Synthesis, and Helix or Hairpin-Turn Secondary Structures of 'Mixed' α/β-Peptides Consisting of Residues with Proteinogenic Side Chains and of 2-Amino-2-methylpropanoic Acid (Aib)

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Twelve peptides, **1**–**12**, have been synthesized, which consist of alternating sequences of α- and β-amino acid residues carrying either proteinogenic side chains or geminal dimethyl groups (Aib). Two peptides, **13** and **14**, containing 2-methyl-3-aminobutanoic acid residues or a 'random mix' of α-, β^2-, and β^3-amino acid moieties were also prepared. The new compounds were fully characterized by CD (*Figs. 1* and *2*), and ^{1}H- and ^{13}C-NMR spectroscopy, and high-resolution mass spectrometry (HR-MS). In two cases, **3** and **14**, we discovered novel types of turn structures with nine- and ten-membered H-bonded rings forming the actual turns. In two other cases, **8** and **11**, we found *14/15*-helices, which had been previously disclosed in mixed α/β-peptides containing unusual β-amino acids with non-proteinogenic side chains. The helices are formed by peptides containing the amino acid moiety Aib in every other position, and their backbones are primarily not held together by H-bonds, but by the intrinsic conformations of the containing amino acid building blocks. The structures offer new possibilities of mimicking peptide–protein and protein–protein interactions (PPI).

1. Introduction. – Since the first report on synthesis, NMR-solution and X-ray-crystal structures, as well as enzymatic stability of short peptides consisting of homologated proteinogenic amino acids (β-homo-amino acids, βhXaa) [1], a whole new area of peptide chemistry has evolved[5]). If we include the oligomers of cyclic β-amino carboxylic acids [3], three different helices (a *14*, a *12*, and a *10*/*12* helix), hairpin turns, and pleated

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[1]) Postdoctoral Researcher, ETH-Zürich, 2002–2004, financed by the *Swiss National Science Foundation*, Project No. 200005-883199.

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[3]) Postdoctoral Fellow at ETH Zürich, 2004–2006, financed by the *Deutsche Forschungsgemeinschaft*, by the *Swiss National Science Foundation*, Project No. 200020-100182, and by *Novartis Pharma AG*, Basel.

[4]) Postdoctoral Research Fellow, ETH Zürich, 2005/2006, financed by *Novartis Institute of Biomedical Research*, Basel.

[5]) For extensive review articles, see [2–5].

sheets have been identified [4][6]). The β-peptidic secondary structures have distinctly different geometries and properties (helicity, macrodipole, directivity), as compared to those of the natural α-peptidic counterparts. Yet, they can be designed in such a way that they mimic α-peptidic compounds in their affinities for protein receptors [4]. These properties, together with the fact that they are stable to enzymatic cleavage and metabolism, make β-peptides candidates for biomedical applications [7–10].

Not least for the reason that α-amino acids are much more readily available, with numerous derivatives being commercially accessible, there has been a recent growing interest in peptides containing α- and β-amino acid residues. Such 'mixed' peptides can also be proteolytically stable [11], and some were tested negative for hemolytic and antimicrobial activities [12]. α/β-Mixed peptides were found to fold to new types of helical secondary structures held together by H-bonded rings containing 9 through 15 atoms [13–19][7]). There are numerous more or less rational ways, in which sequences of α- and β-amino acids can be arranged to form 'mixed' peptides[8]), some of which are shown in **A**–**F**, and there are various reasons for which such 'hybrid' peptides have been studied. Some specific examples are discussed in the following sections.

A $(\alpha)_a-(\beta)_b-(\alpha)_c$
B $(\alpha)_a-(\beta)_b$ or $(\beta)_a-(\alpha)_b$
C $(\beta)_a-(\alpha)_b-(\beta)_c$
D $(\alpha-\beta)_a$ or $(\beta-\alpha)_a$
E $(\alpha-\alpha-\beta)_a$ or $(\beta-\beta-\alpha)_a$
F $(\alpha)_a-(\beta)_b-(\alpha)_c-(\beta)_d-(\alpha)_e$

Almost 80 years ago, it was first proposed by *Abderhalden et al.* [21] that incorporation of a β-amino acid into a peptide (type **A**) should increase its enzymatic stability, *i.e.*, decrease its 'fermentability'. Such an effect was confirmed later. In fact, medicinal chemists are now using β-amino acid-containing compounds in drug design [22]. In recent years, the replacement of a single α-amino acid by a β-amino acid residue, or of a section of two or more α- by β-amino acid moieties in a peptide or protein has been shown to lead to greatly enhanced stability towards enzymatic cleavage, with retention of the secondary and tertiary structures, as well as of the biological activities: the undecapeptide amide *substance P* with one ($\alpha \rightarrow \beta^2$) amino acid exchange binds to the hNK-1 receptor (type **A**, [23]); a β-amino acid residue in the dodecapeptide cleavage-site model of the hemoglobin α-chain prevents proteolysis by plasmepsin II (type **A**, [24]); replacement of the turn components Asn^{113} and Pro^{114} in ribonuclease A by β^2hPro and *ent*-β^2hPro leads to an RNase-A analogue with indistinguishable activity ('protein prosthesis', (type **A**, [25]); substituting the entire N-terminal helix (Xaa^{61-77}) of hIL-8 by a β-peptide capable of forming an amphipathic helix provides an analogue

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[6]) For a recent review article on helical and other secondary structures of β- and γ-peptides, see [5]. For a discussion of the differences between α- and various β-peptidic hairpin-turn structures, see [6].

[7]) For theoretical contributions on helical structures of 'mixed' peptides containing α-, β-, and γ-amino acid residues, see [18][20].

[8]) Note that the β-amino acids incorporated may be β-iso-amino acids, β^2- or β^3-homo-amino acids (β^2hXaa, β^3hXaa), $\beta^{2,3}$-disubstituted, or even $\beta^{2,2,3}$- and $\beta^{2,3,3}$-trisubstituted analogs [4], which increase the number of possible sequences tremendously.

of unchanged inhibition of forskolin-stimulated *c*AMP production and of only slightly reduced affinity for the CXCR1 receptor (type **B**)[9])[10]).

Helical conformations of peptides containing α- and β-amino acid moieties have been studied most extensively [13–20]. Thus, *Balaram* and co-workers have shown that the undecapeptide Boc-Val-Ala-Phe-Aib-β^3hVal-β^3hPhe-Aib-Val-Ala-Phe-Aib-OMe (type **A**), containing three strongly helix-inducing [27] Aib units and two β-amino acid residues, forms a helix in the solid state and in solution, with 13-membered H-bonded rings in the terminal sections, and a 15- and 14-membered ring in the α-β-β, and in the α-α-β or β-α-α segments, respectively [17][11]). The most favored objects of investigations are α/β-mixed peptides with alternating α- and β-amino acid residues (type **D**). With simple proteinogenic side chains, this type of mixed peptides was reported not to fold to helices, which would be stable on the NMR timescale in solution [14][29]. When the incorporated β-amino acids were carbohydrate-derived [19] or conformationally restricted derivatives [13–16], a plethora of helical structures was discovered by X-ray and NMR analysis: a *13*-helix of oligomers containing Ala and *cis*-2-aminocyclopropanecarboxylic acid [16] and 'mixed' helices comprised of H-bonded rings of different sizes[12]); a *9/11*-helix of a C-linked carbo β-amino acid Caa (with Ala) [19]; an *11*-, a *14/15*-, a *10/11/11*-, and an *11/11/12*-helix with diads or triads (type **E**) of *trans*-2-aminocyclopentanecarboxylic acid (ACPC) or *trans*-4-aminopyrrolidinecarboxylic acid (APC), and proteinogenic (Xaa) or non-proteinogenic, α-branched (*cf.* Aib, Cyp, α-MePhe) α-amino acids [13–15].

Hairpin-turn-forming mixed α/β-peptides have, so far, only been described in two papers by *Balaram* and co-workers. They contain the conventional D-Pro-Gly α-peptidic turn motif and carry β-amino acid residues[13]) (βhGly or βhPhe) on opposite sides of the antiparallel sheet part of the hairpins (type **F** [31])[11]). There are also tetrapeptides of the sequence β-α-β-β (type **C**), which are agonists of somatostatin [9], and which may be considered oddities in the context of the present discussion.

We herein describe the synthesis of a number of 'mixed' α/β-peptides consisting exclusively of components with proteinogenic side chains and of the natural, but non-proteinogenic (non-ribosomal), helix-inducing amino acid residue Aib, and we present two helical and two hairpin-turn NMR-solution structures of such peptides.

2. Synthesis of the *α/β*-Mixed Peptides 1–14. – The *Formulae* below show the constitutional and configurational structures of the 14 synthesized α/β-mixed peptides **1**–**14**, and, in *Table 1*, the incorporated amino acids and the various types of mixed pep-

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[9]) Hitherto unpublished results by *R. David*, *R. Günther*, *H.-J. Hofmann*, and *G. Beck-Sickinger* (Universität Leipzig), and *D. Seebach* and *T. Kimmerlin* (ETH-Zürich).

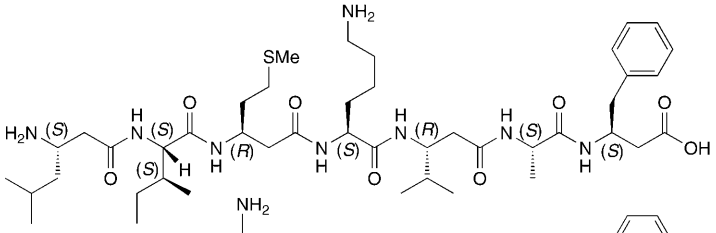
[10]) In a project aimed at testing whether two left-handed β-peptidic helices might be able to force an α-peptidic segment to adopt the *Ramachandran*-allowed (see the discussion in [26]), but rarely seen in proteins, left-handed helical conformation, we have synthesized β/α-mixed peptides of type **C**: *J. V. Schreiber*, *D. Seebach*, ETH-Zürich 2001, hitherto unpublished.

[11]) For other Aib-, βhPhe-, βhGly-, and γhhGly-containing α-peptides, which form helices or hairpin turns in crystal structures and/or in non-protic solutions, see [28] and the discussion in [4], Sect. 3, Fig. 6.

[12]) First discovered in β^2/β^3-alternating 'mixed' β-peptides, which form *10/12*-helices [4][5][30].

[13]) … and also γ- and δ-amino-butyryl and -valeryl residues!

1



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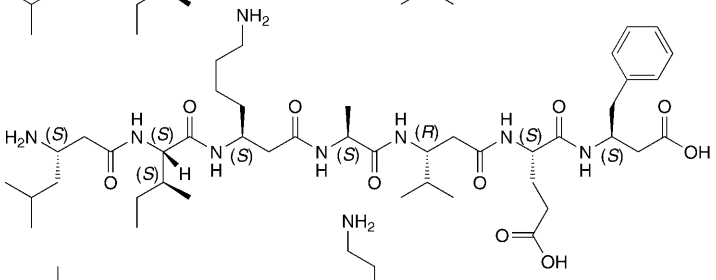

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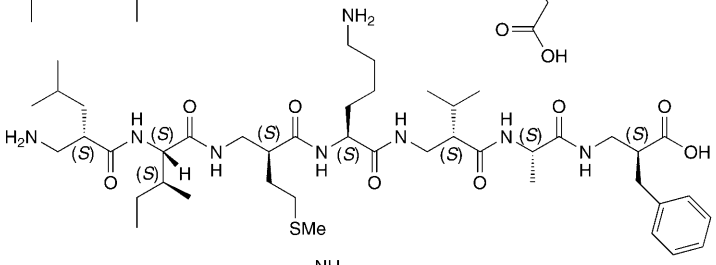

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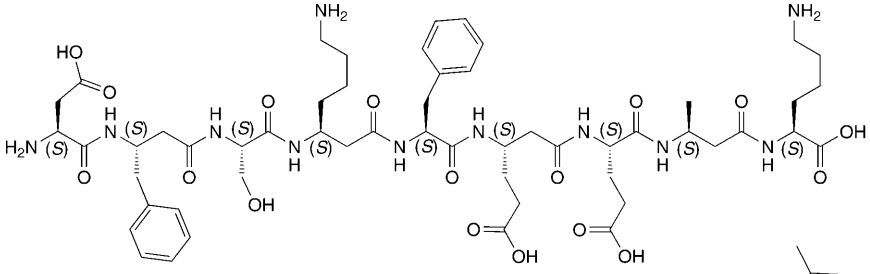

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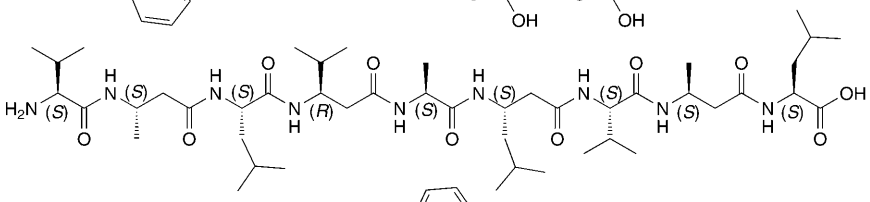

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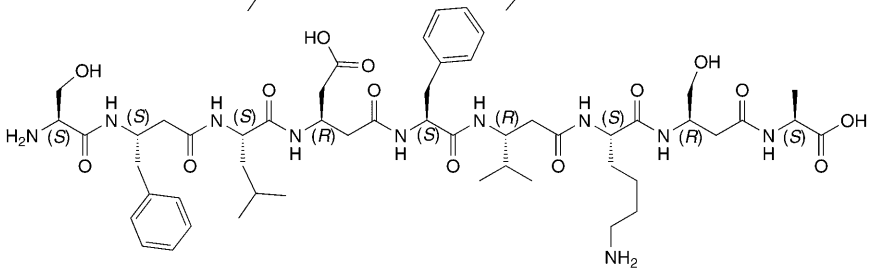

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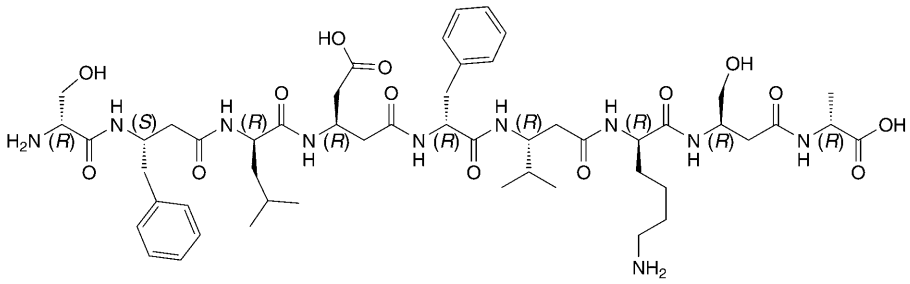

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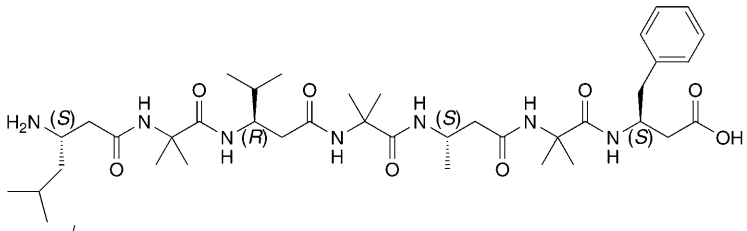

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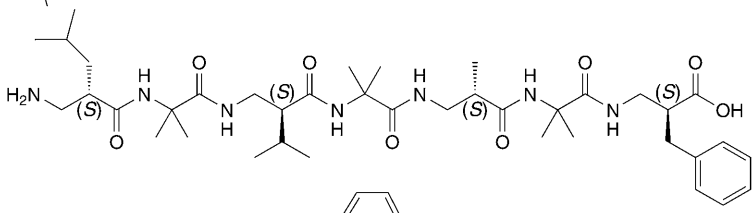

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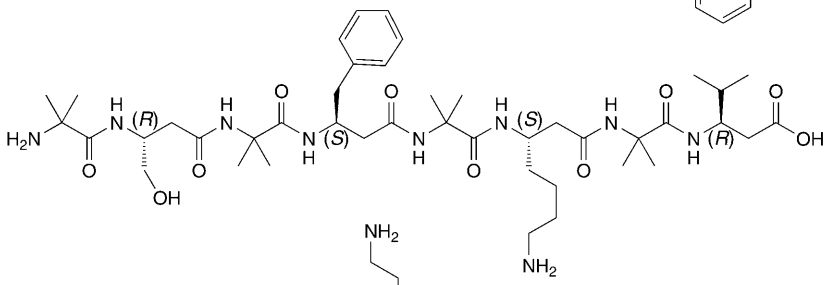

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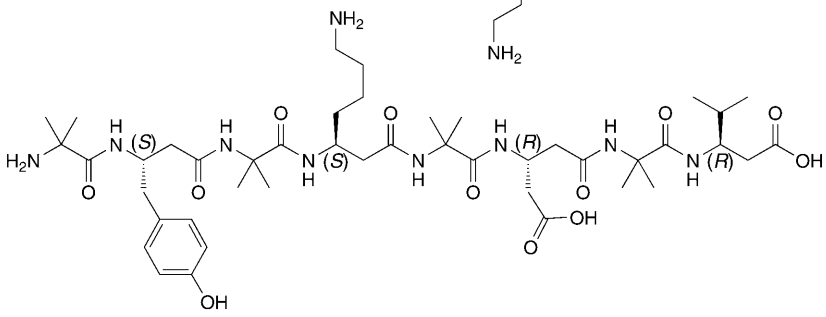

tides are specified. After finding out that none of the simple α/β-peptides **1**, **2**, and **4**–**7** with alternating Lα/Lβ^3, Lβ^3/Lα, or Dα/Lβ^3 building blocks showed any NMR nuclear Overhauser effects (NOEs) between non adjacent residues[14]), we turned to β^2-amino

[14]) Conforming an observation of *Gellman* and co-workers [14].

