

14-Helical Folding in a Cyclobutane-Containing β -TetrapeptideSandra Izquierdo,[†] Marcelo J. Kogan,[‡] Teodor Parella,[§] Albertina G. Moglioni,^{†,||}
Vicenç Branchadell,[†] Ernest Giralt,^{†,⊥} and Rosa M. Ortuno^{*,†}Departament de Química, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain,
Institut de Recerca Biomèdica de Barcelona, Parc Científic de Barcelona, Josep Samitier, 1-5,
08028 Barcelona, Spain, Servei de Ressonància Magnètica Nuclear, Universitat Autònoma de Barcelona,
08193 Bellaterra, Barcelona, Spain, and Departament de Química Orgànica, Universitat de Barcelona,
Martí i Franquès 1-11, 08028-Barcelona, Spain

rosa.ortuno@uab.es

Received February 11, 2004

The efficient synthesis of tetrapeptide **5** containing, in alternation, cyclobutane and β -alanine residues is described. NMR experiments both at low temperature in CDCl_3 and at 298 K in $\text{DMSO-}d_6$ solutions show the contribution of a strong hydrogen bond in the folded major conformation of **5**. Temperature coefficients and diffusion times point out a hydrogen bond involving the NH proton from the cyclobutane residue **1** whereas NOEs manifest the high rigidity of the central fragment of the molecule and are compatible with a 14-membered macrocycle. Theoretical calculations predict a most stable folded conformation corresponding to a 14-helix stabilized by a hydrogen bond between NH_{10} in the first residue and OC_{25} in the third residue. This structure remains unaltered during the molecular dynamics simulation at 298 K in chloroform. All these results provide evidence for a 14-helical folding and reveal the ability of *cis*-2-aminocyclobutane carboxylic acid residues to promote folded conformations when incorporated into β -peptides.

Introduction

The secondary structure of peptides, i.e., helices, turns, and sheetlike conformations, are determinant factors of their biological properties both in α - and in β -amino acid derivatives.¹ Oligomers of β -amino acids (β -peptides) are more resistant to enzymatic hydrolysis and degradation than α -peptides, and this property is of interest for the construction of biomimetic polymers.² Recently, the relevant antimicrobial and hemolytic activity of amphiphilic β -oligomers has been shown, thus prompting the research in this field.³

The design of β -peptides for biological applications has therefore been encouraged. Besides the use of additional functional groups or bulky substituents, hetero⁴ or carbocyclic residues have been employed for trying to

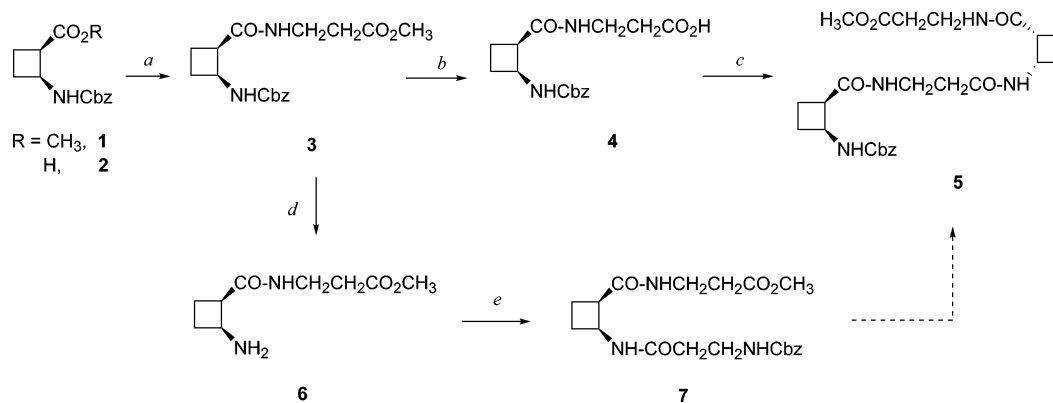
induce the folding of the β -peptidic chains. Foldamers that display specific conformations at short lengths, i.e., less than 10 residues, are especially interesting since they may have medicinal applications such as for disruption of specific protein–protein interactions. Fleet et al. have described left-handed helical structures stabilized by 10-membered hydrogen-bonded rings (10-helix) for oxetane β -amino acid hexamers.^{4e} β -Oligopeptides consisting of 2-aminocyclopentanecarboxylic acid (ACPC)⁵ and 2-aminocyclohexanecarboxylic acid (ACHC)⁶ have been prepared and investigated. Gellman described that oligomers composed of *trans*-ACPC residues adopt a 12-helix conformation,^{5a} whereas Fülöp showed that the pentamer

(3) (a) Werder, M.; Hausre, H.; Abele, S.; Seebach, D. *Helv. Chim. Acta* **1999**, *82*, 1774. (b) Hamuro, Y.; Schneider, J. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **1999**, *121*, 12300. (c) Porter, E. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *124*, 7324. (d) Raguse, T. L.; Porter, E. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *124*, 12774.

(4) (a) Haque, T. S.; Little, J. C.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 6975. (b) Huck, B. R.; Langenhan, J. M.; Gellman, S. H. *Org. Lett.* **1999**, *1*, 1717. (c) Chung, Y. J.; Huck, B. R.; Christianson, L.; Stanger, H. E.; Krauthäuser, S.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **2000**, *122*, 3995. (d) Fisk, J. D.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **2000**, *122*, 5443. (e) Claridge, T. D. W.; Goodman, J. M.; Moreno, A.; Angus, D.; Barker, S. F.; Taillefumier, C.; Watterson, M. P.; Fleet, G. W. J. *Tetrahedron Lett.* **2001**, *42*, 4251. (f) Porter, E. A.; Wang, X.; Schmit, M.; Gellman, S. H. *Org. Lett.* **2002**, *4*, 3317. (g) Langenhan, J. M.; Guzei, I. A.; Gellman, S. H. *Angew. Chem., Int. Ed.* **2003**, *42*, 2402.

