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Inviting C5-Trifluoromethylated Pseudoprolines into Collagen Mimetic Peptides

*Anaïs Terrien,[†] Keyvan Rahgoshay,[‡] Emelyne Renaglia,[‡] Nathalie Lensen,[‡] Yves Jacquot,[#]
Rodrigue Marquant,[†] Thierry Brigaud,[‡] Claire Loison,[§] Grégory Chaume,^{†‡*} Emeric Miclet^{†*}*

[†]Sorbonne Université, Ecole Normale Supérieure, PSL University, CNRS, Laboratoire des Biomolécules, 4 place Jussieu, 75252 Paris Cedex 05, France. [‡]CY Cergy Paris Université, CNRS, BioCIS, 95000, Cergy Pontoise, France. [#]Yves Jacquot – CiTCoM laboratory, Centre National de la Recherche Scientifique (CNRS) Unité Mixte de Recherche (UMR) 8038, Institut National de la Santé et de la Recherche Médicale (INSERM) U1268, Faculty of Pharmacy of Paris, Université Paris Cité, Paris, France. [§]Claire Loison – University of Lyon, Université Claude Bernard Lyon 1, CNRS, Institut Lumière Matière, F-69622, Villeurbanne, France.

ABSTRACT: Numerous Collagen Mimetic Peptides (CMPs) have been engineered using proline derivatives substituted at their C(3) and/or C(4) position in order to stabilize or to functionalize collagen triple helix mimics. However, no example has been reported so far with C(5) substitutions. Here, we introduce a fluorinated CMP incorporating trifluoromethyl groups at the C(5) position of pseudoproline residues. In tripeptide models, our CD, NMR and Molecular Dynamics (MD) studies have shown that, when properly arranged, these residues meet the structural requirements for triple helix assembly. Two host-guest CMPs could be synthesized and were analyzed by CD spectroscopy. The NMR analysis in solution of the most stable confirmed

the presence of structured homotrimers that we interpret as triple helices. MD calculations showed that the triple helix model remained stable throughout the simulation, with all six trifluoromethyl groups pointing outwards from the triple helix. Pseudoproline substituted at the C(5) positions appeared as valuable tools for the design of new fluorinated collagen mimetic peptides.

KEYWORDS: collagen mimetic peptide, fluorine, NMR, CD, Molecular dynamic

INTRODUCTION

Collagen is the most abundant structural protein in mammals. It represents the major component of the extracellular matrix and is widespread all over the body (bones, skin, tendons, cartilages). Purified collagen can be engineered to serve as a valuable material for biomedical applications.¹ Alternatively, Collagen Mimetic Peptides (CMP) are synthesized to mimic the structural properties of natural collagen²⁻⁶ or to give it specific functions.⁷⁻¹¹ At a molecular level, its tertiary structure consists of three individual left-handed polyproline II helices (PPII) folded into a right-handed triple helix which is characterized by a single-residue offset between the three peptide chains and is stabilized by inter-strand hydrogen bonds.¹² Each strand comprises the repeat of the (Xaa-Yaa-Gly) primary sequence where the (2*S*)-proline (Pro) and the (2*S*,4*R*)-4-hydroxyproline (Hyp) are the most prevalent residues at Xaa and Yaa positions, respectively. One of the reasons for this prevalence is that amino acid rings can adopt two conformational states, the C(4)-endo and C(4)-exo ring puckers, which preorganize the suitable ϕ and ψ dihedral angles for the triple helix formation.¹³ Crystal structures of short-length CMPs show a distinct preference for the conformation of the amino acid rings, depending on their position in the collagen chain. C(4)-endo and C(4)-exo puckering are preferred at the Xaa and Yaa positions, respectively.¹⁴⁻¹⁶ This leads to

dissimilar couples of dihedral angles with ($\phi = -73^\circ$, $\psi = 163^\circ$) on average for the Xaa site and ($\phi = -58^\circ$, $\psi = 151^\circ$) for the Yaa. Such a puckering alternation has been proposed as a key feature to stabilize the triple helix and accounts for the high occurrence of the non-proteinogenic Hyp residue at the Yaa position in collagen sequences.^{17,18} While Pro residue is known to slightly favor the C(4)-endo conformation, Hyp residue exhibits a strong preference for the C(4)-exo ring pucker due to a gauche effect of the hydroxyl group. Therefore, replacing the Pro by a Hyp residue at the Yaa position allows a significant improvement of the triple helix thermal stability characterized by an increase of its melting temperature (T_m).²⁻⁵

Following this rationale, numerous CMPs have been engineered using proline derivatives bearing small substituents that stabilize a definite ring pucker through stereo-electronic and/or steric effects (Table 1). For electron-withdrawing substituents, the pseudoaxial orientation is preferred due to the gauche effect. Consequently, (4*S*) or (4*R*) configurations were respectively selected to stabilize the C(4)-endo at the Xaa position or the C(4)-exo at the Yaa position.¹⁹⁻²³ For the non-electron-withdrawing groups, the pseudoequatorial orientation is preferred due to steric repulsions.²⁴ Accordingly, a CMP series has been designed with (4*R*)- or (4*S*)-methylproline at the Xaa and Yaa positions, respectively.

Marked stabilizations of the triple helix were observed for CMP sequences incorporating exo inducer at the Yaa position. In contrast, stabilizing the endo pucker at the Xaa position only slightly improved the CMP thermal stability because this pucker also destabilized the *trans* conformation of the peptide bond which is mandatory in the triple helix assembly.²⁵ Some works of Wennemers *et al.* demonstrated an additional requirement for the stability of the triple helix when bulky C4-amidated prolines were engaged.²⁶⁻²⁹ They showed that these large electron-withdrawing C4-

substituents strongly destabilized the triple helix when grafted on a C4-endo puckered ring at the Xaa position or on a C4-exo puckered ring at the Yaa position. In these examples, steric repulsions were thus able to counteract the benefit of the dihedral angle preorganization achieved by the pyrrolidine puckerings.

C(4)-pucker	C(3)-substitutions ref: 2, 8, 11, 13	C(4)-substitutions ref: 2, 7, 8, 10, 12		C(5)-substitutions ref: 35, 36, 37	
C(4)-endo					
C(4)-exo					

Table 1. Puckering preferences of C(3)-, C(4)- and C(5)-substituted proline derivatives. X accounts for electron-withdrawing substituents as found in hydroxyproline, fluoroproline, chloroproline, methoxyproline, or azidoproline. R accounts for non-electron-withdrawing groups: alkyl, vinyl, or phenyl.

