Pseudopeptide Foldamers: The Homo-Oligomers of Pyroglutamic Acid

Fernando Bernardi,[a] Marco Garavelli,[a] Marco Scatizzi,[a] Claudia Tomasini,*[a] Valerio Trigari,[a] Marco Crisma,[b] Fernando Formaggio,[b] Cristina Peggion,[b] and Claudio Toniolo*[b]

Abstract: As a part of a program evaluating substituted γ-lactams as conformationally constrained building blocks of pseudopeptide foldamers, we synthesized the homo-oligomers of L-pyroglutamic acid up to the tetramer level by solution methods. The preferred conformation of this pseudopeptide series in structure-supporting solvents was assessed by FT-IR absorption, ^{1}H NMR and CD techniques. In addition, the crystal structure of the N^{α}-protected dimer was established by X-ray diffraction. A high-level DFT computational modeling was performed based on the crystallographic parameters. In this analysis, we demonstrated that an αC–H···O=C intramolecular hydrogen bond is responsible for the stabilization of the s-*trans* L-pGlu-L-pGlu conformation by 1.4 kcal mol^{-1}. This effect can be easily detected by ^{1}H NMR spectroscopy, owing to the anomalous chemical shifts of the αCH protons present in all of the oligomers. In summary, we have developed a new polyimide-based, foldameric structure that, if appropriately functionalized, has promise as a rigid scaffold for novel functions and applications.

Keywords: conformation analysis · foldamers · molecular modeling · oligoimides · structure elucidation

Introduction

Foldamers are unnatural oligomers or polymers, based on natural or unnatural building blocks, which generate well-defined three-dimensional structures. Since the stimulating *manifesto* on foldamers by Gellman in 1998,[1] investigation into these new structural scaffolds has blossomed in many laboratories as they hold promise for addressing chemical, physicochemical, and biological problems and represent a new frontier in research.

In this context, we have recently started a program aimed at the expansion of the known set of foldameric oligomers based on pseudopeptide units with homogeneous backbones, that is, built from a single type of monomer.[2] The ability to incorporate different functional groups at defined positions along the main chain will ultimately facilitate the preparation of foldamer combinatorial libraries.

Here, we describe the synthesis, chemical characterization, and conclusions of a detailed conformational analysis in solution performed by FT-IR absorption, ^{1}H NMR and CD techniques on the homo-oligomers (from monomer to tetramer) of L-pyroglutamic acid (L-pGlu = (*S*)-2-pyrrolidone-5-carboxylic acid). Furthermore, we discuss the results of the crystal structure analysis by X-ray diffraction, and of a high-level DFT computational modeling of Boc-(L-pGlu)$_2$-OH (Boc = *tert*-butoxycarbonyl), which demonstrate that a stabilizing αC–H···O=C intramolecular hydrogen bond is present.

Figure 1 shows that pGlu is the γ-lactam dehydrated analogue of Glu and is structurally related to Pro, since both pGlu and Pro are conformationally restricted, five-membered ring systems. L-pGlu is a naturally occurring residue and is frequently found at the N-terminus (but not in an internal position) of bioactive peptides, as it lacks the α-amino functionality of α-amino acids that is replaced by a much more acylation-resistant amide function.

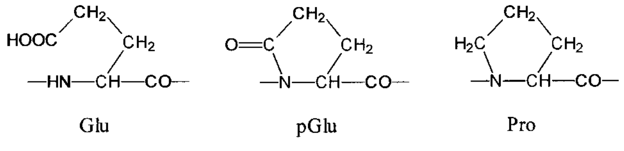

Figure 1. α-Amino acids discussed in this work.

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[a] Prof. C. Tomasini, Prof. F. Bernardi,
Dr. M. Garavelli, Dr. M. Scatizzi, Dr. V. Trigari
Department of Chemistry "G. Ciamician"
Alma Mater Studiorum, University of Bologna
Via Selmi 2, 40126 Bologna (Italy)
Fax: (+39) 051-209-9456
E-mail: tomasini@ciam.unibo.it

[b] Prof. C. Toniolo, Dr. M. Crisma, Prof. F. Formaggio, Dr. C. Peggion
Institute of Biomolecular Chemistry, CNR
Department of Organic Chemistry, University of Padova
Via Marzolo 1, 35131 Padova (Italy)
Fax: (+39) 049-827-5239
E-mail: claudio.toniolo@unipd.it

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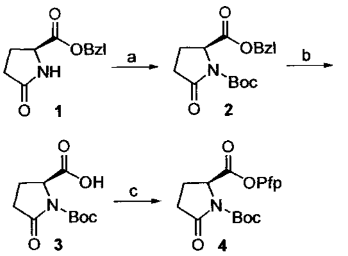
Results and Discussion

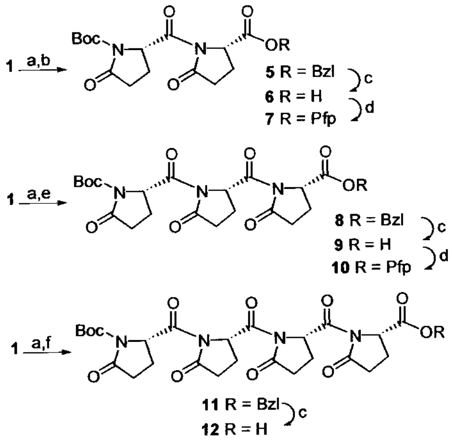
Synthesis and characterization: L-pGlu contains a lactam moiety, which is responsible for the very low reactivity of the NH function. For this reason, very few examples of N-acylation of pyroglutamates have been reported.[3] As L-pGlu homo-oligomers are polyimides, the usual peptide (amide) coupling methods, for example, carbodiimide and 1-hydroxy-1,2,3-benzotriazole,[4] fail. An easy and very convenient approach might involve the use of H-L-pGlu-Cl.[5] We tested the coupling of this very reactive compound with the lithium anion of H-L-pGlu-OBzl (OBzl = benzyloxy) (**1**), which in turn was obtained by reaction of **1** with lithium hexamethyldisilazide (LiHMDS) (Scheme 1). Unfortunately, this straightforward strategy afforded only a complex mixture with no desired product. We therefore moved to an N^{α}-protected form of L-pGlu (Boc-L-pGlu-OH), which forced us to replace the unstable acyl chloride intermediate with a milder activated derivative, such as the pentafluorophenyl (Pfp) ester. For this purpose, L-pGlu derivatives **1**–**4** were prepared by known procedures.[6–8] In this context, we have recently described the synthesis of Boc-(L-Ala)$_2$-L-pGlu-OBzl and other dipeptides by reaction of the lithium anion of H-L-pGlu-OBzl with carboxylic pentafluorophenylesters.[9]



Scheme 1. Synthesis of Boc-L-pGlu-OPfp (**4**). a) Boc$_2$O, DIEA, DMAP (cat.), dry DMF; b) H$_2$, 10% Pd, EtOAc; c) CF$_3$CO$_2$C$_6$F$_5$, pyridine, dry DMF.

Abstract in Italian: *Come parte di un programma volto a utilizzare γ-lattami sostituiti come unità di base conformazionalmente ristrette di foldameri pseudopeptidici, abbiamo sintetizzato, usando metodi in soluzione, gli omo-oligomeri dell'acido* L-*piroglutammico fino al tetrametro. Abbiamo esaminato le loro preferenze conformazionali in solventi strutturanti con l'impiego dell'assorbimento IR, ^{1}H NMR e DC. Inoltre, abbiamo determinato mediante diffrazione dei raggi X la struttura allo stato cristallino del dimero N-protetto. Basandoci sui parametri così ottenuti, abbiamo fatto calcoli di modellistica computazionale DFT. Abbiamo così potuto dimostrare che è presente un legame a idrogeno intramolecolare αC–H···O=C responsabile della stabilizzazione della conformazione* L-*pGlu*-L-*pGlu s-trans di 1.4 kcal mol^{-1}. Questo effetto, che può essere facilmente rilevato mediante analisi ^{1}H NMR a causa degli spostamenti chimici anomali dei protoni αCH coinvolti nei legami a idrogeno, è stato notato in tutti gli oligomeri. In conclusione abbiamo messo a punto una nuova struttura foldamerica poli-immidica che, se funzionalizzata in modo appropriato, potrà essere utilmente impiegata come impalcatura rigida per composti con nuove funzioni e proprietà.*

For the synthesis of the dimer, the first step was the formation of the lithium anion of **1** in dry tetrahydrofuran (THF) at room temperature (Scheme 2). Then, a solution of **4** in dry THF was added. Pure dimer **5** was obtained in 70% yield by application of this method. Milder reaction conditions, such as diisopropylethylamine (DIEA) and catalytic 4-(dimethylamino)pyridine (DMAP) in *N*,*N*-dimethylformamide (DMF) or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DMF, were also tried, but low yields of the desired product **5** were obtained (Table 1).



