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To cite this version:

N Malquin, K Rahgoshay, N Lensen, G Chaume, E Miclet, et al.. CF2H as hydrogen bond donor group for the fine tuning of peptide bond geometry with difluoromethylated pseudoprolines. Chemical Communications, 2019, 55 (83), pp.12487-12490. 10.1039/C9CC05771D . hal-03549249

HAL Id: hal-03549249
https://hal.science/hal-03549249
Submitted on 31 Jan 2022

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COMMUNICATION

CF₂H as hydrogen bond donor group for the fine tuning of peptide bond geometry with difluoromethylated pseudoprolines

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CF₂H-pseudoprolines obtained from difluoroacetaldehyde hemiacetal and serine are stable proline surrogates. The consequence of the incorporation of the CF₂H group is an important decrease of the trans to cis amide bond isomerisation energy and a remarkable stabilisation of the cis conformer by an hydrogen bond.

Because of the unique properties of fluorine atom, fluorinated compounds are increasingly recognised as highly promising for the discovery of new bioactive compounds.1 Considering the specific case of peptides, the incorporation of fluorinated amino acids in peptides has been shown to impart remarkable properties.2 It can modulate the peptide conformation and assembly,3 the local hydrophobicity,4,5 and the resistance towards proteolysis.5 Moreover fluorine atoms are very useful as highly sensitive labels for 19F-NMR spectroscopy because of 100% natural abundance and the absence of background signals in natural and biological media.6 Although several methods have been reported for the synthesis of trifluoromethylated amino acids, the preparation of difluoromethylated one’s is much less common.7,8 It is a lack because the CF₂H group presents unique features and we believe that this group will play an increasing role in peptides chemistry. The main property of the CF₂H group is to act as a lipophilic hydrogen bond donor group bioisostere of a hydroxyl or a thiol.8 These original bioisosteric properties have recently been highlighted in medicinal chemistry.9 Because of its cyclic structure, the proline residue is recognized to play a unique role on peptide backbone conformation. When included into a peptide, the pyrrolidine ring cannot act as an hydrogen bond donor and restrain the diehedral angle to about −60°.10 The Xaa-Pro peptide bond is characterized by a small free energy difference (ΔG°•) between the trans and the cis amide bond conformers, combined with a high activation energy (ΔG‡) for the cis-trans isomerization.11 This isomerisation is considered to play a key role in regulating many important biological processes in proline containing peptides and proteins, including protein folding.12 However, studies of protein folding or control of the peptide or protein function are often hampered by the heterogeneity of the X-Pro peptide bond geometry and the scientific community has constantly been on the lookout for either a cis or a trans stabilizing proline analogue or surrogate.13 In the fluorinated series, CF₂-substituted prolines have recently emerged as very attracting tools for the control of peptides conformation and hydrophobicity.14 On the other hand, very few examples of difluoromethylated prolines have been reported in the literature,15 and to our knowledge the synthesis of enantiopure 5-CF₂H-prolines has not been reported so far. Pseudoprolines (Ψ-Pro) obtained from the condensation of serine and aldehydes appear as very useful surrogates of δ-substituted prolines to control the conformation of peptides.16 Since several years we investigate the scope of trifluoromethylated pseudoprolines (CF₃-Ψ-Pro) in peptide chemistry.17 We reported that the ratio of cis conformation of the Xaa-CF₃-Ψ-Pro peptide bond is increased compared to proline itself. The 2-(S)-CF₃-Ψ-Pro presents a γ-endo puckering of the oxazolidine ring while the 2-(R)-CF₃-Ψ-Pro adopts a γ-exo puckering (Fig. 1).17 Moreover, a remarkable feature of the 2-(R)-CF₃-Ψ-Pro is the decrease of the trans to cis isomerization barrier of the amide bond (-4.17 kcalmol⁻¹ compared to proline).1

Fig. 1 Peptidyl bond isomerization and puckering of CF₃-ΨPro
Considering the growing importance of the difluoromethyl group, we wish to present herein our results dedicated to difluoromethylated pseudoprolines (CF$_2$H-$\psi$Pro). A particular attention is paid to the possibility of interactions through hydrogen bonds.

The oxazolidine ring of the CF$_2$H-pseudoproline was built by condensation of difluoroacetaldehyde ethyl hemiacetal with serine benzyl ester under an acidic catalysis (Scheme 1). Both diastereomers ([S,S]-1 and ([R,S]-1) were conveniently separated by silica gel chromatography. According to our previous investigations in the trifluoromethylated series, the relative configuration of ([R,S]-1) was assigned by nuclear Overhauser effect experiments showing a correlation between the C$_\alpha$ and the C$_\gamma$ protons. In order to investigate the electronic and geometric consequences due to the CF$_2$H group incorporation of CF$_2$H-$\psi$Pro in a peptide chain, the Ac-$\psi$Pro-NHMe model tripeptides ([S,S]-4 and ([R,S]-4) have been synthesized in a multiple-step procedure (Scheme 2). The N-acetylation of ([S,S]-1) with acetic anhydride under iodine catalysis provided a mixture of Ac-$\psi$Pro-OBn ([S,S]-2) and ([R,S]-2). In order to circumvent the epimerization of the C$_\alpha$ atom through an acidic catalysis, the reaction was performed with acetyl chloride in pyridine. These experimental conditions led to the expected N-acetylated oxazolidines ([S,S]-2 and ([R,S]-2) in 81% and 75% yield respectively without epimerisation.

