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Nathan Picois, Lizeth Boderó, Pierre T Milbeo, Thierry Brigaud, Grégory Chaume. Bimodal use of chiral  $\alpha$ -Trifluoromethylalanine in Aib Foldamers: study of the position impact towards the helical screw-sense preference. *Chemistry - A European Journal*, In press, 10.1002/chem.202400540 . hal-04516499

**HAL Id: hal-04516499**

**<https://hal.science/hal-04516499>**

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 **Chemistry  
Europe**  
European Chemical  
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## Accepted Article

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This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

**To be cited as:** *Chem. Eur. J.* **2024**, e202400540

**Link to VoR:** <https://doi.org/10.1002/chem.202400540>

## RESEARCH ARTICLE

# Bimodal use of chiral $\alpha$ -Trifluoromethylalanine in Aib Foldamers: study of the position impact towards the helical screw-sense preference

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**Abstract:** Oligomers of the achiral  $\alpha$ -aminoisobutyric acid (Aib) adopt a  $3_{10}$  helical conformation in which the screw-sense preference can be controlled by a single chiral residue. The use of the fluorinated residue  $\alpha$ -Trifluoromethylalanine ( $\alpha$ -TfmAla) revealed a unique way to both induce and measure the screw-sense preference of such oligomers acting as  $^{19}\text{F}$  NMR probe. This work proposes a systematic study of the effect of this fluorinated chiral inducer on the helical screw-sense preference of poly-Aib oligomers. The impact of the position of the fluorinated residue into pentamers ( $N$ -terminal, central or  $C$ -terminal) as well as the nature of the  $C$ -terminal capping of the peptides was thoroughly studied in light of complete structural analysis. A deeper understanding of the fluorine effect was achieved confirming the unique ability of  $\alpha$ -TfmAla as a helical screw-sense controller.

## Introduction

During the last two decades, considerable attention has been devoted to oligomers of  $\alpha$ -aminoisobutyric acid (Aib) for their ability to stabilize  $3_{10}$  helical structures.<sup>1,2</sup> While such achiral Aib oligomers display an equal population of right-handed ( $P$ ) and left-handed ( $M$ ) helices, it is possible to alter this equilibrium and to promote a screw-sense preference by introducing a chiral residue (controller) along the peptide chains.<sup>2-4</sup> The nature of the chiral residue and its location along the peptide chain were found to play a key role in providing a screw-sense preference. When introduced at the  $N$ -terminus of achiral Aib oligomers, tertiary L-amino acids favour the left-handed ( $M$ ) helix while  $C^\alpha$ -methylated L-amino acids (quaternary amino acids) promote the opposite conformation regardless the  $C$ -terminus moiety (ester or amide).<sup>5</sup> A different trend is observed when the chiral residue is attached at the  $C$ -terminus. In the amide series, a right-handed ( $P$ ) helix is preferred with both tertiary and quaternary L-amino acids. Conversely, the presence of an ester, in particular the largest ones such as the  $-O\text{tBu}$  group, at the  $C$ -terminus gives rise to the destabilising Schellman motif,

favouring the left-handed ( $M$ ) helix.<sup>6,7</sup> The introduction of the chiral residue into Aib oligomers was found to provide higher screw-sense preference when it is located on the  $N$ -terminus. However, very few examples bearing the chiral residue in between two achiral Aib domains were reported.<sup>5</sup>

The assignment of the screw-sense preference relies on circular dichroism (CD) spectroscopy<sup>8,9</sup> and X-ray crystallography. The quantification of the screw-sense preference can be achieved by the incorporation of a reporter as NMR probe in the peptide chain. Different achiral  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{19}\text{F}$  NMR probes have been used to assess the values of the screw-sense preference.<sup>10-12</sup> These reporters do not alter the screw-sense preference and exhibit suitable ratio of anisochronicity at fast and slow exchange regime. Compared to achiral reporter, the use of chiral NMR probes enables the simultaneous assignment of the absolute screw-sense preference by observing the predominant signal position in the NMR spectra.<sup>13-16</sup> For instance, the enantiomerically enriched  $^{13}\text{C}$ -labelled Aib monomer proved to be useful to assess the decay of the helical excess, induced by the presence of tertiary and quaternary chiral amino acids, along the peptide chain.<sup>13,14</sup> The use of chiral fluorinated NMR probes has also been very recently reported by us<sup>15</sup> and others.<sup>16</sup> Recently, Webb et al. reported the use of the ( $R$ )-1-(trifluoromethyl)ethylamido (( $R$ )-TFEA) as  $^{19}\text{F}$  NMR chiral probe.<sup>16</sup> Despite inducing a moderate intrinsic screw-sense preference, this probe allowed to evaluate the overall helical excess induced by several chiral residues at the  $N$ -terminus of Aib-oligomers embedded in vesicle membranes.

