The Effect of Backbone-Heteroatom Substitution on the Folding of Peptides – A Single Fluorine Substituent Prevents a β-Heptapeptide from Folding into a 3_{14}-Helix (NMR Analysis)

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The β-heptapeptides H-βhVal-βhAla-βhLeu-βhAla(X_n)-βhVal-βhAla-βhLeu-OH **3**–**7** with central 3-amino-2-fluoro-, 3-amino-2,2-difluoro-, or 3-amino-2-hydroxybutanoic acid residues (βhAla(X_n)) of *like* and *unlike* configuration were subjected to a detailed NMR analysis in MeOH solution. For the geminal difluoro and for the F- and OH-substituted derivatives of *u*-configuration (see **5**, **4**, and **7**, resp.), 14-helices were found, *i.e.*, with axial disposition of the hetero atoms on the helix. The two compounds containing the central *l*-configured β-amino acid moieties (see **3** and **6**) are not helical over the full lengths of the chains; they have 'quasi-helical' termini and a central turn consisting of a ten-membered H-bonded ring (*Fig. 2*, *d* and *e*). Quantum-mechanical calculations with *l*- and *u*-AcNH-CHMe-CHF-$CONH_2$ confirm the observed preference for a conformation with antiperiplanar arrangement of the F–C and the C=O bond. The calculated energy difference between the observed 'non-helical' geometry of this moiety and a hypothetical helical one is 6.4 kcal/mol (*Fig. 3*).

Introduction. – The influence of a backbone-bound heteroatom (NH_2, OH, SH, F, Cl, Br, *etc.*) on the structure of a peptide or protein is unknown as such compounds are generally unstable, being acetal or ketal derivatives (*Scheme*, *a*).

Still, α-hydroxyglycine-containing peptides have been identified [1] as intermediates of the enzymatic oxidative removal of C-terminal glycines with formation of peptide amides and glyoxylic acid, a most important post-translational modification (*Scheme*, *b*) [1–6]: C-terminal amide groups are, *e.g.*, present in mammalian peptide hormones (oxytocin, gastrin, secretin) and in toxins (mellitin, toxin II, conotoxin, caerulein), containing from 3 to 64 amino acid residues [7–10]. An (*S*)-α-hydroxyglycine-containing tripeptide has been prepared in five steps, under carefully controlled conditions, by *Bogenstätter* and *Steglich* [11]. The hydroxyglycine moiety is rather stable at slightly acidic pH but is cleaved under basic conditions [12]. No information on the secondary structure of a peptide containing this elusive moiety is available.

The same is true of halogen-substituted glycine derivatives ($R^2 = H$, X = F, Cl, Br in equation *a*) in the *Scheme*). Halogenation with *N*-chloro- or *N*-bromosuccinimide (NCS or NBS) of glycine-containing peptides generates α-halogenoglycine residues,

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[1]) Part of the projected Ph.D. thesis of *R. I. M.*, ETH Zürich.

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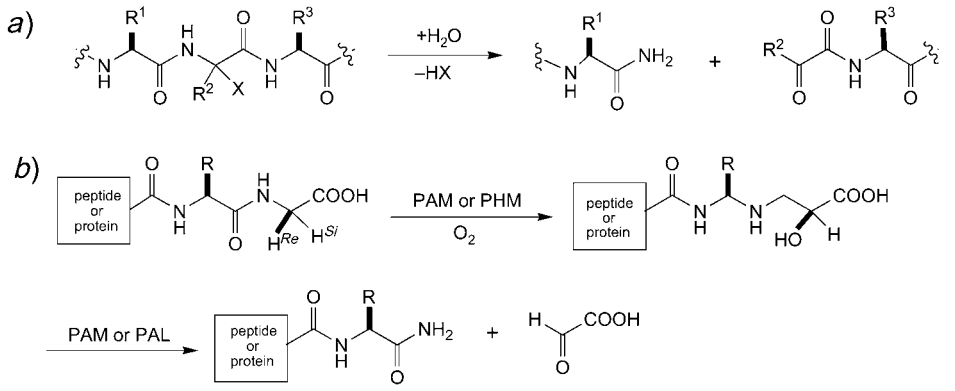
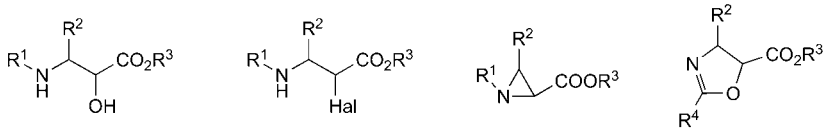
Scheme. a) *Hydrolytic Instability of Peptides or Proteins with Backbone-Bound Heteroatom Substituent X and* b) *Enzymatic Formation of Peptide and Protein Amides by Oxidative Removal of a C-Terminal Glycine Residue* [1–6]



the reactivity of which has been studied extensively by the group of *Steglich* [13] and others [14–17][3])[4]).

In contrast, β-amino-α-hydroxyacids and their derivatives **A** are stable compounds, they occur in natural products (*cf.* bestatin, amastatin, phebestin, taxol [20–24]) and are components of synthetic drugs [25][26]. Also β-amino-α-halogenoacid derivatives **B** should be stable. Although they could cyclize to aziridines **C** or (with *N*-acyl protective groups) to dihydrooxazoles **D**, under certain conditions, this process would be expected to be least likely with amino-fluoro acids. Thus, β-peptides containing such hetero-substituted β-amino acids should offer the unique possibility to study the influence of polar and H-bond-forming substituents on the folding behavior and secondary-structure stability.



