170. Probing the Helical Secondary Structure of Short-Chain β-Peptides

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Dedicated to Professor *Teruaki Mukaiyama*, a dear friend and revered colleague on the occasion of his 70th birthday

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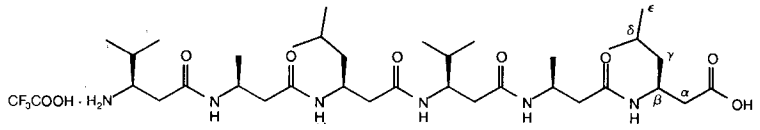
Structural prerequisites for the stability of the 3_1 helix of β-peptides can be defined from inspection of models (*Figs. 1* and *2*): lateral non-H-substituents in 2- and 3-position on the 3-amino-acid residues of the helix are allowed, axial ones are forbidden. To be able to test this prediction, we synthesized a series of heptapeptide derivatives Boc-(β-HVal-β-HAla-β-HLeu-Xaa-β-HVal-β-HAla-β-HLeu)-OMe **13**–**22** (Xaa = α- or β-amino-acid residue) and a β-depsipeptide **25** with a central (*S*)-3-hydroxybutanoic-acid residue (Xaa = –OCH(Me)CH_2C(O)–) (*Schemes 1–3*). Detailed NMR analysis (DQF-COSY, HSQC, HMBC, ROESY, and TOCSY experiments) in methanol solution of the β-hexapeptide H(-β-HVal-β-HAla-β-HLeu)$_2$-OH (**1**) and of the β-heptapeptide H-β-HVal-β-HAla-β-HLeu-(*S*,*S*)-β-HAla(αMe)-β-HVal-β-HAla-β-HLeu-OH (**22**), with a central (2*S*,3*S*)-3-amino-2-methylbutanoic-acid residue, confirm the helical structure of such β-peptides (previously discovered in pyridine solution) (*Fig. 3* and *Tables 1–5*). The CD spectra of helical β-peptides, the residues of which were prepared by (retentive) *Arndt-Eistert* homologation of the (S)- or L-α-amino acids, show a trough at 215 nm. Thus, this characteristic pattern of the CD spectra was taken as an indicator for the presence of a helix in methanol solutions of compounds **13**–**22** and **25** (including partially and fully deprotected forms) (*Figs. 4–6*). The results fully confirm predicted structural effects: incorporation of a single 'wrong' residue ((*R*)-β-HAla, β-HAib, (*R*,*S*)-β-HAla(αMe), or *N*-Me-β-HAla) in the central position of the β-heptapeptide derivatives **A** (see **17**, **18**, **20**, or **21**, resp.) causes the CD minimum to disappear. Also, the β-heptadepsipetide **25** (missing H-bond) and the β-heptapeptide analogs with a single α-amino-acid moiety in the middle (**13** and **14**) are not helical, according to this analysis. An interesting case is the heptapeptide **15** with the central achiral, unsubstituted 3-aminopropanoic-acid moiety: helical conformation appears to depend upon the presence or absence of terminal protection and upon the solvent (MeOH *vs.* MeOH/H_2O).

1. Introduction and Goal. – In a previous paper, we have reported the surprising formation of secondary structures in short-chain β-peptides consisting of β-amino acids (homologated α-amino acids, β-HXaa) exclusively [1]. Thus, the β-hexapeptide H(-β-HVal-β-HAla-β-HLeu)$_2$-OH (**1**) was shown by NMR spectroscopy to exist as a left-handed (or *M*) 3_1 helix of 5-Å pitch in C_5D_5N ((D$_5$)pyridine) (see below, *Fig. 1*). Further-

[1]) Part of the master's thesis of *P. E. C.*, University of Pavia, Italy, carried out at ETH-Zürich 1995/1996 as part of the ERASMUS cooperation programme.

[2]) Postdoctoral research fellow, ETH-Zürich, 1995/1996, financed by a 'Bundesstipendium der Eidgenössischen Stipendienkommission für Ausländische Studierende'. New address: c/o Prof. Dr. *J. H. van Boom*, Gorlaeus Laboratoria, Rijks Universiteit Leiden, Einsteinweg 55, Postbus 9502, NL–2300 RA Leiden.

more, a distinct CD pattern (minimum at 216 nm, maximum at 198 nm) was found for the β-hexapeptide when measured in MeOH; the CD spectrum was hardly dependent upon the presence of terminal protecting groups, upon the solvent, and upon the concentration.



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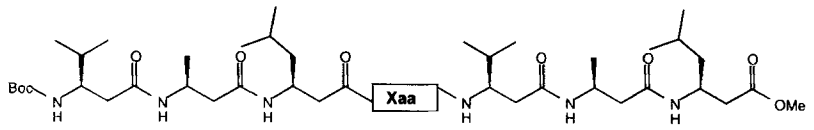

A Xaa = α- or β-amino acid with various substitution patterns

The following question immediately arose from our previous work: what structural changes in the β-peptide sequence will stabilize or destabilize the helical secondary structure? To obtain this kind of information we, first of all, had to synthesize analogs, and we needed to confirm that the CD pattern [2] [3] observed in MeOH is indeed signalling the presence of a helix, so that we could readily determine the effect of stuctural modifications.

Inspection of the model showing a section of a β-peptide helix, generated from the data of the NMR solution structure of **1** leads to the following expectations (see *Figs. 1* and *2*):

a) In the β-hexapeptide **1**, identical side chains are located above each other on the 3_1 helix; incorporation of a seventh β-amino acid in the middle of the sequence (see **A**) will shift the relative position of side chains by 120°, so that different juxtapositions result (*Figs. 1* and *2*,*a* and *b*); the two substituents of β-amino acids *i* and (*i* + 3) are pointing in parallel directions, somewhat similar to those on a β II turn of an α-peptide; in a way, there is a 10-membered ring-type arrangement of the atoms H–C–N–C–O···H–N–C–C–H having a resemblance with the 10-membered ring which is part of β turns (*Fig. 2*,*c*); as shown in *Fig. 2*,*d*, substituents on β-amino acids *i* and (*i* + 2) point away from each other and are far more separate; finally, two substituents on neighboring amino acids protrude from the helix body with an angle of *ca.* 120° (*Fig. 2*,*a* and *b*)[3]), and the section of the helix containing two β-amino-acid residues *i* and (*i* + 1) has, again, a certain resemblance with a β turn (*Fig. 2*,*e*). These structural features of the helix will be confirmed by the NMR solution structure determination of a β-heptapeptide **A**.

b) There is a lateral and an axial position on each tetragonal C-atom along the backbone of the helix; a lateral bond is approximately perpendicular, an axial bond approximately parallel to the helix axis; there may be a substituent other than H in the

[3]) Taking the amide plane as a reference, the angles between substituents on neighboring amino acids in β I, β I′, β II, and β II′ turns are +60, −60, +20, and −20°, respectively [4] [5].

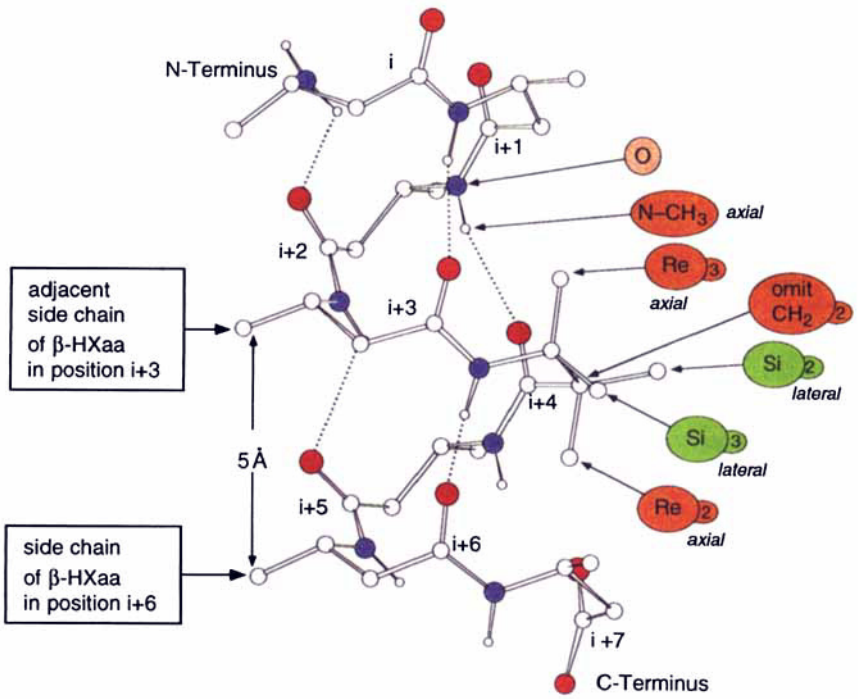

Fig. 1. *Side view of a β-peptide (*M*) 3_1 helix with 5-Å pitch, indicating neighborhoods as well as allowed and forbidden positions of substituents* (MacMoMo presentation; model constructed from data set of the NMR-structure determination of **1** as described [1]). The numbers *i* (N-terminus), $(i + 1)$,...$(i + 7)$ (C-terminus) refer to the position of the *β*-amino-acid residues. The numbers 1, 2, and 3 on the colored labels refer to C(1), C(2), and C(3), resp., of a *β*-amino-acid residue. Color code: carbonyl O-atoms *red*, N-atoms *blue*, labels for sterically forbidden axial substituent positions and for other unfavorable structural modifications *orange*, labels for sterically allowed lateral substituent positions *green*. A lateral bond and substituent on C(3) of each *β*-amino acid is indicated, except on amino acid $(i + 4)$, where all four possible backbone-substituent positions (two lateral and two axial) are pictured (for more details, see text). The topicities *Re* and *Si* are assigned for *CIP* priority sequences C(O) > C(N) > H in the 2-position and N > C(2) > C(4) in the 3-position of the 3-amino-carboxylic-acid moiety (the latter sequence is not correct for *β*-HVal!).

lateral (*Si*) but not in the axial (*Re*)[4]) positions of C(2) and C(3) in a *β*-amino-acid residue of the *M* helix; thus, while incorporation of a geminally disubstituted amino-acid residue stabilizes the helix of α-peptides [6–10], it is expected to prevent formation of a helical secondary structure in a *β*-peptide (*Fig. 1*).

c) The helix is held together by C=O···HN *H*-bonds; *N*-Me substitution will not only lead to loss of a H-bond, but, more importantly, (and as in α-peptides [11]), prevent the helix structure from being formed for steric reasons: on the N-atoms, like on the axial positions of C(2) and C(3) of the amino-acid residues, there is no room for a substituent other than a H-atom in the *β*-peptide helix.

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[4]) Referring to C(3) of the *β*-amino-acid residues, these topicities are correct only if the CH_2 group in the 2-position has *CIP* priority over C(4); see also legend of *Fig. 1*.

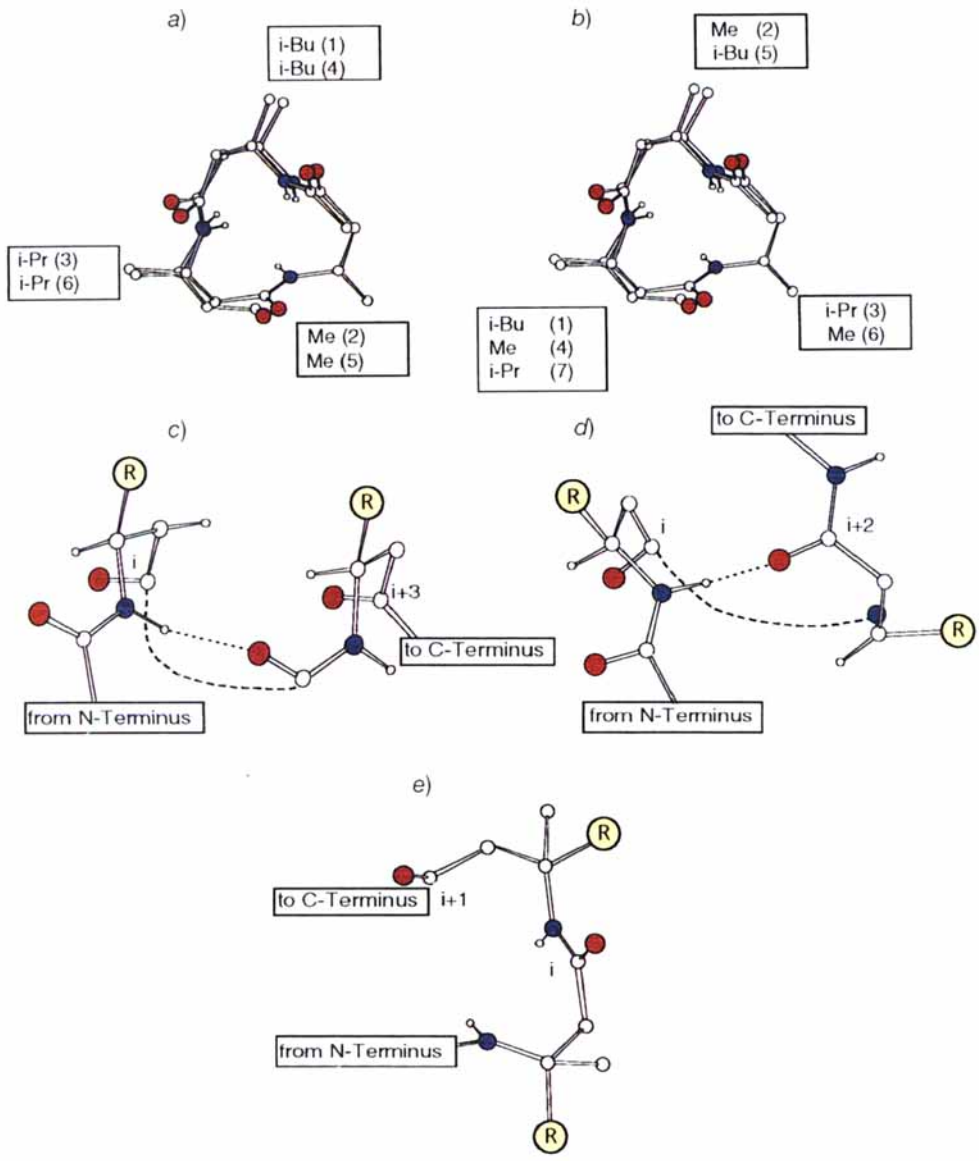


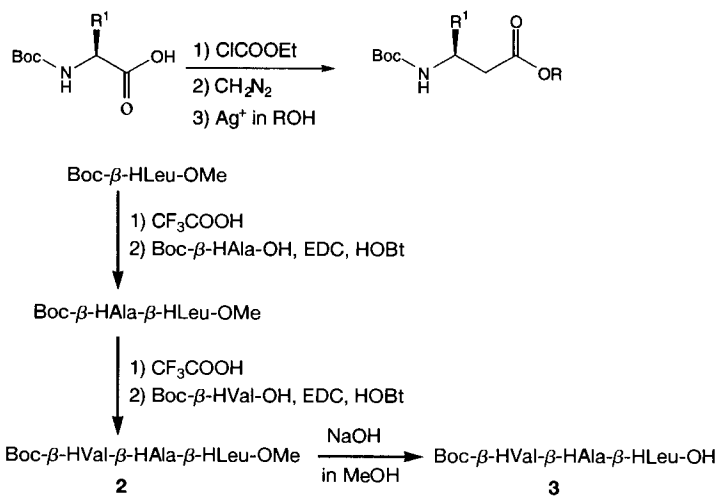
Fig. 2. *Top views along the helix axes of the β-peptide models for H(-β-HVal-β-HAla-β-HLeu)$_7$-OH* (**1**) *and H-β-HVal-β-HAla-β-HLeu-β-HAla-β-HVal-β-HAla-β-HLeu-OH* (**A**, Xaa = β-HAla) *and sections of the helix showing the relative positions of side chains R* (for modeling information and color code, see *Fig. 1*): a) *Top view of the helix of* **1** (the identical side chains Me_2CH (of β-HVal), Me (of β-HAla), and Me_2CHCH_2 (of β-HLeu) are located laterally above each other). b) *Top view of the helix of* **A** *with Xaa = β-HAla* (identical lateral side chains of, *e.g.*, β-HVal[1] and β-HVal[5], are at *ca.* 120° angles (see also text)). c) *Section of the β-peptide* 3_1 *helix* (there is a resemblance of the position of the two side chains R on amino acids *i* and (*i* + 3) with that in a β II-type turn). d) *Another section of the helix showing the relative position of substituents R on amino acids* i *and* (i + 2). e) *Section of the helix with two neighboring β-amino-acid residues* (an arrangement which could be called a 'doubly homologated β turn without H-bond').

d) Replacement of NH by O (in a β-depsipeptide!) will lead to loss of a H-bond, but should, of course, be sterically possible within the helix.

e) Omission of the CH_2 group in α-position to the carbonyl group (in a β-peptide containing a single α-amino acid!) must lead to a breakdown of the helix structure.

