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A New Class of Foldamers Based on *cis*-γ-Amino-L-proline[1,2]

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Abstract: A synthetic method for the preparation of conformationally constrained γ-peptides derived from γ-amino-L-proline is described. The methodology allows the independent buildup of the peptide backbone and the introduction of sequential variations by reactions with the α-amino group of γ-aminoproline. Both alkyl- and acyl-substituted γ-peptides have been prepared and studied by CD and NMR. Conformational restrictions due to the cyclic structure of the monomer give rise to long-range interactions that are indicative of secondary structures even in aqueous solution. Interresidue NOEs suggest a concatenation of turns that, in a permissive solvent, could give rise to an isolated hydrogen bond ribbon, flanked and protected by proline rings.

Introduction

Biopolymers encompass a broad range of structures and are involved in key aspects of our understanding of life. In nature, there are only three major biopolymer backbones, proteins, nucleic acids, and polysaccharides. Therefore, the development of new oligomers that mimic natural biopolymers should provide a powerful tool to obtain compounds with potential applications in life sciences. One particularly relevant aspect of natural biopolymers is the variability introduced by combining a limited set of monomers in a well-defined sequence. The foldamer field is directly inspired by this philosophy. The term foldamer, which was first coined by Gellman,[3] is used to describe those unnatural oligomers that in solution fold into a conformationally ordered state. Some authors have suggested a more rigid definition for this term, adding the restriction that ordered structures formed by these compounds should be stabilized by a collection of noncovalent interactions between nonadjacent monomer units.[4] Foldamers can be classified either by their secondary structure[4] or by the backbone type. Various examples of foldamers of the latter type have been described in the literature, including peptoids,[5] vinylogous polypeptides,[6] peptide nucleic acids,[7] oligoureas,[8] oligopyrrolinones,[9] oligo(phenylene ethylenes),[10] aedamers,[11] guanidines,[12] and β-[3,13] and γ-peptides.[14]

β-Peptides are probably the most extensively studied foldamers.[15] The success of these compounds is a result of three factors: their resemblance to α-peptides, the diverse range of

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(1) A preliminary report describing part of this work was presented at the 17th American Peptide Symposium: Giralt, E.; Royo, M.; Kogan, M.; Crespo, L.; Sanclimens, G.; Farrera, J.; Pons, M.; Albericio, F. Proline: a key building block in "de novo" designed peptide molecules. In *Peptides Proceedings of the 17th American Peptide Symposium*; Houghten, R., Lebl, M., Eds.; American Peptide Society: San Diego, CA, 2001; pp 432−434.

(2) Abbreviations: Ac, acetyl; MeCN, acetonitrile; Ac_2O, acetic anhydride; Amp, *cis*-4-amino-L-proline or (2*S*,4*S*)-4-amino-pyrrolidine-2-carboxylic acid; Boc, *tert*-butoxycarbonyl; Dab, α,γ-diaminobutyric acid; DCM, dichloromethane; DHB, 2,5-dihydroxybenzoic acid; DIEA, *N*,*N*-diisopropylethylamine; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; DMF, *N*,*N*-dimethylformamide; Fmoc, 9-fluorenylmethoxycarbonyl; (2*S*,4*S*)-Boc-Amp(Fmoc)-OH, (2*S*,4*S*)-Fmoc-4-amino-1-Boc-pyrrolidine-2-carboxylic acid; HOAc, acetic acid; HOBt, 1-hydroxy-1,2,3-benzotriazole; HR-ESI: high-resolution electro spray; *i*V, isovaleryl; MALDI-TOF, matrix-assisted laser desorption ionization, time-of-flight; MBHA, *p*-methylbenzhydrylamine; MeOH, methanol; MPLC, medium-pressure liquid chromatography; PhAc, phenylacetyl; RP-HPLC, reversed-phase high performance liquid chromatography; SPOS, solid-phase organic synthesis; SPPS, solid-phase peptide synthesis; TBME, *tert*-butylmethyl ether; TFA, trifluoroacetic acid; TFE, 2,2,2-trifluoroethanol; amino acid symbols denote the L-configuration.

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stabilized secondary structures that they form,[3,16] and the different applications found (e.g., antimicrobials,[17] Trojan carriers,[18] or other biological activities[19]).

Even though γ-peptides represent the natural next step for the generation of a new family of foldamers based on the amide backbone, only a few examples of γ-peptides have been reported in the literature,[20] and these systems are based on linear amino acids with substituents at different backbone positions.[14,21] Different substitution patterns introduce diversity into the different types of secondary structures induced and, as a result, in the possible applications of these materials.[21,22] The increased number of degrees of freedom in γ-peptides, as compared to α- and β-peptides, can be reduced by the use of cyclic monomers,[7b,c,23] which increase the stability of regular secondary structures. The cyclic monomers described to date show less diversity than the linear ones, and there is not much distinction between a conserved backbone and a diversity-carrying side chain.[13g,23g,h,24]

In the work described here, we developed a synthetic strategy to obtain two new families of γ-peptides formed by the cyclic monomer *cis*-γ-amino-L-proline. The backbone in these peptides contains amide/peptide bonds between the carboxyl function and the γ-amino function of successive residues. The α-amino group is left free for the introduction of different substituents, either during the synthesis of the oligomers (obtaining hetero-oligomers) or as a final functionalization step (obtaining homooligomers). The independent buildup of the backbone and side-chain sequences leads to a very high level of synthetic versatility. We explored both acylation and alkylation for the introduction of the side chains to give N^{α}-acyl-γ-peptides and N^{α}-alkyl-γ-peptides, respectively.

CD and NMR studies were used to elucidate the structural features of these new families of compounds in different solvents, and we compared these features with those of acyclic γ-peptides based on α,γ-diaminobutyric acid (Figure 1).

Results and Discussion

Synthesis and Characterization of γ-Hexapeptides. To study the influence on the secondary structure of the different types of linkage between the side chain and the γ-peptide backbone, we prepared two families of γ-peptides: N^{α}-acyl-γ-peptides and N^{α}-alkyl-γ-peptides. An Fmoc/Boc combined solid-phase strategy was chosen, where Fmoc was the temporary protecting group for the γ-amino group of each monomer and Boc was the semipermanent protecting group for the α-amino group through which the side chain was introduced.[25] The same

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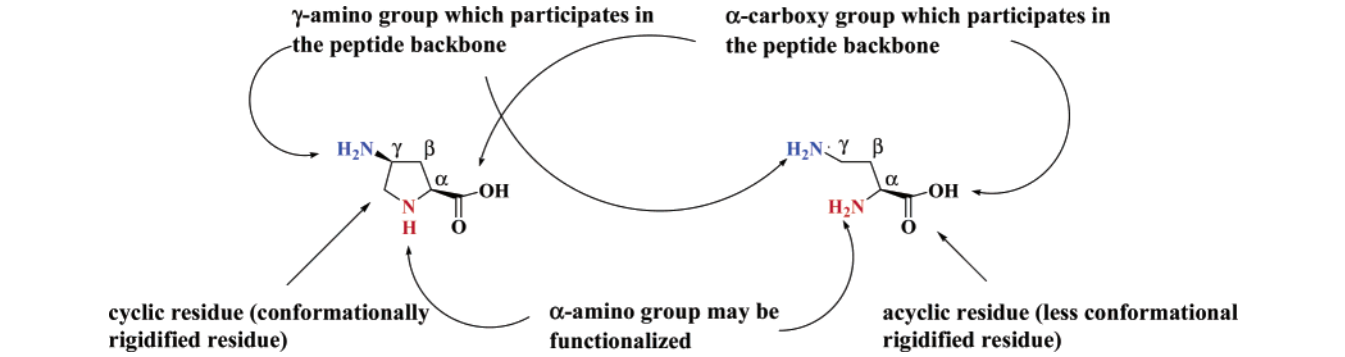

Figure 1. Monomers used in these studies: (left) *cis*-γ-amino-L-proline and (right) L-α,γ-diaminobutyric acid. Peptide synthesis was carried out through the γ-amino group, to give the γ-peptide backbone. The α-amino group allows diversity to be introduced and mimics the protein α-amino acid side chains.