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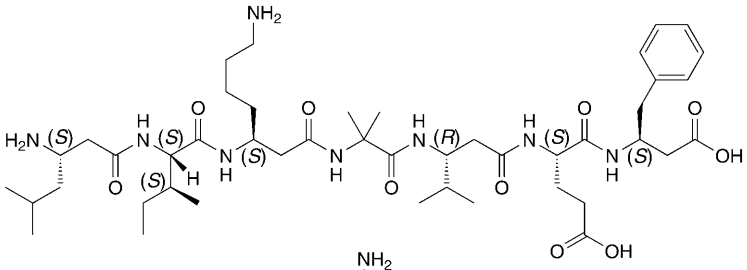

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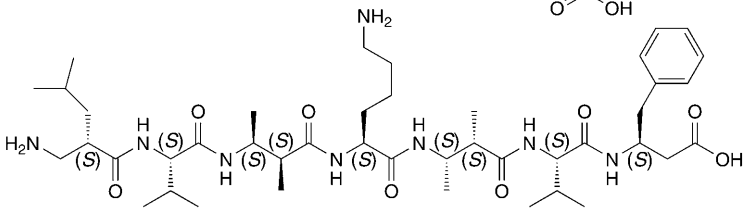

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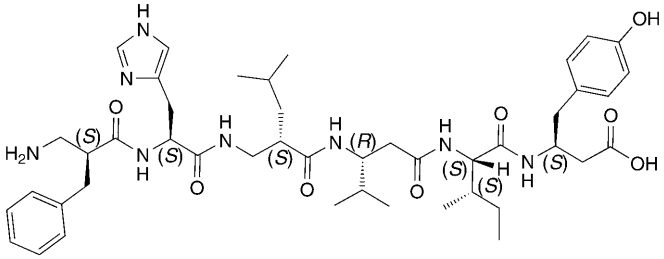

acids- (*i.e.*, **3**, **9**, **13**, and **14**) and Aib-containing (*i.e.*, **8**–**12**) mixed peptides. Among those, we have indeed discovered four, *i.e.*, **3**, **8**, **11**, and **14**, which form secondary structures in MeOH solution (see the turns and helices shown in *Sect. 4*). Thus, the less readily available β^2-homo-amino acids[15]) have again been the key to success in producing new types of hairpin turns[16]).

The α/β-peptides **1**–**14** were synthesized from Fmoc-Xaa(Pg)-OH, Fmoc-Aib-OH, and the previously described acids Fmoc-β^2hXaa(Pg)-OH and Fmoc-β^3hXaa(Pg)-OH by manual solid-phase assembly.

The α-amino acids are commercially available[17]), the β^2-amino acid derivatives were all prepared by the DIOZ-auxiliary approach [35][36]: Fmoc-β^2hAla-OH [35][37], Fmoc-β^2hVal-OH [35][38], Fmoc-β^2hLeu-OH [35], Fmoc-β^2hPhe-OH [35][39], Fmoc-β^2hMet-OH [40]. The $\beta^{2,3}$-disubstituted amino acid (*S*,*S*)-β^3hAla(α-Me)-OH was prepared by α-methylation of Boc-protected esters of (*S*)-β^3hAla-OH through a Li_2 derivative [41][42]. The assembly of the peptides **1**–**14** was carried out by manual solid-phase synthesis on *Wang* resin. The couplings were achieved with HATU [43] and *Hünig* base [44] in DMF, with the excesses of the Fmoc-amino acid

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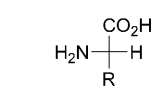
[15]) For a review on β^2-amino acids and peptides, see [32].

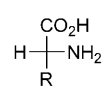
[16]) *Cf.* the novel *10/12*-helix of β^2/β^3-peptides [4][5][30] and the hairpin-turn structures [4][5][6a][8][25][33][34].

[17]) We thankfully acknowledge a generous discount on amino acids by *Fluka*.

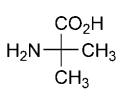
Table 1. *Specifications of the Amino Acids and of the Mixed α/β-Peptides* **1**–**14** (see the accompanying *Formulae*). Behind each short designation of amino acids, as used herein, there is the full specification for the homologated proteinogenic amino acid in parenthesis, as defined in [4]. The mixed peptides are ordered by increasing complexity and/or chain length.



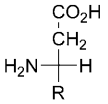
Lα (H-Xaa-OH)



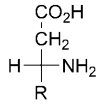
Dα (H-(D)-Xaa-OH)



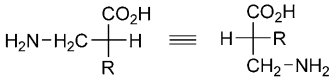
Aib (H-Aib-OH)



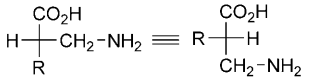
Lβ^3 (H-β^{3}hXaa-OH)



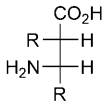
Dβ^3 (H-Dβ^{3}hXaa-OH)



Rβ^2 (H-β^{2}hXaa-OH)



Sβ^2 (H-(S)β^{2}hXaa-OH)



S,Sβ^{2,3} (H-(S,S)β^{2,3}HXaa(αR)-OH)

Peptide No.	Sequence	*n*-mer	Type	Structure
1	(Lβ^3-Lα)$_3$-Lβ^3	7	**D**	
2	(Lβ^3-Lα)$_3$-Lβ^3	7	**D**	
3	(*S*β^2-Lα)$_3$-*S*β^2	7	**D** (β^2)	turn 9
4	(Lα-Lβ^3)$_4$-Lα	9	**D**	
5	(Lα-Lβ^3)$_4$-Lα	9	**D**	
6	(Lα-Lβ^3)$_4$-Lα	9	**D**	
7	(Dα-Lβ^3)$_4$-Dα	9	**D**	
8	(Lβ^3-Aib)$_3$-Lβ^3	7	**D** 3 (Aib)	*14/15*-helix
9	(*S*β^2-Aib)$_3$-*S*β^2	7	**D** 3 (Aib)	
10	(Aib-Lβ^3)$_4$	8	**D** 4 (Aib)	
11	(Aib-Lβ^3)$_4$	8	**D** 4 (Aib)	*15/14*-helix
12	Lβ^3-Lα-Lβ^3-Aib-Lβ^3-Lα-Lβ^3	7	**D** 1 (Aib)	
13	*S*β^2-Lα-*S*,*S*β^{2,3}-Lα-*S*,*S*β^{2,3}-Lα-Lβ^3	7	**D** (β^{2,3})	
14	*S*β^2-Lα-*S*β^2-Lβ^3-Lα-Lβ^3	6	**F**	turn 10

derivatives adjusted to their availability: 5–10 equiv. with the α-amino acids, including Aib, *ca.* 3 equiv. for the β^3-amino acids, and 1.5–2 equiv. for the β^2-amino acids. The success of coupling was checked by the TNBS test [45], and the coupling procedure was repeated in cases of incomplete coupling; this turned out to be especially necessary with the coupling of Aib. Fmoc Deprotection was performed with *ca.* 20% piperidine in DMF, and again the success of this step was checked by the TNBS test.

The deprotective release from the beads was carried out by one of the common cocktails: *i*) TFA/H_2O/TIS 95:2.5:2.5, *ii*) TFA/TIS/H_2O/phenol 90:2.5:5:2.5 for His and Tyr side chain-containing compounds, *iii*) TFA/H_2O/EDT/TIS 94:2.5:2.5:1 for the peptide **1** with a Met side chain. The crude products were first analyzed by analytical RP-HPLC (C_8 or C_{18} columns) to find the best conditions for the subsequent preparative HPLC purification. The purified compounds were characterized by high-resolution mass spectrometry (HR-MS), by CD measurements (*vide infra*, *Sect. 3*), and by NMR spectroscopy (see *Sect. 4*). Of the 14 peptides, 12, **1**–**4**, **6**–**8**, and **10**–**14**, were subjected to a full NMR analysis including DQFCOSY, TOCSY, HSQC, and

HMBC. This means that each and every different type of H- and each C-atom was assigned. For those four peptides, which folded to a predominant helix or turn conformation according to the NMR analysis, we refer to *Sect. 4*. All the other data are given in the *Exper. Part*, which also contains a list of *Abbreviations.*

3. CD Analysis. – The CD spectra of the 14 α/β-mixed peptides were measured in MeOH, and, in some cases, also in H_2O and 2,2,2-trifluoroethanol (TFE). The spectra of those peptides for which we have not found a dominant conformation by NMR are shown in *Fig. 1*; the CD spectra of the four peptides, which have helical or turn structures (see *Sect. 4*), are displayed in *Fig. 2*.

A superficial glance at these spectra does not reveal a general trend or a fundamental difference between those compounds, which – according to NMR – assume a secon-

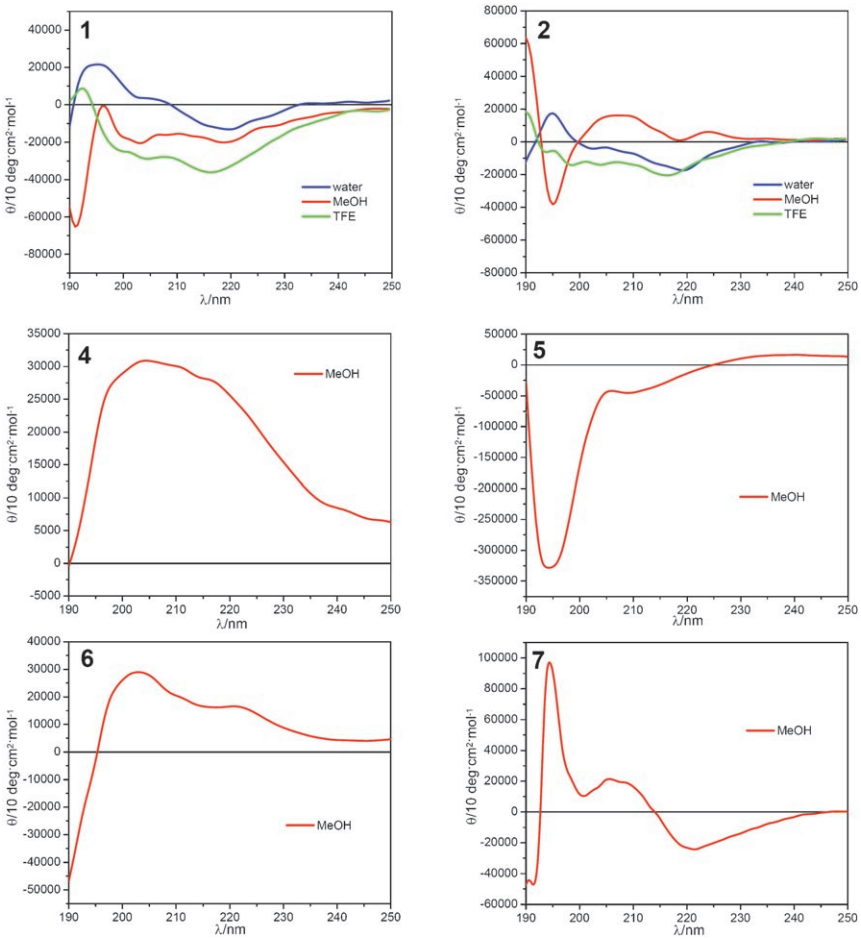


Fig. 1. *Non-normalized CD spectra of the peptides, of which no NMR-solution secondary structure could be determined* (*i.e.*, **1**, **2**, **4**, **5**, **6**, **7**, **9**, **10**, **12**, and **13**), *recorded at 20° in H_2O, 2,2,2-trifluoroethanol* (TFE) *and MeOH* ($c = 0.2$ μM)

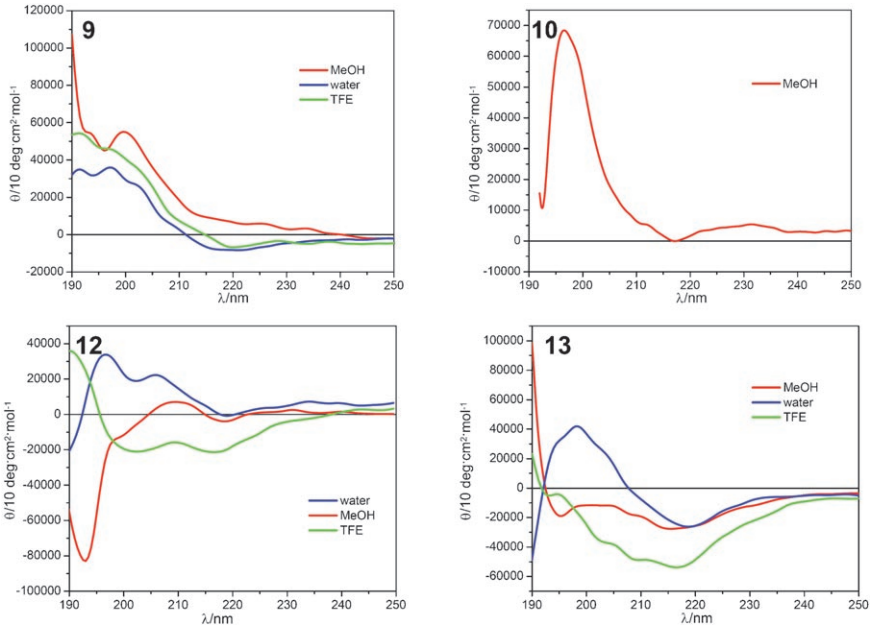


Fig. 1 (cont.)

dary structure (*i.e.*, **3**, **8**, **11**, and **14**) and those, which do not. If we disregard maxima or minima below 200 nm, there are higher-intensity *Cotton* effects of three of the structured peptides, *i.e.*, **3**, **11**, and **14**, as compared to the non-structured ones. Also, the solvent dependence looks very erratic: the classical secondary-structure-inducing solvent 2,2,2-trifluoroethanol gives rise to the most-intensive *Cotton* effect only in two cases, *i.e.*, **1** and **13**, while H_2O, which is normally the worst solvent for observing secondary structures, gives *no Cotton* effect down to 200 nm for compound **3**, whereas, in other cases, the intensity of the CD spectrum in H_2O is comparable to those in MeOH and 2,2,2-trifluoroethanol. Thus, we consider these CD spectra simply as fingerprints of the peptides.