Finally, the simultaneous incorporation of C(4)-substituted prolines at the Xaa and at the Yaa positions was almost always detrimental for the triple-helix formation. Steric clashes between the Xaa and Yaa substituents, or adverse dipolar interactions of small electron-withdrawing groups were responsible for the triple helix disruption.³⁰ The methylproline is one of the rare examples in which a double incorporation ((4*R*) and (4*S*) configurations at Xaa and Yaa positions, respectively) led to an increased T_m .³¹

It is therefore a real challenge to engineer collagen triple helix models in which sequential substituted prolines are present. Raines and coworkers have introduced 3*S*-fluoroproline (3*S*-flp) at the Xaa position in CMP to allow a tight control of the ring pucker without creating repulsive contacts between neighboring fluoro groups.³⁰ The (3*S*-flp-4*R*-Flp-Gly)₇ CMP was then able to

undergo triple helix formation whereas (4*S*-Flp-4*R*-Flp-Gly)₇ failed because of F-F interactions. However, the T_m value was deceptively low because 3*S*-flp weakens the hydrogen bond network within the triple helix, the electron-withdrawing fluoro group being close to the H-bond acceptor C=O. Thus, electronegative substituents at the 3-position are not good candidates to stabilize the triple helix.^{30,32}

So far, no C5-substituted proline analogues have been incorporated in CMP sequences. We envisioned that they represent valuable tools for the design of triple helix models and could be placed sequentially in the CMP sequence. Our group has developed convenient methodologies for the synthesis of various trifluoromethylated pseudoproline bearing a CF₃ group at the C(5) position and their incorporation into peptides.^{33,34} Their marked stereoelectronic properties have been demonstrated and are very relevant for the design of CMP.³⁵⁻³⁸ Nuclear Magnetic Resonance (NMR) studies as well as theoretical calculations showed that both (5*S*)-CF₃-ΨPro (tfmΨP) and (5*R*)-CF₃-ΨPro (TfmΨP) stabilize the PPII backbone conformation in water and efficiently constrain the O(4)-endo and O(4)-exo ring puckers, respectively (Table 1). Moreover, TfmΨP substantially decreases the *cis-trans* rotational barrier which appears as a molecular **switch** for the CMP assembly. Therefore, we anticipated that tfmΨP and TfmΨP pseudoproline residues could be excellent mimics of proline and hydroxyproline, respectively. In this work, our Molecular Dynamics simulations (MD), **Circular Dichroism (CD)** and NMR studies showed that the trifluoromethylated pseudoproline residues meet the structural requirements for the triple helix assembly, even in peptide sequences incorporating two adjacent proline derivatives.

MATERIAL AND METHODS

Synthetic procedures and characterizations. Peptides **P1**, **P2** and **P4** were obtained by solid phase peptide synthesis (SPPS) whereas the synthesis of peptides **P3** and **P5** was performed in solution since coupling reactions at the *N*-terminus of the trifluoromethylated pseudoproline TfmΨP requires the activation of the Fmoc-amino acids partners as acyl chlorides (see the Supporting Information for details).^{33,34} The 21-mer peptide [(Pro-Hyp-Gly)₃-Pro-TfmΨP-Gly-(Pro-Hyp-Gly)₃] **CMP(P3)** was synthesized on a Biotage SyroWave automatic synthesizer from Fmoc-Gly Wang resin and using the Fmoc-Gly-Pro-TfmΨP-OH building block. The 21-mer peptide [(Pro-Hyp-Gly)₃-tfmΨP-TfmΨP-Gly-(Pro-Hyp-Gly)₃] **CMP(P5)** was synthesized on a LibertyBlue CEM automated peptide synthesizer starting from Fmoc-Gly Wang resin and using the Fmoc-Gly-tfmΨP-TfmΨP-OH building block. Detailed synthetic procedures are given in the Supporting Information. For characterization purposes, ¹H NMR (400.00 MHz), ¹³C NMR (100.50 MHz) and ¹⁹F NMR (376.20 MHz) spectra were measured on a BRUKER AVANCE NEO 400 MHz spectrometer in organic solvents. NMR experiments were processed and analyzed with TOPSPIN 4.0.7 program or MestReNova 6.0.2 program. ¹H and ¹³C resonances were readily assigned from the analysis of 2D homonuclear and heteronuclear experiments. NMR spectra and complete characterizations are provided in the Supporting Information. ~~For structural analysis (quantification of the *cis-trans* populations, H-bond studies and analysis of the five membered ring puckerings), NMR experiments were recorded in water (100% D₂O or H₂O:D₂O 9:1) at different temperatures on a spectrometer operating at a ¹H frequency of 500 MHz and equipped with a triple resonance, z-axis pulsed field gradient cryogenic probehead, optimized for ¹H detection.~~

Structural analyses in solution. For NMR structural analysis on **P1-P5** (quantification of the *cis-trans* populations, H-bond studies and analysis of the five membered ring puckerings), NMR

experiments were recorded in water (100% D₂O or H₂O:D₂O 9:1) at different temperatures on a spectrometer operating at a ¹H frequency of 500 MHz and equipped with a TCI triple resonance, z-axis pulsed-field-gradient cryogenic probehead, optimized for ¹H detection. CD and NMR experiments were carried out on **CMP_(P3)** and **CMP_(P5)** solubilized at 10 mM in 50 mM phosphate buffer at pH 7.0. CD spectra were acquired using a Jasco J-815 CD spectropolarimeter equipped with a Peltier temperature-controlled cell holder. Data fitting was performed using the kaleidagraph software. 2D ¹⁹F-¹³C HSQC (heteronuclear single quantum experiment) and ¹⁹F DOSY (diffusion ordered spectroscopy) were recorded at 40°C on a spectrometer operating at a ¹H frequency of 700 MHz and equipped with a TCI-F cryogenic probehead, optimized for ¹H and ¹⁹F detection.

MD procedures. The MD simulations were performed using the GROMACS 2016.4 simulation package³⁹ and the amberff99-idnl all-atom force field, taking into account the correction introduced by A. Aliev *et al.* to describe the proline conformational dynamics.^{40,41} The hydroxyproline (Hyp) and fluorinated pseudoproline (tfmΨP and TfmΨP) topologies and parameters were generated using the ambertool package and the RED server.^{42,43} When unavailable in the initial protein force field, GAFF non-bonded parameters have been used.⁴⁴ The charges were fitted by the standard RESP procedure of the amber force field, fitting the electrostatic potential of the capped amino-acid with constraints on the charges of capping atoms. As in the original force field procedure, the charges of the backbone atoms of the fluorinated pseudoprolines were not constrained. To take into account the specificity of the prolines and the collagen conformation, the charges were fitted on an ensemble of conformations of the capped amino-acids, which was generated by B3LYP/6-31G geometry optimization with Ramachandran

angles fixed, to mimic successively alpha helices ($\Phi \approx -60^\circ$, $\psi \approx -35^\circ$), the Pseudo-Proline-II helices ($\Phi \approx -75^\circ$, $\psi \approx 145^\circ$) and collagen helices ($\Phi \approx -65^\circ$, $\psi \approx 170^\circ$) conformations. For each of these Ramachandran settings, *trans* peptide bonds (dihedral angle $\omega=180^\circ$) were imposed, and various puckering conformations were included. The specific procedures for the simulations performed on peptides **P1** to **P5** and on the triple helices are detailed in the Supporting Information.

RESULTS AND DISCUSSION

Structural studies of tripeptide models. Preliminary structural studies were performed to evaluate the capabilities of trifluoromethylated pseudoprolines to fulfill the conformational features for CMP assemblies: *trans* amide bond, Xaa-endo/Yaa-exo puckering alternation, suitable ϕ and ψ dihedral angles (Figure 1). We started our investigation with three tripeptides incorporating one or two trifluoromethylated pseudoprolines. This series was based on the Xaa-Yaa-Gly triads with acetyl and carboxamide end groups, where Xaa = Pro or tfm Ψ P and Yaa = Hyp or Tfm Ψ P. In addition, Ac-Pro-Pro-Gly-NH₂ (**P1**) and Ac-Pro-Hyp-Gly-NH₂ (**P2**) have been studied as references. To determine the structural propensity of peptides **P1-P5**, we undertook a detailed study combining solution NMR and MD simulations.

