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Development and Conformational Analysis of a Pseudoproline-Containing Turn Mimic†

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The liquid-phase synthesis and the conformational analysis of a small library of fully protected tetramers containing L-pyroglutamic acid (L-pGlu), (4*S*,5*R*)-4-methyl-5-carboxybenzyloxazolidin-2-one (L-Oxd), or (4*R*,5*S*)-4-methyl-5-carboxybenzyloxazolidin-2-one (D-Oxd) as residue i + 1 are reported to test the tendency of these oligomers to assume a β-hairpin conformation. The most promising molecule is Boc-L-Val-D-Oxd-Gly-L-Ala-OBn, which assumes a preferential β-turn conformation in $CDCl_3$, as shown by IR and ^1H NMR analysis. These findings have been confirmed by DFT calculations, which provide an interpretation for the available experimental data and agree with the reported observations.

Introduction

Reverse turns are common motifs in protein structures and account for up to one-third of the residues in globular proteins.[1] A turn can be defined as the site where the peptide changes its overall direction, and different types of turns have been described depending on how many residues are involved in the loop.[2] Among them, the most common naturally occurring type is the β-turn, which involves at least four residues, with a hydrogen-bonded amide proton between the carbonyl of residue i and the NH group of residue i + 3. The γ-turns occur less often and usually involve a hydrogen-bonded amide proton between the carbonyl of residue i and the NH group of residue i + 2. In the de novo design of a reverse-turn conformation, the introduction of conformationally constrained analogues can be very useful.[3] For instance, Lubell pursued two strategies for generating peptide mimics: the first employs the use of bicycles to constrain a dipeptide unit, while the second uses the steric interactions of bulky ring substituents to influence the geometry and conformation of peptide amide bonds.[4] A different approach consists of the introduction of a D-amino acid in the peptide chain: Gellman demonstrated that switching from D-Pro to L-Pro in the linker prevents parallel sheet interactions between L-strand residues,[5] and Balaram built a four-stranded β-sheet structure by introducing D-Pro-Gly units.[6] Imperiali studied sequences including an L-Pro at the (i + 1) position and a D-amino residue at the (i + 2) position and demonstrated that Ac-L-Val-L-Pro-D-Ser-L-His-NH_2 in particular adopts a significant amount of reverse-turn character both in water and in dimethyl sulfoxide.[7]

In this paper, we will describe a study on the synthesis and the conformational analysis of fully protected tetramers containing pseudoprolines as i + 1 residues (Figure 1).

We have recently reported the synthesis and the conformational analysis of homooligomers of L-pyro-

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† Dedicated to Professor Gianfranco Cainelli in the occasion of his 70th birthday.

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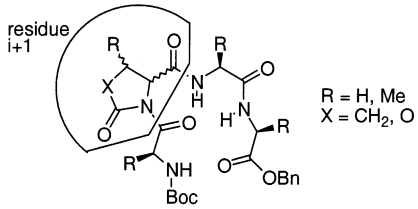

FIGURE 1. General structure of the fully protected tetramers.

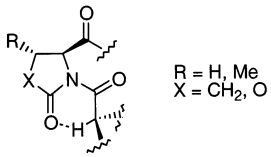

FIGURE 2. Preferential conformation of the imidic bond, which accounts for the anomalous chemical shift of the CH-α proton chemical shift. This effect is due to the tendency of the two carbonyls to lie apart one from the other and is enhanced by the formation of a C═O···H−C hydrogen bond, which stabilizes the structure by approximately 1.4 kcal/mol.[8]

glutamic acid (L-pGlu)[8] and of (4*S*,5*R*)-4-methyl-5-carboxybenzyloxazolidin-2-one (L-Oxd),[9] which have been synthesized up to the tetramer and to the pentamer level, respectively. We have demonstrated that those new polyimides behave as rigid spacers, owing to the presence of the endocyclic carbonyl, which strictly imparts a trans conformation to the adjacent peptide bond. This effect is due to the tendency of the two carbonyls to lie apart one from the other and is enhanced by the formation of a C═O···H−C hydrogen bond, which stabilizes the structure of about 1.4 kcal/mol (Figure 2). X-ray diffraction analysis of Boc-(L-pGlu)$_2$-OH (Boc = *tert*-butyloxycarbonyl, pGlu-OH = pyroglutamic acid) and DFT calculations furnished a confirmation of this finding.[8−10]

Here, we extend our studies on the imidic bond conformational behavior to some short oligopeptides containing L-pGlu, L-Oxd, or D-Oxd (*trans*-(4*R*,5*S*)-4-carboxy-5-methyloxazolidin-2-one) as residue i + 1. In naturally occurring reverse turns, this residue is often L-Pro, so by introducing pseudoprolines, we want to check whether these motifs favor or disfavor the formation of a β-hairpin secondary structure. Some L-Pro-containing tetrapeptides have also been synthesized as reference compounds.

To study and to compare their conformational preferences, conformational analysis and DFT calculations have been performed on the most interesting molecules.

Results and Discussion

Synthesis. We have identified some oligomeric structures as good candidates to check whether the imide moiety favors or disfavors the formation of a β-hairpin conformation. First, we have prepared some oligomers containing L-pGlu as residue i + 1, and we have varied the residue i (L-Ala and L-Val) and the residue i + 2 (Gly and Aib) (Aib-OH = 2-aminoisobutanoic acid). Gly was chosen because of its high occurrence into natural reverse-turn conformations and Aib was chosen because it is highly helycogenic.[11]

L-Pyroglutamic acid is often present in natural polypeptides, but only as an N-terminal amino acid, owing to its low reactivity of the lactam nitrogen.[12] We have recently developed a straightforward method for the N-derivatization of this compound, simply by reacting the lithium salt of H-L-pGlu-OBn (OBn = benzyloxy) with activated esters of protected α-amino acids.[13] The acylation reactions usually occur in high yield. Following this protocol, we have now prepared the tetramers Boc-L-Ala-L-pGlu-Gly-L-Ala-OBn **4a**, Boc-L-Val-L-pGlu-Gly-L-Ala-OBn **4b**, and Boc-L-Val-L-pGlu-Aib-L-Ala-OBn **5** (Scheme 1). After transformation of L-pGlu-OH into L-pGlu-OBn **1**, the product was acylated with Boc-L-Ala-OPfp (OPfp = pentafluorophenyl) or with Boc-L-Val-OPfp to obtain the fully protected dimers **2a** and **2b**, which were hydrogenolyzed by catalyzed reaction with H_2/Pd. The couplings with H-Gly-L-Ala-OBn and H-Aib-L-Ala-OBn afforded the desired tetramers **4a**,**b** and **5** in satisfactory overall yields.

A similar molecule was prepared from L-Oxd by acylation of H-L-Oxd-OBn **6** with Boc-L-Val-OPfp in the presence of diisopropylethylamine (DIEA) and dimethylaminopyridine (DMAP) in DMF (Scheme 2). The yield of this reaction is not very high, but any attempt to ameliorate this result failed. For instance, the protocol utilized for the acylation of H-L-pGlu-OBn **1** (LiHMDS, OPfp ester in dry THF) afforded only a complex mixture. The deprotection of the ester moiety, followed by coupling with H-Gly-L-Val-OBn, afforded the fully protected tetramer **9** in good yield.

A similar approach was followed for the preparation of tetramers Boc-L-Val-D-Oxd-Gly-L-Ala-OBn **13** and Boc-L-Val-D-Oxd-Aib-L-Ala-OBn **14** (Scheme 3).

Finally, we prepared two fully protected tetrapeptides, containing L-Pro as residue i + 1: Boc-L-Val-L-Pro-Gly-L-Ala-OBn **15** and Boc-L-Val-L-Pro-Aib-L-Ala-OBn **16** (Figure 3). These molecules have been synthesized by liquid-phase synthesis, and the couplings have been carried out in good yields following the HOBt/NMM/EDCI protocol [HOBt = 1-hydroxybenzotriazole; NMM = *N*-

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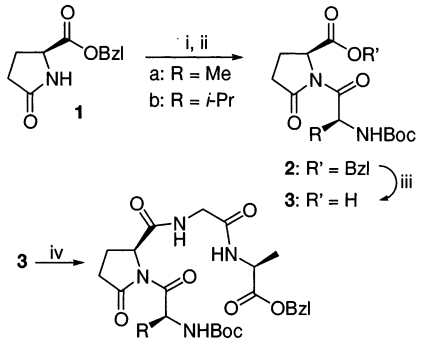
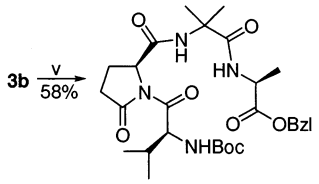
SCHEME 1. Synthetic Routes for the Tetramers 4a,b and 5[a]



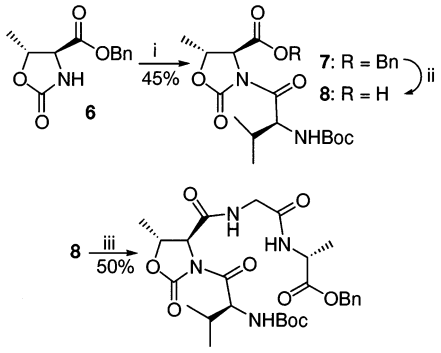
4a: Boc-L-Ala-L-pGlu-Gly-L-Ala-OBzl: 45% yield
4b: Boc-L-Val-L-pGlu-Gly-L-Ala-OBzl: 50% yield



5: Boc-L-Val-L-pGlu-Aib-L-Ala-OBzl

[a] Reaction conditions: (i) LiHMDS (1.2 equiv), dry THF, 0 °C, 30 min; (ii) Boc-L-Ala-OPfp or Boc-L-Val-OPfp (1.5 equiv), dry THF, 0 °C, 1 h; (iii) Pd/C 10% (cat.), MeOH, rt, 2 h; (iv) H-Gly-L-Ala-OBn (2 equiv), NMM (4 equiv), HOBt (1.2 equiv), EDCl (1.2 equiv), dry DMF, rt, 16 h; (v) H-Aib-L-Ala-OBn (2 equiv), NMM (4 equiv), HOBt (1.2 equiv), EDCl (1.2 equiv), dry DMF, rt, 16 h.

