

## Use of trifluoromethylated pseudoprolines for the design of collagen triple helix containing unusual C(5)-substituted proline surrogates

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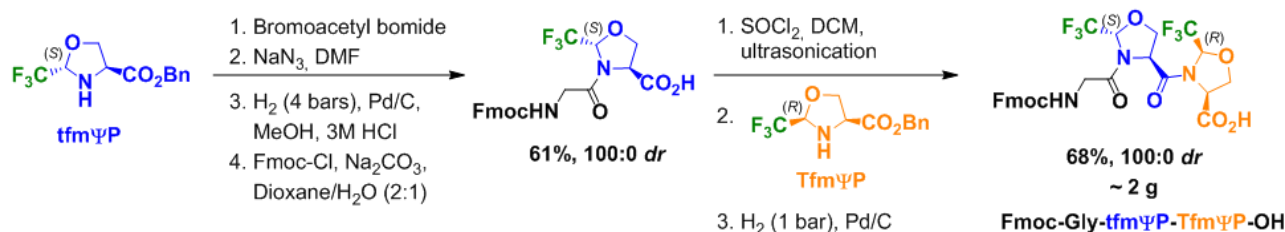
### Introduction

Collagen is the most abundant protein of mammals. This structural protein is omnipresent and represents the major component of the extracellular matrix. Its tertiary structure consists of three individual left-handed polyproline II helices folded into a right-handed triple helix which is stabilized by inter-strand hydrogen bonds with a single-residue offset.[1] Each strand comprises the repeat of the (Xaa-Yaa-Gly primary sequence where the proline (Pro, P and the (4R-4-hydroxyproline (Hyp, O are the most prevalent at Xaa and Yaa positions, respectively).[2] Crystal structures of short-length Collagen Model Peptides (CMPs show that Pro and Hyp residues exhibit preferentially C(4-endo and C(4-exo ring pucker, respectively.[3] This alternation has been proposed to be a prerequisite for the formation of the triple helix (TH since it preorganizes the individual strands in a suitable conformation, thereby decreasing the entropic cost.[4] Following these principles, numerous CMPs have been designed using C(4- and C(3-substituted proline derivatives. It was shown that these latter allowed the formation of the TH, provided that they satisfactorily mimic the conformational properties of Pro and Hyp and do not interfere with the interstrand H-bonds.[1] However, due to the packed nature of the collagen TH, substituents in the close vicinity of the backbone could be very deleterious for the TH stability and, to our knowledge, no work in the literature reports on TH containing C(5-substituted proline derivatives. Our group has developed the synthesis of the CF<sub>3</sub>-pseudoprolines (CF<sub>3</sub>-ψPro) and their incorporation into peptides.[5] We previously demonstrated that these proline analogues allow one to locally tune the peptide bond geometry, the puckering of the 5-membered ring as well as the Φ and Ψ dihedral angles.[6] It was found that the diastereoisomers (S)-CF<sub>3</sub>-ψPro (tfmψP) and (R)-CF<sub>3</sub>-ψPro (TfmψP) act as good mimics of the Pro and Hyp residues, respectively, by adopting the prerequisite puckering necessary for the formation and stabilization of the collagen PPII triple helix. However, we showed that the replacement of Hyp by TfmψP at the Yaa position of the Ac-Xaa-Yaa-Gly-NHMe collagen triplet model strongly affects the PPII-like conformation and perturbs the puckering alternation.[7] This result was confirmed with the fact that TfmψP<sup>11</sup>- [POG]<sub>7</sub> CMP 2 displays a thermal denaturation lowered by 30°C compared to the [POG]<sub>7</sub> 1.[8] In other hand, we demonstrated that the incorporation of both tfmψP and TfmψP at respectively the Xaa and Yaa position of the Ac-Xaa-Yaa-Gly-NHMe collagen triplet model favors the TH conformational requirements.[7] Finally, predictive molecular modeling realized on the [(Pro-Hyp-Gly)-(tfmψP-TfmψP-Gly)-(Pro-Hyp-Gly)] CMP shows that the CF<sub>3</sub> groups point outside of the TH and do not cause interstrand steric penalties. Here, we propose to assess and compare the stability of a collagen TH bearing both tfmψP and TfmψP in the middle of a [POG]<sub>7</sub> sequence.