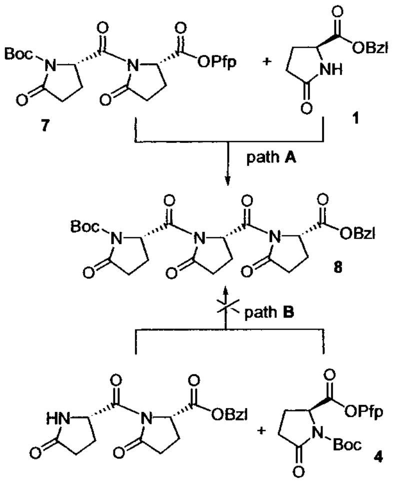
Scheme 2. Synthesis of the Boc-(L-pGlu)$_n$-OR homo-oligomers. a) LiHMDS, dry THF; b) **4** in dry THF; c) H$_2$, 10% Pd, EtOAc; d) CF$_3$CO$_2$C$_6$F$_5$, pyridine, dry DMF; e) **7** in dry THF; f) **10** in dry THF.

Table 1. Reaction conditions and chemical yield for the synthesis of Boc-(L-pGlu)$_2$-OBzl (**5**).

Entry	Solvent	Base (equiv)	t [h]	T [°C]	Yield [%]
1	DMF	DBU (3)	2	0 → RT	25
2	DMF	DIEA (3), DMAP (0.25)	2	0 → RT	30
3	THF	LiHMDS (1.1)	2	0 → RT	70

In principle, to synthesize longer homo-oligomers, main-chain elongation could be performed from the N-terminal side (path **A**) or from the C-terminal side (path **B**) (Scheme 3). To evaluate these two routes, Boc-(L-pGlu)$_2$-OBzl (**5**) was deprotected either at the nitrogen with trifluoroacetic acid (TFA) in methylene chloride, or at the carboxyl group by catalytic hydrogenation. Chain elongation was attempted at both sides, but no trimer was obtained by reaction of the lithium anion of H-(L-pGlu)$_2$-OBzl with Boc-L-pGlu-OPfp (**4**) (path **B**). In this last case, the starting materials decomposed and a complex mixture with no trimer **8** was obtained, probably owing to the low stability of the pseudopeptide ester anion. Therefore, the trimer and the tetramer were synthesized in good yield following path **A**.

Solution conformational analysis: Information on the preferred conformation of the L-pGlu homo-oligomers in solution was obtained in structure-supporting solvents (CDCl$_3$ and MeOH) by FT-IR absorption, ^{1}H NMR and CD techniques.



Scheme 3. Two possible routes for the chain elongation of Boc-(L-pGlu)$_2$-OBzl.

Figure 2 illustrates the FT-IR absorption spectra (C=O stretching region) of the Boc/OBzl-protected pseudopeptide series up to the tetramer level. A strictly comparable trend was observed for the C-deprotected series (not shown). From literature data on imides,[10] and inspection of the spectra in Figure 2, it was evident that the major contribution to all three bands in the 1800–1700 cm^{-1} region was from the carbonyl stretching absorptions of the -CO-N(CH <)-CO- moiety. In particular, the intensity of the two bands at lower wavenumbers increases regularly with oligomer backbone lengthening. The contribution to the spectra in this region from the -COOBzl (in the ester series) and -COOH (in the free carboxylic acid series) groups is expected to be of minor significance and located near 1740 cm^{-1} [11] and 1710 cm^{-1},[10a] respectively. From this FT-IR absorption analysis, we can extract the preliminary information that there is no evidence of any abrupt conformational change in CDCl$_3$ solution as the pseudopeptide main-chain elongates.

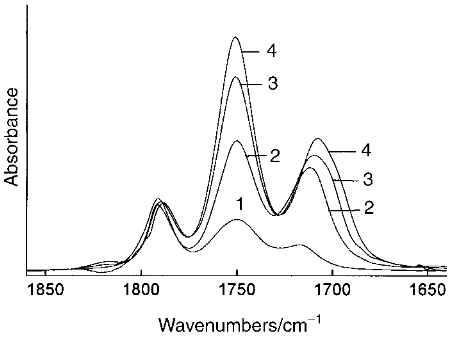


Figure 2. FT-IR absorption spectra in the C=O stretching region of the Boc-(L-pGlu)$_n$-OBzl ($n = 1-4$) series in CDCl$_3$ solution ($c = 1$ mM).

Table 2. Chemical shifts of the αCH protons of the Boc-(L-pGlu)$_n$-OBzl homo-oligomers ($n = 1-4$) in CDCl$_3$ solution (concentration 1 mM).

Compound	n	αCH	αCH	αCH	αCH[a]
2	1				4.57
5	2			5.72	4.89
8	3		5.83	5.74	4.84
11	4	5.87	5.80	5.74	4.83

[a] C-terminal αCH.

In this pseudopeptide (oligoimide) series, the only potentially informative protons for an NMR conformational analysis are the αCH protons. Table 2 lists the chemical shifts of the αCH protons for the Boc-(L-pGlu)$_n$-OBzl ($n = 1-4$) homo-oligomers. In each compound, an unambiguous assignment was made only for the C-terminal αCH proton, by using ^{1}H,^{13}C heteronuclear multi-bond coherence (HMBC) experiments. It is clear that the chemical shifts of all αCH protons, except those assigned to the C-terminal residues, cluster in a region largely downfield relative to common peptide αCH protons (about $\delta = 4.5-5.0$).[12] We have already reported an analogous downfield shift for the Ala αCH proton of the Boc-L-Ala-L-pGlu-OBzl dipeptide.[9] We ascribe this unusual shift to the presence of a γ-lactam carbonyl in the vicinity of the N-terminal and internal αCH protons. This C–H$\cdots$O=C interaction is clearly absent in the case of the C-terminal αCH proton, which resonates in the expected spectral region. The observed insensitivity of all downfield αCH protons[12] to the addition of the strong hydrogen bonding acceptor dimethylsulfoxide[13, 14] (not shown) is consistent with our conformational conclusions. It is also noteworthy that if the αCH proton of a given pGlu residue is proximal to the γ-lactam carbonyl of the following pGlu residue, as in our homo-oligomers, then the exocyclic -CO-N < bond is forced to adopt the s-*trans* conformation. This conclusion: i) is not surprising on energetic grounds as this type of imide conformation (with one exo- and one endocyclic carbonyl)[15] allows the two carbonyl oxygens of our system to be located as far apart as possible, and ii) is indirectly corroborated by the absence of any significant NOE cross-peak between the αCH protons of residues n and n+1 in the homo-oligomers, which would be expected for the s-*cis* imide conformation. A similar phenomenon was observed for the oligomers of *trans*-(4*S*,5*R*)-4-carboxy-5-methyl-oxazolidin-2-one and was attributed to the deshielding effect of the carbonyls of the adjacent heterocyclic rings.[2b] The conformational conclusions extracted from this NMR study fit nicely with those obtained from our FT-IR absorption analysis as discussed above.