2. Synthesis of the β-Heptapeptides and Their Analogs. – The compounds necessary to test the predictions made in *Sect. 1* were prepared from β-amino acids (*Scheme 1*) through the β-tripeptide derivatives **2** and **3** (*Scheme 2*). Boc-protected α-amino acids were homologated by the *Arndt-Eistert* procedure as described previously by us [1] [12] and many others (see recent papers and review articles [13–15]); Boc-protected β-amino acids are thus formed in H_2O, the corresponding methyl esters in MeOH. The Boc-β-HLeu-OMe was *N*-deprotected by CF_3COOH and coupled with Boc-β-HAla-OH (EDC/HOBt)[5]), removal of the Boc group and coupling with Boc-β-HVal-OH led to the Boc-protected β-tripeptide ester **2** and subsequent saponification to the acid **3** (*Scheme 1*).

Scheme 1



The ester **2** was deprotected and coupled with the amino-acid components of interest (see *Scheme 2*): Boc-(*S*)-Ala-OH (→**4**), Boc-Aib-OH (→**5**), Boc-β-HGly-OH (→**6**), Boc-β-HAla-OH (→**7**), Boc-(*R*)-β-HAla-OH (→**8**), Boc-β-HAib-OH (→**9**), (2*S*,3*S*)- and (2*R*,3*S*)-3-amino-*N*-[(*tert*-butoxy)carbonyl]-2-methylbutanoic acid (→**10** and **11**, resp.), and Boc(Me)-β-HAla-OH (→**12**). The tetrapeptides **4**–**12** were then Boc-deprotected and coupled with the acid **3** to give the heptapeptides **13**–**21**. If desired, these could be deprotected either at the N- or at both, the N- and the C-terminus (see, *e.g.* **22** (from **19**) in *Scheme 2*).

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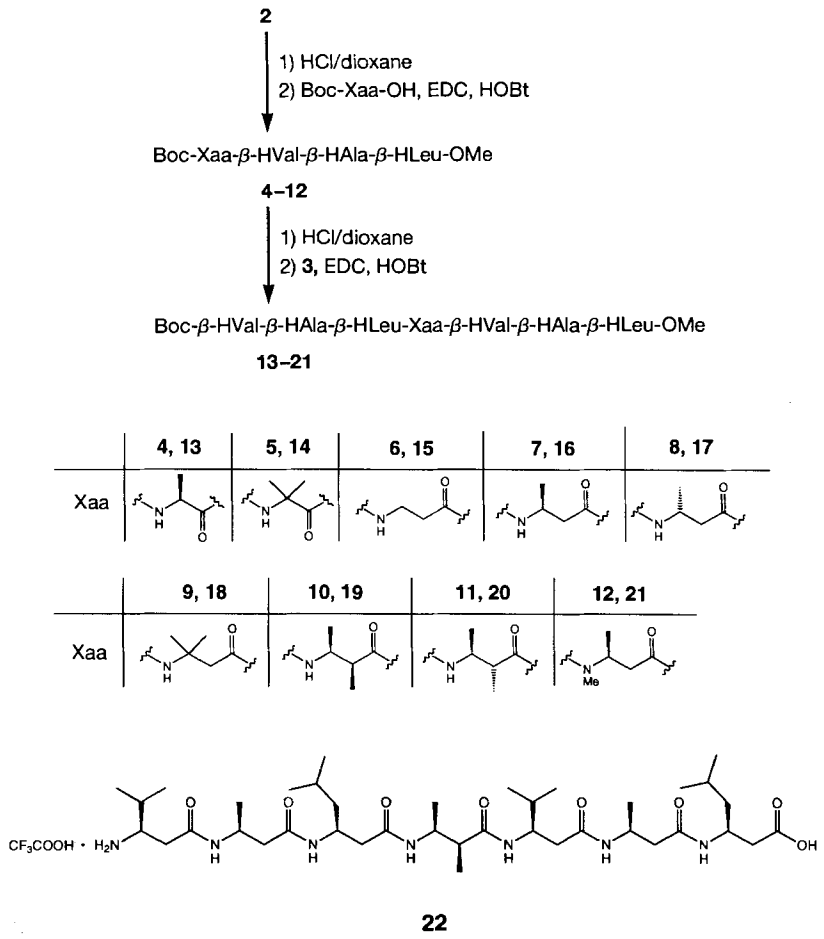
[5]) Standard peptide coupling methods; for full names of abbreviations, see *General* in the *Exper. Part*; ref. to the preparation of specific β-amino acids are given in the *Exper. Part*.

Scheme 2. *Preparation of the Tetra-*(**4**–**12**) *and Heptapeptide Derivatives* **13**–**22**. Xaa = α- or β-amino acid with various substitution patterns.



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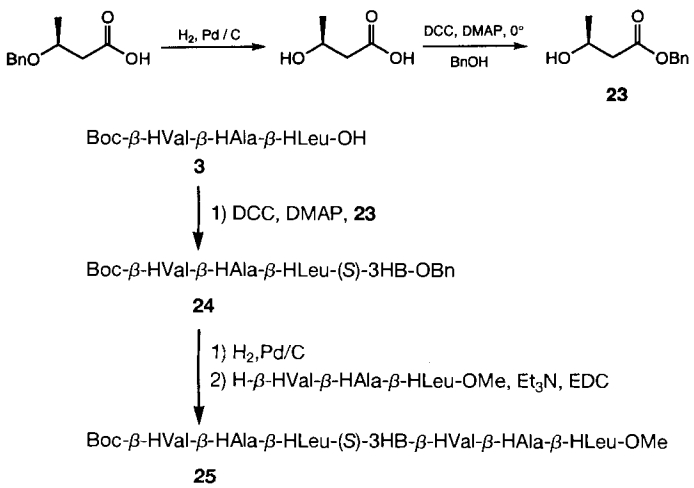
Up to the tripeptide **2**, the yields were excellent (80–90%). Due to poor solubility, the tetra- and heptapeptides had to be purified by washing the solids first with H_2O and then with MeOH (in which they are partially soluble); this usually gave yields in the range of 60–70% of samples which were uniform and pure by NMR analysis[6]), and which were used for further reactions or for structure determinations. The solubility in MeOH increases tremendously when the Boc group is removed (from *ca.* 2 mg/ml to *ca.* 50 mg/ml for the heptapeptides), the compound **22** with both termini unprotected is soluble in MeCN/H_2O 1:1. From the behavior during a melting-point determination of the Boc-

[6]) β-Heptapeptide **19** was also analyzed by hydrolysis to the component β-amino acids which were tranformed to derivatives and shown by GC analysis on a chiral column to be enantiomerically pure. This method is well established for α-peptides [16–18] and was shown previously by us to be applicable to β-peptides as well [1].

protected methyl ester **19**, we got a hint that this compound might be volatile enough for sublimation, and, indeed, we could sublime it at 180°/10^{-5} Torr and obtain a sample which gave a correct elemental analysis[7])!

For the synthesis of the β-depsipeptide **25**, we started from benzyl (*S*)-3-hydroxybutanoate (**23**; (*S*)-3HB) which was obtained as indicated in *Scheme 3* (the (*R*)-enantiomer had been described before, but was prepared differently [19]). The β-tripeptide acid **3** was then coupled with the ester **23**, and the resulting β-tetradepsipeptide **24** was debenzylated and, in turn, coupled with the β-tripeptide ester obtained by Boc-deprotection of **2** (→ **25**). While the β-depsipeptide **24** containing three β-amino-acid residues is well soluble (*e.g.* in CH_2Cl_2 or $CHCl_3$), the depsipeptide **25** is poorly soluble, similar to the β-heptapeptides **13**–**21**.

Scheme 3



3. NMR Solution Structures of the β-Hexa- and β-Heptapeptides 1 and 22 in Methanol. – So far, we have determined the structure of **1** in *pyridine* to be a left-handed helix, but we have not proved that the CD trough at *ca.* 215 nm in *methanol* is also due to a helical secondary structure. We have, therefore, now determined the NMR solution structures of the two β-peptides **1** and **22** in methanol.

The structure of $CF_3CO_2H \cdot H(\text{-}\beta\text{-HVal-}\beta\text{-HAla-}\beta\text{-HLeu})_2\text{-OH}$ (**1** · CF_3COOH) in CD_3OH was determined by NMR spectroscopy following identical procedures as for the previous study of the same molecule in pyridine [1]; DQF-COSY, ROESY, and TOCSY spectra were recorded and fully assigned. Already a qualitative inspection of the patterns of NOEs and couplings showed that the conformation in methanol is very similar to the conformation in pyridine. For example, the characteristic NOEs between the NH of

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[7]) We have pointed out before [1] that we did, so far, not encounter any problems caused by β-elimination in our work with β-amino acids and β-peptides; the sublimation conditions are another demonstration of the stability of β-peptides.

residue i and the H–C(β) of residue $i+2$ and $i+3$ were also observed. One NH/NH NOE each was detectable in the solvents, between residues 3 and 4 in MeOH or between residues 4 and 5 in pyridine. Some other differences of unambiguously assigned NOEs between the two solvents were due to resonance overlaps.

The list of NOEs used for structure calculation in methanol is given in *Table 1*. A total of 24 distance restraints were used (8 sequential restraints (*i.e.*, $|i-j| = 1$), 4 restraints with $|i-j| = 2$, 10 restraints with $|i-j| = 3$, and 2 restraints with $|i-j| = 4$; *Table 1*). In addition, 5 angle restraints were derived from the finding that all coupling constants J(NH,H–C(β)) are larger than 8 Hz. From calculations with the programme X-PLOR [20], 12 conformations were selected. All fulfill the constraints without violations larger than 0.3 Å or 5 degrees. They are shown superimposed onto the structures previously determined in pyridine [1] (*Fig. 3*). The β-peptide folds in both solvents as a 3_1 helix. The preference for a helical conformation of the β-hexapeptide H(-β-HVal-β-HAla-β-HLeu)$_2$-OH (**1**) is, therefore, verified in another, polar solvent. However, in comparison to pyridine, the structures in methanol are less precisely defined, particularly towards the C-terminus. As the number of NOEs used for the structure calculations in methanol is higher than the corresponding number in pyridine, this effect is not due to a difference in spectral quality. It is possible, although not proven at this point, that the apparent increased flexibility of the molecule in methanol is real and could be caused by the stronger competition of intramolecular H-bonds by the solvent.

Table 1. *NOEs as Observed in the ROESY NMR Spectrum of H(-β-HVal-β-HAla-β-HLeu)$_2$-OH* (**1**) *in CD_3OH.* For the structure calculations, the strong, medium, or weak NOEs were translated into upper distance limits of 3.0, 3.5, or 4.5 Å, respectively.

Residue	H-Atom(s)	Residue	H-Atom(s)	NOE
1	H–C(β)	2	NH	weak
1	Me(δ)	2	NH	weak
1	H–C(γ)	4	2H–C(α)	medium
1	2H–C(α)	4	H–C(β)	strong
1	Me(δ)	4	H–C(β)	strong
1	H–C(γ)	4	Me(δ)	weak
1	Me(δ)	4	NH	weak
1	Me(δ)	4	2H–C(α)	weak
1	H–C(γ)	5	2H–C(α)	medium
1	Me(δ)	5	NH	weak
2	Me(γ)	3	NH	weak
2	NH	4	H–C(β)	weak
2	NH	5	H–C(β)	strong
2	2H–C(α)	5	H–C(β)	strong
2	NH	5	Me(γ)	weak
3	H–C(β)	4	NH	weak
3	NH	4	NH	weak
3	NH	5	H–C(β)	medium
3	NH	6	H–C(β)	medium
4	H–C(β)	5	NH	weak
4	Me(δ)	5	NH	weak
4	NH	6	H–C(β)	weak
4	H–C(β)	6	NH	weak
5	Me(γ)	6	NH	weak

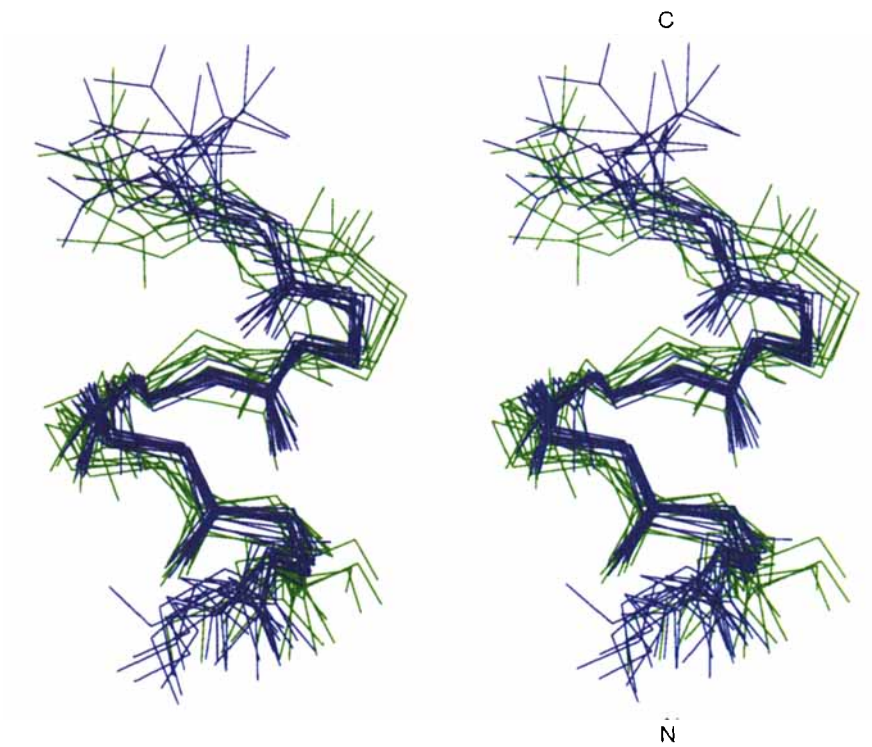

Fig. 3. *Stereo drawings of the conformations of H(-β-HVal-β-HAla-β-HLeu)$_2$-OH* (**1**) *in methanol* (green) *and in pyridine* (blue). The two sets of structures (12 for MeOH, 14 for pyridine [1]) were first best-fitted separately and then overlaid. Only backbone atoms were considered for superposition and display.