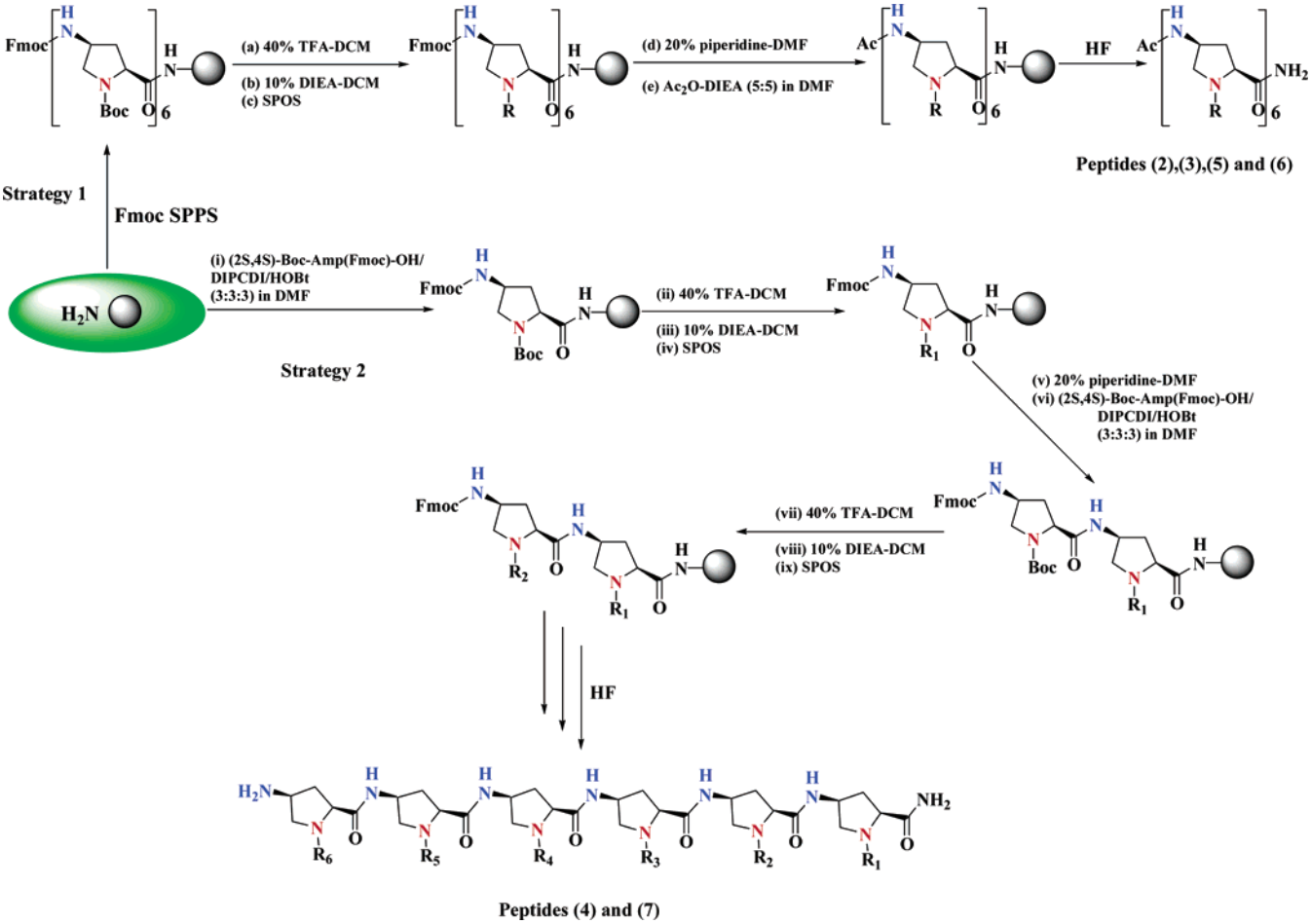

Figure 2. Synthesis of the N^{α}-alkyl-γ-hexapeptides and the N^{α}-acyl-γ-hexapeptides (synthesis of homooligomeric systems and of oligomers with different side chains for both cases). In the case of the homooligopeptides (strategy 1), we first synthesized the peptide backbone using the Fmoc strategy and functionalized the α-amino groups at the end. In the case of the heterofunctionalized γ-hexapeptides (strategy 2), we introduced the corresponding side chain after each monomer coupling. For the acylation of the α-amino groups to obtain N^{α}-acyl-γ-hexapeptides, we used the corresponding acid, DIPCDI, and HOBt in DMF (step c for strategy 1 and steps iv and ix for strategy 2). The N^{α}-alkyl-γ-hexapeptides were obtained by alkylation of the α-amino group of each monomer, through a reductive amination reaction (step c for strategy 1 and steps iv and ix for strategy 2) with the corresponding aldehyde and $NaBH_3$-CN in 1% HOAc/DMF.

strategy allows the synthesis of either homooligomeric γ-peptides (identical side chains) or heterooligomeric γ-peptides (with different side chains). In the case of γ-peptide homooligomers, the backbone was synthesized first, and, after removal of all Boc protecting groups, all of the side chains were introduced at the same time. This strategy was carried out for N^{α}-acyl-γ-peptides and N^{α}-alkyl-γ-peptides (Figure 2, strategy 1). In the case of the sequentially functionalized γ-peptides, different side chains were introduced after the coupling of each monomer (Figure 2, strategy 2).

The γ-peptide backbone was prepared from the protected amino acid (2*S*,4*S*)-Fmoc-4-amino-1-Boc-pyrrolidine-2-carboxylic acid [(2*S*,4*S*)-Boc-Amp(Fmoc)-OH] using DIPCDI with HOBt. The reaction was monitored by the ninhydrin test.[26] The

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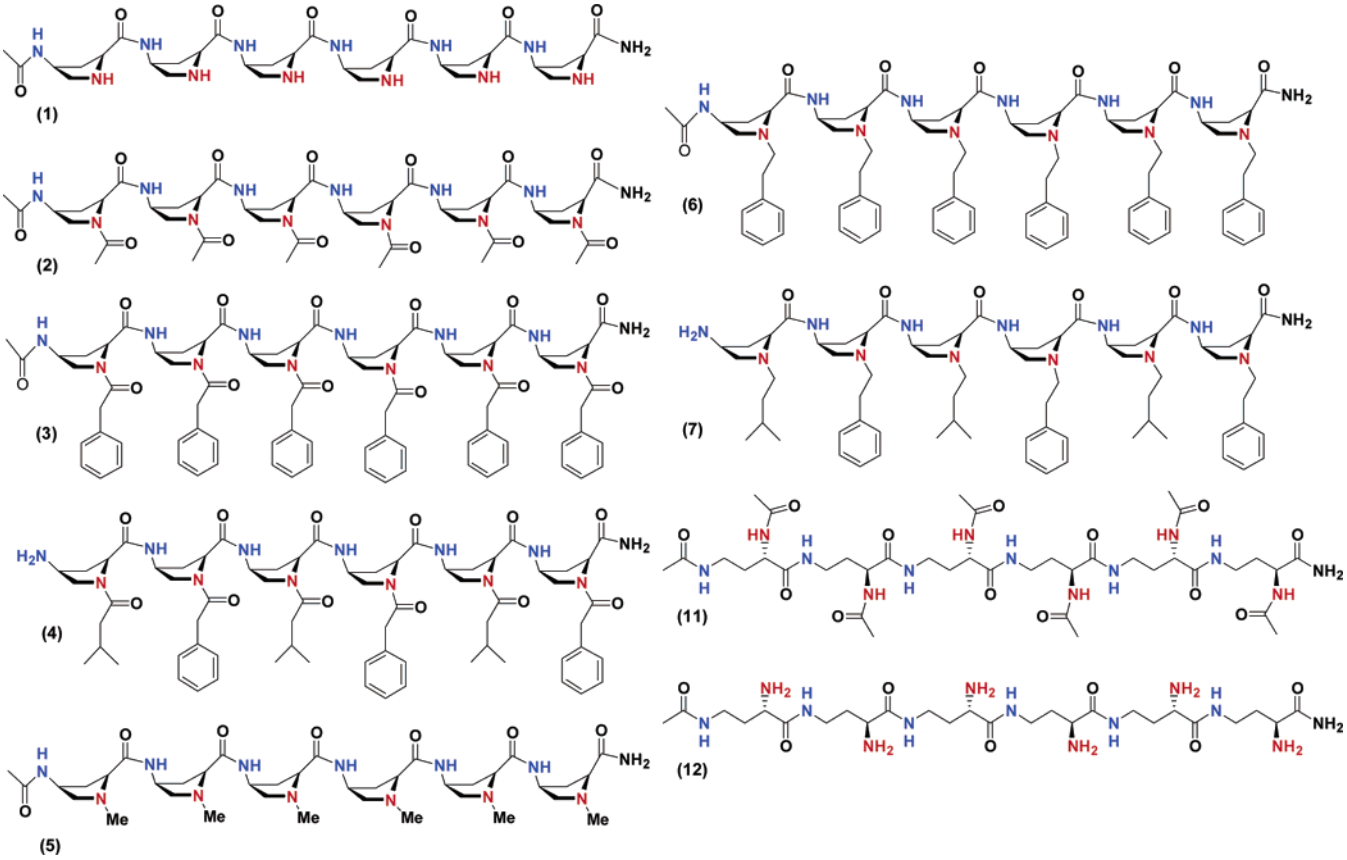

Figure 3. Chemical structures of the *cis*-γ-aminoproline and α,γ-diaminobutyric acid peptide oligomers synthesized in this work.

side chain of N^{α}-acyl-γ-peptides was introduced using the corresponding carboxylic acid and the coupling reagents described above. In this case, the reaction was followed either by the chloranil[27] or by the De Clercq[28,29] tests which detect secondary amines. For N^{α}-alkyl-γ-peptides, the alkyl group was introduced by reductive amination using the corresponding aldehyde and $NaBH_3CN$. This reaction also was monitored by the chloranil test.

