Design of fluorinated Aib foldamers: synthesis, structural study and capacity to interact with amyloid proteins

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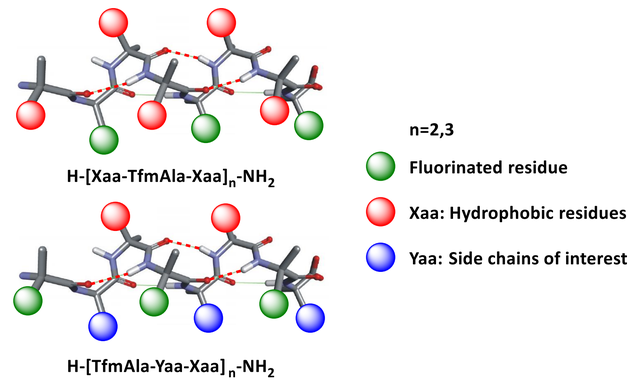
Protein misfolding and **aggregation** and their subsequent dysfunction are associated with more than 30 serious **and incurable human diseases**.[1] **Amyloid protein** aggregation occurs through misfolding and a cascade of protein-protein interactions, leading to oligomeric precursors and **fibrillar species**, both responsible for **cellular dysfunction**.
α-Helical and 3_{10} helical structures have been described for Tau, α-synuclein (α-Syn), amyloid β (Aβ) and human Islet Amyloid PolyPeptide (hIAPP) amyloid proteins and **3_{10} helices** have been proposed as probable **intermediates** in the conversion of **α-helix to β-sheet** during the **$Aβ_{1-40}$ and hIAPP amyloidogenesis**. [2]

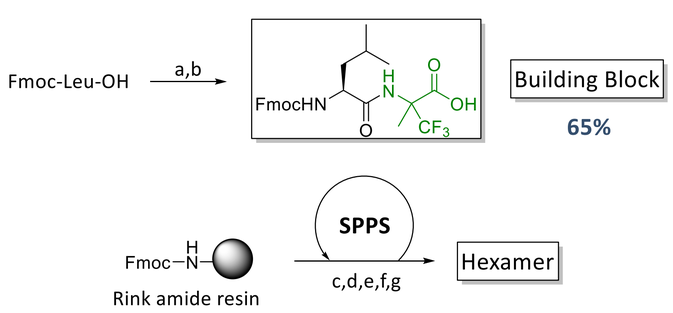
Therefore, the rational design of **peptidomimetic scaffolds** able to promote a **well-defined helical structure** and interact with monomeric or small amyloid protein oligomers to **inhibit** their aggregation constitutes a promising approach.

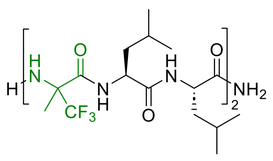
Oligopeptides containing α-aminoisobutyric acid residue (Aib) have been reported as mimics of 3_{10}, mixed 3_{10}/α, or α-helical structure.[3] Our group has recently reported the synthesis of fluorinated Aib foldamers incorporating (*R*)- and (*S*)-α-trifluoromethylalanine (α-TfmAla) and demonstrated their ability to stabilize the 3_{10} helical conformation.[4;5] The 3_{10} helix is characterized by the a 3-fold rotational symmetry with three residues per turn and therefore 3 faces.
In this work, we engineered a promising α-TfmAla-containing peptidomimetic template to interact with amyloid proteins in their helical conformation and inhibit their aggregation. Here, we will present the synthesis of a first generation of fluorinated peptides through adapted SPPS methodology, their conformational studies using NMR and CD spectroscopy as well as our preliminary results on ThT assay of amyloid protein aggregation inhibition.



