Structure and Conformation of β-Oligopeptide Derivatives with Simple Proteinogenic Side Chains: Circular Dichroism and Molecular Dynamics Investigations[1])

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Dedicated to Prof. *Günther Wulff* on the occasion of his 65th birthday

A careful CD analysis (*Figs. 1–3* and *5*; MeOH or H_2O solutions) of β-oligopeptides (**1**–**6**, **B**, **C**) containing four to seven β-amino acids reveals that seemingly small structural changes cause a switch from the CD pattern (maxima of opposite sign near 215 and 200 nm) associated with a 3_{14}-helical structure to the CD pattern (single *Cotton* effect at *ca.* 205 nm) considered characteristic of a so-called *12/10*-helical structure, but also exhibited by a β-peptide adopting a hair-pin conformation with a ten-membered H-bonded ring as the turn motif. Comparison of these CD spectra with those of the *trans*-2-aminocyclohexanecarboxamide oligomers, which give rise to the long-wavelength *Cotton* effect only, suggests that the H-bonded 14-, 12-, and 10-membered ring conformations of the β-peptides, and not just the entire helix structures, might actually generate the *Cotton* effects. This interpretation would be compatible with our previous NMR structure determinations of β-peptides and with previously reported temperature dependences of CD and NMR spectra of β-peptides. To further substantiate this suggestion, we have performed a statistical analysis of the β-peptidic conformations generated by molecular-dynamics calculations (GROMOS96) for a β-hexapeptide (**C**; *the 12/10* helix) and a β-heptapeptide (**6**; *the* 3_{14} helix) in MeOH (*Figs. 6–9*). Up to 400,000 conformations at 0.5-ps intervals were analyzed from up to 200-ns simulations (at 298 to 360 K). The analysis reveals the co-existence of the various H-bonded rings. Remarkably, the central section of the β-peptide **6** (containing a $\beta^{2,3}$-amino-acid residue of *like*-configuration!) adopts a ten-membered-ring conformation for *ca.* 5% of the simulation time, while the central section of the β-peptide **C** adopts a 14-membered-ring conformation for *ca.* 3% of the time, according to this computational analysis. Further experimental and theoretical work will be necessary to find out to which extent the components (H-bonded rings) and the entire helical secondary structures of β-peptides contribute to the observed *Cotton* effects.

1. Introduction. – As for α-peptides (*Fig. 1,a*) CD spectroscopy is a most important method for obtaining a first hint as to whether or not a secondary structure of a β-peptide is present in solution, and which one it might be. Since our first paper on β^3-peptides, consisting entirely of homologated α-amino-acid residues [5], we had collected enough data [6–9] to assign with some confidence a CD pattern in MeOH with a trough between 215 and 220 nm, a zero crossing between 205 and 210 nm, and a

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1) Partially published in preliminary communications [1][2].
2) Part of the projected Dissertation of *J. V. S.*, ETH-Zürich.
3) Part of the Dissertation No. 13203 by *S. A.*, ETH-Zürich, 1999.
4) Correspondence on molecular-dynamics part.

peak near 200 nm (*Fig. 1,b*) to a β-peptidic 3_1 or 3_{14} (*M*)-helix. We also have discovered [10] that seemingly small changes of isomeric positioning of side chains [7][10], of leaving out a single side chain in the center of a protected β-heptapeptide [6], or of having the termini protected or not [6][7][11][12], may cause the β-peptides to exhibit a totally different, more simple CD spectrum with a single, strong *Cotton* effect between 200 and 205 nm (*Fig. 1,c*). The formula of a compound, which gives rise to this latter type of a circular dichroism, is shown in *Fig. 1,c*, together with a model of the NMR solution structure in MeOH, which is a *12/10* (*P*)-helix. The subtle structural differences, causing changes of the CD spectra, have led to publication of a non-reproducible curve by us[5]). In an effort to find out what exactly the structural prerequisites are for a β-peptide derivative with simple aliphatic proteinogenic side chains to exhibit one or the other CD pattern, and with the goal of identifying the conformation or sub-structure that causes the characteristic pattern, we have now measured the spectra of a series of previously prepared and of some new β-tetra-, β-hexa-, and β-heptapeptides **1**–**6**, and we have made a more detailed analysis of the corresponding secondary structures produced by the GROMOS96 molecular dynamics calculations [13-15].

2. Preparation of the β-Peptides 1–5. – The required β^2- and β^3-amino-acid derivatives were all prepared by the previously described methods (*Arndt-Eistert* homologation of α-amino-acid precursors, aminomethylation of *Evans*-type Ti-enolates, α-methylation of β-amino-acid derivatives)[6]). For solution synthesis and fragment coupling, *N*-Boc-protected β-amino acids were employed. For solid-phase syntheses, we have used the Fmoc strategy. Of the β-peptides **1a**–**1f**, **2a** and **2b**, **3a**–**3c**, **4**, and **5**, the synthesis has been described previously for compounds **1a**–**1d** [5], **2b**, and **3a** and **3c** [6]; for these compounds we give some more preparative details in the *Exper. Part*. The new compounds **1e** and **1f**, **2a**, **3b**, **4**, and **5**[1]) are fully described herein. For the preparation of the required $\beta^{2,3}$-amino-acid derivatives **9** and **10**, we used the methylation of dilithium derivatives [17] of the Boc-protected methyl esters **7** and **8**, with chromatographic separation of the *like*- and *unlike*-isomers **a** and **b** [7]. The $\beta^{2,3}$-dimethyl derivative **11** [18], on the other hand, was prepared from *t*-butyl tiglate [19], following the *Davies* procedure [20] (see the *Scheme*)[7]). β-Peptides **1**–**3** were synthesized in solution under standard coupling conditions [5–7] and the β-tetrapeptide and β-hexapeptide derivatives **4** and **5**, respectively, on solid support [1][8][21] (*Rink* and *ortho*-chlorotrityl chloride resin, resp.).

3. CD Spectra of the β-Peptides 1–6. – The CD spectra were measured in MeOH or in aqueous 0.2 mM solutions (*Figs. 1–3*). Spectra of β^3-hexapeptides and β^3-heptapeptides, consisting of homochiral[8]), homologated L-amino-acid residues, are

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5) See the CD of the fully protected hexapeptide in Fig. 4,a, in [6], and compare with *Fig. 2,a*, in *Sect. 3* of the present paper.

6) See the full paper [7], and references cited therein. For a useful new *Evans*-type oxazolidinone, see [16].

7) The two isomers obtained by methylation could only be separated by preparative HPLC [7].

8) We use *homochiral* in *Lord Kelvin*'s definition; see the chapters by *G. Helmchen* in [22], and references cited therein. Unfortunately, the word homochiral is still used by some to refer to enantiomerically pure samples of compounds.

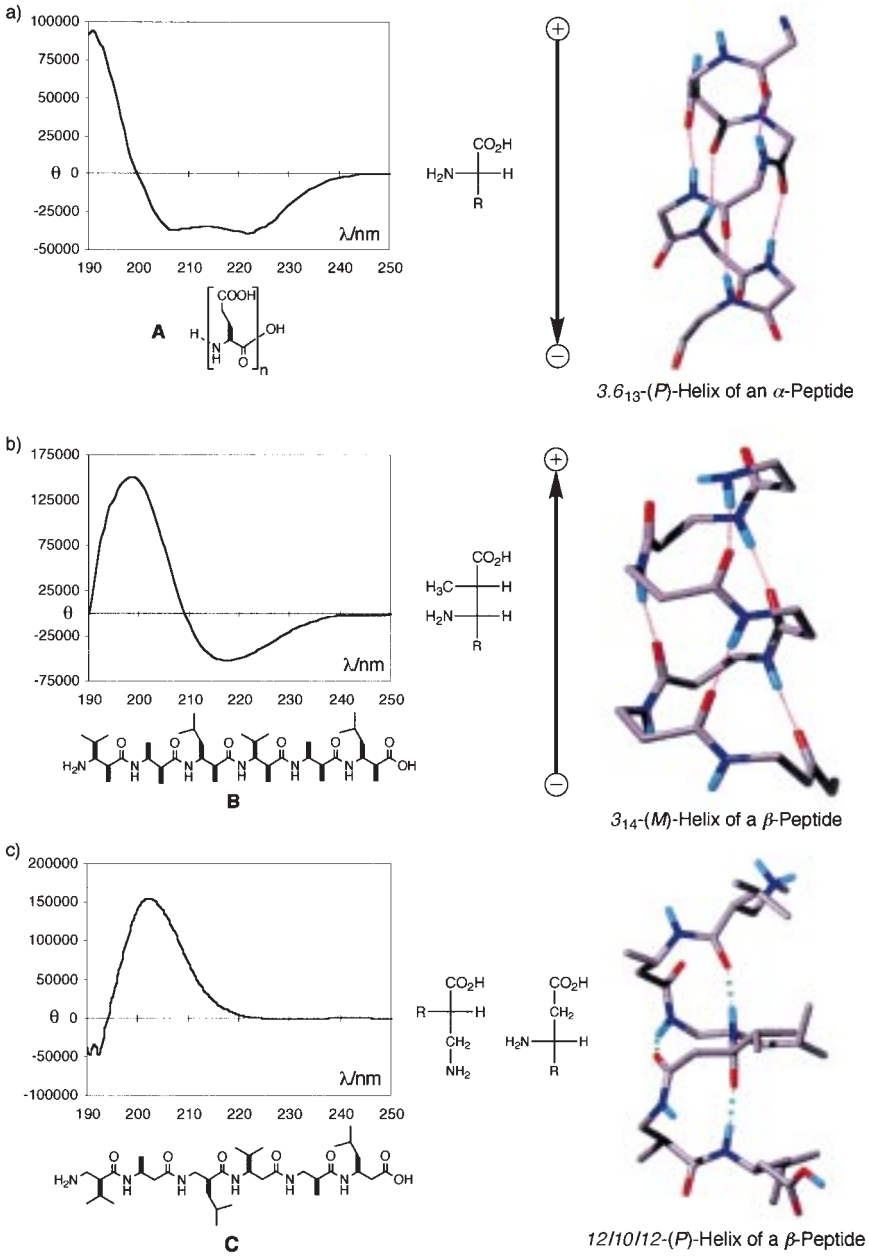
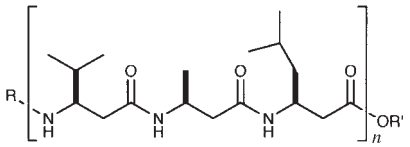
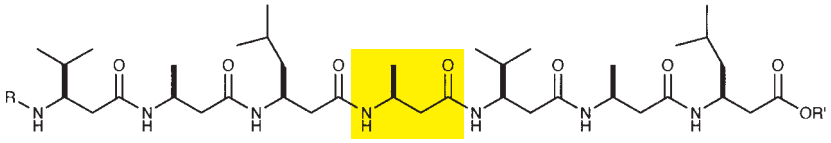


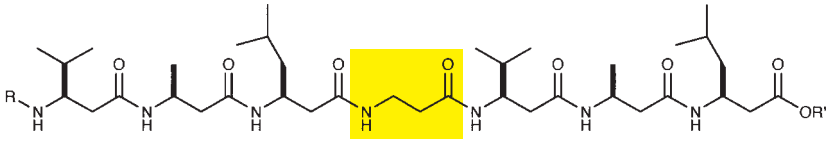
Fig. 1. *Characteristic CD pattern of α- and β-peptides forming helices. a*) CD Spectra of poly(Glu) measured in aqueous solution at pH 4.5 with a concentration of *ca.* 5 mg/ml (θ is on a per amide basis) [3] and the structure of a 3.6_{13}-(*P*)-helix-forming α-octapeptide. The coordinates have been extracted from a protein X-ray structure [4]. *b*) and *c*) CD curves of an all-*like*-$\beta^{2,3}$-peptide and of a mixed β-peptide measured in MeOH (0.2 mM). The coordinates used for the β-hexapeptides correspond to a solution structure examined previously by us [5–7]. The spectra were recorded at r.t. Molar ellipticity [θ] in 10 deg · cm^2 · mol^{-1}. The β-peptides were measured as their TFA salts as obtained after lyophilization.



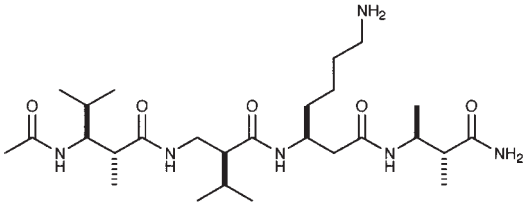
1a $n = 2$ R = H R' = H as trifluoroacetate
1b $n = 2$ R = H R' = Me as trifluoroacetate
1c $n = 2$ R = Boc R' = H
1d $n = 2$ R = Boc R' = Me
1e $n = 2$ R = H R' = Bn as trifluoroacetate
1f $n = 2$ R = Boc R' = Bn



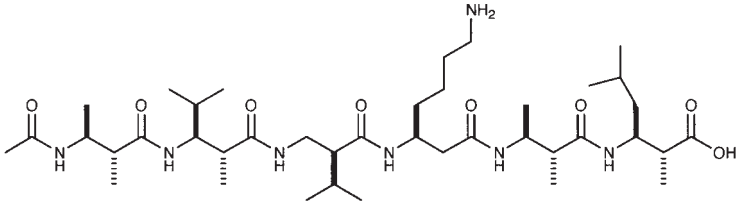
2a R = H R' = H as trifluoroacetate
2b R = Boc R' = Me



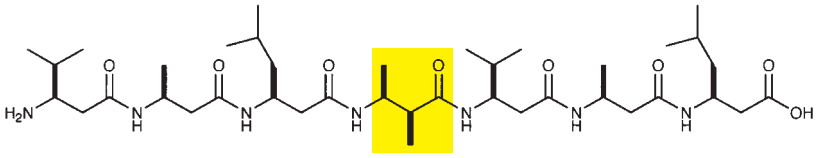
3a R = H R' = H as trifluoroacetate
3b R = Boc R' = H
3c R = Boc R' = Me



4 as trifluoroacetate

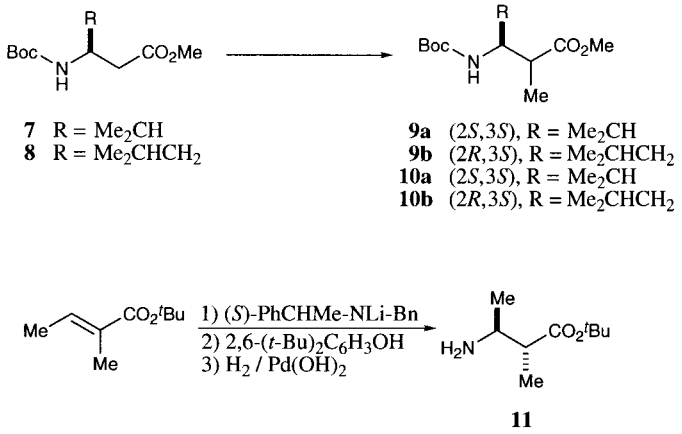


5 as trifluoroacetate



6 as trifluoroacetate

Scheme. *Preparation of the α-Methyl-Substituted β-Amino-Acid Derivatives* **9**–**11**



7 R = Me_2CH
8 R = Me_2CHCH_2

9a (2*S*,3*S*), R = Me_2CH
9b (2*R*,3*S*), R = Me_2CHCH_2
10a (2*S*,3*S*), R = Me_2CH
10b (2*R*,3*S*), R = Me_2CHCH_2

11

presented in *Fig. 2*. It is important to mention at the outset of our discussion that we have no indication for aggregations of the β-peptides included in this investigation, in the concentration range used for CD measurements.