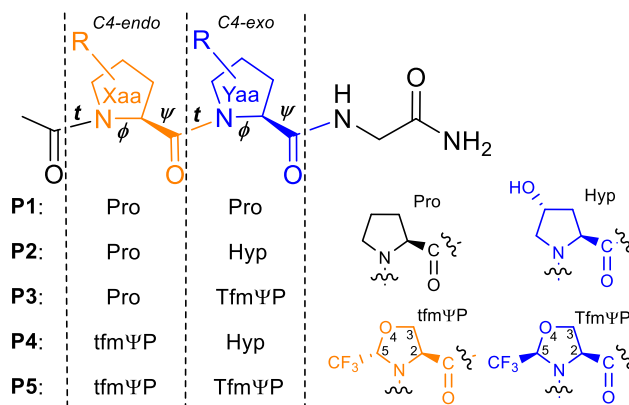


Figure 1. Chemical structures of peptide triads corresponding to established (**P1-P2**) or prospective (**P3-P5**) repeated units of CMPs. Conformational features found in triple helix structures are specified: *trans* peptide bond, C4-endo, $\phi = -73^\circ$ and $\psi = 163^\circ$ for Xaa residue and *trans* peptide bond (*t*), C4-exo, $\phi = -58^\circ$ and $\psi = 151^\circ$ for Yaa residue. Numbering of the atoms is indicated for the trifluoromethylated pseudoproline residues.

Peptide bond geometry. The *cis* or *trans* propensity of the two amide bonds of peptides **P1-P5** can be readily deduced from NMR data, providing the populations of the four possible conformers (noted *cc*, *tc*, *cc*, *tt*, see Table 2).³⁵⁻³⁸ For Yaa = Pro or Hyp (**P1**, **P2** and **P4**), the Xaa-Yaa amide bond was almost entirely in the *trans* conformation whereas for Yaa = TfmΨP, the *cis* amide bond, unsuitable for triple helix assembly, represented as much as 45% for **P3** or 25% for **P5** (*cc* plus *tc* populations). These *cis* amide bond populations contrast with previous observations on small homochiral Fmoc-Pro-TfmΨP-OMe dipeptides which showed no *cis* conformation.³⁶ However, similar increased *cis* populations were found for longer peptides incorporating Xaa-TfmΨP moieties.³⁵ This was correlated with the presence of a $\text{CO}_i \dots \text{HN}_{i+3}$ intramolecular H-bond, as observed in type VI β -turns. Such secondary structure elements were also found in this work for the *tc* conformers in **P3** and **P5**: the chemical shift dependence of their Gly amide proton towards the temperature confirmed the presence of hydrogen bonds (see the Supporting Information, Table S1). However, in CMP triple helix structures, these glycine amide protons act as H-donors for the formation of inter-strand H-bonds. Intramolecular type VI β -turns and triple helix formation are then competing and are directly influenced by the relative stability of these two types of H-bonds. Finally, we observed that the *ct* population was significant in **P4**. This is not surprising since the presence of the CF_3 group at the C5 position in tfmΨP was shown to slightly destabilize the *trans*

geometry of Ac-tfmΨP peptide bond³⁷ but the *cis* population was shown to progressively decrease with increasing size of the preceding amino acid.³⁶ Therefore, a higher *trans* population content is expected for the tfmΨP residue when it is centrally located in a CMP sequence.

Peptide Ac-Xaa-Yaa-Gly	<i>cis</i> Xaa-Yaa		<i>trans</i> Xaa-Yaa	
	<i>cis</i> Ac-Xaa	<i>trans</i> Ac-Xaa	<i>cis</i> Ac-Xaa	<i>Trans</i> Ac-Xaa
<i>conformers</i>	<i>cc</i>	<i>tc</i>	<i>ct</i>	<i>tt</i>
P1 Ac-Pro-Pro-Gly-NH ₂	0	0	20	80
P2 Ac-Pro-Hyp-Gly-NH ₂	0	0	20	80
P3 Ac-Pro-TfmΨP-Gly-NH ₂	5	40	5	50
P4 Ac-tfmΨP-Hyp-Gly-NH ₂	2	3	30	65
P5 Ac-tfmΨP-TfmΨP-Gly-NH ₂	5	20	10	65

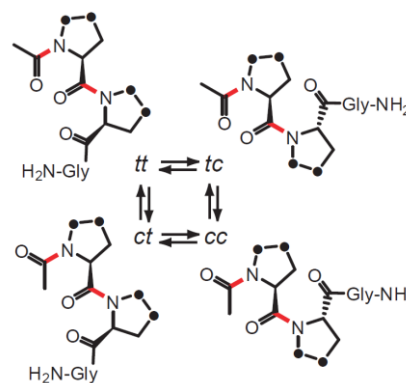


Table 2. *Cis-trans* populations (%) for each peptide **P1-P5**. Measurements have been performed by integration of ¹H signals (and ¹⁹F when available) in water at 25°C. Four conformers (*tt*, *tc*, *ct*, *cc*) are considered for each Ac-Xaa-Yaa-Gly-NH₂ peptide since two amide bonds are subject to *cis-trans* isomerism, as depicted on the right panel.

Ring puckering preferences. Measurements of the ³J_{HαHβ2} and ³J_{HαHβ3} scalar couplings have allowed the calculation of puckering preferences for the main *trans-trans* conformations (Tables 3 and S2-3). Based on a two-conformations X(4)-endo/X(4)-exo jump model (X = C or O), our NMR measurements were in good agreement with the MD simulations and confirmed that the Pro residue is highly flexible within the tripeptide models both at the Xaa and the Yaa positions. The stabilization of the X(4)-exo conformation was also demonstrated for the Hyp and TfmΨP residues at the Yaa positions, whereas the tfmΨP residue considerably enhanced the endo conformation at the Xaa position over the unsubstituted proline residue. Thus, based on the assumption that the

triple helix is stabilized by the amino acids puckering alternation,^{17,18} **P4** and **P5** should be good candidates for the design of new CMP sequences.

Peptide Ac-Xaa-Yaa-Gly	% endo ^a of Xaa		% exo ^b of Yaa	
	NMR ^c	MD ^d	NMR ^c	MD ^d
P1 Ac-Pro-Pro-Gly	52	64	59	29
P2 Ac-Pro-Hyp-Gly	55	63	100	86
P3 Ac-Pro-TfmΨP-Gly	30	67	100	98
P4 Ac-tfmΨP-Hyp-Gly	97	99	100	85
P5 Ac-tfmΨP-TfmΨP-Gly	95	99	100	98

Table 3. Analysis of ring puckers by NMR and MD simulations for the all *trans* conformation (*tt*) of peptides **P1-P5**. [a] % of C(4)-endo ring pucker for **P1-P3** or O(4)-endo ring pucker for **P4-P5**. [b] % of C(4)-exo ring pucker for **P1, P2, P4** or O(4)-endo ring pucker for **P3** and **P5**. [c] For each amino acid ring, populations (%) have been calculated using a two-sites equilibrium model (C4)-endo/(C4)-exo and the corresponding optimized geometries (see also the Supporting Information, Tables S2 and S3).^{37,38} [d] Populations have been determined from 1 μs simulations in explicit water based on the amber99sb-inlp force field, completed by Generalized Amber Force Field (GAFF) for a few of missing parameters. The puckering propensities were calculated through the distributions of the dihedral angle χ_1 N-C(2)-C(3)-X(4) (X = C or O) using a sum of two gaussians, corresponding to the exo ($\chi_1 < -15^\circ$) or the endo ($\chi_1 > 15^\circ$) puckerings.