### Results and Discussion

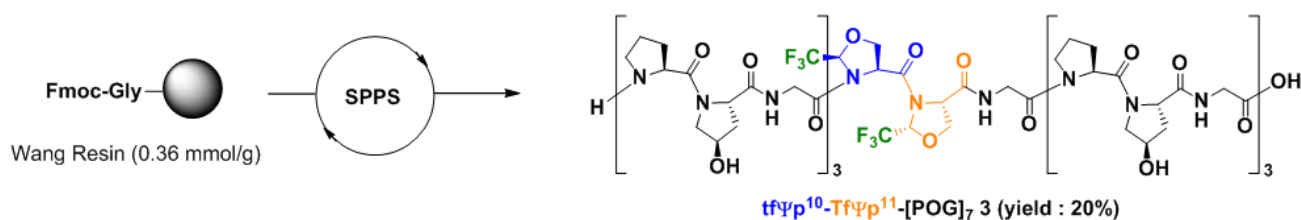
Because of the lack of nucleophilicity of the amino group and the steric bulkiness of the vicinal CF<sub>3</sub>-group, the direct incorporation of the CF<sub>3</sub>-ψPro residues into the [POG]<sub>7</sub> sequence *via* solid-phase peptide synthesis (SPPS) was excluded. Therefore, we planned to incorporate the two CF<sub>3</sub>-ψPro residues into the fluorinated host-guest CMP tfmψP<sup>10</sup>-TfmψP<sup>11</sup>-[POG]<sub>7</sub> 3 using a “ready to use” tripeptide building blocks for SPPS. At this stage, it was anticipated that the use of the Fmoc-tfmψP-TfmψP-Gly-OH building block would be extremely challenging since we previously reported that a complete epimerization of the C(5 stereocenter of the tfmψP residue occurred during peptide coupling reactions at its N-terminus, leading exclusively to the TfmψP moiety.[5b] However, we observed that N-acylation reactions using non-hindered acyl chlorides preserved the integrity of the (S)-configuration at the C(5 position of the tfmψP.[5a] Therefore, the triplet frame of the building block was shifted and the Gly residue at the N-terminal position was introduced sequentially starting from the bromoacetyl bromide acylating reagent (Scheme 1) As expected, the N-acylation of the tfmψP residue proceeded without epimerization of the C(5 stereocenter. Treatment with sodium azide followed by

hydrogenation and Fmoc-protection of the corresponding amine afforded the Fmoc-Gly-tfm $\psi$ P-OH dipeptide. The latter is then activated as acyl chloride and coupled with the Tfm $\psi$ P residue. Subsequent hydrogenolysis of the benzyl ester gave the Fmoc-Gly-tfm $\psi$ P-Tfm $\psi$ P-OH building block as a single diastereomer in large scale.



Scheme 1: Synthesis of the “ready to use” Fmoc-Gly-tfm $\psi$ P-Tfm $\psi$ P-OH building block

The fluorinated host-guest CMP tfm $\psi$ P<sup>10</sup>-Tfm $\psi$ P<sup>11</sup>-[POG]<sub>7</sub> **3** was prepared on Wang resin by automated SPPS under microwave activation using OxymaPure/DIC as a coupling reagent with similar yield (~20%) to those obtained for CMPs **1** and **2** (Scheme 2). This result demonstrates that our CF<sub>3</sub>- $\psi$ Pro residues are compatible with SPPS and TFA cleavage. Nonetheless, it should be stressed that the Fmoc removal step after the insertion of the fluorinated building block required the use of Morpholine:DMF (1:3) mixture in the presence of HOBT (0.1 M) to prevent the tfm $\psi$ P-Tfm $\psi$ P amide bond cleavage.