If we consider the negative *Cotton* effect between 210 and 220 nm as characteristic of the β-peptidic 3_{14} helix, we must draw the following conclusions for β-peptides with simple aliphatic side chains (of Ala, Val, Leu): fully protected (Boc-NH/MeO or BnO) β-hexapeptides, such as **1d** and **1f**, do not form the helix, a seventh β-amino acid with aliphatic side chain is necessary for the 215-nm *Cotton* effect to appear (compare **2b** with **3c**). β-Peptides with Boc-protected N- and unprotected C-terminus (**1c** and **3b**) also exhibit no or only a weakly negative *Cotton* effect in this wavelength range, while a derivative with free N- and esterified C-terminus (such as **1b**) gives rise to the full intensity. All fully deprotected β^3-hexa- and β^3-heptapeptides (**1a**, **2a**, **3a**, and **6**) show the *Cotton* effect with intensities $\theta = -4.0 \cdot 10^4$ to $-9.2 \cdot 10^4$ [$10\ deg \cdot cm^2 \cdot mol^{-1}$]. Besides the fully developed negative *Cotton* effect near 215 nm, these β-peptides also show a more intense ($\theta = +7.7 \cdot 10^4$ to $+1.3 \cdot 10^5$), positive band near 200 nm which is to be considered with due care: it is near the strong $\pi\pi^*$ UV absorption of amides ($\lambda_{max} =$ 204 nm, $\varepsilon = 1.9 \cdot 10^4$ for the unprotected β-peptide **1a**; see *Fig. 4*), and near the limiting wavelength of the instrument and the solvent[9]). On the other hand, the CD spectra lacking the negative band at around 215 nm are all curves with maxima between 200 and 210 nm[10]) (see *Fig. 2*, **1d**, **1f**, **3b**, and **3c**).

Furthermore, both the β-hexapeptide **C** which has been shown by NMR analysis of a MeOH solution to exist mainly as a *12/10* helix (*Fig. 1,c*) [7][10], and the β-hexapeptide **5**, the NMR structure of which is a hairpin, have CD spectra (*Fig. 3,b*),

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[9]) On the borderline of the vacuum-UV part of the spectrum and on the limit for solvents containing heteroatoms with non-bonding electron pairs [23].

[10]) ...just a bit, but clearly above the wavelength of the second band in CD spectra associated with the β-peptidic 3_{14} helix!

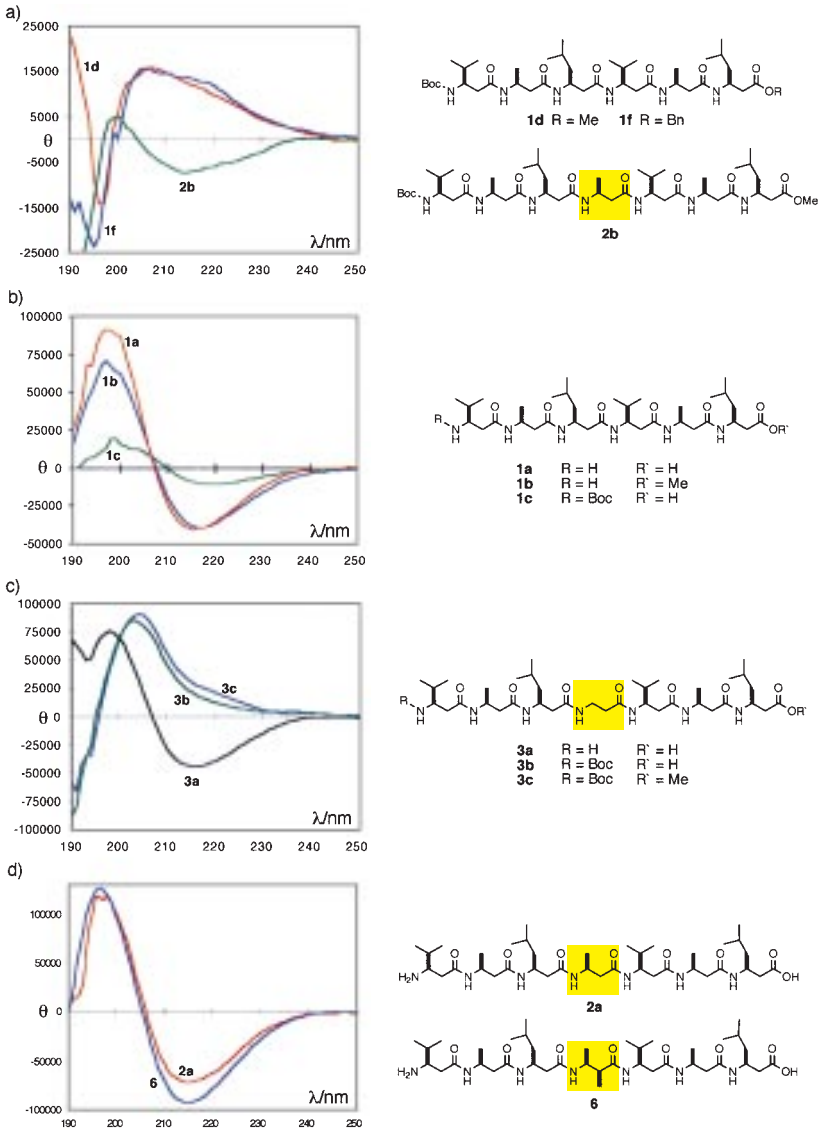

Fig. 2. *CD Spectra of terminally protected and partially and fully unprotected β-hexa- and β-heptapeptides. a*) CD Curves of fully protected β^3-hexapeptides (**1d** and **1f**) and β^3 heptapeptide (**2b**). Considering the negative *Cotton* effect between 210 and 220 nm as characteristic of the β-peptidic 3_{14} helix, we can conclude that a seventh β-amino acid with aliphatic side chain (!) is needed for the characteristic CD pattern to appear. *b*) CD Curves of partially protected and fully unprotected β-hexapeptides **1a**–**1c**. Whereas the *N*-Boc-protected and *C*-unprotected derivative **1c** exhibit only a weak negative *Cotton* effect, the *N*-unprotected and *C*-protected derivative **1b**, as well as the fully unprotected derivative **1a** show the full intensity. *c*) The CD spectra of the fully and partially protected and fully unprotected β-heptapeptides **3a**–**3b** with a central β-homoglycine. In this series, only the fully deprotected derivative **3a** exhibits the typical CD pattern of a 3_{14} helix. *d*) Overlay of the CD spectra of the β-heptapeptides **2a** and **6**. As expected, the additional Me group in β-peptide **6** causes a somewhat more negative *Cotton* effect (*cf.* our model study in [6]). The spectra were recorded at r.t. The concentration was 0.2 mM in MeOH. Molar ellipticity $[\theta]$ in 10 deg·cm^2·mol^{-1}. The deprotected β-peptides were measured as their TFA salts as obtained after lyophilization or drying under high vacuum.

with single maxima between 202 and 208 nm, and so does the simple β-tetrapeptide **4** (*Fig. 3,a*)[11]).

These latter two β-peptides exhibit an opposite dependence from pH of the molar ellipticity in H_2O: **4** gives rise to a somewhat stronger *Cotton* effect at low pH (3.6), whereas **5** gives a *much* more intensive peak at higher pH (11.0) (*Fig. 3*).

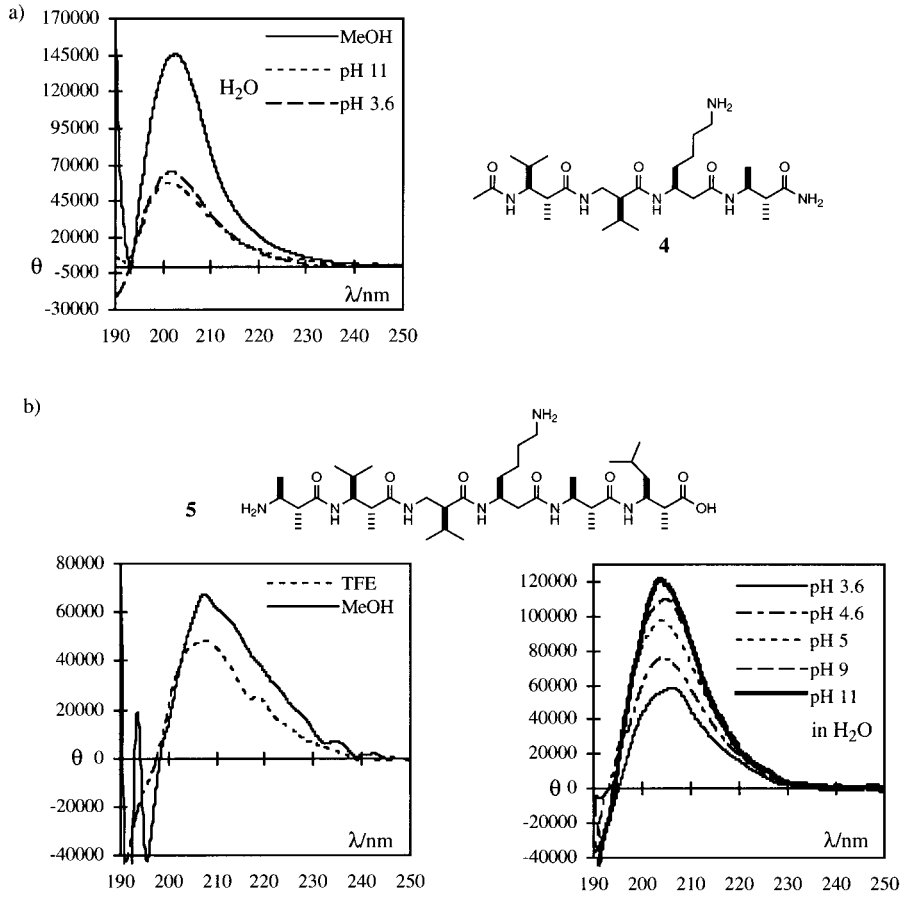

Fig. 3. *CD Spectra of β-peptides constructed so that they cannot form helices. a*) CD Spectra of β-tetrapeptide **4** in MeOH and in H_2O (basic and acidic) solutions. *b*) CD Curves of β-hexapeptide **5** which folds into a hairpin structure in CD_3OH solution as determined by NMR [2]. The curves specified by pH values all refer to aqueous solutions (see *Exper. Part*). The spectra were recorded at r.t., at a concentration of 0.2 mM. Molar ellipticity [θ] in 10 deg·cm^2·mol^{-1}. The deprotected β-peptides were measured as their TFA salts as obtained after lyophilization or drying under high vacuum.

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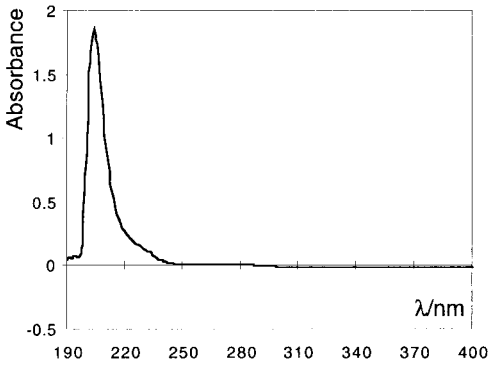
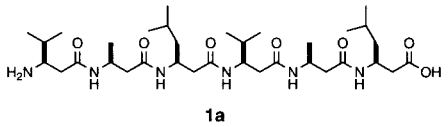
11) It may be concluded from the CD spectrum of **4** that this β-tetrapeptide forms the same hairpin conformation in MeOH solution as does the β-hexapeptide **5** (by NMR analysis [2]), although **4** contains only two 'linear' β-amino acids of *unlike*-configuration. Considering the two additional amide bonds at the *termini* of **4**, this may not be too surprising, after all, because this structural modification (MeCO–NH–CO–NH_2) allows for formation of additional H-bonds, a trick well-known in α-peptide chemistry.





1a

Fig. 4. *UV Spectrum of β-hexapeptide* **1a** *in MeOH*. The Spectrum was recorded at r.t., at a concentration of 0.1 mM. The deprotected β-peptide was measured as its TFA salts as obtained after drying under high vacuum.

We have previously discussed the effects which might influence relative stabilities of the β-peptidic 3_{14} and *12/10* helices (hydrophobic interaction between the aliphatic side chains, charge/pole attractive forces, steric repulsion between substituents on the ten-membered H-bonded ring) [7]. We have now shown that the β-peptides we know to contain the ten-membered H-bonded ring as a secondary structural element (at the site of a dipeptidic section containing a β^2- and β^3-amino-acid sequence), *i.e.*, **C** (*12/10* helix; *Fig. 1,c*) and **5** (hairpin, *Fig. 3,b*), give rise to an intensive, single positive *Cotton* effect between 200 and 210 nm. Furthermore, we notice that β-hexapeptide **D** consisting of (*S,S*)-2-aminocyclohexanecarbonyl residues shows only the negative *Cotton* effect between 215 and 220 nm, and *no* zero-crossing of the CD curve at shorter wavelength [24] (*Fig. 5,a*). It was *Gellman* who questioned recently [24] whether the positive short-wavelength *Cotton* effect exhibited by our β-peptides with conformationally non-restricted backbones might originate from a secondary structure other than the 3_{14} helix[12]). Strikingly, summation of the CD curves of **C** (*12/10* helix) and **D** (3_{14} helix) results in a curve which is, except for the relative intensities of trough and peak, not dissimilar from that measured with the β-peptide **1a** (*Fig. 5,a*). On the other hand, all β-peptides, the NMR structures of which have been shown by us to be 3_{14}-helical, exhibit the two extrema in the CD spectrum, usually with higher intensity of the short-wavelength *Cotton* effect, and, over the years, the measurements have been carried out on at least three different spectrometers, with numerous bottles of MeOH (UV-grade) of different batch numbers.

The following conclusions suggest themselves[13]). *i*) Each type of H-bonded ring (10-, 12-, and 14-membered), which β-peptides form intramolecularly, makes a contribution to the CD spectrum. *ii*) The 14-membered ring's contributions are *Cotton* effects of opposite sign near 215 and near 200 nm. *iii*) The twelve-membered ring's

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12) Theoretical calculations by *Applequist et al.* [25][26] have resulted in CD curves for β-peptides with a single maximum *above* 200 nm.

13) So far, we have no experimental results (such as concentration dependence of CD and NMR spectra) which would indicate that the β-peptides, carrying the simple aliphatic side chains of Ala, Val, Leu, Phe (or the positively charged side chain of Lys), as included in the present investigation, form aggregates in MeOH solutions.