Our group has reported the synthesis of ( $R$ )- and ( $S$ )- $\alpha$ -trifluoromethylalanine (TfmAla) in multi-gram scale and its incorporation into peptides.<sup>17,18</sup> This chiral fluorinated analogue of Aib residue proved to be useful to elucidate the conformation and membrane alignment of Harzianin HK-VI, a member of the antimicrobial peptaibol family, using  $^{19}\text{F}$  solid state NMR spectroscopy.<sup>19</sup> More recently, we reported the use of  $\alpha$ -TfmAla in Aib-oligomers showing that the introduction of the ( $R$ )-TfmAla at the  $N$ -terminal position of Aib pentamers induced a left-handed  $3_{10}$  helix.<sup>15</sup> Interestingly, the screw-sense preference was

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reversed compared to that of a non-fluorinated analogue having an L- $\alpha$ -methylvaline residue, emphasizing the role of the electronic properties of the CF<sub>3</sub> group. More importantly, we also demonstrated that the  $\alpha$ -TfmAla may act not only as a chiral controller but also as a <sup>19</sup>F NMR probe allowing the determination of both the magnitude and the sign of the screw-sense preference. In this work, we consolidate the use of the chiral (*R*)-TfmAla as a bimodal moiety, serving both as inducer and reporter of the helical screw-sense preference in Aib oligomers. The effect of its position along the peptide chain of pentamers (*N*-terminus, central and *C*-terminus) was investigated. A specific focus is given to studying the impact of the *C*-terminal function (amide or ester). Circular dichroism (CD), NMR studies as well as X-ray crystallography were used to unambiguously determine the structuration of these new oligomers and <sup>19</sup>F NMR experiments allowed the determination of the magnitude of the helical screw-sense preference for all the foldamers.

## Results and Discussion

## Design and synthesis of Aib oligomers

Two series of Aib pentamers incorporating a single (*R*)-TfmAla moiety respectively at the *N*-terminus, in the central and at the *C*-terminal position have been designed (Figure 1). They differ at their *C*-terminus with a *tert*-butyl ester (-O*t*Bu) or an amide (-NH*t*Bu), respectively since it has been shown that the presence of the Schellman motif ester may induce a reversal of the helical screw-sense preference.<sup>6,7,20</sup> A Cbz protecting group was introduced at the *N*-terminus as it was reported to maximize the control of the helical preference by quaternary amino acids.<sup>10</sup>

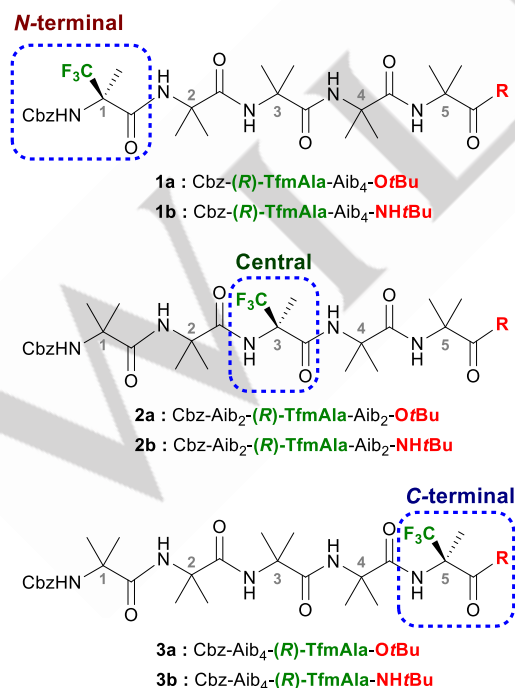


Figure 1. Chemical structure of the targeted fluorinated foldamers 1-3.

