β-Peptide Conjugates: Syntheses and CD and NMR Investigations of β/α-Chimeric Peptides, of a DPA-β-Decapeptide, and of a PEGylated β-Heptapeptide

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β^3-Peptides consisting of six, seven, and ten homologated proteinogenic amino acid residues have been attached to an α-heptapeptide (all D-amino acid residues; **4**), to a hexaethylene glycol chain (PEGylation; **5c**), and to dipicolinic acid (DPA derivative **6**), respectively. The conjugation of the β-peptides with the second component was carried out through the N-termini in all three cases. According to NMR analysis (CD_3OH solutions), the (*M*)-3_{14}-helical structure of the β-peptidic segments was unscathed in all three chimeric compounds (*Figs. 2*, *4*, and *5*). The α-peptidic section of the α/β-peptide was unstructured, and so was the oligoethylene glycol chain in the PEGylated compound. Thus, neither does the appendage influence the β-peptidic secondary structure, nor does the latter cause any order in the attached oligomers to be observed by this method of analysis. A similar conclusion may be drawn from CD spectra (*Figs. 1*, *3*, and *5*). These results bode well for the development of delivery systems involving β-peptides.

1. Introduction. – Many natural products are conjugates made up of a mixture of peptide segments, polyketides, sugars, or fatty acids. While these various structures may not all play a role in promoting a key biological conformation or be part of an essential pharmacophore, they are nevertheless important for the ability to impart stability, solubility, or the correct locational recognition motif to promote optimal biological function. Several pharmaceutical agents also make use of additional conjugation to impart favorable physical properties on an effective but normally short-lived molecule (*cf.* polyethylene glycol (PEG) chains to enhance half-life and increase solubility).

In an effort to explore the possibility of using β-peptides as delivery systems for drugs, we aimed to prepare β-peptidic conjugates of β-peptides (consisting of homologated proteinogenic amino acid residues) that would normally adopt a 3_{14}-helix conformation in solution [1–3] to investigate whether the nonpeptidic segment

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[3]) Part of the Ph.D. Thesis of *J. S.*, ETH Dissertation No. 14298 (2001).
[4]) Postdoctoral Research Fellow at ETH-Zürich (2004–2006), financed by the *Deutsche Forschungsgemeinschaft*, by the *Swiss National Science Foundation* (No. 200020-100182), and by *Novartis Pharma AG*, Basel.

would have an effect on the stability of the helix. The CD- and NMR-solution structure analyses of the β-peptides, with and without the conjugate, would be used to investigate if the stability of the helix was influenced in any way, and whether or not the β-peptidic helix would induce secondary structures in an attached α-peptide or in an oligoethylene glycol moiety.

2. *α/β*-Chimeric Peptides. – Peptides with 'heterogeneous' backbones ('mixed' peptides) consisting of α-, β-, γ-, and/or δ-amino acid residues have recently moved into the focus of interest [4]. We had wondered for a long time[3]) whether an (*M*)-3_{14}-helix-forming β-peptide would influence the folding of an attached α-peptide, *i.e.*, whether the increased helical propensity of the β-peptide portion could somehow promote, or 'force', helicity in the α-peptidic segment of such a chimeric peptide. Since the α-peptide composed of L-amino acid residues folds to a right-handed helix, while the β-peptide consisting of L-β-amino acids folds to a left-handed helix, we decided to perform the test with an α-peptide built of D-amino acid moieties, so that (*M*)-helicity could possibly be attained along the whole length of the 'mixed'-peptide chain[5])[6]).

For structural investigations of such chimeric β/α-peptides that may adopt a $3_{14}/3.6_{13}$-helical motif, an appropriate sequence with intrinsic folding propensity was needed. We have previously shown that β-peptides composed of as few as six L-β-amino acid residues with the side chains of Val, Ala, and Leu fold into a (*M*)-3_{14}-helical structure [1][2], and *Karle et al.* had reported that α-peptides containing α-amino-isobutyric acid (Aib) residues adopt a helical structure (3.6_{13}- and/or 3_{10}-helix) in the solid state [7]. Thus, tridecapeptide **3** was prepared by fragment coupling in solution of the Aib-containing α-peptide **1c** with the β-peptide **2** (see *Exper. Part*). The β-peptidic 'fragment' **2** exhibits a typical CD pattern of a (*M*)-3_{14}-helix [1] (a trough at 215 nm, not shown here), and the α-peptidic 'fragment' **1** consisting of all D-α-amino acids with a central Aib residue gives rise to positive-only CD spectra (*Fig. 1,a*) of various intensities, depending upon its state of protection[7]). The 'mixed' α,β-peptide **3**, on the other hand, shows the expected trough at 215 nm, albeit weak, as compared to a mixture of its precursors, the α-peptide **1c** and the β-peptide **2** (*Fig. 1,b*); a summation of the CD curves of **1c** and **2** results in a very similar picture. With due caution[8]), we may conclude from the CD spectra that there is no influence of the β-peptidic on the α-peptidic secondary structure, neither in a mixture (intermolecular interactions might have shown up) nor in the chimeric α/β-peptide **3** (intramolecular interactions).

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5) Natural peptides can also fold to a left-handed helix, of which short 2- to 3-pitch-long segments are seen in many proteins (see the *Ramachandran* diagram in textbooks of peptide and protein chemistry).

6) Furthermore, there is a right-handed 3_{10}-helix of α-peptide chains. This helix is the preferred folding pattern of peptides consisting of *geminally* disubstituted α-amino-acid residues, such as Aib (aminoisobutyric acid) or Iva (isovaline or 2-amino-2-methylbutanoic acid [5]). For a comprehensive treatment, see the recent monograph 'Peptaibiotics – Fungal Peptides Containing α-Dialkyl α-Amino Acids' [6].

7) A (*P*)-3.6_{13}-helical structure was observed for the fully-protected α-heptapeptide Boc-L-Val-L-Ala-L-Leu-Aib-L-Val-L-Ala-L-Leu-OMe in crystals; however, NMR evidence suggests conformational variability in solution [7].

8) In our experience [2b][8], CD-only analysis of β-peptides can be misleading.

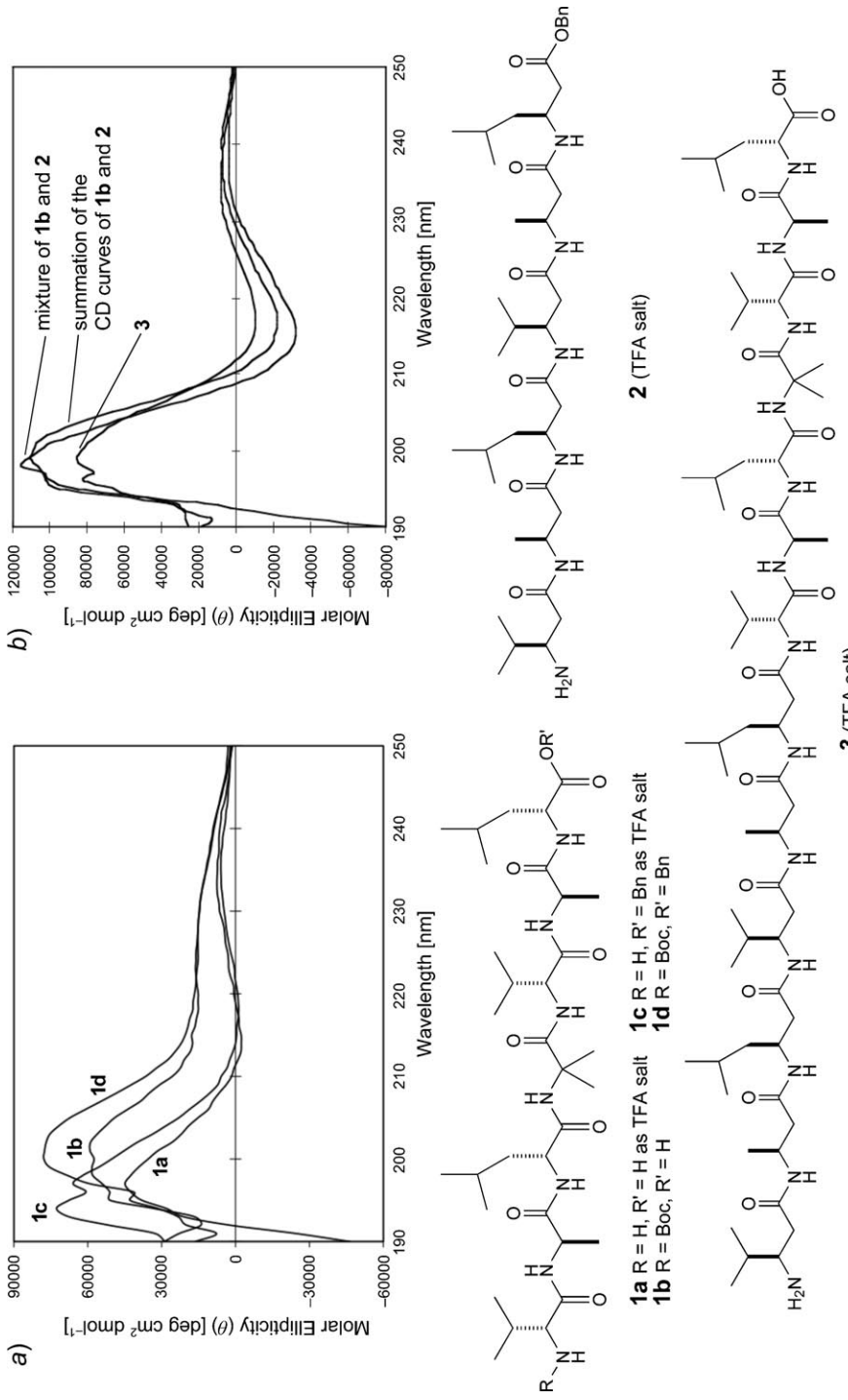

Fig. 1. *Aib-Containing α-heptapeptide* **1**, *β-hexapeptide* **2**, *and chimeric α/β-peptide* **3** *with CD spectra.* The α-peptide **1** consists of D-amino acid residues, and its CD spectrum (*a*) shows a strong *Cotton* effect near 200 nm. The CD spectra (*b*) of the 'mixed' peptide **3** and of mixtures of **1** and **2**.

An NMR-solution structural analysis of the α/β-peptide **3** was not possible, due to the fact that it contains, besides Aib, amino acid residues with only three different types of side chains (Me, i-Pr, i-Bu). We, therefore, prepared (by solid-phase synthesis on *Rink* amide AM resin; see *Exper. Part*) the tridecapeptide **4** with the following features: *i*) there are no two identical residues in the α- and β-peptidic segment; *ii*) there are five functionalized side chains for good solubility in a protic solvent; *iii*) the β-peptidic part carries a Glu and a Lys side chain in (i) and ($i+3$) positions for salt-bridge stabilization of a 3_{14}-helix [2b][9]; *iv*) there are two amino acids (Phe and Trp) with aromatic groups in the side chain for better HPLC detection; *v*) the indolyl group of Trp provides fluorescence; and *vi*) there is a helix-inducing Aib residue in the center of the α-heptapeptidic D-amino acid sequence. A detailed NMR analysis of the tridecapeptide **4** was carried out, including a structure calculation by simulated annealing using the XPLOR-NIH protocol, and distance as well as dihedral angle constraints as derived from the NOE data and coupling constants (for complete assignments of the ^{1}H- and ^{13}C-NMR signals, see *Tables 1* and *2* in the *Exper. Part*). The ten structures lowest in calculated energy, and without NOE or dihedral angle constraint violations are displayed in *Fig. 2*. This structural bundle reveals that all six β^3homo-amino acid residues from the N-terminus to β^3hIle^6 are arranged in two complete turns of a (M)-3_{14}-helix, whereas the NMR data for the seven α-amino acid residues do not constrain this C-terminal part to any of the secondary structures known for α-peptides. This is corroborated by the ^{3}J(HN,Hα) values for residues Phe^7 to Ala-NH$_2^{13}$, which range from 5.9 to 7.8 Hz and indicate a random coil arrangement of the backbone in the α-peptidic part. Therefore, the answer to the question of whether a β-peptide helix can induce helicity in an α-peptide is, in this case, no. This result does, however, provide a unique demonstration of the difference between the folding propensities of α- and β-peptides: even with a helix-inducing Aib residue the α-peptidic portion is disordered, while the β-peptidic portion shows an ordered 3_{14}-helix. How could one better demonstrate this difference than by putting an α- and β-peptide together covalently in the same molecule and examining their conformations 'under identical conditions'.

3. PEGylated β-Peptide. – Attachment of poly- or oligoethylene glycol chains (PEGylation)[9]) to therapeutic proteins, peptides (*cf.* **A**), and small-molecule therapeutics is a well-established method used to influence solubility, stability, immunogenicity, pharmacokinetics, and mode of action [10][10]). Unlike in polyethy-

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[9]) Strictly speaking, polyethylene glycol, prepared by polymerization of ethylene oxide ($n\ C_2H_4O + HX \rightarrow H\text{-}(OCH_2CH_2)_n\text{-}X$) is not a uniform compound, but a mixture of oligoethylene glycols with a *Gaussian* distribution of molecular weights. Our peptide derivative **5c** carries a hexaethylene glycol chain, attached to the N-terminus through a β-hydroxypropanoyl group. Still, following common practice, herein we use the term '*PEGylated* β-heptapeptide'.