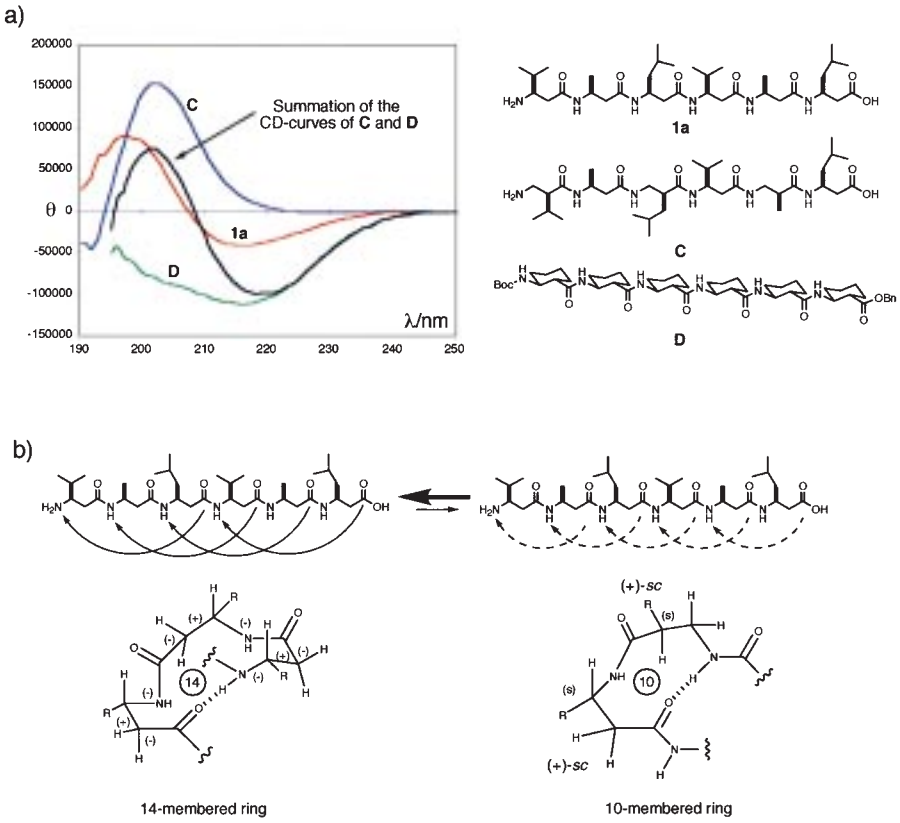

Fig. 5. a) *CD Spectra of β-peptides* **1a**, **C**, *and* **D** *in MeOH, and the summation of the CD curves of* **C** *and* **D**. The deprotected β-peptides **1a** and **C** were measured as their TFA salts as obtained after lyophilization or drying under high vacuum at a concentration of 0.2 mM. For a normalized CD curve of β-peptide **D**, see [24]. In each case, the molar ellipticity $[\theta]$ in 10 deg · cm^2 · mol^{-1} is calculated for the corresponding peptide (not normalized). b) *Interconversion of 14-membered to 10-membered rings in the β-hexapeptide* **1a**.

contribution is probably a weak, short-wavelength effect[14]). *iv*) The ten-membered ring gives rise to a single, strong *ca.* 205-nm *Cotton* effect. *v*) We have no way, at this stage, to determine which, if any, contribution a β-peptidic sheet makes to the CD spectrum[3]). *vi*) CD Spectra of β-peptides can, at least presently, not be used alone to draw conclusions about the secondary structure(s) that may prevail in solution.

4. Analysis of Molecular-Dynamics Simulations for Various H-Bonded Rings. – How could we find independent information about the propensity of β-peptides with different constitutional and configurational substitution patterns to form certain secondary structures? The NMR analyses of our β-peptides in MeOH have, so far, produced the structures of single dominant conformers: either the 3_{14} helix, or the *12/10* helix, or the hairpin. As usual, the analyses were carried out so that for a single

14) See the discussion in the Dissertation of *S.A.* (Footnote [3])), and Fig. 30 on p. 98 therein.

structure best fits with the interproton distances derived from the measured nuclear-*Overhauser*-effect (NOE) data resulted. In the case of the *12/10* helix of β-hexapeptide **C**, we clearly identified a weak and a medium NOE, which were not compatible with the *12/10*-helical structure, but rather with a 3_{14} helix. We concluded that the two secondary structures may be present in an equilibrium, with the *12/10* helix predominating [7]. The presence of mixtures of conformers in β-peptide solutions was also suggested by temperature-dependent CD and NMR measurements [9], showing that the 3_{14} helix folds and unfolds by a non-cooperative mechanism (*unlike* α-peptidic helices). However, a detailed interpretation of the NMR measurements with respect to several conformations in equilibrium was not feasible at the time. On the other hand, our molecular-dynamics calculations [13–15] have confirmed that, in MeOH solution, the β-peptides adopt an ensemble of conformations, with the population distribution depending on structure (amino-acid sequence) and simulation temperature. Thus, we have sought for information about the presence of substructures, such as the 10-, 12-, or 14-membered H-bonded rings, in the trajectories of the molecular-dynamics simulations.

Molecular-dynamics simulations of the β-heptapeptide **6** and the β-hexapeptide **C** in MeOH solution were performed with the GROMOS96 simulation program package in conjunction with the GROMOS force field 43A1 [27]. We note that this is a standard force field for simulation of biomolecular systems and has not been specifically parametrized for β-peptides. We refer the reader to our previous papers [13–15] for details on methodology and simulation setup. The dynamics of the β-heptapeptide **6** was simulated at a constant pressure of 1 atm and a constant temperature of 298 K, 340 K, and 350 K in a rectangular box containing the peptide initially folded in a 3_{14} model helix and 962 MeOH molecules, and at a constant temperature of 360 K in a periodic truncated octahedron containing the peptide initially fully extended along with 1778 MeOH molecules [14]. The dynamics of the β-hexapeptide **C** was simulated at a constant pressure of 1 atm and a constant temperature of 340 K in a periodic truncated octahedron containing the peptide initially fully extended and 1435 MeOH molecules [15]. No bias (*e.g.*, restraints from NOE-derived interproton distances) was used that could favor the experimental fold. The simulation time was 50 ns in all five simulations. Reversible folding of the β-heptapeptide to the experimentally determined left-handed 3_{14} helix was observed at each of the four temperatures, with a clear shift in the equilibrium between folded and unfolded states as a function of temperature (the higher the temperature, the lower the population of the folded state), but with the 3_{14} helix remaining the most populated conformation even at 360 K. Note the common use of the term 'folded' to refer to the experimentally determined conformation, which does not mean that the term 'unfolded' is used as a synonym of unstructured. The melting temperature of the 3_{14} helix of the β-heptapeptide in the force field was estimated from the ratio between folded and unfolded conformations to be 340 K [14]. At 340 K, the β-hexapeptide also had reversibly folded to the experimentally determined right-handed *12/10* helix [15]. Although the *12/10* helix was the most populous conformation, the total ratio between folded and unfolded conformations at this temperature indicated that the melting temperature of the *12/10* helix of the β-hexapeptide had to be lower than 340 K. Interestingly, a clustering analysis of the conformations sampled in the course of the simulation showed the existence of a small percentage (slightly higher than 1% at 340 K) of 3_{14} helix for the β-hexapeptide. This

finding was surprisingly consistent with the experimental observation of a medium NOE between the CH of residue 1 and the CH of residue 4, and a weak NOE between the CH of residue 3 and the CH of residue 6, which are typical for the 3_{14} helix and are violated in the *12/10* helix.

An analysis of the H-bonding preferences of the β-heptapeptide **6** and the β-hexapeptide **C** in the respective simulations at 340 K is presented here. At 340 K, the folded and unfolded states have similar weights, and comparison of the two peptides is facilitated (the β-hexapeptide was simulated only at 340 K). The simulation of the β-heptapeptide has been extended to 200 ns, compared to the 50 ns previously reported [14][15], while results for the β-hexapeptide are based on the 50-ns trajectory discussed above [15]. *Figs. 6* and *7* show the atom-positional root-mean-square deviation

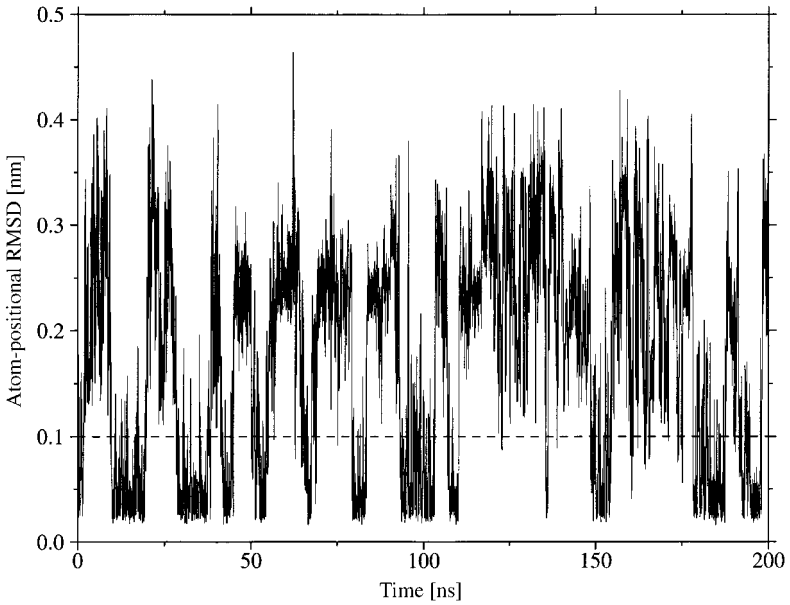

Fig. 6. *Backbone atom-positional root-mean-square deviation (RMSD) from the (3_{14}-helical) initial structure for residues 2 to 6 as a function of simulation time, from a 200-ns molecular-dynamics simulation of the β-heptapeptide* **6** *in methanol at 340 K*. The analysis runs over 20,000 structures extracted at 10-ps intervals from the simulation. Structures with an RMSD below the dashed line unequivocally form a 3_{14} helix.

(RMSD) for the backbone atoms of residues 2–6 (β-heptapeptide) or 2–5 (β-hexapeptide) from the model 3_{14} helix (β-heptapeptide) or *12/10* helix (β-hexapeptide), respectively, as a function of simulation time. The dashed line serves as a (rather conservative) upper limit for identification with the helical model. *Figs. 6* and *7* clearly illustrate the reversibility of the folding, with numerous events of folding and unfolding in the relatively short time scale of the simulations. *Figs. 8* and *9* show the presence of 10-, 12-, and 14-membered H-bonded rings in the β-heptapeptide simulation and in the β-hexapeptide simulation, respectively, as a function of simulation time. The percentage, in which each of the H-bonded rings is present in the simulation, is also shown. A conservative definition of a H-bond has been used (see *Figure* captions).

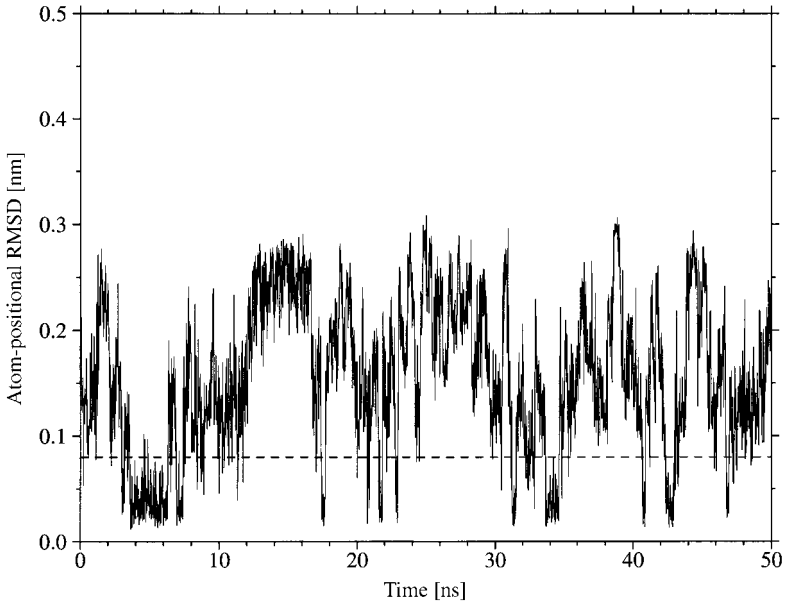

Fig. 7. *Backbone atom-positional root-mean-square deviation (RMSD) from the (*12/10*-helical) initial structure for residues 2 to 5 as a function of simulation time, from a 50-ns molecular-dynamics simulation of the β-hexapeptide* **C** *in MeOH at 340 K.* The analysis runs over 5000 structures extracted at 10-ps intervals from the simulation. Structures with an RMSD below the dashed line unequivocally form a *12/10* helix.

Note that, due to the insufficient resolution of the plots, it is difficult to assess the percentage of presence of a given H-bonded ring from just the amount of color. Nevertheless, the plots help identify where different rings coexist. The β-heptapeptide **6** adopts predominantly the 14-membered H-bonded rings characteristic of the 3_{14} helix. Each of the three central rings (2NH-4O, 3NH-5O, and 4NH-6O) is present for *ca.* 30% of the simulation time, while the two terminal ones (1NH-3O and 5NH-7O) are present for 10–15% of the time. The coexistence of three or more of these rings overlaps well with the regions of low RMSD in *Fig. 6*, as expected. *Fig. 8* also reveals a low but significant presence of ten-membered rings in the simulation, especially the central 4NH-5O, which is present for *ca.* 5% of the simulation time. Twelve-membered H-bonded rings are more rare and localized in time. It is, however, worth noting the presence of a right-handed *12/12/12* helix (5NH-2O, 6NH-3O, 7NH-4O) for a short period of time at around 137 ns. Coexistence of ten- and twelve-membered rings, as in the *12/10* helix of the β-hexapeptide, occurs rarely for the β-heptapeptide. The β-hexapeptide **C** adopts predominantly a combination of ten- and twelve-membered H-bonded rings (*Fig. 9*). Indeed, a full *10/12/10/12/10* helix is sampled at different times in the simulation. The three ten-membered rings (1NH-2O, 3NH-4O, and 5NH-6O) and two twelve-membered rings (4NH-1O and 6NH-3O), characteristic of this conformation, are each present for *ca.* 20–35% of the simulation time, while other possible ten- and twelve-membered rings not belonging to this helical conformation (2NH-3O, 4NH-5O, and 5NH-2O) are rare or not present at all. 14-Membered H-bonded rings occur with a much lower probability, around 1–3% of the time, but are also clearly and

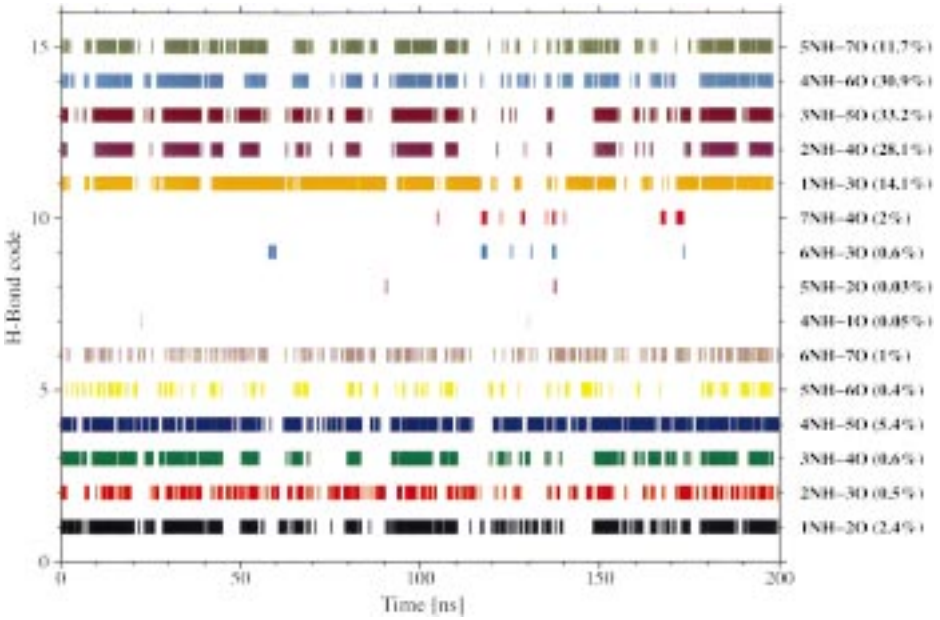

Fig. 8. *Presence of 10-, 12-, and 14-member of H-bonded rings as a function of simulation time, from a 200-ns molecular-dynamics simulation of the β-heptapeptide* **6** *in MeOH at 340 K*. The analysis runs over 400,000 structures extracted at 0.5-ps intervals from the simulation. The geometry of a H-bond is defined by a maximum distance proton-acceptor of 0.25 nm and a minimum angle donor-proton-acceptor of 135°. Each of the individual H-bonds is identified by an integer code from 1 to 15. H-Bond codes 1 to 6 correspond to 10-membered rings, codes 7 to 10 correspond to 12-membered rings, and codes 11 to 15 correspond to 14-membered rings. The residue and the atoms involved in the H-bond are shown at the right-hand side of the plot. The percentage of structures, out of the total ensemble of 400,000, in which each particular H-bond is present, is given within parentheses.

significantly present in the simulation of **C**. Furthermore, complete 3_{14} helices are found at two different times in the simulation of this hexapeptide, at around 25 ns and 39 ns.