SCHEME 2. Synthetic Routes for the Tetramer 9[a]



9: Boc-L-Val-L-Oxd-Gly-L-Ala-OBn

[a] Reaction conditions: (i) DIEA (4 equiv), DMAP (0.7 equiv), Boc-L-Val-OPfp (1.5 equiv), dry DMF, rt, 2 h; (ii) Pd/C 10% (cat.), MeOH, rt, 2 h; (iii) H-Gly-L-Ala-OBn (2 equiv), NMM (4 equiv), HOBt (1.2 equiv), EDCl (1.2 equiv), dry DMF, rt, 16 h.

methylmorpholine; EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride].[14]

Conformational Analysis. Once we obtained a small library of fully protected tetrapeptides, their preferential conformation was analyzed by ^{1}H NMR, IR, and DFT computational modeling. Moreover, some interesting information was obtained by electrospray ionization mass (ESI−MS) analysis. The molecules analyzed were Boc-L-Ala-L-pGlu-Gly-L-Ala-OBn **4a**, Boc-L-Val-L-pGlu-Gly-L-Ala-OBn **4b**, Boc-L-Val-L-pGlu-Aib-L-Ala-OBn **5**, Boc-L-Val-L-Oxd-Gly-L-Ala-OBn **9**, Boc-L-Val-D-Oxd-Gly-L-Ala-OBn **13**, Boc-L-Val-D-Oxd-Aib-L-Ala-OBn **14**; and the reference molecules containing L-Pro at the i + 1 position were Boc-L-Val-L-Pro-Gly-L-Ala-OBn **15** and Boc-L-Val-L-Pro-Aib-L-Ala-OBn **16**.

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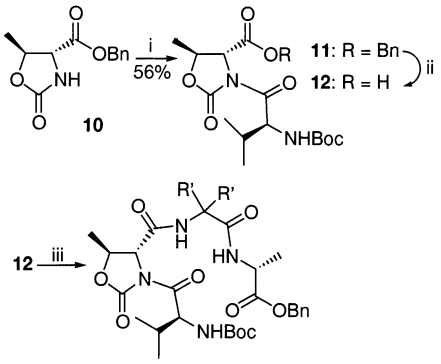
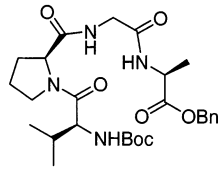
SCHEME 3. Synthetic Routes for the Tetramers 13 and 14[a]

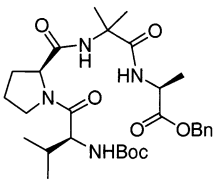


13: R' = H; Boc-L-Val-D-Oxd-Gly-L-Ala-OBn
62% yield
14: R' = Me; Boc-L-Val-D-Oxd-Aib-L-Ala-OBn
53% yield

[a] Reaction conditions: (i) DIEA (4 equiv), DMAP (0.7 equiv), Boc-L-Val-OPfp (1.5 equiv), dry DMF, rt, 2 h; (ii) Pd/C 10% (cat.), MeOH, rt, 2 h; (iii) H-Gly-L-Ala-OBn or H-Aib-L-Ala-OBn (2 equiv), NMM (4 equiv), HOBt (1.2 equiv), EDCl (1.2 equiv), dry DMF, rt, 16 h.



15: Boc-L-Val-L-Pro-Gly-L-Ala-OBn



16: Boc-L-Val-L-Pro-Aib-L-Ala-OBn

FIGURE 3. Tetrapeptides **15** and **16** contain L-Pro as residue *i* + 1 and have been prepared as reference compounds by liquid-phase synthesis, following the HOBt/NMM/EDCI protocol.

IR Analysis. The IR absorption spectra were obtained as a 3 mM solution in methylene chloride: at this concentration, the intramolecular aggregation is usually unimportant. Figure 4 shows the absorption bands between 3500 and 3200 cm^{-1} of all the synthesized compounds and helps us to see if we have non-hydrogen-bonded amide proton bands (above 3400 cm^{-1}) or hydrogen-bonded amide proton bands (below 3400 cm^{-1}).[15] The IR spectra of tetramers **4a**,**b** and **9** are very similar, with a greater band at about 3430 cm^{-1} (non-hydrogen-bonded

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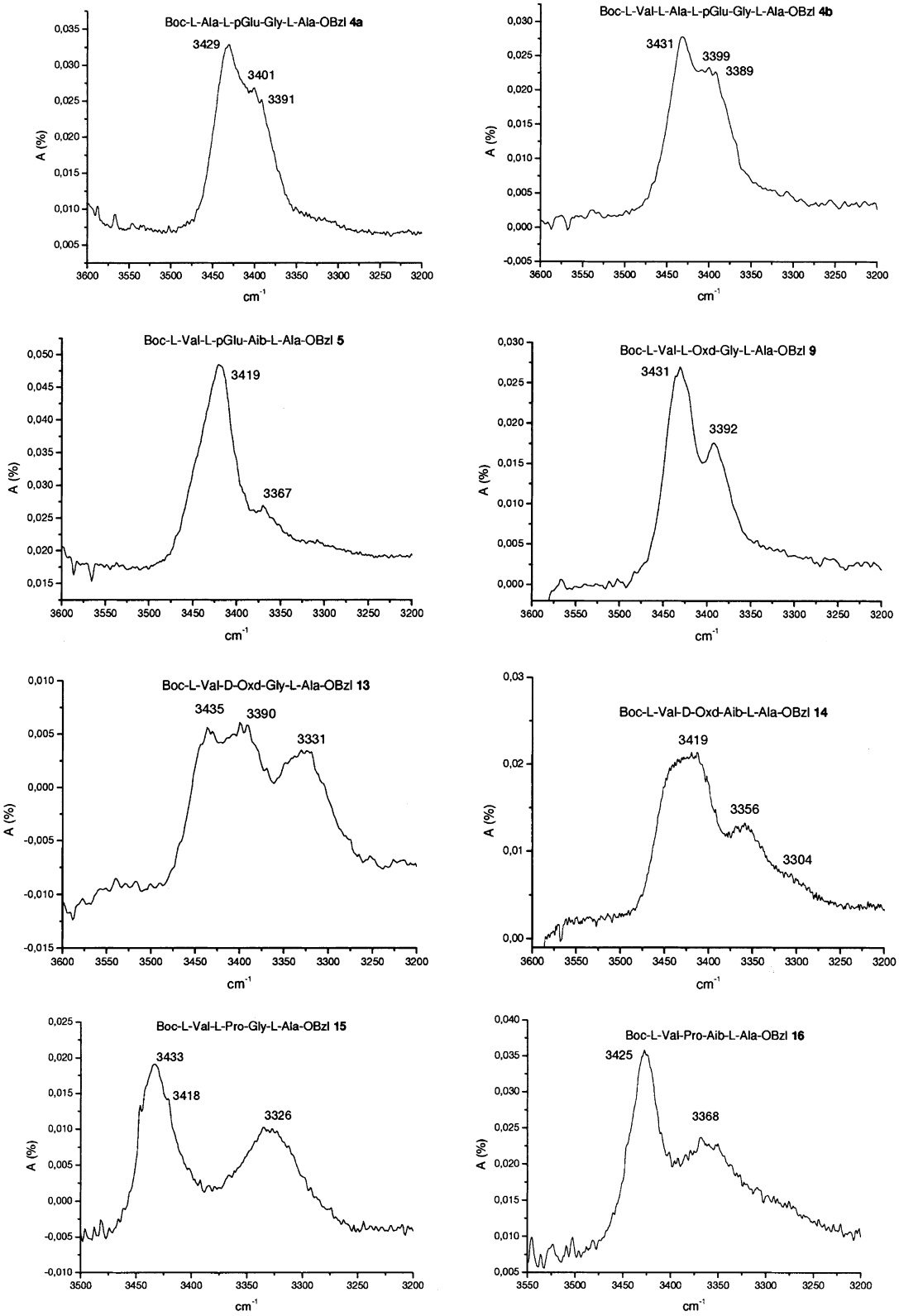


FIGURE 4. N−H stretch region FT-IR data for 3 mM samples of **4a**,**b**, **5**, **9**, and **13**−**16** in pure CH_2Cl_2 at room temperature, after subtraction of the spectrum of pure CH_2Cl_2.

amide proton) and another band at about 3390 cm^{-1} (weakly hydrogen-bonded amide proton). We gather that in these systems we can both vary the nature of the L-amino acid as residue i and utilize L-Oxd or L-pGlu as residue i + 1, without a substantial variation of the IR spectrum and, possibly, of the preferred conformation of the molecule. The IR spectrum of the reference compound Boc-L-Val-L-Pro-Gly-L-Ala-OBn **15** shows a strong band at 3433 cm^{-1} and another longer band at 3326 cm^{-1} (hydrogen-bonded amide proton). The Aib moiety was

TABLE 1. Chemical Shifts of the CHα of Residue i for Tetramers 4a,b, 5, 9, and 13−16

entry	product	residue i	residue i + 1	δ CHα−i (ppm)
1	**4a**	Ala	pGlu	5.34
2	**4b**	Val	PGlu	5.37
3	**5**	Val	PGlu	5.37
4	**9**	Val	L-Oxd	5.40−5.43
5	**13**	Val	D-Oxd	5.33
6	**14**	Val	D-Oxd	5.26
7	**15**	Val	L-Pro	4.25
8	**16**	Val	L-Pro	4.25

introduced as residue i + 2 in two tetramers: Boc-L-Val-L-pGlu-Aib-L-Ala-OBn **5** and the reference compound Boc-L-Val-L-Pro-Aib-L-Ala-OBn **16**. The IR spectra show the presence of a strong band at about 3420 cm^{-1} and a weak shoulder at 3367 cm^{-1}, thus suggesting that in both cases an equilibrium between a β-turn conformation and an open form is present.