4. NMR Analysis of Peptides 3, 8, 11, and 14. – With the exception of **5**, which was insoluble in polar and unpolar solvents, and **9**, which was not investigated beyond standard characterization, comprehensive NMR analysis (CD_3OH, ^1H, DQF-COSY, TOCSY, HSQC, and HMBC) allowed assignment of all H- and C-atoms for peptides **1**–**4**, **6**–**8**, and **10**–**14**. Because the solution of peptide **4** in CD_3OH formed a gel within a few hours, no ROESY data could be acquired. Qualitative inspection of the ROESY spectra of peptides **1**, **2**, **5**, **6**, **7**, **12**, and **13** showed none of the NOE patterns typical for any of the known secondary structures of either α- or β-peptides. Even though a large number of sequential (i, $i+1$) NOEs was present, crucial non-sequential (i, $i+2$, or $i+3$) NOEs were scarce. The ^3J(NH,αH) values of α-amino acid residues were 6–7 Hz, indicating time averaging over several local conformations ('random coil'). This qualitative analysis was confirmed when NOE-derived distance constraints were

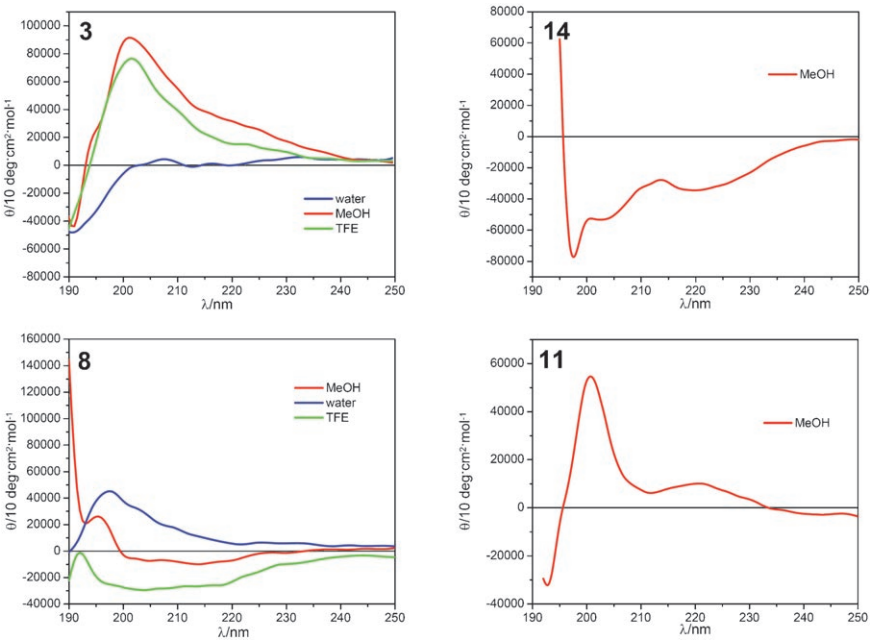


Fig. 2. *Non-normalized CD spectra of the four peptides, of which NMR-solution secondary structures were detected* (*i.e.*, **3**, **8**, **11**, and **14**)*, recorded at 20° in H_2O, 2,2,2-trifluoroethanol* (TFE) *and MeOH* ($c = 0.2$ μM)

used in *Distance Restrained Simulated Annealing* calculations, which confirmed that none of the above mentioned peptides showed a regular secondary structure.

However, for peptide **12**, the resulting bundle of structures with no violations of constraints (not shown) did have a turn at the center of the molecule due to the Aib residue. In the case of heptamer **13**, the resulting bundles (two clusters, not shown) were quite well-defined and showed an S-shaped overall form with two turns induced by the $\beta^{2,3}$-substituted homo-amino acids in positions 3 and 4.

The hexapeptide **14** consists of β^3-homo-amino, β^2-homo-amino, and α-amino acids with a central β^2-β^3 segment, whereas heptapeptide **3** is composed of β^2-homo-amino and α-amino acids in alternating positions. Despite their small size, these two peptides showed a number of (i, $i+n$) NOEs (*Tables 2* and *3* in *Exper. Part*) which allowed the calculation of well-defined structural bundles by *Simulated Annealing*. For peptide **14**, the resulting structures show a turn with a ten-membered H-bonded ring induced by the β^2/β^3 unit. For the mixed peptide **3**, the structures exhibit a turn involving the β^2hMet and Lys units with a (formally) nine-membered H-bonded ring.

In the hybrid α/β^3-alternating peptides **8**, **10**, and **11**, the α-amino acid residues were replaced by Aib residues. The large coupling constants ($^3J(\mathrm{NH},\beta\mathrm{H}) > 8.5$ Hz) observed for the β-homo-amino acid residues of **8**, **10**, and **11** established that the NH and βH protons are in antiperiplanar arrangement. For the success of structure calculations with NOE-derived constraints, it was essential that the signals of the diastereotopic Me groups of each Aib residue could be assigned stereospecifically. This was accom-

plished for peptides **8** and **11** through inspection of the cross-peak intensities of the $HN-C_\alpha(CH_3)_2$ three-bond HMBC correlations in each Aib unit, which allowed to identify the Me group that is antiperiplanar to the NH. Unfortunately, accidental overlap of several Aib Me signals in the spectrum of **10** prevented their diastereospecific assignments and forced us to abandon the attempts at structure calculation for this peptide. Based on the very similar ^3J(NH,βH)-values, we presume that the secondary structure of **10** closely resembles that of the other octapeptide **11** (*cf.* the similar CD spectra of **10** and **11** in *Figs. 1* and *2*). For peptides **8** and **11**, the distance constraints generated from ROESY ($t_m = 300$) cross-peak volumes (*Tables 4* and *5* in *Exper. Part*) were used in slow-cooling-simulated annealing calculations starting from extended conformers. Representative low-energy structures from the resulting bundles with neither NOE nor dihedral-angle constraint violations are shown in *Fig. 3*. Both peptides **8** and **11** form a right handed *14/15*-helix, involving β-homo-amino acid and Aib residues in alternating 14- and 15-membered H-bonded rings, respectively. Close inspection of the formal H-bonds reveals, however, that most of them are unusually long with large deviations from linearity. We, therefore, interpret the formation of regular helical structures in these two peptides as being predominantly due to the cumulative helix-inducing influence of the Aib residues on the local conformation rather than to stabilization through H-bonding.

5. Conclusions. – We have, for the first time, identified mixed α/β-peptides, build of non-cyclic α-, β^2-, β^3-amino acids with proteinogenic side chains and Aib, which fold to a *14/15*-helix or to hairpin-type structures. As in previous investigations of peptides consisting exclusively of β-amino acids, CD spectroscopy turns out not to be useful for obtaining structure-related information. We discovered secondary-structure formation by NMR analysis. There are two major surprises: *i*) the *14/15*-helices of the peptides with alternating β^3-amino acids and Aib are not really 'held together' by H-bonding (too large distances between the NH donor and the C=O acceptor groups), but by the conformational preferences imposed by the *gauche*-ethane bonds of the β^3-amino acid residues and of the Aib moieties (*cf.* the *Thorpe–Ingold* or geminal-dimethyl effect). *ii*) The two turn-structures are both unique, if we look at the details: peptide **3** contains a nine-membered H-bonded ring as the turn element, and in the ten-membered turn-forming ring of peptide **14** there may be a bifurcated H-bonding element like in the natural γ-turns; both turns appear to have only one *trans*-catenate H-bond; also, while **14** contains the well-known turn-inducing β^2-β^3-dipeptide segment [5], the other turn-forming peptide **3** has an α-β^2-segment on the turn part; tetrapeptides of this type had been assumed to form a turn [9] – hitherto without proof.

Incorporation of α-amino acid residues in β-peptidic sequences is not only attractive, because the proteinogenic amino acids are 'cheap', but also because the resulting secondary structures have exhibited a pattern of side-chain arrangement which is different from that of 'pure' β-peptides, so that we will be able to mimic other types of peptide–protein and protein–protein interactions (*cf. c* and *d* in *Fig. 3*). Work on somatostatin-mimicking with peptides of type **3** and **14**, carrying the right substituents on the turn unit (Lys, Trp), is in progress in our laboratory.

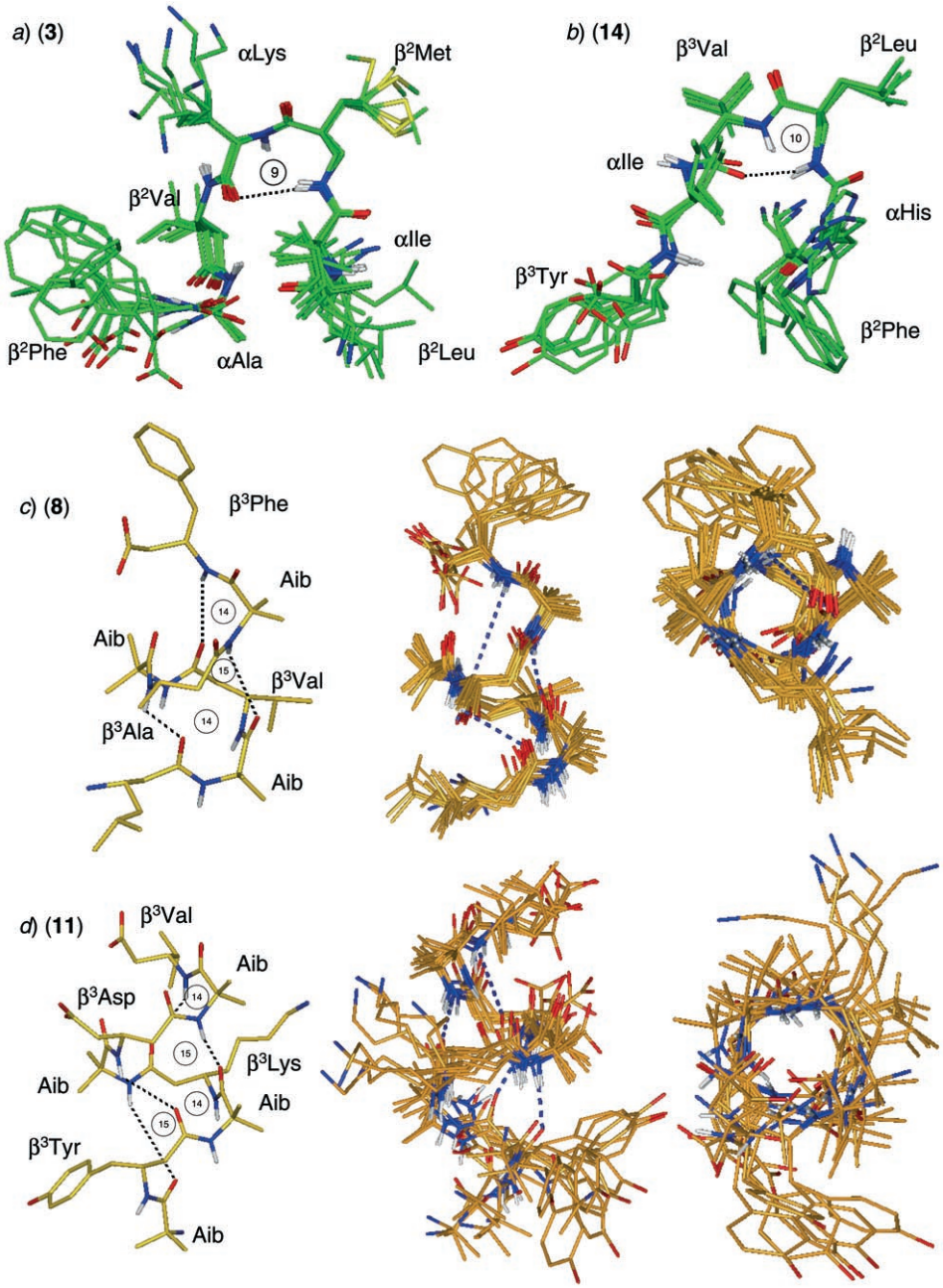


Fig. 3. *Overlay of eight and four lowest-energy NMR-solution structures of the α,β-mixed peptides* **3** (*a*) *and* **14** (*b*)*, respectively. A representative of the* 14/15 *helix of* **8** (*c*) *and* **11** (*d*)*, and bundles of ten and eight, respectively, lowest-energy NMR structures* in CD_3OH.

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Experimental Part

1. *General*. Et_3N and *Hünig* base were distilled from CaH_2 and stored over KOH. Protected Fmoc-amino acids were purchased from *Fluka*. *Wang* resin was purchased from *Novabiochem*. *Abbreviations*: Boc: (*tert*-butoxy)carbonyl, CD: circular dichroism, DMAP: 4-(dimethylamino)pyridine, EDT: 1,2-ethanedithiol, Fmoc: (9*H*-fluoren-9-yl)methoxycarbonyl, h.v.: high vacuum, 0.01–0.1 Torr, HATU: *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, MALDI: matrix-assisted-laser-desorption ionization, MeIm: 1-methyl-1*H*-imidazole, MSNT: 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole, NMM: *N*-methylmorpholine, SPS: solid-phase synthesis, TFA: CF_3COOH, TFE: 2,2,2-trifluoroethanol, TIS: $(i\text{-}Pr)_3SiH$, TNBS: 2,4,6-trinitrobenzenesulfonic acid, Tr: trityl, βhXaa: β-homo-amino acid [1][30][42][46].

Anal. HPLC: on a *Knauer* HPLC system (system 1: pump type *WellChrom K-1000 Maxi-Star*, degasser, UV detector (variable-wavelength monitor), *EuroChrom 2000* integration package; system 2: pump type *64*, UV detector (variable-wavelength monitor), *EuroChrom 2000* integration package) or on a *Merck* HPLC system (*LaChrom*, pump type *L-7150*, UV detector *L-7400*, interface *D-7000*, HPLC manager *D-7000*). *Macherey-Nagel* C_8 column (*Nucleosil 100-5* C_8 (250×4 mm), C_{18} column (*Nucleosil 100-5* C_{18} (250×4 mm), or *Knauer Lichrosolv Si-60,* 7-μm column (250×4 mm). TFA for anal. HPLC was used as UV-grade quality (>99% GC). Prep. HPLC: *Knauer* HPLC system (pump type *64*, programmer *50*, UV detector (variable-wavelength monitor)), *Merck/Hitachi* HPLC system (pump type *L-6250*, UV detector *L-4000*), *Waters* HPLC system (HPLC pump type *515*, dual-wavelength absorbance detector *2487*, or *Merck* HPLC system (*LaChrom*, pump type *L-7150*, UV detector *L-7400*, interface *D-7000*, HPLC manager *D-7000*). *Macherey-Nagel* C_8 column (*Nucleosil 100-7* C_8 (250×21 mm)), C_{18} column (*Nucleosil 100-7* C_{18} (250×21 mm)), or *Knauer Lichrosolv Si-60,* 7-mm column (250×16 mm). TFA for prep. HPLC was used as UV-grade quality (>99% GC). Lyophilization: *Hetosicc* cooling condenser with h.v. pump. or *GAMMA I-20* equipped with a controller *LDC-2M* (*Christ Gefriertrocknungsanlagen*) to obtain the peptides as their TFA salts. These peptides are specified without TFA. NMR Spectra: 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). The multiplicities of signals were determined by the DEPT technique: DEPT: + = primary or tertiary (positive DEPT signal), − = secondary (negative DEPT signal), C_{quat} = quaternary C-atoms. Mass spectra: *VG Tribrid* (EI), *Bruker Reflex* (MALDI), or *IonSpec Ultima 4.7 T FT* Ion Cyclotron Resonance (ICR, HR-MALDI, in a 2,5-dihydroxybenzoic acid matrix) mass spectrometer; in m/z (% of basis peak).

