating medium, such as DMSO, or in the crystalline state. Interestingly, Piv-L-Pro-(*S*,*S*)c_6Phe-NH^{i}Pr, incorporating its cyclohexane analogue with χ^1 fixed at +60°, retains the βI-folded structure under these conditions. Theoretical calculations, supported by the experimental data, indicate that a c_6Phe-*NH* to aromatic π-orbitals interaction has an important influence on the observed β-folding preferences.

Small and medium peptides are generally highly flexible molecules that exist in solution in a dynamic equilibrium of interchanging conformations. As a consequence, most natural peptides with physiological activity cannot be used for therapeutic purposes, since the various conformers available may activate different receptors, thus leading to undesired side effects. The introduction of conformational constraints constitutes one of the most promising approaches to overcome this difficulty. In comparison with native peptides, restricted analogues usually display more favorable pharmacological properties, such as enhanced selectivity and increased metabolic stability. In addition, this strategy offers a valuable means to investigate the relationship between structure and function, the understanding of which is essential for the rational design of peptide-based drugs.

A major approach in the development of conformationally restricted peptides is to decrease the flexibility of the backbone.[1] The different methodologies applied to limit the allowed combinations of the ϕ,ψ torsion angles include short-, medium- and long-range cyclization[1b,c,2] (backbone to backbone, side chain to backbone, side chain to side chain), peptide bond modification,[1a,c,3] incorporation of specific amino acids,[1,4] and use of fragments of a nonpeptide nature that mimic secondary structure elements[1,5] (α-helices, β-sheets, turns).

Although recent years have seen enormous progress in the design of peptides with well-defined backbone conformations, insufficient attention has been paid to the geometry of the side chain moieties. Side chains are, however, essential in peptide-

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receptor recognition processes, to the extent that their interaction with the complementary receptor groups determines biological specificity. Their three-dimensional arrangement is therefore critical for bioactivity, and the elucidation of the precise spatial disposition required for binding to the receptor is of the utmost importance in establishing the so-called bioactive conformation.

The incorporation of side chain constrained amino acids constitutes a powerful tool to explore this important aspect of peptide structure.[6] Restrictions on the χ torsion angles will affect the conformational preferences of the side substituents and have an impact on the rotameric distribution with respect to unmodified residues. The equilibrium among the three low-energy $C^{\alpha}-C^{\beta}$ staggered conformations assumed by proteinogenic amino acids may be shifted to a particular rotamer. Moreover, dispositions not accessible to these residues may be allowed, preferred, or even fixed by adequately restricting the χ values. Thus, the incorporation of amino acids with well-defined χ tendencies into bioactive peptides may provide fundamental insights into the precise conformational requirements of the side chain groups to fit the receptor binding site.

Phenylalanine, in the same way as other aromatic residues, seems to play an important role in recognition processes and is often located in pharmacophoric regions.[7] In addition, a wide variety of constrained phenylalanine analogues with different degrees of control over the benzylic chain orientation has become synthetically available.[8] This amino acid therefore constitutes an appropriate candidate to investigate the structural and biological consequences arising from side chain restriction. The rotational freedom of the side chain can first be influenced by methylation at the α- or β-positions, which has been extensively studied in (αMe)Phe-[4d,9] and (βMe)Phe-containing[6,9f,10] peptides. The presence of more sterically demanding groups, as in (αEt)Phe,[11] (β^{i}Pr)Phe,[6,12] and Dip (diphenylalanine),[13] will further reduce the space available to the phenyl ring. In all these cases, rotation about $C^{\alpha}-C^{\beta}$ is limited by steric interactions between the vicinal substituents. Rigidity can be increased through the introduction of bridges of variable length between different parts of the molecule, and this type of modification has proven extremely useful in the case of Tic (1,2,3,4-tetrahydroisoquinoline carboxylic acid).[13b,d,14]

An attractive series of phenylalanine cyclic derivatives can be obtained by linking the α- and β-carbons through an alkylidene bridge. Rotation about $C^{\alpha}-C^{\beta}$ is then prohibited, the side chain orientation being dictated by both the bridge length (and subsequent ring size) and the stereochemistry at C^{α} and C^{β}. The smallest member of this family, in which an additional methylene unit closes a cyclopropane and which we denote as c_3Phe, has been incorporated into several peptide sequences.[13b,15,16] In this context, we have studied its behavior in the model dipeptides RCO-L-Pro-c_3Phe-NHMe[16] and observed an influence of c_3Phe chirality on the β-turn type accommodated in weakly polar solvents. Of special relevance is the finding that, among the two *cis* derivatives, (*S*,*S*)c_3Phe (analogous to L-Phe) preferentially induces a type I β-turn, whereas (*R*,*R*)c_3Phe (analogous to D-Phe) tends more to βII-folding. It should be noted that for both *cis* stereoisomers the rigid cyclopropane moiety confines the amino and phenyl groups to an eclipsed conformation ($\chi^1 \approx 0°$, Figure 1). On the basis of these observations we have proposed that an attractive interaction between the free c_3Phe-*NH* and the aromatic π-orbitals might play an important role on the observed β-turn tendencies. It appears that this stabilizing intramolecular interaction can be overcompensated by solvation or molecular aggregation, since in DMSO solution and in the solid state both dipeptides incorporating *cis*-c_3Phe accommodate the same βII-turn structure.

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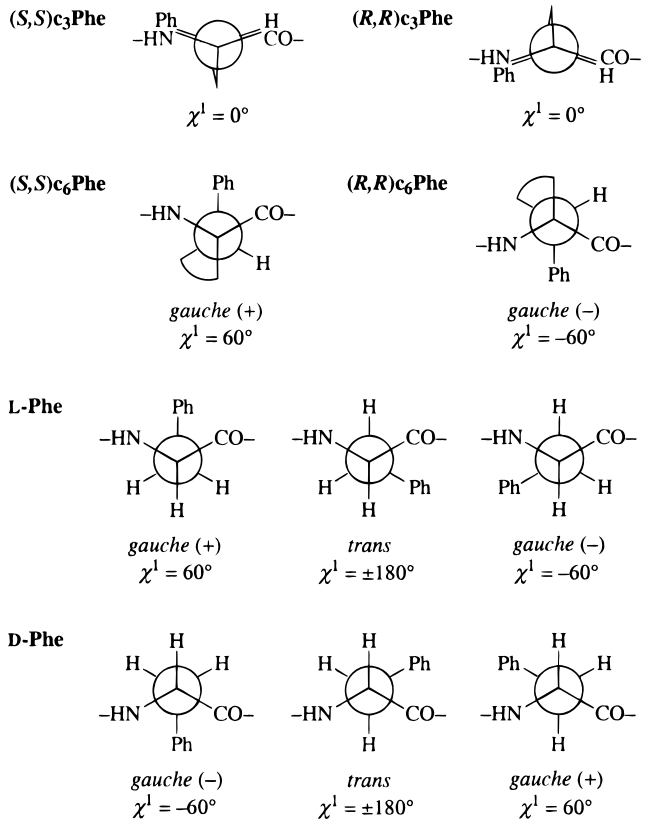

Figure 1. Newman projections of the two *cis* stereoisomers of c_3Phe and c_6Phe (assuming a chair conformation for the cyclohexane ring and an equatorial orientation for the phenyl substituent). Comparison with the three staggered $C^{\alpha}-C^{\beta}$ rotamers in L- and D-Phe.

To further investigate the existence of the aforementioned NH$\cdots\pi$ interaction and in an attempt to evaluate its influence on the β-folding mode, we undertook the study of the *cis* cyclohexane analogues of phenylalanine, which we denote as c_6Phe. Although, in principle, the cyclohexane ring containing C^{α} and C^{β} would be expected to exhibit a certain degree of flexibility, its conformational freedom is in fact reduced by the presence of the aromatic substituent. The six-membered cycle is likely to adopt a chair conformation and the bulky phenyl ring most probably accommodates an equatorial disposition. Assuming that this is the case, the side chain will be confined to be *gauche* (+) in (*S*,*S*)c_6Phe (analogous to L-Phe) and *gauche* (−) in the (*R*,*R*) residue (analogous to D-Phe). As depicted in Figure 1, the aromatic substituent is, in both cases, flanked by the amino and the carbonyl groups, and this disposition corresponds to the sterically most disfavored conformation among the three $C^{\alpha}-C^{\beta}$ staggered rotamers accessible to L- and D-Phe.