[10]) One example is PEGylated interferon alfa-2a (40 kD; commercial name *PEGASYS*), an antiviral drug developed by *F. Hoffmann-La Roche*; it has a dual mode of action – both against viruses and on the immune system. This drug is approved around the world for the treatment of chronic hepatitis C (including patients with HIV co-infection, cirrhosis, 'normal' levels of ALT) and has recently been approved (in the EU, USA, China, and many other countries) for the treatment of chronic hepatitis B (see http://www.pegasys.com/about-pegasys/how-pegasys-works.aspx). For a review article on PEGylated proteins, peptides and non-peptidic drugs, see [10].

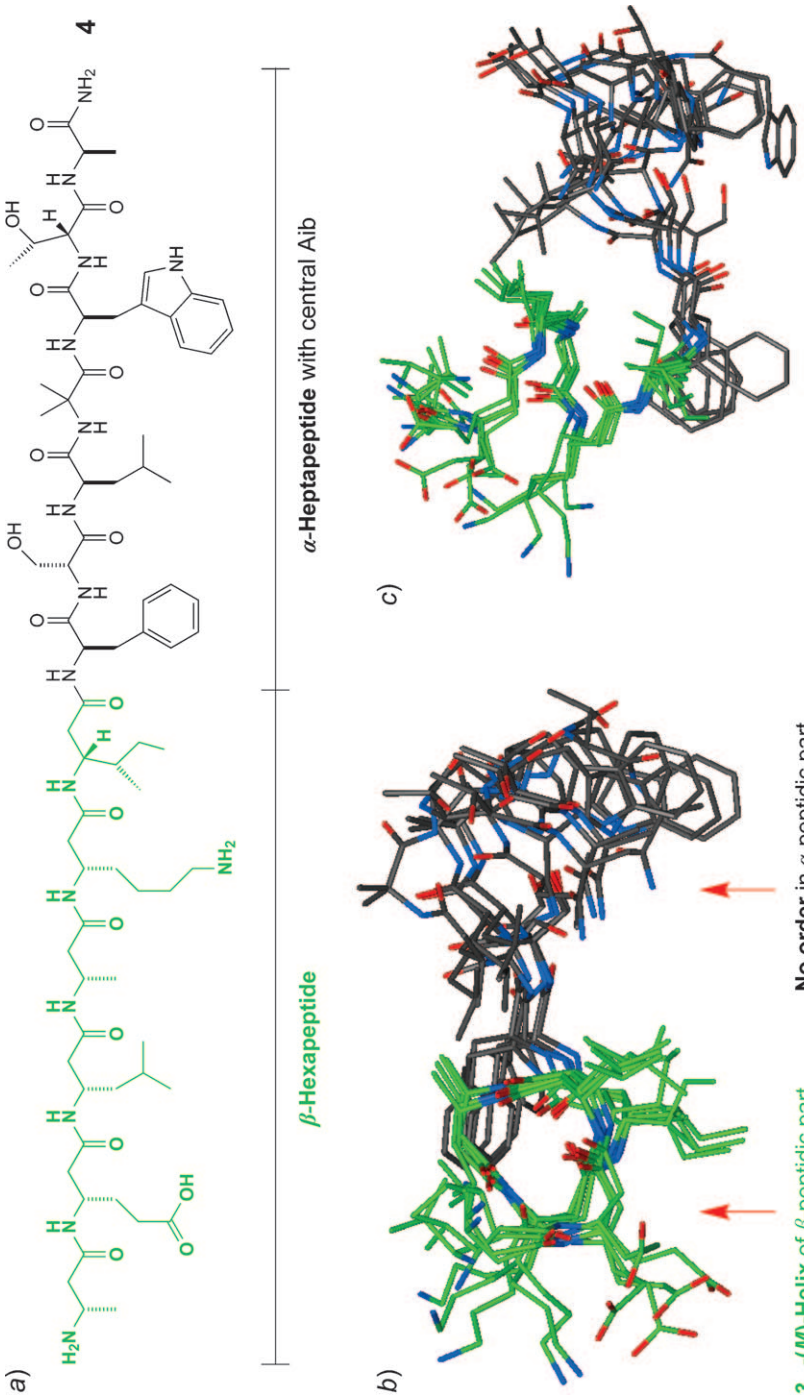
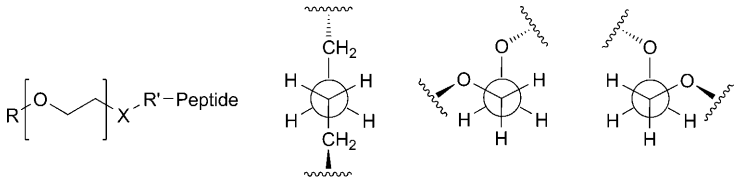


Fig. 2. *Tridecapeptide* **4**. NMR-Solution structure (in CD_3OH) in *a*) top view, and *b*) side view. The β-peptidic section adopts an ordered 3_{14}-(*M*)-helix, while the α-peptidic section has no discernable structure (random conformation). This demonstrates the dramatic difference in the folding propensities of β- and α-peptides.

lene, the chain in PEG is not arranged in a zig-zag conformation **B** but is helical, due to the *gauche* effect [11a], which generally favors *synclinal* conformations of ethane moieties carrying two heteroatoms (X-C–C-Y, see **C**). Of course, the two enantiomeric conformations (+)-*sc* and (–)-*sc* **C** in a PEG chain [11b–d] lead to helices of opposite (*P*)- or (*M*)-helicity, and, in a random PEG sample, there will be equal amounts of the enantiomeric helical conformations in rapid equilibrium at room temperature (low barrier to rotation around the ethane C–C bonds)[11]. We wondered whether there would be interaction between a β-peptidic 3_{14}-helix and a PEG unit in the same molecule, which could lead to an induced helical arrangement of the ethylene glycol chain, and which also could lead to a stabilization of the peptide helix.



The β-heptapeptide **5a** was chosen to look for such an interaction; it was prepared *via* solid-phase peptide synthesis using standard Fmoc strategy (see *Exper. Part*); a PEG chain was attached to its N-terminus (→ **5c**) under peptide-coupling conditions on the resin (HATU/*Hünig* base), using Fmoc-NH$(CH_2CH_2O)_6(CH_2)_2CO_2H$[12]; for comparison, the *N*-Ac derivative **5b** was also prepared.

CD Analysis (*Fig. 3*) of **5a**, **5b**, and **5c** in MeOH (0.2 mM) indicates that all three peptides have helical structures, with characteristic negative *Cotton* effects near 215 nm. The intensity of the *Cotton* effect increases in the order **5a** (free N-terminal amino group) < **5b** (acetylated *N*-terminus) < **5c** (PEG-peptide). This is the more remarkable, since the 'molecular β-peptidic content' decreases from **5a** (MW 849.5) to **5b** (MW 913.5) to **5c** (MW 1184.7), and this might[8]) be interpreted as a helix-stabilizing effect of the PEG appendage.

A detailed NMR analysis of the *N*-Ac derivative **5b** and of the PEGylated β-peptide **5c** was then performed (see *Tables 3–6* in the *Exper. Part*) to see whether interactions could be detected between the peptide and the PEG part of the molecule on the NMR time scale. The NMR structure of the *N*-acetylated β-heptapeptide **5b** (*Fig. 4,a*) in MeOH has the expected 3_{14}-helical folding observed for similar β^3-peptides [1–3]. The PEGylated compound **5c** (*Fig. 4,b*) adopts a helical conformation of the β-peptidic portion, almost identical to that of the *N*-Ac derivative **5b**, which does not appear to interact at all with the covalently attached PEG chain (*i.e.*, no NOEs were observed between NMR signals associated with the PEG portion of the molecule and those originating from the peptide portion). It must be emphasized that the globular-type

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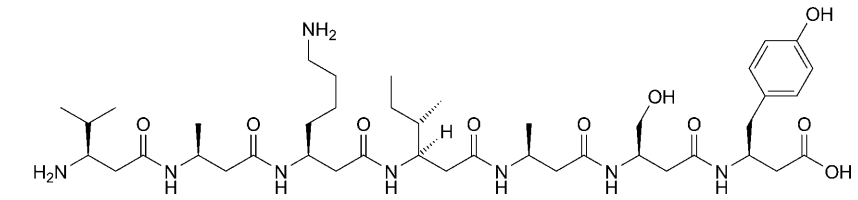
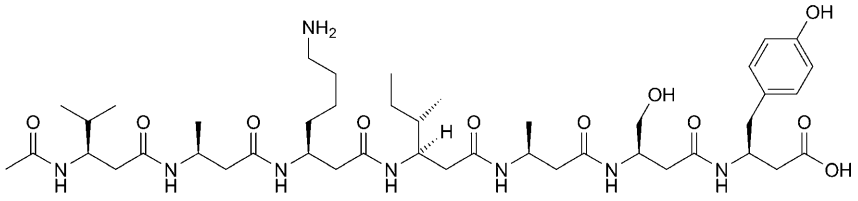
[11]) In a recent communication the synthesis of discrete ethylene glycol oligomers (of up to 48 CH_2CH_2O units) was described; a hexadecamer monomethyl ether gave single crystals consisting of right- and left-handed 3_{10}-helices (X-ray structure analysis) [12a], confirming the structure found by polymer chemists (stretched-fiber X-ray diffraction) a long time ago [12b–d].

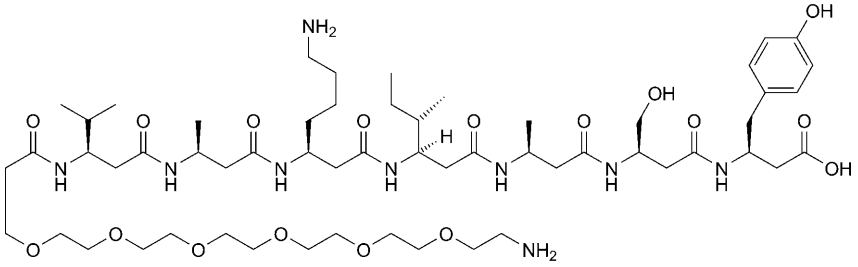
[12]) Commercially available from *Quanta Biodesign Ltd.*



5a



5b



5c

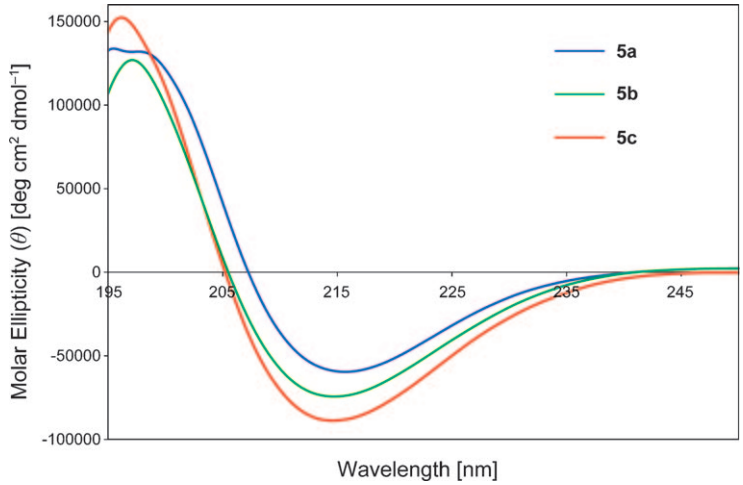


Fig. 3. *CD Spectra of β-heptapeptide derivatives* **5a**–**5c**. The spectra were recorded in MeOH (0.2 mM) and are not normalized.

arrangement of the PEG chain with no defined conformation as shown in *Fig. 4*, *b*, is an artifact of the simulated annealing calculation: since the H-atoms of the OCH_2CH_2O moieties of the PEG chain are almost isochronous in the ^{1}H-NMR spectrum, no dihedral angle or NOE constraints within the PEG chain could be derived experimentally, which leads to a random distribution of all local staggered conformations in the calculations.