2. *General Procedures for Peptide Synthesis.* 2.1. *Anchoring of* N-*Fmoc-Protected Amino Acids on* Wang *Resin; Determination of Loading: General Procedure* 1 (*GP 1*). Esterification of the Fmoc-protected amino acid with *Wang* resin was performed according to [47] by the MSNT/MeIm method. The resin was placed into a dried manual SPS reactor, swelled in CH_2Cl_2 (20 ml/g resin) for 1 h, and washed with CH_2Cl_2. In a separate dry round-bottomed flask equipped with magnetic stirrer, the Fmoc-protected amino acid (5 equiv.) was dissolved in dry CH_2Cl_2 (3 ml/mmol), then MeIm (3.75 equiv.) and MSNT (5 equiv.) were added under Ar. Stirring was continued until the MSNT was dissolved. Thereafter, the soln. was transferred using a syringe to the reaction vessel containing the resin and mixed by Ar bubbling for 2 h. Subsequently, the resin was filtered, washed with DMF (5 ml, 5×1 min) and CH_2Cl_2 (5 ml, 5×1 min), and dried under h.v. for 24 h. The resin substitution was determined by measuring the absorbance of the dibenzofulvene–piperidine adduct: two aliquots of the Fmoc-amino acid resin were weighed exactly (m_1(resin) and m_2(resin)) and suspended in piperidine (20%) in DMF, in volumetric flasks ($V_1 = V_2 = 10$ ml). After 30–40 min, the mixtures were transferred to a UV cell and piperidine (20%) to another UV cell (blank), and the absorbance (A) was measured at 290 nm. The concentrations (c_1 and c_2, [mM]) of the benzofulvene–piperidine adduct in soln. were determined using a calibration curve [47]. 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 attachment to the resin (loading yield) was determined by *Eqn. 2*:

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

2.2. *Capping: General Procedure 2* (*GP 2*). The peptide–resin was covered with DMF (20 ml/g resin), and unreacted OH 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).

2.3. *Fmoc Deprotection: General Procedure 3* (*GP 3*). The Fmoc group was removed by treating the resin with 20% piperidine in DMF (4 ml, 4 × 10 min) under Ar bubbling. After filtration, the resin was washed with DMF (5 ml, 4 × 1 min).

2.4. *Coupling of the Amino Acids on* Wang *Resin: General Procedure 4* (*GP 4*). *GP 4a.* The Fmoc deprotection was carried out according to *GP 3*. 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 the Fmoc-protected amino acid (3 equiv.), HATU (2.9 equiv.), and *Hünig* base (6 equiv.) in DMF (20 ml/g resin) for 60 min. Monitoring of the coupling reaction was performed with the TNBS test [45]. In the case of a positive TNBS test (indicating incomplete coupling), the suspension was allowed to react further for 0.5–4 h, or, after filtration, the peptide-resin was treated again with the same Fmoc-protected amino acid (1–3 equiv.) and with the coupling reagents. The resin was then filtered and washed with DMF (20 ml/g resin, 5 × 1 min) prior to the following deprotection step. For the last coupling, either Boc- or Fmoc-protected amino acids were used. In the second case, the Fmoc group of the last amino acid was removed according to *GP 3*, and the resin was washed with DMF (20 ml/g resin, 5 × 1 min) and with CH_2Cl_2 (20 ml/g resin, 5 × 1 min), and dried under h. v. for 2 h.

GP 4b. As described in *GP 4a*, but coupling with Fmoc-amino acid (5 equiv.), HATU (5 equiv.) and *Hünig* base (10 equiv.) in DMF (5 ml), and capping (Ac_2O (10 equiv.), DMAP (0.2 equiv.) in DMF for 30 min under N_2 bubbling) after every coupling step.

2.5. *Cleavage from the* Wang *Resin and Final Deprotection: General Procedure 5* (*GP 5*). *GP 5a.* The cleavage from the resin and the peptide deprotection were performed according to [48]. The dry peptide–resin was suspended in a soln. of TFA/H_2O/TIS 95 : 2.5 : 2.5 (10 ml) for 2 h. The resin was removed by filtration, washed with TFA (2×), and the org. phase was concentrated under reduced pressure. The resulting oily residue was treated with cold Et_2O, and the formed precipitate was separated. The crude peptide was dried under h. v. and stored at $-20°$ before purification.

GP 5b. As in *GP 5a*, except that a soln. of TFA/H_2O/EDT/TIS 94 : 2.5 : 2.5 : 1 (10 ml) was used for 4 h.

GP 5c. As in *GP 5a*, except that a soln. of TFA/TIS/H_2O/phenol 90 : 2.5 : 5 : 2.5 (10 ml) for 3 h was used.

2.6. *HPLC Analysis and Purification of the Peptides: General Procedure 6* (*GP 6*). *GP 6a. Macherey-Nagel* C_8 column (*Nucleosil 100-5* C_8 (250 × 4 mm)) by using a linear gradient of *A*: 0.1% TFA in H_2O and *B*: MeCN at a flow rate of 1 ml/min. Crude products were purified by prep. RP-HPLC on a *Macherey-Nagel* C_8 column (*Nucleosil 100-7* C_8 (250 × 21 mm)) using gradient of *A* and *B* at a flow rate of 18 ml/min and then lyophilized.

GP 6b. Same as in *GP 6a*, but with a gradient of *A*: 0.1% TFA in H_2O and *B*: MeOH.

GP 6c. Same as in *GP 6a*, but with an anal. *Merck Lichrospher-100* column (C_8, 100 × 4.6 mm), flow rate 1.2 ml/min.

GP 6d. Macherey-Nagel C_{18} column (*Nucleosil 100-5* C_{18} (250 × 4 mm)) by using a linear gradient of *A*: 0.1% TFA in H_2O and *B*: MeCN at a flow rate of 1 ml/min. Crude products were purified by prep. RP-HPLC on a *Macherey-Nagel* C_{18} column (*Nucleosil 100-7* C_{18} (250 × 21 mm)) using gradient of *A* and *B* at a flow rate of 12 ml/min and then lyophilized.

3. *NMR Measurements.* 3.1. *General Remarks.* NMR Spectra of peptides were acquired at 600 MHz (^{1}H)/150.9 MHz (^{13}C) with presaturation of the solvent signal; ^{1}H-NMR: 90-K data points, 128 scans, 5.6-s

acquisition time. {^{1}H}-BB-decoupled ^{13}C-NMR: 80-K data points, 20-K scans, 1.3-s acquisition time, 1-s relaxation delay, 45° excitation pulse. Processed with 1.0-Hz exponential line broadening. The following spectra were used for the resonance assignments: [^{1}H,^{1}H]-DQF-COSY, [^{1}H,^{1}H]-TOCSY, [^{13}C,^{1}H]-HSQC, [^{13}C,^{1}H]-HMBC, and [^{1}H,^{1}H]-ROESY. 2D-NMR: Solvent suppression with presat. DQF-COSY (500 MHz, CD_3OH) with pulsed field gradients (PFG) for coherence pathway selection: acquisition: $2K(t_2)\times512$ (t_1) data points. 10 scans per t_1 increment, 0.17 s acquisition time in t_2; relaxation delay 2.0 s. TPPI Quadrature detection in ω_1. Processing: zero filling and FT to $1K\times1K$ real/real data points after multiplication with a cos^2 filter t_1 and t_2. HSQC with PFG (600, 150 MHz, CD_3OH): acquisition: $2K(t_2)\times512$ (t_1) data points, 48 scans per t_1 increment. ^{13}C-GARP decoupling during t_2; 0.17-s acq. time in t_2. Processing: zero filling and FT to $1K\times1K$ real/real data points after multiplication with a cos^2 filter in t_1 and cos filter shifted by $\pi/2$ in t_2. HMBC with PFG (600, 150 MHz, CD_3OH): acquisition: delay for evolution of long-range antiphase magnetization: 50 ms. No ^{13}C decoupling, otherwise identical to parameters for HSQC. Processing: zero filling and FT to $1K\times1K$ after multiplication with a cos^2 filter in t_2 and a Gaussian filter in t_1; power spectrum in both dimensions. ROESY [49] (600 MHz, CD_3OH). Acquisition: mixing time: 300 ms. CW-Spin lock (2.7 kHz) between trim pulses, $2K(t_2)\times512$ (t_1) data points, 64 scans per t_1 increment. 0.17-s acq. time in t_2, other parameters identical to DQF-COSY. Processing: zero filling and FT to $1K\times512K$ real/real data points after multiplication by a cos^2 filter in t_2 and t_1. Baseline correction with 3rd degree polynomial in both dimensions.

3.2. *Generation of Distance Restraints* (*Tables 2–5*). Assignments and volume integration of ROESY ($t_m=300$ ms) cross-peaks were performed with the aid of SPARKY [50] cross-peaks with a signal to noise ratio ≤1, and peaks overlapping more than 30% at their basis were excluded. Distance constraints and error limits were generated from cross-peak volumes by calibration with known distances (two-spin approximation, ±20% error limits) through a python extension within SPARKY. A distance of 3 Å between backbone NH and HB, and of 1.9 Å between $H_{ax}-\alpha C$ and $H_{eq}-\alpha C$ were used as reference values. The volumes of cross-peaks involving Me groups or other groups of isochronous protons were corrected by division through the number of protons.

ROESY Spectra in CD_3OH and H_2O with mixing time of 300 ms were used. Possible contributions from spin diffusion were excluded from the generation of distance restraints.

Table 2. *NOE Distance Restraints for α,β-Heptapeptide* **3** *in CD_3OH*[a])

Residue	H-Atom	Residue	Atom	d_{NOE} [Å]	Residue	H-Atom	Residue	Atom	d_{NOE} [Å]
1	α	1	β*	3.5	6	α	6	β*	3.5
2	α	2	β	2.6	6	α	6	HN	3.2
2	α	2	γ	3.5	6	β*	6	HN	3.3
2	α	2	HN	3.0	7	β*	7	α	3.4
2	β	2	HN	2.8	7	β*	7	HN	3.5
2	γ*	2	HN	3.3	2	α	3	α	4.9
3	α	2	β*	2.9	2	α	3	HN	2.4
3	α	3	γ*	3.1	2	β	3	HN	3.2
3	α	3	HN	3.3	2	γ*	3	HN	4.5
3	β*	3	HN	3.2	3	α	4	HN	2.4
3	γ*	3	HN	3.7	3	β*	4	HN	4.1
4	α	4	β*	3.1	3	HN	4	HN	3.8
4	α	4	HN	3.2	5	α	6	HN	2.4
5	α	5	β*	2.9	6	β*	7	HN	4.2
5	α	5	HN	3.2	2	α	5	α	4.2
5	γ	5	β*	3.3	2	α	6	β*	4.9
5	HN	5	β*	3.4	3	HN	5	α	5.0
5	HN	5	γ	3.2	4	HN	2	α	3.6

[a]) * = Pseudoatom used for calculation.

Table 3. *NOE Distance Restraints for α,β-Hexapeptide* **14** *in CD_3OH*[a])

Residue	H-Atom	Residue	Atom	d_{NOE} [Å]	Residue	H-Atom	Residue	Atom	d_{NOE} [Å]
2	α	2	β*	3.0	1	β*	2	HN	3.6
2	α	2	HN	3.2	1	β*	2	HN	4.0
3	α	3	γ*	3.1	2	α	3	HN	2.6
3	α	3	HN	3.3	2	HN	3	HN	3.7
3	α	3	β_{Si}	2.7	3	β_{Si}	4	HN	3.7
3	α	3	β_{Re}	3.1	3	β_{Re}	4	HN	2.9
3	HN	3	β_{Si}	3.0	3	HN	4	β*	3.9
3	HN	3	β_{Re}	2.8	3	HN	4	β	4.4
3	HN	3	γ*	4.2	4	α_{Si}	5	HN	2.8
4	β	4	γ	2.6	4	β	5	HN	3.3
4	β	4	HN	3.0	4	HN	3	HN	4.0
4	γ	4	HN	2.9	5	α	6	HN	2.4
5	α	5	β	2.7	5	β	6	HN	3.0
5	γ*	5	HN	3.1	6	β	5	β	4.9
5	α	5	HN	3.1	6	HN	5	γ*	4.2
5	β	5	HN	2.9	2	α	5	α	3.4
6	β	6	HN	3.0	2	α	5	β	5.0
6	α*	6	HN	2.9	2	α	6	HN	4.1
6	α*	6	β	2.8	3	HN	5	α	4.0

[a]) * = Pseudoatom used for calculation.

3.3. *Simulated Annealing (SA) Structure Calculations.* Program XPLOR-NIH v2.9.7 [51]. The standard parameter and topology files of XPLOR-NIH (parallhdg.pro; topallhdg.pro) were modified to accommodate β^2- and β^3-amino acid residues. The SA calculation protocol (adopted from the torsional angle dynamics protocol of *Stein et al.* [52] included 4000 steps (0.015 ps each) of high-temp. torsional-angle dynamics at 20000 K, followed 4000 steps (0.015 ps) of slow cooling to 1000 K with torsion-angle dynamics, 4000 steps (0.003 ps) of slow cooling with cartesian dynamics to 300 K, and a final conjugate gradient minimization. The only non-bonded interactions used were *Van der Waals* repel functions. For each compound, 30 structures were calculated.