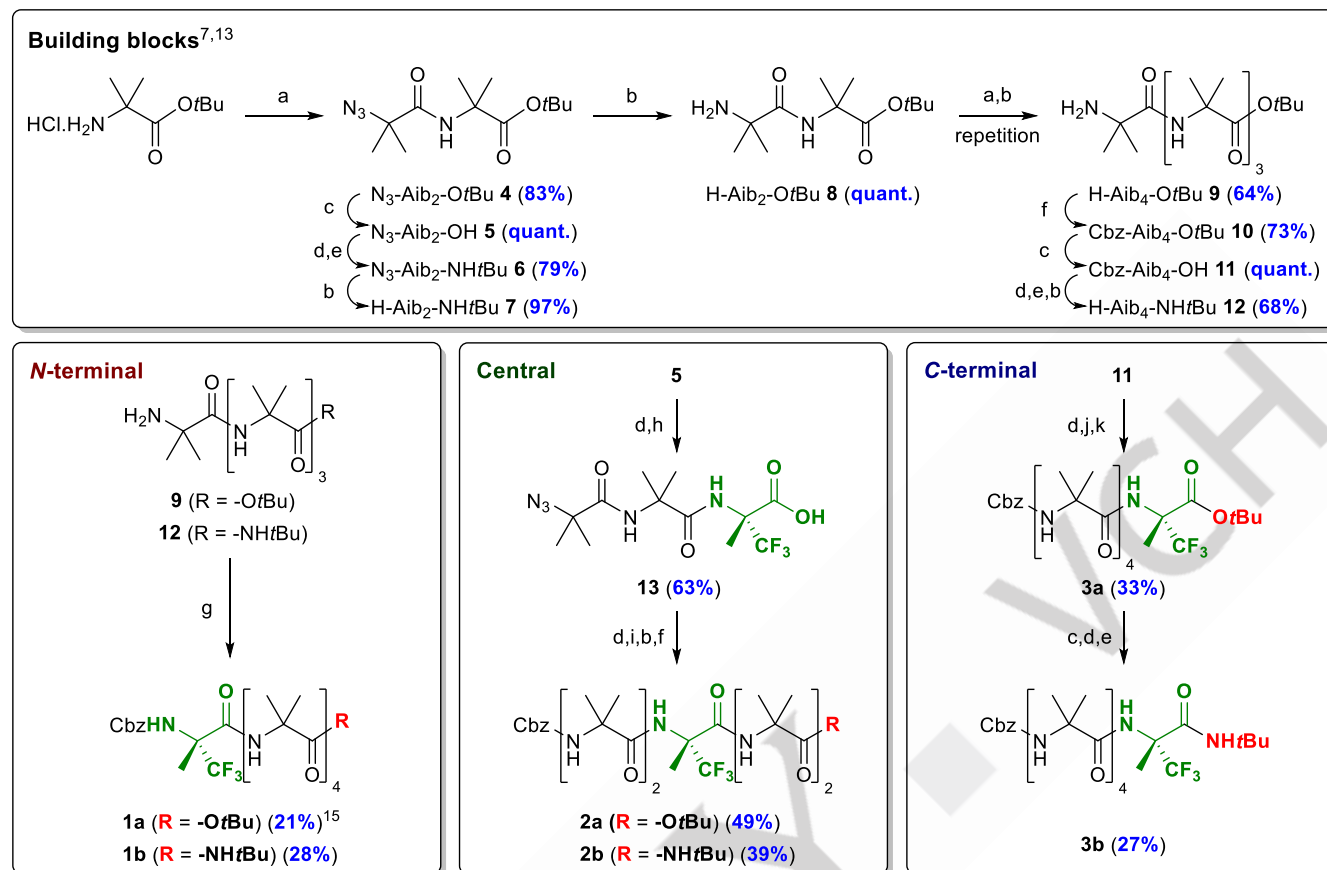
Oligomers 1–3 were synthesized as indicated in Scheme 1. The building blocks were prepared *via* iterative coupling of N<sub>3</sub>-Aib-Cl and reduction of the *N*-terminal azide functional group.<sup>7,13</sup> The synthesis of the *N*-terminal labelled oligomer 1a was performed according to our previously reported procedure by coupling the Cbz protected (*R*)-TfmAla with the Aib tetramer precursor 9 under typical EDC/HOBt conditions in solution.<sup>15</sup>

To access the oligomer 1b, the *tert*-butyl ester 9 was first converted into its amide analogue 12 in few steps and was then coupled with the Cbz-(*R*)-TfmAla-OH in 28% yield. The introduction of the chiral fluorinated amino acid in the central position of the peptide chain was performed starting from dipeptide 5. After its activation using thionyl chloride under sonication, the corresponding acyl chloride was coupled to the unprotected (*R*)-TfmAla-OH under microwave irradiation to give the tripeptide 13 in a good yield. The peptide chain was further elongated with the dipeptide H-Aib<sub>2</sub>-R (R = -O*t*Bu 8 or -NH*t*Bu 7) using acyl chloride activation to afford the two corresponding azide-protected pentamers. Subsequent reduction and protection of the *N*-terminus with a Cbz moiety afforded both compounds 2a and 2b in good yields over 4 steps. The *C*-terminal labelled oligomers 3 were obtained starting from the Cbz-protected tetramer 11. Coupling of the (*R*)-TfmAla was followed by *tert*-butylation of the *C*-terminus moiety to facilitate the isolation of the fluorinated pentamer 3a. After removal of the *tert*-butyl protecting group of 3a and activation as an acyl chloride, the amidation in neat *tert*-butyl amine provided the pentapeptide 3b in moderate yield.

## Structural characterization by NMR analysis

The full characterization of foldamers 1–3 was achieved by NMR using 2D NMR experiments (e.g., HSQC, HMBC, ROESY) in methanol-*d*<sub>3</sub>, a solvent in which the NMR spectra show no concentration-dependence effects (see Supporting Information). The intramolecular H-bond arrangement of the fluorinated peptides 1–3 was determined by measuring the temperature coefficients ( $\Delta\delta/\Delta T$ ) of NH chemical shifts (see Supporting Information). In the *tert*-butyl ester series, compounds 1–3a exhibited  $\Delta\delta/\Delta T$  values greater than -4.6 ppb/K for the NH of residues in position 3, 4 and 5, supporting the existence of intramolecular H-bonds.<sup>21</sup> No perturbation of the intramolecular hydrogen-bonding due to the Schellman motif *tert*-butyl ester at the *C*-terminus was observed.<sup>7</sup> As expected, oligomers of the *tert*-butyl amide series 1-3b displayed an additional intramolecular H-bond due to the presence of the *C*-terminal amide H-bond donor. These results highlight a *i* → *i* + 3 hydrogen bonding pattern consistent with a 3<sub>10</sub> helical structure.<sup>22</sup> Due to the electron withdrawing effects of the CF<sub>3</sub> group, we anticipated that the vicinal N-H amide of the  $\alpha$ -TfmAla displays a stronger H-bond donating effect. This was confirmed when the  $\alpha$ -TfmAla residue was positioned in the central position of the peptide sequence (oligomers 2a-b), with an increase of approximately 1 ppb/K of the  $\Delta\delta/\Delta T$  values of the  $\alpha$ -TfmAla compared to those of Aib<sub>4</sub> and Aib<sub>5</sub> (see Supporting Information). Interestingly, when positioning the  $\alpha$ -TfmAla at the *C*-terminus of the amide series, a drop in the coefficient value of the  $\alpha$ -TfmAla residue was observed, especially compared to neighbouring Aib residues (1-1.5 ppb/K