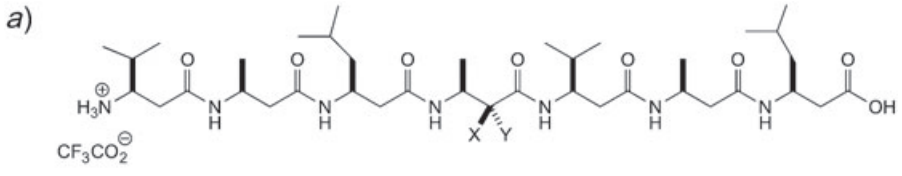
We have previously published – in preliminary form [27][28] – the synthesis and CD spectra of a number of fluoro-[5]) and hydroxy-substituted β-peptides, and we have given

[3]) For a discussion, with references, of the generation and reactions of acyliminium ions, see a review article by *Speckamp* and *de Koning* [18].

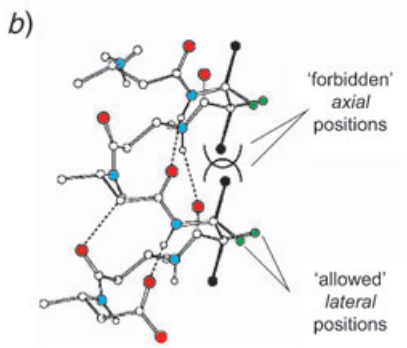
[4]) For an article on peptide-backbone modifications through glycine/sarcosine-derived nucleophilic, electrophilic, and radical intermediates, see an article by *Seebach*, *Beck*, and *Studer* [19].

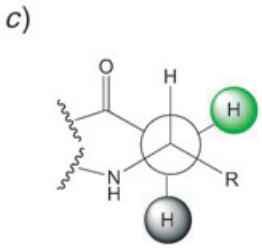
[5]) For a recent review article on the synthesis of fluorinated amino acids, see [29].

full details of the preparation of the β-heptapeptides **1**–**7** (*Fig. 1,a*) [30][31]. The β-heptapeptide **1** with a central βhAla(α-Me) unit of *like* configuration has been shown by CD and NMR analysis [30], as well as by molecular-dynamics simulation [32–36] to form an especially stable 3_{14}-helix, with the two Me groups in lateral positions (*Fig. 1,b* and *c*). Compound **1** (in MeOH solution) gives rise to an intensive negative *Cotton* effect near 215 nm (Θ −70,000) [30], which is considered to be characteristic of the β-peptidic 3_{14}-helical secondary structure. If we would use the intensity of this CD band as a measure for the degree of helicity[6]) [7]) of the compounds **2** [30] and **3**–**7** [27], we



	1	2	3	4	5	6	7
X	Me	H	F	H	F	OH	H
Y	H	Me	H	F	F	H	OH





d)

β-heptapeptide	1	4	7	5	6	3	2
Θ	70	−70	−70	−55	−50	−20	+15

Fig. 1. *β-Heptapeptides with central α-substituted β-amino acid residues of* l- (see **1**, **3**, and **6**) *and* u-*configuration* (see **2**, **4**, and **7**), *the* (M)-3_{14}*-helix of a β-heptapeptide and characteristic CD data. a*) Formulae of the heptapeptides **1**–**7** with βhAla(α-Me), βhAla(α-F), βhAla(α,α-F_2), and βhAla(α-OH) residues. *b*) The 3_{14}-helix (of a *ca.* 5-Å pitch) with 'allowed' (green) and 'forbidden' (black) positions for non-H-atoms (N-atoms blue, O-atoms red). *c*) The (+)-*sc* conformation around the C(α)–C(β) bond in its β-amino acid residues. *d*) Intensities ($\Theta \cdot 10^3$ [10 deg cm^2 dmol^{-1}]; all measurements with of 0.2 mM solutions in MeOH) of the CD band at *ca.* 215 nm (considered to be characteristic of the 3_{14}-helical secondary structure) for the β-peptides **1**–**7**.

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[6]) For a warning note, see [37].

[7]) See also the discussion in the review article on β-peptides (Chapt. 7.6 in [38]).

would have to conclude that the monofluoro derivative **4** and the hydroxyl derivative **7** are 'more helical', with an axial disposition of the heteroatom, than the diastereoisomers **3** and **6**, with lateral heteroatoms! The difluoro-substituted β-heptapeptide **5** would wind up somewhere in between, and **2**, the epimer of **1**, would definitely not be able to form a helix with an axial Me group (*Fig. 1,d*).

We have now performed a complete NMR analysis of the β-heptapeptides **3**–**7**, and the results are described in the following sections.

NMR Analysis of the α-Fluoro- and α-Hydroxy-β-heptapeptides 3–7. – Complete assignment of all ^{1}H- and ^{13}C-NMR resonances for each of the five compounds **3**–**7** in CD_3OH was achieved from the standard set of NMR spectra (1D-^{1}H, 1D-^{13}C, DQF-COSY, TOCSY, HSQC, and HMBC at 500 MHz and 125 MHz, acquired with presaturation of the solvent OH signal). The assignment of identical residues within the sequence was based on $CH_n(\alpha,i) \rightarrow CO(i) \rightarrow NH(i+1)$ correlations in the HMBC spectra. Coupling constants ^3J(NH,CH(β)) were determined from the 1D-^{1}H-NMR spectra, and the qualitative values for ^3J(CH(α),CH(β)), which eventually allowed us to stereoselectively assign the diastereotopic $CH_2(\alpha)$ protons, were estimated from cross-sections of the TOCSY cross-peaks. Chemical shifts, assignments, and coupling constants for β-heptapeptides **3**–**7** are compiled in *Table 1* (see *Exper. Part*).

ROESY Spectra were measured with mixing times of 150 ms and 300 ms, and the cross-peak volumes of the latter were converted into the distance restraints shown in *Table 2* (see *Exper. Part*). Together with dihedral-angle constraints derived from the coupling constants, the distance restraints were used in the simulated-annealing molecular-dynamics calculations (SA) of structural bundles for **3**–**7** with XPLOR-NIH.

