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Control, Quantification and Assignment of Screw-Sense Preference in Helical Aib Foldamers by Introducing the Chiral Constrained α-Trifluoromethylalanine

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Introduction

Foldamers are oligomers with a strong tendency to fold into a defined conformation [1]. Among the different types of foldamers, the oligomers of α-aminoisobutyric acid (Aib) have been widely investigated for their ability to form stable 3_{10}-helices, and their similitude with natural antimicrobial peptides. Because of the achiral nature of Aib, these hydrophobic foldamers do not exhibit axial chirality and present equal population of left- and right-handed conformers. Yet, the equilibrium between the two forms may be altered by incorporating a single chiral residue at *N*- or *C*- terminus, inducing a screw-sense preference on the helical chain [2].

The use of fluorine-containing amino acids is becoming a very promising tool for the design of new bioactive molecules [3,4]. However, their use for the design of fluorinated peptide-based foldamers remains in its infancy with only a few examples reported so far [5-10].

As α-trifluoromethyl-α-amino acids (Tfm-AAs) are interesting to promote the helical secondary structures of peptides [10-12], we propose to investigate the ability of a single fluorinated chiral residue, namely the (*R*)- and (*S*)-α-trifluoromethylalanine (TfmAla) to stabilize the 3_{10}-helical conformation and to act as chiral controller to induce a screw-sense preference when incorporated at the *N*-terminus of short Aib oligomers (Figure 1). We also aim to demonstrate that (*R*)- and (*S*)-TfmAla can serve as efficient ^{19}F NMR probe to reliably determine both the magnitude of the screw-sense preference and its sign assignment. The first series of fluorinated Aib foldamers is based on the introduction of a *C*-terminal ester groups while in the second series, a glycinamide ^{1}H NMR reporter was added at the *C*-terminal position to quantify the induced screw-sense preference of the helix.

Results and Discussion

Synthesis of fluorinated Aib-oligomers

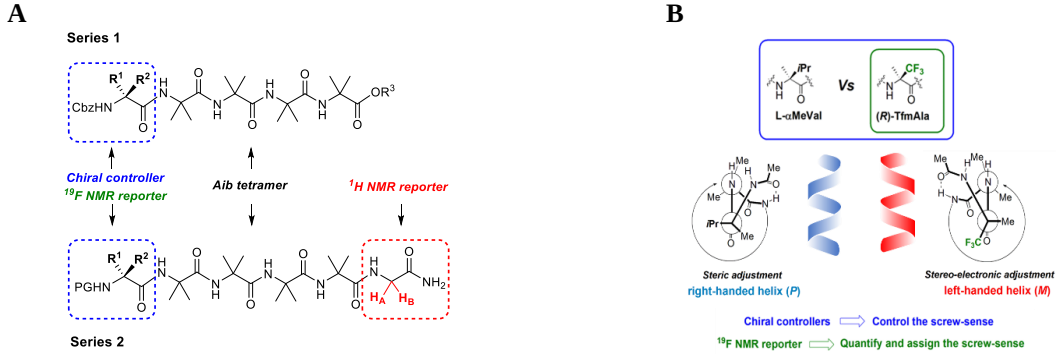
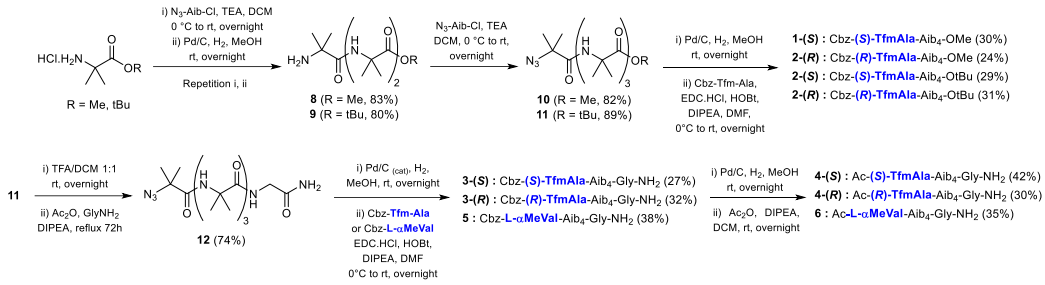


Fig. 1. ***A:*** *Chemical structure of Aib-foldamers and their non-fluorinated analogues;* ***B:*** *Influence of the stereoelectronic properties of chiral TfmAla in the control of screw-sense preference.*



*Scheme. 1. Synthesis of Aib foldamers **1-6**.*

The synthesis of oligomers **1**–**6** started with the preparation of the Aib tetramers precursors **10** and **11**, bearing respectively a methyl or a *tert*-butyl ester at the *C*-terminus, via iterative coupling of N_3-Aib-Cl and reduction of the *N*-terminal azide functional group (Scheme 1) [13]. After the azide reduction of **10** and **11**, the incorporation of the Cbz-protected (*S*)- and (*R*)-TfmAla [14,15] at the *N*-terminal position was carried out under typical EDC/HOBt coupling conditions in solution to afford the final compounds **1**–**2**. Oligomers **3** and **4**, containing a *C*-terminal glycinamide residue, were prepared upon removal of the *tert*-butyl group of **11**, activation with acetic anhydride and reaction with glycinamide hydrochloride. Azide reduction followed by coupling with Cbz-protected (*S*)- and (*R*)-TfmAla afforded the peptides **3**. The substitution of the Cbz group of **3** provided the peptides **4**. Reference compounds **5** and **6**, containing the L-α-methylvaline residue (L-α-MeVal) as chiral controller were also prepared to study the conformations obtained with fluorinated oligomers compared to non-fluorinated ones.