Backbone conformation. Next, we were interested in the distribution of the ϕ and ψ dihedral angles for these five tripeptides in the time course of 1 μs-long MD simulations. Ramachandran plots of Xaa and Yaa are presented in Figure 2 for **P1-P5** and compared with the triple helix canonical geometry. For **P2**, the ϕ and ψ dihedral angles were very close to those measured in the triple helix. This is not surprising since it is composed of the same amino acids as in the triple helix model. As shown on Figure 2a, the replacement of the Pro residue at the Xaa position by the tfmΨP

residue (**P2**→**P4** and **P3**→**P5**) induced an increase of the ϕ -value by ca. 10° without affecting the ψ -value. On the contrary, the replacement of the Hyp by the Tfm Ψ P residue at the Yaa position (**P2**→**P3** and **P4**→**P5**) decreased the Xaa ψ -value by ca. 20° , Xaa ϕ -value being unchanged. Concerning the Yaa residue, small angle shifts were observed for these Hyp→Tfm Ψ P substitutions (-10° for ϕ and $+10^\circ$ for ψ , see Figure 2b). Overall, these small structural deviations compensate within each tripeptide units and could accommodate the triple helix structure.

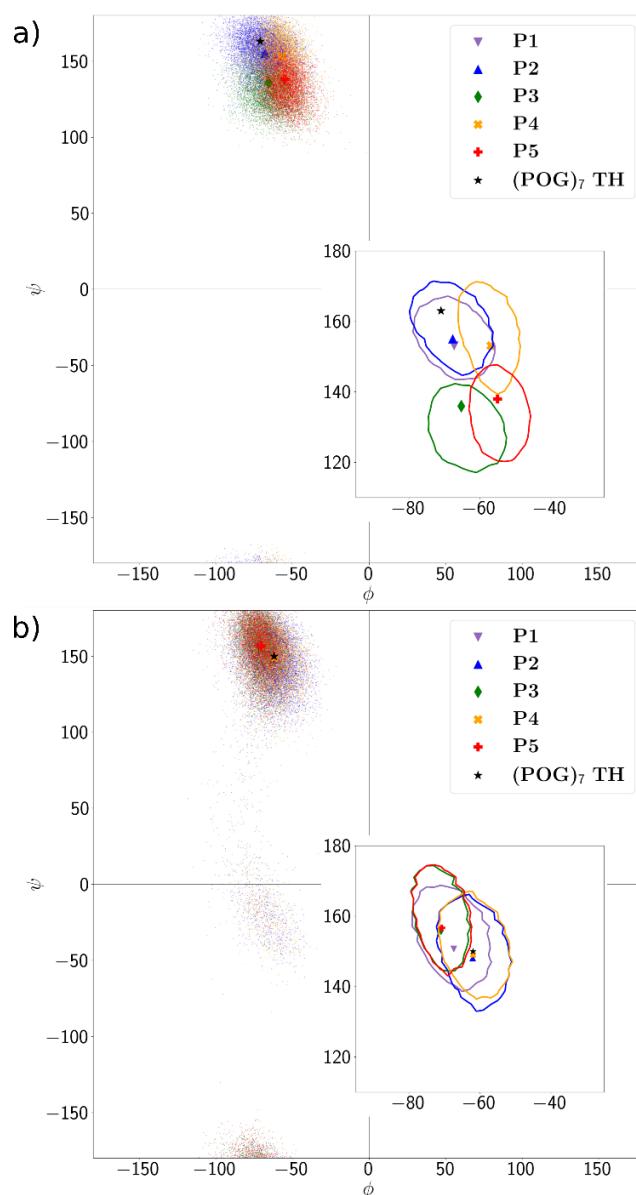


Figure 2. Distribution of the ϕ and ψ dihedral angles obtained by MD simulations for Xaa (a) and Yaa (b) amino acids in peptides **P1-P5** with a carboxamide C-terminal group (see Table S4 for detailed values). For each peptide, 500 000 structures have been extracted from a 1 μ s MD trajectory. The corresponding ϕ and ψ dihedral angles have been plotted on Ramachandran diagrams. The large colored symbols correspond to the average values. On the inserts, the contour lines delineate the 1 σ variation. The star (★) corresponds to the average dihedral angles obtained on a 500 ns-long MD simulation for the central triplet within the (Pro-Hyp-Gly)₇ triple helix (POG₇ TH in the legend).

Structural Studies of fluorinated CMP. To further investigate the behavior of the C(5)-substituted pseudoproline within collagen-mimicking peptides, we designed two host-guest peptides containing the triad found in **P3** or **P5**. **CMP_(P3)** and **CMP_(P5)** were obtained, corresponding to [(Pro-Hyp-Gly)₃-**Pro-Tfm Ψ P-Gly**-(Pro-Hyp-Gly)₃] and [(Pro-Hyp-Gly)₃-**tfm Ψ P-Tfm Ψ P-Gly**-(Pro-Hyp-Gly)₃], respectively. Fmoc-Pro-Tfm Ψ P-Gly and Fmoc-Gly-tfm Ψ P-Tfm Ψ P building blocks were used for the synthesis of **CMP_(P3)** and **CMP_(P5)** by SPPS, with overall yields of 18% and 20%, respectively (see the Supporting Information). To our knowledge, these are the first collagen related peptides incorporating C(5)-substituted proline analogues. CMP folding is usually characterized by circular dichroism (CD) spectroscopy. As shown in Figure 3a, both **CMP_(P3)** and **CMP_(P5)** displayed maxima at 225 nm and minima near 200 nm which correspond to the signature spectra of triple helical assemblies.¹⁷ To investigate their relative stabilities, thermal denaturation studies were performed by monitoring the molar ellipticity at 225 nm as a function of temperature. T_m values of 13°C and 22°C were obtained which are the temperature of the thermal transition curves for which the populations of triple helices and monomers are equal. The lower stability of **CMP_(P3)** could be related to the lower

propensity of **P3** to adopt the all *trans* conformation (Table 2). In addition, it was found that the replacement of the Pro by the tfmΨP residue significantly increased the X(4)-endo ring pucker at the Xaa position (Table 3). Thus, CMPs better accommodate the insertion of the two successive trifluoromethylated pseudoprolines tfmP-TfmP rather than the single TfmP insertion at the Yaa position as found in **CMP_(P3)**. However, other studies have reported T_m values of 36°C for (Pro-Hyp-Gly)₇,² indicating that the local distortions observed in **P3** and **P5** for the ϕ and ψ dihedral angles may singularize the cores of the trifluorinated triple helices.