(5) (a) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Richards, M. R.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 7574. (b) LePlae, P. R.; Fisk, J. D.; Porter, E. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *124*, 6820. (c) Woll, M. G.; Fisk, J. D.; LePlae, P. R.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *124*, 12447. (d) Park, J.-S.; Lee, H.-S.; Lai, J. R.; Kim, B. M.; Gellman, S. H. *J. Am. Chem. Soc.* **2003**, *125*, 8539. (e) Martinek, T. A.; Tóth, G. K.; Vass, E.; Hollósi, M.; Fülöp, F. *Angew. Chem., Int. Ed.* **2002**, *41*, 1718.

[†] Departament de Química, Universitat Autònoma de Barcelona.[‡] Institut de Recerca Biomèdica de Barcelona.[§] Servei de Ressonància Magnètica Nuclear, Universitat Autònoma de Barcelona.^{||} Present address: Departamento de Química Orgànica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina.[⊥] Departament de Química Orgànica, Universitat de Barcelona.(1) For reviews on the β -peptide secondary structure, see: (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173. (b) DeGrado, W. F.; Schneider, J. P.; Hamuro, Y. *J. Peptide Res.* **1999**, *54*, 206. (c) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219. (d) Martinek, T. A.; Fülöp, F. *Eur. J. Biochem.* **2003**, *270*, 3657.(2) (a) Seebach, D.; Overhand, M.; Kühnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 913. (b) Hintermann, T.; Seebach, D. *Synlett* **1997**, 437. (c) Seebach, D.; Abele, S.; Schreiber, J. V.; Martinoni, B.; Nussbaum, A. K.; Schild, H.; Schulz, H.; Henneke, H.; Woessner, R.; Bitsch, F. *Chimia* **1998**, *52*, 734. (d) Rueping, M.; Mahajan, Y.; Sauer, M.; Seebach, D. *ChemBioChem* **2002**, *3*, 257. (e) Schreiber, J. V.; Frackennpohl, J.; Moser, F.; Fleischmann, T.; Kohler, H.-P. E. Seebach, D. *ChemBioChem* **2002**, *3*, 424.

SCHEME 1^a

^a Reagents and conditions: (a) $\text{HCl}\cdot\text{H}_2\text{N}(\text{CH}_2)_2\text{CO}_2\text{Me}$, TEA, HOBt, DEC, DMF, rt, 20 h, 86%; (b) 0.25 M NaOH, 1:10 THF–H₂O, 0 °C, 1 h, 100%; (c) **6**, HOBt, DEC, TEA, DMF, rt, 72 h, 60%; (d) H₂, Pd/C, MeOH, 2 atm, 94%; (e) CbzNH(CH₂)₂CO₂H, HOBt, DEC, DMF, rt, 20 h, 34%.

and heptamer of *cis*-ACPC adopt a self-stabilizing six-strand secondary structure in solution.^{5e} The tetramer of *trans*-ACHC displayed a 14-helix secondary structure both in the solid state^{6b} and in methanol solution.^{6c} Short-lengthed amphiphilic β -peptides prepared by DeGrado et al. have also shown a 14-helix disposition, this conformation being essential for their antibacterial activity.^{3b}

In addition to these results, there are no reports in the literature on the synthesis and structural study of cyclobutane containing β -oligomers in addition to our works.^{7,8} Moreover, although theoretical calculations on the structure of β -peptide models have been reported,⁹ to the best of our knowledge, theoretical studies on β -peptides containing cyclobutane residues have not yet been reported.

According to our research program on the synthesis and structural study of conformationally constrained amino acids and related peptidomimetics,¹⁰ we have synthesized the *cis*-cyclobutane β -amino acid derivative **1**⁷ and its enantiomer (Scheme 1).⁸ We then envisioned the synthesis of cyclobutane β -peptides to test the ability of the cyclobutane unit to constrain conformationally the oligomers derived inducing molecular foldings. Thus, we prepared the dipeptide **3** (Scheme 1) from the amino acid **2** and β -Ala-OMe. X-ray diffraction analysis showed a

hairpin-like disposition of the molecule in the crystal packing, favored by the formation of intermolecular hydrogen bonds.^{7b} This first result was encouraging since it was already known the low propensity of the β -alanine residue for bend formation in the crystal state¹¹ and the formation of a disordered structure without extensive intramolecular hydrogen bonding in aqueous solution.¹² Nevertheless, NMR studies in several solvents did not suggest either the existence of inter- or intramolecular hydrogen bonds or a conformational bias in solution for dipeptide **3**, even though this result was not surprising taking into account its small size.

We decided, therefore, to prepare and study a β -oligomer with extended length. Thus, in this paper, we describe the efficient synthesis of tetrapeptide **5** and its conformational analysis on the basis of NMR experiments and molecular modeling calculations. These studies provide evidence for a 14-helical folding of this molecule.

Results and Discussion

Synthesis of β -Tetrapeptide 5. Two synthetic ways were explored to prepare **5** (Scheme 1). The route involving a linear sequence passes through tripeptide **4**. Thus, benzyl carbamate in **3** was hydrogenolyzed in the presence of palladium on charcoal to afford amine **6**. This compound was coupled with Cbz- β -Ala by treatment with excess 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (DEC) as dehydrating agent and 1 equiv of 1-hydroxybenzotriazole (HOBt) as a catalyst in anhydrous DMF solution at room temperature for 20 h giving **7**, in 34% yield, as a pasty solid difficult to purify. This poor result

(1) (a) Appella, D. H.; Barchi, J. J., Jr.; Durell, S. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 2309. (b) Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 6206. (c) Barchi, J. J., Jr.; Huang, X.; Appella, D. H.; Christianson, L. A.; Durell, S. R.; Gellman, S. H. *J. Am. Chem. Soc.* **2000**, *122*, 2711. (d) Appella, D. H.; LePlae, P. R.; Raguse, T. L.; Gellman, S. H. *J. Am. Chem. Soc.* **2000**, *122*, 4766. (e) Raguse, T. L.; Porter, E. A.; Weisblau, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *124*, 12774. (f) Raguse, T. L.; Lai, J. R.; Gellman, S. H. *J. Am. Chem. Soc.* **2003**, *125*, 5592.