The γ-peptide family based on α,γ-diaminobutyric acid was prepared following the same synthetic strategy using N^{α}-Boc-N^{γ}-Fmoc-L-diaminobutyric acid.

At the end of the synthesis, and after removal of the Fmoc group, the N-terminal amino group was acetylated. However, in a few cases, the terminal amino group was kept free (**4**, **7**) to increase solubility in H_2O. Peptides were cleaved from the resin by acidolytic treatment with anhydrous HF. The purity of the crude γ-peptides ranged from 70% to 95% as determined by HPLC. Compounds were purified to >95% homogeneity by preparative reversed-phase HPLC prior to characterization by electrospray and/or MALDI-TOF mass spectrometry. All compounds gave results consistent with the desired products. The chemical structures of the γ-hexapeptides prepared in this work (**1**−**7**, **11**, and **12**) are shown in Figure 3.

Circular Dichroism. The CD spectra of the γ-peptides reported here were obtained in different solvents: H_2O, MeOH, and TFE in the range 190−250 nm. Peptides were studied in the concentration range 100−1000 μM. The CD spectra are independent of concentration, indicating that aggregation does not occur. The CD spectra of peptides **1**, **2**, **4**, **5**, **11**, and **12** are shown in Figure 4. The largest differences in CD spectra are between γ-peptides derived from γ-aminoproline with acyl or alkyl side chains. Compounds **2** and **4** belong to the N^{α}-acyl-γ-peptide family. The CD spectrum of compound **2** in H_2O shows two minima at 204 and 217 nm and a zero crossing at ca. 200 nm. The CD spectrum of **2** in TFE also shows two minima, but these are slightly shifted to 202 and 223 nm. In MeOH, the CD spectrum shows only one negative maxima at 204 nm. The CD spectrum of peptide **4** in H_2O shows only one negative maxima at 207 nm and a zero crossing at ca. 197 nm; this is similar to the spectrum obtained in MeOH. The CD spectrum in TFE shows a broad negative band with a negative maxima at 207 nm. By comparison with the CD spectrum of **2** in the same solvent, the wide band can be interpreted as resulting from the overlap of the two minima observed for this peptide.[30]

Peptides **1** and **5** are representative of the N^{α}-alkyl-γ-peptides derived from γ-aminoproline and show CD patterns that are markedly different from those of **2** and **4**. The CD spectra of **1** and **5** each show a positive maxima at ca. 200 nm in the three

(26) The γ-amino functions of these building blocks anchored on the resin give a clear positive Kaiser ninhydrin test, so this test is useful in controlling the synthesis of this kind of peptide. Kaiser, E.; Colescott, R L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 594−598.
(27) Christensen, T. *Acta Chem. Scand.* **1979**, *33*, 760−766.
(28) Madder, A.; Farcy, N.; Hosten, N. G. C.; De Muynck, H.; De Clercq, P.; Barry, J.; Davis, A. P. *Eur. J. Org. Chem.* **1999**, *11*, 2787−2791.
(29) Although the De Clercq test seems more sensitive than the the chloranil test (De Clercq gives slightly positive when chloranil gives negative), the final product whose synthesis has been controlled by just the chloranil test is good to excellent.
(30) This different intensities of the CD bands between **2** and **4** could be related to the presence of an aromatic side chain in **4** or to the increased bulkiness of the substituents.

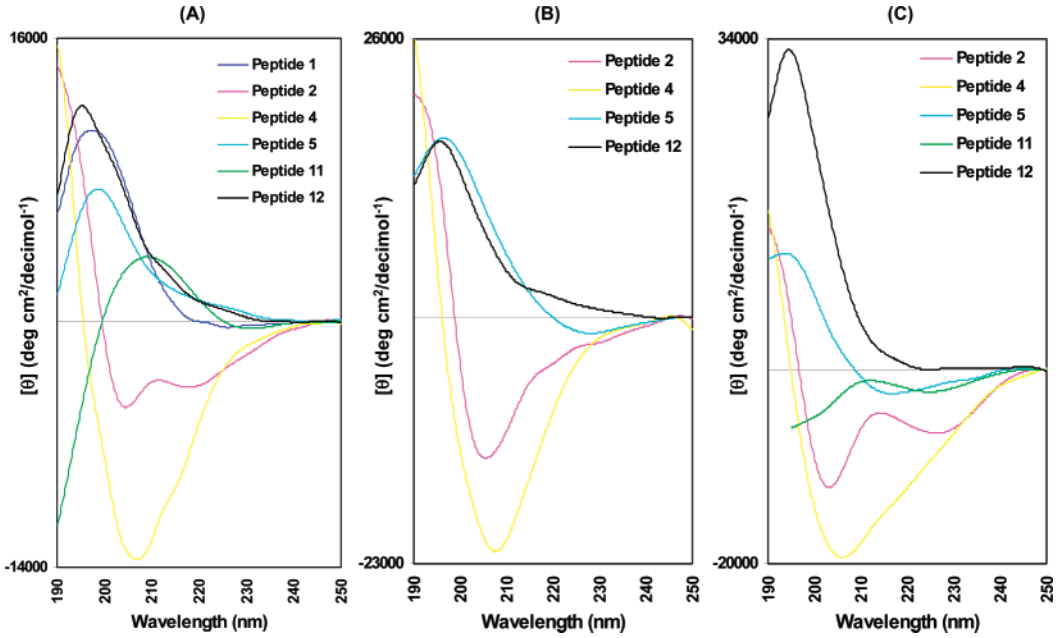

Figure 4. Circular dichroism spectra for peptide hexamers (**1**, **2**, **4**, **5**, **11**, and **12**) in (A) H_2O, (B) MeOH, and (C) TFE at 25 °C. Data were normalized for γ-peptide concentration and number of monomer groups.

solvents studied. Compound **1** in H_2O at pH = 12.1 (results not shown) and compound **5** in methanol both show a weak negative maxima at ca. 220−230 nm. The CD spectrum of peptide **5** in TFE shows a more intense minimum at ca. 220 nm, thus giving a CD pattern more similar to that observed for the N^{α}-acyl-γ-peptide family.

Peptide **12** (derived from α,γ-diaminobutyric acid, with a free α-amino group) presents a CD pattern similar to that of the N^{α}-alkyl-γ-peptides derived from γ-aminoproline. The corresponding acylated form (peptide **11**) has completely different CD spectra in H_2O and TFE. In TFE, peptide **11** gives rise to a CD spectrum resembling that of the acylated γ-peptides derived from γ-aminoproline. In H_2O, on the other hand, **11** shows a completely different spectrum that is characterized by a maximum at ca. 210 nm.

The CD pattern in each family of γ-peptides was compared to those observed for monomeric model compounds that were representative of each class. The CD spectra in H_2O of each γ-peptide family resemble that of its corresponding model compound when the data are normalized in terms of the number of amide chromophores. The CD spectral features observed for alkyl and acyl peptides should therefore be attributed mainly to interactions involving the additional amide chromophore in the second family (Figure 5). This situation is not unexpected given that interactions between chromophores responsible for the CD spectra are distance-dependent and the interactions between the backbone and side-chain amide bonds in the N^{α}-acyl-γ-peptides are equivalent to the interaction between adjacent backbone peptide bonds in α-peptides. It is these latter interactions that give rise to the well-known CD spectra of natural peptides.

Within each family, the CD spectra show significant solvent dependency. In acylated peptide **2**, derived from γ-aminoproline, the long wavelength minimum is shifted from 217 to 225 nm on changing solvent from H_2O to TFE. The corresponding peak appears as a shoulder in MeOH. More strikingly, in alkylated

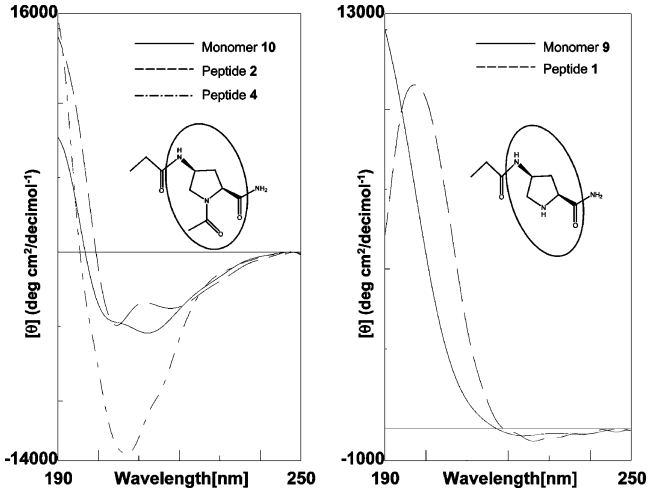

Figure 5. Circular dichroism spectra for the different peptide oligomers in H_2O at 25 °C. The data have been normalized for γ-peptide concentration and number of monomer groups.