1. Piperidine (16 eq), MW\*; 2. DIC (6 eq), Oxyma pure (6 eq), AAs (6 eq), MW; 3. TFA/TIS/H<sub>2</sub>O (95:2.5:2.5)

\* Deprotection conditions for the Fmoc-Gly-tfm $\psi$ P-Tfm $\psi$ P moiety : Morpholine:DMF (1:3), HOBT (0.1 M)

Scheme 2: Synthesis of the fluorinated host-guest CMP **3**

The stability of the triple helix of tfm $\psi$ P<sup>10</sup>-Tfm $\psi$ P<sup>11</sup>-[POG]<sub>7</sub> **3** was then assessed. CD spectroscopy is the most common analytical method for the determination of collagen TH melting temperatures. At 10 mM, the fluorinated CMP **3** exhibits a PPII structuration with the characteristic positive band at 225 nm on the CD spectrum (Figure 1A). The comparison of the CD spectra of CMPs **1**-**3** shows that the non-fluorinated CMP **1** displays the higher  $[\theta]$  maximum. Sigmoidal curves  $[\theta] = f(T)$  were obtained by increasing the temperature as a result of the thermal denaturation of the collagen TH. CMPs **2** and **3** displayed lower melting temperatures ( $T_m$ ) for the TH compared to the reference [POG]<sub>7</sub> **1** (Figure 1B). However, CMP **3** displays a 12°C higher  $T_m$  value compared to CMP **2**, despite the fact that it bears a second CF<sub>3</sub>- $\psi$ Pro residue. As mentioned before, it was observed that when incorporated into the Ac-Xaa-Yaa-Gly-NHMe collagen triplet model, a single Tfm $\psi$ P at the Yaa position strongly affects the puckering of the Xaa=Pro residue, while when Xaa=tfm $\psi$ P and Yaa=Tfm $\psi$ P the puckering alternation required in the TH model was maintained.[7] This result suggests that satisfying the C(4)-endo/C(4)-exo alternation allows a significant increase of the  $T_m$  value in TH models incorporating Pro and Hyp analogues. Therefore, the collagen TH can accommodate large CF<sub>3</sub> groups close to the peptide backbone provided that the puckering alternation is correct and that the CF<sub>3</sub> groups point outside of the TH.

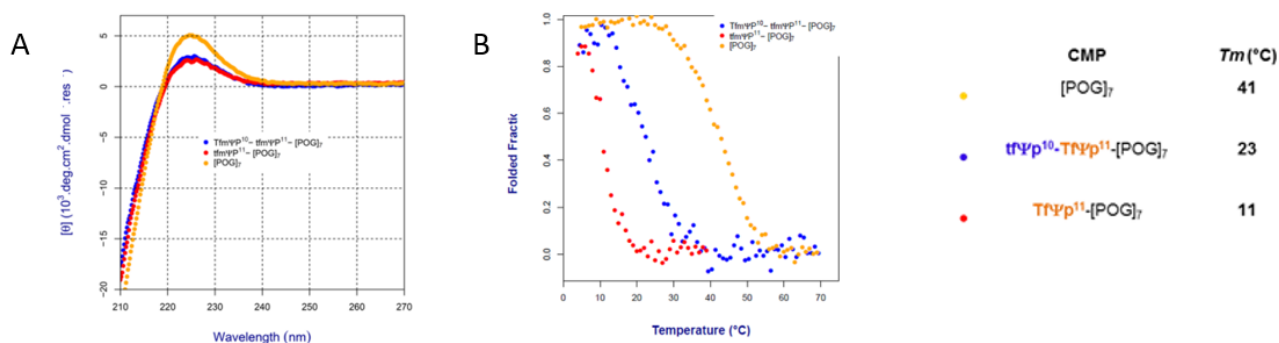


Figure 1: A. CD spectra of CMPs 1-3 (10 mM, phosphate buffer (50 mM), 4°C) showing the maximum positive band at 225 nm; B. Sigmoidal curves obtained from the thermal denaturation of 1-3 at a heating range of 10°C/h.

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