The present paper describes the synthesis and structure elucidation of the model dipeptides Piv-L-Pro-(*S*,*S*)c_6Phe-NH^{i}Pr (**1**) and Piv-L-Pro-(*R*,*R*)c_6Phe-NH^{i}Pr (**2**). Their conformational properties have been studied in the solid state by X-ray diffraction and in solution by ^{1}H NMR and FTIR absorption spectroscopy, and the results compared to those of the analogous dipeptides incorporating L- and D-Phe.[16a,17] With the aim of evaluating separately the effects of the selective orientation of the aromatic side chain and those arising from tetrasubstitution at C^{α}, we have carried out a parallel study on Piv-L-Pro-Ac_6c-NH^{i}Pr (**3**), which contains the unsubstituted 1-aminocyclohexanecarboxylic acid. The existence of an NH to phenyl ring interaction as a discriminating factor between the βI and βII-turns has also been investigated by theoretical calculations. Preliminary results have already been published.[18]

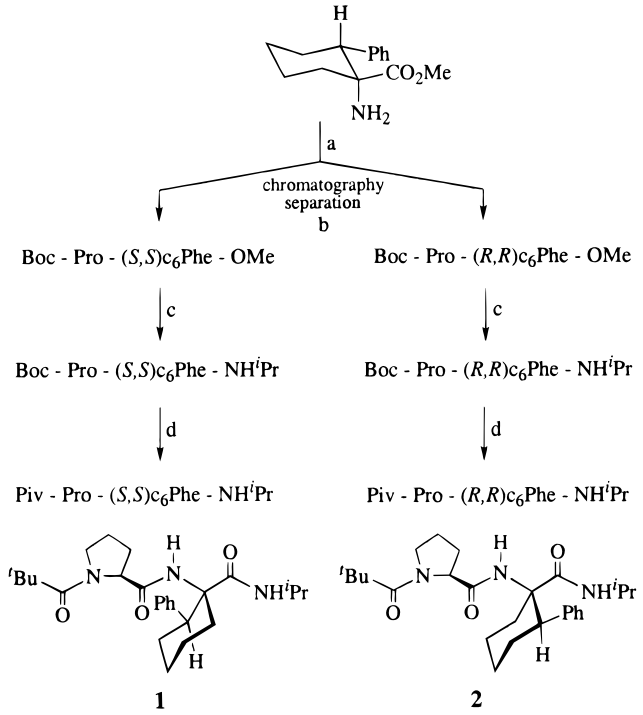

Figure 2. Synthesis of the two Piv-Pro-c_6Phe-NH^{i}Pr dipeptides **1** and **2** from racemic H-c_6Phe-OMe. Conditions: (a) Boc-L-Pro-OH/^{i}BuO-COCl/NMM/CH_2Cl_2, −15 °C 24 h; yield: 85%. (b) Eluent AcOEt/hexanes 1/1; R_f 0.67 (*S*,*S*), 0.50 (*R*,*R*). (c) ^{i}PrNH$_2$/AlMe$_3$/toluene, 0 °C 1 h, 50 °C 36 h; yield 48−50%. (d) 1: TFA/CH_2Cl_2 2/3, room temperature 2 h. 2: Piv_2O/Et_3N/DMAP/CH_2Cl_2, 0 °C 1 h, room temperature overnight; yield 83−85%.

Experimental Section

Synthesis. Figure 2 shows the synthetic route for dipeptides **1** and **2**. The preparation of *cis*-methyl 1-amino-2-phenylcyclohexanecarboxylate by Diels−Alder reaction of 1,3-butadiene and (*Z*)-2-phenyl-4-benzylidene-5(4*H*)-oxazolone has already been reported.[19] The racemic amino ester was classically coupled to *N*-*tert*-butyloxycarbonyl-L-proline by the mixed anhydride procedure using isobutyl chloroformate,[20] and the resulting diastereoisomeric dipeptides were separated by column chromatography on silica gel. Subsequent treatment with isopropylamine in the presence of $AlMe_3$ allowed the transformation of the methyl esters into the corresponding amides.[21] The Boc group was then cleaved by TFA and the amino group was acylated with pivalic anhydride to give enantiomerically pure **1** and **2**. Taking L-proline as a reference, the resolution of their crystal structures revealed the absolute configuration of the c_6Phe residue to be (2*S*,3*S*) in **1** and (2*R*,3*R*) in **2**. The Ac_6c-containing dipeptide **3** was obtained from commercially available 1-aminocyclohexanecarboxylic acid following standard procedures, using Boc as transitory protection and isobutyl chloroformate as the coupling agent. The use of the Piv group in **1**−**3** was preferred to Boc to avoid the *cis*−*trans* isomerization about the Boc-Pro tertiary urethane bond.[22] All intermediate derivatives gave satisfactory analytical

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and spectroscopic data. Elemental analyses were carried out on a Perkin-Elmer 200 C,H,N,S analyzer. Specific rotations were measured in a 10 cm cell at 25 °C using a Perkin-Elmer 241-C polarimeter. Melting points were determined on a Büchi SMP-20 or Büchi 510 apparatus and were not corrected. Solid-state IR spectra were registered on a Mattson Genesis FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX-300 apparatus using the solvent signal as internal standard. C_L^β/C_D^β and C_L^γ/C_D^γ denote the carbon atoms of the cyclohexane ring that occupy positions β and γ, respectively, in an L-/D-amino acid.

Piv-L-Pro-(2*S*,3*S*)c$_6$Phe-NH^{i}Pr (**1**): mp 155 °C. R_f (AcOEt/hexanes 1/1) = 0.22. $[\alpha]_D$ −111.7 (*c* 1.33, CH_2Cl_2). IR (Nujol) 3402, 3373, 3338, 1678, 1641, 1605 cm^{-1}. Anal. Calcd for $C_{26}H_{39}N_3O_3$: C, 70.71; H, 8.90; N, 9.52. Found: C, 70.59; H, 8.94; N, 9.56. ^{1}H NMR ($CDCl_3$, *c* 0.01 M, 300 MHz) δ 0.78 (d, 3H, J = 6.6 Hz, ^{i}Pr-CH_3); 1.04 (d, 3H, J = 6.6 Hz, ^{i}Pr-CH_3); 1.12 (s, 9H, Piv-(CH_3)$_3$); 0.80−2.20 (m, 11H, c$_6$Phe-$C_D^\beta H_{ax}$ + c$_6$Phe-$C_L^\gamma H_2$ + c$_6$Phe-$C_D^\gamma H_2$ + c$_6$Phe-C$^\delta H_2$ + Pro-C$^\beta H_2$ + Pro-C$^\gamma H_2$); 3.06 (bd, 1H, ^2J = −14.7 Hz, c$_6$Phe-$C_D^\beta H_{eq}$); 3.33 (dd, 1H, J = 12.9, 3.0 Hz, c$_6$Phe-$C_L^\beta H$); 3.68 (m, 1H, Pro-C$^\delta H$); 3.79 (m, 1H, Pro-C$^\delta H$); 3.89 (m, 1H, ^{i}Pr-C*H*); 4.36 (dd, 1H, J = 6.3 Hz, J = 5.7 Hz, Pro-C$^\alpha H$); 6.18 (bs, 1H, c$_6$Phe-N*H*); 6.20 (bd, 1H, J = 7.8 Hz, N*H*^{i}Pr); 7.15−7.30 (m, 5H, c$_6$Phe-C$_6H_5$). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.3, 22.1, 22.2, 25.7, 26.4, 26.7, 27.5, 27.7, 30.3, 39.1, 41.3, 48.1, 48.7, 64.5, 64.8, 127.1, 128.3, 128.6, 140.5, 171.1, 171.3, 176.8.

Piv-L-Pro-(2*R*,3*R*)c$_6$Phe-NH^{i}Pr (**2**): mp 192 °C. R_f (AcOEt/hexanes 1/1) = 0.29. $[\alpha]_D$ +8.0 (*c* 1.8, CH_2Cl_2). IR (Nujol) 3373, 3323, 1691, 1631, 1616 cm^{-1}. Anal. Calcd for $C_{26}H_{39}N_3O_3$: C, 70.71; H, 8.90; N, 9.52. Found: C, 70.80; H, 8.87; N, 9.49. ^{1}H NMR ($CDCl_3$, *c* 0.01 M, 300 MHz) δ 0.83 (d, 3H, J = 6.6 Hz, ^{i}Pr-CH_3); 0.98 (d, 3H, J = 6.6 Hz, ^{i}Pr-CH_3); 1.18 (s, 9H, Piv-(CH_3)$_3$); 1.10−2.25 (m, 11H, c$_6$Phe-$C_L^\beta H_{ax}$ + c$_6$Phe-$C_L^\gamma H_2$ + c$_6$Phe-$C_D^\gamma H_2$ + c$_6$Phe-C$^\delta H_2$ + Pro-C$^\beta H_2$ + Pro-C$^\gamma H_2$); 3.08 (bd, 1H, ^2J = −15.0 Hz, c$_6$Phe-$C_L^\beta H_{eq}$); 3.41 (dd, 1H, J = 13.2, 3.0 Hz, c$_6$Phe-$C_D^\beta H$); 3.71 (m, 2H, Pro-C$^\delta H_2$); 3.93 (m, 1H, ^{i}Pr-C*H*); 4.02 (dd, 1H, J = 7.5, 5.1 Hz, Pro-C$^\alpha H$); 5.57 (bs, 1H, c$_6$Phe-N*H*); 6.76 (bd, 1H, J = 7.8 Hz, N*H*^{i}Pr); 7.18−7.33 (m, 5H, c$_6$Phe-C$_6H_5$). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.4, 21.7, 22.4, 25.8, 26.3, 26.6, 27.2, 28.3, 30.2, 38.5, 41.2, 47.8, 48.6, 63.7, 64.9, 127.4, 128.1, 128.7, 140.1, 170.9, 172.4, 177.2.