The solution structure of β-heptapeptide **22** in methanol was investigated by NMR spectroscopy using DQF-COSY, HSQC, HMBC, and ROESY experiments. The DQF-COSY and HSQC spectra allowed to assign the signals of all protons and all H-bound C-atoms to the corresponding β-amino acids β-HVal, β-HLeu, β-HAla(α-Me), or β-HAla. The sequential assignments were obtained through C–H long-range correlations (N*H*–CO–C(α)–C(β)–H) across the peptide bond. The ^{1}H- and ^{13}C-chemical shift and the ^{1}H,^{1}H-coupling constants are reported in *Tables 2* and *3*. The large *J*(NH,H–C(β)) coupling constants, the substantial difference in the chemical shifts of geminal protons at C(α), and the surprisingly slow exchange of NH protons upon dissolution of **22** in CD_3OD immediately suggested a well defined secondary structure similar to that observed for the hexapeptide **1**.

With the exception of the N-terminal NH_3^+ protons, the signal of which is too broad to show resolved coupling, all NH protons exhibit coupling constants *J*(NH,H–C(β)) between 8.7 and 9.7 Hz, which correspond to a nearly *anti*-periplanar arrangement of NH and H–C(β). For all residues, one of the two H–C(α) (labelled H_{ax}–C(α))[8]) shows a large

[8]) The notations H_{ax}–C(α) and H_{la}–C(α) are used in the NMR part to assign the signals of axial and lateral protons (see *Fig. 1*).

Table 2. *^{1}H-NMR Chemical Shifts* (CD_3OH) *and Coupling Constants for the Heptapeptide* **22**. Diastereotopic protons and Me groups are labeled with ′ and ″ in the order of decreasing ^{1}H-chemical shifts.

Residue	β-HVal[1]	β-HAla[2]	β-HLeu[3]	β-HAla(α Me)[4]	β-HVal[5]	β-HAla[6]	β-HLeu[7]
NH, NH_3	7.80 (br.)	8.53 (J(NH,β) = 9.2)	8.45 (J(NH,β) = 9.6)	8.33 (J(NH,β) = 9.3)	7.35 (J(NH,β) = 9.6)	7.64 (J(NH,β) = 8.7)	7.73 (J(NH,β) = 9.5)
H_{la}–C(α)	2.60 (J(αla,β) = 2.8, J(α,α) = 15.8)	2.45 (J(αla,β) = 4.5, J(α,α) = 15.4)	2.38 (J(αla,β) = 4.5, J(α,α) = 14.6)		2.50 (J(αla,β) = 3.9, J(α,α) = 15.2)	2.46 (J(αla,β) = 3.8, J(α,α) = 15.8)	2.57 (J(αla,β) = 4.5, J(α,α) = 16.5)
H_{ax}–C(α)	2.78 (J(αax,β) = 11.5)	2.84 (J(αax,β) = 12)	2.57 (J(αax,β) = 12.3)	2.59 (J(αax,β) = 10.8)	2.22 (J(αax,β) = 12.3)	2.30 (J(αax,β) = 11.6)	2.47 (J(αax,β) = 10.0)
Me–C(α)				1.13 (J(αax,β) = 7.0)			
H–C(β)	3.57 (J(β,γ) = 4.7)	4.41 (J(β,γ) = 6.6)	4.41	4.09 (J(β,γ) = 6.6)	4.23 (J(β,γ) = 7.0)	4.49 (J(β,γ) = 6.7)	4.43
H′–C(γ)	2.06 (J(γ,δ) = 7)		1.38		1.72 (J(γ,δ) = 7)		1.40
Me′(γ)		1.22		1.10		1.12	
H″–C(γ)			1.275 (J = 6.5)				1.30
H–C(δ)			1.58				1.60
Me′(δ)	1.08				0.934		
Me″(δ)	1.075				0.921		
Me′(ε)			0.945 (J(δ,ε) = 6.5)				0.915 (J(δ,ε) = 6.5)
Me″(ε)			0.893 (J(δ,ε) = 6.5)				0.907 (J(δ,ε) = 6.5)

Table 3. *^{13}C-NMR Chemical Shifts* (CD_3OD) *for the Heptapeptide* **22**. C-Atoms of diastereotopic Me groups are labeled with ′ and ″ in the order of decreasing chemical shifts.

	C=O	C(α)	Me–C(β)	C(γ)	C(γ)	C(δ),C′(δ)	C″(δ)	C′(ε)	C″(ε)
β-HVal[1]	171.2	35.8		55.8	32.0	19.1	17.6		
β-HAla[2]	173.2	43.2		43.5	21.4				
β-HLeu[3]	171.8	42.3		45.7	46.8	26.1		23.0	23.4
β-HAla(α Me)[4]	176.6	47.3	18.7	48.5	18.7				
β-HVal[5]	171.9	39.2		52.8	34.4	19.8	19.4		
β-HAla[6]	171.5	42.8		43.2	20.9				
β-HLeu[7]	175.0	40.7		45.4	45.7	26.1		22.8	23.6

coupling constant with H–C(β) (10–12.7 Hz), the other one (H_{la}–C(α), absent in β-HAla(αMe)[4]) only a small one. This, together with a strong intraresidual NOE between H–C(β) and H_{la}–C(α) is consistent with H_{ax}–C(α) being *anti*-periplanar and H_{la}–C(α) *syn*-clinal relative to H–C(β).

In the ROESY spectrum of **22** in CD_3OH, the NH protons of residues 2, 3, and 4 all show four NOEs to H_{ax}–C(α,$i-1$), H_{ax}–C(α,i), H–C(β,$i+2$), and H–C(β,$i+3$) (see *Table 4*). NH_3(1) shows NOEs to H_{ax}–C(α,1), H–C(β,3), and H–C(β,4), while NH(5) shows NOEs to H_{ax}–C(α,4), H_{ax}–C(α,5), and H–C(β,7). Notable are the strong NOEs between H–C(β,$i+3$) and H_{ax}–C(α,i). This characteristic pattern of NOEs, which had been observed for the hexapeptide **1** as well, clearly determines the structure as a 3_1 helix. In contrast to the NMR study of hexapeptide **1** in CD_3OD, where only one sequential d(NH,NH) NOE cross-peak was observed in the ROESY spectrum, an almost complete sequence of d(NH,NH) NOE's (the missing d(NH,NH) between NH(6) and NH(7) may be obscured by the diagonal peaks) was found for heptapeptide **22** (*Table 4*). This may indicate that the additional amino-acid residue in **22** as compared to **1** increases the stability (and thereby the conformational rigidity) of the helix.

Table 4. *Weak* (4.5 Å), *Medium* (3.5 Å), *and Strong* (3.0 Å) *NOEs Observed in the ROESY NMR Spectrum of Compound* **22** *in* CD_3OH

Residue	H-Atom(s)	Residue	H-Atom(s)	NOE	Residue	H-Atom(s)	Residue	H-Atom(s)	NOE
1	NH	1	H–C(β)	strong	4	NH	3	H_{ax}–C(α)	medium
1	NH	1	H_{ax}–C(α)	strong	4	NH	3	H_{la}–C(α)	strong
1	NH	2	NH	weak	4	NH	4	H_{ax}–C(α)[a]	
1	NH	2	H_{ax}–C(α)[a]		4	NH	4	H–C(β)	strong
1	NH	3	H–C(β)	medium	4	NH	4	H–C(γ)	strong
1	NH	4	H–C(β)	medium	4	NH	5	NH	weak
1	H–C(β)	1	H_{ax}–C(α)	strong	4	NH	6	H–C(β)	medium
1	H–C(β)	1	H_{la}–C(α)	strong	4	NH	7	H–C(β)	weak
2	NH	1	H_{ax}–C(α)	strong	4	H–C(β)	1	H_{ax}–C(α)	strong
2	NH	1	H_{la}–C(α)	strong	5	NH	4	H_{ax}–C(α)	strong
2	NH	2	H–C(β)	medium	5	NH	4	Me–C(α)	medium
2	NH	3	NH	weak	5	NH	5	H–C(β)	medium
2	NH	4	H–C(β)	medium	5	NH	5	H_{ax}–C(α)	strong
2	NH	5	H–C(β)	medium	5	NH	6	NH	medium
2	H–C(β)	2	H–C(γ)	strong	5	NH	7	H–C(β)[a]	
2	H–C(β)	2	H_{la}–C(α)	strong	5	H–C(β)	2	H_{ax}–C(α)	strong
3	NH	2	H_{ax}–C(α)	strong	5	H–C(β)	5	H–C(γ)	strong
3	NH	2	H_{la}–C(α)	medium	5	H–C(β)	5	H_{la}–C(α)	strong
3	NH	3	H–C(β)	medium	6	NH	6	H–C(β)	strong
3	NH	3	H_{ax}–C(α)	strong	6	NH	6	H_{ax}–C(α)	strong
3	NH	4	NH	weak	6	NH	5	H_{ax}–C(α)	strong
3	NH	5	H–C(β)	medium	6	H–C(β)	3	H_{ax}–C(α)	strong
3	NH	6	H–C(β)	medium	6	H–C(β)	6	H_{la}–C(α)	strong
3	H–C(β)	3	H–C(δ)	strong	7	NH	6	H_{ax}–C(α)	strong
					7	NH	7	H_{ax}–C(α)	strong

[a] Not integrable.

The kinetics of amide H-exchange in heptapeptide **22** shows that the NH protons of residues 3, 4, and 5 are surprisingly well protected from exchange (see *Table 5*). Fast exchange was observed for residue 2, suggesting either reduced helix stability, or less steric protection from solvent, or both, in the N-terminal region, particularly noticeable is the relatively slow exchange of the two NH protons in residues 6 and 7. Since, according

to the model of a monomeric 3_1 helix, these protons are not expected to participate in H-bonds, their slow exchange may signify a slightly different local backbone conformation and, possibly, H-bonding pattern in the C-terminal part of the peptide. While the available NMR data do not allow to define this conformational irregularity more precisely, we hope that an ongoing detailed molecular-dynamics study using NMR-derived constraints and explicit treatment of solvent will provide answers to this question.

Table 5. *Pseudo First-Order Rate Constants* (k) *for the Exchange of NH Protons in the β-Heptapeptide* **22** *at 24.5° in* CD_3OD

Residue		k [s^{-1}]
NH_3(1)	(N-terminus)	$\geq 10^{-2}$
NH(2)		$2.30 \cdot 10^{-3}$
NH(3)		$6.14 \cdot 10^{-5}$
NH(4)	(central β-HAla(α Me))	$5.01 \cdot 10^{-5}$
NH(5)		$4.40 \cdot 10^{-5}$
NH(6)		$3.16 \cdot 10^{-4}$
NH(7)	(C-terminus)	$2.92 \cdot 10^{-4}$

4. Circular Dichroism Spectra of the β-Heptapeptides in Methanol. – Having shown that the β-hexa- and β-heptapeptides form helical secondary structures in methanol, we are confident that the intensive trough and peak in the CD spectrum of the β-hexapeptide methyl ester [1], is in fact due to the presence of the helix. From the previous investigation [1] and from experiences collected in the meantime, we also know that the general pattern of the CD spectra does not change when we go from protected to unprotected β-peptides (see *Fig. 4, a*, with the spectra of **1**, its methyl ester, and the corresponding Boc-protected methyl ester). Thus, we used the CD spectra of the new β-heptapeptide derivatives and of their analogs for testing the predictions made in the introduction (*Sect. 1*). The following CD investigations were all carried out with solutions in MeOH.

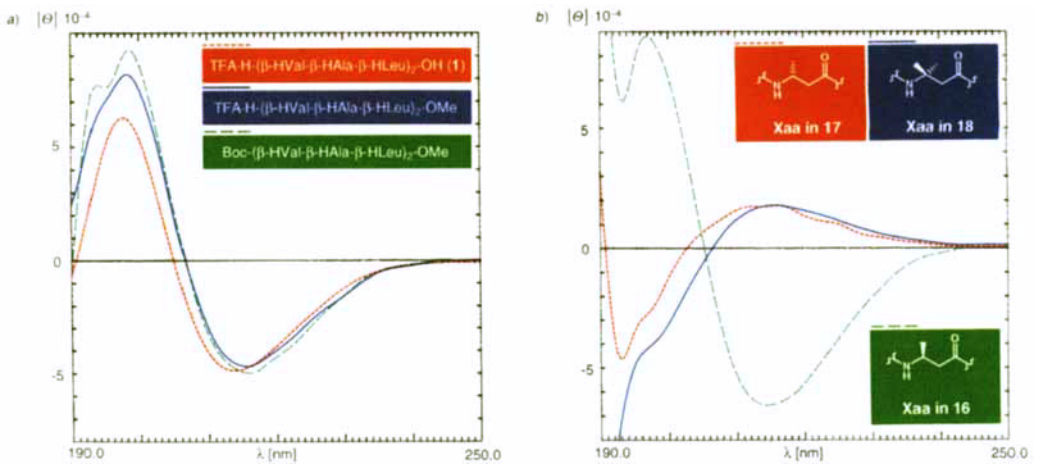

Fig. 4. *CD Spectra of the β-hexa- and β-heptapeptide derivatives* **1** *and* **16**–**18** *in MeOH:* a) *CD curves of β-hexapeptide* **1** [1], *its Me ester* (both as trifluoroacetate salts (TFA)), *and the Boc-protected Me ester* (0.2 mM). b) *Overlay of the CD spectra of the β-heptapeptides with 'correct'* (**16**) *and 'wrong'* (**17**) *configuration of the central β-HAla, and with an additional Me group* (**18**). Molar ellipticity [Θ] in 10 deg cm^2 moi^{-1}.