All these results are quite disappointing because they suggest that the introduction of the pseudoprolines L-pGlu and L-Oxd in the tetramer as residue i + 1 does not facilitate the formation of a reverse-turn conformation more than the introduction of L-Pro.

Much better results have been obtained with the tetramers containing D-Oxd as residue i + 1: the spectrum of Boc-L-Val-D-Oxd-Gly-L-Ala-OBn **13** shows three bands of nearly the same intensity at 3435 cm^{-1} (non-hydrogen-bonded amide proton), 3390 cm^{-1} (weakly hydrogen-bonded amide proton), and 3331 cm^{-1} (strongly hydrogen-bonded amide proton), while the IR spectrum of Aib-containing **14** shows a strong band at 3419 cm^{-1} and a weak band at 3356 cm^{-1}, suggesting that in this case the Aib moiety disfavors the presence of strong hydrogen bonds. From a general insight, the best candidate for a β-hairpin structure should be **13**.

^{1}H NMR Analysis. All the spectra of the molecules containing L-pGlu, L-Oxd, or D-Oxd show a signal between 5 and 6 ppm, which is due to the CHα of the residue i (Table 1). We have already observed and studied this outcome,[8,9] and it was attributed to the presence of the heterocycle carbonyl, which strongly deshields the hydrogen. The reference compounds **15** and **16** (entries 7 and 8), which contain L-Pro as residue i + 1, in fact do not produce this effect.

An evaluation of the NOESY spectra ($CDCl_3$) of the compounds (Figure 5) shows that in any case a significant NOE was observed between the CHα of the residue i + 1 (L-pGlu for **4b** and **5**; L-Oxd for **9**; D-Oxd for **13** and **14**; L-Pro for **15** and **16**) and NH of the residue i + 2 (Gly or Aib). Furthermore, D-Oxd containing compounds (**13** and **14**) show an NOE enhancement between NH-Gly and NH-Ala for **13** and between NH-Aib and NH-Ala for **14** (weak signal).

Those results show that a kind of turn should be formed; in particular, the proximity of the two amide protons is indicative of a compact, rather than extended structure. This outcome has been further detected by investigation of the DMSO-d_6 dependence of NH proton chemical shift.[16] This solvent has a strong hydrogen-bonding acceptor character and, if it bound to a free NH proton, it will be expected to dramatically move its chemical shift downfield. In the case of a β-turn conformation, the amide proton (i + 3)-NH should be hydrogen bonded, while for a γ-turn conformation it should be (i + 2)-NH. The evaluation of inaccessible NH groups by ^{1}H NMR was performed by adding increasing amounts of DMSO-d_6 to 1 mM tetramer solutions in $CDCl_3$. The results are reported in Figure 6.

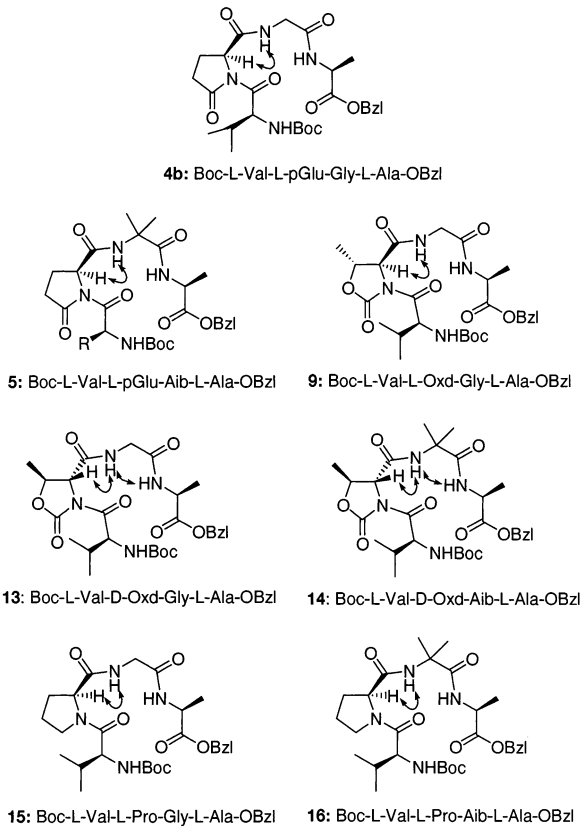


FIGURE 5. Significant NOE enhancements of **4b**, **5**, **9**, and **13−16** obtained by performing the NOESY experiments on 5 mM solutions in $CDCl_3$ and utilizing a mixing time of 900 ms.

The results obtained for **4a**,**b** and **9** are very similar and show that only the i-NH is nearly insensitive to DMSO, thus suggesting that this proton is hydrogen-bonded. This is in agreement with the IR result and show that we can both vary the nature of the L-amino acid at the i position and utilize L-Oxd or L-pGlu, without a substantial variation of the preferred conformation of the molecule. The results obtained from Boc-L-Val-L-Pro-Gly-L-Ala-OBn **15** show a similar trend. From these data we can gather that this group of molecules do not show any preference for a β-hairpin conformation, although they should take a compact conformation, as shown by the NOESY experiments.

The introduction of the Aib moiety gives better results: Boc-L-Val-L-pGlu-Aib-L-Ala-OBn **5** possesses two hydrogen-bonded amide protons i-H and (i + 3)-NH, i.e., NH-Ala and NH-Val and is in agreement with a β-turn structure. The reference molecule Boc-L-Val-L-Pro-Aib-L-Ala-OBn **16** shows a similar trend.

The two compounds of the D series behave in a very different way: Boc-L-Val-D-Oxd-Aib-L-Ala-OBn **14** gives quite good results (Δδ i-NH: 0.24 ppm; Δδ (i + 3)-NH: 0.38 ppm), while the titration of **13** affords a very unusual

(16) (a) Kopple, K. D.; Ohnishi, M.; Go, A. *Biochemistry* **1969**, *8*, 4087. (b) Martin, D.; Hauthal, G. In *Dimethyl Sulphoxide*; Van Nostrand-Reinhold: Wokingham, U.K., 1975.

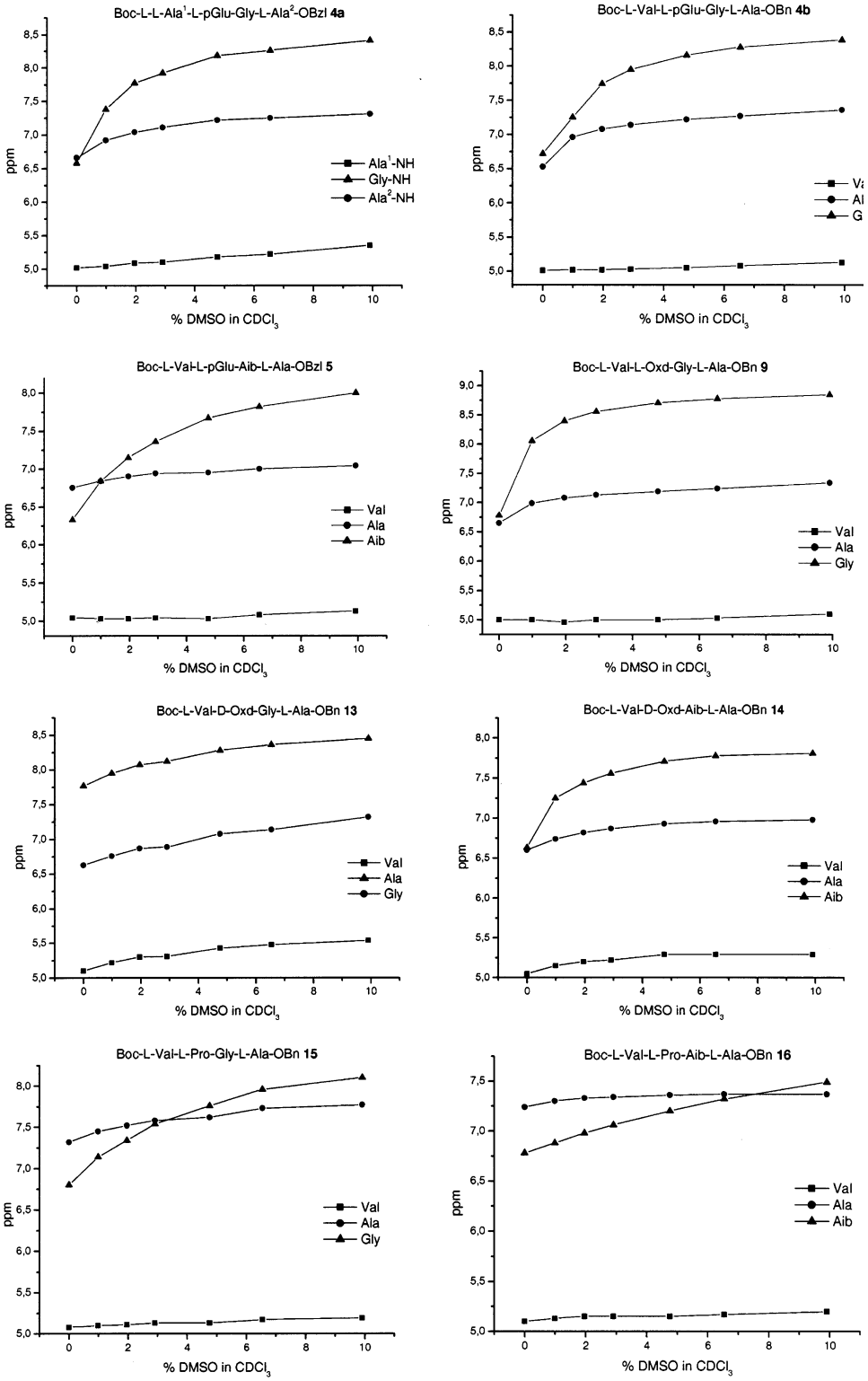


FIGURE 6. Variation of NH proton chemical shift (ppm) of **4a**,**b**, **5**, **9**, and **13**−**16** as a function of increasing percentages of DMSO-d_6 to the $CDCl_3$ solution (v/v) (concentration: 1 mM).

plot of the three NHs ($\Delta\delta$ = 0.44, 0.68, and 0.69 ppm), thus showing that an equilibrium is going on and all the amide protons are involved. This outcome is very disappointing, as **13** shows both promising IR spectrum and NOE enhancements and is the best candidate for a stable β-hairpin conformation. So further analyses were needed to explain the behavior of **13** in solution.

