Synthesis and CD Spectra of Fluoro- and Hydroxy-Substituted β-Peptides

Preliminary Communication

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β-Amino acids **1**–**3** with OH and F substituents in the α-position have been prepared (*Scheme*) from the natural (*S*)-α-amino acids alanine, valine, and leucine, and incorporated into β-hexa- and β-heptapeptides **4**–**12**. The peptide syntheses were performed according to a conventional solution strategy (Boc/Bn protection) with fragment coupling. The new β-peptides with (series **a**) and without (series **b**) terminal protection were isolated in HPLC-pure form and characterized by NMR spectroscopy and MALDI mass spectrometry. The chemical properties as well as the patterns of the CD spectra (*Figs. 3–5*) depend upon constitution (OH, F, F_2 substitution) and configuration (*l* or *u*) of the amino acid residues, upon the total number of OH and F substituents in the peptide chain, and upon the solvent used (H_2O, MeOH, CF_3CH_2OH, $(CF_3)_2CHOH$). No reliable clues regarding the structures can be obtained from these CD spectra. Only a full NMR analysis will be able to answer the questions: *a*) with which known secondary structures (*Figs. 1* and *2*) of β-peptides are the OH and F derivatives compatible? *b*) Are new secondary structures enforced by the polar and/or H-bonding backbone substituents? Furthermore, the β-peptides described here will enable us to study changes in chemical, enzymatic, and metabolic stability, and in physiological properties caused by the heteroatoms.

Structural evidence from NMR, X-ray, molecular-modelling, and CD investigations of β-peptides containing the side chains of proteinogenic amino acids has provided rules for the design of the secondary structures 3_{14} helix and strands [1], while the possible substitution patterns and configurations of amino acids in the β-peptidic hairpin turn and *12/10* helix have not been probed as well [2]. As outlined in *Fig. 1*, steric hindrance should prevent non-H-atoms from occupying axial positions in the 3_{14} helix and in-plane position in a sheet (see black spheres in *Fig. 1*,*a* and *b*), which has been concluded mainly from investigations with Me substituents (**A**, **B**, and **C** in *Fig. 2*). The *Van der Waals* radius of a Me group is of course much larger than that of a H-atom, and we wondered whether an F- or O-atom, or an OH group might fit the forbidden position next to the C=O group (*cf.* the A values in *Fig. 1*,*c*, and **D**, **E**, **F**, and **G** in *Fig. 2*). As compared to the H-atom, these atoms have, of course, not only a somewhat different size but also an electronic effect (*Pauling* electronegativities H 2.2, C 2.6, O 3.4, F 4.0) and the ability of forming H-bonds[4]). Thus, another issue to be addressed by the present study was to see what influence the substituents F and OH might exert on

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[3]) Part of the Dissertation of *M. R.* (No. 14677), ETH-Zürich, 2002.

[4]) For a discussion of the H-bond-acceptor properties of organic fluoride, see [3].

the backbone conformation of a β-peptide when placed in allowed positions (green spheres in *Fig. 1*), an effect that cannot possibly be studied in α-peptides[5][6]. Finally, the F- and OH-substituted β-peptides will offer an opportunity to determine what effects the enhanced electrophilicity of the amide C=O groups has on their biological properties (such as antimicrobial and haemolytic activity, enzymatic and metabolic stability, or biodegradability[7]).

The required enantiomerically pure *N*-Boc-protected 3-amino-2-fluoro and 3-amino-2-hydroxy carboxylic acids with the side chains of alanine, valine, and leucine were prepared as shown in the *Scheme*. The *N,N*-dibenzyl-protected aldehydes prepared from the amino acids by standard procedures [8] were subjected to a *trans*-cyanohydration process (cyanohydrine of acetone/KCN/cat. Bu_4NI [9]) to give mixtures (1:2 to 1:1) of epimeric cyanohydrins. This nonselective reaction was practical in the present investigation, since we needed to have both, the *l*- and the *u*-diastereoisomers of the 3-amino-2-hydroxyalkanoates, and since it did not make any difference which epimer was used for the conversion to the difluoro derivatives. Methanolysis of the epimeric dibenzylamino-cyanohydrins, followed by chromatographic separation of the hydroxy methyl esters, debenzylation, *N*-Boc protection, and saponification, led to the configurationally pure acids **1a**–**1c**. On the other hand, the diastereoisomeric *N,N*-dibenzyl-protected hydroxy methyl esters were converted to the 2-fluoromethyl esters by retentive OH/F substitution with DAST [10][11], saponified, debenzylated, and *N*-Boc-protected to give the acids **2a**–**2c** of either (*R*,*S*)- or (*S*,*S*)-configuration. Oxidation of the epimeric mixtures of 2-hydroxy methyl esters to 2-keto esters, which do not racemize if chromatographic purification is avoided, treatment with DAST [10], saponification, debenzylation, and *N*-Boc protection provided the 2,2-difluoro carboxylic acids **3a**–**3c** (*Scheme*). For the C-terminal amino acid residues of the β-peptides to be prepared (see **4**–**13**) by solution coupling of *N*-Boc-protected hydroxy and fluoro amino acids, the benzyl esters of **1c**, **2c**, and **3c** were required; they were prepared by treatment of the corresponding Cs salts with BnBr [12].