Qualitative inspection of the NOE patterns exhibited by **3**–**7** immediately revealed that the two compounds with *unlike* relative configuration in the central α-substituted βhAla[4] residue (see **4** and **7**) as well as the α-difluoro derivative **5** showed the typical pattern observed for 3_{14}-helical structures. This was confirmed by the full SA calculation, which resulted in well-defined bundles of helical low-energy structures with no NOE violations for **4**, **5**, and **7** (*Fig. 2,a–c*). As observed earlier for 3_{14}-helical β-hexa- and β-hepta-peptides, the C-terminal residue (βhLeu[7]) is less well-defined by NOE constraints and, therefore, appears more frayed out in the calculated bundles[8]).

Surprisingly, the monofluoro derivative **3** with *like* configuration assumes a structure in which the α-fluoro substituent in the βhAla(α-F)[4] residue is again antiperiplanar (*ap*) to the carbonyl C=O bond. As a consequence, the overall structure is not a helix but consists of two quasi-helical termini (residues 1–3 and 5–6) flanking a central turn with a 10-membered H-bonded ring between NH of residue 3 and CO of residue 4 (*Fig. 2,d*).

The structural bundle obtained for the *like* α-hydroxy-β-heptapeptide **6** is the least well-defined one of the five, with the C-terminal residues βhAla[6] and βhLeu[7] being largely unrestrained through NOEs. However, the 15 structures of lowest energy

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[8]) Whether this corresponds to higher conformational freedom of the C-terminus or is only due to the lower density of observable NOEs remains to be determined. Measurements of ^{13}C-relaxation times to answer this question are currently under way.

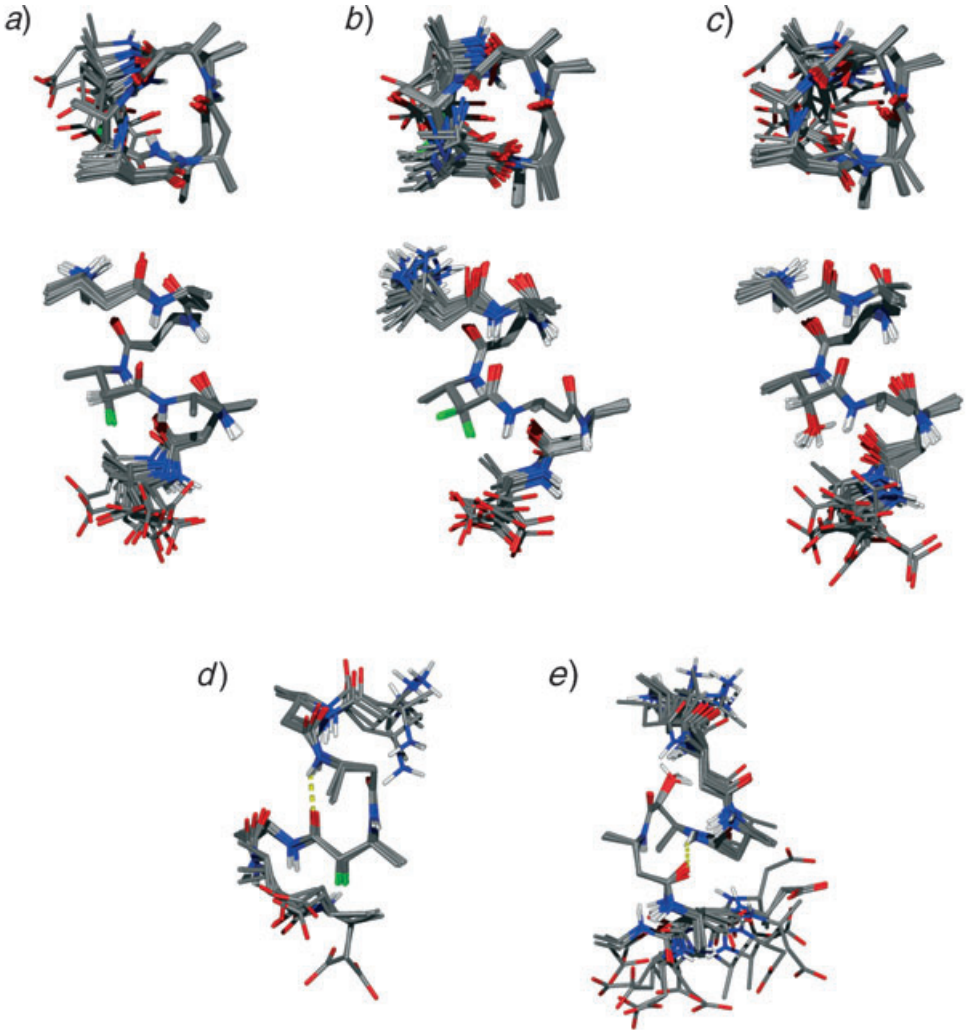

Fig. 2. *NMR-Derived structures of β-heptapeptides* **3**–**7**. *a*) Residue 4 = *u*-βhAla(α-F) for **4**; *b*) residue 4 = βhAla(α,α-F_2) for **5**; *c*) residue 4 = *u*-βhAla(α-OH) for **7**; *d*) residue 4 = *l*-βhAla(α-F) for **3**; *e*) residue 4 = *l*-βhAla(α-OH) for **6**. Shown are bundles of the 15 structures with the lowest energies that do not violate any experimentally derived NOE or dihedral-angle constraints. Superposition of bundles by least-squares fit of *a*, *c*, *d:* all backbone atoms of residues 2–6; *b*, *e:* backbone atoms of residues 3–5.

(*Fig. 2*, *e*) all show the α-hydroxy substituent of residue 4 almost synperiplanar (*sp*) to the C=O bond and a ten-membered H-bonded ring between NH of residue 4 and CO of residue 5. The N-terminal residues are also quite well-defined and have a *quasi*-3_{14}-helical conformation.