(7) (a) Martín-Vilà, M.; Minguillón, C.; Ortuño, R. M. *Tetrahedron: Asymmetry* **1998**, *9*, 4291. (b) Martín-Vilà, M.; Muray, E.; Aguado, G. P.; Álvarez-Larena, A.; Branchadell, V.; Minguillón, C.; Giralt, E.; Ortuño, R. M. *Tetrahedron: Asymmetry* **2000**, *11*, 3569.

(8) Izquierdo, S.; Martín-Vilà, M.; Moglioni, A. G.; Branchadell, V.; Ortuño, R. M. *Tetrahedron: Asymmetry* **2002**, *13*, 2403.

(9) (a) Bode, K. A.; Applequist, J. *Macromolecules* **1997**, *30*, 2144. (b) Wu, Y.-D.; Wang, D.-P. *J. Am. Chem. Soc.* **1998**, *120*, 13485. (c) Möhle, K.; Gunther, R.; Thormann, M.; Sewald, N.; Hofmann, H. *J. Biopolymers* **1999**, *50*, 167. (d) Christianson, L. A.; Lucero, M. J.; Appella, D. H.; Klein, D. A.; Gellman, S. H. *J. Comput. Chem.* **2000**, *21*, 763. (e) Daura, X.; Gademann, K.; Schäfer, H.; Jaun, B.; Seebach, D.; van Gunsteren, W. F. *J. Am. Chem. Soc.* **2001**, *123*, 2393. (f) Lin, J.-Q.; Luo, S.-W.; Wu, Y.-D. *J. Comput. Chem.* **2002**, *23*, 1551. (g) Beke, T.; Csizmedia, I. G.; Perczel, A. *J. Comput. Chem.* **2004**, *25*, 285.

(10) For some selected recent works, see: (a) Martín-Vilà, M.; Hanafi, N.; Jiménez, J. M.; Álvarez-Larena, A.; Piniella, J. F.; Branchadell, V.; Oliva, A.; Ortuño, R. M. *J. Org. Chem.* **1998**, *63*, 3581. (b) Rifé, J.; Ortuño, R. M.; Lajoie, G. *J. Org. Chem.* **1999**, *64*, 8958. (c) Moglioni, A. G.; García-Expósito, E.; Aguado, G. P.; Parella, T.; Moltrasio, G. Y.; Branchadell, V.; Ortuño, R. M. *J. Org. Chem.* **2000**, *65*, 3934. (d) Illescas, B.; Rifé, J.; Ortuño, R. M.; Martín, N. *J. Org. Chem.* **2000**, *65*, 6246. (e) Moglioni, A. G.; Muray, E.; Castillo, J. A.; Álvarez-Larena, A.; Moltrasio, G. Y.; Branchadell, V.; Ortuño, R. M. *J. Org. Chem.* **2002**, *67*, 2402. (f) Aguado, G. P.; Moglioni, A. G.; Ortuño, R. M. *Tetrahedron: Asymmetry* **2003**, *14*, 217.

(11) Pavone, V.; Di Blasio, B.; Lombardi, A.; Isernia, C.; Pedone, C.; Benedetti, E.; Valle, G.; Crisma, M.; Toniolo, C.; Kishore, R. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1233.

(12) Glickson, J. D.; Applequist, J. *J. Am. Chem. Soc.* **1971**, *93*, 3276.

TABLE 1. Chemical Shifts, Coupling Constants, NOEs, and Temperature Coefficients for the NH Protons of the Predominant Conformer of **5**

NH ^a	δ^b (ppm)	J (Hz)	NOE ^c	$\Delta\delta/\Delta T^d$ (ppb/K)
H ₁₀	6.11 (7.20)	9.0	H _{13a} (w), H _{12a} (s), H _{12b} (m)	-2.2
H ₁₆	6.88 (7.65)	6.0	H _{18a,b} (m), H _{17a,b} (s), H ₁₄ (s)	-3.7
H ₂₀	7.18 (7.94)	8.2	H _{22b} (s), H ₂₁ (m), H _{18b} (s), H _{17a,b} (w)	-3.5
H ₂₆	6.40 (7.73)	6.0	H _{28a} (m), H _{27a,b} (s), H ₂₄ (s)	-3.4

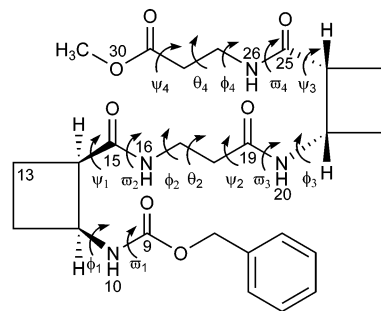
^a See Figure 1 for atom numeration. ^b In CDCl₃ at 260 K and, in parentheses, in DMSO-*d*₆ at 298 K. ^c In CDCl₃ at 260 K and in DMSO-*d*₆ at 298 K. w: weak. m: medium. s: strong. ^d In CDCl₃.

led us to investigate a shorter convergent route to synthesize **5**. With this purpose, the methyl ester in **3** was hydrolyzed by using 0.25 M NaOH in a 1:10 THF–water solution to produce acid **4** without epimerization. This compound was condensed with amine **6** under similar conditions than above to afford the tetrapeptide **5** in 60% yield, which was purified by crystallization from ethyl acetate. Crystals were not suitable for X-ray diffraction analysis.