Figure 7 shows the variation of the NH proton chemical shift as a function of the temperature for **13** and for its epimer **9** (used as reference compound) in $CDCl_3$ and in DMSO-d_6. This test points out the presence of hydrogen-bonded amide protons: in $CDCl_3$ a small temperature coefficient (≤3 ppb/K in absolute value) indicates the presence of a hydrogen-bonded amide proton,[17] while

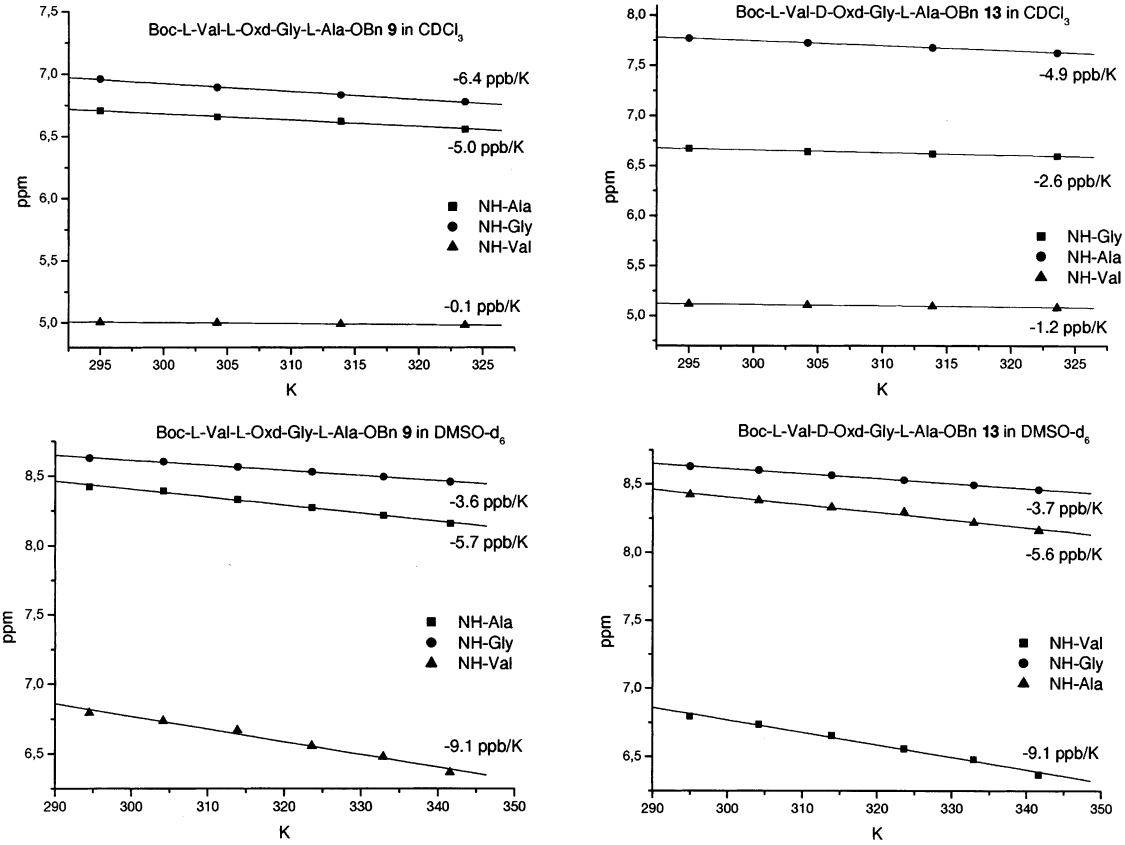


FIGURE 7. Variation of NH proton chemical shift (ppm) of **9** and **13** of as a function the temperature (°C) for 1 mM samples measured both in $CDCl_3$ and DMSO-d_6 (concentration: 1 mM).

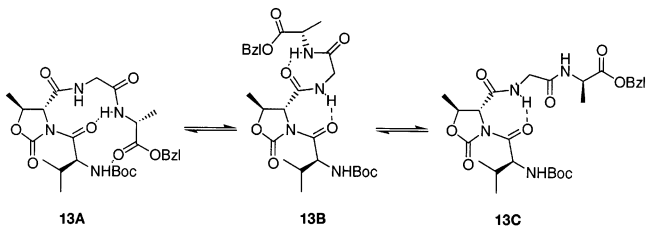


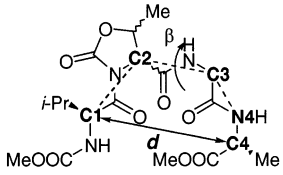
FIGURE 8. Preferred conformations of **13** proposed on the basis of the spectroscopic data.

protons that participate to an equilibrium between hydrogen-bonded and non-hydrogen-bonded state show a larger temperature coefficient. In DMSO-d_6, the temperature coefficients can be bigger, so that an hydrogen-bonded amide proton can have a value of 4 ppb/K or smaller in absolute value.[18]

For **13**, in the two solvents we obtain opposite results. In $CDCl_3$, both protons NH-Val and NH-Ala are hydrogen bonded, thus showing that this molecule assumes a β-turn conformation, like **13A** (Figure 8) in structure-supporting solvents such as chloroform. This outcome is in full agreement both with the IR spectrum (in CH_2Cl_2) and with the NOESY results (in $CDCl_3$). On the contrary, in a competitive solvent like DMSO-d_6, only NH-Gly is a hydrogen-bonded amide proton, thus suggesting that in this solvent a γ-turn conformation is preferred (**13B** or **13C**, Figure 8). Again, DMSO-d_6 favors a γ-turn conformation of **9**, while $CDCl_3$ does not favor any turn of **9**, because only NH-Val is hydrogen-bonded.

The titration outcome of **13** with DMSO-d_6 (Table 2, entry 6) can be easily explained: as $CDCl_3$ favors the formation of a β-turn conformation and DMSO-d_6 favors

TABLE 2. Calculated Energies and Geometrical Parameters for Conformers IA and IB of MeOOC-(L-Val)-(L-Oxd)-Gly-(L-Ala)-OMe and for Conformers IIA and IIB of MeOOC-(L-Val)-(D-Oxd)-Gly-(L-Ala)-OMe



entry	conformer	E_{abs} (hartree)	E_{rel} (kcal mol^{-1})	d^a (Å)	distance O1−H4^b (Å)	β^c (deg)
1	**IA**	−1598.92130	+1.46	5.18	1.93	+8.5
2	**IB**	−1598.92274	+0.55	9.03	5.55	+161.5
3	**IIA**	−1598.92362	0.00	5.31	1.99	−29.6
4	**IIB**	−1598.92227	+0.85	8.81	5.61	−176.6

[a] β-Turns are characterized by d less than 7.00 Å. [b] β-Turns are characterized by a O1−H4 distance less than 2.50 Å. [c] The virtual dihedral angle β indicates a tight β-turn in the range of 0 ± 30°.

(17) (a) Boussard, G.; Marraud, M. *J. Am. Chem. Soc.* **1985**, *107*, 1825. (b) Stevens, E. S.; Sugawara, N.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1980**, *102*, 7048. (c) Gellman, S. H.; Adams, B. R. *Tetrahedron Lett.* **1989**, *30*, 3381. (d) Gellman, S. H.; Dado, G. P.; Liang, G.-B.; Adams B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164. (e) Yang, J.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 9090−9091. (f) Jones, I. G.; Jones, W.; North, M. *J. Org. Chem.* **1998**, *63*, 1505.

(18) Alonso, E.; Lopez-Ortiz, F.; del Pozo, C.; Peralta, E.; Macìas, A.; Gonzalez, J. *J. Org. Chem.* **2001**, *66*, 6333.

a γ-turn conformation, in a mixture of structure-supporting and competitive solvents an equilibrium is going on between open and closed conformations.

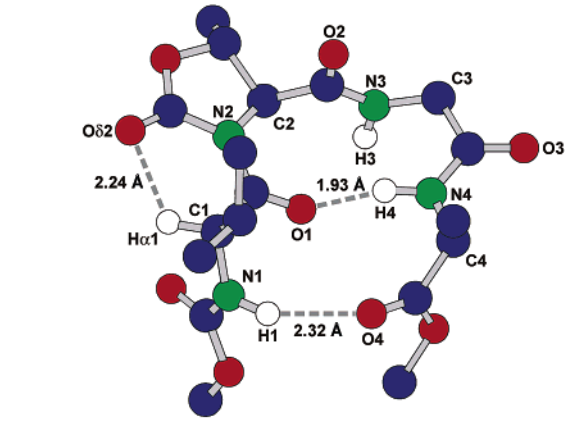
ESI−MS Analysis. Thermospray high-performance liquid chromatography/mass spectrometry (LC/ESI−MS) has been successfully used to analyze nonvolatile and thermally labile compounds. More recently, electrospray ionization (ESI)[19] linked with multiple tandem mass spectrometry has been employed in the structural characterization of a series of nonvolatile products, including oligopeptides.[20]

We have undertaken a systematic analysis of our tetramers to check their purity and to obtain some information regarding their behavior in the gas phase. This information could indeed be very useful for our research because it has been recently demonstrated that the gas-phase proteins should be viewed as tools to examine the interactions that are present in solution.[21] From the mass spectral analysis, the presence of the M + H_2O peak is outlined by a main or an abundant peak for the molecules containing L-pGlu, L-Oxd, or D-Oxd. The tests were repeated avoiding the contact of the samples with water (using dry methanol as solvent), and similar results were obtained. Thus, the M + H_2O peaks should be ascribed to the presence of a water molecule strongly bonded to the tetrapeptide between two or more functional groups. This result is not surprising; indeed, it has been recently observed that the presence of cooperative effects between more than one functional group is crucial for the hydration of a polypeptide chain. Only the molecules containing L-pGlu, L-Oxd, and D-Oxd (**4a**,**b**, **5**, **9**, **13**, and **14**) show this peak, regardless the presence of Gly or Aib. On the contrary, molecules containing L-Pro never show the peak, thus suggesting that these molecules are unable to bond to the water and that the heterocycle carbonyl is crucial for this effect.