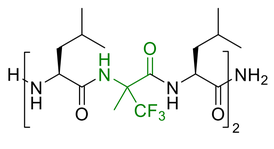
Assessing the impact of the position of TfmAla in hydrophobic hexamers

Synthesis of Leu-oligomers

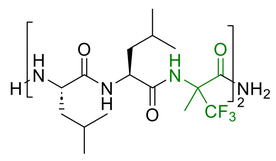




H-[TfmAla-Leu-Leu]$_2$-NH$_2$
1R 18%
1S 22%
***N*-terminal**



H-[Leu-TfmAla-Leu]$_2$-NH$_2$
2R 24%
2S 37%
Middle



H-[Leu-Leu-TfmAla]$_2$-NH$_2$
3R 15%
3S 9%
***C*-terminal**

(a) $SOCl_2$, dry DCM,))), r.t., 1 h 30; (b) H-TfmAla-OH, dry THF, 100 W, 100 °C, 20 min; (c) Fmoc-Leu-OH, HATU, DIPEA, DMF, r.t., 40 min; (d) Piperidine/DMF 20 % r.t., 5 min, 15 min; (e) Building block, PyAOP, NMM, DMF, 50 W, 75 °C, 1 h; (f) Fmoc-TfmAla-OH, PyAOP, NMM, DMF, 50 W, 75 °C, 1 h; (g) TFA:H_2O:TIS 95:2.5:2.5 1h30

Conformational studies

Intramolecular (i ← i+3) **H-bonding pattern** of 3_{10} helix structure has been confirmed by temperature coefficients (Δδ/ΔT) of -NH protons for compounds **1**-**3**. Sequential NH_i-NH_{i+1} and $H\alpha_i$-NH_{i+2} **ROE** correlations are also in support of the **3_{10} helix conformation**.

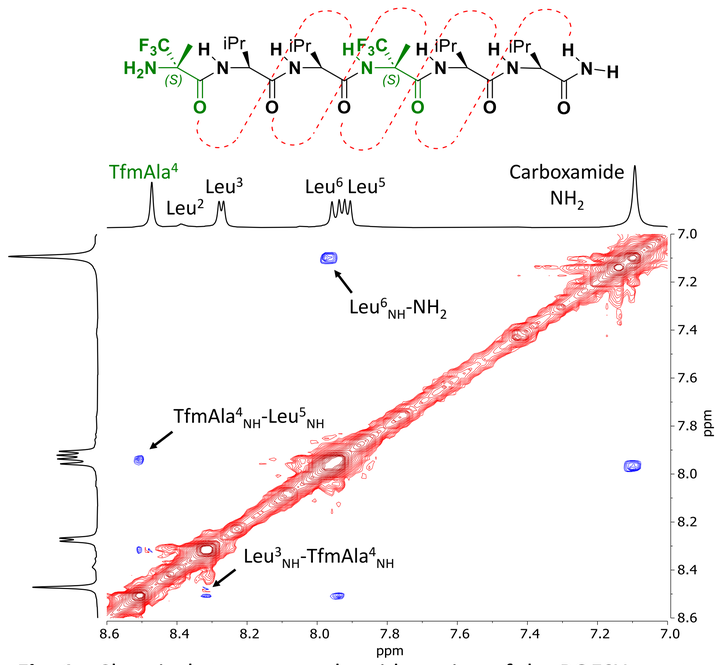


Fig. 1 – Chemical structure and amide region of the ROESY spectrum of peptide **1*S*** (5 mM CD_3OH, 20 °C).

Circular dichroism has been performed on peptides **1-3** and all spectra show the typical **3_{10} helix signature**.[6] A **negative band** around **205 nm** is characteristic of a **right-handed** helicity (*P*) while a positive band refers to a left-handed helicity (*M*).

Perturbations in the CD profile are observed as the TfmAla is moved within the repetition unit. At ***N*-terminal** position, (*S*)- and (*R*)-TfmAla configurations lead to a **difference in curve amplitude**. When placed in the **middle**, the configuration of the TfmAla has **no significant impact** on the CD signature. However, when incorporated in ***C*-terminal** position, (*S*)-TfmAla induces a **inversion of the helicity** of the peptide from a *P* to a *M* helix. Peptide **1*S***, containing the (*S*)-TfmAla in *N*-terminal position seems to have **the greatest helical content**.