Piv-L-Pro-Ac$_6$c-NH^{i}Pr (**3**): mp 240 °C. R_f (CH_2Cl_2/^{i}PrOH 9/1) = 0.50. $[\alpha]_D$ −14.3 (*c* 0.47, MeOH). IR (Nujol) 3351, 3297, 1691, 1639, 1604 cm^{-1}. Anal. Calcd for $C_{20}H_{35}N_3O_3$: C, 65.72; H, 9.65; N, 11.50. Found: C, 65.79; H, 9.67; N, 11.46. ^{1}H NMR ($CDCl_3$, *c* 0.01 M, 300 MHz) δ 1.06 (d, 3H, J = 6.6 Hz, ^{i}Pr-CH_3); 1.13 (d, 3H, J = 6.6 Hz, ^{i}Pr-CH_3); 1.26 (s, 9H, Piv-(CH_3)$_3$); 1.18−2.23 (m, 14H, Ac$_6$c-C$_6H_{10}$ + Pro-C$^\beta H_2$ + Pro-C$^\gamma H_2$); 3.73 (m, 2H, Pro-C$^\delta H_2$); 4.0 (m, 1H, ^{i}Pr-C*H*); 4.29 (dd, 1H, J = 6.8, 5.8 Hz, Pro-C$^\alpha H$); 5.96 (bs, 1H, Ac$_6$c-N*H*); 6.87 (bd, 1H, J = 7.8 Hz, N*H*^{i}Pr). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.3, 21.4, 22.2, 22.5, 25.1, 26.2, 27.3, 27.7, 30.5, 33.1, 38.9, 41.2, 48.7, 60.4, 63.2, 171.8, 173.1, 177.9.

X-ray Diffraction. Colorless single crystals of **1** (monohydrate) and **2** were obtained by slow evaporation from cyclohexane/diisopropyl ether solutions. The X-ray diffraction data were collected at room temperature on a Nonius CAD-4 four circle diffractometer, using graphite-monochromated Cu Kα radiation (λ = 1.54180 Å). The independent reflections were measured in the $\omega/2\theta$-scan mode and in the θ range 1−70°. Unit cell parameters were determined by least-squares refinement of the setting angles of 25 high-angle reflections ($20° < \theta < 30°$). The crystal structures were solved by direct methods using SIR92.[23] The E-maps revealed the whole molecules, except the hydrogen atoms, and the existence of two independent peptide and water molecules per asymmetric unit for **1** (molecules A and B). Structure refinement was performed using SHELXL-93.[24] Heavy atoms were affected by anisotropic thermal factors while hydrogen atoms were located by calculation and affected by an isotropic thermal factor of 5 Å^2. The NH hydrogens were replaced at a distance of 1.03 Å from nitrogen in the direction found by refinement.[25] The main crystallographic data, together with the residual *R* factors, are given in Table 1. Crystal data, fractional coordinates for the hydrogen and non-hydrogen atoms, equivalent thermal parameters and anisotropic temperature parameters for the non-hydrogen atoms, interatomic bond lengths and bond angles, and torsion angles have been deposited as Supporting Information.

Table 1. Crystal Data

	1	**2**
crystal system	triclinic	orthorhombic
space group	*P*1	$P22_12_1$
unit cell dimensions		
a, Å	10.165(2)	17.166(2)
b, Å	10.536(2)	23.613(3)
c, Å	12.664(1)	6.347(1)
α, deg	86.27(1)	
β, deg	78.62(1)	
γ, deg	87.52(1)	
Z	2	4
density$_{calcd}$, g·cm^{-3}	1.151	1.140
no. of reflcns collected	4688	2514
no. of independent reflcns	4688	2514
data/parameters	4688/618	2514/297
residual factors [$I > 2\,\sigma(I)$]	R_1 = 0.0392	R_1 = 0.0391
	wR_2 = 0.1117	wR_2 = 0.0869
goodness-of-fit	1.038	1.125

^{1}H NMR and IR Absorption Spectroscopy. Structural analysis of dipeptides **1**, **2**, and **3** in solution was performed by NMR and IR absorption techniques. IR spectra were run in the Fourier transform mode on a Bruker IFS-25 apparatus at a resolution of 2 cm^{-1}. A cell equipped with CaF_2 windows and with a path length fixed at 0.5 mm was used, and a total of 256 scans were averaged. The sample chamber was flushed continuously with dry air. Measurements were carried out at room temperature in CH_2Cl_2 and DMSO with peptide concentrations of 0.005 M. The absence of solute−solute interactions was confirmed by the fact that unmodified spectra were obtained after further dilution. Absorbance of samples was calculated by subtracting the pure solvent spectrum scanned under the same conditions, and the contribution of residual water was eliminated, if necessary, by correction in the 3500−3600 cm^{-1} region, where the peptide does not absorb. Attention was focused on the most informative frequency domains corresponding to the NH (3200−3500 cm^{-1}) and C′O (1550−1750 cm^{-1}) stretching vibrations. A free secondary amide group is expected to give an NH absorption at 3400−3450 cm^{-1} and a C′O band at 1650−1700 cm^{-1}. When involved in a hydrogen bond these absorptions are shifted to lower frequencies. The tertiary nature of the Pro-preceding amide group, along with the presence of a quaternary carbon adjacent to the carbonyl, results in a very low frequency for the free Piv-*C′O* (about 1620 cm^{-1} both in CH_2Cl_2 and DMSO). Thus, the pivaloyl group has a 2-fold advantage: it excludes the *cis* conformation of the Piv-Pro bond for steric reasons[22] and shifts the Piv-*C′O* frequency out of the usual region for peptide carbonyls, allowing easier interpretation of IR data. The NH and C′O absorption bands for dipeptides **1**, **2**, and **3** were assigned on the basis of previous studies on similar peptides,[16a,17] and of the perturbation induced by Boc/Piv and OMe/NH^{i}Pr exchange. Second-derivative and curve-decomposition of spectra were carried out to obtain the absorption maxima of overlapping bands.

^{1}H NMR spectra were acquired using a Bruker ARX-300 spectrometer with the solvent signal as the internal reference. Solutions of peptides (0.01 M) in $CDCl_3$ and DMSO-d_6 were used. Proton resonances were assigned by COSY and HOHAHA experiments. Nuclear Overhauser enhancement due to the proximity of Pro-C$^\alpha H$/Xaa-N*H* was estimated through 1D-NOESY difference experiments by irradiation at the Xaa-N*H* frequency. The solvent accessibility of the amide protons was investigated by considering their resonance shift in $CDCl_3$/DMSO-

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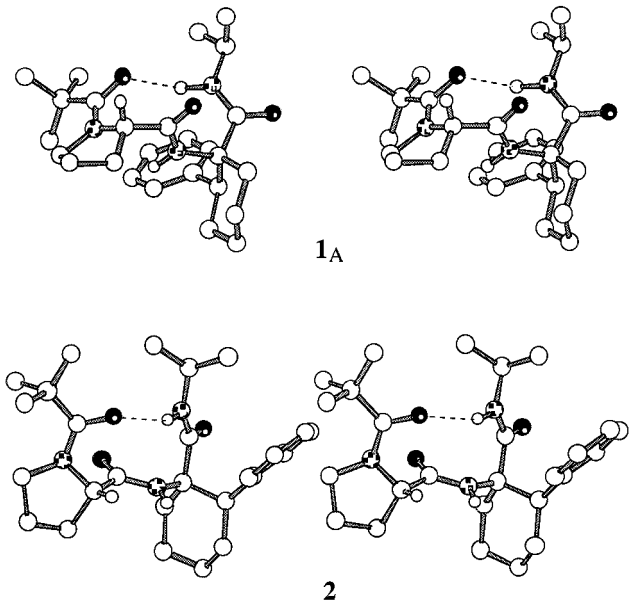