NMR Studies. Complete ¹H and ¹³C resonance assignments were performed using conventional 2D techniques (COSY, HSQC, and HMBC experiments). Moreover, other NMR experiments (variable-temperature 2D NOESY/2D ROESY and measurement of temperature coefficients and apparent self-diffusion coefficients) were performed on **5** in order to state its conformational bias in solution. Two main conformations were observed in its ¹H NMR spectrum. However, variable-temperature NMR experiments in the 230–300 K were needed because of the large line broadening observed due to the strong chemical exchange cross-peaks detected in NOESY spectra at 270–300 K temperature. For the predominant conformation (>95%), the NOE data observed at low temperature (Table 1) suggest hydrogen-bond interactions between the amide protons and carbonyl oxygen atoms. The large proton–proton coupling constants (9 Hz) and the absence of NOE for NH₁₀–H₁₁ and NH₂₀–H₂₁ are in agreement with a spatial anti disposition of the NH and the vicinal cyclobutane CH protons (see Figure 1 for atom numeration). Otherwise, the high chemical shift anisotropy observed for the methylene protons at C₁₇ and C₁₈ (β -alanine residue) accounts for a remarkable rigidity of the C₁₅–N₂₀ segment of the molecule. In contrast, the other β -alanine residue (MeO–C₂₉–C₂₈–C₂₇ fragment) must be rather flexible according to the chemical shifts of the methylene protons (see the Experimental Section).

The temperature coefficients in CDCl₃ solution suggest a stronger intramolecular hydrogen bond for NH₁₀ ($[\Delta\delta/\Delta T] = 2.2$ ppb/K) with respect to the other three NH protons (3.4–3.7 ppb/K).¹³

Chemical-exchange properties of exchangeable NH or hydroxyl spin systems have been also studied using diffusion-based NMR experiments.¹⁴ It has been demon-

**FIGURE 1.** Structure of tetrapeptide **5** where atom numeration and torsional angles are defined.

strated that the observed apparent diffusion behavior of the NH resonances reflects the relative lifetimes of these protons in the peptide and the water environments¹⁵ and, therefore, provides a rapid and useful method for studying NH exchange rates and detect qualitative information about the different exchange sites of the molecule such as hydrogen bond strength and solvent accessibility properties.

In the absence of exchange, all proton resonances belonging to the same molecule present the same diffusion behavior. However, in the presence of exchange, the NHs involved in exchange appear to have an intermediate character, since their apparent diffusion behavior lie between those of the large peptide and the small residual water molecules. The different decays observed for all NH resonances of **5** in 1D pulsed field gradient spin–echo (PFGSE) experiments account for their exchange properties. A series of diffusion experiments recorded with different diffusion times confirm the faster decay for all NH protons except for NH₁₀. In addition, the NH₁₀ proton decays in a similar way as observed for nonexchangeable protons evidencing the stronger hydrogen bonding properties of this proton in close analogy with the measured temperature coefficients (see the Supporting Information).

The minor conformation (less than 5%) was difficult to study due to its low contribution. The major differential feature of its ¹H NMR spectrum with respect to the major conformer is the large upfield shift (+0.9 ppm) of the NH₂₆ proton whereas the other NH protons show a slight downfield shift about -0.2 to -0.5 ppm and a stronger hydrogen bond for NH₁₀ based on the temperature coefficients. This second conformation could be related to the cis–trans isomerism of the amide bonds.

In DMSO-*d*₆ solution, results were similar to those in CDCl₃ although the molecule seems to be slightly more flexible according to the chemical shifts for the protons in the central β -alanine fragment (see the Supporting Information). Otherwise, the observed significant NOEs are the same than in CDCl₃ (Table 1), and diffusion and NOESY experiments at 298 K show that NH₁₀ is involved in a hydrogen bond stronger than the hydrogen bonds with the other amide protons.

Conformational Search. To determine the conformational space available to tetrapeptide **5**, we performed

(13) References for temperature coefficients in peptides and proteins: (a) Rothermund, S.; Weibhoff, H.; Beyermann, M.; Krause, E.; Bienert, M.; Muge, C.; Sykes, B. D.; Sonnichsen, F. D. *J. Biomol. NMR* **1996**, *8*, 93. (b) Baxter, N.-J.; Williamson, M. P. *J. Biomol. NMR* **1997**, *9*, 359. (c) Cierpicki, T.; Otlewski, J. *J. Biomol. NMR* **2001**, *21*, 249. (d) Cierpicki, T.; Zhukov, I.; Byrd, R. A.; Otlewski, J. *J. Biomol. NMR* **2002**, *157*, 178.

(14) (a) Cabrita, E. J.; Berger, S.; Bräuer, P.; Kärgler, J. *J. Magn. Reson.* **2002**, *157*, 124. (b) Cabrita E. J.; Berger, S. *Magn. Reson. Chem.* **2002**, *40*, S122.

(15) Liu, M.; Toms, H. C.; Hawkes, G. E.; Nicholson, J. K.; Lindon, J. C. *J. Biomol. NMR* **1999**, *13*, 25.

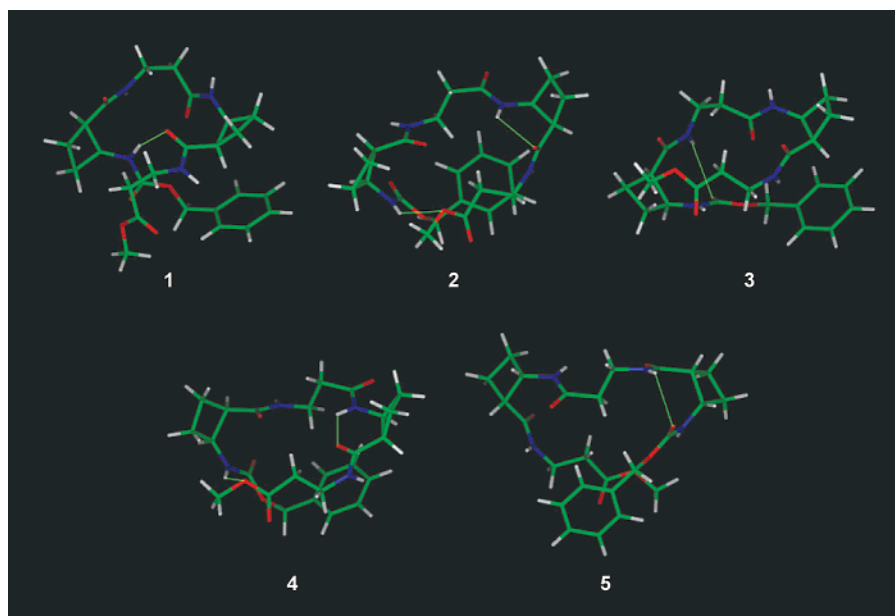


FIGURE 2. Stick conformational representation of minimum energy conformers of tetrapeptide **5** obtained after the ISA conformational search.