With the suitable hydroxy and fluoro amino acids at hand, we synthesized the β-tri-, β-hexa-, and β-heptapeptides **4**–**13** with various numbers of hydroxy or fluoro amino acids incorporated. All syntheses were carried out in solution and according to the same principle: starting from the benzyl ester of the C-terminal Leu-derived hydroxy or fluoro amino acid, a tripeptide was built up[8]), which was then employed for a dimerizing fragment coupling to an *N*- and *C*-protected hexapeptide **6a**, **10a**, **11a**, and **12a**. When a central OH-, F-, or F_2-substituted β-amino acid was to be incorporated, the β-tripeptide with the side chains of Val, Ala, and Leu was first coupled with the corresponding heterosubstituted β-amino acid, and, to the resulting tetrapeptide, another β^3hVal-β^3hAla-β^3hLeu fragment was attached to give the β-heptapeptides **4a**, **5a**, **7a**, **8a**, and **9a**. Full deprotection (Bn and *N*-Boc cleavage) of β-peptides **4a**–**9a**,

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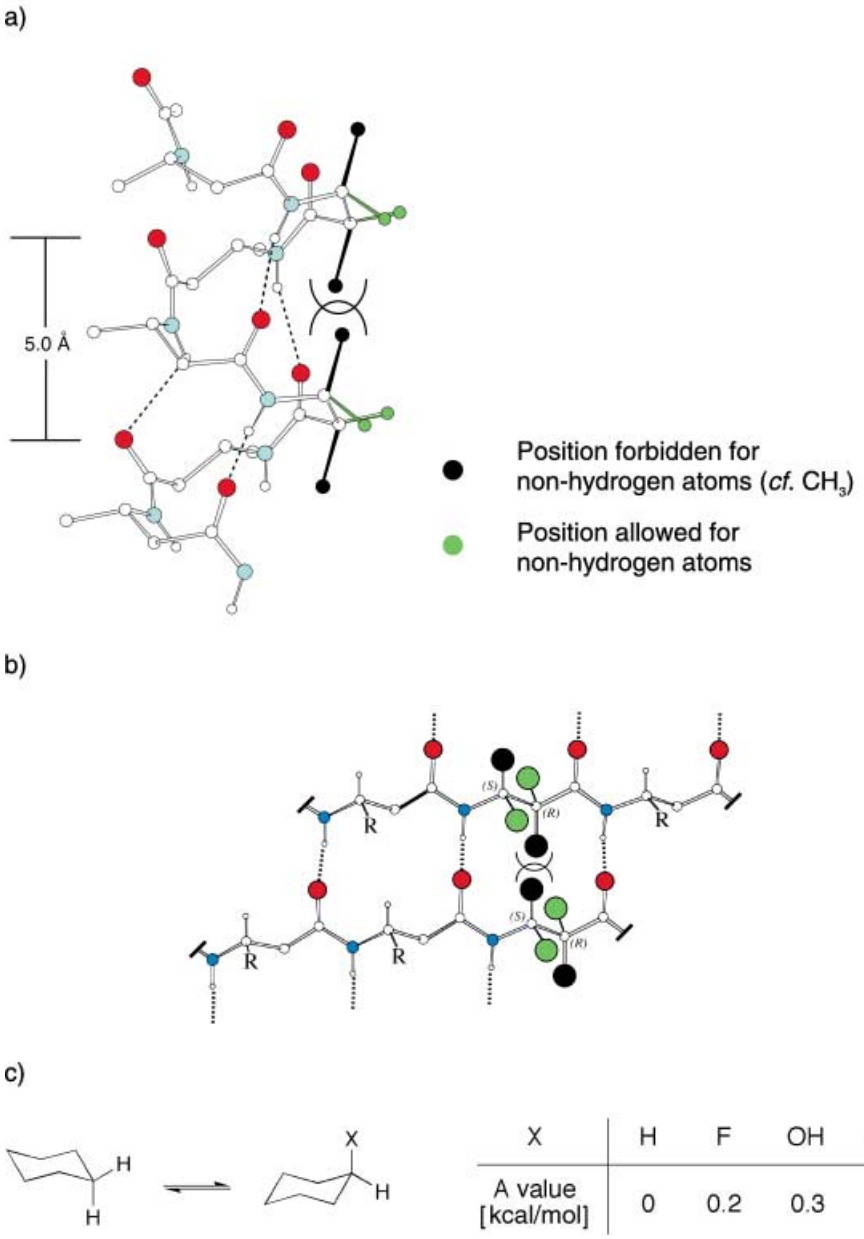
[5]) For a general discussion of OH substitution in β- and γ-peptides, see [4].

