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# Spontaneous Self-Assembly of Fully Protected Ester 1:1 [ $\alpha/\alpha$ - N $\alpha$ -Bn-hydrazino] Pseudodipeptides into a Twisted Parallel $\beta$ -Sheet in the Crystal State

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## Spontaneous self-assembly of fully protected ester 1:1 [alpha/alpha-N<sup>alpha</sup>-Bn-hydrazino] pseudodipeptides into a twisted parallel beta-sheet in the crystal state

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3 **Spontaneous self-assembly of fully protected ester 1:1 [ $\alpha/\alpha$ - $N^\alpha$ -**  
4 **Bn-hydrazino] pseudodipeptides into a twisted parallel  $\beta$ -sheet**  
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6 **in the crystal state**  
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11 Eugénie Romero,<sup>†</sup> Ralph-Olivier Moussodia,<sup>†</sup> Alexandre Kriznik,<sup>‡</sup> Emmanuel Wenger,<sup>§</sup> Samir  
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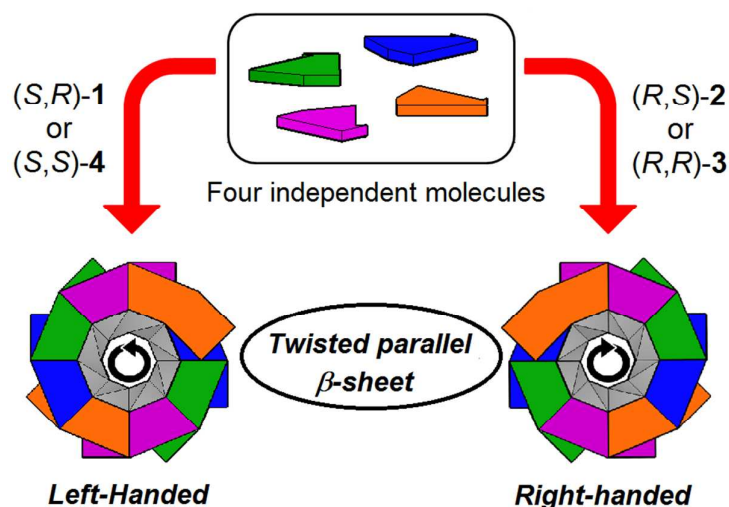
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39 **Keywords:** Self-Assembly, Twisted parallel  $\beta$ -sheet, Hydrazinopeptide, IR, NMR,  
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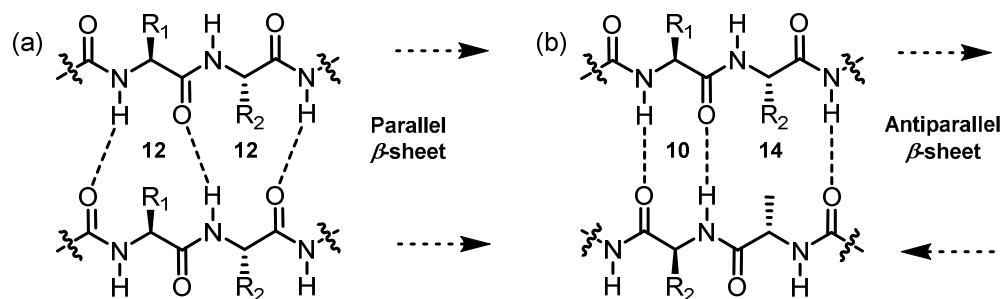
## ABSTRACT

Previous studies have demonstrated that amidic  $\alpha/\beta$ -pseudodipeptides, 1:1 [ $\alpha/\alpha$ - $N^\alpha$ -Bn-hydrazino], have the ability to fold *via* a succession of  $\gamma$ -turn ( $C_7$  pseudocycle) and hydrazinoturn in  $CDCl_3$  solution, their amide terminals enabling the formation of an intramolecular H-bond network. Despite their lack of a primary amide terminals allowing the formation of the hydrazinoturn, their ester counterparts **1-4** were proven to self-assemble into  $C_6$  and  $C_7$  pseudocycles by intramolecular H-bonds in solution state, and into an uncommon twisted parallel  $\beta$ -sheet through intermolecular H-bonding in the crystal state to form a supramolecular helix with eight molecules needed to complete a full  $360^\circ$  rotation. Such self-organization (with eight molecules) has been only observed in specific  $\alpha/\alpha$ -pseudodipeptide, depsipeptide (Boc-Leu-Lac-OEt). Relying on IR absorption, NMR, X-ray diffraction and CD analyses, the aim of this study was to demonstrate that stereoisomers of ester 1:1 [ $\alpha/\alpha$ - $N^\alpha$ -Bn-hydrazino] pseudodipeptides **1-4** are not only able to self-assemble into this  $\beta$ -helical structure. The absolute configuration of the asymmetric  $C^\alpha$ -atom of the  $\alpha$ -amino acid residue influences the left- or right-handed twist without changing the pitch of the formed helix.

## INTRODUCTION

By definition, molecular self-assembly is the spontaneous organization of individual molecules without external guidance into structurally well-defined and rather stable arrangements through a number of non-covalent interactions.<sup>1</sup>

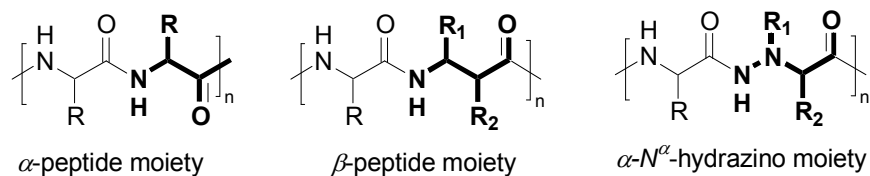
Many crucial biological functions rely on the ability of peptides to display particular folding/self-assembly patterns or secondary structures, called helices, turns and  $\beta$ -sheet, which occur through the formation of H-bonds established between  $\alpha$ -amino acid backbone atoms. Among the various secondary structures, the  $\beta$ -sheet family, also called  $\beta$ -pleated sheet (Figure 1), is the less well studied for small-molecule structures. Their relative lack of study, compared to that of  $\alpha$ -helices, can be considered quite surprising since the formation of supramolecular  $\beta$ -sheet architectures is at the basis of various neurodegenerative diseases such as Alzheimer's,<sup>2</sup> Huntington's,<sup>3</sup> prion protein<sup>4</sup> and other related diseases.<sup>5</sup>



**FIGURE 1.** Hydrogen bond patterns in  $\beta$ -sheets: (a) parallel  $\beta$ -sheet ( $C_{12}$  pseudocycles) and (b) antiparallel  $\beta$ -sheet ( $C_{14}$  and  $C_{10}$  pseudocycles). The dotted arrows represent the direction of the peptide chain.

The use of peptides in biological applications such as drug delivery is severely hampered by their inherent propensity to rapid degradation through proteolysis. As an alternative, biologically active pseudopeptides, *i.e.* peptides with modified backbones, have emerged due to their potential resistance to peptidases and proteases and their ability to adopt well-defined

secondary structures similar to their corresponding peptide analogs.<sup>6</sup> Gellman and Seebach,<sup>7,8</sup> have demonstrated that  $\beta$ -peptides (Figure 2) were able to adopt well-defined arrangement and to display stability against proteases and peptidases.<sup>9</sup>

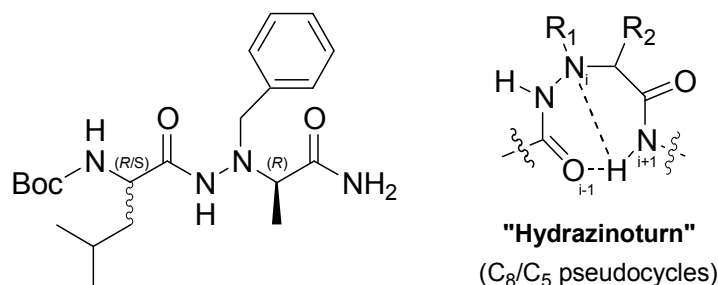


**FIGURE 2.** The backbones of  $\alpha$ -peptide,  $\beta$ -peptide, and  $\alpha$ - $N^\alpha$ -hydrazino moieties.

These pioneering studies have paved the way to the intensive studies of linear and cyclic  $\beta$ -peptides,<sup>7-9</sup> hybrid pseudopeptides consisting of  $\alpha/\beta$ -,  $\alpha/\gamma$ -,  $\beta/\gamma$ -amino acids,<sup>10</sup> together with the less common studies of  $\beta/\delta$ - and  $\gamma/\delta$ -pseudopeptides.<sup>11</sup> The use of heterogeneous backbones in pseudopeptidic designs was indeed justified by studies in the 2000's indicating the relevance of the introduction of a  $\beta$ -amino acid in a peptide to both increase the proteolysis stability<sup>12</sup> and induce a preorganization that could lead to new folding possibilities.<sup>10c</sup>