TABLE 2. Distances^a Between a Selection of NH and OC Atom Pairs for the Energy Minima Conformers (ISA) of Tetrapeptide **5**

family ^b	E^c	NH ₁₀ ⋯OC ₂₅	NH ₁₀ ⋯O ₃₀ C	NH ₂₀ ⋯OC ₂₅	NH ₁₆ ⋯OC ₉
5 ₁	0.00	2.3	4.0	5.7	4.9
5 ₂	0.70	7.1	2.6	2.7	2.9
5 ₃	0.97	5.7	3.8	3.5	2.7
5 ₄	0.99	7.0	2.6	2.7	3.0
5 ₅	1.00	7.2	2.9	3.4	2.7

^aIn angstroms. ^bSee Figure 2. ^cRelative energies in kcal mol⁻¹.

molecular dynamics with iterative simulated annealing¹⁶ (ISA) calculations. For the ISA a heating and cooling protocol was run five times, employing fully extended starting structures of **5** and CFF91 (implemented in DISCOVER 3) as force field, which was parametrized for cyclobutane derivatives and also for proteins.¹⁷

Structures corresponding to the energy minima (within a 2 kcal/mol window above the global minimum) were analyzed on the basis of the relevant geometric parameters, which are diagnostics of folding of the structure. A detailed study of the conformers obtained in the ISA search allows us to classify the minima energy conformers into five families (**5**₁–**5**₅) (Figure 2, Tables 2 and 3). The absolute minimum **5**₁ is clearly more stable (0.7–1 kcal/mol) than the other four conformers **5**_{2–5}. Tables 2 and 3 present the relative energies and values of selected geometric parameters for all these structures. Conformer **5**₁ presents an intramolecular hydrogen bond between NH₁₀ and OC₂₅ which could be indicative of the presence of a 14-helical structure. The participation of NH₁₀ in a hydrogen bond is in agreement with the NMR results (temperature coefficients and diffusion experiments). On the other hand, in conformers **5**_{2–5} weak hydrogen bonds

are present (Table 2, the distances between NH₁₀⋯O₃₀C, NH₂₀⋯OC₂₅, NH₁₆⋯OC₉ are lower than 2.8 Å but higher than 2.5 Å).

The values determined for ω torsion angles suggest a predominant trans disposition for all the amide bonds in **5**_{1–5} (Table 3). We have also analyzed the torsion angles belonging to the N₁₆–C₁₉ central β -alanine and to the N₂₆–C₂₉ terminal β -alanine (Table 3). Conformer **5**₁ adopts a folded conformation in which the distance between the two cyclobutane rings is around 7 Å, while this distance would be 11 Å in an extended hypothetical structure. The value of the torsion angle θ_2 (–67°) corresponds to a gauche conformation, which is in agreement with the θ_2 value of a β -amino acid residue involved in a 14-helical structure.^{1c,9a}

On the other hand, the value $\theta_4 = 60^\circ$ corresponds to a gauche conformation avoiding the steric hindrance between the C₁₁–C₁₂–C₁₃–C₁₄ cyclobutane ring and the MeO–C₂₉–C₂₈ fragment that can be present in the anti conformation.

Even though the θ_2 value in conformer **5**₃ corresponds to a gauche conformation, a hydrogen bond to stabilize a 14-helical structure is not present, and the θ_4 value corresponds to an anti disposition between the substituents. The structures **5**_{2,4,5} present a more extended conformation than **5**₁ since the distances between the cyclobutanes are around 9 Å, as shown in Table 3. The θ_2 and θ_4 torsional angles in these three conformers are close to 180° corresponding to an anti disposition. In summary, regarding the rotation around the two central bonds of both β -alanine residues (θ_2 and θ_4 torsion angles) **5**₁ could be classified as gauche/gauche, **5**₃ as gauche/anti, and the other three conformers **5**_{2,4,5} as anti/anti.

We have also analyzed other conformationally relevant torsional angles (Table 3) and distances (Supporting Information). For all five conformations **5**_{1–5}, protons in the pairs NH₁₀/H₁₁ and NH₂₀/H₂₁ are close to an anti disposition (i.e., ϕ_1 and ϕ_3 values around 140°, Table 3), which is in agreement with the NMR results (large

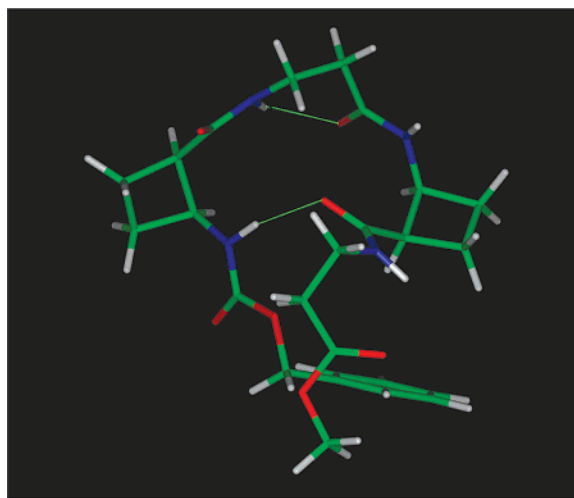
(16) Corcho, F.; Filizola, M.; Pérez, J. J. *Chem. Phys. Lett.* **2000**, *319*, 65.

(17) (a) http://www.accelrys.com/support/life/discover/forcefield/cff9x_refs.html. (b) Mal, B.; Nussinov, R. *J. Mol. Biol.* **2000**, *296*, 1091.

