# 3-$CF_3$/$C_2F_5$ - Substituted Proline Analogs: Synthesis and Proteolytic Stability of Their Amide Derivatives

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## Introduction

• Fluorinated proline analogues are of particular interest for many fields such as medicinal chemistry and peptide sciences.
• The introduction of a $CF_3$-group or another polyfluoroalkyl group into the proline skeleton is particularly challenging and requires different approaches depending on the position in the molecule. For instance, proline analogues bearing a $CF_3$-group in different positions (Fig. 1) were prepared by diverse methods [1-4].
• For 3-$CF_3$ and 3-$C_2F_5$-proline we have recently proposed an effective approach which can be used to prepare gram-scale of *cis*- and *trans*-3-$CF_3$/$C_2F_5$-Pro.[2]

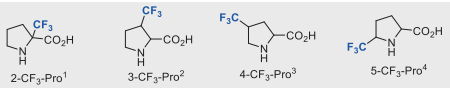
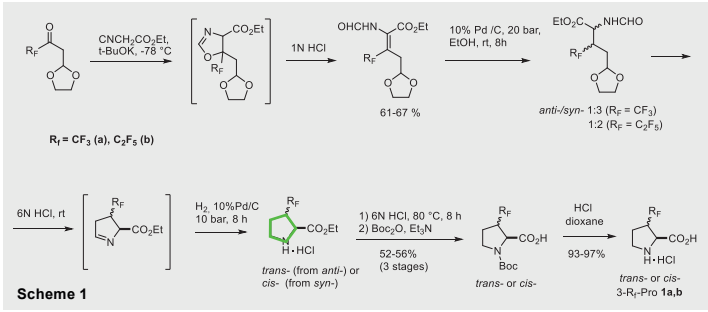


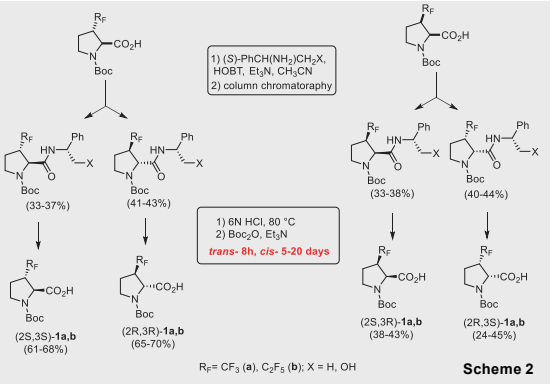

Figure 1




Scheme 1

## Enantiomers of 3-$R_F$-prolines and dipeptides

In order to obtain pure enantiomers of compounds **1** racemate resolution via *N*-Boc amino acid amides ***cis-*** and ***trans-8*** using *(S)*-phenyl ethyl amine or *S)*-phenyl ethanol amine was applied (Scheme 4). The letter approach was found the most effective. In order to obtain cis-enantiomers the remarkable long time of hydrolysis was needed **(up to 20 days!)** in order to reach the full conversion. The absolute configuration for several amides was assigned by X-ray analysis.




Scheme 2

The developed approach allowed us to synthesize and characterize all possible stereoisomers of 3-$CF_3$/$C_2F_5$-Prolines **1a,b (Figure 2).**[5]

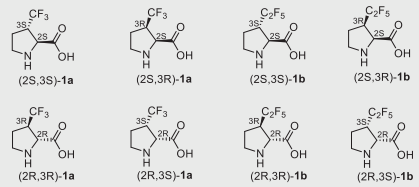
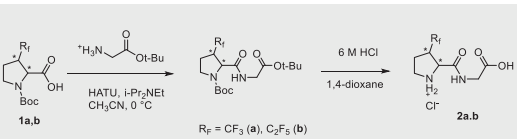



Figure 2

Next, we studied the impact of the fluoroalkyl group on the hydrolytic stability of model dipeptides for the case of $CF_3$- and $C_2F_5$-substituted prolines **1a,b**. The corresponding dipeptides **2a,b** were synthesized by standard procedure.




Scheme 3

## Study of proteolytic stability

Next, mouse plasma stability was measured for the synthesized compounds 2 and compared to that of (S)- and (R)-Pro-Gly hydrochlorides **(Figure 3, Table 1)**.

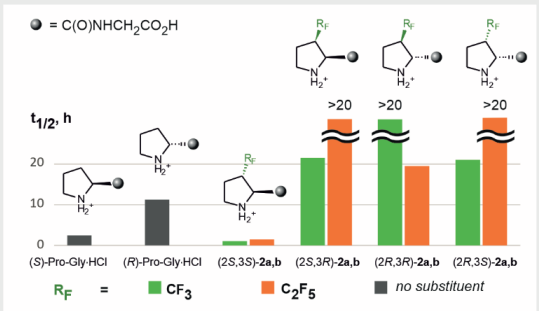



Figure 3

**Table1.** Elimination constants ($k_{el}$) and half-lifes ($t_{1/2}$) of **2** in mouse plasma

| Entry | $R_F$ | 2 | $k_{el}$·10 $^3$, $min^{-1}$ | $t_{1/2}$, h |
|---|---|---|---|---|
| 1 | – | (S)-Pro-Gly· HCl | 4.8 | 2.4 |
| 2 | $CF_3$ | (2S,3S)-**2a** | 11.1 | 1.0 |
| 3 | $C_2F_5$ | (2S,3S)-**2b** | 8.0 | 1.4 |
| 4 | $CF_3$ | (2S,3R)-**2a** | ~0.5[a] | ~20[a] |
| 5 | $C_2F_5$ | (2S,3R)-**2b** | <0.5[a] | >20[a] |
| 6 | – | (R)-Pro-Gly· HCl | 1.0 | 11.2 |
| 7 | $CF_3$ | (2R,3R)-**2a** | <0.5[a] | >20[a] |
| 8 | $C_2F_5$ | (2R,3R)-**2b** | ~0.5[a] | ~20[a] |
| 9 | $CF_3$ | (2R,3S)-**2a** | ~0.5[a] | ~20[a] |
| 10 | $C_2F_5$ | (2R,3S)-**2b** | <0.5[a] | >20[a] |

**General conclusions**

• Most of the compounds derived from D-proline ((2R)-isomers) showed higher hydrolytic stability as compared to their L-counterparts ((2S)-isomers).
• A pair of $CF_3$-substituted *cis*-isomers (2S,3R)-**2a** and (2R,3S)-**2a** had comparable half-life in mouse plasma (~20 h).
• Introduction of the perfluoroalkyl group at the 3rd position of proline led to the improved proteolytic stability for all dipeptide derivatives except for trans-isomers (2S,3S)-**2**.
• For both $CF_3$- and $C_2F_5$-substituted compounds (2S,3S)-**2**, plasma stability was nearly twice lower as compared to parent (S)-Pro-Gly.
• Compounds with $C_2F_5$ group had higher half-life compared to those with the $CF_3$ group.

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