γ-aminoproline peptides, the CD signal at around 220 nm changes from positive (in H_2O) to negative (in MeOH and TFE). Interestingly, the absence of amide chromophores in the side chains indicates that the observed changes in the CD spectra reflect a different conformation of the peptide backbone in H_2O than in TFE or MeOH. Backbone cyclization has a major effect on the structure of γ-peptides, as deduced from the CD spectra of **11** and **2** in H_2O and TFE. The CD spectrum of **11** changes from having a positive maximum in H_2O to one characterized by two minima, while the spectra of **2** show two minima in all solvents studied. This behavior suggests that the spectra with two minima could correspond to a structural feature that is preserved in all solvents in the peptide with a cyclized backbone, but is only present in TFE when the backbone is not restrained.

The large differences in the CD spectra between alkylated and acylated peptides **11** and **12** suggest some structural role

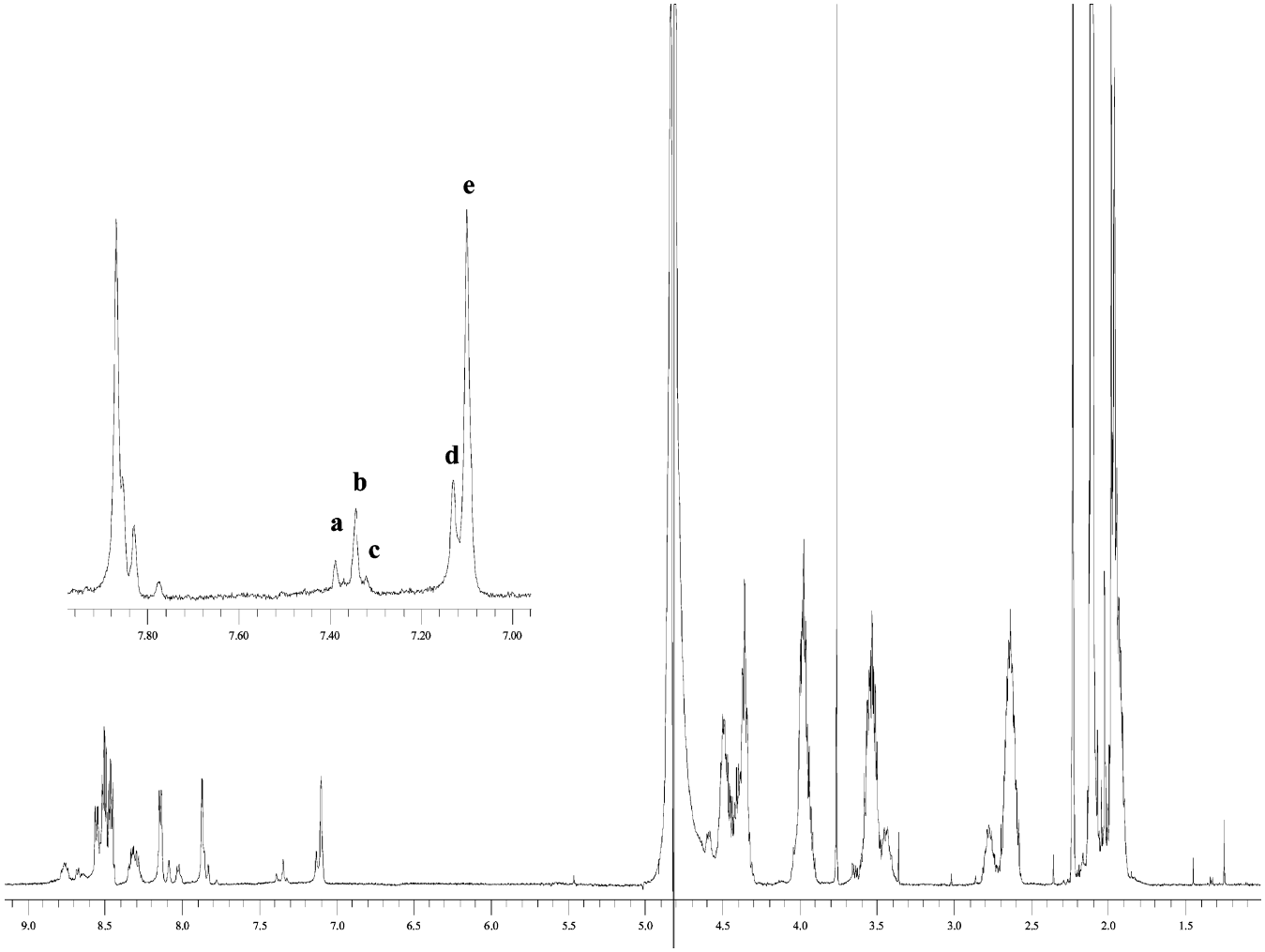

Figure 6. ^{1}H NMR spectrum of γ-peptide **2** in H_2O plus 10% D_2O. The inset shows an expansion of the carboxamide region. Labeled peaks correspond to different isomers. The relative intensities are (a)1:(b)2.8:(c)0.46:(d)3.4:(e)10.8.

for the amide group in the side chain, at least in the peptides formed by noncyclic γ-amino acids.

Nuclear Magnetic Resonance. The ^{1}H NMR spectrum of γ-peptide **2** in H_2O/D_2O (9:1) is shown in Figure 6.[31] The presence of different sets of signals is evident, and this corresponds to different conformations in slow exchange. On considering the carboxamide signals (inset in Figure 6), it can be seen that at least five different conformations are present. The different conformations can be traced to the presence of possible side-chain cis−trans isomers for each of the side-chain amide groups. This situation was confirmed by the observation of two isomers in 3:1 relative populations in the model monomer **10**, which contains an acetyl side chain, and a single population for monomer **9** with a free α-amino group (spectra not shown).

The number of possible isomers in the hexamer is 64 (2^6). However, the chemical shift of the C-terminal carboxamide depends mainly on the conformation of the side chain of the last residues. The relative populations of the four major isomers observed in the carboxamide region are close to 9:3:3:1. These are the expected populations if we consider that the conformations of neighboring side chains are independent of each other and the relative stabilities of the trans and cis isomers are the same as in the monomer (3:1).

The presence of strong hydrogen bonds involving the main-chain amide groups can be ruled out on the basis of the observation of the same temperature coefficients (−6 ppb K^{-1}) for all of the observed amide protons, a fact that suggests a flexible structure.

However, the $^3J_{NH-\gamma}$ couplings of all internal amide protons show values of around 7.5 Hz, which are slightly higher than the expected 6.5 Hz for a freely rotating group, as observed for the N-terminal amide proton. In addition, the presence of a number of interresidue NOEs suggests a preferred average conformation of proline γ-peptides.

NOE spectra were obtained in the rotating frame as the global correlation tune of the peptides studied gave only very weak NOEs in the laboratory frame. In addition, strong overlap due to the highly repetitive sequence of the peptides studied prevented complete sequential assignment of the backbone protons. However, the observation of an NOE in the N^{α}-acyl family between protons located in opposite faces of the proline ring was interpreted as evidence of interresidue interactions. Indirect NOE (three-spin effects) can be ruled out in rotating frame experiments as the resulting cross-peaks would have

(31) Peptides were practically not soluble in $CDCl_3$, but were soluble in H_2O, and some of them were soluble in MeOH and TFE.