We first compared the CD curves obtained with the Boc-β-hexa- and -heptapeptide methyl esters (*Fig. 4, a* and *b*): the pattern is the same (trough 215/216, peak 197/197 nm), but the maxima of the β-heptapeptide are more intense ($\Theta = -4.8 \cdot 10^4$ *vs.* $-6.7 \cdot 10^4$ and $+6.2 \cdot 10^4$ *vs.* $+8.8 \cdot 10^4$). We then tested the effect of configuration reversal from (*S*) to (*R*) on the central residue β-HAla (see **16** *vs.* **17**): the characteristic CD pattern disappears, as it does when we introduce geminal dimethyl groups in the β-position of the central amino acid (see **18**) (*Fig. 4, b*). Thus, the expectation, that an axial substituent in the 3-position of a β-amino-acid residue should not be compatible with the helix structure, was borne out. Next we compared the CD spectra of the β-heptapeptides with (2*S*,3*S*)- and

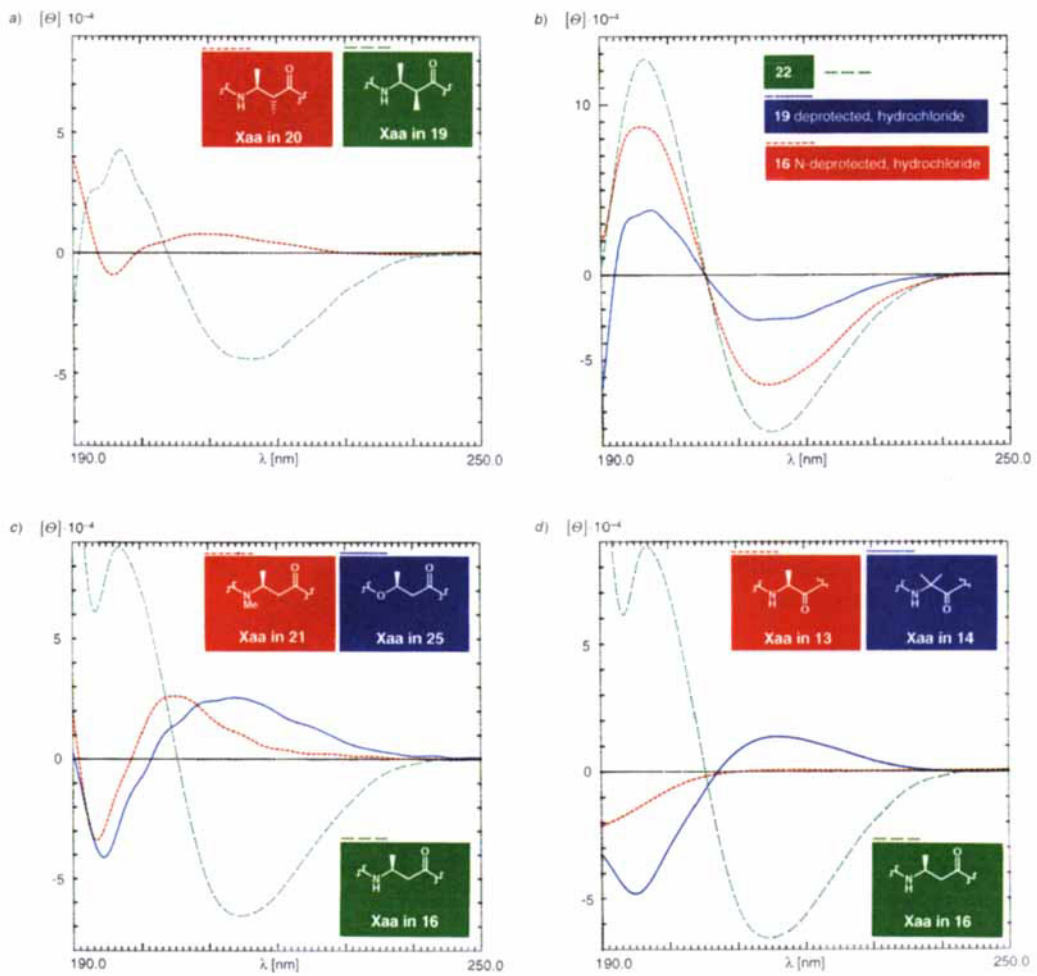

Fig. 5. *CD Spectra of terminally protected and unprotected β-heptapeptide derivatives and their analogs* **13**, **14**, **16**, **19**–**22**, *and* **25**: a) *CD spectra of the two epimers* **19** *and* **20** (additional Me group in the 2-position of the central β-amino acid). b) *CD similarities of fully deprotected β-heptapeptide* **22** (trifluoroacetate), *of the corresponding hydrochloride* (from **19**), *and of an* N-*deprotected β-heptapeptide methyl ester* (from **16**). c) *A CD comparison of the β-heptapeptide* **16** (with central β-HAla), *the NMe derivative* **21**, *and the corresponding depsipeptide* **25**. d) *Overlay of the CD spectra of the heptapeptides with central β-HAla, Ala, and Aib.* Molar ellipticity [Θ] in 10 deg cm^2 mol^{-1}.

(2*R*,3*S*)-3-amino-2-methylbutanoic-acid residues (see **19** *vs.* **20**) in the middle of the chain (*Fig. 5,a*): again, a single inversion of configuration leads to a breakdown of the intensive CD absorptions (with reversal of the signs!). The unprotected β-heptapeptide **22**, which was the subject of one of the NMR structure determinations described in *Sect. 3*, gives rise to an even more intensive CD absorption than its protected precursor ($-9.2 \cdot 10^4$ *vs.* $-4.6 \cdot 10^4$ at 215 and $+1.3 \cdot 10^5$ *vs.* $+4.5 \cdot 10^4$ at 197 nm for **22** and **19**, resp.; *cf. Fig. 5,a* and *b*). This bears with the expectation that we may have a Me group in the 2-position of a β-peptide residue if it is in a lateral (*Si*), but not if it is in an axial (*Re*) position of the *M* helix. To exemplify the already mentioned statement that the CD spectrum hardly changes upon deprotection(s) (*cf. Fig. 4,a*), we have included in the overlay of *Fig. 5,b*, some CD curves of fully and partially deprotected β-heptapeptides (from **16** and **19**).

From the spectra shown in *Fig. 5,c* and *d*, it is evident that the characteristic CD pattern which we assign to the helix structure disappears when NH in the central β-HAla residue of the β-peptide **16** is replaced by O (depsipeptide **25**) or NMe (Me-β-HAla derivative **21**), and also when the α-amino-acid residues Ala (see **13**) or Aib (a helix inducer in α-peptides; see **14**) are incorporated.

A big surprise is the CD spectrum, shown in *Fig. 6*, of the Boc-β-heptapeptide methyl ester **15** with a simple 3-aminopropanoyl unit (β-HGly) in the central position: it does not exhibit the typical trough at 215 nm; but rather a peak at 204 nm with a shoulder at 220 nm. Thus, we are confronted with the fact that β-HGly (or a single missing Me group) can have a pronounced effect on the secondary structure of a β-peptide – an intriguing resemblence with the role of glycine in α-peptides where its presence is, *e.g.*, favoring turn structures [4], and, as a consequence, leads to increased yields of the cyclization step in the synthesis of cyclopeptides [21]. How subtle the effect of the missing Me group is becomes evident from the CD spectra of the fully deprotected peptide obtained from **15** by treatment first with NaOH and then with CF_3COOH: the helix-specific trough is restored when the CD spectrum of the unprotected β-peptide is measured in MeOH, and it disappears when the solvent MeOH/H_2O 1:1 is used. The structure present in a solution of **15** showing the positive CD peak at 204 nm will be determined by NMR measurements and molecular-dynamics analyses and will be reported elsewhere.

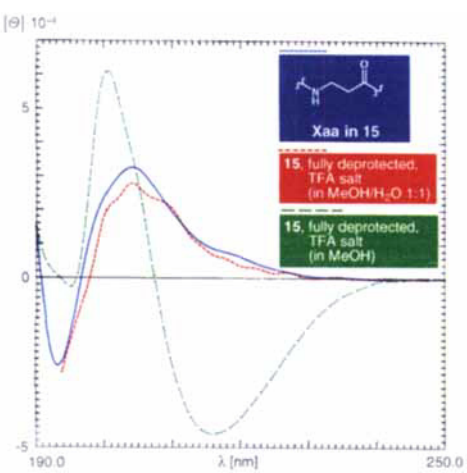

Fig. 6. *Overlay of the CD spectra of* **15** *and the corresponding unprotected β-heptapeptide* (as trifluoroacetate salts (TFA)) *in different solvents*. There is no helix-typical trough in the CD of the fully protected derivative **15**. For the free peptide, however, the presence of the secondary helix structure is indicated by the CD in MeOH. Upon addition of 10% H_2O, the trough intensity decreases to *ca.* 60%, and with 20% H_2O added, the peak at 204 nm has reached *ca.* 70% of the maximum intensity. Molar ellipticity [Θ] in 10 deg cm^2 mol^{-1}.

5. Conclusion and Outlook. – After having shown that the 3_1 helix (*M*) formed by short-chain β-peptides (built of L-β-amino acids) is stable in pyridine and methanol solution, and that it is characterized by a typical CD spectrum, we have tested its stability under the influence of structural modifications. As predicted by simple inspection of a model, axial positions (parallel to the helix axis) at C(2), C(3), and N (backbone atoms) may only be occupied by H-atoms[9]), while lateral positions (perpendicular to the helix axis) may bear larger substituents (*e.g.* Me, Me_2CH, Me_2CHCH_2). Lack of a single H-bond (in a β-depsipeptide) also leads to disappearance of the typical CD pattern.

The most surprising result is the CD spectrum of the β-heptapeptide **15** with no substituents on the central β-amino-acid residue[10]). The disappearance of the trough in this case may be due to a break-up of the helix caused by improved solvent accessibility (decrease of hydrophobic interactions between adjacent side chains).

Our search for secondary solution structures of β-peptides other than helices, *i.e.*, β-sheets, turns, tubes *etc.*, will be guided by the CD spectra reported herein. Thus, one of our next synthetic targets will be β-peptides built from β-amino acids bearing the stereogenic center in the α-position to the carbonyl group, *i.e.*, $A(-HNCH_2CH(R)C(O)-)_nB$ (*cf.* **19** and **20**), and we will determine the NMR solution structures of those compounds showing a distinct CD peak, rather than a trough, at longer wave length (*e.g.* **15** and **18**)[11]). This will eventually lead to the rules governing the structural world of β-peptides, β-proteins, and perhaps β-enzymes.

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Experimental Part

1. *General.* Abbreviations: EDC (1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride), FC (flash chromatography), *GP* (general procedure), HOBt (1-hydroxy-1*H*-benzotriazole), h.v. (high vacuum, 0.01–0.1 Torr), Boc_2O (di(*tert*-butyl) dicarbonate), DCC (*N*,*N*′-dicyclohexylcarbodiimide), DMAP (4-dimethylaminopyridine), BzCl (benzoyl chloride), BnOH (benzyl alcohol), three-letter-amino-acid abbreviations for α-amino acids, β-HXaa for β-homo-amino-acid residue. The diazo ketones of (*S*)- and (*R*)-alanine were prepared according to [12] [22]. THF was freshly distilled over K under Ar before use. DMF was distilled under reduced pressure from CaH_2 and stored over 4-Å molecular sieves. Et_3N and MeCN were distilled from CaH_2 and stored over KOH and 4-Å molecular sieves, resp., $ClCO_2Et$ was distilled and stored at −25°. Solvents for chromatography and for workup were distilled from *Sikkon*. All other chemicals for reactions were used as purchased from *Fluka*. Amino-acid derivatives were purchased from *Bachem*, *Senn*, and *Degussa*. The β-heptapeptide **22** was analyzed by GC, after hydrolysis and transformation to derivatives as described in [16] [18]. TLC: *Merck* silica gel *60* F_{254} anal. plates; detection with UV and I_2. FC: *Merck* silica gel *60* (40–63 μm). GC: *Chirasil-Val* column (*Macherey-Nagel*, 25 m, 0.4 mm); *Carlo-Erba-Fractovap 4160-HRGC*; injector temp. 220°; detector temp. 220° (FID); carrier gas:

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[9]) These axial H-atoms show the strongest nuclear *Overhauser* effects in the NMR spectra: NH on residue *i* with NH on residue *i* + 1 and axial H–C(2) of residue *i* with axial H–C(3) of residue *i* + 3 (see *Sect. 3*).

[10]) Like 'black sheeps in families', the totally unexpected (at first shocking) result is probably the most interesting one in view of eventually understanding the intricate relationship between constitution and secondary structure of β-peptides.

[11]) While the helix-typical CD pattern does normally not change upon dilution (*Sect. 1* and [1]), there are dramatic differences between the CD spectra of **18** in 0.2, 0.04, and 0.02 mM MeOH solutions.

0.5 bar H_2; temp. program: 3 min 85°, 4°/min until 190°. Anal. HPLC: *Kontron* HPLC system (UV detector *Uvicon LCD-75*, programmer *200*, integrator *Shimadzu C-R 1B Chromatopak*), *Macherey-Nagel-C_8* column (*Nucleosil 100-5 C_8*) (250 × 4 mm)). Prep. HPLC: *Knauer* HPLC system (pump typ *64*, programmer *50*, UV detector (variable-wavelength monitor), *Eurospher-80-C_8* column (*Nucleosil 100-5 C_8* (250 × 20 mm)). Melting points: *Büchi 510*; uncorrected. Optical rotations: 10-cm, 1-ml cell, *Perkin-Elmer-241* polarimeter; at r.t. Circular dichroism (CD): *Jobin-Yvon-Mark-III* system: recording between 190 and 300 nm; peptide concentrations: 0.2 mM in MeOH; molar ellipticity $[\theta]$ in deg cm^2dmol^{-1}, λ in nm. IR Spectra: *Perkin-Elmer-782* spectrophotometer. ^{1}H-NMR: *Bruker-AMX-II-500* (500 MHz), *Bruker-ARX-300* (300 MHz)-, or *Varian-Gem-200* (200 MHz) spectrometer; chemical shifts δ in ppm and coupling constants J in Hz. ^{13}C-NMR: *Bruker-AMX-II-500* (125 MHz), *Bruker-AMX-400* (100 MHz), or *Varian-XL-300* (75 MHz) spectrometer. Mass spectra: *VG Tribrid* (EI) and *Hitachi-Perkin-Elmer RMU-6M* (FAB).

2. *Solution Structure Determination of* **1** *in Methanol.* Solution structure determination: *Bruker DMX 500* (500 MHz) with 10 mg of **1** dissolved in 0.5 ml of CD_3OH; DQF-COSY, ROESY, and TOCSY spectra [23] [24] at 25°; structure calculations as in [23] [24]. Assigned peaks in ROESY spectra were conservatively classified into three categories: strong, medium, and weak, representing upper inter-proton distances of 3.0, 3.5, and 4.5 Å, resp. Dihedral restraints for 5 residues were derived from measured ^3J(NH,β) coupling constants and incorporated during the process of structure refinement. The procedure for calculating structures followed standard simulated annealing protocols as described in the X-PLOR Manual 3.0 [20]. A starting structure for X-PLOR runs was generated with the in-house-developed graphics programme WITNOTP [25]. The same programme was used to generate the topology and parameter files required for structure calculations.

3. *NMR Spectroscopy of Heptapeptide* **22**. Sample: 12 mg of **22** dissolved in 0.6 ml of CD_3OD (for ^{13}C-NMR, HSQC) or CD_3OH (for DQF-COSY, HMBC, ROESY).

1D-NMR: ^{1}H-NMR (500 MHz): suppression of the $CD_3O\mathit{H}$ signal with WATERGATE [26]; 32 K data points; 64 scans; 1.6 s acquisition time. ^{13}C-NMR (125 MHz): $\{^1H\}$-broadband decoupled; 80 K data points, 4880 transients, 1.3 s acquisition time; 45° excitation pulse; 1 s relaxation delay; processed with 0.5 Hz exponential line broadening.

2D-NMR: DQF-COSY (500 MHz, CD_3OH): with pulsed field gradients (PFG) for coherence pathway selection [27] and solvent suppression; acquisition: 2 K(t_2) × 512(t_1) data points, 4 scans per t_1 increment, 0.21 s acquisition time in t_2, max. acquisition time in t_1 0.105 s, relaxation delay 2.0 s, TPPI quadrature detection in ω_1; processing: zero filling and FT to 1 K × 1 K real/real data points after multiplication with $\sin^2$ filter shifted by $\pi/3$ in ω_2 and $\pi/2$ in ω_1. HSQC (500, 125 MHz, CD_3OD): with PFG [28]; acquisition: 2 K(t_2) × 512(t_1) data points, 2 scans per t_1 increment, ^{13}C-GARP decoupling during t_2, 0.33 s acquisition time in t_2, 0.022 s max. acquisition time in t_1, 1.5 s relaxation delay; processing: zero filling and FT to 1 K × 1 K real/real data points after multiplication with cos filter in ω_1 and $\cos^2$ filter in ω_2. HMBC [29] (500, 125 MHz, CD_3OH): with PFG; acquisition: solvent suppression by presaturation, no ^{13}C-decoupling, otherwise identical to parameters for HSQC; processing: zero filling and FT to 1 K × 1 K after multiplication with $\cos^2$ filter in ω_2 and gaussian filter in ω_1, power spectrum in both dimensions. ROESY [30] (500 MHz, CD_3OH): acquisition: a series of 4 ROESY spectra with mixing times of 50, 100, 150, and 250 ms was acquired, solvent suppression by presaturation, CW-spin lock (3.8 kHz) between trim pulses, 4 K(t_2) × 460(t_1) data points, 32 scans per t_1 increment, 0.468 s acquisition time in t_2; other parameters identical to DQF-COSY; processing: zero filling and FT to 2 K × 1 K real/real data points after multiplication by $\sin^2$ filter shifted by $\pi/3$ in ω_2 and $\cos^2$ filter in ω_1, baseline correction with 3rd degree polynomial in both dimensions.