The cis/trans ratio of the acetyl-$\psi$Pro bond was determined by $^1$H and $^{19}$F NMR integration in CDCl$_3$. For that purpose, we first assigned each conformer by comparing the $\alpha$, $\beta$, and $\gamma$ chemical shifts of the cis and the trans isomers according to Lubell et al.$^{18}$ For the trans isomer, the $\alpha$ and $\beta$-carbons appear upfield while they appear downfield in the cis isomer. Conversely, the $\delta$ carbon for the trans isomer appears downfield relative to that of the cis isomer. Once acetylated on their N-terminal position, we checked the acidic stability of the CF$_2$H-oxazolidines ([S,S]-2 and ([R,S]-2). Experiments showed that, similarly to the CF$_3$H-$\psi$Pro, they are stable in an acidic medium (5% TFA in CHCl$_3$), whereas pseudoprolines of the non-fluorinated series are generally prone to ring opening.$^{18}$ Even when exposed to more concentrated acidic medium for several hours (3h in TFA/H$_2$O 95:5) no ring opening or epimerization occurred. The CF$_2$H-oxazolidines are therefore suitable for SPPS strategy.

**Scheme 1** Synthesis of H-Ser[2,3,5]Val-$\psi$Pro)-OBn ([S,S]-1 and ([R,S]-1)

**Scheme 2** Synthesis of Ac-$\psi$Pro-NHMe ([S,S]-4 and ([R,S]-4)

After removal of the benzyl ester function by hydrogenolysis in quantitative yields, the N-methyl amides ([S,S]-4 and ([R,S]-4) were obtained in 40% and 33% yield using classical peptide coupling conditions (Scheme 2).

The cis-$\psi$trans bond isomerisation of the CF$_2$H-oxazolidine-containing peptide models ([S,S]-4) and ([R,S]-4) was studied by $^1$H NMR analysis in water at various temperatures. The coalescence temperatures were determined to estimate the rotational barriers for the cis-$\psi$trans isomerisation. The results are reported in the Table 1 and compared with those reported for regular proline$^{18}$ and pseudoproline$^{17c}$ as well as those previously obtained in the CF$_3$ series.$^{13c}$ The difference of isomerisation energy between the two diastereomers of the CF$_3$ series (ca. 2.5 kcal.mol$^{-1}$) is no longer observed in the CF$_2$H series, the energy barriers are low for both CF$_2$H-pseudoprolines. It is interesting to note that the energy barriers for amide isomerisation of both CF$_2$H-pseudoproline is ca 5.0 kcal.mol$^{-1}$ lower than for proline.$^{18}$

The cis amide bond contents were significantly higher when the CF$_3$ substituent was replaced by the CF$_2$H. Indeed in the apolar chloroform solution, the cis population of ([S,S]-4) reached even 78%. This enrichment may be due to the presence of a new H-bond involving the CF$_2$H-group. Conversely, it was previously demonstrated that the trans amide bond conformation of the 2-(R)-CF$_3$-$\psi$Pro analogue was stabilized by a H-bond between the C-terminus amide NH and the carbonyl of the N-terminal acyl group.$^{17c}$ Two antagonist H-bonds may thus compete in the compound ([R,S]-4) (Fig. 2). This balance was slightly in favour of the CF$_2$H H-bond in water (53% cis population) and underlines the hydrophobicity of the fluorinated group which partially preserves the stability of the H-bond in polar solvents such as methanol and water (see supplementary information).

Finally, the puckering of the CF$_2$H-five-membered rings were determined by NMR from the coupling constants between the $\alpha$ and the $\beta$ protons as previously reported in the CF$_3$ series (see supplementary information).$^{17c}$

The results showed that the Ac-((S,S)-2S,4S)-CF$_2$H-$\psi$Pro-NHMe ([S,S]-4) adopted a $\gamma$-endo puckering conformation for the two
geometries of the peptide bond. The same observation was made for the 2-(S)-CF$_2$-Y-Pro analogue.\textsuperscript{17c} Conversely the compound (R,S)-4 underwent a puckering switch when the amide bond geometry was reversed.

