Note: Helix or No Helix of β-Peptides Containing β^3hAla(αF) Residues?

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A remote ^4J(F,H) coupling (F–C(α)–C(O)–N–H) of up to 4.2 Hz in α-fluoro amides with *antiperiplanar* arrangement of the C–F and the C=O bonds (dihedral angle F–C–C=O *ca.* 180°) confirms that previous NMR determinations, using the XPLOR-NIH procedure, of the secondary structures of β-peptides containing β^3hAla(αF) and β^3hAla(αF_2) residues were correct. In contrast, molecular-dynamics (MD) simulations, using the GROMOS program with the 45A3 force field, led to an incorrect conclusion about the relative stability of secondary structures of these β-peptides. The problems encountered in NMR analyses and computations of the structures of backbone-F-substituted peptides are briefly discussed.

To investigate the effect of the backbone-bound F-atom on the secondary structure of β-peptides, we had prepared compounds **1**–**4** (*Fig. 1*) and investigated their secondary structures by CD and 2D-NMR spectroscopy [1][2].

On a β-peptidic 3_{14}-helix, there are axial and lateral positions on the α- and β-carbonyl tetrahedral centers (**A** in *Fig. 2*) [3]. In this helical structure, there seems to be room only for H-atoms in the axial positions [4]. Replacement of an axial H-atom in the α-position by an F-atom (*cf.* heptapeptide **1**) should thus lead to steric hindrance and destabilization of the helix structure. On the other hand, an *antiperiplanar* (*ap*) arrangement **B** of F and C=O O-atom is present in the corresponding helix residue. This conformation **B** has been detected in numerous X-ray crystal structures of α-fluoro amides (*cf.* **E**[2]) in *Fig. 2*), and it was calculated by DFT methods to be by 7–8 kcal more stable than the *synclinal* (*sc*) conformation **C** [1][5], which would be present when a lateral H-atom is replaced by F (*cf.* heptapeptide **2** and tridecapeptide **4**). Rotation around the C(O)–C(α) bond from *sc* to *ap* of the F-substituted amino acid residue in a 3_{14}-helix of these two peptides would 'break' the helix (**C** → **D** in *Fig. 2*).

Our NMR analyses in MeOH solution provided the following conclusions [1][2]: *i*) heptapeptides **1** (axial F) and **3** (one axial, one lateral F) have a helix structure; *ii*) *heptapeptide* **2** is *not* helical, *i.e.*, the F–C–C=O *ap*-effect is stronger than the helix-folding propensity and has caused a flip into the non-helical conformation **D** of the

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[2]) CCDC-809710 contains the supplementary crystallographic data for this work. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/data_request/cif (or from the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