Extending the  $\beta$ -peptide concept has given birth to bis-nitrogenated compounds in which the  $C^\beta$ -atom has been replaced by a nitrogen atom, leading to the new family of "hydrazinopeptides".<sup>13</sup> Previous studies performed in our laboratory have demonstrated the ability of mixed amidic 1:1 [ $\alpha/\alpha$ - $N^\alpha$ -hydrazino]mers to self-assemble *via* a succession of  $\gamma$ -turn ( $C_7$  pseudocycle) and hydrazinoturn (Figure 3). This latter consists in the formation of a bifurcated hydrogen bond through  $C_5$  and  $C_8$  pseudocycles, involving the lone pair of the  $N^\alpha$ -atom. Interestingly, this specific intramolecular H-bond network is formed regardless of the chirality.<sup>14</sup>

Moreover, cyclization of mixed 1:1 [ $\alpha/\alpha$ - $N^\alpha$ -hydrazino]mers led to the corresponding macrocycles which were able to self-assemble into nanotubes *via* the formation of an intermolecular H-bond network.<sup>15</sup>



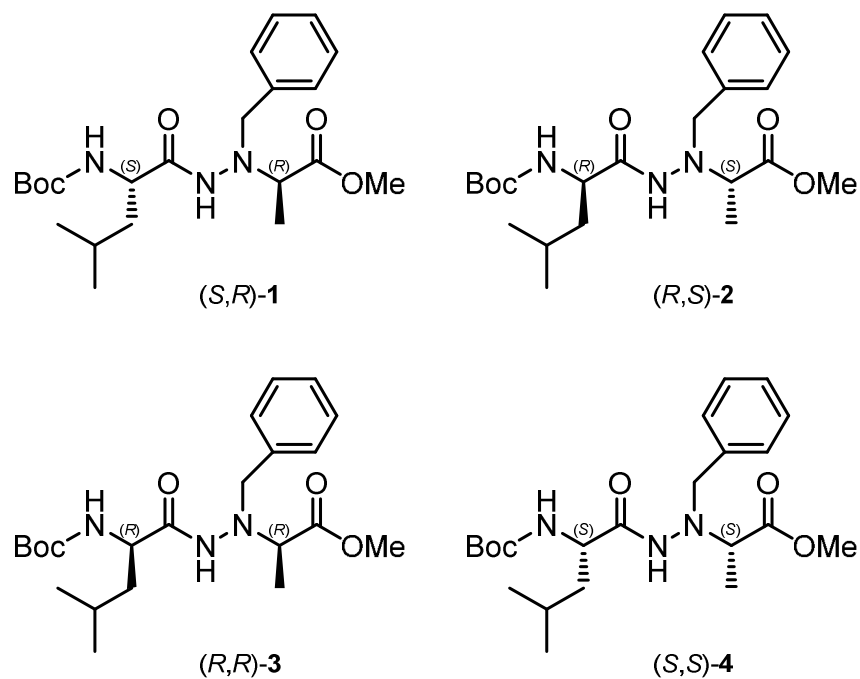
**FIGURE 3.** Self-organization of amidic 1:1[leucine/ $\alpha$ - $N^\alpha$ -Bn-(D)-hydrazinoalanine] pseudodipeptides through the “hydrazinoturn”. C<sub>5</sub> is N<sub>i+1</sub>-H•••N<sub>i</sub> and C<sub>8</sub> is N<sub>i+1</sub>-H•••O<sub>i-1</sub>=C intramolecularly H-bonded.

Such versatile self-organization, *i.e.* succession of  $\gamma$ -turn and hydrazinoturn, emphasized the particular role of the hydrazino link within this pseudopeptide series. These results led us to envision the possibility to access to various secondary structures by wisely manipulating this particular backbone.

As currently employed to simulate the role of the amide bond in protein folding, we decided to replace the amide terminals by an ester through “amide-to-ester mutagenesis”,<sup>16</sup> thereby preventing our newly formed ester 1:1 [ $\alpha/\alpha$ - $N^\alpha$ -Bn-hydrazino] pseudodipeptide from folding into a hydrazinoturn. We propose that this backbone modification should open the door to new “intermolecular” self-assemblies promoted by the intrinsic preorganization of the hydrazinopeptides.<sup>17</sup>

In this study, we thus undertook the synthesis and the conformational analysis of ester 1:1 [ $\alpha/\alpha$ - $N^\alpha$ -Bn-hydrazino] pseudodipeptides **1-4** (Figure 4). In order to evaluate the influence of the chirality on the structuration, we decided to compare the structuration of enantiomers (*S,R*)-**1** and (*R,S*)-**2**, or (*R,R*)-**3** and (*S,S*)-**4** respectively. It is important to note that compounds

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3 **1-4** possess a  $sp^3$  nitrogen atom ( $N^\alpha$ -atom) which can be considered as an extra chiral center  
4 with a non-fixed configuration. The conformational analyses in solution and in solid state will  
5 be studied by using X-ray crystallography, microcrystalline CD, NMR and IR data.  
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33  
34 **FIGURE 4.** Chemical structures of the stereoisomers of ester 1:1[ $\alpha/\alpha'$ - $N^\alpha$ -Bn-hydrazino] pseudodipeptides **1-4**  
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## 39 **RESULTS AND DISCUSSION**

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### 45 **Studies of the self-assembly in solution state by $^1\text{H}$ NMR experiments and FTIR**

### 46 **absorption spectroscopy**

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50 Ester [ $\alpha/\alpha'$ - $N^\alpha$ -hydrazino] pseudodipeptides **1-4** were synthesized according to our previously  
51 described procedure.<sup>14</sup>  
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3 In a first step, the conformation of the stereoisomers **1-4** in solution state was studied through  
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5  $^1\text{H}$  NMR and FTIR absorption techniques. First of all, we showed that no significant  
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7 differences could be pointed out when comparing the spectra of compounds **1-4** (see  
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9 Supporting Information pp S2 to S9). The data obtained for compound (*S,R*)-**1** will be  
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11 described below as a reference.  
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14  $^1\text{H}$  NMR spectra were recorded in  $\text{CDCl}_3$  in a range of concentration varying from  $10^{-4}$  to  $10^{-1}$   
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16 M. No significant variation was observed (Figure 5a) concerning the chemical shifts of the  
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18 carbamidic (NHBoc) and hydrazidic NH protons. This result demonstrated that no  
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20 intermolecular H-bonding occurs in compound (*S,R*)-**1**. On the basis of small model  
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22 compounds described in one of our earlier studies,<sup>18f</sup> we established that the chemical shifts of  
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24 non H-bonded carbamic and hydrazidic NH protons are estimated to be roughly 4.7 and 6.5  
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26 ppm, respectively. Taking into account these data, the carbamidic NH proton of compound  
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28 (*S,R*)-**1** located at 4.8 ppm has been considered as a non H-bonded proton. On the contrary,  
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30 the hydrazidic proton located at 8.0 ppm (instead of 6.5 ppm for free one) has been defined as  
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32 a H-bonded proton.  
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37 To validate these assumptions,  $^1\text{H}$  NMR spectra were also performed in a mixture of  
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39  $\text{CDCl}_3/\text{DMSO-}d_6$  ( $c = 10^{-2}$  M, from 0 up to 10%, Figure 5b) in order to detect the NH protons  
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41 involved in an intramolecular H-bond. Solvents with H-bond acceptor atoms, such as  $\text{DMSO-}$   
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43  $d_6$ , are able to form intermolecular H-bonds producing a downfield shift, which is less  
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45 significant when the H-atom participates in an intramolecular H-bond. This type of  
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47 experiment has been proven to be efficient in evaluating the H-bond strength.<sup>18</sup> As shown in  
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49 Figure 5b, the chemical shift of the hydrazidic NH proton is unchanged whatever the amount  
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51 of DMSO, which means that it is involved in an intramolecular H-bond. Conversely, the  
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53 chemical shift of the free carbamidic NH proton (NHBoc) is sensitive to DMSO.  
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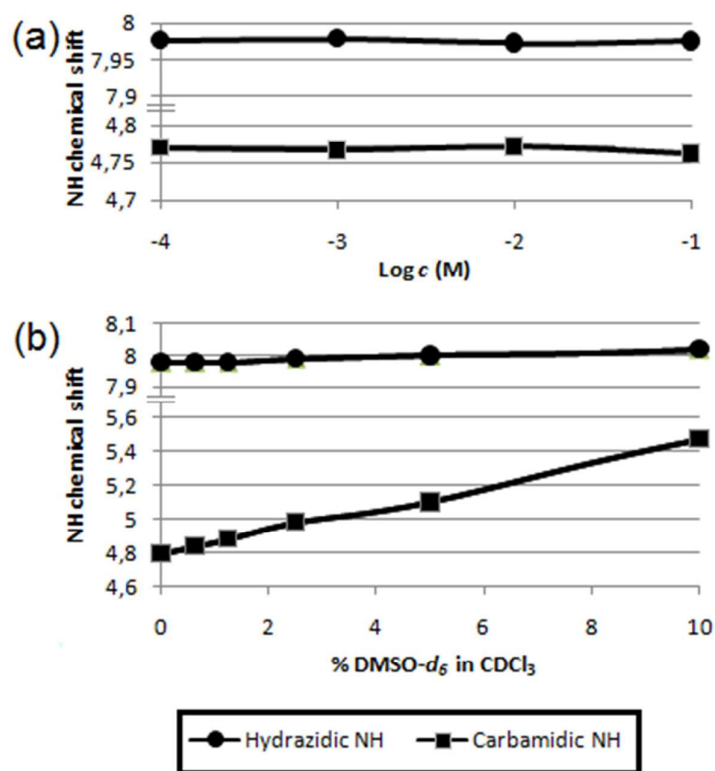