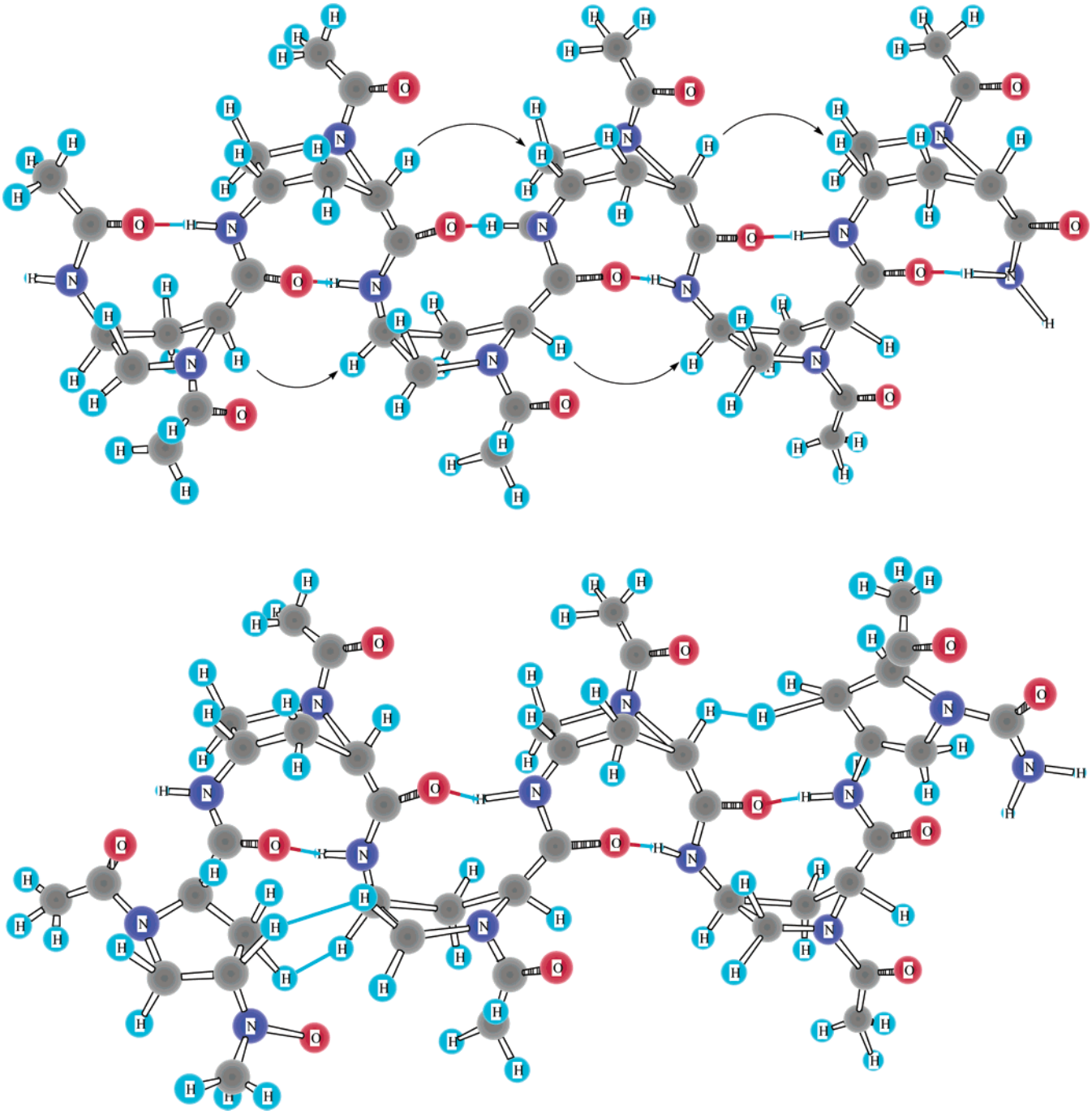

Figure 7. Top: structural model for peptide **2** deduced from NMR data. Bottom: related structure for peptide **2** derived by reorientation of the N- and C-terminal proline residues, with the observed NOE contacts indicated.

opposite signs. Interresidue NOEs are observed between protons γ and β', $\alpha-\beta'$ and $\gamma-\delta'$, where the "prime" symbol denotes the proton trans to the α proton of the proline ring. γ-Peptides containing a free or an alkylated α-amino group show a slightly different pattern of interresidue NOEs. The $\alpha-\beta'$ strong NOE is still observed. However, the $\beta'-\gamma$ NOE is not observed in the free peptide **1** and is very weak in the methylated peptide **5**. Additional strong NOEs are observed between protons β, α, and δ'.

The observed interresidue NOEs cannot be explained as interactions between consecutive residues. However, the NOEs are consistent with short distances between residues i and i+2 in the structures shown in Figure 7 by rotation of the C- and N-terminal proline groups. Due to the evidence of flexibility, no attempt to derive a single structure using restrained minimization was attempted. The suggested structure for peptide **2** in H_2O is an idealized model for the folding of proline-derived γ-peptides. The structure can be described as a series of turns in which the two amide bonds connected to the α and γ positions of each proline are in the same plane, which is perpendicular to the average plane of the proline rings. In aqueous solution, however, hydrogen bonds are not formed, a situation indicated by the temperature coefficients and the coupling constants measured on NH residues. In the minimized structure, protons

α and γ in prolines i and i+2 are at 5 Å. This structure also accounts for the observed coupling constant of 7.5 Hz $^3J_{NH-\gamma}$ as the dihedral angle between the two bonds is close to 0°.

Concluding Remarks

The γ-amino-L-proline is a very convenient building block for the preparation of γ-peptides. The use of two orthogonal protecting groups for both amino groups of the building block leads to a flexible synthesis strategy for alkyl- and acyl-substituted γ-peptides, allowing a convenient method for the preparation of both homo- and heterooligomers.

The CD spectra of alkylated peptides suggest that proline-containing γ-peptides show solvent-dependent secondary structures. In addition, comparison of the CD spectra of γ-peptides prepared from γ-amino-L-proline and γ-aminobutyric acid in different solvents indicates that the proline-derived γ-peptides retain in aqueous solutions a conformation that is present only in TFE solution in the linear analogues. NMR spectra in aqueous solution show interresidue NOEs that are consistent with a folded structure in which vectors perpendicular to the mean plane of adjacent proline rings are alternatively pointing up and down and, moreover, the peptide bonds are in a common plane perpendicular to those of the proline ring.

The turn-like conformation induced by the γ-aminoproline skeleton could stabilize one of the smallest conceivable β-meander structures, which could prove useful as a model for an isolated, β-sheet-like, hydrogen bond ribbon.

Work is in progress to characterize the biological properties of proline γ-peptides which, as shown in this work, are readily accessible from a synthetic point of view with a versatility that opens the way to a variety of applications.

Experimental Section

Materials and Equipment. (2*S*,4*S*)-Fmoc-4-amino-1-Boc-pyrrolidine-2-carboxylic acid and N^{α}-Boc-N^{γ}-Fmoc-L-diaminobutyric acid were obtained from Neosystem (Strasbourg, France), and MBHA resin (0.7 mmol/g) was supplied by Calbiochem-Novabiochem AG. DIPCDI was obtained from Fluka Chemika (Buchs, Switzerland), and HOBt was from Albatross Chem, Inc. (Montreal, Canada). Solvents for peptide synthesis and RP-HPLC equipment were obtained from Scharlau (Barcelona, Spain). Trifluoroacetic acid was supplied by KaliChemie (Bad Wimpfen, Germany). Other chemicals were obtained from Aldrich (Milwaukee, WI) and were of the highest purity commercially available. All commercial reagents and solvents were used as received. HF was obtained from Air Products and Chemicals, Inc. (Allentown, Canada), and the equipment was from Peptide Institute Inc., Minoh, Osaka, Japan. Analytical RP-HPLC was performed using Shimadzu (Kyoto, Japan) or Waters (Milford, MA) chromatography systems with reversed-phase Kromasil C_{18} (250 × 4 mm) 10 μm and Symmetry C_{18} (150 × 4.6 mm) 5 μm columns with UV detection at 220 nm. Semipreparative RP-HPLC was performed on a Waters (Milford, MA) chromatography system using Vydac C_8 (1 × 25 cm, 10 μm) and Symmetry C_8 (3 × 10 cm, 5 μm) columns. Compounds were detected by UV absorption at 220 nm. Mass spectra were recorded on a MALDI Voyager DE RP time-of-flight (TOF) spectrometer (Applied Biosystems, Framingham). DHB was used as a matrix and was purchased from Aldrich.

Circular Dichroism. CD measurements were obtained using a Jasco model 720 spectropolarimeter at 25 °C. Spectra were obtained in fused quartz cells with path lengths in the range 0.1−1 mm. Peptide samples as TFA salts were dissolved in an appropriate amount of the selected solvent [(H_2O (pH in all cases was in the 6.0−6.5 range), MeOH, and TFE)]. A baseline correction was measured with only solvent in the cell. Data are expressed in terms of mean residue ellipticity, [θ] (deg cm^2 $dmol^{-1}$), calculated per mol of total amide groups (chromophores) present in the different molecules.