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**Scheme 1.** Synthesis of oligomers 1-3: (a)  $N_3$ -Aib-Cl,  $Et_3N$ , dry DCM, 0 °C to rt, overnight; (b)  $H_2$  (1 bar), Pd/C (10% w/w), MeOH, rt, overnight; (c) TFA/DCM 1:5, rt, 90 min; (d)  $SOCl_2$ , dry DCM, sonication, rt, 90 min; (e) *tert*-BuNH<sub>2</sub>, rt, 1 h; (f) Cbz-Cl,  $K_2CO_3$ ,  $H_2O$ /dioxane, 0 °C to rt, overnight; (g) Cbz-(*R*)-TfmAla-OH, EDC.HCl, HOBT, DIPEA, dry DMF, 0 °C to rt, 64 h; (h) (*R*)-TfmAla-OH, dry THF, 100 °C, 100 W, 20 min; (i) **7** or **8**, dry THF, rt to 100 °C, 90 min, sealed tube; (j) (*R*)-TfmAla-OH, dry THF, rt to 100 °C, overnight, sealed tube; (k) *tert*-Butyl 2,2,2-trichloroacetimidate (TBTA), dry DCM, rt to reflux, 4 h.

lower), while these coefficients were almost identical in the ester series. Although this lower coefficient still confirms the presence of an intramolecular H-bond, it reveals that the C-terminal amide has a significant impact in the H-bond network of the oligomers. Additionally, ROESY NMR experiments on oligomers 1-3 (see Supporting Information) carried out in methanol-*d*<sub>3</sub> supported the presence of stable  $3_{10}$  helical conformation by displaying cross-peaks between adjacent NH(i)/NH(i+1) together with the absence of ROE correlations between NH protons further apart in the helix.<sup>23</sup> Therefore, the incorporation of a  $\alpha$ -TfmAla residue within Aib oligomers does not hamper the adoption of a  $3_{10}$  helical structure.

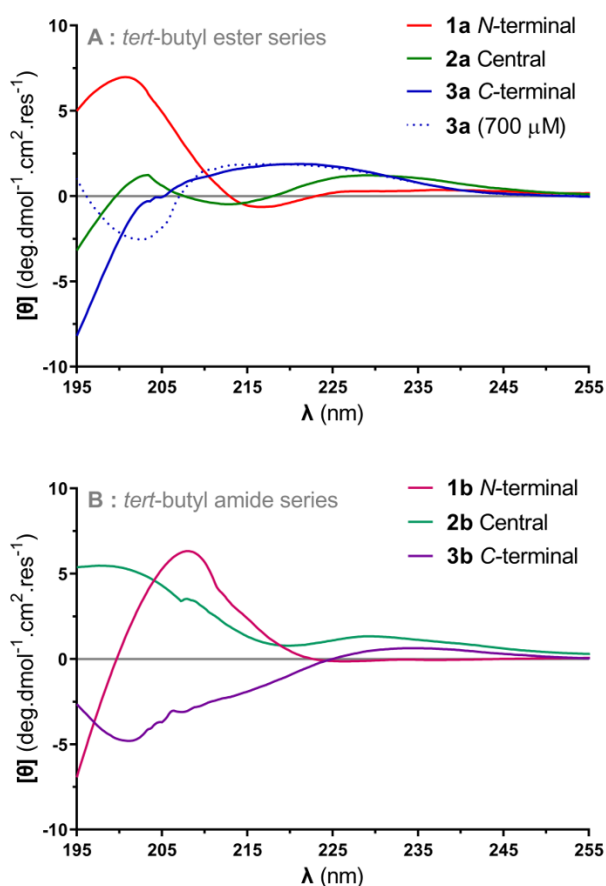
### Assignment of the screw-sense preference and helical excess

Circular dichroism (CD) spectra of 1-3 exhibited a major band around 205 nm accompanied in some cases by a weaker shoulder centered near 222 nm (Figure 2). These features are consistent with a  $3_{10}$  helical conformation.<sup>14,24</sup> The assignment of the absolute screw-sense preference was provided by the sign of the major band (negative in a right-handed (*P*) helix and positive in a left-handed (*M*) helix).<sup>24</sup> Oligomers 1 displayed a positive band at 205 nm regardless the nature of the C-capping (*tert*-butyl

ester **1a** or *tert*-butyl amide **1b**) which is characteristic of the left-handed  $3_{10}$  helix (*M*) (Table 1, entries 1 and 2). It is noteworthy that oligomers **1a** and **1b** exhibit similar magnitude of ellipticity around 205 nm. This result agrees with our NMR data and confirms that the presence of a Schellman motif in **1a** does not destabilize the  $3_{10}$  helical structure compared to its amide analogue **1b** that display an additional intramolecular H-bond. We previously hypothesized on similar poly-Aib oligomers that the reversed screw-sense orientation of fluorinated oligomers bearing a (*R*)-TfmAla at the N-terminal position compared to their non fluorinated counterpart (with L- $\alpha$ -MeVal) could be attributed to the electronic effects conferred by the CF<sub>3</sub> group (Table 1, entries 3 and 4).<sup>15</sup> Oligomers **2** bearing the chiral fluorinated residue in the central position of the peptide chain also displayed the CD signature of the left-handed  $3_{10}$  helix (*M*) regardless the ester or amide functional group at the C-terminus (Table 1, entries 5 and 6). Here again, the screw-sense preference is reversed compared to that observed with the quaternary L-amino acid chiral inducer.<sup>5</sup> It should be noticed that the oligomer **2a** (ester series) displays a significantly lower magnitude of ellipticity to that of **2b** (amide series) around 205 nm. Conversely, the introduction of the



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**Figure 2.** CD spectra of peptides 1-3 (300  $\mu\text{M}$  in MeOH, 20°C), top: ester series (a), bottom: amide series (b).