[6]) We know already that a β-peptide consisting of *unlike*-3-amino-2-hydroxy carboxylic acid moieties (*cf.* **11**) folds to a novel 2_8 helix (probably due to H-bond formation of OH with the neighboring C=O O-atom [5]), rather than adopting a sheet structure [6].

[7]) For investigations of these properties of 'normal' β-peptides, see [7], and refs. cit. therein.

[8]) Boc-β^3hVal-β^3hAla-β^3hLeu-OBn has been used by us many times before [1].

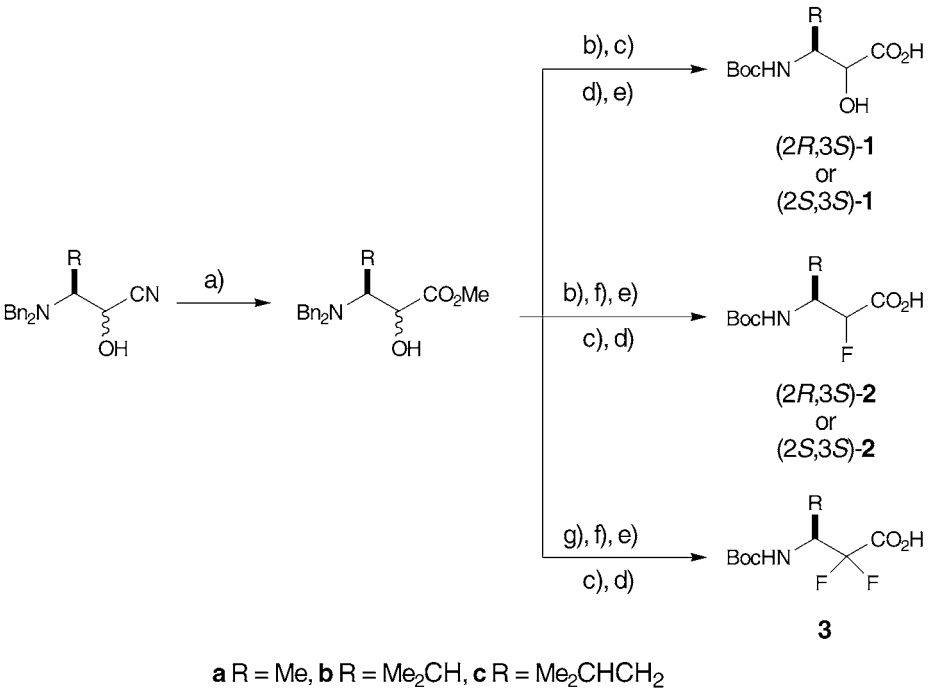


X	H	F	OH	CH_3
A value [kcal/mol]	0	0.2	0.3	1.7

Fig. 1. *Model of the* (M)-3_{14} *helix of a* β*-peptide* (a) *and of the* β*-peptidic parallel sheet structure* (b) *and* A *values for preference of equatorial* vs. *axial position on cyclohexane* (c)

followed by reversed-phase HPLC purification, provided the *N*- and *C*-terminally unprotected β-peptides **4b** – **9b**.