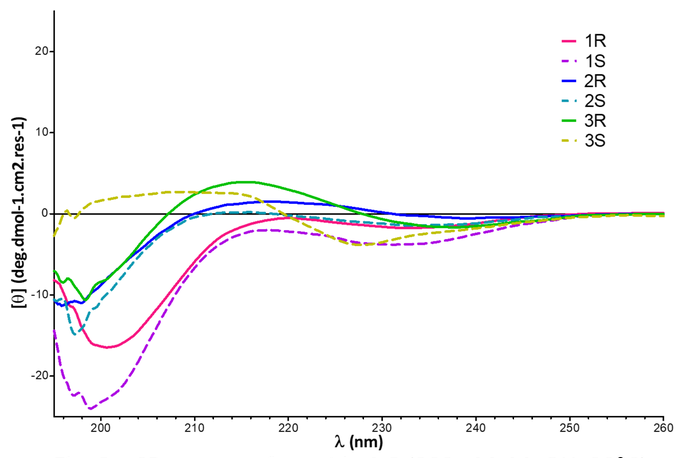


Fig. 2 – CD spectra of peptide **1-3** (300 µM, MeOH, 20°C).

Amphipathic nonamers

Design's rational

The 3_{10} helix is characterized by the a 3-fold rotational symmetry with three residues per turn and therefore 3 faces.

A rational repetition of a **triplet TfmAla-Yaa-Aib** has been designed to lead to original **nonameric foldamers** presenting a highly hydrophobic fluorinated face on the helix. The second face, carrying the Aib residues, is expected to strengthen the helicity of the foldamer. Finally, the third face exposing **selected side chain** of amino acids (Yaa) will be responsible for the **specific** and potent **interactions** (hydrophobic, aromatic or electrostatic) with an amyloid protein.

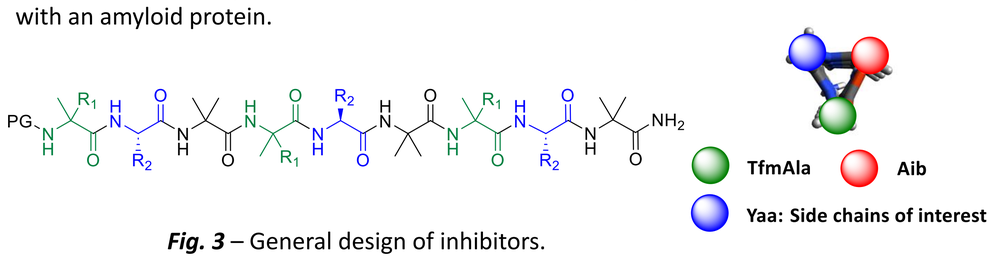


Fig. 3 – General design of inhibitors.

Series	PG	Xaa	Peptide name	Yield
Anionic	H-	(*R*)TfmAla	**4*R***	7 %
		(*S*)TfmAla	**4*S***	6 %
		Aib	**4-Aib**	32 %
	Ac-	(*R*)TfmAla	**5*R***	9 %
		(*S*)TfmAla	**5*S***	7 %
Cationic	H-	(*R*)TfmAla	**6*R***	13 %
		(*S*)TfmAla	**6*S***	12 %
		Aib	**6-Aib**	30 %
		(*R*)TfmAla	**7*R***	17 %
		(*S*)TfmAla	**7*S***	13 %
		Aib	**7-Aib**	23 %

All peptides were synthetized using previously reported SPPS method to obtain generic sequence
PG-[Xaa-Yaa-Aib]$_3$-NH$_2$
(PG = H- or Ac-; R_1 = CF_3, Xaa = TfmAla; R_1= CH_3, Xaa = Aib).

Conformational studies were carried out using both NMR and CD spectroscopy and revealed a **3_{10} helix** or a **mixed α/3_{10}** helix structure for all nonamers.

Interaction with amyloid protein

Thioflavin T (ThT) dye fluorescence is commonly used to quantify the formation and inhibition of amyloid fibrils in the presence of anti-amyloidogenic compounds. When bounded to b-sheet-rich structures, such as those in amyloid aggregates, the dye displays enhanced fluorescence and a characteristic red shift of its emission spectrum.[7]

We first investigated the activity of our inhibitors towards **hIAPP** amyloid protein involved in type II diabetes.

As preliminary results, 10 nonamers were tested via ThT assays at a peptide/hIAPP ratio of 10 : 1. None of the inhibitors tested showed any fluorescence that would indicate the presence of β-sheet-like aggregates.