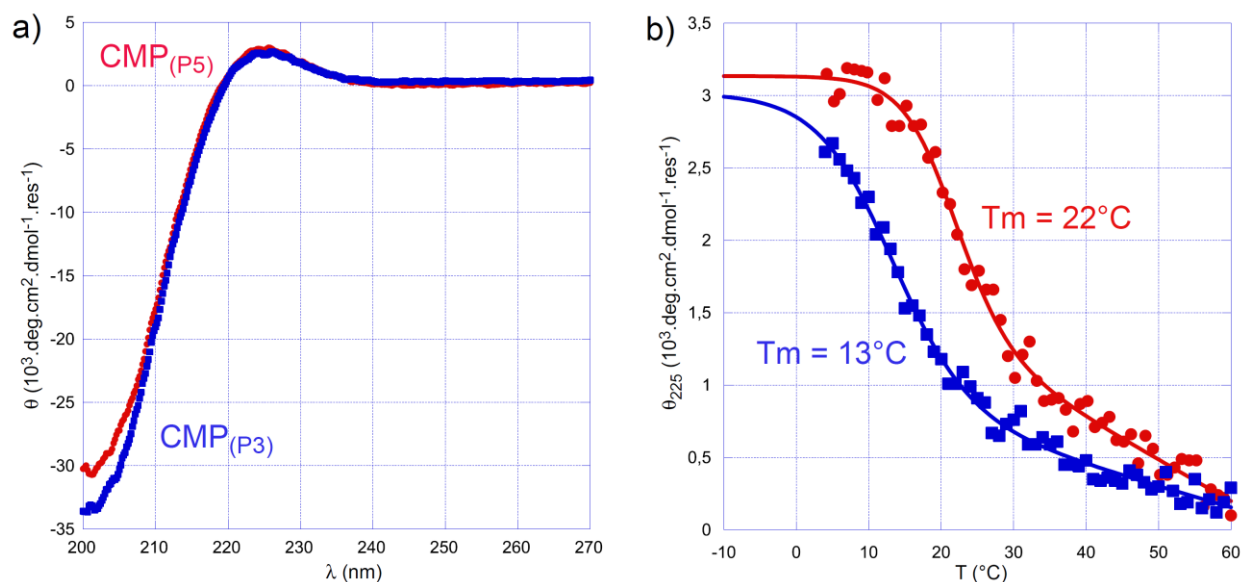


Figure 3. Circular Dichroism spectroscopy of **CMP_(P3)** (blue squares) and **CMP_(P5)** (red circles). (a) CD spectra recorded at 4 °C. (b) Thermal denaturation of CMPs followed by the molar ellipticity at 225 nm. Experimental data were fitted (bold lines) to obtain T_m of 13°C and 22°C for **CMP_(P3)** and **CMP_(P5)**, respectively (see the Supporting Information for the detailed procedure).

In order to further characterize the presence of the **CMP_(P5)** triple helix in solution, we undertook a liquid state NMR study. Since the peptide chain contains two adjacent trifluoromethylated pseudoprolines, ¹⁹F NMR spectra were recorded to get a clear picture of its structural features.

Interestingly, a first set of six resonances was observed between -76.0 and -76.6 ppm, away from a large signal centered at -77.9 ppm. On the ^{19}F - ^{13}C HSQC spectrum, the latter split into two ^{13}C signals at 117.30 and 117.65 ppm (Figure 4b). To better understand these observations, a ^{19}F DOSY spectrum was recorded which indicated that the six downfield resonances shared a same diffusion coefficient ($7.53 \times 10^{-11} \text{ m}^2.\text{s}^{-1}$), whereas the signal at -77.9 ppm corresponded to a significantly larger diffusion coefficient ($1.14 \times 10^{-10} \text{ m}^2.\text{s}^{-1}$). Thus, the DOSY measurements allowed the calculation of a hydrodynamic radius ratio of 1.51 between these two species which was associated to a volume ratio of 3.4 . These data strongly suggested that the six upfield resonances correspond to the triple helix whereas the two signals at -77.9 ppm accounts for the two CF_3 groups of the $\text{tfm}\Psi\text{P}$ - $\text{Tfm}\Psi\text{P}$ motif in the isolated $\text{CMP}_{(\text{P5})}$ peptide strand. Within the triple helix, the one-residue offset between the three chains leads to different environments for each of the six trifluoromethyl groups and lifts the chemical shift degeneracies (Figure 4).

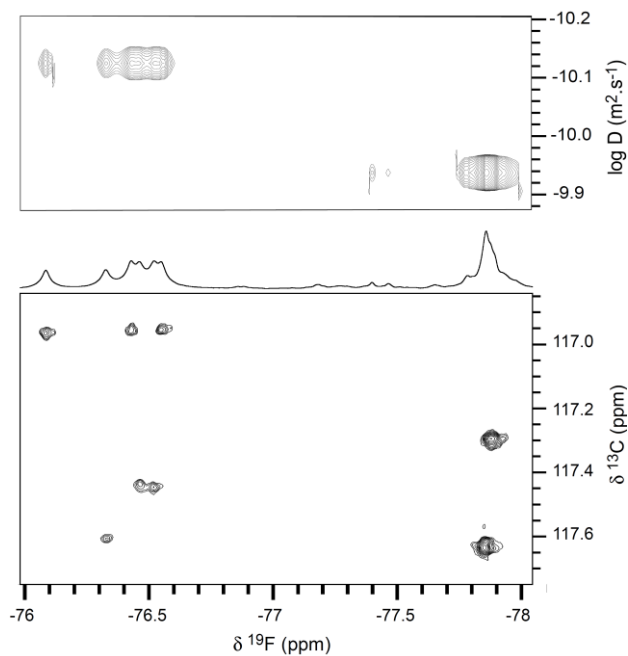


Figure 4. a) ^{19}F DOSY spectrum of the $\text{CMP}_{(\text{P5})}$ peptide. b) 2D ^{19}F - ^{13}C HSQC spectrum together with a ^{19}F 1D projection. Peptide concentration 10mM, pH = 6.8, 40°C, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 90:10.

Therefore, our NMR experiments demonstrated that the $\text{CMP}_{(\text{P5})}$ peptide was in a slow exchange regime between a triple helix and a monomeric state. At 40°C, the integration of the ^{19}F NMR signals indicated a population of 25% for the triple helix composed of $\text{CMP}_{(\text{P5})}$ peptide incorporating two adjacent trifluoromethylated proline analogues with unusual C5-substitutions.

To gain insights into the structural features of the host-guest peptide $\text{CMP}_{(\text{P5})}$ at the atomic level, molecular dynamics simulations were performed. The stable non-fluorinated triple helix of (Pro-Hyp-Gly) $_7$ was chosen as a reference structure. The simulated trimeric structure [$\text{CMP}_{(\text{P5})}$] $_3$ is displayed in Figure 5. The triple-helical structure was well conserved in the time course of the simulation as attested by the low root mean square displacements (RMSD). Relative to the reference crystalline structure (PDB 1K6F),¹⁵ the RMSD along the 500 ns-long trajectories was about 1.2 Å for the backbone atoms of the triple helix both in the absence and in the presence of trifluoromethyl pseudoproline residues (see the Supporting Information Figure S11).