FIGURE 5. (a) Concentration-dependence in CDCl<sub>3</sub> (from 10<sup>-1</sup> to 10<sup>-4</sup> M) and (b) solvent dependence as determined by <sup>1</sup>H NMR (300 MHz) in mixed CDCl<sub>3</sub>/DMSO-*d*<sub>6</sub> (*c* = 10<sup>-2</sup> M) of NH chemical shifts of **1**

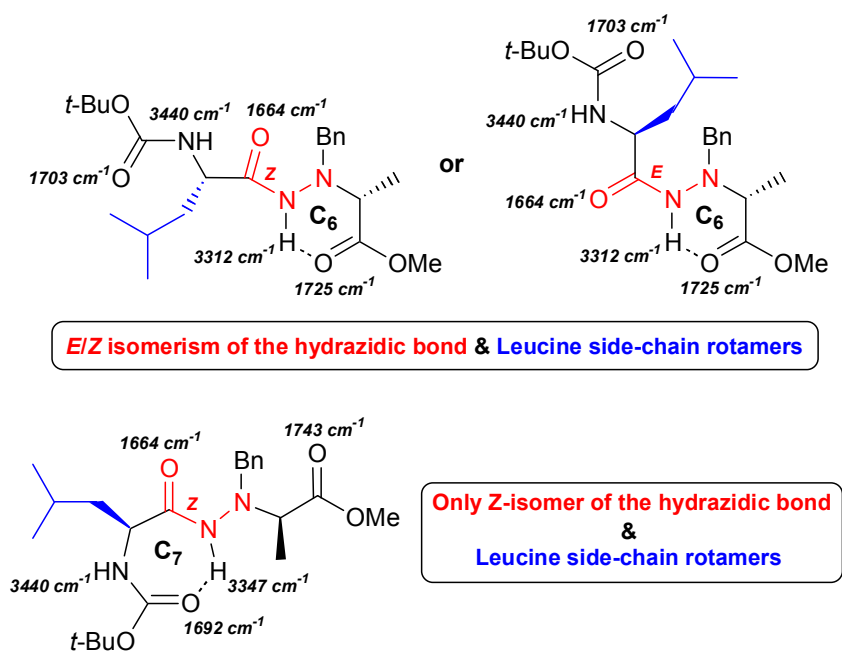


FIGURE 6. Possible conformations of compound **1** in CDCl<sub>3</sub> solution

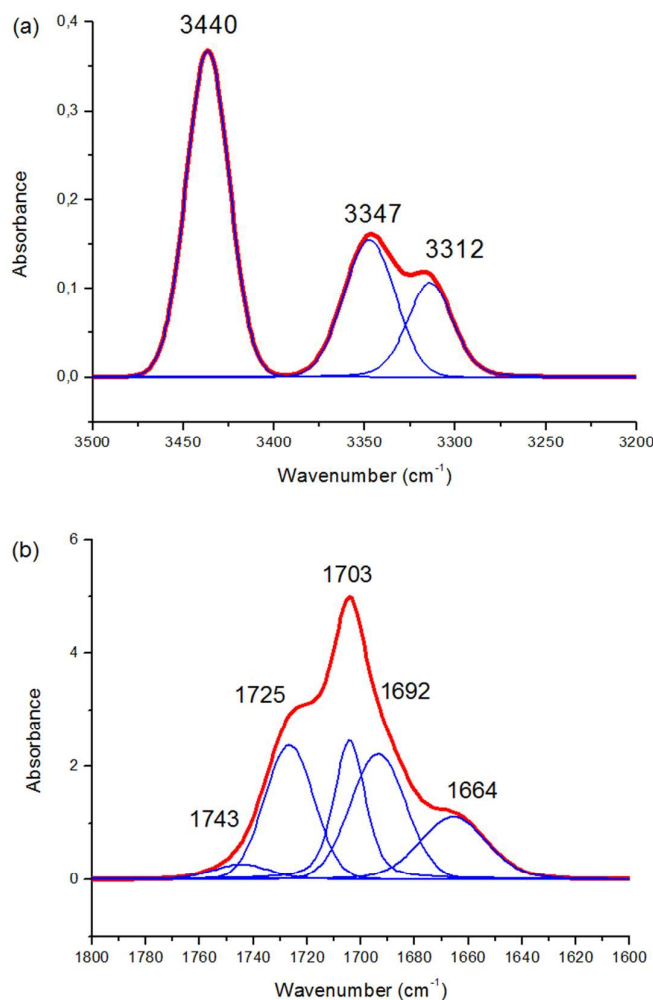
Careful inspection of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of compound (*S,R*)-**1** (see Supporting Information pp S2 to S5), revealed an additional minor set of signals, demonstrating the presence of a several conformations in  $\text{CDCl}_3$  solution. These various conformations were due to the possible presence of *E/Z* isomerism of the hydrazidic bond<sup>18f,19</sup> and leucine side-chain rotamers.<sup>20</sup> At this point, two possible conformations ( $\text{C}_6$  and  $\text{C}_7$  pseudocycles), involving the hydrazidic NH proton, were envisioned (Figure 6).

To complement these NMR results, FTIR absorption spectroscopic studies were also conducted. FTIR absorption spectroscopy is an efficient method to detect several conformers in solution by given differentiable N-H and C=O signals for each conformer.<sup>21</sup> FTIR experiments were conducted in  $\text{CDCl}_3$  at a concentration where intermolecular contacts have been experimentally proved to be undetectable ( $c = 10^{-2}$  M).

Figure 7 presents the IR spectra of N-H and C=O stretching regions of compound (*S,R*)-**1** (three and five bands, respectively). For the N-H stretching region, the first band at  $3440\text{ cm}^{-1}$ , located in the free N-H domain ( $> 3400\text{ cm}^{-1}$ ),<sup>17,21</sup> corresponds to the free NHBoc and the two other bands at  $3347$  and  $3312\text{ cm}^{-1}$  were assigned to the H-bonded hydrazidic NH which is involved in both conformations described above (Figure 6). Among the 5 bands observed in the C=O domain, 4 bands ( $1743/1725\text{ cm}^{-1}$  and  $1703/1692\text{ cm}^{-1}$ ) were assigned to the ester and carbamidic CO (free/bonded) respectively and the last one located at  $1664\text{ cm}^{-1}$  to the free hydrazidic CO, which is not involved in any intramolecular H-bond. These FTIR absorption results are an endorsement of our hypothesis that compound (*S,R*)-**1** adopts both  $\text{C}_6$  and  $\text{C}_7$  conformations in  $\text{CDCl}_3$  solution (Figure 6).

