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Peptides Incorporating 3,4-Dihydroxyprolines: Synthesis and Structural Study †

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Abstract:

Keywords:

1. Introduction

Protein secondary structure is the three-dimensional form taken by local segments of proteins. Although the most common types of structure are helices and sheets, other types of structures, such as turns and loops, are also formed [1]. The type of structure depends on the folding achieved by the protein as a result of the formation of hydrogen bonds between the carbonyl and NH groups of the peptide backbone.

Turns can be defined as the non-repetitive units within the secondary structure of proteins. Among them, the β- and γ-turns are the most studied ones [2]. The third group within the turns are the α-spins, which were characterised and classified on the basis of the X-ray crystallographic study of about 190 proteins.

Turns play an important role, both structurally and functionally. With regard to the structural aspect, they intervene in the folding of the peptide chain, favoring the formation of a specific tertiary, globular or fibrous structure. With regard to the functional aspect, the turns are mainly located in the region of the proteins most exposed to the environment, and therefore influence various processes, such as molecular and cellular recognition, as well as interactions between peptide structures and non-peptide substrates or receptors. In addition, the turns serve as templates for the design of new drugs, antigens and pesticides.

This communication refers to the case of the serine-proline sequence, in terms of its capacity to induce type I β-turns. This dipeptide is present in proteins that regulate gene expression and DNA binding. In addition, this sequence is recognized as a substrate by numerous kinases, representing a preferential site for protein phosphorylation [3].

An analysis of the serine-proline (SP) domains in the protein structures indicated a unique hydrogen bonding pattern. This domain features a hydrogen bond between the carbonyl oxygen of the Ser (i) residue and the amide proton of the i + 3 residue. However, in addition to this classical hydrogen bond, serine can participate in a side-chain-to-main-chain hydrogen bond to form two distinct patterns, called serine-proline turns. In the 6 + 10 pattern (see Figure 1) the serine hydroxyl group forms a hydrogen bond with the main-chain amide proton of residue i + 3 to create a six-membered hydrogen-bonded ring adjacent to the classical 10-membered ring. In the 9 + 10 pattern (see Figure 1) the serine hydroxyl group forms a hydrogen bond with the amide proton of residue i + 2, creating a nine-membered hydrogen bonded ring, which intersects the classical 10-membered ring.

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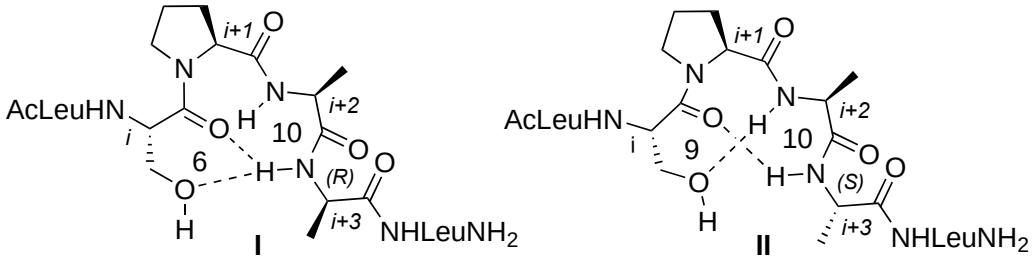


Figure 1. These turns were detected in natural peptides [4]. In addition, Galande described for the first time their presence in synthetic peptides [5], and was able to establish that the following requirements are necessary for β-turns to be generated with the SP subunit: (1) The presence of the serine-proline (SP) dipeptide domain; (2) Hydrophobic amino acids, such as leucine, flanking the SPXX tetrapeptide; (3) A residue other than glycine at positions i + 2 and i + 3.

Under these conditions the peptide has a 6 + 10 type structure.

2. Working Plan

As an application of our work on the synthesis of dihydroxyprolines, we decided to evaluate the ability of the dihydroxyproline-proline domain to induce the formation of β-turns, similar to those induced by the serine-proline pair in synthetic peptides. To this end, it is proposed to incorporate the dihydroxylated-proline dimer highlighted in blue into peptide **III**.

This novel peptide supports the possibility of hydrogen bond formation similar to that of peptide **II**, which incorporates the serine-proline sequence (in blue). They would be:

(1) Main chain hydrogen bonding, between the carbonyl oxygen of the Ser (i) residue and the amide proton of residue i + 3.