(*R*)-TfmAla at the C-terminus of **3** showed an opposite screw-sense preference (*P* helix) compared to compounds **1** and **2** (Table 1, entries 7 and 8). In the case of compound **3a**, the handedness of the helix was clarified by using a higher concentration (700  $\mu\text{M}$ , Figure 2A). The *tert*-butyl ester oligomer **3a** shows an opposite screw-sense preference to that observed with quaternary L-amino acids such as L- $\alpha$ -MeVal, as reported in the literature (Table 1, entries 7 and 9).<sup>6</sup> On the other hand, the *tert*-butyl amide oligomer **3b** shows in solution the same right-handed screw-sense than a L- $\alpha$ -MeVal containing hexameric analogue (Table 1, entries 8 and 10).<sup>6</sup>

In the ester series, it has been reported that the oligomers having a chiral inducer at the *N*- and *C*-terminal position respectively exhibit opposite screw sense preference.<sup>6</sup> However, in the amide series, the handedness of the oligomers is reported to remain identical regardless the position of the chiral inducer along the peptide chain.<sup>8</sup> In our case, the opposite screw-sense observed in the amide **3b** compared to compounds **1b** and **2b** was unexpected and suggests that the (*R*)-TfmAla chiral inducer plays a prominent role in the promotion of the helical handedness to that of the nature of the C-terminal functional group.

Taking benefit of the presence of the CF<sub>3</sub> group on the (*R*)-TfmAla residue, we quantified the magnitude of the screw sense

**Table 1.** Observed screw-sense preference in solution and in solid state.

Entry	Compound	Major screw-sense <sup>[a]</sup>	
		solution	solid state
1	Cbz-( <i>R</i> )-TfmAla-Aib <sub>4</sub> -O <i>t</i> Bu ( <b>1a</b> )	<i>M</i>	—
2	Cbz-( <i>R</i> )-TfmAla-Aib <sub>4</sub> -NH <i>t</i> Bu ( <b>1b</b> )	<i>M</i>	—
3	Cbz-( <i>R</i> )-TfmAla-Aib <sub>4</sub> -GlyNH <sub>2</sub>	<i>M</i> <sup>15</sup>	1:1 <sup>15</sup>
4	Cbz-L- $\alpha$ -MeVal-Aib <sub>4</sub> -GlyNH <sub>2</sub>	<i>P</i> <sup>10,15</sup>	—
5	Cbz-Aib <sub>2</sub> -( <i>R</i> )-TfmAla-Aib <sub>2</sub> -O <i>t</i> Bu ( <b>2a</b> )	<i>M</i>	<i>M</i>
6	Cbz-Aib <sub>2</sub> -( <i>R</i> )-TfmAla-Aib <sub>2</sub> -NH <i>t</i> Bu ( <b>2b</b> )	<i>M</i>	<i>P</i>
7	Cbz-Aib <sub>4</sub> -( <i>R</i> )-TfmAla-O <i>t</i> Bu ( <b>3a</b> )	<i>P</i>	<i>P</i>
8	Cbz-Aib <sub>4</sub> -( <i>R</i> )-TfmAla-NH <i>t</i> Bu ( <b>3b</b> )	<i>P</i>	<i>M</i>
9	Cbz-Aib <sub>5</sub> -L- $\alpha$ -MeVal-O <i>t</i> Bu	<i>M</i> <sup>6</sup>	—
10	Cbz-Aib <sub>5</sub> -L- $\alpha$ -MeVal-NH <i>t</i> Bu	<i>P</i> <sup>6</sup>	—

[a] *M*: left-handed helix, *P*: right-handed helix.

preference of oligomers **1–3** by determining the helical excess through <sup>19</sup>F NMR at very low temperature (213 K) reaching slow regime exchange (see Supporting Information). The helical excesses (h.e.<sub>obs</sub>) of oligomers **1–3** reported in Table 2 were obtained by integrating the isolated resonances<sup>15</sup> and their signs were assigned based on the CD signature.<sup>24</sup> As expected, helical excess increased from ca. 52% to ca. 84% upon repositioning the (*R*)-TfmAla in the Aib pentamers from the *C*- to the *N*-terminus respectively. The values obtained for **1** are very similar to those previously reported for fluorinated pentamer containing a C-terminal glycinamide (Gly-NH<sub>2</sub>) residue.<sup>15</sup> The introduction of the (*R*)-TfmAla in between the Aib chains resulted in intermediate helical excess values (54% and 76% for *tert*-butyl ester **2a** and amide **2b** respectively). Compared to their ester analogues **1–3a**, oligomers bearing a *tert*-butyl amide **1–3b** displayed higher observed helical excess. This can be explained by the presence of an additional intramolecular H-bond expected to increase the stability of the helical conformation. Nevertheless, while oligomers **1** and **3** exhibited a slight difference in helical excess values between the two series (ca. 6%), oligomers **2** displayed a more significant difference (22%). This is consistent with the difference in ellipticity magnitude observed between **2a** and **2b** (Figure 2).