Exchange kinetics of NH protons (^{1}H-NMR, 400 MHz, 24.5°): The sample in CD_3OH was evaporated to dryness, dried overnight, and redissolved in CD_3OD. ^{1}H-NMR spectra were taken at 12 different times (approximately doubling the interval between measurements) after dissolution, and the integrals of the NH signals relative to those of the β-protons were determined. The first-order rate constants were calculated from the slope of the plot of $\ln[I(\text{NH})/I(\text{H}-\text{C}(\beta))]$ *vs.* time.

4. *General Procedures for the Boc-Deprotection of Amino Acids* (*GP 1*). *GP 1a*: According to [31], the Boc-protected amino acid was dissolved in sat. HCl/dioxane (0.25M) at 0° (ice-bath). The mixture was allowed to warm to r.t., then stirred for 1.5 h, and evaporated. The obtained HCl salts were used without further purification.

GP 1b: According to [31], a stirred soln. of the Boc-protected amino acid in CH_2Cl_2 (0.5M) was treated at 0° (ice-bath) under Ar with an equal volume of CF_3COOH. The mixture was allowed to warm to r.t., then stirred for 1.5 h, and evaporated. The residue was dried under h.v. The CF_3COOH salts were used without further purification nor characterization.

5. *General Procedures for Peptide Coupling Using EDC (GP 2). GP 2a*: According to [32] [33], a stirred soln. of the HCl salt of the amino ester (1 equiv.) in $CHCl_3$ (0.2M) at 0° (ice-bath) under Ar was treated successively with Et_3N (3 equiv.), HOBt (1.2 equiv.), the Boc-protected amino acid (1 equiv.), and EDC (1.2 equiv.). The mixture was allowed to warm to r.t. and stirring was continued for 16 h. The mixture was diluted with $CHCl_3$ and washed with 1N HCl (thoroughly), aq. sat. $NaHCO_3$, and NaCl soln. The org. phase was dried ($MgSO_4$) and evaporated and the residue purified using FC.

GP 2b: According to [32] [33], a stirred soln. of the HCl salt of the amino ester (1 equiv.) in $CHCl_3$ (0.2M + 10% DMF) at 0° (ice-bath) under Ar was treated with Et_3N (3 equiv.). HOBt (1.2 equiv.), the Boc-protected amino acid (1 equiv.), and EDC (1.2 equiv.) were then added successively. The mixture was allowed to warm to r.t. and stirring was continued for 16 h. The mixture was evaporated and the residue dried for 4 h under h.v. and subsequently stirred for 12 h in H_2O. The white precipitate was collected by filtration and stirred again for 5 h in MeOH. The product was filtered and dried for 16 h under h.v. over P_2O_5.

*(3S)-3-[(*tert*-Butoxy)carbonylamino]butanoic Acid* (= N-*[(*tert*-Butoxy)carbonyl]-(S)-β-homoalanine;* Boc-(*S*)-β-HAla-OH). A soln. of (*S*)-3-[(*tert*-butoxy)carbonylamino]-1-diazobutan-2-one (9.5 g, 44.6 mmol) in THF (200 ml) containing 10% of H_2O at −25° (bath temp.) was treated with a soln. of silver trifluoroacetate (1.08 g, 4.9 mmol) in Et_3N (18 ml, 129.3 mmol) under Ar with the exclusion of light. The mixture was allowed to warm to r.t. within 3 h in the dark, then diluted with Et_2O (100 ml), and extracted with aq. sat. $NaHCO_3$ soln. The aq. phase was carefully adjusted to pH 2–3 at 0° with 6N HCl and extracted with Et_2O. The org. phase was dried ($MgSO_4$) and evaporated: Boc-(*S*)-β-HAla-OH (7.7 g, 85%). White solid. Spectroscopical data: in agreement with [34], $[\alpha]_D^{r.t.} = -14.1$ ($c = 1.0$, $CHCl_3$) ([34]: $[\alpha]_D^{r.t.} = -14.0$ ($c = 1.0$, $CHCl_3$)).

*(3R)-3-[(*tert*-Butoxy)carbonylamino]butanoic Acid* (= N-*[(*tert*-Butoxy)carbonyl]-(R)-β-homoalanine;* Boc-(*R*)-β-HAla-OH). As described for Boc-(*S*)-β-HAla-OH, using (*R*)-3-[(*tert*-butoxycarbonyl)amino]-1-diazobutan-2-one (1.0 g, 4.7 mmol) in THF (20 ml): Boc-(*R*)-β-HAla-OH (0.8 g, 84%). $[\alpha]_D^{r.t.} = +16.0$ ($c = 1.0$, $CHCl_3$).

*Methyl (3S)-3-[(*tert*-Butoxy)carbonylamino]butanoate* (Boc-(*S*)-β-HAla-OMe). According to [22] [35], a soln. of (*S*)-3-[(*tert*-butoxy)carbonylamino]-1-diazobutan-2-one (1.4 g, 6.5 mmol) in MeOH (25 ml) at −25° (bath temp.) under Ar with the exclusion of light was treated with a soln. of silver benzoate (0.164 g, 0.75 mmol) in Et_3N (2.63 ml, 18.85 mmol). The mixture was allowed to warm to r.t. within 3 h in the dark and then evaporated and the residue dissolved in AcOEt. After washing with aq. sat. $Na_2S_2O_3$, $NaHCO_3$, NH_4Cl, and NaCl soln., the org. phase was dried ($MgSO_4$) and evaporated. FC (hexane/AcOEt 8:2) yielded Boc-(*S*)-β-HAla-OMe (1.1 g, 72%). Colorless oil. NMR: in agreement with [36]. $[\alpha]_D^{r.t.} = -22.1$ ($c = 0.6$, $CHCl_3$) ([36]: $[\alpha]_D^{r.t.} = -20.2$ ($c = 0.54$, $CHCl_3$)).

*Methyl (2S,3S)- and (2R,3S)-3-[(*tert*-Butoxy)carbonylamino]-2-methylbutanoate* (Boc-β-HAla(α Me)-OMe). According to [35] [37], BuLi (8.3 ml, 10.3 mmol) was added to a soln. of $(i\text{-}Pr)_2NH$ (1.47 ml, 10.3 mmol) in THF (9.2 ml) at −78°. After 20 min, a soln. of Boc-(*S*)-β-HAla-OMe (1.1 g, 4.7 mmol) in THF (15 ml) was added and the mixture stirred for 1.5 h at −78°. MeI (1.2 ml, 18.8 mmol) was then added slowly (temp. monitoring (at −78°) with an internal thermometer), and the mixture was stirred for 16 h, subsequently hydrolyzed with a sat. NH_4Cl soln., diluted with Et_2O, and extracted with sat. $NaHCO_3$, NH_4Cl, and NaCl solns. The org. layer was dried ($MgSO_4$) and evaporated. FC (pentane/AcOEt 6:1, gradient) yielded the major diastereoisomer Boc-(*S,S*)-β-HAla(α Me)-OMe as a white solid (0.43 g), the minor diastereoisomer Boc-(*R,S*)-β-HAla(α Me)-OMe as a viscous oil (0.21 g), and mixed fractions (0.11 g) (71%).

Boc-(S,S)-β-HAla(αMe)-OMe: M.p. 49–50°. $[\alpha]_D^{r.t.} = -5.2$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3682, 3435, 3025, 1707, 1502, 1425, 1369, 1179, 1076, 1015. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.88 (*d*, $J = 7.6$, Me); 0.92 (*d*, $J = 7.8$, Me); 1.19 (*s*, *t*-Bu); 2.38–2.44 (*m*, CHCO); 3.44 (*s*, MeO); 3.63–3.66 (*m*, CHN); 5.06 (br. *d*, $J = 9$, NH). ^{13}C-NMR (50 MHz, $CDCl_3$): 13.2; 18.3; 27.8; 43.5; 47.7; 50.9; 78.2; 154.9; 174.9. EI-MS: 233 (0.01, $[M + 1]^+$), 232 (0.1, M^+), 231 (0.2), 216 (0.2), 158 (16), 144 (59), 88 (65), 57 (100), 44 (49).

Boc-(R,S)-β-HAla(αMe)-OMe: $[\alpha]_D^{r.t.} = -37.3$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3682, 3435, 3015, 2430, 1707, 1502, 1441, 1364, 1169, 1020. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.00 (*d*, $J = 6.9$, Me); 1.05 (*d*, $J = 7.1$, Me); 1.34 (*s*, *t*-Bu); 2.49 (*m*, CHCO); 3.58 (*s*, MeO); 3.72 (*m*, CHN); 4.89 (br. *d*, $J = 9.1$, NH). ^{13}C-NMR (50 MHz, $CDCl_3$): 13.2; 18.3; 27.81; 43.5; 47.7; 50.9; 78.3; 154.9; 174.9. EI-MS: 232 (M^+), 188 (3), 176 (3), 158 (22), 144 (67), 88 (58), 57 (100), 44 (47).

*(2S,3S)-3-[(*tert*-Butoxy)carbonylamino]-2-methylbutanoic Acid* (Boc-(*S,S*)-β-HAla(α Me)-OH). Boc-(*S,S*)-β-HAla(α Me)-OMe (70 mg, 0.3 mmol) was dissolved in MeOH (3 ml), treated with 0.62N NaOH (0.5 ml, 0.3 mmol), and then stirred for 36 h. The mixture was acidified to pH 1 with 1N HCl and extracted with AcOEt. The org. layer was dried ($MgSO_4$) and evaporated: Boc-(*S,S*)-β-HAla(α Me)-OH (50 mg, 84%). White solid. M.p. 90–91°. $[\alpha]_D^{r.t.} = -7.4$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3440, 1706, 1502, 1455, 1392, 1367, 1165, 1078, 1014. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.18 (*d*, $J = 7.0$, Me); 1.22 (*d*, $J = 7.7$, Me); 1.43 (*s*, *t*-Bu); 2.5–2.69 (*m*, CHCO); 3.69–3.80 (*m*,

CHN); 5.07 (*d*, $J = 7.1$, NH). ^{13}C-NMR (50 MHz, $CDCl_3$): 13.7; 18.9; 28.1; 43.8; 47.7; 79.1; 155.3; 180.1. FAB-MS: 435 (25, $[2M + 1]^+$), 218 (48, $[M + 1]^+$), 217 (2, M^+), 162 (100), 144 (48), 118 (38).

(2R,3S)-3-[(tert-Butoxy)carbonylamino]-2-methylbutanoic Acid (Boc-(*R,S*)-β-HAla(αMe)-OH). As described above, with Boc-(*R,S*)-β-HAla(αMe)-OMe (0.2 g, 0.9 mmol), MeOH (5 ml), and 0.62N NaOH (1 equiv.) (stirring for 6 h): Boc-(*R,S*)-β-HAla(αMe)-OH (0.18 g, 91%). White solid. M.p. 90–90.5°. $[\alpha]_D^{r.t.} = -15.4$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3445, 2980, 1707, 1503, 1455, 1392, 1367, 1162. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.06 (*d*, $J = 7.3$, Me); 1.12 (*d*, $J = 7.4$, Me); 1.35 (*s*, *t*-Bu); 2.48–2.67 (*m*, CHCO); 3.71–3.82 (*m*, CHN); 5.05 (br. *d*, $J = 7.5$, NH). ^{13}C-NMR (50 MHz, $CDCl_3$): 13.4; 16.7; 28.0; 44.0; 48.0; 79.1; 155.0; 179.4. EI-MS: 218 (0.1, $[M + 1]^+$), 217 (0.02, M^+), 176 (0.2), 158 (4), 144 (46), 88 (30), 57 (100), 44 (33).

Methyl (2S,3S)-3-(Benzoylamino)-2-methylbutanoate (Bz-(*S,S*)-β-HAla(αMe)-OMe). Boc-(*S,S*)-β-HAla(αMe)-OMe (60 mg, 0.26 mmol) was Boc-deprotected according to *GP 1a*. The corresponding HCl salt was then dissolved in CH_2Cl_2 (1.5 ml) and treated at 0° with Et_3N (0.044 ml, 0.31 mmol), BzCl (0.036 ml, 0.31 mmol), and a catal. amount of DMAP. The mixture was stirred for 12 h and washed with sat. NaCl soln. The aq. phase was then extracted with CH_2Cl_2 (2×) and the org. layer dried ($MgSO_4$) and evaporated: Bz-(*S,S*)-β-HAla(αMe)-OMe (43 mg, 70%). $[\alpha]_D^{r.t.} = -32.2$ ($c = 1.0$, $CHCl_3$) ([37]: $[\alpha]_D^{r.t.} = +33.7$ ($c = 0.98$, $CHCl_3$)). Spectroscopic data: identical to that of the compound described in [37], but with opposite sign of optical rotation.

3-[(tert-Butoxy)carbonylamino]-3-methylbutanoic Acid (Boc-β-HAib-OH). For 1 h, 5,6-dihydro-6,6-dimethyluracil (prepared according to [38]; a sample recrystallized from EtOH melted at 204° ([38]: m.p. 202°)) (2.89 g, 0.02 mol) was heated in boiling aq. 1N NaOH (200 ml). The mixture was then cooled, concentrated under reduced pressure, and treated with 11 ml of conc. HCl. A soln. of Boc_2O (5.23 g, 0.024 mol) in dioxane (40 ml) was then added and stirring continued for 12 h. The pH value was adjusted to 9 with 1N HCl and the excess Boc_2O extracted with Et_2O. The aq. phase was then acidified to pH 2 and extracted with Et_2O. The org. layer was dried (MgSO) and evaporated: Boc-β-HAla(βMe)-OH (2.4 g, 65%). NMR: in agreement with [39]. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.40 (*s*, 2 Me); 1.45 (*s*, *t*-Bu); 2.73 (*s*, 2 CHCO). EI-MS: 218 (0.13, $[M + 1]^+$), 217 (0.2, M^+), 202 (15), 158 (28), 144 (22), 102 (100), 57 (65).

Benzyl (3S)-3-Hydroxybutanoate (**23**). A soln. of (3*S*)-3-(benzyloxy)butanoic acid (prepared as for its enantiomer described in [40]; 3 g, 15.4 mmol) in AcOEt (30 ml) was hydrogenated using 200 mg Pd/C (10% Pd) as catalyst. After completion of the reaction (5 h), AcOEt (10 ml) was added, the catalyst removed by filtration through *Celite*, the solvent evaporated, and the solid residue used without further purification. (3*S*)-3-Hydroxybutanoic acid (0.8 g, 7.7 mmol) in $CHCl_3$ (18 ml) was slowly added to a stirred soln. of BnOH (1.6 ml, 15.4 mmol), DCC (1.58 g, 7.7 mmol), and DMAP (93 mg, 0.77 mmol) in $CHCl_3$ (16 ml) at 0°. The mixture was stirred for 6 h, filtered, and evaporated. The oily residue was purified by FC (hexane/AcOEt 7:3) and the excess BnOH removed by bulb-to-bulb distillation (6 h at 60°/h.v.): **23** (1.33 g, 89%). Oil. NMR: in agreement with [19].

Methyl (3S)-{(3S)-3-[(tert-Butoxy)carbonylamino]butanoylamino}-5-methylhexanoate (Boc-β-HAla-β-HLeu-OMe). Following *GP 2a*, methyl (3*S*)-3-[(*tert*-butoxy)carbonylamino]-5-methylhexanoate (prepared as in [1]; 7.8 g, 30.1 mmol) was Boc-deprotected according to *GP 1b*. The obtained crude CF_3COOH salt was treated with Boc-β-HAla-OH (6.9 g, 30.1 mmol) to yield Boc-β-HAla-β-HLeu-OMe (9.2 g, 90%) after FC. M.p. 122–123°. $[\alpha]_D^{r.t.} = -37.8$ ($c = 1.0$, $CHCl_3$). Spectroscopic data: in agreement with [1].

