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Design, Synthesis and Biological Evaluation of Fluorinated Cathepsin D Inhibitors

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Introduction

Aspartic peptidases, like Cathepsin D, are involved in many pathologies affecting human population like hypertension, Alzheimer Disease (AD) and Acquired Immune Deficiency Syndrome (AIDS). Moreover, this enzyme, is considered as a potential target for the treatment of breast cancer. In this context the development of efficient inhibitors and inhibitor screening methods is a major need for the scientific community [1]. Pepstatin A is a penta-peptide, containing two residues of the γ-amino acid Statin, considered as the gold standard among the natural inhibitors of Cathepsin D. Nevertheless, due to its poor selectivity and its poor metabolic stability, no applications have been found for the treatment of the diseases mentioned above with this molecule. The synthesis and the evaluation of peptide-like ligands of Cathepsin D containing fluorine atoms constitutes an innovative strategy that could lead to the development of new molecules as ^{19}F NMR probes and/or new potential inhibitors towards Cathepsin D [2]. The introduction of fluorinated amino acids in the peptide scaffold, in fact, could: *(i)* transform the ligand into an efficient ^{19}F NMR probe opening the way to NMR's based functional assays [3]; *(ii)* help the cellular membrane uptake; *(iii)* improve the bioavailability [4].

Results and Discussion

Structures of the synthesized pepstatin A analogues are summarized in Figure 1. We initially chose to increase locally the hydrophobicity replacing the *N*-terminal isovaleryl moiety of pepstatin A with a trifluoroacetyl group in order to quickly access to the fluorinated analogue **1**. All the syntheses were carried out on solid support *via* classical Fmoc-based solid phase synthesis (SPPS). Chlorotrityl resin was used instead of Wang resin because of the free hydroxyl group of Wang resin and the risk of side reactions due to the presence of the free hydroxyl of the statin residues. A series of classical Fmoc-aa coupling/deprotection reactions yielded the pentapeptide Val-Val-Sta-Ala-Sta, then a trifluoroacetylation was carried out directly on the resin using ethyl trifluoroacetate and the analogue **1** was obtained in an overall yield of 31% after cleavage and purification.

The synthetic pathway for the analogues **2** and **3** incorporating (*R*) and (*S*) enantiomers of *N*- protected trifluoromethyl alanine (Z-TfmAla) in *N*-terminal position is reported in Scheme 1. These modifications should increase the affinity of these inhibitors for Cathepsin D and improve their metabolic stability by the presence of a fluorinated and quaternarized amino acid. For synthetic convenience, *N*-Cbz protected trifluoromethylated amino acid were used and the protecting group was then preserved in the final inhibitor since *N*-Cbz inhibitors of CatD have already been found in the literature. After coupling of the first amino acid, Fmoc-statin, on the chlorotrityl resin in the presence of DIPEA, deprotection with a 20% solution of piperidine in DMF allows to carry out the coupling reaction of the second amino acid in the presence of HBTU and DIPEA. These steps are repeated with each amino acid to be coupled until the tetrapeptide Val-Sta-Ala-Sta is obtained. Finally, the fluorinated analogues **2** and **3** were obtained by coupling the previously synthesized (*R*) or the (*S*) Cbz-TfmAla on the tetrapeptide respectively in 16% and 28% yield.

The synthesis of analogue **4**, with a (*S*)-TFM (TFM = trifluoromethionine) instead of Valine, was therefore considered; we indeed hypothesized that the incorporation of a fluoroalkyl side chain at the P1 position would increase the potency of the inhibitor. During the synthesis, the unexpected epimerization of the TFM allowed us to obtain analogue **5** incorporating a (*R*)-TFM. We initially considered the synthesis of analogue **4** by coupling the isoval-TFM building block to the Val-Sta-Ala-Sta tetrapeptide. However, during the coupling reaction, the undesired isomerization of the TFM led to a mixture (50/50) of diastereomers **4** and **5**. The two diastereomers could be separated, characterized