Figure 3. Stereoviews of the βI- and βII-folded crystal molecular structures of **1** (molecule A) and **2**, indicating the intramolecular hydrogen bond.

d_6 mixtures with increasing DMSO-d_6 content. The signal of a solvent-exposed N*H* is rapidly downfield shifted, whereas that of a solvent-protected proton (most probably by hydrogen bonding) is only weakly affected by DMSO-d_6 N*H*-solvation.[16a,17,26]

Theoretical Analysis. Molecular mechanics calculations on the c_6Phe residue, as well as on the Ac-Pro-Xaa-NHMe dipeptides, were performed using the AMBER 4.1 program[27] with the standard Cornell (parm94) force field set of parameters.[28] Computations were carried out *in vacuo*, using a dielectric constant of 1. Low-energy minima were characterized using the conjugate gradient algorithm of the SANDER module with a convergence criterium of 0.001 kcal/mol Å^{-1} on the energy gradient. *Ab initio* calculations on the c_6Phe residue were performed using the Gaussian94 suite of programs.[29] Hartree−Fock as well as second-order Møller−Plesset (MP2) calculations were carried out using the standard 6−31(d) basis set.[30] Energy decomposition analysis of the formamide−benzene interaction was performed using the constrained space orbital variation method[31] implemented in the HONDO8.5 program.[32] This procedure permits the partition of the different contributions to the SCF interaction energy by computing the frozen core−core contribution, a polarization term, and a charge-transfer term, which is corrected for the basis set superposition error (BSSE) with the counterpoise method.[33] In addition, the dispersion contribution to the interaction energy was computed as the difference between the Hartree−Fock and MP2 energies, once the BSSE had been corrected.

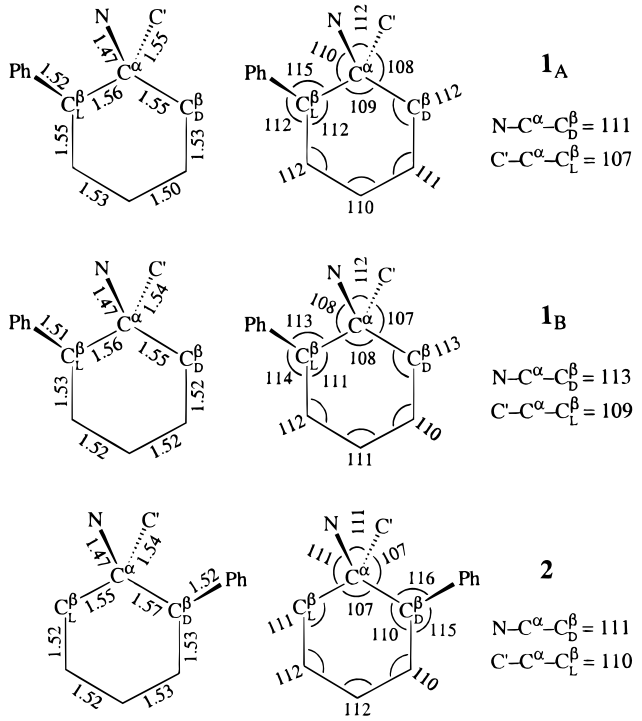

Figure 4. Bond distances (Å) and bond angles (deg) for the cyclohexane ring in the crystal molecular structures of **1** (molecules A and B) and **2**. C_L^β and C_D^β denote the carbon atom in the position corresponding to an L- and D-residue, respectively.

Table 2. Main Torsion Angles[a] (deg) with Standard Deviations for Peptides **1**[b] and **2**

fragment	angle	$\mathbf{1}_A$	$\mathbf{1}_B$	**2**
Piv	ω	−175.3(2)	−163.4(3)	−178.2(3)
Pro	ϕ[c]	−56.2(3)	−68.7(3)	−60.5(4)
	ψ[c]	−38.1(3)	−28.3(3)	144.2(2)
	ω	−175.8(2)	−179.3(2)	169.3(2)
	χ^1	−36.7(3)	22.5(4)	−18.8(4)
	χ^2	41.4(3)	−33.8(4)	33.5(4)
	χ^3	−30.6(3)	31.3(4)	−35.0(4)
	χ^4	7.6(3)	−16.8(3)	24.0(4)
	θ	18.3(3)	−3.1(3)	−3.3(4)
c_6Phe	ϕ[c]	−62.2(3)	−81.4(3)	65.8(3)
	ψ[c]	−17.4(3)	−5.2(3)	19.1(4)
	ω	−178.6(3)	−178.1(2)	174.3(3)
	χ^1[d]	60.3(3)	62.3(2)	−72.2(3)
	χ^2[d]	86.7(3)/−97.1(3)	84.4(3)/−96.0(3)	77.9(4)/−104.1(3)

[a] See ref 34 for definition. [b] Two independent molecules per asymmetric unit (molecules A and B). [c] Values for ideal β-turns are (ref 42) the following: $\phi_{i+1} = -60$ (βI, βII); $\psi_{i+1} = -30$ (βI), 120 (βII); $\phi_{i+2} = -90$ (βI), 80 (βII); $\psi_{i+2} = 0$ (βI, βII). [d] This angle refers to the orientation of the phenyl ring.

Results and Discussion

Crystal Structures. Figure 3 shows the X-ray diffraction structures of **1** (molecule A) and **2**. The dimensions of the cyclohexane ring in the c_6Phe residues are indicated in Figure 4. Table 2 summarizes the most relevant torsion angles.[34] The geometry of the intra- and intermolecular hydrogen bonds is given in Table 3. The ^{t}Bu methyl groups in both **1** and **2**, as well as the ^{i}Pr methyl groups and the water molecules in **1**, are thermally agitated. All amide bonds assume the usual *trans*

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Table 3. Dimensions (Å, deg) with Standard Deviations of the Hydrogen Bonds[a] for Peptides **1**[b] (Monohydrate) and **2**

peptide	donor−H	acceptor	symmetry code	donor···acceptor	H···acceptor	donor−H···acceptor
1[b]	*NH*(^{i}Pr)$_A$	Piv-*C′O*$_A$	*x*; *y*; *z*	3.039(3)	2.05(2)	163(3)
	NH(^{i}Pr)$_B$	Piv-*C′O*$_B$	*x*; *y*; *z*	3.098(4)	2.10(4)	165(3)
	W-*H*$_A$	Piv-*C′O*$_A$	*x*; *y*; *z*	2.966(4)	2.11(4)	144(3)
	W-*H′*$_A$	c$_6$Phe-*C′O*$_B$	*x*; *y*; *z*	2.833(5)	1.93(4)	151(4)
	W-*H*$_B$	W-*O*$_A$	*x*; 1 + *y*; *z*	2.875(6)	1.99(4)	146(3)
	W-*H′*$_B$	c$_6$Phe-*C′O*$_A$	*x*; *y*; *z*	2.828(6)	2.30(5)	113(3)
2	*NH*(^{i}Pr)	Piv-*C′O*	*x*; *y*; *z*	3.354(4)	2.43(3)	151(2)
	c$_6$Phe-*NH*	c$_6$Phe-*C′O*	*x*; *y*; −1 + *z*	3.058(3)	2.05(2)	168(3)

[a] The NH hydrogens have been replaced at 1.03 Å from N in the direction found by refinement.[25] [b] Two independent molecules per asymmetric unit. The subscript A or B refers to molecule A or B, respectively.

conformation,[35] with only Pro-ω in **2** (169°) and Piv-ω in **1**$_B$ (−163°) deviating significantly from planarity. The Piv-Pro amide link is forced to accommodate the *trans* disposition for steric reasons.[22] The proline ring adopts the twisted C$^\beta$-*endo*/C$^\gamma$-*exo* conformation in **1**$_A$, the envelope C$^\gamma$-*endo* conformation in **1**$_B$, and the envelope C$^\gamma$-*exo* conformation in **2**.[36]

