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Design and Structural Analysis of Fluorinated Polyproline-Type Foldamers and their Ability to Interact with Membrane Models

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INTRODUCTION

Foldamers are oligomers with a strong tendency to fold into a well-defined secondary structure.[1] Among peptidic foldamers, oligomers of prolines are known to adopt a **polyproline type II helix (PPII)** in water where amide bonds are **all-*trans*** and a more compact helix (PPI) in organic solvent with all-*cis* amide bonds (fig. 1).

In this work, we report the incorporation of the trifluoromethyl pseudoproline (**CF_3ΨPro**) into a polyproline backbone and the **structural analysis** of the resulting oligomers. Then, in order to increase the interaction with membranes, **amphipathic oligomers** are synthesized with the addition of a guanidyl group on prolines.

all-*trans* PolyProline II helix
Extended left-handed helix
(φ = -75°, ψ = 146°, ω = 180°)
Favored in water

High energy of interconversion ⇌

all-*cis* PolyProline I helix
Compact right-handed helix
(φ = -75°, ψ = 160°, ω = 0°)
Favored in organic solvent (*n*-propanol)

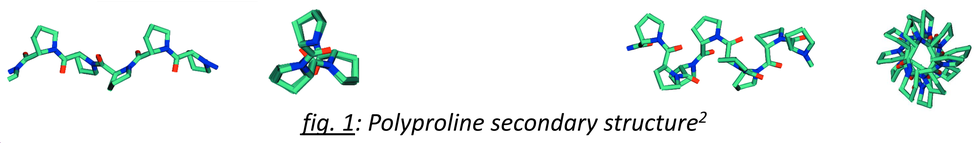
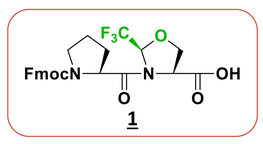
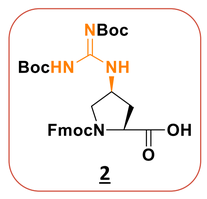


fig. 1: Polyproline secondary structure[2]

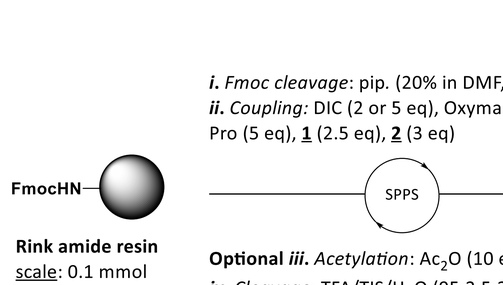
SYNTHESIS



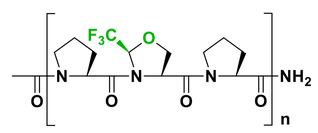


As the CF_3 group decreases the nucleophilicity of the amine, the coupling of the **CF_3ΨPro** is performed in solution to access the ready-to-use **building block 1** for SPPS.[3]

The **guanidylated proline 2** is synthesized as reported.[4]

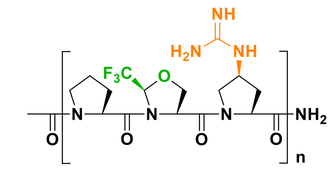


Hydrophobic series



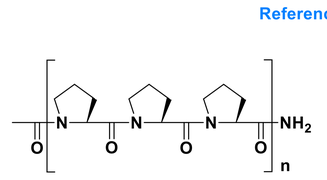
n = 1: 3a, 30%
n = 2: 3b, 31%
n = 3: 3c, 45%

Amphipathic series

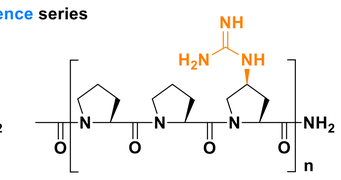


n = 1: 4a, 45%
n = 2: 4b, 40%
n = 3: 4c, 26%

Reference series



n = 1: 5a, 71%
n = 2: 5b, 37%
n = 3: 5c, 25%



n = 1: 6a, 14%
n = 2: 6b, 25%
n = 3: 6c, 34%

Polyproline oligomers are obtained in good yield
(MW activation is used for oligomers 3 and 5)
(Oligomers 4 and 6 are TFA salt)

NMR ANALYSIS

- Determination of the rotamer populations (**^{19}F NMR**).
- Full characterization of fluorinated oligomers (**^{1}H, ^{13}C and 2D NMR)**.
- Assignment of amide bond conformation (**NOESY and ROESY NMR**, fig. 2 and 3).