For both series, fluorinated peptides show slightly better activity compared to their non fluorinated analogs. In the case of anionic series, the configuration of TfmAla does not appear to have a direct influence on the inhibition while it does for the cationic series.

Our **preliminary results** show a **reduction** of hIAPP **fibrils** by our inhibitors **between 40 % and 80 %**.

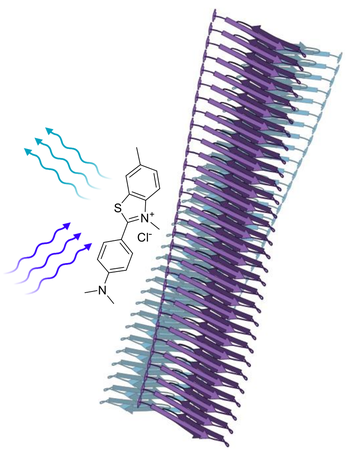


Fig. 4 – ThT binding on amyloid fibrils.

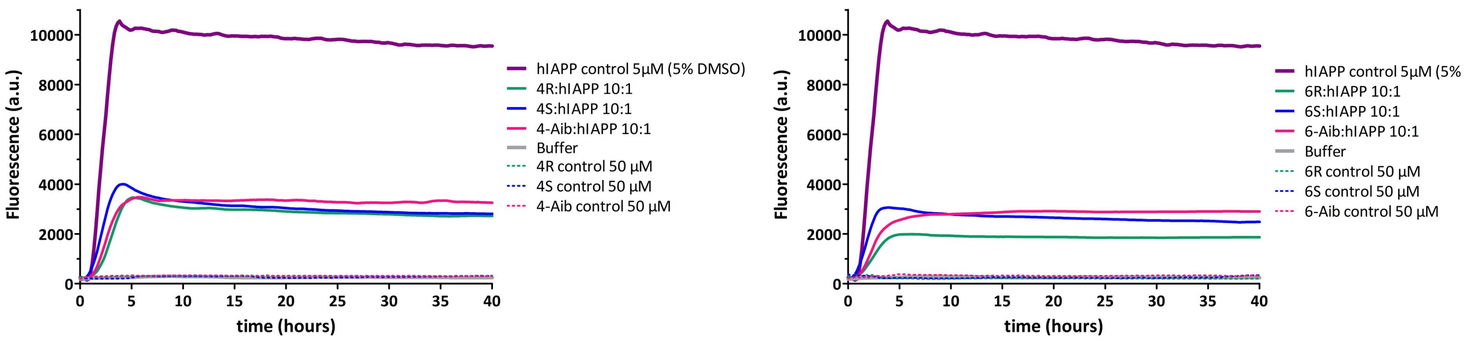


Fig. 5 – Preliminary results of ThT assays on hIAPP fibrillation (Buffer 10 mM Tris/HCl, 100 mM NaCl, pH 7.4). Triplicate average.

Conclusion

- ❑ The introduction of (*R*)- and (*S*)-TfmAla into hydrophobic hexamer using SPPS and microwave activation is a success.
- ❑ Both NMR and CD results tend to a 3_{10} helix. The peptide's conformation is impacted by both the configuration and the position of the fluorinated amino acid.
- ❑ A first generation of nonameric amphipathic inhibitors has been synthetised using the previously optimised SPPS method.
- ❑ The ThT assays evaluating their activity towards hIAPP are promising and other tests will be carried out at different ratios. Inhibition of other amyloid targets will also be tested through these assays.
- ❑ Investigation needs to be done towards the different side chains of Yaa to evaluate the impact regarding the interaction with different amyloid protein.

References

[1] Ke, P. C. et al. *Chem. Soc. Rev.* **2017**, *46* (21), 6492–6531.
[2] Bram, Y. et al. Sci. Rep. 2017, 7, 14031
[3] Jones, J. E. et al *J. Am. Chem. Soc.* **2016**, *138* (2), 688–695.
[4] Bodero, L. et al. *Chem. Eur. J.* **2022**, e202103887.
[5] Picois, N. et al. *Chem. Eur. J.* **2024**, e202400540.
[6] Toniolo, C. et al. *J. Am. Chem. Soc.* **1996**, *118*, 2744–2745.
[7] Khurana, R. et al. *J. Struct. Biol.* **2005**, *151*, 229–238.

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