In conclusion, the difluoro-β-heptapeptide **5** and the β-heptapeptides **4** and **7** with (2*R*,3*S*) or *unlike* configuration of the central amino acid residue form a 3_{14}-helix with

an axial F or OH substituent, while the epimers **3** and **6** with *like* configuration of these residues are not helical over the full length of the molecule. This means that a single F or OH group in the 'wrong' position of a β-heptapeptide is able to prevent the formation of the full helix. There must be a helix-stabilizing effect of an axial F or OH heterosubstituent (*cf.* **4** and **7**) and/or a destabilizing effect of a lateral one (*cf.* **3** and **6**); with a CF_2 group, the helix-stabilizing influence of the axial F prevails (see **5**). The NMR structures also confirm that both substituents, F and OH, are small enough to occupy a 'forbidden' position on the 3_{14}-helix, where a Me group 'sterically destroys' the helix (*cf.* **2**)[9].

Close inspection of the structural bundles reveals that, in the β-heptapeptides studied here, the steric demand on an axial substituent in the 3_{14}-helix might be slightly less in the fourth residue than it would be earlier in the sequence because the axial α-substituent in residue 4 points towards the C-terminal residue. Since, under the conditions of the NMR measurements, the terminal carboxy group is protonated, the H-bond between NH of residue 5 and the carboxy O-atom of residue 7 is expected to be weaker than the H-bonds to amide carbonyl groups earlier in the sequence. Hence, the rise of the last half-turn of the helix may well be increased compared to the central turns and offer the axial α-substituent in residue 4 more room. Although the sparsity of observable NOEs at the C-terminus did not allow us to experimentally verify this 'loosening' of the helix, the fact that β-heptapeptide **2** with a slightly larger Me group at this position is no longer able to form a helix clearly points towards rather strict steric limits for substituents pointing in the axial direction of a 3_{14}-helix.

Discussion. – Since the observed trend for axial polar substituents is opposite to the expectations based on simple steric reasoning, it must be due to stereoelectronic effects, dipole–dipole interaction, H-bonding and/or solvation factors. On the basis of X-ray crystallography and quantum-chemical calculations, *O'Hagan* and co-workers concluded that, in α-fluoroamides, the conformer with the α-fluoro substituent oriented *trans* or *ap* to the C=O bond is preferred by *ca.* 8 kcal/mol over the *gauche* or synclinal (*sc*) conformer (which was not a minimum on the calculated potential-energy surface (PES)). For the vicinal polar C–N and C–F bonds in *N*-(β-fluoroalkyl)amides, the *sc* arrangement was calculated to be *ca.* 2 kcal/mol lower in energy than the *ap* conformer [39–41]. *Rittner* and co-workers [42][43] have investigated the conformations of α-fluoroamides by IR, NMR, and theoretical methods and found that the *sc* conformer becomes a true minimum on the PES if a reaction field solvation model was included in the DFT calculations. While the energy of the *ap* conformer was calculated to be almost insensitive to solvent polarity, the *sc* conformer was lowered in energy with increasing solvent dielectric constant.

In the α-fluoro-β-peptides **3** and **4**, both the α-fluoroamide and the *N*-(β-fluoroalkyl)amide structural motifs are present, and the two conformational effects are expected to be cumulative. To verify that the predicted relative stabilities of the individual conformers stay the same in the combined case of an α-fluoro-*N*-(β-fluoroalkyl)amide moiety, we calculated the (gas phase) energies of (2*R*,3S)- (**8**) and

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[9] The so-called *A* values for destabilization of axial Me, OH, and F on a cyclohexane ring are 1.79, 0.60, and 0.25 kcal/mol, respectively (see text books of stereochemistry).

(2*S*,3*S*)-3-(acetylamino)-2-fluoro-*N*-methyl-butanamide (**9**) as a model for the central residue in β-peptides **3** and **4** on the same theoretical level as that used by *Banks et al.* [39] (B3LYP/6-31 + G(d)DFT geometry optimization). The minimized structures obtained for the two epimers with different starting conformations are shown in *Fig. 3*. Our calculation predicts that the ('helix') conformer **8a** of the *unlike* epimer **8** is *ca.* 7 kcal/mol more stable than the corresponding 'helix' conformer **9a** of the *like* epimer **9** (in this case, the *sc* conformation is a minimum on the PES even without reaction field solvation model), whereas the 'non-helix' conformation **9b** of the *like* epimer **9** found experimentally in β-peptide **3** is only 0.5 kcal/mol less stable than **8a**.