The dimensions of the cyclohexane ring in the c$_6$Phe moieties are quite similar to those reported for the Ac$_6$c residue.[37] It should be noted, however, that the endocyclic C$^\beta_L$−C$^\alpha$−C$^\beta_D$ bond angle is smaller (107−109°), and the distance between C$^\alpha$ and the C$^\beta$ bearing the phenyl group increases with respect to the unsubstituted Ac$_6$c residue. These variations, together with the large exocyclic bond angles corresponding to phenyl branching (112−116°), must be ascribed to the steric interactions arising from β-substitution. In all three molecules the six-membered ring accommodates a chair conformation, with a mean absolute value for the endocyclic torsion angles of about 57°, which is slightly higher than that theoretically predicted for cyclohexane (54.7°).[38] As expected, the phenyl ring and the carbonyl group assume the equatorial disposition, while the amino substituent is oriented axially. It is noteworthy that in all crystal structures containing Ac$_6$c so far described, with the exception of the free amino acid,[39,40d] the amino group occupies the axial position.[37,40] Clearly, in the case of the c$_6$Phe derivatives, the presence of the aromatic substituent in a *trans* relative stereochemistry with respect to the carbonyl group will further stabilize this conformation. As a consequence, and according to our expectations, the rigidly held phenyl ring adopts a *gauche* (+) disposition in **1** ($\chi^1 = 60°$ in **1**$_A$ and 62° in **1**$_B$) and a slightly distorted *gauche* (−) orientation in **2** ($\chi^1 = -72°$), both corresponding to the least favored staggered rotamer in their respective Phe analogues[41] (Figure 1). As for χ^2, which is not restricted by covalent constraints, the values observed in all three cases lie close to ±90°. This χ^2-conformation, which is generally preferred by aromatic residues in peptides and proteins,[41] minimizes steric interactions between the phenyl ring and the backbone atoms. Another notable feature is the proximity between the c$_6$Phe amino and phenyl groups, with the distance between the N atom and the closest aromatic ring C atom (C$_{ipso}$) being 3.02 Å in **1**$_A$, 2.98 Å in **1**$_B$, and 3.19 Å in **2**.

All three molecules are stabilized by an intramolecular *NH*(^{i}Pr) to Piv-*C′O* hydrogen bond that closes a 10-membered pseudocycle typical of a β-turn.[42] The N···O distance of 3.35 Å in **2** is at the upper limit for hydrogen bonding[43] and is larger than the corresponding distance in **1** (3.04 and 3.10 Å). The orientation of the middle amide group, with the proline C′═O and C$^\alpha$−H bonds in the *syn* disposition for **1** and the *anti* disposition for **2**, corresponds to the type I and II β-turn,[42] respectively (Figure 3). It should be noted that, as far as the residue in position $i + 2$ is concerned, the fundamental difference between the βI- and βII-turns lies in the sign of the ϕ angle. Comparison of the torsion angles gathered in Table 2 shows that the βI-folded structures adopted by molecules A and B of dipeptide **1** differ mainly in the conformation of c$_6$Phe. Thus, **1**$_B$ exhibits c$_6$Phe-ϕ,ψ values close to those expected for an ideal type I β-turn, while the βI structure of molecule **1**$_A$ is distorted at the c$_6$Phe residue.

The molecular packing is completely different in the two crystals. The βII-folded molecules of **2** are intermolecularly hydrogen bonded by a c$_6$Phe-*NH* to c$_6$Phe-*C′O* interaction with a N···O distance of 3.06 Å. In **1** monohydrate, the molecules interact by W-*H* to *C′O* hydrogen bonds with two connected water molecules so that the water dyad is in contact with two molecules A and one molecule B. Contrary to the (*R*,*R*)c$_6$Phe-*NH* in **2**, the (*S*,*S*)c$_6$Phe-*NH* in **1** is not involved in any intermolecular interaction. This finding is in line with the known lower accessibility of the Xaa-*NH* site in βI-folded Pro-Xaa sequences.[17]

It is pertinent to mention that both Piv-L-Pro-L-Phe-NHMe[17] and Piv-L-Pro-D-Phe-NHMe[44] have been shown to accommodate a type II β-turn in the solid state, the aromatic side chain lying close to the *gauche* (−) ($\chi^1 = -42°$) and *gauche* (+) ($\chi^1 = 74°$) dispositions, respectively. With regard to Ac$_6$c, the two crystal structures containing the Pro-Ac$_6$c sequence described

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Table 4. Amide NH and C′O Stretching Frequencies[a] (cm^{-1}) for Piv-L-Pro-Xaa-NH^{i}Pr **1**−**3** in CH_2Cl_2 and DMSO Solution (c = 0.005 M) and Comparison with the Analogous Dipeptides Incorporating L- and D-Phe[b]

			Xaa-*NH*		*NH*(^{i}Pr)		Piv-*C′O*				Pro-*C′O*		Xaa-*C′O*
solvent	peptide	Xaa	bonded	free	free	bonded	free	βI-bonded	βII-bonded	γ-bonded	βI-free	βII-free	free
CH_2Cl_2	**1**	(*S*,*S*)c$_6$Phe	3310 vw	3381 m	3435 vw	3359 s		1614 s		1596 vw	1689 s		1648 s
	2	(*R*,*R*)c$_6$Phe		3404 m		3360 s		1613 w	1603 s		1686 w	1701 s	1646 s
	3	Ac$_6$c		3428 m		3362 s		1614 m	1603 m			1693 s	1655 s
		L-Phe		3413 m	3450 w[c]	3355 s[c]	1620 w	1612 s			1685 s		1666 s
		D-Phe		3419 m	3450 vw[c]	3345 s[c]		1614 w	1601 s			1692 s	1666 s
DMSO	**1**	(*S*,*S*)c$_6$Phe		3380 m		3350 s		1610 s			1687 s		1647 s
	2	(*R*,*R*)c$_6$Phe		3400 m		3365 s			1606 s			1689 s	1648 s
	3	Ac$_6$c		3271[d]		3357 s			1605 s			1687 s	1650 s
		L-Phe		3273[c,d]		3337 m[c]	1619 m		1604 m			1685 m	1667 s
		D-Phe		3260[c,d]		3328 s[c]	1619 w		1603 m			1684 s	1666 s

[a] Strong (s), medium (m), weak (w), and very weak (vw) absorption band. [b] See ref 16a. [c] The NH(^{i}Pr) group is replaced by NH(Me). [d] Broad absorption denoting a DMSO-solvated free NH.

Table 5. Chemical Shift and Solvent Sensitivity (ppm) of the Amide N*H* Proton Resonances for Piv-L-Pro-Xaa-NH^{i}Pr **1**−**3** (c = 0.01 M), Nuclear Overhauser Enhancement of the Pro-C$^\alpha$*H* Proton Resonance by Irradiation at the Xaa-N*H* Resonance, and Comparison with the Analogous Dipeptides Incorporating L- and D-Phe[a]

		Xaa-N*H*			N*H*(^{i}Pr)			nOe (%)	
peptide	Xaa	$CDCl_3$	DMSO-d_6	$\Delta\delta$[b]	$CDCl_3$	DMSO-d_6	$\Delta\delta$[b]	$CDCl_3$	DMSO-d_6
1	(*S*,*S*)c$_6$Phe	6.18	6.11	−0.07	6.20	6.55	0.35	4	16
2	(*R*,*R*)c$_6$Phe	5.57	6.74	1.17	6.76	6.44	−0.32	21	47
3	Ac$_6$c	5.96	7.82	1.86	6.87	7.13	0.26	26	55
	L-Phe			1.81			0.97[c]	8	*d*
	D-Phe			2.37			0.56[c]	27	*d*

[a] See ref 16a. [b] Shift of the N*H* proton resonance on going from $CDCl_3$ to DMSO-d_6. [c] The NH(^{i}Pr) group is replaced by NH(Me). [d] Not determined because of Pro-C$^\alpha$*H* and Phe-C$^\alpha$*H* resonance overlapping.

thus far[37b,40b] are also βII-folded. Not surprisingly, in all four cases the Xaa-*NH* sites (Xaa = L-Phe, D-Phe, Ac$_6$c) are intermolecularly hydrogen bonded to carbonyl groups of symmetry related molecules.

Structures in Solution. The conformational tendencies of **1**, **2**, and **3** were investigated by FTIR absorption and ^{1}H NMR in chlorinated solvents of low polarity (CH_2Cl_2, $CDCl_3$) and in the NH-solvating medium DMSO. Table 4 lists the amide stretching frequencies and Table 5 shows the NMR data. Results previously reported[16a] for Piv-L-Pro-L-Phe-NHMe and Piv-L-Pro-D-Phe-NHMe are included for comparison.