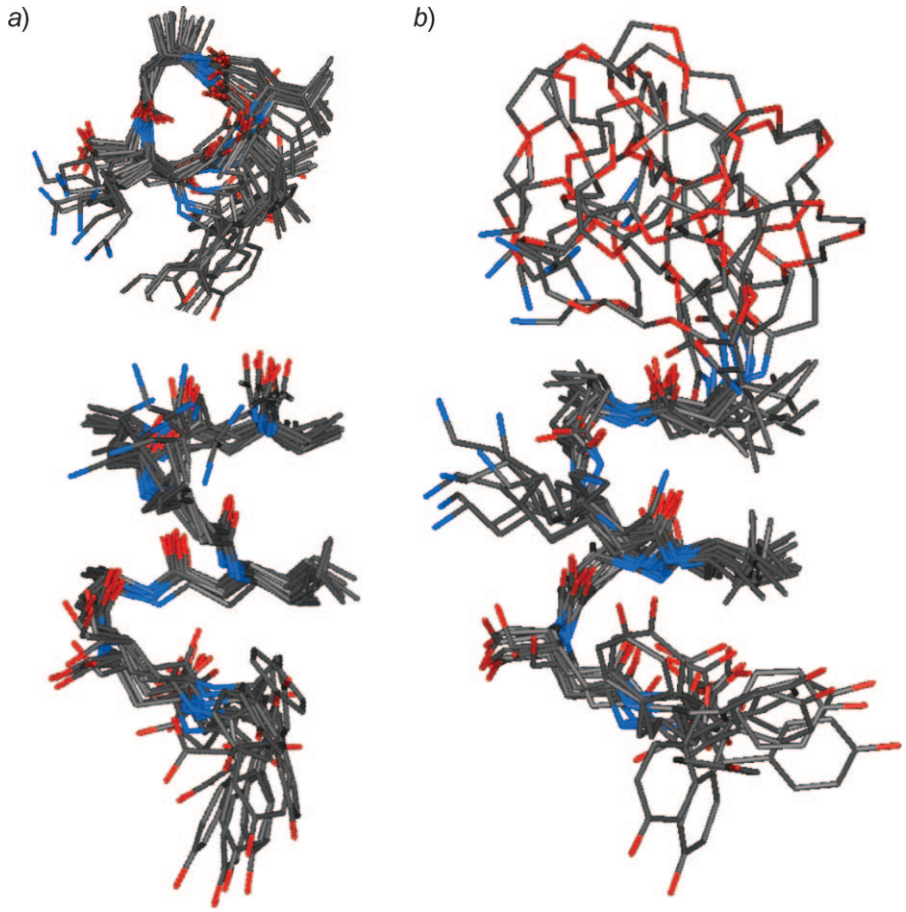


Fig. 4. *NMR-Solution structures of* a) *the acetylated β-heptapeptide* **5b**, *and* b) *the PEGylated β-heptapeptide* **5c**, *in* CD_3OH. The structure of **5b** is shown in top view along the helix axis and in side view perpendicular to this axis. The structure of **5c** is shown perpendicular to the helix axis.

Thus, the NMR analysis was not able to confirm an interaction between the *β*-peptidic and the ethylene glycol parts in compound **5c** that was suggested by the CD analysis. The complete absence of NOEs between the PEG signals and the *β*-peptide signals indicates that the PEG chain does not prolongate the helical turn at the N-terminus of the peptide. However, this does not preclude that, within the PEG chain, a

helical secondary structure such as seen recently in PEG oligomers by X-ray-analysis (see *Footnote 11* above) might be present.

4. The Dipicolinic Acid (DPA) Derivative 6. – Lastly, to explore the potential of small-molecule β-peptide conjugates, we chose to attach pyridine-2,6-dicarboxylic acid (=*dipicolinic acid*; DPA) to a β-decapeptide designed to adopt a 3_{14}-helix in MeOH (compound **6** in *Fig. 5*). The high affinity of DPA for Tb^{3+}, resulting in intense luminescence under UV excitation, forms the basis for a number of biologically relevant assays including cell permeability [13] and bacterial spore detection (*cf. Anthrax* [14]). Such DPA-peptide derivatives have previously been used by us to investigate the permeation of α- and β-oligopeptides through lipid bilayers [15] and also as potential somatostatin mimics [4b]. The β-peptidic segment of the peptide conjugate **6** was designed to include a potential salt bridge between the side chain in positions 5 (βhGlu) and 8 (βhLys) of the sequence to assist in stabilizing helical secondary structure. Compound **6** was prepared *via* solid-phase peptide synthesis using Fmoc strategy. N-Terminal attachment of DPA was carried out under standard coupling conditions (HATU/DIPEA) using pyridine-2,6-dicarboxylic acid mono-benzyl ester [15]. Cleavage of the peptide from the resin, followed by hydrogenolysis of the benzyl group and purification by RP-HPLC, gave the peptide **6** of $>98\%$ purity.

The CD spectrum of peptide derivative **6** in MeOH (0.2 mM) shows an intense negative *Cotton* effect near 215 nm indicative of a helical conformation (*Fig. 5*, *b*). The NMR solution-structure analysis confirmed that compound **6** adopts the expected 3_{14}-helical conformation in CD_3OH (*Fig. 5*, *c* and *d*, and *Tables 7* and *8* in the *Exper. Part*). The fact that NOEs were observed between the H-atoms of the Leu side chain of residue 3 and H–C(3) of the N-terminal pyridine ring (*Fig. 5*, *a*) suggests some additional degree of order at the N-terminus including the pyridine-2,6-dicarboxylic acid moiety.

It is, therefore, clear that covalent attachment of DPA to medium-length β-peptides does not lead to destabilization of the 3_{14}-helix in MeOH (good news for the development of small-molecule-β-peptide conjugates). In turn, for DPA-conjugates such as **6**, the β-peptidic sequence does not interact with the terminal aromatic carboxylic acid group in any way, confirming that, in solution, the DPA moiety adopts a conformation, which may be favorable for use in its associated biological assays.

5. Conclusions. – We have shown that the attachment of conjugates (DPA, PEG, α-peptides) does not appear to affect the helical conformation of β-peptides designed to adopt a 3_{14}-helix in MeOH. This has been demonstrated for the DPA-conjugated β-peptide **6** and for the PEGylated conjugate **5c**. These studies bode well for the future attachment of drug-like molecules (such as antibiotics, DNA and RNA[13]), *etc.*) to β-peptides for development as delivery systems and potential therapeutics.

We have also shown that covalent attachment of an α-peptidic sequence, *via* an amide bond, to a β-peptidic sequence (to give a chimeric β/α-peptide) does not affect the nature or stability of the 3_{14}-β-peptide helical segment. While we had originally designed the chimeric β/α-sequence to induce helicity in the α-peptide segment, using

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[13]) See chapter 10.5 in [2b] and Chapter 3 in [2d], and refs. cit. therein.

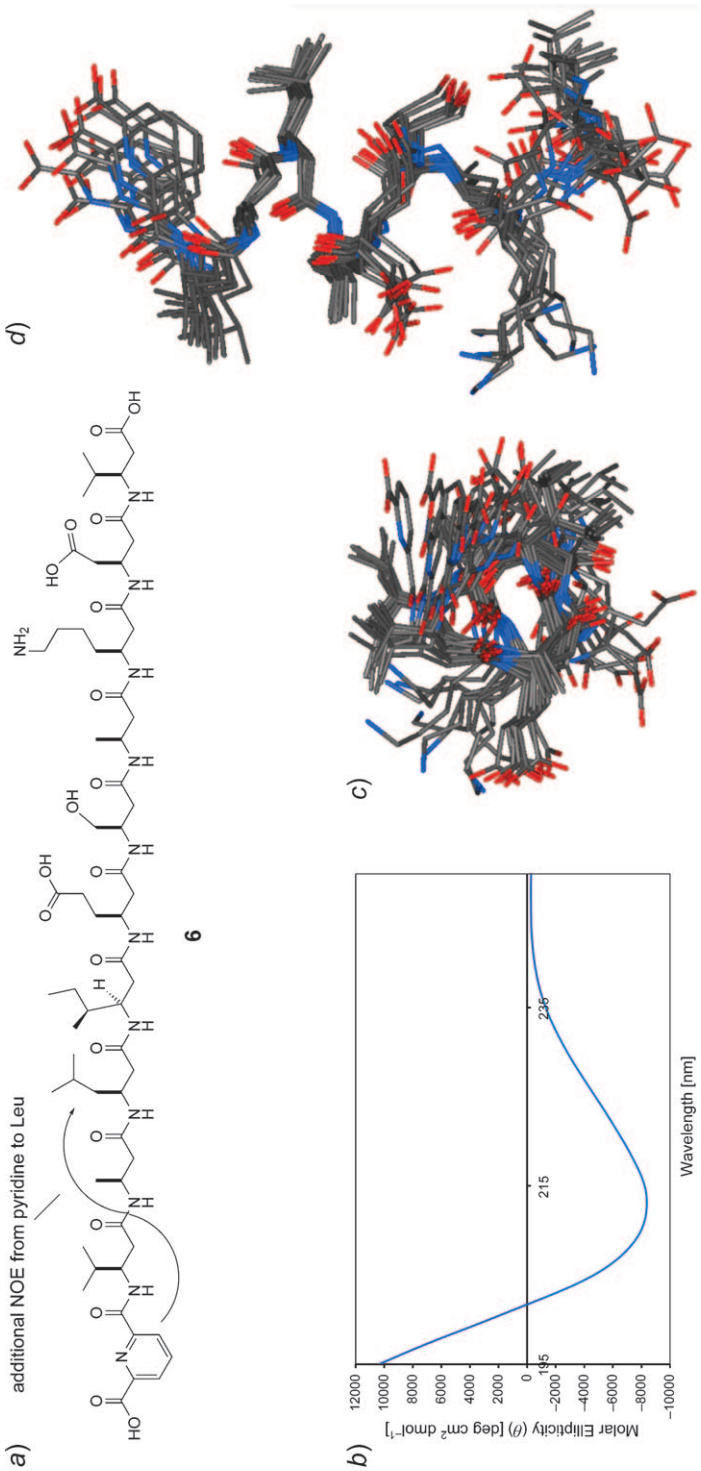

Fig. 5. *Molecular formula of DPA-β-decapeptide conjugate* **6** (*a*), *normalized CD spectrum of* **6** (*b*), *and NMR-Solution Structure of* **6** (*c* and *d*). The CD spectrum was measured in MeOH (0.2 mM). The NMR structure is deduced from measurements in CD_3OH (see *Exper. Part*).

the β-peptide segment, the NMR solution-structure analysis showed that this did not occur. However, this result clearly, and quite dramatically, displays the greater inherent stability of a β-peptide helix over that of an α-peptidic helix, in what could be called an *intramolecular comparison.*

We thank *Albert K. Beck* for his invaluable help in preparing the manuscript of this work.

Experimental Part

Abbreviations. Boc: (*tert*-butoxy)carbonyl, CD: circular dichroism, DIPEA: *N*-ethyl-*N,N*-diisopropylamine ($EtN(i\text{-}Pr)_2$), DMAP: 4-(dimethylamino)pyridine, DPA: pyridine-2,6-dicarboxylic acid, EDC: 1-ethyl-3-[(dimethylamino)propyl]carbodiimide, Fmoc: [(9*H*-fluoren-9-yl)methoxy]carbonyl, HATU: *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HOBt: 1-hydroxybenzotriazole, h.v.: high vacuum (0.01 – 0.1 Torr), MALDI: matrix-assisted laser desorption ionisation, MeIm: 1-methyl-1*H*-imidazole, NMM: *N*-methylmorpholine, SPPS: solid-phase peptide synthesis, TFA: trifluoroacetic acid, TIS: triisopropylsilane ($(i\text{-}Pr)_3SiH$), TNBS: 2,4,6-trinitrobenzenesulfonic acid.

General. Side-chain-protected Fmoc-α- and Fmoc-β^3-amino acids were purchased from *Fluka*. *Wang* resin and *Rink Amide AM* resin were purchased from *Novabiochem*. NMR: Chemical shifts δ are given in ppm relative to resonances of solvent (^1H: 3.31 ppm for CD_3OD; ^{13}C: 49.15 ppm for CD_3OD), coupling constants *J* are given in Hz. MS: *VG Tribrid* (EI), *Bruker Reflex* (MALDI), or *IonSpec Ultima 4.7 TFT* Ion Cyclotron Resonance (ICR, MALDI-HR-MS, in a 2,5-dihydroxybenzoic acid matrix) mass spectrometer; in *m*/*z* (% of basis peak). Anal. HPLC: *Merck* HPLC system (*LaChrom*, pump type *L-7150*, UV detector *L-7400*, interface *D-7000*, HPLC manager *D-7000*). Prep. HPLC: *Merck/Hitachi* HPLC system (pump type *L-6250*, UV detector *L-4000*) or *Merck* HPLC system (*LaChrom*, pump type *L-7150*, UV detector *L-7400*, interface *D-7000*, HPLC00 manager *D-7000*). TFA for anal. and prep. RP-HPLC was UV-grade quality (> 99% GC). Lyophilization: *Hetosicc* cooling condenser with h.v. pump to obtain the peptides as their TFA salts.

Loading of Wang *Resin: General Procedure 1* (*GP 1*). To a soln. of the Fmoc-protected β-amino acid (3 equiv.), in dry CH_2Cl_2 (5 ml) under Ar, was added Melm (2.25 equiv.) and MSNT (3 equiv.) The mixture was stirred for 2 min and then added to the pre-swollen resin (CH_2Cl_2 for 1 h), and the suspension was mixed by Ar bubbling for 4 h. The resin was then filtered, washed with CH_2Cl_2 (4 ml, 6 × 1 min), and dried under h.v. overnight. The loading was determined by measuring the absorbance of the benzofulvene piperidine adduct: two aliquots of the Fmoc-amino acid resin were weighed exactly (m_1(resin) and m_2(resin) [mg]) and suspended in an exact amount of piperidine solution (20% in DMF) in volumetric flasks ($V_1 = V_2 = 10$ [ml]). After 30 min, the mixtures were transferred to a UV cell, and the absorbance (A) was measured relative to a blank piperidine soln. (20% in DMF) at 290 nm. The concentrations (c_1 and c_2, [mM]) of the benzofulvene – piperidine adduct in soln. were determined using a calibration curve [16]. The loading (Subst.) was then calculated according to *Eqn. 1:*

$$\text{Subst}_n\ [\text{mmol/g resin}] = c_n \cdot V_n / \{m_n(\text{resin}) - [c_n \cdot V_n \cdot (\text{MW} - 18)/1000]\} \quad (1)$$

(MW = molecular weight of the Fmoc-protected amino acid).