Methyl (3S)-3-{(3S)-3-{(3S)-3-[(tert-Butoxy)carbonylamino]-4-methylpentanoylamino}butanoylamino}-5-methylhexanoate (Boc-β-HVal-β-HAla-β-HLeu-OMe; **2**). Boc-β-HAla-β-HLeu-OMe (8.0 g, 23.5 mmol) was Boc-deprotected following *GP 1b*. The obtained CF_3COOH salt was treated according to *GP 2a* with Boc-β-HVal-OH (prepared as in [1]) (5.3 g, 23.5 mmol). FC ($MeOH/CHCl_3$, 15:85) yielded **2** (9.1 g, 88%). White microcrystalline solid. M.p. 178–179°. $[\alpha]_D^{r.t.} = -38.1$ ($c = 1.0$, $CHCl_3$). Spectroscopical data: in agreement with [1].

(3S)-3-{(3S)-3-{(3S)-3-[(tert-Butoxy)carbonylamino]-4-methylpentanoylamino}butanoylamino}-5-methylhexanoic Acid (Boc-β-HVal-β-HAla-β-HLeu-OH; **3**). According to [41], a soln. of **2** (0.80 g, 1.75 mmol) in MeOH (1.2M) was treated with 0.75N NaOH (2.8 ml, 2.1 mmol) at r.t. After 4 h, the mixture was adjusted to pH 2–3 with 1N HCl and extracted with AcOEt. The org. phase was dried ($MgSO_4$) and evaporated: **3** (0.78 g, 97%). White microcrystalline solid. M.p. 190–191°. $[\alpha]_D^{r.t.} = -8.87$ ($c = 0.53$, MeOH). Spectroscopical data: in agreement with [1].

Methyl N-[(tert-Butoxy)carbonyl]-(S)-alanyl-(R)-β-homovalyl-(S)-β-homoalanyl-(S)-β-homoleucinate (Boc-Ala-β-HVal-β-HAla-β-HLeu-OMe; **4**). Compound **2** (0.27 g, 0.6 mmol) was Boc-deprotected according to *GP 1a* and the resulting HCl salt coupled with commercially available Boc-Ala-OH (0.12 g, 0.6 mmol) according to *GP 2b*: **4** (0.28 g, 90%). White solid. M.p. 200–201°. $[\alpha]_D^{r.t.} = -34.6$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3428, 1663, 1499, 1368, 1165, 1080. ^{1}H-NMR: (300 MHz, $CDCl_3$): 0.87–0.93 (*m*, 2 Me_2C); 1.17 (*d*, $J = 6.5$, Me); 1.32 (*d*, $J = 6.8$, Me); 1.41 (*s*, *t*-Bu); 1.23–1.86 (*m*, 4 CH); 2.32–2.57 (*m*, 3 CH_2CO); 3.7 (*s*, MeO); 3.89–4.01 (*m*, CHN); 4.14–4.38 (*m*, CHN); 5.05 (br. *d*, $J = 6.8$, NH); 6.33 (br. *d*, $J = 9$, NH); 6.90–7.11 (*m*, 2 NH). ^{13}C-NMR (75 MHz, $CDCl_3$):

14.2; 18.4; 18.7; 19.5; 19.8; 21.1; 22.1; 22.9; 25.0; 28.4; 31.8; 39.2; 42.4; 42.9; 43.1; 44.5; 50.5; 51.8; 52.6; 60.5; 67.2; 80.1; 155.8; 170.9 (2 C); 172.5; 172.9. FAB-MS: 1057 (1.5, $[2M + 1]^+$), 529 (100, $[M + 1]^+$), 528 (7.2, M^+), 430 (12), 429 (34), 314 (14), 245 (12), 229 (17), 185 (14), 182 (14), 160 (13), 101 (5).

*Methyl {2-[(*tert*-Butoxy)carbonylamino]-2-methylpropanoyl}-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucinate* (Boc-Aib-β-HVal-β-HAla-β-HLeu-OMe; **5**). Compound **2** (0.43 g, 1.1 mmol) was Boc-deprotected according to *GP 1a* and the resulting HCl salt coupled with commercially available Boc-Aib-OH (0.22 g, 1.1 mmol) according to *GP 2a*. FC ($CHCl_3$/MeOH 85:15) yielded **5** (0.58 g, 97%). White solid. M.p. 182–183°. $[\alpha]_D^{r.t.} = -29.9$ ($c = 1.0$, $CHCl_3$): 3428, 1721, 1660, 1502, 1368, 1158, 1082. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.88–0.94 (*m*, 2 Me_2C); 1.20 (*d*, $J = 6.7$, Me); 1.23–1.91 (*m*, 4 CH); 1.43 (*s*, *t*-Bu); 1.49 (*s*, 2 Me); 2.19–2.54 (*m*, 3 CH_2CO); 3.67 (*s*, MeO); 3.88–4.05 (*m*, CHN); 4.15–4.42 (*m*, 2 CHN); 5.05 (br. *s*, $J = 6.8$, BocN*H*); 6.42 (br. *d*, $J = 6.9$, NH); 6.99 (br. *d*, $J = 7.1$, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 18.4; 19.5; 20.1; 22.1; 22.9; 25.0; 25.7; 28.3; 32.0; 39.2; 39.4; 43.2; 44.4; 52.1; 56.8; 80.2; 154.6; 170.5; 170.6; 172.2; 174.3. FAB-MS: 1107 (3.5, $[2M + 1 + Na]^+$), 543 (100, $[M + 1]^+$), 542 (7.2, M^+), 443 (31), 358 (27), 245 (12), 199 (14), 102 (10), 101 (5).

*Methyl {3-[(*tert*-Butoxy)carbonylamino]propanoyl}-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucinate* (Boc-β-HGly-β-HVal-β-HAla-β-HLeu-OMe; **6**). Compound **2** (0.22 g, 0.5 mmol) was Boc-deprotected according to *GP 1a* and the resulting HCl salt coupled with commercially available 3-[(*tert*-butoxy)carbonylamino]propanoic acid (95 mg, 0.5 mmol) according to *GP 2b*: **6** (0.18 g, 70%). White solid. M.p. 193–195°. $[\alpha]_D^{r.t.} = -11.5$ ($c = 1.0$ $CHCl_3$). IR ($CHCl_3$): 3312, 1733, 1684, 1638, 1539, 1438, 1366, 1282, 1179, 1140, 1113. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.91–0.98 (*m*, 2 Me_2C); 1.13 (*d*, $J = 6.6$, Me); 1.20–1.83 (*m*, 4 CH); 1.42 (*s*, *t*-Bu); 2.14–2.53 (*m*, 4 CH_2CO); 3.64 (*s*, MeO); 4.09–4.38 (*m*, 5 CHN). ^{13}C-NMR (75 MHz, CD_3OD): 18.6; 19.8; 20.1; 22.2; 23.6; 26.1; 28.8; 33.3; 37.5; 38.3; 40.1; 41.1; 43.6; 44.6; 46.1; 52.3; 53.6; 61.1; 156.4; 172.9; 173.2; 173.7; 173.8. FAB-MS: 529 (77, $[M + 1]^+$), 528 (14, M^+), 429 (100), 407 (12), 322 (13), 245 (9), 170 (11), 128 (21), 111 (8), 101 (2).

Methyl N-*[(*tert*-Butoxy)carbonyl]-(*S*)-β-homoalanyl-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucinate* (Boc-β-HAla-β-HVal-β-HAla-β-HLeu-OMe; **7**). Compound **2** (0.45 g, 0.98 mmol) was Boc-deprotected according to *GP 1a* and the resulting HCl salt treated with Boc-(*S*)-β-HAla-OH (0.21 g, 0.98 mmol) according to *GP 2b*: **7** (0.35 g, 66%). White amorphous solid. M.p. 207–208°. $[\alpha]_D^{r.t.} = -18.5$ ($c = 1.0$ CF_3CH_2OH). IR (KBr): 3302, 2872, 1738, 1683, 1641, 1545, 1437, 1367, 1251, 1178, 1099, 1060, 1031. ^{1}H-NMR (200 MHz, CD_3OD): 0.9 (*d*, $J = 6$, 2 Me_2C); 1.18 (*d*, $J = 8.0$, 2 Me); 1.26–1.83 (*m*, 4 CH); 1.43 (*s*, *t*-Bu); 2.16–2.55 (*m*, 4 CH_2CO); 3.64 (*s*, MeO); 3.86–4.33 (*m*, 4 CHN). ^{13}C-NMR (50 MHz, CD_3OD): 15.5; 16.7; 17.1; 18.0; 19.2; 20.5; 23.1; 25.8; 30.2; 36.9; 37.9; 40.5; 41.0; 41.6; 43.03; 49.2; 50.5; 77.22; 154.2; 169.8; 170.2; 170.3; 170.6. FAB-MS: 1085.8 (1.2, $[2M + 1]^+$), 543.4 (71.7, $[M + 1]^+$), 443.4 (100), 245.2 (40.9), 156.1 (36.6), 114.09 (22.0).

Methyl N-*[(*tert*-Butoxy)carbonyl]-(*R*)-β-homoalanyl-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucinate* (Boc-(*R*)-β-HAla-β-HVal-β-HAla-β-HLeu-OMe; **8**). Compound **2** (0.82 g, 1.8 mmol) was Boc-deprotected according to *GP 1a* and the resulting HCl salt treated with Boc-(*R*)-β-HAla-OH (0.36 g, 1.8 mmol) according to *GP 2b*: **8** (0.65 g, 67%). White amorphous solid. M.p. 230–231°. $[\alpha]_D^{r.t.} = -17.3$ ($c = 1.0$, CF_3CH_2OH). IR (KBr): 3302, 2872, 1738, 1683, 1641, 1545, 1437, 1367, 1251, 1178, 1099, 1060, 1031. ^{1}H-NMR (200 MHz, CD_3OD): 0.90 (*d*, $J = 6.4$, Me_2C); 0.91 (*d*, $J = 6.7$, Me_2C); 1.13 (*d*, $J = 6.7$, Me); 1.14 (*d*, $J = 6.6$, Me); 1.24–1.82 (*m*, 4 CH); 1.41 (*s*, *t*-Bu); 2.10–2.58 (*m*, 4 CH_2CO); 3.64 (*s*, MeO); 3.80–4.39 (*m*, 4 CHN). ^{13}C-NMR (100 MHz, CD_3OD/CF_3CH_2OH): 19.9; 20.0; 21.6; 22.4; 22.5; 23.7; 23.9; 26.1; 29.0; 33.7; 40.4; 41.3; 43.9; 44.5; 45.0; 46.2; 52.6; 53.9; 80.9; 159.0; 173.2; 173.4; 173.7; 174.1. FAB-MS: 565.4 (10.7, $[M + 23]^+$), 543.4 (50.8, $[M + 1]^+$), 542.3 (4.2, M^+), 443.4 (100), 245.2 (15.9), 156.1 (14.0), 114.09 (7.8).

*Methyl {3-[(*tert*-Butoxy)carbonylamino]-3-methylbutanoyl}-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucinate* (Boc-β-HAib-β-HVal-β-HAla-β-HLeu-OMe; **9**). Compound **2** (1.05 g, 2.3 mmol) was Boc-deprotected according to *GP 1a* and the resulting HCl salt coupled with 3-[(*tert*-butoxy)carbonylamino]-3-methylbutanoic acid (0.5 g, 2.3 mmol) according to *GP 2a*. FC ($CHCl_3$/MeOH 4:1) yielded **9** (1.02 g, 85%). White solid. M.p. 188–190°. $[\alpha]_D^{r.t.} = -27.9$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3431, 3005, 2099, 1708, 1656, 1500, 1453, 1367, 1170, 1080. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.90 (*d*, $J = 6.0$, 2 Me_2C); 1.19 (*d*, $J = 6.0$, Me); 1.24–1.82 (*m*, 4 CH); 1.36 (*d*, $J = 2$, 2 Me); 1.41 (*s*, *t*-Bu); 2.13–2.59 (*m*, 4 CH_2CO); 3.67 (*s*, MeO); 3.90–4.34 (*m*, 3 CHN); 5.48 (*s*, NH-Boc); 6.31 (br. *d*, $J = 8.0$, NH); 6.56 (br. *d*, $J = 10$, NH); 7.00 (br. *d*, $J = 10$, NH). ^{13}C-NMR (50 MHz, CD_3OD): 18.8; 19.4; 19.9; 22.1; 22.8; 27.6; 28.5; 31.8; 39.1; 42.0; 43.2; 43.3; 44.4; 47.16; 51.7; 51.9; 79.0; 155.1; 170.4; 170.5; 170.7; 172.3. FAB-MS: 1113 (1, $[2M + 1]^+$), 557 (98, $[M + 1]^+$), 556 (10, M^+), 457 (100), 358 (26), 245 (70), 196 (34), 160 (39).

Methyl N-*[(*tert*-Butoxy)carbonyl]-(2*S*,3*S*)-2-methyl-β-homoalanyl-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucinate* (Boc-(*S*,*S*)-β-HAla(α-Me)-β-HVal-β-HAla-β-HLeu-OMe; **10**). Compound **2** (1.4 g, 3 mmol) was Boc-deprotected according to *GP 1a* and the resulting HCl salt coupled with (2*S*,3*S*)-3-[(*tert*-butoxy)carbonylamino]-2-methylbutanoic acid (0.64 g, 3 mmol) according to *GP 2b*: **10** (1.01 g, 65%). White amorphous solid.

M.p. 183–184°. $[\alpha]_D^{r.t.} = -4.7$ ($c = 1.0$, MeOH). IR (KBr): 3854, 3751, 3676, 3649, 3302, 2972, 1742, 1690, 1646, 1542, 1457, 1367, 1249, 1173, 1081, 1022. ^{1}H-NMR (200 MHz, CD_3OD): 0.88 (*d*, $J = 6.6$, Me_2C); 0.9 (*d*, $J = 6.4$, Me_2C); 1.08–1.17 (*m*, 3 Me); 1.2–1.91 (*m*, 4 CH); 1.41 (*s*, *t*-Bu); 2.25–2.62 (*m*, 7 CHCO); 3.6 (*s*, MeO); 3.63–3.76 (*m*, CHN); 3.91–4.16 (*m*, CHN); 4.17–4.35 (*m*, 2 CHN); 5.86 (br. *d*, $J = 9.9$, NH); 6.27 (br. *d*, $J = 8.7$, NH); 6.80 (*d*, $J = 8.2$, NH); 7.05 (br. *d*, $J = 10$, NH). ^{13}C-NMR (75 MHz, CD_3OD): 14.7; 17.9; 18.8; 19.0; 21.5; 22.5; 24.5; 27.9; 31.5; 38.2; 39.2; 41.9; 42.9; 44.4; 48.7; 51.3; 51.6; 78.8; 155.8; 170.6; 170.9; 171.8; 175.0. FAB-MS: 1113 (1, $[2M + 1]^+$), 1011 (2), 557 (29, $[M + 1]^+$), 556 (4, M^+), 457 (100).