**Table 2.** Observed helical excess (h.e.<sub>obs</sub>) in <sup>19</sup>F NMR.

Compound	<b>1a</b>	<b>1b</b>	<b>2a</b>	<b>2b</b>	<b>3a</b>	<b>3b</b>
<sup>19</sup> F NMR reporter						
h.e. <sub>obs</sub> <sup>[a]</sup> (%)	-80	-88	-54	-76	+50	+54

[a] Calculated by integrating isolated CF<sub>3</sub> resonances (213 K, [C] = 2.5 mM, CD<sub>3</sub>OH). Helical excess (h.e.) is negative in a left-handed helix and positive in a right-handed helix.

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Non-fluorinated hexamer analogues of **3** have been reported to show a significant difference in helical excess values between the *tert*-butyl ester and its amide derivatives.<sup>6,8</sup> Apart from its sign, the inferred helical excess (h.e.)<sup>25</sup> of Cbz-Aib<sub>5</sub>-L- $\alpha$ -MeVal-OtBu (-56%) (Table 1, entry 9) is similar to that obtained for compound **3a** (+50%).<sup>6,8</sup> The helical excess of Cbz-Aib<sub>5</sub>-L- $\alpha$ -MeVal-NHtBu (+92%) (Table 1, entry 10) is significantly higher compared to **3b** (+54%).<sup>6,8</sup> The presence of an extra Aib residue in the peptide chain of the non-fluorinated Cbz-Aib<sub>5</sub>-L- $\alpha$ -MeVal-NHtBu oligomer, giving an additional intramolecular hydrogen bond, may account for the significant increase in helical excess. Another explanation could be that the introduction of the fluorinated (*R*)-TfmAla chiral inducer at the C-terminal position may exert a different behaviour both compared to other positions in the peptide chain and to the L- $\alpha$ -MeVal chiral inducer. Whatever the case, we confirmed that the (*R*)-TfmAla efficiently serves as both a chiral inducer and a highly sensitive <sup>19</sup>F NMR reporter, allowing the assessment of the degree of control exerted by this chiral fluorinated controller itself.

## Crystal structure analysis

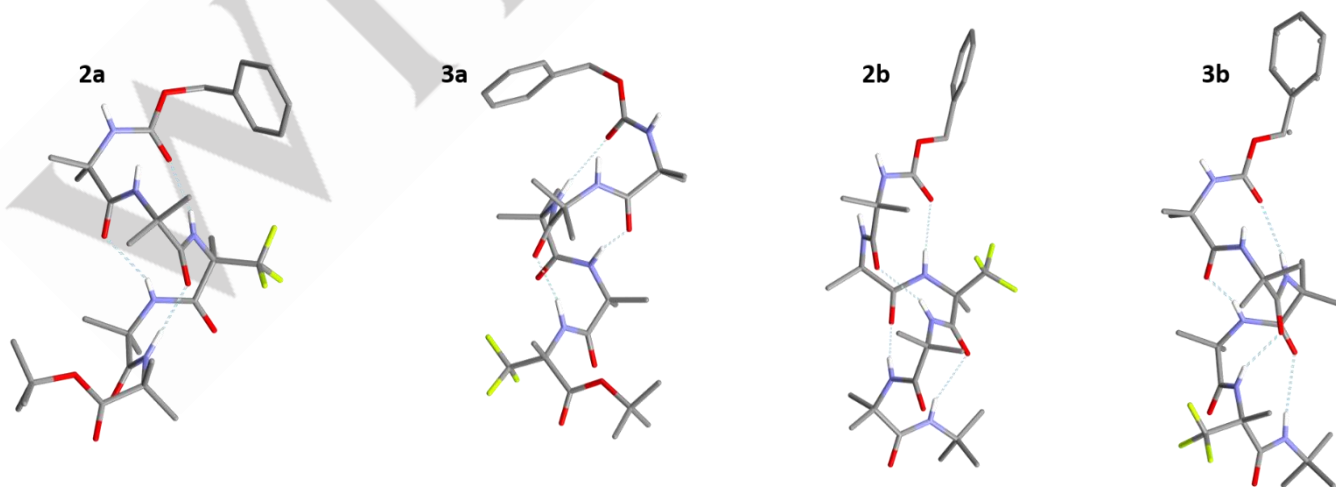
To get more insight into the specific consequences of the incorporation of (*R*)-TfmAla into oligomers **2a-b** as well as **3a-b**