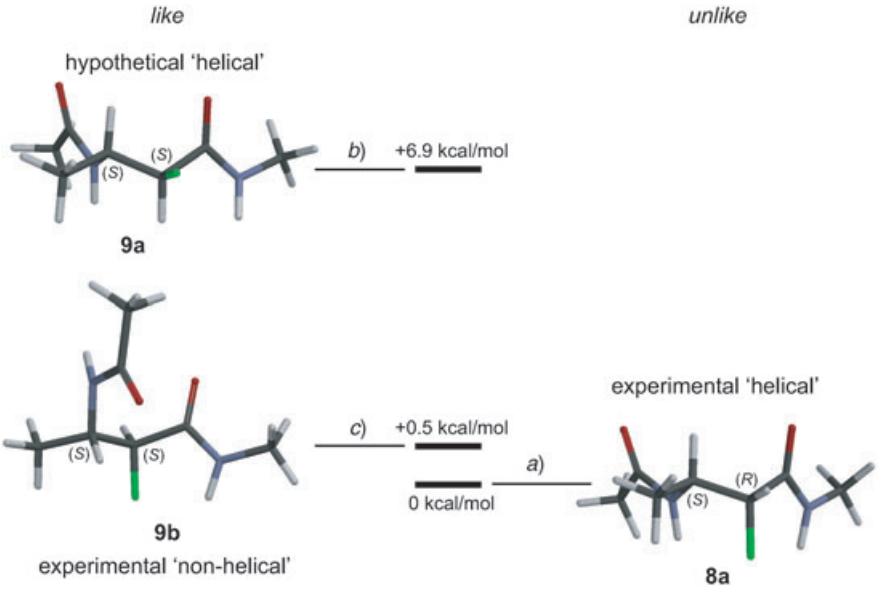

Fig. 3. *Geometry-optimized structures and calculated gas-phase energies* (DFT B3LYP, 6-31 + G(d)) *obtained for the two epimeric models* **8** *and* **9** (AcNH–CH(Me)–CHF–CONHMe) *with different starting conformations. a*) Model **8** in the conformation **8a** as extracted from the central residue of the NMR structure of **4**, *b*) model **9** in the same conformation (see **9a**) (under exchange of the positions of the H- and F-substituents at C(2) in **8**) and *c*) model **9** in the (non-helical) conformation **9b** extracted from the NMR structure of β-peptide **3**.

In the 3_{14}-helical NMR-solution structure of β-peptide **4** (*Fig. 2,a*), the central *unlike* βhAla(α-F)[4] residue shows exactly the local *sc*/*ap* conformation one would expect based on the results of *O'Hagan* and *Rittner*. In the NMR structure of the epimer **3**, the F-substituent is again *ap* to the carbonyl O-atom (which precludes the helix), but the local conformation around the C(α)–C(β) bond of the βhAla(α-F)[4] residue is *ap* and not *sc*. Hence, the energetic preference for the *trans*-arrangement of the C–F and C=O bonds, although predicted to be smaller in MeOH than the 7 kcal/mol calculated for the gas phase, still outweighs the loss of favorable H-bonding and, presumably, side chain interaction energy caused by the interruption of the helix in its central section.

B3LYP-DFT calculations on the same level as described above for models **8** and **9** resulted in a very shallow torsional potential for the corresponding difluoro derivative

10. As shown in *Fig. 4*, all minima and maxima were found within 3 kcal/mol from the lowest-energy conformer, in which one C−F bond is *ap* and one *sc* to the C=O bond ($\theta = 60°$), and the conformer which is structurally closest to the one found in the structures derived from the NMR data ($\theta = 140° \pm 5°$) lies less than 1 kcal/mol higher. Because the driving force for a particular local conformation around the C(α)−CO bond is much lower in the geminal difluoro derivative than in the monofluoro derivatives, the 3_{14}-helix prevails throughout β-peptide **5**.

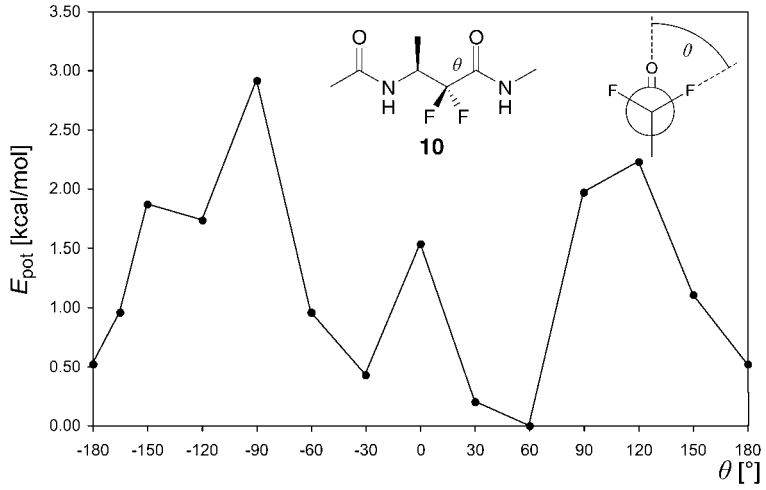

Fig. 4. *Calculated potential energy of model* **10** (AcNH−CH(Me)−CF_2−CONHMe) *as a function of the dihedral angle* θ (F_{Re}−C(α)−C=O). Constrained geometry optimization with DFT B3LYP/ 6-31 + G(d) in steps of 30°.

The evaluation of the factors that lead to the particular structures found for the two epimeric α-hydroxy-substituted β-peptides **6** and **7** is complicated by the possibility of this substituent to act as a H-bond acceptor and donor. Hence, quantum-mechanical calculations of conformational-energy differences without the inclusion of an explicit solvent model are not expected to render a realistic picture. In the case of β-peptide **7** containing the *unlike* βhAla(α-OH)[4] residue, the C−O bond is *ap* to the C=O bond in the structure determined by NMR and, as in the case of the *unlike* monofluoro derivative **4**, this allows formation of a full 3_{14}-helix. In the *like* epimer **6**, the helix is unwound at the central residue as in the fluoro analogue **3**. In contrast to the corresponding C−F bond in **3**, however, the C−OH bond is found to be *sp* to the C=O bond and not *ap*. This indicates that, for the C−O bond of α-hydroxy-β-peptides, the preference for the *ap* arrangement is less pronounced than for the more polar C−F bond in α-fluoro-β-peptides. At the same time, the possibility of a (suboptimal) five-membered H-bonded ring between the OH group and the carbonyl O-atom must be considered for the *sp* orientation. That the factors determining the secondary structure of α-hydroxy-β-peptides are more intricate than in the case of fluoro-peptides is exemplified by the fact that we found a similar *sp*/*sc* arrangement of the OH groups in a protected β-hexapeptide consisting entirely of *u*-configured 3-amino-2-hydroxyacid residues [28].