Molecular-dynamics simulations of β-hexapeptide **5** in MeOH at 298 and 340 K starting from a fully extended conformation have also been performed (manuscript in preparation). Reversible folding of the β-hexapeptide to the experimentally determined hairpin conformation is again observed at either temperature. The turn is closed by a ten-membered H-bonded ring (3NH-4O), which is present for *ca.* 20% of the time. Other ten-membered rings are scarce. Twelve-membered H-bonded rings are more frequently found than 14-membered ones, but both types of rings have a comparatively lower occurrence at 340 K for the β-hexapeptide **5** than for β-hexapeptide **C**. A more detailed analysis of the trajectories of the β-hexapeptide **5** will be presented elsewhere.

Thus, the detailed analysis of our previous MD calculations provides support for the suggestions made above, on the basis of the CD-spectral analysis. Also, *ab initio* calculations by *Wu* and *Wang* [28], and by *Möhle et al.* [29] corroborate the notion that the stabilities of various sizes of H-bonded β-peptide rings are caused by subtle differences in their sequences.

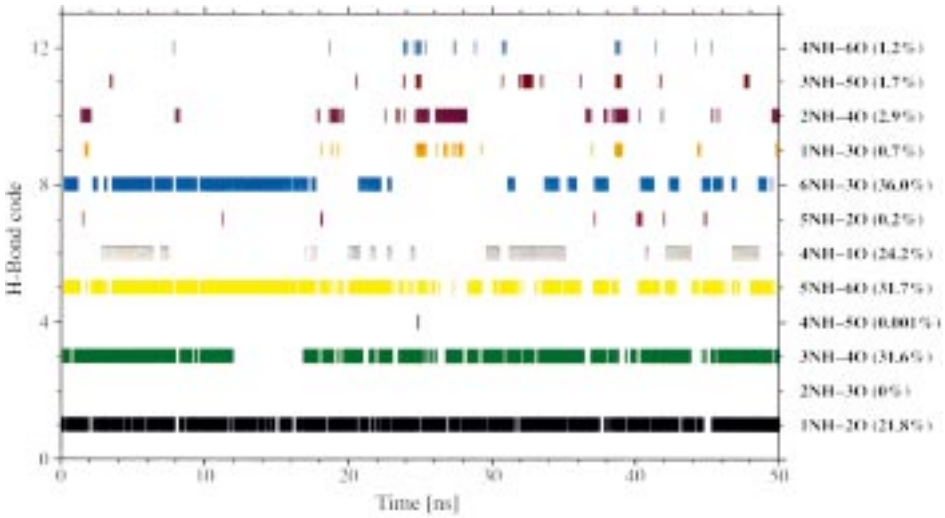

Fig. 9. *Presence of 10-, 12-, and 14-membered H-bonded rings as a function of simulation time, from a 50-ns molecular-dynamics simulation of the β-hexapeptide* ***C*** *in MeOH at 340 K*. The analysis runs over 100,000 structures extracted at 0.5-ps intervals from the simulation. The geometry of a H-bond is defined by a maximum-distance proton-acceptor of 0.25 nm and a minimum-angle donor-proton-acceptor of 135°. Each of the individual H-bonds is identified by an integer code from 1 to 12. H-Bond codes 1 to 5 correspond to 10-membered rings, codes 6 to 8 correspond to 12-membered rings, and codes 9 to 12 correspond to 14-membered rings. The residue and atom names of the atoms involved in the H-bond are shown at the right-hand side of the plot. The percentage of structures, out of the total ensemble of 100,000, in which each particular H-bond is present, is given within parentheses.

Generous supply of amino-acid derivatives by the *Degussa AG*, Wolfgang, is gratefully acknowledged, and we thank the *Novartis Stipendienfonds* for a scholarship granted to *J.V.S. Novartis Pharma AG* (Basel) has also supported this investigation. We appreciate critical discussions with and cautionary comments by *K. Gademann* in the course of preparing the manuscript.

Experimental Part

1. *General*. Abbreviations: BnOH: benzyl alcohol, BOP: (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate, DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene, EDC: 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, FC: flash chromatography, Fmoc-OSu: 9*H*-fluoren-9-ylmethyl *N*-succinimidyl carbonate, GP: General Procedure, HOBt: 1-hydroxy-1*H*-benzotriazole, h.v.: high vacuum, 0.01–0.1 Torr, *β*-HXxx: *β*-homoamino acid [5–7][30], NMM: *N*-methylmorpholin, PE: petroleum ether 40/60, RV: rotatory evaporator, TFA: CF_3COOH, TFE: 2,2,2-trifluorethanol. THF was freshly distilled over Na/benzophenone under Ar before use. DMF and MeCN were distilled under reduced pressure over CaH_2 and stored over 4-Å molecular sieves. Solvents for chromatography and workup procedures were distilled from *Sikkon* (anh. $CaSO_4$; *Fluka*). Et_3N was distilled from CaH_2 and stored over KOH. $ClCO_2Et$ was distilled and stored at +4° under Ar. $(i\text{-}Pr)_2NH$ was freshly distilled over CaH_2. LiCl and LiBr were dried at 150° under h.v. for 16 h. All indicated temp. were monitored with an internal thermometer (*Ebro-TTX-690* digital thermometer). Amino-acid derivatives were purchased from *Bachem*, *Senn*, or *Degussa*. All other reagents were used as received from *Fluka*. The *β*-amino acids were prepared according to literature procedures [5][6][31]. ***Caution:*** The generation and handling of CH_2N_2 requires special precautions [32]. Reactions carried out with the exclusion of light were performed in flasks completely wrapped in aluminium foil. TLC: *Merck* silica gel *60 F254* plates; detection with UV and I_2, or dipping into a soln. of ninhydrin (300 mg), AcOH (3 ml), and Butan-1-ol (100 ml), followed by heating. FC: *Fluka* silica gel *60* (40–63 mm); at *ca.* 0.3 bar. Anal. HPLC: *Knauer* HPLC system (pump type *64*, *EuroChrom 2000* integration package, degasser, UV detector

(variable-wavelength monitor)), *Macherey-Nagel* C_8 column (*Nucleosil 100-5* C_8 (250 × 4 mm)). Prep. HPLC: *Knauer* HPLC system (pump type *64*, programmer *50*, UV detector (variable-wavelength monitor)), *Macherey-Nagel* C_8 column (*Nucleosil 100-7* C_8 (250 × 21 mm)). M.p.: *Büchi-510* apparatus; uncorrected. Optical rotations: *Perkin-Elmer 241* polarimeter (10 cm, 1 ml cell) at r.t. CD Spectra: on a *Jasco J-710* spectropolarimeter from 190 to 250 nm at r.t. in 1-mm rectangular cells. The optical system was flushed with N_2 at a flow rate of *ca.* 10 l/min; parameters: band width 1.0 nm, resolution 0.2–1 nm, sensitivity 100 mdeg, response 0.5 s, speed 50 nm/min, 5 accumulations. All spectra were corrected for the corresponding solvent spectrum. Peptide concentration 0.2 mM. The molar ellipticity $[\theta]$ in $10\,\mathrm{deg}\cdot\mathrm{cm}^2\cdot\mathrm{mol}^{-1}$ (λ in nm) is calculated for the corresponding peptide (not normalized), taking into account the mass of TFA for each free amino group. Smoothing was done by *Jasco* software. Solvents: MeOH (HPLC grade), TFE (*puriss.* ≥ 99.5% GC); aq. buffers: pH 3.6 and 4.6: 0.1M AcONa/AcOH, pH 5.0: NaH_2PO_4/Na_2HPO_4, prepared according to [33]; pH 9.0, 11.0: $NaHCO_3$/NaOH, prepared according to [34]. UV Spectra: on a *Perkin Elmer UV/VIS* spectrometer *Lambda 40* with *PTP-6 Peltier* System at r.t. in 1-cm quartz cells. λ_{max} in nm; solvent: MeOH (HPLC-grade). IR Spectra: *Perkin-Elmer-782* spectrophotometer. NMR Spectra: *Bruker AMX 500* (^{1}H: 500 MHz, ^{13}C: 125 MHz), *AMX 400* (^{1}H: 400 MHz, ^{13}C: 100 MHz), *ARX 300* (^{1}H: 300 MHz), *Varian Gemini 300* (^{1}H: 300 MHz, ^{13}C: 75 MHz), or *Varian Gemini 200* (^{1}H: 200 MHz, ^{13}C: 50 MHz); chemical shifts δ in ppm downfield from internal $SiMe_4$ (= 0 ppm); *J* values in Hz; some compounds show the presence of rotamers which are indicated. MS: *VG Tribrid* (EI) or *Hitachi Perkin-Elmer RHU-6M* (FAB, in a 3-nitrobenzyl-alcohol matrix) spectrometer; in *m*/*z* (% of basis peak). Elemental analyses were performed by the Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich.

2. *Transesterification of β-Amino-Acid Derivatives. General Procedure 1* (*GP 1*). The appropriate methyl ester was dissolved in BnOH (0.5M). A soln. of $Ti(OBn)_4$ in BnOH (0.7–4 equiv., 0.58M) and molecular sieves (4 Å) was added. This mixture was heated at 95° for 40–60 h (NMR control). After filtration over *Celite* and dilution with Et_2O the org. phase was washed thoroughly with aq. KF (pH 1), sat. aq. $NaHCO_3$, and NaCl solns., and then dried ($MgSO_4$). The solvent was removed in RV, and excess BnOH was removed by bulb-to-bulb distillation (100°, 0.1 Torr). The resulting crude product was purified by FC.

3. *Benzyl-Ester Deprotection. General Procedure 2* (*GP 2*). The benzyl ester was dissolved in the appropriate solvent (0.1M), and *ca.* 10% (m/m) Pd/C (10%) was added. The apparatus was evacuated and flushed with H_2 (3×), and the mixture was stirred under an atmosphere of H_2 (1 bar) for 18 h. Subsequent filtration through *Celite* and concentration under reduced pressure yielded the crude carboxylic acid, which was, if not other mentioned, further purified by FC and/or recrystallization.

4. *Boc Deprotection. General Procedure 3a* (*GP 3a*). Similarly to the reported procedure [5], the Boc-protected amino acid was dissolved in CH_2Cl_2 (0.5M) and cooled to 0° (ice-bath). An equal volume of TFA was added, and the mixture was allowed to warm to r.t. and then stirred for further 1.5 h. Concentration under reduced pressure and drying of the residue under h.v. yielded the crude TFA salt, which was used without further purification.

General Procedure 3b (*GP 3b*). The Boc-protected compound was dissolved in TFA (0.25M). After stirring for 2 h at r.t., the mixture was evaporated and the residue dried under h.v.

5. N-*Fmoc-Protection of* $\beta^{2,3}$*-Amino Acids. General Procedure 4* (*GP 4*). A stirred soln. of the TFA salt of the β-amino acid in 0.6M aq. Na_2CO_3 (3 equiv.) was treated with a soln. of Fmoc-OSu (1.1 equiv.) in acetone (0.2M). If necessary, the pH was readjusted to 9–10 with additional Na_2CO_3. After 5 h, the mixture was diluted with H_2O and extracted with Et_2O. The aq. phase was carefully adjusted to pH 1–2 at 0° (ice-bath) with 1N HCl and extracted with AcOEt (3×). The org. layer was washed with H_2O, dried ($MgSO_4$), and concentrated under reduced pressure. FC and/or recrystallization afforded the pure *N*-Fmoc-protected $\beta^{2,3}$-amino acids.

6. *HPLC Analysis and Purification of β-Peptides. General Procedure 5* (*GP 5*). RP-HPLC Analysis was performed on *a Macherey-Nagel* C_8 column/*Nucleosil 100-5* C_8 (250 × 4 mm) or *Macherey-Nagel* C_{18} column/*Nucleosil 100-5* C_{18} (250 × 4 mm) with a linear gradient of *A:* 0.1% TFA in H_2O and *B:* MeCN at a flow rate of 1 ml/min with UV detection at 220 nm. t_R in min. Crude products were purified by prep. RP-HPLC on a *Macherey-Nagel* C_8 column/*Nucleosil 100-7* C_8 (250 × 21 mm) or *Macherey-Nagel* C_{18} column/*Nucleosil 100-7* C_{18} (250 × 21 mm) with a gradient of *A* and *B* at a flow rate of 4 ml/min with UV detection at 214 nm and then lyophilized.

7. *Peptide Coupling with EDC. General procedure 6a* (*GP 6a*). According to [35], a stirred soln. of the TFA salt in $CHCl_3$ (0.5M) at 0° (ice-bath) under Ar was treated successively with Et_3N (3–6 equiv.), HOBt (1.2 equiv.), a soln. of the Boc-protected fragment (1 equiv.) in $CHCl_3$ (0.5M), and EDC (1–1.2 equiv.). The mixture was allowed to warm to r.t., and stirring was continued for 15 h. The mixture was diluted with $CHCl_3$ and washed

with 1N HCl, aq. sat. $NaHCO_3$, and NaCl soln. The org. phase was dried ($MgSO_4$) and evaporated, and the residue was purified by FC and/or recrystallization.

General Procedure 6b (*GP 6b*). A stirred soln. of the TFA salt in CH_2Cl_2 at 0° (ice-bath) under Ar was treated with the Boc-protected fragment (1 equiv.), NMM (2.8 equiv.), HOBt (1.1 equiv.), and EDC (1 equiv.). The mixture was allowed to warm to r.t., and stirring was continued for 15 h. The mixture was diluted with CH_2Cl_2 and worked up as described in *GP 6a*.