The electronic absorption spectra of the imide chromophores of Boc-(L-pGlu)$_n$-OH ($n = 1-4$) in MeOH solution are characterized by two moderately intense ($\varepsilon = 4000-2000$) transitions in the 200–250 nm region centered at about 210 and 220 nm (not shown).[16] The related chirospectroscopic properties are illustrated in Figure 3. While the monomer exhibits a single, positive band (at 215 nm) above 200 nm, the CD spectra of the higher oligomers show dicroic doublets of regularly increasing intensity with increasing backbone length. In the three spectra, the positive lobe is lower in intensity than the negative lobe, and the cross-over point is at 223–225 nm. Also, the three curves give rise to a nearly isodichroic point in the vicinity of 227 nm. The CD spectra of the four benzyl ester oligomers (not shown) strictly compare with those reported in Figure 3. As there is no published systematic investigation of this complex type of imide chromophore,[16] and in view of the lack of a precise

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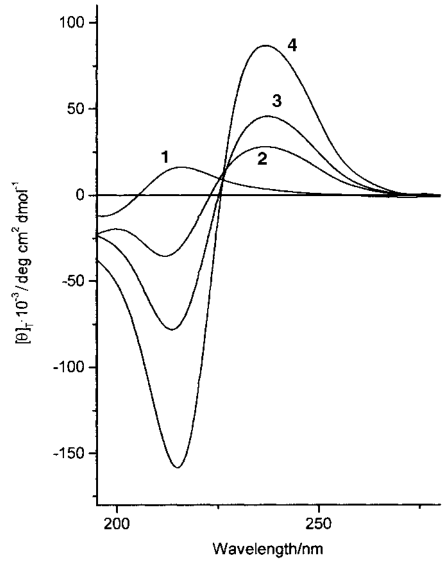


Figure 3. CD spectra of the Boc-(L-pGlu)$_n$-OH ($n = 1-4$) series in MeOH solution ($c = 1$ mM).

correlation of the positions of the UV absorption bands versus the wavelengths of the positive/negative maxima and crossover points in the CD spectra of our homo-oligomers, we made no attempt to assign the observed Cotton effects to any specific electronic transition. However, the shape similarity and the regularly increasing intensity of the CD spectra with main-chain elongation from dimer to tetramer, point to the persistence of the same backbone conformation for these pseudopeptide oligomers in MeOH solution.

Crystal-state conformational analysis: The molecular structure of Boc-(L-pGlu)$_2$-OH (**6**) in the crystal structure obtained by X-ray diffraction analysis is illustrated in Figure 4. The ϕ,ψ backbone torsion angles[17] for both residues 1 and 2 [$\phi_1 = -58.1(6)°$, $\psi_1 = 146.1(4)°$; $\phi_2 = -83.2(5)°$, $\psi_2 = 166.8(4)°$] indicate a partially extended conformation, similar to the type-II poly(L-Pro)$_n$ structure.[18] The ω [C(=O)-N <] torsion angle is s-*cis* [1.1(6)°] in the Boc-L-pGlu^1 segment, but it is (distorted) s-*trans* [−168.2(4)°] in the L-pGlu^1-L-pGlu^2 segment. A tertiary urethane, as in the Boc-N < moiety, in the s-*cis* conformation is not an uncommon observation.[19] The L-pGlu^1-L-pGlu^2 conformation is stabilized by a relatively strong αC–H···O=C intramolecular hydrogen bond,[20] generating a six-membered pseudocyclic form characterized by the following parameters: C1A···O2D 2.827(6) Å, H1A···O2D 2.30(1) Å, and C1A–H1A···O2D 113(1)°. The angle between the normals to the average planes of the two pGlu units is 56.4(2)°.

The ring of the L-pGlu^1 unit adopts a conformation close to a flattened *envelope* (E_3) disposition, with the C1B atom mostly displaced out of the ring (below).[21] The puckering parameters are $Q_2 = 0.156(6)$ Å and $\phi_2 = 248(2)°$. In contrast, the ring of the L-pGlu^2 unit is characterized by an approximately *twist* ($^3T_2$) conformation with the C2B atom above, and the C2G atom below the average ring plane. The puckering parameters are $Q_2 = 0.321(5)$ Å and $\phi_2 = 59.4(8)°$.

In the crystal, the molecules of the dimer are held together in rows along the c direction by OT-HT···O0 ($-x-½$, $-y$, $½+z$) intermolecular hydrogen bonds of normal strength [OT···O0 2.708(5) Å, HT···O0 1.89(1) Å, OT-HT···O0 178.0(3)°].[22, 23]

DFT Computational modeling: In this section, high-level DFT computational results for the Boc-(L-pGlu)$_2$-OH dipeptide (**6**) are presented and compared to the experimental data. In particular, we investigated the occurrence of a non-conventional, weak C–H···O=C hydrogen bond interaction, which has already been recognized as a significant structural feature in both natural and synthetic molecules.[20]

From the structure obtained by X-ray diffraction (see above), we derived the starting geometry for the DFT optimization of **6** (see Experimental Section). The four atoms involved in the hypothetical αC–H···O=C hydrogen bond are C1A, H1A, O2D, and C2D (Figures 4 and 5).

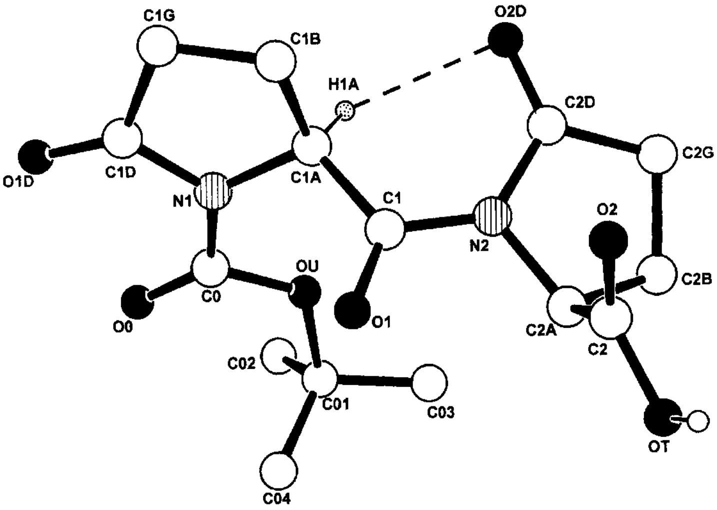
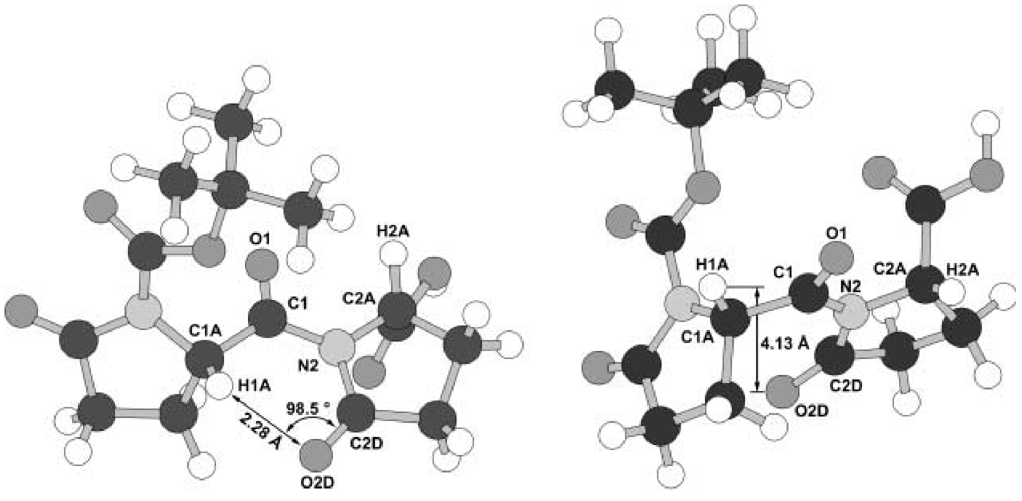


Figure 4. X-ray diffraction structure of Boc-(L-pGlu)$_2$-OH (**6**) with numbering of the atoms. The intramolecular αC–H···O=C hydrogen bond is represented by a dashed line.