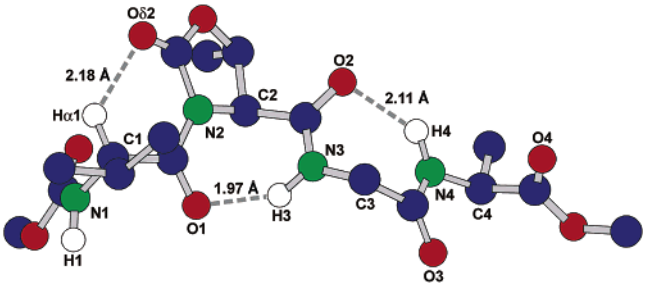
If we try to draw a hypothetical structure to explain this result, we can presume that the water is bonded somehow between the i-NH and the carbonyl of the heterocycle. This outcome could explain the DMSO-d_6 titration trends (Table 2) which highlight that i-NH is always hydrogen-bonded. Further studies are currently being made to check the nature of the interaction between water and oligomers containing L-pGlu, L-Oxd, or D-Oxd and to analyze if the water can be replaced by other molecules.

DFT Computational Modeling. In this section, the results of fully unconstrained DFT optimizations are presented for tetrameric systems analogous to **9** and **13** (**I**, MeOCO-L-Val-L-Oxd-Gly-L-Ala-OMe; **II**, MeOCO-L-Val-D-Oxd-Gly-L-Ala-OMe, respectively) (see also the Experimental Section).

Molecular mechanics and molecular dynamics are the conventional tools used for modeling oligomers and for conformational search and analysis. This is a very convenient approach when big molecular systems are under investigation. However, the reliability of the results depends heavily on the quality of the force field used. This is particularly crucial when hydrogen bonds



MeOOC-(L-Val)-(L-Oxd)-Gly-(L-Ala)-OMe **IA**



MeOOC-L-Val-L-Oxd-Gly-L-Ala-OMe **IB**

FIGURE 9. DFT-optimized structures for the tetrameric systems **I** (MeOCO-L-Val-L-Oxd-Gly-L-Ala-OMe) analogous to **9** with partial atom labeling. For clarity, only the H-bonded hydrogen atoms are shown.

in general, and weak hydrogen bonds in particular (such as C−H···O=C interactions), have to be described, which can be involved in the stabilization of complex molecular architectures. In previous works,[8,9] we have shown how weak intramolecular hydrogen bonds (i.e., C−H···O=C) play an important role in the stabilization of conformational minima for foldameric-type homooligomers. As shown above, ^{1}H NMR analysis has unveiled the presence of these features also in the oligomers investigated here (Table 1). Moreover, the hydrogen bonding network plays a very important role in the design and stability of β-turn mimics. Therefore, despite the increased computational costs, we decided to carry out our calculations at a fully correlated level (i.e., density functional theory) using both a well tested functional and basis set.

At first, a reasonable set of starting structures has been constructed to match the geometrical information revealed by NOESY spectra. Preliminary AM1 optimizations have been performed on this molecular set, and the minimum energy points located within this procedure provided the starting structures for the DFT optimizations and refinements. Although this procedure does not guarantee the location of the lowest energy minima,[22] as we will show below the DFT simulations are in agreement with the experimental evidence, and also provide an interpretation for the available observations. This provides a further validation for this approach and for the reliability of the aforementioned optimized structures.

(19) (a) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse C. M. *Mass Spectrom. Rev.* **1990**, *9*, 37. (b) Whitehouse, C. M.; Dreyer, R. N.; Yamashuta, M.; Fenn, J. B. *Anal. Chem.* **1985**, *57*, 675.
(20) Karas, M.; Hillenkamp, F. *Anal. Chem.* **1988**, *60*, 2299.
(21) Jarrold, M. F. *Acc. Chem. Res.* **1999**, *32*, 360.

(22) Extended molecular dynamics computations on these tetrameric oligomers are currently under investigation and will be reported soon.

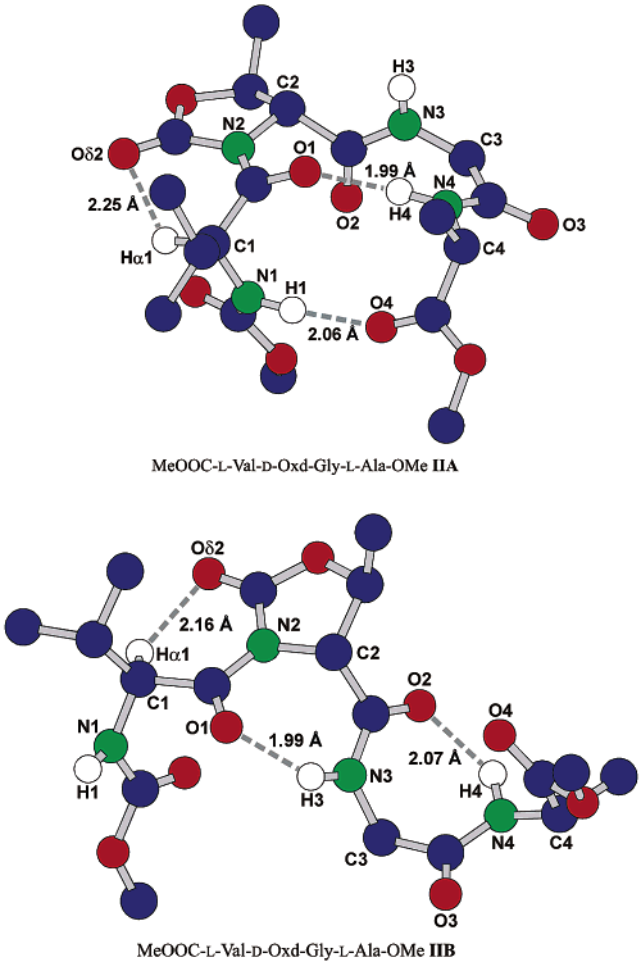


FIGURE 10. DFT-optimized structures for the tetrameric systems II (MeOCO-L-Val-D-Oxd-Gly-L-Ala-OMe) analogous to **13** with partial atom labeling. For clarity, only the H-bonded hydrogen atoms are shown.

Table 2 reports the results for the DFT-optimized lowest energy minima of these conformers: two different minima exist for both molecular systems **I** (Figure 9) and **II** (Figure 10) and are very close in energy. Compounds **IA** and **IIA** represent stable and compact β-hairpin conformations (Figures 9a and 10a), while **IB** and **IIB** are γ-turn like open structures (Figures 9b and 10b). The topography of β-turns and their mimic can be described in terms of a single virtual tortion angle β, defined by Cα1, Cα2, Cα3, and N4 of the tetrapeptide model and the interatomic distance d between Cα1 and Cα4. The DFT geometry-optimized minima **IA**, **IB**, **IIA**, and **IIB** were evaluated according to the rules fixed by Ball:[23] (1) β-turns are characterized by d less than 7.00 Å; (2) the distance between O1 and H4 should be less than 2.50 Å; (3) the virtual torsion angle β (defined by the atoms C1−C2−C3−N4) should be in the range of 0 ± 30°. All these criteria are fulfilled by conformations **IA** and **IIA**, so they are both tight β-turn conformations. Very remarkably, the energies comparison shows that the β-turn structure **IIA** is more stable than the open form **IIB**; on the contrary, for isomer **I** we obtain the opposite result: the open form **IB** is slightly preferred to **IA**.

(23) Ball, J. B.; Hughes, R. A.; Alewood, P. F.; Andrews, P. R. *Tetrahedron* **1993**, *49*, 3467.

These computational results provide a rationalization for the observed preference of **13** to give a β-hairpin structure, while **9** has been shown to prefer a less structured conformation. Furthermore, since both β-hairpin and open-chain conformations have similar energies (Table 2), tuning between the two structures can be easily induced, in principle, by changing external conditions (i.e., the environment). This can be accomplished, for example, by changing the solvents: structure-supporting solvents (such as chloroform) can increase the stability of more compact conformations such as β-turns, while highly coordinating competitive solvents (like DMSO) can invert this stability pattern, as we observed for **13**.

Conclusions

In this paper, we have shown the liquid-phase synthesis and the conformational analysis of a small library of fully protected tetramers containing L-pGlu, L-Oxd, D-Oxd, or L-Pro as residue i + 1 and Gly or Aib as residue i + 2. These compounds have all been analyzed by IR, ^{1}H NMR, and ESI−MS. The molecules containing D-Oxd showed a good propensity to the formation of a β-hairpin conformation. Among them, Boc-L-Val-D-Oxd-Gly-L-Ala-OBn assumed a preferential β-turn conformation in chloroform and a preferential γ-turn conformation in DMSO, while its epimer Boc-L-Val-L-Oxd-Gly-L-Ala-OBn showed a minor propensity to assume ordered forms. These findings have been confirmed by DFT calculations, which provide an interpretation for the available experimental data, and agree with the reported observations. On the other hand, the introduction of an Aib moiety as residue i + 2 enhanced the propensity for the β-turn conformation in the L-series while restraining it in the D-series.

Furthermore, by LC/ESI−MS analysis, we have highlighted that only the tetramers containing L-pGlu, L-Oxd, or D-Oxd show a strong tendency to chelate a molecule of water in the gas phase. This outcome suggests that this behavior is maintained in the liquid phase and that oligopeptides containing these heterocycles show a remarkable tendency to chelate water molecules. On the contrary tetramers containing L-Pro do not follow this behavior.