TABLE 3. Characteristics^a of Energy Minima Conformers (ISA) for Tetrapeptide **5**

family ^b	E^c	ω_1	ω_2	ϕ_2	θ_2	Ψ_2	ω_3	ω_4	ϕ_4	θ_4	Ψ_4	R^d	ϕ_1	ϕ_3
5 ₁	0.00	-179	179	147	-67	-91	-179	179	73	60	-160	7.3	128	-140
5 ₂	0.70	177	-173	-78	173	-83	-179	-176	76	175	-147	9.4	-140	134
5 ₃	0.97	178	167	74	69	153	177	-175	71	178	165	8.9	-128	-149
5 ₄	0.99	177	-175	-77	176	-82	179	-176	77	176	137	9.2	-123	133
5 ₅	1.00	176	-173	70	-177	73	172	-174	79	175	179	9.4	-148	-135

^a See Figure 1. Dihedral angles in degrees. ^b See Figure 2. ^c Relative energies in kcal mol⁻¹. ^d Distance between pseudoatoms in the mass center of each cyclobutane ring in angstroms.

**FIGURE 3.** Structure of tetrapeptide **5** optimized at the B3LYP/6-31G(d) level of calculation.

proton–proton coupling constants (9 Hz) and absence of NOEs for $NH_{10}-H_{11}$ and $NH_{20}-H_{21}$. On the other hand, there is a good agreement between the observed qualitative NOE data (Table 1) and the calculated distances.

We have optimized the geometry of tetrapeptide **5** using the density functional B3LYP¹⁸ method with the 6-31G(d) basis set using the Gaussian-98 program.¹⁹ The absolute minimum obtained in the conformational search with the CFF91 force field **5**₁ has been taken as a starting point for this optimization. The obtained structure, which is shown in Figure 3, maintains the 14-helical structure, with a hydrogen bond distance $NH_{10}\cdots OC_{25}$ slightly shorter (2.01 Å) than the one obtained with CFF91 (2.11 Å). The main differences between the B3LYP and CFF91 structures are in the orientation of the phenyl ring of Cbz group and in the ψ_3 dihedral angle, which differs by 39.4° from one structure to another. Moreover, at the B3LYP level of calculation, the $NH\cdots O$ distances corresponding

to $NH_{16}\cdots OC_{19}$ (2.14 Å) and $NH_{26}\cdots OC_{29}$ (2.32 Å) are notably shorter than the values obtained with CFF91 (see Table 2).

Molecular Dynamics Calculations. To study the dynamic behavior of the most stable family of conformers of **5**, the lowest energy conformer of **5**₁ (resulting from the Iterative Simulated Annealing conformational search) was taken as the starting structure for a 1 ns room-temperature molecular dynamics calculation using an explicit chloroform solvent model. We analyzed along the trajectory those geometric parameters diagnostic for folding. The distance between NH_{10} and OC_{25} found in the resulting conformer **5**₁ remained almost constant, around 2.5 Å, during the molecular dynamics (Figure 4A). On the other hand, conformer **5**₁ showed no major fluctuations of dihedral angles Ψ_2 , θ_2 , ϕ_2 , θ_4 , and Ψ_4 , while ϕ_4 showed a considerable degree of fluctuation (Figure 4B). These results are in agreement with NMR data that strongly suggests that $C_{15}N_{20}$ segment of the molecule is more rigid than the $MeO-C_{29}-C_{28}-C_{27}$ fragment.

Concluding Remarks

The presence of cyclobutane residues in tetrapeptide **5** promotes the adoption of a folded major conformation in solution. Thus, according to theoretical calculations (ISA, DFT), the most stable conformation corresponds to a 14-helix disposition. Moreover, molecular modeling allowed us to establish that the NH_{10} proton is involved in a hydrogen bond with OC_{25} stabilizing the formation of a 14-helical structure. These results are in excellent agreement with the temperature coefficients and data from diffusion-based NMR experiments, which show indeed the participation of NH_{10} in a strong hydrogen bond in the predominant (>95%) conformation in $CDCl_3$ and $DMSO-d_6$ solution. Furthermore, the observed NOEs are compatible with the 14-helix-type structure proposed for **5**.

Experimental Section

Acid 4. A solution of 0.25 M NaOH (7.2 mL, 1.8 mmol) was added to an ice-cooled solution of dipeptide **3**^{7b} (360 mg, 1.1 mmol) in 1:10 THF–H₂O (198 mL). The mixture was stirred at 0 °C for 3 h. Then, the reaction mixture was washed with four portions of dichloromethane, 2 M HCl was subsequently added to the aqueous phase to reach pH 2, and the acid-variant solution was extracted with six portions of dichloromethane. The combined organic extracts were dried over anhydrous MgSO₄, and solvent was evaporated at reduced pressure to afford quantitatively acid **4** (340 mg): crystals; mp 120–122 °C (from ethyl acetate–pentane); $[\alpha]_D -86$ (*c* 1.0, methanol); 500 MHz ¹H NMR ($CDCl_3$) δ 1.89 (m, 1H), 2.33 (m, 1H), 2.47 (m, 2H), 3.27 (m, 1H), 3.35 (m, 1H), 3.62 (m, 2H), 4.46 (q, *J* = 9 Hz, 1H), 5.07 (dd, *J* = 12 Hz, 1H), 5.10 (d, *J* = 12 Hz, 1H), 6.02 (d, *J* = 9 Hz, 1H), 6.83 (broad s, 1H),

(18) (a) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648. (b) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.

(19) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.6; Gaussian, Inc.: Pittsburgh, PA, 1998.