(2) Hydrogen bonding of the side chain with the main chain, the hydroxyl group of the serine and the amide proton of residue i + 3.

It could form bonds, similar to those established by the serine-proline domain (Figure 2).

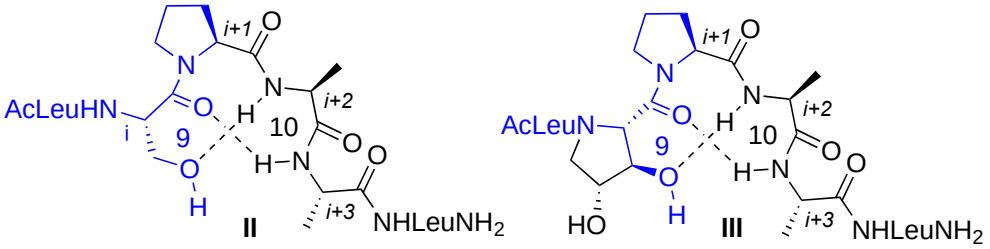
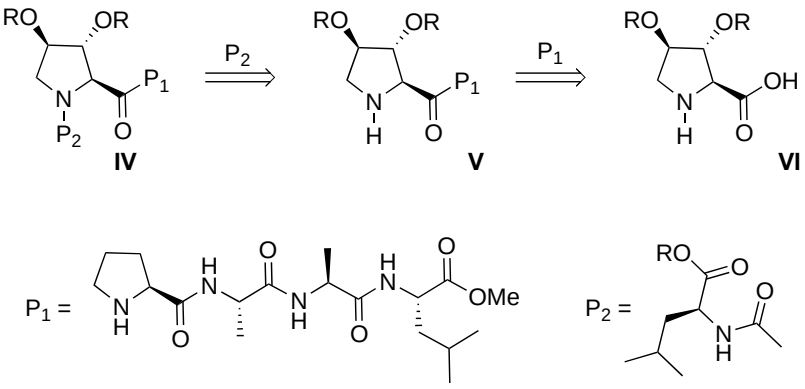
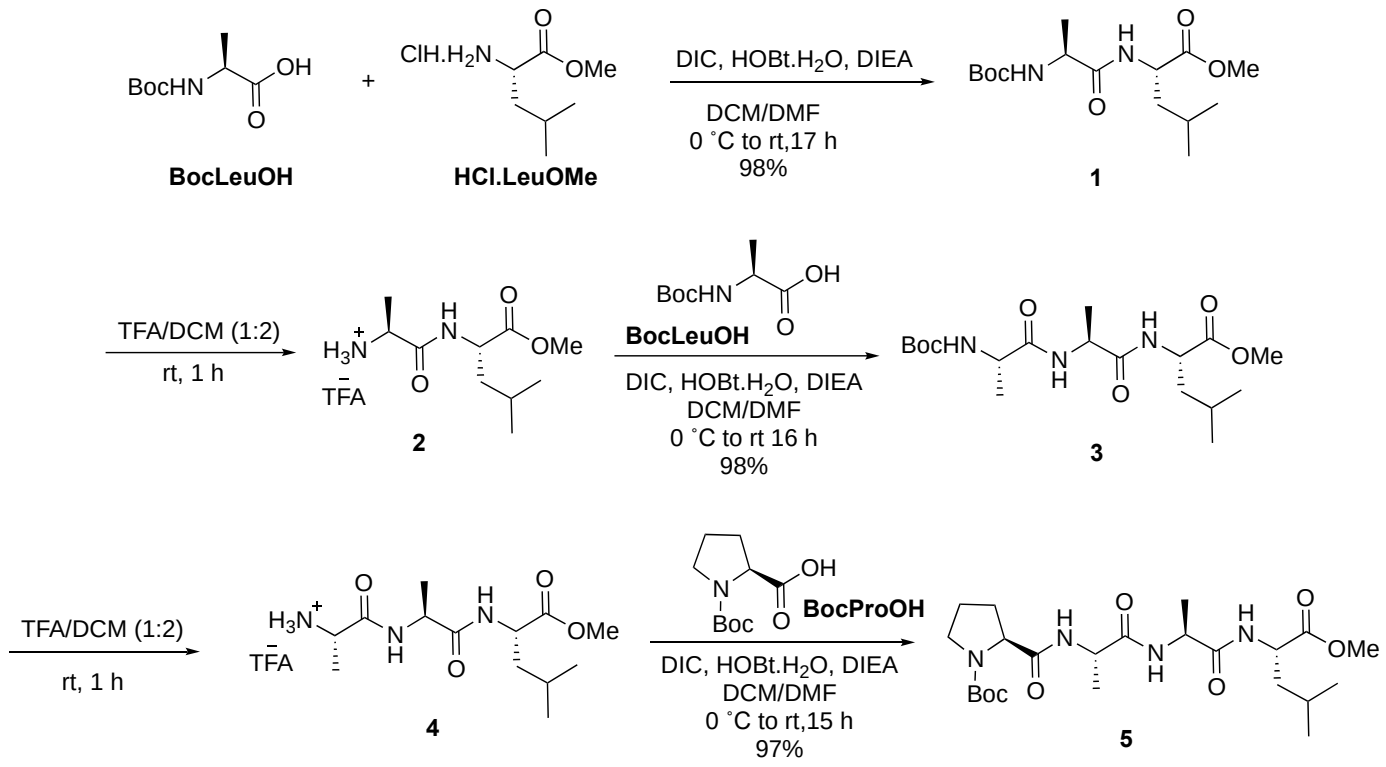