The yield for the resin attachment (loading yield) was determined by *Eqn. 2:*

$$\text{Loading yield} = [(\text{Subst}_1 + \text{Subst}_2)/2]/\text{Subst}_{\text{theor.}} \quad (2)$$

Anchoring of N-*Fmoc-Protected Amino Acids on a* Rink *Amide Resin: General Procedure 2* (*GP 2*). The resin was placed into a dried manual SPS reactor, swelled in DMF (20 ml/g resin) for 1 h, and washed with DMF (6 × 5 ml). The resin was deprotected according to *GP 4* and coupled with the first amino acid according to *GP 5*.

Capping of Free Amino Groups: General Procedure 3 (*GP 3*). The peptide – resin was covered with DMF (20 ml/g resin), and the unreacted amino groups were capped using Ac_2O (10 equiv.) and DMAP

(0.1 equiv.) dissolved in DMF (0.1 ml/mmol Ac_2O) for 1–2 h under Ar bubbling. The resin was then washed with DMF (20 ml/g resin, 5 × 1 min) and with CH_2Cl_2 (20 ml/g resin, 5 × 1 min).

Fmoc Deprotection: General Procedure 4 (*GP 4*). The Fmoc deprotection was carried out using 20% piperidine in DMF (4 ml, 4 × 10 min) under N_2 bubbling. After filtration, the resin was washed with DMF (5 ml, 4 × 1 min) and CH_2Cl_2 (5 ml, 4 × 1 min).

Coupling of Amino Acids on Wang, Rink *Amide*/*Amide AM Resin: General Procedure 5* (*GP 5*). Fmoc Deprotection was carried out according to *GP 4*. Solid-phase synthesis was continued by sequential incorporation of Fmoc-protected amino acids. For each coupling step, the resin was treated with a soln. of Fmoc-protected amino acid (3 equiv.), HATU (2.9 equiv.), and DIPEA (6 equiv.) in DMF (5 ml) for 1–2 h. Monitoring of the coupling reaction was performed with the TNBS test [17]. In case of a positive TNBS test (indicating incomplete coupling), the suspension was allowed to react for a further 1–2 h, or retreated with the same Fmoc-protected β-amino acid (2 equiv.) and coupling reagents. After complete coupling, the resin was washed with DMF (5 ml, 5 × 1 min). The cycle was then repeated until all remaining amino acids were incorporated.

Side-Chain Deprotection and Cleavage of Peptides from Wang/Rink *Amide*/Rink *Amide AM Resin: General Procedure 6* (*GP 6*). The dry peptide/peptide–resin was treated with a soln. of TFA/TIS/H_2O 95 : 2.5 : 2.5 (10 ml) for 3 h. The resin was filtered, washed with TFA (2 × 5 ml), and the org. phase was concentrated under reduced pressure. The crude peptide, which formed upon addition of cold Et_2O to the oily residue, was collected, dried under h.v., and stored at $-20°$ before being purified by RP-HPLC.

HPLC Analysis and Purification of Peptides: General Procedure 7 (*GP 7*). RP-HPLC Analysis was performed with a *Macherey-Nagel* C_{18} column (*Nucleosil 100-5* C_{18} (250 × 4 mm)) or a *Macherey-Nagel* C_8 column (*Nucleosil 100-5* C_8 (250 × 4 mm)) using a linear gradient of *A* (MeCN) and *B* (0.1% TFA in H_2O) at a flow rate of 1 ml/min with UV detection at 220 nm. Retention time (t_R) in min. Aliquots of the crude products were purified by prep. RP-HPLC with a *Macherey-Nagel* C_{18} column (*Nucleosil 100-7* C_{18} (250 × 21 mm)) or a *Macherey-Nagel* C_8 column (*Nucleosil 100-7* C_8 (250 × 21 mm)), using a gradient of *A* and *B* at a flow rate of 10 ml/min with UV detection at 220 nm, and then lyophilized to give the pure peptide as a TFA salt (purity > 95%).

Benzyl Deprotection: General Procedure 8 (*GP 8*). The Bn-protected peptide was dissolved in MeOH (5 ml) under N_2, and Pd/C (10% (*w*/*w*)) was added. The apparatus was evacuated, flushed with H_2 (3 ×), and the soln. was stirred vigorously under H_2 (balloon) for 12 h. The soln. was filtered through *Celite*, which was washed twice with MeOH, and the combined solns. were concentrated under reduced pressure to give the crude product.

Boc Deprotection: General Procedure 9 (*GP 9*). Similarly to the reported procedure [18], the Boc-protected compound was dissolved in CH_2Cl_2 or $CHCl_3$ (0.06–0.55M) 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 1.5–3 h. Concentration under reduced pressure and drying of the residue under h.v. yielded the crude TFA salt, which was, if not otherwise mentioned, used without further purification.

Peptide Coupling with EDC: General Procedure 10 (*GP 10*). *GP 10a*. A stirred soln. of the TFA salt (1 equiv.) in THF, CH_2Cl_2, or $CHCl_3$ (0.1–0.5M) at 0° (ice bath) was treated with the Boc-protected fragment (1.0–2.5 equiv., added as solid or as a soln. in $CHCl_3$ (0.5M)), NMM (2.7–3.5 equiv.), HOBt (1.1–2.5 equiv.), and EDC (0.9–2.5 equiv.). The soln. was either stirred for 1–4 h at 0° (ice-bath) then at r.t. for 26 h–4.5 d, or was directly allowed to warm to r.t., and stirring was continued for 15 h–3 d. The mixture was diluted with CH_2Cl_2 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, if not otherwise mentioned, purified by FC and/or recrystallization.

GP 10b. According to [19], a stirred soln. of the appropriate amino compound, TFA, TsOH, or HCl salt (1.0 equiv.) in $CHCl_3$ (0.1–0.5M) at 0° (ice bath) was treated successively with Et_3N (1.3–5.0 equiv.), HOBt (1.0–3.1 equiv.), the Boc-protected fragment (1.0–2.6 equiv., added as solid or as a soln. in $CHCl_3$ (0.5–0.6M)), and EDC (1.2–3.1 equiv.). The mixture was allowed to warm to r.t., and stirring was continued for 16 h–4 d. Subsequent dilution with $CHCl_3$ was followed by thorough washing with 1N HCl, aq. sat. $NaHCO_3$, and NaCl soln. The org. phase was dried ($MgSO_4$) and then concentrated *in vacuo*. FC and/or recrystallization yielded the pure peptide.

Preparation of **1a**–**1d**. *Boc*-D-*Ala*-D-*Leu-OBn*. According to [20], a soln. of Boc-D-Ala-OH (3.73 g, 19.7 mmol) in THF (40 ml) at −15° was treated with NMM (2.20 ml, 20.0 mmol) and isobutyl chloroformate (2.60 ml, 19.9 mmol). After 15 min, a cold (0° bath temp.) soln. of TsOH · H-D-Leu-OBn (7.28 g, 20.1 mmol) and NMM (2.20 ml, 20.0 mmol) in THF (40 ml) was added dropwise. After 1 h at 0°, the ice-bath was removed, and the soln. was allowed to warm to r.t. Stirring was continued for 23 h. The mixture was then diluted with AcOEt and washed with 5% citric acid, sat. aq. $NaHCO_3$, and sat. aq. NaCl soln. All aq. layers were additionally extracted twice with AcOEt. The combined org. layers were dried ($MgSO_4$) and evaporated to yield Boc-D-Ala-D-Leu-OBn (7.45 g, 96%), which was used without further purification. ^{1}H-NMR Data in agreement with those in [20].

Boc-D-*Val*-D-*Ala*-D-*Leu-OBn*. Boc-D-Ala-D-Leu-OBn (7.30 g, 18.6 mmol) was deprotected in CH_2Cl_2 (37 ml) according to *GP 9* for 3 h. To a soln. of Boc-D-Val-OH (4.03 g, 18.5 mmol) in THF (27 ml), NMM (5.75 ml, 52.2 mmol) and isobutyl chloroformate (2.4 ml, 18.3 mmol) were added at −15°. After 15 min, a cold (0°, bath temp.) soln. of the TFA salt in THF (27 ml) was added dropwise. After 1 h at 0°, the ice-bath was removed, and the soln. was allowed to warm to r.t. Stirring was continued for 22 h. The mixture was then diluted with AcOEt and washed with 5% citric acid, sat. aq. $NaHCO_3$, and sat. aq. NaCl soln. All aq. layers were additionally extracted twice with AcOEt. The combined org. layers were dried ($MgSO_4$) and evaporated. Flash chromatography (FC; AcOEt/PE 1:1) yielded Boc-D-Val-D-Ala-D-Leu-OBn (4.71 g, 52%). ^{1}H-NMR Data in agreement with those in [20].

Boc-D-*Leu-Aib-OBn*. Boc-D-Leu-OH · H_2O (4.92 g, 19.7 mmol) was dissolved in CH_2Cl_2 (36 ml) and heated under reflux using an 'inverse' water separator for 12 h and evaporated. To a soln. of the resulting Boc-D-Leu-OH in THF (100 ml) at −15°, NMM (2.3 ml, 20.9 mmol), isobutyl chloroformate (2.75 ml, 21.0 mmol) were added, and, after stirring for 5 min, a cold soln. (0°, ice bath) of Boc-Aib-OBn · TosOH (7.42 g, 22.0 mmol) and NMM (2.42 ml, 22.0 mmol) in DMF (50 ml) was added. The mixture was allowed to warm to r.t. within 2 h, and stirring was continued for 22 h. The soln. was evaporated under reduced pressure, and the residue was dissolved in AcOEt, washed with 1N HCl (3×), sat. aq. K_2CO_3 (3×), H_2O (3×), and sat. aq. NaCl soln, and dried ($MgSO_4$). Removal of the solvent under reduced pressure gave Boc-D-Leu-Aib-OBn (7.23 g, 89%). For anal. purposes, a sample was recrystallized from $CHCl_3$/hexane. Colorless crystals. $[\alpha]_D = +48.0$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3435*m*, 3007*m*, 2961*m*, 2871*w*, 1734*s*, 1681*s*, 1498*s*, 1455*s*, 1389*m*, 1368*s*, 1277*m*, 1159*s*, 1050*m*, 1023*w*, 871*w*, 656*w*, 630*w*, 619*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.88 (*d*, $J = 6.2$, Me); 0.89 (*d*, $J = 6.5$, Me); 1.41 (*s*, *t*-Bu); 1.53 (*s*, Me); 1.54 (*s*, Me); 1.56–1.66 (*m*, 3 CH); 3.90–4.10 (*m*, CHN); 4.86 (*d*, $J = 8.1$, NH); $\nu_A = 5.11$, $\nu_B = 5.16$ (*AB*, $J_{AB} = 12.3$, CH_2O); 6.77 (br. *s*, NH); 7.32–7.37 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 22.0, 22.9, 24.7 (Me); 24.8 (CH); 28.3 (Me); 41.0 (CH_2); 53.0 (CH); 56.4 (Me); 67.3 (CH_2); 80.1 (C); 128.4, 128.5, 128.7 (CH); 136.0, 156.1, 167.8, 172.0, 174.4 (C). ESI-MS (pos. mode): 445 (25, $[M+K]^+$), 429 (100, $[M+Na]^+$), 407 (17, $[M+H]^+$). Anal. calc. for $C_{22}H_{34}N_2O_5$ (406.52): C 65.00, H 8.43, N 6.89; found: C 64.93, H 8.31, N 7.00.