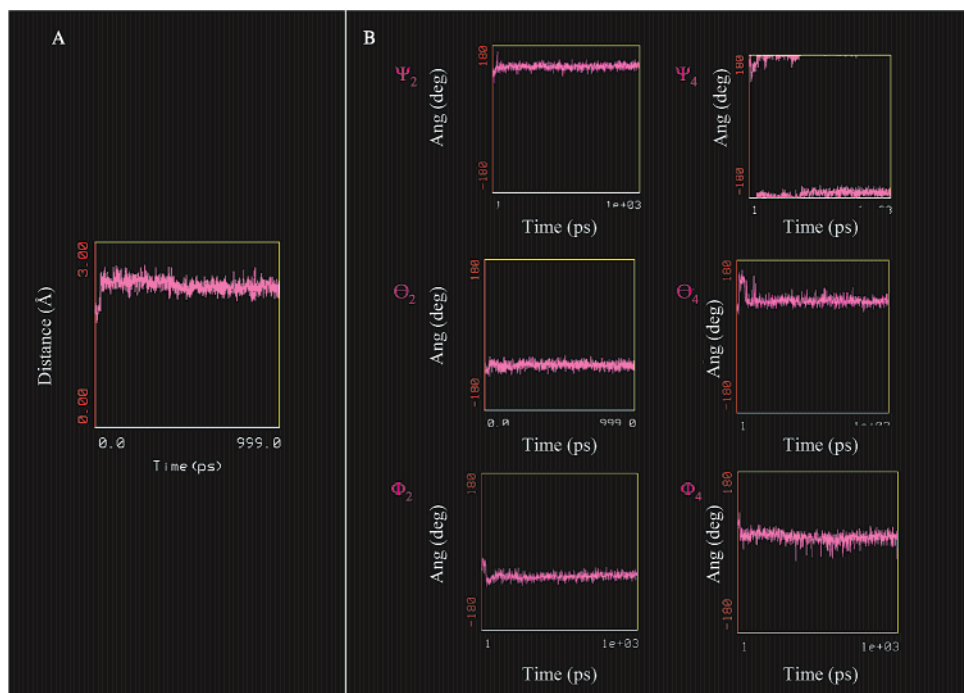


FIGURE 4. Values of a selection of geometric parameters diagnostic for the molecule folding of the conformer 5_1 as a function of time in a molecular dynamics simulation at 298 K in chloroform: (A) distance between $NH\cdots OC_{25}$; (B) torsion angles belonging to the $N_{16}-C_{19}$ central β -alanine and to the $N_{26}-C_{29}$ terminal β -alanine.

7.32 (m, 5H); 62.5 MHz ^{13}C NMR ($CDCl_3$) δ 17.6, 28.7, 34.0, 34.7, 46.2, 46.8, 67.1, 128.0, 128.4, 136.1, 156.1, 173.1, 175.1. Anal. Calcd for $C_{16}H_{20}N_2O_5$: C, 59.97; H, 6.30; N, 8.75. Found: C, 59.93; H, 6.19; N, 8.65.

Amine 6. A mixture of dipeptide 3^{7b} (360 mg, 1.1 mmol) and 10% Pd/C (77 mg) in methanol (15 mL) was stirred at room temperature overnight under 2 atm of hydrogen pressure. The reaction mixture was filtered through Celite that was successively washed with several portions of dichloromethane. Solvents were removed under vacuo from the combined filtrates to afford amine **6** as an oil (220 mg, 100% yield) that was used in the coupling step without further purification: $[\alpha]_D -6$ (c 1.5, MeOH); IR (film) 3439, 1736, 1600 cm^{-1} ; 250 MHz 1H NMR (methanol- d_4) δ 1.57 (m, 2H), 1.96 (m, 2H), 2.20 (m, 1H), 2.52 (m, 2H), 3.32 (m, 1H), 3.41 (m, 2H), 3.66 (s, 3H); 62.5 MHz ^{13}C NMR (methanol- d_4) δ 17.7, 28.7, 34.1, 34.6, 46.3, 46.7, 67.1, 128.1, 128.5, 136.1, 156.1, 172.9, 175.2.

Trapeptide 5. Hydroxybenzotriazole (78 mg, 1.0 mmol) and DEC hydrochloride (535 mg, 3.4 mmol) were successively added to a solution containing acid **4** (370 mg, 1.2 mmol) and amine **6** (230 mg, 1.1 mmol) in freshly distilled dry DMF (14 mL). The light-protected mixture was stirred at room temperature for 72 h under nitrogen atmosphere. Then ethyl acetate (50 mL) was added, and the solution was washed with five portions of saturated aqueous $NaHCO_3$. The organic phase was dried over anhydrous $MgSO_4$, and the solvent was removed at reduced pressure to afford tetrapeptide **6** (310 mg, 60% yield) as a solid that was purified by crystallization: crystals; mp 173–175 °C (from ethyl acetate); $[\alpha]_D -60$ (c 0.5, $CHCl_3$); IR (film) 3308, 1734, 1689, 1642 cm^{-1} ; 500 MHz 1H NMR ($CDCl_3$) δ 1.98 (m, 2H), 2.15 (m, 3H), 2.26 (m, 3H), 2.36 (m, 2H), 2.51 (m, 2H), 3.18 (m, 1H), 3.30 (m, 2H), 3.49 (m, 2H), 3.62 (m, 1H), 3.72 (s, 3H), 4.48 (q, $J = 8.5$ Hz, 1H), 4.63 (q, $J = 8.5$ Hz, 1H), 5.03 (d, $J = 12$ Hz, 1H), 5.11 (d, $J = 12$ Hz, 1H), 6.11 (d, $J = 9.0$ Hz, 1H), 6.40 t, $J = 6.0$ Hz, 1H), 6.88 (t, $J = 6.0$ Hz, 1H), 7.18 (d, $J = 8.2$ Hz, 1H), 7.32 (m, 5H); 62.5 MHz ^{13}C NMR ($CDCl_3$) δ 18.3, 19.0, 28.5, 28.6, 33.7, 34.8, 35.0, 35.7, 45.3, 45.5, 45.9, 46.7, 51.7, 66.4, 128.0, 128.5, 136.5, 155.8, 171.2, 172.7, 172.9, 173.1; HRMS (EI, 70 eV) calcd for $C_{25}H_{34}N_4O_7$ 502.2616, found 502.2428.