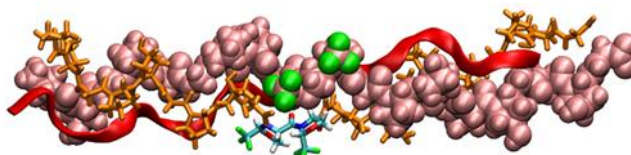


Figure 5. Conformation of the homo-trimer helix of $\text{CMP}_{(\text{P5})}$ after a 500 ns simulation in explicit solvent. Initial structure was derived from the 1K6F PDB structure.¹⁵ The three backbone chains are represented in three different modes: CPK (pink), lines (orange) and ribbons (red). The fluorine atoms are depicted in green. Usual atomic color code is used for the central host peptide in the line representation. Picture was generated using VMD and povray rendering.⁴⁵

In the simulation, the six trifluoromethyl groups rotated around the C(5)-CF₃ bond, indicating that they were not entangled in a bulky environment, and that the structure around them was relaxed. The analysis of the ring puckering confirmed that the tfmΨP residue at the Xaa position greatly stabilized the O(4)-endo conformation within the triple helix ($\chi_1 = 18 \pm 6^\circ$), whereas the Pro residue displayed a high flexibility in the reference triple helix, the endo and exo pucker being almost equally populated (Figure 6a). At the Yaa position, the stabilization of the C(4)-exo by the Hyp residue was clearly demonstrated ($\chi_1 = -30 \pm 8^\circ$) but we observed that the TfmΨP residue was even able to restrict further the dynamic of the 5-membered ring ($\chi_1 = -24 \pm 4^\circ$, Figure 6b).

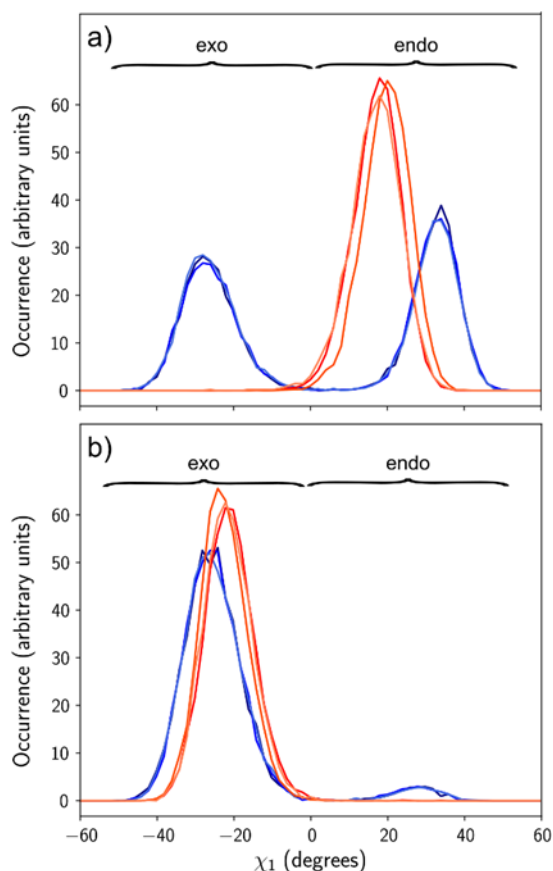


Figure 6. Probability distribution of the dihedral angle χ_1 (N-C(2)-C(3)-C(4)) for the (a) Xaa and (b) Yaa amino acids of the central triplets in the reference triple helix (Pro-Hyp-Gly)₇ (blue curves)

and in the modeled homo-trimer helix of **CMP(P5)** (orange curves). For both triple helices, distributions are given for the three chains along 500-ns long trajectories.

Under close scrutiny, some other differences could be detected between the reference and the fluorinated triple-helix. The pseudoprolines presence shifted the average backbone dihedral angles relative to those of (Pro-Hyp-Gly)₇ (see the Supporting Information, Table S5), as already noticed in model tripeptides (Figure 2). These modifications of the triple helix structure locally distorted the Gly-NH→Pro-CO hydrogen bond network between the three peptide chains. Indeed, the distance between the hydrogen-bond donating N of Gly and the accepting O of Pro or tfmΨP was maintained in the 3.0 ± 0.2 Å range, but the hydrogen bond orientations were less favorable in the central region of the fluorinated triple helix (see the Supporting Information, Figure S12). **Whereas such a local distortion may account for the decreased stability of **CMP(P5)** compared to the reference (Pro-Hyp-Gly)₇ triple helix, the tfmΨP-TfmΨP motifs did not destabilize the overall PPII structure of the three peptide chains during the time course of the MD simulation (see the Supporting Information, Figure S13). In agreement with CD and NMR experimental data, our MD simulation established that a triple helix model could be obtained bearing six trifluoromethyl groups in its central part.**

Conclusion

In this work, we have demonstrated that trifluoromethylated pseudoprolines, bearing a CF₃ group at the C(5) position, represent valuable tools for the design of triple helix models. Our NMR and MD studies showed that peptides **P3-P5**, incorporating tfmΨP and/or TfmΨP residues, meet the structural requirements for the triple helix assembly. In particular, the incorporation of two adjacent tfmΨP and TfmΨP residues preserved a high population of the all-*trans* conformation

(*tt*) which is required for triple helix assembly. Analysis of ring puckers for the *tt* conformers showed that tfmΨP and TfmΨP residues displayed the strongest propensities to stabilize the endo and exo conformation, respectively. This feature provides a strong rationale for selecting trifluoromethylated pseudoproline analogues as proline analogues in CMP sequences.

MD simulations showed that, **in a host guest CMP sequence**, tfmΨP and TfmΨP imposed small deviations relative to the canonical ϕ and ψ angles found in collagen triple helices, which slightly affects the hydrogen bond network in the close neighborhood of the substitutions. Overall, these deviations compensate along the peptide chain and the triple helix remains stable in the MD simulations. **Both CD and ^{19}F NMR spectroscopies were used** to demonstrate the existence of the fluorinated triple helix in solution, **which, according to the literature, was however less stable than its parent CMP**. The analysis of diffusion coefficients was in good agreement with the formation of a trimer. In addition, NMR spectra showed distinct signals for the six trifluoromethyl groups embedded in the triple helix.

Our results pave the way for the development of new collagen mimetic peptides. First it is shown that C(5) substituted proline analogues can be incorporated in CMP sequence without breaking the triple helix structure, even for relatively large groups as trifluoromethyl groups. Using C5-trifluoromethylated pseudoproline analogues, it was possible to cluster six trifluoromethyl groups, representing no less than 18 fluorine atoms, on a small, exposed and well-defined region of the host guest CMP. This opens new possibilities to control the interactions of the collagen triple helix model. A highly fluorinated surface may favor supramolecular assembly between triple helices or could promote biological interactions with collagen partners.

SUPPORTING INFORMATION.

Detailed synthetic procedures for **P1** to **P5**, building block Fmoc-Gly-tfmΨP-TfmΨP-OH and fluorinated host-guest **CMP(p3)** and **CMP(p5)**. NMR spectra and extracted NMR data in water. Detailed procedures for **CD**, NMR and MD simulations.