Experimental Part

NMR Measurements. The trifluoroacetate salts (H_3N^+/COOH form) of β-heptapeptides **3**–**7** were dissolved in CD_3OH ($c \approx 10$ mM). All NMR spectra (*Table 1*) were acquired at 500 MHz (^1H)/125 MHz (^{13}C) with presaturation of the solvent OH-signal.

DQF-COSY with coherence transfer selection by *z*-gradients. TOCSY with 80 ms DIPSI-2 spin lock (8.9 kHz). HSQC with coherence transfer selection by *z*-gradients. HMBC with coherence transfer selection by *z*-gradients. ROESY: clean ROESY [44] with 300 ms and 150 ms CW-spin lock (2.8 kHz). Spectral width 6000 Hz, $2k \times 512$ data points acquired (64 scans/FID) with TPPI. Processed with $\cos^2$ window function to give $1k \times 1k$ real data points. Polynomial baseline correction applied in both dimensions.

Assignments and volume integration of ROESY cross-peaks were performed with the aid of SPARKY [45]. Distance constraints and error limits were generated from cross-peak volumes by calibration with known distances (two-spin approximation, $\pm 20\%$ error limits) through a python extension within SPARKY. The volumes of cross-peaks involving Me groups or other groups of isochronous protons were corrected by division through the number of protons.

Simulated-Annealing (SA) *Structure Calculations.* Data, see *Table 2*. Program XPLOR-NIH v2.9.7 [46]. The standard parameter and topology files of XPLOR-NIH (parallhdg.pro; topallhdg.pro) were modified to accommodate β-amino acid residues. Equilibrium bond lengths and bond angles for the α-fluoro-, α,α-difluoro-, and α-hydroxy-βhAla residues were taken from X-ray structures of the corresponding protected amino acids. Minimized extended zig-zag conformations were used as the starting structures. The SA calculation protocol (adopted from the torsional angle dynamics protocol of *Stein et al.* [47]) included 4000 steps (0.015 ps each) of high-temperature torsional-angle dynamics at 20000 K, followed by 4000 (0.015 ps) steps of slow cooling to 1000 K with torsion-angle dynamics, 4000 steps (0.003 ps) of slow cooling with cartesian dynamics to 300 K, and a final conjugate gradient minimization. The only nonbonded interactions used were *Van der Waals* repel functions. For each compound, 50 structures were calculated.

Quantum-Chemical Calculations of Models **8**–**10**. Program QCHEM v.2.1.0 [48] (HP-UX parallel version with 4 processors). The starting structures of **8a**, **9b**, and **10** were generated by cutting out the central βhAla(α-X) residue of the NMR-derived structures for **3**, **4**, and **5** and replacing the N-terminal chain by an acetyl group and the C-terminal chain by a *N*-methylcarboxamide group (builder mode of program SPARTAN). The dihedral angles within the backbone of the βhAla(α-X) residue were left as found in the average NMR structure. Model **9a** was generated from **8a** by exchange of the α-H and α-F substituents. These starting structures were subjected to geometry optimization in hybrid density-functional calculations (B3LYP, 6-31 + G(d) basis set). For the difluoro-substituted model **10**, a series of B3LYP/6-31 + G(d) geometry-optimization calculations with the dihedral angle $F_{Re}-C(\alpha)-C{=}O$ constrained was performed (steps of 30°, from $-165°$ to 180°).

Table 1. *^{1}H- and ^{13}C-NMR Data and Assignments for β-Heptapeptides* **3**–**7**. n.a. = not assigned.