NMR Spectroscopy. NMR spectra were acquired on a Bruker 500 spectrometer. γ-Hexapeptides were dissolved in H_2O/D_2O (9:1) at concentrations of ∼3 mg/mL at 25 °C apart from the temperature experiments, which were collected between 5 and 30 °C under the control of a Eurotherm variable-temperature unit with an accuracy of 0.1 °C. Two-dimensional spectra (COSY, TOCSY, NOESY, and ROESY) were recorded employing standard pulse sequences with the number of acquisitions typically set to 64 for the COSY, NOESY, and ROESY and 32 for the TOCSY experiments. Presaturation was used to suppress the water resonance. TOCSY spectra were recorded with an isotropic mixing time of 70 ms. NOESY spectra were acquired with mixing times of 150 and 400 ms. ROESY spectra were collected with mixing times of 150 and 200 ms. The temperature coefficients of the amide protons for compound **2** were studied by acquiring monodimensional spectra at six different temperatures between 278 and 303 K in 5° increments and are reported in −ppb/K. All spectra were processed with VNMR on an PC computer.

General Procedures. Solid-Phase Synthesis. Peptide syntheses were performed manually in a polypropylene syringe fitted with a polyethylene porous disk. Solvents and soluble reagents were removed by suction. Washings between deprotection, coupling, and subsequent deprotection steps were carried out with DMF (5 × 1 min) and DCM (5 × 1 min) using 10 mL of solvent/g of resin each time.

Fmoc Group Removal. (i) DMF (5 × 1 min); (ii) piperidine/DMF (2:8) (1 × 1 min + 2 × 15 min); (iii) DMF (5 × 1 min).

Boc Group Removal. (i) DCM (5 × 1 min); (ii) TFA/DCM (4:6) (1 × 1 min + 1 × 30 min); (iii) DCM (5 × 1 min); (iv) DIEA/DCM (5:95) (3 × 3 min); (v) DCM (5 × 1 min).

Solid-Phase γ-Peptide Backbone Elongation. All syntheses were carried out with MBHA resin (1 g) by an Fmoc/Boc combined solid-phase strategy. Couplings of Boc-Amp(Fmoc)-OH (1.58 g, 5 equiv) and Fmoc-Dab-OH (1.53 g, 5 equiv) were carried out with DIPCDI (540 μL, 5 equiv) and HOBt (472 mg, 5 equiv) in DMF for 2 h at 25 °C. After the coupling, the resin was washed with DMF (5 × 1 min) and DCM (5 × 1 min). Couplings were monitored by the Kaiser test.

Synthesis of Homo N^{α}-Acyl-γ-hexapeptides. After the N^{α}-Boc groups had been removed, acylation of the α-amino groups was carried out using RCOOH (30 equiv, 5 equiv for each amine), DIPCDI (540 μL, 30 equiv), and HOBt (472 mg, 30 equiv) in DMF for 2 h at 25 °C. The resin was washed with DMF (5 × 1 min) and DCM (5 × 1 min). The acylation was monitored by the chloranil test.

Synthesis of Hetero N^{α}-Acyl-γ-hexapeptides. Once the monomer had been introduced by coupling of the corresponding protected monomer, the N^{α}-Boc protecting group was removed and the acyl function was introduced as above. After removal of the N^{γ}-Fmoc protecting group, the reactive sequence was repeated.

Synthesis of Homo N^{α}-Alkyl-γ-hexapeptides. After the Boc groups had been removed, alkylation of the α-amino group was performed by on-resin reductive amination using RCHO (30 equiv, 5 equiv for each amine) and $NaBH_3CN$ (232 mg, 30 equiv) in 1% HOAc in DMF for 2 h. After the reductive amination, the resin was washed with DMF (5 × 1 min) and DCM (5 × 1 min). The alkylation was monitored by the chloranil test.

Synthesis of Hetero N^{α}-Alkyl-γ-hexapeptides. This transformation was carried out by the sequential acylation methodology, but in this case the alkylation conditions outlined above were used.

Acetylation. The end terminal N^{γ}-amino Fmoc protecting group was removed and was terminated with Ac_2O (337 μL, 5 equiv) and DIEA (607 μL, 5 equiv) in DMF for 2 h. The resin was washed with DMF (5 × 1 min) and DCM (5 × 1 min).

Acidolytic Cleavage with HF. The peptide resin was washed with MeOH (3 × 1 min), dried, and treated with HF in the presence of 10% anisole for 1 h at 0 °C. Peptides were precipitated with cold anhydrous TBME, dissolved in HOAc, and then lyophilized.

Ac-(γAmp)$_6$-NH$_2$ (1): The crude peptide was purified by preparative HPLC using a linear gradient of MeCN (containing 1% of TFA) and H_2O (containing 1% of TFA). The purity of each fraction was verified by analytical HPLC and MALDI-TOF and showed that the peptides were 95−99% pure. ^{1}H NMR [H_2O/D_2O (9:1), 500 MHz]: δ = 1.848−1.945 (m, 5H; 5Hβ); 1.962 (s, 3H; CH_3); 1.998−2.052 (m, 1H; Hβ); 2.587−2.647 (m, 5H; Hβ'); 2.673−2.719 (m, 1H; Hβ'); 3.017−3.057 (m, 4H; 4Hδ); 3.001 (dd, $J_{\delta-\delta'}$ = 12.0 Hz and $J_{\delta-\gamma}$ = 4.5 Hz; 1H, Hδ); 3.200 (dd, $J_{\delta-\delta'}$ = 12.0 Hz and $J_{\delta-\gamma}$ = 4.5 Hz; 1H, Hδ); 3.334−3.383 (m, 4H; Hδ'); 3.398−3.450 (m, 2H; Hδ'); 4.011 (t, $J_{\alpha-\beta}$ = 7.5 Hz; 4H, 4Hα); 4.081 (t, $J_{\alpha-\beta}$ = 7 Hz, 1H; Hα); 4.190 (dd, $J_{\alpha-\beta}$ = 7 Hz and $J_{\alpha-\beta'}$ = 9 Hz, 1H; Hα); 4.337−4.427 (m, 6H; 6Hγ); 7.274 (s, 1H; carboxamide); 7.895 (s, 1H; carboxamide); 8.138 (d, $J_{NH-\gamma}$ = 5.5 Hz, 1H; N-terminal amide); MS calcd for $C_{32}H_{53}N_{13}O_7$, $[M + H]^+$ 732.4263; HR-ESI found, $[M + H]^+$ 732.4239; MALDI-TOF found, 732.97 $[M + H]^+$, 754.99 $[M + Na]^+$, and 771.97 $[M + K]^+$.

Ac-[γAmp(N^α-Ac)]$_6$-NH$_2$ (2): The crude peptide was purified by preparative HPLC using a linear gradient of MeCN (containing 1% of TFA) and H_2O (containing 1% of TFA). The purity of each fraction was verified by analytical HPLC and MALDI-TOF and showed that the peptides were 95−99% pure. ^{1}H NMR [H_2O/D_2O (9:1), 500 MHz]: 1.900−1.983 (m, 6H; Hβ'); 1.973 (s, 3H; Nt methyl); 2.091−2.105 (m, 18H; side-chain methyls); 2.572−2.685 (m, 6H; Hβ); 3.490−3.570 (m, 6H; 6Hδ); 3.916−4.010 (m, 6H; 6Hδ'); 4.328−4.372 (m, 6H; 6Hα); 4.442−4.499 (m, 6H; 6Hγ); 7.087 (s, 1H; carboxamide); 7.855 (s, 1H; carboxamide); 8.126 (d, $J_{NH-\gamma}$ = 6.5 Hz, 1H; N-terminal amide); 8.499 (m, $J_{NH-\gamma}$ around 7.5 Hz, 5H; central amides); these NMR data correspond to the majority species, although there are other minority species. MS calcd for $C_{44}H_{65}N_{13}O_{13}$, $[M + Na]^+$ 1006.4717; HR-ESI found, $[M + Na]^+$ 1006.4765; MALDI-TOF found, $[M + H]^+$ 984.57, $[M + Na]^+$ 1006.60, and $[M + K]^+$ 1022.56.

Ac-[γAmp(N^α-PhAc)]$_6$-NH$_2$ (3): The crude peptide was purified by preparative HPLC using a linear gradient of MeCN (containing 1% of TFA) and H_2O (containing 1% of TFA). The purity of each fraction was verified by analytical HPLC and MALDI-TOF and showed that the peptides were 95−99% pure. MALDI-TOF calcd for $C_{80}H_{89}N_{13}O_{13}$, $[M + H]^+$ 1439.67; found, $[M + H]^+$ 1440.28, $[M + Na]^+$ 1464.61, and $[M + K]^+$ 1479.57. This peptide could be synthesized, but it proved impossible to obtain the CD and NMR data for solubility reasons.