Scheme. *Preparation of the* N-*Boc-Protected 3-Amino-2-fluoro, 3-Amino-2,2-difluoro, and 3-Amino-2-hydroxy Carboxylic Acids* **1**–**3** *from the Proteogenic Amino Acids Alanine, Valine, and Leucine.* The OH/F substitution with DAST is carried out with the *N,N*-dibenzyl-protected, diastereomerically pure methyl 3-amino-2-hydroxyalkanoates and takes place with retention of configuration (as established by X-ray analysis of one of the products and spectroscopic assigment by analogy). Boc = *t*-(Butoxy)carbonyl, DAST = Et_2NSF_3.



a R = Me, **b** R = Me_2CH, **c** R = Me_2CHCH_2

a) HCl (gas)/MeOH. b) Chromatographic separation of epimers (SiO_2; hexane/AcOEt). c) H_2/Pd-C, MeOH. d) Boc_2O, Et_3N, MeOH, 20°. e) LiOH · H_2O, EtOH. f) DAST, CH_2Cl_2, 0°. g) *Swern* oxidation: DMSO, $SOCl_2$, CH_2Cl_2, −78°.

All hexa- and heptapeptides, of which the formulae are shown herein, have been isolated in high purity (> 97%) and characterized by CD, ^{13}C- and ^1H-NMR spectroscopy, and by MALDI mass spectrometry. The starting materials (amino acid derivatives) and the intermediate di-, tri-, and tetrapeptides have been fully characterized by NMR, IR, MS, $[\alpha]_D$, m.p., and elemental analysis[9]).

Some CD spectra[10]) in 0.2 mM MeOH of the unprotected β^3-heptapeptides, with central F- and OH-substituted amino acid moieties, are shown in *Fig. 3*. They are most puzzling, to say the least, because all show the 3_{14}-helix-typical pattern with a negative *Cotton* effect between 215 and 220 nm, zero crossing between 210 and 205 nm, and a positive *Cotton* effect near 200 nm – no matter whether the F or OH group would occupy a lateral (**4b** and **7b**) or axial position (**5b**, **8b**, and **9b**) on such a 3_{14} helix. As shown in *Fig. 4*, the pattern of the CD spectra of the *u*-mono-hydroxy and of the *u*-

[9]) Full details will be presented in forthcoming full papers.

[10]) All CD spectra shown herein are non-normalized.

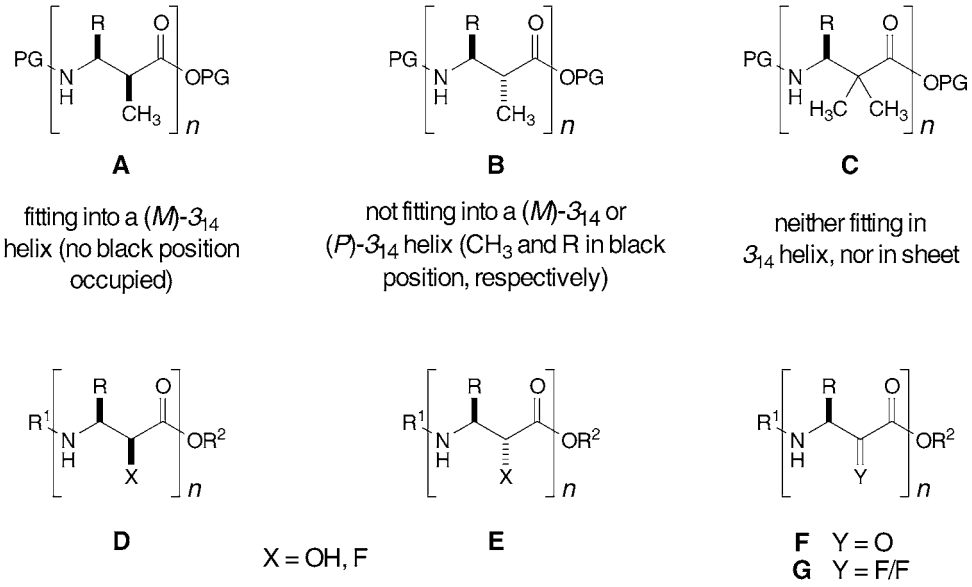


Fig. 2. *General structures of* $\beta^{2,3}$*- and* $\beta^{2,2,3}$*-peptides* **A**–**C** [1] *and of the heterosubstituted* β*-peptides* **D**–**G** *described herein*