Methyl N-*[(*tert-*Butoxy)carbonyl]-(2*R*,3*S*)-2-methyl-β-homoalanyl-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucinate* (Boc-(*R*,*S*)-β-HAla(α Me)-β-HVal-β-HAla-β-HLeu-OMe; **11**). Compound **2** (0.34 g, 0.74 mmol) was Boc-deprotected according to *GP 1a* a and the resulting HCl salt coupled with (2*R*,3*S*)-3-[(*tert*-butoxy)carbonylamino]-2-methylbutanoic acid (0.16 g, 0.74 mmol) according to *GP 2b*: **11** (0.183 g, 60%). White solid. M.p. 285° (dec.). $[\alpha]_D^{r.t.} = -10.8$ ($c = 1.0$, CF_3CH_2OH). IR (KBr): 3302, 1753, 1684, 1646, 1534, 1458, 1368, 1251, 1170, 1072, 1020. ^{1}H-NMR (300 MHz, CD_3OD): 0.88–0.92 (*m*, 2 Me_2C); 1.08–1.20 (*m*, 3 Me); 1.22–1.81 (*m*, 4 CH); 1.43 (*s*, *t*-Bu); 2.23–2.55 (*m*, 7 CHCO); 3.64 (*s*, MeO); 3.69–3.71 (*m*, CHN); 4.04–4.22 (*m*, 2 CHN); 4.27–4.32 (*m*, CHN). ^{13}C-NMR (75 MHz, CD_3OD/CF_3CH_2OH): 21.8; 22.0; 24.0; 25.9; 28.6; 31.2; 35.9; 43.1; 43.4; 46.0; 48.8; 153.4; 175.6; 175.9; 176.7; 180.0. FAB-MS: 1113 (1, $[2M + 1]^+$), 1013 (2), 557 (100, $[M + 1]^+$), 556 (8, M^+), 457 (88).

Methyl N-*[(*tert-*Butoxy)carbonyl]-(S)*-N-*methyl-β-homoalanyl-(R)-β-homovalyl-(S)-β-homoalanyl-(S)-β-homoleucinate* (Boc(Me)-β-HAla-β-HVal-β-HAla-β-HLeu-OMe; **12**). Compound **2** (0.21 g, 0.46 mmol) was Boc-deprotected according to *GP 1a* and the resulting HCl salt coupled with *N*-[(*tert*-butoxy)carbonyl]-*N*-methyl-(*S*)-β-homoalanine (0.1 g, 0.46 mmol) was described in *GP 2a*. FC ($CHCl_3$/MeOH 85:15) yielded **12** (0.21 g, 85%). White solid. M.p. 167–168°. $[\alpha]_D^{r.t.} = -38.4$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3427, 3010, 2965, 2433, 1727, 1666, 1502, 1438, 1367, 1177. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.88 (*d*, $J = 6.7$, Me_2C); 0.89 (*d*, $J = 6.4$, Me_2C); 1.14 (*d*, $J = 7.1$, Me); 1.18 (*d*, $J = 7.2$, Me); 1.23–1.83 (*m*, 4 CH); 1.43 (*s*, *t*-Bu); 2.23–2.56 (*m*, 4 CH_2CO); 2.86 (*s*, MeN); 3.65 (*s*, MeO); 3.92–4.16 (*m*, CHN); 4.19–4.32 (*m*, 2 CHN); 4.45–4.59 (*m*, CHN); 6.51 (br. *d*, $J = 7.1$, NH); 6.8 (br. *d*, $J = 7.2$, NH); 7.06 (br. *d*, $J = 7$, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 17.8; 19.5; 21.5; 22.7; 22.9; 24.6; 28.2; 31.1; 39.4; 42.4; 42.5; 42.8; 44.1; 44.3; 51.3; 76.97; 79.1; 155.2; 170.0; 170.1; 171.6; 171.7. FAB-MS: 1086 (3), 544 (24, $[M + 1]^+$), 543 (68, M^+), 557 (32), 556 (6), 543 (68), 443 (100), 245 (43), 156 (38).

Methyl N-*[(*tert-*Butoxy)carbonyl]-(R)-β-homovalyl-(S)-β-homoalanyl-(S)-β-homoleucyl-(S)-alanyl-(R)-β-homovalyl-(S)-β-homoalanyl-(S)-β-homoleucinate* (Boc-β-HVal-β-HAla-β-HLeu-Ala-β-HVal-β-HAla-β-HLeu-OMe; **13**). Compound **4** (50 mg, 0.1 mmol) was Boc-deprotected according to *GP 1a* and the resulting HCl salt treated with **3** (0.04 g, 0.1 mmol) according to *GP 2b*: **13** (0.07 g, 80%). White amorphous solid. M.p. 274°. (dec.). $[\alpha]_D^{r.t.} = -13.6$ ($c = 0.5$, CF_3CH_2OH). CD (0.2 mM in MeOH): $+1.30 \cdot 10^4$ (216). IR (KBr): 3300, 3080, 1741, 1688, 1640, 1542, 1458, 1367, 1310, 1248, 1174, 1146, 1044, 1021. ^{1}H-NMR (200 MHz, CD_3OD): 0.90–0.93 (*m*, 4 Me_2C); 1.24 (*d*, $J = 5$, 2 Me); 1.33 (*d*, $J = 7$, Me); 1.42 (*s*, *t*-Bu); 1.20–1.82 (*m*, 8 CH); 2.11–2.58 (*m*, 6 CH_2CO); 3.65 (*s*, MeO); 3.70–3.81 (*m*, CHN); 3.97–4.40 (*m*, 6 CHN). ^{13}C-NMR (75 MHz, CF_3CD_2OD): 14.3; 14.5; 14.8; 15.7; 15.8; 16.2; 18.3; 19.4; 19.6; 22.5; 25.0; 30.4; 37.3; 40.9; 41.5; 47.1; 49.3; 50.6; 78.6 (Me_3COCO); 155.6 (Me_3COCO); 170.3 (2C); 170.6; 170.9; 171.9; 172.1 (2 C). FAB-MS: 855 (48, $[M + 1]^+$), 854 (42 M^+), 755 (100), 754 (93), 641 (20), 370 (12), 358 (14), 245 (29), 243 (21), 225 (12), 198 (19), 196 (25), 182 (34), 170 (32), 154 (48), 140 (20), 136 (21), 128 (64), 112 (38), 111 (47), 101 (8).

Methyl N-*[*(tert-*Butoxycarbonyl)-(R)-β-homovalyl-(S)-β-homoalanyl-(S)-β-homoleucyl-(2-amino-2-methylpropanoyl)-(R)-β-homovalyl-(S)-β-homoalalnyl-(S)-β-homoleucinate* (Boc-β-HVal-β-HAla-β-HLeu-Aib-β-HVal-β-HAla-β-HLeu-OMe; **14**). Compound **5** (0.9 g, 1.7 mmol) was Boc-deprotected according to *GP 1a* and the resulting HCl salt treated with **3** (0.72 g, 1.7 mmol) according to *GP 2b*: **14** (0.87 g, 64%). White amorphous solid. M.p. 230° (dec.). $[\alpha]_D^{r.t.} = -7.1$ ($c = 0.5$, CF_3CH_2OH). CD (0.2 mM in MeOH): $+0.30 \cdot 10^4$ (213). IR (KBr): 3443, 1712, 1503, 1454, 1392, 1368, 1166, 1081. ^{1}H-NMR (300 MHz, CD_3OD): 0.86–0.94 (*m*, 4 Me_2C); 1.15 (*d*, $J = 6.6$, 2 Me); 1.21–1.89 (*m*, 8 CH); 1.42 (*s*, *t*-Bu); 1.43 (*s*, Me); 2.17–2.49 (*m*, 6 CH_2CO); 3.64 (*s*, MeO); 3.71–3.79 (*m*, CHN); 3.85–3.95 (*m*, CHN); 4.10–4.33 (*m*, 4 CHN). ^{13}C-NMR (75 MHz, CD_3OD): 18.3; 19.4; 19.7; 19.9; 20.2; 22.1; 23.6; 25.3; 26.0; 28.8; 33.4; 33.7; 39.3; 40.4; 40.9; 43.8; 44.2; 44.5; 44.7; 58.1; 79.9; 172.6; 172.7; 173.0; 173.1; 173.2; 173.3; 176.4. FAB-MS: 868 (60, $[M + 1]^+$), 867 (6, M^+), 768 (100), 655 (28), 358 (12), 341 (38), 326 (28), 255 (36), 245 (45), 225 (24), 168 (25),126 (68), 128 (85).

Methyl N-*[(*tert-*Butoxy)carbonyl]-(R)-β-homovalyl-(S)-β-homoalanyl-(S)-β-homoleucyl-(3-aminopropanoyl)-β-homovalyl-(S)-β-homoalanyl-(S)-β-homoleucinate* (Boc-β-HVal-β-HAla-β-HLeu-β-HGly-β-HVal-β-HAla-β-HLeu-OMe; **15**). Compound **6** (63 mg, 0.12 mmol) was Boc-deprotected according to *GP 1a* and the resulting HCl salt treated with **3** (43 mg, 0.12 mmol) according to *GP 2b*: **15** (0.61 g, 60%). White amorphous solid. M.p. 300° (dec.). $[\alpha]_D^{r.t.} = -4.4$ ($c = 0.5$, CF_3CH_2OH). CD (0.2 mM in MeOH): $+3.0 \cdot 10^4$ (215). IR (KBr): 3304,

1735, 1680, 1645, 1528, 1458, 1368, 1312. ^{1}H-NMR (300 MHz, CD_3OD): 0.90–1.02 (*m*, 4 Me_2C); 1.12–1.16 (*m*, 2 Me); 1.22–1.81 (*m*, 8 CH); 1.42 (*s*, *t*-Bu); 2.12–2.53 (*m*, 7 CH_2CO); 3.65 (*s*, MeO); 3.74–3.82 (*m*, 4 CHN); 4.14–4.21 (*m*, 4 CHN). ^{13}C-NMR (75 MHz, CF_3CD_2OD): 11.4; 12.1; 13.2; 13.1; 15.5; 16.4; 19.7; 21.6; 26.7; 27.0; 33.9; 34.0; 36.4; 37.8; 38.4; 40.5; 40.3; 46.2; 46.3; 47.6; 48.5; 52.7; 74.9; 152.3; 166.5; 166.9 (2C); 167.2; 167.8; 168.4; 168.7. FAB-MS: 854 (11, $[M + 1]^+$), 853 (6, M^+), 754 (100), 641 (15), 182 (18), 154 (11), 128 (23), 113 (10), 110 (15).

Methyl N-*[(*tert-*Butoxy)carbonyl]-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucyl-(*S*)-β-homoalanyl-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucinate* (Boc-β-HVal-β-HAla-β-HLeu-β-HAla-β-HVal-β-HAla-β-HLeu-OMe; **16**). Compound **7** (0.15 g, 0.27 mmol) was Boc-deprotected according to *GP 1a* and the resulting HCl salt treated with **3** (0.12 g, 0.27 mmol) according to *GP 2b*: **16** (0.15 g, 64%). White amorphous solid. M.p. 279° (dec.). $[\alpha]_D^{r.t.} = -18.2$ (*c* = 1.0, CF_3CH_2OH). CD (0.2 mM in MeOH): $+8.78 \cdot 10^4$ (197), $-6.67 \cdot 10^4$ (215). IR (KBr): 3854, 3751, 3676, 3649, 3301, 1740, 1688, 1646, 1540, 1438, 1367, 1314, 1249, 1175, 1144, 1045, 1021. ^{1}H-NMR (300 MHz, CD_3OD): 0.92 (*d*, *J* = 6.4, 4 Me_2C); 1.11–1.18 (*m*, 3 Me); 1.19–1.87 (*m*, 8 CH); 1.44 (*s*, *t*-Bu); 2.12–2.61 (*m*, 7 CH_2CO); 3.65 (*s*, MeO); 3.71–3.88 (*m*, CHN); 4.08–4.41 (*m*, 6 CHN). ^{13}C-NMR (75 MHz, CF_3CD_2OD): 11.2; 11.6; 12.2; 13.0; 13.1; 15.2; 16.1; 19.3; 21.8; 26.6; 27.2; 33.8; 33.9; 34.0; 36.6; 37.8; 38.2; 40.2; 40.3; 46.0; 46.1; 47.2; 48.5; 52.9; 74.8; 152.1; 166.6; 166.8 (2C); 167.0; 167.6; 168.2; 168.6. FAB-MS: 890 (15, $[M + Na]^+$), 869 (4, $[M + 1]^+$), 868 (20, M^+), 768 (100), 655 (20), 182 (18), 128 (29), 111 (16).

Methyl N-*[(*tert-*Butoxy)carbonyl]-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucyl-(*R*)-β-homoalanyl-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucinate* (Boc-β-HVal-β-HAla-β-HLeu-(*R*)-β-HAla-β-HVal-β-HAla-β-HLeu-OMe; **17**). Compound **8** (80 mg, 0.15 mmol) was Boc-deprotected according to *GP 1a* and the resulting HCl salt treated with **3** (65 mg, 0.15 mmol) according to *GP 2b*: **17** (0.08 g, 63%). White amorphous solid. M.p. 290° (dec.). $[\alpha]_D^{r.t.} = -7.8$ (*c* = 0.5, CF_3CH_2OH). CD (0.2 mM in MeOH): $+2.64 \cdot 10^4$ (205). IR (KBr): 3854, 3751, 3676, 3297, 3079, 1740, 1688, 1646, 1543, 1438, 1367, 1311, 1249, 1174, 1145, 1051. ^{1}H-NMR (300 MHz, CD_3OD): 0.88–0.94 (*m*, 4 Me_2C); 1.13–1.17 (*m*, 3 Me); 1.27–1.88 (*m*, 8 CH); 1.41 (*s*, *t*-Bu); 2.15–2.47 (*m*, 7 CH_2CO); 3.64 (*s*, MeO); 3.70–3.81 (*m*, 1 CHN); 4.13–4.40 (*m*, 6 CHN). ^{13}C-NMR (75 MHz, CF_3CD_2OD): 10.4; 10.8; 11.8; 12.3; 12.4; 12.5; 14.4; 15.5; 15.6; 18.6; 21.0; 25.9; 26.4; 32.7; 33.3; 35.8; 36.2; 37.4; 37.6; 38.9; 39.0; 39.7; 45.3; 46.6; 47.7; 52.2; 74.3; 151.8; 165.9; 166.0; 166.1; 166.2; 166.4; 166.8; 167.8. FAB-MS: 890 (19, $[M + Na]^+$), 869 (5, $[M + 1]^+$), 868 (25.5, M^+), 768 (100), 655 (43), 570 (17), 182 (14), 128 (25), 112 (14).

Methyl N-*[(*tert-*Butoxy)carbonyl]-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucyl-(3-amino-3-methylbutanoyl)-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucinate* (Boc-β-HVal-β-HAla-β-HLeu-β-HAib-β-HVal-β-HAla-β-HLeu-OMe; **18**). Compound **9** (0.30 g, 0.54 mmol) was Boc-deprotected according to *GP 1a* and the resulting HCl salt treated with **3** (0.24 g, 0.54 mmol) according to *GP 2b*: **18** (0.31 g, 70%). White amorphous solid. M.p. 220–222° (dec.). $[\alpha]_D^{r.t.} = -22.8$ (*c* = 1.0, CF_3CH_2OH). CD (0.2 mM in MeOH): $+2.53 \cdot 10^4$ (214). IR (KBr): 3854, 3751, 3676, 3649, 3303, 1740, 1686, 1646, 1540, 1458, 1367, 1310, 1249, 1175, 1144, 1050, 1022. ^{1}H-NMR (200 MHz, CD_3OD): 0.88–0.93 (*m*, Me_2C); 1.12–1.17 (*m*, Me_2C); 1.20–1.98 (*m*, 8 CH); 1.37 (*d*, *J* = 6.7, 2 Me); 1.42 (*s*, *t*-Bu); 2.13–2.59 (*m*, 7 CH_2CO); 3.64 (*s*, MeO); 3.68–3.71 (*m*, CHN); 4.10–4.30 (*m*, 5 CHN). ^{13}C-NMR (75 MHz, CD_3OD): 18.3; 18.6; 19.7; 20.2; 22.0; 22.2; 23.6; 25.8; 26.0; 26.6; 27.4; 28.8; 33.1; 33.8; 39.7; 40.0; 40.3; 40.9; 41.4; 43.4; 43.9; 44.1; 44.4; 44.6; 45.9; 46.6; 47.9; 50.4; 50.5; 52.1; 54.8; 79.9; 155.8; 172.4; 172.5; 172.6 (2C); 172.7; 173.1; 173.3. FAB-MS: 882 [31, $M + 1]^+$), 881 (6, M^+), 782 (100), 669 (14).