H-[γAmp(N^α*i*V)-γAmp(N^α-PhAc)]$_3$-NH$_2$ (4): The crude peptide was purified by preparative HPLC using a linear gradient of MeCN (containing 1% of TFA) and H_2O (containing 1% of TFA). The purity of each fraction was verified by analytical HPLC and MALDI-TOF and showed the peptides to be 95−99% pure. ^{1}H NMR [H_2O/D_2O (9:1), 500 MHz]: 0.910−0.959 (m, 18H; isovaleric methyls); 1.270 (s, 1H; primary amine NH_2); 1.800−1.950 (m, 6H; 6Hβ'); 2.037−2.087 (m, 3H; three isovaleric CH); 2.138−2.240 (m, 6H; three isovaleric methylenes CH_2); 2.452−2.567 (m, 6H; 6Hβ); 3.464−3.536 (m, 6H; 6Hδ'); 3.656−3.719 (m, 6H; three CH_2 linkage to phenyl); 3.799−3.896 (m, 6H; 6Hδ); 4.227−4.335 (m, 6H; 6Hα); 4.363−4.478 (m, 6H; 6Hγ); 7.162 (s, 1H; carboxamide); 7.187−7.271 (m, 15H; aromatic protons); 7.811 (s, 1H; carboxamide); 8.561 (d, $J_{NH-\gamma}$ = 7.5 Hz, 1H; amide); 8.616−8.679 (m, 4H; central backbone amides); 8.874 (d, $J_{NH-\gamma}$ = 8 Hz, 1H; amide); these NMR data correspond to the major species, although there are other minor species. MALDI-TOF calcd for $C_{69}H_{93}N_{13}O_{12}$, $[M + H]^+$ 1295.71; found, $[M + H]^+$ 1296.88, $[M + Na]^+$ 1318.88, and $[M + K]^+$ 1334.85.

Ac-[γAmp(N^α-Me)]$_6$-NH$_2$ (5): The crude peptide was purified by preparative HPLC using a linear gradient of MeCN (containing 1% of TFA) and H_2O (containing 1% of TFA). The purity of each fraction was verified by analytical HPLC and MALDI-TOF and showed the peptides to be 95−99% pure. ^{1}H NMR [H_2O/D_2O (9:1), 500 MHz]: 1.986 (s, 3H, CH_3 acetyl); 2.053−2.141 (m, 4H, Hβ'); 2.201−2.211 (m, 2H, Hβ'); 2.824 (s, 9H, 3 × CH_3); 2.845 (s, 3H, CH_3); 2.901 (s, 3H, CH_3); 2.911−2.974 (m, 9H, 6 Hβ and one CH_3); 3.402−3.569 (m, 6H, 6Hδ); 3.612−3.779 (m, 6H, 6Hδ'); 3.991−4.086 (m, 4H, 4Hα); 4.160 (t, $J_{\alpha-\beta,\beta'}$ = 9 Hz; 1H, Hα); 4.226 (t, $J_{\alpha-\beta,\beta'}$ = 9 Hz; 1H, Hα); 4.450−4.511 (m, 1H, Hγ); 4.570−4.645 (m, 5H, 5Hγ); 7.525 (s, 1H, carboxamide); 8.054 (s, 1H, carboxamide); 8.300 (d, $J_{NH-\gamma}$ = 5.5 Hz; 1H, amide Nt); 8.900 (bs.); MS calcd for $C_{38}H_{65}N_{13}O_7$, $[M + H]^+$ 816.5202; HR-ESI found, $[M + H]^+$ 816.5212; MALDI-TOF found, $[M + 3H]^+$ 818.03, $[M + Na]^+$ 840.08, and $[M + K]^+$ 856.09.

Ac-[γAmp(N^α-CH$_2$CH$_2$Ph)]$_6$-NH$_2$ (6): The crude peptide was purified by preparative HPLC using a linear gradient of MeCN (containing 1% of TFA) and H_2O (containing 1% of TFA). The purity of each fraction was verified by analytical HPLC and MALDI-TOF and showed the peptides to be 95−99% pure. ^{1}H NMR (H_2O-D_2O (9:1), 500 MHz): 1.951−2.091 (m, 18H, 6Hβ' and 6 × CH_2); 2.800−3.117 (m, 18H, 6Hβ and 6 × CH_2); 3.268−3.780 (m, 12H, 6Hδ and 6Hδ'); 4.088−4.427 (m, 12H, 6Hα and 6Hγ); 7.252−7.402 (m, 30H, aromatics); 7.464 (s, 1H, carboxamide); 8.007 (s, 1H, carboxamide); 8.264−8.276 (d, $J_{NH-\gamma}$ = 6 Hz; 1H, Nt amide); MALDI-TOF calcd for $C_{80}H_{101}N_{13}O_7$, $[M + H]^+$ 1355.79; found, $[M + H]^+$ 1359.83, $[M + Na]^+$ 1382.07, and $[M + K]^+$ 1397.05.

H-{γAmp[N^α-CH$_2$CH$_2$CH(CH$_3$)$_2$]-γAmp(N^α-CH$_2$CH$_2$Ph)}$_3$-NH$_2$ (7): The crude peptide was purified by preparative HPLC using a linear gradient of MeCN (containing 1% of TFA) and H_2O (containing 1% of TFA). The purity of each fraction was verified by analytical HPLC and MALDI-TOF and showed the peptides to be 95−99% pure. ^{1}H NMR (H_2O-D_2O (9:1), 600 MHz): 0.883−0.919 (m, 18H, 6 × CH_3 isopropylic); 1.494−1.598 (m, 6H, 3 × CH_2 isopropyl); 1.613−1.686 (m, 3H, 3 × CH isopropyl); 2.007−2.157 (m, 6H, 6Hβ'); 2.200−2.267 (m, 6H, 3 × CH_2 phenyl); 2.900−3.144 (m, 12H, 6Hβ and 3 × CH_2 phenyl linkage to N); 3.172−3.315 (m, 6H, isopropylic 3 × CH_2 linkage to N); 3.404−3.659 (m, 6H, 6Hδ); 3.760−3.922 (m, 6H, 6Hδ'); 4.253−4.410 (m, 6H, 6Hα); 4.700−4.900 (m, 6H, 6Hγ); 7.291−7.429 (m, 15H, aromatics); 7.554 (s, 1H, carboxamide); 8.060 (s, 1H, carboxamide); 8.963−8.973 (d, $J_{NH-\gamma}$ = 6 Hz; 1H, amide); 9.016−9.039 (t, $J_{NH-\gamma}$ = 7.2 Hz; 2H, central amides); 9.061−9.072 (d, $J_{NH-\gamma}$ = 6.6 Hz; 1H, amide); 9.184 (bc., 1H, *Nt* amine); MALDI-TOF calcd for $C_{69}H_{105}N_{13}O_6$, $[M + H]^+$ 1211.83; found, $[M + H]^+$ 1212.79, $[M + Na]^+$ 1234.76, and $[M + K]^+$ 1250.37.

H-[γAmp(N^α-Ac)]-NH$_2$ (8): This compound was synthesized in the solid phase using a Boc/Fmoc strategy. The synthesis was carried out on 350 μmol of MBHA resin at a substitution level of 0.7 mmol/g. The Boc-Amp(Fmoc)-OH monomer was first coupled onto the resin by using the same Fmoc/*t*Bu strategy used above. When the monomer was coupled to the resin, the N^α-Boc protecting group was removed and the α-amino group was acetylated using Ac_2O (170 μL, 5 equiv) and DIEA (305 μL, 5 equiv) in DMF for 2 h. The Fmoc group was removed and the peptide cleaved from the resin. The crude peptide was precipitated with anhydrous diethyl ether, dissolved in HOAc, and lyophilized. The crude peptide was not purified by preparative HPLC because the product was obtained in good yield and with excellent purity. The purity of the sample was verified by analytical HPLC and MALDI-TOF. ^{1}H NMR [H_2O/D_2O (9:1), 500 MHz]: 2.010 (s, 3H, CH_3); 2.131 (s, 2H, NH_2); 2.175 (ddd, $J_{\beta-\beta'}$ = 15 Hz, $J_{\beta-\alpha}$ = 4.2 Hz, $J_{\beta-\gamma}$ = 4.2 Hz; 1H, Hβ); 2.745 (ddd, $J_{\beta'-\beta}$ = 15 Hz, $J_{\beta'-\alpha}$ = 9.6 Hz, $J_{\beta'-\gamma}$ = 6.6 Hz; 1H, Hβ'); 3.860 (dd, $J_{\delta-\delta'}$ = 12 Hz, $J_{\delta-\gamma}$ = 2.4 Hz; 1H, Hδ); 4.044 (dd, $J_{\delta'-\delta}$ = 12 Hz, $J_{\delta'-\gamma}$ = 6 Hz; 1H, Hδ'); 4.116 (dddd, $J_{\gamma-\beta'}$ = 6.6 Hz, $J_{\gamma-\delta'}$ = 6 Hz, $J_{\gamma-\beta}$ = 4.2 Hz, $J_{\gamma-\delta}$ = 2.4 Hz; 1H, Hγ); 4.521 (dd, $J_{\alpha-\beta'}$ = 9.6 Hz, $J_{\alpha-\beta}$ = 4.2 Hz; 1H, Hα); 7.291 (s, 1H, carboxamide); 8.017 (s, 1H, carboxamide); MS calcd for $C_7H_{13}N_3O_2$, $[M + H]^+$ 172.1080; HR-ESI found, $[M + H]^+$ 172.1073; MALDI-TOF found, $[M + H]^+$ 171.96, $[M + Na]^+$ 193.94, and $[M + K]^+$ 209.90.