Boc-D-*Ala*-D-*Leu-Aib-OBn*. Boc-D-Leu-Aib-OBn (5.48 g, 13.5 mmol) was Boc-deprotected in CH_2Cl_2 (27 ml) according to *GP 9* for 2 h. A soln. of the resulting TFA salt in $CHCl_3$ (85 ml) was cooled to 0° (ice-bath) and treated with Boc-D-Ala-OH (2.55 g, 13.5 mmol), NMM (4.16 ml, 37.8 mmol), HOBt (2.45 g, 16.2 mmol), and EDC (2.58 g, 13.5 mmol). Stirring was continued for 2 h at 0° (ice bath) and at r.t. for 22 h. The mixture was thoroughly washed with 1N HCl, aq. sat. $NaHCO_3$, and NaCl soln. The org. phase was dried ($MgSO_4$) and then concentrated under reduced pressure to yield Boc-D-Ala-D-Leu-Aib-OBn (5.6 g, 87%). For anal. purposes, a sample was recrystallized from $CHCl_3$/hexane and further purified by FC (MeOH/CH_2Cl_2 3:97). White solid. M.p. 115–118°. R_f (MeOH/CH_2Cl_2 3:97) 0.20. $[\alpha]_D = +62.6$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3678*w*, 3434*m*, 3008*m*, 2982*m*, 2872*w*, 1676*s*, 1603*w*, 1499*s*, 1455*s*, 1389*m*, 1368*m*, 1289*m*, 1159*s*, 1067*w*, 1027*w*, 852*w*, 658*w*, 628*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.87 (*d*, $J = 6.3$, Me); 0.89 (*d*, $J = 6.5$, Me); 1.31 (*d*, $J = 7.1$, Me); 1.43 (*s*, *t*-Bu); 1.45–1.69 (*m*, 3 CH); 1.52 (*s*, Me); 1.54 (*s*, Me); 4.07–4.11 (*m*, CHN); 4.35–4.44 (*m*, CHN); 5.04 (*d*, $J = 6.5$, NH); 5.13 (*s*, CH_2O); 6.56 (*d*, $J = 8.2$, NH); 6.86 (*s*, NH); 7.29–7.37 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.9, 21.8, 22.9 (Me); 24.6 (CH); 24.7, 24.7, 28.2 (Me); 40.4 (CH_2); 50.4, 51.6 (CH); 56.3 (C); 67.1 (CH_2); 80.4 (C); 128.1, 128.2, 128.4 (CH); 135.7, 155.6, 170.9, 172.7, 174.0 (C). HR-MALDI-MS: 500.2729 (81, $[M+Na]^+$, $C_{25}H_{39}N_3NaO^+$; calc. 500.2737), 400.2205 (100, $[M-Boc+Na]^+$, $C_{20}H_{31}N_3NaO_4^+$; calc. 400.2212), 378.2392 (5, $[M+H-Boc]^+$; $C_{20}H_{32}N_3O_4^+$; calc. 378.2393).

Boc-D-*Val*-D-*Ala*-D-*Leu*-*Aib*-*OBn*. Boc-D-Ala-D-Leu-Aib-OBn (4.37 g, 9.15 mmol) was Boc-deprotected in CH_2Cl_2 (18.3 ml) according to *GP 9* for 3 h. The resulting TFA salt was dissolved in $CHCl_3$ (18.3 ml) and treated with Boc-D-Val-OH (1.99 g, 9.16 mmol, added as solid), NMM (2.82 ml, 25.6 mmol), HOBt (1.52 g, 10.0 mmol), and EDC (1.75 g, 9,13 mmol) according to *GP 10a* for 1 h at 0° (ice-bath). THF (3 ml) was added, and the mixture was allowed to warm to r.t. and then stirred for 2.5 d. The mixture was worked up as described in *GP 10b*. FC (AcOEt/PE 4 :1) yielded Boc-D-Val-D-Ala-D-Leu-Aib-OBn (4.12 g, 78%). Colorless glass. R_f (AcOEt/PE 4 :1) 0.54. $[\alpha]_D = +52.1$ ($c = 1.1$, $CHCl_3$). IR ($CHCl_3$): 3430*m*, 3008*m*, 2963*m*, 1675*s*, 1498*s*, 1455*m*, 1389*m*, 1368*m*, 1278*m*, 1157*s*, 984*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.85 (*d*, $J = 6.4$, Me); 0.88 (*d*, $J = 6.5$, Me); 0.92 (*d*, $J = 6.9$, Me); 0.95 (*d*, $J = 6.9$, Me); 1.35 (*d*, $J = 7.1$, Me); 1.39 – 1.65 (*m*, 2 CH); 1.44 (*s*, *t*-Bu); 1.51 (*s*, Me); 1.53 (*s*, Me); 1.67 – 1.74 (*m*, CH); 2.09 – 2.15 (*m*, CH); 3.92 – 3.95 (*m*, CHN); 4.38 – 4.45 (*m*, 2 CHN); 5.10 – 5.16 (*m*, NH, CH_2O); 6.82 (*d*, $J = 6.5$, NH); 6.88 (*d*, $J = 8.0$, NH); 7.01 (br. *s*, NH); 7.28 – 7.36 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.8, 18.2, 19.3, 21.7, 23.0, 24.7 (Me); 24.8 (CH); 24.9, 28.3 (Me); 30.6 (CH); 40.3 (CH_2); 49.4, 51.7 (CH); 56.2 (C); 60.4 (CH); 66.9 (CH_2); 80.6 (C); 128.1, 128.1, 128.5 (CH); 136.0, 156.3, 171.1, 171.8, 172.1, 174.1 (C). FAB-MS: 1154 (10), 1153 (13, $[2M + H]^+$), 578 (29), 577 (100, $[M + H]^+$), 469 (13), 384 (12), 328 (19), 307 (17), 215 (16), 194 (16), 115 (10). Anal. calc. for $C_{30}H_{48}N_4O_7$ (576.73): C 62.48, H 8.39, N 9.71; found: C 62.35, H 8.24, N 9.56.

Boc-D-*Val*-D-*Ala*-D-*Leu*-*Aib*-*OH*. Boc-D-Val-D-Ala-D-Leu-Aib-OBn (2.01 g, 3.48 mmol) was transformed in MeOH (83 ml) according to *GP 8* for 23 h to yield Boc-D-Val-D-Ala-D-Leu-Aib-OH (1.65 g, 98%). Colorless glass. $[\alpha]_D = +62.9$ ($c = 1.1$, $CHCl_3$). IR ($CHCl_3$): 3299*m*, 2966*s*, 1657*s*, 1509*s*, 1463*m*, 1456*m*, 1392*m*, 1368*m*, 1165*s*, 1092*w*, 1044*w*, 1017*w*, 869*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.87 – 0.95 (*m*, 4 Me); 1.32 (*d*, $J = 6.7$, Me); 1.37 – 1.64 (*m*, 3 CH); 1.44 (*s*, *t*-Bu); 1.49 (*s*, Me); 1.53 (*s*, Me); 2.01 – 2.09 (*m*, CH); 4.12 – 4.19 (*m*, CHN); 4.55 – 4.65 (*m*, CHN); 4.70 – 4.80 (*m*, CHN); 5.28 (*d*, $J = 8.6$, NH); 7.35 (br. *s*, NH); 7.76 (br. *s*, NH); 8.13 (*d*, $J = 6.8$, NH); ^{13}C-NMR (100 MHz, $CDCl_3$): 17.8, 19.0, 19.2, 22.1, 22.8, 24.3 (Me); 24.6 (CH); 25.4, 28.2, 28.3 (Me); 31.7 (CH); 40.8 (CH_2); 48.7, 51.6 (CH); 56.2 (C); 59.5 (CH); 80.2, 156.1, 171.6, 171.9, 172.9, 176.2 (C). FAB-MS: 995 (14, $[2M + Na]^+$), 973 (29, $[2M + H]^+$), 747 (13), 725 (23), 625 (16), 510 (15), 509 (53, $[M + Na]^+$), 488 (30), 487 (100, $[M + H]^+$), 469 (12), 431 (14), 387 (22), 384 (22), 328 (34), 217 (35), 215 (31), 171 (11), 155 (13), 154 (27), 138 (14), 137 (21), 136 (24), 116 (21), 111 (10), 106 (11), 103 (12).

Boc-D-*Val*-D-*Ala*-D-*Leu*-*Aib*-D-*Val*-D-*Ala*-D-*Leu*-*OBn* (**1d**). Boc-D-Val-D-Ala-D-Leu-OBn (1.57 g, 3.19 mmol) was Boc-deprotected in $CHCl_3$ (6.4 ml) according to *GP 9* for 2 h. A soln. of the resulting TFA salt in $CHCl_3$ (3.2 ml) at 0° (ice bath) was treated with NMM (0.98 ml, 8.89 mmol), a cold (0°, ice-bath) soln. of Boc-D-Val-D-Ala-D-Leu-Aib-OH (1.55 g, 3.19 mmol) in DMF (3.2 ml), HOBt (0.58 g, 3.83 mmol), and EDC (0.61 g, 3.18 mmol). The mixture was allowed to warm to r.t. After stirring for 27 h, the soln. was evaporated under reduced pressure, and the residue was dissolved in $CHCl_3$ and washed with 1N HCl, sat. aq. $NaHCO_3$, and sat. aq. NaCl soln. All aq. layers were additionally extracted with $CHCl_3$. The combined org. layers were dried ($MgSO_4$) and concentrated. FC (MeOH/CH_2Cl_2 1 :9) yielded **1d** (2.18 g, 79%). Yellowish crystalline solid. M.p. 179 – 181°. R_f (MeOH/CH_2Cl_2 1 :9) 0.59. $[\alpha]_D = +17.0$ ($c = 1.1$, $CHCl_3$). CD (0.2 mM in MeOH): $+7.8 \times 10^4$ (200 nm). IR ($CHCl_3$): 3326*m*, 2963*m*, 2873*w*, 1665*s*, 1526*s*, 1369*w*, 1156*m*, 984*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.87 – 1.04 (*m*, 8 Me); 1.43 – 1.52 (*m*, 4 Me); 1.48 (*s*, *t*-Bu); 1.53 – 1.81 (*m*, 5 CH); 2.09 – 2.16 (*m*, 2 CH); 2.42 – 2.50 (*m*, CH); 3.70 – 3.73 (*m*, CHN); 4.08 – 4.19 (*m*, 3 CHN); 4.48 – 4.63 (*m*, 2 CHN); $\nu_A = 5.14$, $\nu_B = 5.16$ (*AB*, J_{AB} 12.5, CH_2O); 5.66 (*d*, $J = 2.7$, NH); 6.93 (*d*, $J = 6.7$, NH); 7.03 (*d*, $J = 3.8$, NH); 7.26 – 7.39 (*m*, 2 NH, 5 arom. H); 7.54 (*s*, NH); 7.69 (*d*, $J = 7.9$, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.1, 17.3, 17.3, 18.8, 19.3, 19.4, 21.6, 21.7, 22.6, 22.8, 23.2 (Me); 24.5, 24.8 (CH); 27.1, 28.2 (Me); 28.8, 29.6 (CH); 40.1, 40.6 (CH_2); 49.4, 50.9, 51.5, 54.0 (CH); 56.9 (C); 60.1, 62.2 (CH); 66.5 (CH_2); 81.3 (C); 127.8, 127.9, 128.1, 128.4 (CH); 136.0, 157.4, 171.5, 172.5, 173.1, 173.3, 173.4, 173.8, 176.1 (C). FAB-MS: 1058 (121), 883 (20, $[M + Na]^+$), 882 (40), 862 (12), 861 (48, $[M + H]^+$), 861 (100, M^+), 766 (10), 640 (19), 639 (57), 569 (22), 568 (66), 512 (14), 470 (14), 469 (48), 414 (14), 413 (59), 384 (10), 369 (10), 328 (37), 293 (10), 215 (14).

TFA · *H*-D-*Val*-D-*Ala*-D-*Leu*-*Aib*-D-*Val*-D-*Ala*-D-*Leu*-*OBn* (**1c**). Compound **1d** (0.30 g, 0.35 mmol) was Boc-deprotected in CH_2Cl_2 (0.7 ml) according to *GP 9* for 2 h. The residue was co-evaporated with toluene and $CHCl_3$, and dried under h.v. to yield quantitatively **1c** (0.35 g). Colorless glass. $[\alpha]_D = +27.6$ ($c = 1.1$, $CHCl_3$). IR ($CHCl_3$): 3315*m*, 2963*m*, 1737*m*, 1664*s*, 1535*m*, 1469*w*, 1386*w*, 1172*s*, 1038*w*. ^{1}H-NMR

Table 1. *The ^1H- and ^{13}C-Data, and Assignments for the Chimeric β/α-Tridecapeptide* **4**. δ in ppm, *J* in Hz.