Methyl N-*[(*tert-*Butoxy)carbonyl]-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucyl-(2*S*,3*S*)-2-methyl-β-homoalanyl-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucinate* (Boc-β-HVal-β-HAla-β-HLeu-(*S*,*S*)-β-HAla(α Me)-β-HVal-β-HAla-β-HLeu-OMe; **19**). Compound **10** (0.6 g, 1.0 mmol) was Boc-deprotected according to *GP 1a* and the resulting HCl salt treated with **3** (0.443 g, 1.0 mmol) according to *GP 2b*: **19** (0.55 g, 62%). White amorphous solid. For the microanalysis **19** was sublimated at 180°/10^{-5} mbar. M.p. 275° (dec.). $[\alpha]_D^{r.t.} = -16.5$ (*c* = 1.0, CF_3CH_2OH). CD (0.2 mM in MeOH): $+4.48 \cdot 10^4$ (198), $-4.60 \cdot 10^4$ (216). IR (KBr): 3292, 3076, 1733, 1687, 1646 1538, 1446, 1364, 1307, 1241, 1174, 1143. ^{1}H-NMR (300 MHz, CD_3OD): 0.90–0.93 (*m*, 4 Me_2C); 1.08–1.17 (*m*, 4 Me); 1.21–1.56 (*m*, 6 CH); 1.43 (*s*, *t*-Bu); 1.59–1.69 (*m*, CH); 1.71–1.83 (*m*, CH); 2.18–2.63 (*m*, 13 CHCO); 3.68 (*s*, MeO); 3.79–3.85 (*m*, CHN); 4.00–4.16 (*m*, CHN); 4.20–4.42 (*m*, 5 CHN). ^{13}C-NMR (75 MHz, CF_3CD_2OD): 10.8; 11.7; 12.5; 12.8; 14.7; 14.9; 15.8; 18.8; 21.3; 26.3; 26.7; 32.3; 33.1; 33.4; 35.5; 35.8; 37.4; 37.6; 38.9; 39.1; 39.6; 45.5; 46.4; 47.9; 52.5; 71.6; 151.6; 166.0; 166.1; 166.3; 167.1; 168.1; 170.5; 170.6. FAB-MS: 1785 (5, $[2M + 1 + Na]^+$), 882 (13, $[M + 1]^+$), 881 (3, M^+), 904 (64), 782 (100), 196 (20), 128 (37). Anal. calc. for $C_{45}H_{83}N_7O_{10}$ (881): C 61.27, H 9.63, N 11.11; found: C 61.26, H 9.63, N 11.14.

Methyl N-*[(*tert-*Butoxy)carbonyl]-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucyl-(2*R*,3*S*)-2-methyl-β-homoalanyl-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucinate* (Boc-β-HVal-β-HAla-β-HLeu (*R*,*S*)-β-HAla(α Me)-β-HVal-β-HAla-β-HLeu-OMe; **20**). Compound **11** (0.15 g, 0.27 mmol) was Boc-deprotected according to *GP 1a* and the resulting HCl salt treated with **3** (0.12 g, 0.27 mmol) according to *GP 2b*: **20**

(0.11 g, 50%). White amorphous solid. M.p. 303° (dec.). $[\alpha]_D^{r.t.} = -3.1$ ($c = 0.5$, CF_3CH_2OH). CD (0.2 mM in MeOH): $+0.60 \cdot 10^4$ (203). IR (KBr): 3292, 1740, 1646, 1533, 1446, 1369, 1307, 1174, 1138. ^{1}H-NMR (300 MHz, CD_3OD): 0.87–0.92 (*m*, 4 Me_2C); 1.09–1.20 (*m*, 2 Me); 1.23–1.35 (*m*, 2 Me); 1.41 (*s*, *t*-Bu); 1.43–1.85 (*m*, 8 CH); 2.15–2.47 (*m*, 13 CHCO); 3.64 (*s*, MeO); 3.67–3.81 (*m*, CHN); 4.06–4.36 (*m*, 6 CHN). ^{13}C-NMR (75 MHz, CF_3CD_2OD): 10.4; 10.9; 11.8; 12.4; 14.4; 15.5; 18.6; 21.1; 25.9; 26.5; 33.2; 33.4; 35.8; 36.1; 36.8; 36.9; 37.5; 37.6; 39.2; 39.6; 41.4; 41.9; 45.4; 46.3; 47.8; 52.3; 74.6; 150.2; 166.0; 166.2; 166.3; 166.5; 166.9; 167.9; 169.9. FAB-MS: 904 (38, $[M + Na]^+$), 882 (17, $[M + 1]^+$), 881 (8, M^+), 782 (100), 669 (32), 584 (17), 170 (18), 128 (28), 111 (19).

Methyl N-*[(*tert-*Butoxy)carbonyl]-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucyl-(*S*)-*N*-methyl-β-homoalanyl-(*R*)-β-homovalyl-(*S*)-β-homoalanyl)-(*S*)-β-homoleucinate* (Boc-β-HVal-β-HAla-β-HLeu-Me-β-HAla-β-HVal-β-HAla-β-HLeu-OMe; **21**). Compound **12** (0.21 g, 0.37 mmol) was Boc-deprotected according to *GP 1a* and the resulting HCl salt treated with **3** (0.165 g, 0.37 mmol) according to *GP 2b*: **21** (0.20 g, 65%). White amorphous solid. M.p. 200° (dec.). $[\alpha]_D^{r.t.} = -10.9$ ($c = 1.0$, MeOH). CD (0.2 mM in MeOH): $+1.97 \cdot 10^4$ (203). IR (KBr): 3306, 3079, 2480, 1645, 1542, 1457, 1367, 1311, 1249, 1175, 1145, 1052, 1020. ^{1}H-NMR (300 MHz, CD_3OD): 0.89 (*m*, 4 Me_2C); 1.11–1.20 (*m*, 3 Me); 1.27–1.82 (*m*, 8 CH); 1.43 (*s*, *t*-Bu); 2.11–2.60 (*m*, 7 CH_2CO); 2.72 (*s*, 1.5 H, MeN, rotamer); 2.91 (*s*, 1.5 H, MeN, rotamer); 3.64 (*s*, MeO); 3.71–3.87 (*m*, CHN); 3.98–4.38 (*m*, 5 CHN); 4.48–4.65 (*m*, CHN). ^{13}C-NMR (75 MHz, CD_3OD): 17.6; 18.4; 18.6; 18.7; 19.1; 19.7; 20.1; 22.1; 23.7; 23.8; 26.1; 27.2; 28.6; 28.9; 30.5; 30.6; 32.9; 33.3; 33.8; 39.6; 39.7; 40.2; 40.5; 40.6; 41.1; 43.5; 43.6; 43.9; 44.4; 44.5; 44.6; 45.0; 46.0; 46.1; 46.3; 51.4; 52.2; 53.3; 53.5; 54.9; 79.8; 79.9; 158.0; 172.1; 172.3; 172.4; 172.5; 172.6; 173.1; 173.3. FAB-MS: 882 (24, $[M + 1]^+$), 881 (3, M^+), 782 (100), 669 (10), 210 (13), 182 (12), 128 (15), 111 (13).

(R)-β-Homovalyl-(S)-β-homoalanyl-(S)-β-homoleucyl-(2S,3S)-2-methyl-β-homoalanyl-(R)-β-homovalyl-(S)-β-homoalanyl-(S)-β-homoleucine Trifluoroacetate (H-β-HVal-β-HAla-β-HLeu-(*S*,*S*)-β-HAla(αMe)-β-HVal-β-HAla-β-HLeu-OH·CF_3COOH; **22**). A soln. of **19** (0.16 g, 0.18 mmol) in CF_3CCH_2OH (0.125M) was treated with 5N NaOH (100 equiv.) and heated at 80° (bath temp.) for 24 h. The mixture was neutralized with *Dowex-H*$^+$*50×8*. The ion exchanger was removed by filtration and the filtrate evaporated. The residue was then dissolved in CF_3COOH (0.25M) under Ar. After stirring for 2 h at r.t., the mixture was evaporated and the residue dried under h.v. The oily residue was triturated with Et_2O, and a colorless foam was obtained (0.135 g, 87%). The peptide was purified by prep. reversed-phase HPLC (MeCN/H_2O (H_2O containing 0.1% of CF_3COOH) gradient, *i.e.* 30 s 1:9, 12 min 1:1, 16 min 1:1, 20 min 1:9, and 22 min 1:9). M.p. 105° (dec.). $[\alpha]_D^{r.t.} = -2.0$ ($c = 1.0$, CF_3CH_2OH). CD (0.2 mM in MeOH): $+1.28 \cdot 10^5$ (197), $-9.20 \cdot 10^4$ (215). IR (KBr): 3298, 1654, 1559, 1458, 1375, 1201, 1140. ^{1}H-NMR (300 MHz, CD_3OD): 0.88–0.96 (*m*, 4 Me_2C); 1.06–1.83 (*m*, 20 H); 1.98–2.98 (*m*, 13 CHCO); 3.52–3.70 (*m*, CHN); 4.09–4.12 (*m*, CHN); 4.19–4.24 (*m*, CHN); 4.41–4.61 (*m*, CHN); 7.37 (br. *d*, $J = 9.3$, NH); 7.67 (br. *d*, $J = 8.7$, NH); 7.75 (br. *d*, $J = 9.3$, NH); 8.35 (br. *d*, $J = 9.0$, NH); 8.46 (br. *d*, $J = 9.0$, NH). ^{13}C-NMR (75 MHz, CD_3OD): 17.7; 18.7; 19.1; 19.5; 19.9; 21.0; 21.5; 22.8; 23.1; 23.5; 23.6; 26.0; 32.0; 34.46; 35.8; 39.2; 40.7; 42.2; 42.8; 43.3; 43.6; 45.3; 45.7; 46.9; 52.7; 55.8; 171.1; 171.4; 171.7 (2 C); 173.2; 174.9; 176.4. FAB-MS: 868 (8.4, $[M + 1]^+$), 867 (8.3, M^+), 808.4 (12.6), 807.4 (22.8), 806.4 (25.1), 771.5 (23.8), 770.5 (54.8), 769.4 (94.89), 768.4 (100), 154.1 (13.7), 128.1 (21.9).

*Benzyl (3S)-{*N*-[(*tert*-Butoxy)carbonyl]-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucyloxy}butanoate* (Boc-β-HVal-β-HAla-β-HLeu-(*S*)-3HB-OBn; **24**). A soln. of **23** (0.25 g, 1.75 mmol) in $CHCl_3$ (4 ml) and DMF (2 ml) was successively treated with DMAP (0.015 g, 0.18 mmol), DCC (0.26 g, 1.75 mmol), and **3** (0.55 g, 1.75 mmol). The mixture was then stirred for 16 h, diluted with $CHCl_3$ filtrated, and evaporated. FC (100% AcOEt) gave **24** (0.61 g, 80%). White solid. M.p. 135–136°. $[\alpha]_D^{r.t.} = -1.90$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3609, 3434, 1729, 1477, 1465, 1374, 1262, 1162, 1096. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.88 (*d*, $J = 6.7$, 2 Me_2C); 1.19 (*d*, $J = 6.3$, Me); 1.27 (*d*, $J = 6.4$, Me); 1.42 (*s*, *t*-Bu); 1.36–1.85 (*m*, 4 CH); 2.19–2.71 (*m*, 4 CH_2CO); 3.61–3.78 (*m*, CHN); 4.18–4.39 (*m*, 2 CHN); 5.11 (*s*, PhCH_2O); 5.18–5.34 (*m*, CHO); 6.43 (br. *d*, $J = 7.6$, NH); 6.88 (br. *d*, $J = 7.9$, NH); 7.30–7.40 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 18.1; 19.1; 19.4; 19.6; 21.8; 21.9; 22.5; 22.7; 28.1; 31.9; 38.9; 40.2; 41.7; 42.6; 42.8; 43.9; 53.1; 66.2; 67.2; 78.8; 127.9; 128.1; 128.3; 135.2; 155.7; 169.8; 170.2 (2 C); 170.6. FAB-MS: 1240 (2, $[2M + 1]^+$), 620 (48, $[M + 1]^+$), 619 (4, M^+), 522 (11), 520 (100), 407 (12), 322 (13), 243 (9), 170 (11), 128 (21), 111 (8), 101 (2).

*(3S)-{*N*-[(*tert*-Butoxy)carbonyl]-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucyloxy}butanoic Acid* (Boc-β-HVal-β-HAla-β-HLeu-(*S*)-3HB-OH). A soln. of **24** (0.37 g, 0.5 mmol) in AcOEt (3 ml) was hydrogenated using 40 mg Pd/C (10% Pd) as catalyst. After completion of the reaction (3 h), AcOEt (4 ml) was added and the catalyst removed by filtration over *Celite*. The solvent was evaporated and the solid residue (0.23 g) used without further purification.

Methyl N-*[(*tert*-Butoxy)carbonyl]-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucyl-[(*S*)-3-hydroxybutanoyl]-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucinate* (Boc-β-HVal-β-HAla-β-HLeu-(*S*)-3HB-β-HVal-β-HAla-β-HLeu-OMe; **25**). Compound **2** (0.11 g, 0.28 mmol) was Boc-deprotected according to *GP 1a* and

the resulting HCl salt treated with **24** (0.15 g, 0.28 mmol) as described in *GP 2b*: **25** (0.15 g, 65%). White amorphous solid. M.p. 120–122° (dec.). $[\alpha]_D^{r.t.} = -12.9$ ($c = 0.5$, CF_3CH_2OH). CD (0.2 mM in MeOH): $+1.85 \cdot 10^4$ (215). IR (KBr): 3306, 1735, 1685, 1646, 1544, 1458, 1367, 1309, 1177. ^{1}H-NMR (200 MHz, CD_3OD): 0.88–0.93 (*m*, 4 Me_2C); 1.12 (*d*, $J = 6.5$, Me); 1.14 (*d*, $J = 6.5$, Me); 1.23 (*d*, $J = 6.3$, Me); 1.28–1.73 (*m*, 8 CH); 1.43 (*s*, *t*-Bu); 2.16–2.55 (*m*, 7 CH_2CO); 3.65 (*s*, MeO); 3.70–3.87 (*m*, CHN); 4.11–4.34 (*m*, 5 CHN); 5.22–5.32 (*m*, CHO). ^{13}C-NMR (75 MHz, CD_3OD): 18.4; 18.7; 19.8; 20.1; 22.1; 23.7; 26.1; 28.7; 28.9; 33.5; 33.8; 40.2; 40.5; 41.1; 41.8; 43.1; 43.5; 43.7; 44.2; 44.4; 45.9; 52.2; 53.5; 54.9; 69.4; 79.8; 158.0; 171.7; 172.2; 172.3; 172.5; 172.6; 173.1; 173.3. FAB-MS: 869 (42, $[M + 1]^+$), 769 (100), 684 (23), 683 (43), 656 (20), 571 (14), 358 (12), 341 (10), 326 (11), 255 (24), 245 (60), 243 (24).

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