9. *Preparation of the β-Amino Acids. Benzyl (2*R,*3*S*)-3-{[(*tert-*Butoxy)carbonyl]amino}-2,4-dimethylpentanoate* (Boc-(2*R*,3*S*)-$\beta^{2,3}$-HVal(α-Me)-OBn). Methyl ester **9b** (prepared as described in [7]; 1.26 g, 5.12 mmol) was transesterified with $Ti(OBn)_4$ (0.68 equiv.) for 37 h according to *GP 1*. FC (Et_2O/pentane 1:6 → 1:5) yielded Boc-(2*R*,3*S*)-$\beta^{2,3}$-HVal(α-Me)-OBn (1.33 g, 77%). Colorless waxy solid. M.p. 59.5–61.5°. R_f (Et_2O/pentane 1:6) 0.15. $[\alpha]_D^{r.t.} = +3.8$ ($c = 1.34$, $CHCl_3$). IR ($CHCl_3$): 3446*w*, 3036*w*, 3005*w*, 2974*m*, 2923*m*, 2882*w*, 1713*s*, 1503*s*, 1456*m*, 1390*m*, 1369*s*, 1303*m*, 1169*s*, 1097*w*, 1072*w*, 1046*w*, 903*w*, 867*w*, 626*w*. ^{1}H-NMR (400 MHz, $CDCl_3$; signals of rotamers in italics): 0.86 (*d*, *J* = 6.8, Me); 0.90 (*d*, *J* = 6.7, Me); 1.15 (*d*, *J* = 7.0, Me); 1.43 (*s*, *t*-Bu); 1.61–1.69 (*m*, Me_2CH); 2.56–2.67 (*m*, CHCO); 3.79–3.85 (*m*, CHN); *4.06*, 4.38 (*d*, *J* = 10.6, NH); 5.06 (*d*, *J* = 12.3, 1 H, PhCH_2); 5.14 (*d*, *J* = 12.3, 1 H, PhCH_2); 7.29–7.56 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$; signals of rotamers in italics): 12.58, *13.78*, *16.18*, 17.21, 20.21, 28.37 (Me); 30.34, *30.61*, 42.62, *43.13*, *57.37*, 58.83 (CH); 66.47 (CH_2); 79.16 (C); 128.18, 128.32, 128.53 (CH); 135.97, 155.91, 174.65 (C). FAB-MS: 671 (5.4, $[2M]^+$), 336 (67.3, $[M+1]^+$), 280 (100), 236 (80.5), 192 (22.7), 172 (32.8), 116 (30.0). Anal. calc. for $C_{19}H_{29}NO_4$ (335.44): C 68.03, H 8.71, N 4.18; found: C 68.10, H 8.55, N 4.11.

*Benzyl (2*R,*3*S*)-3-{[(*tert-*Butoxy)carbonyl]amino}-2,5-dimethylhexanoate* (Boc-(2*R*,3*S*)-$\beta^{2,3}$-HLeu(α-Me)-OBn). Methyl ester **10b** (prepared as described in [7]; 2.42 g, 8.85 mmol) was transesterified with $Ti(OBn)_4$ (1.5 equiv.) for 45 h according to *GP 1*. FC (Et_2O/pentane 1:5) yielded Boc-(2*R*,3*S*)-$\beta^{2,3}$-HLeu(α-Me)-OBn (2.59 g, 84%). White waxy solid. R_f (Et_2O/pentane 1:5) 0.26. $[\alpha]_D^{r.t.} = -37.4$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3443*w*, 3005*m*, 2964*m*, 2872*w*, 1708*s*, 1503*s*, 1456*m*, 1390*m*, 1369*s*, 1174*s*, 1103*m*, 1041*w*, 908*w*, 872*w*. ^{1}H-NMR (400 MHz, $CDCl_3$; signals of rotamers in italics): 0.92 (*d*, *J* = 6.7, 2 Me); 0.86 (*d*, *J* = 6.6, Me); 0.87 (*d*, *J* = 6.4, Me); 1.11–1.26 (*m*, CH_2, Me); 1.42, *1.64* (*s*, *t*-Bu); 1.69–1.65 (m, Me_2CH); *2.48–2.57*, 2.63–2.69 (*m*, CHCO); *3.70–3.79*, 3.84–3.91 (*m*, CHN); *4.19* (br., NH); 4.57 (*d*, *J* = 9.5, NH); 5.10 (*d*, *J* = 12.3, 1 H, PhCH_2); 5.14 (*d*, *J* = 12.3, 1 H, PhCH_2); 7.30–7.56 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 13.17, 21.60, 23.48 (Me); 24.91 (CH); 28.38 (Me); 41.05 (CH_2); 44.52, 51.02 (CH); 66.31 (CH_2); 79.13 (C); 128.22, 128.28, 128.57 (CH); 136.02, 155.50, 174.39 (C). EI-MS: 350 (0.7, M^+), 192 (22.9), 186 (31), 130 (81.4), 91 (100). Anal. calc. for $C_{20}H_{31}NO_4$ (349.47): C 68.74, H 8.94, N 4.01; found: C 68.78, H 8.84, N 3.96.

*(2*R,*3*S*)-3-{[(*tert-*Butoxy)carbonyl]amino}-2,4-dimethylpentanoic Acid* (Boc-(2*R*,3*S*)-$\beta^{2,3}$-HVal(α-Me)-OH). Boc-(2*R*,3*S*)-$\beta^{2,3}$-HVal(α-Me)-OBn (2.27 g, 7.98 mmol) was debenzylated in MeOH (40 ml) according to *GP 2*. Recrystallization (CH_2Cl_2/hexane) yielded Boc-(2*R*,3*S*)-$\beta^{2,3}$-HVal(α-Me)-OH (1.90 g, 97%). White powder. M.p. 113–114°. R_f (CH_2Cl_2/MeOH 12:1) 0.48. $[\alpha]_D^{r.t.} = +13.3$ ($c = 1.0$, MeOH). IR ($CHCl_3$): 3446*w*, 2980*m*, 2931*m*, 2875*s*, 1714*s*, 1504*s*, 1456*m*, 1392*m*, 1368*m*, 1170*s*, 1092*w*, 1043*w*, 986*w*, 868*w*. ^{1}H-NMR (400 MHz, $CDCl_3$; signals of rotamers in italics): 0.89 (*d*, *J* = 6.8, Me); 0.95 (*d*, *J* = 6.6, Me); 1.17 (*d*, *J* = 7.0, Me); 1.43, *1.45* (*s*, *t*-Bu); 1.59–1.83 (*m*, Me_2CH); 2.57–2.64 (*m*, CHCO); *3.68–3.72*, 3.80–3.84 (*m*, CHN); 4.45, *5.57* (*d*, *J* = 10.5, NH); 10.6 (*br.*, COOH). ^{13}C-NMR (100 MHz, $CDCl_3$; signals of rotamers in italics): *12.77*, 12.95, *16.88*, 17.10, 20.30, 28.28, 28.35 (Me); 30.38, *30.65*, *42.40*, 42.60, 57.20, 58.71 (CH); *79.38*, *80.71*, 156.12, *157.71*, *180.07*, *180.57* (C). EI-MS: 246 (0.5, $[M+1]^+$), 202 (41.4), 172 (25.2), 146 (58.9), 116 (24.2), 102 (100), 84 (31.5), 74 (21.4), 72 (21.1), 57 (26.7). Anal. calc. for $C_{12}H_{23}NO_4$ (245.32): C 58.75, H 9.45, N 5.71; found: C 58.64, H 9.37, N 5.70.

*(2*R,*3*S*)-3-{[(*tert-*Butoxy)carbonyl]amino}-2,5-dimethylhexanoic Acid* (Boc-(2*R*,3*S*)-$\beta^{2,3}$-HLeu(α-Me)-OH). Boc-(2*R*,3*S*)-$\beta^{2,3}$-HLeu(α-Me)-OBn (2.59 g, 7.42 mmol) was debenzylated in AcOEt (37 ml) according to *GP 2*. FC (CH_2Cl_2/MeOH 12:1) yielded Boc-(2*R*,3*S*)-$\beta^{2,3}$-HLeu(α-Me)-OH (1.56 g, 81%). White foam. R_f (CH_2Cl_2/MeOH 12:1) 0.36. $[\alpha]_D^{r.t.} = -44.4$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3443*w*, 3200–2850 (br.), 1707*s*, 1505*s*, 1469*m*, 1392*m*, 1368*s*, 1168*s*, 1103*w*, 1046*w*, 1007*w*. ^{1}H-NMR (400 MHz, $CDCl_3$; signals of rotamers in italics): 0.92 (*d*, *J* = 6.7, 2 Me); 1.16 (*d*, *J* = 7.1, Me); 1.24–1.37 (*m*, CH_2); 1.44, *1.48* (*s*, *t*-Bu); 1.60–1.67 (*m*, Me_2CH); *2.49–2.54*, 2.63–2.66 (*m*, CHCO); 3.84–3.92 (*m*, CHN); 4.76 (br. *d*, *J* = 9.0, NH); *5.50* (br. *s*, NH); 7.52 (br., COOH). ^{13}C-NMR (100 MHz, $CDCl_3$; signals of rotamers in italics): 13.12, 21.59, 23.50 (Me); 24.95 (CH); 28.37 (Me); 40.76 (CH_2); *41.92*, 44.27, 50.78, *51.74* (CH); 79.36; 155.66, 179.89 (C). FAB-MS: 541 (10.2, $[2M+Na]^+$), 282 (45.7, $[M+Na]^+$), 204 (100), 130 (63.8). Anal. calc. for $C_{13}H_{25}NO_4$ (259.34): C 60.21, H 9.72, N 5.40; found: C 60.20, H 9.64, N 5.23.

*(2*R,*3*S*)-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-2,4-dimethylpentanoic Acid* (Fmoc-(2*R*,3*S*)-$\beta^{2,3}$-HVal(α-Me)-OH). Boc-(2*R*,3*S*)-$\beta^{2,3}$-HVal(α-Me)-OH (0.308 g, 1.26 mmol) was Boc-deprotected according to

GP 3a. The resulting TFA salt was transformed according to *GP 4*. Recrystallization (CH_2Cl_2/hexane) yielded Fmoc-(2*R*,3*S*)-$\beta^{2,3}$-HVal(α-Me)-OH (0.397 g, 86%). White powder. RP-HPLC according to *GP 5* (20–80% *B* in 20 min; C_8): t_R 10.6, purity >99%. M.p. 176.5–177.5°. R_f (CH_2Cl_2/MeOH 10:1) 0.37. $[\alpha]_D^{r.t.} = +3.50$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3440*w*, 3150–2860 (br.), 1724*s*, 1513*s*, 1451*m*, 1302*w*, 1095*w*, 1045*w*, 909*w*, 620*w*. ^{1}H-NMR (400 MHz, CD_3COCD_3; signals of rotamers in italics): 0.93 (*d*, *J* = 6.8, Me); 0.93 (*d*, *J* = 6.7, Me); 1.14 (*d*, *J* = 7.0, Me); 1.80–1.90 (*m*, Me_2CH); 2.66–2.71 (*m*, CHCO); 3.84–3.90 (*m*, CHN); 4.22 (*t*, *J* = 7.0, $CHCH_2O$); 4.31–4.41 (*m*, $CHCH_2O$); *3.65*, 6.24 (*d*, *J* = 10.3, NH); 7.30–7.39 (*m*, 2 arom. H); 7.39–7.43 (*m*, 2 arom. H); 7.68–7.71 (*m*, 2 arom. H); 7.86 (*d*, *J* = 7.5, 2 arom. H). ^{13}C-NMR (100 MHz, CD_3COCD_3): 14.22, 17.12, 20.83 (Me); 31.34, 42.89, 48.24, 58.97 (CH); 66.69 (CH_2); 120.79, 126.08, 126.15, 127.89, 127.91, 128.48 (CH); 142.15, 145.18, 145.23, 157.64, 176.41 (C). FAB-MS: 735 (2.0, $[2M]^+$), 368 (38.8, $[M+1]^+$), 178 (100), 165 (23.2). Anal. calc. for $C_{22}H_{25}NO_4 \cdot 0.5H_2O$ (376.46): C 70.19, H 6.96, N 3.72; found: C 70.20, H 6.85, N 3.74.

*(2*R,*3*S*)-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-2,5-dimethylhexanoic Acid* (Fmoc-(2*R*,3*S*)-$\beta^{2,3}$-HLeu(α-Me)-OH). Boc-(2*R*,3*S*)-$\beta^{2,3}$-HLeu(α-Me)-OH (0.87 g, 3.35 mmol) was Boc-deprotected according to *GP 3a*. The resulting TFA salt was transformed according to *GP 4*. FC (CH_2Cl_2/MeOH 20:1 → 10:1) and recrystallization (CH_2Cl_2/pentane) yielded Fmoc-(2*R*,3*S*)-$\beta^{2,3}$-HLeu(α-Me)-OH (1.12 g, 87%). White powder. RP-HPLC according to *GP 5* (20–80% *B* in 20 min; C_8): t_R 13.2, purity >99%. M.p. 184–186° (dec.). R_f (CH_2Cl_2/MeOH 10:1) 0.39. $[\alpha]_D^{r.t.} = -28.4$ ($c = 0.68$, $CHCl_3$). IR ($CHCl_3$): 3436*w*, 3100–2850 (br.), 1716*s*, 1513*s*, 1450*m*, 1331*w*, 1105*w*, 600*w*. ^{1}H-NMR (400 MHz, CD_3COCD_3): 0.90 (2*d*, *J* = 6.7, 6.5, 2 Me); 1.14 (*d*, *J* = 7.1, Me); 1.20–1.29 (*m*, 1 H, CH_2); 1.47–1.54 (*m*, 1 H, CH_2); 1.63–1.71 (*m*, Me_2CH); 2.52 (*quint.*, *J* = 7.2, CHCO); 3.92–4.05 (*m*, CHN); 4.22 (*t*, *J* = 7.0, $CHCH_2O$); 4.38 (*d*, *J* = 6.9, $CHCH_2O$); 6.26 (*d*, *J* = 9.0, NH); 7.29–7.33 (*m*, 2 arom. H); 7.38–7.42 (*m*, 2 arom. H); 7.67–7.70 (*m*, 2 arom. H); 7.85 (*d*, *J* = 7.5, 2 arom. H). ^{13}C-NMR (100 MHz, CD_3COCD_3): 14.26, 21.81, 24.00 (Me); 25.62 (CH); 42.97 (CH_2); 45.76, 48.25, 52.30 (CH); 66.59 (CH_2); 120.77, 126.07, 126.12, 127.86, 127.89, 128.46 (CH); 142.15, 145.11, 145.24, 157.17, 172.20 (C). FAB-MS: 2021 (34.9, $[5M-2H+3K]^+$), 1221 (36.5, $[3M-1H+2K]^+$), 801 (8.5, $[2M+K]^+$), 420 (18.4, $[M+K]^+$), 404 (15.1, $[M+Na]^+$), 382 (29.6, $[M+1]^+$), 178 (100). Anal. calc. for $C_{23}H_{27}NO_4$ (381.47): C 72.42, H 7.13, N 3.67; found: C 72.45, H 7.25, N 3.62.

tert-*Butyl (*E*)-2-Methylbut-2-enoate*. 2-Methylprop-1-ene (41 g, 0.75 mol) was condensed into a 500-ml round-bottom flask containing a soln. of tiglic acid ((*E*)-2-methylbut-2-enoic acid; 15.0 g, 0.15 mol) in CH_2Cl_2 (100 ml) at −20°. Conc. H_2SO_4 (0.8 ml) was added, and the soln. was stirred at r.t. for 56 h. The mixture was cooled to −4°, quenched with sat. aq. $NaHCO_3$ soln., and stirred vigorously to evaporate excess 2-methylprop-1-ene. After drying ($MgSO_4$), the crude product was distilled (90–95°, 79 Torr) to yield tert-*butyl* (E)-*2-methylbut-2-enoate* (12.28 g, 52%). Colorless oil. B.p. 95° (78 Torr). R_f (Et_2O/pentane 1:9) 0.65. Spectroscopic data: in agreement with those in [36].