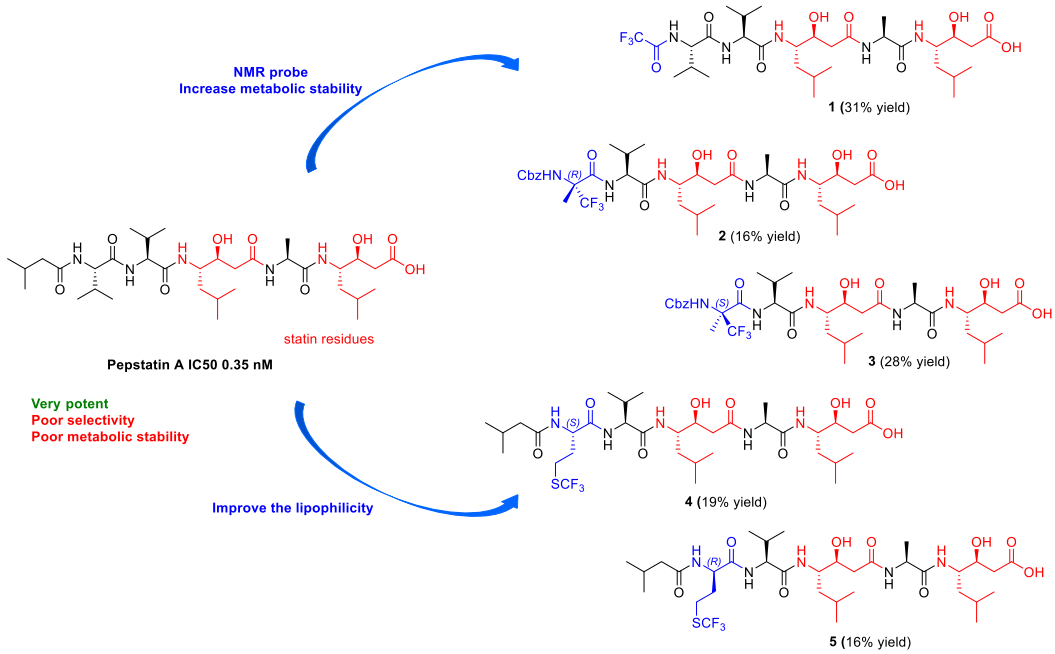
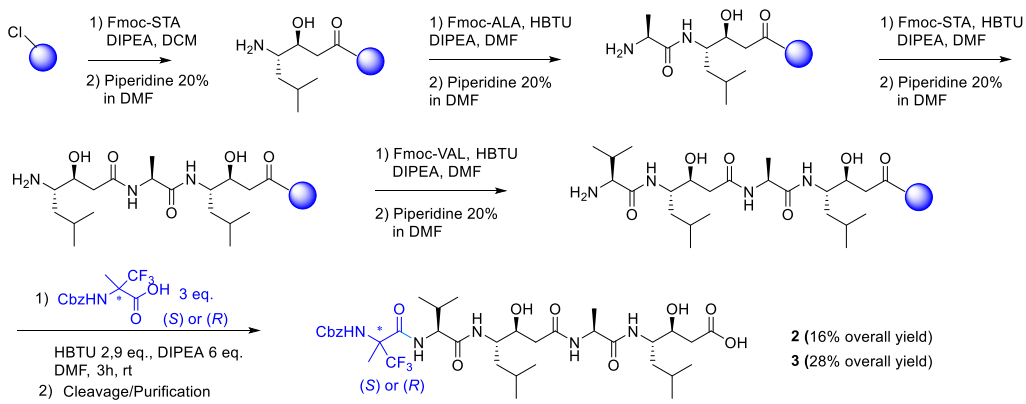


Fig. 1. Structure of the fluorinated analogues of Pepstatin A.

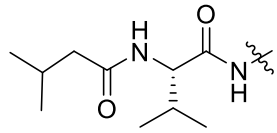
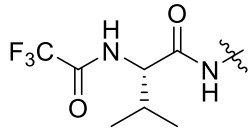
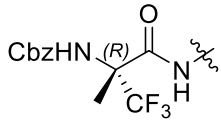
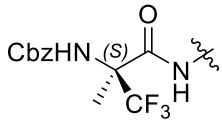
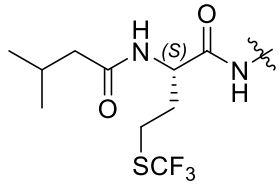
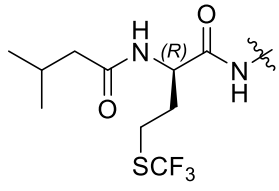
and tested. In order to avoid this epimerization and to be able to identify each diastereomer, we decided to couple the (*S*) Fmoc-protected TFM on the Val-Sta-Ala-Sta tetrapeptide, and then to carry out an acylation of the *N*-terminus by isovaleric chloride. The compound **4** has been cleaved from the resin, purified by semi-prep HPLC, and obtained in 19% overall yield.

Once the fluorinated analogues of Pepstatin A were synthesized, fluorescence tests were carried out in order to evaluate the inhibitory activity of these new analogues. All the tests were initially performed on Pepsin (cheaper and easier to handle) before being carried out on cathepsin D (Table 1). Surprisingly, as shown in Table 1, analogue **5** incorporating an (*R*)-isovaleryl trifluoromethionine in the *N*-terminal position instead of (*S*)-isovaleryl valine, showed a very interesting and better inhibitory activity than that of pepstatin A



*Scheme 1. Synthesis of the fluorinated analogues **2** and **3** of Pepstatin A.*

*Table 1. Inhibitory activities of **1-5** against Pepsin and Cathepsin D.*

Compound	*Structure of the N-terminus fragment*	*IC_{50} (nM) against Pepsin*	*IC_{50} (nM) against Cath D*
Pepstatin A		50	5
1		>4000	>2000
2		375	65
3		48	28
4		775	58
5		31	0.4

References

1. Dash, C., et al. *Crit. Rev. Biochem. Mol. Biol.* **38**, 89-119 (2003), http://dx.doi.org/10.1080/713609213
2. Salwiczek, M., et al. *Chem. Soc. Rev.* **41**, 2135-2171 (2012), https://doi.org/10.1039/C1CS15241F
3. Huhmann, S., et al. *Beilstein J. Org. Chem.* **13**, 2869 (2017), https://doi.org/10.3762/bjoc.13.279
4. Chen, H., et al. *Chem. Soc. Rev.* **42**, 7971-7982 (2013), https://doi.org/10.1039/C3CS60129C
5. Devillers, E., et al. *Amino Acids* **48**, 1457-1468 (2016), https://doi.org/10.1007/s00726-016-2200-9