The optimized H1A–O2D bond length is 2.28 Å, while the optimized H1A-O2D-C2D bond angle and C1A-H1A-O2D–C2D dihedral angle are 98.5° and 51.8°, respectively. Table 3 lists the optimized bond lengths, bond angles, and dihedral angles for **6**. A comparison with the data reported in the literature for related systems[20] confirms that a weak αC–H···O=C hydrogen bond interaction does exist. Indeed, in those peptide-like molecular systems that are similar to our molecule, the mean hydrogen bond length for this kind of interaction is 2.4 Å, and the bond angle varied from 90–180°.

A conformational analysis was then performed to quantitatively explore the stabilizing effect of the non-covalent interaction in **6**. Therefore, a new conformer **6a**, was optimized by the same DFT procedure, starting from optimized conformer **6** and setting the H1A-C1A-C1-O1 dihedral angle to an initial value of 0° [in **6**, the value for this angle is −148.9° (Table 3)]. This rotation was performed to fully break the C1A–H1A···O2D stabilizing interaction. Conformer **6a** (Table 3), with a final value for the H1A-C1A-C1-O1 dihedral angle of 11.2°, is stable, that is, it corresponds to a



Boc-(L-pGlu)$_2$-OH (6) Boc-(L-pGlu)$_2$-OH (6a)

Figure 5. DFT optimized structures of the conformers **6** and **6a** of Boc-(L-pGlu)$_2$-OH with partial numbering of the atoms. The two conformers differ by a rotation about the C1A–C1 bond.

Table 3. Geometrical parameters calculated for conformers **6** and **6a** of Boc-(L-pGlu)$_2$-OH.

	Boc-(L-pGlu)$_2$-OH (**6**)	Boc-(L-pGlu)$_2$-OH (**6a**)
bond lengths [Å]		
H1A–O2D	2.28	4.13
H1A–C1A	1.09	1.09
C1A–C1	1.53	1.54
C1–O1	1.22	1.22
C1–N2	1.41	1.41
N2–C2A	1.46	1.47
N2–C2D	1.41	1.41
C2D–O2D	1.22	1.21
bond angles [°]		
H1A-O2D-C2D	98.5	83.8
C1A-H1A-O2D	112.4	3.1
C1A-C1-N2	118.3	122.5
C2A-N2-C2D	112.5	112.4
N2-C2D-O2D	126.1	126.1
dihedral angles [°]		
C1A-H1A-O2D-C2D	51.8	–121.4
H1A-C1A-C1-O1	–148.9	11.2

minimum on the potential energy surface. Moreover, it was found to be 4.8 kcal mol^{-1} higher in energy than **6** (Table 4).

The quantitative estimate of the hydrogen bond contribution in **6** (as compared to **6a**) involved the study of the two model systems **I** and **II** (Figure 6 A and B), which allowed a more accurate determination of the stabilization energy due to the non-covalent C1A–H1A ··· O2D–C2D interaction. Each of the two models comprises two distinct molecular units, both with appropriate atomic skeletons to match the structure of **6**, which are placed either at hydrogen bond distance (**I**) or at a much longer, non-interacting distance (**II**) (actually a H1A ··· O2D distance of 10 Å is enough for this purpose; see the Experimental Section for further details). Our calculations yield a 1.4 kcal mol^{-1} stabilization energy attributable to the C1A–H1A ··· O2D–C2D hydrogen bond (Table 4), which is within the range reported in the literature for this kind of non-conventional C–H ··· O=C interaction (less than 2.0 kcal mol^{-1}).[20] Consistently, the more stable conformation contains the hydrogen bond both in the real (**6**) and model (**I**) systems. Nevertheless, additional non-covalent contributions (e.g., repulsive steric effects involving the Boc group and other substituents) must be invoked to account for the higher energy difference (4.8 kcal mol^{-1}) computed between conformers **6** and **6a**.

We also investigated the effect induced by the weak H1A ··· O2D interaction on the ^{1}H NMR spectra (Table 5): chemical shift (δ) values for H1A and H2A protons were simulated for **6**[24] and compared to those observed (Table 5).

The experimental results show a $\delta = 4.86$ chemical shift for H2A, but also a downfield single-proton $\delta = 5.74$ signal, which may be tentatively assigned to H1A and to the deshielding effect induced by the H1A ··· O2D hydrogen bond. Simulated values for H1A and H2A chemical shifts ($\delta = 5.40$ and 4.61, respectively) are in good agreement with the experimental data, as the small chemical shift differences may be ascribed to the approximate method used for the calculations. However, the correlation between theoretical and experimental values is not sufficient to assign the non-

Table 4. Calculated energies for conformers **6** and **6a** of Boc-(L-pGlu)$_2$-OH and molecular models **I** and **II**.

Molecule	E_{abs} [Hartree]	E_{rel} [kcal mol^{-1}]
6	–1291.78461	0.00
6a	–1219.77703	+4.76
I	–1122.47107	0.00
II	–1122.46881	+1.42

Table 5. Calculated and experimental chemical shifts (δ) for the H1A and H2A protons of conformers **6** and **6a** of Boc-(L-pGlu)$_2$-OH.

Proton	Calculated (**6**) [ppm]	Experimental (**6**) [ppm]	Calculated (**6a**) [ppm]
H1A	5.40	5.74	4.61
H2A	4.61	4.86	4.60
$\Delta\delta$	0.79	0.88	0.01

covalent H1A$\cdots$O2D interaction as the cause for the observed anomalous H1A chemical shift. Therefore, chemical shift simulations were also performed in parallel, for the H1A and H2A protons of conformer **6a**, which are not involved in hydrogen bond interactions. Indeed, in this case, the computed chemical shifts for H1A and H2A are $\delta = 4.61$ and 4.60, respectively (Table 5). Interestingly, the computed value for H1A is very different from that experimentally determined. In conclusion, it is now possible to ascribe the anomalous downfield chemical shift computed and observed for H1A in Boc-(L-pGlu)$_2$-OH (**6**) to the noncovalent H1A$\cdots$O2D interaction. Finally, the coupling constants ($^3J_{HH}$) for H1A and H2A were simulated by using the procedure introduced by Bagno.[24] Table 6 reports the results of these calculations, which are in excellent agreement with the experimentally detected values. Figure 7 shows a ^{1}H NMR spectral simulation for the observed and computed chemical shifts and coupling constants of the H1A and H2A protons.