*(*S*)-*N*-Benzyl-1-phenylethylamine*. (*S*)-1-Phenylethylamine (15.9 ml, 0.125 mol) was benzylated acccording to [37]. Distillation (122°, 0.12 Torr) yielded (*S*)-N-*Benzyl-1-phenylethylamine* (19.74 g, 75%). Colorless oil. B.p. and spectroscopic data: in agreement with those in [37].

tert-*Butyl (2*R,*3*S,*α*S*)-3-[Benzyl(α-methylbenzyl)amino]-2-methylbutanoate*. *tert*-Butyl (*E*)-2-methylbut-2-enoate (5.0 g, 32 mmol) was transformed with the Li amide derived from (*S*)-*N*-Benzyl-1-phenylethylamine (10.82 g, 51.2 mmol) according to [38]. FC (Et_2O/pentane 1:50) yielded tert-*butyl (2*R,*3*S,*α*S*)-3-[*benzyl(α-methylbenzyl)amino]-2-methylbutanoate* (7.95 g, 68%). Colorless oil. R_f (Et_2O/pentane 1:50) 0.26. Spectroscopic data: in agreement with those in [38].

tert-*Butyl (2*R,*3*S*)-3-Amino-2-methylbutanoate* (*H-(2*R,*3*S*)-β^{2,3}-HAla(α-Me)-O^t-Bu*; **11**). *tert*-Butyl (2*R*,3*S*,*αS*)-3-[benzyl(α-methylbenzyl)amino]-2-methylbutyrate (3.42 g, 9.31 mmol) was dissolved in AcOEt (60 ml), and $Pd(OH)_2$ (0.68 g) was added. The flask was evacuated (3×) and flushed with H_2 (3×), and the mixture was stirred under an atmosphere of H_2 (2 balloons) at r.t. for 42 h. The mixture was filtered through *Celite* and evaporated under reduced pressure (45°, 85 mbar): crude **11** (1.61 g, 99%); yellowish crystals, used directly in the next step. R_f (EtOH/NH_3/H_2O 7:1:1) 0.78. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.14 (*d*, *J* = 6.2, 2 Me); 1.46 (*s*, *t*-Bu); 2.29–2.42 (*m*, CHCO); 2.84 (br. *s*, NH_2); 3.13–3.26 (*m*, CHN).

*(2*R,*3*S*)-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-2-methylbutanoic Acid* (Fmoc-(2*R*,3*S*)-$\beta^{2,3}$-HAla(α-Me)-OH). Amine **11** (1.61 g, 9.29 mmol) was dissolved in TFA (10 ml) and stirred for 3 h at r.t. Evaporation yielded the crude amino acid that was Fmoc-protected according to *GP 11*. FC (Et_2O/pentane/AcOH 6:4:0.1) and recrystallization (AcOEt/hexane) gave Fmoc-(2*R*,3*S*)-$\beta^{2,3}$-HAla(α-Me)-OH (2.32 g, 74%). White powder. M.p. 205–205.5°. R_f (Et_2O/pentane/AcOH 6:4:0.1) 0.19. $[\alpha]_D^{r.t.} = +7.79$ ($c = 0.68$, acetone). IR (KBr): 3327*s*, 3066*m*, 2976*s*, 2889*m*, 2622*w*, 1685*s*, 1544*s*, 1450*s*, 1420*m*, 1380*m*, 1333*m*, 1284*s*, 1256*s*, 1217*s*, 1150*m*, 1107*s*, 1089*s*, 1028*s*, 976*m*, 928*m*, 880*m*, 795*w*, 779*w*, 757*m*, 737*s*, 669*m*, 622*m*, 588*w*, 547*w*, 502*w*, 424*m*. ^{1}H-NMR (400 MHz, CD_3COCD_3): 1.16 (*d*, *J* = 7.1, Me); 1.19 (*d*, *J* = 6.7, Me); 2.58 (*quint.*, *J* = 7.2, CHCO); 2.85–

3.94 (*m*, CHN); 4.21–4.24 (*m*, C*H*CH$_2$O); 4.28–4.33 (*m*, 1 H, CHC*H*$_2$O); 4.37–4.41 (*m*, 1 H, CHC*H*$_2$O); 6.37 (*d*, *J* = 7.9, NH); 7.30–7.34 (*m*, 2 arom. H); 7.39–7.50 (*m*, 2 arom. H); 7.69 (*d*, *J* = 7.5, 2 arom. H); 7.86 (*d*, *J* = 7.5, 2 arom. H); 10.73 (br., COOH). ^{13}C-NMR (100 MHz, CD$_3$COCD$_3$): 14.67, 19.08 (Me); 45.80, 48.18, 49.98 (CH); 66.71 (CH$_2$); 120.81, 126.06, 126.12, 127.90, 127.92, 128.49 (CH); 142.14, 145.17, 145.21, 156.65, 176.07 (C). FAB-MS: 679 (4.7, $[2M]^+$), 340 (100, $[M+1]^+$). Anal. calc. for $C_{20}H_{21}NO_4$ (339.39): C 70.78, H 6.24, N 4.13; found: C 70.65, H 6.44, N 4.10.

10. *Synthesis of the β-Peptides. Boc-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OMe.* Boc-(*S*)-β^3-HLeu-OMe (prepared as described in [5]; 6.77 g, 26.1 mmol) was Boc-deprotected in CHCl$_3$ for 2 h according to *GP 3a*. The obtained TFA salt was treated according to *GP 6a* with Et$_3$N (10.9 ml, 78.2 mmol), HOBt (4.72 g, 31.2 mmol), Boc-(*S*)-β^3-HAla-OH (5.30 g, 26.1 mmol; prepared as in [6]) in CHCl$_3$ (52 ml), and EDC (5.98 g, 31.2 mmol). FC (AcOEt/pentane 1:1 → 2:1) yielded Boc-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OMe (6.83 g, 76%). ^{1}H-NMR Data: in agreement with those in [5].

*Boc-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OBn.* Boc-(*S*)-β^3-HLeu-OBn (prepared as described in [7]; 19.85 g, 59.20 mmol) was Boc-deprotected following *GP 3a*. The obtained TFA salt was treated according to *GP 6b* with Boc-(*S*)-β^3-HAla-OH (12.25 g, 60.27 mmol; prepared as described in [6]), NMM (12.25 ml, 165.63 mmol), HOBt (9.85 g, 65.08 mmol), and EDC (11.35 g, 59.21 mmol). After 20 h, the mixture was worked up. FC (AcOEt/PE 1:1) yielded Boc-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OBn (14.67 g, 59%). ^{1}H-NMR Data: in agreement with those in [7].

*Boc-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OMe.* Boc-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OMe (2.75 g, 8.0 mmol) was Boc-deprotected in CHCl$_3$ following *GP 3a*. The obtained TFA salt was treated according to *GP 6a* with Et$_3$N (4.46 ml, 32.0 mmol), HOBt (1.45 g, 9.6 mmol), Boc-(*R*)-β^3-HVal-OH (1.85 g, 8.0 mmol; prepared as described in [5]) in CHCl$_3$, and EDC (1.84 g, 9.6 mmol). FC (MeOH/CH$_2$Cl$_2$ 3:97 → 5:95) yielded Boc-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OMe (2.93 g, 80%). ^{1}H-NMR Data: in agreement with those in [5].

*Boc-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OBn.* Boc-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OBn (12.06 g, 28.7 mmol) was Boc-deprotected following *GP 3a*. A stirred soln. of the obtained TFA salt in CHCl$_3$ (57 ml) at 0° (ice-bath) under Ar was treated with Boc-(*R*)-β^3-HVal-OH (6.84 g, 29.6 mmol; prepared as described in [5]), NMM (8.9 ml, 80.8 mmol), HOBt (4.78 g, 31.6 mmol), and EDC (5.53 g, 28.8 mmol). The mixture was allowed to warm to r.t., and, after stirring for 5 h, THF (10 ml) was added. After another 12 h, the mixture was diluted with CHCl$_3$ and washed with 1N HCl, aq. sat. NaHCO$_3$ and NaCl solns. The org. phase was dried (MgSO$_4$) and evaporated. FC (MeOH/CHCl$_3$ 3:97 → 1:9) yielded Boc-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OBn (6.18 g, 42%). ^{1}H-NMR Data: in agreement with those in [5].

*Boc-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OH.* From the methyl-ester derivative: According to [39], a soln. of Boc-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OMe (1.98 g, 4.3 mmol) in MeOH (4.6 ml) and THF (2 ml) was treated with aq. 0.75N NaOH (6.9 ml). After 11 h, the pH of the mixture was adjusted to 2 with 1N HCl, and the mixture was extracted with AcOEt. The org. phase was dried (MgSO$_4$) and evaporated: Boc-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OH (1.7 g, 89%). ^{1}H-NMR Data: in agreement with those in [5]. From the benzyl ester derivative: Boc-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OBn (2.51 g, 4.7 mmol) was dissolved in MeOH (113 ml), and Pd/C (10%, 0.31 g) was added. The apparatus was evacuated, flushed with H$_2$ (5 ×), and the mixture was stirred under H$_2$ for 19 h. Subsequent filtration through *Celite* and concentration under reduced pressure yielded Boc-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OH (2.03 g, 97%). ^{1}H-NMR Data: in agreement with those in [5].

*Boc-(*S*)-β^3-HAla-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OMe.* Boc-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OMe (1.00 g, 2.20 mmol) was Boc-deprotected for 2 h according to *GP 3a*. The obtained TFA salt was treated in CH$_2$Cl$_2$ (4.5 ml) according to *GP 6b* with Boc-(*S*)-β^3-HAla-OH (0.44 g, 2.16 mmol; prepared as described in [6]), NMM (0.67 ml, 6.10 mmol), HOBt (0.36 g, 2.38 mmol), and EDC (0.42 g, 2.19 mmol). FC (MeOH/CH$_2$Cl$_2$ 6:94) yielded Boc-(*S*)-β^3-HAla-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OMe (0.11 g, 92%). ^{1}H-NMR Data: in agreement with those in [6].

*Boc-β-HGly-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OMe.* Boc-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OMe (0.24 g, 0.52 mmol) was Boc-deprotected in CHCl$_3$ according to *GP 3a*. The obtained TFA salt was treated according to *GP 6a* in CHCl$_3$ (2.1 ml) with Et$_3$N (0.29 ml, 2.1 mmol), Boc-β-HGly-OH (0.10 g, 0.52 mmol; added as solid), HOBt (0.06 g, 0.64 mmol), and EDC (0.123 g, 0.64 mmol). After 18 h, the mixture was worked up. FC (MeOH/CH$_2$Cl$_2$ 6:94) yielded Boc-(*S*)-β-HGly-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OMe (0.18 g, 63%). ^{1}H-NMR (200 MHz, CDCl$_3$): 0.84–0.98 (*m*, 4 Me); 1.22 (*d*, *J* = 6.9, Me); 1.25–1.68 (*m*, 3 CH); 1.44 (*s*, *t*-Bu); 1.71–1.90 (*m*, CH); 2.20–2.67 (*m*, 4 CH$_2$CO); 3.31–3.47 (*m*, CHN); 3.68 (*s*, MeO); 3.91–4.44 (*m*, 3 CHN); 5.30–5.47 (*m*, NH); 6.28–6.41 (*m*, NH); 6.48–6.59 (*m*, NH); 6.92–7.07 (*m*, NH).

*TFA · H-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*S*)-β³-HAla-(S)-β³-HLeu-OH* (**1a**). Compound **1c** (41 mg, 0.05 mmol) was Boc-deprotected for 2.5 h according to *GP 3b*. Coevaporation with toluene (3×) and CCl_4 (2×) yielded quantitatively **1a** (48.1 mg). ^{1}H-NMR and MS data: in agreement with those in [5].

*TFA · H-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*S*)-β³-HAla-(S)-β³-HLeu-OMe* (**1b**). Compound **1d** (198 mg, 0.25 mmol) was Boc-deprotected with TFA (1 ml) according to *GP 3b*. Coevaporation with toluene and lyophilization (1,4-dioxan) yielded **1b** (150 mg, 74%). ^{1}H-NMR and MS data: in agreement with those in [5].

*Boc-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*S*)-β³-HAla-(S)-β³-HLeu-OH* (**1c**). From the methyl-ester derivative: A soln. of **1d** (366 mg, 0.47 mmol) in TFE (3.7 ml) was treated with 5N NaOH (9.33 ml) and heated at 70° (bath temp.). After 45 min, THF (1 ml) was added, and after 27 h the mixture was diluted with TFE and neutralized with *Dowex-H⁺ 50 × 8*. The ion exchanger was removed by filtration and the filtrate evaporated. Precipitation of the obtained solid from TFE/MeOH yielded **1c** (225 mg, 63%). ^{1}H-NMR and MS data: in agreement with those in [5]. From the benzyl-ester derivative: **1f** (423 mg, 0.49 mmol) was dissolved in TFE (10 ml), and Pd/C (10%, 45 mg) was added. The apparatus was evacuated, flushed three times with H_2, and the mixture was stirred under H_2 for 32 h. Subsequent filtration through *Celite* and concentration under reduced pressure yielded **1c** (376 mg, 99%). ^{1}H-NMR and MS data: in agreement with those in [5].

*Boc-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*S*)-β³-HAla-(S)-β³-HLeuOMe* (**1d**). Boc-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OMe (1.01 g, 2.2 mmol) was Boc-deprotected according to *GP 3a*. A stirred soln. of the obtained TFA salt in $CHCl_3$ (2.2 ml) at 0° (ice-bath) under Ar was treated with Et_3N (1.5 ml, 10.8 mmol), a soln. of Boc-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OH (0.97 g, 2.2 mmol) in DMF (2.2 ml), HOBt (0.40 g, 2.6 mmol), and EDC (0.50 g, 2.6 mmol). The mixture was allowed to warm to r.t., and stirring was continued for 14 h. The mixture was evaporated, the residue was dried for 4 h under h.v. and then stirred for 20 min in MeOH. The white precipitate was collected by filtration, washed with H_2O/MeOH 1:1, and dried under h.v. Precipitation from TFE/EtOH yielded **1d** (1.04 g, 60%). ^{1}H-NMR Data: in agreement with those in [5]. FAB-MS: 806 (10.3, $[M+Na]^+$), 805 (25.2, $[M-1+Na]^+$), 783 (17.0, M^+), 684 (36.0, $[M+1-Boc]^+$), 683 (100, $[M-Boc]^+$), 570 (12.1).