4. *Synthesis of the Peptides. 2 TFA·H-(*S*)-β³hLeu-Ile-(*R*)-β³hMet-Lys-(*R*)-β³hVal-Ala-(*S*)-β³hPhe-OH* (**1**). Fmoc-(*S*)-β^3hPhe-OH (823 mg, 2.05 mmol) was loaded onto the *Wang* resin (500 mg, 0.82 mmol/g, 100–200 mesh) according to *GP 1*. The loading was determined to be 0.58 mmol/g (71%), corresponding to 0.29 mmol of Fmoc-(*S*)-β^3hPhe-OH. After capping (*GP 2*), a portion of this Fmoc-amino acid resin (200 mg) was used for synthesis (*GP 4b*). After cleavage from the resin (*GP 5b*), the crude peptide was purified by prep. RP-HPLC (5–50% *B* in 40 min) according to *GP 6c* to yield 23 mg (18%) of **1** as a TFA salt. White solid. RP-HPLC (5–50% *B* in 40 min, t_R 23.76 min): purity >97%. ^{1}H-NMR (600 MHz, CD_3OH): 0.91 (*d*, *J* = 6.5, Me, Ile); 0.92 (*m*, 2 Me, β^3hVal); 0.96 (*m*, 2 Me, β^3hLeu); 1.19–1.54 (*m*, CH_2, Ile); 1.23 (*d*, *J* = 7.2, Me, Ala); 1.55 (*m*, CH_2, β^3hLeu); 1.72 (*m*, CH, β^3hLeu); 1.78 (*m*, CH, Ile); 1.80 (*m*, CH_2, β^3hMet); 2.04 (*s*, SMe, β^3hMet); 2.32 (*dd*, *J* = 9.4, 15.8, 1 H, CH_2, β^3hVal); 2.43 (*m*, CH_2, β^3hPhe); 2.45 (*m*, CH_2, β^3hMet); 2.49 (*dd*, *J* = 4.1, 16.0, 1 H, CH_2, β^3hVal); 2.55 (*dd*, *J* = 8.1, 16.0, 1 H, CH_2, β^3hLeu); 2.58 (*m*, CH_2, β^3hMet); 2.68 (*dd*, *J* = 4.4, 16.0, 1 H, CH_2, β^3hLeu); 2.84 (*m*, CH_2, β^3hPhe); 3.58 (*m*, CH, β^3hLeu); 4.03 (*m*, CH, β^3hVal); 4.06 (*m*, CH, Ile); 4.15 (*m*, CH, Ala); 4.26 (*m*, CH, Lys); 4.34 (*m*, CH, β^3hMet); 4.38 (*m*, CH, β^3hPhe); 7.17–7.20 (*m*, 3 arom. H, β^3hPhe); 7.22–7.27 (*m*, 2 arom. H, β^3hPhe); 7.86 (*d*, *J* = 9.1, NH, β^3hVal); 7.92 (*d*, *J* = 8.6, NH, β^3hPhe); 8.05 (*d*, *J* = 8.5, β^3hMet); 8.12 (*d*, *J* = 6.5, NH, Ala); 8.13 (*d*, *J* = 7.5, NH, Lys); 8.24 (*d*, *J* = 7.5, NH, Ile). ^{13}C-NMR (150 MHz, CD_3OH): 15.3 (SMe, Met); 16.1 (Me, Ile); 18.2 (Me, Ala); 19.6 (Me, β^3hVal); 22.7 (β^3hLeu); 23.1 (Me, Ile); 32.1 (CH, β^3hLeu); 33.4 (CH, β^3hVal); 35.4 (Ile); 35.5 (CH_2, β^3hMet); 37.7 (CH, Ile); 38.0 (CH_2, β^3hLeu); 39.1 (CH_2, β^3hMet); 39.2 (CH_2, β^3hPhe); 39.9 (CH_2, β^3hVal); 41.0 (CH_2, β^3hPhe);

Table 4. *NOE Distance Restraints for α,β-Heptapeptide* **8** *in CD_3OH*[a])

Residue	H-Atom	Residue	Atom	d_{NOE} [Å]	Residue	H-Atom	Residue	Atom	d_{NOE} [Å]
1	α*	1	β	3.2	5	α*	6	HN	3.2
1	δ	1	β	3.3	5	β	6	HN	3.5
2	HN	2	β	3.3	5	HN	4	β^*_{Si}	3.2
3	γ	3	β	2.8	5	HN	4	HN	3.3
3	γ	3	HN	3.0	6	HN	5	HN	4.0
3	α*	3	HN	3.3	6	HN	5	γ*	4.8
3	β	3	α*	3.2	7	HN	6	β^*_{Si}	3.2
4	β^*_{Si}	4	HN	3.2	7	HN	6	HN	3.4
5	β	5	α*	3.1	1	α*	3	HN	3.4
5	β	5	β*	3.0	1	β	3	HN	5.0
5	β	5	HN	3.0	1	β	4	β^*_{Si}	4.4
5	β*	5	HN	3.5	1	β	4	HN	5.2
5	α*	5	HN	3.2	1	β	5	β	5.0
6	β^*_{Si}	6	HN	3.2	1	δ	5	β	5.2
7	β	7	β*	3.2	2	β^*_{Re}	5	α_{Re}	4.0
7	HN	7	β*	3.3	7	HN	6	β^*_{Si}	3.2
7	β	7	β*	3.0	7	HN	6	HN	3.4
7	β	7	HN	3.0	1	α*	3	HN	3.4
7	HN	7	β*	3.3	3	β	6	β^*_{Si}	4.0
1	β*	2	HN	2.8	3	β	6	HN	4.1
1	β	2	HN	4.2	3	β	7	HN	5.0
2	HN	1	δ	5.2	4	β^*_{Re}	7	HN	4.7
2	HN	3	β*	3.4	5	β	7	α*	5.0
3	HN	2	HN	3.3	4	β^*_{Re}	7	α*	4.6
3	β	4	HN	3.2	5	β	7	HN	4.0
4	HN	3	γ	4.9	6	β^*_{Si}	3	γ	5.1
4	HN	3	β*	2.8	7	HN	5	α*	4.9

[a]) * = Pseudoatom used for calculations.

41.9 (CH_2, β^3hMet); 43.1 (CH_2, β^3hLeu); 48.1 (CH, β^3hMet); 48.9 (CH, β^3hLeu); 49.4 (CH, β^3hPhe); 51.2 (CH, Ala); 53.8 (CH, β^3hVal); 54.8 (CH, Lys); 60.3 (CH, Ile); 127.6 (arom. C); 129.5 (arom. C); 130.4 (arom. C); 138.6 (arom. C); 172.4 (C=O); 172.9 (C=O); 173.5 (C=O); 173.6 (C=O); 173.7 (C=O); 174.6 (C=O); 175.4 (C=O). MALDI-HR-MS: 877.5570/899.5400 (100/45, $[M+H]^+$/$[M+Na]^+$; calc. for $[C_{44}H_{77}N_8O_8S]^+$/$[C_{44}H_{76}N_8O_8SNa]^+$: 877.5580/899.5399).

*2 TFA·H-(*S*)-β³hLeu-Ile-(*S*)-β³hLys-Ala-(*R*)-β³hVal-Glu-(*S*)-β³hPhe-OH* (**2**). Fmoc-(*S*)-β^3hPhe-OH (823 mg, 2.05 mmol) was loaded onto the *Wang* resin (500 mg, 0.82 mmol/g 100–200 mesh) according to *GP 1*. The loading was determined to be 0.58 mmol/g (71%), corresponding to 0.29 mmol of Fmoc-(*S*)-β^3hPhe-OH. After capping (*GP 2*), a portion of this Fmoc-amino acid–resin (200 mg) was used for synthesis (*GP 4b*). After cleavage from the resin (*GP 5a*, 4 h), the crude peptide was purified by prep. RP-HPLC (5–50% *B* in 40 min) according to *GP 6c* to yield 33 mg (26%) of **2** as a TFA salt. White solid. RP-HPLC (5–50% *B* in 40 min, t_R 21.41 min): purity >97%. ^{1}H-NMR (600 MHz, CD_3OH): 0.92 (*m*, 2 Me, β^3hVal); 0.95 (*m*, 2 Me, β^3hLeu); 1.19 (*d*, *J* = 6.5, Me, Ile); 1.29 (*d*, *J* = 7.2, Me, Ala); 1.42 (*m*, CH_2, β^3hLys); 1.51 (*m*, CH_2, Ile); 1.56 (*m*, CH_2, β^3hLys); 1.65 (*m*, CH_2, β^3hLys); 1.73 (*m*, CH, β^3hLeu); 1.79 (*m*, CH, β^3hVal); 1.81 (*m*, CH, Ile); 1.88 (*m*, CH_2, Glu); 2.29 (*m*, CH_2, Glu); 2.36 (*dd*, *J* = 8.0, 16.1, 1 H, CH_2, β^3hVal); 2.42 (*m*, CH_2, β^3hPhe); 2.48 (*dd*, *J* = 4.3, 16.2, 1 H, CH_2, β^3hVal); 2.56 (*dd*, *J* = 8.0, 16.0, 1 H, CH_2, β^3hLeu); 2.68 (*dd*, *J* = 4.7, 16.0, 1 H, CH_2, β^3hLeu); 2.84 (*m*, CH_2, β^3hPhe); 2.92 (*m*, CH_2, β^3hLys); 3.58 (*m*, CH, β^3hLeu); 4.01 (*m*, CH, β^3hVal); 4.12 (*t*, *J* = 7.2, CH, Ile); 4.18 (*m*, CH, β^3hLys); 4.20 (*m*, CH, β^3hPhe); 4.21 (*m*, CH, Ala); 4.24 (*m*, CH, Glu); 7.17–7.21

Table 5. *NOE Distance Restraints for α,β-Octapeptide* **11** *in CD_3OH*[a])

Residue	H-Atom	Residue	Atom	d_{NOE} [Å]	Residue	H-Atom	Residue	Atom	d_{NOE} [Å]
2	HN	2	α*	3.2	2	α_{Re}	2	HN	2.7
2	β	2	γ*	3.1	2	β	3	HN	3.2
2	β	2	α_{Si}	3.0	2	HN	3	HN	4.0
2	β	2	HN	3.0	4	β	5	HN	3.5
2	γ*	2	HN	3.5	4	HN	3	HN	3.3
3	HN	3	β^*_{Si}	3.2	4	HN	3	β^*_{Si}	3.0
4	β	4	α*	3.1	5	HN	4	HN	4.0
4	β	4	HN	2.9	5	HN	4	α*	2.9
4	HN	4	α*	3.4	5	β^*_{Si}	6	HN	3.0
4	HN	4	γ*	3.5	6	HN	5	HN	3.3
6	HN	6	α_{Re}	3.0	6	α_{Re}	7	HN	2.7
6	HN	6	α_{Si}	3.9	6	β	7	HN	3.2
6	HN	6	β	2.9	7	HN	6	HN	3.7
6	β	6	α_{Si}	3.0	7	HN	8	HN	3.2
6	β	6	γ*	3.0	2	β	4	HN	4.0
6	HN	6	γ*	3.4	2	β	5	β^*_{Si}	3.8
8	β	8	α*	3.1	3	β^*_{Re}	6	α_{Re}	3.4
8	HN	8	α*	3.5	4	β	6	HN	4.0
8	β	8	γ	2.7	4	β	7	HN	4.3
8	HN	8	γ	3.0	4	β	7	β^*_{Si}	3.3
8	HN	8	β	2.9	5	HN	2	β	3.0
1	β^*_{Si}	2	HN	3.1	5	β^*_{Re}	8	α*	3.7

[a]) * = Pseudoatom used for calculations.

(*m*, 3 arom. H, β^3hPhe); 7.23–7.28 (*m*, 2 arom. H, β^3hPhe); 7.70 (*m*, *J* = 9.1, NH, β^3hVal); 8.01 (*d*, *J* = 6.0, NH, Glu); 8.03 (*d*, *J* = 7.0, NH, β^3hPhe); 8.05 (*d*, *J* = 8.5, NH, β^3hLys); 8.07 (*d*, *J* = 6.6, NH, Ala); 8.22 (*d*, *J* = 7.5, NH, Ile). ^{13}C-NMR (150 MHz, CD_3OH): 11.2 (Me, Ile); 16.1 (Me, β^3hVal); 18.5 (Me, Ala); 22.7 (Me, β^3hLeu); 23.8 (CH_2, β^3hLys); 25.5 (CH, β^3hLeu); 26.1 (CH_2, Ile); 28.2 (CH_2, β^3hLys); 28.7 (CH_2, Glu); 31.1 (CH_2, Glu); 33.4 (CH, β^3hVal); 34.5 (CH_2, β^3hLys); 37.9 (CH, Ile); 38.3 (CH_2, β^3hLeu); 38.8 (CH_2, β^3hPhe); 39.4 (CH_2, β^3hVal); 41.1 (CH_2, β^3hPhe); 41.7 (CH_2, β^3hLys); 43.1 (CH_2, β^3hLeu); 48.3 (CH, β^3hLys); 48.9 (CH, β^3hLeu); 49.5 (CH, β^3hPhe); 50.9 (CH, Ala); 53.5 (CH, β^3hVal); 54.5 (CH, Glu); 60.1 (CH, Ile); 127.5 (arom. C); 129.6 (arom. C); 130.5 (arom. C); 139.3 (arom. C); 172.5 (C=O); 172.9 (C=O); 173.2 (C=O); 173.4 (C=O); 173.6 (C=O); 174.8 (C=O); 176.7 (C=O). MALDI-HR-MS: 875.5592/897.5432 (100/13, $[M+H]^+/[M+Na]^+$; calc. for $[C_{44}H_{75}N_8O_{10}]^+$/$[C_{44}H_{74}N_8O_{10}Na]^+$: 875.5601/897.5426).

*2 TFA·H-(*S*)-β²hLeu-Ile-(*S*)-β²hMet-Lys-(*S*)-β²hVal-Ala-(*S*)-β²hPhe-OH* (**3**). Fmoc-(*S*)-β^2hPhe-OH (933 mg, 2.33 mmol) was loaded onto the *Wang* resin (500 mg, 0.93 mmol/g 100–200 mesh) according to *GP 1*. The loading was determined to be 0.67 mmol/g (72%), corresponding to 0.34 mmol of Fmoc-(*S*)-β^2hPhe-OH. After capping (*GP 2*), a portion of this Fmoc-amino acid–resin (100 mg) was used for synthesis (*GP 4b*). After cleavage from the resin (*GP 5b*), the thus obtained crude peptide was purified by prep. RP-HPLC (5–50% *B* in 40 min) according to *GP 6c* to yield 19 mg (26%) of **3** as a TFA salt. White solid. RP-HPLC (5–50% *B* in 40 min; t_R 27.66 min): purity > 95%. ^{1}H-NMR (600 MHz, CD_3OH): 0.92 (*d*, *J* = 6.5, Me, β^2hVal); 0.99 (*s*, Me, β^2hLeu); 1.02 (*d*, *J* = 6.8, Me, β^2hVal); 1.10 (*d*, *J* = 6.7, Me, Ile); 1.36 (*d*, *J* = 7.1, Me, Ala); 1.45 (*m*, CH_2, Lys); 1.55 (*m*, CH_2, Ile); 1.64 (*m*, CH_2, Lys); 1.68 (*m*, CH_2, Lys); 1.83 (*m*, CH, β^2hVal); 1.85 (*m*, 1 H, Ile); 1.90 (*m*, CH_2, β^2hMet); 2.06 (*s*, SMe, β^2hMet); 2.20 (*m*, CH, β^2hLeu); 2.30 (*m*, CH, β^2hVal); 2.46 (*m*, CH_2, β^2hMet); 2.68 (*m*, CH, β^2hMet); 2.85 (*m*, CH_2, β^2hPhe); 2.91 (*m*, CH, β^2hPhe); 2.92 (*m*, CH_2, Lys); 3.02 (*m*, 1 H, CH_2, β^2hVal); 3.12 (*m*, CH_2, β^2hMet); 3.20 (*m*, 1 H, CH_2, β^2hPhe); 3.60 (*m*, CH_2, β^2hMet); 3.61 (*m*, 1 H, CH_2, β^2hPhe); 3.70 (*m*, 1 H, CH_2, β^2hVal); 3.77 (*m*,

CH_2, β^2hLeu); 4.21 (*m*, CH, Ile); 4.30 (*m*, CH, Lys); 4.33 (*m*, CH, Ala); 7.16–7.23 (*m*, 3 arom. H, β^2hPhe); 7.24–7.28 (*m*, 2 arom. H, β^2hPhe); 8.19 (*m*, NH, β^2hPhe); 8.21 (*m*, NH, β^2hVal); 8.27 (*d*, *J* = 7.0, NH, Lys); 8.29 (*d*, *J* = 6.5, NH, Ala); 8.30 (*d*, *J* = 6.7, NH, Ile); 8.50 (*m*, NH, β^2hMet). ^{13}C-NMR (150 MHz, CD_3OH): 15.5 (SMe, β^2hMet); 15.9 (Me, Ile); 18.3 (Me, Ala); 22.5 (Me, β^2hVal); 23.3 (Me, β^2hLeu); 23.6 (CH_2, β^2hMet); 26.2 (CH_2, Ile); 29.5 (CH, β^2hVal); 29.9 (CH_2, β^2hMet); 36.9 (CH_2, β^2hPhe); 41.5 (CH_2, β^2hVal); 42.1 (CH_2, β^2hPhe); 43.1 (CH_2, β^2hMet); 46.5 (CH, β^2hMet); 49.1 (CH, β^2hPhe); 51.1 (CH, Ala); 54.6 (CH, β^2hVal); 55.4 (CH, Lys); 60.8 (CH, Ile); 127.6 (arom. C); 129.5 (arom. C); 130.1 (arom. C); 140.3 (arom. C); 174.6 (C=O); 174.7 (C=O); 175.7 (C=O); 175.8 (C=O); 176.5 (C=O); 177.0 (C=O); 177.7 (C=O). ESI-HR-MS: 877.5571/899.5201 (100/10, $[M+H]^+$/$[M+Na]^+$; calc. for $[C_{44}H_{77}N_8O_8S]^+$/$[C_{44}H_{76}N_8O_8SNa]^+$: 877.5580/899.5399).