β-Amino acid	NH	$CH_2(\alpha)$	H–C(β) (^3J(NH,CH(β))	H–C(γ) Me(γ) $CH_2(\gamma)$	H–C(δ) Me(δ)	Me(ε)	CO	C(α)	C(β)	C(γ)	C(δ)	C(ε)
H-βhVal-βhAla-βhLeu-*l*-βhAla(α-F)-βhVal-βhAla-βhLeu-OH (**3**):												
βhVal[1]		2.6	3.47	2.00			171.6	35.77	56.10	32.01	18.83 (2C)	
βhAla[2]	8.092	2.54	4.40	1.20			172.9	43.41	44.16	21.13		
βhLeu[3]	8.099	2.41	4.36	1.29, 1.42	1.61	0.92	172.7	42.72	45.90	45.82	26.03	22.56 (2C)
βhAla(α-F)[4]	8.32	4.92	4.42 (8.56)	1.17			169.0	93.65	47.17	15.28		
βhVal[5]	8.00	2.31	4.16	1.82	0.95		172.2	39.09	53.80	33.67	19.39 (2C)	
βhAla[6]	7.80	2.34	4.30 (8.30)	1.13			172.1	43.36	44.11	20.50		
βhLeu[7]	7.85	2.49	4.38 (9.29)	1.32, 1.43	1.61	0.92	175.2	41.10	45.92	45.15	26.03	23.59 (2C)
H-βhVal-βhAla-βhLeu-*u*-βhAla(α-F)-βhVal-βhAla-βhLeu-OH (**4**):												
βhVal[1]	7.75	2.65	3.57	2.055	1.071, 1.073		171.3	36.10	56.23	32.03	17.5, 18.95	
βhAla[2]	7.99	1.22	4.57 (9.20)	1.22			n.a.	43.30	43.63	21.34		
βhLeu[3]	8.47	2.75	4.39 (9.17)	1.27	1.59		173.1	46.75	45.90	21.34	26.02	23.55 (2C)
βhAla(α-F)[4]	8.48	5.075	4.68 (9.66)	1.25			169.6	94.76	47.01	16.89		
βhVal[5]	7.76	2.48	4.28 (9.17)	1.81	0.95		171.8	38.04	53.29	34.19	19.60 (2C)	
βhAla[6]	7.58	2.33, 2.43	4.48 (8.56)	1.13			171.7	43.19	43.71	20.96		
βhLeu[7]	7.72	2.49	4.43 (8.9)	1.31, 1.40		0.92	174.8	40.45	45.55	45.54	26.05	26.06 (2C)
H-βhVal-βhAla-βhLeu-βhAla(α,α-F_2)-βhVal-βhAla-βhLeu-OH (**5**):												
βhVal[1]	7.78	2.64, 2.67	3.55	2.04	1.057	0.92	174.9	35.73	56.07	32.062	17.50, 18.91	
βhAla[2]	7.87	2.68	4.54 (9.05)	1.22			171.7	43.34	43.80	21.48		
βhLeu[3]	8.43	2.71	4.41 (9.29)	1.29, 1.38	1.59	0.90	171.6	41.69, 41.70	45.72	46.60, 46.70	26.066	22.90, 23.52
βhAla(α,α-F_2)[4]	8.54		4.40 (9.78)	1.21			n.a.		47.32	13.04		
βhVal[5]	8.36	2.46, 2.55	4.22 (8.68)	1.81	0.93, 0.96		173.0	38.36, 38.38	54.06	34.01	19.42, 19.91	
βhAla[6]	7.64	2.33, 2.42	4.45 (8.56)	1.24	7.64	2.33/2.4	173.2	43.25	43.774	20.90		
βhLeu[7]	7.76	2.50	4.39 (9.29)	1.32, 1.41	1.61	0.93	171.4	40.59	45.59	45.46, 45.47	26.06	22.70 (2C)
H-βhVal-βhAla-βhLeu-*l*- βhAla(α-OH)-βhVal-βhAla-βhLeu-OH (**6**):												
βhVal[1]		2.60, 2.68	3.49	2.01			171.5	35.93	56.10	31.90	17.72, 18.74	
βhAla[2]	8.19	2.40, 2.61	4.45 (8.80)	1.21			173.0	43.26	43.98	20.98		
βhLeu[3]	8.14	2.41	4.35 (8.40)	1.28, 1.42	1.60	0.92	172.3	42.81	46.23	45.96	25.92	22.52 (2C)
βhAla(α-OH)[4]	8.12	4.07	4.23 (7.95)	1.11			173.5	75.47	48.68	15.84		
βhVal[5]	7.73	2.30, 2.46	4.16 (10.19)	1.81	0.95		172.3	38.99	53.28	33.70	19.45 (2C)	
βhAla[6]	7.75	2.34	4.33 (9.05)				171.9	43.16	43.92	20.51		
βhLeu[7]	7.82	2.45, 2.51	4.36 (9.17)	1.31, 1.43	1.61	0.91	n.a.	41.01	45.75	45.20	25.98	23.51 (2C)
H-βhVal-βhAla-βhLeu-*u*-βhAla(α-OH)-βhVal-βhAla-βhLeu-OH (**7**):												
βhVal[1]	7.78	2.60, 2.71	3.56	2.06	1.07		171.4	36.12	56.23	32.09	17.64, 18.96	
βhAla[2]	8.16	2.45, 2.78	4.57 (9.17)	1.23			173.2	43.32	43.66	21.33		
βhLeu[3]	8.38	2.39, 2.65	4.39 (9.29)	1.28, 1.39	1.58	0.92	172.7	42.16	45.91	46.78	26.04 (2C)	n.a.
βhAla(α-OH)[4]	7.99	4.08	4.57 (9.05)	1.17			173.9	75.42	48.36	17.65		
βhVal[5]	7.71	2.47	4.23 (9.41)	1.80	0.93		171.9	38.26	53.02	34.11	23.60	
βhAla[6]	7.64	2.36, 2.45	4.43 (8.44)	1.14			172.2	43.04	43.84	20.94		
βhLeu[7]	7.83	2.51	4.44	1.31, 1.44	1.39	0.91	n.a.	40.62	45.63	45.30	26.14	19.4 (2C)

Table 2. *ROESY-Derived Distance Constraints Used in the Simulated Annealing Calculations*

Residue	H-Atom	Residue	H-Atom	Distance [Å]
H-βhVal-βhAla-βhLeu-*l*-βhAla(α-F)-βhVal-βhAla-βhLeu-OH (**3**):				
1	β	1	γ	2.6
2	β	2	NH	2.9
2	NH	2	γ	3.2
3	NH	3	α	2.8
3	NH	3	β	3.0
3	NH	3	δ	3.7
3	NH	3	α_{Si}	3.3
3	NH	3	α_{Re}	5.7
4	γ	4	α_{Re}	3.3
4	NH	4	α_{Re}	2.8
4	NH	4	β	3.1
4	NH	4	γ	2.9
5	NH	5	α_{Si}	3.9
5	NH	5	α_{Re}	3.1
5	NH	5	β	2.9
5	NH	5	γ	2.9
6	NH	6	β	3.0
6	NH	6	γ	3.2
7	NH	7	β	3.0
7	NH	7	γ	3.3
7	NH	7	δ	3.6
1	α	2	NH	2.5
1	β	2	β	4.9
1	δ	2	NH	5.2
2	α_{Re}	3	NH	2.6
3	α	4	NH	2.5
3	β	4	NH	3.1
4	α_{Re}	5	NH	3.2
4	NH	5	NH	5.3
4	β	5	NH	3.2
4	γ	5	NH	4.3
3	NH	4	NH	4.3
6	NH	5	α_{Re}	3.4
H-βhVal-βhAla-βhLeu-*u*-βhAla(α-F)-βhVal-βhAla-βhLeu-OH (**4**):				
1	β	1	δ	2.8
1	β	1	γ	2.3
2	β	2	α_{Si}	2.3
2	NH	2	γ	2.8
3	δ	3	α_{Si}	2.7
3	γ	3	α	2.9
3	NH	3	δ	3.5
4	α	4	γ	3.0
4	NH	4	α	3.5
4	NH	4	γ	3.8
4	NH	4	γ	2.9
5	β	5	δ	2.8
5	NH	5	γ	3.2
5	NH	5	β	2.9
6	α_{Si}	6	γ	3.0
6	NH	6	α_{Re}	2.8
6	β	6	γ	2.6
6	NH	6	β	2.9
6	NH	6	γ	3.5
7	NH	7	γ	3.6
1	β	2	β	4.5
1	β	2	NH	4.4
1	β	2	γ	5.2
1	α_{Si}	2	NH	3.3

Table 2 (cont.)