*TFA · H-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*S*)-β³-HAla-(S)-β³-HLeu-OBn* (**1e**). Compound **1f** (69 mg, 0.08 mmol) was Boc-deprotected with TFA (0.45 ml) according to *GP 3b*. Coevaporation with toluene (3×) and drying under h.v. yielded **1e** (62 mg, 88%). Colorless glass. $[\alpha]_D^{r.t.} = -2.9$ ($c = 0.5$, MeOH). CD (0.2 mM in MeOH): $-3.8 \cdot 10^4$ (216 nm), 0 (207 nm), $+5.2 \cdot 10^4$ (199 nm). IR (KBr): 3291*m*, 3086*m*, 2963*m*, 1734*m*, 1654*s*, 1545*s*, 1458*m*, 1387*m*, 1309*w*, 1262*m*, 1202*s*, 1177*s*, 1143*m*, 799*w*, 721*w*, 698*w*, 598*w*. ^{1}H-NMR (400 MHz, CD_3OD): 0.88–0.95 (*m*, 6 Me); 1.08 (*d*, $J = 6.9$, 2 Me); 1.14 (*d*, $J = 6.7$, Me); 1.19 (*d*, $J = 6.7$, Me); 1.21–1.33 (*m*, 2 CH); 1.37–1.46 (*m*, 2 CH); 1.51–1.73 (*m*, 3 CH); 2.01–2.10 (*m*, CH); 2.16 (*dd*, $J = 14.7$, 11.7, 1 H, CH_2); 2.28–2.49 (*m*, 6 H, CH_2); 2.57–2.77 (m, 5 H, CH_2); 3.49–3.54 (*m*, CHN); 4.18–4.25 (*m*, CHN); 4.33–4.58 (*m*, 4 CHN); $\nu_A = 5.14$, $\nu_B = 5.19$ (*AB*, $J_{AB} = 12.5$, CH_2O); 7.31–7.40 (*m*, 5 arom. H); 7.43 (*d*, $J = 9.2$, NH); 7.83 (*d*, $J = 9.1$, NH); 8.22 (*d*, $J = 9.1$, NH). ^{13}C-NMR (100 MHz, CD_3OD): 18.2, 19.1, 19.3, 19.5, 20.8, 21.1, 22.9, 23.4, 23.7 (Me); 26.0, 26.1, 32.0, 34.1 (CH); 36.1, 39.1, 40.8, 42.0, 43.0, 43.3 (CH_2); 43.5, 43.6, 45.2 (CH); 45.5 (CH_2); 45.8 (CH); 46.4 (CH_2); 52.9, 56.3 (CH); 67.7 (CH_2); 128.9, 128.4, 127.7 (CH); 137.3, 171.6, 171.6, 171.8, 172.7, 173.1, 173.5 (C). FAB-MS: 761 (29), 760 (100, $[M+1]^+$).

*Boc-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*S*)-β³-HAla-(S)-β³-HLeu-OBn* (**1f**). Boc-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OBn (2.11 g, 4.0 mmol) was Boc-deprotected in $CHCl_3$ according to *GP 3a*. A stirred soln. of the obtained TFA salt in $CHCl_3$ (5 ml) was treated with NMM (1.65 ml, 15.0 mmol) and Boc-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OH (1.78 g, 4.0 mmol) in DMF (5 ml). After stirring for 4 h, the mixture was cooled to 0° (ice-bath), and HOBt (0.72 g, 4.8 mmol) and EDC (0.76 g, 4.0 mmol) were added. The mixture was first stirred at 0° (ice-bath) for 2 h and then allowed to warm to r.t. After stirring for 21 h, the mixture was evaporated, and the residue was dried under h.v. The obtained solid was dissolved in $CHCl_3$ and washed with 1N HCl. The org. phase was evaporated, and the obtained residue was washed with acetone. FC (MeOH/$CHCl_3$ 1:9 → 2:8) and precipitation from TFE/MeCN yielded **1f** (0.84 g, 25%). White amorphous solid. M.p. 237.0° (dec.). $[\alpha]_D^{r.t.} = -33.5$ ($c = 0.9$, TFE). CD (0.2 mM in MeOH): $+1.6 \cdot 10^4$ (205 nm), 0 (199 nm). IR (KBr): 3297*s*, 3074*w*, 2960*m*, 2872*w*, 1686*m*, 1646*s*, 1541*s*, 1457*m*, 1367*m*, 1310*m*, 1249*m*, 1173*m*, 1017*w*, 697*w*. ^{1}H-NMR (300 MHz, CD_3COOD; signals of rotamers in italics): 0.87–0.92 (*m*, 8 Me); 1.14–1.20 (*m*, 2 Me); 1.25–1.34 (*m*, 2 CH); 1.44 (*s*, *t*-Bu); 1.47–1.72 (*m*, 4 CH); *1.49* (*s*, *t*-Bu); 1.74–1.85 (*m*, 2 CH); 2.29–2.63 (*m*, 6 CH_2); 3.86–3.91 (*m*, CHN); 4.14–4.42 (*m*, 5 CHN); $\nu_A = 5.11$, $\nu_B = 5.19$ (*AB*, $J_{AB} = 12.3$, CH_2O); 7.34–7.40 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz, CD_3COOD): 18.0, 18.3, 18.8, 19.0, 20.0, 20.2, 21.9, 22.1, 23.1, 23.2 (Me); 25.5 (CH); 28.5 (Me); 33.0, 33.1, 33.7 (CH); 38.9, 39.7, 40.4, 42.0, 42.8, 43.0, 43.9, 44.5 (CH_2); 44.8, 46.0, 46.7, 53.8, 54.8, 55.6 (CH); 67.5 (CH_2); 80.4 (C); 129.3, 129.4, 129.6 (CH); 137.0, 158.1, 173.1, 173.4, 173.5, 173.7, 173.9, 174.1

(C). FAB-MS: 882 (24, $[M+Na]^+$), 881 (54), 860 (23, $[M+1]^+$), 859 (47, M^+), 761 (12), 760 (43), 759 (100), 646 (10). Anal. calc. for $C_{46}H_{78}N_6O_9$ (859.16): C 64.31, H 9.15, N 9.78; found: C 64.19, H 8.99, N 9.66.

*TFA · H-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-(*S*)-β³-HAla-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OH* (**2a**). A soln. of **2b** (26.9 mg, 0.031 mmol) in TFE (0.3 ml) was treated with 5N NaOH (0.62 ml) and heated at 50° (bath temp.). After 25 h, the mixture was diluted with TFE and neutralized with *Dowex-H⁺ 50 × 8*. The ion exchanger was removed by filtration, and the filtrate was evaporated and dried under h.v. The residue was treated with 3 ml of TFA for 1 h 45 min according to *GP 3b*. Purification by RP-HPLC (20–70% *B* in 30 min; C_{18}) according to *GP 5* yielded **2a** (7.3 mg, 31%). White amorphous solid. RP-HPLC (isocratic 40% *B*; C_{18}): t_R 11.5, purity >97%. M.p. <120° (dec.). UV (0.1 mM in MeOH): $1.0 \cdot 10^4$ (204 nm). CD (0.2 mM in MeOH): $-7.1 \cdot 10^4$ (215 nm), 0 (206 nm), $+11.8 \cdot 10^4$ (196 nm). IR (KBr): 3293*s*, 3084*m*, 2964*s*, 2421*w*, 1646*s*, 1548*s*, 1458*s*, 1375*m*, 1311*m*, 1203*s*, 1139*s*, 836*w*, 800*w*, 722*m*. ^{1}H-NMR (500 MHz, CD_3OD): 0.90–0.96 (*m*, 6 Me); 1.07 (*d*, *J* = 6.9, 2 Me); 1.14 (*d*, *J* = 6.7, 2 Me); 1.18–1.44 (*m*, 4 CH); 1.23 (*d*, *J* = 6.6, Me); 1.56–1.64 (*m*, 2 CH); 1.70–1.77 (*m*, CH); 2.03–2.10 (*m*, CH); 2.21–2.33 (*m*, CH_2CO); 2.38–2.62 (*m*, 5 CH_2CO); 2.76 (*dd*, *J* = 15.4, 11.6, 1 H, CH_2CO); 2.89 (*dd*, *J* = 14.9, 11.9, 1 H, CH_2CO); 3.54–3.58 (*m*, CHN); 4.24–4.28 (*m*, CHN); 4.45–4.49 (*m*, 4 CHN); 4.54–4.59 (*m*, CHN); 7.36 (*d*, *J* = 9.3, NH); 7.64 (*d*, *J* = 8.3, NH); 7.74 (*d*, *J* = 9.4, NH); 8.32 (*d*, *J* = 8.8, NH); 8.39 (*d*, *J* = 9.3, NH). ^{13}C-NMR (100 MHz, CD_3OD): 17.57, 19.01, 19.49, 19.85, 20.97, 21.19, 21.34, 22.80, 23.02, 23.51, 23.65 (Me); 26.02, 31.96, 34.19 (CH); 35.97, 39.16, 40.78, 42.38, 42.70, 42.94, 43.12 (CH_2); 43.41, 43.61, 43.75, 45.34, 45.70 (CH); 45.78, 46.93 (CH_2); 53.11, 55.91 (CH); 171.19, 171.48, 171.79, 172.00, 172.03, 173.41, 174.95 (C). FAB-MS: 792 (15), 777 (12, $[M+Na]^+$), 776 (32), 756 (12), 755 (44, $[M+1]^+$), 754 (100, M^+), (100, $[M+1]^+$).

*Boc-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-(*S*)-β³-HAla-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OMe* (**2b**). Boc-(*S*)-β³-HAla-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OMe (80 g, 0.15 mmol) was Boc-deprotected in $CHCl_3$ for 3 h according to *GP 3a*. A stirred soln. of the obtained TFA salt in $CHCl_3$ (0.75 ml) at 0° (ice-bath) under Ar was treated with DMF (0.08 ml), Et_3N (0.06 ml, 0.43 mmol), HOBt (27 mg, 0.18 mmol), Boc-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OH (67 mg, 80.2 mmol), EDC (36 mg, 0.19 mmol), and another amount of DMF (0.5 ml). The mixture was allowed to warm to r.t. After stirring for 20 h, the mixture was evaporated and the residue dried under h.v. The obtained solid was stirred for 11 h in H_2O. The white precipitate was collected by filtration, stirred for 13 h in MeOH, collected by filtration again and dried under h.v.: **2b** (79 mg, 62%). For anal. purposes, **2b** was precipitated from TFE/MeCN. ^{1}H-NMR and MS data: in agreement with those in [6].

*TFA · H-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-β-HGly-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OH* (**3a**). Compound **3b** (28 mg, 0.03 mmol) was dissolved in TFA (2 ml). After stirring for 2 h at r.t., the mixture was evaporated, and the oily residue was coevaporated with toluene and lyophilized (1,4-dioxan). Precipitation of the lyophilisate from EtOH/pentane yielded **3a** (8.8 mg, 36%). White amorphous solid. M.p. 250–252°. $[\alpha]_D^{r.t.} = -26.7$ (*c* = 0.3, TFE). CD (0.2 mM in MeOH): $-4.4 \cdot 10^4$ (216 nm), 0 (207 nm), $+7.7 \cdot 10^4$ (198 nm). IR (KBr): 3293*s*, 3081*m*, 2961*m*, 1645*s*, 1543*s*, 1458*m*, 1369*m*, 1202*m*, 1139*m*, 800*w*, 721*w*. ^{1}H-NMR (500 MHz, CD_3OD): 0.90–0.95 (*m*, 6 Me); 1.05 (*d*, *J* = 6.9, Me); 1.06 (*d*, *J* = 6.9, Me); 1.14 (*d*, *J* = 6.7, Me); 1.22 (*d*, *J* = 6.7, Me); 1.25–1.32 (*m*, 2 CH); 1.35–1.45 (*m*, 2 CH); 1.57–1.63 (*m*, 2 CH); 1.72–1.78 (*m*, CH); 1.98–2.06 (*m*, CH); 2.26–2.52 (*m*, 5 CH_2CO); 2.59–2.70 (*m*, 3 H, CH_2CO); 2.76 (*dd*, *J* = 14.7, 10.6, 1 H, CH_2CO); 3.05–3.10 (*m*, CHN); 3.46–3.50 (*m*, CHN); 3.83–3.90 (*m*, 2 CHN); 4.18–4.23 (*m*, CHN); 4.35–4.42 (*m*, 2 CHN); 4.44–4.51 (*m*, CHN). ^{13}C-NMR (125 MHz, CD_3OD): 18.0, 19.0, 19.2, 19.8, 20.8, 21.1, 22.5, 22.8, 23.5, 23.6 (Me); 26.1, 26.1, 32.0, 34.0 (CH); 35.9, 36.0, 36.6, 39.4, 41.7, 42.6, 43.1, 43.2 (CH_2); 43.9, 43.9 (CH); 45.6 (CH_2); 46.0, 46.1 (CH); 46.3 (CH_2); 53.4, 56.1 (CH); 171.4, 171.9, 172.4, 172.6, 172.9, 173.2, 176.0 (C). FAB-MS: 762 (13), 741 (42), 740 (100, M^+).

*Boc-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-β-HGly-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OH* (**3b**). Compund **3c** (105 mg, 0.12 mmol) was treated at 40° in TFE (1 ml) with 5N NaOH. After stirring for 18 h at this temp., the mixture was neutralized with *Dowex-H⁺ 50 × 8*. The ion exchanger was removed by filtration. Evaporation of the filtrate under reduced pressure yielded **3b** (92 mg, 89%). For anal. purposes, **3b** was recrystallized from MeOH. White amorphous solid. M.p. 258° (dec.). $[\alpha]_D^{r.t.} = -12.1$ (*c* = 0.6, TFE). CD (0.2 mM in MeOH): $+8.6 \cdot 10^4$ (203 nm). IR (KBr): 3302*s*, 3080*m*, 2961*s*, 2872*m*, 1687*s*, 1646*s*, 1542*s*, 1458*s*, 1367*m*, 1308*m*, 1249*m*, 1174*m*. ^{1}H-NMR (500 MHz, DMSO): 0.75–0.85 (*m*, 8 Me); 0.98 (*d*, *J* = 6.5, 2 Me); 1.11–1.20 (*m*, 2 CH); 1.30–1.39 (*m*, 2 CH); 1.36 (*s*, *t*-Bu); 1.51–1.59 (*m*, 2 CH); 1.59–1.68 (*m*, 2 CH); 2.03–2.10 (*m*, 4 H, CH_2CO); 2.14–2.26 (*m*, 9 H, CH_2CO); 2.31–2.36 (*m*, 1 H, CH_2CO); 3.13–3.14 (*m*, CHN); 3.23–3.26 (*m*, CHN); 3.64–3.68 (*m*, CHN); 3.95–4.18 (*m*, 5 CHN); 6.51 (*d*, *J* = 9.4, NH); 7.58–7.73 (*m*, 4 NH); 7.84 (br., NH). ^{13}C-NMR (125 MHz, DMSO): 17.7, 17.8, 19.0, 19.1, 19.5, 21.4, 23.1, 23.2 (Me); 24.2 (CH); 28.1, 28.9 (Me); 31.2, 31.6 (CH); 35.4, 38.4, 40.2, 41.8 (CH_2); 42.0, 42.1 (CH); 42.9, 43.0, 43.7 (CH_2); 44.0, 44.1, 50.8, 50.9, 52.5

(CH); 77.2, 155.0, 169.1, 169.5, 169.6, 169.7, 169.8, 172.3 (C). FAB-MS: 863 (68, $[M+Na]^+$), 862 (100), 840 (8, M^+), 762 (13), 741 (50), 740 (91), 627 (18), 182 (16), 128 (14).