Tripeptide 7. Following the same procedure described above for the preparation of **5**, tripeptide **7** was synthesized from amine **6** (140 mg, 0.7 mmol) and *N*-Cbz- β -Ala (235 mg, 1.0 mmol) as a solid: yield 97 mg (34%); crystals; mp 127–130 °C (ethyl acetate–pentane); $[\alpha]_D -28$ (c 1.0, MeOH); IR (film) 3431, 1730, 1705, 1656 cm^{-1} ; 250 MHz 1H NMR ($CDCl_3$) δ 1.58 (m, 2H), 2.15 (m, 2H), 2.29 (m, 1H), 2.36 (m, 2H), 2.52 (m, 2H), 3.23 (m, 2H), 3.44 (m, 3H), 3.67 (s, 3H), 5.06 (s, 2H), 5.60 (broad s, 1H), 5.97 (broad s, 1H), 6.16 (broad s, 1H), 7.36 (m, 5H); 62.5 MHz ^{13}C NMR ($CDCl_3$) δ 22.4, 28.7, 33.8, 34.8, 35.6, 38.7, 44.6, 45.7, 51.8, 66.5, 127.9, 128.0, 128.5, 136.6, 156.5, 171.3, 172.8, 173.1; MS (EI, 70 eV) m/z 405 (M^+ , 6), 298 (24), 195 (28), 91 (100).

NMR Studies on Tetrapeptide 5. All 1D, 2D (COSY, NOESY HSQC, and HMBC experiments), and diffusion NMR spectra were performed on a 500 MHz spectrometer equipped with a 5 mm triple-resonance inverse probehead incorporating pulsed-field gradients in the z -direction. NMR spectra were registered in $CDCl_3$ or $DMSO-d_6$, and chemical shifts are given in parts per million (δ scale).

NMR diffusion experiments were recorded using the LED pulse scheme with bipolar gradients²⁰ without temperature calibration in order to minimize undesired convection effects.²¹ Similar results were also obtained using the double STE pulse sequence. A series of 1D diffusion experiments were recorded with several diffusion times ranging from 100 ms to 1 s in order to observe the different intensity decay for each NH resonance and thus to extract qualitative information about hydrogen bonding strength. The duration of each z -gradient was set to 1.5 ms and data were acquired with 16 K using a recycle delay of 1.5 s. Data were processed using a line broadening of 0.5 Hz. Complete structural characterization using the described COSY, HSQC, NOESY, and diffusion experiments was performed on both $CDCl_3$ and $DMSO-d_6$ solutions (see the Supporting Information for more details).

(20) Wu, D.; Chen, A.; Johnson, C. S. *J. Magn. Reson. A* **1995**, *115*, 260.

(21) Esturau, N.; Sánchez-Ferrando, F.; Gavin, J. A.; Roumestand, C.; Delsuc, M. A.; Parella, T. *J. Magn. Reson.* **2001**, *153*, 48.

Molecular Modeling Studies. Conformational Search.

All calculations were run on a SGI workstation (R4000, 128 MB RAM, 19 GB hard disk) under an Irix 5.3 operating system. Molecular mechanics calculations were carried out with Spartan v 5.0 and Insight II discover 3.0 v1997.

Iterative Simulated Annealing (ISA). The calculations were carried out within the molecular mechanics using the CFF91 force field implemented in DISCOVER v.97. They were conducted under vacuum with a distance-dependent dielectric constant ($4r$) and a cutoff of 13 Å. Starting from extended structures of the tetrapeptide **5**, the structure was minimized and subsequently heated to 900 K in a very short period of time. The structure was then cooled slowly to 100 K and minimized. In our case, the heating was carried out in steps. At each step the temperature was raised 100 K in 0.1 ps and following heating the system was allowed to stay 1 ps at the new temperature. The system was allowed to stand for 10 ps at 1000 K and then cooled in steps. At each step, the temperature was lowered 100 K in 0.1 ps and after cooling the system was allowed to stay 10 ps at the new temperature. This structure is the starting conformation for another cycle, creating a library of conformations that are rank ordered by energy every 150 cycles. To determine the conformer families, all the backbone atoms from C₉ to C₂₉ were considered. The procedure was repeated until no new conformations appeared after a predetermined number of cycles (in our case five times) within a 2 kcal/mol energy range with respect to the lowest energy structure already found. Heating must be carried out rapidly in order to make the molecule jump to a different region in the space. In contrast, cooling was slow in order to obtain the lowest energy minimum of the region. This protocol was run several times (five), employing fully extended starting structures of **5**.

Molecular Dynamics. The resulting lowest energy conformer of tetrapeptide **5** (resulting from the Iterative Simulated Annealing) was used as starting structure for a molecular dynamics calculation using an explicit solvent model in chloroform. NVT calculation at 298 K was performed using a cubic box of 48 Å side length and 433 chloroform molecules. Periodic bounded conditions were applied. After heating and equilibration of the system for 0.25 ns, evolution time was 0.75 ns. 750 structures were saved periodically for further trajectory analysis.

Acknowledgment. S.I. thanks the UAB for a predoctoral fellowship. Financial support from MCyT-FEDER (BQU2001-1907, BIO2002-2301, and BQU2003-01677) and Generalitat de Catalunya (2001SGR00182, 2001SGR00047) is gratefully acknowledged.

Supporting Information Available: Cartesian coordinates of tetrapeptide **5** obtained by using the CFF91 and B3LYP methods. Distances between hydrogen atoms for energy minima conformers. ¹H and ¹³C assignments in DMSO-*d*₆ at 298 K, ¹H NMR spectrum in CDCl₃ at different temperatures, ¹H NMR spectrum in DMSO-*d*₆ at 298 K, 2D ¹H-¹H spectra, 2D ¹H-¹H NOESY spectra, 2D gradient-enhanced ¹H-¹H COSY and NOESY spectra, 2D gradient-enhanced phase-sensitive ¹H-¹³C HSQC spectra, temperature coefficients, and self-diffusion NMR studies for tetrapeptide **5**. ¹H NMR for amine **6** and tripeptide **7**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

JO0497555