In CH_2Cl_2 solution, the *NH*(^{i}Pr) in **3** displays the typical behavior of a hydrogen-bonded site. It exhibits an intense and broad absorption near 3360 cm^{-1} and its proton resonance experiences a very small shift on going from $CDCl_3$ to DMSO-d_6. In comparison, the free Ac$_6$c-*NH* gives rise to a sharp band at 3428 cm^{-1} and its resonance shows a much higher sensitivity to solvent polarity. In the C═O stretching region, the Piv-*C′O* absorption is shifted to lower frequencies in comparison to that encountered at 1619 cm^{-1} for Piv-Pro-OMe, where it is free from any intramolecular interaction. It ensues that **3** is folded in a β-turn conformation stabilized by an *NH*(^{i}Pr) to Piv-*C′O* intramolecular hydrogen bond. The composite band observed for Piv-*C′O*, with contributions at 1614 and 1603 cm^{-1} of approximate relative intensity 45/55, denotes the occurrence of two different hydrogen-bonded states for this carbonyl and we have identified these as corresponding to the type I and II β-turn, respectively.[16a]

The compound incorporating (*R*,*R*)c$_6$Phe (**2**) also adopts a β-turn structure in CH_2Cl_2 solution, with a clear preference for the βII-disposition. The βI/βII ratio of about 20/80, according to the relative intensities of their respective Piv-*C′O* contributions, is in fact very similar to that encountered for Piv-L-Pro-D-Phe-NHMe.[16a]

The behavior of the (*S*,*S*)c$_6$Phe-containing dipeptide (**1**) in this solvent also parallels the preference of Piv-L-Pro-L-Phe-NHMe for the βI structure,[16a,17] and in both cases the presence of βII-folded molecules can be excluded since no absorption is discernible at about 1603 cm^{-1}. These two systems do, however, show important differences. A nonnegligible percentage of open conformation is present for the latter, as evidenced by the IR bands denoting free Piv-*C′O* and *NH*(Me) vibrators.[16a] The *NH*(^{i}Pr) in **1** also exhibits a minor contribution at high frequency, but the absence of any absorption near 1620 cm^{-1} excludes the existence of free Piv-*C′O* sites. The only possibility compatible with these observations is the occurrence of an (*S*,*S*)c$_6$Phe-*NH* to Piv-*C′O* γ-folded[45] conformer. The involvement of these vibrators in a hydrogen bond is in fact confirmed by the weak contributions at 3310 and 1596 cm^{-1}, which are also found in Piv-Pro-NHMe, where the only folded form can be γ-like. The γ-turn structure is known to be accommodated by some Pro-Xaa sequences in CCl_4 solution, and to disappear in the presence of more polar chlorinated solvents.[17,46]

As stated above, the βI and βII conformations predominantly adopted in CH_2Cl_2 by **1** and **2**, respectively, were attributed on the basis of the Piv-*C′O* stretching frequencies. Two other criteria allowed us to corroborate this assignment. We have proposed[16a,17] that the free Pro-*C′O* stretching frequency should be higher for the type II than for the type I β-turn, respectively translating the *syn* or *anti* disposition of the (i + 1) proline N−C$^\alpha$ and C′═O bonds (Figure 3). Dipeptide **1**, which exclusively adopts the type I β-turn, gives rise to a single Pro-*C′O* absorption at 1689 cm^{-1}, while **2**, which is preferentially βII-folded, presents a major contribution at 1701 cm^{-1} and a weak shoulder at 1686 cm^{-1}. Compound **3**, which exhibits high percentages of both βI- and βII-turns, presents two partially overlapping bands centered at 1693 cm^{-1}. The type I and II β-turns also differ in the Pro-C$^\alpha$*H*/Xaa-N*H* interproton distance, which is shorter for the latter (Figure 3) and should therefore

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be reflected by a stronger nOe effect. In line with the IR absorption results in CH_2Cl_2, the enhancement of the Pro-C$^{\alpha}$*H* signal in $CDCl_3$ solution upon irradiation of the c_6Phe-N*H* resonance proved significantly smaller for **1** than for **2**.

The behavior displayed by the c_6Phe-*NH* in **1** and **2** deserves particular comment. Apart from the minor γ-folded conformer detected for **1** and characterized by a weak absorption at 3310 cm^{-1}, c_6Phe-*NH* is not hydrogen bonded to any carbonyl group in the molecule. However, and contrary to what would be expected for a free NH group,[16a,17,26] the (*S*,*S*)c_6Phe-N*H* proton resonance is unaffected by addition of DMSO-d_6 and that of (*R*,*R*)c_6Phe-N*H* is actually downfield shifted, although to a lesser extent than is usually encountered for free NHs (2−3 ppm). This weak or extremely weak sensitivity to solvation must be interpreted in terms of solvent inaccessibility. Protection from solvent certainly arises from the close proximity to the phenyl ring, which also results in a very low stretching frequency in CH_2Cl_2 for the free c_6Phe-*NH* vibrator at 3381 and 3404 cm^{-1} in **1** and **2**, respectively. Thus, the shift by about 50 cm^{-1} in **1** and 25 cm^{-1} in **2** with reference to the absorption at 3428 cm^{-1} exhibited by Ac$_6$c-*NH* in **3** evidences the existence of an interaction between the free c_6Phe-*NH* and the aromatic ring.

Such an attractive interaction involving the Phe side chain and NH in Pro-Phe dipeptides, and being more intense for homochiral than for heterochiral sequences, has been invoked to explain their respective preferences for the βI- and βII-forms in low-polarity solvents.[17] This interaction decreases the NH frequency by about 15 cm^{-1} for L-Phe and 10 cm^{-1} for D-Phe with respect to that found near 3430 cm^{-1} for L-/D-Ala-*NH* in analogous dipeptides. Moreover, it would also account for the predominance of the Phe C$^{\alpha}$−C$^{\beta}$ *gauche* (+) and *gauche* (−) dispositions respectively observed in $CDCl_3$ for the L-Pro-L-Phe and L-Pro-D-Phe sequences,[17] despite being sterically disfavored orientations[41] (Figure 1). In this solvent the c_6Phe-C$^{\beta}$*H* benzylic proton exhibits one small and one large vicinal coupling constant (3.0 and 12.9 Hz for **1**, 3.0 and 13.2 Hz for **2**), which denote the axial orientation of the C$^{\beta}$−H bond and therefore the equatorial disposition of the aromatic substituent. Consequently, and as occurs in the crystal molecular structures, the cyclohexane moiety forces the phenyl ring into the *gauche* (+) disposition for **1** and the *gauche* (−) orientation for **2**, thus favoring the interaction between the c_6Phe-*NH* and the aromatic side chain.

In DMSO-d_6 solution, the phenyl substituent is also equatorial in both c_6Phe residues. In contrast, DMSO NH-solvation results in a modification of the side chain rotameric preferences in the Pro-Phe sequences,[17] along with the presence of high percentages of open conformers.[16a,17] In addition, the folded form in Piv-L-Pro-L-Phe-NHMe becomes of the βII-type.[16a]

In this solvent the *NH*(^{i}Pr) stretching frequency in **1**−**3** retains practically the same value as in CH_2Cl_2, meaning that this vibrator is still intramolecularly hydrogen bonded, a situation that is in line with the very weak solvent sensitivity of its proton resonance. For all three compounds, Piv-*C′O* gives rise to a single peak, denoting the sole existence of one-folded conformer. This corresponds to the βI-turn in **1** and the βII-turn in **2** and **3**, as evidenced by the magnitude of the Pro-C$^{\alpha}$*H*/Xaa-N*H* nOe. The presence of open forms can be ruled out, since no Piv-*C′O* contribution can be detected near 1620 cm^{-1}.

DMSO is known to interact with accessible NHs and, indeed, the Ac$_6$c-*NH* absorption is actually shifted from 3428 cm^{-1} in CH_2Cl_2 to 3271 cm^{-1}, while the c_6Phe-*NH* in **1** and **2** exhibits the same frequency as in CH_2Cl_2. This result provides evidence for the retention of the NH to π-orbitals interaction in DMSO solution and is in agreement with the moderate or extremely low sensitivity of the c_6Phe-N*H* proton resonance to solvation. The fact that in **2** this resonance is downfield shifted by 1.17 ppm on going from $CDCl_3$ to DMSO-d_6, while there is no IR indication for NH-solvation, suggests that the DMSO molecules are not in direct contact with the (*R*,*R*)c_6Phe-*NH* proton to form a hydrogen bond, but sufficiently close to perturb its magnetization state. It is worth mentioning that the proximity of the phenyl ring to the c_6Phe-*NH* for both **1** and **2** inferred from the observations discussed above constitutes the main argument in favor of the assignment of a type I β-turn structure for **1** and a βII disposition for **2** in DMSO solution.

Theoretical Analysis. The experimental data reveal that dipeptides **1** and **2** display a marked propensity for the βI- and βII-turn, respectively. The relative stability of these structures for each compound has been analyzed by theoretical calculations, and the possible influence of a c_6Phe-*NH* to aromatic ring attractive interaction on the observed β-turn tendencies has been examined.