CH$_3$CH$_2$CO-(γAmp)-NH$_2$ (9): This compound was synthesized in the solid phase using a Boc/Fmoc strategy with 350 μmol of MBHA resin at a substitution level of 0.7 mmol/g. The Boc-Amp(Fmoc)-OH monomer was first coupled onto the resin by a general Fmoc/*t*Bu strategy. The coupling of Boc-Amp(Fmoc)-OH (790 mg, 5 equiv) was carried out with the same protocol used above. When the monomer

had been coupled to the resin, the Fmoc group was removed and the acylation of the γ-amino group was carried out using CH_3CH_2COOH (130 μL, 5 equiv), DIPCDI (270 μL, 5 equiv), and HOBt (236 mg, 5 equiv) in DMF for 2 h. After the coupling was complete, the N^{α}-Boc protecting group was removed. Peptides were cleaved from the resin with anhydrous HF in the presence of 10% anisole for 1 h at 0 °C. The crude peptide was precipitated with anhydrous diethyl ether, dissolved in HOAc, and lyophilized. The crude peptide was not purified by preparative HPLC because the target compound was obtained in good yield and with excellent purity. The purity of the sample was verified by analytical HPLC and MALDI-TOF. ^{1}H NMR [H_2O/D_2O (9:1), 500 MHz]: 1.075 (t, $J_{CH3-CH2}$ = 7.8 Hz; 3H, CH_3); 2.001 (s, 1H, NH); 2.161 (ddd, $J_{\beta',\beta}$ = 14.4 Hz, $J_{\beta',\alpha}$ = 7.2 Hz, $J_{\beta',\gamma}$ = 7.2 Hz; 1H, Hβ′); 2.236 (q, $J_{CH2-CH3}$ = 7.8 Hz; 2H, CH_2); 2.800 (ddd, $J_{\beta,\beta'}$ = 13.8 Hz, $J_{\beta,\alpha}$ = 7.2 Hz, $J_{\beta,\gamma}$ = 7.2 Hz; 1H, Hβ); 3.405 (dd, $J_{\delta-\delta'}$ = 12 Hz, $J_{\delta-\gamma}$ = 5.4 Hz; 1H, Hδ); 3.657 (dd, $J_{\delta'-\delta}$ = 12 Hz, $J_{\delta'-\gamma}$ = 7.2 Hz; 1H, Hδ′); 4.480−4.518 (m, 2H, Hα and Hγ); 7.407 (s, 1H, carboxamide); 7.952 (s, 1H, carboxamide); 8.110 (s, 1H, amide); MS calcd for $C_8H_{15}N_3O_2$, $[M + H]^+$ 186.1237; HR-ESI found, $[M + H]^+$ 186.1232; MALDI-TOF found, $[M + H]^+$ 186.03, $[M + Na]^+$ 208.00, and $[M + K]^+$ 223.97.

CH_3CH_2CO-[γAmp(N^{α}-Ac)]-NH_2 (10): This compound was synthesized in the solid phase using a Boc/Fmoc strategy. The synthesis was carried out on 350 μmol of MBHA resin at a substitution level of 0.7 mmol/g. The coupling of Boc-Amp(Fmoc)-OH (790 mg, 5 equiv) was carried using the same protocol described above. The N^{α}-Boc protecting group was removed, and the acylation of the α-amino group was carried out using Ac_2O (84 μL, 5 equiv) and DIEA (155 μL, 5 equiv) in DMF for 2 h. The Fmoc group was removed, and the acylation of the γ-amino group was carried out using CH_3CH_2COOH (130 μL, 5 equiv), DIPCDI (270 μL, 5 equiv), and HOBt (236 mg, 5 equiv) in DMF for 2 h. The peptide was cleaved from the resin with anhydrous HF in the presence of 10% anisole for 1 h at 0 °C. The crude peptide was precipitated with anhydrous diethyl ether, dissolved in HOAc, and lyophilized. The crude peptide was not purified by preparative HPLC because the compound was obtained in good yield and with excellent purity. The purity of the sample was verified by analytical HPLC and MALDI-TOF. ^{1}H NMR [H_2O/D_2O (9:1), 500 MHz]: 1.081 (t, $J_{CH3-CH2}$ = 7.8 Hz; 3H, CH_3); 1.974−2.019 (m, 1H, Hβ); 2.116 (s, 3H, methyl Nα); 2.212−2.238 (q, $J_{CH2-CH3}$ = 7.8 Hz; 2H, CH_2); 2.618 (ddd, $J_{\beta,\beta}$ = 13.8 Hz, $J_{\beta,\alpha}$ = 9 Hz, $J_{\beta,\gamma}$ = 6 Hz; 1H, Hβ); 3.527 (dd, $J_{\delta-\delta'}$ = 11.1 Hz, $J_{\delta-\gamma}$ = 5.4 Hz; 1H, Hδ); 3.975 (dd, $J_{\delta'-\delta}$ = 11.1 Hz, $J_{\delta'-\gamma}$ = 6.6 Hz; 1H, Hδ′); 4.401−4.449 (m, 2H, Hα and Hγ); 7.098 (s, 1H, carboxamide); 7.778 (s, 1H, carboxamide); 8.011 (d, $J_{NH-\gamma}$ = 5 Hz; 1H, amide). These NMR data correspond to the majority compound; in the NMR spectrum is observed another minority species corresponding to the cis isomer in the side chain with relative population 3:1. MS calcd for $C_{10}H_{17}N_3O_3$, $[M + Na]^+$ 250.1162; HR-ESI found, $[M + Na]^+$ 250.1150; MALDI-TOF found, $[M + H]^+$ 227.92, $[M + Na]^+$ 249.94, and $[M + K]^+$ 265.86.

Ac-[Dab(N^{α}-Ac)]$_6$-NH_2 (11): The crude peptide was purified by preparative HPLC using a linear gradient of MeCN (containing 1% of TFA) and H_2O (containing 1% of TFA). The purity of each fraction was verified by analytical HPLC and MALDI-TOF and showed the peptides to be 95−99% pure. ^{1}H NMR [H_2O/D_2O (9:1), 500 MHz]: 1.788−1.878 (m, 6H, Hβ); 1.971−2.060 (m, 27H; 6Hβ, 3H backbone methyls and 18H side-chain methyls); 3.236−3.325 (m, 12H, Hγ); 4.138−4.180 (m, 4H, Hα); 4.196−4.238 (m, 2H, Hα); 7.093 (s, 1H, carboxamide); 7.695 (s, 1H, carboxamide); 7.966 (t, $J_{NH-\gamma}$ = 5.5 Hz; 1H, amide Nt); 8.128−8.162 (m, 5H, backbone amides); 8.326−8.374 (m, 6H, side-chain amides); MS calcd for $C_{38}H_{65}N_{13}O_{13}$, $[M + Na]^+$ 934.4717; HR-ESI found, $[M + Na]^+$ 934.4741; MALDI-TOF found, $[M + Na]^+$ 936.34 and $[M + K]^+$ 952.30.

Ac-(Dab)$_6$-NH_2 (12): The crude peptide was purified by preparative HPLC using a linear gradient of MeCN (containing 1% of TFA) and H_2O (containing 1% of TFA). The purity of each fraction was verified by analytical HPLC and MALDI-TOF and showed the peptides to be 95−99% pure. ^{1}H NMR [H_2O/D_2O (9:1), 600 MHz]: 2.002 (s, 3H, CH_3 acetyl); 2.031−2.172 (m, 12H, Hβ); 3.290−3.350 (m, 6H, Hγ); 3.399−3.485 (m, 6H, Hγ); 3.537−3.567 (m, 2H, amines); 3.631−3.658 (m, 2H, amines); 3.757−3.793 (m, 2H, amines); 3.959−4.056 (m, 6H, Hα); 7.396 (s, 1H, carboxamide); 7.935 (s, 1H, carboxamide); 8.110 (t, J = 5 Hz; 1H, amide Nt); 8.440−8.700 (m, 5H, backbone amides); MS calcd for $C_{26}H_{53}N_{13}O_7$, $[M + H]^+$ 660.4263; HR-ESI found, $[M + H]^+$ 660.4269; MALDI-TOF found, $[M + H]^+$ 661.87, $[M + Na]^+$ 683.88, and $[M + K]^+$ 699.88.

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