Residue	HN ($^3J_{HN}$)	$CH_2(\alpha)$ H−C(α)	H−C(β), $CH_2(\beta)$, Me(β)	H−C(γ), Me(γ), $CH_2(\gamma)$	H−C(δ), Me(δ), $CH_2(\delta)$	Me(ε), $CH_2(\varepsilon)$	$CH_2(\zeta)$	Ph 2,3,4	1*H*-Indole N(1), C-atoms 2,4,5,6,7	C-terminal NH_2
β^3hVal[1]	–	2.65/2.60	3.52	2.03	1.06/1.05					
β^3hGlu[2]	8.17 (9.2)	2.61/2.43	4.40	1.85/1.75	2.36					
β^3hLeu[3]	8.11 (9.1)	2.28/2.21	4.26	1.33/1.14	1.47	0.88/0.85				
β^3hAla[4]	7.92 (8.6)	2.37/2.15	4.36	1.12						
β^3hLys[5]	6.98 (9.4)	2.43/2.17	4.28	1.51/1.44	1.39	1.63	2.92			
β^3hIle[6]	7.72 (9.1)	2.46/2.33	4.14	1.36	0.80/1.00	0.82				
Phe[7]	8.29 (7.8)	4.58	3.18/2.97					7.26/7.282/7.22		
Ser[8]	7.82 (6.4)	4.41	3.92/3.72							
Leu[9]	8.44 (5.9)	4.25	1.55	1.70	0.97/0.92					
Aib[10]	8.26 (–)	–	1.33/1.26							
Trp[11]	7.43 (5.7)	4.45	3.38/3.29						10.34/7.32/7.53/7.00/7.08/7.31	
Thr[12]	7.80 (7.2)	4.19	4.32	1.41						
Ala[13]	7.68 (7.4)	4.10	0.94							7.55/6.70

Residue	CO	C(α)	C(β)	C(γ)	C(δ)	C(ε)	C(ζ)	Ph C-atoms 1,2,3,4	1*H*-Indole C-atoms 2,3,3a,4,5,6,7,7a
β^3hVal[1]	172.00	35.90	56.13	32.07	17.87				
β^3hGlu[2]	172.90	42.01	47.69	31.78	32.39				
β^3hLeu[3]	172.10	42.73	46.08	46.09	25.93	23.44/23.46			
β^3hAla[4]	172.00	43.75	44.14	21.36					
β^3hLys[5]	171.80	41.97	47.71	35.96	24.36	28.82	41.00		
β^3hiIle[6]	173.70	37.81	51.84	40.14	15.32/26.75	12.21			
Phe[7]	173.80	56.95	38.67					138.9/129.7 130.6/128.1	
Ser[8]	173.50	56.93	63.10						
Leu[9]	175.80	54.83	40.95	25.92	20.16/20.14				
Aib[10]	177.80	58.25	24.30/16.13						
Trp[11]	172.90	57.37	27.77						125.3/110.9/128.7/119.3/ 120.1/122.7/112.7/138.4
Thr[12]	175.60	68.54	50.99	35.95					
Ala[13]	172.90	61.93	22.30						

Table 2. *NOEs for Chimeric β/α-Tridecapeptide* **4**

Residue	H-Atom	Residue	H-Atom	d_{NOE} [Å]
1	γ	1	β	2.5
2	β	2	γ1	3.5
2	γ2	2	HN	3.2
2	β	2	HN	2.9
3	HN	3	α_{Re}	2.9
3	β	3	α_{Si}	2.7
3	β	3	γ*[a])	2.9
3	β	3	HN	2.9
3	δ	3	HN	3.1
3	HN	3	α_{Si}	2.9
4	α_{Si}	4	β	2.6
4	β	4	γ*	2.8
4	β	4	HN	2.9
4	γ*	4	HN	3.3
4	HN	4	α_{Re}	2.8
5	α_{Si}	5	β	2.6
5	HN	5	β	3.0
5	β	5	ε*	3.6
6	α_{Si}	6	β	2.5
6	α_{Re}	6	HN	2.7
6	β	6	γ	2.2
6	β	6	HN	2.9
6	γ	6	HN	2.9
7	α	7	β*	2.9
7	α	7	HN	2.7
7	β_{Si}	7	β_{Re}	1.8
8	HN	8	α	2.8
8	β_{Re}	8	β_{Si}	2.0
8	β_{Si}	8	HN	3.0
9	HN	9	α	2.7
9	HN	9	γ	2.7
11	α	11	H2	3.1
11	α	11	β*	2.9
11	HN	11	α	2.6
12	HN	12	α	3.0
12	HN	12	β	3.6
13	α	13	β*	2.8
13	α	13	HN	2.9
13	β*	13	HN	3.3
2	α_{Re}	3	HN	2.4
3	α_{Re}	4	HN	2.5
3	β	4	HN	3.6
5	HN	4	α_{Si}	3.0
4	HN	3	α_{Si}	2.7
4	HN	3	HN	3.8
5	HN	4	β	3.8
5	HN	4	HN	4.4
5	HN	6	HN	4.3
7	HN	6	α_{Si}	2.7

Table 2 (cont.)

Residue	H-Atom	Residue	H-Atom	d_{NOE} [Å]
6	α_{Re}	7	HN	2.6
7	HN	6	β	3.3
6	HN	5	α_{Re}	2.3
7	α	8	HN	2.4
8	HN	7	β*	3.4
7	HN	8	HN	3.0
9	HN	8	β*	3.5
8	HN	9	HN	3.7
10	HN	9	α	2.5
9	γ	10	HN	4.6
9	HN	10	HN	3.0
10	HN	11	HN	2.9
12	HN	11	α	3.6
13	HN	12	β	3.3
13	HN	12	HN	2.8
1	γ	4	β	4.1
2	α_{Re}	5	β	2.6
3	HN	6	β	3.2
4	α_{Re}	7	α	2.9
4	γ*	7	α	3.7
4	HN	6	β	3.4
4	HN	7	α	3.3
6	β	3	α_{Si}	3.2
9	HN	7	α	4.2
8	β_{Re}	10	HN	4.2
10	HN	8	β_{Si}	4.0
8	β_{Si}	11	H2	3.7
8	HN	4	γ*	4.5
9	α	11	H2	3.8
9	α	11	HN	3.5
10	HN	8	α	4.1
11	α	13	HN	3.0
11	β_{Re}	13	HN	3.9
11	β_{Si}	13	HN	3.8
11	HN	13	HN	2.8
11	HN(1)	9	α	3.9
11	HN(1)	13	β*	4.7
13	β*	9	HN	4.8
13	HN	9	α	3.5

[a]) *: Pseudoatom used for calculations.

(400 MHz, CD_3OD): 0.87 (*d*, *J* = 6.2, Me); 0.90–0.97 (*m*, 5 Me); 1.03 (*d*, *J* = 6.9, Me); 1.05 (*d*, *J* = 7.0, Me); 1.35 (*d*, *J* = 7.2, Me); 1.38 (*d*, *J* = 7.1, Me); 1.44 (*s*, Me); 1.46 (*s*, Me); 1.51–1.78 (*m*, 6 CH); 2.14–2.34 (*m*, 2 CH); 3.65–3.70 (*m*, CHN); 4.10–4.13 (*m*, CHN); 4.16–4.23 (*m*, CHN); 4.33–4.38 (*m*, CHN); 4.41–4.49 (*m*, 2 CHN); ν_A = 5.13, ν_B = 5.15 (*AB*, J_{AB} 12.3, CH_2O); 7.28–7.38 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, CD_3OD): 17.9, 18.0, 18.1, 18.9, 19.8, 21.9, 22.2, 23.3, 23.3, 25.4 (Me); 25.8, 31.3, 31.6 (CH); 41.3, 41.5 (CH_2); 50.3, 50.4, 52.4, 54.3 (CH); 58.0 (C); 59.5, 60.7 (CH); 67.9 (CH_2); 129.3, 129.3, 129.6 (CH);

Table 3. *^{1}H- and ^{13}C-NMR Data and Assignments for Acetyl-β-heptapeptide* **5b**. δ in ppm, *J* in Hz.

Residue	NH	$CH_2(\alpha)$	H−C(β) (^3J(HN,Hβ))	H−C(γ), Me(γ), $CH_2(\gamma)$	Me(δ), $CH_2(\delta)$	$CH_2(\varepsilon/\zeta)$, Me(ε)	C(α)	C(β)	C(γ)	C(δ)	C(ε/ζ)
β^3hVal[1]	8.04	2.52	4.22 (9.60)	1.79	0.94		39.18	53.40	34.06	19.41	
β^3hAla[2]	8.06	2.61	4.38 (NA)	1.14			43.63	44.14	21.30		
β^3hLys[3]	8.07	2.35/2.56	4.33 (NA)	1.52	1.39	1.65/2.92	41.76	47.23	35.95	23.78	28.40/41.0
β^3hIle[4]	7.79	2.37	4.28 (8.95)	1.53	0.91/1.11	0.926	38.80	52.27	40.93	15.7/27.17	19.64
β^3hAla[5]	7.46	2.30/2.40	4.47 (8.52)	1.15			43.43	43.93	21.32		
β^3hSer[6]	7.63	2.32/2.50	4.36 (8.58)	3.43/3.47			38.41	49.62	64.80		
β^3hTyr[7]	7.97	2.47/2.58	4.49 (8.51)	2.64/2.72			NA	49.03	41.17		

Table 4. *NOEs for* N-*Acetyl-β-heptapeptide* **5b**

Residue	H-Atom	Residue	H-Atom	d_{NOE} [Å]
2	β	2	γ	3.1
2	β	2	HN	2.9
3	β	3	γ*	2.9
3	β	3	HN	2.9
4	β	4	ε1	2.7
4	β	4	ε2	2.9
4	β	4	HN	2.9
5	β	5	HN	2.8
5	γ	5	HN	2.5
6	β	6	γ1	2.7
6	β	6	HN	2.9
2	HN	ACE1	α*	3.5
5	β	6	HN	3.9
5	HN	6	HN	3.9
6	β	7	HN	4.0
7	HN	6	αSi	3.1
1	α*[a])	4	β	3.4
2	γ	5	β	3.0
3	HN	6	β	3.4
5	HN	7	β	3.1
5	γ	8	β	2.9
5	γ	8	γ1	3.6
5	γ	8	γ2	3.7

[a]) * = Pseudoatom used for calculations.

137.3, 169.3, 173.3, 173.6, 174.7, 174.8, 175.1, 177.2, 177.2 (C). FAB-MS: 1521 (10), 98 1 (34), 959 (16), 784 (29), 783 (72), 782 (86, $[M+Na]^+$), 763 (10), 762 (36), 761 (82), 760 (100, $[M+H]^+$), 369 (23), 284 (19), 171 (17).

Boc-D-*Val*-D-*Ala*-D-*Leu*-*Aib*-D-*Val*-D-*Ala*-D-*Leu*-*OH* (**1b**). Compound **1c** (0.20 g, 0.23 mmol) was transformed in MeOH (5.6 ml) according to *GP 8* for 18 h to yield **1b** (0.15 g, 85%). Colorless crystalline solid. M.p. 142–144°. $[\alpha]_D = +41.9$ ($c = 0.9$, MeOH). CD (0.2 mM in MeOH): $+5.9 \times 10^4$ (201 nm). IR (KBr): 3320*m*, 2962*m*, 2873*w*, 1654*s*, 1528*s*, 1469*m*, 1388*m*, 1367*m*, 1247*m*, 1168*m*, 1044*w*, 668*w*. ^{1}H-NMR (400 MHz, CD_3OD): 0.89–4.98 (*m*, 8 Me); 1.37 (*d*, $J = 7.2$, Me); 1.42 (*d*, $J = 7.2$, Me); 1.46 (*s*, Me); 1.48 (*s*,

Table 5. *^{1}H- and ^{13}C-NMR Data and Assignments for PEG-β-heptapeptide* **5c**. δ in ppm, *J* in Hz.

Residue	NH	$CH_2(\alpha)$	H–C(β) (^3J(HN,Hβ))	H–C(γ), Me(γ), $CH_2(\gamma)$	Me(δ), $CH_2(\delta)$	$CH_2(\varepsilon/\zeta)$, Me(ε)	C(α)	C(β)	C(γ)	C(δ)	C(ε/ζ)
β^3hVal[1]	8.14	2.40/2.55	4.25 (9.20)	1.80	0.95		38.80	53.24	34.25	19.54	
β^3hAla[2]	8.12	2.36/2.66	4.39 (9.24)	1.14			43.55	43.92	21.02		
β^3hLys[3]	8.09	2.36/2.57	4.34 (8.89)	1.52	1.40	1.64/1.75	41.66	46.91	35.90	23.58	28.31/41.04
β^3hIle[4]	7.79	2.37	4.29 (8.95)	1.53	0.93/1.11	0.928	38.75	52.32	41.07	15.72/27.24	19.70
β^3hAla[5]	7.42	2.29/2.41	4.49 (8.88)	1.15			43.32	43.80	21.17		
β^3hSer[6]	7.62	2.32/2.52	4.38 (8.66)	3.42/3.48			38.37	49.55	64.90		
β^3hTyr[7]	7.80	2.44/2.57	4.50 (9.02)	2.64/2.72			39.12	48.80	41.14		

t-Bu); 1.49 (*s*, Me); 1.56 – 1.77 (*m*, 6 CH); 1.99 – 2.08 (*m*, CH); 2.21 – 2.29 (*m*, CH); 3.78 – 3.82 (*m*, CHN); 4.12 – 4.16 (*m*, CHN); 4.19 – 4.25 (*m*, 2 CHN); 4.35 – 4.46 (*m*, 2 CHN); 6.81 (*d*, *J* = 5.7, NH); 7.34 (*d*, *J* = 7.4, NH); 7.79 (*d*, *J* = 6.7, NH); 7.87 – 7.96 (*m*, 3 NH); 8.31 (*d*, *J* = 5.2, NH). ^{13}C-NMR (100 MHz, CD_3OD): 17.5, 18.0, 18.6, 19.0, 19.6, 19.8, 21.8, 22.2, 23.2, 23.5, 25.0 (Me); 25.8, 25.9 (CH); 26.3, 28.8 (Me); 31.1, 31.6 (CH); 41.4, 41.7 (CH_2); 50.6, 51.6, 52.2, 54.6 (CH); 58.1 (C); 60.9, 61.0, 62.7 (CH); 81.1, 158.8, 173.4, 174.9, 175.0, 175.7, 175.8, 177.6 (C). FAB-MS: 1562 (5, $[2M + Na]^+$), 990 (16), 793 (41), 792 (100, $[M + Na]^+$), 639 (11), 568 (13), 469 (11), 413 (17), 328 (12).