Previously, we assessed the suitability of molecular mechanics calculations to describe the NH$\cdots\pi$ interaction, taking formamide−benzene as a model system. The ability of the π electron density of aromatic compounds to act as a Lewis base and thus interact with proton donors has long been noted,[47] and more recently has been recognized as an important factor in peptide and protein stability.[48] At the theoretical level, complexes between benzene and a variety of HX systems (X = F, Cl, OH, SH, NH_2, CN, CCl_3) have been characterized.[48d,g,49−51] In most cases, a symmetric structure with the HX molecule placed over the center of the phenyl ring has been suggested as the most favorable topology,[48d,g,49] but considerably off-centered geometries with H−X pointing toward the midpoint of a C−C bond have also been reported.[50] Nevertheless, in crystallized proteins the N−H groups are found to lie parallel to aromatic rings rather than adopt a perpendicular disposition.[48d−h]

The interaction curve between formamide and benzene was computed as described in the Experimental Section. Calculations were performed with the SCF-optimized geometries of both

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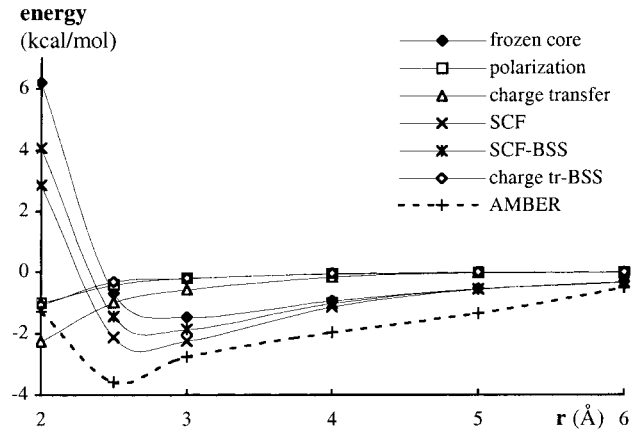

Figure 5. Energy profile and decomposition analysis of the formamide−benzene interaction (see Experimental Section for details). Comparison with the results of AMBER calculations.

Table 6. Geometrical Features of the Minima Obtained for Ac-(*S*,*S*)c_6Phe-NHMe by Molecular Mechanics and *ab Initio* Calculations (ϕ,ψ, deg; ΔE, kcal/mol)

conformation		AMBER			ab initio			
		ϕ	ψ	ΔE	ϕ	ψ	ΔE(SCF)	ΔE(MP2)
eq[a]	A	−56	8	0.0	−74	30	0.0	0.0
	B	47	−10	3.0	51	−1	6.2	5.4
	C	−59	167	5.9	−64	170	3.5	4.0
	D	−179	−179	8.1	180	180	5.9	5.9
ax[a]	A	−63	25	2.6	−76	70	2.9	3.0
	B	61	−10	3.1	74	−72	4.1	3.2

[a] Equatorial (eq) or axial (ax) disposition of the phenyl ring.

molecules. On keeping benzene fixed, two approaching paths for the formamide molecule were investigated: on the center and on a C−C bond of the benzene ring. Both pathways exhibit a low-energy conformation at a distance of about 2.7 Å with a well depth between 2.5 and 2.8 kcal/mol. When the system is completely relaxed the only minimum found at the SCF level shows the formamide molecule approximately on the center of the aromatic ring, with a distance from the closer formamide hydrogen to the plane of the aromatic ring of 2.68 Å. The interaction energy computed with this geometry at the MP2 level and corrected for the basis set superposition error is 2.94 kcal/mol. In an attempt to better characterize the nature of this interaction, the different contributions to the energy were assessed following the constrained space orbital variation method. These contributions are shown in Figure 5, and their analysis reveals the electrostatic term to be the larger. These results suggest that this interaction can be well accounted for in force field calculations. For the sake of comparison, the interaction energy of formamide with benzene computed with the Cornell force field of AMBER is also represented in Figure 5. Although the minimum differs by about 1 kcal/mol from that of the *ab initio* results, the plot clearly shows the ability of the force field calculations to account for this interaction.

Prior to the analysis of the β-turn preferences of the Pro-c_6Phe sequences, we investigated the conformational space available to the c_6Phe residue to confirm that the theoretical predictions agree with the experimental evidence. The Ramachandran maps of Ac-(*S*,*S*)c_6Phe-NHMe with the cyclohexane ring in a chair conformation and the phenyl substituent either in an equatorial or an axial disposition were computed within the molecular mechanics framework. Contour energy maps are shown in Figure 6. Minima, which were characterized by energy minimization, are depicted by letters, and their geometrical features are described in Table 6. With the aim of assessing the

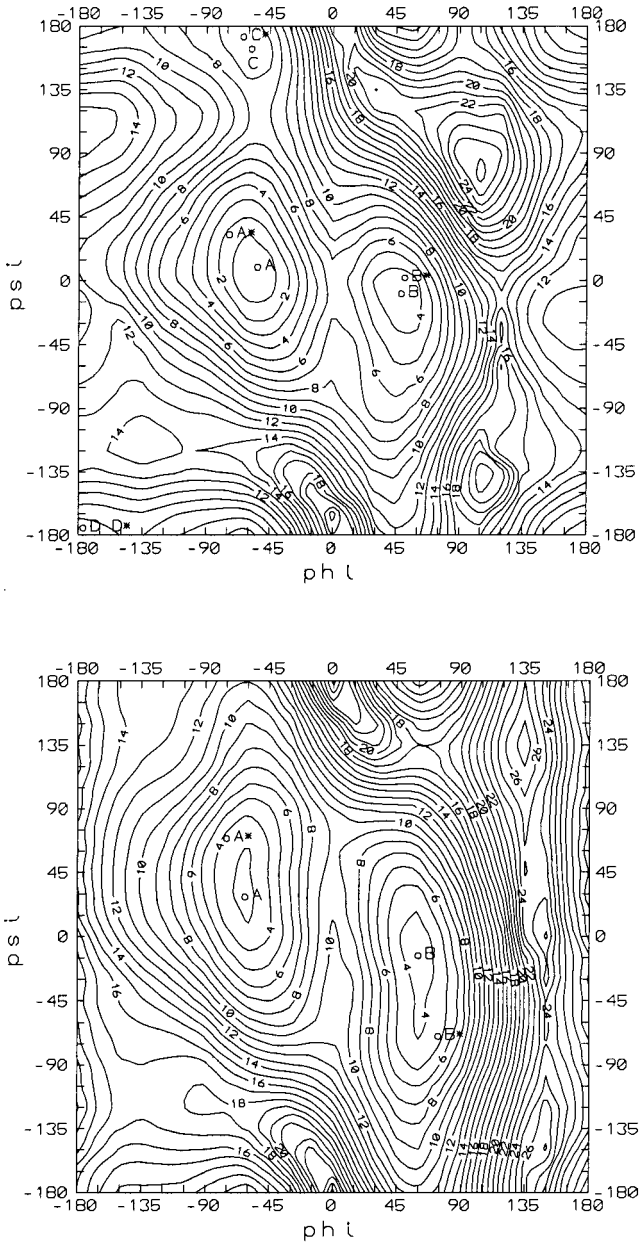

Figure 6. (ϕ,ψ) energy maps obtained by molecular mechanics calculations for Ac-(*S*,*S*)c_6Phe-NHMe with the cyclohexane ring in a chair conformation and the phenyl substituent in an equatorial (up) or an axial (down) disposition. Energy contours are drawn at intervals of 1.0 kcal/mol and minima are depicted by letters. Minima obtained from *ab initio* calculations are indicated with an asterisk.