*2 TFA · H-Asp-(*S*)-β³hPhe-Ser-(*S*)-β³hLys-Phe-(*S*)-β³hGlu-Glu-(*S*)-β³hAla-Lys-OH* (**4**). Fmoc-Lys-OH (223 mg, 0.72 mmol) was loaded onto the *Wang* resin (200 mg, 0.90 mmol/g, 100–200 mesh) according to *GP 1*. The loading was determined to be 0.64 mmol/g (72%), corresponding to 0.13 mmol of Fmoc-Lys-OH. After capping (*GP 2*), the peptide synthesis was performed according to *GP 4a*. Treatment of the peptide–resin according to *GP 5a* afforded the crude peptide **4** (100 mg). Purification of a part of the crude peptide (20 mg) by RP-HPLC (5–15% *B* in 40 min) according to *GP 6a* yielded 10 mg of **4** (calc. overall yield 50%) as TFA salt. White solid. RP-HPLC (5–10% *B* in 5 min, 10–50% *B* in 40 min, 50–95% *B* in 50 min; t_R 33.20 min): purity >95%. ^{1}H-NMR (600 MHz, CD_3OH): 1.12 (*d*, *J* = 6.8, Me, β^3hAla); 1.50 (*m*, CH_2, Lys); 1.55 (*m*, CH_2, β^3hLys); 1.62 (*m*, CH_2, β^3hLys); 1.65 (*m*, CH_2, Lys); 1.67 (*m*, CH_2, β^3hLys); 1.71 (*m*, CH_2, Lys); 1.79 (*m*, CH_2, β^3hGlu); 1.91 (*m*, CH_2, Glu); 2.20 (*m*, CH_2, β^3hGlu); 2.26 (*m*, CH_2, β^3hGlu); 2.27 (*m*, CH_2, β^3hLys); 2.30 (*m*, CH_2, Glu); 2.41 (*m*, CH_2, β^3hAla); 2.52 (*dd*, *J* = 7.5, 14.8, 1 H, CH_2, β^3hPhe); 2.80 (*m*, CH_2, Phe); 2.88 (*m*, 1 H, CH_2, β^3hPhe); 2.90 (*m*, CH_2, Lys); 2.92 (*m*, CH_2, β^3hLys); 3.77 (*d*, *J* = 5.6, CH_2, Ser); 4.05 (*m*, CH, Asp); 4.06 (*m*, CH, β^3hGlu); 4.15 (*m*, CH, β^3hLys); 4.21 (*m*, CH, Glu); 4.23 (*m*, CH, Ser); 4.24 (*m*, CH, β^3hAla); 4.43 (*m*, CH, β^3hPhe); 4.50 (*m*, CH, Phe); 7.15–7.26 (*m*, 3 arom. H, Phe); 7.16–7.25 (*m*, 3 arom. H, β^3hPhe); 7.23–7.30 (*m*, 2 arom. H, Phe); 7.26–7.29 (*m*, 2 arom. H, β^3hPhe); 7.94 (*d*, *J* = 9.0, NH, β^3hLys); 8.05 (*d*, *J* = 8.1, NH, Ser); 8.10 (*d*, *J* = 8.5, NH, β^3hGlu); 8.12 (*d*, *J* = 7.1, NH, Phe); 8.13 (*d*, *J* = 7.3, NH, Glu); 8.24 (*d*, *J* = 8.0, NH, Lys); 8.32 (*d*, *J* = 8.6, NH, β^3hPhe); 8.58 (br. *s*, NH, β^3hAla). ^{13}C-NMR (150 MHz, CD_3OH): 28.0 (CH_2, β^3hLys); 28.1 (CH_2, Lys); 28.4 (CH_2, β^3hGlu); 28.5 (CH_2, Glu); 28.8 (Me, β^3hAla); 31.3 (CH_2, β^3hGlu); 31.6 (CH_2, Glu); 32.1 (CH_2, Lys); 34.4 (CH_2, β^3hLys); 36.4 (CH_2, Asp); 38.7 (CH_2, Phe); 40.8 (CH_2, Lys); 40.9 (CH_2, β^3hLys); 41.0 (CH_2, β^3hPhe); 41.4 (CH_2, β^3hPhe); 41.6 (CH_2, β^3hLys); 41.7 (CH_2, β^3hGlu); 43.1 (CH_2, β^3hAla); 62.9 (CH_2, Ser); 127.8 (arom. C); 129.6 (arom. C); 130.3 (arom. C); 138.4 (arom. C); 127.9 (arom. C); 129.6 (arom. C); 130.4 (arom. C); 139.2 (arom. C); 169.0 (C=O); 172.3 (C=O); 173.0 (C=O); 173.4 (C=O); 173.6 (C=O); 175.4 (C=O); 176.7 (C=O); 177.2 (C=O). MALDI-HR-MS: 1156.5739 ($[M+H]^+$; calc. for $[C_{54}H_{81}N_{11}O_{17}]^+$: 1156.5812).

*2 TFA · H-Val-(*S*)-β³hAla-Leu-(*R*)-β³hVal-Ala-(*S*)-β³hLeu-Val-(*S*)-β³hAla-Leu-OH* (**5**). Fmoc-Leu-OH (318 mg, 0.90 mmol) was loaded onto the *Wang* resin (200 mg, 0.90 mmol/g, 100–200 mesh) according to *GP 1*. The loading was determined to be 0.58 mmol/g (68%), corresponding to 0.12 mmol of Fmoc-Leu-OH. After capping (*GP 2*), the peptide synthesis was performed according to *GP 4a*. Treatment of the peptide–resin according to *GP 5a* afforded the crude peptide **5** (100 mg). Purification of a part of the crude peptide (20 mg) by RP-HPLC (50% *B* in 10 min, 50–95% *B* in 45 min; t_R 30.00 min) according to *GP 6b* yielded 10 mg of **5** (calc. overall yield 50%) as TFA salt. White solid. RP-HPLC (5–10% *B* in 5 min, 10–90% *B* in 5 min, 90–99% *B* in 60 min; t_R 34.52 min): purity >95%. MALDI-HR-MS: 924.6521 ($[M+H]^+$; calc. for $[C_{46}H_{85}N_9O_{10}]^+$: 924.6419).

*2 TFA · H-Ser-(*S*)-β³hPhe-Leu-(*S*)-β³hAsp-Phe-(*R*)-β³hVal-Lys-(*S*)-β³hSer-Ala-OH* (**6**). Fmoc-Ala-OH (311 mg, 1.00 mmol) was loaded onto the *Wang* resin (200 mg, 0.90 mmol/g, 100–200 mesh) according to *GP 1*. The loading was determined to be 0.63 mmol/g (70%), corresponding to 0.12 mmol of Fmoc-Ala-OH. After capping (*GP 2*), the peptide synthesis was performed according to *GP 4a*. Treatment of the peptide–resin according to *GP 5a* afforded the crude peptide **6** (100 mg). Purification of a part of the crude peptide (20 mg) by RP-HPLC (5–15% *B* in 40 min) according to *GP 6a* yielded 10 mg of **6** (calc. overall yield 50%) as TFA salt. White solid. RP-HPLC (5–99% *B* in 30 min; 5% *B* in 20 min, 30% *B* in 30 min, 99% *B* in 30 min; t_R 28.23 min): purity >95%. ^{1}H-NMR (600 MHz, CD_3OH): 0.85 (*m*, 2 Me, β^3hVal); 0.88 (*d*, *J* = 6.5, Me, Leu); 0.92 (*d*, *J* = 6.6, Me, Leu); 1.37 (*d*, *J* = 7.4, Me, Ala); 1.41 (*m*, CH_2,

Lys); 1.50 (*m*, CH_2, Leu); 1.62 (*m*, CH, Leu); 1.65 (*m*, CH_2, Lys); 1.75 (*m*, CH_2, Lys); 1.76 (*m*, CH, β^3hVal); 2.25 (*m*, 1 H, CH_2, β^3hVal); 2.29 (*m*, CH_2, β^3hAsp); 2.43 (*dd*, *J* = 8.4, 14.5, 1 H, CH_2, β^3hPhe); 2.51 (*m*, CH_2, β^3hSer); 2.52 (*m*, 1 H, CH_2, β^3hVal); 2.54 (*m*, 1 H, CH_2, β^3hPhe); 2.64 (*dd*, *J* = 7.7, 14.3, 1 H, CH_2, β^3hAsp); 2.69 (*d*, *J* = 8.8, 16.0, 1 H, CH_2, β^3hAsp); 2.84 (*dd*, *J* = 7.0, 14.0, 1 H, CH_2, β^3hPhe); 2.93 (*dd*, *J* = 6.5, 15.2, 1 H, CH_2, β^3hPhe); 2.94 (*dd*, *J* = 6.0, 14.3, 1 H, CH_2, Phe); 2.96 (*m*, CH_2, Lys); 3.15 (*dd*, *J* = 8.0, 16.0, 1 H, CH_2, Phe); 3.52 (*m*, CH_2, β^3hSer); 3.82 (*m*, CH, Ser); 3.86 (*m*, CH_2, Ser); 4.06 (*m*, CH, β^3hVal); 4.23 (*m*, CH, β^3hSer); 4.24 (*m*, CH, β^3hAsp); 4.28 (*m*, CH, Lys); 4.32 (*m*, CH, Ala); 4.46 (*m*, CH, Phe); 4.50 (*m*, CH, β^3hPhe); 4.62 (*m*, CH, Leu); 7.16–7.23 (*m*, 3 arom. H, Phe); 7.18–7.23 (*m*, 3 arom. H, β^3hPhe); 7.24–7.29 (*m*, 2 arom. H, Phe); 7.26–7.28 (*m*, 2 arom. H, β^3hPhe); 7.95 (*d*, *J* = 8.2, NH, β^3hSer); 8.02 (*d*, *J* = 7.8, NH, Phe); 8.03 (*d*, *J* = 8.1, NH, β^3hVal); 8.10 (*d*, *J* = 7.6, NH, Lys); 8.13 (*d*, *J* = 6.7, NH, Leu); 8.23 (*d*, *J* = 7.7, NH, Ala); 8.25 (*d*, *J* = 8.8, NH, β^3hPhe); 8.49 (*d*, *J* = 7.7, NH, β^3hAsp). ^{13}C-NMR (150 MHz, CD_3OH): 17.9 (Me, Ala); 22.3 (Me, β^3hVal); 23.2 (Me, Leu); 23.6 (CH_2, Lys); 26.1 (CH, Leu); 28.1 (CH_2, Lys); 32.5 (CH_2, Lys); 37.9 (CH_2, β^3hSer); 39.2 (CH_2, β^3hVal); 39.3 (CH_2, Phe); 40.6 (CH_2, β^3hAsp); 40.7 (CH_2, β^3hAsp); 41.1 (CH_2, Lys); 41.2 (CH_2, β^3hPhe); 41.4 (CH_2, β^3hPhe); 41.7 (CH_2, Leu); 47.1 (CH, β^3hAsp); 49.8 (CH, Ala); 50.5 (CH, β^3hSer); 51.3 (CH, β^3hPhe); 54.2 (CH, β^3hVal); 54.3 (CH, Leu); 54.6 (CH, Lys); 56.5 (CH, Ser); 58.1 (CH, Phe); 62.0 (CH_2, Ser); 64.5 (CH_2, β^3hSer); 127.8 (arom. C, β^3hPhe); 127.9 (arom. C, Phe); 129.6 (arom. C, β^3hPhe); 129.7 (arom. C, Phe); 130.3 (arom. C, β^3hPhe); 130.4 (arom. C, Phe); 138.7 (arom. C, β^3hPhe); 139.3 (arom. C, Phe); 168.1 (C=O); 173.0 (C=O); 173.3 (C=O); 173.8 (C=O); 174.1 (C=O); 174.9 (C=O); 175.0 (C=O); 176.5 (C=O). MALDI-HR-MS: 1069.5928 ($[M+H]^+$; calc. for $[C_{52}H_{81}N_{10}O_{14}]^+$: 1069.5856).

*2 TFA·H-(*R*)-Ser-(*S*)-β³hPhe-(*R*)-Leu-(*R*)-β³hAsp-(*R*)-Phe-(*R*)-β³hVal-(*R*)-Lys-(*R*)-β³hSer-(*R*)-Ala-OH* (**7**). Fmoc-(*R*)-Ala-OH (311 mg, 1.00 mmol) was loaded onto the *Wang* resin (200 mg, 1.00 mmol/g, 100–200 mesh) according to *GP 1*. The loading was determined to be 0.62 mmol/g (62%), corresponding to 0.12 mmol of Fmoc-(*R*)-Ala-OH. After capping (*GP 2*), the peptide synthesis was performed according to *GP 4a*. Treatment of the peptide–resin according to *GP 5a* afforded the crude peptide **7** (100 mg). Purification of a part of the crude peptide (20 mg) by RP-HPLC (5–15% *B* in 40 min) according to *GP 6a* yielded 10 mg of **7** (calc. overall yield 50%) as TFA salt. White solid. RP-HPLC (5–99% *B* in 30 min; 5% *B* in 5 min, 30% *B* in 20 min, 99% *B* in 30 min; t_R 23.51 min): purity >95%. ^{1}H-NMR (600 MHz, CD_3OH): 0.65 (*d*, *J* = 6.6, Me, β^3hVal); 0.83 (*d*, *J* = 6.9, Me, β^3hVal); 0.93 (*d*, *J* = 6.5, Me, Leu); 0.96 (*d*, *J* = 6.6, Me, Leu); 1.34 (*d*, *J* = 7.3, Me, Ala); 1.51 (*m*, CH_2, Lys); 1.55 (*m*, CH_2, Leu); 1.62 (*m*, CH, β^3hVal); 1.65 (*m*, CH, Leu); 1.67 (*m*, CH_2, Lys); 1.82 (*m*, CH_2, Lys); 2.34 (*dd*, *J* = 7.4, 14.5, 1 H, CH_2, β^3hVal); 2.46 (*m*, CH_2, β^3hAsp); 2.50 (*m*, CH_2, β^3hSer); 2.55 (*m*, CH_2, β^3hAsp); 2.58 (*m*, CH_2, β^3hPhe); 2.72 (β^3hPhe); 3.40 (*dd*, *J* = 7.8, 12.0, 1 H, CH_2, Ser); 2.48 (*m*, 1 H, CH_2, β^3hVal); 2.92 (*m*, CH_2, Lys); 3.04 (*m*, CH_2, Phe); 3.61 (*m*, CH_2, β^3hSer); 3.63 (*m*, 1 H, CH_2, Ser); 3.80 (*m*, CH, Ser); 3.88 (*m*, CH, β^3hVal); 4.21 (*m*, CH, Lys); 4.25 (*m*, CH, β^3hSer); 4.30 (*m*, CH, Leu); 4.36 (*m*, CH, Ala); 4.50 (*m*, CH, β^3hAsp); 4.53 (*m*, CH, β^3hPhe); 4.55 (*m*, CH, Phe); 7.15–7.24 (*m*, 3 arom. H, β^3hPhe); 7.26–7.28 (*m*, 2 arom. H, β^3hPhe); 7.16–7.23 (*m*, 3 arom. H, Phe); 7.24–7.28 (*m*, 2 arom. H, Phe); 7.97 (*d*, *J* = 8.1, NH, β^3hSer); 8.02 (*d*, *J* = 8.9, NH, β^3hVal); 8.03 (d, *J* = 7.0, NH, Lys); 8.15 (*d*, *J* = 6.9, NH, Leu); 8.24 (*d*, *J* = 7.2, NH, Ala); 8.27 (*d*, *J* = 8.8, NH, β^3hAsp); 8.29 (*d*, *J* = 9.1, NH, β^3hPhe); 8.31 (*d*, *J* = 7.0, NH, Phe). ^{13}C-NMR (150 MHz, CD_3OH): 17.7 (Me, Ala); 19.3 (Me, β^3hVal); 23.3 (Me, Leu); 23.8 (CH_2, Lys); 26.1 (CH, Leu); 28.1 (CH_2, Lys); 32.2 (CH_2, Lys); 39.1 (CH_2, Phe); 40.9 (CH_2, Lys); 41.2 (CH_2, β^3hPhe); 41.5 (CH_2, β^3hPhe); 41.7 (CH_2, Leu); 45.6 (CH, β^3hAsp); 45.7 (CH_2, β^3hAsp); 49.9 (CH, Ala); 50.1 (CH, β^3hPhe); 50.7 (CH, β^3hSer); 54.1 (CH, Leu); 54.4 (CH, β^3hVal); 55.2 (CH, Lys); 56.8 (CH, Ser); 57.2 (CH, Phe); 61.9 (CH_2, Ser); 64.2 (CH_2, β^3hSer); 127.7 (arom. C, β^3hPhe); 127.9 (arom. C, Phe); 129.5 (arom. C, β^3hPhe); 129.6 (arom. C, Phe); 130.4 (arom. C, β^3hPhe); 130.5 (arom. C, Phe); 138.2 (arom. C, β^3hPhe); 139.3 (arom. C, Phe); 167.9 (C=O); 172.7 (C=O); 173.0 (C=O); 173.1 (C=O); 173.5 (C=O); 173.7 (C=O); 174.4 (C=O); 174.5 (C=O); 174.9 (C=O); 176.9 (C=O). MALDI-HR-MS: 1069.5928 ($[M+H]^+$; calc. for $[C_{52}H_{81}N_{10}O_{14}]^+$: 1069.5856).