Experimental Section

General Methods. Routine NMR spectra were recorded with spectrometers at 300 or 200 MHz (^{1}H NMR) and at 75 or 50 MHz (^{13}C NMR). Chemical shifts are reported in δ values relative to the solvent peak of $CHCl_3$, set at 7.27 ppm. Infrared spectra were recorded with an FT-IR spectrometer. Melting points were determined in open capillaries and are uncorrected. Dry CH_2Cl_2 and dry DMF were purchased from a commercial supplier. Peptide bond formation was accomplished via standard solution-phase procedures with 1-hydroxybenzotriazole, *N*-methylmorpholine, and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.[10] The amino groups and the carboxy groups were orthogonally protected with *tert*-butyloxycarbonyl and benzyl groups. H-Gly-L-Ala-OH was purchased from a commercial supplier.

IR Studies. High-quality infrared spectra (64 scans) were obtained at 2 cm^{-1} resolution using a 1 mm NaCl solution cell and a Nicolet 210 FT-infrared spectrometer. All spectra were obtained in 3 mM solutions in dry CH_2Cl_2 at 297 K. All compounds were dried in vacuo, and all the sample preparations were performed in a nitrogen atmosphere.

^{1}H NMR Studies. High-quality ^{1}H NMR spectra were recorded with a Varian Mercury 400. Measurements were carried out in $CDCl_3$ and in DMSO-d_6 using tetramethylsilane as internal standard. Samples for conformational analysis were prepared on the benchtop and were typically between 5 and 10 mM. Proton signals were assigned by COSY spectra. Data for conformational analysis came from NOESY spectra with typical mixing times of 900 ms.

LC−MS Analysis. Acetonitrile and methanol for HPLC were purchased from a commercial supplier. All the samples were prepared by diluting 1 mg in 5 mL of a 1:1 mixture of H_2O and acetonitrile in pure acetonitrile or in pure methanol. The samples were analyzed with a liquid chromatography Agilent Technologies HP1100 equipped with a Zorbax Eclipse XDB-C8 Agilent and Technologies column (flow rate 0.5 mL/min) and equipped with a diode-array UV detector (220 and 254 nm). The MSD1100 mass detector was utilized under the following conditions: mass range 100−2500 uma, positive scanning, energy of fragmentor 50 V, drying gas flow (nitrogen) 10.0 mL/min, nebulizer pressure 45 psig, drying gas temperature 350 °C, capillary voltage 4500 V.

Boc-L-Ala-L-pGlu-OBn (2a). LiHMDS (2.2 mmol, 1M sol. in THF, 2.2 mL) was added to a stirred solution of benzyl (*S*)-pyroglutamate **1**[24] (2.0 mmol, 438 mg) in dry THF (7 mL) under nitrogen atmosphere at 0 °C. The mixture was stirred for 20 min at 0 °C and 40 min at room temperature. A solution of Boc-L-Ala-L-pGlu-OPfp (2.4 mmol) in dry THF (4 mL) was added dropwise at 0 °C. The mixture was stirred 20 min at 0 °C and 3 h at room temperature. Water (10 mL) was added, and the mixture was concentrated (rotary evaporator) to remove THF, the aqueous layer was extracted with ethyl acetate (3 × 10 mL), the combined organic layers were dried over sodium sulfate, and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (cyclohexane/ethyl acetate 8:2 as eluant) and obtained as a liquid in 80% yield: $[\alpha]_D$ −54.2 (c = 1.1 CH_2Cl_2); IR (Nujol) ν 3397, 1739, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.30 (d, 3H, J = 6.9 Hz), 1.41 (s, 9H), 2.01−2.18 (m, 1H), 2.30−2.45 (m, 1H), 2.52−2.75 (m, 2H), 4.85 (dd, 1H, J = 3.3, 9.6 Hz), 5.07 (d, 1H, J = 6.9 Hz), 5.16 (AB, 2H, J = 12.3 Hz), 5.35 (dq, 1H, J = 6.9 Hz), 7.32−7.40 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 17.6, 21.3, 28.2, 31.7, 49.7, 57.7, 67.4, 79.8, 128.2, 128.5, 134.8, 155.1, 170.4, 173.7, 174.5. Anal. Calcd for $C_{20}H_{26}N_2O_6$: C, 61.53; H, 6.71; N, 7.18. Found: C, 61.43; H, 6.79; N, 7.22.

Boc-L-Val-L-pGlu-OBn (2b). For the synthetic procedure from **1**, see the preparation of Boc-L-Ala-L-pGlu-OBn **(2a)** above: liquid, 83% yield; $[\alpha]_D$ = −18.5 (c = 1.0, CH_2Cl_2); IR (CH_2Cl_2) ν 3456, 3377, 1746, 1686 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.76 (d, 3 H, J = 6.9 Hz), 1.05 (d, 3 H, J = 6.9 Hz), 1.43 (s, 9 H), 1.98−2.15 (m, 1 H), 2.15−2.30 (m, 1 H), 2.30−2.48 (m, 1 H), 2.51−2.75 (m, 2 H), 4.85 (dd, 1 H, J = 3.6, 9.6 Hz), 5.09 (bs, 1 H), 5.18 (s, 2 H), 5.41 (dd, 1 H, J = 3.3, 9.6 Hz), 7.30−7.41 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 15.9, 20.1, 21.7, 28.6, 30.3, 32.2, 58.1, 58.5, 67.7, 79.9, 128.5, 128.8, 128.9, 155.8, 171.0, 171.7, 174.1. Anal. Calcd for $C_{22}H_{30}N_2O_6$: C, 63.14; H, 7.23; N, 6.69. Found: C, 63.23; H, 7.27; N, 6.73.

Boc-L-Ala-L-pGlu-OH (3a). Pd/C **(**10%) on charcoal (50 mg) was added to a solution of **2a** (0.51 g, 1.3 mmol) in methanol (10 mL), and the mixture was stirred in a Parr apparatus with hydrogen (3 atm) for 3 h. Then, the catalyst was filtered on a Celite pad, and the mixture was concentrated. The acid **(3a)** was obtained as a waxy solid in 98% yield (0.43 g) without any further purification: $[\alpha]_D$ = −49.5 (c = 1.0, CH_2Cl_2); IR (CH_2Cl_2) ν 3435, 1745, 1720, 1698 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.32 (d, 3 H, J = 6.6 Hz), 1.46 (s, 9 H), 2.05−2.20 (m, 2 H), 2.41−2.55 (m, 1 H), 2.60−2.82 (m, 2 H), 4.85 (dd, 1 H, J = 3.9, 9.0 Hz), 5.20 (d, 1 H, J = 9.9 Hz), 5.36 (dd, 1 H, J = 3.3, 9.9 Hz), 9.52 (bs, 1 H); ^{13}C NMR ($CDCl_3$) δ 16.9, 21.7, 28.7, 31.3, 48.3, 58.0, 80.5, 155.4, 174.3, 174.7, 175.1. Anal. Calcd for $C_{13}H_{20}N_2O_6$: C, 51.99; H, 6.71; N, 9.33. Found: C, 51.90; H, 6.67; N, 9.25.

Boc-L-Val-L-pGlu-OH (3b). For the synthetic procedure from **2b**, see the preparation of Boc-L-Ala-L-pGlu-OH **(3a)** above: yield 97%; mp = 52−57 °C; $[\alpha]_D$ = −16.1 (c = 1.0, CH_2Cl_2); IR (CH_2Cl_2) ν 3430, 1751, 1722, 1698 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.82 (d, 3 H, J = 6.9 Hz), 1.08 (d, 3 H, J = 6.6 Hz), 1.46 (s, 9 H), 2.11−2.25 (m, 2 H), 2.40−2.55 (m, 1 H), 2.55−2.80 (m, 2 H), 4.85 (dd, 1 H, J = 3.6, 9.0 Hz), 5.22 (d, 1 H, J = 9.9 Hz), 5.42 (dd, 1 H, J = 3.3, 9.9 Hz), 9.21 (bs, 1 H); ^{13}C NMR ($CDCl_3$) δ 15.9, 20.1, 21.7, 28.5, 30.3, 32.2, 58.0, 58.2, 80.2, 156.4, 174.0, 174.3, 175.1. Anal. Calcd for $C_{15}H_{24}N_2O_6$: C, 54.87; H, 7.37; N, 8.53. Found: C, 54.92; H, 7.42; N, 8.46.

Boc-L-Ala-L-pGlu-Gly-L-Ala-OBn (4a). H-Gly-L-Ala-OBn (0.26 mmol, 61 mg), NMM (0.52 mmol, 57 μL), HOBt (0.16 mmol, 22 mg), and EDCI (0.16 mmol, 31 mg) were added to a stirred solution of Boc-L-Ala-L-pGlu-OH (**3a**) (0.13 mmol, 39 mg) in dry DMF (2 mL) under nitrogen atmosphere at room temperature. The mixture was stirred at room temperature for 24 h, ethyl acetate (10 mL) was added, and the mixture was washed with 1 M aqueous HCl (2 × 7 mL) and with an aqueous saturated solution of $NaHCO_3$ (1 × 10 mL). The combined organic layers were dried over sodium sulfate, and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (cyclohexane/ethyl acetate 2:8 as eluant), and product **4a** was obtained pure as a white solid in 45% yield: mp = 120−124 °C; $[\alpha]_D$ = −16.8 (c = 0.2, CH_2Cl_2); IR (CH_2Cl_2) ν 3431, 3390, 1746, 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.37 (d, 3 H, J = 7.3 Hz), 1.44 (s, 9 H), 1.45 (d, 3 H, J = 7.2 Hz), 2.08−2.38 (m, 2 H), 2.54−2.66 (m, 1 H), 2.75−2.90 (m, 1 H), 3.81 (dd, 1 H, J = 4.8, 16.8 Hz), 4.18 (dd, 1 H, J = 6.6, 16.8 Hz), 4.62 (dq, J = 6.9 Hz), 4.70 (dd, 1 H, J = 3.0, 8.4 Hz), 5.19 (AB, J = 12.3 Hz), 5.20 (bs, 1 H), 5.34 (dq, J = 7.2 Hz), 7.01 (d, J = 7.2 Hz, 1 H), 7.25 (bs, 1 H), 7.21−7.43 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 18.0, 21.9, 28.6, 32.3, 43.2, 48.6, 50.2, 59.5, 67.5, 80.1, 128.3, 128.7, 128.9, 135.5, 155.8, 168.7, 171.4, 173.1, 174.8, 175.9. Anal. Calcd for $C_{25}H_{34}N_4O_8$: C, 57.90; H, 6.61; N, 10.80. Found: C, 57.99; H, 6.57; N, 10.72.