*TFA · H-*D*-Val-*D*-Ala-*D*-Leu-Aib-*D*-Val-*D*-Ala-*D*-Leu-OH* (**1a**). Compound **1b** (95 mg, 0.12 mmol) was Boc-deprotected in $CHCl_3$ (0.25 ml) according to *GP 9* for 2 h. The residue was co-evaporated with toluene, dissolved in TFE, filtered, and concentrated under reduced pressure to yield **1a** (73 mg, 88%). Colorless glass. $[\alpha]_D = +40.8$ (*c* = 0.5, MeOH). CD (0.2 mM in MeOH): $+4.3 \times 10^4$ (198 nm). IR (KBr): 3315*m*, 2964*m*, 1659*s*, 1540*s*, 1469*m*, 1388*m*, 1203*s*, 800*w*, 722*w*. ^{1}H-NMR (400 MHz, CD_3OD): 0.87 – 0.99 (*m*, 6 Me); 1.04 (*d*, *J* = 6.9, Me); 1.06 (*d*, *J* = 6.9, Me); 1.38 (*d*, *J* = 7.1, Me); 1.39 (*d*, *J* = 7.2, Me); 1.44 (*s*, Me); 1.46 (*s*, Me); 1.51 – 1.79 (*m*, 6 CH); 2.12 – 2.28 (*m*, 2 CH); 3.65 – 3.69 (*m*, CHN); 4.13 – 4.18 (*m*, CHN); 4.20 – 4.25 (*m*, CHN); 4.35 – 4.47 (*m*, 3 CHN); 7.33 (2*d*, *J* = 7.9, 2 NH); 7.98 (*d*, *J* = 8.1, NH); 8.07 (*d*, *J* = 7.3, NH); 8.22 – 8.23 (*m*, NH). ^{13}C-NMR (100 MHz, CD_3OD): 17.9, 18.0, 18.1, 18.9, 19.9, 21.9, 22.2, 23.3, 23.5, 25.6 (Me); 25.8, 25.9, 31.5, 31.6 (CH); 41.5, 41.6 (CH_2); 50.3, 50.4, 52.1, 54.2 (CH); 58.1 (C); 59.6, 60.7 (CH); 169.3, 173.3, 174.6, 174.7, 174.9, 175.7, 177.1, 177.2 (C). FAB-MS: 715 (12), 714 (13), 694 (28), 693 (69), 692 (100, $[M + Na]^+$), 671 (11), 670 (13, $[M + H]^+$).

Preparation of **3**. *Boc-(*R*)-β^3-hVal-(*S*)-β^3-hAla-(*S*)-β^3-hLeu-(*R*)-β^3-hVal-(*S*)-β^3-Ala-(*S*)-β^3-Leu-*D*-Val-*D*-Ala-*D*-Leu-Aib-*D*-Val-*D*-Ala-*D*-Leu-OBn*. A soln. of **1c** (0.13 g, 0.15 mmol) in $CHCl_3$/1,2-dimethoxyethane (DME) 1:1 (1.2 ml) at 0° (ice-bath) was subsequently treated with Et_3N (83 μl, 0.60 mmol), BocTFA · H-(*R*)-β^3-hVal-(*S*)-β^3-hAla-(*S*)-β^3-hLeu-(*R*)-β^3-hVal-(*S*)-β^3-Ala-(*S*)-β^3-Leu-OH [1][21] (0.12 g, 0.15 mmol), HOBt (28 mg, 0.19 mmol), and EDC (37 mg, 0.19 mmol). The mixture was allowed to warm to r.t. After 14 h, $CHCl_3$ (1 ml) was added to the mixture, and stirring was continued for another 10 h. The mixture was then evaporated under reduced pressure and dried under h.v. The residue was dissolved in MeOH under reflux, filtered, and cooled to 4° (cooling room). After stirring for 2 h at 4° (cooling room), the precipitate was filtered and dried under h.v. to yield Boc-(*R*)-β^3-hVal-(*S*)-β^3-hAla-(*S*)-β^3-hLeu-(*R*)-β^3-hVal-(*S*)-β^3-Ala-(*S*)-β^3-Leu-*D*-Val-*D*-Ala-*D*-Leu-Aib-*D*-Val-*D*-Ala-*D*-Leu-OBn (96 mg, 43%). White amorphous solid. M.p. 249° (dec.). $[\alpha]_D = +26.9$ (*c* = 0.6, 2,2,2-trifluoroethanol (TFE)). CD (0.2 mM in MeOH): -0.99×10^4 (218 nm), 0 (212 nm), $+8.5 \times 10^4$ (199 nm). IR (KBr): 3304*s*, 3067*w*, 2960*m*, 2872*w*, 1652*s*, 1538*s*, 1457*m*, 1368*m*, 1249*m*, 1172*m*, 698*w*. ^{1}H-NMR (CD_3OD/$CDCl_3$ 1:3): 0.88 – 0.93 (*m*, 12 Me); 0.95 – 1.03 (*m*, 4 Me); 1.17 (*d*, *J* = 6.7, 2 Me); 1.28 – 1.37 (*m*, 2 CH, 2 Me); 1.40 – 1.51 (*m*, 6 CH); 1.44 (*s*, *t*-Bu); 1.47 (*s*, Me); 1.51 (*s*, Me); 1.53 – 1.83 (*m*, 8 CH); 2.12 – 2.55 (*m*, 6 CH_2); 3.72 – 3.80 (*m*, CHN); 4.01 (*d*, *J* = 6.5, CHN); 4.07 – 4.12 (*m*, 2 CHN); 4.14 – 4.30 (*m*, 6 CHN); 4.38 (*q*, *J* = 7.3, CHN); 4.49 – 4.52 (*m*, CHN); 5.16 (*s*, CH_2O); 6.17 (*d*, *J* = 10.1, NH); 6.95 (*d*, *J* = 7.6, NH); 7.28 – 7.38 (*m*, 5 arom. H); 7.71 (*d*, *J* = 2.0, NH); 8.08 (*d*, *J* = 7.84, NH). ^{13}C-NMR (CD_3OD/$CDCl_3$ 1:3): 17.1, 17.8, 18.3, 18.4, 19.1, 19.6, 19.6, 19.7, 20.1, 20.5, 21.8, 22.1, 22.1, 22.3, 23.0, 23.2, 23.5, 23.7, 23.7, 24.3

Table 6. *NOEs for PEG-β-Heptapeptide* **5c**

Residue	H-Atom	Residue	H-Atom	d_{NOE} [Å]
2	γ	2	α_{Re}	3.2
2	γ	2	α_{Si}	3.5
2	β	2	HN	2.9
2	γ	2	HN	3.2
3	β	3	HN	3.0
3	β	3	γ*[a])	3.0
4	β	4	ε1	3.2
4	β	4	HN	2.9
5	β	5	HN	3.0
5	γ	5	HN	3.0
6	β	6	γ*	2.8
6	β	6	HN	2.9
7	β	7	γ1	2.8
7	β	7	HN	2.9
8	β	8	HN	2.9
8	HN	8	α_{Re}	2.7
8	HN	8	γ1	2.9
2	HN	PEG1	α1	3.0
2	HN	PEG1	α2	2.9
2	HN	PEG1	β*	4.0
5	β	6	β	4.2
5	HN	6	HN	3.7
7	HN	6	A1	3.1
7	HN	6	β	4.2
6	HN	7	HN	4.4
8	HN	7	α_{Re}	2.3
PEG1	α1	4	β	3.1
PEG1	α2	4	β	3.2
3	β	PEG1	HX19	5.0
3	β	PEG1	HX20	4.9
2	γ	5	β	3.3
4	ε1	PEG1	α1	4.3
4	ε2	PEG1	α2	4.4
4	γ*	7	γ1	4.9
5	β	2	α_{Re}	3.4
6	β	3	HN	3.2
7	β	5	HN	3.4
8	β	5	γ	3.0

[a]) * = Pseudoatom used for calculations HX19, HX120 protons next to terminal NH2 in PEG.

(Me); 25.4, 25.7, 25.8 (CH); 26.7, 27.0, 28.8 (Me); 30.1 (CH); 30.4 (CH_2); 30.8, 32.7, 32.8, 33.4 (CH); 40.0, 40.1, 40.7, 41.0 (CH_2); 43.0, 43.3, 43.8, 44.2 (CH_2); 44.2 (CH); 44.3 (CH_2); 46.2, 46.5, 50.4, 51.7, 52.0, 52.9, 54.6, 54.8 (CH); 57.6 (C); 61.0, 61.6 (CH); 67.5 (CH_2); 79.8, 128.3, 128.9, 128.9, 129.2, 135.7 (CH); 136.9, 157.6, 172.1, 172.2, 172.3, 172.4, 172.7, 173.1, 173.2, 173.8, 174.3, 174.7, 174.8, 175.7, 177.4 (C). FAB-MS: 1534 (24), 1533 (100, $[M+Na]^+$), 1412 (12), 1411 (48).

*TFA · H-(*R*)-β³-hVal-(*S*)-β³-hAla-(*S*)-β³-hLeu-(*R*)-β³-hVal-(*S*)-β³-Ala-(*S*)-β³-Leu-*D*-Val-*D*-Ala-*D*-Leu-Aib-*D*-Val-*D*-Ala-*D*-Leu-OH* (**3**). Boc-(*R*)-β³-hVal-(*S*)-β³-hAla-(*S*)-β³-hLeu-(*R*)-β³-hVal-(*S*)-β³-Ala-(*S*)-β³-Leu-D-Val-D-Ala-D-Leu-Aib-D-Val-D-Ala-D-Leu-OBn (61 mg, 40 μmol) was transformed in

Table 7. *^{1}H- and ^{13}C-NMR Data and Assignments for DPA β-Peptide* **6**. δ in ppm, *J* in Hz.

Residue	NH	$CH_2(\alpha)$	H–C(β) (^3J(HN,Hβ))	H–C(γ), Me(γ), $CH_2(\gamma)$	H–C(δ), Me(δ), $CH_2(\delta)$	$CH_2(\varepsilon/\zeta)$, Me(ε)	C(α)	C(β)	C(γ)	C(δ)	$CH_2(\varepsilon/\zeta)$
β^{3}hVal^1	9.04	2.63/2.78	4.48	1.96	1.01		39.89	54.31	34.28	19.74	
β^{3}hAla^2	8.25	2.22	4.31 (8.44)	1.10			43.52	44.12	21.72		
β^{3}hLeu^3	8.06	2.45	4.27 (8.95)	1.23/1.36	1.51	0.83	42.60	52.65	46.55	26.05	23.42
β^{3}hIle^4	8.14	2.40/2.66	4.40 (9.10)	1.60	1.12/1.50	0.902	37.71	52.21	40.45	27.20	19.59
β^{3}hGlu^5	8.16	2.46	4.51 (NA)	1.80/1.88	2.87		NA	47.33	32.61	41.92	
β^{3}hSer^6	8.28	2.492.78	4.51	3.56			38.32	49.98	65.28		
β^{3}hAla^7	8.42	2.41/2.61	4.60 (8.80)	1.16			43.35	43.72	21.49		
β^{3}hLys^8	7.45	2.30/2.51	4.47 (9.10)	1.54	1.46	1.65/2.96	41.92	47.73	36.45	24.65	28.92/41.13
β^{3}hAsp^9	7.62	2.49/2.61	4.88 (9.02)	NA			40.17	44.57			
β^{3}hVal^{10}	7.86	2.50/2.64	4.26 *9.12*	1.78	0.93		41.84	46.08	33.86	1.35	

TFE (1 ml) according to *GP 8* for 18 h. The residue was Boc-deprotected with TFA (2 ml) according to *GP 9* for 2 h. The crude product was co-evaporated with toluene, dissolved in TFE, filtered, and evaporated under reduced pressure to yield **3** (62 mg, quant.). Colorless glass. $[\alpha]_D = +21.5$ ($c = 1.1$, TFE). CD (0.2 mM in MeOH): $+7.9 \times 10^4$ (204 nm). IR (KBr): 3304*m*, 2963*m*, 1652*s*, 1540*s*, 1469*m*, 1387*m*, 1202*s*, 1173*s*, 800*w*, 706*w*. ^{1}H-NMR (400 MHz, CD_3OD): 0.89–0.98 (*m*, 14 Me); 1.06 (*d*, $J = 6.8$, Me); 1.07 (*d*, $J = 6.9$, Me); 1.14 (*d*, $J = 6.7$, Me); 1.18 (*d*, $J = 6.7$, Me); 1.22–1.49 (*m*, 4 CH); 1.37 (*d*, $J = 7.2$, Me); 1.41 (*d*, $J = 7.2$, Me); 1.45 (*s*, Me); 1.48 (*s*, Me); 1.55–1.79 (*m*, 9 CH); 1.98–2.06 (*m*, CH); 2.08–2.18 (*m*, CH); 2.20–2.27 (*m*, CH); 2.34–2.62 (*m*, 6 CH_2); 3.43–3.48 (*m*, CHN); 4.12–4.22 (*m*, 4 CHN); 4.27–4.46 (*m*, 7 CHN); 7.35 (*d*, $J = 7.6$, NH); 7.74 (*d*, $J = 9.5$, NH); 7.86–7.90 (*m*, NH); 7.99–8.09 (*m*, 3 NH); 8.19 (*d*, $J = 6.0$, NH). ^{13}C-NMR (100 MHz, CD_3OD): 17.8, 18.1, 18.2, 18.8, 18.9, 19.0, 19.7, 19.9, 20.7, 20.9, 21.9, 22.3, 22.6, 22.7, 23.3, 23.5, 23.6, 23.8, 25.2 (Me); 25.8, 25.9, 26.0, 31.2, 31.6, 32.0, 33.6 (CH); 35.9, 39.4, 41.3, 41.6, 42.4, 42.8, 43.3 (CH_2); 43.9, 44.0 (CH); 45.1, 45.7 (CH_2); 46.0, 46.3, 50.6, 50.9, 52.1, 53.0, 54.6, 56.2 (CH); 58.1 (C); 60.7, 60.9 (CH); 171.6, 172.0, 172.2, 172.6, 172.8, 173.4, 173.6, 173.8, 174.8, 175.0, 175.5, 175.6, 177.4 (C). FAB-MS: 1366 (17), 1365 (17), 1360 (16, $[M+K]^+$), 1358 (32), 1348 (11), 1345 (16), 1344 (38, $[M+Na]^+$), 1343 (94), 1342 (57), 1329 (11), 1327 (15), 1324 (18), 1323 (14), 1321 (100, M^+), 1320 (43), 750 (19), 439 (11), 411 (16), 397(14).