Same kind of FTIR absorption analysis is obtained at high concentration ( $c = 1$  M,  $\text{CDCl}_3$ ) indicating that this conformational equilibrium is still maintained. It is noteworthy that the

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3 same self-organization has been observed in other solvents ( $c = 10^{-2}$  M) such as toluene and  
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5 methanol (see Supporting Information pp S10 and S11).  
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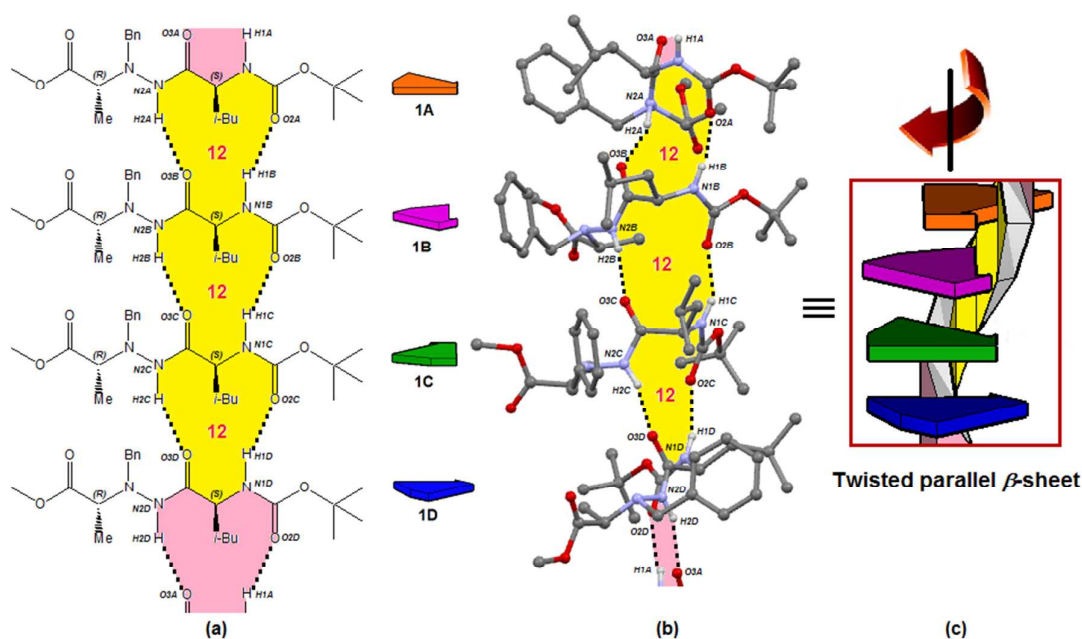


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43 **FIGURE 7.** IR spectra (in red) and deconvoluted IR spectra (in blue) of (a) N-H and (b) C=O stretching regions  
44 in CDCl<sub>3</sub> ( $c = 10^{-2}$  M) of **1**  
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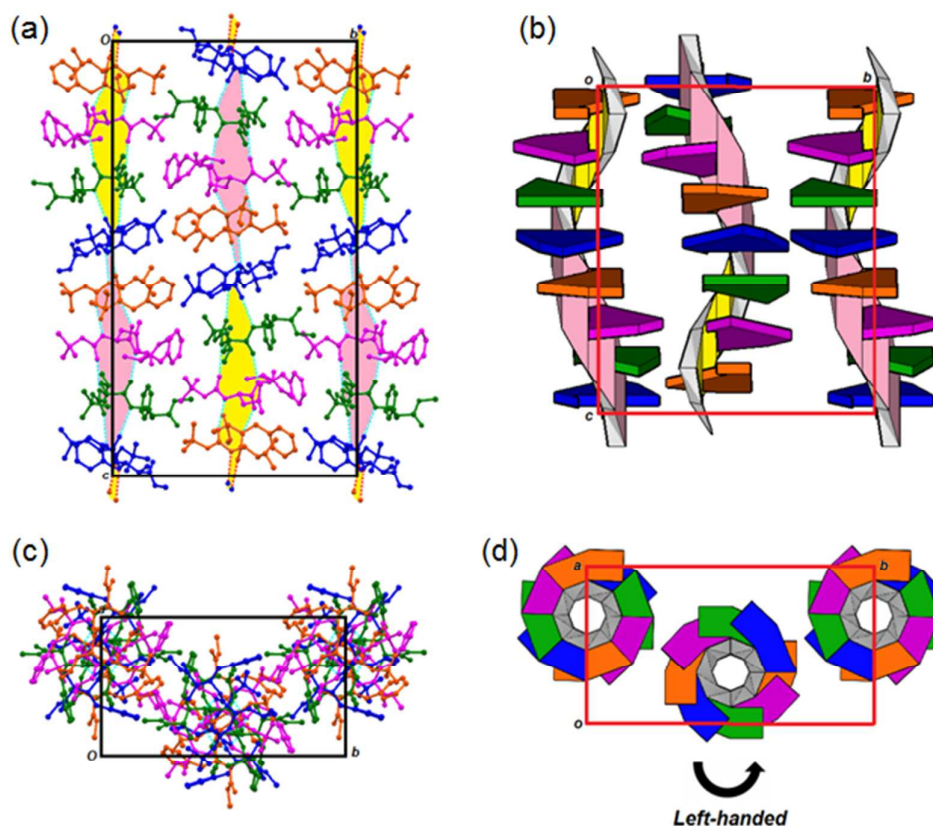
#### 50 **Studies of the self-assembly of 1-4 in the crystal state by X-ray diffraction**

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53 Crystals of compounds **1-4** were grown by slow evaporation from a  
54 dichloromethane/petroleum ether solution and crystallized in the space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> (see  
55 Supporting Information pp S12 to S23 for more details). Crystallographic data and structure  
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refinement parameters are reported in CIF format with CCDC references 1447540 to 1447543 (a copy of CIF formats are available in Supporting Information). The crystal structure determination of (*S,R*)-**1** and (*R,S*)-**2** was obtained from low-resolution X-ray diffraction data collected at 100K (R-factor values of 6.92 and 9.56% respectively). Regarding compounds (*R,R*)-**3** and (*S,S*)-**4**, poor X-ray diffraction data were collected (R-factor values of 17.04 and 12.14% respectively). Consequently, their crystal structures are less resolved but are still sufficient to confirm the packing and the folding.



**FIGURE 8.** (a) Hydrogen bond patterns between four independent molecules (1A-1D), (b) 12-membered hydrogen-bonded rings and (c) cartoon representation of the twisted parallel  $\beta$ -sheet for **1**



**FIGURE 9.** (a,c) Molecular packing and (b,d) cartoon representation of the twisted parallel  $\beta$ -sheet of **1** viewed along the  $a$  and  $c$  axis respectively

The X-ray diffraction structure of the heterochiral ester (*S,R*)-**1** corresponds to sixteen molecules in the unit cell (four molecules 1A-1D in the asymmetric unit) and the  $N^\alpha$ -atom has a pyramidal conformation with (*S*)-configuration (Figures 8 and 9).

The four independent molecules 1A-1D stacked one atop the other form a tetramer block and two of these can interact together to provide an octamer block. This tetrameric/octameric association is maintained through an intermolecular H-bonding network between the carbamidic C=O and NH and the hydrazidic C=O and NH of each molecule 1A-1D, closing a 12-membered H-bonded pseudocycle with a distance  $d_{(C=O \cdots H-N)}$  of 1.98 to 2.16 Å (Figures 8, 9 and Table S2 in Supporting Information) demonstrating a parallel  $\beta$ -sheet arrangement as indicated in Figure 1.

The data collected from the crystal analysis of the intermolecular packing clearly show no intramolecular H-bonds in the crystal lattice, as evidenced by the high nuclear distances. The “ $\beta$ -strand” molecules stack up on top of each other into a parallel fashion through intermolecular H-bonds. However, the most interesting feature of this self-assembly is the presence of a twist that shapes a left-handed helical supramolecular structure. The formation of a helical structure will be referred as a twisted  $\beta$ -sheet. It is noteworthy that the formed helix requires eight molecules to complete a full  $360^\circ$  rotation with a pitch of 38.20 Å. In the CCDC database, there are various examples of acyclic dipeptides with parallel  $\beta$ -sheet structures<sup>22</sup> (flat tapes or twisted with different numbers of molecules per turn) but only one parallel twisted  $\beta$ -sheet was observed with eight molecules per turn (depsidipeptideBoc-Leu-Lac-OEt).<sup>23</sup>

By taking a close look at the torsion angle values ( $\omega$ ,  $\phi$  and  $\psi$ ) of the Leu residue, responsible for the left-handed helical supramolecular structure, we have found that these values are closely related to the left-handed type-II poly(L-Pro)<sub>n</sub> conformation (Table 1)<sup>24</sup> but the supramolecular helix is approximately four times more elongated than type II poly(L-Pro)<sub>n</sub> helix by comparing their helical pitch (Table 1).