Boc-L-Val-L-pGlu-Gly-L-Ala-OBn (4b). For the synthetic procedure from **3b**, see the preparation of Boc-L-Ala-L-pGlu-Gly-L-Ala-OBn **(4a)** above: yield 50%; mp = 53−58 °C; $[\alpha]_D$ = +9.6 (c = 0.5, CH_2Cl_2); IR (CH_2Cl_2) ν 3431, 3383, 1748, 1681 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.77 (d, 3 H, J = 6.8 Hz), 1.05 (d, 3 H, J = 6.8 Hz), 1.43 (s, 9 H), 1.44 (d, 3 H, J = 7.6 Hz), 2.10−2.36 (m, 3 H), 2.56−2.66 (m, 1 H), 2.74−2.86 (m, 1 H), 3.77 (dd, 1 H, J = 4.8, 17.0 Hz), 4.17 (dd, 1 H, J = 6.8, 17.0 Hz), 4.56−4.66 (m, 2 H), 5.03 (d, 1 H, J = 9.6 Hz), 5.18 (AB, 2 H, J = 12.4 Hz), 5.37 (dd, 1 H, J = 3.2, 9.6 Hz), 6.64 (bs, 1 H), 6.77 (d, 1 H, J = 6.4 Hz), 7.20−7.44 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 16.0, 17.8, 19.8, 21.6, 28.3, 30.3, 32.2, 43.0, 48.3, 58.1, 59.4, 67.2, 79.8, 128.0, 128.6, 135.3, 159.1, 168.2, 170.8, 172.5, 174.5. Anal. Calcd for $C_{27}H_{38}N_4O_8$: C, 59.33; H, 7.01; N, 10.25. Found: C, 59.40; H, 7.07; N, 10.18.

Boc-L-Val-L-pGlu-Aib-L-Ala-OBn (5). H-Aib-L-Ala-OBn (0.26 mmol, 69 mg), NMM (0.52 mmol, 57 μL), HOBt (0.16 mmol, 22 mg), and EDCI (0.16 mmol, 31 mg) were added to a stirred solution of Boc-L-Val-L-pGlu-OH **(3b)** (0.13 mmol, 43 mg) in dry DMF (2 mL) under nitrogen atmosphere at room temperature. The mixture was stirred at room temperature for 24 h, ethyl acetate (10 mL) was added, and the mixture was washed with 1 M aqueous HCl (2 × 7 mL) and with an aqueous saturated solution of $NaHCO_3$ (1 × 10 mL). The combined organic layers were dried over sodium sulfate, and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (cyclohexane/ethyl acetate 2:8 as eluant) and product **4a** was obtained pure as a white solid in 58% yield: mp = 176−178 °C; $[\alpha]_D$ = −11.3 (c = 0.9, CH_2Cl_2); IR (CH_2Cl_2) ν 3417, 3370, 1746, 1713, 1693 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.75 (d, 3 H, J = 6.8 Hz), 1.04 (d, 3 H, J = 7.2 Hz), 1.40 (d, 3 H, J = 6.8 Hz), 1.43 (s, 9 H), 1.52 (s, 3 H), 1.56 (s, 3 H), 1.95−2.31 (m, 3 H), 2.56 (ddd, 1H, J = 5.2,

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9.6, 15.2 Hz), 2.74−2.85 (m, 1 H), 4.50−4.60 (m, 2 H), 5.03 (d, 1 H, J = 9.6 Hz), 5.16 (AB, 2 H, J = 12.4 Hz), 5.37 (dd, 1 H, J = 3.2, 9.6 Hz), 6.71 (s, 1 H), 6.83 (d, 1 H, J = 7.2 Hz), 7.30−7.39 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 15.9, 17.7, 19.7, 21.3, 24.3, 25.7, 28.2, 30.2, 32.1, 48.4, 57.2, 57.9, 59.4, 67.0, 79.6, 128.0, 128.3, 128.5, 135.3, 155.8, 167.0, 172.7, 173.4, 174.3. Anal. Calcd for $C_{29}H_{42}N_4O_8$: C, 60.61; H, 7.37; N, 9.75. Found: C, 60.50; H, 7.45; N, 9.81.

Boc-L-Val-L-Oxd-OBn (7). Boc-L-Val-OPfp (2 mmol, 0.76 g) was added to a stirred solution of H-L-Oxd-OBn **(6)** [9] (1.2 mmol, 0.29 g), DIEA (5 mmol, 0.87 mL), and DMAP (0.81 mmol, 99 mg) in dry DMF (2 mL) under nitrogen atmosphere at 0 °C. The mixture was stirred at room temperature for 2 h, ethyl acetate (10 mL) was added, and the mixture was washed with 1 M aqueous HCl (2 × 7 mL) and with an aqueous saturated solution of $NaHCO_3$ (1 × 10 mL). The combined organic layers were dried over sodium sulfate, and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (cyclohexane/ethyl acetate 7:3 as eluant) and obtained in 45% yield as a liquid: $[\alpha]_D$ = −21.2 (c = 1.3, CH_2Cl_2); IR (CH_2Cl_2) ν 3393, 1791, 1752, 1699 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.79 (d, 3 H, J = 7.0 Hz), 1.06 (d, 3 H, J = 6.6 Hz), 1.48 (s, 9 H), 1.55 (d, 3 H, J = 6.2 Hz), 2.05−2.20 (m, 1 H), 4.50−4.62 (m, 2 H), 5.07 (d, 1 H, J = 9.2 Hz), 5.21 (s, 2 H), 5.42 (dd, 1 H, J = 3.6, 9.2 Hz), 7.25−7.42 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 15.7, 19.7, 21.1, 28.3, 30.5, 57.1, 61.7, 68.1, 73.5, 80.0, 128.4, 128.7, 128.8, 134.4, 151.2, 155.8, 167.5, 172.9. Anal. Calcd for $C_{22}H_{30}N_2O_7$: C, 60.82; H, 6.96; N, 6.45. Found: C, 60.71; H, 7.06; N, 6.52.

Boc-L-Val-L-Oxd-OH (8). For the synthetic procedure from **7**, see the preparation of Boc-L-Ala-L-pGlu-OH **(3a)** above: yield 98%; mp = 118−122 °C; $[\alpha]_D$ = −7.4 (c = 1.3, CH_2Cl_2); IR (CH_2Cl_2) ν 3350, 1786, 1706 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.85 (d, 3 H, J = 6.9 Hz), 1.09 (d, 3 H, J = 6.6 Hz), 1.46 (s, 9 H), 1.62 (d, 3 H, J = 6.3 Hz), 2.18−2.42 (m, 1 H), 4.56 (d, 1 H, J = 5.4 Hz), 4.65−4.85 (m, 1 H), 5.27 (d, 1 H, J = 9.3 Hz), 5.32−5.48 (m, 1 H), 9.60−10.02 (bs, 1 H); ^{13}C NMR ($CDCl_3$) δ 16.1, 19.9, 21.4, 28.5, 30.8, 57.6, 61.9, 74.1, 80.7, 151.8, 156.5, 170.5, 171.1. Anal. Calcd for $C_{15}H_{24}N_2O_7$: C, 52.32; H, 7.02; N, 8.13. Found: C, 52.40; H, 7.06; N, 8.18.

Boc-L-Val-L-Oxd-Gly-L-Ala-OBn (9). For the synthetic procedure from **8**, see the preparation of Boc-L-Ala-L-pGlu-Gly-L-Ala-OBn **(4a)** above: yield 50%; mp = 59−63 °C; $[\alpha]_D$ = +18.7 (c = 0.8, CH_2Cl_2); IR (CH_2Cl_2) ν 3423, 3390, 1792, 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.82 (d, 3 H, J = 7.2 Hz), 1.07 (d, 3 H, J = 6.9 Hz), 1.43 (s, 9 H), 1.44 (d, 3 H, J = 6.6 Hz), 1.53 (d, 3 H, J = 6.6 Hz), 2.14−2.22 (m, 1 H), 3.83 (dd, 1 H, J = 4.8, 16.8 Hz), 4.15 (dd, 1 H, J = 6.9, 16.8 Hz), 4.45 (d, 1 H, J = 6.0 Hz), 4.61 (dq, 1 H, J = 7.5 Hz), 4.69 (dq, 1 H, J = 6.0 Hz), 5.10 (d, 1 H, J = 7.0 Hz), 5.19 (AB, 2 H, J = 12.0 Hz), 5.40−5.43 (m, 1 H), 7.04 (d, 1 H, J = 6.9 Hz), 7.30−7.42 (m, 5 H), 7.55 (bs, 1 H); ^{13}C NMR ($CDCl_3$) δ 16.4, 17.9, 19.9, 20.4, 28.6, 30.8, 43.3, 48.6, 57.7, 63.8, 67.5, 74.4, 80.1, 128.3, 128.8, 128.9, 135.5, 151.9, 168.1, 168.4, 173.0, 174.4. Anal. Calcd for $C_{27}H_{38}N_4O_9$: C, 57.64; H, 6.81; N, 9.96. Found: C, 57.71; H, 6.88; N, 9.89.

Boc-L-Val-D-Oxd-OBn (11). For the synthetic procedure from **10**,[9] see the preparation of Boc-L-Val-L-Oxd-OBn **(7)** above: liquid; 56% yield; $[\alpha]_D$ = +3.7 (c = 1.8, CH_2Cl_2); IR (CH_2Cl_2) ν 3383, 1799, 1760, 1706 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.89 (d, 3 H, J = 6.6 Hz), 1.12 (d, 3 H, J = 6.9 Hz), 1.48 (s, 9 H), 1.54 (d, 3 H, J = 6.3 Hz), 2.26−2.44 (m, 1 H), 4.48 (d, 1 H, J = 3.9 Hz), 4.61 (dq, 1 H, J = 3.9 Hz), 5.10 (d, 1 H, J = 9.1 Hz), 5.26 (s, 2 H), 5.46 (dd, 1 H, J = 3.9, 9.1 Hz), 7.29−7.45 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 16.5, 19.5, 21.2, 28.2, 31.1, 58.7, 62.0, 68.0, 73.6, 80.5, 128.3, 128.7, 134.6, 155.5, 167.5, 168.6, 172.9. Anal. Calcd for $C_{22}H_{30}N_2O_7$: C, 60.82; H, 6.96; N, 6.45. Found: C, 60.88; H, 7.03; N, 6.55.