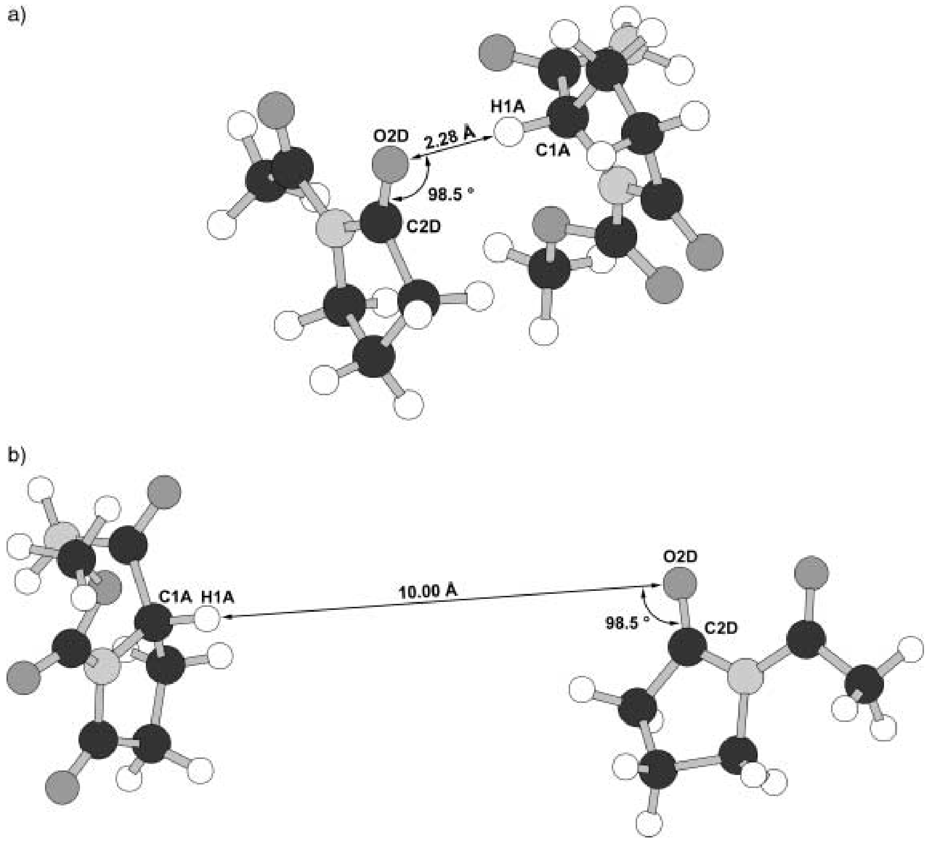


Figure 6. DFT optimized structures of molecular models **I** (A) and **II** (B), which were used to estimate the stabilization energy due to the αC–H$\cdots$O=C hydrogen bond interaction.

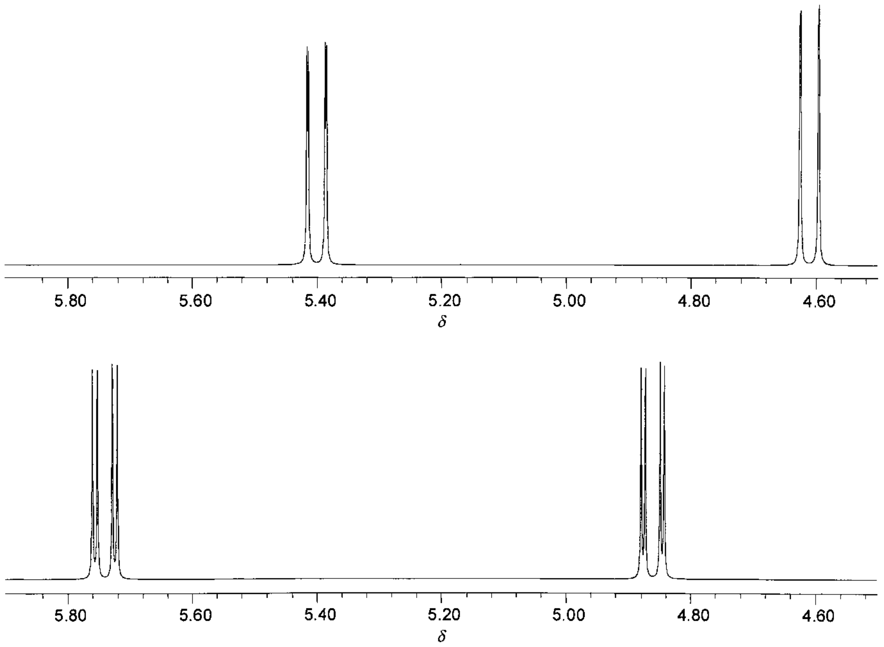


Figure 7. Simulated ^{1}H NMR spectra for the calculated (top) and observed (bottom) chemical shifts (δ) and coupling constants ($^3J_{HH}$) of the H1A and H2A protons of Boc-(L-pGlu)$_2$-OH (**6**) (NMR frequency, 300 MHz; linewidth, 0.5 Hz).

Conclusion

Oligomeric compounds that fold into a predictable, stable and well-defined three-dimensional structure can be exploited as rigid and easily tunable molecular rulers or templates, and help us to learn about energy/electron transfer mechanisms, self-organizing phenomena, and catalytic processes.

In this work we designed, synthesized, and assessed the preferred conformation in solution and in the crystal state of a new pseudopeptide foldameric series based on L-pGlu, which is characterized by a unique, extended conformation stabilized by αC–H$\cdots$O=C intramolecular hydrogen bonds. High-level DFT computational modeling confirmed that this hydrogen bond between H1A and O2D does exist and is responsible for a stabilization of the s-*trans* L-pGlu-L-pGlu conformation of about 1.4 kcal mol^{-1}. Furthermore, we demonstrated that this effect can be easily detected by ^{1}H NMR spectroscopy, owing to the anomalous

Table 6. Calculated and experimental coupling constants ($^3J_{HH}$) for the H1A and H2A protons of Boc-(L-pGlu)$_2$-OH (**6**).

Proton	Calculated [Hz]	Experimental [Hz]
H1A	8.8	9.6
	0.7	2.4
H2A	8.9	9.2
	0.4	2.0

downfield chemical shift of the H1A proton, induced by the deshielding effect of the hydrogen bond. Therefore, from inspection of the ^{1}H NMR spectra of the higher (L-pGlu)$_n$ homo-oligomers, which all show $n-1$ deshielded signals, we can safely conclude that all L-pGlu-L-pGlu bonds are exclusively in the s-*trans* conformation.

We have constructed a computer model, using the WebLab Viewer Pro 3.7 program from Molecular Simulations, of the (L-pGlu)$_n$ ($n=4$) structure based on the ψ_1, ω_1, and ϕ_2 torsion angles of the rigid central part of the L-pGlu^1-L-pGlu^2 system of Boc-(L-pGlu)$_2$-OH. This new foldameric structure is compared in Figure 8 with that of four residues of the type-II poly-(L-Pro)$_n$ helix ($\phi=-78°$, $\psi=149°$, $\omega=180°$).[18] Both are left-handed, elongated ternary helices with an $\alpha C^1 \cdots \alpha C^4$ distance (pitch) of 9.3–9.4 Å. Obviously, the stabilizing $\alpha C{-}H \cdots O{=}C$ intramolecular hydrogen bond characteristic of the (L-pGlu)$_n$ helix is absent in the type-II poly-(L-Pro)$_n$ helix. We might anticipate that for this reason, and for the stable disposition of the two carbonyl groups of each imide system, the (L-pGlu)$_n$ polymer would not exhibit an s-*cis* $\rightleftharpoons$ s-*trans* dynamic equilibrium as observed for the related (L-Pro)$_n$ polymer.[18] As this phenomenon, particularly significant in short homo-oligomers,[25] is responsible for the onset of multiple, coexisting conformers, it is clear that the (L-Pro)$_n$ oligopeptides cannot be safely exploited as spacers or templates with well-predetermined separations. Conversely, the rigid (L-pGlu)$_n$ homo-oligomeric system, if appropriately functionalized in the γ-lactam moiety, may represent an extremely useful tool for future detailed studies at the molecular level within the interface between chemistry and biology. In addition, the ternary nature of (L-pGlu)$_n$ helices will facilitate the construction of multiphilic systems with faces of different polarities. It is evident that, before conducting application studies in aqueous solutions, one should ascertain the stability of the water-soluble analogues of these polyimide systems as a function of pH with respect to both solvolysis and epimerization, including an estimation of the pK_a of the αCH proton involved in the intramolecular hydrogen bonding.