	n = 1	n = 2	n = 3
3	All-*trans* (>95%)	All-*trans* (>95%)	All-*trans* (>95%)
4	All-*trans* (>95%)	All-*trans* (>95%)	All-*trans* (>95%)

fig. 2: Amide bond ratio of synthetized fluorinated oligomers determined in D_2O at 20°C

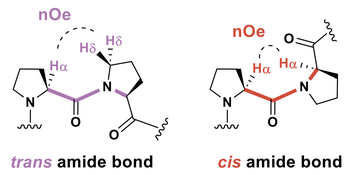


fig. 3: Assignment of amide bonds with nOe effect

all-*trans* amide bond conformation is strongly favored as expected for PPII helix

CIRCULAR DICHROISM (CD) ANALYSIS

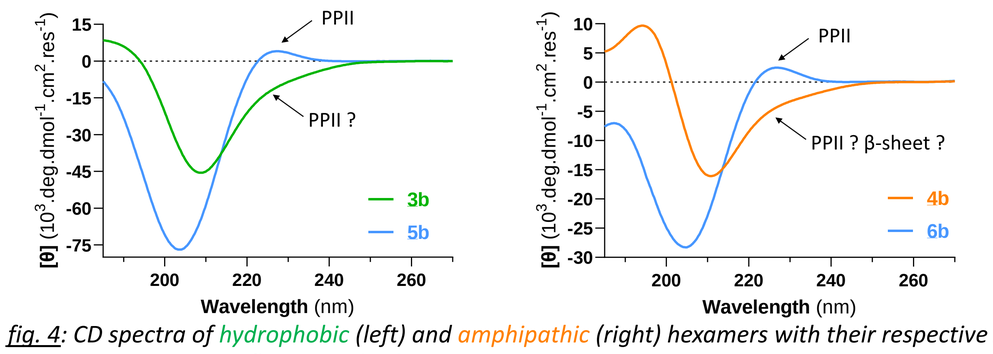


fig. 4: CD spectra of hydrophobic (left) and amphipathic (right) hexamers with their respective non fluorinated reference (100 µM in PBS 20 mM (pH = 7.0) at 4°C)

CD spectra of fluorinated oligomers do not reveal typical PPII signature (absence of positive band at 226 nm)

PPII CD SIGNATURE CONCEPT QUESTIONED FOR OLIGOMERS 3

Impact of the solvant on the CD signature

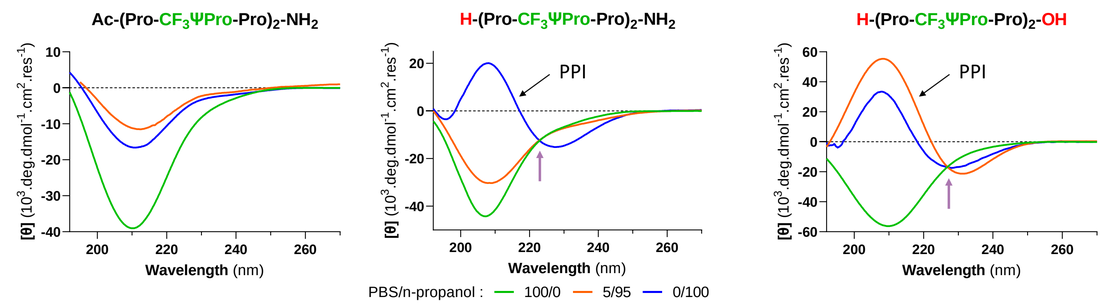


fig. 5: Impact of the n-propanol depending on the termini (100 µM at 4°C); ⟶ : isobestic point

By increasing *n*-propanol content, **PPI signature is observed** with charged termini (*N*-term: H, *C*-term: OH). Neutral termini (*N*-term: Ac, *C*-term: NH_2) are known to stabilize PPII helix.[5] Moreover, the presence of **isobestic points** reveal that **only two conformations** are involved in this equilibrium: **PPI ⇌ PPII**.[6]