Figure 2. The proposed synthesis plan to prepare **IV** involves subjecting the dihydroxylated proline (suitably protected) to the two peptide couplings shown in Scheme 1.



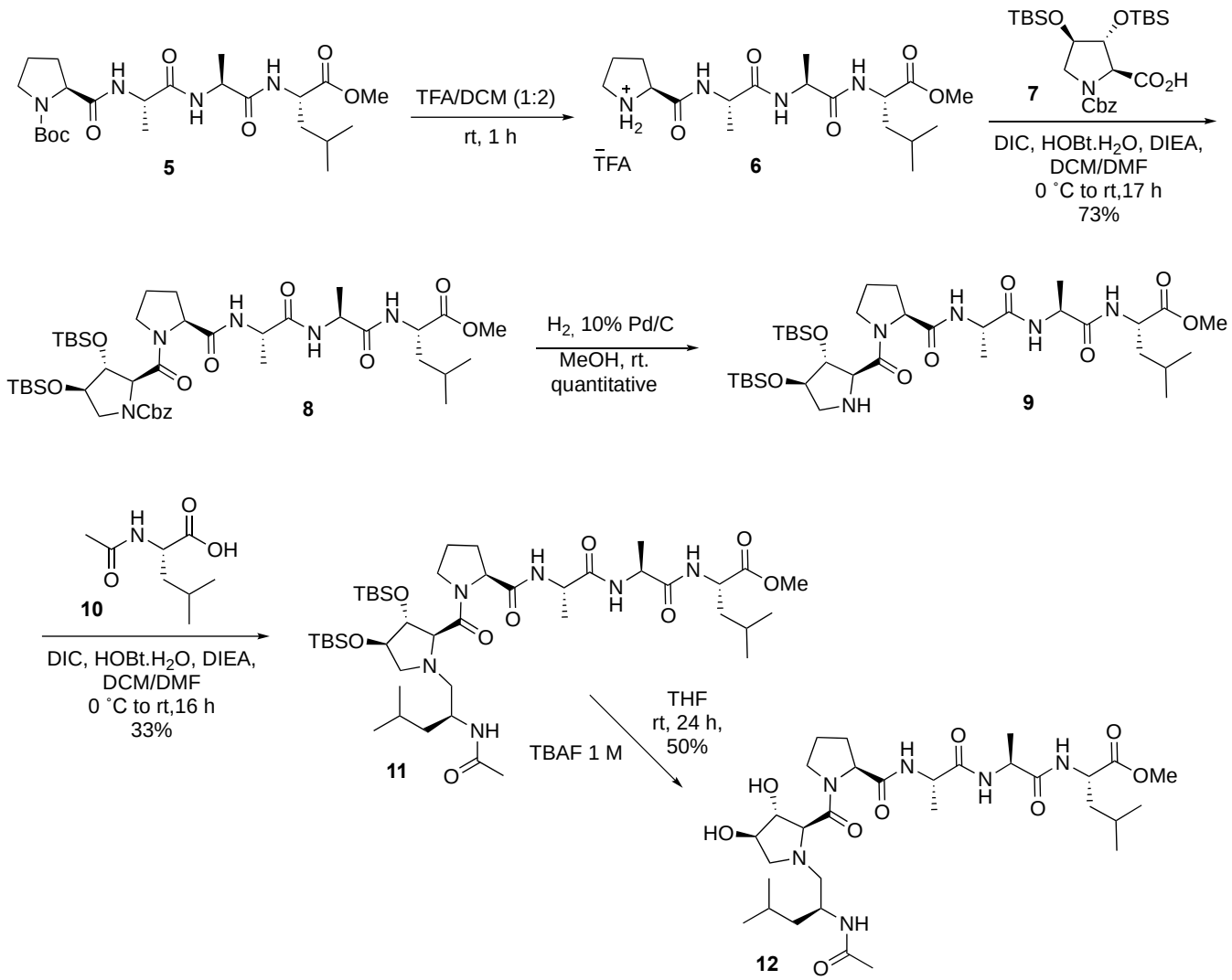
Scheme 1. Results.