Residue	H-Atom	Residue	H-Atom	Distance [Å]
1	α_{Re}	2	NH	2.4
2	β	3	NH	4.1
2	NH	3	NH	3.5
4	α	5	δ	4.2
5	β	6	NH	4.2
5	δ	4	β	4.4
5	β	6	NH	4.2
5	γ	4	α	4.9
5	NH	4	β	3.3
H-βhVal-βhAla-βhLeu-βhAla(α,α-F_2)-βhVal-βhAla-βhLeu-OH (**5**):				
2	NH	1	α	4.3
2	α	3	NH	2.3
2	NH	3	β	4.0
2	β	3	NH	4.2
2	NH	3	NH	4.7
3	α	4	NH	3.6
3	NH	4	NH	3.5
4	NH	5	NH	3.5
5	α1	6	NH	3.2
5	β	6	γ	4.9
5	β	6	NH	4.0
5	α_{Si}	6	NH	5.1
6	α_{Si}	7	NH	2.9
6	α_{Re}	7	NH	2.6
1	α_{Re}	4	β	2.7
2	α_{Re}	5	β	2.4
2	γ	5	β	3.7
2	NH	5	β	3.3
3	α	6	β	2.3
3	NH	5	β	3.6
3	NH	6	β	3.2
3	NH	6	γ	5.1
4	β	1	δ	4.3
4	NH	6	β	3.7
4	NH	7	β	3.2
2	β	5	δ	4.4
2	γ	5	NH	5.1
5	NH	7	β	3.8
7	β	4	γ	3.7
H-βhVal-βhAla-βhLeu-*l*-βhAla(α-OH)-βhVal-βhAla-βhLeu-OH (**6**):				
2	α_{Si}	2	β	2.4
2	β	2	NH	2.9
2	γ	2	NH	3.6
3	β	3	α	2.4
3	β	3	NH	2.9
3	δ	3	NH	3.4
3	γ	3	NH	3.4
4	β	4	NH	3.0
4	γ	4	NH	3.5
4	α	4	NH	2.9
5	α_{Re}	5	NH	3.2
5	α_{Si}	5	NH	2.4
5	β	5	NH	3.0
5	γ	5	NH	3.2
6	β	6	NH	2.9
6	α	6	β	2.4
7	α_{Si}	7	NH	2.6
7	α_{Re}	7	NH	2.8
7	β	7	NH	2.9

Table 2 (cont.)

Residue	H-Atom	Residue	H-Atom	Distance [Å]
1	β	2	NH	4.4
2	α_{Re}	3	NH	2.5
2	β	3	NH	3.6
2	NH	1	α_{Si}	2.6
2	NH	1	α_{Re}	2.3
2	NH	3	β	4.6
4	α	5	NH	2.6
4	β	5	NH	3.2
5	α_{Si}	6	NH	3.1
5	α_{Re}	6	NH	2.3
3	β	2	NH	4.6
7	NH	6	α	2.8
1	α_{Si}	4	β	5.8
1	α_{Re}	4	α	3.3
2	α_{Re}	4	α	3.7
2	α_{Re}	5	β	2.6
2	NH	4	α	4.3
2	NH	4	β	3.7
2	NH	5	β	3.8
3	NH	5	β	3.5
4	γ	2	NH	4.8
4	β	6	α	3.9
H-βhVal-βhAla-βhLeu-*u*-βhAla(α-OH)-βhVal-βhAla-βhLeu-OH (**7**):				
1	α_{Si}	2	NH	3.0
1	α_{Re}	2	NH	2.6
1	β	2	NH	4.1
2	α_{Si}	3	NH	3.8
2	α_{Re}	3	NH	2.5
1	α_{Si}	4	β	3.1
1	α_{Re}	4	β	2.5
1	β	2	β	4.4
1	γ	4	β	3.9
2	β	3	NH	4.2
2	NH	3	β	5.1
2	NH	3	NH	4.3
3	α_{Si}	4	NH	3.8
3	α_{Re}	4	NH	2.5
3	δ	4	β	4.4
3	NH	4	NH	4.0
4	α	5	γ	4.4
4	α	5	NH	3.2
4	β	5	NH	4.2
3	β	4	NH	4.2
4	NH	5	β	5.0
4	NH	5	NH	4.0
4	α	5	α_{Si}	5.2
5	β	6	NH	4.2
6	α_{Si}	7	NH	2.9
6	α_{Re}	7	NH	2.7
1	α_{Si}	4	α	5.1
1	α_{Si}	4	β	3.1
1	α_{Re}	4	NH	2.4
1	β	4	β	5.1
1	δ	4	α	4.6
2	α_{Re}	5	β	2.4
2	α_{Re}	6	γ	4.6
2	γ	5	β	3.6
2	NH	4	α	4.8

Table 2 (cont.)

Residue	H-Atom	Residue	H-Atom	Distance [Å]
2	NH	5	β	3.4
2	NH	5	γ	5.3
3	α_{Re}	6	β	2.3
3	α_{Re}	6	γ	5.6
3	NH	5	β	3.7
3	NH	6	β	3.0
3	NH	6	γ	5.6
4	NH	6	β	3.1
4	NH	6	γ	4.9
5	NH	7	β	3.7

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