*X-ray structure of **3c***

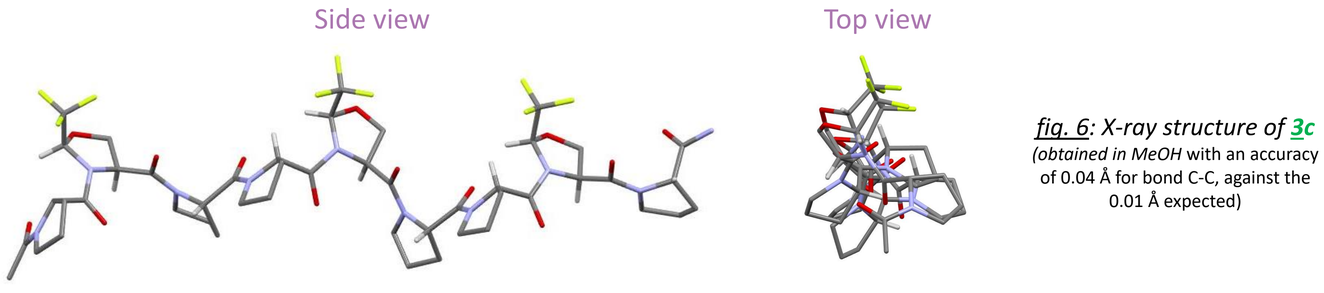


fig. 6: X-ray structure of 3c (obtained in MeOH with an accuracy of 0.04 Å for bond C-C, against the 0.01 Å expected)

X-ray structure of **3c** reveals a **left handed helix** with **slightly distorted C_3 symmetry**.

Conclusion

We have assembled a set of CD and X-ray diffraction data consistent with hydrophobic fluorinated oligomers 3 adopting a helical structure close to a PPII helix

INTERACTION WITH MEMBRANE MODELS MONITORED BY ^{19}F NMR (LEFT) AND DSC (RIGHT)

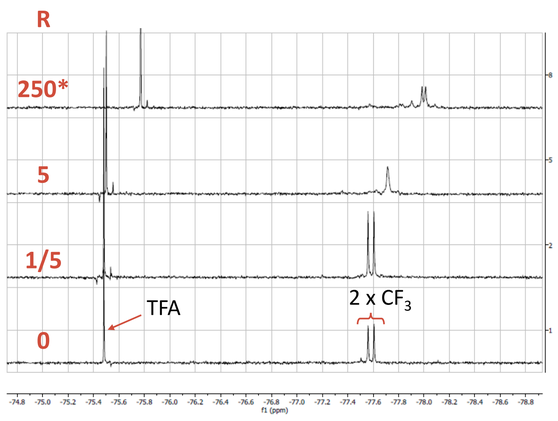


*fig. 7: Interaction with SDS micelles monitored by ^{19}F NMR of 4b with different concentration of SDS (foldamer as TFA salt at 200 µM in H_2O/D_2O (90/10), *: above CMC, R = [SDS]/[foldamer])*

As **^{19}F is very sensitive** to its environment, we assume that the chemical shift displacement is due to **interaction with micelles**. This is confirmed by DOSY ^{1}H-^{19}F assay.

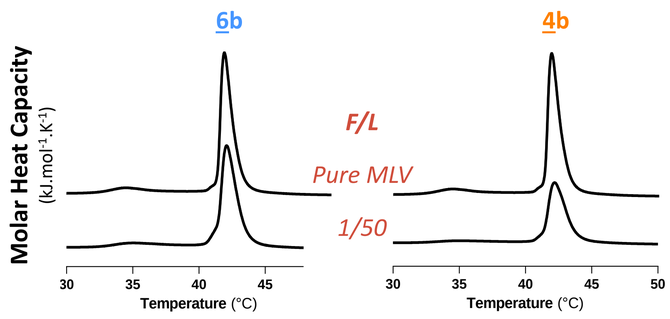


fig. 8: Interaction of hexamers 4b and 6b with Multi-Lamellar Vesicles, MLV, of DPPG (anionic lipid) monitored by DSC (1 mg/mL MLV, F/L: molar ratio of foldamers on lipids)

DSC experiments reveal that **foldamers 6b and 4b disrupt MLV thermal transitions**, with the decrease of the area under the curve, providing evidence of an **interaction**. A **more significant effect of the fluorinated foldamer** can be highlighted.

Foldamer 4b interacts with membrane models, SDS micelles and DPPG MLV

CONCLUSION

- X-ray crystallography, CD and NMR spectroscopies are consistent with **3 adopting a helical secondary structure close to PPII**. The presence of the **CF_3 group** in δ position of the oxazolidine ring **may cause steric clashes** that can partially (or locally) disrupt PPII helix structure.
- Concerning the amphipathic series **4**, more **experiments** have to be done **to decipher the secondary structure**, between **PPII helix** or **β-sheet**.
- The introduction of cationic groups on our fluorinated oligomers leads to **foldamers able to interact with membrane models**. Moreover, **^{19}F NMR** revealed to be a **useful tool** to investigate these **interactions**.

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Acknowledgements

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