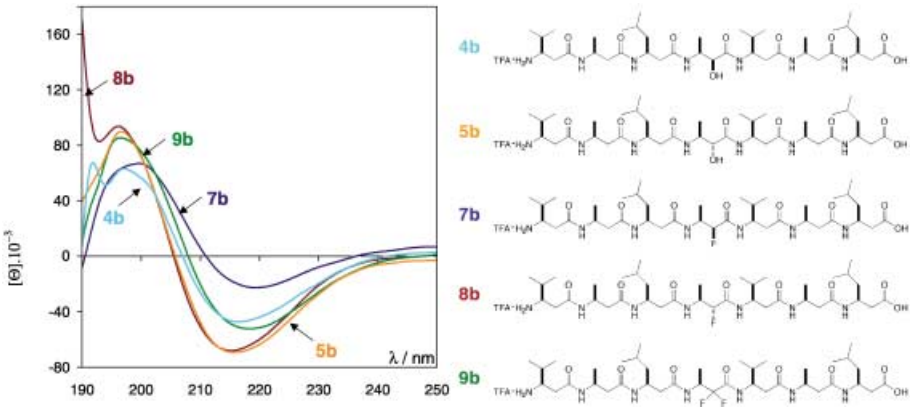


Fig. 3. *Comparison of the CD spectra* (0.2 mM in MeOH) *of unprotected* β*-heptapeptides carrying a central* α*-heterosubstituted* β*-amino acid.* All the new β-heptapeptides exhibit the CD pattern associated to an (*M*)-3$_{14}$-helical structure [1].

mono-fluoro derivatives **5b** and **8b** does not change with the solvent (MeOH, CF_3CH_2OH, $(CF_3)_2CHOH$), except when we switch to aqueous solution, which causes a dramatic effect. Also, the protected and the unprotected[11]) β-peptides of this type may exhibit totally different CD spectra as exemplified by the mirror-image-type

[11]) *Cf.* the stabilization of the 3$_{14}$ helix upon terminal deprotection [1b][13].

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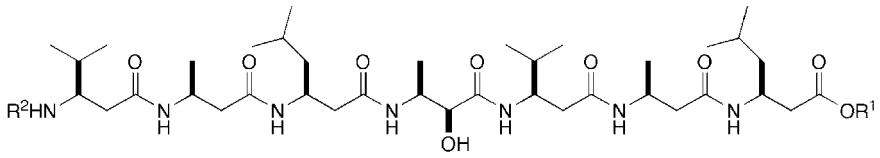

a R^1 = Bn, R^2 = Boc **b** R^1 = R^2 = H (TFA salt)

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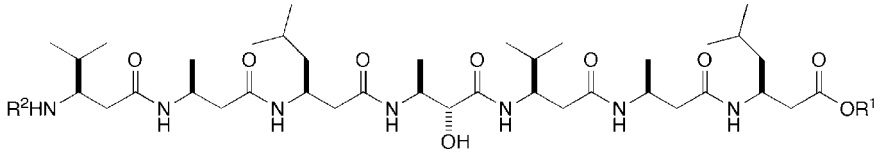

a R^1 = Bn, R^2 = Boc **b** R^1 = R^2 = H (TFA salt)

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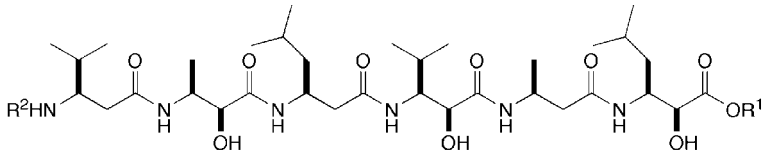

a R^1 = Bn, R^2 = Boc **b** R^1 = R^2 = H (TFA salt)

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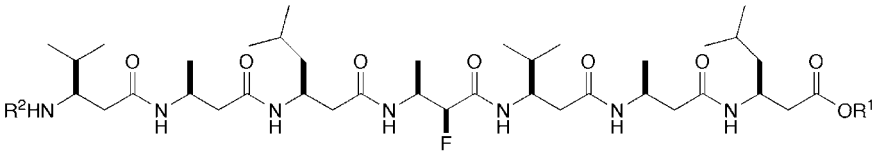

a R^1 = Bn, R^2 = Boc **b** R^1 = R^2 = H (TFA salt)