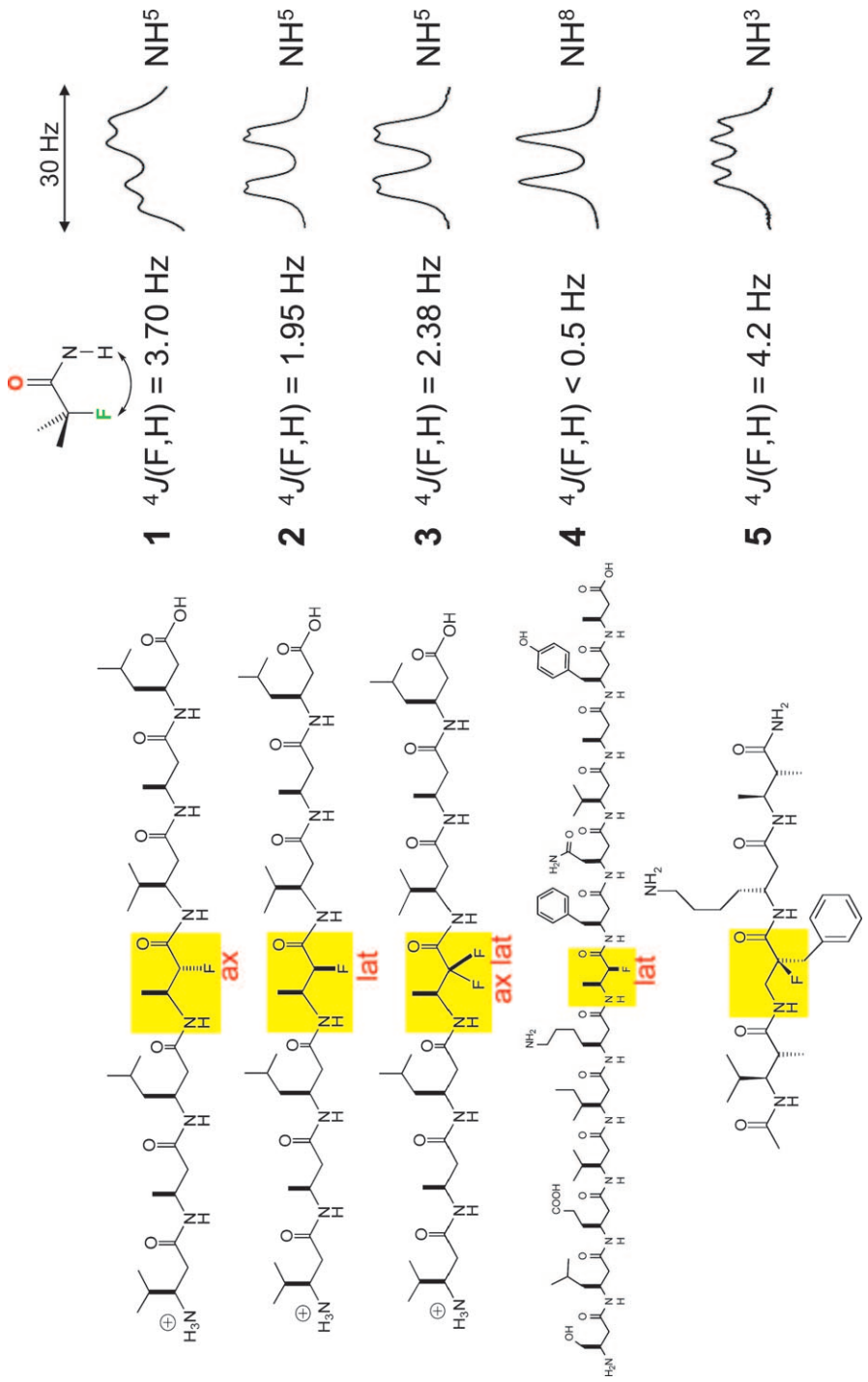

Fig. 1. *F-Substituted β-peptides* **1**–**5** *and NMR signals of the F–C–CO–NH H-atoms in the spectra of* **1**–**5**. In a β-peptidic 3_{14}-helix (*cf. Fig 2*) the F-atoms would be in the axial or lateral positions as indicated in the *Formulae* **1**–**4**. The ^4J(F,H) coupling in **2** establishes that the central, F-substituted amino acid is predominantly in a *non-helical* conformation.