*TFA·H-(*S*)-β³hLeu-Aib-(*R*)-β³hVal-Aib-(*S*)-β³hAla-Aib-(*S*)-β³hPhe-OH* (**8**). Fmoc-(*S*)-β^3hPhe-OH (933 mg, 2.33 mmol) was loaded onto the *Wang* resin (500 mg, 0.93 mmol/g 100–200 mesh) according to *GP 1*. The loading was determined to be 0.72 mmol/g (77%), corresponding to 0.36 mmol of Fmoc-(*S*)-

β^3hPhe-OH. After capping (*GP 2*), a portion of this Fmoc-amino acid–resin (200 mg) was used for synthesis (*GP 4b*). After cleavage from the resin (*GP 5a*, 4 h), the crude peptide (123 mg) was partially (65 mg) purified by prep. RP-HPLC (5–50% *B* in 40 min) according to *GP 6c* to yield 40 mg (60%) of **8** as a TFA salt. White solid. RP-HPLC (5–50% *B* in 40 min; t_R 22.91 min): purity >97%. ^{1}H-NMR (600 MHz, CD_3OH): 0.90 (*d*, *J*=6.6, Me, β^3hVal); 0.93 (*s*, Me, β^3hLeu); 0.94 (*s*, Me, β^3hVal); 1.18 (*d*, *J*=6.6, Me, β^3hAla); 1.33 (*s*, Me, Aib); 1.34 (*s*, Me, Aib); 1.43 (*s*, Me, Aib); 1.44 (*s*, Me, Aib); 1.46 (*s*, Me, Aib); 1.49 (*s*, Me, Aib); 1.50 (*m*, CH_2, β^3hLeu); 1.71 (*m*, CH, β^3hLeu); 1.77 (*m*, CH, β^3hVal); 2.27 (*dd*, *J*=5.0, 14.0, 1 H, CH_2, β^3hAla); 2.34 (*dd*, *J*=7.2, 14.5, 1 H, CH_2, β^3hVal); 2.41 (*m*, 1 H, CH_2, β^3hVal); 2.44 (*m*, 1 H, CH_2, β^3hAla); 2.50 (*m*, CH_2, β^3hPhe); 2.61 (*m*, CH_2, β^3hLeu); 3.58 (*m*, CH, β^3hLeu); 3.91 (*m*, CH, β^3hVal); 4.23 (*m*, CH, β^3hAla); 4.45 (*m*, CH, β^3hPhe); 7.20–7.23 (*m*, 2 arom. H), 7.24–7.28 (*m*, 3 arom. H) (β^3hPhe); 7.63 (*d*, *J*=8.8, NH, β^3hAla); 7.65 (*d*, *J*=9.4, NH, β^3hVal); 7.71 (*d*, *J*=8.8, NH, β^3hPhe); 8.06 (*s*, NH, Aib); 8.08 (*s*, NH, Aib); 8.34 (*s*, NH, Aib). ^{13}C-NMR (150 MHz, CD_3OH): 19.5 (Me, β^3hLeu); 20.1 (Me, β^3hVal); 21.2 (Me, β^3hAla); 25.1 (Me, Aib); 25.3 (Me, Aib); 25.4 (CH, β^3hLeu); 25.4 (Me, Aib); 26.2 (Me, Aib); 26.3 (Me, Aib); 26.7 (Me, Aib); 33.4 (CH, β^3hVal); 39.1 (CH_2, β^3hPhe); 39.6 (CH_2, β^3hVal); 43.8 (CH_2, β^3hAla); 44.7 (CH, β^3hAla); 48.8 (CH, β^3hLeu); 49.5 (CH, β^3hPhe); 54.5 (CH, β^3hVal); 127.5 (arom. C); 129.4 (arom. C); 130.5 (arom. C); 139.7 (arom. C); 171.8 (C=O); 173.2 (C=O); 173.7 (C=O); 175.3 (C=O); 176.1 (C=O); 176.32 (C=O); 176.39 (C=O). ESI-HR-MS: 760.4956/782.4854 (100/65, $[M+H]^+$/$[M+Na]^+$; calc. for $[C_{39}H_{66}N_7O_8]^+$/$[C_{39}H_{65}N_7O_8Na]^+$: 760.4967/782.4787).

*TFA·H-(*S*)-β²hLeu-Aib-(*S*)-β²hVal-Aib-(*S*)-β²hAla-Aib-(*S*)-β²hPhe-OH* (**9**). Fmoc-(*S*)-β^2hPhe-OH (933 mg, 2.33 mmol) was loaded onto the *Wang* resin (500 mg, 0.93 mmol/g, 100–200 mesh) according to *GP 1*. The loading was determined to be 0.67 mmol/g (72%), corresponding to 0.34 mmol of Fmoc-(*S*)-β^2hPhe-OH. After capping (*GP 2*), a portion of this Fmoc-amino acid–resin (150 mg) was used for synthesis (*GP 4b*). After cleavage from the resin (*GP 5b*), the crude peptide was purified by prep. RP-HPLC (5–50% *B* in 40 min) according to *GP 6b* to yield 7 mg (8%) of **9** as a TFA salt. White solid. RP-HPLC (5–50% *B* in 40 min; t_R 25.66 min): purity >95%. ESI-HR-MS: 760.4950/782.4790 (100/65, $[M+H]^+$/$[M+Na]^+$; calc. for $[C_{39}H_{66}N_7O_8]^+$/$[C_{39}H_{65}N_7O_8Na]^+$: 760.4967/782.4787).

*2 TFA·H-Aib-(*R*)-β³hSer-Aib-(*S*)-β³hPhe-Aib-(*S*)-β³hLys-Aib-(*R*)-β³hVal-OH* (**10**). Fmoc-(*R*)-β^3hVal-OH (317 mg, 0.90 mmol) was loaded onto the *Wang* resin (200 mg, 0.90 mmol/g, 100–200 mesh) according to *GP 1*. The loading was determined to be 0.63 mmol/g (70%), corresponding to 0.13 mmol of Fmoc-(*R*)-β^3hVal-OH. After capping (*GP 2*), the peptide synthesis was performed according to *GP 4a*. Treatment of the peptide–resin according to *GP 5a* afforded the crude peptide **10** (100 mg). Purification of a part of the crude peptide (20 mg) by RP-HPLC (5–15% *B* in 40 min) according to *GP 6a* yielded 10 mg of **10** (calc. overall yield 50%) as TFA salt. White solid. RP-HPLC (10% *B* in 5 min, 10–60% *B* in 45 min, 60–95% *B* in 55 min; t_R 33.12 min): purity >95%. ^{1}H-NMR (600 MHz, CD_3OH): 0.90 (*d*, *J*=6.8, Me, β^3hVal); 0.92 (*d*, *J*=7.0, Me, β^3hVal); 1.15 (*s*, 2 Me, Aib); 1.17 (*s*, Me, Aib); 1.33 (*s*, Me, Aib); 1.42 (*m*, CH_2, β^3hLys); 1.43 (*s*, 2 Me, Aib); 1.46 (*s*, 2 Me, Aib); 1.57 (*m*, 1 H, CH_2, β^3hLys); 1.61 (*m*, CH_2, β^3hLys); 1.66 (*m*, 1 H, CH_2, β^3hLys); 1.85 (*m*, CH, β^3hVal); 2.27 (*dd*, *J*=4.7, 14.6, 1 H, CH_2, β^3hLys); 2.37 (*dd*, *J*=4.0, 14.0, 1 H, CH_2, β^3hPhe); 2.47 (*m*, 1 H, CH_2, β^3hLys); 2.48 (*dd*, *J*=8.0, 15.8, 1 H, CH_2, β^3hSer); 2.52 (*dd*, *J*=6.4, 14.4, 1 H, CH_2, β^3hSer); 2.53 (*m*, CH_2, β^3hPhe); 3.61 (*m*, CH_2, β^3hSer); 4.05 (*m*, CH, β^3hVal); 4.18 (*m*, CH, β^3hLys); 4.30 (*m*, CH, β^3hSer); 4.51 (*m*, CH, β^3hPhe); 7.16–7.23 (*m*, 3 arom. H, β^3hPhe); 7.24–7.28 (*m*, 2 arom. H, β^3hPhe); 7.58 (*d*, *J*=9.2, NH, β^3hPhe); 7.68 (*d*, *J*=8.9, NH, β^3hVal); 7.82 (*d*, *J*=9.1, NH, β^3hLys); 7.99 (*d*, *J*=8.1, NH, β^3hSer); 8.03 (*s*, NH, Aib); 8.06 (*s*, NH, Aib); 8.16 (*s*, NH, Aib). ^{13}C-NMR (150 MHz, CD_3OH): 19.6 (Me, β^3hVal); 23.8 (CH_2, β^3hLys); 24.7 (Me, Aib); 24.8 (Me, Aib); 24.9 (Me, Aib); 25.4 (Me, Aib); 26.3 (Me, Aib); 26.6 (Me, Aib); 27.0 (Me, Aib); 27.8 (CH_2, β^3hLys); 34.9 (CH_2, β^3hLys); 37.8 (CH_2, β^3hVal); 40.1 (CH_2, β^3hPhe); 43.1 (CH_2, β^3hPhe); 43.6 (CH_2, β^3hLys); 47.9 (β^3hLys); 49.9 (CH, β^3hPhe); 50.1 (CH_2, β^3hSer); 50.9 (CH, β^3hSer); 53.5 (CH, β^3hVal); 60.4 (CH_2, β^3hSer); 127.6 (arom. C); 129.4 (arom. C); 130.4 (arom. C); 139.5 (arom. C); 172.9 (C=O); 173.1 (C=O); 173.2 (C=O); 175.7 (C=O); 176.4 (C=O); 176.6 (C=O); 177.8 (C=O). MALDI-HR-MS: 875.5539 ($[M+H]^+$; calc. for $[C_{43}H_{73}N_9O_{10}]^+$: 876.5553).

*2 TFA·H-Aib-(*S*)-β³hTyr-Aib-(*S*)-β³hLys-Aib-(*S*)-β³hAsp-Aib-(*R*)-β³hVal-OH* (**11**). Fmoc-(*R*)-β^3hVal-OH (318 mg, 0.90 mmol) was loaded onto the *Wang* resin (200 mg, 0.90 mmol/g, 100–200

mesh) according to *GP 1*. The loading was determined to be 0.61 mmol/g (68%), corresponding to 0.13 mmol of Fmoc-(*R*)-β^3hVal-OH. After capping (*GP 2*), the peptide synthesis was performed according to *GP 4a*. Treatment of the peptide–resin according to *GP 5a* afforded the crude peptide **11** (100 mg). Purification of a part of the crude peptide (20 mg) by RP-HPLC (5–15% *B* in 40 min) according to *GP 6a* yielded 10 mg of **11** (calc. overall yield 50%) as TFA salt. White solid. RP-HPLC (10% *B* in 5 min, 10–60% *B* in 45 min, 60–95% *B* in 50 min; t_R 33.12 min): purity >95%. ^{1}H-NMR (600 MHz, CD_3OH): 0.91 (*m*, Me, β^3hVal); 1.30 (*s*, Me, Aib); 1.41 (*s*, Me, Aib); 1.44 (*s*, Me, Aib); 1.48 (*s*, Me, Aib); 1.50 (*s*, Me, Aib); 1.58 (*m*, CH_2, β^3hLys); 1.61 (*m*, CH_2, β^3hLys); 1.68 (*m*, CH_2, β^3hLys); 1.80 (*m*, CH, β^3hVal); 2.35 (*m*, CH_2, β^3hLys); 2.40 (*dd*, *J*=4.8, 14.4, 1 H, CH_2, β^3hAsp); 2.42 (*m*, CH_2, β^3hTyr); 2.53 (*m*, 1 H, CH_2, β^3hAsp); 2.60 (*m*, 1 H, CH_2, β^3hTyr); 2.62 (*m*, CH_2, β^3hAsp); 2.80 (*dd*, *J*=5.0, 14.0, 1 H, CH_2, β^3hTyr); 2.91 (*m*, CH_2, β^3hLys); 4.02 (*m*, CH, β^3hVal); 4.10 (*m*, CH, β^3hLys); 4.40 (*m*, CH, β^3hTyr); 4.51 (*m*, CH, β^3hAsp); 6.68 (*m*, 2 arom. H, β^3hTyr); 7.12 (*m*, 2 arom. H, β^3hTyr); 7.59 (*d*, *J*=9.0, NH, β^3hVal); 7.6 (br. *s*, NH, β^3hLys); 7.81 (*d*, *J*=8.8, NH, β^3hTyr); 7.85 (*d*, *J*=8.6, NH, β^3hAsp); 8.02 (*s*, NH, Aib); 8.12 (*s*, NH, Aib); 8.14 (*s*, NH, Aib). ^{13}C-NMR (150 MHz, CD_3OH): 19.5 (Me, β^3hVal); 24.0 (Me, Aib); 24.6 (Me, Aib); 25.1 (Me, Aib); 25.2 (Me, Aib); 25.4 (Me, Aib); 26.1 (Me, Aib); 26.3 (Me, Aib); 26.5 (Me, Aib); 27.8 (CH_2, β^3hLys); 34.8 (CH_2, β^3hLys); 37.6 (CH_2, β^3hVal); 40.5 (CH_2, β^3hTyr); 40.9 (CH_2, β^3hLys); 42.3 (CH_2, β^3hTyr); 42.8 (CH_2, β^3hLys); 45.6 (CH, β^3hAsp); 48.1 (CH, β^3hLys); 50.6 (CH, β^3hTyr); 53.5 (CH, β^3hVal); 116.6 (arom. C); 130.2 (arom. C); 131.4 (arom. C); 157.4 (arom. C); 172.6 (C=O); 172.7 (C=O); 172.7 (C=O); 173.2 (C=O); 174.8 (C=O); 175.6 (C=O); 176.4 (C=O); 176.5 (C=O); 176.6 (C=O). MALDI-HR-MS: 920.5469 ($[M+H]^+$; calc. for $[C_{44}H_{73}N_9O_{12}]^+$: 920.5452).