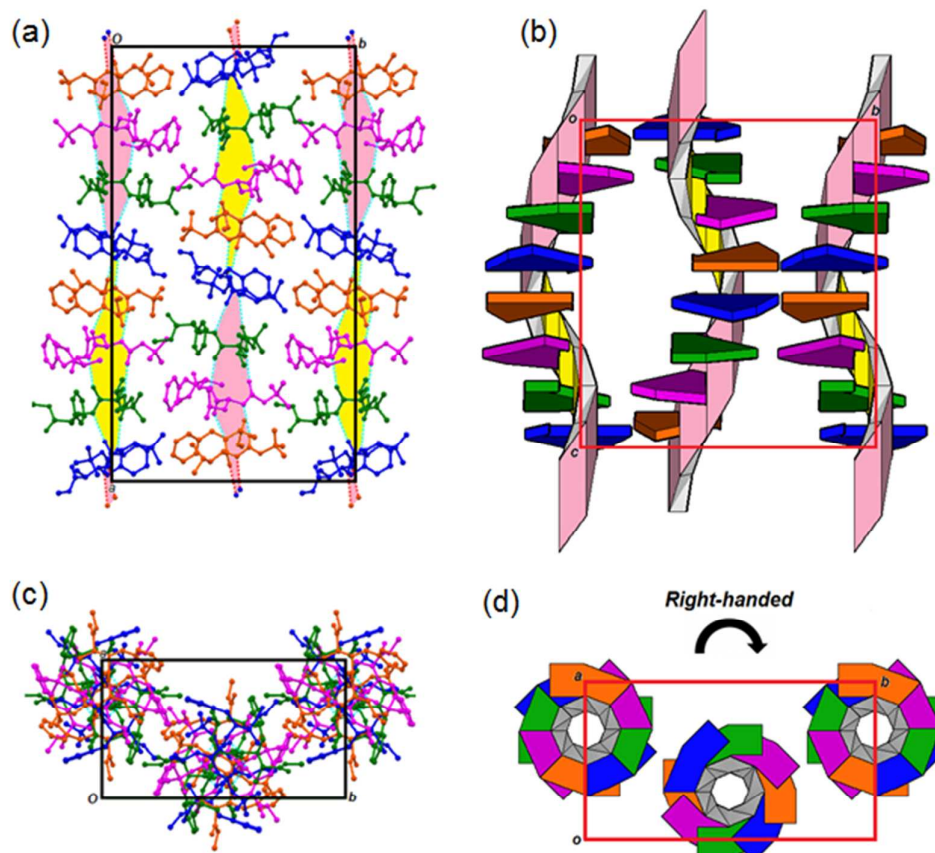
**TABLE 1.** Selected parameters characterizing the left-handed helical supramolecular structure in **1** and the left-handed type-II poly(L-Pro)<sub>n</sub> helix.

	Supramolecular helix in <b>1</b>	type-II poly(L-Pro) <sub>n</sub> helix <sup>24</sup>
$\omega$ (°)	+172 to +180	+180
$\phi$ (°)	-81 to -70	- 80
$\psi$ (°)	+115 to +124	+150
n	8 <sup>a</sup>	3 <sup>b</sup>
Pitch (Å)	38.20	9.3

<sup>a</sup>Number of residues (L-Pro) per helical turn for the type-II poly(L-Pro)<sub>n</sub> helix

<sup>b</sup>Number of molecules per helical turn for the supramolecular helix in **1**

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3 X-ray diffraction analysis of compound (*R,S*)-**2** showed that changing the  $\alpha$ -carbon chirality  
4 of both the  $\alpha$ -amino acid and the  $\alpha$ - $N^\alpha$ -Bn-hydrazino acid residues in our pseudodipeptide  
5 backbone reverses the chirality of the  $N^\alpha$ -atom. As a result, the corresponding heterochiral  
6 (*R,S*)-**2** is the enantiomer of (*S,R*)-**1** and logically leads to a reverse turn of the  $\beta$ -helical  
7 structure, *i.e.* compound (*R,S*)-**2** adopts a right-handed twist with the same helical pitch (38.19  
8 Å, Figure 10). It is known that an achiral pseudodipeptide (CCDC Refcode XEZDUC) is able  
9 to form a parallel twisted  $\beta$ -sheet self-assembly.<sup>25</sup> However, as compounds **1** and **2** possess  
10 two chiral carbons, we decided to analyse the crystal structures of homochiral (*R,R*)-**3** and  
11 (*S,S*)-**4** stereoisomers in order to know which chiral carbon was responsible for the control of  
12 helicity. The results were unambiguous and are presented in Table 2.  
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**FIGURE 10.** (a,c) Molecular packing and (b,d) cartoon representation of the twisted parallel  $\beta$ -sheet of **2** viewed along the *a* and *c* axis respectively



TABLE 2. Summary of the handedness of the helices adopted by **1-4** in the crystal state

Compound	Chirality of the $N^\alpha$ -atom	Twist direction of the helix	Pitch (Å)
( <i>S,R</i> )- <b>1</b>	<i>S</i>	Left-handed	38.20
( <i>R,S</i> )- <b>2</b>	<i>R</i>	Right-handed	38.19
( <i>R,R</i> )- <b>3</b>	<i>R</i>	Right-handed	38.17
( <i>S,S</i> )- <b>4</b>	<i>S</i>	Left-handed	38.21

Homochiral compounds (*R,R*)-**3** and (*S,S*)-**4** (see Supporting Information pp S19 to S24) present the same kind of  $\beta$ -helical structures as those observed with heterochiral compounds with a left- and right-handed twist, respectively. The crystal structure of (*R,R*)-**3** corresponds to sixteen molecules in the unit cell (four molecules of 3A-3D in the asymmetric unit) with the  $N^\alpha$ -atom having a pyramidal conformation with (*R*)-configuration as observed for (*R,S*)-**2**. As previously, the chirality inversion of  $N^\alpha$ -atom between (*R,R*)-**3** and (*S,S*)-**4** occurred, making them an enantiomeric couple, with a  $N^\alpha$ -atom adopting the same configuration as the  $C^\alpha$ -atom of the  $\alpha$ -amino acid residue. Therefore, this alternating switch of the helicity, without changing the helical pitch, can undoubtedly be attributed to the  $\alpha$ -carbon chirality of the  $\alpha$ -amino acid residue by comparison of the crystal structures of stereoisomers **1-4**.

Indeed, a careful consideration of the intermolecular H-bond network points out that only one of the two asymmetric carbons present in the 1:1 [ $\alpha/\alpha$ - $N^\alpha$ -Bn-hydrazino] pseudodipeptide backbone, namely the  $\alpha$ -amino acid  $C^\alpha$ , is involved in the formation of the 12-membered H-bond pseudocycle responsible for the parallel  $\beta$ -sheet formation (Figure 9).