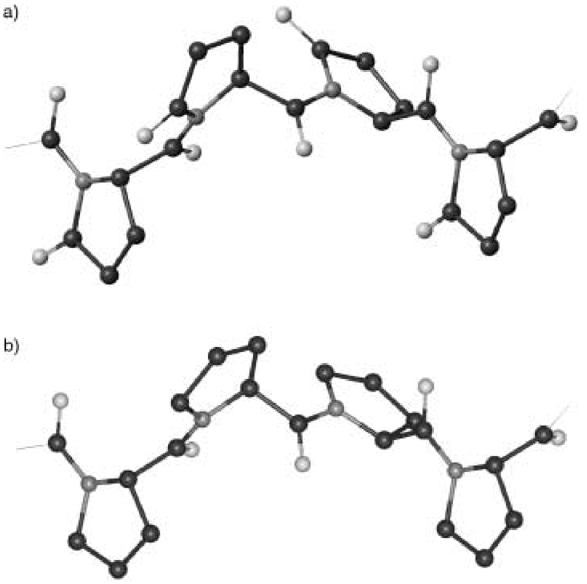


Figure 8. Computer models of: a) The ternary helical structure of the pseudopeptide (L-pGlu)$_4$ segment generated by the repeating ψ_1, ω_1, and ϕ_2 backbone torsion angles of Boc-(L-pGlu)$_2$-OH. b) A (L-Pro)$_4$ segment of the type-II poly-L-(Pro)$_n$ ternary helix.[18]

Experimental Section

Synthesis and characterization: Materials and reagents were of the highest commercially available grade and used without further purification. Reactions were monitored by thin-layer chromatography using Merck silica gel 60 F254-covered plastic plates. Compounds were visualized under a UV lamp or by staining with ceric ammonium molybdate (CAM). Flash chromatography was performed using a Merck silica gel 60 (230–400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 200, a Varian Gemini 300, or a Varian Mercury 400 spectrometer. Chemical shifts are reported as δ values relative to the solvent peak of CHCl$_3$ ($\delta=7.27$). IR absorption spectra were recorded with a Nicolet 210 FT-IR spectrophotometer. Melting points were determined in open capillaries and are uncorrected.

H-(L-pGlu)-OBzl (1): Benzyl bromide (20 mL, 170 mmol) was added dropwise to a stirred solution of H-L-pGlu-OH (20 g, 155 mmol) and triethylamine (TEA) (43 mL, 310 mmol) in acetone (100 mL). The mixture was stirred at room temperature for 24 h, affording a white solid precipitate. Water was added, and acetone was removed and replaced with ethyl acetate (EtOAc). The organic layer was separated, dried over Na$_2$SO$_4$ and concentrated. Flash chromatography (cyclohexane/EtOAc 7:3) gave pure **1** (30 g, 88%). All analytical data were in agreement with those reported in the literature.[6]

Boc-L-pGlu-OBzl (2): A solution of **1** (10 g, 46 mmol), DIEA (8.7 mL, 50.6 mmol), di-*tert*-butyldicarbonate (Boc$_2$O) (21.2 g, 92 mmol), and DMAP (5.5 g, 46 mmol) was stirred in dry DMF (8 mL) under nitrogen at room temperature for 3 h. Then, EtOAc was added (100 mL), and the mixture was washed with 0.1M HCl (2 × 50 mL) and 0.1M NaHCO$_3$ (2 × 50 mL). The organic layer was separated, dried over Na$_2$SO$_4$ and concentrated in vacuo. Flash chromatography (cyclohexane/EtOAc 7:3) afforded pure **2** (13.2 g, 90%). All analytical data were in agreement with those reported in the literature.[7]

Boc-L-pGlu-OH (3): 10% Pd/C on charcoal (1.3 g) was added to a solution of **2** (12.8 g, 40 mmol) in EtOAc (10 mL), and the mixture was stirred in a Parr hydrogenator under hydrogen (3 atm) for 1 h. The catalyst was then filtered through a Celite pad, and the mixture was concentrated. Acid **3** (9.0 g, 98%) was used in the next step without any further purification. M.p. 77–80 °C; $[\alpha]_D^{20}=-33.5$ ($c=0.5$ in CH$_2$Cl$_2$); ^{1}H NMR (300 MHz, CDCl$_3$): $\delta=1.49$ (s, 9H; *t*Bu), 2.05–2.71 (m, 4H; CH_2–CH_2), 4.65 (dd, ^3J(H,H) = 2.7 Hz, ^3J(H,H) = 9.3 Hz, 1H; CHN), 9.03 (brs, 1H; COOH); ^{13}C NMR (75 MHz, CDCl$_3$): $\delta=21.4$, 27.8, 31.2, 58.7, 83.9, 149.2, 173.6, 175.7; IR (CH$_2$Cl$_2$): $\tilde{\nu}=1790$, 1750, 1715 cm^{-1} (C=O); elemental analysis calcd (%) for C$_{10}$H$_{15}$NO$_5$ (229.1): C 52.40, H 6.60, N 6.11; found: C 52.45, H 6.56, N 6.14.

Boc-L-pGlu-OPfp (4): Pyridine (0.3 mL, 3.8 mmol) and then pentafluorophenyl trifluoroacetate (0.65 mL, 3.8 mmol) were added to a stirred solution of **3** (0.72 g, 3.4 mmol) in dry DMF (2 mL). The reaction was stirred for 45 min at room temperature and then diluted with EtOAc (50 mL), washed with 0.1M aqueous HCl (2 × 30 mL) and 5% aqueous NaHCO$_3$ (1 × 30 mL), dried over Na$_2$SO$_4$ and concentrated in vacuo. The pentafluorophenyl ester **4** (1.24 g) was obtained in quantitative yield, but it could not be completely purified by silica gel chromatography. ^{1}H NMR (300 MHz, CDCl$_3$): $\delta = 1.51$ (s, 9H; *t*Bu), 2.11–2.18 (m, 1H; C*H*H), 2.45–2.80 (m, 3H; CH*H*-C*H*$_2$), 4.95 (dd, ^3J(H,H) = 3.0 Hz, ^3J(H,H) = 9.3 Hz, 1H; CHN).

Boc-(L-pGlu)$_2$-OBzl (5): LiHMDS (1.2 mL, 1M in THF, 1.2 mmol) was added to a solution of H-L-pGlu-OBzl (**1**) (0.37 g, 1.15 mmol) in dry THF (8 mL) at room temperature under an inert atmosphere. The mixture was stirred for 30 min, then Boc-L-pGlu-OPfp (**4**) (0.5 g, 1.26 mmol) in dry THF was added at 0°C, and the mixture was stirred for an additional hour at room temperature under an inert atmosphere. THF was removed in vacuo and replaced with EtOAc (50 mL). The organic layer was washed with a saturated NH$_4$Cl aqueous solution (2×30 mL), dried and concentrated in vacuo. Flash chromatography (cyclohexane/EtOAc 7:3) gave dimer **5** (0.34 g, 70%). M.p. 68–70°C; $[\alpha]_D^{20} = -86.6$ ($c = 0.5$ in CH$_2$Cl$_2$); ^{1}H NMR (400 MHz, CDCl$_3$): $\delta = 1.36$ (s, 9H; *t*Bu), 1.85–1.98 (m, 1H; C*H*H), 2.09–2.18 (m, 1H; CH*H*), 2.25–2.48 (m, 4H; $2 \times$ CH$_2$), 2.55–2.73 (m, 2H; CH$_2$), 4.87 (dd, ^3J(H,H) = 2.4 Hz, ^3J(H,H) = 9.6 Hz, 1H; CHN), 5.09 (d, ^2J(H,H) = 12.9 Hz, 1H; C*H*H–Ph), 5.25 (d, ^2J(H,H) = 12.9 Hz, 1H; CH*H*–Ph), 7.18–7.23 (m, 5H; Ph); ^{13}C NMR (100 MHz, CDCl$_3$): $\delta = 20.7$, 21.6, 27.8, 30.7, 31.5, 57.6, 59.4, 67.7, 83.4, 128.3, 128.7, 128.8, 134.7, 149.8, 170.4, 171.5, 173.4, 174.4; IR (Nujol): $\tilde{\nu} = 1780$, 1739, 1703 cm^{-1} (C=O); elemental analysis calcd (%) for C$_{22}$H$_{26}$N$_2$O$_7$ (430.2): C 61.39, H 6.09, N 6.51; found: C 61.35, H 6.13, N 6.47.