Table 7. β-Turn Low-Energy Conformations of Ac-L-Pro-c_6Phe-NHMe (ϕ,ψ, deg; ΔE, kcal/mol)

c_6Phe configuration	Pro-ψ	c_6Phe-ϕ	c_6Phe-ψ	ΔE	structure
(*S*,*S*)	−27	−63	−10	−4.1	βI
	115	47	17	2.4	βII
(*R*,*R*)[a]	111	59	16	−2.2	βII

[a] The βI-turn structure is located in a flat region at about 2.0 kcal/mol but it does not exhibit a stationary point (the lowest energy corresponds to Pro-ψ = 7, c_6Phe-ϕ = −47, c_6Phe-ψ = 4).

performance of the force field parameters in the description of the c_6Phe residue, minima thus obtained were contrasted with results of *ab initio* calculations. The molecular mechanics low-energy structures were used as starting conformations for geometry optimization at the Hartree−Fock level. The results

Table 8. Geometrical Features of the Low-Energy Conformations of Ac-L-Pro-c_6Phe-NHMe Listed in Table 7[a] (d = distance, Å)

c_6Phe	structure	d[Ac-C′*O*···N*H*(Me)][b]	d[c_6Phe-N*H*···Ph][c]	d[Pro-C′*O*···Ph][d]	d[c_6Phe-C′*O*···Ph][d]
(*S*,*S*)	βI	1.99	**2.60**	5.11	3.45
	βII	2.08	3.98	**3.05**	**3.20**
(*R*,*R*)	βII	1.97	**2.64**	5.06	3.56

[a] Short contact distances are indicated in bold. [b] Length of the hydrogen bond closing the C10 cycle. [c] Distance from H to the center of the closest phenyl C—C bond. [d] Distance from O to the closest phenyl C atom.

of these calculations are also listed in Table 6 and minima obtained are shown in Figure 6 with an asterisk. Comparison of results indicates that molecular mechanics and *ab initio* geometries are within a 15° tolerance, with the exception of the ψ dihedral angle in the case of the axial conformer, due to the shallowness of the map in this region. Furthermore, the relative energies of conformations B and C of the equatorial conformer are interchanged in the molecular mechanics and *ab initio* calculations. Although MP2 calculations correct this difference to a certain extent, *ab initio* and molecular mechanics do not provide the same relative ordering of these two minima. Despite these differences, both methods clearly support that the conformation with the benzene ring in the equatorial position is more favorable, as experimentally established both in solution and in the solid state, and suggest an acceptable performance of force field calculations in describing the geometries of the conformations accessible to this residue. Obviously, the maps of Ac-(*R*,*R*)c_6Phe-NHMe exhibit the same features as those of Ac-(*S*,*S*)c_6Phe-NHMe since they are related by the correspondence $(\phi,\psi) = (-\phi,-\psi)$.

We then studied the conformational propensities of Ac-L-Pro-(*S*,*S*)c_6Phe-NHMe and Ac-L-Pro-(*R*,*R*)c_6Phe-NHMe using the set of molecular mechanics parameters assessed above and considering the phenyl substituent to be oriented equatorially. The terminal pivaloyl and isopropylamide groups in **1** and **2** were replaced by acetyl and methylamide for the sake of simplicity. Table 7 lists the low-energy conformations obtained. For the compound incorporating (*S*,*S*)c_6Phe both type I and II β-turns appear as minima, the former being more stable by 6.5 kcal/mol, whereas for the (*R*,*R*)c_6Phe-containing dipeptide only a βII-turn structure can be located as a low-energy minimum. Indeed, the βI-turn is situated on a flat section of the conformational energy map about 4.2 kcal/mol above the βII conformation, but it does not exhibit a stationary point. As a typical feature of β-turns, minima are characterized by an *NH*(Me) to Ac-*C′O* hydrogen bond. Inspection of the structural parameters of the different low-energy conformations (Table 8) suggests that the stability of the structures is modulated by the distance between the c_6Phe-*NH* and the phenyl moiety. Indeed, the two lowest energy conformations exhibit a c_6Phe-N*H* proton to aromatic ring distance of about 2.6 Å, which may result in an attractive NH···π interaction.

In accordance with the experimental results, theoretical calculations reveal the preference of the L-Pro-(*S*,*S*)c_6Phe and L-Pro-(*R*,*R*)c_6Phe sequences for the βI- and βII-turn, respectively. In an attempt to evaluate the contribution of the aromatic π cloud to c_6Phe-*NH* attraction to the stability of the lowest energy conformations obtained, we performed calculations on similar dipeptides where the possibility for this interaction had been eliminated. To this end, we replaced the phenyl ring of the c_6Phe residue by a cyclopentyl group. The cyclopentyl substituent was preferred to the cyclohexyl one because the former deviates from planarity to a lesser extent and was therefore considered to be more appropriate to reproduce the steric effects of the phenyl substituent. The low-energy conformations obtained are listed in Table 9. For the Ac-L-Pro-Xaa-NHMe dipeptide where Xaa is (*S*,*S*)-1-amino-2-cyclopentylcyclohexanecarboxylic acid, the βI structure is more stable than the βII-turn by 4.4 kcal/mol, while in the case of the (*R*,*R*) residue both structures are isoenergetic. Thus, elimination of the NH···π interaction leads to a decrease in the energy difference between type I and II β-turns for both (*S*,*S*) and (*R*,*R*) configurations, suggesting that the attractive interaction between the c_6Phe-*NH* and the benzene ring stabilizes the βI-turn in the L-Pro-(*S*,*S*)c_6Phe sequence and the βII-turn in L-Pro-(*R*,*R*)c_6Phe. In the case of the former, steric factors also seem to favor the βI disposition since this folded form remains preferred in the absence of the NH···π interaction.

Table 9. β-Turn Low-Energy Conformations of Ac-L-Pro-Xaa-NHMe, with Xaa = 1-Amino-2-cyclopentylcyclohexanecarboxylic Acid (ϕ,ψ, deg; ΔE, kcal/mol)

Xaa configuration	Pro-ψ	Xaa-ϕ	Xaa-ψ	ΔE	structure
(*S*,*S*)	−28	−63	2	1.0	βI
	115	50	12	5.4	βII
(*R*,*R*)	−25	−49	−14	3.5	βI
	108	57	7	3.2	βII

Table 10. Comparison of the Conformational Preferences of RCO-L-Pro-Xaa-NHR′ Dipeptide Sequences

	solution		
Xaa	CH_2Cl_2	DMSO	solid state
(*S*,*S*)c_6Phe[a]	βI ≫ γ	βI	βI
(*R*,*R*)c_6Phe[a]	βII ≫ βI	βII	βII
Ac_6c[a,b]	βII ≥ βI	βII	βII
L-Phe[c]	βI ≫ open	open > βII	βII
D-Phe[c,d]	βII ≫ βI	βII > open	βII
(*S*,*S*)c_3Phe[e]	βI ≥ βII	βII	βII
(*R*,*R*)c_3Phe[e]	βII ≫ βI	βII	βII

[a] This work. [b] See refs 37b and 40b. [c] See refs 16a and 17. [d] See ref 44. [e] See ref 16.

Conclusion

The conformational behavior of the Piv-L-Pro-c_6Phe-NH^{i}Pr dipeptides investigated in this work is summarized in Table 10, where it is compared to that of the analogous compounds incorporating Ac_6c, Phe, and c_3Phe. Experimental data provide evidence that the Pro-c_6Phe sequences exhibit a high tendency to β-folding in solution, even in DMSO. The high stability of the folded conformation in the presence of such a strong solvating medium is also typical of the Ac_6c- and c_3Phe-containing dipeptides and seems to be related to the C^α-tetrasubstituted character of these residues. In contrast, the unmodified Pro-Phe sequences exhibit high percentages of open conformers under the same conditions.

The β-folded modes adopted by the Pro-c_6Phe dipeptides are dictated by c_6Phe chirality and are little dependent on the solvent nature and on the solute or crystalline state. The (*S*,*S*)c_6Phe residue, analogous to L-Phe with χ^1 fixed at about 60°, mainly or exclusively occupies the $i + 2$ position of a type I β-turn, while (*R*,*R*)c_6Phe, analogous to D-Phe with χ^1 constrained near −60°, shows a marked preference for the βII structure. We have presented experimental and theoretical arguments showing that the influence of the side chain disposition on the β-turn type is

related to the fact that the aromatic π-orbitals are involved in an attractive interaction with the c_6Phe-*NH*.

In comparison, for the Pro-Ac_6c sequence, which does not have an aromatic substituent, both β-turn types I and II have practically the same occurrence in low-polarity solvents, and the latter becomes strongly favored in DMSO. This βI-to-βII transition characterizes as well the L-Pro-L-Phe dipeptide, which adopts a βI-turn in CH_2Cl_2 and a βII structure in DMSO and in the solid state, and results from the better accessibility of the middle Xaa-*NH* in the βII disposition. Solvation and molecular aggregation involving c_3Phe-*NH* are also able to compensate for the energy difference between type I and II β-turns in the case of the cyclopropane derivatives.

The retention of the βI-folded conformation of Piv-L-Pro-(*S*,*S*)c_6Phe-NH^{i}Pr in DMSO and in the crystalline state is therefore particularly noteworthy and evidences the value of the cyclohexane analogues of phenylalanine in the induction of specific secondary structures. To gain a deeper knowledge of the β-folding modulation by side chain orientation, further investigations on other χ^1-restricted surrogates of phenylalanine are in progress.

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Supporting Information Available: Crystal data, fractional coordinates for the hydrogen and non-hydrogen atoms, equivalent thermal parameters and anisotropic temperature parameters for the non-hydrogen atoms, interatomic bond lengths and bond angles, and torsion angles (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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