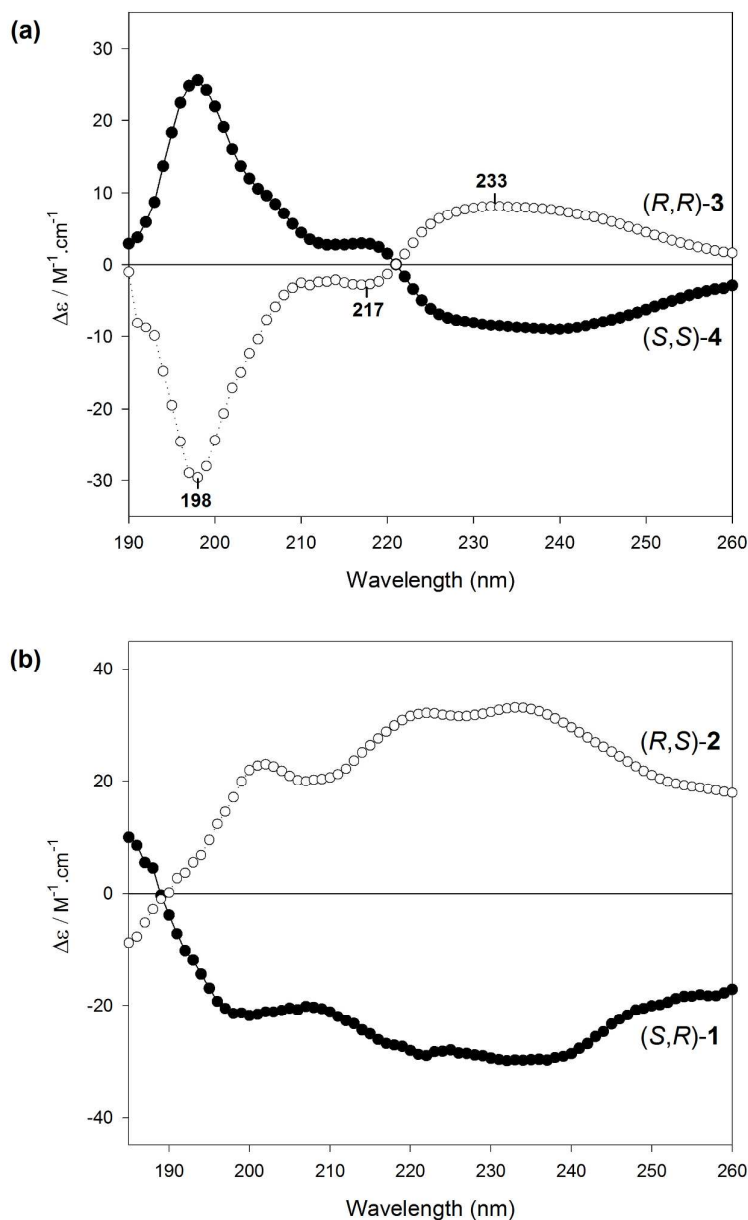
This seems to pinpoint the fact that a D-amino acid in the ester 1:1 [ $\alpha/\alpha$ - $N^\alpha$ -Bn-hydrazino] pseudodipeptide series will induce a right-handed twisted  $\beta$ -sheet, while its L-amino acid counterpart will lead to a left-handed helical structure.

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3 **Studies of the self-assembly of 1-4 in the crystal state by microcrystalline Circular**  
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5 **Dichroism**  
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8 CD spectroscopy has been widely used to investigate structural changes in non-racemic chiral  
9 organic molecules. While it is frequently performed in solution, solid-state CD analysis,  
10 through the microcrystalline technique, also represents a powerful tool that provides crucial  
11 specific supramolecular information.<sup>26</sup> CD spectra were recorded on microcrystals of  
12 compounds **1-4** obtained by crushing the crystals in a mortar.  
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20 With the aim of optimizing results, microcrystals of **1-4** were suspended in Nujol oil ( $c = 25$ -  
21  $50 \text{ mg.mL}^{-1}$ ), which is required to achieve a viscous suspension. Figure 11 depicts  
22 microcrystalline CD spectra of compounds **1-4** and it is worth mentioning that Nujol oil has  
23 no effect on the CD signal.  
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30 As illustrated in Figure 11, the shape of CD curves differs from those conventionally used for  
31 the determination of protein secondary structure ( $\alpha$ -helix,  $\beta$ -sheet or random coil)<sup>27</sup> and  
32 provides a specific signal for both homochiral and heterochiral series. In each series, we  
33 clearly observed an inversion of the CD signal, which confirms the presence of two pairs of  
34 enantiomers as well as the complete chirality control of the  $N^{\alpha}$ -atom in the crystal state.  
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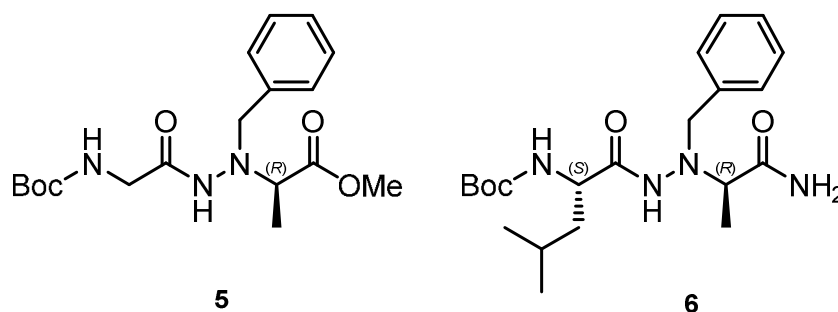
**FIGURE 11.** Microcrystalline normalized CD spectra of pseudodipeptides 1-4, in Nujol mulls ( $c = 25\text{-}50 \text{ mg}\cdot\text{mL}^{-1}$ ), in 0.01 cm quartz flat cells, at 293K; Nujol blank was subtracted from the spectra: (a) Superimposition of homochiral compounds (R,R)-3 and (S,S)-4 CD spectra. (b) Superimposition of heterochiral compounds (S,R)-1 and (R,S)-2 CD spectra

In the homochiral series (Figure 11a), compound (R,R)-3 presents, contrary to its enantiomer (S,S)-4, only one broad positive band centered at approximately 233 nm, and two negative bands estimated at 217 and 198 nm. Analysis of heterochiral series gives too weak signals

when recorded on a CD spectropolarimeter. By the fact, CD spectra of compounds **1-2** were recorded on a SRCD (synchrotron radiation CD) spectropolarimeter.

By correlating the X-ray diffraction and CD results of (*R,R*)-**3** and (*S,S*)-**4**, we can conclude that the shape of the curves of (*R,R*)-**3** and (*S,S*)-**4** refer to right- and left-handed helical  $\beta$ -sheet structures, respectively.

For supporting these conclusions, compounds **5** and **6**<sup>13</sup> (Figure 12) were analyzed by microcrystalline CD. Pseudodipeptide **5** is an analogue of (*S,R*)-**1** and of (*R,R*)-**3** bearing a glycine residue (no chiral carbon center) instead of the leucine one. Compound **6** is a C-terminal amide analogue of (*S,R*)-**1** and is known to fold via an hydrazinoturn which could prevent the self-assembly into  $\beta$ -sheets.

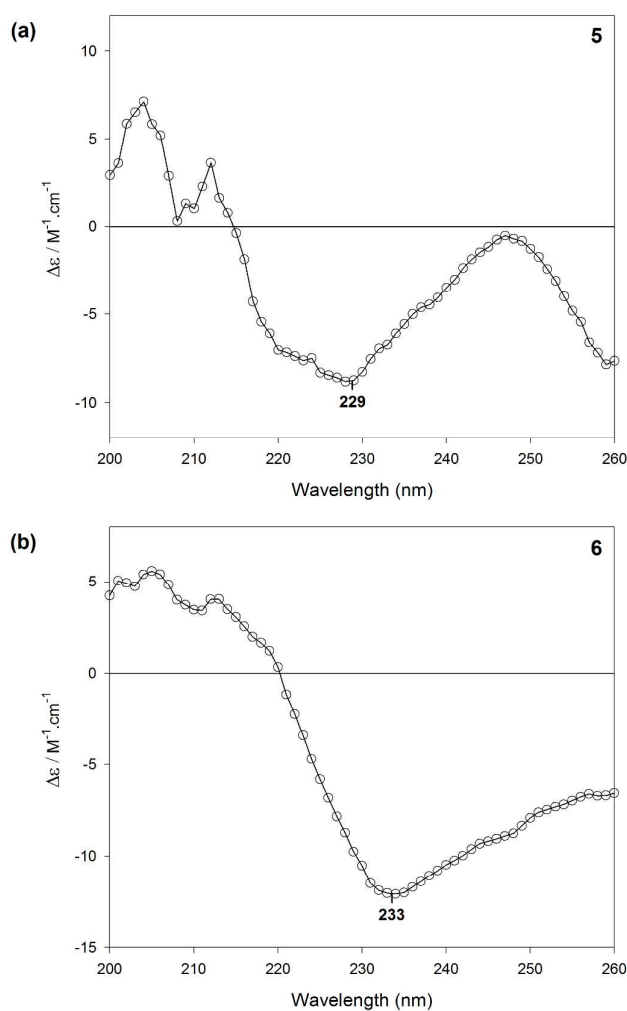


**FIGURE 12.** Chemical structure of pseudodipeptides **5-6**

Crystals of compounds **5** and **6** were grown by slow evaporation from a dichloromethane/petroleum ether mixture, but once again, the poor quality crystals prevented us from performing the X-ray diffraction analysis. However, as noted earlier, the microcrystalline CD spectra of these compounds could be recorded on a CD spectrometer (Figure 13).