*Boc-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-β-HGly-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OMe* (**3c**). Boc-β-HGly-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OMe (175 mg, 0.33 mmol) was Boc-deprotected in $CHCl_3$ according to *GP 3a*. To a stirred soln. of the obtained TFA salt in $CHCl_3$ (1.65 ml) and DMF (0.17 ml) at 0° (ice-bath) under Ar, Et_3N (0.18 ml, 0.13 mmol), $CHCl_3$ (1.3 ml), DMF (0.3 ml), Boc-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OH (150 mg, 0.34 mmol), HOBt (63 mg, 0.42 mmol), EDC (78 mg, 80.4 mmol), and another amount of $CHCl_3$ (1 ml) was added. The mixture was allowed to warm to r.t., and stirring was continued for 13 h. After evaporation, the residue was dried for 5 h under h.v. and subsequently stirred for 2.5 h in H_2O. The white precipitate was collected by filtration and washed with H_2O. FC (MeOH/$CHCl_3$ 8:92 → 15:85) yielded **3c** (154 mg, 55%). ^{1}H-NMR and MS data: in agreement with those in [6].

*Ac-(2*R*,3*S*)-β²,³-HVal(α-Me)-(*S*)-β²-HVal-(*S*)-β³-HLys-(2*R*,3*S*)-β²,³-HAla-NH₂* (**4**). *Rink* amide resin [40][41] (181 mg, 1.00 mmol/g) was swelled in DMF/CH_2Cl_2 1:1 (3.6 ml) for 30 min and Fmoc-deprotected using 20% piperidine in DMF (5.4 ml, 2 × 15 min) under Ar bubbling. A soln. of Fmoc-(2*R*,3*S*)-β²,³-HAla(α-Me)-OH (83.0 mg, 0.244 mmol), BOP (80.1 mg, 0.54 mmol) and HOBt (82.2 mg, 0.54 mmol) in DMF (2 ml), and (i-Pr)$_2$EtN (279 µl, 1.63 mmol) were added successively to the resin, and the suspension was mixed for 60 min by Ar bubbling. Monitoring of the coupling was performed with TNBS [42]. The resin was then filtered and washed (11 ml) with DMF/CH_2Cl_2 1:1 (3 × 3 min). The initial loading of the *Rink* amide resin was used to calculate the amount of the first β-amino acid attached to the resin. The Fmoc group of the first β-amino acid attached to the resin was removed using DBU/piperidine/DMF (9.1 ml; 1:1:48, 2 × 10 min) under Ar bubbling. The resin was then filtered and washed with DMF/CH_2Cl_2 1:1 (9.1 ml/6 × 3 min). Solid-phase synthesis was continued by sequential incorporation of Fmoc-(*R*)-β³-HLys(Boc)-OH (pepared as described in [21]), Fmoc-(*S*)-β²-HVal-OH (pepared as described in [21]), and Fmoc-(2*R*,3*S*)-β²,³-HVal(α-Me)-OH. For each coupling step, a soln. of the Fmoc-β-amino acid (1–3 equiv.), BOP (3 equiv.) and HOBt (3 equiv.) in DMF (2 ml), and (i-Pr)$_2$EtN (9 equiv.) were added successively to the resin, and the suspension was mixed by Ar bubbling for 15–60 min. Monitoring of the coupling reaction was performed with TNBS. In case of a positive TNBS test (indicating incomplete coupling), the suspension was allowed to react further for 15–60 min with an additional equiv. of Fmoc-β-amino acid and coupling reagents. The resin was then filtered and washed (9.1 ml) with DMF/CH_2Cl_2 1:1 (3 × 3 min) prior to the following Fmoc-deprotection step. After the removal of the last Fmoc protecting group, the resin was acetylated at the N-terminus. The Fmoc-deprotected peptide-resin was washed (5.4 ml/mmol) with DMF/CH_2Cl_2 1:1 (5 × 3 min) and treated successively with (i-Pr)$_2$EtN (620 µl, 3.62 mmol) and Ac_2O (171 µl, 1.81 mmol) in DMF/CH_2Cl_2 1:1 (2 ml) under Ar bubbling for 10 min. Monitoring of the acetylation was performed with TNBS. The resin was then washed (5.4 ml) with DMF (5 × 3 min), CH_2Cl_2 (3 × 3 min), Et_2O (5 × 1 min), and dried under h.v. for 12 h. The dry acetylated *Rink* amide peptide-resin was first swelled in CH_2Cl_2 (3.6 ml, 10 min), then treated with CH_2Cl_2/TFA/(i-Pr)$_3$SiH 90:9:1 (3.6 ml, 20 min), then again with CH_2Cl_2/TFA/(i-Pr)$_3$SiH 90:9:1 (3×), and with CH_2Cl_2/TFA/(i-Pr)$_3$SiH 95:4:1 (3.6 ml, 3×), allowing the solvent to pass through the resin bed slowly. The deprotection was completed by stirring the oily residue in TFA/H_2O/(i-Pr)$_3$SiH 95:2.5:2.5 for 10 min. The solvent was evaporated, coevaporated with CH_2Cl_2, and dried under h.v. The precipitate, which formed upon addition of cold Et_2O to the oily residue, was collected by centrifugation and afforded a first fraction of crude **4** as TFA salt (41 mg, 77%, 58% purity (HPLC)). Repeated treatment of the resin as described above yielded an additional fraction of the crude peptide **4** (4.5 mg, 8%, 57% purity (HPLC). Purification by RP-HPLC (2–40% *B* in 30 min; C_8) according to *GP 5* yielded the TFA salt of **4** (23.9 mg, 45%) . White solid. RP-HPLC (2–50% *B* in 20 min; C_{18}): t_R 12.0, purity > 97%. M.p. 267° (dec.). CD (0.2 mM in MeOH): $+1.53 \cdot 10^5$ (202 nm). CD (0.2 mM pH 11): $+6.76 \cdot 10^4$ (201 nm). CD (0.2 mM pH 3.6): $+6.56 \cdot 10^4$ (202 nm). IR ($CHCl_3$): 3634*m*, 3437 (br.), 3007*w*, 2945*m*, 2838*w*, 1713*w*, 1601*w*, 1467*w*, 1333*w*, 1261*m*, 1098*m*, 1016*s*. ^{1}H-NMR (500 MHz, D_2O): 0.84 (*d*, *J* = 6.8, Me); 0.86 (*d*, *J* = 6.8, Me); 0.89 (*d*, *J* = 6.7, Me); 0.97 (*d*, *J* = 6.7, Me); 1.02 (*d*, *J* = 6.9, Me); 1.09 (*d*, *J* = 7.0, Me); 1.12 (*d*, *J* = 6.7, Me); 1.33–1.70 (*m*, 7 H, CH, CH_2); 1.71–1.82 (*m*, CH); 2.02 (*s*, MeCO); 2.12–2.16 (*m*, CHCO); 2.37–2.47 (*m*, 3 CHCO); 2.56–2.61 (*m*, CHCO); 2.95 (*t*, *J* = 7.5, CH_2N); 3.21 (*dd*, *J* = 13.7, 9.6, 1 H, CH_2N); 3.50 (*dd*, *J* = 13.7, 4.2, 1 H, CH_2N); 3.83–3.88 (*m*, CHN); 3.95–4.01 (*m*, CHN); 4.16–4.17 (*m*, CHN); 7.82 (*d*, *J* = 10.4, NH); 8.04 (*t*, *J* = 5.4, NH); 8.11 (*d*, *J* = 8.9, NH). ^{13}C-NMR (125 MHz, D_2O): 15.36, 16.46, 19.06, 20.56, 22.19, 22.23, 22.59, 24.50, (Me); 24.50, 28.99 (CH_2); 31.02, 32.36 (CH); 35.40, 41.90, 42.14, 44.54 (CH_2); 45.04, 48.23, 49.68, 50.31, 55.97, 59.48 (CH); 175.09, 176.87, 178.62, 180.18, 182.87 (C). FAB-MS: 541 (100, $[M+1]^+$).

*H-(2*R*,3*S*)-β²,³-HAla(α-Me)-(2*R*,3*S*)-β²,³-HVal(α-Me)-(*S*)-β²-HVal-(*S*)-β³-HLys-(2*R*,3*S*)-β²,³-HAla-(2*R*,3*S*)-β²,³-HLeu(α-Me)-OH* (**5**). Esterification of the Fmoc-protected β-amino acid with the *ortho*-chlorotrityl chloride resin was performed according to [43][44]. The resin (210 mg; initial loading: 1.00 mmol Cl/g) was

dried under h.v. for 20 min and swelled in CH_2Cl_2 (4.2 ml) for 10 min. A soln. of Fmoc-(2*R*,3*S*)-$\beta^{2,3}$-HLeu(α-Me)-OH (64.0 mg, 0.17) in CH_2Cl_2 (2.1 ml) and $(i\text{-}Pr)_2EtN$ (101 µl, 0.59 mmol) were then added successively, and the suspension was mixed by Ar bubbling for 4 h. Subsequently, the resin was filtered, washed (4.2 ml) with CH_2Cl_2/MeOH/$(i\text{-}Pr)_2EtN$ 17:2:1 (3 × 3 min), CH_2Cl_2 (3 × 3 min), DMF (2 × 3 min), CH_2Cl_2 (3 × 3 min), MeOH (2 × 3 min), and finally dried under h.v. for 12 h. The resin substitution was determined by measuring the absorbance of the dibenzofulvene piperidine adduct:

An aliquot (10–15 mg) of the Fmoc-amino acid resin was washed with MeOH and Et_2O in a small glass tube ('Glührörchen'), dried under h.v. for 20–30 min, and weighed exactly ($m_{resin} = 13.4$ mg). Piperidine (20%) in DMF (2 ml) was added. After 20 min, this soln. was diluted with DMF to 25 ml in a graduated cylinder. The obtained soln. was dispensed in a UV cell, and DMF in another UV cell (blank), and the absorbance (A) was measured at 300, 289, and 266 nm [45]. The loading (Subst) was calculated for each of the three values according to *Eqn. 1*.

$$\text{Subst (mmol/g resin)} = 25000 \cdot A/(\varepsilon \cdot m_{resin}) \quad (1)$$

Extinction coefficients of the dibenzofulvene piperidine adduct: $\varepsilon(300\text{ nm}) = 7800$; $\varepsilon(289\text{ nm}) = 5800$; $\varepsilon(266\text{ nm}) = 17500$; m_{resin} in mg.

The theoretical substitution of the *ortho*-chlorotrityl chloride resin ($Subst_{theor.}$), which corresponds to 100% esterification, is given by *Eqn. 2* [43][15]).

$$Subst_{theor.}\text{ (mmol/g resin)} = n/[1 + 0.001 \cdot n(MW - 36.5)] \quad (2)$$

n = mmol of Fmoc-protected β-amino acid used for esterification per 1 g of resin; MW = molecular weight of the Fmoc-protected β-amino acid.

The yield for the attachment to the resin (loading yield) was determined by *Eqn. 3*.

$$\text{Loading yield} = \text{Subst}/Subst_{theor.} \quad (3)$$

Loading 0.53 mmol/g (85%); 112 µmol of anchored Fmoc-(2*R*,3*S*)-$\beta^{2,3}$-HLeu(α-Me)-OH. The Fmoc group of the first amino acid attached to the *ortho*-chlorotrityl-chloride resin was removed using 20% piperidine in DMF (6.3 ml, 2 × 15 min) under Ar bubbling. The resin was then filtered and washed with DMF (6.3 ml, 6 × 3 min). Solid-phase synthesis was continued by sequential incorporation of Fmoc-(2*R*,3*S*)-$\beta^{2,3}$-HAla(α-Me)-OH, Fmoc-(*R*)-β^3-HLys(Boc)-OH (prepared as described in [21]), Fmoc-(*S*)-β^2-HVal-OH (prepared as described in [21]), Fmoc-(2*R*,3*S*)-$\beta^{2,3}$-HVal(α-Me)-OH, and Fmoc-(2*R*,3*S*)-$\beta^{2,3}$-HAla(α-Me)-OH. For each coupling step, a soln. of the Fmoc-β-amino acid (3 equiv.), BOP (3 equiv.) and HOBt (3 equiv.) in DMF (2 ml), and $(i\text{-}Pr)_2EtN$ (9 equiv.) were added successively to the resin, and the suspension was mixed by Ar bubbling for 15–60 min. Monitoring of the coupling reaction was performed with TNBS [42]. In case of a positive TNBS test (indicating incomplete coupling), the suspension was allowed to react further for 15–60 min. The resin was then filtered and washed (6.3 ml) with DMF (3 × 3 min) prior to the following Fmoc deprotection step. After the removal of the last Fmoc protecting group, the resin was washed (6.3 ml) with DMF (6 × 3 min), CH_2Cl_2 (3 × 3 min), Et_2O (5 × 1 min), and dried under h.v. for 12 h. The dry Fmoc-deprotected peptide-resin was treated for 2 h with 10 ml of a TFA/H_2O/$(i\text{-}Pr)_3SiH$ (95:2.5:2.5) soln. The resin was removed by filtration, washed with TFA, and the org. phase containing the peptide was concentrated under reduced pressure. The precipitate, which formed upon addition of cold Et_2O to the oily residue, was collected by filtration: crude **5** as TFA salt (97.2 mg, 90%), purity 57% (RP-HPLC). Purification by RP-HPLC (5–18% *B* in 10 min, then 18–30% *B* in 25 min; C_8) according to *GP 5* yielded the TFA salt of **5** (18.7 mg, 17%). White solid. RP-HPLC (5–30% *B* in 10 min; then 30–40% *B* in 10 min; C_8): t_R 13.0 min, purity > 98%. M.p. < 250° (dec.). CD (0.2 mM in MeOH): $+6.73 \cdot 10^4$ (208 nm). CD, (0.2 mM pH 11): $+1.21 \cdot 10^5$ (204 nm). CD (0.2 mM pH 3.6): $+5.85 \cdot 10^4$ (206 nm). IR (KBr): 3600–2600 (br.), 1654*s*, 1541*s*, 1458*m*, 1388*w*, 1304*w*, 1271*w*, 1202*s*, 1176*s*, 1138*s*, 836*w*, 799*w*, 722*w*, 668*w*. ^{1}H-NMR (400 MHz, D_2O): 0.80–0.87 (*m*, 4 Me); 0.92–1.05 (*m*, 6 Me); 1.21–1.80 (*m*, 2 Me, 11 CH); 2.11–2.18 (*m*, CHCO); 2.29–2.40 (*m*, 3 CHCO); 2.49–2.64 (*m*, 3 CHCO); 2.91 (*t*, $J = 7.8$, CH_2N); 3.23 (*dd*, $J =$ 13.7, 9.5, CHN); 3.40–3.51 (*m*, 2 CHN); 3.86–3.95 (*m*, 2 CHN); 4.11–4.16 (*m*, 2 CHN); 8.00 (*d*, $J = 9.5$, NH); 8.00–8.02 (*m*, NH); 8.06 (*d*, $J = 8.7$, NH). ^{13}C-NMR (100 MHz, D_2O): 14.60, 16.09, 16.11, 17.32, 18.88, 19.48,

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[15]) This formula does not take into account the small difference in weight between the material with Cl, compared to the material with MeO, the latter being formed on the resin during the capping step.

21.10, 22.27, 22.52, 22.57, 23.23 (Me); 25.04 (CH_2); 25.42 (Me); 27.16 (CH); 29.03 (CH_2); 31.21, 32.30 (CH); 35.44, 41.94, 42.17, 43.42, 44.46 (CH2); 45.23, 46.69, 47.30, 49.25, 59.71, 50.29, 51.91, 52.03 (CH); 55.79, 59.09 (CH); 175.15, 178.48, 178.93, 179.53, 179.95, 182.31 (C). FAB-MS: 763 (27.8, $[M+Na]^+$), 741 (100, M^+).

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