In accordance with this synthetic plan, the synthesis of tetrapeptide **5** was first carried out from the corresponding commercial amino acids (Scheme 2). The protection system used for the amino acids was the Boc/OMe system. For the deprotection of the amino groups, 1:2 mixtures of TFA/DCM were used. For peptide coupling, the DIC/HOBt system, DIEA base and DCM/DMF solvents were used as activating agent/coupling agent. Under these conditions, we proceeded as follows:



Scheme 2. The first coupling of leucine with alanine was carried out by activating BocAlaOH with HOBt and DIC in DCM. It was necessary to add a few drops of DMF to achieve complete solubilization of the reaction mixture. Then, this solution was added to a previously prepared solution of leucine and DIEA in DCM, leaving it under stirring overnight at room temperature. The dipeptide **1** obtained was subjected to a hydrolysis reaction with TFA leading to dipeptide **2**, with its free amino group, which was coupled with another BocAlaOH unit under the above coupling conditions. This led to tripeptide **3** and this to the corresponding tripeptide **4**, with its free amino group, to which BocProOH was readily coupled. This gave the tetrapeptide **5** (designated as P1 in the synthetic plan of Scheme 1).

According to our synthetic plan, we then proceeded to attach a unit of dihydroxyproline **7** to the *N*-terminal end of tetramer **5** (Scheme 3).



Scheme 3. After removal of the Boc group from tetramer **5**, the resulting peptide **6** was coupled with dihydroxyproline **7**, under the indicated conditions. This gave the pentapeptide **8** in 73% yield.

The last programmed coupling consisted of the incorporation of a leucine unit **10** to the *N*-terminal end of pentamer **9**. This coupling was carried out under the peptide coupling conditions used in this section, previously deprotecting the terminal amino group of **8** by catalytic hydrogenation. This led to the hexamer **11**, which was obtained with a yield of only 33%. This low yield may be due to the reactivity problems presented by amino acids with terminal acyl groups.

Finally, the two silylated groups of the dihydroxylated proline subunit were deprotected using TBAF as a fluoride source. This allowed us to obtain the desired hexapeptide **12**.

A preliminary structural study based on the ^{1}H NMR spectrum showed that the synthesized peptide **12** shows multiple conformations in solution. In the aromatic region, between 6.0 ppm and 9.0 ppm, the signals corresponding, among others, to the amide protons appear. Figure 3 shows multiple signals for the same proton.

