Proceedings of the 36th European Peptide Symposium
Michal Lebl (Editor)
European Peptide Society, 2022 *https://doi.org/10.17952/36EPS.2022.267*

Synthesis of Fluorinated Amino Acids for the Design of Injectable Hydrogels

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Introduction

Amphipathic peptides, composed of alternating hydrophobic and hydrophilic amino acids, have been shown to form self-assembled hydrogels. These hydrogels proved adequate drug delivery platforms that can deliver pharmaceutical cargoes in a stable and prolonged manner when injected subcutaneously [1]. Such systems can increase patient compliance by limiting the number of injections required for the efficient treatment of chronic diseases. Currently, however, the drug release window of these peptide hydrogels is limited to a maximum of four days and this window ideally needs to be extendable for optimal use in diverse clinical settings.

As the role of fluorine within medicinal chemistry continues to develop, fluorinated amino acids have shown their utility in promoting and stabilizing well-defined secondary structures, as well as increasing local hydrophobicity and enhancing the biological profile of drug candidates [2]. Thus, the rational introduction of fluorine atoms into peptide hydrogels might provide access to a new class of injectable controlled-delivery systems that can take advantage of the favorable properties of fluorine atoms. Two strategies are investigated for the synthesis of fluorinated peptide hydrogels based on a previously developed hexapeptide consensus sequence. The first relies on the incorporation of fluorinated amino acids along the hydrophobic face of the amphipathic peptide of type 1 (Figure 1a), the second concerns the design of fluorinated β-hairpin peptide hydrogelator of type 2 (Figure 1b). Several Fmoc-protected amino acids were synthesized for introduction into those peptides, including the trifluoromethionine (TFM) and the CF_3-pseudoproline ($CF_3\psi$Pro) (Figure 2).

Results and Discussion

For the design of peptides of type **1**, TFM has been considered for its capacity to increase the hydrophobicity of peptides [3]. The synthesis has been performed by adapting procedures reported in the literature [4]. Starting from L-homocystine, the Fmoc-TFM-OH building block ready-to-use for solid phase peptide synthesis (SPPS) was obtained with an overall yield of 36% over 4 steps (Figure 2a).

Regarding the design of β-hairpins of type **2**, several turn motifs including fluorinated residues were prepared to promote the formation of β-sheet and eventually help the self-assembly process to form hydrogels. To this end, Fmoc-Lys(Alloc)-$CF_3\psi$Pro-OH and Fmoc-Pro-$CF_3\psi$Pro-OH were chosen. Because of the lack of nucleophilicity of the amino group and the steric bulkiness of the vicinal CF_3-group of the $CF_3\psi$Pro residue, the dipeptide building blocks have been synthesized according to our optimized conditions using activated amino acids as acyl chloride (Figure 2b)[5]. A series of the two dipeptides with different configurations were obtained with overall yields between 12-66%.

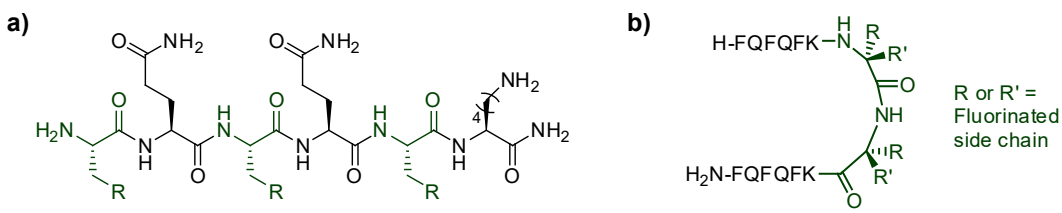

*Fig. 1. a) Type **1**: Hexapeptide sequence; b) Type **2**: β-hairpin sequence.*