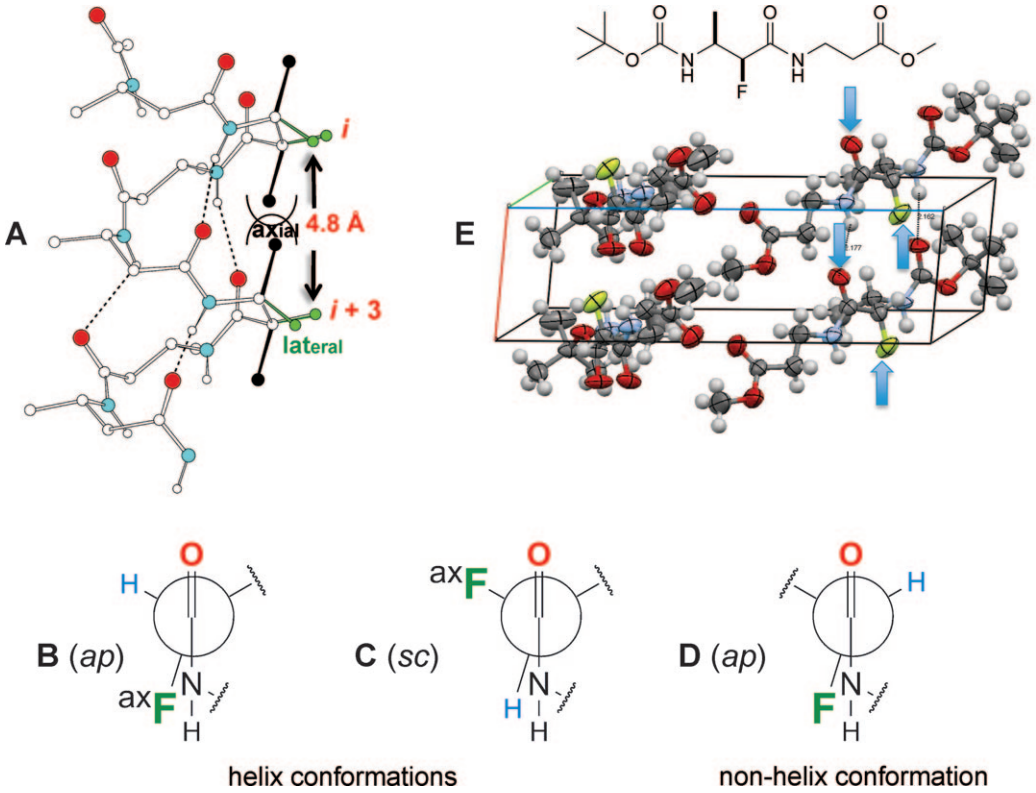

Fig. 2. *Construction principle of the β-peptidic* (M)-3_{14}*-helix*. Axial and lateral positions on the sp^3-centers (**A**); helix conformations **B** and **C** of the CO–C(α) bond of a (2*R*)- and (2*S*)-configured 2-fluoro 3-amino acid, respectively; rotation around the CO–C(α) bond of **C** leads to a non-helical conformation **D**; X-ray crystal structure[2]) of a Boc-*β*-dipeptide methyl ester (**E**) containing the β^3hAla(2-F)-moiety with the typical *ap* arrangement of F and C=O O-atom

central amino acid; *iii*) *β*-tridecapeptide **4**, on the other hand, is helical with an F–C–C=O dihedral angle of *ca.* 90°, *i.e.*, in this longer-chain peptide, the helix propensity overrides the *ap* effect.

The intensity of the *Cotton* effect near 215 nm in the CD spectra of the four peptides (**4** ≈ **1** > **3** > **2**) is in agreement with the results of the NMR analyses [2][3]).

β-Tetrapeptide **5** was synthesized with the intention to stabilize[4]) a *β*-peptidic turn structure by incorporating an appropriate *α*-fluoro-*β*-amino-acid residue (*Fig. 3*). The XPLOR simulated-annealing calculations based on NOE and ^3J(H,H)-derived constraints provided two structural clusters (24 and 6 components) with F–C_α–C=O dihedral angles of *ca.* 180 and *ca.* 150°, respectively. Our conclusion was: '*since no*

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[3]) The CD trough near 215 nm is generally associated with the *β*-peptidic 3_{14}-helix (see the discussion in the review article [6]), but it can be deceiving [7].

[4]) The turn structure was additionally fortified [6][8][9] by two terminal *α*-methyl *β*-amino acid residues of *unlike* [10] configuration.

reliable calibrations for the vicinal $^3J(H,F)$ coupling constants in α-fluoro-β-amino-acid residues are available, we had to abstain from using corresponding constraints in the simulated-annealing (SA) calculations, and this was the major cause for the appearance of two different conformational clusters in the bundle of accepted structures' [2].