Boc-(L-pGlu)$_2$-OH (6): The title compound was obtained from **5** in 97% yield, by application of the synthetic procedure used above for the preparation of Boc-L-pGlu-OH (**3**). M.p. 84–87°C; $[\alpha]_D^{20} = -91.6$ ($c = 1.0$ in CH$_2$Cl$_2$); ^{1}H NMR (400 MHz, CDCl$_3$): $\delta = 1.50$ (s, 9H; *t*Bu), 2.02–2.15 (m, 1H; C*H*H), 2.20–2.75 (m, 7H; CH*H*, $3 \times$ CH$_2$), 4.86 (dd, ^3J(H,H) = 2.0 Hz, ^3J(H,H) = 9.2 Hz, 1H; CHN), 5.74 (dd, ^3J(H,H) = 2.4 Hz, ^3J(H,H) = 9.6 Hz, 1H; CHN); ^{13}C NMR (50 MHz, CDCl$_3$): $\delta = 20.8$, 21.6, 27.8, 30.9, 31.5, 57.5, 59.8, 83.8, 149.5, 171.4, 173.5, 174.7; IR (Nujol): $\tilde{\nu} = 1792$, 1746, 1686 cm^{-1} (C=O); elemental analysis calcd (%) for C$_{15}$H$_{20}$N$_2$O$_7$ (430.2): C 52.94, H 5.92, N 8.23; found: C 52.98, H 5.96, N 8.24.

Boc-(L-pGlu)$_2$-OPfp (7): The title compound was obtained from **6** in 95% yield, by application of the synthetic procedure used above for the preparation of Boc-L-pGlu-OPfp (**4**). ^{1}H NMR (200 MHz, CDCl$_3$): $\delta = 1.48$ (s, 9H; *t*Bu), 1.93–2.16 (m, 1H; C*H*H), 2.35–2.91 (m, 7H; CH*H* + $3 \times$ CH$_2$), 5.19 (dd, ^3J(H,H) = 2.8 Hz, ^3J(H,H) = 9.2 Hz, 1H; CHN), 5.77 (dd, ^3J(H,H) = 2.0 Hz, ^3J(H,H) = 9.2 Hz, 1H; CHN).

Boc-(L-pGlu)$_3$-OBzl (8): The title compound was obtained from the lithium anion of **1** and **7** in 61% yield, by application of the synthetic procedure used above for the preparation of Boc-(L-pGlu)$_2$-OBzl (**5**). M.p. 77–80°C; $[\alpha]_D^{20} = -146.6$ ($c = 1.0$ in CH$_2$Cl$_2$); ^{1}H NMR (400 MHz, CDCl$_3$): $\delta = 1.37$ (s, 9H; *t*Bu), 1.85–2.11 (m, 4H; $2 \times$ CH$_2$), 2.25–2.62 (m, 8H; $4 \times$ CH$_2$), 4.84 (dd, ^3J(H,H) = 2.4 Hz, ^3J(H,H) = 9.6 Hz, 1H; CHN), 5.09 (d, ^2J(H,H) = 12.3 Hz; C*H*H–Ph), 5.28 (d, ^2J(H,H) = 12.3 Hz; CH*H*–Ph), 5.64 (dd, ^3J(H,H) = 2.4 Hz, ^3J(H,H) = 8.8 Hz, 1H; CHN), 5.74 (dd, ^3J(H,H) = 2.8 Hz, ^3J(H,H) = 8.0 Hz, 1H; CHN); ^{13}C NMR (100 MHz, CDCl$_3$): $\delta = 20.8$, 20.9, 21.6, 27.8, 30.9, 31.1, 31.4, 57.6, 58.6, 59.5, 67.7, 83.3, 128.4, 128.7, 128.8, 134.7, 149.6, 170.4, 170.7, 171.7, 173.7, 174.5, 175.1; IR (CH$_2$Cl$_2$): $\tilde{\nu} = 1787$, 1749, 1703 cm^{-1} (C=O); elemental analysis calcd (%) for C$_{27}$H$_{31}$N$_3$O$_9$ (541.2): C 59.88, H 5.77, N 7.76; found: C 59.85, H 5.81, N 7.80.

Boc-(L-pGlu)$_3$-OH (9): The title compound was obtained from **8** in 92% yield, by application of the synthetic procedure used above for the preparation of Boc-L-pGlu-OH (**3**). M.p. 94–97°C; $[\alpha]_D^{20} = -136.8$ ($c = 0.4$ in CH$_2$Cl$_2$); ^{1}H NMR (200 MHz, CDCl$_3$): $\delta = 1.46$ (s, 9H; *t*Bu), 2.02–2.92 (m, 12H; $6 \times$ CH$_2$), 4.81 (dd, ^3J(H,H) = 2.6 Hz, ^3J(H,H) = 9.2 Hz, 1H; CHN), 5.75 (dd, ^3J(H,H) = 2.2 Hz, ^3J(H,H) = 9.2 Hz, 1H; CHN), 5.85 (dd, ^3J(H,H) = 1.8 Hz, ^3J(H,H) = 8.8 Hz, 1H; CHN); ^{13}C NMR (50 MHz, CDCl$_3$): $\delta = 20.8$, 21.0, 21.7, 27.9, 30.1, 31.2, 31.5, 57.4, 58.7, 59.7, 83.6, 149.4, 170.6, 171.5, 173.5, 174.4, 174.7, 175.2; IR (CH$_2$Cl$_2$): $\tilde{\nu} = 3684$ (OH), 1787, 1752, 1703 cm^{-1} (C=O); elemental analysis calcd (%) for C$_{20}$H$_{25}$N$_3$O$_9$ (451.2): C 53.21, H 5.58, N 9.31; found: C 53.26, H 5.55, N 9.35.

Boc-(L-pGlu)$_3$-OPfp (10): The title compound was obtained from **9** in 95% yield, by application of the synthetic procedure used above for the preparation of Boc-L-pGlu-OPfp (**4**). ^{1}H NMR (200 MHz, CDCl$_3$): $\delta = 1.42$ (s, 9H; *t*Bu), 2.05–2.22 (m, 2H; CH$_2$), 2.30–2.88 (m, 10H; $5 \times$ CH$_2$), 5.12 (dd, ^3J(H,H) = 2.8 Hz, ^3J(H,H) = 9.0 Hz, 1H; CHN), 5.75 (dd, ^3J(H,H) = 1.8 Hz, ^3J(H,H) = 8.8 Hz, 1H; CHN), 5.85 (m, ^3J(H,H) = 1.8 Hz, ^3J(H,H) = 8.8 Hz, 1H; CHN).

Boc-(L-pGlu)$_4$-OBzl (11): The title compound was obtained from the lithium anion of **1** and **10** in 48% yield, by application of the synthetic procedure used above for the preparation of Boc-(L-pGlu)$_2$-OBzl (**5**). M.p. 92–95°C; $[\alpha]_D^{20} = -166.8$ ($c = 0.2$ in CH$_2$Cl$_2$); ^{1}H NMR (300 MHz, CDCl$_3$): $\delta = 1.45$ (s, 9H; *t*Bu), 1.98–2.68 (m, 16H; $8 \times$ CH$_2$), 4.81 (dd, ^3J(H,H) = 2.1 Hz, ^3J(H,H) = 9.3 Hz, 1H; CHN), 5.06 (d, ^2J(H,H) = 12.0 Hz, 1H; OC*H*H–Ph), 5.26 (d, ^2J(H,H) = 12.0 Hz, 1H; OCH*H*–Ph), 5.71 (dd, ^3J(H,H) = 2.7 Hz, ^3J(H,H) = 9.6 Hz, 1H; CHN), 5.77 (dd, ^3J(H,H) = 1.5 Hz, ^3J(H,H) = 9.6 Hz, 1H; CHN), 5.84 (dd, ^3J(H,H) = 1.8 Hz, ^3J(H,H) = 9.3 Hz, 1H; CHN), 7.25–7.40 (m, 5H; Ph); ^{13}C NMR (75 MHz, CDCl$_3$): $\delta = 20.8$, 21.0, 21.6, 27.9, 30.8, 31.0, 31.2, 31.4, 57.6, 58.7, 59.5, 67.7, 83.3, 128.3, 128.6, 128.7, 134.6, 149.5, 170.3, 170.5, 170.6, 171.5, 173.6, 174.3, 175.1; IR (CH$_2$Cl$_2$): $\tilde{\nu} = 1790$, 1749, 1707 cm^{-1} (C=O); elemental analysis calcd (%) for C$_{32}$H$_{36}$N$_4$O$_{11}$ (652.7): C 58.89, H 5.56, N 8.58; found: C 58.96, H 5.61, N 8.61.