Boc-L-Val-D-Oxd-OH (12). For the synthetic procedure from **11**, see the preparation of Boc-L-Ala-L-pGlu-OH **(3a)** above: yield 97%; mp = 139−143 °C; $[\alpha]_D$ = +23.8 (c = 0.8, MeOH); IR (CH_2Cl_2) ν 3350, 1786, 1706 cm^{-1}; ^{1}H NMR (CD_3OD) δ 0.83 (d, 3 H, J = 6.9 Hz), 1.00 (d, 3 H, J = 6.9 Hz), 1.44 (s, 9 H), 1.50 (d, 3 H, J = 6.3 Hz), 2.22−2.44 (m, 1 H), 4.48 (d, 1 H, J = 3.6 Hz), 4.72 (dq, 1 H, J = 3.6, 6.3 Hz), 5.38−5.48 (m, 1 H), 9.60−10.02 (bs, 1 H); ^{13}C NMR (CD_3OD) δ 14.3, 18.8, 20.2, 27.6, 31.1, 62.0, 65.8, 74.7, 79.5, 152.2, 154.2, 171.2, 172.9. Anal. Calcd for $C_{15}H_{24}N_2O_7$: C, 52.32; H, 7.02; N, 8.13. Found: C, 52.34; H, 6.99; N, 8.07.

Boc-L-Val-D-Oxd-Gly-L-Ala-OBn (13). For the synthetic procedure from **12**, see the preparation of Boc-L-Ala-L-pGlu-Gly-L-Ala-OBn **(4a)** above: yield 62%; mp = 67−71 °C; $[\alpha]_D$ = +2.3 (c = 0.8, CH_2Cl_2); IR (CH_2Cl_2) ν 3435, 3399, 3332, 1786, 1746, 1706, 1686 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.04 (d, 3 H, J = 6.6 Hz), 1.07 (d, 3 H, J = 6.8 Hz), 1.41 (s, 9 H), 1.45 (d, 3 H, J = 7.6 Hz), 1.55 (d, 3 H, J = 6.2 Hz), 1.98−2.18 (m, 1 H), 3.83 (dd, 1 H, J = 5.8, 16.8 Hz), 4.03 (dd, 1 H, J = 6.2, 16.8 Hz), 4.50 (d, 1 H, J = 4.4 Hz), 4.59 (dq, 1 H, J = 7.4 Hz), 4.82 (dq, 1 H, J = 4.4, 6.2 Hz), 5.12 (d, 1 H, J = 5.6 Hz), 5.20 (s, 2 H), 5.33 (dd, 1 H, J = 5.6 Hz), 6.79 (d, 1 H, J = 7.4 Hz), 7.20−7.40 (m, 5 H), 7.80 (bs, 1 H); ^{13}C NMR ($CDCl_3$) δ 17.7, 18.1, 19.2, 21.2, 28.3, 30.2, 43.4, 48.2, 57.4, 62.9, 67.0, 74.7, 80.8, 127.9, 128.3, 128.5, 135.3, 151.5, 167.7, 168.0, 172.1, 174.2. Anal. Calcd for $C_{27}H_{38}N_4O_9$: C, 57.64; H, 6.81; N, 9.96. Found: C, 57.59; H, 6.84; N, 10.01.

Boc-L-Val-D-Oxd-Aib-L-Ala-OBn (14). For the synthetic procedure from **8**, see the preparation of Boc-L-Val-L-pGlu-Aib-L-Ala-OBn **(5)** above: yield 53%; mp = 64−69 °C; $[\alpha]_D$ = +7.4 (c = 0.5, CH_2Cl_2); IR (CH_2Cl_2) ν 3419, 3352, 1788, 1734, 1688 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.95 (d, 3 H, J = 7.2 Hz), 1.06 (d, 3 H, J = 6.4 Hz), 1.41 (d, 3 H, J = 6.8 Hz), 1.42 (s, 9 H), 1.51 (d, 3 H, J = 6.2 Hz), 1.52 (s, 3 H), 1.55 (s, 3 H), 2.04−2.17 (m, 1 H), 4.35 (d, 1 H, J = 3.6 Hz), 4.59 (dq, 1 H, J = 6.8 Hz), 4.81 (dq, 1 H, J = 3.6, 6.2 Hz), 5.06 (d, 1 H, J = 5.6 Hz), 5.15 (AB, 2 H, J = 12.8 Hz), 5.26 (bs, 1 H), 6.61 (d, 1 H, J = 6.8 Hz), 7.00 (s, 1 H), 7.22−7.37 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 17.4, 18.3, 19.8, 21.4, 25.2, 28.6, 30.3, 31.2, 48.6, 52.6, 57.8, 63.6, 65.7, 67.3, 74.9, 80.6, 91.2, 128.4, 128.6, 128.8, 135.8, 152.0, 156.2, 167.2, 173.0, 173.6, 174.0. Anal. Calcd for $C_{29}H_{42}N_4O_9$: C, 58.97; H, 7.17; N, 9.49. Found: C, 58.89; H, 7.11; N, 9.54.

Boc-L-Val-L-Pro-Gly-L-Ala-OBn (15). This compound was obtained following the NMM, HOBt, and EDCI protocol from commercially available amino acids: mp = 69−71 °C; $[\alpha]_D$ = −23.6 (c = 0.3, CH_2Cl_2); IR (CH_2Cl_2) ν 3423, 3328, 1750, 1716, 1690, 1624 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.88 (d, 3 H, J = 6.8 Hz), 0.95 (d, 3 H, J = 6.8 Hz), 1.42 (d, 3 H, J = 8.0 Hz), 1.43 (s, 9 H), 1.90−2.22 (m, 5 H), 3.58−3.64 (m, 1 H), 3.66 (dd, 1 H, J = 4.4, 17.2 Hz), 3.76−3.88 (m, 1 H), 4.22−4.32 (m, 1 H), 4.62 (dq, 1 H, J = 6.8 Hz), 5.09 (d, 1 H, J = 9.6 Hz), 5.17 (AB, 2 H, J = 12.0 Hz), 6.64 (bs, 1 H), 7.20−7.44 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 17.6, 19.3, 25.3, 28.4, 31.3, 43.1, 47.8, 48.2, 57.1, 60.8, 67.0, 79.1, 128.1, 128.3, 128.6, 135.5, 155.8, 168.8, 171.9, 172.2, 172.8. Anal. Calcd for $C_{27}H_{40}N_4O_7$: C, 60.88; H, 7.57; N, 10.52. Found: C, 60.97; H, 7.62; N, 10.47.

Boc-L-Val-L-Pro-Aib-L-Ala-OBn (16). This compound was obtained following the NMM, HOBt, and EDCI protocol from commercially available amino acids: mp = 152−155 °C; $[\alpha]_D$ = −50.4 (c = 1.0, CH_2Cl_2); IR (CH_2Cl_2) ν 3425, 3364, 1739, 1699 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.89 (d, 3 H, J = 6.4 Hz), 0.96 (d, 3 H, J = 7.2 Hz), 1.38 (d, 3 H, J = 7.2 Hz), 1.39 (s, 3 H), 1.43 (s, 9 H), 1.44 (s, 3 H), 1.90−2.28 (m, 5 H), 3.56−3.64 (m, 1 H), 3.74−3.82 (m, 1 H), 4.25 (dd, 1 H, J = 6.8, 9.2 Hz), 4.35 (dd, 1 H, J = 4.8, 7.2 Hz), 4.54 (dq, 1 H, J = 6.8 Hz), 5.09 (d, 1 H, J = 9.6 Hz), 5.13 (AB, 2 H, J = 12.0 Hz), 6.78 (s, 1 H), 7.20−7.40 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 18.0, 18.2, 19.7, 24.8, 25.6, 26.9, 28.1, 28.7, 31.7, 48.1, 48.7, 61.2, 67.2, 80.0, 128.3, 128.4, 128.7, 154.6, 170.9, 172.5, 172.9. Anal. Calcd for $C_{29}H_{44}N_4O_7$: C, 62.12; H, 7.91; N, 9.99. Found: C, 62.18; H, 7.83; N, 10.05.

Computational Methods. The starting guess structures used for the DFT optimization of the analogous of **9** and **13** (i.e., MeOCO-L-Val-L-Oxd-Gly-L-Ala-OMe **I** and MeOCO-L-Val-D-Oxd-Gly-L-Ala-OMe **II**, respectively) have been preliminary located via AM1 optimizations. A set of reasonable structures has been initially constructed so as to match the geometrical

information revealed by NOESY spectra. AM1 optimizations have then been performed on this molecular set, and the minimum energy points located within this procedure provided the starting structures used for the DFT optimizations and refinements. All calculations were carried out using the tools available in the Gaussian 98[10] package on a SGI Origin 3800 multiprocessor system, using the DFT/B3LYP functional (i.e., Becke's three parameter hybrid functional with the Lee−Yang−Parr correlation functional).[25] This functional has been shown to properly describe both standard hydrogen-bonds,[26] as well as nonclassical weakly bound hydrogen bonds (such as C−H···O═C interactions).[27] In particular, according to the literature,[28] a mixed basis set was used for the correct description of the hydrogen bonding network: a 6-31+G(d) basis set for atoms i-NH, i-CH_α, i-CO, CO-endocyclic, (i + 1)-CO, (i + 2)-NH, (i + 3)-NH, (i + 3)-CO, and a 6-31G(d) basis set for all other atoms. The use of diffuse functions is highly recommended to properly simulate hydrogen bond interactions and the related energetics.[28]

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