*2 TFA·H-(*S*)-β^3hLeu-Ile-(*S*)-β^3hLys-Aib-(*R*)-β^3hVal-Glu-(*S*)-β^3hPhe-OH* (**12**). Fmoc-(*S*)-β^3hPhe-OH (823 mg, 2.05 mmol) was loaded onto the *Wang* resin (500 mg, 0.82 mmol/g 100–200 mesh) according to *GP 1*. The loading was determined to be 0.58 mmol/g (71%), corresponding to 0.29 mmol of Fmoc-(*S*)-β^3hPhe-OH. After capping (*GP 2*), a portion of this Fmoc-amino acid–resin (200 mg) was used for synthesis (*GP 4b*). After cleavage from the resin (*GP 5a*, 4 h), the thus obtained crude peptide was purified by prep. RP-HPLC (5–50% *B* in 40 min) according to *GP 6c* to yield 85 mg (56%) of **12** as a TFA salt. White solid. RP-HPLC (5–50% *B* in 40 min; t_R 26.99 min): purity >97%. ^{1}H-NMR (600 MHz, CD_3OH): 0.92 (*d*, *J*=6.5, Me, β^3hVal); 0.94 (*m*, Me, β^3hVal); 0.95 (*d*, *J*=7.5, Me, Ile); 0.96–0.98 (m, 2 Me, β^3hLeu); 1.22 (*m*, CH_2, Ile); 1.42 (*m*, CH_2, β^3hLys); 1.43 (*s*, Me, Aib); 1.45 (*s*, Me, Aib); 1.61 (*m*, CH_2, β^3hLys); 1.63 (*m*, CH_2, β^3hLys); 1.70 (*m*, CH, β^3hLeu); 1.72 (*m*, CH_2, β^3hLeu); 1.8 (*m*, CH, Ile); 1.82 (*m*, CH, β^3hVal); 1.87 (*m*, 1 H, CH_2, Glu); 1.94 (*m*, 1 H, CH_2, Glu); 2.29 (*m*, CH_2, Glu); 2.34 (*dd*, *J*=6.0, 15.5, 1 H, CH_2, β^3hLys); 2.43 (*m*, CH_2, β^3hVal); 2.44 (*dd*, *J*=4.5, 16.5, 1 H, CH_2, β^3hLys); 2.49 (*m*, CH_2, β^3hPhe); 2.58 (*dd*, *J*=5.0, 16.0, 1 H, CH_2, β^3hLeu); 2.69 (*dd*, *J*=7.6, 15.6, 1 H, CH_2, β^3hLeu); 2.89 (*m*, CH_2, β^3hPhe); 2.92 (*m*, CH_2, β^3hLys); 3.58 (*m*, CH, β^3hLeu); 4.15 (*t*, *J*=7.4, CH, Ile); 4.22 (*m*, CH, Glu); 4.40 (*m*, CH, β^3hPhe); 7.18–7.23 (*m*, 3 arom. H); 7.26–7.28 (*m*, 2 arom. H, β^3hPhe); 7.50 (*d*, *J*=9.5, NH, β^3hVal); 7.99 (*d*, *J*=8.5, NH, β^3hPhe); 8.02 (*d*, *J*=8.3, NH, β^3hLys); 8.03 (*d*, *J*=7.0, NH, Glu); 8.07 (*s*, NH, Aib); 8.24 (*m*, *J*=7.5, NH, Ile). ^{13}C-NMR (150 MHz, CD_3OH): 16.2 (Me, Ile); 19.3 (Me, β^3hLeu); 25.4 (CH, β^3hLeu); 25.5 (Me, Aib); 26.10 (CH_2, Ile); 26.15 (CH_2, β^3hLeu); 26.20 (Me, Aib); 26.23 (Me, β^3hVal); 28.6 (CH_2, Glu); 31.4 (CH_2, Glu); 33.4 (CH, β^3hVal); 37.9 (CH, Ile); 38.3 (CH_2, β^3hLeu); 39.2 (CH_2, β^3hLeu); 39.4 (CH_2, β^3hVal); 41.2 (CH_2, β^3hLeu); 49.0 (CH, β^3hLeu); 49.4 (CH, β^3hPhe); 54.1 (CH, β^3hVal); 55.1 (CH, Glu); 60.1 (CH, Ile); 127.6 (arom. C); 129.4 (arom. C); 130.5 (arom. C); 139.3 (arom. C); 172.5 (C=O); 172.7 (C=O); 173.39 (C=O); 173.43 (C=O); 174.1 (C=O); 175.0 (C=O); 176.5 (C=O); 176.8 (C=O). MALDI-HR-MS: 889.5748/911.5617 (100/58, $[M+H]^+$/$[M+Na]^+$; calc. for $[C_{45}H_{77}N_8O_{10}]^+$/$[C_{45}H_{76}N_8O_{10}Na]^+$: 889.5757/911.5577).

*2 TFA·H-(*S*)-β^2hLeu-Val-(*S*,*S*)-$\beta^{2,3}$Ala(αMe)-Lys-(*S*,*S*)-$\beta^{2,3}$Ala(αMe)-Val-(*S*)-β^3hPhe-OH* (**13**). Fmoc-(*S*)-β^3hPhe-OH (933 mg, 2.33 mmol) was loaded onto the *Wang* resin (500 mg, 0.93 mmol/g 100–200 mesh) according to *GP 1*. The loading was determined to be 0.72 mmol/g (77%), corresponding to 0.36 mmol of Fmoc-(*S*)-β^3hPhe-OH. After capping (*GP 2*), a portion of this Fmoc-amino acid–resin (200 mg) was used for synthesis (*GP 4b*). After cleavage from the resin (*GP 5a*, 4 h), the crude peptide was purified by prep. RP-HPLC (5–50% *B* in 40 min) according to *GP 6c* to yield 59 mg (39%) of **13** as a TFA salt. White solid. RP-HPLC (5–50% *B* in 40 min; t_R 28.59 min): purity >95%. ^{1}H-NMR (600 MHz, CD_3OH): 0.87 (*d*, *J*=6.7, Me, Val); 0.89 (*d*, *J*=6.6, Me, Val); 0.94 (*m*, 2 Me, β^2hLeu); 0.96 (*m*, 2 Me, Val); 1.02 (*d*, *J*=6.8, Me, $\beta^{2,3}$hAla(αMe)); 1.12 (*d*, *J*=7.2, Me, $\beta^{2,3}$hAla(αMe)); 1.13 (*d*, *J*=7.2, Me,

$\beta^{2,3}$hAla(αMe)); 1.14 (*d*, *J* = 6.8, Me, $\beta^{2,3}$hAla(αMe)); 1.29 (*m*, CH_2, β^2hLeu); 1.42 (*m*, CH_2, Lys); 1.62 (*m*, CH, β^2hLeu); 1.63 (*m*, 1 H, CH_2, Lys); 1.65 (*m*, CH_2, Lys); 1.84 (*m*, 1 H, CH_2, Lys); 1.99 (*m*, CH, Val); 2.15 (*m*, CH, Val); 2.40 (*dd*, *J* = 7.8, 15.8, 1 H, CH_2, β^3hPhe); 2.47 (*dd*, *J* = 5.2, 16.2, 1 H, CH_2, β^3hPhe); 2.56 (*m*, CH, $\beta^{2,3}$hAla(αMe)); 2.63 (*m*, CH, $\beta^{2,3}$hAla(αMe)); 2.80 (*dd*, *J* = 7.4, 14.0, 1 H, CH_2, β^3hPhe); 2.87 (*dd*, *J* = 6.7, 12.4, 1 H, CH_2, β^3hPhe); 2.89 (*m*, 1 H, CH_2, β^2hLeu); 2.90 (*m*, CH, β^2hLeu); 2.92 (*m*, CH_2, Lys); 3.15 (*m*, 1 H, CH_2, β^2hLeu); 4.01 (*m*, CH, $\beta^{2,3}$hAla(αMe)); 4.10 (*m*, CH, $\beta^{2,3}$hAla(αMe)); 4.12 (*m*, CH, Val); 4.15 (*m*, CH, Val); 4.31 (*m*, CH, Lys); 4.46 (*m*, CH, β^3hPhe); 7.16–7.23 (*m*, 3 arom. H, β^3hPhe); 7.25–7.28 (*m*, 2 arom. H, β^3hPhe); 7.98 (*d*, *J* = 8.8, NH, $\beta^{2,3}$hAla(αMe)); 7.94 (*d*, *J* = 8.5, NH, $\beta^{2,3}$hAla(αMe)); 8.0 (*d*, *J* = 8.6, NH, Val); 8.02 (*d*, *J* = 8.8, NH, β^3hPhe); 8.19 (*d*, *J* = 7.6, NH, Lys); 8.23 (*d*, *J* = 7.5, NH, Val). ^{13}C-NMR (150 MHz, CD_3OH): 18.9 ($\beta^{2,3}$hAla(αMe)); 19.1 (Me, β^2hLeu); 19.4 (Me, $\beta^{2,3}$hAla(αMe)); 20.1 (Me, Val); 23.3 (Me, Val); 23.8 (CH_2, Lys); 27.1 (CH_2, β^2hLeu); 28.3 (CH_2, Lys); 31.4 (CH, Val); 32.5 (CH_2, Lys); 38.8 (CH_2, β^3hPhe); 40.7 (CH, β^2hLeu); 40.8 (CH_2, Lys); 41.3 (CH_2, β^3hPhe); 43.2 (CH_2, β^2hLeu); 45.0 (CH, $\beta^{2,3}$hAla(αMe)); 46.2 (CH, $\beta^{2,3}$hAla(αMe)); 49.3 (CH, $\beta^{2,3}$hAla(αMe)); 49.4 (CH, $\beta^{2,3}$hAla(αMe)); 49.5 (CH, β^3hPhe); 54.4 (CH, Lys); 60.4 (CH, Val); 61.3 (CH, Val); 127.7 (arom. C); 129.6 (arom. C); 130.4 (arom. C); 139.3 (arom. C); 173.1 (C=O); 173.3 (C=O); 173.5 (C=O); 174.8 (C=O); 175.9 (C=O); 177.2 (C=O); 177.4 (C=O). ESI-HR-MS: 831.5711/853.5393 (100/6, $[M+H]^+/[M+Na]^+$; calc. for $[C_{43}H_{75}N_8O_8]^+/[C_{43}H_{74}N_8O_8Na]^+$: 831.5702/853.5522).

*2 TFA · H-(*R*)-β²hPhe-His-(*S*)-β²hLeu-(*R*)-β³hVal-Ile-(*S*)-β³hTyr-OH* (**14**). Fmoc-(*S*)-β^3hTyr(O^{t}Bu)-OH (705 mg, 1.49 mmol) was loaded onto the *Wang* resin (298 mg, 1.00 mmol/g 100–200 mesh) according to *GP 1*. The loading was determined to be 0.76 mmol/g (76%), corresponding to 0.23 mmol of Fmoc-(*S*)-β^3hTyr(O^{t}Bu)-OH. After capping (*GP 2*), the peptide was synthesized according to *GP 4a*. After cleavage from the resin (*GP 5c*), the crude peptide was purified by prep. RP-HPLC according to *GP 6d* (5–10% *B* in 5 min, 10–50% *B* in 35 min, 50–99% *B* in 10 min) to yield 94 mg (54%) of **14** as a TFA salt. White solid. RP-HPLC (5–10% *B* in 5 min, 10–50% *B* in 35 min, 50–99% *B* in 10 min; t_R 34.24 min): purity >95%. ^{1}H-NMR (600 MHz, CD_3OH): 0.89 (*d*, *J* = 7.2, Me, Ile); 0.90 (*d*, *J* = 6.4, 2 Me, β^3hLeu); 0.93 (*m*, Me, β^3hVal); 1.12 (*m*, CH_2, Ile); 1.23–1.55 (*m*, CH_2, β^2hLeu); 1.57 (*m*, CH, β^2hLeu); 1.70 (*m*, CH, Ile); 1.81 (*m*, CH, β^3hVal); 2.39 (*dd*, *J* = 8.0, 16.0, 1 H, CH_2, β^3hTyr); 2.47 (*m*, 1 H, CH_2, β^3hTyr); 2.48–2.56 (*m*, CH_2, β^3hVal); 2.57 (*m*, CH, β^2hLeu); 2.68–2.74 (*m*, CH_2, His); 2.77 (*m*, CH_2, β^2hPhe); 2.90–3.01 (*m*, CH_2, His); 3.01 (*m*, 1 H, CH_2, β^2hPhe); 3.20 (*m*, 1 H, CH_2, β^2hPhe); 3.23–3.43 (*m*, CH_2, β^2hLeu); 4.10 (*t*, *J* = 7.6, CH, Ile); 4.39 (*m*, CH, β^3hTyr); 4.65 (*m*, CH, His); 7.10 (*m*, 2 H, β^3hTyr); 7.17 (*m*, 2 H, β^3hTyr); 7.21–7.24 (*m*, 2 H, β^2hPhe); 7.25–7.29 (*m*, 3 H); (β^2hPhe); 7.84 (*d*, *J* = 8.7, NH, β^3hVal); 7.90 (*d*, *J* = 8.5, NH, β^3hTyr); 8.01 (*d*, *J* = 8.3, NH, Ile); 8.10 (*t*, *J* = 6.0, NH, β^2hLeu); 8.5 (*d*, *J* = 7.6, NH, His). ^{13}C-NMR (150 MHz, CD_3OH): 15.9 (Me, Ile); 18.6 (Me, β^3hVal); 26.04 (CH_2, Ile); 26.04 (CH, Ile); 27.1 (Me, β^2hLeu); 27.2 (CH, β^2hLeu); 28.1 (CH_2, His); 33.4 (CH, β^3hVal); 37.5 (CH_2, β^2hPhe); 37.9 (CH_2, β^3hVal); 38.9 (CH_2, β^3hTyr); 39.9 (CH_2, β^2hLeu); 40.5 (CH_2, β^3hTyr); 43.0 (CH_2, β^2hLeu); 43.3 (CH_2, β^2hPhe); 49.8 (CH, β^3hTyr); 53.5 (CH, β^3hVal); 53.9 (CH, His); 60.1 (CH, Ile); 127.5 (arom. C); 129.7 (arom. C); 130.2 (arom. C); 130.3 (arom. C); 131.5 (arom. C); 140.5 (arom. C); 172.4 (C=O); 173.2 (C=O); 174.2 (C=O); 174.4 (C=O); 175.2 (C=O); 176.7 (C=O). ESI-HR-MS: 847.5061/869.4842 (100/12, $[M+H]^+/[M+Na]^+$; calc. for $[C_{45}H_{67}N_8O_8]^+/[C_{45}H_{66}N_8O_8Na]^+$: 847.5076/869.4896).

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