CD spectra of compounds **5** (Figure 13a) and **6** (Figure 13b) are closed to aryl benzyl sulfoxide compounds presenting only a particular negative maximum value around 230 nm corresponding to the benzyl part.<sup>28</sup> This observation suggests that the presence of a new

hydrogen bond donor site at the *C*-terminal position (compound **6**) and the absence of a chiral carbon center in the  $\alpha$ -amino acid residue (compound **5**) avoid the supramolecular self-assembly into  $\beta$ -sheets. In the case of **6**, it possibly due to a bifurcated intramolecular H-bond (called hydrazinoturn), as was previously demonstrated in the  $\text{CDCl}_3$  solution state.<sup>14</sup>



**FIGURE 13.** Microcrystalline normalized CD spectra of pseudodipeptides **5** (a) and **6** (b), in Nujol mulls ( $c = 50 \text{ mg} \cdot \text{mL}^{-1}$ ), in 0.01 cm quartz flat cells, at 293K; Nujol blank was subtracted from the spectra

## CONCLUSION

In summary, we highlighted in this work that fully protected 1:1 [ $\alpha/\alpha$ - $N^\alpha$ -Bn-hydrazino] pseudodipeptides **1-4** can self-assemble in a twisted  $\beta$ -sheet supramolecular structure in the

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2  
3 crystal state. The  $N^\alpha$  atom chirality is fixed and adopts the same configuration as the  $C^\alpha$ -atom  
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5 of the  $\alpha$ -amino acid residue. Changing the chirality of the  $C^\alpha$ -atom of the leucine residue lead  
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7 to the inversion of the helicity of the twisted  $\beta$ -sheet, without changing the helical pitch.  
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9 Finally, this supramolecular  $\beta$ -helical structure is not maintained in the solution state, leaving  
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11 in its place a conformational equilibrium between  $C_6$  and  $C_7$  pseudocycles.  
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15 The study of the morphology of pseudodipeptides 1-4 by SEM analyses in order to determine  
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17 if these pseudodipeptides can generate any amyloid-like fibrils through  $\beta$ -sheet mediated self-  
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19 assembly, which is a main cause of the neurodegenerative disease Alzheimer's is currently in  
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21 progress.  
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## 24 EXPERIMENTAL SECTION

### 25 NMR

26  
27 All NMR spectra (1D and 2D) were recorded at 298K on a 300 MHz spectrometer with  
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29 tetramethylsilane (TMS) as the internal standard. Chemical shifts ( $\delta$ ) are reported in ppm  
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31 downfield from  $CDCl_3$  ( $\delta = 7.26$  ppm) for  $^1H$  NMR and relative to the central  $CDCl_3$   
32  
33 resonance ( $\delta = 77.0$  ppm) for  $^{13}C$  NMR spectroscopy.  
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37  $^{13}C$  multiplicity data were obtained from JMOD experiments (C and  $CH_2$  up,  $CH_3$  and CH  
38  
39 down).  
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41 For  $^1H$  NMR spectroscopic data, the coupling constants ( $J$ ) are given in Hz and the  
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43 multiplicity is defined as: s for singlet; d for doublet; q for quartet; m for multiplet; br for  
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45 broad, or combinations thereof.  
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47  $^1H$  and  $^{13}C$  NMR signals were assigned with the help of COSY, ROESY, JMOD, HSQC and  
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49 HMBC experiments.  
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51 For each compound,  $^1H$  and  $^{13}C$  NMR data indicated the presence of multiple conformations  
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53 in  $CDCl_3$  solution due in large part to the  $E/Z$  isomerism of the hydrazidic bond<sup>19</sup> and/or the  
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55 possible rotamers of the leucine side chain.<sup>20</sup> Only the chemical shifts of the major  
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3 conformations are given. Owing to the similarity of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, only one  
4 characterization data is given for each pair of enantiomers (1/2 and 3/4).  
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6  
7 For the concentration dependence experiment in  $\text{CDCl}_3$ , the chemical shift of each NH proton  
8 was measured under four different concentrations ( $10^{-4}$ ,  $10^{-3}$ ,  $10^{-2}$  and  $10^{-1}$  M).  
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11 For the solvent dependence experiment, the chemical shift of each NH proton was measured  
12 at a  $10^{-2}$  M concentration at various percentages of  $\text{DMSO-}d_6$  in  $\text{CDCl}_3$  (0, 0.625, 1.25, 2.5, 5  
13 and 10%).  
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### 16 17 **FTIR absorption**

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19 Infrared spectra were recorded with an attenuated total reflectance Fourier transform infrared  
20 (FTIR) spectrophotometer equipped with Mid-IR source, KBr beamsplitter, and a liquid-  
21 nitrogen-cooled mid-band mercury cadmium telluride (LN-MCT Narrow) detector. Each  
22 spectrum was recorded over 256 scans at 298K and obtained in  $\text{CDCl}_3$  ( $c = 10^{-2}\text{M}$ ) with a  
23  $\text{CaF}_2$  cell of 500  $\mu\text{m}$  path length at a concentration of 10 mM after subtraction of the solvent  
24 spectrum ( $\text{CDCl}_3$ ).  
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30 The deconvolution method was used to resolve the overlapping bands in the NH and C=O  
31 stretching regions and the resulting deconvoluted spectra provided information about the  
32 conformational equilibrium occurring in the  $\text{CDCl}_3$  solution. The deconvolution of N-H and  
33 C=O stretching region bands ( $3500\text{-}3200\text{ cm}^{-1}$  and  $1800\text{-}1600\text{ cm}^{-1}$  respectively) was  
34 performed with the OPUS a software package (version 6.5). Band positions were determined  
35 based on fixed number bands obtained from the second derivatives of the original spectra.  
36 These bands were automatically adjusted by the damped least-squares optimization algorithm  
37 developed by Levenberg-Marquardt. The obtained root mean square error (RMSE) was below  
38 0.0009 for each spectrum.  
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### 45 46 **Circular Dichroism**

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48 Microcrystalline CD spectra were recorded on a CD spectropolarimeter, scanned on the far-  
49 UV range, at 293K with 1 nm steps from 260 to 190 nm and averaged over 3 scans. Between  
50 each scan, a delay of 5 minutes was respected in order to detect any UV induced  
51 modification.<sup>29</sup> For each compound no spectra shape modification was noted. Crystals were  
52 crushed with a mortar to obtain a very small granulation, and the microcrystals were then  
53 suspended in Nujol oil at a final concentration of 25 or 50  $\text{mg}\cdot\text{mL}^{-1}$ . This preparation provided  
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3 an optimum homogeneity of Nujol mull, allowing to avoid depolarization or scattering  
4 effects.<sup>30</sup> Absorbance was simultaneously registered and checked for any variation between  
5 isomers. Moreover, to demonstrate the absence of detectable artefactual signals,<sup>31</sup> several  
6 measurements were done with different angles by inverting engraved 0.01 cm path length cells  
7 by a 180° flip on the y-axis and 90° rotations on the z-axis. With these control conditions, all  
8 measurements remained unchanged, providing similar spectra. Absorbance levels, below 1.5  
9 AU, between heterochiral then homochiral compounds, were superimposable with each other.  
10 Nujol is a mixture of hydrocarbons and exhibits significant absorbance below 200 nm. The  
11 CD spectra of pure Nujol oil as a function of mdeg and high tension (HT) in the wavelength  
12 range from 180 nm to 260 nm have been recorded in order to prove the reliability of our CD  
13 measurements (see Supporting Information pp S25).  
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### 21 X-ray diffraction

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24 Crystals of compounds **1-4** exhibited weak diffracting intensities. Their X-ray data were  
25 collected at 100K with a diffractometer equipped with a copper microsource ( $\lambda = 1.54184 \text{ \AA}$ )  
26 and a CCD detector. The structure was solved by direct methods with SIR2014.<sup>32</sup> The  
27 structure models refinements were conducted using a spherical atom model with ShelXle.<sup>33</sup>  
28 All X-ray data for each compound (**1-4**) are deposited in the CCDC database under access  
29 numbers: 1447540, 1447541, 1447542, and 1447543 respectively. Selected crystallographic  
30 data for **1-4** are provided in the Supporting Information.  
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### 36 HRMS

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38 The ESI-HRMS analysis was conducted using a quadrupole-time-of-flight (QTOF) mass  
39 spectrometer equipped with an ion funnel ESI Electrospray source (1  $\mu\text{L}$  - 1 mL/min).  
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### 44 Characterization of compounds 1-4