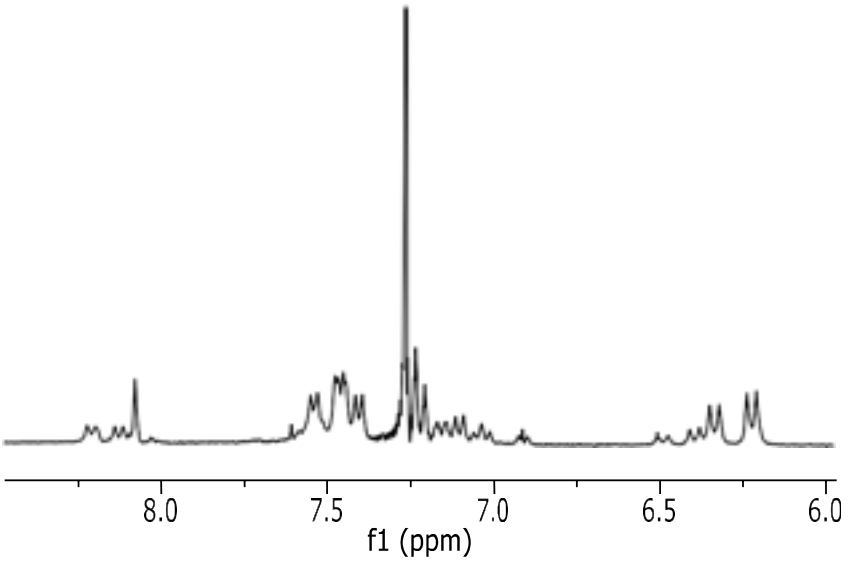


Figure 3. ^{1}H RMN Spectrum of **12** in $CDCl_3$ with the extended aromatic region in which the amide proton signals are present.

A similar situation was observed in DMSO-d6 (see Figure 4), with several signals associated with multiple conformations. In this case, due to the influence of the solvent used, the signals shift considerably, grouping into two regions, one between 5.30 and 5.90 ppm and the other between 7.70 and 8.30 ppm.

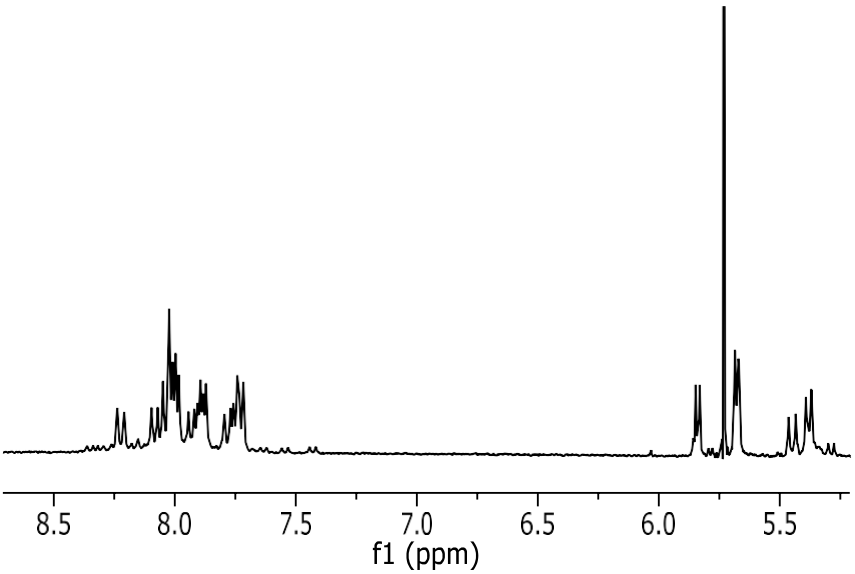


Figure 4. ^{1}H RMN Spectrum of **12** in DMSO-d_6 with the extended aromatic region in which the amide proton signals are present.

Given these results, it can be concluded that the presence of dihydroxylated proline is not capable of stabilizing a predominant structure of the peptide.

References

1. Leszczynski, J.F.; Rose, G.D. *Science* **1986**, *234*, 849–855.
2. Vass, E.; Hollósi, M.; Besson, F.; Buchet, R. *Chem. Rev.* **2003**, *103*, 1917–1954.
3. Villen, J.; Beausoleil, S.A.; Gerber, S.A.; Gygi, S.P. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 1488–1493.
4. Suzuki, M.; Yagi, N. *Proc. Biol. Sci.* **1991**, *246*, 231–235.
5. Bøjstrup, M.; Lundt, I. *Org. Biomol. Chem.* **2005**, *3*, 1738.
6. Song, B.; Bomar, M.G.; Kibler, P.; Kodukula, K.; Galande, A.K. *Org. Lett.* **2012**, *14*, 732–735.