and for a comparison with their non-fluorinated L- $\alpha$ -MeVal containing analogues, crystal structure analyses were performed. Recrystallization of peptides **2a**, **2b**, **3a** and **3b** from ACN gave crystals suitable for solid state structure analysis by X-ray diffraction. While in the ester series, the crystallographic structure of **2a** and **3a** showed a left- and a right-handed  $3_{10}$  helix respectively, in accordance with CD experiments, the X-ray structures of **2b** and **3b** from the amide series display an opposite screw-sense in comparison to observations in solution (Table 1, entries 6 and 8). This discrepancy has been reported in the literature for related Aib compounds.<sup>5,15,16</sup> It is worth mentioning that the X-ray structure of **2a** revealed a twinned crystal composed of two independent left-handed  $3_{10}$  helices rotated approximately 180° relative to each other. Most of the  $\phi$ ,  $\psi$  backbone torsion angle values observed for the first four residues of each foldamer slightly deviate from those typically observed for ideal  $3_{10}$  helix ( $\pm 57^\circ$ ,  $\pm 30^\circ$ ) (Table 3). Nevertheless, the structures show the presence of three and four consecutive *i*→*i*+3 C=O...H-N bonds for the ester and the amide series respectively, consistent with the  $3_{10}$  helical conformation (Figure 3). The incorporation of the (*R*)-TfmAla in the center of the peptide chain does not greatly affect the  $\phi$  and  $\psi$  torsion angles of the adjacent residues. However, the presence of a *tert*-butyl ester at the

**Table 3.** Structural parameters for foldamers **2a**, **3a**, **2b** and **3b** observed in their corresponding X-ray structure.

Residue	<b>2a</b> <sup>[a]</sup>		<b>2b</b>		<b>3a</b>		<b>3b</b>	
	$\phi$ (deg)	$\psi$ (deg)	$\phi$ (deg)	$\psi$ (deg)	$\phi$ (deg)	$\psi$ (deg)	$\phi$ (deg)	$\psi$ (deg)
Aib <sup>1</sup>	56.5/56.5	31.6/32.1	-58.4	-29.1	-62.4	-20.9	54.7	36.5
Aib <sup>2</sup>	49.7/51.5	32.3/31.4	-51.7	-33.9	-47.8	-34.8	49.8	37.4
Aib <sup>3</sup> or ( <i>R</i> )-TfmAla <sup>3</sup>	41.9/41.9	38.3/41.1	-52.5	-33.1	-51.6	-33.8	49.9	42.1
Aib <sup>4</sup>	66.2/70.1	18.0/13.2	-62.7	-23.1	-54.7	-36.7	60.7	21.9
Aib <sup>5</sup> or ( <i>R</i> )-TfmAla <sup>5</sup>	-46.9/-42.3	—	37.8	—	-64.9	-26.5	46.3	36.0

[a] Macled crystal presenting two independent helices.



**Figure 3.** Solid-state structure of **2a** in the first domain (*M* helix), **3a** (*P* helix), **2b** (*P* helix) and **3b** (*M* helix) in their corresponding X-ray structure. Some H atoms have been removed and intramolecular H-bonds are indicated as dashed (light blue).

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C-terminus induces a helix inversion in **2a** and **3a**, as shown by the sign reversal in  $\phi$  torsion angle for the last residue (Table 3). In the case of the peptide **3a**, it has previously been reported that when the chiral controller is located at the C-terminus of Aib oligomers, the inability of C-terminal esters to continue the hydrogen-bond network gives rise to a dipole repulsion leading to a local helical inversion and excluding the stereogenic center from the  $3_{10}$  helix structure.<sup>6,7</sup> In such situation, it is well established that a chiral residue (controller) induces a left-handed screw-sense by lying its bulky substituent eclipsed to its NH group.<sup>6</sup> Interestingly, the positive  $\phi$  angle in **3a** ( $\phi = 37.8^\circ$ ) place the  $\text{CF}_3$  group not eclipsed but perpendicular to its NH group ( $91.8^\circ$ ), inducing a right-handed helix as observed in solution (Figure 4A). Moreover, the  $\text{CF}_3$  group appears to be eclipsed to the  $\alpha$ -TfmAla C=O group (the torsion angle of  $\text{F}_3\text{C}-\text{C}\alpha-\text{C}=\text{O}$  is  $-9.7^\circ$ ) leading to an alignment with the overall helix macrodipole. Peptide **2a** also shows a partial dipole alignment of the  $\text{CF}_3$  and the  $\alpha$ -TfmAla C=O groups (torsion angle of  $\text{F}_3\text{C}-\text{C}\alpha-\text{C}=\text{O}$  is ca.  $-23.5^\circ$ ) which probably helps to stabilize the helical screw-sense. Such dipole alignment could explain the reversal of the helical screw-sense of **2a** and **3a** compared to that of a non-fluorinated analogue, as already observed with oligomers bearing the  $\alpha$ -TfmAla residue at the N-terminal position.<sup>15</sup> In amide series, peptide **2b** does not display such dipole alignment in solid state (torsion angle of  $\text{F}_3\text{C}-\text{C}\alpha-\text{C}=\text{O}$  is ca.  $-94.6^\circ$ ). Nevertheless, because **2b** displays reversed screw-sense in solution and in solid-state, it is difficult to conclude on the exact role of the  $\text{CF}_3$  group in stabilizing the helical

conformation when the  $\alpha$ -TfmAla is located in the central position of the peptide chain. Conversely, the X-ray structure of peptide **3b** shows a  $\text{CF}_3$  group partially eclipsed with its C=O group (the torsion angle of  $\text{F}_3\text{C}-\text{C}\alpha-\text{C}=\text{O}$  is  $-27.6^\circ$ ), inducing a left-handed helix (Figure 4B). This screw-sense is opposite to that observed in solution. The stabilization of the helical screw-sense via the non-optimal dipole alignment does not appear strong enough to be maintained in solution. Therefore, the steric effect should be the main contribution in solution, inducing a reversal of the helical screw-sense preference by placing the  $\text{CF}_3$  group perpendicular to its C=O group, as observed with non-fluorinated oligomers on amide series.<sup>6</sup> The competition between the steric and the stereo-electronic effects of the  $\text{CF}_3$  group may lead to a low energy barrier of the  $3_{10}$  helix interconversion, explaining the low helical excess observed for **3b** (Table 2). These results provide insight into the unique role imparted by the stereo-electronic properties of the  $\text{CF}_3$  group in controlling the helical conformation.