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47 Compounds **1-6** were prepared according to reference 14.  
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50 **Heterochiral pseudodipeptides Boc-[(L)-Leucine- $\alpha$ -N<sup>z</sup>-benzyl-(D)-hydrazinoalanine]-**  
51 **OMe and Boc-[(D)-Leucine- $\alpha$ -N<sup>z</sup>-benzyl-(L)-hydrazinoalanine]-OMe, (*S,R*)-**1** and (*R,S*)-**  
52 **2.** White powder (3.63g, 96%). Characterization data: mp 111-113°C; <sup>1</sup>H NMR (300 MHz,  
53 CDCl<sub>3</sub>, 10 mM) of major conformer  $\delta$  0.89 (d, 6H,  $J = 6.3 \text{ Hz}$ , 2  $\delta\text{CH}_3\text{Leu}$ ), 1.23-1.65 (m, 6H,  
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3  $\beta\text{CH}_3\text{Ala}$ ,  $\beta\text{CH}_2\text{Leu}$ ,  $\gamma\text{CHLeu}$ ), 1.47 (s, 9H, Boc), , 3.72-3.83 (m, 1H,  $\alpha\text{CHAla}$ ), 3.78 (s, 3H,  
4 OCH<sub>3</sub>), 3.88-4.13 (m, 3H, CH<sub>2</sub>,  $N^\alpha\text{-Bn}$ ,  $\alpha\text{CHLeu}$ ), 4.77 (br s, NHBoc), 7.23-7.43 (m, 5H, Ar.),  
5 7.98 (s, 1H, NH hydrazidic); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) of major conformer  $\delta$  16.3  
6 ( $\beta\text{CH}_3\text{Ala}$ ), 22.7 (2  $\delta\text{CH}_3\text{Leu}$ ), 24.6 ( $\gamma\text{CHLeu}$ ), 28.3 (3 CH<sub>3</sub>, Boc), 41.2 ( $\beta\text{CH}_2\text{Leu}$ ), 51.6 (O-  
7 CH<sub>3</sub>), 52.3 ( $\alpha\text{CHLeu}$ ), 60.0 ( $\alpha\text{CHAla}$ ), 60.1 (CH<sub>2</sub>,  $N^\alpha\text{-Bn}$ ), 79.9 (C, Boc), 127.6 (CH, Ar.),  
8 128.3 (2 CH, Ar.), 129.3 (2 CH, Ar.), 136.3 (C, Ar.), 155.3 (C=O, Boc), 171.1 (C=O,  
9 hydrazid), 174.6 (C=O, ester); HRMS (ESI) calculated for C<sub>22</sub>H<sub>35</sub>N<sub>3</sub>O<sub>5</sub> [M+H]<sup>+</sup> *m/z* 422.2655,  
10 found 422.2649 and 422.2646.  
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21 **Homochiral pseudodipeptides Boc-[(D)-Leucine- $\alpha$ - $N^\alpha$ -benzyl-(D)-hydrazinoalanine]-**  
22 **OMe and Boc-[(L)-Leucine- $\alpha$ - $N^\alpha$ -benzyl-(L)-hydrazinoalanine]-OMe, (R,R)-3 and (S,S)-**  
23

24 **4.** White powder (3.03g, 80%). Characterization data: mp 82-84°C; <sup>1</sup>H NMR (300 MHz,  
25 CDCl<sub>3</sub>, 10 mM) of major conformer  $\delta$  0.94 (d, 6H, *J* = 6.3 Hz, 2  $\delta\text{CH}_3\text{Leu}$ ), 1.34-1.56 (m, 6H,  
26 ,  $\beta\text{CH}_3\text{Ala}$ ,  $\beta\text{CH}_2\text{Leu}$ ,  $\gamma\text{CHLeu}$ ), 1.43 (s, 9H, Boc), 3.68-3.79 (m, 1H,  $\alpha\text{CHAla}$ ), 3.75 (s, 3H,  
27 OCH<sub>3</sub>), 3.82-3.93 (m, 1H,  $\alpha\text{CHLeu}$ ), 3.96-4.08 (m, 2H, CH<sub>2</sub>,  $N^\alpha\text{-Bn}$ ), 4.80 (br s, NHBoc),  
28 7.23-7.39 (m, 5H, Ar.), 7.93 (s, 1H, NH hydrazidic); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) of major  
29 conformer  $\delta$  16.1 ( $\beta\text{CH}_3\text{Ala}$ ), 22.6 (2  $\delta\text{CH}_3\text{Leu}$ ), 24.4 ( $\gamma\text{CH Leu}$ ), 28.3 (3 CH<sub>3</sub>, Boc), 41.1  
30 ( $\beta\text{CH}_2\text{Leu}$ ), 51.6 (O-CH<sub>3</sub>), 51.9 ( $\alpha\text{CHLeu}$ ), 60.0 (CH<sub>2</sub>,  $N^\alpha\text{-Bn}$ ), 60.2 ( $\alpha\text{CHAla}$ ), 79.7 (C, Boc),  
31 127.6 (CH, Ar.), 128.2 (2 CH, Ar.), 128.8 (2 CH, Ar.), 136.4 (C, Ar.), 155.5 (C=O, Boc),  
32 171.4 (C=O, hydrazid), 174.5 (C=O, ester); HRMS (ESI) calculated for C<sub>22</sub>H<sub>35</sub>N<sub>3</sub>O<sub>5</sub> [M+H]<sup>+</sup>  
33 *m/z* 422.2655, found 422.2633 and 422.2638.  
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48 **Pseudodipeptide Boc-[Glycine- $\alpha$ - $N^\alpha$ -benzyl-(D)-hydrazinoalanine]-OMe, 5.** Amorphous  
49 solid (3.18g, 97%). Characterization data: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 10 mM)  $\delta$  1.36-1.44  
50 (m, 12H), 3.57-3.65 (m, 3H), 3.73 (s, 3H), 3.99-4.13 (m, 2H), 5.15 (br s, 1H), 7.26-7.39 (m,  
51 5H), 7.56 (s, 1H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  16.0 (CH<sub>3</sub>), 28.2 (CH<sub>3</sub>), 41.3 (CH<sub>2</sub>), 51.7  
52 (CH<sub>3</sub>), 59.7 (CH), 61.9 (CH<sub>2</sub>), 79.8 (C), 127.5 (CH), 127.8 (2 CH), 129.1 (2 CH), 136.1 (C),  
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3 155.5 (C=O), 172.3 (C=O), 174.2 (C=O); HRMS (ESI) calculated for C<sub>18</sub>H<sub>27</sub>N<sub>3</sub>O<sub>5</sub> [M+H]<sup>+</sup>  
4  
5 *m/z* 366.2023, found 366.1998.  
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8 **Heterochiral pseudodipeptideBoc-[(L)-Leucine- $\alpha$ -N<sup>α</sup>-benzyl-(D)-hydrazinoalanine]-NH<sub>2</sub>,**  
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10 **6.** White powder (3.45g, 95%). Characterization data: mp 149-151°C; <sup>1</sup>H NMR (300 MHz,  
11 CDCl<sub>3</sub>, 10 mM)  $\delta$  0.77 (d, 3H, *J* = 6.5 Hz), 0.80 (d, 3H, *J* = 6.5 Hz), 1.35 (d, 3H, *J* = 7.1 Hz),  
12 1.45 (s, 9H), 1.40-1.62 (m, 3H), 3.51 (q, 1H, *J* = 7.1 Hz), 3.79-4.03 (m, 3H), 4.69 (br d, 1H, *J*  
13 = 7.5 Hz), 5.32 (s, 1H), 7.26-7.38 (m, 6H), 8.06 (br s, 1H). The physical data (NMR and  
14  
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19 HRMS) are in agreement with our values reported in Ref. 14.  
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25 **ACKNOWLEDGMENTS**  
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35 fellowship of Ms Eugenie Romero.  
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50 **SUPPORTING INFORMATION AVAILABLE**  
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53 Copies of <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of compounds **1-5**. Copy of <sup>1</sup>H NMR spectrum of  
54 compound **6**. Copies of 2D HSQC and HMBC NMR spectra of compounds **1-4**.  
55 Concentration and solvent dependence <sup>1</sup>H NMR spectra of compounds **2-4**. IR spectra and  
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3 deconvolved IR spectra in CDCl<sub>3</sub> of compounds 2-4. IR spectra in MeOH and toluene of  
4  
5 compound 1. Crystallographic data of compounds 1-4. Crystallographic CIF files of  
6  
7 compounds 1-4. Copies of far UV CD spectra of pure Nujol oil as a function of mdeg and  
8  
9 high tension (HT) in the wavelength range from 180 nm to 260 nm. This material is available  
10  
11 free of charge *via* the Internet at <http://pubs.acs.org>.  
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