*H-(*R*)-β^{3}hVal-(*S*)-β^{3}hGlu-(*S*)-β^{3}hLeu-(*S*)-β^{3}hAla-(*S*)-β^{3}hLys-(*R*)-β^{3}hIle-(*R*)-Phe-(*R*)-Ser-(*R*)-Leu-Aib-(*R*)-Trp-(*R*)-Thr-(*R*)-Ala-NH$_2$* (**4**). *Rink*-Amide AM resin (100 mg, 0.071 mmol, 0.71 mmol/g) was loaded with Fmoc-D-Ala-OH according to *GP 2*. SPPS was continued according to *GP 5*, until all remaining residues were incorporated. For couplings of Fmoc-Aib-OH and Fmoc-(*S*)-β^{3}hLys(Boc)-OH, the coupling time was prolonged to 2 h. After final Fmoc deprotection (*GP 4*), the resin was dried under h.v. for 2 h, and the peptide was cleaved from the resin according to *GP 6* to give 129 mg of crude peptide. A portion (44 mg) was purified by prep. RP-HPLC (34–95% *A* in 50 min: 5 min 34% *A*; 45 min. 40% *A*; 50 min. 95% *A*; 17 ml/min; t_R 18.82 min) according to *GP 7* to give **4** (16.9 mg) as the TFA salt. White solid. Anal. RP-HPLC (5–99% *A* in 60 min: 45 min 50% *A*; 60 min 99% *A*): t_R 37.45 min, purity > 98%. ^{1}H- and ^{13}C-NMR spectra: see *Tables 1* and *2*. MALDI-HRMS: 1545.9452 ($[M+H]^+$, $C_{77}H_{124}N_{16}O_{17}^+$; calc. 1545.9403).

*H-(*R*)-β^{3}hVal-(*S*)-β^{3}hAla-(*S*)-β^{3}hLys-(*S*)-β^{3}hIle-(*R*)-β^{3}hSer-(*S*)-β^{3}hTyr-OH* (**5a**). *Wang* resin (300 mg, 1.1 mmol/g, 0.33 mmol) was esterified with Fmoc-β^3-hTyr(^{t}Bu)-OH (625 mg, 1.32 mmol) according to *GP 1*. Loading was determined to be 83% corresponding to 270 μmol of resin-bound Fmoc-β^{3}hTyr. After capping (*GP 3*) and Fmoc-deprotection (*GP 4*), the peptide–resin was coupled with Fmoc-β^{3}hSer(tBu)-OH (326 mg, 823 μmol), Fmoc-β^{3}hAla-OH (268 mg, 823 μmol), Fmoc-β^{3}hIle-OH (302 mg, 823 μmol), Fmoc-β^{3}hLys(Boc)-OH (397 mg, 823 μmol), Fmoc-β^{3}hAla-OH (268 mg, 823 μmol), and Fmoc-β^{3}hVal-OH (291 mg, 823 μmol) according to *GP 5*. After final Fmoc deprotection (*GP 4*), the resin was washed with CH_2Cl_2 and dried under h.v. for 4 h. A portion of the resin (200 mg)

Table 8. *NOEs for DPA β-Peptide* **6**

Residue	H-Atom	Residue	H-Atom	d_{NOE} [Å]
1	β	1	γ	2.5
1	β	1	HN	2.9
1	γ	1	HN	3.0
2	β	2	γ*[a])	2.9
2	β	2	HN	2.9
3	β	3	γ1	2.8
3	β	3	γ2	2.7
3	β	3	HN	3.1
3	β	3	δ	2.8
4	β	4	HN	3.0
4	γ	4	HN	3.4
5	β	5	γ1	2.8
5	β	5	HN	2.9
6	β	6	γ*	2.8
6	β	6	HN	2.9
7	β	7	γ*	2.7
7	β	7	HN	2.9
8	β	8	HN	3.0
8	δ*	8	HN	4.1
10	β	10	γ	2.3
10	β	10	HN	2.9
10	γ	10	γ	3.0
1	HN	2	HN	5.2
3	δ	4	HN	4.8
5	δ*	6	HN	2.7
6	γ*	7	β	4.0
7	HN	8	HN	4.4
1	γ	4	β	3.2
1	HN	3	β	3.9
1	HN	4	β	3.9
2	HN	4	β	3.6
3	δ	6	γ*	5.1
3	γ1	6	β	3.7
3	HN	6	β	3.1
4	γ	7	β	3.2
5	β	2	HN	3.1
5	δ*	3	HN	5.1
5	δ*	8	β	3.1
7	β	4	HN	3.1
7	HN	10	β	3.0
8	HN	10	β	3.6
8	HN	10	β	3.7

[a]) * = Pseudoatom used for calculations.

was then placed in a SPS reactor, and the peptide was cleaved from the resin according to *GP 6* to give 94 mg of crude peptide. A portion of the crude peptide (50 mg) was purified by prep. RP-HPLC (20% *A* in 5 min, 35–95% *A* in 30 min, C_{18}) according to *GP 7* to give **5a** (16.2 mg) as a TFA salt. Colorless solid. Anal. HPLC (5% *A* in 5 min, 5–95% *A* in 30 min, t_R 26.36). Purity > 95%. MALDI-MS: 887 (10,

$[M+K]^+$), 871 (14, $[M+Na]^+$), 851 (12), 850 (48), 849 (100, $[M+H]^+$). HR-MS: 849.5459 ($C_{42}H_{73}N_8NaO_{10}^+$; calc. 849.5450).

*Ac-(*R*)-β³hVal-(*S*)-β³hAla-(*S*)-β³hLys-(*S*)-β³hIle-(*R*)-β³hSer-(*S*)-β³hTyr-OH* (**5b**). A third portion of peptide–resin was placed in a SPS reactor and acetylated according to *GP 3*. The peptide–resin was then dried under h.v. for 2 h, and the peptide was cleaved from the resin according to *GP 6a* to give 37 mg of crude peptide. Purification by prep. RP-HPLC (20% *A* in 5 min, 20–80% *A* in 30 min, C_{18}) according to *GP 7* gave **5b** (12.1 mg) as a TFA salt. Colorless solid. Anal. RP-HPLC (5% *A* in 5 min, 5–95% *A* in 30 min, t_R 19.33). Purity >95%. ^{1}H- and ^{13}C-NMR spectra: see *Tables 3* and *4*. MALDI-MS: 930.5 (12), 929.5 (29, $[M+K]^+$), 915.5 (16), 914.5 (51), 913.5 (100, $[M+Na]^+$), 892.6 (24), 891.6 (45, $[M+H]^+$). HR-MS: 913.5373 ($C_{44}H_{74}N_8NaO_{11}^+$; calc. 913.5375).

*Amino-PEG$_6$-(*R*)-β³hVal-(*S*)-β³hAla-(*S*)-β³hLys-(*S*)-β³hIle-(*R*)-β³hSer-(*S*)-β³hTyr-OH* (**5c**). A second portion of the peptide–resin (100 mg) was swollen in a SPS reactor (DMF, 4 ml, 30 min) and then washed with DMF (5 ml, 3 × 1 min). The resin was filtered, and a soln. of Fmoc-amido dPEG$_6$ acid (41 mg, 70 μmol), HATU (26 mg, 68 μmol), and DIPEA (24 μl, 140 μmol) in DMF (3 ml) was added, and the soln. was mixed by N_2 bubbling for 3 h. The resin was filtered and washed with DMF (5 ml, 6 × 1 min). After final Fmoc deprotection (*GP 4*), the resin was dried under h.v. for 2 h, and the peptide was cleaved from the resin according to *GP 6* to give 53 mg of crude peptide. Purification by prep. RP-HPLC (20% *A* in 5 min, 20–80% *A* in 30 min, C_{18}) according to *GP 7* gave **5c** (11.1 mg) as a TFA salt. Colorless solid. Anal. HPLC (5% *A* in 5 min, 5–95% *A* in 30 min, t_R 18.98). Purity >95%. ^{1}H- and ^{13}C-NMR spectra: see *Tables 5* and *6*. MALDI-MS: 1223 (26, $[M+K]^+$), 1208 (48), 1207 (72, $[M+Na]^+$), 1187 (66), 1186 (67), 1185 (100, $[M+H]^+$). HR-MS: 1184.7394 ($C_{57}H_{102}N_9O_{17}^+$; calc. 1184.7402).

*DPA-(*R*)-β³hVal-(*S*)-β³hAla-(*S*)-β³hLeu-(*S*)-β³hIle-(*R*)-β³hSer-(*S*)-β³hAla-(*S*)-β³hLys-(*S*)-β³hAsp-(*R*)-β³hVal-OH* (**6**). *Wang* resin (100 mg, 1.1 mmol/g, 0.11 mmol) was esterified with Fmoc-β³-hVal-OH (194 mg, 550 μmol) according to *GP 1*. Loading was determined to be 86% corresponding to 95 μmol of resin-bound Fmoc-β³hVal. After capping (*GP 3*) and Fmoc-deprotection (*GP 4*), the peptide–resin was coupled with Fmoc-β³hAsp(tBu)-OH (121 mg, 284 μmol), Fmoc-β³hLys(Boc)-OH (137 mg, 284 μmol), Fmoc-β³hAla-OH (93 mg, 284 μmol), Fmoc-β³hSer(tBu)-OH (113 mg, 284 μmol), Fmoc-β³hGlu(tBu)-OH (125 mg, 284 μmol), Fmoc-β³hIle-OH (104 mg, 284 μmol), Fmoc-β³hLeu-OH (104 mg, 284 μmol), Fmoc-β³hAla-OH (93 mg, 284 μmol), Fmoc-β³hVal-OH (100 mg, 284 μmol), and pyridine-2,6-dicarboxylic acid monobenzyl ester [17] (73 mg, 284 μmol) according to *GP 5*. The crude peptide was cleaved from the resin according to *GP 6* to give 140 mg of crude Bn-protected peptide. After Bn-deprotection (*GP 8*), a portion of the crude peptide (37 mg) was purified by prep. RP-HPLC (5–35% *A* in 5 min, 35–95% *A* in 30 min, C_{18}) according to *GP 7* to give **6** (12.2 mg) as a TFA salt. Colorless solid. Anal. HPLC (5% *A* in 5 min, 5–95% *A* in 30 min, t_R 25.75). Purity >95%. ^{1}H- and ^{13}C-NMR spectra: see *Tables 7* and *8*. MALDI-MS: 1372.7 (17), 1371.7 (23, $[M+K]^+$), 1356.8 (22), 1355.8 (26, $[M+Na]^+$), 1335.8 (32), 1334.8 (77), 1333.8 (100, $[M+H]^+$). HR-MS: 1333.7639 ($C_{63}H_{105}N_{12}O_{19}^+$; calc. 1333.7619).

Determination of Solution Structures by NMR. All NMR measurements (including DQF-COSY, TOCSY, HSQC, HMBC, and ROESY at τ_m = 150, 300 ms) were conducted at 600 MHz (^{1}H) and 150 MHz (^{13}C) in CD_3OH at 25.6° with solvent suppression by presaturation. NMR Measurements, signal assignment, derivation of distance constraints, and simulated annealing molecular-dynamics calculations were carried out as described previously (see, *e.g.*, [4b]).

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