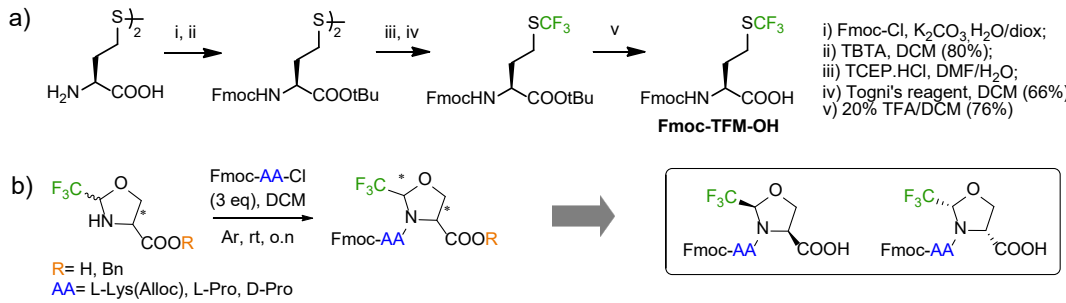

Fig. 2. a) Synthesis of Fmoc-TFM-OH; b) Coupling conditions of the $CF_3\psi$Pro residue using acyl chloride and the prepared building blocks.

Subsequently, a set of linear fluorinated hexapeptides of type **1** has been designed, by substituting the phenylalanine residues from the hydrophobic face with different fluorinated amino acids, including TFM as well as several commercial fluorinated phenylalanine derivatives. After the synthesis, the gelation properties of the resulting fluorinated hydrogelators have been assessed qualitatively using the inverted tube test. The minimum gelation concentration has been determined using PBS buffer as trigger, according to an established protocol [1]. The results suggested that, in general, the substitution of all phenylalanine positions by a fluorinated residue increased the hydrophobicity of the peptide, while decreasing the stiffness of the gels. Also, the single substitution of the phenylalanine at the *C*- terminal position afforded hydrogels with interesting gel properties, as already observed in a previous study [6]. To evaluate the structuration occurring during the self-assembly, FT-IR spectroscopy has been performed and displayed the typical signature of the presence of β-sheet for most of the formed gels.

In parallel, β-hairpin hydrogelators of type **2** have been designed by connecting two strands based on the hexamer consensus sequence, through a β-turn motif based on the well-known D-Pro-Pro motif as β-turn inducer. A set of fluorinated β-hairpin hydrogelators have been successfully synthesized incorporating the $CF_3\psi$Pro residue to functionalize the β-turn motif of the hairpin sequence. Interestingly, several sequences showed promising gel properties and, again, FT-IR characterization showed β-sheet structuration. A more extensive characterization will be performed, including circular dichroism (CD) and nuclear magnetic resonance (NMR), to better understand the self-assembly process of these hydrogelators.

In summary, fluorinated building blocks have been successfully synthesized and introduced into peptides using SPPS. In the next step, quantitative assessment of the gel strength will be performed using dynamic rheometry. Subsequently, the release properties of the most promising sequences will be further investigated *in vitro* using different cargoes.

Acknowledgments

We thank the Eutopia and CY Initiative of Excellence (grant « Investissements d'Avenir » ANR-16-IDEX-0008) for their financial support. SB and CM also acknowledge the Research Council of VUB for the financial support through the Strategic Research Programme (SRP50).

References

1. (a) Martin, C., et al. *J. Med Chem.* **61**, 9784-9789 (2018), https://doi.org/10.1021/acs.jmedchem.8b01282 ; (b) Martin, C., et al. *Mater. Today Chem.* **3**, 49-59 (2017), http://dx.doi.org/10.1016/j.mtchem.2017.01.003
2. Sloand, J.L., et al. *Pept. Sci.* **113**, e24184 (2021), https://doi.org/10.1002/pep2.24184
3. Gadais, C., et al. *ChemBioChem.* **19**, 1026-1030 (2018), http://dx.doi.org/10.1002/cbic.201800088
4. (a) Almaliti, J., et al. *Eur. J. Med. Chem.* **161**, 416-432 (2019), https://doi.org/10.1016/j.ejmech.2018.10.024; (b) McConathy, J., et al. *J. Med. Chem.* **45**, 2240-2249 (2002), https://doi.org/10.1021/jm010241x; Gadais, C., et al. *Eur. J. Org. Chem.* **2**, 246-251 (2017), https://doi.org/10.1002/ejoc.201601318
5. Chaume, G., et al. *J. Org. Chem.* **78**, 10144-10153 (2013), https://doi.org/10.1021/jo401494q
6. Bettens, T., et al. *Mater. Adv.* **2**, 4792-4803 (2021), https://doi.org/10.1039/D1MA00455G