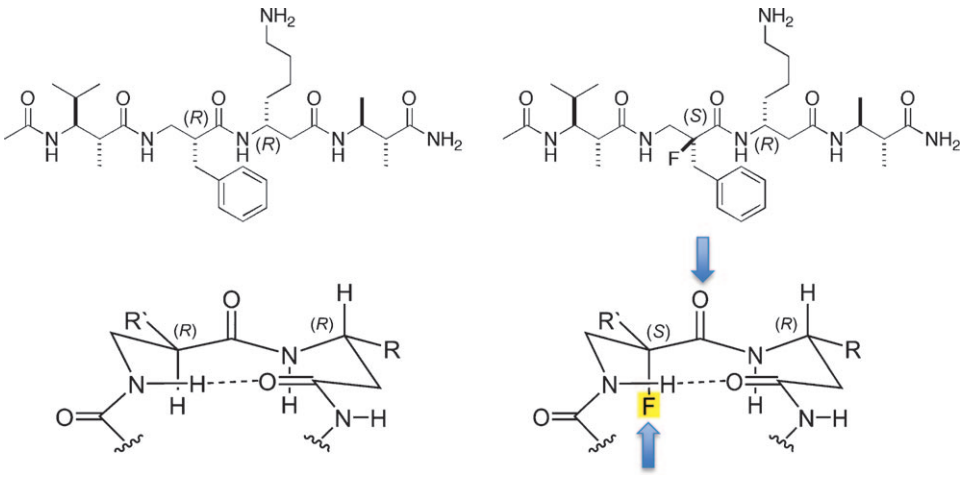

Fig. 3. *Incorporation of an F-substituent into the β²-amino acid residue of a β-peptidic turn as a probe of the F–C–C=O* ap *effect for turn stabilization* [2]

Recent explicit solvent molecular-dynamics (MD) simulations for **1**, **2**, and **3** in methanol by *Gattin* and *van Gunsteren* led these authors to conclude that the order of helix stability of the three β-heptapeptides is **2** > **3** > **1** [11], just the opposite of the order derived from the NMR and CD spectra by us [1][2]. In these MD simulations, the program GROMOS and the GROMOS force field 45A3 [12] were used except for charges and repulsive *Van der Waals* parameters for fluorine, which were taken from a study of *Fioroni et al.* on hexafluoroisopropanol [13]. As in *Fioroni*'s work, no specific torsional potential around the FC(α)–CO bond was included, because an 'ab initio *conformational study with the Gaussian03 program, using the DFT/6-311 + G** method*[5]) *provided torsional-angle energy profiles [….] in good agreement with those obtained by the GROMOS force field*'. The authors did not refer to the previous DFT calculations [1][5], which had provided the *ap*-conformer with 180° torsion angle as the more stable one, and they completely ignored the exclusive presence of this very conformer in X-ray crystal structures (see *Fig. 2*, **E**[2]) and [5]).

We have reported earlier that a long-range coupling ^4J(F,H) between the F-atom at C(α)^i and HN^{i+1} of *ca.* 4 Hz can be observed in fluoro-tetra-β³-peptide **5**, and that this coupling is not detectable in tridecapeptide **4**, for which our NMR analysis evidenced a helix with an F–C(α)–C=O dihedral angle near 90° in the central fluorinated residue. Since theoretical considerations indicate that this four-bond coupling can only be as large as 4 Hz, if the four bonds connecting the two nuclei are coplanar, its size should be

[5]) The model structure was H_2N–C(β)H(Me)–C(α)F_2–CONHMe, a fragment of difluoropeptide **3**.

a valuable diagnostic tool for the (possibly time-averaged) dihedral angle F–C(α)–C=O. We, therefore, revisited our NMR data and looked at the signals of the FC(α)C(O)NH H-atoms in the corresponding β-amino-acid residues of peptides **1**–**5**. In *Fig. 1*, we show the NH signal of the amino-acid moiety, following the α-fluoro-substituted residue for each of the peptides **1**–**5**, and the values of the ^4J(F,H) couplings determined by deconvolution.