## Conclusion

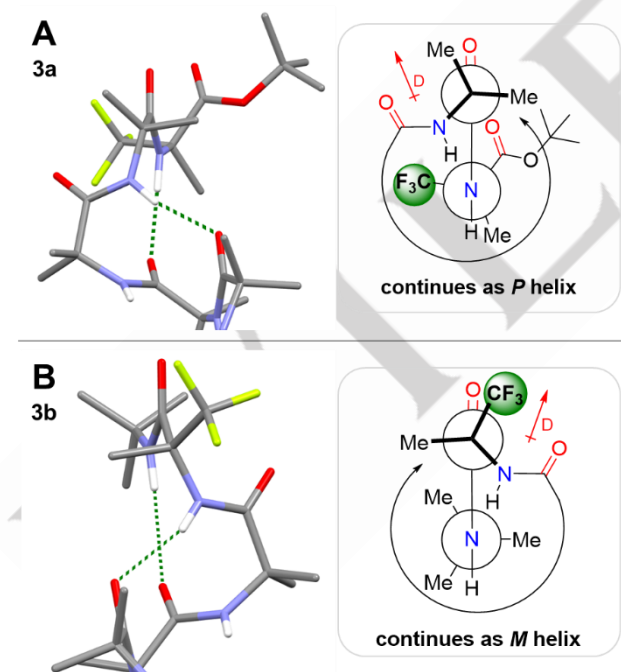
We reported the synthesis of five new short Aib-based foldamers introducing the chiral fluorinated amino acid (*R*)-TfmAla at different positions into the peptide chain. We demonstrated using NMR studies, CD spectrometry and X-ray crystallography that all foldamers exhibited  $3_{10}$  helical structures either in solution or in the solid-state. Due to the stereo-electronic properties of the  $\text{CF}_3$  group, the dipole alignment of the  $\text{CF}_3$  with the helix macrodipole seems to be the driving force enabling the fine control of the helical screw-sense preference. With the exception of peptide **3b**, all fluorinated oligomers displayed a screw-sense preference in solution of the same magnitude but reversed compared to that induced by non-fluorinated quaternary L-amino acids. Finally, we confirmed that the  $\alpha$ -TfmAla can efficiently act as a bimodal moiety, serving both as inducer and  $^{19}\text{F}$  NMR reporter. These results enable the design of new foldamers introducing multiple chiral inducers at various positions to maximize the preferred helical excess. As an extension, we are now focusing on the design of new active foldamers in interaction with various biological partners such as proteins and membrane models.

## Experimental Section

The data supporting the findings of this study are available in the Supporting Information of this article. Deposition Numbers 2256103 (for **2a**), 2256104 (for **2ab**), 2256105 (for **3a**), and 2256106 (for **3b**) contain the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures.

## Acknowledgements

The Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation is thanked for financial support of NP. Authors



**Figure 4.** Last turn conformation as observed in the X-ray crystal structure of peptides **3a** (A) and **3b** (B) showing the C-terminal ester and amide function respectively (the rest of the molecule is omitted for clarity). In frame, the Newman projection of their C-terminus viewed from the N-terminal direction to illustrate the origin of the conformational preference. Green dashed lines indicate intramolecular hydrogen bonds.



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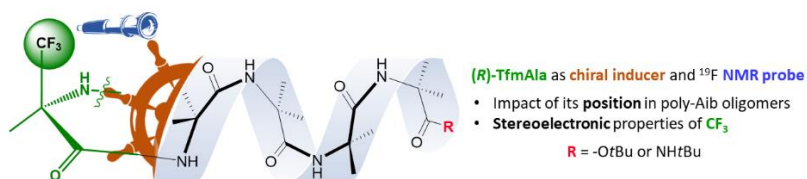
thank Dr. Pascal Retailleau (Departement of X-ray crystallography at Institut de Chimie des Substances Naturelles – UPS, CNRS-UPR2301 – Gif sur Yvette) for his help with the crystal structure solution.

**Keywords:** fluorinated peptides • foldamers • helical structures • conformational analysis • circular dichroism

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- [25] Inferred helical excess corresponds to the values that have been corrected for decay of conformational preference between the chiral inducer and the NMR spectroscopic reporter. It allows to estimate the direct control exerted by the chiral inducer, as outlined in ref. [10].

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## Entry for the Table of Contents



Introduction of a chiral  $\alpha$ -trifluoromethylalanine residue into Aib oligomers proved its effectiveness as a bimodal moiety, acting both as controller and  $^{19}\text{F}$  NMR reporter of the helical screw-sense preference. By varying its position along the chain and the nature of the C-terminal capping, our study revealed the stereoelectronic properties of the  $\text{CF}_3$  group enabling the control of the helical conformation.

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