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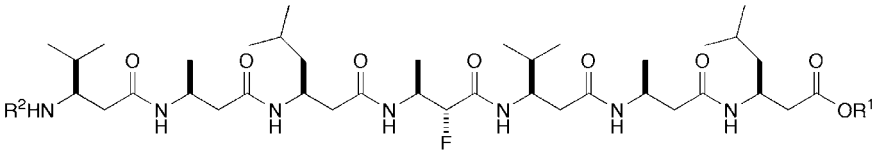

a R^1 = Bn, R^2 = Boc **b** R^1 = R^2 = H (TFA salt)

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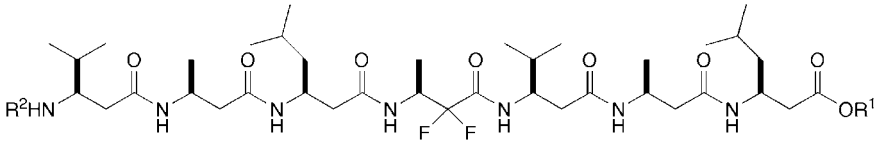
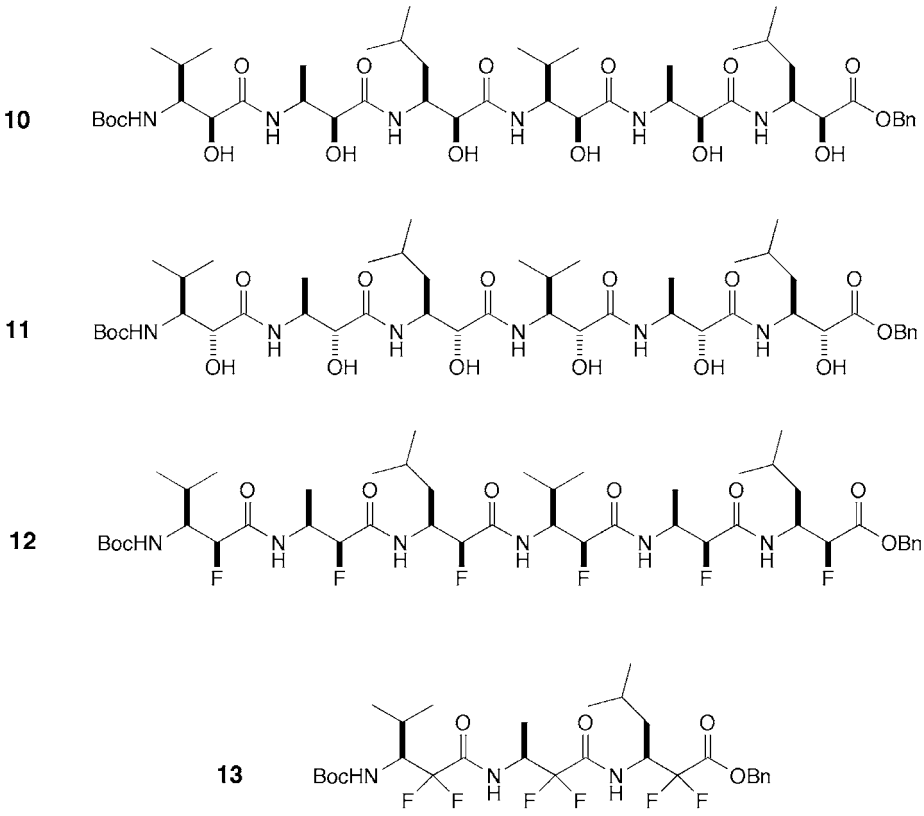


a R^1 = Bn, R^2 = Boc **b** R^1 = R^2 = H (TFA salt)