With ^4J(F,H) = 4.2 Hz, the hairpin-forming peptide **5** shows the largest long-range coupling, very close to the value predicted by high-quality DFT calculations of this coupling constant for a coplanar conformation[6]). Since peptide **5** can form a hairpin with a ten-membered H-bonded ring, where the F-atom is almost ideally *ap* to the C=O group, it is not surprising that this otherwise unconstrained peptide shows the largest long-range coupling. The fact that ^4J(F,H) for peptide **1** is only slightly smaller confirms our earlier conclusion that this peptide predominantly assumes a helical conformation with the nearly axial C–F bond. For peptide **2**, this coupling is distinctly smaller than for **1**, but its value of *ca.* 2 Hz indicates that **2** either assumes a dominant conformation where the F–C(α)–C=O dihedral angle is not far from 180°, or that other conformers with F–C(α)–C=O far from 180° are populated to some extent. For the α,α-difluoro peptide **3**, only one of the F-nuclei couples with HN^{i+1} with an intermediate value for ^4J(F,H), consistent with the NMR-derived structural bundle, which shows values of 140–160° for the dihedral angle F–C(α)–C=O. The β-tridecapeptide **4**, which is clearly a helix over the entire length according to the NOE data, exhibits no ^4J(F,H) coupling at all, consistent with a dihedral angle F–C(α)–C=O of *ca.* 90°.

Hence, analysis of four-bond F,H couplings confirms the previous conclusions from the NMR analyses [1][2]: *i*) conformational flip of peptide **2** into the non-helical conformation **D** with *ap*-arrangement F–C(α)–C=O; *ii*) the necessary *ap*-arrangement of *one* F-atom in the difluoro derivative **3** (the second, non-*antiperiplanar* F on the CF_2 group does not couple with the NH H-atom); *iii*) the *sc*-conformation in the β-tridecapeptide **4**. The conclusion deduced from MD simulations, according to which β-peptide **2** forms the most stable helix (with lateral F-atom; F–C–C=O torsion angle, 80°), is incorrect.

Whenever a single conformer dominates in solution, MD simulations of β-peptides with proteinogenic side chains in explicit MeOH with the GROMOS package and force fields resulted in conformational clusters that were consistent with structural bundles derived by constrained simulated-annealing calculations from NMR data [7b][14]. In cases where several conformers are appreciably populated, the standard NMR procedure, which searches for static structures that are consistent with all the NOE and coupling data, usually fails, because the NMR data consist of weighted time averages, and no single structure is consistent with them. In the past, the reliability of the GROMOS force field for this particular class of molecules and the solvent MeOH has often been useful in such situations. In the present case, however, the discrepancies must have to do with the presence of the F-atom in the central amino-acid residue of

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6) Calculations were performed by Dr. *Marc-Olivier Ebert* in our laboratory. These calculations predict that the ^4J(F,H) coupling drops very steeply when the four involved bonds deviate from coplanarity. The results of this project, which has the goal to determine reliable *Karplus* coefficients for F-containing peptides, will be reported separately.

the β-peptides **1**–**5**[7]). This conclusion is corroborated by a more recent MD simulation (GROMOS 53A6 force field, modified for F according to [13]; explicit MeOH) of fluoro-β-tetrapeptide **5**, which provided results consistent with the NMR data (distance constraints and J values), only if the NMR-derived constraints were included in the simulation as time-average restraints [15].

Both the NMR analysis based on NOE and ^3J(H,H) data alone, and the unrestrained MD simulation failed to produce unambiguous structures for β-peptide **5** (*Fig 3*). This is obviously due to the lack of reliable NMR *and* force-field parameters (*Karplus* coefficients, torsional potential) for molecules of this type[8]). The qualitative use of the ^4J(F,H) coupling as a diagnostic tool is a first step towards the quantitative use of such additional NMR-derived restraints. We are now in the process of collecting X-ray-crystal structures and of obtaining the corresponding NMR spectra of compounds with the structural element of α-fluoro-β-amino acid amides.

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