Boc-(L-pGlu)$_4$-OH (12): The title compound was obtained from **11** in 92% yield, by application of the synthetic procedure used above for the preparation of Boc-L-pGlu-OH (**3**). M.p. 151–154°C; $[\alpha]_D^{20} = -179.2$ ($c = 0.7$ in CH$_2$Cl$_2$); ^{1}H NMR (300 MHz, CDCl$_3$): $\delta = 1.43$ (s, 9H; *t*Bu), 1.98–2.72 (m, 16H; $8 \times$ CH$_2$), 4.76 (dd, ^3J(H,H) = 2.0 Hz, ^3J(H,H) = 9.5 Hz, 1H; CHN), 5.70 (dd, ^3J(H,H) = 1.8 Hz, ^3J(H,H) = 8.7 Hz, 1H; CHN), 5.79 (dd, ^3J(H,H) = 1.5 Hz, ^3J(H,H) = 9.0 Hz, 1H; CHN), 5.83 (dd, ^3J(H,H) = 1.5 Hz, ^3J(H,H) = 9.0 Hz, 1H; CHN); ^{13}C NMR (75 MHz, CDCl$_3$): $\delta = 20.7$, 21.1, 21.7, 27.9, 30.8, 31.2, 31.5, 57.4, 58.8, 58.9, 59.7, 83.7, 149.5, 170.5, 170.6, 171.5, 173.5, 174.5, 174.6, 175.3, 175.4; IR (CH$_2$Cl$_2$): $\tilde{\nu} = 3687$ (OH), 1791, 1752, 1703 cm^{-1} (C=O); elemental analysis calcd (%) for C$_{25}$H$_{30}$N$_4$O$_{11}$ (562.2): C 53.38, H 5.38, N 9.96; found: C 53.35, H 5.33, N 9.91.

FT-IR absorption: FT-IR absorption spectra were recorded with a Perkin–Elmer 1720X spectrophotometer, nitrogen flushed, equipped with a sample-shuttle device, at 2 cm^{-1} nominal resolution, averaging 100 scans. Solvent (baseline) spectra were recorded under the same conditions. Cells with path lengths of 0.1, 1.0, and 10 mm (with CaF$_2$ windows) were used. Spectrograde CDCl$_3$ (99.8% ^{2}H) was purchased from Fluka.

^{1}H NMR spectroscopy: ^{1}H NMR spectra were recorded with a Bruker AM 400 spectrometer. Measurements were carried in deuterochloroform (99.8% ^{2}H from Aldrich) and deuterated dimethylsulfoxide (99.96% D$_6$ from Acros Organics) using tetramethylsilane as the internal standard.

CD spectroscopy: The CD spectra were obtained on a Jasco J-710 spectropolarimeter. Cylindrical fused quartz cells of 10, 1, 0.2, and 0.1 mm path length (Hellma) were used. The values are expressed in terms of $[\theta]_T$, the total molar ellipticity (deg cm^2 dmol^{-1}). Spectrograde MeOH (Baker) was used as solvent.

X-ray diffraction: Crystals of Boc-(L-pGlu)$_2$-OH (**6**) were grown by vapor diffusion from EtOAc/petroleum ether. Diffraction data were collected on a Philips PW 1100 diffractometer. Crystal data and refinement parameters are summarized in Table 7. The structure was solved by direct methods using the SHELXS 97 program.[26a] Refinement was carried out on F^2 by full-matrix block least-squares, using all data, by application of the SHELXL 97 program,[26b] with all non-hydrogen atoms anisotropic, and allowing their positional parameters and anisotropic displacement parameters to refine at alternate cycles. The position of the hydrogen atom of the C-terminal -COOH group was recovered from a difference Fourier map, while the remaining hydrogen atoms were calculated at idealized positions. All hydrogen atoms were refined as riding on their carrying atom with U_{iso} set equal to 1.2 (or 1.5 for the -COOH and methyl groups) times the U_{eq} of the parent atom.

CCDC-172246 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; (fax: (+44)1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk).

Computational details: All calculations were carried out using the tools available in the Gaussian 98[27] 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).[28] For the determination of αC–H···O=C hydrogen bonds, a mixed basis set was used: a 6-31+G(d) basis set for the C1A, H1A, O2D, and C2D atoms, and a 6-31G(d) for all other atoms of Boc-(L-pGlu)$_2$-OH (**6**). Since the O2D lone-pairs strongly interact with H1A, the use of diffuse functions on heavy atoms is fundamental.[29]

Table 7. Crystal data and structure refinement parameters for Boc-(L-pGlu)$_2$-OH (**6**).

formula	$C_{15}H_{20}N_2O_7$
M_r	340.3
T [K]	293(2)
wavelength [Å]	1.54178
crystal system	orthorhombic
space group	$P2_12_12_1$
a [Å]	6.117(3)
b [Å]	13.481(4)
c [Å]	20.089(4)
α [°]	90
β [°]	90
γ [°]	90
V [Å^3]	1657(1)
Z	4
ρ_{calcd} [Mg m^{-3}]	1.37
μ [mm^{-1}]	0.93
F(000)	720
crystal size [mm]	0.30 × 0.10 × 0.05
θ range [°]	5.49 – 59.99
index ranges	$-1 \le h \le 6$, $0 \le k \le 15$, $0 \le l \le 22$
refl. collected	1728
independent refl.	1693 [R(int) = 0.087]
refinement method	full-matrix block least-squares on F^2
data/restraints/parameters	1693/0/217
GoF (on F^2)	1.0
final R indices [$I > 2\sigma(I)$]	$R1 = 0.069$, $wR2 = 0.171$
R indices (all data)	$R1 = 0.080$, $wR2 = 0.181$
largest diff. peak and hole [e Å^{-3}]	0.263; −0.292

From the structure obtained by X-ray diffraction (Figure 4 and Table 7) we derived the geometry for the DFT optimization. The starting geometry used for **6a** optimization was obtained by rotating the optimized dimer system (**6**) about the C1A–C1 bond (until the two atoms H1A and O1 were eclipsed). This choice follows from the fact that C1A–C1 is a true single bond, whilst the C1–N2 bond has a partial double bond character (see bond lengths reported in Table 3).

Molecular models **I** and **II** (Figure 6 A and B, respectively) have been used to estimate the stabilization energy due to the αC–H···O=C hydrogen bond interaction. These systems are composed of two separated fragments: The first fragment contains only the H1A–C1A bond, while the C2D–O2D bond belongs to the second fragment. In model **I**, the H1A···O2D hydrogen bond length was kept frozen at 2.28 Å, and the bond angle H1A···O2D–C2D at 98.5° (this was done to match the values optimized in **6**). Values of 10.00 Å and 98.5° were fixed in model **II** for H1A···O2D and H1A···O2D–C2D, respectively. All of the other variables were left free to relax in both models **I** and **II**. Chemical shift simulation was performed by using the standard tool available in the Gaussian 98 package.[30] A standard 6-31G(d) basis set was used for all the atoms. The computed data do not directly yield the chemical shift value, but only a value for the isotropic magnetic tensor. The chemical shift value is obtained from Equation (1):

$$\delta_H = 32.18 - \sigma_H \qquad (1)$$

in which 32.18 is the calculated isotropic magnetic tensor for the protons in tetramethylsilane, and σ_H is the calculated isotropic magnetic tensor for the investigated proton. The coupling constants ($^3J_{HH}$) for H1A and H2A were also computed according to the procedure suggested by Bagno.[24] The value of $^3J_{HH}$ was calculated using Equation (2):

$$^3J_{HH} = 75740.19 \times \mathrm{BFC} \qquad (2)$$

in which BFC is the Fermi contact term, and 75740.19 is a constant for H–H coupling.

^{1}H NMR spectra for H1A and H2A were simulated using the Mest-Re-C 2.3a program.[31]

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