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REFERENCES

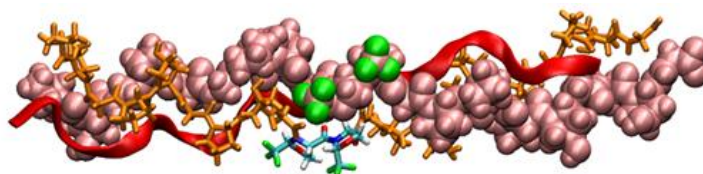
- (1) Gu, L.; Shan, T.; Ma, Y.; Tay, F. R.; Niu, L. Novel Biomedical Applications of Crosslinked Collagen. *Trends Biotechnol.* **2019**, *37* (5), 464–491. <https://doi.org/10.1016/j.tibtech.2018.10.007>.
- (2) Shoulders, M. D.; Raines, R. T. Collagen Structure and Stability. *Annu. Rev. Biochem.* **2009**, *78* (1), 929–958. <https://doi.org/10.1146/annurev.biochem.77.032207.120833>.
- (3) Bella, J. Collagen Structure: New Tricks from a Very Old Dog. *Biochem. J.* **2016**, *473* (8), 1001–1025. <https://doi.org/10.1042/BJ20151169>.
- (4) Kubyshkin, V. Stabilization of the Triple Helix in Collagen Mimicking Peptides. *Org. Biomol. Chem.* **2019**, *17* (35), 8031–8047. <https://doi.org/10.1039/C9OB01646E>.
- (5) Tanrikulu, I. C.; Westler, W. M.; Ellison, A. J.; Markley, J. L.; Raines, R. T. Templated Collagen “Double Helices” Maintain Their Structure. *J. Am. Chem. Soc.* **2020**, *142* (3), 1137–1141. <https://doi.org/10.1021/jacs.9b07583>.

- (6) Hulgán, S. A. H.; Hartgerink, J. D. Recent Advances in Collagen Mimetic Peptide Structure and Design. *Biomacromolecules* **2022**, *23* (4), 1475–1489. <https://doi.org/10.1021/acs.biomac.2c00028>.
- (7) Aronoff, M. R.; Egli, J.; Menichelli, M.; Wennemers, H. γ -Azaproline Confers PH Responsiveness and Functionalizability on Collagen Triple Helices. *Angew. Chem. Int. Ed Engl.* **2019**, *58* (10), 3143–3146. <https://doi.org/10.1002/anie.201813048>.
- (8) Peterson, C. M.; Helterbrand, M. R.; Hartgerink, J. D. Covalent Capture of a Collagen Mimetic Peptide with an Integrin-Binding Motif. *Biomacromolecules* **2022**, *23* (6), 2396–2403. <https://doi.org/10.1021/acs.biomac.2c00155>.
- (9) Ren, Y.; Zhang, H.; Qin, W.; Du, B.; Liu, L.; Yang, J. A Collagen Mimetic Peptide-Modified Hyaluronic Acid Hydrogel System with Enzymatically Mediated Degradation for Mesenchymal Stem Cell Differentiation. *Mater. Sci. Eng. C* **2020**, *108*, 110276. <https://doi.org/10.1016/j.msec.2019.110276>.
- (10) Chattopadhyay, S.; Teixeira, L. B. C.; Kiessling, L. L.; McAnulty, J. F.; Raines, R. T. Bifunctional Peptide That Anneals to Damaged Collagen and Clusters TGF- β Receptors Enhances Wound Healing. *ACS Chem. Biol.* **2022**, *17* (2), 314–321. <https://doi.org/10.1021/acscchembio.1c00745>.
- (11) Aronoff, M. R.; Hiebert, P.; Hentzen, N. B.; Werner, S.; Wennemers, H. Imaging and Targeting LOX-Mediated Tissue Remodeling with a Reactive Collagen Peptide. *Nat. Chem. Biol.* **2021**, *17* (8), 865–871. <https://doi.org/10.1038/s41589-021-00830-6>.
- (12) Fiala, T.; Barros, E. P.; Ebert, M.-O.; Ruijsenaars, E.; Riniker, S.; Wennemers, H. Frame Shifts Affect the Stability of Collagen Triple Helices. *J. Am. Chem. Soc.* **2022**, *144* (40), 18642–18649. <https://doi.org/10.1021/jacs.2c08727>.
- (13) Vitagliano, L.; Berisio, R.; Mastrangelo, A.; Mazzarella, L.; Zagari, A. Preferred Proline Puckerings in Cis and Trans Peptide Groups: Implications for Collagen Stability. *Protein Sci. Publ. Protein Soc.* **2001**, *10* (12), 2627–2632. <https://doi.org/10.1110/ps.ps.26601a>.
- (14) Bella, J.; Eaton, M.; Brodsky, B.; Berman, H. M. Crystal and Molecular Structure of a Collagen-like Peptide at 1.9 Å Resolution. *Science* **1994**, *266* (5182), 75–81. <https://doi.org/10.1126/science.7695699>.
- (15) Berisio, R.; Vitagliano, L.; Mazzarella, L.; Zagari, A. Crystal Structure of the Collagen Triple Helix Model [(Pro-Pro-Gly)(10)](3). *Protein Sci. Publ. Protein Soc.* **2002**, *11* (2), 262–270. <https://doi.org/10.1110/ps.32602>.
- (16) Okuyama, K.; Miyama, K.; Mizuno, K.; Bächinger, H. P. Crystal Structure of (Gly-Pro-Hyp)(9) : Implications for the Collagen Molecular Model. *Biopolymers* **2012**, *97* (8), 607–616. <https://doi.org/10.1002/bip.22048>.
- (17) Holmgren, S. K.; Taylor, K. M.; Bretscher, L. E.; Raines, R. T. Code for Collagen's Stability Deciphered. *Nature* **1998**, *392* (6677), 666–667. <https://doi.org/10.1038/33573>.
- (18) Vitagliano, L.; Berisio, R.; Mazzarella, L.; Zagari, A. Structural Bases of Collagen Stabilization Induced by Proline Hydroxylation. *Biopolymers* **2001**, *58* (5), 459–464. [https://doi.org/10.1002/1097-0282\(20010415\)58:5<459::AID-BIP1021>3.0.CO;2-V](https://doi.org/10.1002/1097-0282(20010415)58:5<459::AID-BIP1021>3.0.CO;2-V).
- (19) Hodges, J. A.; Raines, R. T. Stereoelectronic Effects on Collagen Stability: The Dichotomy of 4-Fluoroproline Diastereomers. *J. Am. Chem. Soc.* **2003**, *125* (31), 9262–9263. <https://doi.org/10.1021/ja035881z>.
- (20) Shoulders, M. D.; Guzei, I. A.; Raines, R. T. 4-Chloroprolines: Synthesis, Conformational Analysis, and Effect on the Collagen Triple Helix. *Biopolymers* **2008**, *89* (5), 443–454. <https://doi.org/10.1002/bip.20864>.