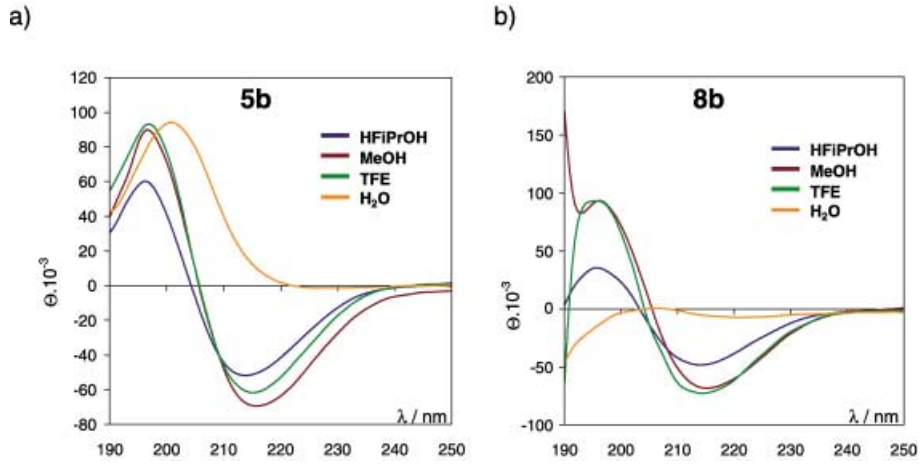


Fig. 4. *Influence of the solvent on CD spectra* (0.2 mM in MeOH, CF_3CH_2OH (TFE), $(CF_3)_2CHOH$ (HF^iPrOH), and H_2O) *of the* u-hydroxy-*β-heptapeptide* **5b** (a) *and of the* u-*fluoro-β-heptapeptide* **8b** (b).

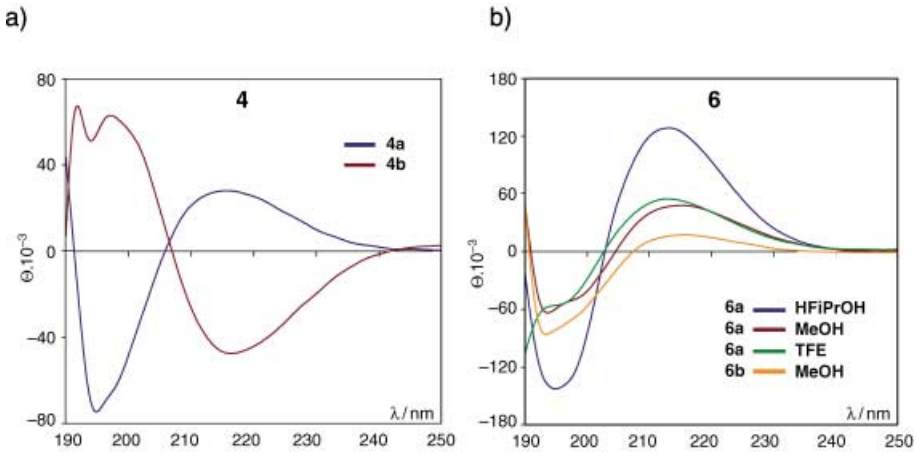


Fig. 5. *CD Spectra of β-peptides* **4** *and* **6** *containing* l-*2-hydroxy-3-amino acid moieties. a*) CD Spectra in MeOH of the terminally protected and unprotected *l*-hydroxy-*β*-heptapeptides, **4a** and **4b**, respectively. *b*) Overlay of the CD spectra of the protected hexapeptide **6a** (in different solvents) and of its unprotected form **6b** (in MeOH).

curves obtained for the *l*-mono-hydroxy derivatives **4a** and **4b** in MeOH (*Fig. 5*,*a*). The all-*l*-trihydroxy-hexapeptide, on the other hand, shows CD spectra of the same general pattern, whether it is protected or unprotected, or whether MeOH or the fluorinated solvents are used (*Fig. 5*,*b*).

Thus, on the basis of the CD spectra, it is impossible to arrive at any safe structural assigment. If we would use analogy with the typical CD spectra of *β*-peptides, which, according to NMR analysis, form a 3_{14} helix [1][13], a *12/10* helix [1][14], or a hairpin turn [6], we would, for instance, have to assign (*M*)-3_{14}-helical structure to **4b**, **5b**, **7b**, **8b**, and **9b**, (*P*)-3_{14}-helical structure to **4a** in MeOH and to **6a** in $(CF_3)_2CHOH$, and hairpin or *12/10*-helical structure to **5b** in H_2O! Thus, CD spectroscopy of *β*-peptides, once again, turns out to be useless for structure determination [15]. At this point, it can provide only a preliminary hint as to which compounds might be most interesting to investigate by NMR spectroscopy first! We have performed or are in the process of performing extensive, state-of-the-art, high-field NMR measurements of most of the novel *β*-peptides mentioned herein. The interpretation of these measurements will require time[12]), and the results will be published in due course. There will be, no doubt, many surprises.

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[12]) It usually takes *ca*. 3.5 days to perform the measurements (1D Presat, DQFCOSY, TOCSY, HSQC $^{13}C/^{1}H$, HMBC $^{13}C/^{1}H$, ROESY with different mixing times in ms), and it may take several weeks or even months for the evaluation of the data.

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