Instead of maintaining an exo-puckered oxazoline ring for both the cis and the trans amide conformers, as observed with 2-(R)-CF$_2$-Y-Pro\textsubscript{17c} the cis rotamer of (R,S)-4 showed a slight preference for the endo puckering to allow the CF$_2$H-H-bond. These structural features can be understood by analyzing the unusual NMR parameters of the CF$_2$H groups. First, a high chemical shift difference ($\Delta\delta$) of the diastereotopic \textsuperscript{19F} from a restricted dynamic of the difluoromethyl group. For the cis conformers, $\Delta\delta$ was above 5 ppm, whereas $\Delta\delta = 3$ ppm for their corresponding trans conformations (see Fig. 3 and supplementary information). Second, the measured vicinal couplings reflected a very limited rotameric averaging of the CF$_2$H group for the cis conformers. In particular the Ac-(2S,4S)-CF$_2$H-Y-Pro-NMe$_2$ (S,S)-4 displayed the following values: $J_{\text{HF,2}} = 3.2$ Hz; $J_{\text{HF,2}} = 18.2$ Hz; $J_{\text{HF,3}} = 1.0$ Hz. These three couplings are compatible with a single H-C-C-H dihedral angle of “SO” (see supplementary information). Such a restricted rotation of the CF$_2$H group likely arose from the H-bonding observed in the cis forms since the oxygen of the preceding acetyl group is facing the positively charged CF$_2$H proton in this conformation (Fig. 3). The endo puckering of the oxazoline ring may be needed for the CF$_2$H H-bonding establishment in the cis conformers, whereas the exo puckering was required for the NH…O=C H-bond in the trans form.\textsuperscript{17c} This provides an explanation for the trans-exo to cis-endo transition observed for the compound (R,S)-4. The (S,S)-4 diastereoisomer adopts preferentially the endo conformation which explains the high stability of the CF$_2$H-O=CH bond in the cis conformer. In the trans conformers, the CF$_2$H group cannot establish any H-bond since the O=C acceptor of the preceding acetyl group is facing the positively charged CF$_2$H. Therefore, several rotamers of the CF$_2$H group at the $\delta$ position of an oxazolinedine unit is detrimental for coupling reactions because of its powerful withdrawing effect. Although the CF$_2$H group is less deactivating, typical protocols using HOBt, EDCI, DIPEA or IBCF reagents failed to achieve the N-coupling reaction of (S,S)-1 and (R,S)-1 with Fmoc-Gly-OH. However, the peptide coupling could be achieved in good yields while using the most electrophilic Fmoc protected glycine and alanine amino acid chloride (Scheme 3). When the reaction was performed in the presence of pyridine, the epimerization of the $\delta$ carbon of the oxazolinidine ring through a dynamic kinetic resolution (DKR) process\textsuperscript{17d} was avoided or limited. The expected dipeptides (S,S)-5, (R,S)-5, (S,S)-6 and (R,S)-6 were obtained in 86%, 76%, 57% and 88% yield respectively (Scheme 3). It should be noticed that in any cases the cis conformer of the amide bond was the major one. The cis/trans conformers ratio of the peptide-Y-Pro bond measured by NMR in CDC$_2$$_2$ were ranging from 58/42 to 83/17. After having successfully solved the challenging coupling reaction at the N-terminal positions of the CF$_2$H-pseudopropines, suitable experimental conditions were investigated for the C-terminal coupling. The removal of the benzyl ester was carried out by palladium mediated hydrogenolysis without impacting the N-Fmoc protecting group. The free carboxylic acid was then used in the next step without further purification. Typical protocols involving HOBT, EDCI, DIPEA at room temperature were not suitable for the coupling reaction between dipeptides 6 and HCl,H$_2$N-Ala-OBu. However, the coupling of the CF$_2$H-dipeptides using BOP-Cl in the presence of TEA afforded the expected tripeptides (S,S)-7 and (R,S)-7 in 56% and 63% yield respectively. $^1$H and $^13$C NMR measurements in CDC$_2$$_2$ revealed a 85/15 cis/trans ratio for (S,S)-7 and 59/41 for (R,S)-7.

In conclusion, original enantiopure (S,S) and (R,S) CF$_2$H-pseudopropines have been synthesized as stable 5-substituted proline surrogates which can be incorporated in a peptide chain. The CF$_2$H group provides a strong decrease of the trans to cis amide bond isomerisation energy and enhances the cis conformation content compared to the CF$_3$ series, by acting as an efficient hydrogen bond donor. These CF$_2$H-proline surrogates will provide outstanding tools for the fine tuning of the dynamic and the secondary structure of peptides, in particular type VI $\beta$-turns.\textsuperscript{13b} Such conformations are known to play important roles in protein structures and molecular recognition. New non-natural amino acids able to stabilize the cis-isomer backbone geometry and/or type VI $\beta$-turns are still in demand for the design of potent and selective prototypes for drug discovery.\textsuperscript{19}

The authors gratefully acknowledge Central Glass Co. for the gift of difluoroacetaldehyde hemiacetal and the Agence Nationale de la Recherche for funding (ANR CH2PROBE and ANR F-LAIR). K. R. thanks the MNREST for a fellowship.

**Conflicts of interest**

There are no conflicts to declare.

**Notes and references**