- (21) Kotch, F. W.; Guzei, I. A.; Raines, R. T. Stabilization of the Collagen Triple Helix by O-Methylation of Hydroxyproline Residues. *J. Am. Chem. Soc.* **2008**, *130* (10), 2952–2953. <https://doi.org/10.1021/ja800225k>.
- (22) Sonntag, L.-S.; Schweizer, S.; Ochsenfeld, C.; Wennemers, H. The “Azido Gauche Effect”-Implications for the Conformation of Azidoprolines. *J. Am. Chem. Soc.* **2006**, *128* (45), 14697–14703. <https://doi.org/10.1021/ja0654938>.
- (23) Doi, M.; Nishi, Y.; Uchiyama, S.; Nishiuchi, Y.; Nakazawa, T.; Ohkubo, T.; Kobayashi, Y. Characterization of Collagen Model Peptides Containing 4-Fluoroproline; (4(S)-Fluoroproline-Pro-Gly)₁₀ Forms a Triple Helix, but (4(R)-Fluoroproline-Pro-Gly)₁₀ Does Not. *J. Am. Chem. Soc.* **2003**, *125* (33), 9922–9923. <https://doi.org/10.1021/ja035997v>.
- (24) Ganguly, H. K.; Basu, G. Conformational Landscape of Substituted Prolines. *Biophys. Rev.* **2020**, *12* (1), 25–39. <https://doi.org/10.1007/s12551-020-00621-8>.
- (25) Hinderaker, M. P.; Raines, R. T. An Electronic Effect on Protein Structure. *Protein Sci. Publ. Protein Soc.* **2003**, *12* (6), 1188–1194. <https://doi.org/10.1110/ps.0241903>.
- (26) Erdmann, R. S.; Wennemers, H. Effect of Sterically Demanding Substituents on the Conformational Stability of the Collagen Triple Helix. *J. Am. Chem. Soc.* **2012**, *134* (41), 17117–17124. <https://doi.org/10.1021/ja3066418>.
- (27) Erdmann, R. S.; Wennemers, H. Functionalizable Collagen Model Peptides. *J. Am. Chem. Soc.* **2010**, *132* (40), 13957–13959. <https://doi.org/10.1021/ja103392t>.
- (28) Erdmann, R. S.; Wennemers, H. Importance of Ring Puckering versus Interstrand Hydrogen Bonds for the Conformational Stability of Collagen. *Angew. Chem. Int. Ed Engl.* **2011**, *50* (30), 6835–6838. <https://doi.org/10.1002/anie.201008118>.
- (29) Egli, J.; Siebler, C.; Köhler, M.; Zenobi, R.; Wennemers, H. Hydrophobic Moieties Bestow Fast-Folding and Hyperstability on Collagen Triple Helices. *J. Am. Chem. Soc.* **2019**, *141* (14), 5607–5611. <https://doi.org/10.1021/jacs.8b13871>.
- (30) Hodges, J. A.; Raines, R. T. Stereoelectronic and Steric Effects in the Collagen Triple Helix: Toward a Code for Strand Association. *J. Am. Chem. Soc.* **2005**, *127* (45), 15923–15932. <https://doi.org/10.1021/ja054674r>.
- (31) Shoulders, M. D.; Hodges, J. A.; Raines, R. T. Reciprocity of Steric and Stereoelectronic Effects in the Collagen Triple Helix. *J. Am. Chem. Soc.* **2006**, *128* (25), 8112–8113. <https://doi.org/10.1021/ja061793d>.
- (32) Jenkins, C. L.; Bretscher, L. E.; Guzei, I. A.; Raines, R. T. Effect of 3-Hydroxyproline Residues on Collagen Stability. *J. Am. Chem. Soc.* **2003**, *125* (21), 6422–6427. <https://doi.org/10.1021/ja034015j>.
- (33) Chaume, G.; Barbeau, O.; Lesot, P.; Brigaud, T. Synthesis of 2-Trifluoromethyl-1,3-Oxazolidines as Hydrolytically Stable Pseudoprolines. *J. Org. Chem.* **2010**, *75* (12), 4135–4145. <https://doi.org/10.1021/jo100518t>.
- (34) Chaume, G.; Simon, J.; Caupène, C.; Lensen, N.; Miclet, E.; Brigaud, T. Incorporation of CF₃-Pseudoprolines into Peptides: A Methodological Study. *J. Org. Chem.* **2013**, *78* (20), 10144–10153. <https://doi.org/10.1021/jo401494q>.
- (35) Chaume, G.; Feytens, D.; Chassaing, G.; Lavielle, S.; Brigaud, T.; Miclet, E. Conformational Properties of Peptides Incorporating a Fluorinated Pseudoproline Residue. *New J. Chem.* **2013**, *37* (5), 1336–1342. <https://doi.org/10.1039/C3NJ41084F>.
- (36) Chaume, G.; Simon, J.; Lensen, N.; Pytkowicz, J.; Brigaud, T.; Miclet, E. Homochiral versus Heterochiral Trifluoromethylated Pseudoproline Containing Dipeptides: A Powerful

- Tool to Switch the Prolyl-Amide Bond Conformation. *J. Org. Chem.* **2017**, *82* (24), 13602–13608. <https://doi.org/10.1021/acs.joc.7b01944>.
- (37) Feytens, D.; Chaume, G.; Chassaing, G.; Lavielle, S.; Brigaud, T.; Byun, B. J.; Kang, Y. K.; Miclet, E. Local Control of the Cis-Trans Isomerization and Backbone Dihedral Angles in Peptides Using Trifluoromethylated Pseudoprolines. *J. Phys. Chem. B* **2012**, *116* (13), 4069–4079. <https://doi.org/10.1021/jp300284u>.
- (38) Park, H. S.; Kang, Y. K. Which DFT Levels of Theory Are Appropriate in Predicting the Prolyl Cis-Trans Isomerization in Solution? *New J. Chem.* **2019**, *43* (44), 17159–17173. <https://doi.org/10.1039/C9NJ02946J>.
- (39) Abraham, M. J.; Murtola, T.; Schulz, R.; Páll, S.; Smith, J. C.; Hess, B.; Lindahl, E. GROMACS: High Performance Molecular Simulations through Multi-Level Parallelism from Laptops to Supercomputers. *SoftwareX* **2015**, *1–2*, 19–25. <https://doi.org/10.1016/j.softx.2015.06.001>.
- (40) Aliev, A. E.; Kulke, M.; Khaneja, H. S.; Chudasama, V.; Sheppard, T. D.; Lanigan, R. M. Motional Timescale Predictions by Molecular Dynamics Simulations: Case Study Using Proline and Hydroxyproline Sidechain Dynamics. *Proteins Struct. Funct. Bioinforma.* **2014**, *82* (2), 195–215. <https://doi.org/10.1002/prot.24350>.
- (41) Lindorff-Larsen, K.; Piana, S.; Palmo, K.; Maragakis, P.; Klepeis, J. L.; Dror, R. O.; Shaw, D. E. Improved Side-Chain Torsion Potentials for the Amber Ff99SB Protein Force Field. *Proteins Struct. Funct. Bioinforma.* **2010**, *78* (8), 1950–1958. <https://doi.org/10.1002/prot.22711>.
- (42) Salomon-Ferrer, R.; Case, D. A.; Walker, R. C. An Overview of the Amber Biomolecular Simulation Package. *WIREs Comput. Mol. Sci.* **2013**, *3* (2), 198–210. <https://doi.org/10.1002/wcms.1121>.
- (43) Dupradeau, F.-Y.; Pigache, A.; Zaffran, T.; Savineau, C.; Lelong, R.; Grivel, N.; Lelong, D.; Rosanski, W.; Cieplak, P. The R.E.D. Tools: Advances in RESP and ESP Charge Derivation and Force Field Library Building. *Phys. Chem. Chem. Phys.* **2010**, *12* (28), 7821–7839. <https://doi.org/10.1039/C0CP00111B>.
- (44) Wang, J.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A. Development and Testing of a General Amber Force Field. *J. Comput. Chem.* **2004**, *25* (9), 1157–1174. <https://doi.org/10.1002/jcc.20035>.
- (45) Humphrey, W.; Dalke, A.; Schulten, K. VMD: Visual Molecular Dynamics. *J. Mol. Graph.* **1996**, *14* (1), 33–38. [https://doi.org/10.1016/0263-7855\(96\)00018-5](https://doi.org/10.1016/0263-7855(96)00018-5).

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We demonstrate the synthesis of a highly fluorinated collagen model peptide using for the first time a proline analogue substituted on the C(5) position. We show its triple helix assembly in solution, in agreement with an extensive MD analysis.