Conformational studies of fluorinated Aib-oligomers

Intramolecular (i → i+3) H-bonding pattern of 3_{10} helix structure has been confirmed by temperature coefficients ($\Delta\delta/\Delta T$) of NH protons for compounds **1-4**. The presence of the Schellman motif at the *C*-terminus of oligomers **1** and **2** did not perturb the intramolecular hydrogen-bonding contacts, even when using the more sterically demanding *tert*-butyl ester. Sequential NH(i)/NH(i+1) ROE correlations and the absence of NH(i)/NH(i+2) cross-peaks support the 3_{10}-helix conformation.

The CD spectra of foldamers **1-4** displayed a major band near 205 nm similar to the spectra of the reference compounds **5**–**6** and consistent with a 3_{10}-helix structure [16]. (*R*)-enantiomers of the fluorinated foldamers **1-4** induce a left-handed helicity (*M*), the mirror image of that induced by their non-fluorinated analogs **5** and **6**.

Finally, X-ray crystallography of **4-(*S*)** showed 2 conformers **4-(*S*)*M*** and **4-(*S*)*P*** in equal population exhibiting a 3_{10}-helix. The CF_3 group in **4-(*S*)*P*** is eclipsed to the C=O group leading to an alignment of their dipoles.

Quantification of screw-sense preference The helical excess (h.e.) was calculated by measuring the ratio of anisochronicity of the Gly diastereotopic protons at fast and slow exchange regime in methanol (h.e. = $\Delta\delta_{fast}/\Delta\delta_{slow}$) [17] (Figure 2A). Fluorinated foldamers **3-4** display slightly lower helical excess (ca. 10%) compared to their non-fluorinated analogues **5-6** (Table 1).

The CF_3 group can also be used as highly sensitive probe for ^{19}F NMR spectroscopy to assess the degree of control exerted by the chiral TfmAla controller itself and the variation of the conformational preference along the

Table 1. Anisochronicity of the Gly diastereotopic protons at slow and fast exchange regime.

Compound	$\Delta\delta_{fast}$ ^{293K} (ppb)[a]	$\Delta\delta_{slow}$ ^{193K} (ppb)[b]	^{1}H NMR reporter h.e.$_{obs}$[c], h.e.$_0$[d] (%)	^{19}F NMR reporter h.e.$_{obs}$[e] (%)
3-(*S*)	261	417	+62, +81	+80
3-(*R*)	264	419	–63, –83	–80
5	275	382	+72, +95 (+52, +68)[f]	
4-(*S*)	198	417	+47, +62	+66
4-(*R*)	198	417	–47, –62	–68

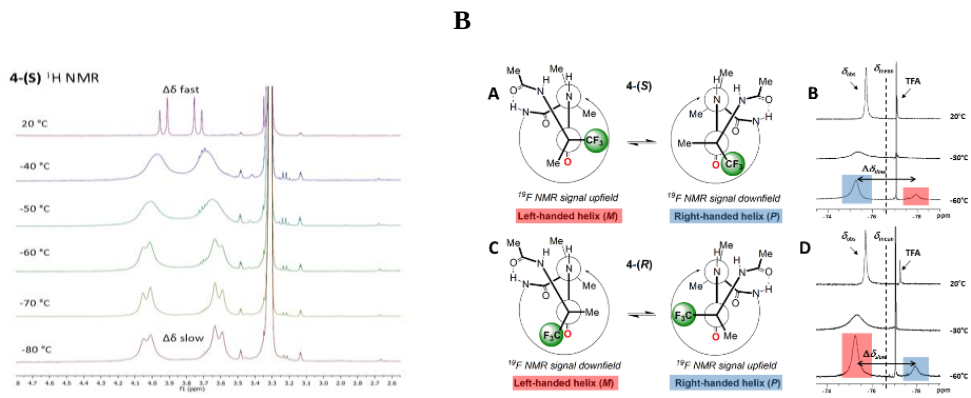


Fig. 2. ***A:*** *NMR spectra of peptides* ***4-(S)*** *showing Gly CH_2 signals and chemical shifts from -80°C to 20°C;* ***B:*** *Newman projections of the N-terminal turn of* ***4-(S)*** *and* ***4-(R)*** *in left-handed and right-handed helices and ^{19}F NMR spectra of* ***4-(S)*** *and* ***4-(R)*** *in CD_3OD. TFA: trifluoroacetic acid.*

peptide chain. Two distinct peaks corresponding to the CF_3 resonances of the (*M*)- and (*P*)-helices were observed when slow regime exchange was reached (Figure 2B). The helical excess ($h.e._{obs}$) of the fluorinated peptides **3** and **4**, obtained by integrating the isolated resonances, were found to be ca. 80% and 67% respectively, in good agreement with the inferred helical excess values ($h.e._0$). We were also able to assign the screw-sense preference by observing whether the predominant CF_3 signal appears upfield or downfield to that of the minor one.

To conclude, we report the synthesis of two series of Aib-based short fluorinated foldamers containing the (*S*)- or (*R*)-α-TfmAla as chiral controller at the *N*-terminus and an ester or glycinamide unit at the C-terminus. NMR conformational studies, circular dichroism and X-ray crystallography were consistent with a 3_{10}-helix type for fluorinated Aib oligomers, indicating a right-handed screw-sense preference for the (*S*)-enantiomers and left-handed screw-sense preference for the (*R*)-enantiomers. The selectivity of the screw-sense preference is reversed compared to that induced by the non-fluorinated L-α-MeVal chiral inducer due to the electronic properties of the CF_3 group. The CF_3 group can also be used as ^{19}F NMR probe allowing the easy determination of both the magnitude of the screw-sense preference and the assignment of its sign.

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